Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 897–904

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Determination of dexmedetomidine in human plasma using high performance liquid chromatography coupled with tandem mass spectrometric detection: Application to a pharmacokinetic study

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ARTICLE INFO

Article history:
Received 3 February 2009
Received in revised form 5 June 2009
Accepted 5 June 2009
Available online 16 June 2009

Keywords:
Dexmedetomidine
HPLC-MS/MS
Human plasma
Pharmacokinetics

ABSTRACT

A rapid, sensitive and selective high performance liquid chromatography-electrospray ionization-tandem mass spectrometry method (HPLC-ESI-MS/MS) was developed and validated for the determination and pharmacokinetic investigation of dexmedetomidine (DMED) in human plasma. Dexmedetomidine and the internal standard (ondansetron) were extracted in a single step with diethyl-ether from 1.0 mL of alkalinized plasma. The mobile phase was a mixture of acetonitrile and 0.5% formic acid solution (30:70, v/v) at a flow rate of 0.2 mL min^{-1}. The detection was performed on a triple quadrupole tandem mass spectrometer in the selected reaction monitoring (SRM) mode using the respective $[M+H]^+$ ions *m/z* 201.0 → 95.1 for DMED and *m/z* 294.1 → 170.1 for the IS. The assay exhibited a linear dynamic range of 5–5000 pg mL^{-1} with the correlation coefficient above 0.9995. The lower limit of quantification (LLOQ) was 5 pg mL^{-1} with a relative standard deviation of less than 15%. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The validated HPLC-MS/MS method has been successfully applied to study the pharmacokinetics of three level doses of DMED in Chinese healthy volunteers.

1. Introduction

Dexmedetomidine (DMED, 4-[(1S)-1-(2, 3-dimethylphenyl)-ethyl]-1H-imidazole), a relatively selective alpha$_2$-adrenoceptor agonist, possesses potent sympatholytic effects with ensuing sedation, hemodynamic stabilization and analgesic effects in patients undergoing surgery. Dexmedetomidine (DMED) played an important role in the clinical application for its distinguished therapeutic effect.

However, very few methods have been published for the quantification of DMED in plasma. Hui et al. [1] developed a GC–MS method for the determination of DMED in plasma over concentration ranges of 0.1–40 ng mL^{-1}. Hence, the GC–MS method was time-consuming and complicated because of the multi-step derivatization procedure. Zhao et al. [2] published a paper on the study of clinical pharmacokinetics of DMED by an HPLC-MS/MS method with a quantitation of DMED over concentration ranges of 22.4–5725.0 pg mL^{-1}. Mallet et al. [3] proposed a method for the determination of DMED by HPLC-MS/MS with an LLOQ of 19 pg mL^{-1}. All of these methods were insufficiently sensitive for the determination of low plasma concentrations in pharmacokinetic studies of DMED. A more sensitive HPLC-MS/MS method with an LLOQ of 5 pg mL^{-1} was also developed and validated in pediatric plasma [4]. However, this method was based on a solid-phase extraction (SPE) procedure, which was laborious, costly and time-consuming. In addition, the preparation of the mobile phase was tedious.

A rapid and highly selective HPLC-MS/MS method for the quantification of DMED in human plasma was developed and validated in this investigation. The procedure exhibited excellent performance in terms of high sensitivity acquisition with a LLOQ of 5 pg mL^{-1} and rapid analytical speed (4.0 min per sample). A comprehensive study of the stability of DMED was also performed during this investigation. The validated HPLC-MS/MS method has been successfully applied to a clinical pharmacokinetic study of intravenous administration of low (0.5 μg kg^{-1}), middle (1.0 μg kg^{-1}) and high (1.5 μg kg^{-1}) dose of DMED to Chinese healthy volunteers.

2. Experimental

2.1. Chemicals and materials

DMED reference standard (purity 99.86%) was supplied by Sichuan Baili Pharmaceutical Company (Chengdu, PR China).

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doi:10.1016/j.jpba.2009.06.012

Ondansetron reference standard (IS, purity 98.70%) was obtained from Chengdu Hengrui pharmaceutical company (Chengdu, PR China). HPLC grade acetonitrile was purchased from VWR International Company (Darmstadt, Germany). Other reagents of analytical grade such as formic acid, diethyl ether and Na_2CO_3 were purchased from Nanjing Chemical Reagent Co., Ltd. Methanol (analytical reagent) was purchased from Jiangsu Hanbon Sci. & Tech. (Nanjing, PR China). Water was distillated twice before use using BSZ-2 water purification system from Botonyc company (Shanghai, PR China). The mobile phase was filtrated through a 0.22 μm cellulose membrane filter before use.

2.2. HPLC-MS/MS instrumentation

The liquid chromatography coupled to tandem mass spectrometric detection system (Thermo Electron Corporation) consisted of a Finnigan™ Surveyor LC pump, an auto-sampler (Finnigan Surveyor), an on-line vacuum degasser and a triple quadrupole TSQ Quantum mass spectrometer (Thermo Electron Corporation). The system was run by Xcalibur 2.0 software (Thermo Electron Corporation).

