

Optimization of Nanoelectrospray with Plasma Derived Samples for Qualitative & Quantitative Biomarker Analysis

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Introduction

The application of on-line nanobore LC with nano-ESI is a predominant analytical method for qualitative and quantitative biomarker analysis. Optimization of both the LC column and nanospray emitter is essential for high-performance analysis of trace mixtures throughout the lifetime of the study. A systematic investigation of contamination that results from the analysis of plasma was conducted. A major source of contamination was identified as thermal decomposition products of small biochemical compounds such as peptides and lipids. Polymer coatings on nanospray emitters consisting of a polymer matrix with electrically conductive composite filler have been studied for capillary electrophoresis-ESI coupling.¹⁻³ Here we investigate the use of non-filled, non-conductive, polymer coatings (polyimide¹, polyethylene²) applied as an overcoat⁴ to tapered fused-silica emitters and combined with various rinsing schemes to evaluate nanospray emitter durability and performance.

Materials & Methods

Mass Spec Instrumentation and Components

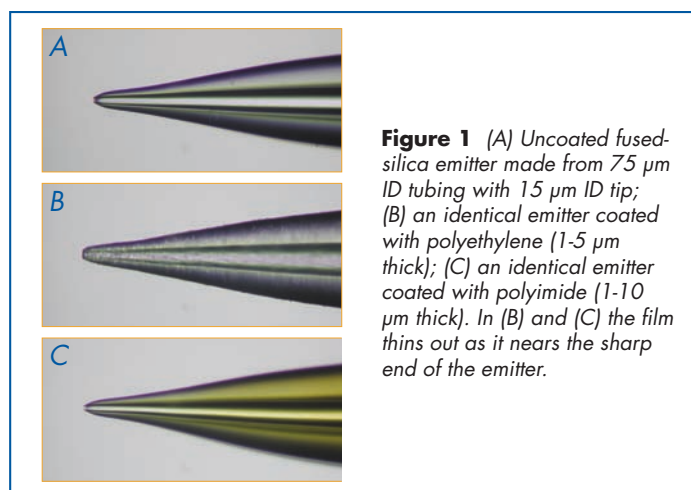
- Ion-trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Nanoflow HPLC Pump (2D NanoLC, Eksigent)
- Water/Acetonitrile, each containing 0.1% formic Acid
- Nanospray source (Digital PicoView® 150, New Objective)
- SilicaTip® emitters (360 µm OD, 75 µm ID, 15 µm tip ID, New Objective) with applied coatings as follows:
 - ◇ Uncoated: Approximately 3-4 mm of exposed fused-silica at tapered end of emitter
 - ◇ Polyethylene coated: 1-5 µm thickness at the tapered end of the emitter
 - ◇ Cured Polyimide: 1-5 µm thickness at the tapered end of the emitter

