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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Solicitud de patente de invención titulada: "METODOS DE TRATAMIENTO Y
FORMULACIONES DE CEFALOSPORINA."

Solicitante: PFIZER PRODUCTS INC.

Nuestra Ref.: P2004/000040

RESPUESTA AL OFICIO No. 10748, NOTIFICADO POR FIJACIÓN EN LISTA No. 274,

DE FECHA 22 DE AGOSTO DE 2007.

(SEGUNDO EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486:

1. En primer lugar, y con el fin de brindar mayor claridad a la presente solicitud, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza por completo el presentado al momento junto con mi memorial de respuesta al primer examen de fondo.

En el Capítulo Reivindicatorio Modificado ahora se incluye el buffer empleado en las composiciones reclamadas en la Reivindicación 2, que puede ser opcionalmente buffer de citratos. Asimismo, se incluye la Reivindicación 5 que especifica que las composiciones comprenden auxiliares de formulación adicionales, como agentes de carga para liofilización.

Valga anotar que en cumplimiento del Artículo 34 de la Decisión 486, las anteriores modificaciones de ninguna manera corresponden a ampliación alguna de la materia inicialmente divulgada.

2. a- El Examinador insiste en que las patentes US6.020.329 (D1) y WO92/01695 (D2), afectan el nivel inventivo de la presente invención.

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El Examinador reitera que la única diferencia entre D1 y la presente invención es el rango específico de pH usado (6,0-7,5). Adicionalmente, el Examinador anota que una persona medianamente versada en el arte derivaría de manera evidente a partir de D1 el desarrollo de formulaciones acuosas de cefalosporinas con un pH específico. A su vez, D2 revela los excipientes adecuados para la formulación de cefalosporinas. De esta forma, la presente invención resultaría obvia a partir de la combinación de los documentos de D1 y D2.

b- i) En el presente caso, D1 y/o D2 no proveen información que sugiera que el Compuesto I es inestable tanto en forma sólida como líquida. Adicionalmente, el arte previo citado no enseña que el Compuesto I no puede estabilizarse por medio de técnicas de cristalización convencionales, como sí es posible con otras cefalosporinas. De otra parte, D1 y D2 se refieren principalmente a compuestos β -lactámicos, y solamente de manera genérica revelan composiciones que contienen estos compuestos; es decir, D1 y/o D2 no indican (i) la combinación de preservativos, (ii) el pH requerido en soluciones acuosas previa liofilización, ni (iii) el contenido de agua tras la liofilización bajo las cuales se alcanza una mayor estabilidad de los compuestos activos de interés.

Así las cosas, no existe sugerencia en D1 y/o D2 que indique que el Compuesto I podía ser estabilizado y formulado para inyección al mantener el rango de pH de 6,0 a 7,5 antes de emplear métodos de liofilización para remover el agua de la forma farmacéutica final.

En caso de mantener su posición, solicito respetuosamente que el Examinador señale los apartes específicos en D1 y/o D2 que sugieren la necesidad de desarrollar formulaciones acuosas del compuesto I en un rango de pH específico entre 6,0 y 7,5 antes de liofilizar.

ii) Vale la pena resaltar que las características esenciales de la presente invención consisten en: i) una forma farmacéutica liofilizada; ii) la estabilización del compuesto activo dentro de un rango específico de pH; iii) la adición de un aglutinante en una cantidad entre 1 y 100; y iv) la preservación de la composición.

Así entonces, el Capítulo Reivindicatorio Modificado reclama composiciones específicas que comprenden la sal sódica del compuesto de Formula I y un preservativo, con un pH entre 6.0 a 7.5 previa liofilización, y cuyo contenido de agua residual después de la liofilización es menor al 1%, en las que el buffer empleado para ajustar el pH es opcionalmente buffer de citratos.

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Volviendo al estado de la técnica citado, D1 revela composiciones de cefalosporina que pueden ser liofilizadas, más no enseña el pH que debe presentar la composición previa liofilización. A partir de D1 no es posible predecir que ajustando el pH entre 6.0 y 7.5, se incrementa la estabilidad del compuesto de Formula I a largo plazo. Por su parte, D2 divulga los excipientes conocidos para formulaciones de β -lactámicos, pero no provee indicaciones directas que pudieran llevar a una persona medianamente versada en la materia a establecer las condiciones específicas que conllevan a mejoras sorprendentes en la estabilidad de formulaciones de cefalosporinas, como las alcanzadas con la presente invención.

iii) Nuevamente, con el fin de ilustrar el aporte de la presente invención al estado de la técnica, me permito citar el documento Connors et.al., Chemical Stability of Pharmaceuticals, (p.305 New York, NY. John Wiley and Sons, 1986), citado durante el examen de patentabilidad en la Oficina de Patentes de Estados Unidos. Este documento presenta una tabla y una gráfica que muestran la velocidad de hidrólisis de cefotaxime a diferentes valores de pH. De esta información es evidente cómo la velocidad de hidrólisis de cefotaxime se incrementa a pHs por debajo de 2 y por encima de 8; por el contrario, la mayor estabilidad es alcanzada en el rango de pH de 3 a 7. Igualmente, allí se establece que el tiempo de vida media ($t_{1/2}$) de cefotaxime a 25°C es típicamente 8.6×10^5 segundos (aproximadamente 10 días), es decir, en una composición neutra de cefotaxime, la mitad de éste se degrada en 10 días.

En contraste, el Compuesto I de la presente invención fue evaluado a una temperatura de 40°C, en donde se esperaría obtener una mayor degradación que a 25°C. La tabla a continuación integra datos de las tablas 3, 4 y 5 de la Descripción de la presente solicitud y muestra que a un pH cercano al neutro, el Compuesto I en las formulaciones de la presente invención, presenta una degradación de aproximadamente 0.8% en 12 semanas a 40°C.

Asimismo, la estabilidad a largo plazo de las composiciones de la presente invención también se ve incrementada, puesto que después de 18 meses a 25°C la degradación del compuesto activo es inferior al 10%. Por el contrario, formulaciones con un pH de 4 presentan porcentajes de degradación del principio activo superiores al 10%, lo que resulta inaceptable en productos farmacéuticos. Igualmente, los datos a continuación demuestran que la estabilidad óptima para las formulaciones que emplean un buffer de citratos se encuentra entre 6,0 a 7,0.

Otro de los aportes de la presente invención constituye la determinación precisa de la relación entre sacarosa/sal sódica de compuesto I (3:1), que conlleva a una estabilidad mejorada (Reivindicación 5). Por el contrario, formulaciones con relaciones sacarosa/sal sódica de

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compuesto I de (2:5) resultaron menos deseables (evaluación efectuada luego del almacenamiento a 40°C hasta por 12 semanas).

TABLA No 1				
Porcentaje del Compuesto I después de 12 semanas de almacenamiento a 40°C y tiempo de anaquel controlado a temperatura ambiente a 25°C				
Formulación #	pH	12 semanas (40°C)	% de degradación después de 12 semanas (40°C)	% de degradación después de 12 semanas (25°C)*
1	3.5	91.3	8.7	19
2	3.9	94.3	5.7	15
3	4.1	95.9	4.1	11
4	5.1	96.9	3.1	8
5	6.0	97.2	2.8	7
6	5	97.5	2.5	7
7	5.5	98.0	2.0	5
8	5.7	98.6	1.4	4
9	6.0	98.7	1.3	3
10	6.2	98.9	1.1	3
11	6.5	99.1	0.9	2
12	6.0	99.2	0.8	2
13	7.8	99.1	0.9	2
14	6.1	99.2	0.8	2

*calculado como se describe en la Descripción

El grado de estabilización alcanzado con la presente invención, indudablemente representa un resultado inesperado. El objetivo ideal a alcanzar con este tipo de composiciones farmacéuticas es que se pueda disponer de una composición liofilizada, que al ser reconstituida, permita su empleo por un periodo de una semana o más. Sin embargo, composiciones con un tiempo de vida media como las conocidas en el estado del arte, no cumplen este objetivo. Desde este punto de vista, Connors et al. enseña que no es conocido un rango de pH en el que cefotaxime presente una estabilidad deseada.

En conclusión, la presente invención no solo ha perfeccionado condiciones conocidas, sino que ha determinado aquellas que no solo eran desconocidas, sino que no se podían derivar de las enseñanzas del estado de la técnica.

iv) Aún cuando el Examinador argumente que la preparación de composiciones liofilizadas resulta derivado del estado del arte, debe recordarse que el Examinador precisamente tiene la carga de la prueba para demostrar por qué el desarrollo de una composición particular, como las reivindicadas en la presente solicitud, no presenta altura inventiva.

El estudio de solicitudes de patente que conduzca a conclusiones anticipadas, como las expuestas por el Examinador, corresponde abiertamente a un análisis conocido en el ámbito de patentes como "análisis hindsight". Para entender el análisis equivocado que realiza la SIC, podemos citar el Manual Andino de Patentes, que indica claramente: "...la búsqueda de anterioridades se efectúa a posteriori, tomando como punto de partida la misma invención, ...el examinador debe realizar el esfuerzo intelectual de colocarse en la situación que ha tenido que afrontar el técnico con conocimientos medios en la materia en un momento en que la invención no era conocida, es decir antes de la invención"¹ (mi subrayado). No es apropiado anticiparse a los resultados presentados por la invención, menos aún basándose solamente en la especulación, ya que así, siempre resultará "obvio" todo objeto de una solicitud de patente, sin dar mayor importancia al trabajo, intelecto y creatividad involucrados en la invención, para llegar a la solución del problema planteado en la solicitud.

Lo anterior hace necesario que ese Despacho brinde más importancia al problema técnico planteado y a la solución propuesta al momento de enfocar su análisis, en vez de limitarse a la simple comparación de las características técnicas del producto con alguna anterioridad, o aún peor, deduciendo que una invención específica es obvia a partir de suposiciones insustentables, o el uso de el estado de la técnica previo como si se tratara simplemente de armar un rompecabezas que coincida con la invención reclamada, sin tener en cuenta enseñanzas y sugerencias que necesariamente conducirían a la persona medianamente versada en la materia en una dirección opuesta a la invención reivindicada.

El Examinador no puede desatender de manera subjetiva, como lo enseña el MAP, que el análisis objetivo de nivel inventivo debe enfocarse en determinar "si teniendo en cuenta el estado de la técnica en su conjunto, existe alguna indicación que lleve a la persona versada en la materia a modificar o adaptar el estado de la técnica más cercano para resolver el problema técnico, de tal forma que llegue a un resultado que estuviera incluido en el tenor de la reivindicación".² De hecho, en el análisis objetivo de patentabilidad reconoce como indicios de patentabilidad los

¹ Manual Andino de Patentes, Secretaría de la Comunidad Andina, OMPI, EPO, 2004. Sección 10.2.1.

