*Electronic Supplemental Information*

# Collision Cross Section Calibrants for Negative Ion Mode Traveling Wave Ion Mobility - Mass Spectrometry

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**Abstract**

Unlike traditional drift-tube ion mobility–mass spectrometry, traveling-wave ion mobility–mass spectrometry typically requires calibration in order to generate collision cross section (CCS) values. Although this has received a significant amount of attention for positive ion mode analysis, little attention has been paid for CCS calibration in negative ion mode. Here, we provide drift-tube CCS values for [M-H]- ions of two calibrant series, polyalanine and polymalic acid, and evaluate both types of calibrants in terms of the accuracy and precision of traveling-wave ion mobility CCS values that they produce.

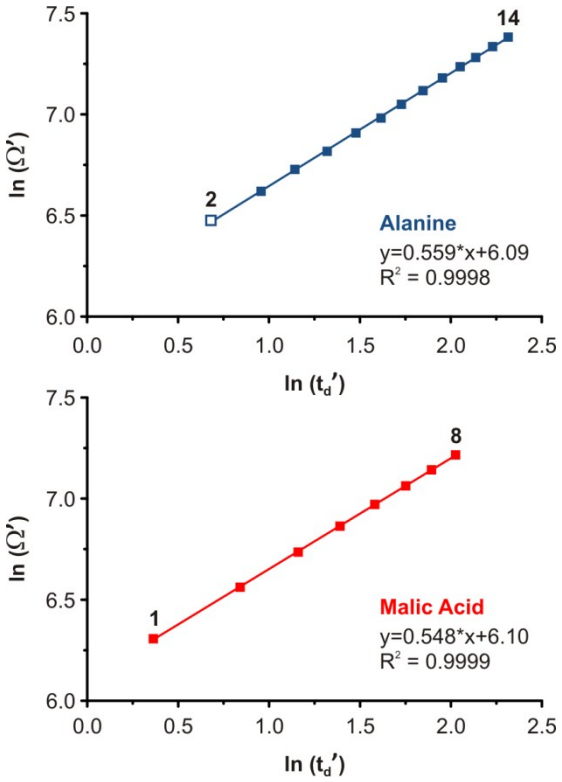


**Figure S1.** TWIM CCS calibration plots for polyalanine (*n*=2-14, blue) and polymalic acid (*n*=1-8, red) using ln ($t_d$') vs. ln (Ω') curves. Normalized CCS values (Ω') correspond to CCS values in Å$^2$ (all DTIM except for dialanine, unshaded) divided by both charge state and the inverse square root of reduced mass. Corrected drift times correspond to mass-independent flight times.

| Calibrant | m/z | DTIM CCS ($N_2$) | TWIM CCS ($N_2$, cross-cal.) | SD (n=5) | % Diff. (TWIM:DTIM) |
|---|---|---|---|---|---|
| $(Ala)_2$ | 159.08 | | 133.0 | 0.2 | |
| $(Ala)_3$ | 230.11 | 150 | 151.1 | 0.6 | +0.76% |
| $(Ala)_4$ | 301.15 | 165 | 165.6 | 0.3 | +0.38% |
| $(Ala)_5$ | 372.19 | 179 | 180.9 | 0.1 | +1.06% |
| $(Ala)_6$ | 443.23 | 195 | 196.2 | 0.1 | +0.62% |
| $(Ala)_7$ | 514.26 | 209 | 210.6 | 0.1 | +0.75% |
| $(Ala)_8$ | 585.30 | 223 | 223.3 | 0.1 | +0.15% |
| $(Ala)_9$ | 656.34 | 238 | 237.8 | 0.1 | -0.10% |
| $(Ala)_{10}$ | 727.37 | 253 | 251.5 | 0.2 | -0.58% |
| $(Ala)_{11}$ | 798.41 | 267 | 265.0 | 0.2 | -0.76% |
| $(Ala)_{12}$ | 869.45 | 279 | 277.0 | 0.3 | -0.71% |
| $(Ala)_{13}$ | 940.49 | 294 | 291.0 | 0.4 | -1.01% |
| $(Ala)_{14}$ | 1011.52 | 308 | 304.7 | 0.5 | -1.07% |
| | | | | | |
| $(MA)_1$ | 133.01 | 114 | 112.1 | 0.5 | -1.64% |
| $(MA)_2$ | 249.02 | 141 | 140.0 | 0.3 | -0.68% |
| $(MA)_3$ | 365.03 | 165 | 164.9 | 0.2 | -0.04% |
| $(MA)_4$ | 481.04 | 186 | 185.8 | 0.2 | -0.10% |
| $(MA)_5$ | 597.06 | 206 | 205.9 | 0.2 | -0.05% |
| $(MA)_6$ | 713.06 | 225 | 225.2 | 0.3 | +0.11% |
| $(MA)_7$ | 829.08 | 243 | 243.5 | 0.2 | +0.19% |
| $(MA)_8$ | 945.08 | 261 | 261.4 | 0.3 | +0.13% |

