# Quantitative Metabolite Profiling of Bioreactor Cultures Using Automated CE-MS Workflows for Bioprocess Optimization

US HUPO 2026
Poster 13.08

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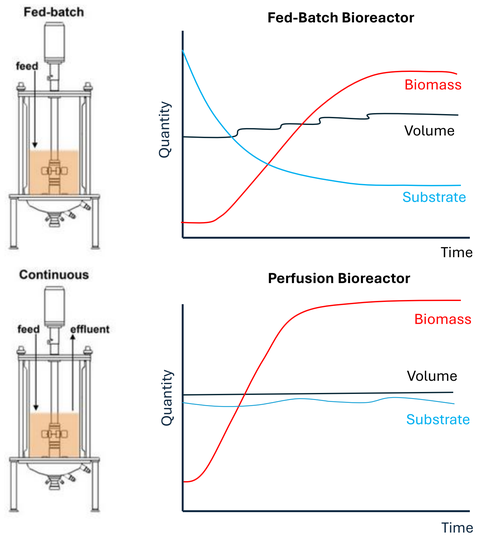
## Introduction

**Motivation.** Understanding the metabolic state of bioreactor cultures is critical for optimizing growth conditions and improving product quality. Traditional metabolomics workflows often require labor-intensive derivatization, complex instrument setup, and expert data interpretation, all of which limit reproducibility and throughput. This work demonstrates a high-throughput, automated CE-MS workflow for quantitative metabolite profiling of cell culture media using MoveKit™ CE reagents and software-guided processing. The approach is designed to reduce manual steps, increase consistency across users, and generate reproducible data for bioprocess characterization.

**Bioreactor Types.** Bioreactor optimization for both fed-batch and perfusion bioreactors rely on optimizing the feed or perfusion media to optimize product yield and quality while minimizing production of undesired byproducts. Generally, substrates (such as glucose and glutamine) will drop rapidly in fed-batch cultures but can be replenished using continuous or bolus feeding; evidence suggests continuous feeding can improve product quality. Perfusion bioreactors aim to keep the substrate concentration constant and remove toxic metabolite accumulation, resulting in overall higher yield.



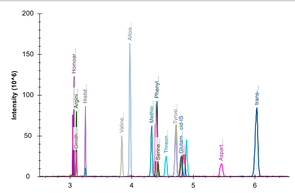

**Aims**. We examine the utility of a high-throughput, automated metabolomics pipeline for rapidly characterizing media from fed-batch and perfusion CHO bioreactors. We use a simple sample preparation with 10x diluted media and methanol precipitation, followed by CE-HRMS analysis and automated processing to compare continuous versus bolus-fed batch reactors, and perfusion bioreactors where one reactor became contaminated.

## MoveKit™ CE Consumables

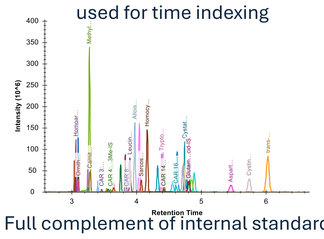

**What's Included:**
Extraction filter and collection 96-well plates
Extraction and Focusing Reagent
Internal standards (50 SIL, custom from CIL)
SST, Blank, Calibration, and QC Samples

**Protocol**
20 µL 10:1 diluted cell culture media
180 µL of extraction + focusing reagent
Shake (15 min)
Filter (spin or vacuum manifold)

**Coverage:**
Library of 300+ polar metabolites
14 metabolite classes; no derivatization
Flexibility to add your own compounds




17 internal standards used for time indexing



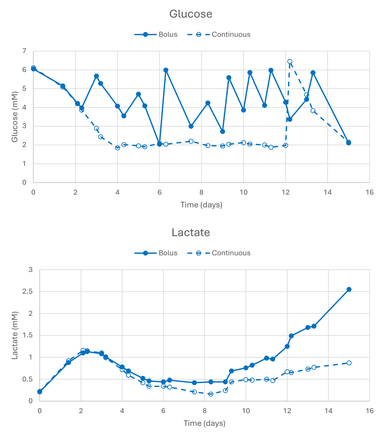

Full complement of internal standards used in the MoveKit CE method

## Materials and Methods

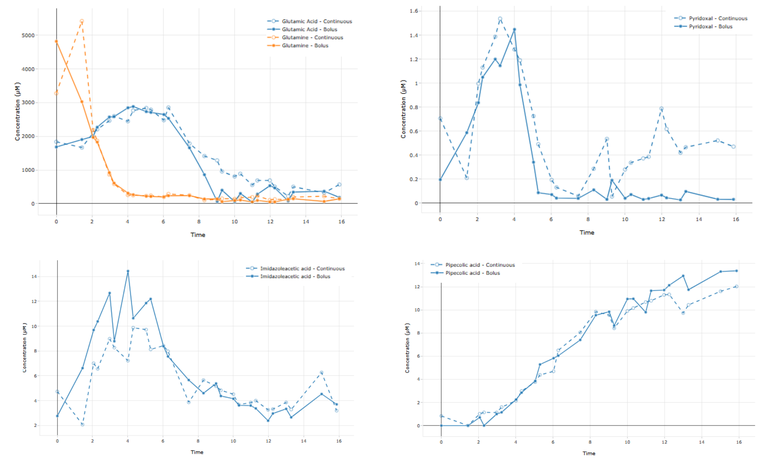
Bioreactor samples were collected from CHO fed-batch or perfusion cultures. Spent media (20 µL after 10x dilution) were processed using MoveKit CE (Move Analytical), which includes pre-formulated reagents, internal standards, and calibration materials. Protein precipitation and filtration were performed in a 96-well format without derivatization or dry-down. Samples were analyzed using a ZipChip CE (Repligen) and an Exploris 240 mass spectrometer (ThermoFisher Scientific). Automated System Suitability Tests (SST) verified migration time precision, mass accuracy, and signal response before running each sample plate. Data were automatically checked for data quality, then processed through a Skyline template with an automated peak assignment model and quantified against external calibration materials prepared on the same plate.

