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Short Communication

High-performance liquid chromatographic separation of ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction

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ABSTRACT

R(−)-Ondansetron and *S*(+)-ondansetron in human serum were resolved and quantified using a stereospecific HPLC method. Each enantiomer and the internal standard prazosin were isolated from serum using a solid-phase extraction procedure on a cyanopropyl column. Recoveries of 97, 96 and 88% were obtained for the *R*(−)-enantiomer, the *S*(+)-enantiomer, and the internal standard, respectively. A cellulose-based chiral analytical column (Chiralcel OD) was used with a mobile phase consisting of hexane–95% ethanol–2-propanol–acetonitrile (65:25:10:1, v/v). Linear calibration curves were obtained for each enantiomer in serum in the concentration range 10–200 ng/ml. The limit of quantitation of each enantiomer was 10 ng/ml. The detection limit for each enantiomer in serum using UV detection at 216 nm was 2.5 ng/ml (signal-to-noise ratio of 3).

INTRODUCTION

Ondansetron, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, is a highly selective and potent 5-HT_3 receptor antagonist with anti-emetic action. It exhibits little or no sedation or extrapyramidal side effects associated with other commonly used anti-emetic drugs such as metoclopramide, prochlorperazine or droperidol [1]. Ondansetron is remarkably free of side effects, but clinical experience has shown that headache and constipation are frequently observed [2].

Ondansetron exists as a racemate and possesses one asymmetric center (see Fig. 1). Stereoselective differences between the *R*- and *S*-enantiomers of ondansetron have yet to be

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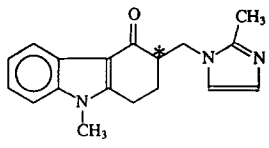
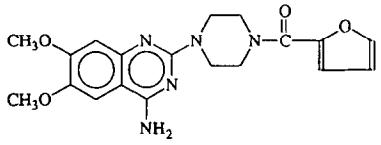


ONDANSETRON



PRAZOSIN

Fig. 1. Chemical structures of ondansetron and prazosin (internal standard).

reported in the literature. However, stereospecific 5-HT_3 receptor blocking has been observed with the *S*-enantiomer of another selective antagonist zacopride [3].

Analysis of racemic ondansetron in plasma has been reported using high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) [4,5]. Resolution of the *R*- and *S*-ondansetron enantiomers and their determination in human serum has not yet been reported. This study reports the chiral separation of *R*(−)- and *S*(+)-ondansetron and determination of the enantiomers in human serum using a Chiralcel OD column and solid-phase extraction. The method was linear over the range 10–200 ng/ml using UV detection at 216 nm. The detection limit of the procedure for each enantiomer was 2.5 ng/ml (at a signal-to-noise ratio $S/N = 3$).

EXPERIMENTAL

Reagents and chemicals

Racemic ondansetron hydrochloride and the *R*(−)- and *S*(+)-enantiomers as maleate salts were supplied by Glaxo (Research Triangle Park, NC, USA). The internal standard prazosin was obtained from Pfizer Labs. (Groton, CT, USA). HPLC-grade hexane, 2-propanol and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ethyl alcohol (95%) was obtained from Florida Distillers (Miami, FL, USA) and absolute ethanol (200 proof) was obtained from Midwest Grain Products (Weston, MO, USA). HPLC-grade triethylamine and drug-free human serum were obtained from Fisher Scientific (Pittsburgh, PA, USA). Cyanopropyl, phenyl, and diol solid-phase extraction columns (100 mg/1 cc size) were obtained from J & W Scientific (Folsom, CA, USA) and the Vac-Elut Vacuum manifold was obtained from Analytichem (Sunnydale, CA, USA).

Chromatographic system

The HPLC system consisted of a Beckman Model 110B pump (Fullerton, CA, USA), a Model 7125 Rheodyne injector (Cotati, CA, USA) equipped with a 100-μl loop, a Kratos Spectroflow 757 variable-wavelength UV detector (Ramsey, NJ, USA) set at 216 nm and a Hewlett-Packard Model 3392 integrator (Palo Alto, CA, USA). The Chiralcel OD column (10 μm, 250 mm × 4.6 mm I.D.) equipped with a Chiralcel OD precolumn (10 μm, 500 mm × 4.6 mm I.D.) were obtained from J.T. Baker (Phillipsburg, NJ, USA). The mobile phase consisted of hexane–95% ethanol–2-propanol–acetonitrile (65:25:10:1, v/v) and was delivered at a flow-rate of 1.0 ml/min. The solution was filtered (0.45-μm nylon membrane) and sonicated prior to use. The chiral and guard columns were operated at ambient temperature (23 ± 1°C).

