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Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid–liquid extraction or solid-phase extraction in combination with high-performance liquid chromatography and fluorescence detection Application to pharmacokinetics

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Abstract

Amisulpride (SOLIAN) belongs to the benzamide series and shows antischizophrenic and antidepressant (antidysthymic) properties in man. Two methods suitable for pharmacokinetic investigations are proposed for the determination of amisulpride in human plasma. For the liquid–liquid extraction (LLE) based method, the plasma, added with the internal standard (an amisulpride analogue) is alkalinised with NaOH and extracted with a diethyl ether–chloroform mixture. The organic phase is removed, evaporated to dryness and redissolved in an acidic phosphate–acetonitrile mixture that, after a back-washing step with *n*-hexane, is injected onto the HPLC column (C_{18} BDS type) connected with a fluorimetric detector. The second method is based on an automatic solid-phase extraction (SPE) performed on an ASPEC device. The plasma sample, diluted with a pH 9 borate buffer, is loaded onto a disposable SPE C_{18} 100-mg column. The analytes of interest (amisulpride and internal standard), after two washing steps with different solvents, are recovered in pure methanol; after evaporation to dryness, the residue is dissolved in an acidic phosphate buffer and injected onto the chromatographic apparatus already described. The limit of quantitation (LOQ) is 0.5 ng ml^{-1} for both methods; a linear correlation between concentrations and detector response has been demonstrated in the range 0.5–640 ng ml^{-1} for LLE, which is the most used method; for SPE methods, less used, linearity has been assessed in the plasma range of 0.5–160 ng ml^{-1}.

Keywords: Amisulpride; Benzamide

1. Introduction

Amisulpride, (*R,S*)-(±)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulphonyl-2-methoxybenzamide, is a benzamide derivative chemically related to sulpiride, commercialized as a CNS agent, effective either as antidysthymic, antischizophrenic or antipsychotic drug depending on the dose: 50–100 mg for the treatment of dysthymics disorders [1,2], over 200 mg for the treatment of negative symptoms in schizophrenic patients [3] and over 600 mg for psychotic disorders [4,5]. In vitro and in vivo studies

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on animal models [6] demonstrate that amisulpride derives its pharmacological properties from a selective antagonist effect on D_2 and D_3 presynaptic dopaminergic receptors. In man, in the same way as other sulpiride derivatives, amisulpride presents a double phase absorption profile, with a first phase limited by intestinal tractus (C_{max}, 1–3 h) and a second phase, evident as a double plasmatic concentration peak (C_{max}, about 4.5 h), dependent on the total administered dose [7]. The protein binding of the drug is only 17% and the mean apparent elimination half-life in plasma, after oral administration, is about 15.5 h [7]. Amisulpride, in man, undergoes to a low metabolisation and the unmodified drug can be recovered in urine and feces [7]. For the determination of amisulpride in human plasma a liquid–liquid extraction (LLE) procedure followed by HPLC analysis with UV absorbance detection has been already proposed [8]. Furthermore, a general solid-phase extraction (SPE) with HPLC and UV absorbance detection method for the determination of several benzamide derivatives in human plasma and urine is also available [9]. Notwithstanding, determination of the compound in human plasma for pharmacokinetic studies and for drug level monitoring in clinical investigations concerning low-medium dosage treatments (anti-dysthymics and anti-schizophrenic treatments), requires more sensitivity. For these reasons two different sample preparation procedures, using the same chromatographic conditions, were developed, validated and compared to each other for the determination of amisulpride in human plasma: a manual LLE with an additional back-washing step to further increase the sample cleaness, and an automatic SPE method utilizing a robotic device (ASPEC).

