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SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

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TÍTULO SOLICITADO: "Nueva sal formiato de O-Desmetilvenlafaxina"

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AP DERADO: ALICIA LLOREDA RICAURTE

TRÁMITE: SUSTENTACIÓN DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, identificado como aparece al pie de la firma, reconocido apoderado del opositor en el trámite de la referencia, estando dentro de los términos de la prórroga solicitada, por medio del presente escrito me permito ratificar y sustentar los argumentos del escrito de oposición radicado dentro del plazo de ley ante esa entidad.

I. RATIFICACIÓN

Reitero a su Despacho la solicitud de negar el beneficio patentario para la totalidad de las reivindicaciones contenidas en el expediente de la referencia, pues se ha demostrado que el principio activo conocido por el nombre genérico de Venlafaxina, incluyendo sus sales y en particular la sal de fumarato, al igual que su principal metabolito, O-desmetil venlafaxina, han sido revelados al menos desde la publicación de la patente norteamericana US 4,535,186, titulada "2-Phenyl-2-(1-

hydroxycycloalkyl or 1-hydroxycycloalk-2-enyl)ethylamine derivatives" en 13-08-1985, esto es, hace más de veinte (20) años.

Si bien es cierto la citada patente US 4,535,186 hace referencia expresa a Venlafaxina *per se*, sus sales, su metabolito principal y su sal "Fumarato", no lo es menos que de la mención hecha en el *abstract* del mismo documento de sus "*Sales farmacológicamente aceptables*" se deriva efectivamente la posibilidad de obtener otro tipo de sales cristalinas del mismo principio activo.

En efecto, en la columna 2, líneas 35 a 42 del documento en cuestión se describe que las sales ácidas de adición farmacéuticamente aceptables de los compuestos básicos allí descritos pueden ser formadas de manera convencional por la reacción de la base libre con una cantidad equivalente de cualquier ácido que forma sales no tóxicas. Entre los ácidos que se mencionan se encuentran ácidos orgánicos e inorgánicos como el clorhídrico, bromhídrico, fumárico, maléico, succínico, sulfúrico, fosfórico, tartárico, acético, cítrico, oxálico y ácidos similares. En consecuencia, pese a no mencionarse explícitamente el ácido fórmico, es claro que existe una amplia gama de ácidos capaces de reaccionar con la sustancia base para dar una sal farmacéuticamente aceptable, tal como se pretende en la solicitud colombiana en trámite.

Ahora bien, frente a la pretensión de obtener privilegio sobre "FORMIATO DE O-DESMETILVENLAFAXINA" como tal, es importante resaltar que en la naturaleza existe un gran número de compuestos orgánicos capaces de adoptar una o más formas cristalinas o, por el contrario, una forma amorfa. Lo anterior para significar que O-desmetilvenlafaxina y su correspondiente sal formiato u otra farmacéuticamente aceptable corresponde, simplemente, a una de las muchas posibles configuraciones espaciales internas que puede tener dicha molécula (ya sea como base o como sal), comprendida en el estado de la técnica, como se ha demostrado, desde 1985.

Por tanto, el Formiato de O-desmetilvenlafaxina cristalino pretendido, como la posible forma amorfa de la misma debería compartir la misma fórmula condensada, $C_{18}H_{25}NO_2 \cdot CH_2O_2$, hecho que corroboraría que en ambos casos se está frente a la misma molécula, con independencia de la configuración espacial interna o aún

externa – hábito cristalino - que ésta pueda adoptar, y sin perjuicio de las eventuales, naturales y predecibles diferencias que puedan presentarse en ciertas propiedades fisicoquímicas de una y otra, **siempre se estará frente a la misma sustancia**. En este punto es necesario hacer notar que la pretensión de obtener patente sobre la forma cristalina de formiato de O-desmethyivenlafaxina no es otra cosa que la pretensión de patentar una forma de presentación de una sustancia conocida, carente por demás de Nivel Inventivo.

