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Short Communication

Determination of remoxipride in human plasma and urine by reversed-phase ion-pair high-performance liquid chromatography

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ABSTRACT

A sensitive method is described for the measurement of remoxipride in human plasma and urine. Remoxipride and its internal standard are extracted from plasma or urine at pH 12 with a mixture of hexane and methyl *tert.*-butyl ether. After washing the organic phase with base, the compounds are extracted into acid and analyzed on a C_{18} column with ultraviolet detection at 214 nm. The mobile phase is composed of acetonitrile and aqueous buffer (sodium perchlorate and phosphoric acid, pH 1.7). The limits of reliable quantitation for remoxipride are 12.5 and 50 ng/ml for plasma and urine, respectively. The run times are 6 min for plasma and 3 min for urine. The method has been successfully used to assay remoxipride clinical study samples. This mobile phase has also been successfully applied to the analysis of other basic drugs such as cimetidine, codeine, diltiazem and quinidine with minor modifications.

INTRODUCTION

Remoxipride, *S*(−)-3-bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide, is a new neuroleptic agent [1], currently under development in the USA. It is a weak, but relatively selective central dopamine D2-receptor antagonist [2,3]. Many current antipsychotic drugs are not selective and it is postulated that some central neuropharmacological effects of the development of adverse effects are due to their blockade of other receptors. Remoxipride appears to have preferential affinity for extrastriatal dopamine D2-receptors and also has marked affinity for central sigma receptors. The chemical structures of remoxipride and the internal standard (I.S.) FLA 913, 3-bromo-N-[(1-propyl-2-pyrrolidinyl)-methyl]-2,6-dimethoxybenzamide, are shown in Fig. 1.

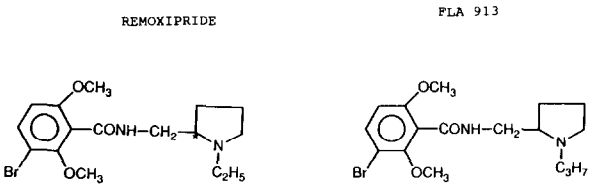

Fig. 1. Chemical structures of remoxipride and its internal standard FLA 913.

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Several methods for the analysis of remoxipride in biological fluids have been reported. One involved the direct injection of the sample onto a precolumn loaded with hexadecyltrimethylammonium bromide followed by post-column extraction and reversed-phase high-performance liquid chromatography (HPLC) [4]. Nilsson [5] reported a solvent extraction method with different procedures for high and low concentration ranges. The method also involved an evaporation step of the extracting organic solvent. To support human pharmacokinetic studies, we have developed a robust method which involves the same liquid–liquid extraction for both plasma and urine for different concentration ranges without an evaporation step.

EXPERIMENTAL

Materials

All reagents were HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). Remoxipride free base and I.S. (Merck Research Labs., Rahway, NJ, USA) were used as reference standards. Disposable glass screw-cap 16 × 150 mm culture tubes and disposable glass screw-cap 15-ml conical tubes were used for extraction (Baxter, Edison, NJ, USA). Heparinized human plasma (Biological Specialty, Landsdale, PA, USA) was used as control.

Equipment

A multi-tube vortexer (VWR, Bridgeport, NJ, USA) was used for mixing. The UV detector was a Spectroflow 783 (Applied Biosystem, Foster City, CA, USA) with the wavelength set at 214 nm. The analytical column was an Analytichem Sepralyte C_{18} (50 mm × 4.6 mm I.D., 3 μm particle size, Harbor City, CA, USA). The HPLC system consisted of HP 1090L with a built-in column heater. The output was recorded on a Spectra-Physiscs 4270 integrator.

Chromatographic conditions

The HPLC mobile phase consisted of 75% (69% for urine) aqueous buffer and 25% (31% for urine) acetonitrile. The aqueous buffer consisted of 0.2 *M* sodium perchlorate and 0.1 *M* phosphoric acid (pH 1.7). The flow-rate was 1.3 ml/min and the column temperature was 40°C.

