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Note

High-performance liquid chromatographic determination of etomidate in plasma

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Etomidate (R16659), (*R*)-(+)-ethyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate (Fig. 1), is a short-acting hypnotic agent used in humans for anaesthesia. Several analytical methods have already been described [1–5] but none of them was completely satisfactory for our needs. Wynants et al. [1] used a gas chromatographic method that requires the extraction of 3 ml of plasma. Van Hamme et al. [2] described a gas chromatographic–mass spectrometric method that is sensitive but requires and expensive analytical instrument. A high-performance liquid chromatographic (HPLC) method was developed by Avram et al. [4] that is sensitive to 20 ng/ml. Ellis and Beck [5] reported an HPLC method with a 4-ml plasma extraction, where expected etomidate concentrations were less than 50 ng/ml.

This paper describes a quantitative method, in human plasma, based on liquid–liquid extraction and HPLC determination. The limit of quantitation was 5 ng/ml, with only 1 ml of plasma sample.

EXPERIMENTAL

Materials

Etomidate and the internal standard (propoxate, R7464), (*R*)-(+) propyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate, were supplied by Janssen Pharmaceutica (Beerse, Belgium). The structures are shown in Fig. 1. Pentane (Spectranal, Riedel de Haën, La Plaine St. Denis, France), methanol (Hypersolv, BDH, Paris, France) and acetonitrile (Chromasolv, Riedel de Haën) were HPLC grade. Ammonia (28%), sulphuric acid (0.5 *M*), sodium

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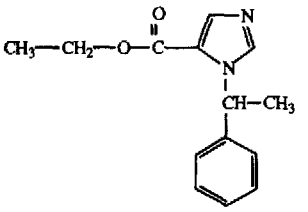
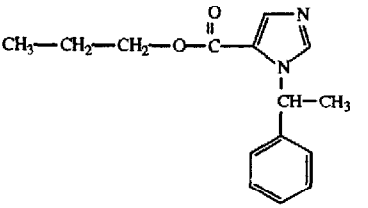


ETOMIDATE



PROPOXATE

Fig. 1. Structures of etomidate and propoxate.

borate and potassium dihydrogenphosphate were of analytical grade (RP Normapur, Prolabo, Paris, France) and prepared in doubly distilled water.

Analytical conditions

The HPLC system was a Kontron 460 automatic injector (St. Quentin en Yvelines, France), a Kratos Spectroflow 400 pump (Roissy, France), a Kontron 430 detector and a Spectra-Physics SP 4100 integrator (Les Ulis, France). The reversed-phase column (25 cm × 4.6 mm I.D.) was packed with Spherisorb (5 μm) coated with ODS-2 (Prolabo). The guard column (3 cm × 4.6 mm I.D.) was a Spherisorb 10-μm RP 18 (Brownlee Labs., Touzart et Matignon, Vitry sur Seine, France). The mobile phase was acetonitrile–methanol–water containing 0.025 *M* phosphate buffer (30:40:30, v/v); the pH was adjusted 8.1 with ammonia (28%). The flow-rate was set at 1.2 ml/min, and the detection wavelength was 242 nm.

Preparation of standard solutions

Stock standard solutions of etomidate and propoxate (1 mg/ml) were prepared in methanol. Working standard solutions (0.1–20 μg/ml) of etomidate were prepared by successive dilutions of the stock solution. Working internal standard solution was set at 4 μg/ml by diluting the stock solution. Solutions were stored at 4 °C and freshly prepared every week.

