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Stereoselective determination of R-(+)- and S-(−)-remoxipride, a dopamine D_2-receptor antagonist, in human plasma by chiral high-performance liquid chromatography

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Abstract

A stereoselective high-performance liquid chromatographic (HPLC) method is described for the selective and sensitive quantitation in human plasma of R-(+)- and S-(−)-enantiomers of remoxipride. Remoxipride was extracted from basified plasma into hexane–methyl-*tert.*-butyl ether (20:80, v/v), washed with sodium hydroxide (1.0 M), then back-extracted into phosphoric acid (0.1 M). A structural analog of remoxipride was used as an internal standard. The sample extracts were chromatographed using a silica-based derivatized cellulose chiral column, Chiralcel OD-R, and a reversed-phase eluent containing 30–32% acetonitrile in 0.1 M potassium hexafluorophosphate. Ultraviolet (UV) absorbance detection was performed at 214 nm. Using 0.5-ml plasma aliquots, the method was validated in the concentration range 0.02–2.0 μg/ml and was applied in the investigation of systemic inversion of remoxipride enantiomers in man. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Remoxipride

1. Introduction

Remoxipride, S-(−)-3-bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl)-2,6-dimethoxybenzamide (Fig. 1), is an antipsychotic agent [1] that has demonstrated stereoselective affinity for central dopamine D_2-receptors [2,3]. Extensive pharmacokinetic studies have been conducted in human subjects [4–6] with remoxipride administered as the S-(−) optical isomer, and a number of its metabolites in plasma and urine have been characterized [7,8]. Remoxipride metabolites in man have been identified as optical isomers of the S-(−) configuration [9] or as achiral compounds [10]. There are no reports of human systemic bioconversion of administered S-(−)-remoxipride to R-(+)-remoxipride in the scientific literature.

Non-stereoselective high-performance liquid chromatographic (HPLC) methods for the analysis of remoxipride and its metabolites in human fluids have been reported [11–13]. A few chiral HPLC methods for the separation of remoxipride enantiomers for enantiomeric purity determinations have been documented [14,15], as well as a brief description of a chiral HPLC assay for R-(+)-remoxipride in human urine [7]; however, no direct chiral HPLC methods

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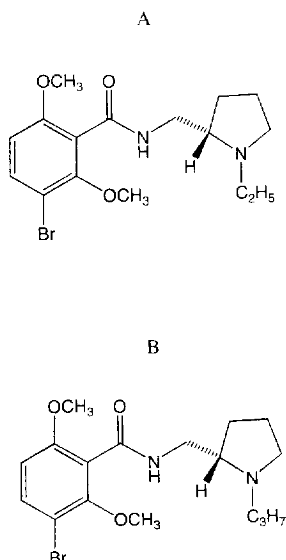

Fig. 1. Chemical structures of (A) remoxipride and (B) internal standard (I.S.).

for the determination of both enantiomers in human plasma have been reported.

Pharmacological studies have shown that enantiomers of many drugs differ in activity [16] as well as in metabolism [17,18]. Bioconversion of optically active isomers to their respective enantiomers has been documented for ibuprofen and other 2-arylpropionic acids [16,17,19]. Clinical evidence in human patients has demonstrated that the therapeutic effect of antipsychotic drugs is directly related to dopamine D_2-receptor affinity [20]. The binding of remoxipride to dopamine D_2-receptors is selective for the *S*-(−) configuration [2]. A study conducted in Sprague–Dawley rats demonstrated that most of the pharmacological activity (blockade of apomorphine-induced hyperactivity) resided in the *S*-(−) enantiomer; not only was *R*-(+)-remoxipride relatively inactive, but it showed a higher toxicity than *S*-(−)-remoxipride [21]. Therefore, it is important to evaluate the occurrence and extent of human bioconversion of *S*-(−)-remoxipride to the inactive *R*-(+) enantiomer.

This paper describes a direct stereoselective method for the determination of remoxipride enantiomers in human plasma using reversed-phase chromatography with a Chiralcel OD-R HPLC column and UV detection. The method was developed in order to investigate the systemic inversion of administered *S*-(−)-remoxipride to *R*-(+)-remoxipride in healthy male volunteers.

2. Experimental

2.1. Chemicals and reagents

S-(−)-Remoxipride was obtained in-house (Merck Research Laboratories, Rahway, NJ, USA). *R*-(+)-Remoxipride hydrochloride monohydrate and the racemic internal standard, (±)3-bromo-N-[(1-propyl-2-pyrrolidinyl) methyl)-2,6-dimethoxybenzamide (I) were obtained from Astra Research Centre (Södertälje, Sweden). The synthetic route for the preparation of optical isomers of remoxipride has been documented [21].

