

# Quantitative Analysis of the Human Metabolome by Differential Dimethyl Labeling of Amine-containing Metabolites Using LC-MS



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## Overview

### Purpose

- We seek a method to globally quantify amine-containing metabolites in human urine and other human body fluids.

### Methods

- Differential dimethyl labeling of amine-containing metabolites in urine using  $^{12}\text{C}$ - and  $^{13}\text{C}$ -formaldehyde.
- Comparative quantification of amine-containing metabolites in human urine sample using a Bruker EsquireLC ion trap LC-MS and a Mariner LC-MS with a TOF analyzer.

### Results

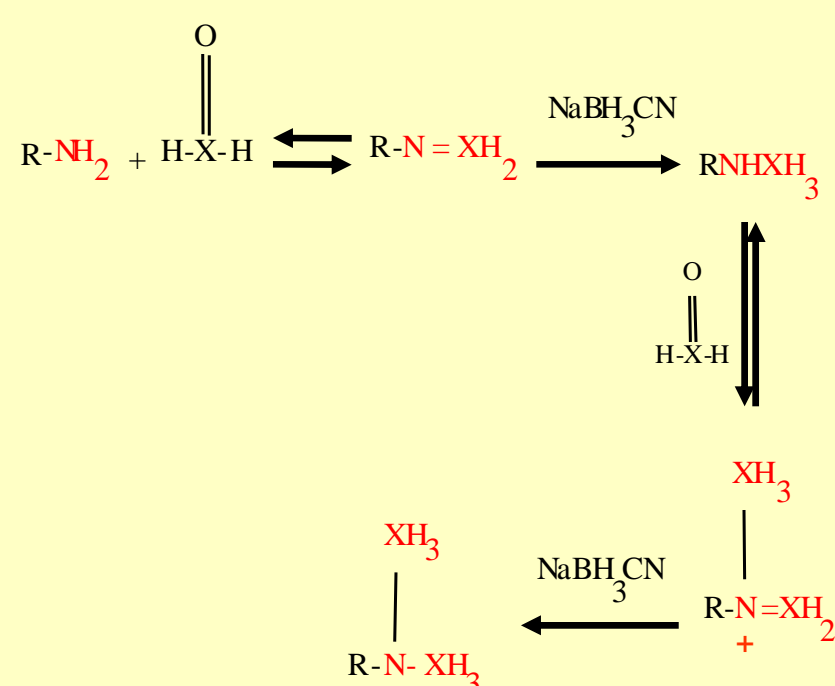
- More than 100  $^{12}\text{C}$ - and  $^{13}\text{C}$ -dimethyl labeled peak pairs were observed.
- The isotope effect observed in both reversed phase and HILIC chromatography was minimum.
- The preliminary identification of metabolites can be obtained by the accurate mass measurement by LC ESI-TOF.

## Introduction

Metabolome analysis is complementary to genomics and proteomics in the elucidation of gene function. In contrast to the proteome or transcriptome, the metabolome is more diverse in chemical and physical properties because of the large variations in atomic arrangements. Studies of the metabolome include the analysis of a wide range of chemical species, from low molecular weight non-polar volatiles to high molecular weight polar glucosides, non-polar lipids and inorganic species.

