Research Article to be submitted to

Analyst

Supplemental Information

Multi-Functional Group Derivatization of Amines, Hydroxyls, and Carboxylates for Metabolomic Investigations of Human Tissue by Electrospray Ionization Mass Spectrometry

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Figure S1. Dimethylaminoacetyl chloride tagging of amine and hydroxyl groups. Time course of tagged glycine and threonine. The signal response was determined from reconstituted ion chromatograms. Results were determined by mean ± SD from 3 independent samples.

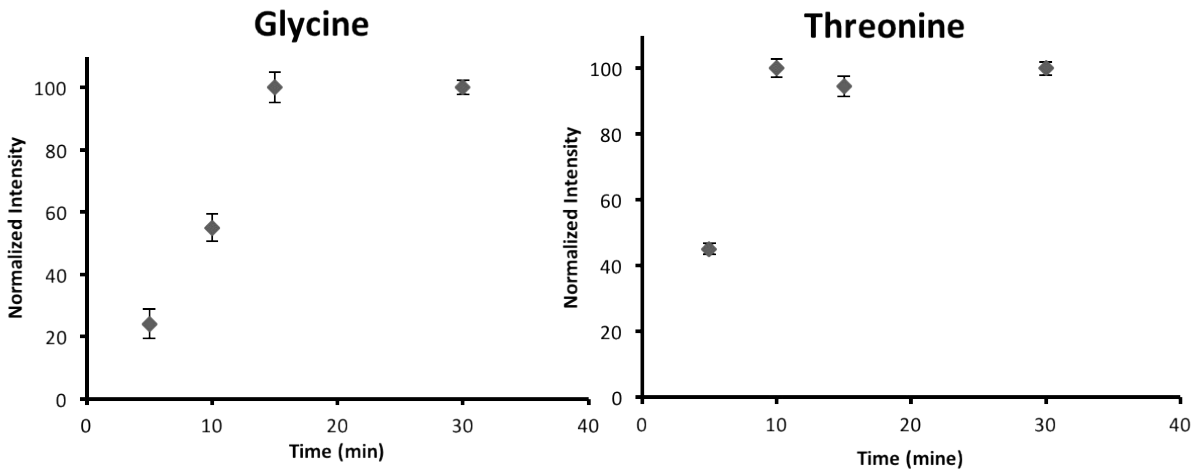

Figure S2. Dimethylaminoacetyl chloride and N, N-Diethylethylenediamine two step tagging of amine and carboxylate groups. Time course of tagged glycine and succinate. The signal response was determined from reconstituted ion chromatograms. Results were determined by mean ± SD from 3 independent samples.

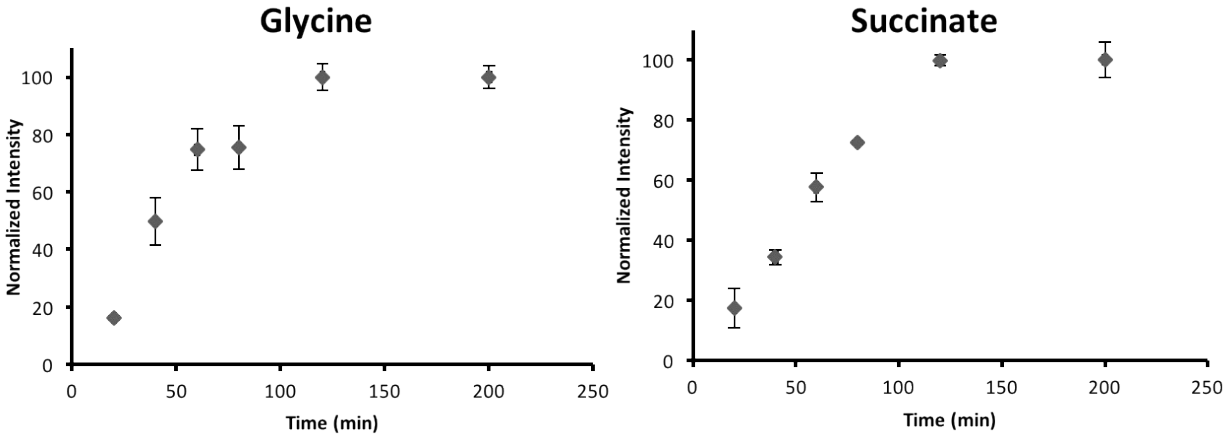

Figure S3. Comparison of Dimethylaminoacetyl chloride tagging and Dimethylaminoacetyl chloride and N, N-Diethylethylenediamine two step tagging. The signal response was determined from reconstituted ion chromatograms. The concentration of standards is 5µM. Results were determined by mean ± SD from 3 independent samples, n=3.

