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Simultaneous determination of dexamethasone and 6β-hydroxydexamethasone in urine using solid-phase extraction and liquid chromatography: applications to in vivo measurement of cytochrome P450 3A4 activity

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Abstract

It has been demonstrated that the formation of the hydrophilic metabolites of dexamethasone, 6α- and 6β-hydroxydexamethasone, correlated with cytochrome P450 (CYP) 3A4 enzyme levels. So, the 6β-hydroxydexamethasone/dexamethasone urinary ratio could be a specific marker for human CYP3A4 activity. We have developed a sensitive and specific high-performance liquid chromatographic method for the simultaneous quantification of urinary free dexamethasone and 6β-hydroxydexamethasone using 6α-methylprednisolone as internal standard. This method involved a solid phase extraction of the three compounds from urine using Oasis HLB Waters cartridges with an elution solvent of ethyl acetate (2 ml) followed by diethyl ether (1 ml). Separation of the three analytes was achieved within 24 min using a reversed-phase Nova-Pak C_{18} analytical column (4 μm, 300 mm × 3.9 mm i.d.). An ultraviolet detector operated at 245 nm was used with a linear response observed from 10 to 100 ng/ml for dexamethasone and from 25 to 1000 ng/ml for 6β-hydroxydexamethasone. Obtained from the method validation, inter-assay precision was below 15% and accuracy ranged from 95.7 to 110%. The extraction efficiency of the assay was approximately of 99% and was constant across the calibration range. The lower limit of quantitation was 10 ng/ml for dexamethasone and 25 ng/ml for 6β-hydroxydexamethasone; at these levels, precision was below 16% and accuracy was 99–109%. This method was applied to in vivo measure of the CYP3A4 activity.

Keywords: Dexamethasone; 6β-Hydroxydexamethasone; Cytochrome P450

1. Introduction

It is widely recognized that most of the anticancer drugs have a narrow therapeutic index. There is substantial inter-individual variability in pharmacokinetic parameters that results in undertreatment with inappropriate therapeutic effect in some patients or overtreatment with unacceptable severe side-effects in others. Factors affecting pharmacokinetic variability include: drug absorption, metabolism and excretion. Among them drug metabolism can be considered as one of the major factors responsible of pharmacokinetic variability.

The cytochromes P450 (CYP) are a superfamily of enzymes responsible for the metabolism of a wide variety of xenobiotics including drugs. Several isozymes of P450 exist and are classified into families and subfamilies [1]. Of the various P450 isozymes, CYP3A4 is present in abundance in human liver microsomes and plays an important role in the metabolism of a large number of anticancer drugs [2]. The activity of CYP3A4 exhibits 5–10-fold interindividual

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variability. Thus, a predictive method of its activity has considerable potential for clinical application [3].

Three major non-invasive in vivo probes for the estimation of the inter-patient variability of CYP3A4 activity have been reported: the erythromycin breath test (EBT), the urinary dapsone recovery test and the measurement of 6β-hydroxycortisol/cortisol ratio [4–6]. The EBT has been shown to be valuable for predicting the clearance of several drugs including the anticancer drug docetaxel in a recent study [7]. The cortisol is only useful for enzyme induction, while dapsone is not useful at all as a CYP3A probe. Dexamethazone would have advantage in that it is non-radioactive (unlike EBT) and will be given to the patient anyway.

Dexamethasone, one of the several derivatives of hydrocortisone, has been widely used for many years as an anti-inflammatory agent. It is also used for prophylactic anti-emetic premedication before antineoplastic drug administration. This drug is metabolized extensively in human liver microsomes to 6-hydroxy and side-chain cleaved products. There were two 6-hydroxylation products, the major metabolite being 6β-hydroxydexamethasone, with 6α-hydroxydexamethasone as a relatively minor metabolite. Hyland et al. [8] have showed that the formation of both 6α- and 6β-hydroxydexamethasone correlated with CYP3A4 levels. So, we have undertaken a study to assess the predictability of the inter-patient variability of CYP3A4 activity estimated by measuring 6β-hydroxydexamethasone/dexamethasone urinary ratio after the administration of dexamethasone before each chemotherapy course.

Some high-performance liquid chromatography (HPLC) methods have been developed to quantify dexamethasone in plasma and tissues [9–14]. A gas chromatography–mass spectrometry method after derivatization of dexamethasone has been also reported [15]. Recently, a liquid chromatography–tandem mass spectrometry has been published for the determination of β-methasone and dexamethasone in bovine liver [16]. However, most of these published methods either did not report assay validation or reported assay validation which were incomplete. To date, the only bio-analytical assay available for the simultaneous determination of dexamethasone and 6β-hydroxydexamethasone in urine has been published by Minagawa et al. [17]. These two compounds were quantified by gas chromatography–mass spectrometry after formation of trimethylsilyl derivatives. However, this method has not been validated by the authors.

