SHIMADZU
Excellence in Science

GCMS-8050™ NX Triple Quadrupole Gas Chromatograph Mass Spectrometer

Application News

GC-CI-MS/MS Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Isomers in Salami

Toshinari Ishii

User Benefits

- CI-MS/MS can isolate difficult to distinguish PAHs isomers, reducing the risk of false positives and expediting the analysis.
- Both separation by specific transition and sensitive analysis by high-intensity transition are possible.
- It is not easily affected by impurities, and good recovery rate can be obtained even with a simple pretreatment method.

■ Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of compounds with two or more benzene rings attached to each other. They are formed by incomplete combustion or pyrolysis of organic substances and can be mixed into food and environmental water. Benzo[a]pyrene, a typical compound, is carcinogenic. Some other PAHs are also suspected to be carcinogenic. For this reason, some PAHs are regulated such as those highlighted in Fig. 1. GC/MS and LC/MS analyses have been adopted as official methods but the separation of isomers can be challenging. Indeed, PAHs have multiple isomers due to different benzene ring bond positions. Due to their stable structures, EI-MS/MS collision-induced dissociation (CID) cannot show differences in mass spectra. In addition, due to the close physical properties such as polarity and boiling point, chromatogram separation is difficult, even with dedicated columns.

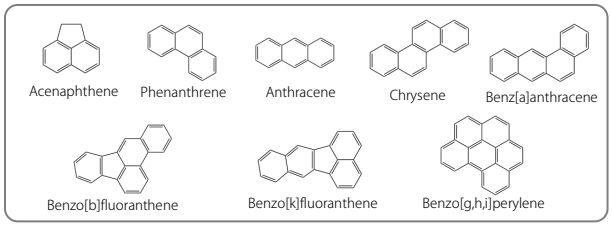

Fig. 1 Examples of regulated PAHs

■ The Analysis Concept CI-MS/MS

In this article, we analyzed chrysene and triphenylene using CI-MS/MS, investigating PAHs' stability changes due to addition reactions. Chrysene and triphenylene are a combination of isomers that are challenging to separate. These separations are particularly important because chrysene is regulated while triphenylene is not.

However, electron-donating groups impact molecules' HOMO-LUMO gap, reducing their stability. In that context, even PAHs with stable structures could be fragmented. Specific fragments were obtained by MS/MS due to the addition of electron-donating groups derived from reagent gases used by CI (Fig. 2). Since the $[M+H]^+$ ion is detected at a high intensity, it can be used for sensitive analysis.

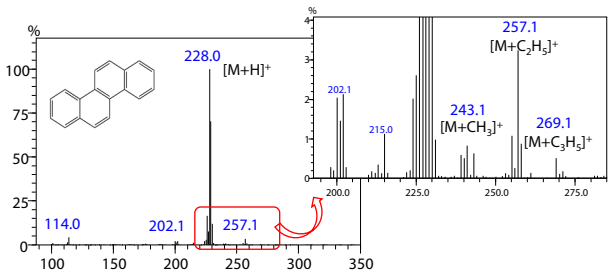

Fig. 2 Mass spectrum of Chrysene

■ PAHs Standard Mixture Analysis

Benz[a]anthracene, triphenylene and chrysene were mixed and analyzed using the conditions detailed in Table 1. Standards mixture was diluted with acetonitrile to 50 ng/mL prior analysis. In addition, although not shown in the chromatogram, it has been confirmed that high-boiling PAHs such as benzo[b]phenanthrene, benzo[k]phenanthrene, and benzo[j]phenanthrene can be separated using this method.

Table 1 Analysis Conditions of GCMS-8050 NX

System	: GCMS-TQ8050 NX
Column	: SH-I-PAH (P/N 227-36074-02) (60 m x 0.25 mm I.D., df = 0.10 µm)
Inlet Temp.	: 250 °C
Injection Volume	: 1 µL
Injection Mode	: Splitless
Carrier Gas	: He
Gas Control	: Linear Velocity (42.5cm/sec)
Oven Temp.	: 50 °C (2 min) → (40 °C/min) → 200 °C → (25 °C/min) → 250 °C (3 min) → (15 °C/min) → 350 °C (5 min) **Total 23 min**
MS Interface Temp.	: 330 °C
Ion source Temp.	: 250 °C
Ionization	: PCI (Methane)
Data Acquisition Mode	: MRM

Fig. 3 shows the analysis of the standards mix. The $[M+C_2H_5]^+$ ion-derived transition is highlighted in pink. As observable on the MRM chromatograms, both product ions were only detected for the regulated compounds benzo[a]anthracene and chrysene. The stability of benzo[a]anthracene and chrysene decreased due to the addition of C_2H_5, improving CID cleavage. On the other hand, thanks to a higher resonance stability and despite ion addition, triphenylene cleavage seems more difficult compared to other isomers, leading to a single response.

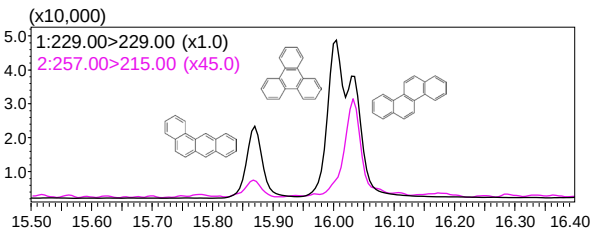

Fig. 3 GC-CI-MS/MS chromatograms of PAHs standards mix

■ Sensitivity and Linearity

The sensitivity and linearity of chrysene were investigated. Standard solutions containing chrysene and benzo[a]anthracene were prepared with acetonitrile at concentrations of 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, and 500 ng/mL for analysis. For each, 10 ng/mL of chrysene-d 12 was added and used as the internal standard.

