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Application Data Sheet

No.86

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Fatty Acids in Food Using PCI-GC-MS/MS

While some fatty acids, such as the n-3 fatty acids EPA and DHA, are beneficial to human health because they lower the amount of blood-borne neutral fat, too much intake of saturated fatty acids raises the risk of some diseases. For this reason, there is a need for the batch analysis of these fatty acids in the life sciences and food engineering sectors. Despite requiring methylation, GC-MS has gained attention because of its suitability for multicomponent batch analyses.

In fatty acid analyses utilizing GC-MS, the EI (electron ionization) method is used for ionization. With the EI method, there are many types of fragment ions, making it easy to select an *m/z* to enable separation by mass from impurities. However, because of the large number of fragment ions, the sensitivity of the individual ions is reduced, making it difficult to detect trace quantities of fatty acids. In contrast, with the PCI (positive chemical ionization) method, protonated molecular ions can be detected, from which molecular weight data can be obtained. Since there is only a small number of fragment ion types, the sensitivity is increased. This means, however, that the ion types that can be selected for monitoring are limited and there may not be any ions that can be separated by mass from impurities.

This application data sheet introduces the results of an investigation of separation from impurities based on the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods. In addition, in Application Data Sheet No. 85, we introduce the results of an investigation of sensitivity in the analysis of fatty acids in foods.

Pretreatment Method

Saury (fish) was used to investigate the separation from impurities in each analysis mode. The fatty acid methylation kit (P/N: 06482) sold by Nacalai Tesque was utilized for the pretreatment. The pretreatment method is shown in Fig. 1.

The edible flesh from the saury was collected and pulverized with a mill, after which 200 mg was measured out. After adding 2 mL of the extraction liquid and agitating, the mixture was centrifuged, and 500 μL of extracted liquid was obtained. The extracted liquid was dried under a nitrogen flow, and 500 μL each of reagents A and B were added. After leaving the mixture to stand for 1 hour at 37 °C, 500 μL of reagent C was added, and it was left to stand at 37 °C for a further 20 minutes. Afterward, 2 mL of the extraction liquid was added, and after centrifuging, the organic phase was collected. Deionized water was used to clean 1 mL of the organic phase, resulting in the test solution.

Refer to Application Data Sheet No. 85 for the analysis conditions for the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods.

Analysis methods included in the GC/MS Metabolite Database Ver. 2 were used for the analysis conditions and monitoring *m/z*.

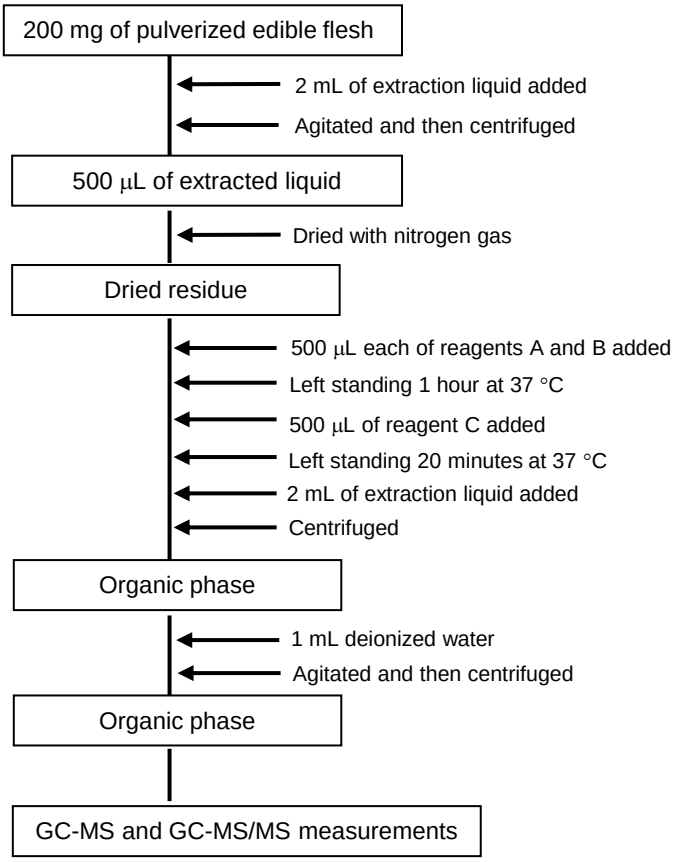
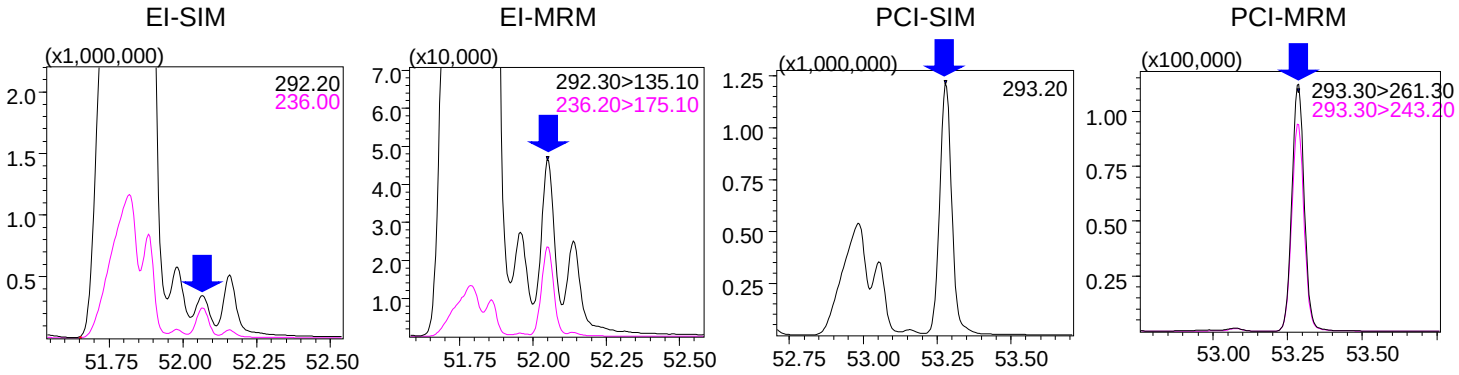