En cuanto a la caracterización de la sustancia Formiato de O-desmetil venlafaxina, en función de su tamaño de partícula, me permito llamar la atención del Despacho a la muy conocida posibilidad de modificar la solubilidad, disolución y biodisponibilidad de principios activos manipulando el tamaño de partícula de los mismos (o su área o su distribución de tamaño). Lo anterior es tan evidente que se ha convertido en una rutina requerida en lineamientos de la FDA cuando se está estudiando una nueva entidad química.

Es bien sabido también que el tamaño de partícula de un polvo es crucial en el desarrollo galénico de formulaciones farmacéuticas, tanto en su empleo en la fabricación de cápsulas, comprimidos, y suspensiones, entre otras formas farmacéuticas.

II. COMPLEMENTO DE INFORMACIÓN.

En adición a lo expuesto en el punto precedente y en el escrito inicial de oposición sobre el artículo "Nonaqueous capillary electrophoresis-mass spectrometry for separation of venlafaxine and its phase I metabolites" publicado en Electrophoresis 2001 Feb; 22(3):491-6, ruego al Despacho se sirva tener en cuenta los siguientes argumentos:

- Que el artículo se refiere a la separación de Venlafaxina y de sus metabolitos simultáneamente por electroforesis capilar acuosa y no acuosa, mediante el uso de un capilar convencional y una mezcla de metanol-acetonitrilo (20/80 v/v) y que contienen 25mM de formato de amonio y 1M de ácido fórmico, lo cual permite suponer que para llevar a cabo una buena separación de dichos metabolitos, estos reaccionan con el formato de amonio y con el ácido fórmico para formar

sales adecuadas de los mismos -entre los que se encuentra la sal de Formiato de O-desmetil venlafaxina-, circunstancia que restaría a la solicitud en curso el indispensable requisito de **Novedad**.

- Que el artículo en mención, copia íntegra del cual adjunto para mayor ilustración del Despacho, ha sido catalogado en el Informe Internacional de Búsqueda practicado por la Oficina Europea de Patentes a la solicitud internacional PCT WO2003/103603 equivalente a la solicitud colombiana en trámite, como de categoría "X", es decir, que afecta la novedad y el nivel inventivo de la solicitud aún tomando el documento en forma independiente. El documento adjunto es particularmente relevante frente a las reivindicaciones 1, 39, 41, 43 y 44, pues hace referencia a los siguientes temas:
 - El compuesto O-desmetilvenlafaxina formiato, por la reacción de O-desmetilvenlafaxina con ácido fórmico;
 - La O-desmetilvenlafaxina está presente en un disolvente (Acetonitrilo/Metanol).

Por lo expuesto, respetuosamente reitero que la solicitud de la referencia, en todas sus reivindicaciones, no cumple con los requisitos dispuestos en la Decisión 486 de 2000, y que por esa razón no puede ser objeto de privilegio de patente.

III. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de que el Despacho considere oficiosamente, en particular de las allegadas al escrito de oposición, la siguiente:

- Copia del artículo: "Nonaqueous capillary electrophoresis-mass spectrometry for separation of venlafaxine and its phase I metabolites", publicado en Electrophoresis 2001 Feb; 22(3):491-6.

Señor Superintendente,



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Nonaqueous capillary electrophoresis-mass spectrometry for separation of venlafaxine and its phase I metabolites

Aqueous and nonaqueous capillary electrophoresis (NACE) were investigated for separation of venlafaxine, a new second-generation antidepressant, and its three phase I metabolites. Working at basic pH, around the venlafaxine pK_a value, was effective in resolving the investigated drugs, but created considerable peak tailing. To overcome electrostatic interactions between analytes and silanol groups, investigations were also carried out at acidic pH. However, despite the addition of up to 50% v/v of organic solvents (*e.g.*, methanol or acetonitrile), complete separation of the studied compounds was not possible. NACE was found to be an appropriate alternative to resolve venlafaxine and its metabolites simultaneously. Using a conventional capillary (fused-silica, 84.5 cm length, 50 μ m inner diameter), and a methanol-acetonitrile mixture (20/80 v/v) containing 25 mM ammonium formate and 1 μ M formic acid, complete resolution of these closely related compounds was performed in less than 3.5 min. Selectivity, efficiency and separation time were greatly affected by the organic solvent composition. As the electric current generated in nonaqueous medium was very low, the electric field was further increased by reducing the capillary length. This allowed a baseline resolution of venlafaxine and its three metabolites in 0.7 min. Selectivity was compared in aqueous and nonaqueous media in relation to the acid-base properties of the analytes as well as to the solvation degree. Finally, the method successfully coupled on-line to mass spectrometry with electrospray ionization interface allowed significant sensitivity enhancement.

