APPLICATION NOTE

1599-9833

Author:
PerkinElmer, Inc.
Waltham, MA

Low Attomole Quantitation of Bio-Therapeutic Peptides in Human Plasma with PerkinElmer QSight® 220 Triple Quad Mass Spec

Introduction

With an increasing number of peptide-based drugs being developed and used in the market, reliable, rapid and sensitive quantitation methods are needed. Although traditional immunoassay based methods can offer sensitive quantitation for larger molecules, they have poor linear dynamic range and utilize antibodies which can detect only a single, specific target, making assays costly and time consuming. Additionally, they may have poor selectivity because of interference from homologous peptides with similar chemical or structural characteristics.

With high selectivity, improved precision and accuracy, the LC-MS/MS technique is favoured in peptide quantitation.

Therapeutic peptides have multiple charge states, extensive fragments, and a small dynamic range which make it challenging to develop a fast and reliable quantitative method. However with recent advancements in tandem quadrupole mass spectrometry and a unique ion source, a simple and sensitive LC-MS/MS method utilizing the QSight® 220 triple quadrupole mass spectrometer has been developed for the quantitation of several therapeutic peptides in human plasma at attomole levels.

PerkinElmer®
For the Better

2. Method

Peptides (Angiotensin-I, Angiotensin II, Arg-vasopressin, and α-Neo-Endorphin(1-7)) and human plasma were purchased from Sigma. All of the chemicals were used as received. A stock solution for each of the peptides was prepared at a 1 mg/mL concentration in methanol and stored at -20°C. The stock solutions were then diluted to the targeted concentrations used in the method.

Human plasma (after being subjected to protein precipitation) was used as a matrix solvent. The protein precipitation procedure used was to mix one volume of human plasma with two volumes of cold acetonitrile (-20°C), and then to centrifuge the mixture for 20 min at 5000 rpm. The last step is to filter the supernatant with a 0.2µ filter.

The targeted peptides were then spiked into the protein-precipitated plasma and sequentially diluted with protein-precipitated plasma in order to prepare concentrations used for the calibration curve (0.001-100 ng/mL).

2.1. Mass Spectrometry Conditions

Table 1: Overview of Settings used on the QSight® 220 Mass Spectrometer

ESI Voltage (V)	5500
HSID Temp (°C)	250
Nebulizer Gas Setting	400
Drying Gas Setting	150
Heating Gas	350
Source Temp. (oC)	325
Dwell Time (ms)	100
Pause Time (ms)	5

2.2. LC Conditions

Utilizing a Shimadzu® Prominence UFLC® system, a 10 µL sample was loaded on a Phenomenex Kinetex® C18 column (50x2.1mm, 2.6µ) at 50°C with a gradient method at 400 µL/min: solvent B (5% water in methanol, 0.1% formic acid and 5mM NH_4OAc) was kept at 5% until 1 min and increased to 95% at 3.1 min, washed about 1.0 min and followed by 1.0 min post separation equilibrium. The LC cycle time was 5.5 min. The solvent A was 5% methanol in water with 0.1% formic acid and 5 mM NH_4OAc.

Quick Facts:

- Quantitative method for α-Neo-Endorphin, Angiotensin-I, Angiotensin-II and Arg-vaso-pressin in human plasma utilizing the QSight® 220 triple quadrupole mass spectrometer.
- LLOQ: (Attomole per µL)
  - α-Neo-Endorphin: 10.7
  - Angiotensin-I: 7.5
  - Angiotensin-II: 74.5
  - Arg-vasopresin: 19.1

3. Results

3.1. Extracted Ion Chromatogram (EIC)

Figure 1: (A) Human plasma blank, The EIC of MRM at m/z 420.8/136.1 (B) α-Neo-Endorphin(1-7) (2+), 10.7 attomol/µL in human plasma, The EIC of MRM at m/z 420.8/136.1.

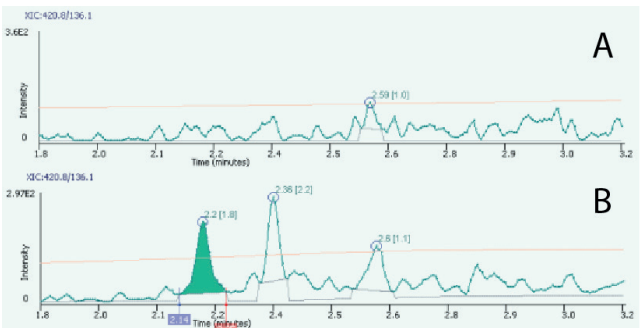


Figure 2: (A) Human plasma blank, The EIC of MRM at m/z 433.0/110.1 (B) Angiotensin I (3+) 7.5 attomol/µL in human plasma, the EIC of MRM at m/z 433.0/110.1.

