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Selective and sensitive determination of amisulpride in human plasma by liquid chromatography–tandem mass spectrometry with positive electrospray ionisation and multiple reaction monitoring

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Abstract

The development of a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with positive electrospray ionisation (ESI(+)) and multiple reaction monitoring (MRM) for the selective and sensitive bioanalytical determination of amisulpride, a substituted benzamide derivative, in human plasma is described. Plasma was cleaned up using a liquid–liquid extraction (diisopropylether:dichloromethane = 1:1 (v/v)) procedure. The chemically related drug sulpiride was used as internal standard (ISTD) and a primary calibration function was established in the concentration range of 0.50–500.52 ng/ml for amisulpride in plasma by triple analysis of the corresponding calibration standards. A linear relationship between concentration and signal intensity (given as peak area ratio analyte/ISTD) was obtained (linear regression: $r = 0.9999$). A lower limit of quantification (LLQ) of 0.50 ng/ml was used during measurement of study plasma samples. Satisfying results of within-day precision (CV = 0.79 to 1.98%) and accuracy (mean relative deviation: −1.68 to 3.58%) and between-day precision (CV = 1.34 to 4.62%) and accuracy (mean relative deviation: −1.73 to −3.77%) as well as of recovery (amisulpride: 81.74 to 84.83%; sulpiride: 58.65%) and selectivity investigations confirmed the high reliability of this established LC-MS/MS method. Sufficient stability of amisulpride in plasma was achieved during freeze–thaw-cycles, for storage periods of 24 h at room temperature and 20 days at <−20 °C as well as in extracts (storage conditions: <−20 °C, 6 days and 7 °C, 6 days) with mean relative deviations between − 2.83 and 2.91%. An example of a pharmacokinetic profile determined after administration of an amisulpride 200 mg dose in a pilot study is given in this paper. A peak plasma concentration (C_{max}) of 522.58 ng/ml was achieved after 3.55 h (t_{max}). Corresponding values of areas under the plasma concentration–time curve (AUC) of 3405.35 ng h/ml ($AUC_{0-\infty}$) and 3306.54 ng h/ml ($AUC_{0-tlast}$) were obtained. The terminal plasma elimination half-life ($t_{1/2}$) was 10.36 h.

Keywords: Amisulpride; Sulpiride; Benzamide derivative; Antipsychotic drug; Human plasma; Liquid chromatography; Tandem mass spectrometry; Positive electrospray ionisation

1. Introduction

Amisulpride, a substituted benzamide derivative, is chemically related to sulpiride and sultopride [1–3] and binds selectively to human cerebral dopaminergic receptor subtypes D_2 (IC_{50} = 21 nM) and D_3 (IC_{50} = 6 nM) in order to effect on dopaminergic neurotransmission by blocking these receptors. Amisulpride does not have any affinity for D_1 receptors or other receptors such as adrenergic, cholinergic, serotonergic or H_1 histaminergic receptors [3–5].

The drug belongs to the so-called atypical antipsychotics and exhibits a dual dopamine receptor-blocking effect at different dose levels [4–6]. Low doses of amisulpride are known to block preferentially presynaptic D_2 and D_3 receptors (facilitating dopamine release and enhancing neurotransmission), whereas higher doses block postsynaptic D_2 and D_3 receptors (inhibiting dopaminergic hyperactivity) [3,5].

Amisulpride demonstrates antischizophrenic, antipsychotic, antidepressant and antidysthymic properties in man [3,5] and is clinically effective at low doses (50–300 mg/day) on the negative symptoms of acute schizophrenic exacerbations [4,5]. High amisulpride doses (400–800 mg/day) inhibit hyperdopaminergic symptomatology and control positive psychotic symptoms [4,5].

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After a single oral dose of 50 mg racemic amisulpride, the drug shows a rapid absorption with an absolute bioavailability of about 50% and a biphasic absorption profile [3–5]. Two distinct plasma concentration peaks are generally observed at about 1 and 3 h (t_{max}) after dosing. Peak plasma concentrations of 38–42 ng/ml (first peak) and of 54–56 ng/ml (second peak) are achieved within about 1 and 3–4 h [3–5] and corresponding AUC values range between 580 and 1400 ng h/ml [3].

Amisulpride shows a large volume of distribution of V_d = 5.8 l/kg (406 l) and weak protein binding (11–17%) [3,5]. The plasma elimination half-life ($t_{1/2,\beta}$) of amisulpride is 7–8 h after i.v. route and about 11–12 h after oral route with a weak increase up to 15.6 h in elderly subjects (remaining within the range of interindividual variability) [3–5]. After a 50 mg oral dose, the renal clearance ranges from about 17–20 l/h and exhibits little variation with the dose administered but correlates linearly with creatinine clearance (the plasma systemic clearance is about 31–42 l/h) [3,5,7]. Amisulpride metabolites are of rather minor relevance, since the drug undergoes minimal metabolism (less than 5% of the dose administered) to inactive metabolites (oxidation of the pyrrolidine ring, *N*-deethylation and hydroxylation) [3–5].

