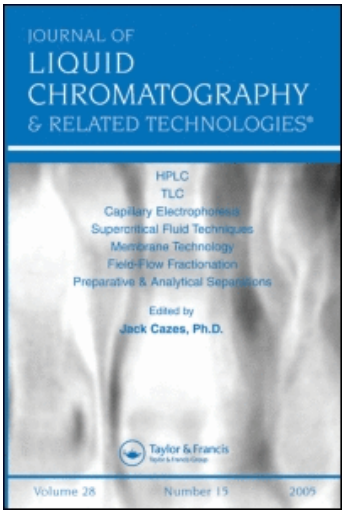


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# A LIQUID CHROMATOGRAPHIC-TANDEM MASS SPECTROMETRIC METHOD FOR THE QUANTITATIVE ANALYSIS OF DEXAMETHASONE IN HUMAN PLASMA

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## ABSTRACT

A simple and specific assay for the rapid quantification of the plasma concentration of dexamethasone from 0.250 to 250 ng/mL has been developed and validated using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method used methyl-*t*-butyl ether (MTBE) to extract dexamethasone and the internal standard (IS) beclomethasone from alkalinized human plasma sample. The chromatographic separation was on a $C_{18}$ column ($50 \times 3$ mm, 5-μm) using acetonitrile-water-formic acid (35 : 65 : 0.1, v/v) mobile phase at a flow rate of 0.500 mL/min. The retention times were approximately 1.8 min for dexamethasone and 2.0 min for the internal standard. The tandem mass spectrometric detection was by monitoring singly charged precursor → production transitions 393 → 373

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($m/z$) for dexamethasone and 409 → 391 ($m/z$) for the internal standard. The validated method used 0.5-mL plasma. The linear correlation coefficients of the calibration curves were greater than, or equal to, 0.9972. The run time for each sample injection was 3.0 min.

The recoveries of the extraction were found to be 88–91% for dexamethasone at three concentration levels and 84.5% for the IS, respectively. The intra-day precision and accuracy of the control samples were of ≤4.8% relative standard deviation (RSD) (n = 6) and −7.6 to 5.6% relative errors (RE). The inter-day precision and accuracy were ≤5.1% RSD (n = 18) and −6.9 to −0.3% RE. The partial volume experiments showed excellent dilution integrity for high concentration level control samples. The stability data illustrated that this compound was stable in plasma at least for three freeze/thaw cycles, or keeping plasma samples at ambient temperature for 24 hours, or storing extracted samples at room temperature for 48 hours, and or storing frozen samples at approximately −20°C for up to three months.

*Key Words*: Dexamethasone; LC-MS/MS; Human plasma



## INTRODUCTION

Dexamethasone (9α-fluoro-11β, 17α, 21-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione, shown in Figure 1a), a corticosteroid with mainly glucocorticoid activity, was found to be effective in many diseases and has a wide medical practice in the treatment of cerebral oedema, malignancy, blood disorders, congenital adrenal hyperplasia, cerebral malaria, nausea, and vomiting induced by cancer chemotherapy, and respiratory disorders caused by premature delivery.[1] Its biological half-life in plasma is about 190 minutes. Due to its stronger potency than other corticosteroids, a lower dose, typically a few milligrams or even half a milligram is given.[1,2] Therefore, a sensitive and accurate assay is needed for supporting pharmacokinetic studies and clinical trials.

By now, major techniques having been used for the determination of dexamethasone in biological matrices include radioimmunoassay (RIA),[3–7] gas chromatography-mass spectrometry (GC-MS),[8–12] and liquid chromatography (LC),[10–23] including immunoaffinity chromatography (IAC).[11,22] The cross-reactivity of possible metabolites of dexamethasone or other endogenous compounds required a highly selective antibody for accurate quantification of

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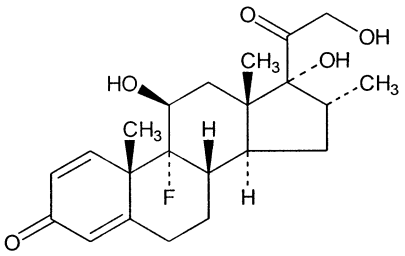
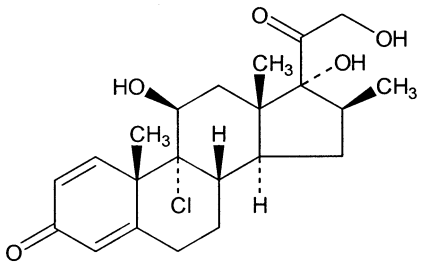



(a) Dexamethasone, m. w. 392.5




(b) Beclomethasone (IS), m. w. 408.9

***Figure 1.*** Chemical structures of (a) dexamethasone, and (b) beclomethasone.

dexamethasone and, thus, limited the use of RIA methods.[13] GC-MS assays had high sensitivity, but usually needed a complicated and time-consuming derivatization step. The LC methods employing conventional detection techniques lacked enough sensitivity, thus, they either required a large sample size[13–19] or had a relatively high detection limit.[20] The chemiluminescence detection for LC had significantly improved the sensitivity and achieved a detection limit of 0.2 ng/mL, however, this technique required a lengthy derivatization (110 min) and column separation (ca. 60 min for each injection)[19] and, thus, had very limited use.

