Fundamental Characteristics of Small Molecule Analysis Using Ultra-Low Dynamic Flow

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Introduction

Ultra-low flow rate (< 20 nL/min) nanospray ionization has demonstrated reduced ion suppression, a trend toward equimolar response, and high ionization and utilization efficiency for small molecule analytes. The majority of these experiments feature the use of offline (static) nanospray. Typically static nanospray is operated in a regime whereby the applied voltage generates or controls the effective through-emitter flow rate of mobile phase. Such static experiments are often difficult to control because the flow rate is a function of applied voltage, mobile phase composition, and emitter geometry. By decoupling through-emitter flow rate from applied voltage, it is possible to retain the benefits of static nanospray in combination with the robustness and repeatability of pumped flow (dynamic) nanospray.

Instruments & Methods

Mass Spectrometer: TSQ Quantum Ultra (Thermo Fisher Scientific)

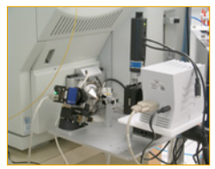
LC Pump: Custom-built, low-pressure (<30 psi) isocratic, feedback-controlled pump (New Objective, Inc.)

Nanospray Source: Digital PicoView DPV-650 (New Objective, Inc.)

A novel custom-built, low-pressure (< 30psi) isocratic, feedback-controlled pump (New Objective, Inc.) was connected to a customized nanospray source (Digital PicoView DPV-650; New Objective, Inc.) mounted to a triple quadrapole mass spectrometer (TSQ Quantum Ultra, Thermo Fisher Scientific). The pump was connected to a liquid nitrogen tank through a series of pressure regulators (VWR; Alicat Scientific), used to control the flow rate of mobile phase. The mobile phase consisted of HPLC grade water that was processed through a vacuum degasser (Shimadzu).

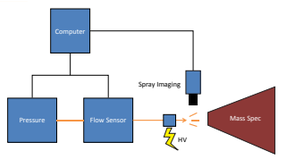
The sample consisted of a four compound suite with equal concentrations (2 µM) into LLE processed plasma extract with a 50% MeOH reconstitution. The compounds were Propranolol, Albendazole, Eucatropine, and Diltiazem.

Sample was infused with a 250 µL syringe (Hamilton Gas-Tight) into a sample line (25 µm ID x 50 cm tubing) connected via a clear elastomer union (PicoClear Union; PCU-360; New Objective, Inc.) to a 10 µm ID tip metal- coated emitter (FS360-20-10-CE-5-C20; New Objective, Inc.). The emitter was positioned approximately 2 mm from the inlet with applied voltages varying from 650 V to 1500 V.



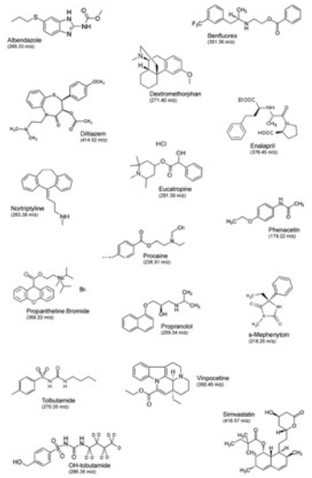
Digital PicoView 650 on a Thermo Scientific TSQ Quantum Ultra mass spectrometer

FIGURE 1



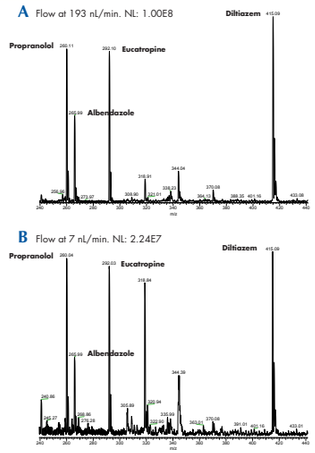
Representative diagram of experimental setup. Pressure, flow rate, and spray imaging data is fed to an open feedback loop to the computer and the operator.

FIGURE 2



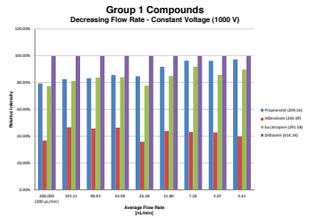
Compound structures for all 16 compounds tested. Group 1 consisted of Albendazole, Diltiazem, Eucatropine, and Propranolol. Note the diversity of chemical space.

FIGURE 3



Representative base peak chromatograms, summed over the duration of each run (1 minute acquisition). A) Average flow rate 193 nL/min. at 2.07 bar (30 psi). B) Average flow rate 7 nL/min. at 0.06 bar (0.9 psi).

FIGURE 4

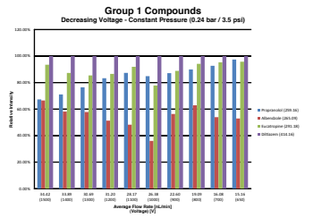


Comparative data plot of relative intensity over a series of flow rate adjustments (fixed ESI voltage) for Group 1 compounds (Propranolol at 259.16 m/z, Albendzole at 265.09 m/z, Eucatropine at 291.18 m/z, and Diltiazem at 414.16 m/z).

