The Development and Application of the Automated Method for the Quantification of 3-MCPD-,2-MCPD-, and Glycidylesters in Edible Oils and Fats

Ralph Zwagerman, 21-06-2017, 3-MCPD Symposium, Berlin

IOI Loders Croklaan

Let's create together

IOI GROUP

Motivation for Development

- Insufficient capacity
- Problems with robustness
- Necessity of processing optimization
- Analysis time
- Goals of automatization
  - **Decreasing analysis time**
  - **Minimizing contact time**
  - **Improving robustness**
  - **Fast QC Service**

Current Recommended Indirect Methods

	AOCS Cd29a-13	AOCS Cd29b-13	AOCS Cd29c-13
Reaction time	16 hours	16 hours	5.5 min max
Reaction conditions	Acidic, 40°C	Alkaline, -25°C	Alkaline, RT
Components quantified	3-MCPDe, 2-MCPDe and GE	3-MCPDe, 2-MCPDe and GE	3-MCPDe and GE (Calculated)
Automation Problems	• Precipitation of salts • Solvent evaporation steps	• Very low temperatures • Solvent evaporation steps	• No direct GE detection • No 2-MCPDe detection • Solvent evaporation steps
Remarks			**Basis for automation**

Adaptions for automation

Step	AOCS Cd 29c-13	Automated method
Sample	Two Aliquots (A & B)	One Aliquot
Reaction	alkaline, RT	alkaline, 5°C
Glycidol conversion[1)]	A: NaCl solution B: NaBr solution	NaBr solution
Sample clean-up	3 x extraction + drying	none
Extraction Derivatives	iso-octane, after solvent evaporation	n-hexane
Analysis	PTV Splitless, GC-MS	PTV Splitless, GC-MS/MS

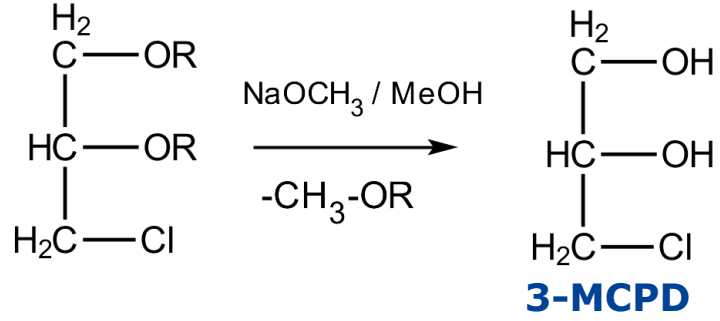
The Automated Method

- First Version Published in July 2016:

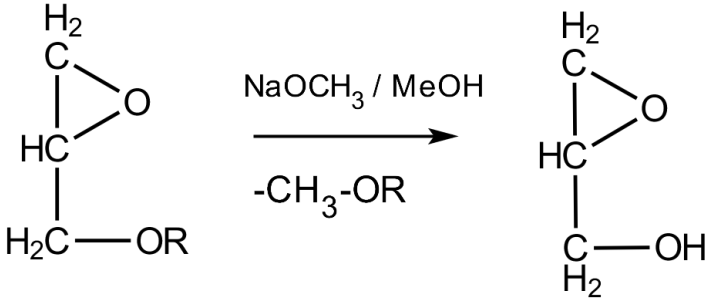
  Eur. J. Lipid. Sci. Technol. 2016, *118*, 997-1006

- Adapted version of AOCS Cd 29c-13
- **"3-in-1" Analysis method**
- Hardware: Thermo Scientific Triplus RSH / Trace 1300 / TSQ8000
- Everything automated after weighing the sample
- **21 samples / 24 hours** or 4 samples / 4.5 hours (1 series)
- Operated by ≤1 FTE

