Source: istock / Kuzihar

# Focusing on 3-MCPD and Glycidol

When fat containing foods are processed and heated, fatty acid esters of 3- and 2-monochloropropanediol (3-MCPD and 2-MCPD), and of glycidol can be formed, all of which are unwanted process contaminants. To ensure a safe food supply, the presence of these compounds in food must be monitored. Given the abundance of food and food products, monitoring should be efficient and therefore automated. GERSTEL offers a wide spectrum of validated, proven automated analysis solutions.

*By Oliver Lerch, Ph.D. and Jasmin Zboron*

3-Monochloropropanediol (3-MCPD), 2-Monochloropropanediol (2-MCPD) and glycidol - as well as their fatty acid esters - are unwanted substances in food due to their health effects. These compounds can all be generated during food processing – especially when heating is involved. The compounds have been found in fatty bakery products, baby food, soy sauce, as well as edible fats and oils [1]. Free 3-MCPD and 2-MCPD can be formed when fat and salt containing food is exposed to high temperatures in the production process. The ester bound form of 2-MCPD, 3-MCPD as well as Glycidol are formed during refining of oil types that are not edible in the native form. Refining, and especially deodorization involves heating the oil or fat to temperatures between 200 and 300 °C to remove off-flavors and contaminant residues.

## Dangerous process contaminants

The International Agency for Cancer Research (IACR) has designated 3-MCPD a potential carcinogen [2]. During food production and processing, it is nearly impossible to completely avoid the formation of 3-MCPD, and therefore the European Food Safety Agency (EFSA) aims to limit the exposure, having defined a tolerable daily intake (TDI) of 0.8 µg of 3-MCPD per kg body weight [3]. For 2-MCPD and its fatty acid esters, an assessment of the health risks is currently not possible due to a lack of toxicological data [1]. The IACR categorizes glycidol as a probable carcinogen and as genotoxic.
The contaminant levels in food must be reduced to minimize consumer health impact – especially for infants who are not breastfed and are instead given

food produced in an industrial process. Reaching such ambitious goals requires use of highly sensitive laboratory instruments for chemical analysis.

### Maximum Levels in certain foods:

The European Union (EU) Commission has defined maximum levels in Directive 2020/1322 [4]:

#### 3-MCPD

- Plant based oils and fats (palm-, sunflower-, rape-, and olive oil etc.): 1250 µg/kg
- Other plant- and animal-based oils / fats: 2000 µg/kg
- Baby food/infant formula and ingredients used: 15 to 750 µg/kg

#### Glycidol

- Plant based oils and fats, fish oil, oil from marine organisms etc.: 1000 µg/kg
- Baby food/infant formula and ingredients used: 6 to 500 µg/kg

## Choosing the right method

In collaboration with food producers and contract laboratories, GERSTEL has developed fully automated analysis solutions that meet the requirements of key international methods for determination of 3-MCPD, 2-MCPD and Glycidol present in their fatty acid ester form. The solutions are being used successfully in the laboratories of leading international companies and of reputable contract laboratories and public institutes.

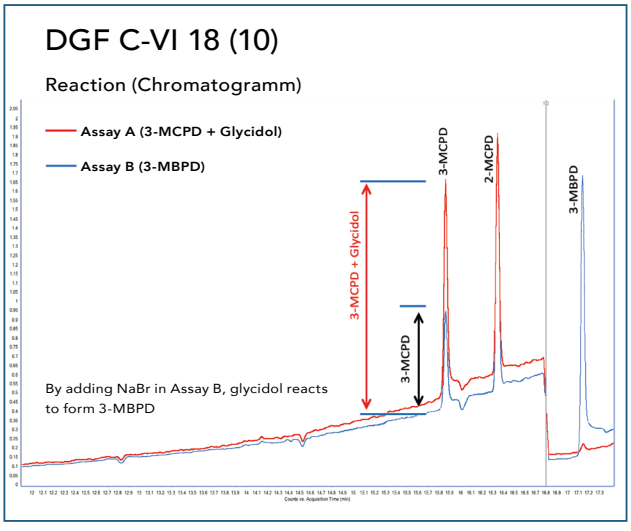
All methods listed and presented in this article are based on the same chemistry through hydrolysis. Further, glycidol is transformed to 3-MCPD or 3-MBPD (brominated form) respectively. After derivatization with phenylboronic acid (PBA), the analytes are finally determined directly or indirectly by GC-MS or GC-MS/MS. Differences between the listed methods are mainly found in the reaction control, the internal standards added, and the data handling and calculations performed. Incidentally, if the sample is a complex food type, such as a bakery product, a nougat cream that contains nuts, a chocolate bar, or similar, a further sample preparation step is required: Extraction of the fat contained in the product. The 3-MCPD, 2-MCPD, and glycidol levels are then determined in the extract.

