Compositional Analysis of Lipids

Thursday 20 - Friday 21 June 2013

Het Pand, Ghent, Belgium

Organised by SCI's Lipids Group in Collaboration with Ghent University and EFL Physical Properties Division

SCI
where science meets business

Recent developments in the analysis of MCPD esters and glycidyl esters in edible oils and fats

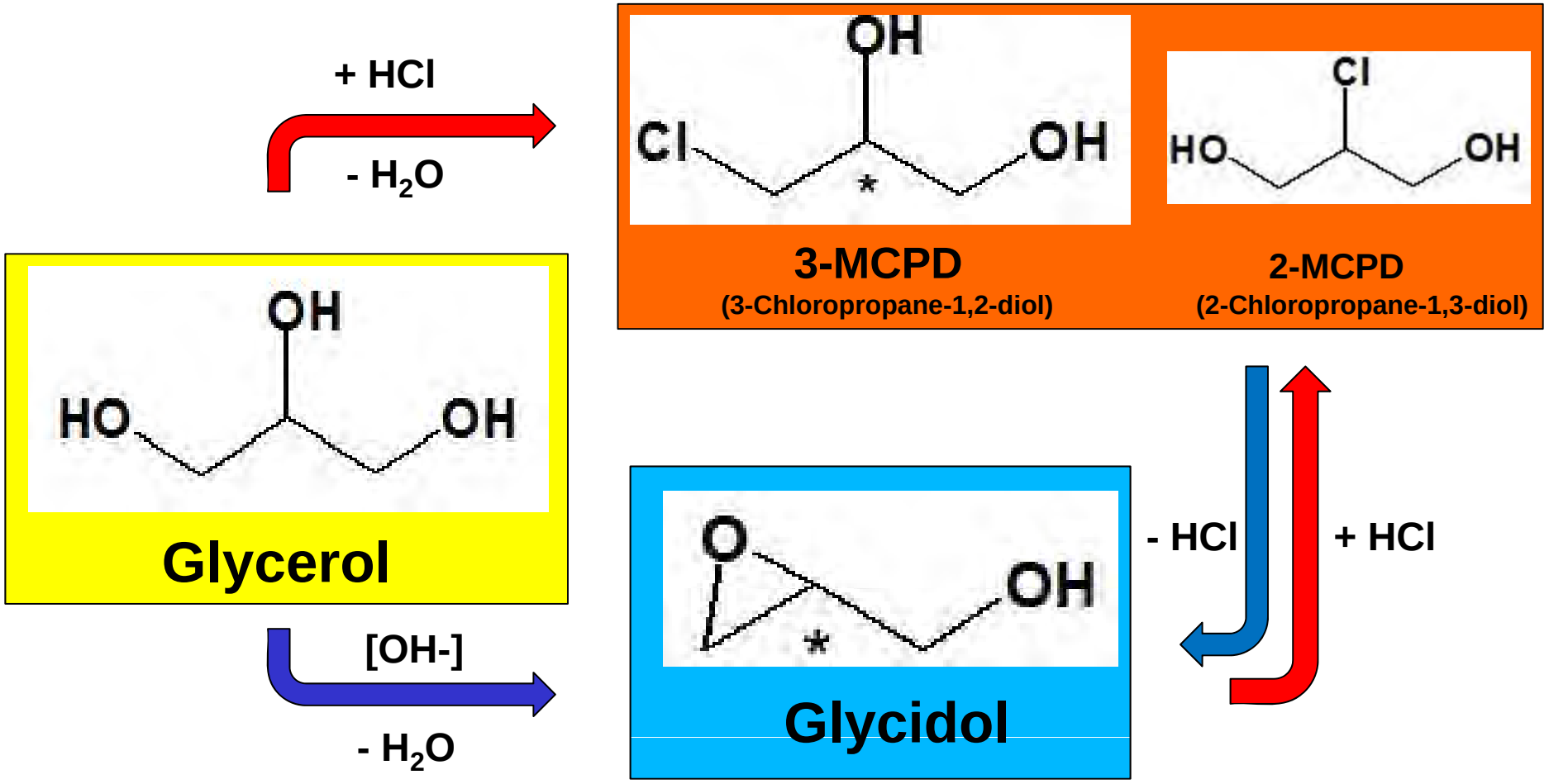
SGS Germany GmbH

J. Kuhlmann

WHEN YOU NEED TO BE SURE

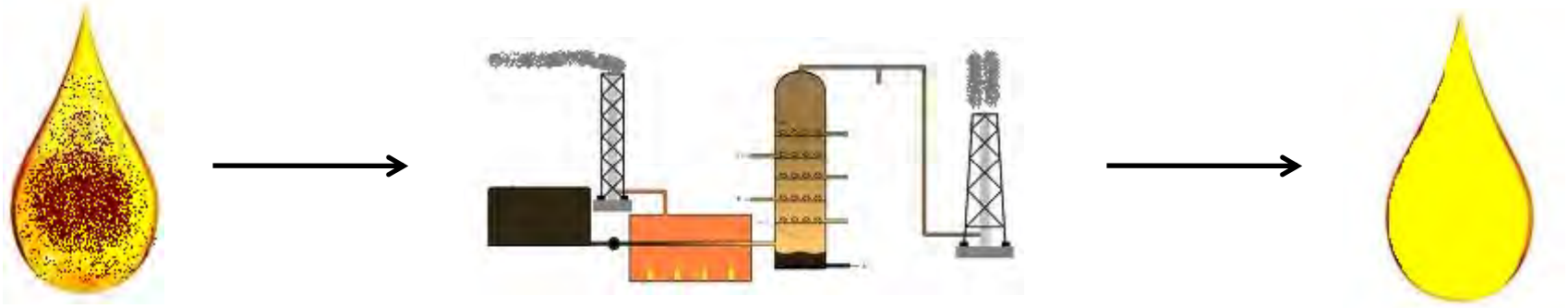
SGS

2-MCPD, 3-MCPD & Glycidol: Derivates of glycerol



Introduction

(fatty acid) bound Glycidol & MCPD in oils & fats



crude oil **refining** **refined oil**

Bound MCPD & Glycidol are generated mainly during deodorisation at high temperatures.

