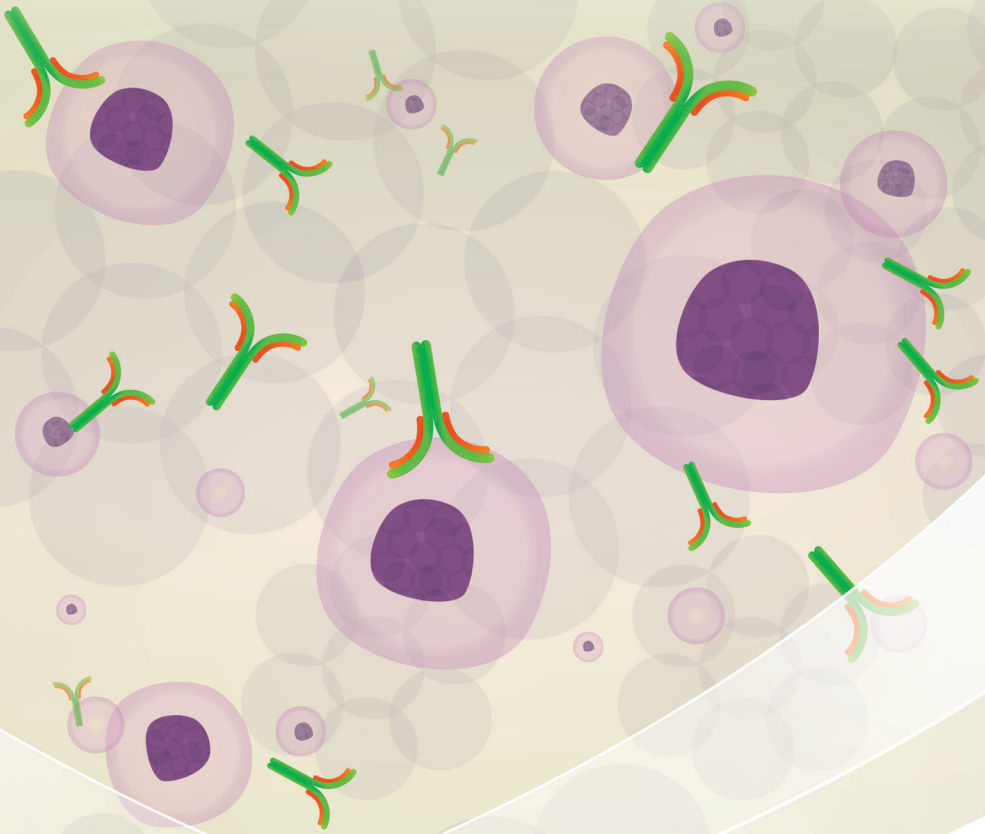
LC-MS/MS Makes Cell Culture Media Analysis Fast, Easy and Effective