² Manual Andino de Patentes, Secretaría de la Comunidad Andina, OMPI, EPO, 2004. Sección 10.1.

efectos sorprendentes e inesperados de una invención cuando éstos no pueden ser evidentemente predecibles del estado de la técnica.³

3. Por todo lo anterior, considero que las objeciones presentadas por el Examinador han sido resueltas y por consiguiente solicito respetuosamente que se proceda con la concesión de las reivindicaciones pendientes en la presente solicitud de patente.

Atentamente,



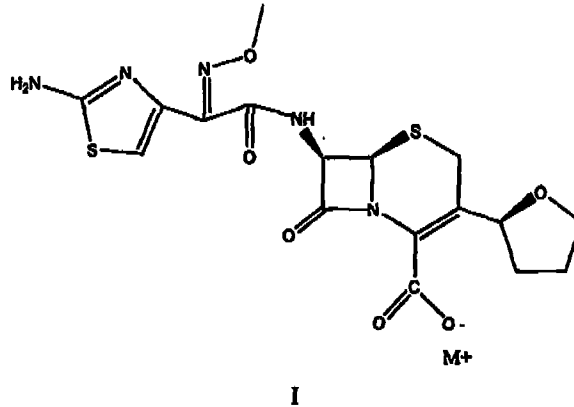
CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295

Anexos: Capítulo Reivindicatorio Modificado.
 Recibo de pago correspondiente a la tasa de modificación.
 Artículo Connors et.al., Chemical Stability of Pharmaceuticals, (p.305
 New York, NY. John Wiley and Sons, 1986).

³ Manual Andino de Patentes, Secretaría de la Comunidad Andina, OMPI, EPO, 2004. Sección 10.4.

CAPITULO REIVINDICATORIO MODIFICADO
(SEGUNDO EXAMEN DE FONDO)

1. Una composición farmacéutica liofilizada que comprende un compuesto de Formula I



en donde M es Na⁺, un buffer opcional farmacéuticamente aceptable, y un preservativo farmacéuticamente aceptable seleccionado entre metil parabeno, propil parabeno, o una combinación de metil y propil parabeno; en donde la composición acuosa, previa liofilización tiene un pH de 6.0 a 7.5, y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado.

2. La composición de conformidad con la Reivindicación 1, en donde el buffer es un buffer de citrato.
3. La composición de conformidad con las Reivindicaciones 1 a 2, en donde la composición después de liofilización tiene un contenido de agua residual menor al 0,5% en peso de liofilizado.
4. La composición de conformidad con las Reivindicaciones 1 a 3, en donde el vehículo es sacarosa y la relación vehículo: compuesto es 3:1.
5. Una composición conformidad con Reivindicaciones 1 a 4, que además comprende un agente de carga para liofilización.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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 Act. 444 RESPUESTAREQUE Folios: 41

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	81240762	04/11/2008	2,519,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
12		MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS

RESPONSABLE :

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of Pharmaceuticals**

A Handbook for Pharmacists

Second Edition

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PH A. CONNORS
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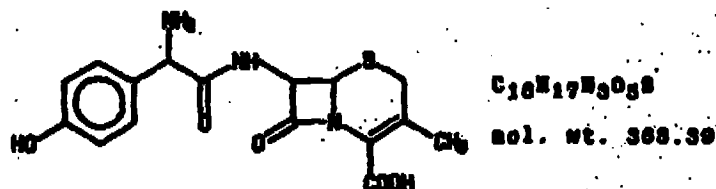
Cefadroxil

GENERAL

Names

(6R,7R)-7-[(R)-2-amino-2-(p-hydroxyphenyl)acetanido]-8-methyl-6-oxo-6-thia-1-azabicyclo[4.2.0]oct-2-ene-3-carboxylic acid; cefadroxil.

Structure



Forms Available

Cefadroxil monohydrate, anhydrous cefadroxil.

Physical Properties

Cefadroxil is soluble in water and slightly soluble in alcohol (1). The apparent pK_a 's for cefadroxil are: pK_{a1} (carboxylic acid) = 2.64; pK_{a2} (α -ammonium group) = 7.80; pK_{a3} (phenolic group) = 9.69 at 35°C, μ = 0.8 (2).

Stability Summary

In aqueous solution, cefadroxil degradation results in the cleaving of the β -lactam moiety by three parallel mechanisms: (1) intramolecular aminolysis; (2) water-catalysed hydrolysis; and (3) nucleophilic attack of hydroxide ion (2). General acid and general base catalysis occur in citrate and phosphate buffer systems (2). The activation energies, at 35°C, are re-

been performed.
side reactions
products result
on the β -lactam

its for intrave-
can affect the
has been found
inactivated when
with multiple B

rbashi, Can. J.

16th ed.),
Pennsylvania,

Sci. Ed., 7, 95

Sci. Ed., 4, 91

m. Sci., 60, 503

Am. J. Hosp.

- Ray W. Wood
(Wisconsin)

16

ported to be 21.4 and 23.8 kcal/mol at pH 7 and 11.8, respectively (2). The apparent activation energy at the alkaline pH may include the heat of ionisation of water, 18.1 kcal/mol (3). The net activation enthalpy of the hydroxide-ion-catalyzed degradation is 10.8 kcal/mol (2). Two other aminoccephalosporins, cephalixin and cephradine, have stability patterns similar to cefadroxil (4).

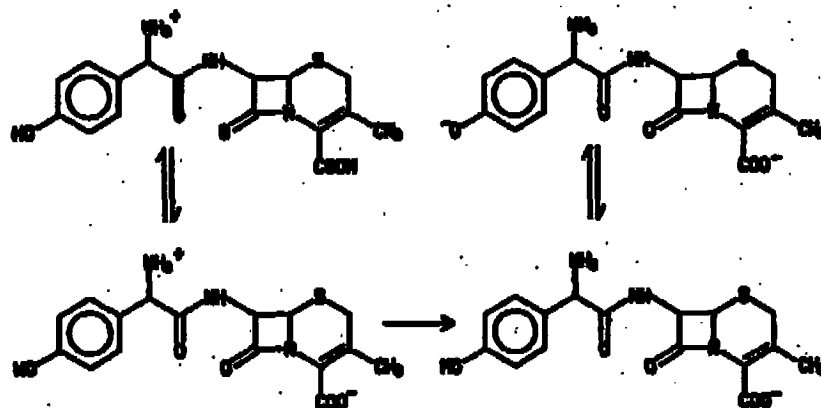
HO-

DRUG KINETICS

Reaction and Rate Equations (2)

In the pH range 2 to 10 cefadroxil exists in four ionic forms: as a cation (IH_3^+), a zwitterion (IH_2^+), an anion (I^-), and a dianion (I^{2-}). The ionization scheme is shown in Scheme 1.

HO-



The degra

$IH_3^+ \rightleftharpoons$

$IH_2^+ \rightleftharpoons$

$IH_2^+ \rightleftharpoons$

$I^- + H^+$

$I^{2-} + H^+$

The rate

rate =

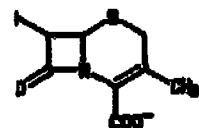
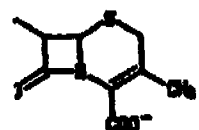
SCHEME 1

In neutral and alkaline solutions, the main degradation products are two piperazine-2,5-diones (II and III) and 3-hydroxy-4-methyl-5(3H)-thiophenone (IV):

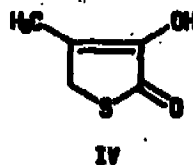
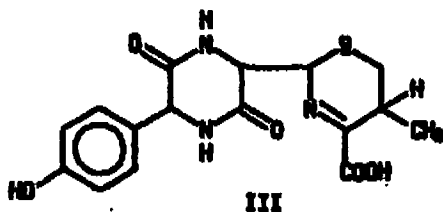
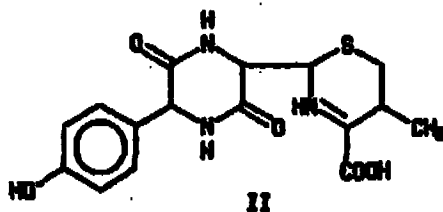
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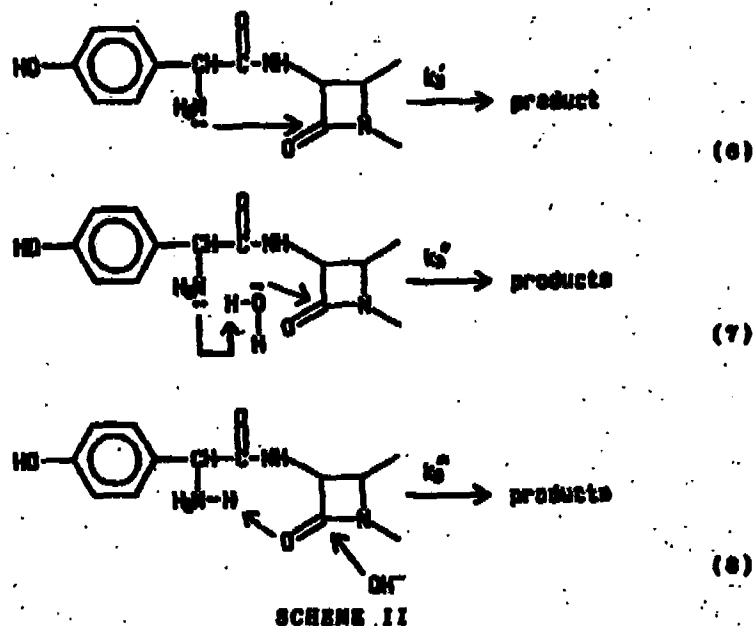


The rate equation for this kinetic scheme is:

$$\text{rate} = k_1[IH_2^+] + k_2[IH^{\pm}] + k_3[I]_T / ([H^+] + K_a) \\ k_4[I^-][OH^-] + k_5[I^{2-}][OH^-]$$

where k_1 and k_2 represent the apparent first-order rate constant for the water-catalysed degradation of cationic and zwitterionic cefadroxil; k_3 is the rate constant of three kinetically indistinguishable mechanisms that occur near pK_{a2} (Scheme II); k_4 and k_5 are

the second-order rate constants for the OH^- catalyzed degradation of both anionic species.



pH-Rate Profile

Figure 1 shows the dependence of the logarithm of the apparent first-order rate constant on pH for the overall loss of cefadroxil at 35°C, $\mu = 0.5$. The rate constants have been corrected to zero buffer concentration (2). The dissociation of the phenolic moiety apparently has no effect on the β -lactam cleavage since the slope of the line near pH 10 is unity. There is an inflection point at pH values near pK_{a2} , indicating that the dissociation equilibria of the side-chain amino group affect the degradation rate (2). Below pH 5, the degradation rate of cefadroxil is pH independent, indicating that the reaction was not influenced by the dissociation of the 4-carboxylic acid group.

