Quantitative Determination of Dexamethasone in Human Plasma by Stable Isotope Dilution Mass Spectrometry

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Abstract □ An analytical method for the quantitation of nanogram to subnanogram amounts of dexamethasone is described. Dexamethasone was isolated from human plasma using a C_{18}-bonded reverse-phase cartridge, purified by subsequent normal-phase HPLC, and the corresponding trimethylsilyl derivative analyzed by gas chromatography–mass spectrometry (GC–MS). The quantitation by isotope-dilution MS was carried out by selected-ion monitoring on the $(M + 1)^+$ ion of the trimethylsilyl derivative of dexamethasone and its stable isotopically labeled diluent, [$^{13}C_6$,$^2H_3$]dexamethasone (681 and 690 *m/z*, respectively). Methane was used as the GC carrier gas and as the chemical-ionization reagent gas. The sensitivity of the method, judged from the lower limit of detection of the mass spectrometer, was at ~100 pg. The inter- and intraassay coefficients of variation (CV) determined at two different concentrations were 3.83 and 3.78% for 2 ng/mL and 2.64 and 1.29% for 5 ng/mL, respectively. Plasma concentration profiles for dexamethasone following a single 1-mg iv and a 2-mg oral dose of dexamethasone administered 24 h apart to two healthy volunteers are presented. The mass fragmentographic method described here is useful for bioavailability and pharmacokinetic studies of the synthetic glucocorticoid.

Keyphrases □ Dexamethasone—quantitative determination in human plasma, stable isotope dilution mass spectrometry □ Stable isotope mass spectrometry—quantitative determination of dexamethasone in human plasma

The assay of steroid hormones in biological fluids has been greatly simplified with the development of highly sensitive RIA techniques. RIA methods were developed for the estimation of plasma dexamethasone (1–3) and used to follow plasma levels of dexamethasone to determine the bioavailability of oral dexamethasone (4) and intravenous dexamethasone esters (5). Bioavailability studies of prednisone tablets have also been performed by an RIA method (6). There are often, however, serious restrictions to the use of RIA techniques in that cross-reactivity with other steroids is a serious problem in achieving acceptable specificity.

More specific and precise HPLC methods have been widely used in determining natural and synthetic corticosteroids in biological fluids. The methods have been applied to bioavailability and pharmacokinetic studies of synthetic corticosteroids such as prednisone, prednisolone (7, 8), and budesonide (9). A test method for determining content uniformity by HPLC has also been described for fludrocortisone acetate (10). The HPLC method offers the advantage that the corticosteroids can be analyzed without derivatization, unlike GC. However, the HPLC method lacks sensitivity and cannot be used to make accurate determinations of plasma concentrations in the nanogram-to-subnanogram range. Moreover, analytical methods such as RIA and HPLC do not have the advantage of the isotope dilution technique in that the correction for losses of a particular substance in various biological samples in the extraction and purification procedure cannot be easily made.

The use of gas chromatography–mass spectrometry (GC–MS) and isotopically labeled drugs as diluents has found broad application in pharmacological studies (11, 12). In this technique, stable isotopically labeled carriers serve as the ideal internal standard to correct for losses of a substance under study in the initial isolation procedures. Pharmacokinetic studies represent one field in which the sensitivity and specificity of GC–MS techniques offer an advantage. The present paper describes the development of an analytical method which involves stable isotope dilution mass spectrometry to follow plasma concentrations of dexamethasone after the administration of a therapeutic dose. The internal standard used was dexamethasone M+9 ([$^{13}C_6$,$^2H_3$]dexamethasone). The method has been applied to the bioavailability study of a commonly used tablet formulation of dexamethasone.

EXPERIMENTAL

Chemicals—Methanol[1] (HPLC grade), methylene dichloride[2] (distilled in glass), hexane[2] (UV grade, distilled in glass), and glacial acetic acid[3] (AR grade) were used without further purification. *N,O*-bis(Trimethylsilyl)trifluoroacetamide[4] (BSTFA) was commercially available, and dexamethasone[5] was a gift. Stable isotopically labeled dexamethasone, [1,2,3,4,10,19-$^{13}C_6$,19,19,19-$^2H_3$]dexamethaone, was synthesized under an FDA contract[6].

Mass Fragmentography—Mass fragmentographic determinations were made with a gas chromatograph–mass spectrometer fitted with a chemical-ionization source and a data system[7]. Methane was used as the GC carrier (15 mL/min) and as the chemical-ionization reagent gas. The chemical-ionization source pressure was maintained at ~0.21 torr, and electron energy of 70 eV. The trimethylsilyl derivatives were monitored at *m/z* values of 681 (nonlabeled dexamethasone) and 690 (labeled dexamethasone) by multiple-ion detection with an integration time of 0.839 s each.

GLC was performed on a glass column (60.96 cm × 2 mm i.d.) packed with 3% SP-2100[8] on Supelcoport[8] (80–100 mesh). The column temperature was 270°C and the temperature of the injector was 285°C. The temperature of the ion source was 270°C.

