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Determination of the enantiomers of ketamine and norketamine in human plasma by enantioselective liquid chromatography–mass spectrometry

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Abstract

A sensitive enantioselective liquid chromatographic assay with mass spectrometric detection has been developed and validated for the simultaneous determination of plasma concentrations of (*R*)- and (*S*)-ketamine, and (*R*)- and (*S*)-norketamine. The compounds were extracted from human plasma using solid-phase extraction and then directly injected into the LC–MS system for detection and quantification. Enantioselective separations were achieved on a liquid chromatographic chiral stationary phase based upon immobilized α_1-acid glycoprotein (the Chiral AGP column). The separations were achieved using a mobile phase composed of 2-propanol–ammonium acetate buffer (10 m*M*, pH 7.6) (6:94, v/v), a flow-rate of 0.5 ml/min and a temperature of 25 °C. Under these conditions, the analysis time was 20 min. Detection of the ketamine, norketamine and bromoketamine (internal standard) enantiomers was achieved using selected ion monitoring at *m*/*z* 238.1, 224.1 and 284.0, respectively. Extracted calibration curves were linear from 1 to 125 ng/ml per enantiomer for each analyte with correlation coefficients better than 0.9993 and intra- and inter-day RSDs of less than 8.0%. The method was applied to samples from a clinical study of ketamine in pain management.

Keywords: Enantiomer separation; Ketamine; Norketamine

1. Introduction

Ketamine (Ket) is a dissociative anaesthetic agent that has also been shown to induce analgesia by non-opioid mechanisms [1–3]. Ket is a chiral molecule that is commercially available as a racemic mixture, i.e., a 50:50 mixture of its enantiomers, (*R*)-Ket and (*S*)-Ket, Fig. 1. However, (*R*)- and (*S*)-Ket have significantly different pharmacodynamic activities [4,5] as the therapeutic potency of (*S*)-Ket is 2–4-times greater than the (*R*)-enantiomer [6,7] and (*S*)-Ket is a more potent analgesic agent than (*R*)-Ket [2]. However, the post-hypnotic stimulatory properties and agitated behavior are associated with (*R*)-Ket [4,6,7].

It has also been reported that (*R*)- and (*S*)-Ket have significantly different pharmacokinetic profiles [4,5]. This may be due to the fact that Ket undergoes extensive first pass metabolism involving N-demethylation to norketamine (Fig. 1) [6,8–10], fol-

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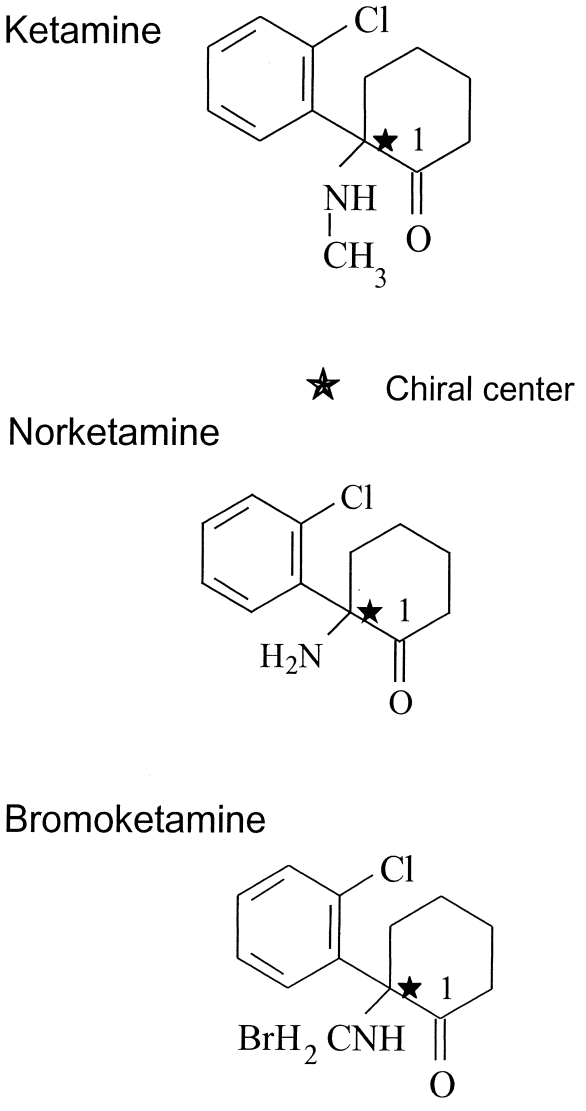

Fig. 1. Chemical structures of ketamine, norketamine and bromoketamine.

lowed by further oxidation to 4-, 5-, and 6-hydroxy-norKet derivatives [9]. (*R*)- and (*S*)-norKet have similar activity to those of the parent drug, but with a shorter duration of action [11,12].