So far, one study has reported about a fast screening procedure for several neuroleptics in plasma using liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization (APCI-LC/MS) [8].

The aim of the present work was to develop and validate a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with positive electrospray ionisation ((ESI(+)) using a triple stage quadrupole mass spectrometer arrangement for the selective and sensitive quantification of amisulpride in human plasma. This method is assumed to be advantageous for a reliable, fast and sensitive measurement of amisulpride plasma concentrations for pharmacokinetic studies [4,5,9].

2. Experimental

2.1. Chemicals and reagents

The standard substance amisulpride (4-amino-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulfonyl)-2-anisamide: CAS no.: 71675-85-9; purity: 100%), was obtained from LGC Promochem, Wesel, Germany. The internal standard (ISTD) sulpiride ((*RS*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-5-sulfamoylbenzamide; CAS no.: 15676-16-1; purity: 100%) was provided by Sigma, Deisenhofen, Germany. Molecular structures of both substances are shown in Fig. 1.

The reagents ammonium formate and potassium carbonate were both purchased from Fluka (Buchs, Switzerland). Formic acid (98–100%), acetonitrile (Lichrosolv), methanol (Lichrosolv), diisopropylether and dichloromethane were obtained from Merck (Darmstadt, Germany). Human pool plasma (formula A USP XX) was provided from German Red Cross Centre, Ulm, Germany. Purified water demin. was supplied from a Millipore System.

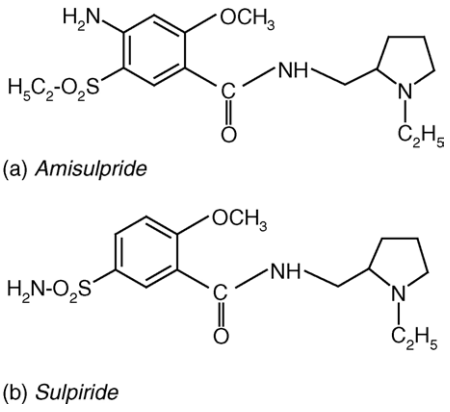

Fig. 1. Molecular structures of amisulpride (a) and internal standard sulpiride (b).

2.2. Solutions

Stock solutions were prepared in methanol for amisulpride calibration (c = 103,200 ng/ml), for quality control (c = 102,800 ng/ml) as well as for ISTD sulpiride (c = 109,600 ng/ml). Additionally, a working solution of sulpiride resulted from dilution of ISTD stock solution with methanol (c = 999.55 ng/ml). For stability tests, further stock solutions were prepared for amisulpride (c = 100,800 ng/ml) as well as for sulpiride (c = 105,600 ng/ml).

2.3. Sample preparation

Frozen plasma samples were thawed in a water bath (20 °C) and mixed for 10 s. To 0.25 ml of plasma 50 μl ISTD and 500 μl 1 M K_2CO_3 were added and homogenized. This mixture was extracted with 4 ml of diisopropylether: dichloromethane = 1:1 (v/v) for 20 min. After centrifugation (5 min, 1000 × *g*), the upper organic layer was removed, placed into another vial and evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 500 μl of the LC-eluent (see Section 2.5). Finally, 10 μl of the solution were used for subsequent LC-MS/MS analysis.

2.4. Apparatus

For LC-MS/MS investigations a triple stage quadrupole mass spectrometer (micromass Quattro Micro setup with API-source and ESI-probe, micromass, Altrincham, UK) together with an LC device (Jasco HPLC pump PU 1580, Jasco Gradient Unit LG 1580-04, Jasco Degasser DG 1580-54, Jasco Germany, Groß-Umstadt, Germany) was used.

2.5. Chromatographic and MS/MS conditions

Chromatographic separation of the analyte amisulpride and the ISTD sulpiride was carried out with a Phenomenex Synergi Polar-RP analytical column (4 μm particle size, 75 mm × 4.6 mm i.d.; Merck, Darmstadt, Germany) in the isocratic mode with 5 mM ammonium formate and acetonitrile (30:70, v/v) as eluent, a flow rate of 0.8 ml/min and an injection volume of 10 μl.

MS/MS conditions were as follows: positive electrospray ionisation (ESI(+), source temperature: 100 °C, desolvation gas: 400 l/h (nitrogen), collision gas: helium. The detection of precursor/product ion pairs was carried out by multiple reaction monitoring (MRM; two mass pairs, two channels). For increased sensitivity and enhanced selectivity, collision-induced dissociation (CID) was employed with helium as collision gas for generation of product ions from precursor ions (gas-cell-pressure: 2.3 Pa). The instrumental parameters were as follows: the cone voltage was set to 40 V for both amisulpride and ISTD sulpiride; the collision energy was set to 100 eV for amisulpride and 95 eV for sulpiride; the dwell-time was set to 0.2 s for both amisulpride and sulpiride. The first quadrupole was set to monitor protonated molecules $(M + H)^+$ at mass/charge ratios (m/z) 370.1 (amisulpride) and m/z 342.1 (sulpiride). Generated product ions were monitored in the third quadrupole at m/z 241.9 (amisulpride) and m/z 112.0 (sulpiride).

