Determination of 90 amine-containing metabolites in biological samples using the SCIEX Triple Quad 4500 system

Chen Huimin, Long Zhimin, Guo Lihai
SCIEX, Asia Pacific Application Support Center, Shanghai, China

ABSTRACT

Amino acids and their amine metabolite derivatives are common biomarkers monitored for understanding human physiological processes in biomarker discovery and biological studies. In this study, we report a metabolome profiling technique for analyzing metabolites containing amines using a targeted analysis method using the SCIEX Triple Quad 4500 system. A chemical derivatization strategy based on dansylation reaction was explored, which increases the retention capacity of amines in reversed-phase chromatography and improves the ionization efficiency in mass spectrometry. We developed a fast quantitative method to detect 90 amine-containing metabolites (including amino acids and their methylated and acetylated products) from complex biological matrices in 15 minutes.

INTRODUCTION

Amino acids and their amine metabolite derivatives are common biomarkers monitored for understanding human physiological processes in biomarker discovery and biological studies. Many studies have found significantly higher levels of polyamines and their metabolites present in the biological fluids and affected tissues of cancer patients and other patients with hyperproliferative diseases. Therapeutic polyamine analogues have been shown to be potentially useful in treating cancer and other hyperproliferative disorders. Quantifying amine containing metabolites could potentially be applied to monitor tumor growth and regression in cancer studies.[1-2]

The challenge for metabolome profiling lies in the analysis of the large portion of highly polar metabolites. Highly polar, hydrophilic compounds are poorly retained on a reversed phase (RP) LC stationary phase and will elute at or near the initial void. Sensitivity of ESI-MS detection near the void may be significantly reduced due to poor ESI desolvation as a result of the high percentage of aqueous mobile phase in the initial RP gradient runs. The alteration of the metabolite chromatographic retention properties and MS detectability may be accomplished through chemical derivatization.

In this study, we report a metabolome profiling technique for analyzing metabolites containing amines using targeted analysis method on the SCIEX Triple Quad 4500 system. A chemical derivatization strategy based on dansylation reaction was explored, which increases the retention capacity of amines in reversed-phase chromatography, improves the ionization efficiency and facilitates MS-based quantification and identification of potentially hundreds of amine-containing metabolites in complex biological matrices.

MATERIALS AND METHODS

Sample preparation: All samples (mouse tissue, plasma and urine) were extracted using methanol. Dansyl chloride acetonitrile solution and buffer were then added in the supernatant. The dansylation reaction was allowed to proceed for 60 min at 60 °C and NaOH was added to the reaction mixture to consume the excess dansyl chloride. The whole process should be as fast as possible and avoid light. The reaction scheme is shown in Figure 1.[3]

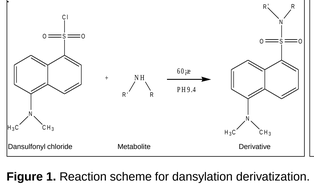

Figure 1. Reaction scheme for dansylation derivatization.

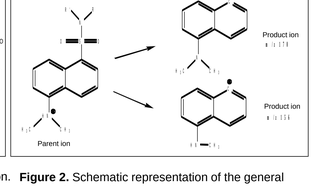

Figure 2. Schematic representation of the general fragmentation pattern of the dansylated analyte.

HPLC conditions: Samples were analyzed on a SCIEX Triple Quad 4500 system coupled with an ExionLC system using multiple reaction monitoring (MRN) analysis. A 15-minute gradient on a Phenomenex Kinetex C18 column (2.1 x 50 mm, 2.6 µm, 100Å) was employed for good separation. The composition of the mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The injection volume was set to 1 µL.

MS/MS conditions: An SCIEX Triple Quad 4500 system with Turbo V ion source and an electrospray ionization (ESI) probe in positive mode was used. The MS source conditions were as follows: curtain gas (CUR), 35 psi; collision gas (CAD), medium; nebulizing gas (GS1), 55 psi; heater gas (GS2), 55 psi; ion spray (IS) voltage, 5500 V; source temperature, 550ºC. Based on the general fragmentation pattern of the dansylated analyte, 90 metabolites were detected using 1 MRM transition per compound (Figure 2).

RESULTS

Based on the related literatures and amino acids standards, a sensitive and reliable UPLC-MS/MS method was developed, optimized and validated for the rapid quantitative analysis of amino acids and their derivatives in biological samples.[3-4] The proposed method was successfully applied for mouse tissues, plasma and urine (Figure 3-5) .The results demonstrated that 72 amine metabolites can be detected in mouse tissues, which were used for target metabolomics analysis to find the potential metabolite biomarkers. In addition, we detected 71 amine metabolites in plasma and 88 metabolites in urine, respectively.

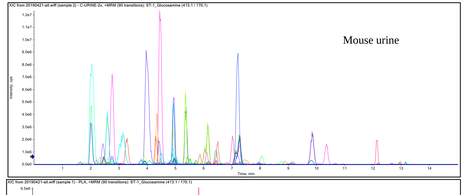

Figure 3. Detection of amine-containing metabolites in mouse urine by LC-MS/MS.

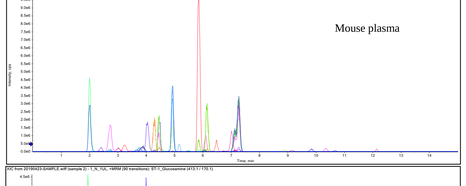

Figure 4. Detection of amine-containing metabolites in mouse plasma by LC-MS/MS.

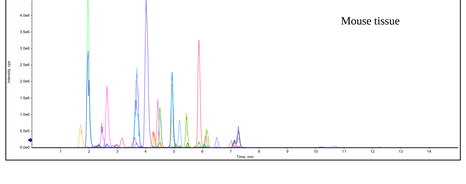

Figure 5. Detection of amine-containing metabolites in mouse tissue by LC-MS/MS.

This method was also used for targeted metabolomics between paracancerous tissue and cancer tissue in model mice. In this study, this semi-quantitative analysis of amine metabolites helped in determining a set of differential compounds. Supervised statistical models using partial least squares discriminant analysis (PLS-DA) are shown in Figure 6. This is depicted in the heat map of the top 25 most significant metabolite changes in Figure 7. Heat map of amine-containing metabolites level differences between cancer tissue and paracancerous tissue is shown in Figure 7. Metabolites (x-axis) are clustered (Pearson's R, complete linkage) according their significance and fold-change (Student's t-test). The media conditions (y-axis) are clustered (Pearson's R, complete linkage) according to similarity in metabolite level of metabolite changes.

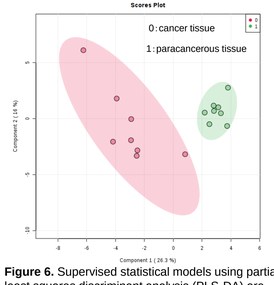

Figure 6. Supervised statistical models using partial least squares discriminant analysis (PLS-DA) are shown (n = 16). PLS-DA scores plot for cancer tissue (red) and paracancerous tissue (green).

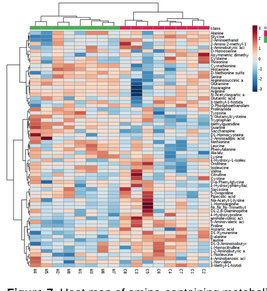

Figure 7. Heat map of amine-containing metabolites level differences between cancer tissue (red) and paracancerous tissue (green).

There were 7 metabolites that had significant changes using a statistical significance filter of p-value <0.05 and a fold change of >2 (Figure 6). The amount of cystathionine, methylguanidine, guanine and histamine in cancer tissue is lower than that in paracancerous tissue. The content of β-alanine, DL-Kynurenine and aminolaevulinic acid in cancer tissue is higher than that in paracancerous tissue (Table 1 and Figure 9).

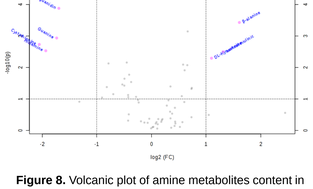

Figure 8. Volcanic plot of amine metabolites content in paracancerous tissue compared with that in cancer tissue.

Name	Fold change	p-value	Variation trend
Cystathionine	0.24103	0.00013145	Down
Histamine	0.2617	0.00036933	Down
Guanine	0.30046	0.0011478	Down
Methylguanidine	0.30888	0.0018332	Down
β-alanine	3.0461	0.0029219	Up
Aminolevulinic acid	2.4787	0.0031365	Up
DL-Kynurenine	2.1398	0.005015	Up

Table 1. Fold change, p-value and variation trend of 7 metabolites that had significant changes in paracancerous tissue compared with that in cancer tissue.

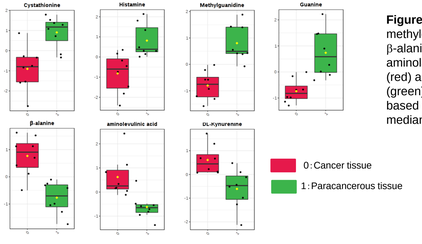

Figure 9. Box plots of cystathionine, methylguanidine, guanine, histamine β-alanine, DL-kynurenine and aminolaevulinic acid for cancer tissue (red) and paracancerous tissue (green) display the distribution of data based on: minimum, 25th percentile, median, 75th percentile and maximum.

CONCLUSIONS

We developed a fast quantitative method for the detection of 90 amine-containing metabolites (including amino acids and their methylated and acetylated products) from complex matrices in 15 minutes. Dansylation alters the chromatographic behaviors of polar and ionic metabolites normally not retainable on a RP column to an extent that they can be retained and separated by RPLC with high efficiency.

This fast, reliable, targeted method can efficiently and accurately detect the metabolite components in biological samples and provide effective differential analysis, enabling high-throughput analysis of the differences of amine metabolites across disease models.

REFERENCES

1. Casero, R. A., Marton, L. J. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nat. Rev. Drug Discovery, 2007, 6, 373–390.
2. Khuhawar, M. Y., Qureshi, G. A. Polyamines as cancer markers : applicable separation methods. J. Chromatogr., B 2001, 764, 385–407.
3. Kevin Guo, Liang Li. Differential 12C-/13C-Isotope Dansylation Labeling and Fast Liquid Chromatography/Mass Spectrometry for Absolute and Relative Quantification of the Metabolome. Anal. Chem, 2009, 81, 3919–3932.
4. Khamis M.M. et al. Comparison of accuracy and precision between multipoint calibration, single point calibration, and relative quantification for targeted metabolomic analysis. Analytical and Bioanalytical Chemistry, 2018.

TRADEMARKS/LICENSING

To receive a copy of this poster:

- Scan the code with your phone camera
- Complete the form