APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 239

Fully Automated Determination of 3-MCPD, 2-MCPD and Glycidol in Edible Oils and Fats based on ISO 18363-4 - Zwagerman/Overman Method

Oliver Lerch, Jasmin Zboron and Marco Wolff

GERSTEL GmbH & Co.KG, Eberhard-Gerstel-Platz 1, 45473 Mülheim an der Ruhr, Germany

Keywords

3-MCPD, 2-MCPD, Glycidol, edible oil and fat, ISO 18363-4, Zwagerman/Overman method, laboratory automation

Abstract

Fatty acid esters of 3- and 2-monochloropropanediol (3-MCPD-e, 2-MCPD-e) and glycidol (Gly-e) are process contaminants that are formed, for example, when edible oils and fats are refined. After ester cleavage during digestion in the human body they pose a relevant health risk and therefore need to be determined in edible oils and fats and in fat containing food.

A series of standardized analysis methods for indirect analysis of 3-MCPDe and Gly-e are currently available, laid down in different standard method documents. GERSTEL previously developed and presented comprehensively automated analysis systems following ISO 18363-1, AOCS Cd 29c-13, DGF C-VI 18 (10) (GERSTEL AppNote 191) and ISO 18363-3, AOCS Cd 29a-13 (GERSTEL AppNote 217). In this document, we present a system for comprehensive automation of the ISO 18363-4 (Zwagerman/Overman) method, the most recent standard method available for 3-MCPD-e, 2-MCPD-e and Gly-e determination.

When performing the analysis using the MultiPurpose Sampler (MPS) along with the necessary modules and options, the only manual steps the user needs to perform are weighing an aliquot of oil into a sample vial, sealing it, and placing it in the MPS sample tray. All other sample preparation steps listed in the norm and the subsequent GC-MS/MS determination are performed automatically. Limits of quantification (LOQs) of 15 µg/kg for 3-MCPD, 44 µg/kg for glycidol and 5.7 µg/kg for 2-MCPD are achieved, well below the 100 µg/kg LOQs required in the ISO standard for all three compounds. It was proven that by integrating an automated evaporation step into the workflow, even lower LOQs can be achieved. Precision of repeat analyses is mainly below 5% relative standard deviation for all three compounds, except for glycidol at low concentrations. Trueness was verified by analyzing a sample from a proficiency test and a sample for which external analysis results were available. When processing real world samples, the system and method were shown to work ruggedly and reliably. The complete setup and method parameters are bundled and made available for end-users enabling rapid method setup and analysis of 3-MCPD, 2-MCPD and glycidol by ISO 18363-4.

Introduction

Fatty acid esters of 3- and 2-monochloropropanediol (3-MCPD-e, 2-MCPD-e) and glycidol (Gly-e) are process contaminants that are present in a variety of fat containing foods. These compounds are formed in fatty foodstuffs in the presence of sodium chloride whenever high temperatures are applied during processing such as, for example, when refining edible oils and fats. During digestion in the human body, ester cleavage occurs and 3- and 2-monochloropropanediol (3-/2-MCPD) as well as glycidol (Gly) are released.

The International Agency for Research on Cancer (IARC) has classified glycidol in category 2A as probably carcinogenic to humans and 3-MCPD in category 2B as possibly carcinogenic to humans [1]. These relevant health risks have caused the European Union (EU) and many countries world-wide to define maximum acceptable concentrations for edible oils and fats and for fatty food [2].

APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 239

For glycidol, the maximum allowable amount in oils and fats is 1000 µg/kg. The maximum levels for food intended for infants and young children are between 6 and 500 µg/kg. For 3-MCPD the permitted levels for different oils and fats are between 1250 and 2500 µg/kg, and for food intended for infants and young children, the maximum allowable concentration is between 15 and 750 µg/kg. Since toxicological assessment for 2-MCPD is not completed yet, there are no maximum levels set for this compound so far.

A couple of standardized analysis methods for indirect analysis of 3-MCPD-e and Gly-e are already available, laid down in different documents of the International Organization for Standardization (ISO), the American Oil Chemists' Society (AOCS) and the German organization Deutsche Gesellschaft für Fettwissenschaft (DGF). All these methods rely on similar chemical processes and workflows, yet the reaction conditions, internal standards used, and calculation methods differ. Fats are saponified under alkaline or acidic conditions, the target analytes are released from their esters and the generated fatty acids are converted to their corresponding methyl esters (FAMEs). The reaction is quenched in the presence of an aqueous sodium chloride or sodium bromide solution, converting glycidol into 3-MCPD or 3-monobromopropanediol (3-MBPD), respectively. In method ISO 18363-3 (equivalent to AOCS Cd 29a-13) these steps are executed in reverse order, first the transformation of glycidol esters to 3-MBPD esters then the ester cleavage. Subsequently FAMEs are removed by extraction with a non-polar solvent while the analytes stay in the aqueous phase. They are derivatized by phenylboronic acid (PBA), and the derivatives extracted by a non-polar solvent and determined by GC-MS(/MS).

