

A Novel Method for Interfacing Capillary Electrochromatography to Mass Spectrometry

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1. Introduction

Capillary electrochromatography (CEC) is often described as a hybrid of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC), utilizing electroosmotic flow to drive mobile phase through the capillary as in CE, and packing the capillary with stationary phase particles similar to those used in HPLC. CEC aims to incorporate the high separation efficiency of CE with the selectivity and sample capacity of HPLC.

Detection in CEC is most simply achieved by absorbance measurements, but the small internal diameter (ID) capillaries used (less than 100 μm) result in relatively poor concentration detection limits (although absolute detection limits are impressive). In this work an ion trap is used in conjunction with a time-of-flight mass spectrometer (TOF/MS). As analytes elute they are first desorbed with a pulsed infrared laser and then ionized using a pulsed ultraviolet laser. Ions are stored in the trap before ejection into the TOF/MS. The ion trap acts as an accumulation stage while the TOF/MS provides a fast pulsed mass analyzer.

2. Methods

Figure 1 shows an overall schematic of the instrument together with an illustration of the sample introduction interface. The CEC column is terminated in a grounded union with a narrower capillary that transfers eluent to the desorption point. The reduction in ID is employed to preserve temporal resolution [1]. The infrared desorption laser is focused on the terminus of the transfer capillary, which is located in the ring electrode of the ion trap, while the ultraviolet ionization laser passes through the center of the trap.

