Sub-Picogram Level Quantitation of Desmopressin in Small Volumes of Human Plasma Using a Trap-Elute Microflow

Using the QTRAP® 6500+ System with OptiFlow™ Turbo V Source and M5 MicroLC System

Rahul Baghla, Khatereh Motamedchaboki, Remco van Soest and Lei Xiong
SCIEX, Redwood City, California, USA

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 the replacement of 8-l-arginine with 8-d-arginine result 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].

Therapeutically, desmopressin reduces urine production, restricts water elimination from the kidneys by binding to the Vasopressin V2 receptors (V2R) in renal-collecting ducts, thereby facilitating increased water reabsorption. The longer half-life of desmopressin over vasopressin offers additional 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.

The sub picogram/mL quantitation of desmopressin in human plasma using an analytical flow HPLC methodology was published in a previously described method[2], in which 1 mL human plasma was used for desmopressin quantification at 0.5 pg/mL. In this current work, microflow LC combined with the OptiFlow Turbo V Source was used to quantitate the desmopressin at the same level 0.5 pg/mL in human plasma with 3.3 times less consumption of plasma samples and injecting 3.3x less volume to ensure enough sample for 5 reinjections. A

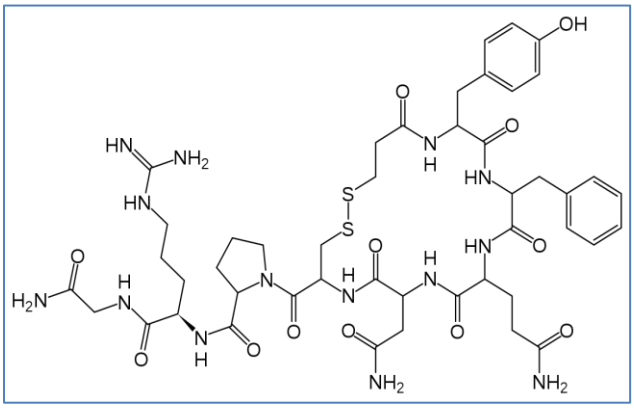

Figure 1. Structure of Desmopressin.

similar LLOQ was observed while injecting 7x less sample on column compared to previous analytical flow assay[2].

Key Features of the SCIEX Microflow LC-MS/MS Solution

- M5 MicroLC system provides:
  - Microfluidic flow control for accurate flow rates down to 1 µL/min
  - Trap-elute option for fast and large volume sample loading
  - Flexibility to couple with any microflow LC column
- OptiFlow™ Turbo V Source on the QTRAP® 6500+ LC-MS/MS system provides:
  - Easy setup with no probe or electrode position optimization
  - Robust performance and long electrode lifetime

Methods

Sample Preparation: The sample preparation method is modified from the previously published technical note[2] to obtain cleaner extracts. Desmopressin spiked human plasma samples in the range from 0.5 to 250 pg/mL, with 25 pg/mL of internal standard were extracted using weak cation exchange cartridges (WCX, Waters). Cartridges were conditioned with 1 mL of methanol followed by 1 mL of 100 mM ammonium acetate in water. 0.3 mL of spiked plasma mixed with 0.3 mL of 5% acetic acid in water was loaded on the pre-conditioned cartridge. After loading, the cartridges were washed with 1 mL of 5% ammonium hydroxide in water followed by 2 mL of methanol. Analytes were eluted using 5% acetic acid in methanol followed by drying under a nitrogen stream at 40 °C. Samples were reconstituted in 0.1 mL of 0.1% acetic acid in water and 15 µL was injected for the LC-MS/MS analysis.

LC-MS Conditions for Microflow Analysis: Separation was performed using the M5 MicroLC system in trap-elute mode. Table 1 describes the chromatographic conditions for analyte trapping. Table 2 describes the chromatographic conditions for analyte separation.

