Targeted analysis of energy metabolites in cells by using SCIEX LC-MS/MS

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ABSTRACT

Cell energy metabolism plays a critical role in sustaining life by enabling cellular growth and function. Its impact on cellular function also means it can play a central role in many diseases, which makes it an important area for research. Here, A method was developed for analyzing more than 300 energy to enable high-throughput targeted analysis for cell energy metabolism research.

INTRODUCTION

As the basic unit of organism structure and function, cells can be used in disease mechanism research, pharmacology and cell energy metabolism research. By studying cell energy metabolism, and with the help of various cell models, researchers can identify the endogenous energy metabolites of cells, which directly reflect the biomarker information of cell life activities. The energy metabolites include many high-energy phosphoric acid bound compounds, such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP). During analysis they are easy to complex with metal ions in the pipeline, resulting in poor peak shape and reduced sensitivity. Here, the mobile phase conditions were optimized, and a method for detecting more than 300 energy metabolites was established using the SCIEX 7500 system to enable high-throughput targeted analysis.

MATERIALS AND METHODS

Chromatography was performed with an ExionLC AD system on a Waters ACQUITY BEH Amide column（100 × 2.1 mm，1.7 µm）at 40℃. A 26 min gradient of mobile phase A (95% water, 20 mM ammonium acetate and 5 µM methylenediphosphonic acid) and mobile phase B (95% acetonitrile, 20 mM ammonium acetate and 5 µM methylenediphosphonic acid) was used at a flow rate of 300 µL/min. Positive mode and negative mode electrospray ionization (ESI) was used to detect 300 energy metabolites.

Binary Gradient

Time(min)	A(%)	B(%)
0	5	95
1.0	5	95
12.0	25	75
15.0	60	40
20.0	60	40
20.1	5	95
26.0	5	95

Mass parameters

Ion source: ESI positive and negative

Scan type: MRM

CUR: 40 psi　　IS: +3000 V / -3000 V

Tem: 500℃　　Gas1: 35 psi

GAS2: 70 psi　　CAD: 10

RESULTS

Figure 1 show an extracted ion chromatogram (XIC) of compounds detected in positive mode, and Figure 2 shows an XIC for compounds detected in negative mode.

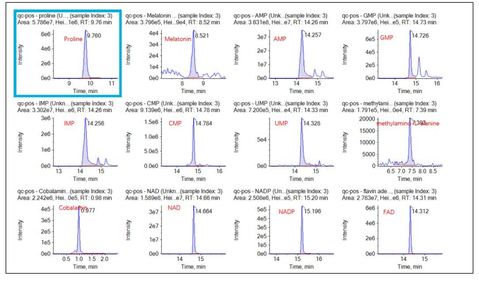

Figure 1. XIC for compounds detected in positive mode.

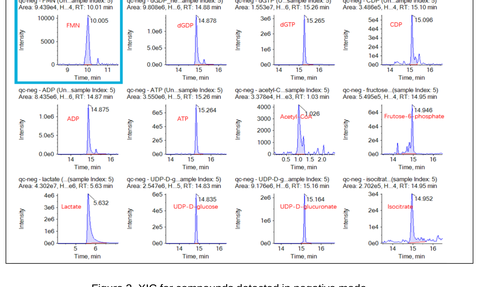

Figure 2. XIC for compounds detected in negative mode.

Twelve cell samples were detected for differential analysis. These samples were divided into 2 groups, 6 cell samples in the drug were administration group and the others were control group. First, partial least squares discriminant analysis (PLS-DA)[1] was performed on the measured peak area results of the 2 sample groups. The 2 groups were clearly distinguished, indicating that there were significant differences in energy metabolites between the drug administration group and the control group(Figure 3).

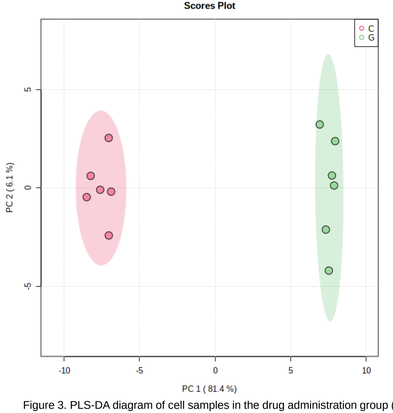

Figure 3. PLS-DA diagram of cell samples in the drug administration group (G) and the control group (C).

To identify the components of energy metabolites with significant changes, a statistical analysis was performed to obtain the p-value and the variable importance in the projection (VIP) value of each metabolite in the 2 sample groups(p-value<0.01 and VIP>1); A total of 92 metabolites showed significant changes. The heat map [1] in Figure 4 shows that there are significant differences between the control group (C) and the drug administration group (G).

Among the top 25 compounds, the contents of 14 substances including (adenosine, guanosine, inosine, uracil and glucose-6-phosphate) increased in the administration group while the contents of 11 substances including (deoxyguanosine triphosphate, glycine, inositol, aspartic acid and sarcosine) decreased.

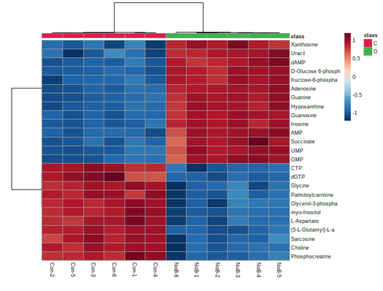

Figure 4. Heat map of cell samples in the control group (C) and the drug administration group (G) with the top 25 compounds detected.

CONCLUSIONS

A method for detecting more than 300 energy metabolites was established using the SCIEX 7500 system. This method can be used for targeted metabolite research in cells. It not only can detect metabolites in samples efficiently and accurately, but also can provide an effective difference analysis, enabling a fast and reliable method for high-throughput analysis of differences in energy metabolites.

REFERENCES

1. Website：https://www.metaboanalyst.ca/

TRADEMARKS/LICENSING