2. Experimental

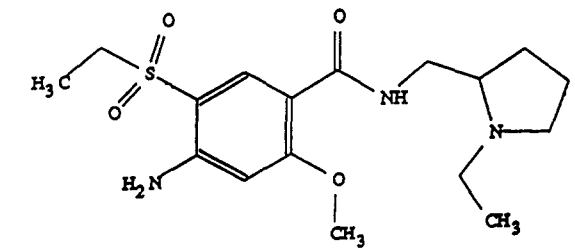
2.1. Reagents, chemicals and standards

Methanol and acetonitrile were HPLC grade (Merck, Darmstadt, Germany), dried diethyl ether and chloroform were analytical grade (Merck); *n*-hexane was for organic residue analysis (Merck); boric acid, Suprapur® (Merck), sodium hydroxide pellets, analytical grade (Merck), potassium chloride, analytical grade (Merck), potassium dihydrogen phosphate anhydrous (KH_2PO_4) analytical grade (Merck), triethylamine (TEA) was analytical grade (C. Erba, Milan, Italy), phosphoric acid, analytical grade (85%) (Merck), pure water was obtained from deionized water then purified on a Milli-Q4 system (Millipore, Bedford, MA, USA). Borate buffer 0.073 *M* (pH 9) for extraction was prepared by dissolving 6.18 g of boric acid and 7.46 g of potassium chloride in 1 l of pure water, then bringing 500 ml of this solution to pH 9 with about 185 ml of 0.1 *M* sodium hydroxide. Phosphate buffer 0.025 *M* (pH 3) was prepared by dissolving 131.1 g of potassium dihydrogen phosphate in 1 l of pure water to obtain a 1 *M* solution, then diluting 25 ml of this solution to obtain a 0.025 *M* solution and adjusting to pH 3 with phosphoric acid solution. The buffer was utilised as an injection solvent for the SPE method, while for the LLE method, a buffer–acetonitrile (90:10, v/v) mixture was prepared and utilised to redissolve the extraction residue and for injection. Diethyl ether–chloroform (95:5, v/v) mixture was obtained by mixing 95 ml of diethyl ether with 5 ml of chloroform. The water–acetonitrile (70:30, v/v) solution for the washing of the SPE columns was prepared by adding 300 ml of acetonitrile to 700 ml of pure water. The mobile phase was prepared by adding to 25 ml of 1 *M* potassium dihydrogen phosphate solution about 950 ml of water and 1 ml of triethylamine; the pH was adjusted to 3 with phosphoric acid and the solution diluted to 1 l with water; 850 ml of this solution were diluted to 1 l with acetonitrile.

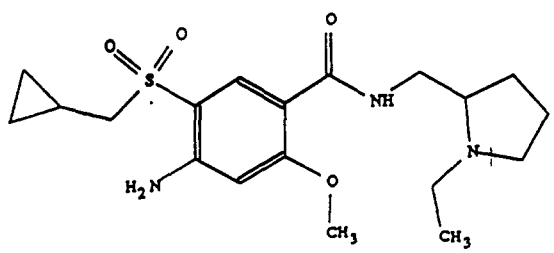
Amisulpride and the internal standard L-(−)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-(cyclopropyl)methylsulphonyl-2-methoxybenzamide (I.S.) (see Fig. 1), were of pharmaceutical grade and obtained from Synthélabo Recherche, Bagneux, France.

2.2. Standard solutions

Stock solutions (1 mg/ml) of amisulpride and the internal standard (I.S.) were prepared in methanol (Table 1); standard solutions were prepared weekly from stock solutions by suitable dilutions with methanol and were used for the preparation of



AMISULPRIDE



INTERNAL STANDARD (IS)

Fig. 1. Chemical structure of amisulpride (I) and its internal standard (I.S.).

plasma standards. Stock solutions were stable for at least one month if stored at 0–5°C. The standard solutions were added to pre-dose human plasma, for the preparation of the plasma standards used for daily calibration.

2.3. Chromatographic system

For the proposed methods, the chromatographic equipment consisted of a constant-flow double piston pump model Isochrom (Spectra-Physics, San Jose, CA, USA), a spectrofluorimetric LC detector Model 821-FP (Jasco, Tokio, Japan), provided with a standard HPLC cell (cell volume: 16 μl) operating at λ_{ex}=280 nm, λ_{em}=370 nm, an automatic sample injector Model 460 (Kontron, Milan, Italy) provided with a six-port automatic valve and 200-μl external loop and an integrator Model Chromjet SP4400 (Spectra-Physics).

