Journal of Chromatography, 162 (1979) 591—595
Biomedical Applications

CHROMBIO. 299

Note

Assay of etomidate in plasma by capillary gas chromatography with nitrogen-selective detection

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(Received October 11th, 1978)

Two procedures have been described in the literature for the determination of the intravenous anaesthetic etomidate in plasma: (1) a gas-chromatographic (GC) method which requires a laborious extraction procedure and is not sensitive enough to determine very low plasma concentrations, unless plasma samples larger than 1 ml plasma are used [1]; (2) a mass-fragmentographic method which requires a gas chromatograph—mass spectrometer and may therefore not be very suitable for routine analysis [2].

In the present communication a method for the assay of etomidate in plasma is described, which is more sensitive and less laborious than the previously published GC method [1] and which can be applied to pharmacokinetic studies in man. The use of a support-coated open tubular (SCOT) column together with a nitrogen-selective detector has proved to be very suitable in this respect. In addition to etomidate it was found that propoxate could be analysed equally well with this method.

EXPERIMENTAL

Materials and methods

Etomidate [(R)-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxyl] and propoxate [(+)-propyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate] were kindly supplied by Janssen Pharmaceutica (Beerse, Belgium). Pentane was from Baker (Phillipsburg, N.J., U.S.A.), redistilled; ethanol, p.a. was from Merck (Darmstadt, G.F.R.). The analysis was performed on a dual-column 5710 (Hewlett-Packard) gas chromatograph with a N—P detector (Model 18789A). Column: SCOT, 10 m × 0.4 mm I.D., Duran 50 glass. Support layer: Tulla-

nox, silanized fumed silica, particle size <10 μm (Cabot Corp., Boston, Mass., U.S.A.). Stationary phase: Carbowax 20M. Temperatures: column 170°, injection port 200°, detector 300°. Gas flow-rates: carrier (helium) 10 ml/min, hydrogen 3 ml/min, air 100 ml/min, auxiliary gas (helium) 15 ml/min.

Extraction procedure

To 1.0 ml plasma in a centrifuge tube were added 25 μl ethanol containing 125.0 ng propoxate as internal standard. After homogenization the mixture was extracted once with 5 ml pentane on a Cenco whirlmixer for 15 sec. After centrifuging for 5 min at 2500 *g*, the upper organic layer was removed with a pasteur pipette and transferred to a conical evaporation tube. The solvent was evaporated to dryness at 40° in a stream of dry nitrogen on a water-bath. The residue was dissolved in 100 μl ethanol and 1—2 μl of this solution was brought on to the needle of the solid GC injection system, which was previously used for the determination of underivatized nitrazepam in plasma [3]. After evaporation of the solvent the residue was injected into the gas chromatograph.

Preparation of calibration curves

The concentration of etomidate was calculated with the aid of calibration curves prepared by adding known amounts of etomidate to 1.0 ml blank plasma. These standard samples were analysed by the same procedure as described previously and the ratios of the peak heights of etomidate to internal standard were plotted against the known concentrations of etomidate. The same procedure was followed for estimating the extraction yield of etomidate from plasma at various concentrations, except that propoxate (125 ng) was used as an external standard. The ratios found were compared to the ratios of standard amounts of the drugs. Before analysis of a sample series, calibration was always carried out using at least two plasma samples containing a known concentration of etomidate.

RESULTS AND DISCUSSION

Fig. 1 shows gas chromatograms of extracts from plasma samples taken at 8 and 240 min after the intravenous injection of 17 mg etomidate to a surgical patient, as well as the chromatogram of a blank extract. There is no interference from endogenous plasma substances or metabolites and retention times are short. Propoxate — which is structurally very closely related to etomidate — was chosen as an internal standard in the assay of etomidate and the two peaks are well separated, both at low and high concentrations.

Identification of the compounds eluting from the gas chromatograph was carried out by means of a combined gas chromatograph—mass spectrometer (LKB-2091 with PDP-11 computer system). The mass spectra of etomidate and propoxate show a parent peak at 244 *m/e* and a peak with a relative intensity of 100% at 105 *m/e*, representing in both cases the removed ethylbenzene moiety. On applying mass chromatography for the analysis of a plasma extract containing etomidate and propoxate, peaks with *m/e* values of 105 and 244 coincide with the GC retention time of etomidate, and peaks with *m/e* values of 105 and 258 have the retention time of propoxate (Fig. 2).

