

GC/FID Analysis of Fatty Acid Methyl Esters without Correction Factors Using the Polyarc® Reactor

Application Note

Food and Flavor

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Abstract

An ARC Polyarc® reactor was used in series with a flame ionization detector (FID) to analyze the composition of fatty acid methyl esters (FAMEs) in a mixture. By using this method, accurate quantification of 24 different FAMEs (C8 to C24) is demonstrated without the use of correction factors (response factors) and with a single internal standard (methyl tricosanoate) with an average error of 2.2%. The quantification error of C8 and C10 esters is significantly reduced compared with an analysis using FID and theoretical correction factors (from 9.7% to 5.6% and 3.1% to 0.3%, for C8 and C10, respectively). These results suggest that the accurate quantification of large mixtures of FAMEs is possible without calibration or theoretical correction factors because of the universal carbon response of the Polyarc/FID.

Introduction

The analysis and quantification of the fatty acid component of lipids in animals, plants and microorganisms pose many challenges to the analyst and modern instrument technology. This is due, in part, to the large number and variety of fatty acids in nature, which include molecules with carbon lengths from 2 to more than 80, various degrees of unsaturation, unique stereochemistries and a vast array of different functional groups [1]. It is estimated that many thousand fatty acids exist in nature with 437 unique fatty acids in cow's milk alone [2].

Fatty acid compositions are typically characterized with gas chromatography (GC) paired with flame ionization detectors (FID). The fatty acids are esterified to form fatty acid methyl ester (FAME) analogues, which are volatile, usually nonreactive and separate well in GC columns. The composition analysis involves (1) the identification of the FAME and (2) the quantification of its concentration in the mixture. The identification of a FAME is obtained readily from the retention time using FID and tabulated data, or using a mass spectrometer and an appropriate database. A large amount of progress has been made to optimize the separation and identification of FAMEs with modern GC columns.

The quantification of FAMEs in a mixture requires the meticulous calibration of the detector response to account for the different sensitivity of the FID (or other detector) to molecules of different sizes and types. Theoretical correction factors (also known as response factors) that attempt to account for variations in FID sensitivity have been tabulated, however, the accuracy of these on instruments with varying equipment, methods and histories is tenuous. Furthermore, the application of these correction factors extends only to those species that have been tabulated, a mere fraction of the large number of potential fatty acids. Unfortunately, the calibrations required to generate correction factors for FID analysis are time consuming, require expensive standards and are impractical for the large number of fatty acids found in many samples.

Here, we apply the Polyarc® reactor from Activated Research Company (ARC) to the GC/FID analysis of FAMEs and show that the accurate analysis of FAME molecules is possible without traditional calibration. The reactor completely and rapidly converts all C8 to C24 FAMEs to methane for a uniform FID response. The universal combustion technology allows the technique to be applicable to other lipid molecules that can be analyzed with GC.



Experimental

An Agilent 7890A GC equipped with a capillary-optimized FID and an ARC Polyarc reactor (PA-RRC-A02) were used for the analysis. Helium (99.999%, Praxair) was used as the carrier and FID makeup gas. Air (ultra-zero grade, Praxair) and H₂ (99.999%, Praxair) were supplied to the ARC electronic flow control module (PA-MFC-A09) and to the FID. The effluent of the GC column was sent directly to the inlet of the Polyarc reactor via a zero-dead volume union (PA-CPM-R46). The reactor effluent was connected directly to the FID. Figure 1 illustrates the

Oven	50 °C (2 min), 10 °C/min to 180 °C (5 min), 5 °C/min to 240 °C (15 min)
Column	HP-5 (30 m × 0.32 mm × 0.25 μm)
Syringe Injection	10 μL
	0.5 μL

