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Automated Analysis of 3-MCPD and glycidol in food

Tobias Uber

MCPD in the news

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Decision of EFSA regarding MCPD/glycidol triggered heated discussions

EFSA: The Panel selected a BMDL10 value for 3-MCPD of 0.077 mg/kg bw per day for induction of renal tubular hyperplasia in rats and derived a tolerable daily intake (TDI) of 0.8 µg/kg bw per day. The mean exposure to 3-MCPD was above the TDI for 'infants', 'toddlers' and 'other children'.

- https://www.efsa.europa.eu/de/efsajournal/pub/4426

Süddeutsche Zeitung
SZ.de Zeitung Magazin

Food manufacturers discuss pros/cons of using palm oil in the light of contamination by MCPDs, glycidol

- Süddeutsche Zeitung on 15th of Nov 2016 http://sz.de/1.3248703

3-Monochloropropane-1,2-diol (3-MCPD)

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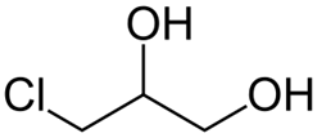
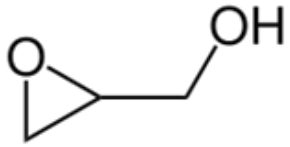
3-MCPD is a suspected carcinogen, the TDI value is 2 µg/kg body weight (WHO 2002), reduced to 0.8 µg/kg by EFSA



3-MCPD

It is present in food mainly as ester. During digestion free 3-MCPD is formed in the human body

3-MCPD is a process contaminant generated when fatty or salted food is heated, e.g. soy sauce



Glycidol

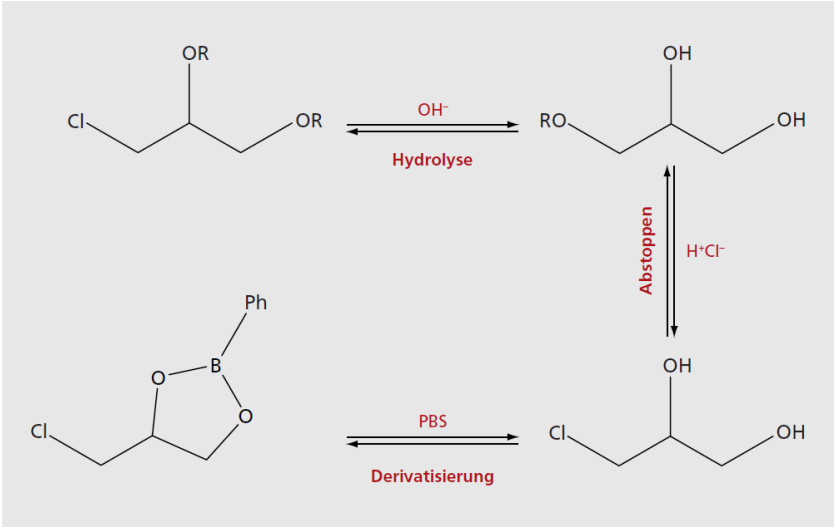
Glycidyl esters are also of interest, since free glycidol is formed during digestion

So far 2-MCPD has not been proven to be carcinogenic

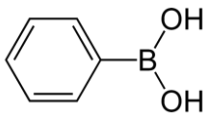
Analysis of 3-MCPD and Glycidolesters

Two ways to determine MCPD

Direct analysis: Every ester is analysed separately, usually done by LC-MS/MS. Because of the large number of possible esters this is a very complex method.



Indirect analysis: During analysis, the esters are hydrolysed and free 3-MCPD is formed. The 3-MCPD is analysed by GCMS after derivatization with phenylboronic acid.



2-MCPD not considered here.

Official methods for MCPDs

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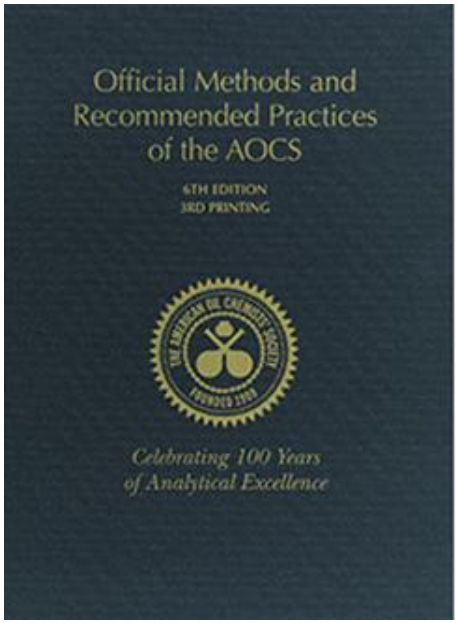
AOCS methods for characterization of 3-MCPD

Cd 29a-13 - 2- and 3-MCPD fatty acid esters and glycidyl fatty acid esters in edible oils and fats by transesterification.



Cd 29b-13 - Determination of bound MCPD and bound 2,3-epoxy-1-propanol (glycidol) by GC/MS.

Cd 29c-13 - Fatty-acid-bound 3-chloropropane-1,2,diol (3-MCPD) and 2,3-epoxi-propane-1-ol (glycidol), determination in oils and fats by GC/MS (differential measurement).

Cd 28-10 - Glycidyl fatty acid esters in edible oils.

Available methods for MCPDs using PAL3

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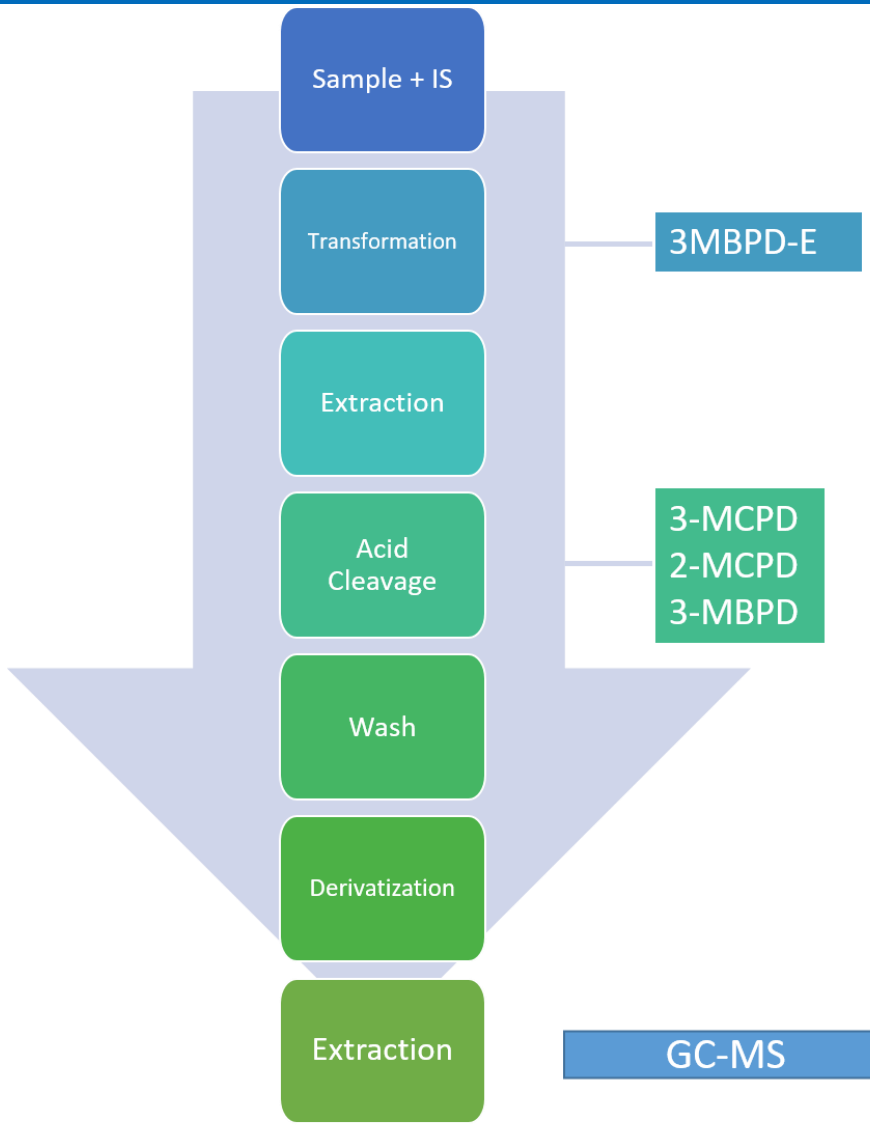
Trivial name	Components	Automation using PAL3 sampler	Official Method Name	Comment
Kuhlmann-Method	3 MCPD,GE (as difference)	100 % 160 cm DHR PAL RSI/RTC	AOCS Cd 29c-13 DGF C-VI 18 (10)	- Optional TQ for higher sample throughput - ~ 48 samples/day
Kuhlmann "3in1" SGS "3in1"	3MCPD,GE, 2MCPD	90 % 160 cm DHR PAL RSI/RTC with manual step	AOCS Cd 29b-13	– Manual step involves placing the tray in the fridge @ -22 °C for 16 h.
Unilever-Method	3MCPD,GE , 2MCPD	100 % 160 cm DHR PAL RSI/RTC with CooledStack & Centrifuge	AOCS Cd 29a-13	- 16 h @ 40 °C - May require ultrasonic bath
Zwagerman-Overman-Method	3 MCPD, GE, 2MCPD	100 % 160 cm DHR PAL RSI/RTC	-	– Requires TripleQuad MS for 2-MCPD
BfR-Methods	3 MCPD, 2MCPD	80 % 160 cm DHR PAL RSI/RTC Requires manual ASE	BfR-Method 22 BfR-Method 23	– Chloride free – Requires ASE (125 °C) – Alkaline (22) or acidic (23)
EFSA-Method JRC-Method	3MCPD, 2MCPD, GE (+ free 3MCPD & 2MCPD)	80 % 160 cm DHR PAL RSI/RTC Requires manual PLE	EFSA EN-779	- Describes two methods - 18h @ 40 °C - Also free 2- and 3-MCPD separate