This paper describes a specific, reliable, and sensitive analytical methods to quantify, simultaneously, dexamethasone and 6β-hydroxydexamethasone (Fig. 1) in urine. This method has an enhanced precision due to the use of an internal standard (6α-methylprednisolone). It was validated according to validation procedures, parameters and acceptance criteria based on USP XXIII guidelines and FDA guidance [18–20]. Moreover, stability tests under various conditions were performed. The developed method was used to quantify dexametasone and 6β-hydroxydexamethasone in urine samples collected from a cancer patient receiving intravenous administration of dexamethasone (20 mg).

2. Experimental

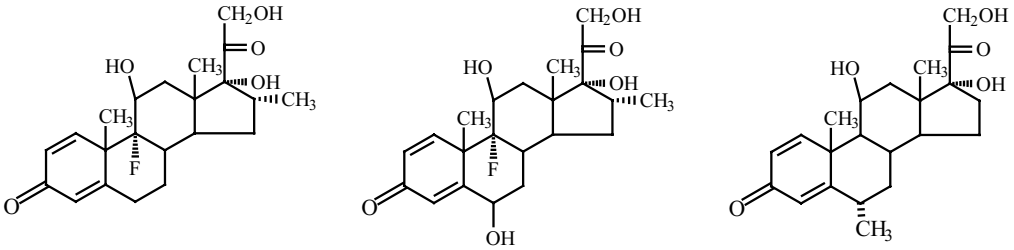
2.1. Reagents

Dexamethasone (9α-fluoro-16α-methylprednisolone) and NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form) were purchased from Sigma (St. Quentin Fallavier, France). The internal standard, 6α-methylprednisolone (6α-methyl-11β,17α,21-trihydroxy-1,4-pregnadien-3,20-dione), was obtained from Pharmacia & Upjohn (St. Quentin-en-Yvelines, France). Ethanol, methanol, acetonitrile, trifluoroacetic and acetic acids, and ammonium acetate (Merck, Darmstadt, Germany), benzoyl chloride, pyridine, and *m*-chloroperbenzoic acid (Acros, Geel, Belgium), ethyl acetate, diethyl ether and chloroform (Aldrich, Saint Quentin Fallavier, France) and sodium hydrogenocarbonate (Rectapur, Paris, France) were all of analytical-reagent grade. Purified water was obtained from Fresenius (France Pharma, Sèvres, France).

Oasis HLB cartridges (60 mg, 3 ml) were supplied by Waters (Saint Quentin, France).

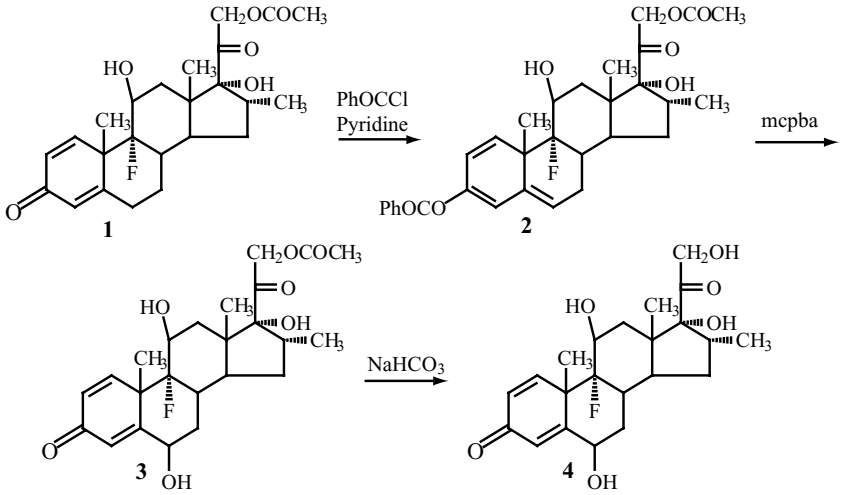
The acetate buffer consisted of 0.77 g ammonium acetate in 1 l of purified water adjusted to pH 4.8 with acetic acid.

For the validation of the method, human urine was obtained from pooled samples collected from healthy volunteers. Aliquots were stored at −20 °C before use.



Dexamethasone **6β-hydroxydexamethasone** **6α–methylprednisolone**

Fig. 1. Chemical structures of dexamethasone and 6β-hydroxydexamethasone.



Scheme 1. Synthesis of 6β-hydroxydexamethasone; dexamethasone 21-acetate: compound **1**; 6β-hydroxydexamethasone: compound **4**; PhOCCl: benzoyl chloride; mcpba: *m*-chloroperbenzoic acid.

