Application Note 21478

Selective and Highly Accurate Analysis of Desmopressin from Human Plasma

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Key Words

SOLAμ WCX, Accucore Vanquish, Vanquish, desmopressin, LC-MS/MS, UHPLC, mixed-mode SPE, solid-phase extraction, micro-elution, peptides, bioanalysis, biopharma

Goal

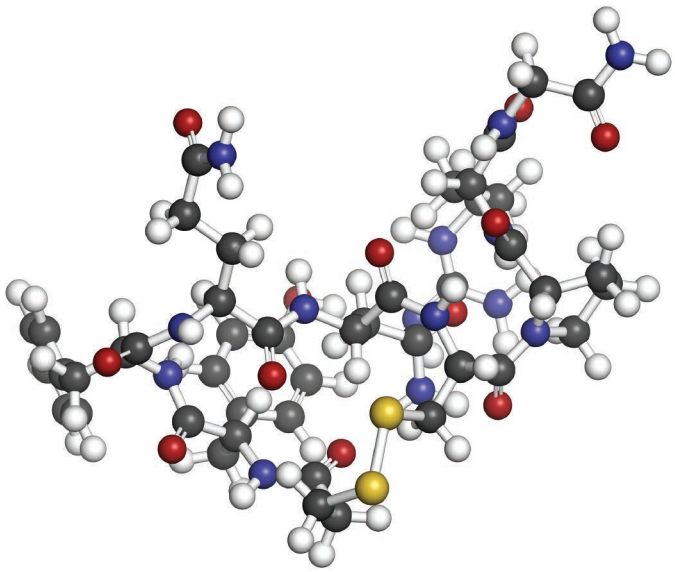
To describe an accurate, precise, high-throughput workflow for the analysis of desmopressin from human plasma utilizing micro-elution solid-phase extraction (SPE), followed by liquid chromatography separation coupled to triple quadrupole mass spectrometry detection (LC-MS/MS).

Introduction

Analysis of peptides presents very specific challenges for the bioanalyst: analyte solubility, non-specific binding to labware, and the ability to selectively detect a specific peptide in the presence of a complex matrix. Analysis can typically require long gradients and extensive sample preparation, which can result in low recovery levels for the peptide in question. Issues with system or column carryover can also be challenging.

Desmopressin (Figure 1) is a synthetic peptide consisting of nine amino acids and is very similar to endogenous peptides present in human plasma. Selective analysis can be achieved by combining micro-elution solid-phase extraction (SPE) with ultra-high pressure liquid chromatography (UHPLC) and ultra fast selective reaction monitoring (SRM) mass spectrometry. Micro-elution SPE provides quick and selective extraction of peptides from biological matrices without the need for post-extraction processing. Utilizing UHPLC technology allows for fast and reproducible separation, and mass spectrometry SRM provides robust, selective, and sensitive detection.

Sample preparation was performed using Thermo Scientific™ SOLAμ™ WCX, a weak cation exchange, mixed-mode, micro-elution SPE product. SOLAμ products provide reproducibility, robustness, and ease of use at low elution volumes by utilizing the revolutionary Thermo Scientific™ SOLA™ solid-phase extraction technology. This removes the need for frits, delivering a robust, reproducible format that ensures highly consistent results at low elution volumes.



SOLAμ products deliver:

- Lower sample failures due to high reproducibility at low elution volumes
- Increased sensitivity due to lower elution volumes
- The ability to process samples which are limited in volume
- Improved stability of bio-molecules by reduction of adsorption and solvation issues

This technique selectively extracts and concentrates desmopressin from human plasma with high recovery and low matrix effects. Octreotide (Figure 2) was used as an analogue internal standard (IS) and was added to the plasma before processing.

