Analytical Methods for the Determination of 3-MCPD Esters in Oils/Fats

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INTRODUCTION

The 3-Monochloropropane-1,2-diol (3-MCPD) occurs in fats and oils in free and esterified forms (with fatty acids); with a major part being bound in the form of diesters. Free 3-MCPD was first identified in acid-hydrolysed vegetable protein and soya sauce (Velisek *et al.*, 1978), and provisional maximum tolerable daily intake (TDI) of 2 µg 3-MCPD kg^{-1} body weight was set by the Joint FAO/WHO Expert Committee on Food Additives. Twenty years later, a group of researchers from the Czech Republic reported on the occurrence of 3-MCPD esters in fried food (Svejkovska *et al.*, 2004) and in vegetable oils (Zelinkova *et al.*, 2006).

Since then, several methods of analysis have been developed by various research organisations. These methods are based on indirect determination of bound 3-MCPD via transesterification in acid (BfR Method 008) or in alkali (BfR Method 009 and 010; DGF, 2009; Weißhaar, 2008). The principle of the indirect method involves the conversion of the esters to free 3-MCPD, and then the 3-MCPD is quantified using Gas Chromatography-Mass Selective Detector (GC-MSD).

Other than that, the direct quantification of 3-MCPD esters has also been developed using Liquid Chromatography-Time-of-Flight/Mass Spectrometry (LC-TOF/MS) (Haines *et al.,* 2011). The direct method involves quantification based on the direct determination of individual esters.

Seefelder *et al.* (2008) developed a rapid method for the determination of the ratio of 3-MCPD monoesters to diesters in fats and oils using GC-MS and isotopically labelled 3-MCPD as internal standard. They demonstrated that 3-MCPD monoesters and diesters have been accepted by intestinal lipase as substrates *in vivo* using simple intestinal model. The paper also reported that 3-MCPD esters in human is unlikely to be completely hydrolysed into 3-MCPD, as triglycerides and phospholipids are hydrolysed in the intestine liberating 2-monoglycerides. From their study, it was found that a maximum of about 15% of the total amount of 3-MCPD bound in esters is present in the monoesterified form. In addition, it was also found that the release of 3-MCPD from 3-MCPD diesters is slower than from mono-esters, therefore suggesting that 3-MCPD esters may contribute only marginally to the overall dietary exposure to 3-MCPD.

Crews (2011) reported the problems which arise with a number and variety of methods as indicated below:

- indirect methods with acid or alkali methanolysis;
- direct methods with and without clean-up;
- direct methods with varied LC-MS technique;
- insufficient agreement between indirect methods;
- insufficient agreement between direct methods; and
- different results with direct *vs.* indirect methods.

These problems are caused by wide differences in analytical approaches; lack of reference compounds; lack of understand-

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ing of indirect method chemistry; large number of analytes for direct methods and problems of matrix effects in direct methods.

ANALYSIS OF 3-MCPD ESTERS – INDIRECT METHOD

Determination of bound 3-MCPD is based on the conversion of individual 3-MCPD esters into a single compound, which is 3-MCPD. *Figure 1* shows the sequence of steps: addition of internal standard to the sample, transesterification (either in acid or alkaline condition), neutralisation and salting out, derivatisation of the extracted 3-MCPD and quantification by GC-MSD.

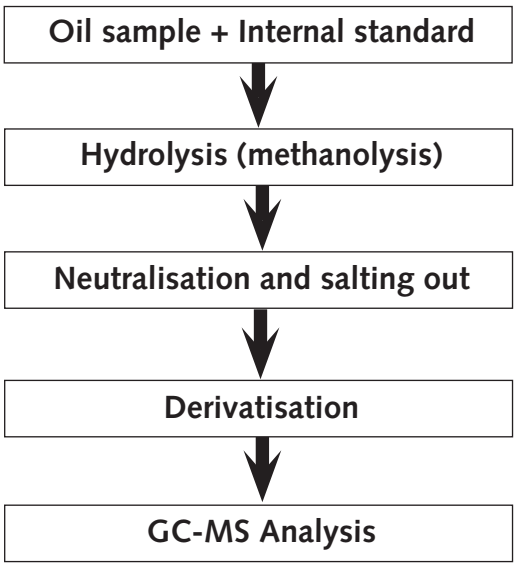

Figure 1. Basic analytical protocol.

