

Improved LC-MRM quantification sensitivity for cyclic peptides from the natriuretic peptide family

Featuring the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software

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Cyclic peptides have been identified as important therapeutic modalities. This is related to their stability in blood and their potential for oral dosing. LC-MS method development to quantify trace levels of cyclic peptides in biological matrices has remained challenging. High baseline interference in matrices requires the method to be highly selective to obtain the desired S/N. They are often resistant to CID fragmentation, due to their knotted tertiary structure and/or non-mobile proton.

The natriuretic peptide (NP) family is a group of genetically distinct cyclic peptides with similar structure, containing an amino acid ring formed by a disulfide bond between two cysteine residues (Figure 1). They have emerged as important candidates for development of diagnostic tools and therapeutic agents in cardiovascular diseases.¹ In this project, two peptides in the NP family, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) were selected as model analytes to evaluate the quantification capability of the SCIEX 7500 System. The OptiFlow® Pro Ion Source, together with the D Jet™ Ion Guide, offers significantly improved sensitivity for cyclic peptide quantification. Improved ion desolvation and ion focusing improves MRM performance. Both ANP and BNP were quantified at 0.05 ng/mL in rat plasma, multiple times lower than the previously published LLOQ.² Outstanding reproducibility, accuracy, and linearity was also achieved, proving the workflow robustness in parallel with the superior sensitivity.

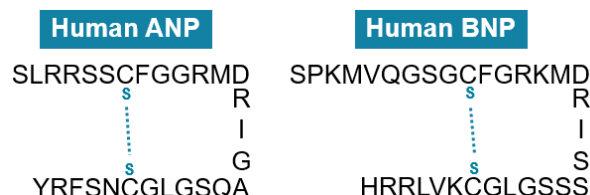


Figure 1. The amino acid sequences of human ANP and BNP. A disulfide bond is formed between the two cysteine residues, (C7-C23 for ANP, C10-C26 for BNP) to form the ring shape.



Key features of cyclic peptide quantification workflows using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

- Quantification of cyclic peptides (ANP and BNP) at 0.05 ng/mL in rat plasma with outstanding reproducibility, precision, accuracy, and linearity
- Hardware improvements including:
 - D Jet Ion Guide—sampling more ions, with no sacrifice in robustness³
 - OptiFlow Pro Ion Source—the next generation ion source with no physical adjustments required, achieve the best sensitivity under all conditions³
 - E Lens™ Technology—delivers greater sensitivity in ESI with increased field strength and ion generation, through more energetic ESI droplet desolvation³
- 5-fold improvements in S/N were shown compared to the SCIEX Triple Quad™ 6500+ LC-MS/MS System
- SCIEX OS Software for data acquisition and processing—single platform for acquisition, processing and management, customizable and easy to use

Methods

Sample preparation: Rat plasma was protein precipitated and loaded onto the mixed mode SPE cartridge. The eluents were diluted 1:1 (v/v) by water and served as the processed biological matrix. Human ANP, human BNP and rat ANP were spiked into the processed rat plasma, endogenous rat ANP was used as the internal standard. Serial dilution with processed plasma was performed to create the calibration curves for analysis.

LC-MS conditions: Samples were analyzed in triplicate by a SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready, coupled with an ExionLC™ system. The method details are summarized in Table 1 and 2. The same sample set was also analyzed using a SCIEX Triple Quad 6500+ LC-MS/MS System, coupled with the same HPLC system, to characterize the performance difference between the two mass spectrometers. All MRM parameters were optimized on both mass spectrometers for accurate performance comparison.

Data processing: Data was processed using the Analytics function in SCIEX OS Software 2.0.

Table 1. Chromatographic conditions.

