Journal of Chromatography B, 878 (2010) 3277–3282

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Dexamethasone quantification in dried blood spot samples using LC–MS: The potential for application to neonatal pharmacokinetic studies

Parul Patel[a], Sangeeta Tanna[a,*], Hussain Mulla[b], Venkatesh Kairamkonda[c], Hitesh Pandya[d], Graham Lawson[a]

[a] Leicester School of Pharmacy, De Montfort University, Leicester, LE1 9BH, UK
[b] Centre for Therapeutic Evaluation of Drugs in Children, University Hospitals of Leicester NHS Trust, LE3 9QP, UK
[c] Neonatal Unit, University Hospitals of Leicester NHS Trust, LE1 5WW, UK
[d] Department of Infection, Immunity and Inflammation, University of Leicester, LE1 5WW, UK

ARTICLE INFO

Article history:
Received 29 July 2010
Accepted 12 October 2010
Available online 20 October 2010

Keywords:
Dried blood spot (DBS)
Dexamethasone
Chronic lung disease
Guthrie card
LC–MS

ABSTRACT

A high-performance liquid chromatography (LC–MS) method has been developed and validated for the determination of dexamethasone in dried blood spot (DBS) samples. For the preparation of DBS samples whole blood spiked with analyte was used to produce 30 μl blood spots on specimen collection cards. An 8 mm disc was cut from the DBS sample and extracted using a combination of methanol: water (70:30, v/v) containing the internal standard, triamcinolone acetonide. Extracts were centrifuged and chromatographic separation was achieved using a Zorbax Eclipse Plus C18 column using gradient elution with a mobile phase of acetonitrile and water with formic acid at a flow rate of 0.2 ml/min. LC–MS detection was conducted with single ion monitoring using target ions at *m/z* 393.1 for dexamethasone and 435.1 for the internal standard. The developed method was linear within the tested calibration range of 15–800 ng/ml. The overall extraction recovery of dexamethasone from DBS samples was 99.3% (94.3–105.7%). The accuracy (relative error) and precision (coefficient of variation) values were within the pre-defined limits of ≤15% at all concentrations. Factors with potential to affect drug quantification measurements such as blood haematocrit, the volume of blood applied onto the collection card and spotting device were investigated. Although a haematocrit related effect was apparent, the assay accuracy and precision values remained within the 15% variability limit with fluctuations in haematocrit of ±5%. Variations in the volume of blood spotted did not appear to affect the performance of the developed assay. Similar observations were made regarding the spotting device used. The methodology has been applied to determine levels of dexamethasone in DBS samples collected from premature neonates. The measured concentrations were successfully evaluated using a simple 1-compartment pharmacokinetic model. Requiring only a microvolume (30 μl) blood sample for analysis, the developed assay is particularly suited to pharmacokinetic studies involving paediatric populations.

1. Introduction

Dexamethasone therapy is used for the treatment and prevention of chronic lung disease (CLD) of the newborn [1,2]. The dosing regimen used in clinical trials has varied considerably with cumulative doses ranging between 0.9 and 7.8 mg/kg [3]. Despite such use, there is little pharmacokinetic (PK) data for the drug in this population and the optimum dose in CLD remains unknown. Dexamethasone therapy in neonates is associated with serious short-term adverse effects as well as long-term complications, namely cerebral palsy [4,5]. PK data is urgently required in order to optimise current dosing regimens. The paucity in data is at least in part due to practical difficulties and ethical considerations surrounding blood collection in neonates. There are a number of reports on the quantification of dexamethasone in blood plasma using liquid chromatography coupled with UV [6–10], mass spectrometry [11–15], or fluorescence detection [16,17], gas chromatography with mass spectrometry [18,19] and immunological [20]. However, most of these assays require a relatively large blood volume (typically >0.5 ml) to generate a sufficient plasma volume for analysis. This prerequisite significantly reduces the feasibility of paediatric PK studies and the problem is most apparent in premature newborns where ethical considerations prohibit large volume sampling.

Abbreviations: CLD, chronic lung disease; PK, pharmacokinetics; DBS, dried blood spot.

