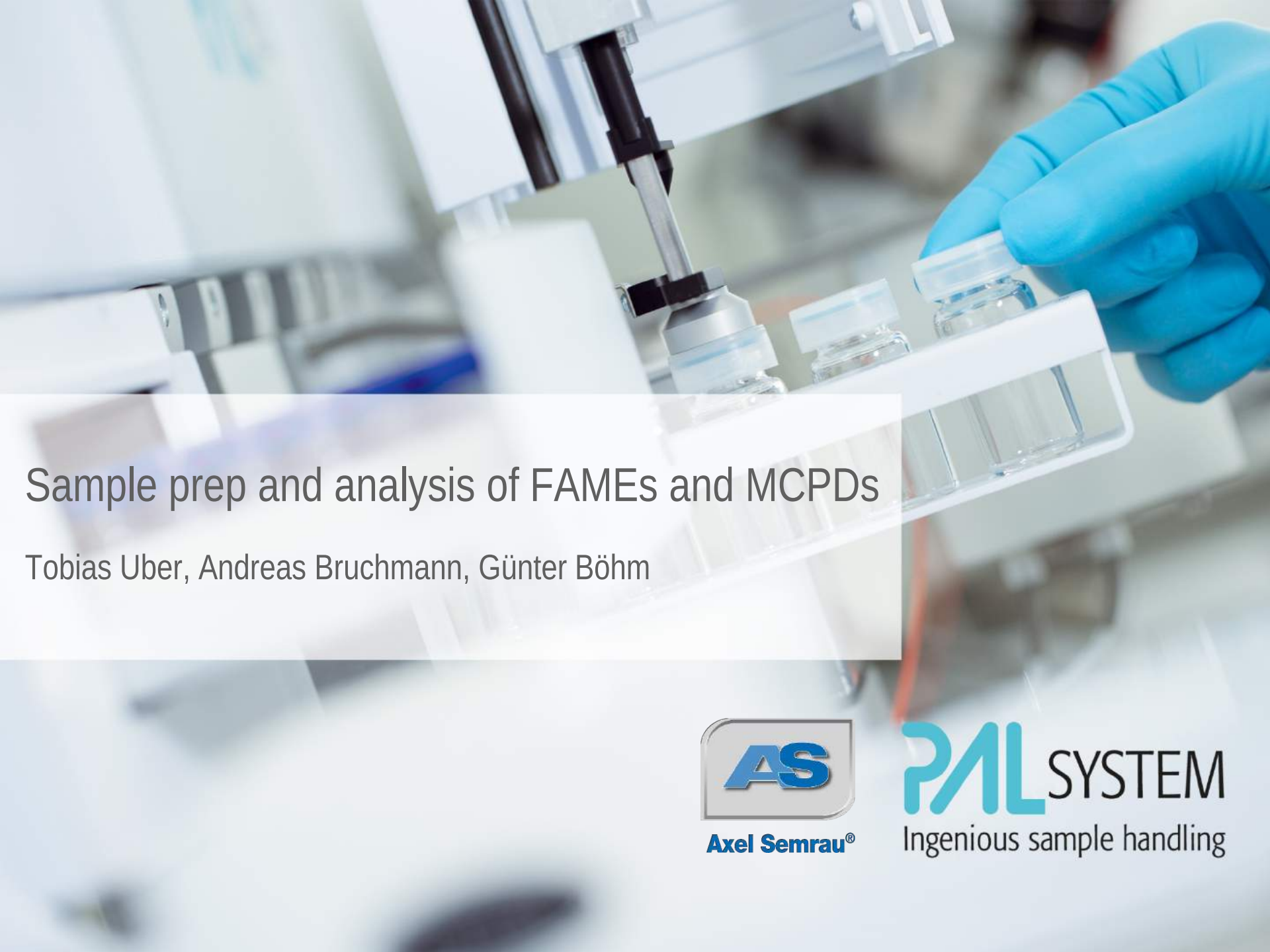

Sample prep and analysis of FAMEs and MCPDs

Tobias Uber, Andreas Bruchmann, Günter Böhm

Axel Semrau®

PAL SYSTEM
Ingenious sample handling

Overview

- Analysis of FAMEs
  - Methods
  - Example data
  - Advantages of automation
- Analysis of MCPDs
  - Methods
  - Example data
  - Advantages of automation
- Summary

Fatty Acids as FAME by GC and GC/MS

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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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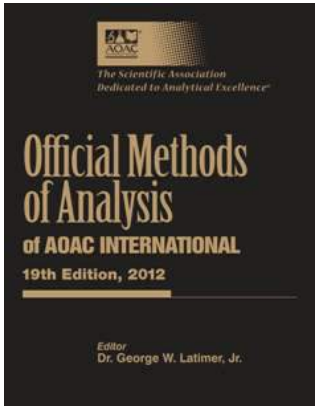
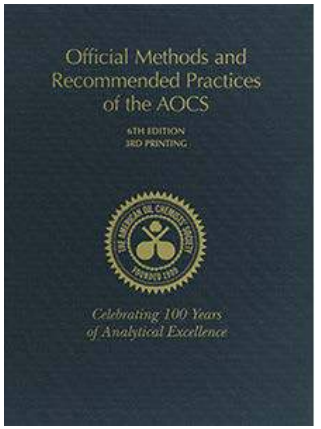
Official methods for different matrices

- 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

Rapid Transesterification (1) — Switzerland 269.1

- 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

Saponification/Esterification — AOCS Ce 2-66

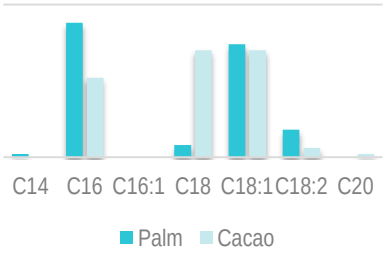
- Weigh fat/oil sample into a vial
- Add 0.5 M methanolic NaOH
- Heat the mixture for 10 min
- Add BF_3, shake and heat for 2 min
- Add heptane and shake
- Add sat. NaCl solution and shake
- Transfer supernatant into vial with Na_2SO_4
- Inject from the heptane phase into GC with FID detection
- Start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