The analytical column, 150×4.6 mm I.D., was filled with 5-μm Hypersil C_{18} BDS material (Shandon, Runcorn, UK) provided with a guard-column, 20×4.6 mm I.D., 40-μm Pelliguard LC_8 (Supelco, Bellefonte, PA, USA). The pump flow-rate was 1.0 ml min^{-1}, the injection volume was 150 μl, the integrator chart speed was 0.5 cm min^{-1} and the attenuation set at 16. Under these conditions the retention times for the investigated compounds were: 4 min for amisulpride and 7 min for the internal standard.

2.4. SPE and ASPEC system

SPE was performed on disposable solid-phase extraction columns, monofunctional C_{18} type, filled with 100 mg of silica packing (I.S.T., Hengoed, UK); all the column activation and sample treatment steps (loading onto the column, purification and

Table 1
Standard solutions used for the determination of amisulpride in human plasma

Standard solution No.	Amisulpride conc. LLE method (ng·20 μl^{-1})	Amisulpride conc. SPE method (ng·20 μl^{-1})	Internal standard conc. (ng·20 μl^{-1})
1	640.0	–	–
2	320.0	–	–
3	160.0	160.0	–
4	80.0	80.0	–
5	20.0	20.0	–
6	5.0	5.0	
7	2.0	–	
8	–	1.0	
9	0.5	0.5	–
10	–	–	50.0 or 100.0[a]

[a]50 ng·20 μl^{-1} for the LLE method and 100 ng·20 μl^{-1} for the SPE method.

elution) were performed by an ASPEC apparatus (Gilson Biolabo, Middelton, WI, USA) by means of a software developed for sample preparation.

2.5. Sample preparation

2.5.1. LLE method

The frozen plasma samples (pre-dose and unknowns) were thawed in a water-bath at 37°C before weighing at room temperature; then 1 g of pre-dose plasma (for each future plasma standard) and unknown samples were weighed and added with 20 μl of standard solutions for each calibration point (Table 1); 20 μl of internal standard solution were also added to all samples and mixed. The samples were then extracted according to the following procedure. All the samples were diluted with 1 ml of pure water and alkalinised with 0.2 ml of 1 *M* NaOH; then the samples were extracted with 7 ml of diethyl ether–chloroform (95:5, v/v) mixture by shaking at 40 rpm for 10 min (tumble extractor). After separation, obtained by centrifuging and freezing the aqueous phase (500 *g* for 8 min at −20°C), the upper organic layer was transferred and evaporated to dryness under a light stream of pure nitrogen at 40°C. The residue was then redissolved in 250 μl of a 0.025 *M*, pH 3, phosphate buffer–acetonitrile (90:10, v/v) mixture and back-washed with 1 ml of *n*-hexane on a tumble extractor (at 20 rpm for 10 min). The aqueous phase was separated from the hexane layer that was discarded. After evaporation of the solvent residue under a light stream of pure nitrogen at 40°C, the final aqueous phase was transferred to conical vials for automatic sample injection. Finally, 150 μl of the solution were injected into the injection port of the HPLC system.

2.5.2. ASPEC SPE method

The sample (standards and unknowns), prepared in the same way as for the LLE method, were diluted with 1 ml of 0.073 *M* borate buffer, pH 9, and vortex-mixed. The diluted 2-ml samples were automatically loaded and processed onto SPE C_{18} type columns, previously activated, by the ASPEC apparatus according to the reported procedure (Fig. 2). At the end of the automated process, the eluates were collected from the SPE column, evaporated to dryness and finally the residues were redissolved in 250 μl of 0.025 *M* phosphate buffer, pH 3. The samples were vortex-mixed and 150 μl of the solutions were automatically injected onto the chromatographic column.

