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Application News

Element Selective Gas Chromatograph Mass Spectrometer

Quantification of Nitrogen-Containing Compounds in Algae-Based Bio-Oil Using ELEM-SPOT™

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User Benefits

- Even in samples with complex matrices, nitrogen-containing compounds (N-compounds) can be detected selectively and with high sensitivity, enabling analysis with an equimolar response.
- With the same setup, qualitative analysis can also be performed as a conventional GC-MS system.
- By using the Gas Selector, oxygen-containing compounds (O-compounds) can also be analyzed using the same setup.

■ Introduction

Expectations for algal biomass are growing as a next-generation key resource toward a carbon-neutral, low-carbon society. In addition to its high CO_2-reduction potential, algal biomass has attracted attention because it can be cultivated without competing with food resources, and its utilization is expected across a wide range of fields including fuels and materials.

Unlike fossil resources, algal biomass contains many amino-acid-derived N-compounds. Because these compounds can cause catalyst poisoning in downstream refining processes, accurate determination of their content and optimization of the process are essential. However, selective and highly sensitive detection of compounds containing heteroatoms (e.g., N and O) in a complex matrix has been challenging with conventional technologies. Our proprietary ELEM-SPOT system combines a GC-MS with the combustion catalytic reactor EL-30, enabling the world's first selective and highly sensitive analysis of N- and O-compounds in complex samples. In this article, we present an application example of analyzing N-compounds in algae-based bio-oil using ELEM-SPOT.

Fig. 1 ELEM-SPOT™

We have signed a comprehensive clean-energy research collaboration agreement with TotalEnergies, Université de Pau et des Pays de l'Adour (UPPA), and the University of Oviedo (UO). This system merges jointly owned patented technology from TotalEnergies, UPPA, and UO with our analytical technology.

*Sales are limited to areas where utilities can be procured and systems can be supported.

■ Detection Principle

In this system, after organic compounds are separated by GC, they are oxidatively decomposed in the combustion catalytic reactor EL-30, and the resulting species are detected by MS operated in SIM mode. By monitoring the signal at *m/z* 30, corresponding to nitric oxide (NO), a nitrogen-derived combustion product, N-compounds can be detected selectively.

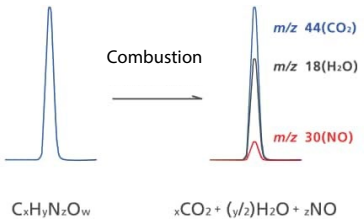

Fig. 2 Detection Principle of N-Compounds

■ Analytical Conditions

The analytical conditions are summarized in Table 1. In ELEM-SPOT, in addition to parameters such as the EL-30 combustion furnace temperature and combustion gas flow rate, there are additional settings that are not available in conventional GC-MS. All these parameters can be easily configured using the LabSolutions™ software.

Analysis Mode Settings

ELEM-SPOT provides two analysis modes:

(i) GC-Combustion-MS (Comb) mode, in which compounds are oxidatively decomposed and N- and/or O-compounds are selectively detected.

(ii) MS Direct (MS) mode, in which measurements are performed in the same manner as a conventional GC-MS without passing through EL-30. If high-abundance components (e.g., the solvent peak or major constituents) are introduced to EL-30, reactor degradation may be accelerated and analytical stability may decrease. Therefore, the analysis is started in MS mode and then switched to Comb mode after solvent elution.

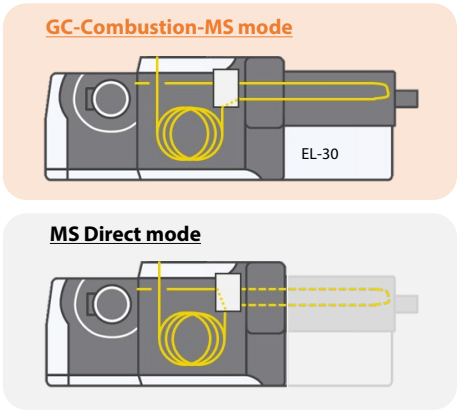

Fig. 3 Two Analysis Modes of ELEM-SPOT

Combustion Gas Settings

$^{16}O_2$/He (1% v/v) was used for the combustion gas of the oxidative decomposition reaction. In addition, by connecting $^{18}O_2$/He (1% v/v) using the Gas Selector, the system can be switched to O-compound analysis as needed without changing the hardware configuration.

