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Quantification of 3-Monochloropropane-1,2-diol Esters in Edible Oils by Large-Volume Injection Coupled to Comprehensive Gas Chromatography–Time-of-Flight Mass Spectrometry

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3-Monochloropropane-1,2-diol (3-MCPD) esters are a group of process contaminants formed during the refining of edible oils and fats. A method for the determination of 3-MCPD esters in such oils and fats based on large-volume injection–comprehensive gas chromatography–time-of-flight mass spectrometry (LVI–GC×GC–ToF MS) is presented. The simplified method for sample preparation consists of alkaline hydrolysis followed by extraction of 3-MCPD from the lipid matrix and derivatization using phenylboronic acid. The limit of detection was 0.8 μg kg^{-1}, reported as free 3-MCPD. The repeatability was better than 2.7% relative standard deviation for levels around 0.5 μg g^{-1} 3-MCPD. The GC×GC–ToF MS system was shown to be stable over 100 analyses.

3-Monochloropropane-1,2-diol (3-MCPD) is carcinogenic, highly suspected to be genotoxic in humans, has male antifertility effects, and is a chemical byproduct that may be formed in foods. It is primarily created in foods by protein hydrolysis through adding hydrochloric acid to speed up the reaction of (soy) protein with lipids at high temperatures. In another method, 3-MCPD can also occur in foods that have been in contact with materials containing epichlorohydrin-based wet-strength resins—used in the production of some tea bags and sausage casings. It has been found in some East Asian and Southeast Asian sauces, such as oyster, hoisin, and soy sauces. The use of hydrochloric acid rather than traditional slow fermentation is a far cheaper and faster method but unavoidably creates carcinogens.

In 2006 Zelinková et al.[1] reported the detection of 3-MCPD fatty acid esters (3-MCPD esters) in edible oils. In native or unrefined fats and oils, no or only traces of 3-MCPD esters were detectable,[2,3] but in nearly all refined fats and oils, concentrations of 3-MCPD esters in the range of 0.2–20 mg kg^{-1} are present. There are several methods available for the determination of 3-MCPD esters, from which gas chromatography coupled to mass spectrometry (GC–MS) is the most common technique.[4] Key challenges when using chromatographic separation for the analysis of 3-MCPD esters are the coelution of compounds of interest with large amounts of matrix constituents, the sensitivity, and system stability.

The current methods for 3-MCPD ester analysis in edible oils and fats actually measure the total 3-MCPD content of the oil or fat after hydrolysis. The procedures consist of a number of subsequent steps starting with hydrolysis, the removal of the fatty acids (as their methyl esters), extraction of the free 3-MCPD with salting out, derivatization with phenylboronic acid, preconcentration by solvent evaporation, and finally GC–MS analysis.[5] Deuterium-labeled [D_5]3-MCPD or esters thereof are used as internal standards. Potential problems in the procedure are 1) degradation of the 3-MCPDs during (alkaline) hydrolysis resulting in higher detection limits, 2) formation of additional 3-MCPDs is possible if chloride salts are used in the salting out extraction steps, and 3) the stability of the mass spectrometer owing to strong source contamination. Limits of detection (LODs) are in the range of 0.5 ppm 3-MCPD ester.

Several studies have been published in which large-volume injection (LVI) methods were used for the GC determination of trace pollutants.[6] The LVI technique enables significant improvement of the sensitivity of the analytical methods. Rather than using splitless injections of 1–2 μL, with LVI it is possible to inject sample volumes of over 100 μL. Another reason to use LVI can be to simplify sample preparation, for example, by taking out concentration steps such as solvent evaporation or salting out.

About a decade ago, a new chromatographic technique for the characterization of complex samples became commercially available: comprehensive two-dimensional gas chromatography (GC×GC), first reported by Phillips et al.[7] GC×GC has a much increased peak capacity and offers significantly improved detection limits through chromatographic optimization.[8,9] Owing to the high peak capacity and the numerous compounds that are resolved in a GC×GC separation, the use of a mass spectrometer is highly desirable for identification and confirmation purposes. Dallüge et al.[10] reported that only MS instruments that can acquire a minimum of 50 full spectra per second allow reliable identification, and subsequent quantification, of the classical narrow peaks in the two-dimensional chromatogram. At present, time-of-flight mass spectrometry (ToF MS) is the method of choice because it provides full mass range spectra at high data acquisition rates.