With the recent emergence of atmospheric pressure ionization techniques, LC coupled with mass spectrometry (LC-MS), especially with tandem mass spectrometry (MS/MS), has become the most powerful approach to identify and quantify drugs and drug metabolites in complex biological matrices.[24,25] Due to

the highly specific mass transition pattern for each given analyte, LC-MS and LC-MS/MS techniques often need only simple sample clean-up and column separation, resulting in a tremendous improvement of analysis speed and throughput. In past years LC-MS methods have been used to study corticosteroids in urine,[22,26–28] plasma,[29] and milk replacers.[30] However, most of these studies were with an emphasis on identification and confirmatory purposes.[26–28,30] Creaser et al. developed an on-line IAC-LC-MS method for the determination of dexamethasone in equine urine, which reached a detection limit of 0.1 ng/mL, but needed 10-mL sample and ca. 20 minutes for each injection.[22] Shibasaki et al. described an LC-Thermospray-MS method for the quantification of cortisol, prednisolone, cortisone, and prednisone in plasma, but dexamethasone was excluded.[29] Recently, Stolker et al. made a systematic comparison of different LC methods that combined various extraction procedures with column liquid chromatography using UV or mass spectrometric detection for the determination of corticosteroids in biological matrices.[23] Previous LC-MS methods mostly focused on urine samples.[22,26–28,30] Use of the more selective and sensitive LC-MS/MS in the quantitative determination of low nanogram of dexamethasone in plasma has not been reported yet.

The purpose of this work is to develop and validate a simple, rapid, and sensitive assay to support pharmacokinetics and clinical studies of dexamethasone in human plasma samples. The validated dynamic range was 0.250 to 250 ng/mL dexamethasone using 0.5-mL plasma. Beclomethasone, as shown in Figure 1b, was used as the internal standard (IS). This method used a simple liquid-liquid extraction and each injection only required 3 min on column separation. The described method is suitable for the determination of dexamethasone in clinical plasma samples.



## EXPERIMENTAL

### Chemicals, Materials, and Apparatus

Dexamethasone with 99.9% purity and beclomethasone (IS) with 99.7% purity were both purchased from Sigma (St. Louis, MO, USA). These reference standards were used without further purification. Formic acid, ACS reagent, was from Aldrich Chemical Company (Milwaukee, WI, USA). Ammonium hydroxide (29.7%), ACS plus, was from Fisher Chemicals (Fair Lawn, NJ, USA). Methyl-*t*-butyl ether (MTBE), acetonitrile (ACN), methanol, and water were of LC grade and were from Fisher Scientific (St. Louis, MO, USA). Trifluoroacetic acid (TFA) was from Sigma. Control blank human plasma with $K_3$-EDTA anticoagulant was from Biochemed Pharmacologicals (Winchester, VA, USA).

A dry ice-acetone bath was used for freezing the aqueous portion after the target compounds were extracted into the organic phase. A water-bath Turbo Vap was used to evaporate the organic solvent in the extracts. An LC system consisting of solvent delivery LC10ADVP, autoinjector SIL10ADVP, Controller SCL10ADVP, and column oven CTO10ASVP was from Shimadzu, Kyoto, Japan. The analytical column used was BDS Hypersil $C_{18}$ 50 mm × 3.0 mm (5-μm, 100 Å) from Keystone Scientific (Bellefonte, PA, USA). A Phenomenex $C_{18}$ 4.0 mm × 3.0 mm was used as guard column. A Supeco 0.5-μm in-line prefilter was also used to prevent particles from entering the analytical column. The ionspray ionization-triple quadrupole mass spectrometer API 3000 made by Sciex (Concord, ON, Canada) was used for detection. Sciex sample control software version 1.4 and MacQuan version 1.6 with a power-Macintosh computer were used for data acquisition and data analysis, respectively.