2.6. Analysis of calibration standards

In order to establish a primary calibration curve, a set of eight calibration standards (covering a concentration range from 0.50 to 500.52 ng/ml) was prepared in human pool plasma and analysed in triplicate. Calibration of analyte was done by establishing a linear regression function after $1/x$ weighting of the analyte/ISTD peak area ratio versus analyte concentration relationship. Lower limits of detection (LLD) and quantification (LLQ) were calculated according to the procedure of Funk et al. [10]. Thereby, the results of analysis of the three lowest calibration standards with concentrations of 0.50–2.00 ng/ml were used to determine LLD and LLQ. Additionally, the reproducibility was determined based on threefold determination of each calibration standard.

A validation of the dilution procedure for plasma samples containing amisulpride concentrations outside the calibration range was carried out by measurement of calibration standard CD (nominal concentration: 1044.29 ng/ml). Correct dilution was checked by measurement of five diluted samples, back-calculation of concentrations and comparison with nominal amisulpride concentration.

2.7. Selectivity

The selectivity of the method was tested by measurement of six blank plasma samples of different origin. Selectivity was verified by the fact that MRM detected unambiguously pairs of signals of product ions m/z 241.9 (from precursor ions m/z 370.1) for amisulpride and m/z 112.0 (from precursor ions m/z 342.1) for sulpiride. The selective determination of amisulpride was illustrated by mass spectra and by representative two-channel MRM chromatograms.

2.8. Quality control

In order to describe within-day and between-day precision and accuracy, three different concentrations of QC samples, one upper concentration (QC-1: 411.20 ng/ml), one in the intermediate range (QC-2: 13.36 ng/ml and one near the lower limit of quantification (QC-3: 0.67 ng/ml) were applied. To evaluate precision and accuracy, coefficients of variation (CV (%)) as well as relative deviations between measured mean values and nominal values were determined.

2.9. Stability tests

Stability tests were performed for analyte-spiked plasma samples under various conditions (repeated freezing and thawing; storage at room temperature for 24 h) by threefold determinations of two calibration standards with different concentrations (upper and intermediate calibration range). Extended stability was investigated after storage at <−20 °C for 20 days by comparison with freshly prepared calibration standards. Thereby, two calibration standards were freshly prepared from the stock solution and implemented into the whole set of calibration standards prepared at study start.

The stability of extracts was confirmed for a storage period of 6 days at <−20° C or 7 °C (cooled autosampler) by comparison with freshly prepared extracts (from calibration standards).

Finally, the stability of amisulpride and ISTD sulpiride in the stock solutions was shown after 21 days storage at <−20 °C by comparison with data resulting from freshly prepared stock solutions.

2.10. Recovery

The recovery of amisulpride was investigated as follows: individual peak area ratios, obtained after triple analysis of three different (ISTD-spiked) recovery samples representing the high, intermediate and low calibration range of the method (i.e. amisulpride concentrations in ng/ml: 500.52; 20.02; 0.50) were measured and related to arithmetic mean peak area ratios of threefold analysed recovery references. Thereby, three different calibration standards were used as recovery samples and treated (sample work up) as described in Section 2.3. For preparing recovery references, recovery working solutions were added to the residue after clean-up of ISTD-spiked blank plasma samples.

Recovery of ISTD sulpiride was determined by mean of an analogous procedure, employing one calibration standard of the intermediate range to which the ISTD was added either before or after sample clean-up.

Calculation of the extraction efficiency was achieved by relating data of recovery samples (analyte or ISTD) to those obtained with recovery references (analyte or ISTD).

2.11. Pharmacokinetic evaluation

Determination of the pharmacokinetic parameters was performed by non-compartmental assessment of data using the computer program WinNonlin (WinNonlin V03.1A, Pharsight Corporation, California, USA). Mean and individual concentration–time profiles (both in the linear and loglinear scale) were generated and used to determine the maximum plasma concentrations (C_{max}) and the time required to attain these maximum concentrations (t_{max}). The area under the plasma

concentration–time curve from $t = 0$ h to the time point of the last quantifiable plasma concentration C_{last} ($AUC_{0-tlast}$) was calculated by the linear trapezoidal rule. The elimination rate constant λ was estimated by log–linear regression of concentrations observed during the terminal phase of elimination. $AUC_{0-\infty}$ was calculated by extrapolation to infinity of $AUC_{0-tlast}$ according to the equation $AUC_{0-\infty} = AUC_{0-tlast} + C_{last}/\lambda$.

