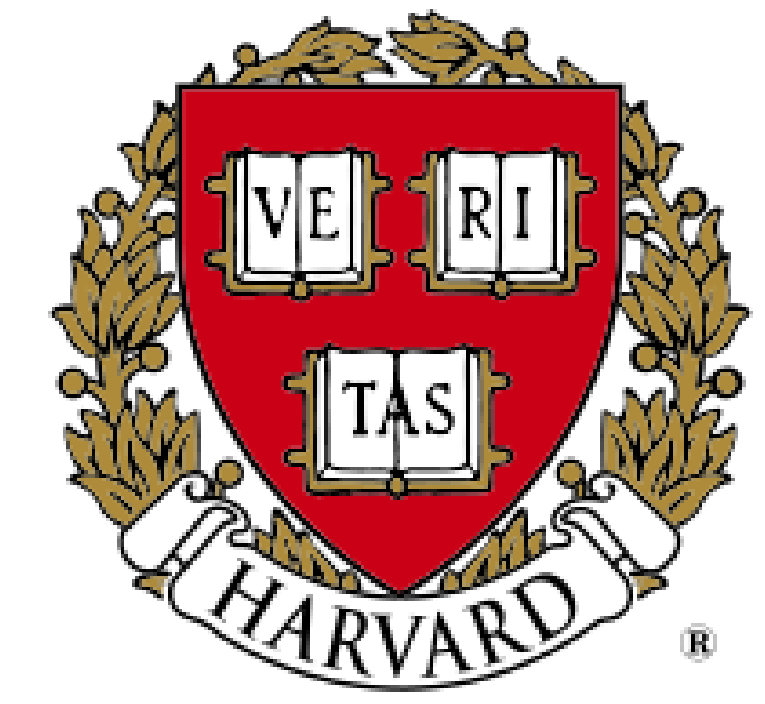




An Active Background Ion Reduction Device (ABIRD) Increases Signal to Noise Ratio, Sensitivity And System Reliability



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Overview

- High sensitivity nanospray mass spectrometry is subject to variable interference from ubiquitous volatile compounds in the air.
- To address this problem an **Active Background Ion Reduction Device (ABIRD)** was developed and tested on several mass spectrometers.
- The **ABIRD** imparts to a mass spectrometry system a consistent low background ion signal with improved peak signal to noise ratios while isolating the instrument from variable ambient conditions in the laboratory with no loss of peptide signal.

Introduction

It has been shown that volatile polydimethylcyclisiloxanes cause the major background signal commonly observed under nanospray ESI conditions¹. These and other intermittent volatile compounds in the lab environment can significantly interfere with high sensitivity LC-MS/MS experiments. An **Active Background Ion Reduction Device (ABIRD)** was constructed which removes these compounds from the ambient lab air at the ESI source. When in use, a significantly lower background ion signal is observed, imparting a low consistent baseline over a chromatographic run, while peak signal to noise ratios improve markedly with no loss of peptide signal.

Methods

- LTQ-Orbitrap, (ThermoFisher Scientific, San Jose CA)
 - NanoAcquity UPLC with 0.1% formic acid buffers, (Waters, Milford MA)
 - Fritted glass capillary trapping and pulled tip analytical columns in Picoview nanospray source, (New Objective, Woburn MA)
 - **ABIRD** configured in an **Open Source** configuration (**Figure 1**).
- Q-TOF Premier, (Waters, Milford MA)
 - NanoAcquity UPLC (Waters) with 0.1% formic acid buffers
 - Symmetry trapping and Acquity analytical columns in nanospray lock mass source
 - **ABIRD** configured in **Semi-Sealed Source** configuration (**Figure 2**).
- Angiotensin peptide standards, (Sigma-Aldrich, St. Louis MO)
- Enolase digest standard, (Waters)

Results

Nanospray background comparisons with ABIRD on LTQ, LTQ-Orbitrap and Q-TOF

- 2 minute full MS scan acquired at initial equilibration conditions.
- **ABIRD** device turned on after 1 minute.
- ~30 seconds each segment summed for background ion intensity comparison.
 - **RESULT: Between 10x and 20x reduction in background ion intensity and composition with device on (Figures 3, 4 and 5).**

Chromatographic peak Signal to Noise comparison on LTQ and LTQ-Orbitrap

- 25 fmol angiotensin peptide mix injected on column.
- 1-51%B / 10 minute fast gradient elution.
- One injection **ABIRD** off, repeat injection with **ABIRD** on.
- Peak areas integrated and signal to noise ratios calculated in Qual Browser, (Thermo)
- ~1 min. summed from middle of each chromatogram for background ion comparison.
 - **RESULT: ~5x increase in peak signal to noise ratios with the device on.**
 - **RESULT: Summed baseline signal during the chromatogram had between 10x and 40x lower background ion intensity with **ABIRD** on. (Figures 6 and 7)**