FIGURE 1. C degradation

Activation E

The Arrhenius at pH 7 and in Figure 2. pH 7 is 21.4 mol (corrected (2)).

Other Data

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FORMULATIONS

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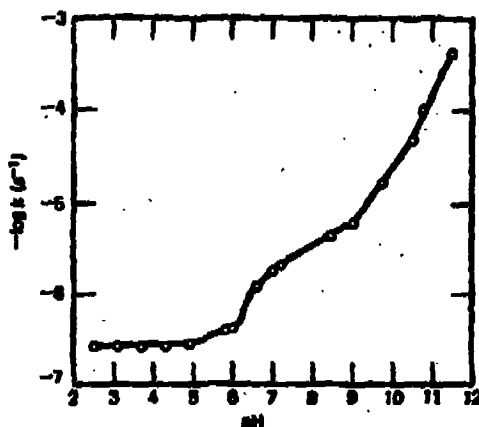


FIGURE 1. Cefadroxil. pH-rate profile for cefadroxil degradation in aqueous solution at 25°C and $\mu = 0.5$.

Activation Energy

The Arrhenius plot for the degradation of cefadroxil, at pH 7 and pH 11.5 and ionic strength 0.5, is shown in Figure 2. The activation energy of cefadroxil at pH 7 is 21.4 kcal/mol, and at pH 11.5 it is 10.7 kcal/mol (corrected for the heat of ionization of water) (3).

Other Data

General acid and general base catalysis occur in citrate and phosphate buffer systems.

Tsuji et al. (2) discussed cefadroxil stability relative to the stabilities of cephalexin, cephadrine, and cephaloglycin.

FORMULATIONS AND COMBINATIONS

Degradation Reactions

Cefadroxil is administered orally in capsules, tablets, and as an aqueous suspension that is reconstituted from a dry powder (5). Hydrolytic reactions of

the solid-state cefadroxil may be possible from moisture in the air. Once the suspension is reconstituted, the product is much less stable owing to hydrolytic and pH effects.

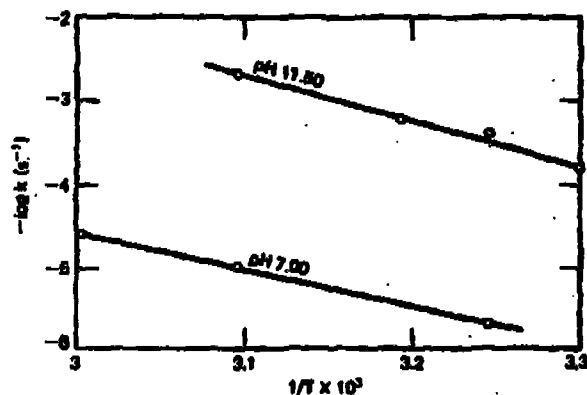


FIGURE 2. Cefadroxil. Arrhenius plot for the apparent first-order rate constants for cefadroxil degradation at pH 7 and 11.50.

Stabilisation Reactions

Since hydrolysis of solid-state cefadroxil may be a cause of degradation, cefadroxil should be kept in a tightly closed container (6,7). After reconstitution, store in a refrigerator and discard any unused portion after 14 days (5).

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- Patrick Sinko
(Michigan)

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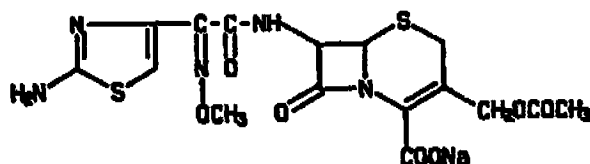
Cefotaxime

GENERAL

Names

Cefotaxime sodium, 7-[2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]cephalosporanic acid (sodium salt).

Structure



$C_{16}H_{18}N_5NaO_7S_2$

mol. wt. 477.48

Forms Available

Cefotaxime sodium.

Physical Properties

Cefotaxime sodium is an off-white powder that decomposes at temperatures of 162-163°C (1). It is fairly soluble in water, producing aqueous solutions light yellow in color (2). Potentiometric data (3) show pK_a values of 2.1, 3.4, and 10.9.

Stability Summary

Cefotaxime in solution is subject to hydrolysis at the ester group and the β -lactam ring. The deacetylated product can then go on to form a lactone. Hydrolysis is both specific acid catalyzed and specific base catalyzed, with neutral pH values being optimum for stability. Many buffers are compatible with cefotaxime, as are most common large-volume parenterals. Dry powder is best stored in a dark, cool, dry environment, and can be sterilized by gamma irradiation.

DRUG KINETICS

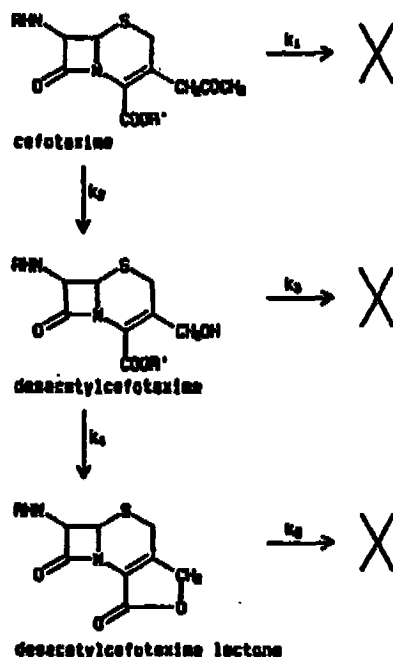
Reaction and R

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DRUG KINETICS

Reaction and Rate Equations

Cefotaxime is a 3-acetoxymethylcephalosporin, as are cephalothin and cephaloglycin, hence the major routes of degradation involve hydrolysis of the β -lactam ring and the acetoxy ester. At pharmaceutically relevant pH, temperature, and concentration values the amide side-chain hydrolysis is negligible (4). De-esterification proceeds more rapidly than the β -lactam ring opening. The observed degradation rate constant is the sum of the rate constants for the two major parallel routes. At pH < 4, an internal ring closure can occur to form the lactone of the de-esterified product. Scheme I illustrates this decomposition pathway, with R representing the amide side chain, X representing products of β -lactam ring opening, and R' representing sodium or hydrogen ion.



SCHEME I

azolyl)-2-meth-
(sodium salt).

$C_{16}H_{18}N_3NaO_7S_2$

mol. wt. 477.46

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304 Cefotaxime

At pH 5.5, the rate of formation of desacetylcefotaxime exceeds that of the lactam hydrolysis product, with $k_2:k_1 = 1.8$ (4). The pseudo-first-order reaction yields parallel log C vs. time plots over a 20-fold concentration range. Typical values in the neutral pH region at 25°C are $k_{obs} = 8 \times 10^{-7} \text{ s}^{-1}$ and $t_{1/2} = 8.6 \times 10^5 \text{ s}$.

The constant k_{obs} is the sum of the rate constants for acid-catalyzed, water-catalyzed, and hydroxide-ion-catalyzed hydrolysis:

$$k_{obs} = k_H[H^+] + k_W + k_{OH}[OH^-] \quad (1)$$

The sum of the two routes (lactam and ester hydrolysis) is followed experimentally, hence $k_{obs} = k_1 + k_2$. Using the previous estimate of k_{obs} in the neutral pH region, and the fact that $k_2:k_1 = 1.8$, yields $k_1 = 2.9 \times 10^{-7} \text{ s}^{-1}$ and $k_2 = 5.1 \times 10^{-7} \text{ s}^{-1}$.

pH-Rate Profile

Figure 1 is a plot of log k_{obs} vs. pH at 25°C and ionic strength = 0.5 M (4). It exhibits the typical U-shape of cephalosporins that are inactive orally (5). Specific acid catalysis occurs below pH 3 and specific base catalysis occurs above pH 8. Between pH 3 and pH 7 the hydrolysis rate is virtually independent of pH. The calculated line uses the estimates of $k_H = 1.15 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, $k_W = 8.51 \times 10^{-7} \text{ s}^{-1}$, and $k_{OH} = 4.49 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ (4). The pH of minimum hydrolysis rate is pH 5.13.

The positive deviation from the calculated line between pH 2 and pH 4 has been postulated (4) to be due to an intramolecular general acid catalysis by the carboxyl group on the deacetylation reaction. A more easily identified shoulder is noted on the k_{obs} vs. pH plot near the pK_a of cefotaxime. It is possible that the ionization may slightly alter the kinetics of degradation.

Activation Energy

Figure 2 shows an Arrhenius plot of cefotaxime degradation of several pH values and ionic strength = 0.5 M (4). Determination of the slopes results in the following data:

pH	E_a (kcal·M ⁻¹)
2.23	21.2
5.52	24.7
8.94	9.4

At pH 8.94, the activation for the heat of ionization, the calculated k_{obs} with the measured experime

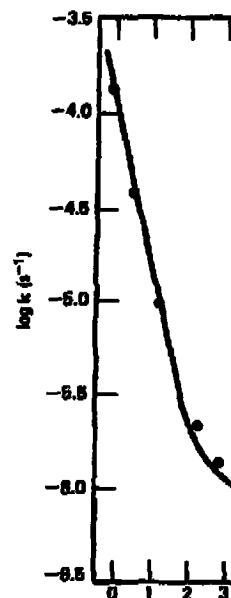


FIGURE 1. Cefotaxime. pH at 25°C, ionic strength =

pH	E_a (kcal·M ⁻¹)	E_a (kJ·M ⁻¹)	25°C; $t_{1/2}$ (hr)
2.23	21.2	88.4	88.6
5.32	24.7	103.5	213.9
8.94	9.4	39.4	27.2

At pH 8.94, the activation energy has been corrected for the heat of ionization of water. At all pH values, the calculated k_{obs} at 25°C was in good agreement with the measured experimental k_{obs} at 25°C.

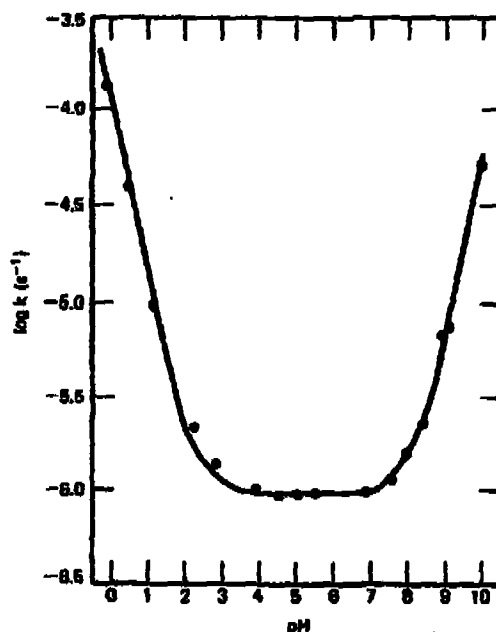


FIGURE 1. Cefotaxime. pH-rate profile for hydrolysis at 25°C, ionic strength = 0.5 M (4).

