Surveying Amino Acid Variability Among CHO Media

Application Note

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Abstract

Chinese hamster ovary (CHO) cells are widely used as the mammalian host to produce therapeutics such as monoclonal antibodies and vaccines. CHO cell culture employs complex media formulations that vary in key nutrients such as vitamins, amino acids, and trace elements.

The PATsmart™ REBEL® XT System is an at-line analyzer designed to measure amino acid concentrations in media samples in less than 15 minutes. In this application note, we present and analyze the diversity of amino acid concentrations across thirteen CHO media samples, demonstrating how REBEL XT can be readily applied to profile both fresh and spent media. By providing rapid amino acid analysis, the REBEL XT System enables process development laboratories to gain timely insights into cell growth and metabolism, without the delays and costs associated with external analysis.

Introduction

CHO cells are widely considered the "gold standard" for manufacturing therapeutic proteins.[1] Finding the correct amount of each amino acid is critical for cell development, as amino acids form the building blocks of proteins and are a primary driver of cell growth.[2] Insufficient levels of amino acids may inhibit protein production or yield a corrupt protein sequence.[3] Excessive concentrations of amino acids may drive cell growth over protein production or alter the charge variant profile of the product.[4,5]

There are numerous CHO cell variants with varying nutrient needs and feeding strategies. This has led to the release of many commercial options for CHO media with varying levels of vitamins, amino acids, sugars, and other trace elements.[5] However, manufacturers often do not provide precise quantities of amino acids present in the formulations.

Traditionally, measuring these nutrients has been accomplished by off-line high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC-MS) systems in a central analytical laboratory, which often takes days or even weeks, delaying critical insights into potential process successes or failures. In contrast, the REBEL XT System is designed for at-line use in a process development laboratory and provides amino acid concentration data rapidly and with minimal sample preparation. Results are reported in millimoles per liter (mM), providing clear and quantitative data in less than 15 minutes.

This application note highlights the use of the REBEL XT System to quantify the variation in amino acid levels found in thirteen commercially available CHO media blends.

Materials and Methods

Thirteen different CHO media were sourced from common cell culture media vendors. The media comprised a variety of powders and pre-prepared solutions. Powders were prepared in solution following vendor guidelines. Each media sample was diluted 100x in REBEL XT diluent (10 µL sample, 990 µL diluent) and stored in autosampler vials. The vials were vortexed to ensure the samples were fully mixed. A batch run sheet was prepared for the thirteen media samples for analysis on a calibrated REBEL XT system. Each vial was analyzed with five replicate measurements.

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Results and Discussion

The amino acid concentration profiles revealed clear variability between media (Figure 1). The concentrations ranged from 0.03 mM (choline) to 19.3 mM (asparagine). The five replicate measurements for each media sample demonstrated good repeatability, with a median RSD of 5.3%.

One critical parameter is amino acid solubility in water, which varies from 0.54 g/kg for tyrosine to 1250 g/kg for proline.[7] Since cell cultures have limited ranges for pH and temperature, low-solubility amino acids such as tyrosine and tryptophan must be formulated in quantities such that no acidic or basic agent will be necessary to dissolve them. Glutamine was detected in only one media sample, which is expected due to its poor shelf stability: glutamine is unstable in aqueous solutions and degrades to form pyrrolidonecarboxylic acid in cell cultures.[7] This has led to the common practice of spiking glutamine into media at the start of a bioreactor run; thus, it would not be expected to find glutamine in most commercially available media.

Alanine was detected in some, but not all, of the samples. Alanine may be produced from excess pyruvate during the exponential growth phase, or it may accumulate as a response to ammonia stress. Alanine can then be consumed as an alternative energy source when glucose becomes depleted. The variability of alanine highlights the complexity of commercial media components and emphasizes the need for media characterization and optimization.

