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Short communication

Resolution and quantitation of pentazocine enantiomers in human serum by reversed-phase high-performance liquid chromatography using sulfated β-cyclodextrin as chiral mobile phase additive and solid-phase extraction

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Abstract

A sensitive and stereospecific HPLC method was developed for the analysis of (−)- and (+)-pentazocine in human serum. The assay involves the use of a phenyl solid-phase extraction column for serum sample clean-up prior to HPLC analysis. Chromatographic resolution of the pentazocine enantiomers was performed on a octadecylsilane column with sulfated-β-cyclodextrin (S-β-CD) as the chiral mobile phase additive. The composition of the mobile phase was aqueous 10 m*M* potassium dihydrogenphosphate buffer pH 5.8 (adjusted with phosphoric acid)–absolute ethanol (80:20, v/v) containing 10 m*M* S-β-CD at a flow-rate of 0.7 ml/min. Recoveries of (−)- and (+)-pentazocine were in the range of 91–93%. Linear calibration curves were obtained in the 20–400 ng/ml range for each enantiomer in serum. The detection limit based on $S/N=3$ was 15 ng/ml for each pentazocine enantiomer in serum with UV detection at 220 nm. The limit of quantitation for each enantiomer was 20 ng/ml. Precision calculated as R.S.D. and accuracy calculated as error were in the range 0.9–7.0% and 1.2–6.2%, respectively, for the (−)-enantiomer and 0.8– 7.6% and 1.2–4.6%, respectively, for the (+)-enantiomer ($n=3$). © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Pentazocine; Cyclodextrins

1. 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 non-narcotic morphine analogue widely used in the management of patients with post-operative pain or initial carcinogenic pain [1]. Pentazocine is a chiral compound which possesses three asymmetric centers at positions 2, 6 and 11 (Fig. 1). The absolute configuration of the (−)-*cis* or α-pentazocine has been determined to be 2*R* 6*R* 11*R* and the (+)-*cis*- or α-enantiomer has the configuration 2*S* 6*S* 11*S* [2,3]. (+)-Pentazocine is the more pharmacologically active enantiomer. The side effects caused by pentazocine are stereospecific. Lightheadedness and relaxation are caused by the (−)-enantiomer, whereas anxiety is produced by the (+)-enantiomer [4].

For quantitation of racemic pentazocine in blood, plasma or serum, various analytical techniques have been reported including fluorometry [5], radioimmunoassay [6], radioreceptor assay [7], mass frag-

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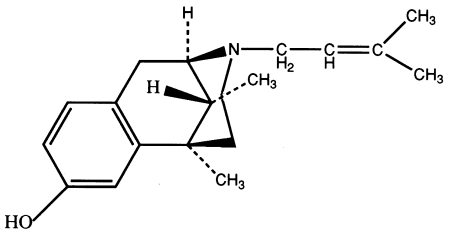
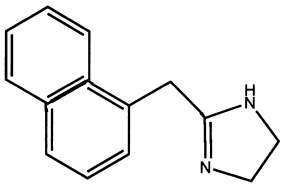


PENTAZOCINE



NAPHAZOLINE

Fig. 1. Chemical structures of pentazocine and the internal standard naphazoline.

mentography [8], gas chromatography (GC) [9] and high-performance liquid chromatography (HPLC) [10,11]. However, only one paper has reported the resolution and determination of pentazocine enantiomers in serum using liquid–liquid extraction for sample clean-up [12].

Cyclodextrins (CDs) and their derivatives have been successfully used as chiral mobile phase additives for a number of chiral compounds [13]. The use of CDs as chiral mobile phase additives provides a flexible alternative for the separation of enantiomers because separations can be performed on conventional columns which generally have higher efficiencies and are less expensive than chiral stationary phases. Derivatization of a CD increases the solubility and also changes the chiral recognition ability as well as hydrogen bonding capability of the CD relative to a native CD [14].

This study reports the chiral separation and determination of pentazocine enantiomers in human serum using sulfated β-cyclodextrin (S-β-CD) as the chiral mobile phase additive and an octadecylsilane column with UV absorbance detection. Solid-phase extraction (SPE) was used as the sample clean-up procedure. The method is linear in the range 20–400 ng/ml and provides the required selectivity and sensitivity for monitoring levels of (−)- and (+)-pentazocine in human serum. To our knowledge, this is the first time S-β-CD has been reported as a chiral mobile phase additive in reversed-phase HPLC although there is reported in the literature a new S-β-CD bonded chiral stationary phase [15].

