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Liquid Chromatograph Mass Spectrometer LCMS™-2050

Application News

Simultaneous Analysis of CHO Cell Culture Supernatant Components Using a Single Quadrupole Mass Spectrometer

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User Benefits

- A single quadrupole LC-MS system can be used to simultaneously analyze amino acids, organic acids, vitamins, and other substances in a culture supernatant.
- Changes in the components in a culture supernatant can be easily visualized as a function of time by analyzing culture supernatant samples acquired over time with the Multi-omics Analysis Package.

■ Introduction

When using cells to produce beneficial substances or manufacture antibody drugs, the pH level of the culture medium, dissolved gases, carbon sources, nitrogen sources, and other factors are monitored to optimize or manage the culturing process. Cell cultures can contain a variety of substances, including not only glucose and glutamine but also vitamins, nucleic acid-related compounds, and metabolites secreted from cells. Therefore, by comprehensively analyzing all the components in a culture medium, useful information about biological processes can be obtained.

This Application News article describes using a single quadrupole LC-MS system to monitor time-course changes in the components in a supernatant from culturing CHO-K1 cells, which are commonly used for antibody production, by simultaneously analyzing all the components. Because single quadrupole LC-MS systems have a simple configuration process, it is easy even for first-time users to simultaneously analyze components in culture supernatants.

Fig. 1 Nexera™ and LCMS™-2050 Systems

■ Samples

CHO-K1 cells were cultured based on the parameter settings indicated in Table 1. After seeding the cultures, samples were obtained by sampling each culture supernatant after 12, 24, 36, 72, 84, 96, 108, 120, and 132 hours.

Table 1 Culturing Parameter Settings

Seeding Density	2.0 x 10^5 cells/mL
Agitation Rate	140 rpm
Temp./Humidity/CO_2	37 °C/80 %/5 %

■ Pretreatment

Samples were deproteinized by adding 800 µL of acetonitrile to 200 µL of each culture supernatant and centrifuging it (10 minutes at 15,000 rpm). Then the centrifuged supernatants were diluted 10-fold with ultrapure water. Also, 2-isopropylmalic acid (final concentration: 10 nmol/mL) and 10-camphorsulfonic acid (final concentration: 5 nmol/mL) were added as internal standards.

■ Instruments and Analysis Conditions

Analysis was performed with a Nexera series HPLC system that was combined with an LCMS-2050 LC-MS system (Fig. 1). Although compact, the LCMS-2050 single quadrupole mass spectrometer offers easy operability and outstanding performance. Equipped with a DUIS heated ion source, which provides the benefits of both ESI and APCI, it can analyze masses ranging from *m/z* 2 to 2000. That makes it well suited for the simultaneous analysis of components in culture supernatants.

The analysis condition settings for HPLC and MS are shown in Table 2. The analysis conditions for LCMS-2050 was configured based on the analysis conditions in the LC/MS/MS Method Package for Cell Culture Profiling Ver. 3. A total of 144 components were targeted, mainly amino acids, vitamins, other basic culture media components, and secreted metabolites.

Table 2 Analysis Conditions

HPLC Conditions (Nexera XR)	
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-2050)	
Ionization:	ESI/APCI (DUIS), Positive and negative mode
Mode:	SIM (146 events)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	500 °C
DL Temp.:	250 °C

■ Analysis Results

Analysis of all culture supernatants detected 59 components, such as amino acids, organic acids, and vitamins. Mass chromatograms for representative components are shown in Fig. 2.

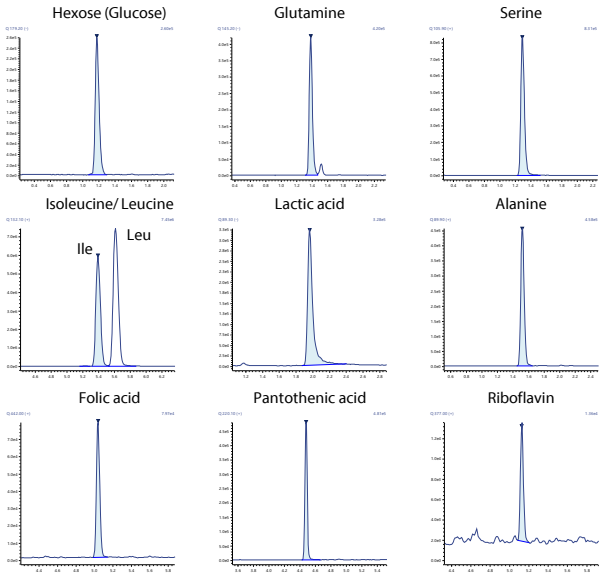

Fig. 2 Mass Chromatograms (Culture Supernatant 72 Hours after Seeding)

To see how the concentration of each component in the culture supernatant changed as culturing progressed, the Multi-omics Analysis Package was used to plot the peak area ratio values (peak area value for each measured component divided by the peak area value for the internal standard 2-isopropylmalic acid) at each sampling time. The results for the representative components are shown in Fig. 3. They show that glucose and glutamine, which are the main source of carbon and nitrogen, and several amino acids decreased as culturing progressed. In contrast, lactic acid and other components secreted as waste matter as the result of glucose consumption increased as culturing progressed.

