A Sub-picogram (0.5 pg/ml) Level Quantification Method for Desmopressin in Human Plasma using Liquid Chromatography Electrospray Mass Spectrometry

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INTRODUCTION

Low-level peptide detection has a number of applications in clinical studies and in pharmacological discovery and development processes, highlighting the increasing relevance of sensitive and selective mass spectrometric platforms in the biolanalytical laboratory. Regulatory requirements demand intensive and rigorous quantitation of therapeutic peptides during pharmacokinetic, bioequivalence and metabolic studies. In addition, drug discovery and development strategies seek to monitor and quantitate peptide biomarkers in complex biological samples, necessitating highly-selective separations of low concentration analytes from high background noise and prominent levels of competing ions. The AB SCIEX Triple Quad™ 6500 LC-MS/MS System, equipped with IonDrive™ Technology for enhanced detector performance, has demonstrated particular strength in the detection of low-level amounts of small molecules, and in this study, we extend the augmented signal-to-noise, broad dynamic range, and the efficient method development capacities of the Triple Quad 6500 System to the detection of sub-picogram levels of a therapeutic peptide under high-throughput conditions.

We have developed a reliable, fast, and sensitive method for the quantification of a nine-amino-acid peptide, desmopressin (1-desamino-8-D-arginine vasopressin). Therapeutically, desmopressin reduces urine production, restricting the elimination of water from the kidneys by binding to the V2 receptors in renal-collecting ducts, thereby facilitating increased water reabsorption. The longer half-life of desmopressin over vasopressin offers some therapeutic advantages, and typical doses of desmopressin to treat diabetes insipidus and bedwetting range between 0.200 to 1.20 mg per day, resulting in very low plasma concentrations. In this bioanalytical study, we have established a sensitive and selective LC-MS/MS method for the quantitation of desmopressin in human plasma, detecting peptide levels as low as 0.500 pg/mL with excellent accuracy and precision.

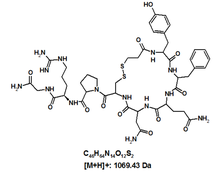
Unique features of the Triple Quad™ 6500 System for low-level peptide detection

- **IonDrive™ Turbo V Source** – Increased ionization efficiency and heat transfer contribute to sensitivity enhancements, including improved signal-to-noise.
- **IonDrive™ QJet Ion Guide** – Increased ion sampling improves method efficiency and ruggedness.
- **IonDrive™ High Energy Detector** – Innovative detector technology boosts dynamic range and sensitivity.

AB SCIEX Triple Quad™ 6500 system

IonDrive™ Turbo V Source IonDrive™ QJet Guide IonDrive™ HE Detector

IonDrive™ Technology Key Innovations



$C_{46}H_{64}N_{14}O_{12}S_2$
[M+H]+: 1069.43 Da

Figure 1: Desmopressin

MATERIALS AND METHODS

Sample Preparation:

Plasma samples (1000 µL) containing 2% desmopressin standard and 50µL internal standard were vortexed and spiked with 50µL of orthophosphoric acid (OPA). Samples were extracted on weak cation exchange cartridges conditioned with methanol followed by 100mM ammonium acetate solution. After loading, samples were washed in three steps: 1) 2% OPA:methanol (80:20 v/v) ; 2) 2% NaOH:Methanol (60:40 v/v); and 3) water:methanol (60:40 v/v). Analytes were eluted with 5% acetic acid in methanol, dried under nitrogen at 40 ºC, and reconstituted with 150 µL of 0.1% acetic acid prior to analysis by mass spectrometry.

HPLC Conditions:

A GL Sciences LC 800 system was used, with a Agilent 300 Extend C18 (150 x 2.1 mm, 3.5µm) analytical column maintained at 40ºC. A gradient elution was employed, consisting of 0.1% acetic acid in water (mobile phase A) and 0.1% acetic acid in Acetonitrile (mobile phase B), at a flow rate of 750 µL/min. The total run-time for the method was 5.0 minutes. The injection volume was set to 50 µL. The rinsing solution was Acetonitrile: Water (50:50 v/v) mixture.

