msFineAnalysis iQ Ver.2 Target Analysis Example Ⅳ

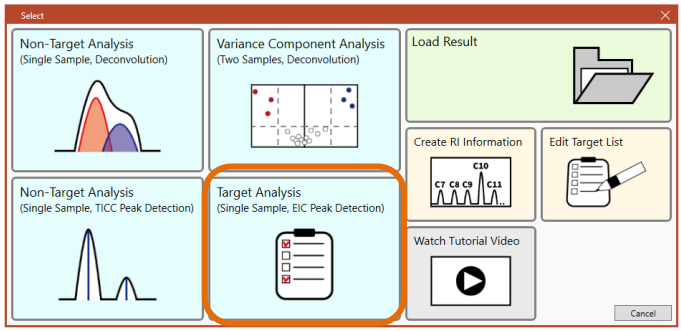
Analysis of Aroma Compounds in "*Hojicha*" using Thermal Desorption GC-MS and Exploration of Pyrazines

Product used：Mass Spectrometer (MS)

1. Introduction

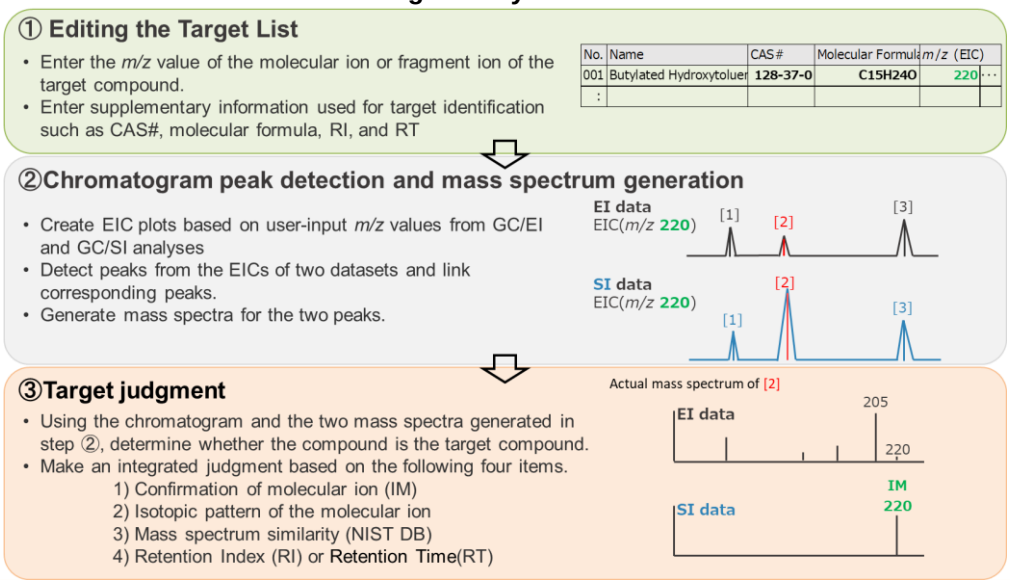
Pyrazines are known as key aroma compounds that characterize the roasted aroma of Hojicha, and in recent years, they have attracted attention for their potential health benefits. Thermal desorption (TD)-GC-MS is an analytical method used for analyzing food aroma components. Although the method enables highly sensitive and comprehensive analysis of aroma compounds in samples, searching for specific target compounds among the many detected components can be time-consuming.

Target Analysis function on the msFineAnalysis iQ is a method for fast and accurate search and evaluation of only the target compounds based on integrated qualitative analysis[1)] with EI and Soft Ionization (SI) data. In this method, it is necessary to register the CAS RN® and/or molecular formula (or molecular weight), fragment composition formula of target compounds in the "Target List" in advance. Based on the registered information, each extracted ion chromatogram (EIC) is plotted for EI and SI measurement data and peaks are detected. The molecular ion peak in the mass spectrum, its isotopic pattern, similarity and retention index with the NIST database are confirmed about the detected peak to determine whether it is the target compound. This time, we analyzed the aroma components of Hojicha using the TD-GC-MS and rapidly identified only pyrazines through Target Analysis.