* Corresponding author.
E-mail addresses: parul-patel@hotmail.co.uk (P. Patel), stanna@dmu.ac.uk (S. Tanna), hussain.mulla@uhl-tr.nhs.uk (H. Mulla), Venkatesh.Kairamkonda@uhl-tr.nhs.uk (V. Kairamkonda), hp28@leicester.ac.uk (H. Pandya), glawson@dmu.ac.uk (G. Lawson).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.10.009

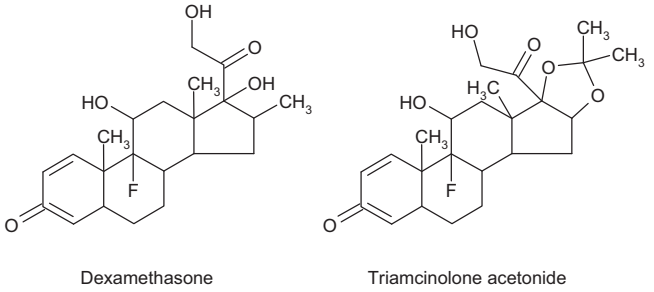

Fig. 1. Chemical structures of dexamethasone and internal standard.

In contrast, dried blood spots (DBS) only require a microvolume blood sample (typically ≤50 μl) for drug quantification. A volume which can be easily collected via a capillary heelstick prick which is already a routine method of blood collection in neonates. These factors make DBS a potentially very useful drug quantification tool in studies involving repetitive sampling (for example in PK studies). The aim of this study was to determine whether DBS in principle, can be used for the measurement of relatively low circulating blood levels of dexamethasone in neonates administered the drug. The advantages of DBS based methods coupled with improved analytical capability has led to a recent rise in the use of this methodology in drug quantification studies; with application to fields including therapeutic drug monitoring [21,22,23], toxicology [24] and pharmacokinetics studies [25–28].

Although there are reported dexamethasone assays compatible with smaller volumes of blood (0.1–0.2 ml plasma), these are still too large for neonates and necessitate complex and time-consuming work-up processes [16,17] making them less practically viable. The objective of the present study was to develop and validate, a simple DBS based method with LC–MS detection for the quantification of dexamethasone in neonatal samples. The developed assay was assessed against internationally accepted validation criteria [29]. Additional tests, such as the effect of sample volume collected and haematocrit were also investigated.

2. Experimental

2.1. Chemicals and materials

Dexamethasone (9α-fluoro-11,17,21-trihydroxy-16-methyl-pregna-1,4-diene-3,20-dione) ≥98% and the internal standard triamcinolone acetonide (9α-fluoro-16α-hydroxyprednisolone 16α,17α-acetonide) ≥99% (Fig. 1) were purchased from Sigma–Aldrich (Poole, UK). Triamcinolone was selected as the internal standard (IS) due to its structural similarity to the analyte of interest. As triamcinolone is not used on the neonatal unit there is no potential for alteration of the analyte to internal standard ratio and therefore to affect dexamethasone quantification. HPLC grade water, acetonitrile and methanol and formic acid ≥98% were obtained from Fisher Scientific (Loughborough, UK). Autosampler vials with 0.3 ml inserts, eppendorf cups and volumetric pipettes were obtained from Fisher Scientific (Loughborough, UK). Specimen collection filter paper type 903 was obtained from Fisher Scientific (Loughborough, UK). Sample glass tubes and polythene bags for storage of blood spot cards were obtained from Richardsons of Leicester (Leicester, UK). An 8 mm diameter punch was obtained from Maun Industries Ltd. (Nottingham, UK). Blank human blood was ethically obtained from healthy adult volunteers following informed consent. Lithium heparin coated capillaries were obtained from Sangius Counting (Nümbrecht, Germany) and blood collection tubes were obtained from Sarstedt (Leicester, UK).

2.2. Preparation of stock, working and extraction solutions

Stock solutions of dexamethasone were prepared by dissolving in methanol:water (80:20, v/v) to produce a 1 mg/ml concentration. A relatively high concentration of methanol was used in the preparation of stock solutions to ensure complete dissolution of the corticosteroid. The stock solution was diluted to produce a concentration of 20 μg/ml in methanol:water (50:50, v/v), this was further diluted with methanol:water (50:50, v/v) to produce different working solutions. All working solutions were prepared freshly on the day of analysis.

Stock solutions of IS were prepared by dissolving in methanol:water (80:20, v/v) to produce a 200 μg/ml concentration. The stock solution was diluted to produce a concentration of 10 μg/ml in methanol:water (50:50, v/v), this was further diluted with methanol:water (70:30, v/v) to produce an extraction solvent containing 5 ng/ml of IS.