Chemistry of the automated method

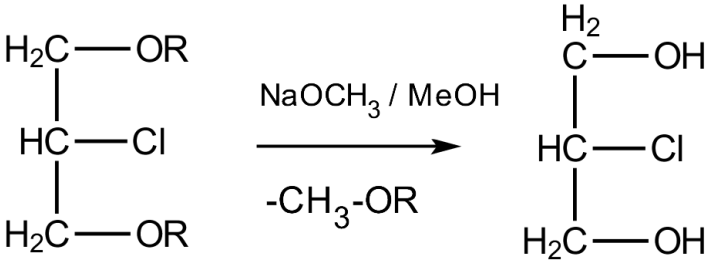


3-MCPD



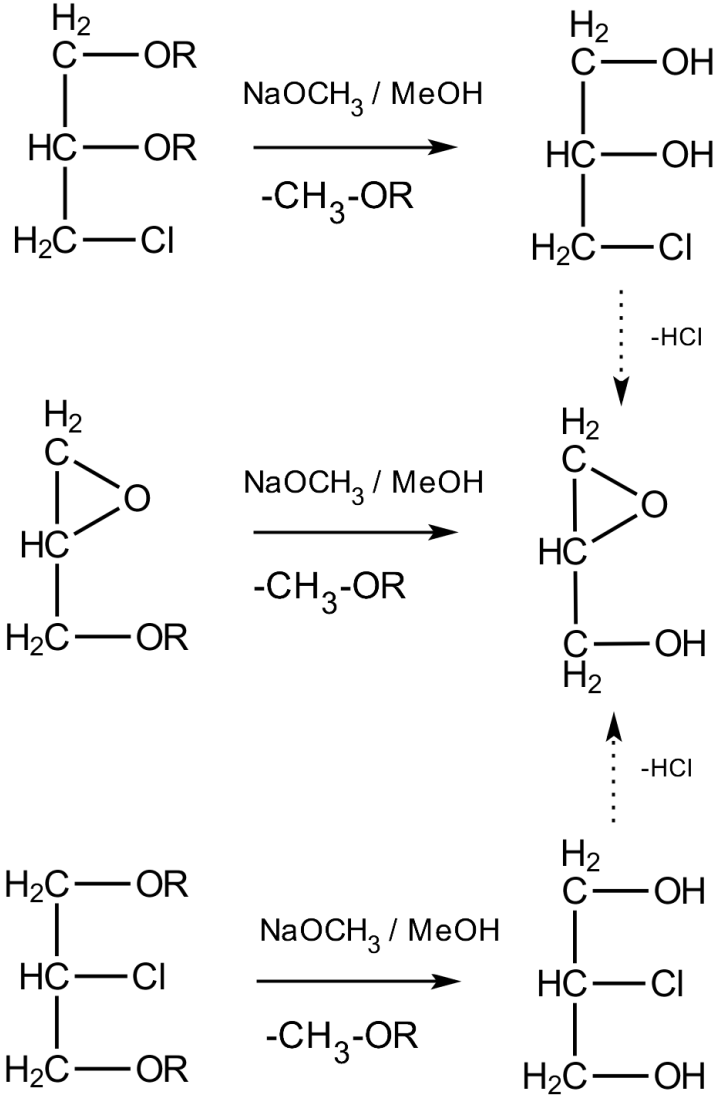
Glycidol

- Conversion of esters to free 3-MCPD, Glycidol and 2-MCPD
- Release of FAMEs



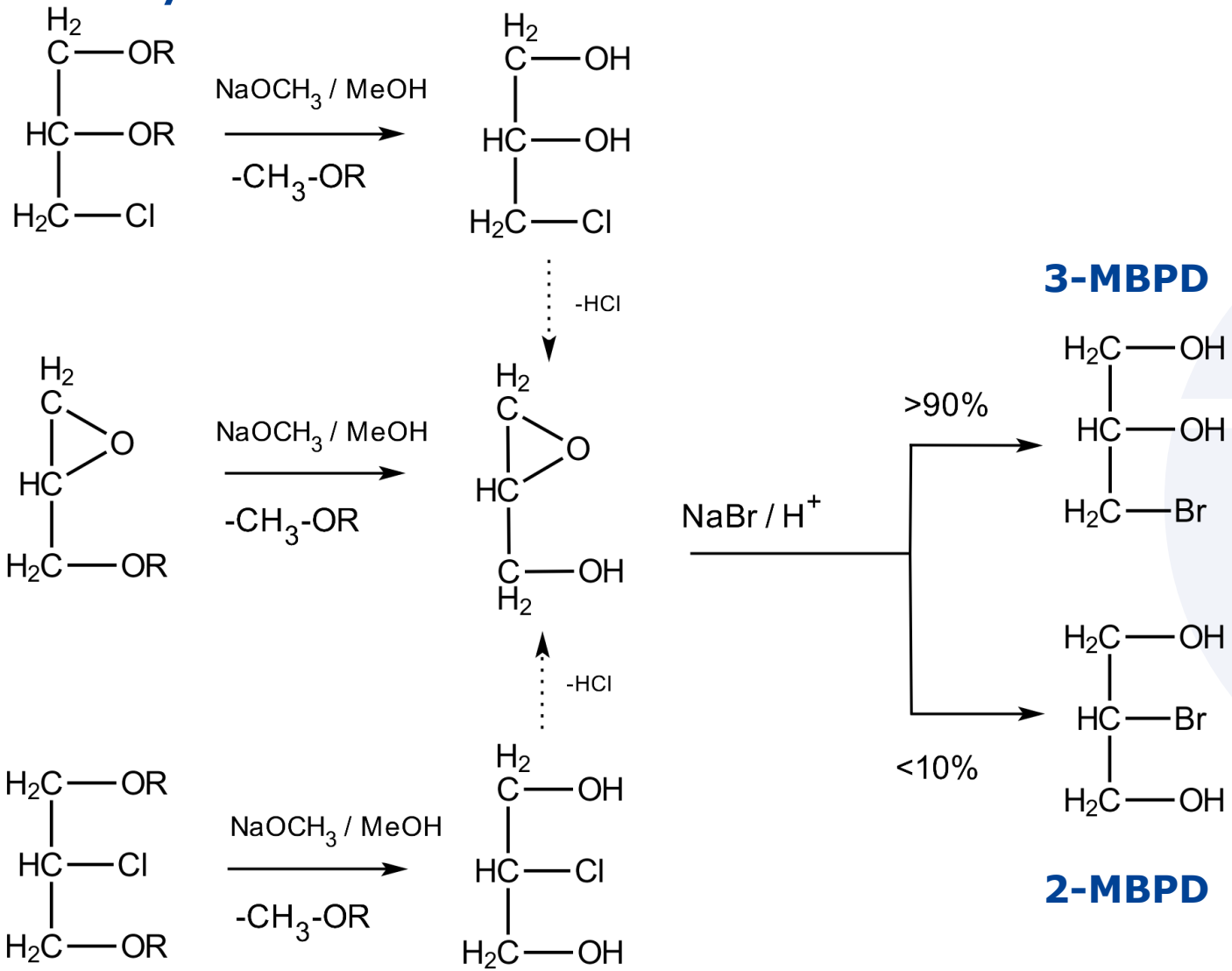
2-MCPD

Chemistry of the automated method

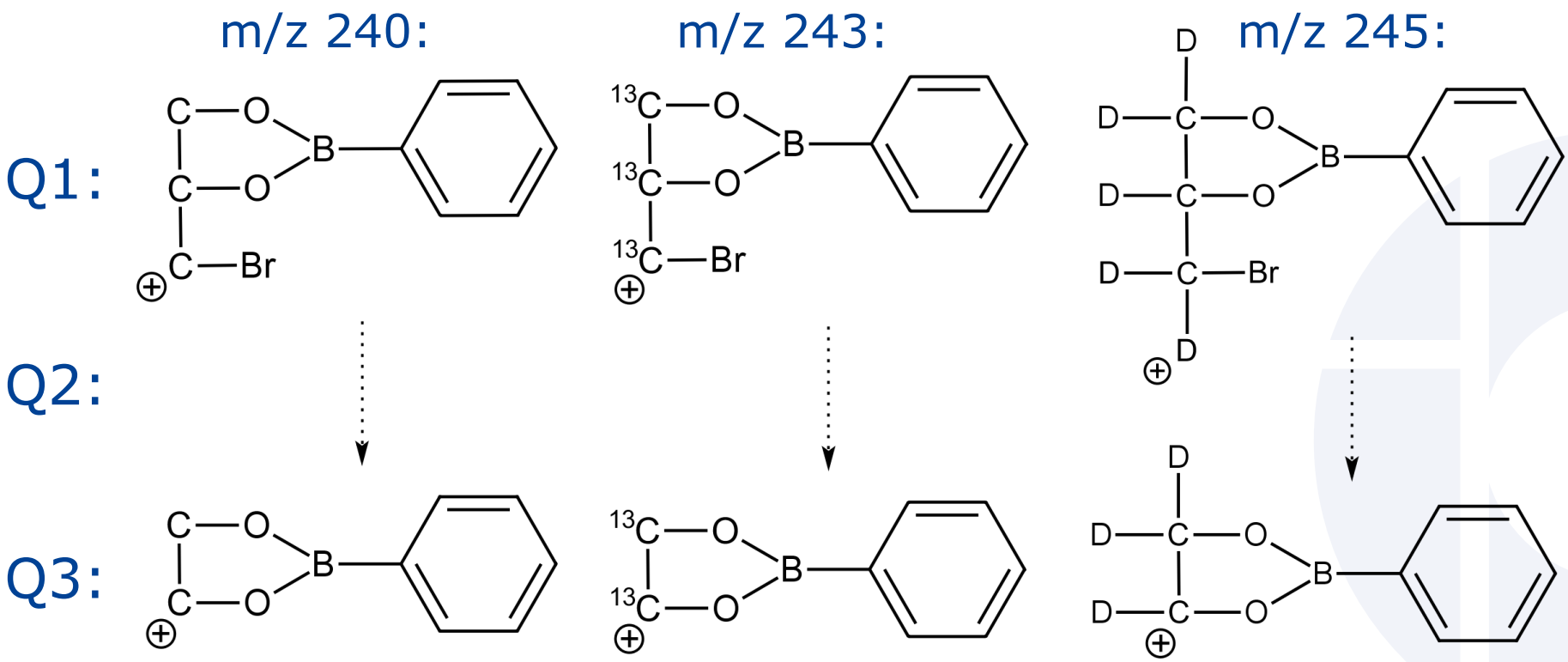


- At -25°C → No conversion
  (Kuhlmann, Eur. J. Lipid. Sci. Technol 2011, 113,335-344)
