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Note

High-performance liquid chromatographic determination of dexamethasone in human plasma

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In the detection of Cushing's syndrome, the interpretation of the dexamethasone suppression test (DST) is sometimes questionnable. Some authors [1, 2] emphasize the interest of simultaneous determination of cortisol and dexamethasone (DXM) in the simplified DST (Nugent test).

Except for radioimmunologic assays [3—5] which require a highly specific antibody in order to avoid cross-reactions with possible metabolites of the DXM, and gas chromatography—mass spectrometry [6, 7] which is time-consuming and not compatible with routine determinations, high-performance liquid chromatographic (HPLC) methods [8—10] do not allow the detection of

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the DXM in the nanogram range. This report describes a reversed-phase HPLC method which is sensitive and specific for the determination of plasma concentrations of DXM below 1 ng/ml.

EXPERIMENTAL

Materials

DXM, equilenine and cortisol were obtained from Sigma (St. Louis, MO, U.S.A.). Acetonitrile, methylene chloride and methanol for chromatography, sodium acetate · $3H_2O$ and acetic acid were purchased from Merck (Darmstadt, F.R.G.). Acetate buffer (0.002 *M*) was prepared in freshly glass-bidistilled water adjusted to pH 4.8 with acetic acid and filtered through a 0.45-μm Millipore filter (Bedford, MA, U.S.A.).

Stock solutions of DXM and cortisol were prepared in methanol at concentrations of 1 and 0.5 g/l for equilenine, respectively. These solutions were stored at 0—5°C. Standard solutions were prepared in methanol at a concentration of 1 mg/l for DXM and 2.5 mg/l for cortisol and equilenine. These solutions were prepared freshly every day.

Preparation of standards

We used cortisol-stripped plasma [11] for the preparation of the DXM standards. To this plasma we added DXM, equilenine (internal standard) and cortisol standard solutions to provide a concentration of 1–20 ng/ml for DXM, 12.5 ng/ml for equilenine and 25 ng/ml for cortisol.

The ratios between the peak heights of DXM and equilenine were plotted versus the concentrations of the standards to obtain a calibration curve. Equations of the computed regression lines and correlation coefficients were calculated.

Extraction procedure

The internal standard (25 ng of equilenine in 10 μl of methanol) and 9 ml of methylene chloride were added to 2 ml of plasma. The mixture was stirred for 1 min on a Vortex Genie mixer (Scientific Industrie, Bohemia, NY, U.S.A.) and centrifuged 5 min at 2600 *g*. The aqueous layer and creamy interface were discarded. The organic phase was transferred into a cone-shaped tube, evaporated to dryness at 60°C under a stream of nitrogen and reconstituted with 30 μl of methanol.

Chromatography

A liquid chromatograph (Varian, Model 5000) equipped with a variable-wavelength detector (Varian, Varichrom) was used in a reversed-phase system with a μBondapak C_{18} column as stationary phase (300 × 3.9 mm I.D.; particle size 10 μm, Waters). The volume of sample injected was 20 μl (Valco Valve). The flow-rate of the mobile phase, acetate buffer—acetonitrile (58:42), was 1 ml/min. The effluent was monitored at 246 nm and the chart-speed was 0.25 cm/min.