Herein, a feasibility study is presented that focuses on the use of LVI coupled to GC×GC–ToF MS for efficient, more relia-

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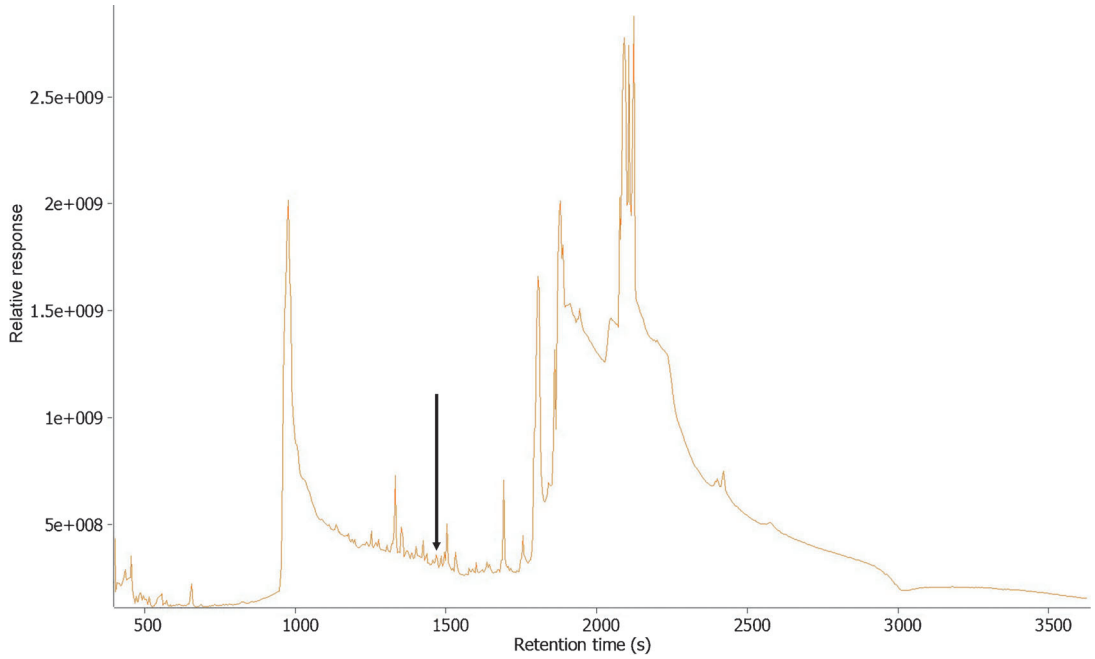

Figure 1. TIC chromatogram of 25 μL palm-oil extract by LVI–GC–ToF MS. The 3-MCPD derivative shows strong coelution with matrix compounds and is not visible in the chromatogram.

ble, and more sensitive 3-MCPD ester analysis in edible oils and fats.

The analytical method as described in the literature[5] is a single-dimension GC procedure. To be able to compare this procedure with that of the comprehensive GC approach, first a run was performed using GC–ToF MS. Figure 1 shows the total-ion-current (TIC) chromatogram of a 25-μL LVI–GC–ToF MS analysis of a palm-oil extract. The arrow in Figure 1 points to the retention time at which the 3-MCPD derivative elutes. However, it immediately becomes clear that the 3-MCPD derivative coelutes with a lot of matrix. As expected the standard 3-MCPD derivative and the labeled [D_5]3-MCPD derivative coelute. In the next set of experiments the analysis was repeated using LVI–GC×GC–ToF MS. Figure 2 shows the TIC contour