## LC-MS/MS Conditions

The analytical column connected with a guard column and pre-filter, was used for sample analysis. The mobile phase used in the experiment was a mixture of acetonitrile (ACN)-water-formic acid at a ratio of 35 : 65 : 0.1(v/v). The flow rate of mobile phase was 0.500 mL/min. The column was operated at ambient temperature. The injection volume was 20 μL. A mixture of ACN-water-TFA at a ratio of 50 : 50 : 0.05 (v/v) was used as the injector wash solution. With the injector wash solution and rinse mode setting at 2, i.e., rinsing injection needle right after each injection, there was no detectable carryover.

The ionspray ionization in positive mode was used to generate molecular ions for mass spectrometric detection. The monitoring fragmentation and conditions for the analyte was optimized by infusing a ca. 0.2 μg/mL pure standard in 1 : 1 methanol–water containing 0.1% formic acid. The multiple reaction mode (MRM) was used to acquire the total ion counts at each time point. A high voltage of 4.5 kV was applied to the sprayer. The source temperature was 500°C. The auxiliary gas flow was 8 L/min. The settings of nebulizing gas, curtain gas, and collision gas flows at the instrument were 12, 9, and 6, respectively. All of the gas used in the experiment was high purity nitrogen ($\geq$ 99.99%) from AGA (Madison, WI, USA). Other optimized parameters were the orifice voltage 31 V, the ring voltage 130 V, Q0 offset −10 V, IQ1 −11 V, ST −16 V, RO1 −11 V, IQ2 −20 V, RO2 −22 V, ST3 −52 V, RO3 −24 V, and DF −400 V. The detection was by monitoring precursor → product transitions at $m/z$ 393 → 373 for dexamethasone, and at $m/z$ 409 → 391 for the internal standard. The dwell time was 500 milliseconds for dexamethasone and 200 milliseconds for the IS. In this assay, both Q1 and Q3 quadrupoles were set at unit resolution. For each injection, the total analysis time was 3 minutes.

Peak areas of chromatograms were integrated and the ratios of the analyte/IS were calculated. The linear regression with a weighted $1/\text{concentration}^2$ was used to obtain a calibration curve from standards and the regression equation of the calibration curve was then used to calculate the concentrations of quality control samples or practical clinical samples.

## Preparation of Standards and Quality Control (QC) Samples

Two separate stock standard solutions (0.500 mg/mL) of dexamethasone were prepared by dissolving the compound in methanol. One was used to make the calibration standards, and the other used for quality control (QC) samples. For the assay validation purpose, two stock standard solutions must agree within 5% in LC-MS/MS response. The internal standard working solution was made at 400 ng/mL in methanol. All stock standard solutions and working standard solutions were stored in a refrigerator with temperature maintained at 2–8°C. The stock standard solution of dexamethasone was confirmed to be stable for at least 3 months when stored in the refrigerator.

Eight calibration standard plasma pools at concentrations of 0.250, 0.500, 1.00, 5.00, 20.0, 100, 200, and 250 ng/mL were prepared by spiking the standard solutions into pooled control blank plasma. The QC samples were prepared using the standard solutions diluted from the second weighing stock solution. Regular QC samples included 0.750 (low-), 75.0 (medium-), and 180 (high-) ng/mL dexamethasone in plasma. An over-the-curve QC at 500 ng/mL and a lower limit of quantitation (LLOQ) QC at 0.250 ng/mL in plasma were also prepared. Each of the plasma standard pools and QC pools was split into pre-labeled 2-mL polypropylene vials (approximately 1.1 mL sample per vial) and stored at approximately −20°C.

## Extraction Procedure

An aliquot of 25.0 μL of the IS working solution (400 ng/mL) was added to each of pre-labeled glass tubes that already contained 0.500 mL of standard plasma samples or QC samples or practical samples. After briefly mixing, a volume of 0.10 mL 1 : 3 ammonium hydroxide-water (v/v) was added to the alkalinized sample. Then 3 mL of methyl-*t*-butyl ether (MTBE) was added to extract the analyte and the IS by vortexing for 3 minutes and centrifuging for 5 minutes at 3,000 rpm (ca. 1750 × g) and 15°C. The aqueous layer was frozen in a dry ice-acetone bath and the organic portion was transferred to clean conical glass tubes. The organic solvent was evaporated to complete dryness at 30°C under a stream of nitrogen at 10 psi in a Turbo-Vap water bath evaporator. The residues

were reconstituted in 0.200 mL of ACN–water–formic acid (30 : 70 : 0.1, v/v/v) by vortexing for 1 minute. After centrifugation at 3,000 rpm and 20°C for 3 minutes, the supernatants were transferred into injection vials. A 20-μL aliquot of extracted sample was injected onto the LC-MS/MS system.

