Supporting information for: Atmospheric Pressure Drift Tube Ion Mobility-Orbitrap Mass Spectrometry: Initial Performance Characterization, by Keelor *et al.*

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Standalone AP-DTIMS Characterization: General Parameters

Before AP-DTIMS-Orbitrap MS performance could be evaluated effectively, basic IMS functionality was tested, and the physical parameters known to have the greatest impact on time-dispersive mobility resolution and sensitivity – drift gas temperature, drift potential, and drift gas flow rate – were optimized. During this assessment, each parameter was systematically adjusted while the DTIMS was operated in single-gate acquisition (Faraday) mode using a gate pulse width of 100 µs. A 25 ppm w/v mixture of standard DTIMS calibration compounds comprising nicotinamide (K_0 = 1.85 cm^2 V^{-1} s^{-1}), 2,6-ditertbutyl pyridine (K_0 = 1.42 cm^2 V^{-1} s^{-1}), and trihexylamine (K_0 = 1.06 cm^2 V^{-1} s^{-1}), were used for analysis. The mobility values reported are from the literature [1], but experimental values were verified through a drift time calibration calculation [$K_{0\text{unknown}} = (t_{d\text{unkown}}/t_{d\text{standard}})*K_{0\text{standard}}$] to be within ±2%.

Drift Gas Flow Rate

Drift gas flow dynamics are one of the principal determinants affecting resolving power and sensitivity. With the ESI source equipped, the MA3100 AP-DTIMS module is partially enclosed. At a standard drift gas temperature (180 ˚C) and operation voltage (nominal entrance electrode potential: +8500 V, effective drift potential: +5750 V), the drift gas flow rate was found to have the most immediate effect on performance. On its own, the volume and rate of gas withdrawn by the spectrometer vacuum near the anode did not appear to significantly affect spectra. Rather, it was obvious that drift gas uniformity was influenced not just by the drift gas flow rate, but the rate of gas evacuated by the anterior (ion source) exhaust pump.