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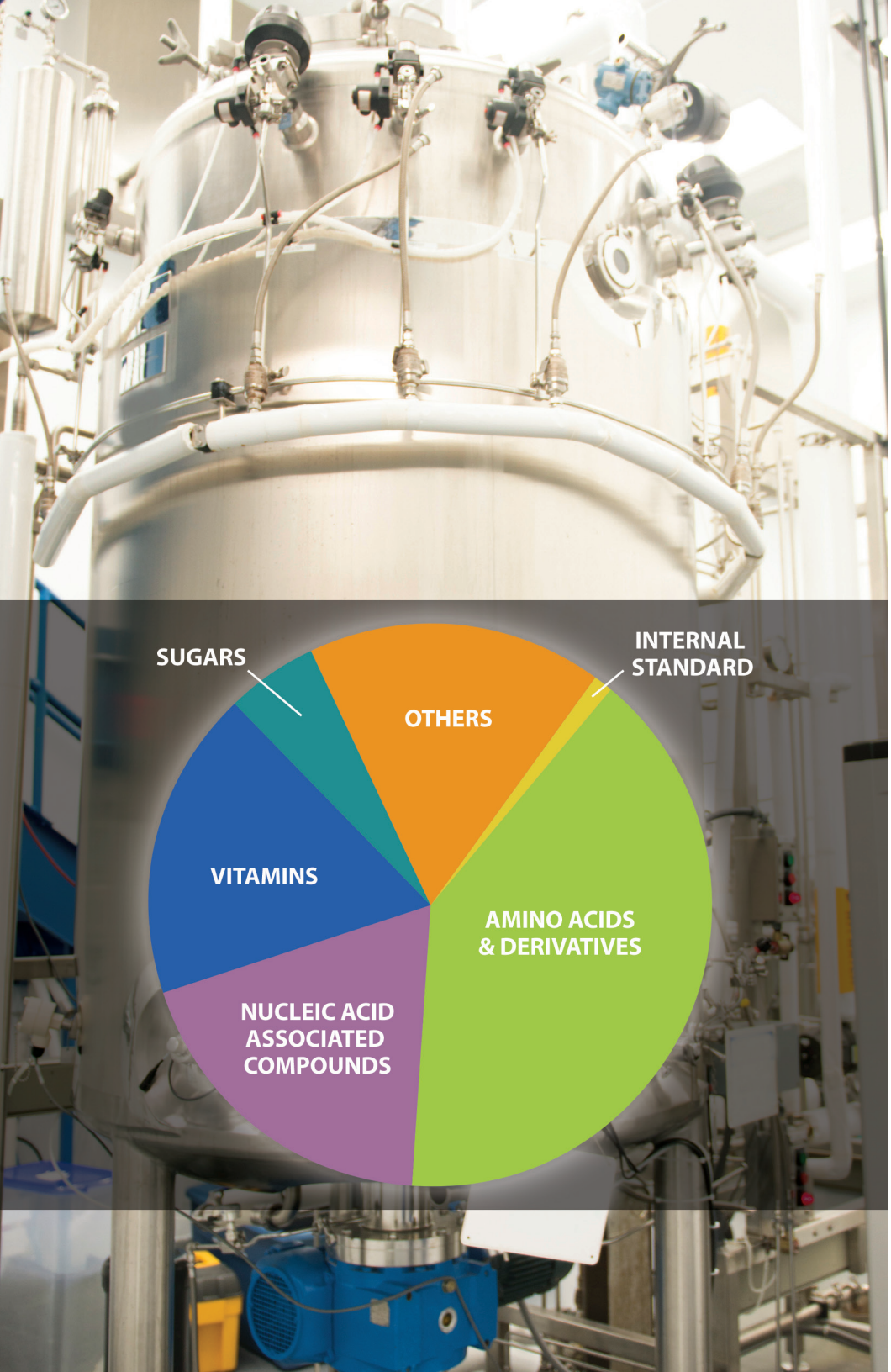


IMPORTANCE OF CELL CULTURE MEDIA TO THE BIOPHARMACEUTICAL INDUSTRY

Research, development and production of biopharmaceuticals is growing rapidly, thanks to the increase of novel therapeutics and biosimilar drugs.

The increased interest in biologics has resulted in significant growth in the cell cultures market, as scientists demand optimal media to ensure the viability of their work.

Cell culture media must contain a precise balance of components, such as glucose, glutamine, nucleic acid, vitamins, other biologically important compounds, and primary metabolites. Ensuring that the cell culture media is comprised of the optimal formulation for growth and is free of impurities is vital to the success of biopharmaceutical development. The quality of the product depends on the quality of the media. Moreover, cell culture media is necessary in calculating product yield and cost of manufacturing.

Ensuring that cell culture media contains a precise balance of components is critical for biopharmaceutical development.

WHO RELIES ON PRECISE CELL CULTURE MEDIA?

