



Experimental Investigation of Charged Nanodroplets in MS Transfer Stages originating from ESI plumes



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Introduction

The most frequently used ionization method at atmospheric pressure in modern mass spectrometry is the Electrospray Ionization (ESI) method although the mechanism is not fully understood. Upon spraying the analyte solution charged droplets are generated, which eventually release the bare ions into the gas phase. It is assumed that just these ions or at most small ion bound solvent clusters containing analyte ions pass the MS-inlet and reach the high vacuum region. This is to be ensured by using counter gas flows and/or other measures at the MS-inlet.

However, current publications suggest that highly charged nanodroplets pass the MS-inlet and reach the mass analyzer, where they constitute contamination issues and thus cause a deterioration in performance of the entire system [1].

By specifically stimulating these often non-detectable nanodroplets, they can disintegrate and thereby release detectable species. Even mass resolved signals of analyte ions are detected, which excludes that the observed effect is due to random noise.

During an experimental investigation of several MS-systems with different inlets (e.g., skimmer systems or capillaries) the general occurrence of this phenomenon is demonstrated. It follows that the genesis of nanodroplets in the ESI-process must be considered. The nanodroplet effects are influenced by varying the ESI conditions such as the spray voltage and/or the source temperature.

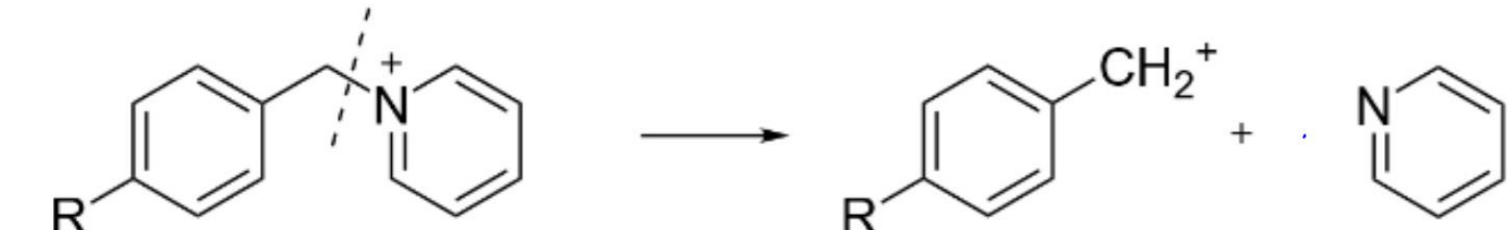
Methods

Instruments:

- 6500 Triple Quad™ with Turbo V™ in ESI-mode (SCIEX)
Inlet: skimmer system
- amaZon ETD Ion Trap with Apollo ESI source (Bruker Daltonics)
Inlet: capillary and funnel system

Chemicals:

- Mixture of five *para*-substituted benzylpyridinium salts in acetonitrile/water (1:1)
- Synthesized from respective benzylpyridinium bromide derivate and pyridine (both from Sigma-Aldrich without further purifications)
- Group of substances with clearly defined fragmentation (fragment spectra and energy)



R = CH₃, CN, Cl, F, NO₂

By manipulating the firmware of the 6500 Triple Quad™ the first quadrupole (Q1, RF-only) can be used as a high-pass filter for the mass range exceeding 1100 Da (cut-off of lower m/z-ratios). Activation in Q2 and subsequent mass resolved detection in Q3 results in spectra with high intensities *below* the Q1 cut-off (see fig. 2). In addition to the typical analyte signals (blue) and their fragment signals (red), an increase of the baseline is detected in the high mass range, which moves like a wave to lower m/z-ratios with increasing collision energy (CE). The presence of fragmentable highly charged nanodroplets in the MS-system is proposed to explain these observations.

With the amaZon ETD ion Trap, the observation can be reproduced. Setting an isolation window between 2450 Da and 2550 Da (apparently only baseline noise in the isolation range) *and* rising the trapping time, releases analyte ions (blue area, see fig. 1). Note the change of the isolated "peak".