ISO 18363-2 (equivalent to AOCS Cd 29b-13) [3] and ISO 18363-3 (equivalent to AOCS Cd 29a-13) [4] are known to yield extremely accurate analysis results with high precision. However, these methods require a significant amount of time, including a transesterification step at -22 to -25 °C or at 40 °C for 16 h. These methods are often applied by contract labs as a kind of gold standard. Both methods can be automated by the GERSTEL MultiPurpose Sample (MPS), see AppNote 217 [5]. ISO 18363-1 (equivalent to AOCS Cd 29c-13 and DGF C-VI 18 (10)) [6] is the most widely used method. It does require two assays per sample, but no overnight incubation and is therefore much faster. The glycidol content is determined via a differential calculation based on the assay A and B results. This calculation is the key drawback of the method since statistical errors of both assays can accumulate for the glycidol determination. In addition, the method is prone to delivering an overestimate of the glycidol content in case of high 3-MCPD concentrations. During the transesterification, 3-MCPD is partly converted to glycidol, and the method does not correct for this. Despite these weaknesses, the method is widely used, especially in production laboratories where rapid results for release of products or delivered raw materials are needed. It has been completely automated using the GERSTEL MPS, see AppNote 191 [7].

ISO 18363-4 [8] is the most recent standard in the ISO series issued in August 2021. It originates from the work of Ralph Zwagerman and Pierre Overman from the Bunge Loders Croklaan company in The Netherlands [9,10]. They were searching for a fast alternative to the AOCS c-method which has drawbacks in the glycidol determination, as explained above. They found a way to determine glycidol directly from the sample and to compensate for deviations caused by glycidol formation from 3-MCPD during the transesterification step. All three analytes are determined in one assay based on calibration curves established before the analysis. To accurately quantify the amount of 3-MCPD that is converted to glycidol, which would otherwise lead to glycidol overestimation, the method applies ^{13}C-correction. In short, a known amount of 3-MCPD-$^{13}C_3$ ester is used to quantify the amount of glycidol-^{13}C3 formed during transesterification. The amount of glycidol-$^{13}C_3$ is then used to calculate the amount of glycidol formed from 3-MCPD enabling the analyst to correct for the overestimation.

This is the complete workflow of ISO 18363-4: The oil or fat sample is dissolved in toluene and methyl-tert-butyl-ether (MTBE). Subsequently, the internal standards 3-MCPD-$^{13}C_3$ diester, as internal standard for 3-MCPD and 2-MCPD, and pentadeuterated glycidyl ester as internal standard for glycidol are added. The sample is then cooled to 10 °C before the alkaline transesterification is initiated by addition of a sodium methoxide solution in methanol. After 12 min incubation at 10 °C, the sample mixture is acidified with an acidic solution of sodium bromide to convert the released glycidol to 3-MBPD. The fatty acid methyl esters generated during the transesterification are removed by extracting twice with iso-octane. The polar analytes remain in the aqueous phase and are derivatized with phenylboronic acid prior to GC-MS/MS determination. Figure 1 shows a representation of the described workflow. The quantification of ester bound 2-MCPD and 3-MCPD is based on the 2-MCPD/3-MCPD-$^{13}C_3$ and 3-MCPD/3-MCPD-$^{13}C_3$ signal ratios, respectively. The quantification of ester-bound glycidol is based on the 3-MBPD/3-MBPD-d_5 signal ratio. The amount

GERSTEL AppNote 239

of 3-MBPD-$^{13}C_3$ formed after the transesterification reaction signifies the amount of released 3-MCPD-$^{13}C_3$ that has degraded to glycidol due to the conditions of the alkaline transesterification. Since no difference in degradation speed between 3-MCPD and 3-MCPD-$^{13}C_3$ has been observed, the observed change in the 3-MCPD-$^{13}C_3$ concentration can be used to correct for overestimation of glycidol caused by degradation of 3-MCPD. Under the conditions used, the 2-MCPD is considered stable and thus will not significantly contribute to possible glycidol overestimation. In contrast to the other parts of the ISO 18363 series, ISO 18363-4 requires GC-MS/MS instrumentation to unambiguously determine the isotopically labelled 3-MBPD as required for correct quantification of the glycidyl ester induced glycidol.

