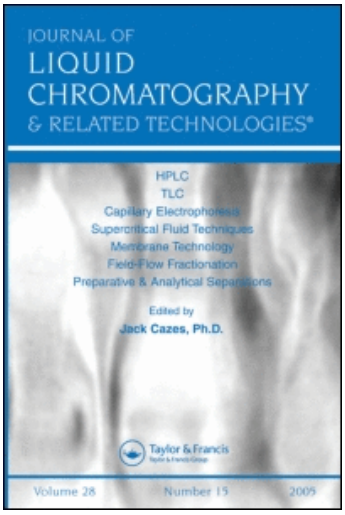

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Quantitative Determination of Pentazocine Enantiomers in Human Serum Using Derivatized β-Cyclodextrin-modified Capillary Electrophoresis and Solid Phase Extraction

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QUANTITATIVE DETERMINATION OF PENTAZOCINE ENANTIOMERS IN HUMAN SERUM USING DERIVATIZED β-CYCLO-DEXTRIN-MODIFIED CAPILLARY ELECTROPHORESIS AND SOLID PHASE EXTRACTION

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ABSTRACT

A high performance capillary electrophoresis (HPCE) assay for the quantitation of (-) and (+) pentazocine in human serum was developed. Resolution of the pentazocine enantiomers was achieved using 15mM methyl-β-cyclodextrin (Me-β-CD) in 100mM phosphate buffer pH 2.5. A 72cm uncoated fused-silica capillary at a constant voltage of 20kV was used for the analysis and 0.03mM hexadecyltrimethylammonium bromide (HTAB) was added to the buffer to decrease the adsorption of endogeneous substances onto the silica wall. The analytes of interest were extracted from serum using a solid phase extraction(SPE) procedure. The phenyl SPE cartridge gave good recoveries in excess of 90% for both (-) and (+) pentazocine, without any interferences.

The detection limits were 25ng/mL and the limits of quantitation were 35ng/mL for each enantiomer. The calibration curves were linear over a range 35-400ng/mL with nalorphine as the internal standard, and the coefficients of determination were greater than 0.999 (n=3). Precision and accuracy of the method were in the range 1.4-7.5% and 1.3-4.7%, respectively, for (-) pentazocine and 1.1-7.8% and 1.4-4.3%, respectively, for (+) pentazocine (n=3).

INTRODUCTION

Pentazocine, (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol) is a central-acting analgesic and a weak narcotic antagonist which is structurally related to morphine. Intravenous administration is widely used in the management of patients with acute or chronic pain, such as cancer, colic fit, or neuralgia.[1] Pentazocine is a chiral compound which possesses three asymmetric centers at positions 2,6 and 11 (Figure 1). The absolute configuration of the (-) cis or α pentazocine has been determined to be 2R 6R 11R and the (+) cis or α enantiomer has the configuration 2S 6S 11S.[2-3] (+)-Pentazocine is the more pharmacologically active enantiomer. The side effects seen with pentazocine are stereospecific. Respiratory depression seems to be caused almost exclusively by the (-) enantiomer. Stereospecific receptor binding sites in lymphocytes have been found to exist in the immune system, whereby (+) pentazocine possesses more binding affinity as compared to the (-) enantiomer. The (+) enantiomer suppresses stimulated lymphocyte proliferation and the (-) pentazocine augments IgM production and induces interleukin-4 production.[4]

The assay of pentazocine enantiomers in serum using HPLC and liquid-liquid extraction as the sample clean-up technique[5] and more recently with solid phase extraction[6] have been reported by this laboratory. Capillary electrophoresis (CE) resolution of pentazocine enantiomers has not been reported thus far in the literature.