FID conditions

Temperature	315 °C
H ₂	1.5 sccm
Air	350 sccm
Makeup	20 sccm (He)
Sampling	100 Hz

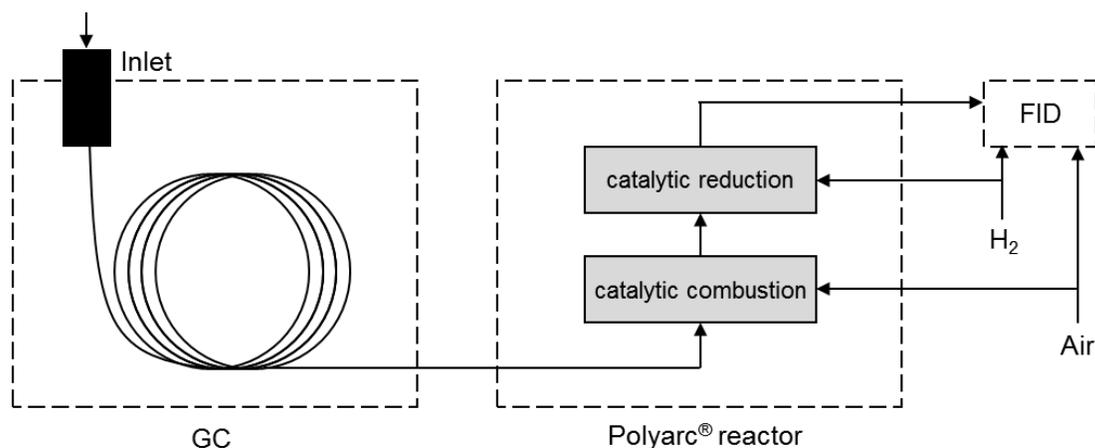


Figure 1. GC-Polyarc/FID configuration

configuration.

A mixture of 25 fatty acid methyl esters (4 wt% of each C8 to C24 FAME, GLC 714, Lot S29-Z; Nu-Check-Prep Inc., 0.038 g) was dissolved in n-heptane (GFS, 0.6415 g). The composition of the sample before dilution was determined gravimetrically and is shown in Table 1.

The sample was also analyzed without a Polyarc reactor on an identical setup, but with the FID H₂ flow rate at 35 sccm and an inlet pressure of 9.5 psi.

GC conditions

Front inlet	Split/splitless
Inlet liner	Agilent P/N 5190-2295
Inlet Temperature	250 °C
Inlet Mode	10:1 Split
Inlet Pressure	11.74 psi
Septum purge flow	3 sccm

Polyarc reactor conditions

Setpoint	293 °C
H ₂	35 sccm
Air	2.5 sccm

Results and Discussion

The separation of 25 FAMES is shown in Figure 2 with the peaks numbered and identified in Table 1 from their relative elution times. With the exception of compounds 10/11 and 15/16, all compounds were baseline separated using the HP-5 column. A different column could be used to completely separate all compounds. Peak resolution, relative retention times and shape are consistent with those found during similar injections directly to the FID without the presence of the Polyarc reactor (Figure 3). The marginal increase in peak tailing observed is the result of the reactions and dispersive mixing that occur in the reactor, the effects of which are attenuated by

Table 1. FAME sample composition before dilution and elution order

Elution	Chain	Name	Weight%
1	C8:0	Methyl octanoate	3.99%
2	C10:0	Methyl decanoate	3.99%
3	C12:0	Methyl laurate	3.99%
4	C13:0	Methyl tridecanoate	3.98%
5	C14:0	Methyl myristate	3.99%
6	C16:0	Methyl palmitate	3.99%
7	C16:1	Methyl palmitoleate	3.99%
8	C17:0	Methyl heptadecanoate	3.99%
9	C18:0	Methyl stearate	3.99%
10	C18:1	Methyl oleate	3.99%
11	C18:2	Methyl linoleate	3.99%
12	C18:3	Methyl linolenate	3.98%
13	C20:0	Methyl arachidate	3.98%
14	C20:1	Methyl 11-eicosenoate	3.98%
15	C20:3	Methyl 11-14-17 eicosatrienoate	3.99%
16	C20:4	Methyl arachidonate	3.98%
17	C20:5	Methyl eicosapentaenoate	3.98%
18	C21:0	Methyl heneicosanoate	3.98%
19	C22:0	Methyl behenate	3.99%
20	C22:1	Methyl erucate	3.98%
21	C22:5n-3	Methyl docosapentaenoate	3.98%
22	C22:6	Methyl docosahexaenoate	3.98%
23	C23:0	Methyl tricosanoate	3.99%
24	C24:0	Methyl lignocerate	3.98%
25	C24:1	Methyl nervonate	3.98%

