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Simultaneous quantitation of dexamethasone palmitate and dexamethasone in human plasma by liquid chromatography/tandem mass spectrometry

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Abstract

A rapid and sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for simultaneous quantitation of dexamethasone palmitate and dexamethasone in human plasma was developed. After sample preparation by protein precipitation and liquid–liquid extraction, the analytes and internal standard (IS) were separated on a Venusil XBP-C_8 column using gradient elution. Multiple reaction monitoring of dexamethasone palmitate, dexamethasone and IS used the precursor to product ion transitions at m/z 631.8 → 373.1, m/z 393.2 → 147.1 and m/z 264.2 → 58.1, respectively. The method was linear over the ranges 1.5–1000 ng/mL for dexamethasone palmitate and 2.5–250 ng/mL for dexamethasone with intra- and inter-day precisions of <10% and accuracies of 100 ± 7%. The assay was applied to a clinical pharmacokinetic study involving the injection of dexamethasone palmitate to healthy volunteers.

Keywords: Dexamethasone; Dexamethasone palmitate; LC/MS/MS; Pharmacokinetics; Human

1. Introduction

Dexamethasone is a potent anti-inflammatory steroid widely used for treating inflammatory conditions, allergic reactions, shock and lymphomas. It is also used to induce precocious fetal lung maturation in threatened preterm labor [1–3]. Dexamethasone is available as the free alcohol and in the form of a variety of C-21 ester prodrugs which are either water-soluble for intravenous (I.V.) administration, e.g. dexamethasone phosphate, or more lipophilic for administration as injectable emulsions, e.g. dexamethasone palmitate [3,4]. Dexamethasone palmitate is two to five times more potent than dexamethasone phosphate as an anti-inflammatory agent in some chronic inflammatory diseases due to its high distribution to inflammatory lesions, high uptake by macrophages and strong suppressive effect on macrophage function [5,6]. Although the delivery and efficacy of lipid formulations of dexamethasone palmitate have been extensively studied, little is known about the clinical pharmacokinetics of dexamethasone palmitate and its rate of conversion to dexamethasone after I.V. administration.

Numerous chromatographic methods have been reported for the determination of dexamethasone in biological matrices based on high-performance liquid chromatography (HPLC) with detection by ultraviolet spectroscopy [7], mass spectrometry (MS) [2,8,9] and tandem mass spectrometry (MS/MS) [3,10–12]. Gas chromatography/mass spectrometry (GC/MS) has also been used but mainly for the analysis of residues in tissues of meat-producing animals [7,13]. For dexamethasone palmitate, however, only a radioimmunoassay method has been reported for its determination in plasma [5,6,14] and the details of the methodology and its validation were lacking. In this paper we describe a simple and sensitive method for the simultaneous determination of dexamethasone palmitate and dexamethasone in human plasma and its application to a pharmacokinetic study

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of dexamethasone palmitate (4 mg) formulated in a lipid emulsion. The main aims of the study were to develop and validate a LC/MS/MS method for simultaneous quantitation of dexamethasone palmitate and dexamethasone in human plasma and investigate the conversion of dexamethasone palmitate to dexamethasone following an I.V. dose of dexamethasone palmitate to Chinese healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Dexamethasone palmitate (purity > 99.0%), dexamethasone (purity > 99.0%) and tramadol hydrochloride (purity > 99.0%) for use as internal standard (IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Dexamethasone palmitate for clinical use was an injectable lipid emulsion (Mitsubishi Pharma Corp.). HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and other chemicals (25% ammonia solution, ammonium acetate, formic acid, isopropanol, potassium chloride, diethyl ether, dichloromethane, hexane and ethyl acetate) were of analytical grade and used as received. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. LC/MS/MS conditions