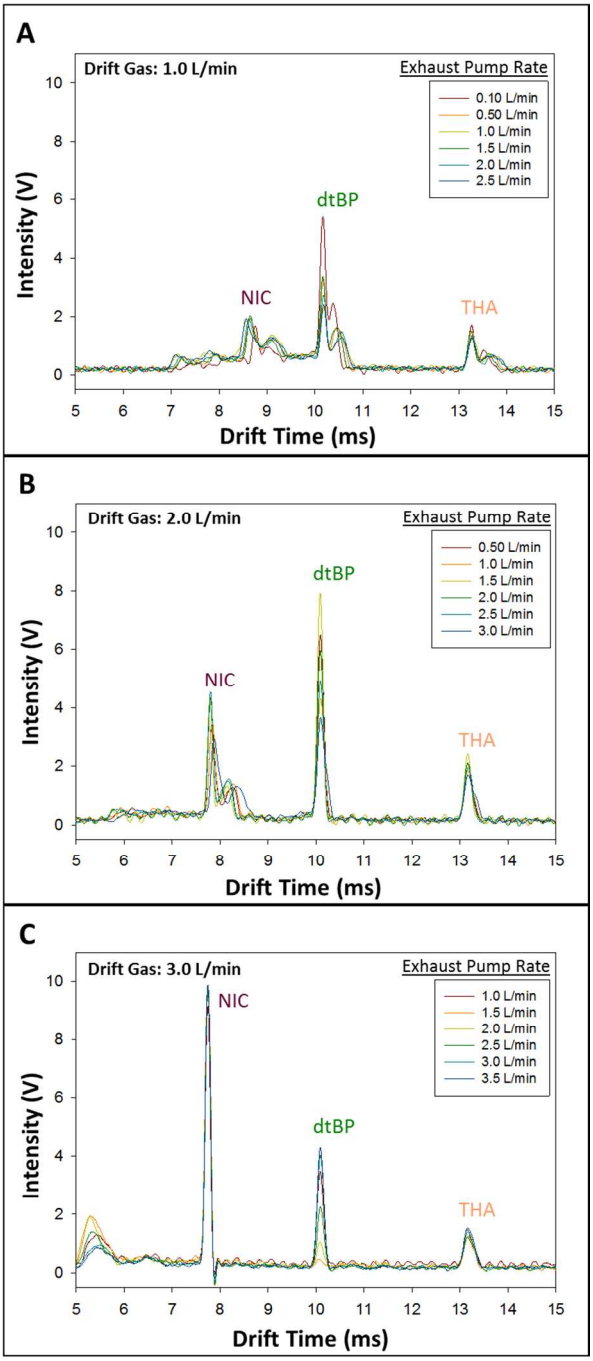

Figure S.1: DTIMS Faraday responses for a standard solution of nicotinamide (NIC), 2,6-dtBP (dtBP), and trihexylamine (THA) 25 ppm w/v in 50:50 methanol/water as a function of drift gas (air) flow rate and exhaust pump rate. Drift gas flow rates were 1.0 L min^{-1} (**A**), 2.0 L min^{-1} (**B**), and 3.0 L min^{-1} (**C**). DTIMS operation potential was set to +8,500 V and drift gas temperature was held at 180℃.

Figure S.1A-C shows Faraday responses for drift air flow rates set between 1.0-3.0 L min^{-1} and different exhaust pumping rates (± 1.0 L min^{-1} from the drift gas rate). For a low drift gas flow rate of 1.0 L min^{-1}, all analyte peaks remained clustered to some extent, as indicated by the broader shoulders detected following each peak, possibly indicative of residual solvation. When selecting for these shoulders with the second DTIMS gate function, the protonated and/or adduct analyte ion masses associated with the adjacent peak drift times (or K_0) were not observed, only background noise, suggesting these shoulders were solvent cluster ions that disappeared when traversing the first differentially pumped stage. Desolvation and peak shape improved with the drift gas held at 2.0 L min^{-1}, where only a small shoulder remained for the first nicotinamide analyte peak. At 2.0 L min^{-1}, if the exhaust pump rate was set higher than the drift gas flow rate, signal was observed to decrease, particularly for the most volatile 2,6-dtBP species. With the drift gas at 3.0 L min^{-1}, desolvation was most effective and signals were clearly resolved. Further increasing the exhaust flow rate had an inconsistent outcome, again, almost singularly affecting signal for 2,6-dtBP. The Faraday response for the 2,6-dtBP analyte largely disappeared with exhaust flow rates between 1-2 L min^{-1}, but was slowly recovered with increasing pump flow rates.

Drift Gas Temperature

As with drift gas flow rate, the drift gas temperature is a critical parameter affecting the gas flow uniformity and ion solvation, which in turn govern resolving power and sensitivity. The MA3100 drift and exhaust gas flow rates were set to intermediate values (2.0 L min^{-1} and 0.5 L min^{-1}, respectively) and the operation voltage was maintained constant (+8500 V). At temperatures below 100℃, Faraday signal was poor to nonexistent for all three analytes, which is likely the consequence of inadequate ESI droplet desolvation. Ramping drift tube temperatures from 100 °C to 240 °C resulted in

an increase in all three analyte responses and a concerted peak shift of ~3 ms to earlier arrival times, as seen in Figure S.2.

