Analysis of Fats in Food Products with GC-TOFMS

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1. 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. As part of this method, the distinction of some *cis* and *trans* isomers is also accomplished, which is desirable information related to the ban on *trans* fats. Here, we combine GC with TOFMS and achieve comparable chromatographic separations based on Method 996.06 for a fatty acid standard and for fatty acids extracted from a variety of butter, margarine, and shortening samples. 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 to see what you are missing in your fats analysis.

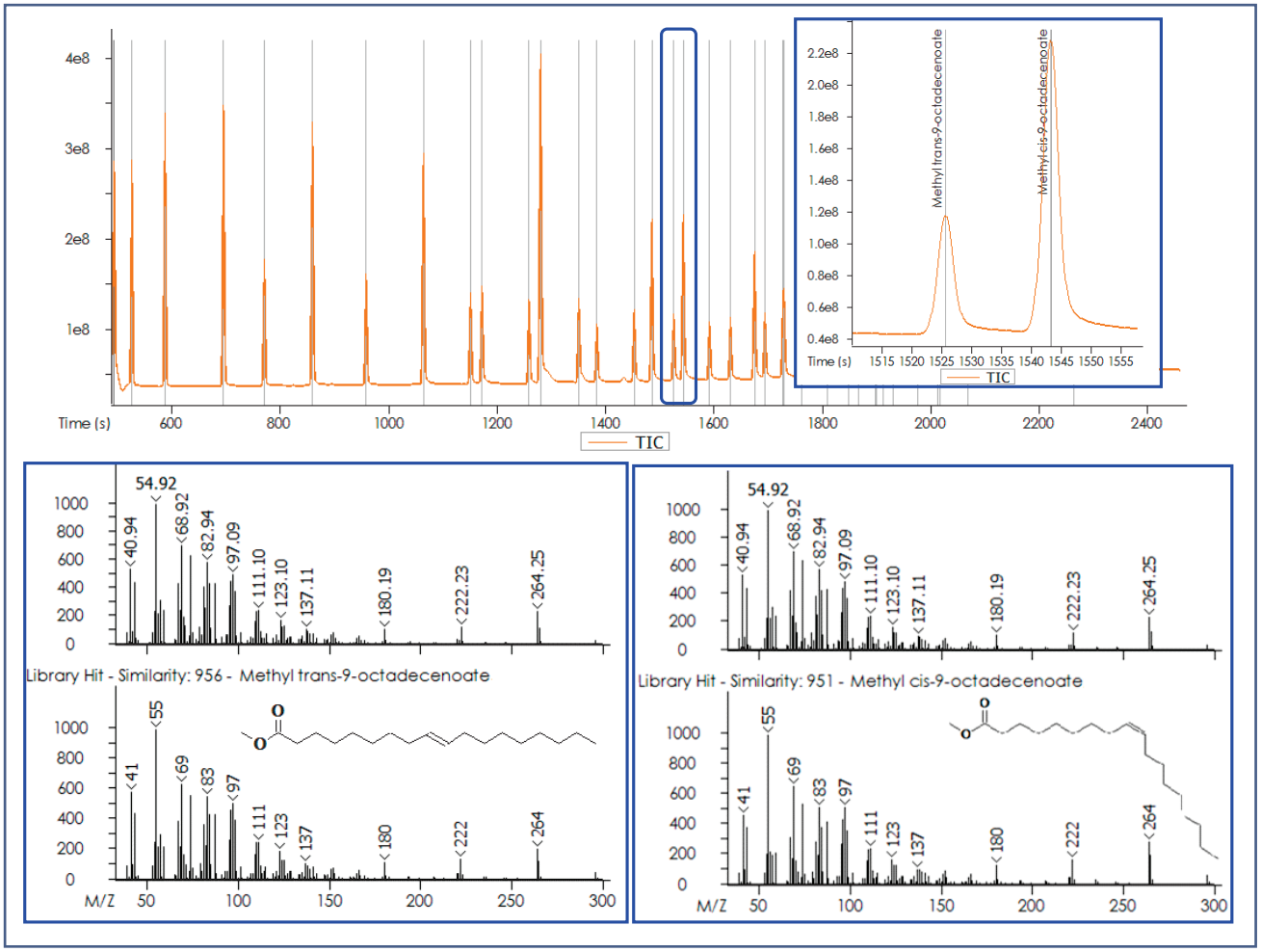

Figure 1. A TIC chromatogram of the FAME standard containing 37 analytes is shown. All of the anticipated analytes were observed and identified with this analytical approach. The cis and trans isomers, methyl trans-9-octadecenoate and methyl cis-9-octadecenoate, are chromatographically separated here and easily distinguished. In cases like this where the mass spectral information is very similar, this chromatographic separation is essential.