Keywords: Venlafaxine / Metabolites / Capillary electrophoresis / Nonaqueous media / Mass spectrometry
EL 4208

1 Introduction

1-2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl cyclohexanol hydrochloride (venlafaxine) is a second-generation antidepressant drug [1–3]. Venlafaxine (Vx) is metabolized into two minor metabolites, *N*-desmethyl venlafaxine (*N*-Vx) and *N,O*-didesmethylvenlafaxine (*NO*-Vx), and into a major active metabolite, *O*-desmethylvenlafaxine (*O*-Vx) which exhibits an activity profile similar to that of Vx [4–7] (see Fig. 1). The analytical methods used for determining Vx include gas chromatography with a nitrogen-phosphorus detector (GC-NPD) [8–10], high-performance liquid chromatography (HPLC) with UV [4, 5, 10], fluorimetric [3], coulometric [11] and mass spectro-

metric [12] detection. Recently, capillary electrophoresis (CE) has become a promising alternative to conventional chromatographic techniques and has been applied to the enantiometric separation of Vx using neutral [13, 14] or negatively charged cyclodextrins [15].

Due to its high efficiency and selectivity, nonaqueous capillary electrophoresis (NACE) has evolved as an interesting alternative to aqueous CE for the separation of closely related compounds (*e.g.*, drug metabolism as well as drug purity studies). Compared to those of water, the different physical and chemical properties (dielectric constant, viscosity, autoprotolysis constant, polarity, volatility, etc.) of organic solvents are particularly interesting in terms of selectivity manipulation and separation time reduction [16–20]. Moreover, organic solvents improve the solubility of hydrophobic compounds and in some cases may reduce the degradation rate of some labile substances [21]. Finally, with a volatile buffer, NACE is suitable for on-line coupling with electrospray ionization-mass spectrometry (ESI-MS) [18, 22–27]. Different NACE and NACE-MS applications are summarized in recent reviews and can be consulted for a more systematic coverage of the field [16–18, 28–30].

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Abbreviations: NACE, nonaqueous capillary electrophoresis; *N*-Vx, *N*-desmethyl venlafaxine; *NO*-Vx, *N,O*-didesmethylvenlafaxine; Vx, venlafaxine; *O*-Vx, *O*-desmethylvenlafaxine; SIM, selected ion monitoring

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This paper focuses mainly on the potential of aqueous and nonaqueous CE for the simultaneous separation of Vx and its three phase I metabolites. Various methanol-acetonitrile mixtures, containing 25 mM ammonium formate and 1 M formic acid, are investigated in NACE and results are compared to those obtained in basic aqueous solutions, around analyte pK_a values as well as in acidic buffer containing up to 50% v/v methanol or acetonitrile. The advantages of nonaqueous media for a rapid separation of the investigated compounds are also discussed. Finally, the potential of the method is also highlighted for the on-line coupling with ESI-MS.

2 Materials and methods

2.1 Chemicals

Vx, O-Vx, N-Vx, and NO-Vx were kindly supplied by Wyeth-Ayerst Research (Princeton, NJ, USA). Analytical reagent grade ammonium formate, formic acid, sodium dihydrogen phosphate, sodium tetraborate, sodium hydroxide, and phosphoric acid (85% w/w) were obtained from Fluka (Buchs, Switzerland). Tris(hydroxymethyl)aminomethane (Tris) was purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade methanol, acetonitrile and isopropanol were supplied by Romil (Kölliken, Switzerland). All other reagents and solvents were analytical grade reagents from Fluka. Ultrapure water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA).