The LC/MS system incorporated an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) interfaced with an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI). Separation was performed on a Venusil XBP-C_8 column (50 mm × 4.6 mm, 5 μm, Agela Technologies Inc., Delaware, USA) maintained at 30 °C protected by a SecurityGuard™ cartridge system (C_8 4.0 mm × 3.0 mm, Phenomenex Inc., USA). The mobile phase which is consisted of mixtures of solvent A (0.5% ammonia solution, pH 8.5, prepared from 25% ammonia solution) and solvent B (acetonitrile) delivered at 1.5 mL/min according to the following linear gradient: 0–0.8 min, 50–40% A; 0.8–1.5 min, 40–10% A; 1.5–4.5 min, 10–5% A; 4.5–5 min, 5–50% A; 5.0–6.0 min, 50% A. Full-scan mass spectra of analytes were obtained by direct infusion of standard solutions (dexamethasone palmitate: 300 ng/mL; dexamethasone: 500 ng/mL) into the ESI source via a syringe pump. The instrument was operated in the positive ion mode with nebulizer gas, curtain gas and collision gas flow rates of 45, 10 and 55 psi, respectively. The ion spray voltage was 5000 V and the source temperature 550 °C. The mass spectrometer was operated at unit resolution for both Q1 and Q3 with a dwell time of 200 ms per channel. Analytes were quantitated by multiple reaction monitoring (MRM) employing the following precursor to product ion transitions and parameters: dexamethasone palmitate, m/z 631.8 → 373.1 with declustering potential (DP) 60 V and collision energy (CE) 23 eV; dexamethasone, m/z 393.2 → 147.1 with DP 53 V and CE 37 eV; IS, m/z 264.2 → 58.1 with DP 29 V and CE 40 eV. Data were collected and processed using the Applied Biosystems Analyst Software.

2.3. Standard and quality control solutions

Stock solutions (1 mg/mL) of dexamethasone palmitate, dexamethasone and IS were prepared in methanol. Standard solutions were prepared by diluting stock solutions with methanol to give concentration of dexamethasone palmitate of 3, 10, 30, 100, 300, 1000 and 2000 ng/mL and of dexamethasone of 5, 10, 20, 50, 100, 200 and 500 ng/mL. Quality control (QC) solutions in methanol were prepared independently with concentrations of 10, 100 and 1000 ng/mL dexamethasone palmitate and of 10, 50 and 200 ng/mL dexamethasone. A 10 ng/mL working solution of the IS in methanol was also prepared. All solutions were stored at 4 °C until required.

2.4. Sample preparation

Frozen human plasma samples were thawed at room temperature and subjected to protein precipitation and liquid–liquid extraction (LLE) as follows. To a tube containing 200 μL plasma were added 100 μL methanol or standard solution or QC solution of dexamethasone palmitate, 100 μL methanol or standard solution or QC solution of dexamethasone and 50 μL IS solution. After mixing, 1 mL isopropanol was added to precipitate proteins. The mixture was then vortex-mixed for 1 min and centrifuged at 10,000 × g for 10 min. The upper clear solution layer was collected and 100 μL water and 100 μL saturated solution of potassium chloride were added. After mixing for 15 s, 3 mL diethyl ether:dichloromethane (60:40, v/v) was added and the sample vortex-mixed for 1 min and shaken for 10 min. After centrifugation at 3500 × g for 5 min, the upper organic layer was transferred to another tube and evaporated under nitrogen at 40 °C. The residue was reconstituted in 200 μL water:acetonitrile (10:90) and 10 μL injected into the LC/MS system.

2.5. Assay validation

The specificity of the method was investigated by analyzing plasma from six different drug-free volunteers. Linearity was assessed by weighted ($1/x^2$) least-squares linear regression of standard curves based on peak area ratios of the seven concentrations of analyte to IS prepared in triplicate on three separate days. Intra- and inter-day precision as relative standard deviation (R.S.D.) and accuracy as relative error (RE) were evaluated by assay of six replicates of each QC sample on three separate days. Matrix effects were evaluated by comparing peak area responses of solutions prepared using 100 μL dexamethasone palmitate QC solutions, 100 μL dexamethasone QC solutions and 50 μL IS solution (total volume 250 μL) to reconstitute residues extracted from six different lots of drug-free human plasma with the mixtures prepared using corresponding QC solutions (100 μL dexamethasone palmitate QC solutions, 100 μL dexamethasone QC solutions and 50 μL IS solution). Recovery of the extrac-

tion procedure was determined by comparing peak areas of QC samples with those of corresponding QC solutions dissolved in the upper organic layer of extracted blank plasma. Stability in plasma and water:acetonitrile (10:90) were evaluated at 20 °C for 4 h. Stability in plasma was evaluated after three freeze/thaw cycles and after storage at −20 °C for 48 days.