Several enantioselective analytical methods have been reported for the analysis of Ket and norKet in plasma. These include assays based on high-performance liquid chromatography (HPLC) using UV detection [13–18] or by gas chromatographic methods [19–23]. The above methods were adequate for pharmacokinetic studies of ketamine. For example, the method described by Yanagihara et al. [18] was capable of measuring plasma Ket levels for 6 h and norKet levels for 1 h after a 50 mg oral dose of racemic-Ket. The lower limits of detection were 5 ng/ml for Ket and 10 ng/ml for norKet. In addition, the retention times for the norKet enantiomers were 25 and 27 min.

Ket is currently undergoing trials as an analgesic agent for the treatment of neuropathic pain. The clinical protocol utilized in this study involved a continuous infusion of racemic-Ket designed to reach plasma concentrations of 60 to 120 ng/ml. It was assumed that the 60 ng/ml aim of the study would produce Ket and norKet plasma levels at or below the previously reported lower limits of detection. Thus, the objective of this study was to develop and validate a highly sensitive enantioselective bioanalytical method to analyze the plasma samples associated with this clinical study.

This manuscript reports the development of the required assay, which is based upon enantioselective liquid chromatography–mass spectrometry (LC–MS) with single ion monitoring (SIM). The lower limit of quantification, LLOQ, of the LC–MS method is 1 ng/ml per Ket enantiomer versus the previously reported LLOQ of 5 ng/ml per enantiomer [18] and the lower limit of detection (LLOD) is 0.25 ng/ml versus the previously reported LLOD of 2.0 ng/ml [16]. The method is reproducible and accurate and has been applied to the analysis of plasma samples from the clinical study.

2. Experimental

2.1. Chemicals and reagents

(+)-(*S*)-Ketamine [(*S*)-Ket]; (−)-(*R*)-ketamine [(*R*)-Ket]; (+)-(*S*)-norketamine [(*S*)-norKet]; (−)-(*R*)-norketamine [(*R*)-norKet]; (*R*,*S*)-ketamine [(*R*,*S*)-Ket]; (*R*,*S*)-norketamine [(*R*,*S*)-norKet] and (*R*,*S*)-bromoketamine [(*R*,*S*)-BrKet] were a kind gift from Dr. Thomas Wolfe from Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA). HPLC-grade 2-propanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-reagent grade ammonium acetate was obtained from J.T. Baker

(Phillipsburg, NJ, USA). Ultra-pure water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). Pooled drug-free human plasma was received from the Apheresis Unit, National Institute on Aging (Baltimore, MD, USA). The extraction cartridges were Oasis HLB 1 ml, 30 mg and Oasis MCX 3 ml, 60 mg (Waters, Milford, MA, USA) and Bond Elut-C_{18}, 1 ml, 50 mg (Varian, Harbor City, CA, USA).

2.2. Apparatus

The analytical system consisted of a Series 1100 LC–MS system (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser (G1322 A), a binary pump (1312 A), an autosampler (G1313A), a thermostated column compartment (G1316 A); a mass-selective detector (G1946 B) supplied with an atmospheric pressure ionization electrospray (API-ES) interface and an on-line nitrogen generation system (Whatman, Haverhill, MA, USA). The chromatographic system was interfaced to a 250 MHz Kayak XA computer (Hewlett-Packard, Palo Alto, CA, USA) using ChemStation software (Rev A.08.03[847], 1990–2000, Hewlett-Packard).

The extractions were performed using a 12-port vacuum manifold, PrepSep from Fisher Scientific (Fair Lawn, NJ, USA).

2.3. Chromatographic conditions

The enantioselective separations of (*R*)- and (*S*)-Ket, (*R*)- and (*S*)-norKet and (*R*)- and (*S*)-BrKet were accomplished using a guard Chiral-AGP column (10×2.0 mm I.D., 5 μm) and an analytical column Chiral-AGP (100×4.0 mm I.D., 5 μm) purchased from Advanced Separation Technologies (Whippany, NJ, USA). The mobile phase consisted of 2-propanol–ammonium acetate buffer [10 m*M*, pH 7.6 (adjusted with ammonium hydroxide)] (6:94, v/v). The flow-rate was 0.5 ml/min, the injection volume was 20 μl and the column temperature was kept at 25 °C.

Mass spectra were recorded using a full scan in the positive ion mode, with a scan range from *m*/*z* 100 to 600. SIM was used to quantitate the target compounds. The chromatograms were monitored at *m*/*z* 238.1 (Ket), *m*/*z* 224.1 (norKet) and *m*/*z* 284.0 (BrKet).

2.4. Optimization of the mass-selective detector parameters

The sensitivity of the Ket and norKet signals were primarily dependent on the MS experimental parameters. In order to identify the optimized condition, the following MS parameters were investigated: fragmentation voltage (50–80 V), capillary voltage (1000–3000 V), nebulizer pressure (40–55 p.s.i.g.; 1 p.s.i.=6894.76 Pa) and drying gas temperature (200–350 °C).

2.5. Preparation of stock solutions

Concentrated stock solutions of (*R*,*S*)-Ket (1.00 mg/ml), (*R*,*S*)-norKet (1.00 mg/ml) and (*R*,*S*)-BrKet (0.40 mg/ml) were prepared in ultra-pure water, placed in capped polypropylene tubes, wrapped in aluminum foil and stored at −20 °C. Spiking standard solutions for the calibration curve and quality control (QC) samples were made by serial dilutions with ultra-pure water starting with their respective concentrated stock solution. These spiking standards were placed in capped polypropylene tubes, wrapped in aluminum foil and stored at 4 °C.

