ThP493

Comprehensive cell culture profiling of iPS cell using LC-QTOFMS: Simultaneous analysis of SIM and Scan mode in a single run

Takanari Hattori[1], Toshiya Matsubara[1], Tsuyoshi Nakanishi[1], Jun Watanabe[1]
1 Shimadzu Corporation, Kyoto, Japan.

1. Overview

We report comprehensive cell culture profiling of an iPS cell by metabolomics based LC-QTOFMS. Simultaneous analysis of SIM and Scan mode in a single run using LC-QTOFMS was effective for cell culture profiling.

2. Introduction

Developing an optimal cell culture bioprocess for the production of biopharmaceuticals requires routine monitoring of medium conditions such as pH, dissolved gas, carbon source (glucose) and nitrogen source (glutamine) for optimization and control of the cell culture process. However, culture medium is composed of various other biologically important compounds such as vitamins, amino acids, nucleic acids and other primary metabolites. Comprehensive analysis of these compounds would lead to more detailed understanding of the bioprocess. Recently, metabolomics has gained a lot of attention for cell culture profiling. In this study, we report comprehensive cell culture profiling of an iPS cell by metabolomics based LC-QTOFMS. SIM and Scan mode were simultaneously used in a single run for comprehensive metabolomics.

3. Methods

3-1. Sample Preparation

Feeder-free iPS cells (1231A3) were maintained in AK02N medium for 6 days. Culture supernatants were collected every 24 hours and stored at -80℃ until use. The cultivation conditions are shown in Table 1. Proteins were removed from the supernatants by adding acetonitrile and centrifugation. Figure 1 shows the detailed procedures.

Table 1 Cultivation Conditions

Cell line	: Feeder-free iPS cells 1231A3
Passage number	: 0P30
Seeding number	: 1.3×10^4 cells/well
Period	: 6 days
Medium	: AK02N
Cell substrate	: iMatrix (0.5 μg/cm2)

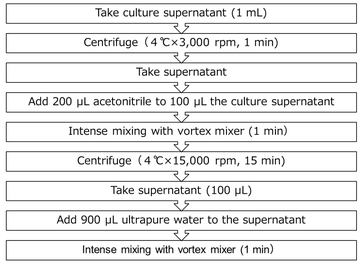

Figure 1 Process flow of sample preparation

3-2. Analytical Conditions

UHPLC (Nexera X2^{TM} system)

Column	: Discovery HS F5 (150 mmL. × 2.1 mmI.D., 3.0 μm)
Mobile phase A	: 0.1% Formate/water
B	: 0.1% Formate/acetonitrile
Flow rate	: 0.35 mL/min
Injection vol.	: 1 μL
Column temp.	: 40℃

MS (LCMS-9030)

Ionization	: ESI (Positive mode)
Mode	: SIM, Scan (m/z 50-500)
DL temp.	: 250℃
HB temp.	: 400℃
Interface temp.	: 400℃
Nebulizing gas	: 3.0 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min

3. Result

3-1. Targeted Analysis Using SIM

In this study, SIM and Scan mode were simultaneously used in a single run. SIM mode is used for targeted metabolomics. The sensitivity of SIM mode is higher than that of Scan mode and it is possible to detect trace amount of metabolites. Scan mode is used for untargeted metabolomics and it is also possible to analyze compounds that are not targets in SIM mode. Therefore, simultaneous analysis of SIM and Scan mode in a single run is effective for comprehensive metabolomics.

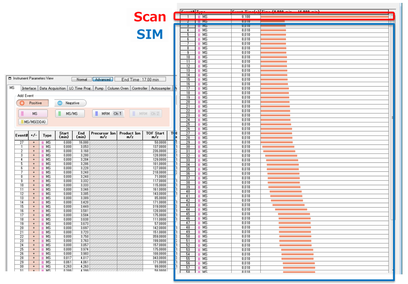

Figure 2 Simultaneous analysis of SIM and Scan mode in a single run

As a result of targeted analysis of culture supernatants of an iPS cell using SIM mode, 27 compounds such as amino acids and vitamins were detected with high sensitivity. By SIMCA 15 software (Umetrics, Sweden), principal component analyses (PCA) were performed. As shown in Figure 3, six clusters were successfully classified with time course.