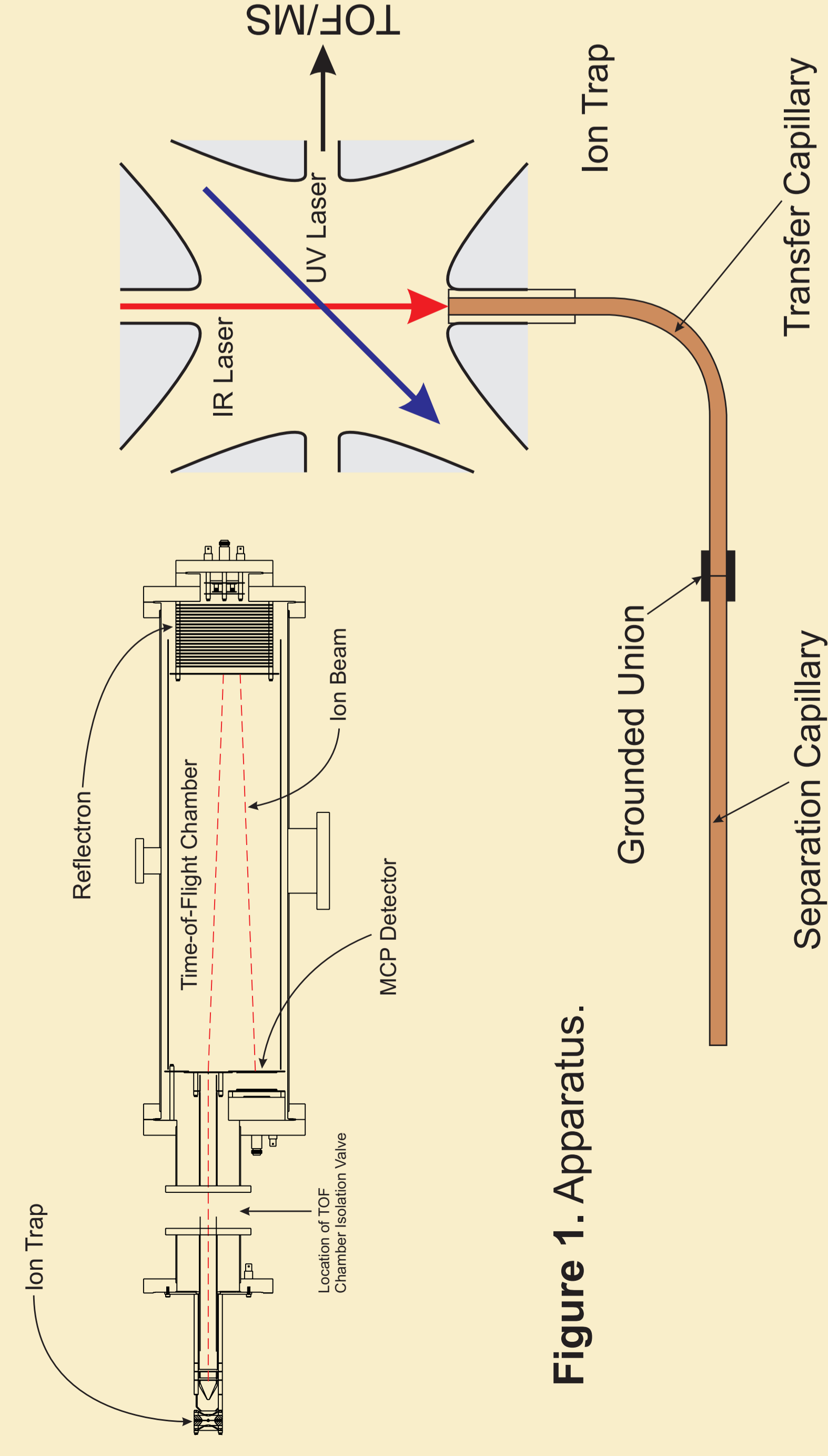


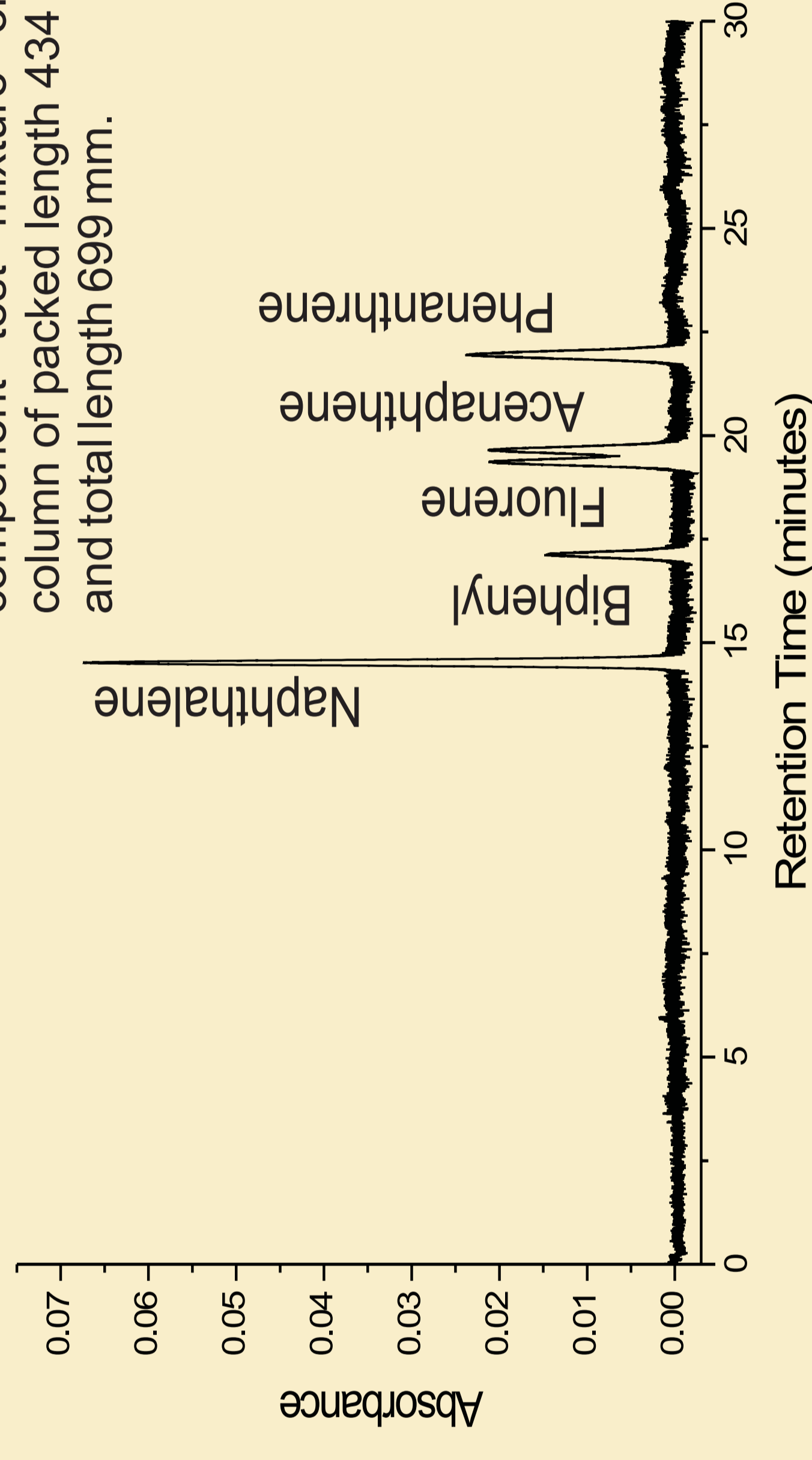
Figure 1. Apparatus.