2. Experimental

2.1. Reagents and chemicals

Racemic pentazocine and the (−)- and (+)-enantiomers as hydrochloride salts were obtained from Research Biochemicals (Natick, MA, USA). The internal standard naphazoline hydrochloride was purchased from Sigma (St. Louis, MO, USA). Absolute ethyl alcohol USP (200 proof) was obtained from Aaper Alcohol and Chemical (Shelbyville, KY, USA) and potassium dihydrogenphosphate, sodium dihydrogenphosphate and ammonium acetate were purchased from J.T. Baker (Phillipburg, NJ, USA). β-Cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution 7.0), methyl-β-cyclodextrin (Me-β-CD, degree of substitution 16.5) and sulfated-β-cyclodextrin (S-β-CD, degree of substitution 13) were kindly supplied by Cerestar USA (Hammond, IN, USA). All solvents were HPLC grade. Drug-free human serum was obtained from Biological Specialty (Colmar, PA, USA). The ethylsilane, octasilane, octadecylsilane, cyanopropyl and phenyl SPE columns (100 mg/cm^3) were obtained from Varian Sample Preparation Products (Harbor City, CA, USA) and the mixed mode SPE disc 15 mg/3 cm^3 (PLUS MP$_3$) was obtained from Ansys (Irvine, CA, USA).

2.2. Instrumentation

The HPLC system consisted of a Beckman Model 110A pump (Beckman, San Ramon, CA, USA) and a Model 728 autosampler (Micromeritics Instruments, Norcross, GA, USA) equipped with a 20 μl loop.

The detector was a Waters Millipore Model 481 LC Spectrophotometer (Milford, MA, USA) and a Spectra-Physics Model 4270 integrator (Spectra-Physics, San Jose, CA, USA) was used to record each chromatogram and peak height responses. Separation of the analytes was achieved on a Prodigy 5 μm ODS(3) 100A column (150×3.2 mm I.D., Phenomenex, Torrance, CA, USA) equipped with a 0.2 μm Opti-solv pre-column minifilter (Optimize Technologies, Portland, OR, USA).

The mobile phase consisted of 10 m*M* potassium dihydrogenphosphate buffer pH 5.8 (adjusted with phosphoric acid)–absolute ethanol (80:20, v/v) containing 20 m*M* S-β-CD delivered at a flow-rate of 0.7 ml/min. The mobile phase was filtered through a 0.45 μm filter (Alltech Associates, Deerfield, IL, USA) and sonicated prior to use. The column was operated at ambient temperature (23±1°C).

2.3. Preparation of stock and standard solutions

Stock solutions (100 μg/ml) of (−)- and (+)-pentazocine and the internal standard naphazoline, 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 prepared to provide 5 μg/ml standard solutions which were used for spiking blank human serum.

2.4. Preparation of spiked human serum

Accurately measured aliquots [5, 15, 30 and 60 μl of the individual 5 μg/ml standard (−)- and (+)-pentazocine solutions] were pipetted into individual 1 ml volumetric tubes and evaporated to dryness with a nitrogen stream. Then 15 μl of the internal standard stock solution were added to each tube and drug-free human serum was added to volume and mixed well to give final concentrations of 25, 75, 150 and 300 ng/ml of each pentazocine enantiomer.

2.5. Assay method

Phenyl SPE cartridges were attached to a vacuum manifold and conditioned with two column volumes of absolute methanol followed by two column volumes of distilled water. Into the cartridges were transferred 1 ml 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 1 ml of methanol–water (60:40, v/v). The pentazocine enantiomers and the internal standard were eluted with 4×250 μl of absolute methanol and evaporated to dryness under a slow nitrogen stream at ambient temperature. The residue was redissolved in 1 ml of the mobile phase, filtered through a 0.2 μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI, USA). Triplicate 20 μl injections were made into the liquid chromatograph.

Linear regression analysis of the peak-height ratios of each pentazocine enantiomer to internal standard versus concentration of each pentazocine enantiomer produced slope and intercept data which were used to calculate concentrations of (−)- and (+)-pentazocine enantiomers in each serum sample.