Additionally, λ and the terminal plasma elimination half-life $t_{1/2}$ ($\ln 2/\lambda$) were determined.

3. Results

3.1. Calibration function and sensitivity of the method

A linear relationship between concentration and signal intensity (given as peak area ratio analyte/ISTD) was obtained for amisulpride in the range of 0.50–500.52 ng/ml ($r = 0.9999$). The calibration line resulting after $1/x$-weighting and linear regression was described by the following equation: $y = (0.1153 \times 10^{-03} \pm 0.9722 \times 10^{-02}) + (0.1763 \times 10^{-01} \pm 0.5104 \times 10^{-04})x$; $r = 0.9999$ (y = peak area ratio, x = plasma concentration in ng/ml, r = coefficient of correlation). The following data were obtained by application of the method described by Funk et al. [10]. LLD and LLQ for determination of amisulpride in plasma were calculated to be 0.13 and 0.20 ng/ml. For practical laboratory purposes, an LLQ of 0.50 ng/ml was used during measurement of study plasma samples with unknown concentrations. For plasma samples containing amisulpride concentrations outside the calibration range, correct dilution was verified by a validation of the dilution procedure as described in Section 2.6.

Considering the results of reproducibility of determination of amisulpride in plasma, the coefficients of variation (CV (%)) for each concentration were calculated from peak area ratios and were well within an acceptable range between 0.47 and 3.38%. Furthermore, values of precision (relative standard deviation) and accuracy (mean relative deviation) of 3.01 and 1.29% were obtained from back-calculated values at LLQ and confirmed the reliability of the method.

With respect to daily recalibration, variations in back-calculated concentrations (given as CV (%)) of all recalibrations were between 0.85 and 5.38%. For all calibration standards, the mean relative deviations between back-calculated and nominal values were between −2.91 and 3.67%.

3.2. Selectivity

The selectivity of the method was tested during method validation by measurement of six blank plasma samples of different origin, without detection of interferences between signals of matrix constituents and signals of spiked calibration standards (data not shown). Additionally, analysis of blank plasma or ISTD-spiked blank plasma confirmed the method's selectivity (data not shown).

Selectivity was ensured by multiple reaction monitoring (MRM) which allows selective determination of product ions of m/z 241.9 (for amisulpride) and of m/z 112.0 (for sulpiride), generated by collision induced dissociation (CID) of precursor ions with 370.1 (amisulpride) and m/z 342.1 (sulpiride). Illustrative product ion mass spectra of amisulpride and sulpiride generated from corresponding $[M + H]^+$-precursor ions are presented in Fig. 2a and b. Representative two-channel MRM chromatograms of a calibration standard (E–H, $c = 0.50$ ng/ml) and MRM chromatograms of a plasma sample of a subject after administration of a 200 mg oral dose (i.e., 12 h post-dose sample) without ISTD-spike are given in Fig. 3a and b and confirmed the method's specificity.

3.3. Recovery

Results of recovery demonstrated that plasma preparation proceeded uniformly throughout the calibration range. Recovery of amisulpride in ISTD-spiked plasma samples was 81.74 ± 5.22, 84.83 ± 3.18 and 81.74 ± 1.91% for the low, intermediate and high concentration range. For ISTD sulpiride, recovery was calculated to be 58.65 ± 2.20%.

3.4. Analytical quality assurance

Satisfying results of within-day precision and accuracy were obtained from five-fold determination for each concentration during the method validation procedure (Table 1). Relative standard deviations (CV (%); $n = 5$) for QC determination between 0.79 and 1.98% reflect the mean within-day precision of the present method. As a measure of within-day accuracy, mean relative deviations ($n = 5$) of −1.68 to 3.58% between measured and nominal QC-concentrations were determined.

In the same manner, the between-day precision and accuracy was demonstrated with data from five-fold determinations for each concentration on 3 different days each (Table 2). Thereby, relative standard deviations (CV (%); $n = 15$) for plasma QC determinations between 1.34 and 4.62% reflect the mean between-day precision, whereas for the between-day accuracy mean relative deviations ($n = 15$) of −1.73 to −3.77% were determined. Also these results fulfilled the tolerance range of acceptable deviations.

3.5. Stability of amisulpride and sulpiride

Stability experiments performed indicated a sufficient stability of amisulpride in plasma during freeze–thaw-cycles as well as for a storage period of 24 h at room temperature (Table 3). Extended stability of amisulpride in plasma was shown for a storage period of 20 days at <−20 °C (Table 3). As given in Table 4, stability of amisulpride in extracts was demonstrated during limited stay in the freezer (<−20 °C, 6 days) and in the cooled autosampler (7° C, 6 days). Finally, stability of amisulpride and sulpiride in the stock solution was confirmed for a storage period of 21 days at <−20 °C (Table 5). As shown in Tables 3–5, the relative deviations between determined and nominal concentrations respectively between peak area ratios of different measurements (stability of stock solutions) remained within the accepted range of accuracy of the method in each case.