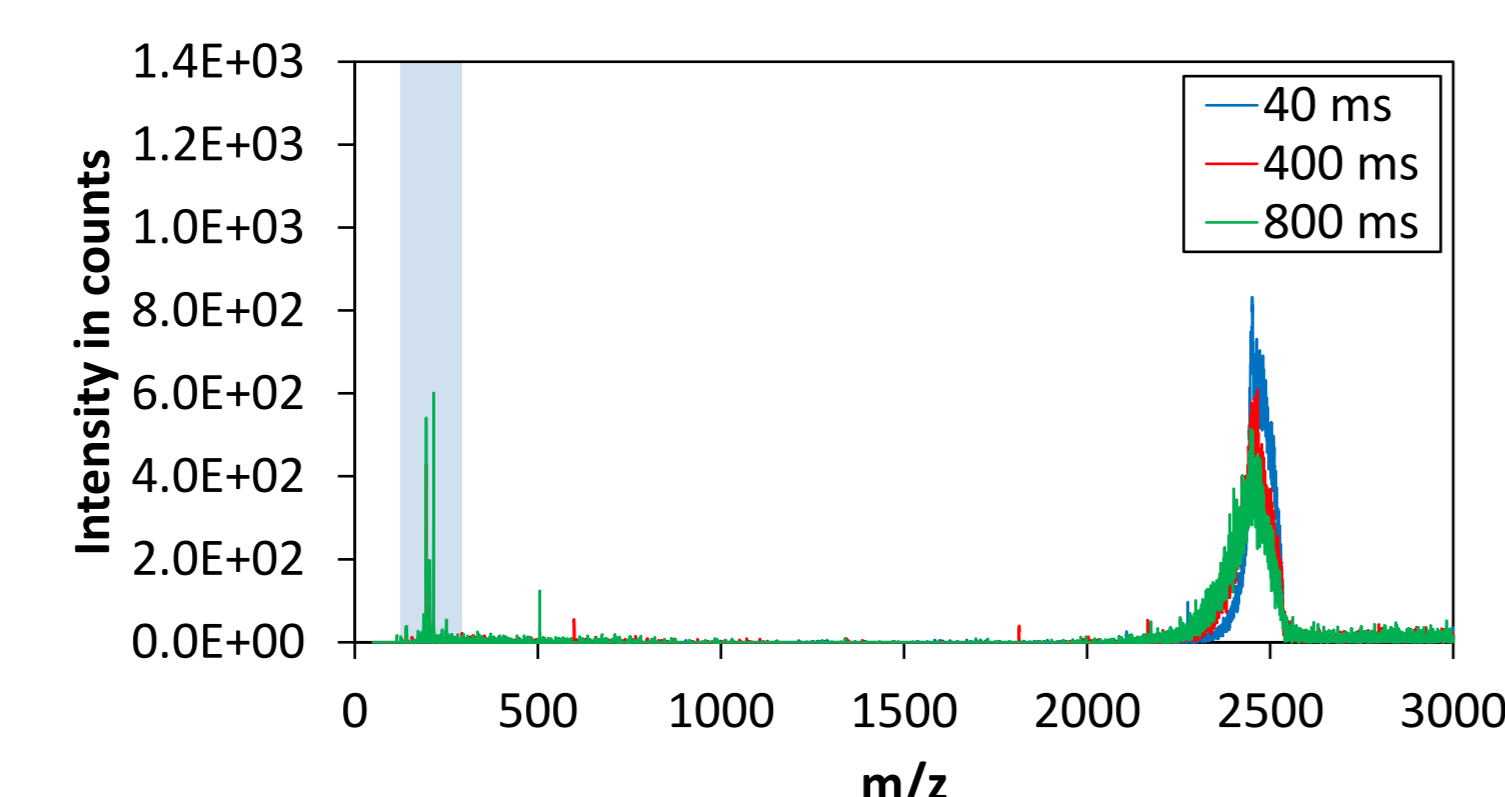


Figure 1: Mass spectra (amaZon ETD Ion Trap) of a trapped m/z-ratio of 2500 Da with a width of 100 Da at several trapping times. Note the area of analyte signals (blue). Source temperature: 50 °C