Parameter	Value
Column	Phenomenex bioZen Peptide XB-C18 50x2.1 mm; 2.6 μ m
Mobile Phase A	Water with 0.1 % formic acid
Mobile Phase B	Acetonitrile with 0.1 % formic acid
Flow Rate	500 μ L/min
Column Temperature	40 °C
Injection Volume	10 μ L

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	95	5
0.1	95	5
5	75	25
5.1	10	90
6.0	10	90
6.1	95	5
7.0	95	5

Table 2. MS parameters on SCIEX 7500 System.

Parameter	Value	Parameter	Value
Curtain gas	40 psi	Source temperature	750 °C
Ion source gas 1	75 psi	Ion source gas 2	70 psi
CAD gas	12 psi	Ion spray voltage	2000 V

Name	Q1	Q3	CE	CXP
ANP human	617	584.1	34	15
BNP human	693.8	756	39	15
ANP rat	613.3	580.9	36	15

Cyclic peptide quantification results

The SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready has multiple novel hardware features to improve instrument sensitivity.³ The OptiFlow Pro Ion Source with E Lens Technology has a universal geometry that supports flow rates from 1 μ L/min to 3000 μ L/min without positional adjustment, using exchangeable probes for micro and high-flow applications.³ The E Lens Technology creates a stronger field at the ESI probe leading to more efficient release of ions from the droplet and deflection of ions towards the orifice for improved sensitivity.³ The D Jet Ion Guide behind the orifice plate efficiently captures and transmits the ions in the higher vacuum region. Its tapered dodecapole geometry, efficiently focuses the ions into the second stage QJet® Ion Guide.³

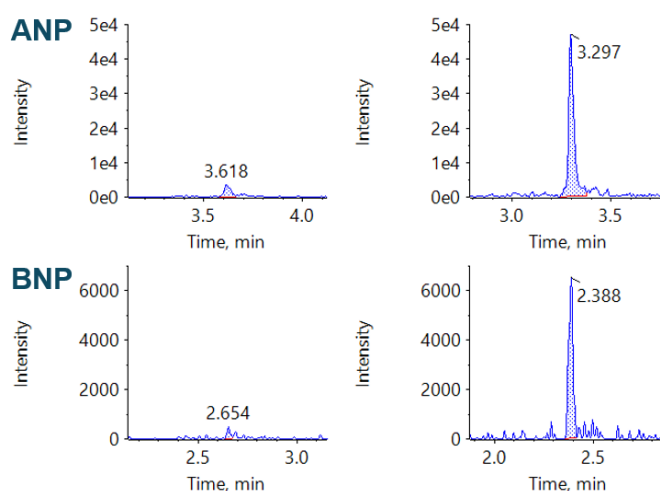


Figure 2. Sensitivity gains for peptide quantification. MRM XIC comparison between SCIEX 7500 System (right) and Triple Quad 6500+ LC-MS/MS System (left) for human ANP and BNP at 0.1 ng/mL. Area gains of >10X and S/N gains for ~5X were observed

Combining these technologies together, greater sensitivity is achieved through gains in the ion generation and ion focusing. To investigate the sensitivity improvement, the same sample set was analyzed on both the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready and the Triple Quad 6500+ LC-MS/MS System. A >10-fold increase in peak area and a 5-fold improvement in S/N was observed (Figure 2).

As shown in Figures 3, 4 and 5, both ANP and BNP are robustly quantified at 0.05 ng/mL in rat plasma, with tight %CV (<11%) and high accuracies (90-114%) across the entire linear dynamic range (0.05-100 ng/mL). 100 ng/mL is the highest concentration prepared in the sample set, the assay ULOQ would potentially be higher than 100 ng/mL as saturation was not yet reached.

