Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for:

Relative quantification of amine-containing metabolites using isobaric *N,N*-dimethyl-leucine (DiLeu) reagents via LC-ESI-MS/MS and CE-ESI-MS/MS

Ling Hao[a], Xuefei Zhong[a], Tyler Greer[b], Hui Ye[c], Lingjun Li*[ab]

[a]School of Pharmacy, University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI, 53705, USA

[b]Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, WI 53706, USA

[c]China Pharmaceutical University, Nanjing, 210009, China

*Corresponding author

Tel.: +1 (608) 265-8491

Fax: +1 (608) 262-5345

E-mail: lingjun.li@wisc.edu

Supporting Information

115	116	117	118
x_{115}= 92.36%	x_{116}= 81.25%	x_{117}= 87.86%	x_{118}= 94.05%
z_{115}= 0.50%	z_{116}= 13.64%	z_{117}= 5.56%	z_{118}= 0.41%
y_{115}= 7.14%	y_{116}= 5.11%	y_{117}= 5.98%	y_{118}= 5.54%

$$S_{115} = x_{115} I_{115} + z_{116} I_{116}$$
$$S_{116} = x_{116} I_{116} + z_{117} I_{117} + y_{115} I_{115}$$
$$S_{117} = x_{117} I_{117} + z_{118} I_{118} + y_{116} I_{116}$$
$$S_{118} = x_{118} I_{118} + y_{117} I_{117}$$

Fig. S1 Correction factors and equations for purity correction of DiLeu reporter ion intensities. x_n is the percent purity of reporter ion, y_n is the percent impurity of -1 Da interference ion, and z_n is the percent impurity of +1 Da interference ion. S_n represents the signal intensity of reporter ion in a raw MS/MS spectrum. I_n represents the actual intensity of reporter ion which can be resolved with the equation implemented in an excel spreadsheet. I_{115}, I_{116}, I_{117}, and I_{118} can then be utilized to calculate the intensity ratios of DiLeu reporter ions for relative quantification.

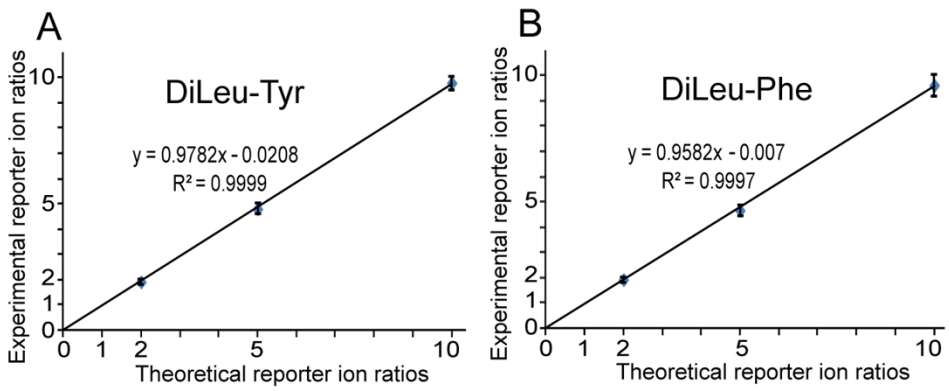

Fig. S2 Examples of linear regression for the experimental to theoretical intensity ratios of DiLeu reporter ions for relative quantification. Each data point with an error bar (standard deviation) represents the average ratio from three replicates obtained from the 1:5:2:10 ratio labeling experiment.

