NANO-SPRAY IONIZATION SOURCE ISOLATION ON PROTEOMIC MASS SPECTROMETERS FOR SILOXANE CONTAMINANT REMOVAL

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ABSTRACT

Direct infusion experiments of Glu-Fibrinopeptide showed a non-stationarity in the signal that appeared to be inversely related to the presence of low mass contaminants, later determined to be polysiloxanes. Monitoring of these contaminants revealed a cyclical temporal profile with a period of approximately 22 minutes. This particular contaminant had the effect of suppressing peptides present at the same time. Further investigation led to the suspected source of the contaminant, the HVAC unit in the building which was also operating at a 22 minutes period. It was suspected that the polysiloxane contaminants ionize by contacting either the high voltage ionization needle or ionized sample. This led to the design and construction of an enclosure to isolate the mass spectrometer's ionization source from the surrounding atmosphere. An airtight enclosure mounted against the inlet of the mass spectrometer effectively sealed off the front end of the instrument. A small port was connected to an activated carbon cartridge to filter the incoming air from contaminants. This design had the effect of removing the polysiloxane from the air, without introducing turbulence or detrimentally impacting the intensity of the signal.

INTRODUCTION

Génome Québec (GQ) Standards

The goal of the GQ Standards initiative was to develop and validate different methodologies to quantify peptides and proteins. The focus of the first phase of this project was to characterize the input-output relationship of a single peptide with varied concentrations. To minimize the number of variables in the experiment a single standard calibrant peptide Glu-Fibrinopeptide B (Glufib) was continuously infused into the mass spectrometer to acquire a "pseudo stable" thirty minute signal. The signal is to be analyzed to determine signal properties in an "ideal" situation.

Problem

Initial analysis of the data showed that signal intensity was highly unstable. Fluctuations of more than two-fold were identified. Analysis of the data showed that major fluctuations coincided with the appearance of "contaminant" ions centered around 355 *m/z* [Figure 1]. Further investigation into the contaminant revealed it to be from the family of polysiloxanes which is reportedly quite prevalent in ambient air (sources include: cosmetics, glue, paint,

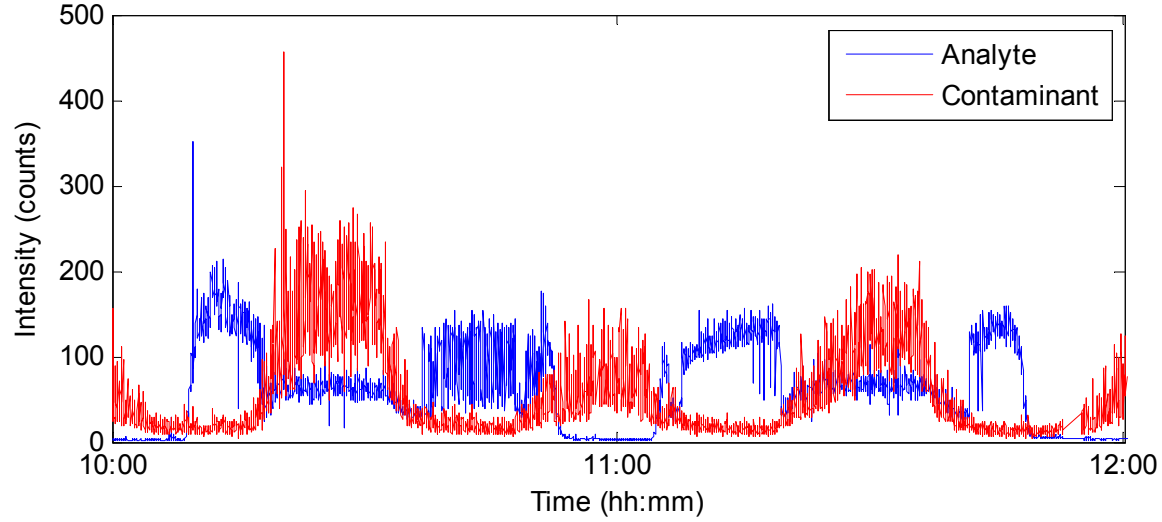

Figure 1: Infusion of analyte at ~785 *m/z* [BLUE] being cannibalized by contaminant signal at 355 *m/z* [RED].