2.3. Preparation of calibration standards and validation samples

Haematocrit levels are initially higher in the newborn and gradually decrease with increasing post-natal age. The observed changes in haematocrit during the neonatal period are also affected by gestational age i.e. prematurity. Dexamethasone therapy is now reserved for use late in the neonatal period and since the majority of patients receiving treatment are pre-term infants lower haematocrit levels are observed in this population. A Haematocrit level of 35% was selected to represent the average haematocrit value expected for the target population planned for study, thereby limiting any haematocrit related effects. Briefly, whole blood was centrifuged at 7000 × *g* for 4 min and the plasma generated transferred to a clean Eppendorf. The erythrocyte suspension was washed with isotonic saline. Finally, the red blood cells and plasma were mixed in proportions (35:65, v/v) to give an adjusted haematocrit of 35%. Calibration standards and validation samples were prepared fresh on a daily basis by diluting the working solutions with blank human whole blood of haematocrit 35%. The final concentration of calibration standards were 15, 50, 100, 250, 500 and 800 ng/ml of dexamethasone in whole blood. The concentrations of calibration standards were selected to reflect the expected blood concentration in neonates receiving dexamethasone treatment. Thirty microlitres of calibration standards and validation samples were spotted directly onto filter card type 903 using a volumetric pipette and allowed to air dry overnight at room temperature prior to processing. A 30 μl volume applied onto filter card gave a spot size of ~9.5 mm in diameter.

2.4. Dried blood spot sample extraction

An 8 mm diameter disc was punched from the centre of the DBS sample and transferred to a clean glass tube. A 250 μl volume of extraction solvent consisting of methanol:water (70:30, v/v) plus IS (5 ng/ml) was added and the tube was shaken at 300 rpm for 20 min. The extract was centrifuged at 13,500 × *g* for 10 min to remove any insoluble residues from the filter card and the supernatant was transferred to an autosampler vial for analysis by LC–MS.

2.5. LC–MS instrumentation and conditions

The chromatographic system consisted of an Agilent 1100 series quaternary solvent delivery pump, autosampler and vacuum degasser. Dexamethasone was analysed on a Zorbax Eclipse Plus (Agilent Technologies, Cheshire, UK) C18 column (150 mm × 2.1 mm i.d., 3.5 μm) attached with a C18 guard column (Phenomenex, Macclesfield UK, 3.0 × 4.0 mm). Gradient elution was used based on a combination of water with 0.13% formic acid

(A) and acetonitrile (B). The mobile phase was initiated at 40% B and maintained for 0.15 min before increasing to 70% B by 3.0 min. A final increase to 80% B was achieved by 6.0 min before returning to 40% B. Thereafter, a 7.5 min post-run at 40% B was maintained prior to the next injection. The flow rate was 200 μl/min and injection volume 25 μl. The column oven temperature was set to 23 °C.

Detection of samples was achieved using an Agilent 1200 mass spectrometer with a positive electrospray ion source. The ionisation source parameters optimised to give maximum analyte signal intensity were: fragmentor voltage, 65 V; drying temperature, 300 °C; capillary voltage, 3500 V; nebuliser pressure, 25 psig; nitrogen gas flow, 7 l/min. The mass spectrometer was set to carry out single ion monitoring for the protonated molecular ion ($M + H^+$) of dexamethasone at m/z 393.1 and triamcinolone acetonide at m/z 435.1. A dwell time of 289 ms was used for each ion. The analytical software Chemstation (series B.1.3, Agilent Technologies) was used to operate the system and aquire all data.

2.6. Validation prodecures

2.6.1. Linearity, selectivity and sensitivity

Calibration standards were prepared in replicate ($n = 5$) and analysed on 3 separate days. A calibration plot of analyte/IS peak area ratio against nominal dexamethasone concentration was produced and an equally weighted linear regression was applied.

The selectivity of the method was determined by analysing DBS samples ($n = 1$) collected from five individual human subjects.

The lower limit of quantification (LLQ) was defined by the lowest concentration that gave a signal to noise ratio equal to or greater than 10 whilst exhibiting an inaccuracy and imprecison of ≤15%.

2.6.2. Accuracy and precision

Inter- and intra-day accuracy and precision for the assay were determined from the analysis of replicate ($n = 5$) calibration standards at six dexamethasone concentrations within the range of 15–800 ng/ml on 3 separate days. The accuracy was expressed as the relative error (RE%) and precision as the coefficient of variation (CV%). A RE and CV of ≤15% at all concentrations was considered acceptable.

