Determination of 3-MCPD and Glycidol in Foodstuff

Fully automated sample preparation via PAL3

in accordance to
DGF C-VI 18(10) // AOCS Cd 29c-13
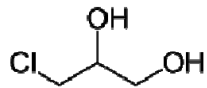
Agilent Technologies

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3-MCPD and Glycidol Esters in Foodstuffs

3-MCPD

Glycidol

Glycidol content of various oils
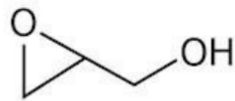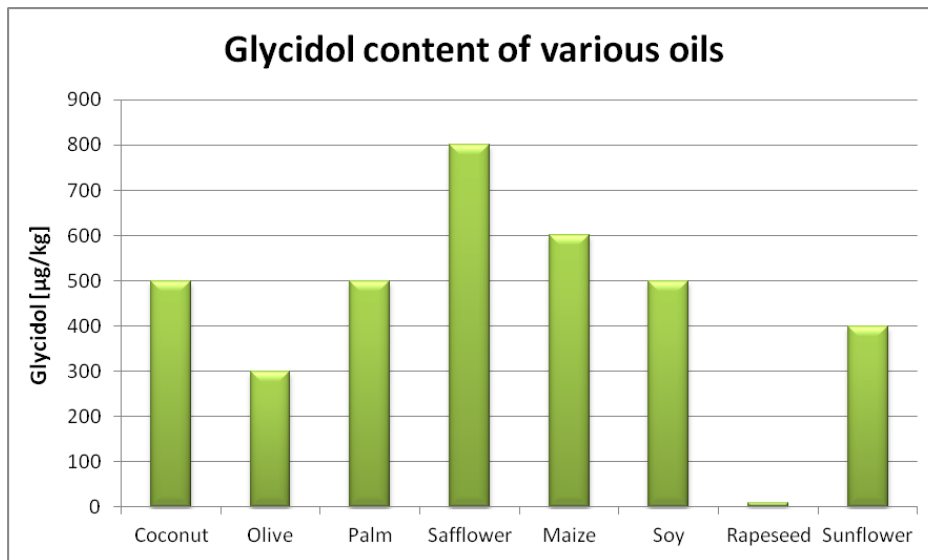
➤ 3-chloro-1,2-propanediol fatty acid esters (3-MCPD-esters) are formed

during deodorization of oils and fats in the course of the refining process
• e.g. sunflower oil

during temperature-treatment of salted foodstuffs
• e.g. soy sauce

➤ TDI of free 3-MCPD:
2.0 µg/kg body weight
(EFSA , 2017)

„What is efficient cargo handling?"

➤ ships loaded with palm oil dock in the harbour and need to unload their product

➤ the laboratory needs to analyse the oil first

➤ release of the product as "good" or "MCPD free"

➤ every waiting hour costs $$

Every hour that a ship is docked at the harbour it costs ~25.000 - 100.000 € depending on its size.

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SiM

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„What is efficient cargo handling?"

➤ **There are two options available:**

- send samples out to be analysed

- have your own lab to analyse the samples

➤ In both cases, depending on the method that is used, a result can take up to 24h or even more.

➤ **"After a few hours of waiting in the harbour, a new MCPD analyzer can be bought"**

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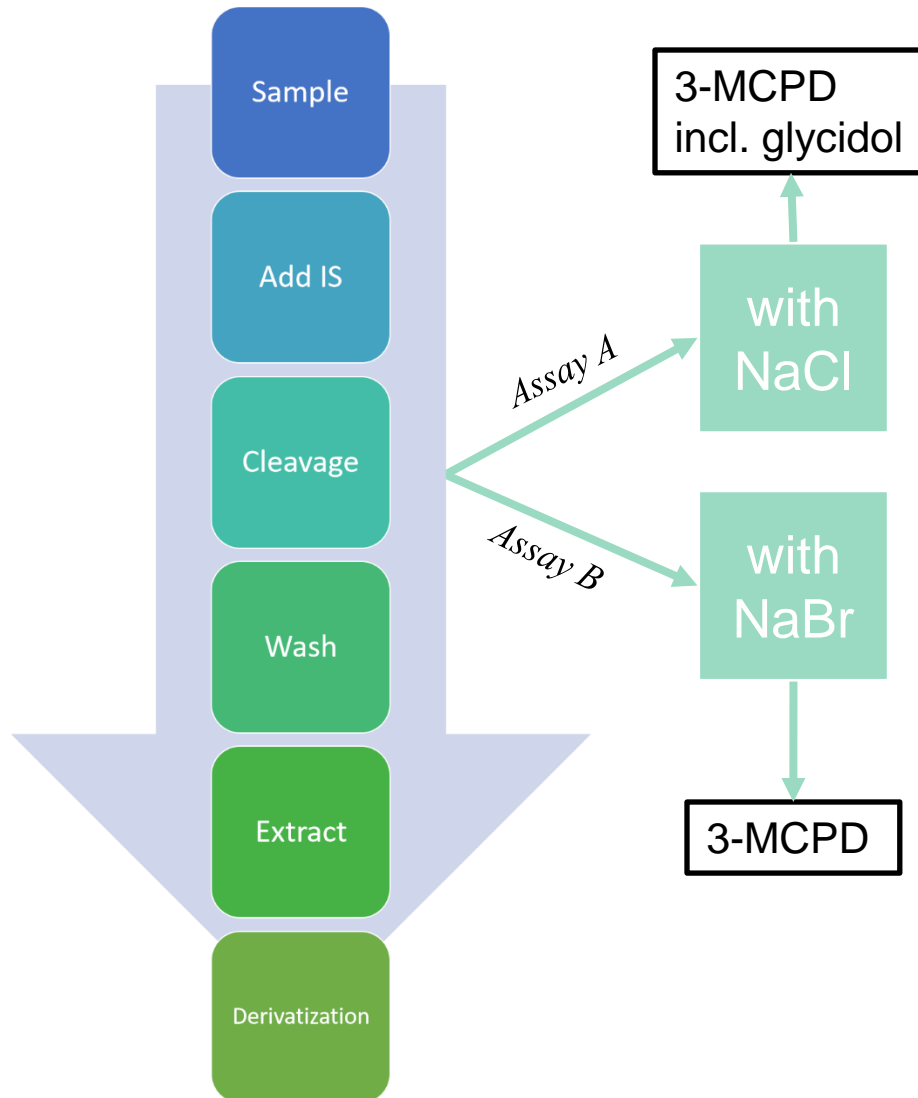
SiM

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Overview:
Available Automations for 3-MCPD Methods

Trivial name	Components	Automation using PAL3 sampler	Official Method Name	Comment
DGF-Method	3 MCPD,GE (as difference), 2MCPD	100 % 160 cm DHR PAL RSI/RTC	AOCS Cd 29c-13 DGF C-VI 18 (10)	- ~ 36 samples/day
Kuhlmann "3in1" SGS "3in1"	3MCPD,GE, 2MCPD	100 % 160 cm DHR PAL RSI/RTC	AOCS Cd 29b-13	- reaction @ -22 °C for 16 h.
Unilever-Method	3MCPD,GE , 2MCPD	100 % 160 cm DHR PAL RSI/RTC	AOCS Cd 29a-13	- reaction @ 40 °C for 16 h. - may require ultrasonic bath
Zwagerman-Overman-Method	3 MCPD, GE, 2MCPD	100 % 160 cm DHR PAL RSI/RTC	-	- requires 13C-labeled standard - a Triple-Quadrupole is required for correct GE quantification - not following official guidlines

➤ all official AOCS methods for 3-MCPD can be automated

➤ for higher sample throughput and instrument longevity

➤ optimization of AOCS Cd 29c-13 in cooperation with Institut Kirchhoff (Berlin)

   AOCS Cd 29c-13/DGFC-VI18(10) (DGF*classic*) ➔ DGF Fast&Clean (DGF F&C)