2.2. Synthesis of 6β-hydroxydexamethasone

Synthesis of 6β-hydroxydexamethasone (9α-fluoro-6β, 11β,17α,21-tetrahydroxy-16α-methyl-1,4-pregnadiene-3,20-dione) was carried out according to the method described by Draper et al. [21]. This was essentially a three step process (Scheme 1) involving: (i) benzoylation of dexamethasone-21-acetate (**1**) using benzoyl chloride in pyridine; (ii) oxydation of dexamethasone benzoate acetate (**2**) using *m*-chloroperbenzoic acid in chloroform; and (iii) removal of the acetate group with saturated sodium bicarbonate.

2.3. Instrumentation and chromatography

2.3.1. HPLC–UV analysis

The HPLC system was composed of a Schimadzu model LC10AT solvent delivery module (Croissy Beaubourg, France), a Shimadzu SIL-10AD automatic sample injection system thermostated at 4 °C, a Schimadzu model SPD-10AV variable-wavelength UV-Vis detector (245 nm) and a Shimadzu integrator model C-R5A (chart speed, 5 mm/min). Reversed-phase HPLC was performed on a Nova-Pak C_{18} column (4 μm, 300 mm × 3.9 mm i.d., Waters, Saint Quentin en Yvelines, France) preceded by a Sentry™ Guard column Nova-Pak C_{18} (20 mm × 3.9 mm i.d., Waters). The mobile phase consisted of a mixture of 0.06% trifluoroacetic acid in ammonium acetate buffer (0.01 M, pH 4.8)–acetonitrile (90:10 (v/v); Solvent A), and 0.06% trifluoroacetic acid in ammonium acetate buffer–acetonitrile (30:70 (v/v); Solvent B). The mobile phases were filtered through a 0.45 μm Millex®-HV filter (Millipore, Bedford, MA, USA) then deaerated ultrasonically prior to use. Chromatography was achieved at ambient temperature (20 °C) with a flow rate of 1 ml/min by the following gradient profile: 0–50% B (linear) for 20 min followed by 50–100% B (linear) for 10 min.

2.3.2. Liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) analysis

The LC/ESI-MS system (SL model) was composed of a Hewlett Packard Agilent 1100 (Les Ulis, France) connected to Hewlett Packard Agilent LC modules (G1312A pump and G1329A autosampler). Reversed-phase HPLC conditions were identical to that described above. The mass spectrometer was calibrated in the positive ion mode using a mixture of NaI and CsI. Voltages were set at +3.0 kV for the capillary and +0.5 kV for the skimmer lens. The source was heated at 100 °C. Nitrogen was used as nebulizing gas (0.25 MPa) and drying gas (720 l/h). The sampling cone voltage was set up at 60 V. The mobile phase was continuously degassed, at a flow rate of 1 ml/min. The eluent was then splitted prior to the mass spectrometer entrance to reach 10 μl/min of mobile phase infused in the source. During all experiments, mass spectra were obtained by scanning from *m/z* 200 to 500.

2.4. Drug solutions

Separate stock solutions of dexamethasone (40 mg/l), 6α-methylprednisolone hemisuccinate (100 mg/l) and 6β-hydroxydexamethasone (100 mg/l) were made in ethanol-purified water (10:90 (v/v)), purified water and methanol–purified water (50:50 (v/v)), respectively; then stored at −20 °C. Working standard solutions of the three compounds were prepared daily from the stock standard solutions by suitable dilutions in drug-free urine. They were used to prepare calibration curves and quality control (QC) samples.

A reference standard solution (300 ng/ml of each compound plus 300 ng/ml of internal standard) was prepared

daily in purified water to check the resolution of the chromatographic system.

2.5. Analytical procedure

2.5.1. Calibration standards and quality control samples

Human urine standards were prepared fresh daily by aliquoting appropriate volumes of drug working solutions into drug-free urine in 1.5 ml polypropylene microtubes (Eppendorf, Polylabo, Strasbourg, France) to produce a concentration series of 10, 20, 30, 40, 60, 80 and 100 ng/ml for dexamethasone and 25, 50, 100, 300, 500, 800 and 1000 ng/ml for 6β-hydroxydexamethasone.

Quality control (QC) samples were independently prepared as described above to yield concentrations of 25, 50 and 90 ng/ml for dexamethasone and 30, 200 and 900 ng/ml for 6β-hydroxydexamethasone.

The sample pretreatment procedure was identical to the one described below. A calibration curve and three QC samples (low, medium, and high; with 2 at each level) were run with every set of 20 unknown samples.