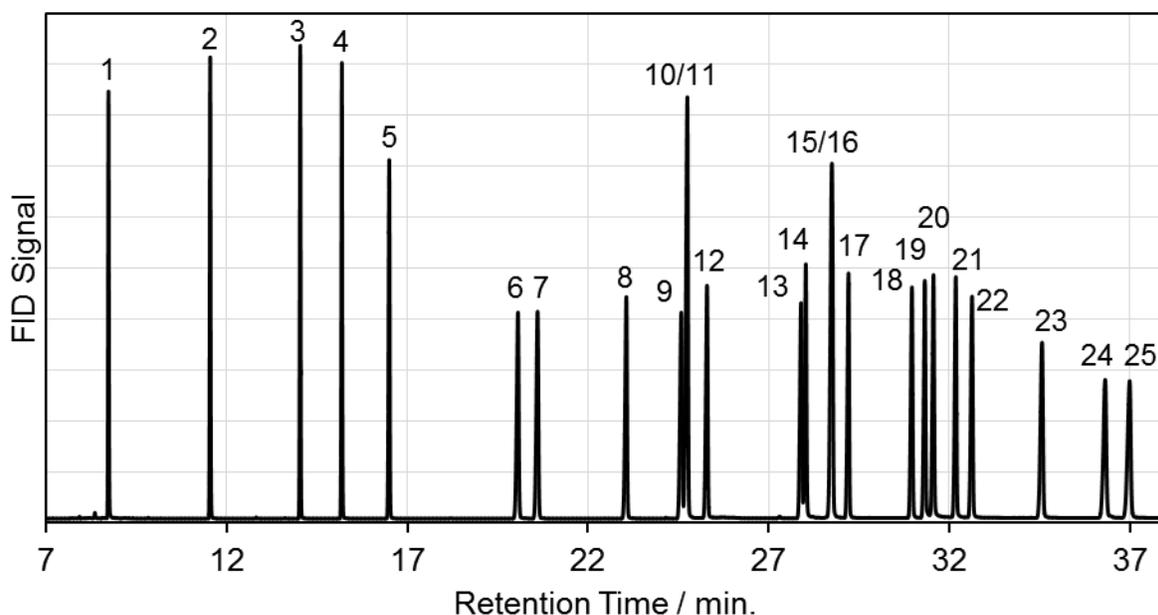


Figure 2. GC-Polyarc/FID chromatogram of 25 component FAME mix.



the unique reactor design with optimized flow dynamics, minimized dead volume and a proprietary catalyst blend.

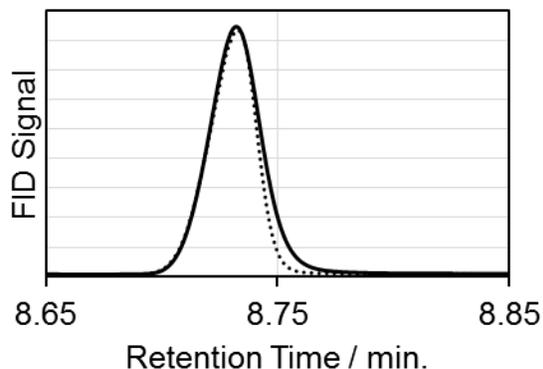


Figure 3. Comparison of the chromatographic resolution of methyl octanoate (C8:0) with (solid) and without (dash) the Polyarc reactor.

The Polyarc reactor converts compounds to methane in a two-step reaction scheme (Figure 1). First, compounds are oxidized until each carbon atom is converted to CO₂. Second, the resulting CO₂ is reduced to CH₄ in the presence of H₂. The resulting CH₄ is measured by the FID, and because non-carbon species are invisible to the FID, the resulting detector response and sensitivity is equivalent on a per carbon basis regardless of the original compound composition and type. The universal and uniform carbon response has been demonstrated on a variety of different molecules. The complete conversion of carbon to methane is the result of the special catalyst blend, reactor flow-path design, and the temperatures and conditions of the Polyarc reactor, and has been verified by mass spectrometry for a number of molecules and concentrations. Thermodynamic calculations ensure that >99.9% of carbon will become methane at equilibrium under the conditions of Polyarc operation [3].