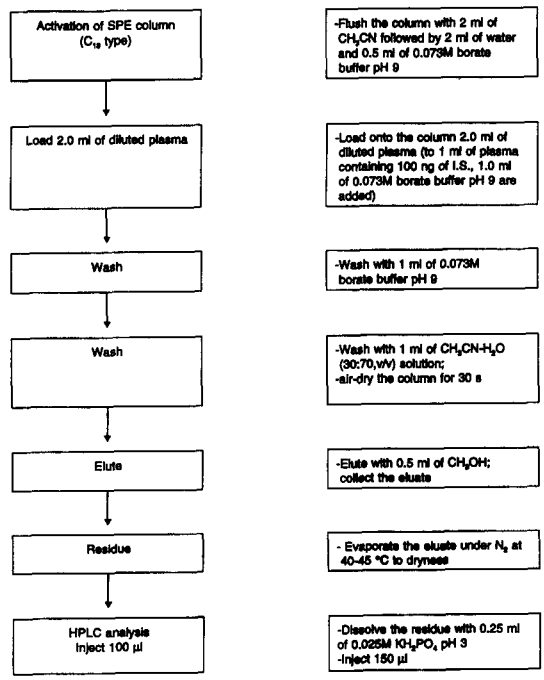


Fig. 2. Scheme of the solid-phase extraction sample preparation for plasma samples containing amisulpride.

2.6. Quantitative determination

Peak-height ratios of amisulpride/internal standard, obtained from human plasma standards, plotted versus the nominal concentration of amisulpride, were used to generate weighted ($1/Y$) linear least square regression lines (calibration equations). The concentrations of amisulpride in the unknown specimens were obtained by interpolation from the calibration equations using peak-height ratios of amisulpride/internal standard, obtained by unknown specimens. All the calculations concerning the quantitative analysis and regressions were automatically performed on a calculator integrator SP 4400 (Spectra-Physics).

3. Results

3.1. Stability

Amisulpride has been shown to be stable at least for 24 h in human plasma maintained either at room temperature or at 37°C; furthermore, it resulted stable in human plasma even if submitted to two freezing–thawing cycles (−20°C/laboratory conditions).

Stability of amisulpride and internal standard in different analytical fluids was also ascertained. In methanolic stock solutions, both amisulpride and internal standard resulted to be stable at least one month when maintained in the refrigerator (0–5°C). Concerning the LLE method, both amisulpride and internal standard have been shown to be stable for at least 3 h in the extraction solvent (diethyl ether–chloroform mixture) at room conditions; moreover, the compounds appeared to be stable, after extraction from plasma, in the HPLC injection solvent for at least 24 h on the autosampler rack (pre-injection conditions).

Concerning the SPE method, both amisulpride and the internal standard were assessed to be stable for at least 24 h at room temperature (pre-extraction conditions) in human plasma diluted with borate buffer as well as in the HPLC injection solvent, at room temperature, on the autosampler rack (pre-injection conditions).

3.2. Recovery

The absolute recovery was evaluated, for both LLE and SPE methods, in pre-dose plasma samples spiked with known amounts of the compounds. Two different concentrations of amisulpride (5 and 80 ng ml^{-1} for LLE and 5 and 40 ng ml^{-1} for SPE) were added to pre-dose human plasma; the samples were extracted according to the described methods, and the internal standard was added to the final injection solvent just before injection. The recovery of the internal standard was evaluated by adding 50 ng ml^{-1} (LLE) or 100 ng ml^{-1} (SPE) to pre-dose plasma using amisulpride as internal standard. The absolute recovery (%) was calculated, for either LLE or SPE methods, from the ratio of the amount of the compound extracted from plasma to the amount added to 250 μl of HPLC injection solution. The absolute recovery for amisulpride is 68.4±2.0 and 68.5±2.1% (mean±C.V.; $n=3$) at 5 and 80 ng ml^{-1} for the LLE method, respectively, while for the SPE method it is 84.7±6.1 and 80±2.4% (mean±C.V.; $n=3$) at 5 and 40 ng ml^{-1}, respectively. For the internal standard the absolute recovery is 72.1±3.7% (mean±C.V.; $n=3$) at 50 ng ml^{-1} for the LLE method and 77.0±2.4% (mean±C.V.; $n=3$) at 100 ng ml^{-1} for the SPE method.

3.3. Selectivity

Several pre-dose plasma samples from different subjects were tested for the absence of interfering compounds. In no case was any chromatographic interference found at the retention times of amisulpride and the internal standard for the LLE method or for the SPE method (see Fig. 3A, Fig. 4A).