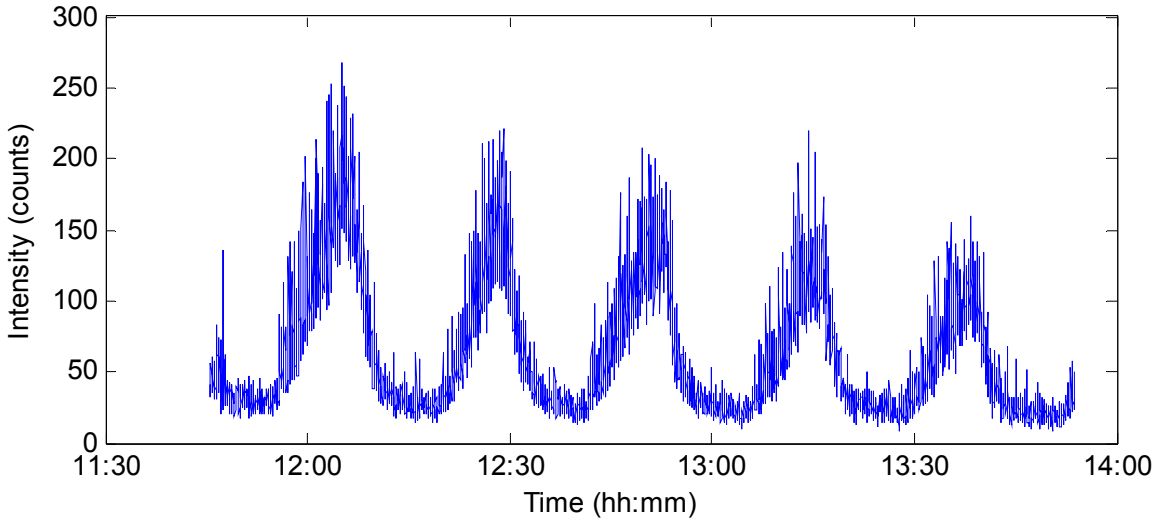

Figure 2: Contaminant signal at 355 *m/z*; with an approximately 22 minute period.

sealants). [1] The particular polysiloxane contaminant at 355 *m/z* monitored in this paper is decamethylcyclopentasiloxane.

Follow-up experiments without injecting the analyte showed a periodic nature to the intensity of the siloxane contaminant. Signal analysis determined the periodicity of the contaminant to be approximately 22 minutes [Figure 2]. The presence of spurious ions in the mass spectrometer is generally not a concern in itself; however the cyclic nature of this siloxane coupled with its depressing effect on the analyte would complicate attempts to make quantitative measurements of peptide intensity.

This paper describes the investigation into the source of the contaminant, the design of a new source to eliminate it and the validation of it.

METHODS

All experiments were run on Q-Tof micro™ mass spectrometers (Micromass, Manchester, U.K.) equipped with a ZSpray™ NanoFlow™ stage (Micromass, Manchester, U.K.). The NanoFlow stage has been modified with a ADPT-MZS nanospray adapter (New Objective, Woburn, MA) to hold the Picofrit® column (New Objective, Woburn, MA).

In the experiments generating Figures 1 and 2, 500 femtomoles/µL of Glufib analyte was infused directly through the PicoFrit column at 50% ACN with a flowrate of 200 nL/min using an 8 µL sample loop.

