INTRODUCTION
Comprehensive chemical profiling and identification of compounds in tobacco and tobacco smoke matrices is a challenging task due to the sample complexity and the wide array of analyses required. LC-MS and GC-MS based methods are typically applied as they offer higher selectivity, sensitivity and peak capacity for complex separations. Recent advancements in ion mobility separation provides an additional dimension of gas phase separation orthogonal to chromatographic separation resulting in high quality mass spectral data. Here we present the use of UPLC separations with IM-MS and novel informatics tools for characterization and confident compound identification in tobacco extracts.

Ion mobility separations differentiate compounds based on size, shape, and charge. Ion mobility data was acquired using Vion IMS QTof. UPLC coupled to ion mobility mass spectrometry (IM-MS) provides accurate mass precursor and fragment ion information, retention time, isotope ratios and averaged collision cross section (CCS) parameters that can be used to build or search mass spectral database. The CCS value provides additional dimension of separation for confident compound identification and is independent of front end chromatography (LC or GC) and matrix effects. The potential of ion mobility for the separation of isomers and chromatographically co-eluting compounds in tobacco and smoke matrices will be presented.

METHODS

ACQUISITION and PROCESSING SOFTWARE: UNIFI (Waters Corporation)

**LC CONDITIONS:**
- LC System: Waters ACQUITY UPLC
- Column: ACQUITY UPLC BEH C18 2.1 x 100 mm, 1.7 µm
- Column Temp: 45 °C
- Sample Temp: 4 °C
- Flow Rate: 0.450 mL/min.
- Injection Volume: 5 µL
- Mobile Phase A: 10 mM ammonium acetate (pH5.0) in Water
- Mobile Phase B: 10 mM ammonium acetate (pH 5.0) in MeOH
- Total Run Time: 17 min.
- Gradient:

**MS CONDITIONS:**
- Instrument: Vion IMS QTof
- Ionization Mode: ESI+
- Collision Energy (LE): 3 eV
- Collision Energy (HE ramp): 20 - 55 eV
- Scan Time: 0.25 sec
- Acquisition Range: 50 - 1200 m/z
- Drift Gas: N₂
- IMS Wave Velocity: 250 m/s
- IMS Wave Height (ramp): 20 - 55 V
- Capillary: 0.8 kV
- Sampling Cone: 20.0 V
- Source Temperature: 120°C
- Source Offset: 80
- Desolvation Temperature: 550°C
- Cone Gas Flow: 0 L/hr
- Desolvation Gas Flow: 1000 L/hr

**RESULTS AND DISCUSSION**

**INCREASE CONFIDENCE IN IDENTIFICATION OF ANALYTES IN SAMPLES AND REDUCE FALSE POSITIVES**
To increase the confidence in compound identification during screening analysis it is beneficial to have several points of identification including retention time, accurate mass for the precursor and fragment ions and collisional cross section (CCS) values to help reduce false positives. Inclusion of CCS measurements provides an important reliable value which is independent of chromatographic retention time, front end chromatography (LC or GC) and is matrix independent. Ion mobility mass spectrometry provides increased peak capacity and resolution for identification of challenging separations including co-eluting isobaric compounds as shown below.

**CONCLUSIONS**

**Mass of Interest**

**SEPARATION AND IDENTIFICATION OF ISOMERS: POLYAROMATIC AMINES**

**USE OF SPECTRAL CLEANUP IN ION MOBILITY MS**
An additional benefit of ion mobility data is the ability to further resolve spectra. This is typical in complex matrices, where the compound of interest is not the most intense component of the spectrum at the observed retention time, and using ion mobility to further resolve the spectral view is a powerful means of dealing with sample complexity.

**Enhanced Peak Capacity**

Based on collision cross section error of less than 2%, it is possible to identify the correct isomer without retention time as an identification point.

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