MS analysis was performed on a SCIEX QTRAP 6500+ system with OptiFlow Turbo V Source with a 25µm SteadySpray™ electrode. The OptiFlow Turbo V Source requires no physical adjustment of the probe or electrode positions. The optimized MS parameters are listed in Table 3. The data was processed using MultiQuant™ Software 3.0.

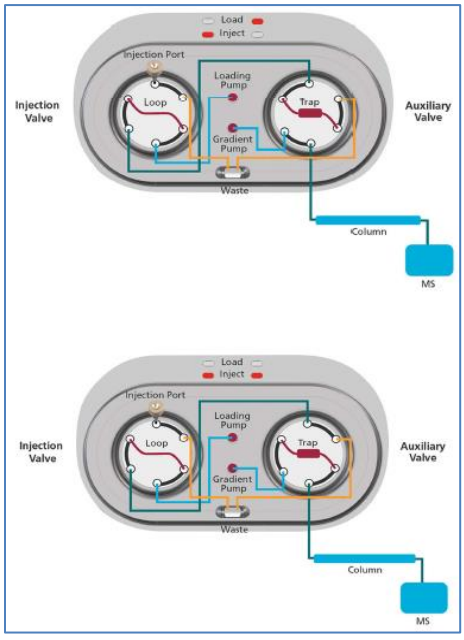

Figure 2. Valve Configuration. Valve configuration for sample loading (top) and elution (bottom) is shown.

Table 1: Chromatographic Conditions for Microflow Analysis: Analyte Trapping.

Parameter	Value
Stationary phase	*Phenomenex Luna 5 µm, C18 Trap Column, 20 x 0.3 mm*
Mobile phase A	*0.1% acetic acid in water*
Mobile phase B	*0.1% acetic acid in acetonitrile*
Flow rate	*40 µL/min*
Column temperature	*Room Temperature*
Injection volume	*15 µL*

Time	Flow Rate (µL/min)	%A	%B
0	40	90	*10*
2	40	*90*	*10*
3	40	*90*	*10*

Table 2: Chromatographic Conditions for Microflow Analysis: Analyte Separation.

Parameter	Value
Stationary phase	*Phenomenex Kinetex 2.6 µm, XB-C18 Column, 50 x 0.3 mm*
Mobile phase A	*0.1% acetic acid in water*
Mobile phase B	*0.1% acetic acid in acetonitrile*
Flow rate	*5 µL/min*
Column temperature	*40 ºC*

Time	Flow Rate (µL/min)	%A	%B
0	5	90	*10*
1	5	*90*	*10*
2.5	5	*60*	*40*
4	5	*60*	*40*
4.5	5	*5*	*95*
10	5	*5*	*95*
10.1	5	*90*	*10*
12	5	*90*	*10*

Table 3. MS Conditions for Microflow Analysis.

Name	Q1	Q3	DP	CE	CXP
Desmopressin_1[1]	535.4	328.2	50	22	15
Desmopressin_2	535.4	794.3	50	27	15
Desmopressin_d5[2]	537.9	328.2	50	22	15

Source/Gas Parameter	Value	Source/Gas Parameter	Value
Curtain gas:	25	CAD gas:	*High*
Ion source gas 1:	20	*Ion spray voltage:*	*4500*
Ion source gas 2:	*20*	*Source temperature:*	*100*

[1]Most suitable transition for quantification

[2]Internal standard transitions

Table 4: Chromatographic Conditions for Analytical Flow Analysis.

