Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: https://saudijournals.com/

A Validated Reversed Phase HPLC Assay for the Determination of Dexamethasone in Human Plasma

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Original Research Article

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Article History
Received: 12.02.2018
Accepted: 20.02.2018
Published: 30.03.2018

DOI:
10.36348/sjmps.2018.v04i03.003

Abstract: A simple and precise reversed-phase high performance liquid chromatography (HPLC) assay for the determination of dexamethasone in human plasma was developed and validated. 1.0 ml plasma samples containing dexamethasone and lansoprazole as internal standard (IS) were deproteinized with 2 ml dichloromethane and 3 ml methyl tert-butyl ether. Separation was achieved on Atlantis dC-18 column with a mobile phase consisted of acetonitrile and water (40:60, v:v), and delivered at a flow rate of 1.0 ml/min. The eluent was monitored spectrophotometrically at 240 nm. No interference in blank plasma or of commonly used drugs was observed. The relationship between the concentration of dexamethasone in plasma and peak height ratio of dexamethasone to the IS was linear over the range of 0.15-8.0 µg/ml. Intra-day and inter-day coefficient of variation (CV) and bias were ≤11.1% and ≤9.5% and ≤9.3% and ≤5.6%, respectively. Mean extraction recovery of dexamethasone and IS was 100% and 97%, respectively. The method was applied to assess dexamethasone stability under various conditions encountered in the clinical laboratory. Dexamethasone stability in processed (24hrs at room temperature, 48hrs at -20°C) and unprocessed (24hrs at room temperature, 8 weeks at -20°C, or after 3 cycles of freeze and thaw) samples was ≥ 90% and ≥ 83%, respectively.
Keywords: Dexamethasone, Lansoprazole, Human plasma, HPLC.

INTRODUCTION

Dexamethasone (CAS: 50-02-2) is a synthetic analogue of cortisol. It is commonly used in the treatment of inflammatory, immunological and allergic disorders.

In addition, it is used as test drug in the diagnosis of Cushing's syndrome [1-3]. Mean bioavailability for large oral doses is more than 60% [4].

Several analytical methods have been reported for the determination of dexamethasone in pharmaceutical formulations [5-7], and biological samples [8-18]. Plasma dexamethasone levels have been mainly determined by either high performance liquid chromatography with UV detection (HPLC-UV) [8-12], or liquid chromatography–tandem mass spectrometric detection (LCMS/MS) [13-17]. Reported methods include liquid-liquid extraction [10-11] solid-phase extraction [13], and protein precipitation procedures [9, 14-17].

In the present study, we describe a simple, precise, rapid, and low-cost HPLC assay for clinically relevant levels of dexamethasone that requires 1.0 ml human plasma. The method was validated and successfully applied to assess stability of dexamethasone under various clinical laboratory conditions.

MATERIALS AND METHODS

Apparatus

Chromatography was performed on a Waters Alliance HPLC 2695 (Waters Associates Inc., Milford, MA, USA) consisting of a quaternary pump, autosampler, column thermostat, photodiode array detector, a reversed-phase Atlantis dC-18 (column 4.6 x 150 mm, 5-µm) and protected by symmetry C-18 (guard column 3.9 x 20 mm, 5 µm). Data were collected with a Pentium IV computer using Empower Chromatography Software.

Chemical and reagents

All reagents were of HPLC grade. Dexamethasone and lansoprazole were purchased from Sigma-Aldrich Co, Steinheim, Germany. Acetonitrile, methanol, dichloromethane, and methyl tert-butyl ether were purchased from Fisher Scientific, Fairlawn, NJ,

USA. HPLC grade water was prepared by reverse osmosis and was further purified by passing through a Synergy Water Purification System (Millipore, Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia.

Chromatographic conditions

The mobile phase consisted of acetonitrile and water (40:60, v:v). The analysis was carried out under isocratic conditions with a flow rate of 1 ml/min at ambient temperature and a run time of 9 minutes. A photodiode array detector set at 240 nm was used.

Preparation of standard and quality control samples

Stock solutions of dexamethasone and lansoprazole (1.0 mg/ml) were prepared in methanol. They were diluted with methanol to produce working solutions of 10 µg/ml. Nine calibration standards in the range of 0.15 – 8.0 µg/ml and four quality control (QC) samples (0.15, 0.45, 4.0, and 7.2 µg/ml) were prepared in human plasma. Calibration standards and QC samples were vortexed for one minute and 1.0 ml aliquots were transferred into teflon-lined, screw-capped, borosilicate, (13 x 100 mm) glass culture tubes and stored at -20 ºC until used.