Proof of Nanodroplets in MS-systems

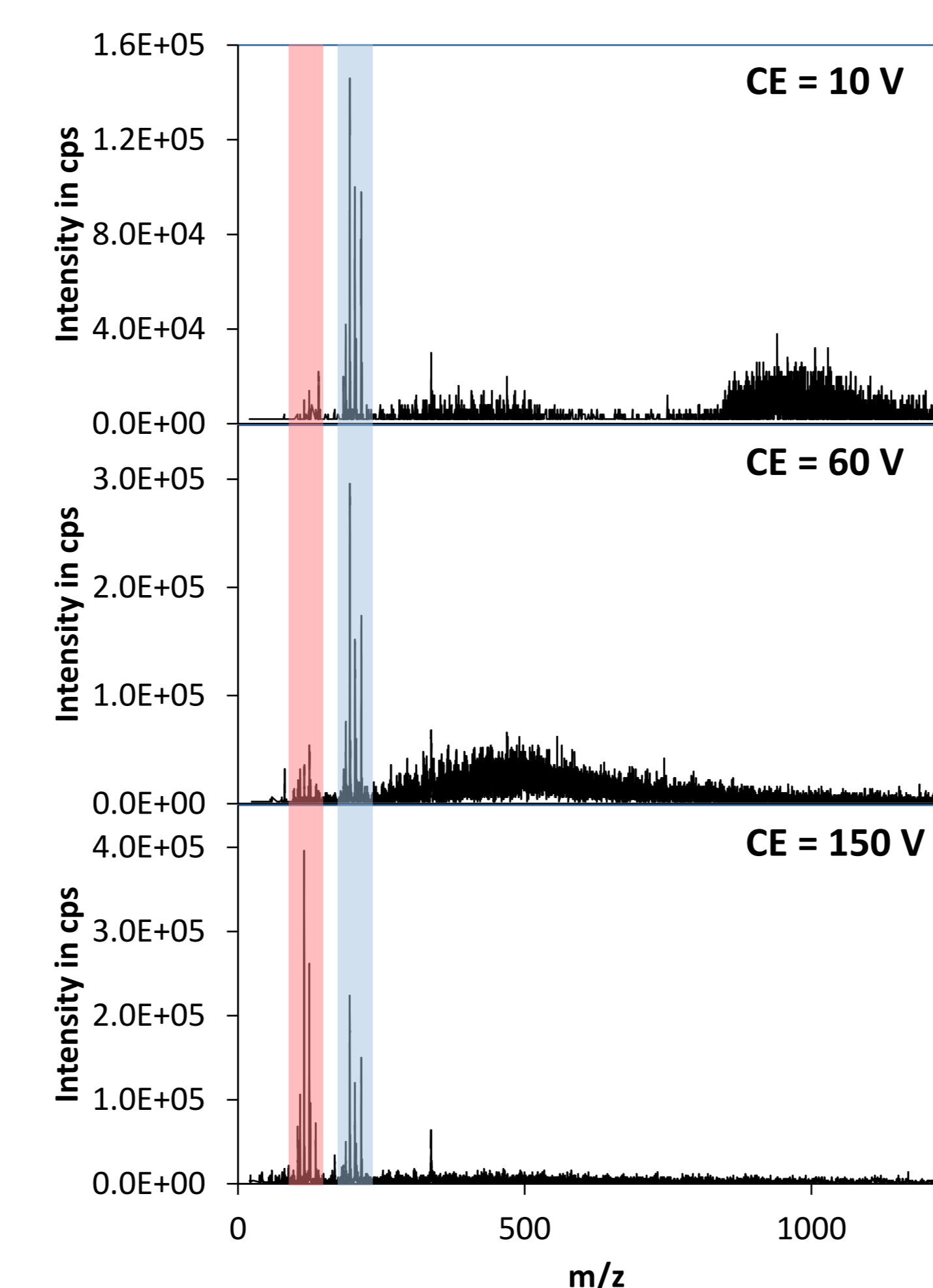


Figure 2: Mass spectra (6500 Triple Quad™) with a cut-off below 1100 Da (Q1) and different collision energies. Note the area of analyte signals (blue) and their typical fragments (red). Source temperature: 30 °C; Ion Spray Voltage: 5500 V

With the triple quad system a maximum of the TIC is recorded by ramping the CE from 0 V to 180 V (see fig. 3, top panel). With a CE of 0 V only noise is present, because the nanodroplets having survived the MS-inlet stage also survive the high vacuum region and can thus not be detected in Q3. The increase of the CE though invokes fragmentation and thus release of detectable species. After the maximum at CE ≈ 60 V the TIC decreases again. This effect is speculated to be the result of droplet fragmentation into ion species, which are too small for the optimal detection range of the used MS-system (fragmentation into small solvent clusters or pure solvent ions). The step-by-step release and subsequent fragmentation of the CN-derivate of the analyte supports this rational (see fig. 3, bottom panel).

All analytes employed, exhibit comparable behavior.

