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Act. 400 OPOSIOBSERVTER Folios: 133 ✓

**FORMULARIO UNICO DE OPOSICION
2000-12**

1	OPOSICION A:		
☒	Patente de Invención	☐	Marca Colectiva
☐	Patente de Modelo de Utilidad	☐	Marca de Certificación
☐	Diseño Industrial	☐	Lema Comercial
☐	Esquema de Trazado para Circuitos Integrados		
☐	Marcas de Productos o Servicios		

2	SOLICITUD PUBLICADA	Número del expediente: 07 028.993 Solicitante: COLL FARMA S.L. Apoderado: DANILO ROMERO RAAD Título de la nueva creación o denominación del signo: UNA COMPOSICIÓN FARMACÉUTICA SÓLIDA QUE COMPRENDE AL TIAZOLIL METIL ESTER DEL ÁCIDO [5S-(5R*,8R*,11R*)]-10-HIDROXI- 2-METIL-5-(1-METILETIL)-1-[2-(1-METILETIL)-4-TIAZOLIL]-3,6-DIOXO- 8,11-BIS(FENILMETIL)-2,4,7,12-TETRAAZATRIDEKAN-13-OICO Y UN PROCEDIMIENTO PARA PREPARARLA. Gaceta 588 de 31 DE ENRO DE 2008, N° de publicación 1630.
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3	OPOSICION PRESENTADA POR:	
OPOSITOR	Nombre: TECNOQUIMICAS S.A., Sociedad comercial organizada y existente a las leyes Colombianas, con domicilio y sede principal en la Ciudad de Cali Valle. Dirección: Calle 23 # 7-39 Cali - Valle Teléfono: 882 5555 Fax: 883 8859 E-mail: Domicilio: Cali - Valle	IDENTIFICACION C.C. ☐ NIT ☒ C.E. ☐ Otro ☐ Número: 890.300.466-5
REPRESENTANTE O APODERADO (74)	Nombre: José Luis Reyes Villamizar Dirección: Cra. 17 No. 88-23, Of. 205 Teléfono: (1) 621.26.31 E-mail: info@reyes-abogados.com Fax: (1) 621.25.42 Domicilio: Bogotá, D.C.	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Número: 79'152.473 de Usaquén TP 44655

4	FUNDAMENTOS DE LA OPOSICION:
	Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000). Se violan los artículos 14, (las patentes de invención deben ser nuevas, tener nivel inventivo y ser susceptibles de aplicación industrial) 16, 18 (una invención tiene nivel inventivo si no es obvia para una persona versada en la materia). Artículo 30 (Reivindicaciones) y artículo 42 (oposiciones) de la Decisión 486 de la Comisión de la Comunidad Andina. Justificación: Ver Anexo

5

Comprobante de pago No. 08-34.138
08-34.139(Oposición) Fecha Abril 28 de 2008
(Prórroga) Fecha Abril 28 de 2008

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Anexos:

Comprobante de pago de la tasa



Poderes, si fuere el caso



Pruebas de los fundamentos de la oposición



Documentos que acrediten el legítimo interés, si fuere el caso



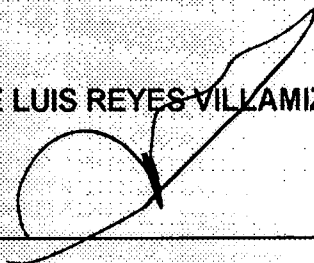
Certificado de existencia y representación legal de las partes, cuando sean personas jurídicas



Copia de la oposición para el traslado al solicitante.

7

NOMBRE: JOSÉ LUIS REYES VILLAMIZAR

FIRMA: 

REYES & REYES

A B O G A D O S

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente 07 028.993

TÍTULO SOLICITADO: UNA COMPOSICIÓN FARMACÉUTICA SÓLIDA QUE
 COMPRENDE AL TIAZOLIL METIL ESTER DEL
 ÁCIDO [5S-(5R*,8R*,11R*)]-10-HIDROXI-2-METIL-5-
 (1-METILETIL)-1-[2-(1-METILETIL)-4-TIAZOLIL]-3,6-
 DIOXO-8,11-BIS(FENILMETIL)-2,4,7,12-
 TETRAAZATRIDEKAN-13-OICO Y UN
 PROCEDIMIENTO PARA PREPARARLA.

PUBLICACIÓN: Gaceta 588 de 31/01/2008, N° de publicación 1630.

PRIORIDAD: AR (AR) 20040103504 de 28/09/2004

SOLICITANTE: COLL FARMA S.L.

APODERADO: DANILO ROMERO RAAD

TRÁMITE: PRESENTACIÓN DE OPOSICION

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al
 pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655
 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la
 sociedad **TECNOQUÍMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42 de
 la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente 07
 028.993, por no cumplir con lo dispuesto por los artículos 14, 16 y 18 de la

Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUÍMICAS S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTO DE LA OPOSICIÓN

La solicitud en curso, que reivindica una composición farmacéutica sólida en forma de comprimidos (con o sin cubierta), capsulas rígidas o suspensión extemporánea, que contiene como principio activo Ritonavir (en su forma polimorfica I), solo o en combinación con otros agentes tales como lopinavir, saquinavir, indinavir, tenofovir, amprenavir, fosamprenavir, atazanavir y tripanavir; carece por completo de los requisitos fundamentales para otorgársele el privilegio de patente de invención, tal y como lo demuestran los siguientes argumentos:

3.1 Siendo Ritonavir un fármaco ampliamente reconocido como antiretroviral del grupo de los inhibidores de la proteasa¹ y siendo utilizado en la terapia recombinante antiretroviral activa de adultos y pacientes pediátricos con infección por VIH-1 y enfermos de SIDA desde que fue aprobado por la FDA el 01 de marzo de 1996², la formulación de dicho fármaco en formulaciones sólidas para administración oral se encuentra revelada con anterioridad en el estado de la técnica, en los términos de las publicaciones que explicarán a continuación:

¹ Literature References: Peptidomimetic HIV-1 protease inhibitor. Prepn: D. J. Kempf et al., WO 94 14436; eidem, US 5541206 (1994, 1996 both to Abbott). Antiretroviral spectrum, pharmacokinetics: idem et al., Proc. Nat. Acad. Sci. USA 92, 2484 (1995). Structural model for drug resistance: M. Markowitz et al., J. Virol. 69, 701 (1995). HPLC determn in biological fluids: R. M. W. Hoetelmans et al., J. Chromatog. B 705, 119 (1998). Review of clinical experience: A. P. Lea, D. Faulds, Drugs 52, 541-546 (1996). Clinical trial with nucleoside analogs in HIV-infected children: S. A. Nachman et al., J. Am. Med. Assoc. 283, 492 (2000).

² <http://www.accessdata.fda.gov/scripts/cder/ob/docs/tempai.cfm>

➤ (FDA ELECTRONIC ORANGE BOOK)

Search results from the "OB_Rx" table for query on "021226³."

Active Ingredient:	LOPINAVIR; RITONAVIR
Dosage Form/Route:	CAPSULE; ORAL
Proprietary Name:	KALETRA
Applicant:	ABBOTT
Strength:	133.3MG;33.3MG
Application Number:	021226
Product Number:	001
Approval Date:	Sep 15, 2000
Reference Listed Drug	Yes
RX/OTC/DISCN:	RX
TE Code:	
Patent and Exclusivity Info for this product ⁴	View

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FDA/Center for Drug Evaluation and Research

Office of Generic Drugs

Division of Labeling and Program Support

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Generic Drug Product Information & Patent Information - **Daily**

Orange Book Data Updated Through February, 2008

Patent and Generic Drug Product Data Last Updated: April 15, 2008

➤ (FDA ELECTRONIC ORANGE BOOK)

Search results from the "OB_Rx" table for query on "020945⁵."

Active Ingredient:	RITONAVIR
Dosage Form/Route:	CAPSULE; ORAL
Proprietary Name:	NORVIR
Applicant:	ABBOTT
Strength:	100MG
Application Number:	020945
Product Number:	001
Approval Date:	Jun 29, 1999
Reference Listed Drug	Yes

³ http://www.accessdata.fda.gov/scripts/cder/ob/docs/obdetail.cfm?Appl_No=021226&TABLE1=OB_Rx

⁴ Patente US 5541206 publicada el 30 de Julio de 1996 y titulada "Retroviral protease inhibiting compounds".

⁵ http://www.accessdata.fda.gov/scripts/cder/ob/docs/obdetail.cfm?Appl_No=020945&TABLE1=OB_Rx

RX/OTC/DISCN:

RX

TE Code:

Patent and Exclusivity Info for this product⁴: [View](#)

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FDA/Center for Drug Evaluation and Research

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Orange Book Data - **Monthly**

Generic Drug Product Information & Patent Information - **Daily**

Orange Book Data Updated Through February, 2008

Patent and Generic Drug Product Data Last Updated: April 15, 2008

- La publicación internacional WO 00/59475 publicada el 12 de octubre de 2000 y titulada "COMPOSITIONS AND METHODS FOR IMPROVED DELIVERY OF IONIZABLE HYDROPHOBIC THERAPEUTIC AGENTS" revela:

The present invention provides pharmaceutical compositions and methods for improved delivery of ionizable hydrophobic therapeutic agents.

In one embodiment, the invention is directed to a pharmaceutical composition including an ionizable hydrophobic therapeutic agent and a carrier. The carrier includes an ionizing agent to ionize the therapeutic agent, and a surfactant. Optionally, the carrier also includes solubilizers, triglycerides and neutralizing agents.

(...)

11. The pharmaceutical composition of claim 9, wherein the hydrophobic therapeutic agent is selected from the group consisting of abacavir, acebutolol, pramipexol, pranlukast, praziquantel, prazosin, procabazine, prochlorperazine, proguanil, propranolol, pseudoephedrine, pyrantel, pyrimethamine, quetiapine, quinidine, quinine, raloxifene, ranitidine, remifentanyl, repaglinide, reserpine, ricobendazole, rifabutin, rifampin, rifapentine, rimantadine, risperidone, ritonavir, rizatriptan, ropinirole, rosiglitazone, roxaditine, roxithromycin, salbutamol, saquinavir, selegiline, sertraline,

(...)

68. The pharmaceutical composition of claim 1 in the form of a preconcentrate, a diluted preconcentrate, a semi-solid dispersion, a solid dispersion, or a sprayable solution.

69. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 1.

70. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 61.

71. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 62.

72. A dosage form comprising a solid particulate carrier coated with or formed from the pharmaceutical composition of claim 1.

74. The dosage form of claim 69, wherein the capsule is a hard gelatin capsule, a soft gelatin capsule, a starch capsule or an enteric coated capsule.

En esta anterioridad se puede apreciar claramente la formulación de ritonavir en formulaciones sólidas para administración oral, específicamente en cápsulas duras de gelatina o en suspensiones, tal y como lo reivindica la solicitud colombiana en trámite, específicamente en sus reivindicaciones 1 a 6. Así mismo menciona la formulación en capsulas blandas, lo cual sobrepasa de manera importante el nivel técnico de lo reivindicado por la solicitud colombiana (que solo involucra cápsulas rígidas), ya que como toda persona medianamente versada en la materia reconoce, la formulación en cápsulas blandas de cualquier fármaco, supone ventajas relacionadas con la biodisponibilidad del mismo.

- La publicación internacional WO 02/096395 publicada el 05 de diciembre de 2002 y titulada "SOFT ELASTIC CAPSULES COMPRISING RITONAVIR AND/OR LOPINAVIR" revela:

The invention relates to a soft elastic capsule, fill and shell compositions of the soft elastic capsule, and pharmaceutical agents contained within the soft elastic capsules. The invention further relates to soft elastic capsules and HIV protease inhibiting compounds contained in the soft elastic capsule. The soft elastic capsule can be used with a broad range of pharmaceutical agents including antibiotics, anti-AIDS pharmaceutical agents, and an array of other medicinally active agents. Important pharmaceutical agents are anti-HIV agents.

(...)

The fill composition for the soft elastic capsule of the invention can include a solubilized HIV protease inhibiting compound or a combination of solubilized HIV protease inhibiting compounds. Preferably, the HIV protease inhibiting compound is a compound of the formula I, formula II, saquinavir, nelfinavir, amprenavir, or indinavir. More preferably the compound is ritonavir, lopinavir, saquinavir, nelfinavir, amprenavir, or indinavir. Most preferably the compound is ritonavir or lopinavir. A combination of solubilized HIV protease inhibiting compounds can also be used. Preferably the combination is ritonavir or nelfinavir and another HIV protease inhibitor, for example, lopinavir, saquinavir, indinavir, amprenavir, or nelfinavir. More preferably, the combination is of ritonavir or nelfinavir and another HIV protease inhibitor, for example, lopinavir, saquinavir, indinavir, amprenavir, or nelfinavir. Most preferably the combination is ritonavir and lopinavir. A preferred ratio of the amount, as measured by weight, of ritonavir to lopinavir in the fill composition is in the range of 2:1 to 1:10; more preferably this ratio is in the range of 1:1 to 1:10; most preferably this ratio is in the range of 1:3 to 1:5, and it is most highly preferred that the ratio is about

(...)

Además reivindica

22. The soft elastic capsule of claim 1 wherein the fill in an initial state comprises:
- is (2S, 3S, 5S)-5-(N-(N-((N-methyl-N-((2-isopropyl-4-thiazolyl)-methyl)amino)carbonyl)-L-valinyl)amino-2-(N-((5-thiazolyl)methoxy-carbonyl)-amino-1,6-diphenyl-3-hydroxyhexane (ritonavir) and (2S, 3S, 5S)-2-(2,6 dimethylphenoxyacetyl)-amino-3-hydroxy-5-(2S-(1-tetrahydropyrimid-2-onyl)-3-methyl-butanoyl)amino-1,6-diphenylhexane (lopinavir) in the range of 10–35% by weight of the fill composition;
 - polyethylene glycol in the range of 3–20% by weight of the fill composition;
 - oleic acid in the range of 50-80% by weight of the fill; and
- wherein the shell in an initial state comprises:
- gelatin in the range of 30–60% by weight of the shell, and
 - Andrisorb in the range of 4–18% by weight of the shell.

De igual manera que en la anterioridad citada atrás, este documento menciona la formulación de Ritonavir en capsulas blandas, lo cual sobrepasa de manera importante el nivel técnico de lo reivindicado por la solicitud colombiana (que solo involucra cápsulas rígidas), ya que como toda persona medianamente versada en la materia reconoce, la formulación en cápsulas blandas de cualquier fármaco, trae *per se* varias ventajas a nivel de la biodisponibilidad del mismo. Así mismo es

importante resaltar que también se menciona la combinación de ritonavir con la mayoría de los fármacos que menciona la solicitud colombiana en trámite, específicamente los mencionados en su reivindicación 15.

3.2 La combinación farmacéutica de ritonavir con los demás compuestos antivirales mencionados por la solicitud colombiana en trámite, específicamente en su reivindicación 15, carece por completo de nivel inventivo, ya que se encuentra suficiente información de carácter científico con anterioridad, que puede dirigir de manera obvia a cualquier persona medianamente versada en la materia a asociar estas sustancias activas en una misma formulación farmacéutica, veamos:

- La publicación científica "STEADY-STATE PHARMACOKINETIC (PK) INTERACTION STUDY OF ATAZANAVIR (ATV) WITH RITONAVIR (RTV) IN HEALTHY SUBJECTS⁶" revela:

Abstract:

ATV is a new protease inhibitor with potent anti-HIV activity. ATV exposure has been substantially increased when combined with RTV. Objective: To assess the PK interaction between ATV (CYP3A substrate/inhibitor) and RTV (CYP 3A inhibitor) at specific doses of 300 and 100 mg, respectively. METHODS: Thirty healthy subjects received open label drugs with a light meal as follows: 300 mg ATV QD on Days 1-10 and then 300 mg ATV and 100 mg RTV QD on Days 11-20. Serial blood samples were collected for PK on Days 10, 15, and 20. RESULTS: Geometric means and ratios of geometric means (90% confidence intervals (CI)) for noncompartmental parameters are listed in the table. Steady state C_{max} and AUC(TAU) of ATV after coadministration with RTV were 1.86 and 3.38 fold higher, respectively, compared to ATV alone. Mean RTV exposure was similar to published data from a previous RTV/ATV combination study. CONCLUSIONS: The combination of RTV and ATV in healthy subjects: substantially increased ATV exposure, was well tolerated and did not affect RTV PK. The ATV/RTV regimen is under evaluation in select HIV+ subjects in Phase III.

⁶ State Pharmacokinetic (PK) Interaction Study of Atazanavir (ATV) with Ritonavir (RTV) in Healthy ...
 Autores: S AGARWALA; R RUSSO; V MUMMANENI; D RANDALL Fuente: Abstr Intersci Conf
 Antimicrob Agents Chemother Intersci ..., 2002 En: Google Scholar

- La publicación científica "PHARMACOKINETICS AND SAFETY OF AMPRENAVIR AND RITONAVIR FOLLOWING MULTIPLE-DOSE, CO-ADMINISTRATION TO HEALTHY VOLUNTEERS⁷" revela:

Abstract:

Objective: To evaluate the safety and pharmacokinetic interaction between amprenavir (APV) and Ritonavir (RTV). Methods: Three open-label, randomized, two-sequence, multiple-dose studies having the same design (7 days of APV or RTV alone followed by 7 days of both drugs together) used 450 or 900 mg APV with 100 or 300 mg RTV every 12 h with pharmacokinetic assessments on days 7 and 14. Safety was monitored as clinical adverse events (AEs) and laboratory abnormalities. Results: Relative to APV alone, RTV co-administration resulted in a 3.3- to 4-fold and 10.84 to 14.25-fold increase in the geometric least-square (GLS) mean area under the plasma concentration±time curve (AUC_{TSS}) and minimum concentration ($Cmin_{ss}$), respectively. APV 900 mg with RTV 100 mg resulted in a 2.09-fold and 6.85-fold increase in the GLS mean AUC_{TSS} and $Cmin_{ss}$, respectively. On day 14, the geometric mean (95% confidence interval) for 450 mg APV AUC_{TSS} ($ig \cdot h/mL$) was 23.49 (19.32±28.57) with 300 mg RTV and 35.42 (30.46±44.42) with 100 ig RTV, and for the 900 mg APV with 100 mg RTV 47.11 (39.47±61.24). The 450 mg APV $Cmin_{ss}$ (ig/ml) were 1.32 (1.05±1.67) and 2.01 (1.70±2.61), and 2.47 (2.08±3.32) for 900 mg APV. The most common AEs were mild and included diarrhea, nausea/vomiting, oral parasthesias, and rash. The triglyceride and cholesterol increased significantly from RTV exposure. Conclusion: Adding RTV to APV resulted in clinically and statistically significant increases in APV AUC and Cmin with variable effects on maximum concentration. The two RTV doses had similar effects on APV but AEs were more frequent with 300 mg RTV.

- La publicación científica "SOLO: 48-WEEK EFFICACY AND SAFETY COMPARISON OF ONCE-DAILY FOSAMPRENAVIR /RITONAVIR VERSUS TWICE-DAILY NELFINAVIR IN NAIVE HIV-1-INFECTED PATIENTS⁸" revela:

Abstract:

Objective: To compare the magnitude and durability of the antiviral response to fosamprenavir (FPV) plus ritonavir (RTV) once-daily (FPV/rQD) with nelfinavir

⁷ Pharmacokinetics and safety of amprenavir and ritonavir following multiple-dose, co-administration ...
Autores: BM Sadler; PJ Piliero; SL Preston; PP Lloyd; Y Lou Fuente: AIDS, 2001 En: Google Scholar

⁸ 48-week efficacy and safety comparison of once-daily fosamprenavir/ritonavir versus twice-daily ...
Autores: JC Gathe Jr; P Ive; R Wood; D Schurmann; NC Bellos Fuente: AIDS, 2004 En: Google Scholar

twicedaily (NFV BID), each administered with abacavir and lamivudine twice-daily. *Methods:* An international, phase III, randomized, open-label study in antiretroviral therapy-naïve, HIV-infected adults. *Results:* Patients with advanced HIV disease received FPV/r QD (n ¼ 322) or NFV BID (n ¼ 327). At week 48, 69% of patients in the FPV/r QD group and 68% in the NFV BID group had plasma HIV-1 RNA (vRNA) , 400 copies/ml, whereas 55% of patients in the FPV/r QD group and 53% in the NFV BID group had vRNA , 50 copies/ml (intent to treat, rebound/discontinuation ¼ failure). More patients in the NFV BID group (17%) experienced virological failure than in the FPV/r QD group (7%). Efficacy of FPV/r QD was maintained in patients with CD4+ cell counts, 50 3 106 cells/l or vRNA > 100 000 copies/ml at entry. At week 48, median CD4+ cell counts were increased to 203 3 106 cells/l (FPV/r QD group) and 207 3 106 cells/l (NFV BID group). Both regimens were generally well tolerated. Diarrhea was more common on NFV BID than on FPV/r QD (16 versus 9%; P ¼ 0.008). Fasting lipid profile results were generally favorable in both treatment arms. FPV/rQD maintained plasma amprenavir (APV) trough concentrations above the mean phenotypic drug-susceptibility (IC50) for wild-type virus for APV. *Conclusion:* As a first choice protease inhibitor with a low daily pill burden, FPV/RQD was well tolerated and provided potent, durable antiviral suppression.

- La publicación científica "PHARMACOKINETIC INTERACTION BETWEEN RITONAVIR AND INDINAVIR IN HEALTHY VOLUNTEERS⁹" revela:

Abstract:

The pharmacokinetic interaction between indinavir and ritonavir was evaluated in five groups of healthy adult volunteers to explore the potential for twice-daily (b.i.d.) dosing of this combination. All subjects received 800 mg of indinavir every 8 h (q8h) on day 2. In addition, subjects in group I received one dose of 800 mg of indinavir on day 1 and 800 mg of indinavir q8h on day 17. Subjects in Groups II and IV each received one dose of 600 mg of indinavir on days 1 and 17, and subjects in groups III and V each received one dose of 400 mg of indinavir on days 1 and 17. During days 3 to 17, ritonavir placebo or ritonavir at 200, 300, 300, or 400 mg q12h was given to groups I, II, III, IV, and V, respectively. Ritonavir at steady state probably inhibited the cytochrome P-450 3A metabolism of indinavir

⁹ Pharmacokinetic Interaction between Ritonavir and Indinavir in Healthy Volunteers Autores: A Hsu; GR Granneman; G Cao; L Carothers; A Japour Fuente: Antimicrobial Agents and Chemotherapy, 1998 En: Google Scholar.

and substantially increased plasma indinavir concentrations, with the area under the plasma concentration-time curve (AUC) increasing up to 475% and the peak concentration in serum (C_{max}) increasing up to 110%. The C_{max}/trough concentration ratio decreased from 50 in standard q8h regimens to less than 14 when indinavir was administered with ritonavir. For a constant Indinavir dose, an increase in the ritonavir dose yielded similar indinavir AUCs, C_{max}s, and concentrations at 12 h (C_{12s}). For a constant ritonavir dose, an increase in the indinavir dose resulted in approximately proportional increases in the indinavir AUC, less than proportional increases in C_{max}, and slightly more than proportional increases in C₁₂. Ritonavir reduced between-subject variability in the indinavir AUC and trough concentrations and did not affect indinavir renal clearance. With the altered pharmacokinetic profile, indinavir likely could be given as a b.i.d. combination regimen with ritonavir. This could potentially improve patient compliance and thereby reduce treatment failures.

- La publicación científica "LOPINAVIR-RITONAVIR VERSUS NELFINAVIR FOR THE INITIAL TREATMENT OF HIV INFECTION"¹⁰ revela:

Abstract:

Background: Lopinavir is a newly developed inhibitor of human immunodeficiency virus (HIV) protease that, when formulated with ritonavir, yields mean trough plasma lopinavir concentrations that are at least 75 times as high as that needed to inhibit replication of wild-type HIV by 50 percent. Methods: We conducted a double-blind trial in which 653 HIV-infected adults who had not received antiretroviral therapy for more than 14 days were randomly assigned to receive either lopinavir-Ritonavir (400 mg of lopinavir plus 100 mg of ritonavir twice daily) with nelfinavir placebo or nelfinavir (750 mg three times daily) with lopinavir-ritonavir placebo. All patients also received open-label stavudine and lamivudine. The primary efficacy end points were the presence of fewer than 400 HIV RNA copies per milliliter of plasma at week 24 and the time to the loss of virologic response through week 48. Results: At week 48, greater proportions of patients treated with lopinavir-ritonavir than of patients treated with nelfinavir had fewer than 400 copies of HIV RNA per milliliter (75 percent vs. 63 percent, $P < 0.001$) and fewer than 50 copies per milliliter (67 percent vs. 52 percent, $P < 0.001$). The time to the loss of virologic response

¹⁰ Lopinavir-Ritonavir versus Nelfinavir for the Initial Treatment of HIV Infection Autores: S Walmsley; B Bernstein; M King; J Arribas Fuente: New England Journal of Medicine, 2002 En: Google Scholar.

was greater in the lopinavir–ritonavir group than in the nelfinavir group (hazard ratio, 2.0; 95 percent confidence interval, 1.5 to 2.7; $P < 0.001$). The estimated proportion of patients with a persistent virologic response through week 48 was 84 percent for patients receiving lopinavir–ritonavir and 66 percent for those receiving nelfinavir. Both regimens were well tolerated, with the rate of discontinuation related to the study drugs at 3.4 percent among patients receiving lopinavir–ritonavir and 3.7 percent among patients receiving nelfinavir. Among patients with more than 400 copies of HIV RNA per milliliter at some point from week 24 through week 48, resistance mutations in HIV protease were demonstrated in viral isolates from 25 of 76 nelfinavir-treated patients (33 percent) and none of 37 patients treated with lopinavir–ritonavir ($P < 0.001$). Conclusions For the initial treatment of HIV-infected adults, a combination regimen that includes lopinavir–ritonavir is well tolerated and has antiviral activity superior to that of a nelfinavir-containing regimen. (N Engl J Med 2002;346:2039-46.)

- La publicación científica “SAQUINAVIR PHARMACOKINETICS ALONE AND IN COMBINATION WITH RITONAVIR IN HIV-INFECTED PATIENTS¹¹” revela:

Abstract:

Objective: The most important hepatic enzyme involved in the metabolism of protease inhibitors is cytochrome P450 3A4 (CYP3A4). Ritonavir (RIT) is a potent inhibitor of CYP3A4 and inhibits saquinavir (SQV) metabolism in healthy volunteers. In this study we investigated the kinetics of SQV when administered alone and in combination with RIT in HIV-infected patients. Design: SQV pharmacokinetics were determined in seven patients who had advanced HIV disease. Steady-state SQV profiles were obtained on two occasions following treatment with SQV 600 mg three times daily alone and when administered with RIT 300 mg twice daily. Methods: Blood samples were obtained at times 0, 1, 2, 4, 6 and 8 h post-dosing. Following centrifugation, separated plasma was heated at 58°C for at least 30 min to inactivate HIV and stored at -80°C until analysis using high performance liquid chromatography. Results: For patients treated with SQV alone there was a 12-fold variability in the area under the SQV concentration–time curve (AUC_{0–8h}) ranging from 293 to 3446 ng·h/ml. When combined with RIT there was a marked increase in the maximum plasma concentration of SQV

¹¹ Saquinavir pharmacokinetics alone and in combination with ritonavir in HIV-infected patients Autores: C Merry; MG Barry; F Mulcahy; M Ryan; J Heavey; JF Fuente: AIDS, 1997 En: Google Scholar.

[median (range), 146 (57–702) versus 4795 (1420–15810) ng/ml; ~95% confidence interval (CI), 2988–6819; $P = 0.0006$, Mann–Whitney U test]. The AUC_{0–8h} for SQV was also significantly increased in the presence of RIT [median (range), 470 (293–3446) versus 27 458 (7357–108 001) ng•h/ml; ~95% CI, 16 628–35 111; $P = 0.0006$]. Conclusions: For some patients, administration of SQV 600 mg three times daily results in very low SQV plasma levels and possibly little antiviral effect. Combination of SQV with RIT results in a significant drug interaction mediated by enzyme inhibition which exposes patients to very high SQV concentrations and potential toxicity. If combination therapy with SQV plus RIT is considered then the dose of SQV should be greatly reduced.

- La publicación científica “INTERACTIONS BETWEEN ATAZANAVIR-RITONAVIR AND TENOFOVIR IN HEAVILY PRETREATED HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS¹²” revela:

Abstract:

The aim of the present study was to assess the pharmacokinetic behavior of atazanavir-ritonavir when it is coadministered with tenofovir disoproxil fumarate (DF) in human immunodeficiency virus (HIV)-infected patients. Eleven patients enrolled in Agence Nationale de Recherche sur le SIDA (National Agency for AIDS Research, Paris, France) trial 107 were included in this pharmacokinetic study. They received atazanavir at 300 mg and ritonavir at 100 mg once a day (QD) from day 1 to the end of study. For the first 2 weeks, their nucleoside analog reverse transcriptase inhibitor (NRTI) treatments remained unchanged. Tenofovir DF was administered QD from day 15 to the end of the study. Ongoing NRTIs were selected according to the reverse transcriptase genotype of the HIV isolates from each patient. The values of the pharmacokinetic parameters for atazanavir and ritonavir were measured before (day 14 [week 2]) and after (day 42 [week 6]) initiation of tenofovir DF and are reported for the 10 patients who completed the study. There was a significant decrease in the area under the concentration-time curve from 0 to 24 h (AUC_{0–24}) for atazanavir with the addition of tenofovir DF (AUC_{0–24} ratio, 0.75; 90% confidence interval, 0.58 to 0.97; $P = 0.05$). There was a trend for a decrease in the minimum concentrations of atazanavir and ritonavir in plasma when they were combined with tenofovir, but none of the differences

¹² Interactions between atazanavir-ritonavir and tenofovir in heavily pretreated human immunodeficiency ...
Autores: AM Taburet; C Piketty; C Chazallon; I Vincent Fuente: Antimicrobial Agents and Chemotherapy, 2004 En: Google Scholar.

reached statistical significance. The median decreases in the HIV RNA loads at week 2 and week 6 were 0.1 and 0.2 log copies/ml, respectively. In summary, our data are consistent with the existence of a significant interaction between atazanavir and tenofovir DF.

- La publicación internacional WO 00/25784 publicada el 11 de mayo de 2000 y titulada "METHOD FOR IMPROVING THE PHARMACOKINETICS OF TRIPANAVIR" revela:

The present invention relates to a novel method for improving the pharmacokinetics of tipranavir, comprising administering to a human in need of such treatment a combination of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.

(...)

Either solid or fluid dosage forms can be prepared for oral administration. Solid compositions are prepared by mixing the compounds of this invention with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methyl cellulose, or functionally similar pharmaceutical diluents and carriers. Capsules are prepared by mixing the compounds of this invention with an inert pharmaceutical diluent and placing the mixture into an appropriately sized hard gelatin capsule. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compounds of this invention with an acceptable inert oil such as vegetable oil or light liquid petrolatum. Syrups are prepared by dissolving the compounds

(...)

Effects of Tipranavir on Ritonavir:

Median plasma ritonavir concentrations following administration of ritonavir 500 mg BID and either tipranavir 600 mg BID or 900 mg BID are depicted in Figure 3. The pharmacokinetics of ritonavir derived from individual subject data are summarized in Table 4. For comparative purposes, the pharmacokinetics of ritonavir when administered alone are included in this table. The combination of ritonavir and tipranavir in this study resulted in an approximate 4-fold decrease in steady-state plasma ritonavir concentrations compared with ritonavir administered alone. Median ritonavir C_{max} values were more than 2-fold lower, and C_{min} values more than 10-fold lower, when ritonavir was dosed with tipranavir. The decrease in plasma ritonavir concentrations, and shortening of the elimination half-life, is consistent with metabolic induction attributable to tipranavir.

(...)

Effects of Ritonavir on Tipranavir:

Mean (SD) plasma tipranavir concentrations following administration of tipranavir alone (1250 mg BID) and in combination with ritonavir (200 mg BID) are shown in Figure 7. The pharmacokinetic estimates derived from individual subject data are provided in Table 9. Median AUC values of tipranavir were approximately 11-fold higher and median tipranavir C_{min} values were about 75-fold higher in subjects receiving tipranavir with ritonavir oral solution than in subjects receiving tipranavir alone. Median C_{max} values increased approximately 5-fold following concomitant dosing with ritonavir. As observed in previous studies in which tipranavir HFC and ritonavir capsules were coadministered, the apparent terminal half-life of tipranavir was not significantly affected by ritonavir.

Median (range) ritonavir pharmacokinetic parameters after administration of ritonavir oral solution 200 mg BID with tipranavir 1250 mg BID are given in Table 10. The median ritonavir AUC, C_{min}, and C_{max} were 12-fold, 54-fold, and 10-fold lower, respectively, for this combination compared to a 500-mg twice-daily dose of ritonavir administered alone. The apparent elimination half-life of ritonavir was also decreased relative to ritonavir given alone, which is consistent with metabolic induction by tipranavir.

(...)

Además reivindica:

1. A method for improving the pharmacokinetics of tipranavir, comprising administering to a human in need of such treatment a combination of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.

Como se puede apreciar en todas estas publicaciones anteriores a la solicitud colombiana, la asociación de dichos principios activos, especialmente para el tratamiento de una infección por VIH, puede arrojar resultados farmacocinéticos positivos, especialmente en el incremento de cantidades y tiempo de permanencia del fármaco en sangre, lo cual favorecería la alternativa de tratar esta clase de enfermedades con la coadministración de los fármacos citados, y por ende, de asociarlos en una formulación farmacéutica para su administración conjunta, como lo hace la solicitud colombiana en trámite, circunstancia que aniquila el nivel inventivo de ésta.

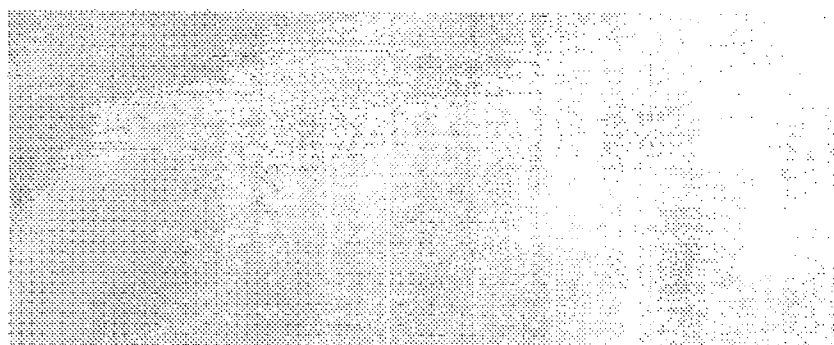
3.3 Por ultimo, la patente americana US 2004/0024031 publicada el 05 de febrero de 2004 y titulada "SOLVATES AND POLYMORPHS OF RITONAVIR AND METHODS OF MAKING AND USING THE SAME" revela:

[0010] This invention encompasses novel solvates and crystal polymorphs of Ritonavir. Specific solvates of the compound include a formamide solvate and a partially desolvated formamide solvate. All of the novel forms exhibit physical and spectroscopic characteristics that differ markedly from those of Forms I and II.

(...)

[0011] The invention further encompasses methods of making novel forms of Ritonavir, as well as methods of making previously known forms. Also encompassed by the invention are methods of using the novel forms of Ritonavir in the treatment of diseases, such as HIV-infection, and for enhancing the pharmacokinetic profiles of other pharmaceutically active compounds. Pharmaceutical compositions and unit dosage forms comprising novel forms of Ritonavir are also encompassed by the invention.

(...)



[0070] Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed., Mack Publishing, Easton Pa. (1990).

(...)

[0096] Preparation of Ritonavir Form I

[0097] NORVIR-brand Ritonavir (Abbott Laboratories, North Chicago, Ill. USA) oral solution was used to obtain Ritonavir Form I. Specifically, approximately 240 mL of NORVIR solution (the contents of a normal prescription bottle, totaling about 19.2 g of Ritonavir) were placed into a 500 mL round bottom flask and vacuum-treated (pumping at high vacuum) for about 6 hours at room temperature on a slightly heated water bath to keep temperature constant. 50 mL of ethyl acetate was added to the viscous residue (dark orange in color).

(...)

Además reivindica:

- d) a method of preparing Ritonavir Form I, which comprises desolvating Ritonavir(IV) for an amount of time sufficient for the formation of Ritonavir Form I;
- f) the method of claim (a), (b), or (c), which further comprises administering a therapeutically effective amount of a second pharmacologically active compound to the patient;
- g) the method of claim (f) wherein the second pharmacologically active compound is a protease inhibitor, a nucleoside reverse transcriptase inhibitor, or a non-nucleoside reverse transcriptase inhibitor, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or polymorph thereof;
- h) the method of claim (g) wherein the protease inhibitor is lopinavir, saquinavir, indinavir, nelfinavir, amprenavir, palinavir, lasinavir, or tipranavir;

Como se puede apreciar claramente, en esta anterioridad se encuentra contenido lo que se refiere a la inclusión de ritonavir en formulaciones sólidas para administración oral, específicamente tabletas y capsulas; la combinación de ritonavir con otros fármacos, específicamente con lopinavir, saquinavir, indinavir, amprenavir y tripanavir; y la utilización (y obtención) de la forma polimorfica I en

dichas formulaciones; con lo cual se ve afectado el contenido del capítulo reivindicatorio que sustenta esencialmente la novedad y el nivel inventivo de la solicitud colombiana en trámite.

Así mismo es importante resaltar, que los compuestos que especifica dicha solicitud colombiana, tales como los silicatos, el óxido de sílice, kollidon, almidones, lauril sulfato de sodio, tablettose 80, estereato de magnesio, lactosa monohidrato y PVP k30, son excipientes comunes y ampliamente utilizados en el estado de la técnica para este tipo de formulaciones sólidas orales, y que, como cualquier persona medianamente versada en la materia reconoce, su elección depende de los resultados obtenidos a través de los respectivos ensayos de preformulación y formulación, por los que toda forma de dosificación de este tipo debe pasar, para asegurar así las características ideales y de calidad del medicamento. En el mismo sentido, la elección e inclusión en la formulación de un polimorfo de un principio activo, en este caso el polimorfo I de Ritonavir, no sustenta alguna novedad y/o nivel inventivo, puesto que dicha selección también se encuentra ligada a los ensayos de preformulación y formulación, en donde el polimorfo escogido debe ser estable y ajustarse a las condiciones de elaboración y a los excipientes que lo van a acompañar, para poder ejercer su actividad farmacológica dentro del medicamento.

En conclusión, se encuentra suficiente información anterior en el mismo estado de la técnica que desvirtúa los argumentos a través de los cuales la solicitud colombiana busca sustentar novedad y nivel inventivo, específicamente a lo que se refiere al uso del polimorfo I de ritonavir, solo o en combinación con otros fármacos, en una formulación sólida para administración oral, con lo cual todo lo contenido en el capítulo reivindicatorio de dicha solicitud resulta insustancial para el caso de una patente de invención.

Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, artículos 14, 16 y 18 (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. SOLICITUD DE PLAZO EXTRA PARA SUSTENTAR LA OPOSICIÓN

En desarrollo de lo dispuesto por el artículo 42, inciso 2º de la Decisión 486, solicito comedidamente a su Despacho el plazo adicional de sesenta días para sustentar la presente oposición. Para los anteriores efectos, me permito anexar la constancia de pago correspondiente.

VI. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de las que puedan presentarse al vencimiento de la prórroga solicitada, y de aquellas que el Despacho considere oficiosamente, las siguientes:

- Abstracts de las publicaciones científicas enunciada en el numeral 3.2 del acápite de fundamentos de la oposición.
- La publicación internacional WO 00/25784 publicada el 11 de mayo de 2000 y titulada "METHOD FOR IMPROVING THE PHARMACOKINETICS OF TRIPANAVIR" paginas 1, 12, 15, 18, 19, 25 y capítulo reivindicatorio.
- Impresión de los resultados de la búsqueda en el "FDA Orange Electronic Book" para Ritonavir.
- La publicación internacional WO 00/59475 publicada el 12 de octubre de 2000 y titulada "COMPOSITIONS AND METHODS FOR IMPROVED DELIVERY OF IONIZABLE HYDROPHOBIC THERAPEUTIC AGENTS" página 3 y capítulo reivindicatorio.
- La publicación internacional WO 02/096395 publicada el 05 de diciembre de 2002 y titulada "SOFT ELASTIC CAPSULES COMPRISING RITONAVIR AND/OR LOPINAVIR" paginas 1, 11, 12 y capítulo reivindicatorio.
- La patente americana US 2004/0024031 publicada el 05 de febrero de 2004 y titulada "SOLVATES AND POLYMORPHS OF RITONAVIR AND METHODS OF MAKING AND USING THE SAME" Páginas 2, 6, 9 y capítulo reivindicatorio.

VII. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos

1. Certificado de existencia y representación legal de Tecnoquímicas S.A.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Constancia de pago para sustentar la solicitud de prórroga a que se refiere el artículo 42, inciso 2º de la Decisión 486.
5. Copia para correr traslado al solicitante.
6. El mencionado en el acápite de pruebas.

VIII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Tecnoquímicas S.A., las recibirá en la secretaría de su Despacho o en su sede localizada en la Calle 23 # 7-39, de la ciudad de Cali.

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR

C.C. 79'152.473 de Usaquén

T.P.A. 44.655 del C.S.J.

LOPINAVIR-RITONAVIR VERSUS NELFINAVIR FOR THE INITIAL TREATMENT OF HIV INFECTION

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 PETER RUANE, M.D., MARGARET JOHNSON, M.D., DAVID JOHNSON, M.D., RICHARD LALONDE, M.D.,
 ANTHONY JAPOUR, M.D., SCOTT BRUN, M.D., AND EUGENE SUN, M.D., FOR THE M98-863 STUDY TEAM*

ABSTRACT

Background Lopinavir is a newly developed inhibitor of human immunodeficiency virus (HIV) protease that, when formulated with ritonavir, yields mean trough plasma lopinavir concentrations that are at least 75 times as high as that needed to inhibit replication of wild-type HIV by 50 percent.

Methods We conducted a double-blind trial in which 653 HIV-infected adults who had not received antiretroviral therapy for more than 14 days were randomly assigned to receive either lopinavir-ritonavir (400 mg of lopinavir plus 100 mg of ritonavir twice daily) with nelfinavir placebo or nelfinavir (750 mg three times daily) with lopinavir-ritonavir placebo. All patients also received open-label stavudine and lamivudine. The primary efficacy end points were the presence of fewer than 400 HIV RNA copies per milliliter of plasma at week 24 and the time to the loss of virologic response through week 48.

Results At week 48, greater proportions of patients treated with lopinavir-ritonavir than of patients treated with nelfinavir had fewer than 400 copies of HIV RNA per milliliter (75 percent vs. 63 percent, $P < 0.001$) and fewer than 50 copies per milliliter (67 percent vs. 52 percent, $P < 0.001$). The time to the loss of virologic response was greater in the lopinavir-ritonavir group than in the nelfinavir group (hazard ratio, 2.0; 95 percent confidence interval, 1.5 to 2.7; $P < 0.001$). The estimated proportion of patients with a persistent virologic response through week 48 was 84 percent for patients receiving lopinavir-ritonavir and 66 percent for those receiving nelfinavir. Both regimens were well tolerated, with the rate of discontinuation related to the study drugs at 3.4 percent among patients receiving lopinavir-ritonavir and 3.7 percent among patients receiving nelfinavir. Among patients with more than 400 copies of HIV RNA per milliliter at some point from week 24 through week 48, resistance mutations in HIV protease were demonstrated in viral isolates from 25 of 76 nelfinavir-treated patients (33 percent) and none of 37 patients treated with lopinavir-ritonavir ($P < 0.001$).

Conclusions For the initial treatment of HIV-infected adults, a combination regimen that includes lopinavir-ritonavir is well tolerated and has antiviral activity superior to that of a nelfinavir-containing regimen. (N Engl J Med 2002;346:2039-46.)

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PROTEASE inhibitor-based combination antiretroviral therapy has led to dramatic improvements in morbidity and mortality associated with human immunodeficiency virus (HIV) infection.¹⁻⁴ However, virologic failure occurs within 12 months in up to 50 percent of patients in whom combination antiretroviral therapy is initiated.^{5,7} Major factors contributing to failure are poor tolerability and toxicity of the drugs, incomplete adherence to the regimen on the part of patients, and pharmacokinetic properties that result in trough concentrations close to or below the levels required to inhibit HIV replication effectively.^{8,9} Lopinavir is a novel peptidomimetic protease inhibitor with potent in vitro activity against HIV¹⁰; it has been formulated with low-dose ritonavir, a cytochrome P450 3A4 enzyme inhibitor, to enhance its pharmacokinetic profile. When administered at a dose of 400 mg of lopinavir and 100 mg of ritonavir twice daily, the resulting mean trough lopinavir concentrations are at least 75 times as high as the protein binding-corrected concentration needed to inhibit the replication of wild-type HIV by 50 percent (EC_{50}).¹¹ The combination of lopinavir and ritonavir was developed to exploit this pharmacokinetic advantage, with the goal of minimizing the risk of treatment failure and maximizing the durability of a treatment response.

The activity of lopinavir-ritonavir in HIV-infected patients was first demonstrated in a phase 2 study of lopinavir-ritonavir in combination with stavudine and lamivudine, in patients who had not previously been treated with antiretroviral drugs. An intention-to-treat analysis showed that HIV RNA was suppressed to fewer than 50 copies per milliliter in 78 percent of these patients after 48 weeks of treatment.¹² On the basis of initial observations from that study,

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*The investigators participating in the M98-863 study are listed in the Appendix.

we conducted a randomized, double-blind, multicenter trial to compare the safety and efficacy of lopinavir–ritonavir with that of nelfinavir.

METHODS

Patients

Patients were recruited from 93 centers in 13 countries in North America, South America, Europe, Africa, and Australia. The patients were at least 12 years old, had more than 400 copies of HIV RNA per milliliter of plasma (measured by Roche Amplicor HIV-1 Monitor), a Karnofsky score over 70, and no prior treatment with stavudine or lamivudine or prior treatment with any other antiretroviral therapy for more than 14 days at any time. There was no restriction on the CD4+ cell count. The patients had not been treated for an active opportunistic infection within 30 days before screening. The exclusion criteria also included pregnancy and alanine aminotransferase or aspartate aminotransferase levels more than three times the upper limit of normal. All patients gave written informed consent.

Study Design

We conducted a randomized, double-blind, comparative study. The primary outcome measures were the proportion of patients with fewer than 400 copies of HIV RNA per milliliter of plasma at 24 weeks and the time to the loss of virologic response through 48 weeks. Secondary efficacy outcomes included the proportion of patients with fewer than 50 copies of HIV RNA per milliliter at weeks 24 and 48 (as determined with the Roche Amplicor HIV-1 Monitor) and changes in the CD4+ cell count. To assess safety, the frequency and severity of treatment-related adverse events, the incidence of laboratory abnormalities, and changes from base line in clinical and laboratory values were compared between the two treatment groups.

Treatment Regimens

Patients were centrally assigned according to a computer-generated randomization schedule in a 1:1 fashion either to receive lopinavir–ritonavir (Kaletra, Abbott Laboratories) at a dose of 400 mg of lopinavir and 100 mg of ritonavir twice daily, plus nelfinavir placebo three times daily, or to receive nelfinavir (Viracept, Agouron) at a dose of 750 mg three times daily plus a lopinavir–ritonavir placebo twice daily. No stratification was used in the randomization. After approval by the Food and Drug Administration of the nelfinavir regimen consisting of 1250 mg twice daily, the protocol was amended to allow nelfinavir to be taken either three or two times daily by patients who had completed at least 24 weeks of study treatment. Thirty patients made this change before week 48. All patients also received twice-daily open-label lamivudine (150 mg; Epivir, GlaxoSmithKline) and stavudine (40 mg; Zerit, Bristol-Myers Squibb) (patients weighing less than 60 kg received 30 mg). If adverse events related to stavudine or lamivudine occurred, the drug could be discontinued and replaced with another nucleoside drug at the discretion of the investigator.

Assessment and Monitoring

The patients were evaluated at base line, every four weeks through week 24, then every eight weeks through week 48. At each visit an interim history was taken and a physical examination was performed, adverse events were recorded, nonfasting hematologic and blood chemistry evaluation were performed, and the HIV RNA level and CD4+ cell count were determined. Peripheral fat wasting, central adiposity, breast hypertrophy, development of a dorsal fat pad, multiple lipomas, and a cushingoid appearance were all considered to be adverse events associated with lipodystrophy or lipoatrophy. Patients' rates of adherence to the regimens were

determined by pill counts of the active protease inhibitor at each study visit.

Viral Genotyping and Phenotypic Analysis

Viral genotyping (GeneSeq, ViroLogic) was performed on samples from all patients who had more than 400 copies of HIV RNA per milliliter at any study visit between weeks 24 and 48. Phenotypic analysis (PhenoSense, ViroLogic) was also conducted on samples from patients treated with lopinavir–ritonavir. For patients with multiple genotypic results, the latest sample was used for data analysis. Resistance to nelfinavir was defined as the presence of a D30N or L90M mutation in HIV protease.¹³ Genotyping was also performed on stored isolates obtained at base line from nelfinavir-treated patients with a D30N or L90M mutation at any time between week 24 and week 48. In the absence of data on new mutations selected by lopinavir *in vivo*, resistance to lopinavir was defined conservatively as the development of any primary or active-site protease mutation (i.e., a mutation at amino acid 8, 30, 32, 46, 47, 48, 50, 82, 84, or 90).¹⁴ Lamivudine resistance was defined as the presence of the M184V/I mutation in reverse transcriptase.

Statistical Analysis

The planned sample size of 330 patients per treatment group provided the study, at the two-sided 0.05 level of significance, with 80 percent power to detect an absolute difference of 10 percent in the proportion of patients with fewer than 400 copies of HIV RNA per milliliter, based on a predicted response rate of 70 percent at week 24 for nelfinavir-treated patients. It also provided 85 percent power to detect an absolute difference of 12 percent (62 percent vs. 50 percent) in the proportion of patients with a virologic response that persisted through week 48. As specified in the protocol, only patients who actually received the study drugs were included in the analyses. The proportion of patients with fewer than 400 or fewer than 50 copies of HIV RNA per milliliter was summarized, with missing values considered to be greater than or equal to 400 copies per milliliter or greater than or equal to 50 copies per milliliter, respectively. Treatment groups were compared by Fisher's exact test. The distribution of values for time to the loss of virologic response through week 48 was estimated with use of a Kaplan–Meier procedure, and the treatment groups were compared by means of the Cox proportional-hazards model. The time of the loss of virologic response was defined as day 0 for patients who never had fewer than 400 copies of HIV RNA per milliliter or as the date of the first of the following: the first of two consecutive HIV RNA values greater than or equal to 400 copies per milliliter after any HIV RNA value of less than 400 copies per milliliter, the use of an antiretroviral agent not allowed by the protocol, or premature discontinuation of the study drug for reasons related to treatment. The rates of incidence of adverse events were compared by Fisher's exact test. A one-way analysis of variance was used to evaluate mean changes from base line. All statistical tests were two-sided.

