High-throughput compound QC workflow with Acoustic Ejection Mass Spectrometry

Chang Liu[1]; Cristiano Veiga[1]; Alandra Quinn[2]; Jeff Chin[2]; Thomas R. Covey[1]; Hui Zhang[2]
[1]SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada; [2]Pfizer Inc., Eastern Point Road, Groton, CT, 06340

ABSTRACT

The druggability assessment requires the addition and incubation of each compound in the biological reaction. The quality of the stock standard directly impacts the assay readout – the impurity and/or the degradation may cause the false positive/negative. Therefore, it is desired to quality control (QC) the compound library. Due to the high sample quantity, the analytical platform used in this workflow is required to provide the high throughput (seconds per sample). Here, we introduce the use of the TOF-based acoustic ejection mass spectrometry (AEMS) system for compound QC with the high analytical throughput and data quality. The generated data could be automatically processed with customizable validation conditions, together with the auto-generated heatmap for data review and visualization.

INTRODUCTION

All pharmalogical screening depends on high-quality compound libraries, and it is highly desired to run fast and reliable QC with the compound library to validate the screening results. LC-MS is widely used for small molecule library QC however the throughput is a bottleneck for big libraries. AEMS is a new platform that provides ultra-high throughput analysis capability [1-3]. In the AEMS system, nanoliter volume samples are introduced from acoustic droplet dispensers into a continuous liquid stream drawn into the ion source by the Venturi effect created with a high velocity pneumatic nebulizer common to ESI. Since its introduction, AEMS has been applied to various high-throughput drug discovery works, including high-throughput pharmacology screening, ADME screening, bioanalysis, and parallel medicinal chemistry. In a recent study, Zhang et.al, reported the use of AEMS system in the Q1 scan mode on a triple quadrupole MS system for compound QC [4]. Herein, we reported the compound QC workflow on a high-resolution version of the AEMS system. The automatic data processing and visualization function is developed to support this workflow.

MATERIALS AND METHODS

The sample plates were prepared in DMSO in the 384-well format. These plates were analyzed on a research prototype of Acoustic Ejection Mass Spectrometry (AEMS) system coupling with the SCIEX TripleTOF 6600 system. The MS was run in the TOF-scan mode. The MS data files each containing 384 ejections were processed with a research-version data splitting algorithm to assign the well-position to each MS signal peak. The split MS data together with the sample information table (the compound ID and/or chemical formular of each well) were imported to the research-version data integration and visualization tool for processing.

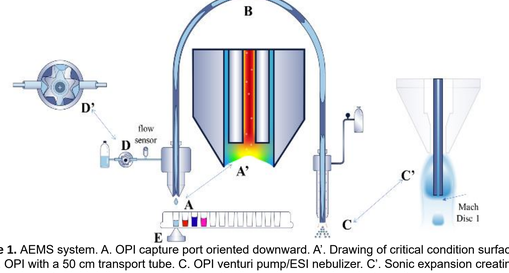

Figure 1. AEMS system. A. OPI capture port oriented downward. A'. Drawing of critical condition surface in "A". B. OPI with a 50 cm transport tube. C. OPI venturi pump/ESI nebulizer. C'. Sonic expansion creating pressure drop. D. Fluid delivery pump. E. Acoustic dispensing upward against gravity.

RESULTS

In AEMS, the MS signal from different ejections was collected as a single data file, as shown in Figure 2. The TOF mass spectra of each ejection could be recorded for the follow up processing.

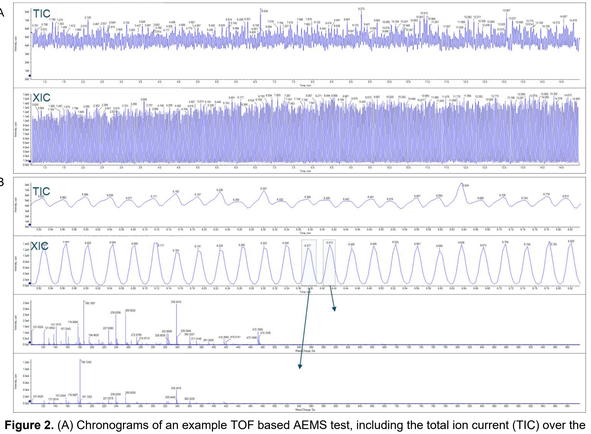

Figure 2. (A) Chronograms of an example TOF based AEMS test, including the total ion current (TIC) over the scanned mass range and the extracted ion current (XIC) of an ion (m/z=255.0) existing in all sample wells. (B) The zoom-in view of the chronograms in (A), and the mass spectra of two adjacent ejections.

To enable the AEMS system for the high-throughput compound QC, the automated data processing capability is essential matching the high-speed data acquisition. In this work, the first step is to correlate the sample well position with each MS signal peak, with the synchronization of the MS signal with the sample ejection log (Figure 3).

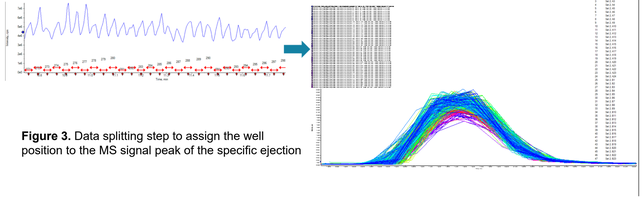

Figure 3. Data splitting step to assign the well position to the MS signal peak of the specific ejection

The split data (MS signal correlated with well position) and the compound information table (formular and charging agent information for each well) could be input into the data processing module for integration, with the results table automatically generated (Figure 4).

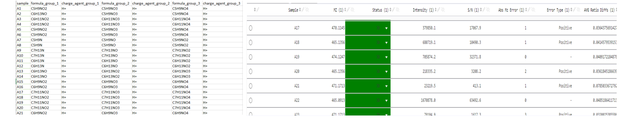

Figure 4. Example of the compound information table and the data processing results. The compound information table could contain multiple target formula per well, and the results includes not only the intensity information, but also mass accuracy, signal to noise ratio, the similarity vs the theoretical isotope MS pattern etc.

In addition to the automatic data processing, the heat-map is generated for the results visualization with the intensity color-coated. In addition, the heat-map is interactive. The mass spectra and the XIC of the target ion of the highlighted well could be reviewed. In the mass spectra window, the thermotical mass with the isotope patten is overlayed for the direct results review/validation (Figure 5).

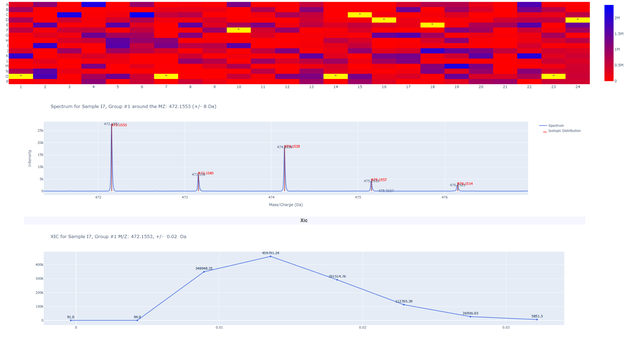

Figure 5. The visualization of the AEMS results for compound QC, including the heat-map, the mass spectra of the clicked well, and the XIC of this well based on the target analyte defined in the compound information table. .

Besides the intensity, several validation rules are considered to confirm the integrated signal is from the target ion or other interference. These validations rules include mass accuracy, signal to noise ratio, and the isotope pattern. The results do not meet these rules will be flagged out in the results table and heat map for users to review (Figure 6).

Figure 6. Example of a sample well (Q23) did not meet the validation rules, with its mass spectra and XIC directly visualized for review

CONCLUSIONS

The high-resolution MS based AEMS system is demonstrated for high-throughput compound QC. In addition to the high-speed data acquisition (~1 sec per sample), the data processing and visualization function is demonstrated, with the interactive data review capability. The system introduces here would enable the library-sized compound QC with high throughput and high accuracy.

REFERENCES

1 Liu, et al. Anal. Chem. (2020) 92, 15818-15826.
2 Zhang, et al. Anal. Chem. (2021) 93, 10850-10861.
3 Häbe, et al. Anal. Chem. (2020) 92, 12242-12249.
4 Zhang, et al. SLAS Technol. (2021), 26, 178-188.

TRADEMARKS/LICENSING