In the electron-impact mode of operation, the electron energy was set to 70 eV and the multiple-ion detection program was set to monitor the ions at *m/z* 680 and 689. The GC column was the same as described above. Helium (20 mL/min) was used as the carrier gas. The column temperature was 240°C, the injector was at 255°C, and the jet separator was at 250°C. The temperature of the ion source was 250°C.

Sample Preparation for Mass Fragmentography—To 1.0 mL of plasma was added 20 ng of dexamethasone M+9 dissolved in 10 μL of methanol, and the plasma was allowed to stand for 30 min. The plasma sample was applied to a C_{18} bonded reverse-phase cartridge[9]. The cartridge was washed with 4 mL of water and then eluted with 5 mL of methanol. The methanol eluate was evaporated to dryness under a stream of nitrogen. To the residue was added 100 μL of the HPLC solvent and the sample was injected with a syringe-loading sample injector[10] into a liquid chromatograph[11] equipped with a

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Burdick & Jackson Laboratories, Inc., Muskegon, Mich.
[3] Mallinckrodt, Inc., St. Louis, Mo.
[4] Pierce Chemical Co., Rockford, Ill.
[5] U.S. Pharmacopeial Convention, Inc., Rockville, Md.
[6] Contract No. 223-79-3011; SRI International, Palo Alto, Calif.
[7] Finnigan Model 4023 with INCOS data system; Finnigan Corp., San Jose, Calif.
[8] Supelco, Inc., Bellefonte, Pa.
[9] C_{18} Sep-Pak Cartridge; Waters Associates, Milford, Mass.
[10] Rheodyne, Inc., Berkeley, Calif.
[11] Waters Model 6000.

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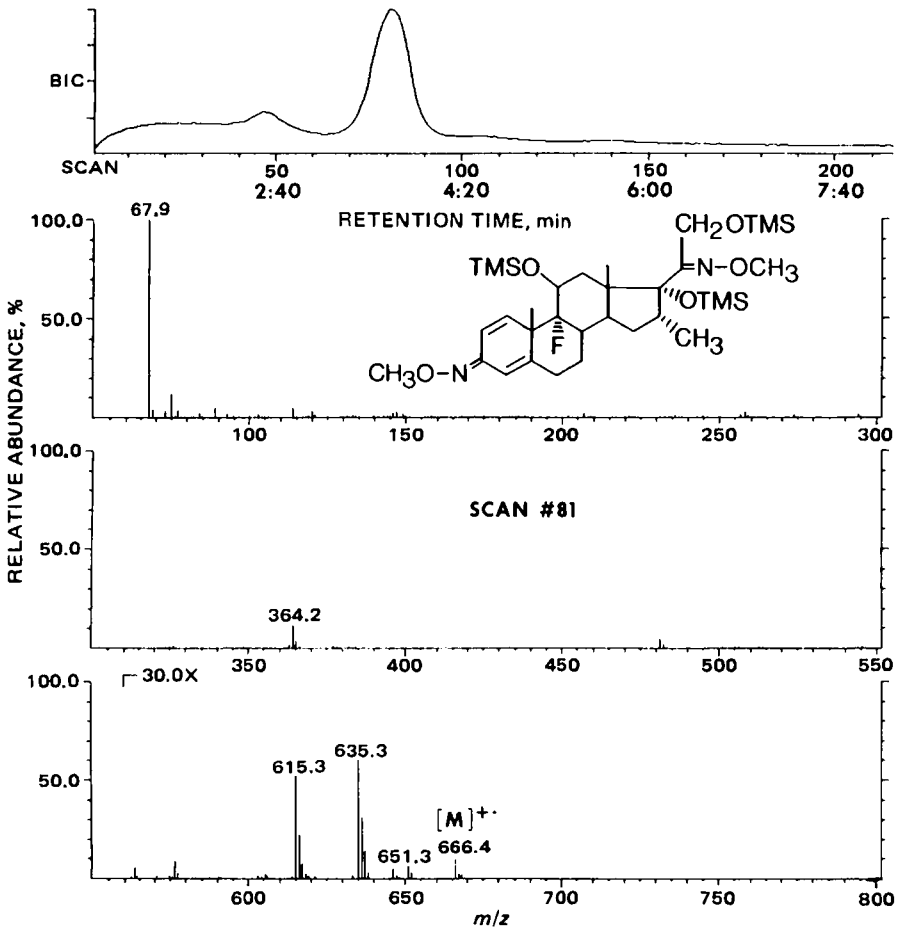

Figure 1—*Mass chromatogram (upper) and mass spectrum (lower) of MO-TMS dexamethasone.*

UV-visible detector[12]. The normal-phase HPLC column[13] (0.25 m × 4.6 mm i.d.) was eluted with 30% methylene dichloride-4% methanol-0.2% acetic acid in *n*-hexane (13) 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 was collected. The solvent was evaporated to dryness under a stream of nitrogen. The trimethylsilyl derivative was formed by reacting the residue with 30 μL of BSTFA in the presence of 10 μg of potassium acetate as a base catalyst (14, 15). The reaction was completed in 0.5–1 h at 80°C. Excess BSTFA was removed under a stream of nitrogen, and the residue was dissolved in 30 μL of *n*-hexane. A 5–10-μL volume of the above *n*-hexane solution was analyzed by GC–MS. In addition, a methoxime-trimethylsilyl (MO-TMS) derivative of dexamethasone was prepared for GC–MS analysis according to a reported method (16).

