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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Sub-Picogram Level Bio-Analytical Method for Quantification of Desmopressin in Human Plasma Using LCMS-8060NX

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User Benefits

- Simple, novel and most sensitive method for Desmopressin determination with LLOQ of 0.25 pg/mL
- Quick and single step sample extraction method can increase sample productivity
- Method offers excellent transferability between different laboratories, ensuring reproducibility of results

1. Introduction

Desmopressin (dDAVP), a synthetic analogue of 8-arginine vasopressin (ADH), is an antidiuretic peptide drug modified by deamination of 1-cysteine and substitution of 8-L-arginine by 8-D-arginine. Desmopressin displays enhanced antidiuretic potency, fewer pressor effects due to V2-selective actions, and a prolonged half-life and duration of action compared to endogenous ADH(1). It has been employed clinically since 1972 and is available in various formulations including intranasal solution, intravenous solution, oral tablet and oral lyophilizate. Current recommendation for (fast and fed) bio-equivalence studies of Desmopressin in human plasma requires a highly sensitive and reproducible method to quantify the analyte at sub-picogram levels(2,3). Shimadzu ADC has developed a unique and novel bio-analytical method for quantification of Desmopressin at LLOQ - 0.25 pg/mL using LCMS-8060NX. The method developed on LCMS-8060NX is free from issues arising due to non-specific binding, extraction recovery etc., and meets the requirements for conducting pharmacokinetic studies as per regulatory requirements at your lab. limited

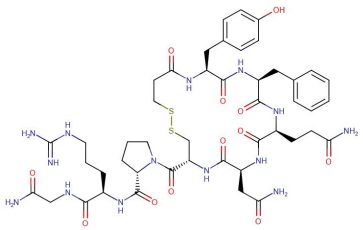

Fig.1 Structure of Desmopressin

2. Salient Features

- Novel and highly sensitive method for estimation of Desmopressin in human plasma was developed (results are presented in Table 1) on LCMS-8060NX.
- Simple extraction procedure enhanced the selectivity of the method
- Optimal plasma volume avoided unnecessary wastage of plasma samples and at the same time increased life of the mass spectrometer as well as HPLC column
- Newly designed IonFocus Unit improved the sensitivity and robustness of the method.
- Heated ESI along with New UF-Qarray™ ion guide technology contributes by increasing ion production and enhancing transmission, respectively. This ensures sensitive and selective quantification of desmopressin at 0.25 pg/mL
- Customized flow gradient method satisfied the peak shape, retention time and background noise
- Method was validated as per US major guidelines for
  - ✓ Linearity
  - ✓ Inter-day and intra-day PA batch
  - ✓ Recovery
  - ✓ Matrix effect
  - ✓ Carry over effect

Table 1 Method Validation Summary

Calibration curve range	0.250-40.000 pg/mL	
Intraday precision and accuracy (For LLOQ-QC)	Accuracy (%Nominal)	116.64
	Precision (% RSD)	8.36
Intraday precision and accuracy (For LQC, MQC, HQC)	Accuracy (%Nominal)	103.15 to 106.12
	Precision (% RSD)	4.16 to 12.27
Global precision and accuracy (For LLOQ-QC)	Accuracy (%Nominal)	117.92
	Precision (% RSD)	13.55
Global precision and accuracy (For LQC, MQC, HQC)	Accuracy (% Nominal)	98.35 to 101.90
	Precision (% RSD)	8.88 to 10.25
Global % recovery	Recovery (%)	76.72
	Precision (% RSD)	4.80
Matrix effect	LQC	1.05
	HQC	0.97

Note: LLOQ QC- Lower Limit of Quantification Quality Control, LQC- Lower Quality Control, MQC- Middle Quality Control, HQC- Higher Quality Control

3. Experimental

3.1. Sample preparation and analytical conditions

Three hundred microliters of extraction buffer was added to plasma samples and vortexed to mix for 30 seconds and processed by using solid phase extraction technique. The sample extraction protocol is mentioned below:

Extraction protocol

Conditioning and equilibration:
1mL methanol followed by 1 mL water

Sample loading

Wash 1: (1.00 mL wash solution 1)

Wash 2: (1.00 mL wash solution 2)

Elution: (1.00 mL of elution solution)

SPE eluent was collected into prelabelled RIA vials and evaporated under stream of nitrogen flow at 50°C.

Evaporated samples were reconstituted in 100 µL of reconstitution solution, vortexed and filled in HPLC vials for injection.

Chromatographic separation was achieved under customized gradient flow conditions on Shim-pack™ GIST-HP C18-AQ column. Detection and quantification of Desmopressin was performed based on multiple reaction monitoring (MRM) under positive electrospray ionization mode on Shimadzu LCMS-8060NX triple quadrupole mass spectrometer.

3.2. Instrument parameters on LCMS-8060NX

Refer to Table 2 for analytical conditions and instrument parameters and Table 3 for MRM transition.