2.6.3. Matrix effects

Replicate ($n = 5$) samples of analyte spiked in extracted blank whole blood at concentrations of 50 and 800 ng/ml were produced to evaluate suppression or enhancement of the detector response due to constituents within dried blood spots. The samples prepared were compared to standards of the same concentration spiked into pure methanol:water (70:30, v/v). The matrix effect was calculated using peak area by the equation $(B/A - 1) \times 100$. Where A represents dexamethasone spiked into pure solvent and B represents dexamethasone spiked into extracted blank whole blood.

2.6.4. Recovery of dexamethasone from dried blood spot

The overall recovery was assessed at concentrations of 50, 250 and 800 ng/ml in replicate ($n = 5$). To determine recovery 30 μl spots were made and allowed to dry. The entire spot on the filter card was then extracted with 250 μl of methanol:water (70:30, v/v). The analytical results from extracted samples were compared to those obtained from the same amount of dexamethasone in solvent methanol:water (70:30, v/v). Recovery was calculated using the following equation: % recovery = peak area of dried blood spot extract/peak area of standard dexamethasone solution × 100.

2.6.5. Stability

DBS samples prepared at concentrations of 50 and 800 ng/ml were investigated following storage at room temperature and 4 °C. To determine stability 30 μl spots were made and allowed to dry. The entire spot on the filter card was then extracted with 250 μl of methanol:water (70:30, v/v). The stability of dexamethasone within dried blood spots was determined by comparing the peak areas obtained from stored samples with fresh samples. DBS samples were considered stable if the difference in peak area between the stored and freshly prepared samples were ≤15%.

The stability of dexamethasone in stock solutions was determined in replicate ($n = 2$) by comparing the peak area obtained after storage at room temperature and 4 °C with those obtained from freshly prepared stock solutions. The stock solution was considered stable if the difference in peak area between the stored and freshly prepared stock was ≤5%.

2.6.6. Spotting device

To determine whether the spotting device has an effect on the formation of blood spots blank whole blood containing 50 and 800 ng/ml dexamethasone was used to produce 30 μl blood spots in replicate ($n = 5$) using a capillary tube or a pipette (Eppendorf). The concentrations of dexamethasone were determined from a calibration line generated from standards spotted using a pipette.

2.6.7. Blood spot size

To assess the effect of blood volume collected on dexamethasone quantification, 25, 30 and 35 μl DBS samples at 50 and 800 ng/ml were prepared in replicate ($n = 5$). An 8 mm diameter disc was punched from the centre of each sample and extracted. The concentrations of extracts were determined using the linear regression equation generated from a calibration produced from 30 μl DBS samples.

2.6.8. Haematocrit

Since haematocrit is directly proportional to the viscosity of blood, it may affect the diffusion properties of the blood spotted onto paper and therefore the amount of drug recovered. This is important to consider as the haematocrit level is known to change considerably during the neonatal period, which may produce unexpected effects [30,31]. To determine the influence of haematocrit on the assay performance, 30 μl DBS samples with an adjusted haematocrit of 30%, 35% and 40% (see Section 2.3) were prepared at 50, 250 and 800 ng/ml in replicate ($n = 5$). An 8 mm disc was punched from each spot and analysed. The concentrations of extracts were determined using the linear regression equation generated from a calibration produced from standards of 35% haematocrit level.

2.6.9. Application of method

The developed methodology was applied to a series of DBS samples collected from two infants receiving a course of dexamethasone. A 10-day dose tapering regimen is used on the Neonatal Unit which entails the following dosing schedule: – 250 μg/kg twice a day for 3 days, then 100 μg/kg twice a day for 3 days, then 50 μg/kg twice a day for 2 days, followed by 25 μg/kg twice a day for 2 days before stopping. The study has received ethical approvals from the Leicestershire, Northampton and Rutland National Health Service Research Ethics Committee and the De Montfort University Research Ethics Committee. DBS samples were collected opportunistically at random time intervals during dexamethasone treatment.

3. Results and discussions

3.1. Linearity, selectivity and sensitivity

The assay showed linearity ($r^2 \geq 0.99$) within the tested concentration range of 15–800 ng/ml. Chromatographic conditions used provided sufficient resolution with elution of dexamethasone and IS at 4.8 and 5.9 min, respectively. There were no significant (≥10%

Table 1
Intra- and inter-day accuracy and precision data for dexamethasone in whole dried blood samples ($n = 5$ at all concentration levels).

