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Application Note

Monitoring Cell Culture Media with the Waters Amino Acid Analysis Solution

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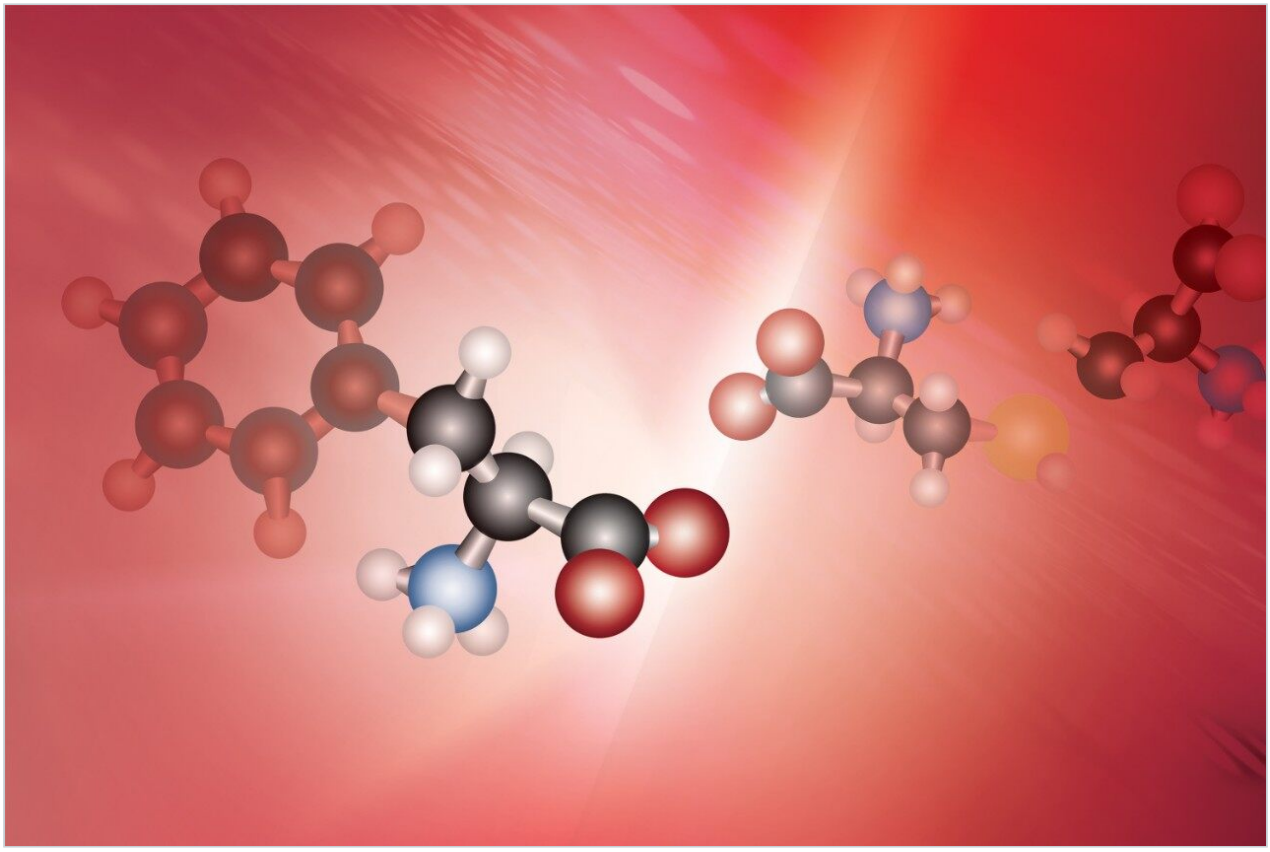

Abstract

Cell culture techniques are routinely used to produce proteins intended for use as biopharmaceuticals. The culture conditions must be optimized to ensure that the protein is produced without structural modification and in the highest possible yield. These preferred conditions will often be different for each clone investigated, so a large number of optimization experiments may be required. This assessment of growth conditions must also consider the changes in the media that occur as a consequence of cell growth, that is, the consumption of nutrients and the release of waste products. The monitoring and optimization are complex because of the large number of physical and chemical parameters that have an effect. The experiments described here are focused on one particular class of components, the free amino acids. We describe our end-to-end analytical method and technology for amino acid analysis.

