Minimization of Atmospheric Background Contaminants in Nanoelectrospray: Identification & Optimization

Benjamin Ngo, Gary A. Valaskovic

New Objective, Inc., Woburn, MA

Introduction

The high-surface area of the (sub)micrometer droplets generated by low-flow nanoelectrospray ionization results in the potential ionization of contaminants present in laboratory air[1]. Neveu[2] and coworkers recently reported on a "bath gas" system designed to reduce the background levels using laboratory air (proceedings of the 56th ASMS Conference, June 1-5, 2008, Denver, Colorado). Here we report on the evaluation of two similar systems for use in combination with on-line nano-LC/MS.

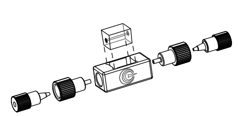
Methods

- Mass Spectrometer: LCQ Deca™ (Thermo Fisher Scientific)
- HPLC Pump: Eksigent nano-LC
- Syringe Pump: Harvard Apparatus® pump with flow sensor

The bath gas system was implemented on a customized digitally-controlled nanospray source (Digital PicoView® DPV-150, New Objective, Inc.) mounted to a conventional 3-D ion trap (LCQ Deca™, Thermo Scientific Inc.) Two different air filtration systems were tested. The first system was driven by a pressure-fan in combination with a high-permeability carbon filtration system. The second system utilized a high quality, dry-air compressor (Jun-Air) that was connected to a flow control system (0-12 L/min.) with an in-line carbon-based organic vapor filter (Supelco) intended for gas chromatography. The flow rate and volume of air delivered at the bath gas outlet was measured with a velocity sensitive air-flow meter (Testo Inc.). The air flow at the outlet of the delivery system could be adjusted from laminar, to sub-turbulent, to turbulent conditions.

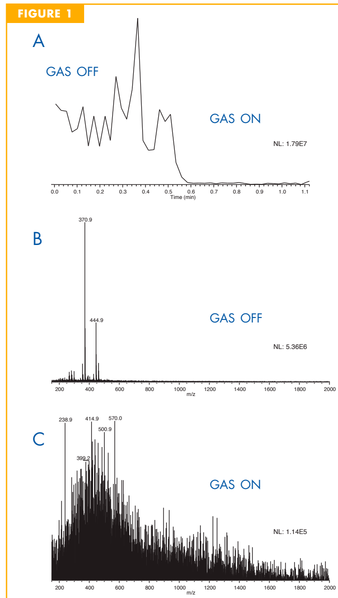
Mobile phase (water, acetonitrile, 0.1% formic acid; Sigma-Aldrich) was delivered by a gradient nanoflow LC pump (Eksigent) or by continuous infusion from a syringe pump (250 μL syringe; Hamilton Gas-Tight). Syringe pump flow rate was measured to within ± 20 nL/min. using an in-line flow sensor (Upchurch Scientific®). The typical flow rate for continuous infusion was 400 nL/min. A 10 μm ID fritted-tip emitter (PF360-50-10-N; New Objective, Inc.) was mounted on the source. High voltage was applied directly to the mobile phase through a clear elastomer PicoClear™ Conductive Union (PCCU-360; New Objective, Inc.). An in-line PEEK™ filter (Upchurch Scientific, P-770) was used to reduce particulate contamination of the emitter.

A three-peptide standard (human angiotensin I, MRFA, bradykinin; Sigma-Aldrich®) was prepared at a concentration of 1 pmol/μL (5 to 50% acetonitrile). All samples contained 0.1% formic acid. Standards of the proposed polysiloxane contaminants (decamethylcyclopentasiloxane and dodecamethylcyclohexasiloxane) were obtained from Gelest, Inc. A 10 μL aliquot of each siloxane was deposited onto the interior surface of a 5 mL glass vial that had been packed with a Kimwipe® tissue. Each vial was positioned in front of the bath gas outlet in order to introduce the volatile compound to the nanospray plume.



Left, Digital PicoView Nanospray Source mounted onto the LCQ Deca; above, PicoClear conductive union

FIGURE 1

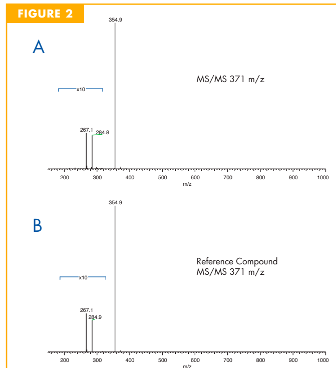


(A) The ion current obtained from cyclosiloxane background ions at m/z 371 and 445 with 0.1% formic acid in water. Bath gas is off for the first 30 sec., on from 30 sec. to 66 sec.