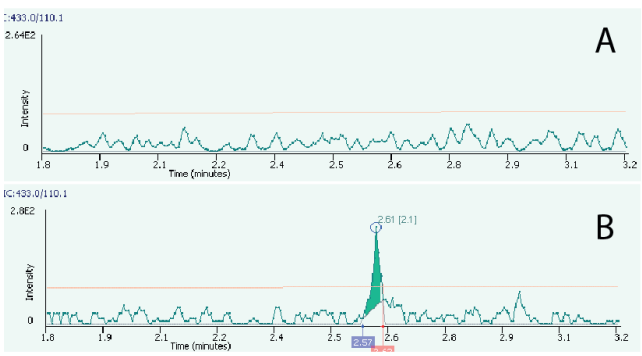


Figure 3: (A) Human plasma blank, The EIC of MRM at m/z 523.9/110.1 (B) Angiotensin II (2+), 74.5 attomol/µL in human plasma, The EIC of MRM at m/z 523.9/110.1.

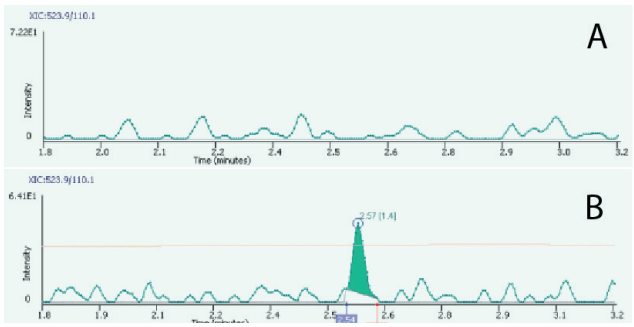
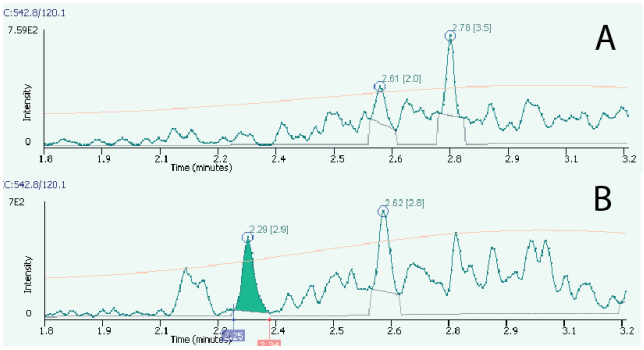
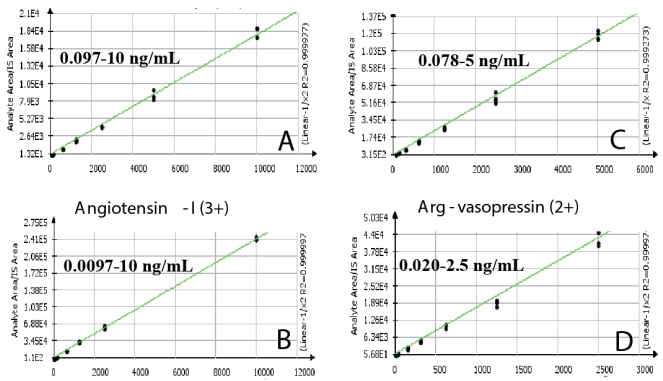


Figure 4: (A) Human plasma blank, The EIC of MRM at m/z 542.8/120.1 (B) Arg-Vasopressin (2+), 19.1 attomol/µL in human plasma, The EIC of MRM at m/z 542.8/120.1.



3.2. Linearity

Figure 5: Calibration curves for (A) α-Neo-Endorphin (1-7), (B) Angiotensin-I, (C) Angiotensin-II and (D) Arg-Vasopressin.



3.3. Quantitation Results

As illustrated below in Table 2, the LLOQs for the four peptides range from 7.5 to 74.5 attomole/µL while accuracies range from 97.1 to 106.1% and CVs range from 11.6 to 16.4%.

Table 2: MRM Transitions, LLOQ levels, Average Accuracy and Central Variance for Measured Peptides

Peptides	Q1	Q2	LLOQ attomol/µl	Av. accuracy (%)	CV %
A-Neo-Endorphin (1-7)	420.8	136.1	10.7	102.2	13.1
Angiotensin I	433.0	110.1	7.5	106.5	11.6
Angiotensin II	523.9	110.1	74.5	106.1	12.3
Arg^8-vassopressin	542.8	120.1	19.1	102.297.1	16.4

4. Conclusion

Based on the comparison of the four peptides in human plasma and solvent, the matrix effect is negligible. Each of the 4 peptides in human plasma has similar quantitation levels to those in solvent. The measured linear dynamic range is up to four orders of magnitude.

This LC-MS/MS method utilizing the PerkinElmer QSight® 220 triple quadrupole mass spectrometer could provide high sensitivity and selectivity for therapeutic peptide quantitation in human plasma, with quantitative capabilities in the low attomole level in clinical research studies.

5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer, Inc.
Wallac Oy
PO Box 10
20101 Turku, Finland
Phone: + 358 2 2678 111
Fax: + 358 2 2678 357

ISO 13485
ISO 9001
CMDCAS
ISO 14001
OHSAS 18001

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

1599-9833