APPNOTE

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GERSTEL AppNote 191 - updated

Fully Automated Determination of 3-MCPD and Glycidol in Edible Oils by GC/MS Based on the Commonly Used Methods ISO 18363-1, AOCS Cd 29c-13, and DGF C-VI 18 (10)

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Keywords

3-MCPD, 2-MCPD, glycidol, edible oil, lab automation, GC-MS, GC-MS/MS, DGF C-VI 18 (10), mid-point backflush

Abstract

3-MCPD, 2-MCPD and glycidol, and especially their fatty acid esters, are process contaminants that are formed, for example, during the refining of edible oil and fats. At least some of the above-mentioned substances are classified as potentially carcinogenic to humans, a fact which has prompted the introduction of rules and regulations that specify tolerable daily intake values and maximum levels in edible oils. Different analytical methods are available for the determination of these compounds. These methods follow two different strategies: Direct determination or, more commonly, indirect differential determination of the contaminants.

This AppNote describes a solution for the fully automated determination of 3-MCPD, 2-MCPD and glycidol in edible oils based on the reliable indirect method DGF C-VI 18 (10), which is essentially identical to the ISO 18363-1 and AOCS Cd 29c-13 methods. The edible oil sample is divided into two parts (assays A and B). In both assays the samples are saponified using a sodium hydroxy methanol solution, but applying different quenching methods. In assay A, free glycidol is converted to 3-MCPD under acidic quenching conditions in the presence of chloride. In contrast, in assay B, the quenching reagent is an acidic chloride-free salt solution in which free glycidol is not converted into 3-MCPD. After derivatization, the 3-MCPD amounts in both samples are determined by GC-MS as phenylboronic acid (PBA) esters. Assay B is used to determine the amount of 3-MCPD in the sample while assay A provides the combined amounts of 3-MCPD and glycidol. The amount of glycidol is determined as the difference between the results of assay A and assay B. 2-MCPD as another possible contaminant is determined in Assay B additional to the requirements of the regulations.

In the work presented here, an automated evaporation step is performed as prescribed in the abovementioned official methods. This ensures that for most matrices the required limits of detection can be reached with a single quadrupole mass spectrometer (MSD) even though this AppNote is based on GC-MS/MS analysis using a triple quadrupole MS. Another important aspect of the method is the evaporation step, which removes excess derivatization reagent that could otherwise accumulate in the GC-MS(/MS) system and influence system stability.

It is demonstrated that the ISO 18363-1 method, which is equal to both the AOCS Cd 29c-13 and the DGF C-VI 18 (10) method, can be automated using the GERSTEL MPS. The results obtained show good correlation with reference data. The excellent standard deviations achieved for the entire sample preparation and analysis workflow speak in favor of automation.

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Introduction

3-Monochloropropanediol (3-MCPD), 2-monochloropropanediol (2-MCPD) and glycidol are contaminants that are present in a variety of food samples. These compounds are formed in fatty and/or salty foods when high temperatures are applied during processing. Significant amounts of MCPD and glycidol fatty acid esters can be formed, for example, during the refining of edible oils, which can be divided into distinct steps as outlined in figure 1.

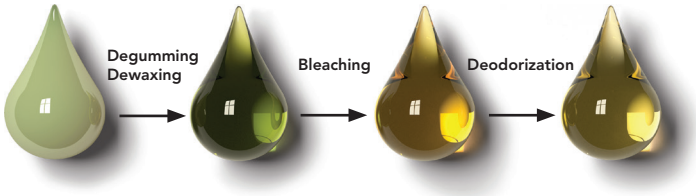

Figure 1: Refining process used in the production of edible oils.

In edible oil refining processes, the final deodorization step is particularly critical and must be carefully controlled to avoid the formation of significant amounts of MCPD and glycidol. The deodorization step is performed to remove unwanted odors and bittering agents from the oil. Varying the applied temperature during the deodorization process often merely changes the ratio of MCPD ester to glycidol ester formed but does not eliminate the formation of these compounds.

While toxicological studies on rats have shown that 3-MCPD causes tumors, the effect of 2-MCPD is less well known. 3-MCPD is labeled as a possible human carcinogen. In contrast glycidol has already been classified as a probable human carcinogen.

Several different methods have been published for the determination of 3-MCPD, glycidol and their esters. The two main approaches for the determination of the esters are the direct method using LC/MS and the indirect methods using GC-MS. The direct method has the disadvantage of having to deal with complex chemical compositions of the esters formed: The fatty acid distribution and the formation of both monoesters and diesters result in a wide variety of MCPD- and glycidyl esters being formed. This means that a lot of individual substances must be quantified to determine the total amount of the contaminants. The situation is further complicated by the fact that quantification standards are unavailable. When assessing the toxicologically relevant part, it must be considered that 3-MCPD-esters are broken down completely into free 3-MCPD during the intestinal resorption process in the human body, and the glycidol esters are converted to free glycidol as well. For these reasons, the indirect methods are currently favored and basically all work according to the same principle. Esters are split into free MCPD and glycidol, derivatized and determined by GC-MS.

This AppNote presents a sample preparation solution based on the GERSTEL MultiPurpose Sampler, which enables fully automated determination of 3-MCPD, 2-MCPD and glycidol in edible oils. It is based on the DGF C-VI 18 (10) [1] method, which in turn is very similar to the ISO 18363-1 [2] and AOCS Cd 29c-13 [3] methods all using differential determination of glycidol and 3-MCPD. The analysis is divided into two assays (A and B). The quenching reaction after the saponification step is the main difference between the two. In assay A, the saponification reaction is stopped by adding an acidic sodium chloride solution. Under these reaction conditions, free glycidol is converted to 3-MCPD and the combined amounts of 3-MCPD and glycidol are determined together as 3-MCPD. In assay B, the quenching reagent is a chlorine-free acidic salt solution, e.g. bromide. In this case free glycidol is not converted to 3-MCPD, which means that only 3-MCPD is determined. The amount of 3-MCPD in both samples is determined by GC-MS after derivatization with phenylboronic acid. The amount of glycidol in the edible oil sample is determined as the difference between the 3-MCPD amounts obtained in assays A and B and appropriately corrected using a calculated conversion factor. A reaction scheme of assays A and B is shown in figure 2.