1) Transesterification

Transesterification Workflow

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

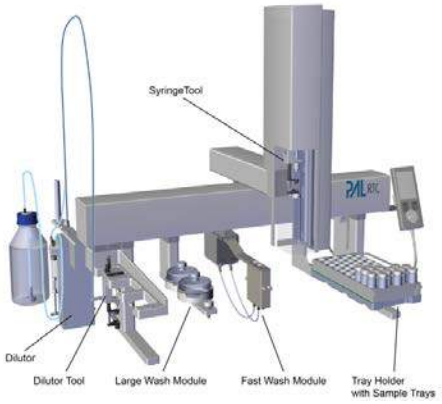
Add citrate

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

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

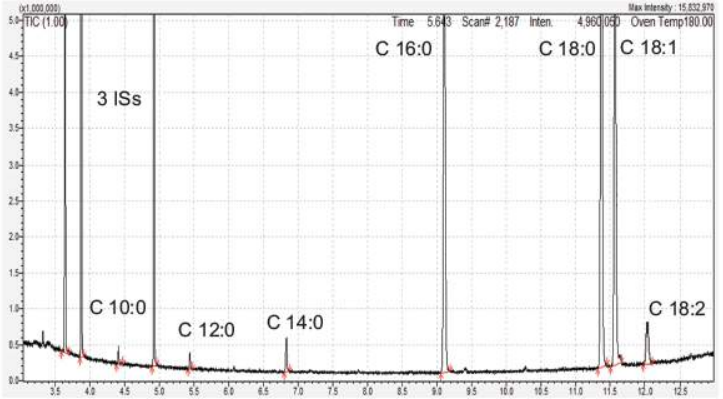
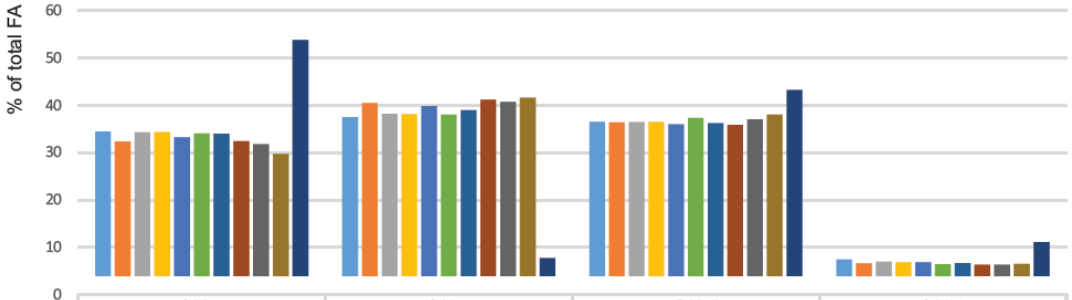


Figure 3: Chromatogramm of FAMEs from a milk chocolate sample



	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

AOAC 996.01

- 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”).

996.01

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 analytical run

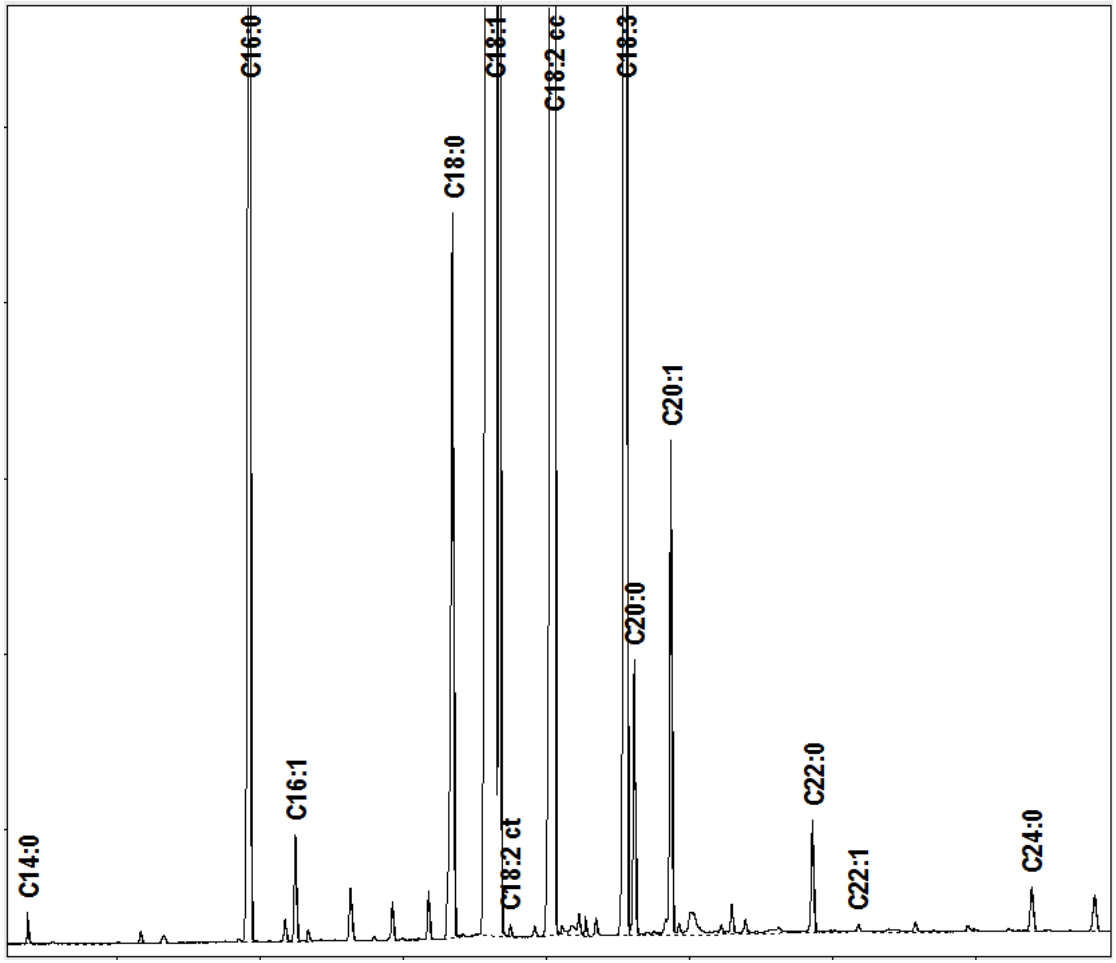
Start next sample

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

Animal feed

AOAC 996.01 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

Axel Semrau®

MCPD Solutions from Axel Semrau

CTC VAR Meeting 9/2017
Tobias Uber / Andreas Bruchmann

MCPD in the news

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

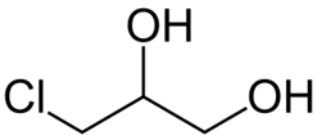
- Food manufacturers discuss pros/cons of using palm oil in the light of contamination by MCPDs, glycidol

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

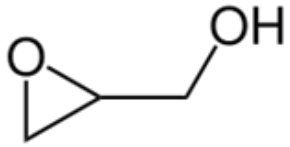
Süddeutsche Zeitung
SZ.de Zeitung Magazin

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

- 3-MCPD is a process contaminant generated when fatty or salted food is heated, e.g. soy sauce
- 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
- It is present in food mainly as ester. During digestion free 3-MCPD is formed in the human body
- Glycidyl esters are also of interest, since free glycidol is formed during digestion
- So far 2-MCPD has not been proven to be carcinogenic