3. Results and discussion

The chemical structures of pentazocine and the internal standard are shown in Fig. 1. Previous work in this laboratory showed that pentazocine enantiomers were resolved on an ovomucoid chiral column using a mobile phase of methanol–acetonitrile–aqueous 10 m*M* potassium dihydrogenphosphate pH 5.8 (20:5.3:74.7, v/v/v) [12]. Liquid–liquid extraction was employed as the sample clean-up technique. Due to the high cost and stability problems associated with chiral protein columns, as well as the use of organic solvents in liquid–liquid extraction, the work reported in this paper using S-β-CD as the chiral mobile phase additive with a classical octadecylsilane column and SPE for sample clean-up offers a faster, cost effective and environmentally safer method for determination of pentazocine enantiomers in human serum.

The technique of utilizing native and derivatized β-CD as chiral mobile phase additives has been successfully investigated in this laboratory [13]. CDs separate enantiomers utilizing the phenomenon of host–guest complexation where a transient diastereomeric complex is formed between the CD and the analyte. 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]. Recently, the application of mixtures of randomly substituted S-β-CD as chiral additives has been reported for the enantioseparation of a number of compounds of pharmaceutical interest [17]. Sulfation of the CD not only confers a negative charge on the CD, but also alters the molecular recognition capabilities of the native CD. In addition, the association between the analyte and the charged CDs could arise from several potential interactions, including electrostatic or ion-pairing interactions as well as hydrophobic interactions and inclusion complexation [15].

Initial studies in this laboratory with various compositions of mobile phases containing either ammonium acetate or sodium dihydrogenphosphate buffers at different pHs and ethanol with native β-CD, HP-β-CD or Me-β-CD did not give baseline resolution of the pentazocine enantiomers on the octadecylsilane column. S-β-CD added to potassium dihydrogenphosphate buffer and ethanol gave resolution of the pentazocine enantiomers. The influences of S-β-CD, ethanol and potassium dihydrogenphosphate concentrations and pH on the resolution of the pentazocine enantiomers were investigated. Typically, when no buffer containing S-β-CD was added to the mobile phase, there was no resolution of the pentazocine enantiomers. When the concentration of the buffer was increased above 10 m*M*, there was partial resolution of the pentazocine enantiomers. Resolution was reduced when the concentration of S-β-CD was increased above 10 m*M* and increasing the concentration of ethanol reduced the retention time but there was a loss of resolution. Increasing the pH above 5.8 also resulted in a loss of resolution.

A mobile phase composition of aqueous 10 m*M* potassium dihydrogenphosphate buffer pH 5.8 (adjusted with phosphoric acid)–ethanol (80:20, v/v) containing 10 m*M* S-β-CD gave baseline resolution of the pentazocine enantiomers with sensitivity in the desired ng/ml range. Typical HPLC chromatograms of blank human serum and serum spiked with 75 ng/ml of each pentazocine enantiomer and 400 ng/ml of the internal standard are shown in Fig. 2. No interferences were observed in blank human serum at the retention times of (−)- and (+)-pentazocine peaks. Baseline resolution of the pentazocine enantiomers was also achieved on a cyanopropyl column with a mobile phase of aqueous 0.85% triethylamine pH 5.8 (adjusted with trifluoroacetic acid)–acetonitrile (85:15, v/v) containing 10 m*M* S-β-CD. Modification of the mobile phase to reduce the retention times on the cyanopropyl column was unsuccessful because of the loss of resolution. Therefore the octadecylsilane column was selected because it not only maintained the resolution of the enantiomers but gave shorter retention times (18–21 min) compared to the cyanopropyl column (25–30 min).

