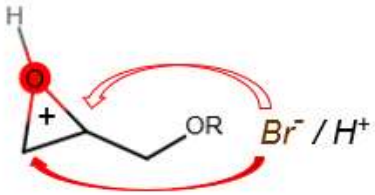
German Federal Institute for Risk Assessment



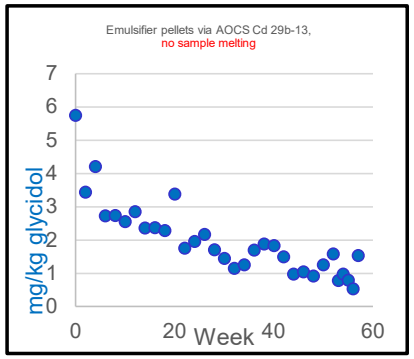
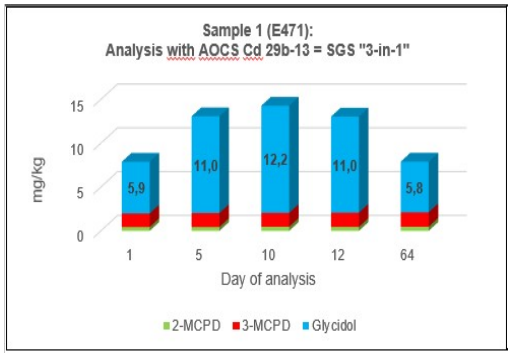


TABLE 5 Summary validation results for RSD_r, RSD_R and HorRat *R* values

	Analyte level	RSD_r (%)	RSD_R (%)	HorRat *R*
Glycidol[a]	0.11 ↔ 28.4 mg/kg	3.3 ↔ 17.1	9.0 ↔ 29.2	0.6 ↔ 1.6
3-MCPD[b]	0.07 ↔ 2.93 mg/kg	2.0 ↔ 16.6	6.7 ↔ 20.7	0.4 ↔ 1.1
2-MCPD[b]	0.02 ↔ 0.72 mg/kg	1.9 ↔ 24.0	10.1 ↔ 28.6	0.5 ↔ 1.0

[a]Glycidyl fatty acid esters expressed as glycidol.
[b]Sum of free MCPD and MCPD fatty acid esters expressed as MCPD.



Solutions to overcome issues in the analysis of 3-MCPD(E) & GE in mono- and diglycerides of fatty acids (E 471)

J. Kuhlmann
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Contents

3-MCPD & glycidol:

EU regulation on food additives

Common analytical concepts & their limitations when applied to E471

Validation of a new analytical method for E471

Recent EU-Regulations
(EU) 2023/1329, (EU) 2023/1428

EU regulations on 3-MCPD & glycidol in food additives

COMMISSION REGULATION (EU) 2023/1329

of 29 June 2023

amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476) and the Annex to Commission Regulation (EU) No 231/2012 as regards specifications for glycerol (E 422), polyglycerol esters of fatty acids (E 475) and polyglycerol polyricinoleate (E 476)

Maximum levels for

- 3-MCPD in E422
- „3-MCPD & glycidol“ in E475, E476

Sum 3-MCPD + 3-MCPD fatty acid esters, expressed as 3-MCPD

= 3-MCPD + 3-MCPDE = 3-MCPD(E)

Glycidyl esters of fatty acids (GE), expressed as glycidol

COMMISSION REGULATION (EU) 2023/1428

of 7 July 2023

amending the Annex to Regulation (EU) No 231/2012 as regards mono- and diglycerides of fatty acids (E 471)

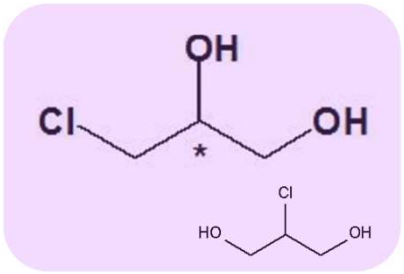
E471: Maximum levels for

- „3-MCPD“ in E471: 0.75 - 2.5 mg/kg
- „ glycidol“: 5 mg/kg

Sum of 3-monochloropropanediol (3-MCPD) and 3-MCPD fatty acid esters, expressed as 3-MCPD	Not more than 0,75 mg/kg (only if added to food for infants and young children) Not more than 2,5 mg/kg (for all uses except for foods intended for infants and young children)
Glycidyl esters of fatty acids, expressed as glycidol	From 30 July 2023 until 30 January 2024, not more than 5 mg/kg if added to food for infants and young children) and not more than 10 mg/kg for all other uses. From 30 January 2024, not more than 5 mg/kg for all uses.

There is an obvious demand for reliable quantitative method(s) for analysis of 3-MCPD(E) & GE in E471!