Preparation of Calibration Curve—To each of 10 standards containing 0.5–200 ng of dexamethasone in 10 μL of methanol was added 20 ng of dexamethasone M+9 in 10 μL of methanol and 10 μg of potassium acetate in 10 mL of methanol. After evaporation of the solvent to dryness under a stream of nitrogen, 30 μL of BSTFA was added to each sample to form the TMS (trimethylsilyl) derivative as described above. A 5–10-μL aliquot of the sample was subjected to GC–MS.

Determination of Accuracy—Dexamethasone in amounts of 2.02, 5.05, and 10.10 ng dissolved in 10 μL of methanol was added to 1.0-mL aliquots of pooled plasma. After preparation of the sample for mass fragmentography as described above, the observed area ratio of *m/z* 681 to *m/z* 690 was determined in triplicate.

Drug Administration—The two healthy adult male volunteers, 39 (subject 1) and 56 (subject 2) years old, weighed 64 and 77 kg, respectively. As a part of the experiment, a complete blood count, urinalysis, and screening blood chemistry battery (SMA) was performed. The laboratory results were found to be within normal limits. After an overnight fast, subjects 1 and 2 received three 0.75-mg dexamethasone tablets[14] orally with 250 mg of water and 1 mg of dexamethasone in its phosphate ester form[15] in 5 mL of saline intravenously over 5 min (1 mL/min), respectively, on the first day. No food was permitted for 4 h after drug administration. On the second day, the drug administration was repeated with each subject receiving the reverse treatment.

On the first day, 20-mL heparinized blood samples were taken from both subjects immediately before drug administration. At the end of injection and 5 min after injection, 10-mL heparinized blood samples were taken from subject 2. At 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 1200, and 1420 min after dosing, 10-mL heparinized blood samples were taken from both subjects. On the second day, 5-mL blood samples were taken rather than 10 mL. The samples were centrifuged at 2000 rpm for 15 min. The plasma samples were placed in culture tubes with plastic caps and stored at −20°C until the time of assay.

RESULTS

Prerequisites for Mass Fragmentographic Analysis—*Derivative Formation*—In the electron-impact or chemical-ionization mass spectrum of dexamethasone, the relative abundance of the molecular or $(M+1)^+$ ion was very small and, unless derivatized, the ionization efficiency of the compound was not sufficient to make possible the analysis of nanogram amounts of dexamethasone. The 3,20-diMO-11β,17α,20-triTMS (MO-TMS) derivative (16) and 11β,17α,20,21-tetraTMS derivative (15) possessed good GC properties and were suitable for use in GC–MS analysis. The electron-impact mass spectra shown in Figs. 1 and 2 indicate that a much higher molecular ion intensity was observed for the tetra TMS derivative at *m/z* 680 than for the MO-TMS derivative at *m/z* 666. When the molecular ion of the tetra TMS derivative was monitored, the sensitivity limit of the assay by GC–MS (electron-impact) was found to be 10 ng of dexamethasone/sample for a standard solution with a signal-to-noise ratio of ~2.5.

Figure 3 shows the chemical-ionization mass spectrum of tetra TMS dexamethasone. An intense $(M+1)^+$ ion was observed at *m/z* 681, with three other predominant peaks in the high mass region >*m/z* 450. This was accomplished by using the chemical-ionization mode for MS analysis and methane as the GC carrier and chemical-ionization reagent gas. The GC effluent was introduced *via* the direct inlet of an MS interface. When the $(M+1)^+$ ion at *m/z* 681 was monitored, the sensitivity limit by GC–MS (chemical-ionization) was found to be 100 pg of dexamethasone for a standard

[12] Tracor 970A.
[13] Spherisorb 5-μm Silica; Phase Sep, Hauppauge, N.Y.
[14] Decadron, 0.75 mg, Lot D0257; Merck Sharp & Dohme.
[15] Decadron phosphate injection, 4 mg/mL, Lot 2371F; Merck Sharp & Dohme.