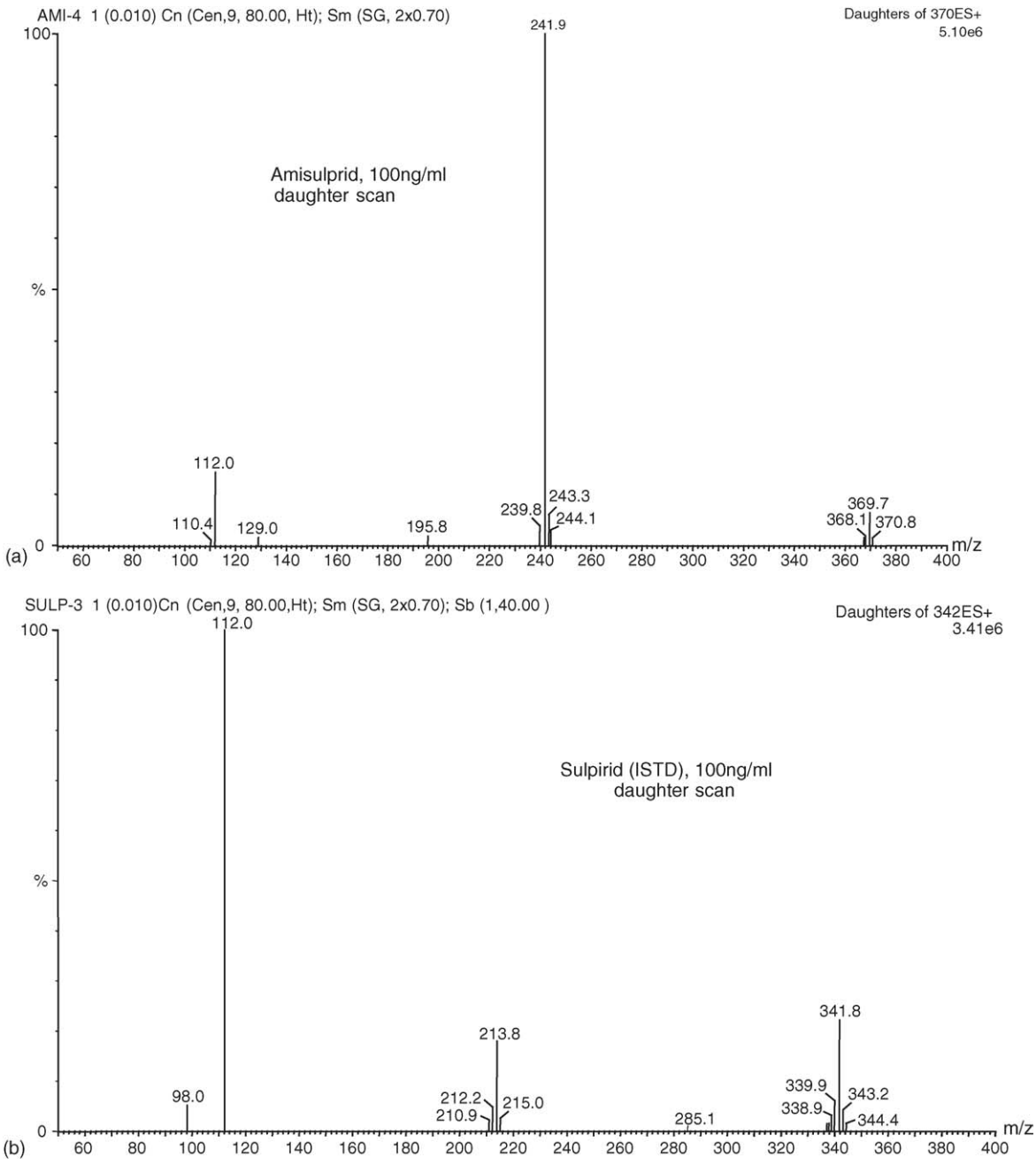

Fig. 2. (a) product ion mass spectrum of amisulpride ($[M + H]^+ = 370.1$) with base peak at m/z 241.9; (b) product ion mass spectrum of sulpiride ($[M + H]^+ = 342.1$) with base peak at m/z 112.0; 50–400 atomic mass units.