**Table S1**. Experimental and theoretical CCS values of polyalanine and polymalic acid using ln ($t_d'$) vs. ln ($\Omega'$) calibration curves as shown in Figure S1. CCS values are reported in Å$^2$ and m/z values correspond to $[M-H]^-$ ions. Polyalanine TWIM values were obtained by using polymalic acid as the calibrant, and polymalic acid TWIM values were obtained by using polyalanine as the calibrant. All differences between TWIM and experimental DTIM ($N_2$) values are less than ± 2.0%. Nevertheless, a systematic trend is observed in which polymalic acid calibration overestimates the CCS of small species and underestimates the CCS of large species. The reverse trend is observed for polyalanine calibration as well, although it is less pronounced – likely due to the wider calibration range of polyalanine. This systematic bias was reduced when using Belehradek power functions to form calibration curves (Figure 3, main text).

| Species | ion | m/z | TWIM CCS (PolyA cal) | TWIM CCS (PolyMA cal) |
|---|---|---|---|---|
| $(LA)_4$ | $[M-H]^-$ | 305.09 | 162.5 | 164.2 |
| $(LA)_5$ | $[M-H]^-$ | 377.11 | 177.6 | 179.0 |
| $(LA)_6$ | $[M-H]^-$ | 449.13 | 192.1 | 193.1 |
| $(LA)_7$ | $[M-H]^-$ | 521.15 | 208.5 | 209.0 |
| $(LA)_8$ | $[M-H]^-$ | 593.17 | 224.7 | 224.8 |
| $(LA)_9$ | $[M-H]^-$ | 665.19 | 239.3 | 238.9 |
| $(LA)_{10}$ | $[M-H]^-$ | 737.21 | 254.3 | 253.3 |
| $(LA)_{11}$ | $[M-H]^-$ | 809.23 | 268.5 | 267.1 |
| $(LA)_{12}$ | $[M-H]^-$ | 881.26 | 282.2 | 280.3 |
| $(LA)_{13}$ | $[M-H]^-$ | 953.28 | 295.4 | 293.0 |
| $(LA)_{14}$ | $[M-H]^-$ | 1025.30 | 308.2 | 305.2 |

**Table S2**. Experimental TWIM CCS values of polylactic acid (*n*=4-14) in $N_2$ drift gas. Polylactic acid is the ester-analog of polyalanine. Polylactic acid was directly infused from a 90% acetonitrile / 10% water solution. CCS values are reported in $Å^2$ and *m/z* values correspond to $[M-H]^-$.

| Species | ion | m/z | TWIM CCS (PolyA cal) | TWIM CCS (PolyMA cal) |
|---|---|---|---|---|
| $(Asp)_4$ | $[M-H]^-$ | 247.06 | 145.6 | 147.7 |
| $(Asp)_5$ | $[M-H]^-$ | 362.08 | 165.9 | 167.6 |
| $(Asp)_6$ | $[M-H]^-$ | 477.11 | 190.5 | 191.6 |
| $(Asp)_7$ | $[M-H]^-$ | 592.14 | 211.5 | 211.9 |
| $(Asp)_8$ | $[M-H]^-$ | 707.16 | 231.0 | 230.8 |
| $(Asp)_9$ | $[M-H]^-$ | 822.19 | 248.3 | 247.6 |
| $(Asp)_{10}$ | $[M-H]^-$ | 937.22 | 264.8 | 263.5 |
| $(Asp)_{11}$ | $[M-H]^-$ | 1052.24 | 285.4 | 283.3 |

**Table S3**. Experimental TWIM CCS values of polyaspartic acid (*n*=4-11) in $N_2$ drift gas. Polyaspartic acid was directly infused from aqueous solution. CCS values are reported in $Å^2$ and *m/z* values correspond to $[M-H]^-$.