Comparison of Drift-tube Ion Mobility and

Structures for Lossless Ion Manipulation(SLIM) for the Characterization of Polysorbate, Polyethers, Surfactants Compounds

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To assess the technical feasibility and analytical capabilities of using a Drift-Tube based Ion Mobility Mass Spectrometry (IM-MS) system with high resolution demultiplexing and the Structures for Lossless Ion Manipulations (SLIM) based High Resolution Ion Mobility device coupled to a 6546 LC/Q-TOF for separation of structurally similar isobaric and isomeric oligomeric Polyether and Polysorbates surfactants.

Excipients such as Polysorbates and Polyethers are an integral part of biotherapeutic manufacturing being used to improve solubility, control pH, boost shelf-life stability and to maintain the preferred conformation of biomolecules (proteins/vaccines). Breakdown products and impurities in the excipients have the potential to cause changes to the biotherapeutic products, impacting shelf-life stability.

The chemical composition of commercial excipients are very complex due to the presence of oligomeric repeat units (PEO, PPO), starting materials (glycerol, sorbitan, isosorbide), the wide variety of fatty ester end-groups and degree of esterification (mono, di, tri) esters. High resolution liquid chromatography mass spectrometry (LCMS) only provides partial characterization.

Ion Mobility mass spectrometry (IMMS) has been shown to be a useful means to characterize polysorbate formulations, using the differences in chemical structure (end-groups, repeat units) to separate co-eluting isomeric/isobaric oligomeric components. To reduce the complexity of the polysorbates, one typically uses long LC gradients (30-60 minutes) separating by fatty acid and degree of esterification. To increase sample through-put one could use high resolution IM (R>200) to eliminate or just reduce the LC gradient to just 3-5 minutes.
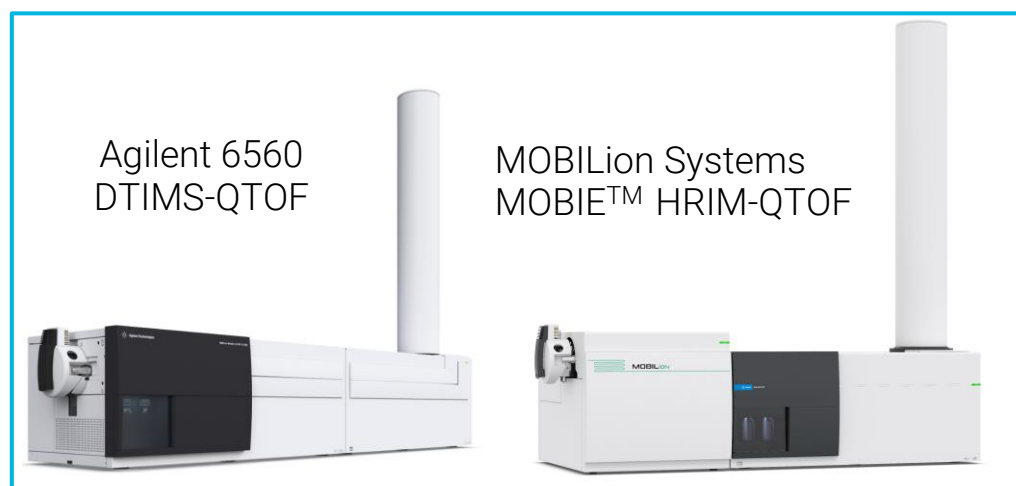
Agilent 6560
DTIMS-QTOF

MOBILion Systems
MOBIE™ HRIM-QTOF

Figure 1: Agilent 6560 DTIMS-QTOF and SLIM based HRIM device coupled to a 6546 LC/Q-TOF

Commercial grade Polysorbates , Polyethers and other surfactants purchased from Sigma Aldrich, prepared at a starting concentration of 1 mg/mL and analyzed using both the Agilent Drift-tube Ion Mobility 6560 IM Q-TOF and SLIM based HRIM device coupled to a 6546 LC/Q-TOF. Both use the Agilent Jet Stream ESI Source operated in positive ion mode (Figure 1). The 6560 Drift-tube system was tuned and calibrated for the mass range of m/z 100 to 1700 and the drift-tube settings (maximum drift time, drift voltage, funnel RF voltages) were adjusted to obtain the highest drift-time resolution. To enhance the analytical sensitivity, in-spectra dynamic range and drift-time resolution the data was collected using 4-bit multiplexing with a 3.9 ms fill and 300 μs release time.