- Overestimation of Glycidylesters

Chemistry of the automated method



The ^{13}C-correction method



m/z 147:
Induced by:
- **Glycidol**
- **3-MCPD**

m/z 149:
Induced by:
- **3-MCPD-$^{13}C_3$**

m/z 150:
Induced by:
- **Glycidol-d_5**

Implicit Hydrogens have been omitted

The ^{13}C-correction method

- **Glycidol-$^{13}C_3$ Result (ppm) =**

$$\left(\frac{Area\ 3\text{-}MBPD\text{-}{}^{13}C_3}{Area\ 3\text{-}MBPD\text{-}d_5}\right) \cdot \text{glycidol-}d_5\ \text{amount (µg)} \cdot \frac{1000}{sample\ (mg)}$$

- **3-MCPD induced glycidol (ppm) =**

$$\left(\frac{\mathbf{3\text{-}MCPD\ Result}\ (ppm)}{\mathbf{C_{3\text{-}MCPD\text{-}}{}^{13}C_3}\ (ppm)}\right) \cdot \text{glycidol-}{}^{13}C_3\ \text{Result (ppm)}$$

- **Glycidyl ester induced glycidol (ppm) =**

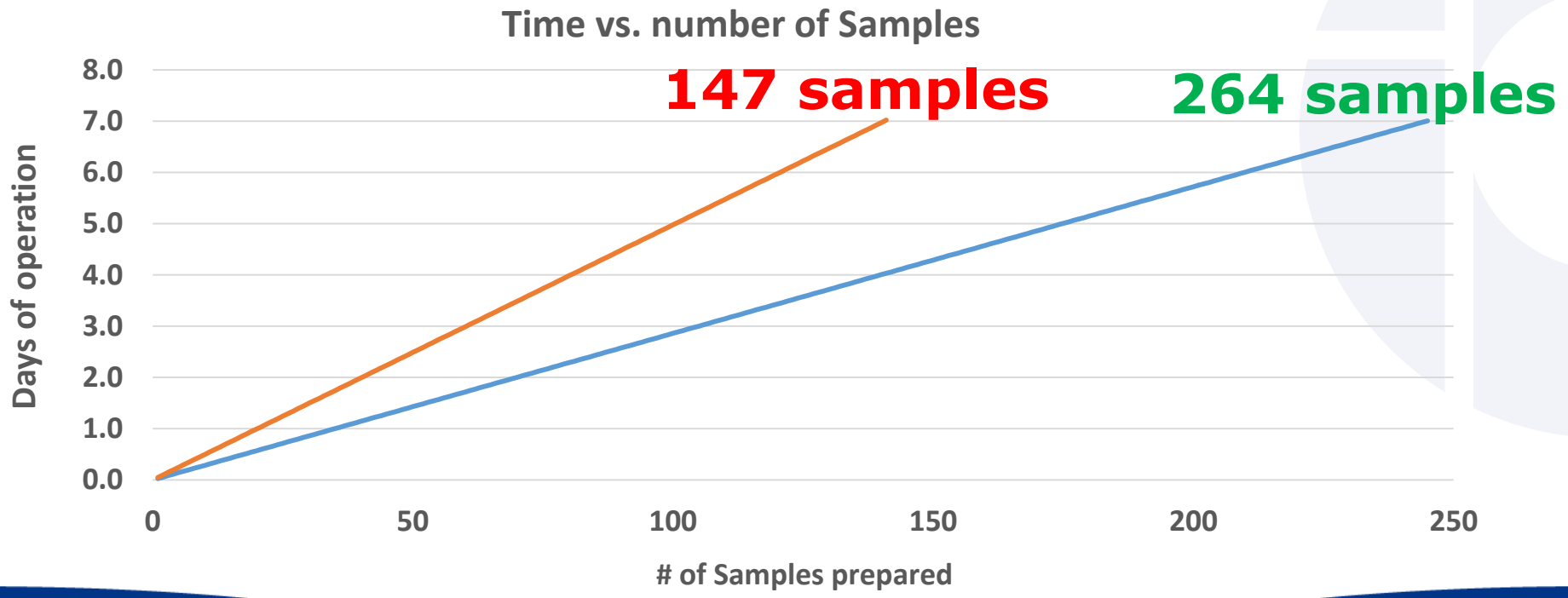
(Glycidol result – 3-MCPD induced glycidol) · Recovery factor

