Analysis of 3-MCPD Esters in Edible Oils Using Large Volume Injection Coupled to Comprehensive Gas Chromatography – Time-of-Flight Mass Spectrometry

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Key Words: GCxGC-TOFMS

1. Introduction

In 2006 Zelinková et al.[1] reported the detection of 3-chloropropane-1,2-diol fatty acid esters (3-MCPD-ester) 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 to 20 mg/kg are present. There are several methods available for the determination of 3-MCPD-esters, from which gas chromatography coupled to mass spectrometry (GCMS) is the most common technique[4]. However, one of the greatest challenges when using chromatographic separation for the analysis of 3-MCPD-ester is the coelution of compounds of interest with large amounts of matrix constituents, 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 the hydrolysis, removal of the fatty acids (as their FAMEs), extraction of the free 3-MCPD with salting out, derivatization with phenylboronic acid, pre-concentration by solvent evaporation and finally GC–MS analysis[5]. Deuterium labelled 3-MCPD-d5 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) stability of the mass spectrometer due to strong source contamination. Limits-of-Detection (LOD) are in the range of 0.5 ppm.

Several studies have been reported in which large-volume injection (LVI) methods were used for the GC determination of trace pollutants[6]. The LVI technique enables significant improvement of sensitivity of the analytical methods. Rather than using splitless injections of 1 to 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, e.g., 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 offering significantly improved detection limits through chromatographic optimisation[8]. Due 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.[9], reported that only MS instruments that can acquire a minimum of fifty full spectra per second allow reliable identification, and subsequent quantification, of the classical narrow peaks in the two-dimensional chromatogram. At present, the time-of-flight mass spectrometer (TOFMS) is the instrument of choice to achieve this since it provides full mass range spectra along with high data acquisition rates.

In this work a feasibility study is presented that focuses on the use of LVI coupled to GC×GC–TOFMS for efficient, more reliable and more sensitive 3-MCPD ester analysis in edible oils and fats.

2. Experimental Conditions

Sample Preparation

100 mg oil in a closed sample tube
- 500 µl MTBE/Acetone (8:2 v/v)
- 1 ml 0.5 M $NaOCH_3$
- 5 µl 3-MCPD-d5 solution (20 µg/ml)
- Shake gently; 10 min, 30°C

Free 3-MCPD
- 3 ml iso-hexane
- 100 µl glacial acetic acid
- 3 ml water (in original method 200 g/l NaCl)
- Agitate 1 min
- Waste iso-hexane layer
- 3 ml iso-hexane
- Agitate 1 min
- Waste iso-hexane layer
- 250 µl phenylboronic acid solution (250 µg/ml in water/acetone (19:1)
- Shake gently; 20 min, 80°C

3-MCPD derivate
- 3 ml hexane
- Agitate 1 min
- Inject 25 µl from hexane layer by LVI (in original method concentrate the hexane and inject 1 µl splitless)

System Parameters

The system used for sample analysis was a Pegasus® 4D GC×GC–TOFMS (LECO, St. Joseph, MI, USA), equipped with a quad-jet thermal modulator (LECO), a second-dimension oven (LECO) and an OPTIC 3 multi purpose programmed temperature vaporizing (PTV) injector (ATAS GL, Eindhoven, the Netherlands) containing a sintered glass liner. The Pegasus 4D instrument was controlled by ChromaTOF (LECO) data acquisition and processing software. The OPTIC 3 injector was controlled by Evolution (ATAS GL) software. The first dimension column was a VF-1ms column of 30 m x 0.25 mm with a film thickness of

0.25 µm (Varian, Middelburg, the Netherlands) and the second dimension column was a VF-17ms 1 m x 0.1 mm column with a film thickness of 0.2 µm (Varian). Sample volumes of 25 µl were injected at a temperature of 40°C. After solvent evaporation (20 s, 50 ml/minute vent flow), the injector was switched to splitless and heated to 300°C at 5°C/second. The 1st dimension GC oven started at a temperature of 40°C where it was held for 1 minute. Following this, it was heated to 190°C at 6°C/minute and then to 280°C (30 minute hold) at 20°C/minute. The 2nd dimension oven was programmed following the 1st dimension oven with an offset of +5°C. The modulation time was set to 4 seconds. Data was acquired in the range of 50 to 500 m/z using an acquisition rate of 200 spectra/second. Helium was used as a carrier gas at a flow rate of 1 ml/minute.