2.3. Chromatographic conditions

Chromatographic separations were carried-out by using a 5.0 μm particle size Agilent ZORBAX SB-CN column (150 mm × 2.1 mm I.D.) whose temperature was maintained at 40 °C. Samples were eluted isocratically with a mobile phase consisting of acetonitrile:0.5% formic acid (30:70, v/v) delivered at a flow rate of 0.2 mL min^{-1}. The auto sampler temperature was maintained at 15 °C and the injection volume was 10 μL. The total HPLC run time was 4.0 min.

2.4. Mass spectrometric condition

The tandem MS system is equipped with an electrospray ionization source (ESI). The mass spectrometer was operated in positive ion and SRM mode with precursor to product qualifier transition *m/z* 201.0 → 95.1 for DMED and *m/z* 294.1 → 170.1 for IS. All the parameters of LC and MS were controlled by Xcalibur 2.0 software. Spray voltage was optimized at 4800 V, transfer capillary temperature at 350 °C, sheath gas and auxiliary gas (nitrogen) pressure at 25 and 10 arbitrary unites (set by the LCQ software, Thermo Electron Corporation), respectively. Argon was used as collision gas at a pressure of 1.5 mTorr and collision energy (CE) was 22 and 32 V for DMED and IS, respectively. The scan width for SRM was 0.01 *m/z* and scan time was 0.3 s. The peak width settings (FWHM) for both Q1 and Q3 were 0.7 *m/z*.

2.5. Preparation of stock solutions

Standard stock solutions of DMED and IS were prepared in methanol at concentrations of 1.0 mg mL^{-1}, respectively. Stock solutions were stored at 4 °C until they were used for working solutions by adding appropriate volume of mobile phase (acetonitrile and 0.5% formic acid solution, 30:70, v/v) for DMED and methanol for IS. Working solutions of different concentration were prepared from above-mentioned stock solution afresh before use.

2.6. Sample preparation

Liquid–liquid extraction was chosen for the sample preparation. All frozen human plasma samples were thawed in a water bath not exceeding 40 °C. Plasma sample (1.0 mL) was extracted with 5.0 mL of diethyl ether after addition of 10 μL IS solution (50 ng mL^{-1} ondansetron in methanol) and 100 μL saturated Na_2CO_3 solution. The mixture was vortexed for 3.0 min, and then centrifuged at 2951 × *g* for 10.0 min. The upper organic layer was transferred and evaporated to dryness under a gentle stream of nitrogen in a water bath not exceeding 40 °C. The dry residue was finally redissolved in 200 μL mobile phase under vortex and centrifuged at 13,772 × *g* for 8.0 min and 10 μL supernatant was injected into the HPLC-MS/MS system.

2.7. Calibration standards and quality control samples

Blank plasma was withdrawn from the deep freezer and allowed to get completely thawed before use. Calibration curves were prepared on five different days by spiking blank plasma with proper volume of one of the working solutions mentioned above to produce the standard curve points equivalent to 5, 10, 50, 100, 500, 1000, 2000 and 5000 pg mL^{-1} of DMED. The following assay procedures were the same as described above. In each run, a blank plasma sample (processed without IS) was analyzed to confirm absence of interference but not used to construct the calibration function.

Quality control (QC) samples were prepared by spiking blank plasma with proper volume of one of the working solution mentioned above to produce a final concentration equivalent to 10 pg mL^{-1} (low level), 500 pg mL^{-1} (middle level) and 2000 pg mL^{-1} (high level) of DMED. The following procedures were the same as that described above.

2.8. Method validation

2.8.1. Linearity and sensitivity

Calibration curves were generated by using the ratios of the analyte peak area to the IS peak area versus concentration and were fitted to the equation $y = bx + a$ by weighted least-squares linearity regression.

The limit of detection (LOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively. Each back-calculated concentration should meet the following acceptable criteria: no more than 20% deviation at LLOQ and no more than 15% deviation above LLOQ.

2.8.2. Specificity

Blank plasma samples of healthy human used for testing specificity of the method were obtained from six different sources. Each of blank plasma was tested for the visible interference. The potential matrix effect on the ionization of DMED and IS were evaluated by comparing the peak area of DMED and IS resolved in the supernatant of the processed blank plasma to that of standard solutions at the same concentration. Three different concentration levels of DMED (10, 500 and 2000 pg mL^{-1} in plasma) were evaluated by analyzing five samples at each set. The matrix effect of IS (500 pg mL^{-1} in plasma) was evaluated by the same method.

2.8.3. Accuracy and precision

The intra-batch precision and accuracy was determined by analyzing five sets of spiked plasma samples of DMED at each QC level (10, 500 and 2000 pg mL^{-1} in plasma) in a batch. The inter-batch precision and accuracy was determined by analyzing five sets of spiked plasma samples of DMED at each QC level in three consecutive batches. The concentration of each sample was calculated using standard curve prepared and analyzed on the same day.