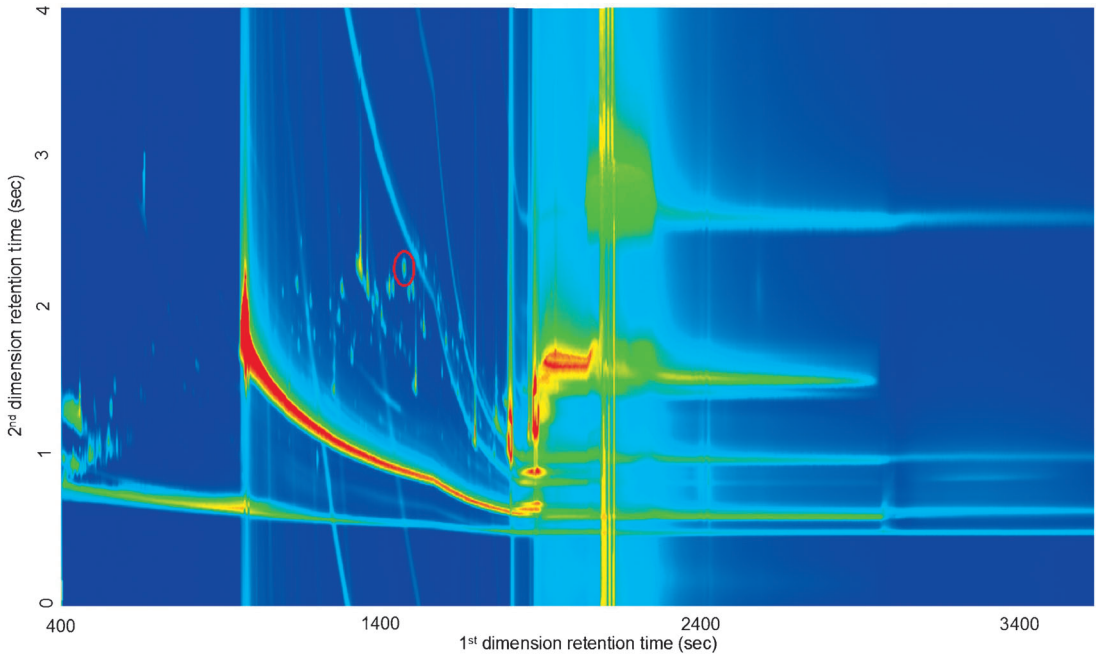

Figure 2. TIC contour plot of 25 μL palm-oil extract by GC×GC–ToF MS. The 3-MCPD derivative (inside red circle) is very well separated from the matrix compounds.

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plot of the LVI–GC×GC–ToF MS analysis and the peak highlighted in the red circle is that of the 3-MCPD derivative.

Here it must be mentioned that despite the chirality of 3-MCPD, only one peak is seen. The selected columns for both the first- and second-dimension separation do not provide chiral separation. The peak for 3-MCPD ester contributes the total amount of all stereoisomers of 3-MCPD. By comparing the GC–ToF MS and GC×GC–ToF MS chromatograms, one can immediately see that the high baseline at which the 3-MCPD derivative elutes originates from tailing of a major, unspecified matrix compound. The peak at about 1000 seconds that is strongly tailing is from the derivatization agent, which was used in excess. The signal at about 2000 seconds could not be identified owing to the saturation of the mass spectrum.

For quantification the extract is spiked with a known amount of $[D_5]$3-MCPD, thus allowing quantification by stable isotope dilution. However, a drawback of this type of quantification is that the 3-MCPD and $[D_5]$3-MCPD derivatives coelute in the first and even almost in the second dimension of the GC×GC separation (see Figure 3; for an explanation of the slightly shorter retention time of the $[D_5]$ derivative, see Žáçek et al.[11]). The resulting mixed spectrum makes proper identification, at least of the 3-MCPD derivative, impossible. However, a powerful advantage of using ToF MS is the ability to perform deconvolution of the mass spectra. Despite the (almost) coelution of the 3-MCPD and the $[D_5]$MCPD derivatives, pure spectra of the analytes can be reconstructed (see Figure 4), now making clear identification of both compounds possible.

Using this strategy, three different palm oil samples were analyzed (for results see Table 1). The amounts of 3-MCPD are calculated using Equation (1):

Table 1. Quantitative analysis of 3-MCPD.

