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Short communication

Enantioselective determination of *S*-(+)- and *R*-(−)-ondansetron in human serum using derivatized cyclodextrin-modified capillary electrophoresis and solid-phase extraction

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Abstract

A high-performance capillary electrophoresis (HPCE) assay method for the quantitation of *S*-(+)- and *R*-(−)-ondansetron in human serum was developed. Resolution was achieved using 15 m*M* heptakis-(2, 6-di-O-methyl)-β-cyclodextrin (DM-β-CD) in 100 m*M* phosphate buffer (pH 2.5). A 72-cm untreated fused-silica capillary, at a constant voltage of 20 kV, was used for the analysis. A 0.03-m*M* cationic detergent was used as a buffer additive to decrease the adsorption of endogenous substances onto the silica wall. The analytes of interest were isolated from endogenous substances using a solid-phase extraction procedure. The cyanopropyl cartridge gave good recoveries in excess of 85% for both *S*-(+)- and *R*-(−)-ondansetron, without any interferences. To decrease the limits of detection of the analytes, an on-capillary sample concentration technique was employed. The detection limit was 10 ng/ml using 2 ml of serum and the limit of quantitation was 15 ng/ml. The calibration curve was linear over a range of 15–250 ng/ml, with procainamide as the internal standard, and the coefficients of determination obtained were greater than 0.999 ($n=3$). Precision and accuracy of the method were 2.76–5.80 and 2.10–5.00%, respectively, for *S*-(+)-ondansetron, and 3.10–6.57 and 2.50–4.35%, respectively, for *R*-(−)-ondansetron. The HPCE method is a useful alternative to existing chiral high-performance liquid chromatographic methods.

Keywords: Enantiomer separation; Ondansetron

1. Introduction

Enantiomers of chiral drugs often show different pharmacological and toxicological properties, so determination of individual enantiomers is necessary for monitoring the pharmacokinetics and pharmacodynamics of chiral drugs. To study individual enantiomers, a wide range of enantioselective analysis methods have been developed. Chiral high-performance liquid chromatography (HPLC) and gas chromatography (GC) are the most commonly used separation techniques [1].

Capillary electrophoresis is a powerful analytical technique for the separation of enantiomers, due to its inherent properties like high efficiency, low sample volume and ease of operation [2–8]. There are four modes of chiral separation in capillary electrophoresis, depending on the type of chiral selector chosen. These are (1) host–guest complexation, (2) natural and synthetic optically active micelles, (3) ligand exchange and (4) proteins. Host–guest complexation is usually performed using

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cyclodextrins (CDs) and more recently 18-crown-6-tetracarboxylic acids [9]. Enantiorecognition is based on inclusion phenomena and leads to the formation of additional interactions with the secondary hydroxyl groups on the rim of the CDs. There are two types of CDs, i.e., neutral and charged. The commonly used neutral CDs are the native α-, β-, γ- and the derivatized dimethyl, trimethyl, hydroxyethyl and hydroxypropyl forms. The charged CDs are carboxymethyl, sulfobutyl ether, sulfated and amino CDs.

Ondansetron, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, is a selective and potent 5-hydroxytryptamine$_3$ ($5HT_3$) receptor antagonist used in the treatment of chemotherapy-induced nausea and emesis [10,11]. Ondansetron possesses one asymmetric center and exists as *S*-(+)- and *R*-(−) enantiomers. Stereospecific 5-HT_3 receptor blocking has been observed with enantiomers of another selective antagonist, zacopride [12]. Assay of ondansetron in plasma has been performed using HPLC, high-performance thin-layer chromatography (HPTLC) and radioimmunoassay [13–16]. A stereoselective assay for the determination of *S*-(+)- and *R*-(−)-ondansetron in human serum has been reported by this laboratory using chiral HPLC [17].

This paper reports the enantiomeric separation of *S*-(+)- and *R*-(−)-ondansetron for the determination of low ng/ml concentrations in human serum using heptakis-2,6-di-O-methyl-β-cyclodextrin-modified capillary electrophoresis and solid-phase extraction. The method is linear in the range of 15–250 ng/ml.

