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Sensitive and specific liquid chromatography–tandem mass spectrometric method for the quantitation of dexmedetomidine in pediatric plasma

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Abstract

Dexmedetomidine (Dex) is a lipophilic imidazole derivative used primarily for the sedation and anxiolysis of adults in the intensive care setting. Dex is being used more frequently in the pediatric intensive care unit. This report describes a selective and highly sensitive assay for Dex in pediatric plasma employing liquid chromatography–tandem mass spectrometry (LC–MS/MS). Dex was extracted from 200 μL of plasma by solid-phase extraction (SPE). High performance liquid chromatography (HPLC) separation was conducted on an YMC ODS-AQ C_{18} column with a flow rate of 0.3 mL/min using a mobile phase comprised of 5 mM ammonium acetate buffer/0.03% formic acid in the solvent mixture of methanol/acetonitrile/water (20:20:60, v/v/v). The intra-day precision (coefficient of variation, % CV) and accuracy for quality control samples, ranged from 1.04 to 6.84% and 90.2 to 100.8%, respectively. The inter-day precision and accuracy ranged from 4.08 to 5.37% and 92.7 to 98.6%, respectively. Stability studies showed that Dex was stable during both the assay procedure and storage. The overall recovery was 76.6–78.3% for Dex in plasma. The analytical method showed excellent sensitivity using a small sample volume (200 μL) with a lower limit of quantitation of 5 pg/mL. This method is robust and has been successfully employed in a pharmacokinetic study of Dex in infants postoperative from cardiac surgery.
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Keywords: Dexmedetomidine; LC–MS/MS; Pediatric; Pharmacokinetics

1. Introduction

Dexmedetomidine (Precedex™) (Fig. 1) is a lipophilic imidazole derivative used primarily for the sedation and anxiolysis of adults in the intensive care setting. It is a relatively selective alpha$_2$-adrenoceptor agonist with centrally mediated sympatholytic, sedative, and analgesic effects [1–4]. Due to its ability to preserve respiratory function at therapeutic doses, the use of dexmedetomidine (Dex) is increasing in the pediatric population. However, there is limited pharmacokinetic data in this population.

One of the barriers to studying the pharmacokinetics of Dex in pediatric patients is the limited volume of plasma that can be sampled from children for Dex quantitation. Methods for quantitation of Dex in human plasma currently published are not suitable for pediatric studies because of the relatively large volumes of plasma needed to reach lower limits of detection [5–7]. We developed and validated a liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) method for the quantitation of Dex using limited plasma sample volumes for use in pediatric pharmacokinetic studies.

2. Experimental

2.1. Materials and chemicals

Dexmedetomidine HCl was obtained from Abbott Laboratories (Chicago, IL, USA) and the internal standard (Tolazoline HCl) was obtained from Spectrum Inc. (New Brunswick, NJ, USA). Human plasma was obtained from The Children's Hospital of Philadelphia (Philadelphia, PA, USA). High performance

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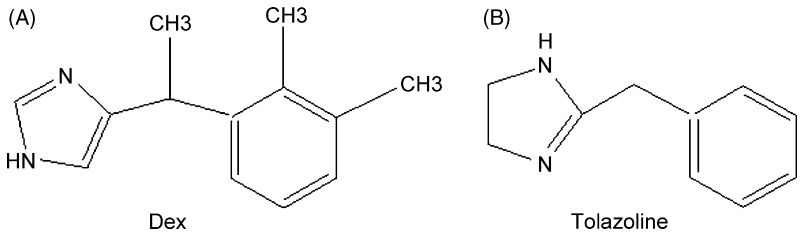

Fig. 1. Chemical structures of (A) Dex and (B) internal standard.

liquid chromatography (HPLC)-grade methanol, acetonitrile, and 2-propanol were purchased from Fisher-Scientific (Pittsburgh, PA, USA). Reagent-grade formic acid (96%) and ammonium acetate were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). De-ionized water was prepared using a Milli-Q water purifying system purchased from Millipore Corp. (Bedford, MA, USA). Extraction cartridges (Oasis HLB 1 cm^3, 30 mg) were obtained from Waters Corp. (Milford, MA, USA).

