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Signatures of Aspirated Charged Droplets in Single Spectra Intensity Distributions in commercial LC-ESI-MS Systems

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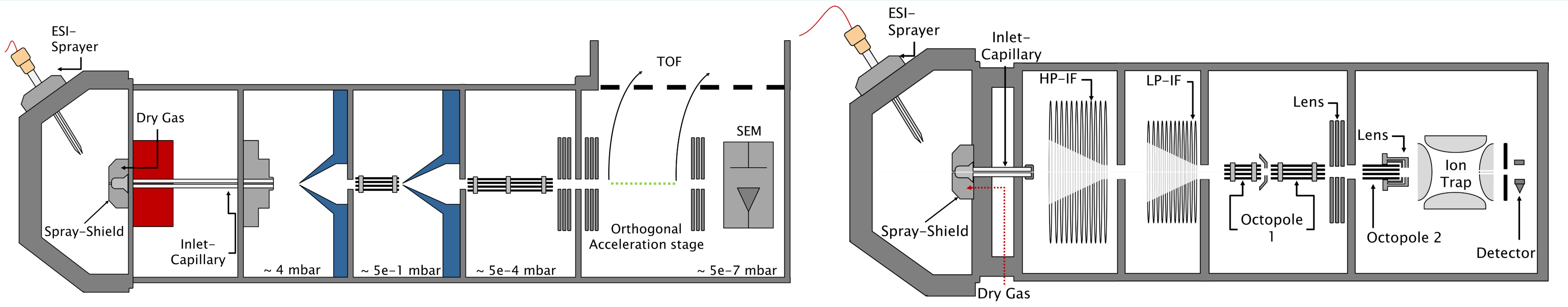
Introduction

Electrospray ionization (ESI) is the most important atmospheric pressure ionization (API) method today, partly due to its characteristic of soft ionization [1]. However, the dynamics of the spray and ionization process is not yet fully understood. Several experimental setups have demonstrated that the use of ESI results in the formation of charged droplets which are aspirated into mass spectrometers (MS). They can pass through the individual vacuum stages of the instruments, causing a variety of adverse consequences [2,3]. Intense bursts of ion signal have been detected in the intensity distribution of non-summed mass spectra. These bursts are limited to ESI: Further experiments with other API methods, without the transfer from the liquid phase, do not yield comparable results. A detailed investigation of recorded individual spectra on various commercially available mass spectrometry devices which considers the impact of different solvent systems provides more information about the underlying mechanism.

Instrumentation



(A) Bruker micrOTOF

(B) Bruker amaZon ETD

Fig. 1 Schematic layout of the utilized mass spectrometers

Method

1. Analyte
- Reserpine, ≥99.0 % HPLC (Sigma Aldrich)

2. LC-System
- Merck Hitachi
  - Binary Pump, L-7200
  - Binary Pump, L-600A

3. Solvents
- Acetonitrile, ≥ 99.8 % HPLC (Fisher scientific)
- Methanol, ≥ 97 % HPLC (Fisher scientific)
- Formic acid, ≥ 98 % puriss. p.a. (ACS reagent)