Extraction procedure

In to 15-ml glass tubes were placed 0.1 ml of working internal standard solution and 0.2 ml of 0.05 *M* borate buffer (pH 10) with 1 ml of plasma. The solutions were extracted with 7 ml of pentane on a rotary mixer (20 min, 24 rpm). The mixture was centrifuged (10 min, 3000 *g*), and the organic layer was transferred to another 15-ml glass tube; 3 ml of 0.5 *M* H_2SO_4 were added and mixed for 10 min. The mixture was centrifuged (5 min, 3000 *g*) and the organic layer discarded. The acid layer was washed with 2 ml of pentane for 5

min. The mixture was centrifuged and the organic layer discarded. The acid layer was made basic with 0.5 ml of ammonia (28%). The solution was extracted twice with 3 ml of pentane on a rotary mixer (10 min, 24 rpm) and centrifuged. The organic layers were transferred and evaporated to dryness at room temperature under a gentle stream of nitrogen.

High-performance liquid chromatography

The dry extract was dissolved into 0.1 ml of mobile phase, and 0.05 ml of the solution was injected into the HPLC column.

Calibration

Using the standard solutions, samples of blank plasma (1 ml) were spiked with etomidate at concentrations in the range 10–2000 ng/ml, with the internal standard at a fixed concentration of 400 ng/ml. All calibration samples were taken through the extraction procedure described above.

Calculations

Ultimate sample concentrations were calculated by determining the peak-area ratio of etomidate relative to the internal standard, and comparing this ratio with the standard curve, obtained after the analysis of the calibration samples.

RESULTS AND DISCUSSION

The extractability from plasma of etomidate, a weak lipophilic organic base, was evaluated for two concentrations (200 and 1000 ng/ml): the recoveries were 98 ± 2 and $95 \pm 3\%$, respectively (mean $\pm$ S.D., $n=4$). At the end of the extraction procedure, the solvent evaporation can be critical because of the low melting point of etomidate base. So each set of samples must be dried at room temperature (20°C).

Fig. 2 shows chromatograms of blank human plasma (A), reference plasma samples, spiked with etomidate (B 200 ng, C 50 ng) and plasma from a patient after intake of etomidate (D). As can be seen, no interfering peaks were encountered at the retention times of etomidate (5.6 min) or the internal standard (7.6 min). Linear relationships were found when the peak-area ratios of etomidate to the internal standard were plotted versus the etomidate plasma concentrations. In order to improve the accuracy at low levels we established a calibration curve between 10 and 200 ng/ml, and for higher levels a calibration curve between 100 and 2000 ng/ml. Tables I and II summarize the precision and accuracy of the method with four replicate extractions at each concentration.

For each set of samples, we established a calibration curve and analysed two quality control samples. These quality controls were stored and taken through