2.6. Preparation of calibration curve and quality control standards

The determinations of Ket and norKet were based on the internal standard method, using BrKet as internal standard. Calibration and QC standards were prepared daily by adding 50 μl of the corresponding spiking standard solution containing Ket, norKet and BrKet to 450 μl drug-free plasma. The seven-point calibration curve ranged from 1.0 to 125.0 ng/ml (1.0, 5.0, 25.0, 50.0, 75.0, 100.0, 125.0 ng/ml) for each ketamine and norketamine enantiomer and a constant concentration of 100.0 ng/ml for each bromoketamine enantiomer. The QC standards were 5.0 ng/ml [low quality control (LQC)], 50.0 ng/ml [medium quality control (MQC)] and 100.0 ng/ml

[high quality control (HQC)]. The concentrations are given per enantiomer.

2.7. Extraction procedure

Sample extraction was performed using solid-phase extraction (SPE). To 15-ml conical polypropylene capped tubes, were added 450-μl aliquots of plasma samples and 50 μl internal standard. The resulting solutions were made alkaline by the addition of 0.5 ml of ammonium acetate buffer (10 m*M*, pH 9.5), vortex-mixed for 2 min, and centrifuged at 1250 *g* (4 °C) for 15 min. The plasma samples were then added to preconditioned 1-ml SPE cartridges (Oasis HLB). The cartridges were conditioned with 1 ml of methanol followed by 1 ml of water and then 1 ml of ammonium acetate buffer (10 m*M*, pH 9.5). After addition of the plasma, the conditioned cartridges were washed with 1 ml of water and the retained compounds were eluted with 0.5 ml methanol. The methanol eluents were transferred to 300-μl autosampler vials and 20-μl aliquots were injected onto the LC–MS system.

2.8. Validation

The intra- and inter-day validation studies for precision and accuracy were performed in quintuplicate with QC standards at 5.0, 50.0 and 100.0 ng/ml per enantiomer. The analyses were carried out over a period of 3 days for the inter-day validation. The curves were constructed by plotting the peak height ratio (*R*)-Ket/(*R*)-BrKet, or (*S*)-Ket/(*S*)-BrKet, or (*R*)-norKet/(*R*)-BrKet or (*S*)-norKet/(*S*)-BrKet against its concentration.

Extraction efficiencies (% recovery) of (*R*,*S*)-Ket, (*R*,*S*)-norKet and (*R*,*S*)-BrKet were determined by comparing peak heights for the QC standards to the peak heights resulting from the chromatography of standard solutions containing the equivalent final concentrations.

Accuracy was determined by comparing the observed concentrations of the QC standards calculated from the calibration curve to their nominal concentrations.

The specificity of the method for each analyte was examined by individually screening Ket, norKet and BrKet after spiking in pooled human plasma.

2.9. Application of the analytical method

The validated method was applied to the analysis of plasma samples obtained from a clinical study on Ket in pain management (Protocol A00-M91-00). conducted in the Anesthesia Research Unit, McGill University Medical Center (Montreal, Canada). After signing consent form, the patients received infusion of Ket (Ketalar, Park-Davis) delivered using a computer-controlled pump (Stanpump, Harvard 22 Basic Syringe Pump; Harvard Apparatus, South Natick, MA, USA). The target plasma concentrations set on the pump for the Ket infusions were 0 ng/ml (baseline), 60 ng/ml (dose 1) and 120 ng/ml (dose 2), during three testing periods of 15 min each. Blood samples were collected at the end of the baseline period and at the beginning and the end of dose 1 and dose 2 periods. Plasma was obtained by centrifugation and the samples were frozen at −80 °C until analysis.

3. Results and discussion

3.1. Optimization of the chromatographic separation

Enantioselective separations on an immobilized α_1-acid glycoprotein chiral stationary phase (AGP-CSP) are affected by the buffer concentration, the type and concentration of organic modifiers and the pH of the mobile phase [18]. Each of these parameters was systematically studied in the development of the enantioselective separation. Temperature also plays a role in separations on a CSP. However, in this study, the temperature was maintained at 25 °C and this parameter was not adjusted.

3.1.1. Selection of the buffer in the mobile phase for an LC–MS application

The buffer selected for this study was ammonium acetate because of its compatibility in LC–MS applications. Buffer concentrations of 10 and 20 m*M* were investigated and there was no significant influence of buffer concentration on the enantioselective separation. Therefore, a 10 m*M* concentration of ammonium acetate was chosen for the study.

