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Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column Application to pharmacokinetics

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Abstract

Amisulpride, a drug belonging to the benzamide series, demonstrates antischizophrenic and antidepressant (antidysthymic) properties in man. For the pharmacokinetic studies of the racemic drug in man, a method of determination based on solid-phase extraction (SPE) from plasma and HPLC on a stereoselective column was developed. For this aim, one millilitre of plasma, after the addition of the internal standard, tiapride or metoclopramide, is diluted with a borate buffer at pH 9, then automatically loaded onto a SPE C_{18} 100-mg column. The column is washed with different solvents, then eluted with 0.5 ml of methanol. After evaporation of the eluted fraction, the residue is reconstituted in 0.25 ml of eluent mixture. An aliquot is injected onto the HPLC column, a Chiralpak AS, equilibrated with an eluent mixture constituted by *n*-hexane–ethanol, (67:33, v/v) containing 0.2% (v/v) of diethylamine (DEA) or *n*-heptane–ethanol, (70:29.8, v/v) containing 0.2% of DEA and connected to a UV detector set at 280 nm or to a fluorimetric detector set at λ_{ex}=280 nm and λ_{em}=370 nm. The limit of quantitation (LOQ) in human plasma is 2.5 ng ml^{-1} for both *S*-(−)- and *R*-(+)-amisulpride isomers with both detection methods. The method has been demonstrated to be linear in the range 2.5–320 ng ml^{-1} for both *R*-(+)- and *S*-(−)-amisulpride in human plasma with both UV and fluorescence detection. Absolute recovery of *S*-(−)- and *R*-(+)-amisulpride enantiomers from human plasma, as well as selectivity, precision and accuracy have been demonstrated to be satisfactory for pharmacokinetics in man and equivalent for both the proposed methods that have been cross-validated on real dosed human plasma samples. The methods have been used for clinical pharmacokinetic studies allowing pharmacokinetic parameters for amisulpride enantiomers in agreement with those obtained for the racemate to be obtained. After dilution with water, urinary samples from subjects treated with amisulpride racemate can be analysed according to the method used for plasma.

Keywords: Enantiomer separation; Amisulpride; Benzamide derivative

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1. Introduction

In the last few years the interest in stereoselective separation of the enantiomers of synthetic drugs has grown continuously [1]. Several articles published in the past decade have strongly indicated that it is imprudent to blithely ignore the consequences of chirality in the metabolism, pharmacokinetics, pharmacodynamics, or toxicity of drugs administered as racemates [2–7].

The pharmacodynamic and pharmacokinetic properties of drug enantiomers have been known for many years, however, only recently have they been considered adequately by both the regulatory authorities and the pharmaceutical industry [8,9]. Such a strong interest in drug chirality has been stimulated and fed by recent progress in analytical methodology in the chromatographic area that has allowed the determination of the enantiomers of a racemic mixture in biological samples [10–13]. Amisulpride is an orthomethoxy benzamide compound with a chemical structure related to two well-known benzamide compounds: sulpiride and sultopride. The pharmacological activity of amisulpride is similar to that of sulpiride with, in addition, more pronounced bipolarity of action found in animals [14]. The compound contains one chiral center (Fig. 1).

A method for the determination of amisulpride optical isomers in human plasma has already been developed [15], based on the separation of the *R*-(+)- and *S*-(−)-enantiomers on a chiral AGP column. The separation is achieved only in the pH range 7.5–8.5, being improved at mildly alkaline conditions. However, under these conditions, the stability of the packing material is very crucial and the performance of the column decreases dramatically with use.

In addition, the inter-reproducibility of the AGP columns is low for such compounds under the reported conditions; when a new column replaces an exhausted one, it is necessary to modify the eluent composition in order to have acceptable retention times for amisulpride enantiomers and the internal standard. For all these reasons we have investigated other analytical possibilities. Here a new stereoselective HPLC method for the determination of the enantiomers of amisulpride is presented.

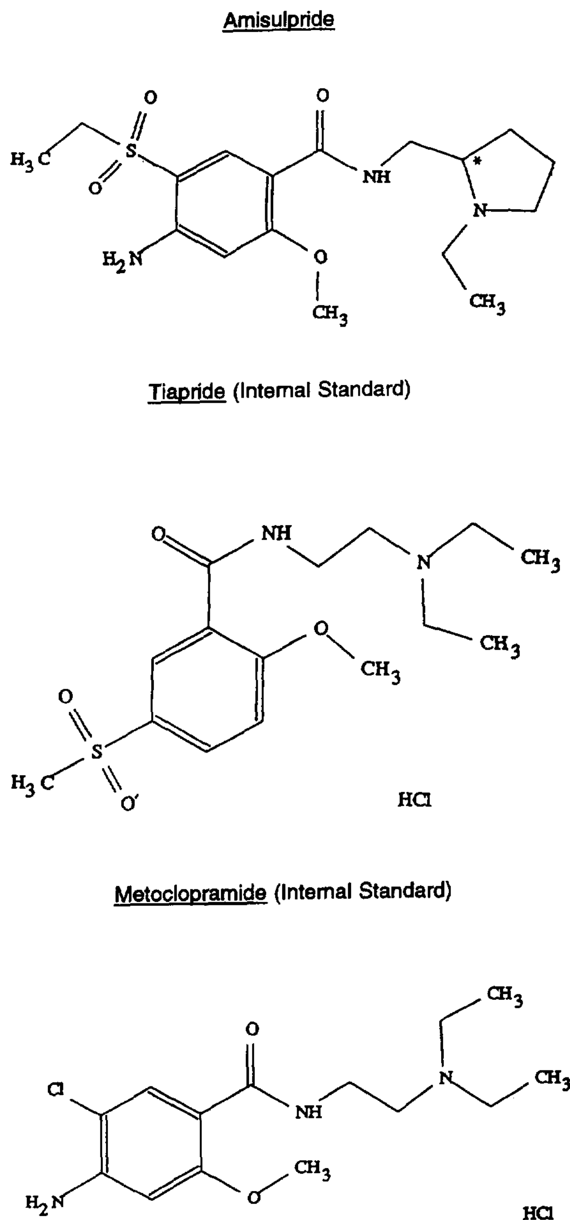

Fig. 1. Chemical structures of amisulpride and internal standards (tiapride and metoclopramide); * asymmetric carbon.