The uniform response of the Polyarc/FID detector to carbon, allows for the quantification of species using,

$$CF = 1 = \frac{area_i / concentration_i}{area_{standard} / concentration_{standard}}, \quad (1)$$

where CF is the relative correction (i.e., response) factor of the species to the internal standard (IS), area is the integrated detector response, and concentration is the molar carbon concentration in the mixture. The CF is unity for all organic molecules when the Polyarc reactor is used. Methyl tricosanoate (C23:0) is used as

the IS for this study, however, any carbon species could be used because of the uniform response of the Polyarc/FID to carbon. The concentration of esters in the sample are thus calculated from the carbon concentration of the IS and the relative peak areas of the IS and the analyte,

$$concentration = \frac{area}{area_{standard}} concentration_{standard} \cdot (2)$$

Sample calculation

The carbon concentration of methyl tricosanoate (MT) in the undiluted sample can be calculated from its weight percent, 3.99 wt%, molecular weight, 368.64 g/mol, and number of carbons per molecule, 24,

$$\frac{3.99 \text{ g MT}}{100 \text{ g sample}} \cdot \frac{1 \text{ mol MT}}{368.64 \text{ g}} \cdot \frac{24 \text{ mol C}}{1 \text{ mol MT}} = 2.6 \cdot 10^{-3} \text{ mol C/g}.$$

The concentration of any other species can now be calculated from its area ratio to MT. For example, the integrated detector response (i.e., area under the curve) of methyl palmitoleate (C16:1; MP) is 829.5, whereas the area of MT is 843.5. From Eq. (2), we find that the carbon concentration of MP is,

$$\frac{829.5 \text{ pA} \cdot \text{s}}{843.5 \text{ pA} \cdot \text{s}} \cdot 2.60 \cdot 10^{-3} \text{ mol C/g} = 2.56 \cdot 10^{-3} \text{ mol C/g}.$$

This equates to a measured weight percent of 4.03% for MP. The gravimetric concentration was 3.99% giving a total measurement vs. gravimetric error of,

$$\frac{|4.03 - 3.99|}{3.99} = 1.00\%$$

Figure 4 shows the results of the FAME analysis using the GC-Polyarc/FID with Eq. (1). Measured concentrations are consistent with those determined using gravimetric sample preparation (Figure 4, top). The absolute errors range from 0.2% to 6.0%, with 70% of the compounds having errors below 3.0%, and 55% below 2.0%. The average error is 2.2% with a standard deviation of 1.6% and a median error of 1.8%. The largest errors occur for C8:0 and C24:0 (5.6 and 6.0%, respectively). These errors probably reflect compound discrimination in the inlet, which is caused by preferential vaporization, reaction or adsorption within the inlet and thus variable on-column loadings for certain compounds. Further improvements in error

may be possible with different inlet liners, splitless injections or on-column injectors, but these details are beyond the scope of this study.

