

**ASMS 2015**

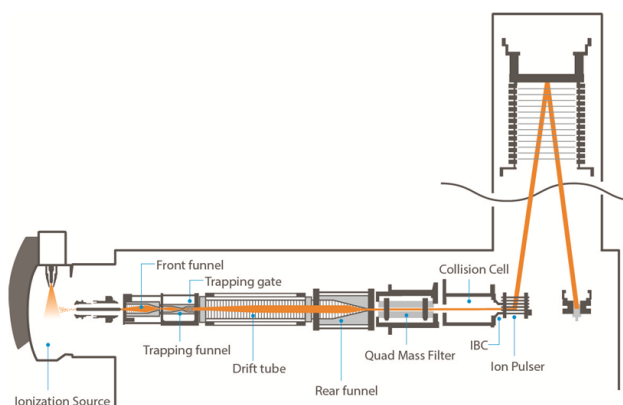
**ThP 641**

Evaluation of the Utility of  
Alternative Drift Gases in a  
Low Pressure  
Conventional Drift Tube  
Ion Mobility MS

Ruwan T. Kurulugama, George  
Stafford, Alex Mordehai, John  
Fjeldsted; Agilent Technologies, Inc.,  
Santa Clara, CA

## Introduction

Helium was used as preferred drift gas in low pressure drift tube ion mobility instruments. This is mainly due to the fact that theoretical collision cross section (CCS) calculations using helium drift gas is relatively well developed and such measurements can be compared with the experimentally obtained CCS values to obtain structural information. Helium provides the most representative CCS values due to its inert nature of the interaction with the analyte ions and its relatively smaller size. Currently, nitrogen is used as the preferred drift gas for commercially available ion mobility-mass spectrometry (IM-MS) instruments. This study evaluates the use of alternative drift gases for complex and difficult to analyze compounds using IM-MS and LC-IM-MS techniques.



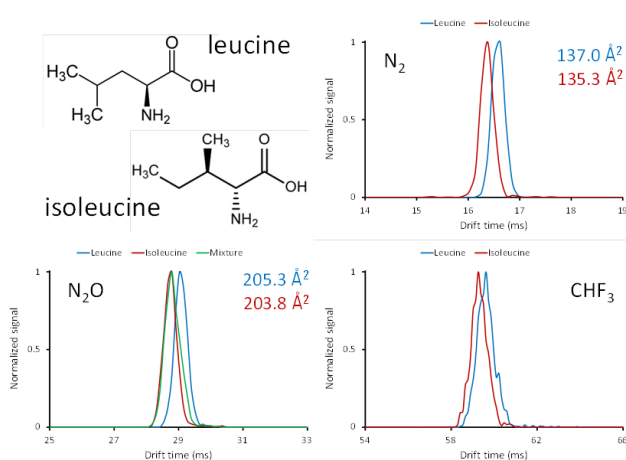
**Figure 1.** Schematic diagram of the Agilent 6560 IM-QTOF mass spectrometer

The IM-MS instrument used in this study is schematically depicted in Figure 1. This instrument consists of a low pressure uniform field drift tube ion mobility apparatus and a high resolution quadrupole time-of-flight (QTOF) mass analyzer. Ions are generated using an AJS source (Agilent Jet Stream, Ion Source). The ions generated at atmospheric pressure are sampled through a single bore glass capillary and transferred into the high pressure ion funnel. This ion funnel efficiently collects the ion signal while allowing to pump away neutrals and gas molecules. The focused ion beam through the transmission geometry ion funnel enters the trapping ion funnel where ions are trapped and periodically released in to the ion mobility cell for drift separation. Mobility separated ions exiting the drift cell are refocused using another ion funnel before the mass analysis. This instrument consists of a quadrupole mass filter for precursor selection and a collision cell for ion fragmentation in All-Ions or tandem MS experiments. The ion mobility resolving power of this instrument for singly charged ions is about 60 (FWHM).

