

MetaboQuan-R for Amino Acids in Human Serum: A Rapid, Targeted UPLC-MS/MS Method for Metabolomic Research Studies

Billy Joe Molloy
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Simultaneous analysis of 29 amino acids in a single analytical run that is under four minutes
- High throughput analysis means larger sample sets can be analyzed rapidly
- Rapid separation of isobaric compounds
- Use of a generic LC-MS configuration yields versatility for switching from one compound class to another

WATERS SOLUTIONS

[AccQ-Tag™ Kit](#)

[CORTECS™ UPLC™ Columns](#)

[ACQUITY™ UPLC I-Class System](#)

[Xevo™ TQ-S micro](#)

[MassLynx™ Software](#)

[TargetLynx™ Software](#)

[Quanpedia™ Software](#)

[MetaboQuan-R™](#)

KEYWORDS

Targeted, amino acids, UPLC, tandem quadrupole, Xevo TQ-S micro, multiple reaction monitoring (MRM), biomarkers, human serum, metabolomics

INTRODUCTION

Amino acids are the constituent building blocks of proteins, and as such are extremely important molecules in human biochemistry. The analysis of these compounds is generally performed using derivatization, followed by flow injection analysis – tandem mass spectrometry (FIA-MS/MS). This method however cannot distinguish isobaric species resulting in limited information acquired from these types of analyses. Here we demonstrate a high-throughput UPLC-MS/MS research method for the semi-quantitative analysis of derivatized amino acids in human serum samples. This application note is also part of a MetaboQuan-R method package.

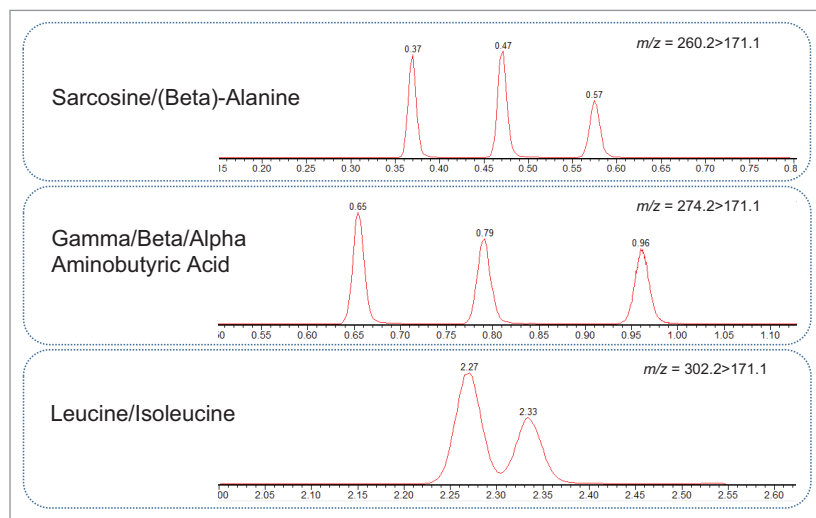


Figure 1. Separation of key isobaric amino acids in human serum.

EXPERIMENTAL

Human serum sample preparation

Human serum samples were prepared using the Waters AccQTag™ Ultra “3X” Derivatization Kit (p/n [186004535](#)). Samples were crashed using sulfosalicylic acid and then derivatized as follows:

- Step 1 Add 50 µL of sample to 1.5 mL eppendorf
- Step 2 Add 50 µL of 10% sulfosalicylic acid
- Step 3 Vortex mix for five seconds
- Step 4 Add 50 µL of water
- Step 5 Vortex mix for five seconds
- Step 6 Centrifuge for 10 minutes at 10,000 rpm @ 5 °C
- Step 7 Add 70 µL of Borate buffer (from AccQTag Kit) to a maximum recovery vial
- Step 8 Transfer 10 µL of supernatant to the maximum recovery vial
- Step 9 Vortex mix for five seconds
- Step 10 Add 20 µL of AccQTag reagent (from AccQTag Kit)
- Step 11 Vortex for five seconds after addition to each sample, allow sample to stand at ambient for one minute
- Step 12 Heat for 10 minutes at 55 °C
- Step 13 Perform a 1 in 10 dilution in 80:20 (water:acetonitrile) (90 µL plus 10 µL sample) in a max recovery vial
- Step 14 Inject 2 µL