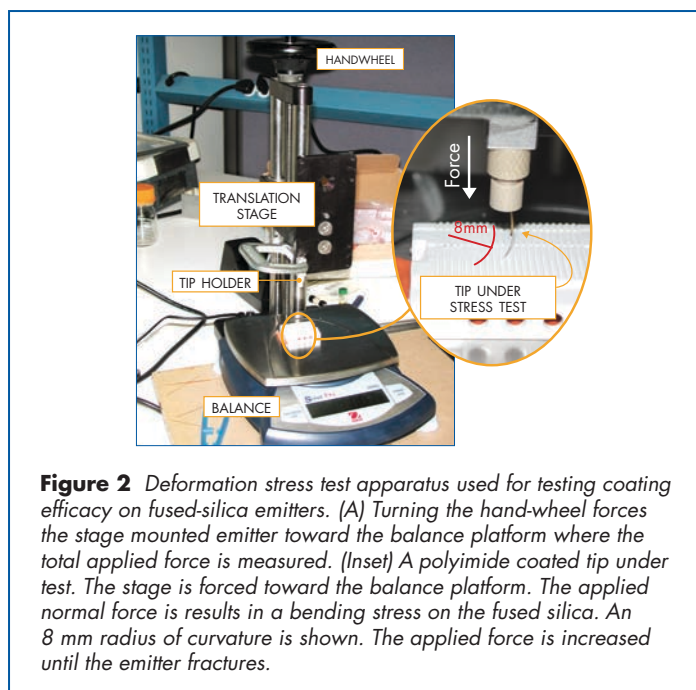
Sample Preparation

- Canine plasma with Heparin (Harlan Bioproducts) was fractionated using two different sample preparation protocols:
 - ◇ Protein Precipitation: 3:1 of ACN; plasma (500 µL) (cold) was vortex mixed and centrifuged (10,000 x g). 1 mL of supernate layer passed through spin filter (0.5 µm) and dried down. Sample reconstituted in 30% ACN (0.1% formic acid) to 1 mL.
 - ◇ Liquid-Liquid Extraction: 500 µL of plasma was vortex mixed with 1,000 µL of MTBE. Organic layer was retained, evaporated to dryness and reconstituted in 30% ACN (0.1% formic acid) to 1 mL.

Bench Top Spray Testing

- Syringe pump (PHD, Harvard Apparatus) with nanoflow sensor (Upchurch Scientific) driving a 250- or 500-µL gas-tight syringe (Hamilton).
- 5.0 kV High voltage power supply (Stanford Research Systems) was driven by a square wave generator (Stanford Research Systems) connected to the syringe pump via a platinum wire embedded in a PEEK™ MicroTee (Upchurch Scientific).
- Dual PEEK in-line microfilters (1 µm) were placed before and after the PEEK microtee. Emitters were connected to the fused-silica outlet of the microtee with a PicoClear Union (New Objective).
- Up to four emitters at a time could be mounted on the stage of a transmitted light microscope for spray observation and documentation purposes. The emitter-to-ground electrode distance was 2.5 mm.





A Deformation Stress Test
 SilicaTip: FS360-75-15-N
 Newtons of Force at Breakage Point

Trial	Plain Fused-Silica	Polyethelene Coated	Polyimide Coated
1	2.94	4.21	3.92
2	3.43	4.12	4.02
3	3.14	2.94	4.51
4	3.53	3.48	4.12
5	3.53	3.63	3.87
Average	3.31	3.68	4.09
STD	0.26	0.52	.25
% Change	—	11%	23%

