SHIMADZU
Excellence in Science

Application News

No. C186

LC/MS

Comprehensive Cell Culture Profiling Using the LCMS™-9030 Quadrupole TOF Mass Spectrometer

In the production of useful substances by fermentation and the manufacturing of antibody drugs, the monitoring of culture media pH, dissolved gases, carbon sources (glucose), and nitrogen sources (glutamine) is implemented in order to optimize and manage the cultivation process. In addition to glucose and glutamine, the culture media components in cell cultures comprise vitamins and nucleic acid related compounds, as well as metabolites and other compounds secreted from cells. Accordingly, the comprehensive analysis of compounds contained in culture media is expected to provide useful information for discussing bioprocesses. Application News No. C106A introduced an example of the monitoring of changes in culture supernatant components in accordance with the hybridoma cultivation process, using a high-performance liquid chromatograph (HPLC) coupled with a triple quadrupole (TQ) mass spectrometer (MS) and a cell culture profiling method package. This Application News introduces an example of the monitoring of changes in culture supernatant components in accordance with the iPS cellular cultivation process using a HPLC coupled with a quadrupole time-of-flight (QTOF) MS. Changes in the components in a culture supernatant can be monitored comprehensively using a combination of targeted SIM and non-targeted full scan analysis via a LC-QTOF-MS.

T. Hattori

LC-QTOF-MS

An LC-QTOF-MS is a LCMS combining a high-performance liquid chromatograph with a quadrupole mass spectrometer and a TOF mass spectrometer (TOF). Shimadzu's first LC-QTOF-MS, the LCMS-9030 (Fig. 1), inherits the high-performance and high ion convergence capabilities cultivated in the LCMS-8000 series of LC-TQ-MS, but integrates this with newly developed TOF technology. It is equipped with Shimadzu's proprietary technology, including UFgrating™, a high strength fine lattice electrode, iRefTOF™, an ideal reflectron, and a high accuracy temperature control system. Data can be acquired with both high sensitivity and high resolution while maintaining consistently stable mass accuracy. Additionally, thanks to high speed measurements at up to 100 Hz, multiple SIM settings can be configured simultaneously with a general scan analysis in the QTOF. In this way, a high sensitivity targeted SIM analysis can be performed at the same time as a comprehensive non-targeted full scan analysis, in a single analysis.

Fig. 1 LCMS™-9030

Cultivation Conditions and Pretreatment

After iPS cell dissemination, the culture supernatant was sampled every 24 hours over a 6 day cultivation period. * The cultivation conditions are shown in Table 1. The sampled culture supernatant was deproteinized by adding acetonitrile. After precipitation of the organic solvent, the centrifuged supernatant was diluted 10-fold with ultrapure water, and was then analyzed as the samples.

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/cm^2)

Analysis Conditions

The HPLC and MS analysis conditions are shown in Table 2. A SIM analysis for 68 compounds and a scan analysis (*m/z* 50 to 500) were performed simultaneously using high speed measurements at up to 100 Hz, a feature of the LCMS-9030.

Table 2 Analysis Conditions

[HPLC conditions] (Nexera™ X2)	
Column	: Reversed-phase column
Mobile phases	: A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Mode	: Gradient elution
Flow rate	: 0.35 mL/min
Injection volume	: 1 μL
[MS conditions] (LCMS-9030)	
Ionization	: ESI (Positive mode)
Mode	: SIM, Scan (*m/z* 50-500)
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 °C
Block heater temp.	: 400 °C
Interface temp.	: 300 °C

Targeted Analysis Using SIM

From the results of the SIM analysis of the culture supernatant, 27 compounds including amino acids and vitamins were detected. Part of the results of plotting peak areas during each sampling time are shown in Fig. 2. It is evident that kynurenine, ornithine, and alanine increased as the cultivation time progressed, while there was a tendency for tryptophan, arginine, and methionine to decrease. The HPLC conditions used in this analysis were those in the LC/MS/MS Method Package for Cell Culture Profiling, which has a proven track record with TQ mass spectrometers. Accordingly, this retention time information is useful with the compounds targeted by the SIM analysis. In this way, it is possible to reliably monitor changes in the compounds in the culture supernatant as indicated in Fig. 2.

* Cultivation of iPS cells was conducted at iPS Portal, Inc.

LCMS, UFgrating, iRefTOF, and Nexera are trademarks of Shimadzu Corporation in Japan and other countries.
Note that TM and ® marks might not be specified in this document.

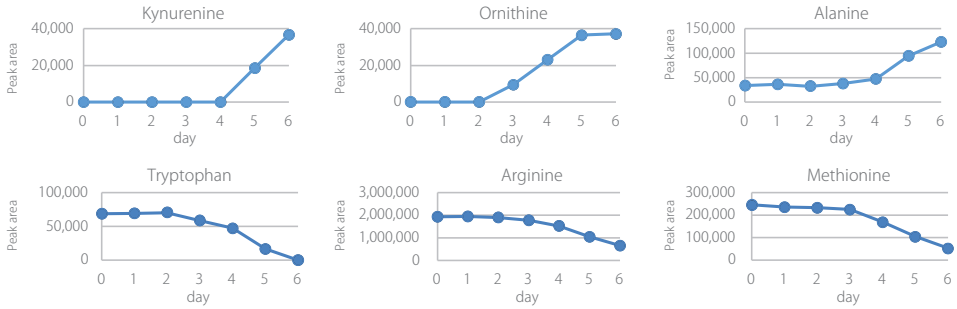

Fig. 2 Changes in Peak Area of the Culture Supernatant Compounds in Accordance with the Cultivation Process