Additionally, the nicotinamide shoulder peak was seen to subside as temperature was raised. These findings were congruent with the arguments made above concerning drift gas flow rate, where 2,6-dtBP was the most persistent signal even at lower drift gas temperatures, and the nicotinamide peak grew to dominate the spectrum with increasing temperature. It is important to note that above ~200 °C, the spectrum baseline was observed to elevate by up to ~2-3 V due to an artificial current induced on the warmed Faraday plate detector. Interestingly, resolving power (2,6-dtBP, R_p~70) did not vary appreciably across the temperature range due to increased band broadening with higher temperatures, although a favorable declustering of nicotinamide above 180 °C was observed. Also, peak-to-peak separation for 2,6-dtBP and trihexylamine showed only a minor decrease between 100-240 °C, with peak spacing reduced only by ~0.6 ms. Assuming a Gaussian distribution, the resolution at 240 ˚C was $R_{p\text{-}p}$~10.1. The separation factor (α ~1.29-1.31) was not seen to vary significantly across temperatures.

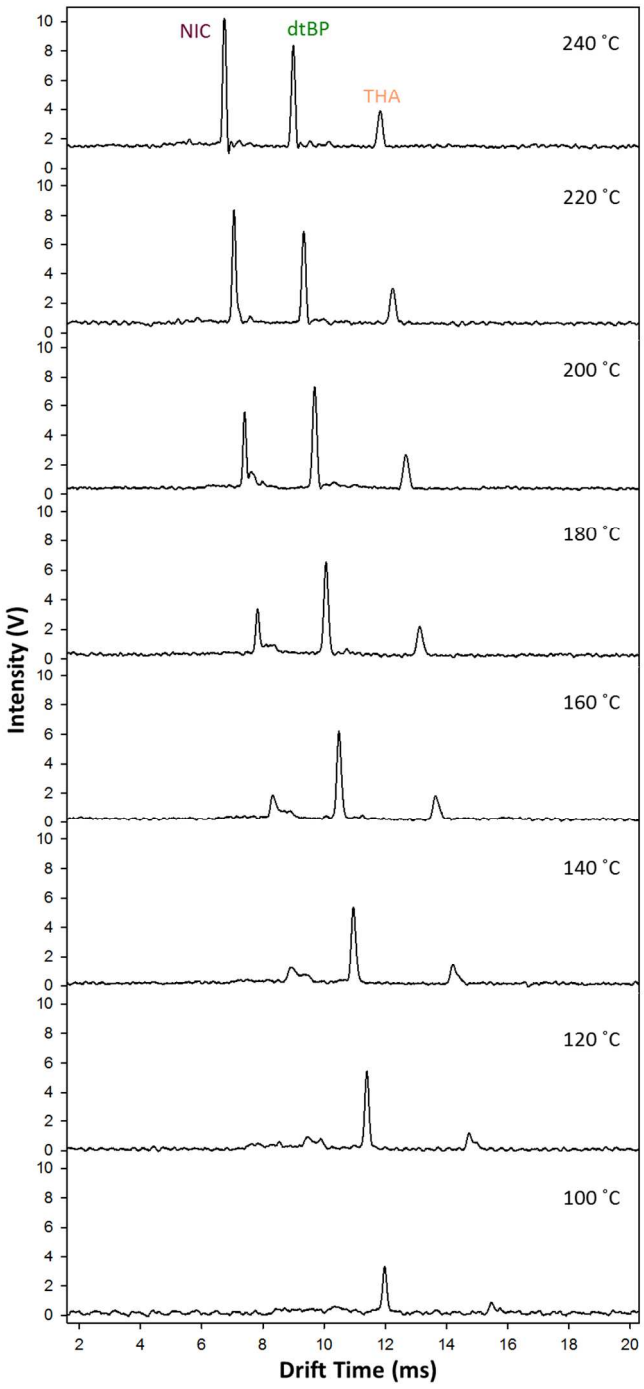

Figure S.2: DTIMS Faraday responses for a standard solution of nicotinamide (NIC), 2,6-dtBP (dtBP), and trihexylamine (THA) 25 ppm w/v in 50:50 methanol/water as a function of drift gas temperature. DTIMS operation potential was set to +8,500 V, and drift gas flow rate and exhaust pump rate were 2.0 L min^{-1} and 0.5 L min^{-1}, respectively.