The vast majority of refined oil & fat contains bound MCPD and/or bound glyicdol *(potential of contaminant formation is dependant on the oil type)*

Also industrial or private frying may cause the formation of bound MCPD !

Introduction

Precursors of bound Glycidol & MCPD in natural oils

Mono*(acyl)*glycerides / Di*(acyl)*glycerides / Tri*(acyl)*glycerides

> 200 ° C

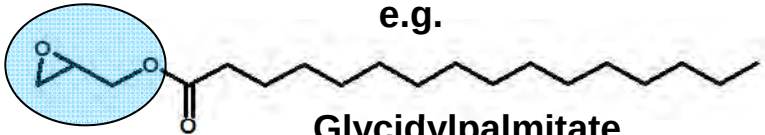
- H_2O/-FA

„*bound glyicdol*“

Glycidyl fatty acid esterS

- **mono esters**

e.g.



Glycidylpalmitate

< 200 ° C

+ HCl*

- H_2O/-FA

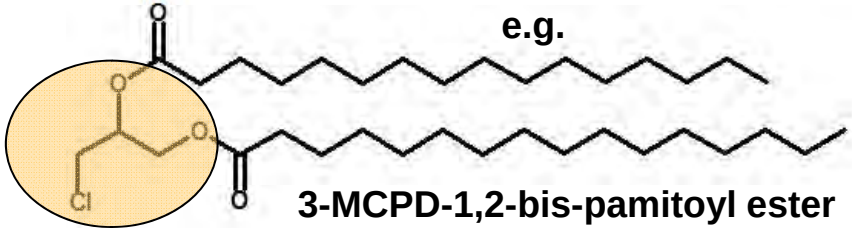
***natural sources**
e.g. $FeCl_x$, chlorinated phytosphingosides[1)]

„*bound MCPD*“

2- & 3-MCPD fatty acid esters

- **1-/2- mono esters & 1,2-/1,3- di esters**

e.g.