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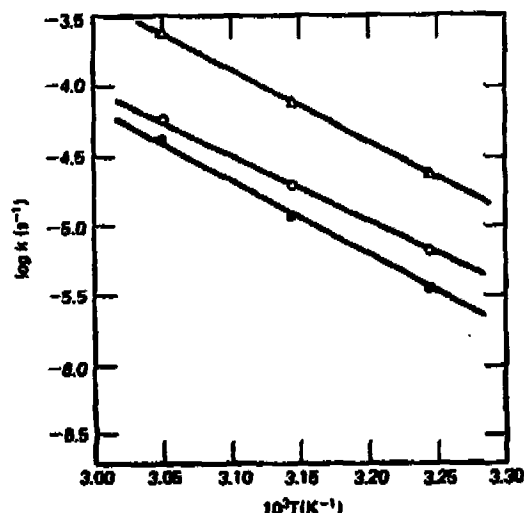


FIGURE 2. Cefotaxime. Arrhenius plots at several pH values (Ref. 4): ionic strength = 0.5 M; pH 2.23 (○); pH 5.82 (●); and pH 8.94 (△).

Other Data

No catalytic effect was seen with citrate or phosphate buffers on the rate of cefotaxime hydrolysis (4). Carbonate and borate buffers have been shown to increase the degradation rate, whereas acetate buffer may slow degradation of cefotaxime (3). An investigation of the primary salt effect using potassium chloride showed no effect in the range 0.2-1.0 M for pH values of 2.2, 5.5, and 8.0 (3,4). Plots at pH 8.9 and 9.5 gave small positive slopes, indicating that two negatively charged species are reacting (3,4).

Jacobs (6) has shown that cefotaxime powders can be sterilized via gamma radiation without breakdown of the drug. Storage of the powder above 30°C causes it to darken (7), the cause being unknown.

FORMULATIONS AND

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Stabilization M

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Das Gupta et al.
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3. H. Fabre, Sci., 73, 6
4. S. M. Berge Pharm. Sci.
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FORMULATIONS AND COMBINATIONS

Degradation Reactions

Cefotaxime sodium should not be diluted with highly basic parenterals, such as sodium bicarbonate injection, or highly acidic parenterals, owing to the acid-catalyzed and base-catalyzed hydrolysis.

Stabilization Methods

Reconstitution of cefotaxime sodium results in a product that will maintain potency for 10 days under refrigeration or 13 weeks when frozen (7). The pH of the solution should be maintained between pH 4 and pH 8 for maximum stability. Solutions prepared with the following injectables maintain potency for 24 h at room temperature: normal saline, 5% dextrose, 10% dextrose, lactated Ringer's solution, sodium lactate injection, 10% invert sugar injection, and dextrose plus normal saline. Dry powders should be stored in a cool, dry place and protected from excessive light.

Das Gupta recently studied the stability of cefotaxime in 5% dextrose and 0.9% sodium chloride solutions at low temperatures (8). The loss in potency was less than 5% after 30 days at 4°C and 224 days at -10°C.

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308 Cefotaxime

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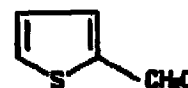
Cephalothin

GENERAL

Names

Cephalothin.

Structure



Forms Available

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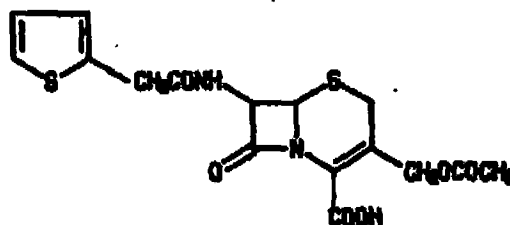
Cephalothin

GENERAL

Names

Cephalothin.

Structure



$C_{16}H_{16}N_2O_6S_2$

mol. wt. 396.43

Forms Available

Sodium cephalothin ($C_{16}H_{15}N_2NaO_6S_2$, mol. wt. 418.41).

Physical Properties

Sodium cephalothin is soluble in water, slightly soluble in alcohol, and insoluble in most organic solvents (1). The pK_a (of the conjugate acid) is 2.22 at 35°C (2).

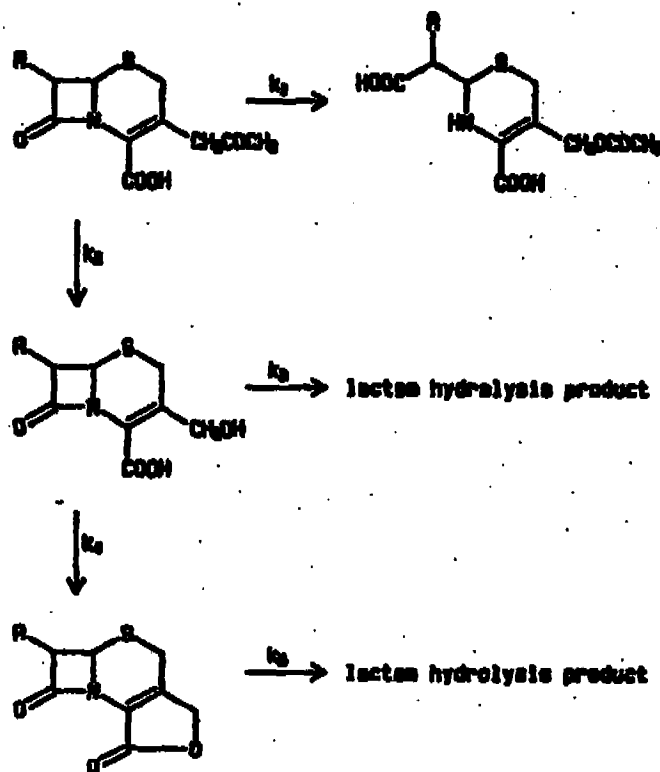
Stability Summary

Cephalothin undergoes hydrolysis of the β -lactam ring and of the 3-acetoxy group. The pH-rate profile reveals specific acid and specific base catalysis, with a broad pH-independent valley from pH 3 to 8. Stability is maximized by ensuring that the pH does not fall outside this range. Frozen aqueous solutions of sodium cephalothin have been shown to be stable for up to 6 weeks.

DRUG KINETICS

Reactions and Rate Equations

Cephalothin is a 3-acetoxymethylcephalosporin and thus has three sites at which hydrolysis can occur: the amide side chain, the β -lactam ring, and the acetoxy ester group. Under conditions of pharmaceutical interest, the lactam and acetoxy hydrolysis routes predominate, the rate behavior being similar to that reported for cephalosporin C (3). In acid medium, hydrolysis of the acetoxy group is followed rapidly by lactonization of the resulting hydroxy group. Letting R represent the amide side chain, these reactions are shown in the following scheme.



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In neutral and basic media the lactonization (the k_4 step) does not take place; the deacetylation (k_2) and lactam opening (k_1 and k_3) steps can be separately studied. Yamana and Tsuji (2) found that the rate constants k_1 and k_3 are about equal, and that the relative rate k_2/k_1 is 1.6. In acidic solution k_4 is larger than k_2 , so the deacetylcephalothin produced in the k_2 step is converted rapidly to the lactone.

The rate of hydrolysis of the lactam ring does not appear to be affected by ionization of the carboxylic acid group (2). The apparent first-order rate constant k_{obs} for hydrolysis of the lactam (from cephalothin, deacetylcephalothin, and deacetylcephalothin lactone) is given by Eq. (1).

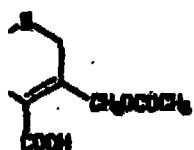
$$k_{obs} = k_2[H^+] + k' + k_{OH}[OH^-] \quad (1)$$

This experimental k_{obs} is equal to k_1 , since this describes the lactam hydrolysis and since k_1 is nearly equal to k_3 .

pH-Rate Profile

Figure 1 shows the plot of $\log k_{obs}$ [defined in Eq. (1)] vs. pH for the loss of the β -lactam ring at 35°C and ionic strength 0.5; the rate constants have been corrected to zero buffer concentration (2). This profile provides evidence that dissociation of the carboxylic acid does not affect the hydrolysis rate, since no inflection is seen at pH values near the pK_a . Acid catalysis is important below pH 3, and base catalysis occurs above pH 8. A very broad pH-independent valley is observed between pH 3 and 8. The pH-rate data are fitted with Eq. (1) and the values $k_2 = 4.6 \times 10^{-6} M^{-1} s^{-1}$, $k' = 3.0 \times 10^{-6} s^{-1}$, and $k_{OH} = 2.9 \times 10^{-1} M^{-1} s^{-1}$.

Figure 1 is the pH-rate profile for the hydrolysis of the β -lactam, that is, for the k_1 step in the reaction scheme. The intact cephalothin molecule, however, is lost more rapidly than this figure indicates, since deacetylation is faster than lactam hydrolysis. Since $k_2 = 1.6 k_1$ and the rate constant for overall loss of intact drug is $k_1 + k_2$, whereas $k_{obs} = k_1$, it appears that the rate constant for loss of drug by both routes is about 2.6 times the values shown in Figure 1.



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olysis product

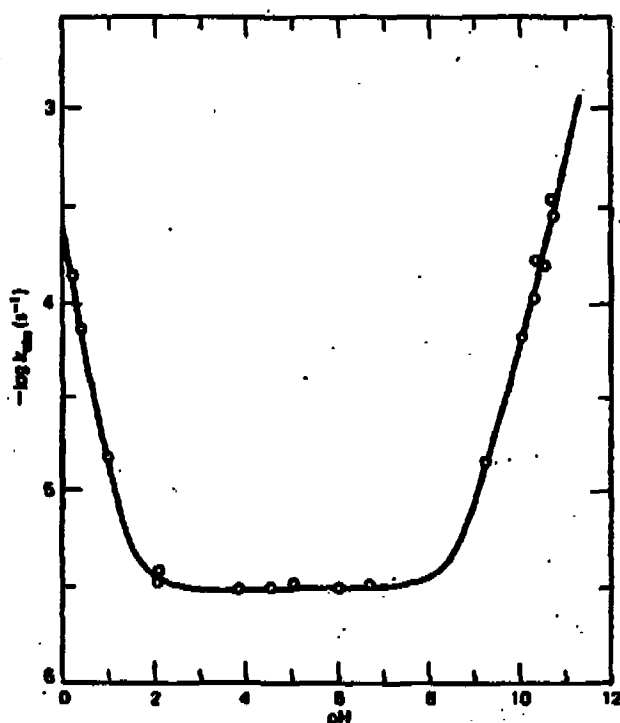


FIGURE 1. Cephalothin. pH-rate profile for hydrolysis of 6-lactam ring in cephalothin at 35°C (2).