The data were gathered at study centers by site investigators and research nurses and forwarded to Abbott Laboratories for data entry and statistical analysis. The principal investigator (Dr. Walmsley) was provided with the data set, drafted the manuscript, and then revised it on the basis of comments from all the other authors. No restrictions were imposed by Abbott on the interpretation of the data or the writing of the manuscript.

RESULTS

Of 859 patients screened, 686 met the eligibility criteria and were randomly assigned to treatment between March 30, 1999, and September 30, 1999. Thirty-three patients (17 assigned to lopinavir–ritonavir and 16 to nelfinavir) never received a study drug;

the remaining 653 patients were included in the analysis. The base-line characteristics were similar in the two study groups (Table 1). A total of 56 patients in the lopinavir–ritonavir group and 77 patients in the nelfinavir group withdrew from the study before week 48. Study drug–related adverse events resulted in discontinuation in 3.4 percent of patients treated with lopinavir–ritonavir and 3.7 percent of those treated with nelfinavir, and discontinuation due to virologic failure occurred more frequently among patients treated with nelfinavir than among those treated with lopinavir–ritonavir (9.2 percent vs. 0.6 percent, $P<0.001$) (Table 2). Although discontinuation of the randomly assigned therapy was not required for patients meeting protocol-defined virologic end points, all patients who discontinued the study with an investigator-specified reason of virologic failure had reached a protocol-defined virologic end point. Unblinding occurred in less than 2 percent of patients and was generally related to the management of clinically significant medical events.

Antiviral Activity

In the primary analysis at week 24, 259 of 326 patients treated with lopinavir–ritonavir (79 percent)

and 233 of 327 nelfinavir-treated patients (71 percent) had fewer than 400 copies of HIV RNA per milliliter ($P<0.05$). The response rates at week 48 were 75 percent for patients treated with lopinavir–ritonavir (245 of 326 patients) and 63 percent for nelfinavir-treated patients (206 of 327 patients; $P<0.001$) (Fig. 1A). The 95 percent confidence interval for the difference in these response rates at week 48 was 5 to 19 percent. At week 48, 67 percent of patients treated with lopinavir–ritonavir and 52 percent of nelfinavir-treated patients had fewer than 50 copies of HIV RNA per milliliter ($P<0.001$) (Fig. 1B).

The primary end point for assessing the durability of the response was the time to the loss of virologic response through week 48. In this analysis, 51 patients in the lopinavir–ritonavir group did not have a persistent response, as compared with 108 patients in the nelfinavir group ($P<0.001$ by the Cox proportional-hazards model) (Fig. 2). The Kaplan–Meier estimates of the proportion of patients with a response that persisted through week 48 were 84 percent for the lopinavir–ritonavir group and 66 percent for the nelfinavir group, corresponding to a hazard ratio of 2.0 (95 percent confidence interval, 1.5 to 2.7). The reasons for the loss of virologic response included viral rebound (which occurred in 21 patients receiving lopinavir–ritonavir and 62 patients receiving nelfinavir), failure to achieve viral suppression (22 and 38 patients, respectively), discontinuation of the regimen

TABLE 1. SUMMARY OF BASE-LINE CHARACTERISTICS.*

CHARACTERISTIC	LOPINAVIR–RITONAVIR PLUS LAMIVUDINE AND STAVUDINE (N=326)	NELFINAVIR PLUS LAMIVUDINE AND STAVUDINE (N=327)
Sex — no. (%)		
Male	260 (80)	264 (81)
Female	66 (20)	63 (19)
Race or ethnic group — no. (%)		
Non-Hispanic white	184 (56)	190 (58)
Non-Hispanic black	81 (25)	83 (25)
Hispanic	48 (15)	37 (11)
Asian or Pacific Islander	8 (2)	12 (4)
Native American or Alaskan Native	3 (1)	3 (1)
Mixed race	2 (1)	2 (1)
Age — yr		
Mean	38.4±9.7	37.3±8.9
Range	19–84	20–68
Weight — kg		
Mean	73.4±14.4	74.4±14.8
Range	38.5–136.1	42.5–149.0
HIV RNA — log ₁₀ copies/ml		
Mean	4.89±0.75	4.92±0.74
Median	5.01	4.98
Range	2.60–6.82	2.79–6.84
CD4+ cell count — cells/mm ³		
Mean	260±214	258±196
Median	232	232
Range	2–944	3–949

*Plus–minus values are means ± SD.

TABLE 2. REASONS FOR STUDY DISCONTINUATION
AT OR BEFORE WEEK 48.

REASON	LOPINAVIR– RITONAVIR PLUS LAMIVUDINE AND STAVUDINE (N=326)	NELFINAVIR PLUS LAMIVUDINE AND STAVUDINE (N=327)
	no. (%)	
Death	5 (1.5)	3 (0.9)
Study drug–related adverse event	11 (3.4)	12 (3.7)
Other adverse event or HIV-related event	5 (1.5)	2 (0.6)
Loss to follow up	13 (4.0)	16 (4.9)
Personal or other reasons	14 (4.3)	10 (3.1)
Noncompliance	7 (2.1)	6 (1.8)
Patient required prohibited medication	1 (0.3)	0
Virologic failure*	2 (0.6)	30 (9.2)
Total†	56 (17.2)	77 (23.5)

* $P<0.001$ for the comparison between the treatment groups. All the patients who discontinued the study with an investigator-specified reason of virologic failure had reached a protocol-defined end point.

†Two patients in each group indicated more than one reason for discontinuation.

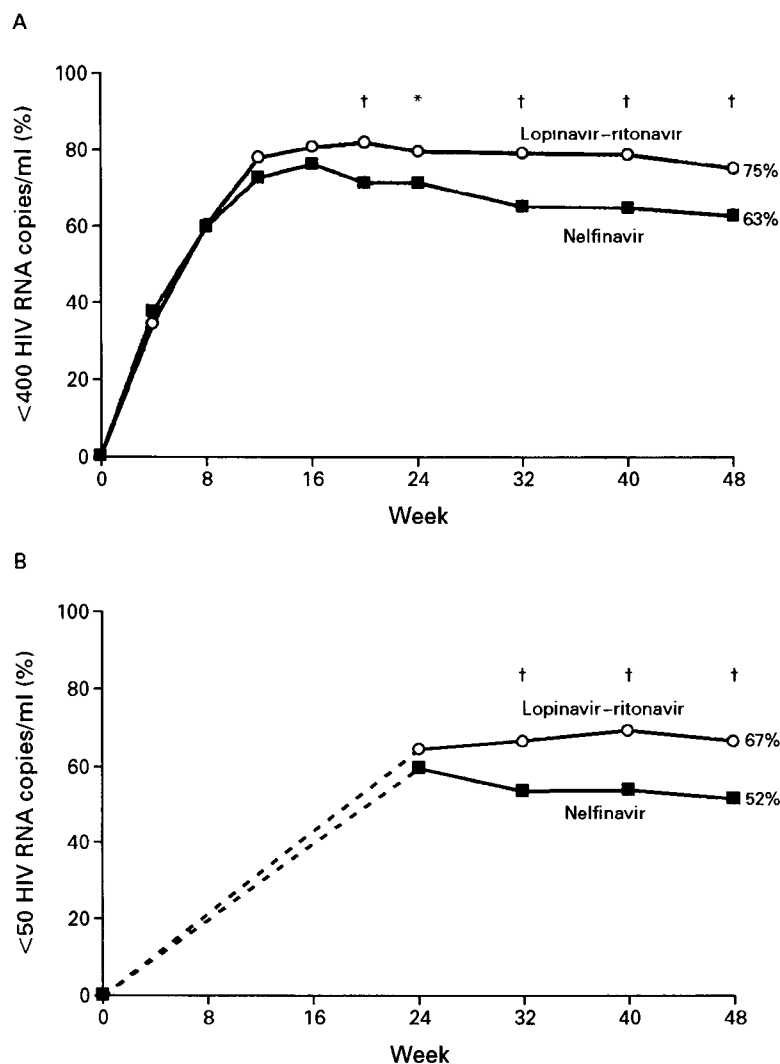


Figure 1. Percentage of Patients with Plasma Human Immunodeficiency Virus (HIV) RNA Levels of Fewer Than 400 Copies per Milliliter (Panel A) and Fewer Than 50 Copies per Milliliter (Panel B).

All the patients who received a study drug are included (326 in the lopinavir-ritonavir group and 327 in the nelfinavir group). The asterisk denotes $P < 0.05$ and the daggers $P < 0.001$ for the comparison between the treatment groups. The dashed portions of the curves represent the period before testing at fewer than 50 copies per milliliter was begun.

because of study drug-related adverse events (7 and 5 patients), and the addition of antiretroviral agents not allowed in the protocol (1 and 3 patients).

Immunologic Changes

Significant mean increases from base line in the CD4⁺ cell count were observed at all visits for each treatment group. Among 517 patients with CD4⁺ cell counts at both base line and week 48, similar mean increases from base line to week 48 were ob-

served in the lopinavir-ritonavir group and the nelfinavir group (207 and 195 cells per cubic millimeter, respectively).

Adverse Events

The most common adverse events of at least moderate severity and with a possible, probable, or unknown relation to protease inhibitor occurred with similar frequency in the two treatment groups (Table 3). Diarrhea was the most common adverse event in

LOPINAVIR-RITONAVIR FOR HIV-1 INFECTION

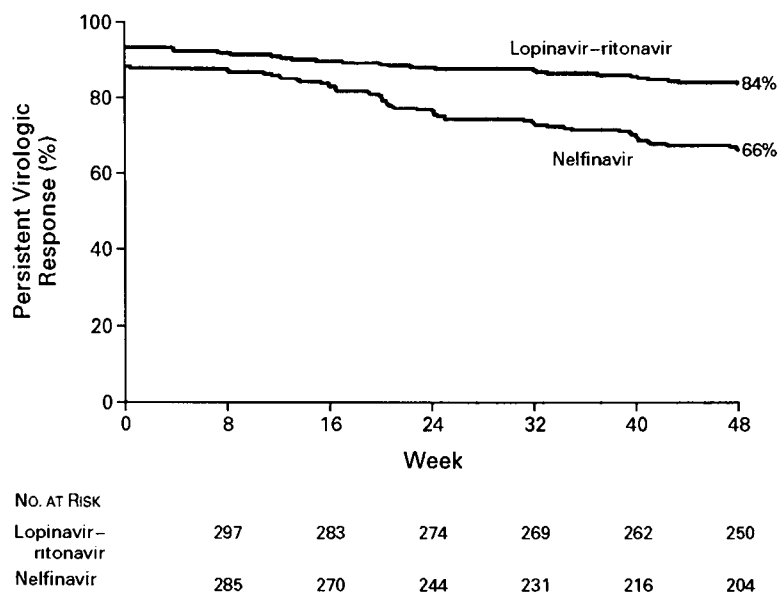


Figure 2. Kaplan-Meier Analysis of the Time to the Loss of Virologic Response through Week 48. $P < 0.001$ for the comparison between the treatment groups, based on the Cox proportional-hazards model.

both treatment groups. Diarrhea and nausea each resulted in the interruption or discontinuation of study drugs in less than 2 percent of patients in both treatment groups. Adverse events consistent with the presence of lipodystrophy or lipoatrophy were reported in 5 percent of patients receiving lopinavir-ritonavir and 6 percent of those receiving nelfinavir. Five patients receiving lopinavir-ritonavir and three receiving nelfinavir died. Four deaths were caused by HIV-associated opportunistic infections or cancers, one by pneumonia, one by coronary artery disease, and one by lactic acidosis. These four deaths were considered unrelated to the study medications. One death due to pancreatitis was considered possibly related to lopinavir-ritonavir; the patient also had lactic acidosis. New acquired immunodeficiency syndrome (AIDS)-defining events (Centers for Disease Control and Prevention class C¹⁵) were reported in 2 percent of patients in each treatment group.

Laboratory Abnormalities

Laboratory measurements were performed without regard to fasting. Grade 3 or 4 elevations in total cholesterol and in triglycerides were more common in patients receiving lopinavir-ritonavir than in those receiving nelfinavir, although the difference was not statistically significant for total cholesterol (Table 3). The mean increases from base line to week 48 in total

cholesterol were 53 mg per deciliter (1.37 mmol per liter) in patients treated with lopinavir-ritonavir and 48 mg per deciliter (1.24 mmol per liter) in those treated with nelfinavir ($P = 0.17$ for the comparison between the groups). The mean increases in triglycerides from base line to week 48 were 125 mg per deciliter (1.4 mmol per liter) in patients receiving lopinavir-ritonavir and 47 mg per deciliter (0.5 mmol per liter) in those receiving nelfinavir ($P < 0.001$ for the comparison between the groups). No patient discontinued the study because of elevated lipid or aminotransferase levels.

Viral Resistance

Fifty-eight patients treated with lopinavir-ritonavir and 102 treated with nelfinavir had at least 400 copies of HIV RNA per milliliter at week 24, 32, 40, or 48. Samples from 37 of the 58 patients receiving lopinavir-ritonavir and 76 of the 102 receiving nelfinavir could be amplified for resistance testing. None of the samples from the 37 patients treated with lopinavir-ritonavir and 25 of 76 samples from those treated with nelfinavir (33 percent) had evidence of genotypic resistance in HIV protease ($P < 0.001$). The absence of resistance to lopinavir in patients treated with lopinavir-ritonavir was confirmed by phenotypic analysis, with all isolates demonstrating wild-type susceptibility. For the 25 nelfinavir-treated subjects

TABLE 3. MOST COMMON ADVERSE EVENTS AND GRADE 3 OR 4 LABORATORY ABNORMALITIES.*

EVENT OR ABNORMALITY	LOPINAVIR- RITONAVIR PLUS LAMIVUDINE AND STAVUDINE (N=326)	NELFINAVIR PLUS LAMIVUDINE AND STAVUDINE (N=327)
	no. (%)	
Abdominal pain	13 (4.0)	10 (3.1)
Asthenia	13 (4.0)	11 (3.4)
Headache	8 (2.5)	6 (1.8)
Digestive problems		
Diarrhea	51 (15.6)	56 (17.1)
Dyspepsia†	7 (2.1)	1 (0.3)
Nausea	22 (6.7)	15 (4.6)
Vomiting	8 (2.5)	8 (2.4)
Laboratory abnormalities‡		
Aspartate aminotransferase or alanine aminotransferase >5 times upper limit of normal	14 (4.5)	17 (5.2)
Total cholesterol >300 mg/dl	28 (9.0)	16 (4.9)
Triglycerides >750 mg/dl§	29 (9.3)	4 (1.3)

*Events listed are of at least moderate severity and of probable, possible, or unknown relation to protease inhibitor. To convert the value for total cholesterol to millimoles per liter, multiply by 0.02586; to convert the value for triglycerides to millimoles per liter, multiply by 0.01129.

† $P < 0.05$ for the comparison between treatment groups.

‡Percentages are based on patients for whom post-base-line values were available (312 treated with lopinavir-ritonavir and 318 treated with nelfinavir).

§ $P < 0.001$ for the comparison between treatment groups.

with resistance, none of the 24 available corresponding base-line isolates demonstrated resistance. Among the samples from week 24 through week 48, resistance to lamivudine was less frequent in patients receiving lopinavir-ritonavir than in those receiving nelfinavir (41 percent vs. 82 percent, $P < 0.001$). There was no statistically significant difference between the treatment groups in adherence or exposure to viral replication (mean HIV RNA at base line or time of genotyping, mean days of treatment with 400 or more copies of HIV RNA per milliliter) among patients with genotypic data.

Adherence

Overall rates of adherence to the active protease-inhibitor regimens were similar in the treatment groups, with 86 percent of patients treated with lopinavir-ritonavir and 83 percent of those treated with nelfinavir having overall adherence above 90 percent. Adherence was strongly associated with treatment response, with 89 percent of all those with a response at week 48 (fewer than 400 copies of HIV RNA per

milliliter) having overall adherence rates above 90 percent, as compared with 61 percent of those without a response at week 48 ($P < 0.001$).

DISCUSSION

Protease-inhibitor therapy can greatly reduce HIV-related morbidity and mortality.^{1,4} Protease inhibitors are highly active in vitro, but their activity in vivo is limited by rapid clearance, resulting in trough levels that are only one to six times above the EC_{50} of wild-type virus.^{8,9,16} Variability among patients in drug concentrations, particularly trough levels,^{17,18} and imperfect adherence also reduce the effectiveness of the antiviral therapy. In order to achieve maximal drug levels, protease inhibitors must be given often and at high doses, potentially compromising tolerability and adherence.^{19,20} Low trough levels of protease inhibitors significantly increase the risk of clinical failure and virologic resistance.^{21,22} More potent protease inhibitors with trough concentrations substantially above the EC_{50} of wild-type virus would be expected to lower rates of viral resistance and improve overall efficacy, provided there is no increased toxicity.

Pharmacologic enhancement of protease inhibitors with ritonavir has been widely employed and has been shown to result in substantial increases in drug concentrations.²³⁻²⁶ However, with the exception of lopinavir-ritonavir, systematic dose-ranging and prospective, randomized, controlled trials have generally not been conducted for these regimens. In this study, lopinavir-ritonavir showed greater efficacy than nelfinavir, according to analyses of the proportion of patients with fewer than 400 or fewer than 50 copies of HIV RNA per milliliter at week 48 and the proportion of patients with a virologic response that persisted through 48 weeks. This improvement was achieved with similar rates of adherence and low rates of study drug-related withdrawals in both treatment groups. The overall response rates observed in the lopinavir-ritonavir group compared favorably with those observed in other randomized clinical trials of highly active antiretroviral therapy.²⁷⁻³⁰ The response seen in the nelfinavir group was similar to that previously reported for this drug.³¹⁻³³ Differences in the frequency of administration of nelfinavir and lopinavir-ritonavir are unlikely to have contributed to the difference in response rates, since the adherence rates were similar in both treatment groups. Furthermore, previous clinical trials comparing nelfinavir given either twice daily or three times daily have shown no significant differences in response rates.^{32,33} These observations suggest that the superior response seen in the patients treated with lopinavir-ritonavir reflected improved antiviral activity and not differences in dosage or adherence.

Additional important considerations in antiretrovi-

ral therapy are the risks and consequences of treatment failure. Significantly more patients had viral rebound and discontinued treatment because of virologic failure in the nelfinavir group than in the lopinavir–ritonavir group. In addition, the consequences of detectable viral load differed in the two treatment groups, in that patients with 400 or more copies of HIV RNA per milliliter were significantly more likely to have protease-inhibitor resistance in the nelfinavir group than in the lopinavir–ritonavir group (33 percent vs. 0 percent). The higher rate of resistance to lamivudine and the development of HIV-protease mutations in nelfinavir-treated patients may also undermine the response to subsequent treatment regimens.³⁴

The double-blind, placebo-controlled design of this study may have resulted in a smaller difference in dropout rates between the groups than in other recent studies of antiretroviral therapy³⁰ and minimized the potential for bias in the reporting of adverse events. Both regimens were well tolerated, with 3.4 percent of patients receiving lopinavir–ritonavir and 3.7 percent of nelfinavir-treated patients discontinuing the study because of study drug–related adverse events. Diarrhea and nausea were the most common adverse events. Laboratory abnormalities were also infrequent, although the incidence of grade 3 or 4 triglyceride elevations was greater in the lopinavir–ritonavir group. These results must be interpreted with caution, since specimens were obtained without regard to fasting. Grade 3 or 4 triglyceride elevations were not associated with acute clinical events. However, longer follow-up will be necessary to determine the clinical significance of elevations in lipid levels, especially as they relate to cardiovascular morbidity and mortality. The incidence of lipodystrophy or lipodystrophy was low and was similar in the two treatment groups. However, because these changes can take longer than 48 weeks to develop, the relative long-term rates remain unknown.

Successful antiviral therapy requires adequate drug potency, good tolerability, and a high barrier to resistance. Pharmacologic improvements in the administration of protease inhibitors are likely to provide more sustained clinical benefit. When antiretroviral therapy is selected for patients not previously treated with antiretroviral drugs, the balance among antiretroviral activity, immunologic recovery, and short- and long-term toxicity must be determined for each patient individually. The findings from this trial show the benefits of therapy with lopinavir–ritonavir, as demonstrated by its superior antiviral activity in comparison with that of nelfinavir and its continued tolerability and high barrier to resistance. These characteristics suggest an important role for lopinavir–ritonavir as an initial protease inhibitor–based treatment for HIV infection.

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APPENDIX

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Saquinavir pharmacokinetics alone and in combination with ritonavir in HIV-infected patients

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Objective: The most important hepatic enzyme involved in the metabolism of protease inhibitors is cytochrome P450 3A4 (CYP3A4). Ritonavir (RIT) is a potent inhibitor of CYP3A4 and inhibits saquinavir (SQV) metabolism in healthy volunteers. In this study we investigated the kinetics of SQV when administered alone and in combination with RIT in HIV-infected patients.

Design: SQV pharmacokinetics were determined in seven patients who had advanced HIV disease. Steady-state SQV profiles were obtained on two occasions following treatment with SQV 600 mg three times daily alone and when administered with RIT 300 mg twice daily.

Methods: Blood samples were obtained at times 0, 1, 2, 4, 6 and 8 h post-dosing. Following centrifugation, separated plasma was heated at 58°C for at least 30 min to inactivate HIV and stored at -80°C until analysis using high performance liquid chromatography.

Results: For patients treated with SQV alone there was a 12-fold variability in the area under the SQV concentration-time curve (AUC_{0-8h}) ranging from 293 to 3446 ng•h/ml. When combined with RIT there was a marked increase in the maximum plasma concentration of SQV [median (range), 146 (57-702) versus 4795 (1420-15810) ng/ml; ~95% confidence interval (CI), 2988-6819; $P = 0.0006$, Mann-Whitney U test]. The AUC_{0-8h} for SQV was also significantly increased in the presence of RIT [median (range), 470 (293-3446) versus 27 458 (7357-108 001) ng•h/ml; ~95% CI, 16 628-35 111; $P = 0.0006$].

Conclusions: For some patients, administration of SQV 600 mg three times daily results in very low SQV plasma levels and possibly little antiviral effect. Combination of SQV with RIT results in a significant drug interaction mediated by enzyme inhibition which exposes patients to very high SQV concentrations and potential toxicity. If combination therapy with SQV plus RIT is considered then the dose of SQV should be greatly reduced.

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Keywords: Saquinavir, ritonavir, pharmacokinetics, HIV, AIDS

Introduction

In 1996, treatment with zidovudine (ZDV) plus didanosine (ddI) or ZDV plus zalcitabine (ddC) was

demonstrated to be more effective than ZDV monotherapy in preventing disease progression and reducing mortality in patients with HIV disease [1,2]. Further progress followed recent studies with three

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reverse transcriptase (RT) inhibitors or two RT inhibitors and a protease inhibitor producing greater antiviral activity with prolonged reductions of 2.0–3.0 log in plasma HIV RNA [3,4]. The HIV protease enzyme is responsible for post-translational processing of Gag and Gag-Pol polyprotein precursors into their functional units and inhibition of the enzyme results in the production of immature, non-infectious virions. Initial studies suggest that the protease inhibitors are very potent anti-HIV drugs with ritonavir (RIT) producing a 1.7-log reduction in plasma viraemia, which is similar to the combination of lamivudine (3TC) plus ZDV. As with the nucleoside analogues, viral resistance may develop with the protease inhibitors; for example, incidence is approximately 45% after 1 year of saquinavir (SQV) monotherapy [5]. Furthermore, cross-resistance to other protease inhibitors has been demonstrated following administration of indinavir suggesting initial therapy with indinavir may limit the benefit of subsequent treatment with others [6]. *In vitro* work demonstrates that isolates from patients treated with RIT may show decreased sensitivity to RIT but remain susceptible to SQV suggesting that combination of these two protease inhibitors may be more effective than monotherapy [7]. However, the administration of protease inhibitors in combination does raise important pharmacokinetic issues in particular clinically relevant drug interactions.

In vitro studies using human liver microsomes have demonstrated that RIT is a potent inhibitor of cytochrome P450 3A4 (CYP3A4) and to a lesser extent other cytochrome P450 isozymes [8]. SQV is extensively metabolised by CYP3A4, and in rats coadministration of RIT increases the area under the concentration–time curve (AUC) of SQV by 36-fold [9]. Single and multiple dose studies in healthy volunteers have also shown that RIT greatly increases the AUC and maximum concentration (C_{max}) of coadministered SQV [10]. We have now determined the magnitude of the RIT–SQV interaction in the clinical setting and report the pharmacokinetics of SQV when administered alone and in combination with RIT in HIV-infected patients.

Methods

Patients

Seven male patients, aged 28–45 years, with sexually acquired HIV infection participated in this study. All patients had advanced HIV disease (Centers for Disease Control and Prevention 1993 criteria, stage IV) with a mean CD4 count of $25 \times 10^6/l$ (range, 10 – $60 \times 10^6/l$). Current medications included co-trimoxazole 960 mg daily as prophylaxis for *Pneumocystis carinii* pneumonia. All patients were receiving triple therapy with ZDV 250 mg twice daily, 3TC 150 mg twice daily and SQV

600 mg three times daily. The mean duration of nucleoside therapy was 2.5 years. All patients were considered for quadruple therapy with the addition of RIT to their current regimen. There was no evidence of significant hepatic (greater than twofold elevation of hepatic transaminases) or renal dysfunction and no medication known to interfere with SQV metabolism (except RIT) was prescribed during the study period. Approval for the study was obtained from the St James's Hospital Ethics Committee and each patient provided written informed consent.

On the first study day, patients attended at 0830 h after an overnight fast. An indwelling intravenous cannula was inserted in the cubital fossa to facilitate blood sampling. At 0900 h a fasting blood sample was taken (time 0 h). Patients ingested ZDV 250 mg, 3TC 150 mg and SQV 600 mg. Blood samples were then taken at 1, 2, 3, 4, 6 and 8 h post-dosing. Prior to the second study day 1 week later, patients had been treated with their usual medications (ZDV, 3TC, SQV) plus RIT 300 mg twice daily for 4 days. The procedure on study day 2 was identical to that of the first day, except for the addition of RIT 300 mg to ZDV, 3TC and SQV prior to sampling. All samples were centrifuged without delay and the separated plasma was heated to 58°C for at least 30 min to inactivate HIV. Plasma was then stored at –80°C until SQV analysis using high performance liquid chromatography (HPLC).

Drug analysis

SQV concentrations in plasma were determined as follows. Plasma samples in duplicate (0.2–0.5 ml, depending on the phase of the study) were pipetted into glass tubes, and sodium hydroxide (0.5 M; 0.3 ml) and internal standard (31-9564; Roche Products, Welwyn Garden City, Hertfordshire, UK) added. Tube contents were mixed thoroughly. Standard curves were prepared containing blank plasma and SQV at a concentration range of 20–400 or 500–8000 ng/ml. Quality control samples for the assessment of precision and accuracy of the assay were prepared by adding known quantities of SQV to blank plasma samples. Test samples, standards and quality control samples were extracted with diethyl ether (5 ml) for 10 min. After centrifuging for 5 min at 3000 rpm the organic phase was transferred to clean tubes and evaporated to dryness. Extracts were reconstituted in the HPLC mobile phase (0.15 ml) and washed with hexane (1 ml). Hexane was subsequently discarded and washed extracts transferred to vials for injection into the HPLC.

SQV and the internal standard were resolved on a Megellen 5C₈ column (5 µm; 250 × 4.6 mm; Phenomenex, Macclesfield, Cheshire, UK) with a mobile phase of acetonitrile : water (63 : 37) at a flow rate of 1 ml/min. Absorbance was monitored at 238 nm. Peaks of interest, SQV (retention time, 10.5 min) and internal standard (retention time, 25 min) were quanti-

fied using a Kontron MT2 data acquisition system (Kontron Instruments, Watford, Hertfordshire, UK).

The limit of quantification was 20 ng/ml. Interassay variability was determined with two different control samples containing nominal concentrations of 100 and 1000 ng/ml. The coefficients of variation were 3.6 and 4.1%, respectively. Intra-assay precision was determined with samples containing 100 and 1000 ng/ml. The coefficients of variation were 8.2 and 3.6%, respectively.

Pharmacokinetic and statistical analysis

SQV concentrations were evaluated for C_{max} , time to C_{max} and AUC to 8 h (AUC_{0-8h}). AUC values were determined by non-compartmental analysis using the TOPFIT computer software (Gustav Fischer Verlag, Stuttgart, Germany).

Differences in SQV pharmacokinetic parameters were compared using the Mann-Whitney U test. A P value < 0.05 was considered statistically significant.

Results

The results are summarized in Table 1. Patients administered SQV 600 mg three times daily demonstrated a 12-fold variability in SQV AUC_{0-8h} ranging from 293 to 3446 ng•h/ml (Fig. 1). When combined with RIT there was a marked increase in the maximum plasma concentration of SQV: median (range), 146 (57–702) versus 4795 (1420–15 810) ng/ml, ~95% confidence interval (CI), 2988–6819; $P = 0.0006$, Mann-Whitney U test (Fig. 2). Because SQV plasma concentrations are shown on an arithmetic scale, the profile of the SQV alone phase appears virtually flat. However, there was an approximate threefold increase in SQV concentration from trough (0 h) to peak (C_{max}). The AUC_{0-8h} for SQV was also significantly increased in the presence of

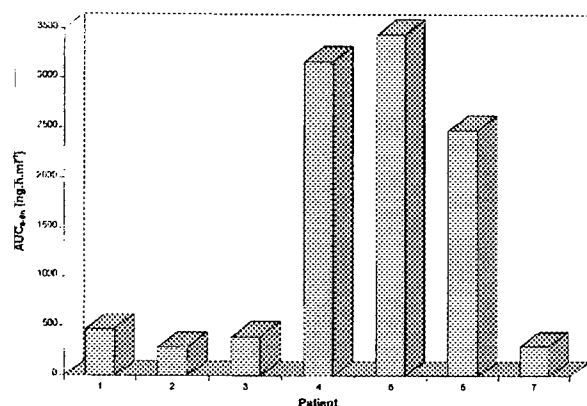


Fig. 1. Area under the concentration-time curve (AUC_{0-8h}) for saquinavir (SQV) in seven patients treated with SQV 600 mg three times daily.

Table 1. Pharmacokinetic parameters for saquinavir (SQV) 600 mg three times daily alone and in combination with ritonavir (RIT) 300 mg twice daily.

	Median (range)		P
	SQV alone	SQV + RIT	
C_{max} (ng/ml)	146 (57–702)	4795 (1420–15810)	0.0006
AUC_{0-8h} (ng•h/ml)	470 (293–3446)	27458 (7357–108001)	0.0006

Significance is defined as $P < 0.05$ (Mann-Whitney U test).

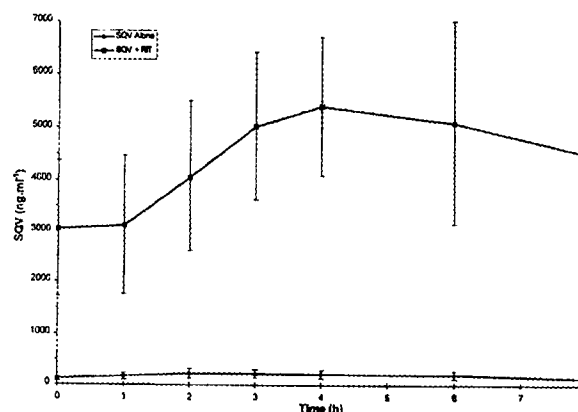


Fig. 2. Mean ($\pm$ SEM) plasma saquinavir (SQV) steady state concentrations following treatment with SQV 600 mg three times daily alone and in combination with ritonavir (RIT) 300 mg twice daily.

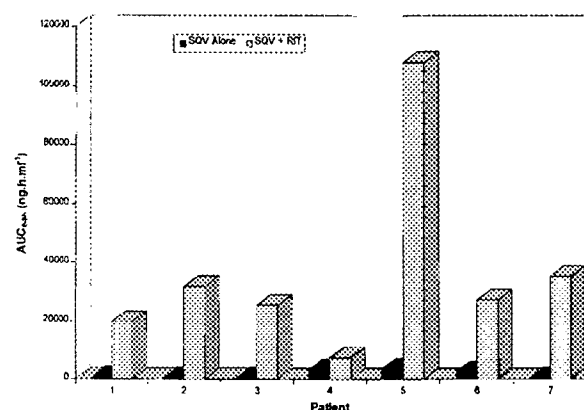


Fig. 3. Area under the concentration-time curve (AUC_{0-8h}) for saquinavir (SQV) in seven patients treated with SQV 600 mg three times daily alone and in combination with ritonavir (RIT) 300 mg twice daily.

RIT: median (range), 470 (293–3446) versus 27 458 (7357–108 001) ng•h/ml; ~95% CI, 16 628–35 111; $P = 0.0006$ (Fig. 3).

Discussion

It is estimated that there are at least 15 human liver cytochrome P450s involved in drug metabolism with the isozymes CYP1A2, CYP3A4, CYP2D6, CYP2C9 and CYP2C19 responsible for the metabolism of the

majority of drugs [11]. The single most important drug metabolising cytochrome P450 is CYP3A and *in vitro* evidence suggests that this is the most influential isoenzyme involved in the metabolism of the protease inhibitors with the isoforms CYP2C9 and CYP2D6 also contributing [9]. If drugs are substrates for a common cytochrome P450 enzyme it may be anticipated that the elimination of one or both may be delayed if the drugs are coadministered. If, as is likely, two protease inhibitors are administered in combination as therapy for HIV we would predict a drug interaction, which is indeed supported by recent *in vitro* and *in vivo* studies. RIT potentially inhibited the metabolism of SQV in rat and human liver microsomes with median inhibitory concentration values of 0.42 and 0.25 μM , respectively [9]. Recent single and multiple dose interaction studies between SQV and RIT in healthy volunteers confirm the increased SQV concentration when coadministered with RIT, whereas RIT pharmacokinetics remained unaltered [10].

In this study we investigated the potential clinical relevance of such an interaction by determining SQV kinetics when administered alone and in combination with RIT in HIV-infected patients attending our clinic. Steady-state SQV profiles were obtained on two occasions following treatment with SQV 600 mg three times daily and when administered with RIT 300 mg twice daily. Our results demonstrate a significant drug interaction between RIT and SQV probably mediated by enzyme inhibition as demonstrated by *in vitro* studies [9]. In the presence of RIT the maximum plasma concentration of SQV was increased 30-fold with a 58-fold increase in the median $\text{AUC}_{0-8\text{h}}$ compared with SQV administered alone. This represents a very potent inhibitory effect of RIT on SQV metabolism exposing patients to very high SQV concentrations. It has been proposed that an interaction between RIT and SQV may be beneficial and overcome problems associated with the poor bioavailability of SQV [12]. Doses of SQV higher than the standard 600 mg three times daily produce a greater and more sustained suppression of HIV viral load and elevation of CD4 counts. Treatment with SQV 3600 mg daily resulted in a maximum mean decrease in plasma HIV RNA levels of 1.06 log RNA copies/ml and a maximum increase in CD4 cell counts of $72 \times 10^6/\text{l}$. Increasing the SQV dose to 7200 mg daily resulted in a mean decrease in HIV RNA of 1.34 log RNA copies/ml with a maximum increase in CD4 counts of $121 \times 10^6/\text{l}$ [13]. Therefore, the ability of RIT to increase SQV levels together with the non-overlapping resistance patterns of SQV and RIT would appear to offer an attractive therapeutic option for the treatment of HIV disease.

Our data show that levels of SQV achieved with SQV 600 mg three times daily plus RIT 300 mg twice daily would be approximately six times greater than those

after SQV 7200 mg daily. Significant toxicity occurred in patients treated with SQV 7200 mg daily with 5% of patients developing severe (grade 3) elevations in liver function tests, 5% developed severe (grade 3) neutropenia, and 5% developed life-threatening (grade 4) elevations in creatinine phosphokinase levels [13]. Therefore, it is not surprising that dosing regimens such as RIT 600 mg twice daily plus SQV 600 mg twice daily, which have been proposed as a therapeutic option and produce SQV levels much higher than monotherapy with SQV 7200 mg daily, result in the appearance of severe (grade 3) or life-threatening (grade 4) elevations in liver function tests in approximately 20% of patients [14]. Therefore, the choice of dosing regimen will be crucial in order to optimize the antiviral efficacy and minimize toxicity with this combination. Preliminary results (unpublished data) from our clinic suggest that the SQV $\text{AUC}_{0-2\text{h}}$ achieved by the combination of SQV 200 mg once daily plus RIT 600 mg twice daily may exceed that following SQV 7200 mg daily, giving some indication of the SQV dose that may suffice when combined with RIT.

As SQV is predominantly metabolised by CYP3A4 present in the gastrointestinal tract and the liver, and as significant intersubject variability in CYP3A4 exists it explains, in part, the 12-fold variability in SQV $\text{AUC}_{0-8\text{h}}$ following SQV 600 mg three times daily. This variability is also seen in the presence of RIT with the SQV $\text{AUC}_{0-8\text{h}}$ ranging from 7357 to 108 001 $\text{ng}\cdot\text{h}/\text{ml}$. As a consequence of this observed variability, for some patients treatment with SQV 600 mg three times daily will result in very low SQV plasma levels and possibly little antiviral effect. When combined with RIT the variability of SQV levels will result in some patients being exposed to very high SQV levels and subsequent toxicity.

As RIT is a potent inhibitor of CYP3A it would be expected to inhibit the metabolism of other CYP3A substrates including protease inhibitors (indinavir, nelfinavir), azole antifungal drugs (ketoconazole, itraconazole), hypnotics (midazolam) and macrolide antibiotics (erythromycin, clarithromycin). Coadministration of RIT and clarithromycin in healthy volunteers produced a 77% increase in clarithromycin AUC and a threefold increase in the elimination half-life [15]. Treatment with RIT 500 mg twice daily plus rifabutin 150 mg daily resulted in a fourfold increase in rifabutin AUC [16]. The ability of RIT to inhibit cytochrome P450 enzymes other than CYP3A is demonstrated by the inhibition of desipramine (CYP2D6) metabolism [17]. As a consequence of the potent inhibition of cytochrome P450 enzymes, RIT is contraindicated in patients receiving some non-sedating antihistamines (astemizole, terfenadine), sedative hypnotics (midazolam) and antiarrhythmics (amiodarone, encainide, flecainide, propafenone, quinidine). However, in addition

to these inhibitory effects, RIT may also induce CYP1A2 [18] and glucuronyl transferase activity [19] thereby increasing the metabolism of drugs such as theophylline (CYP1A2), zidovudine, opiates and ethinyloestradiol (all metabolised by glucuronyl transferase). As the mean AUC of ethinyloestradiol may be reduced by 40% during treatment with RIT 500 mg twice daily, use of alternative contraceptive measures should be considered [19]. The potential for multiple drug interactions mediated predominantly by enzyme inhibition but also by induction, together with the poor tolerability of RIT that we have described in patients with advanced disease (30% discontinuation of therapy [20]), may well limit the use of this potent anti-HIV drug in some patients [21].

This study clearly demonstrates the ability of RIT to inhibit SQV metabolism in the clinical setting. The very high SQV levels as a consequence of this interaction will expose patients to SQV toxicity (increased liver function tests, increased creatinine phosphokinase, neutropenia). If combination therapy with RIT plus SQV is considered, then the dose of SQV should be greatly reduced. Further studies are in progress to determine the optimum dosage.

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Pharmacokinetic Interaction between Ritonavir and Indinavir in Healthy Volunteers

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The pharmacokinetic interaction between indinavir and ritonavir was evaluated in five groups of healthy adult volunteers to explore the potential for twice-daily (b.i.d.) dosing of this combination. All subjects received 800 mg of indinavir every 8 h (q8h) on day 2. In addition, subjects in group I received one dose of 800 mg of indinavir on day 1 and 800 mg of indinavir q8h on day 17. Subjects in Groups II and IV each received one dose of 600 mg of indinavir on days 1 and 17, and subjects in groups III and V each received one dose of 400 mg of indinavir on days 1 and 17. During days 3 to 17, ritonavir placebo or ritonavir at 200, 300, 300, or 400 mg q12h was given to groups I, II, III, IV, and V, respectively. Ritonavir at steady state probably inhibited the cytochrome P-450 3A metabolism of indinavir and substantially increased plasma indinavir concentrations, with the area under the plasma concentration-time curve (AUC) increasing up to 475% and the peak concentration in serum (C_{max}) increasing up to 110%. The C_{max} /trough concentration ratio decreased from 50 in standard q8h regimens to less than 14 when indinavir was administered with ritonavir. For a constant indinavir dose, an increase in the ritonavir dose yielded similar indinavir AUCs, C_{max} s, and concentrations at 12 h (C_{12} s). For a constant ritonavir dose, an increase in the indinavir dose resulted in approximately proportional increases in the indinavir AUC, less than proportional increases in C_{max} , and slightly more than proportional increases in C_{12} . Ritonavir reduced between-subject variability in the indinavir AUC and trough concentrations and did not affect indinavir renal clearance. With the altered pharmacokinetic profile, indinavir likely could be given as a b.i.d. combination regimen with ritonavir. This could potentially improve patient compliance and thereby reduce treatment failures.

Recent advances in the management of human immunodeficiency virus (HIV) infection have led to a new perception of the disease and new treatment paradigms (10). These advances include an improved understanding of the pathogenesis of HIV infection (20, 52), the development of reliable assay methodologies for the detection and quantification of viral load (48, 49), the availability of new, potent antiviral agents, and an appreciation that combination antiretroviral therapy reduces disease progression and mortality risks (7, 8, 16, 19, 37). In this context, indinavir, zidovudine, zalcitabine, and didanosine are the promising new HIV protease inhibitors for the treatment of HIV infection. More potent and sustained suppression of viral replication and durable immunoreconstitution has been shown with the use of dual protease inhibitors or protease inhibitors in combination with nucleoside analogs compared with the suppression achieved with monotherapy (9, 13, 15, 37, 42, 43, 54, 55, 57, 60, 62).

Despite these encouraging advances, anti-HIV therapy remains suboptimal. The durability of the antiviral effect is transient in some individuals. Furthermore, effective combination therapies often demand complex regimens that may involve burdensome frequent dosing, side effects, food restrictions, and hydration requirements. These factors reduce the level of patient adherence to the treatment regimen and may ultimately lead to treatment failure.

Indinavir is a potent HIV protease inhibitor, with an *in vitro*

90% inhibitory concentration (IC_{90}) (HIV-1_{IIIB}) of approximately 0.1 μ g/ml in a system containing 50% human serum and 10% fetal calf serum (1, 46, 47). Indinavir is rapidly metabolized by cytochrome P-450 3A isoenzymes (CYP3A), the major enzymes present in the liver and gastrointestinal tract, with an elimination half-life of 1.8 h (11, 45). The renal clearance (CL_R) of indinavir is reported to be relatively constant over a wide range of concentrations (4, 63). When given as an 800-mg regimen three times a day (t.i.d.) under fasting conditions, the mean steady-state area under the concentration-time curve (AUC) of indinavir for HIV-infected patients over one dose interval was reported to be 18.8 μ g $\cdot$ h/ml (37% coefficient of variation [CV]), and the maximum concentration of drug in serum (C_{max}) and the minimum concentration in serum (C_{min}) were reported to be 7.73 μ g/ml (32% CV) and 0.154 μ g/ml (71% CV), respectively (45). Meals with high fat and protein contents decreased the indinavir AUC and C_{max} by 80 and 85%, respectively (45). To maintain therapeutic concentrations in plasma, indinavir is given at high doses on a strict schedule of once every 8 h (q8h) with rigid meal schedules.

Ritonavir has relatively high bioavailability and an elimination half-life of 3 to 5 h (1, 25, 27, 38). Although it is about 99% protein bound when it is given at a dosage of 600 mg twice daily (b.i.d.) concentrations in plasma in excess of the protein binding-adjusted IC_{90} (2.1 μ g/ml) are usually maintained throughout the dosing interval (14). The bioavailability of ritonavir is minimally affected by food (1).

Ritonavir is a potent CYP3A inhibitor *in vitro* (34), with an estimated K_i of 0.085 μ g/ml for indinavir in experiments with human liver microsomes (33a). In rats, the combined administration of ritonavir and indinavir (10 mg/kg of body weight for

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each drug) resulted in an eightfold increase in the indinavir AUC compared to that for indinavir given alone (26, 28). Recently, we described a clinically significant interaction between ritonavir and saquinavir (22). Probably due to the inhibition of the CYP3A metabolism of saquinavir by ritonavir, saquinavir AUC values were greatly enhanced (>50-fold) when saquinavir was coadministered with ritonavir; the combination regimen given b.i.d. with reduced doses of both drugs has been shown to suppress the level of viral RNA to below quantitation limits (<200 copies/ml) in 90% of the patients treated for 60 weeks (9). Coadministration with ritonavir is expected to significantly alter the pharmacokinetic profile of indinavir in patients. Since the extent of interaction was unknown, to avoid over- or underexposure to protease inhibitors in HIV-infected patients, this study was conducted with non-HIV-infected healthy volunteers.

In contrast to reverse transcriptase inhibitors, significant correlations between antiviral activity and plasma drug concentrations have been demonstrated for HIV protease inhibitors in various clinical studies (2, 6, 14, 17, 27, 59). Suboptimal concentrations in plasma are clearly associated with a rebound in viral titer, indicating the importance of maintaining high concentrations of protease inhibitors in plasma. For indinavir, although the average concentration in plasma from a standard 800-mg q8h regimen is well above the IC_{50} , the mean C_{min} is only slightly above the IC_{50} . Furthermore, C_{min} is highly variable between individuals, suggesting that some patients may have low antiviral coverage for several hours each day. Thus, if ritonavir inhibition of indinavir metabolism can increase the C_{min} of indinavir and allow twice-daily administration, then the combination therapy is expected to reduce treatment failures associated with noncompliance or subtherapeutic lapses in C_{min} .

MATERIALS AND METHODS

Study design. This study evaluated the pharmacokinetics of indinavir in the absence and presence of ritonavir in five groups of non-HIV-infected healthy volunteers by a randomized, multiple-dose, open study design. On day 1, all subjects received an assigned single dose of indinavir (800 mg for group I, 600 mg for groups II and IV, and 400 mg for groups III and V). On day 2, all subjects received 800 mg of indinavir q8h. During days 3 to 17, subjects in groups I, II, III, IV, and V received ritonavir placebo or ritonavir at 200, 300, 300, or 400 mg every 12 h (q12h), respectively. On day 17, in addition to ritonavir doses, subjects in group I (control group) received 800 mg of indinavir q8h, while subjects in groups II and IV each received one dose of 600 mg of indinavir and subjects in groups III and V each received one dose of 400 mg of indinavir.

A total of 30 healthy subjects (8 in groups I, II, IV, and V and 7 in group III), including 14 females, were enrolled in and completed the study. Since a large interaction was expected, the sample size for the study was determined on the basis of the variability observed from previous interaction studies. All subjects were between the ages of 18 and 45 years, tobacco nonusers, and negative for recreational drug and alcohol as determined by screens with an enzyme multiplied immunoassay test kit. No statistically significant differences in demographic characteristics were found among the five treatment groups. Across the groups, the mean $\pm$ standard deviation age, body weight, height, and creatinine clearance were 29.0 ± 7.9 years, 72.0 ± 8.9 kg, 171.6 ± 9.4 cm, and 105 ± 18 ml/min, respectively. All subjects gave written informed consent to participate in this study.

Subjects were confined on study days 1, 2, and 17. Breakfast, morning snack, lunch, dinner, and evening snack were served at approximately 0630, 0930, 1230, 1700, and 2130 h, respectively. Indinavir doses were given at approximately 0700 h on day 1 for all groups and at 0700, 1500, and 2300 h on day 2 for all groups. On day 17, indinavir doses were given at approximately 0700, 1500, and 2300 h for group I and at 0700 h for groups II to V. Ritonavir doses were given at 0700 and 1900 h on day 17. The morning indinavir doses were given after a low-fat breakfast, which consisted of 370 kcal, with 5% of the calories from fat. The afternoon and evening indinavir doses were given between meals. The meals served were identical for days 1, 2, and 17. With the exception of breakfast, all meals and snacks served on days 1, 2, and 17 were regular meals containing approximately 30 to 35% fat. During days 4 and 15, subjects received ritonavir or ritonavir placebo doses at the study site after consuming breakfast and dinner at approximately 0600 and 1800 h, respectively, but were otherwise allowed to be outpatients. During the days that indinavir was given (days 1, 2, and 17), subjects were encouraged to drink at least 1.5 liters of fluid per day. All doses were given

with 240 ml of water. Commercial formulations were used in the study. This study was approved by the Institutional Review Board of Victory Hospital, Waukegan, Ill.

Blood sampling and determination of ritonavir and indinavir concentrations. Blood samples (5 ml on days 1 and 2 and 7 ml on day 17) for the determination of indinavir concentrations were collected on days 1, 2, and 17, and samples for the determination of ritonavir concentrations were collected on days 4, 5, 6, 7, 8, 10, 12, and 14 immediately before administration of the morning doses and on day 17 over a 40-h period. On day 1, samples were collected at 0 (predosing), 0.5, 1, 1.5, 2, 3, 4.5, 6, 8, 10, 12, and 18 h after administration of the morning dose on day 1. On day 2, samples were collected predosing and at 0.5, 1, 1.5, 2, 3, 4.5, 6, 8, 8.5, 9, 9.5, 10, 11, 12.5, 14, 16, 16.5, 17, 17.5, 18, 19, 20.5, 22, and 24 h after administration of the morning dose on day 2. On day 17, blood samples were collected predosing and at 0.5, 1, 1.5, 2, 3, 4.5, 6, 8, 8.5, 9, 9.5, 10, 11, 12.5, 14, 16, 16.5, 17, 17.5, 18, 19, 20.5, 22, 24, 26, 28, 32, 36, and 40 h after the administration of the morning dose for group I. For groups II to V, blood samples were collected predosing and at 0.5, 1, 1.5, 2, 3, 4.5, 6, 8, 10, 11, 12.5, 13, 13.5, 14, 15, 16.5, 18, 20, 22, 24, 28, 32, 36, and 40 h after administration of the morning dose. For the determination of concentrations of ritonavir and indinavir in plasma, a total of 440 ml of blood was collected for group I and 398 ml was collected for groups II to V. In addition, approximately 90 ml of blood was collected throughout the study for clinical laboratory tests. Urine samples (0 to 24 h) for the determination of indinavir concentrations were collected on days -1 (over a 12-h period, as a baseline), 1, 2, 17, and 18 for all groups.

The concentrations of ritonavir and indinavir in plasma were simultaneously measured on the basis of a validated reverse-phase high-performance liquid chromatography (HPLC) procedure with UV detection at 205 nm (44) and with a modified mobile phase. The assay precisions for indinavir and ritonavir were high, with CVs from replicate results ranging from 2.2 to 7.0% for indinavir and 2.9 to 6.7% for ritonavir. The lower limits of quantitation for the assay were 3.63 ng/ml for indinavir and 3.81 ng/ml for ritonavir with 1 ml of plasma.

