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Short Communication

Simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone in plasma by high-performance liquid chromatography

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Introduction

Corticosteroids are commonly used in medical practice and during their application it is necessary to measure the concentration of corticosteroids in body fluids. On the other hand, the amount of endogenous corticosteroids and their metabolites is useful as a diagnostic aid. High-performance liquid chromatography (HPLC) has been widely used for steroid analysis, with adsorption techniques being commonly used for corticosteroids [1, 2]. The advantage of normal-phase HPLC compared with adsorption chromatography has been described by several workers [3, 4]. Toothaker *et al.* [5] studied both normal- and reversed-phase systems for determining corticosteroids. The simplicity and versatility of reversed-phase systems with aqueous methanolic eluents makes this the most likely system to be available in clinical chemistry laboratories. The most widely used stationary phases are non-polar in nature, with octadecyl ligands bonded to microparticulate silicon [6, 7]. A reversed-phase procedure on stationary phases with shorter alkyl chain which is applicable to the determination of corticosteroids has also been described [8, 9]. Recently, several HPLC assays for the simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone in biological fluids have been reported. These methods use normal-phase HPLC systems or gradient elution analysis or complex mobile phases consisting of three or four solvents [3, 4, 9–11].

The purpose of the presented study was to develop a rapid and simple reversed-phase HPLC method for the simultaneous determination of the most frequently used synthetic corticosteroids prednisone, prednisolone and dexamethasone as well as endogenous cortisol.

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Experimental

Reagents and solvents

Analytical standards of prednisone, prednisolone, cortisol and dexamethasone were purchased from WHO Centre for Chemical Reference Substances (Solna, Sweden). Methanol, tetrahydrofuran and methylene chloride (LiChrosolv, Merck, Darmstadt, FRG) were UV grade. All solvents and double-distilled water were filtered through a 0.45-μm pore filter (Millipore, Bedford, MA, USA) and degassed before use.

Apparatus and operating conditions

The analysis was performed by means of a Perkin–Elmer Series 4 liquid chromatograph equipped with a spectrophotometric detector LC-85, automatic sampling system ISS-100 and a prepacked 25 cm × 4.6 mm, i.d. reversed-phase 7 μm C_{18} column (Dr H. Knauer, Oberursel, FRG). Chromatograms were plotted and numerical results calculated by a PE Sigma 15 Chromatography Data Station. All the apparatus used was supplied by Perkin–Elmer Corp., Norwalk, CN, USA. The mobile phase consisted of water/tetrahydrofuran (77:23, v/v). Solvents were continuously degassed using helium. A flow rate of 1.5 ml min^{-1} was used at an input pressure of 15.6–16.6 MPa, the column temperature was 40°C and the detector operated at a wavelength of 245 nm.

Sample preparation

An aliquot (1 ml) of the plasma was transferred to a tube and 0.5 g of sodium sulphate was added, followed by 10 ml of methylene chloride with prednisone as the internal standard (20 ng ml^{-1}). The contents were mixed for 60 s using a vortex-type mixer and centrifuged at 3000 rpm for 10 min. The organic phase was aspirated, transferred to a tube and the solvent was evaporated at 45°C in a stream of nitrogen. The residue was dissolved in 100 μl of methanol, filtered through a 0.45-μm pore filter, and 20 μl injected.

For the determination of prednisone, the described method was modified by substituting dexamethasone as the internal standard.

Measurement of percentage recovery

The percentage recoveries of cortisol and dexamethasone were determined as follows: 10–600 ng of cortisol and dexamethasone was added to 1 ml plasma. Samples were then analysed as described, except that prednisone (20 ng) was added after the extraction procedure. Recovery was calculated by comparing the peak area ratio of a reference mixture of equal amounts of cortisol, dexamethasone and prednisone.

For the measurement of the recoveries of prednisone and prednisolone the same procedure was carried out except that instead of prednisone, dexamethasone was used as the internal standard.

From the peak area of cortisol a blank plasma response was subtracted in order to account for an endogenous serum component of similar retention.