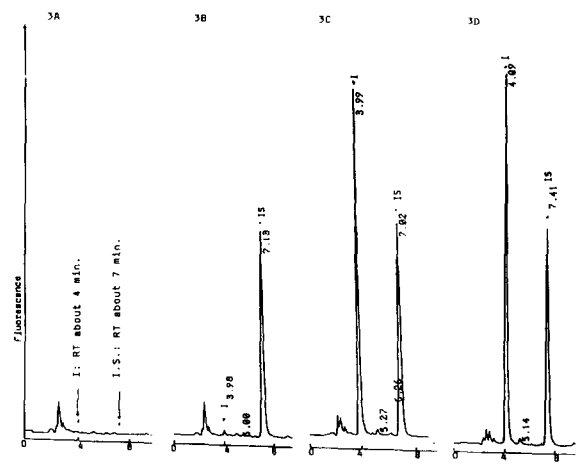


Fig. 3. (A) Chromatogram of pre-dose human plasma, amisulpride=I, internal standard=I.S. (LLE method). (B) Chromatogram showing the LOQ in human plasma: 0.5 ng ml^{-1} (LLE method). (C) Chromatogram of QC sample (40 ng ml^{-1}) (LLE method). (D) Chromatogram of a plasma sample from a healthy subject administered orally with a single dose (50 mg) of amisulpride; sample taken 6 h after administration; level found 56.4 ng ml^{-1} of amisulpride (LLE method).

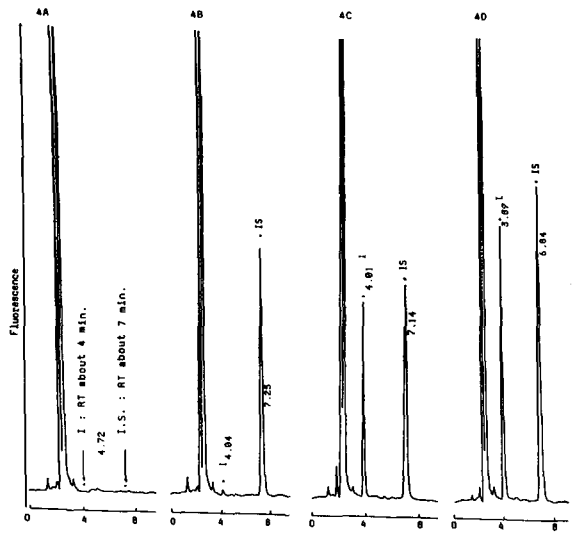


Fig. 4. (A) Chromatogram of pre-dose human plasma, amisulpride=I, internal standard=I.S. (SPE method). (B) Chromatogram showing the LOQ in human plasma: 0.5 ng ml^{-1} (SPE method). (C) Chromatogram of QC sample (40 ng ml^{-1}) (SPE method). (D) Chromatogram of a plasma sample from a healthy subject administered orally with a single dose (50 mg) of amisulpride; sample taken 6 h after administration; level found 50.7 ng ml^{-1} of amisulpride (SPE method).

3.4. Linearity

For both LLE and SPE methods, a linear correlation between peak-height ratio of amisulpride/internal standard (Y) versus amisulpride concentration (x) was found in the range 0.5–160 ng ml^{-1} of amisulpride in human plasma. The study was performed on six concentrations in quadruplicate. Results, expressed as calibration equations and relative coefficient of correlation (r^2), showed a good linear correlation between the amisulpride/internal standard peak-height ratio (x) and the added quantities of amisulpride (y) (weighted $1/Y$ linear regression calibration equation LLE: $y=25.409x - 0.605$, $r^2=1$; SPE: $y=47.412x - 0.481$, $r^2=1$). Moreover, we have demonstrated that the linearity range of the LLE could be extended up to 640 ng ml^{-1} (weighted $1/Y$ linear regression calibration equation: $y=29.907x - 0.208$, $r^2=1$). This extension is useful when pharmacokinetic investigations have to be carried out in subjects treated with high doses (600–1200 mg daily) of amisulpride.

3.5. Limit of quantitation

The limit of quantitation (LOQ), defined according to a confidence limit criterion, is the lowest analyte concentration that can be measured with a coefficient of variation (C.V.) not above 20%. For both LLE and SPE methods it is 0.5 ng ml^{-1} in human plasma (Fig. 3B, Fig. 4B) and it corresponds always to the lowest point of the calibration curve.