3-MCPD



Glycidol

All official AOCS methods automated

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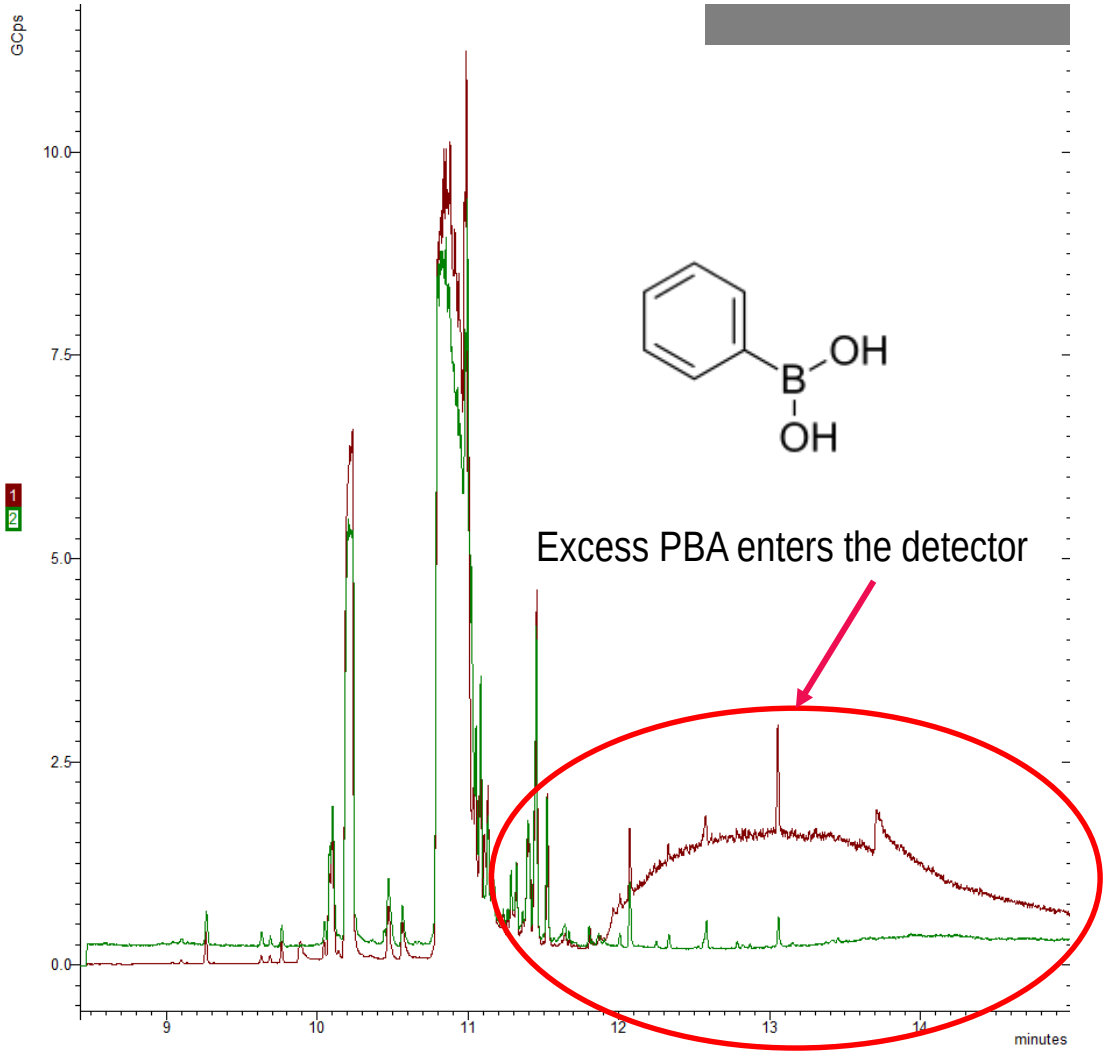
Trivial name	Components	Automation using PAL3 sampler	Official Method Name	Comment
DGF-Method	3 MCPD,GE (as difference), 2MCPD	100 % 160 cm DHR PAL RSI/RTC	AOCS Cd 29c-13 DGF C-VI 18 (10)	- Optional TQ for higher sample throughput - ~ 36 samples/day
Kuhlmann “3in1” SGS “3in1”	3MCPD,GE, 2MCPD	90 % 160 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 - 20 samples per day
Zwagerman-Overman-Method	3 MCPD, GE, 2MCPD	100 % 160 cm DHR PAL RSI/RTC	-	– Requires ^{13}C-Marked standard – 70 samples per day

- Other methods require ASE for sample preparation
- Optimization of AOCS Cd 29c-13 in cooperation with the Institut Kirchhoff Berlin
- Clean technology for higher sample throughput and longevity

Analyzer longevity



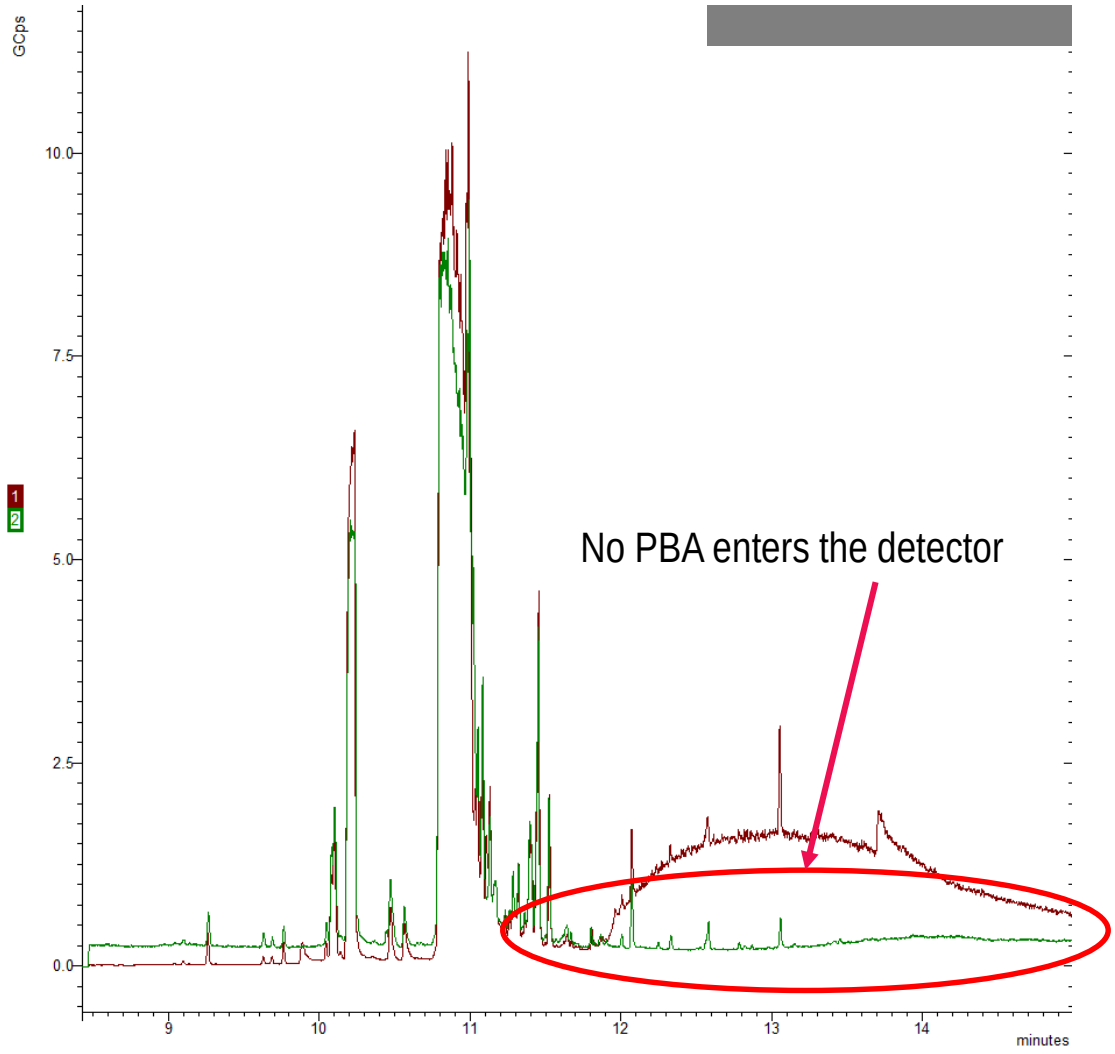
Method adaption

Getting rid of the PBA on the analytical column using a physical and chemical cleaning step.