AOCS Cd 29a-13 workflow



Weigh in ~ 100 mg oil sample

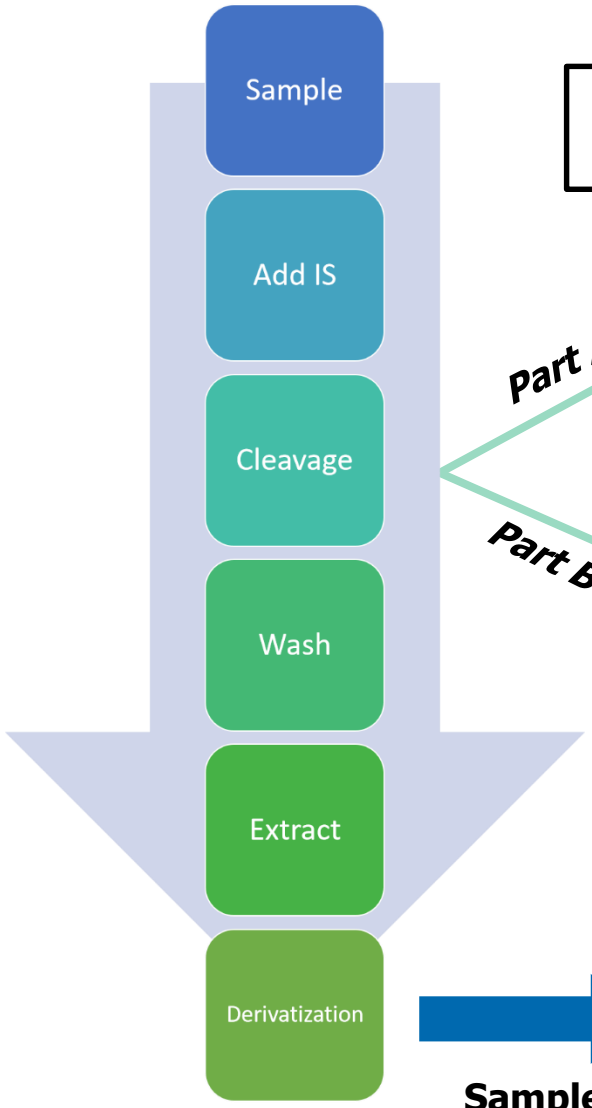
Fully automatic

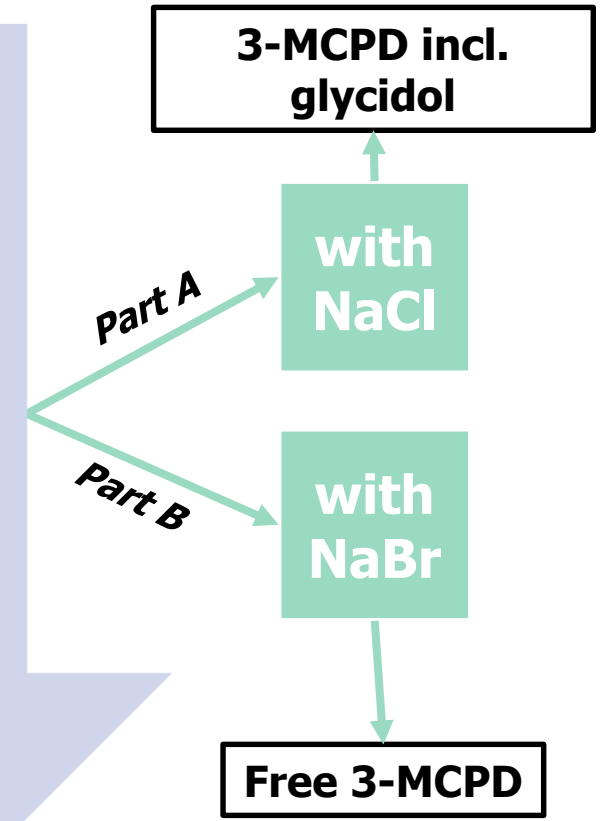
- addition of internal standard
- Glycidol transformation
- cleavage
- derivatization

Injection into GC

Detection by MS

AOCS Cd 29c-13 workflow





Weigh in ~ 100 mg oil sample

Fully automatic

- addition of internal standard
- cleavage
- derivatization

Injection into GC

Detection by MS

Part A: 3-MCPD incl. glycidol
Part B: free 3-MCPD

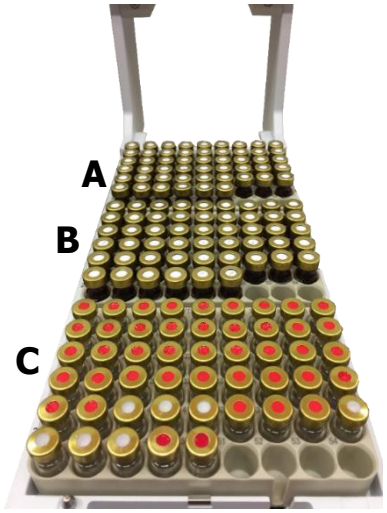
Glycidol = (Part A – Part B) * t

Evaporate & Inject

Sample preparation following the official AOCS/DGF method.

PAL RTC configuration for AOCS 29c-13

Dual Head system with Bruker GC/MS



- **Slot A: Vials with sample**
- **Slot B: Vials with sodiumsulfate**
- **Slot C: Empty vials**

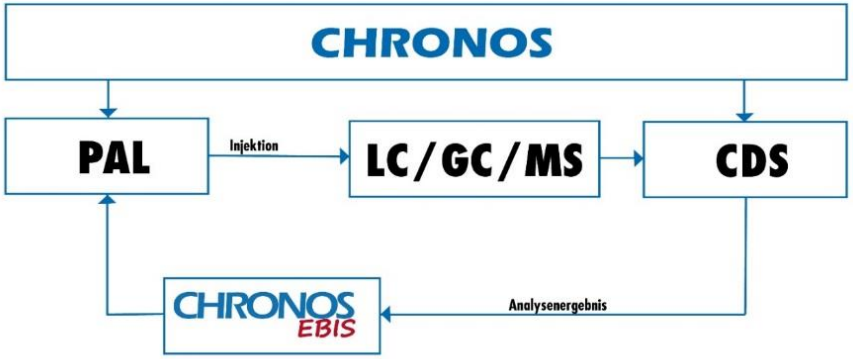
CHRONECT MCPD

The CHRONECT MCPD system does prepare samples following the official AOCS methods

- or with slight adaptions to improve throughput if wanted

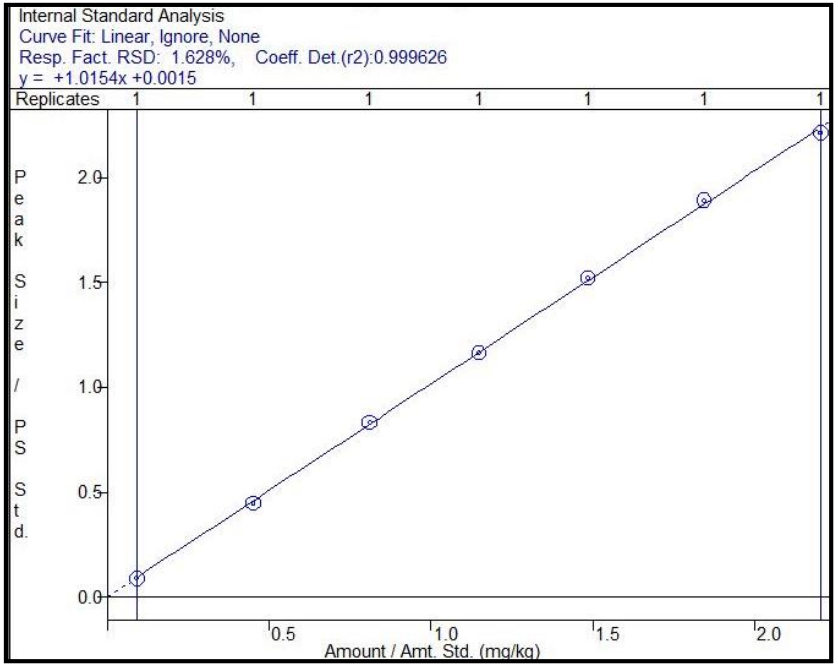
Monitor the robustness of the system using freshly prepared standards