3.6. Precision and accuracy

The precision and accuracy of the methods were evaluated by analysing quality control samples (QC) in human plasma at different concentrations on different days by two analysts; each analyst, after the daily calibration (performed in quadruplicate), analysed a low and medium QC (in quintuplicate or less) over a two-day period. The results for the LLE and SPE method are reported in Table 2. Fig. 3C, Fig. 4C represent chromatograms from QC samples.

3.7. Comparison between LLE and SPE methods

In order to cross-validate the proposed methods, several dosed plasma samples ($n=40$), obtained from in vivo studies were analysed using the two methods. The correlation curve obtained from this comparison showed a good agreement between the two methods (Fig. 5), no statistically significant difference was found between LLE and SPE ($p=0.4082$).

3.8. Application of the method to urinary samples

The described LLE method, with minor modifications, was also used for the analysis of urinary samples collected from healthy subjects administered with amisulpride. For this purpose, to 1 ml of urine, the internal standard (10 μg in 20 μl of methanol) was added, then 0.5 ml were diluted to 50 ml with water and 2 ml of the diluted sample were processed according to the described LLE method; 50 μl of the final extract were injected onto the HPLC system. The stability, checked under the same conditions reported for human plasma, was found to be satisfactory. The selectivity was tested by analysing several blank urine samples for the absence of interfering compounds; no chromatographic interference was

Table 2
Precision and accuracy for amisulpride in human plasma obtained by LLE and SPE methods

	Amisulpride (LLE)		Amisulpride (SPE)	
	4.0 ng ml^{-1}	40.0 ng ml^{-1}	4.5 ng ml^{-1}	40.0 ng ml^{-1}
Precision				
Intra-day R.S.D. (%)	2.6	1.2	3.2	1.6
Inter-day R.S.D. (%)	1.5	1.7	1.6	1.7
Total R.S.D. (%)	3.0	2.0	3.5	2.3
95% upper confidence limit for total R.S.D. (%)	4.5	4.1	5.2	4.2
Accuracy				
Percent recovery ± R.S.D. (%)	99.4±1.9	105.0±1.8	106.4±2.4	105.7±2.0
Number of QC analysed	20	20	20	20

found at the retention times of amisulpride and the internal standard (Fig. 6A). The mean overall absolute recovery of amisulpride in the range 0.2–120 μg ml^{-1} was found to be about 80%. Linearity was found in the range 0.1–150 μg ml^{-1}; LOQ is 0.1 μg ml^{-1} (Fig. 6B). Intra-day precision was evaluated from urinary QC samples at three different concentrations (0.3, 30 and 120 μg ml^{-1}), the mean overall C.V. was about ±4%. Inter-day precision was evaluated from daily calibration curves (n=4) at 7 levels (range 0.1–150 μg ml^{-1}) and the mean overall C.V. was about ±4%.

3.9. Application to pharmacokinetics

The LLE method has been used for the determination of amisulpride plasma levels after oral administration of a single dose of 50 mg to healthy subjects, the mean plasma concentration–time course profile, obtained in fifteen healthy subjects (on a total of eighteen treated), is shown in Fig. 7. The results allowed some pharmacokinetic parameters in human plasma to be obtained, likewise T_{max}, C_{max}, AUC and $t_{1/2\beta}$ (Table 3); urine samples were analysed in

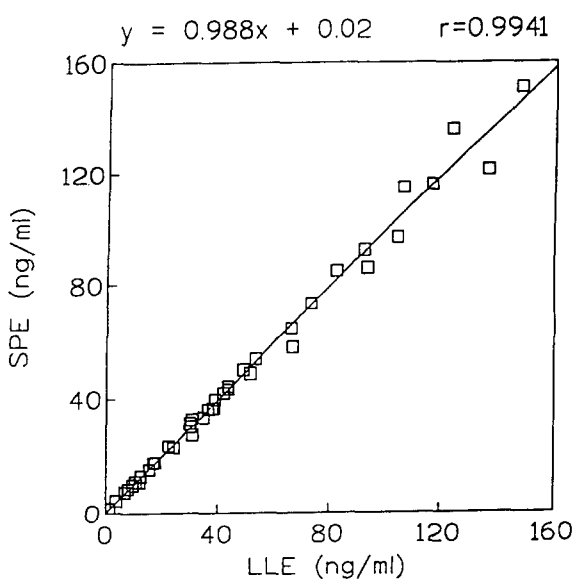


Fig. 5. Comparison of SPE vs. LLE method performed on "real" unknown plasma samples (n=40). Student t test for paired data: t=0.844 (39 df); Probability=0.4082: N.S.

