Determination of MCPD and glycidyl esters in foodstuff

with the CHRONECT Workstation MCPD and the module ISO 18363-1

Application note 1603

CHRONECT Workstation MCPD – Module ISO 18363-1
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Introduction

3-Monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD) and glycidol belong to the group of manufacturing-related contaminants in food. MCPD fatty acid esters can be formed during refining at high temperatures in the presence of chloride-containing salts. However, refining is a necessary chemical and physical refining process in the production of many oils. It is only through this temperature treatment that undesirable odors and flavors, as well as any traces of toxic compounds such as pesticides, heavy metals or mycotoxins, can be removed during further processing. The analysis of these contaminants is becoming increasingly important due to their carcinogenicity.
Laboratory experiments with animals have shown that there is an increased risk of cancer with a sustained elevated intake of free 3-MCPD. As early as March 2016, a new tolerable daily intake value of 0.8 µg/kg body weight for 3-MCPD was derived by the European Food Safety Authority (EFSA). A variety of methods are available today for the analysis of MCPD esters, which can be divided into two groups: direct determination by LC-MS/MS or indirect by GC-MS. Direct analysis is very laborious due to the large number of esters, as each ester is determined individually, so that the indirect method is used more frequently in routine applications. However, manual implementation is time-consuming.
Axel Semrau has therefore automated and optimized the common manual methods.

Automated methods

Axel Semrau recommends the DGF C-VI 18(10), ISO 18363-1 or AOCS Cd 29c-13 method as the most powerful method in routine analysis and has therefore chosen it as the basis for automation. Sample preparation, as shown in Figure 1, is fully automated on the sampler. At the same time, an evaporation step was eliminated, which has been shown not to result in improved measurement accuracy. A further step was integrated with the self-developed Clean-Technology and thus the method "DGF Fast & Clean" was developed.
An application is possible for 3-MCPD as well as for 2-MCPD and for the determination of the glycidol content. The analytes bound to fatty acids are first converted into their respective free form. The release from the fatty acid esters is achieved by transesterification.

According to the DGF method, two approaches are required in sample preparation for the determination of the glycidol content (Figure 1). In approach A, transesterification is stopped by addition of sodium chloride. In the process, MCPD fatty acid esters and glycidyl esters both react to form free MCPD. In approach B, the reaction is stopped with a chloride-free salt solution (sodium bromide). Here, only the MCPD fatty acid esters react to form free MCPD. The free MCPD is then extracted and derivatized in a further step.

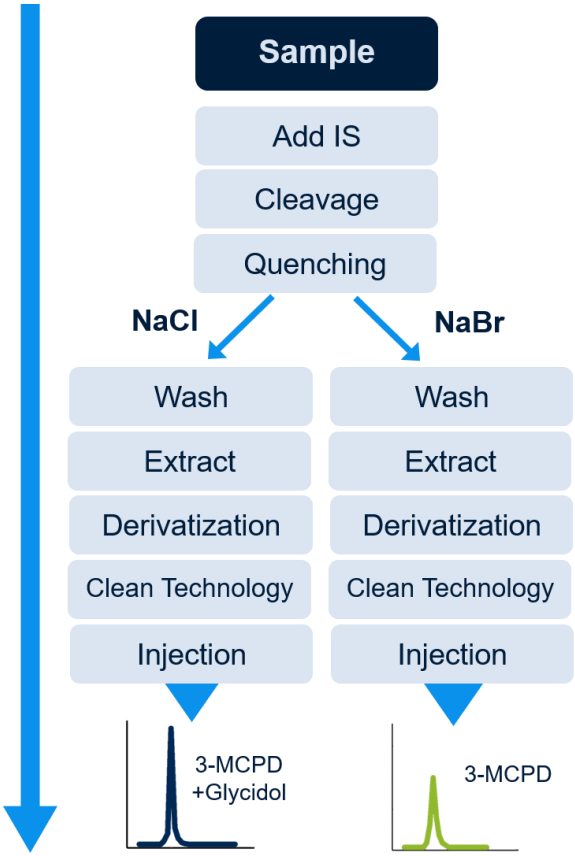

Figure 1: Schematic representation of sample preparation for indirect MCPD analysis with Axel Semrau's "DGF Fast & Clean" - automated with the CHRONECT Workstation MCPD.