FIGURE 5 Constant Voltage

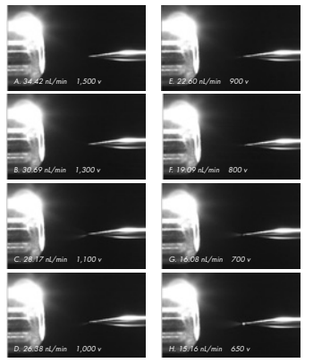
Spray images taken for data in Figure 4 taken with a 10 µm tip ID emitter approximately 2 mm from inlet. Applied voltage was held constant at 1,000 V.

FIGURE 6



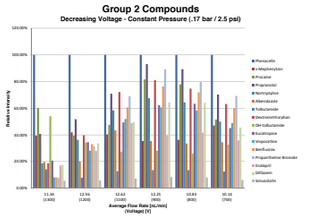
Comparative data plot of relative intensity over applied voltage adjustments for Group 1 compounds. Applied pressure remained consistent at 0.24 bar (3.5 psi), while voltage decreased by 100 volts until loss of signal was observed.

FIGURE 7 Constant Pressure



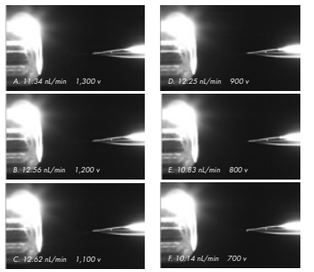
Spray images taken for data in Figure 6 taken with a 10 µm tip ID emitter approximately 2 mm from inlet. Applied pressure was held constant at 0.24 bar (3.5 psi) while applied voltage was adjusted.

FIGURE 8



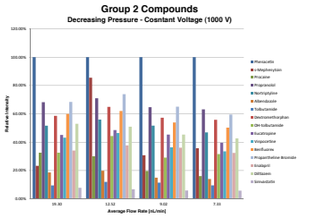
Comparative data plot of relative intensity over average flow rate for a second set of compounds. Group 2 consisted of 16 compounds. Refer to Figure 2 for complete reference.

FIGURE 9 Constant Pressure



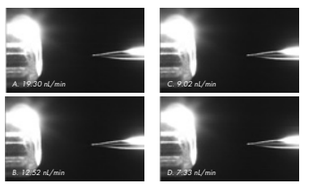
Spray images taken for data in Figure 8 taken with a 10 µm tip ID emitter approximately 2 mm from inlet. Applied pressure was held constant at 0.17 bar (2.5 psi) while applied voltage was adjusted.

FIGURE 10



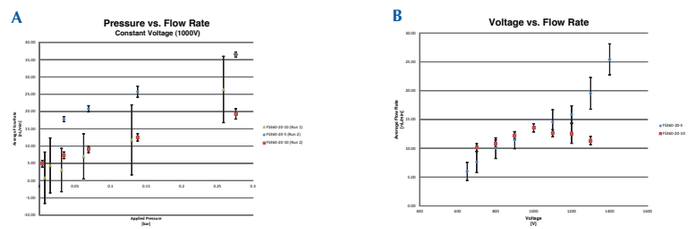
Comparative data plot of relative intensity over change in average flow rate with constant applied voltage for Group 2 compounds.

FIGURE 11 Constant Voltage



Spray images taken for data in Figure 10 with a 10 µm tip ID emitter approximately 2 mm from inlet. Applied voltage was held constant at 1,000 volts while pressure was adjusted.

FIGURE 12 Pressure Plots



A) Applied pressure vs. flow rate of three different emitters, in two sizes, over the course of two days. The applied voltage was kept constant at 1000 V. Note the linear relationship between pressure and flow rate. B) Applied voltage vs. flow rate of two different size emitters. Note the different response of the 5 µm emitter. Error bars plotted to +/- 1 standard deviation.

Results

- A trend towards equimolar response was observed as flow rate decreased. It is particularly pronounced below 10 nL/min. and with an applied voltage between 700 to 900 volts.
- As the spray images show in Figure 7, voltage has a significant impact on spray morphology and flow rate, even at a steady pressure. Multi-jet spray mode, observed at excessive voltage, is inhibitive to the equimolar response trend.
- While there are compounds that perform quite well, there are some that do not, particularly in the second group. The phenacetin had a dominant response over the other compounds. The Simvastatin had a poor response, which could be due to the peak being lost in the noise (Figure 8).

Conclusions

- Nanospray experiments performed in the ultra-low flow rate regime requires the explicit knowledge of flow rate.
- Applied voltage and correlating spray mode has an equally important role in this regime. Applied voltage affects the ionization of different compounds and the resulting droplet size. Thus, an optimized spray imaging system is crucial for verification.
- As flow rate decreases, there is a trend towards equimolar response. But because of the diversity of chemical space and the multi-dimensional relationship between flow rate, applied voltage, and tip geometry, the response is not entirely uniform.

Future Work

- Apply a more structured approach for experimentation by selecting four specific flow rates along with four specific applied voltages to find the "sweet spot" for equimolar response.
- Evaluate a more defined chemical space for industrial utility (metabolite pairs, hydroxinated compounds with parents, etc.)
- Explore a closed feedback loop system where the pressure can be automatically adjusted for a single flow rate, thus further decoupling the electro-osmotic effect of applied voltage.