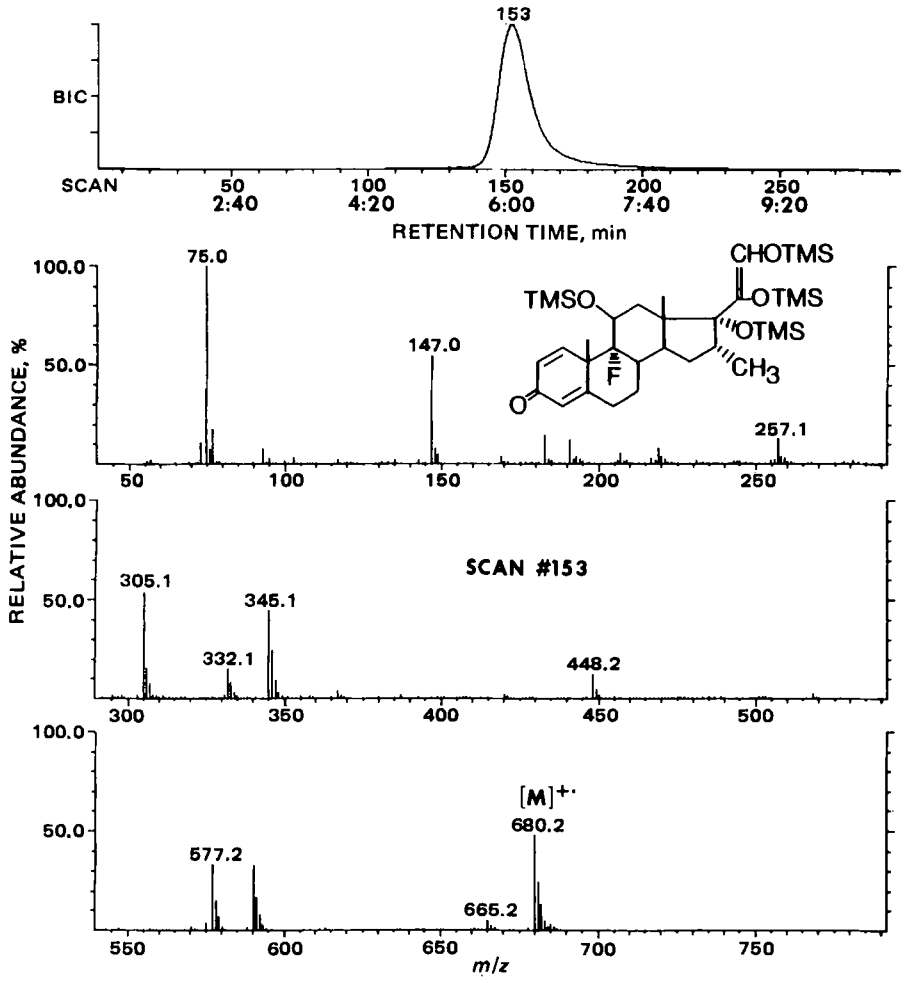

Figure 2—*Mass chromatogram (upper) and mass spectrum (lower) of tetra TMS dexamethasone.*

solution (equivalent to 300 pg/mL of plasma) when a signal-to-noise ratio of ≥2.5:1 was used as a criterion for a significant response.

Selective-ion recordings of tetra TMS dexamethasone showed that no contaminating byproducts with retention times close to that of the TMS derivative were present. After the initial purification procedures, there was no interference with the dexamethasone $(M+1)^+$ ion of the TMS derivative from endogenous compounds in the plasma extract.

Calibration Curve—Dexamethasone M+9 was used as the internal standard in the MS analysis of dexamethasone. The tetra TMS ether of dexamethasone M+9 gave the same fragmentation pattern as the TMS derivative of nonlabeled dexamethasone with the expected mass shifts of 9 amu (Fig. 4). Known mixtures of dexamethasone and dexamethasone M+9 were prepared so that the sample size (5–10 μL) injected into the GC–MS covered the dexamethasone calibrations range of 0.5–200 ng with a fixed amount (20 ng) of dexamethasone M+9. Each mixture was then anlayzed as the TMS derivative, monitoring the $(M+1)^+$ ions at *m/z* 681 for dexamethasone and *m/z* 690 for dexamethasone M+9. There was a good correlation between the mixed molar ratio and the observed peak area ratio. Least-squares analysis of the observed ratio gave a regression line with a slope coefficient of 0.9997.

Accuracy—The accuracy of measurements was determined for dexamethasone added to 1.0-mL aliquots of pooled plasma. The plasma samples contained 20 ng of the internal standard and different amounts (2.02, 5.05, and 10.10 ng) of dexamethasone. The amounts of dexamethasone were measured by the described GC–MS (chemical-ionization) method. The amounts of dexamethasone added were determined and were in good agreement with the amounts of the dexamethasone spiked, the relative error being <3% (Table I).

Precision—As determined by assaying in triplicate six 1.0-mL plasma samples with a dexamethasone concentration of either 2 or 5 ng/mL (Table II), the intraassay coefficients of variation were 3.78% for 2 ng/mL and 1.29% for 5 ng/mL. The interassay coefficients of variation at these two concentrations were 3.83% for 2 ng/mL and 2.64% for 5 ng/mL.