Can react to events within the sample lists using a CHRONOS plugin (EBIS)

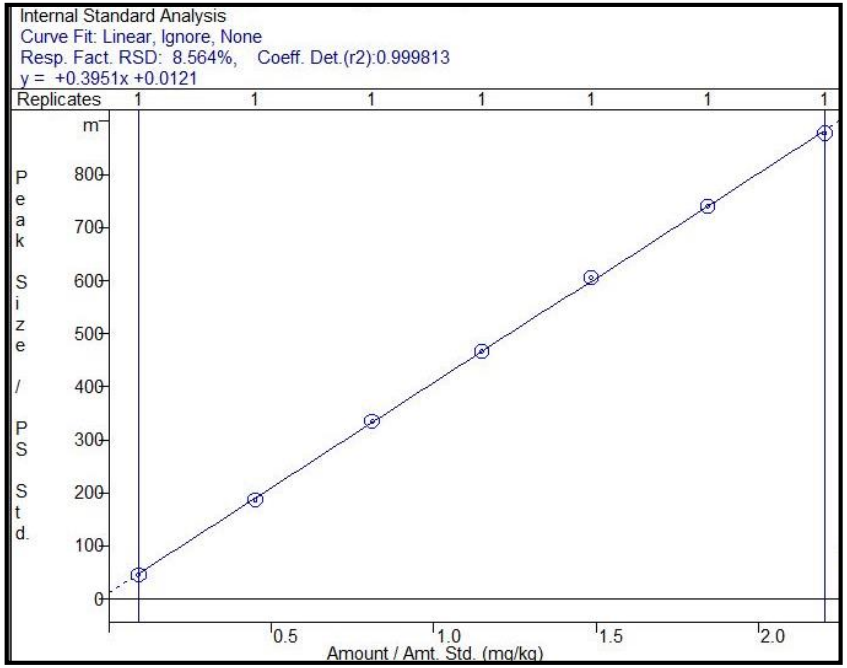


Automatic Calibration

3-MCPD



2-MCPD



- **Calibration between 0.05 mg/kg and 2 mg/kg**

Reproducibility & Stability

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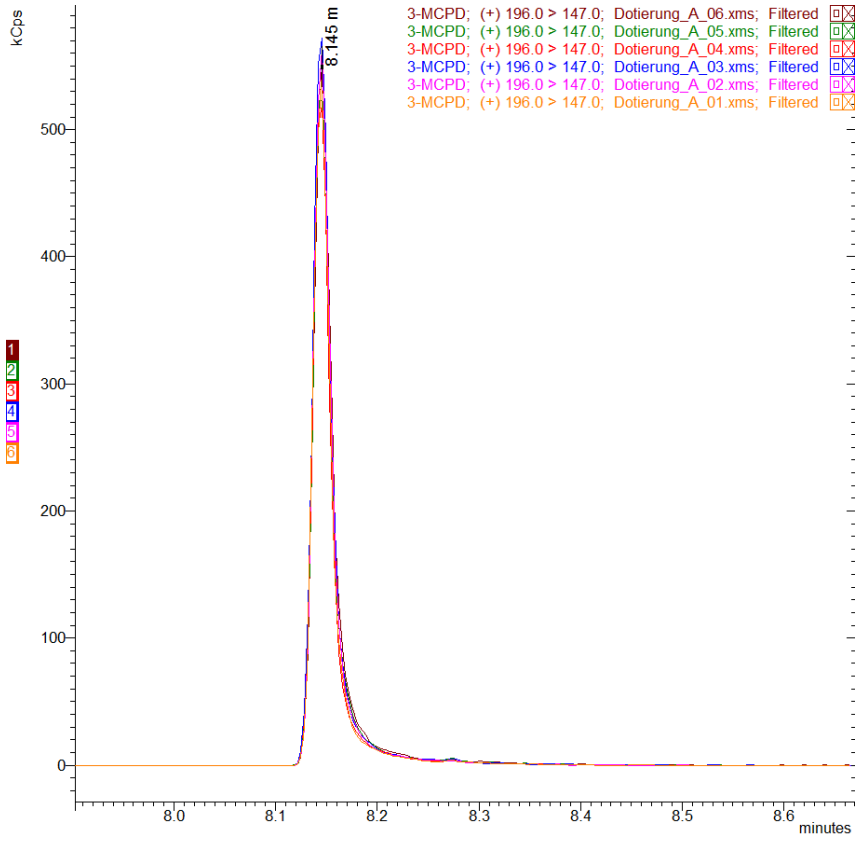
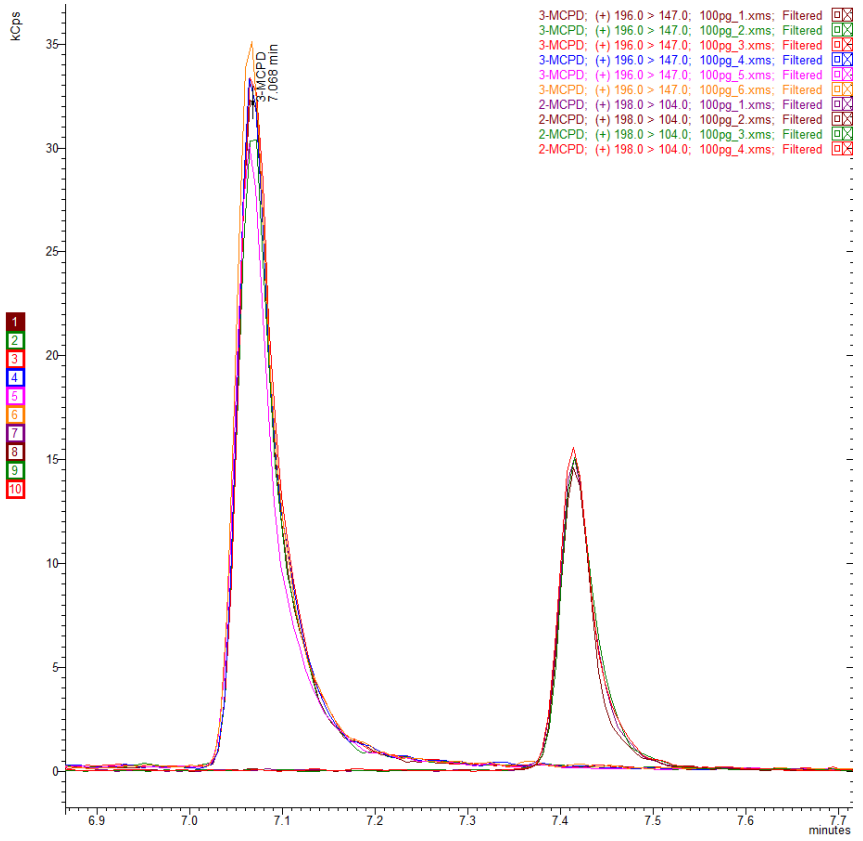
Very good reproducibility at different concentration levels

