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Rapid Analysis of Upstream Bioprocesses Using a Point-of-Need Mass Spectrometer

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INTRODUCTION

Applications of mass spectrometry (MS) in the pharmaceutical industry are well known. The new demands of modern bioprocessing and the implementation of QbD and PAT methods require a deeper understanding of these processes, MS is well placed to provide the 'information rich 'data required. However, traditional mass spectrometers are large, expensive, and require operation by specialists in centralized laboratories. Integrated real-time MS, decentralized and at the point-of-need, could provide important process information quickly and at reduced cost. Key to decentralization and adoption of non-specialists is the use of both deployable mass spectrometers and the simplification of work-flows. Here, the feasibility of using a deployable and compact mass spectrometer for the direct analysis of upstream cell cultures is investigated.

METHODOLOGY

For the feasibility studies a prototype mass spectrometer, based on a Microsaic 4000 MiD® mass spectrometer, and coupled to a Microsaic MiDAS™ sampling interface unit (figure 1) was used.

FIGURE 1. *Prototype mass spectrometer based on the MiD® and MiDAS™ sampling interface unit.*

With an adjustable mass range between 50 and 3200 m/z the monitoring of small molecules in cell culture media and large biologic proteins is possible with this mass spectrometer.

Key to the decentralization and adoption by non-specialists is the simplification of MS work-flows. Direct analysis, without chromatographic separation, was assessed as this significantly reduces workflow complexity and reduces analysis time to under 2 minutes. Spiking of key feed components and metabolites to CD OpticCHO media allowed the feasibility of this direct analysis to be investigated.

The direct analysis of cell culture media with ESI is normally complicated by high concentrations of salts and other involatile compounds which can lead to fouling and instrument downtime. Automated methods, incorporating *in-situ* cleaning, were also devised to allow minimal downtime.

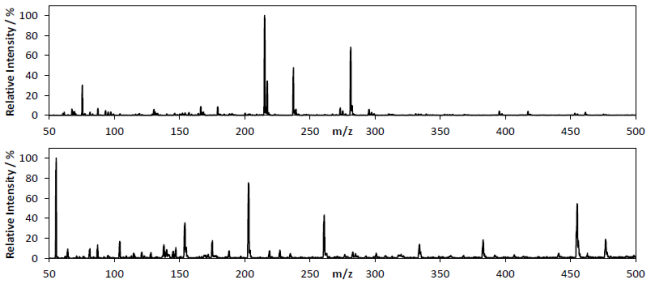

FIGURE 2. *Full scan of CD OptiCHO in negative (top) and positive (bottom) mode.*

RESULTS

Full scans of CD OptiCHO from 50-500 m/z in both positive and negative mode demonstrate the complexity of these media (Figure 2).

Spiking with glucose, lactate, glutamate, glutamine, sorbitol, alanine, glycine and glycerol at a range of concentrations allowed responses to be seen for all samples (Figure 3). Using an internal standard, SIM monitoring of protein and sodium adduct ions allowed the quantification and direct measurement of these species in the cell culture media.