Similar analysis was performed using a triple quadrupole LC-MS (LCMS-8050) system. The results were compared with the results from the described method, which confirmed a similar trend for almost all the detected components, as shown in Fig. 4.

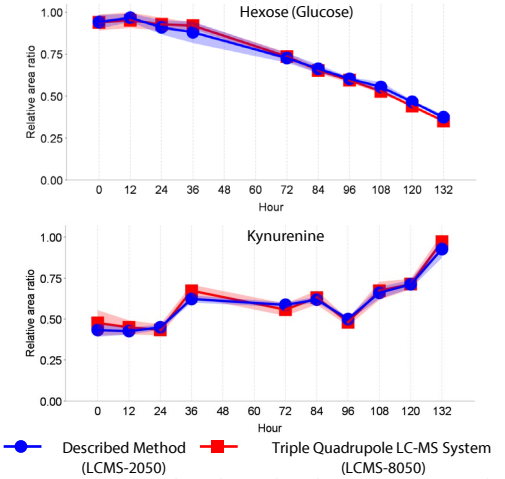

Fig. 4 Comparison with Triple Quadrupole LC-MS System Results

■ Conclusion

As described in Application News No. C106A, if simultaneous analysis of all components is necessary, including trace components secreted from cells, a triple quadrupole LC-MS system is suitable. However, a single quadrupole LC-MS is more than adequate for analyzing the main components in culture media, such as glucose, glutamine, and lactic acid. Widespread use of the single quadrupole LC-MS for simultaneous analysis of the components in culture supernatants can be expected to lead to continuous advancements in cell-related R&D work.

Acknowledgments

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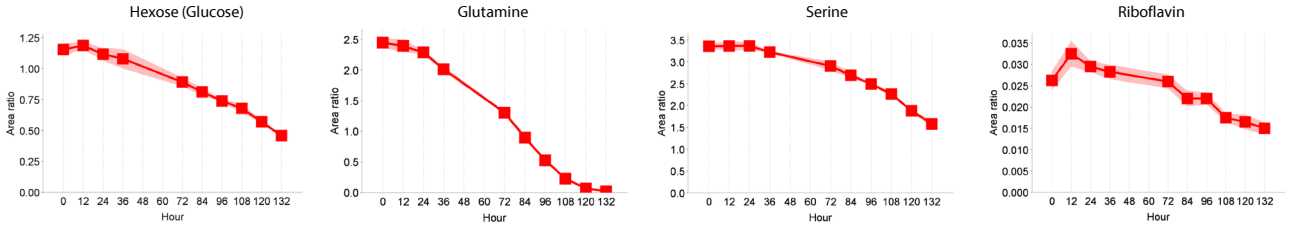
・ Examples of Components with Peak Area Ratios that Decreased as Culturing Progressed



・ Examples of Components with Peak Area Ratios that Increased as Culturing Progressed

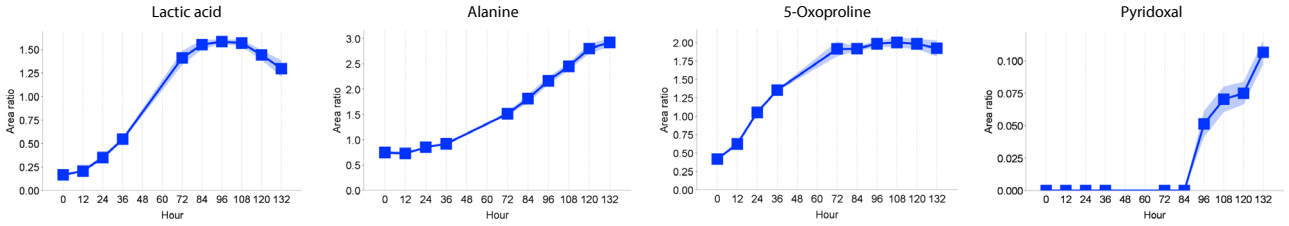


Fig. 3 Changes in Culture Supernatant Components as Culturing Progressed

Related Applications

1. Simultaneous Analysis of Culture Supernatant of Mammalian Cells Using Triple Quadrupole LC/MS/MS Application News No. eC106A

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