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Original Research Article

Highly sensitive assay for the determination of therapeutic peptide desmopressin in human plasma by UPLC–MS/MS

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ABSTRACT

An analytical method based on ultra-performance liquid chromatography with positive ion electrospray ionization (ESI) coupled with tandem mass spectrometry (UPLC–MS/MS) was developed and validated for the determination of therapeutic peptide desmopressin in human plasma. A desmopressin stable labeled isotope (desmopressin d_8) was used as an internal standard. Analyte and the internal standard were extracted from 200 μL of human plasma via solid-phase extraction technique using Oasis WCX cartridges. The chromatographic separation was achieved on an Aquity UPLC HSS T3 column by using a gradient mixture of methanol and 1 mM ammonium formate buffer as the mobile phase. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 1.01–200 pg/mL. Method validation was performed as per FDA guidelines and the results met the acceptance criteria. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. The proposed method was successfully applied to pharmacokinetic studies in humans.

1. Introduction

Desmopressin is a synthetic analog of vasopressin, a natural pituitary hormone with antidiuretic properties. The deamination of vasopressin in the N-terminal 1 position and replacement of 8-L-arginine with 8-D-arginine results in the formation of desmopressin. It has a longer duration of antidiuretic activity than that of the natural hormone and is essentially devoid of other associated pharmacological effects such as vasoconstriction and contraction of smooth muscles in the uterus or in the intestine [1–3]. This prolonged and specific antidiuretic effect makes desmopressin useful for managing a number of enuretic disorders, including nocturia, primary nocturnal enuresis and central diabetes insipidus [4,5]. The oral administration of desmopressin is shown to be safe and effective for treating central diabetes insipidus and primary nocturnal enuresis [6]. The low doses of desmopressin (0.200–1.20 mg per day) may result in very low plasma concentrations. Therefore, a highly sensitive and selective method for the determination of therapeutic levels during clinical studies is required.

According to the literature, few liquid chromatography/mass spectrometric methods (LC–MS) have been reported for the quantitative determination of desmopressin in skin samples [7], blood plasma samples [8] and urine samples [9]. Also, an LC–MS method [10] has been reported for the qualitative detection of desmopressin in human plasma samples for doping control purpose. Most of the analytical methods [7,9,10] reported so far were too insensitive and/or not suitable for quantitative determination of desmopressin in human plasma samples for pharmacokinetic/bioequivalence studies. However, a promising method was reported by Nguyen et al. [8] with an LOQ of 2 pg/mL and employed multi-step solid phase extraction involving many stringent method development protocols with a chromatographic run time of >18 min, which may not be favorable for routine drug analysis. This method utilizes a single-quadrupole mass spectrometry with selected-ion monitoring (SRM) mode to detect the precursor ion. But in the present method a triple-quadrupole mass spectrometry (LC–MS/MS) with multiple reaction monitoring (MRM) mode was used to detect both the precursor ion and fragment ion. It shows that the proposed method is highly specific. Moreover, the method reported by Nguyen et al. [8] does not describe the development process, volume of the sample used, various validation experiments, stability studies and suitability for application to pharmacokinetic/bioequivalence studies. The salient features of chromatographic methods developed for desmopressin in human plasma are summarized in Table 1.

This paper presents, for the first time, the complete development

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Table 1
Salient features of LC–MS methods developed for desmopressin in biological samples.

