CHRONECT by TRAJAN

APPLICATION NOTE

# Determination of MCPD and glycidyl esters in foodstuff

## with the CHRONECT™ Workstation MCPD and the module Draft ISO 18363-4

### Introduction

The determination of 2-MCPD, 3-MCPD and glycidol in oils, fats and fatty foods has become an important analytical method in recent years. Glycidol is classified as a carcinogen and 3-MCPD as a possible carcinogen. Both components are formed, among other things, during the processing of oils and fats at high temperatures. The concentration of these substances is particularly important in the area of baby food and in high-fat foods, where the tolerable daily intake is more easily exceeded. Whether as routine analysis of a contract/commercial laboratory or in a laboratory for the release of produced oils and fats - MCPD and glycidol analysis is increasingly implemented in food relevant laboratories. There are several different methods, which differ mainly in reaction conditions and reaction time. However, in the course of the analysis, both the fatty acid-bound 2-/3-MCPD and glycidol are separated from the fatty acids in all methods and then injected into the gas chromatograph with the aid of a derivatization reagent. The transesterification reaction takes place either in basic or acidic medium, at room temperature, 40 °C (according to ISO 18363-3, also known as the Unilever method or AOCS Cd29a-13) or at - 22 °C (according to ISO 18363-2, also known as the 3-in-1 method or AOCS Cd29b-13). In the case of alkaline transesterification (room temperature), the resulting free 3-MCPD is very rapidly converted to glycidol, which leads to a false result without compensation.

In some methods, this transformation of the glycidol is compensated in different ways. At - 25 °C, it is kinetically controlled, or it is recalculated by adding another halide (sodium bromide) in a second approach. This approach, described in the ISO 18363-1 method (also known as the DGF method or AOCS Cd29c-13), therefore contains the measurement uncertainties of two sample preparations. An alternative is the addition of a $^{13}$C3-labeled 3-MCPD ester standard, which allows the conversion of the 3-MCPD to be directly accounted for in one sample preparation run. Each sample thus requires only one sample preparation (Assay).

This modified method was published in 2015 by Zwagerman *et al.* and represents an elegant and robust alternative for process control. It is currently in the standardization process as Draft ISO 18363-4. As with the official alkaline retesting methods, the internal standard is first added to the sample. Subsequently, the reaction is started at 10 °C. After the reaction has been completed, it is stopped with an acidic sodium bromide solution. The matrix is removed in a further step with a non-polar solvent (hexane or heptane). Subsequently, the free 2-MCPD, 3-MCPD and glycidol (as 3-MBPD) are derivatized using phenylboronic acid (Figure 1). The resulting derivative is extracted with *iso*-octane and transferred to another vial containing sodium sulfate. The solution is then ready for injection into the GC-MS system. In the analysis, the resulting $^{13}$C3-3-MBPD is used to quantify the reverse reaction of glycidol to 3-MCPD.

### Method and device setup

The CHRONECT™ Workstation MCPD is equipped with the extension Draft ISO 18363-4 to perform the complete sample preparation and subsequent analysis fully automatically. The user only has to weigh a certain amount of fat or oil (~ 100 mg) into a 2 mL GC vial and place it into the autosampler. Transesterification, matrix removal, derivatization and injection (Figure 1) are then performed by the autosampler, controlled by the CHRONOS™ software. The core of this variant of the MCPD analysis is the addition of the $^{13}$C3-labeled 3-MCPD ester, which is automatically dosed by the CHRONECT robot Autosampler in the first step. This compensates for any differences due to different matrices or even hardware variations.

In addition to the heating and mixing modules, the autosampler is equipped with a drawer that maintains a constant temperature of 10 °C. Here the transesterification reaction is carried out in a controlled environment. All necessary solvents and reagents are stored in sufficient quantities in the autosampler, so that a sequence can run over several days. Through efficient overlapping of work steps, the result of the GC-MS analysis, including sample preparation and derivatization, is available in 48 minutes.