3-MCPD-1,2-bis-pamitoyl ester

1) K. Nagy et al.: Mass-defect filtering of isotope signatures to reveal the source of chlorinated palm oil contaminants; *Food Addit. Contam.* **2011**, 28, 1492–1500

Toxicological impact of MCPD & Glycidol

- **free 3-MCPD** *in-vivo* toxic effects MRL = **20 µg/**kg in HVP etc., **100 µg/kg** in glycerol [2);3)]
- **bound 3-MCPD:** TDI = **2 µg/kg bw d** *in-vivo* the majority of 3-MCPD is released during digestion [4);5)]
  "Most probably, for the toxicological effects the total available quantity of 3-MCPD in the body is critical" [6)]

  BfR Bundesinstitut für Risikobewertung
- **free & bound 2-MCPD: still no toxicological data available**

- **free glycidol** skin & eye irritation[2] acute oral & inhalative & dermal toxicity[3-4] single-exposure specific target single organ [3] & reproductive toxicity [1B] germ cell mutagenicity[2] carcenogenicity[1B]

  [Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP]]

- **bound glycidol** "Glycidyl esters are completely hydrolyzed during digestion" ***6);7)***
  "In comparison to free glycidol, the glycidol amount resorbed from glycidyl esters is practically identical" ***6);7)***

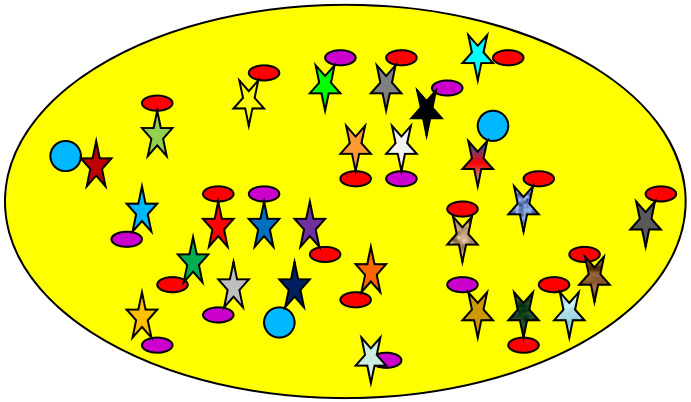
  BfR Bundesinstitut für Risikobewertung

[2),3)] EU Commission Regulations N° 466/2001, N° 232/2012
[4)] EFSA (2011). Scientific report submitted to EFSA 'Comparison between 3-MCPD and its palmitic esters in a 90-day toxicological study' prepared by E. Barocelli, et al. University of Parma, Italy
[5)] K. Abraham, K.E. Appel, E. Berger-Preiss, E. Apel, S. Gerling, H. Mielke, O. Creutzenberg, A. Lampen: Relative oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats. *Arch. Toxicol.* 2013, **87** (4), 649-659
[6)] A. Lampen: Risk assessment of 3-MCPD and glycidyl ester in food; Oral presentation at 8th International Fresenius Conference Contaminants and Residues in Food, April 2013 Mainz Germany
[7)] K.E. Appel, K. Abraham, E. Berger-Preiss, T. Hansen, E. Apel, S. Schuchard, C. Vogt, N. Bakhya, O. Creutzenberg, A.Lampen: Relative oral bioavailability of glycidol from glycidyl fatty acid esters in rats. *Arch. Toxicol.* 2013, Epub ahead of print

Analytical approaches

Direct analysis; determination of the single original esters

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



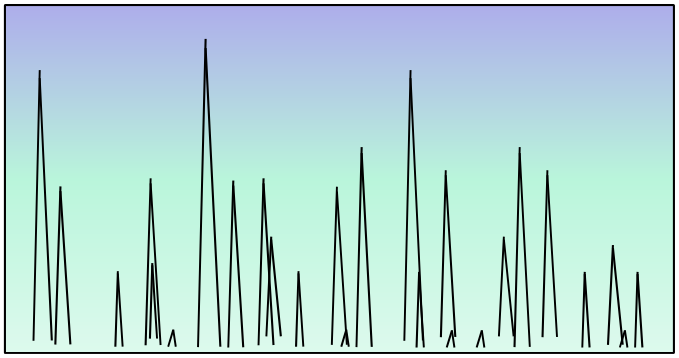
Hypothetic oil

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 (SPE, GPC)



Chromatogram displays up to 27 analytes!