The SLIM based HRIM Device coupled to a 6546 LC/Q-TOF system was tuned and calibrated to optimize the transmission of ions between mass range of m/z 300 and 3200. A series of experiments were carried out to determine the optimum traveling wave parameters required to trap and release ions to obtain the maximum resolution and sensitivity.

LC Conditions

The samples were all analyzed using flow injection and with a very short LC separation using an Agilent RRHD 2.1 x 100 mm 1.9 μm EC-C18 column heated to 45°C, mobile phase solvents of A:Water, B:Methanol and 2.5 mM NH_4Formate and 0.05% Formic acid buffers from 10% B, to 100% B in 3 minutes with a hold of 4-minutes and 0.400 mL/min flow rate. For the concentrated samples, the short LC gradient was used to prevent overloading of the trap and to slightly separate oligomeric components.

Data Analysis Post Processing Drift-Tube

The drift-tube ion mobility multiplexed raw data undergoes a multistep post data acquisition process summarized below.

- Apply Reference Mass Recalibration IM-MS Reprocessor
- Demultiplex using PNNL PreProcessor (v 2022.02.18)
- Apply drift-time calibration using IM-MS Browser
- Find Molecular Features using IM-MS Browser
- Utilize High Resolution Demultiplexing (HRdm) 2.0 for further peak convolution
- Utilize MassProfiler Software to visualize data

Analysis of Polysorbate: Flow Injection.

The flow injection mass spectrum obtained on the MOBIE/LC/Q-TOF shows a series of peaks separated by 44 from the C_2H_4O group with $C_{16}H_{33}O_2$ ester (Figure 2A) and the zoom region of a narrow mass range shows peaks from coeluting oligomers of PEG Sorbitan, PEG Sorbitan Oleate and PEG Sorbitan Palmitate (Figure 2B)
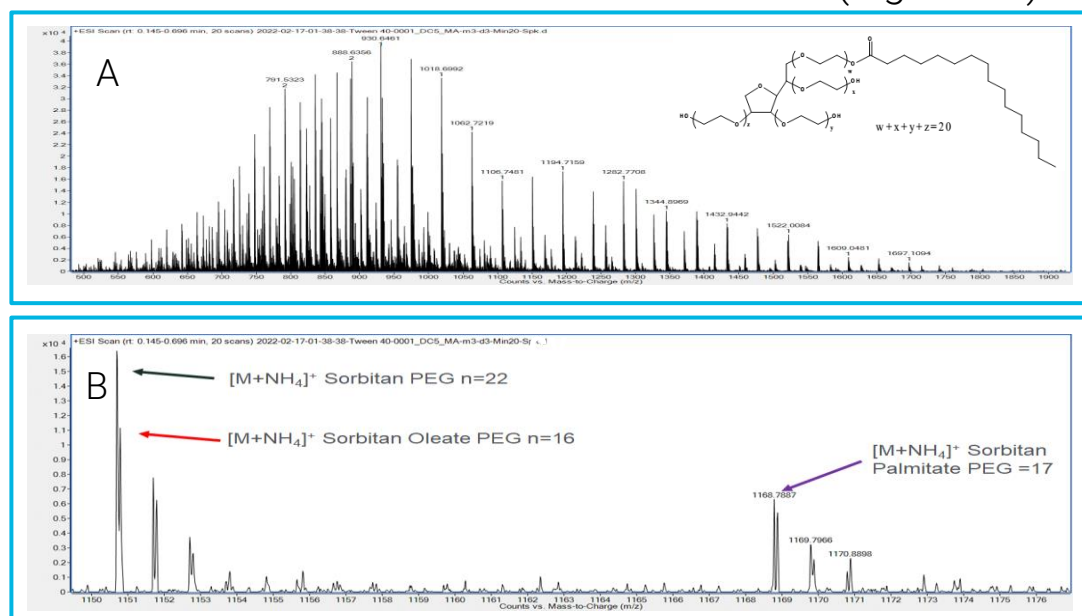
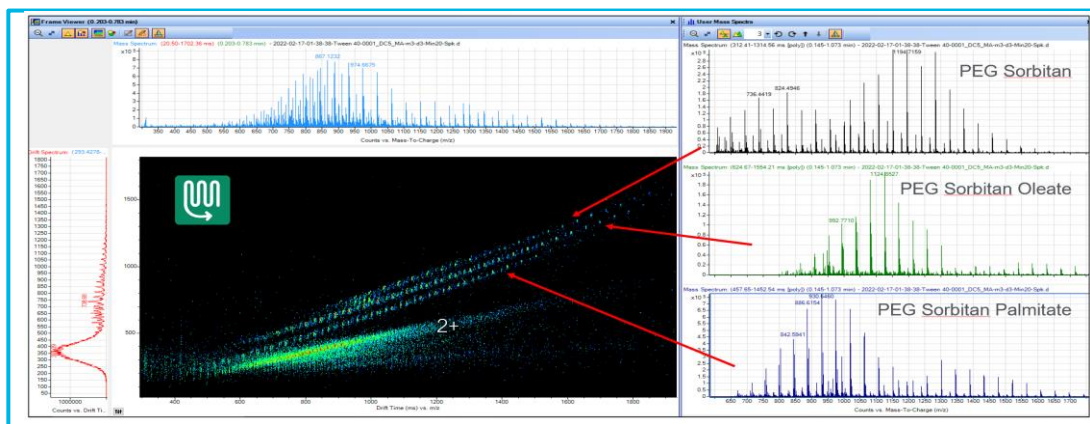Figures 2A-B: Mass Spectrum for Tween 40 showing overlapping isobaric species at *m/z* 1150 and 1168.