The chromatographic mobile phase used in all cases was 25% 50 mM ammonium acetate adjusted to pH 6.0 with acetic acid, 75% acetonitrile. Injection was by application of 5 kV for 5 seconds while 30 kV was applied during separations. Absorbance detection was performed at 214 nm. All CEC columns had an ID of 100 μm and were packed [2] in our laboratory with Hypersil 3 μm mixed mode stationary phase particles. A Waters Capillary Ion Analyzer was used to provide the high potentials and absorbance detection. The chromatographic test mixture consisted of naphthalene, biphenyl, fluorene, acenaphthene and phenanthrene, each at 5 mM in acetonitrile.

The desorption laser (1064 nm) was a Continuum Minilite Nd:YAG operating at fundamental wavelength while the ionization laser (266 nm) was a quadrupled Quanta Brilliant Nd:YAG. Laser pulses were separated by a delay of 25 μs and the repetition rate of the experiment was 10 Hz. Mass spectra were recorded using a Lecroy 9350M digital oscilloscope. The oscilloscope averaged ten spectra and then transferred this data to a PC. Collected data could then be processed to produce selected ion chromatograms.

3. Results

Figure 2. CEC separation of a five component test mixture on a column of packed length 434 mm and total length 699 mm.



The chromatogram resulting from the separation of the test mixture by CEC using absorbance detection is shown in Figure 2. Mobile phase volumetric flow rate was approximately 200 nL/min. The separation efficiency for naphthalene can be expressed as 130,000 theoretical plates per meter. The injection volume was calculated to be in the region of 2 nL, meaning that approximately 10 picomoles of material were injected. Absolute detection limits for this system were in the region of 100 femtomoles. Figure 3 shows CEC/MS data for the same test mixture. In this case the separation efficiency for the naphthalene peak is 100,000. It can clearly be seen that the use of a transfer capillary has not significantly degraded the separation.