LC-MS / LC-MS² / LC-MS-TOF / GC-MS

Analytical approaches

Advantages/disadvantages of direct analysis

In purpose to quantify individual MCPD esters and glycidyl esters:
Direct analysis is the only practicable approach!

Direct analysis in purpose of quantifying the total MCPD & glycidol content

+ ***no chemical transformation***

+ ***additional information***

- ***multi-analyte method***

- ***sophisticated matrix removal and instrumental equipment***

- ***risk of underestimation in case of unexpected or unknown derivatives***

- ***separation becomes really challenging in case of MCPD isomers***

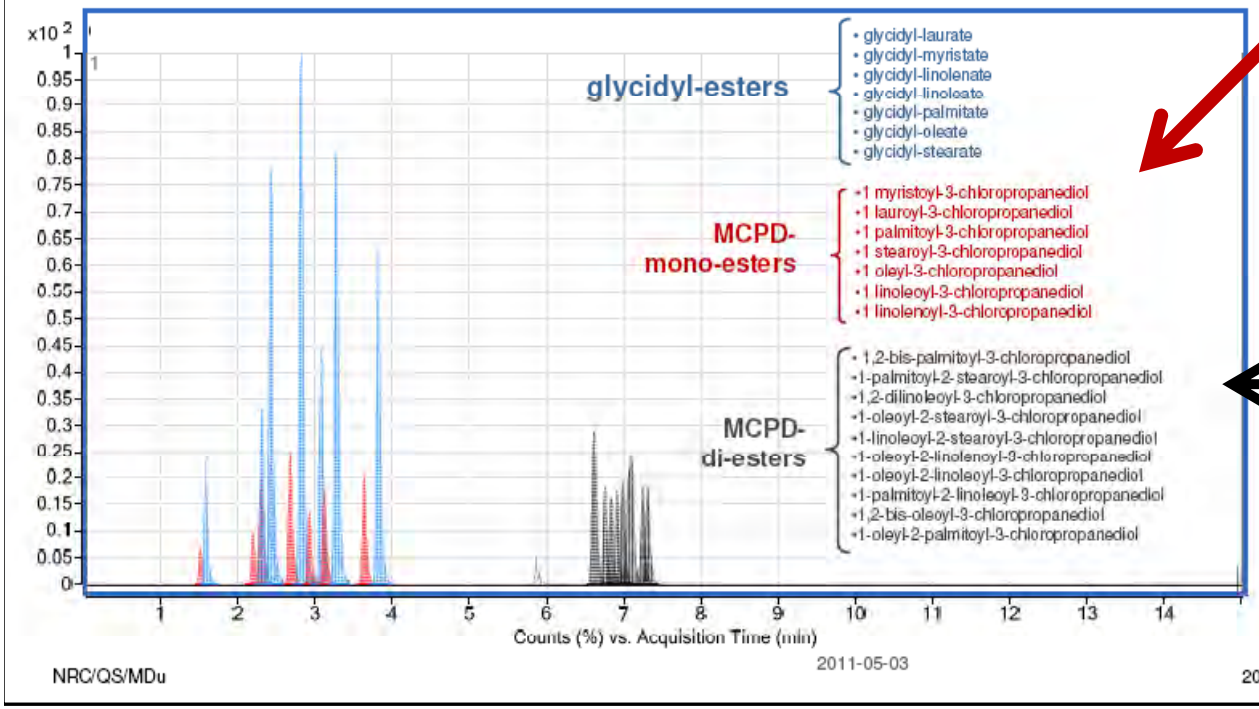
Analytical approaches

Advantages/disadvantages of direct analysis

"*Challenging separation*": what does it mean in practise?