2.8.4. Recovery (extraction efficiency)

The absolute extraction recovery of DMED at three QC samples was determined by measuring the peak area responses from plasma samples spiked with particular working solution of DMED before extraction with those from drug-free plasma samples extracted

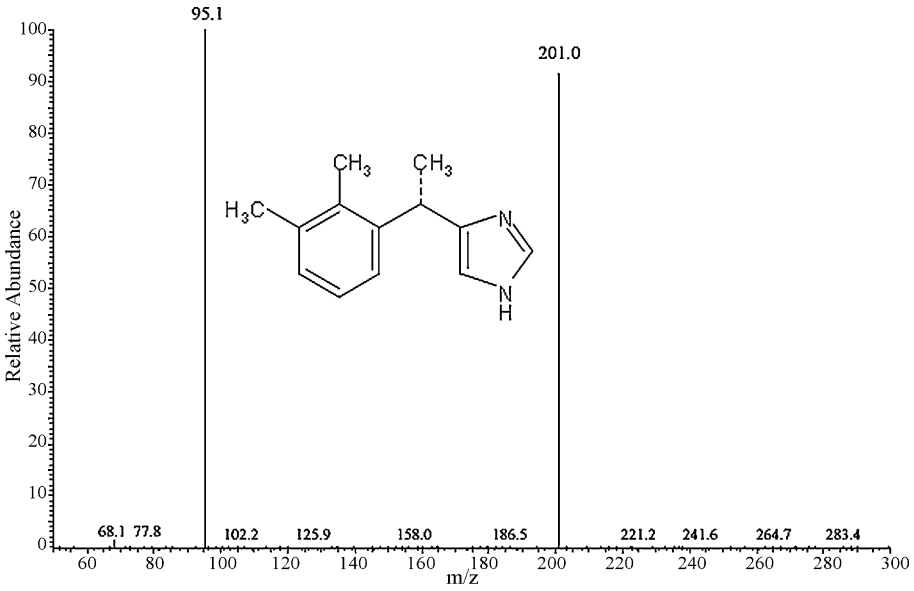

Fig. 1. The positive ion ESI-MS/MS spectrum of DMED and its chemical structure.

and spiked with same concentration of DMED after extraction. The recovery of DMED was determined using five replicates of each QC sample.

2.8.5. Stability study

The QC plasma samples were kept at room temperature for 6 h, extracted and then analyzed for short-term stability study. The QC plasma samples spiked with DMED, which were stored at −20 °C for 48 h, then extracted and analyzed. The post-preparative stability was measured by determining QC samples kept under the auto-sampler condition (15 °C) for 48 h. The freeze thawing stability study was evaluated by comparing the QC plasma samples that had been frozen and thawed three times, with the plasma samples freshly prepared and analyzed.

The stock solution stability of DMED and the IS were evaluated by analyzing their working solutions kept at room temperature for 24 h, respectively.

2.9. Application of the assay

The analytical method proposed and validated here was successfully applied in a pharmacokinetic study of DMED in healthy human volunteers. The study was an open, randomized, crossed study to assess pharmacokinetic behavior of DMED in eighteen healthy male volunteers and ten healthy female volunteers following intravenous administration of low ($0.5\,\mu g\,kg^{-1}$), middle ($1.0\,\mu g\,kg^{-1}$) and high ($1.5\,\mu g\,kg^{-1}$) doses of DMED. Blood samples (3.0 mL) were sampled before intake and at 0.083, 0.17, 0.33, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h after intravenous administration. They were put into lithium heparin tubes and were immediately centrifuged at $2951 \times g$ for 5.0 min. The plasma obtained was frozen at −20 °C until analysis. The plasma samples must be prepared as quickly as possible for its unstability at the room temperature and being frozen.

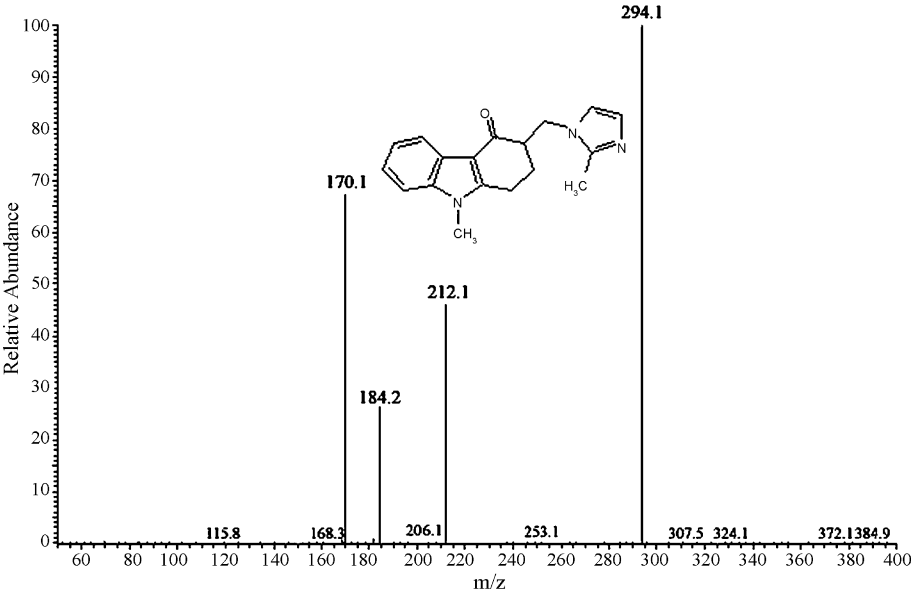

Fig. 2. The positive ion ESI-MS/MS spectrum of IS and its chemical structure.