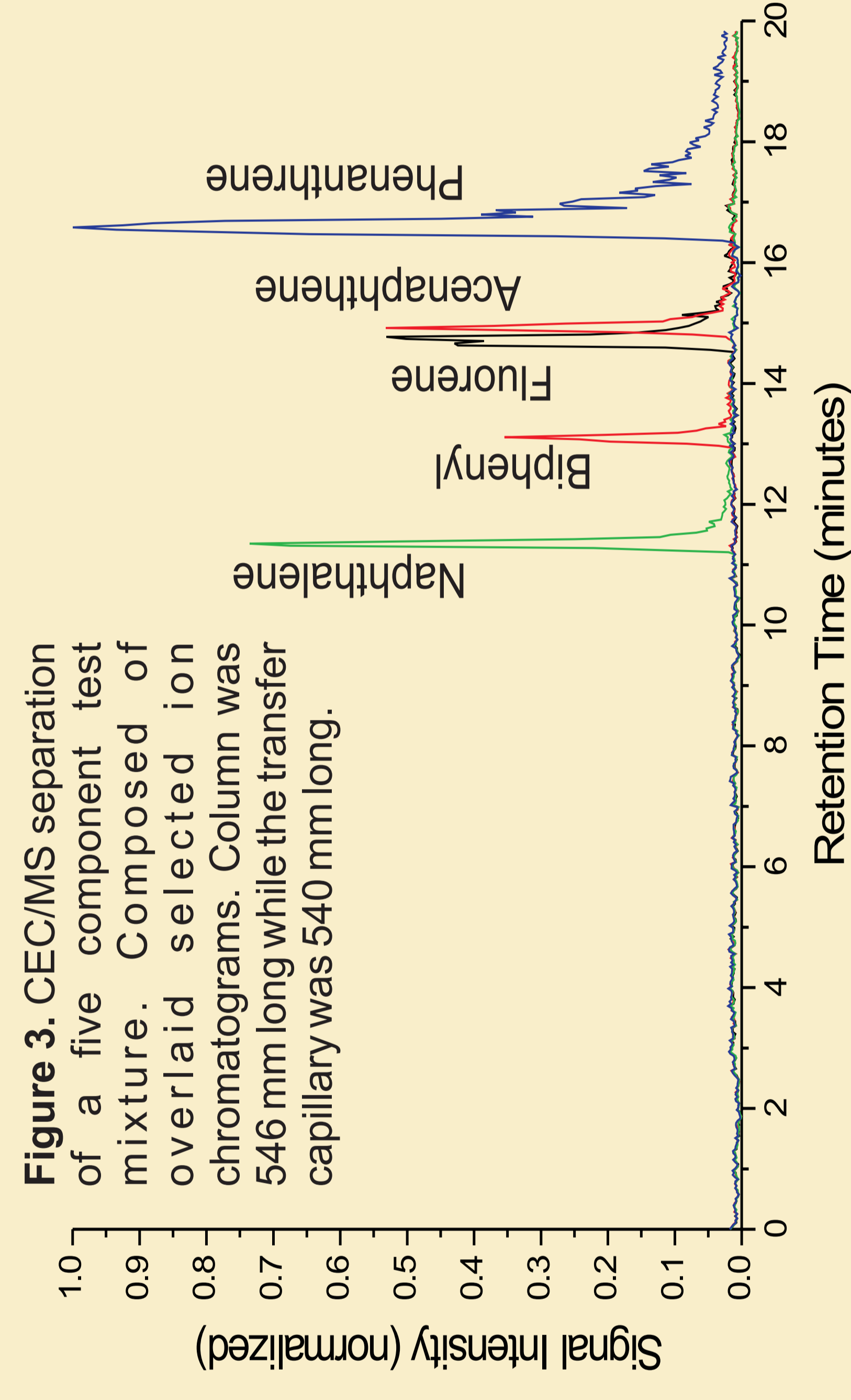


Figure 3. CEC/MS separation of a five component test mixture. Composed of overlaid selected ion chromatograms. Column was 546 mm long while the transfer capillary was 540 mm long.

For instrument optimization and characterization the CEC column and transfer capillary were replaced with a long narrow capillary. Solutions were drawn through this capillary into the vacuum chamber at a similar rate to that for CEC separations in order to provide a continuous analyte source.