2.5.2. Sample pretreatment

The sample pre-treatment procedure involved a solid phase extraction (SPE). The Oasis HLB Waters cartridges were conditioned with 3 ml of methanol and 6 ml of purified water before use. A 1.0 ml aliquot of unknown urine sample or urine standard was mixed with the internal standard (200 ng) and loaded onto individual conditioned SPE column under slight vacuum (approximately 86 kPa). The column was washed with 5 ml of purified water. The compounds of interest were eluted from the column with 2 ml of ethyl acetate followed by 1 ml of diethyl ether by manually applying a slow uniform pressure to the top of the column using nitrogen gas (about 0.2 kg/cm^2). The eluate was evaporated to dryness at 39 °C under nitrogen stream. The residue was dissolved in 250 μl of a mixture of purified water–ethanol (200:50 (v/v)), and filtered through a membrane filter (0.45 μm pore size). An aliquot of 100 μl (or 5 μl) of the filtrate was injected onto the HPLC (or LC/ESI-MS) system for analysis.

2.6. Method validation

For each standard curve an unweighted least squares linear regression of the response (peak area ratios: compound over internal standard) as a function of the nominal concentrations was applied. The parameters of each calibration curve were used: (i) to compute back-calculated concentrations; and (ii) to obtain concentration values for that day's quality control samples and unknown samples. The "Lack of Fit" test was used to confirm the linearity of the method. Moreover, the back-calculated concentrations were compared to the nominal concentrations, and the bias (or mean predictor error) with the 95% confidence interval were computed.

The specificity of the method was investigated by analyzing 10 different batches of drug-free human urine from healthy volunteers to determine whether endogenous constituents coeluted with the different analytes. The retention times of endogenous compounds in the matrix were compared with that of the compounds of interest.

Within- and between-day precision and accuracy of the assay were assessed by performing replicate analyses of QC samples (25, 50 and 90 ng/ml for dexamethasone and 30, 200 and 900 ng/ml for 6β-hydroxydexamethasone) in urine against a calibration curve. The procedure was repeated on different days ($n = 7$) on the same spiked standards to determine between-day repeatability. Intra-day repeatability was determined by treating spiked samples in replicate ($n = 5$) the same day. The accuracy was evaluated as [mean found concentration/theoretical concentration] × 100. Precision was given by the percent relative standard deviation (R.S.D.).

In order to test whether it is possible to apply the described method to samples whose concentrations are higher than the last calibration point, spiked samples at 180 ng/ml for dexamethasone and 1300 ng/ml for 6β-hydroxydexamethasone were prepared. They were diluted four- and 10-fold with drug-free human urine in order to bring concentration within the range of standard curve. Each analysis was performed five times for each concentration, using calibration curves and QC samples. The found concentrations were reported and compared to the nominal one.

Extraction recoveries of dexamethasone and 6β-hydroxydexamethasone from urine were measured three times at each QC sample concentration by calculating the percentage difference between the peak areas of extracted QC samples and those of the authentic (unextracted) standards in the relevant concentration range prepared in a mixture of purified water–ethanol (200:50 (v/v)). The extraction recovery was also computed for the internal standard.

The lower limit of quantitation (LLOQ) was defined as the lowest drug concentration which can be determined with an R.S.D. ≤ 20% and an accuracy between 100 ± 20% on a day-to-day basis [18–20]. Accuracy and precision at the LLOQ were estimated using QC samples. Each QC sample was analyzed versus a calibration curve on 7 consecutive days.

2.7. Stability study

The stability of the two analytes in urine was determined using QC samples at concentrations of 25, 50 and 90 ng/ml for dexamethasone and 30, 200 and 900 ng/ml for 6β-hydroxydexamethasone as follows.

(a) Storing QC samples at room temperature with daylight exposure (about 20 °C) and in a refrigerator at 4 °C for 24 h.
(b) Storing QC samples in urine at −20 °C for 4 months. Prior to their analysis, samples were brought to room temperature and well vortex-mixed.

(c) Storing the alcoholic extracts originating from urine spiked with the two analytes in the autosampler at 4 °C for 24 h.

QC samples were analyzed immediately after preparation (reference values) and at selected time intervals after storage over the studied period. Three replicates were analyzed at every time point. Results are expressed as percent recovery of initial drug concentration. Stability was defined as <10% loss of initial drug concentration.

2.8. Urine specimen from patients

The 6β-hydroxydexamethasone/dexamethasone ratio was computed in metastatic cancer patients receiving intravenous administration of dexamethasone (20 mg) associated to vinorelbine every four weeks. All patients had satisfactory WHO Cooperative Oncology Group scores (0 or 2) and normal biochemical profile including liver enzymes, kidney function and electrolytes. The study procedure was carefully explained, especially the importance of urine collection. The study protocol was reviewed and approved by the institutional review board. All patients gave their informed consent. Urine specimens were collected before drug administration and the following 24 h after dexamethasone administration. The voided urine was collected, the total volume recorded, and two aliquots were placed in vials and stored at −20 °C until analysis. When concentrations exceed the highest value of the standard curve, unknown samples were diluted (4- or 10-fold with drug-free human urine) in order to bring concentration within the range of standard curve.