Further adaption of the official DGF-Method will

- Increase Sample throughput
- Instrument longevity

Clean Technology
physical and chemical



<u>Method adaption</u>

Getting rid of the PBA on the analytical column using a physical and chemical cleaning step.

Further adaption of the official DGF-Method will

- Increase Sample throughput
- Instrument longevity

Reproducibility & Stability

PAL SYSTEM
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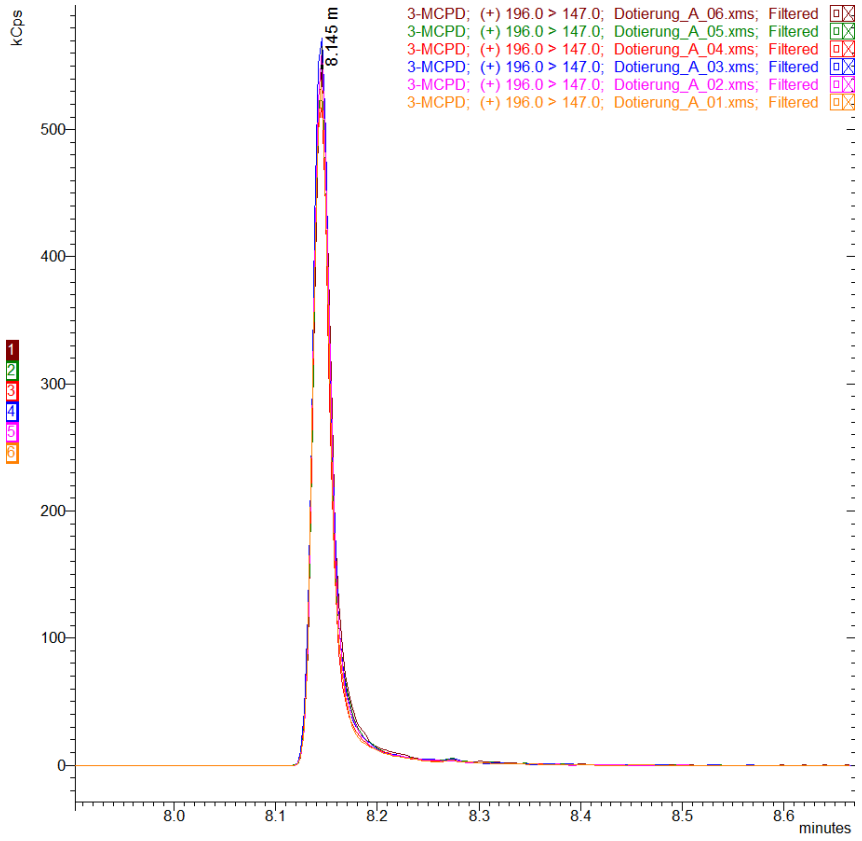
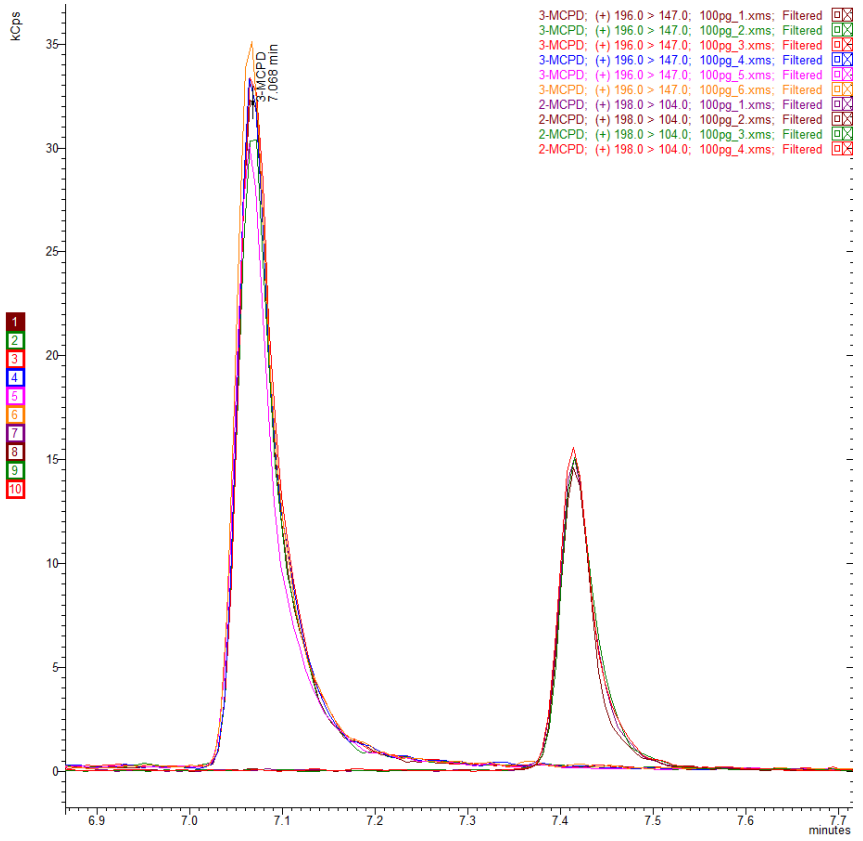
Very good reproducibility at different concentration levels

