

# GC IsoLink II IRMS System: Nitrogen isotope values of amino acids

## Authors

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## Keywords

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Derivatization, Nitrogen,  $\delta^{15}\text{N}$

## Goal

Assess accuracy and precision  
of  $^{15}\text{N}/^{14}\text{N}$  analysis using the  
Thermo Scientific GC IsoLink II  
IRMS System

## Introduction

Amino acids are a significant contributor to organismal biomass. Metabolic pathways influence internal isotopic fractionation in some amino acids, whereas other amino acids can be incorporated into biomass with little change in  $\delta^{15}\text{N}$  values. These isotopic patterns allow the sources of nitrogen as well as organism trophic position to be identified. Nitrogen isotope ratios in amino acids are widely used in biological and medical studies for investigating the structure of food webs in aquatic and terrestrial environments and also metabolic variations of cancer cells.

Amino acids cannot be directly analyzed by GC as they decompose in the injector port and on the GC column. The highly polar amino acid moiety aids their solubility in water but not in organic solvents, which reduces the efficiency of their separation and therefore detection. To overcome this challenge, amino acids can be derivatized to remove the active hydrogen, for example by acetylation.

This note reports  $\delta^{15}\text{N}$  values of amino acids and gathers considerations for nitrogen isotope analysis by gas chromatography/isotope ratio mass spectrometry (GC-IRMS).

## Analytical configuration

Compound specific nitrogen isotope analysis of amino acids were undertaken using a Thermo Scientific™ TRACE™ 1310 GC connected to a Thermo Scientific™ GC IsoLink II™ Conversion Unit, which was interfaced to a Thermo Scientific™ DELTA V™ Isotope Ratio Mass Spectrometer via a Thermo Scientific™ ConFlo IV™ Universal Interface.

The GC IsoLink II Conversion Unit is built on holistic capillary design principle. It provides a seamless GC-IRMS solution with latest innovative GC components incorporating a Micro Channel Device (MCD) (P/N 1372470) for coupling GC column with reactors and a temperature-controlled backflush system (Figure 1). The post-column backflush ensures quantitative removal of solvents in front of the reactors ensuring highest conversion efficiency and increased reactor capacity. The MCD has chemically deactivated internal channels and zero dead volume connections that maintain optimal chromatographic resolution and peak shapes. The MCD is simple to install and leak free when finger tight. With robust, all-metal-sealed GC connections using Ag-ferrules, MCD allows for high temperature GC applications ( $\leq 420$  °C).

The GC IsoLink IRMS System can be readily modified to provide comparable levels of performance to that of the GC IsoLink II IRMS System.

N-containing compounds are separated using the TRACE 1310 GC and subsequently combusted at 1000 °C in a combustion reactor (P/N 1255321) consisting of a NiO tube, CuO, NiO and Pt wires. The reactor incorporates a deactivated capillary attached with a capillary solder joint, which eliminates leakage, isotopic fractionation, and cold spots by direct insertion of reactor into GC oven zone.