Subsequently, the cleaning step developed by Axel Semrau is carried out to protect the GC-MS and allow longer maintenance intervals.
This is followed by the measurement by GC-MS/MS. Finally, the difference between approach A and B is multiplied by a transformation factor (t), which was determined experimentally. This yields the glycidol content.

Device setup for the "DGF Fast & Clean" method

The instrument setup for the "DGF Fast & Clean" method is shown schematically in Figure 2. For the automation of the sample preparation Axel Semrau chooses a 160 cm CHRONECT Robotic DHR, because it offers enough space for all necessary modules and can easily be adapted to all other methods and further applications. However, a variant with a 120 cm CHRONECT Robotic RTC is also possible. The dilutor is equipped with two solvents (*n*-hexane and extraction agent). For this application, the CHRONECT Robotic Autosampler was mounted on a Bruker EVOQ GC-TQ with 456 GC in order to be able to inject the prepared samples directly afterwards. However, the installation can also be carried out with devices from other manufacturers as well as on existing GC-MS.

To prevent the derivatization reagent phenylboronic acid from precipitating so quickly on the analytical separation column and in the ion source of the Triple Quadrupole, the GC system was additionally equipped with clean technology. This contributes to a longer lifetime of the Triple Quadrupole even with more than 3000 injections and ensures robust data. Chemical cleaning removes excess derivatization reagent and physical cleaning removes most of the matrix by backflushing. The entire system is controlled in a user-friendly manner by the CHRONOS software.
This makes even complex procedures easy to use. Axel Semrau's CHRONECT solutions are pre-installed in the application laboratory, tested (Factory Acceptance Test) and installed and tested again directly at the user's site ready for use (Site Acceptance Test). This ensures the fastest possible start of routine measurement operations.

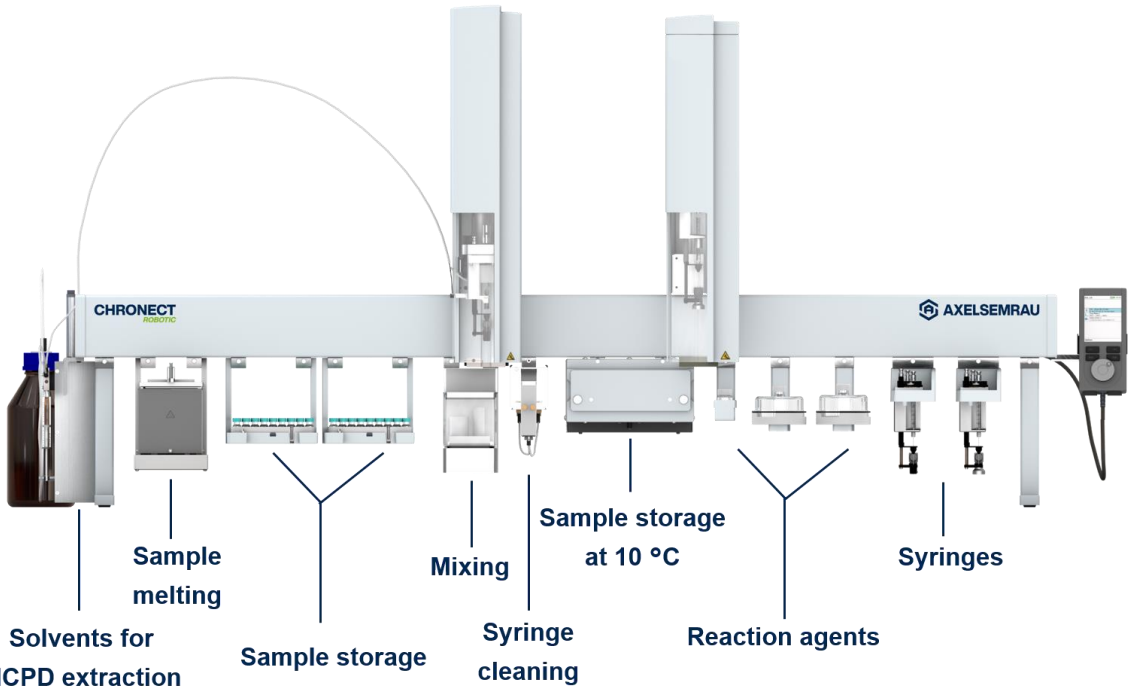

Figure 2: Schematic setup of the CHRONECT Robotic DHR RSI/RTC with modules.

