SEPARATION OF ISOBARIC AMINO ACIDS AND SMALL MOLECULE METABOLITES USING MULTIPASS ION MOBILITY ANALYSIS

Hernando J. Olivos[1], Jorge Smith[1], Eve L. Berger[2]
[1]Waters Corporation, Milford , MA, USA; [2]NASA Johnson Space Center, Houston, TX, USA

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INTRODUCTION

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.

EXPERIMENTAL

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

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

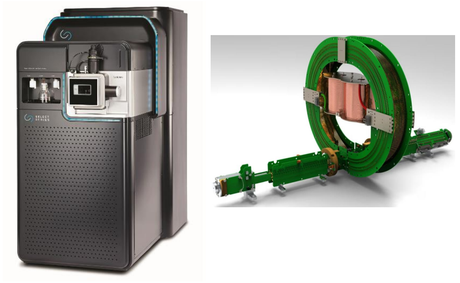
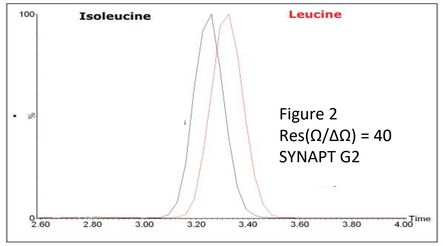
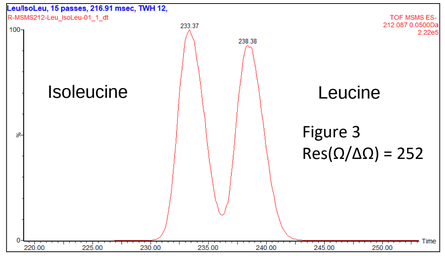


Figure 1. SELECT SERIES Cyclic IMS and its ion optics showcasing the IMS racetrack.

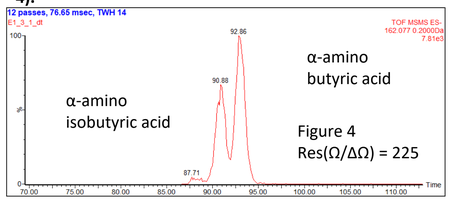
RESULTS

Separation of Leucine and Isoleucine was previously tested in an earlier IMS system (SYNAPT™ G2 , Figure 2) with low IMS resolution (Resolution of 40 Ω/ΔΩ)[1]. Multiple passes around the racetrack of the Cyclic IMS (15 passes, Resolution of 252 Ω/ΔΩ), as the [M+NaAcetate]- cluster, allowed an almost complete separation of them (Figure 3) .

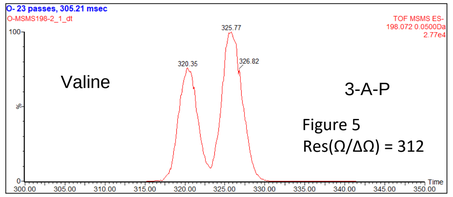




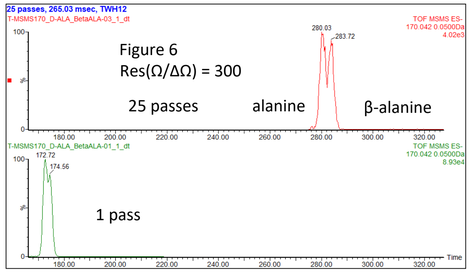
The separation of α-aminoisobutyric acid from α-aminobutyric acid after 12 passes (Resolution of 225 Ω/ΔΩ) as the [M + Acetate]- cluster is shown below (Figure 4).



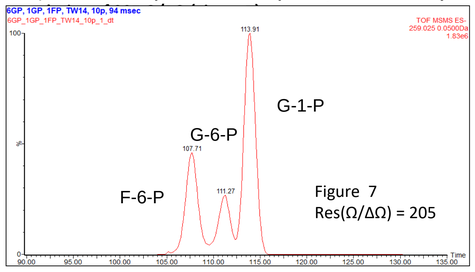
D-valine was separated from 3-A-P after 23 passes (Resolution of 312 Ω/ΔΩ) as the [M+NaAcetate]- cluster (Figure 5).



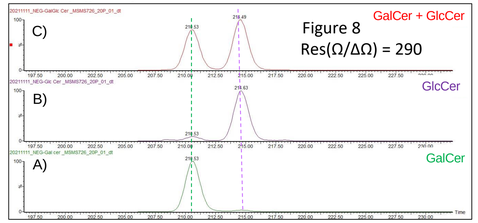
We have been able to have partial separation between alanine and β–alanine after 25 passes around the racetrack of the Cyclic IMS (Resolution of 300 Ω/ΔΩ) as the [M+NaAcetate]- (Figure 6).



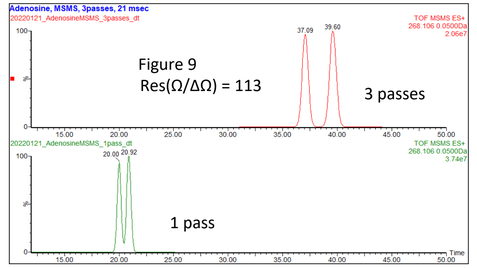
An example of the separation of larger molecular weight metabolites is the case of glucose-6-phosphate (G-6-P) from glucose-1-phosphate (G-1-P), and fructose-6-phosphate (F-6-P) which were separated after 10 passes, with a mobility



We have been able to separate GalCer (d18:1/18:0) (Figure 8-A) from GlcCer (d18:1/18:0) (Figure 8-B) after 20 passes[2] around the racetrack of the Cyclic IMS (Resolution of 290 Ω/ΔΩ) (Figure 8).



During the analysis of several adenosine –based isomers, we found that adenosine itself shows two forms, presumably due to protomeric species (Figure 9). These two forms began to separate with one pass but were completely separated after 2 or 3 passes, with a Resolution of 113 Ω/ΔΩ) for 3 passes.



DISCUSSION

The resolution of 1 pass along the cyclic racetrack is around 65. Each additional pass will increase the ion mobility resolution proportionally to the square root of the number of passes. The losses per pass are between 1 and 2 % on average.

As more passes are allowed, the ions tend to diffuse and, at some point, the faster ion will catch up with the slower ion. This will be the limiting factor for the number of passes per experiment. Each isomeric set has its "sweet spot" for the number of passes.

Two points to consider for future work:

- Calculation of CCS for the species separated by multiple passes.

- Perform IMS^n by slicing one of these peaks out of the racetrack and, after reinjection into the racetrack and multiple passes, verify if one form could potentially convert to the other form. This could be more interesting to evaluate, for example, in the case of the lipids and species like adenosine.

CONCLUSION

- **Several pairs of isomeric species were analyzed by multipass experiments using cyclic ion mobility technology.**
- **Several of these isomers were well resolved showing the power of these multipass experiments.**

References

1. Campuzano I, Giles K, Neeson K, Richardson K., Waters Application note 720003028 EN
2. Isaac G,. Olivos H., Plumb R., Waters Application note 720007539 EN

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