Evaluation of a highly sensitive single platform method for quantification of crucial components from cell culture media (CCM) by LC-MS/MS analysis

Marialuce Maldini[1], Eshani Nandita[2], Zuzana Demianova[2]
[1]SCIEX, Italy; [2]SCIEX, USA

ABSTRACT

Comprehensive identification and quantification of cell culture media (CCM) components is an essential step during the development phases of biotherapeutic production as it can affect the overall quality of the final product. Given the emphasis on quality by design, there is an essential need to develop sensitive, accurate, and robust analytical assays to allow for comprehensive profiling of CCM. The composition of CCM includes various compound classes such as amino acids, sugars, vitamins, nucleobases, fatty acids, and other essential components. Thus, a single platform for the analysis of a mixture of components with a broad range of concentrations can be highly challenging. Herein, a method for highly sensitive quantification of over 110 CCM components using LC-MS/MS is presented.

INTRODUCTION

- Conventional platforms such as biosensor-analyzer, NMR, and Raman spectroscopy are typically unable to offer a single-platform qualitative and quantitative method for CCMA in matrix with high sensitivity, selectivity and throughput.
- In comparison, SCIEX CCMA methods for triple quadrupole and high resolution QTOF systems offer high sensitivity, selectivity, and robustness for the identification and quantification of over 110 compounds in a single analytical method in under 20 mins.
- Herein, a highly sensitive and comprehensive CCMA method on the SCIEX 7500 system coupled to the ExionLC system demonstrates S/N improvements for analytical targets across various classes including amino acids, carbohydrates, vitamins, fatty acids, nucleic acids, and other essential compounds.

MATERIALS AND METHODS

Sample preparation:
Cell culture media samples (CD CHO media, Gibco) with unknown concentrations of analytes were diluted in 100 µL of 50:50 (v:v) 0.1% formic acid (FA) in water and 0.1% FA in acetonitrile and centrifuged. The supernatant was further diluted with 0.1% FA resulting in a total dilution factor of 300. Q0 dissociation (Q0D) was optimized using the QReSS kit (Cambridge Isotope Laboratories, Inc.). Standards were diluted 100-fold in water and a working aliquot was added to each sample and standard dilutions.

LC conditions:
Analytes were separated using a Phenomenex Kinetex F5 column (150 mm × 2.1 mm, 2.6 µm, 100 Å) on an ExionLC system. Total method time was 20 min at a flowrate of 200 µL/min. Mobile phase A was composed of 0.1% FA in water while mobile phase B was composed of 0.1% FA in acetonitrile. Operating column temperature was 40 ºC. Injection volume was 5 µL.

MS conditions:
MRM parameters for more than 110 cell culture components were optimized by chemical standards. For both the SCIEX Triple Quad 6500+ system and the SCIEX 7500 system, the Scheduled MRM Pro algorithm was used to optimize cycle times and maximize dwell times for each MRM transition (Figure 1). By scheduling transitions around the expected retention time of an analyte, the sMRM method enables monitoring of significantly more MRMs simultaneously without sacrificing superior analytical precision.

Two MRM transitions were monitored for each analyte, with a few exceptions when only one MRM was available for analytes ionized in positive and negative mode. This allows for comparisons of ion ratios to help identify potential peak integration issues.

This method contains 178 MRMs in positive and 54 MRMs in negative ionization mode which enables monitoring of over 110 media analytes (Figure 2). For both systems, fast polarity switching allowed analysis in positive and negative ionization modes within one method.

Data processing:
Scheduled MRM algorithm data was processed using the Analytics module in SCIEX OS software 2.0.

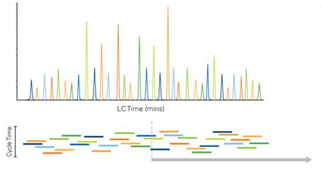

Figure 1. Schematic of the Scheduled MRM Pro algorithm.

- Each MRM transition was monitored in a given retention time window ensuring the simultaneous monitoring of several MRM transitions in a single LC run (Figure 1).
- This approach maintains the maximized dwell times and optimal cycle times for accurate quantification.

SCIEX CCMA method contains >110 media analytes

- The method includes over a 110 CCM analytes which are identified and quantified in under a 20 min run.
- The method is composed of CCM target analytes from classes such as amino acids, vitamins, carbohydrates, fatty acids, nucleic acids, and other essential components (Figure 2).

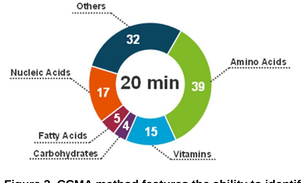

Figure 2. CCMA method features the ability to identify and quantify over a 110 media analytes in under 20 min.