Standard and quality control samples

Remoxipride concentration was expressed as its free base. Stock standards and working solutions were prepared in 0.1 *M* phosphoric acid. The working solution of internal standard was 5 μg/ml in 0.1 *M* phosphoric acid. The plasma standard concentration curves were prepared from 10 to 2000 ng/ml for the high range and 12.5 to 250 ng/ml for the low range. For urine, the standard curves were linear from 1 to 40 μg/ml for the high range and 50 to 1000 ng/ml for the low range. Quality control samples were prepared by adding an appropriate amount of standard to control plasma (urine) at the concentrations close to the highest, middle and lowest standards. These spiked samples were stored frozen and processed with clinical samples on each day of analysis. The concentrations of the quality control samples were 25, 125, 250 and 1000 ng/ml for plasma and 0.125, 0.5, 2.5, 10 and 20 μg/ml for urine.

Preparation of standards

Frozen blank control plasma was thawed at room temperature. For high concentrations, 0.5 ml of plasma or 50 μl of urine was taken and 50 μl of standard working solutions (in 0.1 *M* phosphoric acid) were added. A 200-μl volume of 1 *M* sodium hydroxide solution was added to the samples. Hexane–methyl *tert.*-butyl ether (20:80, v/v) (5 ml) was added. The mixture was vortex-mixed for 2 min and allowed to stand to separate into two phases. The aqueous layer was frozen in a dry ice–acetone bath. The organic layer was transferred to 15-ml screw-cap conical tubes and was washed twice with 250 μl of 1 *M* sodium hydroxide. To the washed organic layer, 500 μl of 0.1 *M* phosphoric acid were added for back-extraction. The mixture was vortex-mixed and allowed to stand 5 min to separate. The organic layer was discarded by aspiration. The small residual organic phase was removed by evaporation under a gently stream of nitrogen. The aque-

ous extract was transferred to an autosampler vial and 50 μl were injected directly onto the HPLC column for analysis. For low concentration curves, 2 ml of plasma or 1 ml of urine was taken instead, vortex-mixed at a lower speed to prevent emulsion and then processed as described.

Patient sample preparation

The patient samples were treated in a similar manner by adding 50 μl of 0.1 *M* phosphoric acid and were processed as described under *Preparation of standards.*

RESULTS AND DISCUSSION

Selectivity

The selectivity of this assay for both plasma and urine was confirmed by running pre-dose and control blank samples. Remoxipride and I.S. can be detected in plasma and urine, respectively, with minimal interference from endogenous compounds. Typical chromatograms for remoxipride and the I.S. in plasma and urine are shown in Figs. 2 and 3. The retention times for remoxipride and the I.S. were 2.8 and 4.5 min, respectively, for plasma samples and 1.8 and 2.6 min, respectively, for urine samples.

Linearity

The small percentage differences between nominal and found concentrations of the standards in the standard curves (Table I) showed that the assay was linear over the concentration ranges investigated.

Precision

The precision of the described procedures for plasma and urine was assessed by calculating the intra-day variation for each point on the standard lines. Intra-day coefficients of variation (C.V.) were all $\leq 10\%$ (Table I).

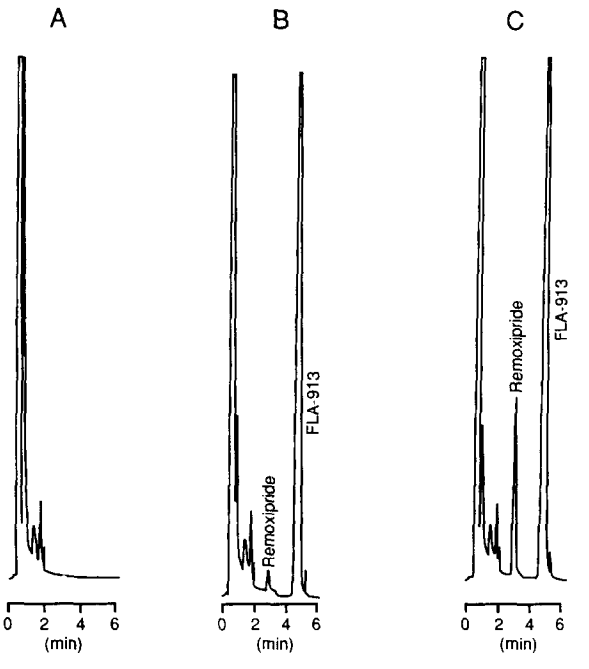

Fig. 2. Typical chromatogram for remoxipride in plasma. (A) Blank; (B) plasma containing 12.5 ng/ml remoxipride; (C) patient plasma containing 78.7 ng/ml remoxipride.