Preparation of standard solutions

Stock solutions of *R*(−)- and *S*(+)-ondansetron were prepared in absolute ethanol and stored protected from light at ambient temperature (23 ± 1°C). A stock solution of prazosin (10 μg/ml) in absolute ethanol was also prepared and stored protected from light at ambient temperature. Appropriate dilutions of the *R*(−)- and *S*(+)-ondansetron stock solutions with absolute ethanol gave 5 and 1 μg/ml solutions which were used for spiking blank human serum.

Preparation of spiked human serum samples

Accurately measured aliquots (10 and 25 μl of the 1 μg/ml standard solutions and 10, 20 and 40 μl of the 5 μg/ml standard solutions) of *R*(−)- and *S*(+)-ondansetron were each added to 1-ml

volumetric tubes followed by the addition of 30 μl of internal standard solution. Blank human serum was added to volume to give standard solutions containing 10, 25, 50, 100 and 200 ng/ml of each enantiomer.

Assay method

To a 1-ml human serum sample containing ondansetron was added 30 μl of internal standard solution. After vortex-mixing for 10 s, the sample was transferred to a cyanopropyl solid-phase extraction column that had been pre-conditioned with 2 column volumes of absolute methanol followed by 2 column volumes of distilled water (Note: do not allow sorbent to dry). After the entire serum sample had been aspirated through the column, the column was washed with one column volume of methanol–water (10:90, v/v). The column was then dried under full vacuum for 20 min. The ondansetron enantiomers and internal standard were eluted with 4–250 μl volumes of absolute methanol containing 0.1% triethylamine. The eluent was evaporated to dryness under a nitrogen stream at ambient temperature. The residue was redissolved in 250 μl of mobile phase and duplicate 100-μl injections were made into the liquid chromatograph. Linear regression analysis of peak-height ratios of each ondansetron enantiomer to internal standard *versus* concentration of each enantiomer produced slope and intercept data which were used to calculate concentrations of *R*(−)- and *S*(+)-ondansetron in each serum sample.

RESULTS AND DISCUSSION

Initial studies in our laboratories concerning the separation of *R*(−)- and *S*(+)-ondansetron had shown that a mobile phase of hexane–ethanol–2-propanol–acetonitrile (70:20:10:0.5, v/v) produced baseline separation ($R_s = 2.04$) of the enantiomers on a Chiralcel OD column with retention times of 13–16 min. The presence of both ethanol and 2-propanol in the mobile phase influenced the peak shape and k' of each analyte. As assay development progressed, it became obvious that the 70:20:10:0.5 mobile-phase composition produced broad yet symmetrical peaks, which was less desirable for obtaining ng/ml sensitivity for each enantiomer. Finally, the composition of the mobile phase was changed to 65:25:10:1. This mixture still provided good resolution of the two enantiomer peaks ($R_s = 1.50$) and, used with the Chiralcel OD column, provided a suitable separation of the ondansetron enantiomers in a run-time of less than 15 min with sensitivity in the desired ng/ml range.

The water content of the ethanol used in the mobile phase had a significant influence on the chiral separation. When a mobile phase of hexane–ethanol–2-propanol–acetonitrile (70:20:10:0.5, v/v) was prepared using 200 proof ethanol, only partial resolution ($R_s = 0.48$) of the enantiomers was observed. When 95% ethanol was substituted for absolute ethanol, the resolution was greatly improved ($R_s = 1.19$). Balmer *et al.* have shown that a mobile phase of hexane–*n*-propanol–diethylamine (95:4:0.1, v/v/v) containing 1500 mg/l of water gave a good separation of the metoprolol enantiomers on a Chiralcel OD column [6]. They found that the k' of the *S*(−)-enantiomer was greatly affected by the water content of the mobile phase but not the k' of the *R*(+)-enantiomer. In this study, the water content of the mobile phase was shown to greatly effect the retention and separation of both *R*(−)- and *S*(+)-ondansetron. The mechanism of these interactions is unknown at present.

The selection of prazosin as internal standard was based on its structural similarity to ondansetron. Both compounds contain a carbonyl functional group, a tertiary amine and a fused aromatic heterocyclic ring with an attached five-member aromatic heterocyclic group. The retention time of prazosin was 7.99 ± 0.24 min and a separation factor α of 1.38 between prazosin and the first eluting ondansetron enantiomer, *R*(−), was obtained. Quantitation was based on peak-height ratios of each ondansetron enantiomer to the internal standard.

