Acoustic Ejection Mass Spectrometry (AEMS) / Differential Ion Mobility for Ultra-High Throughput Mass Spectrometry

Leigh Bedford; Brad Schneider; Eva Duchoslav; Aaron Stella; Subhasish Purkayastha; Chang Liu; Thomas R Covey
SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

INTRODUCTION

Acoustic Ejection Mass Spectrometry (AEMS) is a form of flow injection analysis that achieves sampling rates of greater than 1 Hz and is applicable for quantitative high throughput assays. HPLC is by-passed but the need for chemically based separations to distinguish isobars remains. Low field ion mobility has been suggested but most require pulsed ion beams which introduces a duty cycle loss when coupled to MRM acquisition for quantitation. Mobility measurements must be in the low milliseconds to keep pace. Differential mobility spectrometry (DMS) is unique among forms of mobility as it has all three of the key attributes, millisecond flight times, continuous ion beam operation, and chemically based separations.

MATERIALS AND METHODS

Experiments conducted on a triple quadrupole (QqQ) with the DMS analyzer at atmospheric pressure between the pneumatically-assisted ESI and vacuum aperture as shown in Figure 1. An open port interface (OPI) was positioned over the microtiter plate sample wells and acoustic transducer that dispensed 2.5 nL samples into the OPI. Isobaric opioid and benzodiazepine drugs were analyzed, including morphine, hydromorphone, norhydrocodone, noroxycodone, oxymorphone, dihydrocodeine, mirtazapine, desmethyldoxepin, 7-aminoflunitrazepam, diazepam, 7-aminoclonazepam, oxazepam, chlordiazepoxide, temazepam, olanzapine, desmethylclozapine, flunitrazepam, amoxapine, clonazepam, midazolam, clozapine, fentanyl, and norfentanyl. Compounds were spiked in human urine and analyzed directly or after desalting. DMS separations were optimized by adding isopropanol, acetonitrile, or ethylacetate chemical modifiers to the transport gas to induce the cluster-decluster mechanism which separates molecules based on their unique gas phase ion chemistry.

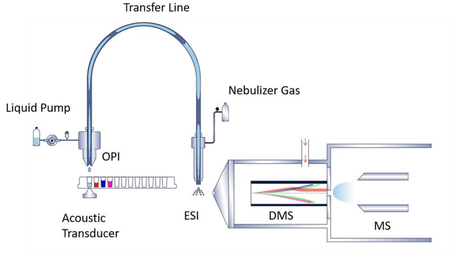


Figure 1. Schematic of an AEMS system with DMS system.

RESULTS

The first step in a high-throughput workflow that utilizes modifier based DMS separations is developing a means to determine optimal separation parameters for the analytes in an automated fashion. For each drug a tuning signal could be generated using an acoustic droplet injection frequency of 20 Hz which consumed sample at 1 µL/min. Figure 2 shows the difference between running the system at a frequency of 10Hz (left side of A) which results in peak widths on the order of 1s for pseudo continuous mode, and 400Hz (right side of A) which results in ejection of a series of 6 droplets over a time period of approximately 2.5 ms to generate a discrete peak comprising 15 nL of analyte diluted in the transport flow stream.

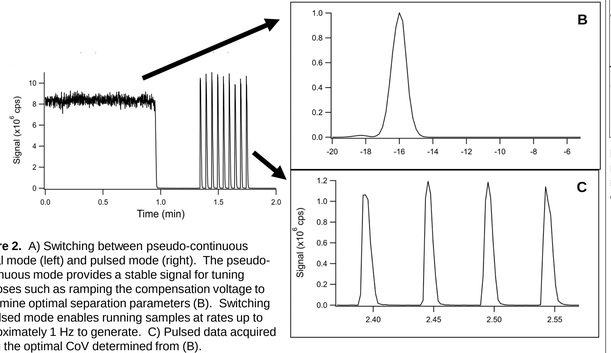
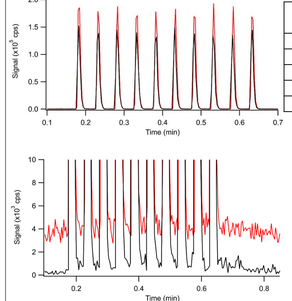


Figure 2. A) Switching between pseudo-continuous signal mode (left) and pulsed mode (right). The pseudo-continuous mode provides a stable signal for tuning purposes such as ramping the compensation voltage to determine optimal separation parameters (B). Switching to pulsed mode enables running samples at rates up to approximately 1 Hz to generate. C) Pulsed data acquired using the optimal CoV determined from (B).

DMS TRANSMISSION



Analyte	Count Rate with DMS Installed (cps)	Count Rate with DMS Removed (cps)	Ratio of Area with DMS Off/On
Reserpine	70,200	90,200	1.3
Minoxidil	43,000	125,000	2.9
Proline	5,680	18,900	3.3
5-Fluorouracil	1,610,000	3,530,000	2.2
Taurocholic Acid	3,100,000	5,090,000	1.6

Table 1. Table of transmission data for 5 different compounds taken with the DMS installed on the MS system and with the DMS removed from the MS system. Typical signal reductions ranged from 1.3 – 3.3X for these samples.