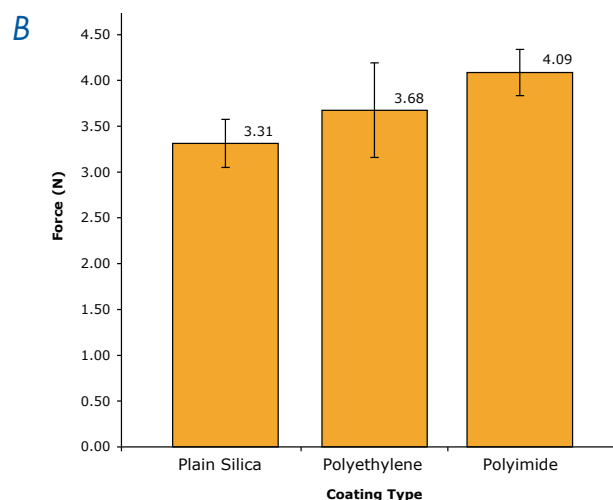
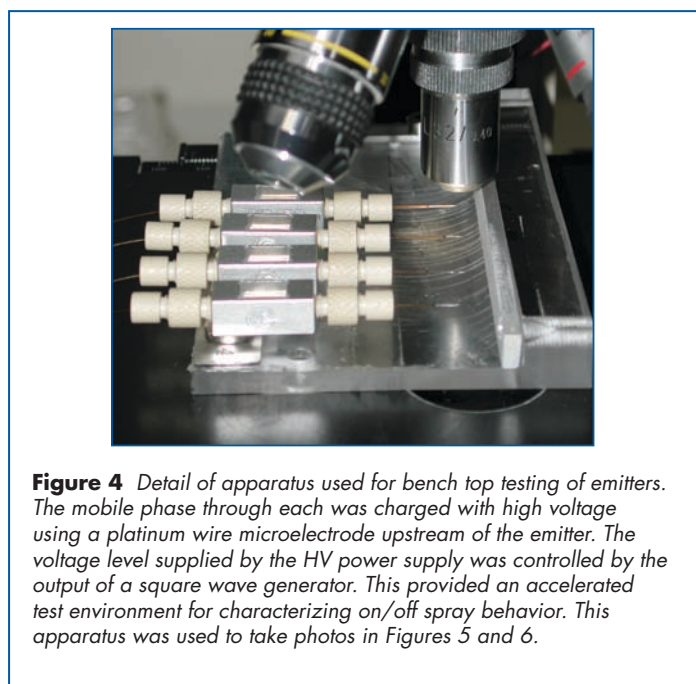
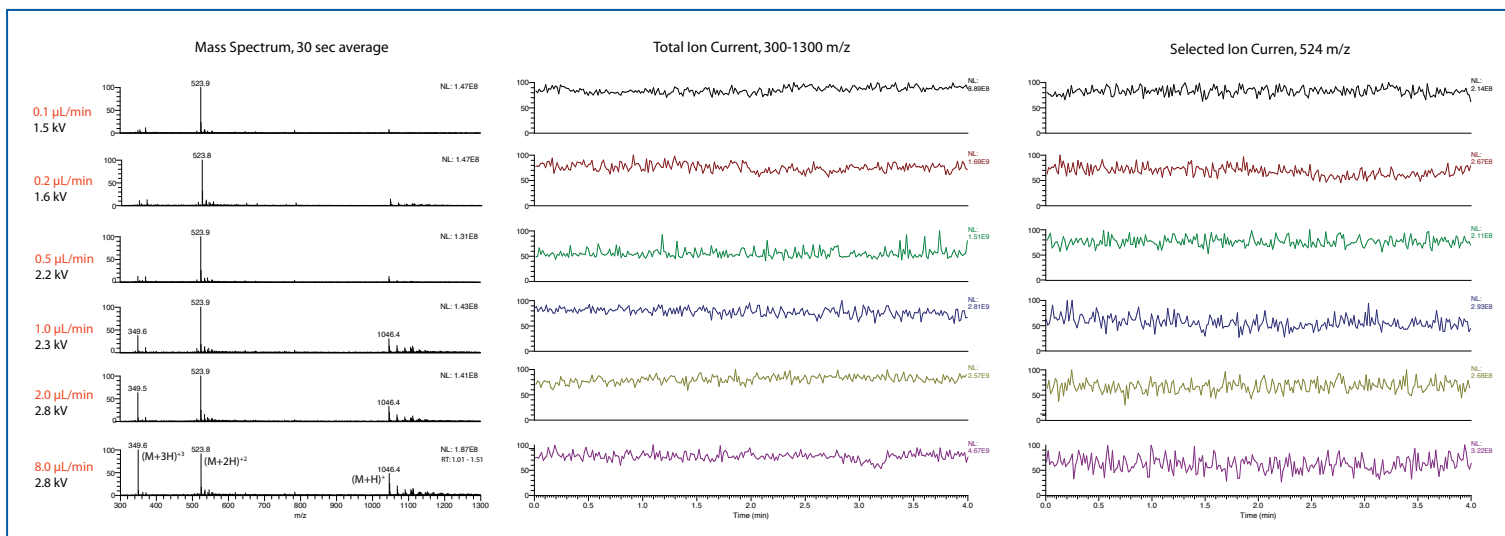
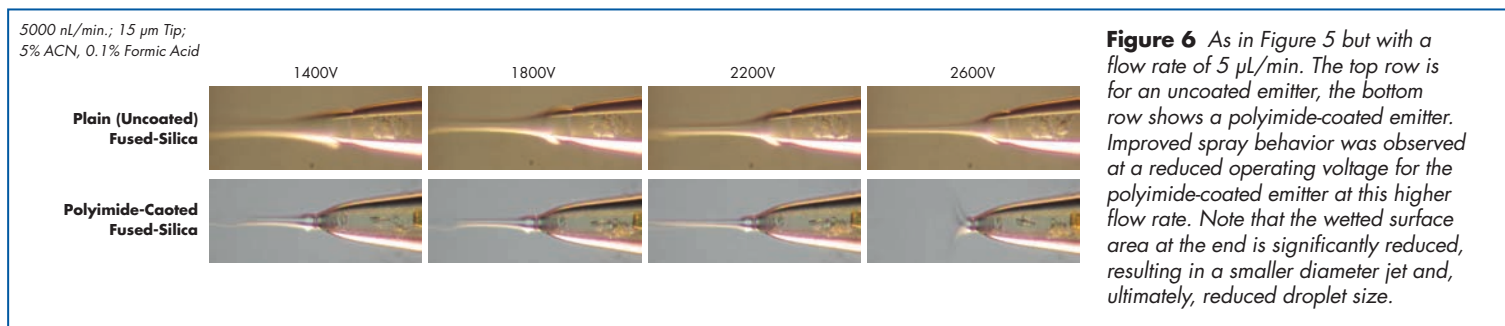
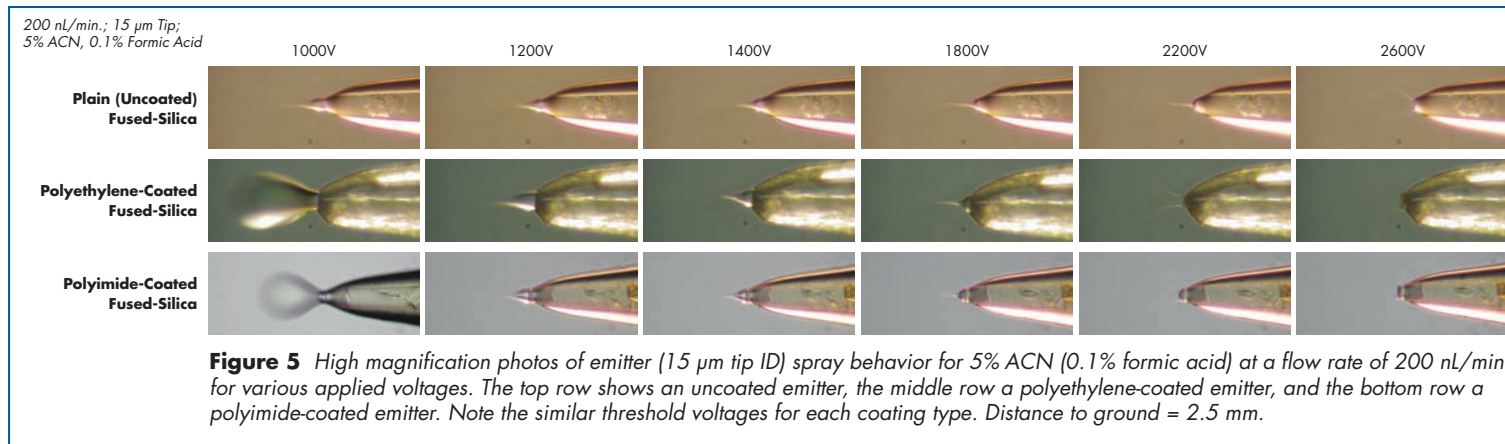