Drug-free heparinized human plasma was purchased from SeraTec Biologicals (New Brunswick, NJ, USA). Potassium hexafluorophosphate was obtained from Aldrich (Milwaukee, WI, USA). HPLC-grade solvents (acetonitrile, methyl-*tert*.-butyl ether and *n*-hexane), phosphoric acid and water were purchased from Fisher Scientific (Pittsburgh, PA, USA). Sodium hydroxide pellets were obtained from Mallincrodt (Paris, KY, USA). All chemicals and reagents were used as received.

2.2. Instrumentation

The HPLC system consisted of a Varian (Sugar Land, TX, USA) Model 5500 pump and a Model 9090 autosampler equipped with a 200-μl sample loop and an Applied Biosystems (Foster City, CA, USA) 783A absorbance detector. The detector was interfaced with a PE Nelson (Cupertino, CA, USA) 900 series A/D for data acquisition and processing using a PE Nelson Access*Chrom gas chromatography–liquid chromatography (GC–LC) data system.

2.3. Chromatographic conditions

The mobile phase consisted of 30–32% acetonitrile in 0.1 *M* potassium hexafluorophosphate, pH 3.5, filtered through a 0.20-μm nylon filter prior to use. The flow-rate of the mobile phase was 0.58–0.70 ml/min through a Chiralcel OD-R analytical

column, 5 μm particle size, 250×4.6 mm I.D. (Chiral Technologies, Exton, PA, USA). The composition and flow-rate of the mobile phase were optimized for each different column. Preceding the analytical column was an in-line prefilter containing a 0.5-μm stainless steel frit (Keystone Scientific, Bellefonte, PA, USA). Ultraviolet (UV) detection was conducted at 214 nm, 0.002 AUFS. Chromatography was performed at ambient temperature using a 100-μl injection volume.

2.4. Preparation of standards

A stock solution of 0.1 mg/ml *R*-(+)-remoxipride, as the free base, was prepared by dissolving and diluting 1.14 mg of the hydrochloride monohydrate reference material in 0.1 *M* phosphoric acid in a 10-ml volumetric flask. A separate stock solution of *S*-(−)-remoxipride was similarly prepared using 1.0 mg of the free base reference material. Separate 20.0 μg/ml *R*-(+) and *S*-(−)-remoxipride working standard solutions were prepared by diluting 5.0 ml of the 0.1 mg/ml stock solutions to 25 ml with 0.1 *M* phosphoric acid.

Working standards of 10.0, 5.0 and 2.5 μg/ml *R*-(+)-remoxipride were prepared by diluting the 20.0 μg/ml working standard with 0.1 *M* phosphoric acid. The 10.0 μg/ml *R*-(+)-remoxipride working standard was diluted with 0.1 *M* phosphoric acid to yield 1.0, 0.5 and 0.2 μg/ml working standards. Separate *S*-(−)-remoxipride working standards were prepared in a similar manner using the 20.0 and 10.0 μg/ml *S*-(−)-remoxipride working standards. Working standards were found to be stable for six weeks at 5°C.

Internal standard stock solution was prepared by dissolving and diluting 1.0 mg of I in 0.1 *M* phosphoric acid in a 10-ml volumetric flask. A 5.0-ml aliquot of the internal standard stock solution was then diluted to 100 ml with 0.1 *M* phosphoric acid to yield a 5 μg/ml working internal standard solution (I.S.).

Plasma standards were prepared by combining 50 μl each of equivalent *R*-(+) and *S*-(−)-remoxipride working standards in 0.5 ml of drug-free human plasma. The concentration range for the resulting standard curve was 0.02 to 2.0 μg/ml.

Unextracted standards were prepared by combining 50 μl each of equivalent *R*-(+)- and *S*-(−)-remoxipride working standards with 200 μl of I.S. and diluting with 200 μl of 0.1 *M* phosphoric acid. Linear regression analysis using peak heights (external standard quantitation) was performed to calculate the concentrations of *R*-(+)- and *S*-(−)-remoxipride recovered from extracted plasma standards versus the corresponding component in the unextracted standard curve.

2.5. Plasma sample preparation and extraction

Liquid–liquid extraction of remoxipride from plasma was based on the sample preparation method used for a non-stereoselective HPLC assay for remoxipride [12].

