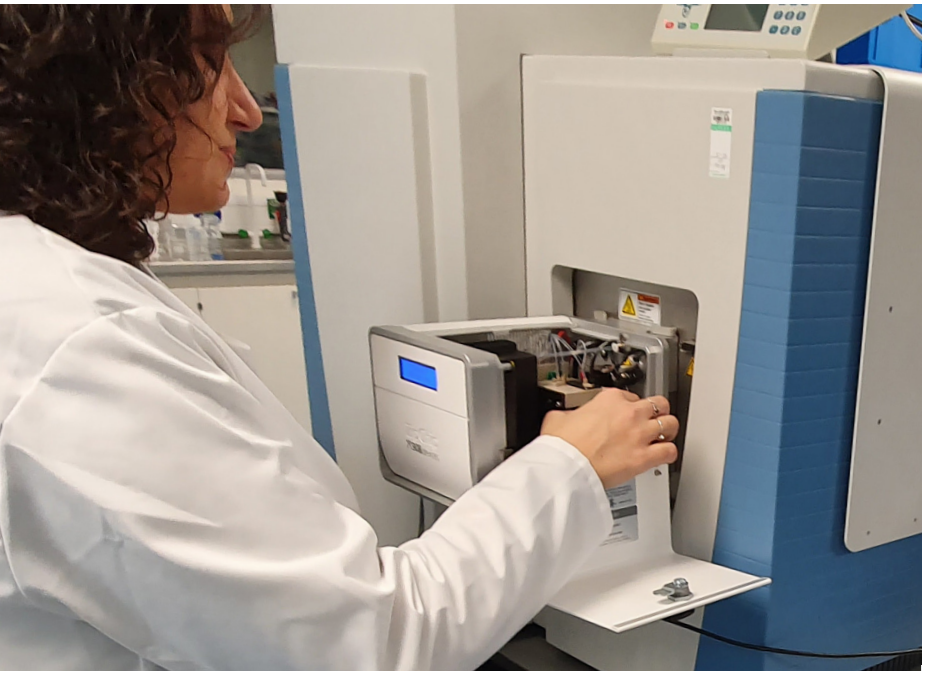
CASE STUDY:

Simple, automated monitoring of amino acids in cell culture media with ZipChip.

HIGHLIGHTS

- The demonstrated ZipChip® method is fast, simple, sensitive and uses the fully-automated microchip electrophoresis platform.
- Cell culture media can be directly analyzed using ZipChip after simple dilution with no further sample manipulation required.
- Amino acids can be monitored in less than 3 minutes using ZipChip, which is a faster alternative than conventional methods.
- Demonstrated ease of use for screening and monitoring amino acids in cell culture media.



> Amino acids can be monitored in **less than 3 minutes using ZipChip**, which is a faster alternative than conventional methods.

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Materials & Methods

Sample Prep:
Spent media samples were prepared following the ZipChip protocol for quantifying amino acids in growth media. Briefly, spent media was diluted 20x with LC-MS grade water and then 10x with sample diluent from the ZipChip Metabolites Kit.

Cell Line and Cell Culture Conditions:
CHO cells were adapted to grow in suspension. The culture experiments were initiated by seeding 0.3 x 10^6 cells/mL in 250 mL polycarbonate Erlenmeyer flasks containing 100 mL of chemically defined CHO cell culture media supplemented with 4mM of L-glutamine on day 0 in triplicate. All cultures were incubated at 37°C during the 10 days. Sampling was made daily by removing 1 mL aliquots, which were centrifuged, filtered and the supernatant stored at -20 °C for further analysis.

ZipChip Protocol	Quantification of Amino Acids in Growth Media
Assay Kit	Metabolites Kit
Chip Type	ZipChip HS
Field Strength	1000 V/cm
Run Time	3 minutes
Mass Spec Type	Thermo Scientific QExactive™ Plus Hybrid Quadrupole Orbitrap mass spectrometer

Introduction

The biopharmaceutical industry has grown significantly over recent years with further expansion predicted based on packed pipelines of recombinant proteins, monoclonal antibodies and peptide drugs. Chinese Hamster Ovary (CHO) cells have been widely utilized for expression of recombinant proteins, which require media to support growth and therapeutic protein production[1,2]. The composition of cell culture media requires a blend of amino acids, vitamins, nucleosides, lipids, carbohydrates, trace elements and other components. Among these classes of compounds, amino acids are crucial as they are the constituent of proteins and are intermediates of many cellular metabolic pathways. As cells grow and produce therapeutic proteins certain amino acids are rapidly consumed. Monitoring the dynamic depletion of amino acids from culture media is important for media design and feeding strategy development. Supplementation with optimal amount of depleted amino acids is required to assure healthy growth, high productivity and to avoid formation of toxic or undesired metabolites[3,4].

Various methods have been employed to analyse amino acids in spent media including LC-UV, LC-MS, GC-MS and CE-MS. However, these methods require sample preparation such as derivatization, solid-phase extraction or microextraction or other steps in the experimental procedure prior to analysis. Derivatisation of some media components is required to enable optical detection using absorbance or fluorescence or for matrix simplification[5,6].

Miniaturized devices and microfluidic techniques offer the advantage of reduced analysis times and reduced consumption of reagents and therefore an environmentally friendly alternative to conventional methods.

In this study, we describe a simple, rapid, and automated microfluidic ZipChip ESI-MS method to monitor amino acids in spent media from an IgG1 producing CHO cell line using batch culture over a period of 10 days. The analysis facilitated identification of amino acids consumed during cell growth.

