SEPARATION OF DIFFERENT TAUTOMERIC FORMS OF A PRECURSOR ION BY A CYCLIC ION MOBILITY SYSTEM

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OVERVIEW

- **Tautomers are isomers of a compound which differ only in the position of the protons and electrons. In the gas phase, ions generated from multifunctional compounds could exist in many tautomeric forms. Often, the fragmentation spectra of different tautomeric ions are significantly different. Spectra recorded without physically separating the tautomeric forms are in fact composites of overlapping spectra. Ion mobility, particularly in conjunction with mass spectrometry provides us a versatile method to separate and characterize gaseous ions of molecules protonated or deprotonated at different sites.**
- **In this study, tautomeric ions of several compounds were separated and their individual spectra were recorded using the SELECT SERIES™ Cyclic™ IMS System.**

INTRODUCTION

Ion mobility in conjunction with mass spectrometry enables the separation and characterization of tautomeric ions. Herein, we explored the possibility of whether tautomeric ions could be separated and isolated, and whether verified tautomeric ions undergo interconversion during extended of period of time that the ions reside during multiple passes possible in the cyclic IMS system.

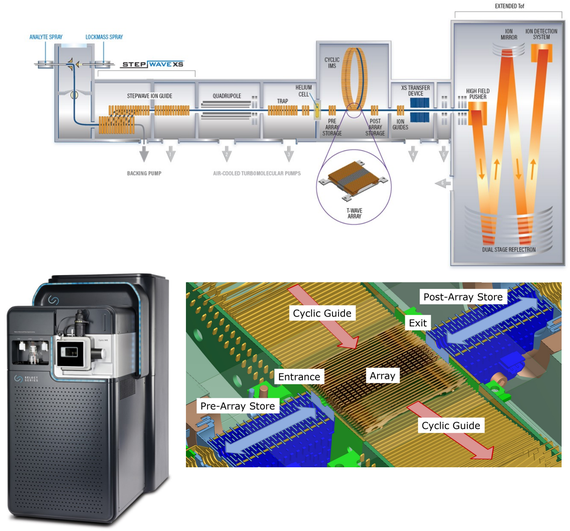

Figure 1. (Top) Schematic of the Cyclic IMS QTOF Instrumentation. It contains three main regions: the trap region, the cyclic ion mobility device, and the transfer region. (Bottom) Zoom in of the cyclic ion mobility device.

RESULTS & DISCUSSION

ION MOBILITY SEPARATION OF PROTOMERS OF ANILINE

Aniline undergoes pronation at two sites (*m/z* 94); either on the amino group (*N*-protomer) or on the ring (Ring protomer). The two protomers were well separated by ion mobility (Figure 2). Several passes through the cyclic system improved the peak resolution (Figure 2B and 2C). The faster arriving ring-protomer was ejected and the slower N-protomer was subjected to several passes in the cyclic system (Figure 3). Since no additional peaks appeared in the mobilogram after three passes (Figure 3B, C, and D), we concluded that no isomerization to the ring-protomer took place within the timeframe of the experiment.

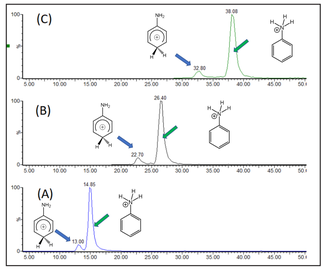

Figure 2. Ion mobility separation of protomers of aniline by one (A), two (B), or three (C) passes in the Cyclic system.

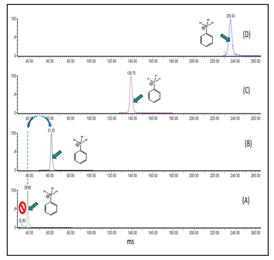

Figure 3. Ion mobility profile of protomers of aniline (A), and those after the faster ring-protomer was eliminated. Panels B, C, and D show arrival time profiles of the selected N-protomer after one (B), two (C), or three (D) passes in the cyclic system.

ION MOBILITY SEPARATION OF TAUTOMERIC ANIONS OF CURCUMIN

Deprotonated curcumin exists as keto and enol tautomeric forms. Mass selected *m/z* 367 ion showed two arrival-time peaks for the tautomeric forms upon ion mobility separation (Figure 4). The faster–eluting tautomer was identified as the keto form.

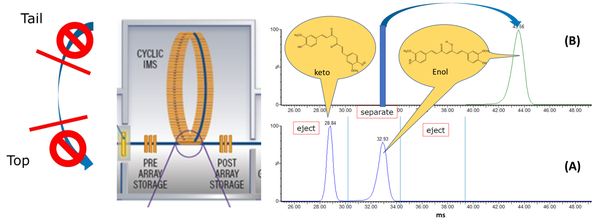

Figure 4. Schematic of a *Top* and *Tail* experiment (left panel); faster and slower ions were ejected. The *m/z* 367 ions generated under negative-ESI conditions from curcumin was subjected to ion mobility separation. The ions in the 30-34 ms were retained and passed for additional separation (B).