2.6. Pharmacokinetic study

The method was used to investigate the plasma profiles of dexamethasone palmitate and dexamethasone in 10 healthy volunteers given an I.V. dose of dexamethasone palmitate (4 mg). Subjects were not allowed to consume alcohol or take any other medication during the study. The clinical protocol was approved by the Ethics Committee of Peking University First Hospital, China. All volunteers read the protocol and gave written informed consent before entering the study. Blood samples were collected into heparinized tubes before the injection and at 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after the injection. Plasma was separated by centrifugation at 2000 × *g* for 10 min and stored at −20 °C until analysis. Pharmacokinetic parameters were calculated.

3. Results and discussion

3.1. MS/MS conditions

In the early stage of method development, the possibility of using ESI or atmospheric pressure chemical ionization (APCI) in the positive ion mode was investigated. ESI in the positive ion mode was found to be linear over a wider concentration range and give lower detection limits (twofold to threefold) than APCI. Full-scan product ion spectra of $[M+H]^+$ for dexamethasone palmitate and dexamethasone are shown in Fig. 1. Fragment ions at *m/z* 373.1, 147.1 and 58.1 were chosen for MRM acquisition of dexamethasone palmitate, dexamethasone and IS, respectively. Because of the lower signal intensity of dexamethasone compared to dexamethsone palmitate, MS parameter optimization concentrated on dexamethasone.

3.2. Chromatographic conditions

In optimizing the chromatography, it was found that on C_{18} columns (Diamonsil, Zorbax extend, Nucleosil and Hypersil) the dexamethasone peak exhibited extensive tailing and the retention time of dexamethasone palmitate (about 6 min) was