To 0.5 ml of plasma (sample or standard) in 150×16 mm glass culture tubes were added 200 μl of the I.S., 50 μl of 0.1 *M* phosphoric acid, 250 μl of 1.0 *M* sodium hydroxide and 5 ml of *n*-hexane–methyl-*tert.*-butyl ether (20:80, v/v). Samples were vortex-mixed for 2 min, and the phases separated on standing. The aqueous layer was frozen in an acetone/dry ice bath and the organic layer was decanted into a clean culture tube containing 250 μl of 1.0 *M* sodium hydroxide. The sample was vortex-mixed again for 2 min and the organic phase was separated. The sodium hydroxide wash procedure was repeated once.

The base-washed organic phase was transferred to a clean culture tube and 500 μl of 0.1 *M* phosphoric acid were added for back-extraction. The sample was vortex-mixed, the layers were separated and the aqueous phase was frozen as before. The organic layer was aspirated to waste. The frozen aqueous layer was washed once by vortex-mixing it with 2 ml of *n*-hexane–methyl-*tert.*-butyl ether (20:80, v/v) for about 10 s; the organic wash solvent was then discarded. Residual *n*-hexane–methyl-*tert.*-butyl ether was evaporated under a stream of nitrogen for 5 min in a water bath at ambient temperature. The extract was transferred to a borosilicate glass autosampler vial for injection into the HPLC system.

2.6. Clinical study design

An open, randomized, five-period crossover study was conducted in fifteen healthy male volunteers to

determine the bioequivalence of 25 and 75 mg remoxipride tablets and capsules and of a 75-mg/ml oral concentrate from two different manufacturers. Only *S*-(−)-remoxipride was administered as single oral doses at weekly intervals. Frequent blood samples were taken by the heparin lock technique for 24 h following each dose. Plasma was harvested after centrifugation and stored at −20°C until assay.

All plasma samples from the study were initially assayed for remoxipride using a non-stereoselective HPLC method [12] to provide data in support of the clinical study. The stereoselective HPLC method was developed and used to analyze both remoxipride enantiomers in selected plasma samples from the study.

3. Results

3.1. Assay specificity

Representative chromatograms of extracts of drug-free human plasma and of a plasma standard containing 0.5 μg/ml of each remoxipride enantiomer are shown in Fig. 2. Comparison of Fig. 2A and Fig. 2B illustrates that no endogenous peaks elute near the retention times of *R*-(+)-remoxipride (t_R=22.6 min), *S*-(−)-remoxipride (t_R=24.1 min) or of the I.S. (t_R=40.6 min). The specificity of the method was further confirmed by the analysis of pre-dose plasma samples from selected study subjects, which showed no interfering peaks.