Thanks to CHRONECT Robotic and CHRONOS, work steps can be nested (Figure 3). With the "DGF Fast & Clean" method, the measurement results of a sample from Part A and Part B are already available after approx. 48 minutes, as Figure 3 shows. Other methods require up to 18 h for the first sample result.

Due to interlaced work steps, 36 samples can thus be measured within 24 hours (72 sample runs). Therefore, in addition to highest precision, this system allows significant time savings. The result obtained with "DGF Fast & Clean" always conforms to the conventional DGF method. However, the modular CHRONECT Robotic System also allows other methods to be automated and integrated into everyday laboratory work.

For example, the 3-in-1 method (AOCS Cd 29b-13 or ISO 18363-2) can be automated. In this case, an additional cooling tray for -22 °C is incorporated. For the Unilever method (AOCS Cd 29a-13 or ISO 18363-3), a centrifuge and an evaporation unit can be added and integrated.

This method, like the Zwagerman method (Draft ISO 18363-4), can thus be easily automated. The older Weisshaar method has also been implemented.

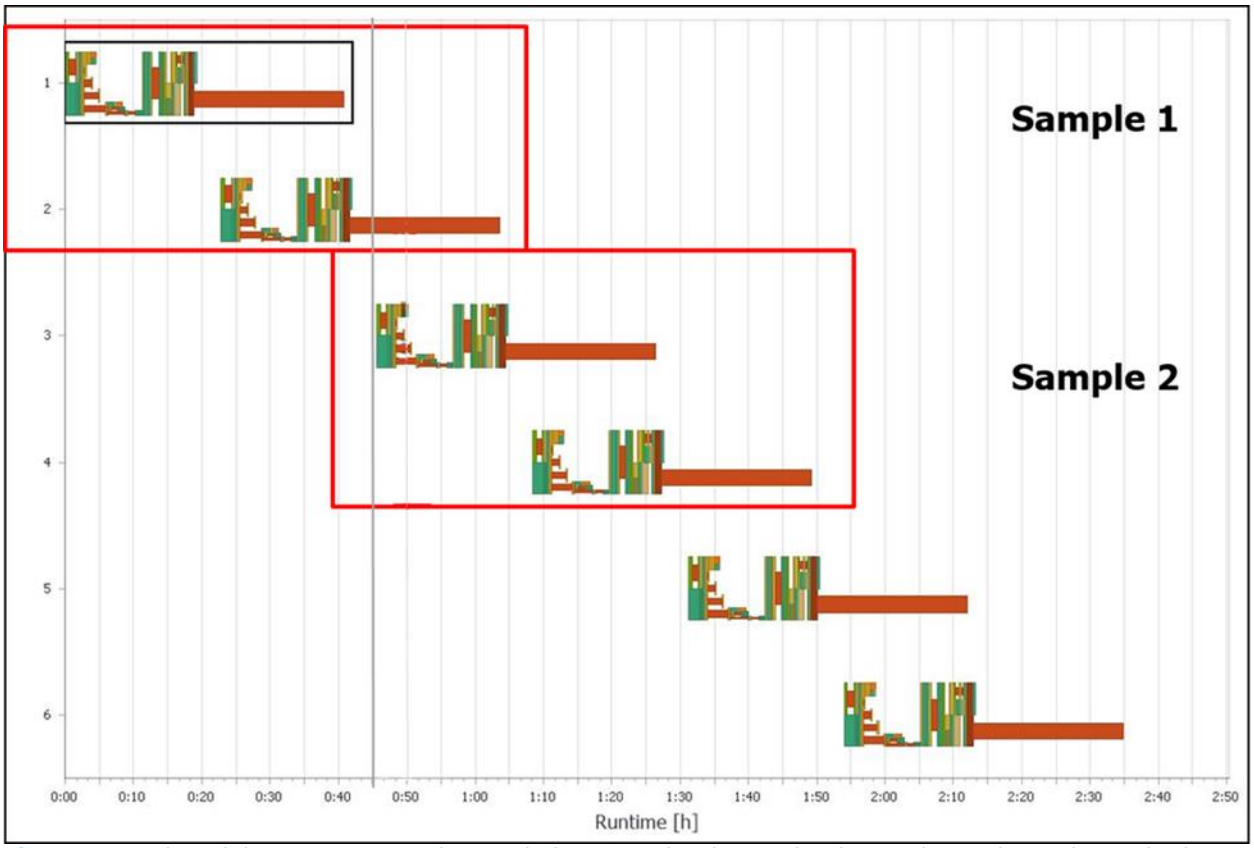

Figure 3: Section of the CHRONOS software platform: Overlapping mode of operation and sample result after 48 minutes.

Measuring parameters and results

Let's take a closer look at the "DGF Fast & Clean". The components 3-MCPD and 2-MCPD and their respective deuterated variants were detected using the triple quadrupole technique. For this purpose, one ion was selected for qualification and one for quantification. The corresponding collision energies for fragmentation of the parent ions after the first quadrupole were determined experimentally.

Important parameters for each component are shown in Table 1. To validate the application, the "DGF classical" method was first validated. In this method, the sample is processed and injected automatically exactly according to the specifications in the DGF norm (including evaporation of the extraction agent). For validation, *rac*-1,2-bis-palmitoyl-3-chloropropanediol and *rac*-1,3-distearoyl-2-chloropropanediol were used.

Table 1: Measuring parameters of the triple quadrupole for the detection of 3-MCPD and 2-MCPD.

