Overview

- **Purpose:** Analyze authentic standards of plant compounds, record collision cross section (CCS) in a searchable library, and Parallel Accumulation Serial Fragmentation (PASEF) analysis in plant extracts.

- **Methods:** CCS values were recorded using UHPLC-TIMS-QTOF-MS/MS. Plants extracts were analyzed using PASEF and reduced separation times.

- **Results:** A CCS library of plant natural products was constructed and improves identification confidence. Isomeric and structurally similar compounds can be differentiated by comparing CCS, which can be used to enhance identification confidence.

Instrumentation & Methods

**Introduction**

Metabolomics is used to quantitatively and qualitatively profile large numbers of small molecule metabolites within a biological sample. The sample relies on (1) the separation of compounds in the sample, (2) and data collected on the metabolites within the sample. Liquid chromatography (LC) separates compounds and provides retention time (RT) information and mass spectrometry (MS) provides mass spectral information. The collected data is then compared with reference libraries and matching scores correspond to the confidence of the unknown compound identifications. The more orthogonal data acquired for each metabolite, the more confident it can be identified.

Ion mobility separates ions based on their size to charge ratio in the gas phase and by analyzing the ion’s mobility through a gas field. Trapped ion mobility (TIMS) is fast and compact permutation enabling higher ion mobility resolution and is suitable for coupling with liquid chromatography (LC) and mass spectrometry (MS). TIMS adds an additional separation domain that can separate co-eluting, isomeric and structurally similar compounds. It provides physical collision cross section (CCS) data for analytes which can be used to enhance identification confidence.

**Instrumentation:** Trapped Ion Mobility Spectrometry

Liquid sample ions are ionized by an electrospray ionization source and the ions travel through a gas capillary into the TIMS. A deflection plate and drift gas direct ions into an ion funnel and then into a 2-stage TIMS analyzer. The concurrent incoming gas continues to push the ions through the TIMS analyzer while an electric field gradient opposes the forward movement of the ions. The first stage TIMS traps ions while the second stage separates ions based upon their mobility. Ions are trapped where the drift gas and electric field forces on the ions are equal. The electric field gradient is gradually decreased to release the ions from the TIMS and the mobility is recorded. The mobility is used to calculate the collisional cross section (CCS).

**Methods for CCS Library Generation of Authentic Compounds**

Authentic compounds were suspended in 85% methanol. The samples were analyzed using Waters Acquity UPLC-I-Class system coupled to a Bruker trapped ion mobility and quadrupole-time of flight mass spectrometers (UHPLC-TIMS-QTOF-MS/MS). Separations were performed using Waters 2.1 x 150 mm, BEH C18, 1.7 µm column and a linear gradient elution of 0.05% formic acid water:acetonitrile. TIMS reversed reduced mobility range from 0.4 to 1.8 ks, was calibrated using Agilent Tune Mix. Mass spectral data were acquired from m/z 100-2000 in negative electrospray ionization mode. Standard compounds were analyzed in triplicate and CCS values were determined for the deprotonated molecular ion ([M-H]-). Adduct formations observed in the isobars were putatively annotated as possible.

**Results**

We have focused on two groups of specialized metabolites: flavonoids and saponins. These compounds can be hydroxylated, methylated, and glycosylated at various positions. We have recorded CCS for over 150 unique compounds. The CCS average, standard deviation, and relative standard deviation (%RSD) were calculated for each compound analyzed in triplicate. An acceptable %RSD is <2% in the field of ion mobility spectrometry. All %RSD from our measurements were <0.50% and the overall average %RSD was 0.10%.

Results & Conclusion

- **Conclusion:**
  - The measured CCS library is implemented in plant natural products which will improve identification confidence in metabolomics.
  - Isomeric and structurally similar compounds can be differentiated by TIMS even when they can not be differentiated on mass alone.
  - TIMS technology and PASEF are promising for improving our depth of coverage into the metabolites of plant extracts.