Activation Energy

The activation energy for the lactam hydrolysis of cephalothin at pH 5.00 is 22.6 kcal/mol, and at pH 10.00 it is 18.4 kcal/mol (corrected for the heat of ionization of water) (2).

Other Data

Buffer components appear to be capable of exerting a moderate accelerating effect on k_{obs} (2).

Yamano and Tsuji (2) have discussed cephalothin stability relative to the stabilities of other cephalosporins and penicillins.

FORMULATION

Degradation

The hydrolysis is important; other nucleophilic degradation of cephalothin is not known.

Aged as been reported included the degradation in by

Stabilization

It is clear maximum change in range 3 to 4. It is a function of probably absolute range

Mean at the stability for injectable room temperature (5°C), and the solution after preparation recommends reconstitution a 10% less than preparation aid of the energy in agreement. Initial concentration

Frozen were found weeks (-20°C or 250 mg/ml) or 5% stabilization of water was

37

FORMULATIONS AND COMBINATIONS

Degradation Reactions

The hydrolysis reactions described earlier are the important routes of drug degradation. Reaction with other nucleophiles, particularly amines, might be anticipated. Color formation in solutions of sodium cephalothin has been reported (4), but its source is not known.

Aged samples of sodium cephalothin injection have been reported to cause pain in mice (5). It was concluded that the pain was caused by acetic acid produced in hydrolysis of the acetoxy group.

Stabilization Methods

It is clear from the pH-rate profile (Fig. 1) that for maximum chemical stability the pH should be in the range 3 to 8. Moreover, in order to forestall precipitation of the free-acid form, the optimal pH range probably should be further restricted to the approximate range 4 to 8.

Munn et al. (4) and Boylan et al. (6) have studied the stability of sodium cephalothin solutions in water for injection, in 0.9% saline, and in 5% dextrose, at room temperature (25°C), refrigerator temperature (5°C), and in the frozen state (-20°C). At 5°C all of the solutions showed about 10% decomposition 14 days after preparation. (The package insert conservatively recommends a maximum storage time of 2 days at 5°C for reconstituted sodium cephalothin solutions.) At 25°C a 10% loss of drug was observed about 34 h after solution preparation. [The t_{90} value calculated with the aid of the pH-rate profile (Fig. 1) and activation energy in the valley region is 33 h, in reasonable agreement.] These solutions were prepared with initial concentrations of 1 g/100 mL.

Frozen solutions of sodium cephalothin in water were found to be stable (<10% decomposition) for 6 weeks (-20°C, pH 4.8 to 6.8, initial concentration 100 or 250 mg/mL) (6). It was recommended that 0.9% saline or 5% dextrose not be used as the solvent when stabilization by freezing is planned, because precipitation of drug on thawing is more frequent than when water was the solvent. Once thawed, solutions should

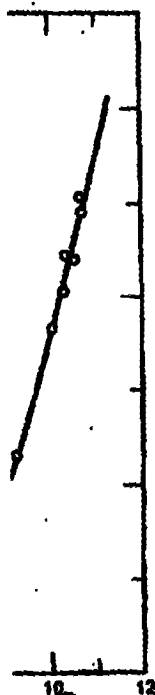


Fig. 1. Rate of hydrolysis of sodium cephalothin at 35°C (2).

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314 Cephalothin

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not be refrozen, since precipitation may result.

Frozen solutions of sodium cephalothin (in 5% dextrose) showed no loss in activity after storage at -20°C for 30 days whether thawed by room temperature exposure or microwave heating (7).

Cephra

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- Kenneth A. Connors
(Wisconsin)

GENERAL

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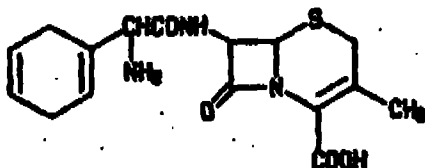
Cephadrine

GENERAL

Names

Cephadrine; 7-[D-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid.

Structure



$C_{16}H_{16}N_2O_4S$

mol. wt. 340.41

Forms Available

Cephadrine monohydrate.

Physical Properties

Melting point 140 to 142°C; pK_1 about 2.6 and pK_2 about 7.8 (1); sparingly soluble in water and solubility at the isoelectric pH is 26 mg/mL (2); very slightly soluble in alcohol; practically insoluble in chloroform and ether.

Stability Summary

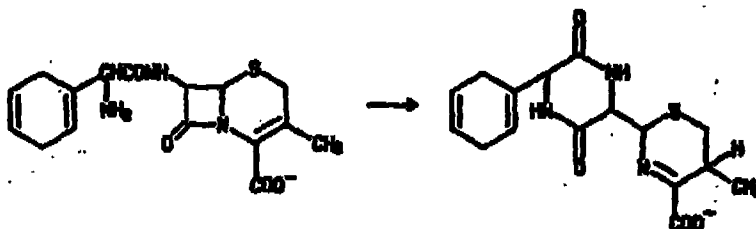
Cephadrine, a deacetoxycephalosporin, undergoes hydrolysis of the β -lactam ring. Cephadrine is fairly acid stable, and is shown to be 25 times more stable than cephalothin, cephaloridine, and cephaloglycin; and 180 times more stable than ampicillin at pH 1.0 (3). Its pH-rate profile shows a pH-independent region below pH 5, specific base catalysis above pH 10.

and an inflection at neutral pH resulting from the hydrolysis of the anion. Stability can be enhanced by keeping the pH below 5.

DRUG KINETICS

Reactions and Rate Equations

Cephadrine is susceptible to β -lactam ring hydrolysis, as is cephalixin. The kinetics and mechanisms of hydrolysis appear to be affected by the dissociation equilibrium of the α -amino group. At pHs between 3 and 5, hydrolysis is by direct water attack. At high pH, hydrolysis is catalyzed by hydroxide ion attack on the anion. At neutral pH, dissociation of the protonated α -amino group occurs, and degradation is catalyzed by the intramolecular nucleophilic attack of the α -amino group upon the β -lactam carbonyl to produce a diketopiperazine derivative. This mechanism is shown in the following scheme.



This diketopiperazine derivative has been isolated as the major alkaline degradation product from an aqueous sodium carbonate solution of cephadrine by Cohen et al. (4).

The apparent first-order rate constant k_{obs} for hydrolysis of the lactam can be given by Eq. (1):

$$k_{obs} = k_H[H^+] + k_{H_2O} + k_{OH}[OH^-] + k_2 \left(\frac{K_2}{K_2 + [H^+]} \right) \quad (1)$$

where k_{H_2O} , k_H , and k_{OH} are the rate constants for the uncatalyzed, hydrogen-ion-catalyzed, and hydroxide-ion-catalyzed reactions, respectively. The spontaneous degradation rate constant of the anionic species

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pH-Rate Profile

Figure 1
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FORMULATION

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is represented by k_2 . The hydrogen-ion-catalyzed reaction is insignificant in the overall kinetic picture.

pH-Rate Profile

Figure 1 shows the plot of $\log k_{obs}$ [defined in Eq. (1)] vs. pH for the hydrolysis of the β -lactam ring at 35°C and ionic strength 0.5; the rate constants have been corrected to zero buffer concentration (3). This profile provides evidence that dissociation of the protonated α -amino group influences the hydrolysis rate, since an inflection is seen at pH values near the pK_2 . Acid catalysis is unimportant, and base catalysis occurs above pH 10. A flat pH-independent region is observed below pH 5. The pH-rate data are fitted to Eq. (1) and the values $k_{H_2O} = 9.00 \times 10^{-7} \text{ s}^{-1}$, $k_{OH} = 1.11 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$, and $k_2 = 2.00 \times 10^{-6} \text{ s}^{-1}$ are obtained. The rate constant k_2 is not determined because of the large contribution by other kinetic terms.

Activation Energy

The activation energy for the hydrolysis of cephradine in aqueous solution has not been reported.

Other Data

Buffer components appear to be capable of exerting a moderate accelerating effect on k_{obs} . Figure 2 shows the dependence of the observed rate constants on total buffer concentration in borate buffer at 35°C (2).

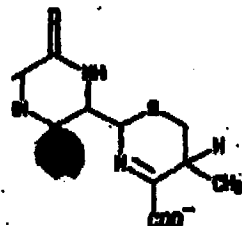
Yanaka and Tanji (3) have discussed cephradine stability relative to the stabilities of other cephalosporins and penicillins.

FORMULATION AND COMBINATIONS

Degradation Reactions

All cephalosporins undergo degradation in aqueous solutions. In a basic aqueous medium, cephradine yields a cyclic product that has undergone migration of the double bond to the C-4,5 position, and protona-

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$$\frac{k_2}{k_2 + [H^+]} \quad (1)$$

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anionic species

318 Cephadrine

tion at the C-3 position as described earlier. Although detailed studies have not been performed in an acidic aqueous medium, cephradine may be expected to undergo reactions similar to cephalixin (5). Reconstituted cephradine solution may vary in color; however, color changes do not affect the potency (6). The origin of color formation is not known.

Stabilisation Methods

It is clear from the pH-rate profile (Fig. 1) that, for maximum chemical stability, the pH should be below 5. However, in an injectable formulation, the acidity

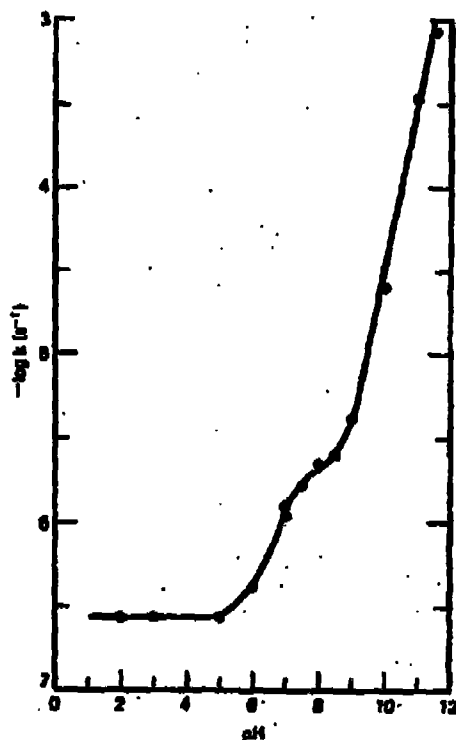


FIGURE 1. Cephradine. pH-rate profile for the hydrolysis of cephradine at 35°C and ionic strength 0.5 M.