3-MCPD at 1 mg/kg

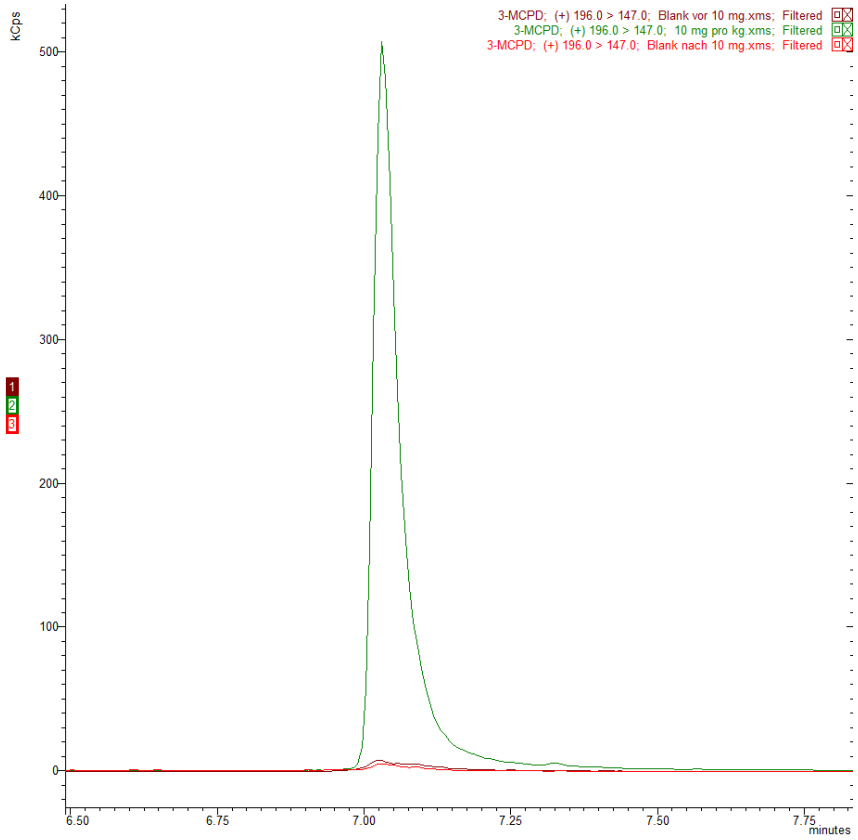


3-MCPD & 2-MCPD at 0.1 mg/kg

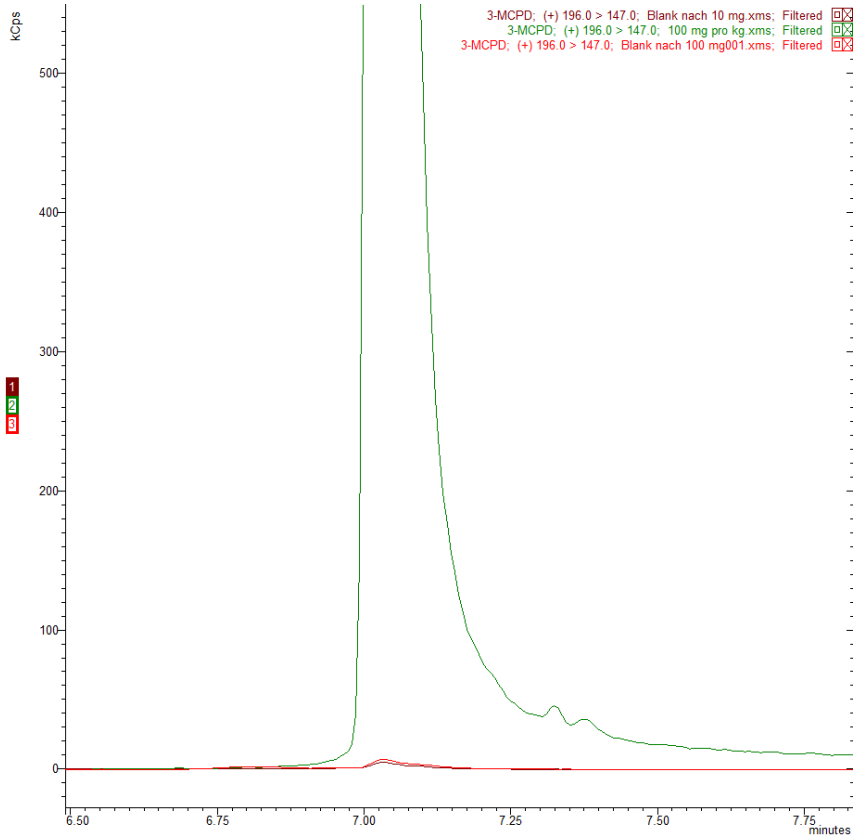


Reproducibility & Stability

10 mg/kg Standard



100 mg/kg Standard



No Carryover visible after highly contaminated samples

High sensitivity

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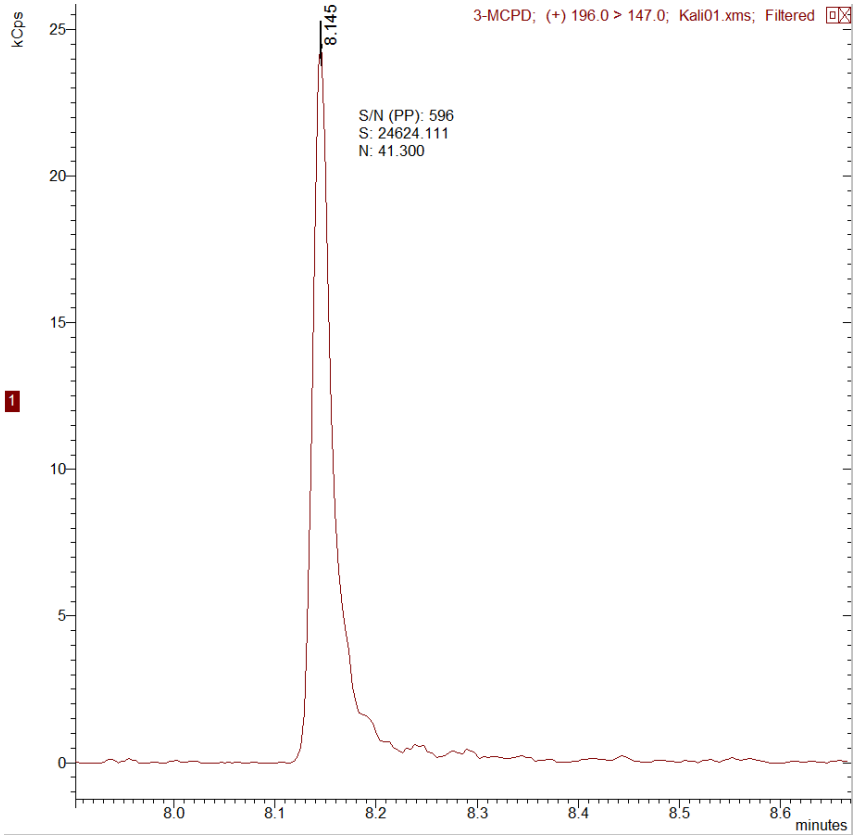
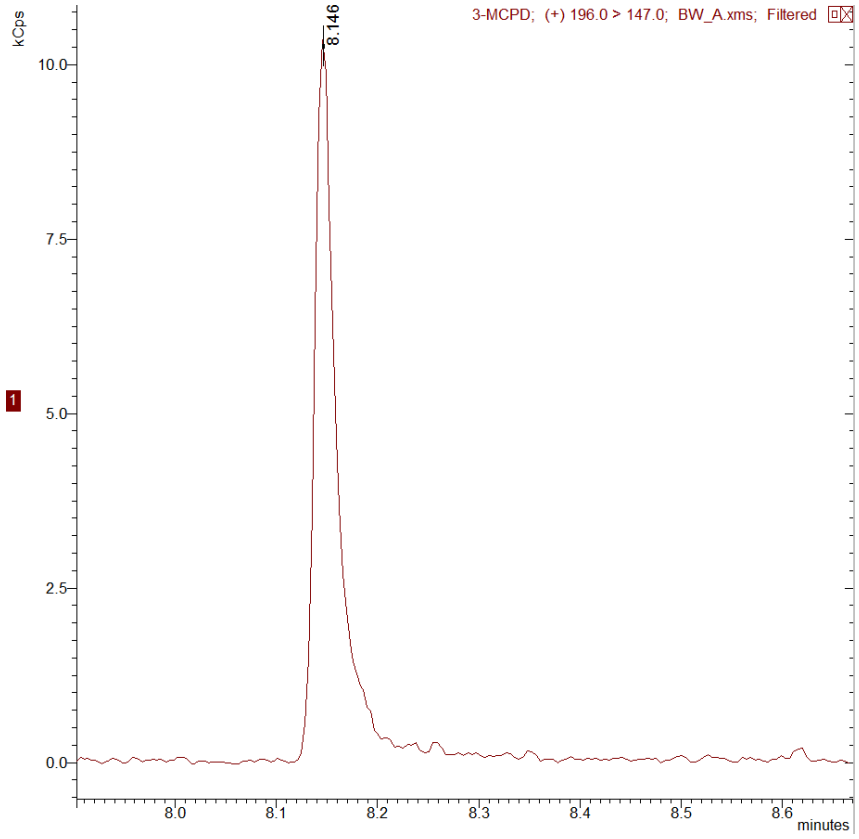
3-MCPD at 0.05 mg/kg



