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VERBAND DER ÖLSAATEN-VERARBEITENDEN INDUSTRIE IN DEUTSCHLAND

BLL
Bund für Lebensmittelrecht und Lebensmittelkunde e.V.

Symposium on MCPD Esters and Glycidyl Esters
Analytics, Toxicology, Risk Assessment, Mitigation –
Where we are today?
20-21 June 2017, Rocket Tower Conference Center, Berlin

Overview on Prevalent Analytical Methods for Fats, Oils and Compound Foodstuffs - What are the Advantages and Drawbacks?

SGS Germany GmbH

J. Kuhlmann

WHEN YOU NEED TO BE SURE

SGS

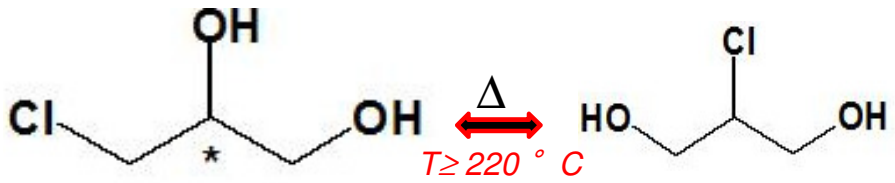
Contents

Structure of the presentation

- **Introduction 2- & 3-MCPD, glycidol**
- **I: Methods for analysis of edible oils and fats**
- **II: Methods for analysis of compound foods**
- **Conclusions/Recommendations**

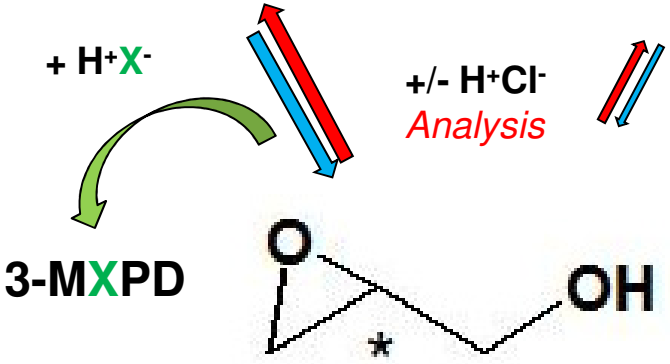
Free & bound 2-MCPD, 3-MCPD & glycidol - structures

Free analytes



3-MCPD
3-*mono*-chloropropane-1,2-diol
3-Chloropropane-1,2-diol

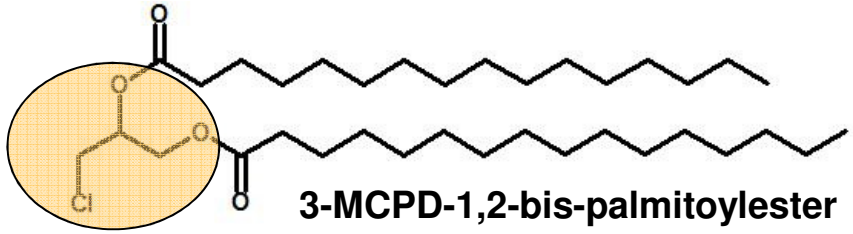
2-MCPD
2-*mono*-chloropropane-1,3-diol
2-Chloropropane-1,3-diol



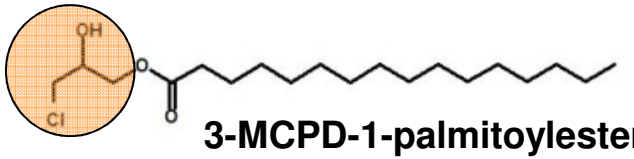
glycidol
(2,3-Epoxi-1-propanol)

(Fatty acid) Bound analytes

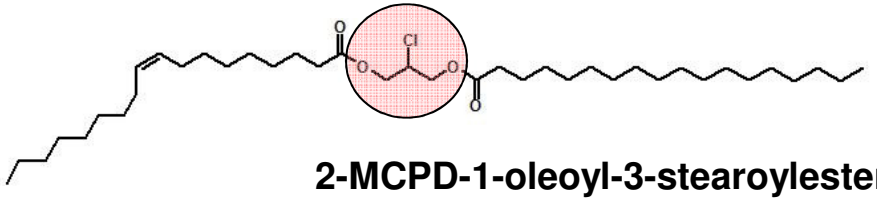
just examples, all fatty acids of an oil/fat might be present



