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High Throughput simultaneous analysis of 39 amino acids in various foods without derivatization using LC-MS/MS

Manabu Yukiyama[1], Keiko Matsumoto[1], Jun Watanabe[1], Itaru Yazawa[2]

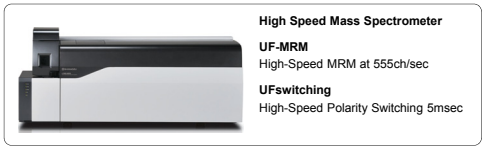
1 Shimadzu Corporation, Kyoto, JAPAN; 2 Imtakt Corporation , Kyoto, JAPAN

1. Introduction

As amino acids in foods are essential components of nutrition and taste, it is expected to develop an easy and efficient analytical method. Amino acids are highly polar compounds, so it needs their derivartization or addition of ion-pair reagent in mobile phase in order to separate them by reversed phase mode. In the case of hydrophilic interaction chromatography, it may be difficult to separate isomers or to analyze comprehensively.
In our previous study[2], we developed a simultaneous analysis method of 20 amino acids by LC-MS/MS with mix-mode column (ion exchange and normal phase), without derivartization. In this study, we tried to increase the number of targeted amino acids (39 amino acids), and detected them in various foods with high sensitivity.

2. Methods and Materials

Amino acid standard regents and food samples were purchased from the market. Standards of 39 amino acids were optimized on each compound-dependent parameter and MRM transition.
As an LC-MS/MS system, HPLC was coupled to triple quadrupole mass spectrometer (Nexera with LCMS-8050, Shimadzu Corporation, Kyoto, Japan). Sample was eluted with a binary gradient system and LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.



High Speed Mass Spectrometer

UF-MRM
High-Speed MRM at 555ch/sec

UFswitching
High-Speed Polarity Switching 5msec

Figure 1 LCMS-8050 triple quadrupole mass spectrometer

3. Result

3-1. Method development

First, MRM method of 19 amino acids was optimized in order to increase the number of analytical amino acids. As a result, all compounds were able to be detected high sensitively with ESI. All amino acids except for methionine sulfone were detected in positive mode. Methionine sulfone was detected in negative mode. Ultra Fast Polarity Switching of 5msec enabled simultaneous analysis of the compounds in both positive and negative modes.

HPLC conditions (Nexera UHPLC system)

Column: Intrada Amino Acid (3.0mmI.D. x 50mm, 3um, Imtakt Corporation, Kyoto, Japan)
Mobile phase:
A: Acetonitrile / Formic acid = 100 / 0.1, B: 100mM Ammonium formate
Time program: B conc.14%(0-3 min) -100%(10min) - 14%(10.01-15min)
Flow rate: 0.6 mL/min Injection volume: 2 uL Column temperature: 40 Celsius

MS conditions (LCMS-8050)

Ionization: ESI, Positive / Negative MRM mode
MRM transition are shown in Table 1.

Mix-mode column “Intrada Amino Acid” provides normal phase separation and ion exchange. In the condition that amino acids were not derivartized and ion-pairing reagent wasn’t used for this analysis, 39 amino acids were retained and separated excellently by controlling pH, salt concentration and acetonitrile ratio. Although alanine and sarcosine are same molecule weight, the mix-mode column enabled these 2 amino acids to be separated chromatographically.

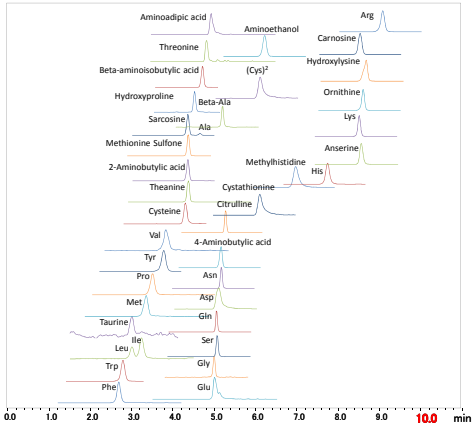


Figure 2 Mass Chromatograms of 39 Amino acids
(concentration of each compound : 10nmol/mL)

The dilution series of these compounds were analyzed. Most of amino acids were detected with good linearity (Table1) and repeatability (Table 2).

Table 1 Linearity of 39 amino acids

	MRM Transition	Linearity Range (nmol/mL)	Linearity Coefficient (r2)		MRM Transition	Linearity Range (nmol/mL)	Linearity Coefficient (r2)
Trp	205.10>188.10	0.01-100	0.995	Theanine	174.50>84.10	1-500	0.988
Phe	166.10>120.10	0.01-100	0.997	Aminoadipic acid	161.90>98.20	0.1-50	0.979
Tyr	182.10>136.00	0.05-100	0.99	Taurine	126.20>44.05	10-500	0.998
Met	150.10>56.10	0.05-200	0.996	Ornithine	133.10>70.10	0.05-100	0.997
Lue,Ile	132.10>86.15	0.01-100	0.995	Cysteine	122.00>76.05	5-500	0.996
Val	118.10>72.05	0.05-100	0.999	Hydroxyproline	132.10>86.05	0.1-500	0.991
Glu	148.10>84.10	0.05-10	0.996	Sarcosine	89.90>43.85	0.05-100	0.999
Pro	116.10>70.10	0.01-50	0.993	β-Ala	89.70>72.10	0.5-500	0.999
Asp	134.20>74.10	0.5-500	0.995	Citrulline	176.10>70.05	0.05-100	0.993
Thr	120.10>74.00	0.1-50	0.992	2-Aminobutyric acid	104.10>58.05	0.5-500	0.996
Ala	90.10>44.10	0.5-500	0.998	Cystathionine	223.00>88.05	0.1-10	0.995
Ser	106.10>60.20	0.5-500	0.998	Aminoethanol	62.00>44.10	0.1-500	0.999
Gln	147.10>84.10	0.05-1	0.995	Anserine	240.70>109.10	0.1-100	0.997
Gly	76.20>29.90	5-200	0.997	Carnosine	227.10>110.05	0.05-100	0.995
Asn	133.10>74.05	0.05-20	0.993	Hydroxylysine	162.90>82.15	0.1-500	0.996
(Cys)2	241.00>151.95	0.05-20	0.99	Methylhistidine	169.90>124.15	0.01-10	0.999
His	156.10>110.10	0.05-200	0.998	3-Aminoisobutyric acid	103.80>86.10	0.05-500	0.991
Lys	147.10>84.10	0.05-5	0.99	4-Aminobutyric acid (GABA)	104.10>87.05	0.05-500	0.995
Arg	175.10>70.10	0.01-100	0.996	Methionine sulfone	180.00>79.20	0.5-100	0.973