In the experiments generating Figure 5, two identical mass spectrometers were set up side by side, under the same HVAC unit, and acquisitions

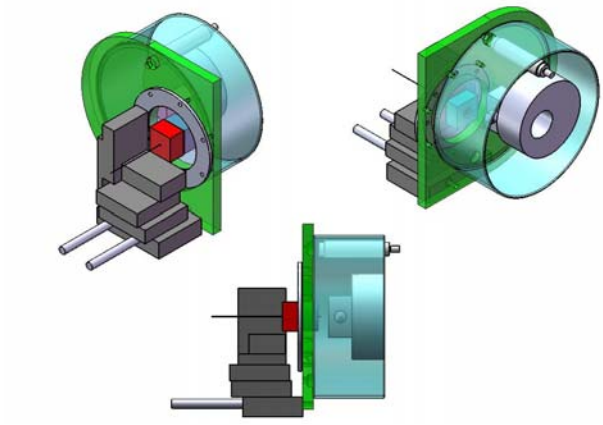

Figure 3: CAD rendering of designed enclosure, with spray stage and mass spectrometer inlet

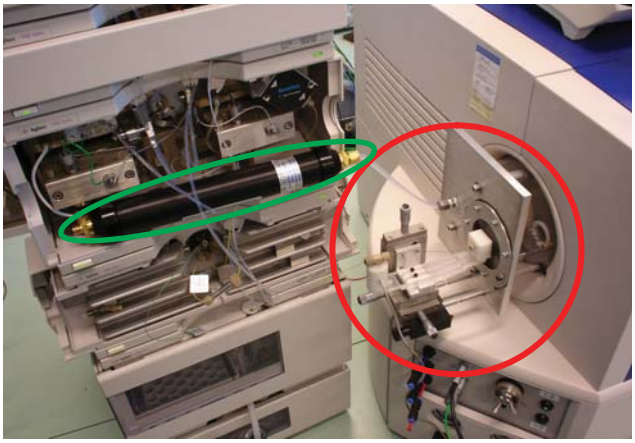

Figure 4: Enclosure assembly (red) mounted on mass spectrometer, activated carbon filter (green)

synchronized. The first instrument was equipped with the new source enclosure, the second without. A 50% ACN solution was infused through the PicoFrit column at 200 nL/min.

SOLUTION

There are several simple criteria for a method of dealing with polysiloxane contamination:

1. The solution should eliminate (or drastically reduce) the siloxane molecules.
2. The signal intensity of the analyte signal should not be affected.

There are currently several solutions to eliminate ambient air contamination from electrospray ionization sources; however, none have been adapted to nanospray sources such as used in our instrument. The solution reported by Schlosser et al. [1] was to blow a purified gas orthogonally to the nano-electrospray source so that ambient contaminants would not contact the high voltage, and therefore not be ionized. This technique was tested in our facilities but the analyte signal was completely obliterated by flow of the gas.

Our solution was to isolate the source from the ambient laboratory air so that siloxane (as well as other) contaminants could not come in contact with the high voltage source. The sealed enclosure was designed such that it completely surrounded the high voltage assembly and the entrance to the mass spectrometer. Figure 3 is the CAD drawing of the enclosure, including the nano-electrospray stage and the inlet to the mass spectrometer.

A small access port was created to connect a nitrogen source in order to create a positive pressure atmosphere inside the enclosure so that leaks would be forced out of the enclosure. In testing however, the vacuum of the mass spectrometer was so intense that the required nitrogen flow rate was beyond the capabilities of our facility. To prevent the contaminant from entering the source, a technique used in gas