The fragment-ion spectra of the two tautomers were significantly different from each other (Figure 5).

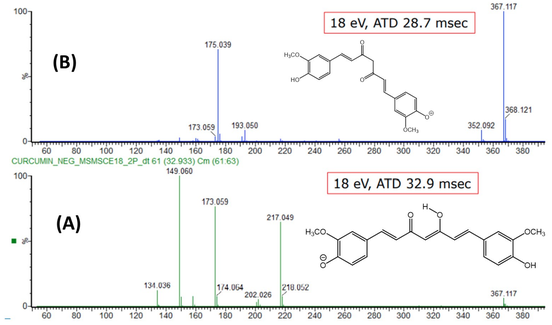

Figure 5. Fragmentation-spectra of enol (A) and keto (B) forms deprotonated curcumin.

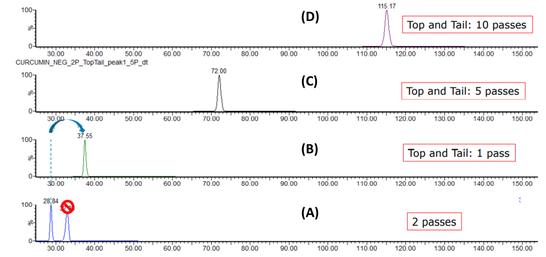

Figure 6. The *m/z* 367 ions generated under negative-ESI conditions from curcumin was subjected to ion mobility separation; The faster eluting ion was selected by a *Top* and *Tail* experiment and subjected to one (B), five (B) or ten (C) passes in the cyclic system.

We performed *Top* and *Tail* experiments with the mobility separated tautomers of curcumin generated from a 100 ppm solution in methanol/water (1:1). Ions representing the first or the second arrival time peak (Figure 6A and 7A) were *sliced* and allowed to run up to 10 additional 10 passes. Since none of the secondary runs produced additional peaks (Figure 6B, C and D, and Figure 7B, C and D), we concluded that no interconversion of keto and enol tautomers occur during time the ions are cycling in the mobility cell.

ION MOBILITY SEPARATION OF PROTOMERS OF 4-NITROANILINE

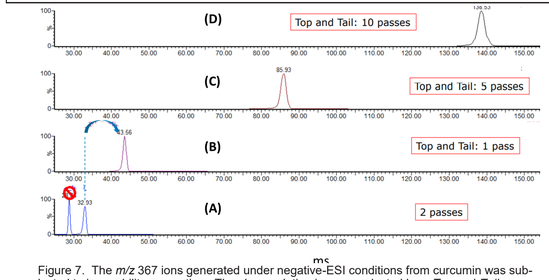

Figure 7. The *m/z* 367 ions generated under negative-ESI conditions from curcumin was subjected to ion mobility separation; The slower eluting ion was selected by a *Top* and *Tail* experiment and subjected to one (B), five (C) or ten (D) passes in the cyclic system.

The mobilogram recorded from the *m/z* 139 ion, generated from a methanolic solution of 4-nitroaniline, under positive-ion electrospray ionization conditions, showed only one arrival-time peak for one pass or two passes within the cyclic ion mobility cell (Figure 8A and C). This peak was recognized to represent the nitro-protonated species. However, when the solution was made in a mixture of methanol and acetonitrile, the mobilogram showed an additional peak (Figure 8B and D). The new peak represents the amino protomer. Evidently, electrospray ionization of 4-nitroaniline shows a pronounced solvent

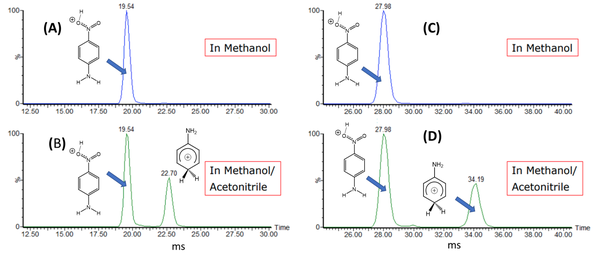

Figure 8. The *m/z* 139 ions generated under positive-ESI conditions from a solution of 4-nitroaniline in methanol (A and C), or a mixture of methanol and acetonitrile (B and D) was subjected to ion mobility separation; Panels A and B represent one pass and panels C and D depicts two passes in the cyclic system.

CONCLUSIONS

- **We performed the separation of tautomeric forms of aniline, curcumin and 4-nitroaniline. Our results demonstrate the power and versatility of the cyclic ion-mobility system for tautomer differentiation.**

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