Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

UPLC-MS/MS Method for Quantitative Determination of Advanced Glycation Endproducts

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Custom Pipettor

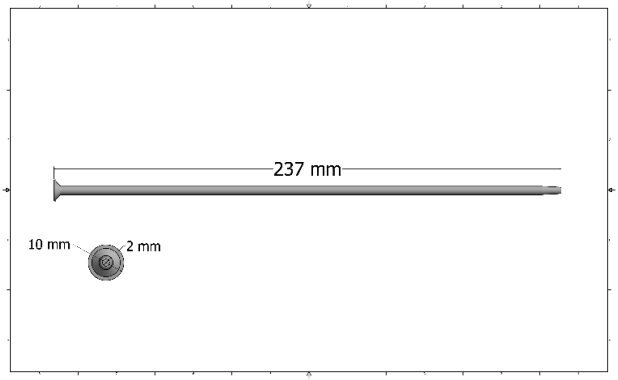

Figure S1. Engineering sketch of custom glass pipettor. The device was customized to fit inside a 6mL Thermo Fisher Scientific vacuum tube to reach the hydrolyzed sample at the bottom. It is made from glass and is 237 mm in length, the interior diameter is 2 mm and the top diameter to attach a rubber pipette bulb is 10 mm.

The custom-made glass pipettor was created out of a need to extract the samples from the acid hydrolysis tubing. Since the samples were at the bottom of a 6 mL vacuum tube and the shape of the tubing did not allow for commonly purchased laboratory equipment, such as 9” Pasteur pipettes, syringes, and serological pipettes, there was no straightforward way to remove the sample without contamination or loss. There are no approved or suggested materials to extract the samples without “free pouring” out into sample collection tubes. Using the design of smaller pipettes and rubber pipette bulbs, the glass shop used the measurements of the vacuum tubing and collaborated to design simple, effective, and reusable glass pipettes that could easily reach the bottom of the tubing and extract the sample. It was important to include glass to limit corrosion overtime with weekly 6M HCl exposure.

Blank Samples

The blank samples after running unknown sample isomers are presented below. There are unknown samples (top) and the corresponding internal standards (bottom) for the three analytes discussed.