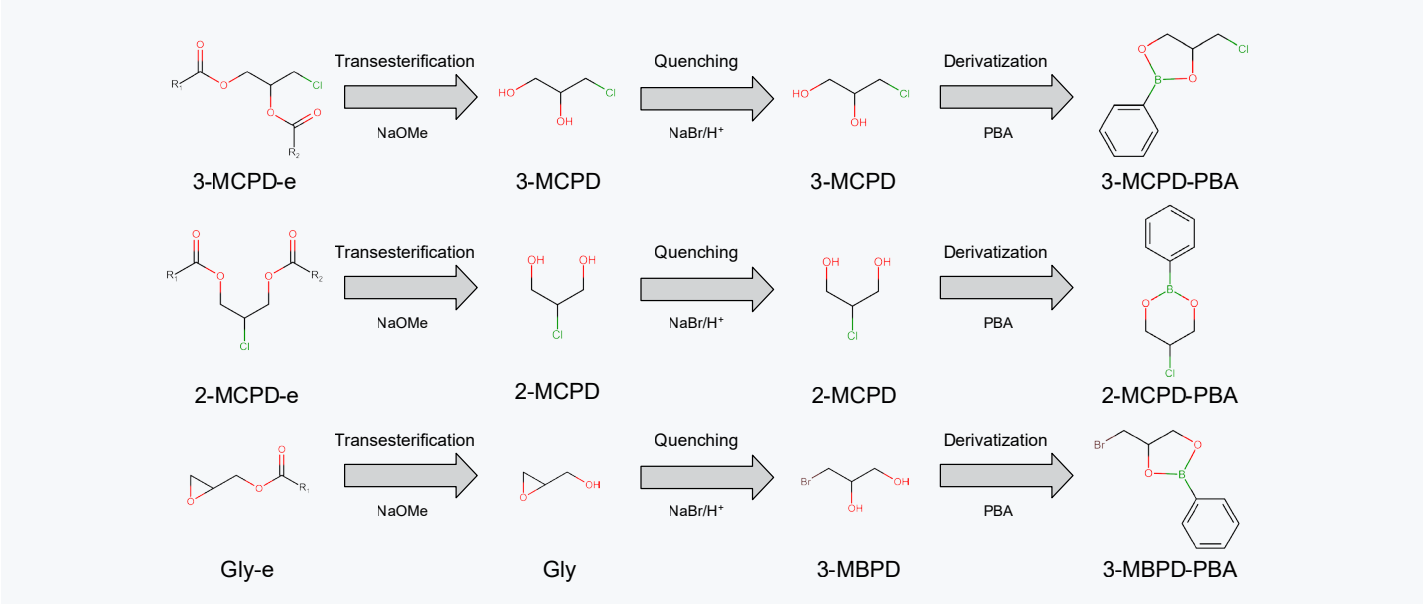

Figure 1: Overview of the chemical reactions performed in the sample preparation workflow of ISO 18363-4.

After weighing in an amount of fat or oil, all remaining sample preparation steps, and the injection into the GC-MS/MS system were performed by the GERSTEL MPS equipped with the necessary modules. The chromatographic system was fitted with a pre-column backflush to keep phenylboronic acid and major matrix constituents from entering and contaminating the analytical column and the mass spectrometer.

Experimental

Materials and Solvents

1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD), 1,3-distearoyl-2-chloropropanediol (SS-2-MCPD), ^{13}C labelled 1,2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD-$^{13}C_3$), glycidyl stearate (Gly-S) and pentadeuterated glycidyl stearate (Gly-S-d_5) each 1000 µg/mL in toluene were purchased from Campro Scientific, Berlin, Germany. Methanol, iso-octane, acetone, toluene, tert-butyl-methyl-ether (MTBE), water and concentrated sulfuric acid, all of analytical grade, were obtained from Merck, Darmstadt, Germany. Phenylboronic acid, sodium bromide and sodium methoxide solution (NaOMe, 25% m/m) were also from Merck.

A 25% (v/v) aqueous sulfuric acid solution was prepared by transferring 25 mL of concentrated sulfuric acid to a 100 mL volumetric flask containing 50 mL of water and topping up to 100 mL with water. The sodium bromide solution was prepared as follows: 600 g sodium bromide was dissolved in 700 mL water, 36 mL of the 25% sulfuric acid was added, and finally the solution was topped up to 1000 mL in a volumetric flask. A 0.35 M NaOMe solution was prepared by adding 20 mL of the 25% NaOMe solution to a 250 mL volumetric flask and filling it to volume with methanol. For a saturated PBA solution 12 g of PBA was dissolved in a mixture of acetone and water (95/5, v/v) under vigorous shaking (the PBA is not dissolved completely).

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. Glycidol at very low µg/kg levels was present in all tested potential blank oils.

GERSTEL AppNote 239

Preparation of Calibration Standards and Samples

As specified in the standard method, three calibration working solutions, one internal standard solution and one spiking solution were prepared. For calibration working solution I (Cal I) 21 µL PP-3-MCPD (1000 µg/mL), 21 µL SS-2-MCPD (1000 µg/mL) and 18 µL Gly-S (1000 µg/mL) were dissolved in 440 µL toluene. To prepare the calibration working solution II (Cal II) the purchased single standard solutions were diluted 1:10 resulting in a concentration of 100 µg/mL. Using these solutions 84 µL of PP-3-MCPD, 84 µL of SS-2-MCPD, and 72 µL of Gly-S were mixed with 260 µL of toluene. Calibration standard III (Cal III) was prepared by mixing 10 µL Cal I with 490 µL of toluene. An additional calibration standard (Cal IV), not mentioned in the norm, was prepared by diluting the purchased single standard solutions 1:100 resulting in a concentration of 10 µg/mL.

The spiking solution was prepared using 1:10 dilutions of the purchased single standards: 56 µL PP-3-MCPD, 48 µL Gly-S and 40 µL SS-2-MCPD dissolved in 856 µL toluene. The internal standard working solution (ISTD) was prepared by mixing 40 µL of the purchased single solution (1000 µg/mL) of Gly-S-d_5, 80 µL of the purchased single solution (1000 µg/mL) of PP-3-MCPD-$^{13}C_3$ and adding 9880 µL of toluene.

Calibration standards were prepared by weighing 100 mg of blank oil into a 4 mL round bottom vial and spiking it with the required volumes of calibration working solution Cal I to III, respectively, as mentioned in table A.1 of the standard. Additional calibrators in the low concentration range were prepared by spiking blank oil with Cal IV solution. The vials were capped with a magnetic screw cap and placed in the MPS sample tray for further processing.