	Concentration (mM)																			
	Ala	Arg	Asn	Asp	Choline	Cystine	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
CHO1	0.06	3.76	3.94	0.22	0.03	0.60		0.20	0.33	0.84	1.88	2.31	2.23	0.63	0.84	0.94	0.32	3.57	0.26	0.90	1.97
CHO2		2.13	6.19	1.62	0.62	0.22		2.17		1.35	2.53	4.69	3.26	0.77	1.39	4.37	7.56	6.18	1.06	1.13	2.94
CHO3	1.13	1.61	7.28	0.67	1.08	0.34		0.84	0.75	0.64	1.25	1.63	1.53	0.35	0.54	1.19	4.00	2.27	0.42	0.63	1.31
CHO4	0.17	1.17	0.24	0.21	0.05	0.38	5.62	0.13	0.35	0.41	1.21	1.45	1.40	0.31	0.39	2.13	0.58	1.72	0.11	0.54	1.14
CHO5	0.24	2.42	0.40	0.27	0.46	0.24		0.32	0.67	0.71	1.21	1.96	1.49	0.34	0.64	1.16	0.54	2.14	0.18	0.77	1.17
CHO6	0.15	1.19	4.33	1.11	0.22	0.25		1.01	0.24	0.77	1.56	2.65	1.90	0.61	0.66	1.80	3.35	2.42	0.70	0.54	1.55
CHO7	0.22	1.07	0.25	0.25	0.42	0.40		0.26	0.43	0.57	0.96	1.36	1.27	0.31	0.48	1.64	1.20	1.70	0.49	0.71	0.99
CHO8		1.66	6.16	12.10	0.55	1.08		3.00		1.45	2.96	5.68	2.94	1.14	1.59	2.34	15.92	6.00	0.93	1.63	3.07
CHO9		2.00	6.40	1.77	0.54	0.27		2.14		1.35	2.33	4.50	3.08	0.71	1.29	3.83	7.47	6.16	1.01	1.05	2.66
CHO10		2.10	6.50	1.78	0.56	0.24		2.26	1.62	1.36	2.36	4.40	3.15	0.74	1.33	3.96	7.94	6.51	1.07	1.10	2.68
CHO11	0.27	3.93	1.23	0.72	0.08	0.26		0.43	0.55	0.49	1.54	1.37	1.14	0.49	0.44	1.23	1.53	0.57	0.14	0.40	0.83
CHO12	0.83	4.15	17.15	8.02	0.46	1.07		0.82	1.19	1.78	4.99	10.69	5.73	1.87	3.81	4.35	8.02	6.15	1.51	1.73	6.43
CHO13		3.85	6.34	19.30	0.91	0.49		4.77	0.11	4.25	6.37	12.87	6.59	1.94	2.56	5.48	16.66	7.09	1.56	1.42	7.16

Figure 1. Amino acid profiles in commercial CHO media measured by the REBEL XT System

Figure 2 shows total amino acid concentrations across the thirteen media samples as measured by the REBEL XT System. Concentrations ranged from 15 mM to 110 mM. The CHO media with the highest total amino acid concentrations, CHO12 and CHO13, were designed for perfusion bioreactors, which support higher cell densities than batch and fed-batch cultures, while CHO1 to CHO11 were designed for fed-batch processes. The perfusion media are designed for longer bioreactor runs with minimal intervention after the run has started. In contrast, fed-batch processes can be supplemented with additional nutrients during the run, requiring lower quantities of amino acids up front. Cell culture media designed for perfusion reactors then must be rigorously characterized, as the high quantities of amino acids and long process time will have greater impacts on the cell culture.[10]

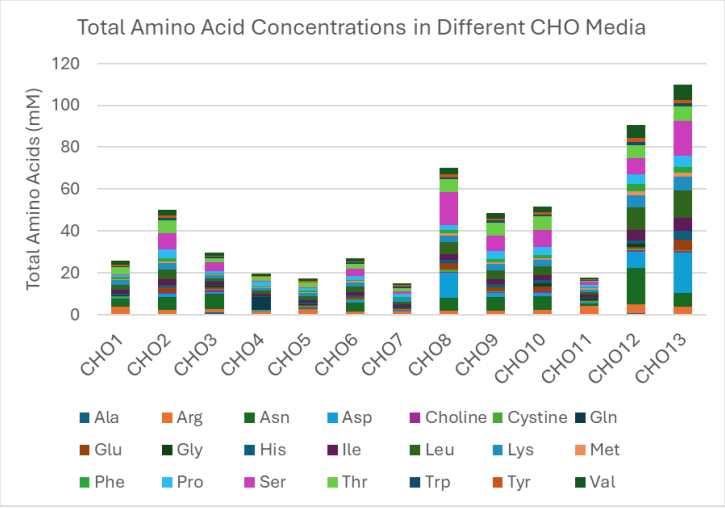

Figure 2. Total amino acid concentrations in commercial CHO media measured by the REBEL XT System

Conclusion

The REBEL XT System was used to analyze amino acid concentrations across thirteen CHO media formulations. Individual amino acid concentrations varied by more than an order of magnitude, while total amino acid concentrations differed by a factor of 7.3 across samples. Measurements exhibited good repeatability and aligned with qualitative expectations based on manufacturer descriptions. These results demonstrate the diversity of commercially available media to grow different CHO cells based on the target therapeutics. Because manufacturers typically do not disclose details of cell media, at-line analysis is an invaluable tool for process optimization. The REBEL XT System provides amino acid concentrations in 15 minutes or less, accelerating media characterization and enabling informed process control.

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