Determination of dexamethasone and dexamethasone sodium phosphate in human plasma and cochlear perilymph by liquid chromatography/tandem mass spectrometry

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ARTICLE INFO

ABSTRACT

A rapid, simple and sensitive liquid chromatography/tandem mass spectrometry (LC–MS/MS) assay was developed for the determination of dexamethasone (Dex) and dexamethasone sodium phosphate (Dex SP) in plasma and human cochlear perilymph. After proteins were precipitated with a mixture of acetonitrile and methanol, Dex, Dex SP and flumethasone, the internal standard, were resolved on a C18 column using gradient elution of 5 mM ammonium acetate and methanol. The three compounds were detected using electrospray ionisation in the positive mode. Standard curves were linear over the concentration range 0.5–500 μg/L ($r > 0.99$), bias was $< \pm 10\%$, intra- and inter-day coefficients of variation (imprecision) were $<10\%$, and the limit of quantification was 0.5 μg/L for both Dex and Dex SP. The assay has been used successfully in a clinical pharmacokinetics study of Dex and Dex SP in cochlear perilymph and plasma.

1. Introduction

Systemic administration of glucocorticoids has been the traditional method of treatment of a number of inner ear conditions including autoimmune inner ear disease, acute postmeningitic labyrinthitis, idiopathic sudden sensorineural hearing loss, and Cogan's syndrome. Recently intratympanic (IT) delivery of glucocorticoids has become an accepted approach for treatment of some of these conditions. The advantages of IT administration include reducing systemic drug concentrations and side effects, and delivering higher doses to the inner ear when compared with systemic administration [1]. Dexamethasone (Dex) is a glucocorticoid that is used for IT delivery, and because of low water-solubility administered in the form of the water-soluble ester prodrug dexamethasone sodium phosphate (Dex SP). This prodrug is hydrolysed rapidly by phosphatases to its active form free Dex [2]. To date, no study has been published looking at the concentrations of Dex and Dex SP in cochlear perilymph and plasma after IT treatment versus IV treatment in humans.

Pharmacokinetic studies of Dex and Dex SP in cochlear perilymph and plasma require a highly sensitive method to analyse the concentrations of Dex and Dex SP because of the extremely small volume of cochlear perilymph samples (~20 μl) available from patients during cochlear implantation, and the low Dex plasma concentration from the low therapeutic dose given by IT route. HPLC with tandem mass spectrometric detection (LC–MS/MS) has been demonstrated to provide high sensitivity for the quantitative determination of drugs and metabolites in biological fluids. Some LC–MS/MS methods for measuring Dex in biological samples have been reported [3–6], in which a relatively large volume of sample was required (>200 μl). For Dex SP analysis, there is one published HPLC–UV–MS method that has been used to report the analysis of Dex SP in cochlear perilymph of guinea pigs [7], in which HPLC with UV detection was used for Dex SP quantification and MS for Dex SP identification. The limit of detection was 100 μg/L. Two LC–MS/MS methods have been reported for the simultaneous determination of Dex and Dex SP in plasma or connective tissue for pharmacokinetics studies [8,9]. The method for plasma [8] required 100 μl of plasma, and protein precipitation by acetonitrile was used for sample preparation. The method for connective tissue [9] required 50 mg of tissue, which was homogenized with water. After centrifugation, the supernatant was filtered and the filtrate was injected into LC–MS/MS. Both methods were sensitive (0.5 ng/ml in plasma and 1 ng/g in connective tissue) but details of the methodology and validation were lacking.

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The aim in this work was to develop and validate a suitable LC–MS/MS method for a pharmacokinetic study of Dex and Dex SP in human cochlear perilymph and plasma, during cochlear implantation.

2. Experimental

2.1. Materials

Dex, Dex SP, flumethasone (FLU) (Fig. 1) and ammonium acetate were purchased from Sigma Co. (Australia). HPLC grade acetonitrile, methanol, and perchloric acid were purchased from BDH (Poole, UK). Distilled, deionised water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). The human plasma used as the assay blank and for the preparation of standards was obtained from New Zealand Blood Services (Christchurch, New Zealand).

2.2. Instrumentation and analytical conditions

The LC–MS/MS system consisted of a Shimadzu LC-20AD HPLC system (Shimadzu Corporation, Kyoto, Japan) interfaced with an API 4000[TM] triple quadrupole mass spectrometer (Applied Biosystems, Foster City, Canada) equipped with a TurboIonSpray® source. Analyst software (Applied Biosystems, Foster City, Canada) was used to control equipment, to coordinate data acquisition, and to analyse data. Dex, Dex SP and the internal standard FLU were separated under gradient elution using a Luna C18 5 μm, 50 mm × 2.0 mm internal diameter analytical column equipped with a C18 4.0 mm × 2.0 mm internal diameter guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of solvent A (5 mM ammonium acetate) and solvent B (methanol). The flow rate was set at 0.3 ml/min. The initial condition was 60% solvent A and 40% solvent B. A linear gradient was performed with mobile phase B increasing from 40% to 90% within 1 min. After 3 min, the mobile phase was returned to the initial condition and re-equilibrated for 3 min. The total analysis time was 6 min.