Solvent Dependency with micrOTOF

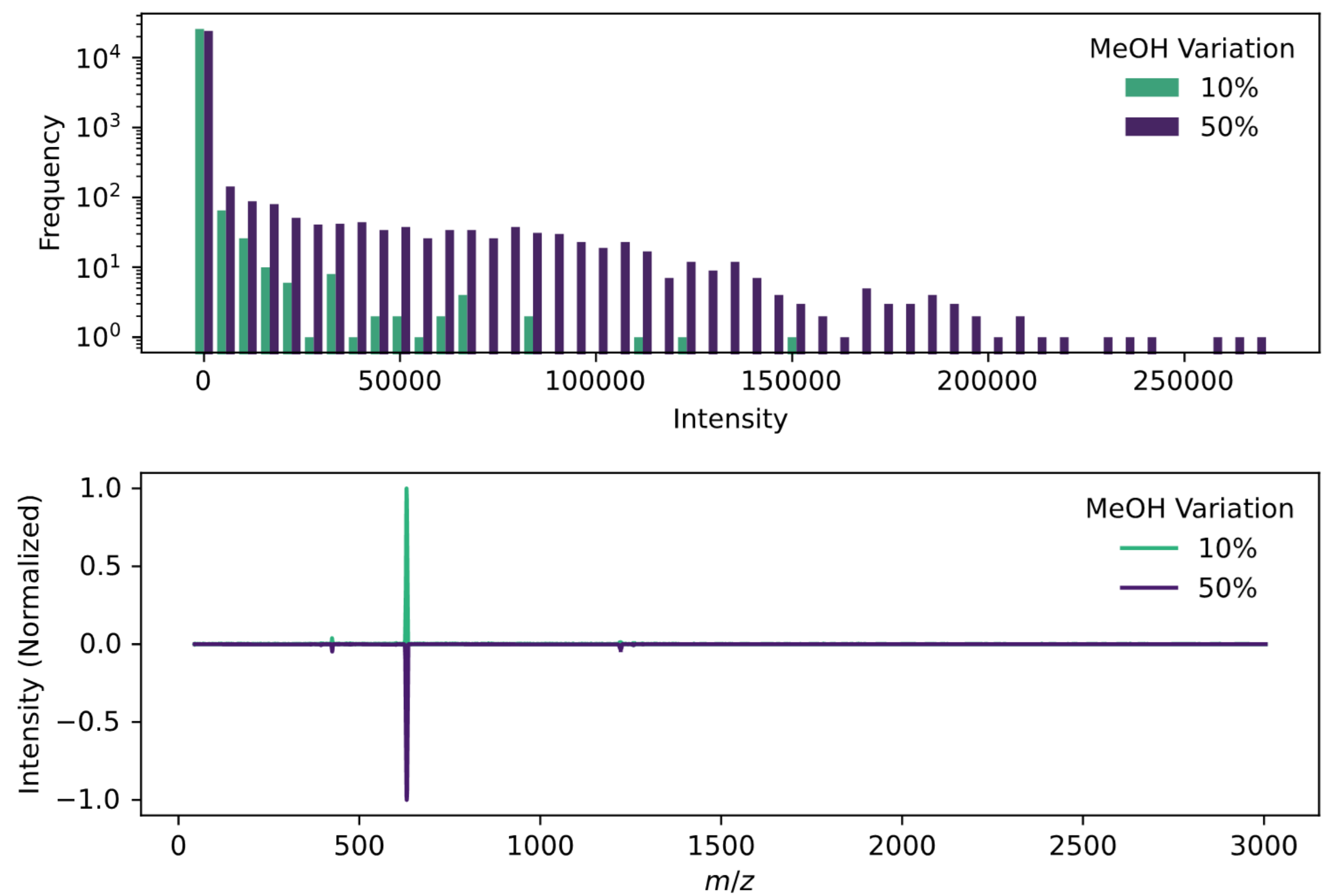


Fig. 2 Intensity distribution and summed mass spectra of single spectra scans made with the micrOTOF (H_2O:MeOH:FA, c(Reserpine) = $8 \cdot 10^{-6}$ M)

Fig. 2 shows a dependence of the signal intensity distribution on the methanol ratio in the solvent mixture. Occasional, very intensive „burst“ spectra are visible with both methanol ratios. Burst spectra frequency and average intensity increases dramatically with methanol ratio.

The histogram in **Fig. 3** shows the intensity distribution of non-summed spectra with respect to the organic solvent under constant conditions. The intensity range is comparable with both solvents, but methanol produces approximately an order of magnitude more burst spectra. There are notably more mass signals in the summed spectra with ACN.

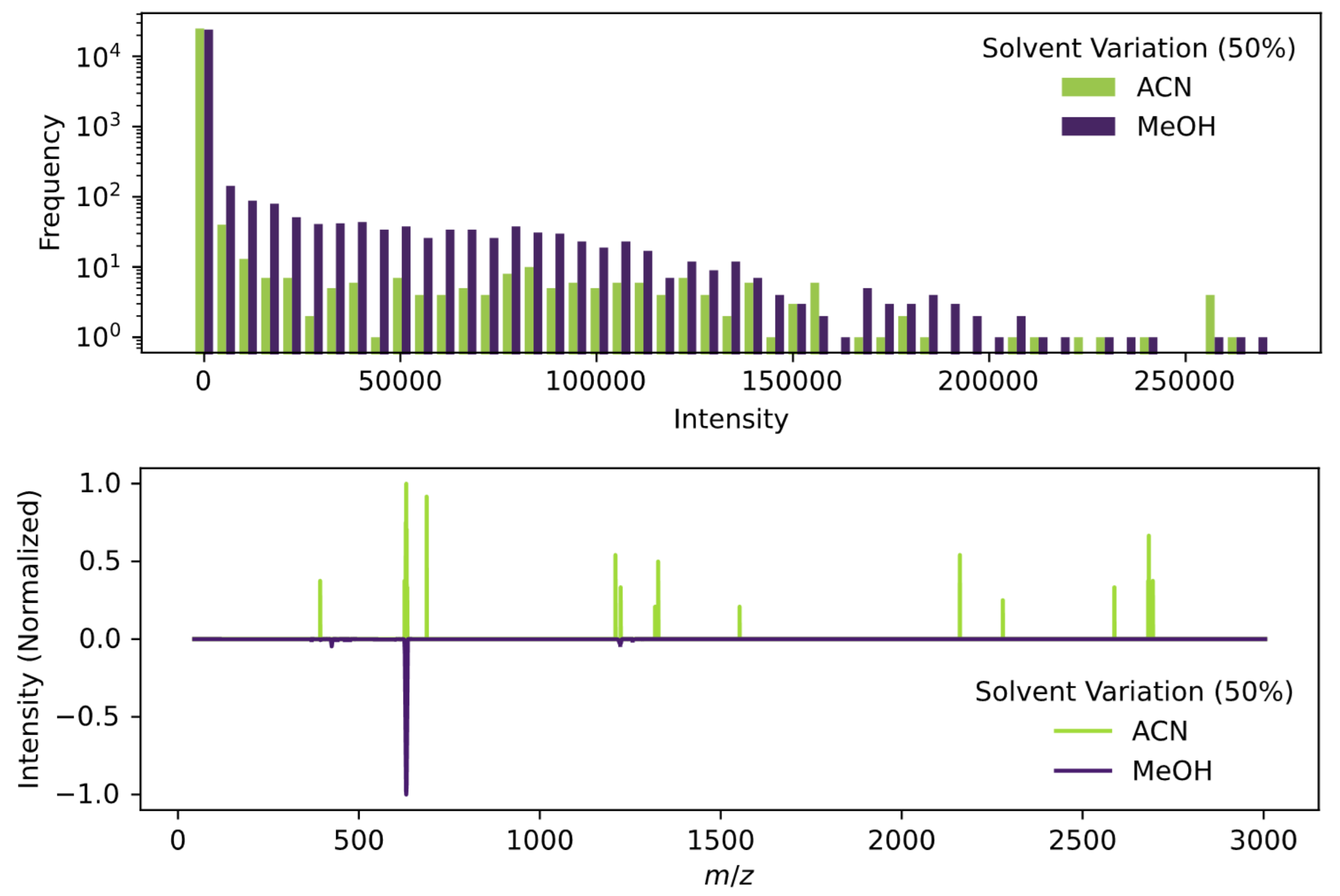


Fig. 3 Intensity distribution and summed mass spectra of single spectra scans made with the micrOTOF (H_2O:MeOH/ACN:FA, c(Reserpine) = $8 \cdot 10^{-6}$ M)