	Nominal conc. (ng/ml)					
	15	50	100	250	500	800
Intra-day						
Run 1						
Mean conc. (ng/ml)	15.8	45.7	91.3	225.4	498.9	777.1
SD	1.8	1.1	4.2	16.7	17.2	33.1
Accuracy (RE%)	5.2	−9.3	−9.6	−10.9	−0.2	−3.0
Precision (CV%)	11.4	2.5	4.6	7.4	3.4	4.3
Run 2						
Mean conc. (ng/ml)	16.7	48.9	99.9	253.0	517.0	758.5
SD	1.5	4.6	5.6	16.8	37.4	43.7
Accuracy (RE%)	10.3	−2.2	−0.1	1.2	3.3	−5.5
Precision (CV%)	9.1	9.2	5.6	6.6	7.2	5.8
Run 3						
Mean conc. (ng/ml)	16.2	52.9	98.2	248.3	565.2	847.7
SD	2.3	6.1	12.9	36.3	27.0	48.7
Accuracy (RE%)	7.5	5.5	−1.9	−0.7	11.5	5.6
Precision (CV%)	14.0	11.5	13.1	14.6	4.8	5.7
Inter-day						
Mean conc. (ng/ml)	16.3	49.2	96.5	242.2	527.1	794.4
SD	1.8	5.1	8.8	25.3	38.4	54.3
Average accuracy (RE%)	9.7	0.3	−1.5	−1.1	7.0	1.2
Overall precision (CV%)	11.0	10.4	9.1	10.5	7.3	6.9

of LLQ area) interferences at either of these retention times and the selectivity of the method is demonstrated by the representative HPLC–MS chromatograms presented in Fig. 2.

The LLQ with a signal-to-noise ratio of ≥10 and acceptable assay accuracy and precision was 15 ng/ml in whole dried blood. A representative LC–MS chromatogram at the LLQ is given in Fig. 2.

3.2. Accuracy and precision

The intra- and inter-day performance of the assay was measured by analysing five spiked samples of dexamethasone at each concentration on 3 separate days (Table 1). Accuracy (RE%) and precision (CV%) values were within the pre-defined 15% limit for all concentrations in each run. The overall variation in assay performance between runs was also within 15%.

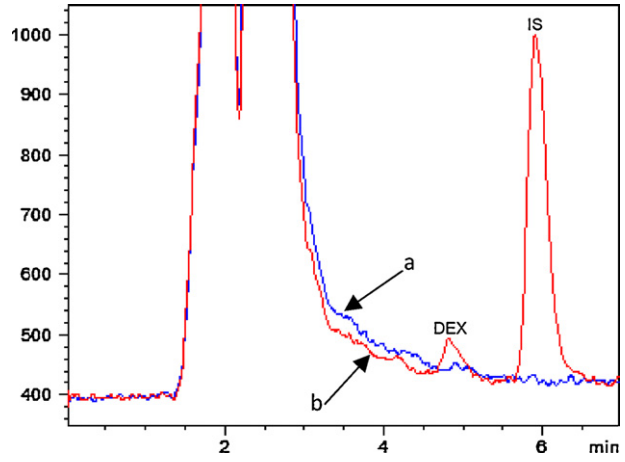

Fig. 2. Representative LC–MS chromatogram of an extracted blank whole blood spot (a) and (b) a whole blood spot spiked with dexamethasone (DEX) 15 ng/ml (LLQ) and extracted with IS.

3.3. Matrix effect

No significant (<5%) ion suppression or enhancement of the analyte signal was observed due to the matrix at the two tested concentrations (Table 2).

3.4. Recovery

The overall recovery of dexamethasone from DBS samples for concentrations 50, 250 and 800 ng/ml was between 98% and 104%. Consistent recovery values at low, medium and high concentrations indicate the extraction process is acceptable across this range. Table 3 gives the overall extraction recoveries obtained for each concentration level. The high recoveries observed indicate analyte stability under the extraction conditions applied and good extraction.

3.5. Stability

Dexamethasone was stable within a DBS sample for at least 7 days at room temperature and 28 days at 4 °C. Differences in peak area between samples stored at 4 °C for 28 days and fresh samples

Table 2
Matrix effect data at two concentrations of dexamethasone ($n = 5$).

