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DETERMINATION OF DEXAMETHASONE IN HUMAN PLASMA AND URINE BY ELECTRON-IMPACT MASS SPECTROMETRY

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SUMMARY

A gas chromatographic—electron-impact mass spectrometric method for the determination of dexamethasone in biological fluids has been developed. Quantitation by isotope-dilution mass spectrometry was carried out by selected-ion monitoring on the molecular ions of the tetra(trimethylsilyl) derivative of dexamethasone and of dexamethasone M+9 (*m/z* 680 and 689, respectively). The sensitivity, specificity, precision and accuracy of the method were demonstrated to be satisfactory for application to pharmacokinetic and bioavailability studies of dexamethasone after administration of a therapeutic dose.

INTRODUCTION

Dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione), a potent synthetic glucocorticoid, has been used for many years as an anti-inflammatory agent and for the diagnosis of Cushing's syndrome. Because of the very small doses used clinically, bioavailability and pharmacokinetic studies of dexamethasone require sensitive, specific and reproducible analytical techniques.

Several studies have employed radioimmunoassay (RIA) techniques to assess the bioavailability of oral dexamethasone and intravenous dexamethasone

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esters [1—3]. RIA, however, suffers from poor specificity and poor reproducibility.

Recently, more specific and precise high-performance liquid chromatographic (HPLC) methods have been widely used in determining natural and synthetic corticosteroids in biological fluids [4–6] and in pharmacokinetic studies of dexamethasone [7, 8]. HPLC offers the advantage that the corticosteroids can be analysed without derivatization. However, it lacks sensitivity and cannot be used for accurate determinations of plasma concentrations after administration of a therapeutic dose in the nanogram to subnanogram range.

The use of gas chromatography—mass spectrometry (GC–MS) and stable isotope-labelled drugs as diluents has found broad application in pharmacological studies [9, 10]. In this technique, stable isotope-labelled carriers serve as an ideal internal standard to correct for losses of the sample substance in the initial isolation procedure. Pharmacokinetic studies represent one field in which the sensitivity and specificity of GC—MS techniques offer an advantage.

In a previous paper [11], we described the determination of dexamethasone in human plasma by gas chromatography—chemical-ionization mass spectrometry (GC—CI-MS). This paper is concerned with the electron-impact (EI) mode of mass spectrometry with selected-ion monitoring (SIM) using dexamethasone M+9 ([$^{13}C_6$,$^{2}H_3$] dexamethasone) as an internal standard for the determination of dexamethasone in human plasma and urine after administration of therapeutic doses.

EXPERIMENTAL

Chemicals and reagents

Dexamethasone was a gift from Sigma (St. Louis, MO, U.S.A.). Stable isotope-labelled dexamethasone, [1,2,3,4,10,19-$^{13}C_6$,19,19,19-$^{2}H_3$] dexamethasone, i.e., dexamethasone M+9, was synthesized under an FDA contract (contract No. 223-79-3011; SRI International, Palo Alto, CA, U.S.A.). N,O-Bis(trimethylsilyl)acetamide (BSA) was purchased from Tokyo Kasey Kogyo (Tokyo, Japan). Methanol, methylene dichloride and *n*-hexane were of HPLC grade. All other chemicals and reagents were of analytical-reagent grade and were used without further purification.

Gas chromatography—mass spectrometry—selected-ion monitoring

GC—MS—SIM measurements were made with a Shimadzu QP1000 gas chromatograph—mass spectrometer system equipped with a data processing system. Gas—liquid chromatography was performed on a glass column (58 cm × 3.0 mm I.D.) packed with about a 20-cm length of 1.5% SP-2100 on Supelcoport (80—100 mesh). The column temperature was 268°C, the injector temperature 275°C and the ion source temperature 280°C. Helium was used as the carrier gas at a flow-rate of about 40 ml/min. The electron energy was set at 20 eV. The multiple-ion detector was focused on the molecular ions at *m/z* 680 for the tetra(trimethylsilyl) (TMS) derivative of non-labelled dexamethasone and at *m/z* 689 for the TMS derivative of labelled dexamethasone.