SIM

Sample Preparation According to DGF C-VI 18(10): designated as "DGF F&C"

Sample

Add IS

Cleavage

Wash

Extract

Derivatization

Assay A

Assay B

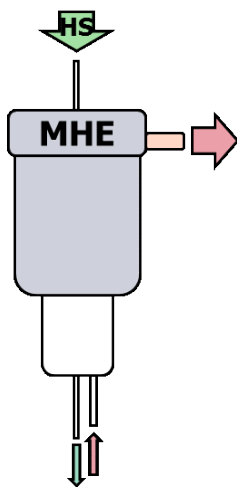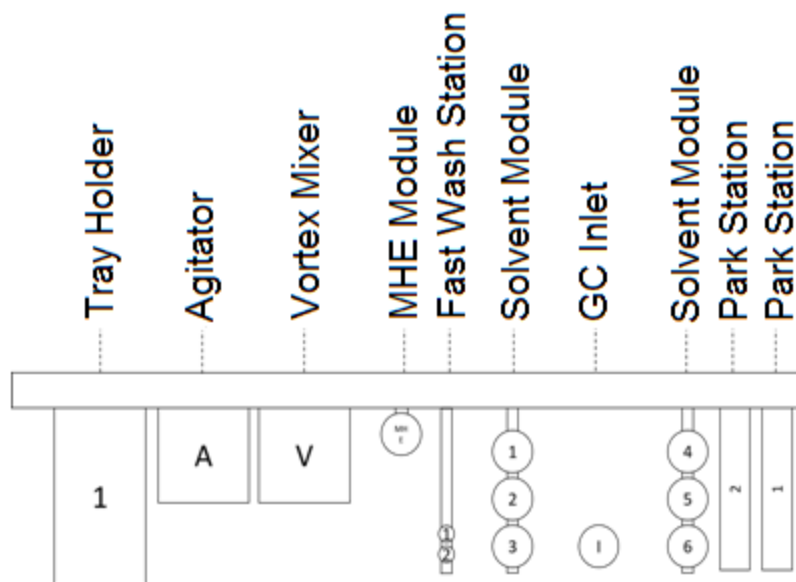with NaCl

with NaBr

3-MCPD incl. glycidol

3-MCPD

➢ weigh in ~ 100 mg oil sample

➢ fully automated
  - addition of internal standard
  - ester cleavage
  - derivatization

➢ injection into GC

➢ detection with MS optional TripleQuad-MS

Assay A: 3-MCPD incl. glycidol
Assay B: Free 3-MCPD

Glycidol = (Assay A – Assay B) * t

t: transformation factor

PAL 3 DHR RSI/RTC Configuration

- PAL 3 DHR RSI/RTC 160 cm

- RSI arm: Dedicated dilutor for save & convenient solvent addition

- Dilutor with hexane as extraction solvent and iso-octane

- Headspace & MHE-tool for evaporation of extraction solvent
  - via N_2 or synthetic air

- Automatic & intelligent interlacing of process steps improves time efficiency
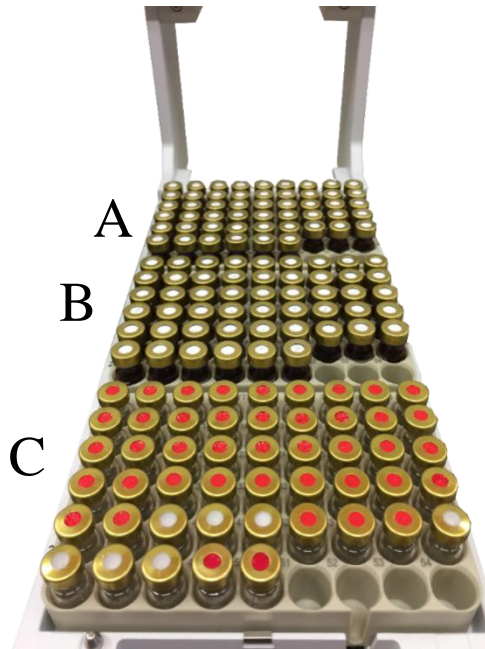
PAL 3 DHR RSI/RTC Configuration
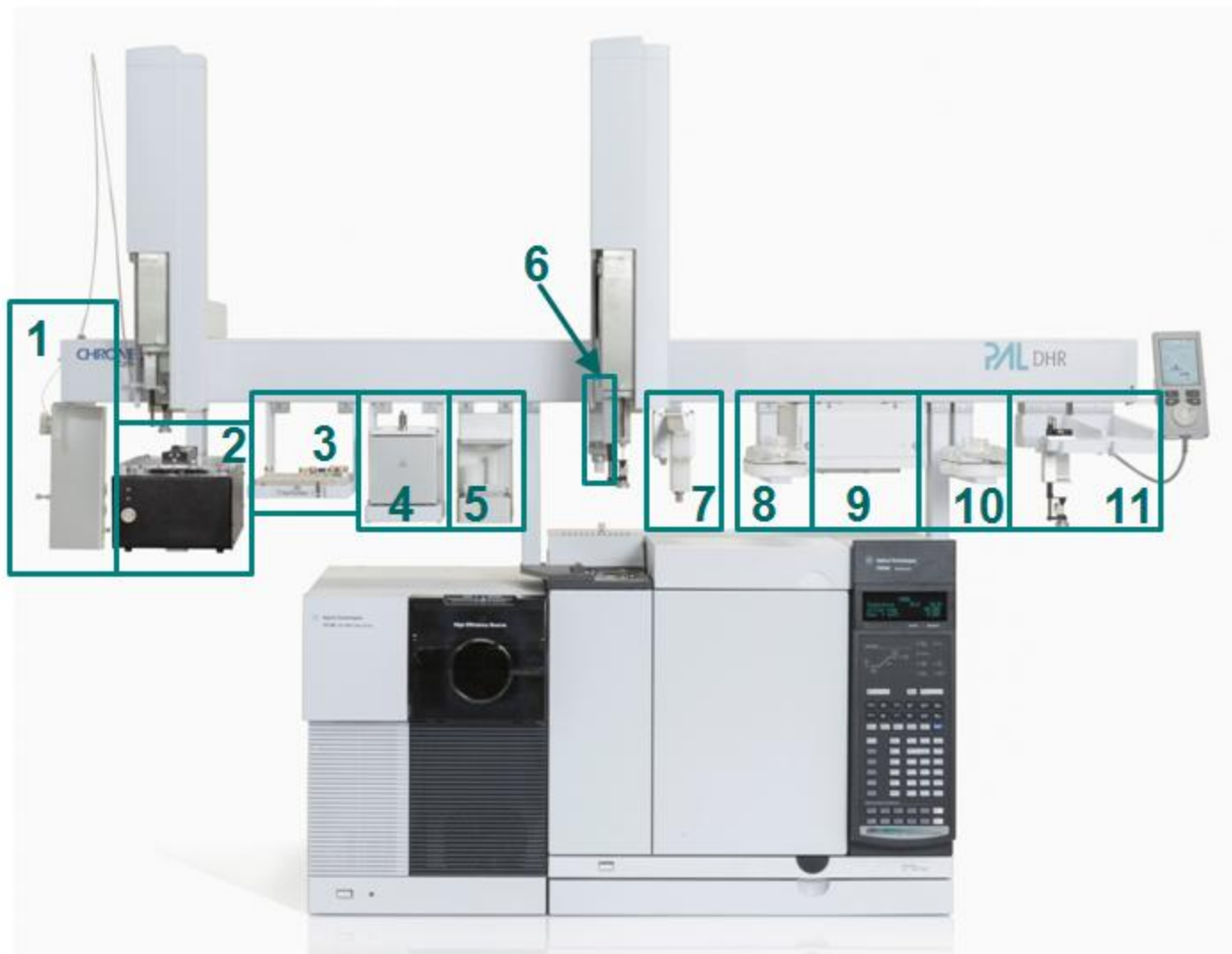
PAL 3 with

1 – Dilutor

2 – Centrifuge

3 – Tray Holder

4 – Agitator

5 – Vortex Mixer

6 – MHE Mod.