2. Experimental

2.1. Chemicals and reagents

Amisulpride racemate (as base): (*R,S*)-(±)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsul-

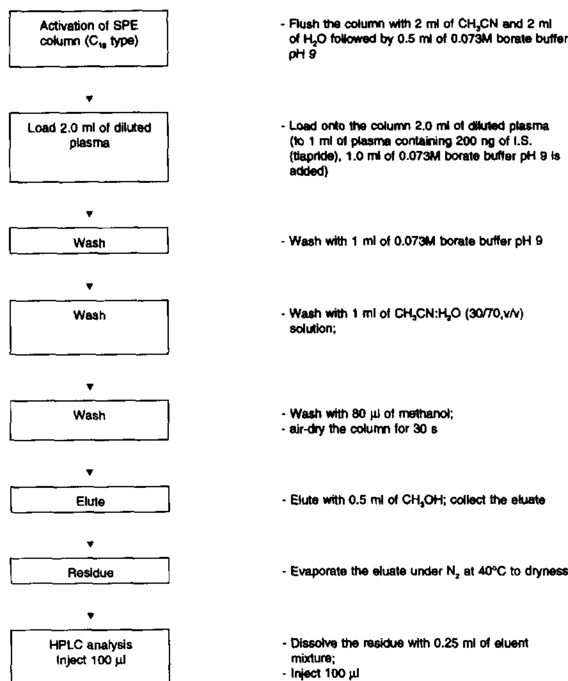

Fig. 2. Scheme of SPE procedure for amisulpride from human plasma.

phonyl-2-methoxybenzamide, tiapride: N-(diethylaminoethyl)-2-methoxy-5-(methylsulphonyl)benzamide, and metoclopramide: 4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxybenzamide (as hydrochloride), both used as internal standards, *R*-(+)- and *S*-(−)-amisulpride enantiomers (both as phosphate) were obtained from Synthélabo Recherche, Chemical Development Department, Paris (France) (see Fig. 1 for structures).

All reagents used were analytical grade or better and were purchased from Merck (Darmstadt, Germany), all solvents were LC grade and were purchased from Merck. Pure water was obtained from deionized water (Milli Ro plus) and next purified on a Milli Q4 system (Millipore, Bedford, MA, USA), 0.1 *M* sodium hydroxide solution was prepared from 58.01 g of pure sodium hydroxide that was dissoved and diluted to one litre with pure water to give 1 *M* solution, then 100 ml were diluted to one litre in order to obtain a 0.1 *M* solution.

Potassium dihydrogen phosphate (1 *M*) was prepared from 136.1 g of pure potassium dihydrogen phosphate and diluted to one litre with pure water.

The borate buffer (pH 9) 0.073 *M* was prepared from 6.18 g of boric acid and 7.46 g of potassium chloride, dissolved in one litre of pure water, then 500 ml of this solution were adjusted at pH 9 with about 185 ml of 0.1 *M* NaOH.

2.2. Standard solutions

The standard solutions of the amisulpride racemate were used for daily calibration; stock solutions (1 mg/ml) were prepared in methanol. Standard solutions were obtained from stock solutions by dilution with methanol (Table 1) and used for the preparation of plasma standards.

2.3. Chromatographic system

The chromatographic system consisted of a double piston pump Spectra Series 200 (Thermo Separation Product, San Jose, CA, USA), a UV detector model UV-975 (Jasco, Tokyo, Japan) set at 280 nm wavelength, an automatic sample injector Promis (Spark Holland, Emmen, Netherlands), an analytical column Chiralpak AS (amylose carbamate coated on silicagel), 25×0.46 cm I.D. (J.T. Baker, Deventer, Netherlands), a guard column, 2×0.46 cm I.D., filled with Pelliguard Si 40 μm (Supelco, Bellefonte, PA, USA) and an integrator

Model Chromjet SP 4400 (Thermo Separation Products). The eluent mixture was constituted by *n*-hexane and ethanol (67:33, v/v) containing 0.2%

Table 1
Methanolic standard solutions used for daily calibration

Standard solution	Amisulpride (racemate) (ng/20 μl)	Tiapride (I.S.) (ng/20 μl)
A	640	–
B	320	–
C	160	–
D	40	–
E	10	–
F	5	–
G	–	200

(v/v) of diethylamine (DEA), which was pumped through the column at flow-rate of 0.5 ml min^{-1}. Since the chromatographic separations performed with such an eluent are very sensitive to laboratory temperature variations, the mobile phase reservoir and the chromatographic column were thermostated at 28°C and 25°C, respectively, in order to guarantee the reproducibility of the retention times of the compounds of interest. The volume that was automatically injected was 0.1 ml. Under these conditions, the retention times were about 12 min for *S*-(−)- amisulpride, 13 min for *R*-(+)-amisulpride and 16 min for tiapride (internal standard). The chromatographic identification of each enantiomer was performed by injecting each single enantiomer.

2.4. SPE and ASPEC system

Disposable solid-phase extraction (SPE) columns were C_{18} type, Isolute MF C_{18}, 100 mg, (International Sorbent Technology, Glamorgan, UK); the activation of the columns as well as the whole sample preparation and manipulations were done automatically by an ASPEC apparatus (Gilson Biolabo, Milan, Italy) with software comprising several pre-stored programs for sample preparation.

2.5. Sample preparation

Aliquots (20 μl) of standard methanolic solutions were added to 1 ml of pre-dose plasma samples; 20 μl of internal standard, tiapride, (200 ng) were added to all the samples [standards, quality control samples (QC_s) and unknowns] which were then diluted with 1 ml of borate buffer (pH 9) and vortex-mixed. A 2-ml volume of the diluted samples was loaded onto the activated SPE columns and automatically processed by the ASPEC apparatus according to the procedure depicted in Fig. 2.