RESULTS

- Optimization of Q0D was performed using the QRESS kit which included isotopically labeled standards. The standards represented compounds from the major classes comprising CCM.

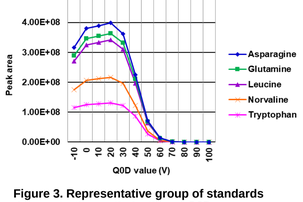

Figure 3. Representative group of standards demonstrating optimization of Q0D.

- Optimization of Q0D demonstrated sensitivity improvements for select compounds (Figure 3).
- Q0D optimization was monitored in the range between -10 V to 100 V in increments of 10 V.
- The optimal Q0D value from the analysis of isotopically labeled standards was applied towards all compounds monitored in the CCMA.

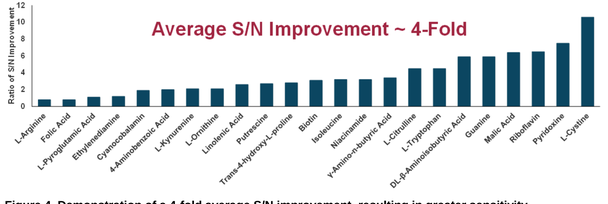

Figure 4. Demonstration of a 4-fold average S/N improvement, resulting in greater sensitivity.

- In a representative collection of 23 compounds, 78% of CCM compounds achieved ≥ 2-fold enhancement in S/N.
- On average, 4-fold improvement in S/N was observed using a SCIEX 7500 system when compared to the SCIEX Triple Quad 6500+ system for compounds in all major CCM classes including amino acids, carbohydrates, vitamins, fatty acids, and nucleic acids (Figure 4).

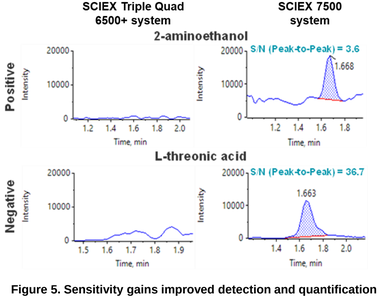

Figure 5. Sensitivity gains improved detection and quantification limits.

- Low level target analytes which were previously not detectable are now within the detectable and quantifiable range (Figure 5).
- Examples of such target analytes include 2-aminoethanol, an essential component for growth of hybridoma cells[1], and L-threonic acid, one of the metabolites of ascorbic acid.[2]
- On the SCIEX 7500 system, 2-aminoethanol was detectable with a S/N of 3.6 while L-threonic acid was well within the quantification range with a S/N of 36.7 (Figure 5).
- Therefore, improvements in both limit of detection (LOD) and limit of quantification (LOQ) were observed across the various compounds in CCM.

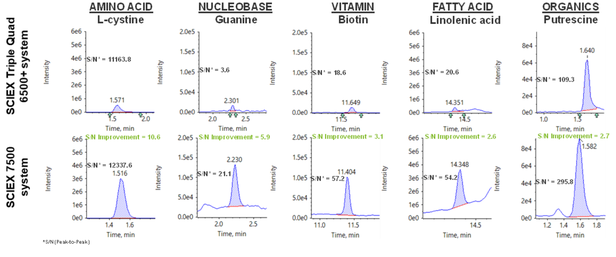

Figure 6. Improvements in S/N were observed across various CCM classes.

- Representative examples from classes such as amino acids, nucleobases, vitamins, fatty acids, and other essential components showed greater S/N improvements on the SCIEX 7500 system (Figure 6).
- All peaks were normalized to respective intensities from the SCIEX 7500 system.
- For example, biotin, which is one of the essential vitamins that plays a role in energy metabolism and fatty acid synthesis,[3] demonstrated a 3-fold improvement in S/N.
- In this manner, boosts in signal sensitivity were observed across various CCM compounds which enabled improved S/N ranging from a factor of 2 to 10-fold on the SCIEX 7500 system.

CONCLUSIONS

- An ultra-sensitive sMRM-based CCMA method has been presented.
- An average of 4-fold improvement in S/N was observed for CCM compounds across various classes.
- For low level target analytes, detection and quantification limits were substantially improved on the SCIEX 7500 system.

REFERENCES

1. Murakami, H. (1982) Growth of hybridoma cells in serum-free medium: ethanolamine is an essential component. PNAS, **79**(4), 1158-1162.
2. Eagle, H. (1959) Amino acid metabolism in mammalian cell cultures. Science, **130**(3373), 432-437.
3. Dakshinamurti K. (1985) Requirement for biotin and the function of biotin in cells in culture. AANALS, 447, 38-55.

TRADEMARKS/LICENSING