Injector	SSL, 1 µL injektion volume, splitless (split 1:30 after 1 min)		
Temperature [°C]	Heating rate [°C/min]	Hold time [min]	Total [min]
85.0		0.10	0.10
200.0	200.0	1.00	1.68
300.0	200.0	10.00	12.18
Pressure regulation	1.5 mL/min constant flow, backflush on after 8.5 min		
Analytical column	2x Rxi-5 MS 15 m, 0.25 mm inner diameter, 0.25 µm film		
Oven program			
Temperature [°C]	Heating rate [°C/min]	Hold time [min]	Total [min]
80.0		1.00	1.00
150.0	10.0	0.00	8.00
320.0	30.0	10.00	23.67
Detector	Transfer line 280 °C, CID gas argon, MRM mode		
Name	Retention time [min]	Precursor ion	Product ion
2-MCPD	7.71	198.00	104.00
		196.00	104.00
2-MCPD-d5	7.66	203.00	107.00
		201.00	93.00
3-MCPD	7.36	196.00	147.00
		196.00	91.00
3-MCPD-d5	7.32	201.00	150.00
		201.00	93.00

Table 2: Recovery (RC in %) and reproducibility (RP in %) of the methods "DGF classic" and "DGF Fast & Clean" for part A and part B measured on four consecutive days.

	„DGF classic“		**„DGF Fast & Clean“**	
	RC	**RP**	**RC**	**RP**
3-MCPD Part A	102.6	3.9	91.6	7.7
3-MCPD Part B	94.3	3.9	101.9	8.8

The two components were added to virgin olive oil in defined amounts and then processed with the CHRONECT Workstation MCPD. Virgin olive oil is suitable as a blank matrix in this case, as it is cold pressed and should therefore not contain any MCPD esters. Corresponding blank measurements confirmed the assumption with a blank value of < 0.02 mg/kg sample for 3-MCPD and 2-MCPD. The results of the validation are shown in Table 2.
A detection limit of 0.011 mg/kg in one hundred percent fat was obtained for 3-MCPD with a limit of quantitation of 0.025 mg/kg in one hundred percent fat. In addition, a reference oil from FAPAS was measured, the 3-MCPD and 2-MCPD contents of which were known from an interlaboratory test. In addition, the method "DGF Fast and Clean" was completely validated in routine according to the EU Commission regulation EU No836/2011. The results of this validation are shown in Table 3.
A further validation of the method is the comparison of the measurement results with those of a reference sample. For this purpose, a reference oil from FAPAS was used, whose measured values originate from an interlaboratory test. Table 4 shows the results of this comparison.

