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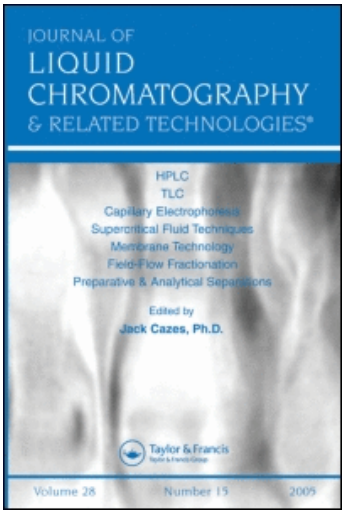

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HPLC Determination of Dexamethasone in Human Plasma

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ABSTRACT

A sensitive high performance liquid chromatography (HPLC) method has been developed for the determination of dexamethasone in human plasma. After 1.5 mL of plasma was extracted with 4 mL of ethyl acetate containing 80 ng/mL of desoxymethasone, analysis of dexamethasone in plasma samples was carried out using a Sphereclone ODS2 column with UV detection for separation and quantification. A mixture of acetonitrile–10 mM phosphate buffer (pH 7.0) (32 : 68, v/v) was used as a mobile phase. The limit of quantitative (LOQ) analysis was 10 ng/mL. The accuracy of the assay was from 96.96% to 106.07%, while the intra- and inter-day coefficient of variation of the same concentration range was less than 15%, except LOQ (<20%). The signals were

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monitored by a UV detector at 240 nm with flow-rate of 1.0 mL/min. The method could be applied, with great success, to evaluate the bioavailability of dexamethasone in human subjects, with excellent selectivity and reproducibility and clinical study.

Key Words: Dexamethasone; HPLC; Human plasma; Bioavailability.

INTRODUCTION

Dexamethasone is a synthetic glucocorticoid with strong anti-inflammatory and anti-allergic activity (Fig. 1a). The drug is used in the diagnosis of adrenal disease in addition to many inflammatory and immunological processes,[1] and sometimes given to premature infants because it could prevent chronic lung disease. However, dexamethasone has a number of potential serious

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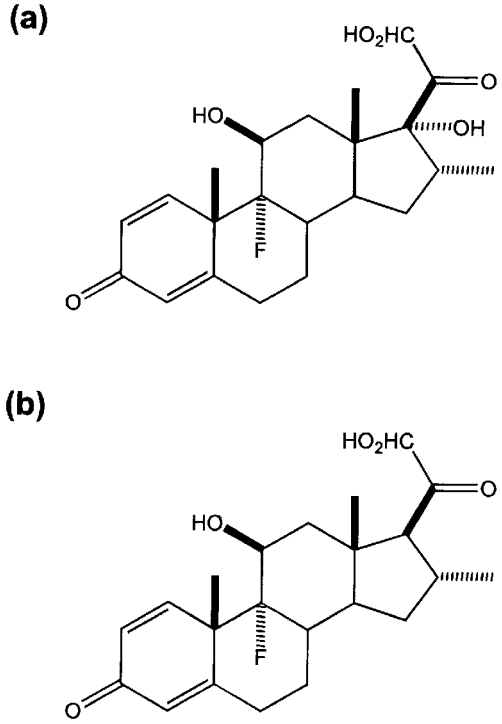

Figure 1. Chemical structures of (a) dexamethasone and (b) desoxymethasone.

adverse effects such as hypertension, adrenal suppression, pneumothorax, hyperglycemia, and increased risk of sepsis. Also, dexamethasone suppresses the body's inflammatory response and often allows infection to occur as a result.[2]

Several methods have been reported in the literatures, including immunological[3,4] and high performance liquid chromatography (HPLC) with ultraviolet[2,3,5–7] and fluorescence,[3,8] mass spectrometry,[3,5,9,10] or gas chromatography with MS detection,[11,12] to detect dexamethasone in biological fluid. Especially, HPLC methods are routinely used for analysis of dexamethasone in biological fluid. A common problem with immunological methods is specific and non-specific interference with antibody-analyte binding.[13–16] In previously published HPLC-MS and GC-MS methods for determination of dexamethasone, processing of samples and using the apparatuses are complex and apparatuses are too expensive. There are also some limitations in previously reported HPLC methods, including pre-column derivatization,[8] no validation in human serum,[6] and no pharmacokinetic data.[2]

The purpose of this study was to develop a sensitive, convenient, and accurate semi-microbore HPLC method using UV detection for the determination of dexamethasone in human plasma, and evaluate the absolute bioavailability of dexamethasone in healthy volunteers after oral administration.