3. Results

Fig. 2 shows the mass spectra of dexamethasone and 6β-hydroxydexamethasone obtained under the analytical conditions. The protonated molecular ion $[M + H]^+$ of the two compounds were observed as base peak at *m/z* 393 and 409, respectively. "Fragment" ions were obtained at *m/z* 373 for dexamethasone, and at *m/z* 389 and 345 for 6β-hydroxydexamethasone.

3.1. HPLC–UV analysis

Representative chromatograms of drug-free urine, urine spiked with dexamethasone, 6β-hydroxydexamethasone and internal standard, and a post-dose urine sample collected from the clinical study are shown in Fig. 3. Under the chromatographic conditions used, the number of theoretical plates (computed on the peak of internal standard) was approximately 127,545. The precolumn was exchanged every 100 sample runs and the column was replaced when the

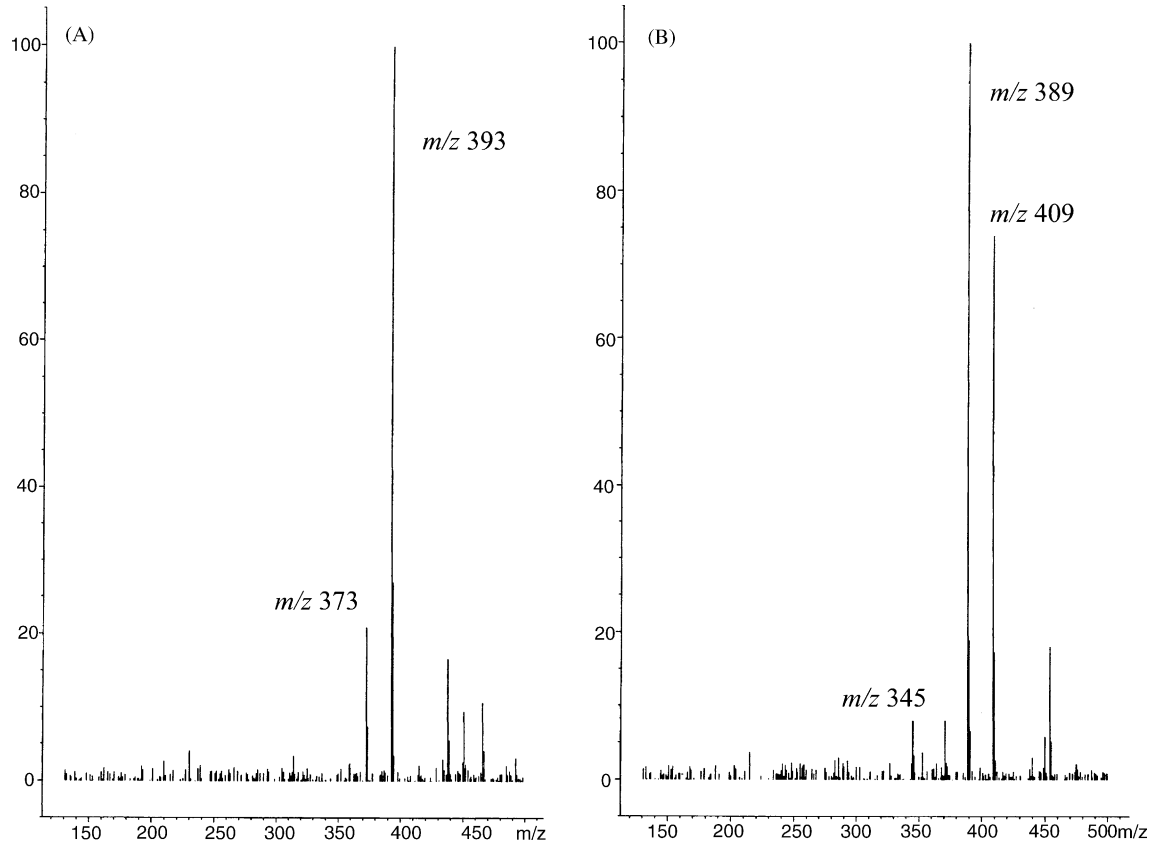


Fig. 2. Mass-spectra for dexamethasone (A) and 6β-hydroxydexamethasone (B).