3. Results and Discussion

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–TOFMS. For this, a 25-µl injection of a palm oil sample extract was analyzed by LVI–GC–TOFMS. From this analysis it became clear that the 3-MCPD derivate coelutes with a lot of matrix. Also 3-MCPD derivate and 3-MCPD-d_5 derivate strongly coelute. In the next set of experiments the analysis was repeated using LVI–GC×GC–TOFMS. Figure 1 shows the TIC contour plot of the LVI–GC×GC–TOFMS analysis and the peak highlighted in the red circle (first dimension retention time 1448 seconds; 2nd dimension time 2.3 seconds) is that of the 3-MCPD derivate. From the same contour plot it is clearly visible that a lot of matrix elutes at the same 1st dimension retention time as 3-MCPD derivate.

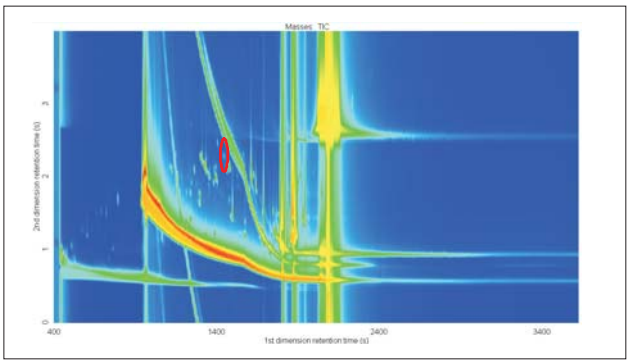

Figure 1: TIC contour plot of 25 µl palm oil extract by GC×GC–TOFMS. The 3-MCPD derivate (inside red circle) is very well separated from the matrix compounds.

For quantification, the extract is spiked with a known amount of 3-MCPD-d5, in that way allowing quantification by stable isotope dilution. However, a drawback of this type of quantification is that the 3-MCPD and 3-MCPD-d5 derivates coelute in the 1st and almost even the 2nd dimension of the GC×GC separation (see Figure 2). The resulting mixed spectrum makes proper identification of the 3-MCPD-derivate impossible. However, a powerful advantage of using TOFMS is the ability to perform True Signal Deconvolution® (TSD®) of the mass spectra. Despite the (almost) coelution of the 3-MCPD and the MCPD-d5 derivates, pure spectra of the analytes can be reconstructed (see Figure 3), now making clear qualification of both compounds possible. Using this strategy, three different palm oil samples were analyzed (for results see Table 1).

Table 1: Qualitative analysis of 3-MCPD

Sample	Weight (g)	Area 3-MCPD-d_5	Area 3-MCPD	Amount 3-MCPD (µg/g)*
064	0.1002	13121818	1979261	0.1505
123	0.1024	18810221	9301420	0.4829
422	0.1013	16923181	5706487	0.3329
123**	0.1044	103775824	90231127	0.8328

** Reported as free 3-MCPD*
*** NaCl used in the sample preparation*

Additionally, sample 123 was also prepared using NaCl during the samples preparation. When comparing the data from the two techniques it could be concluded, based on the peak area of 3-MCPD-d5, that the extraction efficiency is about five times better when using NaCl. However, the reported amount of 3-MCPD is approximately double, due to the formation of the 3-MCPD-ester during sample preparation by the influence of chloride ions. Based on these results, it can be concluded that it is better not to use NaCl during the sample preparation from the standpoint of method precision, despite resulting in a loss of method sensitivity. However, this loss in sensitivity can be easily overcome when using LVI. In this method 25 µl of extract is injected against 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 is shown in Table 2. This resulted in an average reported amount of 0.47 µg/g of 3-MCPD with an average signal-to-noise ratio (S/N) of 1767. Although it is not 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 and the LOQ = (10/1767)*0.47 = 0.00267 µg/g. The % RSD for the calculated amount of 3-MCPD was found to be 2.7%.

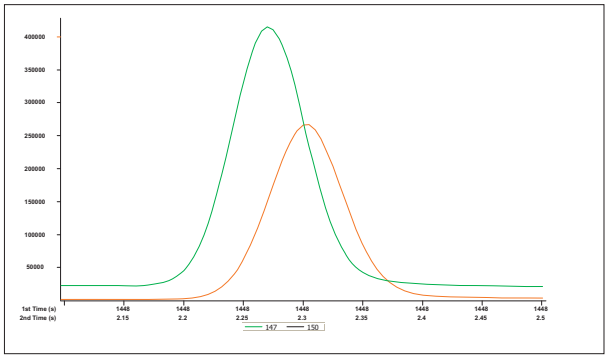

Figure 2: Zoomed extracted-ion-curent of base slice of 3-MCPD derivate (m/z 147, orange), coeluting with 3-MCPD-d5 derivate (m/z 150, green).