Drift Tube Potential

The IMS electric field strength defined by the applied drift tube potential was the last parameter examined having a significant impact on both ionic abundances and resolution. The DTIMS temperature was fixed (180 °C) and the drift/exhaust gas flow rates were set again to the optimum values (2.0 L min^{-1} and 0.5 L min^{-1}, respectively). In positive mode, Faraday signal was essentially absent for analytes below an operation potential of +6000 V (approximate drift potential: +3775 V). Shown in Figure S.3, as the operation potential was raised from +6500 V to a maximum of +10,000 V (max effective drift potential: +6300 V), signal for all three analytes was seen to increase owing to the greater electric field strength (max: ~600 V cm^{-1}) that improved transmission and minimized diffusion. Concomitant with the signal increase for all analytes was an expected shift to lower drift times, totaling ~3.5 ms. Unlike what was seen for drift gas flow rate and gas temperature, the degree of solvent clustering did not appear to be affected by increasing drift potentials, as evidenced by the remaining solvent shoulder on the nicotinamide peak. Resolving power improvement was minimal, only rising from R_p~61 to R_p~66 for 2,6-dtBP across the potential range shown, but the change in peak-to-peak separation was greater. Drift time spacing was decreased by 1.1-1.3 ms between adjacent peaks, while the α term again remained constant at ~1.3, translating to FWHM $R_{p\text{-}p}$~9.4 at 10 kV.

