

Structure Analysis of Unknown Compound in Lemon Juice by SPME-GC-TOFMS and msFineAnalysis AI

Product used: Mass Spectrometer (MS)

Introduction

Food flavor components are known to be an important element of good taste. Gas chromatography-mass spectrometry (GC-MS) is often used to analyze food flavor components. This is because food flavors are highly volatile and complex with numerous components. Electron ionization (EI) is one of the most popular ionization methods used in GC-MS. Consequently, compounds are typically identified by a mass spectral database search using EI mass spectra. Because molecular ions are often weak or absent in 70 eV EI mass spectra, identification of unknowns can be difficult by EI alone. In these cases, soft ionization (SI) can be very helpful for producing and identifying molecular ions. We had developed an integrated qualitative analysis workflow that automatically combines and interprets the information from EI and SI data¹⁾. And then in 2018, we introduced our integrated qualitative analysis software “msFineAnalysis” which uses both EI and SI data to improve compound identification for GC-MS applications. Despite the fact that msFineAnalysis was automatically able to determine the molecular formula and partial structure information from EI fragment ion formulas, the actual structural formulas still required manual analysis using chemical compositions. To address this, we then developed an automated structure analysis software package named “msFineAnalysis AI” which uses artificial intelligence (AI) to predict EI mass spectra from chemical structures²⁾. We have used our newly-developed AI model to create a database of predicted EI mass spectra for around 100 million compounds.

In this MSTips, we report on an example of structure estimation of an unknown compound in lemon juice using msFineAnalysis AI.

Experimental

American lemon juice obtained by squeezing was used as sample. Lemon juice 10 mL was sealed in a 20 mL vial. The SPME mode of the HT2850T autosampler (HTA S.R.L.) was used as the sample preparation device, and volatile components in the headspace area of the vials were targeted for the measurement. A GC-TOFMS (JMS-T2000GC AccuTOF™ GC-Alpha, JEOL Ltd.) was used for the measurement. We performed HS-SPME-GC-TOFMS measurements using both EI and field ionization (FI) modes with a combination EI/FI/FD ion source. The qualitative data processing was performed with msFineAnalysis AI (JEOL Ltd.). Measurement conditions are shown in Table 1.



JMS-T2000GC AccuTOF™ GC-Alpha



HT2850T

Table 1 Measurement

SPME	
SPME Fiber	DVB/CAR/PDMS 2mm (Merck)
Sample amount	10 mL
Extraction temp.	60 °C
Extraction time	30 min
Desorption time	3 min
GC	
Column	ZB-WAX (Phenomenex) 30 m×0.25 mm I.D., df=0.25 μm
Inlet	250 °C, EI=Split 20:1, FI=Splitless
Oven	40 °C (2 min) →10 °C/min→250 °C/min (1 min)
Carrier flow	He, 1.0 mL/min (Constant Flow)
MS	
Ion Source	EI/FI/FD combination ion source
Ionization	EI+:70 eV, 300 μA, FI+:·10 kV, 40 mA
m/z Range	m/z 35 - 800

Results and Discussion

Search for unknown compound

Figure 1 shows TICC of volatile compounds of lemon juice. A total of 37 compounds were detected by deconvolution. Aromatic compounds such as monoterpenes ($C_{10}H_{16}$) like D-Limonene and β -Pinene, and monoterpene alcohols ($C_{10}H_{18}O$) like α -Terpineol and Nerol were mainly detected. Three of the 37 compounds had a similarity score of less than 700 with the library DB, and were presumed to be compounds not registered in the DB (=unknown compounds). Among these, the compound detected around RT 13 min (Unknown compound A, Figure 1) was analyzed in detail, and finally AI structure analysis was performed.