In brief, plasma samples were supplemented with saquinavir prepared in methanol-water (1:1; vol/vol) as the internal standard. The samples were then alkalinized with 0.5 M Na_2CO_3 and extracted with hexane-ethyl acetate (1:1; vol/vol). The organic layer was evaporated to dryness and was redissolved in 300 μ l of the reconstitution solution, a mixture of acetonitrile-methanol-aqueous tetramethylammonium perchlorate solution with trifluoroacetic acid, and washed with an aliquot of hexane. The aqueous layer was injected onto the HPLC system. Separation was achieved on a YMC ODS-AQ column (3-4.5; 50 by 4.6 mm; particle size, 3 μ m) in conjunction with a YMC ODS-AQ cartridge (25 by 4.0 mm; particle size, 5 μ m). The mobile phase for the separation was a mixture of acetonitrile, methanol, and an 0.05 M octanesulfonic acid solution containing 0.1% trifluoroacetic acid (37:5:58, by volume). The flow rate was maintained at 1.5 ml/min.

The extraction recoveries of indinavir and ritonavir at concentrations of 10, 18, 101.8, and 1,018 ng/ml ranged from 85 to 111%. Standard curves were linear over the range of 3.63 to 3038 ng/ml for indinavir and 3.63 to 3,180 ng/ml for ritonavir. Samples with concentrations higher than the upper limit of the standard curve were diluted and reanalyzed. Mean correlation coefficients for the calibration curves for plasma and urine samples ranged between 0.9907 and 0.9999. Interassay CVs ranged from 4.5 to 6.8% for quality control samples for indinavir and from 4.7 to 6.3% for quality control samples for ritonavir for the following three pairs of ritonavir-indinavir concentrations: 20.24-20.36, 202.4-203.6, and 2,024-2,036 ng/ml.

Data analysis. (i) **Noncompartmental analysis.** The pharmacokinetics of ritonavir and indinavir were characterized by standard noncompartmental methods (56). The C_{max} , hour 8 concentration (C_8), hour 12 concentration (C_{12}), and the time to reach C_{max} (T_{max}) were obtained directly from the observed data. The AUC from hour 0 to infinity (AUC_{∞}) was computed as $AUC_t + C_t/\beta$, where t is the time of the last measurable concentration, C_t is the last measurable concentration, and β is the terminal elimination rate constant, which was computed from the last three measurable concentrations. AUC was calculated by using the linear trapezoidal rule. Apparent plasma clearance (CL/F) was computed as the quotient of daily dose and AUC_{0-24} for indinavir. For ritonavir, the day 17 CL/F was computed as dose/ AUC_{24} , where AUC_{24} is the AUC over a 24-h period. CL_R was computed as the quotient of the amount of unchanged indinavir recovered in urine over a time interval (A_{ur}) and AUC_t . The day 17 CL_R for indinavir was computed as $A_{ur,48}/AUC_{48}$, where AUC_{48} is the AUC over a 48-h period. Indinavir plasma concentration-time profiles follow polyexponential decay; to evaluate the effect of ritonavir on the elimination of indinavir, the clinically relevant elimination rate constant (k_{el}) within one dose interval was computed by using the concentrations from hours 4.5 to 8 ($k_{4.5-8}$).

(ii) **Pharmacokinetic modeling.** To derive a greater appreciation of the factors affecting the interaction between ritonavir and indinavir, median ritonavir and indinavir concentration data were fitted with ADAPT II (written by David D'Argenio, University of Southern California, Los Angeles) by using the tenets of the well-stirred model of hepatic metabolism and quasiphenologic parameterization that accounted for the first-pass metabolism of indinavir and CYP3A induction produced by ritonavir (56).

Assuming that a single dose of indinavir had little or no effect on the pharmacokinetics of ritonavir on day 17, ritonavir data for groups II to IV were simultaneously fitted first. The model is similar to the model described previously

TABLE 1. Pharmacokinetic parameters for indinavir and ritonavir administered alone or in combination^a

Study (time) and IDV dose (mg) or dosage	RTV dosage	Group	AUC ($\mu\text{g} \cdot \text{h/ml}$) ^b	C_{max} ($\mu\text{g/ml}$) ^c	C_8 or C_{12} ($\mu\text{g/ml}$) ^d	T_{max} (h) ^e	$t_{1/2}$ ^f	CL/F (liters/h)	CL _R (liters/h)
Indinavir IDV alone (day 1)									
800		I	17 ± 5	5.72 ± 1.62	0.15 ± 0.10	1.9 ± 0.5	1.0	55 ± 29	9.23 ± 2.75
600		II	12 ± 3	4.95 ± 1.06	0.07 ± 0.03	1.8 ± 0.4	1.1	55 ± 14	9.53 ± 4.64
400		III	4 ± 1	1.82 ± 0.80	0.03 ± 0.01	1.6 ± 0.2	1.3	113 ± 37	14.3 ± 7.35
600		IV	10 ± 4	3.83 ± 1.31	0.06 ± 0.02	1.8 ± 0.6	1.0	70 ± 28	10.5 ± 2.94
400		V	4 ± 2	1.66 ± 0.65	0.03 ± 0.02	1.8 ± 0.4	1.3	180 ± 241 ^g	12.0 ± 3.86
IDV alone (day 2)									
800 q8h		I	57 ± 16	7.14 ± 1.11	0.18 ± 0.10	2.0 ± 0.5	1.1	45 ± 14	8.57 ± 3.90
600 q8h		II	51 ± 13	6.86 ± 1.54	0.14 ± 0.05	1.8 ± 0.3	1.0	50 ± 10	9.38 ± 4.28
800 q8h		III	50 ± 15	5.97 ± 1.24	0.11 ± 0.05	1.7 ± 0.3	1.0	51 ± 12	9.17 ± 1.17
600 q8h		IV	54 ± 16	6.97 ± 2.34	0.13 ± 0.04	1.6 ± 0.4	1.0	51 ± 26	9.25 ± 3.49
800 q8h		V	49 ± 13	5.76 ± 1.52	0.13 ± 0.08	1.8 ± 0.5	1.1	58 ± 31	9.11 ± 1.97
IDV with placebo or RTV (day 17)									
800 q8h	Placebo	I	57 ± 15	6.25 ± 1.01	0.15 ± 0.08	1.7 ± 0.4	1.1	45 ± 13	9.59 ± 3.19
600	200 q12h	II	33 ± 5	5.97 ± 1.12	0.43 ± 0.14	2.3 ± 0.5	2.7	18 ± 2	8.36 ± 3.29
400	300 q12h	III	20 ± 3	3.72 ± 0.64	0.26 ± 0.08	1.9 ± 0.2	2.8	21 ± 3	9.92 ± 2.02
600	360 q12h	IV	33 ± 8	5.06 ± 1.16	0.55 ± 0.26	2.3 ± 0.7	2.7	19 ± 5	9.14 ± 3.76
400	460 q12h	V	22 ± 3	3.48 ± 0.52	0.40 ± 0.18	2.4 ± 0.7	2.7	18 ± 2	8.14 ± 2.16
RTV with IDV (day 17)									
600	200 q12h	II	52 ± 12	5.06 ± 1.27	0.71 ± 0.18	2.8 ± 0.5	2.7	9 ± 2	0.24 ± 0.11
400	300 q12h	III	78 ± 22	7.42 ± 1.16	0.89 ± 0.40	2.9 ± 0.4	2.5	9 ± 3	0.18 ± 0.07
600	360 q12h	IV	81 ± 26	6.84 ± 2.45	1.27 ± 0.66	2.9 ± 0.8	3.1	9 ± 4	0.22 ± 0.06
400	460 q12h	V	127 ± 33	8.43 ± 1.07	1.59 ± 0.82	2.9 ± 0.4	2.8	7 ± 2	0.26 ± 0.09

^a Abbreviations: IDV, indinavir; RTV, ritonavir; $t_{1/2}$, half-life.^b AUC₀₋₂₄ for day 1 and AUC₀₋₁₂ for days 2 and 17.^c After the first dose on days 2 and 17.^d C_8 for days 1 and 2 for indinavir, C_{12} for day 17 for indinavir and ritonavir, and day 17 C_8 for indinavir were 1.55 ± 0.33 , 0.89 ± 0.27 , 1.70 ± 0.57 , and 1.08 ± 0.34 $\mu\text{g/ml}$ for groups I to V, respectively.^e Harmonic means; estimated from hour 4.5 to hour 8 concentrations for indinavir and from hour 8 to hour 12 concentrations for ritonavir.^f Excluding subject 120, the CL/F would be 95.3 ± 17.3 liters/h.

(25), with a few modifications. Exploratory analyses revealed that a zero-order input describes the characterization of the absorptive phase better than first-order input does, probably because ritonavir is poorly soluble at neutral pH, with absorption appearing to be dissolution rate limited. Thus, the rate was computed as $\text{dose}/t_{\text{max}}$ with a lag time added as appropriate. Since ritonavir induces its own metabolism, the model estimated the extent of enzyme induction as a function of ritonavir dose. All four groups were fitted with a common value for the initial maximum rate of metabolism [$V_{\text{max(R)}}(\text{initial})$] and the Michaelis-Menten constant [$K_{\text{m(R)}}$], where R represents ritonavir. The effect of CYP3A induction on V_{max} as a function of time was modeled as $V_{\text{max(R)}}(\text{induced}) \cdot [1 - \exp(-k_{\text{CY3A}} \cdot t)]$, where $V_{\text{max(R)}}(\text{induced})$ is the maximum rate of metabolism due to induction at steady state and k_{CY3A} is the degradation rate constant of the CYP3A enzyme. Thus, at any time, the total maximal rate of metabolism is the sum of $V_{\text{max(R)}}(\text{initial})$ and $V_{\text{max(R)}}(\text{induced}) \cdot [1 - \exp(-k_{\text{CY3A}} \cdot t)]$. Since groups III and IV received the same ritonavir dose, these two groups were modeled to have the same $V_{\text{max(R)}}(\text{induced})$. The volumes of distribution were estimated for each of the four groups.

After the extent of enzyme induction was estimated for each ritonavir dose level, the indinavir data for all five dose groups were fitted simultaneously, also with one set of $V_{\text{max(I)}}(\text{initial})$ and $K_{\text{m(I)}}$, where I represents indinavir. The extent of enzyme induction by ritonavir was assumed to be the quotient of $V_{\text{max(R)}}(\text{induced})$ and $V_{\text{max(R)}}(\text{initial})$ since steady state was achieved on day 17. The intrinsic clearance for indinavir [$\text{CL}_{\text{I(I)}}$] in the absence of ritonavir was modeled as $\text{CL}_{\text{I(I)}} = V_{\text{max(I)}}(\text{initial})/[K_{\text{m(I)}} + \text{CP}_{\text{I(I)}}]$, where $\text{CP}_{\text{I(I)}}$ is the observed indinavir concentrations and the fraction of the dose surviving first-pass metabolism was modeled as $1 - \text{ER}_{\text{I(I)}} = 1 - \{[\text{CL}_{\text{I(I)}}/(\text{Q}_h + \text{CL}_{\text{I(I)}})]\}$, where $\text{ER}_{\text{I(I)}}$ is hepatic extraction ratio and Q_h is hepatic plasma flow, which was assumed to be 50 liters/h (56). On day 17, the $\text{CL}_{\text{I(I)}}$ in the presence of ritonavir was modeled as $\text{CL}_{\text{I(I)}}(\text{induced}) = [V_{\text{max(I)}}(\text{initial}) + V_{\text{max(I)}}(\text{induced}) \cdot V_{\text{max(R)}}(\text{induced})/V_{\text{max(R)}}(\text{initial})]/[K_{\text{m(I)}} \cdot (1 + \text{CP}_{\text{I(I)}}/K_{\text{m(I)}}) + \text{CP}_{\text{I(I)}}]$, where $\text{CP}_{\text{I(I)}}$ is the fitted ritonavir concentrations, and the fraction of the dose surviving first-pass metabolism was modeled as $1 - \text{ER}_{\text{I(I)}}(\text{induced}) = 1 - \{[\text{CL}_{\text{I(I)}}(\text{induced})/(\text{Q}_h + \text{CL}_{\text{I(I)}}(\text{induced}))]\}$. A two-compartment model was assumed for indinavir, with $\text{CL}_{\text{I(I)}}$ being assumed to be 9 liters/h for all groups. The two intercompartmental rate constants were highly correlated and weighting dependent. After inspection of residual errors, k_{21} (first-order rate constant for transfer of drug from compartment 2 to compartment 1) was fixed as 0.12 h^{-1} , and k_{12} was estimated by curve fitting. Since indinavir also has limited aqueous solubility, the rate of absorption of indinavir was also computed as $\text{dose}/T_{\text{max}}$ and the volume of distribution was estimated for each dose group. Since only predose ritonavir concentrations were obtained during days 4 to 16, to

characterize the extent of induction, the day 17 plasma ritonavir concentrations were downweighted relative to the predose concentrations at a 1:5 ratio. For fitting of indinavir data, all datum points had a weight of unity. The final model was selected on the basis of R^2 values, weighted sum of residuals, and percent CV for parameter estimates.

(iii) Statistical analysis. For analyses other than paired t tests, ritonavir and indinavir concentration measurements (AUC, C_{max} , C_8 , C_{12} , A_{inf} , and CL_R) were logarithmically transformed to provide approximately normal probability distributions and/or approximately equal variances across groups. With the exception of T_{max} , paired t tests were performed on the differences between day 1 and day 17 indinavir pharmacokinetic parameters to assess the effect of ritonavir on the pharmacokinetics of indinavir. A signed rank test was performed on the changes of indinavir T_{max} . To assess whether indinavir and ritonavir can potentially be coadministered as b.i.d. regimens, indinavir pharmacokinetic parameters between the combination regimens and the standard 800-mg q8h regimen on day 17 were compared by one-way analyses of covariance (ANCOVAs) with effects for group, body weight, an appropriate day 2 indinavir pharmacokinetic variable, and an interaction term of that variable with group. The appropriate day 2 indinavir pharmacokinetic covariate was selected on the basis of the fraction of variability accounted for by the model. Within this ANCOVA framework, all possible pairwise t tests were performed for testing whether group means were the same. In addition, since all subjects received indinavir at 800 mg q8h on day 2, paired t tests were also performed on the differences of indinavir C_{max} , AUC (adjusted for the q12h regimen for day 17 for groups I to V), C_8 (relative to C_8), and CL_R between day 17 and day 2 for each combination-dose group. To assess dose proportionality for indinavir in the presence of a fixed ritonavir dose, ANCOVAs were performed for groups III and IV, with effects for body weight, an appropriate day 2 indinavir pharmacokinetic variable, and its interaction term with indinavir dose level. In all statistical analyses, any P value less than or equal to 0.05 was considered statistically significant. Results with P values of >0.05 and ≤ 0.1 were considered a priori as marginally significant.

RESULTS

Indinavir pharmacokinetics. (i) Effect of ritonavir on indinavir pharmacokinetics. As shown in Table 1 and Fig. 1, ritonavir statistically significantly increased plasma indinavir concentrations compared to those achieved when indinavir was

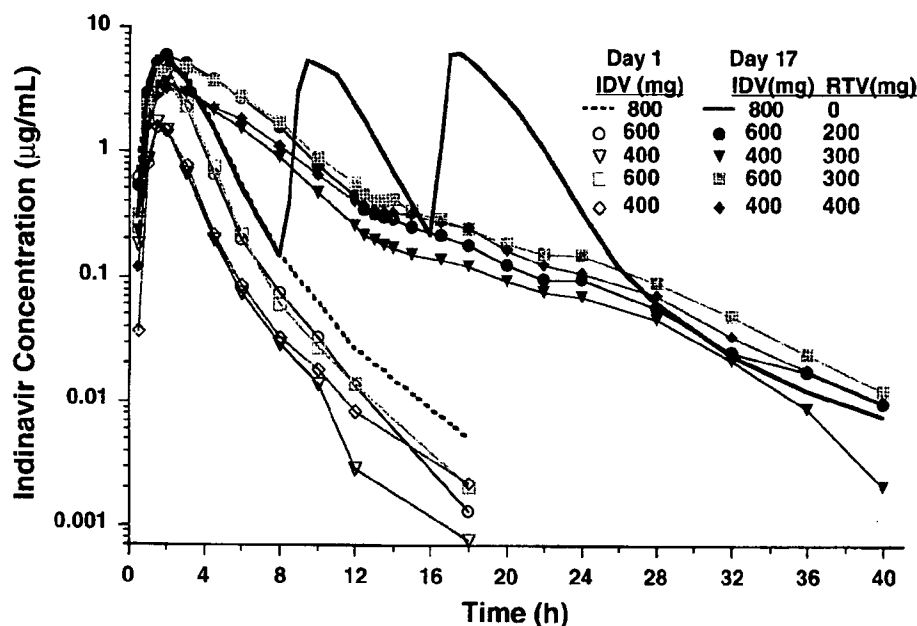


FIG. 1. Mean plasma indinavir (IDV) concentration-time profiles after an administration of single dose of 400 mg (groups III and V), 600 mg (groups II and IV), or 800 mg (group I) alone or in combination with ritonavir (RTV) maintained at 200 mg (group II), 300 mg (groups III and IV), or 400 mg (group V) q12h for 2 weeks.

given alone. Across the four combination regimens, the increases in AUC, C_{max} , and C_{12} ranged from 185 to 475%, 21 to 110%, and 11- to 33-fold, respectively. The k_e values (rate of disappearance from $C_{4.5}$ to C_{24}) decreased by 53 to 67%, with the corresponding half-life increasing from 1.2 ± 0.2 to 2.7 ± 0.1 h. In general, the effects were larger for the 400-mg indinavir dose (groups III and V) than for the 600-mg dose (groups II and IV). The slightly delayed T_{max} (averaging 20 to 40 min later) when indinavir was given with ritonavir was only marginally significant ($P = 0.063$ for groups II and V) or was not statistically significant ($P > 0.125$ for groups III and IV). Also, with the exception of group V (from 12.0 to 8.1 liters/h; $P = 0.030$), indinavir CL_R was not affected by ritonavir. The pharmacokinetic parameters for the control group (group I) determined for the morning dose on day 17 were not statistically significantly different from those determined on day 1.

On the basis of ANCOVA of day 17 data, the regimen of indinavir at 400 mg plus ritonavir at 400 mg, if given q12h, would likely yield AUC values that are only marginally significantly lower ($P = 0.053$) than those achieved with the control regimen of indinavir at 800 mg q8h. The other three combination regimens likely would have AUC values statistically significantly different from that for the standard regimen ($P = 0.003$, 0.005, and 0.003 for groups II, III, and IV, respectively). The two 600-mg indinavir-containing regimens (groups II and IV) would likely yield a slightly higher q12h-adjusted AUC, while the regimen of indinavir at 400 mg plus ritonavir at 300 mg (group III) would likely yield a slightly lower AUC. The mean C_{max} for group II was not statistically significantly different from that for the control group, while those for groups III, IV, and V were all statistically significantly lower than that for the control group ($P < 0.05$ for all comparisons). All four combination-dose groups had statistically significantly higher C_{12} s compared to the C_{12} s for the control group ($P < 0.001$ for all comparisons). The CL_R for all four combination-dose groups was not statistically significantly different from that for the control group (group I), averaging 9.1 liters/h across the five dose groups. Body weight was shown to be a statistically

significant explanatory variable for indinavir C_{max} , AUC, and CL_R in the analyses described above. There was no correlation between the creatinine clearance and CL_R of indinavir given alone or with ritonavir, probably in part due to the small creatinine clearance range for those subjects with normal renal function.

The results of paired t tests comparing the pharmacokinetic parameters determined on day 17 and day 2 are generally consistent with those obtained from ANCOVA. For the control group (group I), the AUC₀₋₂₄, C_{max} , C_{12} , and CL_R values determined on day 2 and day 17 were not statistically significantly different, indicating the lack of any study day effects on the pharmacokinetics of indinavir.

(ii) **Effect of ritonavir dose.** To assess whether an increase in the ritonavir dose had corresponding effects on plasma indinavir concentrations for a fixed indinavir dose, indinavir pharmacokinetics were compared between groups II and IV and between groups III and V in the framework of ANCOVA. The results showed that a 600-mg dose of indinavir yielded similar AUC and trough concentrations ($C_{troughs}$) when it was administered with 200 or 300 mg of ritonavir q12h. However, the C_{max} for group IV was marginally significantly lower than that for group II ($P = 0.051$). For the 400-mg indinavir doses, group V, which received the higher dose of ritonavir, yielded marginally significantly higher $C_{troughs}$ ($P = 0.053$) compared with those for group III but AUCs and C_{max} s similar with those for group III.

(iii) **Dose proportionality of indinavir.** To assess whether plasma indinavir concentrations increased proportionally with indinavir dose for a fixed ritonavir dose, indinavir pharmacokinetics were compared between groups III and IV in the framework of ANCOVA. The results showed that compared to group III, group IV had statistically significantly higher AUC's, C_{max} s, and C_{12} s ($P = 0.0001$, 0.006, and 0.002, respectively). The CL_R s were similar between the two combination regimens. The estimates for AUC and C_{12} ratios for the two combination regimens (1.64 and 1.95, respectively) were slightly higher than the theoretical value of 1.5 (600 mg/400

mg = 1.5); however, the C_{max} ratio (1.27) was slightly less than the theoretical value of 1.5.

Ritonavir pharmacokinetics. The mean steady-state plasma ritonavir concentrations determined in this study were comparable to those of a previous 2-week study of ritonavir with healthy HIV-infected subjects (25). Predose ritonavir concentrations reached a maximum level after dosing for about 2 days; however, due to autoinduction, plasma ritonavir concentrations decreased with time, with stable concentrations reached by the end of 2 weeks.

Pharmacokinetic modeling results. The observed data were well described by pharmacokinetic models that used the principles of the well-stirred model of hepatic metabolism, competitive inhibition, and autoinduction. The R^2 values from the regression for indinavir ranged between 0.87 and 0.93, and those for ritonavir ranged between 0.89 and 0.96. The model estimated that the extents of induction were 107, 148, and 149% for ritonavir dosages of 200, 300, and 400 mg q12h, respectively. The half-life for CYP3A turnover was estimated to be 44.5 h, which is similar to that reported in the literature (36). The V_{max} and K_m for indinavir was estimated to be 86.3 mg/h and 1.11 μ g/ml, respectively. The in vivo K_i was estimated to be 0.10 μ g/ml, which agrees reasonably well with the estimated in vitro K_i of 0.085 μ g/ml. The volume of distribution ranged from 37.9 to 40.1 liters for ritonavir for the four groups and ranged from 54 to 67 liters for indinavir. No apparent correlation was found between the volume of distribution and dose size for either drug. The errors associated with all parameter estimates were under 37%. However, it should be noted that the estimates presented above were based on the median data, which did not account for intersubject variability associated with both drugs.

Intersubject variability. Intersubject variability in indinavir pharmacokinetic parameters was reduced when indinavir was coadministered with ritonavir. Across groups, the CVs for AUC_{0-24} , C_{max} , and C_{12} averaged 30, 21, and 50%, respectively, for the standard indinavir regimen of 800 mg q8h on day 2. When indinavir was given with ritonavir on day 17, the CVs averaged 16, 19, and 39% for AUC_{0-24} , C_{max} , and C_{12} , respectively. For ritonavir, the CVs for day 17 AUC_{0-24} , C_{max} , and predose concentration were 28% $\pm$ 4%, 23% $\pm$ 10%, and 39% $\pm$ 11%, respectively.

Safety assessment. All 39 subjects enrolled in the study completed the study. It should be noted that since the study design does not permit statistical analyses on whether the combination regimen had effects on the safety of either drug given alone, no such analysis was performed. However, it was possible to assess the dose-response relationship for ritonavir during the ritonavir-alone period (day 3 to day 16).

Overall, there were no changes of clinical relevance in vital signs recordings, physical examinations, or electrocardiograms during the conduct of the study. All adverse events observed were mild in nature. The most frequently occurring adverse events that were possibly or probably related to the study drugs were headache and asthenia during indinavir dosing alone and circumoral paresthesia, headache, and nausea after dosing with ritonavir alone or during dosing with indinavir and ritonavir in combination. Circumoral paresthesia was the only event that showed a statistically significant monotonic increasing trend with ritonavir dose during dosing with ritonavir alone. There was no clear correlation between the incidence of adverse events, including circumoral paresthesia, and C_{max} s or AUCs of either drug within the same treatment group.

The results of hematology analyses were generally unremarkable. One subject in group IV exhibited a low hematocrit (31.3%) and hemoglobin (10.7 g/dl) during days 10 through 16.

This was attributed to repetitive blood drawing and resolved in the poststudy follow-up visits.

For abnormal clinical chemistry values, one subject had increased triglyceride levels (435 and 409 mg/dl on days 10 and 19, respectively) and cholesterol levels (292 mg/dl on day 19); one subject had an increased bilirubin level (2.5 mg/dl on days 10 and 12), and three subjects had increased gamma-glutamyl transferase levels (ranging from 106 to 201 IU/liter); all of these were attributed to ritonavir. Two subjects had increased bilirubin levels (1.8 and 2.3 mg/dl on day 3) that were attributed to indinavir. For triglycerides, the ritonavir placebo group (group I) had a statistically significantly lower mean level (90 mg/dl) than the groups receiving ritonavir at 300 mg (groups III and IV; 160 mg/dl) and the group receiving ritonavir at 400 mg (group V; 163 mg/dl) on day 16. Similar results were observed for cholesterol (175, 201, and 211 mg/dl for group I, groups III and IV, and group V, respectively).

DISCUSSION

Ritonavir induces its own metabolism. This study assessed the pharmacokinetics of indinavir in the presence of ritonavir under steady-state (induced) conditions, which are generally achieved after dosing for approximately 2 weeks. It should be noted that since the pharmacokinetics of indinavir alone were assessed under noninduced conditions, the actual magnitude of the inhibitory effect is underestimated in this study.

Indinavir is reported to be primarily eliminated by metabolism via CYP3A, with less than 20% of a dose being recovered as unchanged drug in the urine after oral dosing. The fecal recovery of unchanged indinavir after oral administration of a 400-mg dose was 19%, suggesting that the fraction of dose absorbed is at least 0.8 (45). The results of our study confirmed that the pharmacokinetics of indinavir are moderately nonlinear, with CL/F averaging 104 (excluding one subject who had a CL/F value of 774 liters/h), 63, and 55 liters/h for doses of 400, 600, and 800 mg, respectively. The CL_R of indinavir averaged 9 liters/h after the administration of three 800-mg doses given q8h. Assuming that the fraction of the indinavir dose absorbed and reaching the portal circulation is 0.8 and that the CL_R is 9 liters/h on the basis of well-stirred model of hepatic metabolism (56), the extents of first-pass hepatic metabolism, expressed as percent extraction = $100 \cdot CL_p / (CL_p + Q_h)$, where CL_p is the metabolic clearance from plasma and Q_h is the hepatic plasma flow rate at 50 liters/h, are computed to be 62, 50, and 47% for a 400-, 600-, or 800-mg dose given alone.

Consistent with the interaction effect observed in human liver microsomes and in rats, the results of this study showed that ritonavir increased the AUC of indinavir by 2.9- to 5.8-fold and the C_{max} by 1.2- to 2.1-fold compared to those on day 1 for the indinavir-alone regimens. The net change in AUC could theoretically arise from two main effects: reduction in the systemic clearance and increase in the fraction of the dose systemically available, F . The F term has at least three components: f_{abs} , the fraction of the dose absorbed; f_{gp} , the fraction of the dose not metabolized or otherwise eliminated by gastrointestinal tissue; and f_{hp} , the fraction of the dose surviving first-pass hepatic metabolism.

Effect of ritonavir on F of indinavir. Although the oral bioavailability of indinavir has been theorized to be limited by pH-dependent solubility (3, 4, 12, 40) and regiospecificity (35), the fraction of indinavir dose absorbed was approximately 0.8 (45). Therefore, the observed increases in indinavir AUC cannot be accounted for by an improvement in the f_{abs} of indinavir.

For drugs with high intrinsic clearance, the gut wall may

contribute substantially to the overall first-pass effect (30, 50). It is well known that CYP3A, the major isoform in the metabolism of ritonavir and indinavir, is present in intestinal tissue (31, 32, 41, 50, 61). However, assuming that the enterocyte CYP3A content is not greater than 10% of hepatocytes (50), and the maximal hepatic metabolic rate is 86 mg/h, as determined from the curve fitting described above, then $V_{max,gi}$ is approximately 9 mg/h. Assuming that indinavir is absorbed with zero-order kinetics over a 1.5-h period, then the metabolism in the intestinal epithelial cells represents less than 4% of the administered dose.

However, in considering potential changes in indinavir f_{gi} by ritonavir, the role of glycoprotein P should be addressed. Glycoprotein P is a transmembrane protein associated with a phenotype of multidrug resistance (MDR1). It is capable of actively transporting drug out of intestinal, hepatic, and brain capillary endothelial cells. Recent reports have shown that HIV protease inhibitors are substrates of glycoprotein P and are capable of inhibiting, although not potently inhibiting, the transport of some known glycoprotein P substrates (21, 29, 39). Therefore, it is theoretically possible that ritonavir may increase the f_{gi} of indinavir by inhibiting glycoprotein P transport.

In contrast to f_{gi} , the fraction of the dose surviving first-pass hepatic metabolism (f_h) can be estimated with a higher degree of certainty. When given with ritonavir, CL/F averaged approximately 19 liters/h for the four combinations. The fraction of indinavir dose surviving the first-pass hepatic metabolism when indinavir was given with ritonavir should be greater than 0.8, which is substantially higher than the day 1 f_h under noninduced conditions. Therefore, part of the inhibitory effect of ritonavir should be due to the inhibition of the first-pass metabolism of indinavir.

Effect of ritonavir on the postabsorptive systemic indinavir clearance. As can be seen from Fig. 1, the decline in plasma indinavir concentrations after T_{max} for day 17 during coadministration with ritonavir was significantly slower compared to the decline when indinavir was given alone. The disappearance rate constant during hours 4.5 to 8 decreased about 50 to 63%, suggesting an effect (clearance ratio) of approximately two- to threefold on the basis of indinavir-alone data determined on day 1. Again, since the day 1 data were obtained under noninduced conditions, the comparison presented above probably also underestimates the postabsorptive inhibitory effect exerted by ritonavir.

In vitro-in vivo correlation in K_i . The estimate of the in vivo K_i must be appreciated as being highly model dependent and is limited by factors that are indeterminate. For example, the model did not address the possibility of time delays in inhibition (hysteresis), the protein binding effects, and the potential for inhibitory effects from metabolism in intestinal epithelial cells or glycoprotein P transport. Despite these uncertainties, the estimated K_i value (0.10 $\mu\text{g/ml}$) agrees extremely well with the in vitro K_i (0.085 $\mu\text{g/ml}$).

Factors affecting the ritonavir-indinavir interaction. (i) **Fraction of indinavir dose excreted in urine (f_u).** Since the renal component of indinavir's elimination clearance was not affected by ritonavir, the expected AUC ratio should be larger for subjects who metabolize a relatively large fraction of the dose, f_m (56). Indeed, a positive relationship between AUC ratio and f_m was observed (data not shown), where f_m was estimated as the fraction of dose not excreted in the urine.

(ii) **Indinavir dose.** For a fixed ritonavir dose, an increase in the indinavir dose resulted in a relatively proportional increase in AUC, a more than proportional increase in C_{12} , and a slightly less than proportional increase in C_{max} . For a fixed

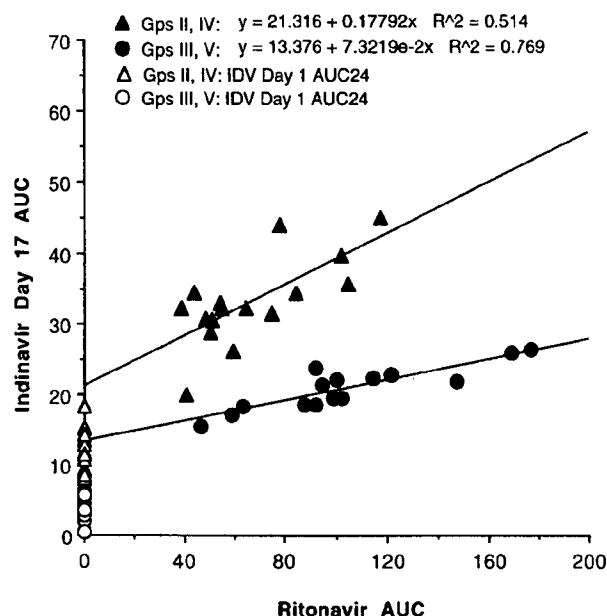


FIG. 2. Relationship between day 17 ritonavir AUC and day 17 indinavir AUC for 400- and 600-mg indinavir doses (subjects in groups II and IV each received 600 mg of indinavir alone or with 200 or 300 mg ritonavir q12h, respectively; subjects in groups III and V each received 400 mg of indinavir alone or with 300 or 400 mg ritonavir q12h, respectively).

indinavir dose (400 or 600 mg), an increase in the ritonavir dose did not have a proportional effect on the concentrations of indinavir in plasma. However, as shown in Fig. 2, there is a shallow but positive correlation between ritonavir AUCs and indinavir AUCs for a fixed indinavir dose. Indinavir metabolic clearance via CYP3A becomes less dominant with increasing ritonavir exposure, and the postabsorptive total clearance asymptotically approaches the non-CYP3A clearance, which is a composite of CL_R and perhaps contributions from glucuronidation (3) (potentially induced) and other noninhibited isoforms. These factors may explain why indinavir AUCs for the two 600-mg and the two 400-mg regimens were relatively similar.

In this study, ritonavir significantly reduced the intersubject variability in indinavir pharmacokinetic parameters, particularly AUCs and C_{trough} s. Since the indinavir AUC was negatively correlated with f_m when it was given alone and since the inhibitory effect by ritonavir was larger for subjects with a higher f_m , it is not unexpected that the intersubject variability in indinavir AUC and C_{trough} was significantly reduced when indinavir was coadministered with ritonavir.

Ritonavir and indinavir combination as potential b.i.d. regimens. Since treatment of HIV infection requires long-term therapy, the requirements of rigid restrictions on dosing and meal schedules in indinavir-containing regimens are expected to result in challenges for patient compliance. This study was conducted to evaluate if combination therapy with indinavir and ritonavir can be given as b.i.d. regimens and therefore improve upon the drawbacks of indinavir therapy. Since no significant accumulation was observed after the administration of multiple doses of indinavir (45), the single-dose data obtained in this study can be used to project multiple-dose exposures.

The results of this study showed that compared to the standard 800-mg q8h regimen, all four combination regimens, if given q12h, will likely have higher C_{trough} s (C_{12} versus C_8 of the standard q8h regimen), lower C_{max} values, and similar CL_R s.

The lower C_{max} values associated with the combination regimens are expected to have a minimal effect on antiviral activity, particularly for combination regimens that yield comparable indinavir AUCs, because the C_{trough} s for combination regimens are expected to be at least 2.5-fold greater than the protein-binding corrected IC_{50} of indinavir. This study also showed that ritonavir significantly reduced the intersubject variability in indinavir AUCs and C_{trough} s. In addition, the results of a recent study showed that the bioavailability of indinavir, when given together with ritonavir, was not affected by regular meals containing 32% of calories from fat (23). Thus, the combination regimens offer several significant practical benefits.

Ritonavir and indinavir combination in renal complications. The CL_R of indinavir, when given alone at 800 mg t.i.d., accounts for roughly 20% of the total clearance. Under such conditions, renal impairment is unlikely to have substantial effects on total clearance. However, in the presence of ritonavir, the CL_R of indinavir approaches 50% of CL/F . Thus, in patients with substantial impairment in renal function, the possibility of a dosage reduction should be entertained.

Due to highly pH-dependent aqueous solubility, nephrolithiasis, which is associated with indinavir crystals, is reported in approximately 10% of patients treated with indinavir (5, 18, 33, 40, 45, 53, 58). The results of this study showed that ritonavir has no overall effect on the CL_R of indinavir. Thus, for combination regimens that maintain the AUCs of the standard indinavir regimen, the total daily urinary excretion of unchanged indinavir should remain unchanged. It should be noted that the results of two recent clinical studies showed that in patients treated with 400 mg of ritonavir plus 400 mg indinavir b.i.d. combination regimen, no nephrolithiasis was reported. Since the combination regimen provides similar indinavir AUC, but substantially lower indinavir C_{max} compared to that provided by the indinavir 800-mg t.i.d. regimen (55, 62), it is possible that the decreased indinavir C_{max} in the combination regimen is responsible for the reduction in the incidence of nephrolithiasis.

Antiviral activities of combination regimens. For protease inhibitor combination regimens, there are concerns about whether low drug concentrations (concentrations less than the IC_{50}) in the combination regimen will favor the emergence of resistant viral strains. The results of our recent *in vitro* experiments with MT4 cells in the presence of 50% human serum and 10% fetal calf serum showed that there are additive or synergistic anti-HIV-1_{IIIB} (wild type) effects between ritonavir and other protease inhibitors, particularly at concentrations near or below the IC_{50} s (24, 46). It should be noted that the ongoing clinical studies show that regimens with dual protease inhibitors have potent antiviral effects (9, 13, 51, 55, 60, 62).

In conclusion, in addition to allowing the use of reduced doses of both drugs, use of the combination of ritonavir and indinavir can also offer the benefit of two potent HIV protease inhibitors. The preliminary results from use of the regimen of 400 mg of ritonavir and 400 mg of indinavir in combination b.i.d. in patients have shown that the regimen may be highly efficacious and well tolerated (55, 62).

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Interactions between Atazanavir-Ritonavir and Tenofovir in Heavily Pretreated Human Immunodeficiency Virus-Infected Patients

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The aim of the present study was to assess the pharmacokinetic behavior of atazanavir-ritonavir when it is coadministered with tenofovir disoproxil fumarate (DF) in human immunodeficiency virus (HIV)-infected patients. Eleven patients enrolled in Agence Nationale de Recherche sur le SIDA (National Agency for AIDS Research, Paris, France) trial 107 were included in this pharmacokinetic study. They received atazanavir at 300 mg and ritonavir at 100 mg once a day (QD) from day 1 to the end of study. For the first 2 weeks, their nucleoside analog reverse transcriptase inhibitor (NRTI) treatments remained unchanged. Tenofovir DF was administered QD from day 15 to the end of the study. Ongoing NRTIs were selected according to the reverse transcriptase genotype of the HIV isolates from each patient. The values of the pharmacokinetic parameters for atazanavir and ritonavir were measured before (day 14 [week 2]) and after (day 42 [week 6]) initiation of tenofovir DF and are reported for the 10 patients who completed the study. There was a significant decrease in the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) for atazanavir with the addition of tenofovir DF (AUC_{0-24} ratio, 0.75; 90% confidence interval, 0.58 to 0.97; $P = 0.05$). There was a trend for a decrease in the minimum concentrations of atazanavir and ritonavir in plasma when they were combined with tenofovir, but none of the differences reached statistical significance. The median decreases in the HIV RNA loads at week 2 and week 6 were 0.1 and 0.2 log copies/ml, respectively. In summary, our data are consistent with the existence of a significant interaction between atazanavir and tenofovir DF.

Human immunodeficiency virus (HIV) type 1 (HIV-1) protease inhibitor (PI)-containing regimens are highly effective in previously untreated patients. Despite the improved outcomes, problems such as failure and toxicity are apparent. The management of patients experiencing failure of different highly active antiretroviral treatment regimens represents a major clinical challenge. Present guidelines recommend that, whenever possible, patients in whom several lines of antiretroviral therapy have failed should receive agents to which they have not previously been exposed (15). Most clinical trials have evaluated the impacts of salvage regimens containing only one investigational drug, but these regimens rarely drive the viral load below the detection limits of current assays. A currently accepted approach to the management of antiviral treatment failure in patients already receiving a PI-containing regimen is to include at least two new antiretroviral agents as part of the salvage regimen. Furthermore, the use of ritonavir-boosted PIs is recommended for these patients (11). Finally, daily dosing can improve adherence (12). In this setting, we hypothesized that combining two newly marketed antiretroviral drugs,

ritonavir-boosted atazanavir and tenofovir disoproxil fumarate (tenofovir DF), could improve the outcome. Tenofovir DF and atazanavir appear to be promising because their pharmacokinetic profiles, activities, safety, and resistance development properties allow once-daily (QD) dosing (12). A phase IIb clinical trial (the Puzzle 2 study, Agence Nationale de Recherche sur le SIDA [ANRS; National Agency for AIDS Research, Paris, France] clinical trial 107) was conducted to evaluate the efficacy of tenofovir DF plus atazanavir-ritonavir in combination with recycled nucleoside reverse transcriptase inhibitors (NRTIs) as salvage therapy in patients who previously failed PI- and non-NRTI (NNRTI)-containing regimens.

Atazanavir is a novel azapeptide PI with a distinct resistance profile (6). The recommended dose of atazanavir is 400 mg QD (7). Like other PIs, atazanavir is a CYP3A substrate, and pharmacokinetic data from studies with healthy volunteers suggest that atazanavir concentrations could be boosted by the addition of ritonavir at a low dose (E. O'Mara, V. Mummamneni, M. Bifano, D. Randall, H. Uderman, L. Knox, and M. Gerald, Abstr 8th Conf. Retrovir. Opportunistic Infect., abstr. 740, 2001; S. Agarwala, R. Russo, V. Mummamneni, D. Randall, M. Gerald, E. O'Mara, Abstr. 42nd Intersci. Conf. Antimicrob. Agents. Chemother., abstr. H1716, 2002). Thus, one may speculate that the pharmacokinetics and antiviral activity of atazanavir could be optimized by the addition of ritonavir at a low dose to the treatment regimens for patients infected with HIV-1 isolates exhibiting high rates of PI muta-

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TABLE 1. Patient demographic and clinical characteristics at the baseline

Characteristic	Values for the 10 patients who completed the study			Values for the patient excluded
	Median	Minimum	Maximum	
Age (yr)	46.4	33.3	59.3	42.2
HIV RNA level (log ₁₀ copies/ml)	5.1	4.1	5.7	5
CD4 count (no. of cells/ μ l)	117.0	19.0	328.0	48
ALT level (IU/liter)	36.0	21.0	70.0	22
AST (IU/liter)	34.0	23.0	58.0	32
Bilirubin concn (μ mol/liter)	7.5	4.0	14.0	17

tions. Tenofovir DF is a prodrug of tenofovir, an NRTI. The recommended dose is 300 mg QD. Tenofovir is eliminated unchanged through the kidney (3).

Although these two drugs do not share the same elimination pathways, pharmacokinetic interactions at other sites cannot be excluded (1).

The aim of the present ANRS trial 107 substudy was to assess the pharmacokinetic behaviors of atazanavir and ritonavir before and after the initiation of tenofovir DF treatment in HIV-infected patients.

(This study was presented in part at the 10th Conference on Retrovirus and Opportunistic Infections, Boston, Mass., 10 to 14 February 2003 [A. M. Taburet et al., 10th Conf. Retrovir. Opportunistic Infect., abstr. 537, 2003].)

MATERIALS AND METHODS

Study design. ANRS trial 107 was a randomized open-label, multiple-dose study with HIV-infected patients who had failed previous antiretroviral therapy. Patients were randomized to receive for the first 2 weeks either unchanged treatments with PIs and NRTIs (group 1) or unchanged treatments with NRTIs in combination with atazanavir (300 mg QD) and ritonavir (100 mg QD) as substitutes for the failing PI therapy (group 2). From week 3 (day 15) to week 26, patients from either group switched to ritonavir-boosted atazanavir plus tenofovir DF at 300 mg QD and NRTIs selected according to the baseline reverse transcriptase genotype of the HIV isolate infecting each patient. Because of potential pharmacokinetic interactions, patients who were receiving an NNRTI at screening stopped taking the drug at least 2 weeks before inclusion in the study.

The pharmacokinetic substudy of ANRS trial 107 was conducted with 11 HIV-infected patients in group 2 at four selected centers.

The objective of the study was to measure the pharmacokinetic parameters of atazanavir when it was administered with ritonavir either before (day 14 [week 2]) or after (day 42 [week 6]) initiation of tenofovir DF in HIV-infected patients in order to detect pharmacokinetic interactions between the two drugs.

Blood samples for atazanavir and ritonavir pharmacokinetic assessments were collected on days 14 (period 1 [week 2]) and 42 (period 2 [week 6]) from a subgroup of patients in group 2. Samples were drawn prior to drug intake in the morning and then after dosing at times of 1, 2, 3, 5, 8, and 24 h. Plasma samples were kept at -20°C until analysis.

Drugs were administered in the morning with a light continental breakfast. The actual times of drug administration and samplings were recorded.

Study population. All patients gave written informed consent to participate in this pharmacokinetic study, which was approved by the Institutional Review Board of Hôpital Saint-Antoine, Paris VI University.

HIV-infected adults were eligible for inclusion if they met the following criteria: no change in antiretroviral treatment within the last month before inclusion in the study; plasma HIV-1 RNA loads of ≥ 10 (100) copies/ml; documented failure of previous treatment with at least two PIs and one NNRTI; and the absence of cardiomyopathy or conduction system disease, defined as QTc interval >450 ms; pause length >3 s on screening electrocardiography; heart rate <40 beats/min; third-degree heart blockage; and clinical symptoms potentially related to heart blockage.

Adverse events were recorded and graded according to the ANRS scale (10).

Drug assays. Plasma samples were assayed for their atazanavir contents at Bristol-Myers Squibb, Saint Nazaire, France, by a validated liquid chromatogra-

phy-mass spectrometry-mass spectrometry assay (13). The lower limit of quantification of the plasma atazanavir concentration was 1 ng/ml. Day-to-day variability for the quality control samples was 5.7% for the three concentrations included in each analytical run.

Plasma was assayed for ritonavir concentrations by a validated reverse-phase high-pressure liquid chromatography method with UV detection, as described by the manufacturer (R. Wieboit, Abbott Laboratories, Abbott Park, Ill.). The lower limit of quantification of the plasma ritonavir concentration was 25 ng/ml. Day-to-day variability for the quality controls was $<5\%$ at the three concentrations included in each analytical run.

Pharmacokinetic analysis. The pharmacokinetic parameters for atazanavir and ritonavir were assessed by noncompartmental methods (WinNonlin; Pharsight Corporation, Mountain View, Calif.). The linear-log trapezoidal method was used to calculate the areas under the concentration-versus-time curves (AUCs) during a dosing interval at steady state (AUC_{0-24}), where t is the time that the last sample was taken, close to 24 h postdosing. The AUCs from 0 to 24 h (AUC_{0-24}) were calculated by extrapolation, whenever the last sample was drawn before 24 h after drug intake and determination of the terminal rate constant (λ_z) was possible. Average concentrations at steady state (C_{avg}) and oral clearances (CL_{FS}) were calculated by the following standard equations: $C_{\text{avg}} = \text{AUC}_{0-24}/\tau$, where τ is the dosing interval, and $\text{CL}_{\text{FS}} = \text{dose}/\text{AUC}_{0-24}$. The maximum concentration observed in plasma (C_{max}), the observed predose concentration (C_{min}), the lowest quantifiable observed concentration before C_{max} (C_{min}) or just before the next dose if the time to C_{min} was <24 h, and the time to the first occurrence of C_{min} (T_{min}) were obtained visually from the plasma concentration-time curves determined on the last day of each study period.

Statistical analysis. This was an observational pharmacokinetic study that assessed for the first time the concentrations of atazanavir when it was combined with ritonavir in the plasma of HIV-infected patients. When this pilot study was designed, there were no data to provide estimates for sample size calculation. Therefore, it was decided that 10 patients would be included to detect important impairments of atazanavir pharmacokinetics.

All statistical analyses were carried out with SAS (version 8.2) software. The pharmacokinetic parameters for atazanavir and ritonavir were summarized by study day by using descriptive statistics. The values of AUC_{0-24} , C_{max} , and C_{min} for atazanavir and ritonavir at week 2 and week 6 were compared by a one-sample t test for paired data. When appropriate, the values of AUC_{0-24} , C_{max} , and C_{min} were log transformed. Two-sided 90% confidence intervals (CIs) were constructed for the ratios of the geometric mean values (day 42 [week 6] versus day 14 [week 2]) of AUC_{0-24} , C_{max} , and C_{min} for atazanavir and ritonavir. For these, the values of AUC_{0-24} , C_{max} , and C_{min} for atazanavir and ritonavir were log transformed; and the resulting points and interval estimates of the means and the mean differences were exponentiated to express the results as geometric means and the ratios of the geometric means on the original scale of measurement.

RESULTS

Patient characteristics. A total of 11 male patients (median age, 46 years) from group 2 of the trial (staggered initiation of atazanavir and tenofovir DF) gave their informed consent to participate to the pharmacokinetic study. Their baseline characteristics are summarized in Table 1, and the changes in their nucleoside analog treatments are presented in Table 2. One patient did not complete the study because of the occurrence of asymptomatic ventricular bigeminy during sampling for the

TABLE 2. Changes in nucleoside analog treatments

Time	No. of patients receiving ^a :					
	Abacavir	Didanosine	Lamivudine	Stavudine	Zalcitabine	Zidovudine
Inclusion	3	4	5	2	1	1
Wk 2 to 6	7	4	7	1	2	4
Introduced at wk 2	5	2	5	1	2	1

^a The patient excluded from the study received lamivudine.

pharmacokinetic study on day 14. The plasma drug concentrations for this patient were excluded from the analysis. Ten patients completed the study. The median baseline HIV RNA load was 5.1 log₁₀ copies/ml. Alanine aminotransferase (ALT) levels were within the normal range in 8 of the 10 patients, and 2 of the 10 patients had mild abnormalities in ALT levels (53 and 70 IU/liter). Aspartate aminotransferase (AST) levels were within the normal range in 6 of the 10 patients, and 4 patients had mild abnormalities in ALT levels (44, 48, 49, and 58 IU/liter, respectively). All patients had normal serum bilirubin levels at the time of inclusion in the study. Plasma creatinine levels were within the normal range (56 to 97 μmol/liter) in all patients. The patients' characteristics did not change significantly during the 6-week study period.

Atazanavir and ritonavir pharmacokinetics. Individual plots of concentrations versus time are shown in Fig. 1. As expected, the concentrations of ritonavir were lower than those of atazanavir. Before dosing the atazanavir trough concentrations were greater than 300 ng/ml in all patients at week 2. Interestingly, the atazanavir AUCs were higher for the four patients who were receiving didanosine at week 2 (AUC₀₋₂₄, 70,676 versus 31,840 ng·h/ml; $P = 0.02$). The pharmacokinetic parameters measured for atazanavir and ritonavir at weeks 2 and 6, in the absence and presence of tenofovir, respectively, are compared in Table 3. The mean C_{max} , C_{min} , and AUC₀₋₂₄ values for

atazanavir at week 2 were 5,233 ng/ml, 862 ng/ml, and 53,761 ng·h/ml, respectively; those for ritonavir were 940 ng/ml, 50 ng/ml, and 7,546 ng·h/ml, respectively. There was a correlation between the atazanavir C_{min} and the ritonavir C_{min} at week 2 ($r^2 = 0.74$; $P = 0.01$). There was a significant decrease in the atazanavir AUC₀₋₂₄ with the addition of tenofovir DF (AUC₀₋₂₄ ratio, 0.75; 90% CI, 0.58 to 0.97; $P = 0.05$). The number of additional NRTIs introduced at week 2 had no effect on this decrease; however, the number of patients enrolled in this pilot study was small. The decrease in the atazanavir C_{min} observed with the addition of tenofovir DF was not significant (C_{min} ratio, 0.77; 90% CI, 0.54 to 1.1; $P = 0.22$), but the interindividual variability was high and the study was underpowered to detect a difference. There was a trend for a decrease in ritonavir concentrations when ritonavir was combined with tenofovir, but none of the differences reached significance.