### Method Validations

The assay validation experiment was designed following "*Guidance for Industry—Bioanalytical Method Validation*" recommended by Food and Drug Administration (FDA) of the United States.[31] The precision and accuracy of the assay were evaluated by running three validation batches on three separate days. Each batch had one set of calibration standards and six replicates of QC samples at low-, medium-, and high-concentration levels. One of the validation batches also included six replicates of over-the-curve QC samples and extra six replicates of diluted high- QC samples. Both over-the-curve and diluted high-QC samples used a partial volume of 0.100 mL, and then was treated with a 5-fold dilution by blank plasma prior to extraction. These partial volume QC samples were used to evaluate the dilution integrity. In one of the batches, six replicates of LLOQ QC samples were processed and analyzed as well. The short-term stability of the plasma samples was also established in one validation batch. The short-term stability included three freeze/thaw cycles and 24-hr room temperature bench-top stability.

To check the storage and injector stability of processed samples, one batch of the extracted samples was re-analyzed using the same system after being stored at room temperature (~22°C) for approximately 48 hours. The 3-month matrix frozen stability was evaluated using a calibration curve obtained from freshly prepared calibration standards to measure the QC plasma samples that had been stored at approximately −20°C for three months.

## RESULTS AND DISCUSSION

### Chromatographic Retention Mechanism and Optimization

As shown in Figure 2, the retention times of both dexamethasone and beclomethasone on the BDS Hypersil $C_{18}$ column decreased with the increase of the organic (ACN) content in the mobile phase. Both the analyte and the IS showed a typical reversed-phase retention mechanism. When a mobile phase contained 45% or higher ACN, the analyte and IS had almost the same, but minimal retention. A weaker mobile phase containing less than 30% ACN drastically increased the retention times for both compounds and made this assay of less

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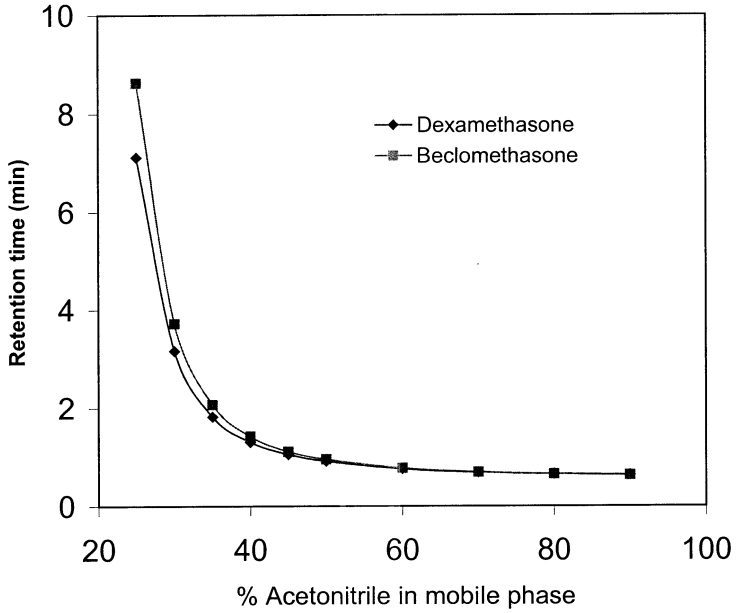


***Figure 2.*** Retention time vs. % acetonitrile in the mobile phase.



practical interest. The mobile phase containing between 30% and 40% ACN showed a 1.3 to 3.5 min retention time and both compounds had similar behavior on the analytical column. Thus, the solution consisting of ACN–water–formic acid 35 : 65 : 0.1 (v/v) was a reasonable choice as the mobile phase for this assay.

The importance of the injection solution composition has been realized and discussed by our research group.[32] An optimized injection solution can improve not only peak shape but also sensitivity. Figure 3 showed the influence of the injection solution composition on the peak shape and detection sensitivity of dexamethasone and beclomethasone. Peaks A, B, C, D, and E were obtained by injecting 5 μL of the same concentration (400 ng/mL for both compounds) with different composition solutions, under the same experimental conditions. If using $\chi$ represented the percentage of ACN in an injection solution, the injection solutions A, B, C, D, and E were corresponded to $\chi = 5\%$, 30%, 35%, 70%, and 90% ACN in a mixture of ACN–water–formic acid at a volume ratio $\chi : (100 - \chi) : 0.1$, respectively. The solution C (35% ACN) was the same as the mobile phase.