7 – Fast Wash

8 – Solvent Mod.

9 – Cold Stack$_{opt.}$

10 – Solvent Mod.

11 – Park Stations

Agilent 7890 GC
with **SQ/TQ MS**

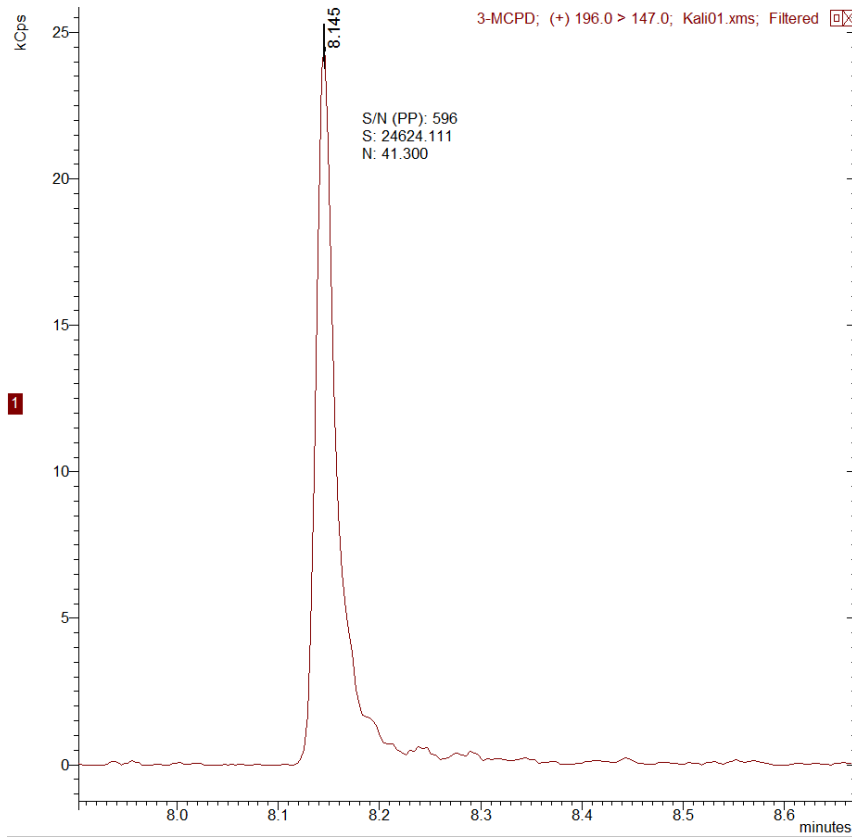# PAL 3 DHR RSI/RTC Configuration

A
B
C

- ➤ Slot A: sample vials
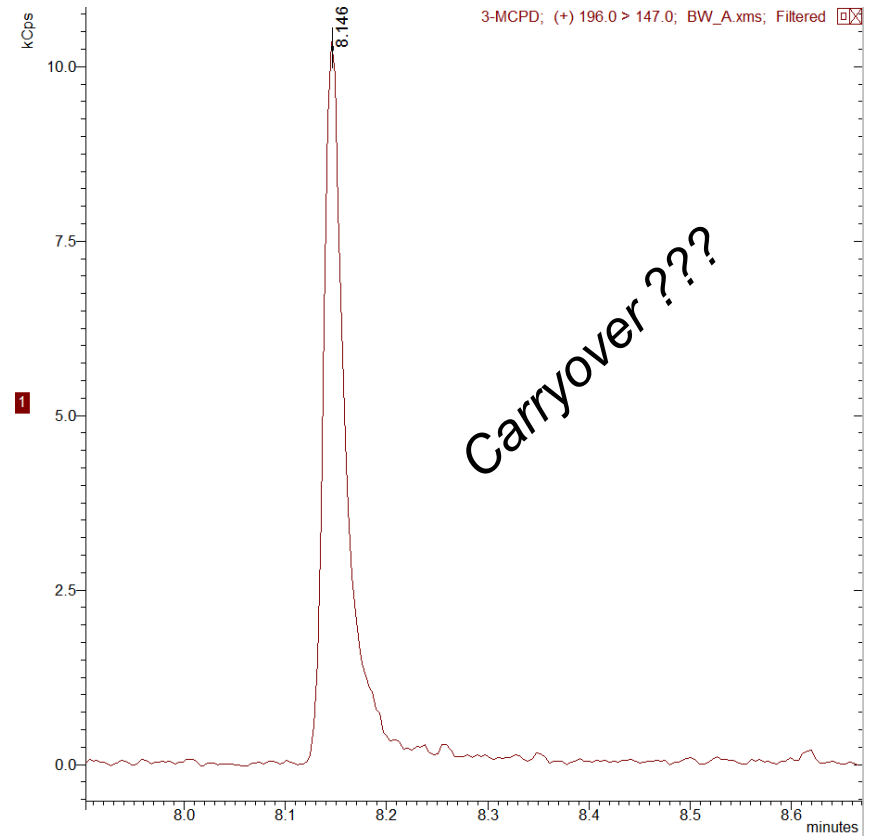- ➤ Slot B: vials with sodium sulfate
- ➤ Slot C: empty vials and standards

- ➤ Dual-Head layout for max. throughput
- ➤ completely autonomous operation
- ➤ large sample capacity
- ➤ flexible, adaptable & expandable