Virologic parameters. At week 2 and week 6, the median decreases in HIV RNA levels from the baseline were not significant and were 0.1 (95% CI, -0.11 to 0.25) and 0.2 (95% CI, -0.02 to 0.90), respectively.

Safety. One patient did not complete the study because of the occurrence of asymptomatic ventricular bigeminy during sampling for the pharmacokinetic study on day 14. This patient had the highest ritonavir C_{max} (1,721 ng/ml) and AUC₀₋₂₄

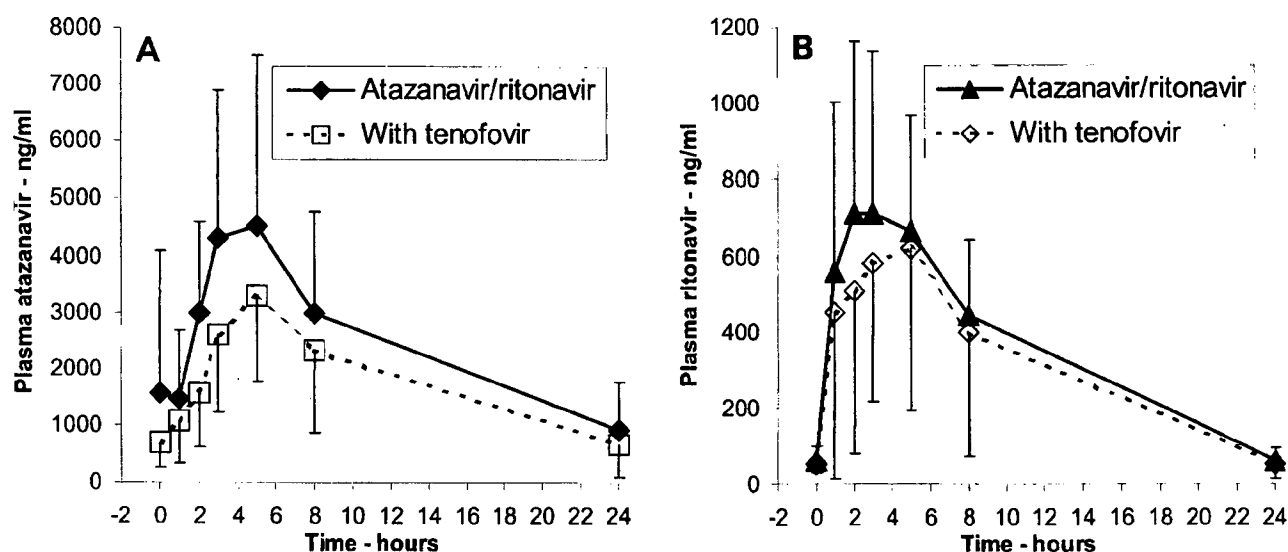


FIG. 1. Mean $\pm$ standard deviation atazanavir (A) and ritonavir (B) concentration-time profiles for patients who received atazanavir at 300 mg QD plus ritonavir at 100 mg QD in the absence (close symbols and solid lines) or the presence (open symbols and dotted lines) of tenofovir DF at 300 mg QD.

TABLE 3. Pharmacokinetic parameters for atazanavir and ritonavir on week 2 (atazanavir-ritonavir) and week 6 (with addition of tenofovir)^a

Drug and time	C _{max} (ng/ml)	T _{max} (h) ^b	C ₀ (ng/ml)	C _{last} (ng/ml)	C _{min} (ng/ml)	AUC ₀₋₂₄ (ng·h/ml)	AUC ₀₋₂₄ (ng·h/ml)	C _{av} (ng/ml)	CL _{CR} (ml/min)	t _{1/2} (h)
Atazanavir										
Wk 2	5,233 ± 3,033	3 (2-5)	1,571 ± 2,524	924 ± 829	862 ± 838	53,761 ± 35,255	53,761 ± 35,255	2,240 ± 1,471	123 ± 61	8.6 ± 2.3
Wk 6	3,443 ± 1,412	5 (1-5)	685 ± 417	665 ± 559	577 ± 367	39,276 ± 23,034	39,276 ± 23,034	1,636 ± 960	164 ± 84	8.6 ± 3.1
R ^c (90% CI)	0.72 (0.56-1.05)				0.77 (0.54-1.10)		0.75 (0.58-0.97)			
P	0.06				0.22		0.05			
Ritonavir										
Wk 2	940 ± 336	3 (2-8)	60 ± 37	59 ± 37	50 ± 31	7,541 ± 3,226	7,541 ± 3,226	314 ± 134	255 ± 98	5.1 ± 0.7
Wk 6	812 ± 447	3 (0-5)	52 ± 22	53 ± 39	42 ± 19	6,603 ± 3,999	6,603 ± 3,999	275 ± 167	507 ± 737	5.3 ± 0.7
R (90% CI)	0.72 (0.43-1.21)				0.91 (0.73-1.13)		0.75 (0.44-1.24)			
P	0.38				0.42		0.41			

^a Data are expressed as means ± standard deviations unless indicated otherwise.^b Data are expressed as medians (ranges).^c R is the ratio of the geometric means for the parameters at week 6 to those at week 2.

(18,794 ng·h/ml), and the atazanavir concentrations were in the upper range (C_{max}: 5,300 ng/ml; AUC₀₋₂₄: 98,217 ng·h/ml), with a slow decline in concentrations versus time (half-life [*t*_{1/2}], 26.7 h). The episode was unrelated to study drug administration, given the absence of a significant increase in the QT interval (<400 ms) following drug administration and the intermittent recurrence of the anomaly 1 month after discontinuation of the study therapy.

DISCUSSION

In patients who have failed therapy with multiple lines of antiretroviral drugs, there is a need to increase PI concentrations to overcome the increase in viral activity. The concentrations of atazanavir obtained when it was combined with ritonavir were two- to fivefold higher than those obtained when atazanavir was administered alone at 400 mg QD (O'Mara et al., Abstr. 8th Conf. Retrovir. Opportunistic Infect., abstr. 740, 2001). The effect of ritonavir on the disposition of atazanavir was studied in healthy volunteers who first received atazanavir at 300 mg QD, followed by atazanavir-ritonavir at 300/100 mg QD (Agarwala et al., 42nd ICAAC). It has been demonstrated that ritonavir increases the level of exposure to atazanavir (AUC) by three- to fourfold; the *t*_{1/2} is increased from 6.5 h (with atazanavir alone) to 15 to 18 h (with the addition of ritonavir), suggesting that the decrease in the hepatic clearance of atazanavir by ritonavir is the major mechanism of the interaction. The values of the pharmacokinetic parameters obtained for the HIV-infected patients in this study are in agreement with those data, although the values for the patients in the present study were slightly lower than those reported for healthy volunteers (Agarwala et al., 42nd ICAAC). The geometric mean AUC₀₋₂₄s for patients after 14 days of therapy and volunteers after 20 days of therapy were 46,073 and 57,039 ng·h/ml, respectively. The *t*_{1/2}s measured during a dosing interval were shorter in patients than in volunteers (8.6 and 16 h, respectively). Several reasons can explain this discrepancy. First, a meal can have an effect, as atazanavir absorption is sensitive to the fat content of meals (for a summary of atazanavir characteristics, see <http://www.fda.gov/cder/approval/index.htm>) and a standard breakfast administered to healthy volunteers in the United States is not the same as the continental breakfast administered in our hospital settings. Second, the duration of therapy can have an effect. Autoinduction was reported with PIs (1) and could explain the lower concentrations after 14 days of therapy compared with those after 10 days of therapy. In keeping with this assumption is the shorter *t*_{1/2}s measured in this study compared to those measured in healthy volunteers (8.6 and 18.1 h, respectively). However, it has been demonstrated in healthy volunteers that the atazanavir steady state is reached within 4 days of therapy when atazanavir is administered either alone or in combination with ritonavir (6). Finally, the rate and extent of absorption could be impaired in HIV-infected patients. Of note, as previously demonstrated with other CYP3A substrates, the plasma atazanavir and ritonavir concentrations were highly variable among the patients included in this study.

Ritonavir concentrations were low, in keeping with the 100-mg QD dosing regimen (4). C_{max}s ranged from 454 to 1,592 ng/ml on week 2 and from 73 to 1,558 ng/ml on week 6.

Such concentrations are below those reported to be efficient against susceptible HIV-1 clinical isolates. Comparison with the data obtained for healthy volunteers (Agarwala et al., 42nd ICAAC) indicates that the ritonavir concentrations were twice those observed in the patients in this study (AUC_{0-24} , 14,844 and 7,011 ng · h/ml, respectively, on week 2), although $t_{1/2}$ s remained unchanged (5.1 and 5.1 h, respectively). The nonreliable absorption of ritonavir in patients or the effect of a meal, as mentioned above for atazanavir, could explain this difference. Despite the low ritonavir concentrations, inhibition of atazanavir metabolism appeared to be sustained during a 24-h dosing interval as a consequence of the high affinity of ritonavir for CYP3A (8).

After the addition of tenofovir DF, there was a trend for lower atazanavir and ritonavir concentrations, but the decrease in the atazanavir AUC_{0-24} was the only difference that reached statistical significance. Interestingly, a significant 25 to 40% decrease in atazanavir concentrations after the addition of tenofovir DF was recently reported from a study with healthy subjects (S. Kaul, K. Bassi, B. Damle, J. Xie, J. Gale, B. Kearne, G. Hanna, Abstr. 43rd Intersci. Conf. Antimicrob. Agents. Chemother., abstr. A-1616, 2003). The mechanism of such an interaction remains to be elucidated. Tenofovir is eliminated unchanged via the kidneys; therefore, an interaction at the biotransformation level is very unlikely (3). Furthermore, it should be pointed out that the atazanavir and ritonavir $t_{1/2}$ s remained unchanged throughout the study (8.6 ± 2.3 and 8.6 ± 3.1 h for atazanavir on week 2 and week 6, respectively, and 5.1 ± 0.7 and 5.3 ± 0.7 h for ritonavir on week 2 and week 6, respectively), suggesting that tenofovir has no effect on atazanavir clearance. Therefore, this interaction likely occurs at the gut level. HIV PIs are substrates of P-glycoprotein (1, 9). The human multidrug resistance P-glycoprotein (ABCB1), which is localized in epithelial cells of the intestine, transports a wide variety of structurally diverse hydrophobic compounds and plays a major role in reducing their bioavailabilities (9). Absorption of the prodrug of tenofovir, tenofovir DF, was reported to involve P-glycoprotein (14); whether modulation of this transporter could explain this interaction remains to be established. It has previously been demonstrated that adefovir, which is closely related to tenofovir, decreased saquinavir concentrations by 50% in HIV-infected patients receiving the combination saquinavir-ritonavir-adefovir compared to those in patients receiving saquinavir-ritonavir-delaquidine (5). Although the exact mechanism underlying this interaction was not elucidated, an induction of P-glycoprotein was hypothesized. Whether adefovir and tenofovir are P-glycoprotein inducers remains to be clearly established. A physicochemical interaction between atazanavir and tenofovir DF when they are simultaneously present in the gut is another postulated hypothesis. The lack of interaction of tenofovir DF with another PI, such as the coformulation of lopinavir-ritonavir, supports the latter assumption (B. P. Kearney, A. Mittan, J. Sayre, J. F. Flaherty, L. Zhong, J. J. Toole, and A. K. Cheng, Abstr. 43rd Intersci. Conf. Antimicrob. Agents. Chemother., abstr. A-1617, 2003). One may therefore speculate that staggered administration of atazanavir-ritonavir and tenofovir DF would avoid such an interaction. However, clinicians are discouraged from using such an approach until data are available to support this hypothesis.

Our data failed to demonstrate a significant correlation between the pharmacokinetic parameters and the viral response. However, this could be explained by the small sample size and the poor overall virologic responses at week 2 and week 6 due to very high levels of resistance mutations among the isolates from the study population at the baseline. The clinical significance of the decrease in atazanavir concentrations when atazanavir treatment was boosted with ritonavir and coadministered with tenofovir DF is unknown. First, atazanavir trough concentrations remained threefold higher than those obtained with atazanavir at 400 mg without ritonavir, and second, data from a recent randomized trial showed that through 16 weeks there was no difference in virologic response whether patients who all received tenofovir DF in combination with one NRTI and one boosted PI were in the arm with the lopinavir-ritonavir coformulation or the arm with the atazanavir-ritonavir coformulation (R. Badaro, E. DeJesus, A. Lazzarin, J. Jemsek, B. Clotet, A. Righiniere, A. Thiry, and R. Wilber, Abstr. 2nd IAS Conf. HIV Pathogenesis Treatment, abstr. 118, 2003). It is now recognized that PI concentrations should be compared and discussed in terms of the concentrations necessary to inhibit the viral replication (2), especially in heavily pretreated patients infected with less susceptible mutant viruses.

One may speculate that the coadministration of tenofovir DF and atazanavir without ritonavir may result in a significant impairment of the virologic response. Further studies are needed to better understand the clinical relevance of this pharmacokinetic interaction in HIV-infected patients.

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Pharmacokinetics and safety of amprenavir and ritonavir following multiple-dose, co-administration to healthy volunteers

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Objective: To evaluate the safety and pharmacokinetic interaction between amprenavir (APV) and ritonavir (RTV).

Methods: Three open-label, randomized, two-sequence, multiple-dose studies having the same design (7 days of APV or RTV alone followed by 7 days of both drugs together) used 450 or 900 mg APV with 100 or 300 mg RTV every 12 h with pharmacokinetic assessments on days 7 and 14. Safety was monitored as clinical adverse events (AEs) and laboratory abnormalities.

Results: Relative to APV alone, RTV co-administration resulted in a 3.3- to 4-fold and 10.84 to 14.25-fold increase in the geometric least-square (GLS) mean area under the plasma concentration-time curve ($AUC_{\tau,ss}$) and minimum concentration ($C_{min,ss}$), respectively. APV 900 mg with RTV 100 mg resulted in a 2.09-fold and 6.85-fold increase in the GLS mean $AUC_{\tau,ss}$ and $C_{min,ss}$, respectively. On day 14, the geometric mean (95% confidence interval) for 450 mg APV $AUC_{\tau,ss}$ ($\mu\text{g} \cdot \text{h/mL}$) was 23.49 (19.32–28.57) with 300 mg RTV and 35.42 (30.46–44.42) with 100 μg RTV, and for the 900 mg APV with 100 mg RTV 47.11 (39.47–61.24). The 450 mg APV $C_{min,ss}$ ($\mu\text{g/mL}$) were 1.32 (1.05–1.67) and 2.01 (1.70–2.61), and 2.47 (2.08–3.32) for 900 mg APV. The most common AEs were mild and included diarrhea, nausea/vomiting, oral parasthesias, and rash. The triglyceride and cholesterol increased significantly from RTV exposure.

Conclusion: Adding RTV to APV resulted in clinically and statistically significant increases in APV AUC and C_{min} with variable effects on maximum concentration. The two RTV doses had similar effects on APV but AEs were more frequent with 300 mg RTV.

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Keywords: Protease inhibitors, amprenavir, ritonavir, safety, pharmacokinetics, drug interaction

Introduction

Combination regimens containing a protease inhibitor (PI) and two nucleoside reverse transcriptase inhibitors can suppress HIV type 1 (HIV-1) replication to levels

below the detection limits of assays, increase CD4+ cell counts, and reduce progression to AIDS [1–5]. Despite these favorable attributes, combination therapies often demand complex treatment regimens involving frequent dosing, food or fluid restrictions, and are

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associated with numerous adverse events [6,7]. The inconvenience of some of these regimens has made it difficult for patients to adhere to treatment, leading to reductions in virological response [8] and the potential for development of drug-resistant variants of HIV. Thus, there is a need for simpler, safer, and more convenient PI-containing regimens that can facilitate adherence.

Amprenavir (APV; Agenerase 141W94; GlaxoSmithKline, Research Triangle Park, North Carolina, USA) is a recently introduced HIV PI. In *in vitro* studies, the mean 50% inhibitory concentration (IC_{50}) of APV against 334 HIV clinical isolates from patients naive to protease inhibitors was shown to be 0.0146 $\mu\text{g/ml}$ [9]. In a similar manner to other HIV PIs, APV binds to plasma proteins ($\approx 90\%$), predominantly to α_1 -acid glycoprotein (AAG), and to a lesser extent, to albumin. Because AAG is an acute-phase protein whose levels change during certain disease states [10,11] these changes in AAG affect the pharmacokinetic profile of APV [12].

Amprenavir, as with the other approved HIV PIs, is metabolized primarily by the hepatic cytochrome P450 enzyme system, specifically CYP3A4 [13,14]. All HIV PIs are inhibitors of CYP3A4, but the HIV PI RTV is the most potent inhibitor of CYP3A4 [14]. Previous studies have indicated significant increases in drug exposure from co-administration of ritonavir (RTV) with indinavir, saquinavir, and nelfinavir [15–21]. In a recent study, co-administration of RTV with APV resulted in significant increases in APV exposures, thus confirming that a significant drug–drug interaction between RTV and APV exists [22]. To address the magnitude of the interaction between APV and RTV and to assess short term safety and tolerance, a series of three randomized, two-sequence, two-period, multiple-dose studies were conducted.

Methods

Participants

Healthy male and female individuals were eligible to participate in each study if they met the following criteria: 18–55 years of age; weighed 55–95 kg; had a body mass index of 20–28 kg/m^2 ; and were seronegative for HIV (confirmed using standard methodologies). All patients provided written informed consent to participate in the trials. Participants were ineligible if they had conditions that would interfere with drug absorption, a history of adverse reactions to protease inhibitors, alcohol or illicit drug use, and abnormal laboratory values outside of prespecified ranges. No concomitant medications were allowed during the study.

Study design

Three multiple-dose, open-label, randomized, two-sequence, two-period drug–drug interaction studies (GlaxoSmithKline protocols PRO10017, PRO10022, and PRO10023) were conducted at single study centers in the United States. All study protocols were reviewed and approved by the Institutional Review Board at the respective study sites. Each period consisted of a screening assessment performed within 14 days prior to dosing, a dosing period during which participants received the study drug, and a follow-up evaluation.

In each study, 18 participants were planned to be randomly assigned to one of two treatment sequences; APV followed by APV+RTV in sequence 1 or RTV followed by APV+RTV in sequence 2 (nine individuals per sequence). In the first study (PRO10017), 450 mg APV every 12 h (q12h) alone (sequence 1) or 300 mg RTV q12h alone (sequence 2) was administered for 7 days. In the following 7 days, all participants received both APV and RTV (APV + RTV) at these same doses. In the subsequent two studies (PRO10022 and PRO10023), the study design remained the same but 450 mg APV q12h and 100 mg RTV q12h (PRO10022), or 900 mg APV q12h and 100 mg RTV q12h (PRO10023) were investigated. Due to the lack of availability of ritonavir capsules at the time, the ritonavir oral solution was used in the first study. Ritonavir capsules were used in the other two studies.

As the affinity of APV for CYP3A4 is similar to that of indinavir [14] and RTV increased the area under the concentration–time curve of indinavir by approximately three-fold [17], a 450 mg dose of APV (approximately one-third the recommended dose) was initially selected to avoid potential exposures in healthy individuals which could greatly exceed those of 1200 mg APV alone (the usual clinical dose). The 300 mg q12h dose of RTV in study PRO10017 was selected to assess the maximal drug interaction with APV, with subsequent dose reduction in PRO10022 and PRO10023 to assess the dependence of the interaction on the degree of RTV exposure.

Participants reported to the study center no later than 1800 h on the evening before dosing days 1, 7, and 14, and received a standardized meal, and with the exception of day 1, were administered their evening dose of study medication(s). After fasting for 8 h overnight, participants were administered their randomized treatment with water (360 ml) on the morning of days 1, 7, and 14. They remained at the center until completion of all post-dosing procedures (24 h on day 1 and 12 h on days 7 and 14). Standard balanced meals were provided during the time that the participants were at the study center.

Assessments

For determination of drug concentrations, 3 to 4 ml blood samples (serum for APV, plasma for RTV) were collected on dosing day 1 at 5 min before the first dose and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 h post-dosing. On dosing days 7 and 14, blood samples were collected up to 12 h post-dosing only. The AAG concentrations were determined from blood samples taken prior to dosing on days 1, 7, and 14. Blood samples were centrifuged within 30 min of collection to separate the serum or plasma, and the samples stored at -20°C until further analysis.

Safety assessments, including medical history, physical examination, hematology, clinical chemistry, urinalysis, and clinical adverse experiences, were carried out on dosing days 1, 7, and 14 before the dose was given. In addition, vital signs and electrocardiograms were performed at the screening evaluation only. Adverse events occurring during the trial were evaluated by the investigator and graded according to protocol toxicity scales and for relationship to study drug.

Bioanalysis of amprenavir, ritonavir and AAG

Serum concentrations of APV were determined by a validated method using protein precipitation followed by high-performance liquid chromatography and detection by tandem mass spectrometry using a positive ion multiple reaction monitoring technique. RTV concentrations in plasma were quantified by PPD-Pharmaco (Richmond, Virginia, USA). Quality control concentrations ranged from 10 to 5000 ng/ml and 25 to 10 000 ng/ml for APV and RTV, respectively. The assay calibration curve was linear from 10 to 5000 ng/ml for APV, and 10 to 15 000 ng/ml for RTV. Accuracy (expressed as % bias) ranged from -3.9 to 5.3% and 0.245 to 5.15% for validation controls for APV and RTV, respectively. Inter-assay precision, expressed as percentage coefficient of variance (%CV), ranged from 4.5 to 8.8% for APV, and 3.97 to 9.19% for RTV. Intra-assay precision ranged from 3.3 to 10.1% for APV. Intra-assay precision and accuracy for RTV ranged from 2.89 to 26.1% and 2.09 to 9.22% , respectively. The AAG assays were performed using a commercially available assay (Quest Diagnostics, Inc., San Juan Capistrano, California, USA).

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from concentration-time data using non-compartmental pharmacokinetic methods (WinNonlin Pro version 1.5; Scientific Consulting Inc., Cary, North Carolina, USA). Single- and multiple-dose pharmacokinetic parameters were calculated from concentration data derived from samples collected on dosing day 1 and days 7 and 14, respectively. The maximum serum concentration ($C_{\max}$, $C_{\max,ss}$) and the time to reach $C_{\max}$ ($T_{\max}$,

$T_{\max,ss}$) were obtained by direct inspection of individual plasma concentration-time data.

Single-dose parameters were estimated for day 1. The elimination half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = \ln(2)/\lambda_z$, where λ_z , the apparent terminal elimination rate constant, was estimated by log-linear regression of the terminal portion of the concentration-time curve. The area under the concentration-time curve ($AUC_{0 \rightarrow t}$) from time zero to the time of the last quantifiable sample (t_{last}) was calculated using the linear trapezoidal rule. $AUC_{0 \rightarrow t}$ was extrapolated from t_{last} to infinity ($AUC_{0 \rightarrow \infty}$) by adding $C_{\text{last}}/\lambda_z$, where C_{last} is the concentration of the last quantifiable sample. Apparent total clearance (CL/F) was calculated as $\text{Dose}/AUC_{0 \rightarrow \infty}$. The apparent volume of distribution during the terminal elimination phase (V_z/F) was calculated as $(CL/F)/\lambda_z$.

Steady-state parameters were estimated for days 7 and 14. $C_{\min,ss}$, the minimum drug concentration at steady state was calculated as $(C_0 + C_t)/2$, where C_0 is the plasma concentration before dosing and C_t is the concentration of the last sample of the steady-state dosing interval. If necessary, $AUC_{0 \rightarrow t}$ was extrapolated to the end of the dosing interval ($AUC_{\tau,ss}$) at time $t = t$, the steady-state dosing interval, using the formula:

$$AUC_{\tau,ss} = AUC_{0 \rightarrow t} + (C_{\text{last}}/\lambda_z) \times (1 - \exp[-\lambda_z(t - t_{\text{last}})]).$$

The apparent total clearance at steady state, CL/F , was calculated as $\text{Dose}/AUC_{\tau,ss}$.

Statistical analysis

Eight evaluable patients per treatment sequence were estimated to be necessary to detect a 40% difference in AUC for APV and a 50% difference in AUC for RTV on a log scale with 80% of power at the 0.05 level. To account for potential dropouts, nine patients per treatment sequence were enrolled.

All pharmacokinetic parameters (except $T_{\max}$) were log-transformed prior to analysis. Comparisons in log-transformed pharmacokinetic parameters ($AUC_{\tau,ss}$, $C_{\max,ss}$, $C_{\min,ss}$, and CL/F) between treatment regimens were analyzed by analysis of variance using the PROC MIXED procedure (SAS, version 6.12; SAS Institute Inc., Cary, North Carolina, USA). The analysis included treatment, period, and sequence as fixed effects and subject-within-sequence as a random effect. AAG concentration was used as a covariate in the analysis (except for PRO10017 where AAG data were not available for analysis when RTV was administered alone due to study error). Geometric least-squares (GLS) mean ratios and their associated 90% confidence intervals (CI) were used for within-subject treatment comparisons of pharmacokinetic parameters. Treat-

ments were considered to have no pharmacokinetic interaction if the 90% CI of the estimated GLS mean differences between the treatments on a log-scale shows less than a 40% difference for APV and a 50% difference for RTV. This indicates that the 90% CIs of the GLS mean ratios fall within 0.67-1.49 for APV and 0.61-1.65 for RTV. Non-parametric methods were used to analyze the 95% CI of median T_{max} values for each treatment. Treatment comparisons of T_{max} values and their associated 90% CI was performed using the Wilcoxon rank sum test.

Results

Participants

The demographic and baseline characteristics of the normal volunteers enrolled in each study were comparable between the studies (Table 1). Eighteen, 18, and 19 individuals were enrolled and 17, 15, and 16 individuals completed studies PRO10017, PRO10022, and PRO10023, respectively.

Pharmacokinetic analysis

The median APV concentration-time curves after administration of APV alone or in combination with RTV are shown in Figure 1. The geometric mean (95% CI) values of selected APV pharmacokinetic

Table 1. Baseline characteristics of study population.

Characteristic	Study		
	PRO10017 (n = 18)	PRO10022 (n = 18)	PRO10023 (n = 19)
Age ^a (years)	33.3 ± 9.6	31.7 ± 9.7	29.3 ± 9.4
Sex			
Male	10	12	9
Female	8	6	10
Race			
White	17	17	18
Other	1	1	1
Weight ^a (kg)	72.4 ± 11.0	74.8 ± 12.5	70.7 ± 9.6

^aValues are given as the mean ± SD.

parameters (C_{max} , AUC , and C_{min}) for each study day in each study and the GLS mean and ratios are shown in Table 2. Single-dose APV pharmacokinetics predicted steady-state pharmacokinetics in all three studies. RTV significantly increased APV $AUC_{\tau,ss}$ and $C_{min,ss}$ compared with those achieved when APV was administered alone. The C_{max} was not uniformly affected by RTV and in only one study (PRO10022) was there a significant effect.

RTV had a complicated inhibition-autoinduction pattern in all three studies (Table 3) consistent with previous reports [16,23,24]. Single-dose RTV pharmacokinetics did not predict steady-state pharmacokinetics. In the first

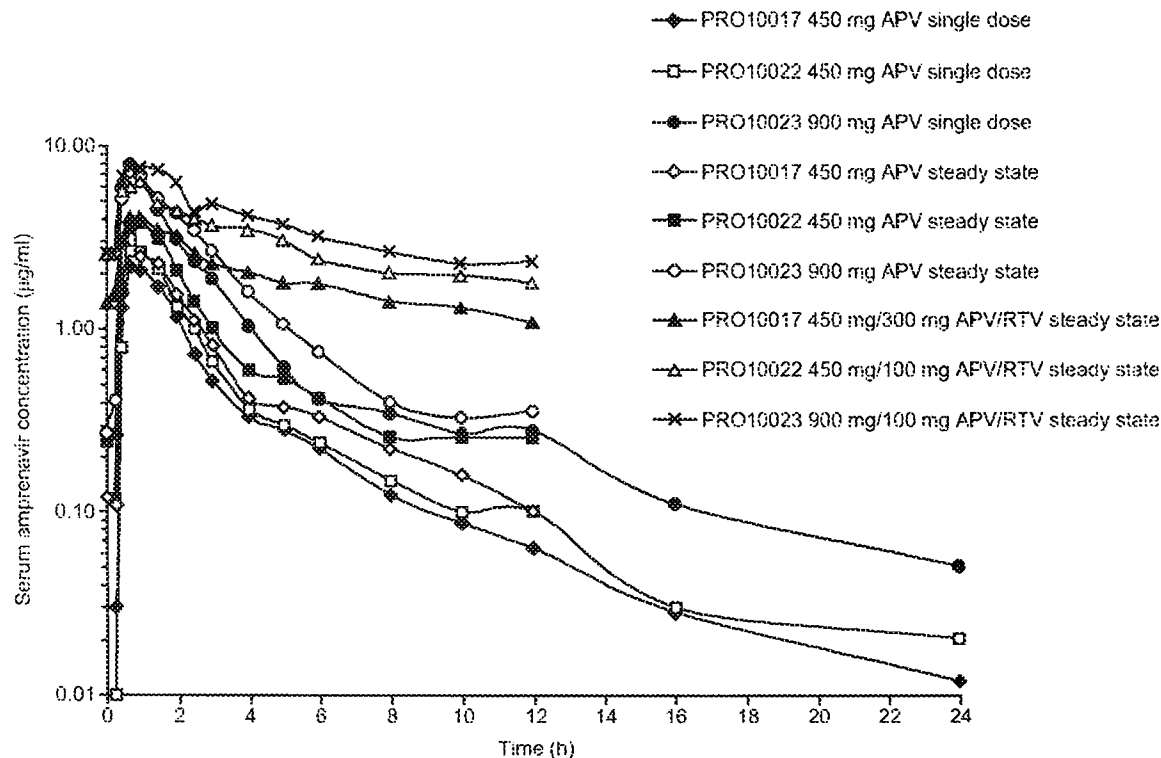


Fig. 1. Median amprevir serum concentration-time curves for the dosing regimens used in each study. APV, amprevir; RTV, ritonavir.

Table 2. Summary of estimated amprenavir (APV) pharmacokinetic parameters^a.

Parameter	Geometric mean (95% CI)			GLS mean ^b	GLS mean ratio (90% CI) for treatment comparison	
PRO10017 (450 mg APV/300 mg RTV)						
	Day 1 (APV) (n = 9)	Day 7 (APV) (n = 9)	Day 14 (APV + RTV) (n = 17)	Day 14 (n = 9)	Day 7 (n = 9)	Day 14 : 7
$AUC_{T,ss}$ (h • µg/ml)	6.02 (4.01–9.04) ^c	7.74 (5.91–10.15)	23.49 (19.32–28.57)	26.65	8.00	3.33 (2.66–4.17)
C_{max} (µg/ml)	3.15 (1.99–4.97)	4.15 (3.19–5.40)	4.89 (4.02–5.94)	4.67	4.28	1.09 (0.85–1.40)
C_{min} (µg/ml)	N/A	0.11 (0.07–0.19)	1.32 (1.05–1.67)	1.63	0.11	14.25 (9.04–22.48)
PRO10022 (450 mg APV/100 mg RTV)						
	Day 1 (n = 10)	Day 7 (n = 8)	Day 14 (n = 15)	Day 14 (n = 8)	Day 7 (n = 8)	Day 14 : 7
$AUC_{T,ss}$ (h • µg/ml)	7.3 (5.58–10.06) ^c	8.70 (5.73–14.21)	35.42 (30.46–44.42)	35.19	8.80	4.00 (3.36–4.76)
C_{max} (µg/ml)	3.48 (2.73–4.58)	3.63 (2.36–6.10)	6.83 (5.79–8.97)	6.19	3.66	1.69 (1.22–2.35)
C_{min} (µg/ml)	N/A	0.19 (0.10–0.42)	2.01 (1.70–2.61)	2.07	0.19	10.84 (7.54–15.58)
PRO10023 (900 mg APV/100 mg RTV)						
	Day 1 (n = 10)	Day 7 (n = 9)	Day 14 (n = 16)	Day 14 (n = 8)	Day 7 (n = 9)	Day 14 : 7
$AUC_{T,ss}$ (h • µg/ml)	21.56 (16.46–30.18) ^c	20.16 (14.57–28.96)	47.11 (39.47–61.24)	45.80	21.86	2.09 (1.86–2.36)
C_{max} (µg/ml)	9.06 (6.32–14.22)	8.66 (4.73–15.54)	9.28 (8.02–11.58)	9.27	9.53	0.97 (0.76–1.24)
C_{min} (µg/ml)	N/A	0.31 (0.18–0.58)	2.47 (2.08–3.32)	2.12	0.31	6.85 (4.99–9.41)

^aAll doses were administered every 12 h. ^bGeometric least-square (GLS) means are within-subject treatment comparisons described in Methods. ^c $AUC_{0-\infty}$. Reference values obtained from pooled estimates of geometric mean C_{max} , $AUC_{0-12\text{ h}}$, and C_{min} for 1200 mg amprenavir twice daily alone in three steady-state studies (PROA1002, PROA1012, and PROA1013, n = 43) are 8.21 µg/ml, 25.5 µg/ml, and 0.326 µg/ml, respectively. CI, confidence interval; RTV, ritonavir; N/A, not applicable.

Table 3. Summary of estimated ritonavir (RTV) pharmacokinetic parameters^a.

Parameter	Geometric mean (95% CI)			GLS mean ^b	GLS mean ratio (90% CI) for treatment comparison	
PRO10017 (450 mg APV/300 mg RTV)						
	Day 1 (n = 9)	Day 7 (n = 9)	Day 14 (n = 17)	Day 14 (n = 9)	Day 7 (n = 9)	Day 14 : 7
$AUC_{t,ss}$ (h • µg/ml)	39.51 (28.17–55.41) [‡]	60.11 (46.80–77.19)	39.65 (32.73–48.04)	43.22	60.11	0.72 (0.60–0.86)
C_{max} (µg/ml)	7.32 (5.65–9.47)	12.13 (9.98–14.75)	8.67 (6.92–10.87)	10.43	12.13	0.86 (0.70–1.05)
C_{min} (µg/ml)	N/A	1.44 (0.85–2.43)	0.99 (0.70–1.40)	1.07	1.44	0.74 (0.60–0.92)
PRO10022 (450 mg APV/100 mg RTV)						
	Day 1 (n = 8)	Day 7 (n = 8)	Day 14 (n = 15)	Day 14 (n = 7)	Day 7 (n = 7)	Day 14 : 7
$AUC_{t,ss}$ (h • µg/ml)	4.45 (3.12–6.50) [‡]	14.11 (10.10–20.40)	5.84 (4.92–7.88)	6.46	13.77	0.47 (0.38–0.58)
C_{max} (µg/ml)	0.69 (0.47–1.06)	2.64 (1.39–2.36)	1.35 (1.10–2.07)	1.47	2.59	0.57 (0.42–0.77)
C_{min} (µg/ml)	N/A	0.38 (0.26–0.58)	0.15 (0.11–0.24)	0.16	0.36	0.45 (0.31–0.66)
PRO10023 (900 mg APV/100 mg RTV)						
	Day 1 (n = 9)	Day 7 (n = 8)	Day 14 (n = 16)	Day 14 (n = 8)	Day 7 (n = 8)	Day 14 : 7
$AUC_{t,ss}$ (h • µg/ml)	4.00 (2.78–6.18) [‡]	13.19 (9.77–18.36)	4.58 (3.30–8.21)	5.04	13.92	0.36 (0.28–0.46)
C_{max} (µg/ml)	0.42 (0.29–0.65)	2.37 (1.75–3.35)	1.10 (0.86–1.78)	1.23	1.82	0.68 (0.41–1.10)
C_{min} (µg/ml)	N/A	0.39 (0.29–0.55)	0.13 (0.07–0.34)	0.14	0.42	0.35 (0.23–0.52)

^aAll doses were administered every 12 h. ^bGeometric least-square (GLS) means are within-subject treatment comparisons described in Methods. ^c $AUC_{0-\infty}$. CI, confidence interval; APV, amprenavir; N/A, not applicable.

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7 days, RTV significantly increased its AUC and C_{max} . In the next 7 days there were significant decreases in C_{max} , AUC , and C_{min} . There was no significant sequence effect on APV concentrations indicating that the interaction effect of ritonavir was maximal despite its own time-dependent pharmacokinetics. There were no significant effects on T_{max} for either APV or RTV.

Safety assessment

The 100 mg RTV q12h dose alone or in combination with APV resulted in fewer adverse events than the 300 mg q12h dose alone or in combination with APV. The occurrence of adverse events regardless of relation to study drug was greater with APV and RTV in combination than with either drug alone (Table 4). The most common drug-related adverse events reported by participants were diarrhea, nausea, and oral paresthesia in PRO10017; nausea/vomiting, headache and dizziness in PRO10022; and diarrhea, nausea/vomiting, headache, and oral paresthesia in PRO10023. In PRO10017 one individual withdrew from the study with rash that progressed after the addition of RTV; in PRO10022 two participants withdrew from the study, one due to rash following the first dose of APV alone, and the other withdrew due to rash and pruritis after APV in combination with RTV; and in PRO10023 three participants withdrew from the study, one due to nausea after the first dose of APV, one due to oral/perioral numbness, rash, edema of the face, ocular swelling and pruritis during APV/RTV, and one due to lightheadedness after RTV alone followed by vomiting and oral lesions.

There were also significant effects found on clinical chemistry changes from pre-therapy (Table 5). The 300 mg dose of RTV in combination with APV resulted in a trend towards increases in serum glucose concentration but was not statistically significant. In the study with 300 mg RTV, the glucose change from baseline for the APV to APV/RTV sequence at day 7 was -1.39 ± 14.12 and at day 14 14.5 ± 22.26 mg/dl ($P = 0.07$), and the RTV to RTV/APV sequence at day 7 was 9.17 ± 24.69 and at day 14 it was 12.72 ± 18.54 mg/dl ($P = 0.07$). In contrast, the 100 mg dose of RTV had no effect on serum glucose concentration in either study (data not shown). There were significant increases in cholesterol and triglyceride values from pre-treatment ($P < 0.05$) with both doses of RTV but the time frame differed (Table 5) suggesting a cumulative exposure effect. There were no other clinically significant laboratory changes observed.

Discussion

The series of interaction studies presented here demonstrated statistically and clinically significant effects of

Table 4. Most common ($\geq 5\%$ incidence) adverse events (all grades) by study.

Adverse event ^a	PRO10017 ^b			PRO10022 ^b			PRO10023 ^b			Total n = 55
	450 mg APV n = 9	300 mg RTV n = 9	450 mg APV + 300 mg RTV n = 18	450 mg APV n = 10	100 mg RTV n = 8	450 mg APV + 100 mg RTV n = 16 ^c	900 mg APV n = 10	100 mg RTV n = 9	900 mg APV + 100 mg RTV n = 17	
No. participants experiencing any adverse event n (%)	3 (33)	3 (33)	14 (78)	5 (50)	1 (13)	6 (38)	4 (40)	4 (44)	8 (47)	39 (71)
Diarrhea	0	0	9 (50)	0	0	2 (13)	0	1 (11)	2 (12)	14 (25)
Nausea/vomiting	1 (11)	1 (11)	5 (28)	2 (20)	0	3 (19)	1 (10)	2 (22)	4 (24)	17 (31)
Headache	0	2 (22)	4 (22)	0	1 (13)	2 (13)	1 (10)	2 (22)	4 (24)	12 (22)
Oral paresthesia/numbness	0	0	5 (28)	0	0	0	3 (30)	1 (11)	4 (24)	12 (22)
Dizziness	1 (11)	0	2 (11)	3 (30)	0	2 (13)	0	1 (11)	0	9 (16)
Rash	1 (11)	0	2 (11)	2 (20)	0	0	1 (10)	0	2 (12)	8 (15)

^aThe number (percentage) of participants reporting an adverse event are given. ^bAll doses were administered every 12 hours. ^cTwo participants were not included in this treatment group because they did not receive this treatment. APV, amprenavir; RTV, ritonavir.

Table 5. Mean $\pm$ SD change in triglyceride and cholesterol concentrations from pre-therapy.

	APV (Day 7)	RTV (Day 7)	Sequence 1 APV $\rightarrow$ APV + RTV (Day 14)	Sequence 2 RTV $\rightarrow$ RTV + APV (Day 14)
PRO10017 (450 mg APV/300 mg RTV)	n = 9	n = 9	n = 8	n = 9
Mean $\pm$ SD change from baseline in:				
Triglyceride (mg/dl)	-8.2 $\pm$ 45.1	123.2 $\pm$ 144.3*	140.9 $\pm$ 136.0*	76.5 $\pm$ 66.2*
Cholesterol (mg/dl)	-3.7 $\pm$ 9	1.2 $\pm$ 11.6	10.6 $\pm$ 12*	11.6 $\pm$ 17.4*
PRO10022 (450 mg APV/100 mg RTV)	n = 9	n = 8	n = 8	n = 8
Mean $\pm$ SD change from baseline in:				
Triglyceride (mg/dl)	46.8 $\pm$ 106.4	102 $\pm$ 101.1*	57.4 $\pm$ 82.6†	93.3 $\pm$ 77.6*
Cholesterol (mg/dl)	-1.5 $\pm$ 16	0.9 $\pm$ 13.2	6.6 $\pm$ 13.4	18.2 $\pm$ 10.7*
PRO10023 (900 mg APV/100 mg RTV)	n = 9	n = 8	n = 8	n = 8
Mean $\pm$ SD change from baseline in:				
Triglyceride (mg/dl)	22.7 $\pm$ 28.8	43.6 $\pm$ 70.4†	40.1 $\pm$ 49.8†	67.5 $\pm$ 102.9*
Cholesterol (mg/dl)	9.4 $\pm$ 14.6	11.4 $\pm$ 15.2†	32.3 $\pm$ 10.7*	19.8 $\pm$ 27.2*

Significant difference from pre-therapy (average of day 1 and screening laboratory result) at * $P \leq 0.05$ and † $0.05 < P \leq 0.09$ level. SD, standard deviation; APV, amprenavir; RTV, ritonavir.

RTV on APV $AUC_{t,ss}$ and $C_{min,ss}$. Given the inconsistent effect on C_{max} these results suggest an effect of RTV on inhibition of APV's metabolism than by an effect on its bioavailability. This is similar to the effects of RTV on indinavir rather than on saquinavir and to a lesser extent nelfinavir, where very large increases in C_{max} result from increases in bioavailability [15–21].

Single-dose APV pharmacokinetics predicted steady-state pharmacokinetics in each of these studies. As the participants were normal volunteers with little change in their AAG concentrations this was expected. The findings are consistent with our study showing changes in values of the area under the concentration–time curve were correlated to changes in AAG concentration both between- and within-subjects [12].

The combination of 450 mg APV q12h with the lower dose of RTV (100 mg q12h) tended to be better tolerated than the combination with the higher RTV (300 mg q12h) dose or the higher APV dose (900 mg q12h) in combination with RTV. As the oral solution and capsule formulations of ritonavir are bioequivalent we would expect most of these differences to persist if capsules were available for use in all the trials.

Gastrointestinal symptoms were the adverse events most commonly reported in participants and were mild to moderate in intensity. Differences in the incidence of adverse events between studies may be attributed to differences in the reporting of adverse events between the three study sites. Serum cholesterol and triglyceride concentrations were elevated to a greater magnitude by both RTV doses in combination with APV than by APV administered alone. These findings are consistent with the results of previous studies that have shown

RTV to be associated with increases in plasma triglyceride and cholesterol concentrations [23,24]. It cannot be ruled out that the higher dose of APV (900 mg q12h) may have contributed to the higher cholesterol concentrations observed in PRO10023.

Co-administration of RTV with APV 450 or 900 mg q12h resulted in APV C_{max} , AUC , and C_{min} values that were similar to or slightly greater than those achieved with the approved 1200 mg twice daily APV dose alone as noted in Table 2. These increases in APV C_{min} are consistent with the magnitude of increases in indinavir C_{min} produced by the co-administration of 100 or 200 mg RTV with 800 or 1200 mg indinavir in healthy volunteers and HIV-1 infected patients [18–20]. Several clinical studies have demonstrated a strong correlation between antiviral activity and plasma trough concentrations of PIs [24–31]. Subtherapeutic concentrations of HIV PIs in plasma can be associated with viral rebound and the development of drug resistance [24–31], indicating the importance of maintaining consistent drug concentrations in plasma. Increased trough concentrations of APV may lead to improved efficacy over time, and minimize development of drug-resistant viral strains.

The pharmacokinetic data from these studies were combined in a nonlinear mixed-effect modelling analysis (NONMEM; NONMEM Project Group, University of California – San Francisco, California, USA) to evaluate potential twice daily and once daily regimens [32]. The details of this population analysis will be described in another manuscript. For the twice daily regimen, consideration was given to the potential use of therapy in populations with viral isolates of reduced susceptibilities [9]. When considering a population with

reduced viral susceptibility, a simulated regimen of 600 mg APV twice daily with 100 mg RTV twice daily gives similar C_{min} to protein binding-adjusted IC_{50} ratios as the 1200 mg APV dose for wild-type isolates [9]. As might be anticipated from the degree of interaction demonstrated in the current studies, once-daily regimens were clearly feasible for patients with susceptible virus.

In summary, co-administration of 100 or 300 mg RTV q12h with 450 or 900 mg APV q12h resulted in statistically and clinically significant increases in APV plasma concentrations. Clinical studies are in progress to evaluate the efficacy, pharmacokinetics and safety profile of various doses and schedules of APV in combination with RTV, with emphasis on 600 mg APV twice daily in combination with 100 mg RTV twice daily [33,34].

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SOLO: 48-week efficacy and safety comparison of once-daily fosamprenavir /ritonavir versus twice-daily nelfinavir in naive HIV-1-infected patients

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Objective: To compare the magnitude and durability of the antiviral response to fosamprenavir (FPV) plus ritonavir (RTV) once-daily (FPV/r QD) with nelfinavir twice-daily (NFV BID), each administered with abacavir and lamivudine twice-daily.

Methods: An international, phase III, randomized, open-label study in antiretroviral therapy-naïve, HIV-infected adults.

Results: Patients with advanced HIV disease received FPV/r QD ($n = 322$) or NFV BID ($n = 327$). At week 48, 69% of patients in the FPV/r QD group and 68% in the NFV BID group had plasma HIV-1 RNA (vRNA) < 400 copies/ml, whereas 55% of patients in the FPV/r QD group and 53% in the NFV BID group had vRNA < 50 copies/ml (intent to treat, rebound/discontinuation = failure). More patients in the NFV BID group (17%) experienced virological failure than in the FPV/r QD group (7%). Efficacy of FPV/r QD was maintained in patients with CD4+ cell counts $< 50 \times 10^6$ cells/l or vRNA $\geq 100\,000$ copies/ml at entry. At week 48, median CD4+ cell counts were increased to 203×10^6 cells/l (FPV/r QD group) and 207×10^6 cells/l (NFV BID group). Both regimens were generally well tolerated. Diarrhea was more common on NFV BID than on FPV/r QD (16 versus 9%; $P = 0.008$). Fasting lipid profile results were generally favorable in both treatment arms. FPV/r QD maintained plasma amprenavir (APV) trough concentrations above the mean phenotypic drug-susceptibility (IC_{50}) for wild-type virus for APV.

Conclusion: As a first choice protease inhibitor with a low daily pill burden, FPV/r QD was well tolerated and provided potent, durable antiviral suppression.

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Keywords: fosamprenavir, ritonavir, nelfinavir, SOLO, protease inhibitor, HIV-1

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Introduction

A significant proportion of HIV-infected patients now seeking to initiate highly active antiretroviral therapy (HAART) have reached an advanced stage of disease [1,2, J.Gill, Seventh International Workshop on HIV Observational Databases, Italy, March 2003]. In order to offer this population optimal treatment options, new effective protease inhibitors (PIs) with flexible dosing options are needed.

Fosamprenavir (GW433908, FPV) is a PI for the treatment of HIV-infection in adults [3–6]. It is rapidly hydrolyzed to its active moiety amprenavir (APV) plus inorganic phosphate upon absorption and can be taken without food or water restrictions. Pharmacokinetic studies showed that co-administration of 1400 mg FPV once-daily (QD) with 200 mg ritonavir (RTV) QD or 700 mg FPV twice-daily (BID) with 100 mg RTV BID led to pharmacoenhancement with elevation of the plasma APV area under the curve (AUC) by approximately two-fold and plasma trough concentration (C_{trss} : the concentration at the end of a dosing interval, τ , at steady state) by four- to six-fold in comparison with other studies of FPV in the absence of RTV [3,6]. The plasma APV trough concentrations achieved in these studies [6] exceeded the previously determined concentration required to inhibit by 50% the replication of wild-type virus [7] and facilitate once-daily dosing which may improve adherence and durability of response.

In this study, we compared the safety and antiviral efficacy of a FPV/ritonavir (FPV/r) QD regimen with nelfinavir (NFV) administered BID, both in combination with the nucleoside reverse transcriptase inhibitors (NRTIs) abacavir (ABC) and lamivudine (3TC) delivered BID in antiretroviral therapy (ART)-naïve adults. The primary objective was to compare the magnitude and durability of the antiviral response over 48 weeks. Secondary objectives were to compare the safety and tolerability of FPV/r QD and NFV BID, and to compare the virological, immunological, and metabolic responses to these regimens.

Methods

Patients

This study recruited ART-naïve HIV-1-infected adults with plasma HIV-1 RNA (vRNA) ≥ 1000 copies/ml. Therapy-naïve status was defined as <4 weeks of therapy with any NRTI and no prior therapy with any non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI. Patients were excluded if they had medical conditions that could have compromised their safety or interfered with drug absorption, required

excluded medications, or had protocol-specified abnormal laboratory values. There were no exclusion criteria based on CD4+ cell counts or HIV disease classification [8].

Study design

This randomized, open-label study was conducted at 101 centers in North America, Europe, South Africa and Australia. The primary endpoint was the proportion of patients with vRNA <400 copies/ml at week 48. Secondary endpoints included the proportion of patients with vRNA <50 copies/ml at week 48, discontinuations of randomized PI due to adverse events, changes from baseline in vRNA, CD4+ cell counts, viral resistance, metabolic abnormalities and pharmacokinetics.