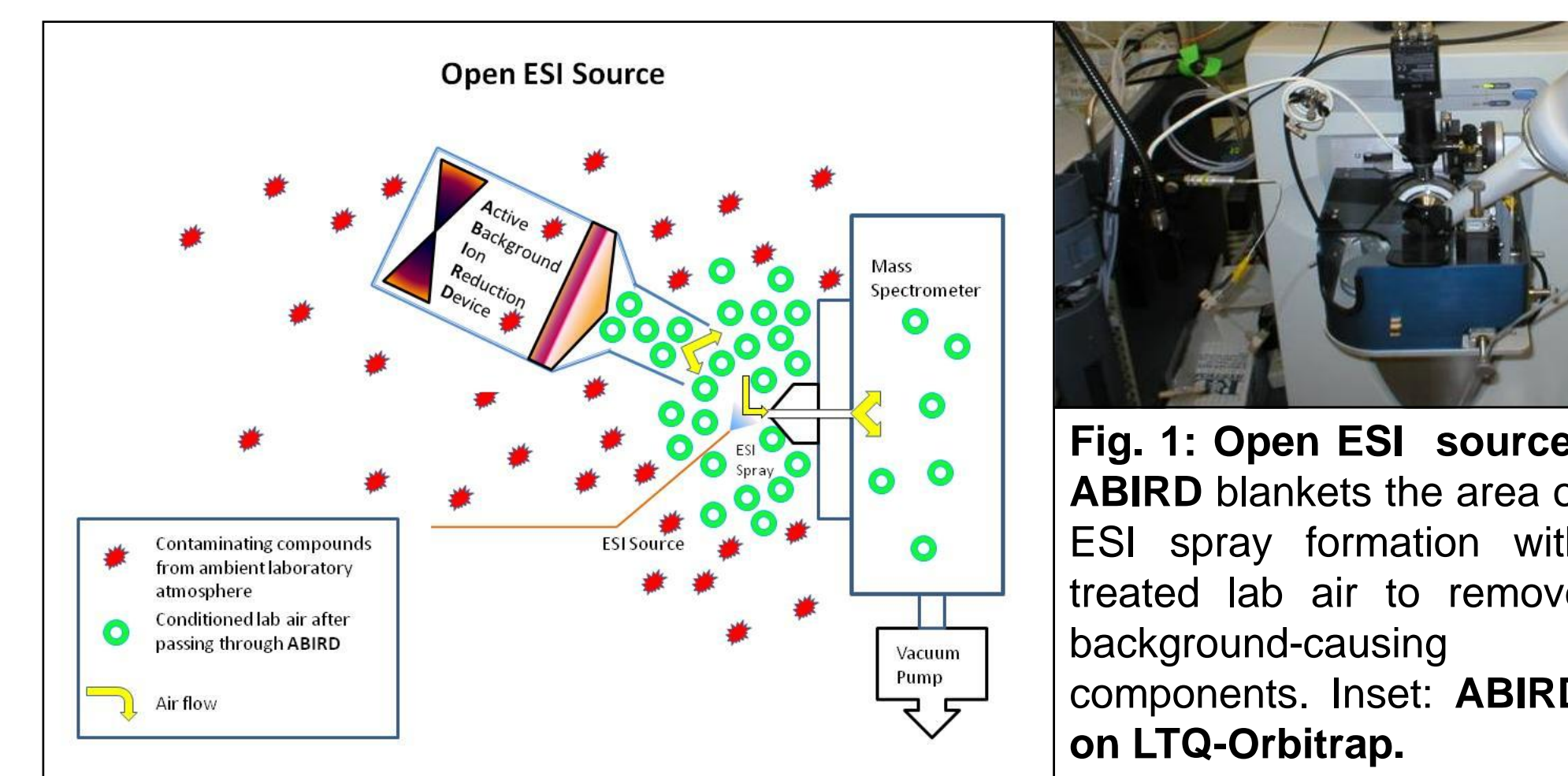


Fig. 1: Open ESI source: ABIRD blankets the area of ESI spray formation with treated lab air to remove background-causing components. Inset: ABIRD on LTQ-Orbitrap.

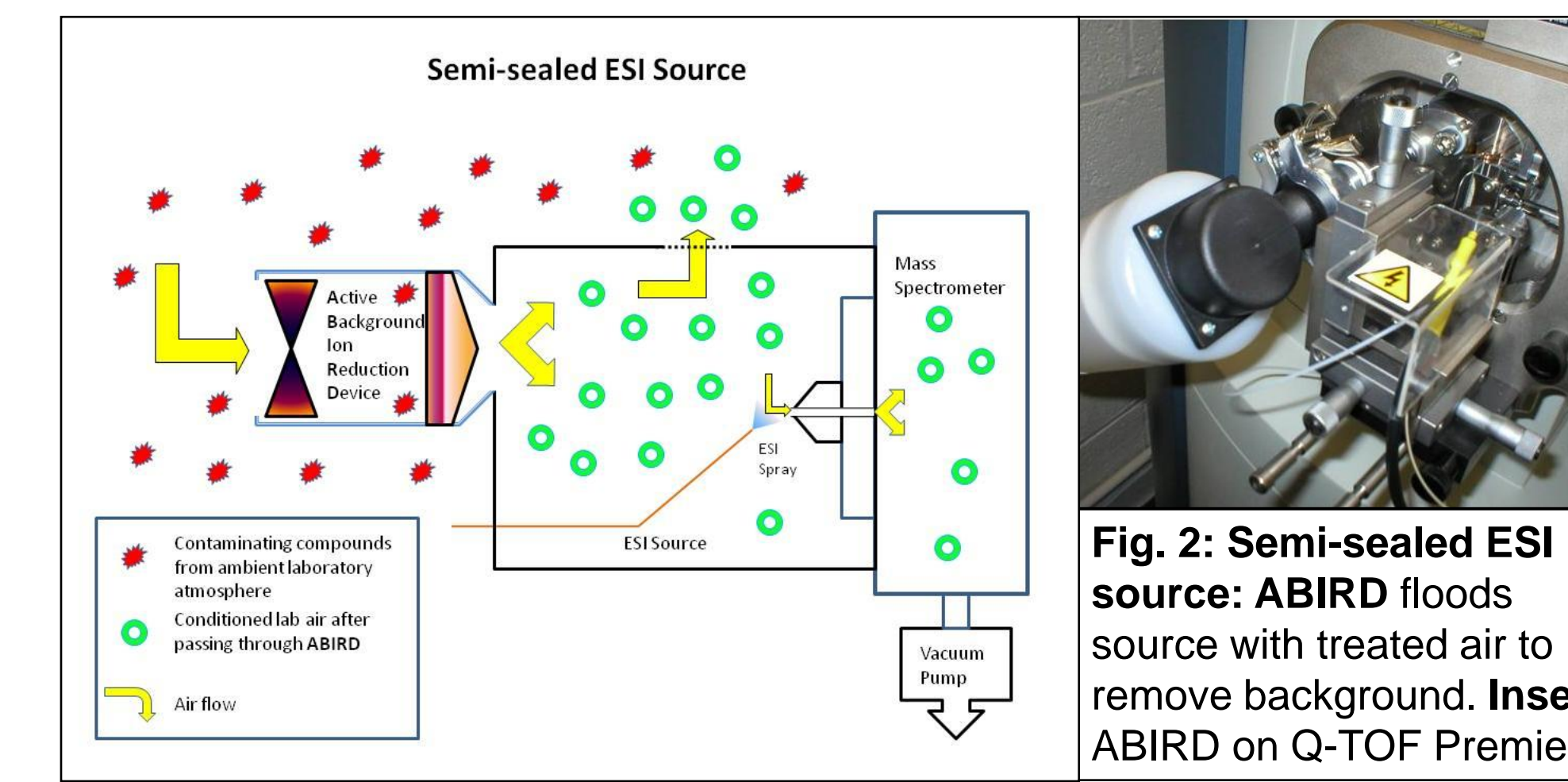


Fig. 2: Semi-sealed ESI source: ABIRD floods the source with treated air to remove background. Inset: ABIRD on Q-TOF Premier.