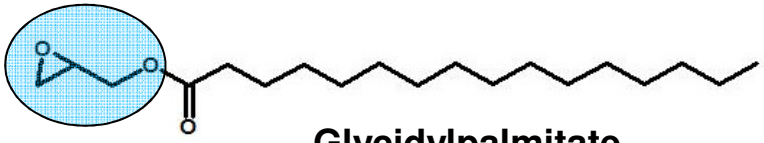
3-MCPD-1,2-bis-palmitoylester



3-MCPD-1-palmitoylester



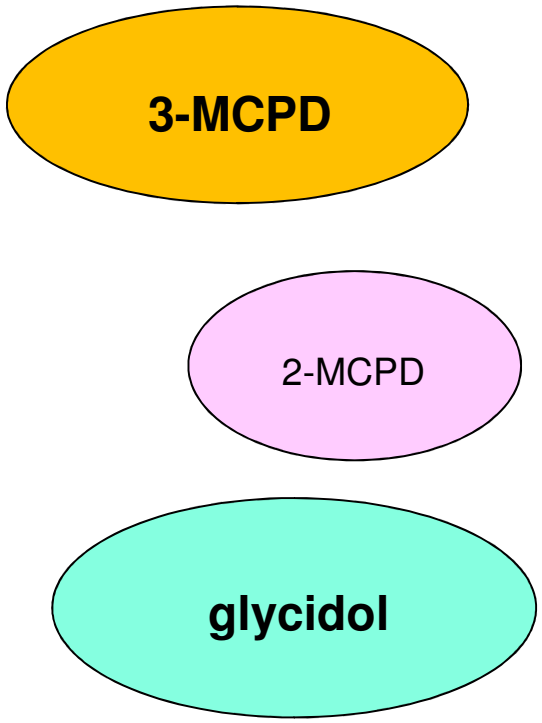
2-MCPD-1-oleoyl-3-stearoylester



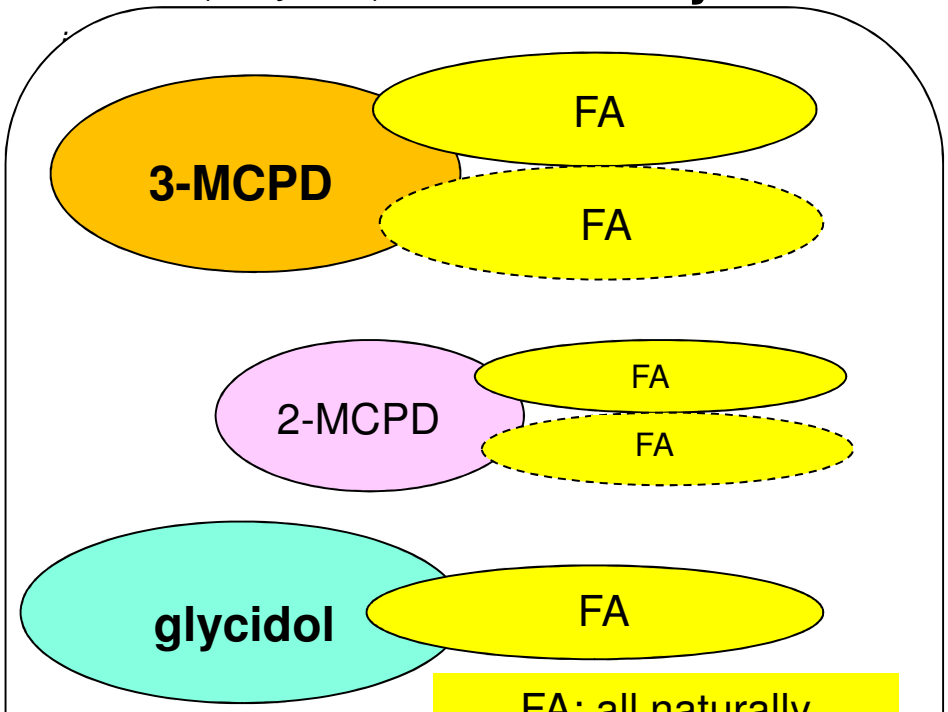
Glycidylpalmitate

Free & bound 2-MCPD, 3-MCPD & glycidol - structures

Free analytes



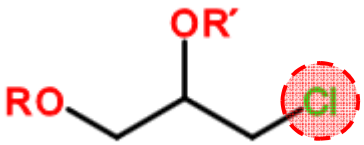
(Fatty acid) Bound analytes



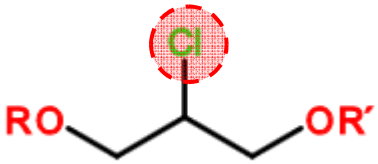
FA: all naturally occurring fatty acids

"*ester-bound 2-/3-MCPD; ester bound glycidol*": **complex group** of contaminants!

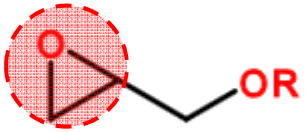
Potential hazards of free & bound 2-MCPD, 3-MCPD & glycidol



3-MCPD



2-MCPD



Glycidol

OR, OR`= OH; various fatty acids

Free analytes: Toxicity is related to **chlorine** or an **epoxy group** at the molecular backbone.

glycidol: ***genotoxic carcinogen*** *2A: probably carcinogenic to humans*

intake should be "As-Low-As-Reasonably-Achievable" (**ALARA**)

3-MCPD: ***non-genotoxic carcinogen*** *2B: possibly carcinogenic to humans*

2-MCPD: *No official classification available*

Bound analytes: During digestion the free analytes are released out of the bound form.

EFSA: "*From toxicological perspective the free and bound analytes are considered to be equivalent on molar base.*"

j.efsa.2016.4426

EU regulations

§

Tolarable Daily Intake (TDI) 3-MCPD			
year	**Organisation/source**	**Analyte**	**TDI [µg/kg bw d]**
5-2016	**EFSA** j.efsa.2016.4426	Free & bound 3-MCPD	**0.8**
11-2016	**JECFA** JECFA/83/SC	Free & bound 3-MCPD	**4**

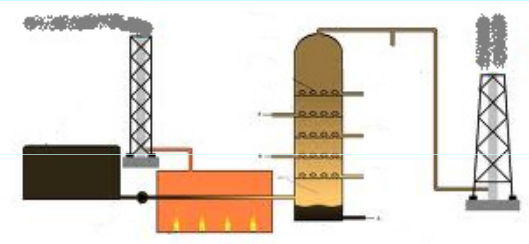
Draft EC regulation based on a TDI of 0.8 µg/kg x bw x d	
Sum of Free 3-monochloropropane-diol (3-MCPD) *and* 3-MCPD fatty acid esters, expressed as 3-MCPD	**Maximum level (µg/kg)**
Vegetable oils and fats intended for direct human consumption or use as an ingredient in food	**2000 ???**
Infant formula and follow-on formula (powder / liquid)	**125/15 ???**
Glycidyl fatty acid esters expressed as glycidol	If achievable lower values for infant formula 2019/2020
Vegetable oils and fats intended for direct human consumption or use as an ingredient in food	**1000**
Infant formula and follow-on formula (powder / liquid)	**75/10**

- *being a draft this information is preliminary and can't be taken as official specification!*

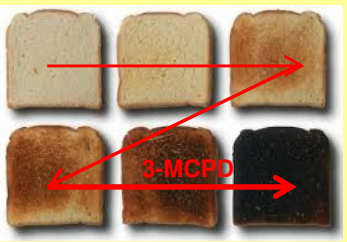
➢ The EC advised EFSA to review the calculation of TDI for 3-MCPD. $MRL_{3\text{-}MCPD}$ might change ↑.

Sources

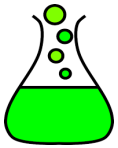
Whenever elevated heat is applied to foods free and/or bound MCPD and bound glycidol might be generated!



Refining, (Deodorisation):
Bound 2-/3-MCPD, bound glycidol
No free MCPD expected!



Frying, smoking, roasting, toasting, barbecueing etc.:
Bound 2-/3-MCPD, bound glycidol
free 2-/3-MCPD



HCl-treatment

Bound 2-/3-MCPD
free 2-/3-MCPD

Other sources:

- HCl treatment
- Migration from contact materials

free 2-/3-MCPD

Part I

- **Methods for analysis of oils & fats**

Methods applicable to oils and fats

Some of the analytical approaches available for the bound analytes in oils and fats.

Indirect determination
(ester cleavage releases the 3 core analytes, GC-MS)

alkaline

Early **DGF C-III 18 (09)**
Σ 3-MCPD + glycidol
DGF C-VI 17 (10); fast

Late **DGF C-III 18 (09) A,B**
A: Σ 3-M + g, B: 3-MCPD
Withdrawn by DGF

BfR method 09
3-MCPD
fast

DGF C-VI 18 (10) A, B
A: Σ 3-M + g, B: 3-MCPD
AOCS Cd 29c-13; fast

Both alkaline based methods would cover free MCPD if present!

SGS **"3-in-1"** Kuhlmann 2011
3-MCPD, 2-MCPD, Glycidol
AOCS Cd 29b-13; slow

acidic

Divinova et al. 2004
Zelinkova et al. 2006
3-MCPD; slow

BfR method 08
3-MCPD
slow

"Unilever"
Ermacora et al. 2013
3-MCPD, 2-MCPD, Glycidol
AOCS Cd 29a-13; slow

The acid based method supposably would not cover free MCPD

Myasaki et al. 2012
3-MCPD, 2-MCPD, Glycidol
fast

Koyama et al. 2015
3-MCPD, Glycidol; fast

enzymic

Validated methods

Validated methods covering 3-MCPD & glycidol

EU Commission recommendation 2014-661:
Use the AOCS Official Methods
Cd 29a,b,c-13
for oils and fats but also for oil- & fat containing foods.
LOQ = 0.1 mg/kg in the oil/fat fraction
LOQ 0.1 ↔ 0.01 mg/kg product in foods containing 100 % ↔ 10 % of fat.