Fig. 1: Pretreatment of the Saury

Analysis Results

The sample extracted from the saury was measured in each analysis mode, and the separation from impurities investigated. Most of the fatty acid methyl esters could be separated from the impurities regardless of the analysis mode. However, a portion of the fatty acids was hard to completely separate from the impurities, both with EI-SIM and EI-MRM. Fig. 2 shows examples of measuring fatty acid methyl esters for which the degree of separation from impurities varied significantly depending on the analysis mode. Methyl linolenate;(Z)18:3n-3 and methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3 were hard to separate from the impurities, both with EI-SIM and EI-MRM. Some degree of separation was possible with PCI-SIM, but there was only one kind of monitoring *m/z*, so problems with peak identification could be expected. In contrast, with PCI-MRM, mass separation excluded impurities eluted nearby, making peak identification easy.

Methyl linolenate;(Z)18:3n-3



Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3

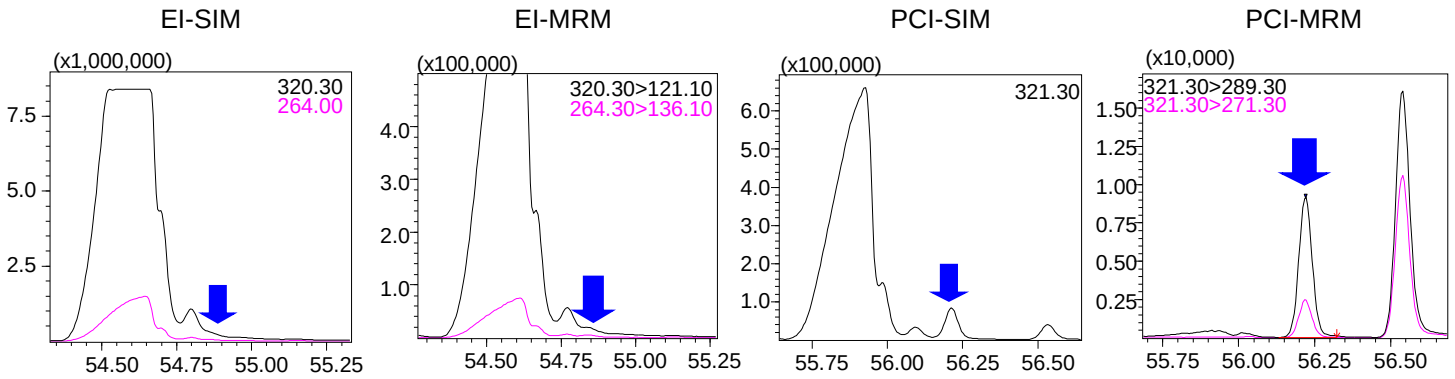


Fig. 2: Mass Chromatograms for Methyl linolenate;(Z)18:3n-3 and Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3 Contained in an Extract of Saury Measured in Individual Analysis Modes

We analyzed fatty acids in foods to investigate mass separation from impurities and sensitivity for the EI-SIM, EI-MRM, PCI-SIM, and PCI-MRM analysis modes. The results revealed that, as shown in Application Data Sheet No. 85, the PCI method is the most sensitive, and for unsaturated fatty acids in particular, provides more sensitive detection than the EI method. Also, PCI-MRM was found to be the most ideal for mass separation from impurities, making peak identification easy.
It is thus evident that the PCI-MRM method is effective for multicomponent batch analyses of fatty acids.

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