Optimization of Method

Step	First version	Second version
Sample	100-120 mg	100-120 mg
Solvents	100 µL internal standard 100 µL toluene	100 µL internal standard 100 µL toluene
Transest.	200 µL of 0.5M $NaOCH_3$/MeOH	200 µL of 0.5M $NaOCH_3$/MeOH
Glycidol Conversion	1000 µL 200g/L acidified NaBr sol.	1000 µL **650g/L** acidified NaBr sol.
FAME extraction	2x 600 µL hexane	2x 600 µL hexane
Derivatization	100 µL PBA solution 10 min at 80°C + shaking	100 µL PBA solution **No heat, vortexing 10s**
Extraction	600 µL of hexane	600 µL of hexane

Sample Capacity Increase

- Code fully re-written
- Customized Cooltray + Agitator, 6 Positions → 20 positions
- **32 samples / 24 hours: 44%** Faster than Original method

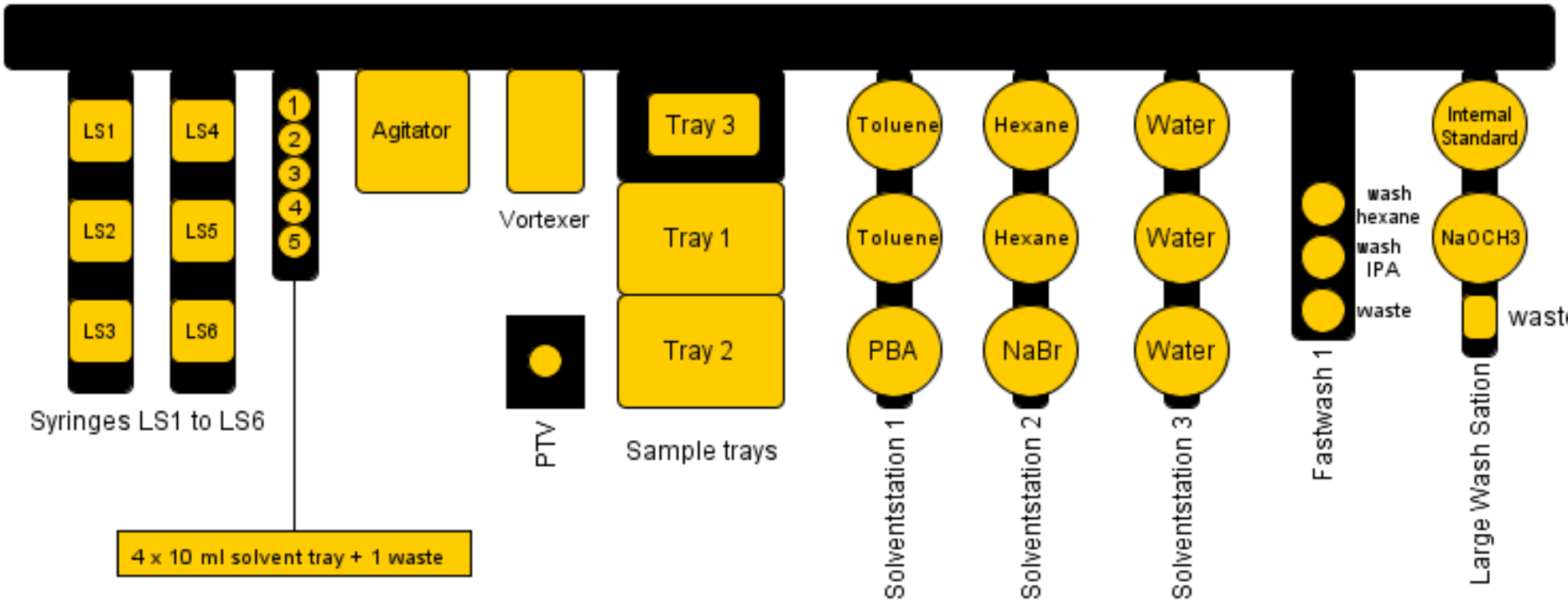


Sample Capacity Increase

	First Version	Second version
Spike samples	2	2
QC samples	1	1
Unknown samples	3	17
Prep time	2 hrs, 35 min	6 hrs, 50 min
GC analysis (inj to inj)	17 min	17 min
Total time	4 hrs, 35 min	12 hrs, 54 min
Per sample	69 min	38 min
Single Sample	n.a.	56 min