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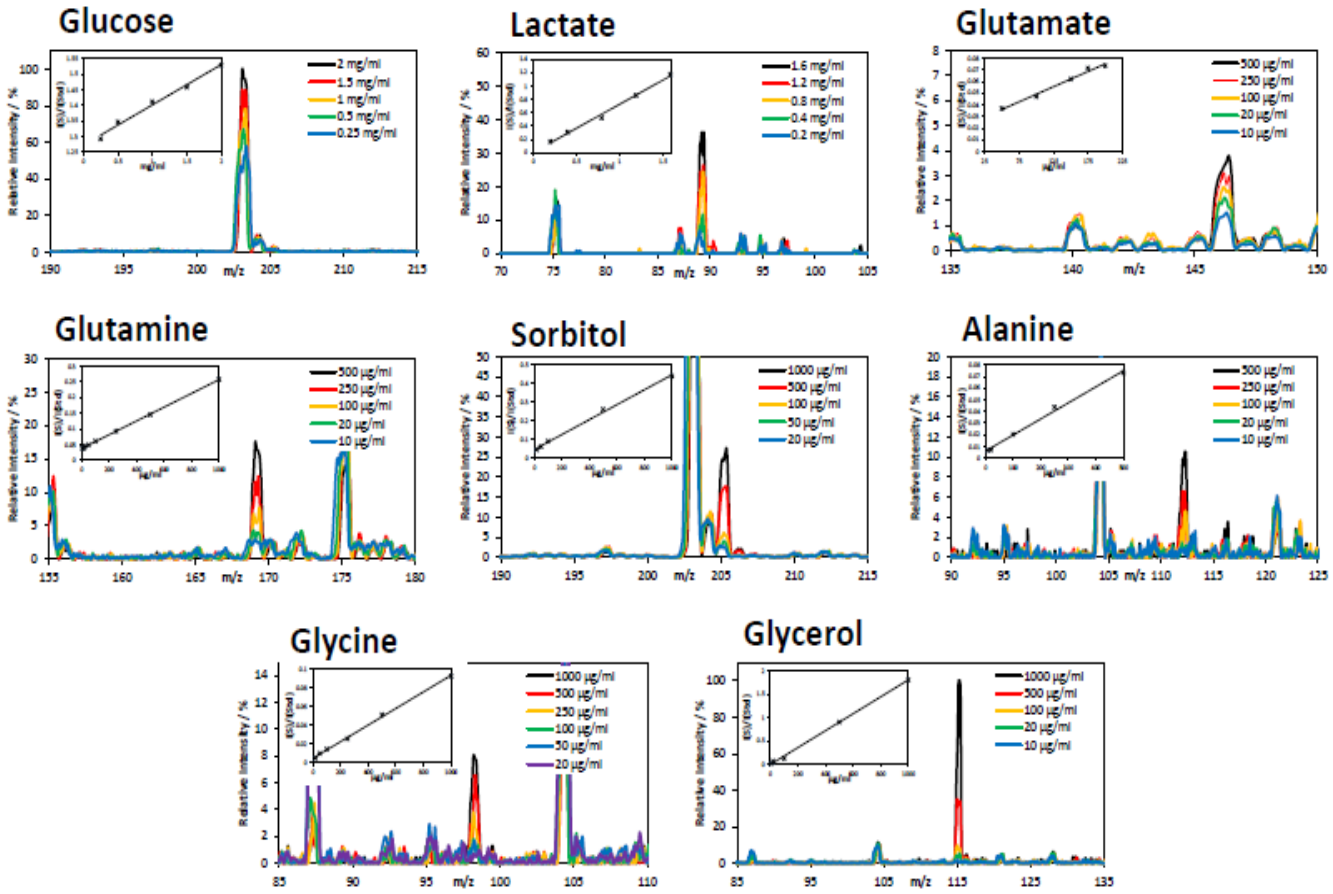

FIGURE 3. *Representative full scans demonstrating changes in intensity with concentration. Inserts show quantification of chemical concentration using SIM mode*

CONCLUSIONS AND FUTURE WORK

These feasibility studies demonstrate the potential of point of need MS to directly monitor key component in upstream cultures. In addition, the use of a microspray ESI source allows small microlitle volumes to be analysed. Work is in progress with our academic and industrial partners to further validate this instrumentation and methodology.

Studies are also on-going to assess point of need MS with integrated dialysis for the monitoring of biologics during upstream processing [1]. Use of automated protein mass tool to convert MS data into simple and easy to understand information is also being assessed as part of this evaluation [2].

Point-of-need MS offers the potential to monitor upstream cell cultures in greater detail, close to real time, and with significantly reduced response times relative to centralized facilities. The ability to analyse microlitle volumes may also allow the monitoring of micro-bioreactors.

REFERENCES

(1) S.Wright, A.I McIntosh; Methods and System for Monitoring Biomolecule Separations by Mass Spectrometry; US Patent US9535042 (2014)

(2) A. I. McIntiosh; A Method for Extracting Mass Information from Low Resolution Mass-to-Charge Ration Spectra of Multiple Charged Species; WO2017202561 (2017)

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