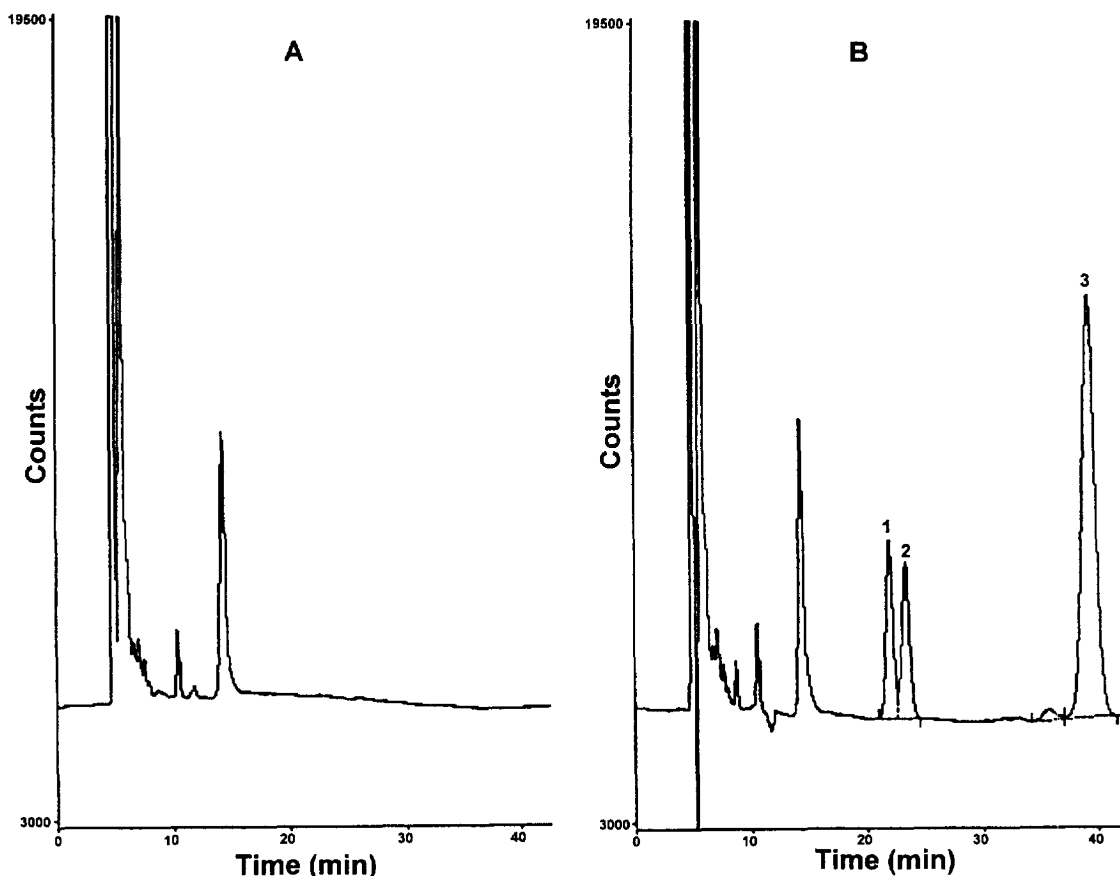

Fig. 2. Representative chromatograms of (A) control human plasma and (B) plasma standard containing 0.5 μg/ml each of *R*-(+)- and *S*-(−)-remoxipride and 2.0 μg/ml of the I.S.. Peaks: 1=*R*-(+)-remoxipride, 2=*S*-(−)-remoxipride and 3=I.S.

3.2. Linearity

Linear regression calibration curves based on seven data points were constructed for each remoxipride enantiomer by plotting the peak height ratio of the enantiomer to the I.S. versus the concentration of remoxipride in plasma standards. The curves were weighted $1/y$ where y=peak height ratio. A typical linear regression equation for *R*-(+)-remoxipride included a slope of 0.8736±0.0054 and a *y*-intercept of 0.0020±0.0012; for *S*-(−)-remoxipride, typical values for slope and *y*-intercept were 0.7517±0.0032 and 0.0009±0.0007, respectively. Correlation coefficients of >0.999 were typically obtained for both enantiomers over the concentration range of 0.02 to 2.0 μg/ml of plasma, demonstrating linearity over the entire standard range. The use of weighted least squares regression resulted in less than 15% deviation between the nominal standard concentration and the experimentally determined standard concentration calculated from the regression equation.

3.3. Extraction recovery

Recoveries of remoxipride enantiomers from plasma were determined by assaying replicate plasma standards (n=6 at each of seven different concentrations) versus an unextracted standard curve. The mean extraction recoveries of *R*-(+)-remoxipride and *S*-(−)-remoxipride were 88.1±10.2 and 85.0±8.6%, respectively, over the concentration range 0.02–2.0 μg/ml of both enantiomers in plasma. Similarly, the mean recovery for the I.S. at 2.0 μg/ml in plasma was 95.6±7.8% (n=64).

3.4. Assay precision and accuracy

Replicate standards (n=6) in human plasma were extracted and analyzed to assess the intra-day variability of the method. Accuracy and precision throughout the standard curve are summarized in Table 1 for both remoxipride enantiomers. The greatest variation was observed at 0.02 μg/ml, the lowest concentration analyzed, where coefficients of variation (C.V.s) for *R*-(+)- and *S*-(−)-remoxipride were 10.6 and 10.4%, respectively. C.V.s at all other standard concentrations were <7%.

Quality control (QC) plasma samples containing 0.1 and 1.0 μg/ml of *R*-(+)- and *S*-(−)-remoxipride were prepared and frozen at −20°C in 0.75 ml aliquots for daily analysis to assess inter-day variability. An additional QC plasma sample was prepared by combining 0.02 μg/ml of *R*-(+)-remoxipride with 2.0 μg/ml of *S*-(−)-remoxipride, representing a 100-fold difference in concentration of *R*-(+)- to *S*-(−)-remoxipride. This mixed concentration QC sample was used to assess accuracy and inter-day variability of a low concentration of *R*-(+)-

Table 1
Intra-day reproducibility data for remoxipride enantiomers in human plasma

Nominal concentration (μg/ml)	*R*-(+)-Remoxipride			*S*-(−)-Remoxipride		
	Mean[a] analyzed concentration (μg/ml)	Accuracy[b] (%)	C.V.[c] (%)	Mean[a] analyzed concentration (μg/ml)	Accuracy[b] (%)	C.V.[c] (%)
0.02	0.020	100.0	10.6	0.019	96.7	10.4
0.05	0.050	99.0	6.9	0.052	103.0	3.4
0.10	0.110	109.7	3.9	0.105	104.8	2.7
0.25	0.276	110.5	2.4	0.264	105.5	0.7
0.50	0.511	102.1	3.4	0.501	100.1	4.6
1.0	1.01	100.9	2.6	1.00	100.0	2.7
2.0	1.93	96.5	6.4	1.94	96.8	6.3
Mean±S.D.		102.7±5.4			101.0±3.6	

[a] n=6.
[b] Calculated as (mean analyzed concentration/nominal concentration)×100.
[c] Coefficient of variation, based on analyzed concentrations.