Conclusions

- An ultra-sensitive MRM based cyclic peptide quantification workflow using SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready has been presented
- Human ANP and BNP were quantified at 0.05 ng/mL in rat plasma with outstanding reproducibility, accuracy and linearity
- Combining the OptiFlow Pro Ion Source with E Lens Technology and D Jet Ion Guide, significant sensitivity improvements were observed compared to a previously published method²
- A greater than 10-fold increase in peak area and a 5-fold improvement in S/N was observed versus the SCIEX 6500+ System

References

1. Das BB, Solinger R (2009) *Cardiovasc. Hematol. Agents Med. Chem.* **7(1)**, 29-42.
2. Ciccimaro E., et al, (2014) *Anal. Chem.* **86**, 11523–11527.
3. Enabling new levels of quantification. [SCIEX technical note RUO-MKT-02-11886-A](https://www.sciex.com/technical-note/RUO-MKT-02-11886-A).

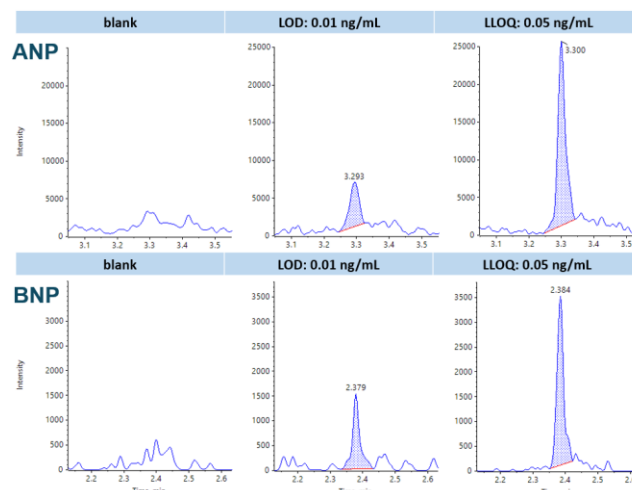


Figure 3. Representative MRM XICs for human ANP and BNP in rat plasma. From left to right: in matrix blank, at 0.01 ng/mL and 0.05 ng/mL.

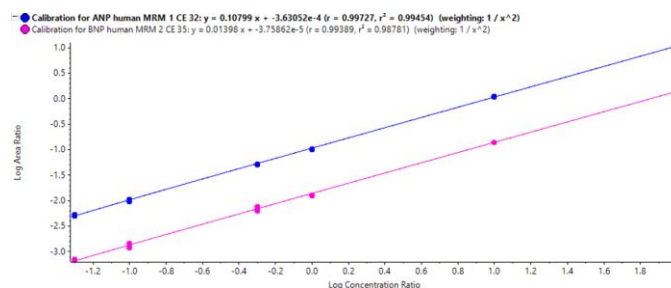


Figure 4. Calibration curves (log-log) for human ANP and BNP from 0.05 to 100 ng/mL in rat plasma.

Component...	Actual Conc...	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
ANP human...	0.05	3 of 3	5.141e-2	1.764e-3	3.43	102.81
ANP human...	0.10	3 of 3	9.565e-2	5.170e-3	5.40	95.65
ANP human...	0.50	3 of 3	4.809e-1	1.419e-2	2.95	96.19
ANP human...	1.00	3 of 3	9.456e-1	2.325e-2	2.46	94.56
ANP human...	10.00	3 of 3	1.022e1	1.085e-1	1.06	102.17
ANP human...	100.00	3 of 3	1.086e2	6.032e0	5.55	108.62

Component...	Actual Conc...	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
BNP human...	0.05	3 of 3	5.110e-2	2.203e-3	4.31	102.19
BNP human...	0.10	3 of 3	9.715e-2	9.789e-3	10.08	97.15
BNP human...	0.50	3 of 3	4.860e-1	4.916e-2	10.12	97.21
BNP human...	1.00	3 of 3	9.017e-1	7.905e-3	0.88	90.17
BNP human...	10.00	3 of 3	9.946e0	1.473e-1	1.48	99.46
BNP human...	100.00	3 of 3	1.138e2	5.558e0	4.88	113.83

Figure 5. Quantification summaries for human ANP and BNP in rat plasma.

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