2.2. Liquid chromatography (LC)

The Shimadzu HPLC system consisted of two LC-20AD delivery pumps, a DGU-20A5 Shimadzu vacuum degasser, a SIL-20AC Shimadzu autosampler and a CBM-20A system controller (Columbia, MD, USA). HPLC separations were achieved using an analytical column, YMC ODS-AQ C_{18} column (2 mm × 100 mm, 3 μm, 120 Å) obtained from Water Corp. (Milford, MA, USA), with a C_8 guard column (2 mm × 4 mm) obtained from Phenomenex (Torrance, CA, USA). HPLC separations were conducted using a flow rate of 0.3 mL/min of an isocratic mobile phase comprised of a 60:40 mixture of solvent A and B: (solvent A) 5 mM ammonium acetate adjusted with 0.03% formic acid and (solvent B) acetonitrile:methanol (50:50, v/v). The following elution program was used: 0–4 min isocratic 40% B (separation of Dex and internal standard), 4–6 min 90% B (regeneration of the column), 6–8 min 40% B (reconditioning of the column). An injection volume of 30 μL was used. The column and autosampler were maintained at room temperature and 4 °C, respectively. When data acquisition was not occurring, an electronic valve actuator with a Rheodyne selector valve was used to divert the LC flow to waste.

2.3. Mass spectrometry analysis

Samples were analyzed with an API 4000 tandem mass spectrometer (Sciex, Toronto, Canada) equipped with TurboIonSpray and a Shimadzu HPLC system. The software used for controlling this equipment and acquiring and processing the data was Analyst Version 1.4 (Sciex, Toronto, Canada). The positive ion mode for MS/MS analyses was selected. Nitrogen was used as the nebulizer, auxiliary, collision and curtain gas. Analytes were detected by tandem mass spectrometry using multiple reaction monitoring (MRM) with a 200 ms dwell time. The mass transitions were: Dex, *m/z* 201 → 95 and IS, *m/z* 161 → 77.

The instrument response was optimized by using a syringe pump infusion of Dex in mobile phase at a constant flow (10 μL/min) into a stream of mobile phase eluting from the LC column. The main working parameters of the mass spectrometer were: Curtain gas, 20 psi; gas 1 (nebulizer gas) 34 psi; gas 2 (heater gas) 60 psi; collision activate dissociation (CAD) gas 8 psi; TurboIonSpray voltage 2500 V; entrance potential (EP) 10 V; collision energy (CE) 28 V; collision cell exit potential (CXP) 5 V; source temperature 600 °C; and declustering potential (DP) 57 V. Positive ion electrospray MS/MS product ion spectra of Dex and Tolazoline HCl (internal standard) are shown in Fig. 2. Product ion mass spectra of Dex and Tolazoline HCl were obtained using a mobile phase with precursor ion, *m/z* 201.0 and 161.2, respectively, with an infusion rate of 10 μL/min. Although the strongest product ion is *m/z* 91 for Tolazoline HCl, the production ion, *m/z* 77, was chosen because it was the most specific determination for the internal standard in plasma.

2.4. Preparation of standard, quality control (QC) samples and internal standard (IS)

Two stock solutions were prepared for each analyte from independent weighings. Standard solutions were prepared from one stock solution and QC samples were prepared from the other. The primary stock solutions of Dex were prepared by dissolv-