Table 3: Results of the validation of a CHRONECT Workstation MCPD in a routine laboratory with a set LOQ of 0.05 mg/kg fat.

Validation parameter	Validation data	Criterion for characteristics of the method	Criteria met
System precision	2.9 %	< 10 %	✓
Calibration 0.05 – 0.5 mg/kg 0.5 – 5.0 mg/kg	R^2: 0.9996 R^2: 0.9999	R^2> 0.99	✓
LOQ 0.05 mg/kg	Part A 7.4 % Part B 9.0 %	RSD < 20 % S/N 1:10	✓
Linearity 0.05 / 2.0 / 10 mg/kg	Part A 4.2 % Part B 3.9 %	R^2> 0.99	✓
Accuracy	Part A 95 % Part B 88 %	75 - 110 %	✓
Reproducibility	Part A 3.9 % Part B 2.8 %	RSDr < 1.5 %	✓

Table 4: Contents of a reference oil from FAPAS (T2646QC) according to the "DGF classic" and "DGF Fast & Clean" methods as well as real samples determined only according to the "DGF Fast & Clean" method. In comparison with values from a customer laboratory with manual sample preparation.

Sample	Measurement	3-MCPD [mg/kg]	2-MCPD [mg/kg]	Glycidol [mg/kg]
Reference oil (FAPAS)	Manual „DGF klassisch“	0.59	0.31	0.26
	„DGF classic “	0.49	0.30	0.23
	„DGF Fast & Clean“	0.50	0.38	0.36
Unknown edible oil	Manual „DGF classic“	0.78	0.39	0.64
	„DGF Fast & Clean“	0.80	0.58	0.73
Rapeseed oil	Manual „DGF classic“	0.14	< 0.10	0.10
	„DGF Fast & Clean“	0.11	0.08	0.13
Sunflower oil-HL	Manual „DGF classic“	0.84	0.39	0.15
	„DGF Fast & Clean“	0.73	0.60	0.29
Sunflower oil-HO	Manual „DGF classic“	0.31	0.15	0.49
	„DGF Fast & Clean“	0.25	0.19	0.58

Evaluation of the results

Internal validation of the methods (DGF classic and "DGF Fast & Clean") was performed using spiked standards to determine recovery and reproducibility of the overall procedure. For the MCPD, a reproducibility of approx. 94 % was determined. For the "DGF Fast & Clean" method, recoveries ranging from 91 to 116 % were determined. With 93 - 96 % reproducibility, the "DGF classic" method is slightly above the "DGF Fast & Clean" method with 87 - 92 %.
Using the respective methods in routine operation will significantly improve the reproducibility values for both variants compared to manual sample preparation. The validation carried out here is

based on measurements on four consecutive days.
The validation of the method "DGF Fast & Clean" according to the EU commission (Table 3) additionally shows the achievement of all necessary criteria for the use of this method in routine e.g. for the release analysis of a production refinery.
Looking at the range of values from the interlaboratory comparison for the reference oil FAPAS 2646 (3-MCPD 0.352 - 0.821 mg/kg), it can be seen that there seem to be significant deviations between laboratories of +/- 40 % in the determination of 3-MCPD. It should be noted that the values from the interlaboratory comparison were determined using various manual methods (DGF, Unilever, 3-in-1). Automation would certainly lead to smaller deviations in the values.