3. Results and discussion

3.1. Sample preparation

Sample extraction techniques such as protein precipitation and liquid–liquid extraction were all attempted, a one-step liquid–liquid extraction can produce a relative clean sample and reduce the possibility of introducing highly polar materials into the column and MS system. A preliminary evaluation of different extraction solvents such as diethyl ether, ethyl acetate or dichloromethane–ethyl acetate (20:80, v/v) under acidifying, alkalizing or neutral conditions showed that extraction with diethyl ether in alkalizing condition exhibited higher extraction efficiency. Then, NaOH, saturated Na_2CO_3 solution and saturated $NaHCO_3$ solution were all attempted. Finally, 100 μL of saturated Na_2CO_3 solution was adopted for sample preparation in terms of precise, consistent and reproducible recoveries.

3.2. HPLC-MS/MS optimization

The HPLC-MS/MS method for the determination of DMED in human plasma was investigated. Tandem mass spectrum analysis was carried out by electrospray ionization. Both the positive and negative modes were investigated and the response of positive ions was stronger than negative ions, which indicate that the positive mode was much more sensitive. Hence, it was employed in the following experiments. In the precursor ion full scan spectra, the most abundant ions were protonated quasimolecular ion $[M+H]^+$ with *m/z* 201.0 for DMED and *m/z* 294.1 for IS. And the product ions scan spectra was also investigated for using SRM mode, the most prominent product ions were *m/z* 95.1 for DMED and *m/z* 170.1 for IS, respectively. Other main mass spectrometry parameters such as spray voltage, capillary temperature, sheath gas and auxiliary gas pressure, source CID, collision gas pressure and collision energy, were also optimized by continuous infusion of a standard solution of DMED (1 μg mL^{-1}) and the IS (1 μg mL^{-1}) with a TSQ Quantum electronically controlled integrated syringe and the TSQ Quantum Tune program. Finally, the transition ions of *m/z* 201.0 → 95.1 for DMED and *m/z* 294.1 → 170.1 for the IS were set as detecting ions for obtaining maximum sensitivity. The positive ion ESI-MS/MS spectrum of DMED and IS are shown in Figs. 1 and 2.

To achieve symmetric peak shape and a short run time for the analysis of DMED and the IS, mobile phases such as acetonitrile–formic acid solution, methanol–formic acid solution, acetonitrile–water, methanol–water, acetonitrile–aqueous ammonium acetate solution (10 mM) and methanol–aqueous ammonium acetate solution (10 mM) with different ratios were investigated for optimization. Employing acetonitrile and 0.5% formic acid solution (30:70, v/v) as the mobile phase, DMED and the IS were eluted rapidly with symmetric peak shape within 4.0 min.

3.3. Assay selectivity and matrix effect

The representative chromatograms of blank plasma (Fig. 3A), blank plasma spiked with DMED at the LLOQ of 5 pg mL^{-1} (Fig. 3B) and volunteer plasma sample at 0.5 h after a single intravenous administration of 28.5 μg of DMED (Fig. 4A) mean that no endogenous interference were present at the retention times of 2.65 min (DMED) and the 2.55 min (IS). All the ratios of the peak area of the analytes dissolved in the supernatant of the processed blank plasmas (from five different sources of human plasma) compared to that of the standard solutions at the same concentration were between 85% and 115%, which means that no co-eluting 'invisible' matrix components could significantly influenced the ionization of DMED and the IS.

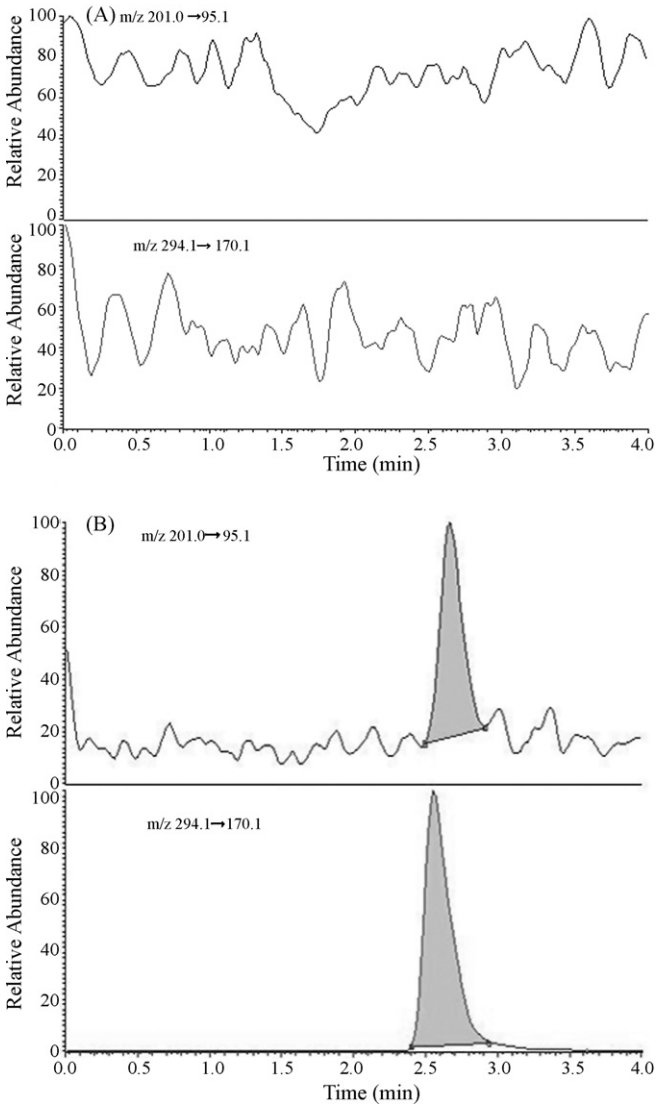

Fig. 3. Representative SRM chromatograms for DMED and the IS resulting from analysis of (A) blank plasma (drug and IS free) and (B) 5 pg mL^{-1} (LLOQ) of DMED in human plasma.