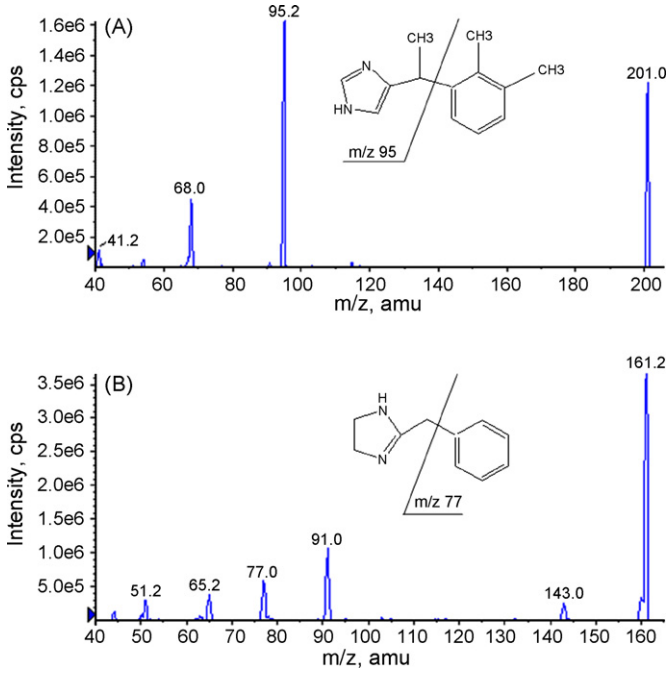

Fig. 2. Representative MRM positive product ion mass spectra for (A) Dex and (B) internal standard.

ing Dex in water producing a concentration of 1.0 mg/mL and were stored at −80 °C. Two stock solutions of concentration 1 μg/mL were freshly prepared by diluting each primary stock solution with water. Working solutions of Dex were freshly prepared by appropriately diluting the respective stock solution with plasma to achieve concentrations of 2.5, 5, and 10 ng/mL for the standard and 12 ng/mL for the QC. Nine standards containing Dex concentrations of 5, 10, 25, 50, 100, 250, 500, 1000 and 1500 pg/mL were prepared by adding the appropriate volumes of working solution into 2.0 mL microcentrifuge tubes containing plasma. Three QC levels were prepared in the same manner by adding appropriate volumes of working solution to obtain concentrations of 15, 300 and 1200 pg/mL, representing low (LQC), medium (MQC), and high (HQC) QCs, respectively.

The internal standard stock solution (1.0 mg/mL) was prepared by dissolving 50 mg of Tolazoline HCl in 5 mL of methanol followed by 45 mL of water and was stored at −4 °C. This solution was further diluted with water to prepare the internal standard working solution of 10 μg/mL of IS. Internal standard solution was prepared by diluting IS working solution with reconstitution buffer (5 mM ammonium acetate and 0.03% formic acid in solvent mixture of 20:20:60, methanol:acetonitrile:water) to a concentration of 150 ng/mL.

2.5. Solid-phase extraction (SPE) procedures

Standard and QC samples were freshly prepared followed by vortexing and centrifuging to ensure homogeneity and absence of large, suspended particles. Waters Oasis HLB 1 mL extraction cartridges were preconditioned with methanol (1 mL) and washed with Milli-Q water (1 mL). The plasma samples (200 μL) were passed through SPE cartridges under centrifugation (1500 rpm, 5 min). The cartridges were washed with 1 mL of 40% methanol in water, and finally analytes were eluted with a solution of 5% 2-propanol, 10% acetonitrile, and 85% methanol. The eluate was dried at 37 °C under nitrogen and reconstituted with 100 μL of 5 mM ammonium acetate and 0.03% formic acid in a solvent mixture of methanol:acetonitrile:water (20:20:60, v/v/v) containing 150 ng/mL internal standard, and 30 μL of samples were injected into the LC–MS/MS system for analysis.

2.6. Method validation

Method validation and documentation were performed according to guidelines set by the United States Food and Drug Administration (FDA) for bioanalytical method validation [8]. This method was validated in terms of linearity, specificity, lower limit of quantitation (LLOQ), recovery, intra- and inter-day accuracy and precision, and stability of analyte during the sample storage and processing procedures. Each analytical run included a double blank sample (without IS), a blank sample (with IS), nine standard concentrations for calibration, and replicate sets ($n = 6$) of QC samples (LQC, MQC and HQC).