Direct determination
(determination of a selected number of contaminant esters)

Dilute & shoot

Blumhorst et al. 2011
GE
LC-MS²

Haines et al. 2011
3-MCPD-E, GE
LC-MS²

SPE or SPE²

Masukawa et al. 2010/11
GE
SPE²; LC-MS:
AOCS Cd 28-10

Granvogl et al. 2011
GE
SPE; LC-MS²

Dubois et al. 2011
3-MCPD-E, 2-MCPD-E, GE
SPE²; LC-MS²

Steenbergen et al. 2013
GE
I/I; LC; GC/MS

MacMahon et al. 2013
3-MCPD-E, 2-MCPD-E, GE
2 x SPE²; 2 x LC-MS²

Limits of quantification

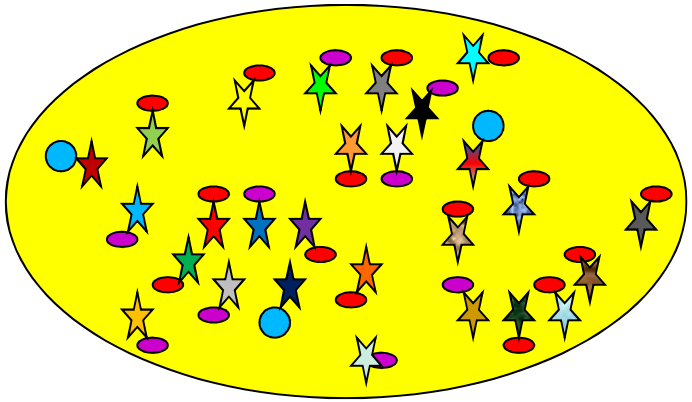
	AOCS Cd 29a-13 *(„Unilever“)*	AOCS Cd 29b-13 *(„SGS 3-in-1“)*	AOCS Cd 29c-13 *(„DGF“)*	Wenzel et al. [5] AOCS Cd 29a-13 modi. *(„Unilever“ modified) in-house validation*	Kuhlmann[4] AOCS Cd 29b-13 modi. *(SGS „3-in-1“$_{Low\text{-}LOQ}$) in-house validation*
In oils & fats	LOQ[2] [mg/kg]	LOQ[3] [mg/kg]	LOQ estimated [mg/kg]	LOQ[5] [mg/kg]	LOQ[4] [mg/kg]
3-MCPD	0.14	0.10	0.15	0.013	0.010
2-MCPD	0.14	0.10	0.15	0.015	0.010
Glycidol	0.19	0.10	0.15	0.031	0.010

2): Ermacora A., Hrncirik K.: A Novel Method for Simultaneous Monitoring of 2-MCPD,3-MCPD and Glycidyl Esters in Oils and Fats. *J. Am .Oil Chem. Soc.* **2013**, 90, 1–8
3): Kuhlmann J.: Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils. *Eur. J. Lipid. Sci. Technol.* **2011**, 113, 335–344.
4): Kuhlmann J.: Analysis and occurrence of dichloropropanol fatty acid esters and related process-induced contaminants in edible oils and fats. *Eur. J. Lipid. Sci. Technol.* **2016**, 118(3), 82-395.
5): Wenzl T, Samaras V, Giri A, Buttinger G, Karasek L, Zelinkova Z: Development and validation of analytical methods for the analysis of 3-MCPD (both in free and ester form) and glycidyl esters in various food matrices and performance of an ad-hoc survey on specific food groups in support to a scientific opinion on comprehensive risk assessment on the presence of 3-MCPD and glycidyl esters in food1. EFSA supporting publication 2015: EN-779, **2015**, 12 (3)

- In oils and fats the official methods limits of quantification seem to be sufficient for future regulations, improvements should be easily feasible e.g. by the use of GC-MS²-techniques.
- **This applies not automatically to other, difficult matrices (e.g. emulsifiers).**
- **This applies not automatically to compound foods.**

Bound analytes **direct analysis**: Determination of the original esters

- glycidol
- 3-MCPD
- 2-MCPD
- fatty acid(s)



Hypothetic oil

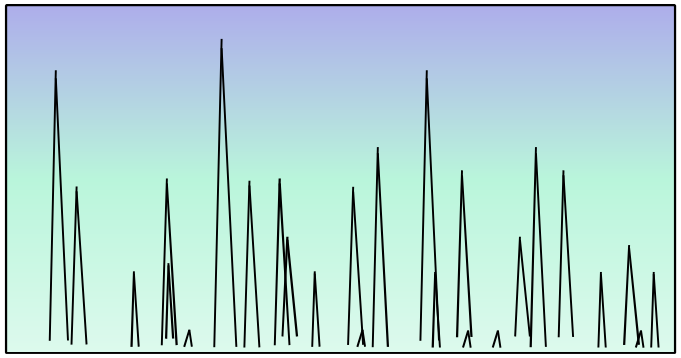
Contains only 3 relevant fatty acids

This yields up to 27 analytes

3 Glycidyl ester
9-MCPD mono-ester
15 MCPD di-ester

Matrix removal in the majority of applications

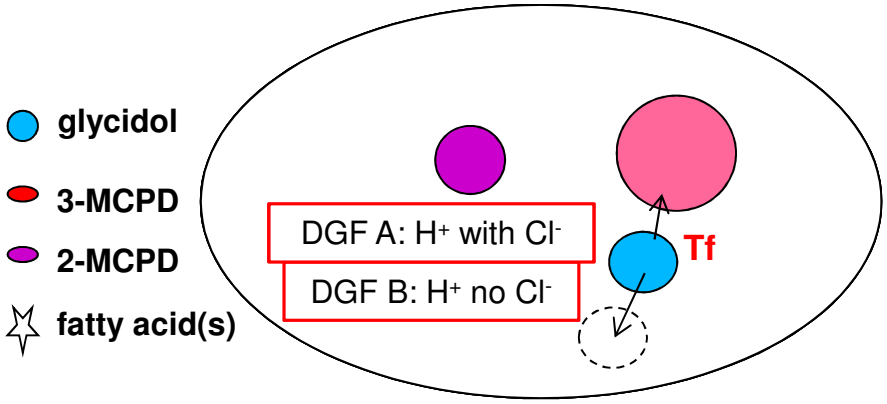
↓ LC-MS / **LC-MS²** / LC-MS-TOF



Chromatogram displays up to 27 analytes!

Direct analysis – indirect quantification:
From every detected ester the amount of core analyte is calculated via molecular weights. Subsequently single 2-MCPD-, 3-MCPD- and glycidol contents are added up.