Table 2 Repeatability of 39 amino acids

Amino acid	%RSD*	Amino acid	%RSD*	Amino acid	%RSD*
Trp	0.90	Gln	17.29	Sarcosine	3.86
Phe	0.73	Gly	9.54	β-Ala	5.36
Tyr	4.97	Asn	8.52	Citrulline	4.92
Met	0.78	(Cys)2	5.49	4-Aminobutyric acid(GABA)	2.68
Lue	0.84	His	3.06	Cystathionine	3.48
Ile	0.90	Lys	1.95	Aminoethanol	1.73
Val	1.90	Arg	8.70	Anserine	0.88
Glu	5.54	Theanine	6.11	Carnosine	1.19
Pro	1.17	Aminoadipic acid	7.63	Hydroxylysine	5.64
Asp	9.19	Taurine	8.23	Methylhistidine	3.91
Thr	3.47	Ornithine	7.53	β-Aminoisobutyric acid	7.36
Ala	2.01	Cysteine	4.76	2-Aminobutyric acid	3.30
Ser	10.67	Hydroxyproline	5.29	Methionine sulfone	15.88

*@ 5nmol/mL : except for Gly 10nmol/mL Taurine 50nmol/mL

3-2. The analysis of 39 amino acids in food samples

Dried Bonito fish (Katsuobushi), pork and wine were analyzed by using this method. Katsuobushi (extracted by hot water) and wine were diluted with 0.1N HCl. Pork was delipidated with n-Hexane after a deproteinizing preparation with 5% sulfosalicylic acid. These were filtered through a 0.2um filter and then analyzed. MRM chromatograms of each food sample are shown in Figure 3,4,5. In the case of Katsuobushi, Histidine carnosine and anserine which are typical substances in a migratory fish were detected with high intensity. It is known that carnosine is contained in muscle and improves muscle function. Carnosine also was plentifully detected in pork. On the other hand, for wine, abundant amino acids detected were proline, glutamic acid, glutamine, arginine and GABA.

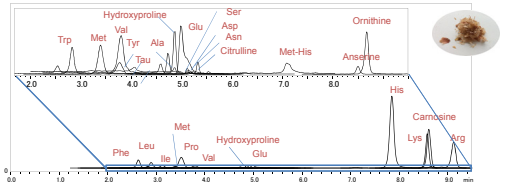


Figure 3 Mass Chromatograms of Extract of Katsuobushi (500 fold dilution with 0.1N HCl)

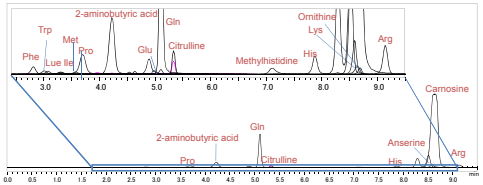


Figure 4 Mass Chromatograms of pork (10 fold dilution with 0.1N HCl)

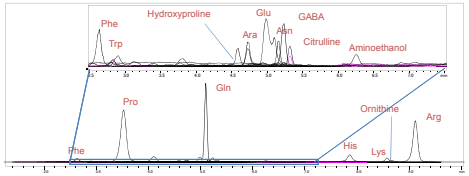


Figure 5 Mass Chromatograms of Wine (100 fold dilution with 0.1N HCl)

Recovery rate was evaluated using analytical data of Katsuobushi and beer spiked with standard amino acids (50nmol/mL). Both Katsuobushi and beer showed good recoveries with almost 70~120% (Figure 6,7).

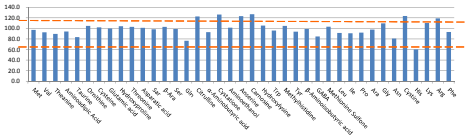


Figure 6 Recovery data (extract of Katsuobushi spiked with standard amino acids)

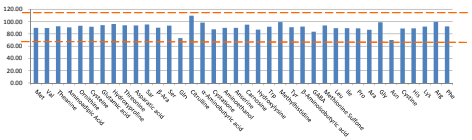


Figure 7 Recovery data (beer spiked with standard amino acids)

4. Conclusions

- ✓ 39 amino acids could be separated without derivatization by using a typical volatile mobile phase suitable for LC/MS analysis and detected with high sensitivity.
- ✓ Ultra Fast Polarity Switching of 5msec enabled simultaneous analysis of the compounds in both positive and negative modes
- ✓ The mix-mode column enabled isomers-separation completely.
- ✓ This method was able to be applied to the analysis of amino acids in various food samples.

5. Reference

1. Imtakt Technical Report T1734E (Imtakt Co., Kyoto)
2. Keiko Matsumoto et al., Poster No.TP510, ASMS2014 in Baltimore, June 15-19, 2014