The eluents were collected from the columns and evaporated to dryness and then the residues were reconstituted with 0.25 ml of the eluent mixture. The samples were vortex-mixed and 100-μl aliquots were automatically injected onto the chromatographic column.

2.6. Quantitative determination

Peak-height ratio of amisulpride enantiomer *R*-(+)- or *S*-(−)-amisulpride/internal standard, obtained from human plasma standards plotted versus the nominal concentration of *R*-(+)- or *S*-(−)-enantiomers, are used to generate the linear least squares regression line.

The concentrations of *R*-(+)- or *S*-(−)-amisulpride in the unknown specimens were obtained by interpolation from the calibration equation using the peak-height ratios of *R*-(+)- or *S*-(−)-amisulpride/internal standard, obtained from unknown specimens. All the operations and calibrations were automatically performed on a Chromjet integrator.

2.7. Use of fluorescence detection

Some minor modifications of the method have been performed in order to also allow the use of fluorimetric detection which in some pre-dose plasma samples was demonstrated to be more selective than UV detection. These modifications are as follows: the use of a fluorimetric detector (Jasco model 821-FP), set at 280 and 370 nm of λ_{ex} and λ_{em} respectively, a new internal standard metoclopramide (800 ng) replacing the non-fluorescent tiapride and a slight modification of the eluent mixture constituted by *n*-heptane–diethylamine–ethanol (70:0.2:29.8, v/v), required to achieve the complete chromatographic separation between the new internal standard and the *S*-(−)-enantiomer of amisulpride. The mobile phase reservoir and the column were thermostated at 34°C and 28°C, respectively.

3. Results

3.1. Stability

The stability of amisulpride enantiomers in methanol and in human plasma, at both ordinary laboratory conditions and 37°C were investigated, as well as stability in human plasma after two freezing-thawing cycles and in human plasma diluted with borate buffer pH 9 (pre-conditions for the automatic SPE process). Stability of the analyte was found to be

satisfactory in all conditions. The stability of both amisulpride enantiomers and the internal standards (tiapride and metoclopramide) were investigated and found satisfactory in the HPLC injection solvent for 24 h under the ordinary pre-injection laboratory conditions.

3.2. Absolute recovery

The absolute recovery of amisulpride and internal standards (tiapride or metoclopramide) was evaluated from pre-dose plasma samples. Amisulpride was added to pre-dose plasma and processed according to the method described, the internal standard was added to the final residue just before reconstitution with injection solvent. Absolute recovery (%) of amisulpride was calculated from the amisulpride/internal standard peak-height ratio in the chromatogram of the plasma sample divided by the amisulpride/internal standard peak-height ratio in the chromatogram of a synthetic solution. The absolute recovery of internal standard was calculated in the same way, by using amisulpride as internal standard for quantitative calculations. The mean overall absolute recovery of amisulpride obtained from QC_s (at 5 and 80 ng ml^{-1}, respectively) was about 80% (n=6), the mean recoveries of both tiapride (at 200 ng ml^{-1}) and metoclopramide (at 800 ng ml^{-1}) were about 77%.

3.3. Selectivity

Several pre-dose human plasma samples from different subjects were tested for the absence of interfering compounds. Use of either the UV or fluorimetric method guaranteed the highest selectivity (Fig. 3a and Fig. 4a).

3.4. Linearity

A linear correlation between peak-height ratio of each amisulpride enantiomer and internal standard (tiapride or metoclopramide) versus the concentration of each enantiomer was found in the range 2.5–320 ng ml^{-1} (at six levels) for each enantiomer; Fig. 3c and Fig. 4c show typical chromatograms of the linearity curve. The equation of the curve, obtained by weighted linear regression ($1/y$), was $y=22.8x+0.28$ and $y=25.8x+0.41$ for the S-(−)- and R-(+)-enantiomer, respectively, by using the UV method. The equation of the curve, obtained by weighted linear regression ($1/y$), was $y=76.15x+0.06$ and $y=86.43x-0.02$ for S-(−)- and R-(+)-enantiomer respectively, by using the fluorimetric method.

3.5. Limit of quantitation

The limit of quantitation (LOQ) for each amisulpride enantiomer in human plasma, with either the UV or the fluorimetric methods, was 2.5 ng ml^{-1} (Fig. 3b and Fig. 4b). The mean coefficient of variation (C.V.) at LOQ, in human plasma samples, processed on the same day, showed a value ca. ±5% (n=4) and ca. ±2.3% (n=6) for both the enantiomers by using the UV and fluorimetric method, respectively.

3.6. Precision and accuracy

Intra-day precision and accuracy of the UV method were evaluated by analysing QC_s in human plasma at concentrations of 7.5 and 30 ng ml^{-1} of each amisulpride enantiomer. The mean overall accuracy was ca. 100% and the mean overall C.V. was ca. ±4% (n=5) for both enantiomers. When inter-day precision was evaluated from the slope of the calibration curves over a one-month period, the mean slope showed a C.V. of ca. ±5.7% (n=10) for both amisulpride enantiomers. Inter-day precision and accuracy of the fluorimetric method were evaluated over a one-month period by analysing QC samples in human plasma at concentrations of 10, 50 and 250 ng ml^{-1} for each amisulpride enantiomer. The mean overall accuracy was 92%, and the mean overall C.V. was ca. ±8% (n=26) for S-(−)-enantiomer, the mean overall accuracy was 93% and the mean overall C.V. was ca. ±8% (n=26) for R-(+)-enantiomer. During the same period the mean slope of the calibration equations (n=15) showed a C.V. of ca. ±7% for the S-(−)-enantiomer and ±9% for the R-(+)-enantiomer.