Introducing 3-MCPD, 3-MCPD esters & glycidol, glycidyl esters



Stable in foods

3-MCPD

Possibly cancerogenic

EFSA: TDI = 2 µg/kg x bw x d

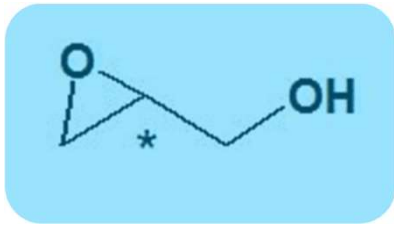
2-MCPD

glycidol

Probably cancerogenic

genotoxic

MoE > 10 000

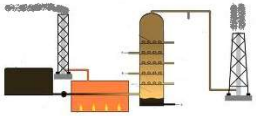


Instable in foods

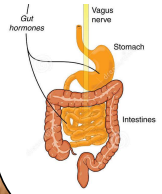
heat-induced food-borne toxicants

refining of edible oils generates fatty acid esters (FA) of 3-MCPD & glycidol:

Oil & fat refining:
Deodorisation:
180 - 250 ° C

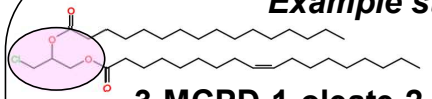


stable in foods: 2-MCPDE > 3-MCPDE > GE



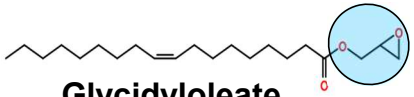
human digestion

Example structures

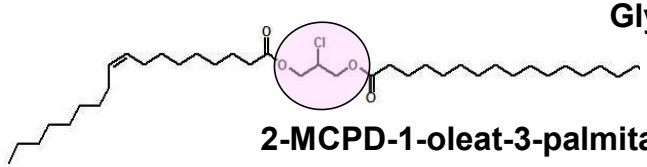


3-MCPD-1-oleate-2-palmitate

3-MCPD-1-palmitate



Glycidyloleate



2-MCPD-1-oleat-3-palmitate

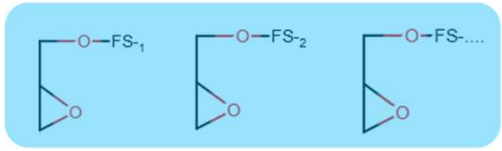
Same toxicity as the free analytes on molar basis

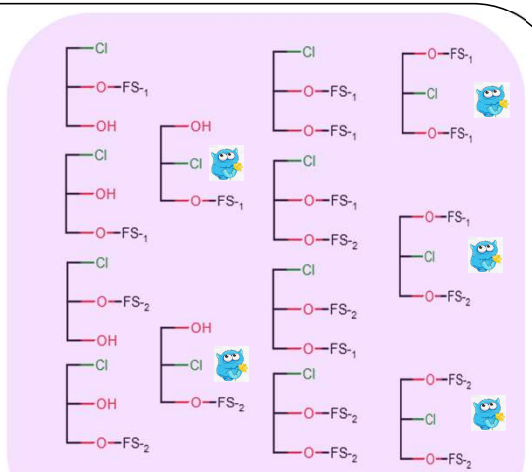
…but various, isomeric MCPDE

n FA = n^2+4n+(Σ1→(n-1)) MCPDE

Just a few GE….

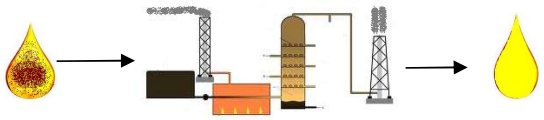
n FA = n GE





e.g. 13 MCPDE from 2 fatty acids

Source of 3-MCPD(E) & GE in E471



Edible oil/fat refining:

$T_{max} \rightarrow 250\ ^{\circ}\ C$

e.g. alkaline catalyzed interesterification

destillation

E471

Mono- & diglycerides

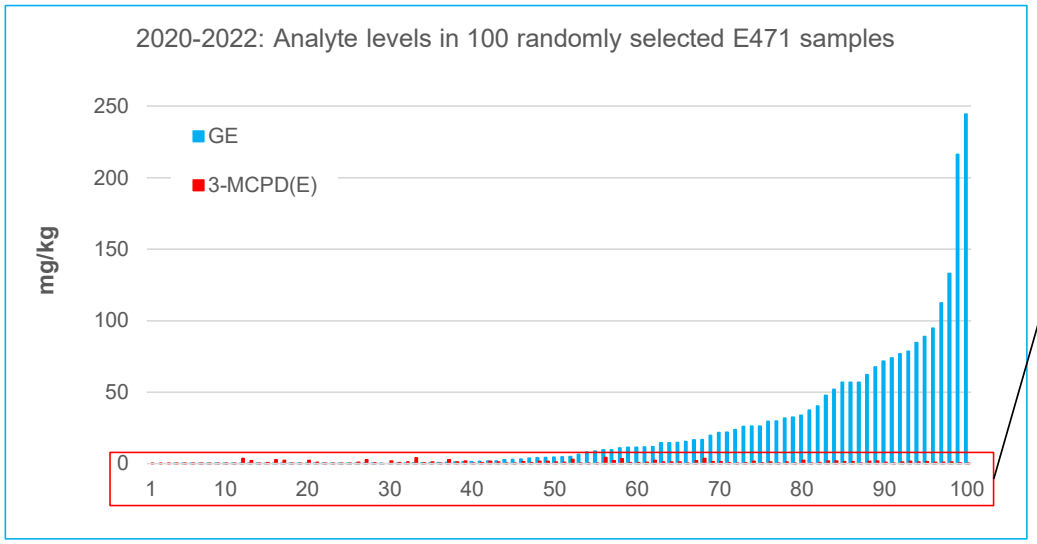
of fatty acids

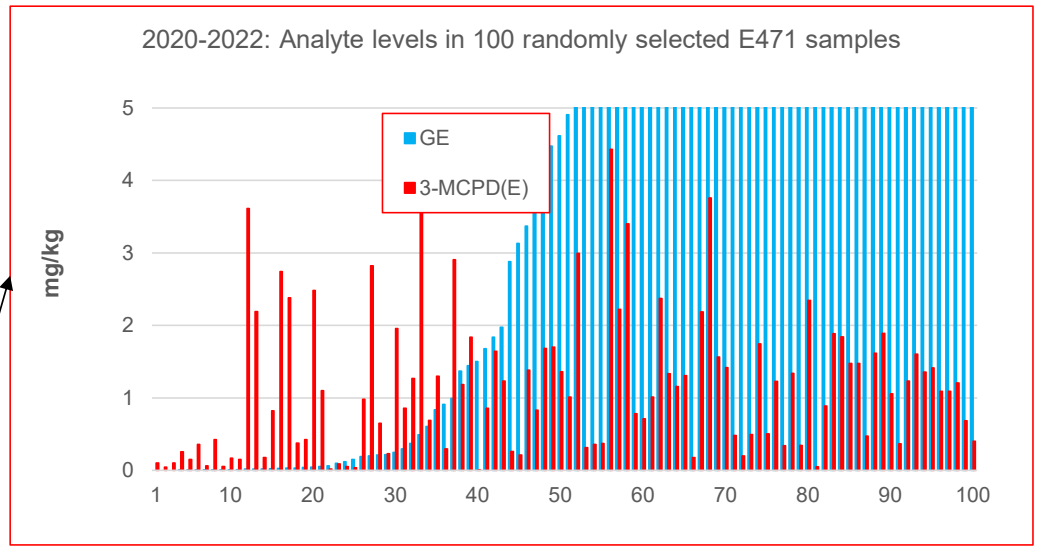
- Alkaline conditions promote GE-Formation
- GE are similar volatile as MAG and might be concentrated by destillation

GE >> MCPDE

↓ Recent mitigation techniques

GE ≅ MCPDE





Principal analytical approaches for 3-MCPD(E)/GE determination

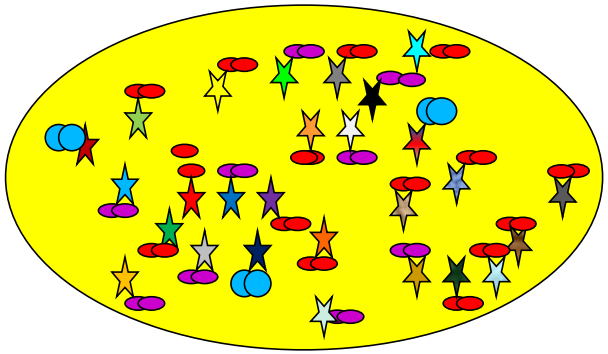
Direct Methods: *Determination of single esters – calculation of core components*

Glycidol

3-MCPD

2-MCPD

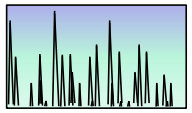
Fatty acids(s)

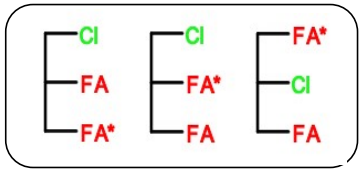


e.g. matrix removal via SPE + LC-MS²

or

dilute & shoot – Orbitrap





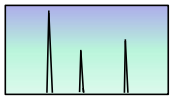
7 FA = 105 analytes:

7 GE + 98 2-/3-MCPE

No routine application due to 2-/3-MCPDE separation issues & limited availability of reference compounds.

➢ *Food emulsifiers do have different properties as edible oils & fats, they are **not in the scope** of any offical method.*

indirect methods:



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

AOCS Cd29-13 b,c,f a d,e

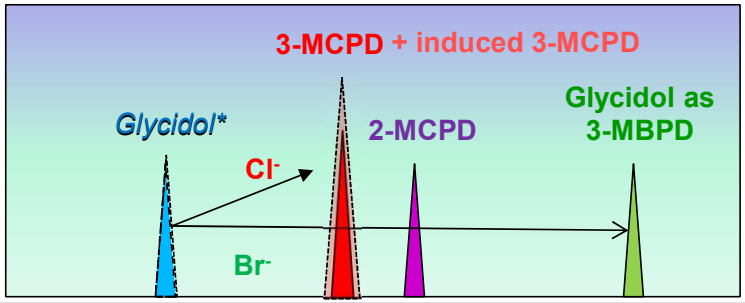
matrix removal ***e.g. l/l-extraction)***

Glycidol: stabilizing transformation *(into MXPD)*

derivatisation*

e.g. PBA

GC-MS



Official Methods for edible oils & fats

AOCS Cd 29 a,b,d,e,f-13/19/21

ISO 18363-2 & 4

18363-3 etc.