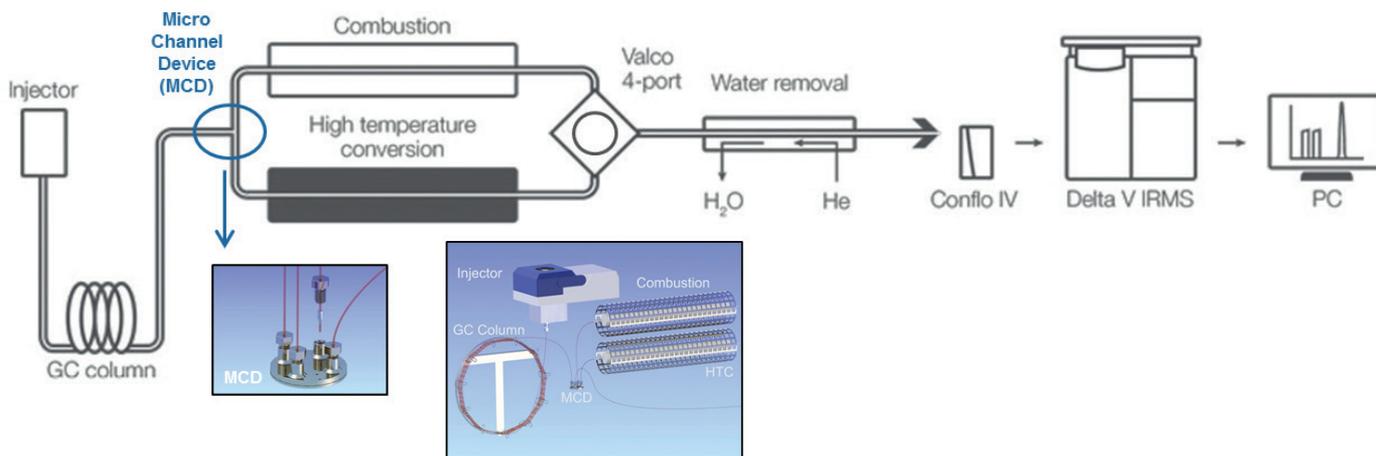
The reactor is a single reactor that provides quantitative oxidation and reduction of  $N_2$  according to the following equation:



$CO_2$  produced from combustion of individual compounds is trapped downstream from the effluent stream using a liquid nitrogen trap. The cryo-trapping is fully automated in the Thermo Scientific™ Isodat™ Software Suite providing automatic release of  $CO_2$ . For the analysis presented here, the GC IsoLink II IRMS System was setup as described in Table 1.

**Table 1. GC and IRMS Conditions.**

TRACE 1310 GC Inlet Parameters	
Injection Volume ( $\mu$ L)	1.0
Liner	Single taper (P/N 453A1345)
Injector ( $^{\circ}$ C)	260 $^{\circ}$ C
Injector Module and Mode	SSL, splitless
Splitless Time and Split Flow	1.5 min, 100 mL/min
Carrier Gas (mL/min)	Helium, 1.4 mL/min
Oven Temperature Program	
Initial Temperature ( $^{\circ}$ C)	80 $^{\circ}$ C
Hold Time (min)	1 min
Rate 1 ( $^{\circ}$ C /min)	20 $^{\circ}$ C/min
Temperature 1 ( $^{\circ}$ C)	170 $^{\circ}$ C
Hold Time 1 (min)	0 min
Rate 2 ( $^{\circ}$ C /min)	8 $^{\circ}$ C/min
Temperature 2 ( $^{\circ}$ C)	270 $^{\circ}$ C
Hold Time 2 (min)	1 min



**Figure 1. GC-IRMS system schematics with Micro Channel Device.**

## Amino acids derivatization

Amino acid standards were derivatized to enhance their volatility and assure optimal separation for stable isotope analysis using GC-IRMS. N-Acetyl-i-propyl (NAIP) esters were prepared according to the method by Corr et al., 2007<sup>1</sup>.

Amino acids were propylated with acedified isopropanol which was subsequently removed under a gentle stream of nitrogen. Dichlormethane (DCM) was added and evaporated at room temperature to remove excess isopropanol and water. Acetylation was performed by addition of acetic anhydride, trimethylamine and acetone. The resulting acetylates were dried under a gentle stream of nitrogen and dissolved in ethyl acetate. Saturated NaCl solution was added to affect the phase separation. The organic phase was dried under a gentle stream of nitrogen; NAIP esters were reconstituted with DCM and transferred into autosampler vials.

Thermo Scientific™ TriPlus RSH™ Autosampler was used for sample introduction to the TRACE 1310 GC, which was configured with a Thermo Scientific™ TraceGOLD™ TG-5MS 30 m × 0.25 mm I.D. × 0.25 μm film capillary column for chromatographic separation.

