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irm-GC/MS: $^{15}N/^{14}N$ Isotope Ratio Monitoring Chlorophyll Derivatives

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Key Words

- **Biomarkers**
- **GC Combustion**
- **Isotope Ratio MS**
- **Polyphyrines**

Introduction

Tetrapyrrole ring structures (porphyrines) are the backbone of important biomolecules like haemoglobin, chlorophyll, and vitamin B_{12}. Because of the inherent stability of porphyrines, they can hold information on biological and environmental processes expressed in their isotope ratios for long periods of time.

Irm-GC/MS allows to read the ^{13}C and ^{15}N information in derivatized porphyrines in the low nmol to pmol range either for natural abundances or by conducting tracer experiments.[1] In *irm*-GC/MS, all compounds eluting from a GC column are converted on-line into CO_2 and/or N_2 in the combustion interface and transferred on-line into an Isotope Ratio Mass Spectrometry (IRMS). ^{15}N determination of compounds with high molecular weight (e.g. M*= 822 g/mol for the chlorophyll derivative described in this report) is a rigorous test of all components of an *irm*-GC/MS system. The GC/C interface and IRMS meet the requirements for highest sensitivity, linearity and stability while maintaining chromatographic integrity.

The ^{15}N determination of porphyrines is made difficult by the relatively low nitrogen content of the compounds and their derivatives. In the current case, the chlorophyll derivative contains 6.8% N, corresponding to 3.4% N_2. Ion statistics and ionization efficiency then ask for 43 times more sample for ^{15}N determination than for ^{13}C measurement with the same precision. In addition, the GC separation of such relatively high molecular weight compounds requires high temperature and high carrier gas flow.

This, together with the limited injection capacity of the GC column, reduces the amount of N_2 transferred to the IRMS and increases the risk of nitrogen background contribution formed by micro leaks in the GC connections.

Experimental

IRMS	Finnigan MAT 252
GC	Hewlett Packard 5890 series II
Injector	On column
Column	HT 5, 12 m, 0.32 mm i.d., film thickness 0.10 µm
GC program	1 min at 40°C; 35°C/min to 80°C/min;
	20°C/min to 275°C/min; 6°C/min to 315°C/min; 10 min at 315°C; constant flow
GC/C interface	Standard GC/C II interface

Results

The chlorophyll derivative was prepared in duplicate (A_1, A_2) to demonstrate the external reproducibility of the entire ^{15}N determination procedure.[1] The injected amounts ranged from 750 pmol to 1.3 nmol corresponding to 120 - 220 pmol N_2 into the IRMS. Although the N_2 background is extremely low and stable, it forms a permanent (rectangular) contribution of about 30% to the integrated porphyrine peak (see Figure 1). Optimized and fully automated background correction algorithms during acquisi-

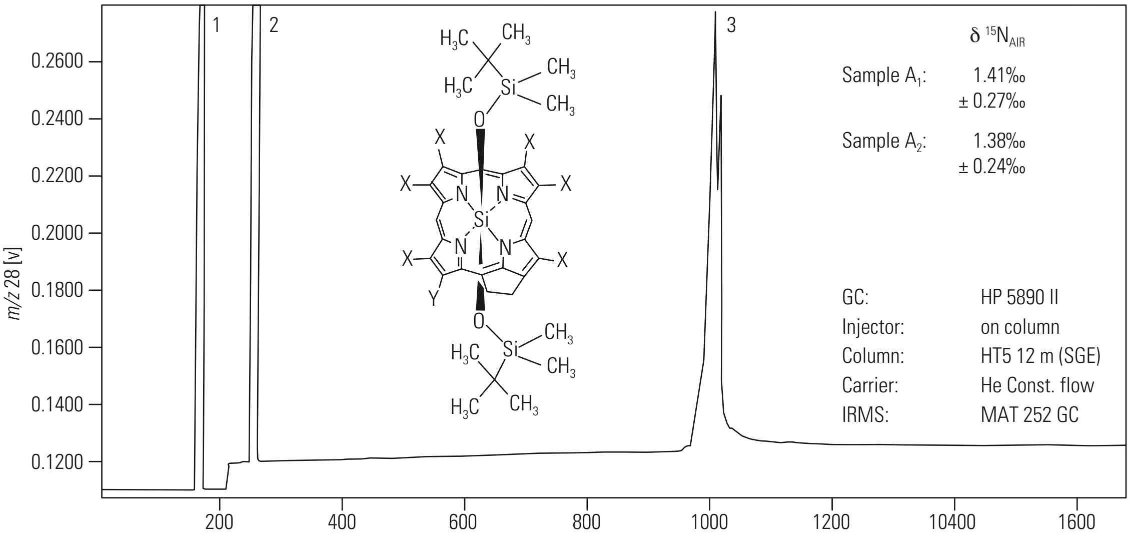

FIGURE 1. *m/z* 28 GC trace of a derivatized Chlorophyll sample.

tion and in post-acquisition data review of the ISODAT data system ensure a correct isotope ratio determination. The $\delta^{15}N$ value versus atm. air (international standard) of sample A^1 was 1.41‰ ± 0.27‰, of sample A_2 1.38‰ ± 0.24‰. The corresponding standard deviation (n = 6) in atom percent was ± 0.00009 at %. The absolute values were within analytical error of the off-line determination. The results prove the high sensitivity in combination with linearity and stability of the MAT 252 IRMS coupled to the GC/C combustion interface.

Similar results can be expected from a DELTA V IRMS or a MAT 253 IRMS coupled with the GC Isolink unit via the ConFlo IV interface.

References

[1] J.P. Sachs, D.J. Repeta, A. Hilkert, Measurement and Assessment of the Carbon and Nitrogen Isotopic Composition of Chlorophyll Derivatives, presented at the *17th International Meeting on Organic Geochemistry*, San Sebastian, Spain, 1995

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