Sample	Weight [mg]	Area $[D_5]$3-MCPD	Area 3-MCPD	Amount 3-MCPD [$\mu g\,g^{-1}$][a]
A	100.2	13121818	1979261	0.15
B	102.4	18810221	9301420	0.48
C	101.3	16923181	5706487	0.33
B[b]	104.4	103775824	90231127	0.83

[a] Reported as free 3-MCPD. [b] NaCl used in the sample preparation.

$$\text{Amount3}-\text{MCPD} = \frac{\text{peakarea3}-\text{MCPD}}{\text{peakarea}[D_5]3-\text{MPCD}} \times \frac{\text{amount}[D_5]3-\text{MCPD}\ (\mu g)}{\text{sampleweight}\ (g)} \quad (1)$$

Additionally, one of the samples, the sample coded B, was also prepared using NaCl during the sample preparation. On comparing the data from the two techniques it could be concluded, based on the peak area of $[D_5]$3-MCPD, that the extraction efficiency is about five times better when using NaCl. However, the reported amount of 3-MCPD is approximately double, most likely because of formation of 3-MCPD esters during sample preparation through the influence of chloride ions. Based on these results, it can be concluded that it is better not to use NaCl during sample preparation from the perspective of method precision, even though this results in a loss of method sensitivity. Fortunately, this loss in sensitivity can be easily overcome if using LVI. In this method, 25 μL of extract is injected compared to 1 μL in the standard method.

To get an impression of the LOD and the limit of quantification (LOQ), the extract of palm oil sample 123 was injected ten times. The analytical data are shown in Table 2. This resulted in an average reported amount of 0.47 $\mu g\,g^{-1}$ of 3-MCPD with an average signal-to-noise ratio (*S/N*) of 1767. Although it is not

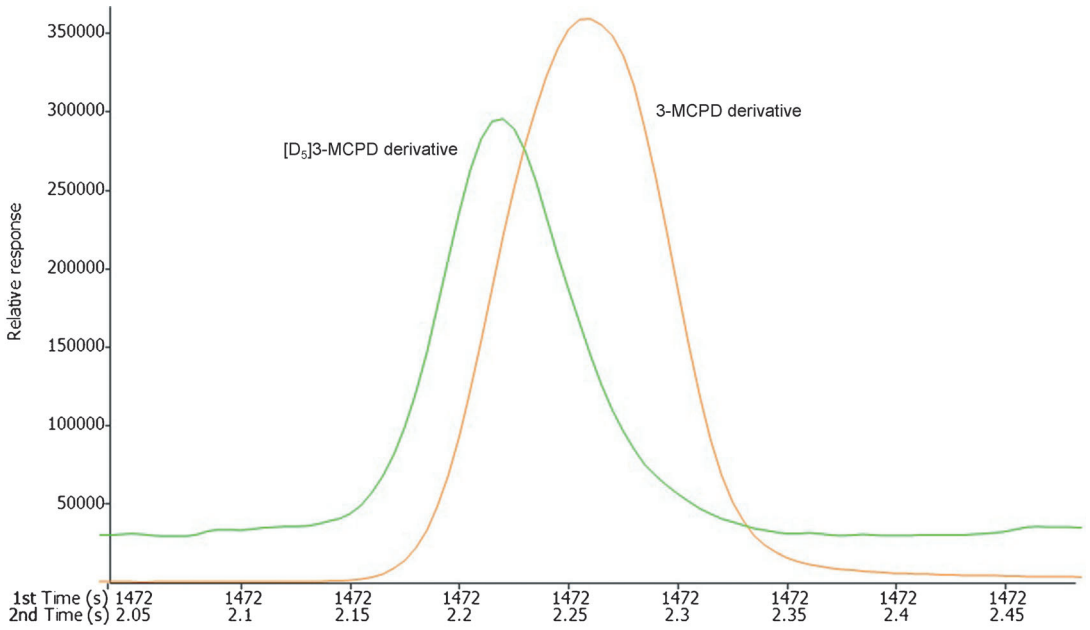

Figure 3. Zoomed extracted ion current of base slice of the 3-MCPD derivative (*m/z* 147, orange), coeluting with the $[D_5]$3-MCPD derivative (*m/z* 150, green).