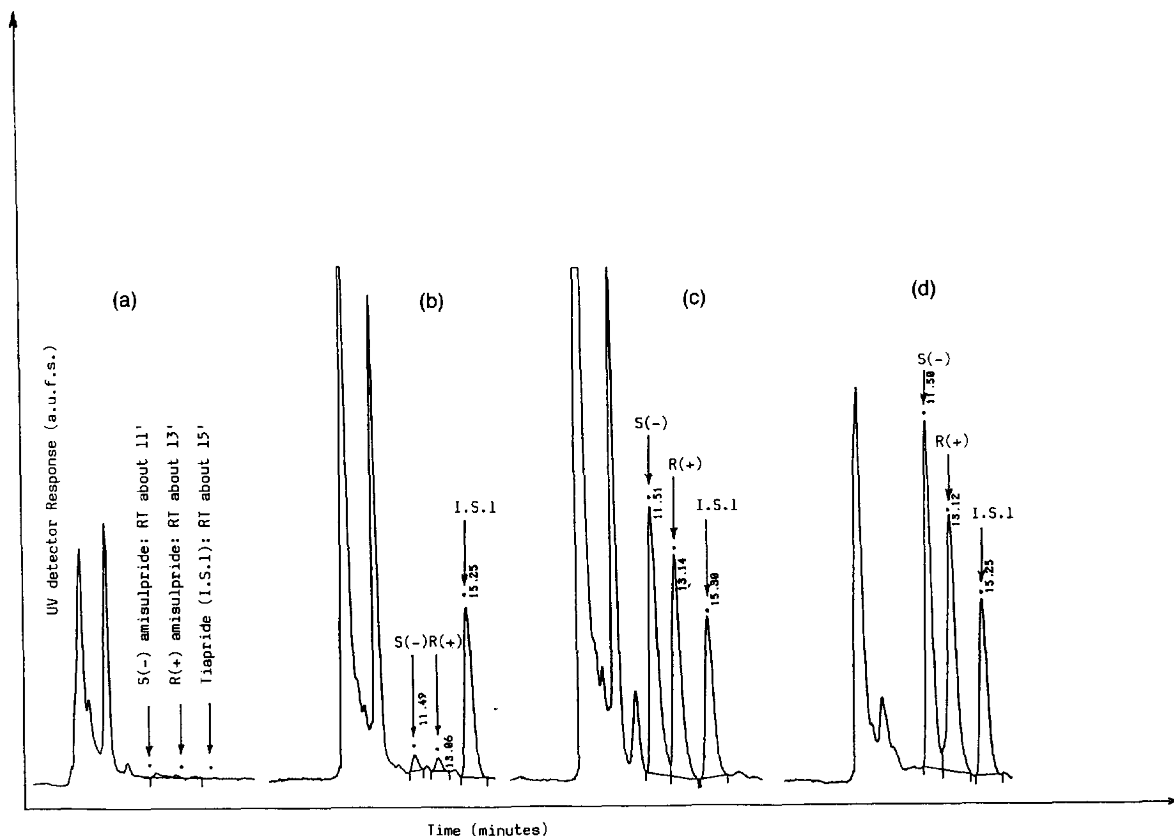

Fig. 3. (a) Chromatogram of pre-dose plasma analysed according to UV method; S(−)=amisulpride S-(−)-enantiomer, R(+)=amisulpride R-(+)-enantiomer, I.S.1=tiapride, internal standard. (b) Chromatogram of LOQ (2.5 ng ml^{-1} for each enantiomer) in human plasma, UV method. (c) Chromatogram of plasma standard (50 ng ml^{-1} for each enantiomer), UV method. (d) Chromatogram of a plasma sample from a healthy volunteer treated by i.v. infusion with 50 mg of amisulpride racemate; UV method, sample collected 1.75 h after the end of infusion. Concentrations found: S-(−)-enantiomer, 74.3 ng ml^{-1}; R-(+)-enantiomer, 62.2 ng ml^{-1}.

3.7. Cross-validation between UV and fluorescence methods

The two detection methods were cross-validated by analysing some unknown plasma samples (n=26) obtained from a clinical pharmacokinetic study. An acceptable agreement was found between the two methods as shown from the linear correlation coefficient (r) (Fig. 5a and b). Concerning the selectivity, usually the fluorimetric method gives rise to cleaner chromatograms than the UV one, where non-fluorescent unknown peaks not affecting the quantitation are detected after the internal standard. However, in several plasma samples obtained from subjects treated either orally or by the intravenous (i.v.) route with amisulpride racemate (50 mg), some unknown substances (not present in pre-dose and in the latest collected samples) co-eluted with S-(−)-amisulpride enantiomer peak detected fluorimetrically, thus affecting its quantitation. In such a case, the UV method can allow a better resolution and quantitation of the S-(−)-enantiomer (Fig. 5a–d).

3.8. Application of the fluorimetric method to human urine samples

Both the described UV and fluorimetric methods could be suitable for the determination of S-(−)- and R-(+)-amisulpride enantiomers in urine samples. However, only the fluorimetric method was used by