S. no.	Column; mobile phase; flow rate; injection volume	Extraction technique; biological matrix; mean recovery	Detection technique; linear dynamic range/LOD; analytical run time; retention time; application/ purpose	Refs.
1	Nucleosil C_{18} (CC 125/2, 120-3); acetonitrile–0.01% formic acid in 1.6 mM ammonium acetate (33:67, v/v); 0.20 mL/min; 10 µL	Extraction with water and methanol/ ethanol (50:50); skin; ND	LC–MS; 0.05–2 µg/mL; 10 min; 2.6 min; transdermal	[7]
2	Phenomenex Luna C_{18} (150 mm×2 mm i.d., 5 µm); methanol–0.05% formic acid, pH 3 (gradient composition); 0.20 mL/min; 50 µL	SPE with Strata-X 8B-S100-TAK C18-E (30 mg) cartridges; blood plasma; 88.67%	LC–MS; 2.00 pg/mL; 18 min; ND; ND	[8]
3	Pyramid–C_{18} (50 mm×2.1 mm i.d., 1.9 µm); acetonitrile–0.1% formic acid (gradient composition); 0.30 mL/min; ND	SPE with STRATA-XCW (30 mg) cartridges; urine; 103%	LC–MS/MS TOF; 50–2000 pg/mL; 13 min; 5.4 min; doping control	[9]
4	Zorbax 300SB C_{18} (50 mm×1.0 mm, i.d., 3.5 µm); acetonitrile–0.1% acetic acid–0.01% triflouro acetic acid (gradient composition); 50 µL/ min; 30 µL	SPE with Oasis® WCX (60 mg) cartridges; plasma; 40%	LC–MS/MS; 50 pg/mL; 25 min; 10 min; qualitative analysis	[10]
5	Aquity HSS T3 (100 mm×2.1 mm i.d., 1.8 µm); methanol–1 mM ammonium formate (gradient composition); gradient flow; 20 µL	SPE with Oasis® WCX (30 mg) cartridges; plasma; 77.3%	LC–MS/MS; 1.01-200 pg/mL; 7 min; 3.3 min; pharmacokinetics	PM

ND, no data available; SPE, solid phase extraction; PM, present method.

and validation of a simple, highly sensitive and selective UPLC-MS/MS method in MRM mode for the quantification of desmopressin in human plasma using desmopressin d_8 as an internal standard (IS). This sensitive method (1.01 pg/mL) requires only 200 µL human plasma for solid-phase extraction (SPE) technique, minimum usage of organic solvents and demonstrates excellent performance in terms of ruggedness with a sample cut off time of 7.0 min. The application of this assay method to a clinical pharmacokinetic study in healthy South Indian male subjects following oral administration of desmopressin is described under fasting condition. The authenticity in the measurement of study data is demonstrated through incurred samples reanalysis.

2. Experimental

2.1. Chemicals and reagents

Reference sample desmopressin (≥97%) and the internal standard (IS) desmopressin d_8 (≥97%) were purchased from Sigma-Aldrich Limited (Bengaluru, India). LCMS grade methanol was purchased from J.T. Baker (Phillipsburg, USA). Analytical grade ammonium formate, formic acid and acetic acid were purchased from Merck Ltd (Mumbai, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). The control human plasma sample was procured from Deccan′s Pathological Labs (Hyderabad, India).

2.2. UPLC–MS/MS instrument and conditions

An UPLC system (Waters Corporation, Milford, USA) consisting of an Aquity HSS T3 column (100 mm×2.1 mm, 1.8 µm; Waters corporation, Milford, USA) equipped with a binary pump and a 96-vial autosampler (Waters, Milford, USA) was used for the study. Aliquots of 20 µL of the processed samples were injected into the column, which was kept at 40 °C. A mobile phase consisting of a mixture of 1 mM ammonium formate buffer (solvent A) and methanol (solvent B) in a gradient proportion was used to separate the analyte from the endogenous components. The gradient program was run from 25% B to 75% B in 3 min and ramped up to 90% B within 0.2 min and held for 1 min and ramped down to initial conditions within 0.25 min and stayed there for 2.0 min. Flow gradient was also performed starting with 0.4 mL/min up to 1.5 min, then 0.2 mL/min within 0.25 min and stayed for 1.75 min and flow came back to initial flow.

Quantification was achieved with MS–MS detection in positive ion mode (ES+) for the analyte and the internal standard using a Waters XEVO TQ-S mass spectrometer (Manchester, United Kingdom). The source temperature, desolvation temperature and desolvation gas flow were set at 150 °C, 500 °C and 1000 L/h, respectively. The capillary voltage, cone voltage and collision energy were 1.6 kV, −30 V, 14 V for desmopressin and 1.6 kV, −25 V, 12 V for the IS. The dwell time for each transition was 75 ms and argon gas was operated at 3.5×10^{-3} bar. Detection of the ions was carried out in the MRM mode, by monitoring the transition pairs of *m/z* 535.5 precursor ion to the *m/z* 328.3 production for desmopressin and *m/z* 539.7 precursor ion to the *m/z* 328.4 product ion for the IS. The analysis data obtained were processed by Masslynx SCN 843 (Version 4.1).