Capillary electrophoresis is a powerful analytical technique for the separation of enantiomers due to its inherent properties of high efficiency, low sample volume, small amounts, if any, of organic modifiers and ease of operation.[7-13] There are four modes of chiral separation in CE depending on the type of chiral selector used. These are (i) host-guest complexation (ii) natural and synthetic optically active micelles (iii) ligand exchange and (iv) proteins. Host-guest complexation is usually performed using cyclodextrins (CD) and, more recently, 18-crown-6-tetracarboxylic acids.[14] Enantio-

recognition is a combination of inclusion phenomenon and additional interactions with the functional groups on the rim of a cyclodextrin. The various derivatized cyclodextrins have increased solubility compared to the native CD and undergo additional interactions with analytes, thereby enhancing chiral recognition.[15]

This paper reports a HPCE method for the resolution and quantitation of (-) and (+) pentazocine in human serum using methyl-β-cyclodextrin (Me-β-CD) as the chiral selector. The method is linear in the range of 35-400ng/mL and provides the required selectivity and sensitivity for monitoring levels of (-) and (+) pentazocine in human serum.

EXPERIMENTAL

Reagents and Chemicals

Racemic pentazocine and the (-) and (+) enantiomers as hydrochloride salts were obtained from Research Biochemicals Incorporated (Natick, MA, USA). The internal standard nalorphine was purchased from Merck & Co. Inc. (Rahway, NJ, USA). Hexadecyltrimethylammonium bromide (HTAB) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphoric acid (85%), sodium dihydrogen phosphate monohydrate were obtained from J.T. Baker (Phillipburg, NJ, USA). α, γ and β-cyclodextrin, heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) and methyl-β-cyclodextrin (Me-β-CD) were also obtained from Sigma. Hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Hydroxyethyl-β-cyclodextrin (HE-β-CD), carbomethyl-β-cyclodextrin (CM-β-CD) and amino-β-cyclodextrin were obtained from Advanced Separation Technologies (Whippany, NJ, USA). Sulfated-β-cyclodextrin (S-β-CD) were kindly supplied by Cerestar USA Inc. (Hammond, IN, USA). Drug-free human serum was obtained from Biological Specialty Corporation (Colmar, PA, USA).

The ethylsilane, octasilane, octadecylsilane, cyanopropyl and phenyl solid phase extraction columns(100mg/1cc) were obtained from Varian Sample Preparation Products(Harbor City, CA, USA) and the mixed mode solid phase extraction disc (15mg/3cc PLUS™.MP$_3$) was purchased from Ansys, Inc. (Irvine, CA, USA). All the solutions were filtered through a 0.2μm nylon filter (Acrodisc 13, Gelman Sciences, Ann Arbor, MI, USA).

Instrumentation

All capillary electrophoresis experiments were performed using an ABI 270A Capillary Electrophoretic System (Applied Biosystems, Foster City, CA, USA) equipped with a UV detector. An uncoated fused-silica capillary (total length, 72cm, effective length, 50cm; 50μm i.d.; Polymicro Technologies, Phoenix, AZ, USA) was used for the analysis. The capillary temperature was kept at 30°C and the applied voltage was 20kV.

The typical running current was 45μA. A 0.5cm-detection window was created by stripping the polyimide coating from the capillary. The detection was towards the cathodic end.

Electrophoretic conditions

The run buffer consisted of an aqueous solution of 100mM sodium dihydrogen phosphate buffer pH 2.5 (adjusted with 100mM phosphoric acid) containing 15mM Me-β-CD and 0.03mM HTAB. The analytes were monitored at 220nm. New capillaries were conditioned by rinsing with 1M sodium hydroxide for 10min followed by 10min each with water and run buffer solutions.

Sample introduction was performed using a vacuum injection (80psi) for 20s. Before each analysis, the capillary was rinsed with 0.1M sodium hydroxide and 2.0min with run buffer.

Preparation of stock and standard solutions

Stock solutions (100μg/mL) of (-) and (+) pentazocine and the internal standard nalorphine, as their free bases, were prepared in absolute methanol and stored, protected from light, at ambient temperature (23±1°C). Appropriate dilutions of the individual pentazocine stock solutions were made to provide 5μg/mL standard solutions which were used for spiking blank human serum.