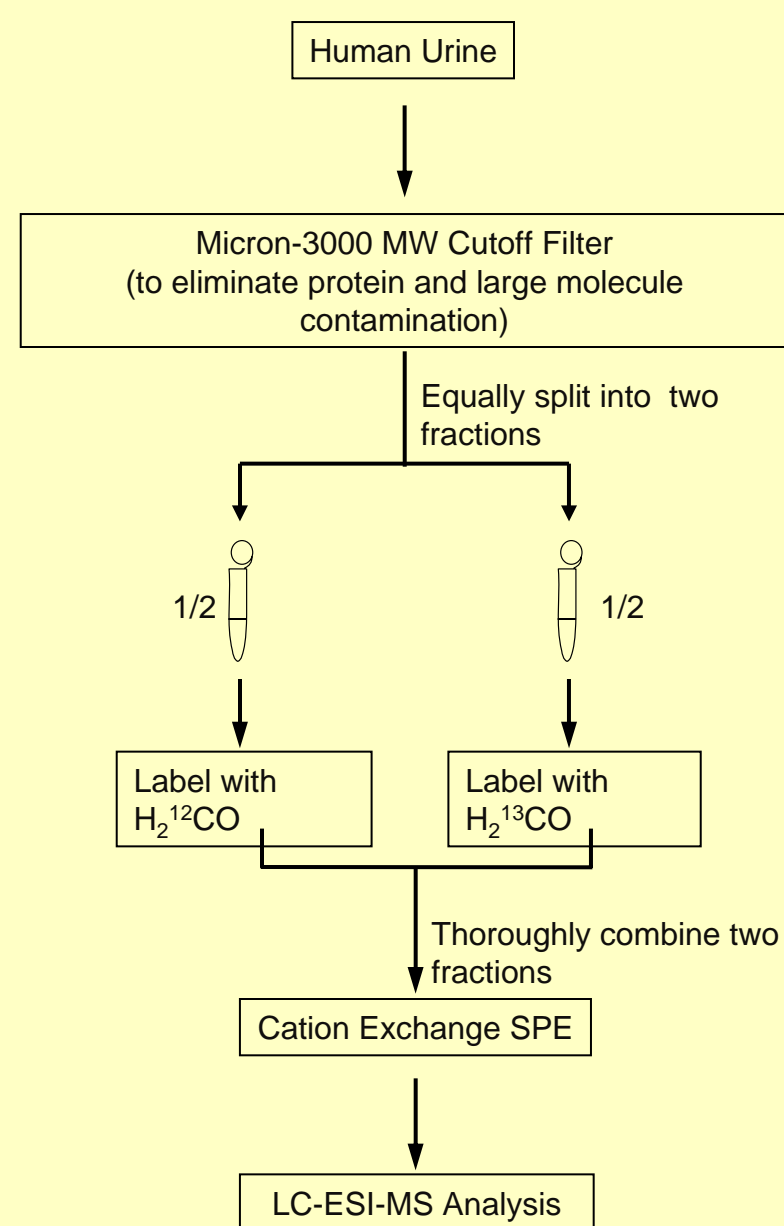
Ion suppression has deleterious effects in quantitation using LC-MS. Stable isotope dilution-based comparative quantification is one of the most important methods for overcoming ionization suppression. The stable isotope dilution method is also useful for normalization of variation in the process of sample extraction and derivatization. We are interested in developing stable isotope dilution-based quantification methods for metabolomics. Differential dimethyl labeling of N-termini peptides with  $\text{d}(0)$  and  $\text{d}(2)$ -formaldehyde combined with LC-ESI or LC-MALDI have been used for quantitation of proteins. In this work, we demonstrate that a similar derivatization method can be used to uniformly label and globally quantify amine-containing metabolites in human urine, including both primary and secondary amines. The labeling reaction is a very simple, fast, and easy chemical derivatization protocol. Using the retention time of known metabolites and high resolution, high mass accuracy instruments such as ESI-TOF or FT-ICR, identification of metabolites can be achieved with high confidence.

## Experimental



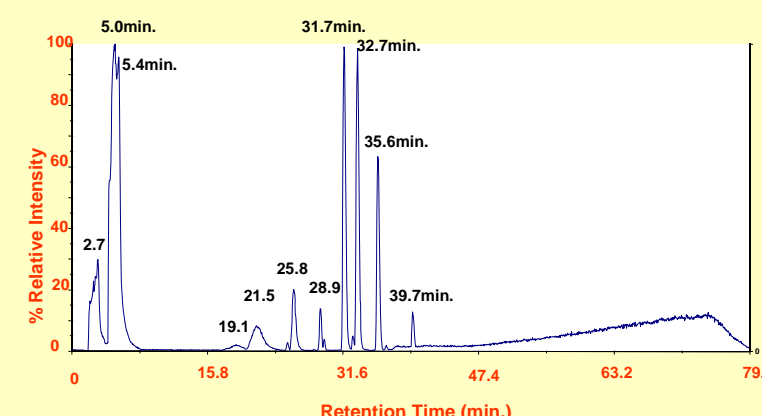
**Figure 1.** Differential Dimethyl Isotopic Labeling Strategy.

X:  $^{12}\text{C}$  or  $^{13}\text{C}$ , Mass difference:  $2.012 \cdot n$   
n: the number of labeling sites

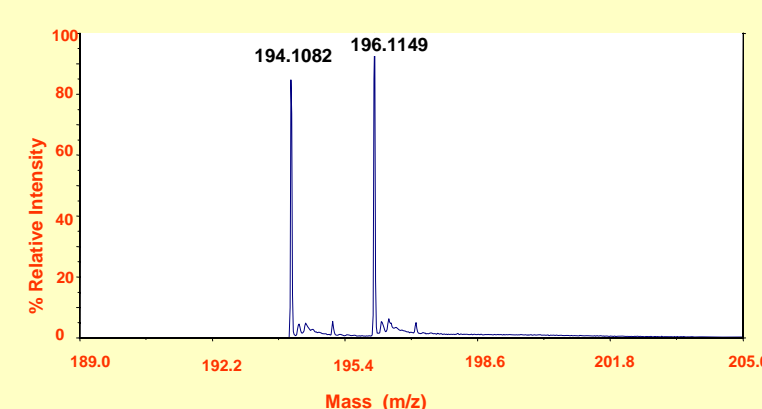


**Figure 2.** Workflow for quantitative analysis of amine-containing metabolites using differential dimethyl isotope labeling and LC-ESI-MS

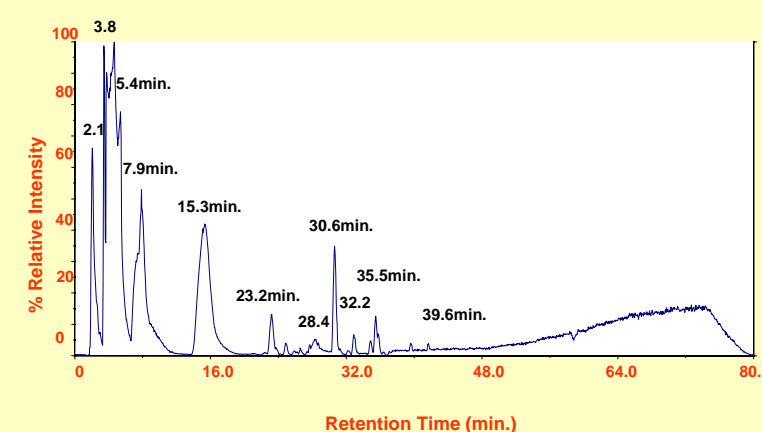
## Results and Discussion



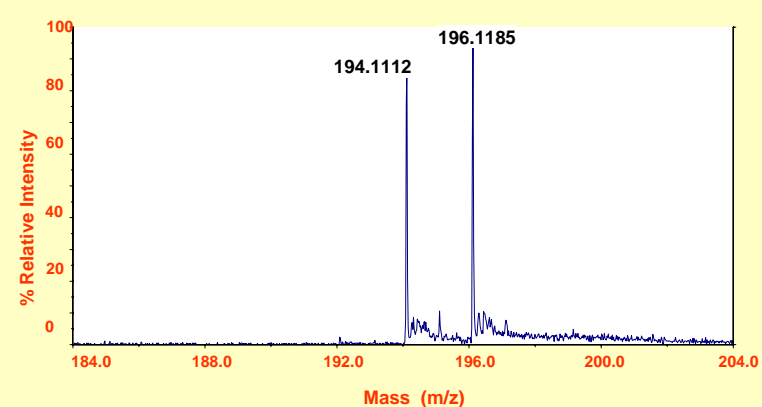
**Figure 3.** RP-HPLC base peak chromatogram of dimethyl labeled amino acid standards.