Figure 3 (A) Results of stress testing plain, polyethylene and polyimide coated emitters using the apparatus in Figure 2. (B) Graphical representation of stress test data. The error bars are set at one standard deviation. Note the 23% increase in strength for the polyimide coated emitters.





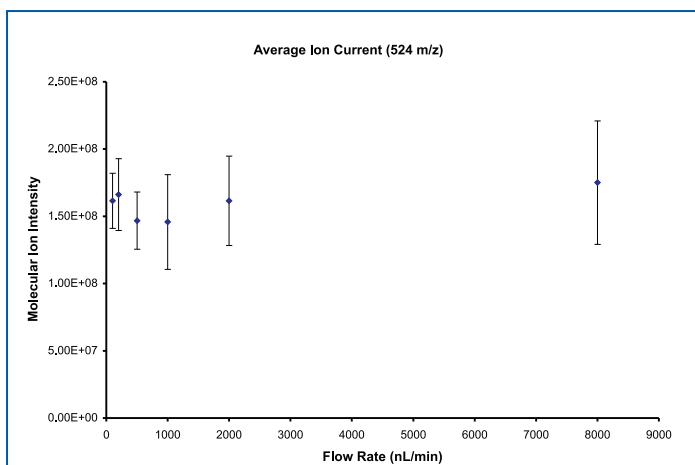


Figure 8 Plot of mean Selected Ion Current (m/z 524) for the doubly protonated species vs. flow rate. Error bars are ± 1 standard deviation.