Real oil and fat samples (all in the amount of 100 mg in a 4 mL round bottom vial) and calibration samples were subjected to the following workflow steps, all performed by the MPS:

- Add 100 µL toluene (or the volume specified in table A.1 for the calibration standards)
- Add 200 µL MTBE
- Add 100 µL ISTD solution
- Agitate at 250 rpm and 80 °C for 120 s to melt or solve the oil or fat
- Homogenize for 10 s in the quickMIX module
- Cool the vial in a cooled tray at 10 °C for 240 s
- Add 200 µL NaOMe solution to start the transesterification
- Homogenize for 10 s in the quickMIX module
- Cool the vial for 12 min at 10 °C
- Add of 700 µL acidic sodium bromide solution to stop the reaction
- Homogenize for 10 s in the quickMIX module
- Keep the sample for 5 min at room temperature for complete conversion of Gly to 3-MBPD
- Add 300 µL iso-octane
- Homogenize for 10 s in the quickMIX module
- Agitate at 400 rpm and 80 °C for 270 s in order to dissolve potentially jellified upper layer
- After 3 min at room temperature remove the upper iso-octane/toluene/MTBE layer with the FAMEs
- Add 600 µL iso-octane
- Homogenize for 10 s in the quickMIX module
- Remove the upper iso-octane layer with the FAMEs
- Add 100 µL PBA solution to derivatize the analytes
- Add 600 µL iso-octane
- Homogenize for 1 min in the quickMIX module to extract the derivatives into the iso-octane layer
- Inject 2 µL of the iso-octane layer into the GC-MS/MS

Instrumentation

They analysis system used is shown in figure 2. 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 for sample introduction (left), the other with a 1 mL syringe for sample preparation (right). From left to right the following modules are included: Two tray holders for sample/extract vials, a 10 mL wash station, several 180 mL solvent reservoirs, an mVAP evaporation station, which in this case is used only for sample heating. The solvent evaporation needed for ISO 18363-1 can be performed using the same instrument. Further, a quickMIX module high power agitator, a high flow fast wash station, a cooled stack, and a syringe module exchange station.

An Agilent Technologies (Waldbronn, Germany) 7890 or 8890 with split-splitless inlet was used in combination with an Agilent Technologies 7010 triple quadrupole mass spectrometer for analysis of the prepared samples.

GERSTEL AppNote 239

Figure 2: Analysis system used for automated determination of 3-/2-MCPD- and glycidol-esters from edible oils and fats according to ISO 18363-4. The MultiPurpose Sampler (MPS) mounted on top of the GC-MS/MS performs all sample preparation steps. The heads are equipped with a 10 µL syringe for sample injection (left) and a 1 mL syringe for sample preparation (right). From left to right the modules included are: two tray holders for sample/extract vials, a 10 mL wash station, solvent reservoirs, an evaporation station (here used for sample heating only, not for solvent evaporation. Needed for ISO 18363-1, which this instrument performs as well), a quickMIX high-power agitator, a high flow fast wash station, a cooled stack and a syringe module exchange station.

Analysis Conditions

GC-MS/MS conditions were mainly set up as specified in the standard, a few parameters were optimized or had to be adapted. The GC was outfitted with a DB-5ms 10 m, 0.32 mm ID, 0.10 µm film pre-column connected to the analytical column, a DB-5ms Ultra Inert 20 m, 0.18 mm ID, 0.18 µm film using a purged ultimate union (all Agilent Technologies). Mid-column backflush was employed to prevent excess PBA and FAMEs from entering and contaminating the analytical column and the mass spectrometer. In contrast to the standard method, in which a 2 m, 0.53 mm ID pre column is mentioned, a 0.32 mm ID precolumn showed better performance in our setup. The following analysis parameters were chosen:

GC Agilent 7890	
Inlet	Split/splitless
Liner	Splitless, double taper, ultra Inert
Injection volume	2 µL
Pneumatics	Splitless for 1.4 min, then 100 mL/min 5 mL/min septum purge
Temperature	350 °C
Pre-column	10 m DB-5ms (Agilent), d_i=0.32 mm, d_f=0.10 µm
Column	20 m DB-5ms ultra inert (Agilent), d_i=0.18 mm, d_f=0.18 µm
Pneumatics	He; constant flow, 1.7 mL/min
Backflush	at 7.3 min, with 25 mL/min
Temperature	70 °C (1 min), 15 °C/min to 120 °C (0.5 min), 40 °C/min to 320 °C (5 min)
MS/MS Agilent 7010	
Ionization	Electron impact (EI) at 70 eV
Mode	Multiple Reaction Monitoring (MRM), for details see table 1
Source Temperature	290 °C
Quadrupole Temperature	150 °C
Collision Gas Flow	N_2, 1.5 mL/min
Quench Gas Flow	He, 2.25 mL/min
Transfer line Temperature	315 °C

GERSTEL AppNote 239

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

Analyte	ISTD	Precursor Ion [Da]	Product Ion [Da]	Purpose
3-MCPD		196	147	Quantification 3-MCPD
3-MCPD		198	147	Qualification 3-MCPD
3-MCPD-$^{13}C_3$	x	199	149	Quantification, ISTD 3-/2-MCPD
3-MCPD-$^{13}C_3$	x	201	149	Qualification, ISTD 3-/2-MCPD
2-MCPD		196	104	Quantification 2-MCPD
2-MCPD		198	104	Qualification 2-MCPD
3-MBPD		240	147	Quantification Gly
3-MBPD		242	147	Qualification Gly
3-MBPD-d_5	x	245	150	Quantification, ISTD Gly
3-MBPD-d_5	x	247	150	Qualification, ISTD Gly
3-MBPD-$^{13}C_3$	x	243	149	Quantification, correction Gly overestimation

Results and Discussion

Sample Preparation Parameters

During development of the automated sample preparation method, we adhered strictly to the volumes, times and other parameters listed in the ISO standard. The complete workflow was conveniently set up in the GERSTEL Maestro software, see figure 3. Each single action was examined thoroughly, and many parameters, such as, for example, aspirating and dispensing speeds and penetration depths were optimized to achieve a fast, rugged, and repeatable workflow.