## Differential method

The "original" 3-MCPD solution, described in GERSTEL Application Note No. 191 (2017) [5] performs fully automated determination of 3-MCPD and glycidol fatty acid esters in plant-based oil following the official AOCS* method Cd 29c-13 [6], the DIN EN ISO 18363-1 method [7], and the DGF** Unified method C-VI 18 (10). The system is available from GERSTEL as customer specific solution based on the GERSTEL MultiPurpose Sampler (MPS). Two sample or extract aliquots are saponified and the reaction stopped in the presence of an acidic chloride- or bromide solution respectively. This step influences the conversion of the ester-bound analytes in their free form and enables the determination of the original levels of 3-MCPD and glycidol in the sample. This method, referred to as the differential method, enables the fast and efficient determination of the analytes. It is the most frequently used method and is implemented worldwide in incoming raw material quality control (QC) as well as quality QC of the final product in oil mills, in the food industry, and in contract laboratories.

**AOCS Cd 29c-13**
**DIN EN ISO 18363-1:2015**
**DGF C-VI 18 (10)**

*The American Oil Chemists' Society
**Deutsche Gesellschaft für Fettwissenschaften




## Unilever method

AOCS Method Cd 29a-13, respectively the DIN EN ISO 18363-3:2017 method also known as the Unilever Method. In collaboration with a customer laboratory, GERSTEL application scientists developed an automated application solution for the Unilever method, described in AppNote 217 [8]. The main difference from the differential method lies in the sample preparation. Just one analysis run per sample is required and the analytes in question are released gradually in steps: Glycidyl esters react with acidified sodium bromide solution to their corresponding 3-Monobromopropanediol-fatty acid esters (3-MBPD). The fatty acid esters are extracted with hexane the extract is then subjected to evaporative concentration, and the residue is taken up in tetrahydrofuran (THF). Subsequently, 3-MBPD-, 2-MCPD- and 3-MCPD fatty acid esters are hydrolyzed to their free form in acidic media at 40 °C for 16 h. Following derivatization with PBA in an ultrasonic bath, the analytes are extracted with hexane. The extract is again evaporated, and the residue taken up in another solvent and finally separated and determined using GC-MS. The automated sample preparation meets the requirements of the standard method and greatly simplifies the analysis thanks to the significant degree of automation.

**AOCS Cd 29a-13**
**DIN EN ISO 18363-3:2017**

## Kuhlmann or SGS 3-in-1 method

Known as the "Volvo" among the 3-MCPD and glycidol fatty acid ester methods, the DIN EN ISO 18363-2:2018, respectively AOCS Cd 29b-13 method, are collectively referred to as the Kuhlmann- or SGS 3-in-1 method [11], a rugged method that delivers good results reliably. It is a relatively slow, but highly precise method that requires sample cooling to between -22 °C and -25 °C. The method including sample preparation and analysis can be fully automated using an integrated GERSTEL system complete with GC-MS.

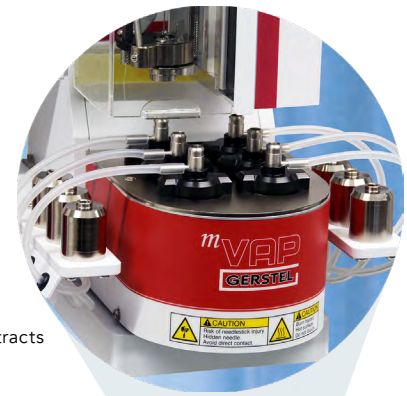
**AOCS Cd 29b-13**
**DIN EN ISO 18363-2:2018**

## Zwagerman / Overman method

ISO 18363-4:2021 [9] is the most recent standard method for the determination of 3-MCPD, 2-MCPD and glycidol fatty acid esters in edible oils. The method was devised by two Dutch scientists, Zwagerman and Overman [10]. Several internal standards are used to compensate for even minor deviations in chemical reactions and in sample preparation. This concerns, among other things, an overestimation of glycidol that has been observed in the presence of large amounts of 3-MCPD. The use of a triple quadrupole GC-MS/MS is required, analytes are determined in MRM Mode. The standard method including all sample preparation steps specified therein have successfully been transferred to a GC-MS/MS system directly coupled to a GERSTEL MPS leading to a fully automated workflow described in GERSTEL AppNote 239 [12]. Important: A high power mixing module is required for efficient vortex-like mixing during extraction, a cooled vial tray for accurate temperature control during transesterification and a fast wash station, all of which are integrated in the automated system.