Cell culture products are used in biopharmaceutical research throughout the drug development process – from discovery to development to manufacturing. Key sectors that rely on precise cell culture media include:

BIOPHARMACEUTICAL COMPANIES

Cell culture media is the basis of research, development and manufacturing of biopharmaceuticals. The media must be able to maintain cells in their desired stage and support cell culture growth. The cell media is specific to each project, extremely labor intensive, and expensive to produce and maintain. Cell growth and productivity are dependent upon precise enhancements to the basal media. Fully defined and characterized media is essential for cultures to progress. Because cell culture media is a key factor in determining product yield, its viability and precision ultimately affect the bottom line, as product yield drives manufacturing capacity, drug availability and drug cost.

CELL CULTURE MEDIA MANUFACTURERS

To stay competitive in this growing market, cell culture manufacturers must invest significant time and money to produce high-quality, customized media. They must conduct significant research to determine the necessary components and properties for each product. And they must select the correct raw materials and use the appropriate production technology in order to meet their customers' needs and ensure quality and batch-to-batch consistency. Cell culture manufacturers must also meet stringent rules and regulations for process manufacturing.

BIOSIMILARS MANUFACTURERS

A challenge for manufacturers of biosimilar biopharmaceuticals is determining the composition of the cell culture media of the original formulation. In addition, they need to prove biosimilarity/comparability to the innovator molecule in order to obtain FDA approval. The biosimilar product must demonstrate high similarity to the reference product. That means there must not be any clinically meaningful difference between the biological product and the reference product in terms of safety, purity and potency. Recently, the FDA has issued three guidance documents for biosimilar drugs that recommend a stepwise approach to showing biosimilarity. These guidelines could ease trial requirements, if the sponsor can demonstrate biosimilarity in earlier steps of the development process. Data from comprehensive media analysis can be correlated to product quality. This can be very useful in driving many of the critical quality attributes like glycosylation to within the specification of the originator reference product.

STEM CELL RESEARCHERS

The unique ability of stem cells to differentiate into other cell types make them valuable in biopharmaceutical discovery, therapeutic development and medical research. Cell culture media is crucial in proliferating and maintaining viable stem cells. The nature of the stem cell research dictates the type of cell culture media, which is optimized for specific applications. Growing interest in stem cell research has added to the growth of the cell culture market.

WHY IT'S IMPORTANT TO MONITOR THE CELL CULTURE PROCESS

Because biopharmaceutical development is highly complex – and maintaining optimal cell culture is exacting, time-consuming and expensive – monitoring is required at every step.

This is especially important because these large molecule biologics are more complex to process than small molecule drugs. Ongoing analysis is vital to cell culture development and media optimization to promote cell growth. It is also necessary to support continuous manufacturing when rebalancing or introducing media components. Monitoring is also essential in determining optimal harvest endpoints in cell development and in developing cost-effective media.

MONITORING METHODS

The current conventional methods for cell culture analysis are performed by biosensor instruments. These methods are limited to certain analytes like glucose, lactate, glutamine, glutamate and ammonium, along with gases (O_2, CO_2), pH and osmolality measurements.

Biosensor monitoring does not indicate how these measurements will affect product quality, such as post-translational modifications (PTMs), glycosylation, charge variants and aggregates profile of products like monoclonal antibodies. Moreover, in order to measure other analytes, the scientist must use additional methods, such as high-performance liquid chromatography (HPLC) or single quadrupole mass spectrometry.

Clearly, this process is fragmented and not a complete solution for analytical groups that are tasked with methods development, sample analysis and methods transfer. Biopharmaceutical developers need to be able to identify all of the media components, PTMs and process impurities that affect product quality.

In addition, as the industry starts to move from traditional batch manufacturing to continuous manufacturing, it needs to monitor product quality in real time throughout the process. Today, biopharmaceutical manufacturers need to grow cells longer in a continuous manner and have the ability to measure all media components with one analytical solution.

A COMPLETE ANALYZER SOLUTION

Because both small and large molecule research is becoming increasingly complex, higher resolution technologies, such as liquid chromatography combined with mass spectrometry (LC-MS/MS), are needed for comprehensive analysis.

To meet this demand, Shimadzu developed the triple quadrupole mass spectrometer LCMS-8060 and Cell Culture Profiling Method Package to make comprehensive cell culture analysis fast, easy and effective.

This complete solution includes:

- Nexera Ultra High Performance Liquid Chromatograph (UHPLC), which seamlessly integrates with the LCMS-8060 for high-quality, high-speed LC-MS/MS analysis
- Optimized Multiple Reaction Monitoring (MRM) libraries, which include analysis conditions such as MRM parameters
- Triple quadrupole mass spectrometer with the highest sensitivity and ultra-fast technologies, including an advanced ion guide design to increase ion production for high sensitivity detection
- Shimadzu's LabSolutions software, an intuitive package that allows for simplified instrument control, diverse data handling and integration with regulatory compliance requirements.

High sensitivity, fast scanning speeds and fast polarity switching enable the collection of high-quality data and information.

The LCMS-8060 provides high-throughput and automated sample preparation with the new Cell Culture Media Analysis Platform (C2MAP-2000). The C2MAP-2000 is the first truly integrated cell culture media sample preparation system for LCMS. It automatically performs all the processes necessary from deproteinization of cell culture media supernatant to dilution and addition of internal standard followed by automatic transfer to the HPLC autosampler. The dedicated C2MAP TRENDS viewer software graphs the component variations across multiple conditions, making comparative analysis of the consumption and depletion of media components easier during the culturing process, as well as the variation in metabolites secreted from cells.

ULTRA-FAST SCANNING AND SWITCHING

The LCMS-8060 *delivers the fastest quadrupole scan speed of up to 30,000 u/second with a 0.1 Dalton step size without the loss of mass accuracy.*

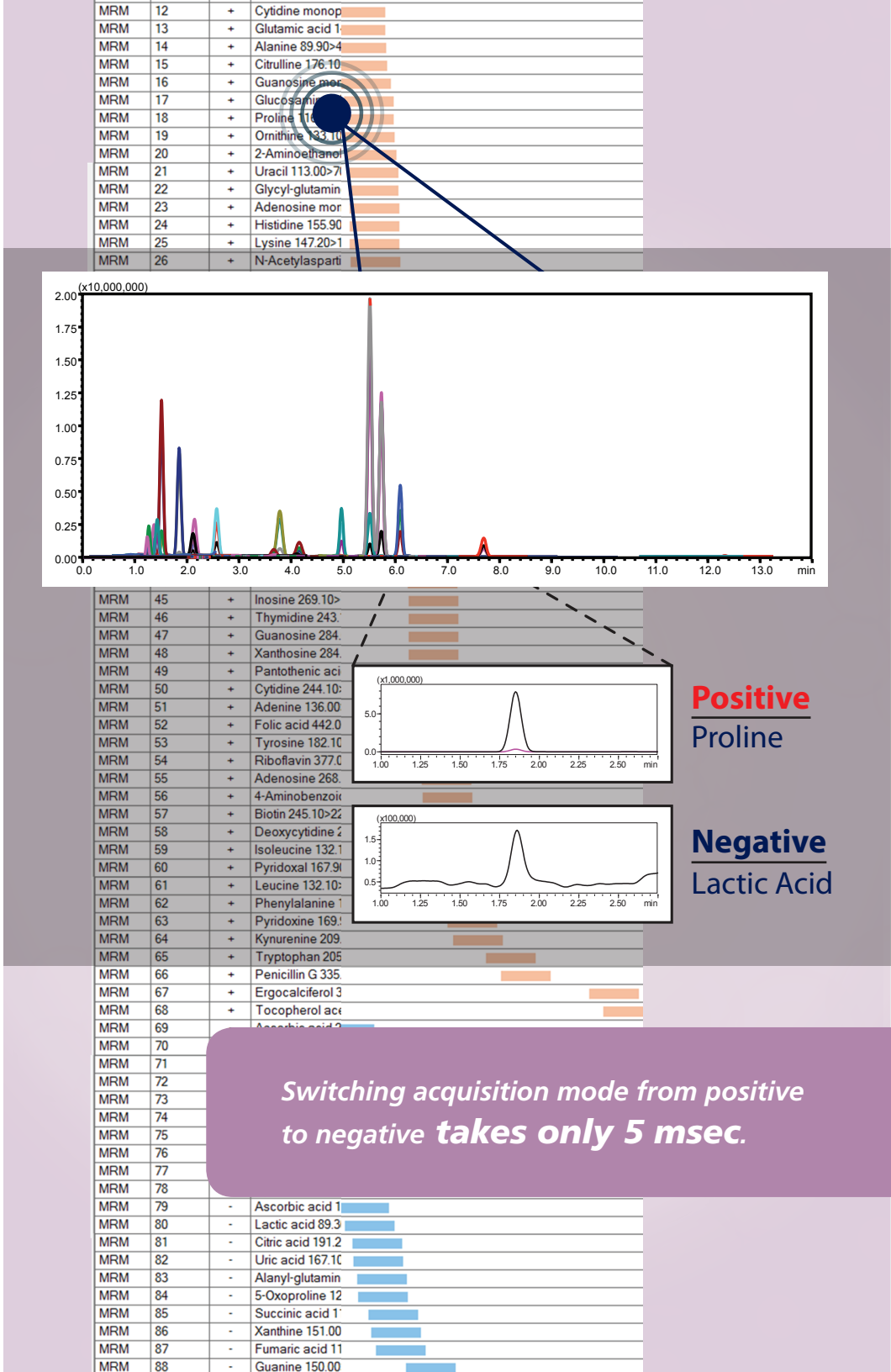
With the fastest polarity switching available, switching acquisition mode from positive to negative only takes 5 msec. Data acquisition occurs so rapidly that triggered MS/MS scans and MRM quantitation can be performed in a single acquisition method.

