

Report

Feasibility study concerning the differentiation of mineral oil hydrocarbons from recycled cardboard and oligomers from resins via multidimensional chromatography

Purchaser: HARRPA (sector group of Cefic)

Outline

Throughout this study, the feasibility of the differentiation of hydrocarbons originating from mineral oils or tackifier resins shall be shown by using two different analytical techniques. On the one hand, the on-line HPLC-GC-FID method for determining mineral oils according to Biedermann et al. (2009)¹ is applied, which is the method of choice for routine analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) according to the BfR-compendium. On the other hand, comprehensive GC (GCxGC-MS/FID) is used as another multidimensional chromatographic technique to characterize hydrocarbon mixtures in detail. The GCxGC method from Biedermann and Grob (2015)² is adapted. The reference samples are contaminated cereals (wheat flakes), which were prepared in the laboratory under controlled conditions (migration via gaseous phase).

Principle

The sample extracts were analysed for saturated hydrocarbons ($\leq C_{24}$), which were eluted in the MOSH fraction, by online HPLC-GC-FID and GCxGC-MS/FID. The quantification of substances and the verification of the HPLC-fractions were done by the help of internal standards. For simplification and comparability of the hump/peak integration, the 'inaccurate' approach excluding all single peaks on top of the hump (e.g. n-alkanes) was used, which is generally applied in routine laboratories. The molecular range of C₁₆-24 refers to the retention times of n-C₁₆ and n-C₂₄.

Experimental

Sample preparation

For the preparation of the reference samples, non-contaminated wheat flakes obtained from a food manufacturer were used (MOSH fraction ≤ 0.1 mg/kg).

- 1) Cereals (wheat flakes) contaminated with mineral oil hydrocarbons from recycled cardboard: 500g of the sample were stored in a recycled cardboard folding box (7dm²) for 12 months at 20°C

¹ Biedermann et al. (2009) J. Agric. Food Chem. 57, 8711

² Biedermann & Grob (2015) J. Chromatogr. A 1374, 146

- 2) Cereals (wheat flakes) contaminated with resin oligomers from a hydrogenated hydrocarbon resin (C9-type; R1100 from Eastman): 10g of the sample were stored in a migration cell with 1g of hydrocarbon resin (pre-melted for 5min at 150°C) for 10d at 60°C
- 3) The mixture of both hydrocarbon contaminants was prepared by mixing the extracts of 1) and 2) in a ratio of 25:75

Internal Standards

Abbreviations, Supplier and functions of Internal standards

Substance	Supplier	Function
n-Undekane (C11)	Fluka	Low-boiler
Cyclohexylcyclohexan (Cycy)	Fluka	Quantification MOSH-fraction
n-Tridekane (C13)	Fluka	Quantification MOSH-fraction
Cholestane (Cho)	Fluka	End of MOSH-fraction
Pentylbenzene (5B)	Fluka	Low-boiler MOAH-fraction
1-Methylnaphtaline (1MN)	Fluka	Quantification MOAH-fraction
2-Methylnaphtaline (2MN)	Fluka	Quantification MOAH-fraction
Tritertbutylbenzene (TBB)	Fluka	Start MOAH-fraction
Perylene (Per)	Fluka	End of MOAH-fraction

The concentrations in the internal standard solution (solvent: toluene) was 300 mg/l for C11, Cycy, 5B, 1MN, 2MN, TBB; 150 mg/l for C13; 600 mg/l for Cho, Per.

Solvents

All solvents used for analysis were GC-MS grade supplied by Merck.

Extraction of cereals

- 10g of the sample were extracted with 20 ml n-hexane (+ 10 µl internal standard solution) for 24h at ambient temperature

HPLC-GC-FID

The supplier of the HPLC-GC-FID system was Brechbuehler AG (Thermo Scientific). 10–90 µl of the sample extract were injected into the HPLC. The normal phase HPLC (silica gel) was used for the separation of saturated (MOSH fraction) and aromatic hydrocarbons (MOAH fraction). Polar components like triglycerides remained on the HPLC-column and were eluted into the waste during the backflush of the column. No interference with MOSH and MOAH occurred. The fraction of interest was transferred (on-line) into GC-FID or collected into a vial for GCxGC analysis.

HPLC setup

Column: Lichrospher Si60 (5µm, 250x2mm, Merck)

Flow: 0.3 ml/min (backflush 0.5ml/min)

Gradient: Eluent A – n-hexane, 100% 0.0-0.1 min, 70% 0.1-6 min, 100% 15-35 min
 Eluent B – dichloromethane, 30% 0.1-6 min, 100% 6-15 min
 Backflush 6-15 min

Transfer: MOSH fraction 2.0 – 3.5 min
 MOAH fraction 3.5 – 5.0 min

GC setup

Column: 7m x 0.53mm ID uncoated precolumn and 15m x 0.25mm ID ZB-1 separation column (Phenomenex)
Injection: On-column (Y-piece)
Carrier: Hydrogen
Pressure: 60 kPa (90 kPa during concurrent solvent evaporation)
Program: 55°C (5 min) – 20°C/min – 350°C (5.0 min)
FID: 360°C, 350 ml/min air, 35 ml/min hydrogen, 20 ml/min nitrogen (make-up)

Further information:

On-line coupled high performance liquid chromatography – gas chromatography (HPLC-GC) for the analysis of mineral oil; Part 1: method of analysis in foods, environmental samples and other matrices. A review; K. Grob, M. Biedermann; Journal of Chromatography A 1255 (2012) 56

GCxGC-MS/FID

The supplier of the GCxGC-MS/FID system was Brechbuehler AG involving a Trace GC Ultra (Thermo Scientific), Zoex-2 modulator (Zeox) and Bench-TOF-MS (Almsco). 8 µl of the reconcentrated sample obtained from a previous HPLC fractionation (MOSH fraction) were injected into the GCxGC twice (FID and MS run). The compounds had been eluted first on a polar column (1st dimension) and were trapped in a loop by a cold-jet. After the release by a hot-jet after a certain modulation time, the substances were separated by a non-polar column (2nd dimension) before detection. For quantification Cycy was used as most robust peak and not affected by coelution

GCxGC setup

Columns: 0.2m x 0.53mm ID uncoated precolumn, 15m x 0.25 mm ID OV-17 (0.15µm film; 1st column) and 1.3m x 0.15mm ID PS-255 (0.05 µm film; 2nd column)
Injection: On-column (PTV splitless)
Carrier: Hydrogen, 60 kPa (FID run); Helium, 1.5 ml/min (MS run)
Program: 50°C (3 min) – 5°C/min – 310°C (1.0 min); modulation time 6s
FID: 350°C, 350 ml/min air, 35 ml/min hydrogen, 20 ml/min nitrogen (make-up)
MS: 40 – 800 amu at 40Hz

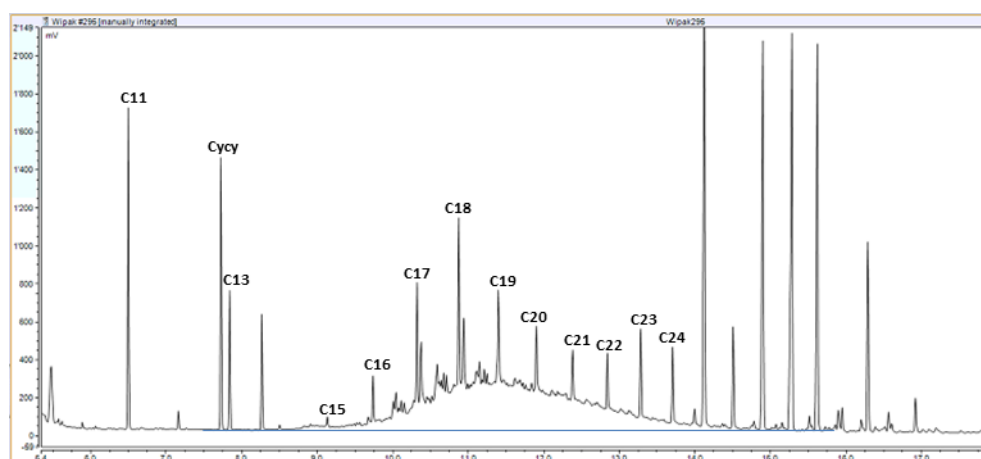
Further information:

Comprehensive two-dimensional gas chromatography for characterizing mineral oils in foods and distinguishing them from synthetic hydrocarbons; M. Biedermann, K. Grob; Journal of Chromatography A 1375 (2015) 146

Results

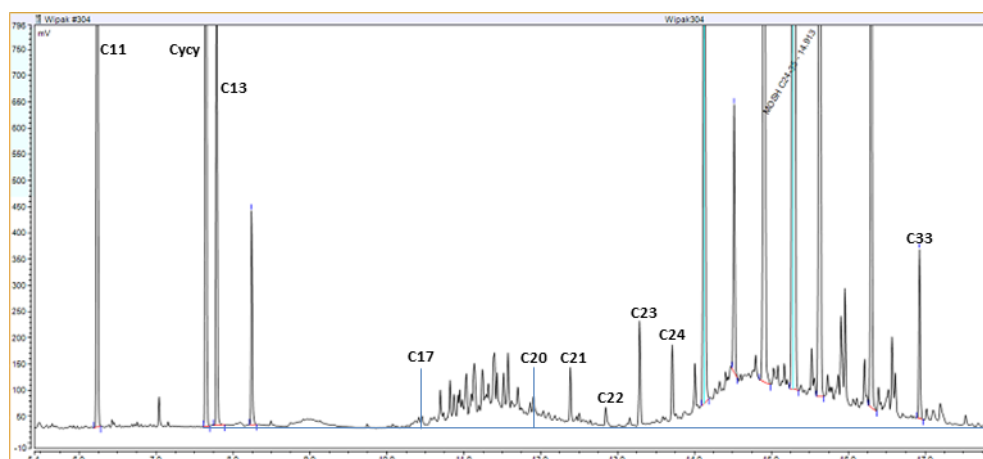
HPLC-GC-FID

The HPLC-GC-FID analysis showed a typical MOSH hump for the cereals contaminated with MOH from recycled cardboard. The migration occurred via gaseous phase, which is displayed by the molecular weight distribution ($\leq C24$). The position of the hump in the chromatogram is between C15 and C24 with a maximum at C19. n-Alkanes from C15-24 (sharp peaks on top of the hump) and pristane/phytane (C17/18) are also characteristic for a MOSH contamination from recycled cardboard.



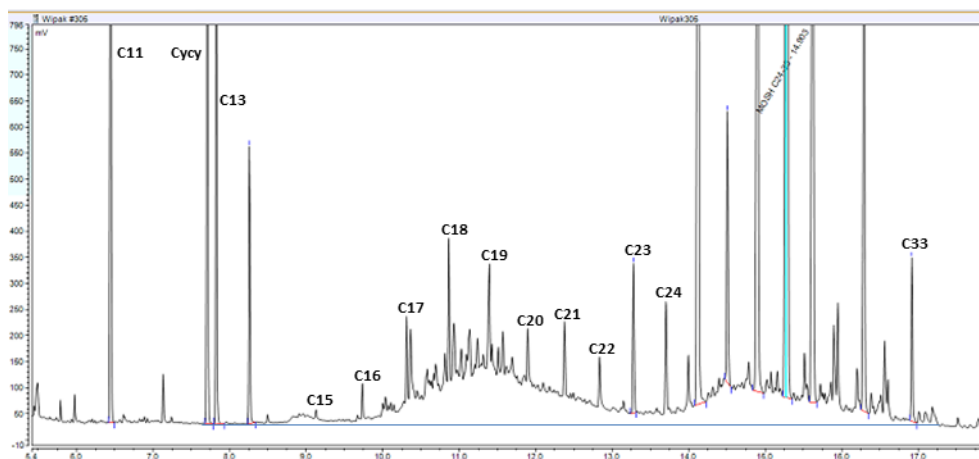
*MOSH fraction cereals contaminated with MOH from recycled cardboard
Concentration C16-24: 9.0 mg/kg*

The cereals contaminated with resin oligomers from a hydrogenated hydrocarbon resin (C9-type) showed two humps of unresolved hydrocarbons in the MOSH fraction. The first hump connected to the migration via gaseous phase ranges between C17 and C21 with a maximum at C18/19. Contrary to the previous shown MOSH hump, the molecular distribution of the oligomer hump is tighter and the n-alkane series is missing. The contamination with oligomers show also own specific sharp peaks of the main structures on top of the hump (ridge). The second hump ranging from C24 until C33 is attributable to an increased transfer at 60°C and is not displaying a real migration via gaseous phase (limited to $\leq C24$). Thus, this hump is not considered for the further investigation.



*MOSH fraction cereals contaminated with resin oligomers
Concentration C16-24: 0.7 mg/kg*

The mixture of both contaminants (MOSH from recycled cardboard and resin oligomers; ratio 25:75) shows also a hump regarding migration via gaseous phase ($\leq C_{24}$). The hump is similar to a typical MOSH contamination from recycled cardboard (shown before) due to the molecular distribution (C_{16-24}) and the corresponding n-alkane series. But with a closer look, a slight change of the hump shape can be observed concerning the maxima and the steeper increase/decrease of the hump. The typical ridge of the oligomer hump between C_{18} and C_{20} can also be noted. However, a quantitative differentiation of both contaminations is not feasible with one-dimensional GC. At least, there are some hints to recognize a mixture of hydrocarbons from different sources in routine analysis using HPLC-GC-FID. A further comprehensive GC analysis would provide more separation power and structural characteristics, respectively.

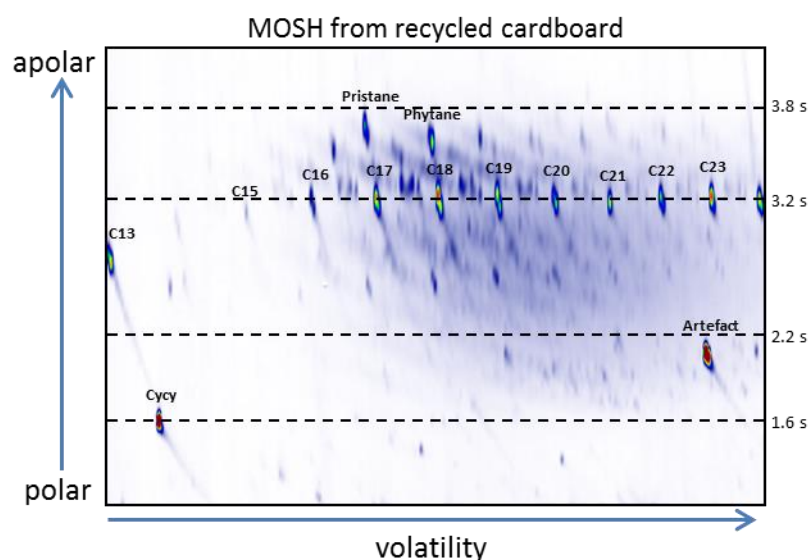


*MOSH fraction mixture of MOSH and resin oligomers
Concentration C16-24: 2.5 mg/kg
(2.0 mg/kg due to MOSH; 0.5 mg/kg due to resin oligomers)*

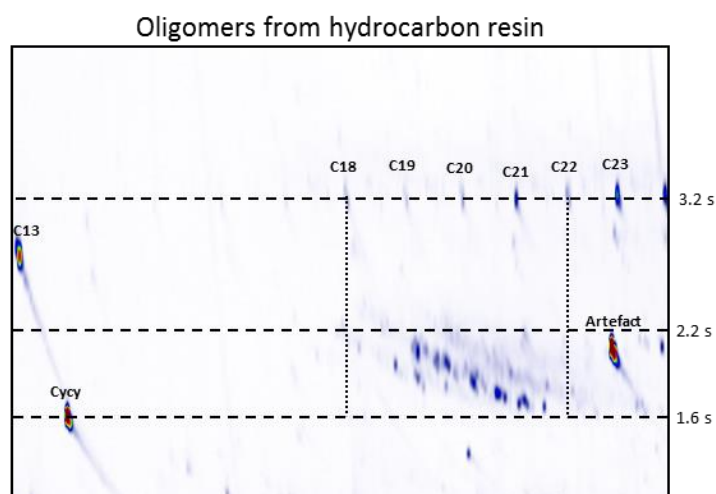
GCxGC-FID/MS

Comprehensive GC is a versatile tool to increase the separation power for mixtures. In this case the reversed setup (1st column: polar; 2nd column: apolar) was chosen to separate saturated hydrocarbons. The navigation in the 2-D plot can be done by the help of the axis: y-axis displays the polarity (from polar to apolar, bottom to top of the plot) and x-axis shows the volatility (from volatiles to semi-volatiles, left to right of the plot). Furthermore the intensity of each peak is displayed by the colour (increase from blue to green to red).

The GCxGC analysis of the cereals contaminated with MOSH from recycled cardboard showed a typical picture. At the top of the plot highly branched compounds, such as pristane and phytane followed by the n-alkane series can be observed. Below the n-alkane line at 3.2s in the 2nd dimension, an unresolved cloud of naphthenic MOSH extends until ca. 1.6s, which is also the ordinate value for the Cicy peak. The quantification of the MOSH contamination by GCxGC-FID leads to similar results compared to routine method (roughly 9 mg/kg, C_{16-24}). In this case, only 4% of the MOSH eluting earlier than 2.2s in the 2nd dimension (0.4 mg/kg).

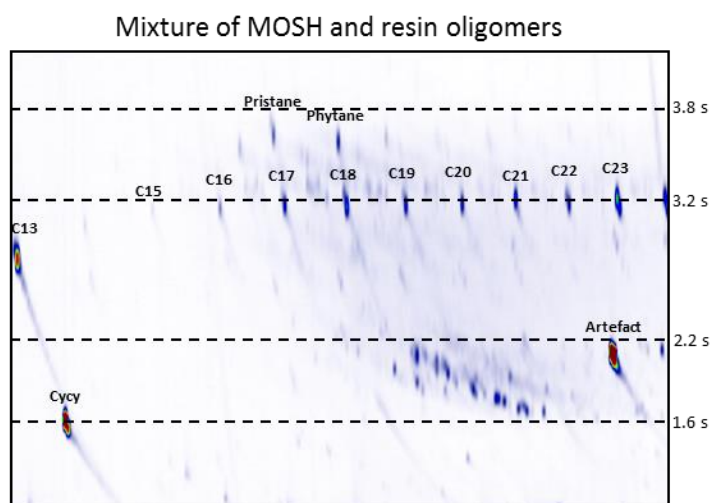


The results of the comprehensive GC analysis of the cereal sample contaminated with oligomers from a hydrocarbon resin show a different picture. The majority of the saturated hydrocarbons (>90%) can be observed in the ordinate range between 1.6 and 2.2s. This effect can be explained by the number of rings ($n \geq 3$) according to Lommatzsch et al. (2016)³, which increases the polarity of the compounds and subsequently decreases the elution time in the 2nd dimension. The oligomers were eluted between n-C18 and n-C22 in the 1st dimension, which is a shift of C1 compared to the findings obtained from HPLC-GC-FID due to a different separation column. The main oligomers are C18- and C19-type (C9-dimers) according to the mass spectra. The quantification of the oligomer contamination by GCxGC-FID lead to similar results compared to routine method (0.6 mg/kg, C16-24). The early elution time in the 2nd dimension will allow a further differentiation of the resin oligomers to MOSH in qualitative and quantitative way. The share of MOSH (<5%) eluting in this region is low and probably negligible.



³ Lommatzsch et al. (2016) Food Addit. Contam. A 33, 473

The test mixture containing MOSH and resin oligomers show a fusion of the previous shown 2D-plots. Most of the resin oligomers can be observed in the region between 2.2 and 1.6s like expected. Thus, a qualitative differentiation of the resin oligomers from MOSH can be performed visually by the help of the retention times. A quantitative determination of the proportion of hydrocarbons from each source can be done as well. The measured values (2.0 mg/kg MOSH & 0.5 mg/kg oligomers) meet the calculated values (2.3 mg/kg MOSH & 0.5 mg/kg oligomers) in this case. In general, a proper quantification of the different hydrocarbon groups is depending on the concentration and the limit of detection, respectively.



The mass spectra obtained from the GCxGC-MS analysis of the oligomers from the hydrogenated hydrocarbon resin (C9-type) show that the data can be used for a further differentiation. This approach could be necessary, if co-elution or an interference with other hydrocarbon mixtures occur during comprehensive GC analysis.

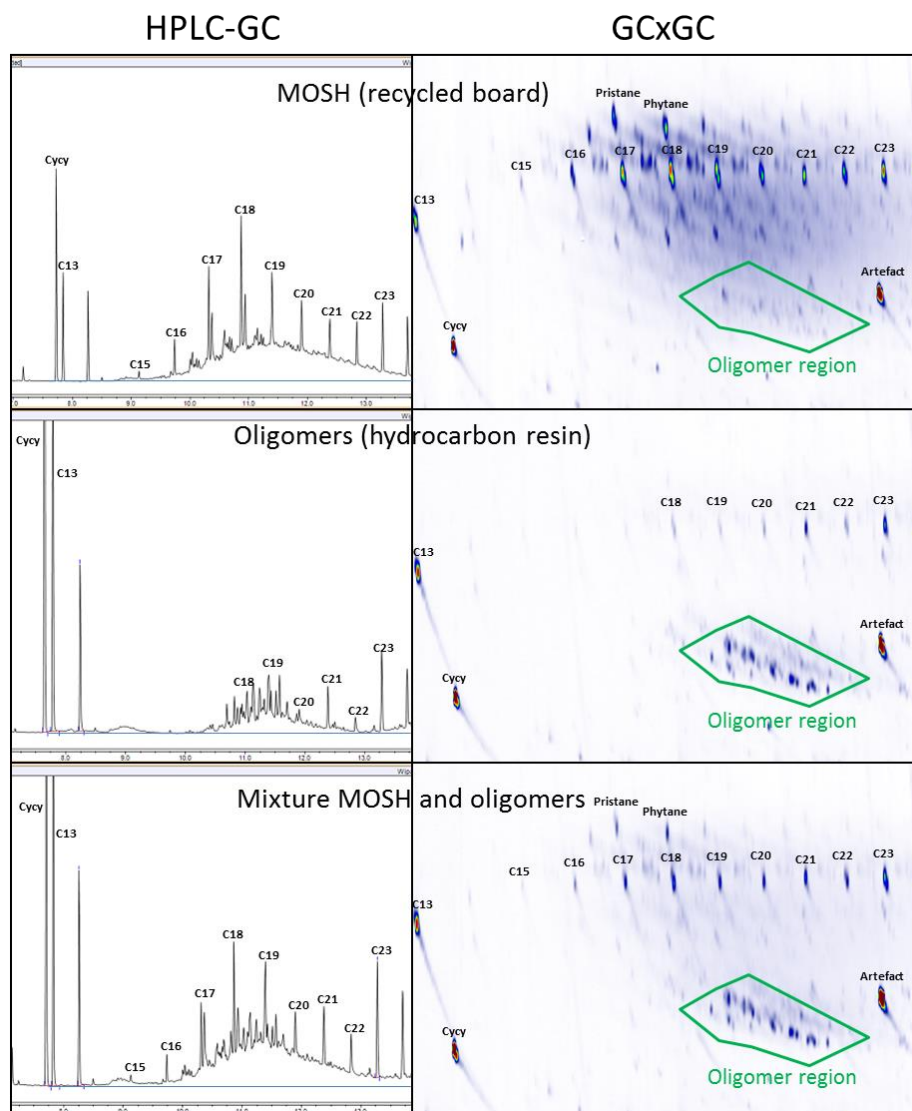
Fragments 121, 122, 135 and 150 m/z seem to be most promising to differentiate these C9-oligomers to MOSH from recycled cardboard. In fact, the SIM mode offers the possibility to visualize only these oligomers by using the mentioned fragments. Examples for mass spectra and SIM mode plots can be found in the appendix.

Summary and Conclusion

The feasibility of differentiating MOSH migrated from recycled cardboard and oligomers migrated from hydrogenated hydrocarbon resin (transfer via gaseous phase) was shown throughout this study. The HPLC-GC-FID method can be used for an identification and quantification of both hydrocarbon types as a pure contamination. This method, which is generally intended to determine saturated and aromatic hydrocarbons, is limited for mixtures of hydrocarbons from different sources. This means that the concentrations of hydrocarbons in the different fractions can be determined, but a qualitative evaluation of their sources is hardly feasible. In this case, it was demonstrated that oligomers from hydrocarbon resins can be misinterpreted as MOSH and could overestimate the measured "MOSH"-concentration. Since this method is used for the routine analysis of MOSH/MOAH, some samples could lead to false positive results regarding the origin of the measured hydrocarbons. A further

analytical technique should be applied, if a definitive identification was impossible and hints regarding a cross-contamination were noted.

Comprehensive GC is able to differentiate these two types of hydrocarbons due to their structural moieties and elution time in the 2nd dimension, respectively. In this case, a quantitative determination of the different shares/concentrations of MOSH and resin oligomers can also be performed.



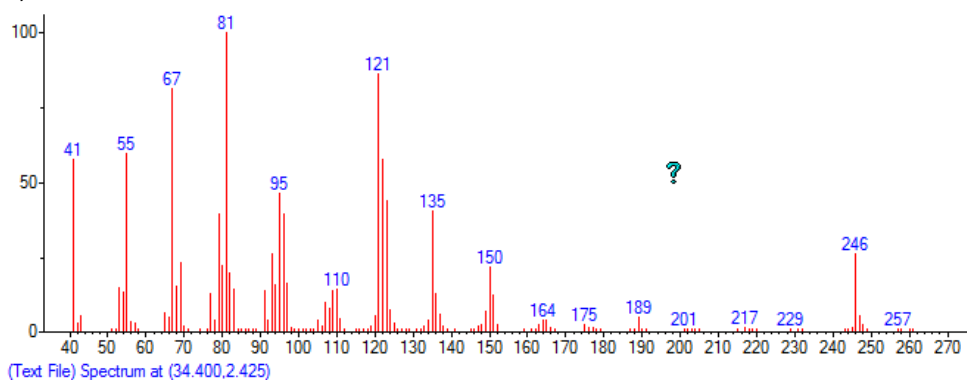
Cologne, 7th April 2017

Martin Lommatzsch

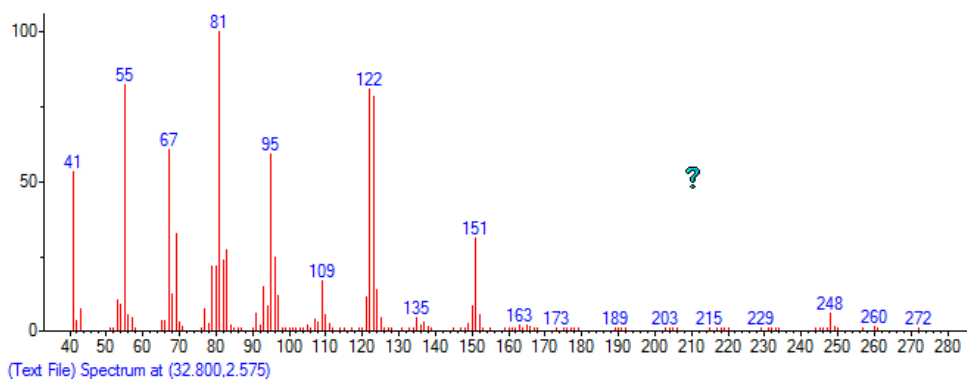
Appendix

Exemplary mass spectra of oligomers from the hydrogenated hydrocarbon resin (C9-type)

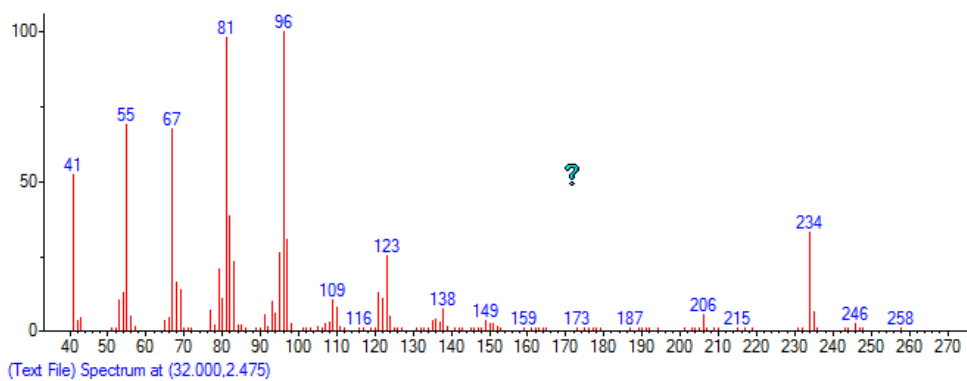
1)



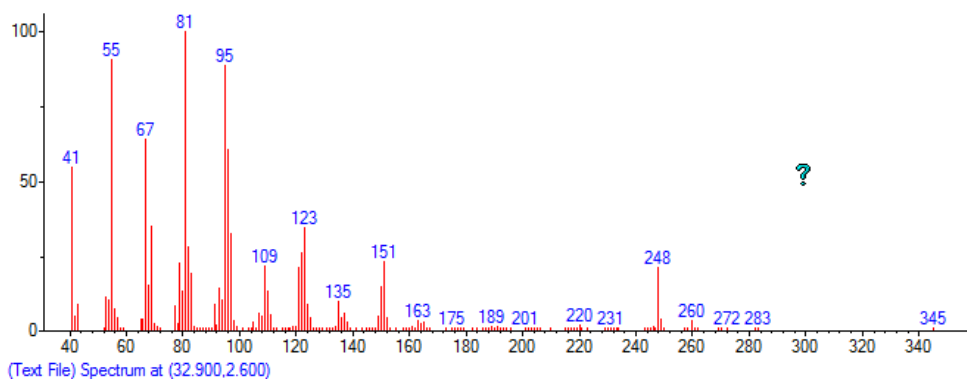
2)



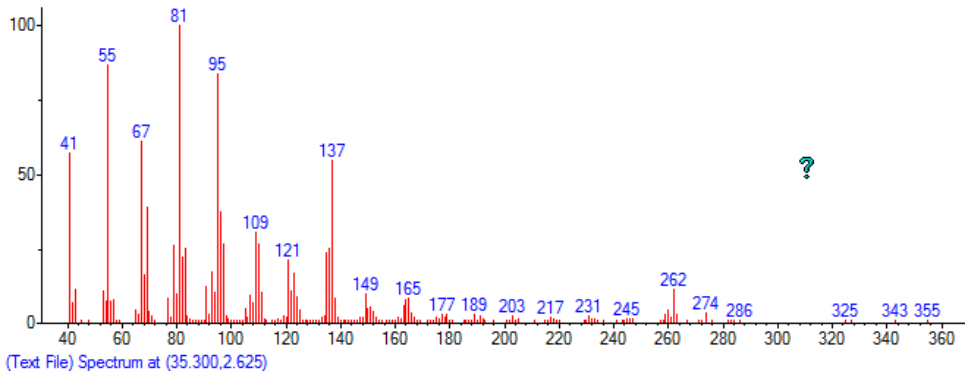
3)



4)

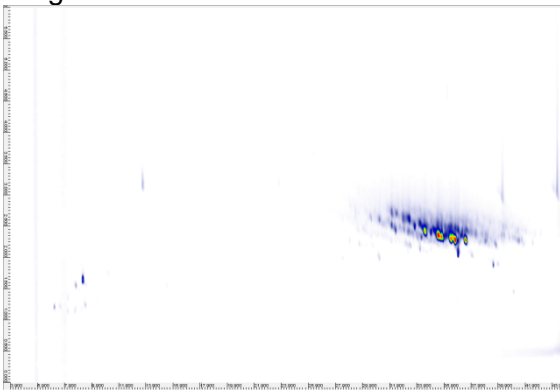


5)

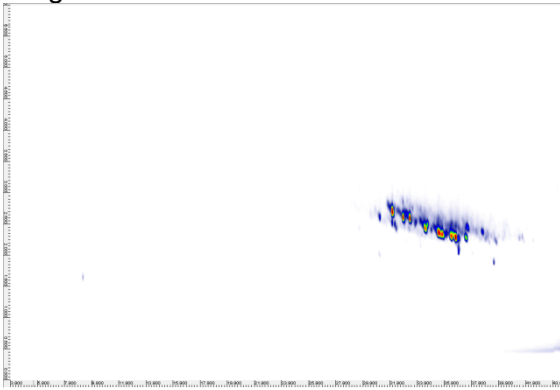


Exemplary SIM mode plots of sample 3 (mixture of MOSH and resin oligomers)

