SHIMADZU

ThP 178

Quantitation of free amino acids in human plasma by Single Quadrupole LC-MS

Tuna Oncu[1], Oben Arslan [1], Ugur Seker [1], Huseyin Akpinar[1]
(1) Shimadzu Middle East and Africa FZE Istanbul, Turkey.

1. Introduction

Amino acids are organic compounds that combine to form proteins. They are typically known as the building blocks of protein. Quantitative analysis of amino acids from biological fluids is of great importance in the clinical diagnosis of inborn errors of amino acid metabolism. The assessment of amino acids in body fluids is needed for the diagnosis and treatment of metabolic diseases. We have here performed a high-throughput method for analysis of amino acids in plasma samples. The method combines classical protein hydrolysis and derivatization with fast separation by UHPLC and detection by a single quadrupole mass spectrometer.

2. Methods

LC-MS instruments are effective systems used in the assessment of amino acid related disorders and can provide quantitative measurement of amino acids with a short sample preparation procedure. Compared to triple quadrupole LC/MS systems, single quadrupole LC/MS systems are cheaper and offer simple analytical conditions and MS optimization. Therefore, it allows even users with basic MS experience to easily operate instrument. More than 30 amino acids were analyzed quantitatively in SIM mode by Shimadzu LCMS-2050 single quadrupole mass spectrometer. Sample preparation is based on Trivitron Bome Trimaris Quantitative Amino Acids Analysis Kit. LCMS-2050 single quadrupole instrument provides results in 20 minutes with minimal sample preparation.

Column	Trimaris Quantitative Amino Acids Column
Mobile phase	Trimaris Mobile Phase A/B
Flow rate	0.25 mL/min
Gradient	35%B (0 min.) → 65%B (12 min.) → 95%B (12.01) → 95%B (16 min.) → 35%B (16.01 min.) → 35%B (20 min.)
Injection Volume	0.5 µL
Column Temperature	40 °C
Ionization	DUIS (positive)
Scan Mode	SIM
DL Temperature	200 °C
Drying Gas Flow Rate	5 L/min
Nebulizing Gas Flow Rate	2 L/min
Heating Gas Flow Rate	7 L/min

Fig. 1 Shimadzu LCMS-2050 and method parameters.

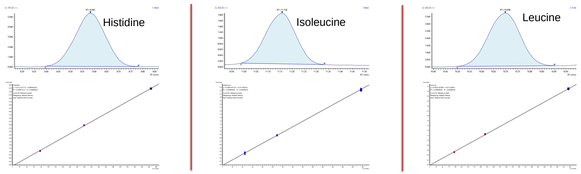
3. Results

In this study, free amino acids were extracted from human plasma samples by using acidic extraction and deproteinization steps. After the extraction, these amino acids were derivatized by reagents. Then derivatized amino acids were separated by analytical column with gradient elution and analyzed by Shimadzu LCMS-2050. The method uses deuterated internal standards for quantitative concentration of all amino acids. 3 level plasma matrix was plotted based on Trivitron Bome Trimaris quantitative amino acid sample preparation protocol. A good linear relationship between analyte peak area/internal standard peak area and analyte concentration/internal standard concentration was obtained for all compounds. (R^2 >0.995). Two quality control (QC) materials were analyzed at high and low levels. The intra-day and inter-day reproducibility of the studies were determined.

Sample Preparation

1. Take 100 µL of sample, QC or standard into the sample preparation tube.
2. Add 100 µL of Reagent 1 and vortex for 10 seconds.
3. Add 200 µL of Reagent 2 and vortex for 10 seconds.
4. Add 200 µL of Reagent 3 and vortex for 15 seconds. Incubate at room temperature for 3 min.
5. Add 200 µL of Reagent 4 and vortex for 15 seconds. After mixing wait for a minute.
6. Centrifuge for 5 min at 8000 rpm.
7. Transfer the clear supernatant to 200 µL of insert vial.

Amino acid derivatization prior to mass spectrometry has the advantage that the chromatographic separation of the compound is more manageable and increases its specific sensitivity. On the other hand, derivatization did not result in a significant loss of time in sample preparation. Leucine and isoleucine amino acids with similar m/z charge ratios were separated chromatographically.