3.4. Linearity of calibration curves

The method exhibited excellent linear response over the selected concentration range of 5–5000 pg mL^{-1} by weighted ($1/x^2$) least-squares linear regression analysis. The mean standard curve was typically described by the equation: $y = 466.52x - 0.20314$, $r = 0.9995$, where x corresponds to the peak area ratio of DMED to the IS and y refers to the concentration of DMED added to plasma. The use of the weighted regression resulted in less than 10% deviation between the nominal and experimental concentrations calculated from the equations. Results of five representative standard curves for LC–MS/MS determination of DMED are given in Table 1.

The lower limit of quantification for DMED proved to be 5 pg mL^{-1}, and the lower limit of detection was 3 pg mL^{-1}.

3.5. Precision and accuracy

An assessment of intra-batch and inter-batch precisions was conducted by analyzing quality control (QC) samples at three levels. Data of precisions are presented in Table 2. The QC concentrations

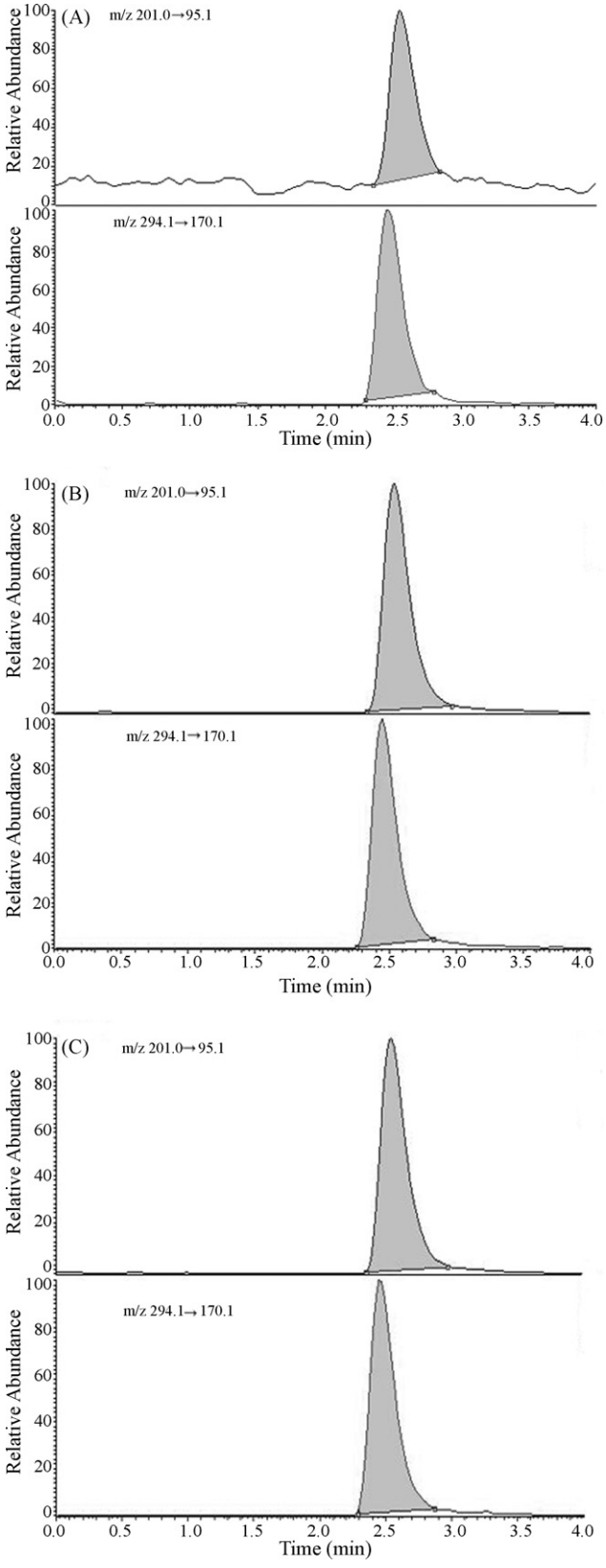

Fig. 4. The chromatograms of the plasma samples at 0.5 h after a single intravenous administration of (A) 28.5 μg (0.5 μg kg^{-1} × 57 kg), (B) 60.0 μg (1.0 μg kg^{-1} × 60 kg) and (C) 93.0 μg (1.5 μg kg^{-1} × 62 kg) dose of DMED.

were calculated from calibration curve and the intra-batch and inter-batch precision was less than 10% and the accuracy deviation values for intra-batch and inter-batch were all within (100 ± 15)% of the actual values at each QC level. The results revealed good precision and accuracy.