2.6.1. Linearity and sensitivity

For the evaluation of the linearity of the standard calibration curve, the analyses of Dex in plasma samples were performed on 3 independent days using fresh preparations. The calibration curves were prepared over a concentration range of 5–1500 pg/mL at nine concentrations: 5, 10, 25, 50, 100, 250, 500, 1000, and 1500 pg/mL. The concentration range was selected to cover the range of concentrations expected in pediatric plasma samples. Each calibration curve consisted of a double blank sample, a blank sample and nine calibrator concentrations. Another double blank sample was analyzed immediately following the highest concentration standard in each run to monitor the carry-over of Dex or the internal standard.

The calibration curve was developed using the following criteria: (1) the mean value should be within ±15% of the theoretical value, except at LLOQ, where it should not deviate by more than ±20%; (2) the precision around the mean value should not exceed a 15% coefficient of variation (CV), except for LLOQ, where it should not exceed a 20% CV; (3) at least 75% of the non-zero standards of each nominal concentration should meet the above criteria; and (4) the correlation coefficient (r) should be greater than or equal to 0.98.

Each calibration curve was constructed by plotting the analyte to internal standard peak area ratio (y) against analyte concentrations (x). The calibration curves were fitted using a quadratic regression model, $y = ax^2 + bx + c$, weighted by $1/x$ using the Analyst® software. The resulting a, b, and c parameters were used to determine back-calculated concentrations, which were then statistically evaluated.

2.6.2. Specificity

The specificity was defined as non-interference at retention times of Dex from the endogenous plasma components and no cross-interference between Dex and IS using the proposed extraction procedure and LC–MS/MS conditions. Six different lots of Dex-free plasma were evaluated with and without internal standard to assess the specificity of the method.

2.6.3. Accuracy and precision

The intra- and inter-assay precisions were determined using the CV (%), and the intra- and inter-assay accuracies were expressed as the percent difference between the measured concentration and the nominal concentration:

$$\%\text{accuracy} = \left(\frac{\text{measured concentration}}{\text{nominal concentration}}\right) \times 100$$

Intra-assay precision and accuracy were calculated using replicate ($n = 6$) determinations for each concentration of the spiked plasma sample during a single analytical run. Inter-assay precision and accuracy were calculated using replicate ($n = 6$) determinations of each concentration made on 3 separate days.

2.6.4. Recovery (extraction efficiency)

The extraction efficiency of Dex was determined by comparing the peak areas of Dex added into blank plasma and extracted using the SPE procedure with those obtained from Dex spiked directly into reconstitution buffer at three concentrations (15, 300, 1200 pg/mL). The following formula was used to calculate

the extraction efficiency:

$$\%\text{recovery} = \left(\frac{\text{measured plasma concentration}}{\text{spiked buffer concentration}}\right) \times 100$$

2.6.5. Stability study

The stability of Dex in human plasma was assessed by analyzing replicates ($n = 6$) of QC samples at concentrations of 15, 300 and 1200 pg/mL, during the sample storage and processing procedures. Freshly prepared and analyzed samples were used for comparison. The short-term stability was assessed after exposure of the plasma samples to room temperature for 24 h. The long-term stability was assessed after storage of the plasma samples at −80 °C for 90 days. The freeze/thaw stability was determined after four freeze/thaw cycles (room temperature to −80 °C). The sample stability in the autosampler tray was evaluated by measuring samples prior to and 24 h after storage in the autosampler tray at 4 °C. This sample stability evaluation mimics the residence time of the samples in the autosampler for each analytic run. The stability of Dex in dried extract residue was assessed after storage at −80 °C for 2 days. The concentrations obtained were compared with the freshly prepared QC samples, and the percentage concentration deviation was calculated. The analytes were considered stable in human plasma when the concentration difference was less than 15% between the freshly prepared samples and the stability testing samples.

3. Results

3.1. Linearity and sensitivity

Correlation coefficients (r) from inter-day analyses over eight calibration curves are greater than 0.998. A representative calibration curve for Dex is shown in Fig. 3. The LLOQ was determined to be 5 pg/mL, with precision and accuracy less than 20% CV and the signal-to-noise ratio (S/N) considerably higher than 10 (Table 1). Representative chromatograms of double blank, blank, LLOQ and the upper limit of quantification (ULOQ) samples are shown in Fig. 4. No carry-over peaks were observed at the retention times and the ion channels of either Dex or the internal standard.