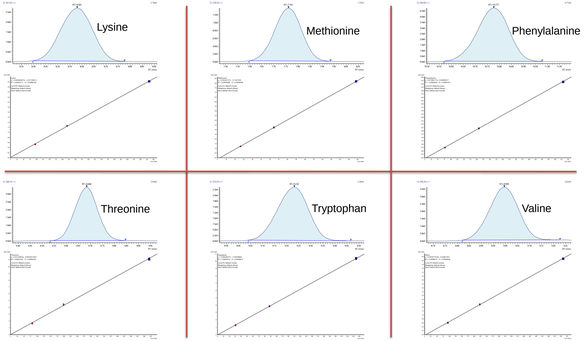


Fig. 2 SIM chromatograms & calibration curves of 9 essential amino acids

No.	Compounds	m/z	R^2	Rt	Cal Range (nmol/mL)			Low QC RSD%	High QC RSD%
1	Phosphoethanolamine	228.1	0.9998	2.7	47.3	156.1	523.5	3.2	1.1
2	Ethanolamine	148.1	0.9964	2.7	24.6	65.7	168.5	0.4	0.8
3	Arginine	303.2	0.9996	2.8	57.2	137.8	353	0.7	0.9
4	Glutamine	275.1	0.9992	3.5	188	527.5	1452.2	1.6	0.7
5	Citrulline	304.0	1.0000	3.6	30.8	88.5	237.9	2.3	1.6
6	Hydroxylysine	317.2	0.9997	3.9	13.6	42.3	118.6	2.5	1.4
7	Serine	234.3	0.9999	4.0	119.4	273.8	617.5	1.4	1.1
8	Asparagine	243.3	0.9992	4.0	37.7	107.7	282.2	4.5	1.0
9	Hydroxyproline	260.2	0.9999	4.3	23	68.3	195.8	6.8	2.4
10	5-hydroxytryptophan	349.1	0.9999	4.3	9.1	29.8	69.9	6.8	1.9
11	Glycine	204.3	0.9998	4.5	186.5	497.2	1310	1.5	1.7
12	Threonine	248.1	0.9993	4.7	81.4	198.1	518	2.1	0.6
13	Argininosuccinic acid	443.2	0.9989	5.3	3	10	26.9	8.3	2.6
14	Alanine	218.3	0.9980	5.7	146.8	354.5	904.1	-	8.1
15	Histamine	284.1	1.0000	5.9	20.4	63	192.5	2.8	2.1
16	gamma-aminobutyric acid	232.2	0.9999	6.0	13.7	39.4	117.7	-	1.1
17	beta-aminoisobutyric acid	232.2	0.9999	6.7	12.6	37	105.3	-	2.3
18	alpha-aminobutyric acid	232.2	1.0000	7.2	11.7	35	100.2	-	1.3
19	Ornithine	347.3	1.0000	7.5	35.8	110.6	311.7	7.0	1.7
20	Proline	244.1	0.9995	7.6	95.4	217.8	558.9	2.9	0.8
21	Methionine	278.2	1.0000	7.8	26.2	64.3	158.3	9.2	0.8
22	Thiaproline	262.1	1.0000	8.4	11	32.6	110.3	9.1	1.2
23	Aspartic acid	304.2	0.9993	8.6	49.1	107.7	249	7.2	0.7
24	Histidine	370.2	0.9997	8.6	76.6	199.2	384.9	1.4	0.5
25	Lysine	361.2	0.9998	8.6	95	217	532.9	1.1	0.5
26	Valine	246.2	0.9999	9.0	92.5	222.5	558.7	1.4	0.8
27	Glutamic acid	318.2	0.9959	9.1	115.4	271.9	636.3	3.5	1.5
28	Tryptophan	333.2	1.0000	9.2	30.4	88	234.4	4.0	0.8
29	alpha-aminoadipic acid	332.2	0.9992	10.3	14.8	47.5	133.6	-	1.3
30	Leucine	260.2	0.9997	10.7	95.6	215.5	542.9	1.1	1.2
31	Phenylalanine	294.0	0.9999	10.8	77.4	206.6	530.7	2.8	0.9
32	Isoleucine	260.2	1.0000	11.2	44.5	108.7	276.4	3.0	1.0
33	alpha-aminopimelic acid	346.2	0.9975	11.8	12.2	37.4	105.2	-	1.4
34	Cystathionine	479.2	0.9998	12.2	7.2	22.3	60.3	0.8	0.8
35	Cystine	497.2	1.0000	12.7	15.6	46.9	163.32	1.0	0.4
36	Tyrosine	396.2	1.0000	13.3	42.2	108.1	293	4.8	1.9
37	Homocysteine	525.3	0.9994	14.8	4.1	12.4	42.8	7.2	1.5

Table 1: Summary of calibration linearity & repeatability.

Quantitative results were evaluated by LabSolutions Insight software. Amino acid reference ranges vary depending on the age of the patient. Lab Solutions Insight contains QA/QC flagging criteria for retention times, reference ion ratios, concentration limits, and more. Configurable color-coding is available to highlight results that are near or outside of limits. Flagging criteria in the evaluation of the patient's result is important in terms of faster evaluation of the result.

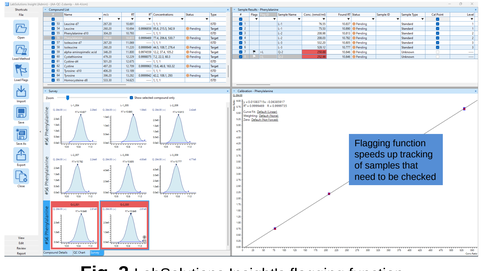


Fig. 3 LabSolutions Insight's flagging function

4. Conclusion

- Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) analysis of amino acids is becoming the method of choice by more and more laboratories because of its speed, sensitivity, and increased specificity. Although LC-MS/MS instruments are more selective and sensitive than LC-MS single quadrupole instruments, LC-MS can also be used in the quantitative analysis of amino acids when the necessary conditions are provided.
- The required sensitivity and chromatographic separation were achieved with the single quadrupole LC-MS.
- The method can quantitatively analyze 37 Amino acids in biological fluids such as plasma in less than 20 minutes.

The authors are affiliated and funded by Shimadzu Corporation.