Preparation of spiked human serum

Accurately measured aliquots (10, 30 and 50μL of the individual 5μg/mL standard (-) and (+) pentazocine standard solutions) were pipetted into individual 1mL volumetric tubes and evaporated to dryness with a nitrogen stream. Then 15μL of the internal standard solution were added to each tube and drug-free human serum was added to volume and mixed well to give final concentrations of 50, 150, and 250ng/mL of each pentazocine enantiomer.

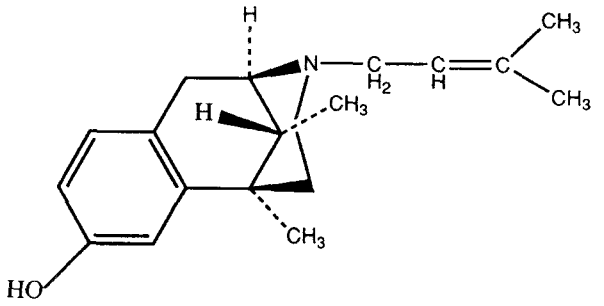
Assay Method

Phenyl solid phase extraction (SPE) cartridges were attached to a vacuum manifold and conditioned with 2 column volumes of absolute methanol followed by 2 column volumes of distilled water. Into the cartridges were transferred 1mL of spiked human serum samples and the vacuum was applied. After the entire sample had been aspirated through the cartridge, the cartridge was washed with 1mL of methanol-water (60:40 v/v). The pentazocine enantiomers and the internal standard were eluted with 4x250μL of absolute methanol. The eluate were filtered through a 0.2μm filter followed by evaporation under a slow nitrogen stream. The residue was reconstituted in 50μL of deionized water after which it was vacuum injected into the capillary for 20s. Absolute recovery of each analyte was calculated by comparing drug peak-area of the spiked analyte samples to unextracted analyte stock solution that had been injected directly into the electrophoretic system. Calibration curves were constructed in the range of 35-400ng/mL of each pentazocine enantiomer. Linear regression analysis of the peak-height ratios of each pentazocine enantiomer to internal standard versus concentration of each enantiomer produced slope and intercept data which were used to calculate concentrations of (-) and (+) pentazocine enantiomers in each serum sample.

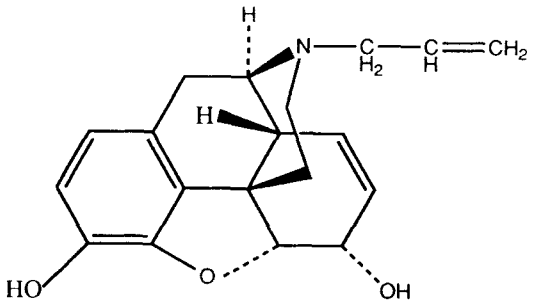
RESULTS AND DISCUSSION

The chemical structures of pentazocine and the internal standard are shown in Figure 1. Cyclodextrins separate enantiomers utilizing the phenomenon of host-guest complexation. The affinity of the analyte for the CD is due to the hydrophobic interactions between the analyte and the CD cavity and the hydrogen bonding of the analyte to the hydroxyl groups or introduced functional groups on the CD ring.[16] Various native and derivatized cyclodextrins were evaluated for their capacity to separate the pentazocine enantiomers. The neutral cyclodextrins were β, α, γ, CDs, DM-β-CD, TM-β-CD, HP-β-CD, HE-β-CD, Me-β-CD, HP-α-CD, HP-γ-CD. The charged CDs investigated were CM-β-CD, amino-β-CD and S-β-CD.