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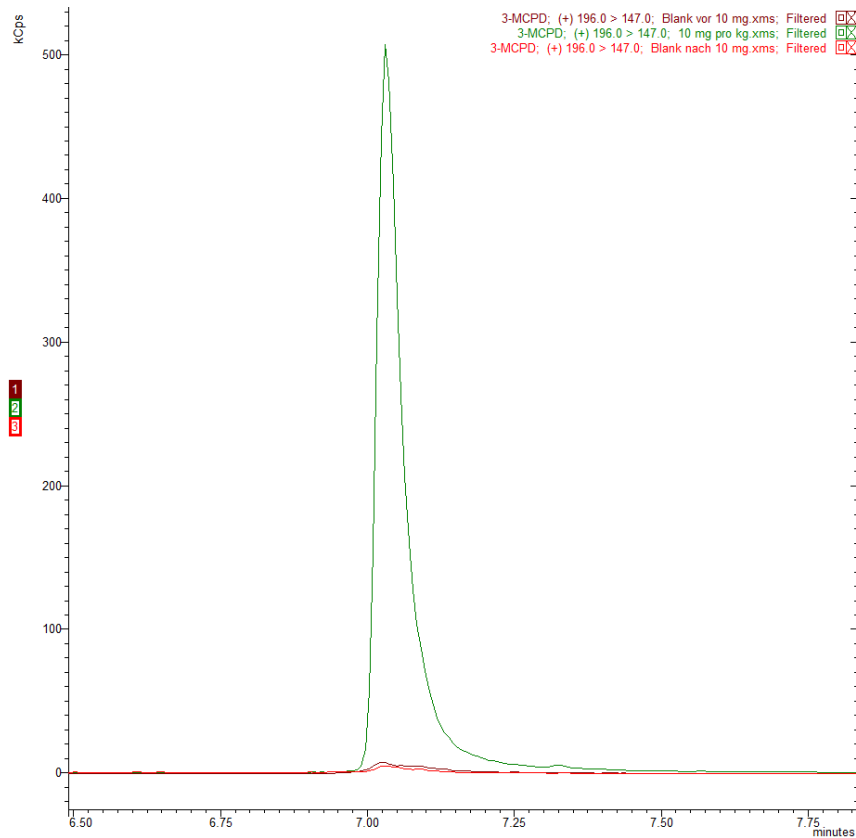# High sensitivity
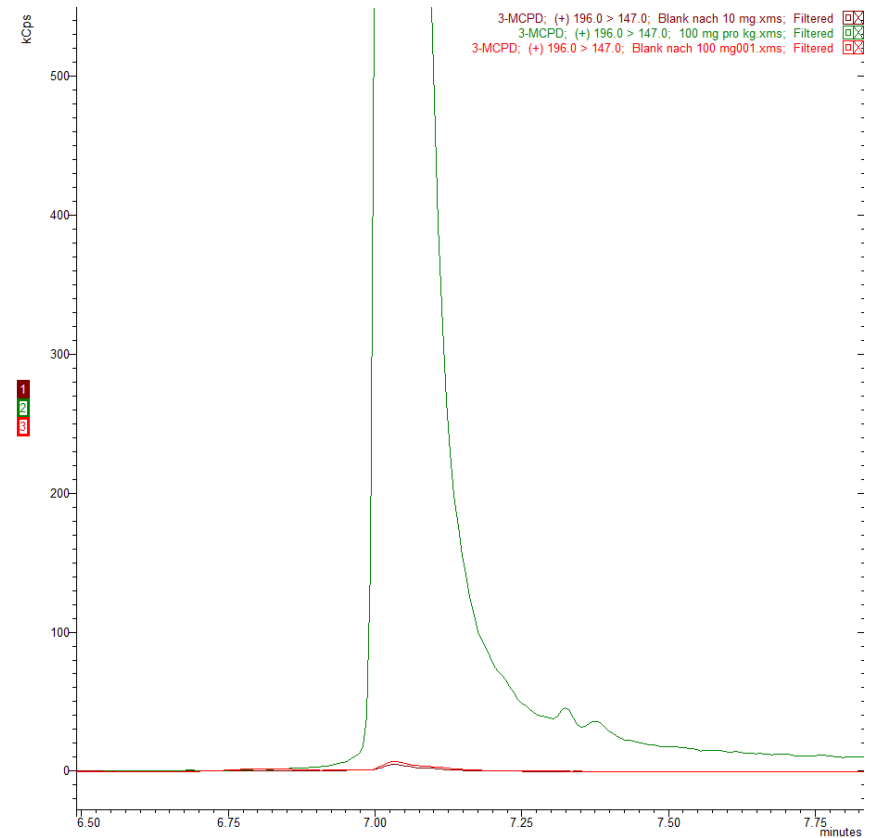

3-MCPD at 0.05 mg/kg

Background ~ 0.02 mg/kg

Investigating Carryover

Standard 10 mg/kg

Standard 100 mg/kg


➔ no visible carryover after highly concentrated samples

High sensitivity?

3-MCPD; (+) 196.0 > 147.0; Ohne Internen Standard.xms; Filtered
3-MCPD; 147.0 (TIC); Blindöl_A.xms; Filtered
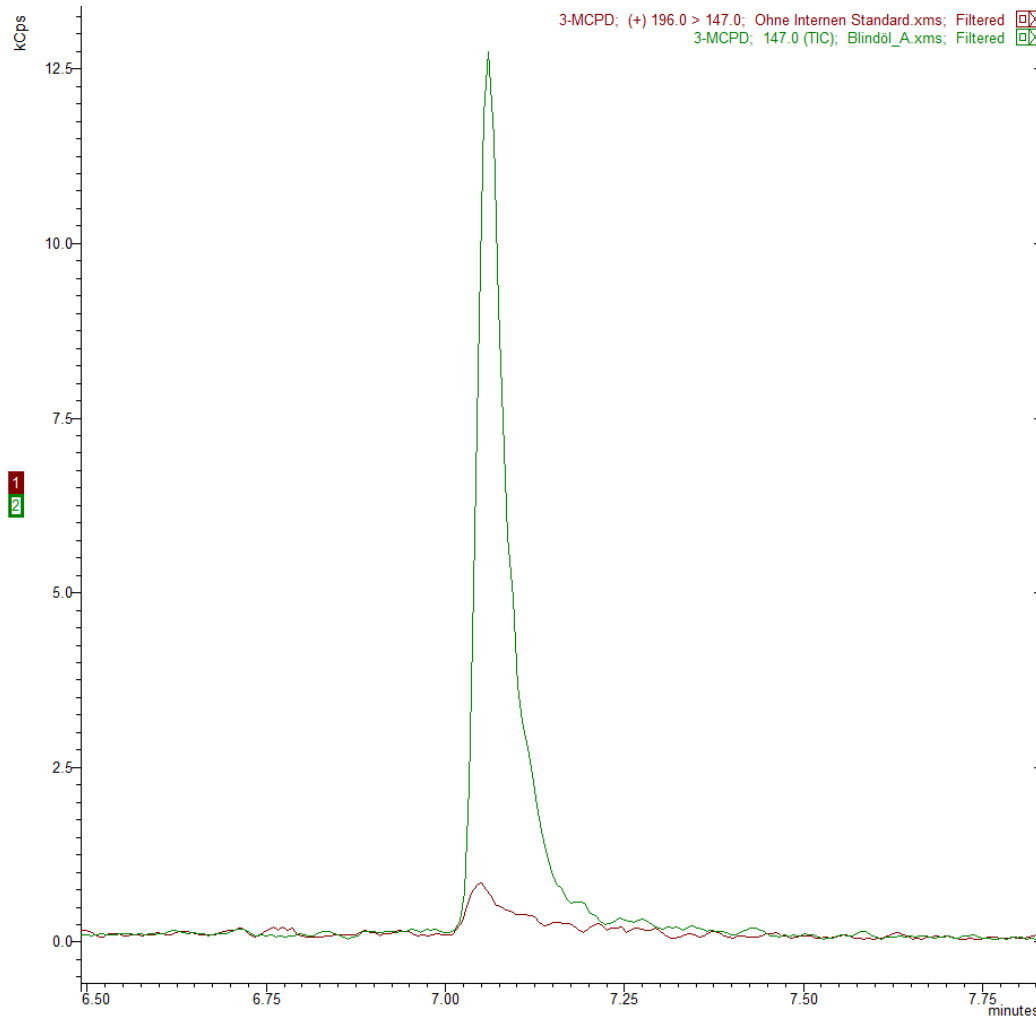
analysis of 3-MCPD ester and a blank sample,
each spiked with int. standard (deuterated 3-MCPD ester)