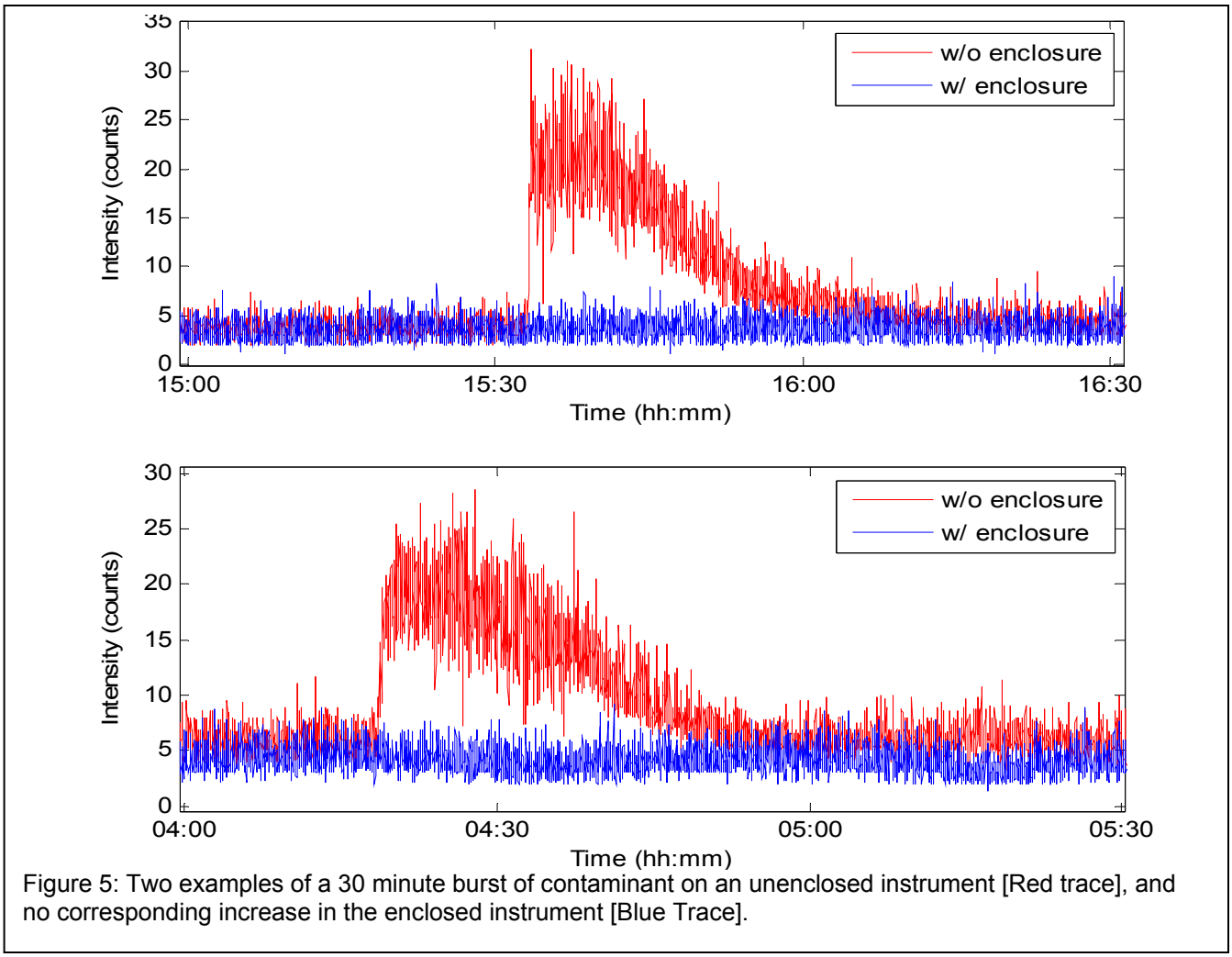

Figure 5: Two examples of a 30 minute burst of contaminant on an unenclosed instrument [Red trace], and no corresponding increase in the enclosed instrument [Blue Trace].