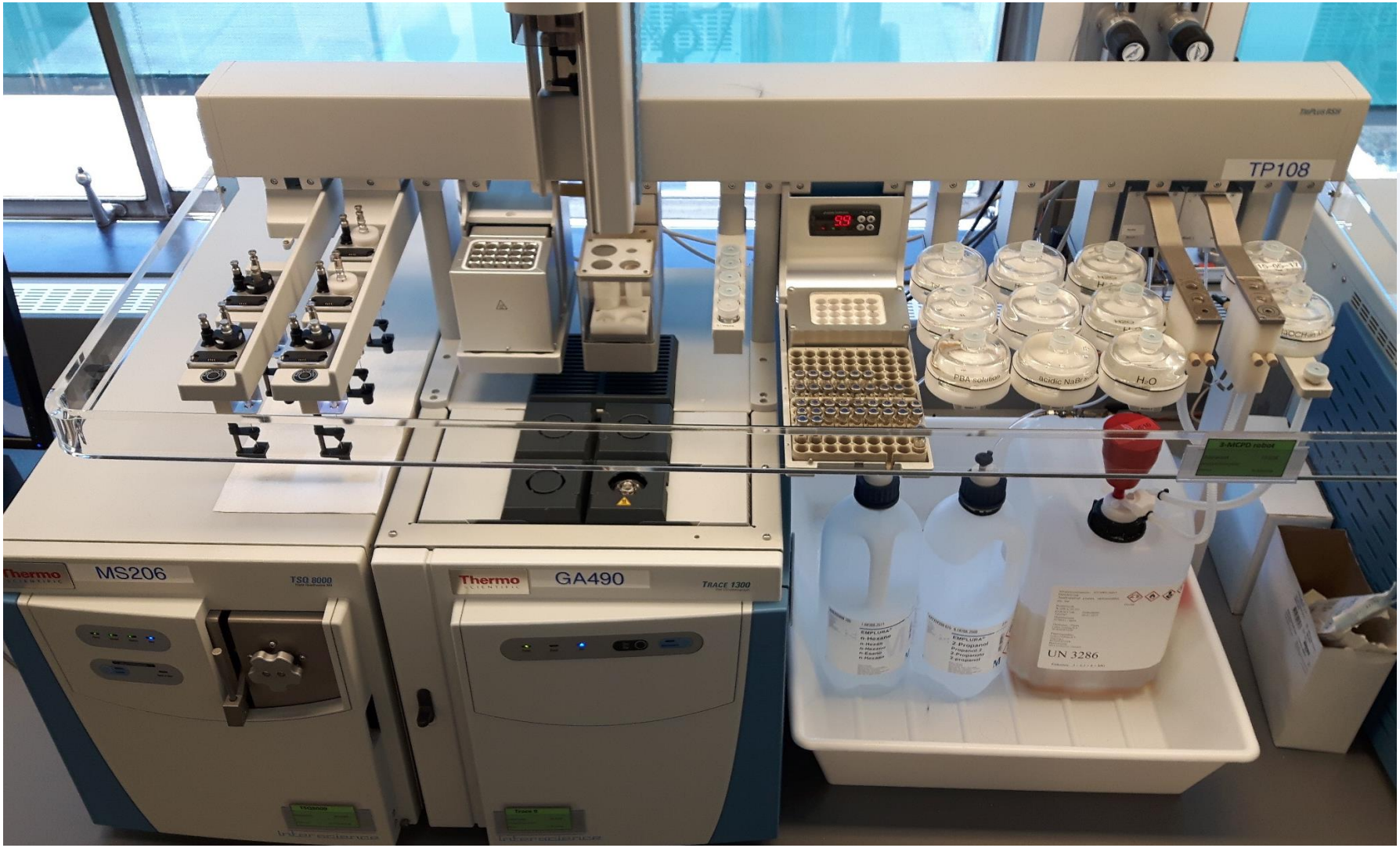
- Smart programming reduces reagent and time waste
- Samples are more clustered

Robot set-up



- Six automatically selectable syringes
- Three sample trays, one temperature controlled
- Agitator, vortexer and fast wash station

Robot set-up



Global Method Uniformity

- Production location
- Sales / Regional office
- Bulk oil terminal

Annual turnover > €1 billion

6 production locations / 9 sales offices

3 million MT of refining capacity / Over 1,000 employees

Global Control Sample

3-MCPDe (ppm)				
	avg	SD	n	remark
The Netherlands	0.55	0.02	97	
Malaysia	0.52	0.02	28	
USA	0.54	0.03	5	Insufficient data
SGS Hamburg	0.55	-	2	

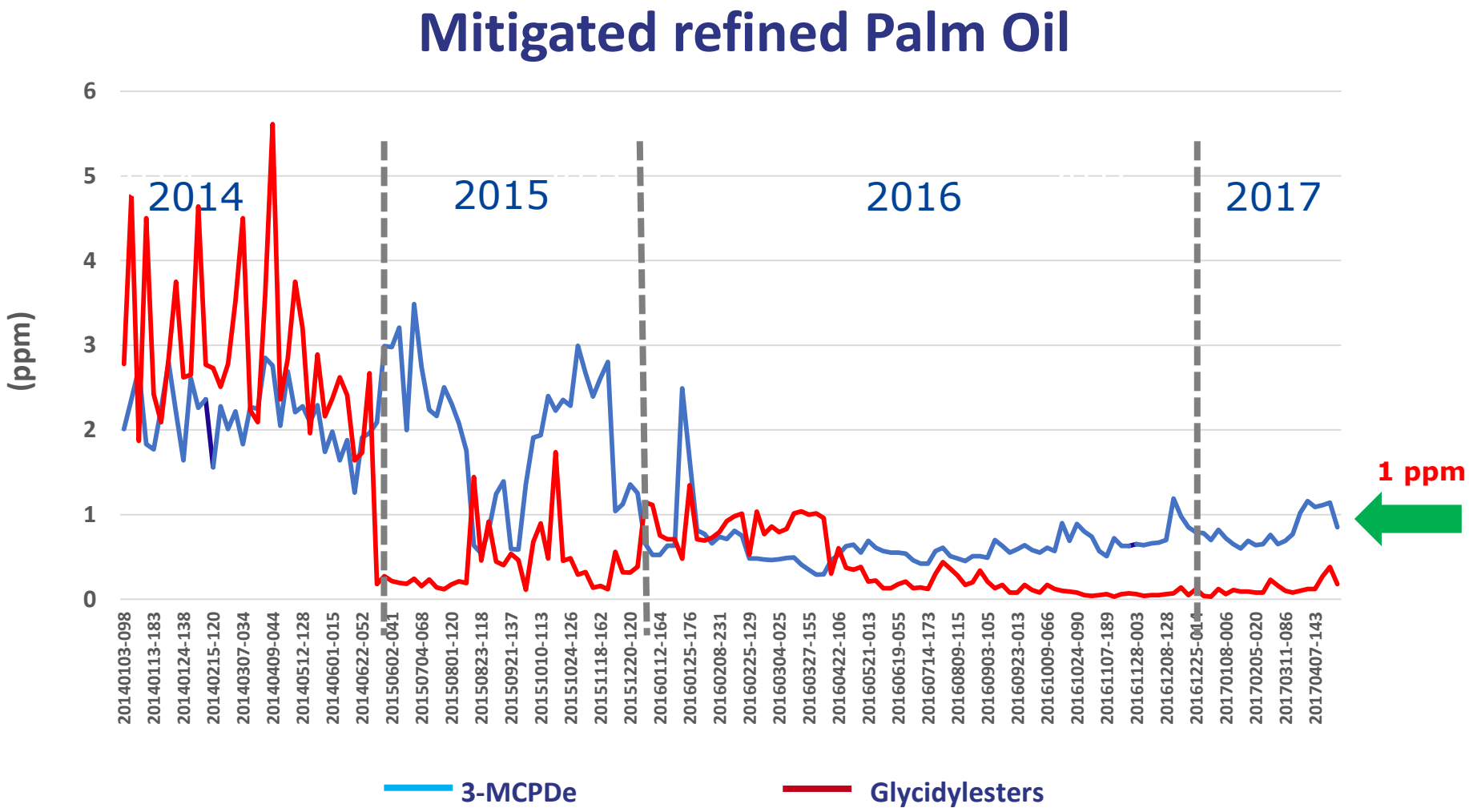
Glycidyl esters (ppm)				
	avg	SD	n	remark
The Netherlands	0.06	0.02	97	
Malaysia	0.07	0.02	28	
USA	0.03	0.03	5	Insufficient data
SGS Hamburg	<0.10	-	2	