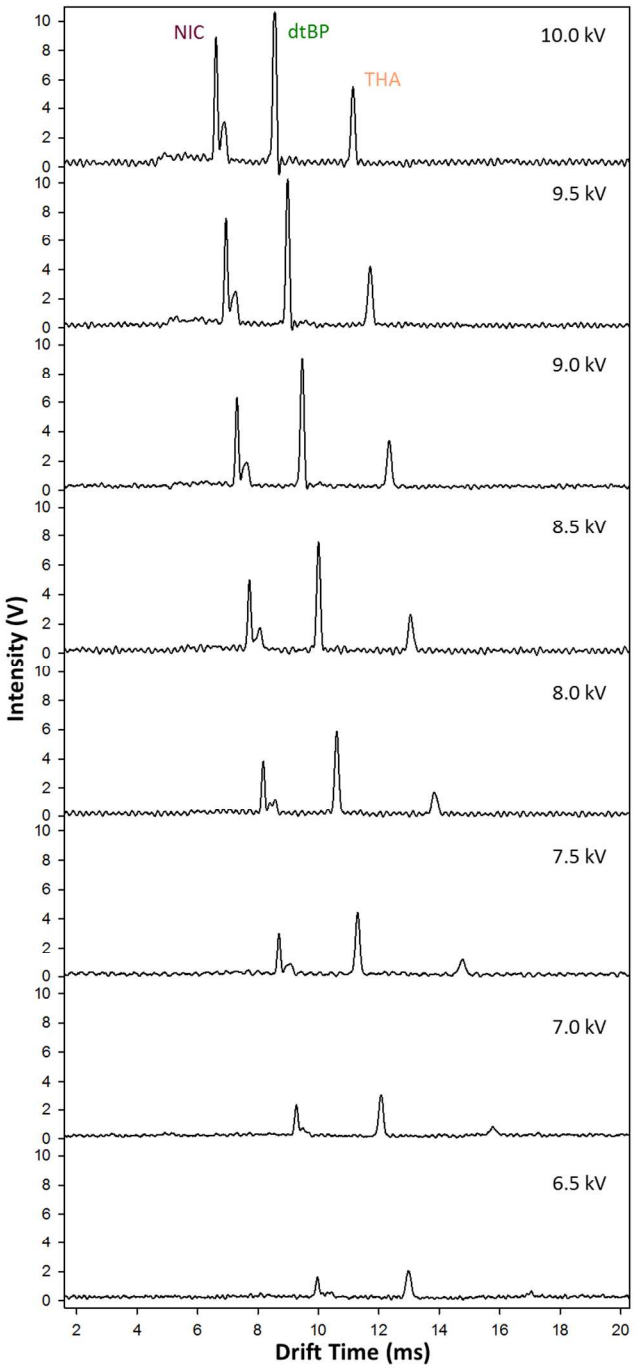

Figure S.3: DTIMS Faraday responses for a standard solution of nicotinamide (NIC), 2,6-dtBP (dtBP), and trihexylamine (THA) 25 ppm w/v in 50:50 methanol/water as a function of DTIMS operation potential. Drift gas temperature was set to 180 ˚C, and drift gas flow rate and exhaust pump rate were 2.0 L min^{-1} and 0.5 L min^{-1}, respectively.

AP-DTIMS-Orbitrap MS Characterization: Effect of Orbitrap Automatic Gain Control and Injection Time

Ideally, for a set mobility scan period of ~20 ms and an injection time of 100 ms, approximately 5 mobility bins for citric acid could be accumulated before mass analysis, while for an IT of 1000 ms, about 50 mobility bins could be accumulated and measured in a single Orbitrap scan. However, during these measurements it is assumed the Orbitrap mass spectrometer acquired continuously at the selected 12 Hz (~83 ms) scan rate determined by the set mass resolution (R=17,500). Therefore the mass analyzer must be measuring "blank" spectra or incomplete (e.g. partial-fill) scans when not exactly synchronized with an injection. Accordingly, the signal intensities appear independent of the IT parameter in Figure S.4 because the average signal intensities (reported over ~110 spectral averages) were essentially the same for either frequent low intensity ion injections (e.g. IT = 100 ms) or fewer higher intensity ion injections (e.g. = 1000 ms). More obvious was the influence of AGC target ion number on the MS signal. For a low target ion number (2.0E+04), higher analyte concentrations triggered fluctuations below the nominal injection time value (IT = 1000 ms), which resulted in more signal detection events per analytical cycle and a larger reported average signal intensity (Figure S.5).