Pharmacokinetics and Bioavailability of Dexamethasone—A single oral dose of three 0.75-mg dexamethasone tablets and a single intravenous dose of 1 mg of dexamethasone in its phosphate ester form were administered 24 h apart to two healthy male volunteers (subjects 1 and 2). Time courses of dexamethasone concentrations in plasma were followed for 24 h by the present

Table I—Accuracy of Mass Fragmentographic Analysis of Dexamethasone in Plasma

Amount Added, ng	Amount Measured, ng				Relative Error, %
	Individual Values[a]			Mean ± *SD*	
2.02	1.96	2.01	1.94	1.97 ± 0.03	−2.47
5.05	5.11	4.97	4.86	4.98 ± 0.10	−1.39
10.10	10.41	9.73	10.08	10.07 ± 0.28	−0.30

[a] Each value represents the mean of triplicate measurements.

Table II—Precision of Mass Fragmentographic Analysis of Dexamethasone in Plasma

Run No.	Amount Determined, ng				*CV*, %
	Individual Values			Mean ± *SD*	
	Amount Added, 2 ng				
1	2.30	2.05	2.12	2.16 ± 0.11	5.09
2	1.96	2.08	2.03	2.02 ± 0.05	2.48
3	1.90	1.96	2.14	2.00 ± 0.10	5.10
4	2.22	2.16	2.11	2.16 ± 0.05	2.32
5	1.88	2.00	2.04	1.97 ± 0.07	3.55
6	1.92	1.86	2.05	1.94 ± 0.08	4.12
				Mean 2.04	3.78
				CV (%) 3.83	
	Amount Added, 5 ng				
1	5.40	5.53	5.34	5.42 ± 0.08	1.48
2	5.08	5.02	5.16	5.08 ± 0.05	0.98
3	5.14	5.26	5.12	5.17 ± 0.07	1.35
4	5.02	5.12	4.95	5.03 ± 0.07	1.39
5	4.94	4.87	4.99	4.93 ± 0.05	1.01
6	5.32	5.10	5.18	5.20 ± 0.08	1.54
				Mean 5.14	1.29
				CV (%) 2.64	

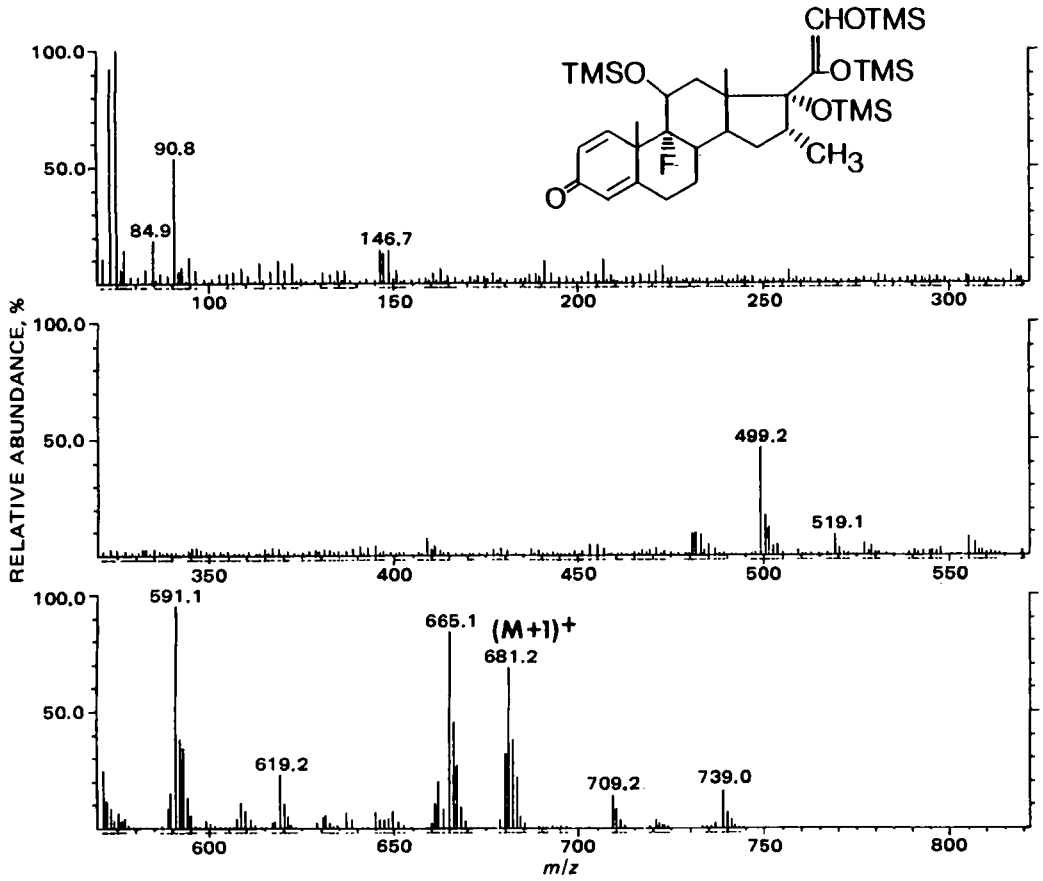

Figure 3—*Chemical-ionization mass spectrum of tetra TMS dexamethasone.*

method. Figure 5 shows a typical example of the selected-ion chromatogram for dexamethasone from the plasma sample.