2. Experimental

2.1. Reagents and chemicals

Racemic ondansetron hydrochloride and the *S*-(+)- and *R*-(−) enantiomers (as maleate salts) were a gift from Glaxo (Research Triangle Park, NC, USA). The internal standard, procainamide, and hexadecyltrimethylammonium bromide (HTAB) were obtained from Sigma (St. Louis, MO, USA). Phosphoric acid (85%), sodium dihydrogenphosphate monohydrate and ammonia solution (35%, v/v) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Native α-, β- and γ-cyclodextrins, heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) were obtained from Sigma. Hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were obtained from Aldrich (Milwaukee, WI, USA). Hydroxyethyl-β-cyclodextrin (HE-β-CD), carboxymethyl-β-cyclodextrin (CM-β-CD) and amino-β-cyclodextrin were obtained from Advanced Separation Technologies (Whippany, NJ, USA). Sulfated β-cyclodextrin was a gift from American Maize Products (Hammond, IN, USA). Drug-free human serum was obtained from Biological Specialty (Colmar, PA, USA). Cyanopropyl and C_{18} solid-phase extraction columns (100 mg/1 ml size) and the Vac-Elut vacuum manifold were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). All the solutions were filtered through a 0.2-μm nylon filter (Acrodisc 13, Gelman Sciences, Ann Arbor, MI, USA).

2.2. Preparation of stock and standard solutions

Individual stock solutions were prepared in absolute methanol to give concentrations of 100 μg/ml of *S*-(+)- and *R*-(−)-ondansetron and 100 μg/ml of procainamide (internal standard). Appropriate volumes of the individual *S*-(+)- and *R*-(−)-ondansetron stock solutions were pipetted into 2 ml volumetric flasks and evaporated. Then 2 ml of serum and 6.5 μl of the internal standard solution were added to the tubes and mixed well. A stock solution of sodium dihydrogenphosphate was prepared in double distilled, deionized water and the pH was adjusted to 2.5 using 100 m*M* phosphoric acid.

2.3. Electrophoretic system

All capillary electrophoresis experiments were performed using an ABI 270A capillary electrophoretic system (Applied Biosystems, Foster City, CA, USA) equipped with an UV detector. An uncoated fused-silica capillary (total length, 72 cm; effective length, 50 cm; 50 μm I.D; Polymicro Technologies, Phoenix, AZ, USA) was used for the analyses. The capillary temperature was kept at 30°C and the voltage applied was 20 kV. The typical running current was 65 μA. A 0.5-cm detection

window was created by stripping the polyimide coating from the capillary. The detection was towards the cathodic end.

The run buffer consisted of an aqueous solution of 100 m*M* sodium dihydrogenphosphate, pH 2.5 (adjusted with 100 m*M* phosphoric acid), containing 15 m*M* DM-β-CD and 0.03 m*M* HTAB. The analytes were monitored at 254 nm.

2.4. Electrophoretic conditions

New capillaries were conditioned by rinsing them with 1 *M* sodium hydroxide for 10 min followed by 10 min each with water and run buffer solution. Sample introduction was performed using a vacuum injection (80 p.s.i.) for 20 s. Before each analysis, the capillary was rinsed for 2 min with 0.1 *M* sodium hydroxide and for 2.5 min with run buffer solution.

3. Assay procedure

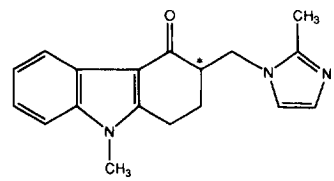
Sample clean-up was performed using a solid-phase extraction (SPE) method reported previously [14,16], with minor changes. To 2 ml of serum containing *S*-(+)- and *R*-(−)-ondansetron were added 6.5 μl of the internal standard, followed by mixing. The cyanopropyl (CN) SPE cartridge was conditioned using 1×1000 μl of methanol followed by 1×1000 μl of 1% ammonia in 2-propanol and 1×1000 μl of deionized water. The cartridge was not allowed to dry between the washing and sample application steps. A 100-μl volume of 0.5 *M* hydrochloric acid was added to the cartridge followed by the serum sample. The column was washed with 2×1000 μl of water and 2×1000 μl of acetonitrile, with the cartridge being allowed to dry between and after washes. The analytes were eluted with 4×500 μl of a 1% ammonia solution in 2-propanol. The eluate was filtered using a nylon syringe filter prior to evaporation using a nitrogen stream. The samples were reconstituted in 90 μl of distilled water and the sample was vacuum injected onto the capillary for 20 s.

Calibration curves were constructed using concentrations of 15, 75, 150 and 250 ng. Linear regression analysis of D/I.S. peak-area ratios versus concentration gave slope and intercept data for each analyte, which were used to calculate the concentration of each analyte in the serum samples. The absolute recovery was calculated by comparing drug peak-area of the spiked analyte samples to unextracted analyte stock solutions that had been injected directly into the electrophoretic system.