Thermo SCIENTIFIC

Separation was achieved on a Thermo Scientific™ Vanquish™ UHPLC system with a Thermo Scientific™ Accucore™ Vanquish C18+ analytical column. Accucore Vanquish C18+ UHPLC columns use Solid Core Technology to facilitate fast and highly efficient separations. This next-generation column features 1.5 µm solid core particles, which are not totally porous but instead have a solid core and a porous outer layer. The optimized phase bonding creates a high-coverage, robust phase. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks. The tightly controlled 1.5 µm diameter of Accucore Vanquish particles, in combination with controlled manufacturing processes, results in a column that delivers the increased chromatographic performance required for rapid screening methods.

The Accucore Vanquish UHPLC column and Vanquish UHPLC system were designed in combination to achieve the best possible chromatographic performance. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. By exploiting the 1500 bar high-pressure capability of the Vanquish UHPLC system, the flow rate used with the Accucore Vanquish column can be increased while maintaining peak capacity, resulting in shorter method times and increased assay throughput.

Selective reaction monitoring was used for detection on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer with positive electrospray ionization. A linear range of 10 to 5000 pg/mL was achieved. Quality control (QC) samples were prepared at 10, 30, 2500, and 4000 pg/mL levels and demonstrated excellent accuracy and precision.

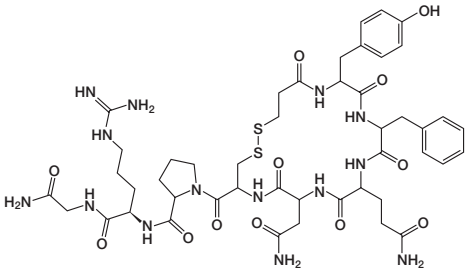


Figure 1. Desmopressin structure.

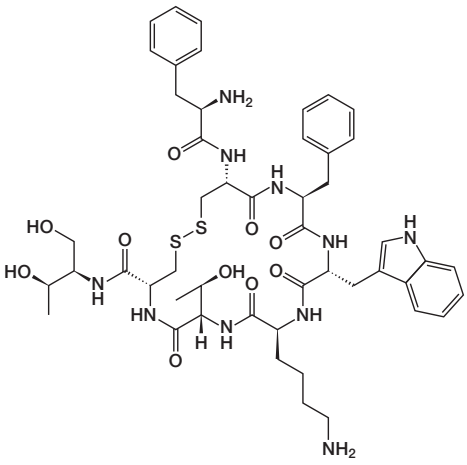


Figure 2. Octreotide (IS) structure.

Experimental

Consumables

- SOLAµ WCX plate (P/N 60209-004)
- Accucore Vanquish C18+ 1.5 µm, 100 × 2.1 mm (P/N 27101-102130)
- Thermo Scientific™ Webseal™ 96-well square well microplate (P/N 60180-P212)
- Webseal mat (P/N 60180-M120)
- Fisher Scientific™ LC-MS grade water (P/N 10095164)
- Fisher Scientific LC-MS grade acetonitrile (ACN) (P/N 10055454)
- Fisher Scientific LC-MS grade methanol (MeOH) (P/N 10636545)
- Fisher Scientific analytical grade formic acid (HCOOH) (P/N 10063427)
- Fisher Scientific Optima™ LC-MS trifluoroacetic acid (TFA) (P/N A116-50)
- Fisher Scientific 2,2,2-trifluoroethanol (TFE) (Peptide Synthesis) (P/N BP622-100)
- Fisher Scientific phosphoric acid (H_3PO_4) (P/N A365-1)
- Fisher Scientific acetic acid (CH_3COOH) (P/N A465-50)
- Fisher Scientific ammonium bicarbonate (NH_4HCO_3) (P/N BP2413500)

Sample Handling Equipment

- Thermo Scientific™ HyperSep™ 96 well vacuum manifold (P/N 60103-351)
- Thermo Scientific vacuum pump, European version (P/N 60104-241)
- Thermo Scientific vacuum pump, North American version (P/N 60104-243)

Sample Pretreatment

Desmopressin and octreotide were obtained from a reputable source in accurately pre-weighed ampoules. An appropriate volume of methanol/deionized water/acetic acid (30:60:10 v/v/v) was added in order to make a 1 mg/mL stock solution of each. Further dilutions of desmopressin were prepared in the same solvent to produce working solutions at the appropriate concentrations.