3.1.2. Selection of the organic modifier in the mobile phase

The mobile phase concentration of 2-propanol was varied between 3 and 10% and the enantioselective separations of Ket, norKet and BrKet were highly dependent on the 2-propanol content. The optimum enantioselective separations of the three compounds were achieved with 2-propanol–buffer (3:97, v/v). Under these conditions, the observed enantioselectivities (α) were 1.14 (Ket), 1.71 (norKet) and 1.34 (BrKet). However, the analysis took 35 min. When the mobile phase concentration of 2-propanol was increased to 10%, the analysis took only 13 min, however, while the observed α values were acceptable for norKet and BrKet, 1.27 and 1.13, respectively, the enantioseparation of Ket was essentially lost (α=1.03). The best balance between enantioselectivity and analysis time was found with a mobile phase composed of 2-propanol–buffer (6:94, v/v). Under these conditions the observed α values were 1.09 (Ket), 1.46 (norKet) and 1.22 (BrKet) and the analysis time was 18 min. This mobile phase composition was used in the remaining optimization studies.

The addition of acetonitrile to the mobile phase has been shown to often improve the enantioselectivity [24]. In this study, the addition of acetonitrile (0.5 to 1.0%, v/v) to the mobile phase had no significant effect on the chromatography, although there was a slight decrease in retention time (0.25 to 0.5 min difference). The mobile phase selected for the validation and clinical studies did not contain acetonitrile.

3.1.3. Optimization of buffer pH

The effect of pH on the enantioselective separation of Ket and norKet was studied using the previously determined mobile phase composition of 2-propanol–ammonium acetate buffer (10 m*M*) (6:94, v/v). The pH of the buffer was varied over the range 4.0 to 7.0, in intervals of 0.5 units and from pH 7.0 to pH 8.5 in 0.1 intervals. At all pH values, an enantioselective separation was observed for norKet and BrKet. At pH 7.0 and higher, baseline enantioselective separations were achieved with observed α values of 1.33–1.86 (norKet) and 1.16–1.57 (BrKet).

An adequate enantioseparation of Ket was harder to achieve. From pH 4.0 to 6.5 no enantioselective separation of (*R*,*S*)-Ket was observed. Between pH 7.0 and pH 7.5, the observed α for Ket ranged from 1.04 (pH 7.0) to 1.09 (pH 7.5) with a maximum resolution (R_s) of 0.69 obtained at pH 7.5. Between pH 7.6 and pH 8.5, the observed α increased from 1.17 to 1.37, the R_s values from 0.83 to 1.90.

On the basis of these studies, the pH of the buffer was set at 7.6. Although the best chromatographic separations were achieved at pH 8.5, the stability of the AGP-CSP is reduced when the pH of the mobile phase is >7 [25]. Thus, the selected pH was a compromise between chromatographic separation and column life.

Based upon these results, the mobile phase composition for the validation and clinical study were set at 2-propanol–ammonium acetate buffer (10 m*M*, pH 7.6) (6:94, v/v). Under these conditions, the analysis was completed in less than 20 min. The relative retentions (k') of (*S*)-Ket and (*R*)-Ket were 9.0 and 10.5, respectively, and the observed α was 1.17 (Fig. 2A), for (*S*)-norKet and (*R*)-norKet the k' values were 5.2 and 8.2, respectively, and the observed α was 1.58 (Fig. 2B), while for BrKet the k' values for (*S*)-BrKet and (*R*)-BrKet were 9.8 and 12.8, respectively, and the observed α was 1.31 (Fig. 2C). The enantiomeric elution orders were established by chromatographing the separate enantiomers.

Previous Ket assays utilizing the AGP-CSP have reported a rapid deterioration of the CSP [16]. Under the described chromatographic conditions, the method was stable and reproducible, allowing us to analyze over 350 plasma standards and patient samples on a single analytical AGP column with replacement of the guard AGP column after an average of 100 plasma samples analysis.

3.2. Optimization of the mass spectrometric detection

The chromatograms were monitored using single ion monitoring for Ket at m/z 238.1, for norKet at m/z 224.1 and BrKet at m/z 284.0 (BrKet). Each compound was injected individually and a full scan mass spectra was obtained and the signals were monitored at each of the specific m/z values. The specific ion data was collected on three separate channels and analyzed. The results of these studies