Based upon reversed-phase principle, the solutions A and B, containing more water, were "weaker" than the used mobile phase. The solutions D and E,

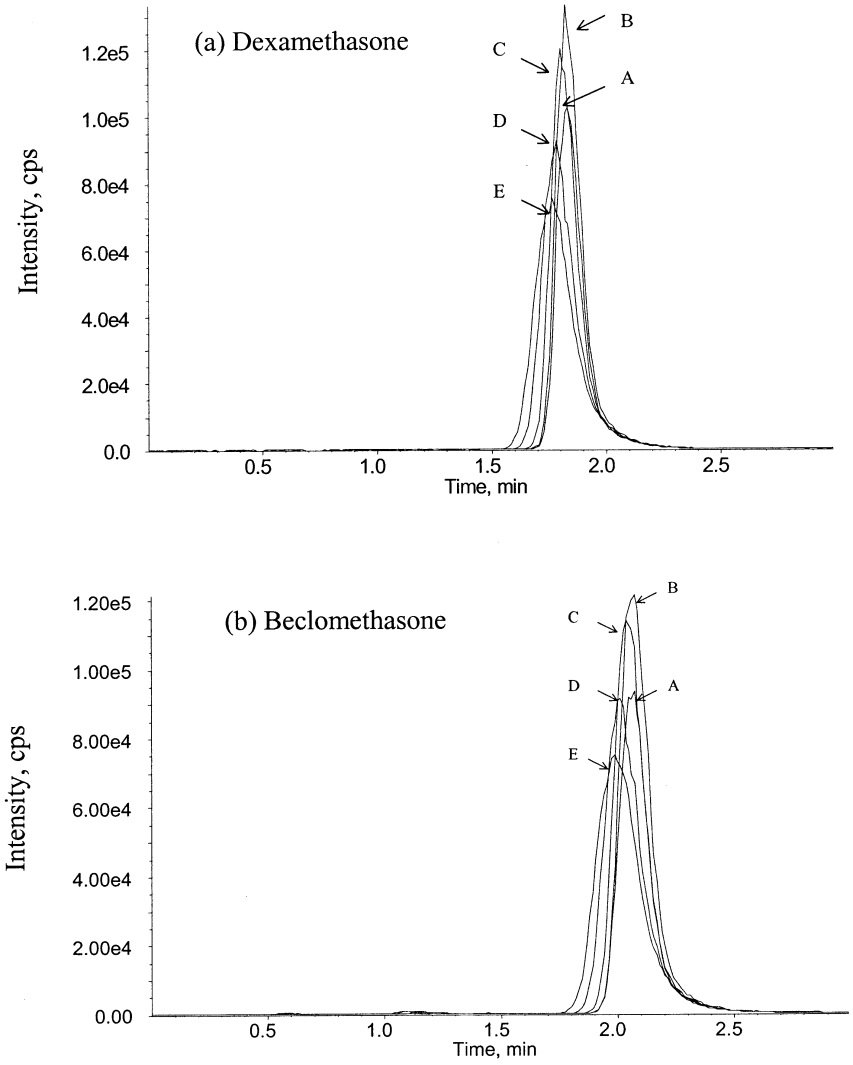


***Figure 3.*** Effects of the injection solution composition (a) dexamethasone, and (b) beclomethasone. The concentration was 400 ng/mL for both compounds. The injection volume was 5 microliters. The injection solutions A, B, C, D, and E corresponded to $\chi = 5\%$, 30%, 35%, 70%, and 90% ACN in the mixture of ACN–water–formic acid at a volume ratio $\chi : (100 - \chi) : 0.1$, respectively.

contained more ACN, and were "stronger" than the mobile phase. With the decrease of % ACN in injection solution, the retention times showed a slight increase for both compounds even when only a small volume (5 μL) was injected. It illustrated that a weaker injection solution tended to keep the compounds on the column longer. As shown in Figure 3, the 90% ACN solution (E) gave a broader peak, but only about 60% of the peak height compared with the one obtained from the 30% ACN solution (B). The 70% ACN solution (D) had about 70% of the peak height of the 30% ACN solution. The 30% ACN solution, a slightly weaker solution than the mobile phase, produced a sharper peak with an absolute peak-height response ~5% higher than that of the solution composition, same as the mobile phase. The 5% ACN solution, a much weaker solution, gave less sensitivity even though the peaks were narrow. This was probably because the compounds had relatively poor solubility in a 95% water solution. Thus, in the current assay, a mixture of 30 : 70 : 0.1 ACN–water–formic acid (v/v), was chosen as the reconstitution solvent to achieve the best peak shape and highest sensitivity.

Under the above optimized chromatographic conditions, the retention times of dexamethasone and IS were approximately 1.8 minutes and 2.0 minutes, respectively. Figure 4 showed representative mass chromatograms of extracts from the human plasma blank, and control 0, a lower limit of quantitation (LLOQ, 0.250 ng/mL) sample, and an upper limit of quantitation (ULOQ, 250 ng/mL) sample obtained on a BDS Hypersil $C_{18}$ 50 mm × 3 mm (5-μm) column.