Plasma concentrations of dexamethasone after intravenous administration appeared to decrease in a biexponential fashion (Fig. 6). The terminal phase of plasma concentrations of dexamethasone after oral administration paralleled that after intravenous administration in each subject. After oral administration the appearance of dexamethasone in the plasma was very rapid. The peak dexamethasone concentrations (15.4 ng/mL for subject 1 and 14.1 ng/mL for subject 2) were observed at 45 and 60 min, respectively, after a single oral dose of 2.25 mg. Semilogarithmic plots of the plasma concentration *versus* time (Fig. 6) indicate that after absorption, a distribution phase was observed in both subjects. The terminal decline phase was monoexponential at time points later than 2 h for subjects 1 and 2.

The observed set of plasma data was fitted to the polyexponential equation ($C_p = \Sigma C_i e^{-\lambda_i t}$) describing the plasma concentration–time curves and parameters characterizing the absorption and disposition of oral and intravenous dexamethasone were obtained *via* a computer[16] by a least-squares estimation using the steepest descent method. The estimate of the elimination half-life ($t_{1/2}$) was obtained from the terminal slope (λ_i): $t_{1/2} = 0.693/\lambda_i$. The total plasma clearance (CL_{pl}) was expressed as the intravenous dose divided by the area under the plasma concentration–time curve (AUC). The AUC from t = 0–10 h ($AUC_{10\ h}$) or t = 0 to infinity (AUC_∞) was measured by the trapezoidal rule. In the case of intravenous administration, the time point of 15 min was taken to be the first data point to measure the AUC. The AUC beyond the last detectable plasma dexamethasone concentration was calculated by dividing the last detectable plasma concentration (10-h point) by the terminal elimination rate constant, λ_i. The volume of distribution (Vd, area) was calculated by dividing the clearance by the terminal rate constant: Vd, area = CL_{pl}/λ_i. These pharmacokinetic parameters are summarized in Table III.

Bioavailability—The absolute bioavailability of oral dexamethasone was calculated from a comparison of dexamethasone concentrations after oral dexamethasone and intravenous dexamethasone-21-phosphate administration based on the AUC. It was assumed that after intravenous injection dexamethasone-21-phosphate rapidly and quantitatively hydrolyzed to its free alcohol (5). The calculated values for bioavailability were 54.7% ($AUC_{10\ h}$) and 54.9% (AUC_∞) for subject 1 and 71.7% ($AUC_{10\ h}$) and 71.2% (AUC_∞) for subject 2.

[16] CBM Model 2001-32.

DISCUSSION

The use of isotope dilution techniques coupled with isotope ratio determination by MS is rapidly becoming the method of choice in the analysis of steroids in biological samples (17–19). Since bioavailability and pharmacokinetic studies of synthetic corticosteroids require sensitive, specific, and reproducible analytical techniques, attempts were made in the present study to devise procedures that would allow analysis of nanogram-to-subnanogram amounts of dexamethasone in human blood by isotope dilution MS.

Generally, the MS analysis of corticosteroids in biological extracts is difficult due to their thermal instability and the problems associated with isolating sufficiently pure material for analysis. Recently, sample purification procedures using a C_{18} bonded reverse-phase cartridge for the quantitative analysis of corticosteroids in adrenal cell cultures by HPLC or GC–MS have been described (20). The present isotope-dilution MS method involves spiking the plasma sample with stable isotopically labeled dexamethasone M+9 and then purifying it by the use of a C_{18} bonded reverse-phase cartridge and normal-

Table III—Parameters Characterizing the Disposition of Dexamethasone

Parameter	Subject 1	Subject 2
	Intravenous Administration	
C_1, mg/mL	481.6	155.8
λ_1, h^{-1}	15.17	10.37
C_2, ng/mL	12.2	8.2
λ_2, h^{-1}	0.38	0.18
$t_{1/2}$, h	1.82	3.85
$AUC_{10\ h}$, h·ng/mL	29.85	34.26
AUC h·ng/mL	30.68	39.21
CL_{pl}, L/h	33.9 (0.53 L/h/kg)	21.82 (0.28 L/h/kg)
Vd, L	89.2 (1.4 L/kg)	121.2 (1.5 L/kg)
	Oral Administration	
C_1, ng/mL	−292.5	−76.4
λ_1, h^{-1}	1.60	2.05
C_2, ng/mL	278.4	52.6
λ_2, h^{-1}	1.54	1.38
C_3, ng/mL	14.1	11.7
λ_3, ng/mL	0.35	0.18
$t_{1/2}$, h	1.98	3.85
$AUC_{10\ h}$, h·ng/mL	35.24	55.95
AUC, h·ng/mL	36.45	63.10

