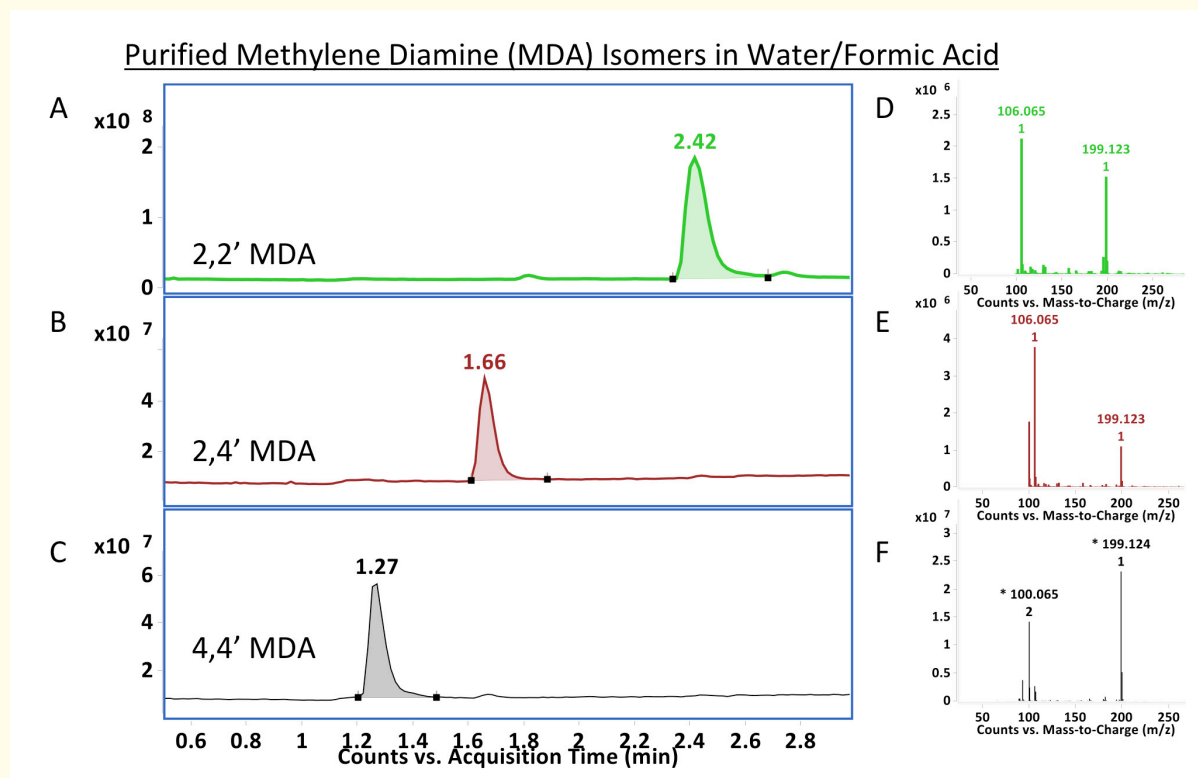


## Supplemental Materials and Methods

### Development of a standard curve for quantifying different isomers of methylene diphenyl diamine (MDA)

A standard curve for quantifying different MDA isomers was developed using purified 2,2', 2,4' and 4,4' MDA obtained from the International Isocyanate Institute (Boonton, New Jersey). Stock solutions of different MDA isomers (10 mg/mL) were prepared by dissolution in acetonitrile, and standard curves were generated by spiking 0.001 to 100  $\mu$ M MDA into quenched samples (1:10 dilution in water/0.1% formic acid) of respective MDI-GSH reactions (120 minute time point) as described in the main Materials and Methods section 3A. The area under the curve (AUC) of extracted ion chromatograms (EIC) for the expected 199.12  $m/z$   $[M+H]^+$  with the defined retention time for each isomer (see figure 15) was plotted, following subtraction of baseline signal from the GSH-MDI reactions. Linear regression of  $\log_{10}$  transformed data from four separate experiments yielded an excellent fit over a roughly 6-log fold range (0.001 to 100  $\mu$ M). Limit of detection was defined based on signal-to-noise ratio  $> 3.0$  as recommended by MacDougall, *et al* [33]. Assessment of MDA levels in reactions of 2,2' and 2,4' MDI with GSH were based on the AUC of 199.12  $m/z$   $[M+H]^+$  ions with retention time defined by standards (1.69 - 1.74 for 2,4' MDA and 2.43 - 2.45 for 2,2' MDA). Analysis excluded a neighboring 199.03  $m/z$   $[M+H]^+$  ion present in control samples without MDI. Analyses obtained similar results when focused on  $[M+H]^+$  ions with a 106.06  $m/z$ , based on prior publications [34] suggesting that 2,2' and 2,4' MDA isomers may fragment during LC-MS analysis, yielding higher amounts of a 106.06  $m/z$   $[M+H]^+$  fragment.



**Figure 15:** LC-MS TIC of purified of methylene diphenyl diamine standards. (A-C) Different MDA isomer standards as labeled were first diluted in acetonitrile and then to 10  $\mu$ M in water/formic acid for LC-MS analysis, and preliminary assessment of their expected retention times in water/formic acid buffer. Note retention time for 2,2' MDA  $>$  2,4' MDA  $>$  4,4' MDA. (D-E) MS analysis of the  $[M+H]^+$  ions eluting at the peak retention time defined in the corresponding LC-MS TIC on left. Note by LC-MS, the 2,2' and 2,4' MDA are detectable intact as a 199.123  $[M+H]^+$   $m/z$ , along with a 106.07  $[M+H]^+$   $m/z$  fragment as previously described by Forsythe *et al* [34], upon direct injection. The 4,4' MDA is detectable as its intact 199.12  $m/z$   $[M+H]^+$  ion and as its corresponding  $[M+2H]^{2+}$  ion with the same nominal mass.