## Specificity and Matrix Effect

Six lots of blank plasma were tested for matrix effects and the assay specificity. For each lot of plasma, a plasma blank (free of both analyte and IS), control 0 (plasma blank spiked with IS only), control 0.250 (spiked with 0.250 ng/mL analyte), and control 20.0 (spiked with 20.0 ng/mL analyte) were used to check interference and lot-to-lot matrix variation. The measured values and statistics are given in Table 1. For all of the six lots plasma, the regions of the analyte and the IS peaks were found to be free of interference. When these blank plasmas were spiked with dexamethasone at 0.250 ng/mL, the measured mean, relative standard deviation (RSD), and relative error (RE), were 0.269 ng/mL, 2.0%, and 7.6%, respectively. For the blank plasma spiked with 20.0 ng/mL of dexamethasone, the measured mean, RSD, and RE were 19.5 ng/mL, 7.3% RSD, and −2.5% RE, respectively. The results demonstrated that the tested plasma had no significant lot-to-lot matrix variation. In addition, matrix effect was tested by comparing post-extraction spiked samples to the pure solutions of the same concentrations. No substantial matrix suppression (less than 5%) was observed for both the analyte and the IS.

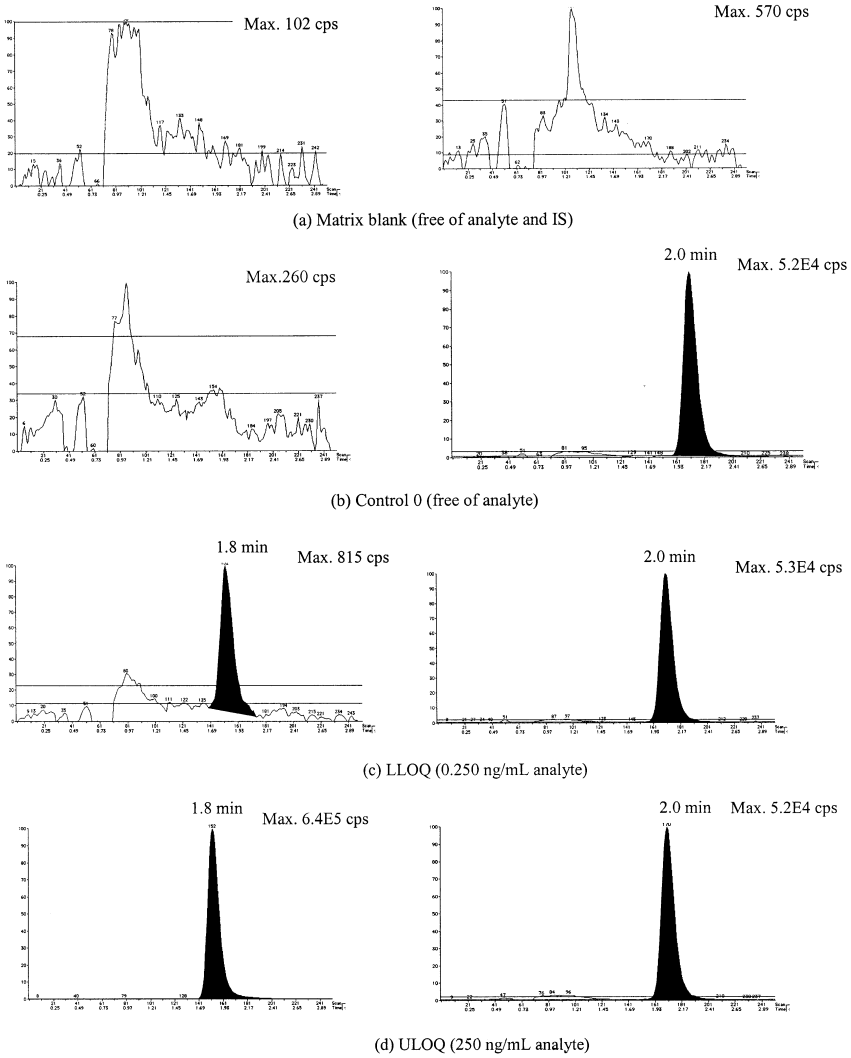


***Figure 4.*** Mass chromatograms of the extracted samples. (a) control plasma blank, (b) control 0 (blank plasma spiked IS only), (c) LLOQ (0.250 ng/mL dexamethasone in plasma), and (d) ULOQ (250 ng/mL dexamethasone in plasma) (left panel – dexamethasone; right panel – beclomethasone).