Chromatographic separation – ToF detection

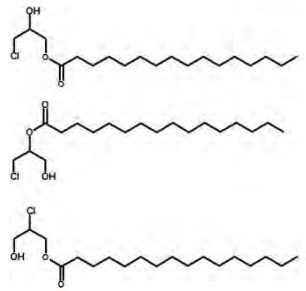
- Column: Waters Acquity HSS T3 (50x2.1mm, 1.8µm)
- Elution: A MeOH:H2O 75:25 (10mM ammonium formate , 0.1% HCOOH)
  B: Isopropanol (10mM ammonium formate, 0.1% HCOOH)
- Run time: 15 min between injection



Not displayed:

Isomeric sn-2 3-MCPD-mono-esters & 2-MCPD-mono-esters

e.g.



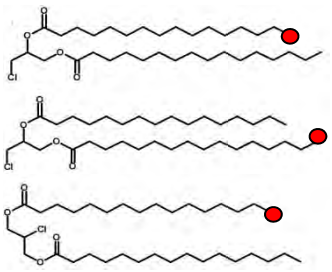
1-fa-3-MCPD

2-fa-3-MCPD

1-fa-2-MCPD

Isomeric 3-MCPD-1,2-di-esters & 2-MCPD-1,3-di-esters

e.g.



1-fa*,2-fa-3-MCPD

1-fa,2-fa*-3-MCPD

1-fa,3-fa*-2-MCPD

M. Dubois; Oral presentation AOCS Annual Meeting 2011, Cincinnati, Ohio

Analytical approaches

Direct analysis; determination of the single original esters

Selection of direct methods

e.g. **glycidyl esters**

Masukawa et al. 2010/2011 = **AOCS/JOCS Cd 28-10** ***(double SPE)*** ***validated***

Blumhorst et al. 2011 = **ADM** ***(dilute & shoot)***

Granvogl et al. 2011 = **DFA** ***(SPE)***

Hrncirik & Ermacora 2013 = **direct Unilever method in progress** ***(GC-MS)***

e.g. **MCPD & glycidyl esters**

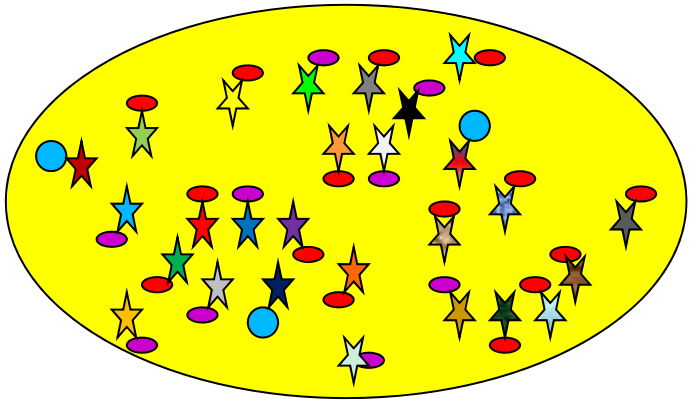
Dubois et al. 2011 = ***Nestle ´ (double SPE for Mono-ester, SPE for Di-ester = 2 Assays)***

Haines et al. 2011 = ***ADM (dilute & shoot)***

MacMahon et al. 2013 = ***FDA***
(2 double SPE assays, 2-MCPD esters considered, progress in isomer separation)

