# The Distinction of *cis* and *trans* Fatty Acids in Food Products with a Novel Benchtop Time-of-Flight GC-MS System

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## Introduction

Reporting the fat content of food on packaging material is required for many products. For this reason, the ability to detect and distinguish different types of fats is important. The AOAC has an official method, Method 996.06, for the detection of fats (total, saturated, and unsaturated) that uses GC paired with FID to separate fatty acids, derivatized with methylation. Some *cis* and *trans* isomers are also distinguished chromatographically as part of this method, which is desirable for the ban on *trans* fats. Here, we combine GC with TOFMS to achieve comparable chromatographic separations based on this method. A fatty acid standard and fatty acids extracted from a variety of butter, margarine, and shortening samples were analyzed. While FID is a standard detector for these analyses, we present some scenarios where MS detection, in particular TOFMS with deconvolution capabilities, offers key benefits.

## Methods

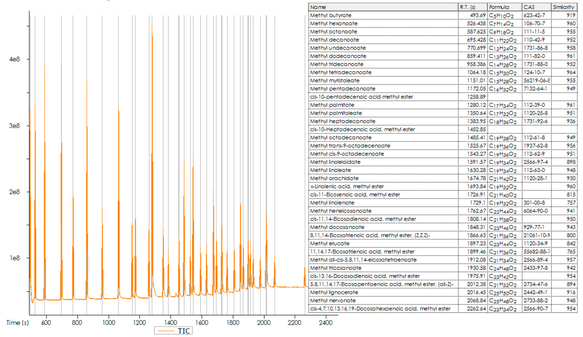
A FAME standard, containing some *cis* and *trans* isomers was purchased for analysis (part CRM47885 from Supelco). Fats were also extracted from a collection of butters, margarines, and shortenings and derivatized to FAMEs, based on the protocol in AOAC Method 996.06. Approximately 185 mg (±5 mg) of sample were dissolved in a solution containing 2 mL each of chloroform and ether. The samples were evaporated to dryness under $N_2$ at 40°C prior to derivatization, which was accomplished with the addition of 2 mL of 7% $BF_3$ in methanol + 1 mL of toluene, followed by heating at 100°C for 45 minutes with shaking every 10 minutes. After cooling to room temperature, 5 mL $H_2O$ + 1 mL hexane + 1 g $Na_2SO_4$ were added. After shaking, the top layer was transferred to a clean vial containing 1 g $Na_2SO_4$ and subsequently analyzed with GC-TOFMS. Instrument conditions are listed in Table 1.

**Table 1. GC-TOFMS Instrument Conditions**

| Gas Chromatograph | Agilent 7890 with Agilent 7693 Autosampler |
|---|---|
| Injection | 1 µL split 200:1 with inlet @ 250°C |
| Carrier Gas | He @ 0.6 ml/min, Constant Flow |
| Column | SP 2560, 75 m x 0.18 mm i.d. x 0.14 µm coating (Supelco) |
| Oven Program | 6 min at 140°C, ramp 4°C/min to 240°C hold 10 min |
| Transfer Line | 250°C |
| Mass Spectrometer | LECO Pegasus® BT |
| Ion Source Temperature | 250°C |
| Mass Range | 35-650 m/z |
| Acquisition Rate | 6 spectra/s |

## Separation of Standard

A routine separation, based on Method 996.06, was achieved with the Pegasus BT. A standard mix, containing 37 FAMEs, was analyzed, and the anticipated analytes were observed and identified. A chromatogram and table with information on the standards are shown in Figure 1.