***Table 1.*** Measured Dexamethasone Concentrations in Spiked Individual Lot Plasma

| | Measured Plasma Concentration (ng/mL) | |
|---|---|---|
| Lot No. | Spiked 0.250 ng/mL | Spiked 20.0 ng/mL |
| 1 | 0.265 | 19.8 |
| 2 | 0.270 | 18.1 |
| 3 | 0.275 | 22.0 |
| 4 | 0.262 | 18.2 |
| 5 | 0.275 | 19.4 |
| 6 | 0.266 | 19.2 |
| Mean (ng/mL) | 0.269 | 19.5 |
| RSD (%) | 2.0 | 7.3 |
| RE (%) | 7.6 | −2.5 |



## Linearity, Sensitivity and Extraction Efficiency

The standard curve range was 0.250 to 250 ng/mL for dexamethasone calculated based on 0.5-mL plasma. This validated curve had a lower limit of quantitation (LLOQ) of 0.250 ng/mL in plasma that gave a signal-to-noise (S/N) ratio of greater 20. Over the above range, the linear correlation coefficients were found to be greater than or equal to 0.9972. The results were given in Table 2. MTBE was chosen as the extraction solvent. The tests showed that the addition of some ammonium hydroxide to the plasma, prior to the extraction, produced a significant improvement of extraction efficiency (ca. 50% higher than that without ammonium hydroxide, data not shown). Although the compounds are neutral, basic condition might help to breakdown drug-protein binding. The recovery was determined by comparing the peak areas of the analyte and IS extracted from plasma with those of post-extraction spiked plasma blanks at corresponding concentrations. As shown in Table 3, the average recoveries were 88–91% for dexamethasone at three concentration levels and 84.5% for the internal standard beclomethasone, respectively.

## Precision and Accuracy

As shown in Table 2, for all three validation curves, the back calculation results for all calibration standards showed $\leq 5.0\%$ RSD and $-5.3$ to 6.9% RE, respectively. The precision and accuracy for quality control samples are given in Table 4. For the low-concentration (0.750 ng/mL) level QC samples, the

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***Table 2.*** Calibration Curve Data for Dexamethasone in Plasma (n = 3)

| Theoretical (ng/mL) | Dexamethasone in Plasma (ng/mL) | | | | | | | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | 0.250 | 0.500 | 1.00 | 5.00 | 20.0 | 100 | 200 | 250 | |
| Day 1 | 0.247 | 0.501 | 1.03 | 5.16 | 20.3 | 102 | 191 | 241 | 0.9995 |
| Day 2 | 0.252 | 0.494 | 0.970 | 5.65 | 20.5 | 93.2 | 184 | 256 | 0.9972 |
| Day 3 | 0.250 | 0.490 | 1.02 | 5.22 | 20.9 | 98.2 | 193 | 239 | 0.9992 |
| Mean (ng/mL) | 0.250 | 0.495 | 1.01 | 5.34 | 20.6 | 97.8 | 189 | 245 | – |
| RSD (%) | 1.0 | 1.1 | 3.2 | 5.0 | 1.5 | 4.5 | 2.5 | 3.8 | – |
| RE (%) | 0.0 | −1.0 | 0.6 | 6.9 | 2.8 | −2.2 | −5.3 | −1.9 | – |

***Table 3.*** Recovery Data

| Compound | Recovery % (mean ± RSD) |
|---|---|
| Dexamethasone (n = 6) | |
| 0.750 ng/mL | 88.1 ± 6.6 |
| 75.0 ng/mL | 90.6 ± 5.6 |
| 180 ng/mL | 88.6 ± 7.2 |
| Beclomethasone (n = 18) | |
| 20.0 ng/mL | 84.5 ± 12.0 |

precision and accuracy were 4.0% RSD and −3.0% RE for intra-day assays (n = 6), and 5.1% RSD and −0.3% RE for inter-day assays (n = 18), respectively. For the medium-concentration level QC samples (75.0 ng/mL), the precision and accuracy were 4.8% RSD and −3.6% RE for intra-day assays (n = 6), and 4.7% RSD and −2.2% RE for inter-day assays (n = 18), respectively. For the high-concentration level QC samples, i.e., 180 ng/mL, the precision and accuracy were found to be 1.4% RSD and −7.6% RE for intra-day assays (n = 6), and 2.6% RSD and −6.9% RE for inter-day assays (n = 18), respectively. The intra-day precision and accuracy were 2.3% RSD and 1.1% RE for of LLOQ QC sample. These results demonstrated excellent precision and accuracy.