Table 2 Analytical conditions and instrument parameters

Parameter	HPLC
Column	Shim-pack™ GIST C18 AQ, 1.9 µm 50 × 2.1mm, (P/N: 227-30807-01)
Mobile Phase	A: 1 mM Ammonium formate in water B: Methanol
Flow Rate	0.5 mL/min with splitter
Oven Temp	60 ˚C
Injection volume	50 µL
Parameter	**MS**
Interface	ESI
Interface temp and Voltage	1 kV and 100 ˚C
MS Mode	MRM, Positive
Heat Block Temp	500 ˚C
DL Temp	300 ˚C
CID Gas	350 kPa
Nebulizing Gas	3 L/min
Drying Gas	5 L/min
Heating Gas	10 L/min

Table 3 MRM transition and parameters of Desmopressin on LCMS

Compound	MRM (m/z)	CE (V)
Desmopressin	535.0-328.0	-21.0

Fig.2 Nexera™ X2 with LCMS-8060NX system

4. Result and Discussion

4.1. Method Development

Developing a sensitive and selective bioanalytical method involves careful choices of chromatography column, mobile phase, and organic solvent to ensure proper resolution and sensitivity while minimizing ion suppression. Once column, mobile phase pH, and organic solvent are established, parameters like gradient slope, flow rate, column temperature, and buffer type and concentration can be adjusted for optimal response. Methanol and 1 mM ammonium formate were found to be the ideal mobile phase, offering superior sensitivity and peak shape compared to other solvents.

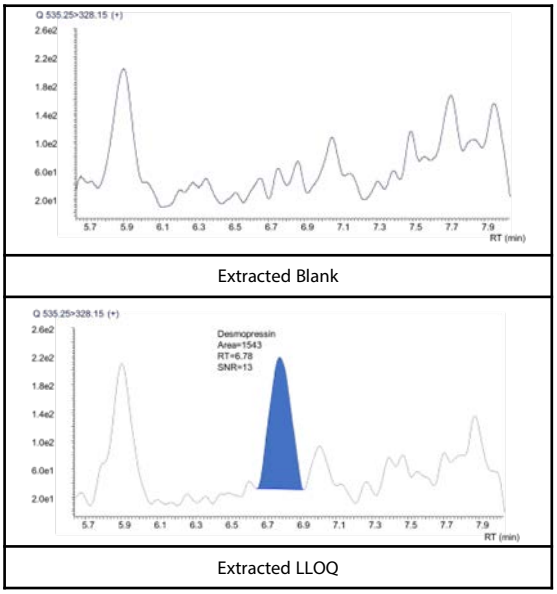

Fig.3 Chromatograms (Ext Blank and Extracted LLOQ – 0.25pg/mL)

For chromatography, a Shim-pack C18 column was chosen due to its good peak shapes and low retention times (3.3 min) for the analyte.

Mass parameters were optimized for positive and negative ionization modes using electrospray ionization. The positive mode yielded higher intensity responses due to the analyte proton acceptance ability. The protonated form [M+H]+ ion served as the parent ion (Q1) for the Q3 product ion spectra. The most sensitive mass transitions were observed at m/z 535.0 to 328.0 for desmopressin. Refer to Table 2 for analytical conditions and instrument parameters. LCMS-8060NX system offers robust sensitivity and selectivity, making it ideal for pharmacokinetic studies. Thus, the MRM technique was selected for assay development.

Extraction methods like protein precipitation and liquid-liquid extraction were initially tested, but inconsistency and ion suppression led to unsatisfactory results. Subsequently, solid-phase extraction (SPE) using SPE C18 cartridges yielded clear extracts with minimal matrix effects and enabled quantitative extraction of the analyte. To improve peak shape and response at lower concentrations, the eluent was evaporated and reconstituted using a mixture of 1 mM ammonium formate and methanol (70:30, v/v). The overall mean recoveries of analyte was consistent, precise and reproducible at all QC levels. This extraction methodology was deemed suitable for the PK study sample analysis.

4.2. Method Validation

Method validation for quantification of desmopressin using LCMS was performed as per the US FDA major guidelines.

Selectivity

The selectivity of the method was evaluated by extracting and analyzing 6 different lots of blank human plasma. Blank matrices from six different lots showed no significant interference at the retention time and MRM transition of desmopressin. Results are presented in Table 4. Representative chromatogram is shown in Fig.3.

Table 4 Selectivity

Plasma lot no.	Desmopressin		
	Blank Plasma	LLOQ area	% Interference
V12349	44	1673	2.63
V12350	0	2014	0.00
V1102	46	1858	2.48
V11491	60	1844	3.25
V9203	101	1800	5.61
V6432	73	1851	3.94

Linearity

A seven-point calibration curve displayed linearity within the concentration range of 0.25 - 40.00 pg/mL for desmopressin (refer to Fig.4) . Comparing weighting models (1/x and $1/x^2$), the regression equation with a $1/x^2$ weighting factor exhibited the optimal fit for the concentration-detector response relationship. The weighted calibration curves achieved a mean correlation coefficient > 0.99 during partial validation. Intra-day and inter-day precision and accuracy results in plasma quality control samples are summarized in table 5 and table 6. Both intra-day and inter-day precision deviation values were < 15% of the relative standard deviation (%RSD) at low, middle, and high-quality control levels, and < 20% at the Low Limit of Quantification (LLOQ) QC level. Intra-day and inter-day accuracy deviation values were within 100% ± 15% of the actual values at low, middle, and high-quality control levels, and within 100% ± 20% at the LLOQ QC level. The findings indicated strong precision and accuracy.

