

Nanoelectrospray Ruggedness for Qualitative and Quantitative Biomarker Analysis Using an Automated Emitter Positioning and Rinsing System

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Overview

- Regular tip washing enables robust and reproducible nanospray analyses
- Stable nanospray provides excellent signal and reproducibility
- The Digital PicoView source enables automatic tip rinsing

Materials & Methods

Instrumentation

- Linear ion-trap mass spectrometer (LCQ Deca™; Thermo Fisher) equipped with syringe pump (Harvard Biosciences)
- Nanospray source (Digital PicoView® 150; New Objective, Inc.)
- Capillary pump operated in normal mode (1100; Agilent)
- Fritted emitter (PicoFrit® Self-Pack-360 µm OD, 75 µm ID, 15 µm tip ID; New Objective, Inc.)

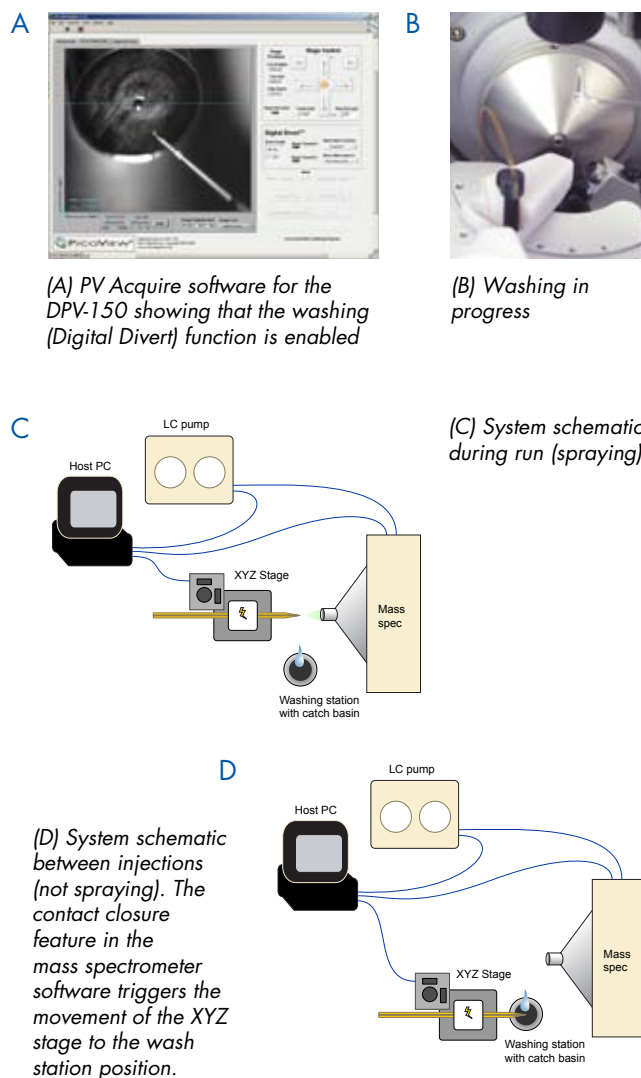
Reagents

- 0.1% Formic acid in water (JT Baker)
- 0.1% Formic acid in Acetonitrile (JT Baker)
- Methanol (JT Baker)
- Caffeine solution, 1 mg/ml in methanol MW 194.19 Da (Sigma-Aldrich)
- Bradykinin fragment 1-7, MW 756.39 Da (Sigma-Aldrich)
- Angiotensin I human acetate salt hydrate, MW 1296.48 Da (Sigma-Aldrich)
- Neurotensin, MW 1672.92 Da (Sigma-Aldrich)
- tert-Butyl methyl ether (MTBE, Sigma-Aldrich)
- Canine plasma with heparin (Harlan Bioproducts)