	Nominal conc. (ng/ml)	
	50	800
Matrix effect % (mean)	−0.4	3.4
Precision (CV%)	4.3	4.9

Table 3
Recovery data from dried blood spots at three concentrations ($n = 5$).

	Nominal conc. (ng/ml)		
	50	250	800
Recovery (%)	94.3	105.7	97.8
SD	13.0	6.1	5.7
Precision (CV%)	13.8	5.8	5.8

Table 4
Effect of spotting device used to apply blood onto filter card at two concentration levels ($n = 5$).

	Spotting device			
	Pipette		Capillary	
Nominal conc. (ng/ml).	50	800	50	800
Mean conc. (ng/ml)	45.6	838.5	50.6	822.8
SD	6.5	54.6	7.5	34.4
Accuracy (RE%)	−9.6	4.6	1.7	2.8
Precision (CV%)	14.2	6.5	14.7	4.2

were −7.5% and −1.6% at 50 and 800 ng/ml, respectively. Moreover, extracted DBS samples were stable for at least 2 days at room temperature. Further tests are underway to determine long-term stability of analyte on filter card.

Stock solutions of dexamethasone were stable for at least 37 days stored at 4 °C with a difference of less than 5% between peaks areas obtained from stored and fresh solutions.

3.6. Spotting device

A comparison of the accuracy and precision of dexamethasone quantification when samples were spotted using either a pipette or capillary is given in Table 4. An acceptable assay performance was observed for both spotting devices used.

3.7. Blood spot size

The accuracy and precision data as shown in Table 5 was within the 15% limit for 25 and 35 μl spot sizes at the two tested concentrations. Furthermore, the maximum variation in accuracy between spot sizes of 25–35 μl was 7%, indicating that the amount of blood spotted did not significantly affect the distribution of dexamethasone across the filter paper and therefore drug quantification.

3.8. Haematocrit

Haematocrit value has been shown to influence drug concentration measurements in DBS sampling [30,31]. This is particularly important to consider in neonatal PK studies due to the considerable inter- and intra-individual variation in haematocrit values observed during the first 28 days of life [32]. The results for the haematocrit investigation gave RE and CV values within the predefined limit of ≤15% at all haematocrit levels for each tested concentration. However, a haematocrit effect was noticeable. Higher haematocrit levels were associated with an increase in the amount of drug recovered at all three concentrations, indicating an altered distribution of analyte across the filter card. The blood spot size decreased (data not shown) with increasing haematocrit, therefore blood viscosity may in part explain the observed differences in concentration of analyte recovered from an 8 mm punch. Similar observations relating haematocrit and area of paper covered by consistent volumes of blood have been reported by others [30,31]. Although haematocrit was found to influence the quantification of dexamethasone the maximum difference in accuracy determined when changing haematocrit from 30% to 40% was less than 15%. There is some evidence to suggest the haematocrit effect is likely to be influenced by paper type and the chemical properties of the compound of interest [30,31,33,34]. Investigations on the effect of haematocrit are warranted during the DBS method development stage, particularly in cases where the target population is likely to exhibit a large degree of variation in haematocrit value.

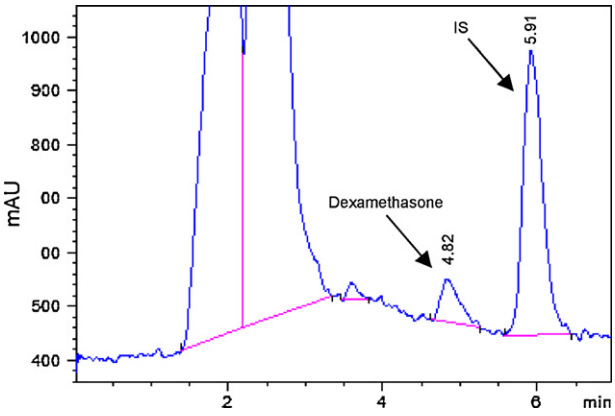

Fig. 3. LC–MS chromatogram of an extracted dried blood spot collected from an infant approximately 7 h following the administration of a 50 μg/kg intravenous dose of dexamethasone.

3.9. Application of assay

An example of a chromatogram obtained from the extract of a DBS sample collected from a premature infant following the administration of dexamethasone is given in Fig. 3. The peak obtained corresponded to a concentration of 22.9 ng/ml of dexamethasone determined from a 30 μl blood sample.

