We developed an isocratic, selective, and very sensitive HPLC method for the determination of ketamine and its two main metabolites in plasma. The compounds were extracted from plasma by a liquid–liquid extraction with a dichloromethane:ethyl acetate mixture followed by an acidic back-extraction. Separation was achieved on a new stationary phase, Purospher RP-18 end-capped, with a mobile phase containing acetonitrile:0.03 mol/L phosphate buffer (23:77 by vol) adjusted to pH 7.2. Because of the high column efficiency and the significant improvement of peak symmetry, the quantification limit could be down to 5 μg/L for ketamine and norketamine (NK). The intraday and interday CVs ranged from 1.7% to 5.8% and 3.1% to 10.2% for all compounds respectively. The method is sensitive enough for monitoring ketamine, NK, and dehydroketamine in plasma during pharmacokinetic studies after an intravenous bolus of a low dose of ketamine.

Ketamine [R,S-2(O-chlorophenyl)-2-(methylamino)cyclohexanone] is a dissociative anesthetic agent that has been widely used in clinical practice for >25 years [1]. Ketamine is also a potent analgesic at subanesthetic doses [2]. Ketamine undergoes an extensive liver metabolism by CYP-450 N-demethylation to norketamine (NK). The cyclohexanone ring also undergoes oxidative metabolism to form the second metabolite dehydronorketamine (DHNK) (Fig. 1). These two metabolites, especially NK, may contribute to the pharmacological effect of ketamine [3].

However, early studies on the effect of ketamine on intracranial pressure have shown that ketamine increased intracranial cerebral pressure, so ketamine has been given up in neuroanesthesia [4]. Moreover, ketamine has been reported to interact with the phencyclidine binding site of the N-methyl-d-aspartate receptor-associated ion channel. This binding may confer neuroprotective properties in cerebral ischemia to ketamine [5]. These findings suggest that ketamine may be used as a sedative agent in cerebral disorders in the intensive care unit. Several analytical methods for the determination of ketamine in plasma or urine with gas chromatography technique with electron capture [6–9] and mass spectrometric detection (GC-MS) [10–12] have been reported. HPLC methods for the analysis of ketamine and its metabolites have also been described in human plasma [13–17] and in equine serum [18]. However, the methods described require derivatization [13] or column thermostabilization [17], or lack sensitivity for an accurate determination of ketamine, NK, and DHNK during pharmacokinetic studies [7, 10, 15, 16, 18].

To investigate the relation between the pharmacokinetics of ketamine and those of its main metabolites and the analgesic and blood flow effect of the drug, we proposed to develop a simple, sensitive, and selective isocratic HPLC method with a high-purity reversed-phase sorbent for the determination of ketamine and its two metabolites in plasma over a 24-h period after an intravenous bolus of a low analgesic dose of ketamine.

Materials and Methods

Chemicals
Ketamine, NK, DHNK, and nortilidine were generously supplied by Parke-Davis. Lyotrol drug-free serum was used for calibration curve (Bio-Merieux).

Acetonitrile, dichloromethane (Uvasol, grade), boric acid (pro analysi), and potassium dihydrogen phosphate
anhydrous (suprapur) were purchased from Merck. Ethyl acetate (HPLC grade) was from Sigma.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS
The chromatographic system consisted of Hewlett Packard 1050 series with a computer HP Vectra 846/33 M using HP Chem software and HP Desk Jet 510 (Hewlett Packard). Separation was achieved with a new kind of reversed-phase Purospher RP-18e (5 μm) 125 × 4 mm (Merck). The silica gel is end-capped and contains fewer metal impurities than a conventional C18 column.

The mobile phase consisted of acetonitrile:0.03 mol/L phosphate buffer (23:77 by vol) adjusted to pH 7.2. The flow rate of the mobile phase was 1.5 mL/min. The detection wavelength was 210 nm. The system was used at ambient temperature (20 °C).