Method of calculation

Prednisone, prednisolone, cortisol and dexamethasone concentrations were calculated using the expression:

$$\text{Concentration of substance of interest} = (A/B) \times (C/D) \times (E/F), \qquad (1)$$

where A is peak area of substance of interest, B the peak area of the internal standard, C the recovery of internal standard, D the recovery of substance of interest, E the concentration of internal standard and F the relative response factor of substance of interest.

The overnight dexamethasone suppression test

The overnight dexamethasone suppression test was used to test the ability of the method to detect endogenous cortisol in plasma of depressed patients. The test was performed on 16 depressed patients (52–89 kg; 28–64 years of age). Plasma samples were taken from a patient 12 h before the oral administration of 0.5 mg of dexamethasone (Dexamethason®, Pliva, Zagreb, Yugoslavia), and next morning, 12 h after the oral administration. Samples were analysed by the described method.

Results and Discussion

The separation of the four corticosteroids by the described system is illustrated in Fig. 1.

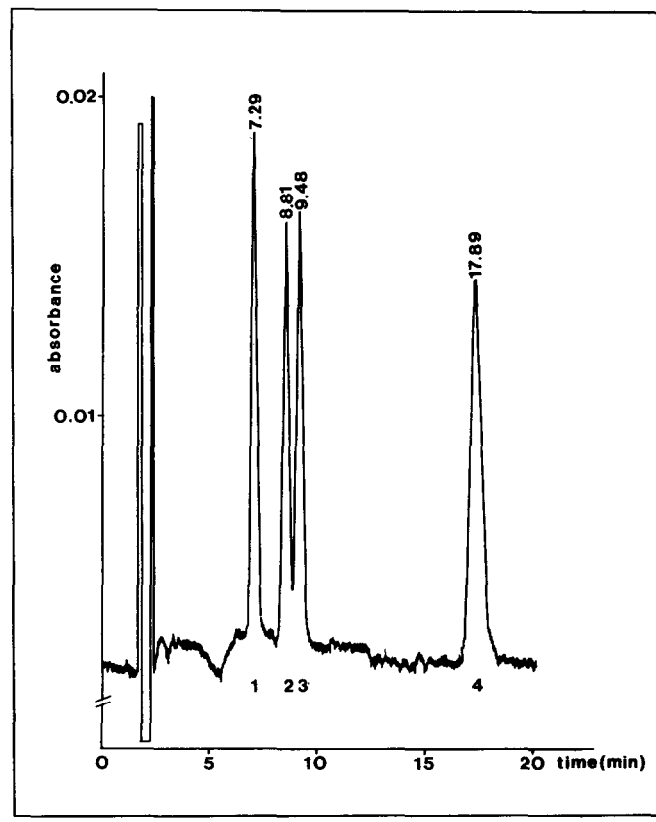

Figure 1
Chromatogram obtained for a standard solution (10 μg ml^{-1} in methanol) of the following: (1) prednisone, (2) prednisolone, (3) cortisol and (4) dexamethasone (20 μg ml^{-1}). The mobile phase was water/tetrahydrofuran (77:23) at a flow rate of 1.5 ml min^{-1}, column temperature 40°C, detector wavelength 245 nm, sample volume 20 μl, detector sensitivity 0.02 AUFS.

Relative response factors

The relative response factors were found to be 0.785 for prednisone, 1.076 for prednisolone, 1.079 for cortisol, and 1.081 for dexamethasone.

Precision

Between day (n = 10) relative standard deviations (RSD) of 4.6 and 1.9% were obtained at plasma concentrations of 50 and 250 ng ml^{-1} of prednisone, respectively. For cortisol at the 45 and 245 ng ml^{-1} level, RSD values of 9.7 and 1.9%, respectively, were obtained. RSD values of 8.0 and 2.5% were obtained at plasma concentrations of 50 and 250 ng ml^{-1} of prednisolone, respectively, which for dexamethasone at the same plasma concentration RSDs of 3.0 and 0.5% were observed.

In all the cases tested, the plots of peak–area ratio versus corticosteroid concentrations were linear over the 40–600 ng ml^{-1} range, with correlation coefficients of 0.9958 for prednisone, 0.9911 for prednisolone, 0.9938 for cortisol, and 0.9945 for dexamethasone.