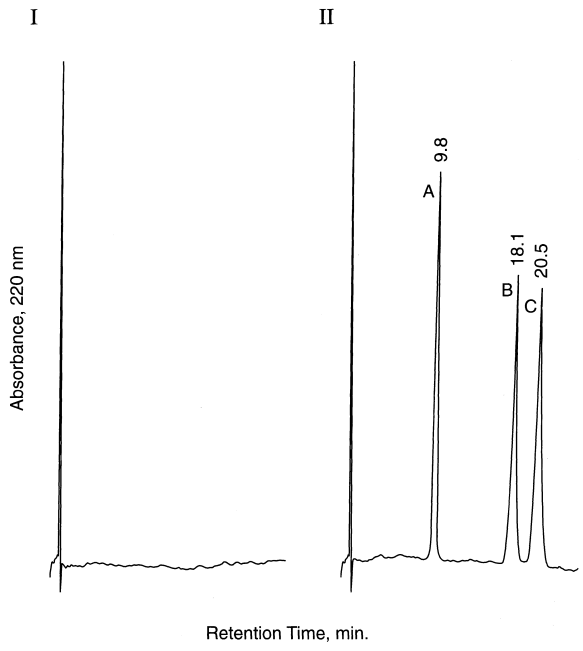


Fig. 2. Typical chromatograms of (I) blank serum and (II) serum spiked with 400 ng/ml of internal standard naphazoline (A) and 75 ng/ml each of (−)-pentazocine (B) and (+)-pentazocine (C) (see Section 2.2 for chromatographic conditions).

Five SPE cartridges (ethylsilane, octasilane, octadecylsilane, cyanopropyl and phenyl) and one mixed-mode SPE disc (PLUS^{TM} MP_3) were investigated for serum sample clean-up prior to the HPLC assay. All the cartridges and disc except phenyl gave recoveries of about 50% or less for both pentazocine enantiomers. The phenyl cartridge was chosen since it provided best results in terms of sample clean-up and recoveries of the pentazocine enantiomers. With absolute methanol as eluent, the mean absolute recoveries using the phenyl cartridge were

91.3±2.8% for (+)-pentazocine, 92.2±3.4% for (−)-pentazocine and 90.8±4.9% for the internal standard (n=5).

Linear calibration curves were obtained in the 20–400 ng/ml range for each enantiomer. Expected serum or plasma levels are in the 50–200 ng/ml range, but are erratic [18]. Standard curves were fitted to a first degree polynomial, $y=ax+b$, where y is the concentration of pentazocine enantiomers, x is the ratio of drug/internal standard peak areas and a and b are constants. Typical regression parameters of a (slope), b (y-intercept) and correlation coefficients were calculated to be 0.00172, 0.00681 and 0.9994, respectively, for (−)-pentazocine and 0.00152, 0.00184 and 0.9996, respectively, for (+)-pentazocine (n=3). The precision (R.S.D.) and accuracy (error) of the method were determined by using spiked human serum samples containing 25, 75, 150 and 300 ng/ml of each pentazocine enantiomer. The data indicated that intra-day precision was in the range 0.8–7.2% (n=3) and the intra-day accuracy was in the range 1.2–6.2% (n=3) for both pentazocine enantiomers and the inter-day precision was in the range 1.0–7.6% (n=9) and the inter-day accuracy was in the range 1.2–4.6% (n=9) for both pentazocine enantiomers. Detailed results are shown in Table 1. The minimum detectable concentration for each enantiomer was determined to be 15 ng/ml (S/N=3). The limits of quantitation were found to be 20 ng/ml for each enantiomer.

In conclusion, an HPLC method has been developed and validated for the assay of (−)- and (+)-pentazocine enantiomers in human serum using S-β-CD as the chiral mobile phase additive on an octadecylsilane column with a phenyl SPE column used as sample clean-up. The method is suitable for the separation and quantitation of each pentazocine enantiomer in the 20–400 ng/ml range.

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Table 1
Accuracy and precision data for pentazocine enantiomers in spiked human serum samples

Analyte	Concentration added (ng/ml)	Concentration found[a] (ng/ml)	Error (%)	R.S.D. (%)
Intra-day				
(−)-Pentazocine	25	23.45±1.46	6.2	6.2
	75	73.22±2.15	2.4	2.9
	150	153.11±3.07	2.1	2.0
	300	304.25±2.72	1.4	0.9
(+)-Pentazocine	25	24.25±1.75	3.0	7.2
	75	73.10±2.45	2.5	3.4
	150	153.24±3.17	2.2	2.1
	300	303.72±2.45	1.2	0.8
Inter-day				
(−)-Pentazocine	25	24.10±1.70	3.6	7.0
	75	73.60±2.52	2.0	3.4
	150	154.10±3.80	2.7	2.5
	300	303.65±3.15	1.2	1.0
(+)-Pentazocine	25	23.87±1.85	4.6	7.6
	75	73.45±2.10	2.1	2.9
	150	153.23±2.87	2.2	1.9
	300	304.16±3.65	1.4	1.2

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

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