The measured concentrations in DBS samples collected from patient 2 were fitted using a 1-compartment pharmacokinetic model. The estimated values of clearance (162 ml/h) and volume of distribution (996 ml) are within expected range for the gestational age of the patient [20,35]. With the exception of a single point all the observed concentrations of dexamethasone in blood lie within the ranges predicted by the PK model for the dosing regimen outlined in Section 2.6.9 (Fig. 4). This suggests that the assay is performing well and that the current DBS method can be used successfully for the measurement of dexamethasone levels in premature infants. Results indicate a greater degree of sensitivity is required to adequately detect dexamethasone levels during the last 2 days of treatment where the dose administered is tapered to 25 μg/kg twice a day before discontinuation. This could be achieved by slight modification of the extraction method. Drying DBS extracts to a residue followed by reconstitution with a smaller volume of solvent prior to injection onto the LC–MS would significantly improve the current LLQ. Further work would include using a tandem mass spec-

Table 5
Effect of varying blood spot size on accuracy and precision of assay at two concentrations ($n = 5$).

	Nominal conc. (ng/ml)					
	50			800		
Volume	25 μl	30 μl	35 μl	25 μl	30 μl	35 μl
Mean conc. (ng/ml)	52.0	48.9	51.1	807.3	758.5	804.0
SD	2.0	4.5	3.4	35.1	43.7	47.6
Accuracy (RE%)	3.8	−2.2	2.1	0.9	−5.5	0.5
Precision (CV%)	3.9	9.2	6.7	4.3	5.8	5.9

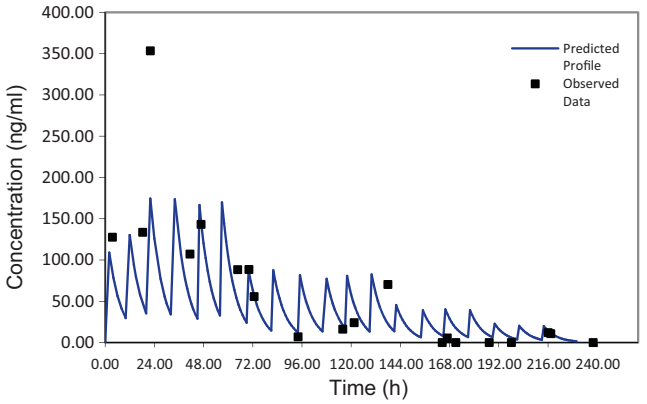

Fig. 4. Predicted PK profile for dexamethasone in pre-term neonates during a 10-day tapering dose of 250 μg/kg twice a day for 3 days, then 100 μg/kg twice a day for 3 days, then 50 μg/kg twice a day for 2 days, followed by 25 μg/kg twice a day for 2 days before stopping, and the observed PK profile generated from DBS samples collected from patient 2.

trometer for greater selectivity and potentially increased detection sensitivity.

4. Conclusion

A simple and sensitive LC–MS method utilising DBS technology has been successfully developed for the quantification of dexamethasone in human whole blood. The validated method has been shown to be accurate and precise with a RE and CV ≤15% at all tested concentrations. The degree of flexibility around blood volume collection and the spotting device used is particularly advantageous to test sites where accurate pipetting may be difficult to achieve. Stability of dexamethasone within DBS samples has been shown following storage at room temperature for 7 days and up to 28 days for samples kept in a refrigerator.

Requiring only a 30 μl blood sample, the method is well suited for application to paediatric populations. In addition, simplified storage conditions and ease of handling samples make DBS an attractive proposition in the conduct of multicentre paediatric PK studies, which may be necessary given the often limited sampling pools available for study. The developed DBS method has been successfully applied to clinical samples collected from pre-term infants and is being used to support an on-going PK study of dexamethasone in newborns.

Conflict of interest

None.

