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Note

Determination of dihydroergocristine and dihydroergotamine in plasma by high-performance liquid chromatography with fluorescence detection

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Dihydroergocristine (DHEC) is used in the treatment of cerebral and peripheral vascular diseases, and dihydroergotamine (DHET) in the treatment of migraine. The analytical procedures commonly used for the determination of these ergot derivatives in plasma are not selective or sensitive enough for pharmacokinetic studies.

The determination of total radioactivity after the administration of labelled compound is highly sensitive but lacks selectivity since ergot alkaloids are extensively metabolized [1—4].

Several radioimmunoassay (RIA) methods have also been described recently [5—9] but although these methods are generally rather sensitive they do not always distinguish between the parent compound and its metabolites. Thin-layer chromatographic and fluorimetric methods [10, 11] are not sensitive enough for pharmacokinetic studies.

Although several high-performance liquid chromatographic (HPLC) methods for the separation of ergot alkaloids in pharmaceutical preparations have been described [12—18], only a few studies report the application of HPLC to pharmacokinetic studies of ergot derivatives [19—21].

The present report describes a rapid, sensitive and selective HPLC method for the determination of DHEC and DHET in plasma and its application to pharmacokinetic studies in rats.

EXPERIMENTAL

Reagents and standard solutions

DHEC methanesulphonate was supplied by Roussel-Maestretti (Milan, Italy) and DHET methanesulphonate was obtained from Sandoz (Basle, Switzerland).

Stock solutions were prepared in methanol at a concentration of 1 mg/ml.

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Working standard solutions with a concentration of 1 μg/ml were prepared daily by dilution in water.

Acetonitrile and chloroform for liquid chromatography were obtained from Merck (Darmstadt, G.F.R.). All other chemicals and reagents were of analytical grade (Merck, and Carlo Erba, Milan, Italy).

Chromatographic apparatus and conditions

Perkin-Elmer (Norwalk, CT, U.S.A.) series 2/2 high-performance liquid chromatograph equipped with either a Perkin-Elmer LC-75 variable-wavelength UV detector and autocontrol system or with a Perkin-Elmer 650-10S spectrophotofluorimeter was used. Samples were introduced by means of a syringe into a Rheodyne 7105 (Berkeley, CA, U.S.A.) injection valve with a 150-μl loop. An RP-8 Hibar column (10 μm particle size; 25 cm × 4.0 mm I.D.) from Merck was operated at room temperature. The mobile phase was acetonitrile—pH 7.2 phosphate buffer (9 m*M* NaH_2PO_4 and 9 m*M* Na_2HPO_4) (60:40) and the flow-rate was 1.0 ml/min. The mixture was degassed at room pressure in an ultrasonic bath for a few minutes. The column effluent was monitored at 223 nm with the UV detector. The spectrophotofluorimeter was employed with an excitation wavelength of 295 nm, an emission wavelength of 350 nm and band widths for both excitation and emission of 10 nm.

Procedure

A 1-ml volume of plasma, 50 μl of internal standard solution and 30 μl of 5 *M* sodium hydroxide solution were placed into a 12-ml tapered glass tube. After adding 7 ml of chloroform, the tubes were shaken on a reciprocal shaker for 10 min. After centrifugation at 2000 *g* for 15 min, the aqueous phase and the emulsion layer were totally aspirated off, and the organic phase transferred into fresh tubes and evaporated to dryness under a flow of nitrogen at 40°C. The residues were reconstituted in 100 μl of the mobile phase and 10—30 μl injected into the chromatograph.

In addition to the unknown samples, plasma calibration standards containing 5, 10, 25 and 50 ng of either DHET or DHEC and 50 ng of internal standard (DHEC or DHET, respectively) were run.

Calibration curves were constructed by plotting the DHEC or DHET concentrations versus the ratio of the peak heights of compounds to those of their respective internal standards.

Recovery

The percentage recovery was calculated by comparing the peak height ratios for DHEC and DHET standards prepared in mobile phase, with those obtained after plasma extracts at the same concentrations were injected.