2.2 Instrumentation

CE experiments were performed in an HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A CE Chemstation (Hewlett-Packard) was used for both CE and MS instrument control, data acquisition, and data handling. Unless otherwise stated, separation was performed in a fused-silica capillary (Composite Metal Service, Worcestershire, UK) with 50 μ m inner diameter and 84.5 cm total length (56 cm from inlet to UV detector). An alignment interface was used with an optical slit matched to the internal diameter, and detection wavelength was set at 195 nm with a bandwidth of 10 nm. All experiments were carried out in cationic mode (anode at the inlet and cathode at the outlet). A constant voltage of 30 kV was applied during analysis. The capillary was thermostated at 20°C. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 50 mbar for 5 s (6 nL injection volume). Before its first use, the fused-silica capillary was sequentially washed with 1 M sodium hydroxide, 0.1 M sodium hydroxide, Millipore water and separation buffer for 5 min each. Between anal-

yses, the capillary was flushed with the running buffer for 2 min. When not in use, it was washed with 0.1 M sodium hydroxide, water, and then dry stored.

2.2.1 ESI-MS

ESI-MS measurements were carried out in positive ionization mode and performed in a single quadrupole HP Series 1100 MSD (Hewlett Packard, Palo Alto, CA, USA), with an upper mass limit of 3000 amu. On-line coupling of the CE instrument to the mass spectrometer detector was achieved with a commercial coaxial sheath liquid interface (Hewlett Packard), which is orthogonally positioned at the MS entrance. CE-MS experiments were performed in a fused-silica capillary (Composite Metal Service) with a 50 μ m inner diameter and 75 cm total length. To maintain stable electrospray, a 20 mm portion of the polyimide coating was removed from the outlet edge of the capillary. This procedure was found effective in providing better mixing characteristics at the probe tip. The drying and nebulizing gases were both nitrogen. ESI capillary was set at + 4.0 kV. Nebulizing pressure and drying gas flow rate were set at 4 psi and 4 L/min, respectively. Gas temperature was set at 150°C and fragmentor voltage at 50 V. The coaxial sheath liquid, consisting of isopropanol-water (50/50 v/v) in presence of 0.5% formic acid was delivered by a Harvard Model 22 syringe pump (South Natick, MA, USA) at 3 μ L min⁻¹. MS detection was carried out in selected ion monitoring (SIM) mode for positive molecular ions. The three selected masses were acquired with a dwell time of 68 ms each.

2.3 Buffer and sample preparation

2.3.1 Buffer solutions

The nonaqueous electrophoretic medium was, unless otherwise specified, a mixture of MeCN/MeOH (80/20 v/v) containing 25 mM ammonium formate and 1 M formic acid. For aqueous experiments, the acidic buffer consisted of 50 mM phosphoric acid solution adjusted to pH 2.5 with a concentrated Tris solution (6 M). For buffer pH investigations, electrolytes were prepared by mixing sodium dihydrogen phosphate, sodium tetraborate and sodium hydroxide solutions in an appropriate ratio to give suitable pH values (between 8 and 10.5) and an ionic strength of 50 mM. Before use, electrolyte solutions were filtered through an 0.40 μ m microfilter (Supelco, Bellefonte, PA, USA) and degassed in an ultrasonic bath for 10 min.

2.3.2 Standard solutions

Stock standard solutions of Vx and its metabolites were prepared by dissolving each compound in methanol to reach a concentration of 1 mg/mL and were then stored

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at 4°C. The drug mixture was prepared by dissolving individual compounds in water and methanol, for aqueous and nonaqueous experiments, respectively.

3 Results and discussion

As shown in Fig. 1, Vx and its metabolites present structural differences which consist of a series of demethylations on the phenyl moiety or on the amine group of the parent drug. Observation of the chemical structure of these analytes allows to classify them as *O*-methylated compounds (Vx and *N*-Vx) and *O*-desmethylated compounds (*O*-Vx and *NO*-Vx) or *N*-methylated compounds (Vx and *O*-Vx), which are tertiary amines, and *N*-desmethylated compounds (*N*-Vx and *NO*-Vx), which are secondary amines.

