# Well-based method scheduling for high-throughput mass spectrometry

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## INTRODUCTION

In high-throughput MS, the MS signal from each sampling is continuously recorded as a single data file, where the same MS data acquisition method is typically utilized. However, for assays when different target analytes within separated sample wells are required to be analyzed in the MS/MS mode, a well-specific MS acquisition method is required. This challenge is more significant for high-throughput MS when the signal duration (peak-width) is short, limiting the number of MS/MS data acquisition methods being monitored simultaneously. In this work, we introduce the concept of selectively activating a limited number of methods at a given time during data acquisition. The time is correlated with the sample well being analyzed, and thus enables dynamic adjustment of methods.

## MATERIALS AND METHODS

In this study, we used the acoustic ejection mass spectrometry (AEMS) as the example high-throughput MS platform. Sample plates in 384- and 1536- well formats were analyzed on a research prototype of AEMS system coupling with either a triple quadrupole, or a QTOF system. The MS data acquisition was in the MS/MS mode (MRM on triple quadrupole, and MRM$^{HR}$ and IDA on QTOF) with the prototype SCIEX OS software for data acquisition and processing. The well-specific MS/MS data acquisition information was input as the MS method. The automatic data processing was triggered in SCIEX OS once the data acquisition was finished.

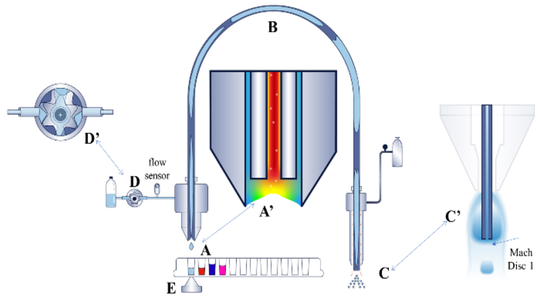



**Figure 1.** AEMS system. A. OPI capture port oriented downward. A'. Drawing of critical condition surface in "A". B. OPI with a 50 cm transport tube. C. OPI venturi pump/ESI nebulizer. C'. Sonic expansion creating pressure drop. D. Fluid delivery pump. E. Acoustic dispensing upward against gravity.

## RESULTS

In AEMS, each ejection results in a one-second baseline-wide, near-Gaussian-shaped signal peak, and the signal from each sample well of the microtiter plate is continuously recorded as a single data file as shown in Figure 2.

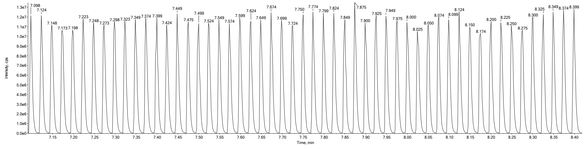


**Figure 2.** Chronograms of an example AEMS data collected from multiple sample wells from the same microtiter plate.

In order to maintain the high data reproducibility of peak area that is required for the accurate quantitation without using the internal standard, more than 8 data points across the signal peak is suggested to maintain the AEMS peak area CV less than 10% (as shown in Figure 3). Therefore, a maximum data acquisition cycle time ~125 msec could be used for the 1-sec wide signal duration. This cycle time limits the number of distinct MS/MS transitions to be monitored simultaneously. For some assays requiring analyzing different targets across samples in the MS/MS mode, MS method adjustment on-the-fly is essential to keep the enough data points across a shape signal peak.

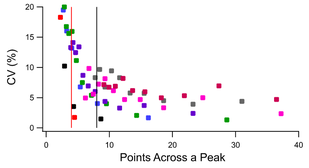
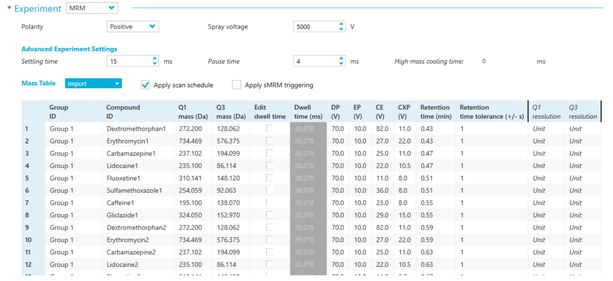



**Figure 3.** Coefficient of variation (CV) vs. points across a peak for penbutolol measured from a complex sample with various dwell time settings from 1 ms to 200 ms (N=30 injections). The data show that to achieve CV better than 15%, we need to measure at least 4 points across a peak. Once we have at least 8 points across a peak, there is no significant further improvement in CV.

In an earlier study, an approach of scheduled method activation was introduced to enable the method switching on-the-fly. The predicted signal appearance time of each sample during a plate run was used to control the method scheduling (as shown in the retention time column in Figure 4). Although it proved successful, the time prediction requires the effort of a pre-run, and the failed-ejection wells where the acoustic ejection module spends a different duration can cause the method activation of following samples due to the off-alignment issue.




| | Group ID | Compound ID | Q1 mass (Da) | Q3 mass (Da) | Edit dwell time | Dwell time (ms) | DP (V) | EP (V) | CE (V) | CXP (V) | Retention time (min) | Retention time tolerance (+/- s) | Q1 resolution | Q3 resolution |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Group 1 | Dextromethorphan1 | 272.200 | 128.062 | | 20.278 | 70.0 | 10.0 | 82.0 | 11.0 | 0.43 | 1 | Unit | Unit |
| 2 | Group 1 | Erythromycin1 | 734.469 | 576.375 | | 20.278 | 70.0 | 10.0 | 27.0 | 22.0 | 0.43 | 1 | Unit | Unit |
| 3 | Group 1 | Carbamazepine1 | 237.102 | 194.099 | | 20.278 | 70.0 | 10.0 | 25.0 | 11.0 | 0.47 | 1 | Unit | Unit |
| 4 | Group 1 | Lidocaine1 | 235.100 | 86.114 | | 20.278 | 70.0 | 10.0 | 22.0 | 10.5 | 0.47 | 1 | Unit | Unit |
| 5 | Group 1 | Fluoxetine1 | 310.141 | 148.120 | | 20.278 | 70.0 | 10.0 | 11.0 | 8.0 | 0.51 | 1 | Unit | Unit |
| 6 | Group 1 | Sulfamethoxazole1 | 254.059 | 92.063 | | 20.278 | 70.0 | 10.0 | 36.0 | 8.0 | 0.51 | 1 | Unit | Unit |
| 7 | Group 1 | Caffeine1 | 195.100 | 138.070 | | 20.278 | 70.0 | 10.0 | 23.0 | 8.0 | 0.55 | 1 | Unit | Unit |
| 8 | Group 1 | Gliclazide1 | 324.050 | 152.970 | | 20.278 | 70.0 | 10.0 | 29.0 | 15.0 | 0.55 | 1 | Unit | Unit |
| 9 | Group 1 | Dextromethorphan2 | 272.200 | 128.062 | | 20.278 | 70.0 | 10.0 | 82.0 | 11.0 | 0.59 | 1 | Unit | Unit |
| 10 | Group 1 | Erythromycin2 | 734.469 | 576.375 | | 20.278 | 70.0 | 10.0 | 27.0 | 22.0 | 0.59 | 1 | Unit | Unit |
| 11 | Group 1 | Carbamazepine2 | 237.102 | 194.099 | | 20.278 | 70.0 | 10.0 | 25.0 | 11.0 | 0.63 | 1 | Unit | Unit |
| 12 | Group 1 | Lidocaine2 | 235.100 | 86.114 | | 20.278 | 70.0 | 10.0 | 22.0 | 10.5 | 0.63 | 1 | Unit | Unit |

**Figure 4**. Example of method switching based on the defined activation time. Total cycle time is 100 msec.

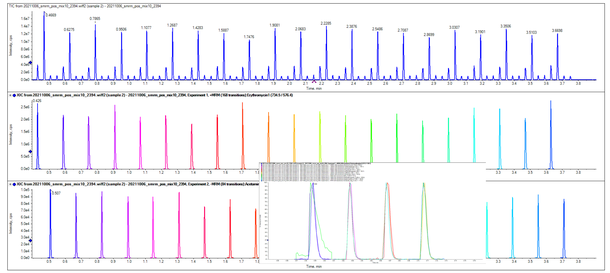


**Figure 5.** Twelve compounds were monitored in sequence for 90 replicates (15 nL ejections). Transitions were activated on an as needed basis for detection to optimize duty cycle using scheduled MRM algorithm (inset shows MRM activation based on detection needs, three MRM's per ejection).

To solve these challenges from the "scheduled" activation method, a new approach has been developed with the correlation of each MS/MS transition with a sample well position (Figure 6). The successful acoustic ejection from a specific sample well passes the triggering signal to the MS data acquisition control module for the activation of the specific MS methods associated with the sample well. With a defined OPI condition, the delay time between the sample ejection and the appearance of the MS signal is relatively stable (Figure 7), showing the robustness of this method.

| | Compound ID | Precursor Ion (Da) | Fragment Ion (Da) | Accumulation Time (sec) | DP (V) | CE (V) | ScheduledWells |
|---|---|---|---|---|---|---|---|
| 1 | Prometon | 226.17000 | 226.16620 | 0.0200 | 80 | 12 | A1 |
| 2 | Ametryn | 228.13000 | 228.12800 | 0.0200 | 80 | 12 | A2 |
| 3 | Simazine | 202.09000 | 202.08600 | 0.0200 | 80 | 12 | A3 |
| 4 | Prometryn | 242.14000 | 242.14400 | 0.0200 | 80 | 12 | A4 |
| 5 | Propazine | 230.12000 | 230.11800 | 0.0200 | 80 | 12 | A5 |
| 6 | Atrazine | 216.10000 | 216.10140 | 0.0200 | 80 | 12 | A6 |

**Figure 6**. Example of method switching based on the sample well position.

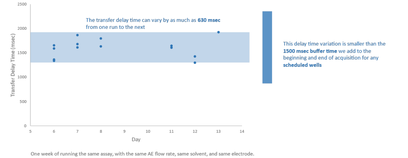



**Figure 7.** The transfer delay time is constant after 1 week of use without re-calibration

This approach has been applied for the analysis of different analytes cross wells in the mode of MRM (Triple Quad MS), MRM HR (QTOF MS), and IDA (with well based inclusion list, as shown in Figure 8)

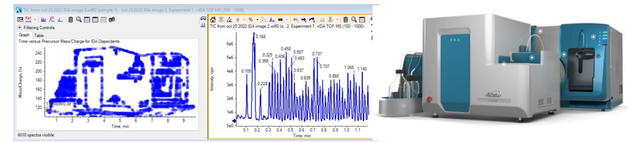


**Figure 8.** The IDA viewer shows dots based on the acquisition time and m/z in the inclusion list.

## CONCLUSIONS

A novel MS method scheduling approach is introduced here for the acoustic ejection mass spectrometry system, allowing the convenient setting of different MS/MS method for distinguished samples. Both data quality and analytical throughput could be achieved simultaneously. It has been applied in multiple MS/MS modes on triple quadrupole and QTOF MS platforms.

## TRADEMARKS/LICENSING



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