Sample preparation

Aliquots of 1.0 ml of calibration standards or QC samples were allowed to equilibrate to room temperature. To each tube, 80 µl of the IS working solution (10 µg/l) was added and the mixture was vortexed for 10 seconds. After the addition of 2 ml of dichloromethane and 3 ml of methyl tert-butyl ether, the mixture was vortexed again for 5 min and then centrifuged for 20 min at 4200 rpm at room temperature. The organic layer was carefully collected into a clean tube and dried under a gentle stream of nitrogen, and the residue was reconstituted in 200 µl mobile phase and centrifuged at 13000 rpm for 5 min at room temperature. The supernatant was transferred into an auto-sampler vial and 100 µl were injected into the HPLC system.

Stability studies

A total of 40 aliquots of each QC samples (0.15, 0.45, and 7.2 µg/ml) were used for stability studies. Five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed (counter stability, 24 hours at room temperature), five aliquots were stored at –20 °C for eight weeks before being processed and analyzed (long term freezer storage stability), and five aliquots were processed, reconstituted, and stored at room temperature for 24 hours or at –20 °C for 48 hours before analysis (autosampler stability). Finally, fifteen aliquots of each QC sample were stored at –20 °C for 24 hours. They were then left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest returned to –20 °C for another 24 hours. The cycle was repeated three times (freeze-thaw stability).

Method validation

The method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance [18]. The validation parameter included: specificity, linearity, accuracy, precision, recovery and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Under the optimal experimental conditions, consisting of a mobile phase of acetonitrile and water (40:60, v: v) and flow rate of 1.0 ml/min, dexamethasone, lansoprazole, and plasma components exhibited a well-defined chromatographic separation within a nine minutes run. The retention times of dexamethasone and lansoprazole (IS) were around 6.1 and 7.6 minutes, respectively.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. No endogenous plasma component co-eluted with dexamethasone or the IS. Figure 1 depicts a representative chromatogram of drug free human plasma used in preparation of calibration standards and QC samples.

Linearity, Accuracy and Precision

Linearity of dexamethasone was evaluated by analyzing ten curves of nine standard concentrations over the range of 0.15-8.0 µg/ml. Figure 2 represents an overlay of chromatograms of extracts of 1.0 ml human plasma spiked with the IS and one of nine concentrations of dexamethasone. The peak height ratios were subjected to regression analysis (regression equation, Y = 0.3616 X - 0.0314). The suitability of the calibration curves was confirmed by back-calculating the concentration of dexamethasone in human plasma from the calibration curves (Table 1). All calculated concentrations were within the acceptable limits. Precision and bias were also determined for four QC samples (0.15, 0.45, 4.0, and 7.2 µg/ml). The intra-day (n=10) and inter-day (n=20, over 3 consecutive days) precision was ≤ 11.1% and ≤ 9.5%, respectively. The intra-day and inter-day bias was in the range of ≤ 9.3% and ≤5.6%, respectively. The results are summarized in Table 2.

Recovery

The recovery of dexamethasone was assessed by direct comparison of peak height from plasma and

methanol samples, using five replicates for each of the four QC samples (0.15, 0.45, 4.0, and 7.2 µg/ml). Similarly, the recovery of the IS was determined by comparing the peak height of the IS in 5 aliquots of 1.0 ml human plasma spiked with 80 µl of IS (100 µg/ml) with the peak height of equivalent samples prepared in methanol. The results are presented in **Table 3**. Mean recovery of dexamethasone and the IS were 100% and 97%, respectively.

Stability

Dexamethasone (0.15, 0.45 and 7.2 µg/ml) stability in processed and unprocessed plasma samples was investigated. Dexamethasone was stable in processed samples for at least 24 hours at room temperature (≥ 92%) or 48 hours at -20 °C (≥ 90%). Dexamethasone in unprocessed plasma samples was stable for at least eight weeks at -20 °C (≥ 83%), 24 hours at room temperature (≥ 95%) and after three freeze-and thaw cycles (≥ 84%). IS (1mg/ml) in methanol was stable for at 24 hours at room temperature (97%), and 8 weeks at -20°C (96%), respectively.

CONCLUSION

The described HPLC assay is accurate, precise, and rapid. It requires only 1.0 ml plasma and utilizes a simple and liquid-liquid extraction for sample preparation. The assay was applied to monitor stability of dexamethasone under various conditions encountered in the clinical laboratories.