3.1 Aqueous CE

In aqueous CE, selectivity is generally better around the pK_a values of the investigated analytes. Since the pK_a of Vx is about 9.3, the pH was investigated between 8 and 10.5. As expected, effective electrophoretic mobilities of analytes decreased with increasing pH (Fig. 2). *O*-methylated compounds (Vx, *N*-Vx) had higher effective mobilities than *O*-desmethylated analytes whatever the pH was. The best separation was achieved at pH 9.5 and the migration order was $N\text{-Vx} < \text{Vx} < \text{NO-Vx} < \text{O-Vx}$. At high pH values, *O*-desmethylated analytes were electrically neutral and migrated with EOF.

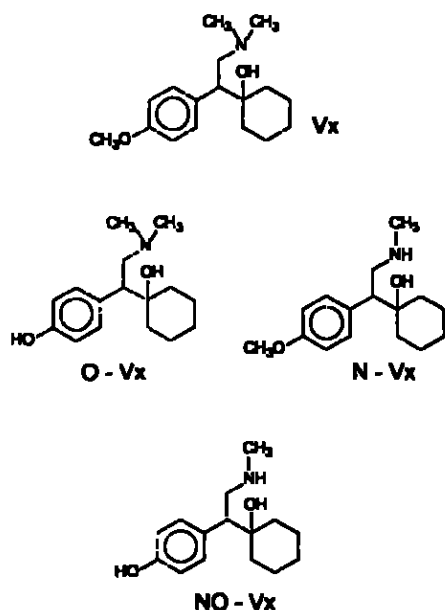


Figure 1. Chemical structure of Vx, *O*-Vx, *N*-Vx, and *NO*-Vx.

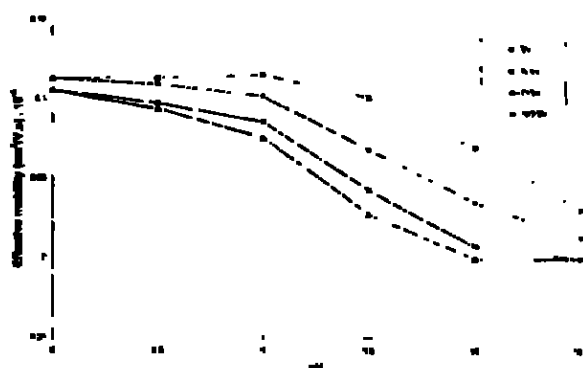


Figure 2. Effect of buffer pH on effective mobilities of investigated compounds. Electrophoretic medium, 50 mM phosphate-borate buffer. Uncoated fused-silica capillary, $L = 64.5$ cm, $l = 58$ cm, 50 μ m ID. Applied voltage, 30 kV; sample injection, 25 mbar for 10 s; temperature, 20°C; detection at 195 nm. Peak assignment as in Fig. 1.

However, under basic pH, strong interactions occurred between the negatively charged silanol groups on the silica surface and positively charged analytes and led to serious peak tailing (data not shown). Thus, further investigations were performed with acidic buffer consisting of 50 mM Tris-phosphate at pH 2.5 where silanol groups are protonated. As shown in Figs. 3a, b, no separation was achieved under acidic conditions. Since the addition of an organic modifier to the BGE may improve selectivity and resolution [31–33], the effect of methanol and acetonitrile on analyte migration behavior was examined. Figures 3a, b report the variation of Vx and metabolites migration times as a function of the organic modifier percentage. Increasing the organic modifier percentage from 0 to 50% improves resolution. In addition, compound migration times have a maximum value around 20 and 50% v/v acetonitrile and methanol, respectively. These findings have been attributed to changes in buffer viscosity and dielectric constant as well as in the zeta potential of the silica wall [16]. However, despite the addition of a high percentage of organic modifier (up to 50%), baseline resolution of Vx and its three metabolites was not achieved. Only a slight separation was observed at an elevated acetonitrile percentage, as shown in Fig. 3b, with the migration order $\text{Vx} \approx \text{N-Vx} < \text{O-Vx} \approx \text{NO-Vx}$.