(B) Bath Gas off. Full scan mass spectra for 0.1% formic acid showing the dominant background ions at 371 and 445 m/z .

(C) Bath gas on. Full scan mass spectra for 0.1% formic acid with the background components removed to baseline level.

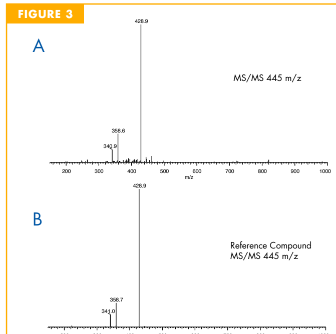
FIGURE 2



(A) The MS/MS product ion spectrum of the 371 m/z protonated molecular ion present in the ambient laboratory air.

(B) The MS/MS product ion spectrum of the 371 m/z protonated molecular ion from the reference compound, decamethylcyclopentasiloxane, introduced into the outlet stream of the bath gas. Note the identical fragments at 267.1, 284.8 and 354.9 m/z.

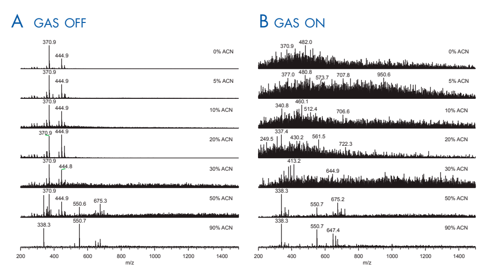
FIGURE 3



(A) The MS/MS product ion spectrum of the 445 m/z protonated molecular ion present in the ambient laboratory air.

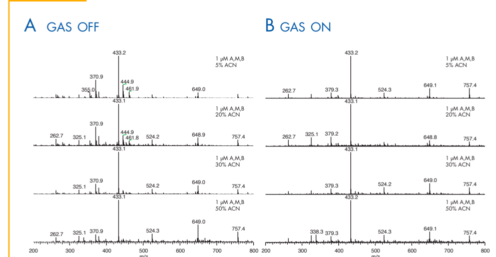
(B) The MS/MS product ion spectrum of the 445 m/z protonated molecular ion from the reference compound, dodecamethylcyclohexasiloxane, introduced into the outlet stream of the bath gas. Note the identical fragments at 340.9, 358.6 and 428.9 m/z.

FIGURE 4 *Mobile phase background*



(A) Representative full-scan mass spectra for increasing percentage of ACN (0.1% formic acid); bath gas off. (B) Representative full-scan mass spectra for increasing percentage of ACN (0.1% formic acid); bath gas on. Note effective removal of 371, 445 m/z background ions. Background ions at 338, 550, m/z etc. that appear for high % ACN appear to be intrinsic to the mobile phase and not atmospheric contaminants.

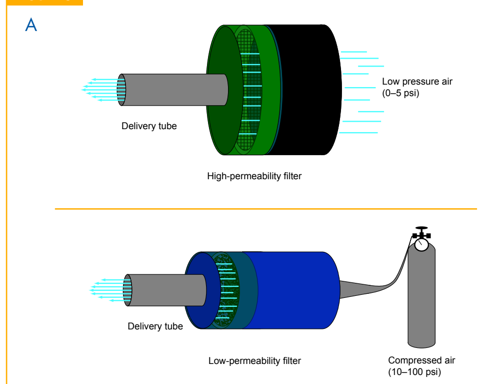
FIGURE 5 *Mobile phase background with analyte (1μM angiontensin, MRFA, bradykinin)*



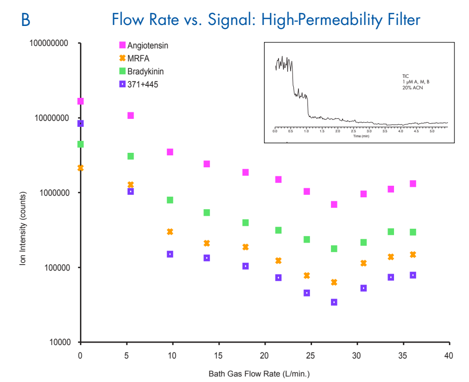
(A) Representative full-scan mass spectra for increasing percentage of ACN (0.1% formic acid); bath gas off.

(B) Representative full-scan mass spectra for increasing percentage of ACN (0.1% formic acid); bath gas on. Note effective removal of 371, 445 m/z background ions. Note the simplification of full-scan spectra for low percent ACN.

