

Assessment of reproducibility in high-throughput experiments by generating over 10,000 data points in an overnight run



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INTRODUCTION

Keeping up with high-volume sample demands has become a need in many types of laboratories. Plate readers are the traditional high-throughput approach for measuring biochemical assays, but some fluorescents or radiolabels can produce data artifacts, disrupt enzyme activity in enzymatic assays or require strict regulations for use. Acoustic Ejection Mass Spectrometry (AEMS) enables the introduction of nanoliter sample volumes with speeds of up to 1 sample per second while using native enzymes and substrates for measurement. With that sampling rate, reproducibility and robustness over large datasets could be a concern. Using the Echo® MS system, with the SCIEX Triple Quad 6500+ mass spectrometer, over 10,000 data points were collected in an 8.5-hour run to assess robustness. A second experiment was done over 4 days, collecting over **56,000 data points**, to performance over multiple days.

METHODS

EXPERIMENT 1:
Small molecule pharma mix was diluted to 1 µg/mL in 10% methanol in a falcon tube. In a Labcyte 384-well plate, 50 µL was pipetted into each well. LC-MS/MS-grade methanol was used as the carrier solvent at a flow rate of 425 µL/min with an ejection droplet volume of 2.5 nL. Two compounds, caffeine and erythromycin, were monitored by MRM in positive mode.

The plate was run 28 times, each run was approximately 17 minutes and the total time to run the study was 8 hours. The result was 10,752 data points from 5,376 sample ejections.

EXPERIMENT 2:
50 µL of 1 µM ADP prepared in a 10x dilution of Simple Buffer with BSA was pipetted into each well of a Labcyte 384-well plate. 70% LC-MS/MS-grade acetonitrile with medronic acid and ammonium formate buffer was used as the carrier solvent at a flow rate of 500 µL/min and an ejection droplet volume of 10 nL. One MRM for ADP was monitored in negative mode.

The plate was run 146 times over 4 days with a time of 10 minutes to run one full plate. The result was 56,064 sample ejections with one data point per ejection.

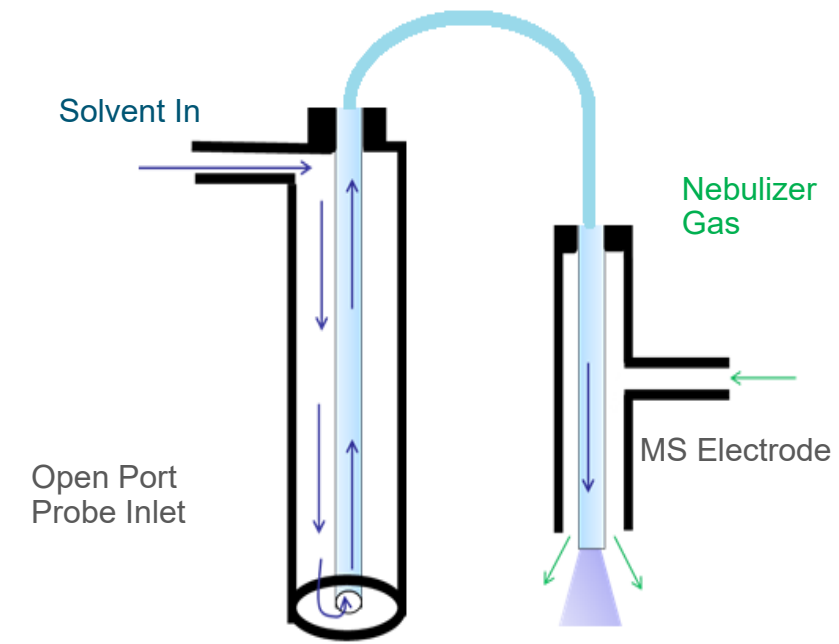


Figure 1. Echo® MS Open Port Interface. Both experiments were performed on a SCIEX Triple Quad 6500+ mass spectrometer using SCIEX OS software for data acquisition. On the Echo® MS system, LC-MS/MS-grade methanol was used as the carrier solvent for introduction of the sample into the OptiFlow Pro ion source using electrospray ionization (ESI).

That’s a lot of data points

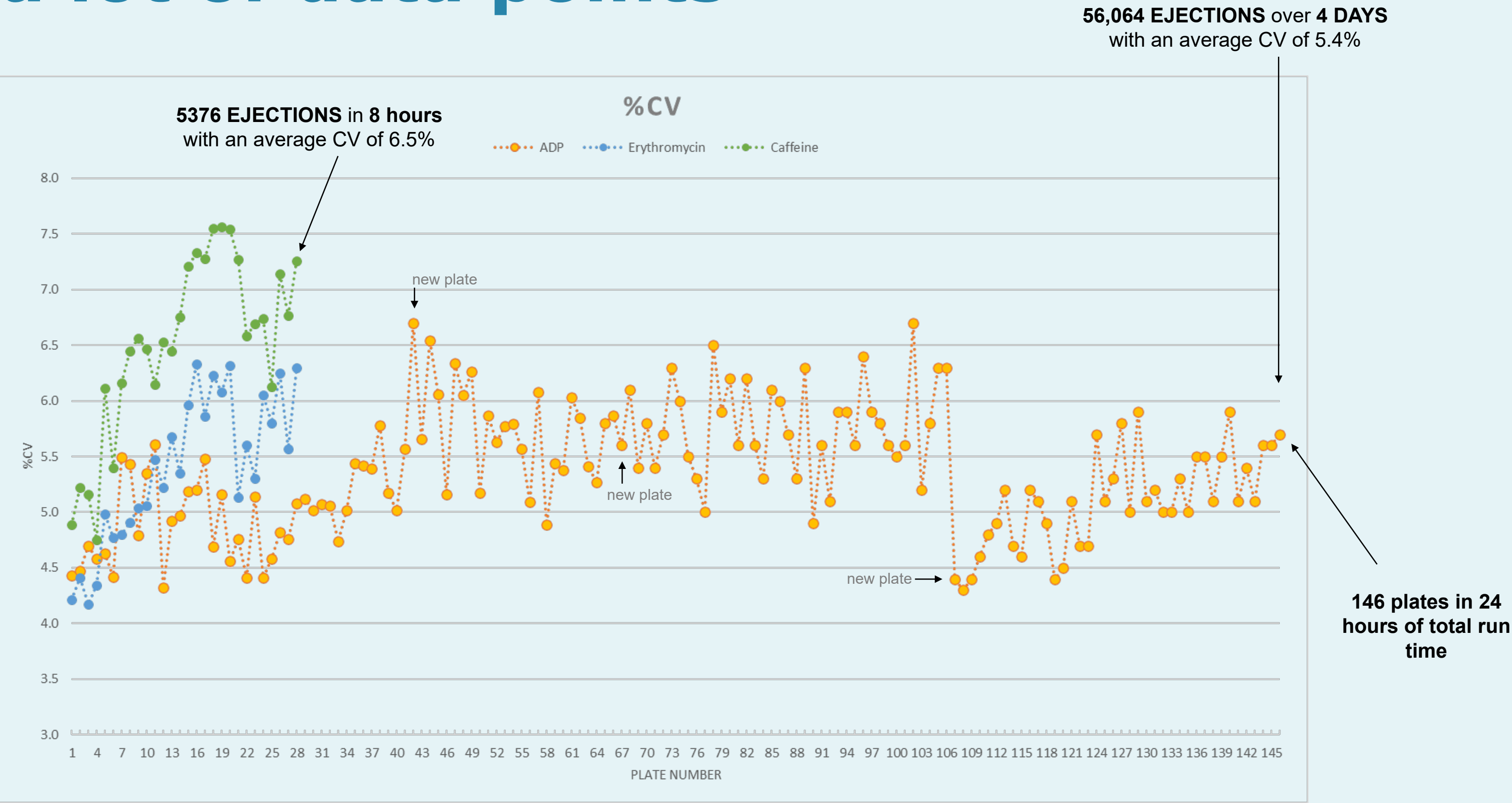
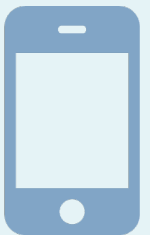


Figure 2. Reproducibility data across experiments 1 and 2. The average %CV across the plate of 384 ejections was computed for each plate run. This %CV is then plotted here per plate to highlight the system reproducibility across extended acquisition.



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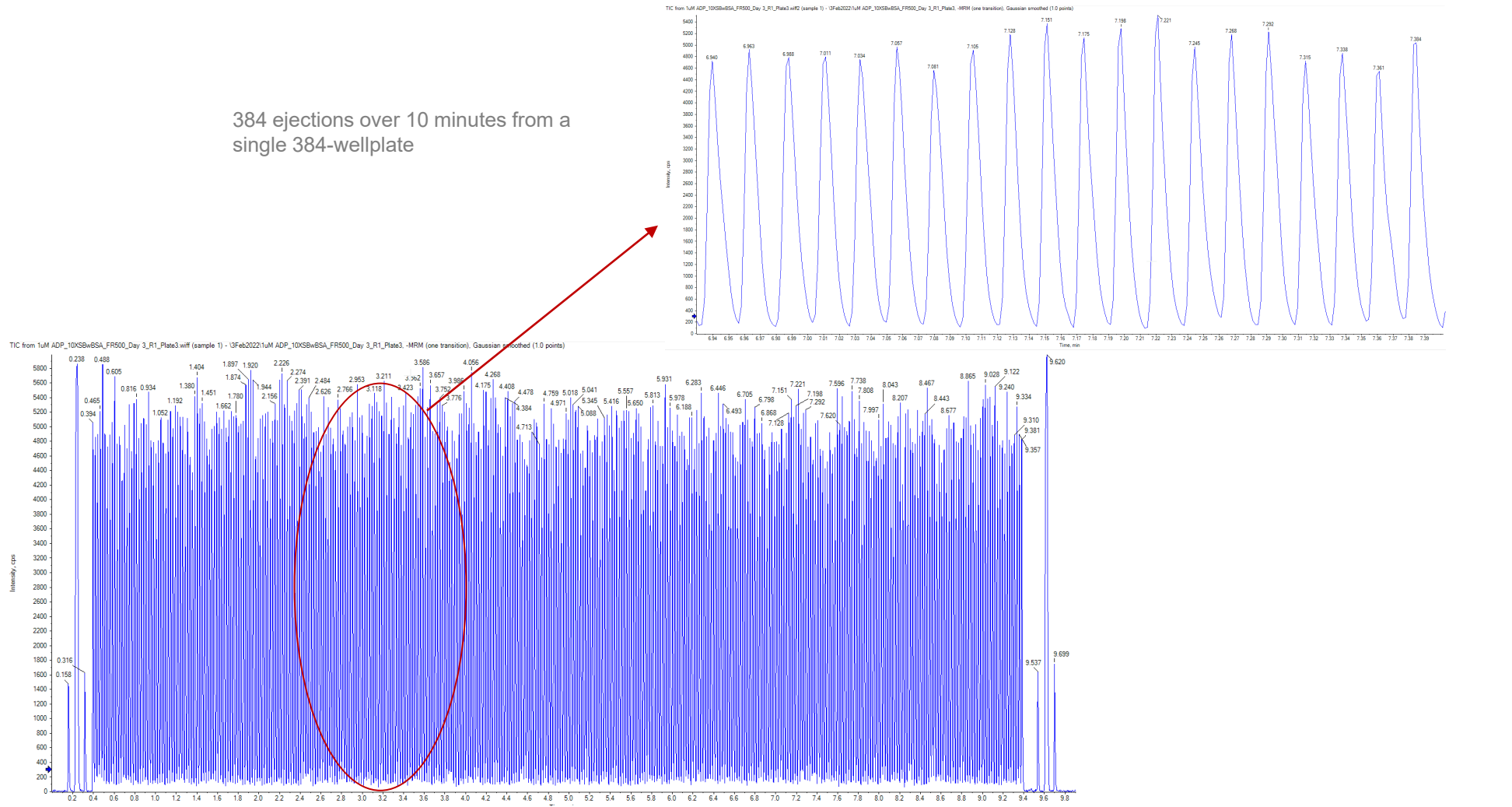


Figure 3. Example ejections across one 384 well plate. In just 10 mins, 384 quantitative measurements of ADP were taken with an %CV across all measurements of ~5% on average. (Zoom) At 1 second per sample, near baseline resolution was achieved between measurements using this flow injection acquisition technique.

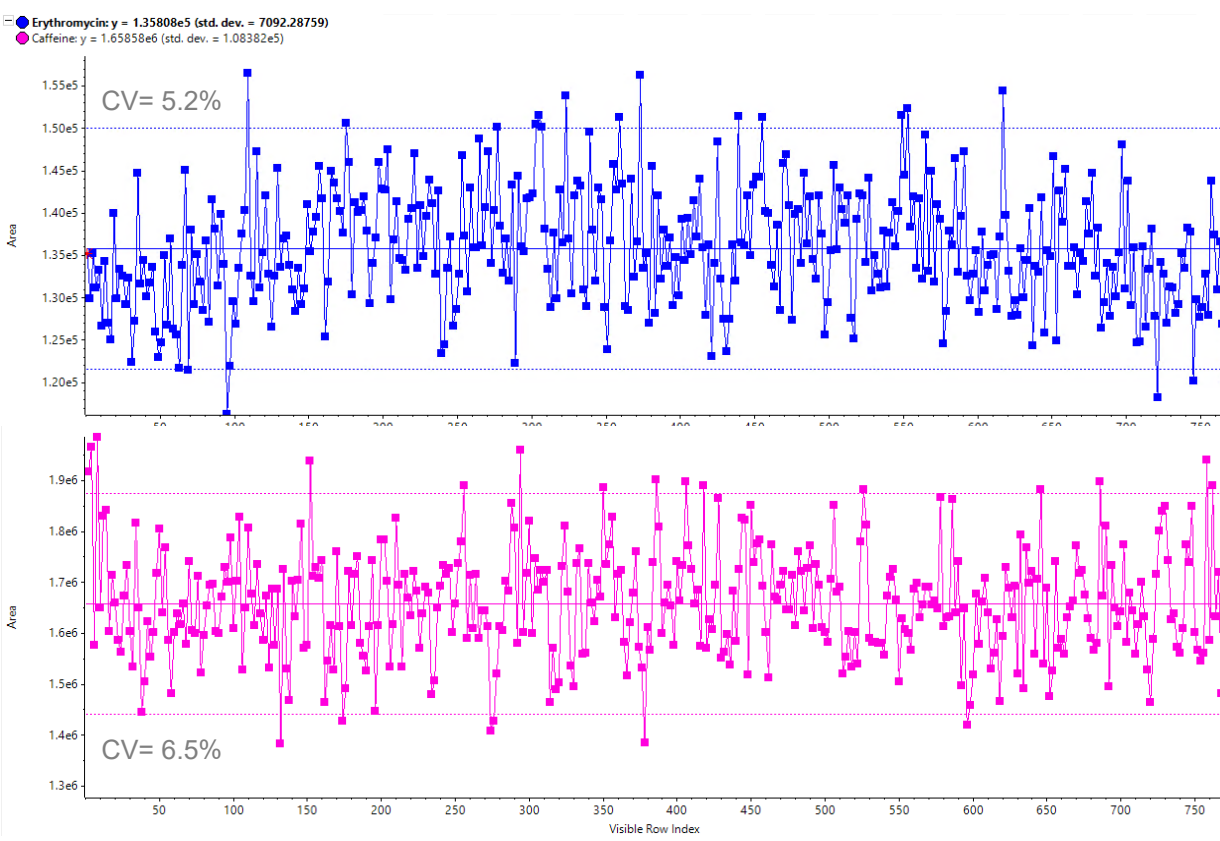


Figure 4. Raw area counts across one 384 well plate. Highly reproducible measurement are obtained with AEMS on two compounds across a plate.

CONCLUSIONS

- Acoustic ejection mass spectrometry (AEMS) using the Echo® MS system provides extremely fast analysis of compounds, measuring a 384-well plate in just 10 mins
- Baseline resolution between ejection peaks ensure no carry-over between measurements
- Very high reproducibility is observed across a single plate and when measuring many plates in series, with average %CV of 5 across 146 well plates on multiple days of analysis.