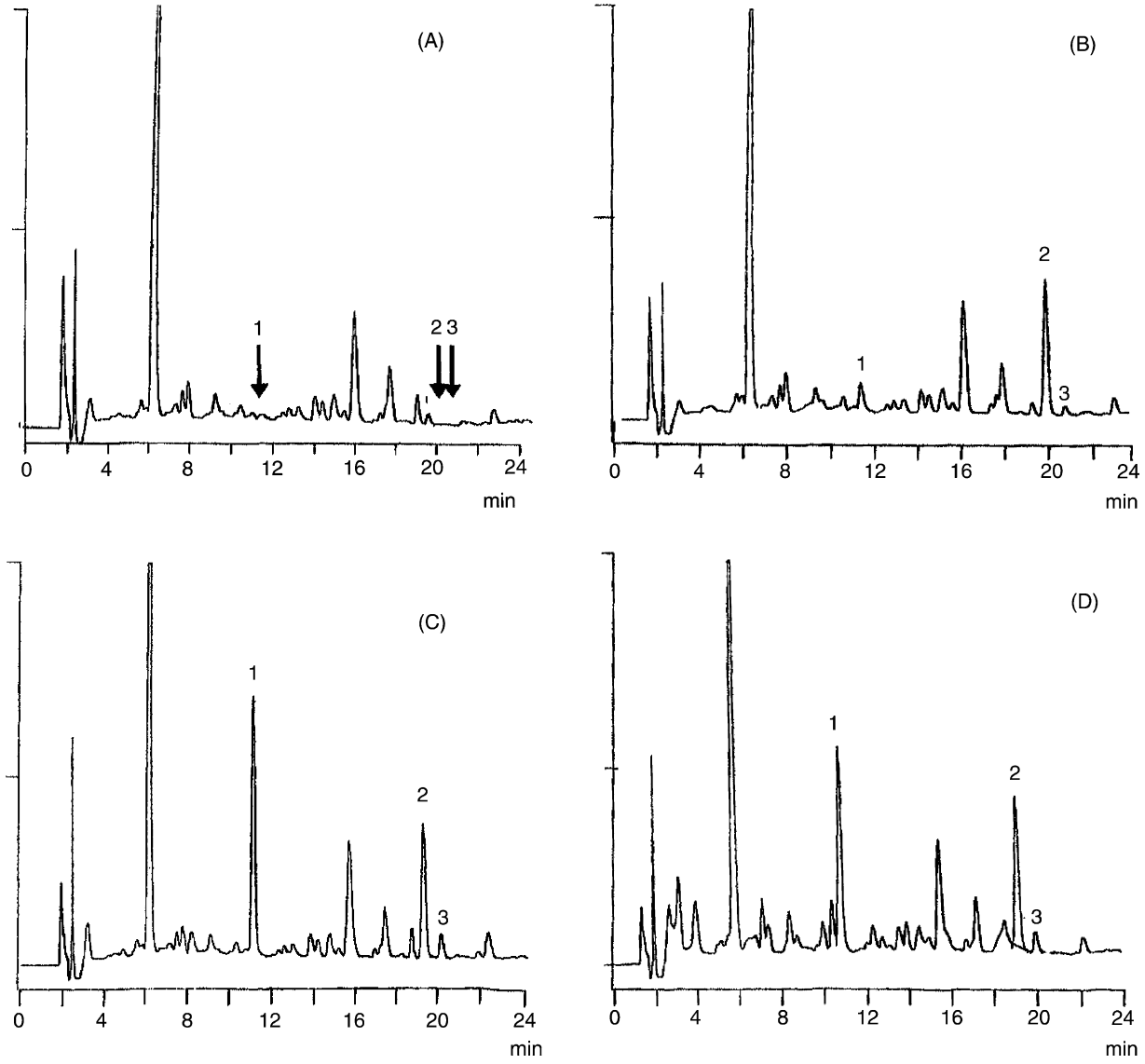


Fig. 3. Chromatograms of blank urine (A) of urine spiked with 20 ng/ml of dexamethasone and 50 ng/ml of 6β-hydroxydexamethasone (B) and 60 ng/ml of dexamethasone and 500 ng/ml of 6β-hydroxydexamethasone (C) and of urine from a patient (D) receiving 20 mg of dexamethasone (urinary concentrations 181 ng/ml of dexamethasone and 1304 ng/ml of 6β-hydroxydexamethasone). Peak 1: 6β-hydroxydexamethasone, Peak 2: internal standard; and Peak 3, dexamethasone. AUFS = 0.032. For chromatographic conditions see text.

number of theoretical plates had decreased below 32,000. 6β-hydroxydexamethasone (retention time $t_{r1} = 11.4 \pm 0.05$, $n = 13$), internal standard (retention time $t_{r2} = 19.9 \pm 0.14$, $n = 13$) and dexamethasone (retention time $t_{r3} = 20.9 \pm 0.07$, $n = 13$), exhibited well peaks separated ($\alpha_{2,3} = 1.3$) under the chromatographic conditions described. The k'-values were 4.9, 9.2 and 9.7 for the three analytes, respectively.

No endogenous interfering peaks were visible at the retention times of the different analytes (Fig. 3A).

3.2. Method validation

The calculated peak–area ratios and the added concentrations of each analyte displayed linear relationship over the selected concentration range with consistent slopes and coefficients of determination (r^2) higher than 0.99 throughout the validation runs (Table 1). The "Lack of Fit" test showed no significant deviation from linearity. For each point of calibration standards, the concentrations were back-calculated from the equation of the linear regression curves (Table 2). A linear regression of the back-calculated concentrations versus the nominal ones provided a unit slope and an intercept equal to 0 (Student's *t*-test). The distribution of the residuals (difference between nominal and back-calculated concentrations) shows random variations, the number of positive and negative values being approximately equal. Moreover, they were normally distributed and centered around zero. The bias (0.15 for dexamethasone and −1.70 for 6β-hydroxydexamethasone) was not statistically differ-

Table 1
Assay linearity of the method

	Determination coefficient of the linear regression analysis[a] ($r^2 \pm$ S.D.)	a (slope $\pm$ S.D.)	b (intercept $\pm$ S.D.)
Dexamethasone			
Intra-day reproducibility ($n = 5$)	$0.997 \pm 8.9 \times 10^{-4}$; R.S.D. = 0.090%	$4.09 \times 10^{-3} \pm 3.9 \times 10^{-4}$; R.S.D. = 9.5%	-0.0006 ± 0.0076
Inter-day reproducibility ($n = 13$)	$0.997 \pm 8.2 \times 10^{-4}$; R.S.D. = 0.082%	$4.28 \times 10^{-3} \pm 7.4 \times 10^{-4}$; R.S.D. = 17.3%	-0.0179 ± 0.0496
β-OH-dexamethasone			
Intra-day reproducibility ($n = 5$)	$0.996 \pm 3.3 \times 10^{-3}$; R.S.D. = 0.33%	$3.43 \times 10^{-3} \pm 0.7 \times 10^{-4}$; R.S.D. = 1.9%	0.0056 ± 0.0251
Inter-day reproducibility ($n = 13$)	$0.997 \pm 1.8 \times 10^{-3}$; R.S.D. = 0.18%	$3.48 \times 10^{-3} \pm 5.3 \times 10^{-4}$; R.S.D. = 15.4%	0.0368 ± 0.0438

r^2: determination coefficient; n: number of replicates.
[a] Linear unweighted regression, formula: $y = ax + b$.

ent from zero (Student's t-test) and the 95% confidence interval (−0.35/0.66 for dexamethasone and −5.36/3.94 for 6β-hydroxydexamethasone) included the zero value.

Table 3 lists the accuracy and precision results. The dilution has no influence on the performance of the method. After a 10-fold dilution, the back-calculated concentrations averaged 196 ng/ml (precision, 0.75%; accuracy, 109%) for dexamethasone and 1415 ng/ml (precision, 3.4%; accuracy, 109%) for 6β-hydroxydexamethasone. After a four-fold dilution, they were 191 ng/ml (precision, 2.2%; accuracy, 106%) and 1329 ng/ml (precision, 5.2%; accuracy, 102%), respectively.

Mean extraction recoveries of dexamethasone and 6β-hydroxydexamethasone were 99.3 ± 1.2% ($n = 9$) and 98.8 ± 4.3% ($n = 9$), respectively. The extraction efficiency was independent of concentration over the range studied. For the internal standard the extraction recovery was 94.2 ± 0.9% ($n = 4$).

The LLOQ was 10 ng/ml for dexamethasone and 25 ng/ml for 6β-hydroxydexamethasone. Using QC samples, R.S.D. did not exceed 16% and accuracy was 90–110%.

3.3. Stability

Frozen QC samples tested over a 4 month period showed no sign of either degradation or loss. For all concentrations

Table 2
Back-calculated concentrations from calibration curves

	R.S.D. (%)	Recovery (%)
Dexamethasone		
Intra-day ($n = 5$)		
10	10.6	104
20	2.8	102
30	5.4	99
40	5.3	99
60	4.1	98
80	1.3	101
100	2.2	100
Inter-day ($n = 13$)		
10	7.6	102
20	8.2	95
30	6.6	97
40	4.8	98
60	5.9	103
80	4.2	99
100	2.7	100
β-OH-dexamethasone		
Intra-day ($n = 5$)		
25	15.6	109
50	7.2	107
100	3.0	103
300	6.7	97
500	5.4	99
800	3.6	100
1000	1.9	100
Inter-day ($n = 13$)		
25	15.0	99
50	9.1	98
100	9.5	103
300	7.6	98
500	6.8	104
800	4.8	101
1000	3.5	99

n: number of replicates.