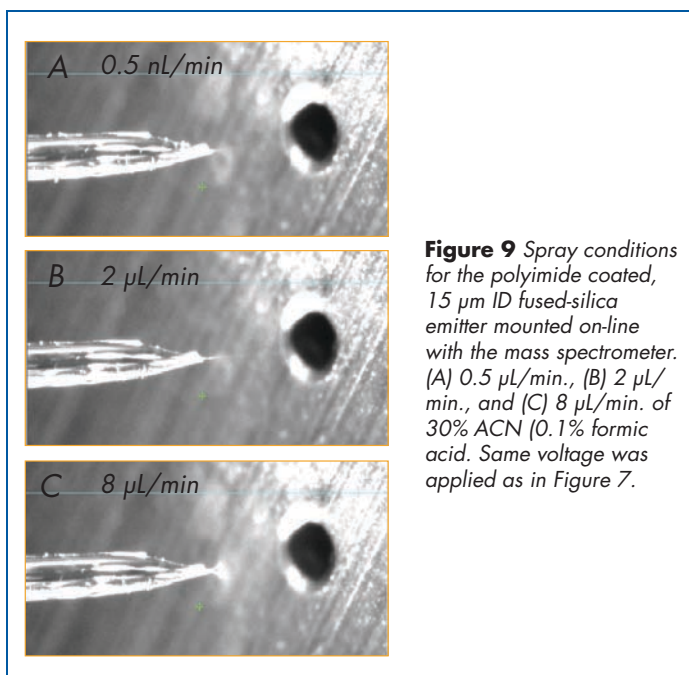


Figure 9 Spray conditions for the polyimide coated, 15 μm ID fused-silica emitter mounted on-line with the mass spectrometer. (A) 0.5 $\mu\text{L}/\text{min}$., (B) 2 $\mu\text{L}/\text{min}$., and (C) 8 $\mu\text{L}/\text{min}$. of 30% ACN (0.1% formic acid). Same voltage was applied as in Figure 7.

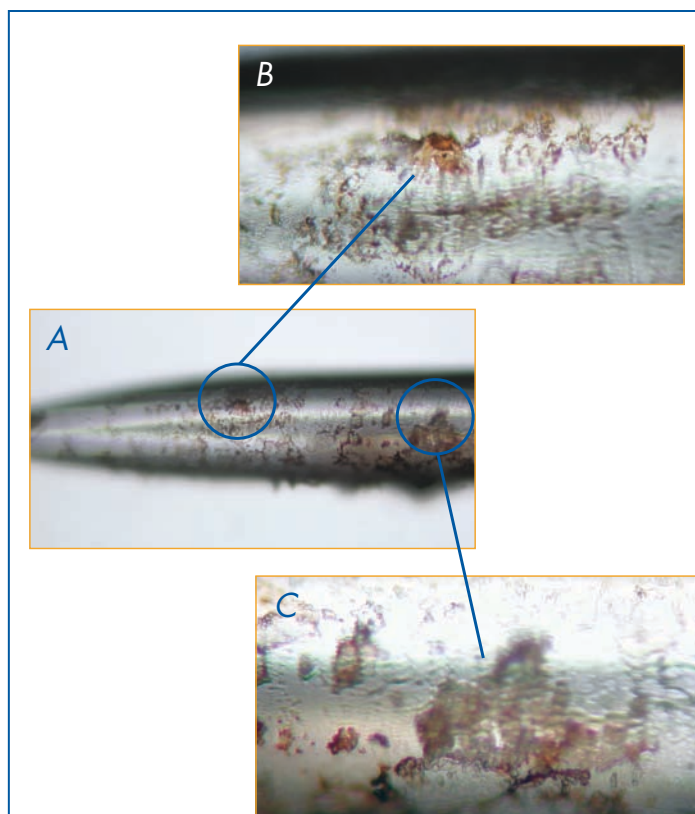


Figure 10 A 15 μm ID plain fused silica emitter was tested for performance spraying (300 nL/min) prepared plasma matrix. Canine plasma matrix was prepared by protein precipitation and reconstituted (30% ACN, 0.1% formic acid). High voltage (2200 V) was turned on and off at a period of on=30 sec., off=30 sec. Total delivered matrix volume was 145 μL (8 hours). The tip was then exposed to a 200° C source for 30 minutes. (A) Low-magnification and (B, C) high-magnification photos of the thermal decomposition products deposited on the surface of the emitter.