Figure 3. AEMS data for reserpine ions taken with (black traces) and without DMS installed (red traces). The bottom pane shows a zoom of the baseline.

The AEMS process samples directly from wells of a sample plate and therefore does not include provisions for LC separation. As a result, the general applicability of this approach is limited by the presence of chemical interferences. DMS separation can substitute for a traditional LC separation on a time-scale that is compatible with AEMS analysis (~ 1 sec per sample).

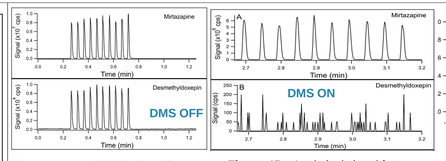


Figure 4A. Analytical signal for mirtazapine (top pane) and interference measured in desmethyldoxepin MRM channel (DMS off, bottom pane).

Figure 4B. Analytical signal for mirtazapine (top pane) and interference measured in desmethyldoxepin MRM channel (DMS on, bottom pane)

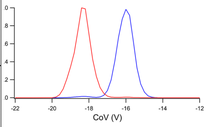


Figure 4C. DMS separation of Mirtazapine (blue) and Desmethyldoxepin (red).

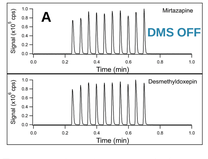


Figure 5A. Analytical signal for desmethyldoxepin (bottom pane) and interference measured in mirtazapine MRM channel (DMS off, top pane).

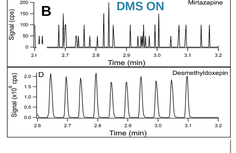


Figure 5B. Analytical signal for desmethyldoxepin (bottom pane) and interference measured in mirtazapine MRM channel (DMS on, top pane).

The elimination of isobaric interferences for these species enables accurate quantitation for either species in the presence of the other. Figure 6A and B shows examples of quantitation of mirtazapine (A) and desmethyldoxepin (B) from a desalted urine matrix without an internal standard, using an injection volume of 50 nL.

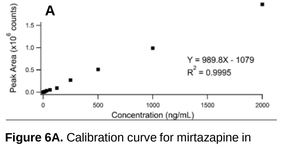


Figure 6A. Calibration curve for mirtazapine in desalted urine at an injection volume of 50 nL.

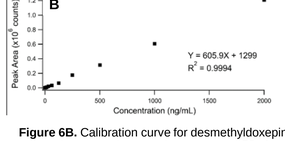


Figure 6B. Calibration curve for desmethyldoxepin in desalted urine at an injection volume of 50 nL.

Linear calibration curves were generated from a total of 10 replicates for the blanks and desalted urine samples. High quality data were generated with LOQs of 0.6 ng/mL and 0.4 ng/mL for mirtazapine & desmethyldoxepin, respectively.

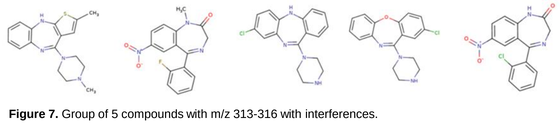


Figure 7. Group of 5 compounds with m/z 313-316 with interferences.

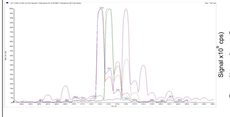


Figure 8A. Q1 spectral overlap for group of 5.

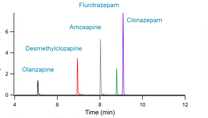


Figure 8B. LC-MS chromatogram for group of 5

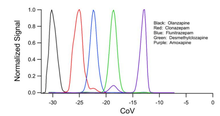


Figure 8C. DMS separation for group of 5

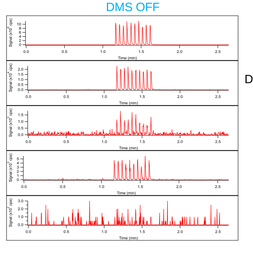


Figure 9A. AEMS data for injection of olanzapine standard (top pane) in the absence of DMS or LC separation.

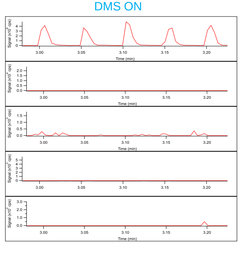


Figure 9B. AEMS data for olanzapine for the group of five with a DMS installed for separation.

CONCLUSIONS

High throughput screening approaches that target analysis of tens of thousands of samples per day with throughput on the order of 1 Hz or greater are not presently compatible with liquid chromatography separations. The elimination of LC significantly reduces analytical selectivity, substantially increasing the likelihood of isobaric interferences in a tandem mass spectrometry measurement from species endogenous or exogenous to the sample matrix. While its peak capacity is generally lower than LC approaches, DMS presents an alternative method to augment selectivity for high throughput screening. Limits of quantitation in the ng/mL range, CVs < 10%, and three orders of magnitude calibration curve linearity were achieved for all compounds including those that would otherwise interfere with each other's MRM transitions.

TRADEMARKS/LICENSING