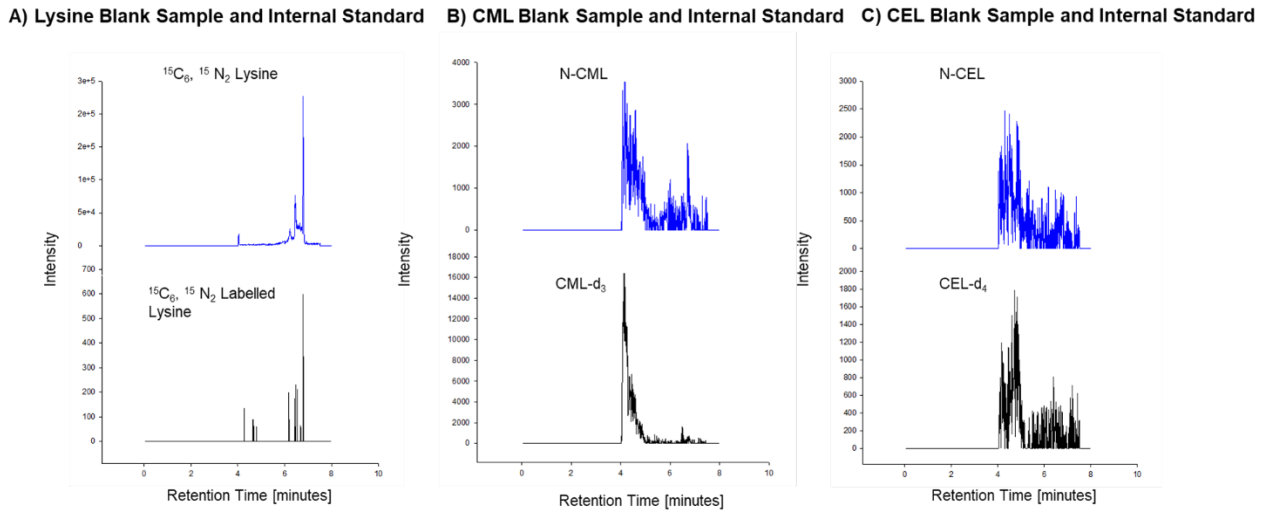

Figure S2. Chromatograms for blank analyte samples and internal standards showing analytes were successfully removed off the column in the LC-MS/MS method. **A)** Lysine chromatogram. *Above* unknown lysine has noticeably less signal than from previous standards and samples with additional isomer peaks showing unclear separation. *Below* labelled lysine internal standard eluting at the same time as above unknown sample in blank with significantly less peak intensity. **B)** CML chromatogram. *Above* unknown CML eluting does not show a clear analyte peak, indicating a lack of sample remaining on the column. *Below* labelled CML eluting with significantly less peak intensity than previous standards and samples. **C)** CEL chromatogram. *Above* unknown CEL does not show clear separation or retention time for analyte. *Below* labelled CEL eluting with significantly less peak intensity than previous standards and samples.

Selectivity

The selectivity of the method for the separation of the isomers is presented below. There is a clear separation of the analytes, the retention time correlated to the labeled IS, and shows good peak shape.

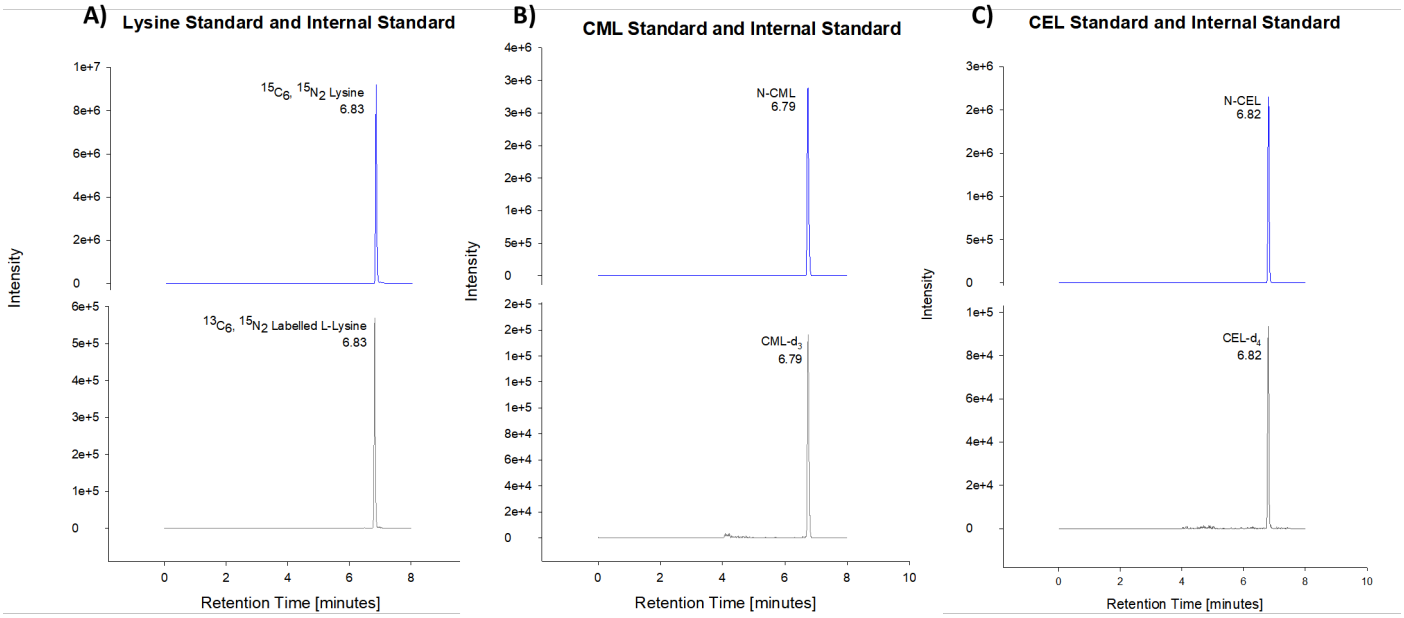

Figure S3. Chromatograms for analyte standards and internal standards showing excellent selectivity of LC-MS/MS method. **A)** Lysine chromatogram for 512 nM calibration curve. *Above* standard lysine eluting at 6.83 minutes with good peak shape and limited tailing. *Below* labelled lysine internal standard eluting at the same time of 6.83 minutes with good peak shape and limited tailing. **B)** CML chromatogram for 512 nM calibration curve. *Above* standard CML eluting at 6.79 minutes with good peak shape and limited tailing. *Below* labelled CML eluting at the same time of 6.79 minutes with good peak shape and limited tailing. **C)** CEL chromatogram for 512 nM calibration curve. *Above* standard CEL eluting at 6.82 minutes with good peak shape and limited tailing. *Below* labelled CEL eluting at the same time of 6.82 minutes with good peak shape and limited tailing.