GROUNDBREAKING TECHNOLOGY INCREASES SENSITIVITY

The newly developed UF-Qarray ion guide incorporates groundbreaking technology that increases LC-MS/MS sensitivity by enhancing ion signal intensity and reducing noise. By improving the ion sampling device, the ion guide, and the vacuum efficiency, the LCMS-8060 delivers a new standard for sensitivity. That yields ultra-low detection limits, allowing for trace quantitation.

In addition to obtaining high-quality data with extraordinary sensitivity, the LCMS-8060 delivers qualitative information concurrently. Product ion scans can be triggered by a preset threshold.

This enables the simultaneous collection of multiple reaction monitoring (MRM) events for each component with full scan product ion spectra at different collision energies.



DEDICATED CELL CULTURE PROFILING METHOD PACKAGE

Shimadzu's Method Package for Cell Culture Profiling is a fully optimized methods package ***using the LCMS-8060.***

Users can quickly and easily implement complex methods without costly, time-consuming methods development by utilizing ready-to-use sample preparation protocols, LC separation conditions and MS acquisition parameters.

This culture medium analysis platform enables the simultaneous analysis of up to 95 components (plus 1 internal standard). Users can acquire detailed data concerning cell culture profiles based on the targeted analyte groups, such as sugars, nucleic acid associated compounds, amino acid derivatives, vitamins, antibiotics and a few chemicals.

Compounds, such as amino acids and vitamins, are commonly analyzed by each compound group, which makes analysis of a culture medium time-consuming. By providing conditions for efficient multi-component analysis, this methods package enables simultaneous analysis in 17 minutes. Because there is no saturation of the signal with high-concentration components, such as glucose or amino acids, it is possible to measure a variety of culture medium components using the same vial.

Analyze up to 95 components simultaneously.

FEATURES

Simultaneous analysis method for 95 components

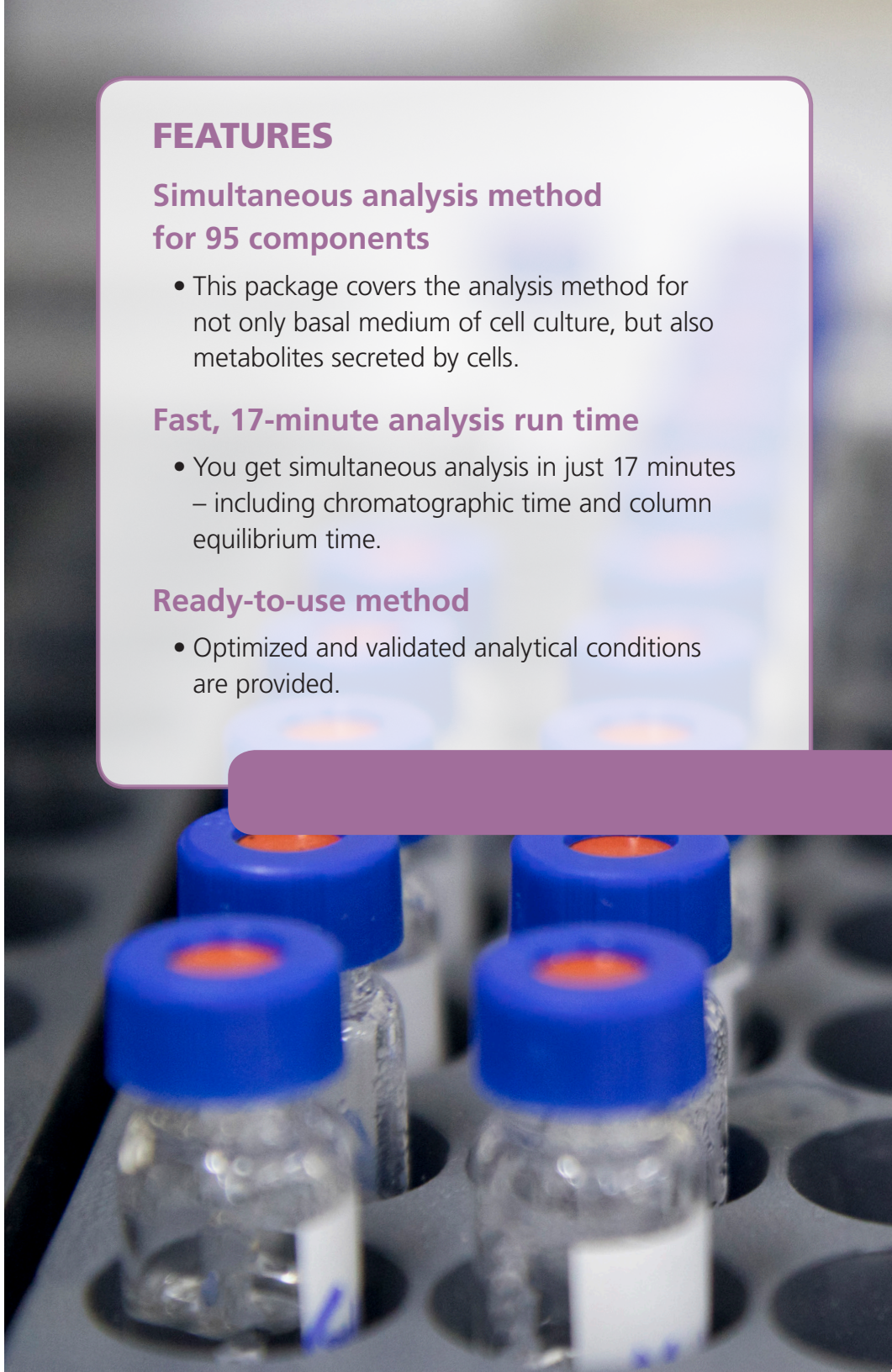
- This package covers the analysis method for not only basal medium of cell culture, but also metabolites secreted by cells.