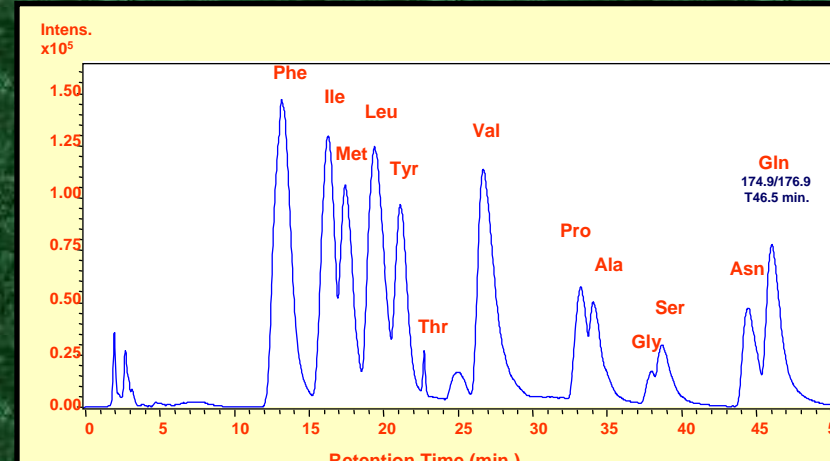
**Figure 4.** Mass spectrum of phenylalanine at retention time of 35.6 min. for dimethyl labeled amino acid standards.



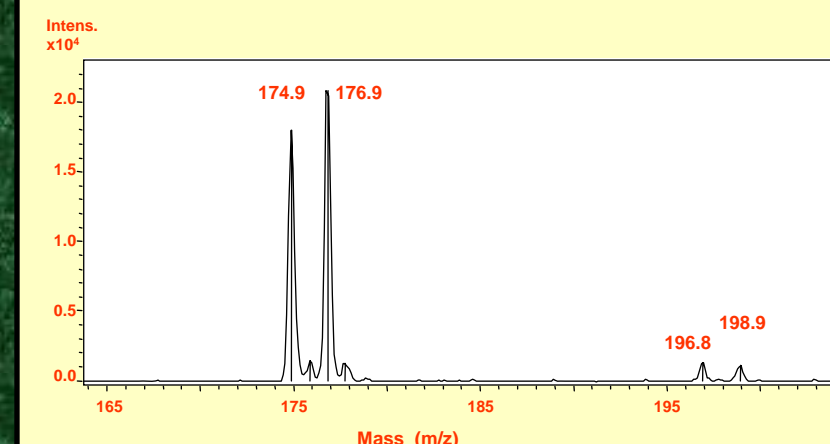
**Figure 5.** RP-HPLC base peak chromatogram of dimethyl labeled human urine after cation exchange SPE purification.



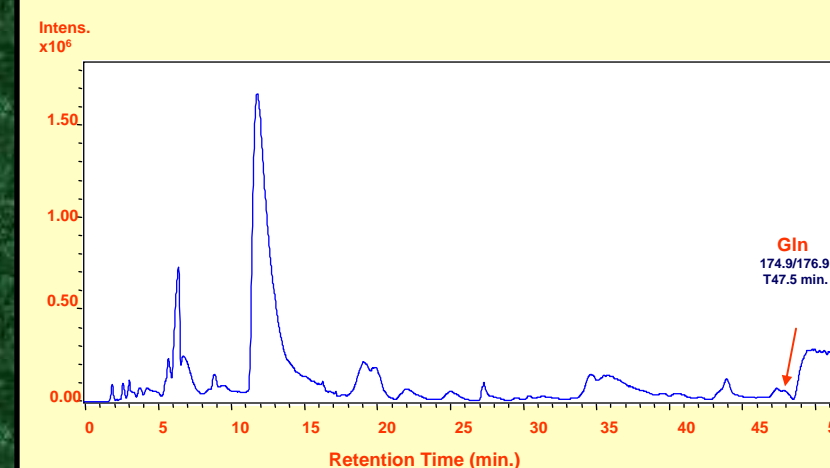
**Figure 6.** Mass spectrum at retention time of 35.5 min. (identify as phenylalanine) for the dimethyl labeled human urine after cation exchange SPE purification.