Injection and Backflush Parameters

Different inlet liners were tested for providing the most intense peaks and good peak shapes. A splitless, double taper, Ultra Inert liner turned out to be the best in this respect. Additionally, injection parameters were varied and peak areas and repeatability of five injections were recorded. It was established, that a sandwich injection with the sample between two air segments in a gas tight 10 µL syringe was best suited. The fast injection cycle was not used and waiting times with the needle in the hot injector were found to be non-beneficial. Finally, the splitless time was optimized to be long enough for achieving a complete transfer of all analytes onto the analytical column, on the one hand, and on the other hand as short as possible to prevent excessive transfer of PBA, FAMEs, or other matrix constituents to the analytical column. A splitless time of 1.4 min was found to be the optimum, in contrast with the 2 min mentioned in the ISO standard.

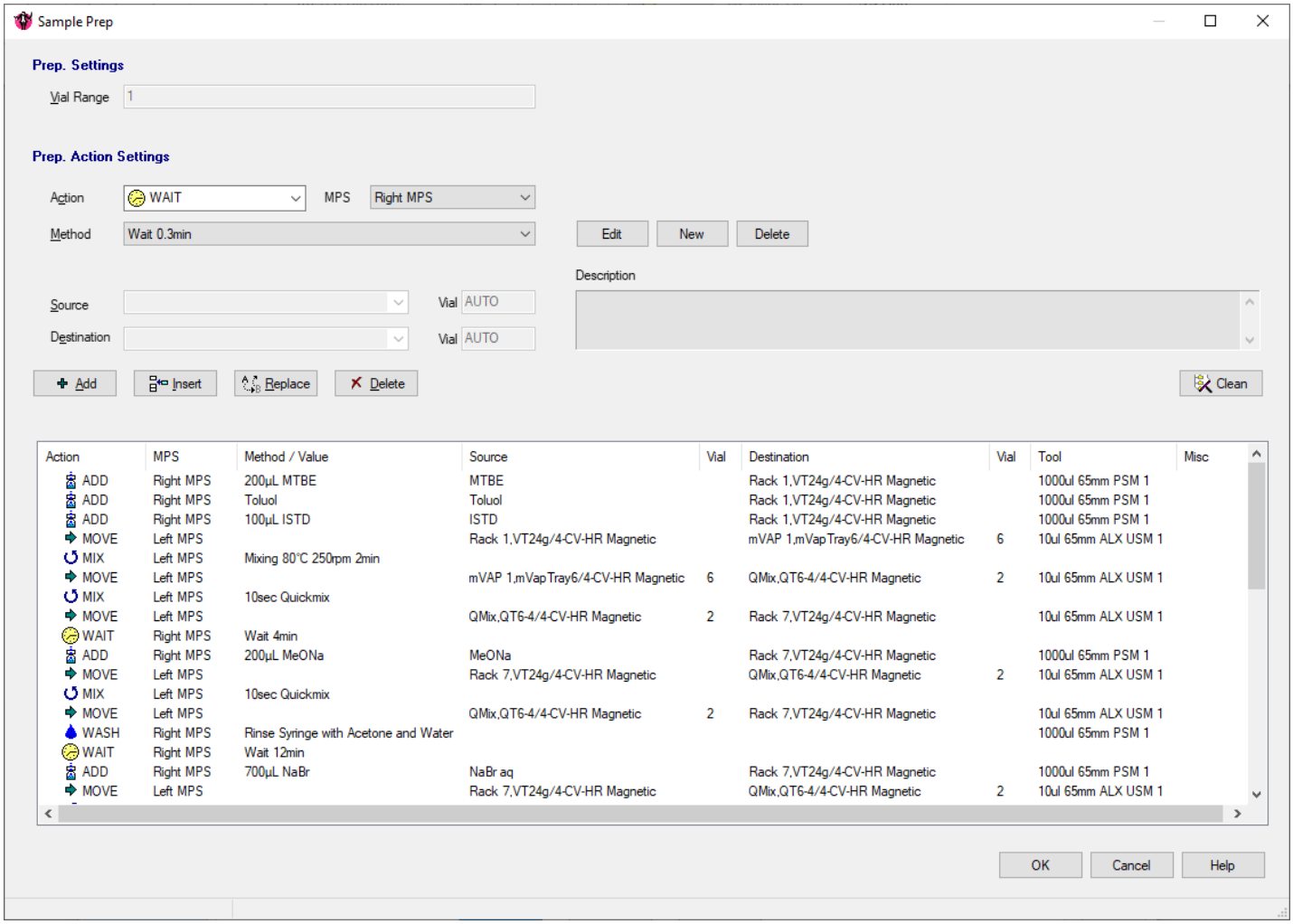

Figure 3: Excerpt of the Maestro sample preparation sequence.

To implement pre-column backflush, at first a 2 m, 0.53 µm ID, 0.1 µm film pre-column was tested, as recommended in the standard. It was determined that this column diameter did not result in stable pressure conditions during the analytical GC run since the pre-column did not have enough restriction. Very likely the backflush described in the ISO standard is an injector backflush only since this is mentioned in the publications of Zwagerman and Overman [9,10]. This means that the pre-column is not backflushed and therefore its restriction is not relevant for the GC pneumatics. With a 10 m, 0.32 mm ID, 0.1 µm film pre-column reliable pressure conditions could be achieved, and pre-column backflush was successfully set up.

GERSTEL AppNote 239

A midpoint backflush between the pre-column and the analytical column is more effective in protecting the analytical column and the mass spectrometer from unwanted matrix. At the time-point at which backflushing is initiated, all analytes must have been transferred onto the analytical column, which was determined to be 7.3 min (see figure 4).