Maillard Reaction for Formation of CML and CEL

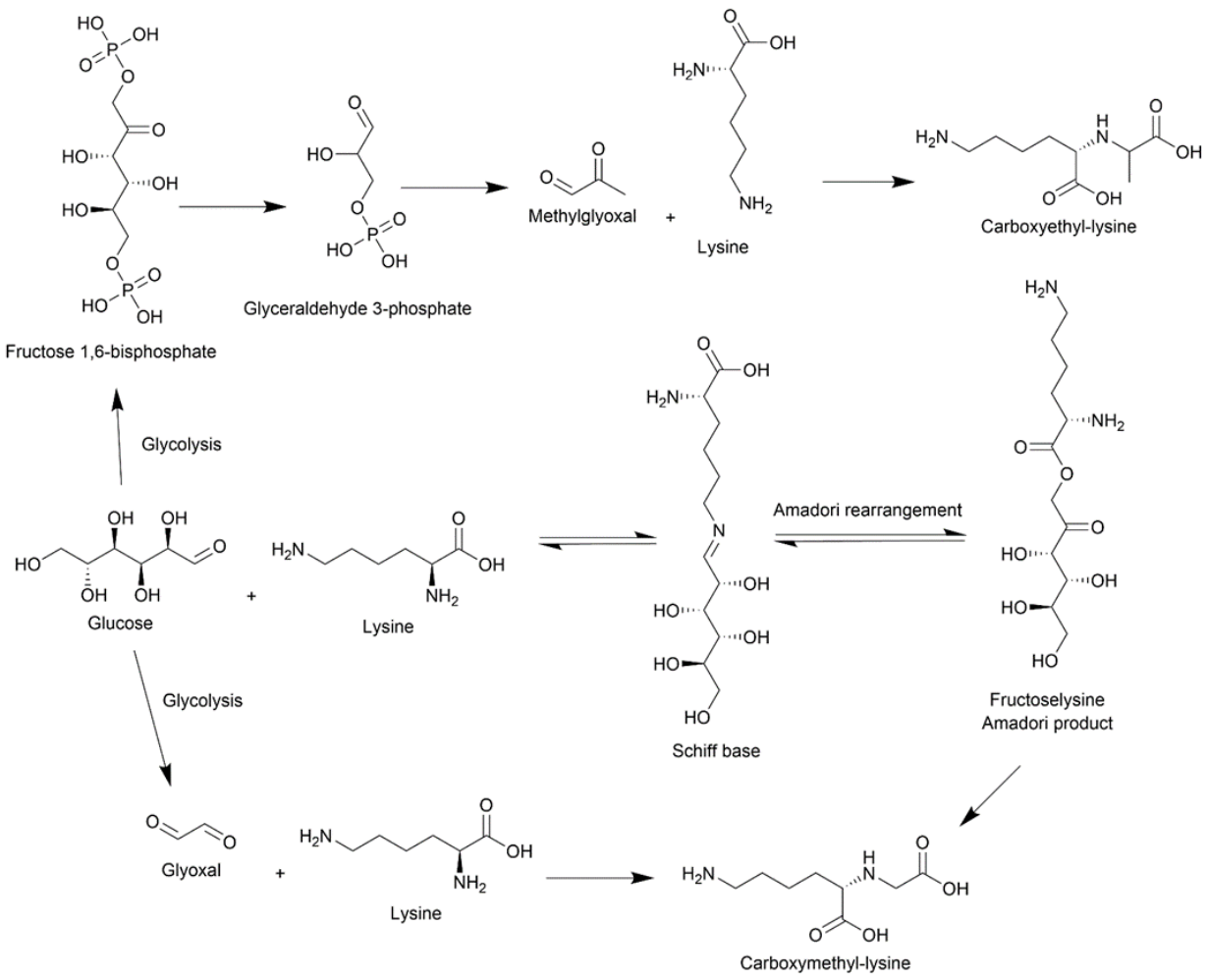

Figure S4. Proposed major pathways of AGE formation. CML formation from glyoxal intermediate and fructoselysine and CEL formation from methylglyoxal intermediate and lysine.