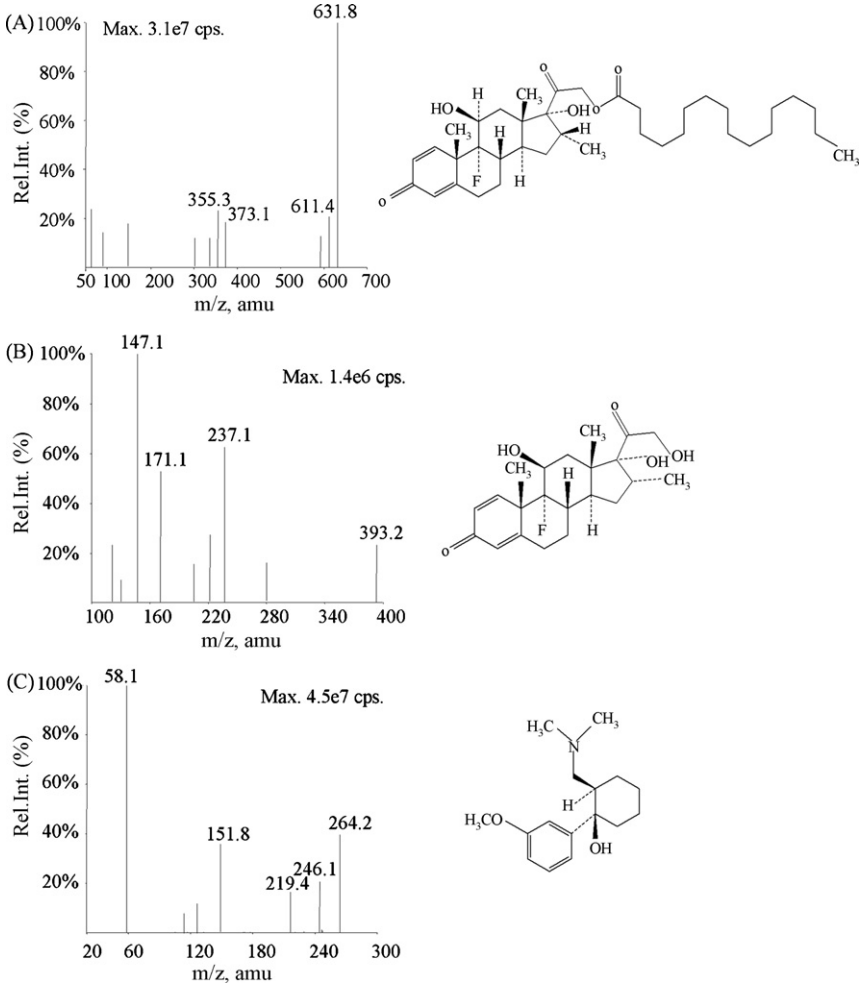

Fig. 1. Full-scan product ion mass spectra of $[M+H]^+$ ions for dexamethasone palmitate (A), dexamethasone (B) and tramadol hydrochloride (C).

relatively long irrespective of the content of organic modifier or the choice of pH modifier (ammonium acetate, formic acid and ammonia solution) in the mobile phase. However, on a C_8 column, a mobile phase containing a high proportion of water gave a symmetrical dexamethasone peak and a mobile phase containing a high proportion of acetonitrile gave a suitably short retention time for dexamethasone palmitate. Thus gradient elution was adopted and found to provide adequate separation, sensitivity, good peak shape and a short run time. It was also found that the inclusion of 0.5% ammonia solution in the mobile phase was crucial to obtaining a high signal intensity and that a high flow rate of 1.5 mL/min produced good peak shapes and gave a short chromatographic run time.

3.3. Sample preparation

Different methods of sample preparation including solid-phase extraction, protein precipitation and LLE with various organic solvents (diethyl ether, dichloromethane, hexane, isopropanol and ethyl acetate) were evaluated. It was found that protein precipitation followed by LLE produced clean extracts with high extraction efficiency irrespective of pH adjustment when a saturated solution of potassium chloride was added to plasma. LLE without prior protein precipitation resulted in low recovery of dexamethasone palmitate presumably because of its tight binding to plasma protein. The function of the saturated potassium chloride was largely to enhance the recovery of dexamethasone.

3.4. Assay validation

3.4.1. Specificity

No significant interference from endogenous substances was observed in the chromatograms of drug-free plasma from six different volunteers at the retention times of the analytes and IS. Representative LC-MRM chromatograms of blank and spiked plasma are shown in Fig. 2A and B. Fig. 2C shows the chromatograms of plasma samples from a healthy volunteer after administration of dexamethasone palmitate.

3.4.2. Matrix effects

Matrix effects relate to the suppression or enhancement of analyte response due to co-eluting endogenous components in biological samples. In this study, the ratios of the peak responses of dexamethasone palmitate were $95.1 \pm 3.2\%$, $90.4 \pm 2.2\%$ and $92.2 \pm 1.9\%$ (six different lots of human plasma at each concentration) at 5, 50 and 500 ng/mL, respectively. Ratios of peak responses of dexamethasone were $82.3 \pm 1.3\%$, $85.6 \pm 1.0\%$ and $81.9 \pm 3.7\%$ (six different lots of human plasma at each concentration) at 5, 25 and 100 ng/mL, respectively. The ratio for the IS was $95.3 \pm 2.4\%$ ($n = 6$). The results indicate that no endogenous substances significantly influenced the ionization of the analytes in plasma.

3.4.3. Linearity and sensitivity

Standard curves for dexamethasone palmitate and dexamethasone were linear over the concentration ranges 1.5–1000 ng/mL and 2.5–250 ng/mL, respectively with correlation coefficients >0.9950. Linear equations for dexamethasone palmitate and dexamethasone were $y = 0.00784x - 0.00335$ and $y = 0.00539x - 0.00461$, respectively. The LLOQ, defined as the lowest concentration analyzed with accuracy and precision within ±20% corresponded to the lowest points on the respective standard curves for each analyte (1.5 ng/mL for dexamethasone palmitate with 7.49% as R.S.D. and 0.13% as RE, and 2.5 ng/mL for dexamethasone with 2.23% as R.S.D. and 0.78% as RE, $n = 6$). The LODs (signal/noise >3) were 0.1 ng/mL for dexamethasone palmitate and 0.6 ng/mL for dexamethasone.