Analytical approaches

Indirect analysis; determination of the released analytes

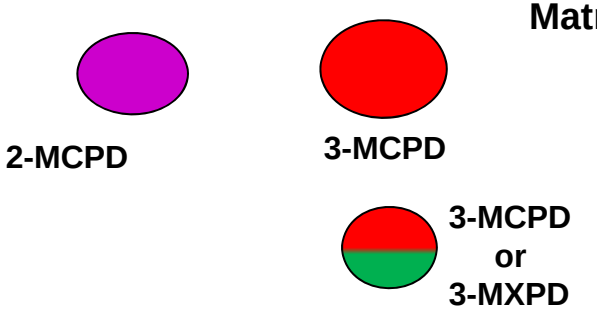


Hypothetic oil

Contains only 3 relevant fatty acids

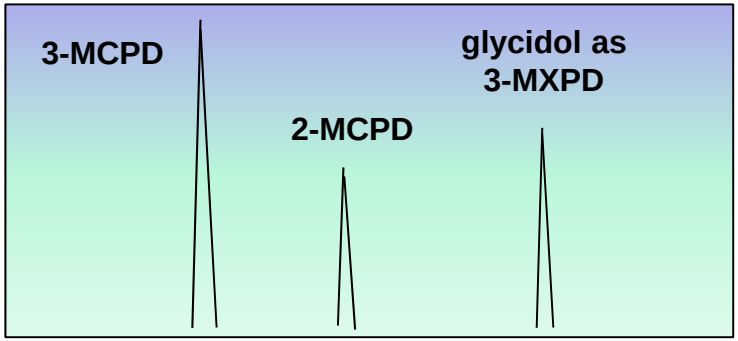
Ester cleavage (ec) (*alkaline / acidic / enzymatic; 3 min – 16 h*)

Matrix removal (*(l/l) extraction*)



Derivatisation
(e.g. HFBA/Acetone/PBA)

GC-MS



Chromatogram displays up to 3 core analytes,

LOQs 0.1 – 0.25 mg/kg

Advantages/disadvantages of indirect GC-MS analysis

Indirect analysis in purpose to quantify the total MCPD & glycidol content

+ ***only 3 reference compounds & iStds***

+ ***less sophisticated matrix removal & instrumental equipment***

+ ***low risk of underestimation***

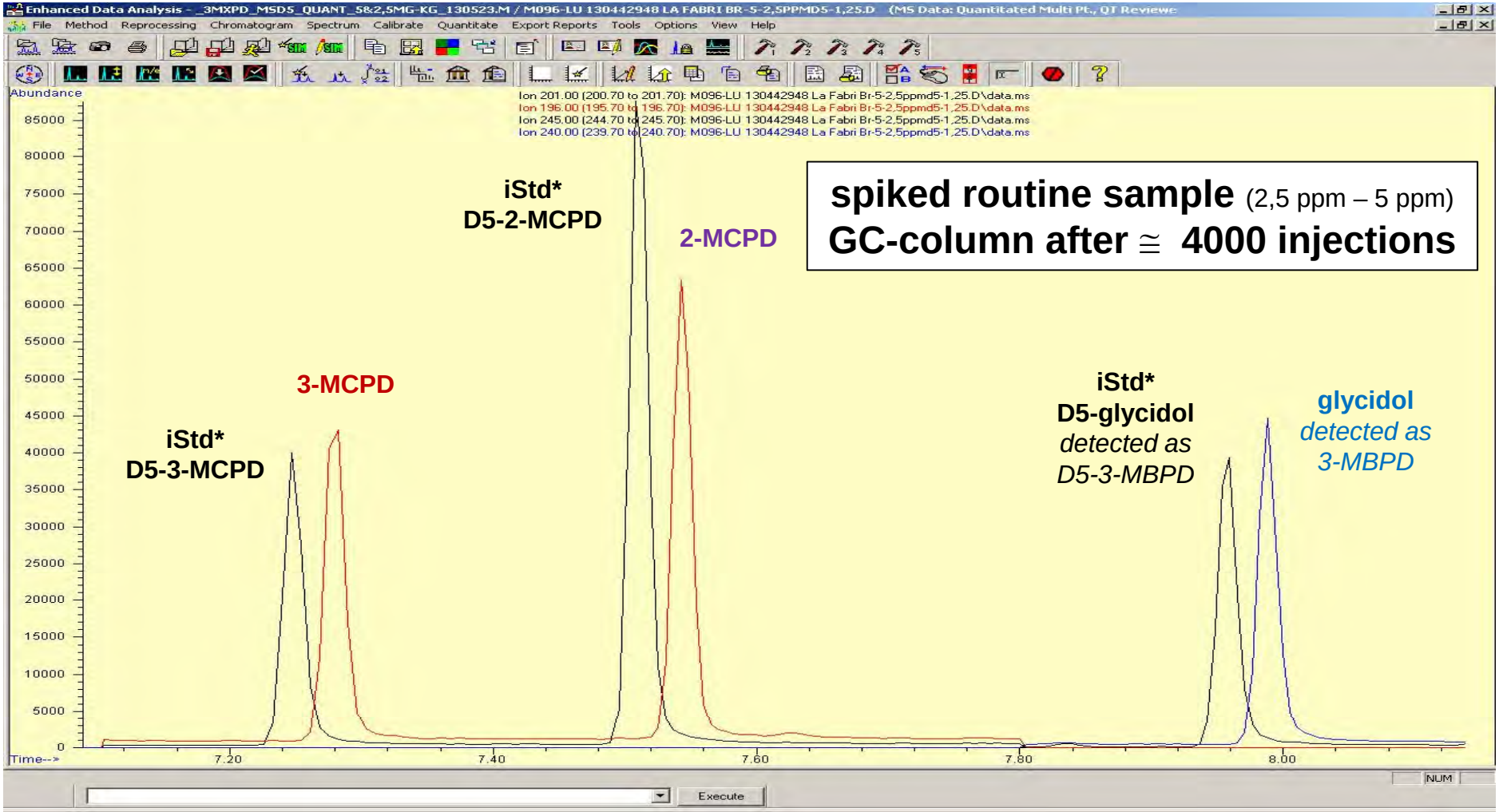
+ ***no problems in separation at all***