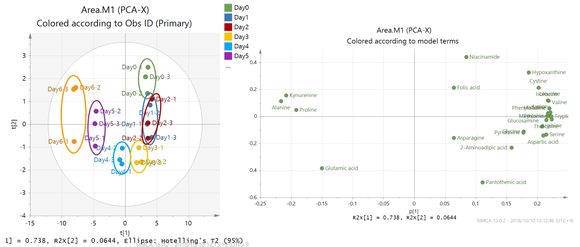

Figure 3 Score plot and loading plot

Part of the results of plotting peak areas during each sampling time are shown in Figure 4. Kynurenine, ornithine, alanine and several compounds have increased with time course. In contrast, tryptophan, arginine, methionine and several compounds have decreased with time course.

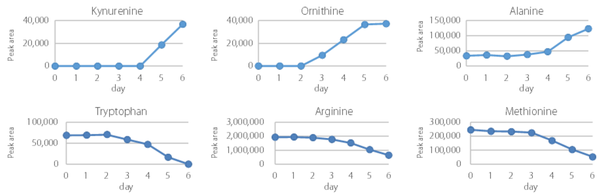

Figure 4 Metabolic fluctuations of the culture supernatant components with time course

3-2. Untargeted Analysis Using Scan

Untargeted analysis was performed from Scan data to search compounds that were not targets in SIM mode. Signpost MS^{TM} (Reifycs Inc., Japan) was used for data analysis (Figure 5). As a result of untargeted analysis, some unknown compounds were detected and their amount have increased or decreased with time course. As one of the unknown compounds, the compound with retention time 5.25 min and m/z 237.0870 increased over 5 days and then decreased on day 6 (Figure 6).

Figure 5 Untargeted data analysis by Signpost MS

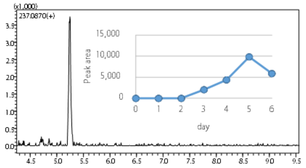

Figure 6 Example of unknown metabolite

As a result of chemical formula prediction, the chemical formula of the unknown compound was estimated as $C_{11}H_{12}N_2O_4$ with high accuracy (score: 99.53/100 and difference from theoretical mass value: 0.111 mDa). Database search for $C_{11}H_{12}N_2O_4$ was conducted using the Human Metabolome Database and formylkynurenine was hit as one of candidate compounds. Next, the MS/MS spectrum of the unknown compound was compared with the theoretical MS/MS spectrum of N'-formylkynurenine. Figure 7 shows the result of the MS/MS spectrum comparison. The main fragment peaks were in agreement with the fragment peaks of theoretical MS/MS. From the above mentioned results, the unknown metabolite was estimated to be N'-formylkynurenine. N'-formylkynurenine is an intermediate metabolite between tryptophan and kynurenine in the kynurenine pathway. Figure 8 shows the metabolic fluctuations in kynurenine pathway.

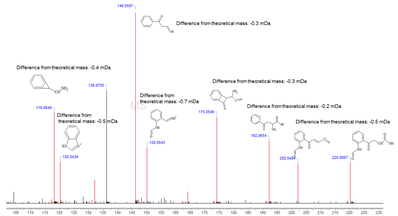

Figure 7 MS/MS spectrum comparison with theoretical MS/MS spectrum of N'-formylkynurenine

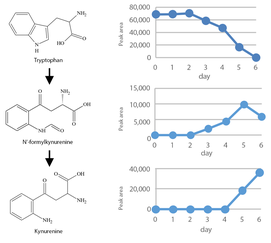

Figure 8 Metabolic fluctuations in kynurenine pathway

5. Conclusions

- We analyzed culture supernatants of an iPS cell for cell culture profiling by LC-QTOFMS that SIM and Scan mode were simultaneously used in a single run for comprehensive metabolomics.
- Some compounds were detected and their amount have increased or decreased with time course and metabolic fluctuations in kynurenine pathway were found.
- Simultaneous analysis of SIM and Scan mode in a single run was effective for comprehensive cell culture profiling.