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Enhanced Detection of Oligomers, Polymers and Additives Using Ion Mobility MS and new Data Mining and Differential Analysis Tools

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Introduction

The analysis of polymeric/oligomer materials using mass spectrometry has been around since the early 80's using methods such as Field Desorption (FD), Laser Desorption (LD), Matrix-Assisted Laser Desorption Ionization (MALDI) and LC/MS interfaced with a various API methods (APCI, ESI, DESI, ASAP). The complex nature of these materials: (polydisperse, multiple monomers, end groups, degree of branching, MW ranges); challenges determining what stationary phase, mobile phase and gradients to use. In addition, the polymer mass spectral data with overlapping series of multiply charged species and adducts makes manual data analysis especially challenging. The low electric field ion mobility mass spectrometer (IMS) utilizing separation in the fourth dimension provided a means to simplify the analysis of several classes of structural polymers and also enhanced the detection polymer additives and dyes that were extracted from a packing materials. A new 4D data mining algorithm integrated into differential analysis software can be used to find unique components present in 'GOOD' and 'BAD' groups of samples. Ion mobility provides a novel means to identify impurities (e.g. extractable, leachable, degradants) in pharmaceutical and consumer products.

Structural Information from Drift

Ion mobility mass spectrometry (IMS) provides insight into the confirmation of metabolites, proteins, glycans, lipids and carbohydrates. For polymers, the adducts used for ionization (H, NH_4, Na, Cs, Cl), the chain length and the degree of branching can lead to confirmation changes as reported by many research groups[1-4]. Star shaped poly(ethylene glycol) PEG polymers are the simplest class of branched polymers with a general structure consisting of several linear chains connected to a central core. These compounds have unique rheological properties that make them useful in drug delivery, thermoplastics and biologics. Polymer standards: hydroxyl terminated PEG 2K 4-arm and 10K 8-arm (Figure 1) and Phenyl T and MQ Siloxane resins were analyzed in this study. Confirmation changes were observed as a function of chain length and adducts for these standards.

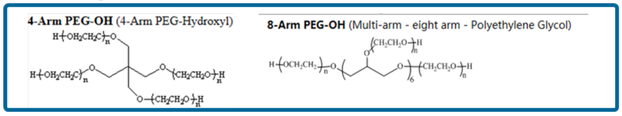


Figure 1: Structures of the 4-arm and 8-arm PEG standards

The extractable/leachable packaging materials extracts samples were prepared using THF and methylene chloride solvents. The Beclomethasone dipropionate impurity sample was obtained from a commercial source without additional sample preparation.

Experimental Conditions

The Agilent 6560 uniform low field ion mobility mass spectrometer (drift ion schematic, Figure 2) interfaced with an Agilent 1290 high pressure liquid chromatograph was used for all experiments. Reverse phase separations were carried out using Agilent Stable-Bond-C8 and C18; 2.1 mm x 150 mm, 1.8 µm columns. The mobile phases of water and acetonitrile with 0.1% formic acid were used from 1.0% B and ramping to 99% B in 15.0 min, with a 5 min hold. The flow rates were varied to optimize separation from 0.450 to 1.0 mL/min. The thermally assisted electrospray ionization source operated in positive-ion mode was used for all the experiments. For comparison, data was collected in both QTOF and IM modes.

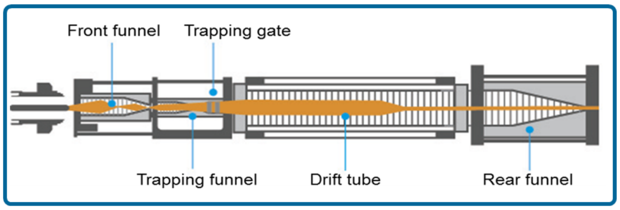


Figure 2: Schematic of Ion Mobility System with Front Ion Funnels, Trap, Drift Tune and Rear Focusing Ion Funnel

Polymer Trend Lines

A major objective in mass spectrometry of oligomeric and polymer materials is determining the chemical composition of the monomers, end-groups, and information on chain length and molecular weight. Industrial grade materials are rarely "Pure", but are often structurally complex mixtures (e.g. linear, branched, cyclic). As John McLean's research group[5] and many others have shown biomolecules of similar class follow drift time trend lines. Oligomers of the same charge state, adduct, end-group composition also follow trend lines and mass spectral data can be extracted along these lines and unique regions using IM Browser software (Figure 3).

