A Fully Automated Method For MCPD – and GE- Esters And The Importance of Glass Quality Of the used Autosampler Vials

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ABSTRACT AND INTRODUCTION

Monochloropropanediol (MCPDe) and glycidyl esters (GE) are the central point of attention of the food industry. Compounds are unwanted by-products of oil refinery, and predominately occur in palm oil, and great care should be taken in processing the oils. Nonetheless, any oil can contain MCPDe and GE when it is not processed with the utmost care. In the oil matrix, MCPDe and GE occur as esters, but they are analyzed after transesterification and derivatization in order to report total MCPD and GE contents.

In this poster an automated method will be presented based on a method from AOCSCd29c-13 or the so-called Zwagerman method showing excellent recoveries. One of the critical steps in the automation is the choice of autosampler vials. Vials with lower glass quality will have an effect on recovery as the esters tend to absorb at the free silanol groups on the glass walls.

When talking about vial quality, normally it is related to 1st hydrolytic class, the percentage of free silanol groups on the surface of the glass. The lower the amount, the better the glass quality and as less analytes will stick to it. This poster will show advantages of using vials with the lowest coefficient of mean linear thermal expansion. In theory the highest quality of vials is expected from 33 type vials, as the coefficient of mean linear thermal expansion is the lowest for the basic tubes used for vial manufacturing, as well as the hydrolytic resistance acc. To ISO 719, acid resistance acc. To DIN 12116 and alkali resistance acc. To ISO 695. The expansion coefficient actually describes the activity of the surface of the glass wall, which relates to the amount of free silanol groups present that can react with analytes and bind them to the glass surface.

Especially for MCPDe when they are put on the automation system before they are derivatized, the glass quality is a key factor for success. This is the most critical step, since at this point in the process, polarity of analytes plays a central role. During this step it is key to make sure, that they do not stick on the glass wall in order to reach the necessary detection limits.

An overview about the automation and the used systems, including glass vials, will be given, as well as a method utilizing a GC Triple Quadrupole for detection.

THE IMPORTANCE OF GLASS QUALITY

Today's vials in Europe are normally made of 1st hydrolytic class glass, according to all the different Pharmacopeias. But there are differences in the glass qualities meeting the Pharmacopeia requirement! A European 51 type glass (51 one percent of free silanol groups on the surface of the glass) is in specs, but for polar compounds, in ultra traces, the adsorption can be significant, up to 75% AND the value may vary from Lot to Lot or even from vial to vial!

A glass surface is never plain. There are particles, scratches and holes. There the polar analytes can stick. In figure 1. an enlargement of different glass surfaces is shown.

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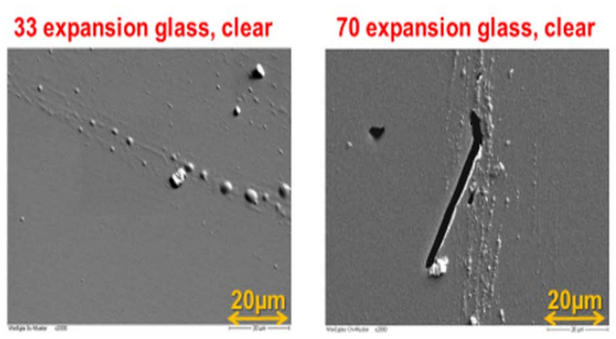
Thermo Scientific™ Chromacol™ GOLD grade vials are the solution. These vials meet every Pharmacopeia´s requirements and provide a significant better low adsorption surface compared to all standard vials on the market with no surface treatment.

Figure 1. Chromacol GOLD grade inert vials



MATERIALS AND METHODS

For the automated sample preparation a Thermo Scientific™ TriPlus™ RSH™ autosampler was used, equipped with a modified heated tray and programmed by SampleQ.

All samples were analyzed using a Thermo Scientific™ TRACE™ 1310 gas chromatograph with an I connect split/splitless injector able to perform backflush. Detection was performed with a Thermo Scientific™ TSQ™ 9000 MS/MS detector.

In the automation method is the complete sample preparation, but in addition, the calibration curve is made, the QC sample is being spiked and prepared and a blanc oil is also prepared

Data reviewing was performed by the Thermo Scientific™ Chromeleon™ software and the complete control of blanc, QC sample, calculations and all classic controls of GC-MS such as retention time, IS recovery and ion ratio's are being monitored automatically.

For separating the compounds a TraceGOLD TG-5SilMS GC Column, 30 m*0.23mmID *0.25um df was used, linked to a 2m precolumn of 0.53mmID.

The GC and the MS method are described in the tables below.

The automated sample preparation process is described in the schematics.

Sample preparation for the analist is limited to weighing in 100-150 mg of oil, and the final injection volume is 2 ul.

Parameter	
Initial Row	70° C
Initial Hold Time	1 min
Ramp 1 rate	15° C/min
Ramp 1 final temperature	120° C
Ramp 2 hold time	0.5 min
Ramp 2 rate	40° C/min
Final temp	350° C
Hold time	2.5 min
S/SL method	
S/SL mode	Splitless
Temperature	350° C
Splitless time	2 min
Split flow:	100 mL/min
Backflush time	6 min
Purge flow	5 mL/min
Carrier mode	Constant Flow
Carrier flow	1.7 mL/min
Vacuum compensation	On

Table 1. GC oven and injection method

	Quantifier	Qualifier
3-MCPD	196>147 @ 8 eV	198>147@ 8eV
3-MCPD-$^{13}C_3$	199>148@ 8eV	
2-MCPD	196>104@14eV	198>104@14eV
3-MBPD	240>147@ 8eV	242>147@8eV
3-MBPD-$^{13}C_3$	243>149@8eV	
3-MBPD-d5	245>150@8eV	247>150@8eV

Table 2. MS Settings

Figure 2. Thermo Scientific RSH Robotic Sampler

3-MCPD-13C3 correction method

Alkaline conditions cause a fraction of the 3-MCPD to convert to glycidol. If this is not corrected the formed glycidol will be overestimated and the results will not be correct. Adding
3-MCPD 13C ester as internal standard will allow quantifying glycidol-13C formed during the reaction, therefor correcting the glycidol result.

Please note that during the transesterification step glycidol is converted to MBPD.

Figure 3. Transitions of native and labelled compounds

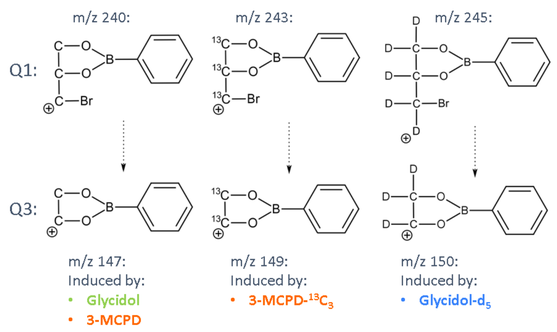
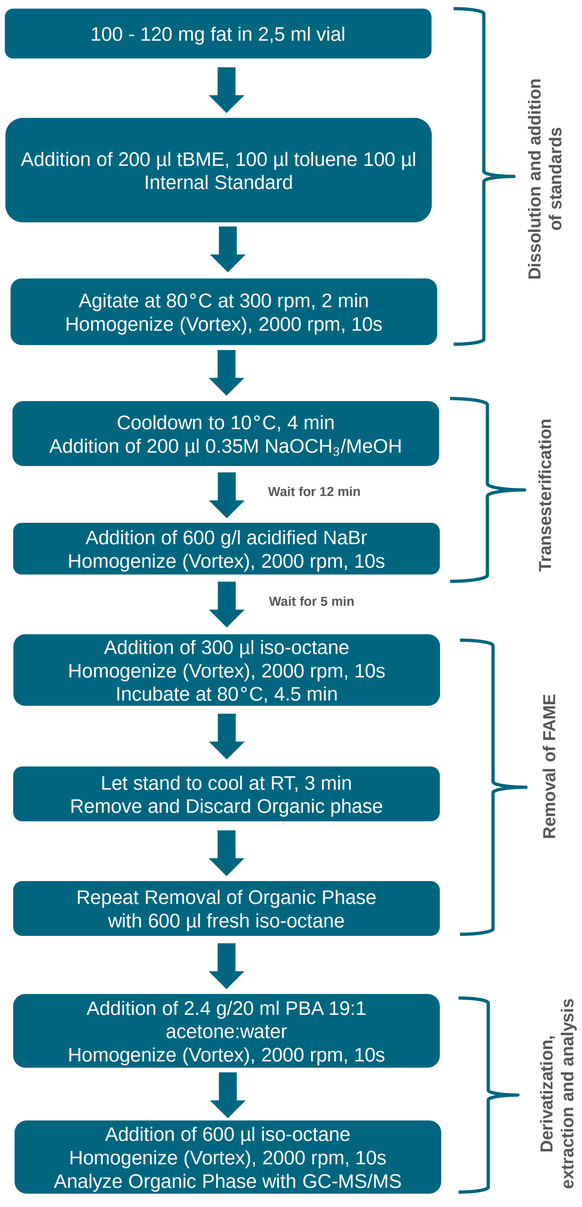


Figure 4. Sample preparation scheme



RESULTS AND DISCUSSION

LOD/LOQ

For determining the detection and quantification limits (LOD/LOQ) extra virgin olive oil was prepared 10 times with the MCPD-robot for determining the blanc levels and standard deviations. The same oil was subsequently spiked with reference material 5 times at concentrations between 1 and 50 ppb. Each level was therefor prepped and analyzed 5 times and compared to the blanc virgin olive oil.

For 3-MCPD the LOD was determined at 10 ug/kg and the LOQ at 15 ug/kg; glycidol LOD was 18.5 ug/kg and LOQ 41 ug/kg, finally the 2-MCPD LOD was 2.7 ug/kg and LOQ was 6.9 ug/kg.

Figure 5. Chromatogram of all analytes at 250 ug/kg

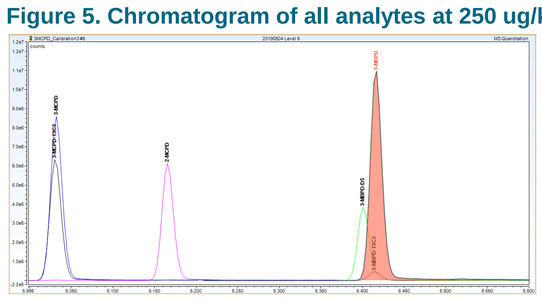
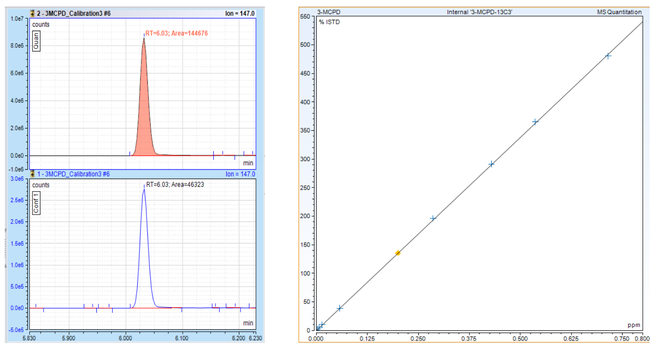
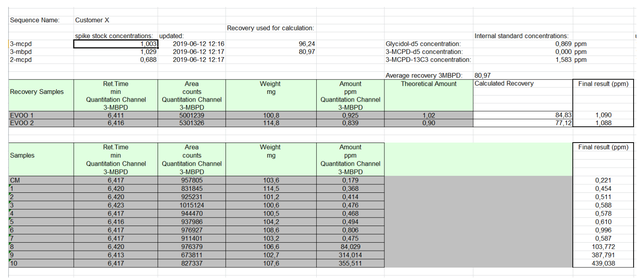


Figure 6. Chromatogram of both transitions of 3 MCPD and the calibration curve from 10 to 500 250 ug/kg



CALCULATION

All data reviewing, calculations and reporting are performed using Chromeleon software. Also the weight of the sample is included so the results are immediately reported as concentration in the samples, not in the extracts. Quality checks such as recovery, QC control, blanc values, calibration curves and ion ratio's are included in the report section of Chromeleon for fast data reviewing without compromising the quality of the results.



Sequence Name: Customer X

Recovery used for calculation:

spike stock concentrations: updated: — Internal standard concentrations:

3-mcpd	1,003	2019-06-12 12:16	96,24	Glycidol-d5 concentration:	0,869 ppm
3-mbpd	1,029	2019-06-12 12:17	80,97	3-MCPD-d5 concentration:	0,000 ppm
2-mcpd	0,688	2019-06-12 12:17		3-MCPD-13C3 concentration:	1,583 ppm

Average recovery 3MBPD: 80,97

Recovery Samples	Ret.Time min Quantitation Channel 3-MBPD	Area counts Quantitation Channel 3-MBPD	Weight mg	Amount ppm Quantitation Channel 3-MBPD	Theoretical Amount	Calculated Recovery	Final result (ppm)
EVOO 1	6,411	5001239	100,8	0,925	1,02	84,83	1,090
EVOO 2	6,416	5301326	114,8	0,839	0,90	77,12	1,088

Samples	Ret.Time min Quantitation Channel 3-MBPD	Area counts Quantitation Channel 3-MBPD	Weight mg	Amount ppm Quantitation Channel 3-MBPD	Final result (ppm)
CM	6,417	957805	103,6	0,179	0,221
1	6,420	831845	114,5	0,368	0,454
2	6,420	925231	101,2	0,414	0,511
3	6,423	1015124	100,6	0,476	0,588
4	6,417	944470	100,5	0,468	0,578
5	6,416	937986	104,2	0,494	0,610
6	6,417	976927	108,6	0,806	0,996
7	6,417	911401	103,2	0,475	0,587
8	6,420	976379	106,6	84,029	103,772
9	6,413	673811	102,7	314,014	387,791
10	6,417	827337	107,6	355,511	439,038

Figure 7. Example of Chromeleon data reviewing

CONCLUSIONS

The approach for MCPD and GE analysis enables

- Limiting the manual labor to weighing in the sample
- Analyzing a full scope of the MCPDe and GE in one single injection
- Short reaction time without evaporation
- Short analysis time
- GE, 2-MCPDe and 3-MCPDe are measured
- High productivity: 40 real samples per 24hrs from prep-to-rep to 80 samples per 24hrs in off-line mode
- Automated calibration curves and addition of internal standards and automated QC control
- Automated calculation, data evaluation and LIMS export
- Complete sequencing, data reviewing and reporting in one single software.

REFERENCES

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