Sample preparation for GC—MS—SIM

To 1.0 ml of plasma were added 20 ng of dexamethasone M+9 dissolved in 10 μl of methanol, and the plasma sample was applied to a Sep-Pak C_{18} cartridge (Waters Assoc., Milford, MA, U.S.A.). The cartridge was washed with 8 ml of distilled water and then eluted with 2 ml of methanol. The methanol eluate was evaporated to dryness under a stream of nitrogen. The residue was dissolved with 100 μl of 10% methanol in methylene dichloride and injected into a liquid chromatograph (Hitachi Model 655) equipped with a variable-wavelength UV detector. The normal-phase HPLC column packed with LiChrosorb Si 100 (5 μm) (0.25 m × 4.0 mm I.D.) was eluted with 30% methylene dichloride—2.5% methanol–0.5% ethanol–0.2% acetic acid in *n*-hexane at the rate of 2.5 ml/min, and the column effluent was monitored at 240 nm. Approximately 5 ml of HPLC eluate of the appropriate fraction were collected in a glass tube. The solvent was concentrated under a stream of nitrogen and the concentrated sample was transferred into a small reaction vial. The solvent was evaporated and to the residue were added 10 μg of sodium acetate in 10 μl of methanol. After evaporation of the methanol solution to dryness under a stream of nitrogen, 50 μl of acetone were added to the residue and the solution was evaporated completely to dryness under a stream of nitrogen.

The TMS derivative was formed by reacting the residue contained 10 μg of sodium acetate as a catalyst with 2.0 μl of BSA in the presence of 5.0 μl of pyridine as a solvent. The reaction was completed in 1.0—1.5 h at 90°C. After cooling, 10 μl of *n*-hexane were added to the reaction mixture and the mixture was refluxed using an auto-mixer for about 10 sec. Excess of reagent was removed under a stream of nitrogen and the residue was dissolved in 20 μl of *n*-hexane. A 2—4 μl aliquot of the *n*-hexane solution was subjected to GC—MS.

Preparation of calibration graph

Known amounts of dexamethasone (0.5–200 ng) and 20 ng of dexamethasone M+9 dissolved in 10 μl of methanol were added to 1.0-ml portions of human blank serum. Each sample was prepared in duplicate. The samples were then carried through the entire procedure as described above and the peak-height ratios (*m/z* 680 to *m/z* 689) were measured.

Determination of accuracy

Dexamethasone in amounts of 2.00 and 5.01 ng dissolved in 10 μl of methanol was added to 1.0-ml portions of human blank serum. After preparation of the sample for GC—MS—SIM as described above, the peak-height ratio (*m/z* 680 to *m/z* 689) was determined in triplicate.

Drug administration

A healthy adult male volunteer, 40 years old, weighing 64 kg, received after an overnight fast six 0.75-mg dexamethasone tablets orally with 200 ml of water on the first day. On the second day, he received 1 mg of dexamethasone in its phosphate ester form in 2 ml of saline intravenously over 5 min. No food was permitted for 4 h after drug administration. On the first day, 5 ml of heparinized blood sample were taken immediately before the oral dose and at

15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720 and 1440 min after dosing. On the second day, 5 ml of heparinized sample were taken immediately after the end of injection and at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 375, 480, 600 and 720 min after the end of injection. The samples were centrifuged at 2000 rpm for 10 min. The plasma samples were placed in culture tubes with plastic caps and stored at −20°C until the time of assay. Urine samples were collected at 0—4, 4—8, 8—12 and 12—24 h after each dosing. The urine volumes were measured and the samples were stored at −20°C until the time of assay.

RESULTS AND DISCUSSION

The use of isotope dilution techniques coupled with isotope ratio determination by MS is rapidly becoming the method of choice in the determination of steroids in biological samples [11, 12—14]. As bioavailability and pharmacokinetic studies of synthetic corticosteroids require sensitive, specific and reproducible analytical techniques, attempts were made to devise procedures that would allow the determination of nanogram to subnanogram amounts of dexamethasone in human blood and urine by isotope-dilution MS.

Several derivatives of glucocorticoids for GC analysis have been introduced in the past [15–18]. It has been suggested that in order to produce stable silylated derivatives of corticosteroids it is necessary first to protect the ketone groups at C-3 and C-20 to form the methoxime (MO) [19—21]. From a quantitative point of view, the MO-TMS derivative has been shown to be the most satisfactory owing to its thermal stability and good GC behaviour [22] and has been used for the determination of synthetic glucocorticoids such as prednisone and prednisolone in biological fluids using GC—MS [23].