The mass spectrometer was operated in the positive ion mode with curtain gas, Gas 1 and Gas 2 flow rates of 20, 45 and 60 psi, respectively. The ion spray voltage was 5000 V and the source temperature was 500 °C. Data acquisition was performed via multiple reaction monitoring (MRM). The optimized precursor-to-product ion transitions monitored for Dex [M+H]$^+$, Dex SP [M+3H−2Na]$^+$ and FLU [M+H]$^+$ were m/z 393 → 373 with declustering potential (DP) 66 V and collision energy (CE) 13 V, m/z 473 → 435 with DP 61 V and CE 17 V, and m/z 411 → 253 with DP 81 V and CE 23 V, respectively.

2.3. Standards

The stock solutions of Dex and Dex SP (both 1.0 mg/ml as free base) were prepared by dissolving 10 mg of the respective standards in 10 ml of methanol. Two sets of the same standard stock solutions were prepared for standard curves and for quality control (QC) samples respectively. The intermediate standard solution of Dex and Dex SP (20 μg/ml of Dex and Dex SP) was prepared by diluting 200 μl of Dex stock solution and 200 μl of Dex SP stock solution to 10 ml of methanol in a volumetric flask. The stock internal standard FLU solution (1.0 mg/L) was prepared by dissolving 10 mg of FLU in 10 ml of methanol. A working solution of the internal standard (0.05 μg/ml) was prepared by diluting the stock solution with water.

2.3.1. Plasma assay

The standard curves of Dex and Dex SP were constructed by spiking drug-free human plasma with the intermediate standard solution of Dex and Dex SP, giving a calibration range of 0.5–500 μg/L for both Dex and Dex SP. Dex and Dex SP plasma quality control (QC) standards were prepared in single 5 ml aliquots in concentrations of 0.5, 10, 100 and 500 μg/L and stored at −30 °C until analysed.

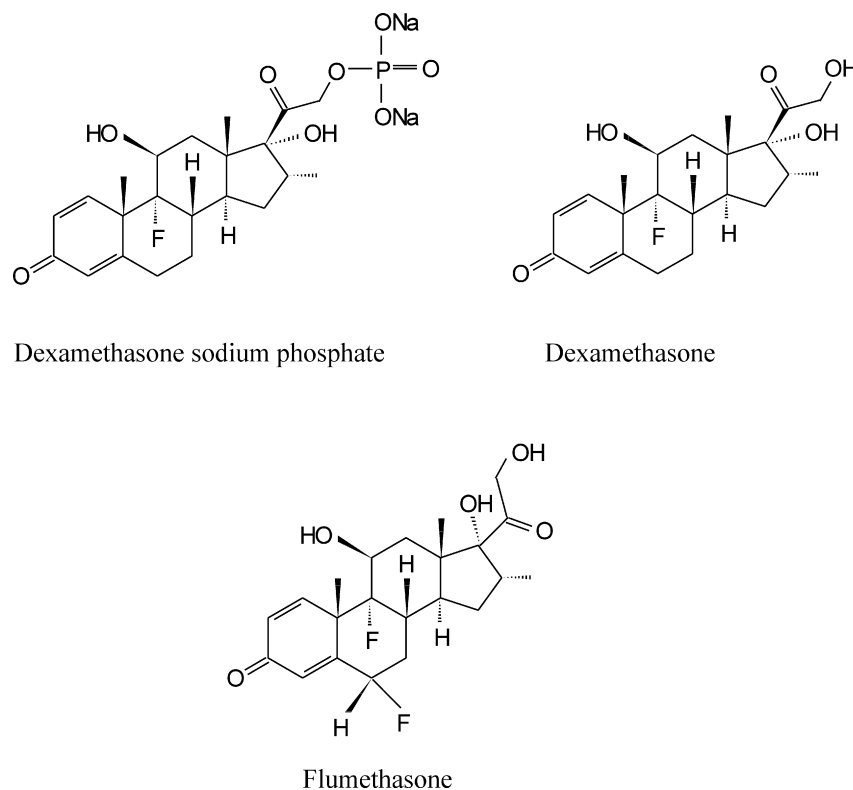### 2.3.2. Cochlear perilymph assay

Because cochlear perilymph has very limited availability (i.e. only sample at the time of the surgical procedure), proxy perilymph [the filtrate of plasma prepared by ultrafiltration (2600 × g for 30 min at 37 °C) using a Diaflo® ultrafiltration membrane, YMT DISCS, 30K NMWL, 14 mm (Amicon Inc., Beverly, USA)] was used to substitute for the blank perilymph for the preparation of standard curves and QCs. The perilymph standard curves of Dex and Dex SP were constructed by spiking proxy perilymph with the intermediate standard solution of Dex and Dex SP, giving the same calibration range as that of the plasma standard curves. Dex and Dex SP proxy perilymph QC standards were prepared in single 5 ml aliquots in the same concentrations as that of plasma QCs, and stored at −30 °C until analysed.

