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Simultaneous quantitative analysis of 20 amino acids in food samples without derivatization using LC-MS/MS

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1. Introduction

In order to detect many kinds of amino acids with high selectivity in food samples, the LC/MS analysis have been used widely. Amino acids are high polar compound, so they are hard to be retained to reverse-phased column such as ODS (typical method in LC/MS analysis). It needs their derivartization or addition of ion pair reagent in mobile phase to retain them. For easier analysis of amino acids, it is expected to develop the method without using reagents mentioned above.
This time, we tried to develop a simultaneous high sensitive analysis method of 20 amino acids by LC/MS/MS with mix-mode column (ion exchange, normal-phase) and the typical volatile mobile phase suitable for LC/MS analysis.

2.Methods and Materials

Amino acid standard regents and food samples were purchased from the market.
Standards of 20 kinds of 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 20 amino acids was optimized. As a result, all compounds were able to be detected high sensitively and were detected in positive MRM transitions. As the setting temperature of ESI heating gas was found to affected on the sensitivity of amino acids, it was also optimized. Even though amino acids were not derivartized and ion-pairing reagent wasn't used, 20 amino acids were retained by using a mixed-mode stationary phase structure and separated excellently on the below-mentioned condition.

HPLC conditions (Nexera system)
Column: Intrada Amino Acid (3.0mmI.D. x 50mm, 3um, Imtakt Corporation, Kyoto, Japan)
Mobile phase
Case1
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)
Case2 (High Resolution condition)
A: Acetonitrile / Tetrahydrofuran / 25mM Ammonium formate / formic acid =9/75/16/0.3
B: 100mM Ammonium formate / Acetonitrile = 80 / 20
Time program: B conc.0%(0-2 min) -5%(3min) - 30%(6.5min) -100%(12min) - 0%(12.01-17min)
Flow rate: 0.6 mL/min Injection volume: 2 uL Column temperature: 40 C

MS conditions (LCMS-8050)
Ionization: ESI, Positive MRM mode
MRM transition are shown in Table 1.

Case1

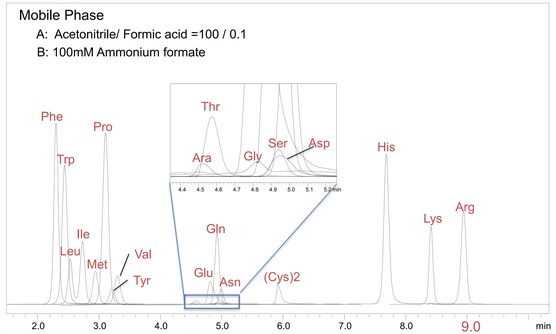

Figure 2 Mass Chromatograms of 20 Amino acids (concentration of each compound : 10nmol/mL)

Case2 (High Resolution condition)

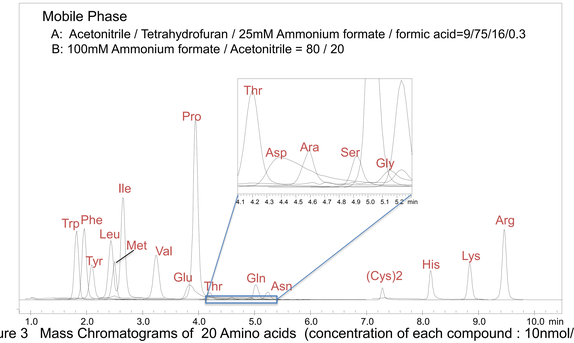

Figure 3 Mass Chromatograms of 20 Amino acids (concentration of each compound : 10nmol/mL)

In this study, two conditions of mobile phase were investigated. It was found that 20 amino acids were separated with higher resolution in case2.

As the mobile phase condition of case1 is more simple and the result of case1 was sufficiently well, case1 analytical condition was used for quantitative analysis. The dilution series of these compounds were analyzed. All amino acids were detected with good linearity and repeatability (Table1).