3.2 NACE

Prompted by the above results obtained with methanol and acetonitrile, NACE was conducted for the separation of Vx and its metabolites. A mixture of methanol, an amphiprotic solvent, and acetonitrile, generally classified among dipolar aprotic solvents, was found to be appropriate to achieve NACE separations [18, 21, 34]. While the electrolyte concentration was kept constant (25 mM

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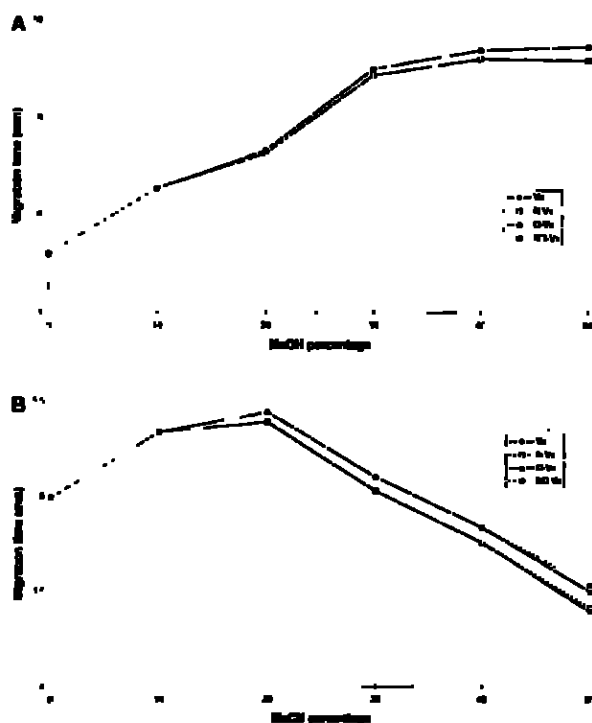


Figure 3. Effect of organic modifier percentage on migration times of investigated compounds: (A) methanol, (B) acetonitrile. Electrophoretic medium, 50 mM Tris-phosphate buffer, pH 2.5. Other conditions as in Fig. 2.

ammonium formate and 1 M formic acid), different methanol-acetonitrile mixtures were investigated. After increase of the methanol percentage in acetonitrile from 0 to 100%, migration times of the studied drugs present a minimum value around 20% methanol (Fig. 4). As already reported [17], this behavior is mainly attributed to concomitant changes of viscosity (η) and dielectric constant (ϵ) of the electrophoretic medium. Indeed, since both electrophoretic and electroosmotic mobilities are directly proportional to the ϵ/η ratio, which is maximum at 20% MeOH, a MeOH-MeCN mixture around this level results in rapid separation.

The different selectivities observed depend on the organic solvent composition. In particular, the migration order of *O*-Vx and *N*-Vx is inverted above 60% methanol. Because electrophoretic mobility is mainly governed by the size and shape of positively charged drugs, inversion of the migration order can be ascribed to changes in solvation degree. Therefore, 20% methanol was selected as a good compromise in terms of selectivity, efficiency and separation time. Under the chosen conditions, a baseline resolution of Vx and its three metabolites was achieved in less than 3.5 min without introducing any additive (e.g., micelle, cyclodextrins) as illustrated in Fig. 5a. Further-

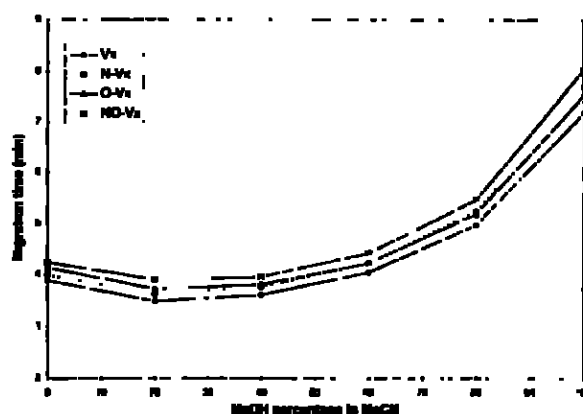


Figure 4. Effect of methanol percentage in the methanol-acetonitrile mixture on the migration time of investigated compounds. Electrophoretic conditions: 25 mM ammonium formate and 1 M formic acid in organic solvent. Other conditions as in Fig. 2.