Principle of prevalent indirect methods.



ester cleavage (***alkaline***)

matrix clean-up *(e.g. l/l-extraction)*

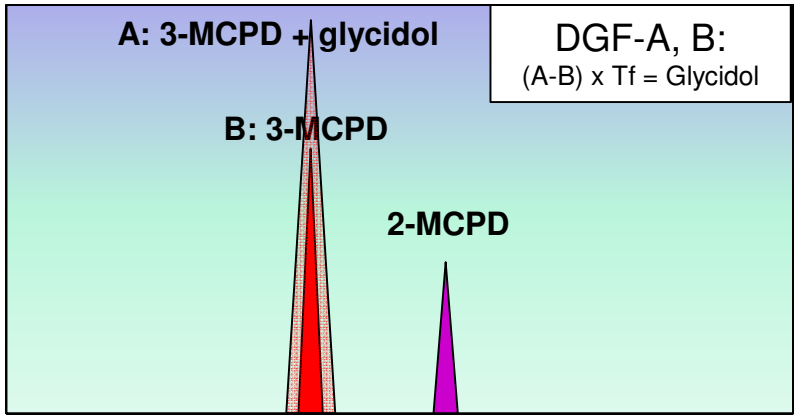
glycidol conversion *(into MXPD)*

Derivatisation*
(e.g. HFBA/acetone/PBA)

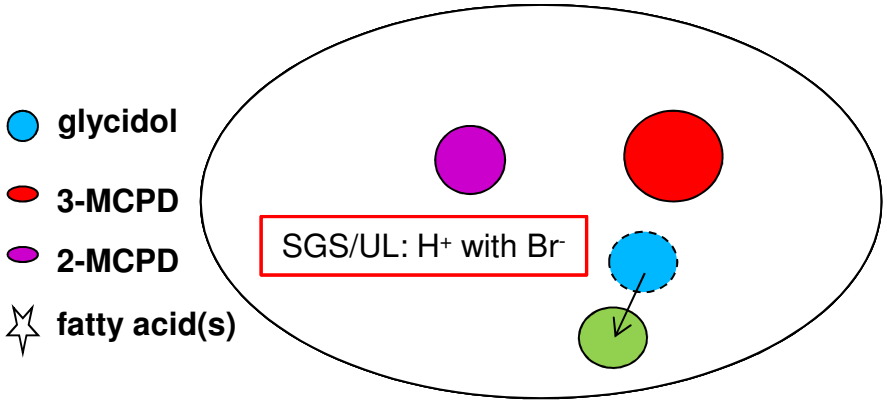
GC-MS

DGF & AOCS Cd29-13 methods, validated for analysis of oils/fats:

Cd29c-13: "DGF" A/B



Principle of prevalent indirect methods.



ester cleavage (***alkaline***, ***acidic***)

matrix clean-up *(e.g. l/l-extraction)*

glycidol conversion *(into MXPD)*

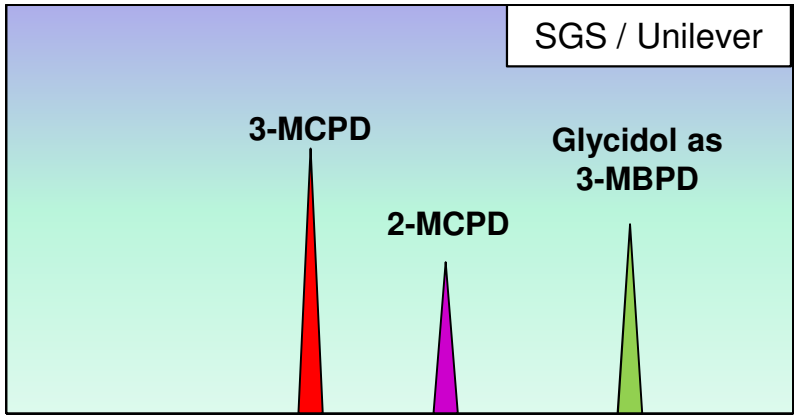
Derivatisation*
(e.g. HFBA/acetone/PBA)

GC-MS

DGF & AOCS Cd29-13 methods, validated for analysis of oils/fats:

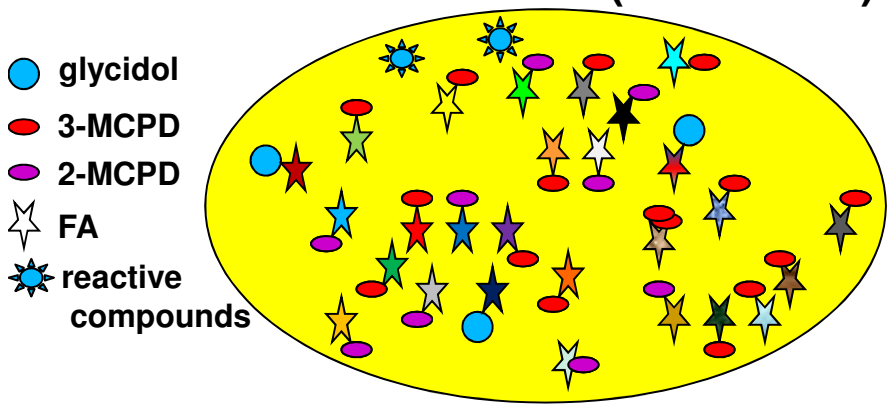
Cd29a-13: "Unilever"
Cd29b-13: "SGS 3-in-1"

Indirect analysis – direct quantification:
The target analytes can be quantified directly via internal standards

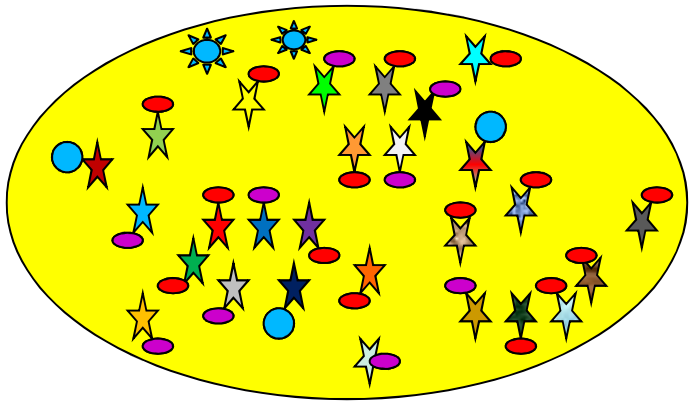


Differences between AOCS Cd29a-13 & Cd29b-13

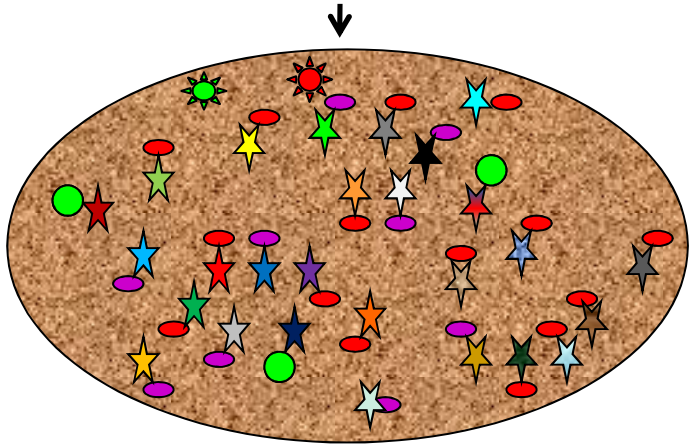
AOCS Cd 29a-13 (“Unilever”)



AOCS Cd 29b-13 (“3-in-1”)



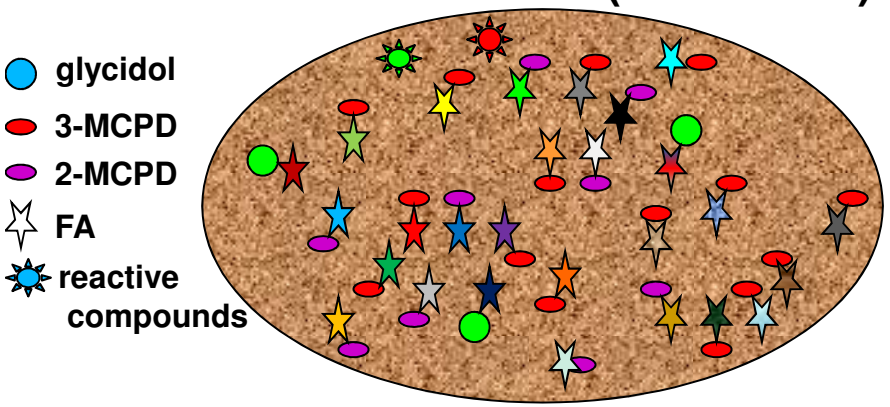
Glycidyl ester conversion *(into MBPD esters)*