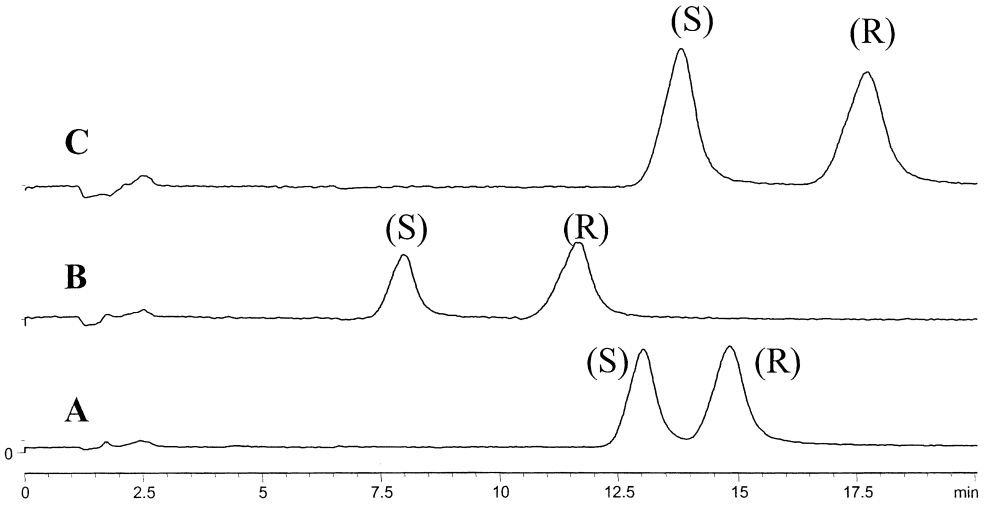

Fig. 2. Representative chromatogram of the low quality control (LQC) plasma sample containing 5 ng/ml of (*R*)-Ket, (*S*)-Ket, (*R*)-norKet and (*S*)-norKet and 100 ng/ml of (*R*)-BrKet and (*S*)-BrKet; where: (A) the chromatographic trace obtained using single ion monitoring at *m*/*z* 238.1 (Ket); (B) the chromatographic trace obtained using single ion monitoring at *m*/*z* 224.1 (norKet); and (C) the chromatographic trace obtained using single ion monitoring at *m*/*z* 284.0 (BrKet).

demonstrated that there were no overlaps in the mass spectra of the compounds at the *m*/*z* values chosen for the monitoring. In addition, the analysis of drug-free blank plasma at these *m*/*z* values detected no interfering peaks, Fig. 3.

The optimization of the mass spectrometer parameters was carried out using flow injection analysis (FIA) in which Ket and norKet standard solutions (50 ng/ml) were injected every 2 min. The Ket and norKet signals were optimized by the evaluation of changes in their peak height at their respective *m*/*z* value in response to changes in fragmentor voltage, capillary voltage, nebulizer pressure and drying gas temperature. The peak heights of (*R*,*S*)-Ket and (*R*,*S*)-norKet varied with the fragmentor voltage and the optimum response was obtained at 60 V. The value for the fragmentor voltage was set to 60 V and the other parameters were evaluated. In this way, the

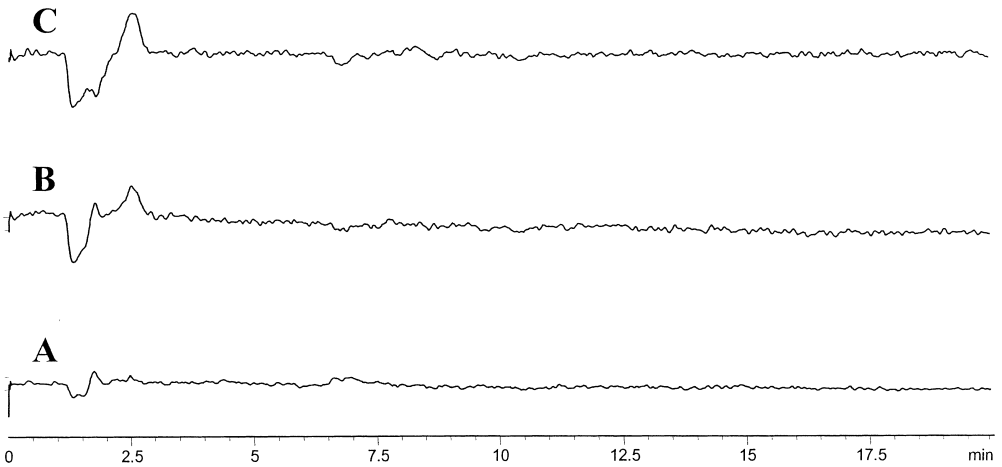

Fig. 3. Representative chromatograms of drug-free plasma obtained using single ion monitoring at *m*/*z* 238.1 (A), *m*/*z* 224.1 (B) and *m*/*z* 284.0 (C).

Table 1
Summary of validation statistics for ketamine

	LQC (5.0 ng/ml)		MQC (50.0 ng/ml)		HQC (100 ng/ml)	
	R	*S*	*R*	*S*	*R*	*S*
Intra-day						
N	5	5	5	5	5	5
Mean	4.9	5.0	48.5	48.4	97.4	97.3
SD	0.1	0.1	1.8	1.4	3.2	3.3
RSD (%)	1.6	2.2	3.8	2.8	3.2	3.4
Inter-day						
N	15	15	15	15	15	15
Mean	5.0	5.0	50.0	49.8	99.8	99.8
SD	0.3	0.4	2.4	2.3	3.7	4.9
RSD (%)	5.8	7.5	4.8	4.5	3.7	4.9
Accuracy (%)	100.2	100.8	99.9	99.6	99.8	99.8
Recovery (%)	95.0	95.5	98.0	98.6	99.5	99.6

optimum conditions based on the maximum peak height were: drying gas flow-rate, 11.0 l/min; nebulizer pressure, 40 p.s.i.g.; drying gas temperature, 350 °C and capillary voltage, 1000 V.

3.3. Extraction efficiency (% recovery)

During the development of the extraction method, three SPE cartridges were tested. The cation-exchange cartridges (Oasis MCX, 3 ml/60 mg) gave low recoveries in the range 60–75% for the 3 compounds as did the Bond Elute C_{18} (1 ml/30 mg), which yielded recoveries of 59–65%. The best recoveries were obtained using the Oasis HLB (1 ml/30 mg) where the recoveries ranged from 95.0 to 99.6%, Tables 1 and 2.