To prepare calibration standards in the range of 10 to 5000 pg/mL, 5 µL of individual working solutions of desmopressin were added to 295 µL of human plasma. The same approach was used to prepare QC samples at 10, 30, 2500, and 4000 pg/mL to be used as the LLOQ, Low, Mid, and High QC, respectively. A working solution of octreotide was prepared in methanol/deionized water/acetic acid (30:60:10 v/v/v) at a concentration of 250 ng/mL. Then, 10 µL of this was added to each standard and QC sample.

Zero standard samples were prepared by adding 5 µL of solvent and 10 µL of octreotide working solution to 295 µL of blank plasma. Double blank samples were prepared by adding 15 µL of solvent to 295 µL of human plasma.

Prior to SPE application each standard, QC sample, zero standard, and double blank were diluted with 300 µL 4% phosphoric acid and mixed well.

Sample Preparation

Compound: Desmopressin
Internal standard: Octreotide
Matrix: Human plasma
Plate type: SOLAµ WCX
Flow rate: 1 drip per second

SPE Procedure

Condition	Add 200 µL of ACN to each well. Apply vacuum to draw all liquid through the plate.
Equilibrate	Add 200 µL of 4% H_3PO_4 (aq) to each well. Apply vacuum to draw all liquid through the plate.
Sample Load	Transfer 600 µL of each sample into individual wells. Apply vacuum to draw all the liquid through the plate.
Wash 1	Add 200 µL of 10 mM NH_4HCO_3 to each well. Apply vacuum to draw all liquid through the plate.
Wash 2	Add 200 µL of 20:80 (ACN/10 mM NH_4HCO_3) to each well. Apply vacuum to draw all liquid through the plate.
Manifold Set-up	Discard all effluent collected and place a fresh collection plate under the SPE device.
Elution	Add 25 µL of 60:39:1 (ACN/water/TFA) to each well. Apply a slow vacuum until all liquid has passed through the plate. Repeat this step once (total volume 50 µL)
Dilution	Remove the collection plate from the manifold and add 50 µL of water to each well. Cap and mix well.

Method Optimization

Multiple SPE chemistries were screened during method development. WCX was chosen, as the results indicated the highest analyte recovery. Small amounts of carryover were initially observed during chromatography development, however, the addition of 1% TFE in the mobile phase and autosampler wash reduced the carryover to zero.

Optimization of the ions used in the SRM detection method was performed. Sensitivity increased by a factor of two when methanol was used in the mobile phase instead of acetonitrile. Upon investigation it was found that in the presence of acetonitrile, desmopressin was observed in both +1 and +2 charge states in roughly equal proportions. In the presence of methanol almost all of the desmopressin existed in the +2 charge state, increasing the sensitivity of the chosen precursor ion. Other publications of this analysis have achieved additional sensitivity by summation of ions[1]; however, this was not adopted in our approach.

Separation Conditions	
Instrumentation	Analyses were performed using a Vanquish UHPLC System consisting of:
	• System base (P/N VH-S01-A)
	• Binary pump H (P/N VH-P10-A)
	• Split sampler HT (P/N VH-A10-A)
	• Column compartment H (P/N VH-C10-A)
Column	Accucore Vanquish C18+, 1.5 µm, 100 × 2.1 mm
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile/methanol/TFE (49:50:1 v/v/v)
Gradient	See Table 1
Flow Rate	0.4 mL/min
Temp.	50 °C
Mobile Phase Pre-heater	50 °C
Injection Details	10 µL
Injection Wash Solvent	Acetonitrile/water/acetic acid/TFE (60:30:9:1 v/v/v/v)

Table 1. LC gradient conditions.

Time (min)	A (%)	B (%)
0	85	15
0.5	85	15
3.5	40	60
3.5	10	90
4	10	90
4	85	15
5	85	15

MS Conditions	
Instrumentation	TSQ Quantiva MS
Polarity	Positive
Spray Voltage	3500 V
Vaporizer Temp.	317 °C
Sheath Gas Pressure	40 Arb
Aux Gas Pressure	12 Arb
Capillary Temp.	333 °C
Collision Pressure	1.5 mTorr
Dwell Time	75 ms
Scan Time	Auto
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7
Transition details are listed in Table 2.	

