Application Note 30052

GC-IRMS: $\delta^{13}C$ in Fatty Acid Methyl Esters (FAME)

Andreas W. Hilkert, Dieter Juchelka, Thermo Fisher Scientific, Bremen, Germany;
Charles B. Douthitt, Thermo Fisher Scientific, Arizona, USA

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Compound Specific Isotope Ratio, Fatty Acid Methyl Esters, Isotope Ratio MS

Introduction

Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS) measurement of the $\delta^{13}C$ content of the methyl esters of fatty acids (FAME) has been used to study natural food webs[1,2] and, with the use of highly $\delta^{13}C$ -enriched precursors, fatty acid metabolism *in vivo*[3].

The use of biolabeled fatty acids to study metabolism is a relatively new technique that combines tracer methodology with natural abundance measurements by using naturally labeled compounds as tracers[4].

Food adulteration often leads to unintended biolabeling; the differences in natural abundance of specific compounds allow detection and authenticity control[5].

A prerequisite, especially for real life applications like FAME, is high GC performance. Figure 1 shows a GC-IRMS chromatogram of FAME separated from the phospholipid fraction of 500 µl serum from neonates[6].

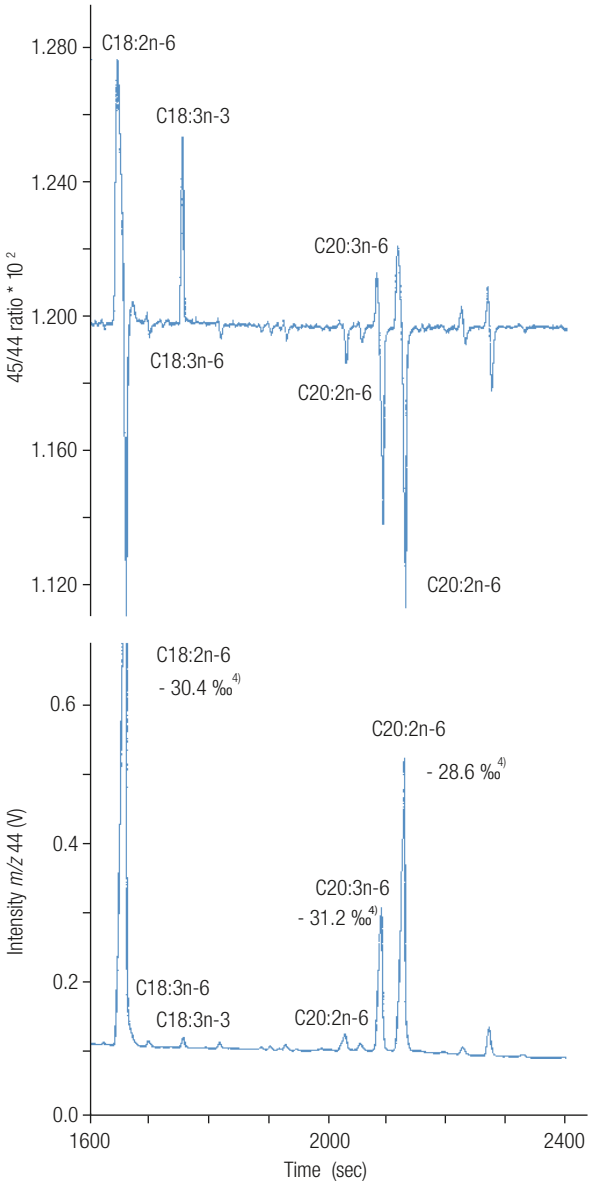

Figure 1. GC-IRMS chromatogram of FAME.