Non-summed Spectra Intensity Distribution

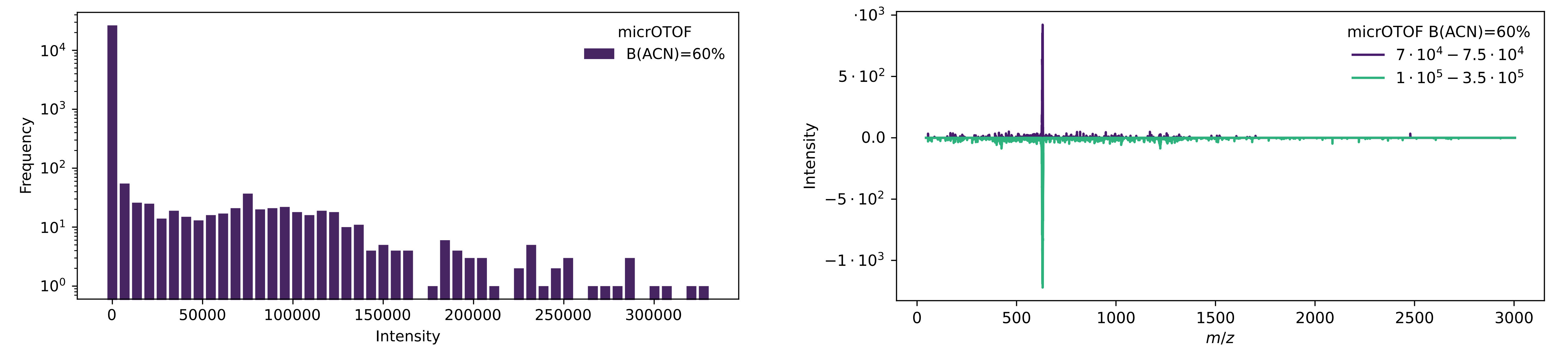


Fig. 4 Single spectrum intensity distribution with the micrOTOF with ACN-Water (left): A large number of the single spectra are entirely empty while a small fraction has very intensity. Right panel: Summed spectra of the very intense individual spectra (intens. 1 - 3.5 · 10^5) and the less intense spectra (intens. 7 - 7.5 · 10^4). The spectra are very similar, with subtle differences in the low intensity mass peaks.

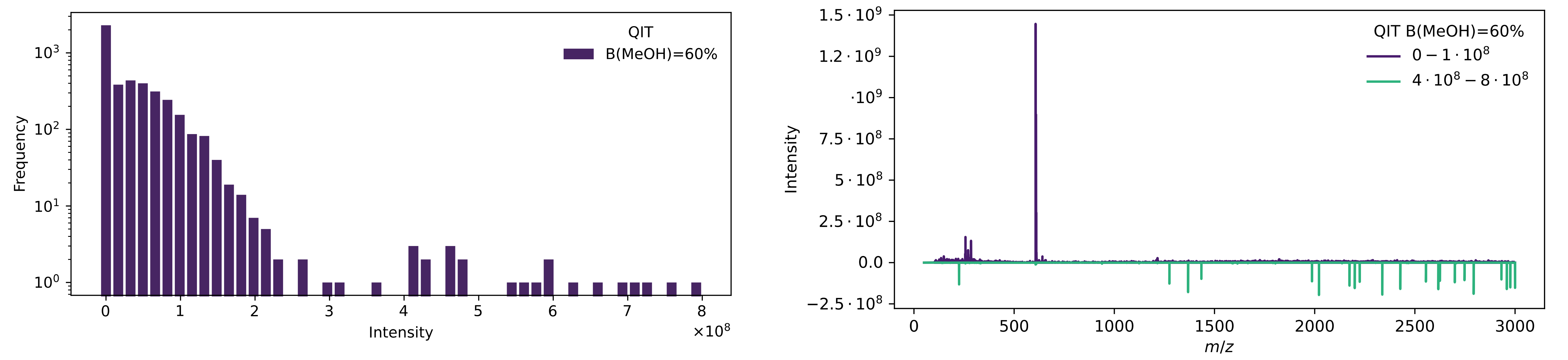


Fig. 5 Single spectrum intensity distribution with the QIT with methanol-water (left). Right panel: Summed spectra of the of the high intensity (intens. 4 – 8 · 10^8) and low intensity mass spectra (< 1 · 10^8). Remarkably, the high intensity single spectra produce an entirely different sum mass spectrum than the low intensity single spectra.