CALIBRATOR SOLUTIONS
Stock solutions of ketamine, NK, and DHNK were prepared at a concentration of 150 mg/L and nortilidine (internal calibrator) at a concentration of 25 mg/L by dissolving each compound in distilled water. These solutions were pooled and divided into 2-mL aliquots and then frozen at -80 °C.

Working solutions were freshly made from the frozen calibrator solutions by appropriate dilutions.

SAMPLE COLLECTION
Blood samples (5 mL) were collected into heparinized tubes and centrifuged without delay at low temperature. Plasma was decanted and stored at -20 °C until analysis.

EXTRACTION PROCEDURE
Plasma (1 mL) containing 20 μL of internal calibrator was alkalinized with 350 μL of 0.2 mol/L borate buffer, pH 13, and then extracted with 5 mL of dichloromethane:ethyl acetate (80:20 by vol) by mixing for 10 min at 60 rpm. After centrifugation at 1500g for 3 min at 15 °C, the organic layer was transferred into a conical glass tube.

The extraction step was repeated with 3 mL of dichloromethane:ethyl acetate (80:20 by vol). The combined organic layer was evaporated to dryness under a gentle nitrogen stream.

The dry residue was redissolved in 500 μL of dichloromethane:ethyl acetate (80:20 by vol) and back-extracted with 2 mL of 2 mol/L HCl. The organic layer was discarded and the acid layer was evaporated to dryness at 45 °C. Lastly, the dry residue was reconstituted in 100 μL of mobile phase and 60 μL were injected into the column.

Results
Chromatogram of a plasma sample supplemented with ketamine, NK, DHNK, and nortilidine at a concentration of 500 μg/L is shown Fig. 2. The separation of the four compounds was achieved in about 15 min.

Analytical recovery for each compound was determined by comparison of peak height obtained from plasma supplemented at known concentrations and those obtained by direct injection of calibrators. Results are displayed in Table 1.

Calibration curves were fitted by plotting the peak height ratio (compounds of interest/internal calibrator) vs the concentration. They were linear in the range 5–5000 μg/L for ketamine and in the range 5–750 μg/L for NK and 10–750 μg/L for DHNK. The correlation coefficient was >0.999 for the three compounds. The within-day and day-to-day accuracy and precision of the method were assessed by replicate analysis of plasma samples supplemented with the compounds of interest at three different concentrations. The results are shown in Table 1.

The minimum detectable amount defined as the signal-to-noise ratio of 4 was found to be 3 ng for each compound, with an injection volume of 60 μL. The quantification limit was 5 μg/L for ketamine and NK and 10 μg/L for DHNK.
μg/L for DHNK with a CV <15% for a 1-mL sample volume.

The Purospher column has demonstrated a long lifetime: ~600 samples were injected without any deterioration. There was no interference with related compounds for drugs commonly used in the intensive care unit such as diazepam, phenobarbital, omeprazole, buprenorphine, atropine, phytoemadione, nalbuphine, dopamide, furosemide, and propofol.

The method was used for monitoring ketamine and its two main metabolites in intensive care patients. Typical chromatograms of a blank and plasma samples obtained 15 min after an intravenous bolus of 150 mg of ketamine are shown in Fig. 3. Representative plasma concentration vs time profiles of ketamine and its metabolites after an intravenous bolus of 2 mg/kg followed by a 2-h infusion of 2 mg/kg per hour are reported in Fig. 4.

**Discussion**

Ketamine, NK, and DHNK are weak basic compounds. Amino groups can interact with residual silanol groups, which cause tailing or irreversible adsorption of the analytes. To minimize this nondesired polar interaction on reversed stationary phase, a new stationary phase Purospher RP 18 end-capped was prepared with a metal-free silica gel and derivatization technique, leading to homogeneous and densely ordered octadecyl groups on the silica surface. This column provides higher efficiency and improves significantly the peak shape of the compounds compared with a conventional reversed-phase column.