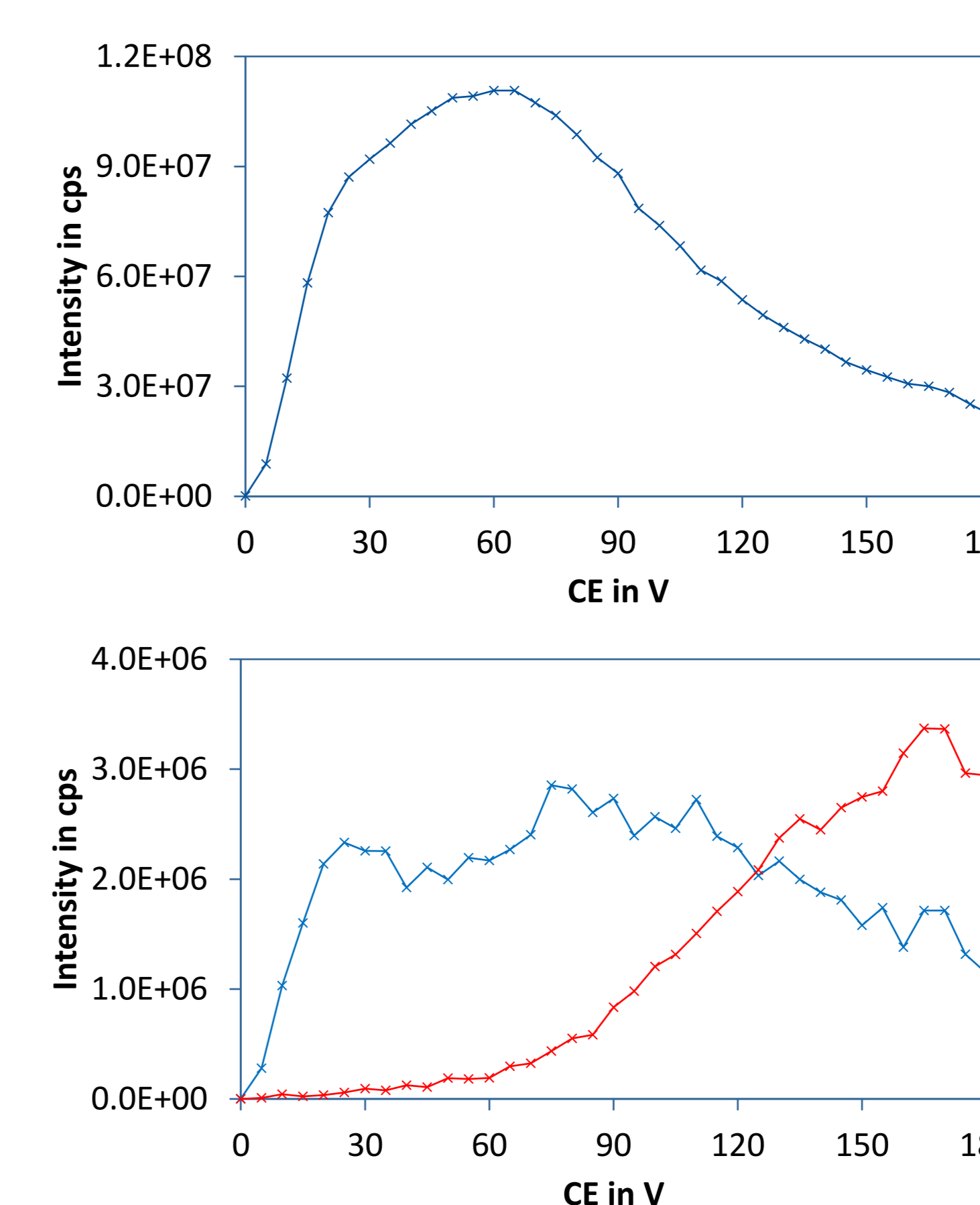


Figure 3: TIC (6500 Triple Quad™) in dependence of the CE (top panel) and intensity of the CN-derivate of the analyte (bottom panel, blue trace) and a characteristic CN fragment (red trace). Source temperature: 30 °C; Ion Spray Voltage: 5500 V
Top panel: Note the maximum of the TIC at CE ≈ 60 V, which fits with the most pronounced signal "wave" in figure 2.
Bottom panel: Note the cross over of the traces, which is representative for all analytes/fragment pairs.

Effects by varying the ESI-conditions

Varying the ESI-temperature

The nanodroplets in MS transfer stages are influenced by varying the ESI-conditions. Increasing the ESI-temperature should have an influence on the generated nanodroplets. The TIC (6500 Triple Quad™) confirms this hypothesis. The intensity increases to a maximum value and then a decline of intensity is recorded (see fig. 4). Note the splitting into two significant maxima from a temperature of 400°C, which indicates a change of the character of the nanodroplets (e.g., droplet size or stability).

The analyte signals also exhibit an intensity increase followed by a nearly complete signal loss as result of the new droplet behavior (see fig. 5).