➢ internal standard contains ~ 0.01 % 3-MCPD

➢ > 90 % of the background are due to the internal standard

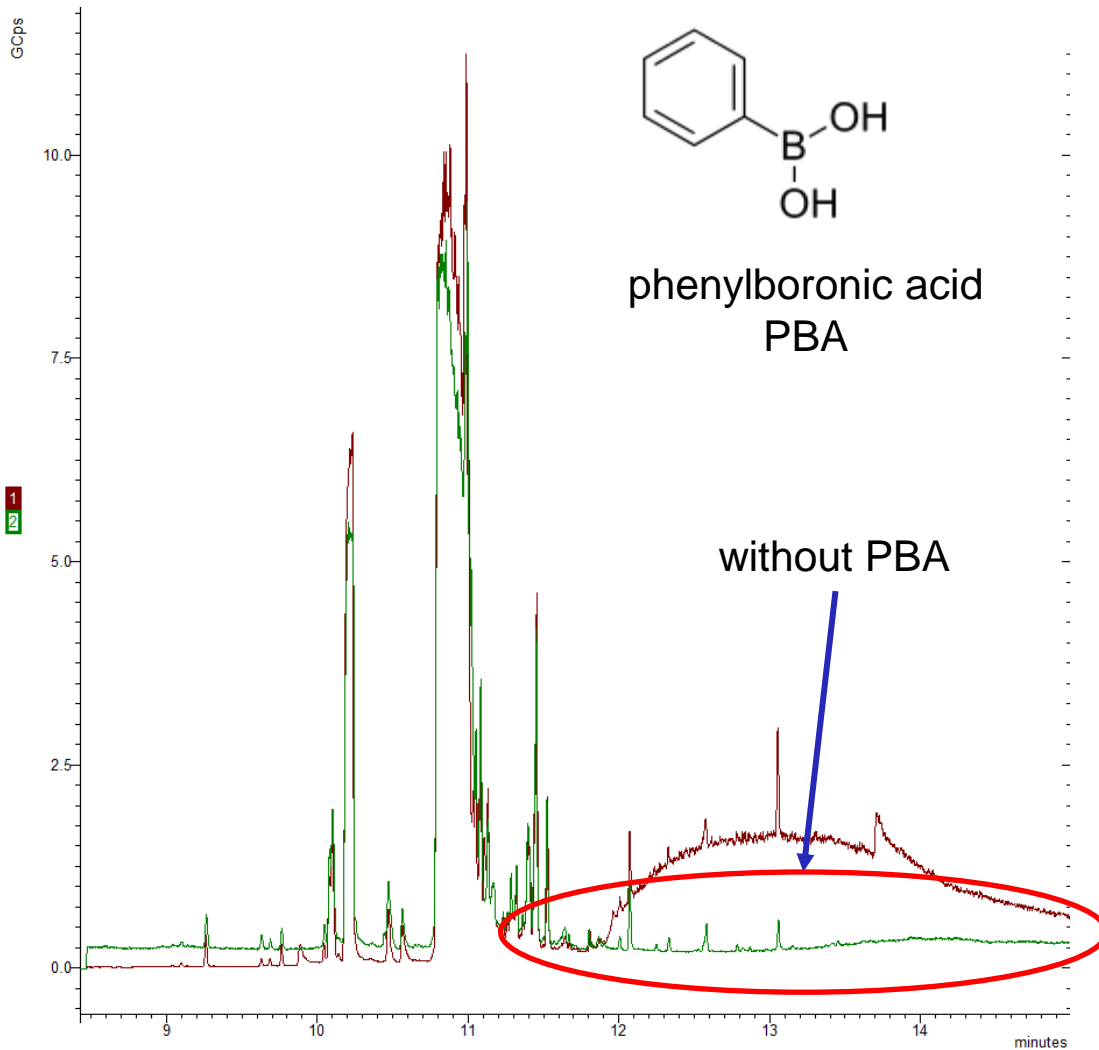➔ reducing the amount of internal standard will increase sensitivity

Analyzer Longevity
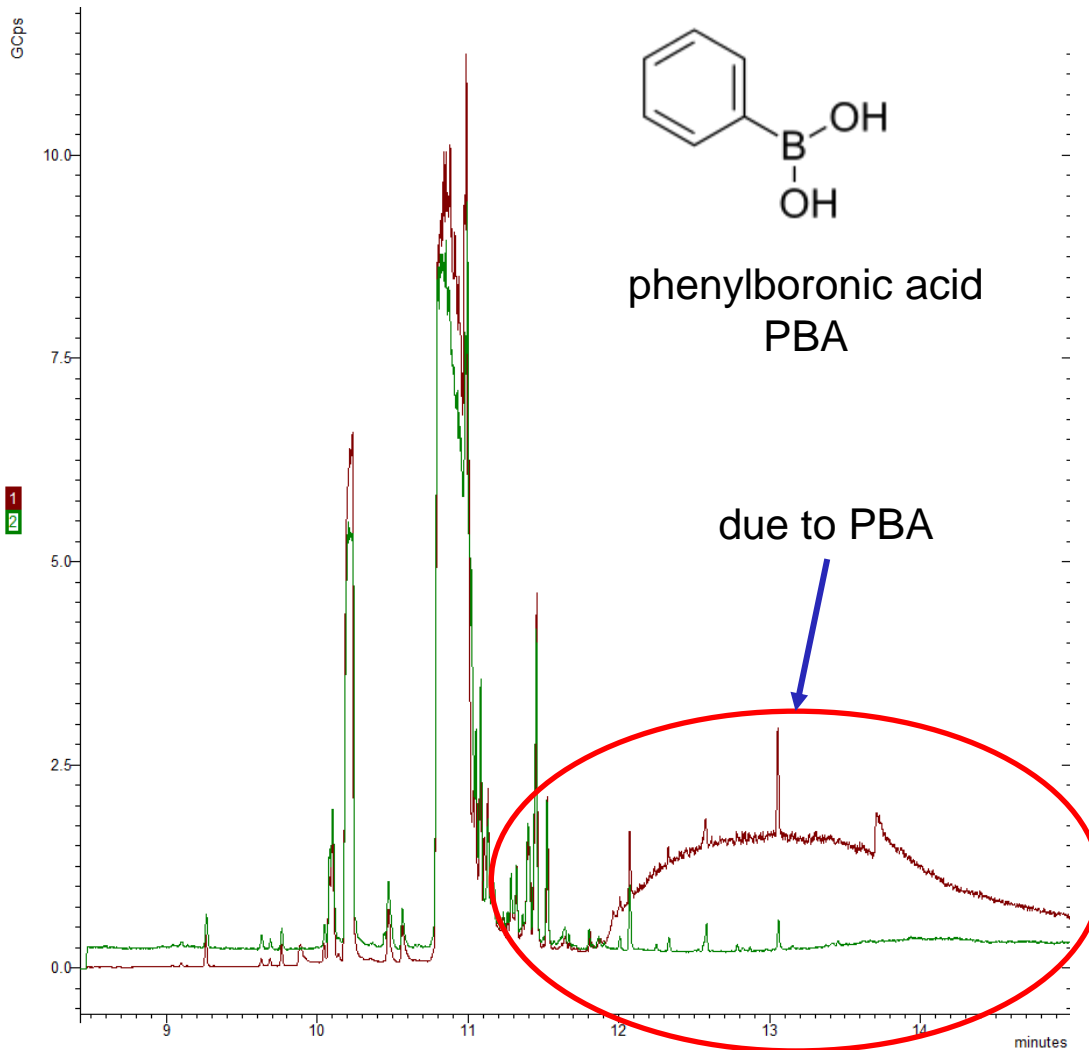
phenylboronic acid
PBA

due to PBA

➤ excess PBA reaches the detector

➤ method adaption:
getting rid of PBA before chromatographic separation using a physical and chemical cleaning step