Animals

Charles-River rats weighing 150—180 g, kept in makrolon cages at constant room temperature (21—22°C) and humidity (60%) were used. At various times after drug administration, the rats were killed by decapitation and the blood collected in plastic tubes containing 0.1 ml of sodium heparin. After centrifugation at 2200 *g* for 15 min the plasma was frozen and kept at −30°C until analysis.

Drugs were given orally in solution by gavage (5 mg/kg, 1.0 ml/kg) or by intraperitoneal injection (1 mg/kg, 1.0 ml/kg).

RESULTS

Detection

With UV detection, the absorbance spectra for DHEC and DHET showed two peaks at 282 and 223 nm. As the highest sensitivity and specificity were achieved at 223 nm, this wavelength was chosen for plasma monitoring. The minimal detectable amount of pure compounds was about 1 ng. The lower limit of detection in plasma extracts was 5—10 ng/ml.

Using fluorescence detection, the excitation and emission peak readings, recorded in spectra by stopping the column outflow into the cell were 295 and 350 nm, respectively. The minimal detectable amount of pure compounds was 0.1 ng. The selectivity and high sensitivity of this detection method increased the sensitivity for measurement of the compounds in plasma extracts to 0.5—0.7 ng/ml.

Extraction and chromatography

Both DHEC and DHET are relatively polar and quantitative extraction can be obtained only with a rather polar solvent. On the other hand, it is advisable to use a solvent with as low a polarity as possible to avoid extraction of interfering plasma substances. In our hands chloroform was a good compromise and gave extracts that were sufficiently clean, particularly for fluorescence detection; the recovery was high even after a single extraction (91 ± 4%, n =

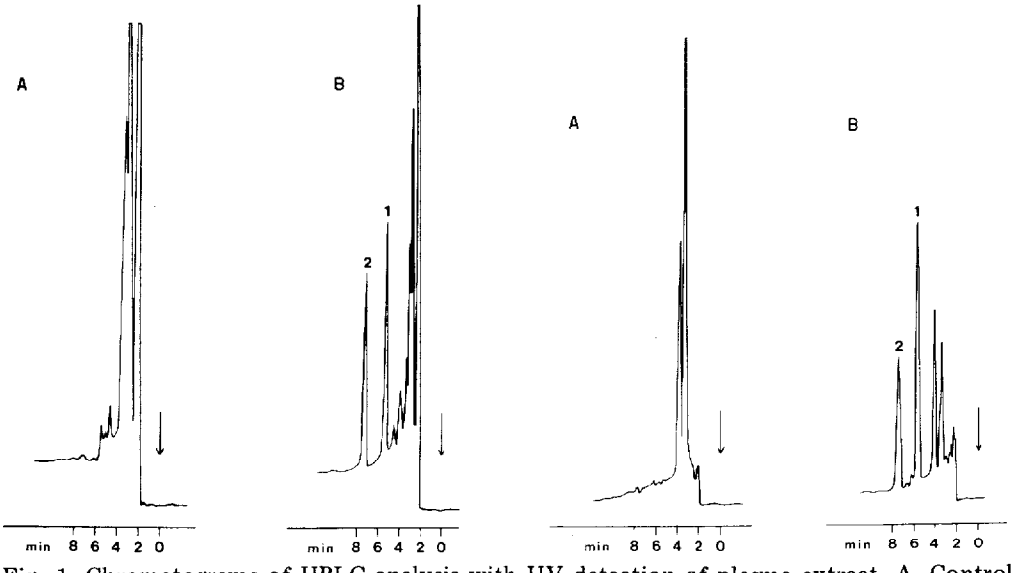

Fig. 1. Chromatograms of HPLC analysis with UV detection of plasma extract. A, Control plasma; B, control plasma containing 100 ng/ml of both DHEC (2) and DHET (1).