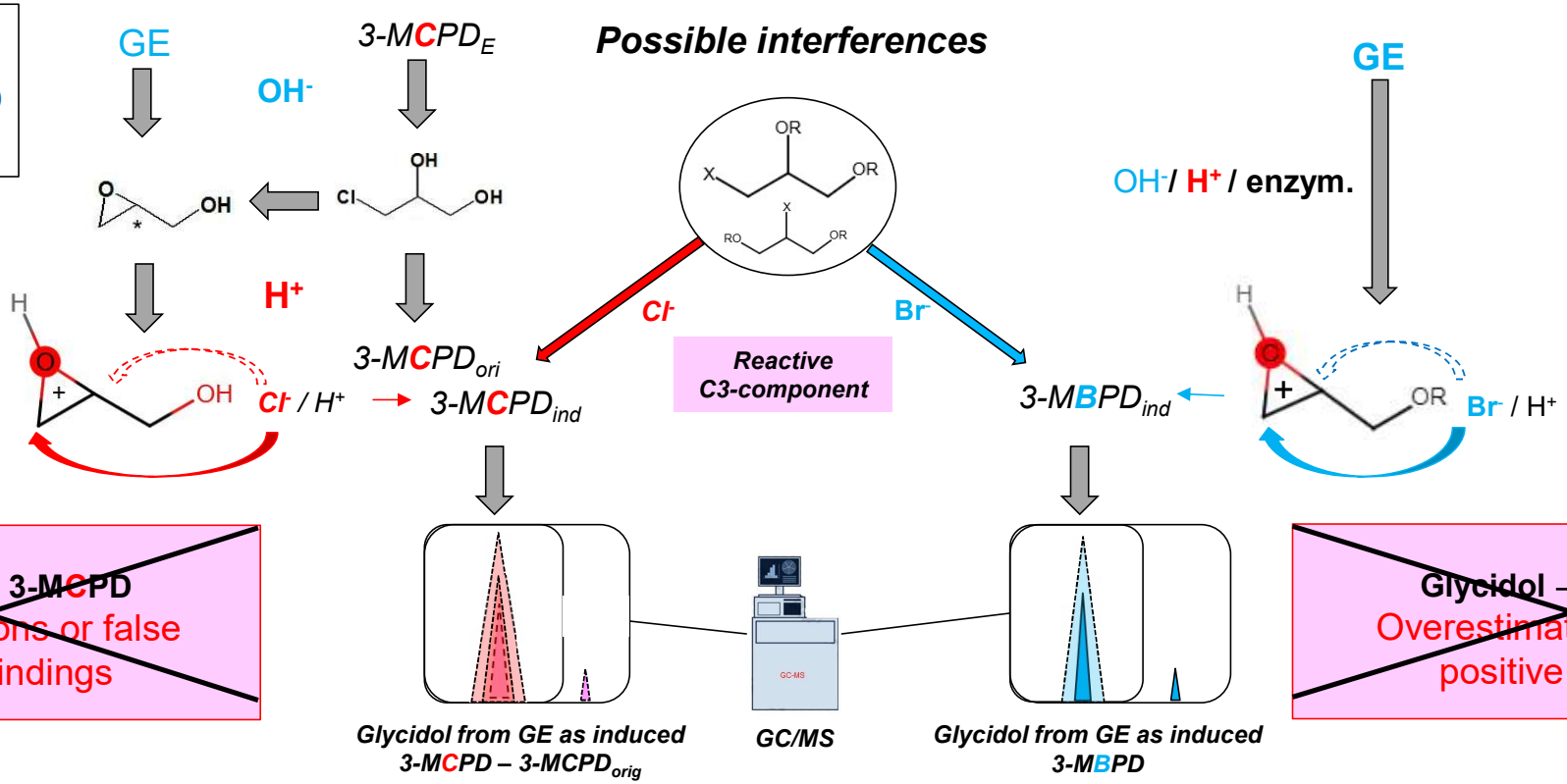
DGF C-VI 17 & 18_A (10)

AOCS Cd 29c-13_A

ISO 18363-1_A

Potential interferences in the indirect determination of GE

Official methods with chloride
DGF C-VI 17 & 18$_A$ (10)
AOCS Cd 29c-13$_A$
ISO 18363-1$_A$



Official methods with bromide
AOCS Cd 29a-13
AOCS Cd 29b-13
AOCS Cd 29d-19
AOCS Cd 29e-19
AOCS Cd 29f-21
„JRC-Methode“
ISO 18363-2,3,4

~~**Cl$^-$**~~
~~**Glycidol → 3-MCPD**~~
~~Overestimations or false positive findings~~

~~**Br$^-$**~~
~~**Glycidol → 3-MBPD**~~
~~Overestimations or false positive findings~~

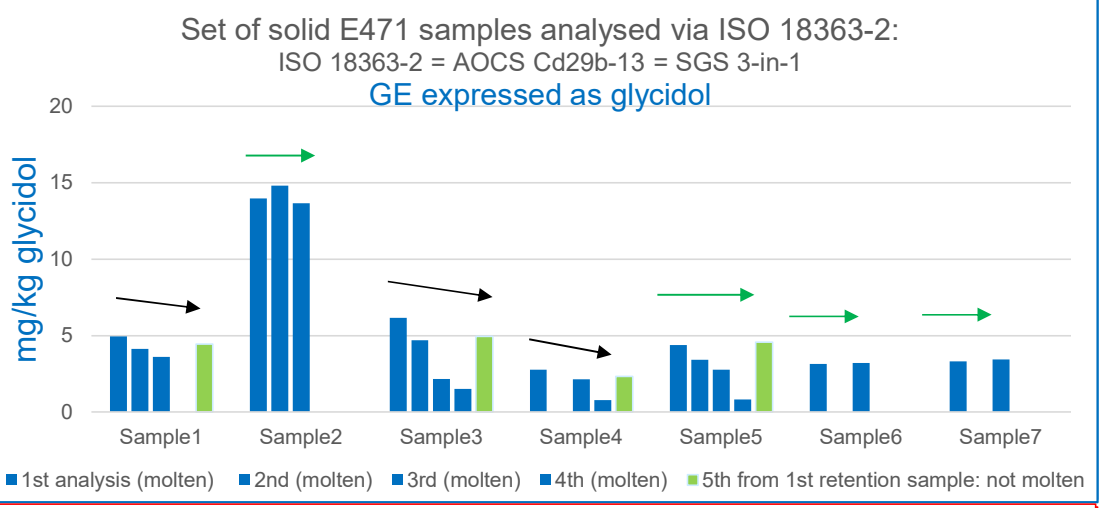
All indirect methods for GE-Analysis are based on the assumption that beside glycidol no further reactive components are present that react with chloride or bromide to 3-MCPD or 3-MBPD.