Table 2
Inter-day variability for quality control plasma samples containing *R*-(+)- and *S*-(−)-remoxipride

R-(+)-Remoxipride			*S*-(−)-Remoxipride		
Nominal concentration (μg/ml)	Mean[a] analyzed concentration (μg/ml)	C.V.[b] (%)	Nominal concentration (μg/ml)	Mean[a] analyzed concentration (μg/ml)	C.V.[b] (%)
0.1	0.104	4.4	0.1	0.096	2.9
1.0	1.04	2.8	1.0	0.981	2.8
0.02	0.022	5.0	2.0	1.99	1.2

[a] $n=8$.
[b] Coefficient of variation, based on analyzed concentrations.

remoxipride in the presence of a high concentration of *S*-(−)-remoxipride. The results, given in Table 2, indicate the low inter-day variability of the method, with C.V.s of 5% or lower for both enantiomers over all QC concentrations.

3.5. Limits of detection and quantitation

The lower limit of quantitation (LLOQ) of the assay was 0.02 μg/ml for both *R*-(+)- and *S*-(−)-remoxipride. Fig. 3 illustrates that in the presence of

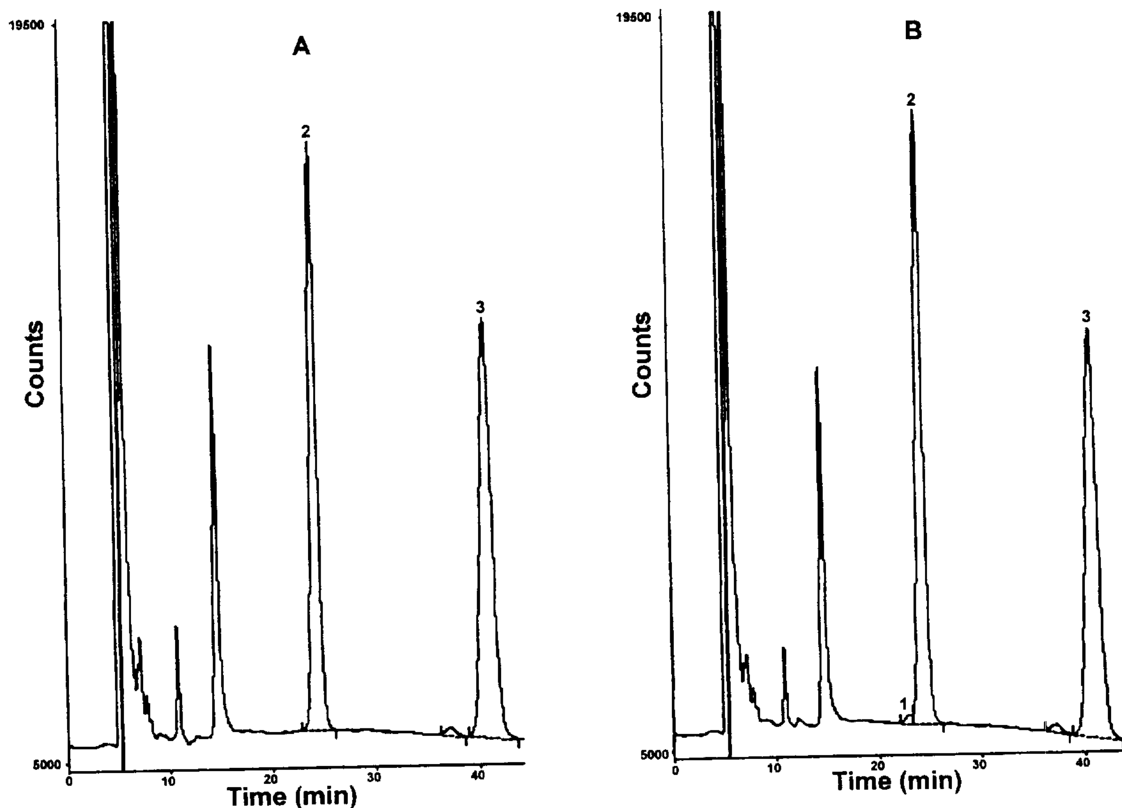

Fig. 3. Representative chromatograms of (A) plasma containing 2.0 μg/ml *S*-(−)-remoxipride and (B) plasma containing 2.0 μg/ml *S*-(−)-remoxipride and 0.02 μg/ml (1.0%, w/w) *R*-(+)-remoxipride (both samples also contained 2.0 μg/ml I.S.). Peaks: 1=*R*-(+)-remoxipride, 2=*S*-(−)-remoxipride and 3=I.S.