References

[1] M.N. Samtani, W.J. Jusko, Int. J. Pharm. 301 (2005) 262.
[2] A.H. Jobe, N. Engl. J. Med. 350 (2004) 1349–1351.
[3] W. Onland, M. Offringa, A.P. De Jaegere, A.H. van Kaam, Pediatrics 123 (2009) 367–377.
[4] A.H. Jobe, E. Bancalari, Am. J. Respir. Crit. Care Med. 163 (2001) 1723–1729.
[5] H.L. Halliday, R.A. Ehrenkranz, L.W. Doyle, Cochrane Database Syst. Rev. (1) (2010), doi:10.1002/14651858, Art. No.: CD001146 pub3.
[6] P.M. Plezia, P.L. Berens, Clin. Chem. 31 (1985) 1870–1872.
[7] P.N. Schild, B.G. Charles, J. Chromatogr. B 658 (1994) 189–192.
[8] E. Grippa, L. Santini, G. Castellano, M.T. Gatto, M.G. Leone, L. Saso, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 738 (2000) 17–25.
[9] Y.K. Song, J.S. Park, J.K. Kim, C.K. Kim, J. Liq. Chromatogr. Related Technol. 27 (2004) 2293–2306.
[10] V. Kumar, M. Mostafa, M.W. Kayo, E.P. Goldberg, H. Derendorf, Pharmazie 61 (2006) 908–911.
[11] Y.L. Chen, X. Jiang, W. Naidong, J. Liq. Chromatogr. Related Technol. 25 (2002) 1317–1334.
[12] R.L. Taylor, S.K. Grebe, R.J. Singh, Clin. Chem. 50 (2004) 2345–2352.
[13] Y. Luo, C.E. Uboh, L.R. Soma, F. Guan, J.A. Rudy, D.S. Tsang, Rapid Commun. Mass Spectrom. 19 (2005) 825–832.
[14] J. Qu, Y. Qu, R.M. Straubinger, Anal. Chem. 79 (2007) 3786–3793.
[15] G. Damonte, A. Salis, L. Rossi, M. Magnani, U. Benatti, J. Pharm. Biomed. Anal. 43 (2007) 376–380.
[16] M. Katayama, Y. Masuda, H. Taniguchi, J. Chromatogr. 612 (1993) 33–39.
[17] S. Wu, H. Wu, S. Chen, Anal. Chim. Acta 307 (1995) 103–107.
[18] J. Girault, B. Istin, B. Fourtillan, Biol. Mass Spectrom. 19 (1990) 292–302.
[19] O.H. Hidalgo, M.J. Lopez, E.A. Carazo, M.S.A. Larrea, T.B.A. Reuvers, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 788 (2003) 137–146.
[20] R.A. Lugo, M.C. Nahata, J.A. Menke, R.E. McClead Jr., Pharmacokinet. Dispos. 49 (1996) 477–483.
[21] J. Déglon, A. Thomas, A. Cataldo, P. Mangin, C. Staub, J. Pharm. Biomed. Anal. 49 (2009) 1034.
[22] P.M. Edelbroek, J. van der Heijden, L.M.L. Stolk, Ther. Drug Monit. 31 (2009) 327–336.
[23] W. Li, F.L.S. Tse, Biomed. Chromatogr. 24 (2010) 49–65.
[24] M. Barfield, N. Spooner, R. Lad, S. Parry, S. Fowles, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 870 (2008) 32–37.
[25] G. Lawson, S. Tanna, H. Mulla, H. Pandya, J. Pharm. Pharmacol. 61 (2009) A33.
[26] P. Patel, G. Lawson, S. Tanna, H. Mulla, J. Pharm. Pharmacol. 61 (2009) A112.
[27] M.F. Suyagh, K.P. Laxman, J. Millership, P. Collier, H. Halliday, J.C. McElnay, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (2010) 769–776.
[28] N. Spooner, R. Lad, M. Barfield, Anal. Chem. 81 (2009) 1557–1563.
[29] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551–1557.
[30] M. Holub, K. Tuschl, R. Ratschmann, K.A. Strnadova, A. Muhl, G. Heinze, W. Sperl, O.A. Bodamer, Clin. Chim. Acta 373 (2006) 27–31.
[31] A.J. Wilhelm, J.C.G. den Burger, A. Chahbouni, R.M. Vos, A. Sinjewel, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 877 (2009) 3916–3919.
[32] J. Jopling, E. Henry, S.E. Wiedmeier, R.D. Christensen, Pediatrics 123 (2009) 333–337.
[33] J.V. Mei, R.J. Alexander, B.W. Adam, W.H. Hannon, J. Nutr. 131 (2001) 1631S–1636S.
[34] T. Minematsu, E. Sugiyama, M. Kusama, S. Hori, Y. Yamada, H. Ohtani, Y. Sawada, H. Sato, T. Takayama, Y. Sugawara, M. Makuuchi, T. Iga, Transplant. Proc. 36 (2004) 1506–1511.
[35] B. Charles, P. Schild, P. Steer, D. Cartwright, T. Donovan, Dev. Pharmacol. Ther. 20 (1993) 205–210.