3-MCPD at 1 mg/kg



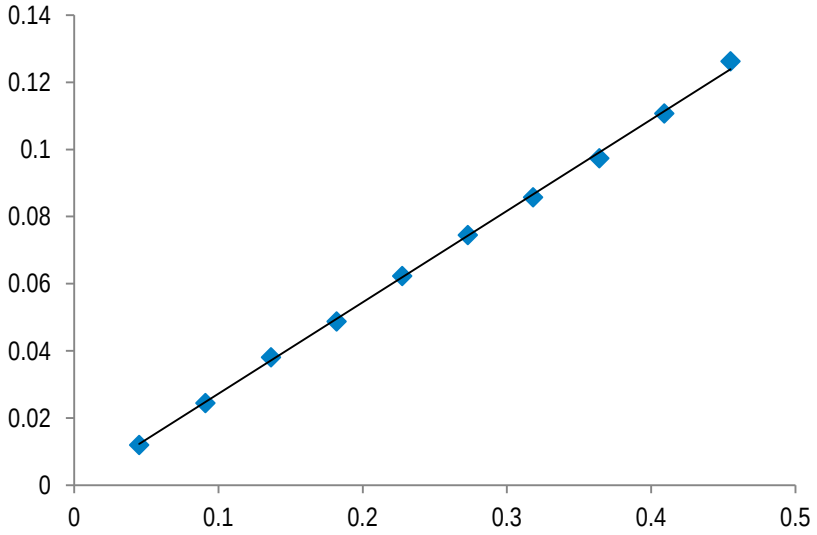
3-MCPD & 2-MCPD at 0.1 mg/kg



Linearity

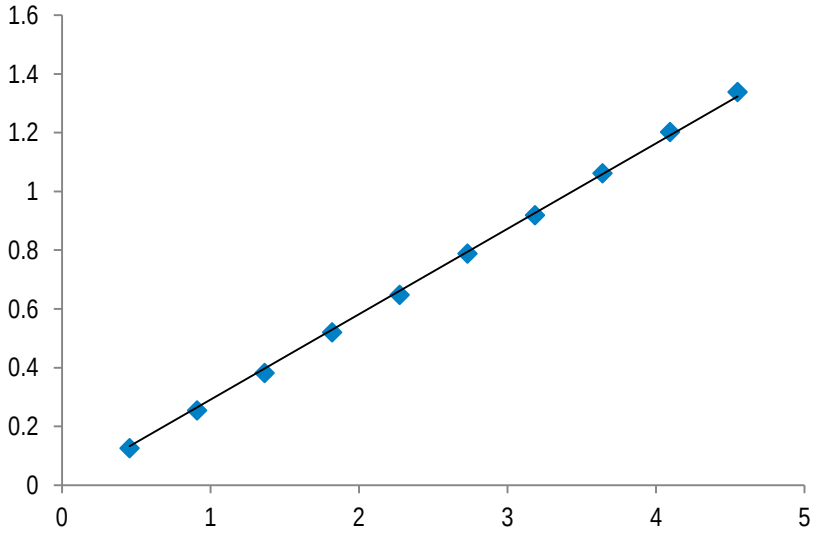
Calibration: 0,05 - 0,5 mg/kg

Correlation coefficient:	**0,9996**
Variation coefficient:	**1,74%**
Calibration:	**Linear**
Equation:	**y = -0.0003 + 0.2733x**



Calibration: 0,5 - 5 mg/kg

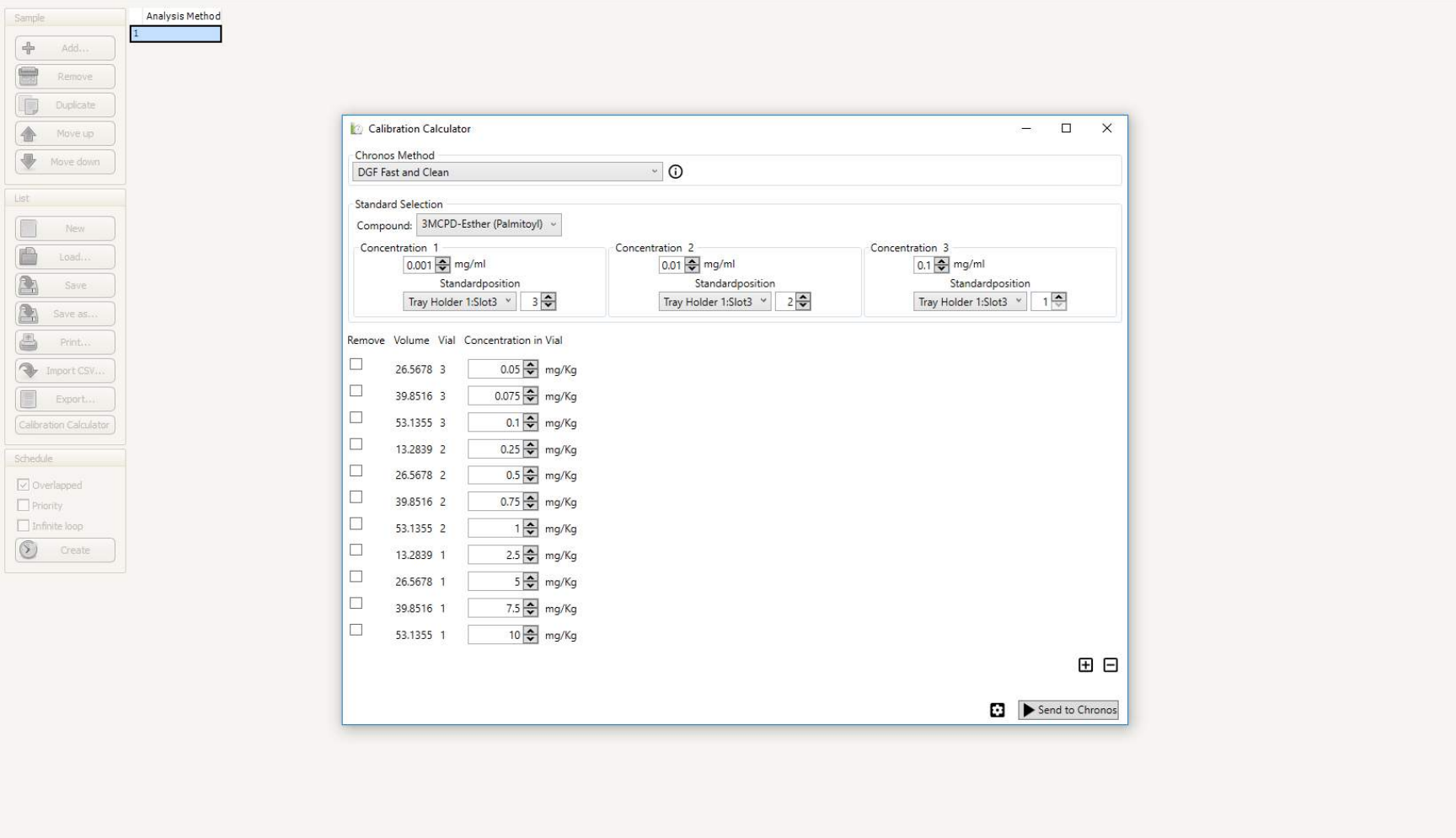
Correlation coefficient:	**0,9999**
Variation coefficient:	**0,88%**
Calibration:	**linear**
Equation:	**y = -0.0183 + 0.2967x**



- Quantification can also be done using only the internal standard, as written in the official AOCS norm