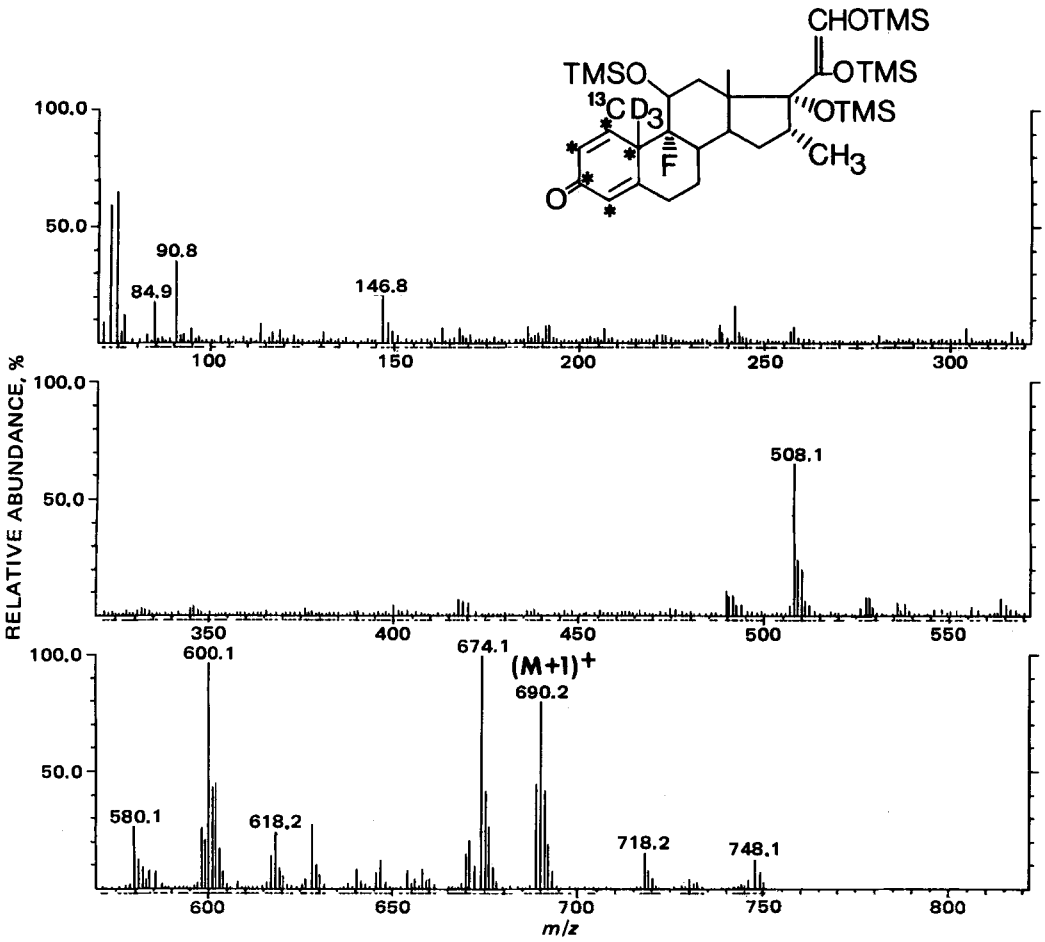

Figure 4—*Chemical-ionization mass spectrum of tetra TMS dexamethasone M+9.*

phase HPLC. The steroid sample was then derivatized and analyzed by GC–MS. The absolute recovery of the purification procedures varied from 80 to 90%, and coextracted endogenous material did not present any problems in the final GC–MS analysis.

For each MS method developed, the choice of derivative and mode of MS analyses must be made to reduce the possibility of interferences and enhance the sensitivity. Several derivatives of corticosteroids for GC analysis have been introduced in the past (14, 21–24), and from a quantitative point of view the double MO-TMS derivative has been shown to be the most satisfactory (23). MO-TMS derivatives have been used for the quantitative determination of prednisolone and prednisone in human plasma using GC–MS (chemical-ionization) with methane as the chemical-ionization reagent gas (25).

It has also 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 (26–28). This step has now, however, been shown not to be necessary in the case of dexamethasone when the silylation reaction was carried out with BSTFA in the presence of a base catalyst such as anhydrous potassium or sodium acetate (15). In the present study potassium acetate was used as a base catalyst, the C-20 ketone group of dexamethasone was converted to an enol ether, and all hydroxyl groups were converted to TMS ether groups under reaction with BSTFA at 80°C for 0.5–1 h. A single product was obtained, and the tetra TMS derivative showed excellent thermal stability and good GC behavior. The MO-TMS derivative of dexamethasone was also thermally stable and suitable for GC analysis. This derivative was recently used for the qualitative determination of dexamethasone along with other synthetic corticosteroids in horse urine samples by combined HPLC-negative-ion chemical-ionization MS using ammonia as the reagent gas (29).