## δ<sup>15</sup>N of amino acids

Figure 2 shows chromatographic separation of a NAIP amino acid esters mixture by GC-IRMS, which represents approx. 600 ng of amino acid derivate on column, with complete peak resolution. The measured δ<sup>15</sup>N values range from -2.5‰ to +15.1‰.

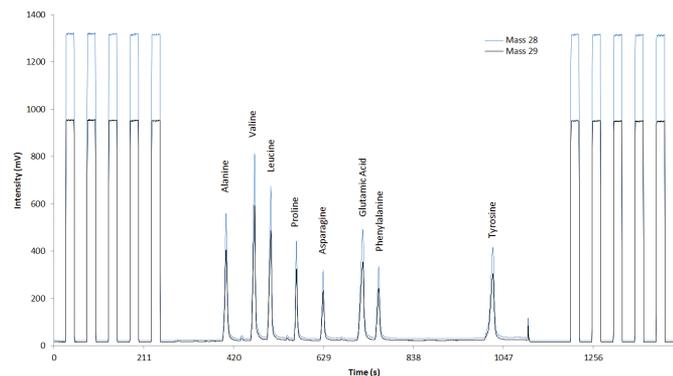


Figure 2. GC-IRMS chromatogram of NAIP derivatives of amino acids mixture.

To cross-check the accuracy of GC-IRMS δ<sup>15</sup>N values, individual amino acids were measured independently using the Thermo Scientific™ EA IsoLink™ IRMS System, configured with a single reactor for nitrogen isotope analysis, comprising Cr<sub>2</sub>O<sub>3</sub>, reduced Cu and AgCoO<sub>4</sub>.

The GC- and EA-IRMS systems used the same calibrated N<sub>2</sub> reference gas tank. The δ<sup>15</sup>N values measured on the EA IsoLink IRMS System were calibrated against USGS-42 (Tibetan Human Hair) and IAEA-N1 (Ammonium Sulfate). GC-IRMS δ<sup>15</sup>N values were calibrated against the calibrated N<sub>2</sub> reference gas tank.

The δ<sup>15</sup>N values of individual amino acids from GC- and EA-IRMS measurements are reported in Table 1 and plotted for comparison in Figure 3. From Table 1, the data precision of individual GC (1σ <0.3‰) and EA (1σ <0.2‰) measurements is recorded. Figure 3 shows the accuracy (r<sup>2</sup> = 0.99) between the measured GC- and EA-IRMS values, indicating not only agreement between the two approaches using the same reference gas tank, but also the fidelity of the GC IsoLink II IRMS System, for nitrogen isotope analysis.

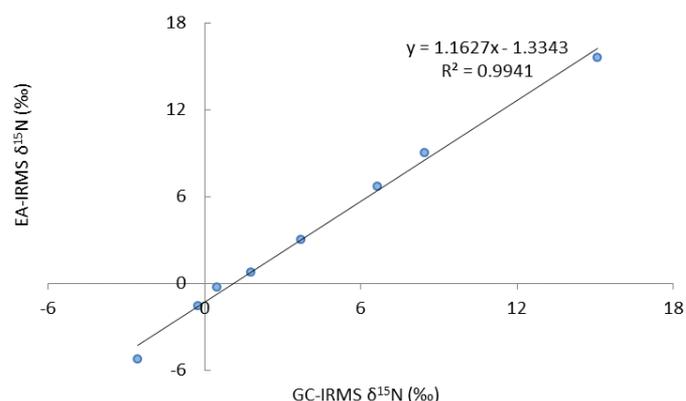


Figure 3. Correlation of δ<sup>15</sup>N values of amino acids NAIP esters obtained by EA-IRMS and GC-IRMS analysis.

Table 2. Measured δ<sup>15</sup>N values of amino acids NAIP esters and the reference material USGS42 and IAEA N1.