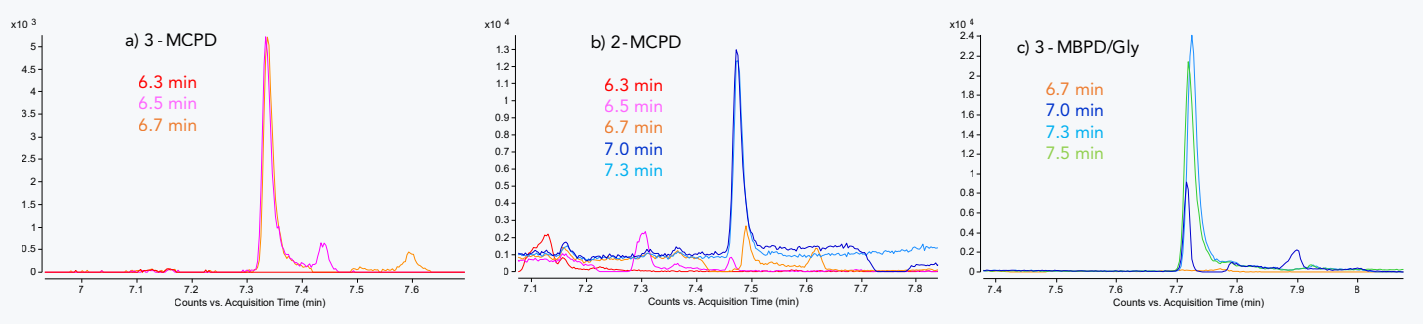

Figure 4: Optimization of backflush activation time. Starting the backflush at 6.3 min or less results in loss of 3-MCPD (a), starting the backflush at 6.7 min or less results in loss of 2-MCPD (b), and starting the backflush at 7.0 min or less results in loss of 3-MBPD/Gly (c). A backflush activation time of 7.3 min was chosen for reliable and complete analyte transfer to the analytical column, on the one hand, and minimal matrix transfer on the other hand.

Figure 5 reveals the protecting effect of backflushing the pre-column. Full scan chromatograms of an extracted olive oil sample with and without pre-column and inlet backflush were recorded. By employing backflush, large amounts of matrix are prevented from entering the analytical column and the mass spectrometer, enabling extended maintenance intervals, and ensuring overall method ruggedness.

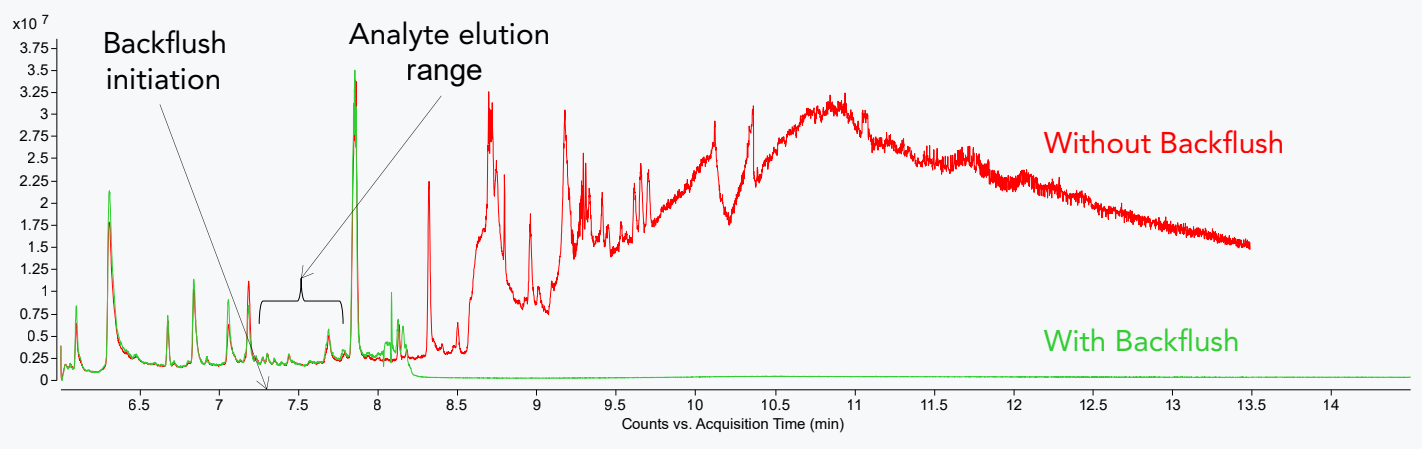

Figure 5: Illustrating the protective effect of pre-column and inlet backflush. Full scan chromatograms of an extracted olive oil sample with (green) and without (red) pre-column and inlet backflush. With backflush large amounts of matrix compounds are prevented from entering the analytical column and the mass spectrometer, enabling extended maintenance intervals, and ensuring overall method ruggedness.

GERSTEL AppNote 239

Validation

Figure 6 shows a typical chromatogram. All analytes are well separated while the deuterated internal standard elutes slightly before the respective analyte and the ^{13}C-marked internal standards elute exactly with the native compound. No interfering matrix peaks are visible in the vicinity of the analyte or internal standard peaks.