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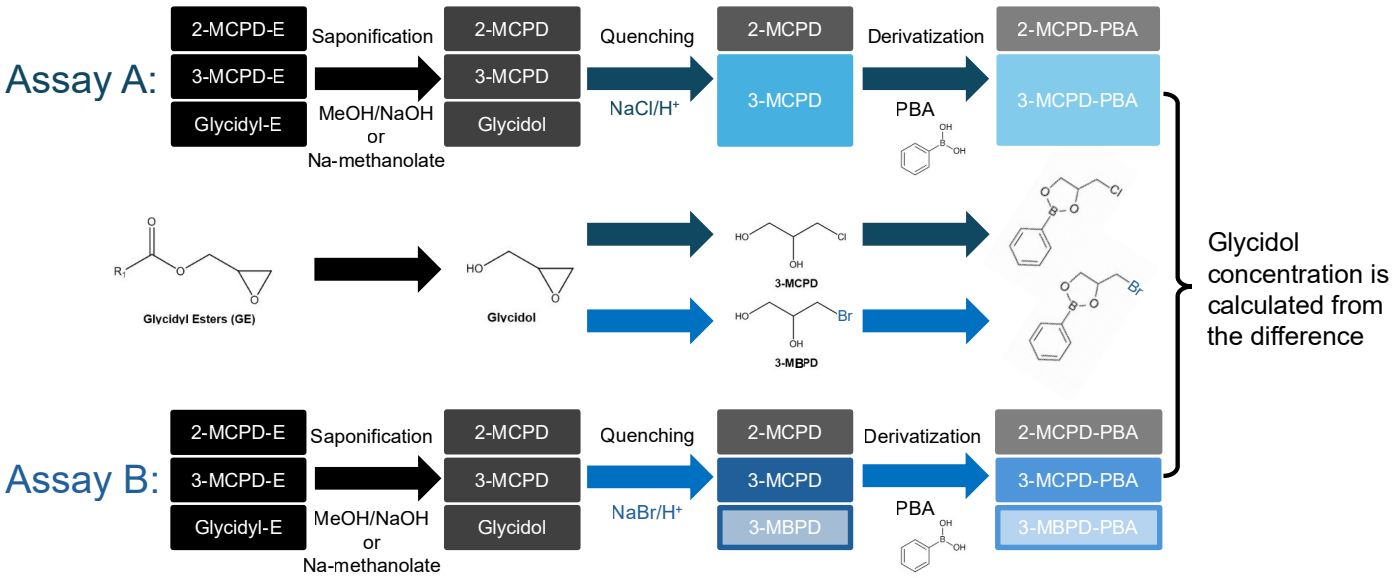

Figure 2: Reaction scheme of assays A and B.

Experimental

Instrumentation

The sample preparation system used is shown in figure 3. The dual head MPS mounted on top of the GC-MS/MS performs all sample preparation steps. One head is equipped with a 10 µL syringe (Universal Syringe Module - USM) for sample introduction (left), the other with a 2.5 mL syringe (Preparative Syringe Module - PSM) and a 250 µL Syringe (USM) for sample preparation (right). From left to right the following modules are included: Two tray holders for sample/extract vials, 10 mL wash station, high flow fast wash station, three 180 mL solvent reservoirs, quickMIX module for liquid-liquid extraction, mVAP evaporation station for solvent evaporation, 6-position heated agitator, and syringe module exchange station. A GERSTEL Universal Peltier Cooling system (UPC) is used to cool the GERSTEL Cooled Injection System (CIS), PTV-type GC inlet for the following analysis run. The heated agitator can be used to initially melt solid oils and fats. An Agilent Technologies (Waldbronn, Germany) 8890 gas chromatograph with CIS inlet was used in combination with an Agilent Technologies 7000E triple quadrupole mass spectrometer for analysis of the prepared samples.

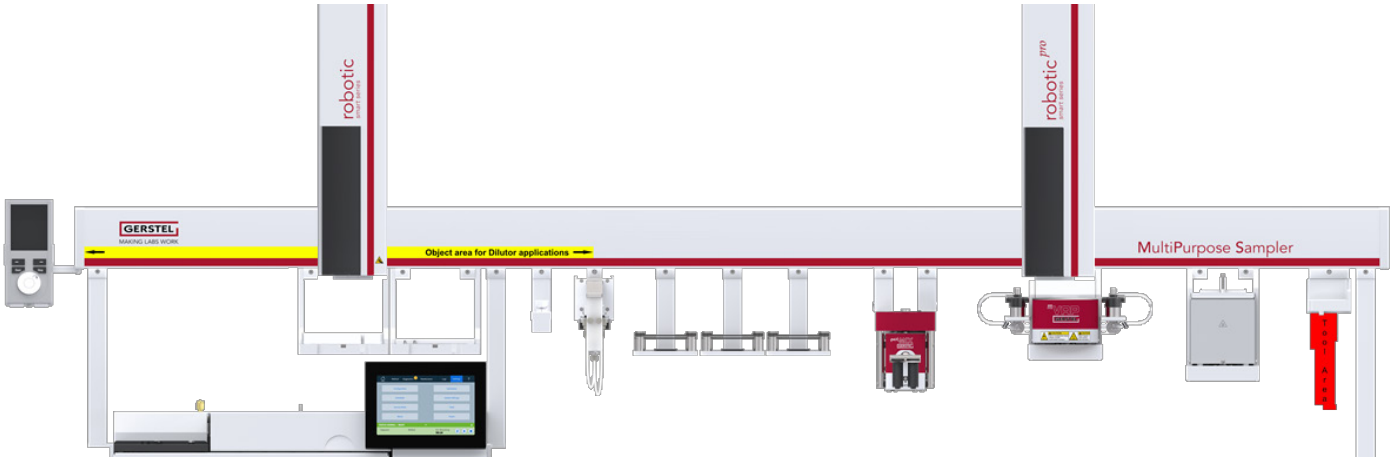

Figure 3: GERSTEL MPS used for automated sample preparation of edible oils prior to GC-MS/MS determination of 2-MCPD, 3-MCPD and glycidol.