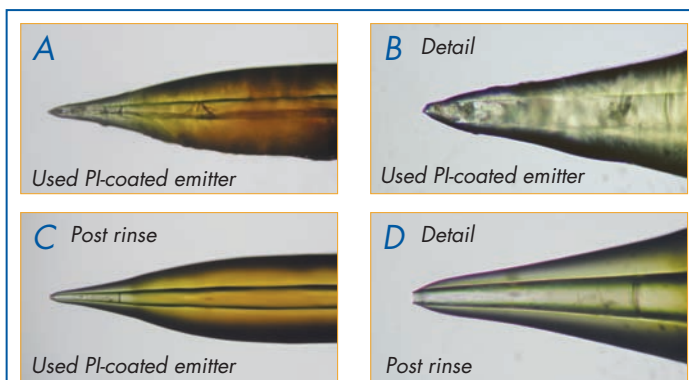


Figure 11 A 15 μm ID polyimide-coated fused-silica emitter was tested under identical conditions as in Figure 10. (A) Low-magnification and (B) high-magnification photos of the decomposition residue. (C) Same emitter as in (B) after ultra-sonic rinsing (30 sec.) and wiping with isopropanol. The tip was then exposed to a 200° C source for 30 minutes. (A) Low magnification and (B, C) high-magnification photos of the thermal decomposition products deposited on the surface of the emitter.

Results

Long-term robustness is a critical element in any quantitative bioanalytical study. Plasma derived samples are of considerable complexity and often contain a high fraction of non-protein/peptide species. To gain insight into the long-term effects on nanospray emitter performance, an accelerated aging test protocol was developed. Cycling the ESI voltage (on-off) as a square wave allowed the rapid build-up surface contamination residue on fused-silica emitters. This process simulates the long-term analytical use of a nanospray emitter. Spraying reconstituted canine plasma matrix for 8 hours at 300 nL/min. (145 μ L total volume) resulted in a thick residue (>5 μ m) with a gum-like appearance on the emitter surface (Figs. 10-11). The residue transformed into a hard reddish-brown film when the emitter (≈ 2 mm) was positioned in front of an inlet at 200° C for 30 minutes with no flow. The emitters rapidly clogged when the ESI voltage was cycled with exposure to increased temperature. Identical emitters were coated with a 1-5 μ m film of polyethylene and polyimide and evaluated in a similar fashion (Figure 1). The polyimide (PI) coated emitters were found to increase the mechanical strength compared to fused-silica by more than 20% (stress breakage testing, Figures 2-3). Furthermore, the PI coated emitter withstood aggressive cleaning procedures that resulted in the restoration of spray performance (Figure 10). PI coated emitters were found to optimize the spray stability for low percent organic mobile phase (5%) across a broad range of flow rates from 100 nL/min. to more than 8 μ L/min. (Figures 5-6). Intra-day RSD's for spray stability were found to be better than 10% and 15% for total ion and selected ion currents respectively at flow rates less than 500 nL/min. (Figures 7-8). The long-term spray stability and ion signal repeatability of PI coated emitters in combination with nanobore LC and an automated in-source tip rinsing system is under investigation.

Conclusions

- Re-coating the tapered portion of the nanospray emitter with a polymer film increases the bulk strength of fused-silica emitters by more than 20%
- Polyethylene and polyimide coatings improve the high flow rate spray performance of 15 μ m ID emitters, even at very low (5%) organic compositions
- Thermal decomposition of organic material in sample (peptides, lipids etc.) exiting the emitter is the source of commonly observed "brown matter" that collects on the outer surface of an emitter
- On-off cycling of the electrospray high voltage while exposed to the high temperature of the MS inlet results in observable residue build-up, eventually blocking emitter flow
- Polyimide-coated emitters withstood aggressive cleaning regimens, including ultrasonication in isopropanol and mechanical wiping with a moistened wipe
- Polyimide coated emitters exhibited the most stable spray current at flow rates below 500 nL/min., but were quite usable at flow rates above 8 μ L/min.

References

- 1) Barnidge, Nilsson, Markides *Anal. Chem.* 1999, 71, 4115-4118.
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- 3) Zhu, Thiam, Valle, Warner *Anal. Chem.* 2002, 74, 5405-5409.
- 4) Valaskovic, McLafferty U.S. Patent 5,788,166