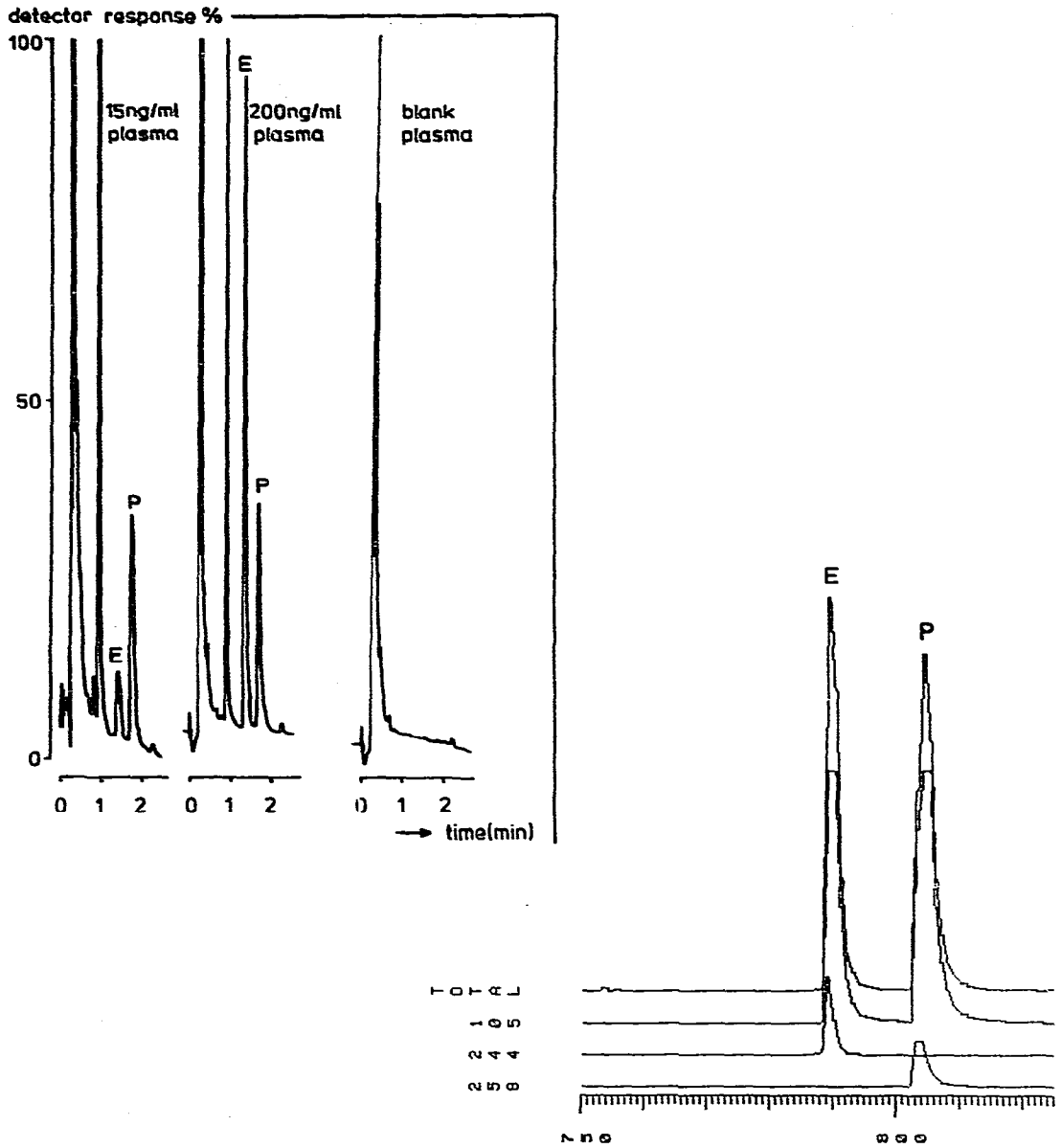

Fig. 1. Gas chromatograms of a plasma extract obtained from a male surgical patient immediately before (right), and 8 min (centre; 200 ng etomidate per ml plasma) and 240 min (left; 15 ng etomidate per ml plasma) after receiving 17 mg etomidate intravenously. E = etomidate, P = propoxate (internal standard; 125 ng/ml plasma).