"Clean Technology"
additional *physical and chemical steps*

phenylboronic acid
PBA

without PBA

- ➢ excess PBA reaches the detector

- ➢ method adaption:
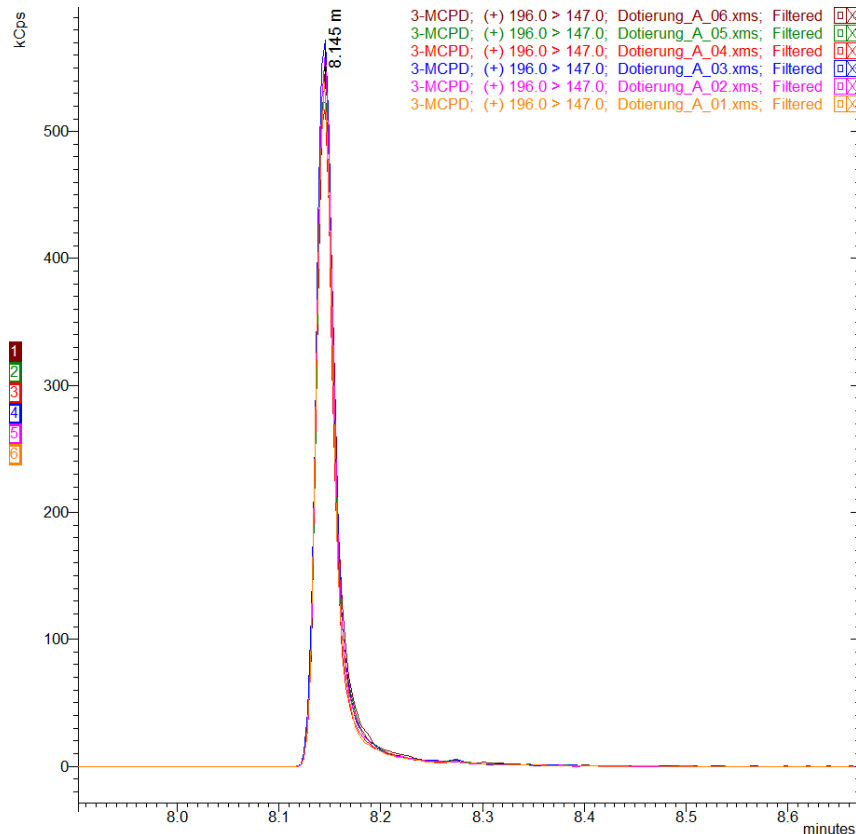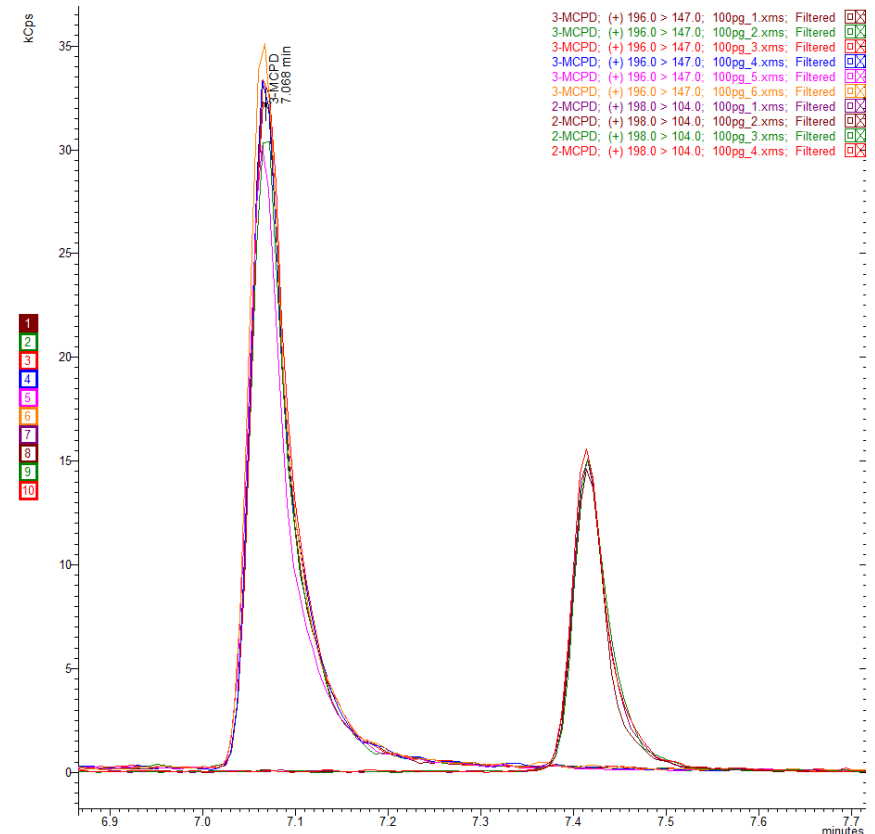 getting rid of PBA before chromatographic separation using a physical and chemical cleaning step

- ➔ further adaption of the official DGF-Method will increase
  - sample throughput
  - instrument lifetime

DGF F&C: Reproducibility & Stability

3-MCPD at 1 mg/kg:
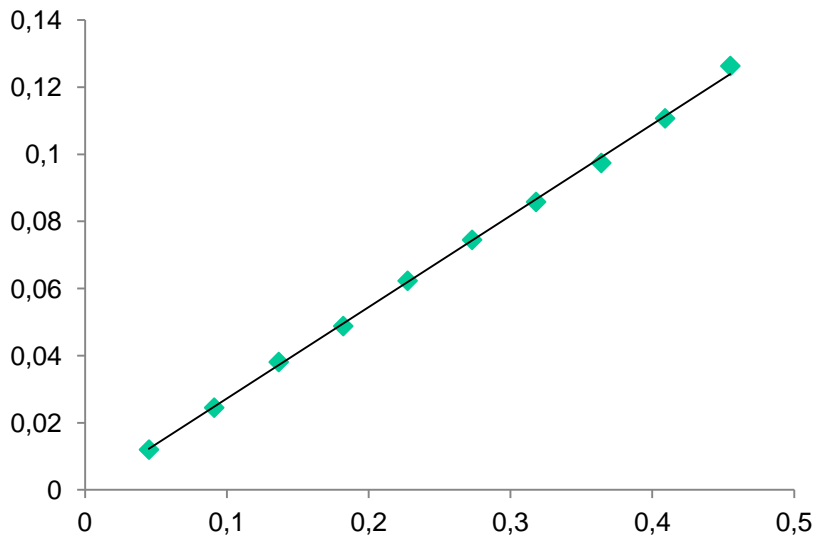
3-MCPD & 2-MCPD at 0.1 mg/kg:

➜ very good reproducibility at different concentration levels

DGF F&C:
External Calibration with 3-MCPD Ester

Calibration: 0.05 – 0.5 mg/kg

Correlation coefficient:	0.9996
Variation coefficient:	1.74 %
Calibration:	linear
Equation:	y = -0.0003 + 0.2733x

Calibration: 0.5 - 5 mg/kg

Correlation coefficient:	0.9999
Variation coefficient:	0.88 %
Calibration:	Linear
Equation:	y = -0.0183 + 0.2967x
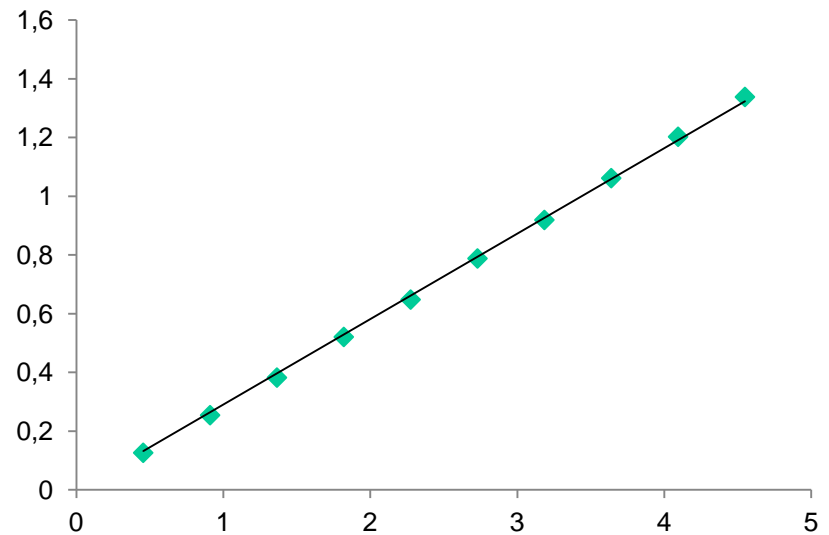
➜ Quantification can also be done using only the internal standard, as written in the official AOCS norm (DGF*classic*).