3.4. Linearity and detection limits

Calibration curves were generated by weighted $(1/x)$ least-squares linear regression. The linear relationships between peak height ratio and drug–enantiomer concentrations in the range of 1 to 125 ng/ml were described by the following equations: $y = 0.0315x + 0.0058$, $r^2 = 1$ [(*R*)-Ket]; $y = 0.027x - 0.0018$, $r^2 = 0.9994$ [(*S*)-Ket]; $y = 0.1259x + 0.0419$, $r^2 = 0.9995$ [(*R*)-norKet]; $y = 0.0688x + 0.0062$, $r^2 = 0.9993$ [(*S*)-norKet]. The data

Table 2
Summary of validation statistics for norketamine

	LQC (5.0 ng/ml)		MQC (50.0 ng/ml)		HQC (100 ng/ml)	
	R	*S*	*R*	*S*	*R*	*S*
Intra-day						
N	5	5	5	5	5	5
Mean	5.0	4.7	49.5	48.9	97.2	97.1
SD	0.2	0.2	0.6	0.9	2.7	2.9
RSD (%)	3.0	4.0	1.2	1.8	2.8	2.9
Inter-day						
N	15	15	15	15	15	15
Mean	5.1	5.1	49.9	49.5	99.9	99.4
SD	0.2	0.4	3.1	2.7	6.6	5.9
RSD (%)	3.9	7.2	6.3	5.5	6.7	5.9
Accuracy (%)	101.4	102.2	99.7	99.0	99.9	99.4
Recovery (%)	99.3	99.2	99.0	99.0	99.5	99.0

were based on nine replicates of a seven-point calibration curve.

The chromatogram of the LQC (5.0 ng/ml per enantiomer of Ket and norKet) is presented in Fig. 2. The LLOQ is the concentration of the drug, in the matrix, which could be determined with a percentage accuracy within acceptable limits (80–100%). LLOQ per enantiomer for Ket and norKet was 1.0 ng/ml ($n = 15$) with an acceptable precision and accuracy, respectively, for each enantiomer as follows: (*R*)-Ket 8.6 and 97.8%, for (*S*)-Ket 9.2 and 97.4%, for (*R*)-norKet 7.5 and 98.8% and for (*S*)-norKet 8.7 and 99.3%. The LLOD was defined as the concentration of the compound at which the signal versus noise ratio (S/N) was equal to 3. For each enantiomer, the LOD for Ket and norKet was 0.25 ng/ml.

