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 NH₄F, 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).





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.

▼ Expe	iment MRM	v												•	Experir	ment MF	RM 👻 —												
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	Group ID	Compound ID	Q1 mass (Da)	Q3 mass (Da)	Edit dwell time	Dwell time (ms)	DP (V)	EP (V)	CE ((V) (CXP Ret (V) tim	etention me (min)	Retention time tolerance (+/- s)	Q1 resolution	Q3 resolut		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 0.4	43	1	Unit	Unit	1	Group2	G6P_1	258.900	97.000		20.278	-35.0	-10.0	-30.0	-15.0	0.43	1	Unit	Unit
2	Group 1	Erythromycin1	734.469	576.375		20.278	70.0	10.0	27.0	22.0 0.4	43	1	Unit	Unit	2	Group 1	Sulfamethoxazole_1	252.200	155.900		20.278	-40.0	-10.0	-21.0	-15.0	0.47	1	Unit	Unit
3	Group 1	Carbamazepine1	237.102	194.099		20.278	70.0	10.0	25.0	11.0 0.4	47	1	Unit	Unit	3	Group 1	Acetaminophen_1	150.100	106.900		20.278	-30.0	-10.0	-25.0	-15.0	0.51	1	Unit	Unit
4	Group 1	Lidocaine1	235.100	86.114		20.278	70.0	10.0	22.0	10.5 0.4	47	1	Unit	Unit	4	Group 1	Gliclazide_neg_1	322.100	170.000		20.278	-45.0	-10.0	-30.0	-15.0	0.55	1	Unit	Unit
5	Group 1	Fluoxetine1	310.141	148.120		20.278	70.0	10.0	11.0	8.0 0.5	51	1	Unit	Unit	5	Group 1	G6P_2	258.900	97.000		20.278	-35.0	-10.0	-30.0	-15.0	0.59	1	Unit	Unit
6	Group 1	Sulfamethoxazole1	254.059	92.063		20.278	70.0	10.0	36.0	8.0 0.5	51	1	Unit	Unit	6	Group 1	Sulfamethoxazole_2	252.200	155.900		20.278	-40.0	-10.0	-21.0	-15.0	0.63	1	Unit	Unit
7	Group 1	Caffeine1	195.100	138.070		20.278	70.0	10.0	23.0	8.0 0.5	55	1	Unit	Unit	7	Group 1	Acetaminophen_2	150.100	106.900		20.278	-30.0	-10.0	-25.0	-15.0	0.67	1	Unit	Unit
8	Group 1	Gliclazide1	324.050	152.970		20.278	70.0	10.0	29.0	15.0 0.5	55	1	Unit	Unit	8	Group 1	Gliclazide_neg_2	322.100	170.000		20.278	-45.0	-10.0	-30.0	-15.0	0.71	1	Unit	Unit
9	Group 1	Dextromethorphan2	272.200	128.062		20.278	70.0	10.0	82.0	11.0 0.5	59	1	Unit	Unit	9	Group 1	G6P_3	258.900	97.000		20.278	-35.0	-10.0	-30.0	-15.0	0.75	1	Unit	Unit
10	Group 1	Erythromycin2	734.469	576.375		20.278	70.0	10.0	27.0	22.0 0.5	59	1	Unit	Unit	10	Group 1	Sulfamethoxazole_3	252.200	155.900		20.278	-40.0	-10.0	-21.0	-15.0	0.79	1	Unit	Unit
11	Group 1	Carbamazepine2	237.102	194.099		20.278	70.0	10.0	25.0	11.0 0.6	63	1	Unit	Unit	11	Group 1	Acetaminophen_3	150.100	106.900		20.278	-30.0	-10.0	-25.0	-15.0	0.83	1	Unit	Unit
12	Group 1	Lidocaine2	235.100	86.114		20.278	70.0	10.0	22.0	10.5 0.6	63	1	Unit	Unit	12	Group 1	Gliclazide_neg_3	322.100	170.000		20.278	-45.0	-10.0	-30.0	-15.0	0.87	1	Unit	Unit
13	Group 1	Fluoxetine2	310.141	148.120		20.278	70.0	10.0	11.0	8.0 0.6	67	1	Unit	Unit	13	Group 1	G6P_4	258.900	97.000		20.278	-35.0	-10.0	-30.0	-15.0	0.91	1	Unit	Unit
14	Group 1	Sulfamethoxazole2	254.059	92.063		20.278	70.0	10.0	36.0	8.0 0.6	67	1	Unit	Unit	14	Group 1	Sulfamethoxazole_4	252.200	155.900		20.278	-40.0	-10.0	-21.0	-15.0	0.95	1	Unit	Unit
15	Group 1	Caffeine2	195.100	138.070		20.278	70.0	10.0	23.0	8.0 0.7	71	1	Unit	Unit	15	Group 1	Acetaminophen_4	150.100	106.900		20.278	-30.0	-10.0	-25.0	-15.0	0.99	1	Unit	Unit
16	Group 1	Gliclazide2	324.050	152.970		20.278	70.0	10.0	29.0	15.0 0.7	71	1	Unit	Unit	16	Group 1	Gliclazide_neg_4	322.100	170.000		20.278	-45.0	-10.0	-30.0	-15.0	1.03	1	Unit	Unit
F i 0.	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.

		Replicate
Solvent	Compound	Number
MeOH	Dextromethorphan	60 of 60
	Carbamazepine	60 of 60
	Lidocaine	60 of 60
	Caffeine	60 of 60
	G6P	60 of 60
	Sulfamethoxazole	60 of 60
	Acetaminophen	60 of 60
	Gliclazide	60 of 60
FA_MeOH	Dextromethorphan	60 of 60
	Carbamazepine	60 of 60
	Lidocaine	60 of 60
	Caffeine	60 of 60
	G6P	60 of 60
	Sulfamethoxazole	60 of 60
	Acetaminophen	60 of 60
	Gliclazide	60 of 60
NH4F_MeOH	Dextromethorphan	60 of 60
	Carbamazepine	60 of 60
	Lidocaine	60 of 60
	Caffeine	60 of 60
	G6P	60 of 60
	Sulfamethoxazole	60 of 60
	Acetaminophen	60 of 60
	Gliclazide	60 of 60





Mean Peak Area	Std.	Percent				Replicate		Std.	Percent	
(cps)	Dev	CV	FWHM (sec)	Solvent	Compound	Number	Mean Peak Area (cps)	Dev	CV	FWHM (sec)
6.40E+05	4.44E+04	6.94	0.548	NH4F_70ACN	Dextromethorphan	60 of 60	7.16E+05	4.13E+04	5.76	0.572
9.72E+05	4.83E+04	4.96	0.566		Carbamazepine	60 of 60	5.90E+05	2.28E+04	3.87	0.575
7.99E+05	4.51E+04	5.65	0.536		Lidocaine	60 of 60	5.95E+05	2.69E+04	4.52	0.561
6.31E+05	2.90E+04	4.60	0.550		Caffeine	60 of 60	1.44E+05	1.19E+04	8.27	0.571
4.21E+04	3.78E+03	8.96	1.043		G6P	60 of 60	1.82E+04	2.08E+03	11.42	0.600
5.89E+04	6.06E+03	10.28	0.531		Sulfamethoxazole	60 of 60	2.35E+04	1.15E+03	4.88	0.528
4.93E+03	3.70E+02	7.51	0.930		Acetaminophen	60 of 60	3.10E+04	1.76E+03	5.68	0.546
9.28E+04	9.70E+03	10.46	0.558		Gliclazide	60 of 60	2.97E+04	2.22E+03	7.48	0.516
1.12E+06	6.78E+04	6.03	0.564	NH4F_80ACN	Dextromethorphan	60 of 60	6.72E+05	3.16E+04	4.71	0.460
1.46E+06	6.33E+04	4.35	0.584		Carbamazepine	60 of 60	4.05E+05	1.53E+04	3.78	0.467
1.55E+06	8.81E+04	5.67	0.561		Lidocaine	60 of 60	4.78E+05	1.52E+04	3.18	0.446
8.59E+05	3.58E+04	4.17	0.563		Caffeine	60 of 60	9.73E+04	7.41E+03	7.62	0.445
1.90E+04	5.21E+03	27.4	0.840		G6P	60 of 60	1.65E+04	1.44E+03	8.75	0.570
4.23E+03	3.69E+02	8.73	0.558		Sulfamethoxazole	60 of 60	2.02E+04	1.02E+03	5.06	0.441
5.10E+02	6.04E+01	11.85	0.569		Acetaminophen	60 of 60	2.20E+04	8.09E+02	3.68	0.449
9.87E+03	8.29E+02	8.39	0.570		Gliclazide	60 of 60	2.32E+04	1.05E+03	4.51	0.439
				 XIC from Neg, NH4F Mach, 419, 15cd, ma XIC from Neg, Net4P / 700244, A19, 15cd, reality, XIC from Neg, Net4P / 700247, 419, 15cd, reality, XIC from Neg, Neuh, 310, 140, reality, avet8 (XIC from Neg, FA, Mech, 370, TBrl, matrix, 2 ml NIC from Neg, FA, Mech, 370, TBrl, matrix, 2 ml 	trix_2_with2(sample 1)-202110000000_NH4P_D0x01_410_The 1 onR2(seconds 1)-2021100000000_NH4P_D0x0000_410_The 100000_NH4P_D0x000000_10000000_0000000_000000000000	 Mod. matrix, Z. 2000 (4 transitions) glicitaride (2022). John, "matrix," A 2010 (4 transitions) glicitaride (2211 / 170 2. 10n1, matrix, "100ma, "MITM (4 transitionale (2221 / 170 d) (5 transitional) glicitaride (221 / 170 d) ZMRM (4 transitional) glicitaride (222. 1 / 170 d) 	1700 09 3322.1 / 170.0)			
8.04E+05	4.99E+04	6.21	0.588	95% - 90% -	Α	Λ.			Giiciaz	
				80% - 1.198	()	TK Λ	1 1270		1.319	٨
1.19E+06	5.17E+04	4.35	0.617	205. 205.				1	$\wedge \wedge$	
9.97E+05	5.83E+04	5.85	0.579	5 00%.	$h \mid 1$			\backslash		$\uparrow \land$
8.54E+05	3.25E+04	3.8	0.589	[2] 00% 48%						V \
1.76E+04	1.47E+03	8.37	0.754	40% 38%	W \	I IVI W			/ NI	X \
2.57E+04	1.72E+03	6.71	0.593	265	X \				/ IN	
4.34E+04	1.95E+03	4.49	0.606	15%			$\wedge \qquad 1 \qquad $	$\langle \rangle$		
2.73E+04	1.70E+03	6.21	0.589		20 121 122	125 124 125	1.56 1.27 1.20	02.1 02.1	1.51 1.5	2 1.55 1.54

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).



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 $NH_{4}F$ 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.

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

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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 NH₄F is added to the carrier solvent (blue, pink, red) in contrast to a suppressed region observed without the addition of NH_4F (green, teal).