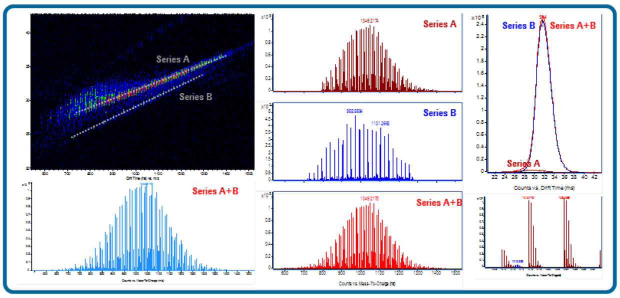


Figure 3. Series A and B from 4-Arm PEG separated by Drift Time and extracted manually along trend line.

Structure/Confirmation Examples

Ion mobility drift heat map 8-arm PEG sample (Figure 4) clearly shows changes in drift time (bends) as a function of molecular weight and chain length. The low molecular weight singly charged linear PEG (shown in red) clearly separates from the higher molecular weight +3 charge (shown in green) and +4 charge states (shown in blue) branched species.

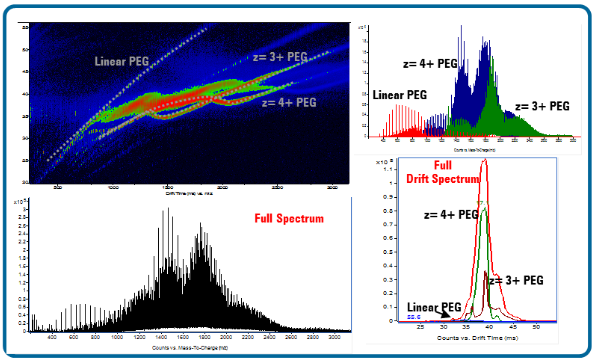


Figure 4: Drift Heat Map showing change in confirmation as a function of charge state and molecular weight 8 arm PEG sample

Data Analysis 4D Feature Finding

Manual extraction of mass spectral data is useful but not practical, because it's not automated, may lead to biased reporting and not applicable to applications like proteomics, metabolomics and impurity profiling. A new 4-dimenstional (4D) unsupervised data mining algorithm was developed that is based on the 3D Molecular Feature Extraction (MFE) algorithm, which separates ions as a function of retention time, mass/charge, abundance and drift time^6. Ions from the same isotopic distribution are grouped together, while the adducts are grouped separately with drift time. More details about the algorithm is found in Poster MP168^7. Results from LCMS separation of the 4-arm PEG sample is shown below (Figure 5).

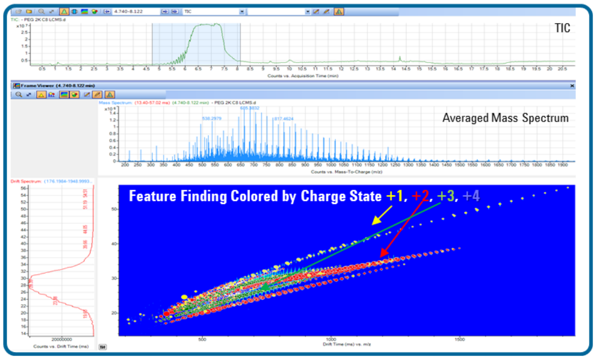


Figure 5: 4D MFE results for LCMS C8 Separation of 4-arm 2K PEG standard

Siloxane Resins

Commercially available siloxane resins, like other oligomers contain a mixture of repeating units and terminal groups. These compounds often contain cyclic (D4, D5, D6 etc.) structures that may leach from packing materials. Unlike PEG's these compounds typically will only form singly charged species so detection over a wide mass range is dependent on high mass ion transmission efficiency of the mass spectrometer. Confirmation differences as a function of drift time was observed for the Siloxane MQ Resin. The lower series varies by alternating mass difference of 86.0191 (SiC_3H_6O) and 104.0298 ($SiC_3H_8O_2$) (Figure 6).