Figure 3: Spectra for Tween 40 Shows Separation of the Modified PEG Sorbitan Components by IM Trend Lines
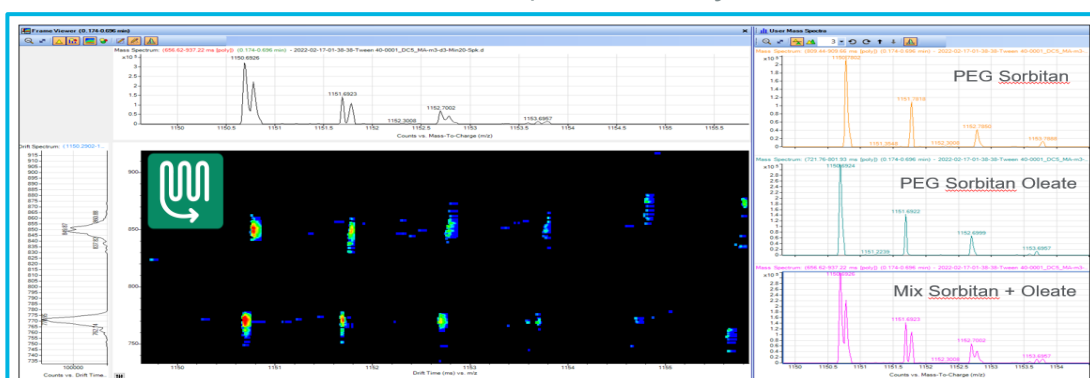
Figure 4: HRIM Easily Separates Isobaric Species
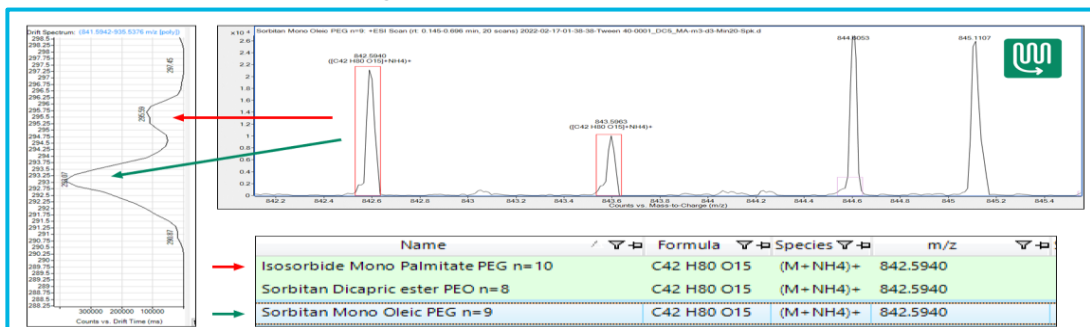
Figure 5: HRIM Able to Separate Isomeric Species by Drift

