In order to detect many kinds of amino acids with high selectivity in food samples, the LC/MS analysis has been used widely. Amino acids are high polar compound, so they are hard to be retained in reversed-phase column such as C18 (typical method in LCMS analysis). This degradation or addition of ion-pair reagent in mobile phase to retain them. For easier measurement, the mobile phase was optimized without using reagent.

This time, we tried to develop a simultaneous high sensitive analysis method of 20 amino acids by LCMS/MS with mis-mi-mole column (on exchange, normal-phase) and the typical volatile mobile phase suitable for LCMS analysis.

2. Methods and Materials
Amino acid standard reagents 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 (Scheme 5, 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.

3. Result

3-1. Method development
First, MRM method of 20 amino acids was optimized. As a result, all compounds were paired reagent wasn’t used, 20 amino acids were retained by using a mixed pairing reagent. Case1 and Case2 were optimized. 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 (Table 1).

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 case 1 is more simple and the result of case 1 was sufficiently well, case 1 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).

In this study, two conditions of mobile phase were investigated. It was found that 20 amino acids were separated with higher resolution in case 2.

3.2. The analysis of 20 amino 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. Furthermore, Japanese Sake, Beer and sweet cooking rice wine (Mirin) were analyzed using this method. Japanese Sake and Beer were diluted with 1 N HCl, sweet cooking rice wine was diluted through a 0.2um filter and then analyzed. MRM chromatograms of each food samples are shown in Figure 5. Amino acids of each sample were detected with high sensitivity.

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4. Conclusions
20 amino acids could be separated without derivatization using a typical volatile mobile phase suitable for LCMS analysis and detected with high sensitivity.

This method was able to be applied to the analysis of amino acids in various food samples.

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