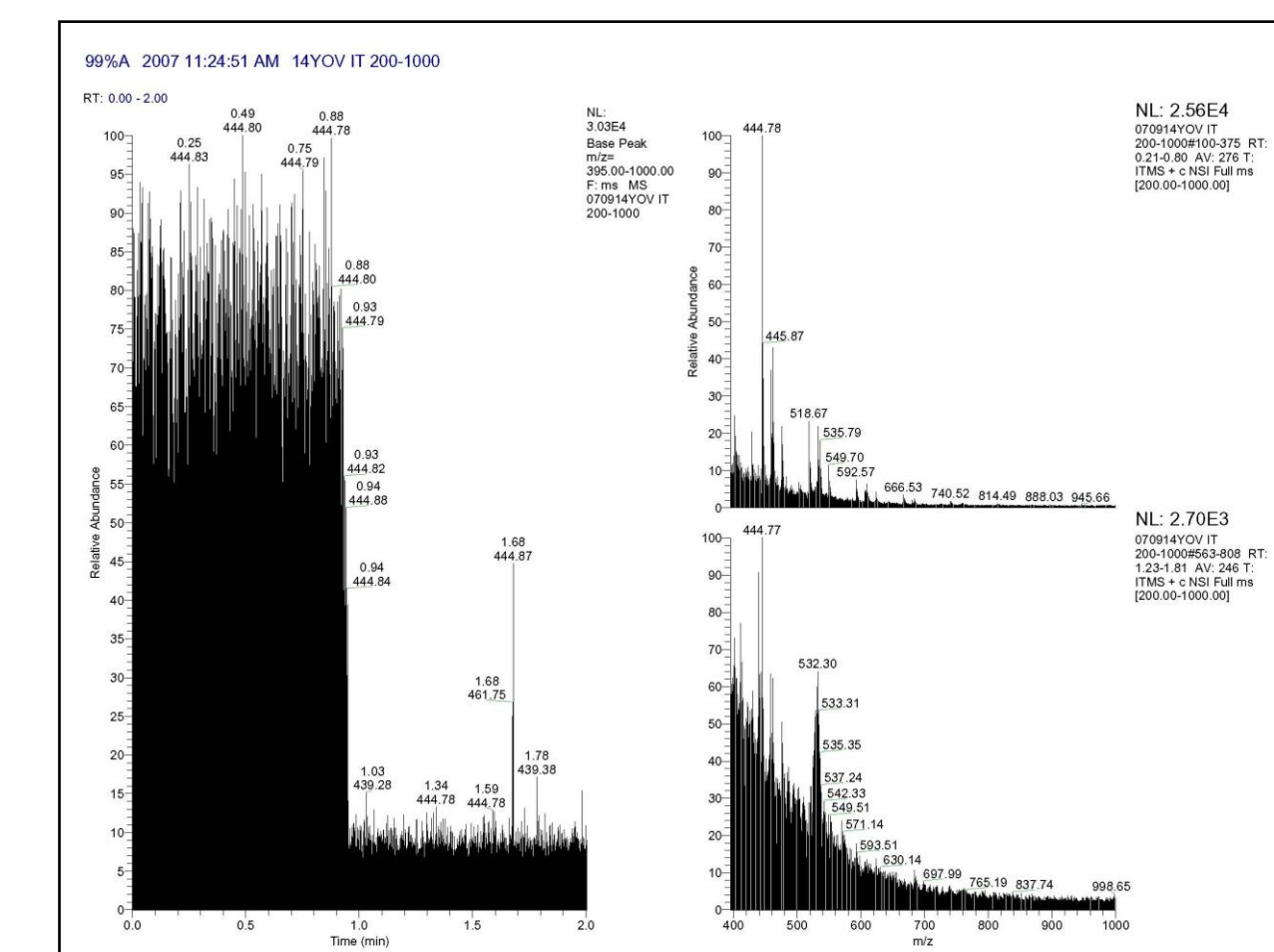


Figure 3: Nanospray background comparisons with ABIRD on LTQ. The summed spectrum show ~10x lower background ion signal observed with the device in operation.