Plugin for Calibration List

Easy to use plugin to create calibration curves



Plugin for Calibration List

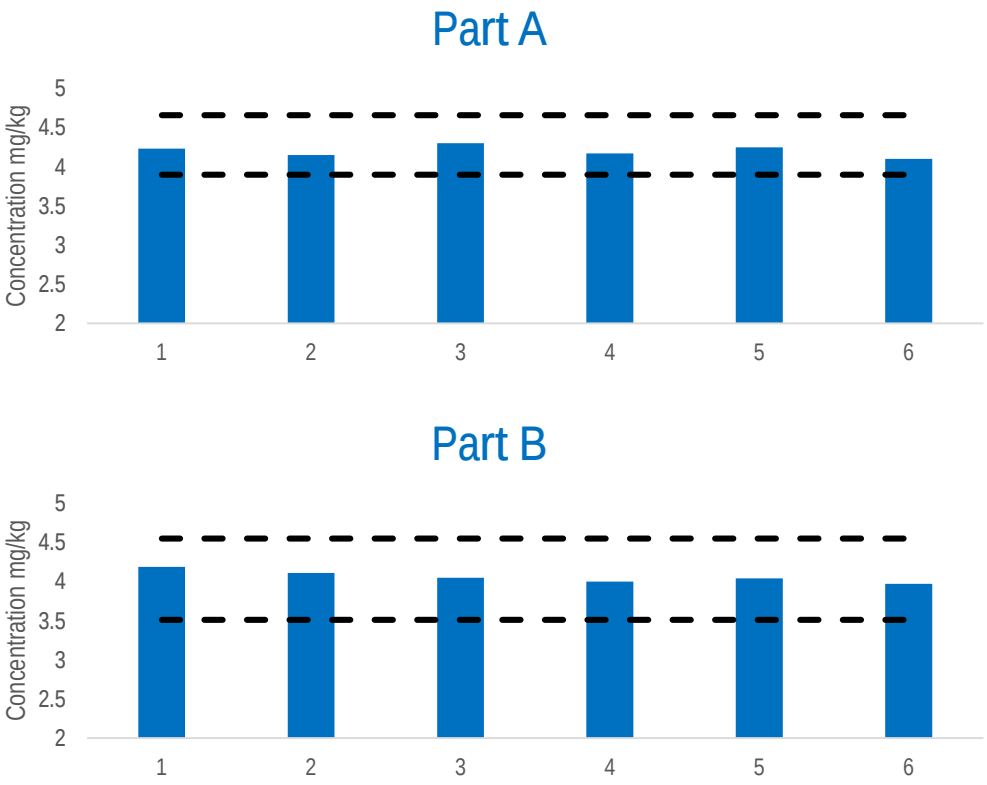
PAL SYSTEM
Ingenious sample handling

Easy to use plugin to create calibration curves

	Analysis Method	Source Tray	Source Vial	Salzart	Calibration?	Standard Volumen [µl]	Calibrationtray	Calibrationvial	Einwaage [mg]	Filename
1	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	26.568	Tray Holder 1:Slot1	3	100	101
2	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	39.8516	Tray Holder 1:Slot1	3	100	101
3	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	53.1355	Tray Holder 1:Slot1	3	100	101
4	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	13.2839	Tray Holder 1:Slot1	2	100	101
5	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	26.5678	Tray Holder 1:Slot1	2	100	101
6	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	39.8516	Tray Holder 1:Slot1	2	100	101
7	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	53.1355	Tray Holder 1:Slot1	2	100	101
8	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	13.2839	Tray Holder 1:Slot1	1	100	101
9	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	26.5678	Tray Holder 1:Slot1	1	100	101
10	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	39.8516	Tray Holder 1:Slot1	1	100	101
11	C:\Use...n.cam	Tray Holder 1:Slot1	1	NaCl	☑	53.1355	Tray Holder 1:Slot1	1	100	101

Method validation

Repeatability and Recovery of *DGF F&C* for 3-MCPD and 2-MCPD on consecutive days



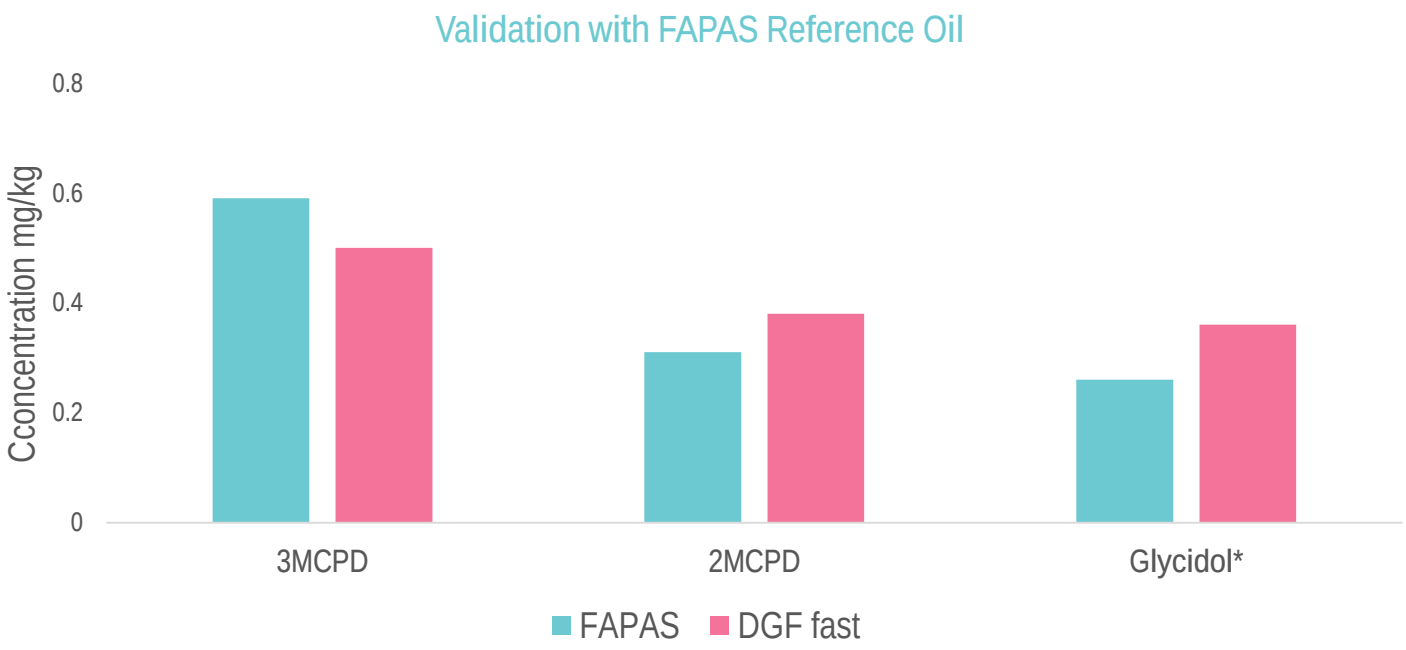
	Recovery %	Reproducibility %
3-MCPD part A	91.6	7.7
3-MCPD part B	101.9	8.8
2-MCPD part B	116.2	8.9