Introduction

Cell culture techniques are routinely used to produce proteins intended for use as biopharmaceuticals. The culture conditions must be optimized to ensure that the protein is produced without structural modification and in the highest possible yield. These preferred conditions will often be different for each clone investigated, so a large number of optimization experiments may be required. This assessment of growth conditions must also consider the changes in the media that occur as a consequence of cell growth, that is, the consumption of nutrients and the release of waste products. The moni-toring and optimization are complex because of the large number of physical and chemical parameters that have an effect. The experiments described here are focused on one particular class of components, the free amino acids.

Amino acids are important as the constituents of proteins, but they also serve as intermediates in many metabolic pathways. They are provided as individual amino acids in the growth media to satisfy both types of nutritional requirements. The concentration of amino acids in the media changes both from consumption of some amino acids and release of others by the growing cells. Monitoring these dynamic conditions is part of the optimization process, and the observed changes in concentration can be used to schedule a “feeding” of the culture or replacement of the medium. The Waters UPLC Amino Acid Analysis Solution (Figure 1) provides a suitable way to monitor these changing nutrient levels.

The Waters UPLC Amino Acid Analysis Solution is a turnkey offering that encompasses instrumentation, derivatization chemistry, separa-tion chemistry, software, and support. The solution includes defined conditions suitable for the assay of the amino acids commonly found in mammalian cell culture media. We show here the use of this defined method in monitoring a growing culture.

Experimental

Conditions for derivatization and analysis are described in detail in the Waters UPLC Amino Acid Analysis Solution System Guide.

Samples of serum-free cell culture medium were obtained at daily time intervals from a bioreactor that was actively producing a biopharmaceutical protein. The medium was diluted 1:4 with 0.1M HCl. A 10 µL aliquot of the dilution, with no additional sample preparation, was derivatized using the standard AccQ▪Tag Ultra protocol.

Figure 1. Waters UPLC Amino Acid Analysis Solution.