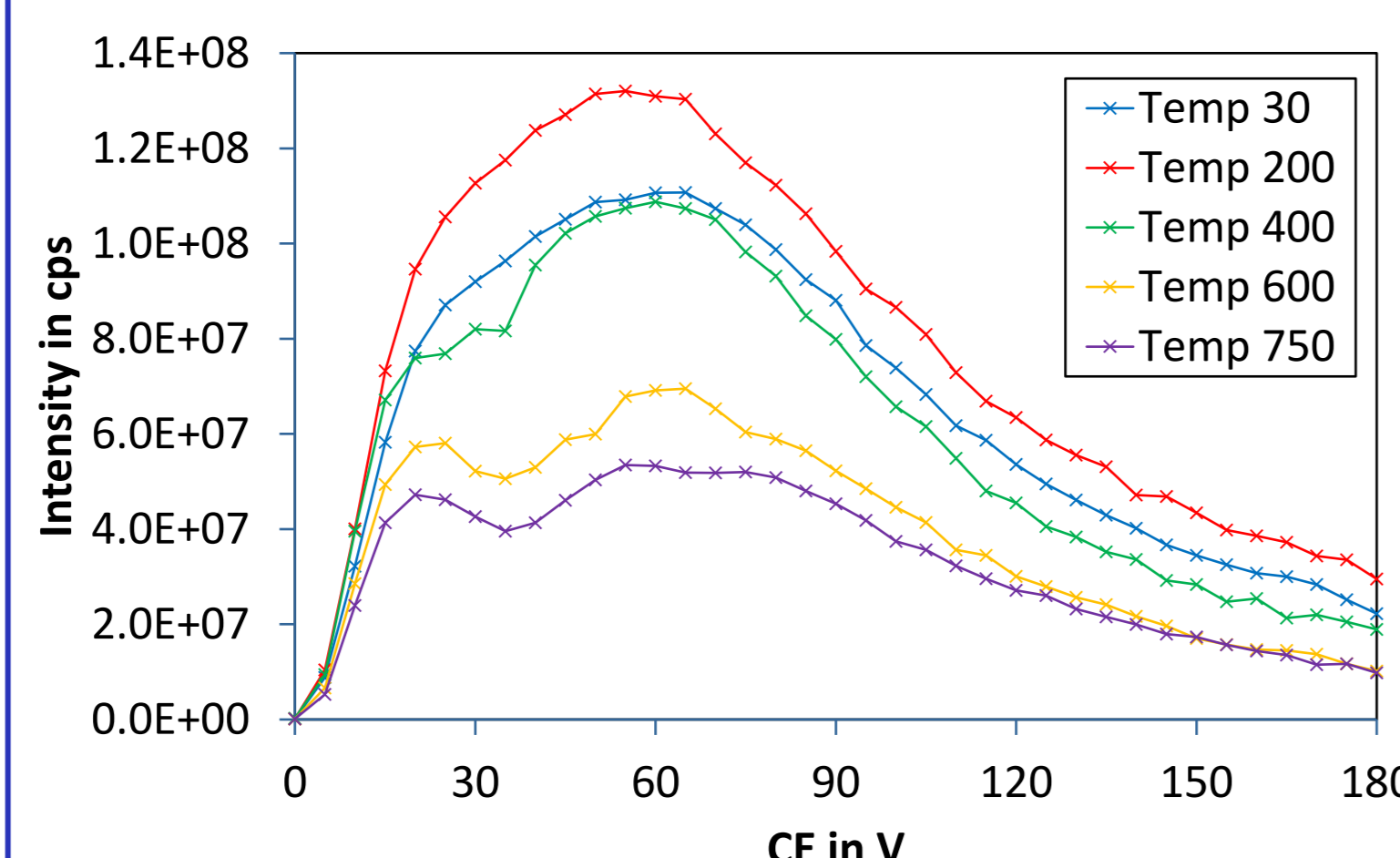


Figure 4: Effect of the source temperature on the TIC (6500 Triple Quad™); Ion Spray Voltage: 5500 V