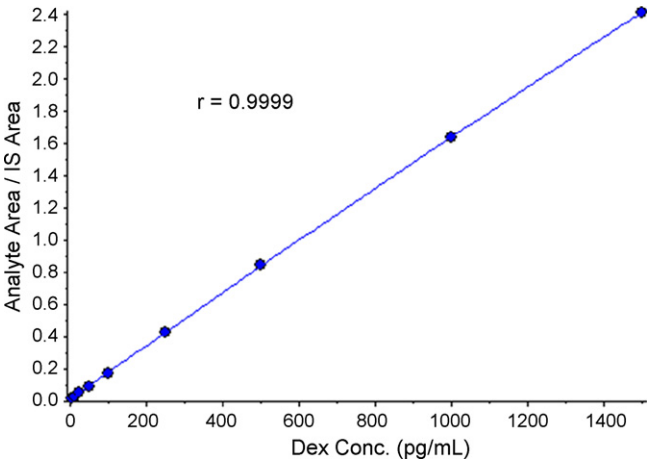

Fig. 3. Representative calibration curve for Dex in human plasma.

3.2. Assay specificity

No significant interfering peaks from endogenous plasma components were found at the retention time corresponding to Dex or the internal standard. Representative chromatograms for double blank control samples (a), blank control samples spiked

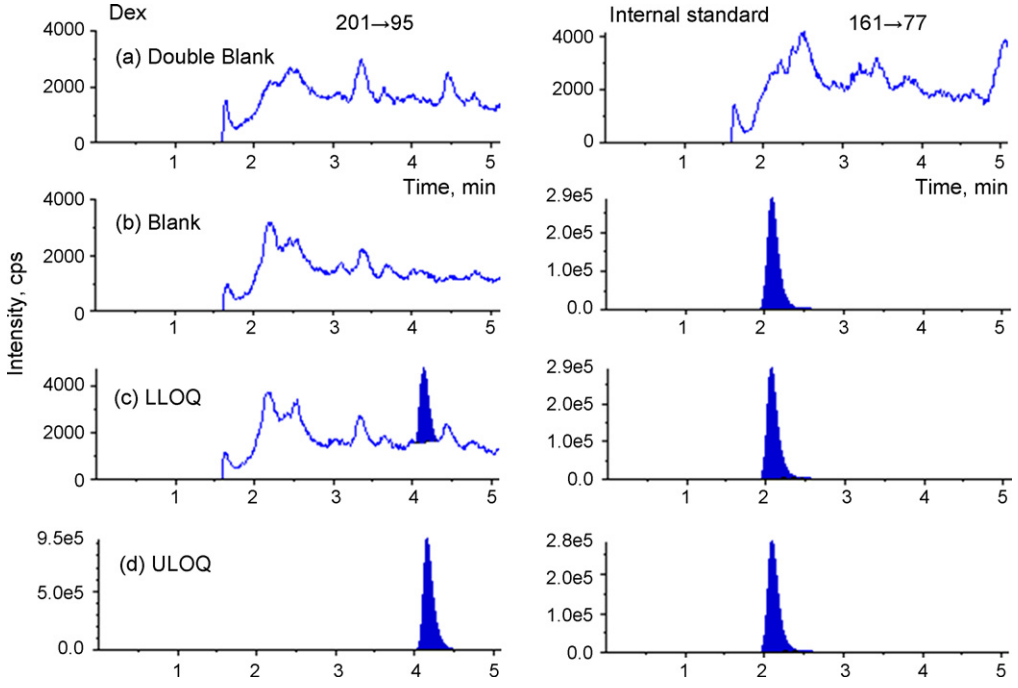

Fig. 4. Representative MRM chromatograms of Dex in human plasma: (a) double blank plasma; (b) blank plasma; (c) LLOQ, 5 pg/mL; and (d) ULOQ, 1500 pg/mL. Dex (left panels, a–d) and its IS (right panels).