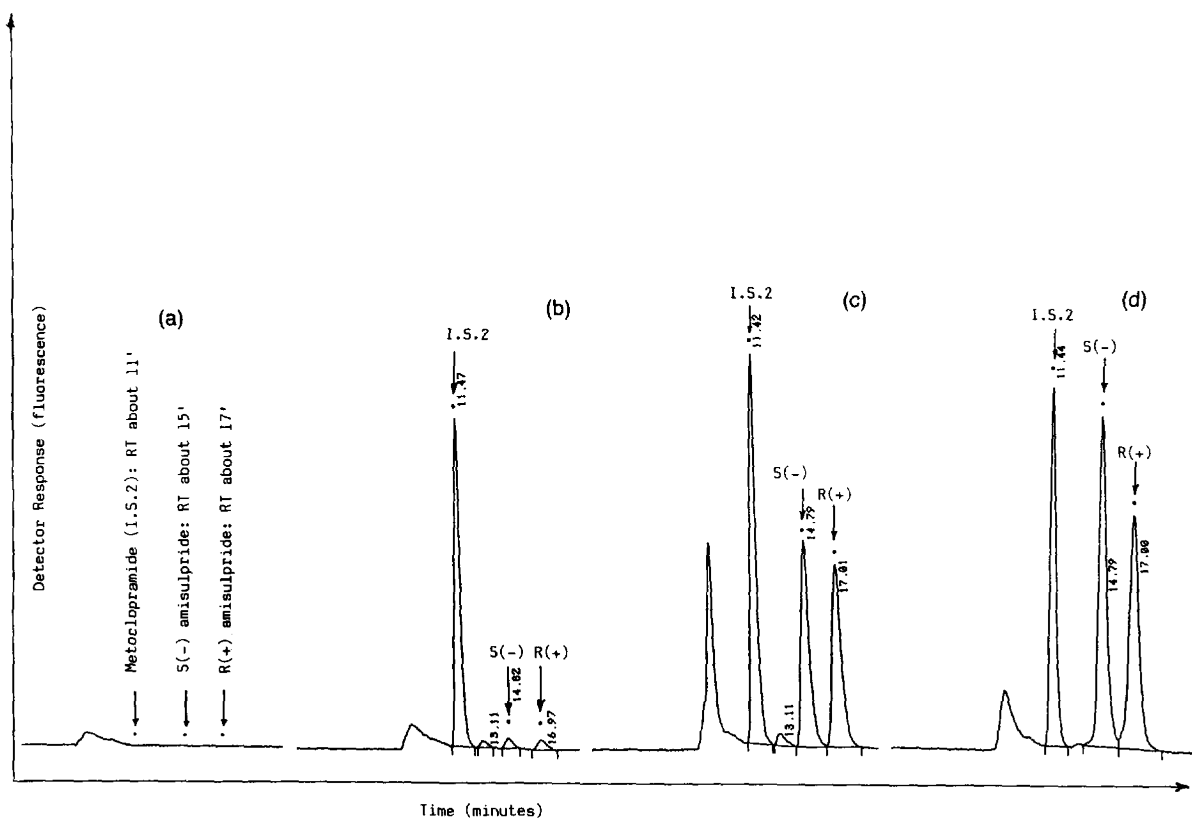

Fig. 4. (a) Chromatogram of pre-dose plasma analysed according to the fluorimetric method; $S(-)$=amisulpride S-(−)-enantiomer, $R(+)$=amisulpride R-(+)-enantiomer, I.S.2=metoclopramide, internal standard. (b) Chromatogram of LOQ (2.5 ng ml^{-1} for each enantiomer) in human plasma, fluorimetric method. (c) Chromatogram of a plasma standard (50 ng ml^{-1} for each enantiomer), fluorimetric method. (d) Chromatogram of an unknown plasma sample from a healthy volunteer treated by i.v. infusion with 50 mg of amisulpride racemate; fluorimetric method, sample collected 2 h after the end of infusion. Concentrations found: S-(−)-enantiomer, 69.4 ng ml^{-1}; R-(+)-enantiomer, 55.7 ng ml^{-1}.

diluting 1 ml of urine and 40 μg of metoclopramide with 25 ml of water and then processing 1 ml according to the described method. The stability of amisulpride enantiomers and metoclopramide in urine under conditions similar to those observed for plasma (see Section 3.1.) was found satisfactory.

The absolute recovery of amisulpride enantiomers and internal standard from human urine is similar to that observed in plasma. Concerning the selectivity, several pre-dose human urinary samples from different subjects were tested for the absence of interfering compounds. In no case were chromatographic interferences found at the retention times of S-(−)- and R-(+)-amisulpride enantiomers and internal standard (Fig. 6a). Linearity between the peak height ratio of each amisulpride enantiomer and internal standard and the concentration of each enantiomer was assessed in the range 0.05–25 μg ml^{-1} by means of weighted linear regression ($1/y$) at six levels. The mean regression equations were $y=3.61x+0.0004$ and $y=4.10x-0.0001$ ($n=4$, obtained on four different days) for S-(−)- and R-(+)-enantiomers, respectively.

LOQ of each enantiomer in human urine was 50 ng ml^{-1} (Fig. 6b). Accuracy and precision were evaluated from urinary QCs (at 0.2, 5 and 20 μg ml^{-1}) analysed in duplicate on four different days. Mean overall accuracy ($n=24$) was 97% and 99% for S-(−)- and R-(+)-enantiomers respectively, and mean overall C.V. ($n=24$) was ±5.4% and ±4.4%