Table 2. Compound transition details.

Compound	Desmopressin	Octreotide
Precursor (*m/z*)	535.4	510.4
Products (*m/z*)	328.2	159.2
Collision energy	18	34

Data Processing

The Thermo Scientific™ Xcalibur™ v3.0, with SII 1.1 for Xcalibur Data System was used for data acquisition and analysis.

Results and Discussion

Extraction recovery was assessed at the LLOQ, Low, Mid, and High QC levels and was found to be greater than 97% in all cases, as described in Table 3 and Figure 3. Matrix effects were assessed and found to be less than 4% in all cases, as described in Table 4 and Figure 4.

Table 3. Compound transition details.

Compound	% Recovery at LLOQ	% Recovery at QCL	% Recovery at QCM	% Recovery at QCH	Average % Recovery
Desmopressin	97.7	98.3	97.8	98.6	98.2
Octreotide	98.6	98.5	98.5	100	99.0

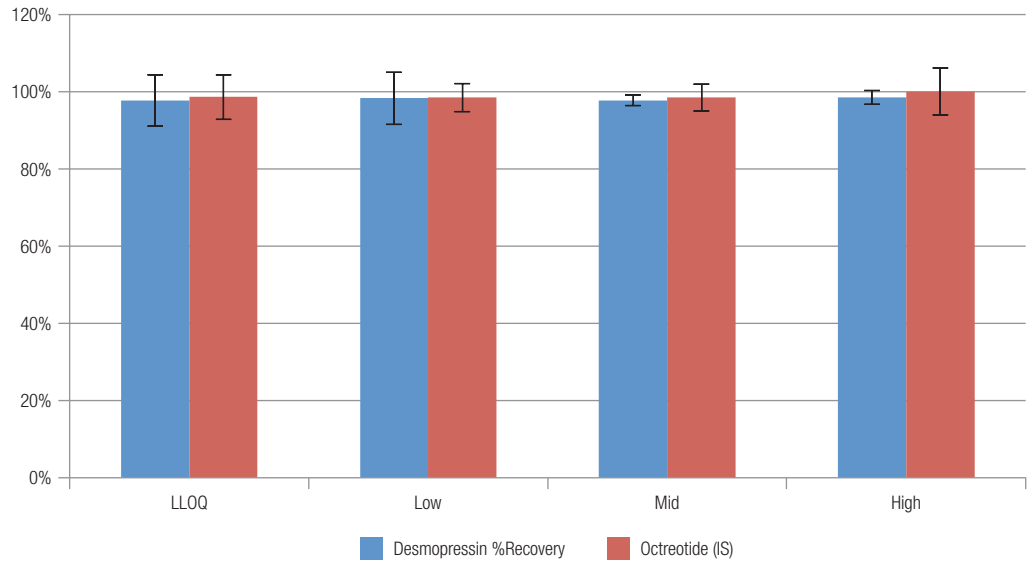


Figure 3. SPE recovery data.

Table 4. Matrix effects data.

Compound	% Matrix Effects at LLOQ	% Matrix Effects at QCL	% Matrix Effects at QCM	% Matrix Effects at QCH
Desmopressin	-3.86	-0.0543	-1.72	-1.23
Octreotide	-1.38	0.373	-2.89	-1.64