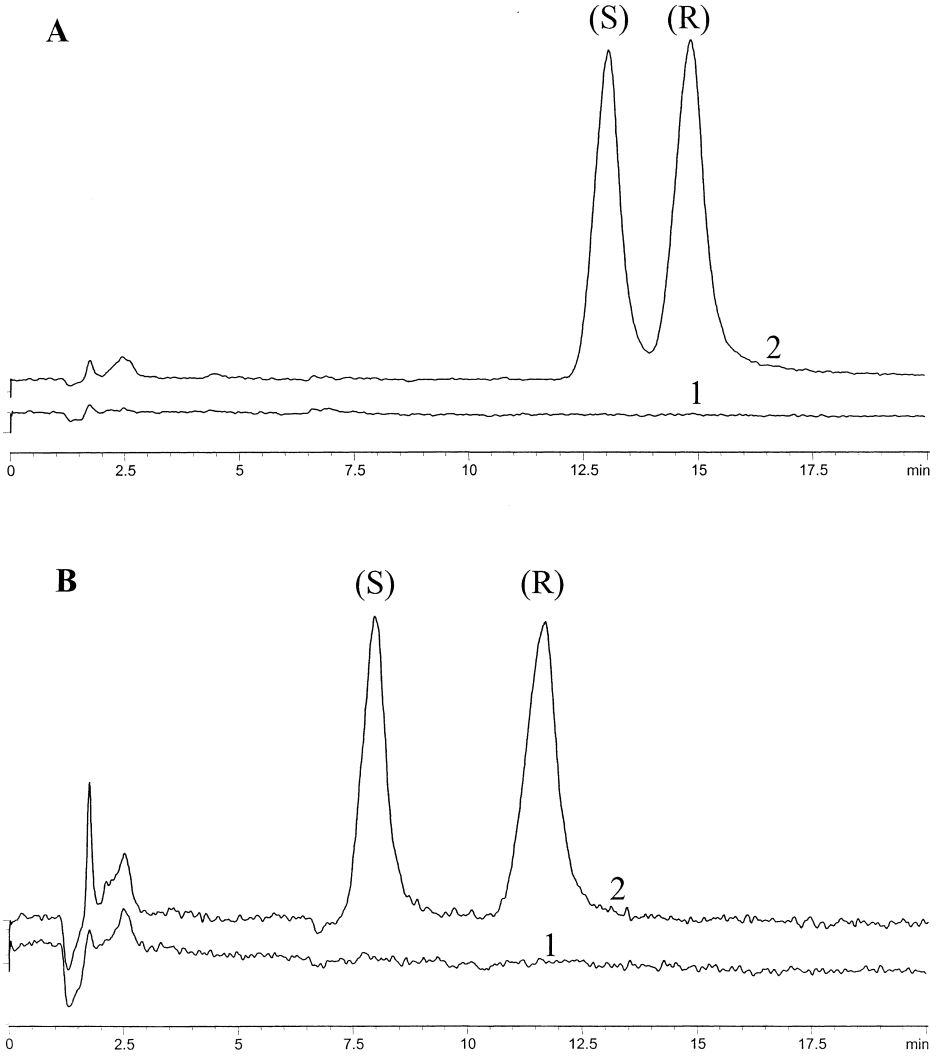

Fig. 4. The chromatograms from the analysis of plasma samples from a patient obtained before the administration of Ket and 15 min after the administration of an infusion of 60 ng/ml, where: (A) the chromatographic traces obtained using single ion monitoring at m/z 238.1 (Ket), trace 1—before administration, trace 2—after administration; (B) the chromatographic trace obtained using single ion monitoring at m/z 224.1 (norKet), trace 1—before administration, trace 2—after administration. The calculated concentrations were: (*S*)-Ket 27.1 ng/ml, (*R*)-Ket 28.9 ng/ml, (*S*)-norKet 3.4 ng/ml, (*R*)-norKet 2.5 ng/ml.

3.5. Accuracy and precision

Accuracy and precision of the method for Ket and norKet were evaluated from quintuplicate analysis of each QC standard levels (LQC, MQC and HQC) repeated for 3 days. The calculated accuracy was 100.0% for (*R*)-Ket, 100.1% for (*S*)-Ket, 100.3% for (*R*)-norKet and 100.2% for (*S*)-norKet, Tables 1 and 2.

The intra-day and inter-day precisions of the method, determined as the relative standard deviation (RSD) for the LQC, MQC and HQC for Ket and norKet were ≤5.8% for (*R*)-Ket, ≤7.5% for (*S*)-Ket, ≤6.7% for (*R*)-norKet and ≤7.2% for (*S*)-norKet. The results of the validation studies demonstrate that the method has excellent accuracy, recovery and precision.

3.6. Stability

Hijazi et al. [26], have previously reported that Ket and norKet were stable in aqueous solutions at −80 °C for at least 6 months, and that plasma samples could be transported at 4 °C within 2 days and can be stored at −20 °C for 10 weeks without any change in the concentrations of Ket and norKet. The results from this study were consistent with the observation by Hijazi et al. [26], as no significant degradation of the Ket and norKet standards or plasma samples was observed.

4. Application to clinical samples

The validated method has been applied to the analysis of plasma samples obtained from a clinical study on Ket in pain management. A representative chromatogram of a patient plasma sample obtained before administration of the drug and 15 min after the end of dose 1 (where the target plasma concentrations was 60 ng/ml) is presented in Fig. 4. The measured concentrations were: (*S*)-Ket 27.1 ng/ml, (*R*)-Ket 28.9 ng/ml, (*S*)-norKet 3.4 ng/ml, (*R*)-norKet 2.5 ng/ml. The plasma concentrations of (*R*)-Ket and (*S*)-Ket in two patients measured before and after doses 1 and 2 are presented in Fig. 5. In these two patients, the norKet levels were below our limit of detection during the first dosing period. At the start of dosing period 2, norKet was undetectable in patient 1 and 1.0 ng/ml of (*R*)-norKet was

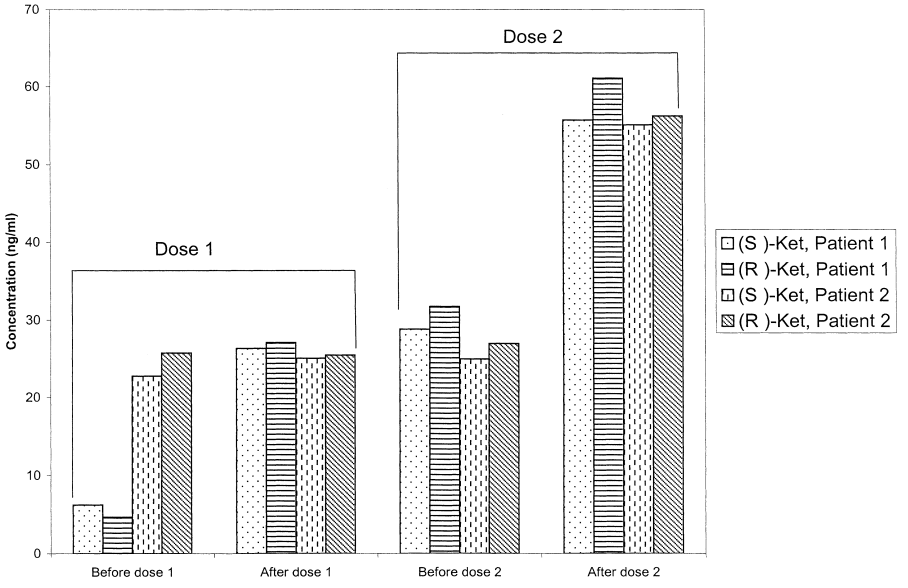

Fig. 5. Plasma concentrations of the enantiomers of ketamine, (*R*)-Ket and (*S*)-Ket, in two patients before and after dosing period 1 and dosing period 2, see the Experimental section for description of the clinical protocol.

observed in patient 2, while at the end of dosing period 2 (*R*)-norKet and (*S*)-norKet were measured in the plasma from patient 1 (2.7 and 1.0 ng/ml, respectively) and patient 2 (2.7 and 2.8 ng/ml, respectively).