Ion Mobility Characterization of Isobaric Oligomers

A mixture of Poly(ethylene glycol)dimethyl ether M_n=500 and Poly(ethylene glycol)bis(carboxymethyl)ether M_n=600 (Figure 6) was prepared to a concentration of 1.0 mg/mL to test the HRIM capabilities of the Drift-tube and MOBIE systems to separate isobaric species.

| *Isobaric Overlap* | Poly(ethylene glycol) **dimethyl ether** | ▲ | $C_2H_6O + (C_2H_4O)_n$ $46.0419 + (44.0262)_n$ | H_3C—[O]—OCH_3 |
| | Poly(ethylene glycol) bis(carboxymethyl) ether (**diacid**) | ● | $C_4H_6O_5 + (C_2H_4O)_n$ $134.0215 + (44.0262)_n$ | |

Figure 6: The chemical structure of Isobaric Oligomeric PEG Dimethyl ether ▲ and PEG diacid ● surfactants
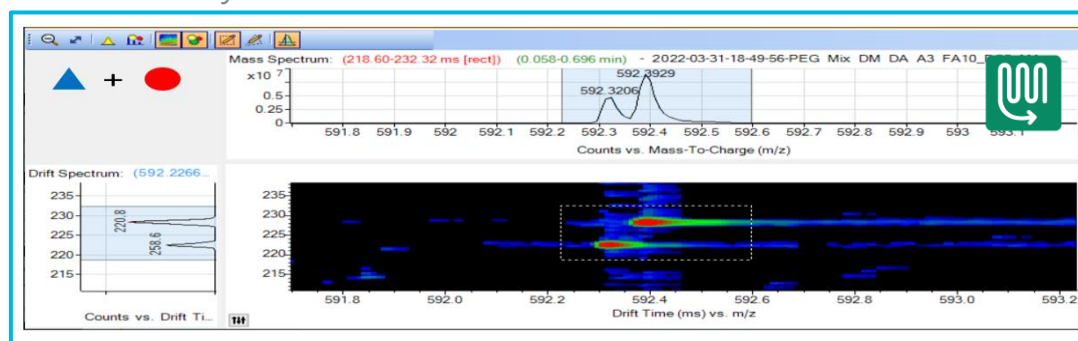

Figure 7: Flow Injection HRIM separations of Isobaric Oligomeric PEG Dimethyl ether ▲ and PEG diacids ●
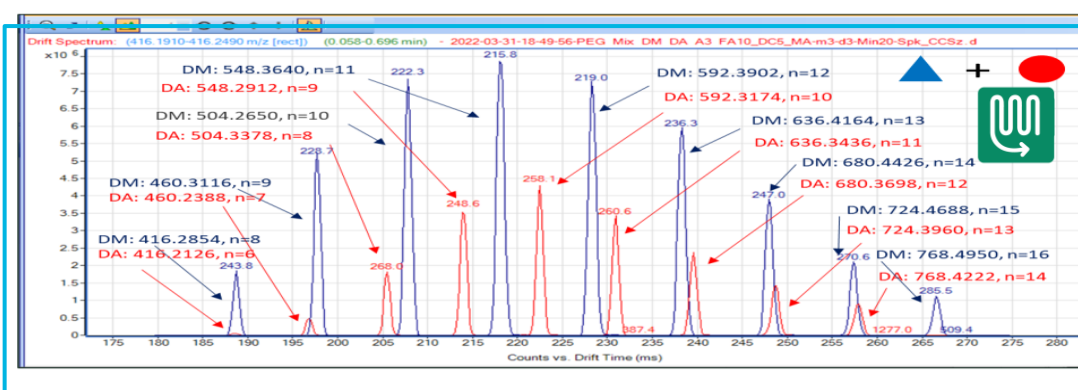