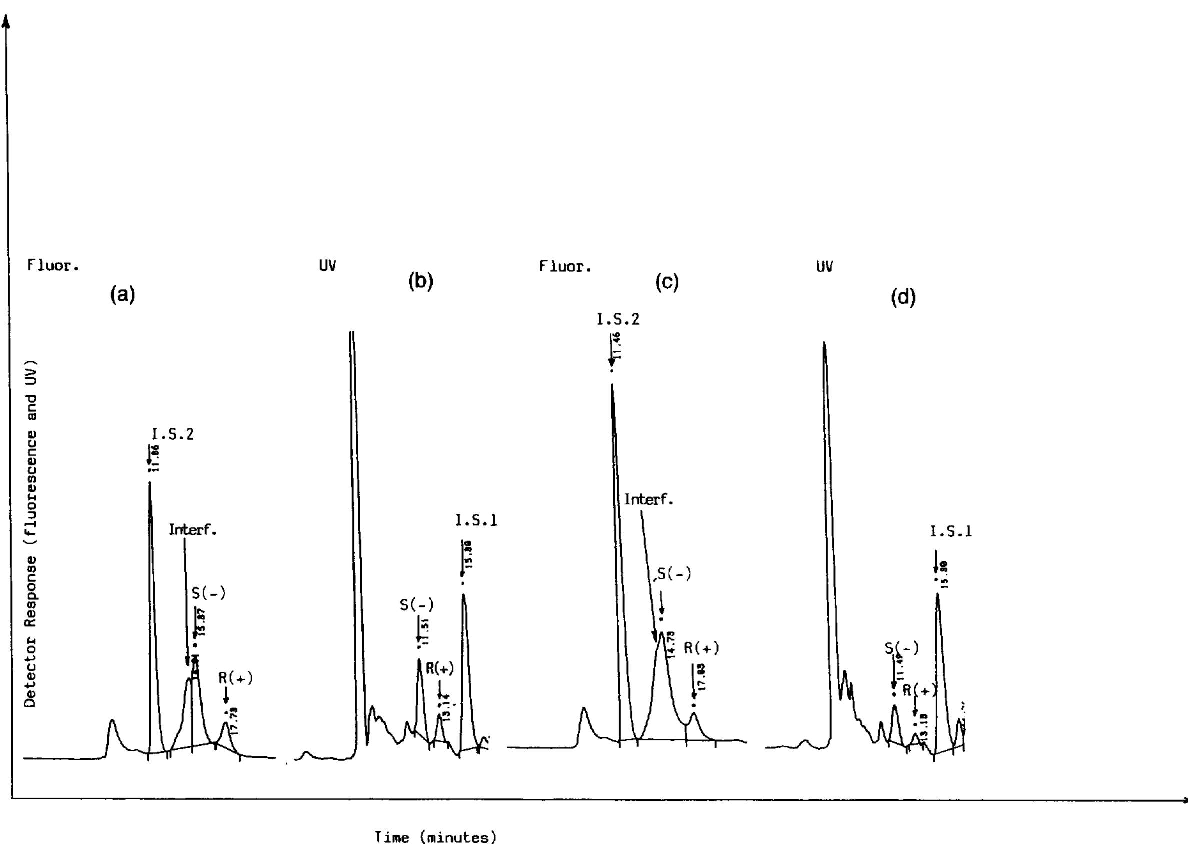

Fig. 5. (a) Chromatogram of a plasma sample from a healthy volunteer treated orally with 50 mg of amisulpride racemate; fluorimetric method, sample collected 6 h after drug intake. The quantitation of $S(-)$ is affected by a coeluted interference. (b) Sample shown in (a) analysed with the UV method. Concentrations found: S-(−)-enantiomer, 14 ng ml^{-1}; R-(+)-enantiomer, 6 ng ml^{-1}. (c) Chromatogram of a plasma sample from a healthy volunteer treated by i.v. infusion with 50 mg of amisulpride racemate; fluorimetric method, sample collected 12 h after the end of infusion. The quantitation of $S(-)$ is affected by a coeluted interference. (d) Sample of (c) analysed with the UV method. Concentrations found: S-(−)-enantiomer, 11.2 ng ml^{-1}; R-(+)-enantiomer, 4.1 ng ml^{-1}.

for S-(−)- and R-(+)-enantiomers, respectively. An example of a chromatogram of a 5-μg ml^{-1} QC sample is shown in Fig. 6c.

3.9. Application to pharmacokinetics

The reported methods have been used for the determination of amisulpride enantiomers in plasma and urine samples of healthy subjects involved in pharmacokinetic studies with amisulpride racemate after various oral doses or after a 50 mg i.v. infusion dose. Representative chromatograms, obtained by UV or fluorimetric methods are shown in Fig. 3d and Fig. 4d. In addition, a chromatogram showing a urine sample is given in Fig. 6d.

The results refer to the plasma levels of racemic amisulpride, obtained by achiral HPLC [16] as well as the corresponding S-(−)- and R-(+)-enantiomer levels, obtained by the UV method. The comparison between the plasma levels versus time course profile of both the enantiomers and amisulpride racemate show that the pharmacokinetic plasma profile of enantiomers parallels that of racemate (Fig. 8) and the sum of S-(−)- and R-(+)-enantiomers plasma concentrations is quantitatively similar to racemate plasma concentrations (Fig. 9). In addition, some pharmacokinetic parameters (T_{max}, C_{max}, $t_{1/2}$ β, $t_{1/2}$ Abs and AUC) calculated from enantiomer plasma levels showed a good agreement with those obtained from the analysis of the racemic mixture