2.3. Preparation of plasma standards and quality controls

Standard stock solution of desmopressin and the IS (0.1 mg/mL) was prepared in water. Working solutions for calibration and controls were prepared by appropriate dilution in water. The IS working solution (1 ng/mL) was prepared by diluting its stock solution with water.

The above working solutions (50 µL) were added to drug-free plasma (950 µL) as a bulk, to obtain desmopressin concentration levels of 1.01, 2.35, 5.37, 11.2, 40.1, 80.1, 120, 160, and 200 pg/mL as a single batch at each concentration. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drug, at concentrations of 1.01 (LLOQ), 3.05 (low), 78.14 (middle) and 156.23 pg/mL (high) as a single batch at each concentration. The calibration and control bulk samples were divided into aliquots in microcentrifuge tubes (Tarson, 2 mL) and stored in the freezer at −20 ± 5 °C until analyses.

2.4. Sample processing

All frozen subject samples, calibration standards and quality control samples were thawed and allowed to equilibrate at room temperature prior to analysis. The samples were vortexed to mix for 10 s prior to spiking. A 200 µL aliquot of human plasma sample was mixed with 25 µL of the internal standard working solution (1 ng/mL of desmopressin d_8). To this, 200 µL of 2% acetic acid solution was added after vortex mixing for 10 s. The sample mixture was loaded onto an Oasis WCX 1 cm^3 (30 mg/ mL) that was pre-conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The extraction cartridge was washed with 1.0 mL of 5% ammonia solution followed by 1.0 mL of methanol. Analyte and IS were eluted with 1.0 mL of 2% formic acid in methanol and evaporated at 45 °C under a gentle stream of nitrogen. The dried extract was reconstituted with 200 µL mixture of 1 mM ammonium formate and methanol (70:30, v/v). Aliquot of 20 µL of the extract was injected into the chromatographic system.

2.5. Bioanalytical method validation

The validation of the above method was carried out as per US FDA guidelines [11]. The parameters determined were selectivity, specificity, sensitivity, matrix effect, linearity, precision, accuracy, recovery, dilution integrity and stability. Selectivity was assessed by comparing the chromatograms of eight different batches of blank plasma obtained from six different sources including one lipemic and hemolyzed plasma. Sensitivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the calibration curve concentrations. Carry-over experiment was performed to verify any carryover of analyte and IS, which may reflect in subsequent runs. The design of the study comprised the following sequence of injections i.e., blank plasma sample→six samples of LLOQ→blank plasma sample→ upper limit of quantitation (ULOQ) sample→blank plasma samples to check for any interference due to carry-over.

Matrix effect was checked with six different lots of K_2 EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma (36 QC samples in total). For checking the linearity standard calibration curves containing at least 9 points (non–zero standards) were plotted. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences. To determine intra-day accuracy and precision, a calibration curve and six replicates of LLOQ QC, LQC, MQC and HQC were analyzed on the same day. Inter-day accuracy and precision were assessed by analyzing three batches of samples on two consecutive days. Recoveries of analyte and IS were determined by comparing the peak area of extracted analyte standard with the peak area of non-extracted standard. Recovery of desmopressin was determined at a concentration of 3.05 (low), 78.1 (middle) and 156.23 (high) pg/mL, whereas for IS it was determined at concentration of 1.00 ng/mL. Dilution integrity was performed to extend the upper concentration limit with acceptable precision and accuracy. Six replicates each at a concentration of about 1.80 times of the uppermost calibration standard were diluted two- and four-fold with blank plasma. The diluted samples were processed and analyzed.

Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The stock solution stability at room temperature and refrigerated conditions (2–8 °C) was performed by comparing the area response of the analyte (stability samples) with the response of the sample prepared from fresh stock solution. Bench top stability (6 h), processed samples stability (autosampler stability for 48 h, wet extract stability for 45 h and reinjection stability for 24 h), freeze–thaw stability (three cycles), long-term stability (30 days) were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (± 15% SD) and precision (≤15% RSD).