Sample Preparation

- Liquid-Liquid Extraction
 - Vortex mixed 500 µL plasma with 1,000 µL of MTBE
 - Centrifugation at 12,000 x g for 5 minutes to separate layers
 - Transferred 500 µL supernatant to ultrafree-MC centrifugal filter units with 0.22 µm Durapore™ PVDF Membrane for spin filtration
 - Evaporated to dryness
 - Reconstituted to 500 µL with 25%MeOH / 5%ACN / 70% H₂O Standard Spike

FIGURE 1



- 250 ng/µl Caffeine
- 5 pmol/µl Angiotensin I
- 5 pmol/µl Neurotensin
- 5 pmol/µl Bradykinin fragment 1-7

On-Line Infusion

A 500- μ L gas-tight syringe (Hamilton) set to deliver a 0.5 μ L/min constant flow rate was used to infuse a standard-spiked LLE canine plasma. An in-line nanoflow sensor (Upchurch Scientific) monitored the flow rate for accuracy and consistency. Triplicate 0.5 μ m PEEK™ MicroTight Microfilters (Upchurch) followed by dual nanofilters with 1 μ m titanium frits were plumbed in-line to minimize clogging.

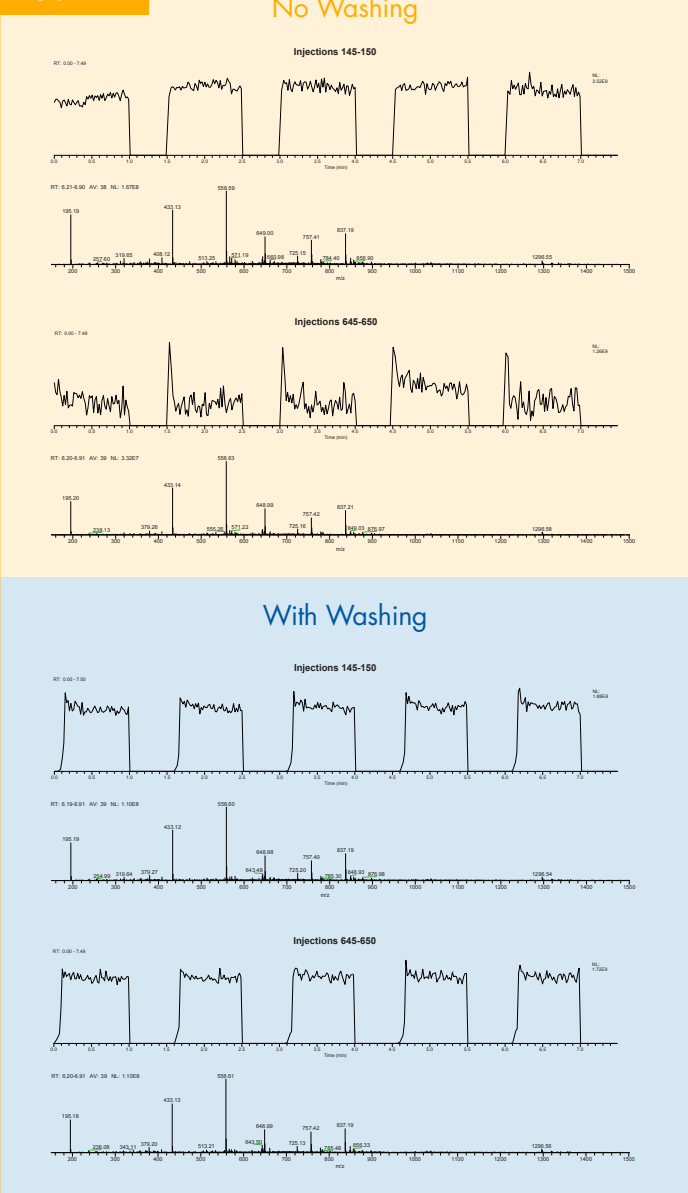
The spray voltage was cycled between 1.7 kV (On) for 1 minute, followed by a spray voltage of 0 kV (Off) for 0.5 minutes; the cycle from 1.7 kV to 0 kV representing an 'injection'.