a high concentration of S-(−)-remoxipride, the R-(+) enantiomer was readily detected at 0.02 μg/ml, a concentration representing only 1.0% (w/w) of that of the S-(−) enantiomer. Inter-day analysis of mixed concentration QC plasma samples confirmed accurate quantitation of 0.02 μg/ml R-(+)-remoxipride in the presence of 2.0 μg/ml S-(−)-remoxipride (Table 2).

The lower limit of detection (LLOD) of R-(+)-remoxipride was slightly lower than the LLOQ. Concentrations of 0.01 μg/ml R-(+)-remoxipride were detected (signal-to-noise ratio=3) in plasma containing 2.0 μg/ml S-(−)-remoxipride; however, the accuracy of the assay for R-(+)-remoxipride was poor. For S-(−)-remoxipride, the LLOD and LLOQ were equivalent.

3.6. Stability in plasma

Storage stability of both remoxipride enantiomers in human plasma was evaluated by analysis, in duplicate, of QC plasma samples during repetitive cycles of freezing and thawing. QC samples were frozen at −20°C in 10 ml aliquots and were thawed and assayed on four different days. Mean values for the four freeze–thaw cycles ranged from 95.1 to 99.6% for R-(+)-remoxipride, and from 95.7 to 96.9% for S-(−)-remoxipride, confirming stability in plasma for both enantiomers at three different concentrations. The highest C.V. (13.1%) was obtained for 0.02 μg/ml R-(+)-remoxipride in combination with 2.0 μg/ml S-(−)-remoxipride. All other C.V.s were 7.2% or lower.

3.7. Bioinversion

Plasma samples from subjects containing various concentrations of S-(−)-remoxipride, based on a non-stereoselective HPLC determination [12], were chosen for stereoselective assay. For some subjects, samples taken near the beginning and the end of the 12 h dosing interval were analyzed.

Typical chromatograms of pre-dose and post-dose plasma samples are given in Fig. 4. As illustrated in Fig. 4B, no R-(+)-remoxipride was detected (LLOD=0.01 μg/ml) in a subject sample containing 1.67 μg/ml S-(−)-remoxipride.

Fig. 5 illustrates a representative profile of plasma concentrations of S-(−)-remoxipride following a single 75 mg oral dose of S-(−)-remoxipride. R-(+)-Remoxipride was not detected in any of the samples assayed stereoselectively, indicating that no bioinversion of S-(−)-remoxipride to the R-(+)-enantiomer following oral administration of 75 mg doses of S-(−)-remoxipride had occurred.

The plot in Fig. 5 includes data for the same samples assayed non-stereoselectively. Agreement between plasma concentrations of S-(−)-remoxipride obtained using the stereoselective method and those of remoxipride determined non-stereoselectively was greater than 96% for all samples from the subject profiled.

4. Discussion

The primary objective of this study was to evaluate the occurrence and extent of bioconversion of S-(−)-remoxipride to its R-(+) enantiomer by detecting and quantitating low plasma levels of the R-(+) enantiomer in the presence of high concentrations of S-(−)-remoxipride. A sensitive and reliable non-stereoselective HPLC method existed for the determination of remoxipride in human plasma [12]. Starting with the same liquid–liquid extraction procedure, a stereoselective assay was developed using a Chiralcel OD-R column, a novel stationary phase designed for the separation of basic and acidic chiral compounds, using a mobile phase similar to that of the non-stereoselective assay.

Different eluents and gradients were evaluated in order to maximize resolution between the remoxipride enantiomers. Resolution factors, calculated using peak widths at half height [22], ranged from 0.4 to 0.9 for all of the chromatographic conditions investigated. Acceptable separation was accomplished by combining acetonitrile with potassium hexafluorophosphate (a strong anion) under isocratic conditions to induce ion-pair chromatography. The resulting peaks were sharp and tailing was minimized, possibly due to reduction of secondary interactions between the analytes and the stationary phase. The improved peak shape increased resolution between enantiomers and enhanced the sensitivity of the method for R-(+)-remoxipride. It should be noted that although the internal standard is racemic,

