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Short communication

Stereoselective determination of apomorphine enantiomers in serum with a cellulose-based high-performance liquid chromatographic chiral column using solid-phase extraction and ultraviolet detection

Emmanuel Ameyibor, James T. Stewart*

Department of Medicinal Chemistry, College of Pharmacy, The University of Georgia, Athens, GA 30602-2352, USA

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Abstract

A novel and rapid method for the separation and determination of *R*-(−)- and *S*-(+)-enantiomers of apomorphine in serum by high-performance liquid chromatography with UV detection is reported. The method involved a solid-phase extraction of the *R*-(−)- and *S*-(+)-enantiomers of apomorphine and the internal standard *R*-(−)-propylnorapomorphine from serum using a C_8 Bond-Elut column. The HPLC system consisted of a reversed-phase cellulose-based chiral column (Chiralcel OD-R, 250×4.6 mm I.D.) with a mobile phase of 35:65 (v/v) acetonitrile–0.05 *M* sodium perchlorate (pH 2.0, adjusted with 60–62% perchloric acid) at a flow-rate of 0.5 ml/min with UV detection at 273 nm. The detection and quantitation limits were 10 ng/ml for each enantiomer using 1 ml of serum. Linear calibration curves from 10 to 1000 ng/ml for both *R*-(−)- and *S*-(+)-enantiomers show coefficient of determination of more than 0.9995. Precision calculated as %R.S.D. and accuracy calculated as % error were 0.2–4.7 and 3.1–6.9%, respectively, for the *R*-(−)-enantiomer and 1.3–4.2 and 0.3–6.8%, respectively, for the *S*-(+)-enantiomer.

Keywords: Enantiomer separation; Apomorphine

1. Introduction

Apomorphine (10,11-dihydroxyaporphine) is a drug that has been used for treatment of a variety of neurological disorders (e.g. Parkinsonism, Huntington's chorea, schizophrenia and thalamic pain), resulting from dopamine imbalances in the brain [1–4]. The *R*-(−)-enantiomer is a potent dopamine receptor agonist whereas the *S*-(+)-enantiomer is inactive as an agonist [5]. Even though apomorphine is administered to humans as the *R*-(−)-enantiomer, there is a need in biomedical research to measure serum concentrations of the *R*- and *S*-enantiomers since apomorphines have been found to inhibit amphetamine-induced locomotor activity in the mouse [6] and lower serum prolactin levels in the rat [7].

Several methods has been reported for the determination of apomorphine including HPLC, gas chromatography, spectrofluorimetry, mass spectrometry with selected ion monitoring, enzyme-radioisotopic methods and electrochemical detection [8–12]. However, no HPLC methods have been reported for the determination of apomorphine enantiomers in serum.

In this paper, a rapid and sensitive chiral HPLC

*Corresponding author.

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method is described which will measure nanogram concentrations of the *R*- and *S*-enantiomers of apomorphine in serum with solid-phase extraction clean-up and UV detection.

2. Experimental

2.1. Reagents and chemicals

Powdered samples of *R*-(−)- and *S*-(+)-apomorphine hydrochloride and the internal standard *R*-(−)-propylnorapomorphine hydrochloride (Research Biochemical International, Natick, MA, USA) were used in the preparation of standard solutions. Blank bovine serum (Cat No. 3160-34) was obtained from Instrumentation Lab (Lexington, MA, USA) Sodium perchlorate was purchased from Fisher Scientific, (Fair Lawn, NJ, USA). The HPLC solvents, acetonitrile and 60–62% perchloric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. HPLC conditions

Chromatography was performed on an isocratic HPLC system consisting of a Beckman Model 110A solvent delivery module (Beckman, San Ramon, CA, USA) a Waters 712 WISP autosampler (Waters Associates, Milford, MA, USA) and a Waters Model 481 variable wavelength UV–Vis detector set at 273 nm. Data acquisition was performed on a HP Model 3290 integrator (Hewlett-Packard, Avondale, PA, USA).

