Determination of MCPD and glycidol esters in foodstuff

with the CHRONECT Workstation MCPD and the module ISO 18363-2

Application note 2001

CHRONECT Workstation MCPD – Module ISO 18363-2

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Introduction

3-Monochloropropanediol (3-MCPD), 2-monochloropropanediol (2-MCPD) and their fatty acid esters and glycidyl (GE) fatty acid esters are process-related impurities and can be formed when fatty foods are heated in the presence of salts. The fatty acid-bound esters of 2-MCPD, 3-MCPD, and glycidol are formed mainly during the refining of vegetable oils and edible fats by the intense heating of triglycerides in the presence of chlorine-containing compounds. They are formed mainly during the final refining step, the removal of odorants and aromas (deodorization). All foods produced on the basis of refined vegetable oils and fats, including margarine, bakery products, infant formula, may also be contaminated. Even when flawless raw materials are used, the contaminants may be formed during subsequent food preparation, e.g., intense heating.

3-MCPD and glycidol show considerable toxic effects in animal studies. 2-MCPD is suspected of being carcinogenic and is therefore being monitored further. In the current assessments of the IARC ("International Agency for Research on Cancer"), 3-MCPD is classified as "potentially carcinogenic to humans (Group 2B)" and glycidol as "probably carcinogenic to humans (Group 2A)". Therefore, both compounds (but especially glycidol) in food are subject to the minimization principle (ALARA principle - "As Low As Reasonably Achievable"). The tolerable daily intake (TDI) of 3-MCPD has been set by the European Food Safety Authority (EFSA) at 2 µg per kg body weight. Especially for infants with their low body weight, this limit can be quickly reached when using infant formula. It is therefore not surprising that a health risk is currently seen, especially for younger populations.

Normally, analysis of 2-/3-MCPD and glycidol is performed from fat or oil samples. If the food is a compound, the first step is to isolate the fat from the samples, which can be done by accelerated solvent extraction. To release the analytes, the fat must be transesterified. After conversion to the form required for measurement (derivatization), the analytes are analyzed by gas chromatography-mass spectrometry.

This method, also known as the 3-in-1 method, is based on a slow alkaline release of MCPD and glycidol from the ester derivatives. The procedure takes 16 hours at -22 °C, stopping the cleavage with acidic sodium bromide solution and forming 3-MCPD, 2-MCPD, and 3-MBPD, as in other methods. By GC-MS, 2-MCPD, 3-MCPD and 3-MBPD are also quantified together in one step after derivatization with PBA. In this work, we present a fully automated workstation to run the official AOCS Cd29b-13 method with little user interaction.

Instrument setup

The use of an autosampler equipped with a sample rack capable of maintaining -22 °C to -25 °C for 16 hours is key to fully automating the official AOCS Cd29b-13 method. The CHRONECT Robotic Autosampler is equipped with such a refrigerated tray and also with tray holders for storing the weighed sample (oil or fat), an agitator for heating, and a vortex mixer for rapid mixing (Figure 1). In addition, it is equipped with a syringe washing station, solvent reservoirs to provide all necessary reagents, and a parking station with various tools and syringes. The CHRONECT workstation is completed by a GC-MS to allow direct sample injection after sample preparation. With two robotic arms that can work simultaneously, an efficient overlapping of the work steps is achieved.