Patients were randomized to receive either FPV (GlaxoSmithKline, Ware, Hertfordshire, UK) 1400 mg QD (two 700 mg tablets) plus RTV (Abbott Laboratories, North Chicago, Illinois, USA) 200 mg QD (two 100 mg capsules), or NFV (Pfizer Pharmaceuticals, New York, USA) 1250 mg BID (five 250 mg tablets). Both treatments were administered with ABC and 3TC (GlaxoSmithKline) BID. Patients were stratified according to screening vRNA: ≥ 1000 –10 000, $>10\,000$ –100 000 and $>100\,000$ copies/ml.

Efficacy and safety assessments

At each visit, patients were assessed for HIV-associated conditions, CD4+ cell counts, and plasma HIV-1 RNA using the Roche Amplicor HIV-1 Monitor test version 1.5 (Roche Diagnostics, Basel, Switzerland) [ultrasensitive assay lower limit of detection (LLOD) = 50 copies/ml, upper limit of detection (ULOD) = 75 000 copies/ml; standard assay LLOD = 400 copies/ml, ULOD = 750 000 copies/ml]. Samples with HIV-1 RNA $>75\,000$ copies/ml using the ultrasensitive assay were re-tested using the standard assay.

Adverse events (AEs) were recorded throughout the study. Abnormal laboratory findings or other abnormal assessments the investigator judged to be clinically significant were recorded as AEs. Drug-related events were reported as related to the entire study drug regimen (PI plus NRTI).

In addition to standard clinical chemistry assessments throughout the study, fasting triglycerides, glucose, total cholesterol and high-density lipoprotein (HDL)/low-density lipoprotein (LDL) cholesterol were assessed at day 1 and at weeks 8, 16, 24, and 48.

Self-reported adherence

Treatment adherence was assessed by a self-reported Patient Medication Adherence Questionnaire, administered to 268 patients in five English-speaking countries. The questionnaire recorded adherence behavior

for each study medication during each of the last 3 days and the weekend prior to weeks 1, 12, 24, and 48. Subjects who reported no missed doses received a 'perfect' adherence score.

Pharmacokinetic and phenotypic drug-susceptibility assessments

At selected centers, patients receiving FPV/r QD underwent $C_{tr,ss}$ pharmacokinetic sampling at weeks 4, 8, and 12 to assess steady-state plasma APV concentrations [9].

The phenotypic drug-susceptibility (IC_{50}) of virus isolated from these same patients at baseline was determined by ViroLogic Inc. (South San Francisco, California, USA) using the PhenoSense assay. [10]

Statistical methods

The planned sample size of 624 patients provided approximately 85% power at the 2.5% significance level to detect differences in proportions of patients achieving vRNA < 400 copies/ml at week 48, using a 12% margin to assess non-inferiority.

For the primary endpoint, the two-sided 95% confidence interval (CI) around the difference in proportions between the FPV/r QD and the NFV BID groups was calculated. If the lower limit of the 95% CI lay above -12%, the non-inferiority of FPV/r QD to NFV BID would be established, whereas if it lay above 0, this would imply superiority of FPV/r QD to NFV BID. The confidence interval was stratified to reflect the vRNA randomization strata using Mantel-Haenszel weights. To provide a fuller description of the variability around the differences in proportions of virological and non-virological failures (the two sub-components of the primary endpoint), descriptive 95% CIs were calculated using the same method as above.

The intent-to-treat (ITT) population included all patients who received at least one dose of randomized treatment. The primary strategy for analyses of proportion endpoints was ITT rebound/discontinuation = failure (RD = F) where positive responders were those patients with confirmed vRNA levels < 400 copies/ml. Treatment failures comprised those patients whose vRNA remained $\geq$ 400 copies/ml, had a confirmed vRNA rebound to $>$ 400 copies/ml irrespective of subsequent viral suppression, or discontinued the study for any reason. Patients with missing values were considered to have vRNA $\geq$ 400 copies/ml if the patient had vRNA $\geq$ 400 copies/ml on the previous or subsequent visit. The primary endpoint was also analyzed using an ITT missing/discontinuation = failure (M = F) approach where treatment failures comprised patients who had vRNA $\geq$ 400 copies/ml on one or more occasions, premature discontinuations for any reason, or who had missing data regardless of

values on previous and subsequent visits. For both ITT analyses, patients who switched background NRTIs for reasons of intolerance were not considered treatment failures. The per protocol analysis included data collected while on randomized PI and excluded data following major protocol deviations.

The safety population consisted of all patients who received at least one dose of randomized treatment. Fisher's exact tests were performed to compare AE incidence rates and generate *P*-values (*P* < 0.01 was considered significant). For fasting triglycerides, HDL, LDL total cholesterol, fasting glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lipase, a Wilcoxon rank sum test was performed to compare the median change from baseline between the treatment groups at week 48 with *P*-values < 0.01 considered significant. In addition to analysis of lipid parameters by toxicity grade, median values and changes from baseline were assessed according to National Cholesterol Education Program (NCEP) lipid categories [11].

Plasma APV concentrations were considered troughs ($C_{tr,ss}$) if the plasma sample was collected within 20–28 h after the previous dose of FPV/r. Amprenavir $C_{tr,ss}$ values for a patient were averaged and these individual values were used to produce the overall summary and $C_{tr,ss}/IC_{50}$ ratios.

Results

Baseline characteristics

A total of 649 patients were randomized and received treatment: 322 in the FPV/r QD group and 327 in the NFV BID group. Baseline characteristics were similar between groups (Table 1). The study population was ethnically diverse and included a relatively high proportion of females (27%). Median vRNA levels (4.8 log₁₀ copies/ml) were high and median CD4⁺ cell counts (170×10^6 cells/l) low. Of note, 20% of patients had CD4⁺ cell counts $< 50 \times 10^6$ cells/l whereas 22% had a history of CDC Class C (AIDS) events.

Efficacy analyses

At week 48, analysis of the proportions of patients achieving vRNA < 400 copies/ml showed the FPV/r QD group (69%) to be non-inferior to the NFV BID group (68%), with a stratified treatment difference (FPV/r QD minus NFV BID) of 1% (95% CI: -6%, 8%) (Fig. 1a, ITT RD = F). The ITT M = F analysis provided similar results: 68% of patients in the FPV/r QD group and 65% of patients in the NFV BID group had vRNA < 400 copies/ml at week 48 (stratified treatment difference 3%, 95% CI: -4%, 10%).

Table 1. Baseline characteristics (intent-to-treat population).

Characteristic	FPV/r QD n = 322	NFV BID n = 327
Sex; n (%) female	96 (30)	78 (24)
Age in years; median (range)	36 (18–69)	36 (18–68)
Ethnicity, n (%)		
White	163 (51)	178 (54)
Black	122 (38)	109 (33)
American Hispanic	24 (7)	25 (8)
vRNA log ₁₀ copies/ml; median (range)	4.8 (2.7–7.3)	4.8 (3.1–6.7)
vRNA > 100 000 copies/ml; n (%)	136 (42)	144 (44)
CD4+ cells × 10 ⁶ /l; median (range)	166 (1–813)	177 (1–1055)
CD4+ cell counts < 50 cells × 10 ⁶ /l; n (%)	62 (19)	69 (21)
CDC classification C; n (%)	67 (21)	73 (22)
Hepatitis B test ^a positive; n (%)	26 (8)	23 (7)
Hepatitis C test ^a positive; n (%)	57 (18)	60 (18)

^a Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C (anti-HCV) antibodies, respectively. FPV/r QD, FPV/rifonavir once-daily; NFV BID, nelfinavir twice-daily; CDC, Centers for Disease Control and Prevention.

Analysis of the per protocol population supported these observations. 95% of patients receiving FPV/r QD and 91% of patients receiving NFV BID had vRNA < 400 copies/ml at week 48, giving a stratified treatment difference of 4% (95% CI: 0%, 9%).

In each treatment group, similar proportions of patients had vRNA < 50 copies/ml at week 48 by ITT RD = F (Fig. 1a, FPV/r QD: 55%; NFV BID: 53%) and ITT M = F analyses (FPV/r QD: 56%; NFV BID: 52%). For the per protocol population, 78% of patients receiving FPV/r QD and 72% of NFV BID recipients had vRNA < 50 copies/ml at week 48.

More patients experienced virological failure in the NFV BID group (17%) than the FPV/r QD group (7%); a difference of 10% (95% CI: 5%, 15%) in favor of FPV/r QD; reasons for virological failure are summarized in Table 2 (ITT RD = F). Conversely, there were more non-virologic failures in the FPV/r QD group (24%) versus the NFV BID group (15%); a difference of 9% (95% CI: 3%, 15%) in favor of NFV; slightly more patients receiving FPV/r QD discontinued the study due to adverse events or withdrawn consent, lost to follow-up and other reasons.

Treatment response, assessed by vRNA strata at study entry, indicates similar efficacy between groups and across strata. However, a higher percentage of patients with entry vRNA > 500 000 copies/ml achieved vRNA < 400 copies/ml in the FPV/r QD group (73%) than in the NFV BID group (53%). In a sub-hoc analysis of response at week 48 by baseline CD4+ cell counts, FPV/r QD maintained antiviral activity amongst those who entered with CD4+ cell counts < 50 × 10⁶ cells/l and ≥ 50 × 10⁶ cells/l (73 and 68% respectively, Fig. 1b, ITT RD = F). However, in the NFV group, a reduced proportion achieved vRNA

< 400 copies/ml amongst patients with a baseline CD4+ cell count of < 50 × 10⁶ cells/l (51%) compared to those having ≥ 50 × 10⁶ cells/l (72%).

CD4+ cell counts increased for both treatment groups through 48 weeks. The median change from baseline was 203 and 207 × 10⁶ cells/l for the FPV/r QD and NFV BID groups, respectively.

Self-reported adherence

The percentage of patients reporting perfect adherence for all study drugs and at each visit assessed was 78% in the FPV/r QD group and 67% in the NFV BID group. Perfect adherence rates were consistently higher in the FPV/r QD arm at week 24 (FPV/r QD: 86%; NFV BID: 80%) and week 48 (FPV/r QD: 90%; NFV BID: 84%), although statistical significance was not assessed.

Safety analyses

Overall, 41% of patients receiving FPV/r QD and 39% receiving NFV BID experienced grade 2–4 AEs considered to be drug-related (Table 3). Diarrhea, the only AE to be statistically significantly different in incidence between treatment groups, was significantly more common in NFV recipients than FPV/r QD recipients (16 versus 9%, $P = 0.008$).

Although 41 subjects were classified as non-virological failures in Table 2 for premature discontinuation of study drug due to adverse events, the ITT RD = F efficacy analyses only considers the first reason for treatment failure. Overall, 44 subjects withdrew from the study due to an AE: 28 (9%) FPV/r recipients versus 16 (5%) NFV recipients. Adverse events that led to withdrawal from the study in at least 1% of subjects in either treatment group were: suspected ABC hypersensitivity (FPV/r QD $n = 4$; NFV BID $n = 3$), diar-

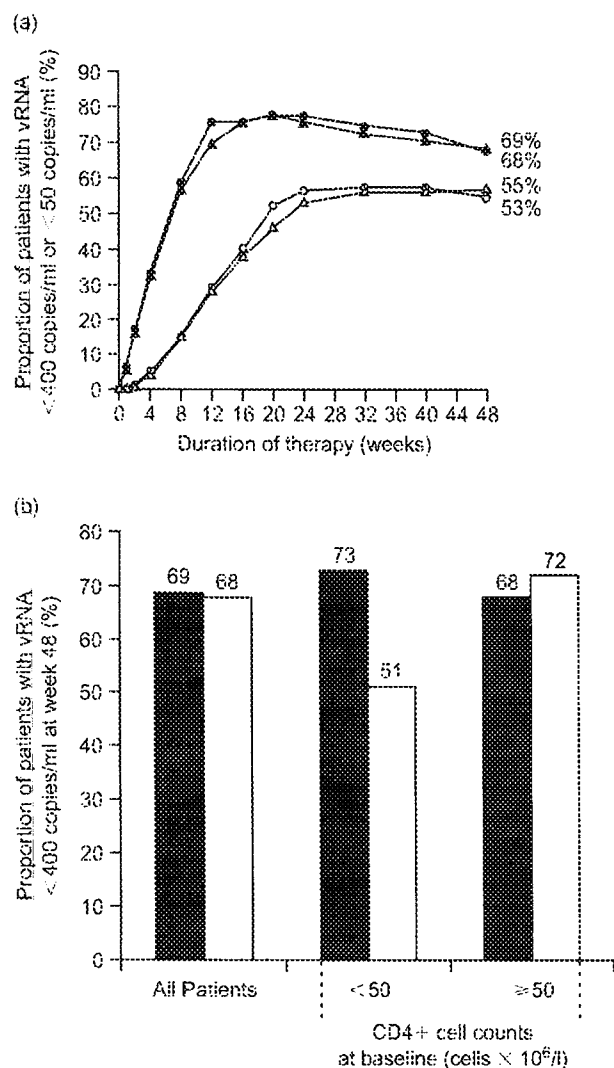


Fig. 1. (a) For the intent-to-treat (ITT) rebound/discontinuation = failure (RD = F) analysis, proportions of patients with vRNA < 400 copies/ml, FPV/r QD (filled triangles) was similar to NFV BID (filled circles) giving a stratified treatment difference (FPV/r QD minus NFV BID) of 1% (95% CI: -6%, 8%). For the ITT RD = F analysis, proportions of patients with vRNA < 50 copies/ml, FPV/r QD (open triangles) was similar to NFV BID (open circles) giving a stratified treatment difference of 2% (95% CI: -6%, 10%). (b) Proportions of patients with vRNA < 400 copies/ml at week 48 for all patients and those with baseline CD4+ cell counts < 50×10^6 cells/l or $\geq 50 \times 10^6$ cells/l for the FPV/r QD (filled area) and NFV BID groups (open area) (ITT RD = F analysis).

thea (FPV/r QD $n = 4$; NFV BID $n = 1$) and nausea (FPV/r QD $n = 4$; NFV BID $n = 1$).

The overall incidence of patients discontinuing ABC for a suspected hypersensitivity reaction was the same in both treatment groups (FPV/r QD: 8%; NFV BID:

8%). Subjects who discontinued ABC for suspected hypersensitivity reactions were allowed to continue on study with their randomized PI and substitute another NRTI for ABC.

Incidences of treatment-emergent grade 3/4 clinical chemistry and hematology abnormalities were generally low and comparable between treatment groups, although the incidence of grade 3 elevations in triglycerides and serum lipase was slightly higher in the FPV/r QD group (Table 4). Serum lipase elevations were observed in both groups and were predominantly asymptomatic. Elevations in liver enzymes were more commonly observed in patients entering the study with evidence of co-infection with hepatitis B and/or C: grade 3/4 ALT increases were observed in 3% (FPV/r QD) and 4% (NFV BID) of subjects without co-infection compared with 24% co-infected with hepatitis B and/or C in both treatment groups.

A slightly greater but insignificant median increase from baseline in triglycerides was observed in the FPV/r QD group (baseline 116 mg/dl; change + 58 mg/dl) compared to the NFV BID group (baseline 130 mg/dl; change + 41 mg/dl). Median baseline cholesterol values were: total 162 and 158 mg/dl; LDL 96 and 94 mg/dl and HDL 37 and 36 mg/dl for the FPV/r QD and NFV BID arms, respectively. Although median increases in total, LDL and HDL cholesterol were observed in both groups, there was no appreciable change in the total/HDL cholesterol ratio during the course of the study in either treatment group (median 4.3 for FPV/r QD and 4.5 for NFV BID at baseline vs 4.8 in both groups at Week 48). All median fasting cholesterol levels at Week 48 remained below (total, LDL) or above (HDL) the recommended NCEP intervention guidelines.

Pharmacokinetic analysis

The FPV/r QD regimen delivered a median plasma APV $C_{t,ss}$ of 1.43 $\mu\text{g/ml}$ ($n = 38$; range: 0.44, 3.41 $\mu\text{g/ml}$). The median IC_{50} value unadjusted for protein binding for APV against HIV-1 was determined in 34 of these patients with plasma amprenavir $C_{t,ss}$ data and was 0.0054 $\mu\text{g/ml}$ (interquartile range: 0.0036, 0.0069 $\mu\text{g/ml}$). Assuming protein binding of 90% for APV, the median protein-binding adjusted plasma APV $C_{t,ss}/\text{IC}_{50}$ value achieved in this study was 27.70 (interquartile range: 14.48, 49.06).

Discussion

The long-term therapeutic success of HAART in HIV patients is dependent on several factors including adherence [12], the patient's immunological and virological status [13–15], drug concentrations [16,17],

Table 2. Study outcomes at week 48 (intent-to-treat rebound/discontinuation = failure)^a.

Outcome	FPV/r QD n = 322 n (%)	NFV BID n = 327 n (%)
Virological success		
vRNA < 400 copies/ml	221 (69)	221 (68)
Virological failure	22 (7)	57 (17)
vRNA rebound (> 400 copies/ml) ^b	20 (6)	28 (9)
Prematurely discontinued as met protocol switch criterion ^c	1 (< 1)	23 (7)
Never achieved viral load suppression (< 400 copies/ml)	1 (< 1)	6 (2)
Non-virologic failure (premature discontinuation)	77 (24%)	47 (15%)
Adverse event	26 (8)	15 (5)
Consent withdrawn	14 (4)	10 (3)
Lost to follow-up	17 (5)	7 (2)
Protocol violation	4 (1)	4 (1)
Other	16 (5)	11 (3)
Missing data	2 (< 1)	2 (< 1)

^aThis table captures the first reason for failure in those cases where more than one type of failure has occurred. ^bvRNA > 400 copies/ml at two consecutive time-points having been previously below 400 copies/ml. ^cvRNA > 1000 copies/ml at two consecutive time-points post week 12. FPV/r QD, FPV/ritonavir once-daily; NFV BID, nelfinavir twice-daily.

regimen tolerability, and risk of emergence of drug resistant virus in treatment failures [18,19].

Fosamprenavir/ritonavir QD is a promising treatment option facilitated by the pharmacoenhancement of APV exposure with low dose RTV [6,7]. This is the first randomized, 48-week comparison of the efficacy and tolerability of FPV/r QD with NFV BID in ART-naïve patients. The NFV comparator was chosen on the basis of its well-characterized safety profile [20] and its position as the leading PI at the time of study design and initiation.

Patients treated in SOLO are consistent with the population of patients currently initiating HAART in clinical practice [1,2,21, J. Gill, personal comm.]. However, the low median baseline CD4+ cell counts observed in both arms of SOLO (166 and 177×10^6 cells/l) illustrate that this population was more immune-compromised than the populations in which atazanavir (ATV) and lopinavir/ritonavir were recently evaluated in comparison with efavirenz and NFV, respectively [22,23]. In these studies, median baseline CD4+ cell counts were 286 and 232×10^6 cells/l, respectively.

The virologic response achieved with FPV/r QD in this population was potent as well as durable as indicated by the proportions of patients achieving < 400 copies/ml and < 50 copies/ml, as well as the maintenance of this response through week 48. Similar efficacy was observed by ITT analysis between the FPV/r QD and NFV arms since the higher number of NFV virologic failures was counterbalanced by more non-virologic failures in subjects receiving FPV/r QD.

The antiviral response to FPV/r QD was maintained in patients entering the study with a severely compro-

mised immune system (< 50×10^6 CD4+ cells/l) or with high viral loads (> 500 000 copies/ml). This was in contrast to the NFV recipients, in which subjects with these same baseline characteristics did not respond as well as subjects with higher CD4+ cell counts or lower viral loads. A similar finding was also noted in the ACTG 384 trial where among subjects who had high viral loads at screening (vRNA > 5.0 log₁₀), those receiving efavirenz, zidovudine and lamivudine had a better response than those receiving nelfinavir plus the same background drugs [24].

The overall response to FPV/r QD compares favorably with those observed for other PI regimen. In A1424034, 70% of patients receiving 400 mg of atazanavir QD achieved vRNA levels of < 400 copies/ml and 32% achieved < 50 copies/ml after 48 weeks [22], although the accuracy of these study results has been questioned due to potential confounders relating to sample collection methods. When comparing the responses seen in SOLO with those observed in M98-863 in which 75 and 67% lopinavir/ritonavir recipients achieved < 400 copies/ml and < 50 copies/ml after 48 weeks respectively [23], it should be noted that this population had a higher median CD4+ cell count at entry (232×10^6 cells/l) than that observed in SOLO (170×10^6 cells/l).

Although cross study comparisons are not ideal, the range of efficacy reported in studies evaluating NFV plus dual NRTIs in therapy-naïve individuals is notable. In contrast with SOLO where 68% of NFV recipients achieved vRNA < 400 copies/ml at 48 weeks, the proportion achieving this response was 55% in NFV511 (750 mg three times daily (TID)), 61 and 58% in NFV542 (1250 mg BID and 750 mg TID, respectively) [20], 63% in M98-863 (750 mg TID) [23] and 56% in A1424-007 (750 mg TID) at 48 weeks [25].

Table 3. Summary of most frequently reported grade 2–4 drug-related adverse events.

Adverse event	FPV/r QD n = 322 n (%)	NFV BID n = 327 n (%)	Fisher exact test P-value
Diarrhea	28 (9)	51 (16)	0.008*
Drug hypersensitivity to abacavir	24 (7)	19 (6)	0.433
Nausea	21 (7)	16 (5)	0.401
Vomiting	19 (6)	12 (4)	0.201
Fatigue	11 (3)	6 (2)	0.229
Headache	8 (2)	9 (3)	1.000
Abdominal pain	8 (2)	6 (2)	0.601
Rash (NOS)	5 (2)	5 (2)	1.000

*Difference statistically significant. FPV/r QD, FPV/ritonavir once-daily; NFV BID, nelfinavir twice-daily; NOS, not otherwise specified.

Table 4. Summary of selected grade 3–4 laboratory abnormalities.

Laboratory parameter	FPV/r QD n (%)		NFV BID n (%)	
	Grade 3	Grade 4	Grade 3	Grade 4
Clinical chemistry				
Alanine transaminase	16 (5)	9 (3)	14 (4)	12 (4)
Aspartate transaminase	14 (4)	5 (2)	15 (5)	9 (3)
Serum lipase	16 (5)	4 (1)	9 (3)	3 (< 1)
Hypertriglyceridemia	14 (5)	2 (< 1)	6 (2)	1 (< 1)
Hyperbilirubinemia	3 (< 1)	0	1 (< 1)	1 (< 1)
Hypercholesterolemia	1 (< 1)	0	0	0
Hyperglycemia	1 (< 1)	0	0	1 (< 1)
Hypoglycemia	1 (< 1)	0	0	0
Hematology				
Neutropenia (< 750 × 10 ⁶ cells/4)	8 (3)	3 (< 1)	11 (3)	3 (< 1)
Thrombocytopenia	3 (< 1)	2 (< 1)	2 (< 1)	0
Leucopenia	2 (< 1)	1 (< 1)	2 (< 1)	0

FPV/r QD, FPV/ritonavir once-daily; NFV BID, nelfinavir twice-daily.

Clinically relevant differences between the treatment groups were observed with respect to the risk of resistance in the treatment failures [26,27] impacting future PI and NRTI treatment options. Consistent with observations from other studies of boosted PI regimen [26], no primary or secondary protease (PRO) mutations were selected in patients experiencing therapy failure on FPV/r QD compared to 50% of NFV recipients ($P < 0.001$) [27,28]. Equally important, significantly fewer patients experiencing therapy failure in the FPV/RTV QD group (13%) developed mutations associated with resistance to NRTIs compared to patients in the NFV group (57%, $P < 0.001$). Detailed information on the resistance profile of FPV/r are reported elsewhere [29].

As observed with other regimens [12], the higher ($\geq 80\%$) rates of perfect self-reported adherence in the FPV/r QD group at weeks 24 and 48 were consistent with the lower rate of virologic failure, significantly less grade 2–4 drug-related diarrhea, absence of food restrictions, lower pill count, and QD dosing of FPV/r compared with NFV BID.

FPV/r QD was well tolerated, with most adverse

events being mild or moderate in intensity, and only a small proportion of patients withdrew from the study due to AEs. The incidence of grade 2–4 drug-related diarrhea in the FPV/r QD group was significantly less than that observed in the NFV group, and lower than that recently reported with other boosted PIs [23].

It is not clear why there were slightly more discontinuations due to diarrhea in the FPV/r QD arm than the NFV arm. One explanation may be that physicians and patients were more aware of management of diarrhea as a side effect of NFV and thus drug discontinuation was seldom warranted whereas the lesser known safety profile of FPV/r led to more cautious management of diarrhea events.

Rashes not considered by investigators to be part of ABC hypersensitivity reaction were reported with low incidence in both arms. The incidence of suspected ABC hypersensitivity reaction reported in this study (8% in each group) is higher than the approximate 5% previously reported in clinical trials [30]. This supports the fact that there is a heightened awareness to ABC hypersensitivity reaction and treating physicians are

adopting an appropriately conservative approach to diagnosis [31].

The low incidences of grade 3/4 laboratory abnormalities compare favorably with incidences reported with other boosted PIs [23]. In this population, generally comparable fasting lipid profiles were observed in both the FPV/r QD and NFV treatment groups. The slight elevation of triglyceride levels in the FPV/r QD group may possibly be attributed to use of ritonavir. Further lipid analyses have been presented elsewhere [32].

In conclusion, the results of this study in ART-naïve, HIV-infected patients with high viral load and low CD4+ cell counts, demonstrate the potency, durability, and tolerability of the FPV/r QD regimen. No FPV/r QD recipients developed PRO mutations and significantly fewer recipients developed mutations in RT throughout the 48 weeks of treatment, hence, preserving future treatment options. Furthermore, the combination of fosamprenavir and ritonavir provides the benefits of no food restrictions, a low daily pill burden, once-daily dosing and a favorable lipid profile. Overall, these data support the use of FPV/r QD in a first line regimen or early in the treatment continuum.

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Steady-State Pharmacokinetic (PK) Interaction Study of Atazanavir (ATV) with Ritonavir (RTV) in Healthy Subjects.

AGARWALA S, RUSSO R, MUMMANENI V, RANDALL D, GERALDES M, O'MARA E.

Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother. 2002 Sep 27-30; 42: abstract no. H-1716.

Bristol-Myers Squibb Company, Princeton, NJ

BACKGROUND: ATV is a new protease inhibitor with potent anti-HIV activity. ATV exposure has been substantially increased when combined with RTV. **Objective:** To assess the PK interaction between ATV (CYP3A substrate/inhibitor) and RTV (CYP 3A inhibitor) at specific doses of 300 and 100 mg, respectively. **METHODS:** Thirty healthy subjects received open label drugs with a light meal as follows: 300 mg ATV QD on Days 1-10 and then 300 mg ATV and 100 mg RTV QD on Days 11-20. Serial blood samples were collected for PK on Days 10, 15, and 20. **RESULTS:** Geometric means and ratios of geometric means (90% confidence intervals (CI)) for noncompartmental parameters are listed in the table. Steady state C_{max} and AUC(TAU) of ATV after coadministration with RTV were 1.86 and 3.38 fold higher, respectively, compared to ATV alone. Mean RTV exposure was similar to published data from a previous RTV/ATV combination study. **CONCLUSIONS:** The combination of RTV and ATV in healthy subjects: substantially increased ATV exposure, was well tolerated and did not affect RTV PK. The ATV/RTV regimen is under evaluation in select HIV+ subjects in Phase III.

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(21) International Application Number: PCT/US99/21469 (22) International Filing Date: 29 October 1999 (29.10.99) (30) Priority Data: 60/106,963 4 November 1998 (04.11.98) US (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FERRY, James, J. [US/US]; 8343 Phoebe Street, Kalamazoo, MI 49009 (US). BALDWIN, John, R. [US/US]; 7823 Ashton Woods Drive, Portage, MI 49024 (US). BORIN, Marie, T. [US/US]; 4615 Carver Drive, Kalamazoo, MI 49009 (US). (74) Agent: YANG, Lucy, X.; Intellectual Property Legal Services, Pharmacia & Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD FOR IMPROVING THE PHARMACOKINETICS OF TIPRANAVIR (57) Abstract The present invention relates to a novel method for improving the pharmacokinetics of tipranavir, comprising administering to a human in need of such treatment a combination of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.		

lowest dose of ritonavir tested (100 mg) and assuming a target trough tipranavir concentration equivalent to that observed for the highest dose combination tested (tipranavir 1350 mg BID/ritonavir 500 mg BID) was desired, then a tipranavir dose of 6750 mg (5 x 1350 mg) would be expected to be required.

5 Thus, these combined results of clinical studies used to investigate the pharmacokinetic drug-drug interaction involving tipranavir and ritonavir support the following limits of dosing: administration BID of a ritonavir dose of between about 30 mg to about 1000 mg, and of a tipranavir dose of between about 200 mg to about 6750 mg. Similarly, the tipranavir/ritonavir combination may be administered once a day as follows: a
10 ritonavir dose of between about 30 mg to about 2000 mg, and of a tipranavir dose of between about 200mg to about 13500 mg.

 Those skilled in the art would know how to formulate the compounds of this invention into appropriate pharmaceutical dosage forms. Examples of the dosage forms include oral formulations, such as tablets or capsules, or parenteral formulations, such as
15 sterile solutions.

 Either solid or fluid dosage forms can be prepared for oral administration. Solid compositions are prepared by mixing the compounds of this invention with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methyl cellulose, or functionally similar
20 pharmaceutical diluents and carriers. Capsules are prepared by mixing the compounds of this invention with an inert pharmaceutical diluent and placing the mixture into an appropriately sized hard gelatin capsule. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compounds of this invention with an acceptable inert oil such as vegetable oil or light liquid petrolatum. Syrups are prepared by dissolving the compounds
25 of this invention in an aqueous vehicle and adding sugar, aromatic flavoring agents and preservatives. Elixirs are prepared using a hydroalcoholic vehicle such as ethanol, suitable sweeteners such as sugar or saccharin and an aromatic flavoring agent. Suspensions are prepared with an aqueous vehicle and a suspending agent such as acacia, tragacanth, or methyl cellulose.

30 In a preferred embodiment, the dosage form used is a self-emulsifying drug delivery system (SEDDS) microemulsion formulation. Details about SEDDS may be found in PCT Patent Applications, International Publication Nos. WO 99/06044 and WO 99/06043, both published on 11 February 1999. The SEDDS formulation allows tipranavir in an

METHOD FOR IMPROVING THE PHARMACOKINETICS OF TIPRANAVIR

Field of the Invention

The present invention relates to a novel method for improving the pharmacokinetics of tipranavir, comprising administering to a human in need of such treatment a combination
5 of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.

Background of the Invention

Since the first description of the malady in the early part of this decade, acquired
10 immunodeficiency disease syndrome (AIDS) and its devastating consequences have been subjects of continuous and intense coverage in both the lay and scientific press. The literature on the disease and the virus is already so vast as to defy thorough citation.

Human immunodeficiency virus (HIV) has long been recognized as the causative agent in AIDS, although a minority opinion to the contrary has been expressed (e.g., P.
15 Duesberg, Proc. Natl. Acad. Sci., USA, 86:755-764 (1989)). Sequence analysis of the complete genomes from several infective and non-infective HIV-isolates has shed considerable light on the make-up of the virus and the types of molecules that are essential for its replication and maturation to an infective species. The HIV protease is essential for the processing of the viral gag and gag-pol polypeptides into mature virion proteins. L.
20 Ratner, et al., Nature, 313:277-284 (1985); L.H. Pearl and W.R. Taylor, Nature, 329:351 (1987). HIV exhibits the same gag/pol/env organization seen in other retroviruses. L. Ratner, et al., above; S. Wain-Hobson, et al., Cell, 40:9-17 (1985); R. Sanchez-Pescador, et al., Science, 227:484-492 (1985); and M.A. Muesing, et al., Nature, 313: 450-458 (1985).

Reverse transcriptase (RT) is an enzyme unique to retroviruses that catalyzes the
25 conversion of viral RNA into double stranded DNA. Blockage at any point during the transcription process by an aberrant deoxynucleoside triphosphate incapable of elongation, such as AZT (zidovudine), should have dramatic consequences relative to viral replication. Much work on the RT target is in progress based, in large measure, upon the fact that nucleosides like AZT are easily delivered to cells. However, the inefficiency of phosphorylation steps to
30 the triphosphate, and the lack of specificity and consequent toxicity, constitute major drawbacks to use of AZT and similar nucleosides having a blocked, or missing, 3'hydroxyl group.

Pharmacokinetic parameters such as AUC, C_{max}, t_{max}, oral clearance, and terminal half-life were determined using standard noncompartmental techniques. Treatment effects on pharmacokinetic parameters were assessed using Wilcoxon's Signed Rank Test.

Results:

5 *Effects of Ritonavir on Tipranavir:*

Mean (SD) plasma tipranavir concentrations following administration of tipranavir alone and in combination with ritonavir are shown in Figure 1. The pharmacokinetic estimates derived from individual subject data are provided in Table 2. The median tipranavir C_{max} value increased approximately 5.6-fold in the presence of ritonavir, whereas
10 median tipranavir C_{min} values increased 45-fold. On average, mean steady-state plasma tipranavir concentrations (C_{ss}) increased 12-fold following dosing with ritonavir. Of note, the increase in tipranavir concentrations was not accompanied by a prolongation of the elimination half-life ($t_{1/2}$). This suggests the possibility that the effect of ritonavir to increase plasma tipranavir concentrations involves, in part, a pre-systemic mechanism. Speculatively,
15 this could be due to an inhibitory effect of ritonavir on P-glycoprotein resulting in enhanced tipranavir absorption, and/or ritonavir inhibition of gut wall tipranavir metabolism.

Effects of Tipranavir on Ritonavir:

Mean (SD) plasma ritonavir concentrations following administration of ritonavir alone and in combination with tipranavir are shown in Figure 2. The pharmacokinetic
20 estimates derived from individual subject data are provided in Table 3. Although only 500 mg twice daily doses of ritonavir were administered in this study in healthy volunteers, the plasma ritonavir concentrations observed following dosing with ritonavir alone were slightly higher than those reported in HIV-infected patients receiving ritonavir 600 mg doses twice daily. This result supports the clinical relevance of ritonavir exposure following the doses
25 employed in this study.

The combination of tipranavir and ritonavir dosing resulted in an approximate 5-fold decrease in mean steady-state ritonavir concentrations compared with ritonavir administered alone. The median ritonavir C_{max} value decreased 3.8-fold following coadministration with tipranavir. In association with a shorter apparent half-life of elimination, the median
30 ritonavir C_{min} concentration was more than 10-fold lower when ritonavir was dosed with tipranavir compared with ritonavir dosed alone. For comparative purposes, it is interesting

Plasma samples were assayed for ritonavir (A-84538) concentrations using a validated, sensitive and specific isocratic HPLC-UV method. Ritonavir and the internal standard (IS) were extracted from human plasma by liquid-liquid extraction with an ethyl acetate mixture. The retention times of the primary analytes were ~7.0 minutes (ritonavir),
5 and ~10.0 minutes (IS). Mean recoveries for ritonavir and the IS were 101% and 91.4%, respectively.

Pharmacokinetic and Statistical Methods:

Pharmacokinetic parameters such as AUC, C_{max}, t_{max}, oral clearance, and terminal half-life were determined using standard noncompartmental techniques.

10

Results:

Effects of Tipranavir on Ritonavir:

Median plasma ritonavir concentrations following administration of ritonavir 500 mg BID and either tipranavir 600 mg BID or 900 mg BID are depicted in Figure 3. The
15 pharmacokinetics of ritonavir derived from individual subject data are summarized in Table 4. For comparative purposes, the pharmacokinetics of ritonavir when administered alone are included in this table. The combination of ritonavir and tipranavir in this study resulted in an approximate 4-fold decrease in steady-state plasma ritonavir concentrations compared with ritonavir administered alone. Median ritonavir C_{max} values were more than 2-fold lower,
20 and C_{min} values more than 10-fold lower, when ritonavir was dosed with tipranavir. The decrease in plasma ritonavir concentrations, and shortening of the elimination half-life, is consistent with metabolic induction attributable to tipranavir.

Effects of Ritonavir on Tipranavir:

Median tipranavir concentrations following tipranavir 600 mg BID administered
25 alone and in combination with ritonavir 100 mg BID or ritonavir 500 mg BID are depicted in Figure 4. Pharmacokinetic parameters of tipranavir derived from individual subject data are provided in Table 5. The quantitative effect of ritonavir on plasma concentrations of tipranavir was dose-related. Median AUC values of tipranavir increased approximately 9-fold following concomitant dosing of ritonavir 100 mg BID and approximately 14-fold for
30 dosing with ritonavir 500 mg BID. Median trough tipranavir concentrations increased

approximately 9-fold following dosing with ritonavir 100 mg BID, and approximately 40-fold for ritonavir 500 mg BID. Compared with tipranavir 600 mg BID administered alone, median C_{max} values increased 5-fold and 7-fold, respectively, following concomitant dosing of ritonavir 100 mg and 500 mg BID, respectively. As in a previous study which assessed the pharmacokinetic drug-drug interaction of tipranavir 1350 mg BID and ritonavir 500 mg BID, no prolongation of the apparent terminal half-life of tipranavir was observed following concomitant dosing.

Median tipranavir concentrations following tipranavir 900 mg BID administered alone and in combination with ritonavir 100 mg BID and ritonavir 500 mg BID are depicted in Figure 5. Pharmacokinetic parameters of tipranavir derived from individual subject data are provided in Table 5. As observed for the tipranavir 600 mg BID data, the quantitative effect of ritonavir on plasma concentrations of tipranavir was dose-related. Median AUC values of tipranavir increased approximately 8-fold following concomitant dosing of ritonavir 100 mg BID and approximately 20-fold for dosing with ritonavir 500 mg BID. Median trough tipranavir concentrations increased approximately 7-fold following dosing with ritonavir 100 mg BID, and approximately 45-fold for ritonavir 500 mg BID. Compared with tipranavir 900 mg BID administered alone, median C_{max} values increased 5-fold and 10-fold, respectively, following concomitant dosing of ritonavir 100 mg and 500 mg BID, respectively. No prolongation of the apparent terminal half-life of tipranavir was observed following concomitant dosing.

Discussion:

A significant pharmacokinetic drug-drug interaction affecting both ritonavir and tipranavir was shown in a previous study in which tipranavir 1350 mg BID and ritonavir 500 mg BID were dosed concomitantly. The pharmacokinetic aspects of this drug interaction were further explored in this study, utilizing a combination of ritonavir and tipranavir doses. Ritonavir concentrations significantly and substantially declined following co-administration of the lowest tipranavir dose tested (600 mg BID). This finding is consistent with the results of previous studies showing that tipranavir induces its own metabolism over a wide dosage range.

Further, the effect of tipranavir to reduce plasma ritonavir concentrations was similar for doses of tipranavir ranging from 600 to 1350 mg BID, suggesting that enzyme induction of tipranavir, while substantial, appears to reach a plateau effect which occurs at or below

Pharmacokinetic and Statistical Methods:

Pharmacokinetic parameters such as AUC, C_{max}, t_{max}, oral clearance, and terminal half-life, were determined using standard noncompartmental techniques.

5 **Results:***Effects of Ritonavir on Tipranavir:*

Mean (SD) plasma tipranavir concentrations following administration of tipranavir
10 alone (1250 mg BID) and in combination with ritonavir (200 mg BID) are shown in Figure
7. The pharmacokinetic estimates derived from individual subject data are provided in Table
9. Median AUC values of tipranavir were approximately 11-fold higher and median
tipranavir C_{min} values were about 75-fold higher in subjects receiving tipranavir with
ritonavir oral solution than in subjects receiving tipranavir alone. Median C_{max} values
15 increased approximately 5-fold following concomitant dosing with ritonavir. As observed in
previous studies in which tipranavir HFC and ritonavir capsules were coadministered, the
apparent terminal half-life of tipranavir was not significantly affected by ritonavir.

Median (range) ritonavir pharmacokinetic parameters after administration of
ritonavir oral solution 200 mg BID with tipranavir 1250 mg BID are given in Table 10. The
20 median ritonavir AUC, C_{min}, and C_{max} were 12-fold, 54-fold, and 10-fold lower,
respectively, for this combination compared to a 500-mg twice-daily dose of ritonavir
administered alone. The apparent elimination half-life of ritonavir was also decreased
relative to ritonavir given alone, which is consistent with metabolic induction by tipranavir.

25 **Discussion:**

The significant effect of ritonavir on the pharmacokinetics of tipranavir previously
observed with different forms of the two drugs (tipranavir disodium salt HFC and ritonavir
capsule) was also demonstrated with the combination of tipranavir SEDDS SEC and the
ritonavir oral solution. The 200 mg dose of ritonavir, which is three times lower than the
30 dose used in the treatment of HIV-1 infection, increased tipranavir steady-state
concentrations by more than 10-fold compared with the same dose (1250 mg BID) of
tipranavir given alone. This effect is observed despite the fact that ritonavir concentrations
in the presence of tipranavir are greatly decreased.

Claims

We claim:

1. A method for improving the pharmacokinetics of tipranavir, comprising
 5 administering to a human in need of such treatment a combination of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein said therapeutically effective amount of
 10 tipranavir is between about 200 mg and about 6750 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 mg and about 1000 mg of ritonavir.
3. The method of claim 1, wherein said therapeutically effective amount of
 15 tipranavir is between about 200 and about 900 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 500 mg of ritonavir.
4. The method of claim 1, wherein said therapeutically effective amount of
 20 tipranavir is between about 200 and about 900 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 300 mg of ritonavir.
5. The method of claim 1, wherein said therapeutically effective amount of
 tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 500 mg of ritonavir.
 25
6. The method of claim 1, wherein said therapeutically effective amount of
 tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 300 mg of ritonavir.
7. The method of claim 1, wherein said therapeutically effective amount of
 30 tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 100 mg of ritonavir.

8. A method for increasing human blood levels of tipranavir, comprising administering to a human in need of such treatment a combination of a therapeutically effective amount of tipranavir or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of ritonavir or a pharmaceutically acceptable salt thereof.

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9. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 mg and about 6750 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 mg and about 1000 mg of ritonavir.

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10. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 and about 900 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 500 mg of ritonavir.

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11. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 and about 900 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 300 mg of ritonavir.

12. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 500 mg of ritonavir.

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13. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 300 mg of ritonavir.

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14. The method of claim 8, wherein said therapeutically effective amount of tipranavir is between about 200 and about 600 mg of tipranavir, and said therapeutically effective amount of ritonavir is between about 30 and about 100 mg of ritonavir.

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(21) International Application Number: PCT/US00/07342 (22) International Filing Date: 16 March 2000 (16.03.00) (30) Priority Data: 09/287,043 6 April 1999 (06.04.99) US (71) Applicant: LIPOCINE, INC. [US/US]; Suite 314, 800 North 350 West, Salt Lake City, UT 84103 (US). (72) Inventors: CHEN, Feng-Jing; 201 East South Temple #420, Salt Lake City, UT 84111 (US). PATEL, Manesh, V.; 1515 South Preston, Salt Lake City, UT 84108 (US). (74) Agent: REED, Dianne, E.; Reed & Associates, 3282 Alpine Road, Portola Valley, CA 94028 (US).			(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: COMPOSITIONS AND METHODS FOR IMPROVED DELIVERY OF IONIZABLE HYDROPHOBIC THERAPEUTIC AGENTS			
(57) Abstract The present invention is directed to a pharmaceutical composition including a hydrophobic therapeutic agent having at least one ionizable functional group, and a carrier. The carrier includes an ionizing agent capable of ionizing the functional group, a surfactant, and optionally solubilizers, triglycerides, and neutralizing agents. The invention further relates to a method of preparing such compositions by providing a composition of an ionizable hydrophobic therapeutic agent, an ionizing agent, and a surfactant, and neutralizing a portion of the ionizing agent with a neutralizing agent. The compositions of the invention are particularly suitable for use in oral dosage forms.			

1 bioavailable form. Such difficulties necessarily result in increased costs, and
compromised patient safety and therapeutic effectiveness.

Thus, there is a need for versatile and effective pharmaceutical compositions that
overcome these deficiencies in the prior art.

SUMMARY OF THE INVENTION

5 The present invention provides pharmaceutical compositions and methods for
improved delivery of ionizable hydrophobic therapeutic agents.

In one embodiment, the invention is directed to a pharmaceutical composition
including an ionizable hydrophobic therapeutic agent and a carrier. The carrier includes
10 an ionizing agent to ionize the therapeutic agent, and a surfactant. Optionally, the carrier
also includes solubilizers, triglycerides and neutralizing agents.

In another embodiment, the invention is directed to a pharmaceutical composition
including a hydrophobic therapeutic agent having at least one ionizable functional group,
and a carrier. The carrier includes an ionizing agent capable of ionizing the functional
15 group, a surfactant, and a triglyceride.

In another embodiment, the invention is directed to a pharmaceutical composition
including a hydrophobic therapeutic agent having at least one ionizable functional group
and a carrier, wherein the carrier includes an ionizing agent capable of ionizing the
ionizable functional group and present in a pre-reaction amount of greater than about 1.5
20 mole equivalents per mole of ionizable functional group, and a surfactant. In a further
aspect of this embodiment, the composition further includes a neutralizing agent capable
of neutralizing a portion of the ionizing agent.

In another embodiment, the invention is directed to a pharmaceutical composition
including a hydrophobic therapeutic agent having at least one ionizable functional group,
and a carrier, wherein the carrier includes an ionizing agent capable of ionizing the
25 ionizable functional group, a surfactant, and a solubilizer present in an amount of greater
than about 10% by weight, based on the total weight of the composition. In this
embodiment, the surfactant includes at least one compound from the group consisting of
alkylglucosides; alkylmaltosides; alkylthioglucosides; lauryl macrogolglycerides;
polyoxyethylene alkyl ethers; fatty acids; lower alcohol fatty acid esters;
30 polyoxyethylene alkylphenols; polyethylene glycol fatty acids esters; polypropylene
glycol fatty acid esters; glycerol fatty acid esters; acetylated glycerol fatty acid esters;
polyethylene glycol glycerol fatty acid esters; polyglyceryl fatty acid esters;

- 1 1. A pharmaceutical composition comprising:
 - (a) a hydrophobic therapeutic agent having at least one ionizable functional group; and
 - (b) a carrier, said carrier comprising:
 - 5 (i) an ionizing agent capable of ionizing the ionizable functional group;
 - (ii) a surfactant; and
 - (iii) a triglyceride.
- 10 2. The pharmaceutical composition of claim 1, wherein the ionizable functional group is an acidic functional group, and the ionizing agent is a pharmaceutically acceptable base.
- 15 3. The pharmaceutical composition of claim 2, wherein the acidic functional group is selected from the group consisting of carboxylic acids, imidazolidinediones, thiazolidinediones, pyrimidinetriones, hydroxyheteroaromatics, phenols, phosphoric acids, sulfuric acids, sulfonic acids, sulfonamides, aminosulfones, sulfonylureas, tetrazoles and thiols.
- 20 4. The pharmaceutical composition of claim 2, wherein the hydrophobic therapeutic agent is selected from the group consisting of acetazolamide, acetohexamide, acrivastine, alatrofloxacin, albuterol, alclofenac, aloxiprin, alprostadil, amodiaquine, amphotericin, amylobarbitol, aspirin, atorvastatin, atovaquone, baclofen, barbitol, benezepril, bezafibrate, bromfenac, bumetanide, butobarbitol, candesartan, capsacin, captopril, cefazolin, celecoxib, cephradine, cephalixin, cerivistatin, cetirizine, chlorambucil, chlorothiazide, chlorpropamide, chlorthalidone, cinoxacin, ciprofloxacin, clinofibrate, cloxacillin, cromoglicate, cromolyn, dantrolene, dichlorophen, diclofenac, dicloxacillin, dicumarol, diflunisal, dimenhydrinate, divalproen, docusate, dronabinol, enoximone, enalapril, enoxacin, enrofloxacin, epalrestate, eposartan, essential fatty acids, estramustine, ethacrynic acid, ethotoin, etodolac, etoposide, fenbufen, fenoprofen, fexofenadine, fluconazole, flurbiprofen, fluvastatin, fosinopril, fosphenytoin, fumagillin, furosemide, gabapentin, gemfibrozil, gliclazide, glipizide, glybenclamide, glyburide, glymepiride, grepafloxacin, ibufenac, ibuprofen, imipenem, indomethacin, irbesartan, isotretinoin, ketoprofen, ketorolac, lamotrigine, levofloxacin, levothyroxine, lisinopril, lomefloxacin, losartan, lovastatin, meclofenamic acid, mefenamic acid, mesalamine, methotrexate, metolazone, montelukast, nalidixic acid, naproxen, natamycin, nimesulide,
- 30

1 nitrofurantoin, non-essential fatty acids, norfloxacin, nystatin, ofloxacin, oxacillin,
oxaprozin, oxyphenbutazone, penicillins, pentobarbital, perfloxacin, phenobarbital,
phenytoin, pioglitazone, piroxicam, pramipexol, pranlukast, pravastatin, probenecid,
2 probucol, propofol, propylthiouracil, quinapril, rabeprazole, repaglinide, rifampin,
5 rifapentine, sparfloxacin, sulfabenzamide, sulfacetamide, sulfadiazine, sulfadoxine,
sulfamerazine, sulfamethoxazole, sulfafurazole, sulfapyridine, sulfasalazine, sulindac,
sulphasalazine, sulthiame, telmisartan, teniposide, terbutaline, tetrahydrocannabinol,
tirofiban, tolazamide, tolbutamide, tolcapone, tolmetin, tretinoin, troglitazone,
trovafloxacin, undecenoic acid, ursodeoxycholic acid, valproic acid, valsartan,
10 vancomycin, verteporfin, vigabatrin, vitamin K-S (II), zafirlukast, and pharmaceutically
acceptable salts thereof.

5. The pharmaceutical composition of claim 4, wherein the hydrophobic
therapeutic agent is selected from the group consisting of acetohexamide, acrivastine,
alatrofloxacin, albuterol, alclofenac, amodiaquine, amphotericin, aspirin, atorvastatin,
15 atovaquone, baclofen, benezepril, bezafibrate, bromfenac, butobarbital, candesartan,
capsacin, captopril, celecoxib, cerivistatin, cetirizine, chlorambucil, chlorpropamide,
chlorthalidone, clonofibrate, cinoxacin, ciprofloxacin, clonofibrate, cloxacillin,
cromoglicate, cromolyn, dantrolene, diclofenac, dicumarol, divalproen, docusate,
dronabinol, enalapril, enoxacin, epalrestate, eposartan, etodolac, etoposide, fenbufen,
20 fenoprofen, fexofenadine, fluconazole, flurbiprofen, fluvastatin, fosphenytoin,
fumagillin, gabapentin, gemfibrozil, gliclazide, glipizide, glyburide, glymepiride,
grepafloxacin, ibufenac, ibuprofen, imipenem, indomethacin, irbesartan, isotretinoin,
ketoprofen, ketorolac, lamotrigine, levofloxacin, levothyroxine, lisinopril, lomefloxacin,
losartan, lovastatin, mesalamine, methotrexate, montelukast, naproxen, nimesulide, non-
25 essential fatty acids, norfloxacin, ofloxacin, oxaprozin, phenytoin, pioglitazone,
piroxicam, pramipexol, pravastatin, probucol, propofol, rabeprazole, repaglinide,
rifampin, rifapentine, sparfloxacin, sulfadiazine, sulfamethoxazole, sulfasalazine,
sulindac, sulphasalazine, telmisartan, teniposide, terbutaline, tetrahydrocannabinol,
tirofiban, tolazamide, tolbutamide, tolcapone, tolmetin, tretinoin, troglitazone,
30 trovafloxacin, undecenoic acid, valproic acid, valsartan, vancomycin, verteporfin,
vigabatrin, vitamin K-S (II), zafirlukast, and pharmaceutically acceptable salts thereof.