Non-Targeted Analysis Using Full Scan

A non-targeted analysis was implemented from the full scan analysis data, in order to search for compounds other than the SIM targeted compounds for which quantitative fluctuations were observed. In this data analysis, a search for unknown compounds for which quantitative fluctuations were observed was performed using Signpost MS, non-targeted analysis software from Reifycs Inc. (Fig. 3) From the results of the analysis, it was confirmed that of the compounds not included in the SIM targets, multiple components experienced quantitative fluctuations. This section introduces an example of compound estimation, in this case an unknown metabolite (Fig. 4) with a retention time of 5.25, and a *m/z* ratio of 237.0870. Firstly, when structure estimation software was used to estimate the structure of this unknown metabolite, the structure was estimated to be that of C11H12N2O4 with high probability, given its score of 99.53, and its deviation from the theoretical value of 0.111 mDa. Next, a search for C11H12N2O4 was conducted using the Human Metabolome Database. The results showed a hit for the compound formylkynurenine. Accordingly, a comparison was made between the MS/MS spectrum for the unknown metabolite and the theoretical MS/MS spectrum for N'-formylkynurenine. The comparison with the theoretical MS/MS spectrum used MS Workbook Suite from ACD/Labs.

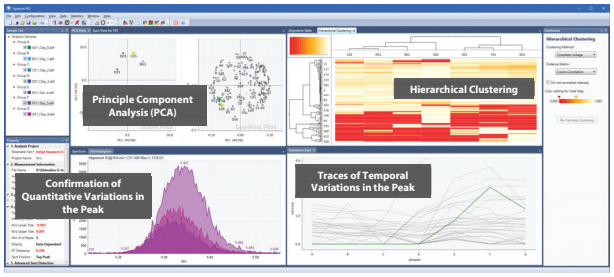

Fig. 3 Non-Targeted Analysis by Signpost MS

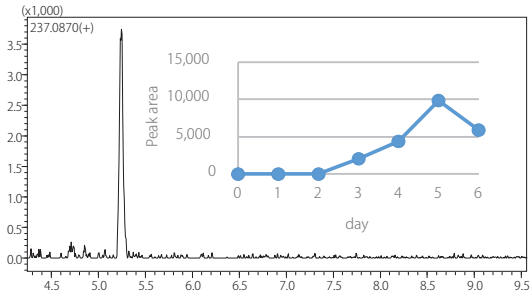

Fig. 4 Unknown Metabolite Fluctuating Quantitatively in Accordance with the Cultivation Process

From these results, as shown in Fig. 5, there was a congruence between the main fragment peaks and the fragment peaks obtained theoretically (fragment peaks congruent with the theoretical MS/MS spectrum are indicated in red). The difference from the theoretical value was 1 mDa max. From the above mentioned results, the unknown metabolite was estimated to be N'-formylkynurenine.

N'-formylkynurenine is an intermediate metabolite of tryptophan and kynurenine in the kynurenine pathway. From the results for tryptophan and kynurenine, analyzed with SIM, and the results for N'-formylkynurenine from the non-targeted analysis, it is believed that tryptophan is taken into cells from the culture media in accordance with the cultivation process, reducing its concentration in the culture media. The metabolites of this, N'-formylkynurenine and kynurenine, increase in concentration in the culture media through secretion to the cell exterior (Fig. 6). The decrease in N'-formylkynurenine on the sixth day is believed to be because the tryptophan within the culture media was depleted. In this way, it is possible to perform comprehensive cell culture profiling by combining targeted SIM with non-targeted full scan analysis.

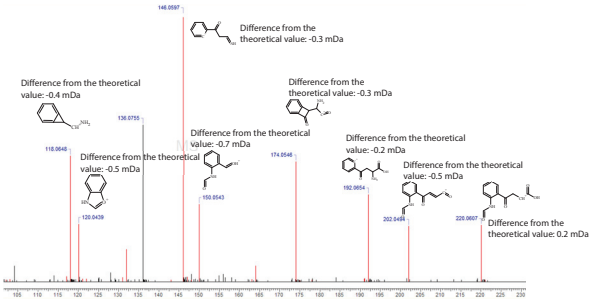

Fig. 5 Confirmation of Congruence with the Theoretical MS/MS Spectra

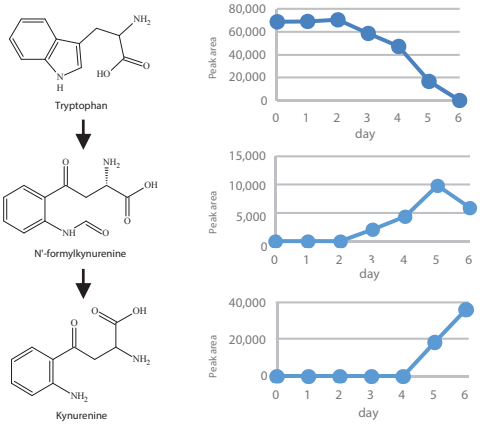

Fig. 6 Metabolic Fluctuations in the Kynurenine Pathway

First Edition: Mar. 2019

Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedure.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Shimadzu disclaims any proprietary interest in trademarks and trade names used in this publication other than its own. See http://www.shimadzu.com/about/trademarks/index.html for details.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2019