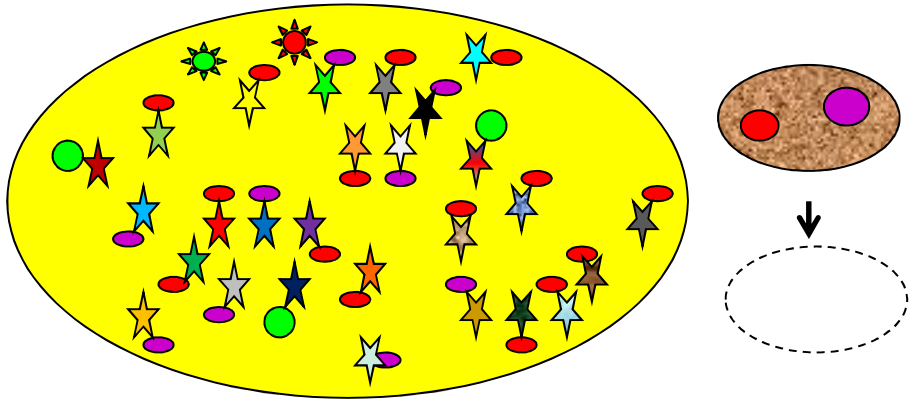
„If reactive components being present in the sample they might react under acidic conditions with the introduced bromide or naturally occurring chloride to glycidol or 3-MCPD artefacts in course of AOCS Cd29a-13 .“

Differences between AOCS Cd29a-13 & Cd29b-13

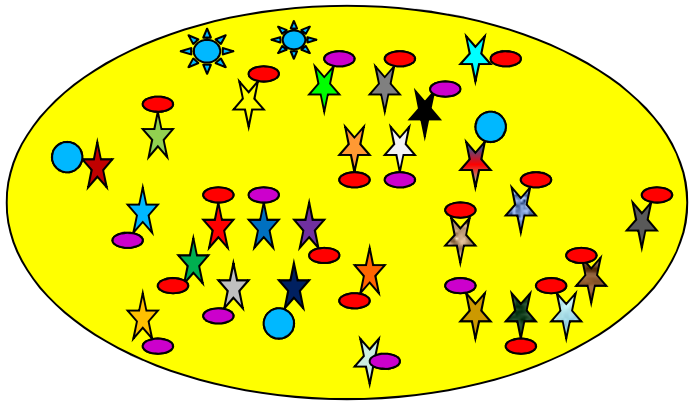
AOCS Cd 29a-13 ("Unilever")



I/I Extraction (*to remove polar agents/solvents*)

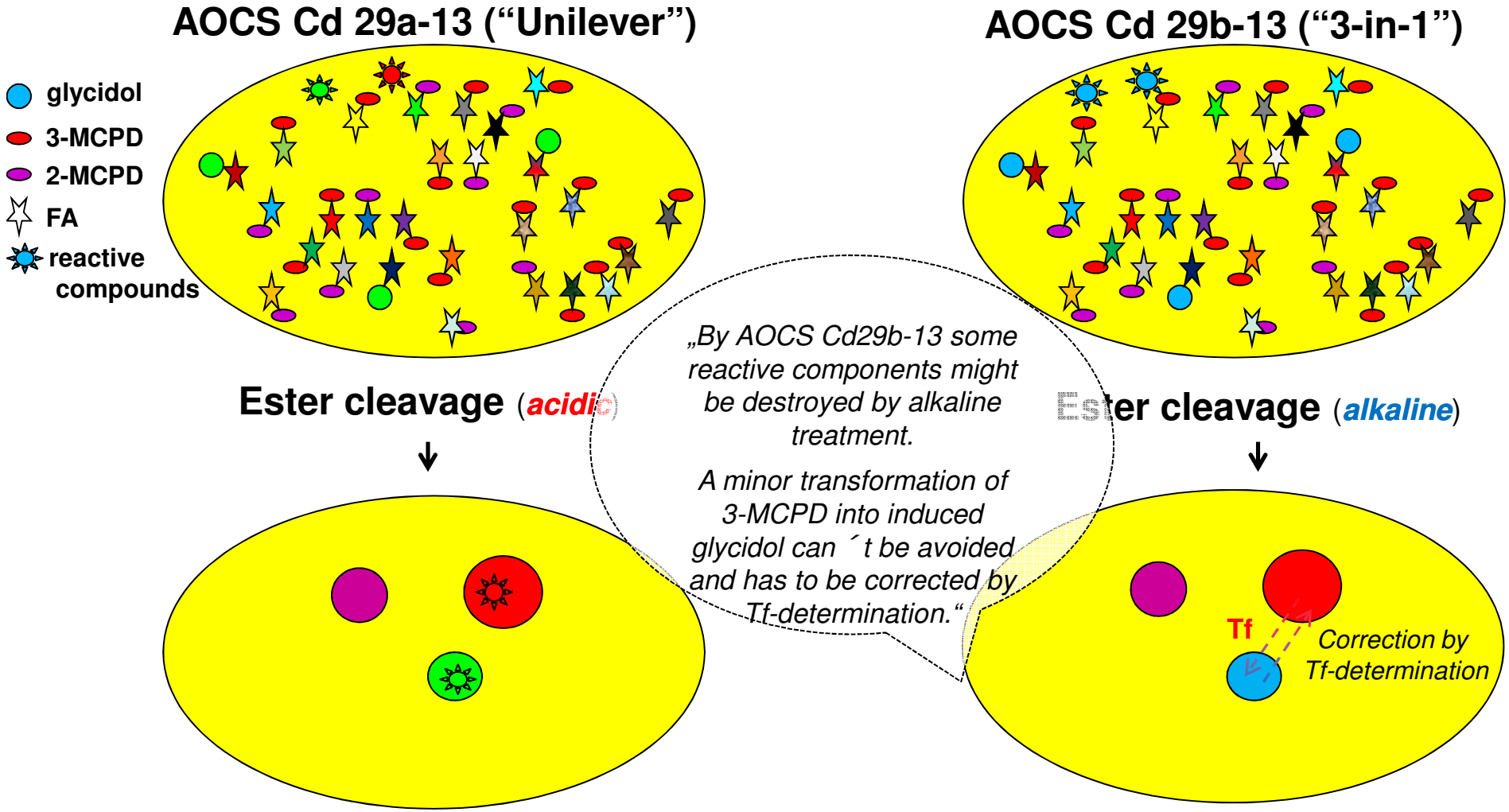


AOCS Cd 29b-13 ("3-in-1")



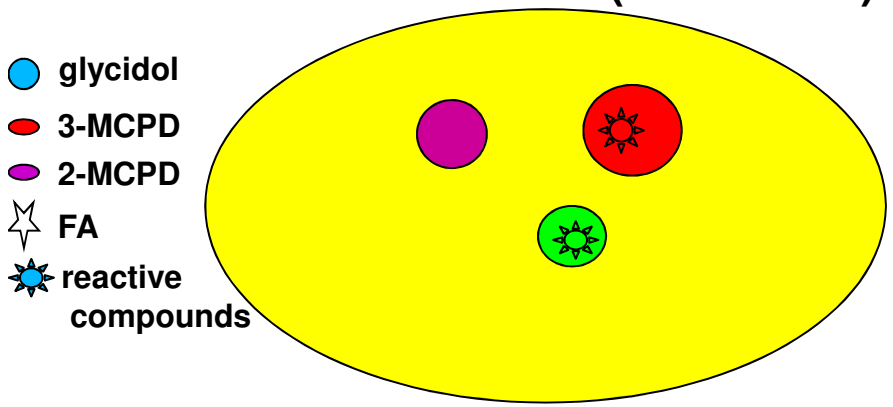
„ In course of AOCS Cd29a-13 free MCPD, if being present in the sample, would presumably be removed together with the polar agents and would not contribute to the results."