| Name | R.T. (s) | Formula | CAS | Similarity |
|---|---|---|---|---|
| Methyl butyrate | 493.69 | $C_5H_{10}O_2$ | 623-42-7 | 919 |
| Methyl hexanoate | 526.420 | $C_7H_{14}O_2$ | 106-70-7 | 960 |
| Methyl octanoate | 587.620 | $C_9H_{18}O_2$ | 111-11-5 | 955 |
| Methyl decanoate | 693.420 | $C_{11}H_{22}O_2$ | 110-42-9 | 952 |
| Methyl undecanoate | 770.699 | $C_{12}H_{24}O_2$ | 1731-86-8 | 958 |
| Methyl dodecanoate | 859.411 | $C_{13}H_{26}O_2$ | 111-82-0 | 961 |
| Methyl tridecanoate | 958.386 | $C_{14}H_{28}O_2$ | 1731-88-0 | 952 |
| Methyl tetradecanoate | 1066.18 | $C_{15}H_{30}O_2$ | 124-10-7 | 964 |
| Methyl myristoleate | 1151.01 | $C_{15}H_{28}O_2$ | 56219-06-8 | 955 |
| Methyl pentadecanoate | 1179.08 | $C_{16}H_{32}O_2$ | 7132-64-1 | 949 |
| cis-10-pentadecenoic acid methyl ester | 1258.89 | | | |
| Methyl palmitate | 1280.12 | $C_{17}H_{34}O_2$ | 112-39-0 | 961 |
| Methyl palmitoleate | 1350.64 | $C_{17}H_{32}O_2$ | 1120-25-8 | 951 |
| Methyl heptadecanoate | 1383.93 | $C_{18}H_{36}O_2$ | 1731-92-6 | 936 |
| cis-10-Heptadecenoic acid, methyl ester | 1452.85 | | | |
| Methyl octadecanoate | 1483.41 | $C_{19}H_{38}O_2$ | 112-61-8 | 949 |
| Methyl trans-9-octadecenoate | 1525.67 | $C_{19}H_{36}O_2$ | 1937-62-8 | 956 |
| Methyl cis-9-octadecenoate | 1543.27 | $C_{19}H_{36}O_2$ | 112-62-9 | 951 |
| Methyl linoleidate | 1591.57 | $C_{19}H_{34}O_2$ | 2566-97-4 | 898 |
| Methyl linolenate | 1630.28 | $C_{19}H_{32}O_2$ | 112-63-0 | 948 |
| Methyl arachidate | 1674.78 | $C_{21}H_{42}O_2$ | 1120-28-1 | 930 |
| cis-Eicosenoic acid, methyl ester | 1693.84 | $C_{21}H_{40}O_2$ | | 960 |
| cis-11-Eicosenoic acid, methyl ester | 1726.91 | $C_{21}H_{40}O_2$ | | 815 |
| Methyl hexadecanoate | 1729.1 | $C_{17}H_{34}O_2$ | 301-00-8 | 757 |
| Methyl henicosanoate | 1762.67 | $C_{22}H_{44}O_2$ | 6064-90-0 | 941 |
| cis-11,14-Eicosadienoic acid, methyl ester | 1808.14 | $C_{21}H_{38}O_2$ | | 930 |
| Methyl docosanoate | 1848.31 | $C_{23}H_{46}O_2$ | 929-77-1 | 943 |
| 8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)- | 1866.63 | $C_{21}H_{36}O_2$ | 21061-10-9 | 800 |
| Methyl erucate | 1897.23 | $C_{23}H_{44}O_2$ | 1120-34-9 | 842 |
| 11,14,17-Eicosatrienoic acid, methyl ester | 1899.46 | $C_{21}H_{36}O_2$ | 55682-88-7 | 765 |
| Methyl cis-5,8,11,14-eicosatetraenoate | 1912.08 | $C_{21}H_{34}O_2$ | 2566-89-4 | 937 |
| Methyl tricosanoate | 1930.58 | $C_{24}H_{48}O_2$ | 2433-97-8 | 942 |
| cis-13,16-Docosadienoic acid, methyl ester | 1975.91 | $C_{23}H_{42}O_2$ | | 954 |
| 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- | 2012.38 | $C_{21}H_{32}O_2$ | 2734-47-6 | 894 |
| Methyl lignocerate | 2016.45 | $C_{25}H_{50}O_2$ | 2442-49-1 | 916 |
| Methyl nervonate | 2068.84 | $C_{25}H_{48}O_2$ | 2733-88-2 | 948 |
| cis-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester | 2262.64 | $C_{23}H_{34}O_2$ | 2566-90-7 | 954 |

