

# **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 researchversion 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.



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



# Acoustic Ejection Mass Spectrometry for high-throughput compound QC

# Chang Liu<sup>1</sup>; Alandra Quinn<sup>2</sup>; Gordana Ivosev<sup>1</sup>; Thomas R. Covey<sup>1</sup>; Brendon Kapinos<sup>2</sup> <sup>1</sup>SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8, Canada; <sup>2</sup>Pfizer Inc., Eastern Point Road, Groton, CT, 06340, USA

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.

MMMMMMM total total 283 283 285 285 287 288 289 290 275 276 277 278 279 280 **1**88 **\* \* 108 \* \* 110\* \* 111 \* \* 192 \* \* 113\* \* \* 114 \* \* 115 \* \* 116\* \* \* 117\* \*** 



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).

gent_group_1	formula_group_2	charge_agent_group_2	formula_group_3	charge_agent_group_3									
	C5H9NO3	H+	C5H9NO4	H+	\$1	Sample 🗘 🖉 🔍	MZ (1) 🗘 🖉 🔍	Status (1) 🗘 🔍 🔍	Intensity (1) 🖘 🔍	S/N (1) 🗘 🔍	Abs Mz Error (1) 🕏 🖉 🔍	Error Type (1) 🗘 🔍	AVG Ratio Diffs (1) 🕯
	C5H9NO3	H+	C5H9NO4	H+									
	C6H11NO3	H+	C6H11NO4	H+									
	C6H11NO3	H+	C6H11NO4	H+	0	A17	470.1145	OK 👻	379850.1	17067.9	1	Positive	0.0364375691427
	C5H9NO3	H+	C5H9NO4	H+									
	C5H9NO3	H+	C5H9NO4	H+									
	C5H9NO	H+	C5H9NO2	H+	0	A18	465.1356	OK 👻	680719.1	10490.3	1	Positive	0.0414579539153
	C5H9NO	H+	C5H9NO2	H+									
	C7H13NO	H+	C7H13NO2	H+			474.1247	ж 🔻	705874.2	32371.8	0	-	0.0409172184878
	C7H13NO	H+	C7H13NO2	H+	0	A19							
	C6H13NO	H+	C6H13NO2	H+									
	C6H13NO	H+	C6H13NO2	H+		A20	465.1356	0K 🔻	218335.2		2	Positive	0.0361845186639
	C6H13NO2	H+	C6H13NO3	H+	0					3208.2			
	C6H13NO2	H+	C6H13NO3	H+									
	C6H9NO3	H+	C6H9NO4	H+		A21	471.1713	08 👻	23219.5	412.1	1	Positive	0.0785033672792
	C6H9NO3	H+	C6H9NO4	H+	U					415.1			
	C7H11NO3	H+	C7H11NO4	H+		A22	465.0913	DK 👻	1670878.0	63492.6	0		0.0485186411713
	C7H11NO3	H+	C7H11NO4	H+									
	C7H11NO3	H+	C7H11NO4	H+	0								
	C7H11NO3	H+	C7H11NO4	H+									
	C6H9NO3	H+	C6H9NO4	H+	$\cap$	∆23	471 1713	NY 🚽	79104 9	1617 3	3	Positive	0 0320025385586

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.



theoretical.

**Confidential - Comp** 

**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

	Found	Not found	Verify	Figure 7.
Found	9506	41	35	value (Fou
Not Found	5	444	7	compound

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.



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.



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.

# TRADEMARKS/LICENSING

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to www.sciex.com/diagnostics. All other products are For Research Use Only. Not for use in Diagnostic Procedures Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd. or their respective owners in the United States and/or certain other countries (see www.sciex.com/trademarks).

© 2023 DH Tech. Dev. Pte. Ltd. RUO-MKT-10-15439-A



The correlation between the standard characterized with the Pund P ≥ 0.5, Verify 0.4 < P < 0.5, Not (0.4) with the manually confirmed status (found vs not found).

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

Vell	-spec	ific ior	1 					Target mz – Sample 13 – Sample 93
100	200	300	400	500	600	700	800	900
0 <sup>5</sup> Ba	ckaroun	d subtra	cted Spe	ectra i	mz=387.	15		
0 <sup>5</sup> Ba	ckaroun '	d subtrad	cted Spe	ectra i	mz=387.	15		

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

To receive a copy of this poster:

To receive a copy of this poster:
Scan the code with your phone camera
Complete the form