2.6. Pharmacokinetic study design

A pharmacokinetic study was performed in healthy male subjects (n=6) and an ethical vote was obtained from the local ethics commission. The subjects were fasted 9 h before administration of the drug formulation. Blood samples were collected following oral administration of 0.4 mg (2×0.2 mg) desmopressin at pre-dose and 0.167, 0.333, 0.5, 0.667, 0.833, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 8, 10, 12, and 16 h, in K_2 EDTA vacutainer collection tubes (BD, Franklin, NJ, USA). The tubes were centrifuged at 3200 rpm for 10 min and the plasma was collected. The collected plasma samples were stored at −20 ± 5 °C till their use. Plasma samples were spiked with the IS and processed as per the extraction procedure described earlier. Plasma concentration–time profile of desmopressin was analyzed by non-compartmental method using WinNonlin Version 5.1. An incurred sample reanalysis was also conducted by selecting the 12 subject samples (two samples from each subject) near C_{max} and the elimination phase. The percent change in the value should not be more than ± 20% [12,13].

3. Results and discussion

3.1. Mass spectrometry

Mass parameters were tuned in both positive and negative ionization modes using electrospray ionization source. The intensity response obtained in positive mode was much higher than those in negative ion mode since the analyte and IS have the ability to accept protons. Protonated form of analyte and IS $[M+H]^+$ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 535.5 to 328.3 for desmopressin and from m/z 539.7 to 328.4 for the IS. LC–MRM is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods [14]. Thus, the MRM technique was chosen for the assay development.

3.2. Method development

The development of sensitive and selective bioanalytical method requires the judicious selection of chromatography column, mobile phase and organic solvent. These parameters should be carefully monitored to produce the required resolution from endogenous components which in turn affect sensitivity and reproducibility of the analytical method by ion suppression. Once chromatographic column, mobile phase pH and organic solvent are set then gradient slope, flow rate, column temperature and buffer type and concentration can be manipulated for optimal response.

Organic solvents such as acetonitrile and methanol were tried in different volume ratio with buffers like ammonium acetate, ammonium formate as well as acid additives like acetic acid and formic acid in varying strength. It was observed that methanol and 1 mM ammonium formate buffer as the mobile phase at gradient composition was most appropriate to give best sensitivity, efficiency and peak shape. Acetonitrile showed more of singly charged spectrum compared to methanol, where doubly charged species was dominating, so methanol has been chosen as an organic solvent as shown in Fig. 1. Initially, separation was tried on different columns like Waters Aquity BEH C18, HSS T3, CSH C18, etc. The use of an Aquity HSS T3 column (100 mm×2.1 mm, 1.8 μm) gave good peak shapes and response even at the lowest concentration level for the analyte and IS. The mobile phase was operated at a gradient flow mode. The retention time of analyte and the IS was low enough (3.3 and 3.3 min) allowing a run time of 7.0 min.

To develop a highly sensitive (pg level) analytical method in human plasma samples, one should have a proper extraction method which can produce good recovery with minimal or no matrix effect. Initially both the extraction methodologies protein precipitation (PP) and liquid–liquid extraction (LLE) were tried with acetonitrile, acetone and methanol for PP and different organic solvents like ethyl acetate, hexane, dichloromethane, diethyl ether and methyl *tert*-butyl ether (MTBE) for LLE. The recovery results obtained were inconsistent with ion suppression in both the occasions. Moreover, the response was not enough to quantify the analyte at LLOQ level.

But for the purpose to develop a highly sensitive and specific method, SPE was tested. Moreover, SPE technique provides clear extracts than the PP and LLE and the influence on sensitivity is considerably small. Hence SPE was tried using Oasis HLB, MCX, MAX, and WCX cartridges. Among the different cartridges tested, WCX cartridges gave clear extracts with minimum matrix effect and quantitative extraction was possible for the analyte and IS. Addition of acetic acid solution to the plasma samples in different volume ratios helped in obtaining consistent and reproducible response. When eluent was injected directly into the UPLC system, the peak shape of analyte was