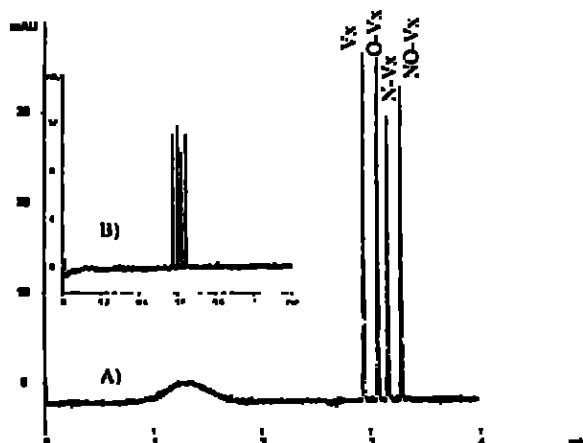


Figure 5. Typical electropherograms obtained under optimized NACE conditions. Electrophoretic conditions: MeOH-MeCN (20/80 v/v) containing 25 mM ammonium formate and 1 M formic acid. (A) Conventional capillary, $L = 64.5$ cm, $l = 56$ cm, 50 μ m ID; applied voltage, 30 kV ($E = 465$ V/cm; $i = 24$ μ A). (B) Short capillary, $L = 32.5$ cm, $l = 24$ cm, 50 μ m ID; applied voltage, 30 kV ($E = 870$ V/cm; $i = 52$ μ A). Sample concentration, 10 μ g/mL each. Other conditions as in Fig. 4.

more, in a nonaqueous solvent, analyte interactions with the silica wall are decreased and allow a better peak shape; the electric current is also generally lower than in aqueous media. As a result, a high electric field can be applied without excessive Joule effect. Since the applied voltage was already set at its maximum value (30 kV), the electric field can be increased from 465 to 923 V/cm by reducing the capillary length to 32.5 cm [20, 35]. In this way, the separation time of Vx and its three metabolites

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was significantly decreased without loss of resolution (Fig. 5b). The minimum capillary length is dependent on CE instrumentation. Under these conditions, the electric current generated (54 μ A) was acceptable and allowed efficient heat dissipation.

3.3 Aqueous versus NACE selectivity

Selectivity was greatly affected by the nature of the electrolyte. In fact, under aqueous conditions and basic pH, the migration order of Vx and metabolites was mainly governed by the solvation degree and pK_a values. Separation between Vx and *N*-Vx (*O*-Vx and *NO*-Vx, respectively) at pH 9.5 was attributed to a pK_a difference due to an additional electron donor group on nitrogen, which decreased the cationic charge of Vx (*O*-Vx). For compounds with identical nitrogen substitution such as Vx and *O*-Vx (*N*-Vx and *NO*-Vx, respectively), the migration order can be explained by higher hydration of *O*-Vx (*NO*-Vx) due to a polar group on the phenyl moiety. At acidic pH and in presence of organic modifiers, only the solvation effect can be considered. In NACE, the migration order observed is $Vx < O-Vx < N-Vx < NO-Vx$, which is contrary to the expected migration order based on the molecular weight. These results indicate that electrophoretic mobility in nonaqueous media is mainly dictated by the solvation degree and/or possible pK_a changes.

3.4 NACE-ESI-MS

One of the main drawbacks of NACE coupled to UV detection is a relatively low sensitivity due to the short optical pathlength of the capillary as well as to the high UV absorbance of organic solvents. In this context, on-line coupling of NACE to MS is a promising combination of two powerful techniques. In contrast with the buffers commonly used in CE (e.g., phosphate, borate, micelles, etc.), organic solvents with volatile electrolytes present MS compatibility. Therefore, as illustrated in Fig. 6, the method described was coupled on-line to ESI-MS and was successfully applied to the analysis of Vx and its metabolites. For instrumental reasons, a long capillary (75 cm) was selected and allowed a separation time of ca. 8 min. An isopropanol-water mixture (50/50 v/v) in the presence of 0.5% formic acid as sheath liquid, delivered at 3 μ L/min, was found to be appropriate to achieve a stable electrospray as well as a high signal-to-noise ratio.