**Figure 1. GC-TOFMS data were collected for a FAME standard, containing 37 analytes. A TIC chromatogram with peak markers for all FAMEs and peak table for the standard are shown.**

## Chromatography + Deconvolution

The separation of the analytes in the standard was achieved with both chromatography and deconvolution. In cases where the spectral information of the analytes is very similar, chromatographic separation is essential. For example, chromatographic separation of the *cis* and *trans* versions of methyl octadecenoate with very similar spectra is shown in Figure 2. In other cases, deconvolution of the TOFMS data provided additional separation to distinguish coeluting analytes with distinct spectra. Figure 3 shows the successful deconvolution of methyl erucate and eicosatrienoic acid, methyl ester. This capability adds information that may be missed in a standard FID analysis.

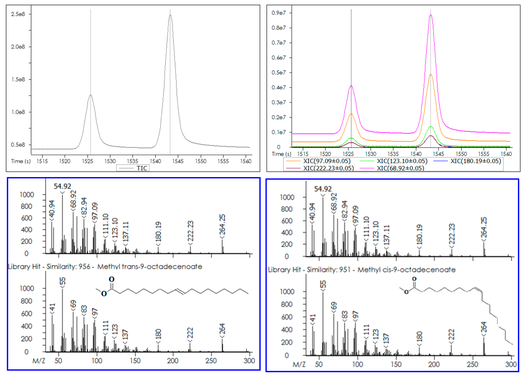



**Figure 2. The *cis* and *trans* isomers, methyl trans-9-octadecenoate and methyl cis-9-octadecenoate, are separated chromatographically and easily distinguished. In cases like this where the mass spectral information is very similar, this chromatographic separation is essential.**

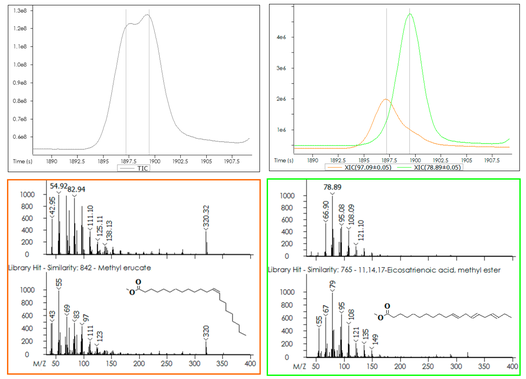



**Figure 3. Deconvolution provides additional information when mass spectral differences are present between the coeluting analytes. Here, two target fatty acids chromatographically overlap, but are mathematically separated from each other with deconvolution. The individual chromatographic profiles can be observed with extracted ion chromatograms (XICs) of m/z ions unique to each analyte. In the TIC these analytes are overlapped with each other, as they would be with FID.**

## Sample Screening

A variety of butter, margarine, and shortening samples were also analyzed and compared to the standard. TIC chromatograms for each are shown in Figure 4. The samples were screened for the target analytes in the standard. A zoom-in view of the *cis* and *trans* isomers of methyl octadecenoate, previously shown in Figure 2, is highlighted in Figure 4. All of the samples contained the *cis* version of this analyte, but only one of the margarines (blue trace, the only one to list trans fat on the nutrition label) also contained the *trans* version, which is likely to be a series of coeluting isomers, at a large concentration. General characterization information on the fat profile can also be observed from this analysis. The most intense peak in each sample, methyl palmitate in butter and methyl linoleate in all others, are shown in Figure 5.