The formation of the MO derivative, however, has been shown not to be necessary with dexamethasone when the silylation reaction was carried out with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of anhydrous potassium acetate as a base catalyst [11]. In this study, sodium acetate was used as a base catalyst and the tetra-TMS derivative of dexamethasone was obtained by reaction with a small volume of BSA instead of BSTFA at 90°C for 1—1.5 h in the presence of pyridine as a solvent.

Fig. 1 shows the gas chromatogram and EI mass spectrum of tetra-TMS-dexamethasone. A single product was obtained, and the tetra-TMS derivative showed good GC behaviour. The EI mass spectrum of tetra-TMS-dexamethasone demonstrated that the relative intensity of the molecular ion at *m/z* 680 was prominent. When the molecular ion *m/z* 680 was monitored, the sensitivity limit of the GC—MS—SIM assay was found to be 100 pg per injection. A signal-to-noise ratio of $\geqslant$ 2.5 was used as a criterion for a significant response. This sensitivity limit was comparable to that of the CI-MS method described previously [11].

The selected-ion recordings of tetra-TMS-dexamethasone at *m/z* 680 and tetra-TMS-dexamethasone M+9 at *m/z* 689 after processing from plasma sample shown in Fig. 2 indicate that no interfering substances with retention times close to those of the TMS derivatives were present.

Generally, the MS analysis of corticosteroids in biological fluids requires two or more purification steps owing to the problems associated with isolating

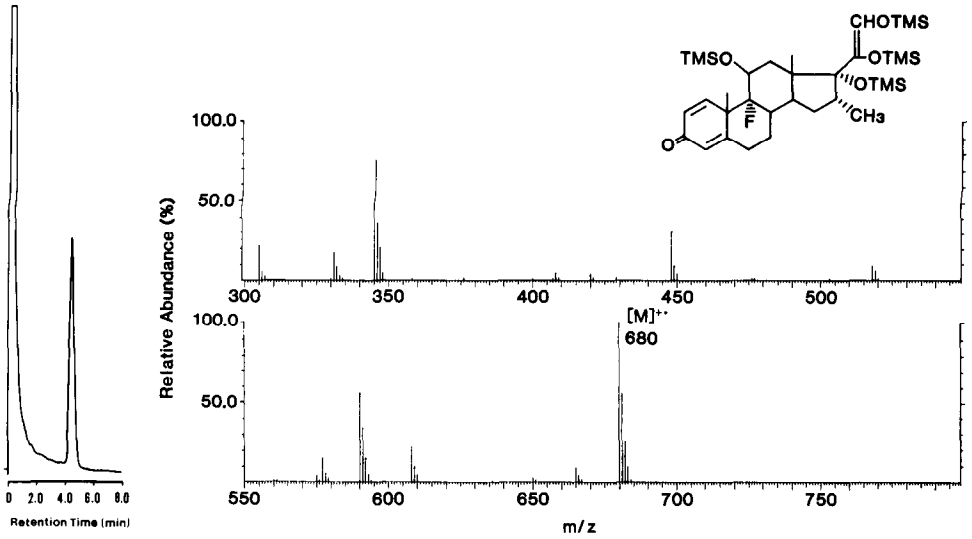

Fig. 1. Gas chromatogram (left) of tetra-TMS-dexamethasone with a 1.5% SP-2100 column and EI mass spectrum (right) of tetra-TMS-dexamethasone.

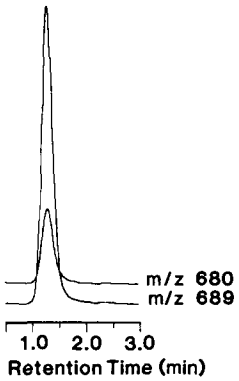

Fig. 2. Mass fragmentogram of tetra-TMS-dexamethasone (*m/z* 680) and tetra-TMS-dexamethasone M+9 (*m/z* 689) after processing from a plasma sample.

sufficiently pure material. Recently, a simple sample purification procedure using a Sep-Pak C_{18} cartridge for the extraction of urinary steroids has been described [24]. The present stable isotope-dilution MS method used a Sep-Pak C_{18} cartridge and normal-phase HPLC in the purification step to enhance the sensitivity and to avoid the possibility of interferences. The absolute recovery of known amounts of dexamethasone (20—100 ng) extracted from human serum with a Sep-Pak C_{18} cartridge was satisfactory (87–95%) and coextracted endogenous material did not interfere with the final GC—MS analysis. The present method provided sensitive quantitation with a detection limit of 500 pg/ml of dexamethasone in serum.

