Liquid Chromatography Mass Spectrometry

A Novel Cell Culture Media Analysis Platform for Culture Process Development

Takashi Suzuki1, Kohei Yamamoto1, Tomonori Nozawa1, Tatsuya Nishio1, Kenichi Toyoda1, Tairo Ogura2, Yasuhiro Mito1, Hajime Bungo1, Masatoshi Takahashi1

1 Shimadzu Corporation, Kyoto, Japan; 2 Shimadzu Scientific Instruments, Columbia, MD

**Introduction**

Optimization and control of cell culture processes are essential to increase production efficiency of biopharmaceuticals. In the field of cell therapy including regenerative medicines, enhanced control of the culture process is also becoming important to reduce cell variability and improve consistency of mass production of the cells. Comprehensive monitoring of culture supernatant components gives researchers useful information for these purposes. However, current technologies for process monitoring are limited to measurement of pH, dissolving gases, and some small compounds such as glucose, glutamine, lactate, and ammonia in culture supernatant.

We have developed a “Cell Culture Media Analysis Platform, C2MAP system” that combines automated pretreatment module for culture supernatant samples with LC/MS/MS. This system can perform automated sample pretreatment and simultaneous analysis of up to 95 compounds including basal medium components and secreted metabolites (The list of target compounds is shown in the column to the right.). This system contains a software that can visualize temporal change in each culture supernatant components through the cell culture.

In this application, we present features of the C2MAP system and its applications.

![Figure 1: Overview of C2MAP system](image-url)
After removal of the cells from culture fluid, vials containing cell culture supernatant (400 to 500 mL) are set into the sample rack of C2MAP-2000 (Max. 65 samples). Pretreatment and measurement flow of C2MAP system are shown in Figure 2.

A dedicated software, C2MAP software, can control both the pretreatment module and LC/MS/MS system, making it possible to carry out seamless analysis and to associate the treated sample and the measurement results easily because pretreatment and analysis are carried out with the common sample ID. The progress of pretreatment and analysis is easily confirmed (Figure 3).

Temporal changes in each component can be graphed with the dedicated viewer software, C2MAP TRENDS, using LC/MS/MS data set. Analysts can monitor variations in basal media components and secreted metabolites during cultivation, as well as display graphs of component comparisons with samples from different culture series. These observations can provide useful insights into considerations of the optimal culture conditions and the culture process.
Results and Discussion

Pluripotent stem cells (PSCs) have a feature maintaining undifferentiated state. In this experiment, C2MAP system was used to compare the temporal changes in the culture supernatant components in undifferentiated human iPS cells and its differentiated counterparts. As a result, significant difference could be found in the time course of some compounds (Fig.5). We think these compounds can be marker candidates for culture process management.

Fetal bovine serum (FBS) often affect cell growth. In this experiment, detection of component amount variation among the product lots was tested. Three different lots of FBS were analyzed by C2MAP system. We could detect 56 compounds from FBS sample. Overall pattern of mass chromatogram from each lot was similar, whereas significant differences were detected in some compounds (Fig.6).

Conclusion

Through multicomponent monitoring of the culture supernatant using C2MAP system, various useful information can be obtained. This information provides useful insights into optimization of the culture media composition and the culture process.
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