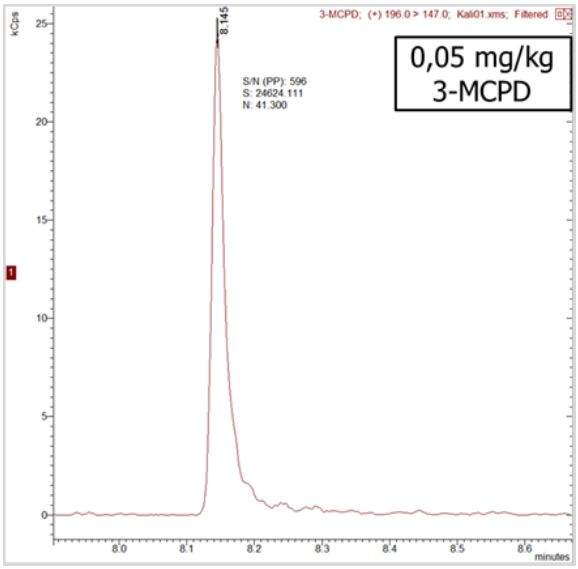

Figure 4: Chromatogram of the measurement of 0.05 mg/kg 3-MCPD in olive oil.

Example chromatograms

The chromatogram in Figure 4 demonstrates that high sensitivity and a clean peak are guaranteed even at low concentrations. As an example, 0.05 mg/kg 3-MCPD was detected in olive oil. The chromatograms in Figure 5 show excellent reproducibility of the automated measurement of 2- and 3-MCPD at different concentrations.

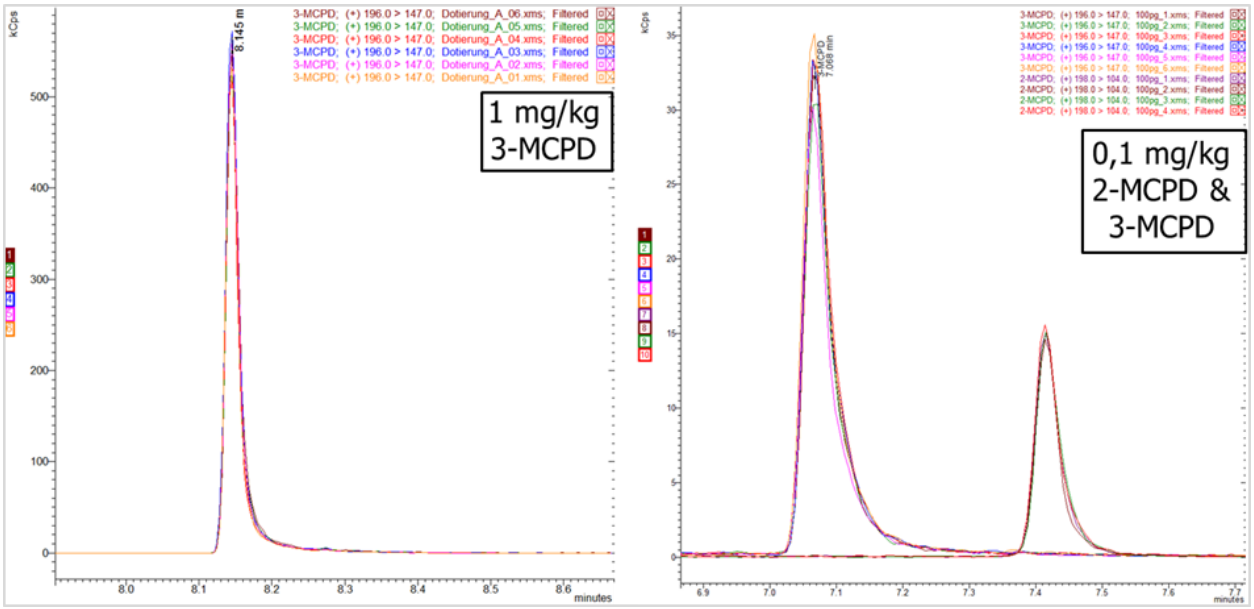

Figure 5: Chromatograms of measurements of several samples of 2- or 3-MCPD at different concentrations in olive oil.

Example measurements

Figure 6 and Table 5 show an excellent comparability of the methods "DGF classic", "DGF Fast & Clean" and the 3-in-1 method. Compared to manual sample preparation, "DGF Fast & Clean" in particular leads to considerable time savings. In the meantime, more than 100 different matrices have been successfully measured with the "DGF Fast & Clean" in routine applications.