3.6. Extraction efficiency

The data of extraction efficiency measured for DMED and the IS in human plasma was consistent, precise and reproducible. The mean absolute extraction recovery of DMED at each QC level (10, 500 and 2000 pg mL^{-1}) was ranged from 87.23 to 89.46%, while the precision values ranged from 9.25 to 9.92%, respectively. Data of extraction recoveries of DMED are presented in Table 3.

3.7. Stability

The stability experiments were performed thoroughly to evaluate the stability of DMED and IS in stock solutions and in plasma samples under different conditions. Each stability test in plasma included three replicates of three levels (10, 500 and 2000 pg mL^{-1}) of QC samples. The stability of spiked QC samples was compared with freshly prepared quality control samples. The results obtained were well within the acceptable limits.

The standard stock solutions of DMED and IS were stable at 4 °C for 30 days and the working solutions of DMED and IS were stable at room temperature for 24 h. The spiked plasma samples of DMED and IS stored at −20 °C were found stable for 48 h. The freeze-thaw stability was also examined. After three freeze-thaw cycles, the samples analyzed were found stable.

Post-preparative stability was determined by analyzing the extracted QC plasma samples kept in auotsampler at 15 °C for 48 h. For the unstability and lower concentration of DMED, the short-term stability of spiked QC samples was examined at 2, 4, 6 and 8 h, respectively. The data showed the above samples were stable at room temperature for 6 h.

Tables 4a–4d summarize the results of stability experiments of DMED. The data showed the reliable stability behavior of DMED under the tested condition.

3.8. Application of the analytical method to a pharmacokinetic study

The validated method was successfully applied for a pharmacokinetic study of dexmedetomidine in twenty-eight healthy volunteers after intravenous administration of 0.5, 1.0 and

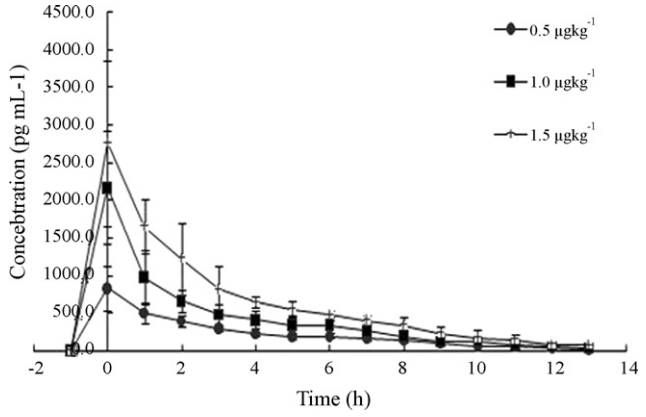

Fig. 5. Mean drug plasma concentration–time curve of DMED from the twenty-eight subjects (three level doses) after intravenous injection.

Table 1
The results of five calibration curves for determining DMED in human plasma.

Concentration added (pg mL^{-1})	Assay	5	10	50	100	500	1000	2000	5000
	1	4.60	10.19	42.63	93.58	500.56	930.88	1951.35	5332.51
	2	5.85	11.69	53.48	93.24	494.01	803.08	1764.38	4494.08
Concentration found (pg mL^{-1})	3	5.89	10.30	47.74	93.79	474.25	1161.71	2201.64	5738.78
	4	6.24	11.24	51.97	83.91	550.79	880.23	1869.29	4820.65
	5	4.85	9.81	45.71	86.16	574.26	901.41	2020.49	5046.88
Mean (pg mL^{-1})		5.49	10.64	48.31	90.14	518.77	935.46	1961.43	5086.58
SD (pg mL^{-1})		0.71	0.79	4.46	4.73	41.93	135.04	164.78	476.76
Precision (%)		13.01	7.38	9.22	5.24	8.08	14.44	8.40	9.37
Accuracy (%)		109.71	106.44	96.61	90.14	103.75	93.55	98.07	101.73

Table 2
The precision and accuracy of the method for determining DMED in human plasma.

Concentration added (pg mL^{-1})	Inter-batch (n = 15)			Intra-batch (n = 5)		
	Concentration found (mean ± SD, pg mL^{-1})	Accuracy (%)	Precision (%)	Concentration found (mean ± SD, pg mL^{-1})	Accuracy (%)	Precision (%)
10	10.54 ± 0.78	105.38	7.37	10.37 ± 0.57	103.70	5.51
500	546.28 ± 37.87	109.26	6.93	560.64 ± 13.15	112.13	2.35
2000	2200.84 ± 114.40	110.04	5.20	2241.60 ± 93.63	112.08	4.18

Table 3
The extraction recoveries of DMED in human plasma (n = 5).

Concentration (pg mL^{-1})	Recovery of extraction (%)					Mean ± SD (%)
10	81.33	89.48	94.40	78.34	97.24	88.16 ± 8.16
500	82.02	99.04	83.96	83.01	99.27	89.46 ± 8.88
2000	78.71	99.85	86.48	81.23	89.87	87.23 ± 8.30

Table 4a
The post-preparative stability of DMED in human plasma (n = 3, auto sampler, 15 °C).