Table 1 shows the method of classification of two indirect methods: (a) acidic transesterification and (b) alkaline transesterification. Generally, only two reagents are used for derivatisation, which are phenylboronic acid (PBA) and heptafluorobutyrylimidazole (HFBI). The salting out reagent is either sodium chloride (NaCl) or sodium sulphate (Na_2SO_4).

(a) Acid-based Transesterification

Divinova *et al.* (2004b) reported an improved routine, simple and sensitive method for the determination of free and bound 3-MCPD in different foods using acid-based transesterification method and quantification by GC-MS. Hrncirik *et al.* (2011) reported that the transesterification in acid medium avoids problems with selectivity and has greater robustness compared to the alkaline transesterification method. The Federal German Institute for Risk Assessment (BfR) also developed a method based on the acid transesterification which is the 'BfR Method 008' (BfR Method_82_FC-008-01). The method involves a hydrolysis step which took 16 hr incubation time. From a collaborative study which was conducted in 2009, MPOB has verified this method in Food Safety Laboratory (*Figure 2*) and the percentage recoveries of bound 3-MCPD spiked into blank sample ranged from 90%-110%.

Further work by Ermacora and Hrncirik (2012) resulted in an improved indirect method for the analysis of 3-MCPD esters based on acid transesterification. They carried out a number of methods which involved both indirect and direct determination of the esters. Their study showed that the indirect method is of better sensitivity, although the series of chemical reactions that take place during sample preparation may affect reliability of results. Their study

Figure 2. Gas Chromatography-Mass Selective Detector (GC-MSD) in Food Safety Laboratory, Malaysian Palm Oil Board.

TABLE 1. METHOD CLASSIFICATION FOR INDIRECT METHOD

Transesterification	Acidic		Alkalic		
Derivatisation	**PBA**	**HFBI**	**PBA**		**HFBI**
Salting out	NaCl/ Na_2SO_4	-	NaCl	Na_2SO_4	Na_2SO_4
Method	BfR 82_FC-008	-	DGF C-III 18 (09)	BfR 82_FC-009	BfR 82_FC-010

also showed that the interference of chloride ions can be eliminated by a single extraction step of the sample before the analysis. In addition, transesterification time can also be reduced from 16 to 4 hr without any significant reduction of the accuracy and repeatability.

Method Performance - Acidic Transesterification

MPOB has verified the 'BfR Method 008' through a collaborative study which was conducted in 2009. Blank oil (crude palm oil) was spiked with different levels of 3-MCPD esters and the percentage recoveries were found to range from 97%-108%, with relative standard deviation (RSD) between 0.02%-0.12% (*Table 2*). Quantitative analysis was carried out by monitoring characteristic ions at m/z 91, 147 and 196, respectively, for derivatised 3-MCPD; while for d_5-3-MCPD, characteristic ions were at m/z 93, 150 and 201. Qualifier ions were m/z 147 towards m/z 150 (*Figure 3*). Calibration curve was constructed and linearity was verified ($r^2 \geq 0.999$) within the concentration range (*Figure 4*).

Alkaline-based Transesterification

Weißhaar in 2008 reported the alkaline-based transesterification method based on the indirect determination of total 3-MCPD in edible oils/fats. The 3-MCPD was released from 3-MCPD esters by transesterification with sodium methoxide in methanol. This method is adopted by the German Society for Fat Science (DGF) and is known as 'Method DGF C-III 18 (009)'. In this method, other compounds forming 3-MCPD under the conditions of analysis are also detected, and the most important 3-MCPD esters forming compound is glycidol (oxirane-2-methanol). This means that the method is not applicable for oils with high glycidol compounds. The method is also used as an indirect method to measure glycidol compounds by subtracting the 3-MCPD esters from the total result.