6. The pharmaceutical composition of claim 5, wherein the hydrophobic
therapeutic agent is selected from the group consisting of acrivastine, alatrofloxacin,

1 albuterol, alclofenac, aspirin, atorvastatin, atovaquone, baclofen, benezepril, bezafibrate,
bromfenac, butobarbital, celecoxib, cerivistatin, cetirizine, chlorpropamide, ciprofloxacin,
cromoglicate, cromolyn, dantrolene, diclofenac, dicumarol, divalproen, dronabinol,
enoxacin, epalrestate, etodolac, etoposide, fenoprofen, fexofenadine, fluconazole,
5 flurbiprofen, fluvastatin, fosphenytoin, gemfibrozil, glipizide, glyburide, glymeperide,
grepafloxacin, ibufenac, ibuprofen, isotretinoin, ketoprofen, ketorolac, lamotrigine,
levofloxacin, levothyroxine, lomefloxacin, lovastatin, methotrexate, montelukast,
naproxen, nimesulide, non-essential fatty acids, norfloxacin, ofloxacin, oxaprozin,
phenytoin, pioglitazone, piroxicam, pravastatin, probucol, rabeprazole, repaglinide,
10 rifampin, rifapentine, sulfamethoxazole, sulfasalazine, teniposide, tetrahydrocannabinol,
tolcapone, tolmetin, tretinoin, troglitazone, trovafloxacin, valproic acid, vancomycin,
vitamin K-S (II), zafirlukast, and pharmaceutically acceptable salts thereof.

7. The pharmaceutical composition of claim 6, wherein the hydrophobic
therapeutic agent is selected from the group consisting of alclofenac, aspirin,
15 atorvastatin, atovaquone, benezepril, bromfenac, celecoxib, cromoglicate, cromolyn,
diclofenac, dronabinol, epalrestate, etodolac, fexofenadine, flurbiprofen, glymeperide,
ibufenac, ibuprofen, isotretinoin, ketorolac, levothyroxine, naproxen, non-essential fatty
acids, oxaprozin, phenytoin, pioglitazone, rabeprazole, repaglinide, teniposide,
tetrahydrocannabinol, tolmetin, tretinoin, troglitazone, trovafloxacin, vitamin K-S (II),
20 and pharmaceutically acceptable salts thereof.

8. The pharmaceutical composition of claim 2, wherein the base is an amino
acid, an amino acid ester, ammonium hydroxide, potassium hydroxide, sodium
hydroxide, sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate,
magnesium hydroxide, magnesium aluminum silicate, synthetic aluminum silicate,
25 synthetic hydrotalcite, magnesium aluminum hydroxide, diisopropylethylamine,
ethanolamine, ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, or a
salt of a pharmaceutically acceptable cation and acetic acid, acrylic acid, adipic acid,
alginic acid, alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid,
butyric acid, carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic
30 acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic
acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid,
salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid,
toluenesulfonic acid, and uric acid, or a mixture thereof.

1 9. The pharmaceutical composition of claim 1, wherein the ionizable
functional group is a basic functional group, and the ionizing agent is a pharmaceutically
acceptable acid.

 10. The pharmaceutical composition of claim 9, wherein the basic functional
5 group is selected from the group consisting of aliphatic amines, aromatic amines, C-
substituted aromatic amines, N-substituted aromatic amines, heterocyclic amines, C-
substituted heterocyclic amines and N-substituted heterocyclic amines.

 11. The pharmaceutical composition of claim 9, wherein the hydrophobic
therapeutic agent is selected from the group consisting of abacavir, acebutolol,
10 acrivastine, alatrofloxacin, albuterol, albendazole, alprazolam, alprenolol, amantadine,
amiloride, aminoglutethimide, amiodarone, amitriptyline, amlodipine, amodiaquine,
amoxapine, amphetamine, amphotericin, amprenavir, amrinone, amsacrine, astemizole,
atenolol, atropine, azathioprine, azelastine, azithromycin, baclofen, benethamine,
benidipine, benzhexol, benznidazole, benztropine, biperiden, bisacodyl, bisanthrene,
15 bromazepam, bromocriptine, bromperidol, brompheniramine, brotizolam, bupropion,
butenafine, butoconazole, cambendazole, camptothecin, carbinoxamine, cephadrine,
cephalexin, cetirizine, cinnarizine, chlorambucil, chlopheniramine, chloproguanil,
chlordiazepoxide, chlorpromazine, chlorprothixene, chloroquine, cimetidine,
ciprofloxacin, cisapride, citalopram, clarithromycin, clemastine, clemizole, clenbuterol,
20 clofazimine, clomiphene, clonazepam, clopidrogel, clozapine, clotiazepam, clotrimazole,
codeine, cyclizine, cyproheptadine, dacarbazine, darodipine, decoquinate, delavirdine,
demeclocycline, dexamphetamine, dexchlorpheniramine, dexfenfluramine, diamorphine,
diazepam, diethylpropion, dihydrocodeine, dihydroergotamine, dilitazem,
dimenhydrinate, diphenhydramine, diphenooxylate, diphenylimidazole, diphenylpyrallin,
25 dipyridamole, dirithromycin, disopyramide, dolasetron, domperidone, donepezil,
doxazosin, doxycycline, droperidol, econazole, efavirenz, ellipticine, enalapril, enoxacin,
enrofloxacin, eperisone, ephedrine, ergotamine, erythromycin, ethambutol, ethionamide,
ethopropazine, etoperidone, famotidine, felodipine, fenbendazole, fenfluramine,
fenoldopam, fentanyl, fexofenadine, flecainide, flucytosine, flunarizine, flunitrazepam,
30 fluopromazine, fluoxetine, flupentixol, flupentixol decanoate, fluphenazine, fluphenazine
decanoate, flurazepam, flurithromycin, frovatriptan, gabapentin, granisetron,
grepafloxacin, guanabenz, halofantrine, haloperidol, hyoscyamine, imipenem, indinavir,
irinotecan, isoxazole, isradipine, itraconazole, ketoconazole, ketotifen, labetalol,

1 lamivudine, lanosprazole, leflunomide, levofloxacin, lisinopril, lomefloxacin,
 looperamide, loratadine, lorazepam, lormetazepam, lysuride, mepacrine, maprotiline,
 mazindol, mebendazole, meclozine, medazepam, mefloquine, melonicam, meptazinol,
 mercaptopurine, mesalamine, mesoridazine, metformin, methadone, methaqualone,
 5 methylphenidate, methylphenobarbital, methysergide, metoclopramide, metoprolol,
 metronidazole, mianserin, miconazole, midazolam, miglitol, minoxidil, mitomycins,
 mitoxantrone, molindone, montelukast, morphine, mortriptyline, moxifloxacin, nadolol,
 nalbuphine, naratriptan, natamycin, nefazodone, nelfinavir, nevirapine, nicardipine,
 nicotine, nifedipine, nimodipine, nimorazole, nisoldipine, nitrazepam, nitrofurazone,
 10 nizatidine, norfloxacin, nystatin, ofloxacin, olanzapine, omeprazole, ondansetron,
 ornidazole, oxamniquine, oxantel, oxatomide, oxazepam, oxfendazole, oxiconazole,
 oxprenolol, oxybutynin, oxyphencylamine, paroxetine, pentazocine, pentoxifylline,
 perchlorperazine, perfloxacin, perphenazine, phenbenzamine, pheniramine,
 phenoxybenzamine, phentermine, physostigmine, pimozide, pindolol, pizotifen,
 15 pramipexol, pranlukast, praziquantel, prazosin, procarbazine, prochlorperazine,
 proguanil, propranolol, pseudoephedrine, pyrantel, pyrimethamine, quetiapine, quinidine,
 quinine, raloxifene, ranitidine, remifentanyl, repaglinide, reserpine, ricobendazole,
 rifabutin, rifampin, rifapentine, rimantadine, risperidone, ritonavir, rizatriptan, ropinirole,
 rosiglitazone, roxaditine, roxithromycin, salbutamol, saquinavir, selegiline, sertraline,
 20 sibutramine, sildenafil, sparfloxacin, spiramycins, stavudine, sulconazole,
 sulphasalazine, sulpiride, sumatriptan, tacrine, tamoxifen, tamsulosin, temazepam,
 terazosin, terbinafine, terbutaline, terconazole, terfenadine, tetramisole, thiabendazole,
 thioguanine, thioridazine, tiagabine, ticlopidine, timolol, tinidazole, tioconazole,
 tirofiban, tizanidine, tolterodine, topotecan, toremifene, tramadol, trazodone, triamterene,
 25 triazolam, trifluoperazine, trimethoprim, trimipramine, tromethamine, tropicamide,
 trovafloxacin, vancomycin, venlafaxine, vigabatrin, vinblastine, vincristine, vinorelbine,
 vitamin K₅, vitamin K₆, vitamin K₇, zafirlukast, zolmitriptan, zolpidem, zopiclone, and
 pharmaceutically acceptable salts thereof.

12. The pharmaceutical composition of claim 11, wherein the hydrophobic
 30 therapeutic agent is selected from the group consisting of abacavir, acebutolol,
 acrivastine, alatrofloxacin, albendazole, albuterol, alprazolam, amiodarone, amlodipine,
 amodiaquine, amphetamine, amphotericin, amprenavir, astemizole, atenolol,
 azathioprine, azelastine, azithromycin, baclofen, benzotropine, bisacodyl, bromazepam,

1 bromperidol, brompheniramine, bupropion, butenafine, butoconazole, cambendazole,
 camptothecin, carbinoxamine, cetirizine, cinnarizine, chlopheniramine, chlorambucil,
 chlorpromazine, cimetidine, ciprofloxacin, cisapride, citalopram, clarithromycin,
 clemastine, clemizole, clomiphene, clonazepam, clopidrogel, clozapine, clotiazepam,
 5 clotrimazole, codeine, cyclizine, delavirdine, dexamphetamine, dexchlorpheniramine,
 diamorphine, diazepam, diethylpropion, dihydrocodeine, dihydroergotamine, diltazem,
 diphenhydramine, diphenylimidazole, diphenylpyrallin, dipyridamole, dirithromycin,
 disopyramide, dolasetron, domperidone, donepezil, doxazosin, droperidol, econazole,
 efavirenz, ellipticine, enalapril, enoxacin, eperisone, ergotamine, famotidine, felodipine,
 10 fenfluramine, fenoldopam, fexofenadine, fentanyl, flecainide, flunarizine, fluopromazine,
 fluoxetine, frovatriptan, gabapentin, granisetron, halofantrine, imipenem, indinavir,
 irinotecan, isoxazole, isradipine, itraconazole, ketoconazole, ketotifen, labetalol,
 lamivudine, lanosprazole, leflunomide, levofloxacin, lisinopril, lomefloxacin,
 loperamide, loratadine, lorazepam, lormetazepam, mazindol, mebendazole, mefloquine,
 15 mercaptopurine, mesalamine, metformin, methadone, methaqualone, methylphenidate,
 methysergide, metoclopramide, metoprolol, metronidazole, miconazole, midazolam,
 miglitol, minoxidil, mitoxantrone, montelukast, naratriptan, nelfinavir, nevirapine,
 nicardipine, nicotine, nifedipine, nimodipine, nimorazole, nisoldipine, nizatidine,
 norfloxacin, ofloxacin, olanzapine, omeprazole, ondansetron, oxamniquine, oxiconazole,
 20 paroxetine, perchlorperazine, phenbenzamine, pheniramine, phentermine, physostigmine,
 pizotifen, pramipexol, prazosin, prochlorperazine, pseudoephedrine, quetiapine,
 quinidine, raloxifene, ranitidine, remifentanyl, repaglinide, rifabutin, rifampin,
 rifapentine, rimantadine, risperidone, ritonavir, rizatriptan, rosiglitazone, roxaditine,
 saquinavir, sibutramine, sildenafil, sparfloxacin, stavudine, sulphasalazine, sumatriptan,
 25 tacrine, tamoxifen, tamsulosin, terazosin, terbinafine, terbutaline, terconazole,
 terfenadine, tiagabine, ticlopidine, tinidazole, tioconazole, tirofiban, tizanidine,
 tolterodine, topotecan, toremifene, tramadol, trazodone, trovafloxacin, vancomycin,
 venlafaxine, vigabatrin, vinblastine, vincristine, vinorelbine, vitamin K₃, vitamin K₆,
 vitamin K₇, zafirlukast, zolmitriptan, zolpidem, zopiclone, and pharmaceutically
 30 acceptable salts thereof.

13. The pharmaceutical composition of claim 12, wherein the hydrophobic therapeutic agent is selected from the group consisting of abacavir, acrivastine, alatrofloxacin, albuterol, amiodarone, amlodipine, amphetamine, amprenavir, astemizole,

1 atenolol, azathioprine, azelastine, azithromycin, baclofen, benztropine, bisacodyl,
 bromazepam, bromperidol, brompheniramine, bupropion, butenafine, butoconazole,
 cambendazole, camptothecin, carbinoxamine, cetirizine, cinnarizine, chlopheniramine,
 chlorpromazine, cimetidine, ciprofloxacin, cisapride, clarithromycin, clemastine,
 5 clemizole, clonazepam, clopidogrel, clotrimazole, codeine, dexchlorpheniramine,
 dihydrocodeine, dihydroergotamine, diphenhydramine, diphenylimidazole,
 diphenylpyrallin, dirithromycin, dolasetron, domperidone, doxazosin, econazole,
 efavirenz, ellipticine, enoxacin, eperisone, ergotamine, famotidine, fenoldopam, fentanyl,
 fexofenadine, flunarizine, fluoxetine, frovatriptan, granisetron, grepafloxacin,
 10 halofantrine, indinavir, irinotecan, isradipine, itraconazole, ketoconazole, ketotifen,
 lamivudine, lansoprazole, leflunomide, levofloxacin, loperamide, loratadine, metformin,
 methadone, methylphenidate, methysergide, metronidazole, miconazole, midazolam,
 miglitol, mitoxantrone, montelukast, naratriptan, nelfinavir, nicotine, nifedipine,
 nimorazole, nizatidine, norfloxacin, ofloxacin, omeprazole, perchloperazine,
 15 phenbenzamine, physostigmine, pizotifen, pseudoephedrine, quetiapine, quinidine,
 raloxifene, ranitidine, remifentanyl, repaglinide, rifabutin, rifampin, rifapentine,
 rimantadine, ritonavir, rizatriptan, rosiglitazone, roxaditine, saquinavir, sibutramine,
 sildenafil, stavudine, sumatriptan, tacrine, tamoxifen, tamsulosin, terazosin, terbinafine,
 tinidazole, tizanidine, tolterodine, topotecan, toremifene, tramadol, trovafloxacin,
 20 vancomycin, vinblastine, vincristine, vinorelbine, vitamin K₅, vitamin K₆, vitamin K₇,
 zafirlukast, zolmitriptan, zolpidem, and pharmaceutically acceptable salts thereof.

14. The pharmaceutical composition of claim 13, wherein the hydrophobic
 therapeutic agent is selected from the group consisting of amlodipine, astemizole,
 brompheniramine, bupropion, carbinoxamine, cetirizine, cimetidine, cisapride,
 25 clemastine, clemizole, dihydroergotamine, diphenhydramine, diphenylimidazole,
 diphenylpyrallin, domperidone, eperisone, famotidine, fexofenadine, frovatriptan,
 granisetron, itraconazole, ketoconazole, ketotifen, lansoprazole, leflunomide,
 loperamide, loratadine, methysergide, miglitol, montelukast, naratriptan, nizatidine,
 omeprazole, ondansetron, phenbenzamine, pseudoephedrine, raloxifene, ranitidine,
 30 repaglinide, rifabutin, rimantadine, ritonavir, rizatriptan, rosiglitazone, roxaditine,
 saquinavir, sibutramine, sildenafil, sumatriptan, tamsulosin, terbinafine, tizanidine,
 tramadol, trovafloxacin, vitamin K₅, vitamin K₆, vitamin K₇, zafirlukast, zolmitriptan,
 zolpidem, and pharmaceutically acceptable salts thereof.

1 15. The pharmaceutical composition of claim 9, wherein the acid is a pharmaceutically acceptable inorganic acid.

 16. The pharmaceutical composition of claim 15, wherein the inorganic acid is selected from the group consisting of hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, carbonic acid, nitric acid, boric acid and phosphoric acid.

5 17. The pharmaceutical composition of claim 9, wherein the acid is a pharmaceutically acceptable organic acid.

 18. The pharmaceutical composition of claim 17, wherein the organic acid is selected from the group consisting of acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, uric acid, and mixtures thereof.

15 19. The pharmaceutical composition of claim 1, wherein the hydrophobic therapeutic agent is present in an amount that is solubilized by the carrier.

 20. The pharmaceutical composition of claim 1, wherein the hydrophobic therapeutic agent is present in a first amount that is solubilized by the carrier and a second amount that is suspended but not solubilized in the carrier.

20 21. The pharmaceutical composition of claim 1, wherein the surfactant is a hydrophilic surfactant or a mixture of hydrophilic surfactants.

 22. The pharmaceutical composition of claim 21, wherein the hydrophilic surfactant is a non-ionic hydrophilic surfactant having an HLB value greater than or equal to about 10.

25 23. The pharmaceutical composition of claim 22, wherein the non-ionic surfactant is selected from the group consisting of alkylglucosides; alkylmaltosides; alkylthioglucosides; lauryl macrogolglycerides; polyoxyethylene alkyl ethers; polyoxyethylene alkylphenols; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglyceryl fatty acid esters; polyoxyethylene glycerides; polyoxyethylene sterols, derivatives, and analogues thereof; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction

1 mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; sugar esters, sugar ethers; sucroglycerides; and mixtures thereof.

24. The pharmaceutical composition of claim 22, wherein the non-ionic
5 hydrophilic surfactant is selected from the group consisting of polyoxyethylene alkylethers; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; polyglyceryl fatty acid esters; polyoxyethylene glycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction
10 mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

25. The pharmaceutical composition of claim 24, wherein the glyceride is a monoglyceride, diglyceride, triglyceride, or a mixture thereof.

26. The pharmaceutical composition of claim 24, wherein the reaction
15 mixture comprises the transesterification products of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

27. The pharmaceutical composition of claim 24, wherein the polyol is glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol
20 or a mixture thereof.

28. The pharmaceutical composition of claim 22, wherein the hydrophilic surfactant is PEG-10 laurate, PEG-12 laurate, PEG-20 laurate, PEG-32 laurate, PEG-32 dilaurate, PEG-12 oleate, PEG-15 oleate, PEG-20 oleate, PEG-20 dioleate, PEG-32 oleate, PEG-200 oleate, PEG-400 oleate, PEG-15 stearate, PEG-32 distearate, PEG-40
25 stearate, PEG-100 stearate, PEG-20 dilaurate, PEG-25 glyceryl trioleate, PEG-32 dioleate, PEG-20 glyceryl laurate, PEG-30 glyceryl laurate, PEG-20 glyceryl stearate, PEG-20 glyceryl oleate, PEG-30 glyceryl oleate, PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-40 palm kernel oil, PEG-50 hydrogenated castor oil, PEG-40 castor oil, PEG-35 castor oil, PEG-60 castor oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-60 corn oil, PEG-6 caprate/caprylate glycerides, PEG-8
30 caprate/caprylate glycerides, polyglyceryl-10 laurate, PEG-30 cholesterol, PEG-25 phyto sterol, PEG-30 soya sterol, PEG-20 trioleate, PEG-40 sorbitan oleate, PEG-80 sorbitan laurate, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-

1 10 oleyl ether, POE-20 oleyl ether, POE-20 stearyl ether, tocopheryl PEG-100 succinate, PEG-24 cholesterol, polyglyceryl-10 oleate, Tween 40, Tween 60, sucrose monostearate, sucrose monolaurate, sucrose monopalmitate, PEG 10-100 nonyl phenol series, PEG 15-100 octyl phenol series, a poloxamer, or a mixture thereof.

5 29. The pharmaceutical composition of claim 22, wherein the hydrophilic surfactant is PEG-20 laurate, PEG-20 oleate, PEG-35 castor oil, PEG-40 palm kernel oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, polyglyceryl-10 laurate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, PEG-30 cholesterol, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, PEG-24 cholesterol, sucrose monostearate, 10 sucrose monolaurate, a poloxamer, or a mixture thereof.

30. The pharmaceutical composition of claim 22, wherein the hydrophilic surfactant is PEG-35 castor oil, PEG-40 hydrogenated castor oil, PEG-60 corn oil, PEG-25 glyceryl trioleate, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polysorbate 20, polysorbate 80, tocopheryl PEG-1000 succinate, PEG-24 15 cholesterol, a poloxamer, or a mixture thereof.

31. The pharmaceutical composition of claim 21, wherein the hydrophilic surfactant is an ionic surfactant.

32. The pharmaceutical composition of claim 31, wherein the ionic surfactant 20 is selected from the group consisting of fatty acid salts, bile salts, phospholipids, phosphoric acid esters, carboxylates, sulfates, sulfonates, and mixtures thereof.

33. The pharmaceutical composition of claim 21, wherein the hydrophilic surfactant is a mixture of at least one ionic surfactant and at least one non-ionic hydrophilic surfactant.

25 34. The pharmaceutical composition of claim 1, wherein the surfactant is a hydrophobic surfactant or mixture of hydrophobic surfactants having an HLB value of less than about 10.

35. The pharmaceutical composition of claim 34, wherein the hydrophobic surfactant is selected from the group consisting of alcohols; polyoxyethylene alkylethers; 30 polyglyceryl fatty acid esters; fatty acids; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower alcohol fatty acids esters; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; lactic acid derivatives of mono/diglycerides; propylene

1 glycol diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters;
polyoxyethylene-polyoxypropylene block copolymers; transesterified vegetable oils;
sterols; sterol derivatives; sugar esters; sugar ethers; sucroglycerides; polyoxyethylene
vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of
5 polyols and at least one member of the group consisting of fatty acids, glycerides,
vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

36. The pharmaceutical composition of claim 34, wherein the hydrophobic
surfactant is selected from the group consisting of fatty acids; lower alcohol fatty acid
esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid
10 esters; polyoxyethylene glycerides; glycerol fatty acid esters; acetylated glycerol fatty
acid esters; polyglyceryl fatty acid esters; lactic acid derivatives of mono/diglycerides;
sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-
polyoxypropylene block copolymers; polyoxyethylene vegetable oils; polyoxyethylene
hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the
15 group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils,
and sterols; and mixtures thereof.

37. The pharmaceutical composition of claim 34, wherein the hydrophobic
surfactant is selected from the group consisting of lower alcohol fatty acids esters;
polypropylene glycol fatty acid esters; propylene glycol fatty acid esters; glycerol fatty
20 acid esters; polyglyceryl fatty acid esters; acetylated glycerol fatty acid esters; lactic acid
derivatives of mono/diglycerides; sorbitan fatty acid esters; polyoxyethylene vegetable
oils; and mixtures thereof.

38. The pharmaceutical composition of claim 34, wherein the hydrophobic
surfactant is a glycerol fatty acid ester, a polyglyceryl fatty acid ester, an acetylated
25 glycerol fatty acid ester, or a mixture thereof.

39. The pharmaceutical composition of claim 38, wherein the glycerol fatty
acid ester is a monoglyceride, diglyceride, or a mixture thereof.

40. The pharmaceutical composition of claim 39, wherein the fatty acid of the
glycerol fatty acid ester is a C₆ to C₂₀ fatty acid or a mixture thereof.

30 41. The pharmaceutical composition of claim 34, wherein the hydrophobic
surfactant is a reaction mixture of a polyol and at least one member of the group
consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and
sterols.

1 42. The pharmaceutical composition of claim 41, wherein the reaction mixture is a transesterification product of a polyol and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols.

5 43. The pharmaceutical composition of claim 42, wherein the polyol is polyethylene glycol, sorbitol, propylene glycol, pentaerythritol or a mixture thereof.

 44. The pharmaceutical composition of claim 34, wherein the hydrophobic surfactant is selected from the group consisting of myristic acid; oleic acid; lauric acid; stearic acid; palmitic acid; PEG 1-4 stearate; PEG 2-4 oleate; PEG-4 dilaurate; PEG-4 dioleate; PEG-4 distearate; PEG-6 dioleate; PEG-6 distearate; PEG-8 dioleate; PEG 3-16
10 castor oil; PEG 5-10 hydrogenated castor oil; PEG 6-20 corn oil; PEG 6-20 almond oil; PEG-6 olive oil; PEG-6 peanut oil; PEG-6 palm kernel oil; PEG-6 hydrogenated palm kernel oil; PEG-4 capric/caprylic triglyceride, mono, di, tri, tetra esters of vegetable oil and sorbitol; pentaerythrityl di, tetra stearate, isostearate, oleate, caprylate, or caprate; polyglyceryl 2-4 oleate, stearate, or isostearate; polyglyceryl 4-10 pentaoleate;
15 polyglyceryl-3 dioleate; polyglyceryl-6 dioleate; polyglyceryl-10 trioleate; polyglyceryl-3 distearate; propylene glycol mono- or diesters of a C₆ to C₂₀ fatty acid; monoglycerides of a C₆ to C₂₀ fatty acid; acetylated monoglycerides of C₆ to C₂₀ fatty acid; diglycerides of C₆ to C₂₀ fatty acids; lactic acid derivatives of monoglycerides; lactic acid derivatives of diglycerides; cholesterol; phytosterol; PEG 5-20 soya sterol; PEG-6 sorbitan tetra,
20 hexastearate; PEG-6 sorbitan tetraoleate; sorbitan monolaurate; sorbitan monopalmitate; sorbitan mono, trioleate; sorbitan mono, tristearate; sorbitan monoisostearate; sorbitan sesquioleate; sorbitan sesquisteate; PEG 2-5 oleyl ether; POE 2-4 lauryl ether; PEG-2 cetyl ether; PEG-2 stearyl ether; sucrose distearate; sucrose dipalmitate; ethyl oleate; isopropyl myristate; isopropyl palmitate; ethyl linoleate; isopropyl linoleate;
25 poloxamers; and mixtures thereof.

 45. The pharmaceutical composition of claim 34, wherein the hydrophobic surfactant is selected from the group consisting of oleic acid; lauric acid; glyceryl monocaprate; glyceryl monocaprylate; glyceryl monolaurate; glyceryl monooleate; glyceryl dicaprate; glyceryl dicaprylate; glyceryl dilaurate; glyceryl dioleate; acetylated
30 monoglycerides; propylene glycol oleate; propylene glycol laurate; polyglyceryl-3 oleate; polyglyceryl-6 dioleate; PEG-6 corn oil; PEG-20 corn oil; PEG-20 almond oil;

1 sorbitan monooleate; sorbitan monolaurate; POE-4 lauryl ether; POE-3 oleyl ether; ethyl
oleate; poloxamers; and mixtures thereof.

46. The pharmaceutical composition of claim 1, wherein the surfactant is a
mixture of at least one hydrophilic surfactant and at least one hydrophobic surfactant.

5 47. The pharmaceutical composition of claim 1, wherein the triglyceride is a
pharmaceutically acceptable oil, hydrogenated oil, partially hydrogenated oil, medium
chain triglyceride, long chain triglyceride, structured triglyceride, or a mixture thereof.

48. The pharmaceutical composition of claim 1, which further comprises a
solubilizer.

10 49. The pharmaceutical composition of claim 48, wherein the solubilizer is
selected from the group consisting of alcohols, polyols, amides, esters, propylene glycol
ethers and mixtures thereof.

50. The pharmaceutical composition of claim 49, wherein the alcohol or
polyol is selected from the group consisting of ethanol, isopropanol, butanol, benzyl
15 alcohol, ethylene glycol, propylene glycol, butanediols and isomers thereof, glycerol,
pentaerythritol, sorbitol, transcitol, mannitol, dimethyl isosorbide, polyethylene glycol,
polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other
cellulose derivatives, maltodextrins, cyclodextrins and cyclodextrin derivatives, and
mixtures thereof.

20 51. The pharmaceutical composition of claim 49, wherein the amide is
selected from the group consisting of 2-pyrrolidone, 2-piperidone, ϵ -caprolactam, *N*-
alkylpyrrolidone, *N*-hydroxyalkylpyrrolidone, *N*-alkylpiperidone, *N*-alkylcaprolactam,
dimethylacetamide, polyvinylpyrrolidone, and mixtures thereof.

25 52. The pharmaceutical composition of claim 49, wherein the ester is selected
from the group consisting of ethyl propionate, tributylcitrate, acetyl triethylcitrate, acetyl
tributyl citrate, triethylcitrate, ethyl oleate, ethyl caprylate, ethyl butyrate, triacetin,
propylene glycol monoacetate, propylene glycol diacetate, ϵ -caprolactone and isomers
thereof, δ -valerolactone and isomers thereof, β -butyrolactone and isomers thereof, and
mixtures thereof.

30 53. The pharmaceutical composition of claim 48, wherein the solubilizer is
selected from the group consisting of ethanol, isopropanol, butanol, benzyl alcohol,
ethylene glycol, propylene glycol, butanediol and isomers thereof, glycerol,
pentaerythritol, sorbitol, transcitol, mannitol, dimethyl isosorbide, polyethylene glycol,

95
1 polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other
cellulose derivatives, maltodextrins, cyclodextrins and derivatives thereof, ethyl
propionate, tributylcitrate, acetyl triethylcitrate, acetyl tributyl citrate, triethylcitrate,
ethyl oleate, ethyl caprylate, ethyl butyrate, triacetin, propylene glycol diacetate, ϵ -
5 caprolactone and isomers thereof, δ -valerolactone and isomers thereof, β -butyrolactone
and isomers thereof, 2-pyrrolidone, 2-piperidone, ϵ -caprolactam, *N*-methylpyrrolidone,
N-ethylpyrrolidone, *N*-hydroxyethyl pyrrolidone, *N*-octylpyrrolidone, *N*-
laurylpyrrolidone, dimethylacetamide, polyvinylpyrrolidone, glycofurol, methoxy PEG,
and mixtures thereof.

10 54. The pharmaceutical composition of claim 48, wherein the solubilizer is
selected from the group consisting of ethanol, isopropanol, benzyl alcohol, ethylene
glycol, propylene glycol, 1,3-butanediol, glycerol, pentaerythritol, sorbitol, transcitol,
glycofurol, dimethyl isosorbide, polyethylene glycol, polyvinylalcohol, hydroxypropyl
methylcellulose, methylcellulose, ethylcellulose, hydroxypropylcyclodextrins, sulfobutyl
15 ether derivatives of cyclodextrins, ethyl propionate, tributylcitrate, triethylcitrate, ethyl
oleate, ethyl caprylate, triacetin, β -butyrolactone and isomers thereof, 2-pyrrolidone, *N*-
methylpyrrolidone, *N*-ethylpyrrolidone, *N*-hydroxyethylpyrrolidone, *N*-octylpyrrolidone,
N-laurylpyrrolidone, dimethylacetamide, polyvinylpyrrolidone, and mixtures thereof.

20 55. The pharmaceutical composition of claim 48, wherein the solubilizer is
triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, dimethylacetamide, *N*-
methylpyrrolidone, *N*-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropyl
methylcellulose, hydroxypropyl cyclodextrins, ethanol, polyethylene glycol 200-600,
transcitol, glycofurol, propylene glycol, dimethyl isosorbide, or a mixture thereof.

25 56. The pharmaceutical composition of claim 48, wherein the solubilizer is
triacetin, ethanol, polyethylene glycol 400, glycofurol, propylene glycol or a mixture
thereof.

57. The pharmaceutical composition of claim 1, wherein the ionizing agent is
present in a pre-reaction amount of greater than about 1.5 mole equivalents per mole of
ionizable functional group.

30 58. The pharmaceutical composition of claim 57, which further comprises a
neutralizing agent capable of neutralizing a portion of the ionizing agent.

1 59. The pharmaceutical composition of claim 58, wherein the ionizable functional group is an acidic functional group, the ionizing agent is a pharmaceutically acceptable base, and the neutralizing agent is a pharmaceutically acceptable acid.

5 60. The pharmaceutical composition of claim 58, wherein the ionizable functional group is a basic functional group, the ionizing agent is a pharmaceutically acceptable acid, and the neutralizing agent is a pharmaceutically acceptable base.

10 61. The pharmaceutical composition of claim 58, wherein the neutralizing agent is present in a pre-reaction amount sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than 1.5 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

15 62. The pharmaceutical composition of claim 61, wherein the pre-reaction amount of the neutralizing agent is sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than about 1.0 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

20 63. The pharmaceutical composition of claim 1, wherein the ionizing agent is present in a pre-reaction amount of greater than about 1.0 mole equivalents per mole of ionizable functional group.

 64. The pharmaceutical composition of claim 1, wherein the ionizing agent is present in a pre-reaction amount of greater than about 0.1 mole equivalents per mole of ionizable functional group.

25 65. The pharmaceutical composition of claim 1, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.5 mole equivalents per mole of ionizable functional group.

30 66. The pharmaceutical composition of claim 1, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.0 mole equivalents per mole of ionizable functional group.

 67. The pharmaceutical composition of claim 1, which further comprises an antioxidant, a preservative, a chelating agent, a viscomodulator, a tonicifier, a flavorant, a colorant, an odorant, an opacifier, a suspending agent, a binder, or a mixture thereof.

1 68. The pharmaceutical composition of claim 1 in the form of a
preconcentrate, a diluted preconcentrate, a semi-solid dispersion, a solid dispersion, or a
sprayable solution.

5 69. A dosage form comprising a capsule filled with the pharmaceutical
composition of claim 1.

70. A dosage form comprising a capsule filled with the pharmaceutical
composition of claim 61.

71. A dosage form comprising a capsule filled with the pharmaceutical
composition of claim 62.

10 72. A dosage form comprising a solid particulate carrier coated with or
formed from the pharmaceutical composition of claim 1.

73. A dosage form comprising the pharmaceutical composition of claim 1
formulated as a solution, a cream, a lotion, an ointment, a suppository, a spray, an
aerosol, a paste or a gel.

15 74. The dosage form of claim 69, wherein the capsule is a hard gelatin
capsule, a soft gelatin capsule, a starch capsule or an enteric coated capsule.

75. The pharmaceutical composition of claim 1, which further comprises
water or an aqueous solution.

20 76. A pharmaceutical composition comprising:
 (a) a hydrophobic therapeutic agent having at least one ionizable
functional group; and
 (b) a carrier, said carrier comprising:
 (i) an ionizing agent capable of ionizing the ionizable
functional group and present in a pre-reaction amount of
25 greater than about 1.5 mole equivalents per mole of
ionizable functional group; and
 (ii) a surfactant.

77. The pharmaceutical composition of claim 76, which further comprises a
neutralizing agent capable of neutralizing a portion of the ionizing agent.

30 78. The pharmaceutical composition of claim 77, wherein the ionizable
functional group is an acidic functional group, the ionizing agent is a pharmaceutically
acceptable base, and the neutralizing agent is a pharmaceutically acceptable acid.

1 79. The pharmaceutical composition of claim 77, wherein the ionizable functional group is a basic functional group, the ionizing agent is a pharmaceutically acceptable acid, and the neutralizing agent is a pharmaceutically acceptable base.

5 80. The pharmaceutical composition of claim 77, wherein the neutralizing agent is present in a pre-reaction amount sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than 1.5 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

10 81. The pharmaceutical composition of claim 80, wherein the pre-reaction amount of the neutralizing agent is sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than about 1.0 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

15 82. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 80.

83. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 81.

20 84. The dosage form of claim 82, wherein the capsule is a hard gelatin capsule, a soft gelatin capsule, a starch capsule or an enteric coated capsule.

85. The pharmaceutical composition of claim 76, which further comprises water or an aqueous solution.

25 86. The pharmaceutical composition of claim 76, which further comprises a solubilizer.

87. A pharmaceutical composition comprising:

- (a) a hydrophobic therapeutic agent having at least one ionizable functional group; and
- (b) a carrier, said carrier comprising:
 - 30 (i) an ionizing agent capable of ionizing the ionizable functional group;
 - (ii) a surfactant selected from the group consisting of alkylglucosides; alkylmaltosides; alkylthioglucosides;

- 1 lauryl macrogolglycerides; polyoxyethylene alkyl ethers;
fatty acids; lower alcohol fatty acid esters;
polyoxyethylene alkylphenols; polyethylene glycol fatty
acids esters; polypropylene glycol fatty acid esters;
5 glycerol fatty acid esters; acetylated glycerol fatty acid
esters; polyethylene glycol glycerol fatty acid esters;
polyglyceryl fatty acid esters; polyoxyethylene glycerides;
polyoxyethylene sterols, derivatives, and analogues
thereof; polyoxyethylene vegetable oils; polyoxyethylene
10 hydrogenated vegetable oils; reaction mixtures of polyols
and at least one member of the group consisting of fatty
acids, vegetable oils, hydrogenated vegetable oils, and
sterols; sugar esters; sugar ethers; sucroglycerides; fatty
acid salts; bile salts; phospholipids; phosphoric acid esters;
15 carboxylates; sulfates; and sulfonates; and
(iii) a solubilizer present in an amount of greater than about
10% by weight, based on the total weight of the
composition.

88. The pharmaceutical composition of claim 87, wherein the solubilizer is
20 selected from the group consisting of alcohols, polyols, amides, esters, propylene glycol
ethers and mixtures thereof.

89. The pharmaceutical composition of claim 87, wherein the solubilizer is
selected from the group consisting of ethanol, isopropanol, butanol, benzyl alcohol,
ethylene glycol, propylene glycol, butanediol and isomers thereof, glycerol,
25 pentaerythritol, sorbitol, mannitol, dimethyl isosorbide, polyethylene glycol,
polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other
cellulose derivatives, cyclodextrins, clodextrins and derivatives thereof, ethyl propionate,
tributylcitrate, acetyl triethylcitrate, acetyl tributyl citrate, triethylcitrate, ethyl oleate,
ethyl caprylate, ethyl butyrate, triacetin, propylene glycol diacetate, ϵ -caprolactone and
30 isomers thereof, δ -valerolactone and isomers thereof, β -butyrolactone and isomers
thereof, 2-pyrrolidone, 2-piperidone, ϵ -caprolactam, *N*-methylpyrrolidone, *N*-
ethylpyrrolidone, *N*-hydroxyethyl pyrrolidone, *N*-octylpyrrolidone, *N*-laurylpyrrolidone,

1 dimethylacetamide, polyvinylpyrrolidone, glycofurol, methoxy PEG, and mixtures thereof.

90. The pharmaceutical composition of claim 87, wherein the solubilizer is present in an amount of at least about 15% by weight, based on the total weight of the composition.

5 91. The pharmaceutical composition of claim 87, wherein the ionizing agent is present in a pre-reaction amount of greater than about 1.5 mole equivalents per mole of ionizable functional group.

92. The pharmaceutical composition of claim 87, wherein the ionizing agent is present in a pre-reaction amount of greater than about 1.0 mole equivalents per mole of ionizable functional group.

93. The pharmaceutical composition of claim 87, wherein the ionizing agent is present in a pre-reaction amount of greater than about 0.1 mole equivalents per mole of ionizable functional group.

15 94. The pharmaceutical composition of claim 87, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.5 mole equivalents per mole of ionizable functional group.

95. The pharmaceutical composition of claim 87, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.0 mole equivalents per mole of ionizable functional group.

20 96. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 94.

97. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 95.

25 98. A pharmaceutical composition comprising:
(a) a hydrophobic therapeutic agent having at least one ionizable functional group; and
(b) a carrier, said carrier comprising:
30 (i) an ionizing agent capable of ionizing the ionizable functional group;
(ii) a surfactant selected from the group consisting of alkylglucosides; alkylmaltosides; alkylthioglucosides;

- 1 lauryl macroglycerides; fatty acids; lower alcohol fatty
acid esters; polyoxyethylene alkylphenols; polyethylene
glycol fatty acids esters; polypropylene glycol fatty acid
5 esters; glycerol fatty acid esters; acetylated glycerol fatty
acid esters; polyethylene glycol glycerol fatty acid esters;
polyglyceryl fatty acid esters; polyoxyethylene sorbitan
fatty acid esters; polyoxyethylene glycerides;
polyoxyethylene sterols, derivatives, and analogues
10 thereof; polyoxyethylene vegetable oils; polyoxyethylene
hydrogenated vegetable oils; reaction mixtures of polyols
and at least one member of the group consisting of fatty
acids, vegetable oils, hydrogenated vegetable oils, and
sterols; sugar esters; sugar ethers; sucroglycerides; fatty
acid salts; bile salts; phospholipids; phosphoric acid esters;
15 carboxylates; sulfates; and sulfonates; and
(iii) a solubilizer comprising at least one compound selected
from the group consisting of alcohols, polyols, amides,
esters, and propylene glycol ethers, the alcohol or polyol
being selected from the group consisting of ethanol,
20 isopropanol, butanol, benzyl alcohol, ethylene glycol,
propylene glycol, butanediols and isomers thereof,
glycerol, pentaerythritol, sorbitol, mannitol, dimethyl
isosorbide, polypropylene glycol, polyvinylalcohol,
hydroxypropyl methylcellulose and other cellulose
25 derivatives, maltodextrins, and cyclodextrins and
cyclodextrin derivatives.

99. The pharmaceutical composition of claim 98, wherein the ionizing agent is present in a pre-reaction amount of greater than about 1.5 mole equivalents per mole of ionizable functional group.

- 30 100. The pharmaceutical composition of claim 98, wherein the ionizing agent is present in a pre-reaction amount of greater than about 1.0 mole equivalents per mole of ionizable functional group.

1 101. The pharmaceutical composition of claim 98, wherein the ionizing agent is present in a pre-reaction amount of greater than about 0.1 mole equivalents per mole of ionizable functional group.

5 102. The pharmaceutical composition of claim 98, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.5 mole equivalents per mole of ionizable functional group.

10 103. The pharmaceutical composition of claim 98, wherein the ionizing agent is present in a pre-reaction amount of about 0.1 to about 1.0 mole equivalents per mole of ionizable functional group.

10 104. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 102.

105. A dosage form comprising a capsule filled with the pharmaceutical composition of claim 103.

15 106. A method of preparing a pharmaceutical composition of an ionizable hydrophobic therapeutic agent, the method comprising

(I) providing a pharmaceutical composition comprising:

(a) a hydrophobic therapeutic agent having at least one ionizable functional group; and

(b) a carrier, the carrier comprising:

20 (i) an ionizing agent capable of ionizing the ionizable functional group; and

(ii) a surfactant; and

(II) providing a neutralizing agent in an amount sufficient to neutralize at least a portion of the ionizing agent.

25 107. The method of claim 106, wherein the ionizing agent is present in the carrier in a pre-reaction amount of greater than about 1.5 mole equivalents per mole of ionizable functional group.

30 108. The method of claim 106, wherein the neutralizing agent is present in a pre-reaction amount sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than 1.5 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before

1 reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

5 109. The method of claim 107, wherein the pre-reaction amount of the neutralizing agent is sufficient to neutralize the ionizing agent so that the amount of ionizing agent is less than about 1.0 mole equivalents per mole of ionizable functional group, based on the amounts of ionizing agent and ionizable functional groups present before reaction with each other but after reaction of the ionizing agent and the neutralizing agent.

10 110. A method of treating an animal with an ionizable hydrophobic therapeutic agent, the method comprising:

- 15 (I) providing a dosage form of a pharmaceutical composition comprising:
 - (a) a hydrophobic therapeutic agent having at least one ionizable functional group; and
 - (b) a carrier, said carrier comprising:
 - (i) an ionizing agent capable of ionizing the ionizable functional group; and
 - (ii) a surfactant; and
- (II) administering said dosage form to said animal.

20 111. The method of claim 110, wherein the pharmaceutical composition further comprises a triglyceride.

112. The method of claim 110, wherein the dosage form is a capsule, a solution, a cream, a lotion, an ointment, a suppository, a spray, an aerosol, a paste or a gel.

25 113. The method of claim 110, wherein the dosage form is administered by an oral, parenteral, topical, transdermal, ocular, pulmonary, vaginal, rectal or transmucosal route.

114. The method of claim 110, wherein the animal is a mammal.

115. The method of claim 114, wherein the mammal is a human.

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(54) Title: **SOFT ELASTIC CAPSULES COMPRISING RITONAVIR AND/OR LOPINAVIR**

(57) Abstract: A soft elastic capsule is described containing a pharmaceutical agent or a mixture of pharmaceutical agents. The soft elastic capsule is prepared from a fill composition and a shell composition that impart acceptable physical characteristics to the soft elastic capsule when in an equilibrium state. The pharmaceutical agents of the fill composition are also soluble when the soft elastic capsule is in the equilibrium state.

SOFT ELASTIC CAPSULES COMPRISING RITONAVIR AND/OR LOPINAVIR

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Technical Field

The invention relates to a soft elastic capsule, fill and shell compositions of the soft elastic capsule, and pharmaceutical agents contained within the soft elastic capsules. The invention further relates to soft elastic capsules and HIV protease inhibiting compounds contained in the soft elastic capsule. The soft elastic capsule can be used with a broad range of pharmaceutical agents including antibiotics, anti-AIDS pharmaceutical agents, and an array of other medicinally active agents. Important pharmaceutical agents are anti-HIV agents.

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Background

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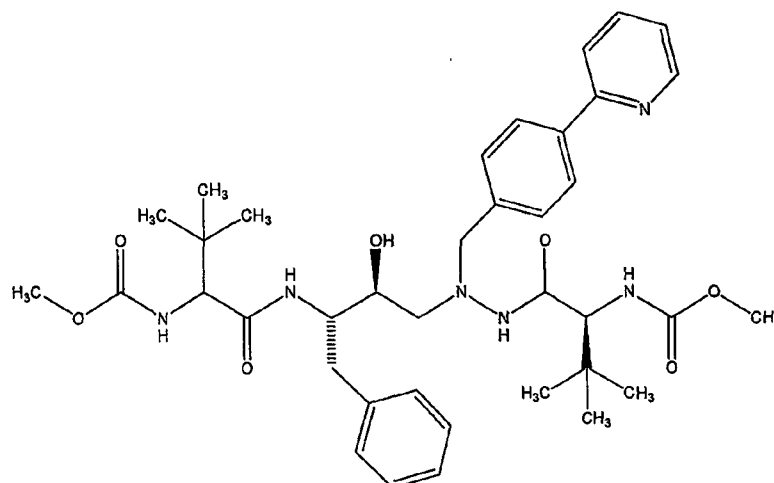
Inhibitors of human immunodeficiency virus (HIV) protease have been approved for use in the treatment of HIV infection for several years. A particularly effective HIV protease inhibitor is (2S, 3S, 5S)-5-(N-((N-methyl-N-((2-isopropyl-4-thiazolyl)-methyl)amino)carbonyl)-L-valinyl)amino-2-(N-((5-thiazolyl)methoxy-carbonyl)-amino)-1,6-diphenyl-3-hydroxyhexane (ritonavir), which is marketed as NORVIR®. Ritonavir is known to have utility for the inhibition of HIV protease, the inhibition of HIV infection, and the enhancement of the pharmacokinetics of compounds which are metabolized by cytochrome P₄₅₀ monooxygenase. Ritonavir is particularly effective for the inhibition of HIV infection when used alone or in a combination with one or more reverse transcriptase inhibitors and/or one or more other HIV protease inhibitors. Ritonavir and processes for its preparation are disclosed in U.S. Patent No. 5,541,206, issued July 30, 1996, the disclosure of which is herein incorporated by reference. Crystalline Form II of ritonavir and processes for its preparation are disclosed in International Patent Application WO00/04016, published January 27, 2000, which is incorporated herein by reference.

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A HIV protease inhibitor useful in combination with ritonavir is lopinavir which has a chemical name of (2S,3S,5S)-2-(2,6-dimethylphenoxyacetyl)-amino-3-hydroxy-5-(2S-(1-tetrahydropyrimid-2-onyl)-3-methyl-butanoyl)amino-1,6-diphenylhexane. Lopinavir is an inhibitor of the HIV protease and prevents cleavage of the HIV Gag-Pol protein, resulting in the production of immature, non-infectious viral particles. The preparation of lopinavir is disclosed in U.S. Patent No. 5,914,332, issued June 22, 1999, the disclosure of which is



for example, BMS-232,623 (3S-(3R*, 8'R*, 9'R*, 12R*)))-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-((4-(2-pyridinyl)-phenylmethyl)-2,5,6,10,13-pentaazatetradecanedioic acid, dimethyl ester) and related compounds disclosed in International Patent Application No. WO99/36404, published July 22, 1999 and International Patent Application No. WO97/40029, published October 30, 1997, both herein incorporated by reference; or a pharmaceutically acceptable salt of any of the above.

Other pharmaceutical agents that can be used in the current invention include, but are not limited to, anti-viral compounds, cell growth inhibitors, antibiotics, antihistamines, analgesics, food supplements, nutrients, vitamins, steroids, and anesthetics. The current invention relates to any physiologically or pharmacologically active substance that produces a local or systemic effect.

Fill Composition

The fill composition for the soft elastic capsule of the invention can include a solubilized HIV protease inhibiting compound or a combination of solubilized HIV protease inhibiting compounds. Preferably, the HIV protease inhibiting compound is a compound of the formula I, formula II, saquinavir, nelfinavir, amprenavir, or indinavir. More preferably the compound is ritonavir, lopinavir, saquinavir, nelfinavir, amprenavir, or indinavir. Most preferably the compound is ritonavir or lopinavir. A combination of solubilized HIV protease inhibiting compounds can also be used. Preferably the combination is ritonavir or nelfinavir and another HIV protease inhibitor, for example, lopinavir, saquinavir, indinavir, amprenavir, or nelfinavir. More preferably, the combination is of ritonavir or nelfinavir and another HIV protease inhibitor, for example, lopinavir, saquinavir, indinavir, amprenavir, or

nelfinavir. Most preferably the combination is ritonavir and lopinavir. A preferred ratio of the amount, as measured by weight, of ritonavir to lopinavir in the fill composition is in the range of 2:1 to 1:10; more preferably this ratio is in the range of 1:1 to 1:10; most preferably this ratio is in the range of 1:3 to 1:5, and it is most highly preferred that the ratio is about 1:4.