Observations in GE-determination when applying official oil & fat-methods to E471

Part I

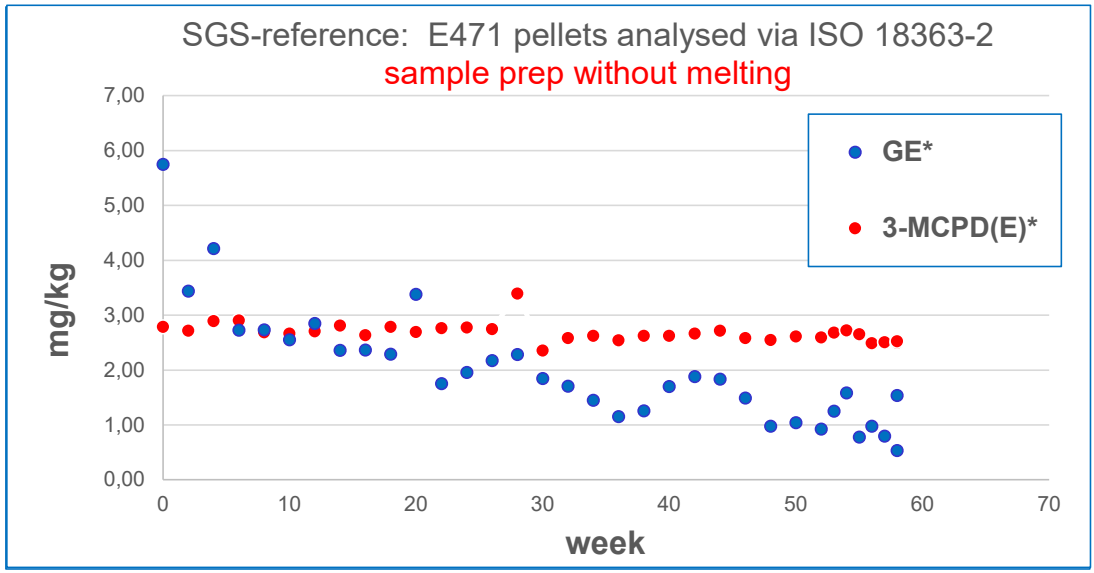
With all official methods, non-liquid samples have to be molten during sample preparation.

(Emulsifiers are **not(!)** in the scope of official methods)



When analysing on subsequent days, the GE-contents in samples 1,3,4,5 decrease significantly. Reason: Analyte-Instability. Accelerated decomposition caused by sample melting.

GE-decomposition not predictable. Levels in some samples unchanged.



GE-decomposition might also occur at room temperature!

Larger variation in results as seen in oils/fats!

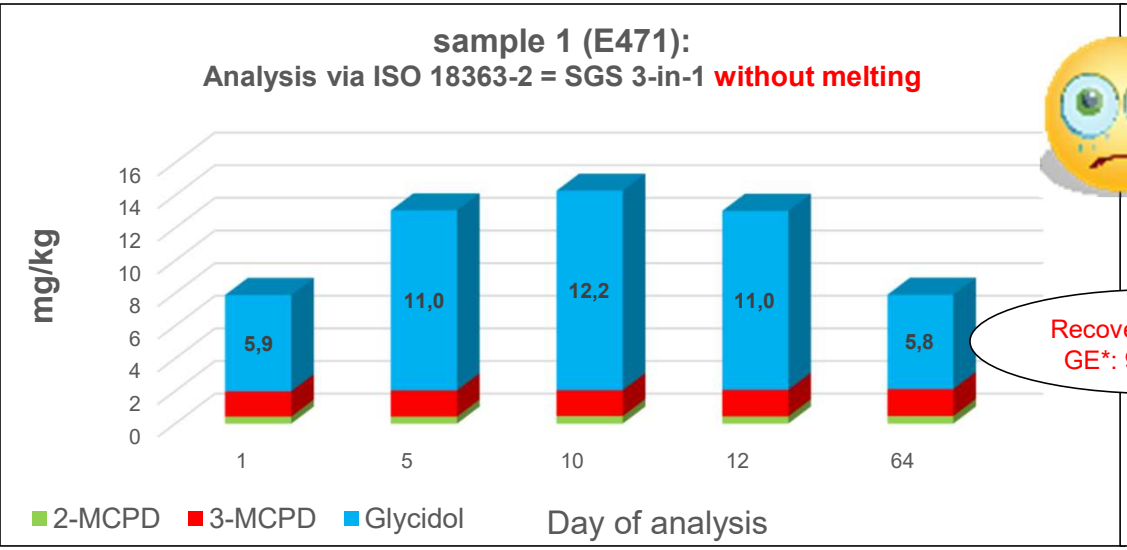
Here there is no interference in the actual sense - but application of the official methods can lead to significant GE underestimations.