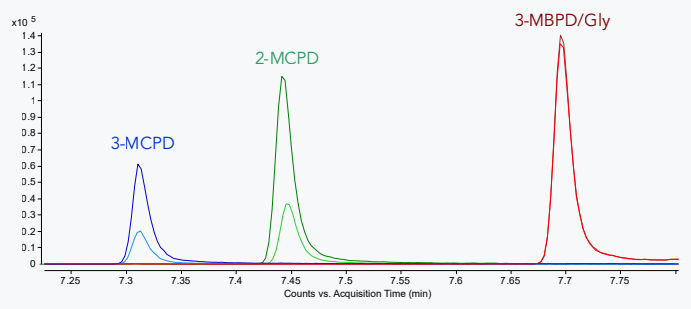

Figure 6: Representative chromatogram of a medium concentration calibration sample with 3-MCPD at 630 µg/kg, 2-MCPD at 570 µg/kg and Gly at 630 µg/kg.

Limits of quantification (LOQs) and limits of detection (LODs) were calculated according to DIN 32645 [11] employing a calibration from spiked blank oils with equally distanced points around the expected LOQ. Resulting LOQs and LODs are summarized in table 2. They were well below the 100 µg/kg level required by the ISO standard. Figure 7 shows representative chromatograms near the determined LOQs for all analytes. No interfering peaks are visible and quantifier/qualifier ratios are adequate. Evaporation of the final extract with the GERSTEL multi-position evaporation station (mVAP) and reconstitution in a smaller volume was tested and can lead to even lower LOQs.

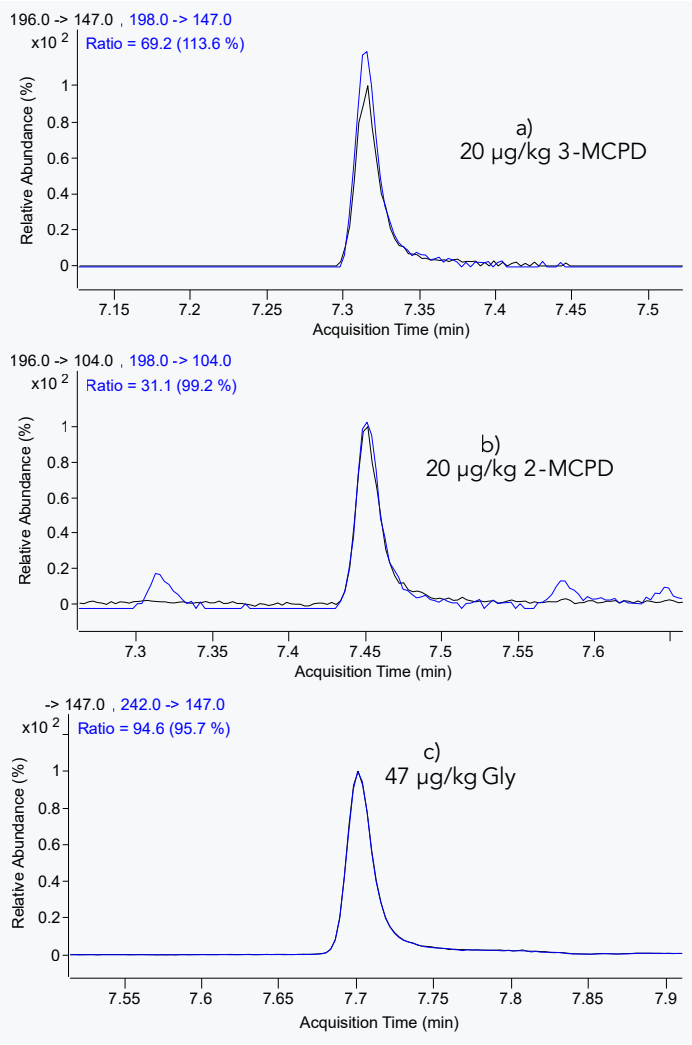

Figure 7: Typical chromatogram traces, quantifier and qualifier, for each compound near the respective limits of quantification. 3-MCPD a) at 20 µg/kg, 2-MCPD b) at 20 µg/kg and Gly c) at 47 µg/kg.

Table 2: Collected validation data for the automated analysis method. Repeat analyses for determination of precision and trueness were performed (n=8). Referred concentrations are: "Low" 50 µg/kg 3-MCPD, 50 µg/kg 2-MCPD, 80 µg/kg Gly; "High" 4740 µg/kg 3- MCPD, 4330 µg/kg 2-MCPD, 4700 µg/kg Gly; "High Oleic Sunflower Oil" 1180 µg/kg 3-MCPD, N/A 2-MCPD, 1150 µg/kg Gly.

Compound	LOQ [µg/kg]	Calibration range [µg/kg]	Coefficient of Determination r^2	Precision low/high RSD [%]	Trueness low/high [%]	Precision High Oleic Sunflower Oil RSD [%]	Trueness High Oleic Sunflower Oil [%]
3-MCPD	15	15 - 7900	0.9999	2.0	103	2.4	102
				0.9	99		
2-MCPD	5.7	15 - 7220	0.9988	1.6	94	6.5	N/A
				1.7	102		
Gly	44	47 - 7830	0.9996	32	77	7.9	101
				2.1	110		

GERSTEL AppNote 239

Typical calibration curves are shown in figure 8. Coefficients of determination are larger than 0.999 for 3-MCPD and Gly and larger 0.99 for 2-MCPD. Calibrations are linear from the LOQ to the highest calibration level mentioned in the standard.