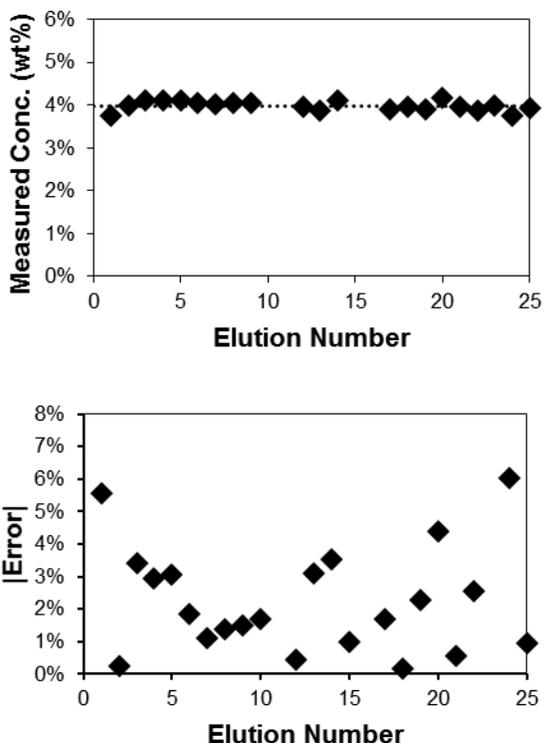


Figure 4. Results of FAME analysis using GC-Polyarc/FID and Eq. (1). Measured concentration (top) of C8 to C24 FAMES (Table 1) using GC-Polyarc/FID and Eq. (1), and the absolute quantification error relative to gravimetric sample preparation (bottom).

Compounds 10 and 11, and 15 and 16 co-elute using this column so that individual concentrations cannot be measured (Figure 2). Using the GC-Polyarc/FID setup, however, the carbon concentration of the 'lump' sum of compounds 10/11 and also 15/16 is determined with 1.7% and 1.0% accuracy, respectively.

An analogous examination of the FAME mixture using only FID (i.e., without the Polyarc reactor) and theoretical correction factors [4] led to absolute errors that range from 0.1% to 9.7%, with 63% having errors below 3.0%. The average error of the analysis is 3.1% and is 41% larger than the error using the Polyarc reactor without correction factors. This FID-only analysis led to 7 samples with errors above 5.0%, compared with only 2 samples above 5.0% error with the Polyarc reactor. These results indicate that the quantification of FAME with the Polyarc reactor exceeds the accuracy obtained using correction factors.

The assumption of uniform carbon response using the data from the FID-only analysis leads to a further decrease in accuracy compared with the use of theoretical correction factors. The average error for this method is 3.25% and the measurement error of C8:0 increases to 15.2%.

Sample calculation of an unknown peak

The concentration of unknowns can also be determined with a high degree of accuracy using the GC-Polyarc/FID setup. Let us assume, for the sake of argument, that compound 8 is an unknown. Even though the compound identity is unknown, the carbon concentration can be calculated from Eq. (2) and the area ratio to the IS with high accuracy,

$$\frac{831.9 \text{ pA} \cdot \text{s}}{843.5 \text{ pA} \cdot \text{s}} \cdot 2.60 \cdot 10^{-3} \text{ mol C/g} = 2.56 \cdot 10^{-3} \text{ mol C/g}.$$

If more information is required, we can estimate the number of carbons by looking at the compounds surrounding 8 in the chromatogram. Compound 7 is C16:1 and 9 is C18:0, so it is likely, on this HP-5 column, that compound 8 has 16, 17 or 18 carbons. Using this information, we can estimate a molar concentration range of the species from,

$$2.56 \cdot 10^{-3} \text{ mol C/g} \cdot \frac{1 \text{ mol}}{17 \text{ mol C}} = 1.5 \cdot 10^{-4} \text{ mol/g},$$

to,

$$2.56 \cdot 10^{-3} \text{ mol C/g} \cdot \frac{1 \text{ mol}}{16 \text{ mol C}} = 1.6 \cdot 10^{-4} \text{ mol/g}.$$

These results indicate that an accurate analysis of fatty acid profiles can be obtained in a single injection with a GC-Polyarc/FID. The quantification of hundreds of FAME compounds is possible with this method, because theoretical correction factors and calibrations are not required. The accuracy of the analysis is an improvement to the analysis using theoretical correction factors with the FID-only and is consistent with the accuracy of typical calibrations. Even in the absence of identification, this method was shown to be able to estimate the concentration of unknowns with a high degree of accuracy. Taken together, these results indicate this method will allow for the quantitative analysis of large mixtures of fatty acids in a single GC injection.

Conclusions

A 25 component mixture of fatty acid methyl esters was quantified with an average error of 2.2% and a maximum error of 6.0% using a GC-Polyarc/FID setup, no calibration and a single injection. This marks an improvement in accuracy from the use of theoretical correction (response) factors. These results imply that large numbers of fatty acids, even those that remain unidentified, can be accurately quantified, allowing for a more complete understanding of industrially-relevant mixtures.

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