Fast, 17-minute analysis run time

- You get simultaneous analysis in just 17 minutes – including chromatographic time and column equilibrium time.

Ready-to-use method

- Optimized and validated analytical conditions are provided.



TRIPLE QUADRUPOLE LC-MS/MS IN ACTION

Shimadzu scientists conducted a simultaneous analysis of culture supernatant of mammalian cells using triple quadrupole LC-MS/MS and the methods package for cell culture profiling.

They demonstrated the change in abundance of culture medium components associated with hybridoma growth.

A murine hybridoma cell line was cultured in Dulbecco's Modified Eagles Media (DMEM) and its culture supernatant was sampled every 24 hours for five days after inoculation. The LCMS sample was prepared by adding an internal standard to the sample and then removing proteins by taking the supernatant after mixing with acetonitrile, which was further diluted with ultrapure water prior to injection. 1 µL was injected to LCMS for simultaneous MRM quantitation of 96 compounds.

The analysis showed that glucose, glutamine and a few other amino acids, which are the primary sources of carbon and nitrogen, decreased in abundance with growing cell numbers. In contrast, lactic acid increased in abundance over time as a result of glucose consumption for anaerobic respiration. A similar pattern of increase was observed for a few other compounds. No change in relative abundance was observed for essential amino acids and some vitamins.

Simultaneous Analysis of Culture Supernatant of Mammalian Cells Using Triple Quadrupole LC-MS/MS

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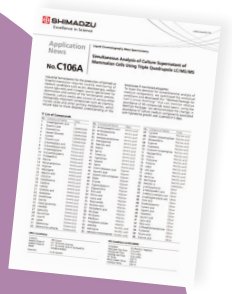
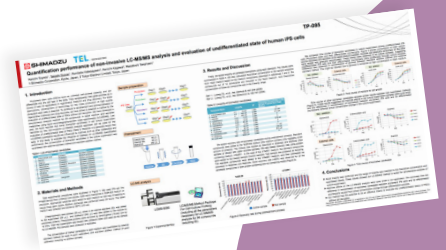


STEM CELL METABOLITE PROFILING

Researchers at Shimadzu also conducted an evaluation of undifferentiated state of human iPS cell line PFX#9 derived from umbilical cord bloods.

They adopted a non-invasive LC-MS/MS analysis approach using cell culture supernatant as samples and the LC-MS/MS Method Package for Cell Culture Profiling. They concluded that the method was suitable for comprehensive and quantitative analysis for cell culture supernatant. An LC-MS/MS analysis approach using the method was able to distinguish the undifferentiated state and differentiated state of pluripotent stem cells (PSCs) in the early stages of cultivation. The method has the potential to be an effective means to evaluate the undifferentiated status of PSCs without cell disruption.



Evaluation of Undifferentiated State of Human iPS Cell Line PFX#9 Derived From Umbilical Cord Bloods

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CONCLUSION

Cell culture media development is complex, and the products are notoriously difficult to analyze. Nonetheless, media analysis is crucial for assuring quality and for calculating product yield and manufacturing costs.

In order to get these cutting-edge biopharmaceuticals to market faster without impacting quality, drug developers need an analytical process that provides complete measurement of the media without having to identify and quantify every component, as in biosensor methods.

Moreover, typical workflows in biopharmaceutical research involve many steps, including cell culture and purification process development, formulation, stability studies, and quality assurance. For efficiency, cost containment, and quality control, manufacturers need a process that reduces the number of steps within the workflow without impacting quality.

Triple quadrupole liquid chromatograph mass spectrometry (LC-MS/MS) with a dedicated cell culture profiling methods package is a complete, holistic solution that makes comprehensive cell culture analysis fast, easy and effective.

For more information on how Shimadzu can help improve your cell culture media analysis, visit our website at www.LabTotalBio.com.

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