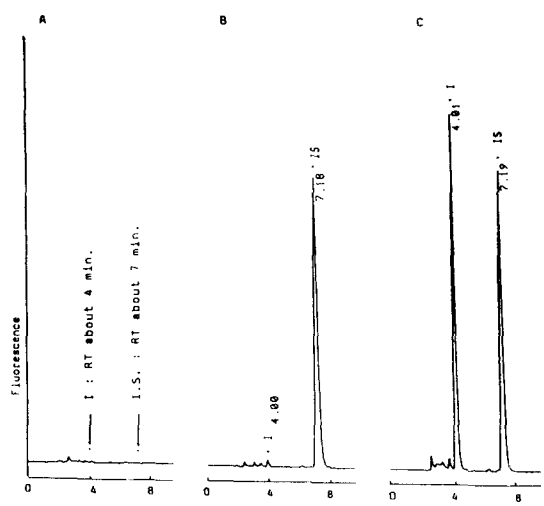


Fig. 6. (A) Chromatogram of pre-dose human urine, amisulpride= I, internal standard=I.S. (B) Chromatogram showing the LOQ in human urine: 0.1 μg ml^{-1}. (C) Chromatogram of a urine sample from a healthy subject administered by i.v. infusion with a single dose (50 mg) of amisulpride; urine collection interval: 6–128 h after administration; level found 6.6 μg ml^{-1} of amisulpride.

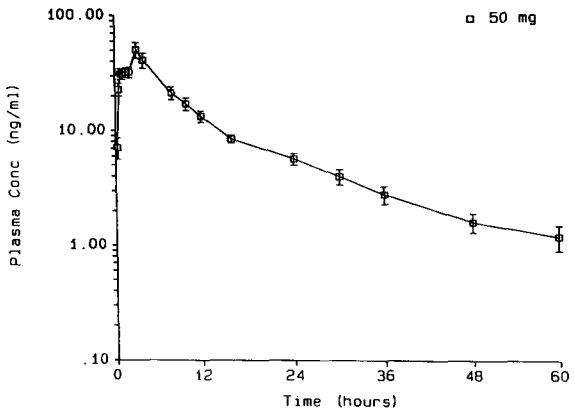


Fig. 7. Mean plasma concentrations–time course (±S.E.M.) of amisulpride in fifteen healthy subjects (on a total number of eighteen) following a single oral o.d. administration of amisulpride.

order to obtain urinary excretion parameters; an sample chromatogram is shown in Fig. 6C. Urinary excretion of unchanged amisulpride (as a percentage of the administered dose) ranged about 23%.

4. Discussion

The aim of the present work was to develop a suitable method for amisulpride determination in bioanalytical laboratories equipped with basic or advanced sample preparation systems. Although time consuming and subject to unpredictable human errors, manual LLE procedure still remains the most traditional approach for isolating non polar xenobiotics from biological matrices, in conventional bioanalytical laboratories where sophisticated automatic sample preparation equipments are not available [10–12]. Several robots, on the other hand, are available to partially or fully automate SPE sample preparation processes and analysis, thus improving the quality of the obtained data (by fully standardising the sample preparation steps), and increasing the sample throughput and the productivity of the analytical instruments [13–19]. For these reasons we have developed, besides an LLE method, an automatic SPE sample preparation procedure on ASPEC, which is one of the most popular SPE automatic sample preparator devices. Moreover, the availability of two different sample preparation methods can be useful, in our experience, when it is necessary to solve selectivity problems related to chromatographic interferences derived from xenobiotics, such as in the case of politherapy or drug interaction studies. In order to achieve a suitable sensitivity for the quantitative determination of the compound in human plasma following low-medium dosage treatments (anti-dysthymic and anti-schizophrenic treatments), both LLE and SPE methods deal with a final step where the analytes of interest in ionised form are concentrated in a small volume of an acidic aqueous buffer. The non-eluting power of this aqueous phase allows the injection of a very large amount of the final concentrated extract (150 μl) without the problem of a band broadening effect (on-column focusing technique). Furthermore, fluorescence detection, more selective towards co-extracted interferences than low-wavelength UV detection described in previous methods [8,9], allows a more favourable signal-to-noise ratio and thus a higher sensitivity to be achieved. The stability studies on the analytes of interest in all the SPE analytical fluids (pre-extraction diluted plasma and eluate), carried out for the longest time (24 h) that a sample can spend in each sample processing phase in a sample sequence, demonstrate that no degradation can be expected. Furthermore, the cross-validation with the LLE method, carried out on real unknown plasma samples from a clinical study analysed according to both LLE and SPE methods, demonstrates that automatic SPE can give rise to results that have a precision, accuracy, and reliability equivalent to LLE measurements. These methods have been successfully used during phase I clinical studies and in phase II–III