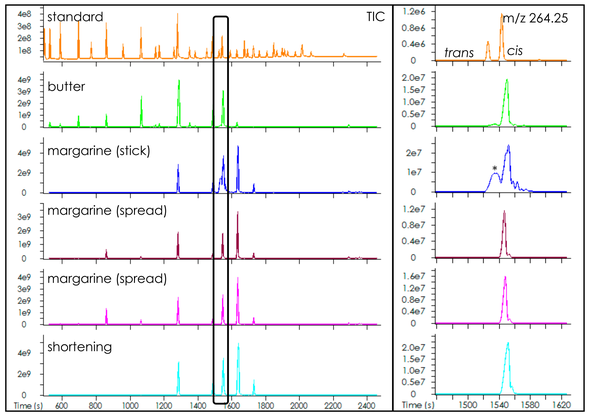



**Figure 4. TIC chromatograms for the standard and each of the screened samples. Butter, stick margarine, two margarine spreads, and a shortening sample were all analyzed. General fat profile information is provided with this screen, as well as information on specific analytes. The *cis* and *trans* fatty acids shown in Figure 2 are highlighted here with XIC 264.25 shown. The *cis* version is observed in all samples, while the *trans* version is observed at high levels only in the sample that listed trans fats on the label (indicated with an asterisk).**

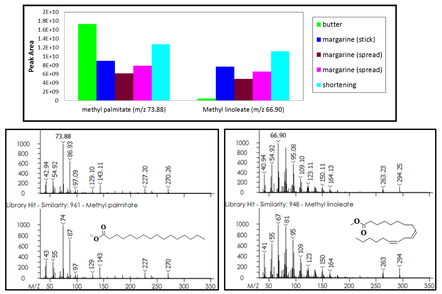



**Figure 5. Insight to the general profile of the fats in each sample can be gained from this data as well. Methyl palmitate is the most intense peak in the butter sample, while methyl linoleate is the most intense peak observed in all of the margarine and shortening samples. The relative amounts of each are shown.**

## Non-Target Characterization

In addition to deconvolution, a benefit of TOFMS detection relative to FID, is the ability to tentatively identify analytes that are observed in the samples that were not present in the standard. Two examples of this are shown in Figure 6. In each case, these analytes are likely to be observed with FID, but would not easily be identified. With MS, the observed spectra were searched against library databases to identify these other important analytes that may have been missed. In Figure 6, a FAME that was not present in the standard was observed. This analyte and others like it may have implications on how the fats should be reported on the nutrition label. Benzaldehyde is observed and shown in Figure 6. Benzaldehyde is known to be present in butter, and may contribute to the taste and aroma of the product, so it is of interest, but it would not need to be included in the fat content on the label.

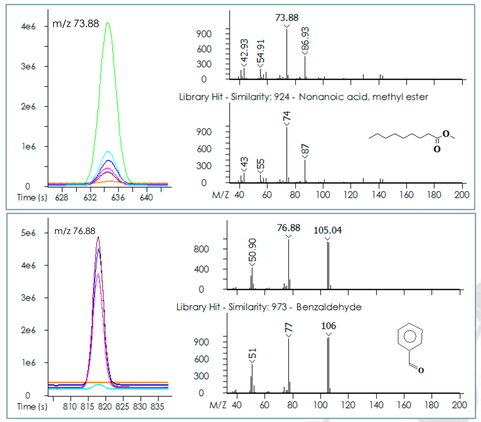



**Figure 6. Nonanoic acid, methyl ester, and benzaldehyde are non-target analytes that were observed and tentatively identified through spectral matching to library databases. These analytes were observed in all of the samples, but were not present in the standard. With FID, it would be difficult to know their identity and whether they needed to be included when determining the fat content.**

## Conclusion

The Pegasus BT is well-suited for routine screening applications, like the analysis of fats in foods. Applications that typically use FID can benefit from MS, as demonstrated here. Chromatographically coeluting analytes can sometimes be distinguished with deconvolution of the MS data and analytes that are not present in the standard can be tentatively identified through searching of spectral databases. A FAME standard was analyzed, and a variety of butter, margarine, and shortening samples were screened for the target analytes. Distinction of some *cis* and *trans* fats was accomplished, and the observations were consistent with the reported nutrition label information for each sample.

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