## Experimental

A commercially available drift tube ion mobility mass spectrometer (Agilent Technologies, 6560 IM-QTOF) was used for all experiments [1]. This instrument was fitted with an optional electronic flow controller for automatic pressure regulation and capacitance diaphragm gauges for accurate pressure measurements when using different drift gases. The drift cell is typically operated at slightly higher pressure than the trapping funnel region. This allows to maintain high purity of the drift gas in the drift cell. The drift tube pressure was maintained at 3.94 Torr for all experiments. The pressure difference between the trap funnel and the drift tube was adjusted for each drift gas (150-220 mTorr) to obtain the highest partial pressure of the selected drift gas in the drift tube while maintaining the optimum sensitivity. The LC experiments were carried out using an Agilent 1290 series Infinity II UHPLC system and a 2.1 x 150 mm ZORBAX column with 1.8 micron particles. All samples were purchased from Sigma-Aldrich (St. Louis, MO) or Agilent technologies (Santa Clara, CA). Pesticide samples were prepared in water:MeOH (70:30%, v/v) solution with 100 ppb concentration. Direct infusion experiments were performed for isomeric compounds using pure samples as well as mixtures. Mobility experiments were performed using helium, nitrogen, nitrous oxide, sulfur hexafluoride and  $\text{CHF}_3$  drift gases.

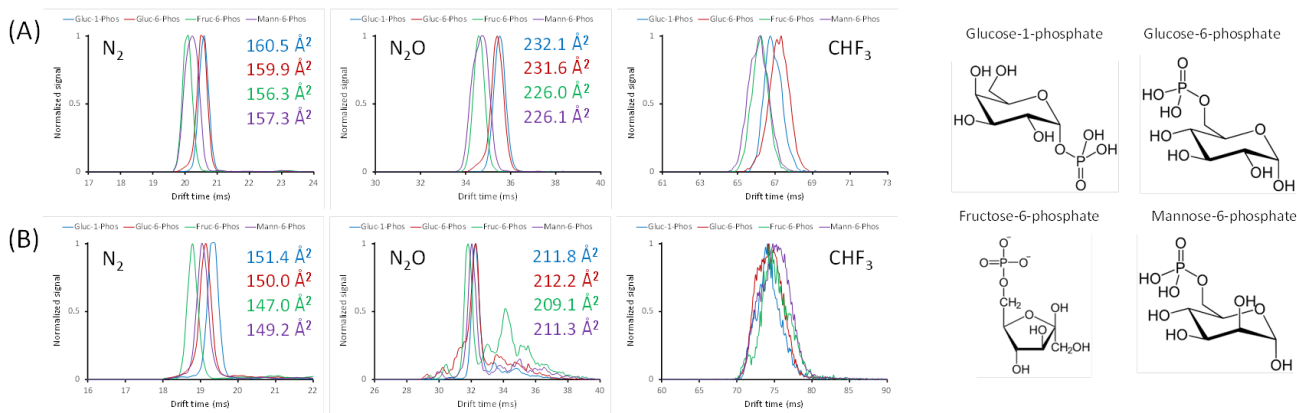
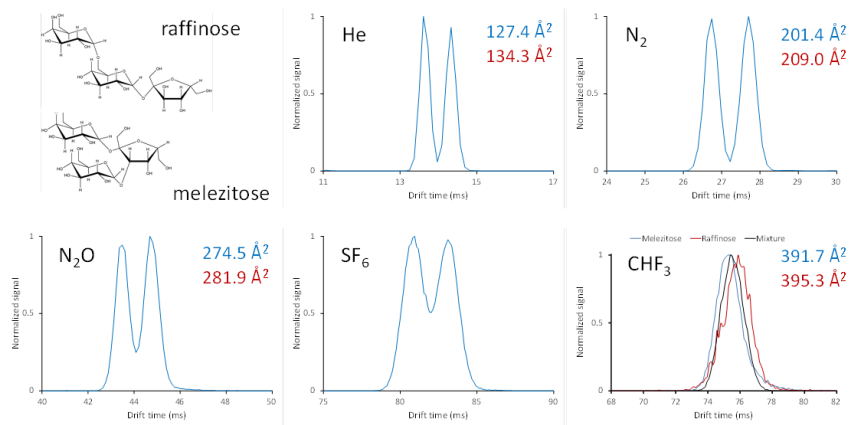
Figure 2 shows the drift time profiles for leucine and isoleucine ( $\text{C}_6\text{H}_{13}\text{NO}_2$ , 131.17 g/mol) singly charged cations using different drift gases. For these two isomeric compounds, nitrogen drift gas provided the best separation, however, in a mixture these two compounds were not separated using any gas employed in this study, as shown in the data for the  $\text{N}_2\text{O}$  experiment. Leucine showed a slightly larger CCS value in all gases.