Parameter	Value
Stationary phase	*Phenomenex Kinetex C18 column, 50 x2.1mm*
Mobile phase A	*0.1% acetic acid in water*
Mobile phase B	*0.1% acetic acid in acetonitrile*
Flow rate	*0.5 mL/min*
Column temperature	*40 ºC*
Injection volume	*15 µL*

Time	Flow Rate (ml/min)	%A	%B
0.0	0.5	95	*5*
1.0	0.5	*95*	*5*
2.5	*0.5*	*60*	*40*
3.0	*0.5*	*60*	*40*
3.5	*0.5*	*5*	*95*
8.0	*0.5*	*5*	*95*
8.1	*0.5*	*95*	*5*
10.0	*0.5*	*95*	*5*

LC-MS Conditions for Analytical Flow Analysis: To identify the sensitivity difference between analytical flow and microflow analysis, each sample was analyzed using a QTRAP 6500+ system coupled with an ExionLC™ AD HPLC system. Table 4 describes the liquid chromatography conditions for analytical flow analysis. The MRM parameters are identical as the microflow analysis (Table 3). The source/gas parameters were optimized at 0.5 mL/min flow rate and summarized in Table 5. The data were processed using MultiQuant Software 3.0.

Table 5: MRM Source / Gas Parameters for Analytical Flow Analysis.

Source/Gas Parameter	Value	Source/Gas Parameter	Value
Curtain gas:	35	CAD gas:	*High*
Ion source gas 1:	55	*Ion spray voltage:*	*5500*
Ion source gas 2:	*60*	*Source temperature:*	*500*

Results

In order to achieve the desired assay sensitivity with less sample volume, a microflow chromatographic technique and ion exchange SPE based sample preparation method were implemented. A 5 µL/min LC flow rate was applied for improved ionization efficiency. The MRM parameters for desmopressin and desmopressin-d5 internal standard were optimized. Ion exchange SPE based sample preparation was optimized to achieve cleaner samples resulting in minimum matrix effect in lower flow rates.

With the enhanced method condition, the microflow assay achieved a LLOQ of 0.5 pg/mL for desmopressin quantitation in 300 µL human plasma. This method showed good selectivity: matrix blank samples showed no interference in human plasma (Figure 4). As summarized in Table 6, the assay accuracy is 91.82-104.34% and CV% are well within the acceptance criteria as per FDA bioanalysis guidelines for all tested samples. The calibration curve covered 3 orders of magnitude (0.5-250 pg/mL) (Figure 5) and displayed linearity with a regression coefficient (r) of 0.997 using a weighting of 1/x2 (Figure 5).

Analyte retention time and internal standard peak retention times were consistent, with both eluting at approximately 3.3 min for microflow and 2.3 min for analytical flow analysis.

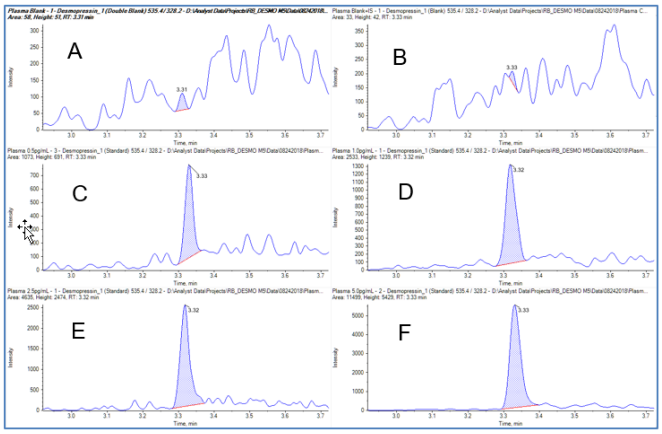

Figure 4. Extracted Ion Chromatograms of Desmopressin in Extracted Human Plasma using Microflow LC. Data is shown for the A) double blank; B) blank; C) 0.5 pg/mL; D) 1.0 pg/mL; e) 2.5 pg/mL f) 5.0 pg/mL.

