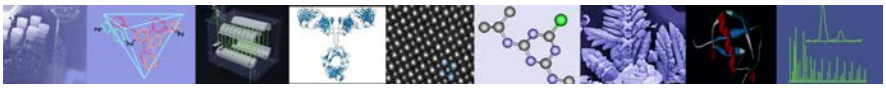


Applications Note

AccuTOF-GCx Series

Comprehensive Analysis + Unknown Component Analysis of Coffee Samples Using Headspace GC-MS

Characteristic Component Extraction by Multiple Classification PCA and Component Identification by High Resolution MS

Introduction

Advances in mass spectrometry are enabling analysis of micro samples and unknown components that were not observable before. As the volume of information acquired from mass spectrometry increases, researchers are calling for simple techniques to analyze numerous components observed, and as a result, there is a rise in demand for comprehensive analytical techniques including multiple classification analysis.

In this work, we will introduce a new technique of non-targeted analysis, which combines comprehensive analysis using high resolution GC-MS and unknown component analysis using soft ionization and EI.

Experiment

Table 1 shows the measurement conditions. Four different types of commercial coffee (A: Indonesian; B: Ethiopian; C: Guatemalan; D: Brazilian) were selected as samples. Each sample was measured 5 times (n=5). The samples were prepared as follows:

1) One gram of coffee beans was loaded into a 22 mL headspace (HS) vial, 15 mL of 100°C water was added, and then the vial was sealed.

2) After the sample had cooled to room temperature, 10 mL of the supernatant was loaded into another HS vial, and 2 µL of an internal reference (p-Bromofluorobenzene) was added to the sample.

3) Finally, 2 mL of the above solution was sealed in a vial and used as a sample.

Table 1. Measurement Conditions

Instruments	JMS-T200GC *AccuTOF GCx* (JEOL Ltd.) MS-62070 STRAP (Headspace autosampler: JEOL Ltd.)
Headspace conditions	
Mode	Trap mode
Extract	3 times
Heating condition	60˚C, 15min
GCTOFMS conditions	
Inlet mode	Split 30:1
Inlet temp.	250˚C
GC column	ZB-WAX (30 m x 0.18 mm, firm thickness 0.18 µm)
Oven temp. program	40˚C (3min) => 30˚C/min => 250˚C (10min)
Carrier gas flow	1.0 mL/min (He, Constant flow)
Ionization method	EI(+) : 70 eV, 300 µA FI(+) : -10 kV, 8mA (Carbotec 5µm)
Spectrum recording interval	0.3 sec/spectrum
m/z range	35 ～ 600
Software	AnalyzerPro (Spectralworks Ltd.)

MS-080318

Results

Figure 1 shows the TIC chromatograms acquired. Among the components observed in each sample, differences between the components detected at high intensity were visible. However, it will be an extremely lengthy process to manually examine all of the detected components. Also, because analysis of micro components hidden under the TIC baseline is likely to produce different results by different operators, auto analysis software capable of peak detection under the same conditions is more effective in comparing the components between samples. For comprehensive analysis of volatile components in coffee, SpectralWorks AnalyzerPro was used.

AnalyzerPro initially extracts the components in question from the chromatogram through deconvolution. The program automatically searches the NIST libraries for all mass spectra of the components selected by deconvolution. The results are tabulated, and the resulting data is subjected to PCA and diffusion analysis.

Figure 2 shows the results of PCA. The PCA score plot classified the measured data according to where they were grown. Specifically, the 1st principal component axis separated the Indonesian coffee (A) from those produced elsewhere. Next, a PCA loading plot was created to identify the components that contributed to the positive separation of the 1st principal component, that is, characteristic components of the Indonesian coffee (A). Figure 3 shows a magnified view of the 1st principal component axis on the positive side in the PCA loading plot (area within a red circle).

Four components shown in Figure 3 contributed the most to the positive separation of the 1st principal component. Of these, 3 components were identified through NIST library search. For example, pyridine is known as an aromatic component of coffee. The content of pyridine in the Indonesian coffee (A) is twice or more higher than those from elsewhere, indicating that it is a characteristic component in Indonesian coffee.