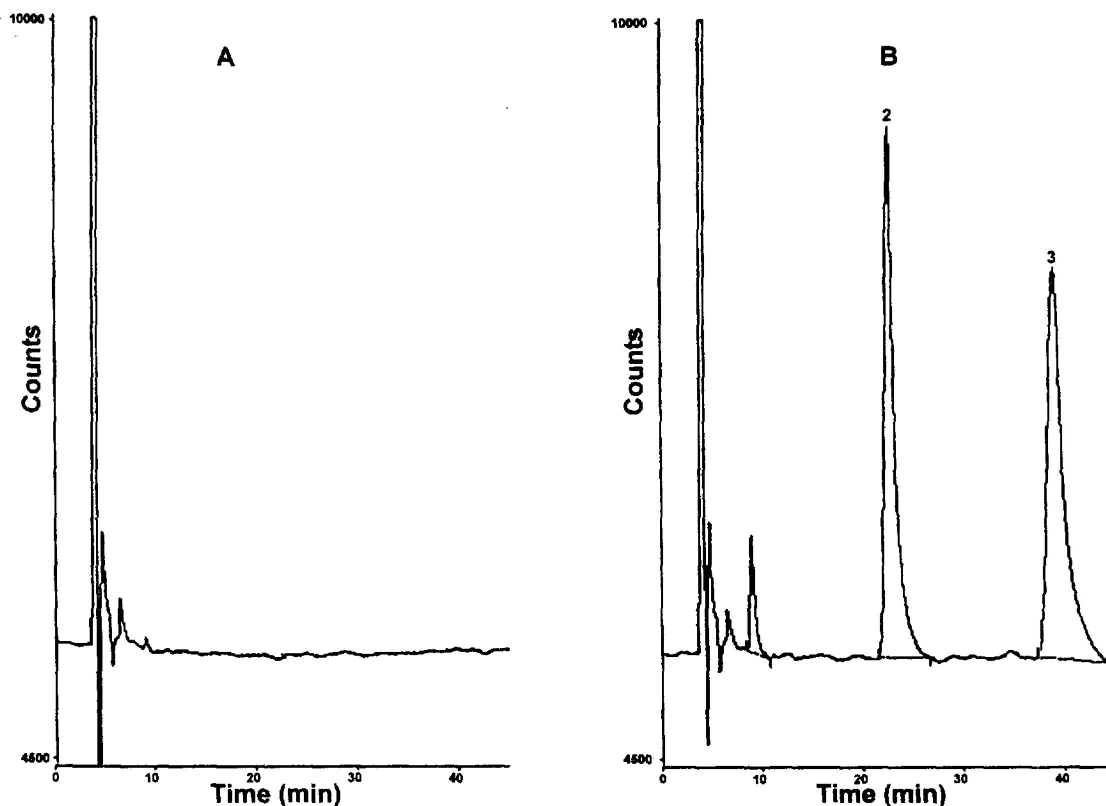

Fig. 4. Representative chromatograms of (A) a pre-dose plasma sample from a subject and (B) a plasma sample obtained from a subject 1 h after oral administration of 75 mg of *S*-(−)-remoxipride. Peaks: 2=*S*-(−)-remoxipride and 3=I.S. The concentration of *S*-(−)-remoxipride in the subject's sample was equivalent to 1.67 μg/ml; no *R*-(+)-remoxipride was detected (LLOD=0.01 μg/ml).

no separation between its enantiomers was observed under the described chromatographic conditions.

The Chiralcel OD-R column required equilibration with mobile phase and drug prior to routine use. The first injection of unextracted standard, used as a system suitability test solution, yielded misshapen peaks that were poorly resolved. The column was conditioned daily with duplicate injections of the highest unextracted standard prior to sample analysis. All subsequent chromatograms were highly consistent in terms of peak retention, shape and resolution.

The Chiralcel OD-R column used in the development of the stereoselective assay was stored at ambient temperature in 100% methanol for more than two years before being used again. After storage, minimal effort was required to obtain the same retention times, peak response and resolution as obtained previously during method development. This demonstrates the remarkable stability of the chiral stationary phase with minimal care.

The bioconversion of *S*-(−)-remoxipride in man has been addressed briefly in the literature. Widman et al. [7] described a normal-phase chiral HPLC method, which was used to analyze urine from excretion experiments in rats, dogs and man. As in the work presented here, *R*-(+)-remoxipride was not found. Both studies provide conclusive evidence that *in-vivo* inversion of remoxipride does not occur to any appreciable extent.