Partial separations were achieved with 20mM HP-β-CD or HE-β-CD or HP-γ-CD in 100mM phosphate buffer pH 2.5 (adjusted with phosphoric acid). Me-β-CD and CM-β-CD gave baseline resolution of the two pentazocine enantiomers. However, under the same electrophoretic conditions, the migration times using CM-β-CD were longer than Me-β-CD, so the latter was used as the additive for this study. Optimization of the run buffer was performed by studying the effects of phosphate buffer concentrations, Me-β-CD



PENTAZOCINE



NALORPHINE

Figure 1. Chemical structures of pentazocine and nalorphine (internal standard).

concentration, pH of the phosphate buffer and applied voltage. The analyte peak shapes improved in symmetry as the phosphate buffer concentration was increased with no significant effect on migration time. An increase in the Me-β-CD concentration increased migration time and concentrations over 15mM resulted in loss of resolution. As the pH was increased above pH 6.0, there was a complete loss of resolution and a decrease in migration time. As the voltage

increased, there was a decrease in migration time and loss of resolution at voltages higher than 20kV. The final run buffer consisted of an aqueous solution of 100mM sodium dihydrogen phosphate buffer pH 2.5 (adjusted with phosphoric acid) containing 15mM Me-β-CD and 0.03mM HTAB. Figure 2-I shows an electropherogram of blank human serum and Figure2-II shows the electropherogram of serum spiked with (-) and (+) pentazocine and the internal standard nalorphine. In addition to the spikes shown, the electropherograms in Figure2 contain a few unknown peaks. The relatively clean electropherograms are typical of the type of results obtained in our laboratory working with solid phase extraction of human serum and the addition of hexadecyl-trimethylammonium bromide (HTAB) to the run buffer. The cationic detergent HTAB reduces the nonspecific interaction of endogeneous substances in serum with the silanol groups on the walls of the capillary. In this separation, 0.03mM HTAB was added to the run buffer.

Five solid phase extraction cartridges (ethylsilane, octasilane, octadecylsilane, cyanopropyl and phenyl) and one mixed mode solid phase extraction disc (PLUS^{TM}.MP$_3$) were investigated for serum sample clean-up prior to the HPCE assay. All the cartridges and disc except phenyl gave recoveries of about 50% or less for both pentazocine enantiomers.

The phenyl cartridge was selected for use since it provided the best data in terms of sample clean-up and recoveries of both pentazocine enantiomers. Absolute methanol was used as the eluent and mean absolute recoveries using the phenyl cartridge were 90.3 ± 2.5% for (+)-pentazocine, 91.2± 3.9% for (-)-pentazocine and 94.5±2.3% for the internal standard nalorphine (n=5). The validation of the HPCE assay was performed by evaluating limits of detection, limits of quantitation, linearity of detector response, method precision and accuracy. The limits of detection were 25ng/mL and the limits of quantitation were 35ng/mL for each enantiomer. Expected serum or plasma levels are in the 50-200ng/mL range, but are erratic.[17]

The calibration curves showed good linearity in the range 35-400ng/mL for (-) and (+) pentazocine. The coefficients of determination were greater than 0.999 (n=3). Representative linear regression equations obtained for (-) pentazocine and (+) pentazocine were y= 0.004880x + 0.002234 (standard error = 0.00390) and y= 0.004700 + 0.003120 (standard error =0.01175), respectively, where y and x were the drug-to-internal standard peak- area ratios and the concentration of each analyte, respectively. The intra-day precision and accuracy (n=3), as expressed by %RSD and %error were in the range 1.5-7.5% and 1.6-3.5% respectively, for the (-) pentazocine and 1.1-7.8% and 1.4-4.3%, respectively, for the (+) pentazocine.