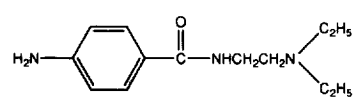
4. Results and discussion

The chemical structures of *S*-(+)- and *R*-(−)-ondansetron and procainamide (I.S.) are shown in Fig. 1. Baseline separation of ondansetron was achieved using 15 m*M* DM-β-CD as the chiral selector. Resolution of *S*-(+)- and *R*-(−)-ondansetron was performed using various neutral and charged CDs. The various neutral CDs investigated were native α-, β- and γ-cyclodextrins, DM-β-CD, TM-β-CD, HP-β-CD, HP-α-CD, HP-γ-CD and HE-β-CD. The charged CDs investigated were CM-β-CD, amino-β-cyclodextrin and sulfated-β-cyclodextrin.

No separations were obtained using any of the CDs except for CM-β-CD and DM-β-CD in 100 m*M* phosphate buffer at pH 2.5. DM-β-CD was chosen as the chiral selector in the run buffer because CM-β-CD provided less resolution than DM-β-CD at similar concentrations. A 15-m*M* concentration of DM-β-CD gave baseline separation of ondansetron enantiomers. Optimization of the run buffer was



ONDANSETRON



PROCAINAMIDE

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

performed by studying the effects of phosphate buffer, DM-β-CD and pH. The analyte peak shapes improved in symmetry as the phosphate buffer concentration increased, with no significant effect on migration time. An increase in DM-β-CD concentration increased migration time without a significant increase in resolution. As the pH was increased above 6.5, there was a complete loss of enantiomer resolution, accompanied by a decrease in migration times. Fig. 2A shows an electropherogram of blank serum and Fig. 2B shows the electropherogram of spiked *S*-(+)- and *R*-(−)-ondansetron and the I.S., procainamide. Since ondansetron is widely used in patients receiving chemotherapy, co-migration of doxorubicin, idarubicin, cyclophosphamide, dacarbazine, cytarabine and etoposide was investigated.

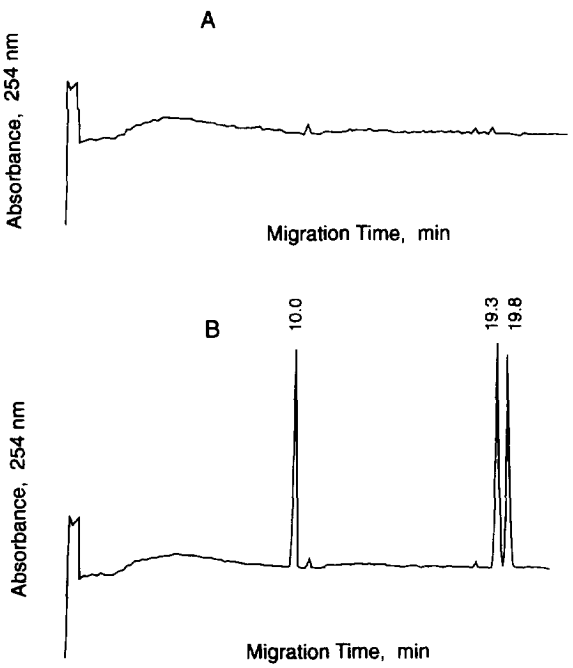


Fig. 2. Electropherograms of (A) serum blank and (B) serum spiked with *S*-(+)-ondansetron (19.3 min), *R*-(−)-ondansetron (19.8 min) and procainamide (internal standard, 10.0 min). Electrophoretic conditions: uncoated fused-silica capillary (50 μm I.D, 72 cm total length, 50 cm effective length), the run buffer consisted of an aqueous solution of 100 m*M* sodium dihydrogen phosphate, pH 2.5 (adjusted with 100 m*M* phosphoric acid), containing 15 m*M* heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and 0.03 m*M* HTAB. Voltage, 20 kV; temperature, 30°C; vacuum injection (80 p.s.i.), 20 s; detection, at the cathodic end.

There were no interferences from these drugs in the electropherograms. This shows that the method is specific for ondansetron in the presence of these co-administered drugs.

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 OH groups or introduced functional groups on the CD ring [18]. Previous studies reported by Schmitt et al. [19] and Chankvetadze et al. [20] have shown that a significant advantage of charged chiral selectors is their strong electrostatic interaction with the charged solute, which can have a significant influence on the resolution of the analyte. The counter-current flow of the chiral additive and the solute may also contribute to a higher enantioselectivity.