Separations were accomplished on a reversed-phase cellulose-based chiral column (Chiralcel OD-R, 250×4.6 mm I.D., 10 μm, Chiral Technologies, Eaton, PA, USA) at ambient temperature (23±1°C). The mobile phase consisted of 35:65 (v/v) acetonitrile–0.05 *M* aqueous sodium perchlorate (pH 2.0, adjusted with 60–62% perchloric acid). It was filtered through a 0.45-μm Nylon-66 filter (MSI, Westborough, MA, USA) and was deaerated by sonication prior to use. The flow-rate was set at 0.5 ml/min. Quantitation was based on linear regression analysis of peak height of analyte versus analyte concentration.

2.3. Preparation of standard solutions

Individual stock solutions of *R*-(−)- and *S*-(+)-apomorphine and the internal standard *R*-(−)-propylnorapomorphine were prepared in 0.01 *M* HCl to give concentrations of 100 μg/ml. Appropriate volumes of the two analytes and the internal standard were pipetted into a 1-ml volumetric flask and serum added to volume to give final serum concentrations of 10, 100, 250 and 500 ng/ml of each analyte and 300 ng/ml of the internal standard.

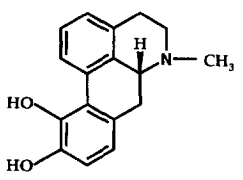
2.4. Assay procedure

Bond-Elut C_8 solid-phase extraction (SPE) columns were attached to a vacuum manifold (Vac-Elut, Varian Sample Preparation Products, Harbor City, CA, USA). The column was conditioned with 2 ml of methanol followed by 2 ml of distilled water. After addition of 0.5 ml potassium dihydrogen phosphate buffer (pH 7.4) and equilibration for 1 min, the serum sample was applied to the column with an additional 0.5 ml of buffer used to rinse the tube. Then, the column was washed with 2 ml of pH 7.4 buffer–methanol (7:3, v/v) followed by 2 ml water. The analytes were eluted with 4×250 μl pH 3.0 phosphate buffer–methanol (3:7, v/v). The eluate was evaporated to dryness in a nitrogen stream and reconstituted in 1 ml of mobile phase prior to analysis by HPLC. A 100-μl sample was injected into the HPLC system.

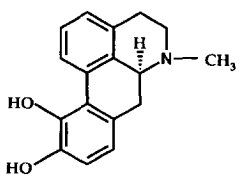
For absolute recovery experiments, spiked samples were compared to unextracted stock solutions. Extracted to unextracted peak-height ratios for each enantiomer were used to calculate the extraction recoveries.*

3. Results and discussions

The chemical structures of the compounds studied are shown in Fig. 1. Attempts were made earlier to separate apomorphine into its *R*-(−)- and *S*-(+)-enantiomers using various Pirkle type chiral columns and derivatization techniques with *tert.*-butyl, phenyl and naphthyl isocyanates. All of these procedures proved to be unsuccessful in this laboratory. Based on the successful chiral resolution of some com-



R(-) - APOMORPHINE



S(+) - APOMORPHINE

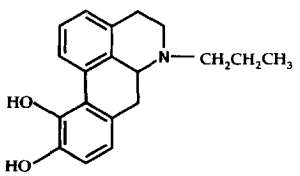


Fig. 1. Chemical structures of apomorphine enantiomers and internal standard.

pounds from different drug classes, the Chiralcel OD-R column was investigated [13]. The Chiralcel column contains a cellulose-tris(3,5-dimethyl phenylcarbamate) stationary phase and is designed for operation in the reversed-phase mode. The recommended mobile phase composition for this column includes a sodium perchlorate buffer system and acetonitrile [13]. The use of the Chiralcel OD-R column enabled the separation of the apomorphine enantiomers within a reasonable chromatographic run time (<20 min).

In the course of developing a solid-phase extraction procedure for serum sample clean-up, an octadecyl (C_{18}) SPE column was initially used. However, the internal standard peak could not be adequately resolved from a large unknown serum peak. It was determined that an octyl (C_8) SPE column was more effective in eliminating the interfering serum peak and gave very good recoveries of the two enantiomers. Fig. 2 shows the chromatograms of blank serum (A) and *R*-(−)- and *S*-(+)-apomorphine added to serum (B).