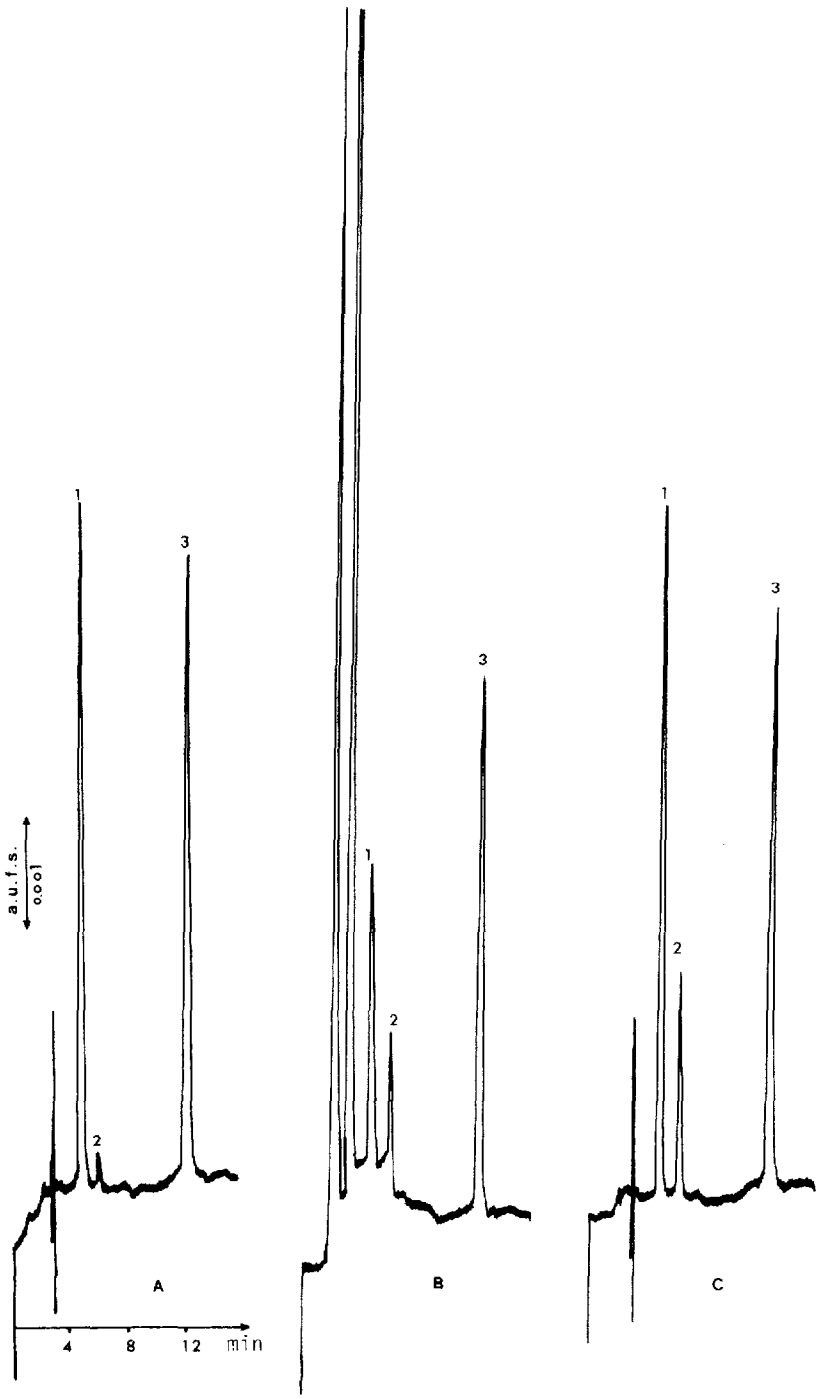

Fig. 1. Chromatograms of (A) charcoal-stripped plasma extract spiked with 25 ng/ml cortisol (1), 1 ng/ml DXM (2) and 12.5 ng/ml equilenine (3). (B) Plasma extract from a patient 8 h after a single dose of 1 mg of DXM. (C) Charcoal-stripped plasma extract spiked with 25 ng/ml cortisol (1), 10 ng/ml DXM (2) and 12.5 ng/ml equilenine (3).

RESULTS

Typical chromatograms are shown in Fig. 1.

Precision

The within-day and between-day precisions were evaluated by analysing cortisol-stripped plasma spiked with known amounts of DXM. The results are summarized in Table I. Both coefficients of variation are ca. 5% for the lower concentrations and < 5% for the highest.

TABLE I

PRECISION OF THE METHOD

All variability statistics are based on five measurements.

	Concentration (ng/ml)	Coefficient of variation (%)
Intra-day	2.5	5.4
	5	3.6
	10	3.5
Inter-day	1.25	5.1
	2.5	3.9
	5	3.6
	10	4
	20	3.7

Extraction efficiency

The recoveries for DXM and equilenine were determined by adding known amounts of each component before and after the extraction and calculated as follows:

For DXM:

$$\frac{\text{Peak height of DXM extracted}}{\text{Peak height of equilenine extracted}} \times \frac{\text{Peak height of equilenine extracted}}{\text{Peak height of DXM non-extracted}} \times 100$$

For equilenine:

$$\frac{\text{Peak height of equilenine extracted}}{\text{Peak height of DXM extracted}} \times \frac{\text{Peak height of DXM extracted}}{\text{Peak height of equilenine non-extracted}} \times 100$$

The results for extraction efficiency are presented in Table II.

TABLE II

EXTRACTION EFFICIENCY OF DEXAMETHASONE AND EQUILENINE

Compound	Concentration (ng/ml)	*n*	Extraction efficiency (mean ± S.D.) (%)
Dexamethasone	2.5	3	89.1 ± 4
	10.0	3	92.2 ± 1.8
Equilenine	12.5	5	91.5 ± 4.3

Linearity

A linear relationship was observed between the ratio of DXM to equilenine (y) and the amount of DXM added to plasma (x, ng/ml): $y = 0.039x + 0.013$; $r = 0.9977$; $n = 25$; concentration range 1.25—20 ng/ml; 0.01 a.u.f.s.

Detection limit

No interfering substances were present in chromatograms spiked with the following drugs: triamcinolone, prednisone, betamethasone, hydrocortisone. The limit of detection in plasma (2 ml), measured at a detector sensitivity of 0.01 a.u.f.s. and allowing a signal-to-noise ratio of 2, was 0.5 ng/ml.