*However, a method, in which the analyte might be destroyed (in an uncontrolled manner) is **not valid**!*

Observations in GE-determination when applying official oil & fat-methods to E471

Part II

„strange results“ when re-analysing E 471-samples.



Recovery of spiked
GE*: 99 – 101 %

GE is a group of heat-induced processing contaminants. The levels should not increase during storage at room temperature.

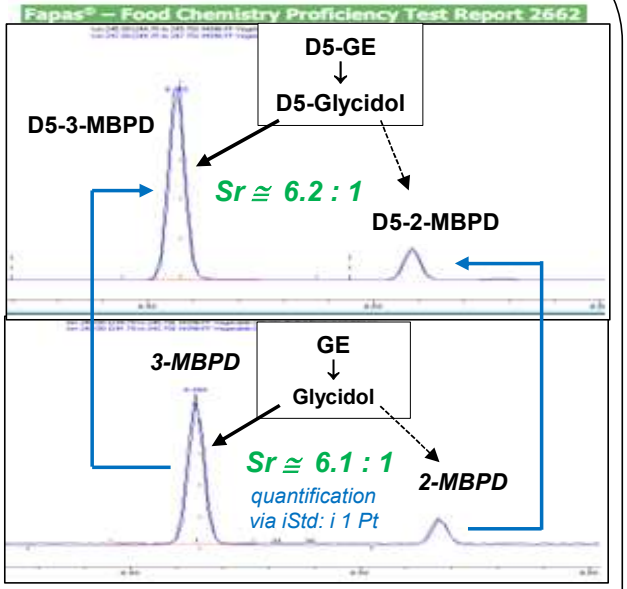
This must be strange reaction kinetics.

Assumption: opposing combination of **GE-decomposition** ↔ **artefact formation** during analysis.

Solutions for identification and compensation of GE artefact formation.

Approach: control of GE-induced 3-MBPD : 2-MBPD ratio

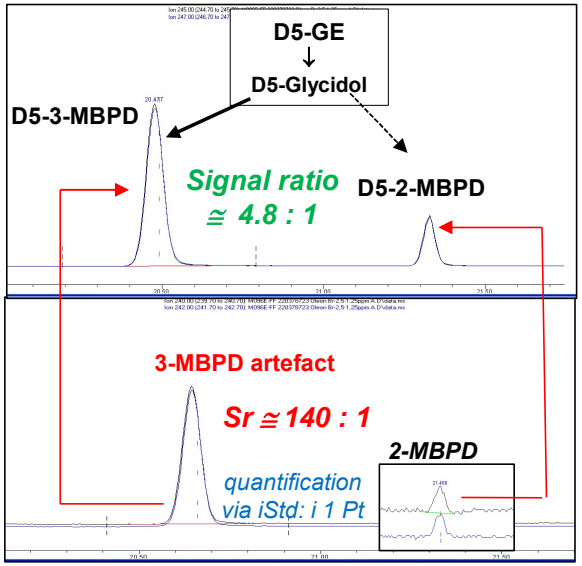
Reference: FAPAS Coconut oil 2662
GE* via ISO 18363-2 = SGS 3-in-1



GE* via D_5-3-MBPD : 496 µg/kg

In one sample, D_5-glycidol & glycidol must react identically.
GE-quantification via 2-MBPD is less sensitive but might be used for verification purpose*

E471 Emulsifier
GE* via ISO 18363-2 = SGS 3-in-1



GE* via D_5-3-MBPD: 480 µg/kg
GE* via D_5-2-MBPD: < 20 µg/kg = < LOQ

If 2-MBPD is underrepresented:
Indication for artefact formation!
The lower value is more meaningful.

Official„3-MCPD(E)/GE-methods are not suitable for emulsifier analysis!
They are not valid for these matrices!

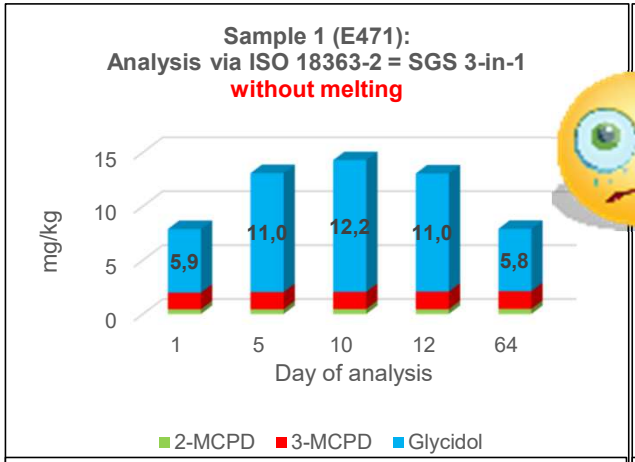
…that ´s not new:

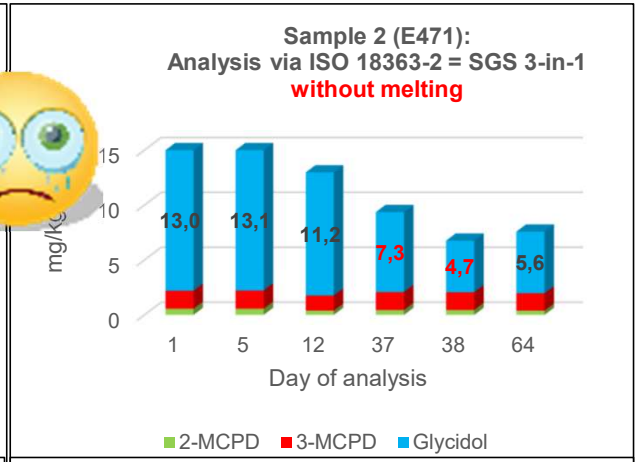
A.Ermacora, K. Hrncirick: J Am Oil Chem Soc **2013**, 90, 1–8
J. Kuhlmann, oral presentation: AOCS Expert Panel on Process Contaminants, **2013**, Montreal, Canada
Z. Zelinkova, A. Giri, T. Wenzel: Food Control, 77, **2017**, 65-75:

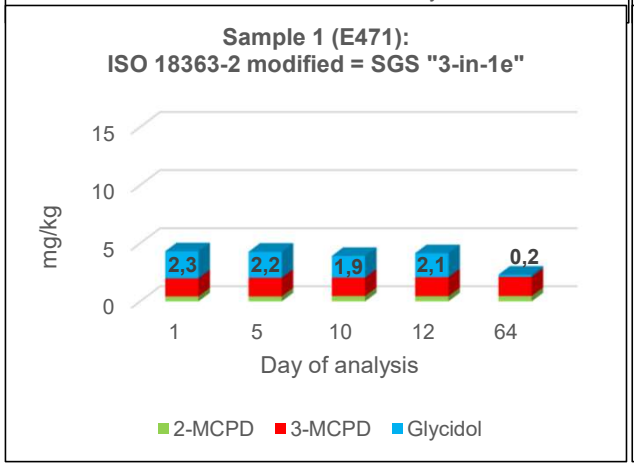
GE quantification via 2-MBPD could work, if sensitivity could be improved and if the melting of samples is avoided.

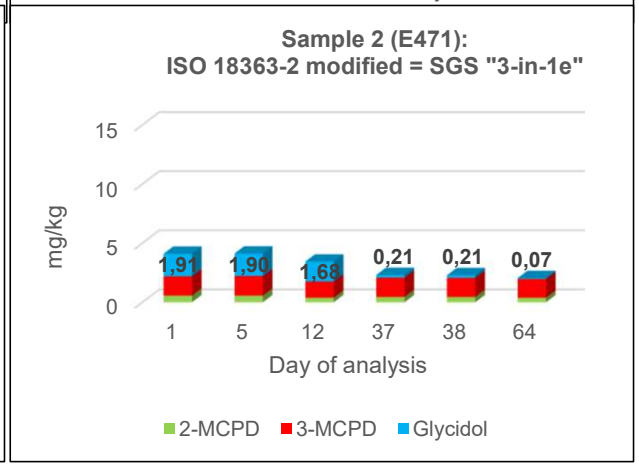
A new method on basis of ISO 18363-2:
Change in sample weight & solvents
Change in raw data evaluation
No sample-melting
„SGS 3-in-1 emulsifier-method“

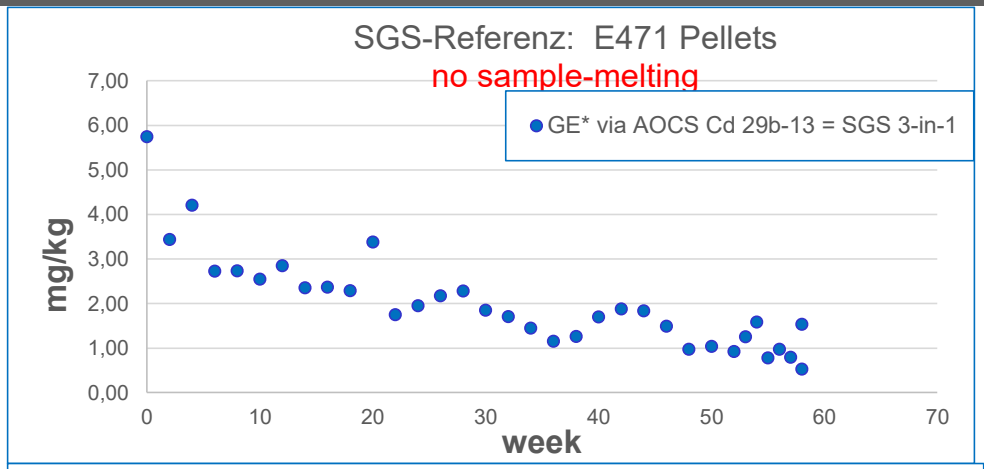
Application of the SGS 3-in-1e emulsifier method: practical examples