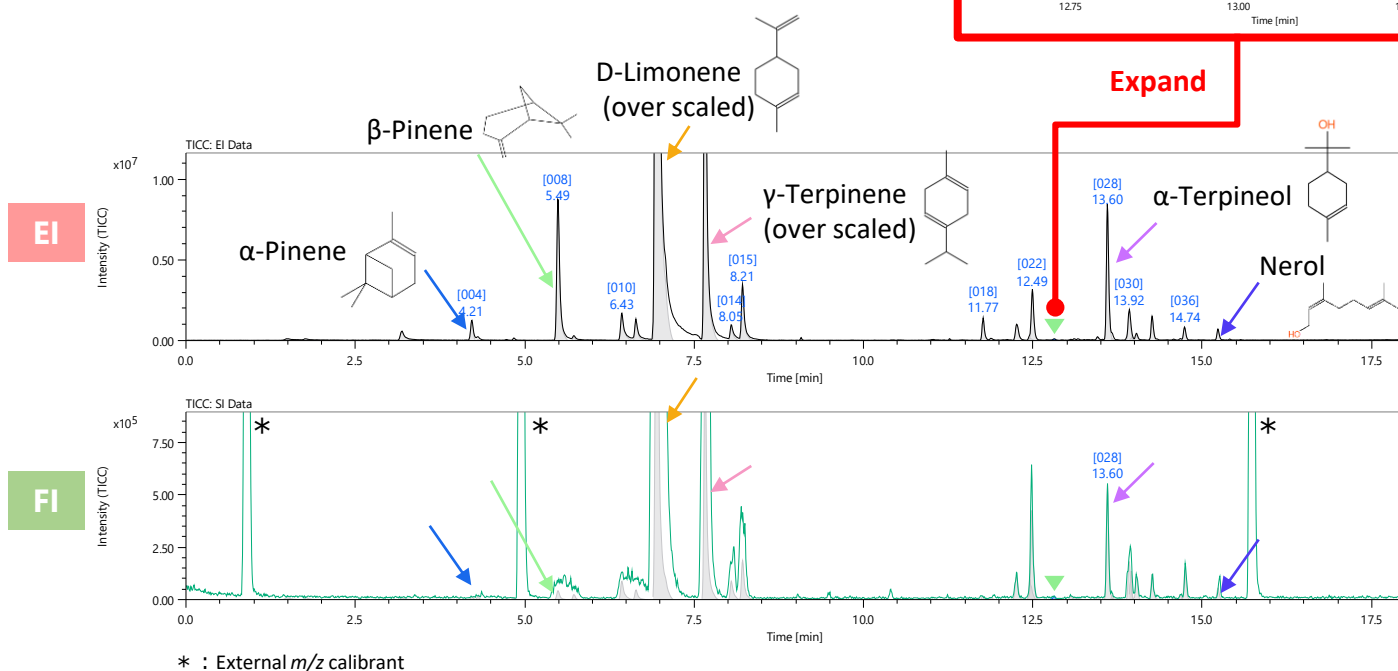
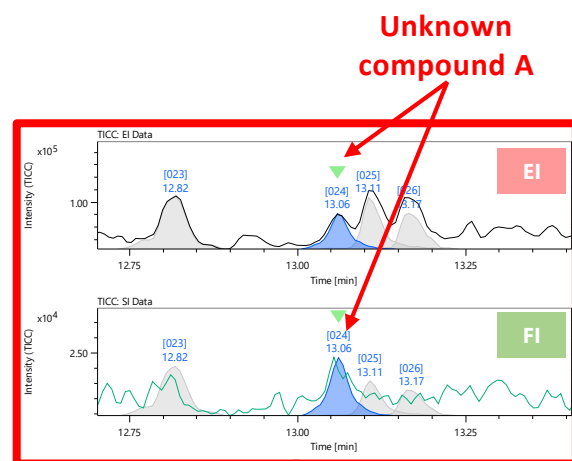


Figure 1 TICC of volatile compounds of lemon juice

Mass spectra and elemental composition estimation results of unknown compound A

Figure 2 shows mass spectra of unknown compound A. For this compound, molecular ion m/z 150 was detected in the mass spectra of both the EI and FI data (IM indicate molecular ion in Figure 2). However, in the FI data, the relative intensity of the molecular ion was high and detected as the base peak. The molecular formula estimated from the molecular ion in the FI mass spectrum was $C_{10}H_{14}O$.