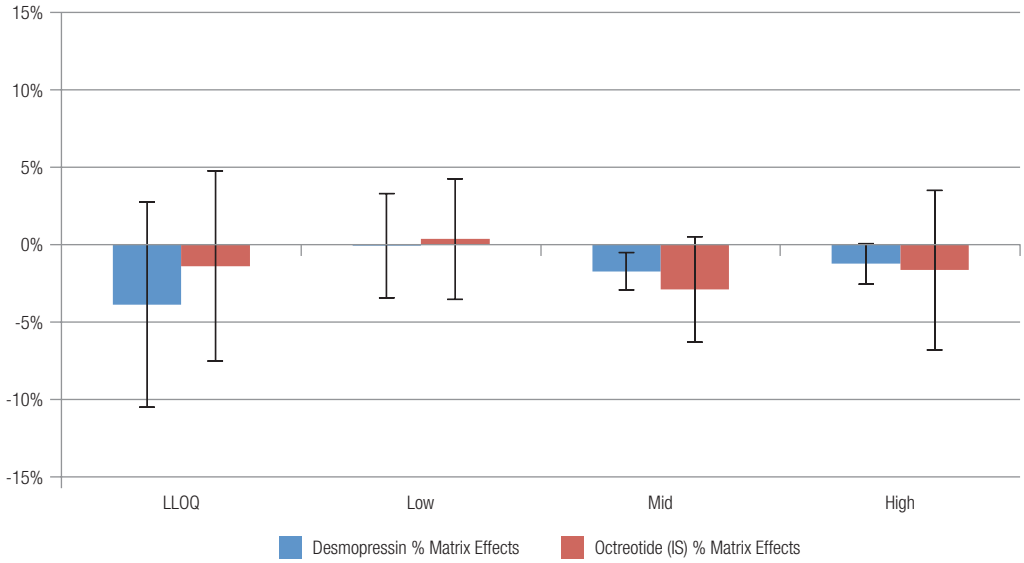


Figure 4. Matrix effects data.

Excellent linearity was observed with a coefficient of determination (r^2) value of 0.9981 over the concentration range of 10 to 5000 pg/mL. Linear 1/x weighting was employed (Figure 5 and Table 5).

The precision and accuracy was measured at LLOQ, Low, Mid, and High QC values (10, 30, 2500, and 4000 pg/mL, respectively) and are shown in Tables 5 and 6.

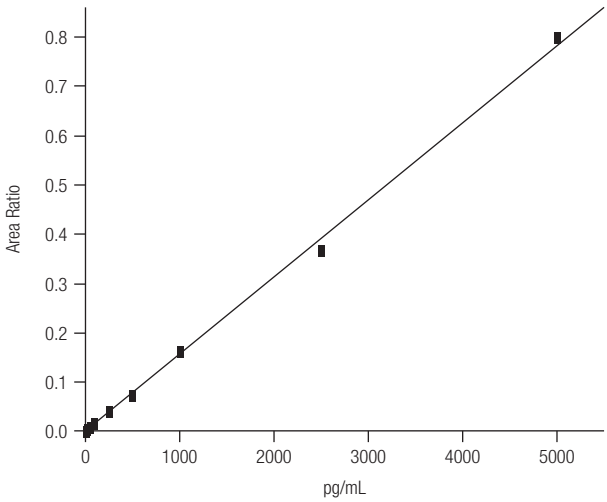


Figure 5. Desmopressin calibration line (10 to 5000 pg/mL).

Table 5. Desmopressin accuracy data.

Compound	Linearity Range (pg/mL)	Coefficient of Determination (r^2)	Mean Relative Error (%) at QCLLOQ (n=6)	Mean Relative Error (%) at QCL (n=6)	Mean Relative Error (%) at QCM (n=6)	Mean Relative Error (%) at QCH (n=6)
Desmopressin	10 to 5000	0.9981	0.347	3.74	3.24	3.82

Table 6. Desmopressin precision data.

Compound	% RSD at QCLLOQ at 10 pg/mL (n=6)	% RSD at QCL at 30 pg/mL (n=6)	% RSD at QCM at 2500 pg/mL (n=6)	% RSD at QCH at 4000 pg/mL (n=6)
Desmopressin	8.41	7.41	2.59	4.24

By exploiting the high pressure capabilities of the Vanquish UHPLC system, in conjunction with the Accucore Vanquish C18+ UHPLC column and a simple binary gradient, good separation of desmopressin, internal standard, and matrix components was achieved within 2.8 minutes with excellent peak shape. A typical chromatogram for the internal standard is presented in Figure 6. Chromatograms of the ULOQ (5000 pg/mL) and LLOQ (10 pg/mL) are presented in Figures 7 and 8.