Recovery

The mean percentage recoveries and RSDs (n = 6) were found to be 76% (2.0) for prednisone, 92% (1.6) for prednisolone, 97% (1.9) for cortisol, and 95% (1.4) for

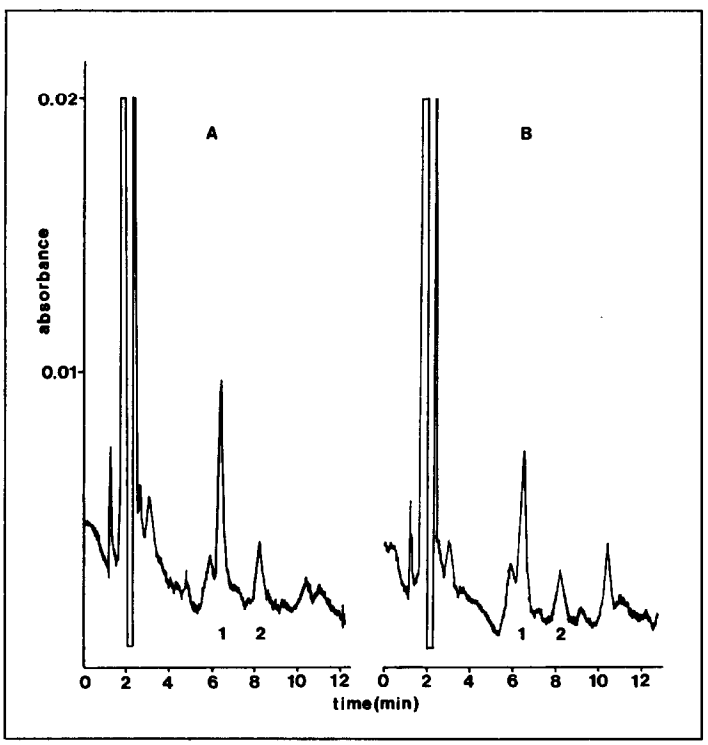

Figure 2
Chromatogram obtained for the plasma of depressed patients 12 h before oral administration of dexamethasone. The plasma concentration of cortisol was calculated to be 108 ng ml^{-1} (A). The plasma chromatogram of the same subject 12 h after oral administration of 0.5 mg of dexamethasone. The plasma concentration of cortisol was calculated to be 155 ng ml^{-1} (B). Both samples were spiked with 200 ng ml^{-1} of prednisone as internal standard. Key: (1) prednisone (internal standard), (2) cortisol. Conditions are as in Fig. 1.

dexamethasone. These values are used in equation (1) when calculating the corticosteroid concentrations in unknown samples.

Sensitivity

Plasma concentrations of 40 ng ml^{-1} of steroids can be reliably analysed by this procedure. In the above analysis, a baseline signal-to-noise ratio of 2 corresponds to a minimum detection limit of 10 ng ml^{-1} of corticosteroids.

The overnight dexamethasone suppression test

An example of the chromatogram obtained for the plasma of depressed patients 12 h before oral administration of dexamethasone is shown in Fig. 2, along with the plasma chromatogram of the same subject sampled 12 h after oral administration of 0.5 mg of dexamethasone.

The morning level of cortisol in plasma of depressed patients was not decreased below the physiological value after administration of dexamethasone for 13 of the 16 patients examined as shown in Table 1.

Table 1
Concentrations of cortisol in plasma of 16 depressed patients 12 h before and 12 h after oral administration of 0.5 mg of dexamethasone

Patient	Plasma concentration of cortisol (ng ml^{-1})	
	Before administration of dexamethasone	After administration of dexamethasone
1	39	—
2	102	195
3	108	155
4	141	—
5	106	35
6	160	108
7	96	111
8	99	102
9	92	98
10	90	112
11	88	135
12	81	82
13	152	140
14	67	122
15	128	118
16	105	104

The described method offers a simple, rapid and reliable simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone. Using described conditions, a satisfactory resolution of prednisolone and cortisol was obtained and the possibility of the quantitative analysis in plasma demonstrated.

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