Integrated qualitative analysis software
msFineAnalysis iQ Ver.2

Target Analysis flow



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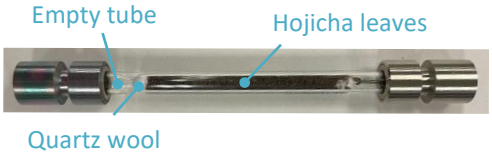
2. Experiment

A Hojicha leaves (Yabukita cultivar) produced in Shizuoka Prefecture weighing approximately 200 mg was used as a sample and placed into a sample tube. The measurement was performed using GC-MS (JMS-Q1600GC, JEOL Ltd.) equipped with TD pre-treatment device (TD-Xr100, MARKES International Ltd.). The ionization methods used were EI and PI as SI. The data obtained were analyzed using the "Target Analysis" function in the integrated qualitative analysis software called msFineAnalysis iQ. The measurement conditions are shown in Table 1.



Sample

TD100-Xr,
JMS-Q1600GC UltraQuad™ SQ-Zeta

Table 1 Measurement Condition

TD	TD-100xr (Markes International Ltd.)
Sample tube type	Empty
Flow path temp	250 °C
Dry(tube)-purge	1 min, 50 mL/min
Tube desorb	80 °C, 60 min, 50 mL/min
Focusing trap	U-T11GPC-2S
Trap cooling	0 °C
Trap-purge	1 min, 50 mL/min
Trap desorb	300 °C, 5 min
TD split	11:1 (split flow:20 mL/min)
GC	8890 GC (Agilent Technologies)
Inlet temp.	250 °C
Column	DB-5MS (Agilent Technologies), 30 m x 0.25 mm, 0.25 µm
Oven temp.	40 °C(1 min)-10 °C/min-300 °C(3 min)
Column flow	2.0 mL/min (He)
MS	JMS-Q1600GC (JEOL Ltd.)
Interface temp.	250 °C
Ion source temp.	250 °C
Ionization	EI (70 eV, 50 µA), PI
Scan range	*m/z* 19～500

3. Results

3.1 Non-target analysis

The TICC of TD-GCMS measurement is shown in Figure 1. 2-Furanmethanol and DDMP which are known to be produced by the Maillard reaction (roasting) and contribute to the roasted aroma, (E,E)-2,4-Heptadienal and Neophytadiene which contribute to the grassy aroma of tea leaves, and caffeine were detected as major peaks. Therefore, it was shown that the analysis of aroma compounds in Hojicha is possible by using TD-GC-MS.

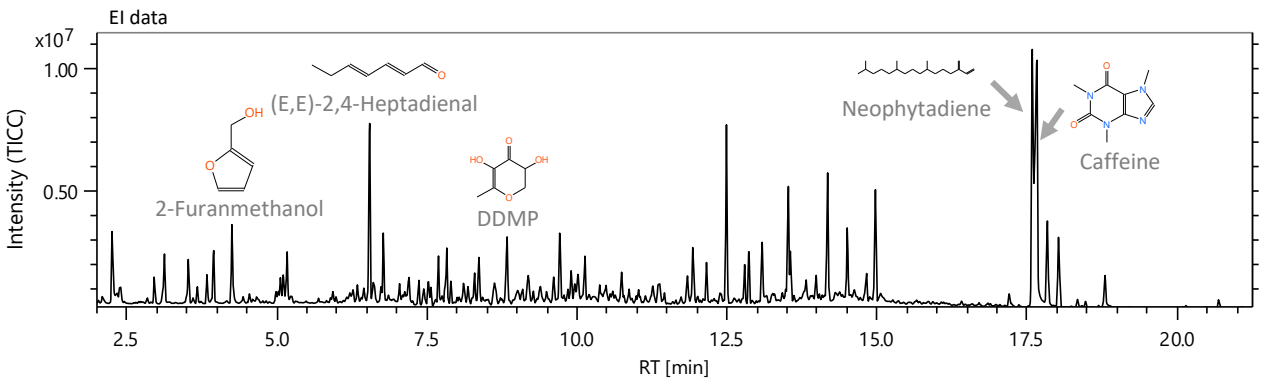


Figure 1 TIC chromatogram of GC/EI data

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3.2 Editing the target list

Table 2 shows the target list registering eight types of pyrazines. In Target Analysis, compounds can be searched using information such as molecular formula, fragment formula, or *m/z* value. In this study, molecular formula information was used.