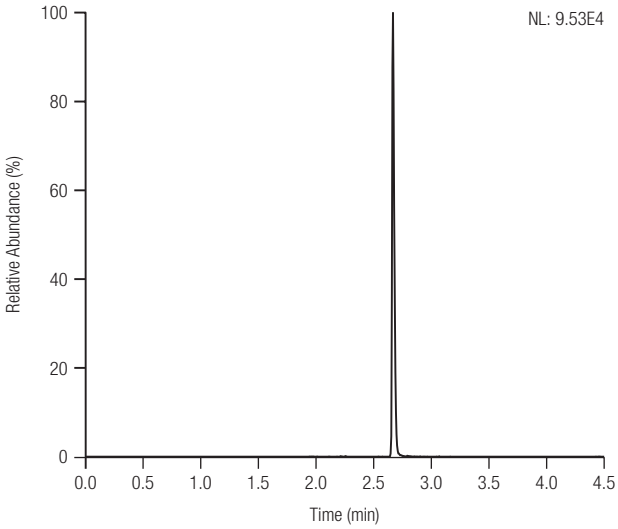


Figure 6. Octreotide (IS) at 5000 pg/mL.

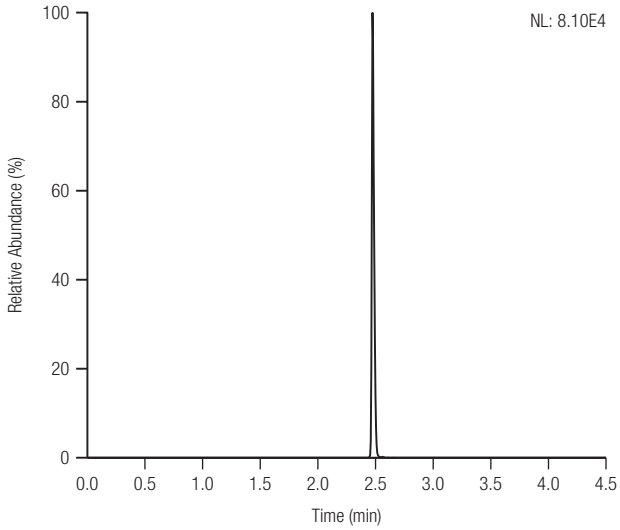


Figure 7. Desmopressin ULOQ sample (5000 pg/mL).

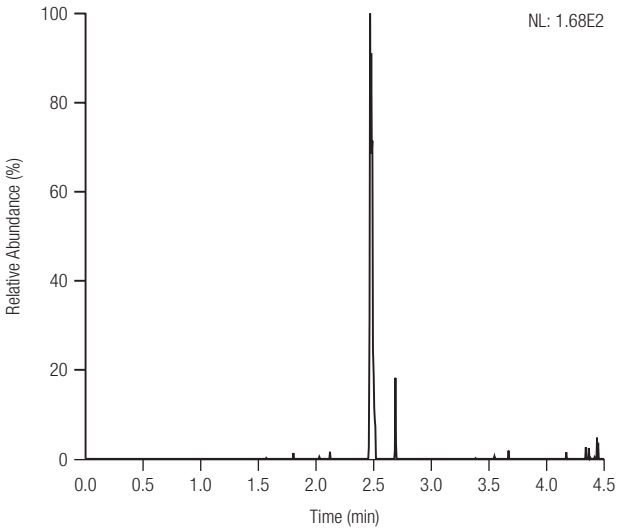


Figure 8. Desmopressin LLOQ sample (10 pg/mL).

Following the extraction of blank human plasma, a small background peak was observed at the same retention time as desmopressin (2.5 minutes). This peak was also present in the blank extracts from pooled plasma (Figure 9) and from six individual plasma sources (data not shown) to approximately the same level. The peak is less than 10% by area than that of the LLOQ sample and so is not considered to interfere with the assay.