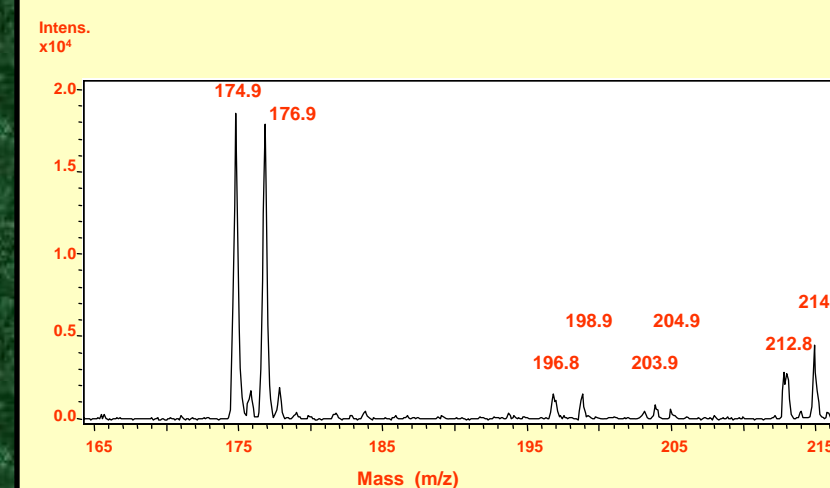
**Figure 7.** HILIC base peak chromatogram of dimethyl labeled amino acid standards.



**Figure 8.** Mass spectrum of glutamine at retention time of 46.5 min. for dimethyl labeled amino acid standards.



**Figure 9.** HILIC base peak chromatogram of dimethyl labeled human urine after cation exchange SPE purification.



**Figure 10.** Mass spectrum at retention time of 47.5 min. (preliminarily identify as glutamine) for the dimethyl labeled human urine after cation exchange SPE purification.

- The relative quantification of metabolites can be performed by calculating the relative intensities of the peaks for  $^{12}\text{C}$  and  $^{13}\text{C}$  dimethylated amine-containing metabolites.
- Hydrophilic interaction chromatography (HILIC) was used to separate highly polar metabolites that hardly retain on conventional RP stationary phases. The enhanced ESI-MS sensitivity of polar metabolites was observed.
- Absolute quantification of the known metabolites can be performed if metabolite standards are available.
- More than 100 of  $^{12}\text{C}$  and  $^{13}\text{C}$  dimethylated peak pairs are observed. Therefore, potentially more than 100 of amine-containing metabolites in human urine could be quantified using this protocol based on one dimensional LC-MS.
- A comparison work using a deuterium labeled methylation method showed an adverse isotope effect on reverse phase chromatography of up to 30 second difference in retention time. The  $^{12}\text{C}$  and  $^{13}\text{C}$ -dimethylated amine-containing metabolites were shown to be eluted with the same retention time, indicating no isotope effect during both reversed phase and hydrophilic interaction chromatography.
- Ionization efficiency of the  $^{12}\text{C}$  and  $^{13}\text{C}$  dimethylated-isotopomers was also investigated. Experimental data showed that the degree of ionization efficiency for the  $^{12}\text{C}$  and  $^{13}\text{C}$ -isotopomers is essentially the same in urine matrix.
- Ionization efficiency of some of metabolites was observed to be enhanced after dimethyl derivatization.
- The preliminary identification of unknown metabolites can be obtained by accurate mass measurement by Mariner EIS-TOF LC/MS. The highly reliable characteristic of reversed phase LC retention time of metabolites can be used as additional information to confirm the identification.
- The dimethyl labeling method can also provide additional information about the functional groups of metabolites. This can improve the confidence of metabolite identification.

## Conclusions

- The matrix effect can be dramatically reduced in LC-MS using this stable isotope dilution-based method. This offers an improvement in quantitative analytical accuracy, especially for samples with a complex matrix.
- This differential dimethyl labeling method is a simple, fast, and easy chemical derivatization protocol and can be readily adopted for other biological fluids in the qualitative and quantitative study of amine-containing metabolites. This can potentially facilitate biomarker discovery by combining information from genomics and proteomics.

## Acknowledgement

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