Table-1: Back-calculated dexamethasone concentrations from ten calibration curves

Nominal Level (µg/ml)	Calculated Level (µg/ml)		*CV (%)	**Accuracy (%)
	Mean	SD		
0.15	0.158	0.009	5.5	105
0.25	0.253	0.022	8.9	101
0.4	0.421	0.018	4.3	105
0.8	0.878	0.031	3.5	110
1.6	1.523	0.128	8.4	95
2.0	1.899	0.109	5.7	95
4.0	3.664	0.163	4.4	92
6.0	5.487	0.313	5.7	91
8.0	8.427	0.235	2.8	105

*Coefficient of variation (CV) = standard deviation (SD) divided by mean measured concentration x 100.** Accuracy = measured level divided by nominal level x 100.

Table-2: Intra- and inter-day precision and bias of dexamethasone assay

Nominal Level (µg/ml)	Measured Level (µg/ml)		CV (%)	Bias (%)
	Mean	SD		
Intra-day (n=10)				
0.15	0.150	0.011	7.6	0.2
0.45	0.492	0.055	11.1	9.3
4.0	4.236	0.132	3.1	5.9
7.2	7.400	0.800	10.8	2.8
Inter-day (n=20)				
0.15	0.157	0.013	8.4	5.0
0.45	0.475	0.045	9.5	5.6
4.0	4.155	0.176	4.2	3.9
7.2	7.390	0.574	7.8	2.6

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.
Bias, measured level - nominal level divided by nominal level x 100.

Table-3: Recovery of dexamethasone and lansoprazole (internal standard) from 1.0 ml Of human plasma

Concentration (µg/ml)	Mean Peak Height (SD)*		Recovery** (%)
	Human Plasma	Methanol	
Dexamethasone 0.15	13326 (436)	13429 (370)	99
0.45	39564 (722)	39216 (627)	101
4.0	429324 (7016)	429802 (11140)	100
7.2	652963 (11561)	650532 (8086)	100
Internal standard 4.0	223571 (15884)	230605 (1579)	97

* Mean peak height (SD), n = 5. * Recovery is equal to mean peak height in human plasma Divided by mean peak height in water x 100

Table-4: Stability of dexamethasone under various clinical laboratory conditions

Nominal	Unprocessed		Processed		Freeze-Thaw		
Level	24 hrs	8 wks	24 hrs	48 hrs	Cycle		
(µg/ml)	RT	-20 °C	RT	-20 °C	1	2	3
0.15	100	90	97	90	101	90	94
0.45	95	85	92	92	101	90	104
7.2	99	83	98	93	99	86	84

Stability (%) = mean measured concentration (n=5) at the indicated time divided by mean measured concentration (n=5) at baseline x 100. Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), after freezing at –20 ºC for 8 weeks (8 wks, –20 ºC), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs, RT) or 48 hours at –20 ºC (48 hrs, -20 ºC).

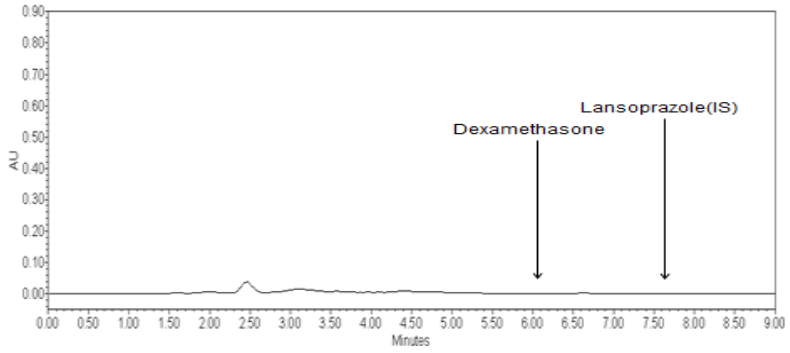

Fig-1: Representative chromatogram of dexamethasone-free and lansoprazole-free human plasma. The arrows indicate the retention times of dexamethasone and lansoprazole

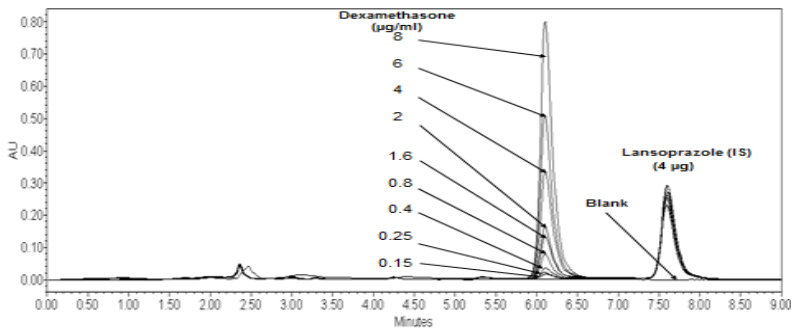

Fig-2: Overlay of chromatograms of extracts of 1.0 ml human plasma spiked with the internal standard (IS) and one of nine concentrations of dexamethasone

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