For an assessment of carryover, blank reagent was subjected to the extraction procedure and injected onto the analytical system following a ULOQ sample extracted from human plasma. No peak was observed at or around the retention time for desmopressin, which indicated a carryover-free assay (Figure 10).

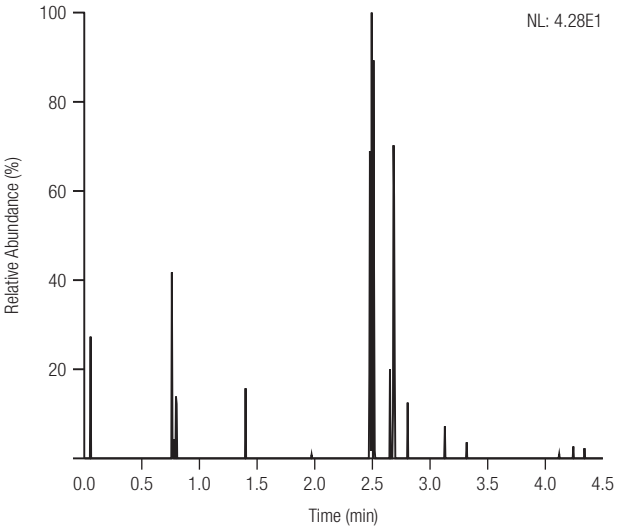


Figure 9. Desmopressin extracted blank + IS sample.

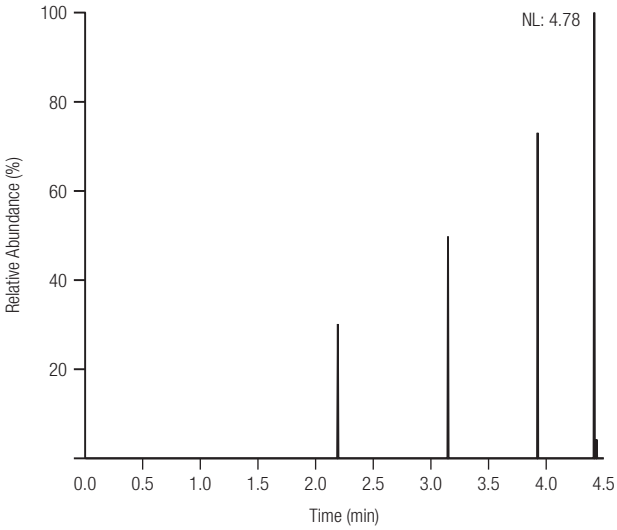


Figure 10. Reagent carryover blank injected after the highest calibration sample (5000 pg/mL).

Conclusion

The results presented demonstrate the successful development of an accurate, precise, and selective SPE-LC-MS/MS method for the analysis of desmopressin from human plasma. Use of micro-elution SPE allowed for concentration of the sample prior to analysis without the need for post-extraction processing. Utilizing SOLAμ mixed-mode SPE provided both a clean and repeatable extract across the concentration range.

- SOLAμ WCX SPE provides a fast, robust, and high-throughput method of extraction of desmopressin from human plasma samples.
- High levels of recovery and low levels of matrix effects were observed, together with high levels of accuracy and precision across the concentration range.
- Use of the Vanquish UHPLC System with an Accucore Vanquish C18+ 1.5 µm analytical column allowed for a fast separation from similar matrix components.

References

1. Neudert, L.; Zaugg, M.; Wood, S.; Struwe, P. A High Sensitivity Dual Solid Phase Extraction LC-MS/MS Assay for the Determination of the Therapeutic Peptide Desmopressin in Human Plasma (White Paper 2011), http://celerion.com/wordpress/wp-content/uploads/2011/09/A-High-Sensitivity-Dual-Solid-Phase-Extract-LC-MSMS-Assay-for-the-Determination-of-the-Therapeutic-Peptide-Desmopressin-in-Human-Plasma_2011.pdf

Useful Links

AppsLab Library

The eWorkflow and the Chromeleon Backup (cmbx) file can be downloaded at AppsLab Library: https://appslab.thermoscientific.com/