In addition to 'BfR Method 009', there is also 'BfR Method 010'. The principle of 'BfR Method 009' is: (a) alkaline hydrolysis with sodium methylate solution, (b) neutralisation and salting out using ammonium sulphate and sulphuric acid, (c) derivatisation using PBA and (d) quantification by GC-MS. The only difference between the 'BfR Method 009' and the 'BfR Method 010' is that the latter uses heptafluorobutyric anhydride (HFBA) as a derivatising reagent. Latest report by Hrncirik *et al.* (2011) showed that the method based on alkaline transesterification is particularly prone to variations in conditions, which is transesterification time and pH value of different salts.

TABLE 2. PERCENT RECOVERY FOR FOUR 3-MCPD ESTER CONCENTRATION LEVELS (BLANK SAMPLE SPIKED WITH 3-MCPD)

Concentration (mg kg^{-1}), $n = 6$	Recovery (%)	RSD (%)
0.25	97.6	0.02
1.0	103.8	0.03
4.0	107.7	0.12
6.0	107.9	0.11

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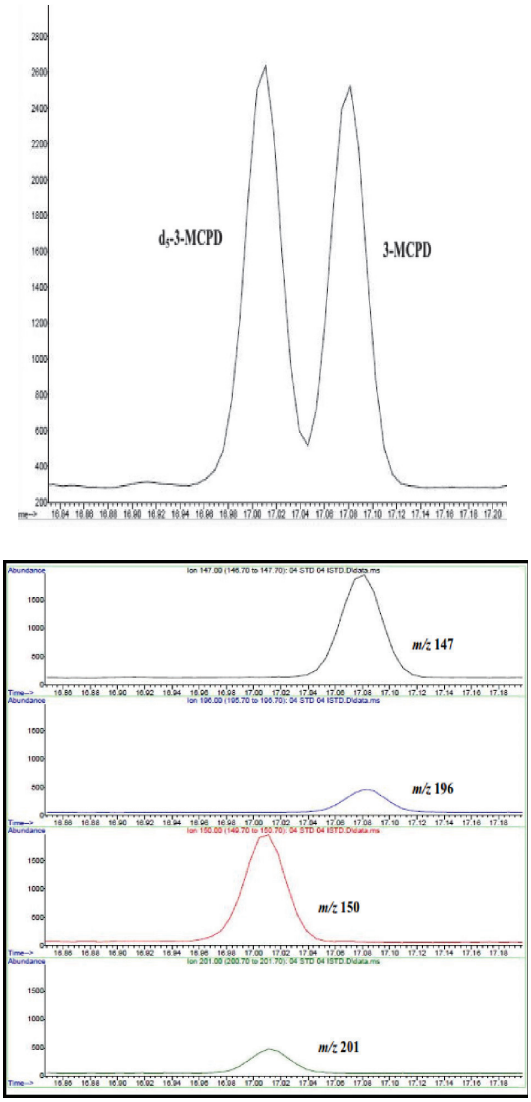

Figure 3. TIC and m/z for 3-MCPD and d_5-3-MCPD in the sample.