Table 1
Specificity and limit of quantitation of Dex in human plasma: lot-to-lot variation

Nominal conc. LLOQ (5 pg/mL)						
Lot #	Measured	*n*	Mean	S.D.	% CV	% Accuracy
Lot 1	4.87	3	4.87	0.69	14.11	97.40
Lot 2	5.36	3	5.36	0.48	8.88	107.27
Lot 3	5.53	3	5.53	0.46	8.25	110.67
Lot 4	5.31	3	5.31	0.49	9.29	106.27
Lot 5	5.67	3	5.67	0.19	3.27	113.33
Lot 6	5.42	3	5.42	0.62	11.37	108.33
n	18					
Mean	5.36					
STDEV	0.27					
% CV	5.07					
% Accuracy	107.21					

with internal standard (b), and samples spiked with analyte (c & d) are shown in Fig. 4.

3.3. Precision and accuracy

The intra- and inter-day precision and accuracy data for Dex was summarized in Table 2. The intra-day precision and accuracy, based on the relative standard deviation replicate for QC samples, ranged from 1.04 to 6.84% and 90.2 to 101%, respectively. The inter-day precision and accuracy ranged from 4.08 to 5.37% and 92.7 to 98.6%, respectively. Table 3 showed the inter-day precision and accuracy of calibration standards ranged from 0.60 to 6.17% and 95.5 to 103%. These data confirm that the present method has a satisfactory accuracy, precision and reproducibility for the quantification of Dex throughout a wide dynamic range.

3.4. Recovery (extraction efficiency)

At 15, 300 and 1200 pg/mL concentration levels ($n = 6$), mean extraction recoveries were $78.32 \pm 6.73\%$, $77.59 \pm 1.43\%$, and $76.59 \pm 2.12\%$, respectively (Table 4). The % CV for all recoveries was less than 8.59% throughout the entire standard concentration ranges, indicating that the extraction efficiency for Dex using SPE was satisfactory and was not concentration-dependent.

3.5. Analyte stability

The stability data from the freeze/thaw and long-term stability tests, as well as for the various process procedures for Dex were summarized in Table 5. The stability experiments of the Dex were designed to cover expected conditions during all of the sample storage, preparation and analysis periods. The precision and accuracy for these freeze–thaw samples ranged from 1.66 to 4.62% and 90.2 to 98.6%, respectively. The results indicated that the analyte was stable in plasma for four cycles when stored at −80 °C and thawed to room temperature. The precision and accuracy for long-term stability samples ranged from 1.15 to 3.40% and 93.2 to 100.7%, respectively. The results of long-term storage stability data indicated that the plasma samples were stable at −80 °C over 90 days. The precision ranged from 1.44 to 4.59% and accuracy ranged from 99.9 to 100.9% for autosampler

Table 2
Intra- and inter-day accuracy and precision of Dex in plasma QC samples

	Nominal conc. (pg/mL)		
	QC low (15)	QC mid (300)	QC high (1200)
Day 1 ($n = 6$)			
Intra-day mean	13.83	272.67	1210.00
S.D.	0.77	18.66	72.66
% CV	5.54	6.84	6.01
% Accuracy	92.22	90.89	100.83
Day 2 ($n = 6$)			
Intra-day mean	14.33	295.33	1165.00
S.D.	0.45	3.83	33.91
% CV	3.14	1.30	2.91
% Accuracy	95.56	98.44	97.08
Day 3 ($n = 6$)			
Intra-day mean	13.53	296.83	1175.00
S.D.	0.70	3.54	12.25
% CV	5.20	1.19	1.04
% Accuracy	90.22	98.94	97.92
Inter-day ($n = 18$)			
Inter-day mean	13.90	288.28	1183.33
S.D.	0.70	15.49	48.26
% CV	5.05	5.37	4.08
% Accuracy	92.67	96.09	98.61