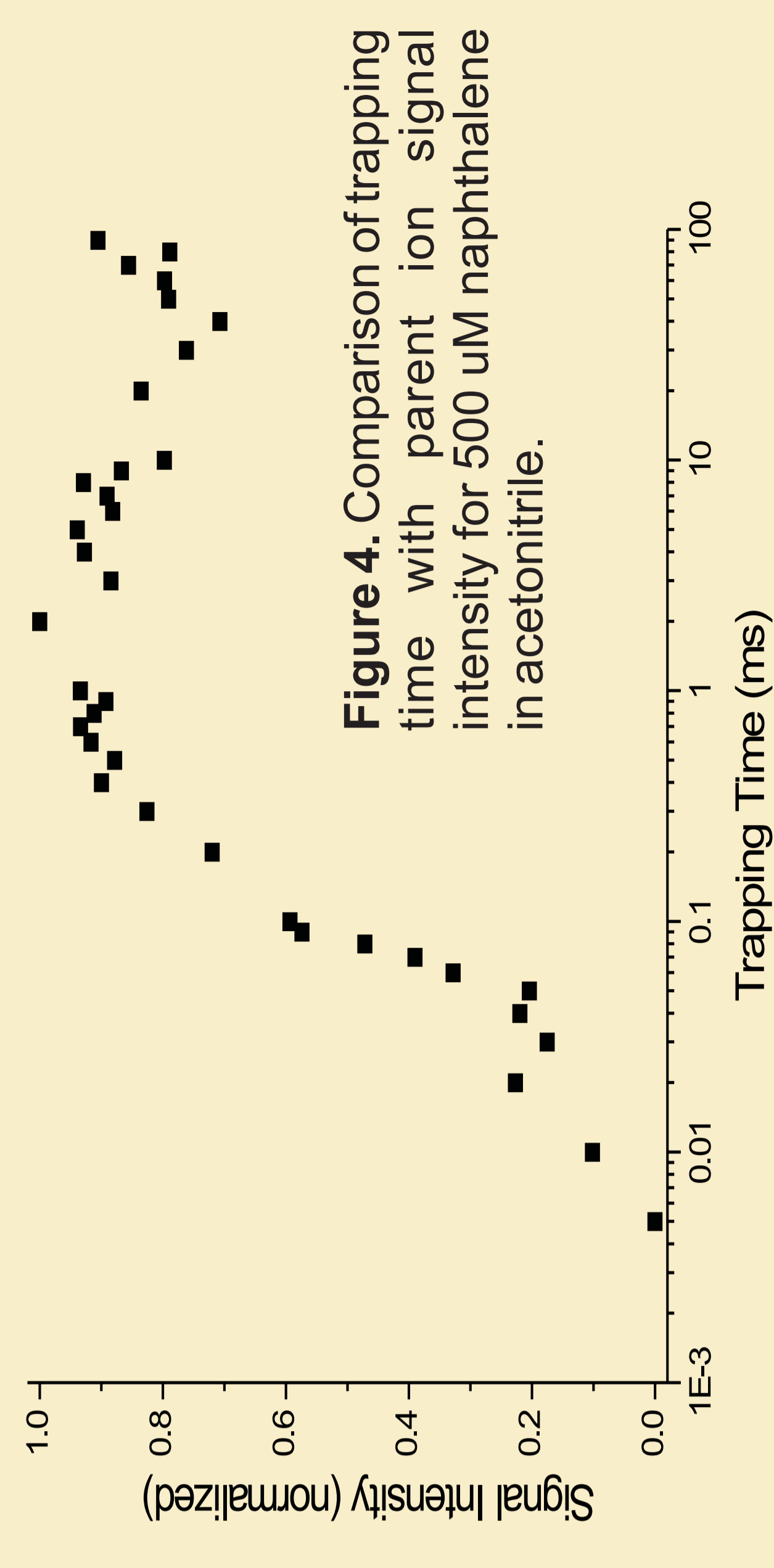


Figure 4. Comparison of trapping time with parent ion signal intensity for 500 μM naphthalene in acetonitrile.

Figure 4 illustrates the effect of ion trapping time, showing that it is necessary to wait a short time before ejection of ions into the TOF/MS. At low concentrations this time lengthens, hence a trapping time of 10 ms was used for all chromatographic studies. When trapping times were increased to over one desorption ionisation cycle (i.e. over 100 ms) there was a slight improvement at low concentrations. Figure 5 shows that it is possible to detect 100 nM solutions of naphthalene in acetonitrile. The rate of entry of naphthalene solution into the instrument was 20 femtomoles/minute, giving single femtomole detection limits.