FIGURE 2. against to pH 9.05; B

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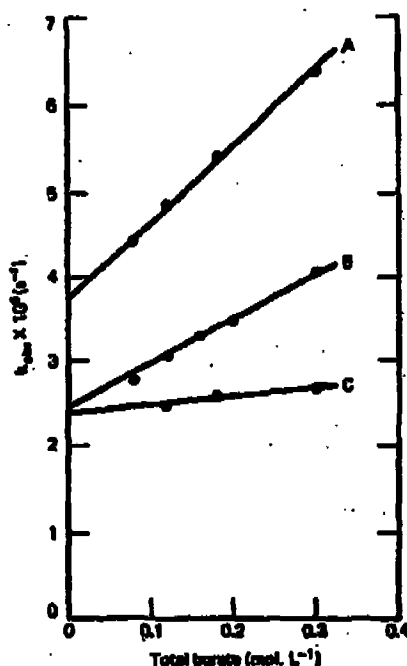


FIGURE 2. Cephadrine. Plot of observed rate constant against total borate buffer concentration at 35°C (A, pH 9.05; B, pH 8.46; C, pH 8.02).

of cephradine must be neutralized by an alkaline substance such as sodium carbonate to provide sufficient solubility and physiological acceptability. As a result, the manufacturer recommends a maximum storage time of 2 h at room temperature, or of 24 h if refrigerated at 5°C for reconstituted cephradine solutions (7). If frozen immediately after reconstitution with sterile water for injection, solutions can retain their potency in the original container up to 2 weeks at -20°C (7). It is also recommended that calcium-containing solutions, such as lactated Ringer's injection, not be used as diluents because cephradine for injection contains sodium carbonate (7).

Wang and Monkhouse (8) have studied the solution stability of two formulations of cephradine—one using

L-arginine and the other sodium carbonate as the neutralizer at 25°C. The degradation rates of 1% cephradine solutions in 0.9% saline, lactated Ringer's, Ringer's, Normosol-R, 5% dextrose, and sterile water were all lower for the arginine-neutralized product than the sodium carbonate-neutralized product. For example, when 0.9% normal saline is used as the diluent, a 3.85% loss of drug is observed with the cephradine-arginine solution, whereas an 8.53% loss of drug is observed with the cephradine/sodium carbonate solution. The enhanced stability with the arginine formulation may be attributable to the lower pH values of the solutions. However, at 5 and 25% cephradine concentrations, the differences in stability between the two formulations are not substantial.

Although the kinetics of decomposition of cephradine in aqueous solution have been described, little information is available on the stability of cephradine in its solid state. Conine et al. (8) have studied the stabilities of commercial cephradine capsules at 5, 25, and 40°C (and 40°C at 75% relative humidity) in various packages. At 5°C, cephradine capsules show little degradation under all conditions studied. However, decomposition occurs at higher temperatures, especially at high humidity. When cephradine capsules (250 mg) are repackaged in amber glass containers, the percentage loss in potency after 6 months at 25, 40, and 40°C (at 75% R.H.) are 2.7, 9.4, and 23.2%, respectively. It appears that cephradine capsules are somewhat unstable at high temperatures. Conditions of high humidity promote further decomposition at the same temperature. This problem can be reduced by storing the drug at low temperatures in completely moisture-tight containers.

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a, *J. Pharm.*

methyl and propyl parabens during freeze-drying is dependent on many factors, including the vacuum, length of drying cycle, temperature, and the amount and chemical entity of the materials present.

Novelty:

There is no disclosure in any of the prior art documents of a lyophilised pharmaceutical composition for parenteral administration comprising a compound of Formula (I), wherein M^+ is Na^+ , with a preservative selected from methyl paraben, propyl paraben and a mixture of methyl and propyl parabens, and wherein the aqueous composition prior to lyophilisation has a pH in the range of 6.0 to 7.5.

Claim 1 is therefore novel, as already acknowledged by the Examiner. Claims 2-6 are dependent on claim 1 and are therefore also novel.

Inventive step

D1 discloses a compound of Formula (I) as in claim 1, and generally discusses that the compounds of the invention can be administered parenterally using a lyophilised formulation which is diluted prior to use. D1 is the closest prior art.

The differences between claim 1 and the disclosure in D1 are:

- i. the use of methyl paraben, propyl paraben, or a mixture of both, as components of a lyophilised composition suitable for parenteral administration.
- ii. the aqueous composition prior to lyophilisation has a pH in the range of 6.0 to 7.5.

The objective problem is the provision of a stable formulation comprising a compound of Formula (I), which is suitable for commercial manufacture and use. The solution provided by the invention of claim 1 is to provide a composition comprising methyl and/or propyl parabens, and to ensure that the aqueous composition prior to lyophilisation has a pH in the range of from 6.0 to 7.5.

The claimed invention unexpectedly provides a highly favourable solution to the problem from 2 different perspectives:

1. The surprisingly favourable properties of the claimed composition enable it to be produced by a particularly efficient lyophilisation process. Such properties could not have been predicted at the filing date of the present application.
2. The claimed aqueous composition pH range prior to lyophilisation is surprisingly favourable because it provides optimum solution stability and lyophilised composition stability. It is noted that optimum solution pH does not necessarily result in the optimum solid state acidity of the lyophilised composition, the solid state acidity being measured by pH equivalent "pHeq". Since the formulation is stable, degradation of the compound of Formula (I) is minimised.

Regarding point 1, the use of parabens in a lyophilised product is not obvious, considering the knowledge of the skilled person at the filing date of the invention. None of the prior art documents D1-D10 disclose parabens as a component of a lyophilised composition suitable for parenteral

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administration, comprising a cephalosporin compound. Furthermore, D10 teaches away from the use of parabens in a lyophilised product, in view of the potential loss of parabens during the lyophilisation process. The option of reducing the drying temperature to 0°C is not practical for the compound of Formula (I), because it would result in a higher residual water content and poor long-term stability of the composition. However, in view of the unexpectedly strong molecular interaction between methyl or propyl parabens and the compound of Formula (I), the inventors surprisingly found there was no loss of methyl or propyl paraben during lyophilisation of the claimed composition.

2 There are also unexpected solubility benefits for parabens in the presence of the compound of Formula (I). At the filing date of the invention the skilled person was aware that parabens are difficult to dissolve, and a standard procedure would be to dissolve parabens at elevated temperature, usually around 70°C. The need for elevated temperatures complicates the manufacturing process and requires the use of specialised equipment for the manufacture of the drug product. Furthermore, temperature sensitive products such as the compound of Formula (I) should not be exposed to high temperatures, in view of the risk of degradation.

3 The inventors were surprised to find that methyl or propyl parabens dissolve readily in aqueous solution containing the compound of Formula (I) at room temperature. After further investigation, they found that the solubility of methyl or propyl parabens in water increased by at least a factor of 5 in the presence of the compound of Formula (I). The solubility of methyl paraben and propyl paraben in water and in the presence of the compound of Formula (I) were 11.5 and 3.0 mg/ml respectively, compared with solubility in water alone of approximately 2.5 and 0.4 mg/ml respectively.

4 The enclosed chart shows the relative solubility of methyl paraben in the presence of different solutes in aqueous solutions, $S_{\text{solution}}/S_{\text{water}}$, where S_{solution} is concentration of methyl paraben in solution in the presence of other solute, and S_{water} is solubility of methyl paraben in pure water. Such an enhancement of solubility for methyl and propyl parabens in the presence of the compound of Formula (I) (cefovecin) enabled the preparation of pre-lyophilisation solution of much higher concentration than was previously thought possible. This enabled a significant reduction (2.5 times) of the amount of water in the pre-lyophilisation solution, which in turn reduced the amount of water to be removed during lyophilisation and resulted in a more efficient drying process.

5 In summary, the prior art does not motivate the skilled person to select methyl and propyl parabens for lyophilised formulations comprising the compound of Formula (I). In fact, the inventors have overcome prejudice in the art which teaches away from using parabens for lyophilised products. There are many options available to the skilled person seeking to provide stable compositions, including the use of alternative preservatives or the use of sugars to stabilise cephalosporin compositions. However, the inventors found that the use of methyl and/or propyl parabens in combination with the compound of Formula (I) is surprisingly effective in producing a product that has both favourable manufacturing characteristics and stability. The choice of methyl and propyl parabens over all other pharmaceutically acceptable preservatives for a parenterally administered, lyophilised, product is inventive. Furthermore, the surprising increase in the solubility of methyl and propyl parabens in water when in the presence of the compound of Formula (I), and the unexpected retention of parabens in the composition during lyophilisation, enable efficient manufacture. Inventive step must therefore be acknowledged for amended claim 1.

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Turning now to point 2, the commercial product needs to be stable in the solid lyophilised form and in the reconstituted solution form. Both solution pH and solid-state acidity therefore have to be optimised in order to achieve the desired product stability. Of the prior art documents D1-D10, only D5 discusses the relationship between formulation stability and solution pH. However, D5 does not even suggest that solid state acidity should be considered. Therefore there is no motivation in the prior art for the skilled person to seek an optimum solution pH that also leads to optimum solid state acidity of the lyophile.

Optimum pH in solution was found to be at a pH range of from 6.0 to 7.5, as shown by the data in Table 1 below, and presented in the application as filed. The degree by which the compound of formula (I) is stabilised, and the pH range at which the compound is stabilised, could not have been predicted at the filing date. D5 shows that the optimum pH ranges of cephalosporin compounds vary, thus cannot be predicted.

Table 1 shows the % degradation of formulations of the compound of Formula (I) at different solution pH:

TABLE 1				
Percent Compound I after 12 weeks storage at 40°C and estimated shelf life at controlled room temperature 25°C				
Formulation #	pH	% Degradation		
		12 weeks (40°C)	after 12 weeks (40°C)	% Degradation after 18 months (25°C)*
1	3.5	91.3	8.7	19
2	3.9	94.3	5.7	15
3	4.1	95.9	4.1	11
4	5.1	96.9	3.1	8
5	6.0	97.2	2.8	7
6	5	97.5	2.5	7
7	5.5	98.0	2.0	5
8	5.7	98.6	1.4	4
9	6.0	98.7	1.3	3
10	6.2	98.9	1.1	3
11	6.5	99.1	0.9	2
12	6.0	99.2	0.8	2
13	7.8	99.1	0.9	2
14	6.1	99.2	0.8	2

*calculated as described in the application

Furthermore, formulating the product at optimum solution stability does not guarantee optimum stability in the lyophilised state, since lyophilisation can result in significant pH changes (L.N. Bell et

al, Potential pH implications in the freeze-dried state, *Cryo-Lett.* 12, 234-244 (1991); Strickley et al, *Pharm Res.* 1989, 6, 11, 971-975).

Measurements of solid state acidity are therefore critical in order to find the optimum pH for the composition. Hammett acidity function, also known as pHeq (Hammett et al, *J. Am Chem Soc.* 1932, 54, 2721-2739;; Glombitza et al, Surface acidity of solid pharmaceutical excipients II. Effect of the surface acidity on the decomposition rate of acetylsalicylic acid *Eur. J. Pharm Biopharm.* 1995, 41, 114-119) can be used to express acidity in complex systems where direct pH measurements cannot be performed, and to predict the rate of acid-base chemical reactions in such systems.

