

Separation of Isobaric Amino acids and Small Molecule Metabolites Using Multipass Ion Mobility Analysis

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Amino acids along with small molecule metabolites are important biomarkers for the study and detection of diseases that are initially analyzed in untargeted omics fashion. Amino acids and many metabolites are isomeric, and their specific form can have a significant impact on biological function. Chromatographic separation of isomers is challenging, and they cannot be resolved by mass spectrometry alone.

Ion mobility is a technique that allows the separation of ions based on their size, shape, and charge. Here we present the results of the separation of isobaric amino acids and small molecule metabolites using a system that allows for multi-pass ion mobility separation which, in turn, enhances the ion mobility resolution of the separation.

Amino acid standards and small molecule metabolites commercially available were infused directly into a SELECT SERIES™ Cyclic™ IMS system. Solutions of individual and mixture of the isobaric species were used and the mobility conditions were optimized for multiple passes for each corresponding set of isobaric species.

Both ionization polarities and various solvent adducts were tested to provide the best signal intensity and separation.

Amino acids such as leucine and isoleucine have previously been separated with ion mobility in a system with lower ion mobility resolution (SYNAPT™ G2 mass spectrometer) rendering about 90% of valley and mobility resolution of around 40 $\Omega/\Delta\Omega$. With the enhanced ion mobility resolution using the cyclic IMS technique we have obtained almost complete separation of those amino acids rendering a valley of about 10% after 15 passes. For this case, the mobility resolution is around 250 $\Omega/\Delta\Omega$.

Another example is the separation of glucose-6-phosphate from glucose-1-phosphate, and fructose-6-phosphate which were separated after 10 passes. For this case, the mobility resolution is around 205 $\Omega/\Delta\Omega$.

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Enhanced Ion Mobility separation of small isobaric amino acids and other small metabolites