

In-Source Reactivity Mass Spectrometry: Revealing Reactive Species through High-Resolution Characterization

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Overview

- The unique dual electrospray ionization source design, equipped in the time-of-flight mass spectrometer LCMS-9050, was utilized for the first time to investigate chemical reactions and ion production mechanisms in the gas phase.
- The integration of high-resolution ISR-MS with tandem mass spectrometry further enhances its analytical capabilities, enabling comprehensive molecular characterization with exceptional precision.

1. Introduction

In-source reactivity mass spectrometry (ISR-MS) revolutionizes chemical analysis by enabling the direct characterization of reactive species and real-time monitoring of complex chemical reactions with high resolution. This poster presents a selection of interesting case studies that highlight the immense potential of high-resolution ISR-MS in various fields such as organic synthesis, organometallic synthesis and charge state modification.

Three case studies were selected:

- Formation of cyclic acetals.** The acetalization of benzaldehyde with aliphatic glycols is catalyzed in the liquid phase by the presence of a proton. In this case study, the high availability of protons at the surface of the positively charged microdroplets was investigated for their potential use as chemical reactors.
- Investigation of a chelated metal ion oxidation state.** The addition of a ligand can alter the oxidation state of an iron cation. In this case study, the introduction of hydrogen peroxide at the microdroplet level was closely scrutinized.
- Supercharging of single-stranded oligonucleotides.** Meta-nitrobenzyl alcohol (mNBA), when combined with single-stranded oligonucleotides in an aqueous ammonium acetate solution, is known to enhance their charge state in negative ionization mode. In this case study, the introduction of mNBA was implemented at the microdroplet level.

2. Instrumentation

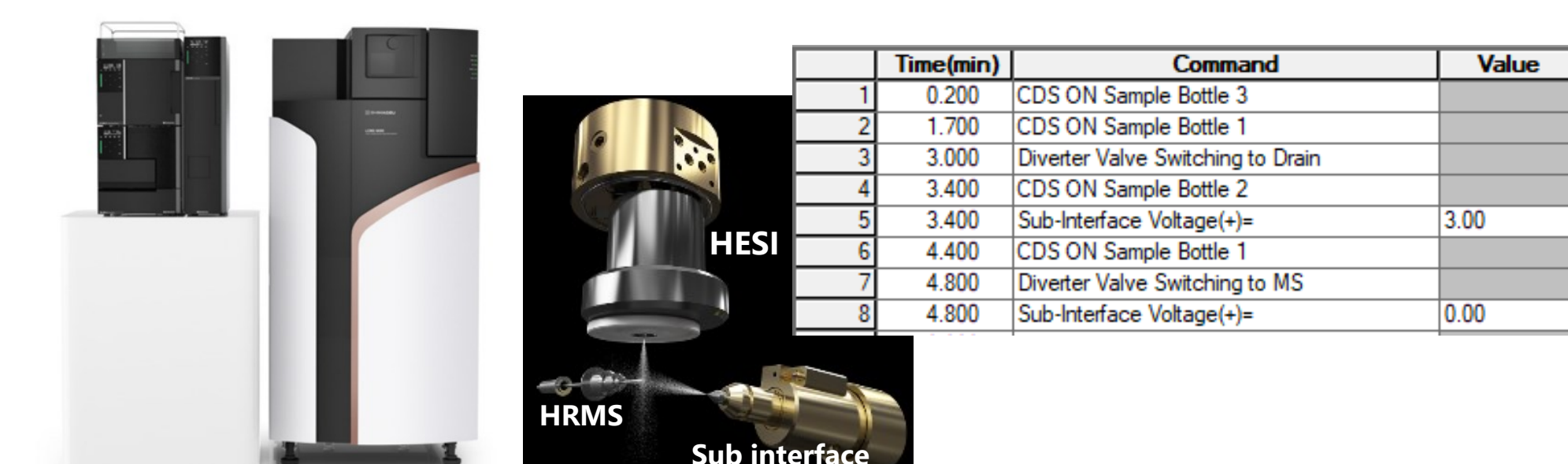


Figure 1. The time-of-flight mass spectrometer LCMS-9050, equipped with a dual electrospray ionization source, was utilized for these investigations. The acquisition software, Labsolution, allows complete control of each parameter of both ESI probes at any given moment during the analysis. Among five possible additives, three bottles were operated.

3. Results

3.1 Formation of cyclic acetals

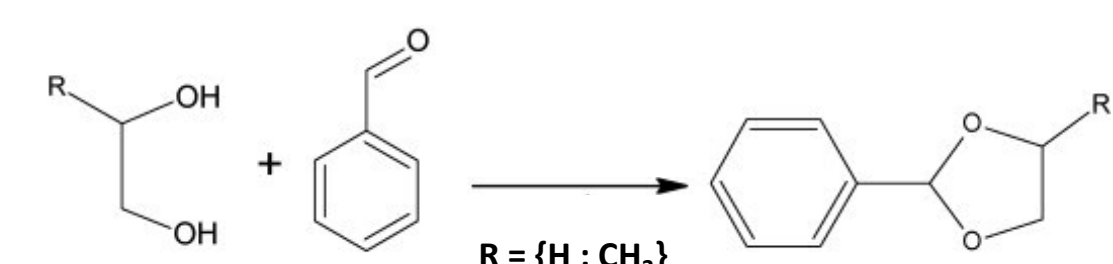


Figure 2. Acetalization is an equilibrium reaction in the liquid phase that is favored in acidic media. The aliphatic glycols were introduced by flow injection analysis (FIA) through the main ESI probe (HESI). Concurrently, the secondary ESI probe (Sub interface) was programmed to infuse benzaldehyde for a specific duration; outside of this period, it was dispensing methanol.