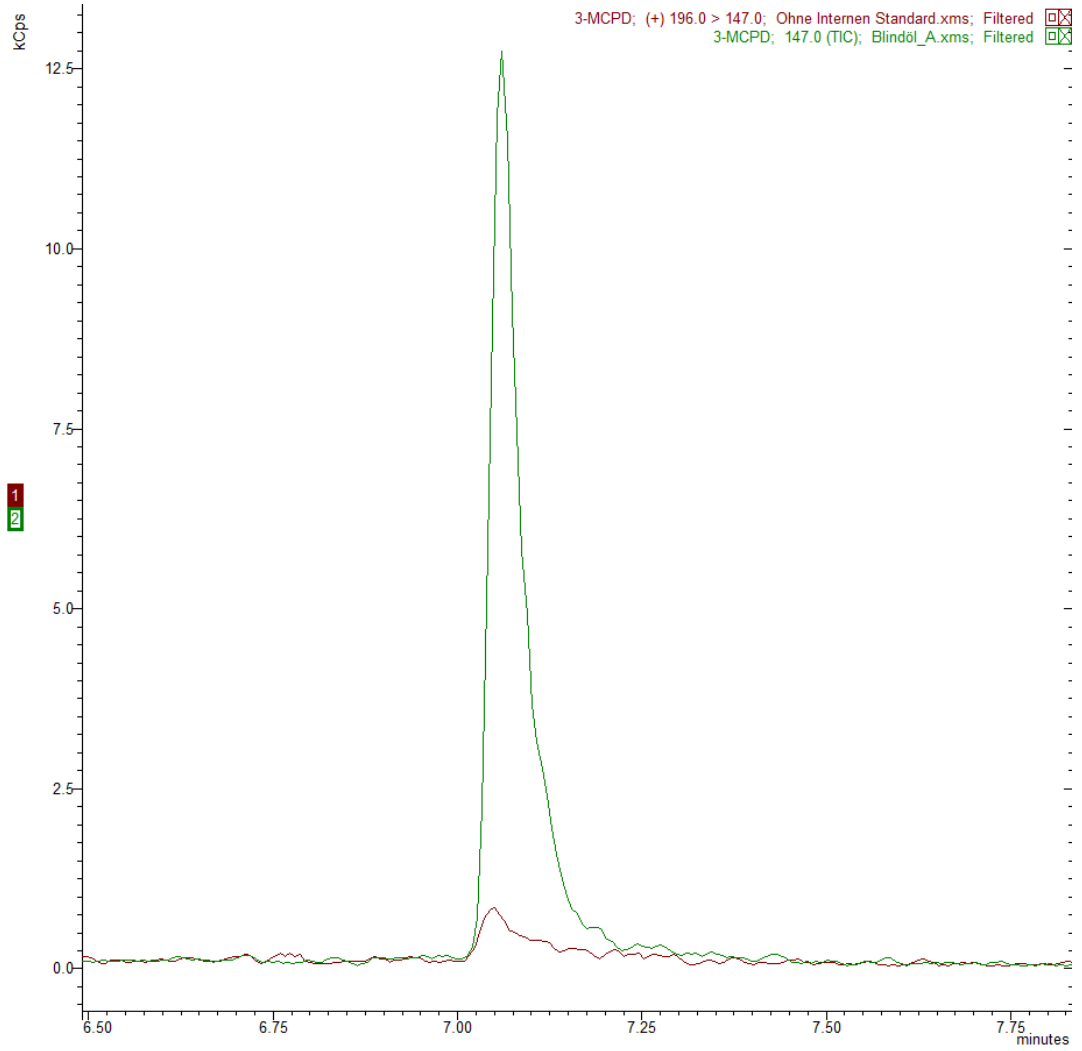
Background ~ 0.02 mg/kg



High sensitivity

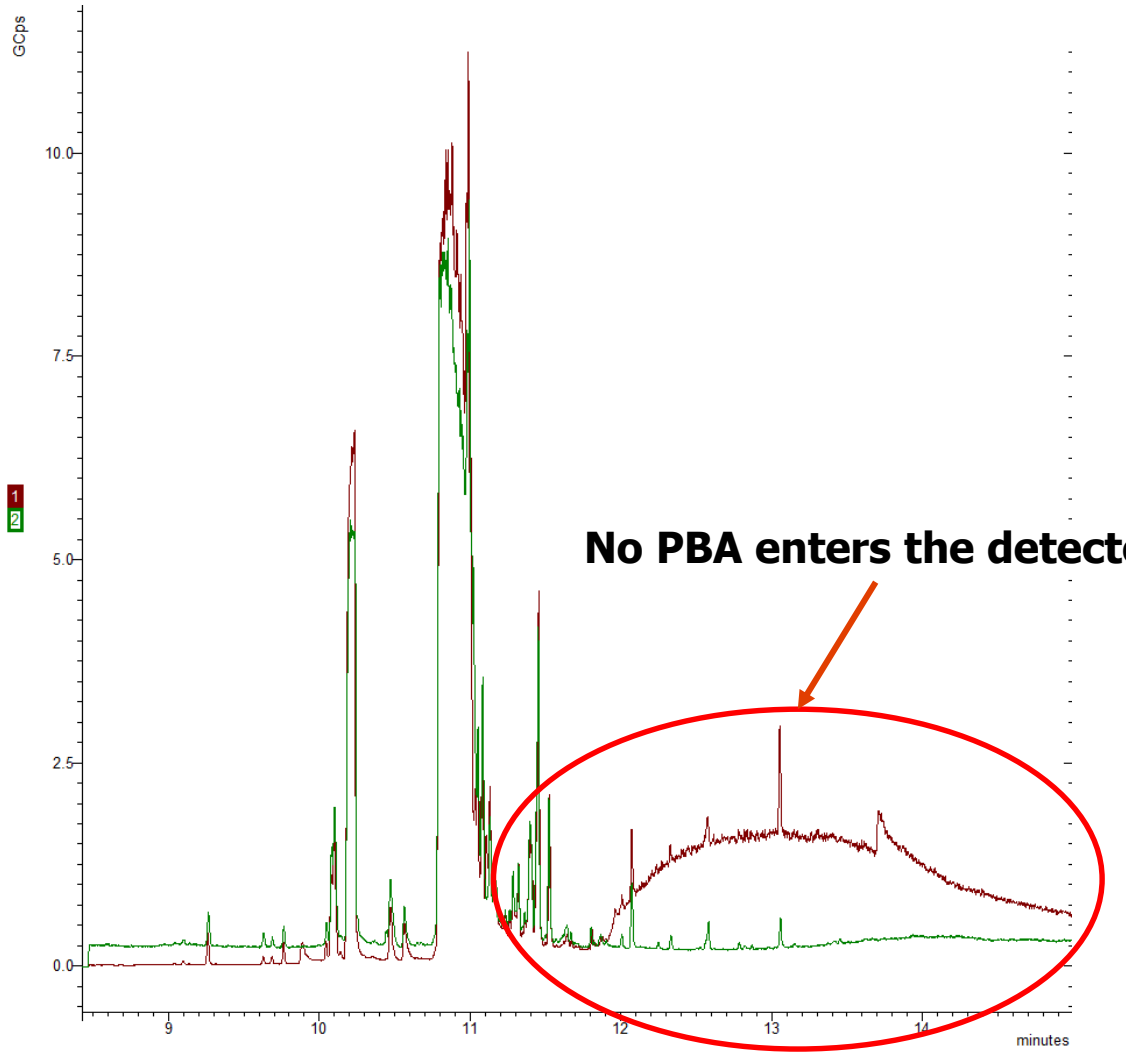
The internal Standard contains ~ 0.01 % 3-MCPD

=> Reducing the amount of internal Standard will increase sensitivity



> 90 % of the background are from the internal Standard

AS Method adaption



Method adaption

Getting rid of the PBA on the analytical column using a Backflush system

Further adaption of the official DGF-Method will

- Increase Sample throughput
- Instrument longevity

Method Adaption DGF Fast

Real samples vs. round robin results

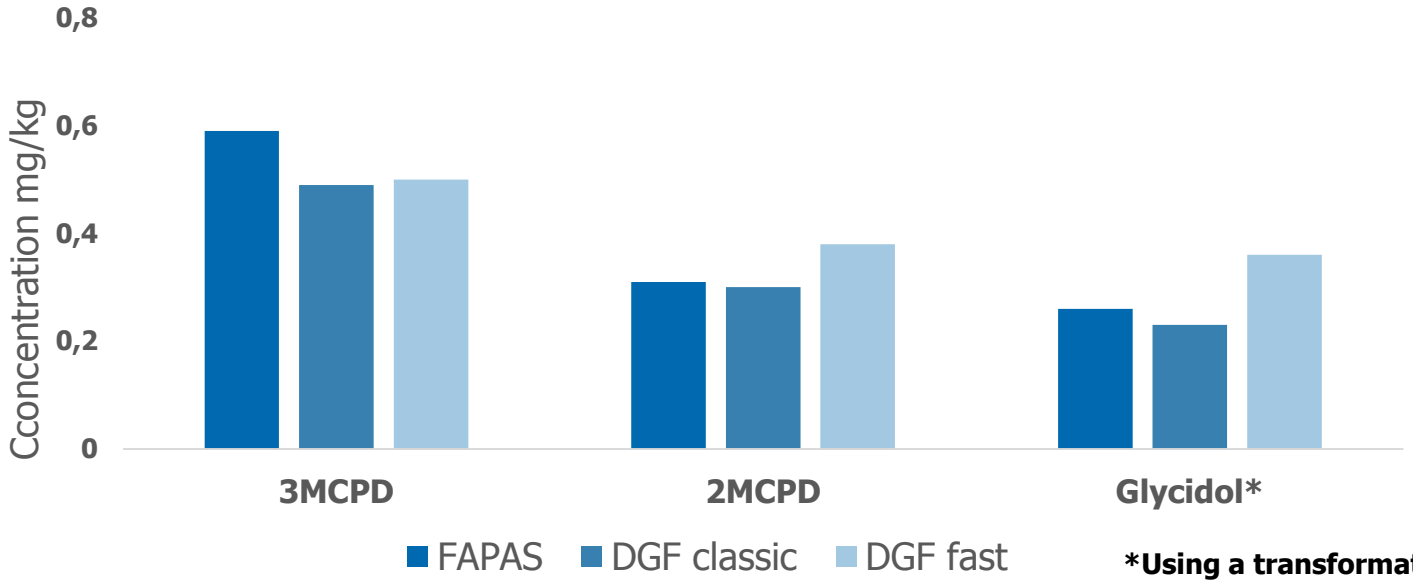
Comparison of the DGF/AOCS method with a faster DGF/AOCS method