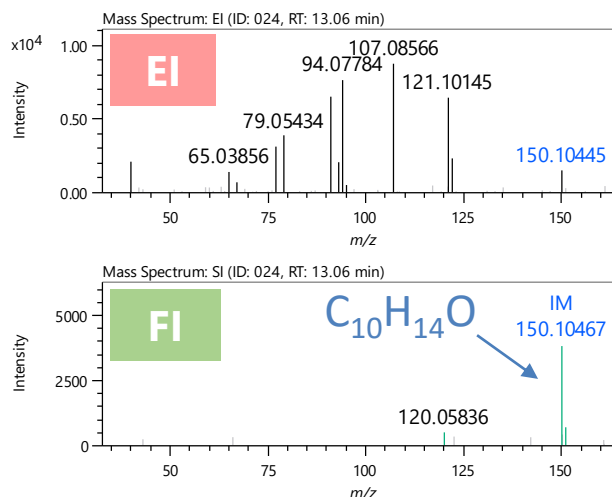


Figure 2 Mass spectra of unknown compound A

AI structure analysis result

Figure 3 shows AI structure analysis results (Top 18 candidates). Among the wide variety of structural formulas, the No. 1 candidate (with the highest similarity score with the AI library) in the AI structure analysis results was a monocyclic monoterpene aldehyde-like compound (Figure 4). Reference 3 states that “*m/z* 79, 93, 107, and 121 occur as fragment ions characteristic of terpenes and terpenoids,” which was confirmed by the EI mass spectrum of this compound (Figure 5). Furthermore, the unknown compound A had a structure similar to safranal, a monocyclic monoterpene aldehyde that is the main component of saffron's aroma (Figure 6). Safranal is known to be produced by hydrolysis of the monoterpene glycoside picrocrocin⁴⁾ (Figure 6). Therefore, unknown compound A may be an aroma component as well as safranal.

As shown in the above results, the structure of unknown compound A could be estimated by AI structural analysis.