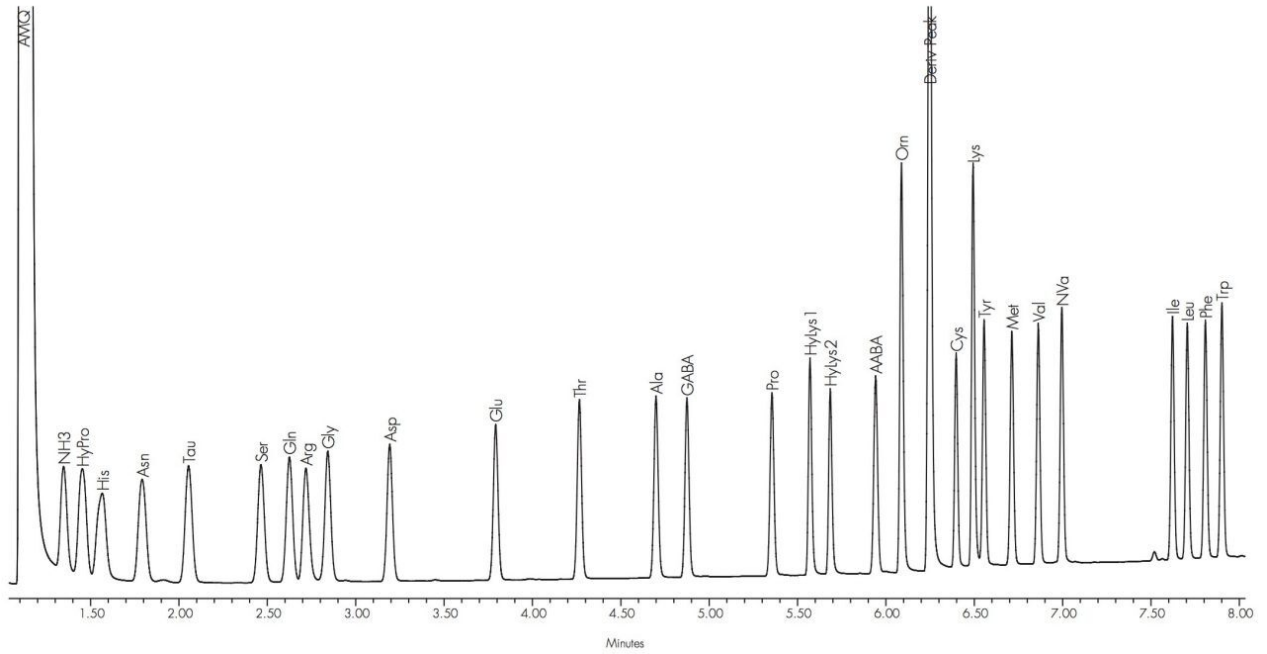

Figure 2. Analysis of standards of amino acids commonly found in cell culture media.

Results and Discussion

An analysis of amino acid standards representing the compounds commonly found in cell culture media is shown in Figure 2. This separation is obtained using the mobile phases and separation conditions that are part of the standard UPLC Amino Acid Analysis Solution. No adjustment of mobile phase pH or changes in composition are required. The resolution and reproducibility are sufficient for unambiguous peak identification and for reliable quantitation.

This method was applied to samples taken from an active bioreactor at daily intervals. These results are overlaid in Figure 3, and a second overlay in Figure 4 magnifies the region of the chromatogram which includes the amino acids that change most significantly during this growth experiment. The chromatographic characteristics observed with the standards are preserved with the authentic samples. The significant amino acids are readily identi-fied and are sufficiently well-resolved for quantitation. There are a few small unidentified peaks that do not interfere with the amino acids.

The comparison of the 1, 3, and 6 day samples clearly shows the decline in concentration for some amino

acids, notably glutamine, and the increase in others, such as alanine. These changes can be expressed quantitatively as plotted in Figure 5. All the amino acids can be quantitated, but only a few are shown as examples, including glutamine which increases in concentration with feeding.

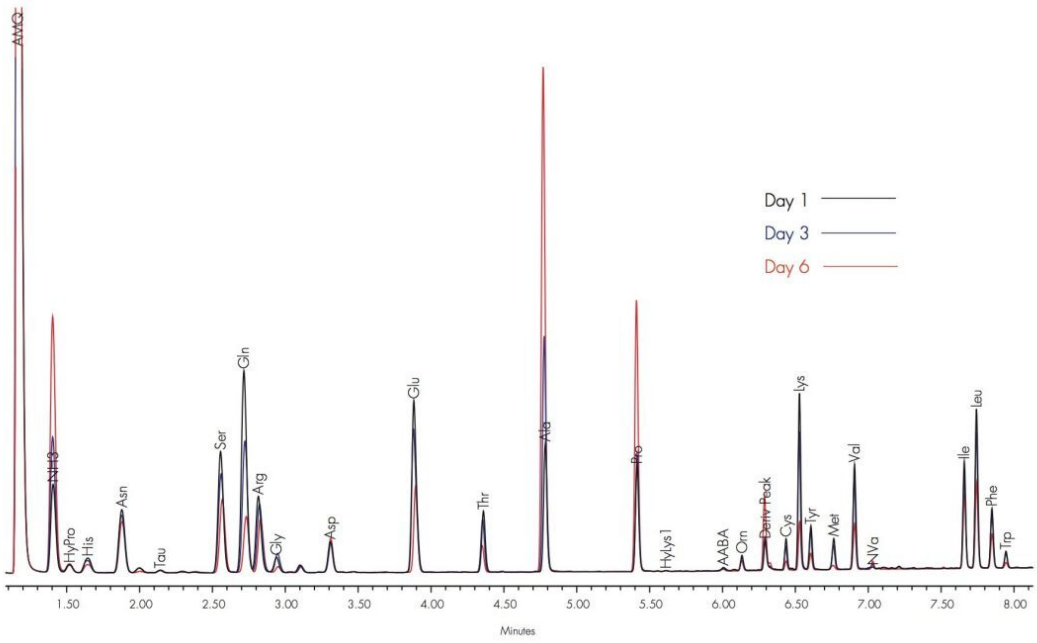

Figure 3. Analysis of amino acids in cell culture media after 1, 3, and 6 days of culture.

