A Combined MRM and SIM Method for Direct Quantitative Determination of Amino Acids in Various Samples on LC/MS/MS

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1. Introduction

Quantitative analysis of amino acids in biological samples, food and nutrition products are often required in various fields from research to manufacturing [1]. Recently, Imtakt introduced a new Intrada Amino Acid column, which is composed of a mixed stationary phase of ion exchange and normal phase, for direct separation and detection of amino acids on LC-MS without the need for pre- or post-column derivatization [2]. This new method not only simplifies the analysis of amino acids drastically, but also reduces the running cost and enhances the applicability for various kinds of samples. We describe the applications of the Intrada Amino Acid column with using a combined MRM-SIM mode for direct analysis of 20 amino acids in a variety of samples on LC/MS/MS. The samples include from human plasma, serum, urines to wines, beers, vinegar, sports water & amino acid drink. The aim of using a combined SIM and MRM method is to increase the detection sensitivity of certain amino acids which have low sensitivities in MRM mode, mainly Glycine and a few other amino acids.

2. Experimental

Twenty amino acid standards in powders were obtained from Sigma Aldrich. They were dissolved in 0.1N HCl solution to obtain individual stock solutions, except for cystine and glutamine, which were dissolved in 1.0N HCl solution. A mixed standard was prepared from the stocks and was diluted using pure water serially to various concentrations as calibrants. Two categories of samples, i.e., biological and beverage samples were collected and analyzed. The sample was de-proteinized by adding MeOH/ACN (1:1) solvent in a ratio of 1:3 or 1:4, followed by vortex and centrifugation at 13,000rpm for 10 mins. The supernatant was transferred and filtered before LC/MS/MS analysis. An LCMS-8040 triple quadrupole coupled with an UFLC system (Shimadzu Corporation) was employed in this work. The detailed conditions are compiled in Table 1.

Table 1: Analytical conditions of twenty amino acids on LCMS-8040: HPLC (left) and MS/MS (right)

Column	Intrada Amino Acid (100 x3 mm, 3μm)
Flow rate	0.6 mL/min
Mobile phase	A: ACN/THF / 25mM ammonium formate /FA = 9 / 75 /16 / 0.3 (v) B: ACN / 100mM ammonium formate = 20 / 80
Elution mode	Gradient elution, 0-3min (0% B) ➔ 9min (17% B) ➔ 16-18min (100% B) ➔ 18.5min (0% B)
Oven temp.	35ºC
Injection vol.	2.0 μL

Interface	ESI
MS mode	Posi, MRM-SIM
Block temp.	400ºC
DL temp.	300ºC
CID gas	Ar (230kPa)
Nebulizing gas flow	N_2, 3 L/min
Drying gas flow	N_2, 15 L/min

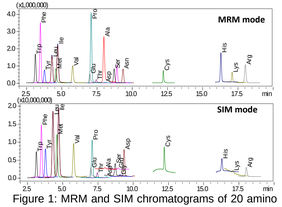

Figure 1: MRM and SIM chromatograms of 20 amino acids mixed standard (50 nmol/mL, 1uL inj).

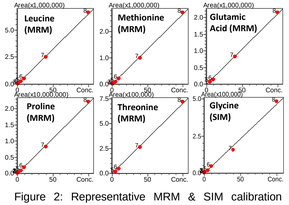

Figure 2: Representative MRM & SIM calibration curves of eight levels at 0.05-100 nmol/mL.

3. Results and Discussion

3.1 Establishment of a combined MRM-SIM method for 20 amino acids

With the Intrada column, both MRM method and SIM method were applied for analysis of amino acids on LC/MS/MS and LC/MS independently [1,2]. Figure 1 shows typical MRM chromatograms of a mixed standard of 20 amino acids on LCMS-8040 following the Imtakt method [1]. However, it was observed that glycine (m/z76) exhibited very low peak intensity in MRM mode (76 > 30). As a result, the detection sensitivity remained poor. In this work, a combined MRM-SIM mode method was adopted instead of only MRM. The SIM data was acquired by the Q3 (Q1-q2-Q3) simultaneously with MRM data in the same analysis on LCMS-8040.

Several pairs of MRM and SIM peaks of amino acids are displayed in Figure 3. It can be seen that, the MRM peak of glycine was very small at 50 nmol/mL and disappeared below the concentration. While the SIM peak of glycine exhibits high intensity at the same concentration and could be detected at 5 nmon/mL in clear solution. Thus, quantitation of glycine could be relied on SIM mode for lower concentration levels. In addition, a few more amino acids, namely, Thr, Asp, Ala, Ser, and Cys, exhibit higher SIM mode intensity and detection sensitivity than MRM mode in clear solutions (Figure 3). Based on these results, a combined MRM-SIM method was established for quantitation of amino acids rather than only MRM method. The details of the MRM-SIM method established are summarized in Table 2. The accuracy and repeatability of the methods (not shown in the table) were evaluated and satisfied results were obtained. A few selected calibration curves are displayed in Figure 2.