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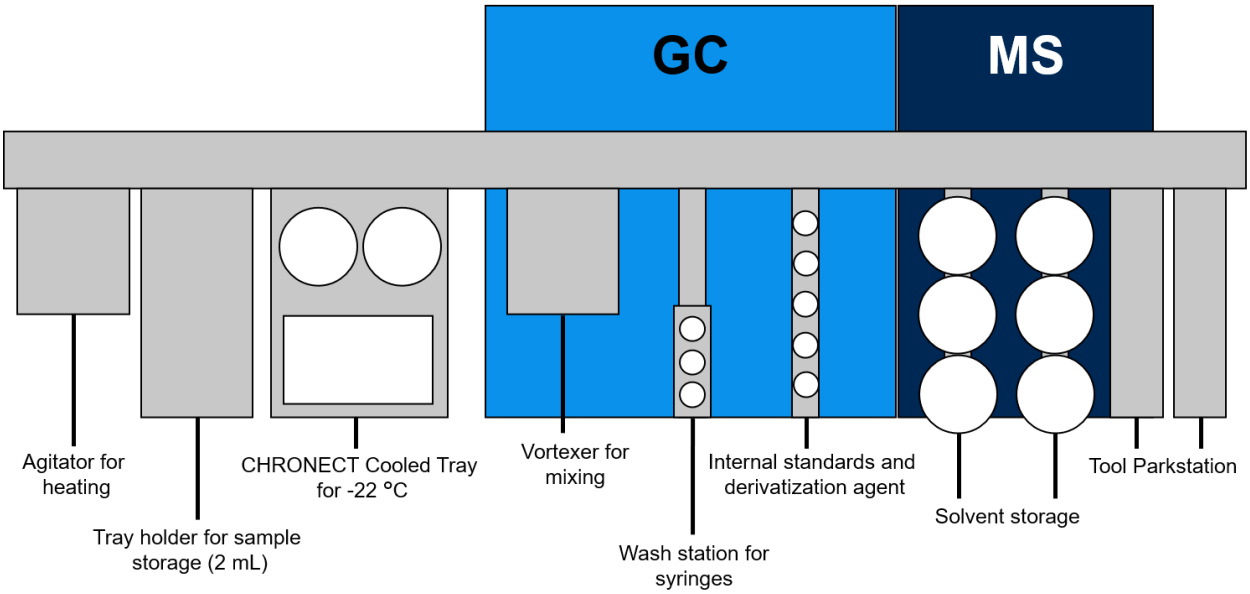

Figure 1: Schematic configuration of the CHRONECT Workstation MCPD - Module ISO 18363-2 mounted on a GC-MS system.

Experimental results

Using the sophisticated CHRONOS software, the CHRONECT workstation can prepare up to 48 samples simultaneously within a single batch. A batch of 30 samples can be analyzed within 24 hours using the same workflow. Using the CHRONECT Robotic's ability to automatically concentrate samples, a calibration curve was generated for all three compounds (2-/3-MCPD and glycidol) with 10 calibration points ranging from 50 µg/kg to 2500 µg/kg. In addition, ten vials containing 100 mg of oil were spiked with all three compounds as a blank to determine reproducibility and recovery.

For additional evaluation of the robustness of the CHRONECT workstation, three different sample types were analyzed ten times each (Figure 2). Virgin olive oil, a vegetable oil from the FAPAS ring test and an alkaline refined palm oil. Results for 2-/3-MCPD and glycidol were compared between all samples to demonstrate reproducibility for different sample types.

Discussion

Spiking of virgin oil samples (extra virgin olive oil) at different concentration levels resulted in very good calibration curves showing the excellent linearity (R^2 > 0.99) for all calibrated compounds. Even at the low concentrations, no significant deviations were seen, indicating even linearity below 50 µg/kg.

Background contamination from these measurements averaged (n = 6) less than 5 ppb for 2-MCPD and glycidol and less than 19 ppb for 3-MCPD for the three compounds. Successive sample preparations and analyses of virgin olive oil fortified to 500 µg/kg of 2-/3-MCPD and glycidol gave overall good recoveries between 94 % and 103 % (Table 1). Automated sample preparation is therefore a robust alternative to manual sample preparation, with almost no operator interaction.