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Materials

3-MCPD-1,2-propanediol-dipalmitate-d_5 (PP-3-MCPD-d_5), 3-MCPD-1,2-propandiol-dipalmitate (PP-3-MCPD), glycidyl stearate (Gly-S), 3-MCPD, 2-MCPD-1,3-propanediol-distearate-d_5 (SS-2-MCPD-d_5) and 2-MCPD-1,3-propanediol-distearate (SS-2-MCPD) each 1000 µg/mL in toluene were purchased from Campro Scientific, Berlin, Germany.

Methanol, acetone, toluene, MTBE (2-methoxy-2-methylpropane), ethyl acetate, isohexane, isooctane (trimethylpentane), distilled water, sodium methoxide solution or sodium hydroxide, sodium chloride, sodium bromide (chlorine-free!!!), phenylboronic acid and sulfuric acid 25%, all of analytical grade, were obtained from Merck, Darmstadt, Germany.

To prepare the sodium methanolate solution dilute sodium methoxide solution to 25 g/L, e.g. 15 mL sodium methoxide solution and 135 mL methanol. For the acidic salt solution with chloride for assay A dissolve 200 g sodium chloride in water, fill up to 1 L with water and add 35 mL 25% sulfuric acid. pH has to be adjusted, so that a mixture of 600 µL sodium chloride solution with 200 µL methanolic sodium methoxide solution results in a pH of 1-2. For the acidic chloride-free salt solution for assay B dissolve 600 g sodium in water, fill up to 1 L with water and add 35 mL 25% sulfuric acid. pH has to be adjusted, so that a mixture of 600 µL sodium bromide solution with 200 µL methanolic sodium methoxide solution results in a pH of 1-2. For the extraction solution mix 90 mL MTBE with 60 mL ethyl acetate (3/2 (v/v)). For the derivatization solution fill 0.2 g PBA up with MTBE in a volumetric 10 mL flask.

A non-thermally treated, cold pressed olive oil was used as blank oil. It was checked for possible traces of the analytes before using it. Nevertheless, glycidol was present at very low µg/kg levels in all tested potential blank oils.

Sample Preparation

- Weigh separate 100 mg samples into two vials
- Aspirate 250 µL MTBE and dispense 100 µL each to assays A and B to dilute the samples
- Aspirate 250 µL ISTD-Solution and dispense each 100 µL to assay A and B
- Shake at 50 °C in the ^{m}VAP and mixing in the ^{quick}Mix
- Aspirate 450 µL sodium methanolate and dispense 200 µL each to assays A and B
- Shake both assays for 4.5 min in the ^{quick}Mix
- Add acidic NaCl solution to assay A and acidic NaBr to assay B
- Aspirate 1300 µL isohexane and dispense 600 µL each to assays A or B, respectively
- Shake both assays for 1 min in ^{quick}Mix
- Wait 5 min for reaction and phase separation
- Discard the isohexane phases to remove FAMES
- Repeat the washing step with isohexane two times
- Aspirate 1300 µL MTBE/EtAc 3/2 (v/v) and dispense 600 µL each to assays A and B
- Shake for 0.4 min in ^{quick}Mix
- Wait 1 min for phase separation and transfer each assay extract to a fresh 4 mL vial
- Repeat the extraction of the analytes two times
- Aspirate 100 µL PBA-Solution (0.2 g phenyl boronic acid in 10 mL MTBE) and dispense 25 µL each to the cumulative extracts of assays A and B for analyte derivatization
- Evaporate to dryness in the ^{m}VAP for 4.5 min at 50 °C
- Aspirate 1100 µL isooctane and dispense 500 µL each to assays A and B
- Reconstitute each assay by shaking in the ^{quick}Mix
- Inject to GC-MS/MS

