Acoustic Ejection Mass Spectrometry for high-throughput compound QC

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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 – impurity or the degradation can cause a false positive or negative. For this reason, it is good practice to run quality control (QC) on the compound library. Due to the high sample quantity, the analytical platform used in this workflow must provide high throughput (seconds per sample). Here, we introduce the use of a TOF-based Acoustic Ejection Mass Spectrometry (AEMS) system for compound QC with the high analytical throughput and data quality and the ability to automatically process the generated data

INTRODUCTION

All pharmacological screening depends on high-quality compound libraries, and it is highly desired to run fast and reliable QC on the compound library to validate the screening results. While liquid chromatography (LC) with UV or MS detection is used for small molecule library QC, however the throughput is a bottleneck for big libraries. AEMS enables ultra-high-throughput analysis. Here, we introduce the use of AEMS system with a high-resolution MS analyzer for the ultra-high-throughput compound QC. An automated data-processing function was developed to generate the compound confidence score according to multiple attributes along with the description of the purity.

MATERIALS AND METHODS

The compound QC test plates were prepared in dimethylsulfoxide (DMSO) using the 384-well format. These plates were analyzed on a research prototype AEMS system coupled with the SCIEX Triple TOF 6600. The MS was run in 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 formula of each well) were imported to the research-version data processing tools for analysis.

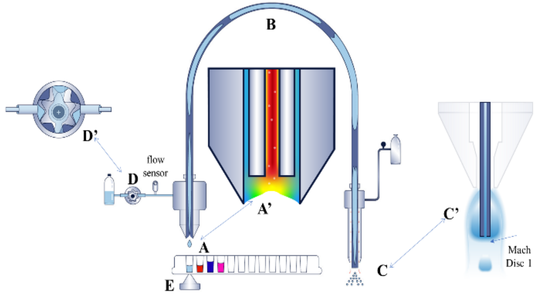

Figure 1. AEMS system. A. OPI capture port oriented downward. A'. Drawing of critical condition surface in "A". B. OPI with a 50 cm transport tube. C. OPI venturi pump/ESI nebulizer. C'. Sonic expansion creating pressure drop. D. Fluid delivery pump. E. Acoustic dispensing upward against gravity.

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 can be recorded for the follow-up processing.

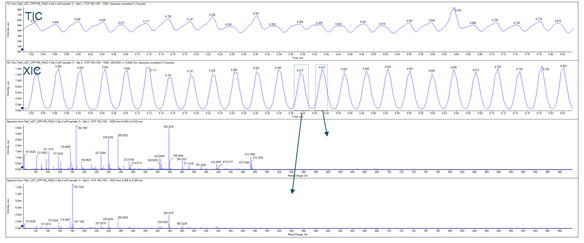

Figure 2. Chronograms of an example TOF-based AEMS test, including the total ion current (TIC) over the scanned mass range, the extracted ion current (XIC) of an ion (m/z=255.0) existing in all sample wells, and the mass spectra of two adjacent ejections.

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).

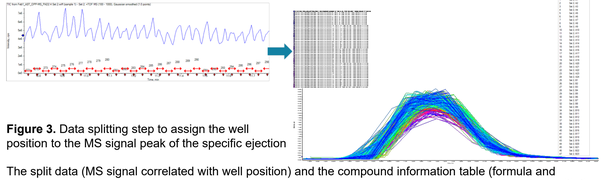

Figure 3. Data splitting step to assign the well position to the MS signal peak of the specific ejection

The split data (MS signal correlated with well position) and the compound information table (formula 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).

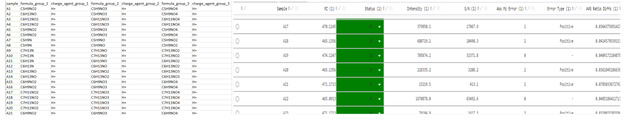

Figure 4. Example of the compound information table and the data processing results. The compound information table could contain multiple target formula per well, and the results includes not only the intensity information, but also mass accuracy, signal to noise ratio, the similarity vs the theoretical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

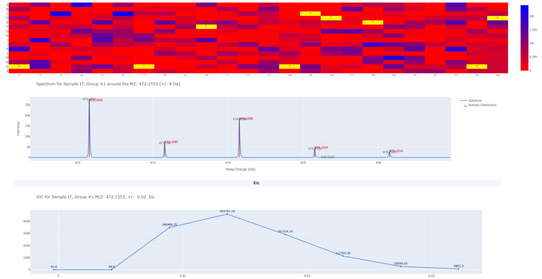

Figure 5. The visualization of the AEMS results for compound QC, including the heat-map, the mass spectra of the clicked well, and the XIC of this well based on the target analyte defined in the compound information table. .

In this tool, four different parameters are automatically calculated describing each sample: intensity of target compound, mass accuracy of target compound (in ppm or Da), signal to noise ratio, and average ratio difference. Each category has a different numerical range of values to characterize the target. Analysis of 20k+ compounds show overlap in compounds that fail (verify) and compounds that pass (OK) in all categories. Therefore, a new parameter is required to describe the overall compound quality considering these different descriptions.

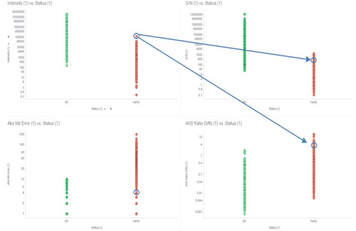

Figure 6. The correction of the compound status with individual description parameters.

The new parameter (P) ranges between 0 and 1 containing the contribution from the two sensitive factors to the compound quality, the signal-to-noise ratio, and the description of isotope distribution comparing with the theoretical.

	Found	Not found	Verify
Found	9506	41	35
Not Found	5	444	7

Figure 7. The correlation between the compound standard characterized with the P-value (Found P ≥ 0.5, Verify 0.4 < P < 0.5, Not Found P ≤ 0.4) with the manually confirmed compound status (found vs not found).

The mass spectra of each ejections from the same plate (with the similar background ions) are cross-compared for the automatic identification of background ion m/z, as shown in Figure 8.

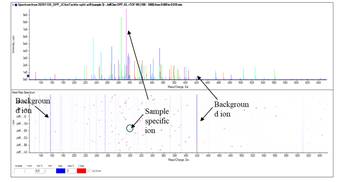

Figure 8. Identification of background ions from sample specific ions.

The identification and subtraction of the background ions from mass spectra could greatly simplify the MS spectra and provide the direct information for the assessment of compound purity. For example, Figure 9 shows two example compounds from two resources. The first compounds also showed different mass spectra from the two runs, but these distinguished ions are from the background. On the other hand, a major impurity ion was discovered on another compound.

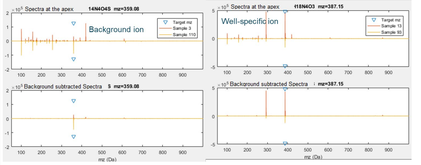

Figure 9. Original and background subtracted mass spectra for two compounds from different resources.

The purity information could then be calculated for the compound standard using the background-processed mass spectra.

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.

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