Table 3
Assessment of the accuracy and precision of the method

	Back-calculated concentrations (ng/ml)	Precision (%)	Accuracy (%)
Dexamethasone			
Intra-day ($n = 5$)			
25	23.2	14.2	93
50	48.3	7.0	97
90	90.1	5.0	100
Inter-day ($n = 7$)			
25	24.4	13.9	97.6
50	55.0	4.7	110
90	96.6	6.6	107
β-OH-dexamethasone			
Intra-day ($n = 5$)			
30	32.2	6.9	107
200	194	4.2	97.0
900	949	2.9	105
Inter-day ($n = 7$)			
30	32.1	13.4	107
200	212	12.1	106
900	861	7.8	95.7

n: number of replicates.

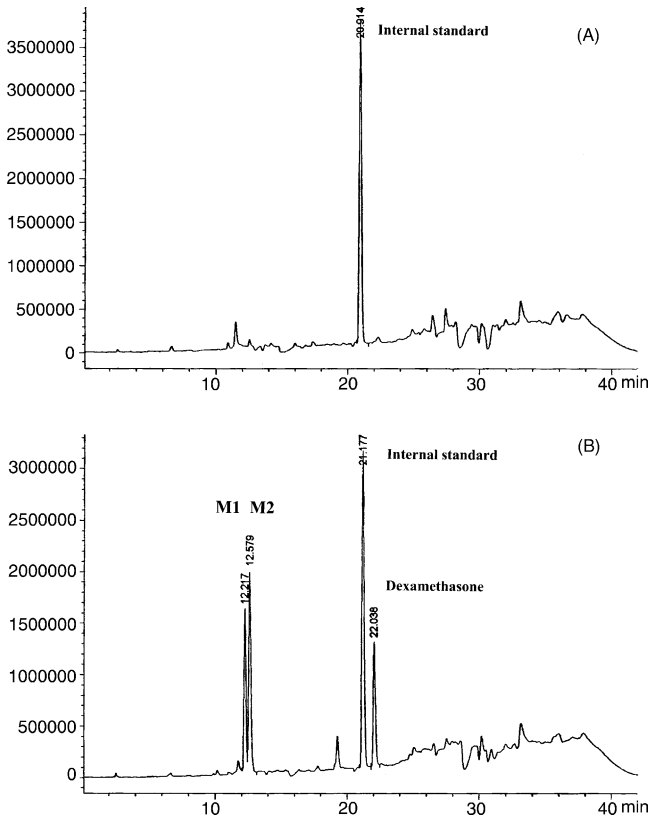


Fig. 4. Typical chromatograms (LC/ESI-MS) illustrating the metabolism of dexamethasone by a patient receiving 20 mg of dexamethasone. Urine extract before dexamethasone administration (A); urine extract after dexamethasone administration (B).

studied no significant difference appeared between times 0–4 months ($P > 0.05$). Urine samples spiked with the two analytes showed no sign of decrease in the nominal starting concentration after 24 h of storage at both room temperature and 4 °C. In alcoholic extracts, dexamethasone and 6β-hydroxydexamethasone were stable for at least 24 h at 4 °C.

3.4. Urine specimen from a patient

The main metabolite of dexamethasone was identified by LC/ESI-MS by comparison with standard. Fig. 4 shows the chromatogram of an urinary extract obtained from a patient entering the clinical study. Two major metabolites were identified: M1 (retention time, 12.2 min) and M2 (retention time, 12.6 min). For both M1 and M2, the putative pseudomolecular ion ($[M + H]^+$) of a monohydroxylated product was seen at *m/z* 409. For the compound M2, the fragmentation pattern (ions at *m/z* 389 and 345) and the retention time value were exactly the same as the authentic 6β-hydroxydexamethasone. The compound M1 was not fully characterized but could be consistent with the 6α-hydroxydexamethasone. For this patient, the M1/dexamethasone and M2/dexamethasone ratios after dexamethasone administration were 1.0 and 1.4, respectively.

4. Discussion and conclusion

In this paper, we described a sensitive reversed-phase HPLC assay to quantify simultaneously dexamethasone and 6β-hydroxydexamethasone in human urine. The validation of this analytical method indicated excellent reproducibility. Distinct advantages include the simplicity and rapidity of sample preparation and chromatography, good resolution between the parent drug and its metabolite, good resolution of the analytes from endogenous compounds, accurate assay of large numbers of samples and the requirement of only common instruments. Furthermore, this method has an enhanced precision due to the use of an internal standard (6α-methylprednisolone). The extraction efficiency was independent of concentration over the range studied, an observation which contributes to the good precision and accuracy of the method. For dexamethasone, the lower limit of quantitation was estimated to 10 ng/ml; it was 25 ng/ml for 6β-hydroxydexamethasone.

As previously reported [17,22], the CYP3A4 was involved in the oxidative metabolism of dexamethasone. This drug undergoing less hepatic biotransformation than cortisol could be a simpler probe for CYP3A4 in vivo. In this study, we have developed an assay that will facilitate further investigations as to whether the ratio is useful as an index of CYP3A4 activity.

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