LC conditions

UPLC separation was performed with an ACQUITY UPLC I-Class System (fixed loop), equipped with a CORTECS T3 2.7 µm (2.1 × 30 mm) analytical column. A sample of 2 µL was injected at a flow rate of 1.3 mL/min. Mobile phase A was 0.01% formic acid_(aq) containing 0.2 mM Ammonium Formate and mobile phase B was 50% isopropanol in acetonitrile containing 0.01% formic acid and 0.2 mM Ammonium Formate. The derivatized amino acids were eluted from the column and separated with a gradient of 1–8% Mobile phase B over 2.4 minutes, followed by a 0.9 minute column wash at 98% Mobile phase B. The column was then re-equilibrated to initial conditions. The analytical column temperature was maintained at 60 °C.

MS conditions

Multiple Reaction Monitoring (MRM) analyses were performed using a Xevo TQ-S micro mass spectrometer. All experiments were performed in positive electrospray ionization (ESI+) mode. The ion source temperature and capillary voltage were kept constant and set to 150 °C and 2.0 kV respectively. The cone gas flow rate was 50 L/hr and desolvation temperature was 650 °C.

Informatics

Method information was imported onto the LC-MS system using the Quanpedia functionality within MassLynx. This extendable and searchable database produces LC and MS methods as well as processing methods for use in TargetLynx for compound quantification.

RESULTS

The 29 amino acids detailed in Table 1 were separated and detected using the LC-MS platform and extraction protocol described herein. Figure 1 shows example chromatograms for the separation of key isobaric compounds achieved using the UPLC method detailed above.

Table 1. List of MS/MS conditions and retention times for derivatized amino acids.

| Amino acid | MRM transition | RT (min) | Cone voltage (V) | Collision energy (eV) |
|-----------------------------|----------------|----------|------------------|-----------------------|
| 4-hydroxyproline | 302.20>171.10 | 0.17 | | |
| Alanine | 260.20>171.10 | 0.57 | | |
| α -Aminobutyric acid | 274.20>171.10 | 0.96 | | |
| β -Aminobutyric acid | 274.20>171.10 | 0.79 | | |
| γ -Aminobutyric acid | 274.20>171.10 | 0.65 | | 20 |
| Aminoadipic acid | 332.20>171.10 | 0.70 | | |
| Asparagine | 303.20>171.10 | 0.20 | | |
| Beta-alanine | 260.20>171.10 | 0.47 | | |
| Citrulline | 346.20>171.10 | 0.41 | | |
| Ethanolamine | 232.20>171.10 | 0.34 | | 14 |
| Glutamine | 317.10>171.10 | 0.30 | | 20 |
| Glycine | 246.20>171.10 | 0.30 | | 25 |
| Histidine | 326.20>171.10 | 1.31 | | |
| Homocitrulline | 360.20>171.10 | 0.67 | | |
| Isoleucine | 302.20>171.10 | 2.27 | 30 | 20 |
| Kynurenine | 379.20>171.10 | 2.07 | | |
| Lysine | 244.10>171.10 | 1.59 | | 25 |
| Methionine | 320.20>171.10 | 1.32 | | 20 |
| Ornithine | 237.10>171.10 | 1.32 | | 25 |
| Phenylalanine | 336.20>171.10 | 2.30 | | 20 |
| Proline | 286.20>171.10 | 0.74 | | 25 |
| Sarcosine | 260.20>171.10 | 0.37 | | 20 |
| Serine | 276.20>171.10 | 0.25 | | 15 |
| Taurine | 296.20>171.10 | 0.24 | | 20 |
| Threonine | 290.20>171.10 | 0.47 | | 25 |
| Tryptophan | 375.20>171.10 | 2.43 | | 20 |
| Tyrosine | 352.20>171.10 | 1.30 | | 25 |
| Valine | 288.20>171.10 | 1.49 | | 20 |
| Leucine | 302.20>171.10 | 2.33 | | |

CONCLUSIONS

A rapid UPLC-MS/MS methodology has been developed for the research analysis of derivatized amino acids. This method has been demonstrated to be suitable for the analysis of physiologically relevant levels of these analytes in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics), meaning it can be applied as part of a suite of analyses that are part of a targeted multi-omics workflow.

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

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters, The Science of What's Possible, ACQUITY, UPLC, MassLynx, TargetLynx, MetaboQuan-R, Quanpedia, and Xevo are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2019 Waters Corporation. Produced in the U.S.A. February 2019 720006271EN AG-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com