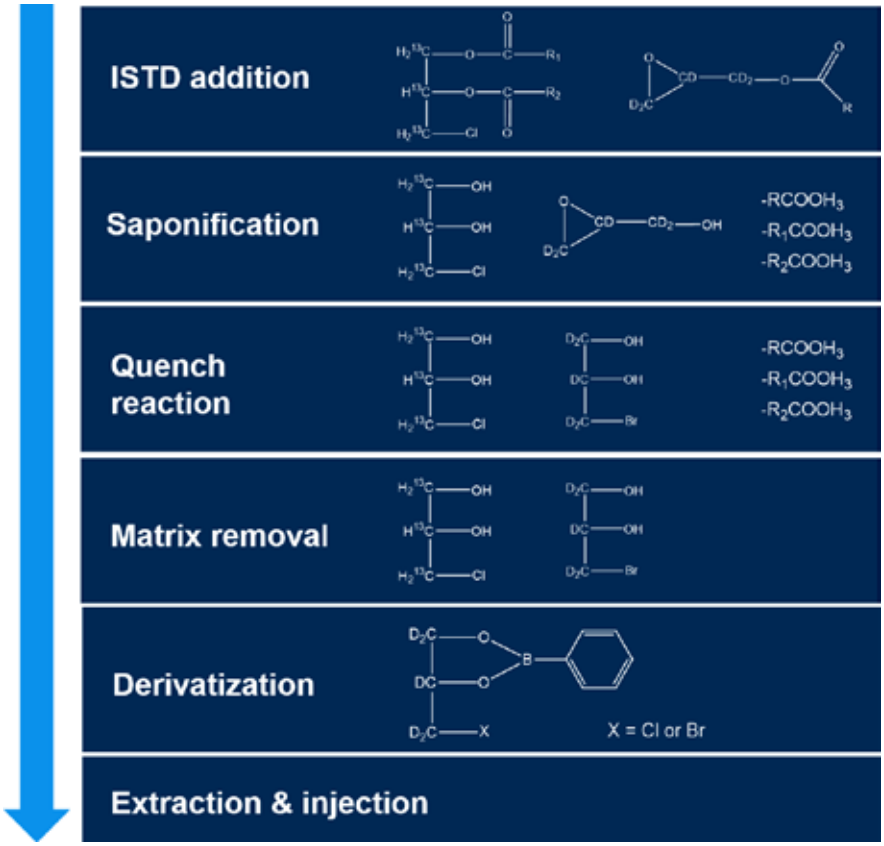


*Figure 1. Schematic sequence of reactions during sample preparation using the two internal standards.*