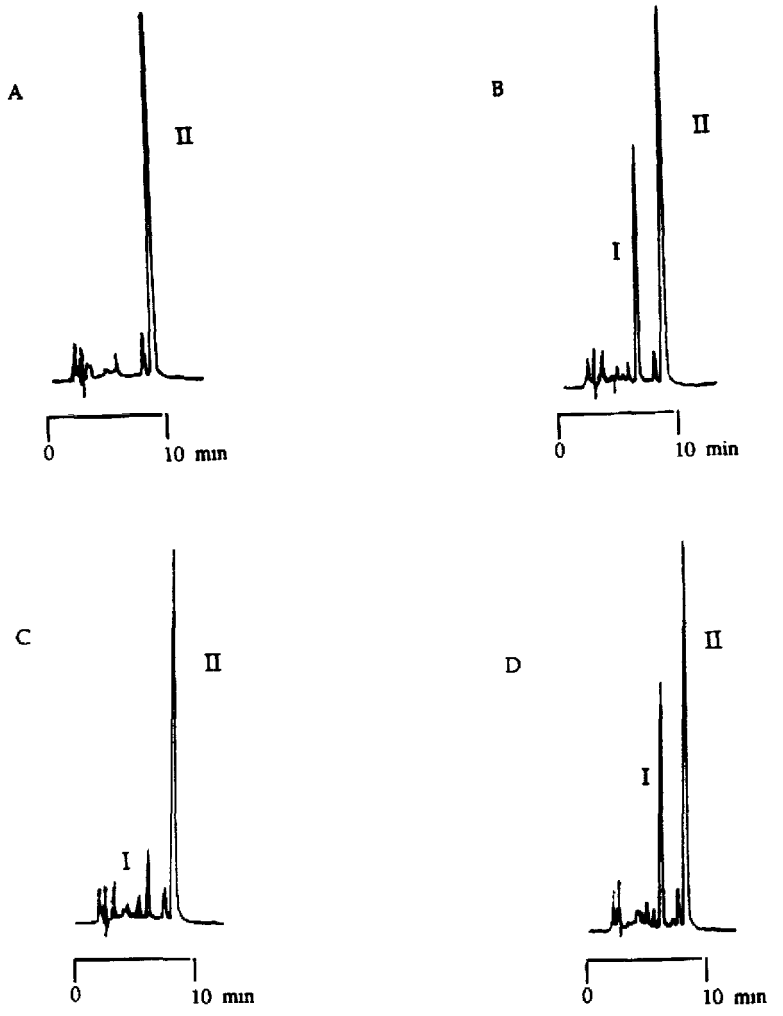


Fig. 2. Chromatograms of reference plasma samples and from a patient. (A) Internal standard (400 ng); (B) etomidate (200 ng) and internal standard (400 ng); (C) etomidate (50 ng) and internal standard (400 ng); (D) sample from a patient 9 min after administration of 18 mg of etomidate: etomidate found, 224 ng. Peaks: I = etomidate; II = internal standard.

the same extraction procedure as the study samples. Their theoretical concentrations were 100 and 500 ng/ml. Calculated quality control concentrations are listed in Table III. The results show the between-day reproducibility over a period of six weeks. The precision was 5.6 and 5.8% at plasma levels of 100 and 500 ng/ml, respectively. According to our results, the method is suitable for monitoring plasma levels during pharmacokinetic studies down to 10 ng/

TABLE I

LINEARITY, ACCURACY AND PRECISION OF THE METHOD BETWEEN 10 AND 200 ng/ml

$y = 0.002\,768x + 0.005\,956$; correlation coefficient = 0.9993.

Concentration (ng/ml)	Peak-area ratio (mean ± S.D., $n = 4$)	Calculated concentration (mean ± S.D., $n = 4$) (ng/ml)	Coefficient of variation (%)	Relative error (%)
10	0.0331 ± 0.0030	9.8 ± 1.0	10.6	−2.0
20	0.0603 ± 0.0034	19.6 ± 1.2	6.1	−2.0
50	0.147 ± 0.0072	51.4 ± 2.6	5.1	+2.0
100	0.281 ± 0.015	99.4 ± 5.4	5.5	−0.6
200	0.560 ± 0.004	200.0 ± 1.5	0.7	0.0

TABLE II

LINEARITY, ACCURACY AND PRECISION OF THE METHOD BETWEEN 100 AND 2000 ng/ml

$y = 0.003\,037x - 0.035\,83$; correlation coefficient = 0.9998.

Concentration (ng/ml)	Peak-area ratio (mean ± S.D., $n = 4$)	Calculated concentration (mean ± S.D., $n = 4$) (ng/ml)	Coefficient of variation (%)	Relative error (%)
100	0.281 ± 0.015	104.2 ± 4.9	4.7	+4.2
200	0.560 ± 0.004	196.2 ± 1.7	0.9	−1.9
500	1.466 ± 0.013	494.7 ± 4.3	0.8	−1.1
1000	3.022 ± 0.020	1007 ± 6.6	0.6	+0.7
2000	6.033 ± 0 086	1998 ± 28.3	1.4	−0.1

TABLE III

BETWEEN-DAY REPRODUCIBILITY FOR ETOMIDATE OVER A PERIOD OF SIX WEEKS

Concentration (ng/ml)	n	Calculated concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Relative error (%)
100	12	93.9 ± 5.3	5.6	−6.1
500	13	457.3 ± 27.6	5.8	−4.9

ml. Thousands of samples have been analysed without changing the column, and no interference has been observed.

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