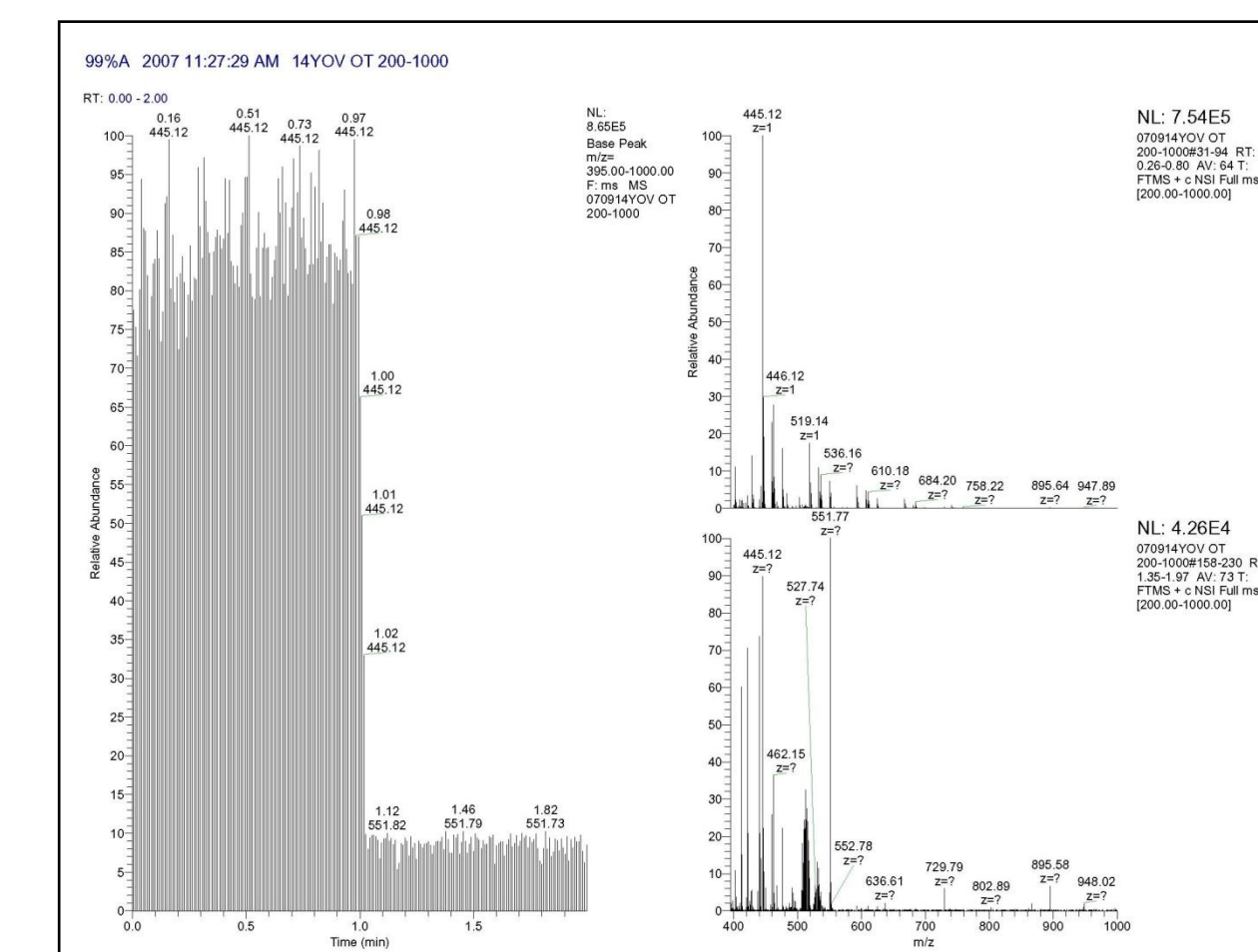


Figure 4: Nanospray background comparisons with ABIRD on LTQ-Orbitrap. The summed spectrum show ~20x lower background ion signal observed with the device in operation.

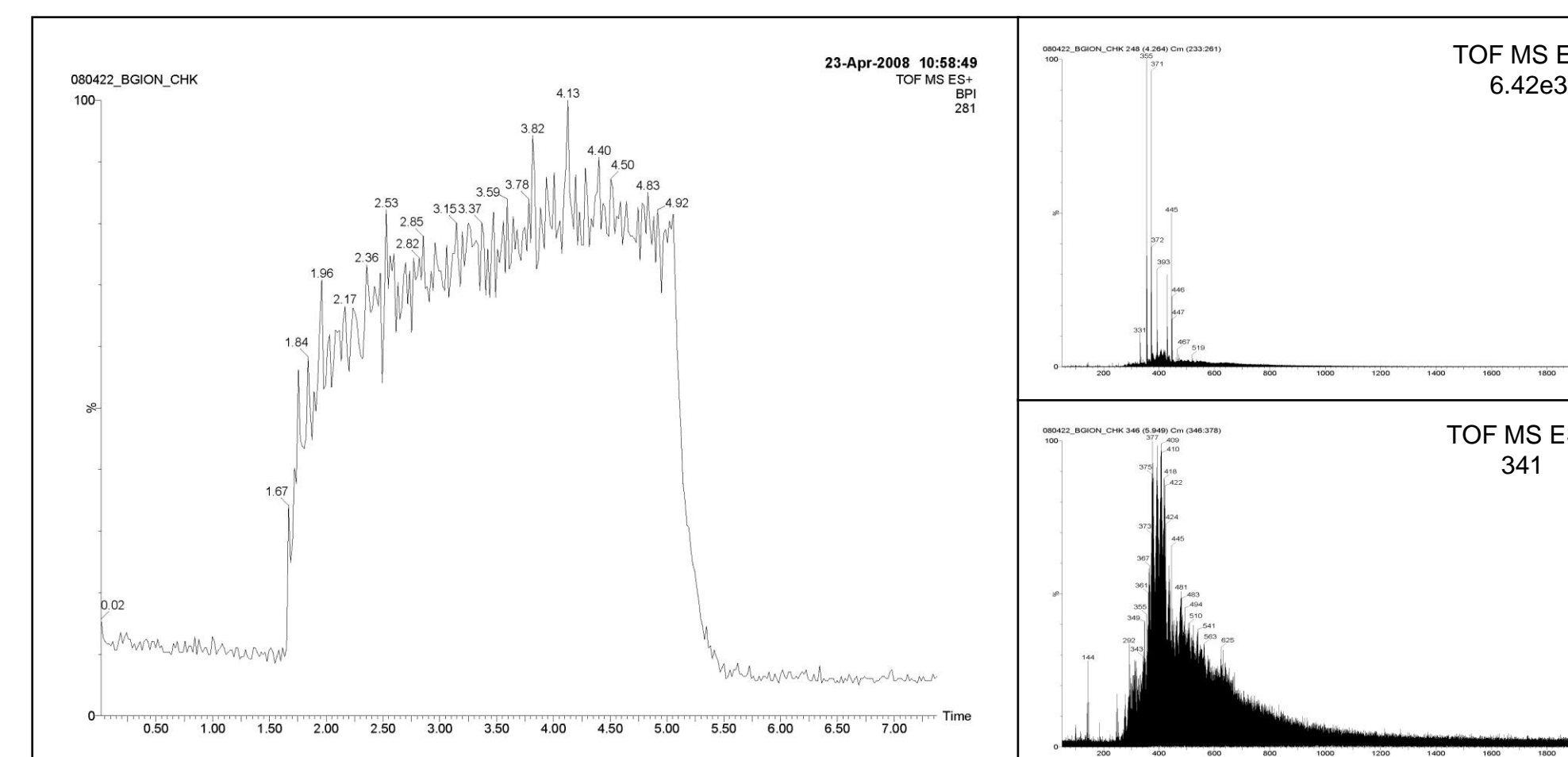


Figure 5: Nanospray background comparisons with ABIRD on Q-TOF Premier. The summed spectrum show ~20x lower background ion signal observed with the device in operation.