To decrease the non-specific interactions of the endogenous substances with the silanol groups, a cationic detergent was used. Cationic detergents cover the negatively charged silica wall of a capillary, forming a net positive charge, thereby reducing the protein adsorption on the wall while the cationic ondansetron is repelled. Under the acidic conditions used in the experiment and employing the cationic detergent, the electromigration of positively charged species towards the cathode was the principle force in effect and the electroosmotic flow became negligible [21]. The addition of HTAB to the background electrolyte system at a concentration of 0.035 m*M* produced stable migration times and peak shapes, suggesting a role in minimizing the amount of irreversible adsorption. Migration time reproducibility was very high with a R.S.D. of less than 0.5% for all three peaks ($n=10$). The effect of voltage on peak resolution and migration time was investigated and it was found that a voltage of 20 kV gave optimum resolution, with an acceptable migration time.

For the extraction of the analytes from a serum sample, SPE was used, which allowed recoveries greater than 85% for both *S*-(+)- and *R*-(−)-ondansetron. The mean absolute recoveries using the CN SPE cartridge were 86.3±3.8% for *S*-(+)-ondansetron, 85.9±4.5% for *R*-(−)-ondansetron and 74.7±4.9% for procainamide ($n=5$).

To decrease the detection limits, on-capillary sample concentration was employed. The sample was prepared in a solution (water) that had a lower conductivity than that of the electrolyte solution. Upon application of the voltage, a greater field will develop across the sample zone, causing the ions to migrate faster. When the ions reach the run buffer, the field decreases and they migrate more slowly. This process occurs until the analytes are compressed into a smaller zone [22,23]. Another analyte stacking method was also investigated. The sample was reconstituted with a solution with an ionic concentration that was identical to that of the run buffer. Before the sample was injected, a plug of water was injected, but there was no significant improvement in the limits of detection. Thus, the sample was reconstituted in water. The limit of detection, based on a signal to-noise-ratio of three, was 10 ng/ml and the limit of quantitation was 15 ng/ml.

The calibration curves showed good linearity in the range of 15–250 ng/ml for *S*-(+)- and *R*-(−)-ondansetron. The coefficients of determination were greater than 0.999 ($n=3$). Representative linear regression equations obtained for *S*-(+)- and *R*-(−)-ondansetron were $y=0.005990x+0.002644$ (standard error=0.00422) and $y=0.005980x-0.00359$ (standard error=0.013750), 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 R.S.D. (%) and % error were 2.76–3.80 and 2.10–5.00%, respectively, for *S*-(+)-ondansetron and 3.10–3.67 and 4.00–4.35%, respectively, for *R*-(−)-ondansetron. The inter-day precision and accuracy ($n=9$, over three days), expressed as R.S.D. (%) and % error were 5.07–5.80 and 2.90–3.42%, respectively, for *S*-(+)-ondansetron and 4.90–6.57 and 2.50–3.35%, respectively, for *R*-(−)-ondansetron. Detailed data are listed in Table 1.

The limits of detection for this CE method are comparable to those reported for chiral HPLC methods. The limits of detection might be improved by using either a bubble or Z-shaped detection cell or by decreasing the reconstitution volume after solid-phase extraction to as low as 15 μl, which is sufficient for repeated injections.

In summary, the HPCE assay described herein is sensitive and suitable for the simultaneous determination of *S*-(+)- and *R*-(−) enantiomers of ondansetron in serum. The solid-phase extraction provided good sample clean-up, with no endogenous interferences. The method showed good linearity and precision within the linear range of 15–250 ng/ml. This HPCE method shows that capillary electrophoresis is an useful alternative to chiral HPLC for the determination of enantiomeric drugs in a biological matrix such as serum.

Table 1
Accuracy and precision of serum samples with added *S*- and *R*-ondansetron

	Concentration added (ng/ml)	Concentration found (ng/ml)	R.S.D. (%)	Error (%)
Intra-day				
S	20	19.0±0.72	3.8	5.0
	225	220.3±7.89	2.8	2.1
R	20	19.13±0.59	3.1	4.4
	225	216.0±7.9	3.7	4.0
Inter-day				
S	20	19.42±1.13	5.8	2.9
	225	217.3±11.02	5.1	3.4
R	20	19.33±1.27	6.6	3.4
	225	219.3±10.69	4.9	2.5

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

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