The HIV protease inhibiting compound or a combination of solubilized HIV protease inhibiting compounds can be present in the fill composition, as measured by weight, in a range of 10-35%. More preferably, the HIV protease inhibiting compound or compounds are present in the range of 12.5-22.5%. Most preferably, the HIV protease inhibiting compound or compounds are present in the range of 15- 20%.

This HIV protease inhibiting compound or a combination of HIV protease inhibiting compounds can be solubilized in a pharmaceutically acceptable organic solvent. Preferably, the pharmaceutically acceptable organic solvent comprises from about 75% to about 90% by weight of the fill composition. More preferably, the pharmaceutically acceptable organic solvent or mixture of pharmaceutically acceptable organic solvents comprises from about 77.5% to about 87.5% by weight of the fill composition. This pharmaceutically acceptable organic solvent of the fill composition can include (1) a pharmaceutically acceptable medium and/or long chain fatty acid or mixtures thereof; (2) an alcohol, preferably propylene glycol; and (3) a pharmaceutically acceptable surfactant. Optionally, water can be included in the fill composition and can be present preferably in a percentage by weight that is relatively low as compared to the other components of the fill composition.

The fill composition can be prepared with a percentage by weight of organic solvent sufficient to solubilize the pharmaceutical agent or combination of pharmaceutical agents, for example, the HIV protease inhibitors, contained in the initial fill composition. In one embodiment of the invention, the initial fill composition contains an increased percentage by weight of alcohol, for example, propylene glycol, sufficient to solubilize the pharmaceutical agent or combination of pharmaceutical agents. The percentage of alcohol, for example, propylene glycol, is increased relative to the other components in the initial fill composition. The alcohol percentage is increased greater than the amount of alcohol necessary to solubilize the pharmaceutical agents, for example HIV protease inhibitors. In this embodiment, the propylene glycol is preferably increased by 100-200% of the minimal amount of propylene glycol necessary to solubilize the HIV protease inhibitors in the initial fill composition.

WE CLAIM:

1. A soft elastic capsule having a fill and a shell, the fill comprising:
a pharmaceutical agent or combination of pharmaceutical agents,
an alcohol,
a fatty acid, and
the shell comprising:
gelatin, and
at least one plasticizing agent,
wherein said shell has an initial state and an equilibrium state, wherein said initial state shell is underplasticized and said fill contains an alcohol amount in excess of an alcohol amount necessary to solubilize said pharmaceutical agent and said alcohol is not present in the initial state shell composition, and wherein said equilibrium state said shell becomes plasticized by the alcohol from said fill to provide suitable capsule hardness and said fill retains sufficient alcohol to maintain said pharmaceutical agent in solution.
2. The soft elastic capsule of claim 1 wherein the amount of alcohol present in said fill in initial state is in the range of 100-200% greater than the amount necessary to solubilize said pharmaceutical agent.
3. The soft elastic capsule of claim 1 wherein a portion in the range of 40-50% of the amount of alcohol present in said fill in initial state migrates into the shell upon equilibrium state.
4. The soft elastic capsule of claim 1 wherein said pharmaceutical agent comprises a solubilized HIV inhibiting compound or a combination of solubilized HIV inhibiting compounds.
5. The soft elastic capsule of claim 4 wherein said HIV inhibiting compound is (2S, 3S, 5S)-5-(N-(N-((N-methyl-N-N-((2-isopropyl-4-thiazolyl)- methyl)amino)carbonyl)-L-

valinyl)amino-2-(N-((5-thiazolyl)methoxy-carbonyl)-amino-1,6-diphenyl-3-hydroxyhexane (ritonavir).

6. The soft elastic capsule of claim 4 wherein said combination of solubilized HIV inhibiting compounds is (2S, 3S, 5S)-5-(N-(N-((N-methyl-N-N-((2-isopropyl-4-thiazolyl)-methyl)amino)carbonyl)-L-valinyl)amino-2-(N-((5-thiazolyl)methoxy-carbonyl)-amino-1,6-diphenyl-3-hydroxyhexane (ritonavir) and (2S, 3S, 5S)-2-(2,6-dimethylphenoxyacetyl)-amino-3-hydroxy-5-(2S-(1-tetrahydropyrimid-2-onyl)-3-methyl-butanoyl)amino-1,6-diphenylhexane (lopinavir).
7. The soft elastic capsule of claim 4 wherein said solubilized HIV inhibiting compound or said combination of solubilized HIV inhibiting compounds is present in said fill in a range of 10–35% by weight.
8. The soft elastic capsule of claim 1 wherein said alcohol is propylene glycol or ethanol.
9. The soft elastic capsule of claim 8 wherein propylene glycol or ethanol are present in said fill in initial state in a range of 3–20% by weight.
10. The soft elastic capsule of claim 1 wherein said alcohol concentration in the fill decreases by not more than 20% after reaching said equilibrium state.
11. The soft elastic capsule of claim 10 wherein after reaching said equilibrium state said shell has acceptable physical characteristics.
12. The soft elastic capsule of claim 11 wherein the acceptable physical characteristics of said shell comprise
 - a hardness in the range of about 4.5 to about 9 Newtons,
 - a total weight of said capsule within 95% of initial weight of capsule and total weight of said fill within 85% of initial weight of said fill,
 - a moisture content of not more than 2% of said fill weight of said capsule,

a disintegration time of not more than 30 minutes, and
an alcohol concentration of at least 40 mg per gram of said fill.

13. The soft elastic capsule of claim 1 wherein said fatty acid is oleic acid.
14. The soft elastic capsule of claim 13 wherein said oleic acid is present in said fill composition in initial state in the range of 50-80% by weight.
15. The soft elastic capsule of claim 1 wherein said fill further comprises a surfactant.
16. The soft elastic capsule of claim 15 wherein said surfactant is Polyoxyl 35 castor oil (Cremophor EL[®]).
17. The soft elastic capsule of claim 15 wherein said surfactant is present in the range of 0-10% by weight of the fill.
18. The soft elastic capsule of claim 1 wherein the gelatin in said capsule is present in the range of 30-60 % by weight of the shell.
19. The soft elastic capsule of claim 1 wherein the plasticizing agent is sorbitol, glycerin, macrogols, or Andrisorb[™].
20. The soft elastic capsule of claim 19 wherein the plasticizing agent is present in initial state in the range of 4-18% by weight of the shell.
21. The soft elastic capsule of claim 1 wherein the ratio of the weight percentage of gelatin to plasticizing agent at said equilibrium state is in the range of 2:1 to 4:1.
22. The soft elastic capsule of claim 1 wherein the fill in an initial state comprises:
is (2S, 3S, 5S)-5-(N-(N-((N-methyl-N-((2-isopropyl-4-thiazolyl)-methyl)amino)carbonyl)-L-valinyl)amino-2-(N-((5-thiazolyl)methoxy-carbonyl)-amino-1,6-diphenyl-3-hydroxyhexane (ritonavir) and (2S, 3S, 5S)-

2-(2,6 dimethylphenoxyacetyl)-amino-3-hydroxy-5-(2S-(1-tetrahydropyrimid-2-onyl)-3-methyl-butanoyl)amino-1,6-diphenylhexane (lopinavir) in the range of 10–35% by weight of the fill composition; polyethylene glycol in the range of 3–20% by weight of the fill composition; oleic acid in the range of 50–80% by weight of the fill; and wherein the shell in an initial state comprises: gelatin in the range of 30–60% by weight of the shell, and Andrisorb in the range of 4–18% by weight of the shell.

23. A method for making soft elastic capsules containing a pharmaceutical agent or a combination of pharmaceutical agents, the method comprising the steps of:
- preparing a fill composition wherein the fill composition comprises
 - a pharmaceutical agent or combination of pharmaceutical agents,
 - a fill alcohol, and
 - a fatty acid;
 - preparing a shell composition wherein the shell composition comprises
 - gelatin, and
 - at least one plasticizing compound;
 - forming an soft elastic capsule wherein said shell has an initial state and an equilibrium state, wherein said initial state shell is underplasticized and said fill contains an alcohol amount in excess of an alcohol amount necessary to solubilize said pharmaceutical agent and said alcohol is not present in the initial state shell composition; and
 - equilibrating said soft elastic capsule wherein equilibrium state said shell becomes plasticized by the alcohol from said fill to provide suitable capsule hardness and said fill retains sufficient alcohol to maintain said pharmaceutical agent in solution.
24. The method of claim 23 wherein said pharmaceutical agent comprises a solubilized HIV inhibiting compound or a combination of solubilized HIV inhibiting compounds.
25. The method of claim 23 wherein said alcohol is propylene glycol or ethanol.

26. The method of claim 23 wherein said fatty acid is oleic acid.
27. The method of claim 23 wherein said fill further comprises a surfactant.
28. The method of claim 23 wherein the plasticizing agent is sorbitol, glycerin, macrogols, or Andrisorb™.



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(54) **SOLVATES AND POLYMORPHS OF
RITONAVIR AND METHODS OF MAKING
AND USING THE SAME**

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(57) **ABSTRACT**

Novel solvates and crystal polymorphs of Ritonavir are disclosed, as well as methods of making them. Specific solvates of the compound include a formamide solvate and a partially desolvated solvate. Also disclosed are methods of making previously known forms of Ritonavir. Methods of using the novel forms of Ritonavir for the treatment of diseases, such as HIV-infection, are disclosed, as are pharmaceutical compositions and unit dosage forms comprising the novel forms of Ritonavir.

a "drug cocktail"). Current therapies for HIV infection focus on inhibiting the activity of viral enzymes that are critical in the life cycle of the virus, such as reverse transcriptase and protease. Antiretrovirals that are presently in use are generally grouped into three classes: nucleoside reverse transcriptase inhibitors ("NRTIs"); non-nucleoside reverse transcriptase inhibitors ("NNRTIs"); and protease inhibitors ("PIs"). Combination therapies using such compounds have been shown to reduce the incidence of opportunistic infections and to increase survival time. It is possible that these and other benefits of combination therapies may be further improved by the use of new crystalline forms of Ritonavir.

3. SUMMARY OF THE INVENTION

[0010] This invention encompasses novel solvates and crystal polymorphs of Ritonavir. Specific solvates of the compound include a formamide solvate and a partially desolvated formamide solvate. All of the novel forms exhibit physical and spectroscopic characteristics that differ markedly from those of Forms I and II.

[0011] The invention further encompasses methods of making novel forms of Ritonavir, as well as methods of making previously known forms. Also encompassed by the invention are methods of using the novel forms of Ritonavir in the treatment of diseases, such as HIV-infection, and for enhancing the pharmacokinetic profiles of other pharmaceutically active compounds. Pharmaceutical compositions and unit dosage forms comprising novel forms of Ritonavir are also encompassed by the invention.

3.1. BRIEF DESCRIPTION OF THE FIGURES

[0012] Aspects of the invention can be understood with reference to the following non-limiting figures:

[0013] FIG. 1 is a Raman spectrum of Ritonavir Form I in the solid state.

[0014] FIG. 2A is a powder X-ray diffraction pattern of Ritonavir Form I, and FIG. 2B contains a peak table for that diffraction pattern.

[0015] FIG. 3 contains a Raman spectrum of Ritonavir Form II in the solid state.

[0016] FIG. 4A is a powder X-ray diffraction pattern of Ritonavir Form II, and FIG. 4B contains a peak table for that diffraction pattern.

[0017] FIG. 5 is a DSC trace of Ritonavir Form III.

[0018] FIG. 6 is a TGA trace of Ritonavir Form III.

[0019] FIG. 7 is a Raman spectrum of Ritonavir Form III in the solid state.

[0020] FIG. 8A is a powder X-ray diffraction pattern of Ritonavir Form III, and FIG. 8B contains a peak table for that diffraction pattern.

[0021] FIG. 9 is a DSC trace of Ritonavir Form IV.

[0022] FIG. 10 is a TGA trace of Ritonavir Form IV.

[0023] FIG. 11 is a Raman spectrum of Ritonavir Form IV in the solid state.

[0024] FIG. 12A is a powder X-ray diffraction pattern of Ritonavir Form IV, and FIG. 12B contains a peak table for that diffraction pattern.

[0025] FIG. 13 is a DSC trace of Ritonavir Form V.

[0026] FIG. 14 is a TGA trace of Ritonavir Form V.

[0027] FIG. 15 is a Raman spectrum of Ritonavir Form V in the solid state.

[0028] FIG. 16A is a powder X-ray diffraction pattern of Ritonavir Form V, and FIG. 16B contains a peak table for that diffraction pattern.

[0029] FIG. 17 shows, by DSC trace, the conversion of Ritonavir Form III to Form IV, and subsequently to Form I while being washed or incubated in aqueous media.

[0030] FIG. 18 provides a comparison of powder X-ray diffraction patterns of Ritonavir Forms I, II, III, IV, and V.

[0031] FIG. 19 shows the effect of leaving Ritonavir Form V in the crystallization mixture for about 16 hours at 5° C. In particular: (A) is a representative X-ray diffraction pattern of Ritonavir(I); (B) is an X-ray diffraction pattern of a sample of Ritonavir(V) after being left in the crystallization mixture for approximately 16 hours, with arrows indicating peaks that signify the presence of Ritonavir(I); and (C) is a representative X-ray diffraction pattern of Ritonavir(V).

[0032] FIG. 20 also shows the effect of leaving Ritonavir Form V in the crystallization mixture for about 16 hours at 5° C. In particular: (A) provides a DSC trace of Ritonavir(I); (B) provides a DSC trace of a sample of Ritonavir(V) after being left in the crystallization mixture for approximately 16 hours, wherein two peaks are observed corresponding to Forms I and V; and (C) is a representative DSC trace of Ritonavir(V).

4. DETAILED DESCRIPTION OF THE INVENTION

[0033] This invention is based, in part, on a discovery that the protease inhibitor ("PI") Ritonavir can be obtained in novel crystalline forms. Advantageously, these new forms have lower melting points than previously known forms of Ritonavir (i.e., Forms I and II), which have poor bioavailability, and may thus be used to provide improved dissolution of Ritonavir. Forms of this invention may also be used to enhance the pharmacokinetics of other drugs (e.g., retroviral agents and reverse transcriptase inhibitors). The new forms of Ritonavir may also be used as intermediates in the manufacture of Forms I and II, as well as in the manufacture of pharmaceutical compositions and dosage forms comprising Ritonavir (e.g., dosage forms comprising dissolved Ritonavir). A further advantage of this invention is that methods of preparing the novel forms disclosed herein can be used to prepare those forms on a variety of scales, from microgram to milligram, gram, and even kilogram quantities.

[0034] A first embodiment of the invention encompasses what is referred to herein as "Ritonavir Form III" or "Ritonavir(III)." Ritonavir(III) has a melting point in the range of from about 78° C. to about 82° C., as exemplified by the Differential Scanning Calorimetry ("DSC") trace of the form shown in FIG. 5, which shows a melting point of about 79° C. A typical Thermal Gravimetric Analysis ("TGA") trace of Ritonavir(III) is provided in FIG. 6. Typical Raman spectra and powder X-ray diffraction patterns of Ritonavir(III) are provided in FIGS. 7 and 8,

are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients can be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose or other mono- or di-saccharides. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient.

[0064] Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmacopeia (USP) 25-NF20 (2002). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

[0065] This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. Water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

[0066] Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

[0067] An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials) with or without dessicants, blister packs, and strip packs.

[0068] The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

[0069] Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise Ritonavir in an amount of from about 50 mg to about 1000 mg, preferably in an amount of from about 75 mg to about 750 mg, and most preferably in an amount of from about 100 mg to about 500 mg.

4.4.1. ORAL DOSAGE FORMS

[0070] Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed., Mack Publishing, Easton Pa. (1990).

[0071] Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, stabilizers, and disintegrating agents.

[0072] Because of their ease of administration, tablets, caplets, and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets and caplets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

[0073] For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[0074] Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to,

sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and propylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

[0095] Certain aspects and embodiments of the invention are illustrated by the following non-limiting examples.

5.1. EXAMPLE 1

[0096] Preparation of Ritonavir Form I

[0097] NORVIR-brand Ritonavir (Abbott Laboratories, North Chicago, Ill. USA) oral solution was used to obtain Ritonavir Form I. Specifically, approximately 240 mL of NORVIR solution (the contents of a normal prescription bottle, totaling about 19.2 g of Ritonavir) were placed into a 500 mL round bottom flask and vacuum-treated (pumping at high vacuum) for about 6 hours at room temperature on a slightly heated water bath to keep temperature constant. 50 mL of ethyl acetate was added to the viscous residue (dark orange in color).

[0098] The resulting solution was placed into a 2 liter flask and while stirring, diethyl ether was added to a final volume of about 1800 mL. The solution was stirred for 30 minutes at room temperature to allow precipitation to occur. The ether phase was poured through a 150 mL sintered glass, coarse frit funnel to clarify the ether. Successive aliquots of approx. 300 mL of filtrate were placed into a 500 mL round bottom flask and the solvent was removed by rotary evaporation in vacuo (water aspirator) until a slightly off-white residue remained.

[0099] Ether aliquots were added to the residue in the round bottom flask until all the ether had been evaporated. The final residue was transferred into a 500 mL Erlenmeyer flask and diluted with methylene chloride to a final volume of about 150 mL. A total of about 350 mL deionized water was added to the methylene chloride solution by gently pouring down the edge of the flask, while the solution was stirred by a magnetic stirbar on a stirplate at approximately 200 rpm.

[0100] After about 1.5 hours of stirring, the mixture was poured into a separatory funnel. After settling, the methylene chloride fraction (bottom layer) was separated into an Erlenmeyer flask, and allowed to dry over anhydrous sodium sulfate for 15 minutes (during which time the organic solvent went from opaque to clear).

[0101] After filtering the methylene chloride solution, the solvent was removed by rotary evaporation. The resulting residual syrup was placed in a -20° C. freezer overnight to allow crystals to form. The crystals were isolated, and then the solid compound was dissolved in approximately 350 mL

of ethyl acetate at about 44° C. Approximately 175 mL of hexane was added with stirring until product began to precipitate.

[0102] A sealed vial containing the mixture was placed in a refrigerator at 4° C. overnight to crystallize. Recrystallization in ethyl acetate/hexane was repeated once more to give the final product, which was dried in vacuo for about 12 hours.

[0103] The identity of the sample as Ritonavir Form I was established by powder X-ray diffraction (distinguishing peaks at 3.3, 6.8, 8.4, and 21.6 2-theta) and purity by elemental analysis (Calcd. %—C: 61.66, H: 6.66, N: 11.66; Obsd. %—C: 61.74, H: 6.78, N: 11.41).

5.2. EXAMPLE 2

[0104] Preparation of Ritonavir(III)

[0105] In order to prepare Ritonavir(III), 10 mg of Ritonavir Form I obtained in Example 1 above, 37.5 μ L of formamide, and 12.5 μ L of toluene (50 μ L solvent total, containing 75% formamide) were deposited into a crimp-top 200 μ L capacity glass vial. The vial was sealed with a crimp top and subsequently incubated at 70° C. until the Ritonavir solid dissolved. The vial was then cooled at a rate of approximately 5° C./minute to 5° C. The vial was incubated at 5° C. until crystals were observed. The crystals in the vial were removed from the supernatant by filtration, air dried, and characterized as described herein.

[0106] Ritonavir(III) was also prepared using the same process conditions from the following mixtures: 10 mg of Ritonavir Form I, 37.5 μ L of formamide, and 12.5 μ L of butyl acetate; and 10 mg of Ritonavir Form I, 37.5 μ L of formamide, and 12.5 μ L of acetone.

5.3. EXAMPLE 3

[0107] Preparation of Ritonavir(IV)

[0108] 50 mg of Ritonavir(III) obtained in Example 2 above was placed on filter paper in a Buchner funnel, which was fitted to a vacuum flask connected to an aspirator. With the vacuum on, 10 mL of deionized water was slowly poured over the solid Ritonavir(III). The resulting material on the filter was dried over vacuum for approximately 15 minutes, and characterized as described herein.

5.4. EXAMPLE 4

[0109] Preparation of Ritonavir(V)

[0110] 10 mg of Ritonavir Form I obtained in Example 1 above, 37.5 μ L of butyl acetate, and 12.5 μ L of acetonitrile (50 μ L solvent total, containing 25% acetonitrile) were deposited into a crimp-top 200 μ L capacity glass vial. The vial was sealed with a crimp top and subsequently incubated at 70° C. until the Ritonavir solid dissolved. The vial was then cooled at a rate of approximately 5° C./minute to 5° C. The vial was incubated at 5° C. until crystals were observed. The crystals in the vial were removed from the supernatant by filtration, and were subsequently air dried, and characterized as described herein.

[0111] Ritonavir(V) was also prepared using the same conditions from the following mixtures: 10 mg of Ritonavir

distinctive of each form using a dispersive Near-IR laser-based Raman spectrometer are listed below in Table 2:

TABLE 4

Form	Unique Raman Peaks (cm ⁻¹)
I	428.1, 821.3, 928.1, 966.3, 982.7, 1033.2, 1452.5, 1444.2, 1461.9, 1647.9
II	417.4, 443.4, 579.9, 954.1, 1029.1, 1662.0
III	691.13, 1393.29
IV	1404.73
V	814.6, 963.7

[0121] The distinct nature of each of the forms of Ritonavir is also evident from powder X-ray diffraction data. Graphical and tabular X-ray diffraction data for each of the three forms of the compound are provided in FIGS. 8, 12, and 16. Several peaks that are distinctive to each form are listed below in Table 3:

TABLE 3

Form	Unique X-ray Diffraction Peaks (degrees 2-Theta)
III	3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, 13.1
IV	3.1, 6.1, 9.2, 12.1, 13.8
V	3.4, 6.4, 9.9, 12.7, 13.6, 15.3, 15.8, 17.5

[0122] The difference between the forms is even more apparent from FIG. 18, which provides a comparison of diffractograms obtained for each of the forms of Ritonavir. These data show that Ritonavir(III) and Ritonavir(IV) exhibit highly similar XRD patterns, which is consistent with a conserved structure that is solvated in one form (Ritonavir(III)) and partially (or essentially fully) desolvated in another form (Ritonavir(IV)).

5.5. EXAMPLE 5

[0123] Conversion of Form V to Form I

[0124] Ritonavir(V) can transform into other forms of Ritonavir under certain conditions. For example, if Ritonavir(V) is prepared as above in Example 3, it was found to form a mixture of Forms V and I when left in the crystallization mixture for a prolonged period of time (hours to days). In a particular instance, Ritonavir(V) that was left in the crystallization mixture for approximately 16 hours at the nucleation temperature of 5° C. formed Ritonavir(I) as evidenced by X-ray diffraction (FIG. 19) and DSC (FIG. 20).

[0125] While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as recited by the appended claims.

What is claimed is:

1. A composition comprising:

a) ritonavir(III);

b) a form of Ritonavir which has a melting point of about 78° C. to about 82° C.;

c) a form of Ritonavir which has a powder X-ray diffraction pattern substantially the same as that shown in FIG. 8A;

d) a form of Ritonavir which has at least one unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

e) a form of Ritonavir which has at least two unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

f) a form of Ritonavir which has at least three unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

g) a form of Ritonavir which has at least four unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

h) a form of Ritonavir which has at least five unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

i) a form of Ritonavir which has at least six unique powder X-ray diffraction peaks selected from the group of: approximately 3.1, 6.8, 7.7, 8.3, 9.2, 10.5, 12.1, and 13.1 degrees 2-Theta;

j) ritonavir (IV);

k) a form of Ritonavir which has a melting point of about 97° C. to about 101° C.;

l) a form of Ritonavir which has a powder X-ray diffraction pattern substantially the same as that shown in FIG. 12A;

m) a form of Ritonavir which has at least one unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

n) a form of Ritonavir which has at least two unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

o) a form of Ritonavir which has at least three unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

p) a form of Ritonavir which has at least four unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

q) a form of Ritonavir which has at least five unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

r) a form of Ritonavir which has at least six unique powder X-ray diffraction peaks selected from the group consisting of: approximately 3.1, 6.1, 9.2, 12.1, and 13.8 degrees 2-Theta;

- s) ritonavir(V);
 - t) a form of Ritonavir which has a melting point of from about 114° C. to about 118° C.;
 - u) a form of Ritonavir which has a powder X-ray diffraction pattern substantially the same as that shown in **FIG. 16A**;
 - v) a form of Ritonavir which has unique powder X-ray diffraction peaks at approximately 3.4, 6.4, 9.9, 12.7, 13.6, 15.3, 15.8, and 17.5 degrees 2-Theta;
 - w) formamide, an immiscible or partially miscible solvent, and Ritonavir; or
 - x) acetate, acetonitrile, and Ritonavir.
2. A method selected from:
- a) a method of preparing Ritonavir(III), which comprises dissolving Ritonavir in a solvent system comprised of formamide and an immiscible or partially miscible solvent to provide a mixture, and reducing the solubility of Ritonavir in the mixture under conditions sufficient to provide Ritonavir(III);
 - b) a method of preparing Ritonavir(IV), which comprises partially desolvating Ritonavir(III) to an extent sufficient to form Ritonavir(IV);
 - c) a method of preparing Ritonavir(V), which comprises dissolving Ritonavir in a solvent system comprised of an acetate and acetonitrile to provide a mixture, and reducing the solubility of Ritonavir in the mixture under conditions sufficient to provide Ritonavir(V);
 - d) a method of preparing Ritonavir Form I, which comprises desolvating Ritonavir(IV) for an amount of time sufficient for the formation of Ritonavir Form I;
 - e) the method of claim (d), which comprises incubating Ritonavir(IV) in an aqueous environment for an amount of time sufficient for the formation of Ritonavir Form I; or
 - f) a method of preparing Ritonavir Form II, which comprises incubating Ritonavir(IV) in an aqueous environment for an amount of time sufficient for the formation of Ritonavir Form I and further incubating the Ritonavir Form I for an amount of time sufficient for the formation of Ritonavir Form II.
3. A method selected from:
- a) a method of inhibiting HIV-1 protease, which comprises contacting HIV-1 protease with Ritonavir(III), Ritonavir(IV), or Ritonavir(V);
 - b) a method of inhibiting HIV-2 protease, which comprises contacting HIV-2 protease with Ritonavir(III), Ritonavir(IV), or Ritonavir(V);
 - c) a method of treating, ameliorating, or managing HIV-infection, which comprises administering to a patient in need of such treatment a therapeutically effective amount of Ritonavir(III), Ritonavir(IV), or Ritonavir(V);
 - d) the method of claim (a), (b), or (c), wherein the form of Ritonavir is Ritonavir(IV) or Ritonavir(V);
 - e) the method of claim (d) wherein the form of Ritonavir is Ritonavir(V);
 - f) the method of claim (a), (b), or (c), which further comprises administering a therapeutically effective amount of a second pharmacologically active compound to the patient;
 - g) the method of claim (f) wherein the second pharmacologically active compound is a protease inhibitor, a nucleoside reverse transcriptase inhibitor, or a non-nucleoside reverse transcriptase inhibitor, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or polymorph thereof;
 - h) the method of claim (g) wherein the protease inhibitor is lopinavir, saquinavir, indinavir, nelfinavir, amprenavir, palinavir, lasinavir, or tipranavir;
 - i) the method of claim (g) wherein the nucleoside reverse transcriptase inhibitor is zidovudine, zalcitabine, lamivudine, didanosine, abacavir, didoxil, stavudine, adefovir, adefovir dipivoxil, or fozivudine;
 - j) the method of claim (g) wherein the non-nucleoside reverse transcriptase inhibitor is delavirdine, efavirenz, immunocal, loviride, nevirapine, or oltpiraz;
 - k) a method for improving the pharmacokinetics of a pharmacologically active compound, which comprises administering to a human in need of the pharmacologically active compound a combination of a therapeutically effective amount of Ritonavir(III), (IV), or (V), or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of the pharmacologically active compound, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or polymorph thereof, wherein the pharmacologically active compound is metabolized by a cytochrome P450 monooxygenase;
 - l) the method of claim (k) wherein the pharmacologically active compound is an antiretroviral compound;
 - m) the method of claim (l) wherein the antiretroviral compound is a protease inhibitor, a nucleoside reverse transcriptase inhibitor, or a non-nucleoside reverse transcriptase inhibitor, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or polymorph thereof;
 - n) the method of claim (m) wherein the protease inhibitor is lopinavir, saquinavir, indinavir, nelfinavir, amprenavir, palinavir, lasinavir, or tipranavir;
 - o) the method of claim (m) wherein the nucleoside reverse transcriptase inhibitor is zidovudine, zalcitabine, lamivudine, didanosine, abacavir, didoxil, stavudine, adefovir, adefovir dipivoxil, or fozivudine;
 - p) the method of claim (m) wherein the non-nucleoside reverse transcriptase inhibitor is delavirdine, efavirenz, immunocal, loviride, nevirapine, or oltpiraz;
 - q) a method of preparing a pharmaceutical composition comprising Ritonavir, which comprises contacting Ritonavir(III), Ritonavir(IV), or Ritonavir(V) with an aqueous solution; or
 - r) a method of preparing a pharmaceutical composition comprising Ritonavir, which comprises contacting Ritonavir (III), Ritonavir (IV), or Ritonavir (V) with an alcohol-based solution.

4. A pharmaceutical composition comprising Ritonavir(V).

5. A single unit dosage form comprising the composition of claim 4.

6. A kit comprising a single unit dosage form of Ritonavir(V) and a single unit dosage form of an antiviral compound.

7. The kit of claim 6 wherein the antiretroviral compound is a protease inhibitor, a nucleoside reverse transcriptase inhibitor, or a non-nucleoside reverse transcriptase inhibitor, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or polymorph thereof.

* * * * *



ESCRITURA NUMERO CUATRO MIL QUINIENTOS -
 TREINTA Y NUEVE (No. 4.539) -----
 En la ciudad de Cali, Capital del Depar-
 tamento del Valle del Cauca, República de
 Colombia, a los VEINTITRES (23) días -
 del mes de OCTUBRE del año DOS MIL UNO --

(2.001), ante mí, JORGE ENRIQUE CAICEDO ZAMORANO, Notario
 T E R C E R O (3o.) del Círculo de Cali, - - - - -

compareció JUAN MANUEL BARBERI OSPINA mayor de edad, vecino de Cali,
 identificado con la cédula de ciudadanía número 14.949.561 de Cali, y dijo: ----

PRIMERO.- Que obra en su carácter de Representante Legal de en nombre y
 representación de TECNOQUIMICAS S.A. sociedad comercial organizada y existente
 conforme a las leyes colombianas, con domicilio y sede principal en Cali constituida

mediante escritura pública número 2670 de la Notaría Segunda de fecha 12 de
 junio de 1957, reformada en distintas oportunidades, el cual consta en el certificado
 expedido por la Cámara de Comercio de Cali

SEGUNDO: Que en tal carácter, por este público instrumento, concedo Poder Especial
 pero amplio y suficiente a JOSE LUIS REYES VILLAMIZAR, mayor de edad, vecino de
 Bogotá, identificado con C.C. # 79.152.473 quien, abogado en ejercicio, portador

de la T.P 44655 del C.S. de la J., domiciliado en Bogotá, D.C., República de Colombia
 con dirección carrera 7 No. 88.23 oficina 205, de la misma ciudad, como su representante
 con poder especial, amplio y suficiente, para obtener la protección de nuestra propiedad

industrial y contra la competencia desleal, a cuyo efecto lo autorizo, para que nos
 represente ante cualesquiera entidad o persona, ejerzan o no jurisdicción, especialmente
 ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo

concerniente a los siguientes asuntos: a) Obtención, formulación de observaciones y
 oposición a patentes de invención, modelos y dibujos industriales y el registro de
 licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad,

medidas cautelares, concesión de licencias y en general los procesos civiles, penales,
 administrativos o contencioso-administrativos relacionados con estos derechos u otros de
 diferente naturaleza; acciones y procesos que promovamos o se nos promuevan; y c) para

Conc. Notario, cédulas de este Círculo
 hago constar que esta fotocopia es
 a la vista.
 13 AGO 2007
 WILLY VALEK MORA
 NOTARIO VENTIDOS
 Bogotá D.C., Colombia

4539
 OCT 23

LUZ MERY CORREA
 ELABORACION DE ESCRITURAS
 Notaría 3ª de Cali

DECLARACION DE AUTENTICACION
 El suscrito Notario, en virtud de la
 diligencia practicada en el Círculo de
 Bogotá, D.C., HA CONSTATADO
 la copia fotostática precedida por el
 original, que fue tomada y que fue a
 vista, en Bogotá, D.C., el 23 de
 octubre de 2007.
 REPUBLICA DE COLOMBIA
 Notario Noveno

que en todos los asuntos arriba determinados, , tanto en los términos señalados por los artículos 42 y complementarios de la Decisión 486 de la Comunidad Andina como del artículo 70 del C.P.C pueda sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistir, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos y ceder a título gratuito y/o oneroso, licenciar, inscribir contratos de cesión y de licencia, inscripción de prendas, levantamiento de prendas, inscribir derechos de autenticación de general todos los asuntos que se deban tramitar ante la Superintendencia de Industria y Comercio.

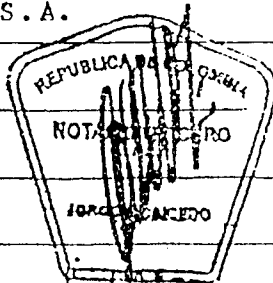
Leído el presente instrumento al otorgante, lo aprobó y en constancia firma el Notario de todo lo cual doy fe.

Se agregan comprobantes. Derechos \$30.000.00 Decreto 1681 de Septiembre 16 de 1996. Reformado mediante Resolución No. 5839 de Diciembre 27 del año 2.000 y Decreto 1326 de Junio 19 de 2001. Se corrió en la hoja número AA 3286877. ENMENDADO "OCTUBRE" VALE.

Juan m Barber

JUAN MANUEL BARBERI OSPINA

En rep. de TECNOQUIMICAS S.A.



Como Notario Veintitres de este Circulo hago constar que esta fotocopia coincide con su fotocopia autentica que he tenido a la vista.

13 AGO 2007

WILLY VALEK MORA
NOTARIO VEINTITRES
Bogota D.C., Colombia

El Notario Tercero de Cali

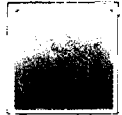
Dr. JORGE ENRIQUE CAICEDO ZAMORANO



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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 1

EN JUNIO DE ESTE AÑO SE ELEGIRÁ JUNTA DIRECTIVA Y REVISOR FISCAL DE LA CAMARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACION DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL TELEFONO 8861339.

CERTIFICA:

NOMBRE: TECNOQUIMICAS S A
DOMICILIO: CALI VALLE
DIRECCION COMERCIAL : ---C 23 7 39
DIRECCION NOTIFICACION JUDICIAL: ---C 23 7 39
CIUDAD: CALI
MATRICULA MERCANTIL NRO. 7049-4 FECHA MATRICULA : 01 DE JULIO DE 1957
AFILIADO

CERTIFICA:

NIT : 890300466-5

CERTIFICA:

QUE POR ESCRITURA NRO. 2670 DEL 12 DE JUNIO DE 1957 NOTARIA SEGUNDA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 01 DE JULIO DE 1957 BAJO EL NRO. 16627 DEL LIBRO IX , SE CONSTITUYO LA SOCIEDAD DENOMINADA COMPANIAS TECNOQUIMICAS LIMITADA

CERTIFICA:

QUE POR ESCRITURA NRO. 8438 DEL 30 DE DICIEMBRE DE 1970 NOTARIA SEGUNDA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 07 DE ENERO DE 1971 BAJO EL NRO. 42260 DEL LIBRO IX , LA SOCIEDAD SE TRANSFORMO DE SOCIEDAD LIMITADA EN SOCIEDAD EN COMANDITA POR ACCIONES BAJO EL NOMBRE DE COMPANIAS TECNOQUIMICAS LIMITADA COMANDITA POR ACCIONES (SIC)

CERTIFICA:

QUE POR ESCRITURA NRO. 2139 DEL 21 DE ABRIL DE 1972 NOTARIA SEGUNDA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 26 DE ABRIL DE 1972 BAJO EL NRO. 794 DEL LIBRO IX , LA SOCIEDAD CAMBIO SU NOMBRE DE COMPANIAS TECNOQUIMICAS LIMITADA

*** CONTINUA ***

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 2

COMANDITA POR ACCIONES (SIC) . POR EL DE TECNOQUIMICAS LIMITADA & CIA S.C.
A. .

CERTIFICA:

QUE POR ESCRITURA NRO. 3255 DEL 30 DE AGOSTO DE 1983 NOTARIA DECIMA DE CALI ,
INSCRITA EN LA CAMARA DE COMERCIO EL 31 DE AGOSTO DE 1983 BAJO EL NRO. 62343
DEL LIBRO IX ,LA SOCIEDAD SE TRANSFORMO DE SOCIEDAD EN COMANDITA POR ACCIONES
EN SOCIEDAD AN?NIMA BAJO EL NOMBRE DE TECNOQUIMICAS S A .

CERTIFICA:

QUE POR ESCRITURA NRO. 5664 DEL 12 DE DICIEMBRE DE 2005 NOTARIA TERCERA DE
CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 19 DE DICIEMBRE DE 2005 BAJO EL
NRO. 14102 DEL LIBRO IX ,SE APROBO LA FUSION POR ABSORCION ENTRE (ABSORBENTE)
TECNOQUIMICAS S.A. Y (ABSORBIDA (S)) DISTRIBUIDORA FARMACEUTICA CALOX
COLOMBIANA S.A. - EN LIQUIDACION- ,
ORGANIZACION FARMACEUTICA AMERICANA S.A. OFA - EN LIQUIDACION- .

CERTIFICA:

REFORMAS DOCUMENTO INS LIBRO	FECHA.DOC	ORIGEN	FECHA.INS	NRO.
E.P. 4075 16922	05/09/1957	NOTARIA SEGUNDA DE CALI	27/09/1957	
E.P. 5718 17242	28/11/1957	NOTARIA SEGUNDA DE CALI	30/12/1957	
E.P. 5507 17529	31/12/1957	NOTARIA PRIMERA DE CALI	13/03/1958	
E.P. 3124 18365	16/07/1958	NOTARIA PRIMERA DE CALI	17/10/1958	
E.P. 3325 18366	29/07/1958	NOTARIA PRIMERA DE CALI	17/10/1958	
E.P. 5726 19258	17/12/1958	NOTARIA PRIMERA DE CALI	12/05/1959	
E.P. 2374 19368	12/05/1959	NOTARIA PRIMERA DE CALI	03/06/1959	
E.P. 2793 22616	20/06/1961	NOTARIA PRIMERA DE CALI	07/07/1961	
E.P. 2794 22617	20/06/1961	NOTARIA PRIMERA DE CALI	07/07/1961	
E.P. 6365 23489	30/12/1961	NOTARIA PRIMERA DE CALI	23/01/1962	
E.P. 1383	21/03/1962	NOTARIA PRIMERA DE CALI	28/03/1962	

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PAGINA No. 3

23850						
E.P.	3396	28/05/1963	NOTARIA	PRIMERA	DE CALI	11/06/1963
26016						
E.P.	8853	30/12/1963	NOTARIA	PRIMERA	DE CALI	23/01/1964
27107						
E.P.	5718	28/11/1957	NOTARIA	SEGUNDA	DE CALI	10/02/1964
27181						
E.P.	2743	29/04/1964	NOTARIA	PRIMERA	DE CALI	06/05/1964
27681						
E.P.	2744	29/04/1964	NOTARIA	PRIMERA	DE CALI	06/05/1964
27682						
E.P.	7057	21/10/1964	NOTARIA	PRIMERA	DE CALI	22/10/1964
28591						
E.P.	1693	26/04/1965	NOTARIA	PRIMERA	DE CALI	28/04/1965
29569						
E.P.	1799	10/05/1966	NOTARIA	PRIMERA	DE CALI	11/05/1966
31623						
E.P.	2498	18/11/1966	NOTARIA	CUARTA	DE CALI	25/11/1966
32757						
E.P.	2792	27/12/1966	NOTARIA	CUARTA	DE CALI	05/01/1967
33009						
E.P.	2209	25/09/1967	NOTARIA	CUARTA	DE CALI	04/10/1967
34591						
E.P.	6933	20/12/1967	NOTARIA	SEGUNDA	DE CALI	28/12/1967
35082						
E.P.	6363	14/11/1968	NOTARIA	SEGUNDA	DE CALI	26/11/1968
37141						
E.P.	7711	31/12/1968	NOTARIA	SEGUNDA	DE CALI	04/02/1969
37615						
E.P.	5597	30/09/1969	NOTARIA	SEGUNDA	DE CALI	18/10/1969
39341						
E.P.	5211	27/08/1970	NOTARIA	SEGUNDA	DE CALI	04/09/1970
41379						
E.P.	6576	07/10/1971	NOTARIA	SEGUNDA	DE CALI	14/10/1971
44154						
E.P.	7286	08/11/1971	NOTARIA	SEGUNDA	DE CALI	10/11/1971
44367						
E.P.	8042	03/12/1971	NOTARIA	SEGUNDA	DE CALI	21/12/1971
44716						
E.P.	6209	05/10/1973	NOTARIA	SEGUNDA	DE CALI	15/10/1973
5505	IX					
E.P.	8741	31/12/1973	NOTARIA	SEGUNDA	DE CALI	08/03/1974
7636	IX					
E.P.	9270	31/12/1974	NOTARIA	SEGUNDA	DE CALI	31/01/1975
11772	IX					
E.P.	9504	31/12/1974	NOTARIA	SEGUNDA	DE CALI	04/04/1975
12441	IX					
E.P.	7566	05/12/1975	NOTARIA	SEGUNDA	DE CALI	09/01/1976

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PAGINA No. 4

15581 IX					
E.P. 2671	09/05/1980	NOTARIA	SEGUNDA	DE CALI	22/10/1980
41529 IX					
E.P. 779	10/03/1982	NOTARIA	DECIMA	DE CALI	03/08/1982
54655 IX					
E.P. 4324	10/11/1982	NOTARIA	DECIMA	DE CALI	30/06/1983
61172 IX					
E.P. 1269	18/03/1986	NOTARIA	DECIMA	DE CALI	01/04/1986
83730 IX					
E.P. 5089	24/09/1986	NOTARIA	DECIMA	DE CALI	29/09/1986
87933 IX					
E.P. 7064	04/11/1987	NOTARIA	DECIMA	DE CALI	19/11/1987
2552 IX					
E.P. 7065	04/11/1987	NOTARIA	DECIMA	DE CALI	19/11/1987
2553 IX					
E.P. 5929	14/07/1989	NOTARIA	DECIMA	DE CALI	26/07/1989
20225 IX					
E.P. 8569	29/09/1989	NOTARIA	DECIMA	DE CALI	02/10/1989
22250 IX					
E.P. 10571	04/12/1992	NOTARIA	DECIMA	DE CALI	15/12/1992
60929 IX					
E.P. 10249	18/12/1995	NOTARIA	DECIMA	DE CALI	20/12/1995
10214 IX					
E.P. 2359	22/05/1997	NOTARIA	TERCERA	DE CALI	27/05/1997
3813 IX					
E.P. 2624	30/06/2000	NOTARIA	TERCERA	DE CALI	07/07/2000
4740 IX					
E.P. 3814	15/09/2000	NOTARIA	TERCERA	DE CALI	18/09/2000
6381 IX					
E.P. 3588	30/07/2004	NOTARIA	TERCERA	DE CALI	04/08/2004
8542 IX					
E.P. 2224	24/05/2006	NOTARIA	TERCERA	DE CALI	30/05/2006
6565 IX					

CERTIFICA:

VIGENCIA: 01 DE ENERO DEL AÑO 2057

CERTIFICA:

OBJETO SOCIAL. LA SOCIEDAD TIENE POR OBJETO: A) LA FABRICACION, EL ENVASADO, LA IMPORTACION, LA EXPORTACION, LA VENTA Y DISTRIBUCION DE PRODUCTOS BIOLOGICOS Y QUIMICOS, MEDICAMENTOS O PRODUCTOS FARMACEUTICOS, PERFUMERIA O EFECTOS DE TOCADOR, PRODUCTOS VETERINARIOS, AGRICOLAS Y ALIMENTOS NATURALES Y/O PRODUCTOS DE CONSUMO POPULAR DE CUALQUIER NATURALEZA Y SERVICIOS EN

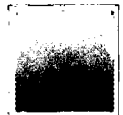
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PAGINA No. 5

GENERAL. B) INVERTIR EN SOCIEDADES O EMPRESAS DE CUALQUIER NATURALEZA O ESPECIE DE LA SOCIEDAD MERCANTIL, NACIONAL O EXTRANJERA, QUE TENGAN COMO ACTIVIDAD O NEGOCIO PROPIO DE SU OBJETO LA FABRICACION, DISTRIBUCION Y COMERCIO EN GENERAL DE PRODUCTOS BIOLOGICOS Y QUIMICOS, MEDICAMENTOS O PRODUCTOS FARMACEUTICOS, PERFUMERIA O EFECTOS DE TOCADOR, PRODUCTOS VETERINARIOS, AGRICOLAS, ALIMENTOS NATURALES Y/O PROCESADOS, PRODUCTOS DE CONSUMO POPULAR, SERVICIOS EN GENERAL O LA EXPLOTACION DE ESTABLECIMIENTOS DE COMERCIO DEDICADOS A DICHO GIRO. C) ADQUIRIR ACCIONES, CUOTAS SOCIALES, BONOS CEDULAS, TITULOS U OTROS VALORES EN COMPANIAS DE CUALQUIER NATURALEZA O TIPO LEGAL, DEDICADAS A LA EXPLOTACION DE CUALQUIER ACTIVIDAD ECONOMICA LICITA Y ENAJENARLAS. D) LA EXPLOTACION DEL NEGOCIO DE LA PUBLICIDAD Y EL ESTABLECIMIENTO DE OFICINAS DEDICADAS A ESTE NEGOCIO O A LA CREACION DE ORGANOS DE PUBLICIDAD HABLADOS O ESCRITOS. E) GARANTIZAR LAS OBLIGACIONES PROPIAS DE LOS ACCIONISTAS O DE TERCEROS. F) LA ADQUISICION DE BIENES MUEBLES, INMUEBLES Y DE TODA CLASE DE EQUIPO Y MAQUINARIA PARA SU ARRENDAMIENTO O PARA LA COMERCIALIZACION DE ELLOS. EN DESARROLLO DEL OBJETO SOCIAL PODRA OBTENER PRIVILEGIOS INDUSTRIALES, CONCESIONES, PATENTES DE INVENCION, DE MEJORAS O PROCEDIMIENTOS, MARCAS DE FABRICA O DE COMERCIO, YA SEA EN NOMBRE PROPIO O DE TERCEROS, OTORGAR A TERCEROS, DENTRO O FUERA DEL PAIS CONCESIONES O USOS DE EXPLOTACION SOBRE ESTOS DERECHOS, EJECUTAR TODA CLASE DE CONTRATOS DE COMPRAVENTA, DISTRIBUCION DE MATERIAS PRIMAS, DE PRODUCTOS SEMIELABORADOS O ELABORADOS EN EL MERCADO INTERNO O EN EL EXTERIOR Y EN GENERAL, LA CELEBRACION DE TODO ACTO O CONTRATO SOBRE BIENES MUEBLES O INMUEBLES DE COMERCIO, INDUSTRIALES O DE OTRA INDOLE QUE SE REFIERAN DIRECTAMENTE A LAS ACTIVIDADES QUE PERSIGUE LA SOCIEDAD.

CERTIFICA:

?RGANOS DE DIRECCI?N Y ADMINISTRACI?N. PARA LOS FINES DE SU DIRECCI?N, ADMINISTRACI?N Y REPRESENTACI?N, LA SOCIEDAD TIENE LOS SIGUIENTES ?RGANOS: A) ASAMBLEA GENERAL DE ACCIONISTAS. B) JUNTA DIRECTIVA. C) PRESIDENTE EJECUTIVO. D) TRES VICEPRESIDENTES EJECUTIVOS. E) VICEPRESIDENCIAS ADMINISTRATIVAS.

LA DIRECCI?N DE LA SOCIEDAD CORRESPONDE, EN PRIMER TERMINO A LA ASAMBLEA GENERAL DE ACCIONISTAS Y EN SEGUNDO LUGAR, A LA JUNTA DIRECTIVA COMO DELEGADA DE AQUELLA.

LA REPRESENTACI?N LEGAL DE LA SOCIEDAD Y LA GESTI?N DE LOS NEGOCIOS SOCIALES, ESTAR?N A CARGO DEL PRESIDENTE EJECUTIVO Y DE LOS TRES VICEPRESIDENTES EJECUTIVOS.

FUNCIONES DE LA ASAMBLEA DE ACCIONISTAS. ENTRE OTRAS LAS SIGUIENTES: 1) ACORDAR LA FUSI?N DE LA SOCIEDAD, SU TRANSFORMACI?N, LA ENAJENACI?N O EL ARRENDAMIENTO DE LA EMPRESA SOCIAL, LA DISOLUCI?N O LA PRORROGA Y CUALQUIER REFORMA, AMPLIACI?N MODIFICACI?N DEL CONTRATO SOCIAL. 9) AUTORIZAR LA ADQUISICI?N DE ACCIONES PROPIAS, CON SUJECI?N A LOS REQUISITOS ESTABLECIDOS POR LA LEY. 10) ADOPTAR EN GENERAL, TODAS LAS MEDIDAS QUE RECLAMEN EL

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PAGINA No. 6

CUMPLIMIENTO DE LOS ESTATUTOS O EL INTER?S DE LA SOCIEDAD.