Concentration of DXM in human plasma

Dexamethasone alcohol (2 × 0.5 mg, Decadron®; Merck, Sharp & Dohme) was administered orally at midnight to seven healthy volunteers (three women, four men; mean age 22.7 years; average body weight 61 kg). Blood samples (10 ml) were collected hourly between 0 and 8 h in tubes containing sodium heparin. The plasma was immediately separated and stored frozen (−20°C) until analysis. Fig. 2 presents the concentration—time profile of dexamethasone (mean ± S.D. of the seven volunteers). We found a terminal phase half-life of 4 h 43 min ± 2 h 12 min (mean ± S.D.). The peak of DXM appears between 1 and 2 h after administration, with a concentration of 7.7 ± 2.9 ng/ml.

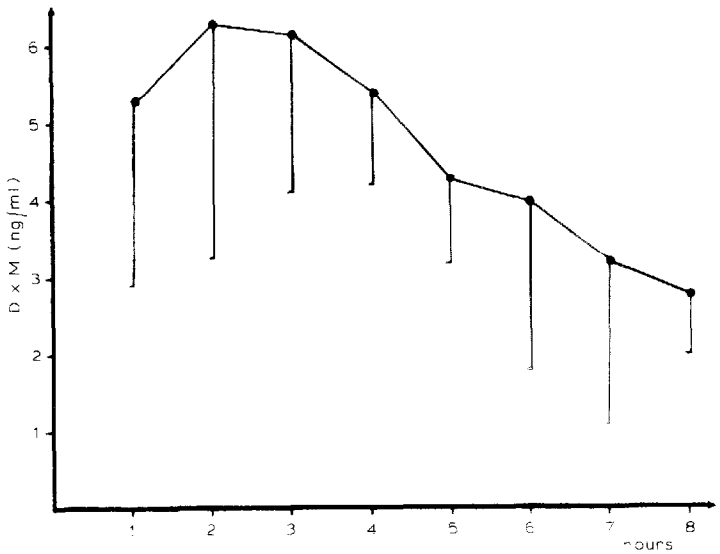

Fig. 2. Plasma dexamethasone concentrations (mean ± S.D.; $n = 7$) after a single oral dose of 1 mg of dexamethasone alcohol given at midnight.

DISCUSSION

We found a maximal UV absorbance at 246 nm for DXM. The use of a 2-ml plasma sample permitted a detection limit as low as 0.5 ng/ml, which was sufficient for kinetic study of the drug after a single oral dose of 1 mg. This detection limit was comparable to those found by different authors in the

determination of synthetic steroids other than DXM by HPLC methods [12, 13]. We did not find differences in the extraction efficiency when we used distilled water for preparation of the standards, and the coefficients of the regression line were similar. We used equilenine as an internal standard because this drug is not extensively used in human pathology and it has an extraction coefficient similar to that of DXM. The measurement of DXM by this HPLC technique is efficient, precise, sensitive and selective; furthermore, it can be used for the simultaneous determination of DXM and cortisol, which is useful in the DST.

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REFERENCES

1 A.W. Meikle, Clin. Endocrinol., 16 (1982) 401.
2 G.F. Johnson, G. Hunt, K. Kerr and I. Caterson, Psychiatr. Res., 13 (1985) 305.
3 D.E. Duggan, K.C. Yeh, N. Matalia, C.A. Ditzler and F.G. McMahon, Clin. Pharmacol. Ther., 18 (1975) 205.
4 L.H. Hare, K.C. Yeh, C.A. Ditzler, F.G. McMahon and D.E. Duggan, Clin. Pharmacol. Ther., 18 (1975) 330.
5 S. Miyabo, T. Nakamura, S. Kuwajima and S. Kishida, Eur. J. Clin. Pharmacol., 20 (1981) 277.
6 Y. Kasuya, J.R. Althaus, J.P. Freemann, R.K. Mitcham and J.P. Skelly, J. Pharm. Sci., 73 (1984) 446.
7 K. Minagawa, Y. Kasuya, S. Baba, G. Knapp and J.P. Skelly, J. Chromatogr., 343 (1985) 231.
8 S.E. Tsuei, J.J. Ashley, R.G. Moore and W.G. McBride, J. Chromatogr., 145 (1978) 213.
9 B.E. Cham, B. Sadowski, J.M. O'Hagan, C.N. de Wytt, F. Bochner and M.J. Eadie, Ther. Drug. Monit., 2 (1980) 373.
10 J.C.K. Loo and N. Jordan, J. Chromatogr., 143 (1977) 314.
11 J.Q. Rose and W.J. Jusko, J. Chromatogr., 162 (1979) 273.
12 M.L. Rocci, Jr. and W.J. Jusko, J. Chromatogr., 224 (1981) 221.
13 W.F. Ebling, S.J. Szefler and W.J. Jusko, J. Chromatogr., 305 (1984) 271.