3.6. Pharmacokinetic application

Fig. 4 shows the mean concentration–time characteristic in both the linear (Fig. 4a) and the log–linear (Fig. 4b) scale (±standard error of the mean; S.E.M.) of amisulpride in plasma obtained after oral administration of a 200 mg dose ($n = 10$ subjects). A peak plasma concentration (C_{max}) of 522.58 ng/ml was achieved after 3.55 h (t_{max}). Correspond-

Table 1
Summary of within-day precision and accuracy of amisulpride ($n = 5$), as assessed during method validation with quality control samples

QC-samples (ng/ml)	Mean value ± S.D. (ng/ml)	CV (%)	Mean relative deviation ± S.D. (%)	Total number of QC-samples
411.20	417.76 ± 3.28	0.79	1.60 ± 0.80	5
13.36	13.14 ± 0.26	1.98	−1.68 ± 1.94	5
0.67	0.69 ± 0.01	1.45	3.58 ± 1.70	5

S.D., standard deviation; CV (%), relative standard deviations.

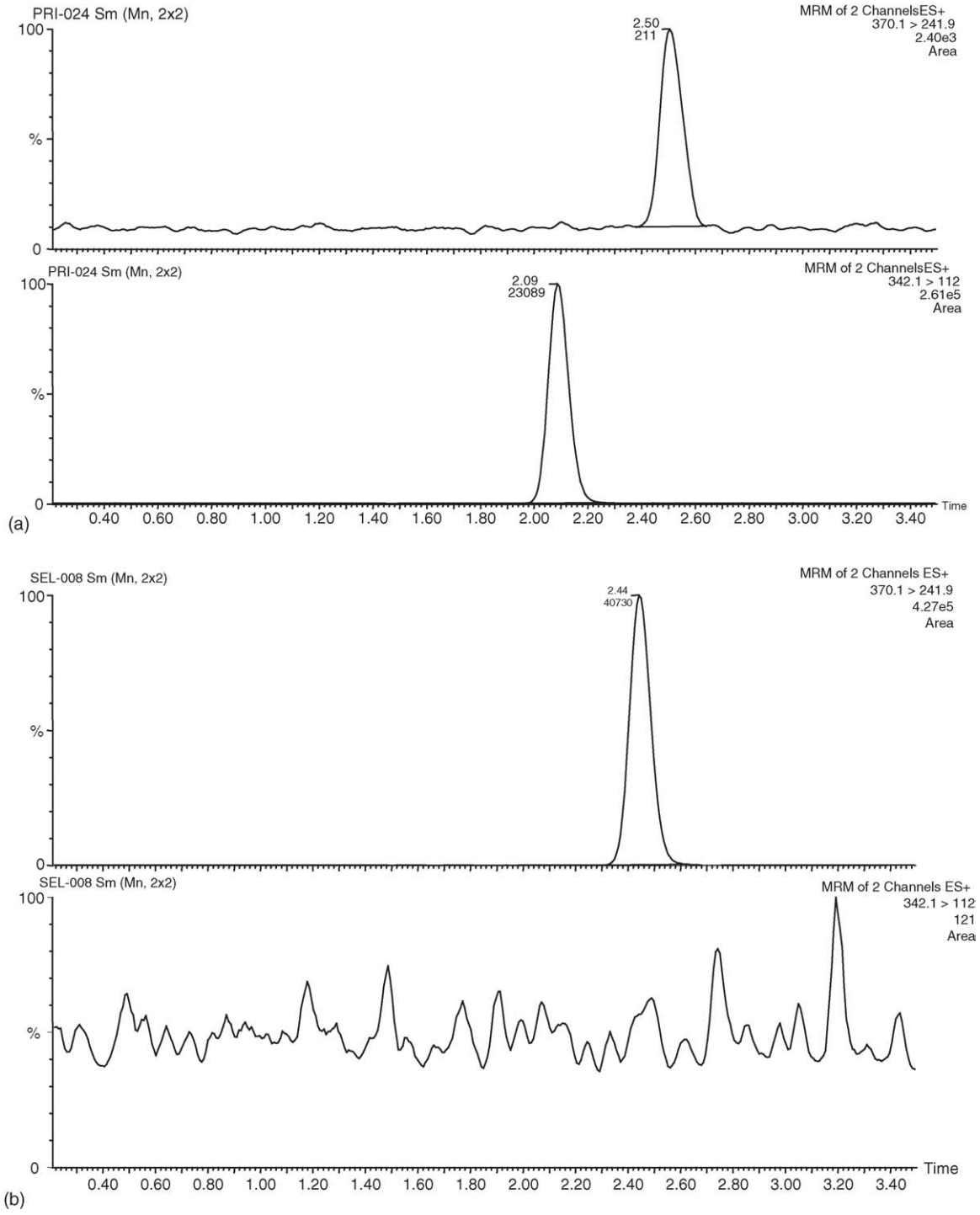

Fig. 3. (a) MRM chromatograms of an ISTD-spiked calibration standard E–H (c = 0.50 ng/ml), ISTD signal see lower chromatogram at retention time of 2.09 min (sulpiride), analyte signal see upper chromatogram at retention time of 2.50 min (amisulpride); (b) MRM chromatograms of a plasma sample of a subject after administration of a 200 mg oral dose (i.e., 12 h post-dose sample) without ISTD-spike, analyte signal at retention time of 2.44 min (upper chromatogram).