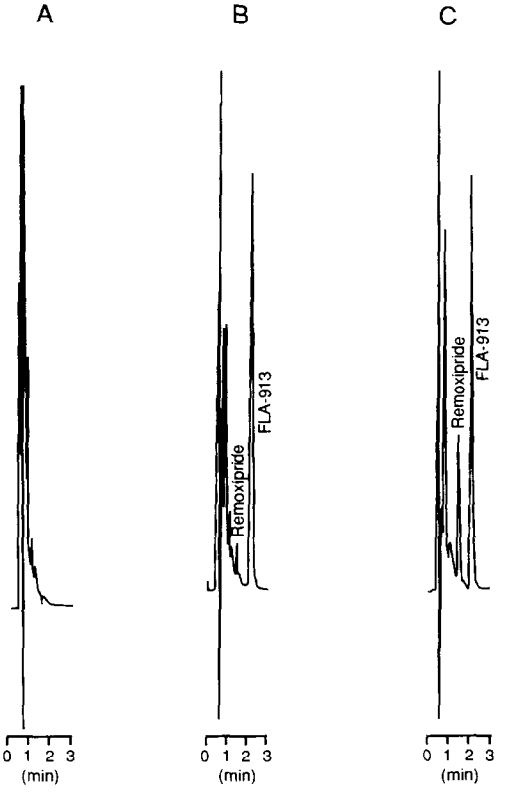

Fig. 3. Typical chromatogram for remoxipride in urine. (A) Blank; (B) urine containing 50 ng/ml remoxipride; (C) patient urine containing 238.4 ng/ml remoxipride.

TABLE I

INTRA-DAY PRECISION OF PLASMA AND URINE STANDARD CURVES

Peak-height ratios used to calculate C.V. (n = 5).

Plasma						Urine					
High concentration range			Low concentration range			High concentration range			Low concentration range		
Nominal (ng/ml)	Found (ng/ml)	C.V. (%)	Nominal (ng/ml)	Found (ng/ml)	C.V. (%)	Nominal (μg/ml)	Found (μg/ml)	C.V. (%)	Nominal (ng/ml)	Found (ng/ml)	C.V. (%)
100	104.1	8.1	12.5	13.1	9.7	1	1.1	8.3	50	53.8	10.4
250	253.0	4.7	25	24.9	0.6	2.5	2.5	8.0	125	124.9	6.1
500	485.6	5.4	62.5	61.6	3.7	5	5.1	3.0	250	245.0	0.6
1000	1008.9	1.7	125	125.2	1.7	10	9.9	1.5	500	500.5	1.2
2000	1998.4	5.2	250	250.1	1.8	20	19.8	3.1	1000	1000.7	0.1
						40	40.1	1.9			
Linear regression line, $1/x$ weighting, all data:											
$y = 1601.2x + 13.3$			$y = 381.9x + 2.6$			$y = 14.8x + 0.1$			$y = 762.6x + 16.2$		
$r^2 = 0.99987$			$r^2 = 0.99997$			$r^2 = 0.99992$			$r^2 = 0.99993$		

Accuracy

The accuracy of this method was established by preparing quality control samples for both plasma and urine. These samples were frozen prior to the analysis. On any given day the standard and quality control samples were run together with clinical samples. The data summarized in Table II show less than 6% variation from nominal values.

Recovery

The extraction recovery of remoxipride from plasma and urine was checked by spiking the appropriate working standards into the corresponding acid and acetonitrile volumes to give a direct standard line as control. The results of extracted plasma and urine standards were compared with those of the direct standards. The recovery was 80% for urine and 75% for plasma within the concentration range (Table III).