For specific transitions, a S/N of 11.3 at 5 ng/mL (Fig. 4) and 2.6 at 1 ng/mL were considered good enough. For the high-intensity transition, S/N is 59.4 at 1 ng/mL (Fig. 5), with a standard deviation σ of 0.01. The lower limit of quantification is estimated to be around 0.1 ng/mL (10σ). For both linearities, R^2 exceeded 0.999, confirming results reliability.

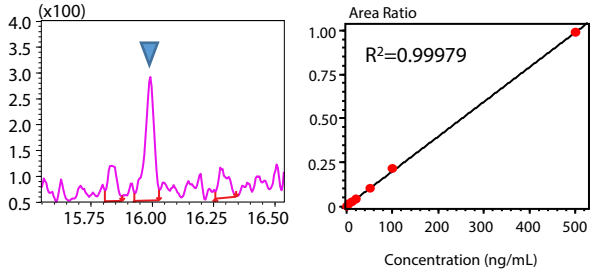

Fig. 4 Sensitivity of chrysene-specific transitions (5 ng/mL) and calibration curve

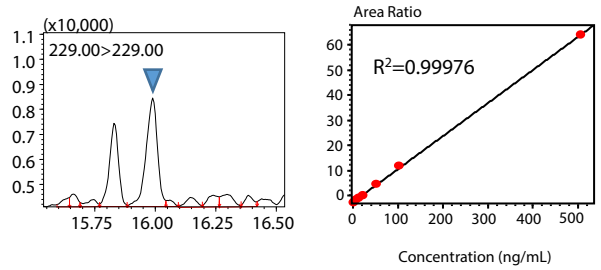

Fig. 5 Sensitivity of high-intensity transitions (1 ng/mL) and calibration curve

■ Food Sample Preparation

Analysis were performed using salami as a sample and PAHs were added as standards. The preparation flow is shown in Fig. 6. First, 5.0 mL of water was added to 10.0 g of crushed salami. Then, chrysene, benzo[a]anthracene, triphenylene standards (STD) and chrysene-*d*12 (ISTD) were added to reach a sample weight of respectively 25 and 10 ng/mL. Extraction was performed using the non-buffered QuECHERS method, using 10.0 mL of acetonitrile. After centrifugation, only 3.0 mL of the upper layer was collected and purified by dispersive solid-phase extraction, thanks to 0.75 g of Z-sep (Supelco). Finally. purified supernatant was analyzed.

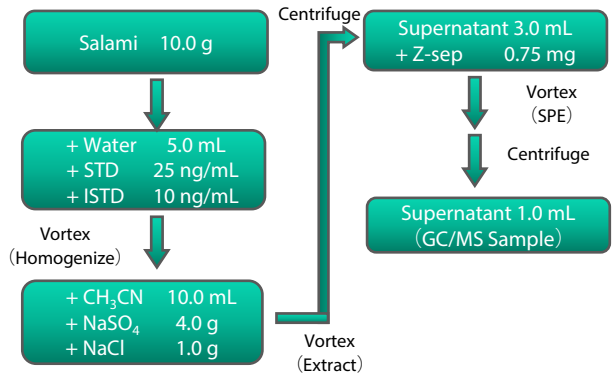

Fig. 6 Workflow of sample preparation

■ Result

The result of the analysis are represented in Fig. 7. Only benzo[a]anthracene and chrysene were detected at the specific transition, while the high-intensity transition included sample-derived contaminant peaks. The inset displayed that chrysene and triphenylene are well separated.

The recovery rate of chrysene was calculated from the area ratio of each transition. The high-intensity transition should be used to detect the triphenylene and peak to measure area value. As a result, the area value is smaller than the actual value, so the recovery rate of chrysene was calculated to be 73.7%. However, when a specific transition was used, it was not affected by triphenylene and a better recovery rate of 105.9% was obtained.

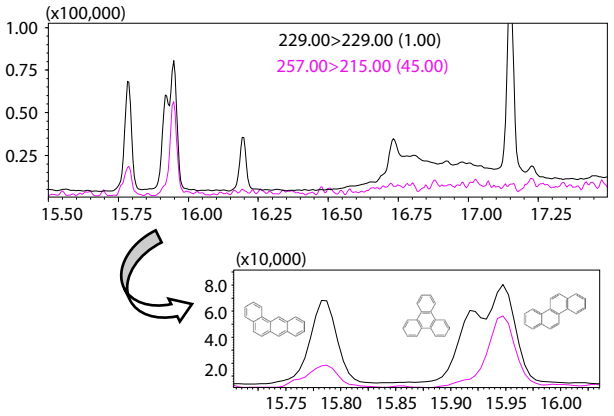

Fig. 7 GC-CI-MS/MS chromatograms of food sample analysis

■ Conclusion

Thanks to CI-MS/MS with methane using triple-quadrupole GC-MS (Fig. 8), we were able to separate chrysene and triphenylene. Indeed, their different resonance stability impacted their cleavage patterns, allowing to distinguish both isomers. In this method, product ion selectivity is improved compared to conventional one. Previous analyses of PAHs using GC-MS/MS utilized an ion whose mass was nearly identical to that of the precursor ion. As their difference is only based on the hydride loss without further fragmentation, it was difficult to improve selectivity. The novel method with specific transition improved both selectivity and sensitivity even for complex samples like food matrices.

As a result of the examination, it was found that the ionic strength ratio of each isomer of benzo[b]phenanthrene, benzo[k]phenanthrene, and benzo[j]phenanthrene, which have higher molecular weights, differs greatly due to additional ions other than C_2H_5.

Fig. 8 GCMS-TQ™ 8050 NX

01-00628-EN First Edition: Jan. 2024

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/