Table 3
Mean pharmacokinetic parameters (±S.E.M.) of amisulpride in plasma of fifteen healthy subjects (on a total number of eighteen treated) following a single oral o.d. administration of 50 mg of amisulpride

Dose (mg)	T_{max} (h)	C_{max} (ng ml^{-1})	$AUC_{0\rightarrow\infty}$ (ng h ml^{-1})	$t_{1/2\beta}$ (h)
50	2.2±0.3	54.6±7.3	541.0±47.7	13.1±1.6

investigations for assessing a patient's compliance. The LLE method was also used successfully for the determination of unchanged amisulpride in human urine.

References

[1] J.A. Costa and E. Silva, Ann. Psychiatr., 5 (1990) 242.
[2] A. Agnoli, L. Ravizza and R. Torta, Proceedings of the XXXVII Italian Psychiatry Society Congress, Rome, 6–11 February 1989, CIC International Editors, Rome, 1989, p. 3.
[3] F. Josserand and F. Weber, Ann. Psychiatr., 3 (1988) 306.
[4] B. Ziegler, in A.J. Puech (Editor), Amisulpride, Expansion Scientifique Française, Paris, 1989, p. 73.
[5] P. Pichot and P. Boyer, in A.J. Puech (Editor), Amisulpride, Expansion Scientifique Française, Paris, 1989, p. 83.
[6] P. Carnoy, S. Ravard, D. Hervé, J.P. Tassin and P. Soubrié, in A.J. Puech (Editor), Amisulpride, Expansion Scientifique Française, Paris, 1989, p. 25.
[7] A. Dufour and C. Desanti, in A.J. Puech (Editor), Amisulpride, Expansion Scientifique Française, Paris, 1989, p. 43.
[8] M. Bohbot, L. Doare and B. Diquet, J. Chromatogr., 416 (1987) 414.
[9] A.P. De Jong, A.J. Wittebrood, W. Du Chatinier and J. Bron, J. Chromatogr., 419 (1987) 233.
[10] G. Schill, Separation Methods for Drugs and Related Organic Compounds, Swedish Academy of Pharmaceutical Sciences, Stockolm, 1978, p. 182.
[11] M.A. Schwartz and J.A.F. De Silva, in J. Blanchard, R.J. Sawchuk and B.B. Brodie (Editors), Principles and Perspectives in Drug Bioavailability, Karger, Basle, 1979, p. 90.
[12] J.A.F. De Silva, in E. Reid (Editor), Trace-organic Sample Handling, John Wiley, New York, 1980, p. 192.
[13] M. Zief and B. Kiser, Solid-phase Extraction for Sample Preparation, J.T. Baker, 1988.
[14] L. Jordan, LC·GC Int., 6 (1993) 594.
[15] R.A. Felder, in G.W. Fong and S.K. Lam (Editors), HPLC in the Pharmaceutical Industry, M. Dekker, New York, 1991, p. 185.
[16] R.E. Majors and B.D. Holden, LC·GC Int., 6 (1993) 530.
[17] S. Ahuja, in J.D. Winefordner and I.M. Kolthoff (Editors), Trace and Ultratrace Analysis by HPLC, J. Wiley, New York, 1992, p. 121.
[18] R.E. Majors, LC·GC Int., 4 (1991) 10.
[19] R.E. Majors, LC·GC Int., 6 (1993) 130.