- *DGF classic* = AOCS CD 29c-13 // *DGF fast* = based on AOCS CD 29c-13, but **shorter**

FAPAS: Manually prepared samples from a round robin test

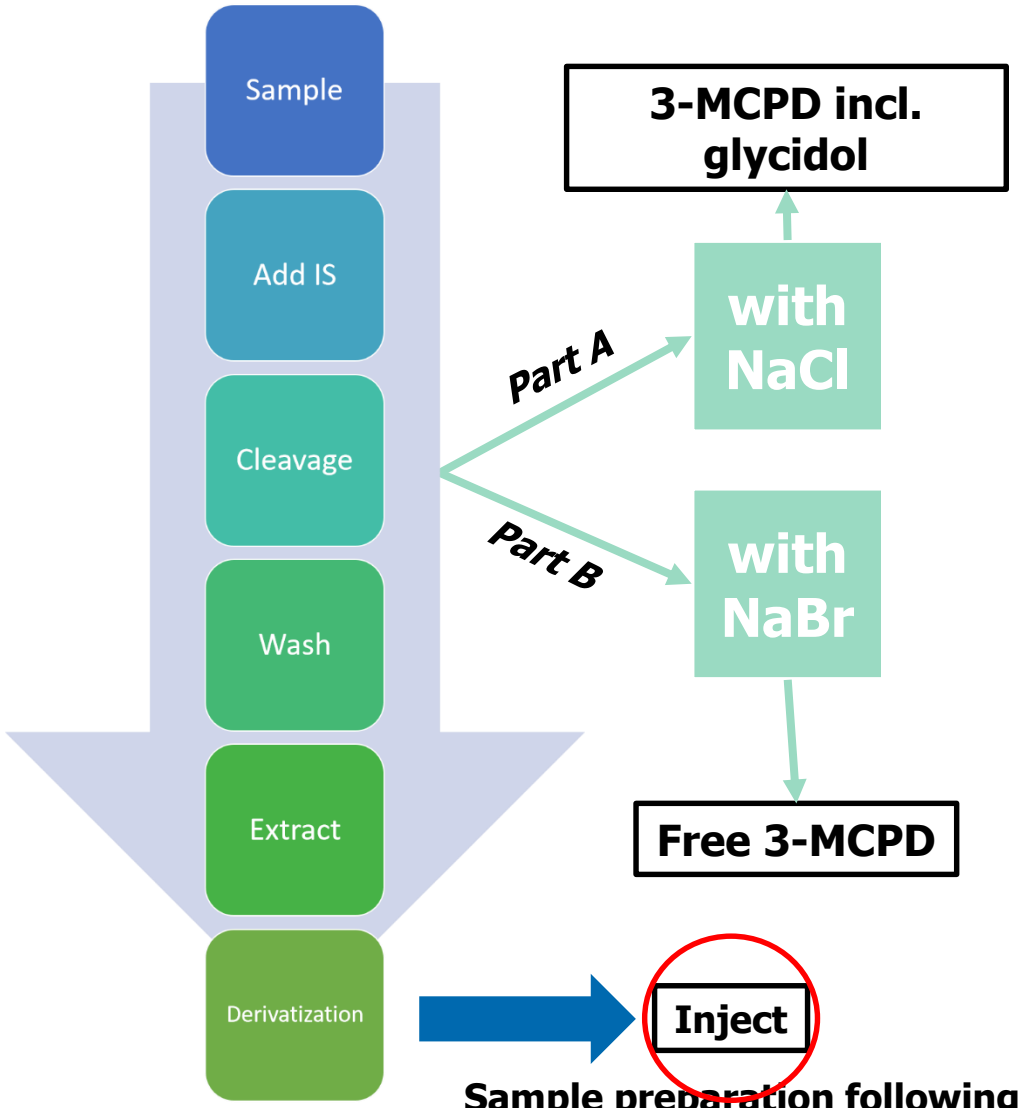
- Processed according to AOCS Cd 29a-13, AOCS Cd 29b-13 or AOCS Cd 29c-13

Validation with FAPAS Reference Oil



***Using a transformation factor of 1.**

AOCS Cd 29c-13 workflow



Weigh in ~ 100 mg oil sample

Fully automatic

- addition of internal standard
- cleavage
- derivatization

Injection into GC

Detection by MS

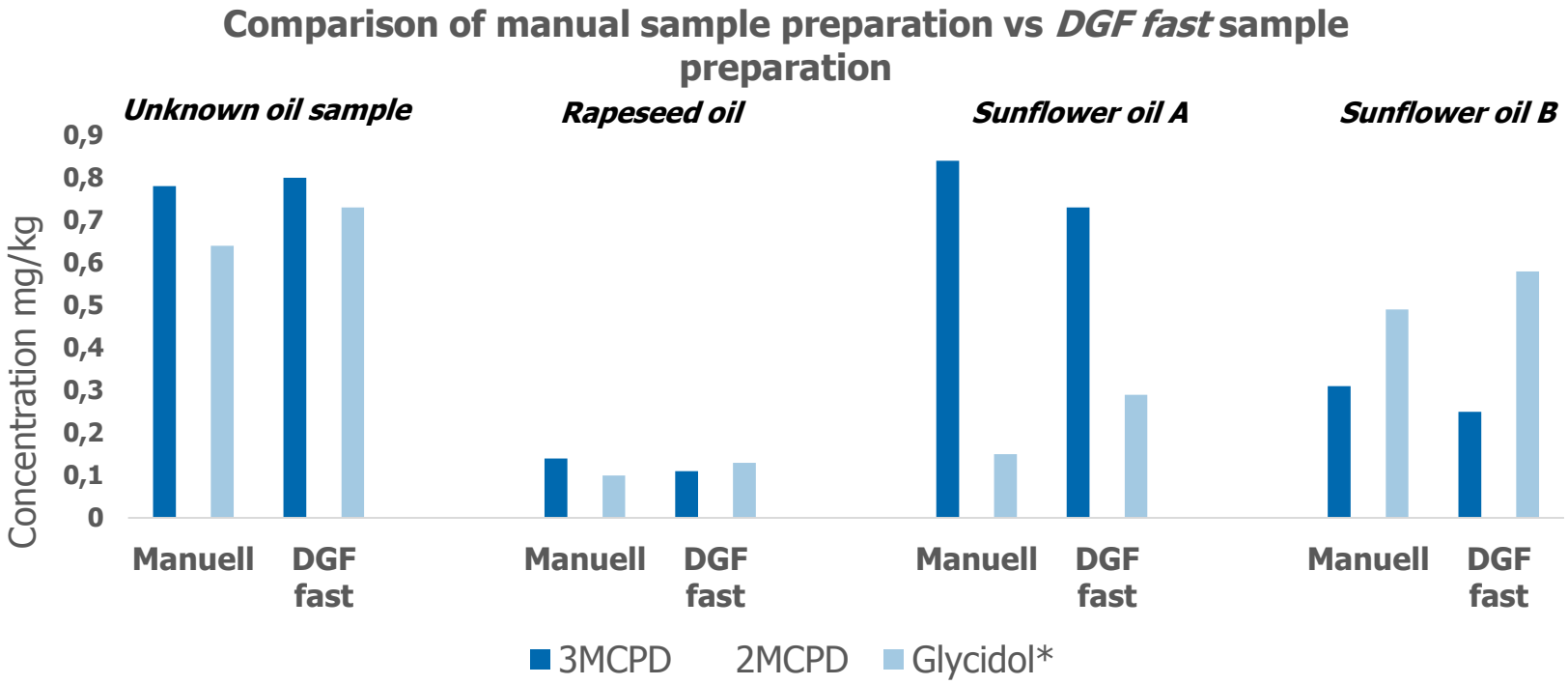
Part A: 3-MCPD incl. glycidol
Part B: free 3-MCPD

Glycidol = (Part A – Part B) * t

Sample preparation following the official AOCS/DGF method.

Method validation

Comparison of manual sample handling according to AOCS Cd 29b-13 with the automated process *DGF fast*



*Using a transformation factor of 1.

Summary MCPDs

Axel Semrau®

Accuracy and precision are comparable to manual results.

20 samples can be processed in 24 hours

(Part A & B).

The automated sample preparation delivers "clean" samples to ensure detector longevity.

Offline or online approach possible.

Coupling to a GC Triple Quadrupol allows low limits of detection.

The modular PAL system platform allows to adapt the system to many methods, e.g. Unilever or SGS.