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2. Experimental

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 was 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 (*Pegasus* BT) Conditions

Gas Chromatograph	**Agilent 7890 with Agilent 7693 Autosampler**
Injection	1 µL with inlet @ 250°C, split 200:1
Carrier Gas	He @ 0.6 mL/min
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

3. Results and Discussion

The *Pegasus* BT is well-suited for routine screening applications, such as this analysis for fats in foods. A standard mix, containing 37 FAMEs, was analyzed with GC-TOFMS, and the anticipated analytes were observed and identified. Chromatography was essential for separating some isomers, such as *cis* and *trans* versions of methyl octadecenoate, shown in Figure 1. In other cases, chromatographic coelution still occurred and deconvolution of the TOFMS data provided additional separation to distinguish the analytes. Figure 2 shows the successful deconvolution of methyl erucate and eicosatrienoic acid, methyl ester, two coeluting analytes that would be difficult to distinguish with FID.

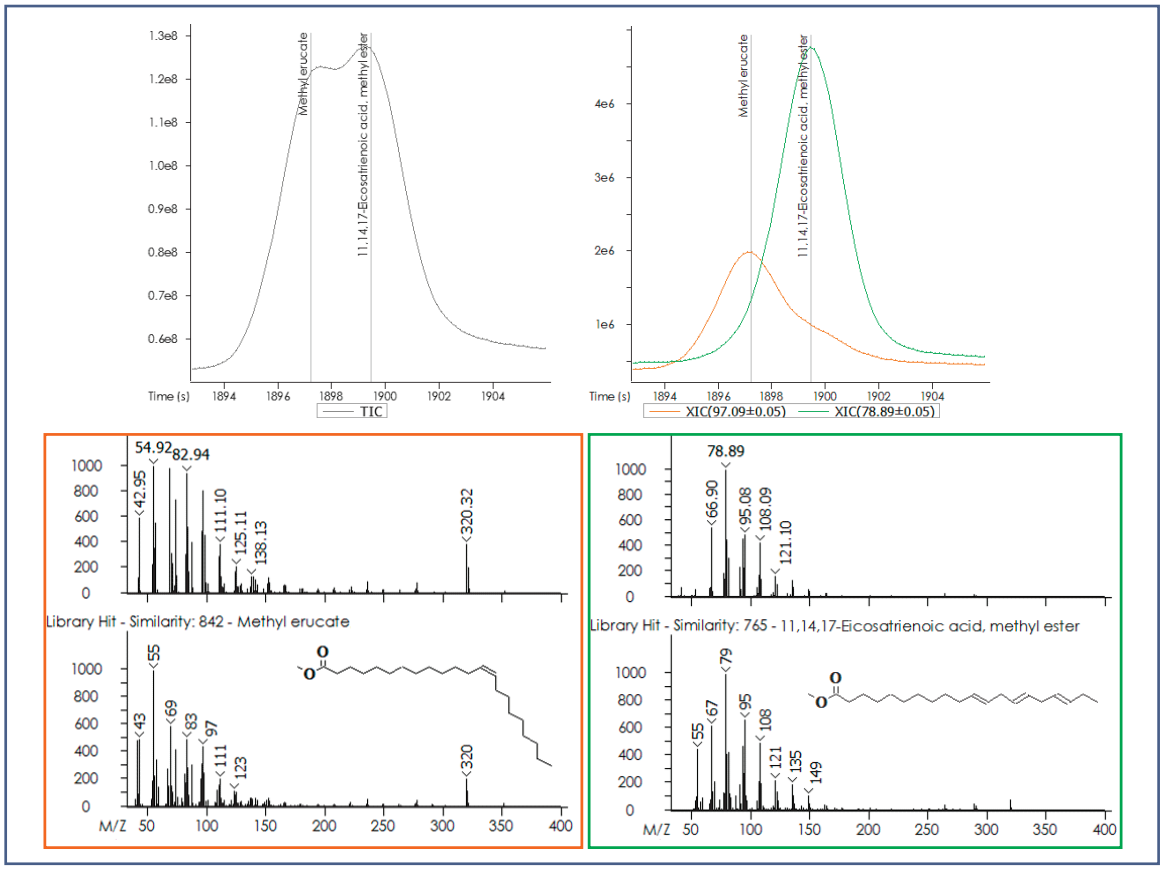

Figure 2. Deconvolution provides additional information when mass spectral differences are present between the coeluting analytes. Here, two target fatty acids chromatographically overlap, but are mathematically separated from each other with deconvolution. The individual chromatographic profiles can 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.