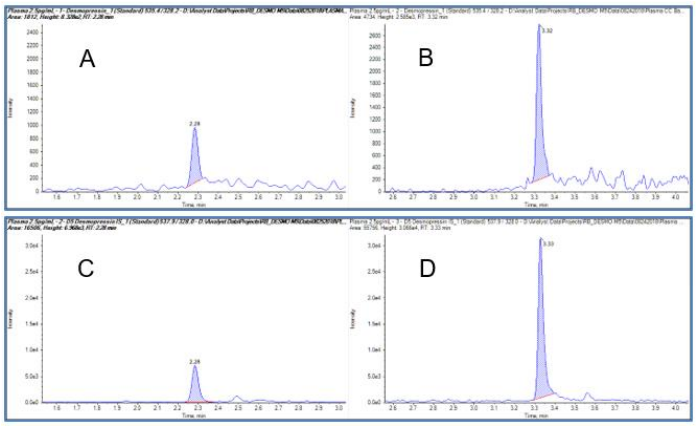

Figure 6. Comparing Analytical Flow Results to the Microflow Results. Extracted ion chromatograms of desmopressin and desmopressin d-5 in extracted human plasma are shown. Analytical flow (A) and microflow (B) XICs for 2.5pg/mL Desmopressin can be compared with Desmopressin-d5 internal standard XICs at analytical flow (C) and microflow (D) rates.

To determine the sensitivity difference between the microflow and analytical flow analysis, the same set of samples were analyzed with both microflow and analytical flow LC-MS systems with the same injection volume.

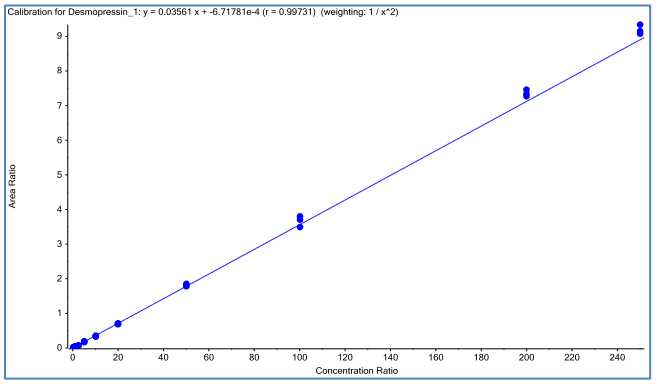

Figure 5. Calibration Curve for Quantitation of Desmopressin in Human Plasma using Microflow LC. Very good linearity was observed for the concentration range of 0.5 pg/mL to 250pg/mL (r = 0.997).

Conclusion

A microflow LC-MS/MS method for the highly sensitive quantitation of desmopressin in human plasma was successfully demonstrated. The QTRAP 6500+ LC system with OptiFlow Source coupled with a M5 MicroLC system provides reliable quantitation of desmopressin at the 0.5 pg/mL level with high reproducibility, high throughput and minimum source optimization requirements. The developed microflow LC method allowed for a reduction of the amount of plasma used with a factor of 3x due to improved sensitivity compared to a previously described method[2] using analytical flow LC, while achieving the same LLOQ.

Table 6: Quantitation Summary for the Microflow LC Experiment.

Actual Conc. (pg/mL)	Calculated Conc. (pg/mL)	Accuracy (%)	CV (%)
0.5	0.5	99.85	15.10
1.0	1.0	104.34	6.46
2.5	2.3	91.82	1.94
5	4.9	97.86	8.40
10	9.7	96.64	1.01
20	19.6	98.03	2.22
50	51.0	101.94	1.58
100	103.0	103.04	4.47
200	206.5	103.25	1.29
250	258.1	103.23	1.49

References

1. Gudlawar SK., Pilli NR., Siddiraju S., & Dwivedi J. (2017). Highly sensitive assay for the determination of therapeutic peptide desmopressin in human plasma by UPLC–MS/MS. *Journal of Pharmaceutical Analysis*, **7(3)**, 196–202. http://doi.org/10.1016/j.jpha.2013.11.002
2. Baghla R., Guttikar S., et al. A Sub-picogram quantification method for desmopressin in plasma using the SCIEX Triple Quad™ 6500 System, SCIEX Technical Note

Document number: RUO-MKT-02-8508-A

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices