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Challenges in Detecting Hydroxyl Radicals Generated in Water Droplets with Mass Spectrometry

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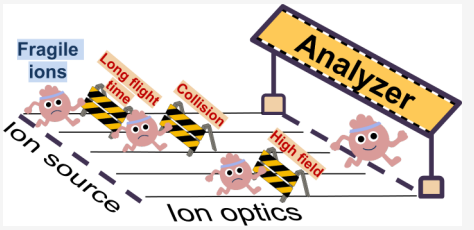
ABSTRACT: Water is sprayed into the air using three distinct methods: (1) nanoelectrospray ionization, (2) a vibrating membrane nebulizer, and (3) a pneumatic nebulizer. The resulting droplets are analyzed by three different mass analyzers: (a) a linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual-pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* system. We searched for hydroxyl radical adducts with hydronium ions (OH•−H_3O^+) or reaction products with caffeine dissolved in water and with melatonin dissolved in water. These experiments were repeated in several different laboratories, and all results were the same. The oxidation products of caffeine and melatonin were not detected when using the Orbitrap mass analyzer having a much longer holding time in the ion trap (500 ms) but could be observed with reduced intensity at much shorter holding times (<10 ms). The signal of OH•−H_3O^+ was also significantly reduced when using the *Velos Pro* dual-pressure linear ion trap mass analyzer. These results suggest that ion signals from fragile radicals may be diminished or lost depending on the mass detection system and operating conditions employed, and there may be a risk of obtaining spurious results when using an Orbitrap mass spectrometer.



INTRODUCTION

Water droplets differ from bulk water in many features, such as the acceleration of many chemical reactions and even in causing new reaction pathways that do not occur in bulk water.[1,2] One striking feature is the production of hydroxyl radicals (OH•). In 2019, Lee et al.[3] reported the production of hydrogen peroxide (H_2O_2) and offered evidence that H_2O_2 resulted from the recombination of OH•. In 2022, Xing et al.[4] presented evidence for the capture of hydroxyl radicals by hydronium ion (H_3O^+) to form $H_4O_2^+$, and Cooks and coworkers[5] have shown that $H_4O_2^+$, which they attribute to the water dimer cation, plays an important role in causing many redox reactions. A major question has been raised concerning what causes the hydroxyl radical to be formed. It has been attributed to contact electrification at the water-hydrophobe interface,[6] to the strong electric field at this interface,[7] and to electron transfer from H_3O^+ to OH^- at the interface.[8,9] Further confirmation for the formation of hydrogen peroxide at the water-hydrophobe interface has been provided in electrochemical studies.[10]

Recently, Chen and Williams[11] utilized nanoelectrospray (nESI) and a vibrating mesh nebulizer (VMN) to generate microdroplets, and an *Orbitrap Elite* mass spectrometer to detect the reaction products. They reported that no signals were observed from the OH•−H_3O^+ ion (m/z 36) or the oxidation products of caffeine (Caf, m/z 212) and melatonin (Mlt, m/z 250). They concluded that the microdroplets generated by VMN and nESI were inactivated compared to the microdroplets generated by pneumatic nebulization (PN). This work has motivated us to reexamine the reported formation of OH• by these three different methods and repeat these experiments in several different laboratories. While we agree with the experimental findings of Chen and Williams, we offer a different interpretation that involves the failure of Orbitrap mass spectrometers to detect fragile ions. Since this work was submitted, another manuscript has been accepted for publication that argues against the notion that microdroplets need to be activated for reactions to occur.[12]

EXPERIMENTAL SECTION

Water microdroplets were generated by nESI, VMN, and PN and the detection system used was respectively a linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer operating at a resolution of 30,000. The VMN that generates 4 μm mean

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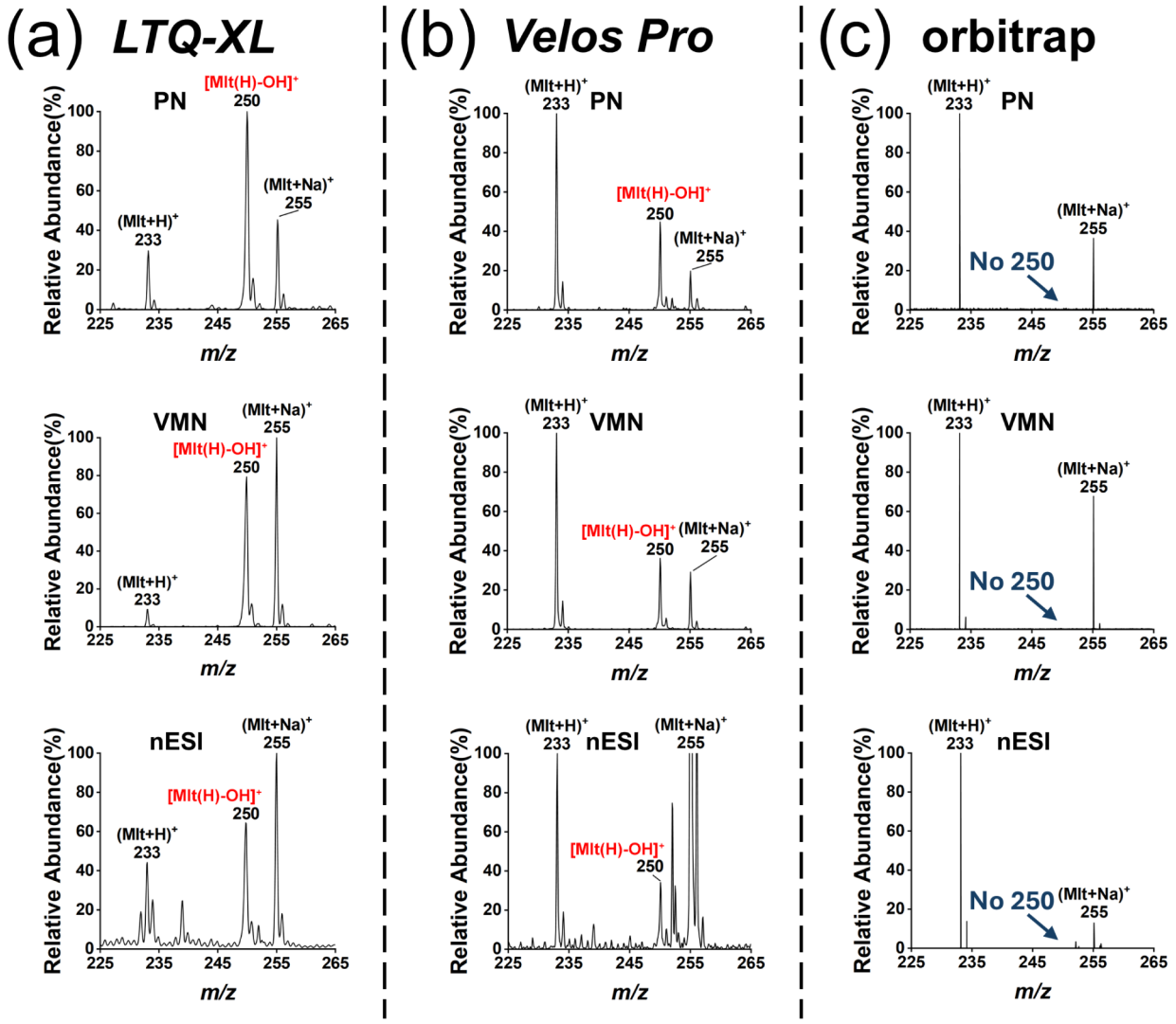

Figure 1. Mass spectra obtained using PN, VMN, and nESI from 100 μM aqueous Mlt solutions using (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer.

diameter droplets was purchased from TEKCELEO, Mougins, France. The nESI tip was pulled using the same method described by Chen and Williams.[11] Water, chemicals, solution concentrations and mass spectrometer settings were also kept consistent with their previous work.[11] More information about the experimental procedure is presented in the Supporting Information.

RESULTS AND DISCUSSION

Figure 1 presents the mass spectra obtained using PN, VMN, and nESI from 100 μM aqueous Mlt solutions using (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer. With *LTQ-XL*, the oxidation product, $(Mlt(H) + OH)^+$, was observed for all three nebulization methods (Figure 1a). Although the relative intensity of $(Mlt(H) + OH)^+/(Mlt + H)^+$ varies among these three methods, the variation does not exceed 50%. Because the reactivity depends on the microdroplet size and reaction time (distance), such a variation cannot reflect the activation difference in the droplets generated by the three methods. In Figure 1b, using the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, the same type of instrument as Chen and Williams used,[11] we can still observe the oxidation products for all three methods, but their intensities were much lower than those obtained on *LTQ-XL*. In Figure 1c, using the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer, we failed to observe any signal from the oxidation products with all the three atomization methods, agreeing with results reported by Chen and Williams.[11]

Figure 2 presents the mass spectra obtained using PN, VMN, and nESI from 100 μM aqueous Caf solutions using again (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer. With *LTQ-XL*, the oxidation product, $(Caf(H) + OH)^+$, was observed for all three nebulization methods (Figure 2a). Although the relative intensity of $(Caf(H) + OH)^+/(Caf + H)^+$ varies among these three methods, the variation does not exceed 50%. Like the case of Mlt, such a variation cannot

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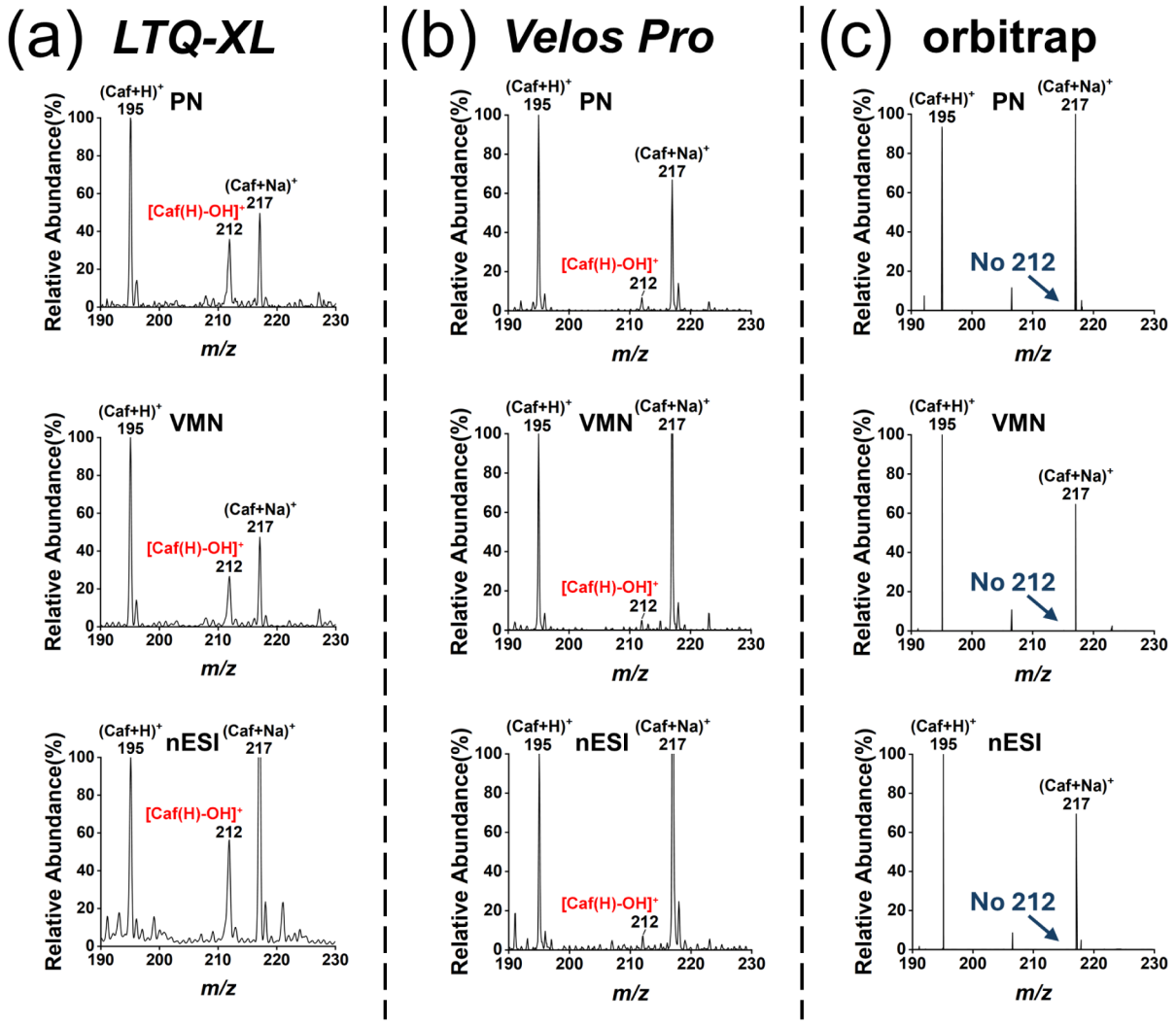

Figure 2. Mass spectra obtained using PN, VMN, and nESI from 100 μM aqueous Caf solutions using (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer.

reflect the activation difference in the droplets generated by these three methods. In Figure 2b, using the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, we still observed the oxidation products for all three methods, but their intensities were much lower than those obtained on the *LTQ-XL*. In Figure 2c, using the Orbitrap mass analyzer, we failed to observe any signal from the oxidation products with all the three atomization methods, once again confirming the results reported by Chen and Williams.[11] We also repeated these experiments at Texas A&M University and the results are presented in Figures S1 and S2, which agree closely with what are shown in Figures 1 and 2. We also recorded the results for pure water paying attention to the OH•$-H_3O^+$ ion signal at m/z 36. With *LTQ-XL*, the OH•$-H_3O^+$ peak was observed for all three nebulization methods (Figure 3a), but with the *Velos Pro* dual pressure linear ion trap mass analyzer, the signal at m/z 36 was much weaker, as shown in Figure 3b.

We agree with the observations of Chen and Williams[11] that the combination of the *Velos Pro* linear ion trap with the Orbitrap mass analyzer is unable to observe the reactions of the hydroxyl radicals with Caf or with Mlt, which are known to be good scavengers for OH•. We believe, however, that these findings point to a failing of the detection system rather than microdroplet inactivation. A similar problem was identified previously in attempting to detect $C_5H_5N^-$, which was called the pyridyl anion.[13] Spraying pyridine dissolved in water readily gave $C_5H_5N^-$, which was detected with an *LTQ-XL* mass spectrometer but failed to be detected with an *Orbitrap Elite* mass spectrometer. We suggested that this failure was caused by the much longer travel time in the Orbitrap device before ion detection.

We propose that a similar phenomenon explains the failure of the Orbitrap mass analyzer. Indeed, as we have noted, the *Velos Pro* gave a reduced signal intensity compared to that of the *LTQ-XL*, as shown in Figures 1−3. Based on these results, this discrepancy could be caused by the different mass spectrometers used rather than the atomization methods. The *LTQ-XL* mass spectrometer is equipped with a single linear ion trap (Figure 4a), but the *Velos Pro* mass analyzer on the *Orbitrap Elite* mass spectrometer has two linear ion traps (Figure 4b): a high-pressure trap (5×10^{-3} Torr) and a low-pressure trap (4×10^{-4} Torr).[14,15] Ions measured by the *Velos Pro* must pass through the high-pressure trap, where they

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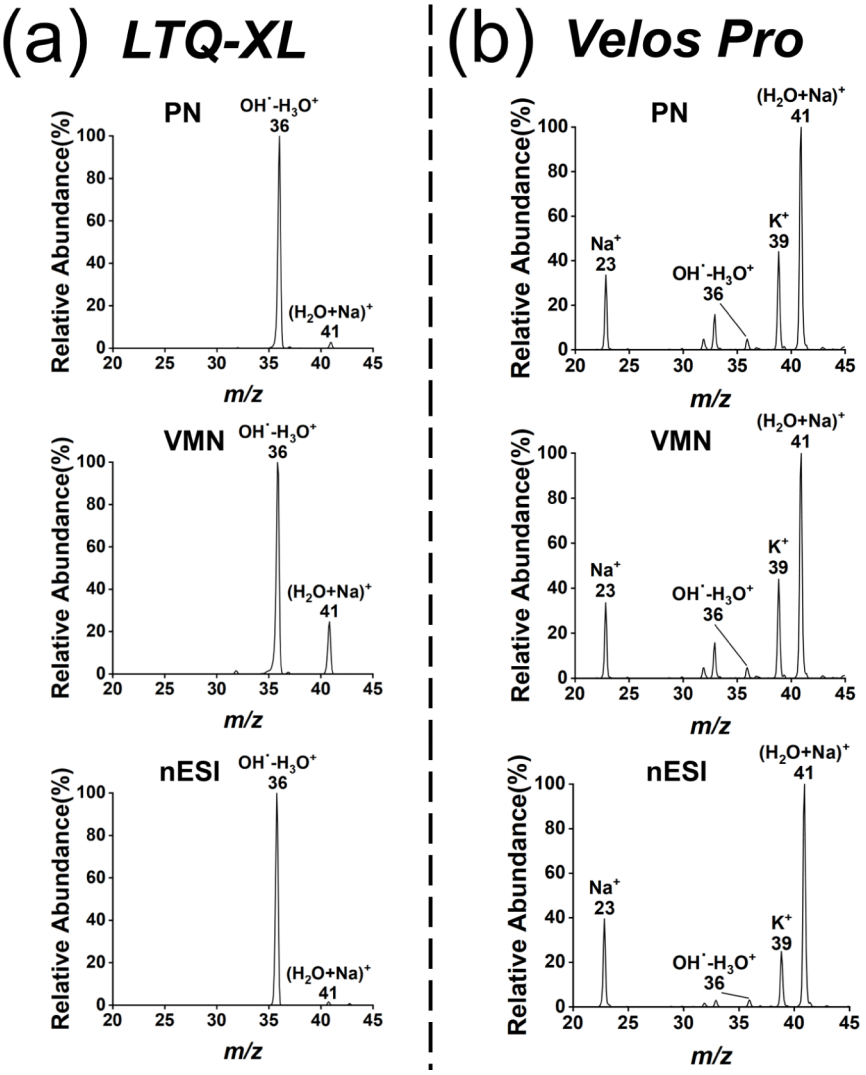

Figure 3. Mass spectra obtained from pure water using PN, VMN, and nESI to generate droplets that are detected with (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer and (b) the *Velos Pro* dual pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer.

collide with He atoms and cool down before mass analysis.[14−16] This process improves the resolution possibly at the cost of the loss of fragile ions. Note that all the ions that Chen and Williams[11] failed to detect were radicals, which are fragile. Figure 4c illustrates the Orbitrap analyzer on the *Orbitrap Elite* mass spectrometer. After passing the *Velos Pro* dual-pressure traps, the ions need to fly a much longer distance, including a mass filter and a C-trap before entering the Orbitrap.[14] During this process, the fragile ions might not survive the longer flight time. Thus, we conclude that care must be taken in interpreting the lack of ion signals when using different mass spectrometers.

Further confirmation is provided by varying the holding time of ions in the ion trap of the Orbitrap, as shown in the Figures S3 and S4. At 500 ms holding time, which is the usual operating conditions, no signal is observed whereas when the holding time is shortened to less than 10 ms ion signals do appear but with reduced intensity. These results show that (1) the path length in the LTQ-Orbitrap is too long for fragile radicals to be detected and (2) confirm that these are fragile ions so the time needed from ionization to detection is an important parameter.

In summary, three different atomization methods were used to generate water droplets. We did not observe significant differences in the generation of the OH•−H_3O^+ ion (m/z 36) or the oxidation products of caffeine (m/z 212) and melatonin (m/z 250). However, the signal intensity differences between the three mass analyzers in detecting fragile ions are vast. The linear ion trap mass analyzer on the *LTQ-XL* mass spectrometer demonstrated the best ability in detecting the fragile products. The *Velos Pro* dual pressure linear ion trap mass analyzer on the *Orbitrap Elite* mass spectrometer performed much worse in detecting the same ions, which was probably caused by the employment of a high-pressure ion trap. The Orbitrap mass analyzer on the *Orbitrap Elite* mass spectrometer failed to detect any fragile ions across all the atomization methods used when operated at what are the recommended normal settings but can detect ions with reduced intensity when the holding time of the ions in the ion trap is reduced by a factor of one hundred. These experiments were repeated in several laboratories with the same results. We conclude that the microdroplets generated by the three methods do not show significant differences in activation, at least in the three cases studied here and in the ∼10,000 examples reported in a previous study.[17] We

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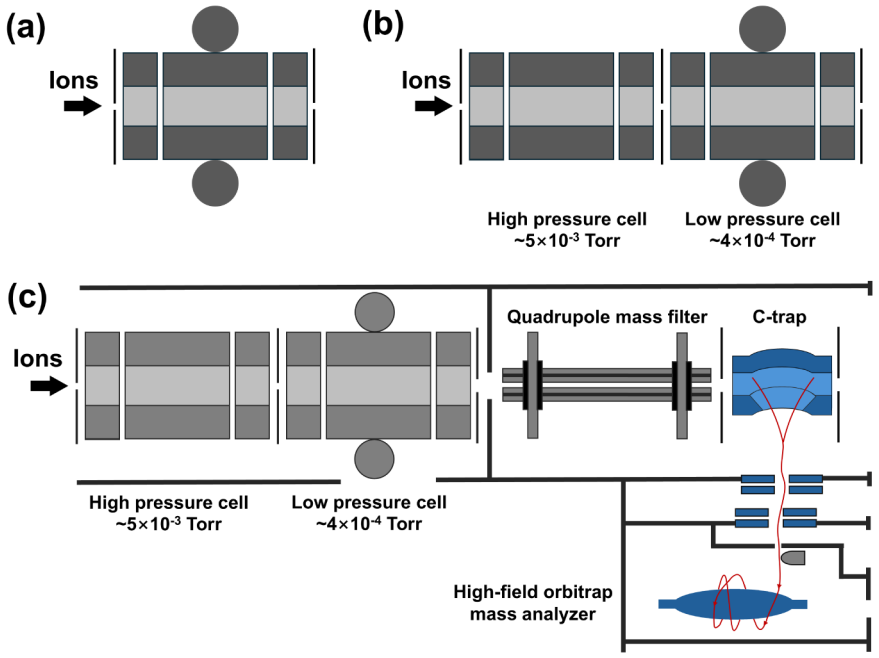

Figure 4. Schematic drawing of (a) the linear ion trap mass analyzer on an *LTQ-XL* mass spectrometer, (b) the *Velos Pro* dual-pressure linear ion trap mass analyzer on an *Orbitrap Elite* mass spectrometer, and (c) the Orbitrap mass analyzer on the same *Orbitrap Elite* mass spectrometer.

recommend that researchers exercise extra caution when selecting mass analyzers and operating conditions for detecting fragile ions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.5c00386.

Experimental methods; reproducibility of results; ion injection time effect; mass spectra for reproducibility of results (Figures S1 and S2); ion chromatograms and mass spectra for the study of the ion injection time effect (Figures S3 and S4) (PDF)

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Notes

The authors declare no competing financial interest.

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