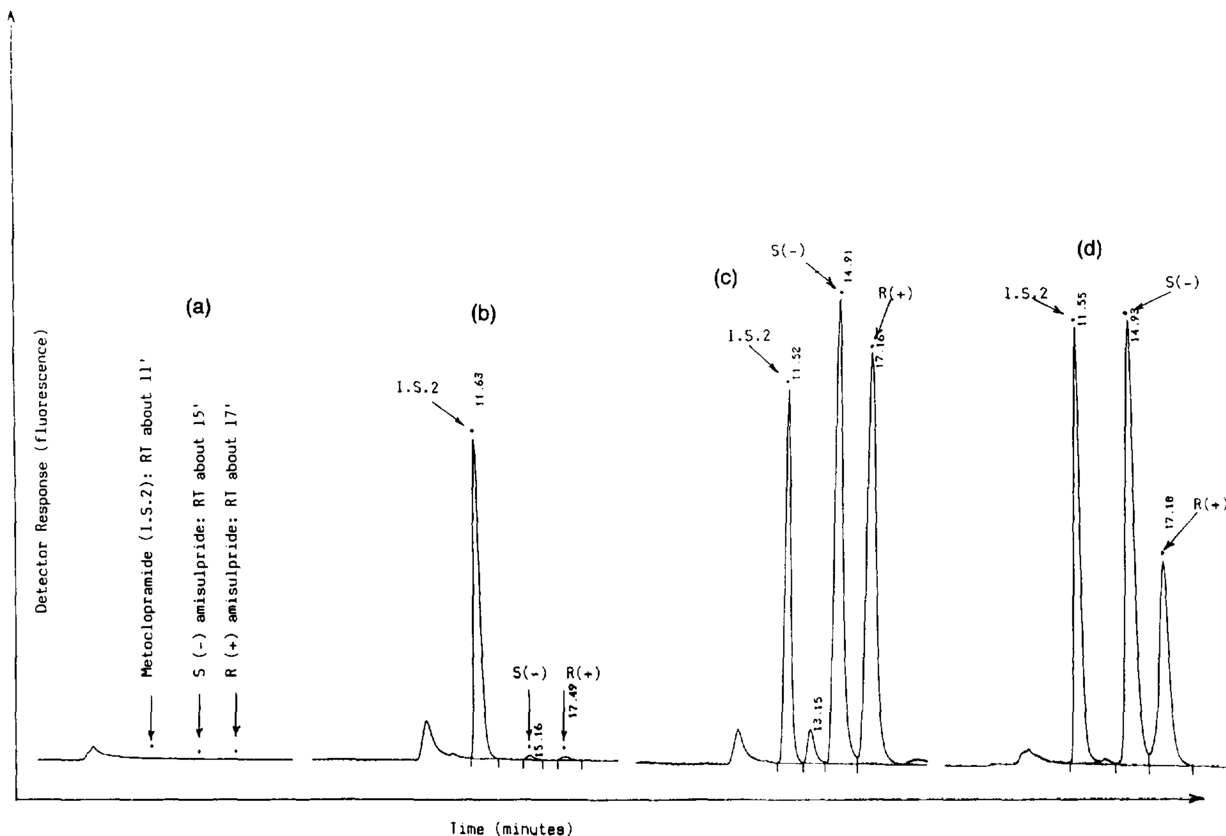

Fig. 6. (a) Chromatogram of pre-dose urine analysed according to fluorimetric method; $S(-)$=amisulpride S-(−)-enantiomer, $R(+)$= amisulpride R-(+)-enantiomer, I.S.2=metoclopramide, internal standard. (b) Chromatogram of LOQ (50 ng ml^{-1} for each enantiomer) in human urine. (c) Chromatogram of a urine standard (50 μg ml^{-1} for each enantiomer). (d) Chromatogram of a urine sample from a healthy volunteer treated orally with 50 mg of amisulpride racemate; 12–24 h fraction. Concentrations found: S-(−)-enantiomer, 3.7 μg ml^{-1}; R-(+)-enantiomer, 1.9 μg ml^{-1}.

(Table 2). Higher C_{max} and AUC values for the S-(−)-enantiomer in comparison with R-(+), suggest that there is a slight difference in drug disposition between the $S(-)$ and $R(+)$ enantiomers. This does not influence the elimination rate constant of amisulpride as a racemate or as an enantiomer.

Concerning the urinary levels of both enantiomers, the amounts excreted at different time intervals, showed a good agreement between the values obtained from racemate measurements as well as those obtained from the sum of enantiomers (Fig. 10).

4. Discussion

Several types of chiral packings were tested and found unsuitable for the chromatographic resolution of R-(+)- and S-(−)-enantiomers of amisulpride.

Chiral AGP with a phosphate buffer eluent at pH between 7.5–8.5 allowed a satisfactory separation of the enantiomers, but the weak alkaline condition (essential for the resolution) caused a rapid degradation of the column after just 100–150 injections. This type of column in addition, at the mentioned conditions, showed a dramatic variability in the retention times of the substances under study due to poor inter-column reproducibility. Another protein (ovomucoid) bonded to silica phase, Ultron, used in a pH range between 3 and 8 did not give satisfactory results, as well as an immobilized β-cyclodextrin phase or β-cyclodextrin used as chiral selector on different packings (C_8, C_{18}, CN, Hypercarb) utilized according to manufacturer's suggestions.

Changing strategy, by moving to normal-phase stereoselective chromatography, Chiracel OD was tried with n-hexane–ethanol (90:10, v/v) as eluent in

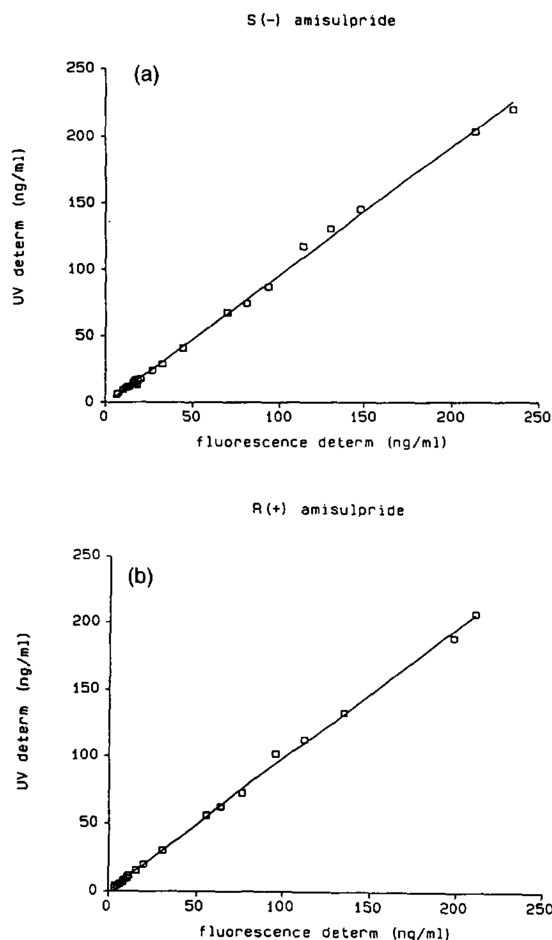

Fig. 7. (a) Correlation between UV and fluorimetric determinations, for $S(-)$ amisulpride enantiomer, performed on plasma samples (n=26) from a subject treated by i.v. infusion and oral administration of 50 mg of amisulpride racemate. $y=0.9648x-0.691$, r=0.9989 (24 df), ($p<0.001$). (b) Correlation between UV and fluorimetric determinations, for $R(+)$ amisulpride enantiomer, performed on plasma samples (n=26) from a subject treated by i.v. infusion and oral administration of 50 mg of amisulpride racemate. $y=0.9808x-0.055$, r=0.9993 (24 df), ($p<0.001$).