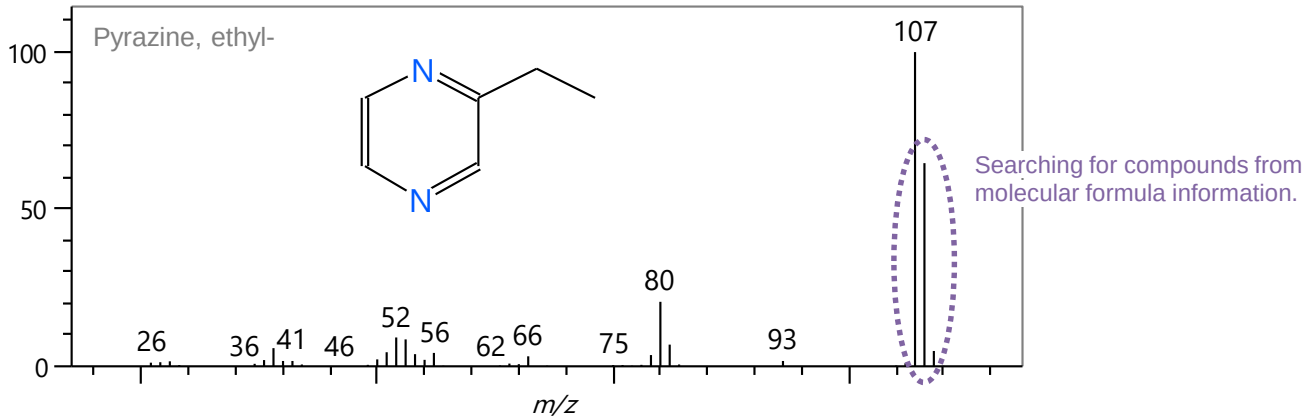

Figure 2　EI mass spectrum of Ethyl pyrazine

Table 2 Target List

Edit Target List

Load Target List...　Create New Target List　Setting of CAS Number Search...

Add from existing Target List...　Add by specifying the number of rows...　Add row

	No.	Name	CAS#	NIST Library	EIC Creation	Molecular Formula	Fragment Formula	m/z (EIC)	RT [min]	RI [iu] Non-Polar	RI [iu] Semi Non-Polar	RI [iu] Polar	Description	Spectrum
☐	001	Pyrazine	290-37-9	✓	Molecular Ion	C4H4N2		80		711	736	1212		View
☐	002	Pyrazine, methyl-	109-08-0	✓	Molecular Ion	C5H6N2		94		801	829	1266		View
☐	003	Pyrazine, 2,5-dimethyl-	123-32-0	✓	Molecular Ion	C6H8N2		108		889	916	1320		View
☐	004	Pyrazine, trimethyl-	14667-55-1	✓	Molecular Ion	C7H10N2		122		980	1004	1402		View
☐	005	Pyrazine, tetramethyl-	1124-11-4	✓	Molecular Ion	C8H12N2		136		1066	1087	1469		View
☐	006	Pyrazine, ethyl-	13925-00-3	✓	Molecular Ion	C6H8N2		108		893	920	1337		View
☐	007	Pyrazine, 2-ethyl-5-methyl-	13360-64-0	✓	Molecular Ion	C7H10N2		122		976	1005	1387		View
☐	008	Pyrazine, 3-ethyl-2,5-dimethyl-	13360-65-1	✓	Molecular Ion	C8H12N2		136		1055	1082	1443		View

Delete selected　Save As...　Close

3.3 Target Analysis Results of pyrazines

Figure 3 shows the results of Target Analysis. The presence of List ID : [T001]、[T002]、[T003]、 [T004]、[T007]、 [T008] was confirmed from the target information shown in the lower-left section of the figure (blue background). To search for the selected compound [T001] Pyrazine, *m/z* value of 80 was registered in the target list. A candidate compound peak was detected in the extracted ion chromatogram (EIC, *m/z* 80) of both EI and SI data.

Figure 4 shows the result of Target Judgment for the peak at RT 2.73 min (▼). The presence of the target compound was judged by the following results. First, each molecular ion peak was observed in both EI and PI data. Next, the difference between the measured RI value and the database RI value (ΔRI: within ±30 is acceptable) was -8 [iu]. Regarding spectrum similarity score (maximum 999), since it decreased due to the low ion intensity of this peak, the spectrum was judged NG. However, since the reverse similarity(*) was 801, indicating a favorable result, Target Judgement was manually changed to [OK] in this case.（ ） From the above, it was confirmed that pyrazine was detected at RT 2.73 min.

Therefore, Target Analysis allows for the identification of compounds with very low ion intensities that may be missed in non-target analysis.