**DIN EN ISO 18363-4:2021**

The only manual step that remains is to weigh the sample in an autosampler vial and transfer it to the MPS tray. To keep the system clean and reduce memory effects to a minimum, a backflush system prevents high-boiling matrix residue and reagent from reaching the analytical column and the MS/MS system.
The method was successfully validated and implemented for the analysis of plant- and animal-based oils and fats. Successful participation in round robins has demonstrated the high quality of the automated sample preparation process and the analysis systems. Relative Standard Deviations were between 0.1 and 10 % for all analytes in different matrices, with only a few values above 5 %. The Limit of Determination of 0.1 mg/kg required in the ISO method was reached. Lower LOQs are possible if a Multi-Position Evaporation module ($^{m}$VAP) is installed on the MPS. The chromatograms are simple, "clean ", and easy to interpret due to the high selectivity of the triple quadrupole GC-MS/MS that largely eliminates nontarget signal. Automation enables 24/7 operation and priority samples can easily be inserted into the running sequence as needed, adapting to urgent changes.




The GERSTEL mVAP enables automated evaporative concentration of extracts as specified in standard methods. Up to 6 extracts can be concentrated in one batch.


AOCS Cd 29c-13 · ISO 18363-1
Differential method

## Selecting a method

Compared with DIN EN ISO 18363-1, the 18363-4 (Zwagerman/Overman) method is somewhat faster (one assay per sample and calibration is required) and it is much faster than the DIN EN ISO 18363-2 and -3 methods. The DIN EN ISO 18363-4 method relies on multiple expensive internal standards but it delivers highly accurate and precise results with good trueness, for example, compared with DIN EN ISO 18363-1. The methods DIN EN ISO 18363-1 and -4 are most often used where results are needed quickly in a production laboratory or for incoming or outgoing quality control, accepting or releasing a shipment; the ISO 18363- 1 (differential method) is used more widely than the ISO 18363-4 (Zwagerman/Overman method), mainly because DIN EN ISO 18363-1 was accepted as a standard method much earlier. Many operations are reluctant to change from a well-known method that delivers good results in a reliable manner – even when a new method offers advantages. The transition to the Zwagerman/Overman method is therefore typically a drawn-out process – unless the analytical requirements involved are changed and updated. This means that laboratories are well advised to prepare and broaden their offering to meet ever changing market demands. If you are interested in learning more about updating your lab to perform automated 3-MCPD analysis, please contact GERSTEL to discuss which solution will best meet the requirements of your organization and how to best approach the analysis of your particular samples.

## LITERATURE REFERENCES

1. Bundesinstitut für Risikobewertung (BfR), Possible health risks due to high concentrations of 3-MCPD and glycidyl fatty acid esters in certain foods BfR Opinion No 020/2020 issued 20 April 2020 https://www.bfr.bund.de/cm/349/possible-health-risks-due-to-high-concentrations-of-3-MCPD-and-glycidyl-fatty-acid-esters-in-certain-foods.pdf
2. Y. Grosse, R. Baan, B. Secretan-Lauby et al., Carcinogenicity of chemicals in industrial and consumer products, food contaminants and flavourings, and water chlorination byproducts, The Lancet Oncology 12 (2011) 328-329, https://doi.org/10.1016/S1470-2045(11)70088-2
3. EFSA, Process contaminants in vegetable oils and foods https://www.efsa.europa.eu/en/press/news/process-contaminants-vegetable-oils-and-foods
4. COMMISSION REGULATION (EU) 2020/1322 of 23.9.2020 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, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R1322&from=PL
5. D. Lucas, A. Hoffmann, C. Gil, 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). GERSTEL AppNote 191 https://gerstel.com/en/3-MCPD-Glycidol-in-oils-fats-AOCS-Cd-29c-13-ISO-18363-1
6. https://www.aocs.org/attain-lab-services/methods/methods/search-results?method=118272
7. https://www.beuth.de/en/standard/iso-18363-1/241591453
8. C. Choo, M. Sabri, S. Goh, Fully Automated Determination of 3-MCPD & Glycidol based on the AOCS Cd 29a-13 Method, AppNote 217 https://gerstel.com/en/3-MCPD-Glycidol-in-oils-fats-AOCS-Cd-29a-13-ISO-18363-3
9. ISO 18363-4:2021, 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
10. 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
11. J. Kuhlmann, Collaborative Study for the Quantification of Total Contents of 2- and 3-MCPD and Glycidol in Food Emulsifiers by GC-MS, Authorea, 29. Juni 2021, https://doi.org/10.22541/au.162499187.75255939/v1
12. O. Lerch, J. Zboron and M. Wolff, Fully Automated Determination of 3-MCPD, 2-MCPD and Glycidol in Edible Oils and Fats based on ISO 18363-4 - Zwagerman/Overman Method. GERSTEL AppNote 239. https://gerstel.com/en/3-MCPD-Glycidol-in-oils-fats-ISO-18363-4