MS parameters:

An AB SCIEX Triple Quad™ 6500 LC-MS/MS system equipped with IonDrive™ Turbo V source was used, in positive Electrospray Ionization (ESI) mode. The optimized MRM for the analyte and internal standard are summarized in Table 1. The source parameters were optimized in T infusion mode. Quadrupole mass analyzers (Q1 and Q3) were set at unit resolution for quantitative analysis. The multiple reaction monitoring was done by selecting the doubly charged molecular ions ($M+2H)^{2+}$ at m/z 535.4 instead of the singly charged ones.

The mass spectrometer was operated in positive ionization mode with electrospray voltage +5500 V and source temperature of 600ºC. Nitrogen was used as nebulizing gas (GS1), drying gas (GS2) and curtain gas at 50, 60 and 40 arbitrary unit, respectively. Valco valve was used to divert the flow to avoid the interference during the chromatographic run.

Analyte	MRM	Dwell Time (ms)	DP	EP	CE	CXP
Desmopressin	535.4/328.0	200	50	10	23	15
Desmopressin d^5	537.9/328.0	200	71	10	25	15

Table 1: MRM transitions and optimised MS parameter for desmopressin

RESULTS

The best ionization was achieved in ESI positive mode. The mass spectrometric parameters for both are given in Table 1. A linear calibration curve was constructed using the $1/X^2$ regression. The calibration curve for desmopressin was linear over a dynamic range of 0.500 to 100.760 pg/mL in plasma sample (Fig 5) with an r value 0.9996. Samples were extracted using solid phase extraction by weak cation exchange chemistry and recovery of Desmopressin from Plasma at three different levels LQC, MQC and HQC was found to be 92.72% and for Desmopressin d5 it was 77.89%.

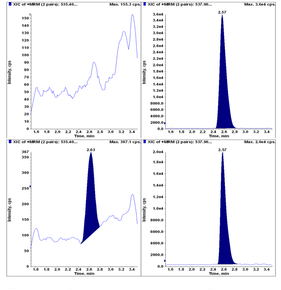


Figure 2: The chromatograms for Desmopressin in zero blank plasma sample (upper pane) and 0.500 pg/mL spiked in plasma sample (lower pane)

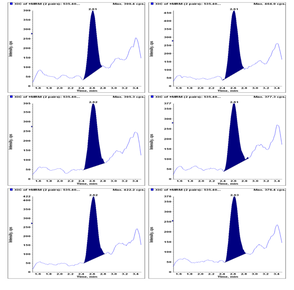


Figure 3: Chromatograms of 6 LLOQ QC (0.502 pg/mL) samples from Precision and Accuracy Batch

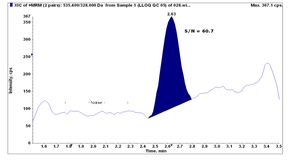


Figure 4: Signal to noise calculation for Desmopressin in extracted plasma sample at LLOQ level (0.500 pg/mL S/N = 60.7)

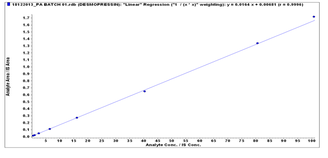


Figure 5: Calibration curve of Desmopressin in plasma from 0.500 pg/mL to 100.760 pg/mL. The method has shown excellent linearity over the concentration range with r = 0.9996.

Desmopressin	LLOQ	LQC	MQC	HQC
Nominal Concentration (pg/mL)	0.502	1.492	40.692	81.384
N	18	18	18	18
Calculated Concentration (pg/mL)	0.5376	1.4678	39.7269	79.933
SD (+/-)	0.05691	0.14051	0.73499	1.36511
%CV	10.56	9.59	1.85	1.71
% Nominal	107.57	98.53	97.64	98.21

Table 2. Inter Day Precision and Accuracy for Desmopressin for three batches

CONCLUSIONS

1. A highly sensitive method for Desmopressin was developed and validated using AB SCIEX Triple Quad™ 6500 LC-MS/MS system in human plasma. The developed method is sensitive (0.5 pg/ml), reproducible and cost effective for bioanalytical laboratory with good precision and accuracy.
2. Recovery of the developed and validated method is 92.7%.
3. The chromatography was developed only for 5min make this highly sensitive, reproducible and high through put method, a valuable tool for CRO/Bio analytical industry

REFERENCES

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Acknowledgements

The authors are indebted to Dr.Venu Madhav, Chief Operating Officer (COO), Veeda Clinical Research, India, for his encouragement and support for this work.