Table 1 Analytical Conditions for Comb Mode

System		: ELEM-SPOT (GCMS-QP2020 NX + EL-30)
Auto-injector		: AOC-30i
Column		: HP1-MS (50 m×0.25 mm I.D., 0.50 µm)
[GC]	Inj. Temp.	: 250 ˚C
	Inj. Mode	: Splitless
	Sampling Time	: 1.5 min
	Carrier Gas	: He
	Flow Mode	: Column Flow (1.5 mL/min)
	Purge Flow	: 3.0 mL/min
	Oven Temp.	: 40 ˚C (3 min) - 2 ˚C/min - 250 ˚C (20 min)
	Inj. Volume	: 1 µL
[Comb]	Interface Temp.	: 250 ˚C
	Oven Temp.	: 1050 ˚C
	Makeup Gas Flow	: He 0.6 mL/min
	Purge Gas Flow	: He 0.6 mL/min
	Combustion Gas Flow	: 0.4 mL/min (^{16}O/He, 0.3% v/v)
	Mode Switch Time	: 13.5 min
[MS]	Interface Temp.	: 250 ˚C
	Ion Source Temp.	: 200 ˚C
	Acquisition Mode	: SIM (*m/z* 28, 30, 44)
	Event Time	: 0.3 sec
	Ionization Voltage	: 0.94 kV
	Detector Start Time	: 14 min

■ Analysis of Algae-Based Bio-Oil

As a real sample, algae-based bio-oil was analyzed. The sample was diluted 1:300 in hexane, and N,N-diethylaniline was added as a standard of known concentration. ELEM-SPOT exhibits an equimolar response, i.e., a linear response proportional to the number of atoms (C, H, and N; and O when $^{18}O_2$ is used as the combustion gas) introduced into the detector. Therefore, it is not necessary to prepare a calibration curve for each compound; the N concentration of other peaks can be calculated using a standard of known concentration.

Figure 4 shows the extracted ion chromatogram at *m/z* 30 and *m/z* 44 obtained in Comb mode. The *m/z* 30 signal corresponds to NO, indicating N concentration, enabling detection of N-compounds. In contrast, the *m/z* 44 signal corresponds to CO_2, indicating C concentration, and provides a chromatographic profile similar to those obtained using GC-FID or GC-MS. The *m/z* 44 chromatogram demonstrates that the sample is a complex multicomponent mixture, suggesting that it would be difficult to detect N-compounds using a universal detector. By monitoring *m/z* 30 in ELEM-SPOT Comb mode, N-compounds were selectively detected.

The quantitative value calculated using the known-concentration standard was 11,140 ppmN. The total N concentration in the same sample determined by a chemiluminescence method was 11,000 ppm N, indicating good agreement. In addition, ELEM-SPOT enables calculation of not only the total N concentration but also that for each chromatographic peak.

Furthermore, the same sample was measured in MS mode and qualitative identification of N-compounds was attempted with library searching. Among the peaks judged as N-compounds by Comb mode, 16 components could be identified as shown in Table 2.

Table 2 List of Identified N-Compounds* (MS Mode) and Calculated N Concentrations (Comb Mode)

Peak	Compound	ppmN
1	4,6-dimethylpyrimidine, 5-methyl-2-pyridinamine, etc.	-
2	Aniline	49
3	2,6-diethylpyrazine etc.	-
4	*N*-ethylsuccinimide, 2-isobutyl-3-methylpyrazine etc.	-
5	3,5-diethyl-2-methylpyrazine	34
6	2,5-dimethyl-3-propylpyrazine	61
7	5*H*-2,5-dimethyl-6,7-dihydrocyclopentapyrazine	164
8	3-ethyl-4-methyl-1*H*-Pyrrole-2,5-dione	53
9	2-butyl-3-methylpyrazine	52
10	2-methyl-3-propylpyrazine	57
11	3-methylquinoline	90
12	5-methylindole	86
13	1-(2'-phenylethyl)-pyrrole	64
14	5,6,7-trimethyl-1*H*-indole	16

* In Reference 1, after N-compound peaks were screened and quantified using ELEM-SPOT, further qualitative identification was performed by GC×GC-MS). As a result, compounds accounting for approximately 70% of the total N concentration were identified.