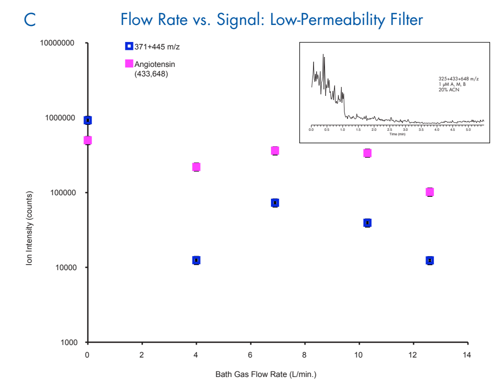
FIGURE 6



(A) Set up of the two systems for delivery of bath gas. Top: Pressure-fan delivery combined with a low-permeability carbon filtration system. Bottom: Compressed dry air delivery with an inline low-permeability carbon-based organic vapor filter.

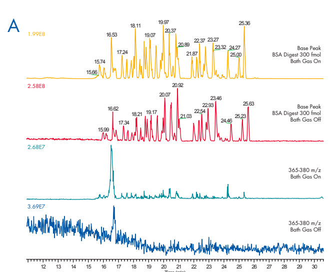


(B) Bath gas flow rate vs. ion intensity for increasing flow rate using a high permeability carbon filter. The flow rate of the bath gas was increased every 30 seconds, with no flow from 0–30 sec. Inset shows the TIC data used to generate the plot.

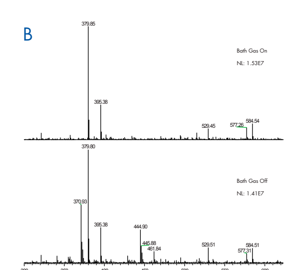


(C) Bath gas flow rate vs. ion intensity for increasing flow rate using a low permeability carbon filter. Note that the background ion intensity falls off much faster than the analyte ion intensity at low bath gas flow rates. Inset shows the TIC data used to generate the plot.

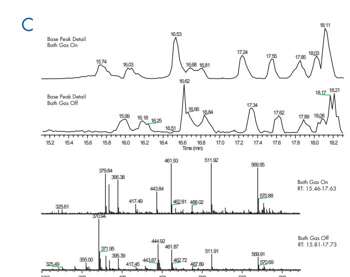
FIGURE 7 *Gradient elution nanobore LC-MS of a BSA digest standard, 300 fmol on-column injection*



(A) Comparison of reconstructed base-peak chromatograms comparing bath gas on with bath gas off. Note that selected ion current for peptide ions in proximity to the 371 and 445 m/z background ions is much improved.



(B) Full scan mass spectra of a BSA peptide (379 m/z) with gas on (top) and off (bottom).



(C) Detail of the base-peak chromatograms from Figure (A) and summed full scan spectra. Note the simplification of the mass spectra.

1. Volkmer-Engert R.,Schlosser A. *J. Mass Spectrom.* 2003; 38: 523-525
2. Neveu, J.A., Ngo, B., Bodnik, B.A., Valaskovic, G.A., Lane, W.S. "An Active Background Ion Reduction Device (ABIRD) Increases Signal-to-Noise Ratio, Sensitivity, and System Reliability" Proceedings of the *56th Annual American Society for Mass Spectrometry Meeting*, Denver, Colorado USA, June 2008

Results

The commonly observed background ions at m/z 371 and 445 were positively identified as cyclosiloxane compounds through the use of reference standards. A systematic study of operational parameters (filter type, gas flow rate, composition, etc.) was conducted to minimize background ion current without compromising analyte signal. A reduction in the background current from siloxane ions was reduced by 100-fold when using either filtration system. Reduction of these commonly observed background ions improves the appearance of the data and may simplify visual interpretation. The real-world impact on the qualitative or quantitative interpretation has yet to be determined. Air was found to be superior to nitrogen (not shown) for use with gradient elution LC as air exhibits a higher breakdown voltage and therefore reduces the chance of corona discharge at the nanospray emitter.

Conclusions

- The background ions at m/z 371 and 445 are confirmed by MS/MS to be cyclosiloxane compounds.
- Minimization of these cyclosiloxane compounds in the atmosphere surrounding the inlet of the mass spectrometer can be achieved with a low-flow bath gas.
- Either low- or high-permeability carbon filters may be used to remove cyclosiloxane compounds from laboratory air.
- Compressed air from a conventional laboratory air compressor can be suitably "scrubbed" for use as a bath gas by a packed bed carbon filter.
- Background levels are typically reduced by 100-fold (i.e. to baseline spectrometer noise levels).
- Bath gas flow rates that are too high (i.e. turbulent) may result in the decrease of analyte ion intensity.