Results with DGF F&C

Real samples vs. round robin results

- FAPAS: Manually prepared samples from a round robin test

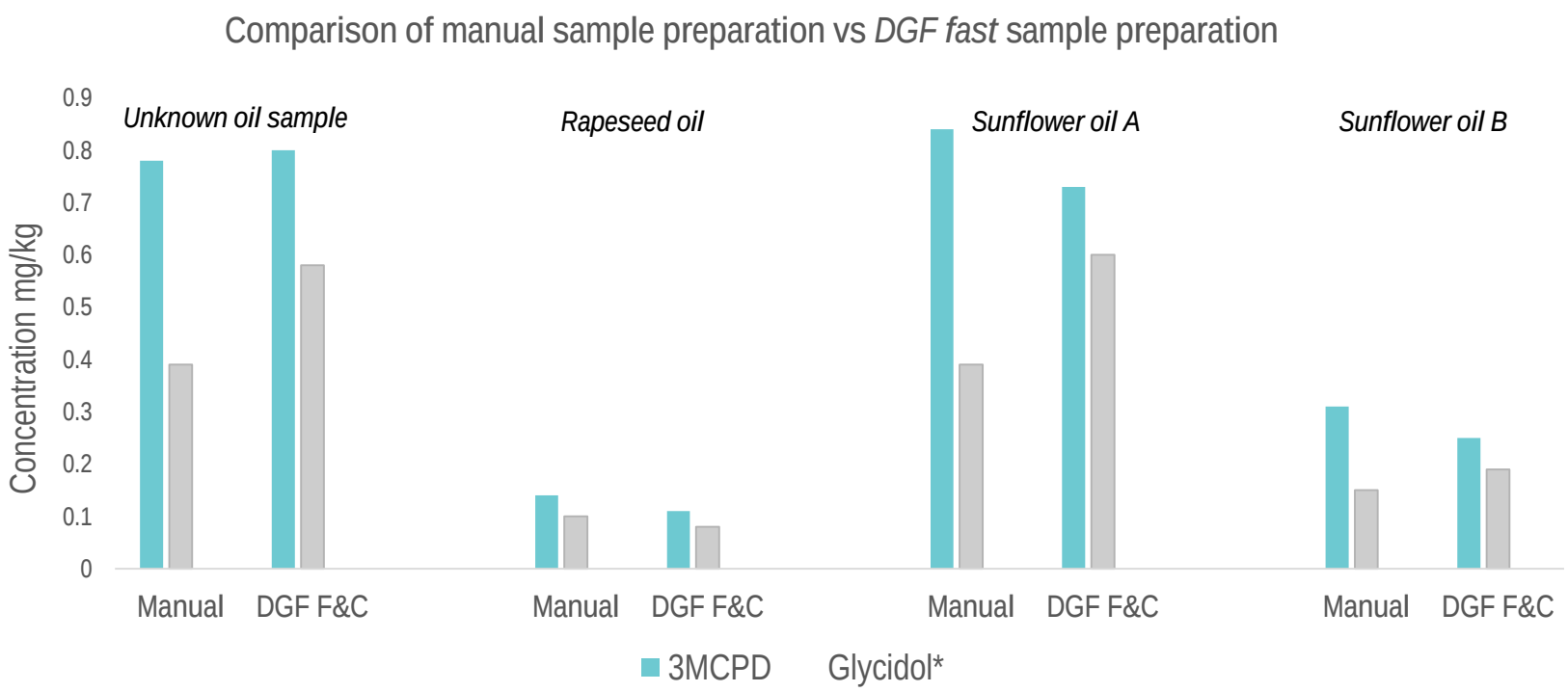
  Processed according to AOCS Cd 29a-13, AOCS Cd 29b-13 or AOCS Cd 29c-13



*Using a transformation factor of 1.

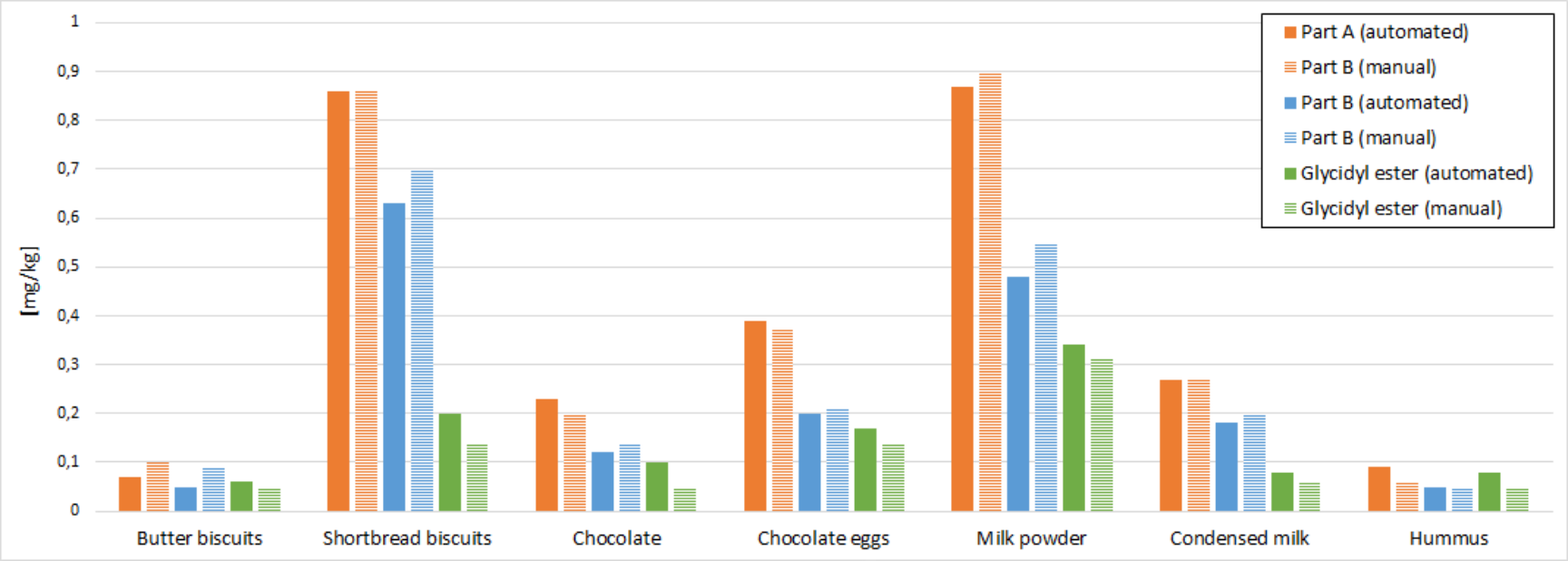
Method validation

Comparison of manual sample handling according to AOCS Cd 29b-13 with the automated *DGF F&C*



*Using a transformation factor of 1.

Comparison of manual sample handling according to AOCS Cd 29c-13 with the automated sample preparation *DGF fast & clean* in different matrices