2.4. Sample preparation

The same sample preparation procedure was used for plasma and perilymph samples. FLU, 50 μl of 0.05 μg/ml, was added to 50 μl of each of blank, standard, QC or patient samples. The mixture was vortexed briefly. After adding 25 μl of 1.0 M perchloric acid to acidify the sample, 200 μl of the mixture of methanol and acetonitrile (1:4) was added to precipitate the proteins. After centrifugation at 15,000 × g for 5 min, a 50 μl aliquot of clear supernatant was mixed with 200 μl of mobile phase (5 mM ammonium acetate and methanol, 50:50) and transferred to the autosampler 96 well plate. A volume of 10 μl was injected into the LC–MS/MS system. Because of the limited availability of cochlear perilymph from patients, the volumes of all the cochlear perilymph samples were ≤20 μl. The volume of patient cochlear perilymph sample was made up to 50 μl with proxy perilymph before sample preparation. The original volume of patient cochlear perilymph sample was noted and the original concentrations of Dex and Dex SP in cochlear perilymph were determined by backcalculating dilution factors.

2.5. Validation

The standard curves were the plot of the peak area ratios (analyte/internal standard) of Dex and Dex SP versus the corresponding concentrations of Dex and Dex SP. The linearity of the standard curves was evaluated by preparing at least 6 calibration curves on different days and determining the correlation coefficient of the curves. To evaluate the assay recoveries and matrix effects, three sets of standards were prepared using a modification of the method of Matuszewski et al. [10] for both Dex and Dex SP at concentrations of 0.5, 10, 100 and 500 μg/L, and FLU at 0.05 μg/ml, the concentration used in the assay. For the plasma assay, the first set was prepared in plasma from six different sources, the second set in the extracts of plasma after-protein precipitation of blank plasma from the same six different sources as in first set, and the third set in mobile phase. Every set included six samples at each concentration. Absolute recoveries at each concentration were measured by comparing the peak areas of Dex, Dex SP and FLU in plasma to those in the spiked after-protein precipitation blank plasma extracts at the corresponding concentrations (n = 6) [Absolute recovery = (peak area of analyte from the spiked plasma sample)/(peak area of analyte from the spiked after-protein precipitation blank plasma extract sample) × 100%]. The matrix effects were assessed by comparing the peak areas of Dex, Dex SP and FLU from the spiked after-protein precipitation blank plasma extracts

Dexamethasone sodium phosphate

Dexamethasone

Flumethasone

Fig. 1. Chemical structures of dexamethasone sodium phosphate, dexamethasone and flumethasone.

with the response of standard solution at the same concentration in the mobile phase ($n = 6$). For cochlear perilymph assay, only the second and third sets of standards were prepared for the matrix effect evaluation. The procedure was the same as for plasma assay but using proxy perilymph instead of plasma. The proxy perilymph was comparatively clear and no protein precipitation was observed after adding extraction solution (25 μl of 1.0 M perchloric acid and 200 μl of the 4:1 mixture of acetonitrile and methanol). Therefore, there was no difference between the prepared proxy perilymph standard samples and the spiked proxy perilymph extract samples and it was not necessary to evaluate the recoveries of Dex, Dex SP and FLU from proxy perilymph. Quality control was assessed by the analysis of six samples at each concentration on the same day (intra-day) and of one sample at each concentration on six different days (inter-day). Bias was determined as the measured minus the actual concentration, expressed as a percentage of the actual concentration. Imprecision was measured as intra- and inter-day coefficients of variation. The lowest concentration for both Dex and Dex SP standard curves was considered to be the lower limit of quantitation (LLOQ), at which the concentration of Dex and Dex SP could be determined with acceptable accuracy and precision. According to the US Food and Drug Administration guidance for bioanalytical method validation [11], the mean value determined at LLOQ should not deviate by more than 20% of the actual value, and the precision determined at LLOQ should not exceed 20% of the coefficients of variation (CV). The limit of detection (LOD) was defined as the lowest concentration that can be detected in a sample but not necessarily quantified. The LOD was calculated as 3 times the response compared to blank response.

The effects of freezing and thawing on the concentrations of Dex and Dex SP were studied using QC samples at 0.5, 10, 100 and 500 μg/L, which were subjected to four freeze–thaw cycles before analysis. The stability of QC samples at −30 °C was evaluated by concentration analysis at monthly intervals for ten months. The stability of the stock standard solutions of Dex and Dex SP at 4 °C

for 10 months was evaluated by comparing the response with that of the freshly prepared standard solutions. The stability of the processed samples at 4 °C (the temperature of the autosampler) for two days was evaluated by comparing the results with the original results. In all cases, Dex and Dex SP were considered to be stable as long as degradation was <10% of the concentration at day 0.

3. Results and discussion

3.1. Mass spectrometry and chromatography

The MS/MS parameters were optimized to produce maximum responses for Dex, Dex SP and the internal standard FLU using electrospray ionisation in the positive ion mode. The protonated molecular ions $[M+H]^+$ were m/z 393 for Dex and m/z 411 for FLU. The transitions yielding the most abundant product ions were $393 \rightarrow 373$ for Dex and $411 \rightarrow 253$ for FLU. For Dex SP, the mass spectrum contains 3 signals at m/z 517 $[M+H]^+$, m/z 495 $[M+2H-Na]^+$ and m/z 473 $[M+3H-2Na]^+$. Comparison of all the product ion abundance for the ions at m/z 517, m/z 495 and m/z 473 showed that the precursor–product ion transition m/z 473 $\rightarrow$ 435 offered the greatest potential for use in quantitative analysis of Dex SP. The product ion spectra of $[M+3H-2Na]^+$ for Dex SP and $[M+H]^+$ for Dex and FLU are shown in Fig. 2.

To achieve optimized chromatographic resolution, peak sharpness and signal intensity, a variety of LC analytical columns, including Phenomenex Gemini C6-Phenyl and Luna C18 and C8 columns, and mobile phase compositions, including 5 mM ammonium acetate, 0.05% formic acid and methanol, were evaluated. Both Phenomenex Luna C18 and C8 columns gave the best chromatographic resolution and sharp peaks. The mobile phase consisting of 5 mM ammonium acetate and methanol gave higher signal intensity than the mobile phase consisting of 0.05% formic acid and methanol. Changes in percentage of methanol in the mobile phase were found to have a profound influence on reten-
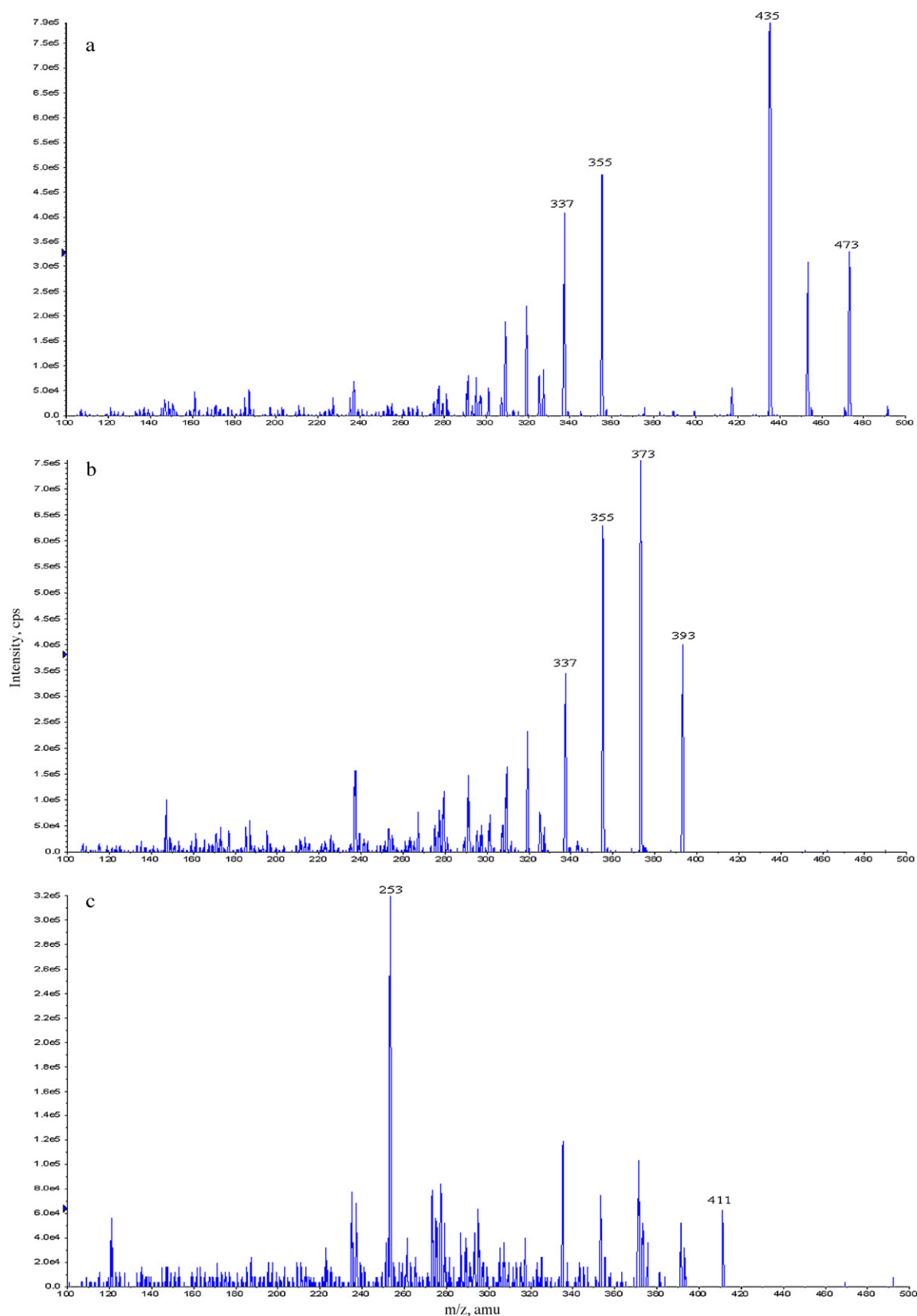
Fig. 2. Product ion mass spectra of (a) dexamethasone sodium phosphate [M+3H−2Na]⁺, (b) dexamethasone [M+H]⁺ and (c) flumethasone [M+H]⁺.

tion time and peak shape. With higher percentage of methanol, the retention time decreased and the peak shape improved. The optimized LC condition chosen was therefore a mobile phase consisting of 5 mM ammonium acetate and methanol with gradient

elution on a Phenomenex Luna C18 column. Under these conditions, the retention times were approximately 2.79, 2.93, and 3.21 min for Dex, Dex SP and the internal standard FLU, respectively (Figs. 3 and 4). Blank plasma samples and proxy perilymph sam-
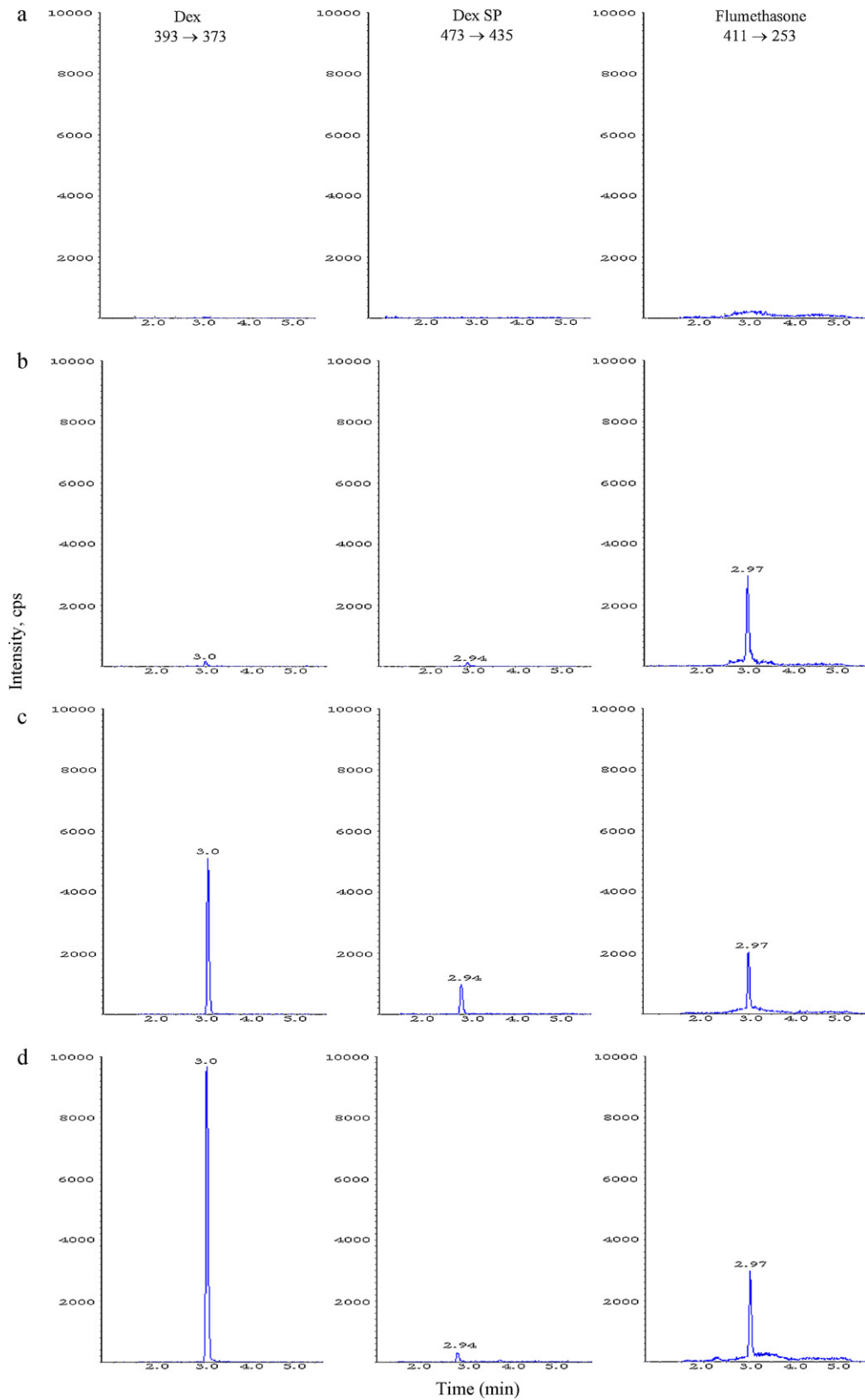
Fig. 3. Representative MRM chromatograms of (a) blank plasma, (b) plasma sample spiked with dexamethasone (Dex) and dexamethasone sodium phosphate (Dex SP) at 0.5 μg/L, (c) plasma sample spiked with Dex and Dex SP at 50 μg/L and (d) plasma sample from a patient after intravenous administration of Dex SP (Dex concentration = 66 μg/L and Dex SP concentration = 17 μg/L). In all cases, except blank plasma, the internal standard flumethasone (FLU) concentration was 0.025 μg/L.

ples from more than six different sources of the same matrix were tested for interference, and Dex, Dex SP and the internal standard peaks were free of interference from any other peaks present in the blanks (Figs. 3 and 4).

3.2. Sample preparation

Protein precipitation is the simplest and most rapid method of plasma and perilymph sample preparation for the measure-
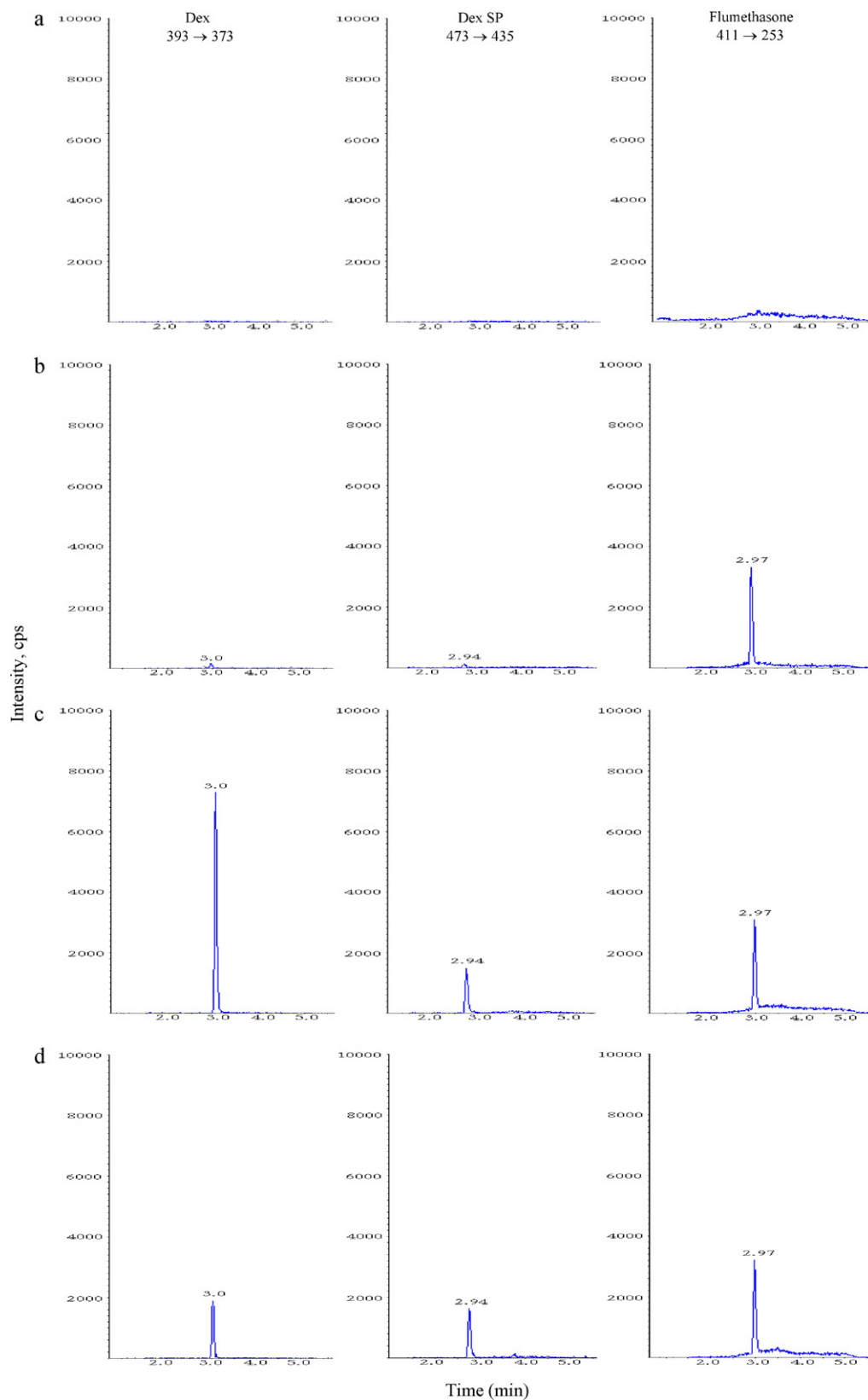
Fig. 4. Representative MRM chromatograms of (a) blank proxy perilymph, (b) proxy perilymph sample spiked with dexamethasone (Dex) and dexamethasone sodium phosphate (Dex SP) at 0.5 µg/L, (c) proxy perilymph sample spiked with Dex and Dex SP at 50 µg/L and (d) cochlear perilymph sample from a patient after intratympanic administration of Dex SP (Dex concentration = 12.8 µg/L and Dex SP concentration = 55.9 µg/L). In all cases, except blank proxy perilymph, the internal standard flumethasone (FLU) concentration was 0.025 µg/L.

Table 1
Intra-day assay variance of the determination of dexamethasone and dexamethasone sodium phosphate in plasma ($n = 6$).

Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± SD)	Bias (%)	Imprecision CV (%)
Dexamethasone				
LLOQ	0.5	0.53 ± 0.01	−6.0	1.9
QC1	10	9.3 ± 0.34	−7.0	3.7
QC2	100	103 ± 4.1	3.0	4.0
QC3	500	494 ± 11.0	1.2	2.2
Dexamethasone sodium phosphate				
LLOQ	0.5	0.48 ± 0.04	−4.0	8.3
QC1	10	10.8 ± 0.93	8.0	8.6
QC2	100	102 ± 4.3	2.0	4.2
QC3	500	509 ± 6.0	1.8	1.2

Table 2
Inter-day assay variance of the determination of dexamethasone and dexamethasone sodium phosphate in plasma ($n = 6$).

Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± SD)	Bias (%)	Imprecision CV (%)
Dexamethasone				
LLOQ	0.5	0.52 ± 0.05	−4.0	9.7
QC1	10	10.1 ± 0.52	1.0	5.1
QC2	100	101 ± 3.7	1.4	3.7
QC3	500	504 ± 12.4	0.87	2.5
Dexamethasone sodium phosphate				
LLOQ	0.5	0.54 ± 0.02	8.0	4.4
QC1	10	10.5 ± 0.53	5.0	5.1
QC2	100	99.8 ± 2.8	−0.2	2.8
QC3	500	490 ± 16.8	−2.0	3.4

Table 3
Intra-day assay variance of the determination of dexamethasone and dexamethasone sodium phosphate in perilymph ($n = 6$).

Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± SD)	Bias (%)	Imprecision CV (%)
Dexamethasone				
LLOQ	0.5	0.51 ± 0.03	2.0	5.7
QC1	10	9.8 ± 0.38	−2.0	3.9
QC2	100	101 ± 3.3	1.0	3.3
QC3	500	520 ± 13.8	4.0	2.7
Dexamethasone sodium phosphate				
LLOQ	0.5	0.47 ± 0.05	−6.0	9.8
QC1	10	10.1 ± 0.16	1.0	1.6
QC2	100	103 ± 4.4	3.0	4.3
QC3	500	527 ± 19.0	5.4	3.6

ment of drug concentrations. To find the most efficient precipitant for sample preparation, three widely used precipitating agents (acetonitrile, methanol and perchloric acid) were compared. Precipitation with acetonitrile or perchloric acid gave better sample clean-up, but the recoveries for Dex, Dex SP and the internal standard FLU were lower. Precipitation with methanol gave high extraction efficiency but the sample clean-up was less efficient and the supernatant was not very clear. Acidifying plasma samples with a small volume of 1.0 M perchloric acid and then extracting with a mixture of acetonitrile and methanol (4:1) provided the cleanest samples and the highest recoveries for Dex, Dex SP and the internal standard FLU.

3.3. Method validation

Standard curves of Dex and Dex SP were linear ($r > 0.999$) for both plasma and perilymph over the concentration range of 0.5–500 μg/L. The intercept with the y-axis was not significantly different from zero. The mean linear regression equations of standard curves for plasma samples were $y = 0.044x + 0.055$ for Dex and $y = 0.019x + 0.032$ for Dex SP. The mean linear regression equations of standard curves for perilymph samples were $y = 0.040x + 0.035$ for Dex and $y = 0.010x + 0.041$ for Dex SP, where y represents the ratio of the analyte peak area to that of the internal standard, and x represents the plasma concentration of the analyte. The coeffi-

Table 4
Inter-day assay variance of the determination of dexamethasone and dexamethasone sodium phosphate in perilymph ($n = 6$).

Sample	Concentration spiked (μg/L)	Concentration found (μg/L) (mean ± SD)	Bias (%)	Imprecision CV (%)
Dexamethasone				
LLOQ	0.5	0.51 ± 0.04	2.0	8.3
QC1	10	10.8 ± 0.59	8.0	5.4
QC2	100	101 ± 5.4	1.0	5.4
QC3	500	496 ± 35.9	−0.8	7.2
Dexamethasone sodium phosphate				
LLOQ	0.5	0.53 ± 0.04	6.0	7.0
QC1	10	9.7 ± 0.47	−3.0	4.9
QC2	100	98.6 ± 8.1	−1.4	8.2
QC3	500	502 ± 16.0	0.4	3.2

Table 5
Matrix effect data for dexamethasone (Dex) and dexamethasone sodium phosphate (Dex SP) ($n = 6$, from six different sources).