**Figure 2.** Drift time profiles of leucine and isoleucine using nitrogen,  $\text{N}_2\text{O}$  and  $\text{CHF}_3$  drift gases.

## Results and Discussion

**Figure 3.** Drift time profiles for melezitose and raffinose infused as a mixture using helium, nitrogen, nitrous oxide, sulfur hexafluoride and  $\text{CHF}_3$  drift gases. The drift time profiles shown for  $\text{CHF}_3$  gas was obtained using pure compounds as well as a mixture. These two tri-saccharides have exact same mass and were analyzed as singly charged sodium adducts. Melezitose has a more compact structure and therefore resulted smaller CCS values. The best separation for these two compounds were obtained using helium and nitrogen drift gases. The highest peak resolution was observed for nitrogen (1.31). The peak resolution was calculated using the following equation:  $R_s = 1.18(t_{D2} - t_{D1}) / (w_{h1} + w_{h2})$  where  $t_{D1}$ ,  $t_{D2}$ ,  $w_{h1}$  and  $w_{h2}$  are drift time of peak 1 and peak 2, and FWHM of peak 1 and peak 2 respectively. Here, peak 2 has the longer drift time. The peak resolutions observed for helium,  $\text{N}_2\text{O}$  and  $\text{SF}_6$  are 1.30, 1.01 and 0.65, respectively. No peak separation was observed for  $\text{CHF}_3$  drift gas. However, infusion of pure samples showed that each compound has slightly different drift time, also indicated by the difference in CCS values. The ion mobility resolving power for each of the two peaks with nitrogen drift gas is 61. These results indicate that highly polar drift gases may reduce the selectivity for compounds with similar chemical composition, active groups and surface charge distributions.

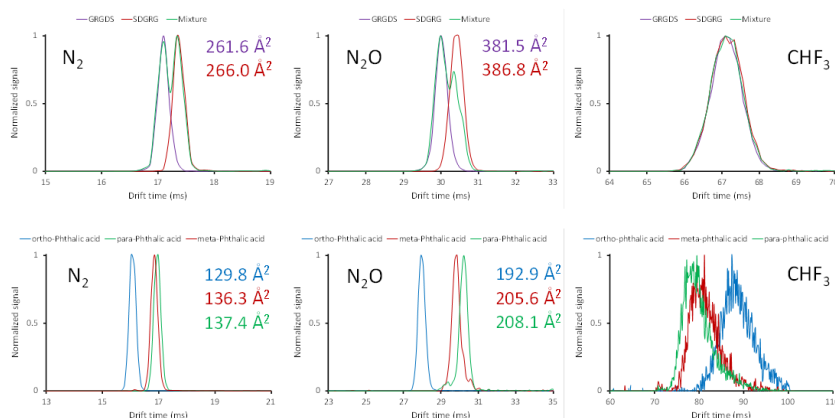
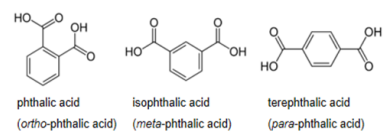


**Figure 4.** Drift time profiles for glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate and mannose-6-phosphate using nitrogen,  $\text{N}_2\text{O}$  and  $\text{CHF}_3$  drift gases, obtained using pure samples. Figure (A) shows data for positive ion mode (singly charged sodiated adduct) and Figure (B) shows data for negative ion mode (singly charged deprotonated). These four compounds are structural isomers with exact same mass ( $\text{C}_6\text{H}_{13}\text{O}_9\text{P}$ , 260.14 g/mol).