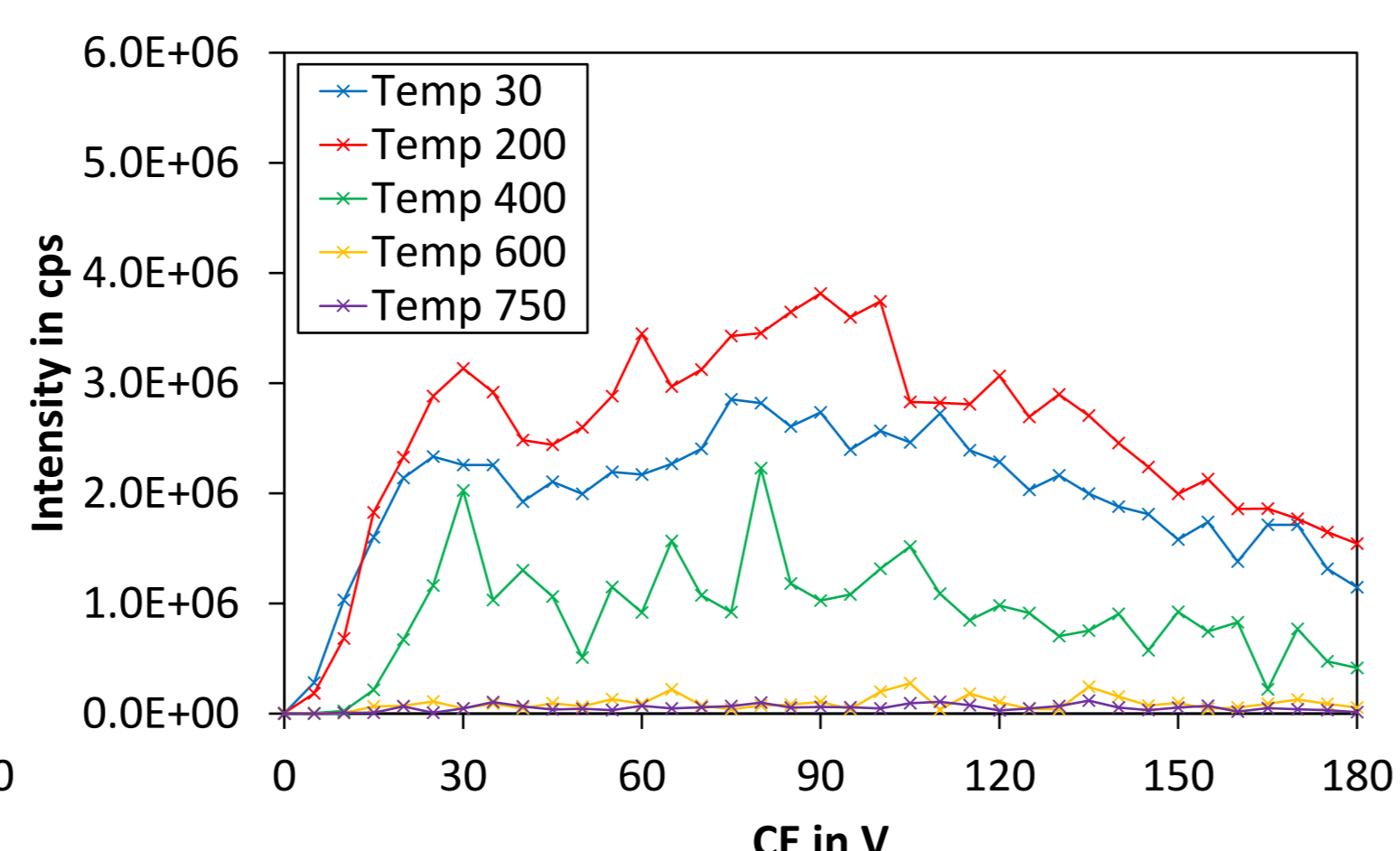


Figure 5: Effect of the source temperature on the ion signal of the CN-derivate of the analyte (6500 Triple Quad™); Ion Spray Voltage: 5500 V

