HPLC and SFC Analyses of Trimer Fatty Acids

John Kimmel, Lisa Zang and Sue D'Antonio

5301 Stevens Creek Blvd., Agilent Technologies, Santa Clara, CA 95051

HPLC 2011
P1-S-422-TU

Agilent Technologies

Introduction

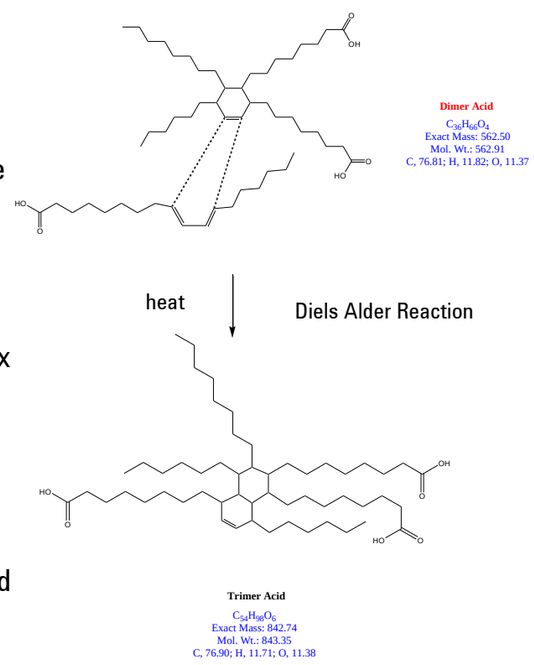
Dimer and Trimer Fatty Acids have extensive applications in the areas of polyamide resins for inks and adhesives, lubricants, greases, oilfield chemicals, fuel additives, corrosion inhibitors, sealants, polymer intermediates and personal skin care products. The polymerization of C18 based fatty acids produces complex mixtures of dimeric, trimeric and higher molecular weight acids including their isomers. It is important to determine the distribution among monomers, dimers, and trimers in these fatty acid components. The conventional separation methods for analyzing these mixtures involve lengthy normal phase HPLC gradients or derivatizing the acids into esters and then analyzing indirectly with GC. Our goal was to find better and faster HPLC and SFC methods using commercially available instruments to analyze trimers fatty acids for quality control without derivitization.

In this study, we investigated alternative HPLC methods using Agilent 1200 Infinity HPLC systems including a 1260 SFC (supercritical fluid chromatography) system.

Experimental

Materials and Sample Preparation

The fatty acid trimer samples and reference standards were generously provided by Chemco Systems. As shown in the figure, the trimer acid is formed via Diels Alder mechanism where the double bond migrations on Linoleic acid (monomer) can result in formation of dimer isomers and trimer isomers. The sample matrix is complex due to the presents of various isomers of the same molecular weights. In addition, the trimer acid samples can only be dissolved in acetone or alcohol type of organic solvents.



For our HPLC studies, samples were dissolved in isopropyl alcohol or in HPLC mobile phase B without any treatment.

Instruments

Agilent 1200 Infinity HPLC binary systems including a 1260 SFC (supercritical fluid chromatography) system were used in the study. For detection of these highly saturated fatty acid trimers, Agilent ELSD and Single Quadruple MS detector were used with an APCI source.

The HPLC system is consisting of:
G1379B micro vacuum degasser
G1312B 1260 binary pump
G1367C 1260 high performance autosampler
G1316C Thermostatted column compartment
Agilent PL-385 evaporative light scattering detector (ELSD) and/or 1260 Infinity Evaporative Light Scattering Detector
G6150 MS Detector with APCI source

Agilent SFC Instrument is consisting of:
Aurora SFC Fusion A5 module
G1312B Infinity 1260 Binary Pump
G4303A Infinity 1260 SFC Autosampler
G1316C Infinity 1260 Thermostatted Column Compartment
G1315C Infinity 1260 Diode Array Detector with 6mm 5ul 120 bar SFC flow cell
Agilent PL-385 evaporative light scattering detector (ELSD) and/or 1260 Infinity Evaporative Light Scattering Detector
G6150 MS Detector with APCI source

HPLC Methods and Columns

Reverse Phase Separation used 75:25 ACN/Acetone(A) and IPA (B) since no separation was achieved with 100% Methanol or IPA. Various C8 and C18 columns were tested.

Normal Phase Separation used Cyclohexane with 0.2%Acetic Acid (Sovent A) and 85% Cyclohexane+15% IPA with 0.2% AA (Solvent B), Flow rate was 1.2 ml/min, column temperature was 45C. Column was Zorbax Sil 4.6x250mm, 5 um. Two gradient methods were tested.

Supercritical Fluid Chromatography Conditions:
Solvent A liquid CO2, Solvent B Methanol, Flow rate 3 ml/min, column 4.6x100mm 1.8um ZORBAX RX SIL, 30C, TCC at 30C. SFC A5 module parameters are System Pressure 100 bar, Nozzle Temperature 60C.

Results and Discussion

Reverse Phase Test

Although reverse phase separation has become a popular LC technique with huge selections of columns commercially available, the separation of Trimer Acids are hindered by its poor solubility in ACN and it is not water soluble at all. Example of that using Zorbax 300A SB C8 4.6x150 mm, 3.5 um is shown in Fig.1 indicating some sample might be trapped on column.

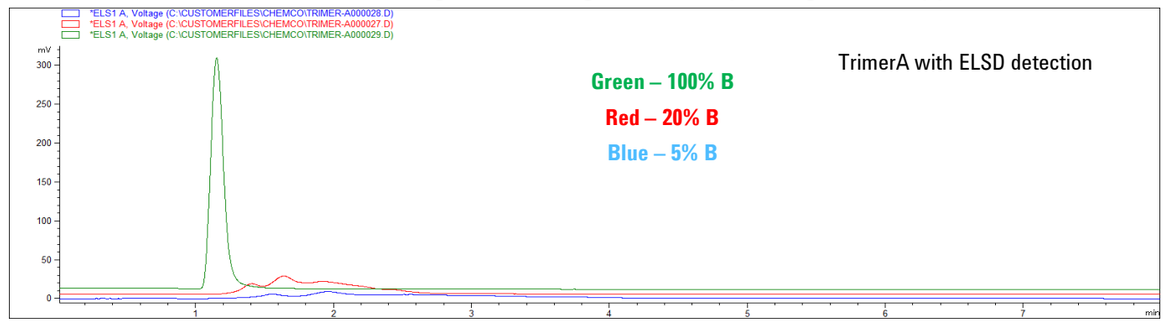
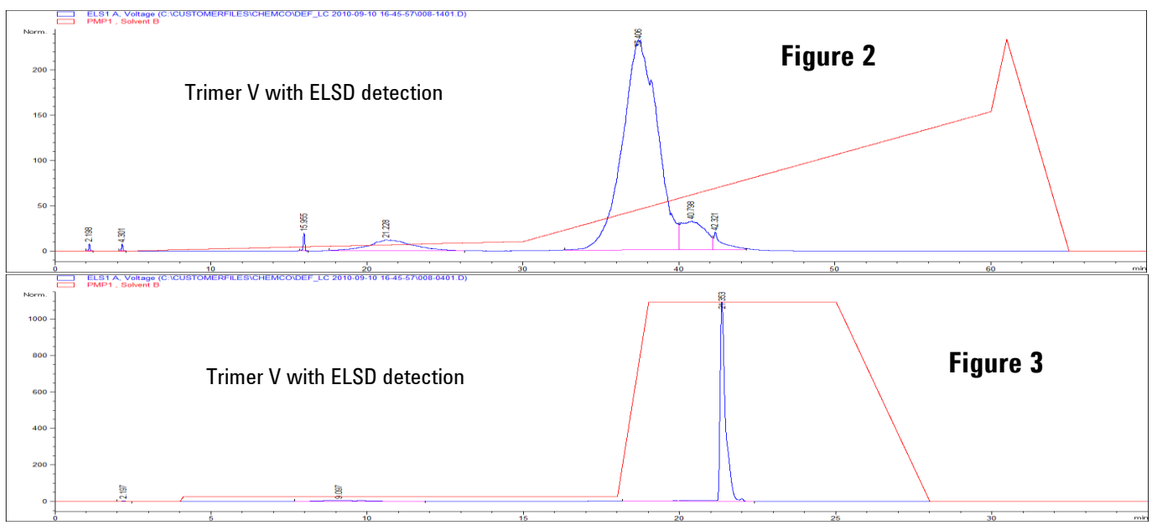


Fig. 1. Isocratic Reverse Separation. Three runs with 1 ul injection varying IPA%.

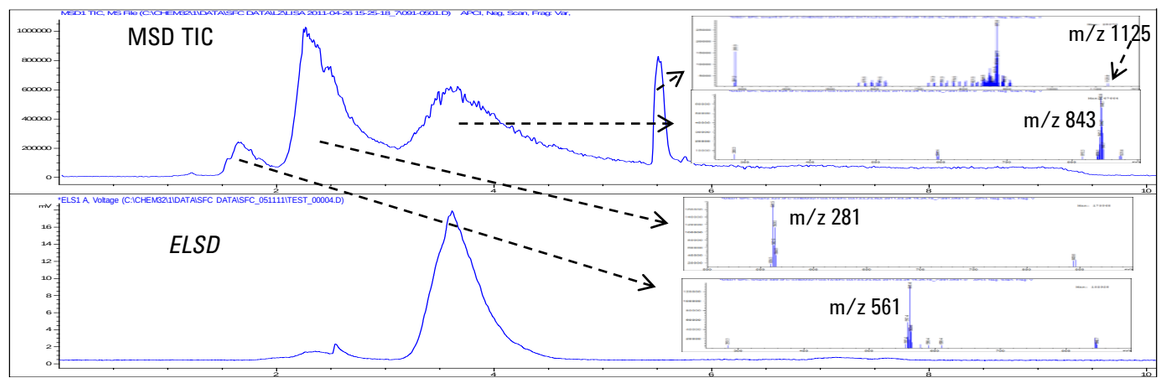
Normal Phase Test

Conventionally, normal phase method has been used to analyze dimer/trimer acids. Step gradients are used to separate dimer acids from trimers and high MW isomers. We tested a long two-slope gradient method to attempt to further separate dimer and trimer isomers. Only partial separation was achieved with a very long run time of 70min (Fig.2). The step gradient method remains a better way to separate dimer isomers from others with a reasonable run time (Fig.3)



SFC Test

SFC has shown its unique ability in separating chiral compounds and in delivering fast separation at every low system pressure. In addition, with running liquid CO2, solvent consumption and waste are greatly reduced. We were able to run SFC split to MS detector, or to ELSD. With MSD we were able to identify dimers, trimers and higher molecular weight ions in the complex trimer samples. Figure 4 shows the MS spectra obtained at different retention times. The separation pattern is similar to that of normal phase but with much shorter time. The work is in progress to improve separation as well as to understand the ionization patterns for accurate identifications using MSD. ELSD can be used for quantitation.



Conclusions

We have compared the separation results from different liquid chromatographic techniques. SFC study with MSD showed its potential to improve trimer acids analysis with significantly reduced run time and with more green mobile phases. Further optimization for reliable quantitative analysis using SFC technique with ELSD or MSD is in progress.

Acknowledgements

Authors would like to thank Dr Kreisler Lau at Chemco Systems for providing samples and for many helpful discussions.