Table1 Linearity and Repeatability of 20 amino acids

	MRM Transition	Linearity		Repeatability*
		Range (nmol/mL)	Coefficient (r2)	%RSD
Trp	205.10>188.10	0.01-100	0.9950	1.4
Phe	166.10>120.10	0.01-100	0.9971	1.2
Tyr	182.10>136.00	0.05-100	0.9900	1.7
Met	150.10>56.10	0.05-200	0.9963	0.1
Lue,Ile	132.10>86.15	0.01-100	0.9955	0.7
Val	118.10>72.05	0.05-100	0.9991	1.9
Glu	148.10>84.10	0.05-10	0.9965	4.5
Pro	116.10>70.10	0.01-50	0.9933	1.5
Asp	134.20>74.10	0.5-500	0.9953	1.4
Thr	120.10>74.00	0.1-50	0.9923	4.5
Ala	90.10>44.10	0.5-500	0.9989	16.2
Ser	106.10>60.20	0.5-500	0.9988	6.5
Gln	147.10>84.10	0.05-1	0.9959	3.9
Gly	76.20>29.90	5-200	0.9974	11.0
Asn	133.10>74.05	0.05-20	0.9939	6.1
(Cys)2	241.00>151.95	0.05-20	0.9909	2.3
His	156.10>110.10	0.05-200	0.9983	1.7
Lys	147.10>84.10	0.05-5	0.9908	0.9
Arg	175.10>70.10	0.01-100	0.9956	0.5

*@ 0.5nmol/mL : except for Gly, 5nmol/mL : for Gly

3-2. The analysis of 20amino acids in food samples

The analysis of the amino acids contained in sports beverage on the market was carried out. In the case of sports beverage, all amino acids written in the package were detected.

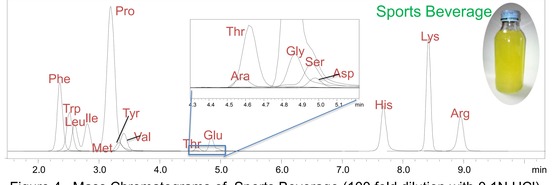

Figure 4 Mass Chromatograms of Sports Beverage (100 fold dilution with 0.1N HCl)

Furthermore, Japanese Sake, Beer and sweet cooking rice wine (Mirin) were analyzed using this method. Japanese Sake and Beer were diluted with 0.1N HCl. Sweet cooking rice wine was diluted in the same way after a deproteinizing preparation. These were filtered through a 0.2um filter and then analyzed. MRM chromatograms of each food samples are shown in Figure 5,6,7. Amino acids of each sample were detected with high sensitivity.

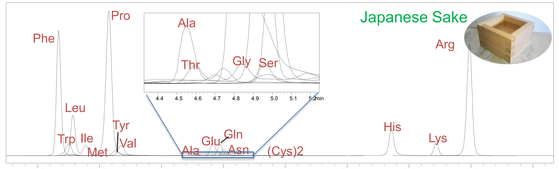

Figure 5 Mass Chromatograms of Japanese Sake (100 fold dilution with 0.1N HCl)

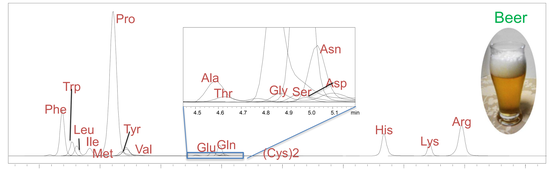

Figure 6 Mass Chromatograms of Beer (10 fold dilution with 0.1N HCl)

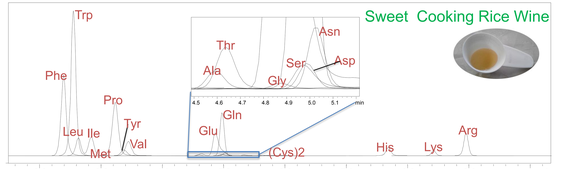

Figure7 Mass Chromatograms of Sweet Cooking Rice Wine (100 fold dilution with 0.1N HCl)

4. Conclusions

- 20 amino acids could be separated without derivatization using a typical volatile mobile phase suitable for LC/MS analysis and detected with high sensitivity.
- This methods was able to be applied to the analysis of amino acids in various food samples.