Using the claimed optimal solution stability range of pH 6 to 7.5 for pre-lyophilised solutions, lyophilised formulations with citrate buffer were observed to have a pHeq of 6.9 to 7.5, and with phosphate buffer the pHeq was observed to be from 6.1 to 6.8. Hence, formulations comprising a compound of formula (I) and with either citrate or phosphate buffer produced the desired target solid state acidity range of pHeq 6 to 7.5, when prepared from the claimed solution stability range. Such close correspondence between optimal solution pH and solid state pHeq could not have been predicted at the priority date.

Table 2 illustrates the differences between solution pH and solid state acidity for sucrose/buffer formulations without the compound of formula (I), and shows that an optimal solution pH of 6.0 to 7.5 would correspond to the pHeq ranging from 5.0 to 7.2, which is outside the optimal solid state stability. This illustrates that optimum pH does not always lead to optimum pH equivalent.

Table 2. pHeq of lyophilized sucrose formulations prepared with either citrate or phosphate buffer.

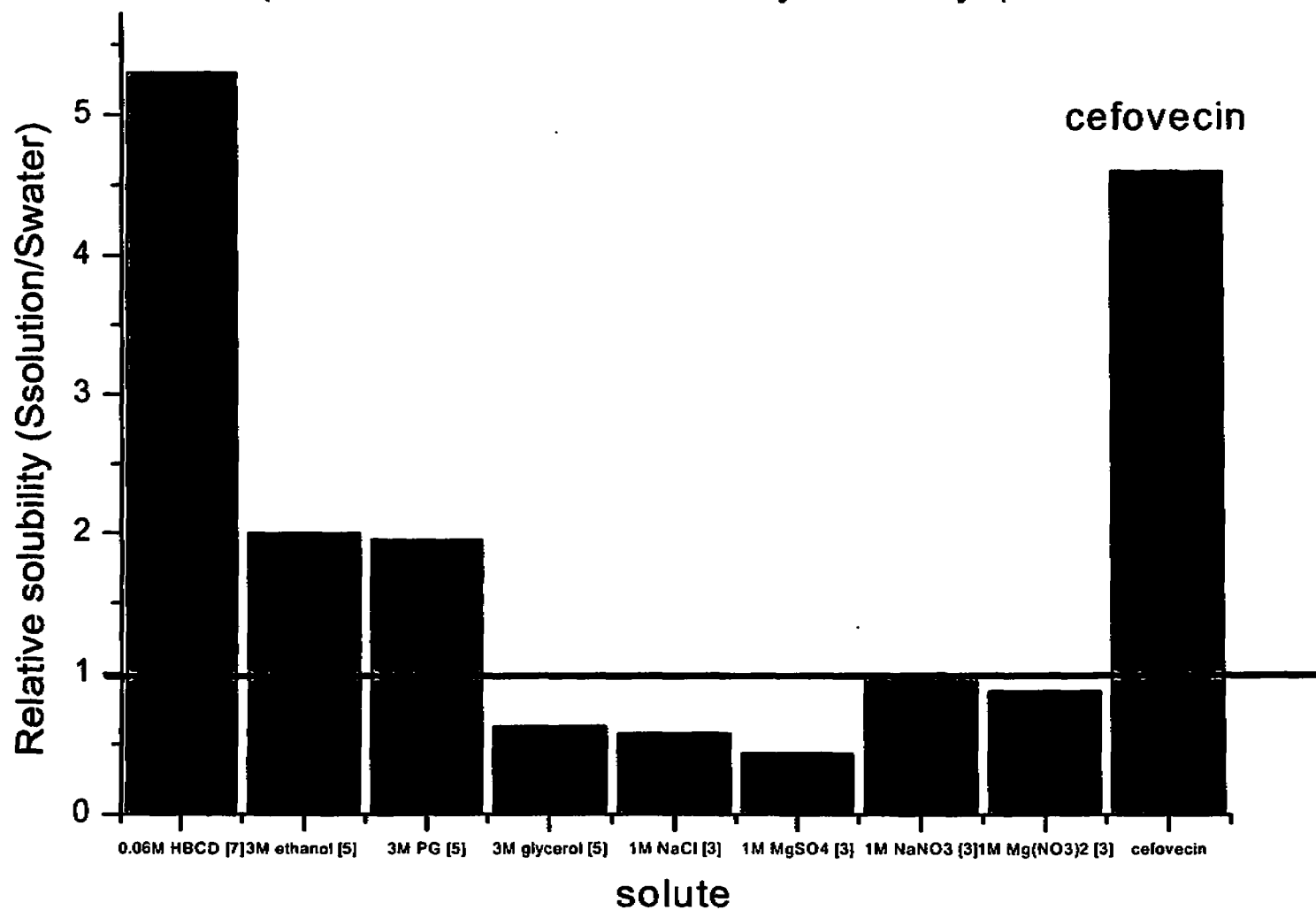
pre-lyophilization solution pH	pHeq	
	citrate	phosphate
5.5	5.13±0.10	4.48±0.04
6	5.47±0.10	4.97±0.05
6.5	5.85±0.07	5.37±0.09
7	6.88±0.16	6.57±0.09
7.5	7.20±0.10	6.83±0.12

Further evidence that the effect of pH on solid-state stability cannot be predicted is found in Li, Bei et al Effects of Acidic N + 1 Residues on Asparagine Deamidation Rates In Solution and in the Solid State, *Journal of Pharmaceutical Sciences* (2005), 94(3), 666-675.

The prior art does not lead the skilled person to arrive at a composition comprising a compound of Formula (I), with a pH of from 6.0 to 7.5. The advantages of this pH range for stability, both of the composition in solution and as lyophilised, could not have been predicted at the priority date. Inventive step must therefore be acknowledged for the selection of an optimum pH range.

Overall, the composition as claimed in claim 1 is inventive over the prior art. The inventors have overcome prejudice in the art to arrive at a composition which has particularly favourable properties for manufacturing and has highly favourable solid state and solution state stability. Since

Impact of a solute on solubility of methyl paraben



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COMMUNICATIONS

The loss of paraben preservatives during freeze drying

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Recently the loss of two preservatives, methylparaben (MP) and propylparaben (PP) was encountered during freeze drying of an aqueous solution in our laboratory. Avis (1970) has noted that volatile antibacterial preservatives (benzyl alcohol, phenol and chlorobutanol) are not suitable for lyophilized products. However, we are unaware of any reports describing the loss of MP or PP during lyophilization. This report describes the factors influencing the loss of these antibacterial preservatives during freeze drying.

Freeze-drying. Solutions containing 0.8 mg ml⁻¹ MP (Amend Drug & Chem. Co., Inc., New York, NY), 0.2 mg ml⁻¹ PP (U.S.P.H.S. S.S.C., Perry Point, MD) and various amounts of mannitol (Aldrich Chem. Co., Milwaukee, WI) or sodium chloride in distilled water were prepared and dispensed (5.0 ml) into 10 ml flint vials. The vials were placed on the prechilled shelf (about -40 °C) of pilot scale, 1 ft³, freeze drying unit (Model 10-800, Virtus Co. Inc., Gardiner, NY). After the contents of the vials were frozen, the product was processed through various drying cycles detailed in Table 1 and 2. Samples were removed and stored at -15 °C until the completion of an experiment at which time all analyses were performed. Also, samples of one lot of calcium leucovorin (BV-79-205) an investigational product containing 4 mg MP and 1 mg PP per vial, were reconstituted with 5.0 ml of distilled water and freeze dried again in our pilot scale unit.

Analysis. MP and PP were determined in the presence of mannitol or calcium leucovorin by reverse phase high pressure liquid chromatography (h.p.l.c.). MP, PP, internal standard (benzophenone) and calcium leucovorin were readily separated (retention volumes = 3.8 ml, 5.5 ml, 7.4 ml, respectively) on a C-18 (10 µm) column by elution with methanol-isopropanol-water (55:10:35, v/v) delivered at 1 ml min⁻¹. The eluted compounds were detected at 254 nm. Mannitol was not detected as it lacks u.v. absorbance. The freeze-dried samples were reconstituted for analysis with 5.0 ml of distilled water. A 125 µl aliquot was diluted to 10 ml with mobile phase containing internal standard, and a 10 µl aliquot was injected for h.p.l.c. analysis. Samples of calcium leucovorin (Ben Venue Labs., Bedford, Ohio, Lots BV-77-225, BV-78-255 and BV-79-205) were similarly diluted and analysed. These also contained 4 mg of MP and 1 mg of PP per vial.

The results in Table 1 indicate a substantial loss of both MP and PP during freeze drying in the pilot unit at 10-20 µm Hg. The losses became progressively greater as the drying time and product temperature increased. The paraben-containing calcium leucovorin product that was processed in a production freeze dryer was analysed and there was little or no apparent loss of MP or PP in the three lots. The content of MP and PP ranged between 97.2-99.7 and 98.2-98.3% of label, respectively. Freeze drying logs for typical batches indicate that the chamber pressure during the 62 h drying cycle varied from 90 to 200 µm Hg. An attempt

* Correspondence.

Table 1. Paraben content of products freeze-dried with vacuum of ~20 µm or 175 µm Hg

Drying cycle (h)	Temp. Range °C	% remaining (s.d.)							
		~20 µm				~175 µm			
		MP	n	PP	n	MP	n	PP	n
24	-30 to 0	95.7 (3.7)	12	95.9 (2.8)	6	98.4 (0.6)	6	99.7 (1.8)	
24	-30 to 0								
24	0 to 5	94.0 (1.9)	11	94.9 (1.5)	7	97.8 (0.8)	7	98.7 (1.3)	
24	-30 to 0								
24	0 to 5	58.1 (3.2)	10	66.5 (2.5)	7	90.0 (1.4)	7	93.7 (1.2)	
24	5 to 25								

* Temperatures were recorded at the beginning and at the end of each 24 h.