Calibration graphs were prepared by spiking 1.0 ml of blank serum with various amounts (0.5—200 ng) of dexamethasone and a constant amount (20 ng) of dexamethasone M+9. Each sample was then analysed as the TMS deriva-

TABLE I

ACCURACY AND PRECISION OF THE DETERMINATION OF DEXAMETHASONE BY GC—EI-MS

Added (ng/ml)	Found (ng/ml)							C.V. (%)	Relative error (%)
	Individual values*						Mean ± S.D.		
2.00	2.29	2.12	1.97	2.00	1.83	1.88	2.02 ± 0.15	7.43	1.0
5.01	4.73	5.30	5.07	4.98	5.20	5.02	5.05 ± 0.18	3.56	0.8

*Each individual value represents the mean of triplicate measurements.

tive, monitoring the molecular ions at *m/z* 680 for dexamethasone and *m/z* 689 for dexamethasone M+9. There was a good correlation between the mixed amount ratio and the observed peak-height ratio. Least-squares analysis of the observed ratio gave a regression line with a slope coefficient of 0.9999.

The accuracy of measurement was determined for dexamethasone added to 1.0-ml aliquots of pooled serum. The serum samples contained 20 ng of the internal standard and different amounts (2.00 and 5.01 ng) of dexamethasone. The amounts of dexamethasone were measured by the proposed GC—MS method. Table I shows that the amounts of dexamethasone determined were in good agreement with the actual amounts added, the relative error being less than 1%. The intra-assay coefficients of variation were 2.80% for 2.00 ng/ml and 2.40% for 5.01 ng/ml and the inter-assay coefficients of variation were 7.43% and 3.56%, respectively.

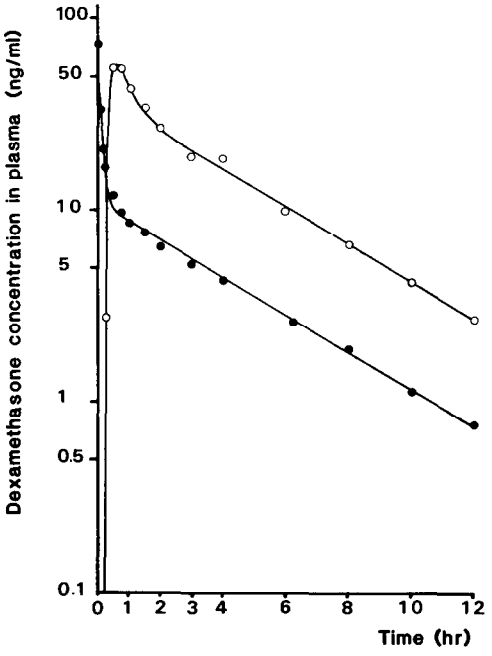

Fig. 3. Semi-logarithmic plot of plasma concentration of dexamethasone versus time after single 1-mg i.v. (•) and 4.5-mg oral (○) doses of dexamethasone.

The proposed method was applied to the determination of the plasma concentration of dexamethasone after administration of 4.5 mg orally and 1 mg intravenously to a healthy subject. Plasma concentrations of dexamethasone could be followed up to 12 h and the plasma concentration versus time curve is shown in Fig. 3.

The proposed method was also applied to the determination of dexamethasone in human urine. The analytical procedures were essentially the same as those described for the plasma samples. Mass fragmentograms of tetra-TMS-dexamethasone after initial purification of the urine sample showed that there was no interference from endogenous compounds with retention times close to those of the molecular ions of tetra-TMS-dexamethasone (*m/z* 680 and 689).

Therefore, the proposed method is also applicable to the determination of dexamethasone in urine.

The proposed method provides a simple, sensitive and reliable technique for determining plasma levels of dexamethasone with good accuracy and precision. The method can be applied to pharmacokinetic and bioavailability studies of dexamethasone after administration of a few milligrams of the drug.

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