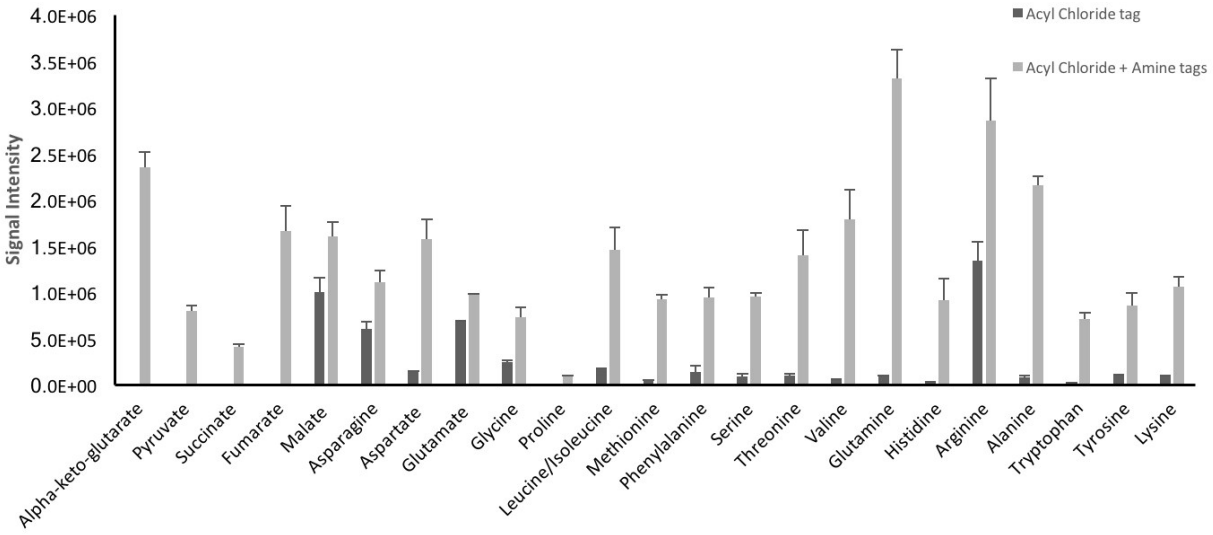

Figure S4. Reconstituted ion chromatogram of LC-MS/MS parent ion dependent scan (TSQ Quantum Access MAX, Thermo Scientific) for methionine and methionine-d_3 (S-methyl-d_3). Black denotes total ion chromatogram of fragmentation for parent ions range from 150-300 m/zs with product scanning mass of 73 and 86 m/z. These m/z (73 and 86) are indicative of compounds which contain the tag. Green and red denotes reconstituted ion chromatograms of both deuterated and non-deuterated methionine. Green denotes expected methionine-d_3 (25 µM) and red denotes methionine (75 µM) parent masses. Chromatograms A and B are normalized to the highest intensity peak of the respective chromatogram and set to 100%. (A) Dimethylaminoacetyl chloride reaction of methionine and methionine-d_3 (S-methyl-d_3). Peaks at 12.5 and 13.7 min are hydrolyzed tag. Peaks at 20.5 min shows detection of both deuterated and non-deuterated methionine. (B) Dimethylaminoacetyl chloride and N, N-Diethylethylenediamine two step tagging of methionine and methionine-d_3 (S-methyl-d_3). Peaks at 13.4 and 16.3 are the result of the acyl chloride tag and the amine tag reacting with each other. Each chromatographic peak was carefully examined for a difference of 3Da. Only derivatized analytes of interests showed 3 Da difference. Taken together, these data indicate that no detectable analyte side reactions are occurring in this system. Chromatograms C and D correspond to A and B above, but without normalization. (C)Normalized Chromatograms of Dimethylaminoacetyl chloride labeled deuterated methionine and methionine. (D) Normalized chromatograms of Dimethylaminoacetyl chloride and N, N-Diethylethylenediamine two step tagged deuterated methionine and methionine.