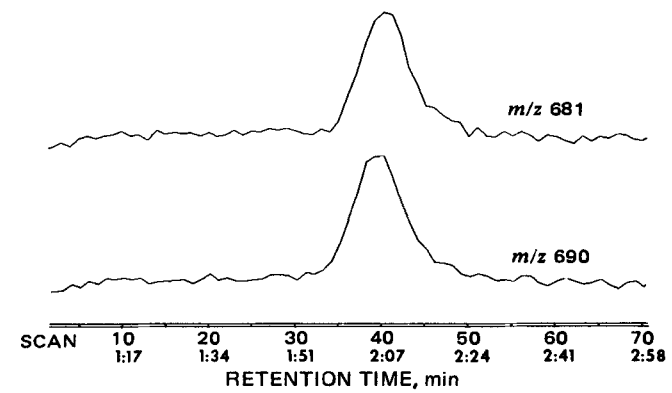

Figure 5—*Mass fragmentograms of tetra TMS dexamethasone and tetra TMS dexamethasone M+9 after processing from a plasma sample.*

Quantitation by MS requires the availability of isotopically labeled compounds to function as internal standards. It was expected that the molecular ion for tetra TMS dexamethasone would have a significant contribution to M+1, M+2, and M+3 because of the presence of four TMS groups. This multiplicity of the molecular ion imposes certain restrictions on the choice of a GC–MS internal standard. For a stable isotopically labeled dexamethasone to serve as an internal standard in the present study, it must be substituted with a large number of stable isotopes so that its molecular ion cluster will not overlap with the molecular ion cluster of nonlabeled dexamethasone.

Dexamethasone M+9, used as the internal standard, was suitable for the present purpose. The standard curve prepared by plotting the peak area ratio of the $(M+1)^+$ ions of the tetra TMS derivatives *versus* the amount of nonlabeled dexamethasone added to 20 ng of dexamethasone M+9 was linear at a concentration range of 0–200 ng, a concentration range commensurate with the clinical levels in the bioavailability study of dexamethasone. Furthermore, accuracy and precision provided by the present method were satisfactory.

The applicability of the method was shown by the determination of plasma dexamethasone levels in two healthy human volunteers after administration of a 2-mg oral or 1-mg iv dose of dexamethasone. This study indicated that most of orally administered dexamethasone was absorbed within 60 min. The terminal half-lives ($t_{1/2}$) calculated from plasma concentration data after oral and intravenous administration were in close agreement in both subjects. The half-life was somewhat short in subject 1 (~2 h) and was ~3.5 h in subject 2. These values were similar to results reported by other workers, who measured total plasma radioactivity after administering radiolabeled dexamethasone intravenously (30) or plasma levels by RIA (4).

An assumption was made that the total plasma clearance (CL_{pl}) was constant for each subject from oral to intravenous dose or *vice versa*, and the values for bioavailability of the oral preparation were estimated by a model-

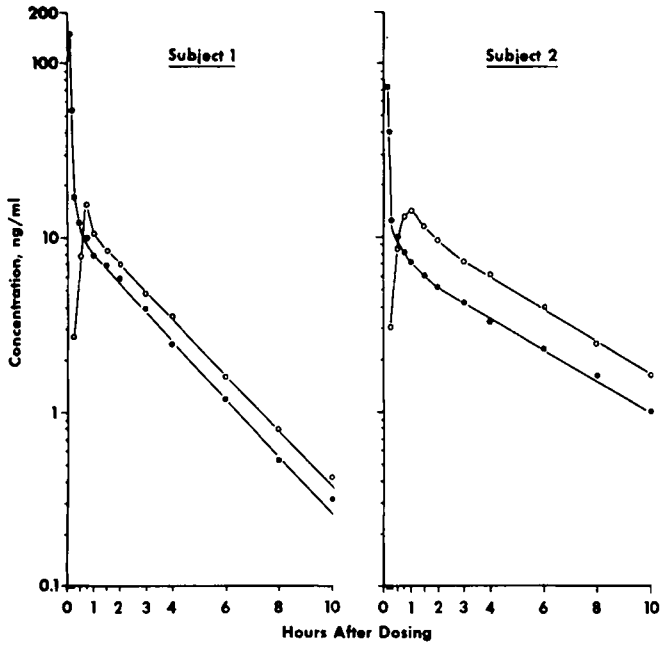

Figure 6—*Semilogarithmic plot of plasma concentrations of dexamethasone* versus *time after single 1-mg iv (●) and 2-mg oral (○) doses of dexamethasone.*

independent comparison of dexamethasone levels after oral and intravenous administration based on the $AUC_{10\ h}$ values of ~55 and 71% for subjects 1 and 2, respectively. A comparison of AUC_{∞} resulted in only a small difference in the bioavailability values from those obtained above.

CONCLUSION

The isotope dilution MS method described here afforded a sensitive and reliable technique to measure nanogram-to-subnanogram amounts of dexamethasone in plasma. Specificity was high because the GC–MS system was used to separate and to detect the specific compound in question after initial purification by a C_{18} bonded reverse-phase cartridge and normal-phase HPLC. It was possible to apply the present method for the accurate determination of low concentrations of dexamethasone in human plasma after administering only a few milligrams of the drug.

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