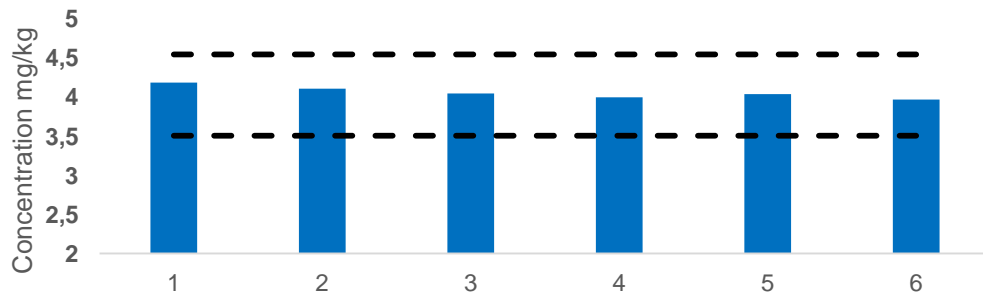**SIM**

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DGF F&C: Method Validation

Recovery and reproducibility for 3-MCPD and 2-MCPD on consecutive days:

Part A
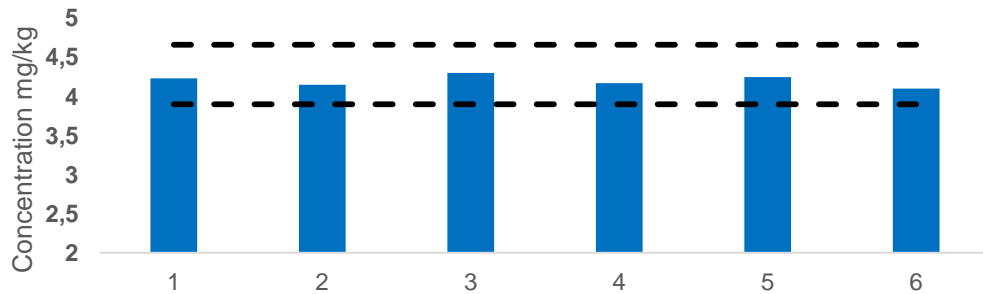
Part B

	Recovery %	Reproducibility %
3-MCPD part A	91.6	7.7
3-MCPD part B	101.9	8.8
2-MCPD part B	116.2	8.9

Automation Comparison: Assay A & Assay B

Assay	Components	Reproducibility	Recovery	Blank
A	3-MCPD	7.7 %	102.63 %	0.016 mg/kg
B	3-MCPD	8.8 %	94.34 %	0.010 mg/kg
	2-MCPD	8.9 %	133.20 %	0.017 mg/kg

➢ prolonged maintenance cycle of the ion source
➔ achieved by backflush of GC column

➢ minimized carry-over
➔ achieved by dual-head autosampler with thorough
rinse cycle routines

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DGF F&C:
Efficient Overlapping with CHRONOS
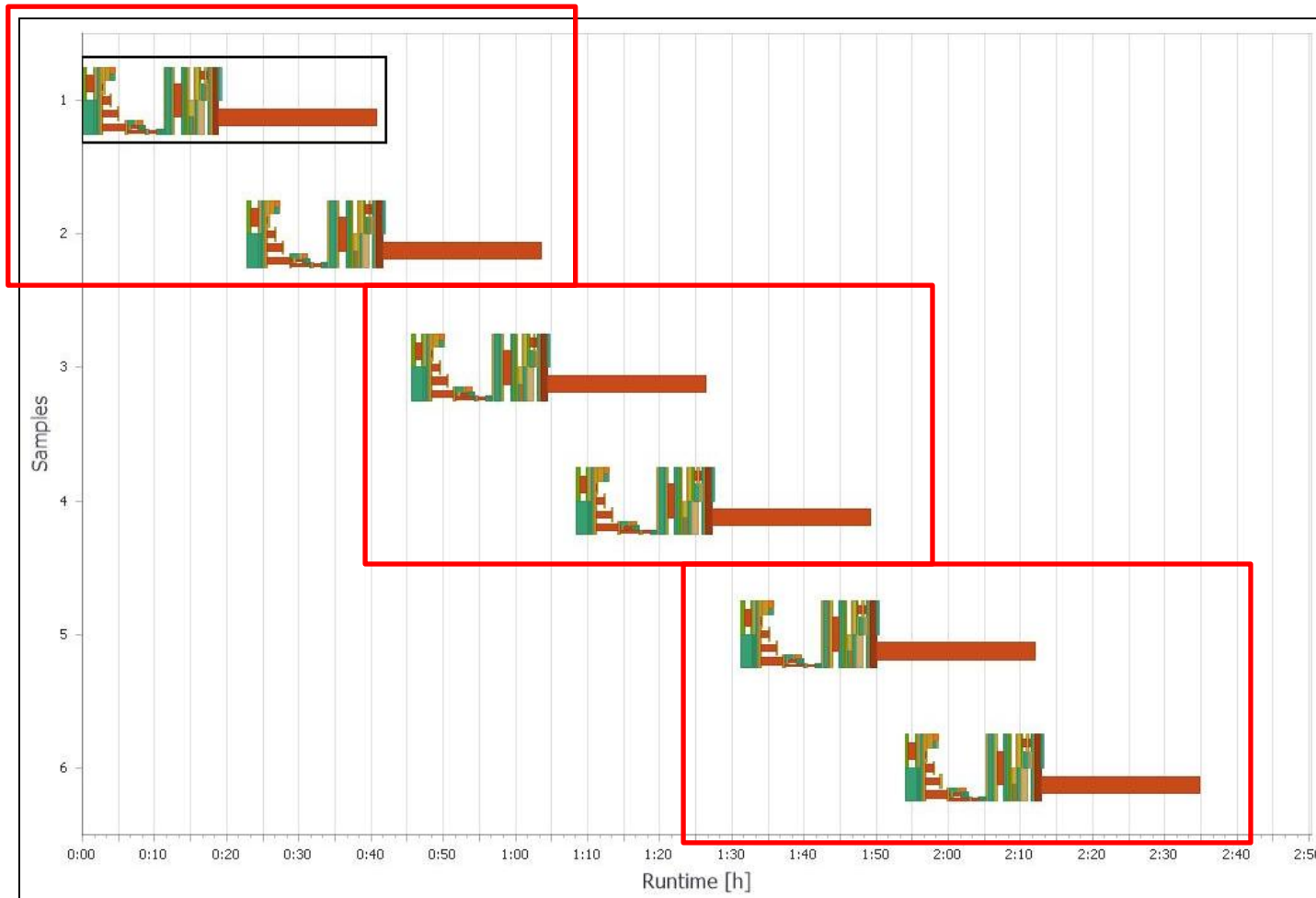
Sample 1