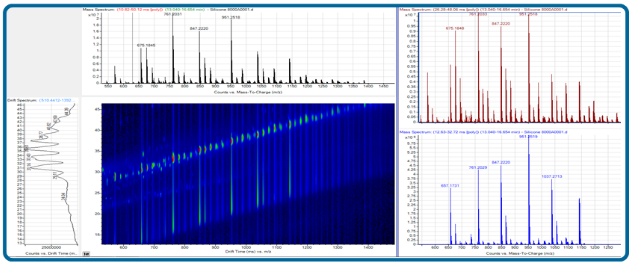
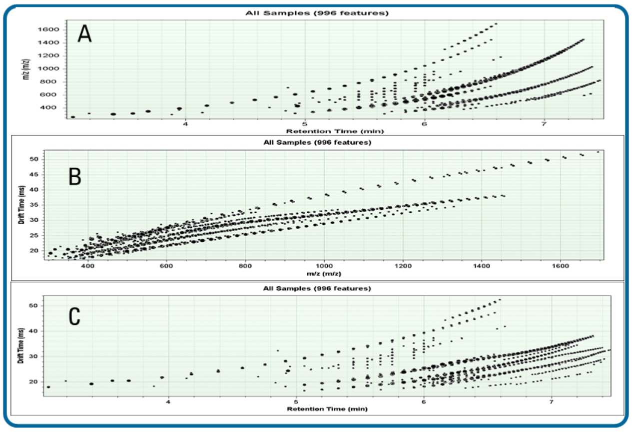


Figure 6: Drift Heat Map showing change in confirmation for Siloxane MQ Resin with mass differences corresponding to SiC_3H_6O and $SiC_3H_8O_2$ groups

Graphical Display Drift Data

For polymer analysis, pattern recognition is commonly used to identify oligomer repeat units as a function of mass difference where these differences may indicate changes in end-groups and confirmation. These patterns can be observed using the Mass Profiler program to plot data as a function of: *m/z* versus RT (**Figure 7A**), Drift Time versus *m/z* (**7B**), and Drift Time versus RT (**7C**). For the 4-Arm PEG we clearly see the presence of minor impurities and drift time changes around RT 6.5 minutes and *m/z* 600-1000.



Figures 7A-C: Graphical Display 4-Arm PEG as function of Drift time, RT, m/z

Results and Discussion

Extractable Leachable Examples

A major challenge in Extractable Leachable analysis is to identify the presence of low level potentially toxic polymer additives, impurities and dyes in the presence of oligomeric surfactants and polymers. Polymers of similar charge state, adducts, end-groups and composition will follow the same trend lines and mass spectral data can be extracted along those trend lines but additives will be single components and will have different drift times. Mass Profiler was used to identify unique dyes and additives extracted from two different packing materials. A custom accurate mass Extractable Leachable accurate mass database was used to identify Copper Phthalocyanine $C_{32}H_{16}N_8Cu$. The average mass spectrum at RT 8.8 shows the presence of two compounds with overlapping isotope pattern but resolvable using difference in drift times (Figure 8).