Accuracy, precision, productivity

CHRONECT YOUR LAB

Axel Semrau®

Automated Sample preparation for analysis of FAMEs using a PAL3

Tobias Uber

Fatty Acids as FAME by GC and GC/MS

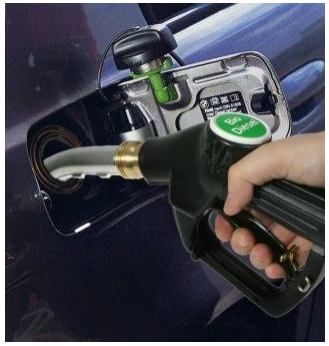
Axel Semrau®

Main applications: food & fuel

Determination of fatty acid composition and content in food

- The saturated fatty acid (SFA) content in the daily diet should be < 10 % to reduce the risk of diabetes
- Requirement to declare fat content / composition on packaging label → EU regulation No 1169/2011 coming into force in Dec 2016

Determination of biodiesel composition to ensure product quality



- EN 14103: Determination of total FAMEs and linolenic methyl ester (C18:3)
-

FAMEs, Official Methods

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Official methods for different matrices

AOAC official methods (GC-FID methods)

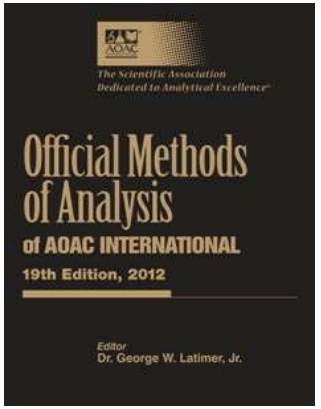
- 996.01 Fat in cereals (e.g. infant formula)
- 996.06 Fat in food (general, saturated and unsaturated)
- 985.21 trans-fatty acids/PHO in margarine
- 991.39 PUFA in fish oils
- 969.33 Fatty acids in oils and fats

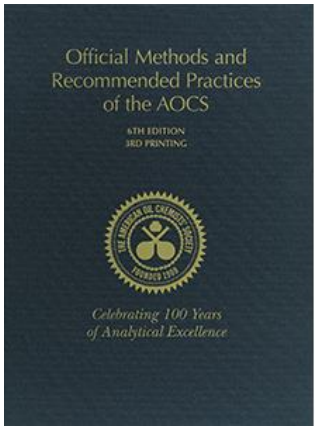
AOCS official methods (GC-FID methods)

- Ce 1i-07 PUFA in fish oils
- Ce 1h-05 cis/trans, SAFA, MUFA, PUFA in oils and fats
- Ce 2b/c-11 Fat in food, beverages, tissues, oils
- Ce 2-66 Fatty acids in oils and fats
- Ce 1e-91 FAMEs of long-chain fatty acids

European official methods

- 12966 – 1.-4. ISO method for animal and vegetable fats and oils
- 2001:1352 European Pharmacopoeia method
- 269.1 Ministry of Health, Switzerland





FAMEs Sample Preparation

Two official methods – two workflow examples

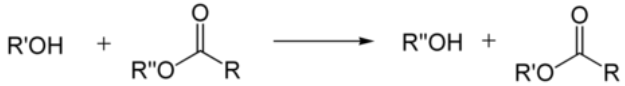
Switzerland 269.1

- Rapid Transesterification (1)
- Weigh fat/oil sample into a vial
- Add dioxane (or MTBE).
- Add methoxide in methanol
- Wait 1 min
- Add heptane and shake
- Add citrate and shake
- Inject from the heptane phase into GC with FID detection
- Start analytical run

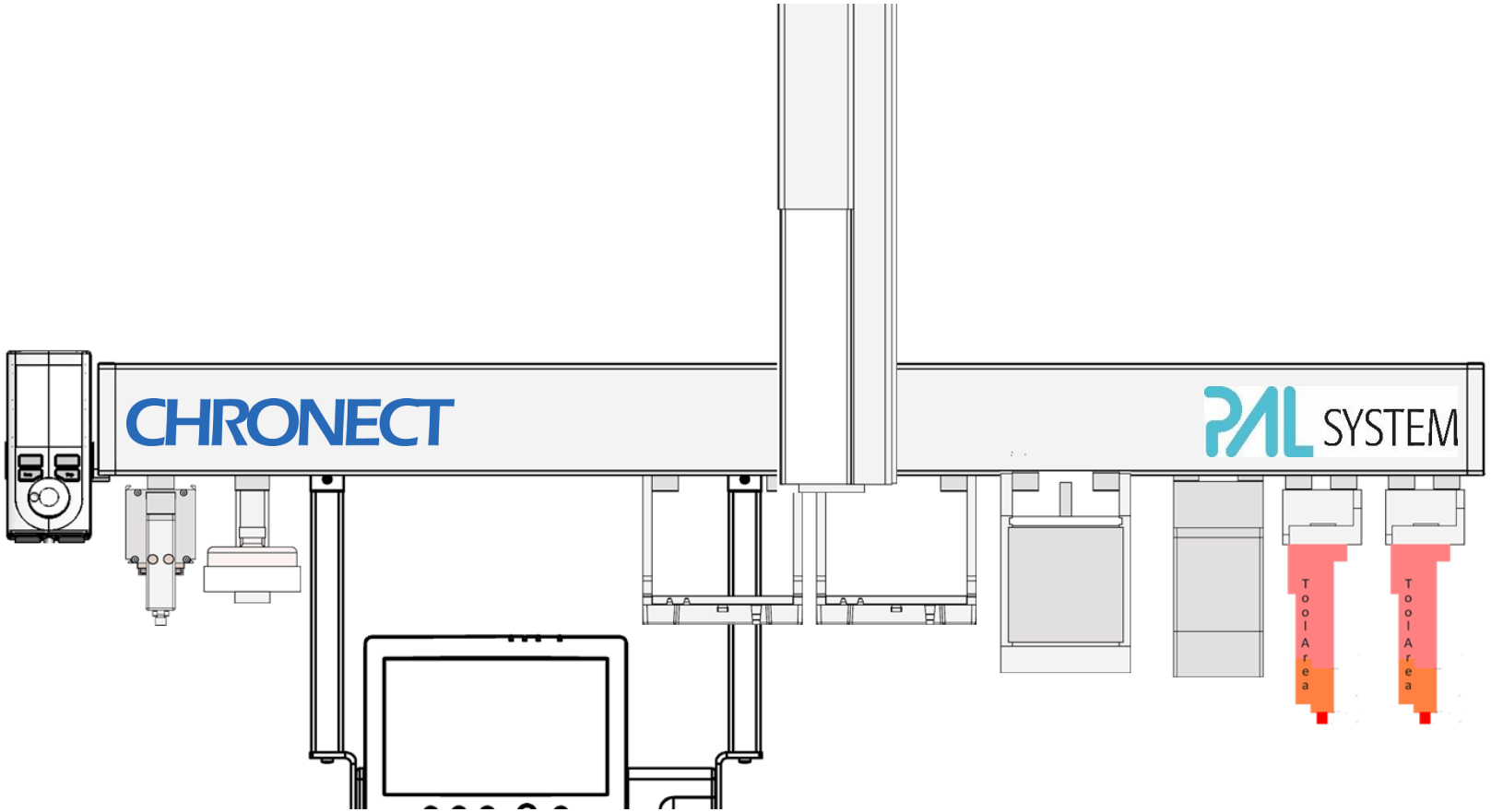
AOCS Ce 2-66

- Saponification/Esterification
- Weigh fat/oil sample into a vial
- Add 0.5 M methanolic NaOH
- Heat the mixture for 10 min
- Add BF_3, shake and heat for 2 min
- Add heptane and shake
- Add sat. NaCl solution and shake
- Transfer supernatant into vial with Na_2SO_4
- Inject from the heptane phase into GC with FID detection
- Start analytical run

1) Transesterification



Instrument Configuration



Transesterification Workflow

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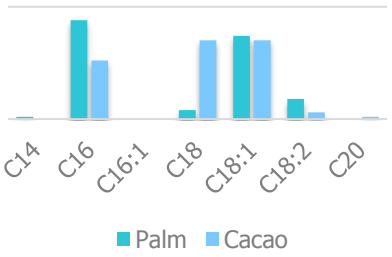
Fat content/composition of 10 different chocolates

Transesterification of fatty acid esters with Na-methoxide is a fast (90 sec), efficient and very robust method.

The PAL RTC allows full automation of the FAME sample preparation, including injection into the GC. This improves process safety and minimizes handling errors.

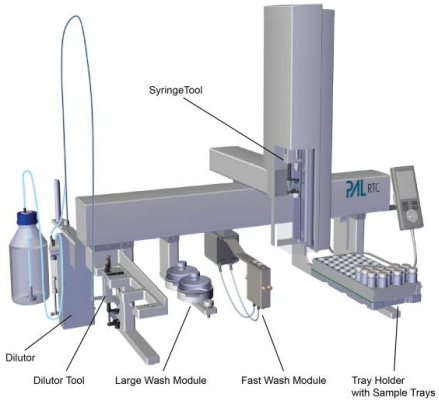
75 samples can be analyzed automatically in 24 hours (limiting factor = GC runtime). The system can process one sample while an other sample is being analyzed ("prep ahead").