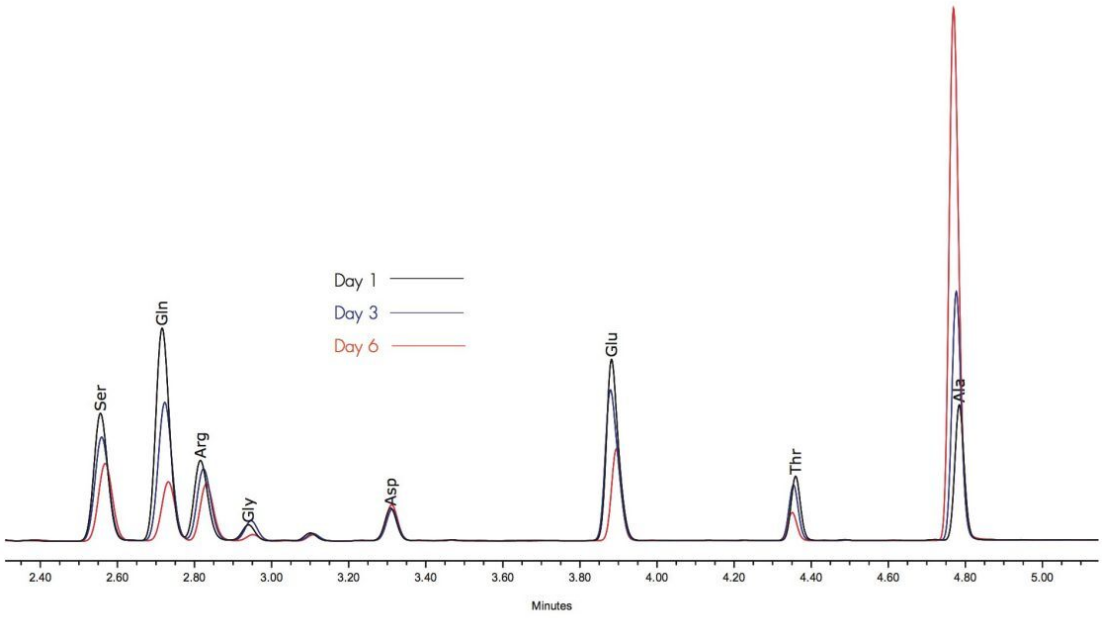

Figure 4. Analysis of critical amino acids in cell culture media after 1, 3, and 6 days of culture.