***Table 4.*** Intra-day and Inter-day Precision and Accuracy of Quality Control Samples

| Compound | Dexamethasone in Plasma (ng/mL) | | | | | |
|---|---|---|---|---|---|---|
| QC Sample | LLOQ 0.250 | Low- 0.750 | Medium- 75.0 | High- 180 | High-* 180 | Over-the-Curve* 500 |
| Intraday (n = 6) | | | | | | |
| Mean (ng/mL) | 0.253 | 0.728 | 72.3 | 166 | 176 | 528 |
| RSD (%) | 2.3 | 4.0 | 4.8 | 1.4 | 3.7 | 2.1 |
| RE (%) | 1.1 | −3.0 | −3.6 | −7.6 | −2.3 | 5.6 |
| Interday (n = 18) | | | | | | |
| Mean (ng/mL) | – | 0.747 | 73.4 | 168 | – | – |
| RSD (%) | – | 5.1 | 4.7 | 2.6 | – | – |
| RE (%) | – | −0.3 | −2.2 | −6.9 | – | – |

*Five-fold dilution QC samples.

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### Dilution Integrity, Stability, and Method Ruggedness

A 5-fold dilution for over-the-curve QC sample and high concentration QC sample by matrix blank, prior to extraction, was used to evaluate dilution integrity. Six replicates of partial volume over-the-curve QC and high QC samples were processed in one of the validation batches. The data were also included in Table 4. The results showed that taking the partial volume and diluting with matrix blank did not give significant deviation for the analytical data.

The stability experiments aimed at testing all possible conditions that the practical samples might experience during the sample shipping and handling, such as freezing/thawing, and a short storage at room temperature (bench-top), and during analysis such as extracted samples sitting in sample tray or refrigerator. These were performed as described in the method validation section. All stability results are summarized in Table 5. Three freeze/thaw cycles and 24-hr room temperature storage for QC samples had no substantial effect on the

***Table 5.*** Stability Data

| Compound | Dexamethasone in Plasma (ng/mL) | | |
|---|---|---|---|
| Nominal Concentration (ng/mL) | 0.750 | 75.0 | 180 |
| 3 freeze/thaw cycles (n = 6) | | | |
| Mean (ng/mL) | 0.754 | 75.6 | 169 |
| RSD (%) | 8.9 | 4.4 | 4.4 |
| RE (%) | 0.6 | 0.8 | −6.1 |
| Bench-top ambient ~24 hrs (n = 6) | | | |
| Mean (ng/mL) | 0.701 | 73.4 | 172 |
| RSD (%) | 7.0 | 5.8 | 9.5 |
| RE (%) | −6.5 | −2.2 | −4.4 |
| Extracts stored ambient ~48 hrs (n = 6) | | | |
| Mean (ng/mL) | 0.749 | 72.8 | 167 |
| RSD (%) | 6.1 | 4.9 | 2.5 |
| RE (%) | −0.1 | −2.9 | −7.2 |
| 3-month matrix storage at −20°C (n = 3) | | | |
| Mean (ng/mL) | 0.781 | 86.5 | 193 |
| RSD (%) | 4.2 | 1.2 | 1.1 |
| RE (%) | 4.2 | 15 | 7.4 |

results. Keeping extracts at room temperature for up to 48 hrs did not affect the quantitative determination of dexamethasone in sample.

The three-month storage stability of quality control samples had also been tested and the data were included in Table 5. The results showed a smaller than 4.2% RSD ($n = 3$ for each level QC) and $\leq 15\%$ RE which met the acceptance criteria, indicating that the QC samples were stable for at least three months if stored frozen at approximately $-20^{\circ}C$.

One set of the validation samples, including calibration standards and regular QC samples, were re-analyzed on another LC-MS/MS system using another analytical column. For the data obtained from the original system, the standard curve had a correlation coefficient ($r^2$) of 0.9972 and QC sample results showed $\leq 3.7\%$ RSD ($n = 6$) and $-8.5\%$ to 0.1% RE. The data obtained from another system showed a standard curve with $r^2 = 0.9977$ and QC data with $\leq 4.6\%$ RSD ($n = 6$) and $-7.2\%$ to 1.5% RE. The results obtained from two separate systems were comparable and both met the acceptance criteria suggested by FDA guidance[31] ($\leq 15\%$ RSD and $\leq 15\%$ RE). In addition, no significant back-pressure increase or other evidences on column degradation were observed even after one thousand injections onto a single analytical column.



## CONCLUSIONS

A simple and rapid LC-MS/MS method for the determination of dexamethasone in human plasma has been developed and validated. This method used a simple one-step liquid-liquid extraction procedure and a $C_{18}$ column coupled with MS/MS for separation and detection. The validated assay used 0.5-mL plasma sample and the standard curve range was 0.250 to 250 ng/mL in human plasma, which covered the concentration range in clinical study samples. The method has been successfully transferred to another facility and has been applied to support the pharmacokinetic studies and clinical trials.

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