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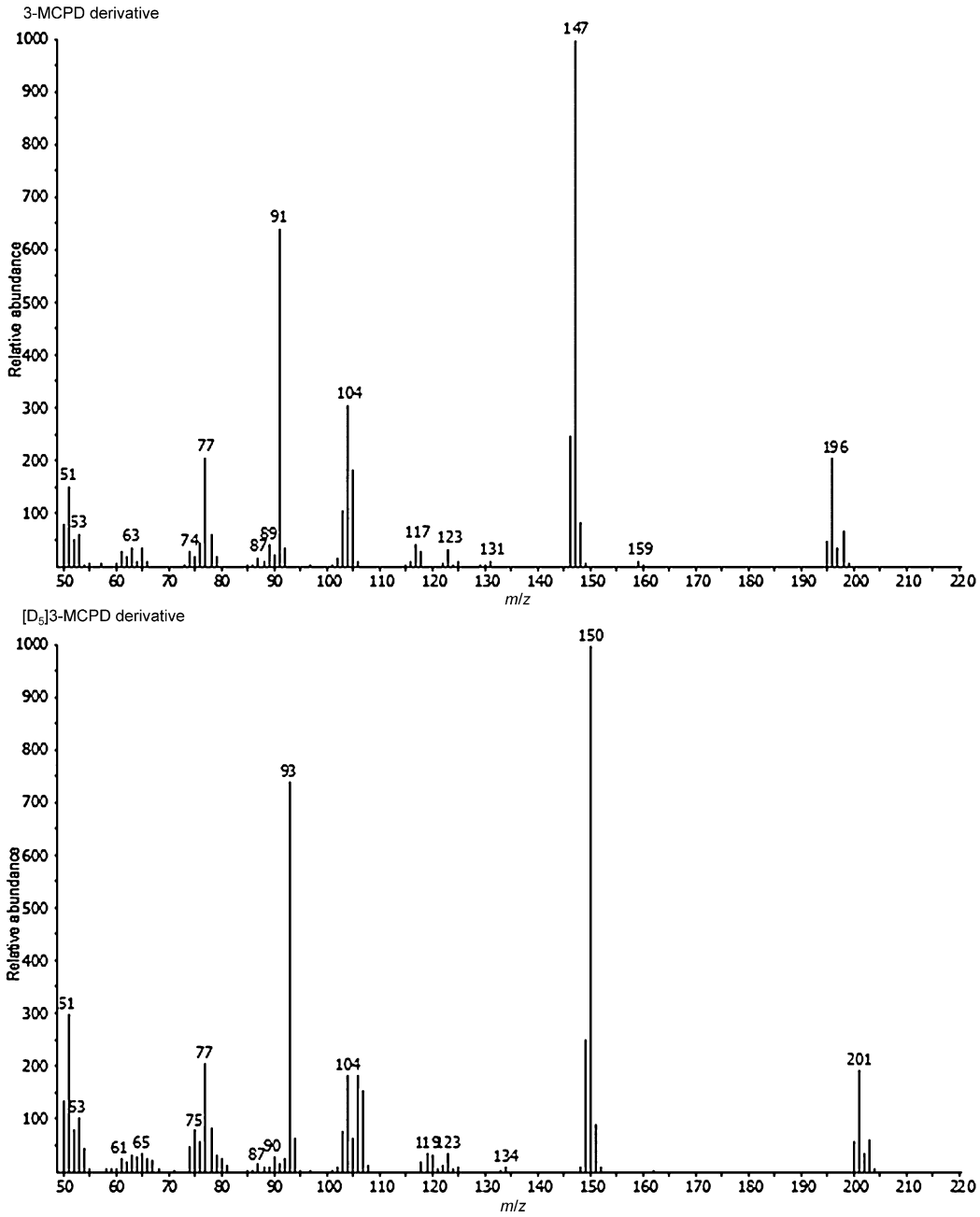

Figure 4. Deconvoluted peak true spectra of the 3-MCPD derivative (top) and [D_5]3-MCPD derivative (bottom).

an exact calculation, it was estimated that the *S/N* behaves linearly with the amount of 3-MCPD. Based on this estimation the LOD = (3/1767) × 0.47 = 0.00080 μg g^{-1} and the LOQ = (10/1767) × 0.47 = 0.00267 μg g^{-1}. The % relative standard deviation (RSD) for the calculated amount of 3-MCPD was found to be 2.7%.

System stability is another topic of interest when using the modified method. It is known from the field that this is often a problem and that the ion source of, for example, quadrupole MS instruments requires frequent maintenance and cleaning, usually after only some ten analyses. To test the Pegasus system stability using the demonstrated method/instrumentation, a total of 100 analyses were performed. After 100 runs the following data were obtained:

- Weight (g): 0.1024
- Area [D_5]3-MCPD: 17579599
- Area 3-MCPD: 8551150
- Amount 3-MCPD (μg g^{-1}): 0.48
- *S/N* 3-MCPD: 1798

From these results it can be concluded that after 100 analyses, the system was still performing like in the first run.