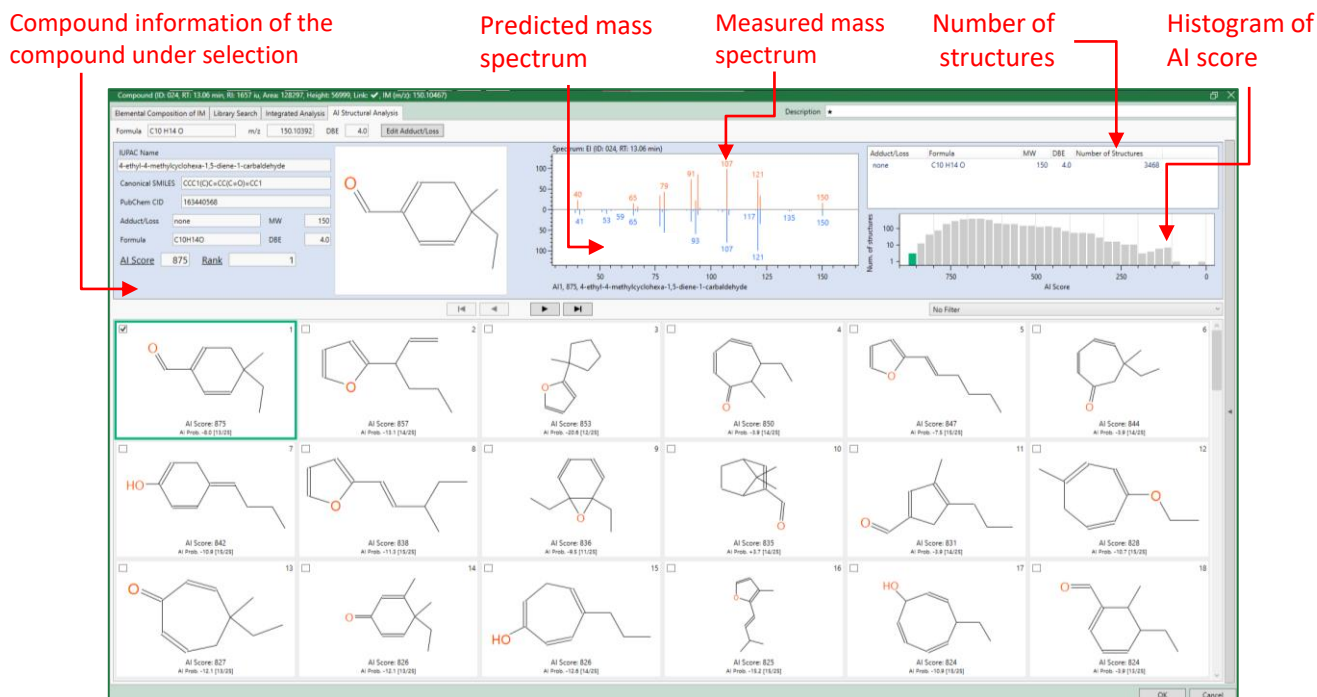


Figure 3 AI structure analysis result of unknown compound A (top 18 candidates)

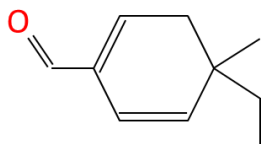


Figure 4 Structural formula of No. 1 candidate of AI structure analysis

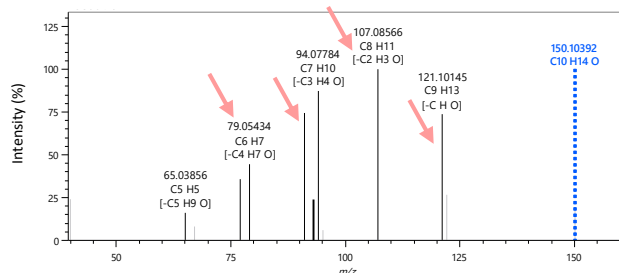


Figure 5 EI mass spectrum of unknown compound A (The blue peak indicates the position of molecular ion)

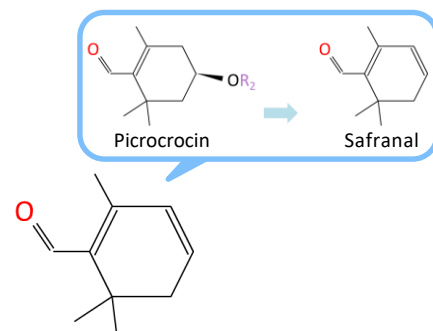


Figure 6 Structural formula of Safranal

Conclusions

In this MSTips, we introduced an example of structure estimation of an unknown compound in lemon juice using msFineAnalysis AI. Manual structure analysis requires a lot of mass spectrometry knowledge and analysis time, but msFineAnalysis AI enables rapid structural estimation. This software is expected to be used for the analysis of unknown compounds in food flavor using GC-TOFMS.

Reference

- 1) M. Ubukata et al, Rapid Commun Mass Spectrom. **2020**; 34:e8820.
- 2) A. Kubo et al, *Mass Spectrometry*, **2023**, 12, A0120.
- 3) Written by F.W. McLafferty. Translated by T. Ueno., Interpretation of Mass Spectra. (Written in Japanese)
- 4) [Kobe Pharmaceutical University, Medicinal Botanical Garden Letter. Issued on 11/22/2022.](#) (Written in Japanese)

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