Table 4
Recovery of Dex in human plasma

	Nominal conc. (pg/mL)		
	QC low (15)	QC mid (300)	QC high (1200)
% Recovery	78.32	77.59	76.59
S.D.	6.73	1.43	2.12
% CV	8.59	1.84	2.77
n	6	6	6

Table 3
Inter-day accuracy and precision of calibration standards in human plasma

Nominal concentration (pg/mL)	5	10	25	50	100	250	500	1000	1500
Measured concentration (pg/mL)									
Inter-day mean	4.78	10.29	25.17	49.70	101.00	253.14	495.43	987.00	1501.43
S.D.	0.29	0.58	0.71	1.94	6.21	14.75	14.81	41.47	9.00
% CV	6.17	5.67	2.82	3.91	6.15	5.83	2.99	4.20	0.60
% Accuracy	95.51	102.90	100.69	99.40	101.00	101.26	99.09	98.70	100.10
n (day)	7	7	7	7	7	7	7	7	7

Table 5
Stability data for Dex under various conditions

Storage period and storage condition	Nominal conc. (pg/mL)	Mean	S.D.	% CV	% Accuracy	*n*
Freeze/thaw stability						
Four freeze/thaw cycles, −80 °C	15	13.53	0.63	4.62	90.22	6
	300	292.67	5.09	1.74	97.56	6
	1200	1183.33	19.66	1.66	98.61	6
Long term stability						
3 Months, −80 °C	15	15.10	0.51	3.40	100.67	6
	300	290.33	3.33	1.15	96.78	6
	1200	1118.33	26.39	2.36	93.19	6
Autosampler stability						
Re-injected samples at 4 °C; 24 h	15	15.13	0.69	4.59	100.89	6
	300	300.00	6.51	2.17	100.00	6
	1200	1198.33	17.22	1.44	99.86	6
Process sample stability						
Plasma sample at RT; 24 h	15	13.12	0.81	6.16	87.44	6
	300	273.33	10.69	3.91	91.11	6
	1200	1103.33	28.05	2.54	91.94	6
Process sample stability						
Final extract, dry residue, −80 °C; 48 h	15	13.50	0.11	0.81	90.00	6
	300	264.00	0.59	0.22	88.00	6
	1200	1067.00	6.21	0.58	88.92	6

stability study. The result suggested that Dex could be analyzed over 24 h in the autosampler tray at 4 °C with acceptable precision and accuracy. The precision and accuracy for the short-term stability (process sample stability) test ranged from 2.54 to 6.16% and 87.4 to 91.9%, respectively. It indicated reliable stability behavior under the experimental conditions of the analytical runs. The precision and accuracy for the extracted dry residue ranged from 0.22 to 0.81% and 88.0 to 90.0%, respectively. The result suggested that the extracted dry residue could be stored at −80 °C for at least 48 h during the sample processing procedures without compromising the integrity of the sample. The results of stability experiments showed that no stability-related problems occurred during sample storage, extraction and chromatography processes for Dex in plasma samples. In addition, standard stock solution of Dex was stable over 90 days at −80 °C.

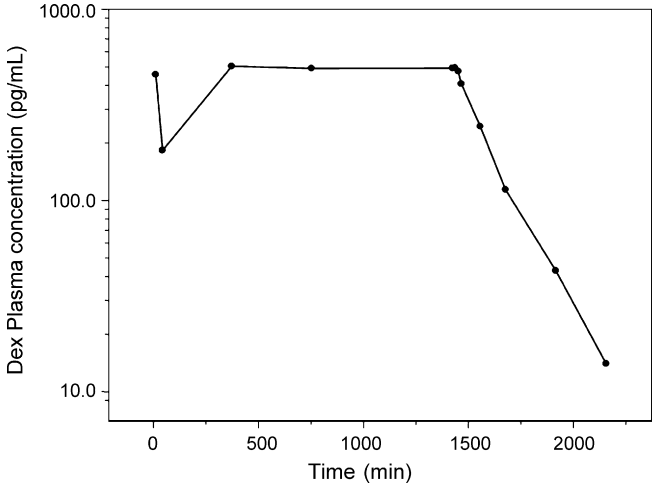

Fig. 5. Representative Dex concentration–time curve.