Sample name	GC-IRMS		EA-IRMS	
	δ <sup>15</sup> N		δ <sup>15</sup> N	
	Average (‰, n=3)	SD (‰)	Average (‰, n=3)	SD (‰)
Phenylalanine (Phe)	0.5	0.19	-0.2	0.13
Tyrosine (Tyr)	6.6	0.08	6.7	0.20
Asparagine (Asp)	3.7	0.26	3.0	0.02
Proline (Pro)	15.1	0.28	15.6	0.18
Leucine (Leu)	1.8	0.29	0.8	0.12
Valine (Val)	8.5	0.09	9.0	0.20
Glutamic Acid (Glu)	-2.5	0.10	-5.2	0.00
Alanine (Ala)	-0.3	0.03	-1.5	0.19
USGS42	-	-	8.1	0.05
IAEA N1	-	-	0.4	0.09

## Considerations for compound specific $\delta^{15}\text{N}$ analysis

- There is no multi-purpose derivatization protocol for nitrogen analysis that covers all amino acids. The derivatization method must be selected according to the purpose of analysis as it can significantly influence reproducibility and data quality<sup>2,3</sup>.
- Parameters to condition the combustion reactor using oxygen must be balanced in order to:
  - a) ensure quantitative combustion to  $\text{CO}_2$  because lack of oxygen leads to  $\text{CO}$  formation that subsequently interferes with the determination of  $\delta^{15}\text{N}$  values, and;
  - b) maintain reductive conditions and prevent breakthrough of nitrogen oxides.
- Recommended pre-oxidation parameters are given in Figure 4.

Parameter	Value	Unit / Default
Pre Ox. start in Seq.	<input type="checkbox"/>	after pre ox. flag resets automatically.
Oxidation Temp 1	600	[°C] ; Default Value: 600°C
Oxidation Time 1	480	[min] ; Default Value: 480 min
Vent Time 1	1	[min] ; Default Value: 1 min
Backflush Time 1	1	[min] ; Default Value: 1 min
Oxidation Temp 2	900	[°C] ; Default Value: 900°C
Oxidation Time 2	240	[min] ; Default Value: 240 min
Vent Time 2	1	[min] ; Default Value: 1 min
Backflush Time 2	1	[min] ; Default Value: 1 min
Oxidation Temp 3	1000	[°C] ; Default Value: 1000°C
Oxidation Time 3	120	[min] ; Default Value: 120 min
Vent Time 3	1	[min] ; Default Value: 1 min
Backflush Time 3	60	[min] ; Default Value: 60 min

Figure 4. Pre-Oxidation parameters for combustion reactors.

- Recommended re-oxidation parameters (Figure 5):
  - Condition with  $\text{O}_2$  for 30 minutes
  - He Backflush after  $\text{O}_2$  conditioning for 120 minutes
  - Purge conditioning line with He for 3 minutes

Parameter	Value	Unit / Default
Oxidize with $\text{O}_2$	30	[min] ; Default Value: 60 min
He Backflush after Oxidation	120	[min] ; Default Value: 60 min
Purge Conditioning Line	3	[min] ; Default Value: 3 min (180/160: 120 min)

Figure 5. Re-Oxidation parameters for combustion reactors.

- Permanent use of the liquid nitrogen trap is not possible as frozen  $\text{CO}_2$  accumulates in the coil. Trapped  $\text{CO}_2$  needs to be released to prevent capillary blockage, which is achieved automatically under software automation at the end of each sample analysis.

## Conclusion

Gas chromatography/isotope ratio mass spectrometry performed using the Thermo Scientific GC IsoLink II IRMS System provides accurate and precise compound specific  $\delta^{15}\text{N}$  analysis of individual amino acids.

This reliable solution is underpinned by optimal GC resolution, complete conversion of sample to  $\text{CO}_2$  and  $\text{N}_2$  gas and quantitative removal of  $\text{CO}_2$ , offering fully automated and a unique combustion/reduction system to meet analytical challenges of nitrogen analysis.

## References

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