For Research Use Only. Not for use in diagnostic procedures.

Low-pg/mL quantification of complex disulfide-rich peptides in rat plasma using microflow LC-MS/MS

Eshani Nandita, Remco van Soest, Zoe Zhang and Elliott Jones
SCIEX, USA

ABSTRACT

Cyclic peptides exhibit enhanced biological activity compared to traditional peptides, given their disulfide-rich composition, which confers structural stability and conformational rigidity. As a result, cyclic peptides have become crucial therapeutic candidates and successful therapeutic agents in cardiovascular diseases. With the current advancement of cyclic peptide therapeutics, there is an equivalent drive toward the development of highly robust and sensitive quantitative methods. Existing LC-MS based bioanalytical methods lack the sensitivity necessary to reliably quantify cyclic peptides. This is primarily due to their complex tertiary structure and high baseline interference with the application of single MS mode. In this study, low-pg/mL quantification for complex cyclic peptides was achieved at an LLOQ of 0.01 ng/mL using a microflow LC-MS/MS platform.

INTRODUCTION

Cyclic peptides are polypeptides held in a ring configuration by chemically stable bonds, such as disulfide linkages. For example, the natriuretic peptide (NP) family is a group of genetically distinct cyclic peptides that contains an amino acid ring formed by disulfide bonds (Figure 1). The unique structure of these peptides confers structural stability and conformational rigidity. As a result, cyclic peptides can exhibit enhanced biological activity compared to traditional peptides. These features have helped identify cyclic peptides as important therapeutic candidates and successful therapeutic agents in cardiovascular diseases.[1]

With emerging interest in the advancement of cyclic peptide therapeutics, there is an equivalent drive towards the development of highly robust and sensitive quantitative methods. Current bioanalytical methods lack the sensitivity necessary to reliably quantify cyclic peptides. For LC-MS based methods, high baseline interference in single MS mode and resistance to CID in MS/MS mode, given the tertiary structure, have an impact on overall sensitivity.

In this study, human ANP was selected as a model analyte to evaluate improvement in sensitivity with the application of microflow LC. Low-level quantification was achieved for human ANP at an LLOQ of 0.01 ng/mL.

The application of microflow LC yielded excellent accuracy, precision and linearity, while providing outstanding quantitative performance in parallel with high sensitivity.

MATERIALS AND METHODS

Sample preparation:

Rat plasma was protein precipitated and the supernatant was diluted 1:1 (v/v) with water which served as the processed biological matrix. Human ANP and a labeled cyclic peptide, internal standard (IS), were spiked into the processed rat plasma. The IS concentration was 10 ng/mL. Serial dilution with processed plasma was performed to create the calibration curves for analysis.

LC conditions:

A SCIEX M5 MicroLC system was used for separation in trap-and-elute mode. A volume of 20 µL of sample was loaded onto the trap column for analysis. The mobile phase A consisted of 0.1% formic acid in water and the organic phase B was composed of 0.1% formic acid in acetonitrile.

Chromatographic conditions for analyte separation is summarized in Table 1. For analyte trapping, the operating flow rate was set to 50 µL/min using a Phenomenex Luna C18(2) column (20 x 0.3 mm, 5 µm, 100 Å). The column was operated at room temperature. For analyte separation, the operating flow rate was 5 µL/min using a Phenomenex Kinetex XB-C18 column (50 x 0.3 mm, 2.6 µm, 100 Å). The column oven temperature was 40ºC.

Mass spectrometry conditions:
Sample analysis was performed using a SCIEX 7500 system in MRM mode. Source was operated in positive ion mode. A summary of the source and MS parameters is displayed in Table 2. The MRM transition and method parameters for human ANP is outlined in Table 3. MRM data was processed using the Analytics function in SCIEX OS software 2.0 using the MQ4 integration algorithm. A weighting of 1/x2 was used for quantification.

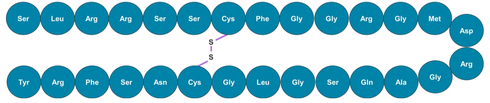

Figure 1. Amino acid sequence of human ANP. The cyclic peptide is composed of 28 amino acids and 1 disulfide-bridge between cysteine residues.

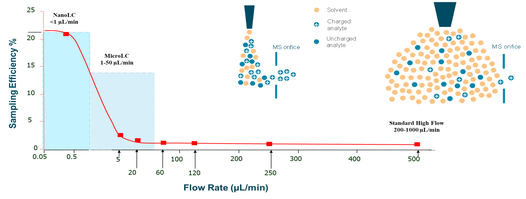

Figure 2. Higher sensitivity achieved using microflow with greater sampling efficiency. Generation of a more confined spray results in a more efficient ionization of the target analytes.