No-washing data was collected continuously over 650 injections; the fritted emitter maintained at fixed XYZ coordinates.

Data collected during the wash run used the digitally controlled stage positioning software (Digital Divert) of the digital nanospray source to shuttle the emitter between two sets of user-defined XYZ coordinates—the spray position and the wash position. The XYZ coordinates for the spray position and the wash position were defined within the PV Acquire™ software prior to initiating automated data acquisition within XCalibur™ (Thermo Fisher).

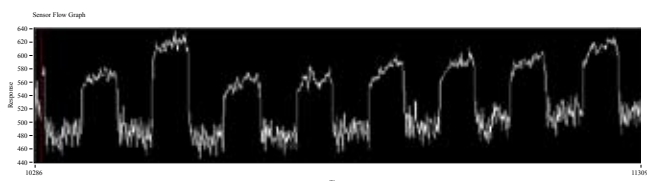
The standard peripheral controls on the mass spectrometer provided the necessary contact closure-control signal required to activate the Digital Divert™ function within PV Acquire. Timing control of the contact closure was established within XCalibur using the Contact Closure tab in Instrument Method. The method dictated movement of the stage to the wash position every time the spray voltage cycled to 0 kV. In the wash position, the exterior of the emitter was washed with a constant flow of 50% MeOH and water at 80 μ L/min delivered by a capillary pump operated in normal mode.

FIGURE 2



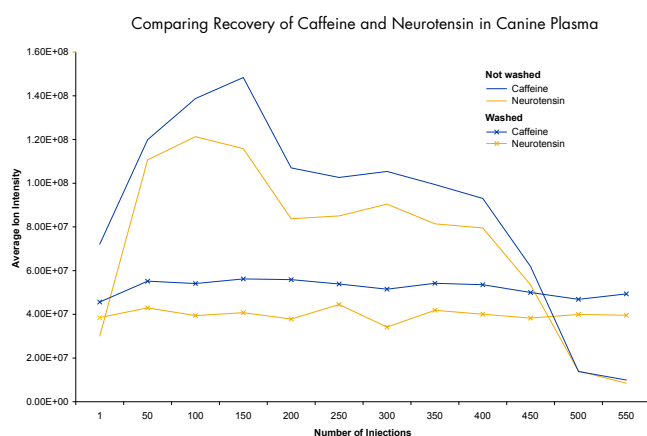
Total Ion Current plots and summed scan spectra for both sets of experimental conditions: with tip washing and without tip washing. At the outset (injections 145 – 150) the TIC for both experiments is similar, as indicated in Panel A and Panel C. Panel B details the TIC and its corresponding summed scan spectra for injections 645 – 650 with no tip washing. An overall intensity decrease is apparent and the degradation in stability is indicated by the jaggedness of the TIC. Contrary to this change in TIC between injections 145 – 150 and 645 – 650 for data collected with no tip washing, Panel D shows the TIC for injections 645 – 650 for data collected with tip washing. The TIC in Panel D is identical to the TIC in Panel C. Regular washing of the emitter exterior has maintained the intensity and stability of the TIC throughout the duration of the experiment. The summed scan spectra shows that while the intensity decreased from injection 150 to injection 650 with no tip washing, the overall profile of the spectrum has not changed.

FIGURE 3



Output from nanoflow sensor. Consistent periodic fluctuation in the flow corresponds to changes in flow rate as the syringe pump turned ON and OFF. The graph demonstrates the accuracy and precision of the flow delivered by the syringe pump.

FIGURE 4



Comparative data plot of Average Intensity per injection for two ions, Neurotensin MH2+ at 837.2 Da and Caffeine MH+ at 195.2 Da. The change in average intensity for each ion over multiple injections is dramatically different for the data set collected with washing versus the data set collected with no washing. The signal intensity collected with emitter washing is highly reproducible whereas the signal intensity with no emitter washing exhibits dramatic fluctuation. The decrease in intensity per injection indicates that eventually the value will go to zero.