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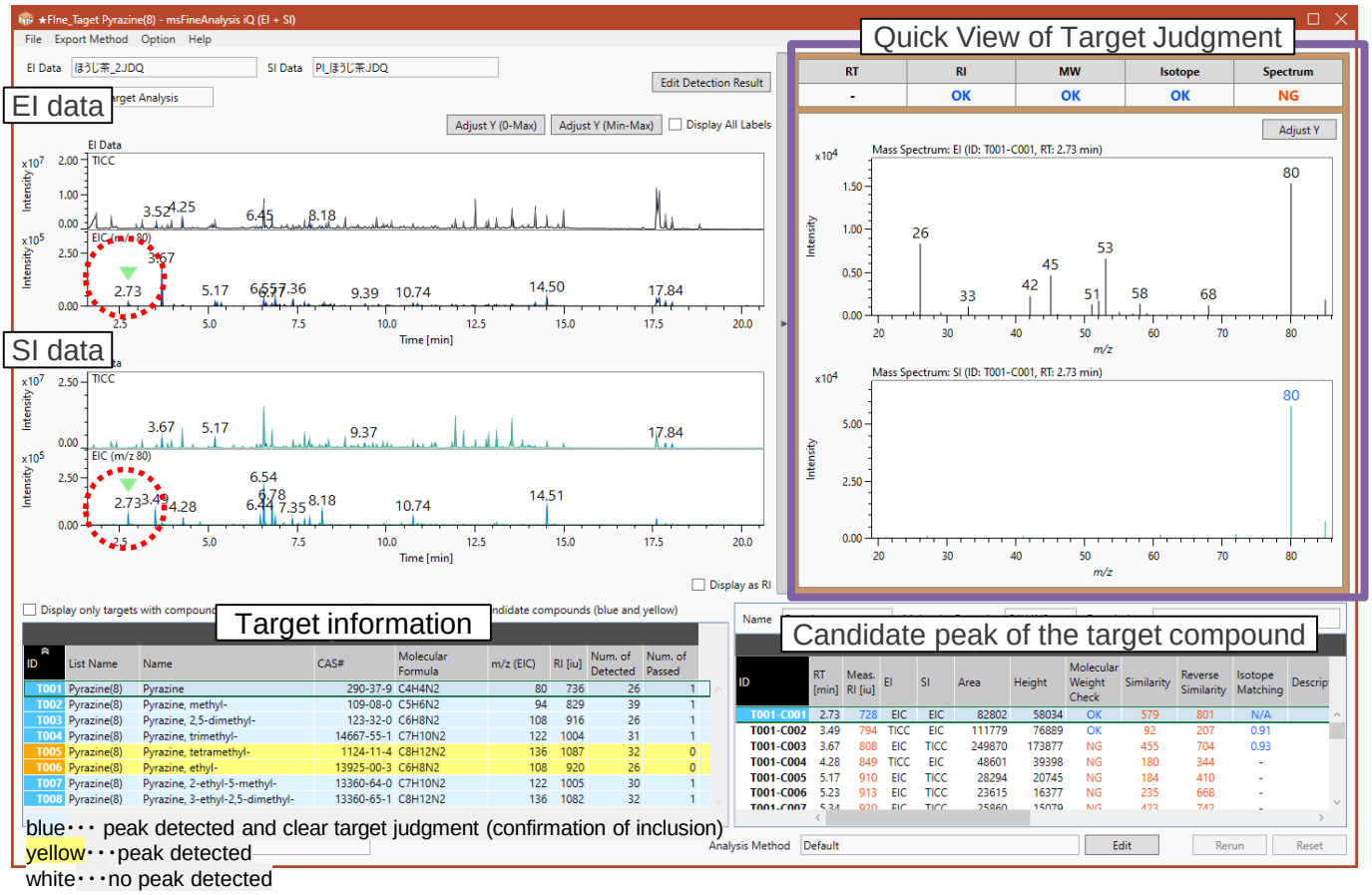

Figure 3 Result of Target Analysis

*While the similarity score is calculated based on all peaks in the measured mass spectrum, the reverse similarity score is calculated using only the peaks present in the library spectrum. The reverse similarity score tends to increase more than the similarity score in the presence of interfering peaks.

Figure 4 Result of Target Judgment

Conclusion

Using the TD-GC-MS method, we measured the aroma components of Hojicha and performed Target Analysis to search for pyrazines. As a result, the presence of pyrazines was rapidly confirmed. Among them, pyrazine had such a low ion intensity that it could have been missed in non-target analysis, but its presence was clearly confirmed through Target Analysis. This report shows that Target Analysis can rapid and accurately identify specific compounds.

References : 1) M. Ubukata et al, Rapid Commun Mass Spectrum., 34 (2020). DOI: 10.1002/rcm.8820

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