TABLE II

INTER-DAY ACCURACY OF PLASMA AND URINE QUALITY CONTROLS

Plasma				Urine			
Nominal concentration (ng/ml)	Found concentration (ng/ml	C.V. (%)	n	Nominal concentration (μg/ml)	Found concentration (μg/ml)	C.V. (%)	n
25	26.6	2.7	2[a]	0.125	0.125	0.1	2[a]
125	123.7	1.1	2[a]	0.5	0.495	0.5	2[a]
250	246.7	4.4	4[b]	2.5	2.6	3.6	3[b]
1000	1020.7	4.2	4[b]	10	9.8	3.2	3[b]
				20	19.2	2.8	3[b]

[a] For low concentration range over separate days.

[b] For high concentration range over separate days.

TABLE III

EXTRACTION RECOVERIES OF THE STANDARDS

n = 3 at each concentration.

Plasma		Urine	
Nominal concentration (ng/ml)	Recovery (%)	Nominal concentration (ng/ml)	Recovery (%)
25	80.6	25	84
50	73.6	50	84
125	73.6	125	82
250	75.1	250	84
500	73.8	500	81

Limit of quantification

The limit of quantification was defined as the lowest concentrations of remoxipride (n = 5) where the peak-height ratio had a C.V. $\leq$10%. The limit of quantification of remoxipride was 12.5 and 50 ng/ml in plasma and urine, respectively.

Analysis of clinical samples

The method described here has been effectively demonstrated for its reproducibility and accuracy by analyzing plasma and urine samples originated from a bioavailability study in which healthy volunteers were given a single oral 75-mg dose of remoxipride as formulations A and B. Representative plasma profiles for one subject are shown in Fig. 4. The amounts of remoxipride excreted in urine (collected over a 48-h period) in the same subject was 14.7 and 17.1 mg for formulation A and formulation B, respectively.

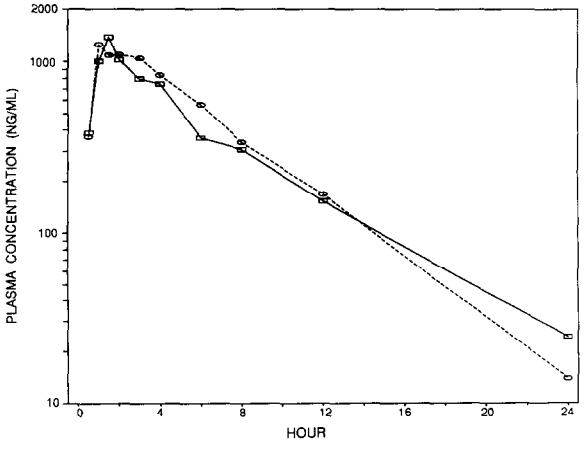

Fig. 4. Plasma concentrations of remoxipride following the administration of 75 mg of oral formulations A (□) and B (○) in a healthy subject.