Table 2. Effect of weight of excipient on the loss of parabens

Freeze-drying Cycle		Excipients* (mg of each component per vial)								No Excipients	
		90 mg Mannitol		45 mg Mannitol 45 mg NaCl % remaining (s.d.) (n = 4)		45 mg Mannitol		22.5 mg Mannitol			
h	Temperature* Range (C°)	MP	PP	MP	PP	MP	PP	MP	PP	MP	PP
24	-30 to 0°	95.5	96.8	99.9	98.4	97.4	95.0	99.5	99.0	93.0	100.3
24	-30 to 0°	(2.4)	(2.6)	(0.5)	(1.5)	(0.4)	(2.6)	(0.2)	(2.2)	(2.3)	(2.4)
24	0 to 25°	78.6	81.1	77.1	78.5	70.7	71.7	75.7	79.5	67.0	73.7
24	0 to 25°	(3.6)	(5.6)	(2.4)	(2.1)	(2.6)	(4.6)	(3.7)	(3.0)	(2.2)	(2.8)
24	0 to 25°	66.6	71.2	56.1	64.8	53.6	58.3	46.3	54.7	34.3	40.0
24	25°	(2.8)	(1.6)	(5.6)	(3.9)	(2.3)	(0.6)	(9.9)	(10.0)	(5.7)	(3.6)

* All formulations initially contained 4 mg/vial MP and 1 mg/vial PP in addition to the excipients listed.

* Temperatures recorded at the beginning and at the end of each 24 h.

to reproduce a vacuum in this range in our freeze drying units using the mannitol-paraben product gave the other results seen in Table 1. Although the loss of MP and PP under these conditions was substantially less than observed at 10-20 μ m Hg, some loss was evident. The reconstitution and subsequent repeat freeze drying at 10-20 μ m Hg of the calcium leucovorin production lot (BV-79-205) resulted in the surprising retention of both MP (93.7%) and PP (95.9%). The drying cycle was identical to the longest cycle detailed at the foot of Table 2. Since the calcium leucovorin product contained 45 mg of sodium chloride in addition to the parabens and 50 mg of drug, the possibility that the increased bulk might account for the retention of the parabens was investigated. The data in Table 2 indicate that the total amount of other material present does reduce the loss of MP and PP. However, the effect is not enough to explain the retention of parabens in the calcium leucovorin product. A possible explanation can be seen in the u.v. spectra of a solution containing 10.9 μ g ml⁻¹ calcium leucovorin, 2.2 μ g ml⁻¹ MP and 0.55 μ g ml⁻¹ PP. The λ_{max} of calcium leucovorin is shifted 5 nm (from 283 to 278 nm) in the presence of parabens indicating the possible formation of a complex containing drug and parabens. Complexation between other compounds and hydroxybenzoates has been described previously (Poochikian & Cradock 1979; Higuchi & Kristiansen 1970; Lachman 1968). The reduced volatility of such a complex could explain the retention of the parabens in this product. Further support for this observation was sought with adriamycin hydrochloride, an antitumour agent supplied commercially as a freeze-dried product. This drug was

chosen as a likely candidate based on physical and structural considerations (lipophilicity, planarity and aromaticity) to retain parabens by a similar mechanism. A formulation containing 10 mg adriamycin hydrochloride, 4 mg MP and 1 mg PP was freeze-dried in a pilot unit under the same conditions as described for the reconstituted calcium leucovorin product. Analysis indicated an average retention of 97.4% for MP and 95.4% for PP in the adriamycin product.

Although the loss of parabens was not evident in the calcium leucovorin or adriamycin product, the possibility that such a loss may occur with other drugs cannot be ignored. The results of this study show that the loss of MP and PP during freeze drying is dependent on the vacuum of the system, the length of drying cycle, the temperature of the product, and the amount and chemical entity of materials present. Analysis of paraben-containing freeze-dried products for both active ingredient and antibacterial preservatives appears warranted.

March 5, 1980

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

Radicación No. 04 043501

Clasificación Internacional (IPC): A61K47/00; A61K31/546, A61K9/19.

Como resultado del examen definitivo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados

Descripción: Folios: 53 a 80 (como se radicó inicialmente)

Reivindicaciones: Folios: 81 a 83 (como se radicó inicialmente)
Folio : 115 Primera Modificación.
Folio: 127 Segunda Modificación.

Prioridad: Folios 1 (30-11-2001, US60/338,536).
(26-07-2002, US60/398,932).

Respuesta del solicitante: Folios: 111 a 114. Primera respuesta.
Folios: 121 a 126 Segunda respuesta.

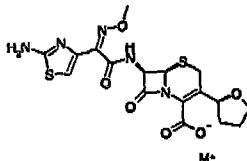
Se acepta para estudio el capítulo reivindicatorio del folio 127.

Objeto de la invención

Título de la solicitud: Métodos de tratamiento y formulaciones de cefalosporina.

El objeto de la presente solicitud de patente de invención consiste en:
Una formulación farmacéutica liofilizada estable que contiene cefovecina sódica.

Reivindicaciones 1-5 : Una composición farmacéutica liofilizada que comprende :



- Un compuesto de fórmula I.
- Un preservativo seleccionado de metilparabeno, propilparabeno o una combinación de ellos.
- En donde la composición acuosa previa liofilización tiene un pH de 6.0 a 7.5 y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado.

El problema planteado en esta solicitud es la necesidad de formulaciones liofilizadas que incrementen la estabilidad a largo plazo de la cefovecina sódica.

Para solucionar este problema técnico la solicitud en estudio proporciona Una composición farmacéutica liofilizada de cefovecina sódica que comprende : un preservativo seleccionado de metilparabeno, propilparabeno o una combinación de ellos, en donde la composición acuosa previa liofilización tiene un pH de 6.0 a

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7.5 y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado.

De la solicitud de Patente

Descripción (artículo 28 D. 486)

La descripción de los folios 53 a 80 con el requisito de claridad o comprensión

Reivindicaciones (artículo 30 D 486)

El capítulo reivindicatorio del folio 127 según los requerimientos establecidos en el artículo 30 es claro y conciso y además está lo suficientemente sustentado por la descripción .

Unidad de invención (artículo 25 D 486)

El capítulo reivindicatorio del folio 127 cumple con el requisito de unidad de invención.

No es una invención (artículo 15 D 486 literal a)-f))

El capítulo reivindicatorio del folio 127 sí es una invención.

Excepción a la patentabilidad (artículo 20 D486 literal a)-d))

El capítulo reivindicatorio del folio 127 sí es patentable.

Reivindicaciones relativas a un uso o a un uso distinto (artículos 14 y 21 D 486)

El capítulo reivindicatorio del folio 127 sí es una invención.

Análisis de la(s) Oposición(es): (artículo 43 D. 486):

No se presentaron oposiciones.

Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es 30-11- 2001, se consultaron las bases de datos y los archivos con que cuenta la entidad con fecha de publicación anterior: No se encontró documento alguno que afecte la patentabilidad del objeto de la presente solicitud.

Análisis de patentabilidad (artículo 14 D486)

Novedad (artículo 16 D486)

Las reivindicaciones 1 a 5 se refieren a una composición farmacéutica liofilizada que comprende : Un compuesto de fórmula I (cefovecina sódica) , un preservativo seleccionado de metilparabeno, propilparabeno o una combinación de ellos, en donde la composición acuosa previa liofilización tiene un pH de 6.0 a 7.5 y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado.

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Se realizó el estudio de novedad comparando elemento por elemento la composición oral reivindicada en la presente solicitud con la presente en el estado de la técnica relacionado.

Como resultado de dicho estudio se encontró que la composición farmacéutica liofilizada de (cefovecina sódica) reclamada en la actual solicitud se diferencia con respecto al estado de la técnica (US6020329), en que en la actual solicitud la composición liofilizada de cefovecina sódica dentro de su formulación contiene un preservativo seleccionado de metilparabeno, propilparabeno o una combinación de ellos, la composición acuosa previa liofilización tiene un pH de 6.0 a 7.5 y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado; por lo tanto las reivindicaciones 1 a 5 cumplen con el requisito de novedad de acuerdo con el artículo 16 de la decisión 486.

Nivel inventivo (artículo 18 D486)

Las reivindicaciones 1 a 5 se refieren a una composición farmacéutica liofilizada que comprende: Un compuesto de fórmula I (cefovecina sódica), un preservativo seleccionado de metilparabeno, propilparabeno o una combinación de ellos, en donde la composición acuosa previa liofilización tiene un pH de 6.0 a 7.5 y la composición después de liofilización tiene un contenido de agua residual menor al 1% en peso de liofilizado.

El estado de la técnica (documento anexado por el solicitante, Connors et al, folios 129 a 161) muestra una tabla y gráfica donde se muestra la velocidad de hidrólisis de cefotaxima a diferentes valores de pH. El estudio concluye que a pH menores de 2 y mayores de 8 la velocidad de hidrólisis de cefotaxime se incrementa y que a pH de 3-7 hay una mayor estabilidad. En los folios 144-145 (páginas 304-305) se concluye que a un pH neutro la cefotaxima a 25°C tiene un tiempo de vida de media de 8.6×10^5 segundos. Es decir que la cefotaxima se degrada a la mitad en aproximadamente 10 días.

Ahora de acuerdo a la tabla del folio 124 en donde se muestra que el compuesto cefovecina sódica de la invención tiene a un pH neutro una degradación del 0.8% a 40°C y del 2% a 25 °C en 12 semanas de estudio y teniendo en cuenta que Connors nos muestra que los rangos de pH óptimos de los compuestos de cefalosporinas puede variar se considera que la solución de la presente invención no es obvia.

Y por último en el documento presentado en fase internacional el 09-08-2007 por el solicitante (Ver folios 162 a 166) se establece que es conocido también en el estado del arte que el uso parabenos dentro de las formulaciones de liofilizados no es adecuada ya que durante el proceso de liofilización hay pérdida de estos, para el experto en la materia no sería obvio utilizar dichos preservantes en la formulación (ver folios 167 a 167 A, documento The loss of paraben preservatives during freeze drying y los argumentos del solicitante del folio 163, último párrafo).

Además de los anteriores argumentos también explica que teniendo en cuenta la dificultad para disolver los parabenos a temperatura ambiente y que para este efecto se tiene que utilizar temperaturas a 70 °C, se encontró que el compuesto cefovecina sódica incrementa la solubilidad de dichos preservantes a temperatura ambiente (Ver folio 163 segundo, tercero y cuarto párrafo y Folio 166 gráfica), lo cual también evidentemente constituye una ventaja y una solución inesperada nada obvia para una persona medianamente versada en la materia.

Aplicación industrial (artículo 18 D 486)

El capítulo reivindicatorio del folio 127 sí tiene Aplicación Industrial.

Respuesta a los argumentos del solicitante:

Los argumentos expuestos por el solicitante en los folios 121 a 126 son aceptados como respuesta al artículo 45 de los folios 117 a 120, en donde se demuestra de manera clara y evidente el nivel inventivo de la solicitud.

Conclusión:

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Conceder** las reivindicaciones: 1 a 5 (folios: 127).

Con base en las reivindicaciones 1-5, este Despacho expresa el título de la solicitud en los siguientes términos: Formulación farmacéutica liofilizada estable que contiene cefovecina sódica” .

Bogotá, D.C.,

CONCEPTO TECNICO No. 1838	EXAMINADOR TECNICO Código No.202075
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