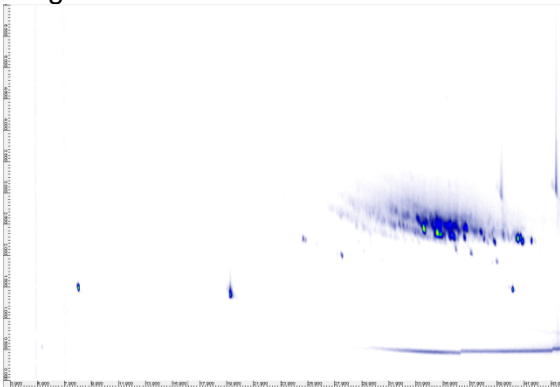
Fragment m/z 121:



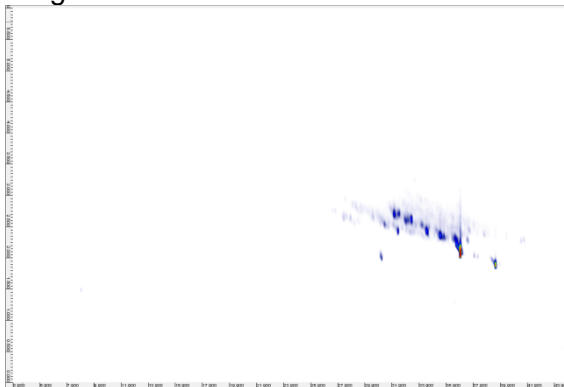
Fragment m/z 122:



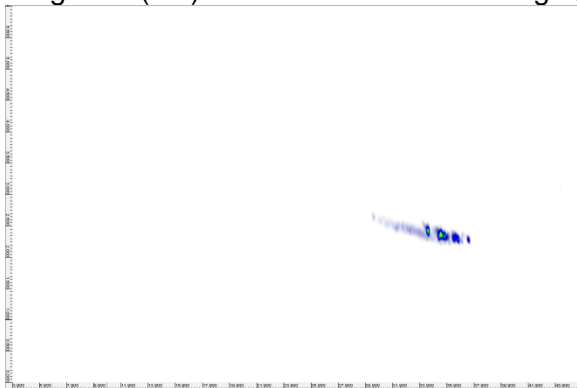
Fragment m/z 135:



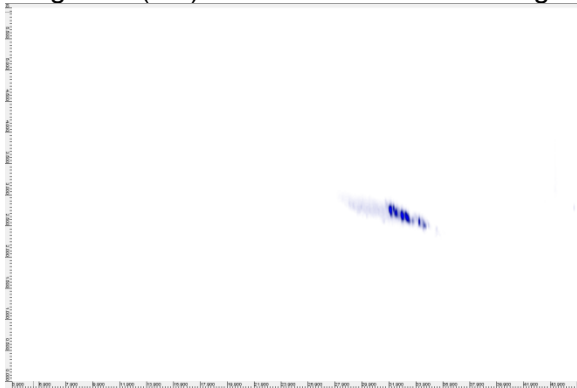
Fragment m/z 150:



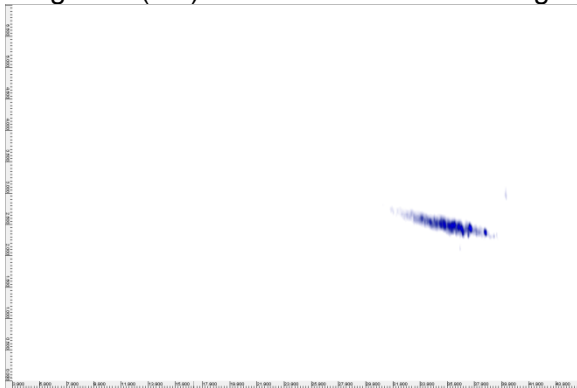
Fragment (M+) m/z 246: Dimer with 4 rings ($C_{18}H_{30}$)*



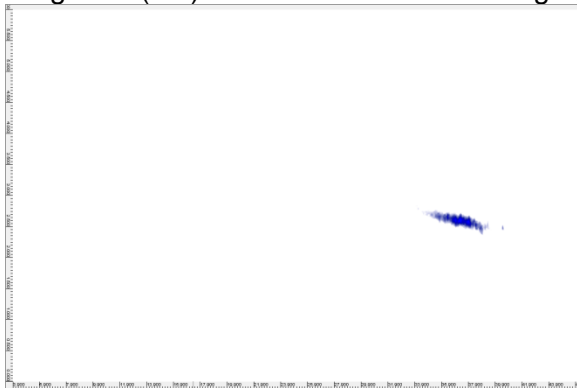
Fragment (M+) m/z 248: Dimer with 3 rings ($C_{18}H_{32}$)*



Fragment (M+) m/z 260: Dimer with 4 rings ($C_{19}H_{32}$)*



Fragment (M+) m/z 274: Dimer with 4 rings (C₂₀H₃₄)*



* According to Lommatzsch et al. (2016) *Food Addit. Contam. A* 33, 473