FIGURE 5

Average Intensity Data

No Tip Washing																
Analyte	m/z (Da)	Injection Number														
		1	50	100	150	200	250	300	350	400	450	500	550	600	650	
Caffeine, MH+	195.19	7.20E+07	1.20E+08	1.39E+08	1.48E+08	1.07E+08	1.03E+08	1.05E+08	9.94E+07	9.30E+07	6.19E+07	8.11E+07	1.38E+07	9.94E+06	1.52E+07	
Angiotensin I, MH3+	433.12	6.46E+07	1.53E+08	1.65E+08	1.64E+08	1.43E+08	1.31E+08	1.32E+08	1.25E+08	1.10E+08	9.12E+07	9.80E+07	2.06E+07	1.37E+07	2.13E+07	
Neurotensin MH3+	558.61	8.65E+07	2.35E+08	2.33E+08	2.33E+08	2.15E+08	1.88E+08	2.05E+08	1.89E+08	1.59E+08	1.37E+08	1.51E+08	3.14E+07	2.03E+07	3.22E+07	
Angiotensin I, MH2+	649.02	3.87E+07	1.03E+08	9.88E+07	1.04E+08	7.82E+07	7.41E+07	7.87E+07	7.00E+07	6.44E+07	4.43E+07	5.14E+07	1.13E+07	7.84E+06	1.17E+07	
Bradykinin 1-7 MH+	757.39	2.73E+07	6.77E+07	7.97E+07	7.69E+07	6.21E+07	6.07E+07	6.07E+07	6.00E+07	4.67E+07	3.59E+07	4.47E+07	8.91E+06	5.51E+06	7.90E+06	
Neurotensin MH2+	837.14	3.00E+07	1.11E+08	1.21E+08	1.16E+08	8.37E+07	8.50E+07	9.04E+07	9.04E+07	8.14E+07	7.95E+07	5.34E+07	7.02E+07	1.40E+07	8.48E+06	1.17E+07

DPV Tip Washing Enabled															
Analyte	m/z (Da)	Injection Number													
		1	50	100	150	200	250	300	350	400	450	500	550	600	650
Caffeine, MH+	195.19	4.56E+07	5.52E+07	5.41E+07	5.62E+07	5.59E+07	5.39E+07	5.15E+07	5.42E+07	5.35E+07	5.00E+07	5.03E+07	4.69E+07	4.93E+07	4.90E+07
Angiotensin I, MH3+	433.12	6.43E+07	7.66E+07	7.50E+07	7.46E+07	7.64E+07	7.45E+07	6.73E+07	7.75E+07	7.79E+07	6.73E+07	7.52E+07	7.42E+07	7.49E+07	7.21E+07
Neurotensin MH3+	558.58	8.47E+07	1.13E+08	1.13E+08	1.10E+08	1.10E+08	1.17E+08	1.07E+08	1.12E+08	1.04E+08	1.06E+08	1.05E+08	1.11E+08	1.03E+08	1.09E+08
Angiotensin I, MH2+	649.03	3.98E+07	3.70E+07	3.88E+07	3.65E+07	3.94E+07	3.71E+07	3.77E+07	3.51E+07	3.47E+07	3.57E+07	3.64E+07	4.20E+07	3.84E+07	3.48E+07
Bradykinin 1-7 MH+	757.41	2.57E+07	2.89E+07	2.98E+07	2.94E+07	3.08E+07	2.95E+07	2.88E+07	3.01E+07	3.02E+07	2.83E+07	3.07E+07	2.90E+07	2.96E+07	3.14E+07
Neurotensin MH2+	837.21	3.85E+07	4.30E+07	3.94E+07	4.08E+07	3.78E+07	4.45E+07	3.41E+07	4.19E+07	4.01E+07	3.83E+07	4.17E+07	3.99E+07	3.96E+07	3.67E+07

Average intensity data for all four standards without washing (top) and with tip washing (bottom).