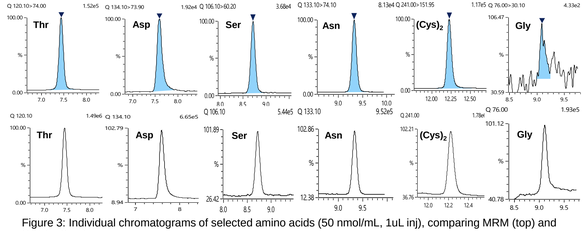

Figure 3: Individual chromatograms of selected amino acids (50 nmol/mL, 1uL inj), comparing MRM (top) and SIM (bottom) modes.

Table 2: Summary of calibration linearity, LOQ and repeatability (%RSD) of the SIM-MRM quantification method.

No	Name	RT (min)	MRM Method (nmol/mL)				SIM Method (nmol/mL)			
			m/z	Range	R^2	LOQ	m/z	Range	R^2	LOQ
1	Tryptophan	3.42	205.1>188.2	0.1-100	0.996	0.1	205.1	0.1-100	0.997	0.1
2	Phenylalanine	3.74	166.1>120.1	0.1-100	0.998	0.1	166.1	0.1-100	0.997	0.1
3	Tyrosine	4.06	182.1>136.2	0.5-100	0.998	0.5	182.1	0.5-100	0.999	0.5
4	Leucine	4.69	132.1>86.3	0.1-100	0.999	0.1	150.1	0.5-100	0.997	0.5
5	Methionine	4.91	150.1>56.1	0.5-100	0.999	0.5	132.1	0.1-100	0.998	0.1
6	Isoleucine	5.1	132.1>86.3	0.1-100	0.999	0.1	132.1	0.5-100	0.998	0.5
7	Valine	6.09	118.2>72.1	0.5-100	0.998	0.5	118.2	0.5-100	0.998	0.5
8	Glutamic Acid	7.06	148.1>84.1	0.5-100	1.000	0.5	148.1	0.5-100	0.998	0.5
9	Proline	7.29	116.1>70.1	0.1-100	0.999	0.1	116.1	0.1-100	0.999	0.1
10	Threonine	7.68	120.1>74.0	1.0-100	1.000	1	120.1	0.5-100	0.996	0.5
11	Aspartic acid	8.05	134.1>73.9	5.0-100	0.996	5	134.1	1.0-100	0.997	1.0
12	Alanine	8.25	90.1>44.1	5.0-100	0.994	5	90.1	0.5-100	0.993	0.5
13	Serine	8.94	106.1>60.2	5.0-100	0.999	5	106.1	1.0 -100	0.997	1.0
14	Glutamine	9.13	147.1>84.1	0.5-100	0.992	0.5	147.1	10-100	0.907	10
15	Glycine	9.38	76.0>30.0	25-100	0.993	25	76	5-100	0.993	5
16	Asparagine	9.56	133.1>74.1	5.0-100	0.999	5	133.1	5-100	0.998	5
17	Cystine	12.31	241.0>151.9	1-100	0.999	1	241	1-100	0.996	1
18	Histidine	16.57	156.1>110.1	0.5-100	0.992	0.5	156.1	0.5-100	0.991	0.5
19	Lysine	17.15	147.0>84.1	0.1-100	0.999	0.1	147	5-100	0.999	5
20	Arginine	18.1	175.1>70.1	0.5-100	0.999	0.5	175.1	0.1-100	0.998	0.5

3.2 Analysis of amino acid in biological and beverage samples

One of the purposes of this study is to evaluate the robustness of the method for different samples (biological and beverage samples). Without additional clean-up except deproteinization and filtering, the liquid sample was injected to LCMS-8040. The results of six representative samples are compiled into Table 3 and the chromatograms of four samples are shown in Figure 4. From the analyses of 20 different samples (3 plasma, 1 serum, 7 urine, 9 beverage samples), we could summarize the results and the method robustness in a few key points. First, the quantitative results by MRM and SIM calibrations are well in agreement with each other. Second, glycine could be detected and quantified only by SIM method in all the samples. Third, two amino acids could not be detected by MRM, but were detected by SIM mode (Asp in urine, Cys in vinegar).

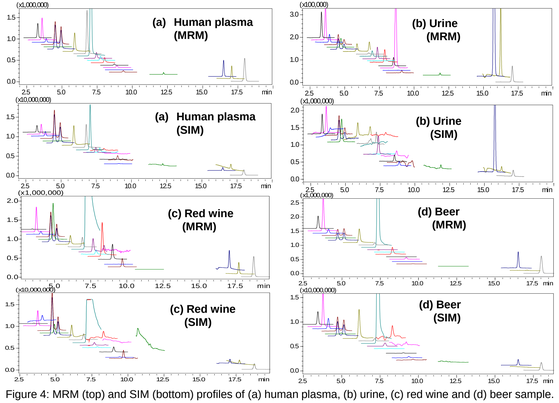

Figure 4: MRM (top) and SIM (bottom) profiles of (a) human plasma, (b) urine, (c) red wine and (d) beer sample.