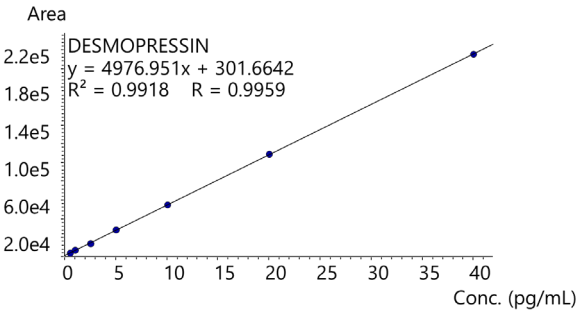

Fig.4 Calibration curve

Matrix effect

Matrix effect assessment aimed to evaluate the influence of different plasma lots on the back-calculated values of QC nominal concentration. The matrix factor and precision for desmopressin were determined to be 1.05% and 11.33% at the low-Quality Control (LQC) concentration, and 0.97% and 3.43% at the high-Quality Control (HQC) concentration. The findings indicated that no significant matrix effect was observed across six batches of human plasma for both low and high-quality control levels. The extraction method demonstrated robustness, delivering consistent and accurate results when applied to real subject samples.

Table 5 Intra-day precision and accuracy

Intra-day (n=6)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (%RSD)
LLOQ QC (0.250 pg/mL)	0.29	116.64	8.36
LQC (1.010 pg/mL)	1.04	103.15	12.27
MQC (5.000 pg/mL)	5.18	103.45	6.41
HQC (20.000 pg/mL)	21.23	106.12	4.16

Table 6 Global precision and accuracy

Inter-day (n=12)			
Nominal Conc (pg/mL)	Observed Conc (pg/mL)	Accuracy (%)	Precision (%RSD)
LLOQ QC (0.250 pg/mL)	0.29	117.92	13.55
LQC (1.010 pg/mL)	1.03	101.90	10.25
MQC (5.000 pg/mL)	5.04	100.63	8.88
HQC (20.000 pg/mL)	19.68	98.35	9.99

Recovery

Recovery determination for desmopressin involved preparing six replicates at low, middle, and high-quality control concentrations. Desmopressin exhibited a global recovery of 76.72% with precision of 4.80%. The recovery of desmopressin was found precise, consistent and reproducible at all QC levels. Consequently, the assay's robustness has been confirmed for high throughput bioanalysis.

Table 8 Matrix factor

Desmopressin	Aqueous sample	Post extracted sample	Matrix factor
LQC	4,257	4,571	1.07
	3,698	4,029	1.09
	4,460	3,624	0.81
	4,357	4,755	1.09
	4,097	4,710	1.15
	3,956	4,228	1.07
Mean			1.05
SD			0.12
%RSD			11.33

Desmopressin	Aqueous sample	Post extracted sample	Matrix factor
HQC	87,269	80,745	0.93
	85,934	83,180	0.97
	85,194	82,434	0.97
	86,663	81,715	0.94
	85,109	84,034	0.99
	83,159	84,816	1.02
Mean			0.97
SD			0.03
%RSD			3.43

Table 7 Recovery

Sr.No.	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample
	LQC		MQC		HQC	
1	4,571	5,929	20,733	24,395	80,745	89,144
2	4029	5,671	20,100	25,173	83,180	98,327
3	3624	6,305	20,016	27,075	82,434	1,01,421
4	4755	5,966	19,989	28,744	81,715	1,10,202
5	4,710	5,708	21,319	30,447	84,034	1,09,018
6	4228	5,585	21,446	27,596	84,816	1,06,688
AVERAGE	4,320	5,861	20,601	27,238	82,821	1,02,467
STD DEV	443.14	264.00	665.54	2239.08	1500.87	7953.60
%RSD	10.26	4.50	3.23	8.22	1.81	7.76
%Recovery	73.70		75.63		80.83	
Note: Read Ext-Sample as extracted sample and PE-Sample as post extracted sample						

Carry-over effect

Carryover was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was present/observed at the retention time and MRM transition of the analyte in the extracted blank sample following the highest standard calibrator.

5. Conclusion

The outcomes presented herein showcase the successful creation and partial validation of an exceptionally sensitive and specific LC-MS/MS technique designed for quantifying desmopressin in human plasma samples. This method exhibits remarkable sensitivity and employs minimum plasma volumes for sample processing. It is deemed suitable for life science studies. Based on the results of all the parameters, it is evident that the developed approach holds potential for bioavailability and bioequivalence (BA/BE) investigations, along with routine basic medical study for drug monitoring, offering the sought-after precision and accuracy.

6. References

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06-SAIP-LC-062-EN First Edition: Oct. 2023

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