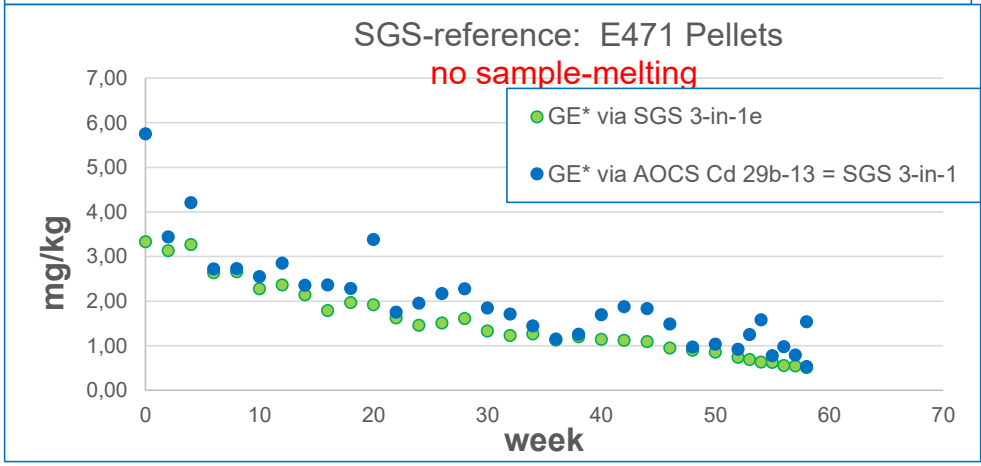












A routine method has to undergo method validation according to international standards before being accepted by official bodies & the scientific community.

Method validation trial on basis of ISO 5725-2 : 2019

Joint venture SGS & EFEMA

➢ 2 x 10 samples (double-blind)

- **All relevant E471 sub-classes covered**
- **Blank materials included (E475, E476)**
- **Analyte combinations** high GE ↔ low 3-MCPDE // low GE ↔ high 3-MCPD
- **Variable habitus**: solid block, pellets, powders, flakes, liquid

➢ **Six participating laboratories / 8 data sets:**

- 3 x Denmark / 1 x France /1 x Ireland / 1 x Germany (3 data sets)

TABLE 5 Summary validation results for RSD_r, RSD_R and HorRat *R* values

	Analyte level	RSD_r (%)	RSD_R (%)	HorRat *R*
Glycidol[a]	0.11 ↔ 28.4 mg/kg	3.3 ↔ 17.1	9.0 ↔ 29.2	0.6 ↔ 1.6
3-MCPD[b]	0.07 ↔ 2.93 mg/kg	2.0 ↔ 16.6	6.7 ↔ 20.7	0.4 ↔ 1.1
2-MCPD[b]	0.02 ↔ 0.72 mg/kg	1.9 ↔ 24.0	10.1 ↔ 28.6	0.5 ↔ 1.0

[a]Glycidyl fatty acid esters expressed as glycidol.
[b]Sum of free MCPD and MCPD fatty acid esters expressed as MCPD.

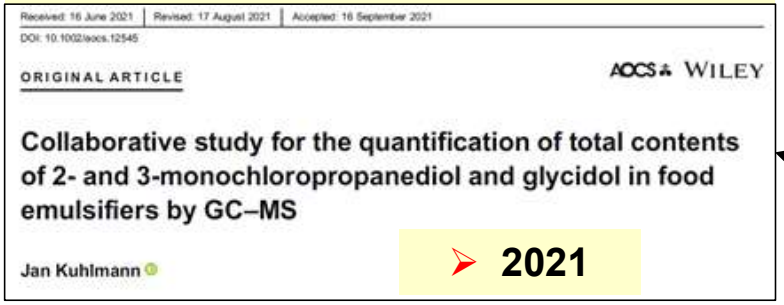
Requirements for achieving acceptance

Method suitable for monitoring & official food control?

	Method LOQ**	Max levels***
2-MCPD(E)*	≤ 0.02 mg/kg	not set
3-MCPD(E)*	≤ 0.07 mg/kg	0.75 – 2.5 mg/kg
GE*	≅ 0.01 mg/kg	5 mg/kg

* Expressed as 2- or 3-MCPD or glycidol / ** lowest included analyte level with RSD_R ≤ 30 %
*** EU 2023/1329 & EU 2023/1428

Peer-reviewed publication


Received: 16 June 2021 | Revised: 17 August 2021 | Accepted: 16 September 2021
DOI: 10.1002/aocs.12545

ORIGINAL ARTICLE

AOCS WILEY

Collaborative study for the quantification of total contents of 2- and 3-monochloropropanediol and glycidol in food emulsifiers by GC–MS

Jan Kuhlmann

➢ **2021**

Acceptance ?

EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 1 April 2022

doi: 10.2903/j.efsa.2022.7308

Follow-up of the re-evaluation of polyglycerol esters of fatty acids (E 475) as a food additive

...

References

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Kuhnle GG, Leblanc J-C, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Boon P, Chrysafidis D, Gürtler R, Mosesso P, Parent-Massin D, Tobback P, Rincon AM, Zs H and Lambré C, 2017a. Scientific Opinion on the re-evaluation of polyglycerol esters of fatty acids (E 475) as a food additive. EFSA Journal 2017;15(12):5089, 32 pp. https://doi.org/10.2903/j.efsa.2017.5089

...

EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2009b. Scientific opinion on Arsenic in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980

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summary

1. Official methods for analysis of 2-/3-MCPD(E)/GE in edible oils/fats have not been designed nor systematically tested for food emulsifiers and might give wrong results for GE.
   Overestimation = artefact formation ↮ decomposition = underestimation

2. In food emulsifieres, GE might show significant decomposition during transport/storage/sample prep..
   These effects have not been observed similarly in foods.

3. On basis of ISO 18363-2, a new routine method for analysis of MCPD(E) & GE in E471/E475/E476 was successfully validated by an international ring trial.

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WHEN YOU NEED TO BE SURE

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