Fig. 2. Chromatogram of a plasma extract containing etomidate and propoxate. The number on the X-axis refers to the numbers of the mass spectra taken during the particular GC—MS run. E = etomidate, P = propoxate.

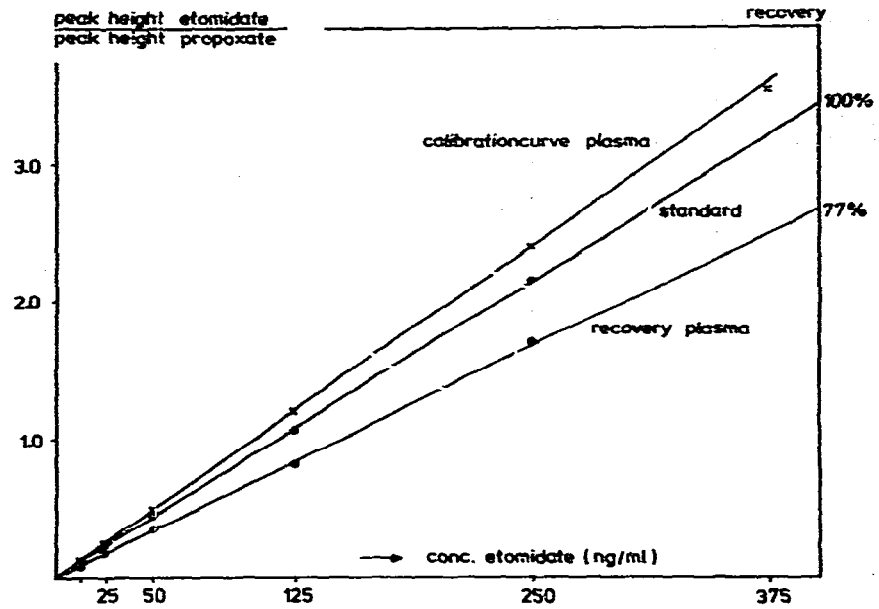

Fig. 3. Peak height ratio of etomidate to propoxate (125 ng) as a function of known etomidate concentrations. The standard curve was obtained with stock solutions of the two compounds. The recovery line was obtained by extraction of etomidate from plasma, using propoxate as external standard. The calibration graph was obtained by extraction of etomidate from plasma, using propoxate as internal standard.

It can be concluded that etomidate and propoxate both leave the GC column unchanged, so that they are being determined in intact form. According to the standard curve (Fig. 3) there is a linearity between the detector response ratio (peak height etomidate:peak height propoxate) and the concentration of etomidate between 12.5 and 375 ng/ml plasma. In Fig. 3 the extraction yields for etomidate in the concentration range 12.5—250 ng/ml plasma are also given. In spite of the short extraction time (15 sec) the recovery of etomidate from plasma is satisfactory (77%) and constant over the whole concentration range. The reproducibility of the whole procedure is reflected in the mean calibration curve of Fig. 3 (n = 5; S.D. $\leqslant$4.9%). It appears that the present method permits the accurate and specific determination of underivatized etomidate and propoxate in plasma in relatively low concentrations. The detection limit is about 5 ng etomidate per ml plasma. The use of the capillary Carbowax 20M SCOT column appears to be a definite improvement in the analysis of etomidate and many samples can be analysed in a short time. A solid injection system is required in order to prevent deterioration of the column support, caused by organic solvents.

Preliminary results have been obtained with surgical patients [4].

ACKNOWLEDGEMENTS

The authors wish to thank Drs. N.P.E. Vermeulen for taking the mass spectra, Mr. L. Bontje and co-workers for drawing the capillary columns, and Mr. L.W. Gerrése and co-workers for constructing the solid injection system.

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