SHIMADZU

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Sub-picogram level Bio-analytical method for quantification of Desmopressin in human plasma using LCMS-8060NX

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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 (refer fig.1). 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. 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. In this work, we present a unique and novel method for the accurate quantification of desmopressin in human plasma using Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled with Nexera X2 UHPLC.

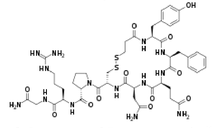

Fig. 1 Structure of Desmopressin [(1)]

2. Materials and methods

2-1. Sample Preparation

Calibration standards and quality control samples were prepared in K2 ETDA human plasma with sample concentrations ranging between 0.25-40.00 pg/mL and 0.25-20.00 pg/mL respectively.

Three hundred microliters of extraction buffer was added to plasma samples to enhance the binding of the analyte while performing the solid phase extraction. SPE cartridges were conditioned with 1 mL methanol and equilibrated with 1 mL water. Plasma samples were added to the cartridges and allowed to pass under gravity. 1 mL of 5% ammonia in water and 5% methanol in water effectively removed the interferences from SPE cartridges. Desmopressin was eluted using 1 mL methanol of and was blown under nitrogen gas before reconstitution of the dried extract with 100 µL of 1 mM ammonium formate in water: methanol-(50:50, V/V).

3. LC-MS/MS analysis

LCMS-8060NX coupled with Nexera™ X2 UHPLC system (Shimadzu Corporation), was used to acquire the data in MRM mode. The instrumental conditions used during the analysis are presented below in Table 1

Table 1 Instrument Parameters for analysis of Desmopressin

UHPLC condition (Nexera™ X2)	
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	0.50 mL/min
Elution mode	Gradient
Column temp	60 ℃
MS parameters (LCMS-8060NX)	
MS interface	Electro Spray Ionization (ESI)
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 5 L/min
Zero air flow	Heating gas- 10 L/min
MS temp	Desolvation line- 300 °C; Heating block- 500 °C; Interface- 100 °C

4. Results

4-1. Selectivity

The selectivity of the method was evaluated by extracting and analyzing 6 different lots of blank human plasma. Results are presented in Table 2. Representative chromatogram is shown in fig.2.

Table 2 Selectivity

	Desmopressin		
Lot no.	Area in blank matrix	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

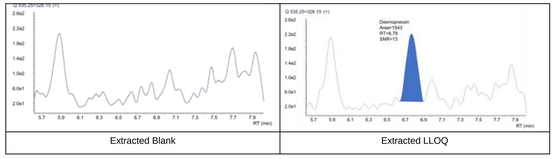

Fig. 2 Chromatograms of extracted blank and extracted LLOQ (0.25 pg/mL)

4-2. Linearity

A seven-point calibration curve with a 1/x^2 weighting factor displayed linearity within the concentration range of 0.25–40.00 pg/mL for desmopressin (refer fig.3) resulted in mean correlation coefficient > 0.99.

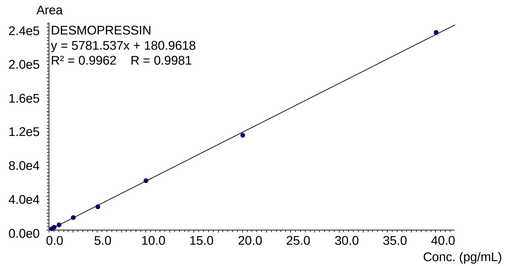

Fig. 3 Representative calibration curve of Desmopressin

4-3. Intra-day and Inter-day Precision and accuracy

Intra-day and inter-day precision and accuracy results in plasma quality control samples are summarized in table 3 and table 4 and were found within the acceptance criteria

Table 3 Intra-day Precision and Accuracy

QC level (n=6)	Mean Conc.	% Accuracy	% CV
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 4 Global Precision and Accuracy

QC level (n=12)	Mean Conc.	% Accuracy	% CV
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

4-4. 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. Results of Recovery statistics and global recovery is presented in Table 5 and Table 6 respectively.

Table 5 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						

Table 6 Global Recovery

QC level	**Recovery**
LQC (n=6)	73.70
MQC (n=6)	75.63
HQC (n=6)	80.83
Mean	**76.72**
SD	**3.68**
% RSD	**4.80**

4-5. Matrix effect

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 as shown in Table 7. The findings indicated that no significant matrix effect was observed across six batches of human plasma for both low and high quality control levels.

Table 7 Matrix Factor

Desmopressin	**AQ-sample**	**PE-sample**	**Matrix factor**	**Desmopressin**	**AQ-sample**	**PE-sample**	**Matrix factor**
LQC	4,257	4,571	1.07	**HQC**	87,269	80,745	0.93
	3,698	4,029	1.09		85,934	83,180	0.97
	4,460	3,624	0.81		85,194	82,434	0.97
	4,357	4,755	1.09		86,663	81,715	0.94
	4,097	4,710	1.15		85,109	84,034	0.99
	3,956	4,228	1.07		83,159	84,816	1.02
Mean			**1.05**				**0.97**
SD			**0.12**				**0.03**
% RSD			**11.33**				**3.43**
Note: Read AQ-Sample as aqueous sample and PE-Sample as post extracted sample							

4-6. 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 human clinical 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 therapeutic drug monitoring, offering the sought-after precision and accuracy.

Reference

1) https://www.chemspider.com/Chemical-Structure.4470602.html (accessed May 04, 2023).