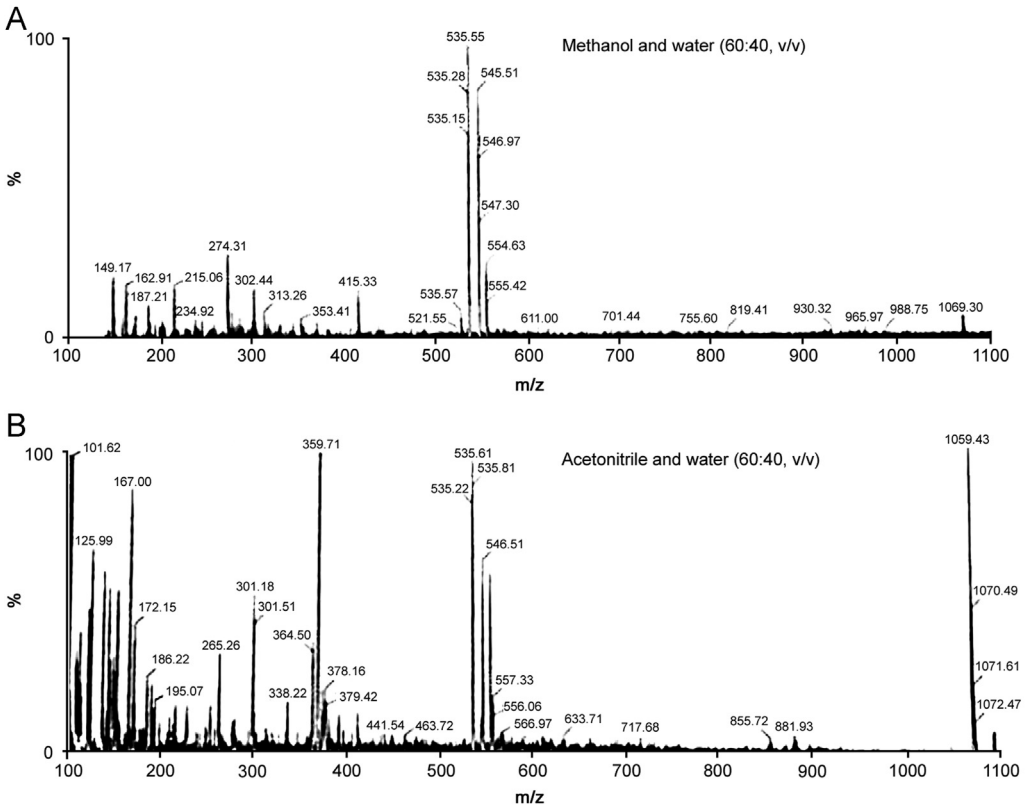

Fig. 1. Effect of organic solvent on multiply charging. (A) methanol and (B) acetonitrile.

unacceptable at lower concentration levels and also the response was insufficient to quantify the analyte. Hence eluent was evaporated and the residue was reconstituted using a mixture of 1 mM ammonium formate and methanol (70:30, v/v). The overall mean recoveries of analyte and the IS were good and reproducible. Moreover, the validation results and subject sample analysis study support this extraction methodology and hence it was accepted in the present study.

Stable labeled isotopes of the analyte as an internal standard (IS) is recommended for bioanalytical assays to increase assay precision and limit variable recovery between analyte and the IS [15,16]. Also for LC–MS/MS analysis, using stable isotope-labeled drugs as internal standards proves to be helpful when a significant matrix effect is possible. At the initial stages of this work, several compounds were investigated to find a suitable IS and finally desmopressin stable labeled isotope desmopressin d_8 was found to be best for the present purpose.

3.3. Method validation

3.3.1. Chromatography, selectivity and carryover effect

The selectivity of the method was examined by analyzing blank human plasma extract (Fig. 2A) and an extract spiked only with the IS (Fig. 2B). As shown in Fig. 2A, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free human plasma at the retention time of the analyte and IS. Similarly, Fig. 2B shows the absence of direct interference from the IS to the MRM channel of the analyte. Fig. 2C depicts a representative ion-chromatogram for the LLOQ sample (1.01 pg/mL). A representative chromatogram resulting from the analysis of subject blank plasma sample and 1.25 h subject plasma sample after the administration of a 0.4 mg oral single dose of desmopressin is shown in Fig. 3. No area of analyte was observed in blank plasma samples run after ULOQ, which suggests no carry-over of the analyte in subsequent runs.

3.3.2. Sensitivity

The lowest limit of reliable quantification for the analyte was set at the concentration of the LLOQ (1.01 pg/mL). The precision and accuracy of analyte at LLOQ concentration were found to be 4.10% and 107.8%, respectively.

3.3.3. Matrix effect

Matrix effect assessment was done with the aim to check the effect of different lots of plasma on the back calculated value of QC's nominal concentration. The precision and accuracy for desmopressin at LQC concentration were found to be 2.05% and 101%, and at HQC level they were 1.21% and 101%, respectively. Results revealed that no significant matrix effect was observed in all the eight batches of human plasma for the analyte at low and high quality control concentrations. Also, the extraction method was rugged enough and gave accurate and consistent results when applied to real subject samples.