5. Conclusions

The bioanalytical assay reported in this manuscript is a simple, sensitive and reproducible method for the enantioselective and simultaneous determination of Ket and norKet in human plasma by LC–MS. The assay has greater sensitivity than previously reported methods and a run time of less than 20 min. Including calibration and quality control standards (10 samples), up to 60 patient samples/day can be analyzed by this method. The method has been utilized in the analyses of plasma samples from a clinical study of racemic-Ket in pain management. The results from this study will be reported elsewhere.

References

[1] P.F. White, J. Ham, W.L. Way, A.J. Trevor, Anesthesiology 52 (1980) 231.
[2] I. Oye, O. Paulsen, A. Maurset, J. Pharmacol. Exp. Ther. 260 (1992) 1209.
[3] P. Klepstad, A. Maurset, E.R. Moberg, I. Oye, Eur. J. Pharmacol. 187 (1990) 513.
[4] P.F. White, W.L. Way, A.J. Trevor, Anaesthesiology 56 (1982) 119.
[5] D.L. Reich, G. Silvay, Can. J. Anaesth. 36 (1989) 186.
[6] M.P. Marietta, W.L. Way, N. Castagnoli, A.J. Trevor, J. Pharmacol. Exp. Ther. 202 (1977) 157.
[7] P.F. White, J. Shuttler, A. Shafer, D.R. Stanski, Y. Horai, A.J. Trevor, Br. J. Anaesth. 57 (1985) 197.
[8] J. Wieber, R. Gugler, J.H. Hengstmann, H.J. Dengler, Anaesthesist 24 (1975) 260.
[9] J.D. Adams, T.A. Baillie, A.J. Trevor, N. Castagnoli, Biomed. Mass Spectrom. 8 (1981) 527.
[10] A.J. Trevor, T.F. Woolf, T.A. Baillie, J.D. Adams, N. Castagnoli, Stereoselective metabolism of ketamine enantiomers. In: J.M. Kamenta, E.F. Domino, P. Geneste (Eds.), Phencyclidine and related arylcyclohexylamines: Present and future applications, NPP Books, Ann Arbor, MI, 1983, p. 279.
[11] S.C. Hong, J.N. Davisson, J. Pharm. Sci. 71 (1982) 912.
[12] M.L. Cohen, A.J. Trevor, J. Pharmacol. Exp. Ther. 189 (1974) 351.
[13] G. Geisslinger, S. Menzel-Soglowek, J. Chromatogr. 568 (1991) 165.
[14] G. Geisslinger, W. Hering, P. Tomann, R. Knoll, H.D. Kamp, K. Brune, Br. J. Anaesth. 70 (1993) 666.
[15] P. Hartvig, J. Valtysson, K.J. Linder, J. Kristensen, R. Karlsten, L.L. Gustafsson, J. Persson, J.O. Svensson, I. Oye, G. Antoni, G. Westerberg, B. Langstrom, Clin. Pharmacol. Ther. 58 (1995) 165.
[16] J.O. Svensson, L.L. Gustafsson, J. Chromatogr. B 678 (1996) 373.
[17] S.R. Edwards, L.E. Mather, Life Sci. 69 (2001) 2051.
[18] Y. Yanagihara, M. Ohtani, S. Kariya, K. Uchino, T. Aoyama, Y. Yamamura, T. Iga, J. Chromatogr. B 746 (2000) 227.
[19] T. Chang, A.J. Glazko, Anaesthesiology 36 (1972) 401.
[20] J.A. Clements, W.S. Nimmo, Br. J. Anaesth. 53 (1981) 27.
[21] J. Wieber, R. Gugler, J.H. Hengstmann, H.J. Dengler, Anaesthesist 24 (1975) 260.
[22] J.L. Pedraz, M.B. Calvo, A.R. Gascon, R. Hernandez, C. Muriel, L.M. Torres, A. Dominguez-Gil, Br. J. Anaesth. 67 (1991) 310.
[23] J.N. Davisson, J. Chromatogr. 146 (1978) 344.
[24] I.W. Wainer, in: I.W. Wainer (Ed.), Drug Stereochemistry: Analytical Methods and Pharmacology, 2nd ed., Marcel Dekker, New York, 1993, p. 167.
[25] Instruction Manual Chiral-AGP, ChromTech. February 2002.
[26] Y. Hijazi, M. Bolon, R. Boulieu, Clin. Chem. 47 (2001) 1713.