Collision energy, source and MS parameters were optimized to achieve sensitive MS/MS quantification.

Table 1. LC gradient conditions.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	60	40
5	40	60
5.2	10	90
6.8	10	90
7	60	40
8.5	60	40

Table 2. Source and MS conditions.

Parameter	Value	Parameter	Value
Curtain gas	35 psi	Source temp.	300°C
Ion source gas1	30 psi	Ion source gas 2	80 psi
CAD gas	11	Ion spray voltage	4000 V

Table 3. MRM method parameters.

Compound	Q1 mass (m/z)	Q3 mass (m/z)	CE (V)	CXP (V)
Human ANP	617.1	584.1	34	15

RESULTS

In this workflow, a sensitive LC-MRM method was developed for the quantification of cyclic peptides in rat plasma. Human ANP was spiked into processed rat plasma at concentrations ranging from 0.01 ng/mL to 200 ng/mL. Calibration curves were measured in triplicates.

The LLOQ was determined based on the requirements that the %CV is below 20% and accuracy is between 80% and 120%. For concentrations above the LLOQ, the %CV was required to be below 15%, with accuracy between 85% and 115%.

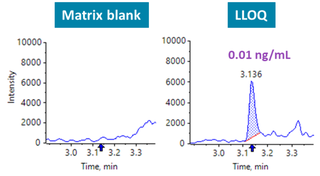

Figure 3. XICs of matrix blank and LLOQ of human ANP. No matrix interferences were observed at the retention time of the analyte.

An LLOQ of 0.01 ng/mL was achieved, as shown in Figure 3. No significant matrix interferences were observed at the retention time of the analyte.

The implementation of microflow LC resulted in a 5-fold improvement in sensitivity, compared to prior implementations of analytical flow LC on a SCIEX 7500 system.[2]

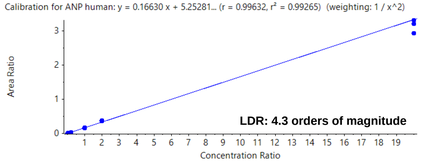

Figure 4. Calibration curve for human ANP. The linear range covered 0.01 ng/mL to 200 ng/mL with an overall linear dynamic range (LDR) of 4.3 orders of magnitude.

Table 4. Concentration, accuracy and precision for human ANP.

Concentration (ng/mL)	Accuracy (%)	CV (%)
200	94.60	6.24
20	108.94	3.36
10	100.94	8.60
2	107.67	3.91
1	101.18	3.24
0.5	93.99	9.26
0.2	99.51	12.30
0.1	93.08	3.20
0.02	98.62	8.44
0.01	101.47	4.10

Calculated concentrations for all calibration points were within ±15% of the nominal value (Table 4). As shown in Table 4, the precision was less than 12.5%, demonstrating high reproducibility.

Overall, a highly sensitive method for the quantification of cyclic peptides was demonstrated. For human ANP, quantification at low-pg/mL levels was achieved.

CONCLUSIONS

- An ultra-sensitive microflow LC-MRM based cyclic peptide quantification workflow using SCIEX 7500 system has been demonstrated in this work
- Compared to previously published data acquired using analytical flow LC on a SCIEX 7500 system[2], a 5-fold improvement in LLOQ was achieved with the implementation of a microflow LC workflow
- Low-level quantification was achieved for human ANP at an LLOQ of 0.01 ng/mL with exceptional reproducibility, accuracy, and linearity
- The combination of the D Jet ion guide, OptiFlow Pro ion source, and E Lens probe enabled a cumulative gain in sensitivity through improvement in ion generation, capture and transmission

REFERENCES

1. Das BB, Solinger R (2009) *Cardiovasc. Hematol. Agents Med. Chem.* **7(1)**, 29-42.
2. Improved LC-MRM quantification sensitivity for cyclic peptides from the natriuretic peptide family. SCIEX technical note, RUO-MKT-02-11883-A.

TRADEMARKS/LICENSING

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to www.sciex.com/diagnostics. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd. or their respective owners in the United States and/or certain other countries (see www.sciex.com/trademarks).

© 2022 DH Tech. Dev. Pte. Ltd. RUO-MKT-10-14632-A

To receive a copy of this poster:

- Scan the code with your phone camera
- Complete the form