This includes normal oils and fats as well as extracts of compound food as shown in Figure 6. In order to make the method even more attractive for further matrices, we are continuously working on improvements of the method. This was most recently implemented in the adaptation of the method for the analysis of hardened fats. Feedback from users is actively incorporated in this process.

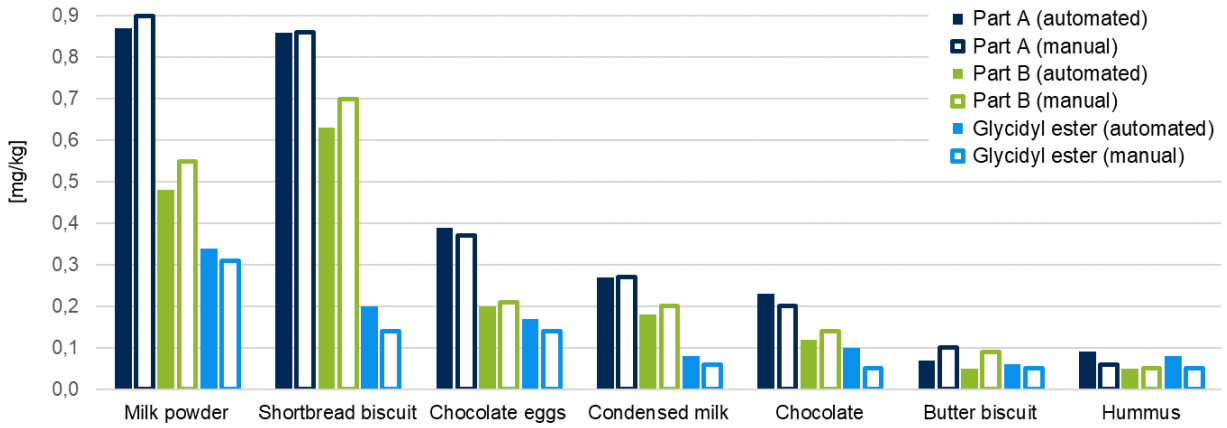

Figure 6: Results of an example measurement for the 2-/3-MCPD and glycidol content of selected food products.

Table 5: Results from an oil mixture with sunflower and rapeseed oil using the "DGF Fast & Clean" method compared with the manual DGF method and the 3-in-1 method.

	Oil mixture I		Oil mixture II	
	3-MCPD ester [mg/kg]	**Glycidyl ester [mg/kg]**	**3-MCPD ester [mg/kg]**	**Glycidyl ester [mg/kg]**
“DGF Fast & Clean”	0.14	0.05	0.11	< 0.05
DGF manual	0.15	0.08	0.13	0.05
3-in-1 method	0.14	< 0.05	0.1	< 0.05

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Summary

2- and 3-MCPD as well as glycidol are present as contaminants in our food. Due to their high carcinogenicity, their analysis is becoming more and more important. The rapidly growing demand requires an automation of analytical methods in order to increase throughput and reproducibility.
The implementation shown here in the form of the "DGF Fast & Clean" method enables a statement to be made in a short time, approx. 48 minutes for a sample with Part A and Part B. Axel Semrau therefore recommends "DGF Fast & Clean" as the
most powerful method for the routine analysis of 3-MCPD, 2-MCPD and glycidol.
Thanks to the modular CHRONECT Robotic system, however, other methods can also be fully automated. Examples are ISO 18363-2, ISO 18363-3 and Draft ISO 18363-4 or the Weisshaar method.

CHRONECT Robotic, in this case equipped with either the Dual-Head technology or a Single-Head and CHRONOS, enables the parallel execution of individual work steps. Therefore, the system can be operated continuously and measurements can be performed in a time-saving manner.
The Clean-Technology developed by Axel Semrau leads to an excellent robustness of the system, as the GC-MS is protected and maintenance intervals are extended. Thus, the fully automated application developed here is excellently suited for operation in routine analysis, as incoming goods inspection or also in online analysis. It leads to excellent results in the analysis of 2-MCPD as well as 3-MCPD and glycidol.

The CHRONECT Workstation MCPD and module ISO 18363-1 are developments by Axel Semrau.

Subject to technical changes

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