Sample 2

Sample 3

➜ DGF F&C allows the analysis of 36 samples (A&B) in 24 hours
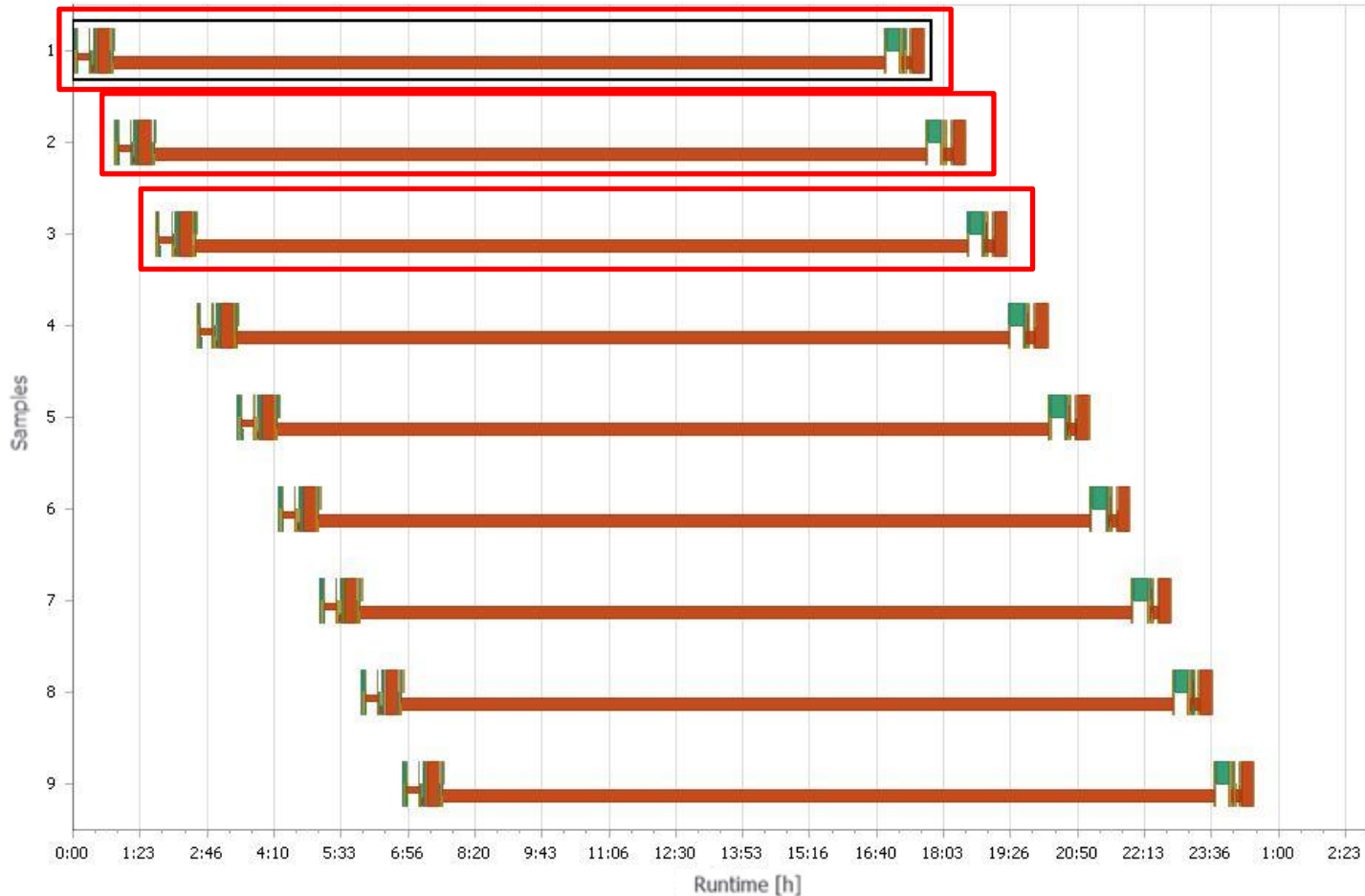
AOCS Cd29a-13:
Efficient Overlapping with CHRONOS

➡ CHRONOS also increases the efficiency of other 3-MCPD methods

Sample Preparation of Oils: Automated vs Manual

Real Samples	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol* (mg/kg)
Sample X	0.78	0.39	0.64
DGF F&C	0.80	0.58	0.73
Sample Rapeseed Oil	0.14	< 0.10	0.10
DGF F&C	0.11	0.08	0.13
Sunflower Oil 1	0.84	0.39	0.15
DGF F&C	0.73	0.60	0.29
Sunflower Oil 2	0.31	0.15	0.49
DGF F&C	0.25	0.19	0.58

Reference Oil	3-MCPD (mg/kg)	2-MCPD (mg/kg)	Glycidol* (mg/kg)
FAPAS	0.59	0.31	0.26
DGF classic	0.49	0.30	0.23
DGF F&C	0.50	0.38	0.36

*at a transformation factor of 1.

➜ accuracy and precision of automated sample preparation comparable to manual results

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Sample Preparation of Different Foodstuffs: Automated vs Manual

Comparison: automated sample preparation (DGF F&C) with manual sample preparation (DGF*classic*)
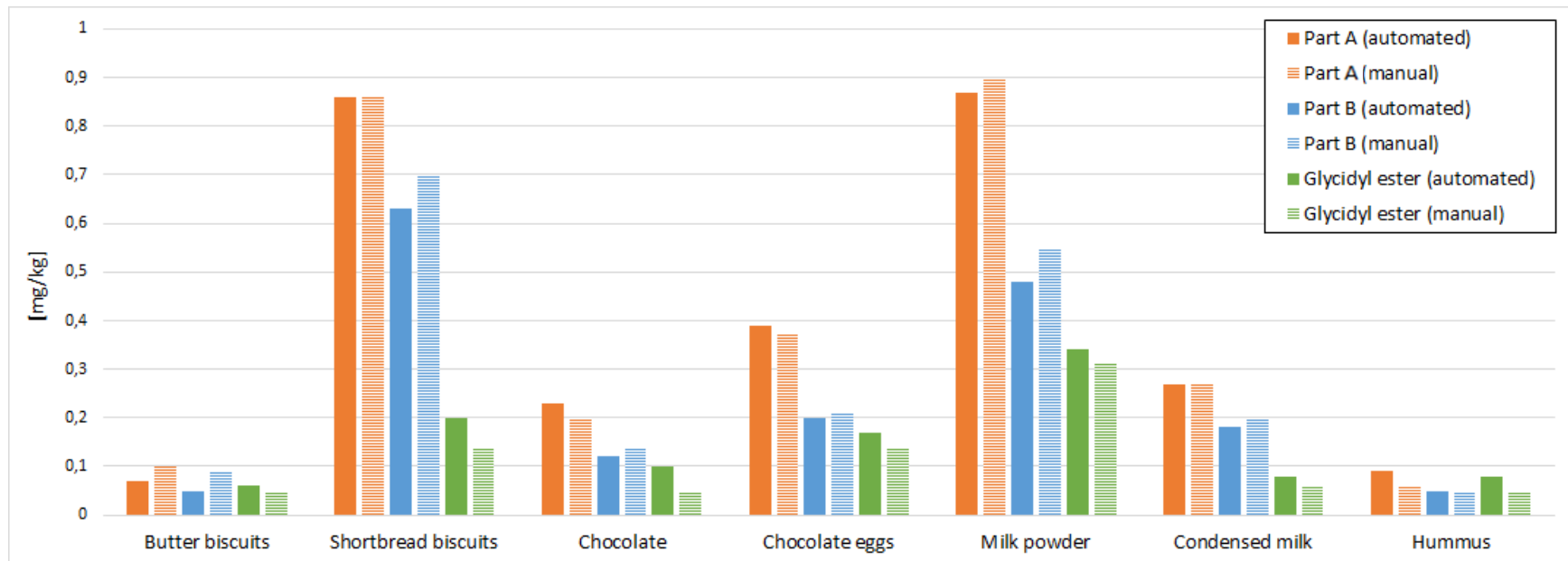
Advanced Automation Features

➤ quantification without external calibration

  • using the ISTD as written in the official AOCS norm

➤ automated addition of spiked samples at LOQ to monitor recovery and LOQ/LODs

➤ control samples can be analyzed as a control chart with every sequence

➤ runtime without user interaction: ~ 2 days

➤ only one manual step: weighing the sample into a 2 mL vial

Summary & Conclusion

➢ 3- & 2 MCPD sample preparation
  • in 45 min according to
  DGF C-VI 18 (10) for assay A & assay B

➢ 36 samples in 24 hours (assay A & assay B)
  due to efficient overlapping with CHRONOS

➢ 24 h-mode: continuous analysis without
  mandatory user interaction

➢ robust data & no carryover

➢ multitude of further methods for MCPD & other applications are also
  available

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Customer's Vote

➢ *"The introduction of the MCPD workbench has improved both the efficiency and the robustness of our MCPD analyses.*
Both, cleanliness of the equipment and the workbench environment and regular care for the different components of the system have shown to be the key to operating our system successfully." (M. Salden, Nofalab)

©NofaLab

Customer's Lab: MCPD Workbench with
Agilent 7890 GC/TQ MS, PAL3 and CHRONOS

MCPD Workbench

more information about
sample preparation and analysis
with PAL/Agilent Chromatography
Systems:

Agilent Technologies

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