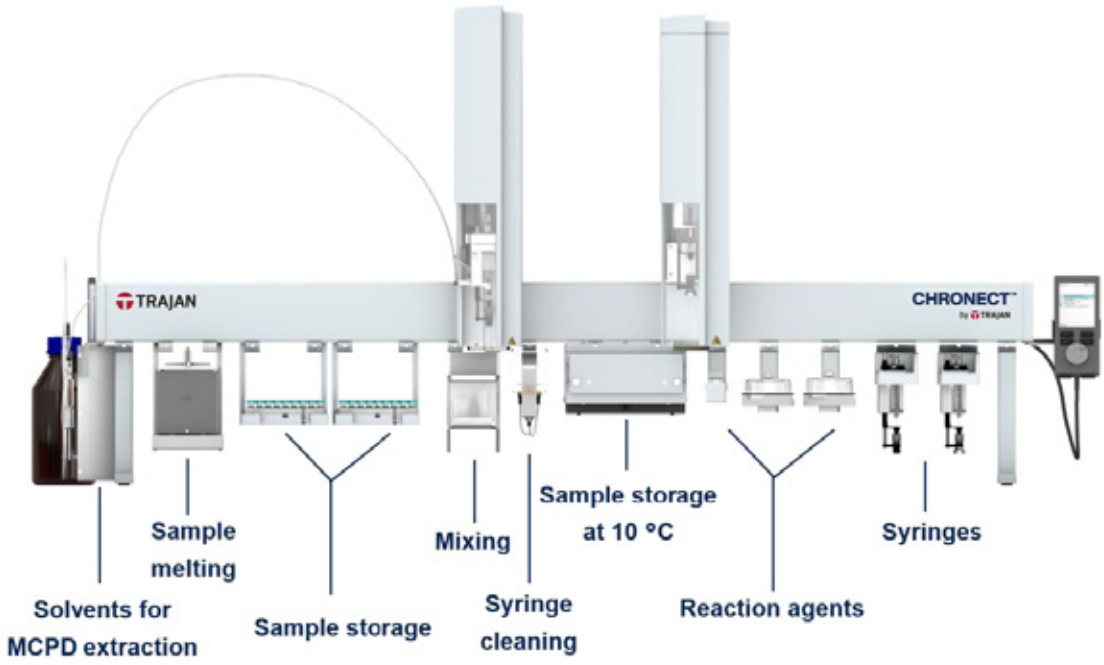


*Figure 2. Schematic structure of the CHRONECT™ robot DHR RSI or RTC with modules for MCPD analysis according to Draft ISO 18363-4.*

## Results

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

| **Injector** | SSL, 1 µL injection volume, spitless (split 1:20 after 1 minute) | | |
|---|---|---|---|
| Temperature [°C] | Heat rate [°C/min] | Hold time [min] | Total [min] |
| 250,0 | isotherm | 15,00 | 15,00 |
| **Gas control** | 1,5 mL/min constant flow, backflush after 8 min | | |
| **Initial temperature** | 2x Rxi-5 MS meter, 0,25 mm ID, 0,25 µm film | | |
| **Oven program** | | | |
| Temperature [°C] | Heat rate [°C/min] | Hold time [min] | Total [min] |
| 70,0 | | 2,00 | 2,00 |
| 200,0 | 20,0 | 0,00 | 8,50 |
| 300,0 | 40,0 | 4,00 | 15,00 |
| **Detector** | Transfer line 280 °C, CID-Gas Argon, MRM-Mode | | |
| Name | Precursor-Ion | Product-Ion | Mode |
| 2-MCPD | 198,00 | 104,00 | Quantifier |
| | 196,00 | 104,00 | Qualifier |
| 2-MCPD-d5 | 203,00 | 107,00 | Quantifier |
| | 201,00 | 93,00 | Qualifier |
| 3-MCPD | 196,00 | 147,00 | Quantifier |
| | 196,00 | 91,00 | Qualifier |
| 3-MCPD-d5 | 201,00 | 150,00 | Quantifier |
| | 201,00 | 93,00 | Qualifier |
| 3-MBPD | 245 | 150 | Quantifier |
| | 242 | 147 | Qualifier |
| 3-MBPD-d5 | 245 | 150 | Quantifier |
| | 247 | 150 | Qualifier |
| 3-MBPD-13C3 | 243 | 149 | Quantifier |
| | ** | ** | Qualifier |

** Not possible due to interference with Glycidol-d5 internal standard.

*Table 2: Validation parameters from measurements with spiked oil and reference material.*

| Parameter | Target | Is |
|---|---|---|
| Recovery (%) | 750 - 100 | 104 |
| Reproducibility (RSD %) | < 10 | 2,6 |
| LOQ* (µg/kg) | < 50 | 32 |
| LOD** (µg/kg) | ≤10 | 10 |

*Limit of quantification; ** Limit of detection.