- ***chemical reactions may cause analyte isomerisation or transformation, MCPD ↔ glycidol conversion or artefact formation***

-*derivatisation for GC-analysis required*

Analytical approaches

Advantages/disadvantages of indirect GC-MS analysis
“no separation problems at all”: what does it mean in practise?



Analytical approaches

Indirect analysis – selection of recent methods

♦ **bound MCPD** ♦

Divinova et al. 2004 ***(slow acidic ec / glycidol destroyed)***
BfR modification 2010 **BfR method 8** ***(validated)***

Kuhlmann 2010 = **DGF C-VI 18 (10) B** ***(fast alkaline ec / validated)***
BfR modifications 2010 **BfR method 9** ***(validated for oils&fats)*** *2010-13* **BfR method 22** ***(validated for foods)***

♦ **sum (!) of [bound MCPD & bound glycidol] detected as 3-MCPD** ♦

Weißhaar et al. 2010 = **DGF C-VI 17 (10)** ***(fast alkaline ec / validated)***

Kuhlmann 2010 = **DGF C-VI 18 (10) A** ***(fast alkaline ec / validated)***

♦ **bound MCPD & glycidol** ♦

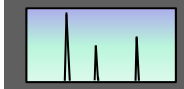
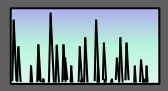
Kuhlmann 2010 = **DGF C-VI 18 (10) A & B** ***(A-B x Tf = glycidol / validated)***

Kuhlmann 2010 = **SGS "3-in-1" method** ***(slow alkaline ec / glycidol → 3-MBPD / in validation)***

Miyazaki et al. 2012 = "**enzymatic method"** ***(enzymatic ec / glycidol → 3-MBPD)***

Ermacora et al. 2013 = "**improved Unilever method"** ***(GE → 3-MBPD-E / slow acidic ec / in validation)***

Method comparison

reliability of recent methods

The imperfect early DGF method C-III 18 (09) *(withdrawn in 2011),* **complex chemistry and in single cases improper method application raised doubts in the reliability of indirect methods in general**

"DGF Method still gives positive results even when MCPD and glycidyl esters are not present." **2010**

"DGF method predicts much higher MCPD concentrations than LCMS when MCPD esters are present." **2010**

"The harsh chemistry of the DGF method creates incorrect results in the analysis of MCPD and glycidyl esters." **2010**

"The critical steps in the analysis of 3-MCPD esters in oil samples are linked to the method of esters hydrolysis and instrument calibration." **2010**

"differential DGF method just a rough"estimation" **2011**

"Chemistry capable of transesterifying oils needs to be avoided in analysis of MCPD and glycidyl esters" **2010**

"The existing indirect methods, however, may yield unreliable results …" ***2012***

Method comparison

2 studies on the comparability and trueness of recent methods

November **2012**

AOCS Your Global Fats and Oils Connection

"**<u>method comparison study</u>** of direct and indirect methods for MCPD-ester and glycidyl-ester "