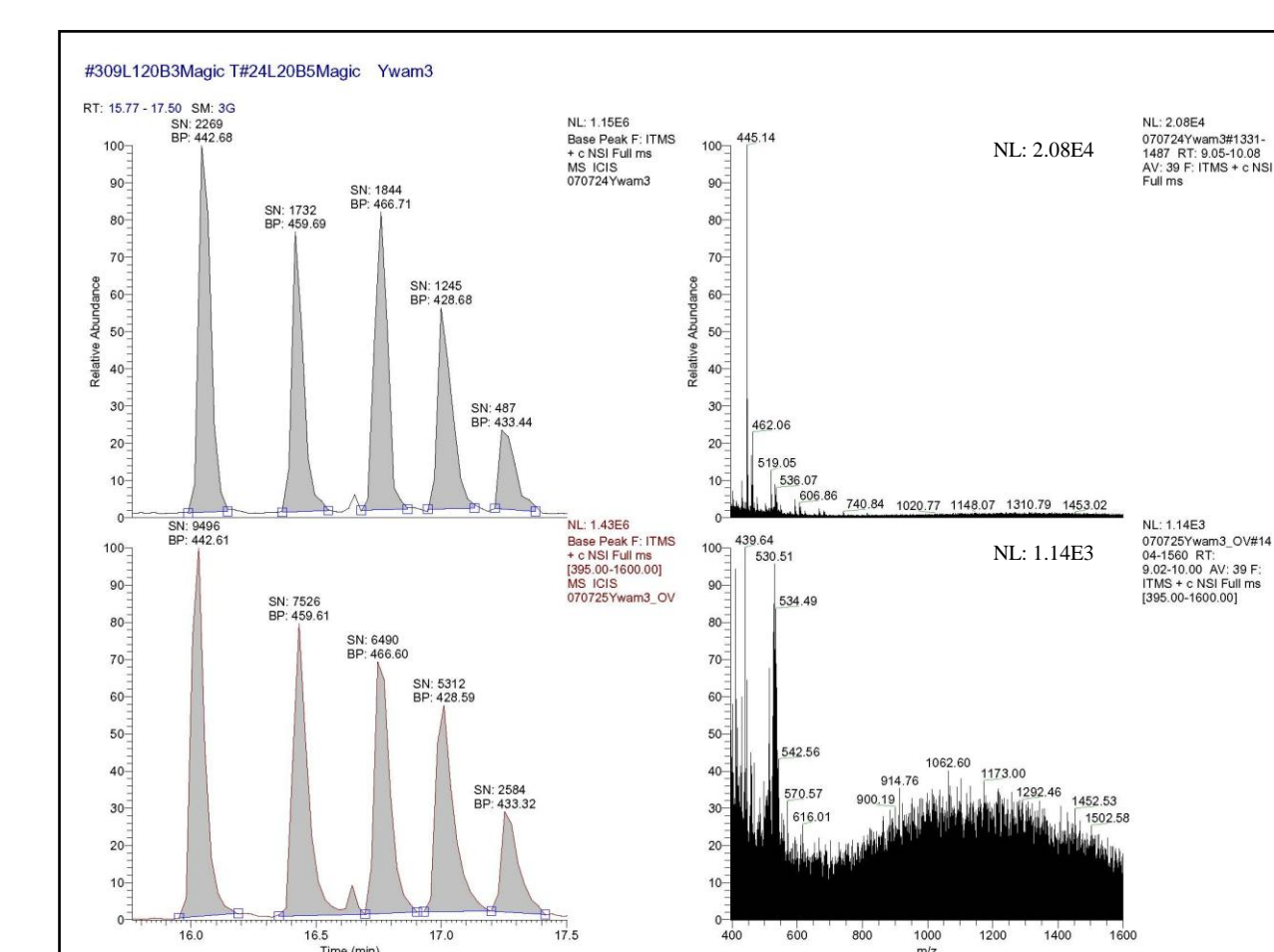


Figure 6: Chromatographic peak S/N ratio comparison on LTQ. Calculated S/N ratios improved ~4x. Mid-gradient summed spectra show a decrease in background ions ~20x.

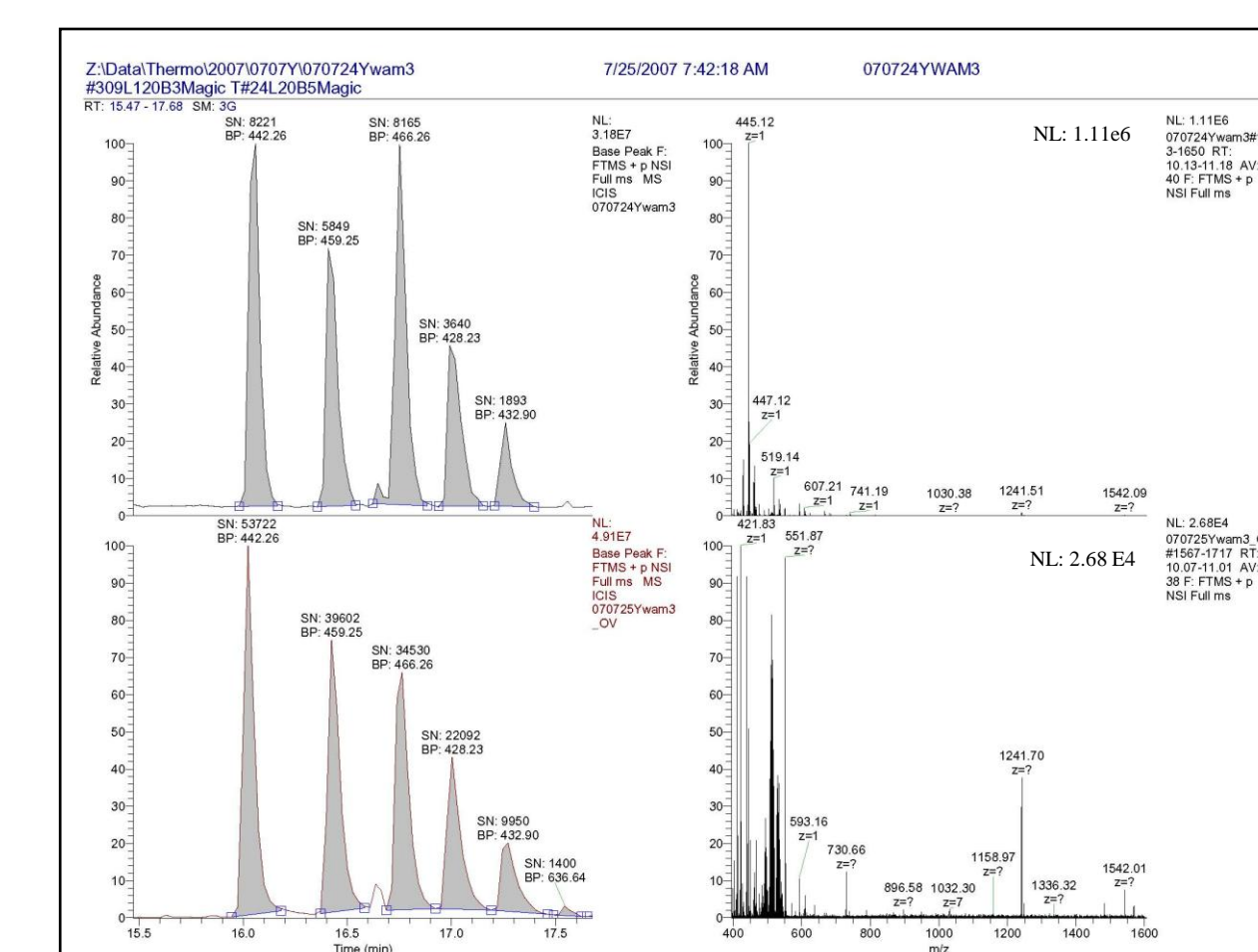


Figure 7: Chromatographic peak S/N ratio comparison, LTQ-Orbitrap. S/N improved ~5x. Summed spectra from the middle of the gradient show a decrease in background ions ~40x.