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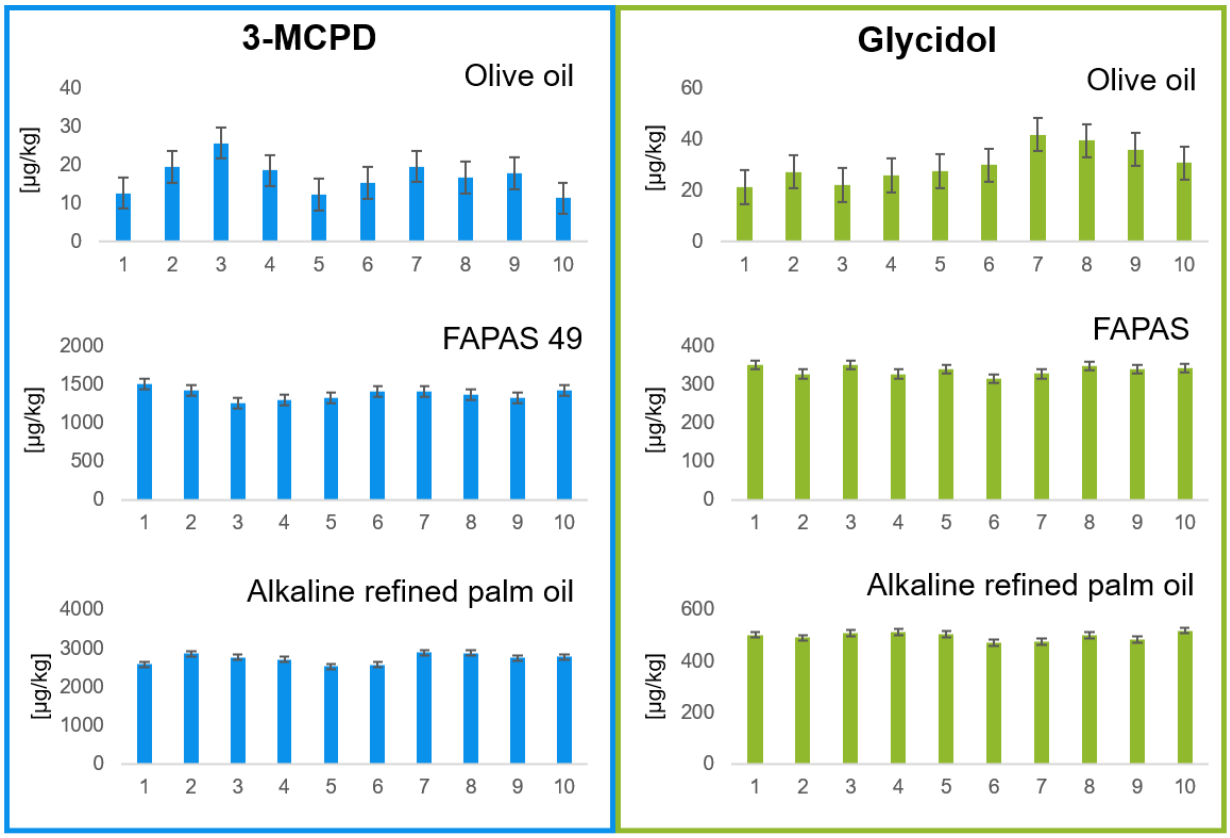

Figure 2: Reproducibility measurements of different sample types in different concentration ranges.

Table 1: Results of the spiked virgin olive oil with 2-MCPD, 3-MCPD and glycidol at 500 µg/kg.

	Concentration [µg/kg]			Recovery [%]		
	3-MCPD	Glycidol	2-MCPD	3-MCPD	Glycidol	2-MCPD
Spiked-1	484	506	507	96.8	101.2	101.4
Spiked-2	478	511	485	95.7	102.2	97.0
Spiked-3	506	485	515	101.1	96.9	103.0
Spiked-4	503	514	497	100.5	102.7	99.3
Spiked-5	499	473	502	99.8	94.5	100.5
Spiked-6	473	486	489	94.6	97.1	97.8
Average	**490.4**	**495.5**	**499.3**	**98.1**	**99.1**	**99.9**
SD	**12.5**	**15.3**	**10.3**	**2.5**	**3.1**	**2.1**
RSD [%]	**2.5**	**3.1**	**2.1**	**2.5**	**3.1**	**2.1**