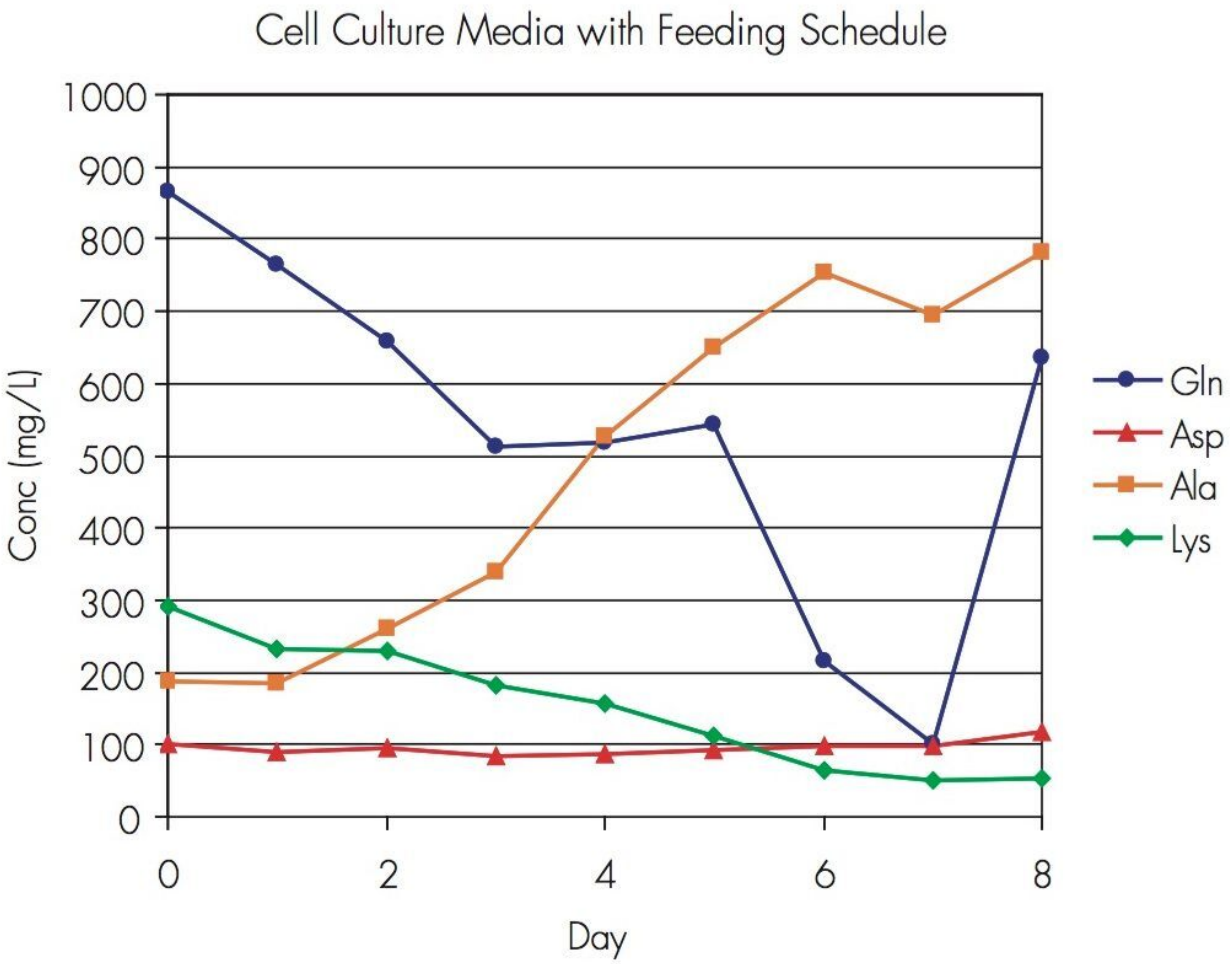

Figure 5. Quantitative trends in amino acid concentration during cell culture.

Conclusion

The Waters UPLC Amino Acid Analysis Solution has been used for the analysis of mammalian cell culture media. The standard method provides the chromatographic resolution required for peak identification. No sample preparation beyond simple dilution is required.

The analysis proves rugged and reproducible over a series of samples. No interferences are observed. The quantitative analysis is suitable for monitoring changes in concentration over time and for recognizing the proper time for a scheduled feeding. These analytical results are obtained with a short run time compatible

with the high throughput requirements for optimizing growth conditions.

The Waters UPLC Amino Acid Analysis Solution provides a complete turnkey analytical method for monitoring amino acids in mammalian cell culture media. The pre-tested column, eluents and reagents ensure that the user will not spend time adjusting the method. The small amount of sample required for good analyses contributes to long column life and minimizes the chance of failure during a series of runs. The high resolution ensures reliable peak identification and quantitation so that runs need not be repeated. These analytical benefits are obtained with a short analysis time for the high throughput required for the optimization of cell culture conditions.

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