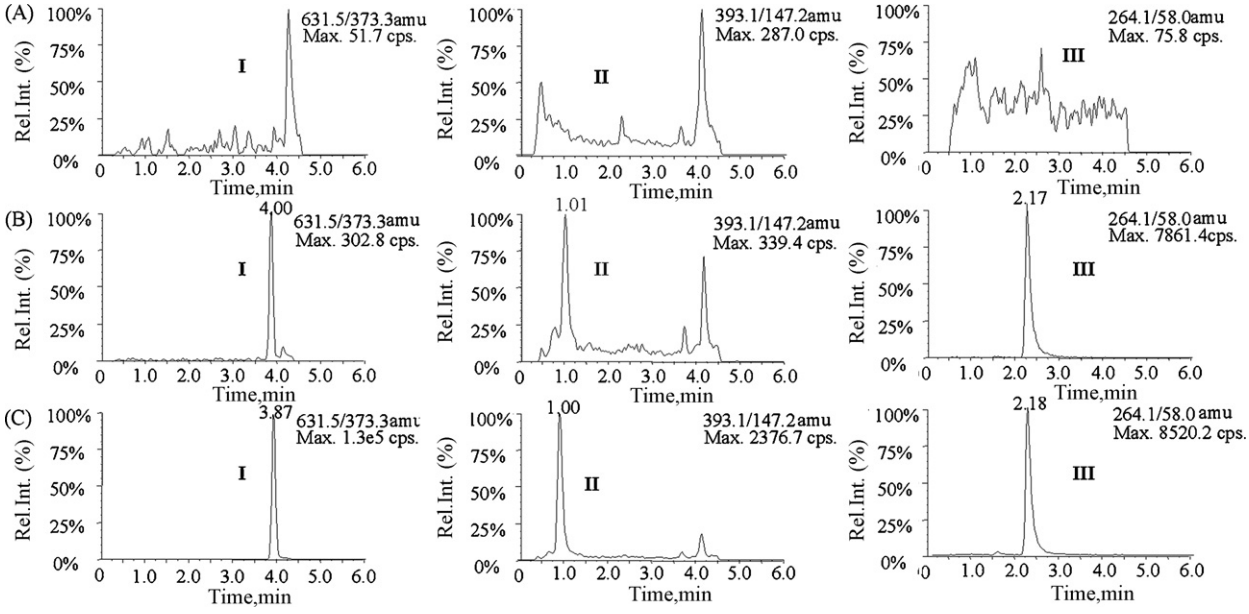

Fig. 2. Representative LC-MRM chromatograms for dexamethasone palmitate (I), dexamethasone (II), and IS (tramadol hydrochloride, III) in human plasma. (A) Blank plasma, (B) blank plasma spiked with dexamethasone palmitate (1.5 ng/mL), dexamethasone (2.5 ng/mL), and IS and (C) a volunteer plasma sample 0.5 h after an I.V. injection of dexamethasone palmitate (4 mg).

Table 1
Accuracy and precision for the determination of dexamethasone palmitate and dexamethasone in human plasma (data are based on assay of six replicates on three different days)

	Concentration (ng/mL)		R.S.D. (%)		RE (%)
	Nominal	Mean found	Intra-day	Inter-day	
Dexamethasone palmitate	5.00	5.12	2.98	2.72	2.37
	50.00	50.29	4.15	2.65	0.59
	500.00	508.50	4.39	4.16	1.70
Dexamethasone	5.00	5.33	4.75	8.12	6.52
	25.00	25.33	1.99	8.40	1.33
	100.00	99.89	1.58	5.98	−0.11

3.4.4. Precision and accuracy

The values were calculated by analysis of variance. Precision and accuracy data are presented in Table 1. Intra- and inter-day precisions were <15% with accuracies of 100 ± 15% at each QC concentration.

3.4.5. Recovery

The extraction recoveries of the two analytes were: dexamethasone palmitate 87.5 ± 1.5%, 84.9 ± 2.3% and 86.3 ± 1.1% (six samples at each concentration) at concentrations of 5, 50 and 500 ng/mL, respectively; dexamethasone 91.5 ± 3.4%, 87.5 ± 5.6% and 89.6 ± 0.3% (six samples at each concentration) at concentrations of 5, 25 and 100 ng/mL, respectively; IS 87.2 ± 4.3% (n = 6).

