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Note

Sensitive and automated gas chromatographic method for the determination of etomidate in plasma samples

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Etomidate (Hypnomidate®, Janssen, Neuss, F.R.G.) is an intravenous (i v.) short-acting hypnotic agent that has been routinely used for many years for the induction of neuroleptanalgesia and inhalation anaesthesia.

Only a few methods for the determination of etomidate levels in plasma have been described. Gas chromatographic (GC) methods [1–3] provide detection limits as low as 12.5 ng/ml. One high-performance liquid chromatographic (HPLC) method allows a minimum detection level of 20 ng/ml [4], and Hebron *et al.* [5] reported a detection limit of 8 ng/ml by HPLC using a 2-ml aliquot of the sample

Recently, a clinical study demonstrated that etomidate is effective in severe endogenous hypercortisolism due to Cushing's syndrome. Serum cortisol and urinary free cortisol excretion dropped from the hypercortisolemic range to normal values [6–8]. For this therapeutic approach [6] lower doese (0.04–0.05 mg/kg per h) of etomidate have to be administered than those used in i.v. anaesthesia (0.1–0.3 mg/kg per h) [9]. Therefore a sensitive method for the determination of low plasma levels is required.

We have developed a faster and more sensitive method than those previously published, which is easily applicable for the automated injection of series of plasma samples down to 0.5 ng/ml plasma levels.

EXPERIMENTAL

Chemicals

Analytical-grade etomidate, (*R*)-(+)-ethyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate, and propoxate (internal standard), (*R*)-(+)-propyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate, were obtained from Janssen Pharmaceutica (Beerse, Belgium). Their structures are shown in Fig. 1.

Chemicals and organic solvents were of the best grade commercially available.

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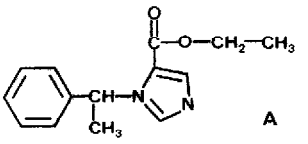
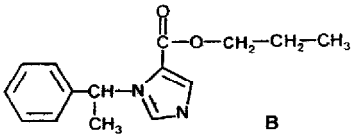


A



B

Fig. 1. Structures of etomidate (A) and the internal standard (B).

Standard solutions

A stock solution, corresponding to 0.1 mg of etomidate per ml of methanol, was prepared. Standard solutions were obtained by diluting the stock solution to concentrations ranging from 0.5 to 250 ng/ml of methanol.

For the internal standard (I.S.), a stock solution of 0.1 mg of propoxate per ml of ethanol was prepared. To spike the samples, this stock solution was further diluted to 50 ng/ml.

Extraction procedure

A 1-ml volume of plasma was transferred to a 15-ml glass centrifuge tube, spiked with 50 ng of I.S. and buffered with 1 ml of phosphate buffer solution (pH 7). After the addition of 2 ml of heptane–isoamyl alcohol (98.5.1.5, v/v) the tube was carefully rotated for 10 min and then centrifuged (15 min, 1900 *g*). The upper organic layer was transferred to a second centrifuge tube, and the plasma was extracted again with 2 ml heptane–isoamyl alcohol. The combined organic layers were back-extracted with 2 ml of 0.05 *M* sulphuric acid and removed after centrifugation. The remaining acidic phase was made alkaline with 0.1 ml of concentrated ammonia and extracted twice with 2-ml aliquots of heptane–isoamyl alcohol. The combined organic layers were finally evaporated to dryness under a gentle stream of nitrogen using a heating block at 50°C. The residue was dissolved in 50 μl of ethanol, from which samples of 5 μl were taken and injected automatically into the gas chromatograph.

Chromatographic conditions

The chromatographic analyses were performed on a Hewlett-Packard Model 5890 A gas chromatograph (HP, Ratingen, F.R.G.) equipped with a nitrogen–phosphorus detector. A WCOT fused-silica capillary column (25 m × 0.32 mm I.D.) coated with 0.11-μm FFAP phase was used (Chrompack, Mülheim, F.R.G.). The injected sample was split in a ratio of 1:5. Helium was used as

carrier gas at a flow-rate of 1.6 ml/min. The make-up gas (nitrogen) flow-rate was 29 ml/min. The injector and detector temperatures were maintained at 250 and 300°C, respectively, with isothermal separation at 195°C. The GC instrument was equipped with a Model 7673 A automatic sampler, which was programmed to wash the syringe properly to prevent carry-over. A Spectra Physics Model 4270 integrator linked to a LABNET data system was used (Spectra-Physics, Darmstadt, F.R.G.). The retention times were 9.13 min for etomidate and 11.28 min for the I.S. (relative retention time 0.81).