Results

During cell growth, amino acids are depleted from the culture media. Monitoring amino acid behaviour is crucial to understand the dynamic conditions and to adjust the concentration for each amino acid in the cell culture media to obtain the maximum possible production of monoclonal antibody. A simple, rapid and automated microfluidic

ZipChip ESI-MS method was developed to monitor amino acids in spent media from IgG1 producing CHO cells growing over a period of 10 days using batch culture.

The base peak electropherogram for the separation of 17 amino acids in water (reference standard) and in cell culture media employing ZipChip ESI-MS is shown in Figure 1. The separation is based on the difference of electrophoretic mobility, related to the charge and size of the analytes. The amino acid migration order observed was the highest positively charged amino acids migrate first, followed by the neutral amino acids and finally the acidic amino acids. The separation of 17 amino acids in 3 minutes showed symmetric peaks and good resolution. Some of the amino acids are unresolved, co-migration of certain amino acid pairs are commonly described in CE applications. However, with high resolution MS detection, co-migrating pairs can be easily distinguished based on differences in m/z.

The method was applied to study samples of conditioned cell culture media collected from flasks over the course of the 10-day batch culture. The culture media is rich in essential amino acids. Figure 2 shows the decline of signal intensity for the majority of the amino acids from day 0 to day 10.

All the amino acids can be quantified by this method. Figure 3 shows the patterns of leucine across the ten days of cell culture. As expected, the amount of the leucine is reduced during the 10-day culture.

Results

ZipChip ESI-MS facilitates rapid quantitative analysis of amino acid in cell culture media using an automated platform generating excellent separation within 3 minutes. The method provides good resolution, selectivity and requires minimal sample preparation and sample handling.

The ZipChip is suitable for screening and monitoring changes in cell culture media during therapeutic protein production with a short analysis time with reliable peak identification due to the coupling of CE with MS.

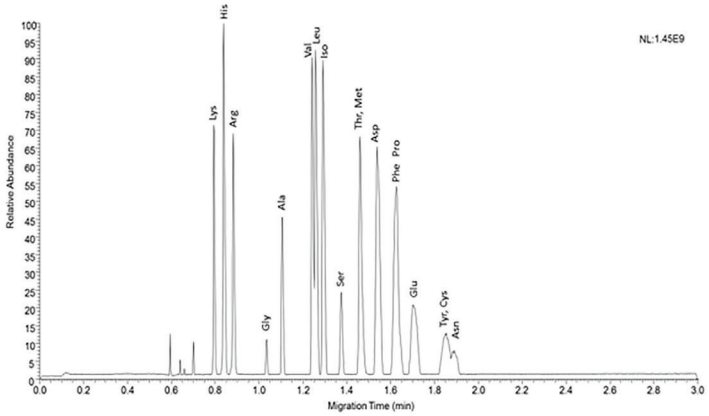

Figure 1: A mix of amino acids was diluted in CE buffer to optimize the separation via chip CE-ESI-MS. (a) Base peak electropherogram for a 500µM of amino acids mix standard.

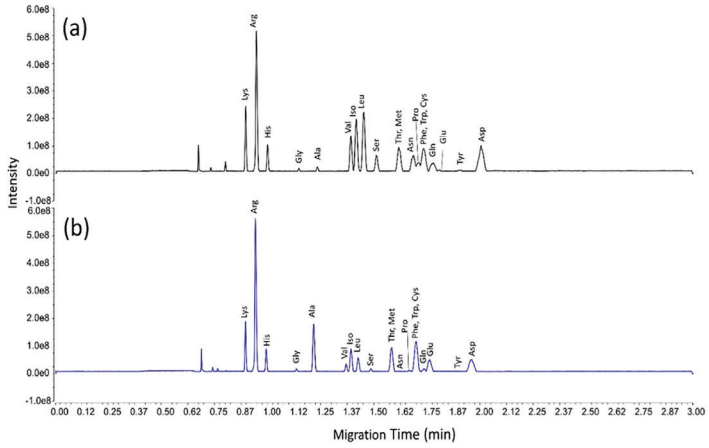

Figure 2: Base peak electropherograms of cell culture growth from different days. (a) Day 1 and (b) Day 10

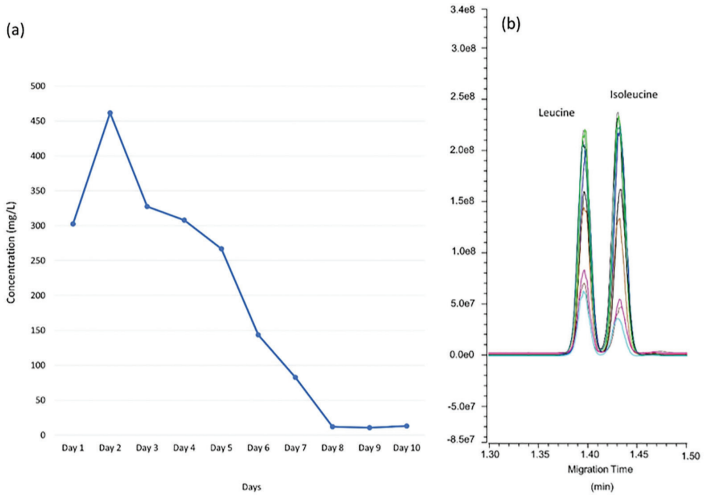

Figure 3: (a) Pattern of consumption of leucine during 10 days of cell culture growth (b) Extracted ion electropherograms of Leucine during 10 days of cell culture growth.

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