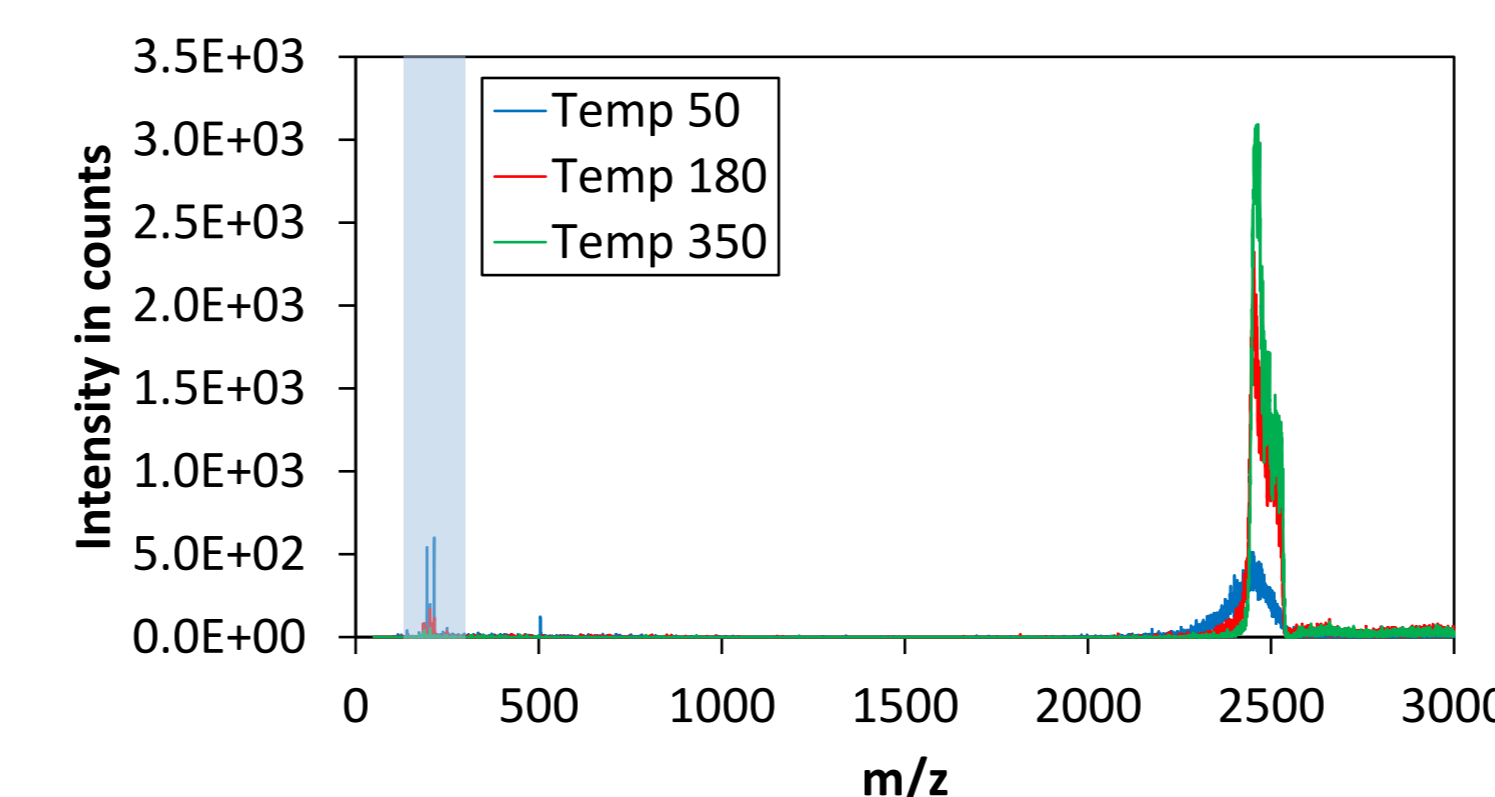


Figure 6: Mass spectra (amaZon ETD Ion Trap) of nanodroplet fragments with different source temperatures. Note the loss of analyte signals (blue area) with rising temperature. Trapping time: 800 ms

The amaZon ETD mass spectra also show the signal loss of analytes with rising temperature (see fig. 6). Simultaneously, the signal in the isolation window shows strong temperature-dependence. Note the higher intensity and sharper edges at higher ESI-temperatures. This indicates a higher droplet stability, which would explain the increasing maximal intensity at this temperatures in fig. 4, too.

Varying the Ion Spray Voltage

Varying the Ion Spray Voltage (6500 Triple Quad™) seems to have less impact on the nanodroplets (see fig. 7). The TIC shows the same general shape, however a deviation at 4500 V is clearly visible. Whether this is a result of nanodroplet changes is subject to further investigations.