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EXPERIMENTAL

Materials

Dexamethasone and desoxymethasone (internal standard, IS) were obtained from Sigma Chemical Co. (St. Louis, MO). Methanol and acetonitrile were HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ). All other chemicals were analytical grade and used without further purification.

Preparation of Standards

Stock solutions of dexamethasone (1 mg/mL) were made by dissolving in methanol and diluted to a concentration of 100 μg/mL. Standard solutions of dexamethasone in human plasma were prepared by spiking the appropriate volume (<10 μL per mL) of various diluted stock solutions, giving trial concentrations of 10, 20, 50, 100, and 200 ng/mL. Internal standard, desoxymethasone, structurally similar to dexamethasone, as shown in Fig. 1, was

dissolved in ethyl acetate to make a stock solution at a final concentration of 80 ng/mL.

Preparation of Samples

Each 1.5 mL of plasma was pipetted into a glass tube and extracted by vortex-mixing for 3 min, with 4.0 mL of ethyl acetate containing 80 ng/mL of desoxymethasone. After centrifugation at 1000 g for 15 min, clear supernatants were transferred into other glass tubes and evaporated under nitrogen at 50–60°C. The residue was reconstituted in 150 μL of mobile phase. Then, the reconstituted samples were filtered through a 0.2-μm reg cellulose syringe filters (National Science, Seoul, Korea). 90 μL of filtered aliquot was injected, with an autosampler into the HPLC system for analysis.

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Apparatus

The determination of dexamethasone was carried out using a semi-microbore HPLC system, which consisted of a Nanospace SI-1 (Shiseido, Tokyo, Japan), equipped with two 2001 pumps, a 2002 UV-VIS detector, a 2003 autosampler, a 2004 column oven, and a 2009 degassing unit. The separation was performed on a Sphereclone ODS2 column (250 mm × 4.6 mm I.D., 5 μm, Phenomenex Inc., Torrance, CA). A guard column (4 mm × 3 mm I.D.) packed with guard cartridge C18 (Phenomenex Inc.) was used.

Chromatographic Conditions

A mixture of acetonitrile and 10 mM sodium phosphate dibasic buffer (32 : 72, v/v, pH 7.0), was used as a mobile phase. The flow-rate was 1.0 mL/min and the injection volume 90 μL. The signals were monitored using a UV detector at 240 nm, and recorded by ds-Chrom (Donam Instruments Inc., Korea). All the analyses were performed at room temperature.

Validation of the Method

Evaluation of the reversed-phase HPLC method was based on proportionality (linearity assay), precision, and accuracy.

Specificity

Drug-free human plasma was tested for interference using the proposed HPLC method, and the result was compared with those obtained from dexamethasone and the internal standard.

Linearity

The calibration curve consisted of the five concentrations: 10, 20, 50, 100, and 200 ng/mL for dexamethasone. The calibration curves were obtained by linear regression; the ratio of dexamethasone peak area to internal standard peak area was plotted vs. dexamethasone concentration in ng/mL.

Precision and Accuracy

The intra- and inter-day precision (coefficients of variation, CV%) and inter-day accuracy (bias%) of the assay procedure, were determined by the analysis of five samples at each concentrations in the same day, and one sample at each concentrations in five different days, respectively.

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Sensitivity

The limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by CV% was lower than 20%, the accuracy expressed by bias% was within 80–120%, and ratio of signal to noise was better than 10.

Recovery

The absolute recovery of dexamethasone from human plasma was performed in the three concentration ranges 10, 50, and 200 ng/mL. This was established by comparing the peak area ratio to internal standard obtained from the standard solutions of human plasma containing dexamethasone with those of non-extracted standards, which represent 100% recovery.

Stability

Freeze and Thaw Stability

Human plasma samples containing 10, 50, and 200 ng/mL of dexamethasone were prepared. The samples were stored at -70°C for 24 hr, subjected to three thaw and freeze cycles and analysed by HPLC.

Short Term Stability

Human plasma samples containing 10, 50, and 200 ng/mL of dexamethasone were exposed to room temperature for 4 hr and analysed by HPLC.

Long Term Stability

Human plasma samples containing 10, 50, and 200 ng/mL of dexamethasone were stored in the deep freezer at −70°C for 10 days and analysed by HPLC.

Standard Solution Stability

Stock solution of dexamethasone was left at room temperature for 6 hr and human plasma samples containing 10, 50, and 200 ng/mL of dexamethasone were stored in the deep freezer at −70°C for 10 days and analysed by HPLC.

Processed Sample Stability

Human plasma samples containing 10, 50, and 200 ng/mL of dexamethasone were left in the autosampler at ambient temperature (ca. 20°C) for 9 hr and analysed by HPLC.

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Preparation of Biological Samples

The validated method was applied to evaluate the bioavailability of dexamethasone. Eight (8 male) healthy volunteers were selected for this study according to medical history, physical examination, and standard laboratory test results (blood cell count, biochemical profile, and urinalysis). The demographic data of these volunteers were: mean age 25.1 years, mean height 176.5 cm, and mean weight 69.5 kg.

After an overnight fast, a catheter was introduced in a forearm superficial vein and a pre-dosing blood sample was collected. Each volunteer was then orally administered 16 tablets (8 mg) of dexamethasone, namely Dexamethasone tablet 0.5 mg® (YuhanMedica, Seoul, Korea) with 240 mL of water. The volunteers continued to fast for 4 hr, after which a standard lunch was served. Venous blood samples (8 mL) were collected before administration and at designated time intervals, i.e., 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, and 24 hr post-dosing. The collected blood was separated by centrifugation at 2000 × g for 20 min, and then, plasma was stored at −70°C until assayed for the dexamethasone content. The pharmacokinetic parameters

were calculated by a bioavailability analytical program, BA Calc 2002 provided by College of Pharmacy in Seoul National University.[17]

RESULTS AND DISCUSSION

HPLC

Selectivity

Drug-free human plasma was screened, and no endogenous interference was observed at the retention time of dexamethasone and internal standard. The Sphereclone ODS2 column has been used as the analytical column since it has 80 Å pore size, which limits the access of large molecules such as proteins, retains drug molecules longer, and improves the sensitivity.[18] A chromatogram of extracted blank human plasma sample, a representative chromatogram of extracted plasma sample containing 100 ng/mL dexamethasone, a representative chromatogram of extracted plasma sample containing 100 ng/mL dexamethasone and 80 ng/mL internal standard, as well as dexamethasone in plasma collected at 1.5 hr after oral administration of 8 mg to human subjects, are shown in Fig. 2.

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Linearity

The calibration curves were linear in the studied range. The mean equation of the calibration curve consisting of five points was $y = 0.0036 (\pm 0.0003)x + 0.0077(\pm 0.0087)$ with correlation coefficient $R^2 = 0.9996 (\pm 0.0010)$, where y represents the ratio of dexamethasone peak area and the internal standard one, and x represents the dexamethasone concentration in ng/mL.

Precision and Accuracy

The intra- and inter-day precision and accuracy results are shown in Table 1. The values obtained were lower than the limits required for biological samples, $\pm 20\%$ for the precision and inaccuracy of the LOQ (10 ng/mL), and $\pm 15\%$ for both of the other concentrations.

Sensitivity

The LOQ of dexamethasone was 10 ng/mL. This method was sufficiently sensitive, with a quantification limit lower than the minimum concentration

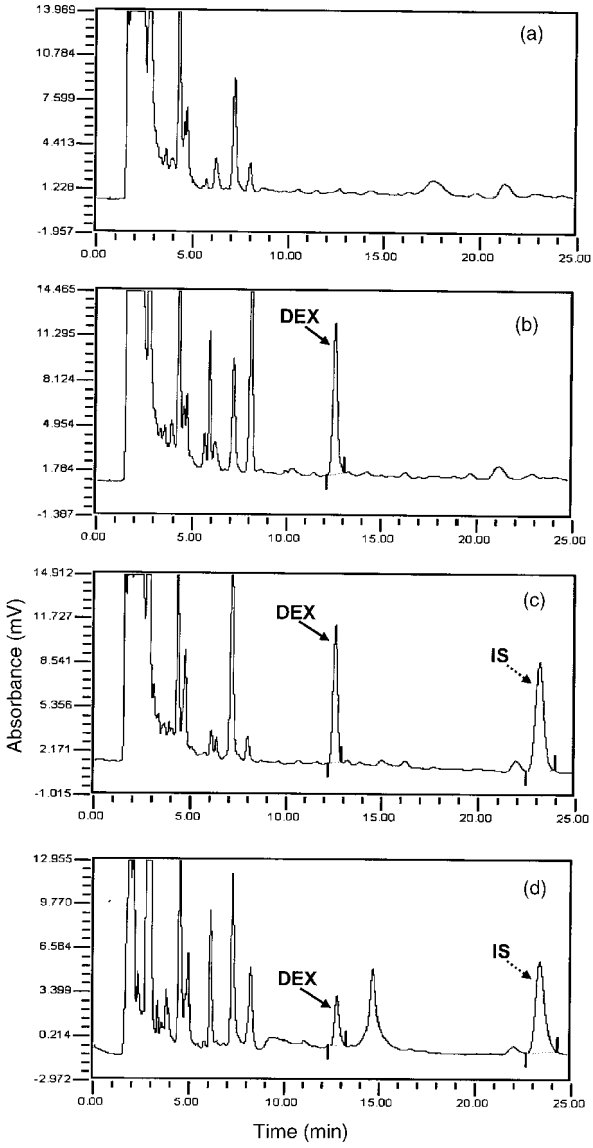

Figure 2. Chromatograms of (a) blank plasma, (b) blank plasma spiked with dexamethasone (100 ng/mL), (c) blank plasma spiked with dexamethasone (100 ng/mL) and desoxymethasone as internal standard (80 ng/mL) and (d) plasma sample from a human subject at 1.5 hr after an oral administration of 8 mg dexamethasone. DEX and IS in chromatograms represent dexamethasone and internal standard, respectively.

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Table 1. Reproducibility of dexamethasone determination in human plasma ($n = 5$).

Dexamethasone concentration (ng/mL)	Precision (CV%)		Accuracy
	Intra-day	Inter-day	
10 (LOQ)	8.82	18.25	106.07
20	10.87	11.93	104.52
50	9.62	4.09	97.95
100	6.94	7.09	96.96
200	6.58	6.92	99.79

recommended for plasma samples obtained after the administration of 8 mg of dexamethasone. The sensitivity of dexamethasone is shown in Fig. 3.

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Recovery and Stability

The results are shown in Table 2. Thus, this assay method for the determination of dexamethasone in human serum has sufficient recovery for extraction, and stability in human serum for the bioavailability assessment of dexamethasone.

Application to the Bioavailability of Dexamethasone

The mean plasma dexamethasone concentration–time profile from 16 tablets of dexamethasone tablet 0.5 mg is shown in Fig. 4. The pharmacokinetic parameters of dexamethasone were $325.31 \pm 109.30\,\text{ng hr/mL}$ of $AUC_{24\,h}$, $111.49 \pm 32.49\,\text{ng/mL}$ of C_{max}, $0.81 \pm 0.46\,\text{hr}$ of T_{max}, $0.20 \pm 0.02\,\text{hr}^{-1}$ of K_e, and $3.47 \pm 0.36\,\text{hr}$ of mean terminal half, which were very similar to the previous reports.[19]

The present method offers practical advantages over the immunological, HPLC-MS and GC-MS in the viewpoint of speed and sample throughput. Moreover, compared with previously reported liquid chromatographic determination of dexamethasone, this method improved sample throughput and presented the pharmacokinetic data of 8 mg of dexamethasone.

CONCLUSION

A determination method of dexamethasone from plasma samples has been developed using semi-microbore HPLC. This analytical method

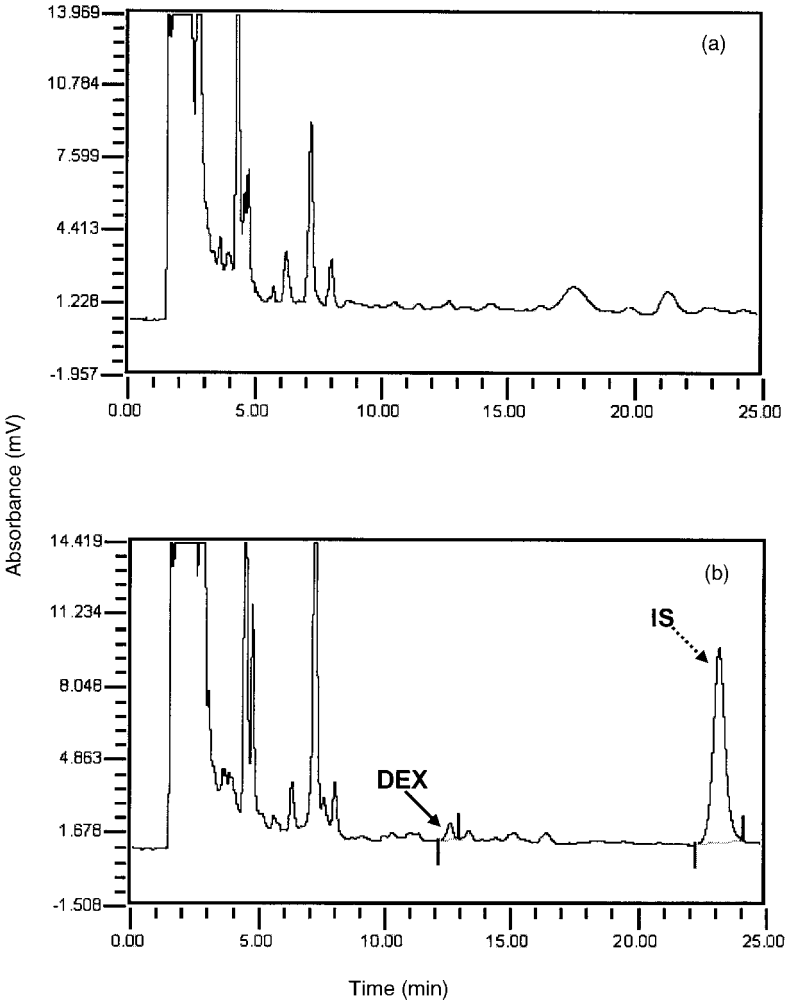

Figure 3. Chromatograms of (a) blank plasma and (b) blank plasma spiked with dexamethasone (10 ng/mL). DEX and IS in chromatograms represent dexamethasone and internal standard, respectively.

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Table 2. Recovery and stability of dexamethasone determination in human plasma ($n = 3$).

Dexamethasone concentration (ng/mL)	Recovery (%)	Freeze–thaw stability (%)	Short term stability (%)	Long term stability (%)	Standard solution stability (%)	Processed sample stability (%)
10	119.8	79.3	120.7	109.6	115.9	85.1
50	79.4	122.4	133.2	116.7	123.1	127.9
200	88.8	77.4	81.1	126.6	105.3	85.5

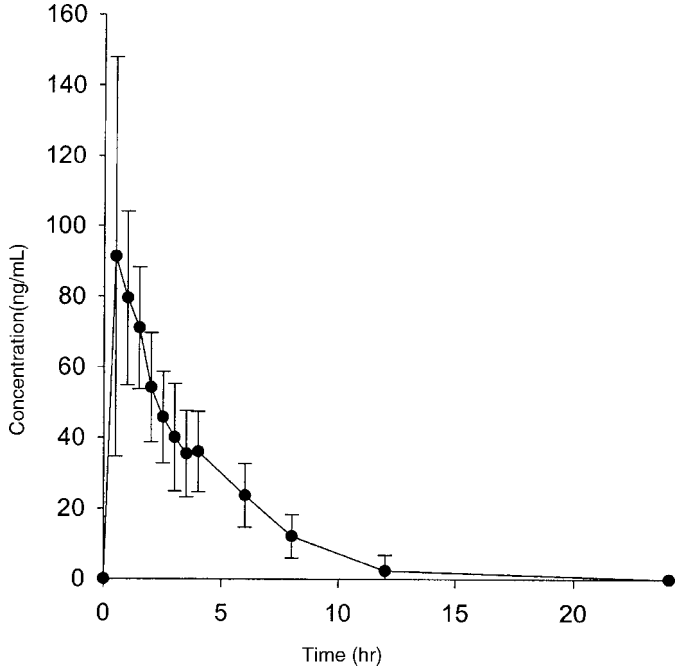

Figure 4. Mean plasma concentration of dexamethasone in human subjects after oral administration of 8 mg dexamethasone. The results represent the mean ± s.d. ($n = 8$).

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showed sensitivity, speed, specificity, and reproducibility using the plasma sample. This method could be successfully applied to evaluate the bioavailability of dexamethasone in healthy volunteers.

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