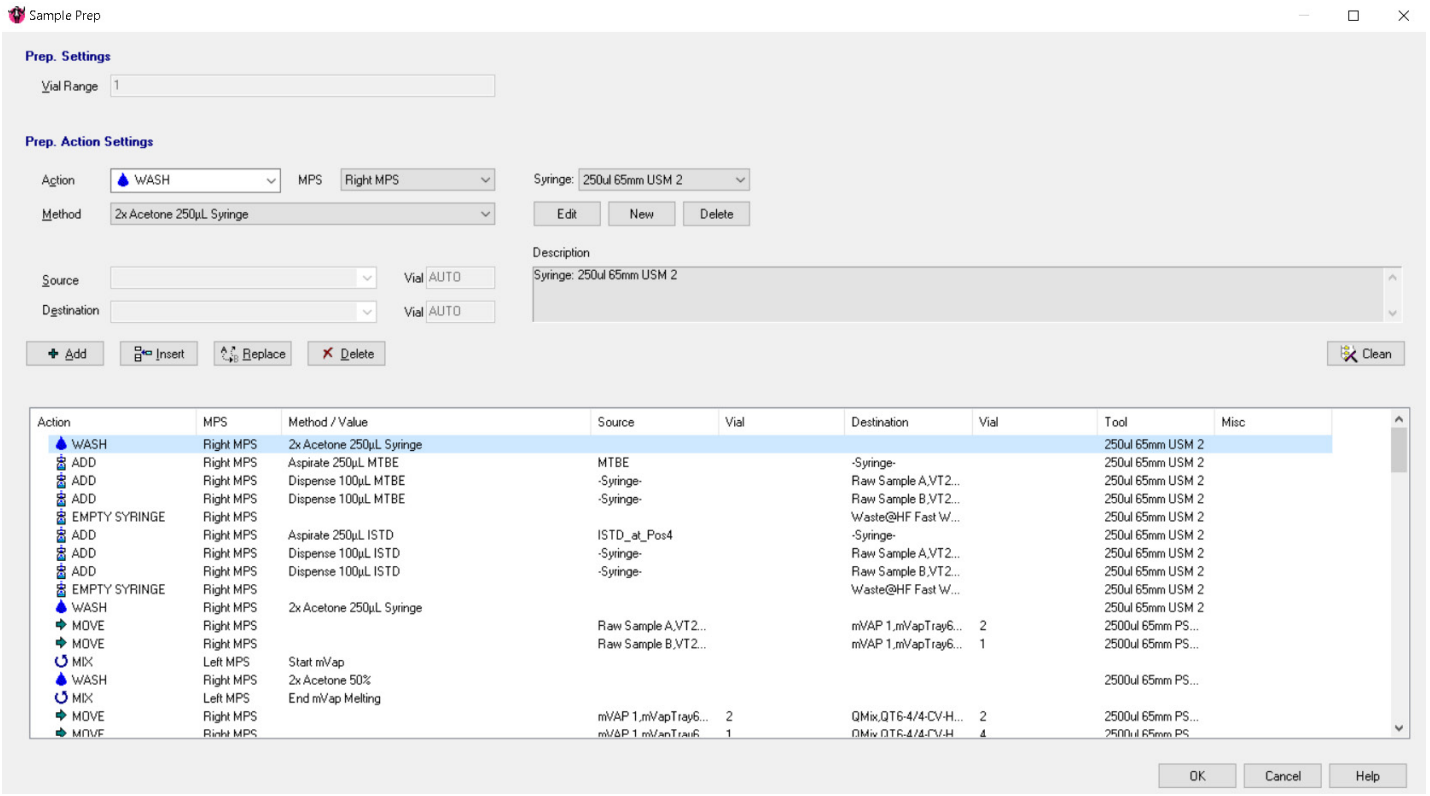

Figure 4: Excerpt of the Maestro sample preparation sequence.

Analysis Conditions

MPS

Injection volume	1 µL

CIS 4

Liner	baffled
Pneumatics mode	splitless
Splitflow	50 mL/min @ 1.0 min
Temperature	85 °C (0 min), 5 °C/sec to 165 °C (12.5 min), 5 °C/sec to 275 °C (10 min)

GC Agilent 8890 Parameters

Pre-column	5 m DB-5ms (Agilent), d_i=0.25 mm, d_f=0.0 µm
Pneumatics	He; ramp flow 1.0 mL/min
Main column	30 m DB-17ms ultra inert (Agilent), d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He; constant flow 1.2 mL/min
Temperature	85 °C (10.5 min), 6 °C/min to 150 °C (0 min), 12 °C/min to 180 °C (0 min), 25 °C/min to 280 °C (7 min)
Pneumatics	Midpoint Backflush start at 1.0 min with -4 mL/min

MS/MS Agilent 7000E Parameters

Ionization	Electron impact (EI) at 70 eV
Solvent delay	6 min
Mode	Multiple Reaction Monitoring (MRM), for details see table 1
Source temp.	230 °C
Quad 1 temp.	150 °C
Quad 2 temp.	150 °C
Collision Gas Flow	N_2, 1.5 mL/min
Quench Gas Flow	He, 2.25 mL/min
Transfer line temp.	280 °C

Table 1: List of compounds with their mass spectral parameters.

Analyte	ISTD	Precursor Ion [Da]	Product Ion [Da]	Purpose
3-MCPD-d_5	x	201	93	Qualification ISTD 3-MCPD
2-MCPD-d_5	x	201	104	Quantification ISTD, 2-MCPD
3-MCPD-d_5	x	201	150	Quantification ISTD, 3-MCPD
2-MCPD-d_5	x	201	93	Qualification ISTD, 2-MCPD
2-MCPD		198	104	Quantification 2-MCPD
3-MCPD		196	91	Qualification 3-MCPD, glycidol
3-MCPD		196	147	Quantification 3-MCPD, glycidol
2-MCPD		196	91	Qualification 2-MCPD

Results and Discussion

The first step for determination of 3-MCPD and glycidol based on the DGF C-VI 18 (10) method is to evaluate the transformation from glycidol to 3-MCPD. Figure 2 shows the conversion factor for the transformation.

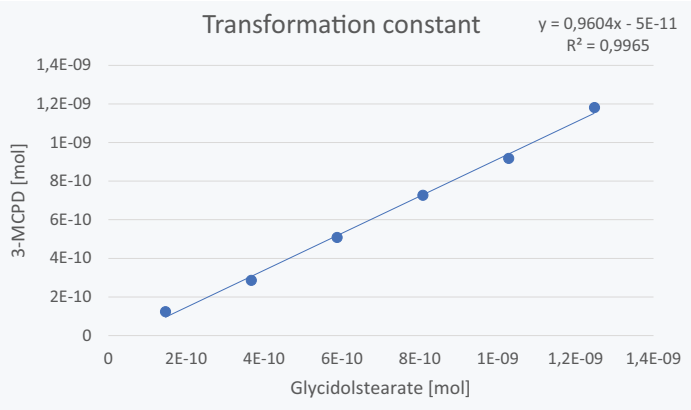

Figure 5: Conversion factor of glycidol to 3-MCPD.

The transformation constant (T) describes the efficiency of the conversion following the method used for assay A. The calculated amount of glycidol (x) is plotted against the measured amount of 3- MCPD (y). A linear regression of the type y = mx + b is performed, with the reciprocal slope (1 / m) giving the conversion factor (t). Multiplying the conversion factor (t) by 0.67 (theoretical value of the molar mass ratio of glycidol and 3-MCPD) results in the transformation constant (T) as a calculated value. The following example in figure 5 shows the amount of 3-MCPD formed as a function of the amount of glycidol (in the form of glycidyl stearate) in a spiked blank oil at six different concentrations.

