Novel methods for increasing throughput and detection of Acoustic Ejection Mass Spectrometry (AEMS)

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ABSTRACT

Acoustic Ejection Mass Spectrometry (AEMS) is starting to augment high volume assays and high-throughput laboratories with quantitative MS data at plate reader speeds. This change in data format broadens compound coverage and reduces preparatory requirements. Analytical chemists are now challenged with detecting and parsing larger data sets in the same time scale. A more efficient series of steps must be developed to minimize the chemists' interactions and refinement with sample introduction and detection while providing the highest degree of compound coverage afforded by electrospray ionization (ESI).

INTRODUCTION

High Throughput Screening (HTS) and High Throughput ADME (HT-ADME) environments are tasked with translating a compound library into lead profiles for drug candidates. Current technologies provide different advantages to this process. Optical plate readers provide a throughput advantage for large targeted screens while creating higher risk for false negatives.[1] Conventional LC-MS/MS increases hit detection and accuracy as well as Z-Prime scoring at the expense of time.[1] Similar examples exist in the rapidly developing field of synthetic biology and small vessel bioreactors. Being able to screen and quantitively detect changes in product yield based on strain modification or enzymatic efficiency allow synthetic biology companies to qualify lead strains or processes with greater confidence and reduce reactor overhead.[2] Current technologies such as short runtime UHPLC-MS/MS and multiplexed LC-MS/MS begin to bridge the gap between sample throughput and quantitative accuracy at approximately 8-35 seconds per sample.[3,4] AEMS technology reduces runtimes further by averaging 1-3 seconds per sample whilst maintaining data quality equivalent to mass spectrometry based detection techniques at the time of this publication.[1-5]

A challenge facing the above assays is how to best optimize an AEMS platform for sample introduction, ionization, compound specificity, and detection. With AEMS, the importance of carrier solvent choice increases due to the removal of chemically incompatible components such as pH sensitive stationary phases, immiscible volatile liquid mixtures or chemically interactive surfaces like needles or needle seats. Carrier solvent selection may now contain higher pH modified solvents like 5mM NH_4F, 0.4-1% formic acid in organic, additions of µM concentrations of phosphonic acids to the above solvents or the use of nonpolar solvents to encapsulate polar ejections.

Another challenge facing scientists is how to quantitate multiple compounds across a plate while minimizing the duty cycle of a triple quadrupole mass spectrometer to maintain data fidelity and low ejection to ejection CV. By utilizing the Scheduled MRM algorithm, AEMS technologies can switch transitions dynamically to acquire the proper data per well without the needing to perform multiple passes over a well-plate.

MATERIALS AND METHODS

Sample preparation: Matrix prepared samples consisting of 5mM HEPES buffer pH 7.0, 20mM NaCl, 1mM MgCl2, 0.0004% Tween, 0.025mM EDTA spiked with Verpamil, Lidocaine, Carbamazepine, Dextromethorphan, Caffeine, Sulfamethoxazole, Acetaminophen, Gliclazide, and Glucose 6-Phosphate in 18.75% Methanol.

AEMS conditions: A SCIEX Echo® MS system with various carrier solvents (mobile phases) was used with Labcyte PPL-0200 384 well plates at flow rates between 370-410 µL/min. Sample droplet volume was fixed at 15nL.

MS/MS Conditions:
A SCIEX Triple Quad 6500+ mass spectrometer with the OptiFlow ion source and an electrospray ionization (ESI) probe was used. A series of small molecules were detected using a single MRM transition per compound provided quantitation in both positive and negative polarities. Data was collected in replicate ejections with an n=60 (Figure 1, Figure 2).

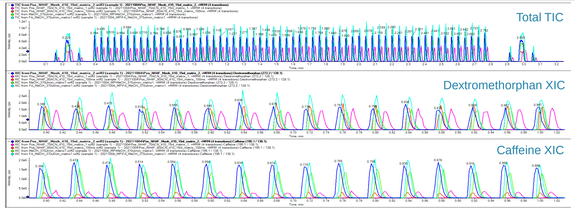

Figure 1. Ionization efficiency varies based on organic solvent and modifier selection as well as compound specificity. Methanol (blue, teal, green) carrier solvents demonstrates enhanced detection for caffeine compared to acetonitrile (pink, red).

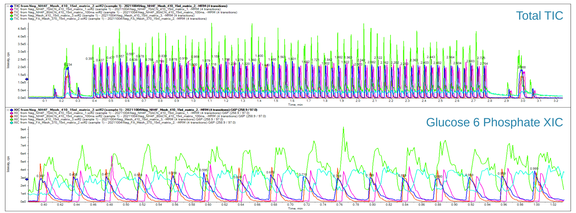

Figure 2. Compound detection is quantitatively improved when NH_4F is added to the carrier solvent (blue, pink, red) in contrast to a suppressed region observed without the addition of NH_4F (green, teal).

The AEMS method that utilizes the Scheduled MRM algorithm uses a time delineated experiment with polarity switching where compound transitions are concurrently activated in 2.4 second (0.04 min) intervals based on the ejection time the acoustic transducer is over a given well. A pre-scan of the plate determines the initial ejection time of the first well whereby all subsequent wells are offset based on the firing delay of the transducer (0.04min). The MRM table is generated in advance of the acquisition and saved as the MS method. A batch file is created with the well sequence matching the MS method. AE firing is set to the scheduling interval of 2400ms. All data is acquired into a single data file to be post processed.

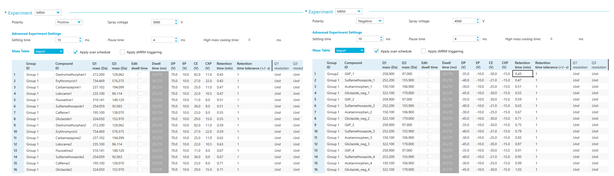

Figure 3. ESI+ and ESI- acquisition method using Scheduled MRM algorithm with a total cycle time of 0.100sec.

RESULTS

Analysis of eight compounds in matrix through both positive and negative ESI polarities indicated a series of optimal carrier solvents for use against most small molecules. The addition of ammonium fluoride at 5mM into the carrier yielded strong detection properties and greater signal to noise for negative mode polar metabolites that are matrix suppressed (Figure 2) while slightly improving positive mode molecules when compared to just methanol. The addition of formic acid enhanced positive mode ionization but dramatically reduced negative mode sensitivity (~10x loss in signal compared to methanol) (Table 1).

When comparing methanol to acetonitrile:water mixtures (70% or 80% ACN), the ACN mixtures suffered a loss in sensitivity from 2-10x (Carbamazepine, Lidocaine, and Caffeine) in positive modes while remaining consistent across negative mode compounds. The most notable change to the peaks was through their FWHM on a time scale (Table 1). Ejections performed with 70% and 80% ACN had decreased FWHM peaks relative to their methanol counterparts in methanol.

Solvent	Compound	Replicate Number	Mean Peak Area (cps)	Std. Dev	Percent CV	FWHM (sec)
MeOH	Dextromethorphan	60 of 60	6.40E+05	4.44E+04	6.94	0.548
	Carbamazepine	60 of 60	9.72E+05	4.83E+04	4.96	0.566
	Lidocaine	60 of 60	7.99E+05	4.51E+04	5.65	0.536
	Caffeine	60 of 60	6.31E+05	2.90E+04	4.60	0.550
	GSP	60 of 60	4.21E+04	3.78E+03	8.95	1.043
	Sulfamethoxazole	60 of 60	5.89E+04	6.06E+03	10.28	0.531
	Acetaminophen	60 of 60	4.93E+03	3.70E+02	7.51	0.930
	Gliclazide	60 of 60	9.28E+04	9.70E+03	10.46	0.558
FA_MeOH	Dextromethorphan	60 of 60	1.12E+06	6.78E+04	6.03	0.564
	Carbamazepine	60 of 60	1.46E+06	6.33E+04	4.35	0.584
	Lidocaine	60 of 60	1.55E+06	8.81E+04	5.67	0.561
	Caffeine	60 of 60	8.59E+05	3.58E+04	4.17	0.563
	GSP	60 of 60	1.90E+04	5.21E+03	27.4	0.840
	Sulfamethoxazole	60 of 60	4.23E+03	3.68E+02	8.73	0.558
	Acetaminophen	60 of 60	5.10E+02	6.04E+01	11.85	0.569
	Gliclazide	60 of 60	9.87E+03	8.29E+02	8.39	0.570
NH4F_MeOH	Dextromethorphan	60 of 60	8.04E+05	4.99E+04	6.21	0.588
	Carbamazepine	60 of 60	1.19E+06	5.17E+04	4.35	0.617
	Lidocaine	60 of 60	9.97E+05	5.83E+04	5.85	0.579
	Caffeine	60 of 60	8.54E+05	3.25E+04	3.8	0.589
	GSP	60 of 60	1.76E+04	1.47E+03	8.37	0.754
	Sulfamethoxazole	60 of 60	2.57E+04	1.72E+03	6.71	0.593
	Acetaminophen	60 of 60	4.34E+04	1.95E+03	4.49	0.606
	Gliclazide	60 of 60	2.73E+04	1.70E+03	6.21	0.589
NH4F_70ACN	Dextromethorphan	60 of 60	7.16E+05	4.13E+04	5.76	0.572
	Carbamazepine	60 of 60	5.90E+05	2.28E+04	3.87	0.575
	Lidocaine	60 of 60	5.95E+05	2.69E+04	4.52	0.563
	Caffeine	60 of 60	1.44E+05	1.19E+04	8.27	0.571
	GSP	60 of 60	1.82E+04	2.08E+03	11.42	0.600
	Sulfamethoxazole	60 of 60	2.35E+04	1.15E+03	4.88	0.528
	Acetaminophen	60 of 60	3.10E+04	1.76E+03	5.68	0.546
	Gliclazide	60 of 60	2.97E+04	2.22E+03	7.48	0.516
NH4F_80ACN	Dextromethorphan	60 of 60	6.72E+05	3.16E+04	4.71	0.460
	Carbamazepine	60 of 60	4.05E+05	1.53E+04	3.78	0.467
	Lidocaine	60 of 60	4.78E+05	1.52E+04	3.18	0.446
	Caffeine	60 of 60	9.73E+04	7.41E+03	7.62	0.445
	GSP	60 of 60	1.65E+04	1.44E+03	8.75	0.570
	Sulfamethoxazole	60 of 60	2.02E+04	1.02E+03	5.06	0.441
	Acetaminophen	60 of 60	2.20E+04	8.09E+02	3.68	0.449
	Gliclazide	60 of 60	2.32E+04	1.05E+03	4.51	0.439

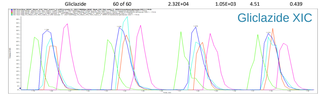

Table 1. Mean peak areas and statistics of 8 compounds in ESI+ and ESI- polarities as impacted by carrier solvent selection, with Gliclazide as an example provided by inset. Colors denote different carrier solvents.

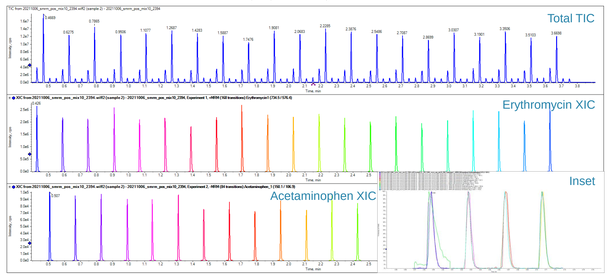

Figure 4. Twelve Compounds were monitored in sequence for 90 replicates (15nL ejections). Transitions were activated on an as needed basis for detection to optimize duty cycle using scheduled MRM algorithm (inset shows MRM activation based on detection needs, three MRM's per ejection).

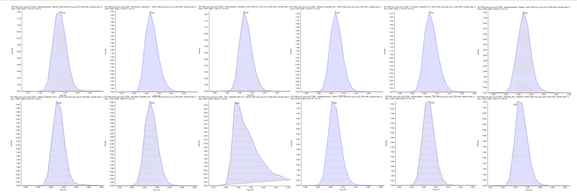

Figure 5. Integrated sonograms from the first cycle of the time scheduled AEMS method.

Compound	Mean Peak Area (cps)	Std. Dev	Percent CV	Compound	Mean Peak Area (cps)	Std. Dev	Percent CV
Dextromethorphan	4.40E+05	21158	4.80	G6P	1.52E+04	2466	16.27
Erythromycin	8.24E+05	59934	7.27	Gliclazide_neg	2.57E+04	1366	5.31
Acetaminophen	2.87E+04	1424	4.96	Gliclazide	1.87E+05	14420	7.70
Caffeine	1.35E+05	12643	9.37	Lidocaine	7.47E+05	32456	4.34
Carbamazepine	3.40E+06	214590	6.31	Sulfamethoxazole_neg	2.41E+04	1479	6.14
Fluoxetine	7.75E+04	3184	4.11	Sulfamethoxazole	2.73E+05	14503	5.31

Table 2. Mean Peak Area and statistics of 12 compounds collected via the time scheduled AEMS over 21 replicate ejections of 15nL.

Time scheduled AEMS data acquisition was able to capture 90 replicate ejections over 21 repeat cycles of the experiment table with CV's less than 10% aside from Glucose 6-Phospate (Table 2). Data was integrated inside SCIEX OS software (Figure 5) and exported to Microsoft Excel for further manipulation.

CONCLUSIONS

Acoustic Ejection Mass Spectrometry is able to produce data on a plate reader timescale while maintaining current LC-MS/MS data fidelity. Utilizing ionizing agents such as formic acid or ammonium fluoride to augment the properties of organic carrier solvents improves detection limits across both polarities with matrix present in a sample. In some cases, the addition of NH_4F allows the detection of suppressed compounds to break free from matrix effects allowing detection when it was previously not possible. By combining these carrier solvents with Scheduled MRM algorithm, the duty cycle of the mass spectrometer can be utilized more efficiently to monitor the compounds present in a well without wasting time seeking compounds not present. This technique has demonstrated equivalency in data quality to conventional AEMS acquisition.

REFERENCES

1 D. McLaren, SLAS 2020
2 R. Baghla, Application Note, DH Tech. Dev Pte. Ltd, (2020, RUO-MKT-02-11695-A)
3 H. Zhang et al, bioRxiv, doi: https://doi.org/10.1101/2020.01.28.923938
4 R. Simon, SLAS 2020
5 A. Wagner et al, SLAS 2020

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