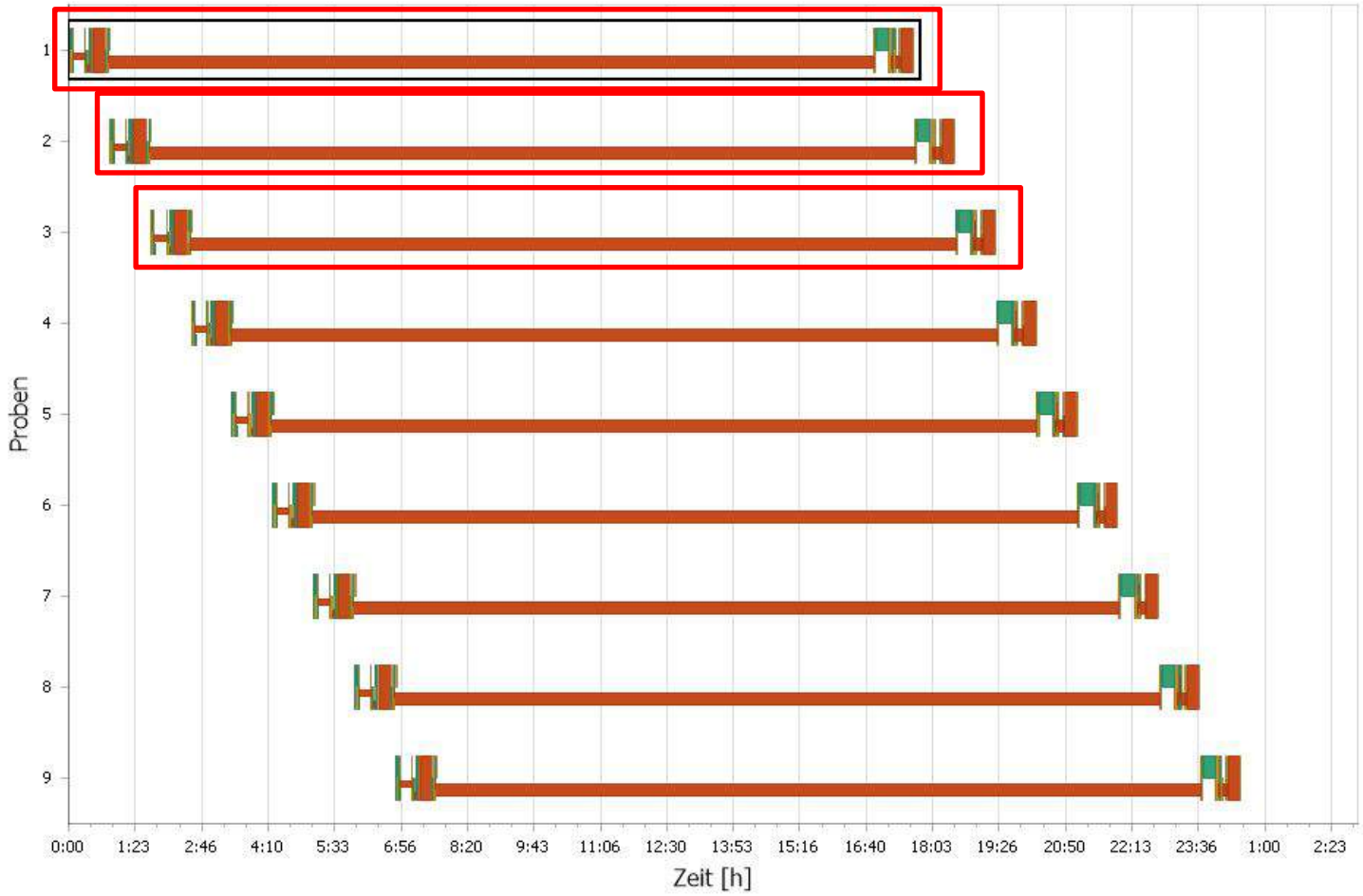


Comparing to other 3-MCPD methods

Results of a mixture of sunflower oil and rapeseed oil with DGF F&C, DGF manual and SGS 3in1

	Fat mixture 1		Fat mixture 2	
	3-MCPD-Ester (mg/kg)	Glycidyl-Ester (mg/kg)	3-MCPD-Ester (mg/kg)	Glycidyl-Ester (mg/kg)
„DGF F&C“	0.14	0.05	0.11	<0.05
„DGF" manual	0.15	0.08	0.13	0.05
“SGS 3- in 1-method” manual	0.14	<0.05	0.10	<0.05

Efficient Overlapping with CHRONOS



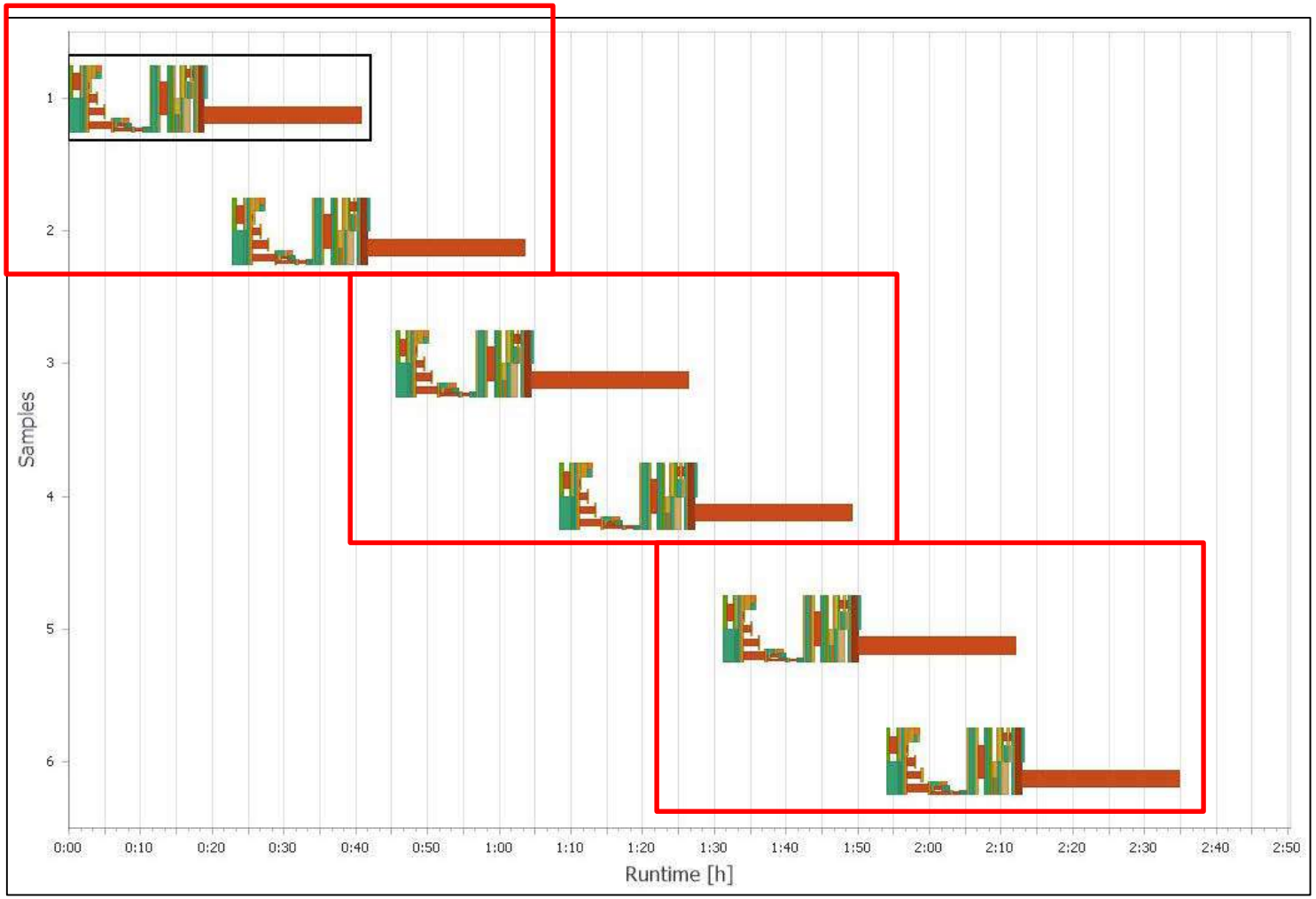
Sample 1

Sample 2

Sample 3

AOCS Cd 29a-13

Efficient Overlapping with CHRONOS



Sample 1

Sample 2

Sample 3

DGF F&C

Contact for further information

CHRONECT solutions are available through:

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45549 Sprockhövel
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- info@axelsemrau.de
- uber@axelsemrau.de