3.3.4. Linearity, precision and accuracy

The nine-point calibration curve was found to be linear over the concentration range of 1.01–200 pg/mL for desmopressin. After comparing the two weighting models ($1/x$ and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration was found to produce the best fit for the concentration–detector response relationship. The mean correlation coefficient of the weighted calibration curves generated during the validation was ≥0.99.

The results for intra-day and inter-day precision and accuracy in plasma quality control samples are summarized in Table 2. The intra-day and inter-day precision deviation values were all within 15% of the relative standard deviation (RSD) at low, middle and high quality control levels, whereas within 20% at LLOQ QCs level. The intra-day and inter-day accuracy deviation values were all within 100% ± 15% of the actual values at low, middle and high quality control levels, whereas within 100% ± 20% at LLOQ QCs level. The results revealed good

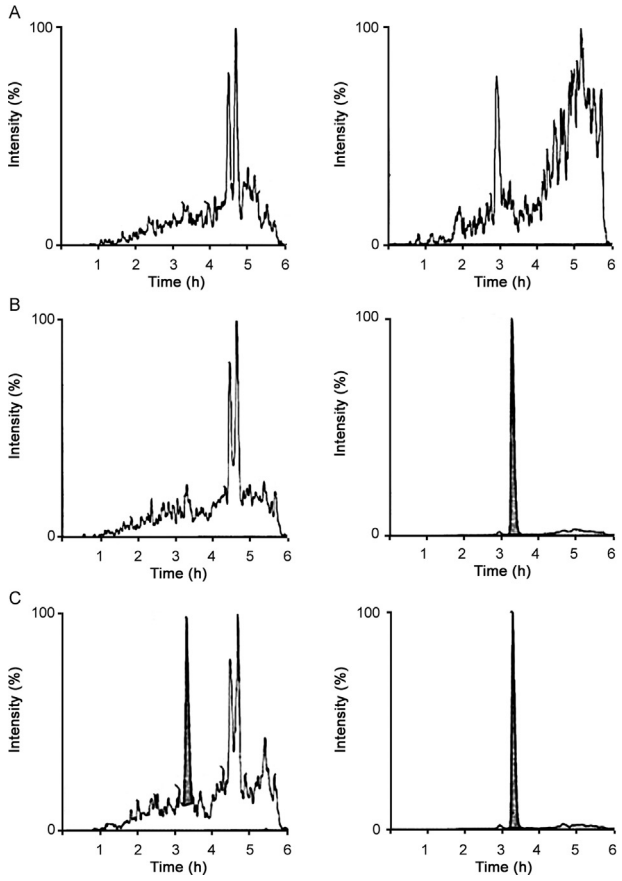

Fig. 2. Typical MRM chromatograms of desmopressin (left panel) and IS (right panel) in (A) human blank plasma, (B) human plasma spiked with IS, and (C) an LLOQ sample along with IS.