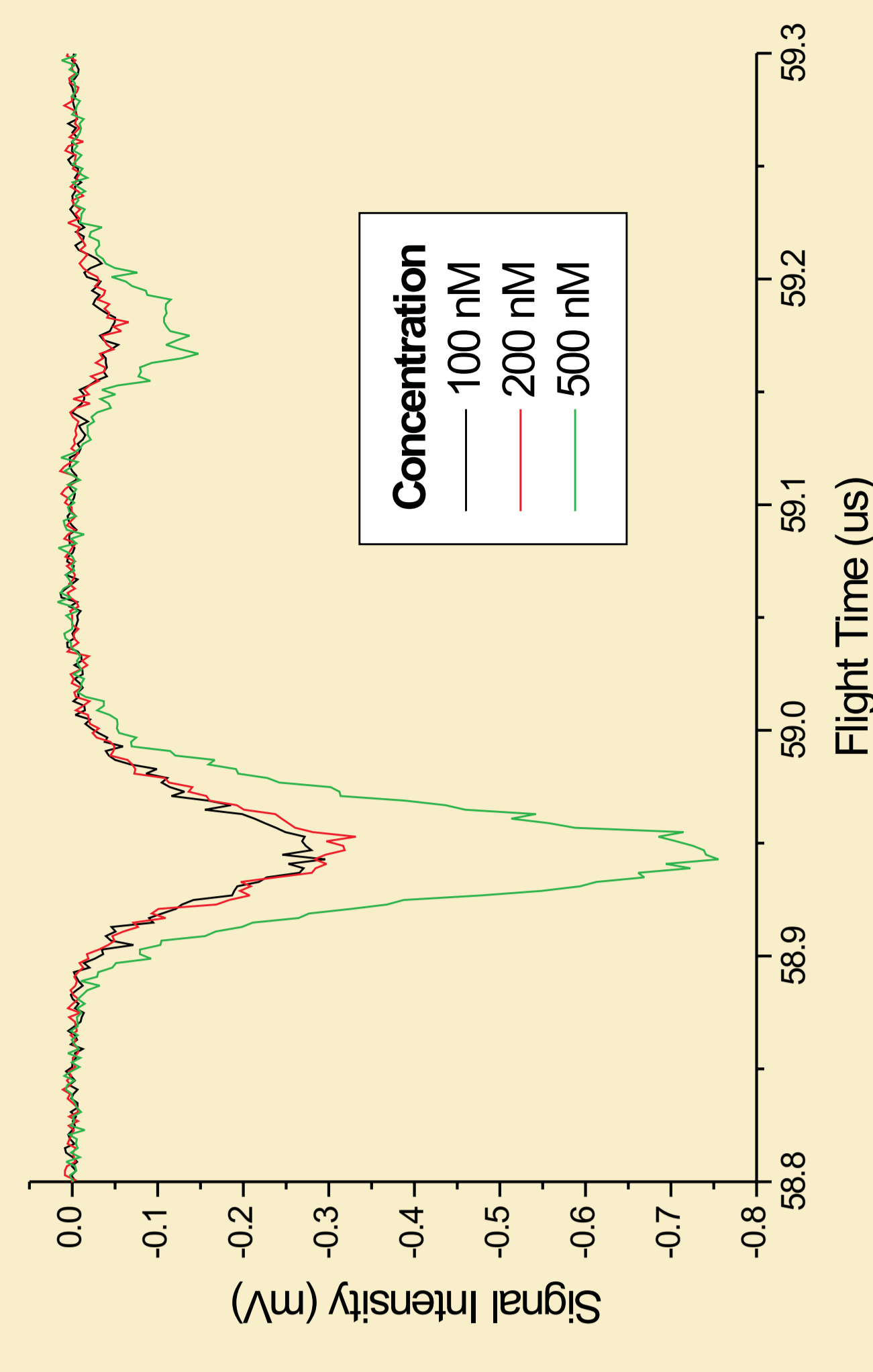


Figure 5. Parent ion peak for naphthalene. Comparison of concentration with signal obtained.

4. Conclusion

The use of this mass spectrometer offers a two order of magnitude increase in sensitivity over absorbance detection.

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5. References

- [1] Boughtflower et al., J. Chromatogr. A, 2000, 887, 409
- [2] Boughtflower et al., Chromatographia, 1995, 41, 398