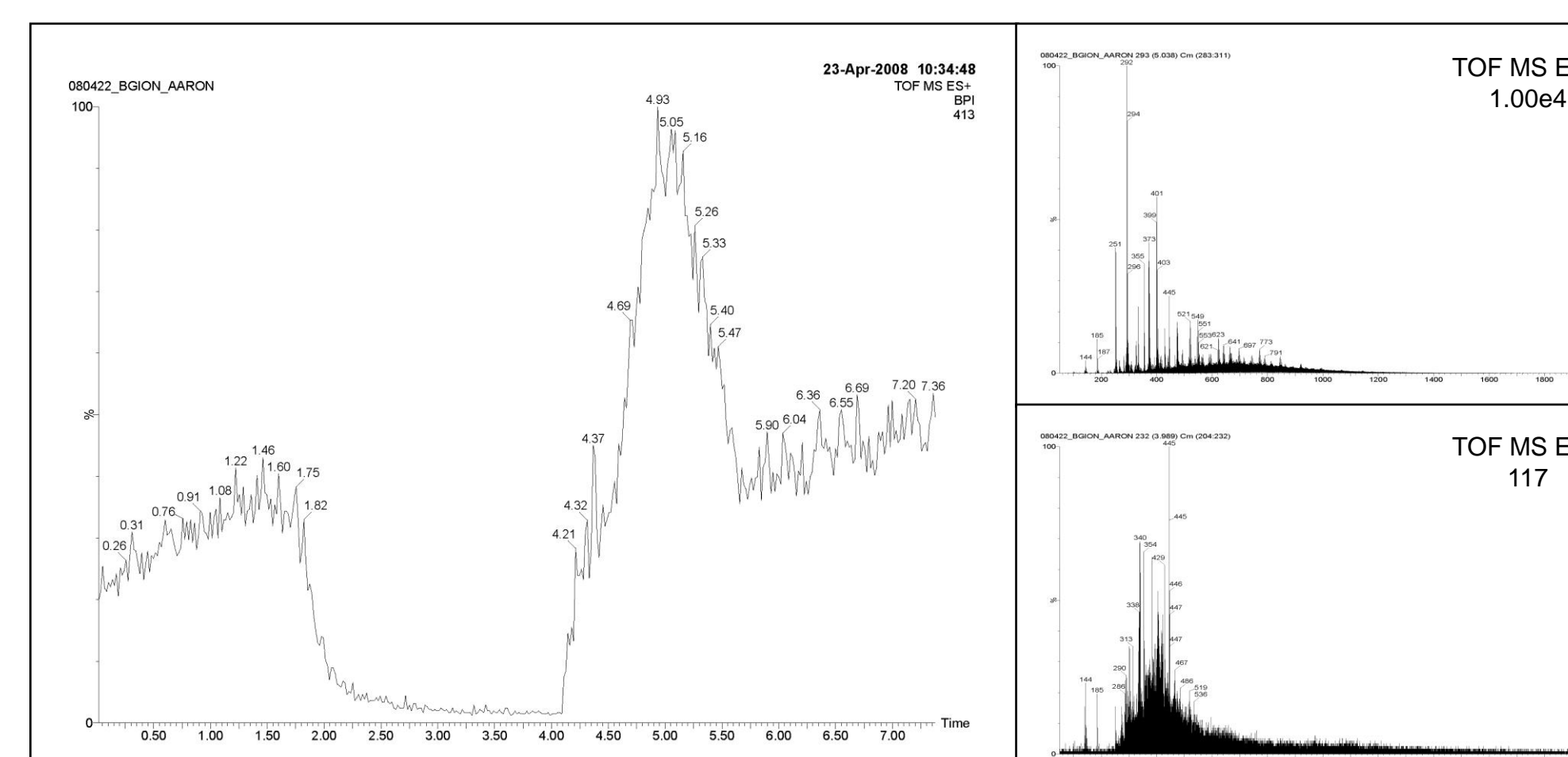


Figure 9: Isolation from transient volatile contamination on Q-TOF Premier: Background ion signal from a teenager, who was wearing a popular deodorant. Note the low mass ions in the spectrum which linger until the bored boy leaves area.