3 SOPs of indirect methods supplied:
<u>Improved Unilever / SGS "3-in-1"</u>
<u>DGF C-VI 18 (10)</u>
direct methods allowed

7 spiked & 1 non-spiked RBD canola oil
1 RBD palm oil

<u>Participation</u>

Indirect methods: 9 to 12 laboratorys each

Direct glycidyl ester: 4 labs

Direct MCPD ester: 1 lab 4 methods

Summary

•All 3 of the indirect methods tested gave comparable results

•In general the direct methods agreed with the indirect methods.

•Methods, either direct or indirect, did not give reliable results if total MCPD concentrations or glycidol concentrations were below ~1 ppm.

M.W. Collison; Oral presentation, AOCS Annual Meeting 2013, Montreal /Ca

It is planned that all three tested indirect methods should become official AOCS methods

Method comparison

2 studies on the comparability and trueness of recent methods

January
2013

European Commission
JRC & IRMM
Joint Research Center
Institute for Reference Materials and Measurements

"**inter-laboratory comparison study** on the determination of MCPD esters and glycidyl esters in edible oils and fats"

***free method choice* / experienced participants**

7 spiked blanks & non-spiked refined oils/fats
(palm oil, palm kernel oil, coconut oil, soy oil, cocoa butter)

Conclusions

Consolidation regarding analysis methods

- Trend towards methods alowing distinction between three classes of substances
  - DGF C-VI 18 (10); Kuhlmann (3 in 1); Ermacora (2012)

Laboratories prefer indirect methods

- Especially for MCPD ester

Performance of direct and indirect methods for the determination of glycidyl esters comparable

Study showed that there is a couple of methods suitable for the monitoring of MCPD esters and glycidyl esters in edible oils!

T. Wenzel; Oral presentation, AOCS Annual Meeting 2013, Montreal - Canada

Some examples of foodstuff containing free/bound MCPD and/or glycidol

French fries, fried potatoes, chips, mayonnaise

Infant formula

Coffee creamer

Chocolate & nut-nougat spreads

Smoked fish & meat

Fish sticks, Fish ´ n ships

Ice cream

Spreads, dressings, margarine

Cookies, cakes, cruller

Dietary supplement oils

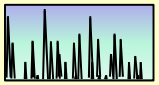
Instant soups

Tofu meals vegetarian sausage/lard/etc.

Puff pastry

Limitations in practise

nobody is perfect - no method is perfect



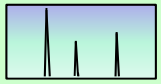
direct methods:

1) due to missing reference substances/iStds not applicable if analytes are bound to:
- **polyunsaturated fatty acids (e.g. fish oils)**
- **other rare fatty acids (MCT oils, rare plant oils)**

2) hardened fats & emulsifiers might impact the SPE sample preparation efficiency

3) the direct MCPD quantification remains questionable until separation problems have been solved

All difficulties might be solved by technical method improvements



Indirect methods based on **Alkaline ester cleavage** (DGF methods / SGS "3-in-1")

4) Due to neutralisation of transesterification reagent not applicable to acidic samples
(e.g. free fatty acids)

Solution: enlarging the amount of ester cleavage agent

based on **Acidic sample pre-treatment** (Improved Unilever method)

5) Does not cover free MCPD

6) LOQ bound glycidol = 0.2 mg/kg

7) Indications of significant glycidol overestimation upon do novo MBPD formation in oils processed after the refining step.

Solution: ??

Summary

Conclusions

- **Indirect methods are more commonly in use for routine analysis of bound MCPD & glycidol**
- **Recently the most common methods showed satisfying comparability and trueness in simple oils & fats**

direct AOCS Cd-28 10, Indirect DGF C-VI 18 (10) / Improved Unilever method / SGS "3-in-1" method)

- **Some new applications have appeared** *e.g. direct GC-MS method, enzymatic ester cleavage, acidic pre-treatment to convert glycidyl esters into MBPD esters*
- **The applicability of the above mentioned methods for other than the tested matrices has to be verified**

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Thank you for your kind attention!

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