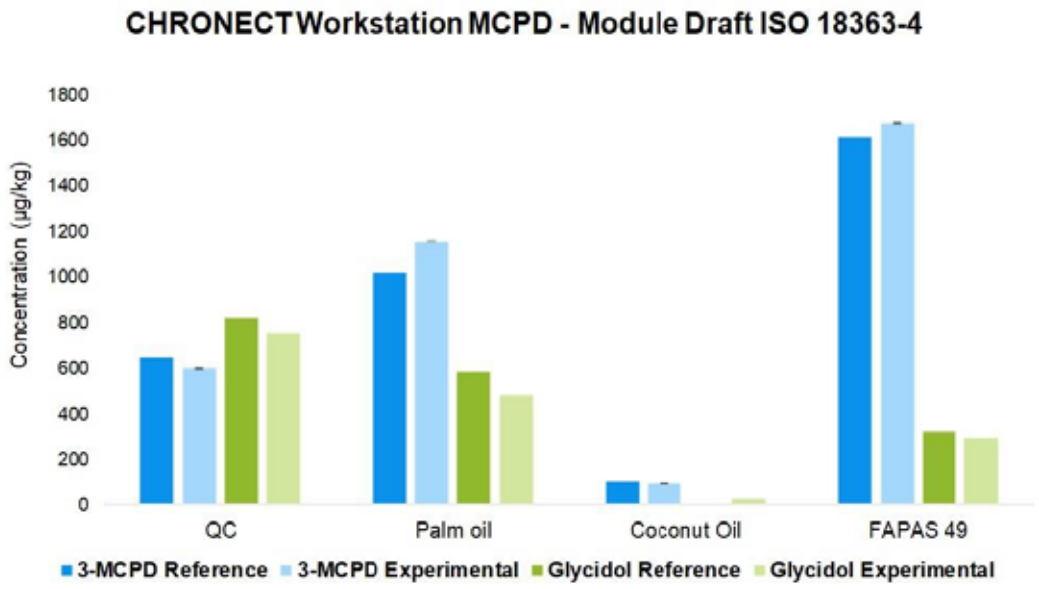


*Figure 3: Comparative values of different reference materials of the automated method Draft ISO 18363-4.*

## Evaluation of the results

Validation of the methods was performed using doped vegetable oils and reference materials to determine the recovery and reproducibility of the overall procedure (Table 2). For recovery, 104% was determined using spiked olive oil and the "Draft ISO 18363-4" method (Table 2). For the free 3-MCPD, a reproducibility of 97.4% was determined. With a blank value < 10 µg/kg, the limit of quantification for the total system is 32 µg/kg. Provided that a smaller blank value is achieved, even lower limits of determination < 25 µg/kg are possible. In this case, the quantity of the internal standard and the general cleanliness of all chemicals used have a major influence on the measurement.

The measurement of the reference materials (Figure 3) shows a similar recovery compared to the theoretical values (3-MCPD experimental and Glycidol experimental) determined with the 3-in-1 method, both in the high mg/kg range and in the low µg/kg range. Both methods, Zwagerman and 3-in-1, follow alkaline transesterification, although in the case of the 3-in-1 method the reaction proceeds at ~ -22 °C for 16 h.

Thanks to the $^{13}$C3-3-MCPD ester and d5-glycidol ester used as internal standards for quantification and correction, only one measurement is required to determine the content of 3-MCPD, 2-MCPD and glycidol.

With the help of the "one-piece workflow", the user receives the sample result after about 48 minutes and can already prepare the next sample with the help of the CHRONOS™ software during the GC run. This ensures efficient utilization of the GC-MS system. In addition, since the results for all three analytes are available after only one sample analysis, the influence of several measurement uncertainties is eliminated.

Other methods with alkaline transesterification for MCPD analysis each require two sample preparations and GC-MS measurements per sample. The time and experimental effort involved is considerably higher than for the method published by Zwagerman but can be compensated for by the automated approach.

## Summary

The automatic determination of 2-MCPD, 3-MCPD and glycidol in fats and oils according to the method of Zwagerman *et al.* represents an elegant and efficient alternative to the previously official ISO methods 18363-1, ISO 18363-2 and ISO 18363-3. The results compare very well with the theoretical reference method Cd29b-13 and show only small deviations in the lower and higher concentration range. Determination and detection limits are similar to the official method (3-in-1), with the largest influence being only the blank value from the solvents and reagents.

For more information visit www.trajanscimed.com or contact techsupport@trajanscimed.com

*The CHRONECT™ Workstation MCPD – Module Draft ISO 18363-4 is a development by Trajan.*
*Subject to technical changes.*