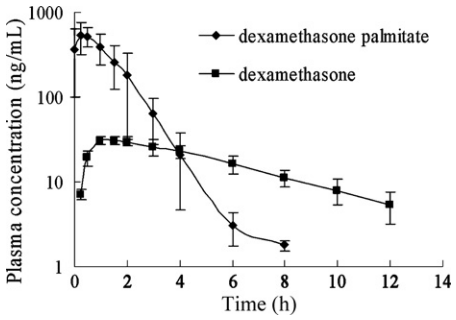

Fig. 3. Mean plasma concentration–time profiles for dexamethasone palmitate and dexamethasone after I.V. administration of 4 mg dexamethasone palmitate. Data are mean ± S.D. for 10 healthy volunteers.

3.4.6. Stability

Stability data are shown in Table 2. The analytes were stable in water:acetonitrile (10:90), in plasma at 20 °C for 4 h, in plasma after three freeze–thaw cycles and in plasma on refrigerated storage at −20 °C for 48 days.

3.5. Pharmacokinetic study

Mean plasma concentration–time profiles for dexamethasone palmitate and dexamethasone after I.V. administration of dexamethasone palmitate (4 mg) are shown in Fig. 3. Pharmacokinetic parameters are as follows: dexamethasone palmitate, C_{max} 616.4 ± 194.8 ng/mL, $t_{1/2}$ 0.73 ± 0.12 h, AUC_{0-t} 925.8 ± 211.0 ng h/mL; dexamethasone,

Table 2
Stability of dexamethasone palmitate and dexamethasone under various storage conditions (n = 3)

Storage conditions	Drug	Concentration (ng/mL)		Percentage change (%)
		Nominal concentration	Mean found concentration ± S.D.	
Stability at −20 °C for 48 days	Dexamethasone palmitate	5.00	5.07 ± 0.74	1.40
		50.00	54.28 ± 2.83	8.57
		500.00	547.24 ± 24.66	9.45
	Dexamethasone	5.00	5.33 ± 0.42	6.53
		25.00	26.70 ± 0.80	6.80
		100.00	99.09 ± 3.00	−0.91
Freeze–thaw stability	Dexamethasone palmitate	5.00	4.92 ± 0.15	−1.67
		50.00	48.71 ± 2.33	−2.57
		500.00	482.99 ± 9.28	−3.40
	Dexamethasone	5.00	5.29 ± 0.27	5.80
		25.00	24.21 ± 2.21	−3.17
		100.00	110.81 ± 2.99	10.81
Autosampler stability at 20 °C for 4 h	Dexamethasone palmitate	5.00	5.33 ± 0.30	6.67
		50.00	55.73 ± 1.20	11.47
		500.00	524.77 ± 10.92	4.95
	Dexamethasone	5.00	5.67 ± 0.05	13.47
		25.00	26.87 ± 1.15	7.48
		100.00	99.93 ± 3.56	−0.07
Stability in plasma at 20 °C for 4 h	Dexamethasone palmitate	5.00	4.91 ± 0.19	−1.80
		50.00	51.63 ± 3.76	3.26
		500.00	516.88 ± 15.92	3.38
	Dexamethasone	5.00	5.04 ± 0.19	0.87
		25.00	24.91 ± 0.78	−0.37
		100.00	111.99 ± 1.74	11.99

C_{max} 32.31 ± 2.62 ng/mL, $t_{1/2}$ 4.02 ± 1.54 h and AUC_{0-t} 198.42 ± 25.78 ng h/mL. The results indicate that, as expected for an I.V. injection, the concentration of dexamethasone palmitate declines rapidly after administration whereas the concentration of dexamethasone declines comparatively slowly.

4. Conclusions

A sensitive, specific and rapid method for the simultaneous determination of dexamethasone palmitate and dexamethasone in human plasma has been developed and validated. An attractive procedure with relative simple sample preparation procedure was made to simultaneously determine dexamethasone palmitate and dexamethasone in clinical pharmacokinetic studies.

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