$^{15}N/^{14}N$ Isotope Ratio Analysis of N-Acetyl O-Propyl Amino Acid Esters by GC-IRMS

Andreas Hilkert, Thermo Fisher Scientific, Bremen, Germany

Key Words

Compound Specific Isotope Analysis, Amino Acids, GC Combustion, Isotope Ratio MS

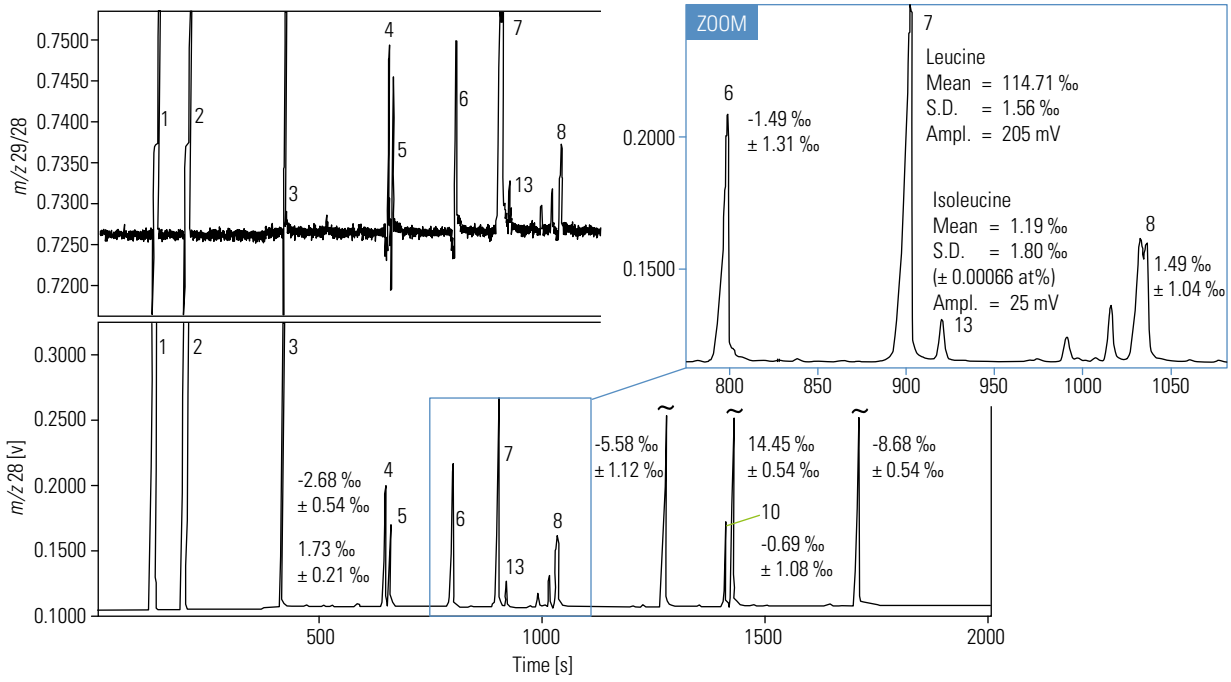

FIGURE 1. *m/z* 28 chromatogram of N-acetyl O-propyl derivatives of amino acids.

Introduction

The analysis of amino acids is an important topic in medical and biological studies. GC-IRMS is a powerful technique for the investigation of metabolic pathways either by conducting tracer experiments or by studying the natural abundance of ^{15}N or ^{13}C in amino acids.

In GC-IRMS, all compounds eluting from a GC are converted on-line into CO_2, and/or N_2 in a combustion interface. N_2 or CO_2 are then introduced into an isotope ratio mass spectrometer (IRMS). Preservation of GC resolution and high overall sensitivity without isotopic fractionation are the most important requirements.

As the ^{15}N isotopic information is not diluted by either the carbon backbone or by the necessary derivatization, the study of nitrogen isotope ratios offers interesting advantages over the determination of the carbon isotopes. Additionally, there is no interference from compounds which do not contain nitrogen. This results in high specificity of the method even with complex mixtures.

Due to the usually low nitrogen content of organic compounds, the determination of $^{15}N/^{14}N$ ratios is much more demanding than the determination of carbon isotope ratios. However, due to advances in the design of the Thermo Scientific™ GC Combustion Interfaces as well as in coupling techniques and IRMS ion sources, these measurements can now be regarded as routine.

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Experimental

Injector	Split/splitless
Cap. column	Ultra 2, 50 m, 0.32 mm i.d, film thickness 0.17 mm
GC program	1 min at 100°C, 15°C/min to 140°C, 4°C/min to 190°C, 8°C/min to 290°C
GC/C interface	Standard GC/C II interface, oxidation reactor at 940°C, reduction reactor at 600°C

Results

The chromatogram in Figure 1 shows a ^{15}N analysis of a mixture of N-acetyl O-propyl amino acid esters (lower: *m/z* 28-trace, upper: isotope ratio trace). The sample contained labeled leucine (1.2 nmol on column) and isoleucine at natural abundance (90 pmol on column).

In the enlarged part of Figure 1, there is a clear separation of the two leucines and no memory or cross-talk from the labeled leucine to the unlabeled isoleucine.The standard deviation for the enriched leucine was ± 1.56‰ (0.00057 at%, n = 3); for the much smaller amount of isoleucine at natural abundance a standard deviation of ± 1.8‰ (0.00066 at%, n = 3) is reported.
These data demonstrate the chromatographic integrity and the sensitivity of the interface coupled to a Thermo Scientific delta S IRMS. Similar results can be expected from a Thermo Scientific DELTA V™ IRMS or Thermo Scientific MAT 253™ IRMS coupled with the Thermo Scientific GC Isolink™ II via the Thermo Scientific ConFlo IV™ universal interface.

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