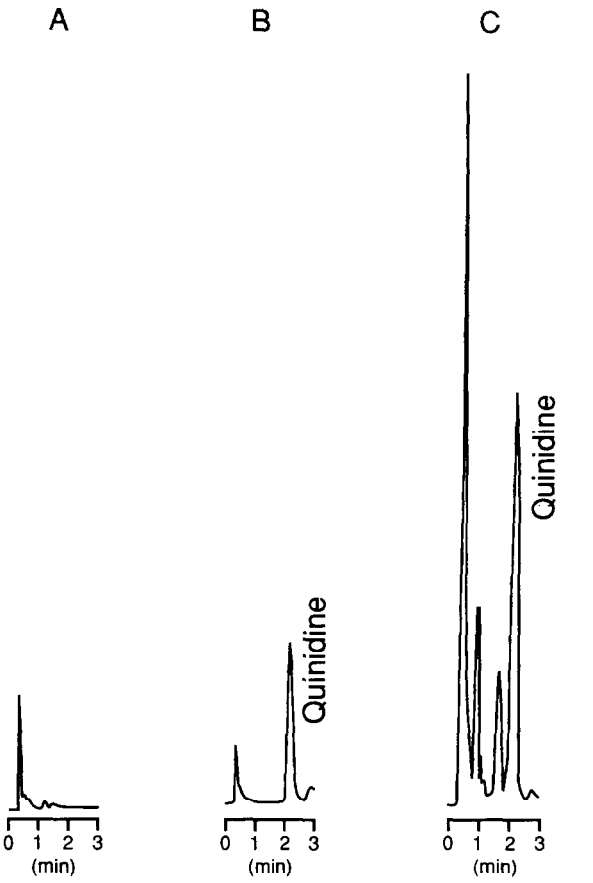

Fig. 5. Typical chromatogram for quinidine in plasma. (A) Blank; (B) plasma containing 0.5 μg/ml quinidine; (C) patient plasma containing 1.4 μg/ml quinidine. Fluorescence detector excitation and emission wavelengths were 340 and 418 nm, respectively.

Application to the analysis of other basic drugs with the same mobile phase

The composition of this particular mobile phase (sodium perchlorate and phosphoric acid

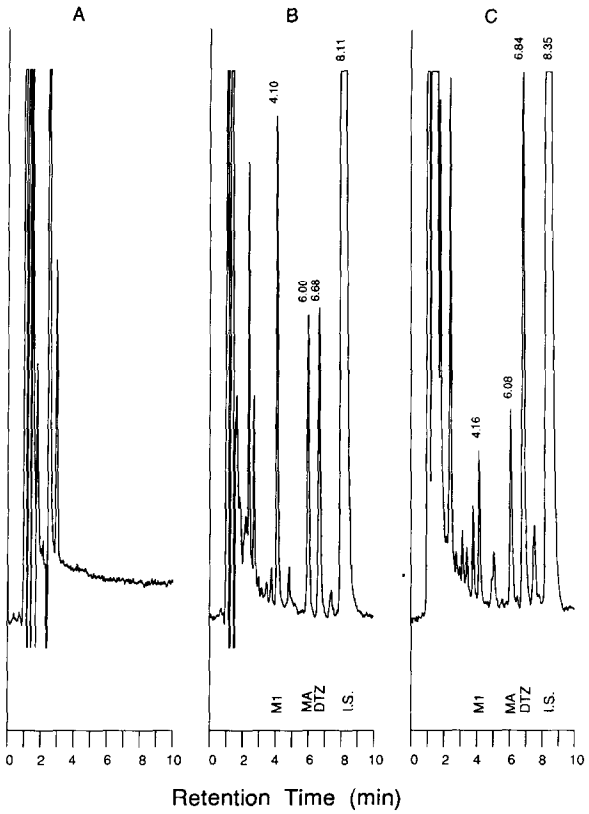

Fig. 6. Typical chromatogram for diltiazem in plasma. (A) Blank; (B) plasma containing 20 ng/ml desacetyldiltiazem (M1), N-monodemethyldiltiazem (MA) and diltiazem (DTZ); (C) patient plasma sample containing 6.4 ng/ml M1, 13.6 ng/ml MA and 56.8 ng/ml DTZ. UV wavelength set at 237 nm.

buffer) has been utilized previously for the analysis of several other basic compounds. By using the same analytical column, codeine [6], cimetidine [7] and quinidine were quantified with minimal interferences from endogenous components. Plasma quinidine was detected fluorometrically following HPLC separation. The mobile phase composition was acetonitrile–aqueous perchlorate buffer (20:80) (Fig. 5). The flow-rate was 1 ml/min and the column temperature was 60°C. The same mobile phase also has been applied to the analysis of diltiazem and its metabolites (Fig. 6). The column used was a C_6 column (100 mm × 4.6 mm I.D., 3 μm particle size, ES Industries, Marlton, NJ, USA) and the mobile phase composition was acetonitrile–aqueous perchlorate buffer (55:45). Diltiazem and its metabolites were monitored by UV detection at 237 nm. The details of the diltiazem method will be published elsewhere.

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