Since no *R*-(+)-remoxipride was found, plasma concentrations of *S*-(−)-remoxipride in subjects' samples that were assayed stereoselectively should be comparable to those for remoxipride obtained using the non-stereoselective method. Linear regression analysis comparing stereoselective and non-

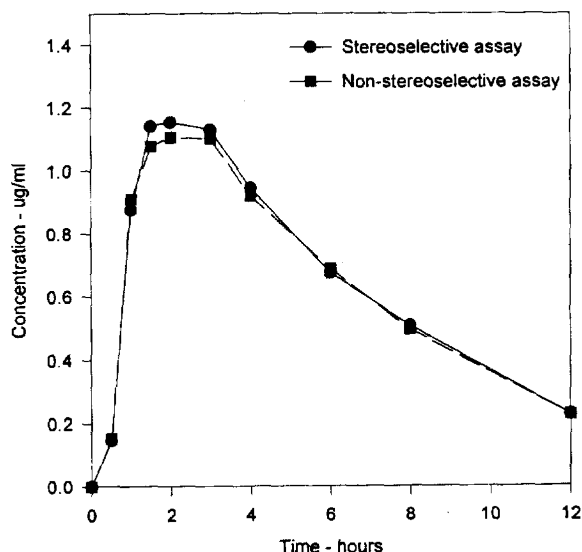

Fig. 5. Plasma profile of *S*-(−)-remoxipride for Subject 14 after oral administration of 75 mg of *S*-(−)-remoxipride; no *R*-(+)-remoxipride was detected (LLOD=0.01 μg/ml) in any sample assayed stereoselectively.

stereoselective data yielded a slope of 0.9620, a *y*-intercept of 0.0123 and a correlation coefficient of 0.9956, demonstrating that the concentrations obtained with both methods were equivalent.

Acknowledgments

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References

[1] A.N. Wadworth, R.C. Heel, Drugs 40 (1990) 863.

[2] C. Köhler, A.C. Radesäter, G. Karlsson-Boethius, B. Bryske, M. Widman, J. Neural Transm. (GenSect) 87 (1992) 49.

[3] L. Farde, C. Von Bahr, Acta Psychiatr. Scand. 82(Suppl. 358) (1990) 67.

[4] M. Grind, M.-I. Nilsson, L. Nilsson, G. Oxenstierna, G. Sedvall, A. Wahlen, Psychopharmacology (Berlin) 98 (1989) 304.

[5] D.J. King, N. Devaney, S.J. Cooper, M. Blomqvist, M.J. Mitchell, J. Psychopharmacol. 4 (1990) 83.

[6] G. Movin-Osswald, M. Hammarlund-Udenaes, Br. J. Clin. Pharmacol. 32 (1991) 355.

[7] M. Widman, L.B. Nilsson, B. Bryske, J. Lundström, Arzneim.-Forsch./Drug Res. 43(3) (1993) 287.

[8] G. Movin-Osswald, J. Boelaert, M. Hammarlund-Udenaes, L.B. Nilsson, Br. J. Pharmacol. 35 (1993) 615.

[9] M. Widman, L.B. Nilsson, B. Bryske and J. Lundström, 12th Eur. Workshop Drug Metab., Basel, Switzerland, 1990, p. 197.

[10] L. Gawell, C.-E. Hagberg, T. Högberg, M. Widman, Acta Chem. Scand. 43 (1989) 476.

[11] L.B. Nilsson, J. Chromatogr. 526 (1990) 139.

[12] R.H.-Y. Chiou, Man-Wai Lo, J. Chromatogr. 581 (1992) 300.

[13] G.H. Gu, C.K. Lim, J. Chromatogr. 515 (1990) 183.

[14] D. Haupt, C. Pettersson, D. Westerlund, Chirality 5 (1993) 224.

[15] C. Pettersson, C. Gioeli, Chirality 5 (1993) 241.

[16] K. Williams, E. Lee, Drugs 30 (1985) 333.

[17] N.P.E. Vermeulen, in A.F. Harms (Editor), Innovative Approaches in Drug Research, Elsevier, Amsterdam, 1986, p. 393.

[18] G. Mikus, M. Eichelbaum, C. Fischer, S. Gumulka, U. Klotz, H.K. Kroemer, J. Pharmacol. Exp. Ther. 253 (1990) 1042.

[19] D.R. Abernathy and N.S. Andrawis, in I.W. Wainer (Editor), Drug Stereochemistry: Analytical Methods and Pharmacology, Dekker, New York, 2nd ed., 1993, p. 386.

[20] L. Farde, F.-A. Wiesel, C. Halldin, G. Sedvall, Arch. Gen. Psychiatry 45 (1988) 71.

[21] L. Florvall, S.-O. Ögren, J. Med. Chem. 25 (1982) 1280.

[22] L.R. Snyder, J.L. Glajch and J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, 1988.