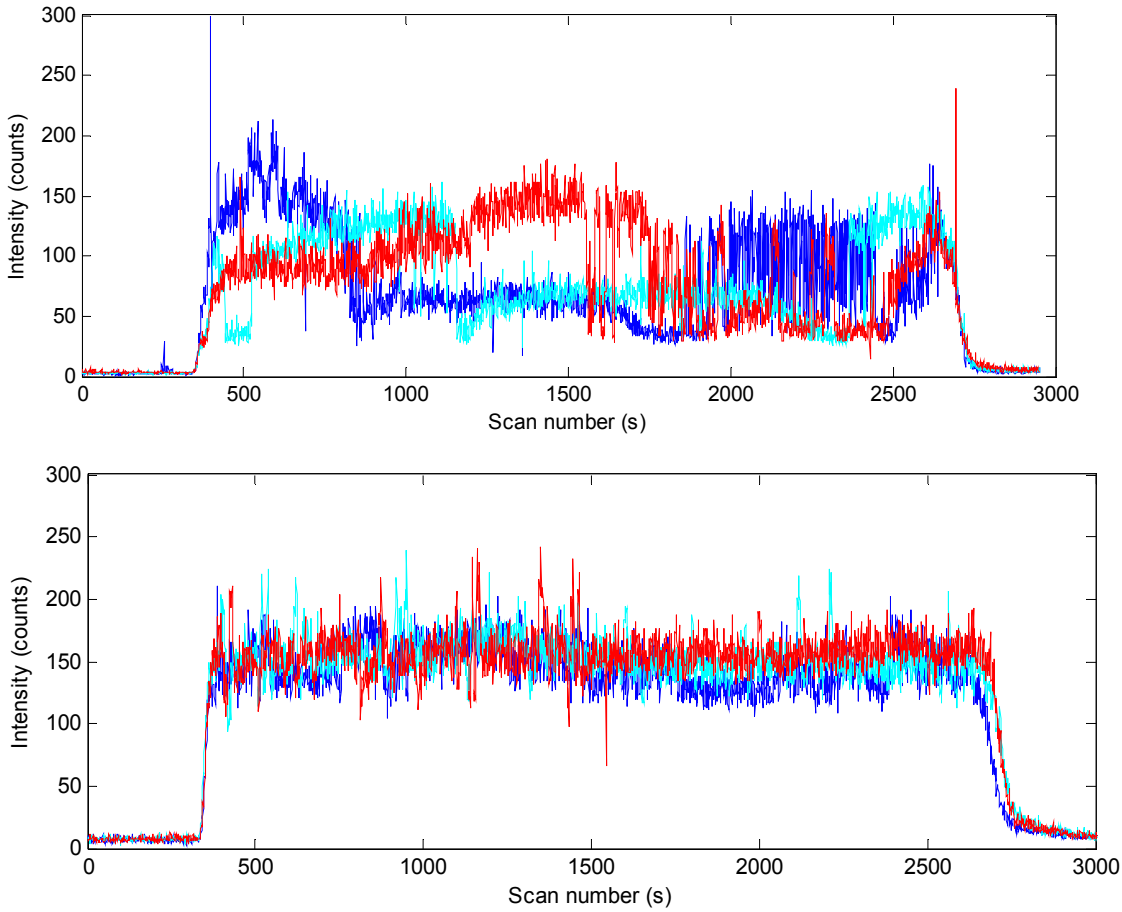

Figure 6: Top 3 traces show direct infusion of Glufib analyte on an unenclosed instrument. Bottom 3 traces show the same experiment on the same instrument with the new enclosure.

chromatography mass spectrometry was adapted; an activated carbon cartridge was attached to the access port to remove contaminants from the ambient air before entering the enclosure. Figure 4 shows the prototype installed on the instrument along with the activated carbon filter connected via tubing.

RESULTS

To evaluate the effectiveness of the new source, we carried out a set of experiments in which two identical instruments were run side by side; one with the new source, and one without. Figure 5 shows that the signal from the first instrument, without the enclosure [Red trace], contains 30 minute bursts of contamination, while the enclosed instrument [Blue trace] shows no signal change.

The average signal intensity of analyte actually increases with the enclosure in place. Figure 6 shows 3 infusions of Glufib analyte without an enclosure, and 3 infusions with the new enclosure showing higher stability and increase in average intensity. The increase in intensity is thought to occur because of the slight vacuum inside the enclosure which helps analyte ion desolvation.

ACKNOWLEDGEMENTS

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