Fig. 2. Chromatograms of HPLC analysis with fluorescence detection of plasma extracts. A, Control plasma; B, control plasma containing 25 ng/ml of DHET (1) and 25 ng/ml of DHEC (2).

6 with an intra-assay coefficient of variation, C.V., of 3.4% for DHEC and 93 ± 6%, n = 6, with a C.V. of 2.8% for DHET). the inter-assay C.V. was found to be of 4.8% for DHEC and 5.2% for DHET. Separation of DHEC and DHET with chromatographic system described above was good (retention times: 7.9 and 5.2 min, respectively) and no interfering peaks were present after either UV or fluorescence detection (Figs. 1 and 2).

Plasma levels

The method described above has been used in our laboratory for the determination of the pharmacokinetics of DHEC and DHET. Peak concentrations after 1 mg/kg i.p. and 5 mg/kg oral administration of the drugs are shown in Table I and typical chromatograms of plasma extracts in Figs. 2 and 3. Peak concentrations were reached 10 min after i.p. and 1 h after oral administration.

TABLE I

PEAK PLASMA LEVELS (ng/ml) OF DHEC AND DHET AFTER ORAL (5 mg/kg) AND INTRAPERITONEAL (1 mg/kg) ADMINISTRATION

Values are mean ± S.E. of five determinations.

Drug	Oral	i.p.
DHEC	3.8 ± 0.9	59.9 ± 6.3
DHET	3.2 ± 0.8	53.0 ± 5.8

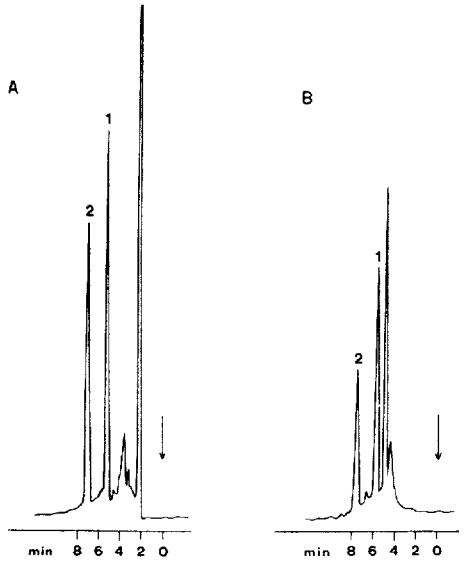

Fig. 3. Chromatograms of HPLC analysis with fluorescence detection of plasma extracts. A, Plasma extract from a rat administered 1 mg/kg i.p. of DHEC (actual concentration 52 ng/ml) (2) and containing 50 ng/ml of DHET (1) as internal standard. B, Plasma extract from a rat administered 1 mg/kg i.p. of DHET (actual concentration 33 ng/ml) (1) and containing 25 ng/ml of DHEC (2) as internal standard.

DISCUSSION

Fluorescence detection proved to be clearly more selective and sensitive

than UV detection. In the present work the sensitivity for DHET was higher than that found by Edlund [21]. This may possibly be due to the greater efficiency of the fluorimeter we used, which is equipped with two monochromators instead of filters. In fact, in our experience filters absorb as much as 70% of the light energy, while monochromators absorb less than 10%. The present chromatographic method can be used to separate the dihydro derivatives of ergotamine, ergocornine, ergocristine and ergocryptine. However, to obtain a complete separation of these compounds a chromatographic column with higher resolution efficiency such as 5 μm RP-8, has to be used. Our HPLC—fluorescence method allows us to measure plasma concentrations of DHEC and DHET after parenteral or oral administration to rats. We do not consider, however, that the sensitivity obtained with this method is great enough for kinetic studies of ergot alkaloids after oral administration of therapeutic doses to humans. Our data confirm the low oral bioavailability of ergot derivatives, the plasma concentrations after oral administration being only about 6% of those after parenteral administration. In fact, using the HPLC—fluorescence method, Ibraheem et al. [20] could measure the plasma levels of ergotamine after parenteral but not after oral administration, indicating a very low oral bioavailability of the drug.

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