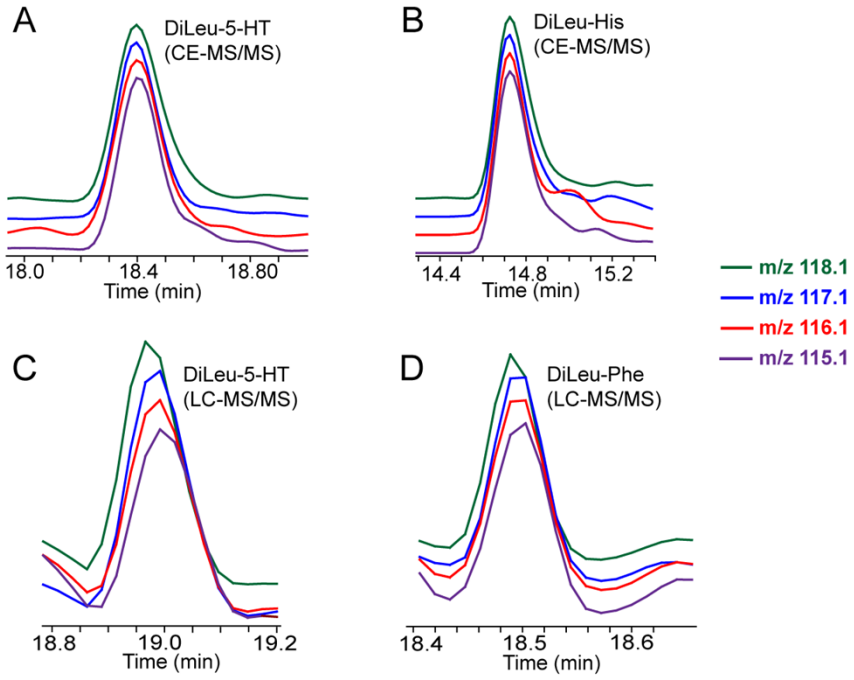

Fig. S3 Extracted ion electropherograms (A, B) and extracted ion chromatograms (C, D) of reporter ions (*m/z* 115.1, 116.1, 117.1, 118.1) in CE-SRM and LC-SRM analysis of DiLeu labeled metabolites. For clarity, each trace offsets by 10% along the y-axis of intensity. 4-plex DiLeu labeled metabolites co-migrated during CE separation (A, B). Negligible retention time difference among the 4-plex DiLeu labeled metabolites was observed in LC separation (C, D).

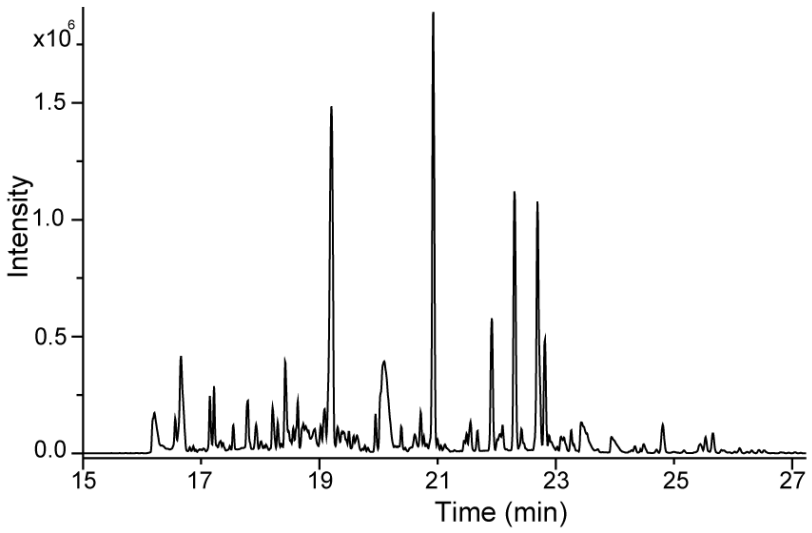

Fig. S4 Base peak ion chromatogram of 4-plex DiLeu labeled mouse urine.

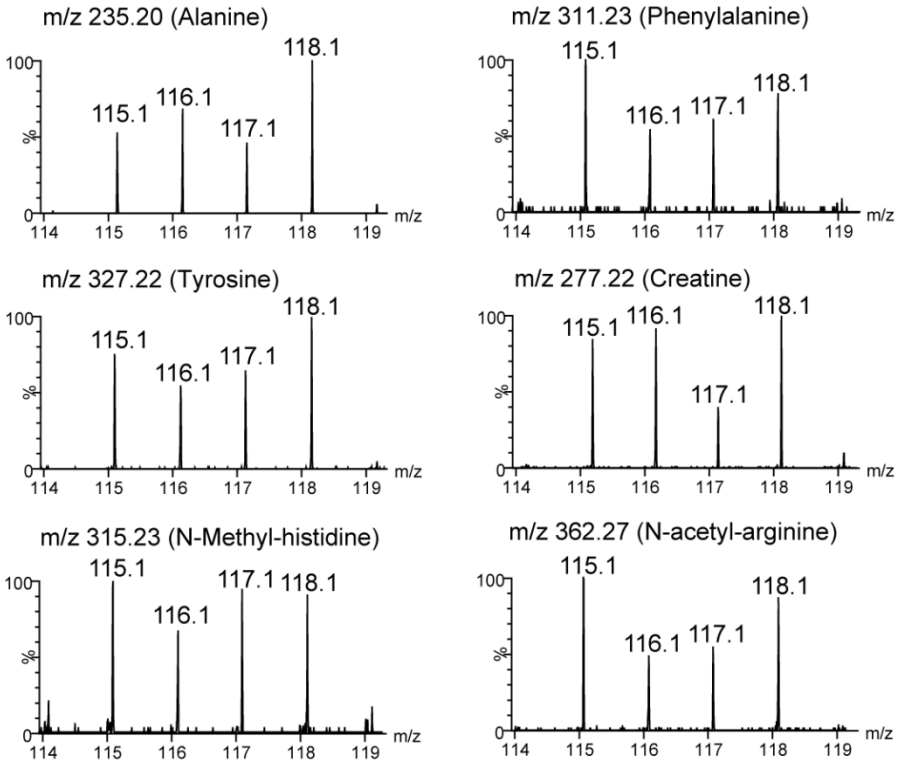

Fig. S5 Examples of MS/MS spectra (reporter ions region) of DiLeu labeled metabolites from mouse urine samples.