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EVALUATION OF A HIGHLY SENSITIVE SINGLE PLATFORM METHOD FOR QUANTIFICATION OF CRUCIAL COMPONENTS FROM CELL CULTURE MEDIA (CCM) BY LC-MS/MS ANALYSIS

ABSTRACT
Comprehensive identification and quantification of cell culture media (CCM) components is an essential step during the development phase of high impact products and critical clinical products. Great emphasis on the quality of media, in terms of ingredients, purity, and critical cellular attributes is desirable for comprehensive profiling of CCM. Herein, an ultra sensitive and selective LC-MS/MS method is applied to quantify over 110 CCM components. This approach maintains the maximized dwell time for each MRM transition allowing for improved S/N within the quantification range with a S/N of 36.7 (Figure 5).

INTRODUCTION
Conventional platforms such as biosensor, NMR, and Raman spectroscopy are typically applied to offer a single-platform qualitative and quantitative method for CCMs in terms of high sensitivity, selectivity, and throughput for the identification and quantification of over 110 compounds in a single method using LC-MS/MS. In comparison, SCIEX CMA methods for triple quadrupole and high resolution QTOF systems offer high sensitivity, selectivity, and quantification of over 110 compounds in a single method using LC-MS/MS.

MATERIALS AND METHODS
Sample preparation:
Cell culture media samples (LC-CHI media, GIBCO) were diluted in the media dilution system using water and a working aliquot was added to each sample and standard dilutions. The solution was equilibrated at room temperature (40 ºC). Injection volume was 5 µL.

LC conditions:
An Acquity UPLC™ HSS T3 column (150 mm x 2.1 mm, 2.6 µm, 100 Å) on an ExionLC™ analyzer was used for separation of a broad range of analytes. The mobile phase was composed of 51% H2O in water and a working aliquot was added to each sample and standard dilutions. Operating column temperature was 40 ºC. Gradient elution is el-0

MS conditions:
The SCIEX Triple Quad 6500+ system was used for compounds in all major CCM classes including amino acids, carbohydrates, vitamins, fatty acids, and nucleic acids (Figure 4). SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For Research Use Only. Not for use in diagnostic procedures.

RESULTS
Optimization of Q0D was performed using the QRESS kit which included isotopically labeled standards. The scheduled MRM algorithm data was processed using the Analytics module in SCIEX OS software 2.0.

• Each MRM transition was monitored in a given retention time for accurate quantification.
• The approach maintains the maximized dwell time for each MRM transition allowing for improved S/N within the quantification range with a S/N of 36.7 (Figure 5).

Figure 1. Schematic of the Scheduled MRM Pro algorithm.

Figure 2. CCM method features the ability to identify and quantify over 110 media analytes in under 20 min. Each MRM transition was monitored in a given retention time for accurate quantification.

Figure 3. Representative group of standards demonstrating extremely sensitive Q0 dissociation (Q0D).

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

• A representative collection of 25 compounds. 79% of CCM compounds achieved ≥2-fold enhancement in S/N.
• Each MRM transition was monitored in a given retention time for accurate quantification.

Figure 5. Average S/N Improvement = 4-Fold

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 vitamin plays a role in energy metabolism and fatty acid synthesis, 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 and selective CCM method had been presented.

• As an example of improved S/N, 34 compounds were observed in CCM across various classes.

• A low level target analytes which were previously not detectable were quantifiable across various classes.

• For low level target analytes, detection and quantification limits were substantially improved on the SCIEX 7500 system.

REFERENCES

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