The retention of ketamine was significantly influenced by the pH of the mobile phase in the range 6.0–7.4, whereas the retention of the two metabolites is less affected. The concentration of the organic modifier (acetonitrile) strongly influenced the retention behavior of the three compounds. The optimal separation was achieved at pH 7.2 with 23% of acetonitrile in the mobile phase.

Considering the extraction step, the use of a mixture of dichloromethane:ethyl acetate (80:20 by vol) as extraction solvent improves analytical recovery of NK and DHNK compared with the use of cyclohexane as previously reported [15]. Acidic back-extraction is required to obtain a clean plasma extract. Recoveries >85% for each compound were obtained with 2 mL of 2 mol/L HCl without interfering peaks.

The specificity of the method was improved by the use of a photodiode array detector. Peak identity was confirmed through library matching by comparison of unknown peak to reference spectra. The improvement of column efficiency and peak symmetry contributes to decrease the quantification limit to 5 μg/L with acceptable accuracy and variability [19]. The sensitivity of this method is better than those previously reported with the GC-MS method [12].

Adams et al. [20] previously reported that DHNK is a methodological artifact resulting from thermal dehydration of hydroxynorketamine. This transformation may be due to the high temperature reached during GC analysis. Human plasma samples analyzed after liquid–liquid extraction and after ultrafiltration exhibit similar concentrations of DHNK. With the ultrafiltration method, there is no risk of transformation of hydroxynorketamine owing to stressing conditions such as acidic or basic conditions.

**Table 1.** Precision, accuracy, and recoveries.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration added, μg/L</th>
<th>Recovery, %</th>
<th>Concentration found, μg/L</th>
<th>Relative error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraassay (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>10</td>
<td>9.9 ± 0.3</td>
<td>504.0 ± 8.6</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2470.0 ± 127.4</td>
<td>9.8 ± 0.4</td>
<td>2.4</td>
</tr>
<tr>
<td>NK</td>
<td>10</td>
<td>9.8 ± 0.4</td>
<td>492.0 ± 8.4</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>753.0 ± 18.1</td>
<td>10.0 ± 0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>DHNK</td>
<td>10</td>
<td>10.0 ± 0.6</td>
<td>503.0 ± 13.1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>503.0 ± 13.1</td>
<td>749.0 ± 33.7</td>
<td>0.1</td>
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<tr>
<td><strong>Interassay (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>10</td>
<td>92.4 ± 3.5*</td>
<td>9.5 ± 1.0</td>
<td>6.5</td>
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<tr>
<td></td>
<td>500</td>
<td>90.3 ± 3.6</td>
<td>498.0 ± 28.4</td>
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<tr>
<td></td>
<td>2500</td>
<td>50.8 ± 4.0</td>
<td>2385.0 ± 169.3</td>
<td>4.6</td>
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<tr>
<td>NK</td>
<td>10</td>
<td>91.8 ± 6.5</td>
<td>10.3 ± 0.7</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>90.3 ± 3.9</td>
<td>490.0 ± 20.6</td>
<td>2.4</td>
</tr>
<tr>
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<td>750</td>
<td>94.5 ± 2.8</td>
<td>725.0 ± 22.5</td>
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<tr>
<td>DHNK</td>
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<tr>
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<td>500.0 ± 23.0</td>
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<tr>
<td></td>
<td>750</td>
<td>85.3 ± 3.8</td>
<td>733.0 ± 24.2</td>
<td>3.6</td>
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</tbody>
</table>

* Mean ± SD.

Relative error: (concentration found – concentration added) x 100/(concentration added).
and an evaporation step. Our results confirm that DHNK is a metabolite recovered in human plasma at significant concentrations and should not be considered an artifact.

The method described presents all the characteristics—simple, selective, and very sensitive—required for monitoring plasma drug concentrations after administration of low doses of ketamine.

References