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Table 2. Analytical data for 3-MCPD analysis.

Sample[a]	Area [D_5]3-MCPD	Area 3-MCPD	Amount 3-MCPD [$\mu g\,g^{-1}$][b]	*S/N* 3-MCPD
B:1	18810221	9301420	0.48	1548
B:2	20186242	9268336	0.45	1634
B:3	20314729	9837198	0.47	1661
B:4	19742597	9622891	0.48	1940
B:5	19607596	9287701	0.46	1866
B:6	19160417	9154108	0.47	1589
B:7	18324274	8998403	0.48	1613
B:8	18015697	8450171	0.46	1615
B:9	21695114	10796004	0.49	2077
B:10	19660950	9816324	0.49	2125
		Average	0.47	1767
		SD	0.01	
		%RSD	2.74	
		LOD	0.00080	
		LOQ	0.00267	

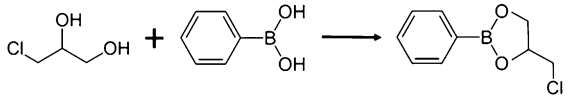
[a] Weight was 102.4 mg for each sample. [b] Reported as free 3-MCPD.

Experimental Section

Sample preparation

The homogenized sample (ca. 100 mg) was weighed into a screw-capped tube, and *tert*-butyl methyl ether/ethyl acetate (8:2 v/v, 500 μL), internal standard solution (250 μL, free [D_5]3-MCPD, 20 $\mu g\,mL^{-1}$ in *tert*-butyl methyl ether), and 0.5 M $NaOCH_3$ solution (1 mL) were added. After 10 min of gentle shaking, isohexane (3 mL), glacial acetic acid (100 μL), and NaCl solution (3 mL, 200 $g\,L^{-1}$) were added (the addition of NaCl solution was omitted for the LVI analysis). After 1 min of agitation, the upper layer was removed with a pipette and discarded. The aqueous layer was extracted with a new portion of isohexane; the upper layer was again removed and discarded.

For derivatisation (see Scheme 1), derivatisation reagent (250 μL; 5 g phenylboronic acid dissolved in 19 mL acetone and 1 mL water) was added to the aqueous phase.[2] Next the tube was



Scheme 1. Derivatization reaction of 3-MCPD.

heated to 80 °C for 20 min under gentle shaking. After cooling to room temperature, the phenylboronate derivative of 3-MCPD was extracted by shaking with *n*-hexane (3 mL). After concentration of the extract by solvent evaporation (not needed in the case of LVI analysis), the *n*-hexane layer was separated and injected into the GC×GC–ToF MS system.

System parameters

The system used for sample analysis was a Pegasus 4D GC×GC–ToF MS instrument (LECO, St. Joseph, MI, USA), equipped with a quad-jet thermal modulator (LECO), a second-dimension oven (LECO), and a CIS4 programmed-temperature vaporizing injector (Gerstel, Mülheim a/d Ruhr, Germany) containing a baffled glass liner. The Pegasus 4D instrument was controlled by ChromaTOF (LECO) data acquisition and processing software. The CIS4 injector was controlled by Maestro (Gerstel) software. The first-dimension column was an Rxi-1 SIL ms column of size 30 m×0.25 mm with a film thickness of 0.25 μm (Restek, Bellefonte, PA, USA) and the second-dimension column was an Rxi-17 SIL ms 1 m×0.18 mm column with a film thickness of 0.18 μm (Restek). Sample volumes of 25 μL were injected at a temperature of 40 °C. After solvent evaporation, the injector was switched to splitless and heated to 300 °C at 5 $°C\,s^{-1}$. The first-dimension GC oven started at a temperature of 40 °C, at which it was held for 1 min. Following this, it was heated to 190 °C at 6 $°C\,min^{-1}$ and then to 280 °C (30 min hold) at 20 $°C\,min^{-1}$. The second-dimension oven was programmed following the first-dimension oven with an offset of +5 °C. The modulation time was set to 4 s. Data were acquired in the range of 50–500 *m/z* using an acquisition rate of 200 $spectra\,s^{-1}$. Helium was used as the carrier gas at a flow rate of 1 $mL\,min^{-1}$.

Keywords: esters · fats and oils · gas chromatography · mass spectrometry · sample preparation

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