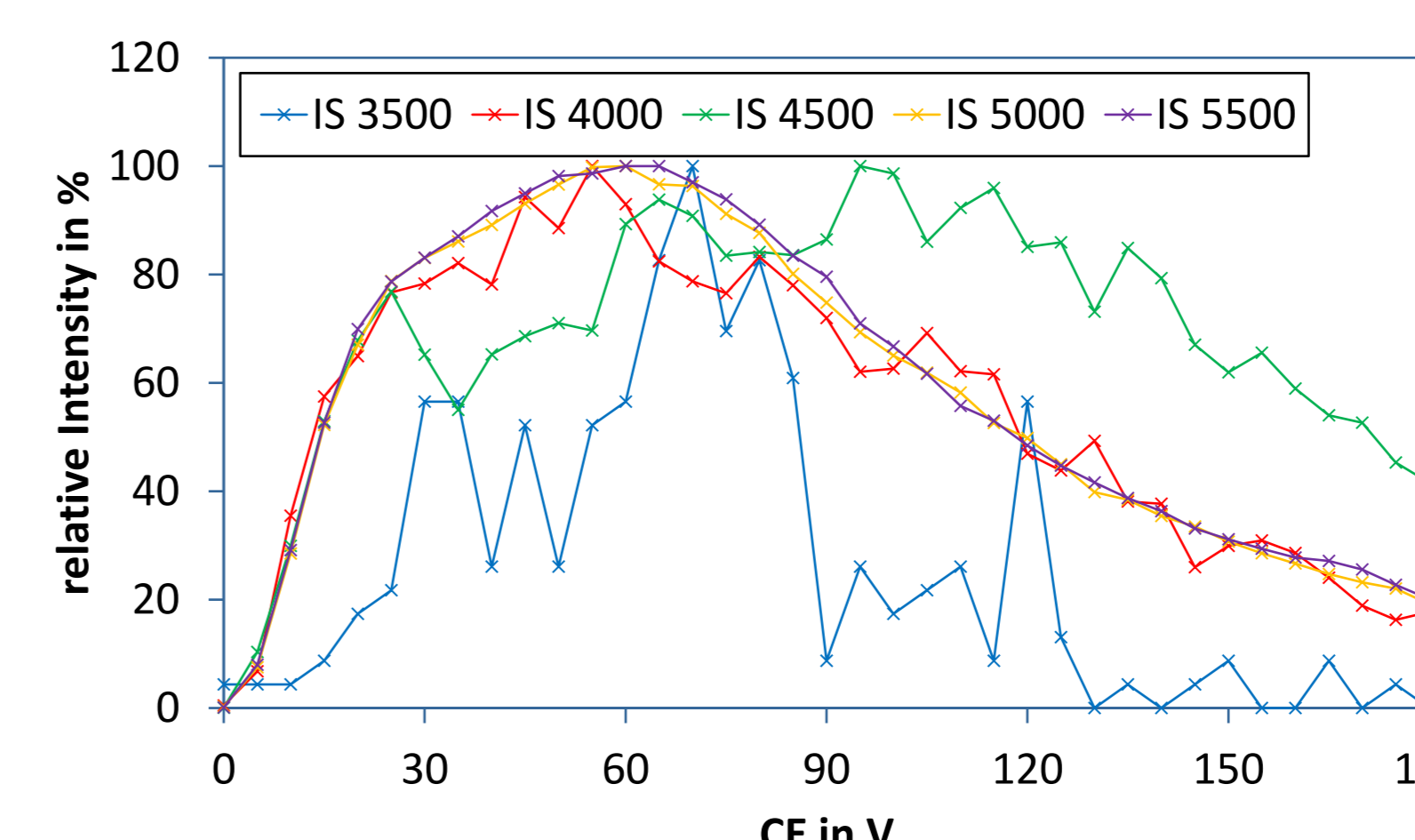


Figure 7: Effect of the Ion Spray Voltage (IS) on the TIC (6500 Triple Quad™); source temperature: 30 °C

Conclusion and Outlook

Conclusion:

- The presence of charged nanodroplets in deep stages of MS transfer/analyzer systems of commercial ESI-MS-systems appears to be almost certain. This needs to be considered in the ESI process theory.
- The individual API inlet system is not critical for this effect. Nanodroplets are detected using a skimmer instrument (SCIEX 6500 Triple Quad™) as well as a capillary/skimmer (Bruker Esquire ion trap) and capillary/funnel instrument (Bruker amaZon ETD ion trap).
- The application of a serious curtain gas flow aimed at defending contamination [2] cannot suppress the presence of nanodroplets in the deep vacuum system.
- Nanodroplets are fragmented upon reaching a threshold energy. This is accomplished in this work upon adjusting the collision energy in a triple quad system or longer trapping times in an ion trap.
- Upon droplet fragmentation, an *unresolved* baseline increase (→ solvent clusters) in conjunction with *mass resolved* ion signals (→ released analyte ions) are detected.
- Varying the ESI source temperature has a significant effect on the spray behavior and thus on the nanodroplets, as has been shown.
- Varying the spray voltage results in far less pronounced effects.

Outlook:

- Analyze more combinations of analytes and solvents
- Larger changes of the ESI-conditions
- Application of further instruments (nanodroplets were also detected with a 6538 UHD Accurate-Mass Q-ToF (Agilent))
- Comparison with previously published [3] und new MD simulations of nanodroplets (refer to poster "Simulations of Collision Induced Evaporation Processes of Nanodroplets in MS Inlet Stages"; TP 278)

Literature

- Kang, Y.; Schneider, B. B.; Covey, T. R.; *On the Nature of Mass Spectrometer Analyzer Contamination*; Journal of the American Society for Mass Spectrometry; 28; 2384-2392 (2017).
- AB Sciex; *6500 and 6500+ Series of Instruments*; System User Guide (2015).
- Markert, C.; Wißdorf, W.; Kersten, H.; Benter, T.; *Simulation of charged Nanodroplets in MS-Transfer-Stage Ion-Guides*; 67th ASMS Conference on Mass Spectrometry and Allied Topics; Atlanta; Georgia; USA (2019).

Acknowledgement

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