It is noteworthy that the high selectivity of NACE allowed the separation of both *O*-Vx and *N*-Vx isomers. Thus, no further fragmentation or MS/MS experiments are required to differentiate these compounds. SIM mode results in a very high signal-to-noise ratio allowing the detection of these compounds at 50 ppb which is much higher than

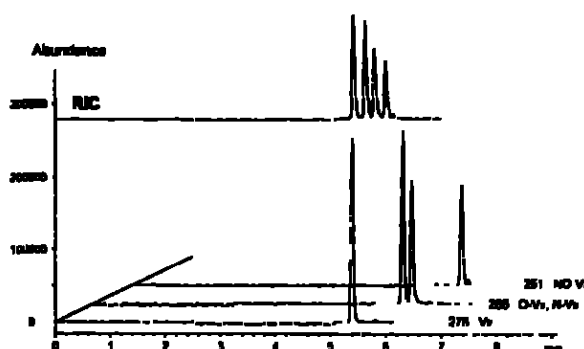


Figure 6. NACE-ESI-MS simultaneous separation of Vx and its three phase I metabolites (1 μ g/mL each). NACE conditions: running buffer, MeOH-MeCN (20/80 v/v) containing 25 mM ammonium formate and 1 M formic acid; fused-silica capillary, 75 cm total length $\times$ 50 μ m ID, 360 μ m OD; pressure injection, 50 mbar for 6 s; applied voltage, 30 kV; temperature, 20°C. MS conditions: SIM positive mode (three ions); capillary voltage, 4 kV; fragmentor, 50 V; drying gas N_2 flow and temperature, 4 L/min and 150°C; nebulizer pressure, 4 psi; sheath liquid, 0.5% formic acid in water-isopropanol (50/50 v/v); sheath flow, 3 μ L/min. Peak identification as in Fig. 1. For other conditions, see Section 2.2.

the sensitivity observed with UV (ca. 1 ppm). This detection limit corresponds to the therapeutic concentration of Vx and makes the method suitable for the determination of the drug in biological fluids. Moreover, the MS signal depends on the structure of the investigated drugs. Tertiary amines showing a higher proton affinity than secondary amines and induce therefore a higher signal abundance for Vx and *O*-Vx than for *N*-desmethylated homologues (*N*-Vx and *NO*-Vx, respectively).

4 Concluding remarks

Aqueous and NACE methods were developed for separation of Vx and its three phase I metabolites. Complete separation of the studied analytes was achieved at basic pH, around the Vx pK_a value. However, considerable peak tailing was observed owing to electrostatic interactions between positively charged analytes and negatively charged silanol groups. To suppress silanol ionization, subsequent investigations were conducted at acidic pH. Introducing up to 50% v/v methanol or acetonitrile in the acidic background electrolyte was not sufficient to achieve a complete separation of the studied drugs. Simultaneous resolution of analytes was also investigated in nonaqueous media with various methanol-acetonitrile mixtures using 25 mM ammonium formate and 1 M formic acid. The composition of the organic solvent determined different selectivities and migration times.

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Vx and its metabolites were simultaneously resolved with a conventional capillary (fused silica, 65 cm length, 50 μ m inner diameter) and a methanol-acetonitrile mixture (20/80 v/v). Since the Joule effect is minimized with a non-aqueous buffer, a high electric field can be applied by reducing the capillary length. This results in rapid and efficient separation. The observed migration order in the investigated electrolytes can be explained by different solvations and/or pK_a changes. Finally, the nonaqueous method described was coupled to ESI-MS. Compared with UV, the combination of a highly selective nonaqueous method and a mass spectrometer detector allows a significant gain in sensitivity.

Received June 2, 2000

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Doctora:
Alicia Lloreda Ricaurte
Apoderada

Oficio N°: 11688

ASUNTO:	Radicación PCT N°	04-122509
	Trámite	011
	Evento	379
	Actuación	440
	Folios	040

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por el doctor José Luis Reyes Villamizar, en su calidad de apoderado de Tecnoquímicas S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha, 13 OCT. 2006.

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