Global Control Sample

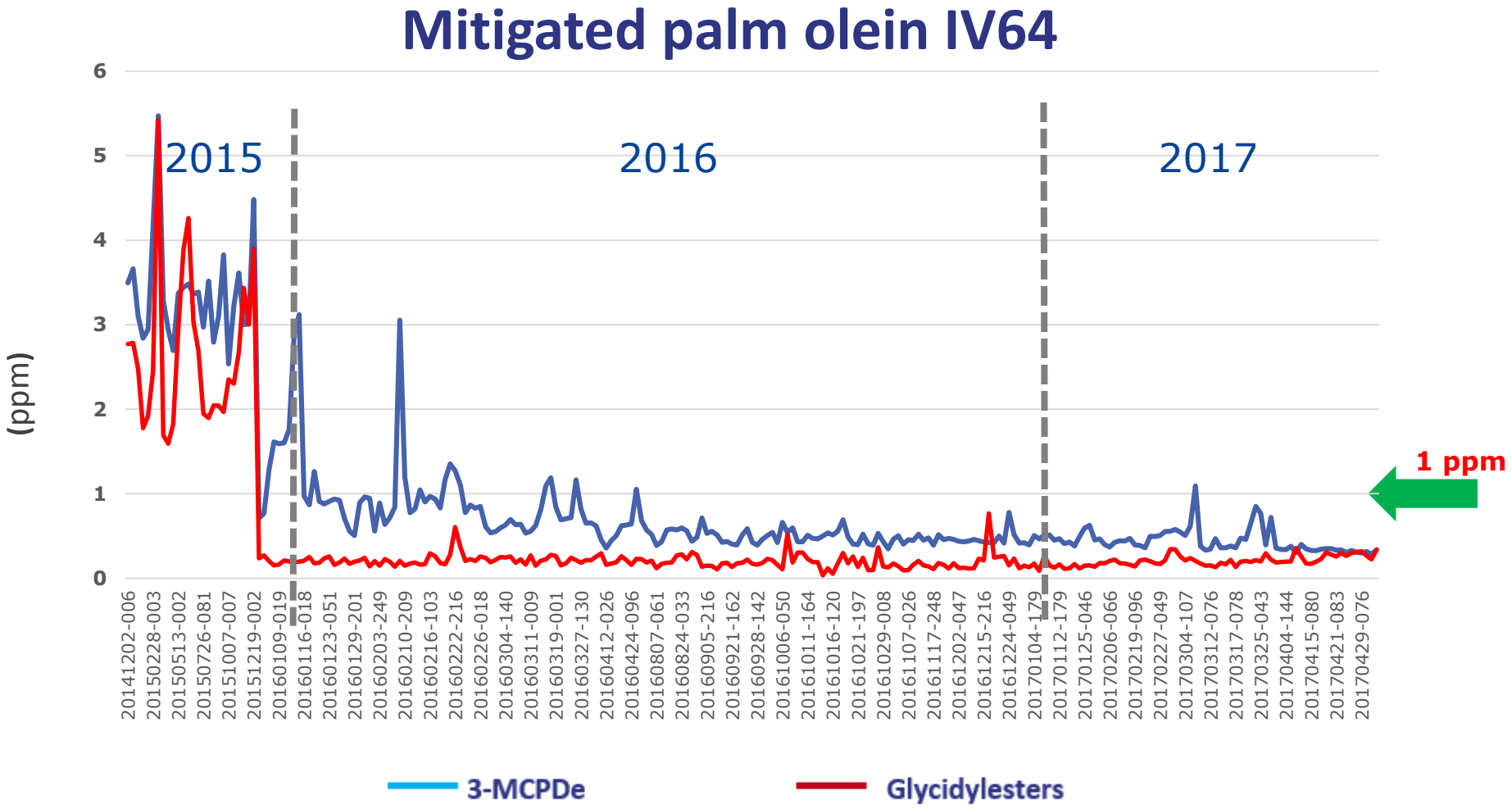
2-MCPDe (ppm)				
	avg	**SD**	**n**	**remark**
The Netherlands	0.30	0.02	97	
Malaysia	0.32	0.02	28	
USA	n.a.	n.a.	-	**Not reported**
SGS Hamburg	0.28	-	2	

- Initial comparison looks very good
- Not enough data for US yet to compare
- **Next: Extended Validation including all departments**

Practical Implications



Practical Implications



Further development

- Goal: Decreasing LOD/LOQ
  - Improving accuracy at current LOQ levels
  - Large Volume Injection (2µL → 50-100µL?)
  - Removal of large quantities of PBA from GC system

    → experimental injector
  - Impurities in internal standard might limit LOD

    → decrease internal standard to counteract contribution

    → cheaper
  - ***Development still ongoing***

Thank you for your attention