The absolute recoveries of the apomorphine enantiomers from serum were determined by comparing the analyte peak height obtained after extraction of spiked samples to the peak height of known amounts of the unextracted analyte. Absolute recoveries of >95% were obtained for both enantiomers. The mean absolute recoveries using the octyl (C_8) SPE were 97.6±2.2% for *R*-(−)-apomorphine and 96.3±2.7% for *S*-(+)-apomorphine (n=3).

The limits of detection and quantitation were 10 ng/ml for both *R*-(−)- and *S*-(+)-enantiomers. The 10–1000 ng/ml range for both enantiomers is within the levels that would be useful for biomedical research [14]. The calibration curves showed good linearity in the 10–1000 ng/ml range and the coefficients of determination were more than 0.9995 (n=9) for both enantiomers. Linear regression equations obtained for *R*-(−)- and *S*-(+)-enantiomers

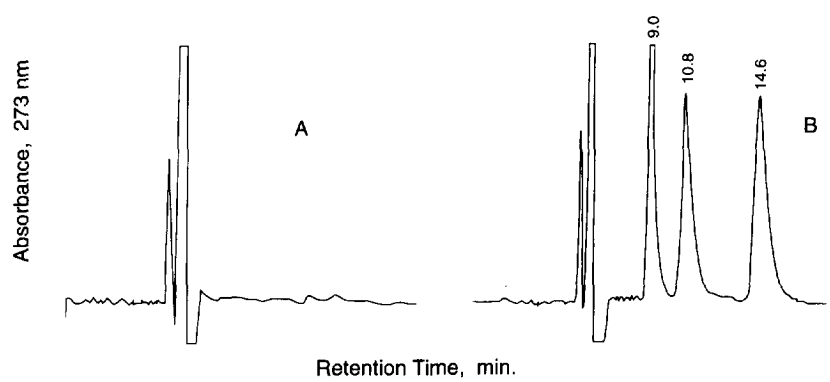


Fig. 2. Typical chromatograms of (A) blank serum and (B) serum spiked with *R*-(−)-apomorphine (9.0 min), *S*-(+)-apomorphine (10.8 min), and internal standard (14.6 min) on a chiracel OD-R column using a 35:65 (v/v) acetonitrile–aqueous sodium perchlorate (pH 2) mobile phase with detection at 273 nm.

Table 1
Assay of apomorphine enantiomers added to serum

	Concentration added (ng/ml)	Concentration found (ng/ml)	Error (%)	R.S.D. (%)
Intra-day (n=3)				
R-(−)-Apomorphine	50.0	53.5±1.3[a]	6.9	3.5
	350.0	366.0±0.6	4.6	0.2
S-(+)-Apomorphine	50.0	52.4±1.3	4.8	3.6
	350.0	348.3±4.2	0.5	1.3
Inter-day (n=9)				
R-(−)-Apomorphine	50.0	51.5±1.9[b]	3.1	4.7
	350.0	367.0±1.2	4.9	0.5
S-(+)-Apomorphine	50.0	53.4±1.6	6.8	4.2
	350.0	349.8±5.7	0.3	2.1

[a] Mean±S.D. based on $n=3$.
[b] Mean±S.D. based on $n=9$.

were $y=0.008699x-0.01317$ and $y=0.004288x+0.023628$, respectively, where y and x are the drug to internal standard peak-height ratios and the concentrations of the analytes, respectively.

Percent error (accuracy) and precision (%R.S.D.) of the serum assays were evaluated using spiked concentrations of *R*-(−)- and *S*-(+)-apomorphine in serum. The intra-day precision and accuracy ($n=3$) were 0.2–3.5% and 4.6–6.9%, respectively, for the *R*-(−)-enantiomer, and 1.3–3.6% and 0.5–4.8%, respectively, for the *S*-(+)-enantiomer. The inter-day precision and accuracy ($n=6$) were 0.5–4.7% and 3.1–4.9%, respectively, for the *R*-(−)-enantiomer and 2.1–4.2% and 0.3–6.8%, respectively, for the *S*-(+)-enantiomer. The detailed data is listed in Table 1.

In summary, a precise, accurate and rapid chiral HPLC method using solid-phase extraction has been developed for the assay of *R*-(−)- and *S*-(+)-apomorphine in serum.

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