Calibration and calculation procedure

Using the etomidate standard solutions, separate series of blank (control) plasma samples (1 ml) were spiked with concentrations ranging from 0.5 to 250 ng/ml and with the I.S. at a fixed concentration of 50 ng/ml. These calibration samples were extracted as described above.

Unknown sample concentrations were calculated by determining the peak-area ratio of etomidate to the I.S. and comparing this ratio with the standard curve obtained by the analysis of the calibration samples.

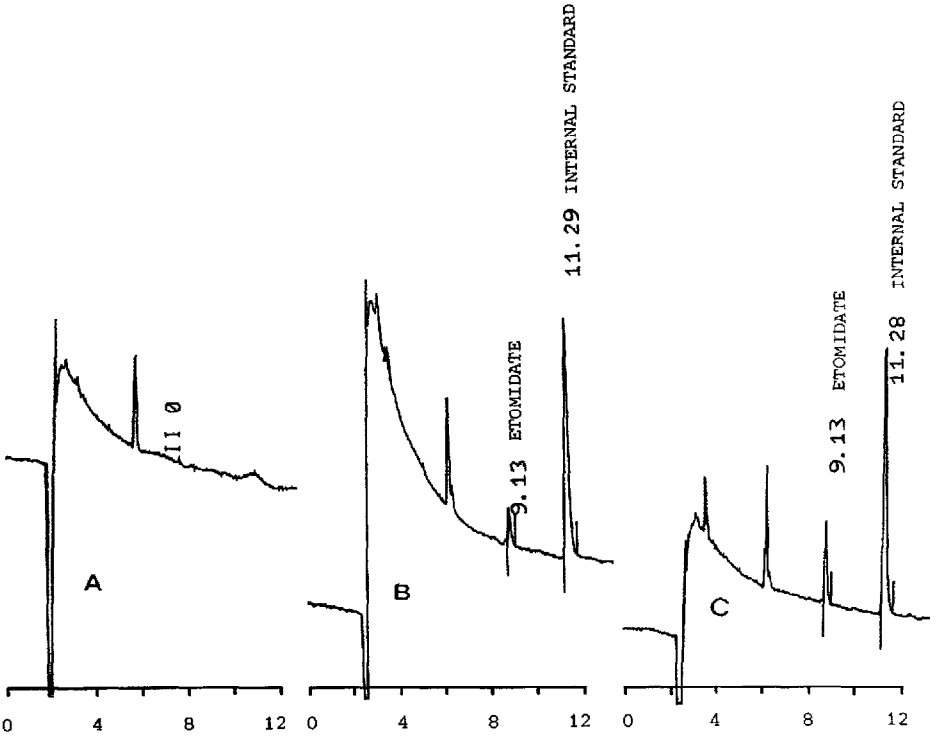


Fig. 2. Chromatograms of blank (control) plasma (A), plasma spiked with etomidate (5 ng/ml) (B) and plasma from a patient (plasma level. 9.8 ng/ml) (C).

RESULTS AND DISCUSSION

The extraction recovery for etomidate and the I.S. from 1 ml of spiked control plasma was sufficiently high: 75%.

Fig. 2 shows chromatograms of blank (control) plasma (A), plasma spiked with etomidate (B) and plasma from a patient (C). All samples were spiked with an I.S. concentration of 50 ng/ml. Good linear relationships were found when the peak area of etomidate to the I.S. were plotted *versus* the etomidate plasma concentrations. Least-squares regression analysis measured with the WCOT capillary column yielded the regression equation $y = 1.056x - 3.828$ ($r^2 = 0.9979$). The accuracy and reproducibility of the method were ascertained by replicate analysis of blank plasma samples spiked with concentrations ranging from 0.5 to 250 ng/ml etomidate. The results are presented in Table I.

TABLE I

ACCURACY AND PRECISION FOR THE DETERMINATION OF ETOMIDATE IN SPIKED PLASMA

Concentration added (ng/ml)	Measured concentration (mean ± S.D.)(ng/ml)	*n*	Coefficient of variation (%)	Accuracy (%)
0.50	0 54 ± 0.13	8	24.1	108.0
1.00	0.94 ± 0.15	7	16.0	94 0
1 50	1.29 ± 0.08	6	6.2	86.0
2.50	2.64 ± 0.12	6	4 5	105 6
5.00	5 28 ± 0.16	9	3 0	105.6
10.00	10.77 ± 0.12	6	1.1	107 7
25 00	25.43 ± 0.39	4	1.5	101.7
50.00	42.56 ± 1.18	6	2.8	85.1
100.00	105.21 ± 2.05	8	1 9	105 2
250.00	249.90 ± 4.25	9	1.7	100 0

The analytical method described here is suitably sensitive and specific and is applicable to the determination of low levels of etomidate in plasma. Additionally, the favourable injection and isothermal operating conditions allow the use of an autosampler.

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