Table 2
Summary of between-day precision and accuracy (3 days; n = 15) of amisulpride, as assessed during method validation with quality control samples

QC-samples (ng/ml)	Mean value ± S.D. (ng/ml)	CV (%)	Mean relative deviation ± S.D. (%)	Total number of QC-samples
411.20	404.10 ± 5.42	1.34	−1.73 ± 1.32	15
13.36	12.86 ± 0.23	1.79	−3.77 ± 1.70	15
0.67	0.65 ± 0.03	4.62	−2.59 ± 5.12	15

S.D., standard deviation; CV (%), relative standard deviations.

Table 3
Stability tests of amisulpride in plasma; three-fold determinations were performed with two calibration standards with nominal concentrations of 500.52 and 20.02 ng/ml and two freshly prepared calibration standards with nominal concentrations of 499.97 and 20.00 ng/ml

Freeze–thaw-cycles	Nominal value 500.52 ng/ml		Nominal value 20.02 ng/ml	
	Mean observed value ± S.D. (ng/ml)	Mean relative deviation ± S.D. (%)	Mean observed value ± S.D. (ng/ml)	Mean relative deviation ± S.D. (%)
First thaw ($n = 3$)	493.95 ± 4.67	−1.31 ± 0.93	19.45 ± 0.05	−2.83 ± 0.25
Third thaw ($n = 3$)	504.16 ± 24.87	0.73 ± 4.97	19.61 ± 0.35	−2.03 ± 1.73
Storage at room temperature, 24 h ($n = 3$)	495.44 ± 3.35	−1.02 ± 0.67	19.82 ± 0.21	−0.98 ± 1.07
	Nominal value 499.97 ng/ml		Nominal value 20.00 ng/ml	
Storage at <−20° C, 20 days ($n = 3$)	499.74 ± 11.26	−0.05 ± 2.25	19.64 ± 0.49	−1.78 ± 2.45

S.D., standard deviation.

Table 4
Stability tests of amisulpride in extracts; three-fold determinations were performed with two calibration standards with nominal concentrations of 500.52 and 20.02 ng/ml

	Nominal value 500.52 ng/ml		Nominal value 20.02 ng/ml	
	Mean observed value ± S.D. (ng/ml)	Mean relative deviation ± S.D. (%)	Mean observed value ± S.D. (ng/ml)	Mean relative deviation ± S.D. (%)
Storage at <−20° C, 6 days ($n = 3$)	515.10 ± 19.85	2.91 ± 3.96	19.61 ± 0.33	−2.05 ± 1.66
Storage in the autosampler, 7° C, 6 days ($n = 3$)	497.29 ± 7.49	−0.65 ± 1.50	19.68 ± 0.18	−1.72 ± 0.88

S.D., standard deviation.

Table 5
Stability of stock solutions of amisulpride and ISTD sulpiride after a storage of 21 days at <−20 °C; measurements ($n = 3$) were carried out with working solutions

	Stored stock solution		Freshly prepared stock solution		
	Mean value ± S.D. (PAR)	CV (%)	Mean value ± S.D. (PAR)	CV (%)	Mean relative deviation ± S.D. (%)
Amisulpride ($n = 3$)	2.3311 ± 0.0310	1.33	2.1694 ± 0.0249	1.15	7.45 ± 1.43
Sulpiride ($n = 3$)	0.4290 ± 0.0057	1.32	0.4397 ± 0.0047	1.06	−2.43 ± 1.29

S.D., standard deviation; CV (%), relative standard deviations; PAR, peak area ratio.

ing AUC values as measure of extent of absorption were 3405.35 ng h/ml ($AUC_{0-\infty}$) and 3306.54 ng h/ml ($AUC_{0-tlast}$). The terminal plasma elimination half-life ($t_{1/2}$) was 10.36 h. Furthermore, values of the percentage residual area (AUC%-extrapol) demonstrated the validity of the extrapolation procedure. Thereby, individual AUC%-extrapol values were between 0.34 and 6.37% (data not shown). Results of the main pharmacokinetic parameters are summarized in Table 6.

Table 6
Main pharmacokinetic parameters (±S.D.) of amisulpride after oral administration of a 200 mg dose; $n = 10$ subjects

$AUC_{(0-\infty)}$ (ng h/ml)	3405.35 ± 830.61
$AUC_{(0-tlast)}$ (ng h/ml)	3306.54 ± 806.62
C_{max} (ng/ml)	522.58 ± 143.43
t_{max} (h)	3.55 ± 1.14
λ (1/h)	0.0722 ± 0.0218
$t_{1/2}$ (λ) (h)	10.36 ± 2.98

4. Discussion

The present results of within-day and between-day precision and accuracy confirmed the high reliability of this established LC-MS/MS method. Our results were in good agreement with values obtained in other studies using HPLC (with UV or fluorescence detection) [2,9,11,12] or LC-MS (APCI-LC-MS) [8] methods for quantification of amisulpride. Also stability experiments indicated a sufficient stability of amisulpride in plasma and in extracts after sample preparation, with acceptable mean relative deviations under different storage conditions.