A variety of butter, margarine, and shortening samples were also screened and compared to the reference standard. TIC chromatograms for each are shown in Figure 3. General characterization information on the fat profile can be observed, and the ChromaTOF® brand software also contains analysis tools to rapidly compare analytes in the samples to the known standards based on spectral similarity and retention time similarity. This "Reference Feature" is essentially a one-point calibration that provides quantification information, as well as when the concentration of the analyte in the reference is known. An example of the type of screening information that can be achieved is shown in Figure 3. A zoom-in view of the *cis* and *trans* isomers of methyl octadecenoate, previously shown in Figure 1, is highlighted. All of the samples contain the *cis* version of this analyte, and one of the margarines (blue trace) contains the *trans* version at a large concentration as well. The *trans* peak is likely a series of coeluting isomers. This margarine was the only sample that had *trans* fats listed on the nutrition label.

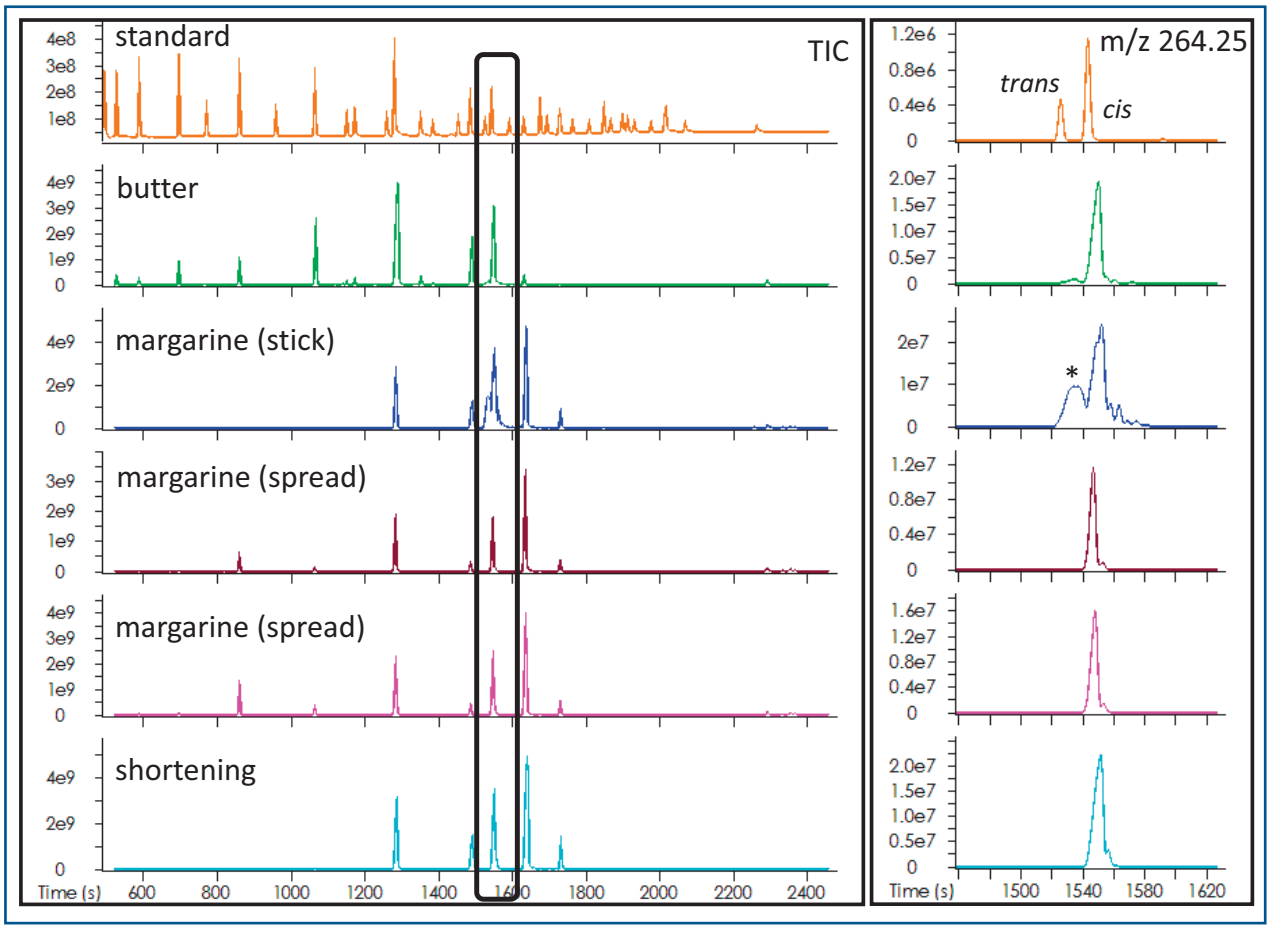

Figure 3. TIC chromatograms for the standard and each of the screened samples. Butter, stick margarine, two margarine spreads, and a shortening sample were all analyzed. General fat profile information is provided with this screen, as well as information on specific analytes. The cis and trans fatty acids shown in Figure 1 are highlighted here with XIC 264.25 shown. The cis version is observed in all samples, while the trans version of observed at high levels only in the sample that listed trans fats on the label (indicated with an asterisk).