Differences between AOCS Cd29a-13 & Cd29b-13



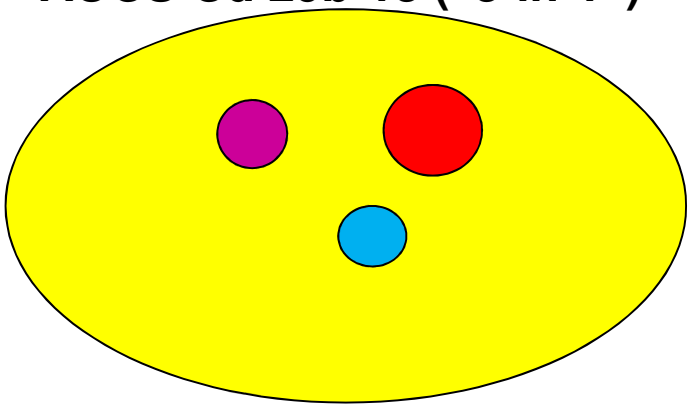
Differences between AOCS Cd29a-13 & Cd29b-13

AOCS Cd 29a-13 ("Unilever")



Matrix removal (l/l-extraction)

AOCS Cd 29b-13 ("3-in-1")



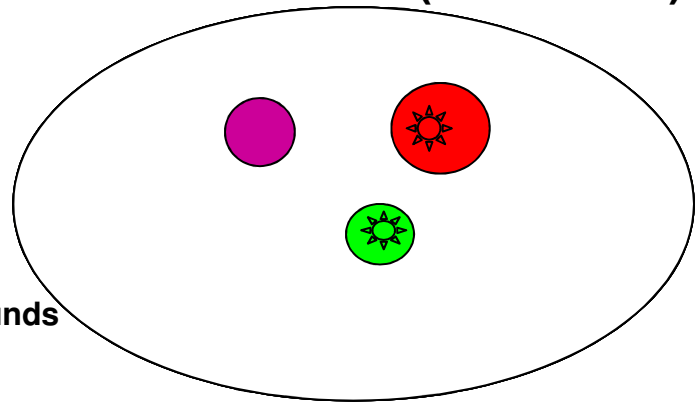
Matrix removal (l/l-extraction)
glycidol conversion into MBPD

„In course of AOCS Cd29b-13 the conversion of released free glycidol into MBPD is carried out after matrix removal in aqueous phase. Low risk of artefact formation"

Differences between AOCS Cd29a-13 & Cd29b-13

AOCS Cd 29a-13 (“Unilever”)

- glycidol
- 3-MCPD
- 2-MCPD
- FA
- reactive compounds



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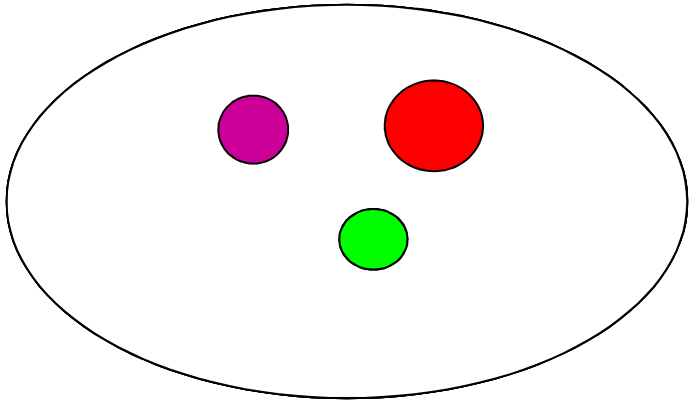
Derivatisation: PBA *in aquous phase*

↓

GC-MS

AOCS Cd 29b-13 (“3-in-1”)



↓

Derivatisation: PBA *in organic phase*

↓

GC-MS

„Derivatisation in an organic phase requires less amounts of derivatisation agent and might reduce the required frequency of cleaning the GC-MS equipment.“