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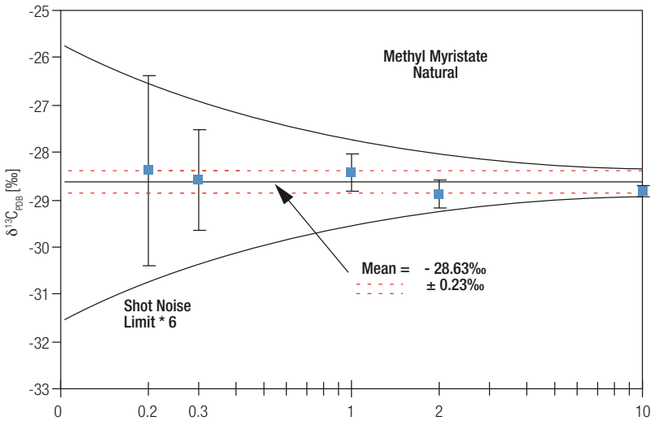

Figure 2. FAME C14:0 natural abundance, sample amount in nanogram vs. $\delta^{13}C_{PDB}$.

Methyl Myristate	
MM:	$C_{15}H_{30}O_2$
MW:	242 ng/nmol
Carbon content:	74.4%
	⇒ 10 ng MM = 0.041 nmol MM
	⇒ = 7.44 ng carbon
	⇒ = 0.62 nmol carbon

Fame on column [ng]	[pmol]	CO_2 [pmol]	Mean to IRMS $\delta^{13}C$ [‰]	S.D. ± [‰]	Repli-cates
10	41.25	154.7	- 28.83	0.13	5
2	8.25	30.94	- 28.89	0.30	6
1	4.13	15.47	- 28.44	0.40	6
0.3	1.24	4.64	- 28.58	1.06	8
0.2	0.83	3.09	- 28.39	2.02	6

Table 1. FAME C14:0 natural abundance.

Natural Abundance

A sample of methyl myristate with natural $\delta^{13}C$-abundance was measured in a dilution series ranging from 10 ng to 0.2 ng on column (41 pmol to 0.8 pmol) (Fig. 2). The reference CO_2 peak was 2 V for all analyses. While the precision of isotope measurement is expected to decrease with decreasing amount of analyte because of counting statistics (precision will decrease as $1/\sqrt{N}$) and because of an increase in the relative importance of chemical background (decrease in S/N), all of the error bars fall within an envelope defined by 6 x shot noise limit[7].

The mean $\delta^{13}C$ of all samples is within ± 0.23‰, with no detectable drift. This experiment shows high precision measurements of $\delta^{13}C$, 0.13‰ (1σ) for 10 ng, with precision within a factor of six of the calculated shot noise limit over the entire range of sample sizes (Table 1). While the published specification for $\delta^{13}C$ is ± 0.2‰ for 10 ng of FAME (0.62 nmol C on column), clearly the integrated GC-IRMS has sub-picomole detection limits, capable of high precision measurements all the way to the end of the dilution series, at 0.8 pmol of methyl myristate on column.

The absence of systematic drift in $\delta^{13}C$ between 0.2 to 10 ng methyl myristate on column establishes that, for Thermo Scientific™ systems, variation in $\delta^{13}C$ due to nonlinearity is less than analytical uncertainty over a dynamic range of at least 50. High precision, sub-picomole detection limits, and the high linearity ensure that GC-IRMS systems deliver to the analyst the largest possible useful working range.

^{13}C Enriched Tracer

A moderately ^{13}C-enriched sample of methyl decanoate (1.43 atom% or 292‰) was measured in a dilution series from 10 ng to 0.3 ng on column (54 to 1.6 pmol of methyl decanoate) (Figure 3), relative to reference CO_2 pulses at natural abundance.

While it is not straightforward to define the shot noise limit for enriched samples, the % standard deviations of the mean (SDM) are excellent, ranging from 0.02% for a 10 ng sample to 0.17% for a 0.3 ng sample (Table 2).

Fame on column [ng]	[pmol]	CO_2 [pmol]	Mean to IRMS $\delta^{13}C$ [‰]	S.D. ± [‰]	SDM
10	53.763	147.85	1.4308	0.0003	0.0229
1	5.376	14.78	1.4297	0.0014	0.0977
0.3	1.613	4.44	1.4319	0.0024	0.1676

Table 2. FAME C14:0 natural abundance.