Concentration added (pg mL^{-1})	Concentration found (mean, pg mL^{-1})					Mean (pg mL^{-1})	SD (pg mL^{-1})	Precision (%)	Accuracy (%)
	Time = 0 h	Time = 6 h	Time = 12 h	Time = 24 h	Time = 48 h				
10	9.56	10.06	11.10	9.98	10.71	10.13	1.36	13.38	101.32
500	557.27	583.61	568.68	464.96	574.02	546.49	51.55	9.43	109.30
2000	2092.00	2290.76	2295.43	2260.29	2229.54	2233.60	149.74	6.70	111.68

Table 4b
The stability of DMED stored at −20 °C (n = 3).

Concentration added (pg mL^{-1})	Concentration found (mean, pg mL^{-1})			Mean (pg mL^{-1})	SD (pg mL^{-1})	Precision (%)	Accuracy (%)
	Time = 0 h	Time = 24 h	Time = 48 h				
10	9.56	10.36	11.27	10.40	1.39	13.39	104.00
500	557.27	562.72	560.76	560.25	33.72	6.02	112.05
2000	2092.00	1938.04	2006.61	2012.22	178.80	8.89	100.61

Table 4c
The short-term stability of DMED in human plasma (n = 3, 20 °C).

Concentration added (pg mL^{-1})	Concentration found (mean, pg mL^{-1})				Mean (pg mL^{-1})	SD (pg mL^{-1})	Precision (%)	Accuracy (%)
	Time = 0 h	Time = 2 h	Time = 4 h	Time = 6 h				
10	9.56	10.63	9.94	10.69	10.21	1.02	9.97	102.06
500	557.27	569.34	566.59	572.11	566.33	18.42	3.25	113.27
2000	2092.00	2289.22	2287.92	2279.00	2237.03	176.80	7.90	111.85

Table 4d
The freeze and thaw stability of DMED in human plasma (n = 3, 3 cycles, −20 °C).

Concentration added (pg mL^{-1})	Concentration found (mean, pg mL^{-1})		Mean (pg mL^{-1})	SD (pg mL^{-1})	Precision (%)	Accuracy (%)
	0	3 cycles				
10	9.56	9.99	9.78	0.84	8.62	97.79
500	557.27	551.88	554.57	34.43	6.21	110.91
2000	2092.00	2215.89	2153.95	172.99	8.03	107.70

Table 5a
The Pharmacokinetic parameters of low dose (0.5 μg kg^{-1}) DMED.

PK parameter	T_{max} (h)	C_{max} (pg mL^{-1})	AUC_{0-8} (pg h mL^{-1})	$AUC_{0-\infty}$ (pg h mL^{-1})	MRT (h)	$t_{1/2}$ (h)	CL (L h^{-1})	V (L)
1	0.00	341.99	919.71	940.00	2.51	1.10	28.19	44.83
2	0.00	450.53	966.25	1040.71	2.83	1.70	28.83	70.50
7	0.00	897.38	1113.12	1297.57	2.48	2.95	23.12	98.31
15	0.00	1043.68	961.37	1115.73	2.62	3.02	25.99	113.44
16	0.00	997.21	1036.57	1207.84	2.83	2.58	24.84	92.33
18	0.083	574.92	1129.67	1247.99	2.71	2.10	27.64	83.94
19	0.00	969.65	870.23	1040.12	2.22	3.69	35.57	189.64
20	0.00	973.13	803.50	815.90	2.26	1.18	34.93	59.52
30	0.00	1238.78	1202.10	1268.67	2.37	1.91	19.71	54.31
Mean	0.0092	831.92	1000.26	1108.26	2.54	2.25	27.65	89.65
SD	0.028	302.58	130.58	163.65	0.23	0.88	5.15	43.60

Table 5b
The pharmacokinetic parameters of middle dose (1.0 μg kg^{-1}) DMED.

PK parameter	T_{max} (h)	C_{max} (pg mL^{-1})	AUC_{0-8} (pg h mL^{-1})	$AUC_{0-\infty}$ (pg h mL^{-1})	MRT (h)	$t_{1/2}$ (h)	CL (L h^{-1})	V (L)
3	0.00	3542.57	1776.81	1988.23	2.20	2.60	29.17	109.46
4	0.00	2739.64	1653.96	1721.63	2.22	1.40	35.43	71.45
5	0.00	1486.51	1346.21	1425.37	2.26	1.99	53.32	152.94
14	0.00	1933.90	1018.46	1125.21	1.95	2.68	55.99	216.43
21	0.00	2579.20	1603.58	1664.84	2.22	1.66	36.04	86.18
23	0.00	1221.21	1261.62	1334.45	2.50	1.64	56.20	132.63
24	0.00	2627.19	1914.87	1948.70	2.23	1.37	27.71	54.80
25	0.00	2595.76	2598.72	2928.84	2.40	2.64	26.63	101.37
28	0.00	1389.04	1365.12	1459.89	2.25	2.10	41.78	126.43
29	0.00	1621.93	1238.36	1297.62	2.44	1.52	39.30	86.02
Mean	0.00	2173.69	1776.81	1297.62	2.44	1.52	39.30	86.02
SD	0.00	752.73	1653.96	1666.84	2.27	1.86	40.38	110.70

Table 5c
The Pharmacokinetic parameters of high dose (1.5 μg kg^{-1}) DMED.