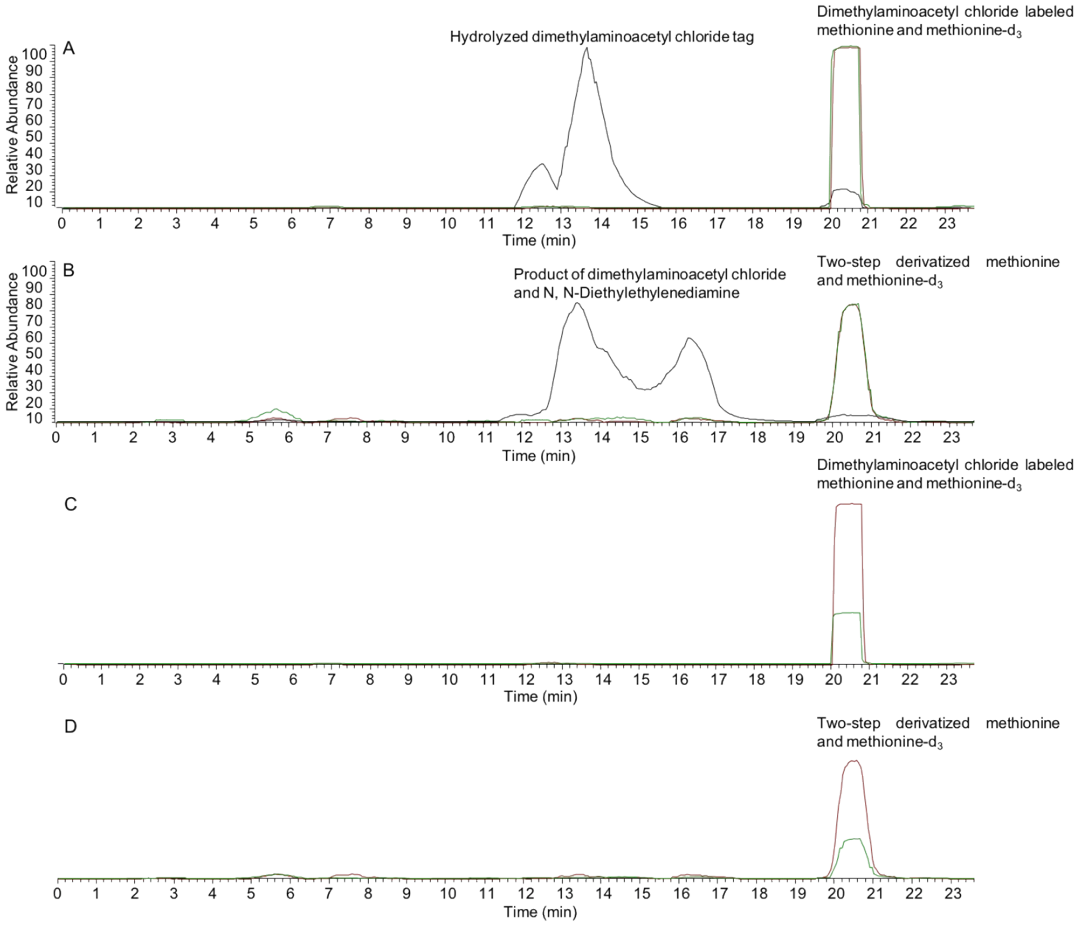

Table S1: Interfering solvent and reagent m/z and interfered analytes

Interfering solvent and reagent	m/z	Interfered analytes
DMSO	157.50-158.50	2 tagged succinate m/z 158.14, fumarate m/z 157.13
		2 tagged leucine/isoleucine m/z 158.14
HOAt	136.50-137.50	2 tagged alanine m/z 137.12

Table S2: Number of tags and number of functional groups that were labeled in each metabolite

	number of tags	number of hydroxyl/primary amine/carboxylate groups
Alpha-keto-glutarate	2	2
Pyruvate	1	1
Succinate	2	2
Fumarate	2	2
Malate	3	3
Asparagine	2	2
Aspartate	3	3
Glutamate	3	3
Glycine	2	2
Proline	1	1
Leucine/Isoleucine	2	2
Methionine	2	2
Phenylalanine	2	2
Serine	3	3
Threonine	3	3
Valine	2	2
Glutamine	2	2
Histidine	2	2
Arginine	2	2
Alanine	2	2
Tryptophan	2	2
Tyrosine	3	3
Lysine	3	3