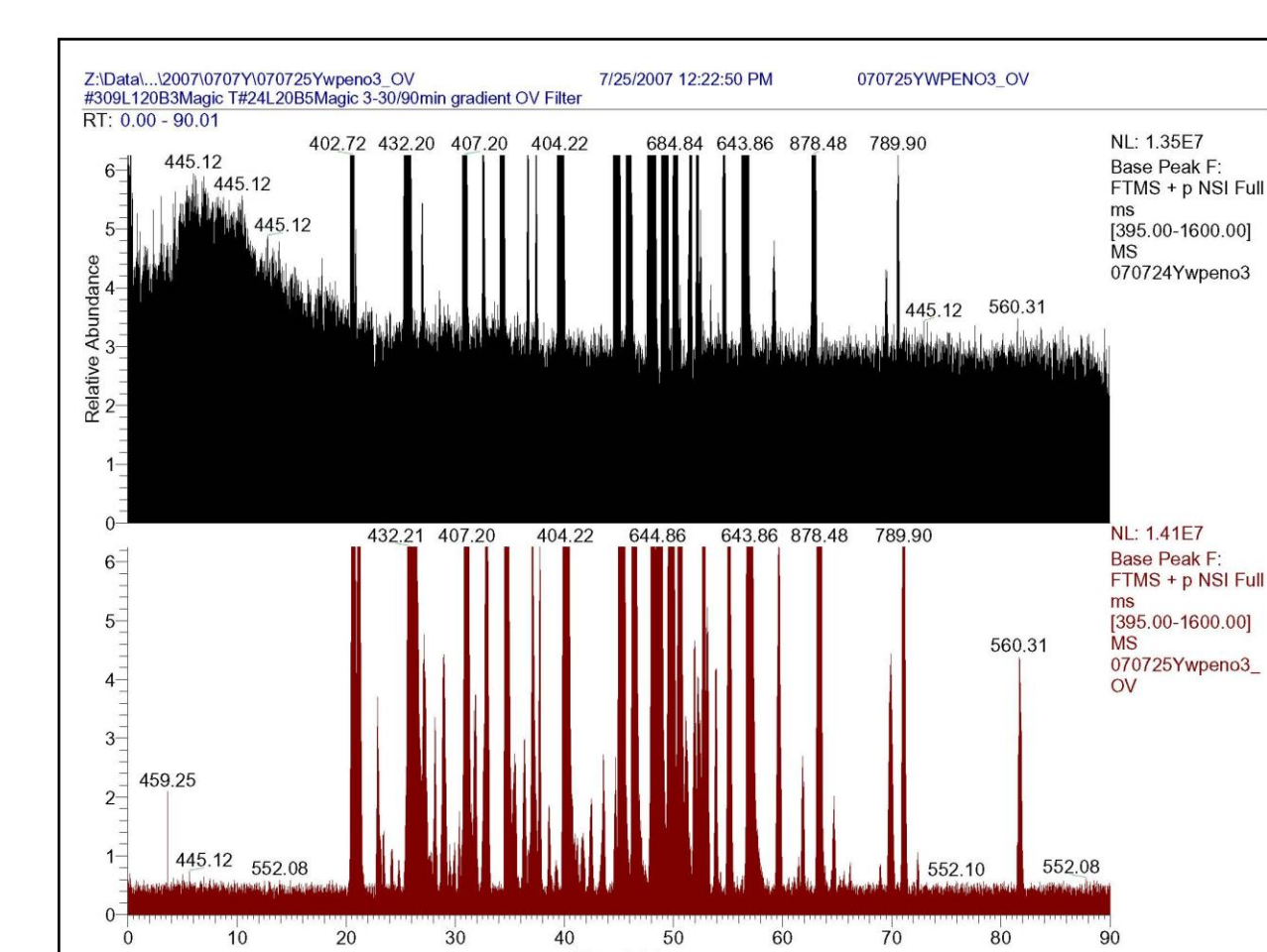


Figure 8: Chromatographic baseline stability and sensitivity on LTQ-Orbitrap. Magnification of the MS BPI chromatogram shows stable low level background ion signal afforded by **ABIRD.**

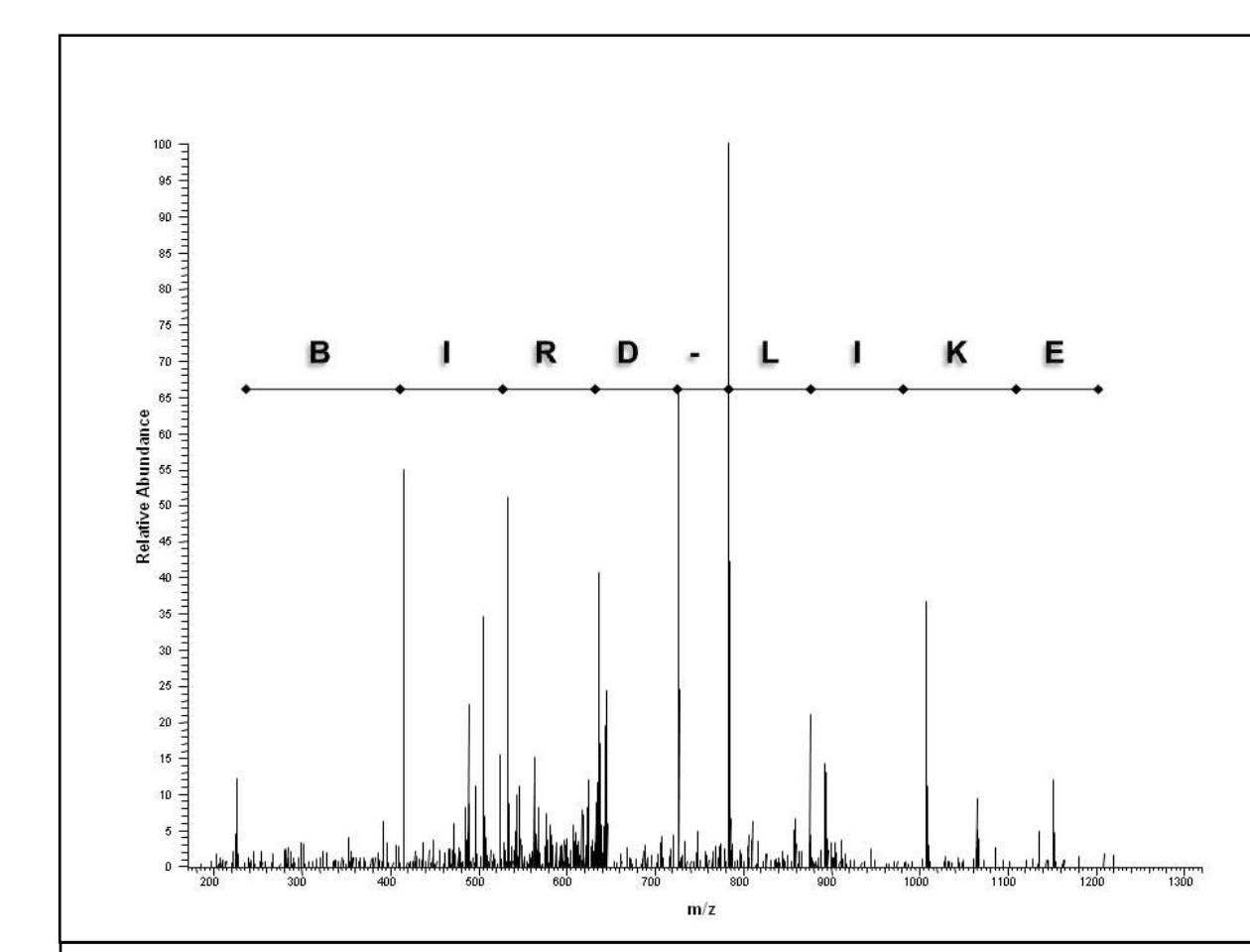


Figure 10: ABIRD has been used for high sensitivity analyses for one year. Installed on the LTQ-Orbitrap, this MSMS spectrum was obtained from ancient dinosaur bone extract. (Unpublished spectrum with example sequence.)