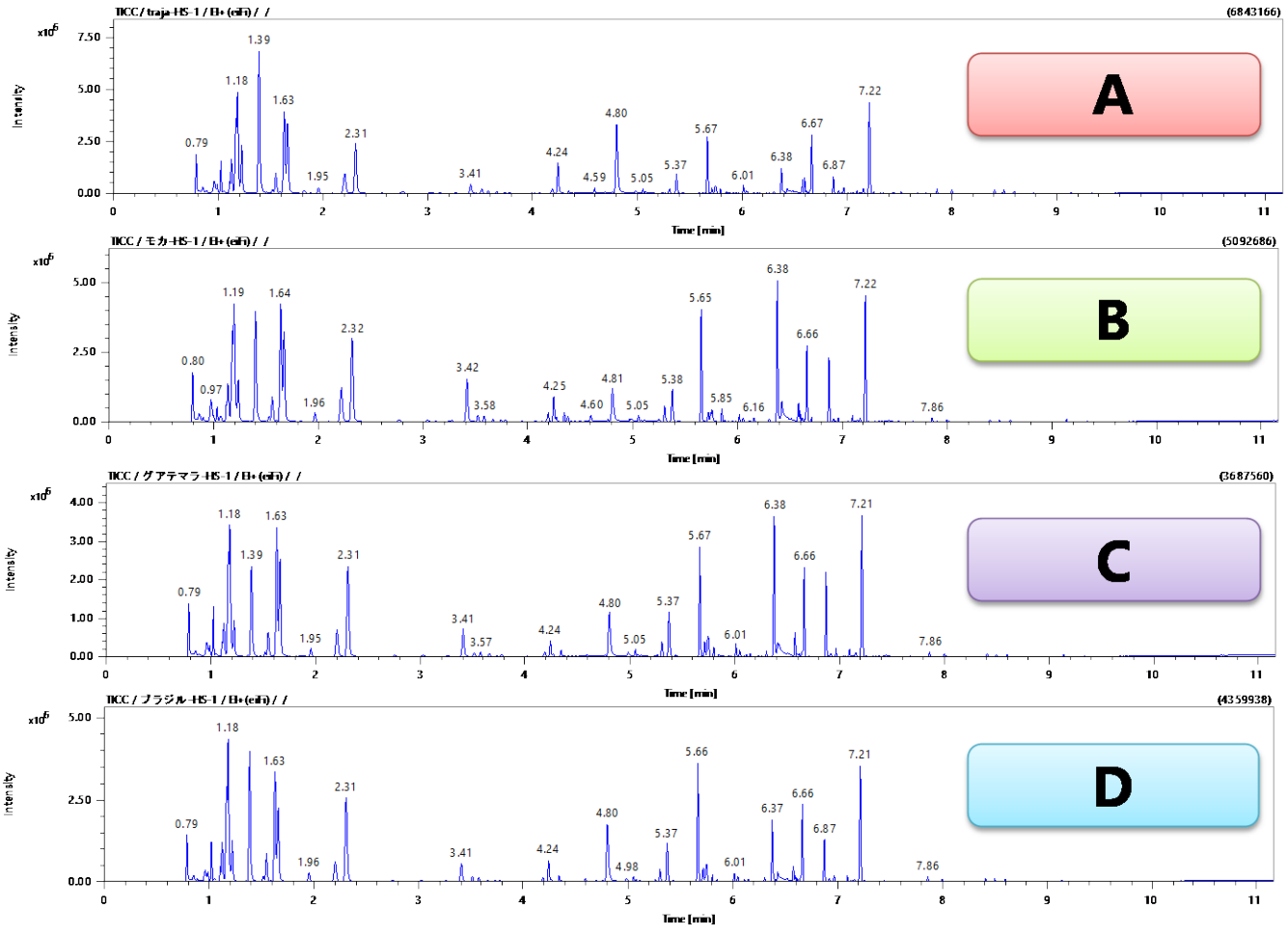


Fig 1. TIC chromatograms

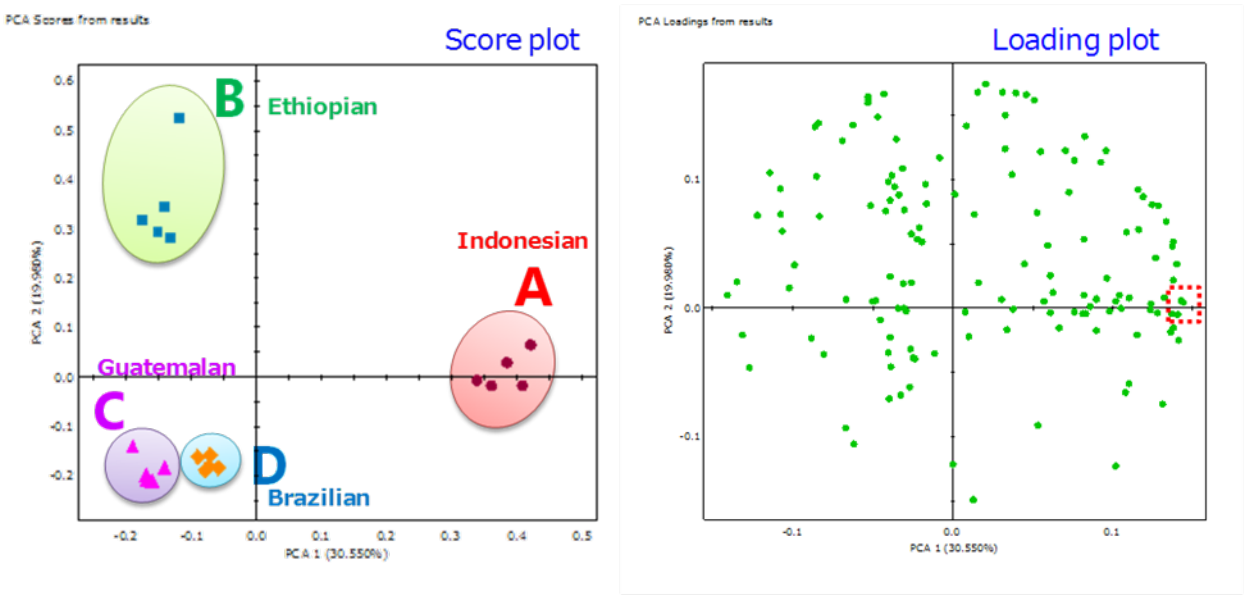


Fig 2. PCA score plot and loading plot