## Bioreactor Metabolomics Measurements

**Bolus vs Continuous Glucose, Fed Batch.** Samples were collected twice daily from a fed-batch CHO bioreactor producing NIST mAb, where glucose was either fed at 6 mM every 2 days or held continuous at 2 mM throughout the majority of the culture.

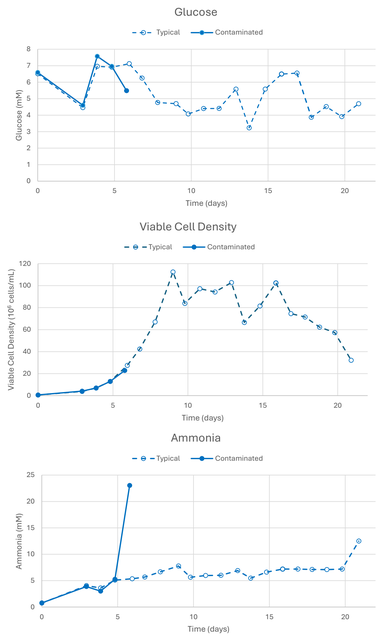



Nova Flex® assay results for glucose and lactate, showing accumulation of lactate in the bolus fed bioreactor.

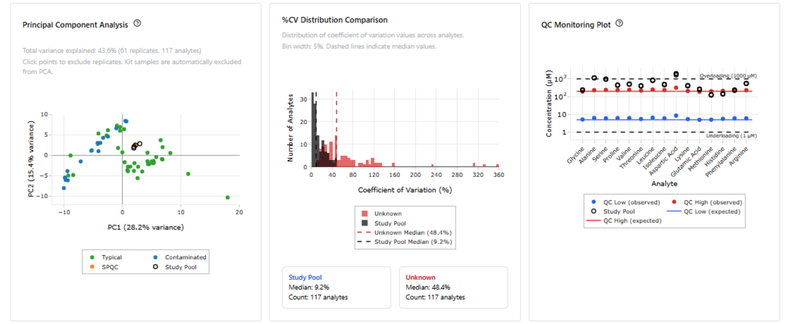



Selected MoveKit™ CE results showing differential dynamic trends in metabolite concentration for amino acids, vitamins, and accumulating byproducts.

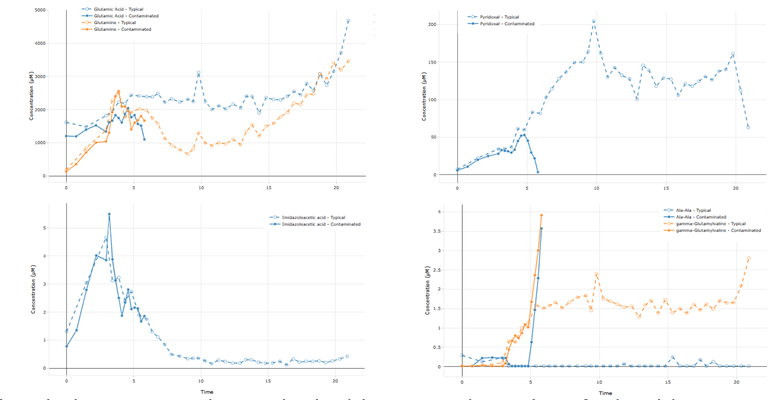
**Typical versus Contaminated Perfusion Bioreactor.** Samples were collected from two perfusion bioreactors producing a trastuzumab biosimilar. One of the bioreactors ran as typical for 21 days while the other became contaminated with bacteria and the reactor was stopped at 6 days. Metabolomics was used to investigate early indication of contamination.




Glucose, VCD, and ammonia from the timepoints used for metabolomics, showing similar glucose and VCD but a spike in ammonia in the contaminated bioreactor on Day 6.




Quality control plots from MoveApp™ for this study.




Trend plots comparing typical with contaminated perfusion bioreactors. While some metabolites show small or no differences, others show large drops (e.g. pyridoxal) or spikes (e.g. dipeptides) with contamination.

## Acknowledgements and Conflicts

All authors are cofounders and own interest in Move Analytical. We gratefully acknowledge our continued collaborations with Repligen Corporation, in particular Ethan Bossange and Erin Redman for providing the bioreactor samples analyzed for this poster.

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