Integration of GC/MS Instrumentation into the Undergraduate Laboratory: Separation and Identification of Fatty Acids in Commercial Fats and Oils

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Capillary gas chromatography with mass spectrometric detection (GC/MS) is generally accepted as one of the most powerful analytical tools available for complex organic mixtures. Its widespread use is a direct result of its ability to provide quantitative and qualitative analysis at the same time, when combined with computerized data acquisition. Only over the last few years, with the decline in prices for bench-top instruments, has developing or adapting laboratory experiments to take advantage of GC/MS capabilities begun. The majority of these have involved using GC/MS for monitoring products in organic reactions (*1–9*); only two have dealt with biochemical applications (*10, 11*).

The experiment described here could be used in either a biochemistry or an instrumental analysis course. It is based on a widely used biochemistry experiment, where fatty acid methyl esters (FAMEs) produced from the saponification of commercial fats and oils with BF_3/methanol are separated and identified by gas chromatography (*12*). It takes advantage of mass selective detection to simultaneously identify and quantitate the component FAMEs. With mass selective detection in the scan mode, the identification and quantitation steps can be done for a mixed standard. This saves time in the laboratory, making the experiment suitable for larger laboratory sections. The experiment can be used to illustrate extraction, derivatization to increase volatility, temperature programming, and mass spectral interpretation.

Experimental Procedure

Materials

Methanol, hexane, sodium hydroxide, anhydrous magnesium sulfate, and sodium chloride were ACS reagent grade (Fisher Chemical Company). The 12% BF_3 in methanol solution was obtained from Supelco, Inc., and stored at 4 °C until used. All standard fatty acid methyl esters were obtained from Sigma and were stored at 4° until used. Fat and oil samples were purchased from local grocery stores, with the exception of nutmeg oil, which was extracted from nutmeg (*12*).

Equipment

An HP5890 Series II Plus gas chromatograph equipped with a 5972 mass selective detector was used for analysis of the FAME mixture from each fat or oil. The instrument is software controlled (Hewlett Packard, ChemStation 2.02.65). Chromatograms were acquired in SCAN mode and the components identified based on software matching with library mass spectra.

Isolation of Fatty Acid Methyl Esters

Caution: This part of the experiment should be carried out in a hood, with no open flames nearby. Also, owing to the low boiling point of the methanol/BF_3 solution, reaction containers should have a glass bead at the bottom and should be monitored closely to avoid bumping.

A 50–75-mg sample of fat or oil was weighed into an 18×150 mm test tube. Three milliliters of 0.5 N methanolic sodium hydroxide was added and the sample was heated in a water bath (60 °C) until a homogeneous solution was obtained. Five milliliters of BF_3/methanol (12% by weight BF_3) was then added to the mixture and the sample was boiled for 3 min. After cooling, the sample was quantitatively transferred to a separatory funnel containing 25 mL of hexane and 20 mL of saturated NaCl. After shaking, the layers were allowed to separate. The water layer was retained for a second extraction and the hexane layer was drained off through anhydrous $MgSO_4$ into a 50-mL volumetric flask. The water layer was again extracted with hexane and the dried hexane layer combined with the first extract. The combined extract was diluted to volume with hexane and mixed thoroughly. A 1.0-mL aliquot of this solution was then diluted to 50 mL with hexane. If the samples were not analyzed immediately, they were stored in a conventional freezer until used.

GC/MS Analysis

GC and MS operating conditions were as outlined in Table 1. The diluted extracts contained about 2–10 μg/mL of each fatty acid methyl ester. A 2-μL injection required injection in split mode to avoid overloading the column, but provided sufficient ion counts to obtain fragmentation patterns for identification by the data acquisition software. After the separation was complete, the students printed out the total ion chromatogram (TIC) and a summary of the peak area percent with the results of the library search for each component. A standard solution containing known masses of each methyl ester was injected and response ratios calculated, using the following formula:

$$\text{response factor} = \frac{\text{peak area, given standard}}{\text{peak area, palmitic acid standard}} \times \frac{\text{concentration, palmitic acid standard}}{\text{concentration, given standard}}$$

(The response factor for small unidentified peaks was assumed to be 1.00.)