Furthermore, the amino acid profiles in different samples are outlined below. (a) In human plasma and serum, the contents of Glu and Ala are the highest, and Met is the lowest. (b) Amino acid contents in urine are varied greatly across the 7 individuals. But Gln and Pro are detected consistently as the highest and lowest, respectively. (c) Most amino acids are found in high contents except Typ and Cys in all three types of wines (red, white and Chinese rice wine). (d) In beer samples, Pro is in highest content and Cys is the lowest. (e) The Sports Water and Amino Acid Drink bought from supermarket are with labeled contents of amino acids in the product bottles. The quantitative results of amino acids are closed to the contents on labels. The Sports Water contains Leu, Ile and Val. The Amino Acid Drink contains Try, Val, Leu, Ile, Thr and Lys.

Table 3: Amino Acid profiles in biological and beverage samples determined by MRM-SIM method on LCMS-8040

ID#	Name	m/z	Biological Sample Conc. (nmol/mL)			Beverage Sample Conc.(nmol/mL)		
			Human Plasma	Human Serum	Urine	Red Wine	Beer	Vinegar
1	Tryptophan	205.1	141.5	34.8	109.9	3.0	111.1	ND
		205.1>188.2	134.8	31.5	100.5	2.5	87.7	ND
2	Phenylalanine	166.1	122.1	90.7	110.6	87.7	173.8	1010.0
		166.1>120.1	122.2	89.6	93.5	79.0	159.6	766.1
3	Tyrosine	182.1	117.6	93.9	91.8	92.4	135.5	47.9
		182.1>136.2	113.8	85.9	116.4	57.9	110.7	31.1
4	Leucine	132.1	285.9	466.4	57.8	180.8	59.4	2988.2
		132.1>86.3	274.6	445.4	53.4	176.2	57.2	3248.1
5	Methionine	150.1	3.6	0.9	111.1	33.7	7.2	90.6
		150.1>56.1	2.5	0.7	112.6	35.1	6.7	83.1
6	Isoleucine	132.1	163.6	101.5	19.2	60.1	48.1	2357.2
		132.1>86.3	151.2	85.7	17.3	56.0	43.1	2010.5
7	Valine	118.2	360.0	375.9	67.3	110.6	255.7	3627.7
		118.2>72.1	383.3	323.2	66.4	99.2	240.5	2783.3
8	Glutamic Acid	148.1	1353.3	907.4	27.4	162.1	60.0	1942.5
		148.1>84.1	1358.1	904.5	18.6	167.5	56.5	1735.6
9	Proline	116.1	496.1	421.7	6.1	14724	3546.9	2851.6
		116.1>70.1	495.7	418.8	6.9	19555	2699.9	3555.1
10	Threonine	120.1	403.1	437.1	769.5	224.0	(235.0)*	2960.4
		120.1>74.0	303.0	375.7	549.2	183.8	5.7	1655.5
11	Aspartic acid	134.1	85.9	285.2	109.2	247.2	53.0	478.7
		134.1>73.9	65.6	288.6	ND	296.8	42.0	116.3
12	Alanine	90.1	937.5	1003.2	1184.1	997.9	1129.2	13420.1
		90.1>44.1	1053.1	1540.6	946.8	689.5	874.0	9828.1
13	Serine	106.1	350.0	574.1	1086.0	156.4	17.5	3205.1
		106.1>60.2	344.8	631.0	1023.8	160.3	15.1	2721.7
14	Glutamine	147.1	195.6	155.7	3007.5	(56.5)*	210.1	10.3
		147.1>84.1	254.0	282.8	3598.2	1.8	116.7	12.1
15	Glycine	76	642.6	493.5	1515.2	488.6	366.3	5215.5
		76.0>30.1	ND	ND	ND	ND	ND	ND
16	Asparagine	133.1	34.7	24.1	250.7	76.5	37.6	561.3
		133.1>74.1	27.5	18.6	188.5	79.1	16.7	456.7
17	Cystine	241	18.9	7.4	73.8	ND	ND	7.9
		241.0>152.0	20.8	6.5	72.4	ND	ND	ND
18	Histidine	156.1	238.4	45.1	2547.4	67.1	117.0	67.2
		156.1>110.1	246.3	49.6	2645.5	49.9	126.3	58.7
19	Lysine	147	551.6	312.6	709.3	101.2	4.9	2135.3
		147.0>84.1	584.2	309.3	797.6	147.3	7.7	3374.8
20	Arginine	175.1	923.3	171.2	86.3	152.9	197.4	1260.9
		175.1>70.1	924.0	170.4	85.2	161.3	194.0	1339.8

*Note: ND = Not Detected; * The SIM peak has co-eluted component and the result is not accurate.*

4. Conclusions

By using the Intrada Amino Acid column, a combined MRM-SIM method has been established for detection and quantification of 20 amino acids in biological and beverage samples. The Intrada Amino Acid column can separate effectively the amino acids without need of pre-column derivatization, which allow direct analysis of amino acids on LC-MS. The advantages of a combined MRM-SIM method on LC/MS/MS are the higher sensitivity for glycine in SIM mode and overall enhanced reliability, robustness and accuracy in comparison with the only MRM method or SIM method.

References

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2. Matsumoto K., Watanabe J., Yazawa I., “Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS”, ASMS 2014, TP 510