The suitability of the system for the separation of the ondansetron enantiomers was based on the following chromatographic parameters and the performance of the method throughout the validation procedure. The retention times of *R*(−)- and *S*(+)-ondansetron and the internal

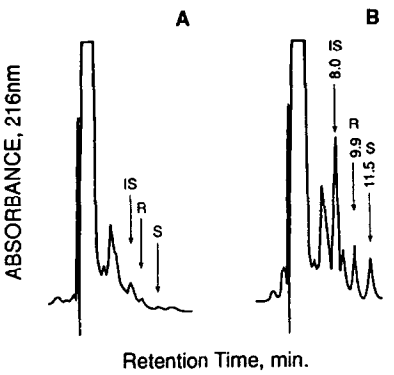


Fig. 2. Typical chromatograms of (A) blank serum and (B) serum spiked with 20 ng/ml each of *R*(−)- and *S*(+)-ondansetron at 216 nm. Internal standard (I.S.) concentration was 300 ng/ml. See Experimental section for chromatographic conditions.

standard prazosin were 9.96 ± 0.22, 11.56 ± 0.27 and 7.99 ± 0.24 min, respectively (n = 5). Capacity factors (k') for the *R*(−)- and *S*(+)-enantiomers were 2.52 ± 0.28 and 3.08 ± 0.37, respectively (n = 5). The respective numbers of theoretical plates for *R*(−)- and *S*(+)-ondansetron were 2181 ± 62 and 2940 ± 52 per 25 cm column (n = 4). Relative retention of the *R*(−)- and *S*(+)-enantiomers was expressed by the separation factor α, calculated to be 1.25 ± 0.02 (n = 4) from the ratio of the capacity factors. Resolution (R_s) of the internal standard and first eluting *R*(−) peaks and for the *R*(−) and *S*(+) peaks were 1.59 and 1.98, respectively.

No interferences were observed in blank human serum at the retention times of *R*(−)- and *S*(+)-ondansetron. Fig. 2 shows typical chromatograms for blank human serum and serum spiked with 20 ng/ml of each enantiomer.

Three solid-phase extraction columns (phenyl, diol and cyanopropyl) were investigated for serum clean-up prior to the chiral HPLC assay. The phenyl column showed an interfering endogenous serum peak at 9.4 min, which co-eluted with *R*(−)-ondansetron. The diol column gave a broad interfering peak that co-eluted with *S*(+)-ondansetron. The cyanopropyl column was found to provide good recoveries of both enantiomers with little or no interference from serum components with the drug or internal standard peaks. The absolute recoveries of *R*(−)- and *S*(+)-ondansetron and prazosin (internal standard) from human serum using the cyanopropyl column were 96.7 ± 2.6, 96.3 ± 2.6 and 88.1 ± 5.4%, respectively, at 500 ng/ml levels (n = 6). The recoveries of the analytes and internal standard were calculated by a comparison of the peak heights of extracted to unextracted analyte or internal standard.

Linear calibration curves were obtained in the 10–200 ng/ml range for each enantiomer. Standard curves were fitted to a first degree polynomial, $y = ax + b$, where y is the ratio of drug/internal standard peak heights, a and b are constants, and x is the ondansetron concentration. Typical values for the regression parameters a (slope), b (y-intercept) and correlation coefficient were calculated to be 0.0025, 0.024 and 0.9999 for *R*(−), and 0.0027, 0.0018 and 0.9998 for *S*(+), respectively (n = 6). The precision and accuracy (percent error) of the method were determined using samples spiked at 25 and 100 ng/ml levels. The data shown in Table I indicates that intra-day precision was in the 2–10% range and intra-day accuracy in the 1–7% range for both ondansetron enantiomers. In

TABLE I

ACCURACY AND PRECISION DATA FOR ONDANSETRON ENANTIOMERS IN SPIKED SERUM SAMPLES

Analyte	Concentration added (ng/ml)	Concentration found (mean ± S.D., n = 3) (ng/ml)	Error (%)	R.S.D. (%)
R(−)	25	25.73 ± 3.58	3.4	10.2
	100	106.96 ± 2.77	6.8	2.4
S(+)	25	25.46 ± 1.43	1.4	5.6
	100	102.56 ± 7.72	2.6	7.5

separate studies, inter-day precision was in the 1.8–5.6% range ($n = 3$) and inter-day accuracy in the 2–4.5% range ($n = 4$) for both enantiomers.

The minimum detectable concentration of each enantiomer was determined to be 2.5 ng/ml ($S/N = 3$). The lowest quantifiable level was found to be 10 ng/ml for each enantiomer: $R(-)$, 4.73 %R.S.D., 3.7% error; $S(+)$, 4.65 %R.S.D., 5.4% error. The Chiralcel OD column was used daily for a period up to 3.5 months without any sign of deterioration due to injections of serum sample extracts.

In conclusion, an HPLC method has been developed and validated for the assay of $R(-)$- and $S(+)$-ondansetron in human serum. The method is suitable for the separation and quantification of each enantiomer in the 10–200 ng/ml range.

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