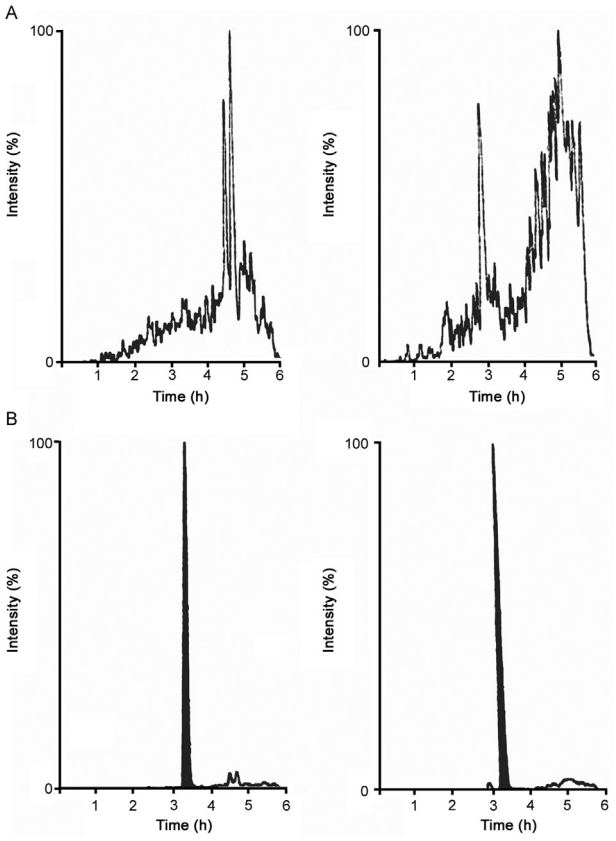

Fig. 3. MRM chromatograms resulting from the analysis of (A) subject blank plasma sample and (B) 1.25 h subject plasma sample, after the administration of a 0.4 mg oral single dose of desmopressin tablet. The sample concentration was determined to be 36.10 pg/mL.

precision and accuracy.

3.3.5. Extraction efficiency

Six replicates at low, middle and high quality control concentrations for desmopressin were prepared for recovery determination. SPE with WCX cartridges proved to be robust and provided the cleanest samples. The mean overall recovery of desmopressin was 77.3% ± 2.38% with the precision range of 3.21%–5.28% and the recovery of IS was 77.7% ± 2.90% with the precision range of 1.06%–1.63%. The recoveries of analyte and IS were good and reproducible. Therefore, the assay has been proved to be robust in high throughput bioanalysis.

3.3.6. Effect of dilution factor

The upper concentration limit of desmopressin can be extended to 360 pg/mL for by 1/2 and 1/4 dilutions with screened human blank plasma. The mean back-calculated concentrations for 1/2 and 1/4 dilution samples were within 85%–115% of their nominal value. The coefficients of variation (%CV) for 1/2 and 1/4 dilution samples were less than 15%.

3.3.7. Stability studies

In the different stability experiments carried out, namely, bench top stability (6 h), autosampler stability (48 h), repeated freeze–thaw cycles (three cycles), reinjection stability (24 h), wet extract stability (45 h at 2–8 °C) and long term stability at −20 ± 5 °C for 30 days the mean% nominal values of the analyte were found to be within ± 15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 3). Thus, the results were found to be within the acceptable limits during the entire validation.

Stock solutions of desmopressin and IS were found to be stable for 15 days at 2–8 °C. The percentage stability (with the precision range) of desmopressin and IS was 104% (2.11%–3.24%) and 99.6% (0.81%–1.56%), respectively.

3.4. Pharmacokinetic study results

In order to verify the sensitivity and selectivity of this method in a real-world situation, the present method was used to test for desmopressin in human plasma samples collected from healthy male volunteers (n=6). The mean plasma concentrations versus time profile of desmopressin is shown in Fig. 4. The maximum concentration (C_{max}) in plasma (35.0 ± 8.10 pg/mL) for desmopressin was attained at 1.40 ± 0.48 h (t_{max}). The area under the plasma concentration–time curve from time zero to last measurable time point (AUC_{0-t}) and area under the plasma concentration–time curve from time zero to infinity time point (AUC_{0-inf}) for desmopressin were 149 ± 46 and 157 ± 51 pg h/mL, respectively. The terminal half-life ($t_{1/2}$) was found to be 3.48 ± 0.50 h.

3.5. Incurred sample reanalysis

In order to assess the suitability of the validated LC–MS/MS method incurred samples were analyzed and data were then compared using incurred sample reproducibility (ISR) procedure and criteria [12,13]. The ISR was performed using two plasma samples from each subject and re-assayed in a separate batch run. The differences in concentrations between the ISR and the initial values for all the tested samples were less than 20% (Table 4), indicating good reproducibility of the present method.

Table 2
Precision and accuracy data for desmopressin.

Quality control	Run	Concentration found (mean ± SD; pg/mL)	Precision (%)	Accuracy (%)
Intra-day variations[a]				
LLOQ	1	1.10 ± 0.04	4.54	109.22
	2	0.98 ± 0.03	3.16	97.21
	3	1.08 ± 0.06	5.08	107.23
LQC	1	2.98 ± 0.21	7.28	97.82
	2	3.18 ± 0.29	9.27	104.44
	3	3.03 ± 0.09	3.24	99.50
MQC	1	81.45 ± 2.40	2.18	104.23
	2	80.51 ± 1.63	2.51	103.03
	3	80.57 ± 1.60	2.82	103.14
HQC	1	159.53 ± 3.22	1.71	102.11
	2	161.29 ± 0.82	0.55	103.24
	3	159.89 ± 4.83	2.04	102.34
Inter-day variations[b]				
LLOQ		1.09 ± 0.06	5.11	108.22
LQC		3.12 ± 0.01	2.90	102.27
MQC		77.08 ± 3.12	4.10	98.64
HQC		155.17 ± 4.64	2.03	99.32