Figure 8: PEG Diacid Shows a Faster Change in Drift as the PEG Chain Length Increases than for the Simpler PEG Dimethyl Ether
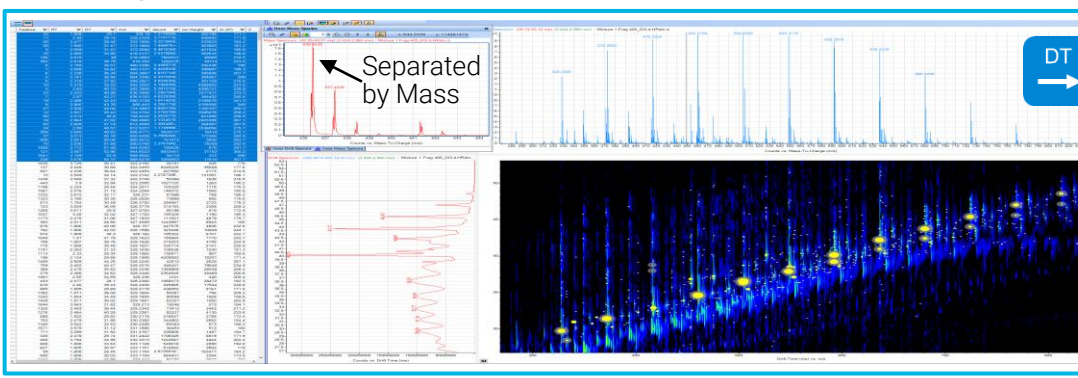

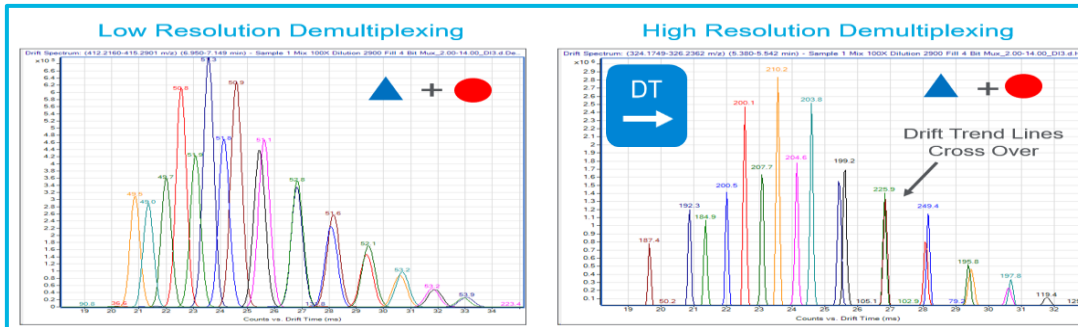Figure 9: Isobaric Oligomers Separated by Mass and DT

Figure 10: HRdm Improves Separation of Isobaric Oligomers

Ion Mobility Characterization of Isomeric Oligomers

A mixture of Poly(ethylene glycol)diglycidyl ether M_n=500 and Poly(ethylene glycol)methacrylate M_n=500 Figure 11 at 1.0 mg/mL used to test HRIM capabilities of the Drift-tube and MOBIE systems to separate isomeric species.
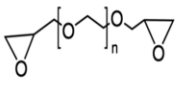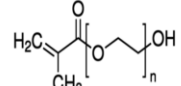

Figure 11: PEGdiglycidyl ether ■ PEGmethacrylate ◆

Data was collected individually for the Poly(ethylene oxide) diglycidyl ether and Poly(ethylene oxide) methacrylate and the 1:1: Mixture on the MOBIE system in Flow Injection and with a short 3-minute LC gradient. An overlay of the Drift spectra show (Figure 12) that very little difference in structure is seen once the poly(ethylene oxide) chain extends to 9 to 10 units as seen in the Drift Spectrum from the 1:1 Mixture (Figure 13).
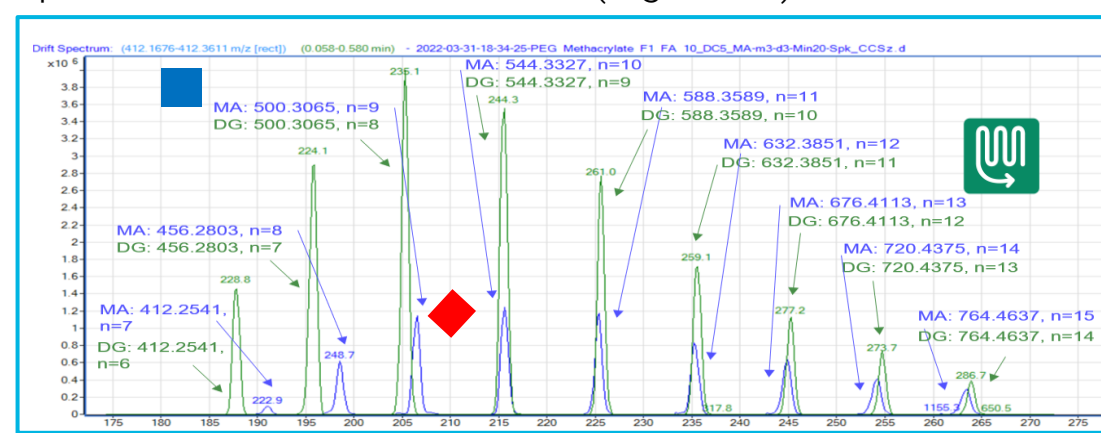