- Add ISTD
- Add NaOMe
- Vortex vial
- Wait (derivatization)
- Add heptane
- Vortex vial
- Add citrate
- Vortex vial
- Inject sample

Start analytical run

- Start next sample



Cis/trans fatty acids
Blending
Counterfeit
Total fat content
PUFA (EPA/DHA)

Fat content/composition of chocolate

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Boehm *et al.*, Poster for ISCC 2016, Riva, Italy

10 chocolate samples

Cocoa butter & palm oil standard

Fatty acid composition from C10:0 to C18:2

Check for presence of palm oil

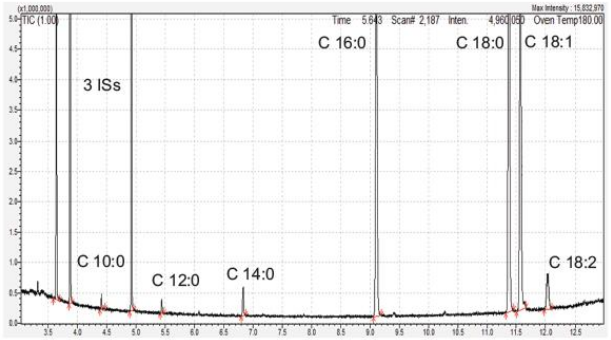
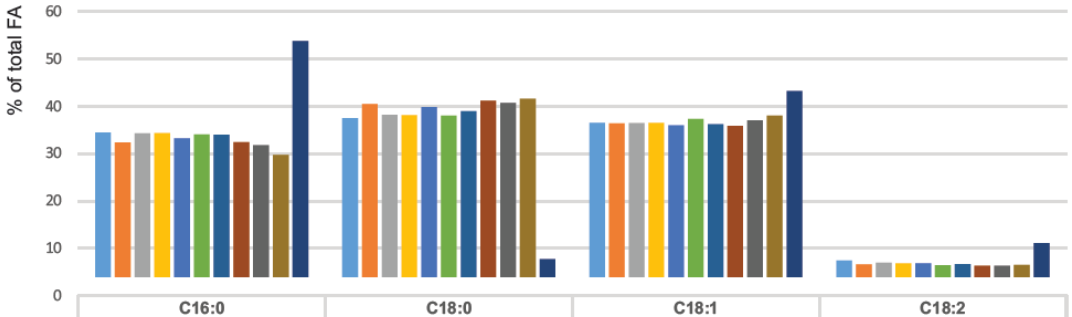


Figure 3: Chromatogramm of FAMEs from a milk chocolate sample



	C16:0	C18:0	C18:1	C18:2
01_Meiji_black chocolate	30.5	33.5	32.5	3.5
02_Meiji_milk chocolate	28.4	36.5	32.4	2.7
03_Meiji_himilk chocolate	30.3	34.2	32.5	3.0
04_Meiji_white chocolate	30.4	34.2	32.5	2.9
05_Lotte_Ghana milk chocolate	29.3	35.9	32.0	2.9
06_Lotte_Zero	30.1	34.0	33.3	2.5
07_Hersheys_milk chocolate	30.0	35.0	32.3	2.8
08_Migros_Budget Milchschokolade	28.4	37.2	31.9	2.4
09_Migros_Noir Special 72%	27.8	36.7	33.1	2.4
Kakaobutter Referenz KL ZH	25.8	37.6	34.1	2.5
Aldrich_Palmoil standard	49.8	3.8	39.3	7.1

Figure 4: Fatty acid profile of different chocolate samples,as well as cocoa butter and palm oil reference samples.

AOCS Workflow

AOCS Ce 2-66

Saponification of fatty acid esters with methanolic NaOH

Esterification with methanolic BF_3

- For fatty acids with an acid value < 2 methanolic KOH is used without meth. BF_3

With PAL RTC the FAME preparation, including handling of the hazardous BF_3 and injection into the GC, can be fully automated.

This improves operational and process safety and minimizes handling errors.

50 samples can be analyzed in 24 hours (limiting factor = GC runtime & phase separation time). The system can process one sample while an other sample is being analyzed ("overlapping").

Ce 2-66

Add methanolic NaOH

Incubate 10 min

Add BF_3

Incubate 2 min

Add heptane

Incubate 1 min

Add saturated NaCl solution

Vortex vial

Transfer supernatant into vial with Na_2SO_4

Inject sample

Start next sample

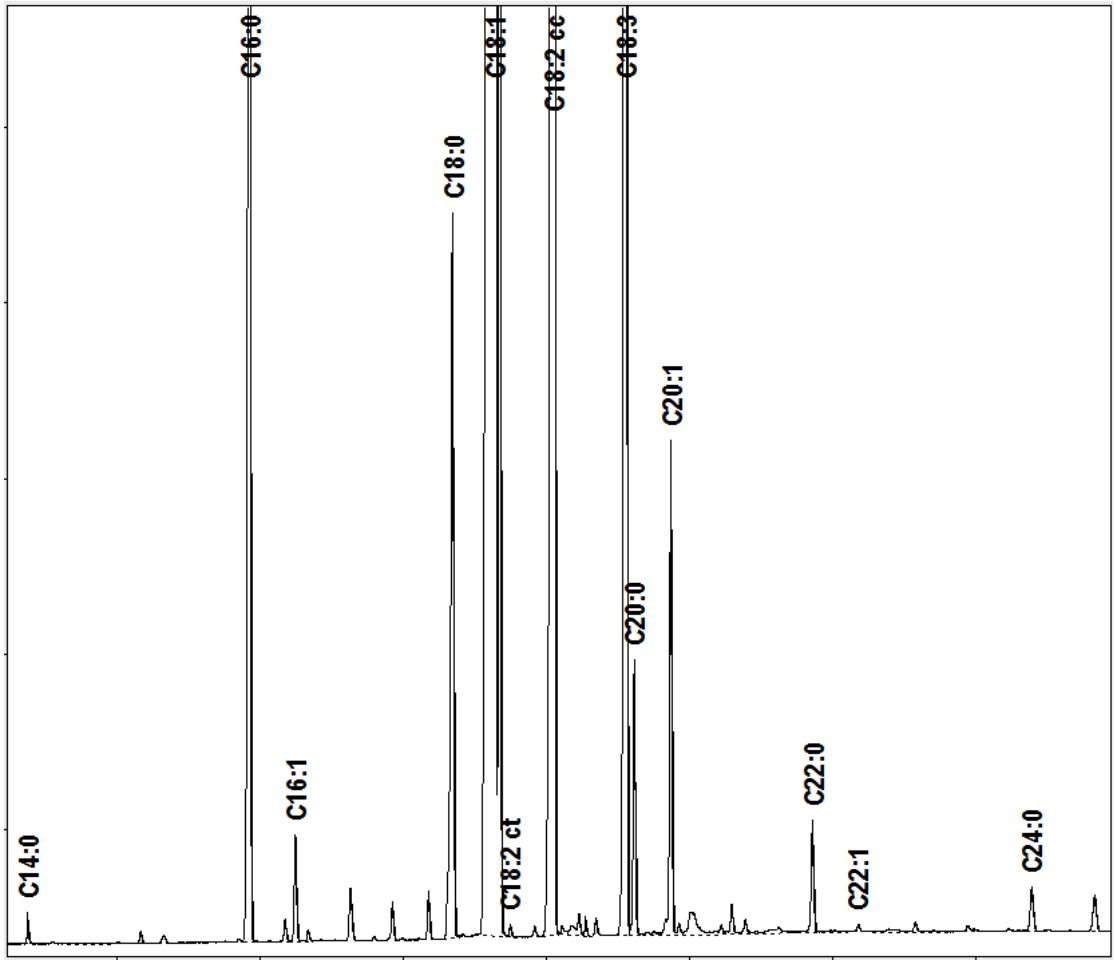
Start analytical run

Cis/trans fatty acids
Blending
Counterfeit
Total fat content
PUFA (EPA/DHA)

Axel Semrau®

AOCS Ce 2-66 method

Fatty acid composition from C14:0 to C24:1



Compound	Measured Area (%)	Desired Area (%)
C14:0	0.06	0.07
C16:0	4.30	4.29
C16:1	0.22	0.27
C18:0	1.94	1.97
C18:1 c	63.66	63.86
C18:2 ct	0.02	0.04
C18:2 cc	18.26	18.42
C18:3 t	0.16	0.15
C18:3 ccc	8.15	8.25
C20:0	0.56	0.56
C20:1	1.03	1.02
C22:0	0.28	0.27
C24:0	0.12	0.13
C24:1	0.13	0.13

Summary FAMEs

Productivity & process safety

High productivity, 50 samples in 24 h, unattended

Process safety

Occupational safety

Further standard methods can be implemented on the same system:

- For example determination of free hexane in animal feed according to AOCS Ba 13-87 und AOCS Ca 3b-87

Contact for further information

CHRONECT solutions are available through:

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