FIGURE 6

Frittited emitter after 650 injections without washing

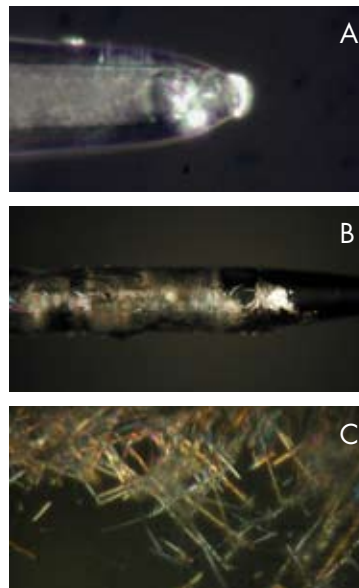


Frittited emitter at 4X magnification using optical microscopy. Sufficient material had accumulated on the emitter where a droplet would form at 0 kV to be clearly visible by the naked eye.

Tip of fritted emitter at 10X magnification. Obvious accumulation of particulates and non-volatiles.

FIGURE 7

Photo microscopy of fritted emitter taken after 650 injections without washing using cross-polarized illumination

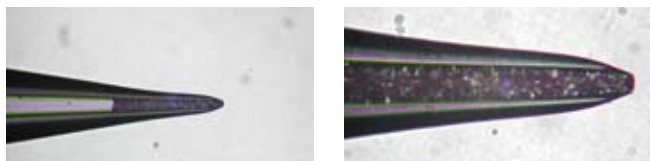


(A) 20X Magnification of accumulated matter at tip of emitter

(B) 20X Magnification of accumulated matter at site of droplet formation

(C) 40X Magnification of accumulated matter at site of droplet formation. Accumulated matter appears crystalline.

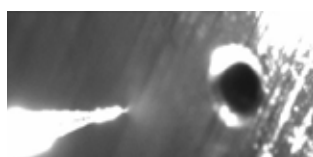
FIGURE 8 Fritted emitter after 650 injections with washing



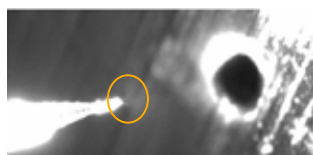
4X Magnification: Clean emitter free of particulates and accumulated matter

10X Magnification

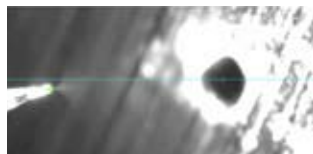
FIGURE 9 Spray images taken during analysis at 2X magnification



(A) Spray image taken at 5.0 ul/min 1.7 kV after 650 injections with no washing. At the higher flow rate, the spray morphology has improved. The experimental parameters are identical in A and B – same tip, same XYZ position, same voltage, same mobile phase composition.



(B) Spray image taken at 0.5 ul/min 1.7 kV after 650 injections with no tip washing. The spray is split, indicating the spray voltage is too high at this flow rate (highlighted area).



(C) Spray image taken at 0.5 ul/min 1.7 kV after 650 injections with washing. Under similar conditions the spray remains robust, when the emitter is submitted to regular washing between sample injections.

Conclusions

- Analyte recovery from a complex matrix and emitter performance is finite and variable when regular automated tip washing is not enabled
- Remote controlled automated tip washing at regular intervals using the Digital PicoView nanospray source enabled stable and robust nanospray and reproducible data
- The ability to generate reproducible data using the Digital PicoView nanospray source offers promising solutions for quantitative analyses

Future Work

- Determine duration of fritted emitter long term spray and signal stability with tip washing
- Evaluate effect of flow rate on analyte recovery in plasma matrix at lower flow rates
- Evaluate effect of analyte concentration on recovery in plasma matrix
- Evaluate tip washing effect on recovery from nanobore chromatographic separation of standard-spiked plasma