Spiked concentrations of LLOQ, LQC, MQC, and HQC are 1.01, 3.05, 78.14, 156.23 pg/mL, respectively.
[a] Six replicates at each concentration.
[b] Eighteen replicates at each concentration.

Table 3
Stability data for desmopressin in plasma (n=6).

Stability test	QC (spiked concentration; pg/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/stability (%)
Process[a]	3.05	2.90 ± 0.21	4.24	95.11
	156.23	152.68 ± 6.25	5.16	97.73
Process[b]	3.05	2.89 ± 0.22	6.03	94.84
	156.23	154.98 ± 3.25	3.41	99.20
Bench top[c]	3.05	2.92 ± 0.13	4.22	95.67
	156.23	146.56 ± 7.26	9.88	93.81
FT[d]	3.05	2.83 ± 0.52	8.26	92.70
	156.23	142.39 ± 1.56	1.13	91.14
Reinjection[e]	3.05	3.05 ± 0.23	3.60	99.96
	156.23	158.04 ± 3.10	2.62	101.06
Long-term[f]	3.05	3.33 ± 0.17	5.22	109.12
	156.23	144.89 ± 4.41	3.18	92.74

[a] After 48 h in autosampler at 10 °C.
[b] After 45 h at 2–8 °C.
[c] After 6 h at room temperature.
[d] After three freeze and thaw cycles.
[e] After 24 h of reinjection.
[f] At −20 °C for 30 days.

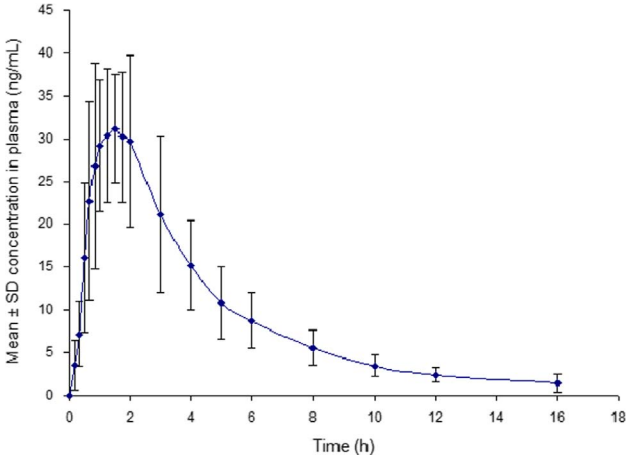

Fig. 4. Mean plasma concentration–time profile of desmopressin in human plasma following oral administration of desmopressin 0.4 mg to healthy volunteers (n=6).

Table 4
Incurred samples re-analysis data of desmopressin.

Sample	Initial conc. (pg/mL)	Re-assay conc. (pg/mL)	Difference[a] (%)
1	35.20	36.26	−2.96
2	4.01	4.15	−3.33
3	24.49	22.85	6.93
4	3.58	3.19	11.41
5	27.86	26.87	3.60
6	3.18	3.24	−1.81
7	29.30	30.25	−3.22
8	3.18	3.29	−3.37
9	35.03	34.23	2.32
10	3.04	2.69	12.43
11	33.45	32.84	1.85
12	3.81	3.47	9.54

[a] Expressed as [(initial conc.−re-assay conc.)/average]×100%.

4. Conclusions

The results presented here demonstrate the successful development and validation of a highly sensitive and selective UPLC–MS/MS method for the determination of desmopressin in human plasma samples. To the best of the authors' knowledge, this is the first UPLC–MS/MS report describing the complete method development and validation process for the determination of desmopressin in human plasma. This method is highly sensitive and employs 200 μL plasma volumes for sample processing. The method showed suitability for clinical studies in humans. From the results of all the validation parameters, we can conclude that the developed method can be useful for bioavailability and bioequivalence (BA/BE) studies and routine therapeutic drug monitoring with the desired precision and accuracy.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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