Another 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 4. In each case, these analytes are likely to also be observed with FID, but need MS detection for identification. With MS, the observed spectra were searched against library databases to identify other important analytes that may have been missed. In Figure 4A, 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. In Figure 4B, benzaldehyde is observed. Benzaldehyde is known to be present in butter and may contribute to the taste and aroma of the product, but would not need to be included in the fat content on the label.

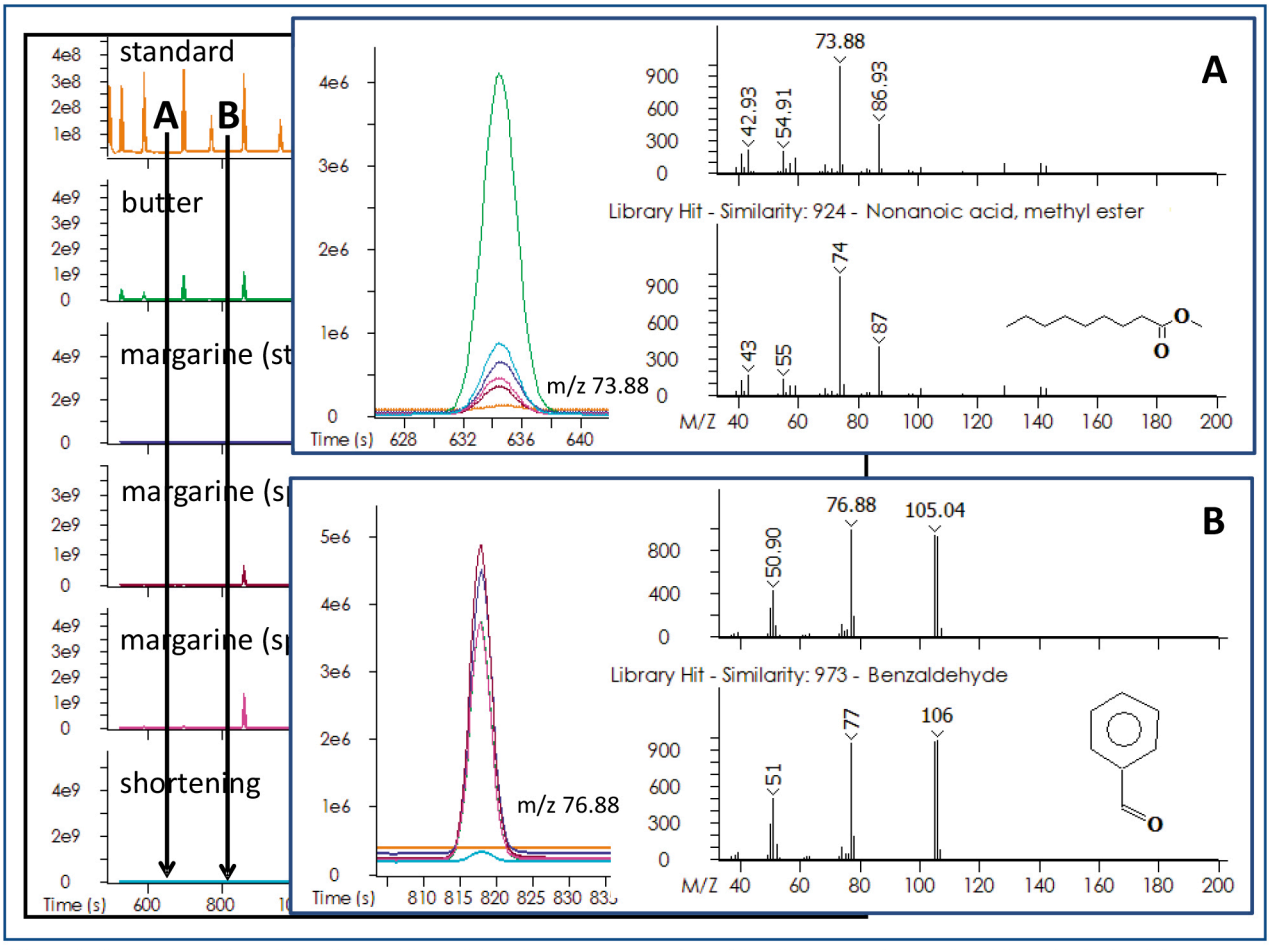

Figure 4. Nonanoic acid, methyl ester, and benzaldehyde are non-target analytes that were observed and tentatively identified through spectral matching to library databases. These analytes were observed in all of the samples, but were not present in the standard. With FID, it would be difficult to know their identity and whether they needed to be included when determining the fat content.

4. 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 switching to 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. The *Pegasus* BT gives you the opportunity to see more in your standard analysis.