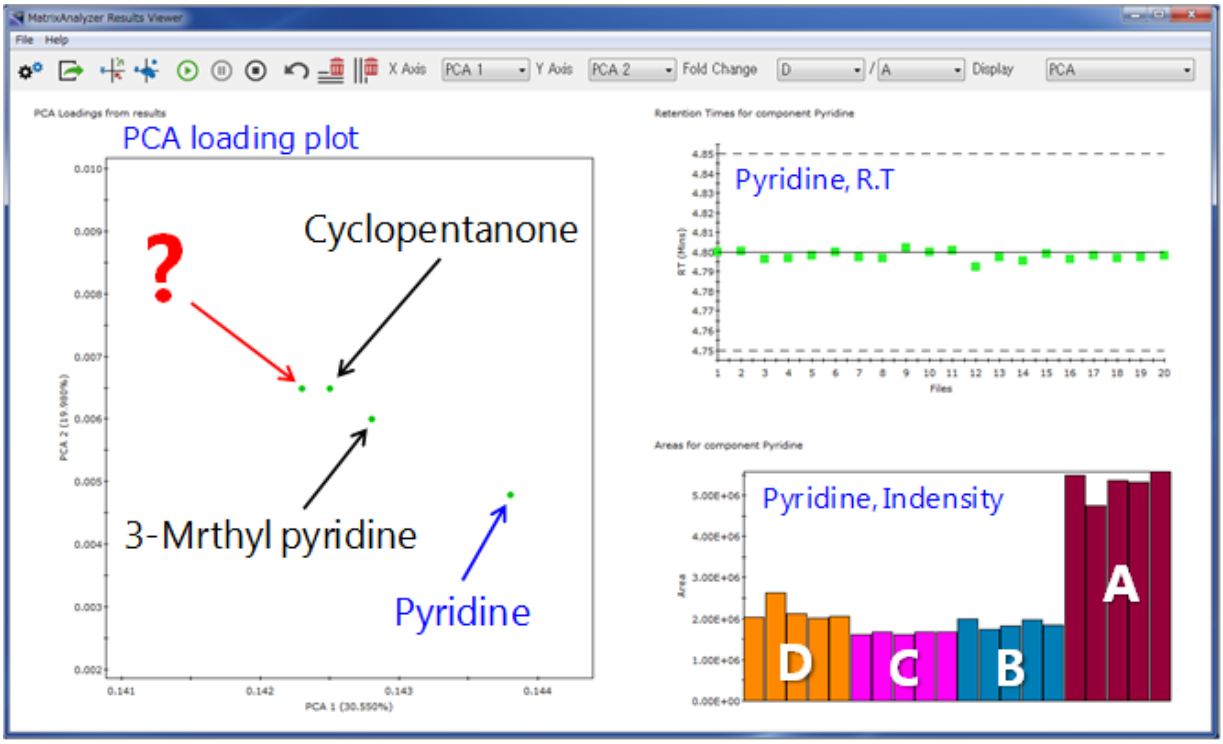
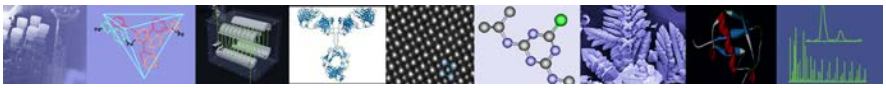
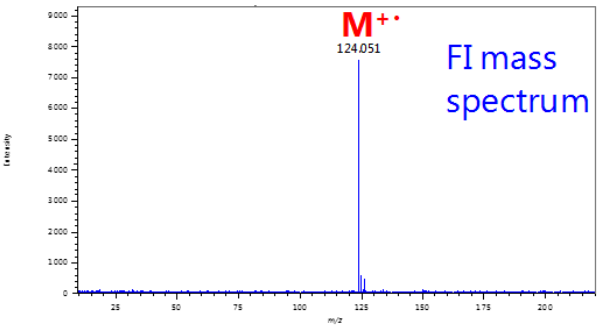
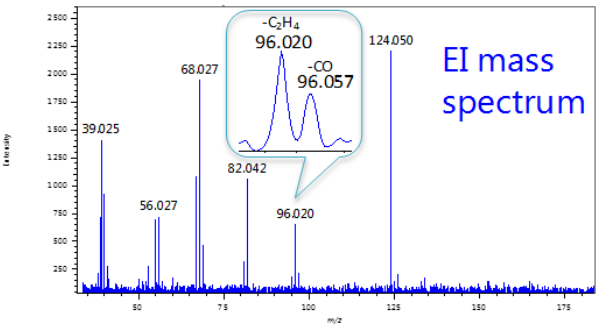