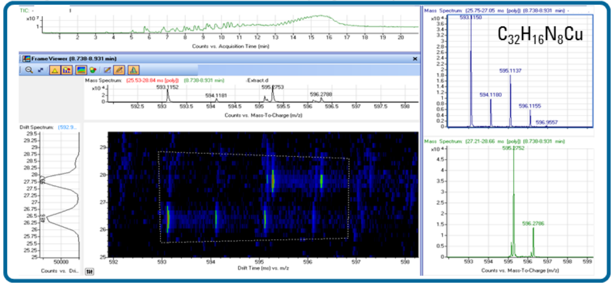


Figure 8: Separation of Possible Copper Phthalocyanine from other extractable using drift and m/z.

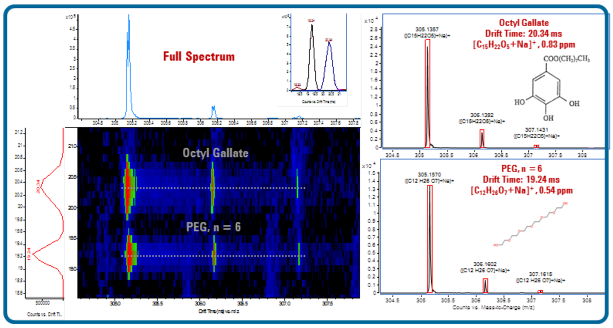


Figure 9: Separation of PEG surfactant and Octyl Gallate antioxidant extractable from expired medication.

Conclusions

Separation by drift time enabled compounds with overlapping isotope patterns to be separated using ion mobility. Ion Mobility in combination with high resolution accurate mass and isotope fidelity using UHPLC separation and data mining with a new 4-D molecular feature extraction (MFE) provides enhanced detection and identification of polymers, oligomers and additives (dyes, antioxidants, pigments) from complex mixtures.

Impurity Profiling Example

Impurity profiling experiments generally rely on extensive LC method development and/or 2D LC to optimize the separation of low concentration degradation products from the Active Pharmaceutical Ingredient (API). Using Ion Mobility mass spectrometry, two degradation products of Beclomethasone diproprionate ($C_{28}H_{37}ClO_7$), that overlap chromatographically and have overlapping isotope patterns were separated by drift time. Beclomethasone ($C_{22}H_{29}ClO_5$), and 21-Dehydro Beclomethasone ($C_{22}H_{27}ClO_5$) previously not detected using standard LCMS conditions (Figure 10).

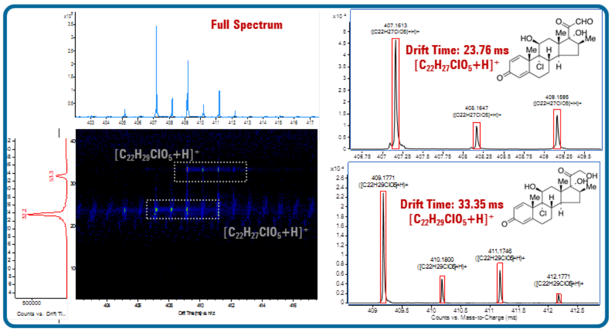


Figure 10: Separation of Beclomethasone ($C_{22}H_{29}ClO_5$), and 21-Dehydro Beclomethasone ($C_{22}H_{27}ClO_5$) by drift time

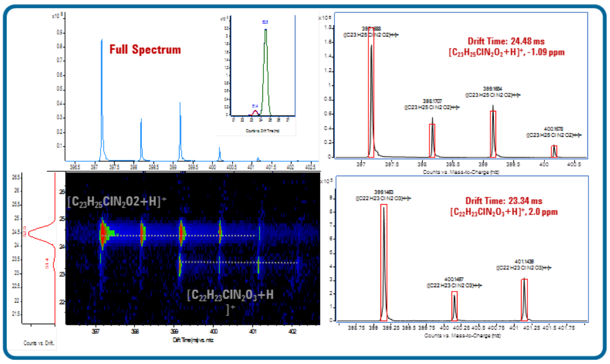


Figure 11: LC/MS Co-elution of degradation products of Loratadine $C_{23}H_{25}N_2O_2Cl$ and $C_{22}H_{23}N_2O_3Cl$

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