Results and Discussion

The software for controlling the instrument and data acquisition gives the results of the assay in the form of both a total ion chromatogram (TIC) and a summary report, which lists the three most likely identities for each peak. Since most tabulated data for fatty acid composition are given by common names and the spectral library catalogs spectra with their IUPAC names, students must correlate these. In the process, they find out which fatty acids are

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Table 1. GC and MS Conditions

Variable or Component	Value or Setting
	GC
Column	HP-5 (5% phenyl, 95% methyl polysiloxane), 0.25 mm × 30 m
Carrier	99.999% He, 0.566 mL/min (27.5 cm/s)
Split ratio	50:1
Injector temp	250 °C
Detector temp	280 °C
Column temp program	50–260 °C @ 5 °C/min; hold 5 min at 260 °C (total run time: 25 min)
	MS
Solvent delay	3.5 min
Mass range	50–500

saturated and which are unsaturated. They are also forced to make informed choices on the likelihood of finding given types of carbon chains (odd number of carbons vs. even, *E* vs. *Z* isomers) in natural fats and oils.

Analysis of FAME Mixture

The experiment has been tested in two different laboratory courses. A sample total ion chromatogram is shown in Figure 1. Using the response factors calculated for each of the standards, the actual percentage was calculated as

$$corrected\ area = \frac{peak\ area\ for\ given\ component}{response\ factor\ for\ that\ component}$$

$$percentage = 100 \times \frac{corrected\ area\ for\ given\ component}{total\ of\ corrected\ areas}$$

The fatty acid profiles found by the students are compared with literature values in Table 2 (*13*, *14*). The values obtained are in line with the usual ranges seen. Variations from the literature values are not unexpected, since climate and nutrients play a large role in the distribution of fatty acids (*13*).

To assess the reproducibility of results, fatty acid profiles for corn oil from February 1994 and from April 1995 were compared. The results were as follows: linoleic acid, 57% vs. 54%; oleic acid, 26% vs. 24%; palmitic acid, 14% vs. 16%; and stearic acid, 2% vs. 2%. These variation are well within the variation expected from batch to batch of corn oil (*13*).

Variations on a Theme

If used for an instrumental analysis course, this experiment can serve to introduce at least two other topics. Any peaks not fully resolved can form the basis for a discussion of improving resolution of the components. In addition, the mass spectra obtained for each component can also be used to study fragmentation patterns. For example, there are noticeable differences in going from saturated to monounsaturated to diunsaturated fatty acids even when their carbon chains are the same length.

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Table 2. Fatty Acid Composition

Type of Oil	Fatty Acid	Lit. Value (%)	Exp. Value (%)
Corn oil	palmitic	8–12	16
	oleic	19–49	28
	linoleic	34–62	54
	stearic	2–5	2
Nutmeg oil	myristic	75–100	96
	palmitic	0–10	4
	other	0–13	—
Peanut oil	palmitic	8	18
	linoleic	26	24
	oleic	56	54
	stearic	3	1
	other	—	3
Safflower oil	palmitic	3–6	16
	linoleic	60–90	84
	oleic	13	—
	stearic	1–3	1

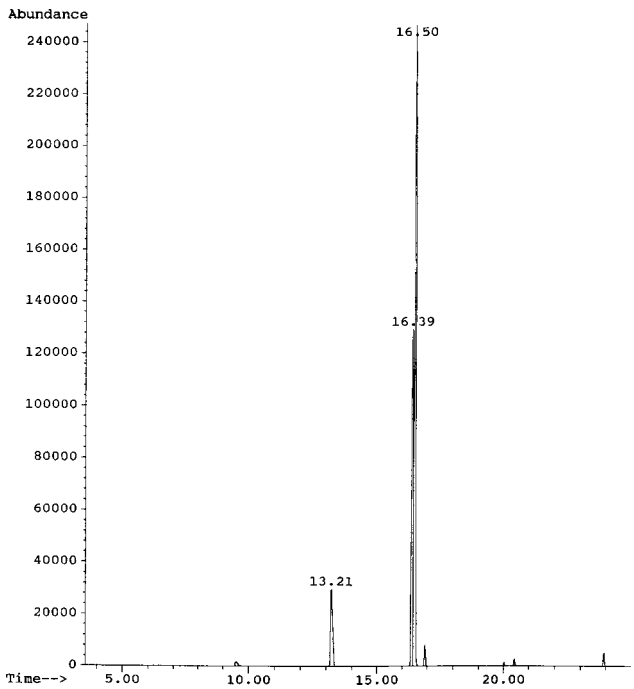

Figure 1. Total ion chromatogram of peanut oil.

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