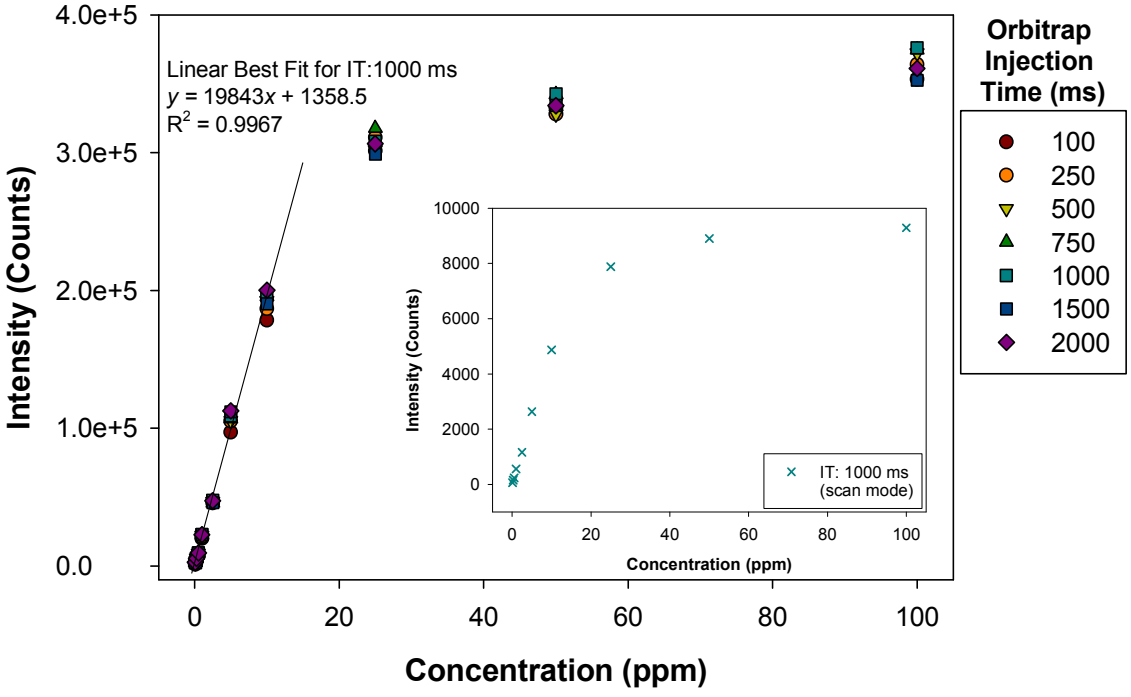

Figure S.4: Citric acid concentration curve for dual-gate AP-DTIMS-Orbitrap MS as a function of Orbitrap maximum injection time (IT). DTIMS gate #1 pulse width was set to 100 µs and gate #2 was fixed open from 8.5-11 ms (2.5 ms window). MS automatic gain control (AGC) was held at 5.0E+06 counts. Each point is the average of over ~110 Orbitrap acquisition scans. A trend-line is drawn for the linear portion of the data (0.1-10 ppm). The inset shows intensities for a scanned-gate acquisition over a 2.5 ms window (gate #1 and #2: 100 µs, scan step: 25 µs).

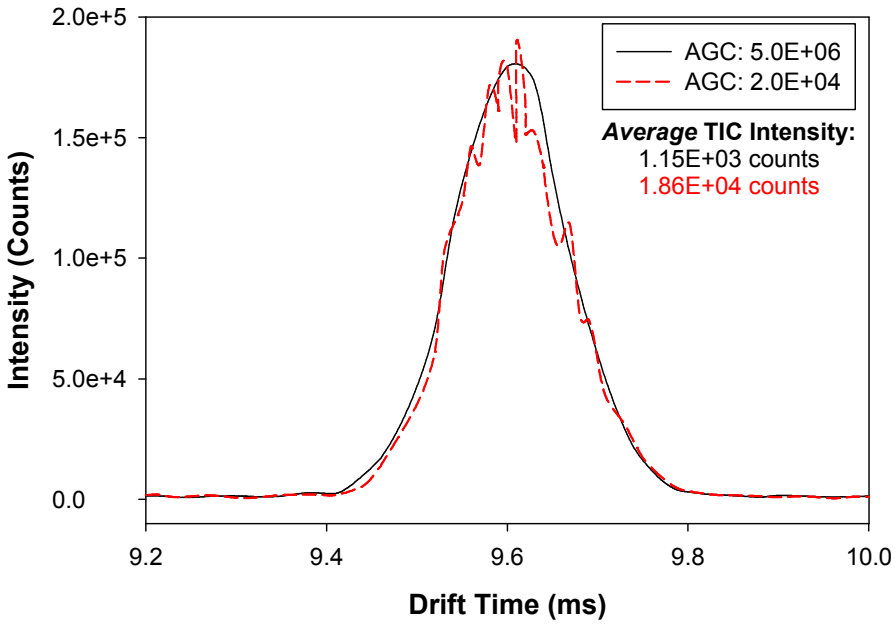

Figure S.5: MS signal traces for scan mode analysis of 25 ppm w/v citric acid using high (5.0E+06) and low (2.0e+04) target ion number settings and a maximum injection time of 1000 ms. The scan window was 2.5 ms (gate #1 & #2: 100 µs, scan step: 25 µs).

References

1. G. Kaur-Atwal, G. O'Connor, A. Aksenov, V. Bocos-Bintintan, C. L. Paul Thomas, C. Creaser. Chemical standards for ion mobility spectrometry: a review. *Int. J. Ion Mobil. Spectrom.* **2009**, *12*, 1-14.