EN LA JUNTA DIRECTIVA SE ENTIENDE DELEGADO EL MAS AMPLIO MANDATO PARA ADMINISTRAR LA SOCIEDAD Y POR CONSIGUIENTE, TENDR? ATRIBUCIONES SUFICIENTES PARA ORDENAR QUE SE EJECUTE O CELEBRE CUALQUIER ACTO O CONTRATO COMPRENDIDO DENTRO DEL OBJETO SOCIAL Y PARA ADOPTAR LAS DETERMINACIONES NECESARIAS EN ORDEN A QUE LA SOCIEDAD CUMPLA SUS FINES Y DE MANERA ESPECIAL, TENDR? LAS SIGUIENTES FUNCIONES ENTRE OTRAS: C) NOMBRAR Y REMOVER LIBREMENTE AL PRESIDENTE EJECUTIVO, A LOS VICEPRESIDENTES EJECUTIVOS Y A LOS VICEPRESIDENTES ADMINISTRATIVOS. E) DISPONER EL ESTABLECIMIENTO O LA CLAUSURA DE SUCURSALES O AGENCIAS, DENTRO O FUERA DEL DOMICILIO SOCIAL. F) FIJAR LAS POL?TICAS DE LA SOCIEDAD EN LOS DIFERENTES ORDENES DE SU ACTIVIDAD, ESPECIALMENTE EN MATERIA FINANCIERA, ECON?MICA Y LABORAL; APROBAR PLANES DE INVERSI?N Y DE PRODUCCI?N; DECIDIR SOBRE NUEVAS LINEAS DE PRODUCCI?N Y VENTA, ESTABLECER NORMAS GENERALES EN MATERIA DE PRECIOS Y DE VENTAS DE LOS PRODUCTOS ELABORADOS POR LA SOCIEDAD O DE LOS MATERIALES Y ~~DEM?S~~ ELEMENTOS EN QUE ELLA NEGOCIE Y DICTAR NORMAS Y REGLAMENTOS PARA LA ORGANIZACI?N Y EL FUNCIONAMIENTO DE TODAS LAS DEPENDENCIAS DE LA SOCIEDAD. G) ~~DETERMINAR~~ LA APLICACI?N QUE DEBA DARSE A LAS UTILIDADES QUE, CON EL CAR?CTER DE RESERVAS DE INVERSI?N, HAYAN SIDO APROPIADAS POR LA ASAMBLEA DE ACCIONISTAS. H) AUTORIZAR PREVIAMENTE LAS OPERACIONES QUE TENGAN POR OBJETO: 1) ADQUIRIR, HIPOTECAR Y EN CUALQUIER OTRA FORMA GRAVAR O LIMITAR EL DOMINIO DE BIENES RA?CES CUYA CUANT?A SEA SUPERIOR A \$100.000.000.00 MONEDA CORRIENTE; 2) CONSTITUIR PRENDA SOBRE BIENES SOCIALES O DARLOS EN ANTICRESIS PARA ACTOS O CONTRATOS CUYA CUANT?A SEA SUPERIOR A \$100.000.000.00 MONEDA CORRIENTE; 3) AUTORIZAR INVERSIONES EN BIENES DE CAPITAL O EFECTUAR REPARACIONES O MEJORAS DE LOS MISMOS, CUANDO UNAS U OTRAS IMPLIQUEN, EN CADA CASO, COSTOS O DESEMBOLSOS EN CUANT?A O SUPERIOR A CIENTO MIL D?LARES AMERICANOS (\$US100.000.00). 4) AUTORIZAR LOS CONTRATOS DE MUTUO QUE PUEDAN CELEBRAR LA SOCIEDAD LOS REPRESENTANTES LEGALES CUANDO SU CUANT?A SEA SUPERIOR A \$100.000.000.00 MONEDA LEGAL COLOMBIANA O A US\$300.000.00. L) AUTORIZAR AL REPRESENTANTE LEGAL PARA QUE SOLICITE, LLEGADO EL CASO, QUE SE ADMITA A LA COMPA??A A LA CELEBRACI?N DE CONCORDATO PREVENTIVO CON SUS ACREEDORES. M) SERVIR DE ?RGANO CONSULTIVO Y ASESOR DE LOS REPRESENTANTES DE LA COMPA??A.

LA ADMINISTRACI?N INMEDIATA DE LA COMPA??A, SU REPRESENTACI?N LEGAL Y LA GESTI?N DE LOS NEGOCIOS SOCIALES ESTAR?N A CARGO DEL PRESIDENTE EJECUTIVO Y DE LOS TRES VICEPRESIDENTES EJECUTIVOS, DESIGNADOS POR LA JUNTA DIRECTIVA PARA PERIODOS DE UN A?O, REELEGIBLES INDEFINIDAMENTE Y REMOVIBLES LIBREMENTE POR ELLA EN CUALQUIER TIEMPO, QUIENES COMO REPRESENTANTES LEGALES PODR?N ACTUAR CONJUNTA O SEPARADAMENTE CON IGUALDAD DE FUNCIONES Y CALIDADES. TODOS LOS EMPLEADOS DE LA COMPA??A CON EXCEPCI?N DE LOS DESIGNADOS POR LA ASAMBLEA DE ACCIONISTAS Y LOS DEPENDIENTES DEL REVISOR FISCAL, SI LOS HUBIERE, ESTAR?N SOMETIDOS EN EL DESEMPE?O DE SUS CARGOS A LOS REPRESENTANTES LEGALES.

LOS REPRESENTANTES LEGALES SON MANDATARIOS CON REPRESENTACI?N INVESTIDOS DE FUNCIONES EJECUTIVAS Y ADMINISTRATIVAS Y COMO TALES TIENEN A SU CARGO LA REPRESENTACI?N LEGAL DE LA COMPA??A, LA GESTI?N COMERCIAL Y FINANCIERA, LA RESPONSABILIDAD DE LA ACCI?N ADMINISTRATIVA, LA COORDINACI?N Y LA SUPERVISI?N

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GENERAL DE LA EMPRESA, LAS CUALES CUMPLIR?N CON ARREGLO A LAS NORMAS DE LOS
ESTATUTOS Y A LAS DISPOSICIONES LEGALES Y CON SUJECI?N A LAS ORDENES E
INSTRUCCIONES DE LA JUNTA DIRECTIVA.

COMO REPRESENTANTES LEGALES DE LA COMPA??A EN JUICIO Y FUERA DE JUICIO, LOS
REPRESENTANTES TIENEN FACULTADES PARA EJECUTAR O CELEBRAR SIN OTRAS
LIMITACIONES QUE LAS ESTABLECIDAS EN LOS ESTATUTOS EN CUANTO SE TRATE DE
OPERACIONES QUE DEBAN SER PREVIAMENTE AUTORIZADAS POR LA JUNTA DIRECTIVA O POR
LA ASAMBLEA DE ACCIONISTAS, TODOS LOS ACTOS O CONTRATOS COMPRENDIDOS DENTRO
DEL OBJETO SOCIAL O QUE TENGAN CAR?CTER SIMPLEMENTE PREPARATORIO, ACCESORIO O
COMPLEMENTARIO PARA LA REALIZACI?N DE LOS FINES QUE PERSIGUE LA SOCIEDAD Y LOS
QUE SE RELACIONAN DIRECTAMENTE CON LA EXISTENCIA Y EL FUNCIONAMIENTO DE LA
MISMA. LOS REPRESENTANTES QUEDAN INVESTIDOS DE PODERES ESPECIALES PARA
TRANSIGIR, ARBITRAR Y COMPROMETER LOS NEGOCIOS SOCIALES; PROMOVER O COADYUVAR
ACCIONES JUDICIALES, ADMINISTRATIVAS O CONTENCIOSO ADMINISTRATIVAS EN QUE LA
COMPA??A TENGA INTER?S E INTERPONER TODOS LOS RECURSOS QUE SEAN PROCEDENTES
CONFORME A LA LEY; DESISTIR DE LAS ACCIONES O RECURSOS QUE INTERPONGAN; NOVAR
OBLIGACIONES O CR?DITOS; DAR O RECIBIR BIENES EN PAGO; CONSTITUIR APODERADOS
JUDICIALES O EXTRAJUDICIALES, DELEGARLES FACULTADES REVOCAR MANDATOS Y
SUSTITUCIONES.

PARAGRAFO: LOS REPRESENTANTES LEGALES DE LA SOCIEDAD TIENEN AMPLIAS
FACULTADES, SIN NINGUNA LIMITACI?N Y SIN REQUERIR AUTORIZACI?N PREVIA DE LA
JUNTA DIRECTIVA, PARA ACTUAR EN NOMBRE DE LA SOCIEDAD EN LICITACIONES
PUBLICAS, PRIVADAS O CUALQUIER OTRA MODALIDAD DE CONTRATACI?N, PRESENTAR
PROPUESTAS, ACEPTAR ADJUDICACIONES Y CONTRATAR SIN LIMITACI?N DE CUANT?A
ALGUNA ANTE LAS ENTIDADES PUBLICAS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL
ESTADO, SOCIEDADES DE ECONOM?A MIXTA, BIEN SEAN ELLAS DEL ORDEN NACIONAL,
DISTRITAL, DEPARTAMENTAL O MUNICIPAL.

CERTIFICA:

DOCUMENTO: ACTA No. 393 DEL 20 DE ENERO DE 1993

ORIGEN: JUNTA DIRECTIVA

INSCRIPCION: 26 DE ENERO DE 1993 No. 62203 DEL LIBRO IX

FUE (RON) NOMBRADO(S):

PRESIDENTE EJECUTIVO
FRANCISCO JOSE BARBERI OSPINA
C.C.14445880

VICEPRESIDENTE EJECUTIVO
JUAN MANUEL BARBERI OSPINA
C.C.14949561

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PAGINA No. 8

VICEPRESIDENTE EJECUTIVO
JUAN BAUTISTA DE LOS RIOS GUTIERREZ
C.C.17108621

VICEPRESIDENTE EJECUTIVO
EMILIO SARDI APARICIO
C.C.14434582

CERTIFICA:

DOCUMENTO: ACTA No. 38 DEL 03 DE FEBRERO DE 1992
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 05 DE JUNIO DE 1992 No. 54016 DEL LIBRO IX

DOCUMENTO: ACTA No. 54 DEL 31 DE MARZO DE 2000
ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS
INSCRIPCION: 24 DE MAYO DE 2000 No. 3622 DEL LIBRO IX

DOCUMENTO: ACTA No. 65 DEL 27 DE JULIO DE 2006
ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS
INSCRIPCION: 04 DE AGOSTO DE 2006 No. 9191 DEL LIBRO IX

FUE (RON) _NOMBRADO(S)

JUNTA DIRECTIVA

PRINCIPALES

PRIMER RENGLON
FRANCISCO JOSE BARBERI OSPINA
C.C.14445880

SEGUNDO RENGLON
JUAN MANUEL BARBERI OSPINA
C.C.14949561

TERCER RENGLON
JUAN BAUTISTA DE LOS RIOS GUTIERREZ
C.C.17108621

CUARTO RENGLON
EMILIO SARDI APARICIO
C.C.14434582

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PAGINA No. 9

QUINTO RENGLON
DIEGO HUMBERTO QUIJANO REISSNER
C.C.16615708

SUPLENTES

PRIMER RENGLON
GUSTAVO ADOLFO SANCHEZ LESMES
C.C.16625960

SEGUNDO RENGLON
VICTOR RICARDO ROSA GARCIA
C.C.6811102

TERCER RENGLON
ANTONIO JAIRO RAMIREZ ECHAVE
C.C.16741040

CUARTO RENGLON
ERNESTO TRUJILLO PEREZ
C.C.14984104

QUINTO RENGLON
ANA MARIA ALVAREZ
C.C.31170896

CERTIFICA:

DOCUMENTO: ACTA No. 59 DEL 31 DE MARZO DE 2003
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 23 DE ABRIL DE 2003 No. 2845 DEL LIBRO IX

FUE(ON) NOMBRADO(S) :

REVISOR FISCAL
DELOITTE & TOUCHE LTDA D & T LTDA
NIT.860005813-4

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 26 DE AGOSTO DE 2004
ORIGEN: DELOITTE & TOUCHE LTDA D & T LTDA

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PAGINA No. 10

INSCRIPCION: 30 DE AGOSTO DE 2004 No. 9417 DEL LIBRO IX

FUE(RON) NOMERADO(S) :

REVISOR FISCAL PRINCIPAL
LUIS FERNANDO QUICENO BENAVIDES
C.C.6106227

REVISOR FISCAL SUPLENTE
JESUS DARIO LOPEZ CASELLA
C.C.16789097

CERTIFICA:

QUE POR ESCRITURA NRO. 5235 DEL 19 DE JULIO DE 1996 NOTARIA DECIMA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 25 DE JULIO DE 1996 BAJO EL NRO. 144 DEL LIBRO V MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, AMPLIO Y SUFICIENTE A CARLOS ALBERTO QUINTERO, MAYOR DE EDAD, VECINO DE ESTA CIUDAD, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO.

14.440.778 DE CALI, PARA QUE EN NOMBRE Y REPRESENTACION DE LA SOCIEDAD, CUYA PERSONERIA OSTENTA, EL REPRESENTANTE EN LOS SIGUIENTES ACTOS: A) PRESENTAR EN LA ADMINISTRACION DE IMPUESTOS Y ADUANAS NACIONALES O DEL ORGANISMO QUE HAGA SUS VECES Y FIRMAR EN REPRESENTACION DE LA SOCIEDAD, LAS DECLARACIONES DEL IMPUESTO SOBRE LA RENTA Y COMPLEMENTARIOS QUE CORRESPONDA A LA SOCIEDAD, Y TODAS LAS DEMAS DECLARACIONES QUE LA SOCIEDAD DEBA PRESENTAR ANTE DICHA ENTIDAD, TALES COMO LA DE VENTAS, RETENCION EN LA FUENTE, TIMBRE Y CUALESQUIER OTRA DECLARACION QUE POR LEY LA SOCIEDAD ESTE OBLIGADA A PRESENTAR.

B) PARA PRESENTAR Y SUSCRIBIR TODOS LOS DOCUMENTOS QUE OBLIGUEN A TECNOQUIMICAS S.A., COMO DECLARANTE DEL IMPUESTO DE INDUSTRIA Y COMERCIO Y EL COMPLEMENTARIO DE AVISOS Y TABLEROS EN LOS MUNICIPIOS EN QUE LA SOCIEDAD ESTE OBLIGADA AL PAGO DE LOS CITADOS IMPUESTOS, PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE SE DERIVEN DE LOS MENCIONADOS TRIBUTOS. C) PARA PRESENTAR Y SUSCRIBIR TODOS LOS DOCUMENTOS QUE OBLIGUEN A TECNOQUIMICAS S.A. COMO DECLARANTE DEL IMPUESTO PREDIAL Y COMPLEMENTARIOS EN LOS MUNICIPIOS DONDE TENGA BIENES INMUEBLES, PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE SE DERIVEN DEL CITADO IMPUESTO. D) PARA DAR RESPUESTA A LOS REQUERIMIENTOS ORDINARIOS Y A LAS SOLICITUDES DE INFORMACION EN RELACION CON LOS IMPUESTOS ADMINISTRADOS POR LA DIRECCION DE IMPUESTOS Y ADUANAS NACIONALES Y POR LAS DEPENDENCIAS MUNICIPALES QUE ADMINISTREN LOS IMPUESTOS DE CARACTER TERRITORIAL. E) Y EN GENERAL, PARA QUE ASUMA LA PERSONERIA EN NOMBRE DE TECNOQUIMICAS S.A., SIEMPRE QUE LO ESTIME CONVENIENTE, DE MANERA QUE EN NINGUN CASO LA SOCIEDAD QUEDE SIN REPRESENTACION EN LOS ASUNTOS RELACIONADOS CON LAS FACULTADES OTORGADAS.

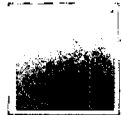
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 11

CERTIFICA:

QUE POR ESCRITURA NRO. 2873 DEL 10 DE JULIO DE 2001 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 06 DE SEPTIEMBRE DE 2001 BAJO EL NRO. 213 DEL LIBRO V MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, AMPLIO Y SUFICIENTE A MARIA TERESA CANON DE GUZMAN, IDENTIFICADA CON LA CEDULA DE CIUDADANIA NRO. 31.238.104 DE CALI, CARLOS ARTURO PENA NIETO IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 19.483.439 DE BOGOTA Y ANGELA MARIA CORREA CARDONA IDENTIFICADA CON LA CEDULA DE CIUDADANIA No.66.835.555 DE CALI, LA PRIMERA COMO PRINCIPAL Y EL SEGUNDO COMO PRIMER SUPLENTE Y LA TERCERA COMO SEGUNDA SUPLENTE, PARA QUE REPRESENTEN A LA SOCIEDAD EN TODOS LOS ASUNTOS CAMBIARIOS, CON FACULTAD PARA FIRMAR DECLARACIONES DE CAMBIOS SEA POR CREDITOS EN MONEDA EXTRANJERA, POR MOVIMIENTOS DE LAS CUENTAS CORRIENTES DE COMPENSACION, IMPORTACIONES DE BIENES Y SERVICIOS, TRANSFERENCIAS Y OTROS CONCEPTOS, REGISTRO DE PRESTAMO EN MONEDA EXTRANJERA OTORGADOS A LA EMPRESA EN SU CONDICION DE RESIDENTE Y TODOS AQUELLOS DOCUMENTOS O DECLARACIONES QUE DEBAN HACERSE ANTE EL BANCO DE LA REPUBLICA U OTRAS ENTIDADES GUBERNAMENTALES RESPECTO DE OPERACIONES EN EL MERCADO CAMBIARIO. LOS APODERADOS SUSCRIBIRAN TODOS LOS DOCUMENTOS Y REALIZAR TODOS LOS ACTOS QUE OBLIGUEN A TECNOQUIMICAS S. A. ANTE LAS AUTORIDADES CAMBIARIAS, PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS ADMINISTRATIVOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE SE DERIVEN DE DICHA ACTIVIDAD Y EN GENERAL PARA QUE ASUMA LA PERSONERIA A NOMBRE DE TECNOQUIMICAS S.A., SIEMPRE QUE LO ESTIMEN CONVENIENTE, DE MANERA QUE EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y CUMPLIMIENTO DE OBLIGACIONES DE CARACTER CAMBIARIO Y EN LOS ASUNTOS CONCERNIENTES PARA LAS MATERIAS DE QUE TRATA ESTE PODER. LOS APODERADOS SE CENIRAN RIGUROSAMENTE A LOS TERMINOS EXPRESADOS EN ESTE MANDATO QUE SE OTORGA POR TERMINO INDEFINIDO Y CUALQUIER NOTIFICACION O REVOCATORIA SE HARA EXPRESAMENTE POR ESCRITURA PUBLICA.

CERTIFICA:

QUE POR ESCRITURA NRO. 307 DEL 31 DE ENERO DE 2002 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 13 DE FEBRERO DE 2002 BAJO EL NRO. 298 DEL LIBRO V MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, AMPLIO Y SUFICIENTE A JORGE ROBERTO OBANDO RODRIGUEZ, MAYOR DE EDAD, VECINO DE ESTA CIUDAD, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 14.960.183 DE CALI, PARA QUE EN NOMBRE Y REPRESENTACION DE LA SOCIEDAD, CUYA PERSONERIA OSTENTA: 1o.) PROMUEVA O DESISTA DE ACCIONES ANTE CORPORACIONES, ENTIDADES O FUNCIONARIOS DEL ORDEN JUDICIAL O GUBERNATIVO, YA SEA EN PROCESOS, ACTUACIONES O ACTOS SIMPLES, DILIGENCIAS O GESTIONES EN QUE TECNOQUIMICAS S.A. TENGA INTERES COMO ACTOR, DEMANDANDO O TERCERO INTERVINIENTE EN RELACION CON LOS ASUNTOS DE CARACTER LABORAL, POLICIVO, PENAL O DE FISCALIA, EN QUE LA EMPRESA SEA PARTE ACTIVA O PASIVA, CON CAPACIDAD PARA NOTIFICARSE Y CONTESTAR Y HACERSE PARTE DE ELLOS EN NOMBRE DE TECNOQUIMICAS S.A. 2o.) SUSCRIBA Y DE POR TERMINADO LOS CONTRATOS DE

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

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Operacion : 02CP20428039

PAGINA No. 12

 TRABAJO DEL PERSONAL VINCULADO A LA EMPRESA. 3o.) CONSTITUYA APODERADOS EN DESARROLLO DE ESTA REPRESENTACION LABORAL, REVOQUE Y SUSTITUYA DICHOS PODERES CUANDO LO ESTIME PROCEDENTE. 4o.) TRANSIGA, CONCILIE, DESISTA Y COMPROMETA A LA EMPRESA EN TODOS LOS TRAMITES ADMINISTRATIVOS Y PROCESOS JUDICIALES, DE CARACTER LABORAL, POLICIVO, PENAL O DE FISCALIA EN LOS QUE LA EMPRESA SEA PARTE DEMANDANTE, DEMANDADA O TERCERO INTERVINIENTE. PARAGRAFO. LA FACULTAD PARA TRANSIGIR O CONCILIAR SE LIMITA A LA SUMA DE QUINIENTOS (500) SALARIOS LEGALES MENSUALES. 5o.) OTORQUE PODERES Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES JUDICIALES DE CARACTER CIVIL, COMERCIAL Y PENAL TENDIENTES A LA RECUPERACION DE LOS CREDITOS A FAVOR DE LA SOCIEDAD, BIEN SEA REPRESENTADOS EN FACTURAS, CHEQUES, TITULOS VALORES O CUALQUIER OTRO DOCUMENTO REPRESENTATIVO DE CREDITO, HASTA POR SUMA EQUIVALENTE A QUINIENTOS (500) SALARIOS MINIMOS LEGALES MENSUALES, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE TECNOQUIMICAS S.A. A LAS AUDIENCIAS DE CONCILIACION QUE PROGRAMEN LOS JUZGADOS CIVILES MUNICIPALES Y DEL CIRCUITO, CON AUTORIZACION PARA TRANSIGIR, DESISTIR CONCILIAR O COMPROMETER A LA SOCIEDAD HASTA POR LA CUANTIA INDICADA. 6o. REPRESENTA A TECNOQUIMICAS S.A. DENTRO DE LOS TRAMITES DE CONCORDATOS, PROCESOS DE REESTRUCTURACION ECONOMICA PREVISTO EN LA LEY 550 DE 1999 Y LAS NORMAS QUE LA ADICIONEN O MODIFIQUEN Y LIQUIDACIONES OBLIGATORIAS QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE LAS ENTIDADES PERTINENTES CUANDO SE TRATE DE OTRA CLASE DE ACREEDORES O ANTE LOS JUZGADOS CIVILES, CON EXPRESAS FACULTADES PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER, NOMBRAR APODERADOS, PRESENTAR LOS CREDITOS, APORTAR LAS PRUEBAS QUE ESTIME NECESARIAS, ASISTIR A LAS AUDIENCIAS PRELIMINARES Y DEFINITIVAS, ACORDAR FORMULAS DE PAGO, RECIBIR Y EN GENERAL PARA EJERCER TODAS AQUELLAS GESTIONES NECESARIAS PARA OBTENER EL RECONOCIMIENTO Y PAGO DE LOS CREDITOS PRESENTADOS. LAS FACULTADES AQUI ESTABLECIDAS ESTAN LIMITADAS PARA LOS CREDITOS HASTA POR CUANTIA DE QUINIENTOS (500) SALARIOS MINIMOS LEGALES MENSUALES. PARAGRAFO. IGUALMENTE QUEDA FACULTADO PARA ACEPTAR Y REPRESENTAR A TECNOQUIMICAS CUANDO LA EMPRESA SEA NOMBRADA EN JUNTA DE ACREEDORES O EN ORGANOS EQUIVALENTES O SIMILARES, EN LOS PROCESOS QUE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE LAS ENTIDADES PERTINENTES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES. 7o.) REPRESENTA A TECNOQUIMICAS S.A. EN LOS PROCEDIMIENTOS DE CONCILIACION QUE LOS DEUDORES DE LA SOCIEDAD CONVOQUEN ANTE LAS ENTIDADES FACULTADAS PARA ELLO CON EL FIN OBTENER UN ACUERDO EN EL PAGO DE LAS ACREENCIAS, CON EXPRESAS FACULTADES PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER Y RECIBIR, FACULTADES QUE SE LIMITAN HASTA POR LA SUMA DE QUINIENTOS (500) SALARIOS MINIMOS LEGALES MENSUALES. 8o.) REVISE LOS EXPEDIENTES DE LOS PROCESOS JUDICIALES DE CARACTER EJECUTIVO EN QUE LA SOCIEDAD SEA PARTE DEMANDANTE, EN TODO EL TERRITORIO NACIONAL, PARA LA RECUPERACION DE SUS ACREENCIAS. 9o.) APRUEBE Y SUSCRIBA ACUERDOS PRIVADOS DE PAGO Y CONCILIACIONES DE OBLIGACIONES A FAVOR DE TECNOQUIMICAS POR LA VENTA DE SUS PRODUCTOS HASTA POR LA SUMA DE (500) SALARIOS MINIMOS LEGALES MENSUALES.

CERTIFICA:

QUE POR ESCRITURA NRO. 310 DEL 31 DE ENERO DE 2002 NOTARIA TERCERA DE CALI ,

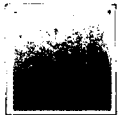
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 13

INSCRITA EN LA CAMARA DE COMERCIO EL 13 DE FEBRERO DE 2002 BAJO EL NRO. 301 DEL LIBRO V MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A CARLOS ORLANDO MUTIS, MAYOR DE EDAD, VECINO DE BUCARAMANGA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA No. 91.200.418 DE BUCARAMANGA, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL ORIENTE DE LA SOCIEDAD, CON SEDE EN BUCARAMANGA, PARA EL EJERCICIO DE LAS SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO, Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES, TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD.

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 14

C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

CERTIFICA:

QUE POR ESCRITURA NRO. 309 DEL 31 DE ENERO DE 2002 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 13 DE FEBRERO DE 2002 BAJO EL NRO. 300 DEL LIBRO V MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A DANIEL AMEZQUITA, MAYOR DE EDAD, VECINO DE BARRANQUILLA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 8.681.962 DE BARRANQUILLA, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL DE LA ZONA ATLANTICA DE LA SOCIEDAD, CON SEDE EN BARRANQUILLA, PARA EL EJERCICIO DE LA SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES, TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VENTILE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN

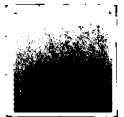
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 15

LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES
LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE
COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR
TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR
DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE
INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES
ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA
REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA
ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES
CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS
MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN
DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL
DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y
PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD
CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

CERTIFICA:

QUE POR ESCRITURA NRO. 308 DEL 31 DE ENERO DE 2002 NOTARIA TERCERA DE CALI ,
INSCRITA EN LA CAMARA DE COMERCIO EL 04 DE MARZO DE 2002 BAJO EL NRO. 318 DEL
LIBRO V MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A
HERNANDO ANGULO MOYANO, MAYOR DE EDAD, VECINO DE BOGOTA, IDENTIFICADO CON LA
CEDULA DE CIUDADANIA NRO. 19.322.139 DE BOGOTA, GERENTE DEL DISTRITO DE VENTAS
DE LA REGIONAL CUNDINAMARCA DE LA SOCIEDAD, CON SEDE EN BOGOTA, PARA EL
EJERCICIO DE LAS SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS
S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y
PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O
EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL,
ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES,
SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO,
ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO
DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE.

PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE
LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES
MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS
ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA
CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN
NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA
TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS
PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO
EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO
CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA
QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS
CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE
SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS
SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES,
TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

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Operacion : 02CP20428039

PAGINA No. 16

EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

CERTIFICA:

QUE POR ESCRITURA NRO. 4.202 DEL 31 DE MAYO DE 1995 NOTARIA DECIMA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 24 DE SEPTIEMBRE DE 2003 BAJO EL NRO. 134 DEL LIBRO V SE CONFIERE PODER ESPECIAL A ANTONIO JAIRO RAMIREZ ECHAVE, ABOGADO TITULADO Y EN EJERCICIO, CON TARJETA PROFESIONAL No. 58.401 DEL MINISTERIO DE JUSTICIA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NUMERO 16.741.040 DE CALI PARA QUE REPRESENTA A TECNOQUIMICAS S.A., EN LOS SIGUIENTES ACTOS: A) SOLICITAR Y ADELANTAR ANTE EL INSTITUTO COLOMBIANO AGROPECUARIO TODOS LOS TRAMITES ATINENTES A LA OBTENCION DE LICENCIAS DE VENTA Y LICENCIAS DE FUNCIONAMIENTO, RENOVACIONES, CERTIFICACIONES, AMPLIACIONES, INSCRIPCIONES Y MODIFICACIONES EN GENERAL; B) TODOS LOS TRAMITES ANTE EL INVIMA ATINENTES A LA OBTENCION DE REGISTRO SANITARIO, RENOVACION DEL MISMO, REFORMULACIONES, ADICIONES DE NUESTRA LINEA DE PRODUCTOS, CAMBIOS DE TITULAR Y DE FABRICANTE, CESIONES, CERTIFICACIONES DE FORMULA Y EXCLUSIVIDAD, MODIFICACIONES DE FABRICAR Y VENDER A IMPORTAR Y VENDER Y VICEVERSA, E IGUALMENTE CERTIFICACIONES SOBRE LA LINEA COMPLETA DE NUESTROS PRODUCTOS, A CUYO OBJETO LO FACULTO PARA DAR ANTE DICHAS AUTORIDADES TODOS LOS PASOS NECESARIOS, PRESENTAR SOLICITUDES, HACER DECLARACIONES, PAGAR DERECHOS, PROBAR EXPLOTACIONES, SOLICITAR TESTIMONIOS, RECIBIR DOCUMENTOS, FORMULAR APELACIONES Y RECLAMOS, DESISTIR Y PERCIBIR. ASI MISMO DICHO APODERADO ESTA AUTORIZADO

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 17

PARA HACER CUANTO FUERE NECESARIO ANTE LAS AUTORIDADES ADMINISTRATIVAS PARA
LOS FINES ARRIBA INDICADOS CON TODAS LAS DEMAS FACULTADES GENERALES Y
ESPECIALES QUE FUEREN NECESARIAS INCLUIDAS LAS DE SUSTITUIR Y DELEGAR EL
PRESENTE PODER SI LO ESTIMARE CONVENIENTE Y EN CASO NECESARIO REVOCAR DICHAS
SUSTITUCIONES. PUDIENDO ATENDER ASI MISMO LAS NOTIFICACIONES QUE DIRECTA O
INDIRECTAMENTE SE GENERE DE LOS ACTOS, ACTUACIONES, DILIGENCIAS O GESTIONES
QUE TENGAN RELACION DE CAUSALIDAD POR LOS SUSODICHOS TRAMITES Y 3) EN
GENERAL PARA QUE ASUMA LA PERSONERIA EN NOMBRE DE TECNOQUIMICAS S.A., SIEMPRE
QUE LO ESTIME CONVENIENTE, DE MANERA QUE EN NINGUN CASO QUEDE SIN LA DEBIDA
REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES EN LOS
ASUNTOS CONCERNIENTES PARA LAS MATERIAS DE QUE TRATA ESTE PODER. ANTONIO
JAIR RAMIREZ ECHAVE SE CENIRA RIGUROSAMENTE A LOS TERMINOS EXPRESADOS EN ESTE
MANDATO QUE SE OTORGA POR TERMINO INDEFINIDO Y CUALQUIER MODIFICACION O SU
REVOCATORIA SE HARA EXPRESAMENTE POR ESCRITURA PUBLICA.

CERTIFICA:

QUE POR ESCRITURA NRO. 6224 DEL 15 DE DICIEMBRE DE 2004 NOTARIA TERCERA DE
CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 17 DE ENERO DE 2005 BAJO EL NRO. 3
DEL LIBRO V MEDIANTE LA CUAL CONCEDE PODER ESPECIAL PERO AMPLIO Y SUFICIENTE
A DIEGO PEREZ MENDOZA MAYOR DE EDAD, VECINO DE BOGOTA, IDENTIFICADO CON CEDULA
DE CIUDADANIA NUMERO 16.820.530 DE CALI, COMO SU REPRESENTANTE CON PODER
ESPECIAL, AMPLIO Y SUFICIENTE, PARA REALIZAR TODOS LOS TRAMITES RELACIONADOS
CON EL PROCEDIMIENTO DE OTORGAMIENTO DE LICENCIA DE VENTA DE LOS PRODUCTOS
AGRICOLAS DE LA SOCIEDAD, CON FACULTAD PARA FIRMAR TODO TIPO DE DOCUMENTOS,
SOLICITUDES, REQUERIMIENTOS, MEMORIALES RESPUESTAS, ACLARACIONES BIEN SEA ANTE
EL INSTITUTO COLOMBIANO AGROPECUARIO ICA, MINISTERIO DEL MEDIO AMBIENTE,
MINISTERIO DE PROTECCION SOCIAL O ANTE LAS AUTORIDADES GUBERNAMENTALES
COMPETENTES EN LO RELACIONADO CON LOS TRAMITES QUE PERMITAN LA
COMERCIALIZACION DE ESE TIPO DE PRODUCTOS. LA FACULTAD TAMBIEN COMPRENDERA LA
DE NOTIFICARSE, RESPONDER E INTERPONER RECURSOS DE TODO TIPO DE ACTO
ADMINISTRATIVO. EL APODERADO SUSCRIBIRA TODOS LOS DOCUMENTOS Y REALIZAR TODOS
LOS ACTOS QUE OBLIGUEN A LA SOCIEDAD ANTE LAS AUTORIDADES MENCIONADAS,
PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS
ADMINISTRATIVOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE SE DERIVEN DE
DICHA ACTIVIDAD Y EN GENERAL, PARA QUE ASUMA LA PERSONERIA A NOMBRE DE
TECNOQUIMICAS S.A., SIEMPRE QUE LO ESTIME CONVENIENTE, DE MANERA QUE EN NINGUN
CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y
CUMPLIMIENTO DE OBLIGACIONES EN LOS ASUNTOS CONCERNIENTES PARA LAS MATERIAS DE
QUE TRATA ESTE PODER. EL APODERADO SE CE?IRA RIGUROSAMENTE A LOS TERMINOS
EXPRESADOS EN ESTE MANDATO QUE SE OTORGA POR TERMINO INDEFINIDO Y CUALQUIER
MODIFICACION O REVOCATORIA SE HARA EXPRESAMENTE POR ESCRITURA PUBLICA.

CERTIFICA:

QUE POR ESCRITURA NRO. 4265 DEL 16 DE SEPTIEMBRE DE 2005 NOTARIA TERCERA DE

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BOGOTA, D.C. - 129

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

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PAGINA No. 18

CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 04 DE OCTUBRE DE 2005 BAJO EL NRO. 168 DEL LIBRO V EL SE?OR JUAN BAUTISTA DE LOS RIOS GUTIERREZ MAYOR DE EDAD, DOMICILIADO EN CALI, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NUMERO 17108621 DE BOGOTA, QUIEN ACTUA EN SU CALIDAD DE REPRESENTANTE LEGAL DE TECNOQUIMICAS S. A. SOCIEDAD COMERCIAL ORGANIZADA Y EXISTENTE CONFORME A LAS LEYES COLOMBIANAS, CON DOMICILIO Y SEDE PRINCIPAL EN CALI. EN DICHA CONDICION CONFIERE PODER AMPLIO Y SUFICIENTE A LAS SIGUIENTES PERSONAS: CLAUDETTE VERNOT HERNANDEZ ABOGADA TITULADA Y EN EJERCICIO, IDENTIFICADA CON LA CEDULA DE CIUDADANIA No. 51722517 DE BOGOTA, PORTADORA DE LA TARJETA PROFESIONAL DE ABOGADO No. 57111 DEL C.S. DE LA J., ANDRES FELIPE ZAPATA OCAMPO, IDENTIFICADO CON LA CEDULA DE CIUDADANIA No. 79598546 DE BOGOTA, PORTADOR DE LA TARJETA PROFESIONAL DE ABOGADO No. 107240 DEL C.S. DE LA J. Y JUAN MANUEL SERRANO CASTILLO, IDENTIFICADO CON LA CEDULA DE CIUDADANIA No. 79875670 DE BOGOTA, PORTADOR DE LA TARJETA PROFESIONAL DE ABOGADO No. 124953 DE C.S. DE LA J., TODOS CON DOMICILIO EN LA CIUDAD DE BOGOTA, D.C., PARA QUE EN NOMBRE DE MI REPRESENTADA ACTUEN EN TODOS LOS ASUNTOS DE PROPIEDAD INTELECTUAL Y LOS DERIVADOS DE ELLA, CON FACULTAD PARA RECIBIR NOTIFICACIONES, NOMBRAR APODERADOS JUDICIALES O EXTRAJUDICIALES EN ESTOS ASUNTOS.

EL PRESENTE PODER SE CONFIERE PARA QUE LOS APODERADOS ACTUEN ANTE CUALQUIER PERSONA O AUTORIDAD ADMINISTRATIVA DEL ORDEN NACIONAL, DEPARTAMENTAL, DISTRITAL Y LOCAL; ANTE LAS AUTORIDADES CONTENCIOSO ADMINISTRATIVAS Y JUDICIALES, PARA OBTENER LA PROTECCION DE NUESTRA PROPIEDAD INTELECTUAL Y CONTRA LAS PRACTICAS RESTRICTIVAS DE LA COMPETENCIA Y LOS ASUNTOS DE COMPETENCIA DESLEAL, A CUYO EFECTO LA AUTORIZO, EN TODO LO CONCERNIENTE A LOS SIGUIENTES ASUNTOS: A) OBTENCION DE PATENTES DE INVENCION, DE MODELO DE UTILIDAD, ESQUEMAS DE TRAZADOS DE CIRCUITOS INTEGRADOS, DISE?OS INDUSTRIALES, EL REGISTRO DE MARCAS DE PRODUCTOS O SERVICIOS; LEMAS COMERCIALES; DEPOSITO DE NOMBRES Y ENSE?AS COMERCIALES, INDICACIONES GEOGRAFICAS; RENOVACION DE SIGNOS DISTINTIVOS, RENOVACIONES, AFECTACIONES AL REGISTRO, INSCRIPCION DE CONTRATOS, SOLICITUD Y RENOVACION DE NOMBRES DE DOMINIO; B) PRESENTACION DE OPOSICIONES AL REGISTRO DE MARCAS DE TERCERO, PRESENTACION DE ALEGATOS EN DEFENSA DE OPOSICIONES AL REGISTRO, ACCION DE CANCELACION, ACCION POR INFRACCION DE DERECHOS, REVOCATORIA DIRECTA, ACCION DE NULIDAD, ACCION DE NULIDAD Y RESTABLECIMIENTO DEL DERECHO, ACCION REIVINDICATORIA, ACCIONES DE TUTELA, POPULARES, DE GRUPO, DE CLASE; ACCIONES Y PROCESOS QUE PROMOVAMOS O SE NOS PROMUEVAN; C) AGOTAMIENTO DE LA VIA GUBERNATIVA, EJERCICIO DEL DERECHO DE PETICION ANTE CUALQUIER PERSONA O AUTORIDAD; D) SOLICITUD DE MEDIDAS CAUTELARES, PRESENTACION DE ACCIONES MEDIANTE PROCESOS CIVILES, PENALES, ADMINISTRATIVOS O CONTENCIOSO ADMINISTRATIVOS RELACIONADOS CON ESTOS DERECHOS U OTROS DE DIFERENTE NATURALEZA, MEDIDAS EN FRONTERA, ACCIONES Y PROCESOS QUE PROMOVAMOS O SE NOS PROMUEVAN; E) OBTENER Y TRAMITAR TODO LO RELACIONADO CON EL RECONOCIMIENTO DE DERECHOS DE AUTOR, DERECHOS CONEXOS Y NUEVAS TECNOLOGIAS Y SU PROTECCION LEGAL; F) SOLICITUD DE REGISTRO Y RENOVACION DE CONTRATOS DE TRANSFERENCIA DE TECNOLOGIA; G) OBTENER Y TRAMITAR TODO LO RELACIONADO CON LICENCIAS DE FUNCIONAMIENTO, REGISTROS SANITARIOS, AUTORIZACION DE CERTIFICADOS DE LIBRE VENTA, AUTORIZACION DE PUBLICIDAD ANTE EL MINISTERIO DE PROTECCION SOCIAL O EL INVIMA O ANTE CUALQUIER AUTORIDAD SANITARIA; H)

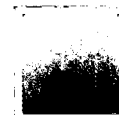
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 19

OBTENCION DE CERTIFICADOS DE CARENCIA DE INFORMES POR TRAFICO DE
ESTUPEFACIENTES.RENOVACIONES, AUTORIZACIONES EXTRAORDINARIAS ANTE LA DIRECCION
NACIONAL DE ESTUPEFACIENTES O EL CONSEJO NACIONAL DE ESTUPEFACIENTES; I)
SOLICITUD DE AUTORIZACIONES PARA PROMOCIONES, RIFAS Y JUEGOS ANTE ETESA O ANTE
LAS AUTORIDADES COMPETENTES DEL ORDEN LOCAL, DISTRITAL O DEPARTAMENTAL; Y EN
GENERAL CUALQUIERA OTRA PETICION ANTE LAS AUTORIDADES ADMINISTRATIVA, ASI COMO
PARA NOTIFICARSE DE CUALQUIER PROVIDENCIA Y RENUNCIAR A TERMINOS. EN TODOS LOS
ASUNTOS ARRIBA DETERMINADOS LA APODERADA QUEDA FACULTADA PARA SUSTITUIR TOTAL
O PARCIALMENTE ESTE MANDATO, TRANSIGIR, CONCILIAR EXTRAJUDICIAL O
JUDICIALMENTE, ADMITIR LOS HECHOS DEL PROCESO PERO NO PARA CONFESAR, DESISTIR,
CANCELAR, RECIBIR, RENUNCIAR Y RATIFICAR LOS ACTOS DE AGENTES OFICIOSOS.

CERTIFICA:

CAPITAL AUTORIZADO: \$27,200,000,000
NUMERO DE ACCIONES: 54,400,000
VALOR NOMINAL: \$500
CAPITAL SUSCRITO: \$27,123,804,000
NUMERO DE ACCIONES: 54,247,608
VALOR NOMINAL: \$500
CAPITAL PAGADO: \$27,123,804,000
NUMERO DE ACCIONES: 54,247,608
VALOR NOMINAL: \$500

CERTIFICA:

QUE POR DOCUMENTO PRIVADO DEL 17 DE NOVIEMBRE DE 2005, INSCRITO EN LA CAMARA
DE COMERCIO EL 23 DE NOVIEMBRE DE 2005 BAJO EL NRO. 13.158 DEL LIBRO IX, EN LA
CUAL CONSTA LA SITUACION DE CONTROL EJERCIDA POR TECNOQUIMICAS S.A.

CONTROLANTE: TECNOQUIMICAS S.A.
DOMICILIO: CALI
NACIONALIDAD: COLOMBIANA

CONTROLADA: INDUGRAFICAS S.A.
DOMICILIO: CALI
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE INDUGRAFICAS S.A. CORRESPONDEN AL DESARROLLO DE
NEGOCIOS RELACIONADOS CON LAS ARTES GRAFICAS, LA DISTRIBUCION DE LAS MISMAS YA
SEA DE SUS PRODUCTOS PROPIOS O POR CONTRATO, TANTO EN EL MERCADO NACIONAL COMO
EN EL INTERNACIONAL.

UNIDAD DE PROPOSITO Y ADMINISTRACION. ENTRE TECNOQUIMICAS E INDUGRAFICAS S.A.
EXISTE UNIDAD DE PROPOSITO Y DIRECCION YA QUE LOS PLANES Y OBJETIVOS DE ESTA

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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PAGINA No. 20

ULTIMA ESTAN DETERMINADAS POR LA PRIMERA.

DE IGUAL MANERA SE CONCRETA EL CONTROL ADMINISTRATIVO SOBRE LA FILIAL A TRAVES DE LA GERENCIA Y LA JUNTA DIRECTIVA CUYOS MIEMBROS PRINCIPALES HACEN PARTE IGUALMENTE DE LA JUNTA Y ADMINISTRACION DE LA MATRIZ.

CERTIFICA:

QUE POR DOCUMENTO PRIVADO DEL 17 DE NOVIEMBRE DE 2005, INSCRITO EN LA CAMARA DE COMERCIO EL 23 DE NOVIEMBRE DE 2005 BAJO EL NRO. 13.159 DEL LIBRO IX, EN LA CUAL CONSTA LA SITUACION DE CONTROL EJERCIDA POR TECNOQUIMICAS S.A.

CONTROLANTE: TECNOQUIMICAS S.A.
DOMICILIO: CALI
NACIONALIDAD: COLOMBIANA

CONTROLADA: TECNOFAR TQ S.A.
DOMICILIO: VILLA, RICA DEPARTAMENTO DEL CAUCA
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE TECNOFAR TQ S.A. CORRESPONDEN A LA PRODUCCION Y COMERCIALIZACION DE PRODUCTOS BIOLOGICOS, QUIMICOS, MEDICAMENTOS Y PRODUCTOS FARMACEUTICOS, PRODUCTOS VETERINARIOS Y AGRICOLAS, ALIMENTOS, COSMETICOS Y DE TOCADOR, PRODUCTOS DE ASEO PARA EL HOGAR Y LA FABRICACION Y DISTRIBUCION DE ENVASES PLASTICOS PARA TODO TIPO DE USO.

UNIDAD DE PROPOSITO Y ADMINISTRACION. ENTRE TECNOQUIMICAS Y TECNOFAR, EXISTE UNIDAD DE PROPOSITO Y DIRECCION YA QUE LOS PLANES Y OBJETIVOS DE ESTA ULTIMA ESTAN DETERMINADAS POR LA PRIMERA.

DE IGUAL MANERA SE CONCRETA EL CONTROL ADMINISTRATIVO SOBRE LA FILIAL A TRAVES DE LA GERENCIA Y LA JUNTA DIRECTIVA CUYOS MIEMBROS PRINCIPALES HACEN PARTE IGUALMENTE DE LA JUNTA Y ADMINISTRACION DE LA MATRIZ.

CERTIFICA:

QUE EL 27 DE MARZO DE 2001 BAJO EL NRO.1979 DEL LIBRO IX, SE INSCRIBIO EN LA CAMARA DE COMERCIO UN DOCUMENTO PRIVADO DE FECHA MARZO 21 DE 2001, EN LA CUAL CONSTA LA SITUACION DE CONTROL EJERCIDA POR LA SOCIEDAD TECNOQUIMICAS S.A.

MATRIZ: TECNOQUIMICAS S.A.
DOMICILIO: SANTIAGO DE CALI.
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE TECNOQUIMICAS CORRESPONDEN A LA PRODUCCION Y COMERCIALIZACION DE PRODUCTOS BIOLOGICOS, QUIMICOS, MEDICAMENTOS Y PRODUCTOS FARMACEUTICOS, PANALES, PRODUCTOS VETERINARIO Y AGRICOLAS, ALIMENTOS, COSMETICOS Y DE TOCADOR Y PRODUCTOS DE ASEO PARA EL HOGAR.

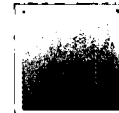
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

Hora Certificado 12:15:21

Operacion : 02CP20428039

PAGINA No. 21

SUBORDINADA: ADHESIVOS INTERNACIONALES S.A.- ADHINTER S.A. DOMICILIO: CALI VALLE DEL CAUCA

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE ADHINTER S.A. SE DESARROLLAN EXCLUSIVAMENTE EN LA ZONA FRANCA DEL PACIFICO CORRESPONDEN A LA PRODUCCION Y COMERCIALIZACION EN EL MERCADO NACIONAL E INTERNACIONAL DE ADHESIVOS MEDICINALES TALES COMO CURAS, VENDITAS, ESPARADRAPOS, PARCHES, VENDAS; ADHESIVOS PARA USO EN OFICINA TALES COMO CINTAS, PEGANTES, PAPELES ADHESIVOS Y CINTAS ADHESIVAS PARA USO INDUSTRIAL.

PRESUPUESTO DE CONTROL: TECNOQUIMICAS S.A., TIENE SUSCRITAS EN LA SOCIEDAD ADHESIVOS INTERNACIONALES S.A. ADHINTER S.A., LA CANTIDAD DE DOS MILLONES CUATROCIENTOS TREINTA MIL (2.430.000) ACCIONES ORDINARIAS DE VALOR NOMINAL DE CIEN PESOS (\$100.00) SOBRE UN TOTAL DE TRES MILLONES (3.000.000) DE ACCIONES ORDINARIAS SUSCRITAS QUE REPRESENTA UNA PARTICIPACION DIRECTA DEL 81% SOBRE EL TOTAL DEL CAPITAL SUSCRITO Y PAGADO DE LA SOCIEDAD.

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.7050-2 ESTABLECIMIENTO DE COMERCIO: TECNOQUIMICAS
UBICADO EN: ---C 23 7 39 DE CALI
RENOVO : POR EL A?O 2008

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.311234-2 ESTABLECIMIENTO DE COMERCIO: TECNOQUIMICAS
UBICADO EN: -K 7 22 122 DE CALI
FECHA MATRICULA : 22 DE ABRIL DE 1992
RENOVO : POR EL A?O 2008

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.311235-2 ESTABLECIMIENTO DE COMERCIO: TECNOQUIMICAS
UBICADO EN: -C 23 4 66 DE CALI
FECHA MATRICULA : 22 DE ABRIL DE 1992
RENOVO : POR EL A?O 2008

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

Fecha : 20080428

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Operacion : 02CP20428039

PAGINA No. 22

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.606730-2 ESTABLECIMIENTO DE COMERCIO: TECNOQUIMICAS S A JAMUNDI
UBICADO EN: --CARRETERA JAMUNDI KM. 23 DE JAMUNDI
FECHA MATRICULA : 14 DE ABRIL DE 2003
RENOVO : POR EL A?O 2008

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.606736-2 ESTABLECIMIENTO DE COMERCIO: TECNOQUIMICAS S A YUMBO
UBICADO EN: --CL. 15 NRO. 30 98 AUTOPISTA CALI-YUMBO KILOMETRO 4 DE YUMBO
FECHA MATRICULA : 14 DE ABRIL DE 2003
RENOVO : POR EL A?O 2008

CERTIFICA:

QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 29 DE
MARZO DE 2008 .

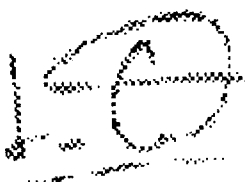
CERTIFICA:

QUE NO FIGURAN OTRAS INSCRIPCIONES QUE MODIFIQUEN TOTAL O PARCIALMENTE EL
PRESENTE CERTIFICADO.

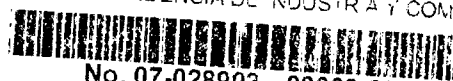
LOS ACTOS Y DOCUMENTOS REGISTRADOS QUEDAN EN FIRME CINCO (5) DIAS HABILES
DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO DENTRO DE DICHO
TERMINO NO SEAN OBJETO DE RECURSOS EN LA VIA GUBERNATIVA.

DE CONFORMIDAD CON EL DECRETO 2150 DE 1.995 Y LA AUTORIZACION IMPARTIDA POR
LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO LA FIRMA MECANICA QUE APARECE A
CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES.

DADO EN CALI A LOS 28 DIAS DEL MES DE ABRIL DEL A?O 2008 HORA: 12:04:38



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-028993- -00003-0000

Fecha 2008-04-28 15:40:47 Dep 2020 NULCREACIONE
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Act. 400 OPOSIOBSERVTER Folios. 133

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 34,138
FECHA : ABRIL 28 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Mr. PAGO
REYES Y REYES ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	542943	28/04/2008	682,000.00

***** CONCEPTO *****

Nº.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES	
12		MARCAS Y LEMAS COMERCIALES	266,000.00
		TOTAL :	\$ 266,000.00

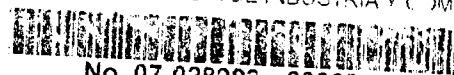
SON: DOSCIENTOS SESENTA Y SEIS MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

133
220

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-028993- -00003-0000

Fecha: 2008-04-28 15:40:47 Dep: 2020 NUECREACIONE
Tra: 11 PATENTEIYII Eve: 378 FASENAC ONALI
Act: 400 OPOSIOBSERVTER Folios: 133

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 34,139
CHA : ABRIL 28 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
REYES Y REYES ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754307	542943	28/04/2008	682,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-08	PRORROGA DE TERMINOS	75,000.00
		31 PRORROGA DE TERMINOS	75,000.00
		TOTAL :	75,000.00

SON: SETENTA Y CINCO MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____