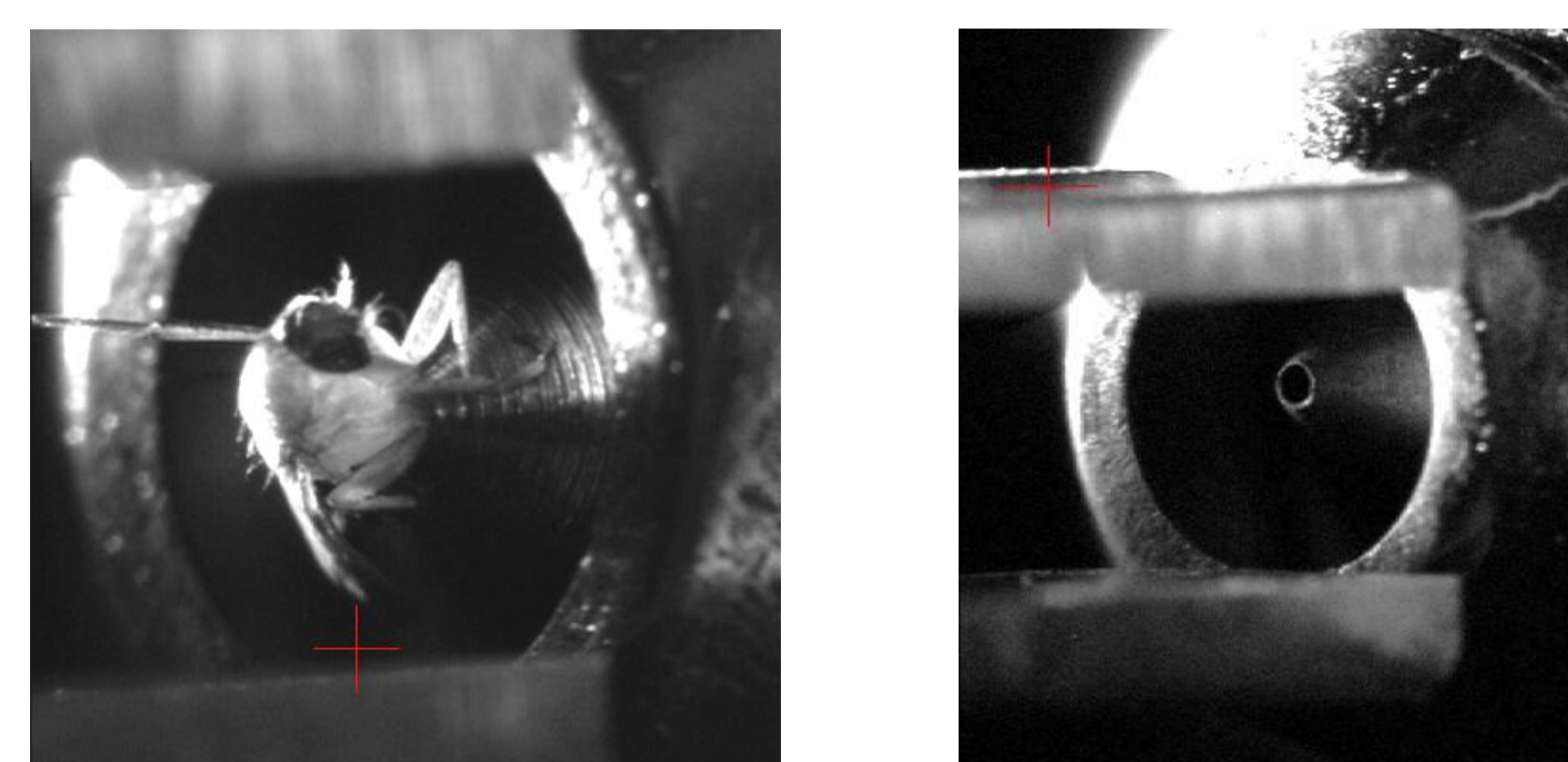


Figure 11: Particulate contamination. Left hand image: D. melanogaster became immobilized on the sample cone of the Q-TOF Premier. **ABIRD significantly reduces the openings in a source to prevent this situation from occurring. Right hand image: unobstructed sample cone.**

Results (continued)

Chromatographic baseline stability and sensitivity on an LTQ-Orbitrap

- 50 fmol enolase tryptic digestion standard injected on column.
- 3 - 30 %B / 90 minute gradient elution.
- One injection **ABIRD** off; repeat injection with **ABIRD** on.
- Full BPI chromatograms magnified to show baseline stability and sensitivity.
 - **RESULT: Chromatographic baseline is more stable and background ions reduced with the **ABIRD** on.**
 - **RESULT: Low level peaks are observed above baseline only with the **ABIRD** on. (Figure 8)**

Isolation from transient volatile contamination on a Q-TOF Premier

- Full MS scan acquired over several minutes at initial equilibration conditions.
- **ABIRD** turned on then off again.
- Significant transient contamination from teenage boy near the instrument observed until bored teenager left the room.
 - **RESULT: The instrument is protected from transient changes in the lab environment while the device is on. (Figure 9)**

ABIRD configured source in a semi-sealed configuration less prone to particulate contamination

- Prior to installation of **ABIRD**, the open source allowed easy access to the inlet sample cone.
 - **RESULT: With **ABIRD** installed the source is more protected from physical contamination of all types. (Figure 10)**

ABIRD has been used for high sensitivity analyses for nearly a year.

- A sample from multi-million year old dinosaur bone has been successfully analyzed.
 - **RESULT: **ABIRD** can be used for all analysis types, and is especially helpful at very high sensitivity. (Figure 11)**

Conclusions

The Active Background Ion Reduction Device (**ABIRD**) has been shown to provide:

- **Reduced background ions**
- **Improved signal to noise ratio**
- **Insulation from unpredictable sources of ambient contamination**
- **No loss of peptide signal**

References

Volkmer-Engert R., Schlosser A. Volatile polydimethylcyclisiloxanes in ambient laboratory air identified as source of extreme background signals in nano-electrospray mass spectrometry. *J. Mass Spectrom.* 2003; **38**:523-525

Acknowledgements

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