PK parameter	T_{max} (h)	C_{max} (pg mL^{-1})	AUC_{0-8} (pg h mL^{-1})	$AUC_{0-\infty}$ (pg h mL^{-1})	MRT (h)	$t_{1/2}$ (h)	CL (L h^{-1})	V (L)
6	0.00	1978.14	2248.80	2531.62	2.49	2.56	34.37	126.72
9	0.00	2314.59	2508.98	2703.73	2.39	2.09	37.17	111.91
10	0.00	2237.63	3036.56	3418.20	2.83	2.00	27.21	78.60
11	0.00	1743.24	2116.68	2268.72	2.17	2.19	37.69	118.84
12	0.00	4535.72	2378.37	2607.46	2.17	2.43	28.76	100.76
17	0.00	3325.75	3060.50	3403.48	2.37	2.12	32.17	98.41
22	0.083	1926.60	2155.64	2273.63	2.00	1.82	42.88	112.81
26	0.00	2908.28	2338.87	2527.29	1.98	2.53	42.73	155.95
27	0.00	4336.57	3040.95	3251.35	2.52	1.74	27.68	69.56
Mean	0.0092	2811.84	2538.73	2772.08	2.33	2.16	34.56	108.34
SD	0.028	1046.87	396.92	462.35	0.27	0.29	6.01	25.68

1.5 μg kg^{-1} dose of DMED, respectively. The chromatograms of the plasma samples at 0.5 h after a single intravenous administration of 28.5 μg (0.5 μg kg^{-1} × 57 kg), 60.0 μg (1.0 μg kg^{-1} × 60 kg) and 93.0 μg (1.5 μg kg^{-1} × 62 kg) of DMED are depicted in Fig. 4 and the mean concentration–time pharmacokinetic profiles for DMED in plasma of the twenty-eight subjects (three level doses) are shown in Fig. 5.

In all approximately 477 samples including four calibration curves, twenty-one QC samples and volunteer samples were run and analyzed successfully within 32 h of the entire analysis.

Table 6
The pharmacokinetic parameters of three doses DMED (males and females).

Dose (μg kg^{-1})	Gender	PK parameter	T_{max} (h)	C_{max} (pg mL^{-1})	AUC_{0-8} (pg h mL^{-1})	$AUC_{0-\infty}$ (pg h mL^{-1})	MRT (h)	$t_{1/2}$ (h)	CL (L h^{-1})	V (L)
0.5 (low)	Male	Mean	0.01	822.23	1012.90	1158.36	2.62	2.67	27.66	108.02
		±SD	0.03	247.53	99.44	119.11	0.23	0.71	4.37	42.47
	Female	Mean	0.00	851.30	974.96	1008.05	2.38	1.40	27.61	52.89
		±SD	0.00	460.64	205.05	234.02	0.12	0.45	7.63	7.44
1.0 (middle)	Male	Mean	0.00	2162.45	1665.28	1772.13	2.30	1.90	40.80	110.58
		±SD	0.00	934.52	494.36	515.48	0.12	0.42	11.85	33.70
	Female	Mean	0.00	2190.56	1443.59	1508.89	2.21	1.81	39.76	110.89
		±SD	0.00	493.59	395.76	369.25	0.20	0.59	11.87	71.88
1.5 (high)	Male	Mean	0.00	2850.16	2705.35	2972.18	2.43	2.17	33.56	106.88
		±SD	0.00	878.80	382.34	430.89	0.27	0.32	5.91	31.91
	Female	Mean	0.03	2735.19	2205.50	2371.88	2.12	2.15	36.57	111.25
		±SD	0.05	1562.00	121.96	174.75	0.10	0.30	6.94	8.49

The pharmacokinetic parameters were calculated using the drug and statistics (DAS) version 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). All of the main pharmacokinetic parameters of three level doses of DMED are shown in Tables 5a–5c. The data showed a proportionate increase in C_{max} and $AUC_{0-\infty}$ with dose strength and the correlation coefficient of $AUC_{0-\infty}$ and dose was $r = 0.9897$. However, $t_{1/2}$, MRT, T_{max}, CL_z did not change with the dose increasing which means the distribution and the elimination rate were independence of dose. All these results demonstrated that ADME was consistent with linear kinetic process.

The pharmacokinetic parameters of eighteen males and ten females listed in Table 6, respectively. No significant differences were observed between males and females in terms of C_{max}, AUC_{0-8}, $AUC_{0-\infty}$, CL_z, T_{max}, MRT or $t_{1/2}$ values.

4. Conclusions

A HPLC-MS/MS method was developed and validated for the quantification of DMED in human plasma. The method was rapid, selective and highly sensitive with an LLOQ at 5 pg mL^{-1} for DMED and offering a wide range of linearity allowed quantification over the range 5–5000 pg mL^{-1}. The analysis time, only 4.0 min for each run, was significantly decreased comparing to conventional HPLC methods. The simple preparation, speed of separation, and reproducibility of analysis are the most outstanding characteristics of this method. The method was successfully applied to evaluate the pharmacokinetics of DMED following intravenous administration of three level doses to healthy Chinese volunteers.

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