Nebulizer Pressure Variation

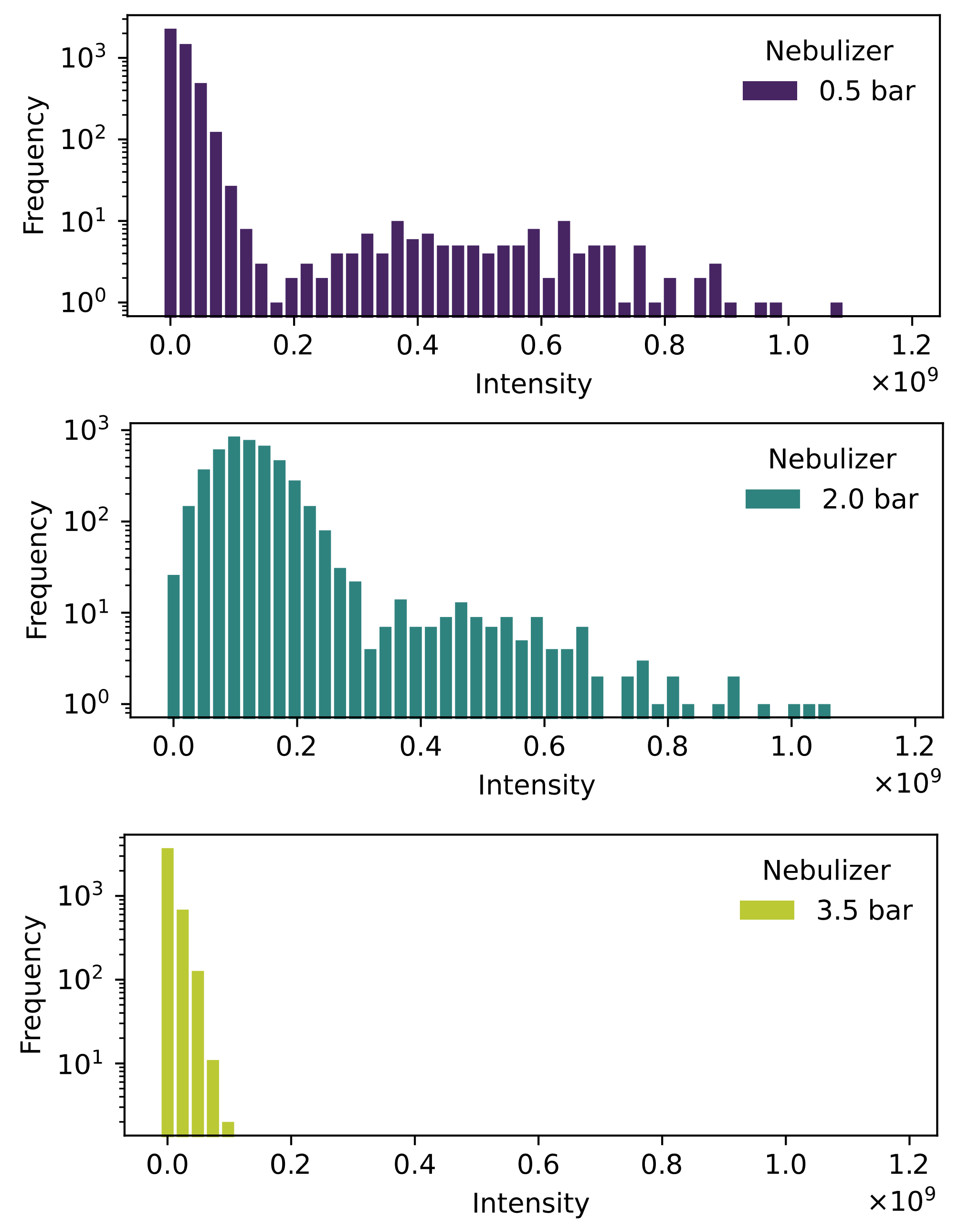


Fig. 6 Single spectrum intensity distribution with the QIT with 1:1 water methanol solvent mixture with the addition of 0.1 % FA, with variation of the nebulizer gas flow rate. The Nebulizer flow significantly changes the intensity distribution.

Conclusion

- Intense spectra which are related to the ion bursts can be detected in various MS-systems
- Inlet-parameters such as the nebulizer pressure and the solvent mixture have noticeable impact
- Intensity distribution changes with solvent mixture (solvent types and organic ratio) (Fig. 2 – 3)
- Distribution shifts to an approximate normal function by increasing the nebulizer pressure (Fig.6)
  - Reduction of the number of empty spectra and high intensity mass spectra
- In some experiments, the summation of single spectra with a high intensity yields a very different mass spectrum than those with low total intensity (Fig. 4 - 5)

Reference

[1] Cole, R. B. (2010) Electrospray and MALDI Mass Spectrometry. Fundamentals, Instrumentation, Practicalities, and Biological Applications, 2nd ed.; John Wiley & Sons Incorporated: Hoboken

[2] Markert, C.; Thinius, M.; Lehmann, L.; Heintz, C.; Stappert, F.; Wissdorf, W.; Kersten, H.; Benter, T.; Schneider, B. B.; Covey, T. R. Observation of charged droplets from electrospray ionization (ESI) plumes in API mass spectrometers, *Analytical and bioanalytical chemistry.* **2021**, *413,* pp. 5587–5600

[3] Kang, Y.; Schneider, B. B.; Covey, T. R. On the Nature of Mass Spectrometer Analyzer Contamination, *Journal of the American Society for Mass Spectrometry.* **2017**, *28,* pp. 2384–2392