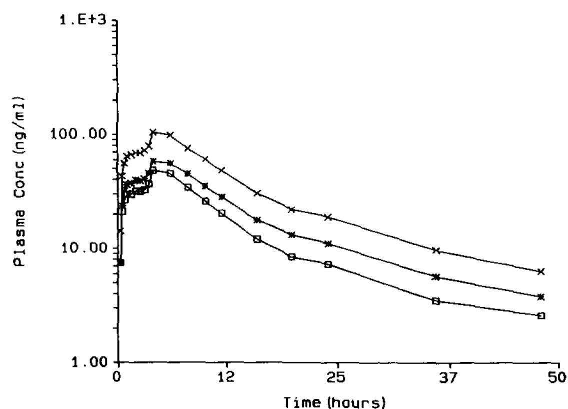

Fig. 8. Mean plasma concentration–time course plot of S-(−)-, R-(+)-amisulpride enantiomers and amisulpride racemate (RAC) in eight healthy volunteers treated orally with 100 mg of amisulpride racemate. (□) $R(+)$; (*) $S(-)$; (×) RAC.

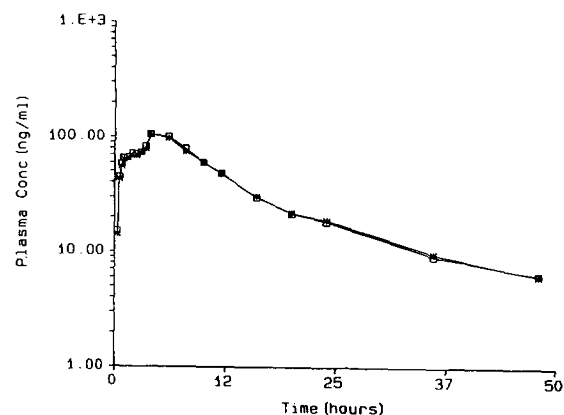

Fig. 9. Mean plasma concentration–time course plot of the sum of S-(−)- and R-(+)-amisulpride enantiomers ($R+S$) and amisulpride racemate (RAC) in eight healthy volunteers treated orally with 100 mg of amisulpride racemate. (□) $R+S$; (*) RAC.

the presence of 0.1% of DEA. Under these conditions a separation between the R-(+)- and S-(−)-enantiomers of amisulpride was achieved. However, the retention times of the substances were over 70 min and different attempts to shorten the chromatography time resulted in diminished resolution.

Finally the choice of a Chiralpak AS packing, an amilose derivative coated on silica gel, permitted us to obtain a nice separation in a short time. The manufacturer's recommendations to avoid the use of strong solvents such as water, acetonitrile, methanol, were strictly observed. The Chiralpak column has been utilized for more than 2000 injections of plasma extracts over a total of four months, in different periods, without any loss of its chromatographic efficiency.

The reproducibility of the retention times of the analytes and the internal standard showed a C.V. of ca. 3%, in the period of use, in comparison to the initial conditions of the new column. Another Chiralpak AS column, from a different batch, showed a very similar chromatographic behaviour.

The proposed sample preparation, according to the SPE technique, allowed us to obtain a sample extract that was fully compatible with the chromatographic

Table 2
Mean pharmacokinetic parameters of amisulpride enantiomers and racemate in six healthy volunteers, after a single oral administration of 100 mg of amisulpride racemate

Amisulpride	$t_{1/2}$ Abs (h)	$t_{1/2}$ Elim (h)	AUC_{0-48} (ng h ml^{-1})	C_{max} (ng ml^{-1})	T_{max} (h)
S-(−)-	1.1	14.4	951.0	67.1	4.0
R-(+)-	0.9	14.1	698.5	58.4	4.0
$\sum(-)(+)$	1.0	14.3	1649.8	125.4	4.0
RAC[a]	0.3	14.1	1748.6	135.8	4.0

[a] RAC=amisulpride racemate.

conditions of the method. Indeed, the high content of *n*-hexane in the mobile phase allowed the column to be always free from lipids that could otherwise have accumulated on the top of the column.

The possibility of using either the UV method or the fluorimetric one for dosed plasma samples allows great improvement in the selectivity of the method and to face interference problems that can originate from different sources: endogenous compounds whose in vivo concentrations are influenced by drug administration, unknown metabolites, food, extralaboratory or inlaboratory contamination.

The cross-validation, performed on real ex in vivo samples points out that the UV and fluorimetric method are equivalent. Such a possibility of choice has been extremely useful to us in the case of an interference peak affecting the fluorimetric quantitation of *S*-(−)-amisulpride enantiomer in several dosed plasma samples which, inexplicably, belonged to a set of samples (from specific subjects) not showing such an interference under UV conditions (Fig. 5a–d).

Urine samples can be easily analysed, after dilution, with the same method used for plasma. Plasma samples previously analysed for amisulpride racemate, when re-analysed for *S*-(−)- and *R*-(+)-enantiomers with the chiral UV method, provided an optimal agreement between amisulpride plasma levels as racemate and as sum of enantiomers. This test is usually considered very important to validate the data obtained from in vivo samples obtained with a stereospecific analytical method.

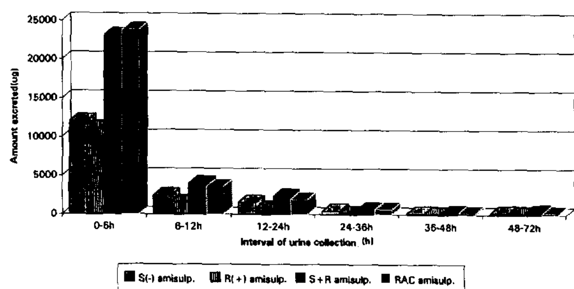

Fig. 10. Urinary amount excretion (μg) of amisulpride as *S*-(−)-enantiomer, *R*-(+)-enantiomer, *R*+*S* enantiomers and racemate (RAC) vs. time, in a subject treated with 50 mg of a single i.v. infusion of amisulpride racemate.

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