During the saponification reaction with sodium methanolate, 3-MCPD is partially converted to glycidol, which is a known side reaction in the standard method. Since the internal standard reacts in the same way, a small proportion of 3-MCPD-d_5 is converted to glycidol-d_5. In assay A the generated glycidol-d_5 is converted back to 3-MCPD-d_5 by the adding acidic NaCl, resulting in the original number of 3-MCPD-d_5 molecules. In assay B the formed glycidol-d_5 is converted to 3-MBPD-d_5 by the adding acidic NaBr. As a result, a part of the 3-MCPD-d_5 is not detected in assay B and the corresponding peak is smaller than in assay A (figure 6). Instead, a 3-MBPD peak appears, which, however, is normally not detected.

The scenario described is illustrated in the chromatogram below:

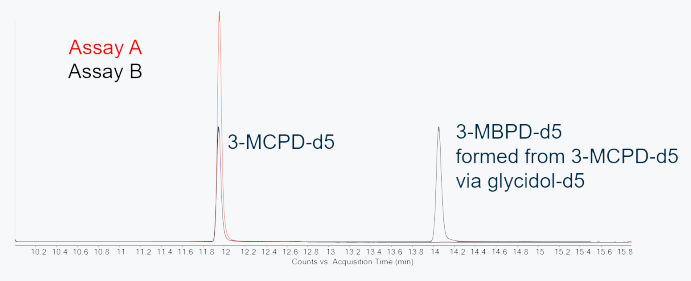

Figure 6: Peak area difference between assay A and assay B for the internal standard 3-MCPD-d_5 due to different chemical reactions in assay A (red chromatogram) and assay B (black chromatogram).

Sample Preparation Parameters

When developing the automated sample preparation method, we strictly adhered to the volumes, times and other parameters listed in the standards. The entire workflow was conveniently set up in the GERSTEL MAESTRO software, see figure 4. Each single action was thoroughly evaluated and parameters, such as aspirating and dispensing speeds and penetration depths, were optimized to achieve a fast, rugged, and repeatable workflow.

Injection and Backflush Parameters

The injection parameters are based on the standard methods, whereby the hold time at 165 °C in the CIS (PTV-type inlet, as listed in the standards) was modified from 10 min to 12.5 min. The final temperature of 320 °C specified in the standard methods was set to the maximum temperature of 275 °C specified for the deactivated liner used in this analysis method.

The analytes are transferred to the column during the specified first ramp of 165 °C, while the derivatizing agent phenylboronic acid and the matrix are mainly retained. The main separation column and the mass spectrometer are thus protected in the best possible way. Backflush is not explicitly mentioned in the standards, but it is a proven, rugged, and widely used method to significantly shorten the analysis time and protect the main separation column and the mass spectrometer by eliminating matrix residues, as can be seen in figure 7. A significant added benefit is removal of the phenylboronic acid (PBA) reagent. PBA causes build-up on ion source surfaces leading to a destabilization of the analysis system and added downtime associated with ion source cleaning [4].