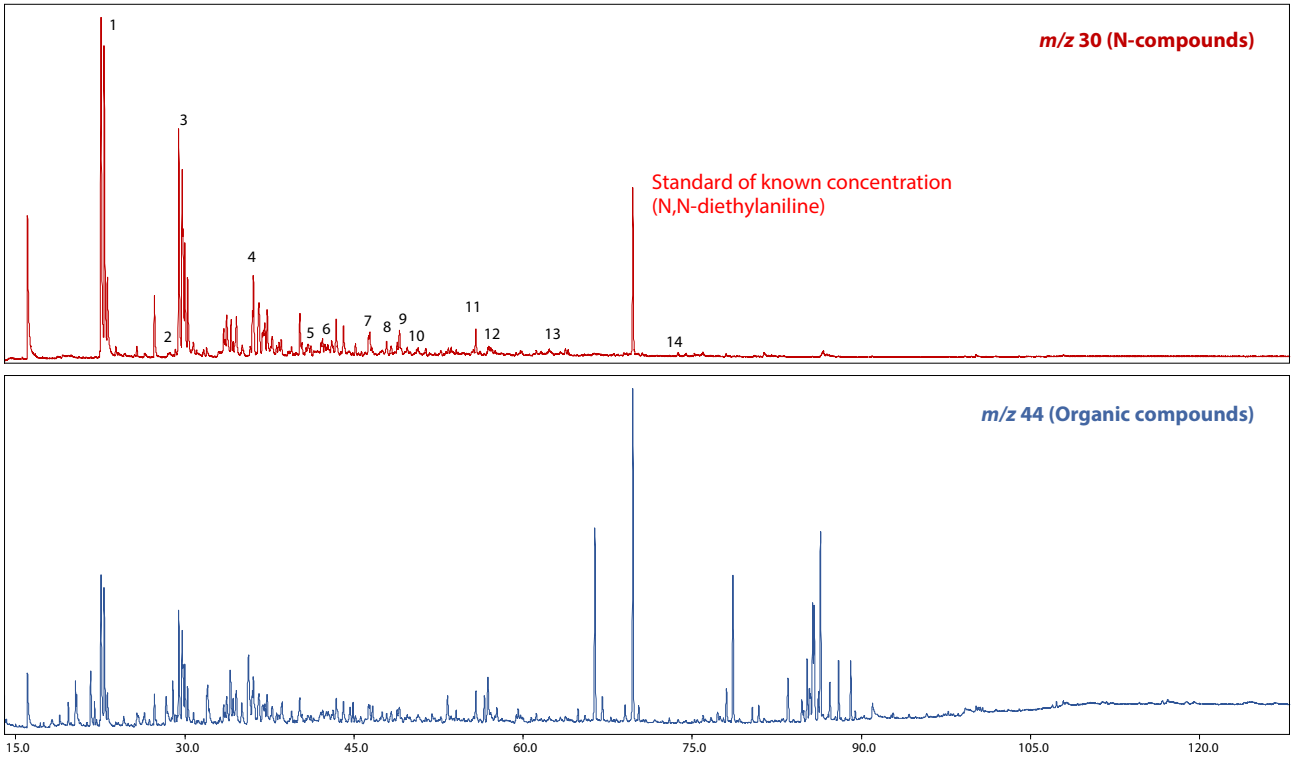

Fig. 4 Chromatograms of Algae-Based Bio-Oil Analysis

*Correction for $C^{18}O$-Derived Peaks

In the *m/z* 30 chromatogram acquired in Comb mode, minor unspecific signals may be observed even for compounds that do not contain N. The main cause is $C^{18}O$ (molecular weight of 30) generated from trace amounts of ^{18}O present in the combustion gas, etc. To correct for this contribution, the following equation can be used:

$$corrected\ m/z\ 30 = m/z\ 30 - \mathrm{m/z}\ 28 \times \frac{\mathrm{Ab}^{18}O}{\mathrm{Ab}^{16}O}$$

Molecular weight of $C^{16}O$

Molecular weight of $N^{16}O$ and $C^{18}O$

Isotopic abundance ratio of ^{18}O and ^{16}O (0.2/99.76)

In this analysis, although we applied this correction, no noticeable differences were observed for peaks originating from non-N-containing compounds; therefore, uncorrected *m/z* 30 results were used.

■ Conclusion

Biomass feedstocks derived from algae contain N-compounds, and removal processes are important when they are used for producing fuels and chemicals. In this article, we presented an application example in which N-compounds in algae-based bio-oil were selectively detected and quantified using ELEM-SPOT. With this system, we were able to specifically detect only N-compounds among hundreds of components. In addition, the total N concentration calculated using a standard of known concentration was in good agreement with the result obtained by a chemiluminescence method.

<Acknowledgements>

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<References>

1) García-Bellido, J.; Freije-Carrelo, L.; Redondo-Velasco, M.; Piparo, M.; Zoccali, M.; Mondello, L.; Moldovan, M.; Bouyssiere, B.; Giusti, P.; Encinar, J. R. "Potential of GC-Combustion-MS as a Powerful and Versatile Nitrogen-Selective Detector in Gas Chromatography" Anal. Chem., vol. 95, no. 31, pp. 11761–11768. July. 2023.

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