3.6. Application

This LC–MS/MS assay has been successfully used to determine the pharmacokinetic profile of Dex in the infant population. Infants post-operative from open heart surgery enrolled in this study were administered a bolus dose of Dex (0.35, 0.5 or 1 mcg/kg) followed by a continuous intravenous infusion (0.25, 0.5 or 0.75 mcg/kg/h). Up to 14 blood samples (1 mL each) were obtained for determination of Dex plasma concentration. A representative plasma concentration–time profile is represented in Fig. 5.

4. Discussion

Differences in drug disposition between children and adults are well recognized. Without the knowledge of drug disposition in these specialized populations, young children continue to receive drugs off-label, carrying the risk of excessive toxicity and sub-optimal therapy. The absence of quantitation assays suitable for the analyses of drug concentrations using small plasma volumes limits the study of drug disposition in small children. With a median weight of 3.5 kg (range 2.5–4.3) for healthy newborns and 12.4 kg (range 10.2–15.2) for healthy infants at 2 years of age [9], pharmacokinetic blood sampling in these age groups must be minimized to avoid complications of iatrogenic anemia. Furthermore, Dex is primarily used in critically ill populations who often already suffer from anemia and frequent phlebotomies for laboratory analysis. It is even more imperative that limited blood sampling is applied to this vulnerable population when designing pharmacokinetic studies.

Several analytical methods have been published to quantify Dex in plasma. These methods include gas chromatography with tandem mass spectrometry (GC/MS) [5], LC–MS/MS [6], and

radioreceptor assays [7]. Unfortunately, these published methods are not suitable for quantifying Dex in pediatric plasma samples. The GC–MS method is a sensitive method developed successfully for the determination of Dex in human plasma with a LLOQ of 50 pg/mL. Unfortunately, this assay requires the use of 1 mL of plasma for each sample analysis. With an average neonatal hematocrit of 50% (range 42–66%) [10], it would require a minimum of 2 mL of blood for each pharmacokinetic sample. Current limitations on blood sampling for research in neonates would allow for no more than six samples to be drawn for pharmacokinetic evaluation with no margin of error for fluctuations in hematocrit or losses while obtaining and handling the specimens. Furthermore, this assay requires chemical derivation for sample preparation, a method not easily applied for the analysis of biological specimens. Recently, a specific and sensitive LC–MS/MS method has been reported with LLOQ of approximately 20 pg/mL. In contrast to the GC-MS method, this assay does not require chemical derivation for sample preparation. However, this assay requires a plasma volume of 900 μL for each specimen analysis. Again, this volume is not suitable for pharmacokinetic studies in young children. The radioreceptor assay has been described for the concentration determination of Dex using small plasma volumes in rats. This radioreceptor assay was used to quantitate Dex levels in rat plasma using volumes of only 200 μL, and reported a lower limit of quanitification of 24 pg/mL. However, the routine use of radioactive labels for pharmacokinetics studies has several disadvantages including health hazards, appropriate elimination of radioactive waste, and special licensing requirements.

The LC–MS/MS method that we developed overcomes the pharmacokinetic sampling limitations in performing pediatric pharmacokinetic studies of Dex using previously described methods. Our method is accurate and validated and does not require chemical derivation or the use of radioactive material. More importantly, the small sample volumes (200 μL) used in this method allow for a five-fold increase in specimens for analysis. Through the use of small sample volumes, pharmacokinetic modeling and selective pharmacokinetic sampling techniques, an accurate and informative pharmacokinetic profile of Dex in neonates and infants can be developed while minimizing the risks of excessive blood sampling in this vulnerable population.

5. Conclusion

A sensitive, accurate and reproducible LC–MS/MS method has been developed and validated for the measurement of Dex in human plasma using limited sample volumes. Using a plasma volume of only 200 μL, this method has a lower limit of quantitation of 5 pg/mL, a linear range of 5–1500 pg/mL, and is well-suited for use in pediatric pharmacokinetic studies.

Acknowledgements

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