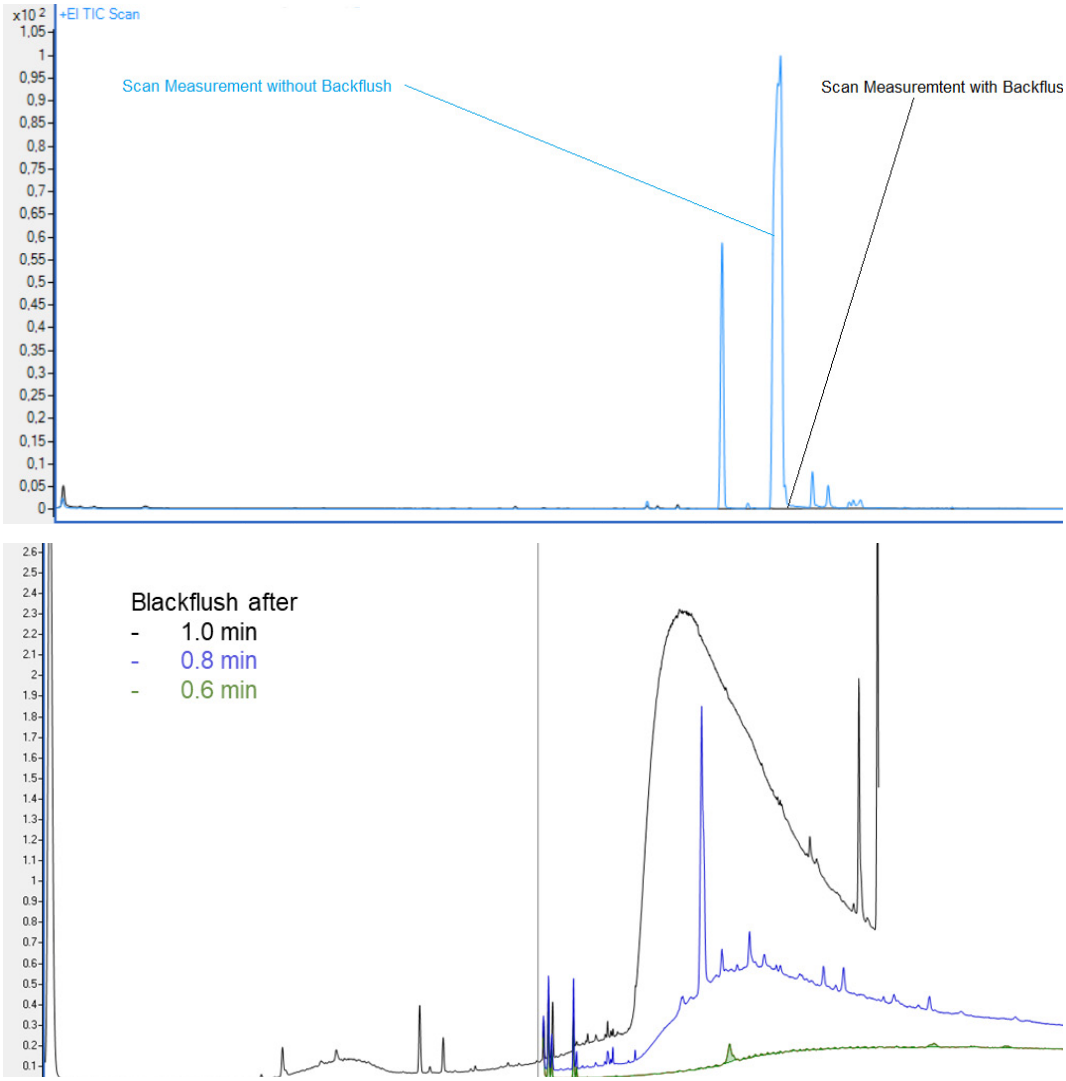

Figure 7: Illustrating the protective effect of pre-column and GC inlet backflush, full scan chromatograms of an extracted oil sample with (black) and without (blue) pre-column backflush are shown in the upper picture. More detailed chromatograms representing a range of backflush times are shown in the bottom picture with backflush after 1 min (black), 0.8 min (blue) and 0.6 min (green), respectively. Backflush prevents the bulk of matrix compounds and derivatization reagent from entering the analytical column and the mass spectrometer, enabling extended maintenance intervals, and ensuring overall ruggedness of the method.

The advantage of a pre-column backflush is that the analytes can be quickly transferred to the main column, while the matrix and reagent are largely retained. Maintenance of a pre-column backflush is easy since the pre-column is connected between the inlet and the purge union and the main separation column is connected between the purge union and the mass spectrometer. It is not nessecary to shut-down the mass spectrometer for pre-column and inlet maintenance. A flow diagram for a pre-column backflush system is shown in figure 8.

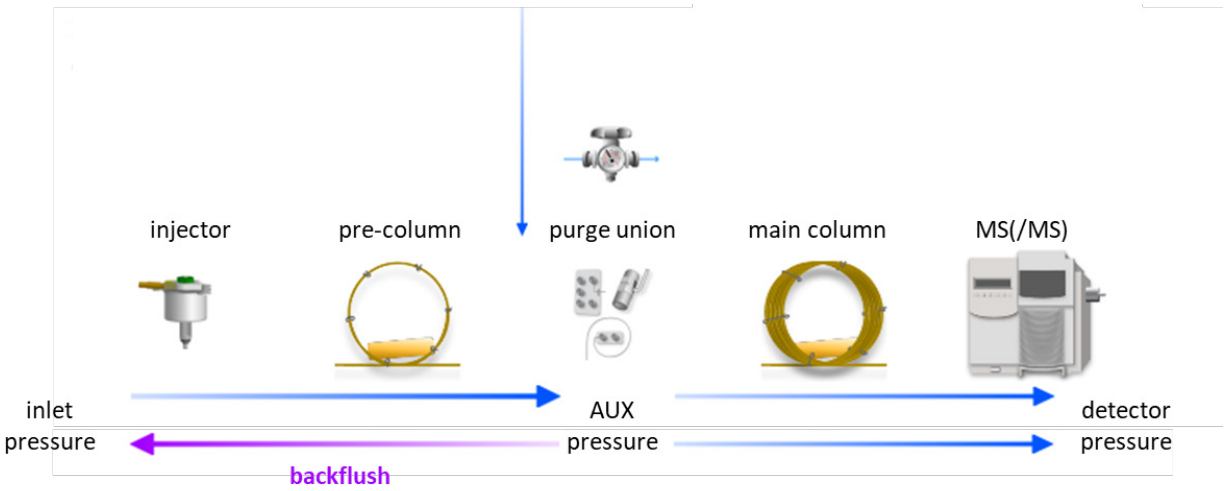

Figure 8: Flow diagram for pre-column backflush: The blue arrows from the injector to the purge union indicate the flow direction during analyte transfer to the main column for separation and MS detection. After a defined time, the flow direction from the purge union to the injector (purple arrow) is changed via the pressure control of the auxiliary pressure and the matrix and reagent residues still remaining on the pre-column are backflushed.

Validation

Figure 9 shows a typical chromatogram. All analytes are well separated, and the deuterated internal standard elutes slightly earlier. No interfering matrix peaks are visible in the vicinity of the analyte or internal standard peaks.

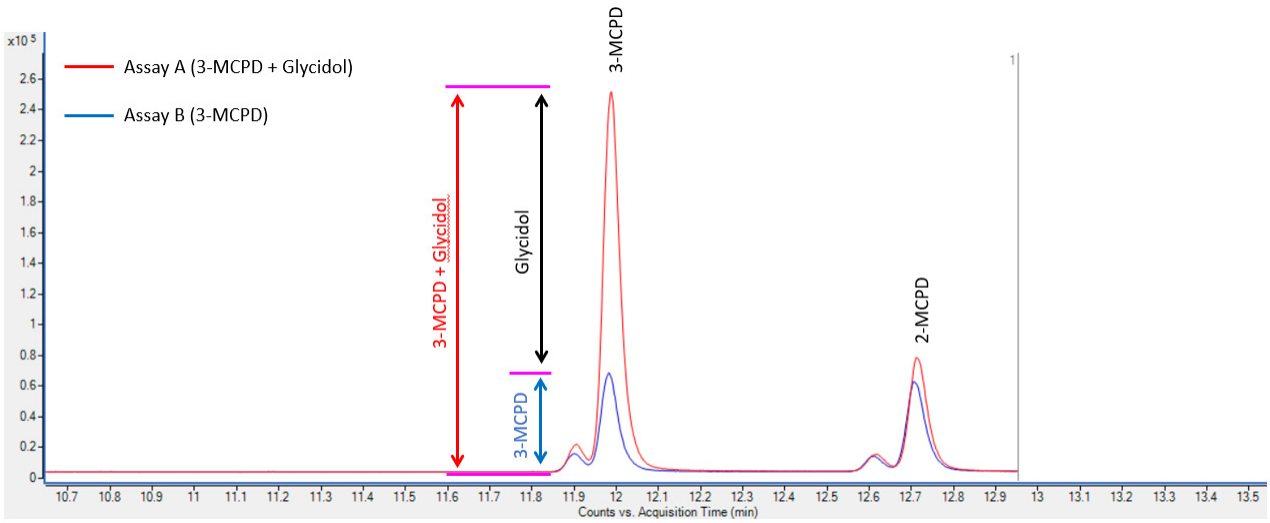

Figure 9: Representative chromatogram: The red chromatogram shows assay A with 3-MCPD, glycidol and 2-MCPD; the blue chromatogram shows assay B with 3-MCPD and 2-MCPD.

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The limits of quantification (LOQs) and limits of detection (LODs) were calculated according to DIN 32645 (11) using a calibration of spiked blank oils with points evenly distributed around the expected LOQ. The resulting LOQs and LODs are summarized in Table 2. They were well below 100 µg/kg. Figure 10 shows representative chromatograms close to the determined LOQs for all analytes. No interfering peaks are visible and the quantifier/qualifier ratios are within the required range.

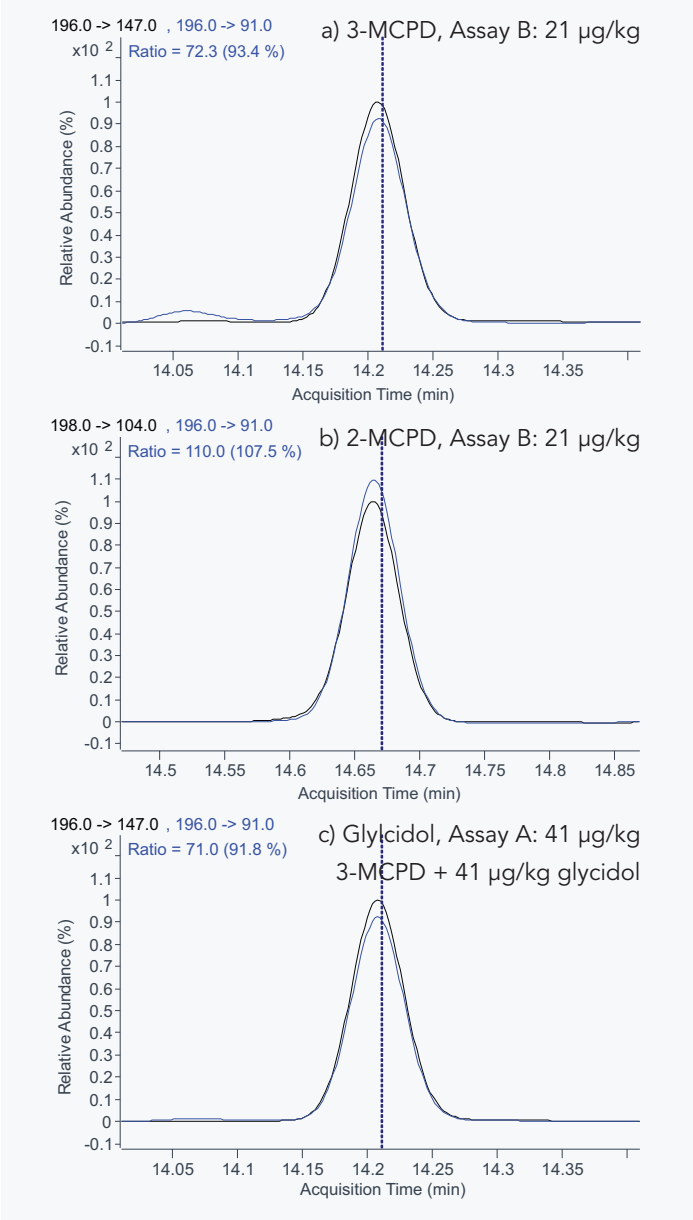

Figure 10: Typical chromatogram traces, quantifier and qualifier, for each compound near their respective limits of quantification. a) 3-MCPD at 21 µg/kg, b) 2-MCPD at 21 µg/kg and c) glycidol at 41 µg/kg.

Typical calibration curves are shown in figure 11. Coefficients of determination are larger than 0.999 for 2-MCPD, 3-MCPD and glycidol. Calibrations are linear from the LOQ to the highest calibration level mentioned in the standard.

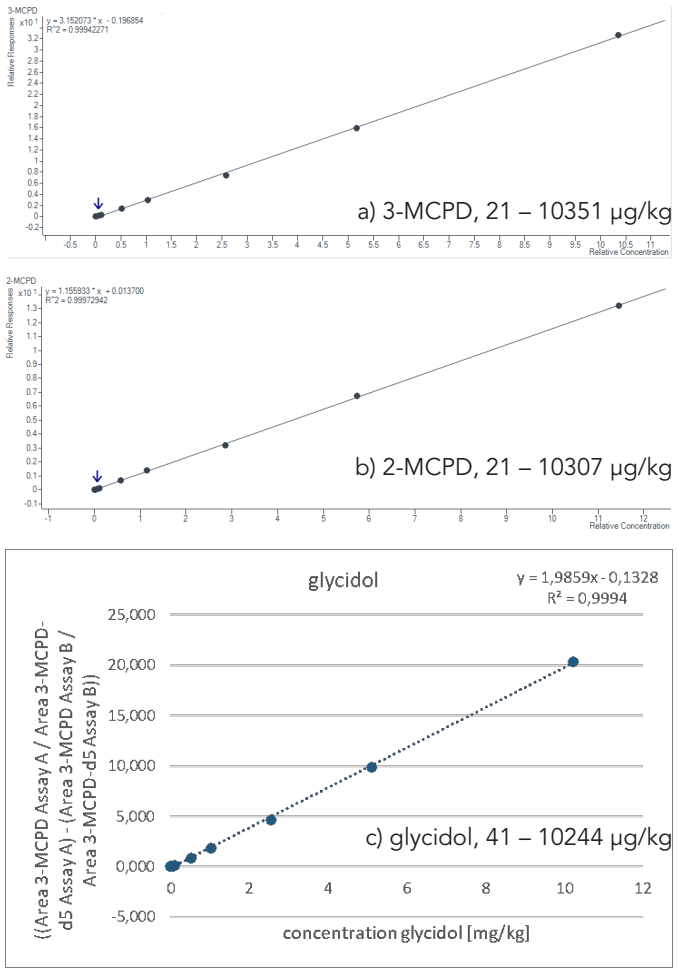

Figure 11: Typical calibration curves for each compound, a) 3-MPCD, b) 2-MCPD, c) glycidol.