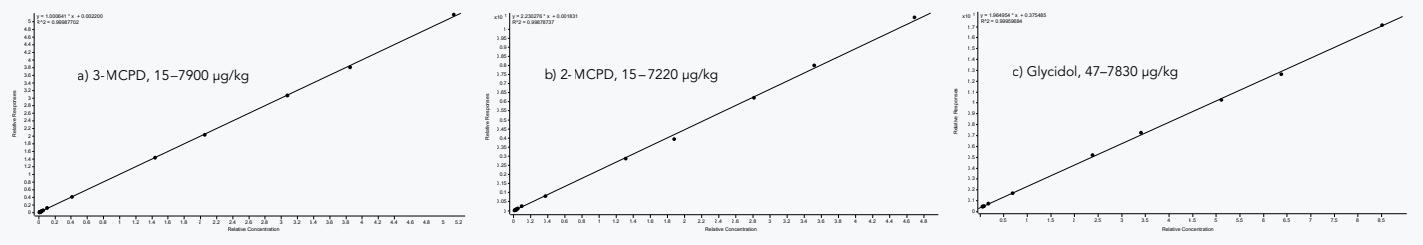

Figure 8: Typical calibration curves for each compound, a) 3-MPCD, b) 2-MCPD, c) Gly.

Application to Real Samples

More than 30 different real samples were analyzed with the developed method. No interfering matrix peaks or other chromatographic or mass spectral issues could be observed, and the sample preparation method worked ruggedly. Analyte concentrations were calculated according to chapter 9.2 and 9.3 of the standard. Analysis results for a proficiency test sample and for a sample analyzed by an external laboratory corresponded well with our results as can be seen in table 3. Additionally, in this table some more selected results for samples without a reference value are presented. All samples from the supermarket comply with EU regulations, except for 1847 µg/kg glycidol in the olive oil margarine which by far exceeds the 1000 µg/kg limit.

Table 3: Analysis results for real samples examined according to ISO 18363-4 with the developed automated workflow. Reference values are given in case they are available.

Sample	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
Sunflower oil 1	331	400 +/- 70	153	170 +/- 130	6040	5030 +/- 1790	Proficiency test
Sunflower oil 2	1197	1180	525	N/A	1175	1150	External sample
Sunflower oil 3	177	N/A	46	N/A	5148	N/A	External sample
Olive oil 1	70	N/A	30	N/A	116	N/A	Purchased in supermarket
Olive oil 2	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Olive oil margarine	134	N/A	53	N/A	1847	N/A	Purchased in supermarket
Rapeseed oil	110	N/A	46	N/A	507	N/A	External sample
Palm oil	221	N/A	82	N/A	206	N/A	External sample
Frying oil	144	N/A	54	N/A	271	N/A	Purchased in supermarket
Lineseed oil	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Pumpkin seed oil	< LOQ	N/A	< LOQ	N/A	170	N/A	Purchased in supermarket
Avocado oil	262	N/A	112	N/A	< LOQ	N/A	Purchased in supermarket
Clarified butter	< LOQ	N/A	< LOQ	N/A	< LOQ	N/A	Purchased in supermarket
Fish oil	323	N/A	45	N/A	143	N/A	Purchased in supermarket

Conclusions

In this work we have shown that the ISO 18363-4 method can be automated comprehensively using a GERSTEL MultiPurpose Sampler MPS equipped with adequate modules. Limits of quantification are well below 100 µg/kg as required by the method, relative standard deviations achieved were mainly well below 5% and trueness between 90 and 110% except for glycidol near the LOQ. The pre-column backflush setup keeps the analytical column and the mass spectrometer clean enabling extended maintenance intervals and ensuring overall method ruggedness. Analysis results obtained correlate well with reference data.

Acknowledgements

Dominik Lucas from GERSTEL GmbH & Co. KG is acknowledged for fruitful discussions during the project.

References

[1] https://monographs.iarc.who.int/list-of-classifications (accessed June 2nd, 2022)

[2] COMMISSION REGULATION (EU) 2020/1322 of 23 September 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of 3-monochloropropanediol (3-MCPD), 3-MCPD fatty acid esters and glycidyl fatty acid esters in certain foods, Official Journal of the European Union (2020) L 310/2-L 310/5

[3] ISO 18363-2:2018-08, Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 2: Method using slow alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol

[4] ISO 18363-3:2017-12, Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 3: Method using acid transesterification and measurement for 2-MCPD, 3-MCPD and glycidol

[5] C.M. Choo, M.P.A. Sabri, S. Goh, Fully Automated Determination of 3-MCPD & Glycidol in Edible Oils & Fats based on the AOCS Cd 29a-13 Method, GERSTEL Application Note No. 217 (2020), www.gerstel.com

[6] ISO 18363-1:2015-08, 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

[7] D. Lucas, A. Hoffmann, C. Gil, Fully Automated Determination of 3-MCPD and Glycidol in Edible Oils by GC/MS based on the Commonly Methods ISO 18363-1, AOCS Cd 29c-13, and DGF C-VI 18 (10), GERSTEL Application Note No. 191 (2017), www.gerstel.com

[8] ISO 18363-4:2021-08, Animal and vegetable fats and oils - Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS - Part 4: Method using fast alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol by GC-MS/MS

[9] R. Zwagerman, P. Overman, A novel method for the automatic sample preparation and analysis of 3-MCPD-, 2-MCPD-, and glycidylesters in edible oils and fats, European Journal of Lipid Science and Technology, 118 (2016) 997-1006

[10] R. Zwagerman, P. Overman, Optimized Analysis of MCPD- and Glycidyl Esters in Edible Oils and Fats Using Fast Alkaline Transesterification and ^{13}C-Correction for Glycidol Overestimation: Validation Including Interlaboratory Comparison, European Journal of Lipid Science and Technology, 121 (2019) DOI:10.1002/ejlt.201800395

[11] DIN 32645:2008-11, Chemical analysis - Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation