Polymer/Oligomer Characterization using High Resolution Mass Spectrometry

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Agilent Technologies
Outline

• **General Overview:**
  – Resolution, Mass Accuracy and High Mass Performance

• **Recent Hardware Innovations**
  – *Increased Ionization Efficiency:* Jet Stream
  – Increased Sensitivity: Ion Funnel 6550 QTOF
  – *Enhanced Separations:* Ion Mobility 6560 QTOF

• **Recent Data Analysis Innovations**
  – Unbiased Data Mining: Molecular Feature Extraction (MFE)
  – Accurate Mass MS/MS libraries Polymer Additive Libraries
  – Differential Analysis: Mass Profiler

• **Questions**
Size Exclusion Chromatography For The Analysis Of Dental Polymers

Copolymers of vinylmethyl ether and maleic anhydride are widely used in a range of dental applications including denture bio-adhesive and a toothpaste additive which helps toothpaste to remain active between brushing. Batch to batch variations of such copolymer systems strongly influence performance in dental applications. In this note, size exclusion chromatography (SEC) was used to analyze two batches of commercial copolymer, one which worked well in formulation and one which failed. The figure shows an overlay of molecular weight distributions determined for the two dental polymers which clearly indicates a batch difference between the two. View application note
Instrumentation for Polymer/Oligomer Analysis

- 7000B GC/QQQ
- 5975C GC/MS
- Hi-DEF Q-TOF 6500 series
- 7700 GC/QTOF
- SFC/MSD/QTOF
- QQQ 6400 Series
- TOF 6200 series
High Resolution LCMS Examples:

- **Raw Material Analysis:** End Groups/MW Distributions
- **Extractable/Leachable:** Polymer Additive Databases
- **Impurity Analysis:** Differential Analysis (Mass Profiler)
- **High Mass Compounds:** Extended Mass Range
- **GPC-Q(TOF):** Enhancement with Post Column Addition!
- **Complex Surfactant Analysis:** MFE and High Res
- **Impurity Analysis:** Cyclic Impurities in TPU Resins
6550 QTOF Instrument Technologies

Comprehensive Performance Enhancements

- Mass Resolution >40,000
- 50 spectra/sec MS and 33 spectra/sec MS/MS
- 5 orders of linear dynamic range
- <1 ppm MS mass accuracy; <2 ppm MS/MS
- Unrivalled sensitivity

New - Ion Beam Compression and Shaping (resolution + sensitivity)

Dual Agilent JetStream orthogonal spray (robust and stable mass calibration)

New - iFunnel (10X sensitivity gain)

Hexapole axial focusing collision cell (faster MS/MS spectra)

Longer Invar flight tube (resolution + stable mass accuracy)

New embedded processor (50 spectra/sec)

4 GHz digitizer + ADC detector (resolution, mass accuracy, dynamic range)
Agilent’s Molecular Formula Generation Software

Scoring based on

- Monoisotopic mass (varies in ppm)
- Isotope distribution (varies in %)
- Isotope spacing (varies in ppm)

Mass Match + Abund. Match + Spacing Match = Overall Score
Mass Accuracy at Trace Level Detection (APCI+)

+APCI Scan (12.94-13.08 min, 36 Scans) Frag=125.0V DEHP 25pg-r001.d Subtract

**[M+H]**

0.07 ppm

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Accurate Mass MS/MS → Structural Information

Degradation Product of Irganox 1010 [C_{56}H_{84}O_{10}]
MS/MS of \([M+H]^+\) \(C_{24}H_{24}O_{10}\) RT 6.9 minutes

Extractables and Leachables Examples

Phthalate

C$_2$H$_4$O Losses

C$_{22}$H$_{21}$O$_9$

C$_{18}$H$_{13}$O$_7$

C$_{10}$H$_9$O$_4$

C$_{10}$H$_7$O$_3$

C$_8$H$_5$O$_3$

C$_{16}$H$_9$O$_6$

C$_{14}$H$_7$O$_4$

C$_{18}$H$_9$O$_6$

C$_{10}$H$_9$O$_4$

C$_{16}$H$_9$O$_6$

C$_{14}$H$_9$O$_4$
MS/MS of [M+H]^+ C_{24}H_{24}O_{10} at RT 7.27 mins
Personal Compound Databases (PCDL)

Polymer Additive Database contains over 1065 Compounds
Building MS/MS Searchable Library

Accurate Mass and MS/MS Library Searchable Databases
Importance of High Resolution MS
Isotopically Resolve Charge States up to +13
Importance of High Resolution

Detect Small Changes in Mass

![Graph showing mass spectra with retention times RT 11.7 min, 12.1 min, and 12.55 min.]

- RT 11.7 min
- RT 12.1 min
- RT 12.55 min
Blow-up of Mass Range \( m/z \) 1319-1344

10’s mDalton mass differences
Mass Differences of 0.162 (+6)
Smaller at higher Charge States
High Mass Capabilities: Charge Reduction +6 to +2
Infusion Sample F68 with $C_{16}H_{33}NMe_2$
Charge Reduction Reduces Complexity of MS Data
+2 Charge State – High Mass Transmission

![Graph showing [C₂H₄O]ₙ 22 Delta Mass]

+ESI Scan (1.453-1.603 min, 8 Scans) F68 Infusion C16Me2N 0001.d

Counts vs. Mass-to-Charge (m/z)

Agilent Technologies
ASTS Houston High Resolution Analysis of Oligomeric and Polymeric Materials
Isotope Resolved Deconvolution Result
F68 MW average 10K
QTOF Ion Sources

Dual-Spray Electrospray Source (ESI)

Agilent Jet Stream Electrospray Technology (AJS)

Atmospheric Pressure Chemical Ionization (APCI)

**Multimode:** Operates in ESI-only, APCI-only, or mixed mode (ESI+APCI)

Atmospheric Pressure Photoionization (APPI)

DART (IonSense)

MassTech AP-MALDI
G3212A - GC APCI Interface

- Increases MS flexibility by enabling both GC and LC compatibility
- Analysis of wider range of compounds
- Proven QTOF LC/MS platform
- Proven 7890 GC performance
- Excellent sensitivity and mass accuracy performance
- Dedicated GC QTOF offers electron impact (EI) ionization
Increased Ionization Efficiency: Jet Stream

- Higher temperatures increases evaporation rate of mobile phase so formation of aerosol and evaporation of droplets will occur more quickly.

- Concentric orientation reduces dispersion

- Introduces many more ions into the MS.

- Reduces number of neutral solvent clusters.

- Result: stronger signals with lower relative standard deviation (RSDs) at the limit of detection.
Irganox 1010: Jet Stream versus Dual ESI

> 10X Enhancement in Ionization → Lower Detection
Increased Sensitivity using Hexabore Capillary

• **SIX** bores
• **HALF** as long

*Six bores, Half the restriction means…*

• 6 times the amount of atmospheric gas sampled

AND

• 10X the number of ions sampled over wide mass range.

*But how do we handle all the extra gas molecules?*
Two Stage Ion Funnel Manages High Gas Load

Stage 1 offset deflects the high quantity of gas exiting the hexabore capillary

Stage 1: 8-12 Torr
Stage 2: 1-3 Torr

RF Ion Guide
Agilent Ion Mobility (IMS) 6560 QTOF Overview

- Based on 6550 Q-TOF
  - Maintains QTOF performance
  - Inserted trapping funnel and drift tube
  - Trapping funnel & gate
  - Matches IMS duty cycle with Q-TOF analysis

- Precursor ions are separated by drift time and
- Product ions are analyzed by QTOF.
The Agilent Ion Mobility System

- Nitrogen buffer gas
- Funnels drive sensitivity
- Uniform Field Drift Tube allows direct determination of $\Omega$
- Resolution approaches theoretical limit:
  \[
  \text{Mobility Resolution} \propto \sqrt{LEZe}
  \]
- Fragmentation after IMS means parents and fragments have common drift times
It’s All About Separation

Peak Capacity = \(60 \times 40,000 \times 14\% = 336,600 \sim 8\text{-fold increase}\)

Dwivedi P, Schultz AJ, Hill HH, 
Metabolic profiling of human blood by high-resolution ion mobility mass spectrometry (IM-MS). 
Int J Mass Spectrom 298:78–90. 2010
Ion mobility separation of MMA and SA (Dimer):

<table>
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<tr>
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<th>CCS (Monomer)</th>
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Ion mobility separation of o-, m- & p-Phthalic acid

- m-Phthalic acid
- p-Phthalic acid
- o-Phthalic acid
Ion Mobility of Polymeric Ink Dispersants
Ion Mobility of Diesel (Hydrocarbons) Sample
Ion Mobility of Diesel (Hydrocarbons) Sample

Enable the extraction of ion series of interest for further study
Molecular Feature Extraction (MFE)
Automated Data Reduction Software

Finds Features in TOF/QTOF Data

Data Reduced sum intensities of isotopes, adducts, clusters and multiply charges ions together.

3D Plot Before Coeluting Features

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Polyetherol Sample

**Graphs:**

- **TIC**
- **TCC**

The graphs illustrate the analysis of a Polyetherol sample using high-resolution mass spectrometry. The plots show the counts versus acquisition time (min) for different scans, highlighting the fragmentation patterns and molecular distributions of the sample.
Individual Components by MFE for Polyetherol
Mass Profiler View of Data
Mixture of Surfactants

Multiple Features (Mass vs. RT) Graphed
58 Mass Separation by RT and
44 Mass Separation minor change

Mass vs. Retention Time

\[ \Delta 58 \quad C_3H_6O \]

\[ \Delta 44 \quad C_2H_4O \]
Differential Analysis
Impurity Profiling of Surfactants
Comparison of Lots 546 and 838 on C18 Column
Extracted Chromatograms overlapped 546 and 838
What is Mass Profiler Software?

• MassHunter Mass Profiler software operates on the compound data of different samples stored in .cef (compound exchange file) format files that are produced by MassHunter Qualitative Analysis software. It lets the user:
  - Investigate similarities and differences in features across multiple analyses and samples
  - Align and normalize features different samples
  - Identify features using the MassHunter ID Browser program

• Mass Profiler does two comparison sets (A vs. B) of data and allows the user to run relatively simple statistics

• Used for differential analysis, i.e. treated vs. untreated
Mass Profiler Software
208 Features with RI > 1.0%

Multiple Features (Mass vs. RT) Graphed
Mass Profiler Mass versus Retention Time Plots
Detect 58 PPO Repeat Pattern (RI > 0.2%)

Impurity in Sample A

Display Surfactant A versus Surfactant B
Log/Log Plot Showing Differential Analysis

![Log/Log Plot](image)
Analysis of TPU Resin
Analysis of Thermoplastic Urethane (TPU)
Find Compounds by Molecular Feature Extraction

Cyclic PTA Oligomers

Resin
Blow-up Retention Times 1.6 to 6 minutes

Resin

Average Spectra
Irganox 1010 and Irganox 1024

Additives

Irganox 1024 higher in different resin
Cyclic PTA Oligomers n=2-8 in TPU Resin

N=6
Cyclics Higher in 87AOB versus Natural

N=6
Summary

- QTOF Hardware Performance provides routine accurate mass 1 ppm in MS and 2 ppm in MS/MS over wide dynamic range utilized to provide molecular formula and structure information for known and unknowns.
- Robust Easy to Use and LEARN!!!
- Molecular Feature Extraction unique unsupervised Data Mining of high resolution LCMS and MS/MS data.
- Mass Profiler Software: Differential analysis visualization software.