Limits of quantification (LOQs) and limits of detection (LODs) were calculated according to DIN 32645 employing a calibration based on spiked blank oils with equidistant concentration points around the expected LOQ. Resulting LOQs and LODs are summarized in Table 2.

Table 2: Collected validation data for the automated analysis method.

Compound	LOQ [µg/kg]	Calibration range [µg/kg]	Coefficient of Determination r^2
3-MCPD	26	21 - 10351	0.9994
2-MCPD	22	21 - 10307	0.9996
Glycidol	44	41 - 10244	0.9994

Table 3: Repeat analyses for determination of precision and trueness were performed (n=5). FAPAS reference material 2672 are: "Low" 174.0 µg/kg 3-MCPD, 62.0 µg/kg 2-MCPD, 99.9 µg/kg glycidol; Mix of refined and crude vegetable oils: 75.8 µg/kg 3- MCPD, 28.8 µg/kg 2-MCPD, 115.6 µg/kg glycidol; Used frying oil: 601.2 µg/kg 3-MCPD, 266 µg/kg 2-MCPD, 85.1 µg/kg glycidol.

Compound	Precision FAPAS 2672 RSD [%]	Trueness FAPAS 2672 [%]	Precision vegetable oils* RSD [%]	Trueness vegetable oils* [%]	Precision used frying oil* RSD [%]	Trueness used frying oil* [%]
3-MCPD	0.4	106	3.3	111	2.8	117
2-MCPD	3.0	117	2.2	123	2.2	119
Glycidol	1.7	92	0.9	99	16.5	91

* Samples "Mix of refined and crude vegetable oils (Sunflower and others)" and "Used Frying Oil" are DGF round robin test samples (29th DGF Proficiency Test on Fat Analysis 2023), which we passed with full marks.

Table 4: Content of 3-MCPD, 2-MCPD and glycidol in real samples.

	3-MCPD [µg/kg]	3-MCPD reference value [µg/kg]	2-MCPD [µg/kg]	2-MCPD reference value [µg/kg]	Gly [µg/kg]	Gly reference value [µg/kg]	Comment
FAPAS 2672	184	174	72	62	92	99.9	Proficiency test
Pasta oil	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Walnut oil	774	N/A	375	N/A	792	N/A	Purchased in supermarket
Germ oil	301	N/A	149	N/A	390	N/A	Purchased in supermarket
Omega 3 capsules	549	N/A	79	N/A	163	N/A	Purchased in supermarket
Frying and rapeseed oil	52	N/A	< LOQ	N/A	64	N/A	Purchased in supermarket
Sunflower oil	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Frying oil	< LOQ	N/A	< LOQ	N/A	156	N/A	Purchased in supermarket
Linseed oil	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Pumpkin seed oil	99	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Rapeseed oil	79	N/A	< LOQ	N/A	< LOQ		Purchased in supermarket
Olive oil	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Clarified butter	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Lard	90	N/A	37	N/A	< LOQ	N/A	Purchased in supermarket
Sample A (29th DGF Proficiency Test)	97	75.8	32.5	28.8	112.5	115.6	Round robin test 2023 DGF proficiency test
Sample B (29th DGF Proficiency Test)	10 (< LOQ)	N/A	3 (< LOQ)	5.8 (< LOQ)	26 (< LOQ)	38.7 (< LOQ)	Round robin test 2023 DGF proficiency test
Sample C (29th DGF Proficiency Test)	665.5	601.2	293	266	106	85.1	Round robin test 2023 DGF proficiency test

Conclusion

In this work, we have shown that the combination of the two methods AOCS Cd 29c-13 and DGS C-VI 18 (10) can be fully automated using the GERSTEL MultiPurpose Sampler MPS equipped with appropriate modules. The limits of quantification are well below 100 µg/kg, the relative standard deviations achieved were well below 5% with the exception of the used frying oil sample for glycidol and the accuracy mostly between 90 and 120%. The work presented here includes an automated evaporation step as required in the above-mentioned official methods. Another important aspect of the evaporation step is that it removes excess derivatization reagent that could otherwise accumulate in the GC-MS(/MS) system and compromise system stability. In addition, the pre-column backflush device shortens the analysis time and improves throughput, while keeping the analytical column and the mass spectrometer clean, enabling significantly longer maintenance intervals and improving the overall ruggedness of the method.

Literature

[1] DGF C-VI 18 (10), Supplementary notes March 2022. Link: livelinkfunc=ll2&objId=41603694&objAction=xmlexport&nodeinfo&committeeinfo&attributeinfo&transform&stylesheet=9619825 (Accessed on 20241107).

[2] DIN EN ISO 18363-1:2022-04 Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol (ISO 18363-1:2015); German version EN ISO 18363-1:2021

[3] AOCS Official Method Cd 29b-13 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by GC/MS (Difference Method)

[4] Eva Walter, Walter Rau Neusser Öl und Fett AG, a Bunge Company: Automated Solutions for Determining 3-MCPD and Glycidyl Fatty Acid Esters in Edible Oils and Fats. Webinar and executive summary. Link: https://webinar.sepscience.com/form/automated-solutions-for-determining-3-mcpd-and-glycidyl-fatty-acid-esters-in-edible-oils-and-fats (accessed on 20241107).