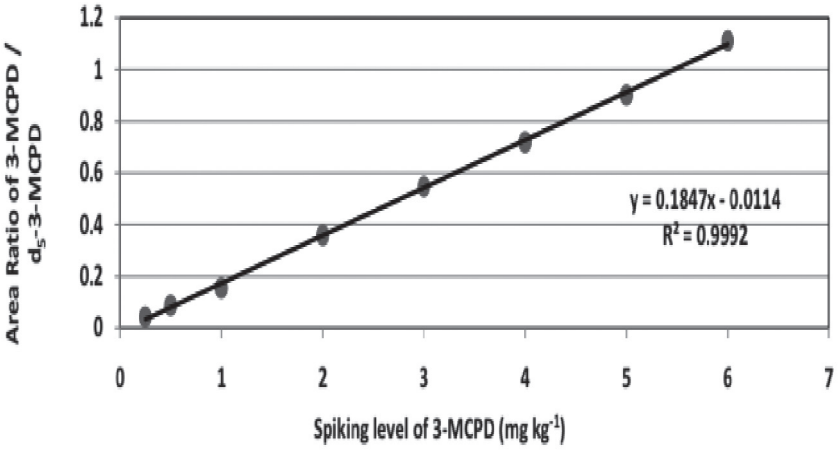

Figure 4. Calibration curve of the 3-MCPD spiked in a blank sample.

ANALYSIS OF 3-MCPD ESTERS – DIRECT METHOD

The first direct method was presented in 2010 by Collison and Haines (ADM Research), using Liquid Chromatography-Time-of-Flight/Mass Spectrometry (LC-TOF/MS) as a quantification tool. The method was developed to provide direct analysis of 3-MCPD monoesters and di-esters without chemical modifications which can give incorrect results and also provides very specific correct identification of the analytes. The principle of the method is based on the direct 'dilute and shoot' method (Pinkston and Stoffolano, 2011). Standards are required for the monoesters and di-esters corresponding to the known fatty acid composition of the vegetable oils.

However, the method requires frequent instrument disassembly and cleaning. There are also other groups working on direct methods such as Granvogl *et al.* (TU München); Mathieu (Nestlé); and S. Macmahon *et al.* (FDA). They are currently developing a direct method for determination of 3-MCPD esters without issues related to instrument fouling. *Table 3* shows a list of direct methods reported by various organisations/ research institutes.

Haines *et al.* (2011) reported on the direct determination of 3-MCPD esters in vegetable oils by LC-TOF/MS. They developed this method because the DGF method gave inconsistent results when salting out conditions varied. They compared the results of the LC-TOF/MS method with the DGF method and results showed that the DGF method consistently gave

TABLE 3. DIRECT METHODS

Method of analysis	Reference
LC-TOF/MS	• Collison and Haines (AOCS Meeting, 2010) • Haines *et al.* (2010)
LC-TOF/MS or LC-Q/TOF	• Mathieu (AOCS Meeting, 2011)
LC-MS/MS	• Pinkston and Stoffolano (AOCS Meeting, 2011) • MacMahon *et al.* (AOCS Meeting, 2011)
LC-MS/MS with Stable Isotope Dilution analysis	• Granvogl and Schieberle (AOCS Meeting, 2011)

TABLE 4. SUMMARY OF METHODS OF ANALYSIS

Indirect methods	Direct methods
• Simple to perform • High uncertainty (results are dependent on sample composition) • Number of methods available • Suitable for routine analyses (total bound 3-MCPD) • Good sensitivity • Trueness is questionable	• Several methods presented (other under development) • Provides a full profile, but is difficult for routine analyses • Substantial challenges (standards, instrumentation, sensitivity)

results that were greater than the LC-TOF/MS method. However, there are a few challenges with the LC-TOF/MS method. The sodium in the mobile phase has detrimental effects on the MS system, and thus requires quick cleaning everyday prior to use. Also, certain parts in the ESI source corrode quickly and require frequent replacement. Furthermore, the need for individual standards is costly and not many laboratories can afford LC-TOF/MS equipment, thus the usage of indirect method may be limited to only a few laboratories. A comparison of the indirect and direct method of analysis is shown in *Table 4.*

CONCLUSION

The indirect methods of analysis avoid the preparation of a series of standards; the methods are also sensitive and easy to perform. Quantification is based only on the total content of the 3-MCPD (free and bound form). The acid hydrolysis method has been found to be more robust, and measures only the 3-MCPD esters, whereas alkaline methods may also form artefacts depending on the chemical being used, resulting in higher values than expected. On the other hand, the direct method offers a full profile quantification of the esters, but it remains difficult for routine analysis.

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