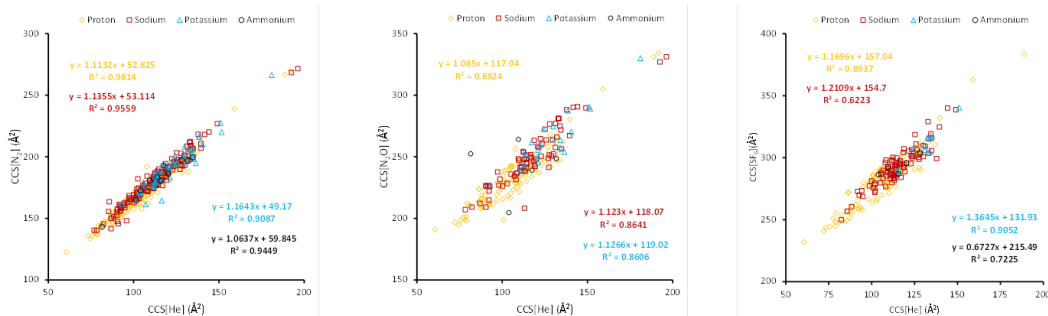
Glucose-1-phosphate, glucose-6-phosphate and mannose-6-phosphate have same six membered ring structure while fructose-6-phosphate has a five membered ring structure resulting a relatively smaller CCS values in nitrogen and  $\text{N}_2\text{O}$  drift gases. However, with the use of more polar  $\text{CHF}_3$  drift gas, the relative drift times and thereby the CCS values for these compounds have changed compared to nitrogen drift gas experiments. Based on the drift time profiles shown here, negative mode experiments in nitrogen drift gas would result the best separation for these compounds in a mixture. Negative mode experiments with  $\text{CHF}_3$  gas resulted broad drift peaks with similar drift times and low signal intensity. For all compounds, negative mode experiments with  $\text{N}_2\text{O}$  gas showed multiple drift peaks in addition to the primary peak.

## Results and Discussion

**Figure 5.** Drift time profiles for GRGDS and SDGRG peptides, analyzed as  $[M + 2H]^{2+}$  ions. These two compounds were separated in a mixture using both nitrogen and  $N_2O$  drift gases. However, the separation obtained for  $N_2O$  is slightly less than that was observed for nitrogen. No separation was observed with  $CHF_3$  drift gas.



**Figure 6.** Drift time profiles for ortho-, meta-, and para-phthalic acids using nitrogen,  $N_2O$  and  $CHF_3$  drift gases. These three compounds are structural isomers with exact same mass ( $C_8H_6O_4$ , 166.14 g/mol). These compounds were analyzed as singly charged deprotonated anions. The best separation was obtained using  $N_2O$  drift gas. Meta and para compounds were not separated from a mixture using any drift gas used in this study. The CCS values for these three compounds in  $N_2$  and  $N_2O$  vary in the following order: ortho < meta < para. However, drift time order is reversed with the  $CHF_3$  drift gas. Although drift time order relative to nitrogen drift gas for individual compounds has shifted with  $CHF_3$  gas, the separation between compounds were not improved.



**Figure 7.** Correlation plots for CCS values between nitrogen, nitrous oxide, and sulfur hexafluoride versus helium drift gas for 275 standard pesticides. The plots include ions containing proton, sodium, potassium and ammonium adducts. Linear regression results for each adduct type are shown. Based on the  $R^2$  values, the nitrogen versus helium plot provided the best correlation. Both carbon dioxide (data not shown) and nitrous oxide had similar correlations with helium CCS values. A lower coefficient of determination was obtained with sulfur hexafluoride versus helium which indicates the possibility of improved selectivity for some compounds. The CCS values for pesticides were obtained using a recently developed single electric field method where a calibration curve is used for rapidly generate CCS values using drift time information.

## Conclusions

In this study, an ion mobility Q-TOF mass spectrometer with the capability to utilize alternative drift gases was used to study the feasibility of employing polarizable drift gases for complex and isomeric sample analyses. The use of highly polarizable drift gases to separate a melzitose and raffinose mixture and two isomeric peptides showed that the separation is not improved for chemically similar ions. This can be explained by the strong interaction between the analyte ions and the drift gas molecules. This interaction essentially reduces the separation achieved purely due to structural differences between analyte ions.

Reference: [1] J. C. May, C. R. Goodwin, N. M. Lareau, K. L. Leaptrot, C. B. Morris, R. T. Kurulugama, A. Mordehai, C. Klein, W. Barry, E. Darland, G. Overney, K. Imatani, G. C. Stafford, J. C. Fjeldsted and J. A. McLean, *Anal. Chem.*, 2014, 86, 2107-2116.