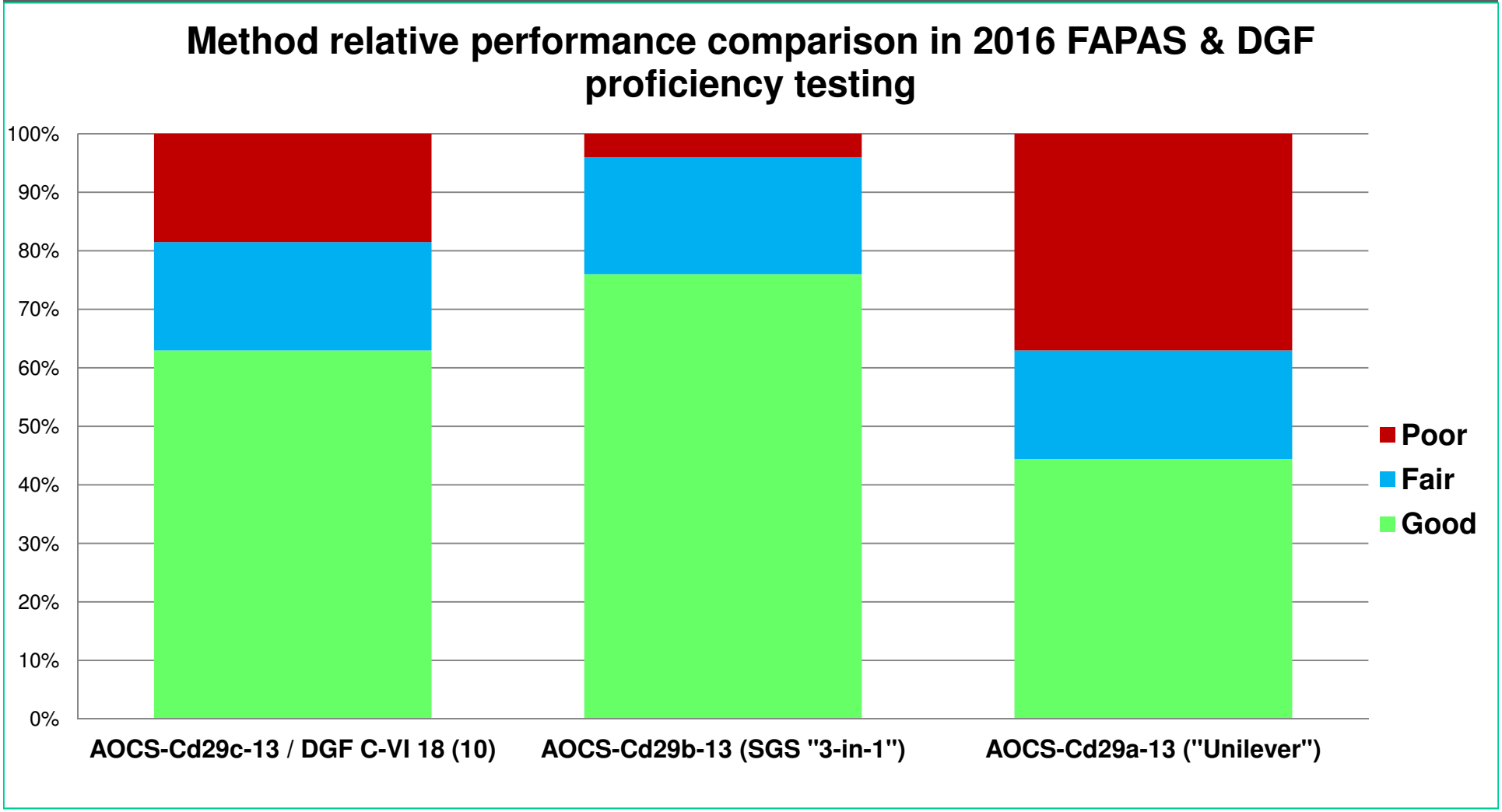
Methods applicable to oils and fats

Performance of the validated methods in interlaboratory comparison.

FAPAS PT 2649: 2016	glycidol				3-MCPD				2-MCPD			
sample: vegetable oil	Participants (n) of 35	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Participants (n) of 45	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Participants (n) of 32	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSDR
AOCS-Cd29a-13 (Unilever)	6	0	**0.31**	8	6	1	**1.48**	21	6	0	**0.74**	13
AOCS-Cd29b-13 (SGS "3-in-1")	7	1	**0.33**	10	7	1	**1.73**	11	6	0	**0.74**	12
AOCS-Cd29c-13 (DGF)	7	4	**0.33**	11	7	0	**1.69**	14	5	0	**0.80**	8

DGF-22 LVU: 2016	glycidol				3-MCPD				2-MCPD			
sample 1: Olive Oil, Blend	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSDR
AOCS-Cd29a-13 (Unilever)	3	0	**0.96**	14	2	1	**0.06**	-	3	1	**0.03**	13
AOCS-Cd29b-13 (SGS "3-in-1")	2	0	**1.18**	11	1	0	**0.03**	-	1	0	0.02	-
AOCS-Cd29c-13 (DGF)	9	1	**0.99**	16	3	1	**0.04**	8	4	1	**0.03**	35
sample 2: Used Frying Oil	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSD_R	Results > LOQ (n)	Outliers (|z| > 2)	**Mean (mg/kg)**	% RSDR
AOCS-Cd29a-13 (Unilever)	2	2	*(0.70)*	-	3	2	**0.35**	-	3	1	**0.20**	23
AOCS-Cd29b-13 (SGS "3-in-1")	2	0	**0.46**	4	2	0	**0.31**	21	2	0	**0.22**	6
AOCS-Cd29c-13 (DGF)	9	2	**0.47**	18	9	3	**0.29**	25	9	1	**0.28**	26
Good	**Mean against the both other methods: < 10 %**							**RSD_R: < 20 %**	**Outliers: < 20 %**			
Fair	**Mean against the both other methods: 10 % - 20 %**							**RSD_R: 20 - 30 %**	**Outliers: 20 % - 30 %**			
Poor	**Mean against the both other methods: > 20 %**							**RSD_R: > 30 %**	**Outliers: > 30 %**			
	Outliers excluded *(except 0.70)*							**Outliers excluded**				

Performance of the validated methods in interlaboratory comparison.



Methods applicable to oils and fats

Pros and cons

Comparison of official methods AOCS Cd 29a,b,c-13

Official method	ec time	Analytes covered	comments
AOCS Cd29a-13 „Unilever“	16 h 40° C	Bound 2-/3-MCPD Bound glycidol *(Free MCPD???)*	**Easy, simple to establish method:** Works well with pure, clean oils/fats. **Overestimations of glycidol** observed in aged or extracted oils and fats. (likely being caused by presence of monoacylglycerides [1,2]) **Overestimations of 3-MCPD** oberserved when chloride is present during conversion of glycidyl esters, e.g. when the method is applied directly to foods. [3]
AOCS Cd29b-13 „SGS 3-in-1“	16 h ca. - 22° C	Free & bound 2-/3-MCPD Bound glycidol	**Rugged but demanding method**: works well for all kinds of oils/fats, margarine, can be applied also to many emulsifiers, can be applied directly to many foods without fat extraction. **2 Assays: Double sample preparation. Needs experienced lab staff.**
AOCS Cd29c-13 „DGF“	3.5-5.5 min RT	Free & bound 3-MCPD Bound glycidol	**Fastest method**, may serve for production control. **Less precise data for glycidol** due to calculative approach. **2 Assays: Double sample preparation. Not validated for 2-MCPD. Not as sensitive as AOCS Cd29a,b-13.**

[1]: J. Kuhlmann, oral presentation: AOCS Expert Panel on Process Contaminants, **2013**, Montreal, Canada
[2] Z. Zelinkova, A. Giri, T. Wenzel: Food Control, 77, **2017**, 65-75
[3] J. Kuhlmann, oral presentation: 10th International Fresenius Conference / Contaminants and Residues in Food / 27th and 28th October **2015** Cologne/Germany

Part II: Methods for analysis of compound foods

General approaches for the analysis of compound foods

Two principal ways might be used for routine analysis of complex matrices:

Fat extraction
prior to analysis with any AOCS Cd 29-13 method.
Extraction efficiency?
Impact on ruggedness/trueness?
Free MCPD included?

No fat extraction:
taking whole samples into an alkaline based AOCS Cd 29-13 method.

Impact on ruggedness/trueness?

AOCS Cd 30-15
"Analysis of 2- and 3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Oil-Based Emulsions"
Free MCPD supposably not included.

In-house SGS "3-in-1" compound foods
Works well for most matrices (spreads, bakery ware, fish, fries, chips) but not for infant formula.
Free MCPD included.

Some points have to be checked!

Methods applicable to compound foods

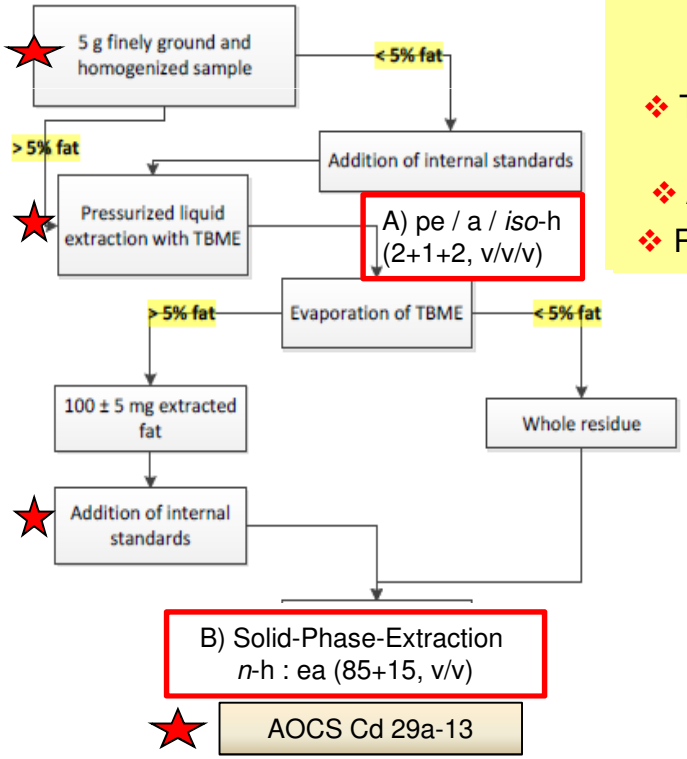
JRC approach for compound foods

EU reference laboratory JRC/IRMM:

"In-house-validated method for the separate analysis of free 2-/3-MCPD & bound 2-/3-MCPD and glycidol in foods[4]

[4]: EFSA supporting publication 2015: EN-779: T. Wenzel et al.

bound 2-/3-MCPD/glycidol



Points to consider for routine analysis:

- Use of liquid nitrogen for grinding (safety).
- PLE works serially & is laborious.
- TBME has a weak extraction efficiency – this method is not applicable to infant formula.
- Addition of iStds after extraction is not best practice.
- Risk of glycidol-overestimation with AOCS Cd 29a-13.

Validation trial including modifications in progress.

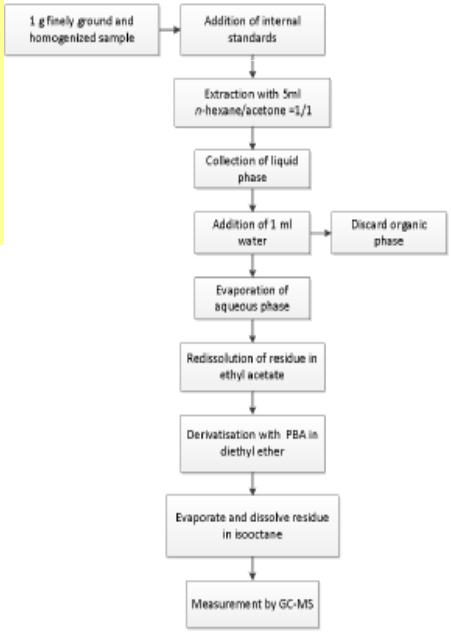
Recent modifications:

A) Other solvents for infant formula.

B) Additional SPE

Fit for purpose as routine method?

Free 2- & 3-MCPD



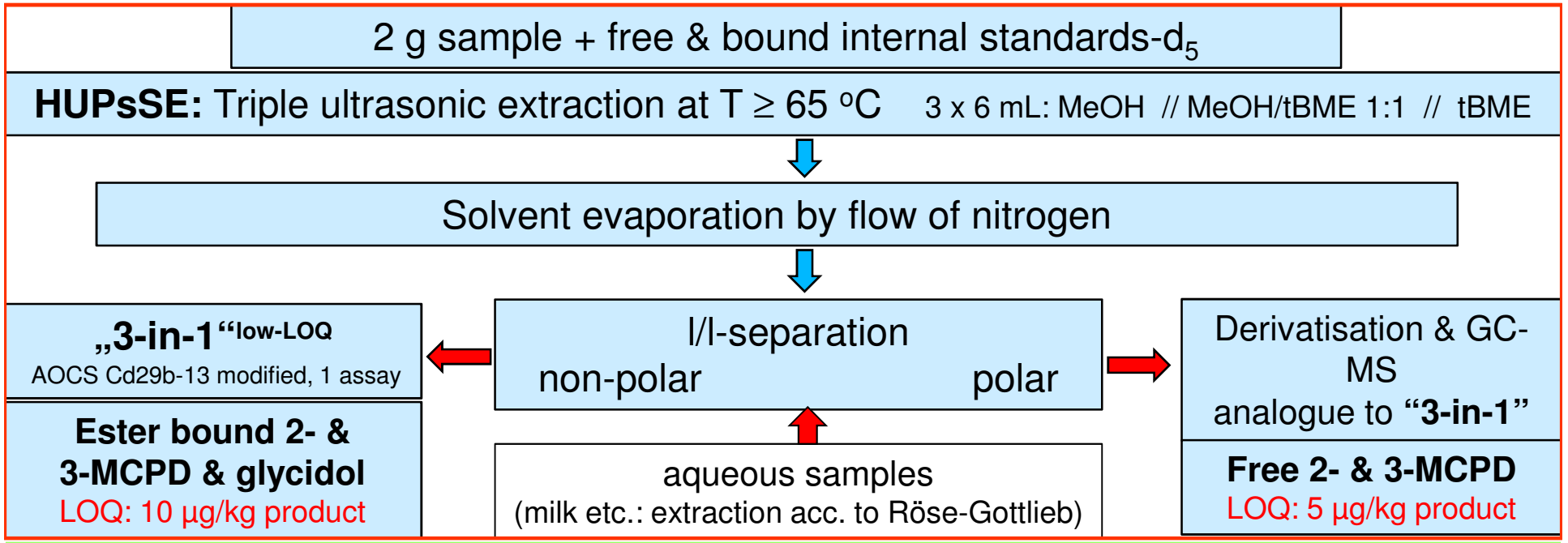
Methods applicable to compound foods

In-house validated SGS "5-in-2" low LOQ approach for compound foods

Extraction* + „3-in-1"low-LOQ

***HUPsSE: H**eat-**U**ltrasonic-**P**ressure **s**upported **S**olvent **E**xtraction

Applicable to compound foods as **spreads, bakery ware, chips, fries, fish, infant formula**.



➢1050 food samples including > 200 infant formulae from the German market analysed in 2016 on behalf of the *Federal German Ministry of Food and Agriculture**. Results reported to EFSA. * this conference presentation by Klara Jirzik

➢Final report at www.ble.de: (https://service.ble.de/ptdb/index2.php?detail_id=56944&site_key=141&stichw=2815HS002&zeilenzahl_zaehler=1#newContent)

Methods applicable to compound foods

New methods on the horizon.

- **Automatisation of the officially validated methods has been realised* or is in development.**

In case of significant deviations from the original protocol the official validation does not apply any longer !

* this conference presentations by Tobias Uber / Ralph Zwagermann

- The **FDA** is working on a **new l/l extraction technique** for infant formula and compound foods in combination with direct LC-MS^2 analysis.

- March, 2017: On behalf of the Infant Nutrition Council of America (INCA) **AOAC International** has established a working group on methods for analysis of free and bound 2- & 3-MCPD and glycidyl esters in infant formula and adult nutritionals.

Conclusions & Recommendations

- **All derivatives** of glycidol and MCPD should be considered as relevant food contaminants as they have attracted increasing attention by authorities & NGOs.
- In regard to food control methods should cover **bound and free** 3-MCPD.
- There is an increasing demand for **higher sensitivity.**
- **Validated methods for oils and fats** *(AOCS Cd 29a,b,c-13)* **do show different applicability and cover different sets of analytes.**
  - Evaluate results under this perspective.
  - Choose the method that fits best for your purpose.
- **When new methods are applied for compound foods, it should be checked:**
  - If the applied extraction technique is sufficient.
  - If free MCPD is included or not.
  - If co-occurring matrix components might have an impact on accuracy/trueness.
- **Verify new applications by parallel testing with other established/accepted techniques.**

Alternative use of empty solvent bottles!

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Thank you for your kind attention!