Figure 12: Overlay of Arrival Spectra from PEGDiglycidyl and PEGMethacrylate from the MOBIE system via FIA
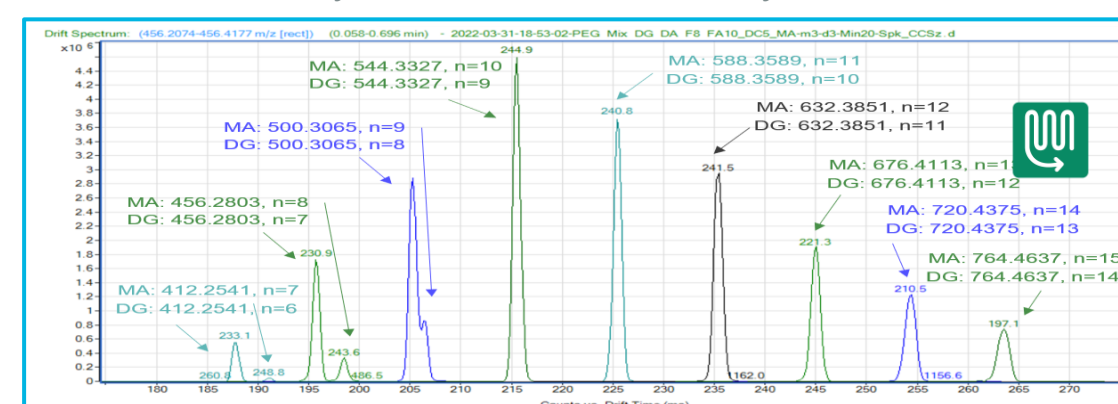

Figure 13: Arrival Spectra Differences Between Isomers too Small to Detect For Longer PEG Chains (>n=9)
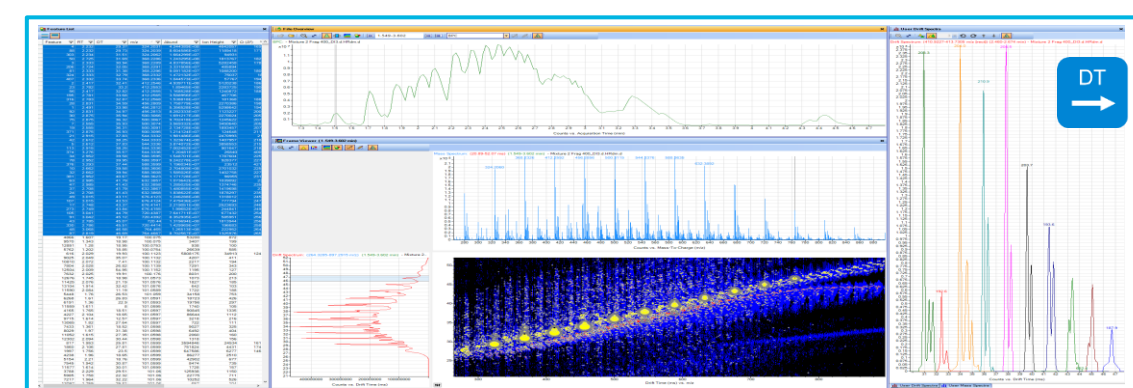

Figure 14: Drift-tube using HRdm to Resolve PEG Isomers with short LC Gradient

https://explore.agilent.com/asms

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Not So Pure… Using HRIM to Detect Minor Impurities

Breakdown products and minor impurities in commercial oligomeric surfactants can impact their performance as stabilizing agents in biotherapeutics. The 4-D feature finding and visualization software (Agilent Mass Profiler) provides a means to identify impurities based on mass and CCS value. The drift time versus retention time (RT) plot for PEGMethacrylate (Figure 15) from Drift-tube data shows the presence of PEG and PEG Dimethacrylate, singly and doubly charged ions. Differences between the surfactant mixtures can be visualized as shown for PEG Digylcidyl and PEG Methacrylate mixture (Figure 16).
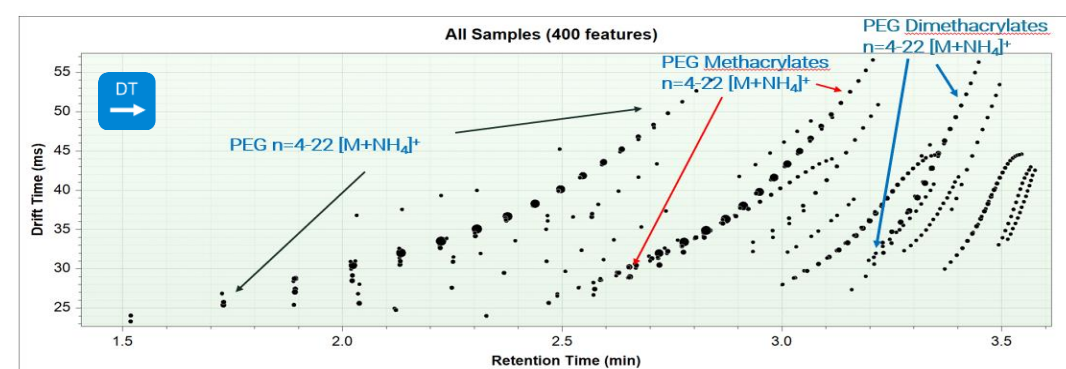

Figure 15: Impurities of PEG and PEG Dimethacrylate Easily Detected in MassProfiler Drift Time vs RT Plot.
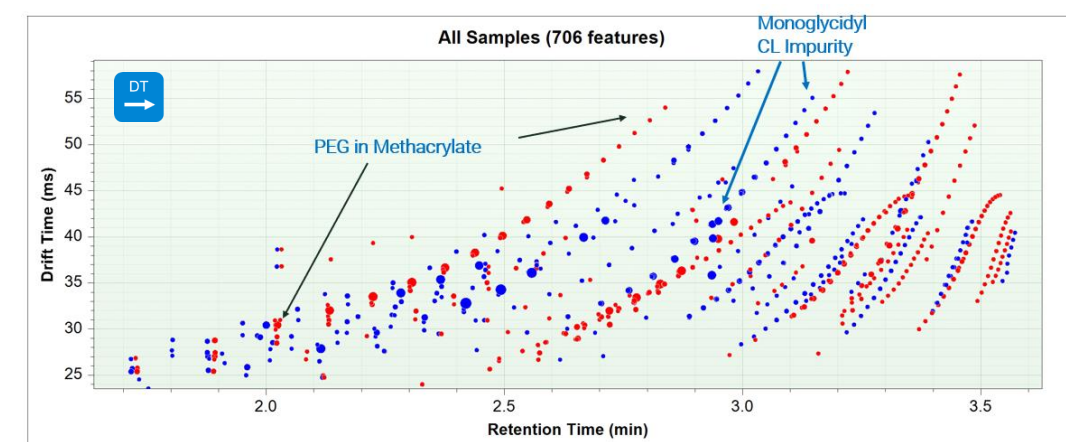

Figure 16: Drift Time vs RT Plot Separation of PEG Isomers and Detection of Impurities in the Standards.

Conclusions

Full Spectrum ion mobility separations provides significant advantages for analysis of complex polymer samples

- Ability to Separate Coeluting Compounds
- Ability to Observe Trend Lines in Oligomeric Compounds
- Ability to Identify Minor Components in Presence of Complex Polymer Matrix
- Ability to improve identifications based on Collision Cross Section conversion
- Existing Software Solutions Enable 4-D Feature Finding & Processing