Pharmacokinetic data obtained in a pilot study were compared with published pharmacokinetic profiles of amisulpride after a 50 mg oral dose. Pharmacokinetic parameters such as time to reach maximum plasma concentration ($t_{max} = 3.55$ h) as well as the terminal plasma elimination half-life ($t_{1/2} = 10.36$ h) were well comparable with values from literature [3–5]. Also a biphasic absorption behaviour with two distinct plasma concentration peaks, as already known for amisulpride [3–5], was evident in individual concentration–time curves (not shown)

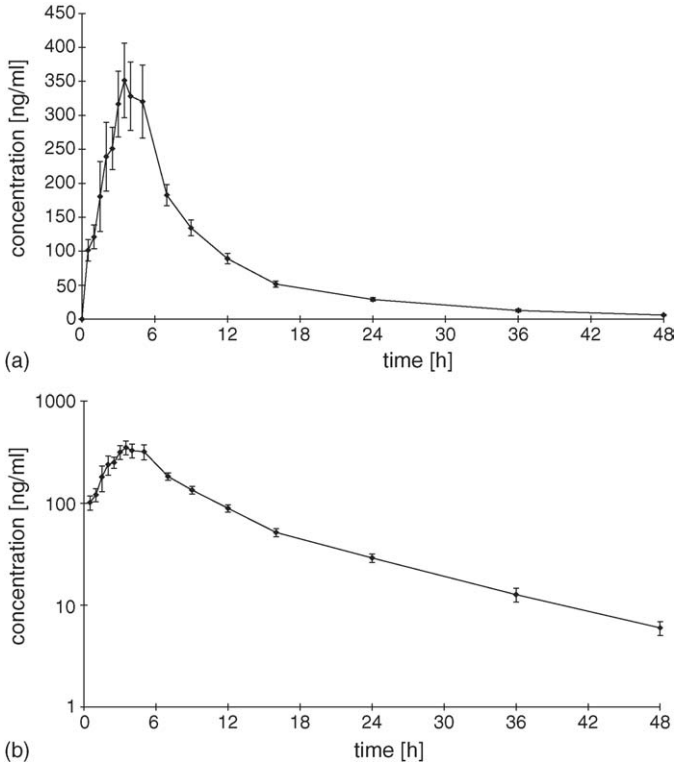

Fig. 4. Pharmacokinetic profile (±S.E.M.) of amisulpride after administration of a 200 mg oral dose: (a) linear plot, (b) log–linear plot.

and is also indicated in the mean concentration–time curve in Fig. 4. The peak plasma concentration (C_{max}) of 522.58 ng/ml, obtained after an oral dose of 200 mg, was higher than theoretically expected under the assumption of linear pharmacokinetics and for healthy volunteers with normal renal function [5]. However, this phenomenon should be discussed carefully since most pharmacokinetic data have been reported for 50 mg dosages so far [3–6].

Generally, interpretation of the mass spectra data might be hampered by interferences from metabolites. However, this might play a rather minor role for the present study, since amisulpride is practically not metabolised [3–5]. Moreover, the specificity of the method was verified by the fact that MRM detected unambiguously pairs of signals of product ions for amisulpride and the ISTD sulpiride.

As another analytical issue, ion suppression effects should be discussed critically with respect to the use of mass spectrometry with electrospray ionisation for quantification of analyte concentrations in plasma. Although no separate experiments have been performed for the present study to investigate a possible influence of matrix effects, the selectivity and the reliability of the method were demonstrated. Acceptable variations in back-calculated concentrations with good results for mean relative deviations of all calibration standards with known analyte concentrations were obtained for the method validation data as well as for the validation data generated during study sample measurements.

Nevertheless, the liquid–liquid extraction procedure might be more advantageous as compared with protein precipitation in order to avoid the influence of unknown matrix constituents on the analyte signal. Moreover, the selectivity of the method was tested during method validation by measurement of six blank plasma samples of different origin, without detection of interferences between signals of matrix constituents and signals of spiked calibration standards. Additionally, analysis of blank or ISTD-spiked blank plasma, of calibration standards, as well as of non-ISTD-spiked subject plasma samples confirmed the method's selectivity.

Altogether, our LC-MS/MS method for determination of amisulpride can be considered sensitive and reliable, especially with regard to quantification of low amisulpride concentrations in human plasma. Moreover, the examples of mass chromatograms illustrate the level of selectivity achieved and confirm a selective and valid determination of the amisulpride in plasma. In addition to the method validation data, the obtained pharmacokinetic data underline the high reliability of the established LC-MS/MS method.

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