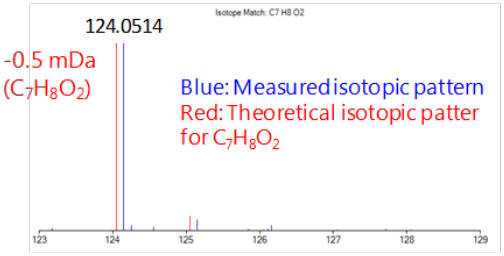
Fig 3. Enlarged view of the PCA loading plot







FI Accurate mass measurement result



EI Accurate mass measurement result

Mass	Formula	Calculated Mass	Mass Error [mDa]	DBE
39.0246	C3 H3	39.0229	1.7	2.5
56.0270	C3 H4 O	56.0257	1.3	2.0
68.0268	C4 H4 O	68.0257	1.1	3.0
82.0416	C5 H6 O	82.0413	0.3	3.0
96.0204	C5 H4 O2	96.0206	-0.2	4.0
96.0571	C6 H8 O	96.0570	0.1	3.0

Fig 4. EI and FI mass spectra and exact mass measurement results for the unknown component in the Indonesian coffee

When a NIST library search was used for one of the 4 components shown in Figure 3 (marked by ? In the figure), the Match Factor was low at 682 for the top candidate, suggesting that this component is not registered in the NIST library database. Thus, the molecular formula of this component was estimated by soft ionization (FI). The structural formula was also estimated by calculating the composition of fragment ions observed by EI.

Figure 4 shows the FI mass spectrum, its isotopic pattern and exact mass analysis results as well as the EI mass spectrum and its exact mass analysis results. The peak at *m/z* 124 observed in the FI mass spectrum was subjected to exact mass and isotopic pattern analysis and was estimated to have a formula of $C_7H_8O_2$ and an unsaturation level of 4. Because the level of unsaturation was an integer, it was determined that the peak at *m/z* 124 was the molecular ion. When the EI fragment ions were subjected to exact mass analysis using each element and its quantity of $C_7H_8O_2$, the compositions of all fragment ions were determined. This also suggests that the formula for this unknown component is $C_7H_8O_2$. The structure shown in Figure 5 was estimated from the formulas of the EI fragment ions. The peak at *m/z* 96 observed as an EI fragment ion was a doublet peak produced by desorption of CO and ethylene from the molecular peak. High

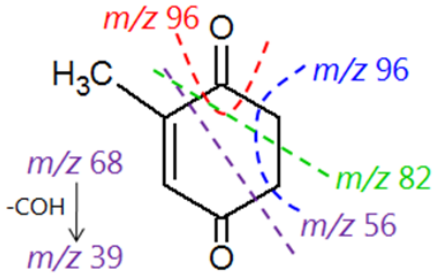


Fig 5. Estimated structural formula for the unknown component in Indonesian coffee

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resolution MS can identify fragment ions having the same integer value (CO and ethylene are both 28 u) by determining their exact masses. The results demonstrate that accurate composition determination for the EI fragment ions makes it possible to estimate the structure of an unknown component.

Conclusion

FI showed strong molecular ions for all compounds measured in this application. PI also showed molecular ions but also produced more fragment ions relative to FI. Also, PI is sensitive for the measurement of aromatic compounds, making it particularly useful for looking at polycyclic aromatics. This application note confirms that PI and FI are soft ionization techniques that can be used to complement the EI results.