Calibration relative to a $\delta^{13}C$-enriched reference gas would increase the measurement precision even further, to the levels shown in Figure 2: any nonlinearity is not measurable; both experiments show that ^{13}C-enriched samples can be measured with precision and linearity comparable to natural abundance measurements; previous work has shown that heavily labeled ^{13}C compounds can be detected at significantly lower levels, comparable to tracers labeled with ^{14}C[3].

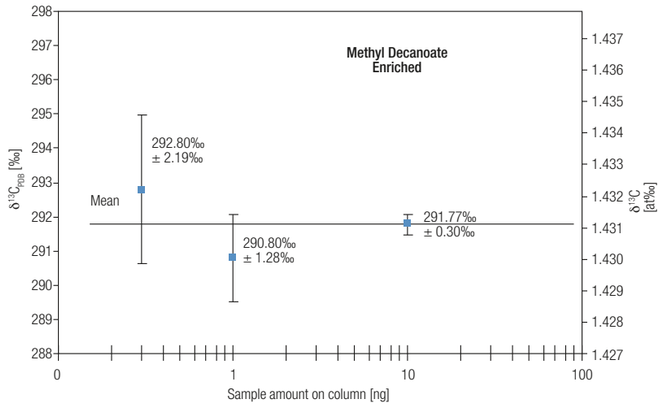

Figure 3. FAME C10:0 enriched abundance, sample amount vs. $\delta^{13}C_{PDB}$.

Biolabeling with $\delta^{13}C$ Natural Abundance Tracers

The $\delta^{13}C$ of fatty acids from plants is a function of the photosynthetic pathway used, with a difference of approximately 15 - 20‰ between C_3 and C_4 plants. The high precision and high sensitivity of GC-IRMS systems allow biolabeled compounds to be used as tracers of fatty acid metabolism. Table 3 shows the $\delta^{13}C$ change for the full term synthesized arachidonic acid methyl esters (C20:4n-6) in the serum lipids of neonates with C_3 diet when changing their dietary fat to corn oil (C_4).

Infant	Corn oil tracer (C_4)	Infant
(C_3 diet)	added to diet	day 4
$\delta^{13}C_{PDB}$: -30.1‰		-25.8‰

Table 3. Biolabelling of arachidonic acid with a corn oil tracer[4].

Conclusions

GC-IRMS systems allow highly precise measurement at natural and enriched abundance of $\delta^{13}C$ in FAME, which, among other things, allows the use of biolabeled "natural abundance tracers" as an alternative to heavily labeled tracers. Detection limits for typical FAME compounds are < 1 picomole on-column. Systematic studies of dilution series establish that non-linearity does not contribute significantly on GC-IRMS systems to analytical uncertainty over the accessible dynamic range.

Linearity refers to the dependence of the measured $\delta^{13}C$ value on sample size; if the measured value is independent of sample size or, alternatively, of the difference in signal intensity between sample and standard, then a mass spectrometer is said to be linear. Non-linearity translates directly into inaccuracy of measured $\delta^{13}C$.

Loose usage of basic terms, including linearity, sensitivity, detection limits and precision, has contributed to confusion concerning the definition and significance of GC-IRMS instrumental specifications. To avoid unnecessary confusion and to facilitate comparisons between instruments, specifications should be clearly defined and closely related to fundamental performance characteristics. It is suggested that, as done in this report (Table 4),

(1) precision should be defined relative to the shot noise limits defined by counting statistics,

(2) sensitivity and detection limits should be defined in terms of amount of sample on column, and

(3) linearity should be specified over a dynamic range relative to a reference peak height.

	Resolution	⇒	maximum
GC	Fractionation	⇒	none
	Memory	⇒	none
	Combustion	⇒	quantitative
C	Resolution	⇒	maximum
	Reference Gas	⇒	precise
	Sensitivity	⇒	maximum
IRMS	Linearity	⇒	maximum
	Precision	⇒	maximum
	Stability	⇒	maximum

Table 4. Definition of performance characteristics for a GC-IRMS system.

Similar results are obtained from all successors of the delta S, e.g. the Thermo Scientific DELTA V™ isotopes ratio mass spectrometer coupled to the recently introduced Thermo Scientific GC Isolink™ II.

References

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Thermo Fisher Scientific (Bremen) GmbH
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