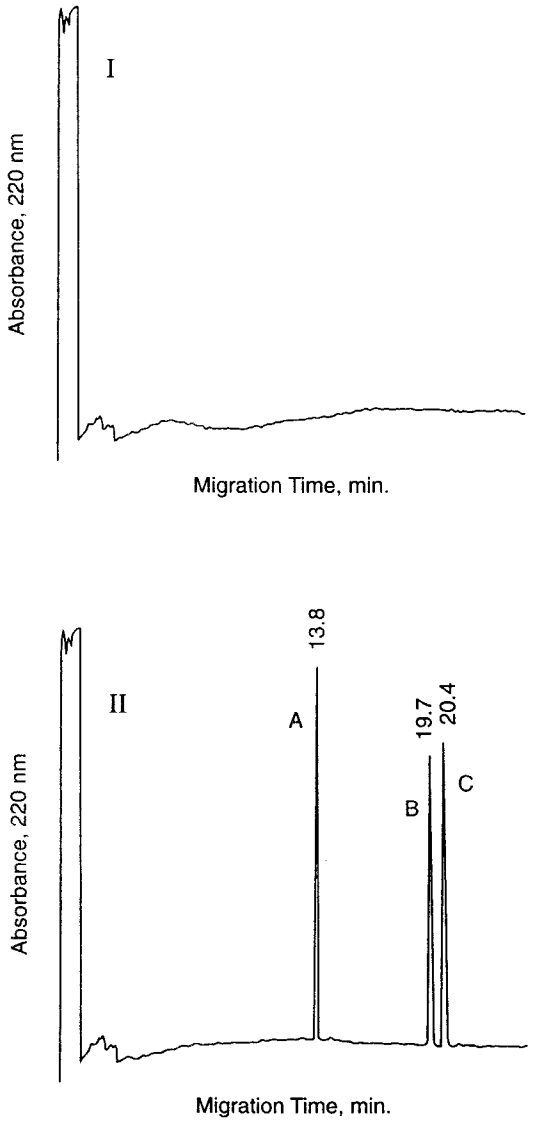


Figure 2. Electropherograms of (I) serum blank and (II) serum spiked with (A) nalorphine (400ng/nL, 13.8min), (B) (-) pentazocine (150ng/mL,19.7min) and (C) (+) pentazocine (150ng/mL, 20.4min). (See Experimental Section for electrophoretic parameters).

Table 1

Accuracy and Precision Data for Pentazocine Enantiomers in Spiked Human Serum Samples

Analyte	Conc. Added (ng/mL)	Conc. Found[a] (ng/mL)	Error (%)	RSD (%)
INTRA-DAY				
(-) Pentazocine	50	48.24 ± 3.61	3.5	7.5
	150	153.67 ± 4.20	2.5	2.7
	250	254.19 ± 3.90	1.6	1.5
(+) Pentazocine	50	47.85 ± 3.72	4.3	7.8
	150	154.25 ± 3.40	2.8	2.2
	250	253.34 ± 2.72	1.4	1.1
INTER-DAY				
(-) Pentazocine	50	47.65 ± 2.95	4.7	6.2
	150	153.76 ± 3.10	2.5	2.0
	250	253.24 ± 3.61	1.3	1.4
(+) Pentazocine	50	48.28 ± 3.16	3.4	6.6
	150	154.16 ± 2.94	2.7	1.9
	250	253.94 ± 2.75	1.6	1.1

[a]Based on n=3 for intra-day and n=9 for inter-day assay.

The inter-day precision (%RSD) and accuracy (%error) were in the range 1.4-6.2% and 1.3-4.7%, respectively, for (-) pentazocine (n=9), and 1.1-6.6% and 1.6-3.4%, respectively, for (+) pentazocine (n=9). See Table 1 for additional data.

In summary, the HPCE assay described herein is sensitive and suitable for the simultaneous determination of (-) and (+) pentazocine in serum. The phenyl solid-phase extraction method provided good sample clean-up with no endogenous interferences. The method showed good linearity and precision within the linear range of 35-400ng/mL. The HPCE method shows that capillary electrophoresis is a useful alternative to chiral HPLC for the determination of enantiomeric drugs in a biological matrix such as serum.

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