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应用纪要

MetaboQuan-R for Amino Acids in Human Serum

Billy J. Molloy

Waters Corporation

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Abstract

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.

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

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.

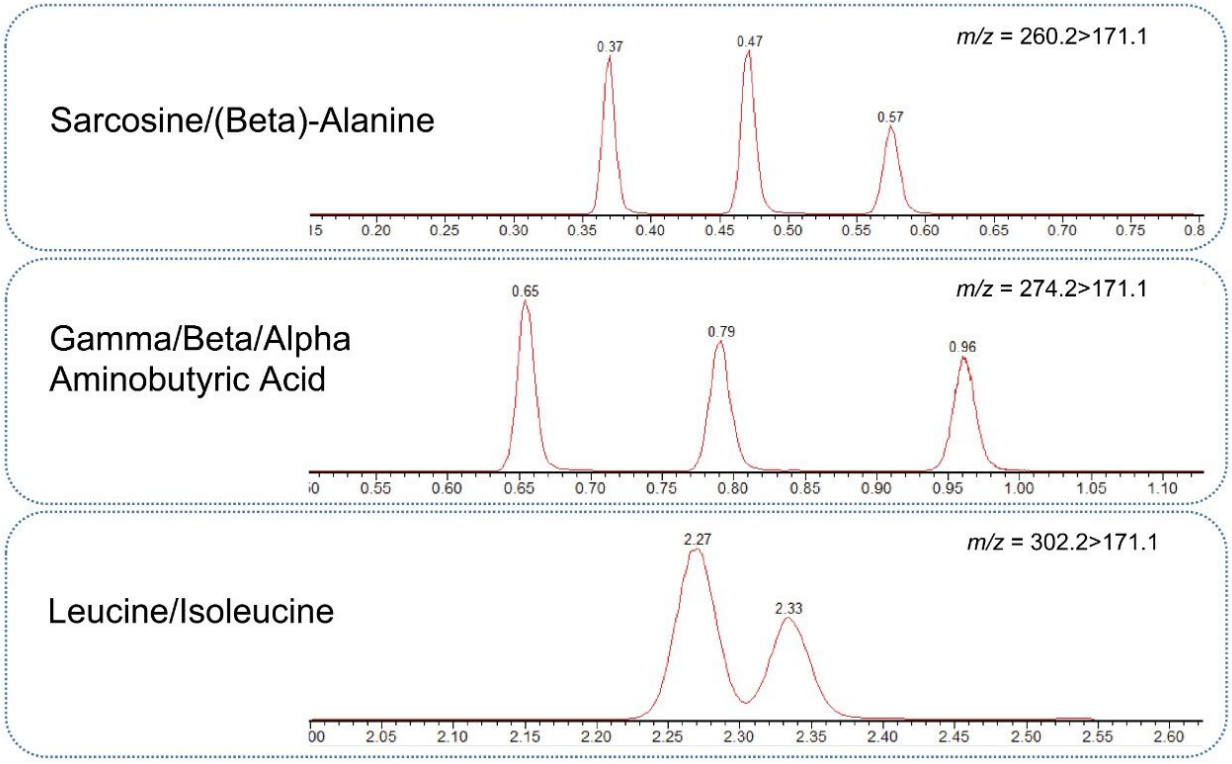

Figure 1. Separation of key isobaric amino acids in human serum.

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 uL 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 ÅãC

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 and Discussion

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.

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		
Histidine	326.20>171.10	1.31		25
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		
Proline	286.20>171.10	0.74		20
Sarcosine	260.20>171.10	0.37		25
Serine	276.20>171.10	0.25		20
Taurine	296.20>171.10	0.24		15
Threonine	290.20>171.10	0.47		20
Tryptophan	375.20>171.10	2.43		25
Tyrosine	352.20>171.10	1.30		
Valine	288.20>171.10	1.49		20
Leucine	302.20>171.10	2.33		

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

Conclusion

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.

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MetaboQuan-R <https://www.waters.com/waters/Targeted-Omics/nav.htm?cid=134980821>

Available for purchase online

AccQ-Tag Ultra "3X" Derivitization Kit <
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CORTECS T3 Column, 120Å, 2.7 µm, 2.1 mm X 30 mm <
https://www.waters.com/waters/partDetail.htm?partNumber=186008481>

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