Concentration spiked (μg/L)	Dex (mean ± SD%)	Dex SP (mean ± SD%)
Plasma		
0.5	106 ± 8.2	114 ± 4.0
10	101 ± 7.1	108 ± 6.6
100	106 ± 5.9	116 ± 9.0
500	103 ± 3.6	112 ± 4.4
Proxy perilymph		
0.5	103 ± 6.8	106 ± 9.2
10	97.4 ± 6.7	111 ± 7.6
100	95.0 ± 7.7	104 ± 6.5
500	96.0 ± 5.8	101 ± 6.9

cients of variation for the slopes of the six standard curves were <3.5% for both Dex and Dex SP in both plasma and perilymph matrices. The corresponding coefficients of variation for the intercepts were <16%. The LOD was around 0.2 μg/L and the LLOQ was around 0.5 μg/L for both Dex and Dex SP in both plasma and perilymph matrices. The accuracy and precision were assessed at LLOQ and the low, medium and high level QCs. For both Dex and Dex SP in both plasma and perilymph matrices, there was no constant direction to the bias (i.e. + or −) for LLOQ and QCs and the mean values were within ±10% of the spiked values (Tables 1–4). Imprecision was small, as indicated by both intra- and inter-day coefficients of variation of <10% at concentrations of LLOQ and QCs (Tables 1–4). The absolute recoveries of Dex and Dex SP from plasma at concentrations of 0.5, 10, 100 and 500 μg/L were similar and consistent, with mean values of around 100%. The absolute recovery of the internal standard FLU at the concentration employed was 105 ± 4.0% ($n = 6$).

Matrix effects were assessed by comparing the response of Dex and Dex SP and the internal standard FLU from the spiked after-protein precipitation blank plasma extracts or proxy perilymph extracts with the response of standard solution at the same concentration in the mobile phase [Matrix effect = (peak area of analyte spiked in protein precipitated blank plasma or proxy perilymph extracts)/(peak area of analyte spiked in mobile phase) × 100%]. A value of 100% indicates that the responses in the mobile phase and in protein precipitated plasma were the same and no absolute matrix effect is observed. A value of >100% indicates ionisation enhancement and a value of <100% indicates ionisation suppression. The matrix effects (mean ± SD%) for both plasma and perilymph determined at concentrations of 0.5, 10, 100 and 500 μg/L for Dex and Dex SP are shown in Table 5. The matrix effect for the internal standard FLU was 98.7 ± 6.8% ($n = 6$). No significant matrix effects were evident.

Dex and Dex SP were found to be stable in plasma and proxy perilymph for at least four freeze–thaw cycles when stored at −30 °C. The QC samples for both plasma and proxy perilymph at concentrations of 0.5, 10, 100 and 500 μg/L were stable for at least 10 months at −30 °C. Stock standard solutions of Dex and Dex SP remained

stable for at least 10 months at 4 °C. The processed samples were stable for at least three days at 4 °C. In all cases, the concentrations of Dex and Dex SP of stored samples deviated <5% from the freshly prepared samples.

3.4. Application of the assays

The method has been used in a clinical study to investigate delivery of Dex and Dex SP into cochlear perilymph after intratympanic or intravenous administration of Dex SP using measurement of the concentrations of Dex and Dex SP in the cochlear perilymph of the human ear and in plasma. The plasma standard curves of both Dex and Dex SP covered the entire range of concentrations in patient samples. Because IT administration delivered much higher doses to the inner ear when compared with systemic administration, there were some concentration values of Dex and Dex SP in cochlear perilymph above the perilymph standard curves. The samples with abnormally high concentrations of Dex and Dex SP were diluted and reanalysed according to the US Food and Drug Administration guidance for bioanalytical method validation [11]. To ensure the accuracy and reproducibility of the method for patient samples, 23% of the incurred plasma samples were reanalysed. All the repeat values were ≤17% difference from the initial value. A paper arising from this clinical study is in preparation.

4. Conclusions

A validated LC–MS/MS method for the determination of Dex and Dex SP in the cochlear perilymph of the human ear and in plasma has been described. The method has been used in a clinical study and proven to be rapid, sensitive, specific and accurate.

References

[1] P.A. Bird, E.J. Begg, M. Zhang, A.T. Keast, D.P. Murray, T.J. Balkany, Otol. Neurotol. 28 (2007) 1124.
[2] P. Rohdewald, H. Möllmann, J. Barth, J. Rehder, H. Derendorf, Biopharm. Drug Dispos. 8 (1987) 205.
[3] Y. Luo, C.E. Uboh, L.R. Soma, F. Guan, J.A. Rudy, D.S. Tsang, Rapid Commun. Mass Spectrom. 19 (2005) 825.
[4] M.N. Samtani, W.J. Jusko, Biomed. Chromatogr. 21 (2007) 585.
[5] R. DiFrancesco, V. Frerichs, J. Donnelly, C. Hagler, J. Hochreiter, K.M. Tornatore, J. Chromatogr. B 859 (2007) 42.
[6] Y. Yang, H. Li, K. Gao, M. Liu, Y. Sun, T. Yan, J.P. Fawcett, Y. Cui, J. Gu, J. Chromatogr. B 862 (2008) 119.
[7] H. Liu, X. Chen, S. Zhang, L. Qu, Y. Zhao, H. Liu, M. Dong, J. Chromatogr. B 805 (2004) 255.
[8] A.B. Gurney, D.C. Wascher, Am. J. Sports Med. 36 (2008) 753.
[9] K. Hosseini, D. Matsushima, J. Johnson, G. Widera, K. Nyam, L. Kim, Y. Xu, Y. Yao, M. Cormier, J. Ocul. Pharmacol. Ther. 24 (2008) 301.
[10] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[11] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2001.