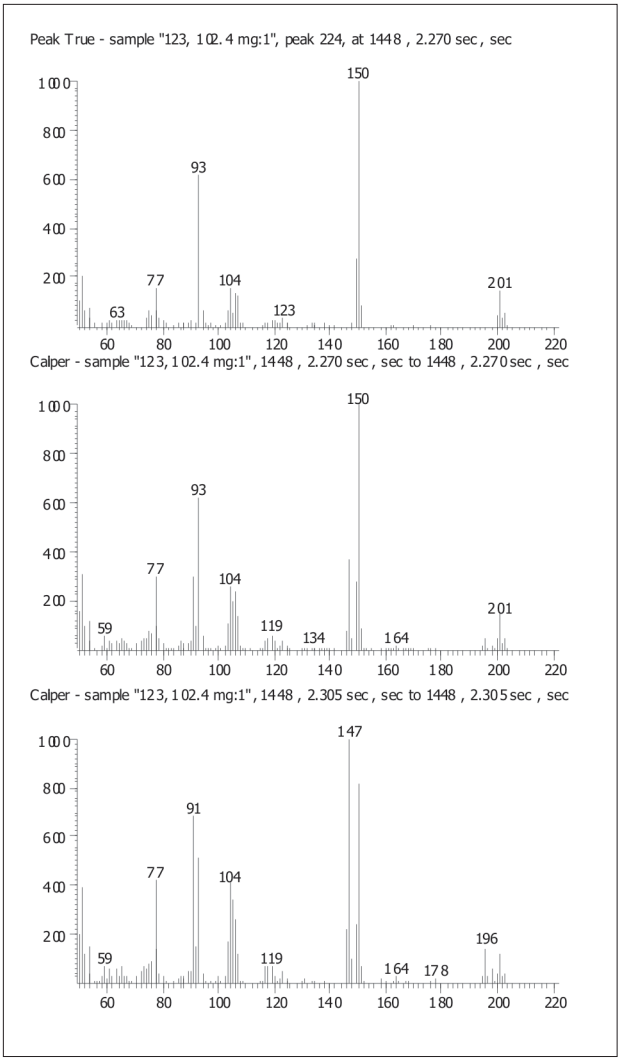

Figure 3: Peak spectra of deconvoluted 3-MCPD-d5 derivate (top), deconvoluted 3-MCPD derivate (middle) and non-deconvoluted mix-spectrum due to coelution of both compounds (bottom).

Table 2: Analytical data for 3-MCPD analysis.

Sample	weight (g)	Area 3-MCPD-d5	Area 3-MCPD	Amount 3-MCPD (µg/g)*	S/N 3-MCPD
123:1	0.1024	18810221	9301420	0.48	1548
123:2	0.1024	20186242	9268336	0.45	1634
123:3	0.1024	20314729	9837198	0.47	1661
123:4	0.1024	19742597	9622891	0.48	1940
123:5	0.1024	19607596	9287701	0.46	1866
123:6	0.1024	19160417	9154108	0.47	1589
123:7	0.1024	18324274	8998403	0.48	1613
123:8	0.1024	18015697	8450171	0.46	1615
123:9	0.1024	21695114	10796004	0.49	2077
123:10	0.1024	19660950	9816324	0.49	2125
			Average	0.47	1767
			SD	0.01	
			% RSD	2.74	
			LOD	0.00080	
			LOQ	0.00267	

** Reported as free 3-MCPD*

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, a quadrupole requires frequent maintenance and cleaning after a maximum of ten analyses. To test the Pegasus® system's 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 3-MCPD-d5: 17579599
- Area 3-MCPD: 8551150
- Amount 3-MCPD (µg/g): 0.48
- S/N 3-MCPD: 1798

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

4. Conclusions

Sample extract aliquots up to 25 µl were injected. As a result of this, the salting out step is less critical: low detection limits were obtained even at low extraction recoveries and thus the side reaction with the chloride can be eliminated. Additionally, the final pre-concentration step could also be eliminated. Together this results in a faster, more economical method, reducing the amount of manual sample handling hence reducing potential error and reducing the cost-per-analysis. A clear advantage of using comprehensive GC×GC is the substantially improved resolution of the GC separation leading to the elimination of interferences, even at very low 3-MCPD levels. Additionally, due to the open source design of the Pegasus TOFMS, the system is extremely stable when considering instrument maintenance. Over 100 samples can be analyzed without any system break down. True Signal Deconvolution greatly supported the pure identification of both 3-MCPD derivate and 3-MCPD-d5 derivate, resulting in more reliable qualification and quantification.

5. References

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