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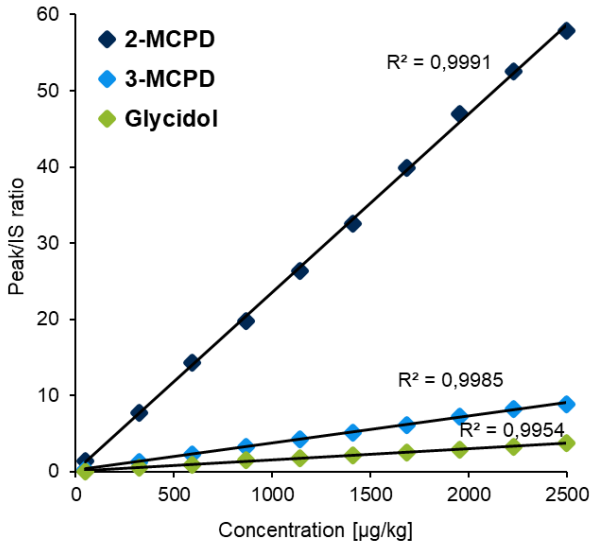

Figure 3: Calibration curves for 2-MCPD, 3-MCPD and glycidol between 50 µg/kg and 2500 µg/kg.

To further evaluate the CHRONECT workstation, three different sample types were analyzed 10 times each to see how different matrix types affect robustness. A virgin olive oil blank, a vegetable oil from the FAPAS interlaboratory test (FAPAS T2649QC) with a good database as reference, and an alkaline refined palm oil from a refinery were analyzed.

A relative standard deviation (RSD) above 20 % was observed for the blank, although the values were all below the lowest calibration point of 50 and below the LOQ of the system. The reference material showed very good agreement with the data obtained from the interlaboratory tests ($z < 2$), as did the RSD. The same was true for the physically refined palm oil with an RSD for all three compounds below 6 %.

Table 2: Reproducibility measurements for three different types of oils.

#	Blank oil			FAPAS oil 49			Physically ref. palm oil		
	3-MCPD [µg/kg]	Glycidol [µg/kg]	2-MCPD (µg/kg)	3-MCPD [µg/kg]	Glycidol [µg/kg]	2-MCPD [µg/kg]	3-MCPD [µg/kg]	Glycidol [µg/kg]	2-MCPD [µg/kg]
1	13	21	1	1507	350	795	2594	504	1466
2	20	27	3	1421	327	774	2877	493	1596
3	26	22	7	1261	351	703	2781	511	1547
4	19	26	3	1294	327	711	2715	516	1507
5	12	28	1	1322	340	708	2545	506	1432
6	15	30	1	1408	316	760	2588	473	1453
7	20	42	1	1406	328	763	2906	478	1668
8	17	39	1	1368	348	718	2884	502	1702
9	18	36	1	1329	340	709	2765	486	1591
10	11	31	1	1428	343	757	2788	520	1571
Average	**17.6**	**30.2**	**2.2**	**1368.3**	**336.4**	**737.9**	**2739.4**	**496.6**	**1551.4**
SD	**4.1**	**6.5**	**1.8**	**69.9**	**11.3**	**31.8**	**124.2**	**15.1**	**85.6**
RSD	**23.2**	**21.7**	**79.4**	**5.1**	**3.3**	**4.3**	**4.5**	**3.0**	**5.5**

With this good reproducibility and sensitivity, a limit of quantification (LOQ) below 25 µg/kg can be achieved when the amount of 3-MCPD and glycidyl in the blank is reduced so that baby food grade samples (LOQ < 6 µg/kg) can be analyzed. The CHRONECT Workstation can be equipped with an additional evaporation unit recently used for other methods such as AOCS Cd29a-13 and AOCS Cd29c-13 to allow measurements even on a single quadrupole mass spectrometer with comparable LOQs.

The CHRONECT Workstation MCPD - Module ISO 18363-2 is an excellent addition to your laboratory in a routine environment with reliable data. The method presented has the advantage that a sample can be analyzed for its 2-/3-MCPD and glycidol content in a single run.

The CHRONECT Workstation MCPD with module 18363-2 is a development by Axel Semrau.

Subject to technical changes

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