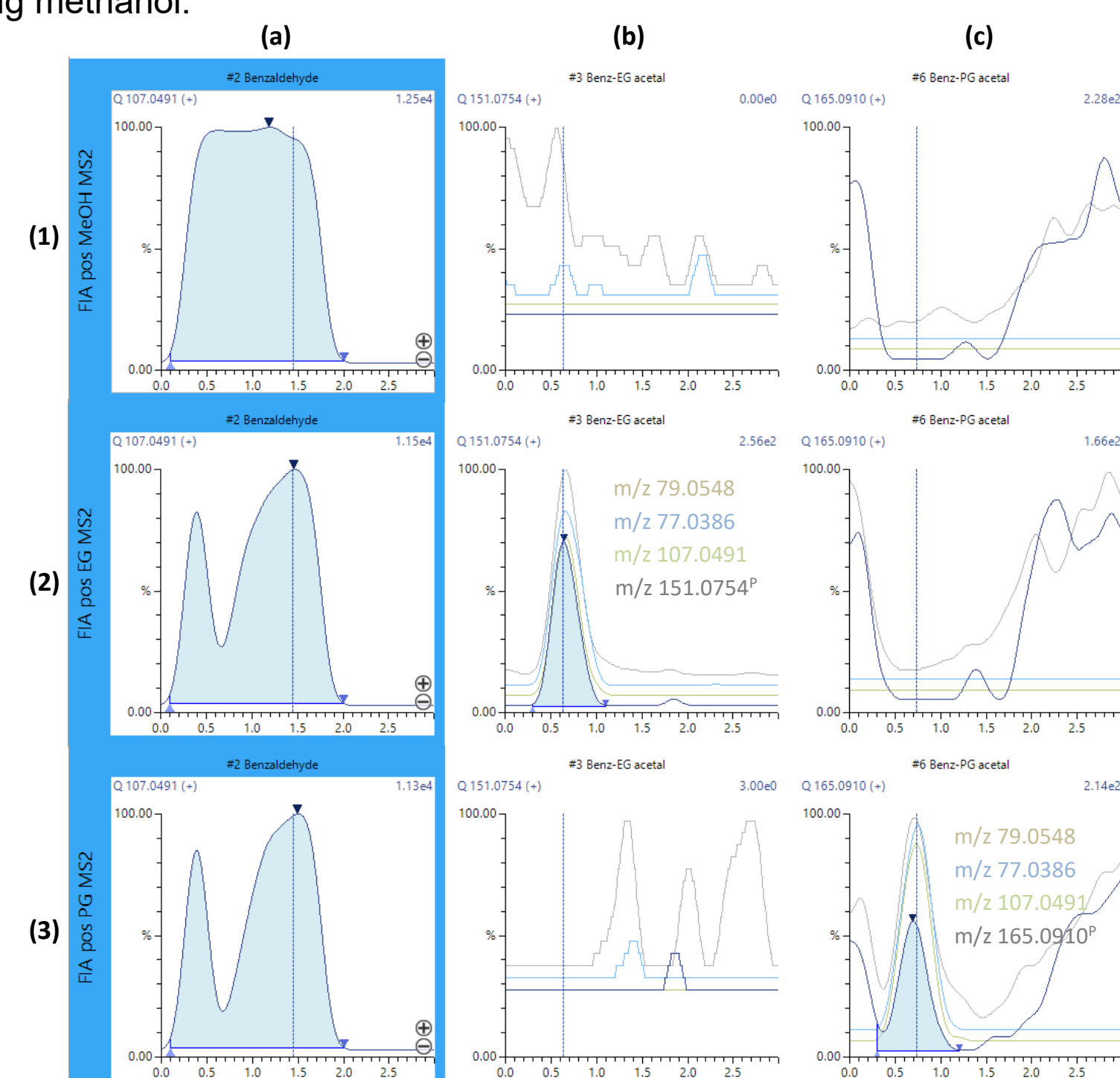


Figure 3. Extracted ion chromatograms at 5 ppm of (a) protonated benzaldehyde infused from 0.2 min to 1.7 min, (b) protonated acetal synthesized from ethylene glycol and its MS² fragment ions, and (c) protonated acetal synthesized from propylene glycol and its MS² fragment ions for the injection at 0.3 min of (1) MeOH, (2) ethylene glycol in MeOH, and (3) propylene glycol in MeOH.

- The chromatograms clearly shows the production of cyclic acetal ions concurrent with the consumption of the reagent detectable in positive ion mode. This demonstrates the efficient merging of both sprays in the early stages of microdroplets evaporation, ensuring that both reagents can be mixed in the same microdroplet before the excess of positive charge at the surface repels them.
- The increasing availability of protons, coupled with the departure of the produced water molecules in the later stages of microdroplets evaporation/desolvation, shifts the reaction equilibrium towards the production of cyclic acetals. This makes the microdroplet a perfectly suited chemical reactor.

3.2 Investigation of a chelated metal ion oxidation state

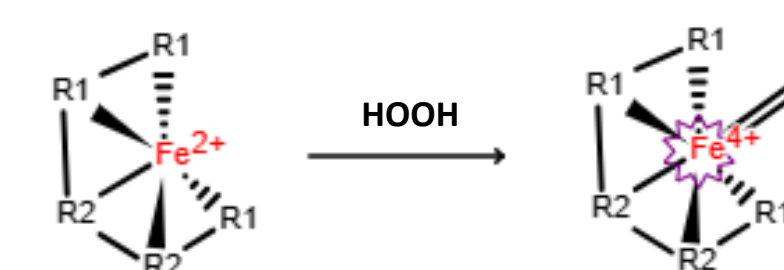


Figure 4. Oxidation of chelated iron. The doubly charged iron, chelated with a non-reactive pentadentate ligand involving five non-binding nitrogen doublets, was introduced by FIA through the HESI. Concurrently, the Sub interface was programmed to infuse hydrogen peroxide for a specific duration; outside of this period, it was dispensing methanol.

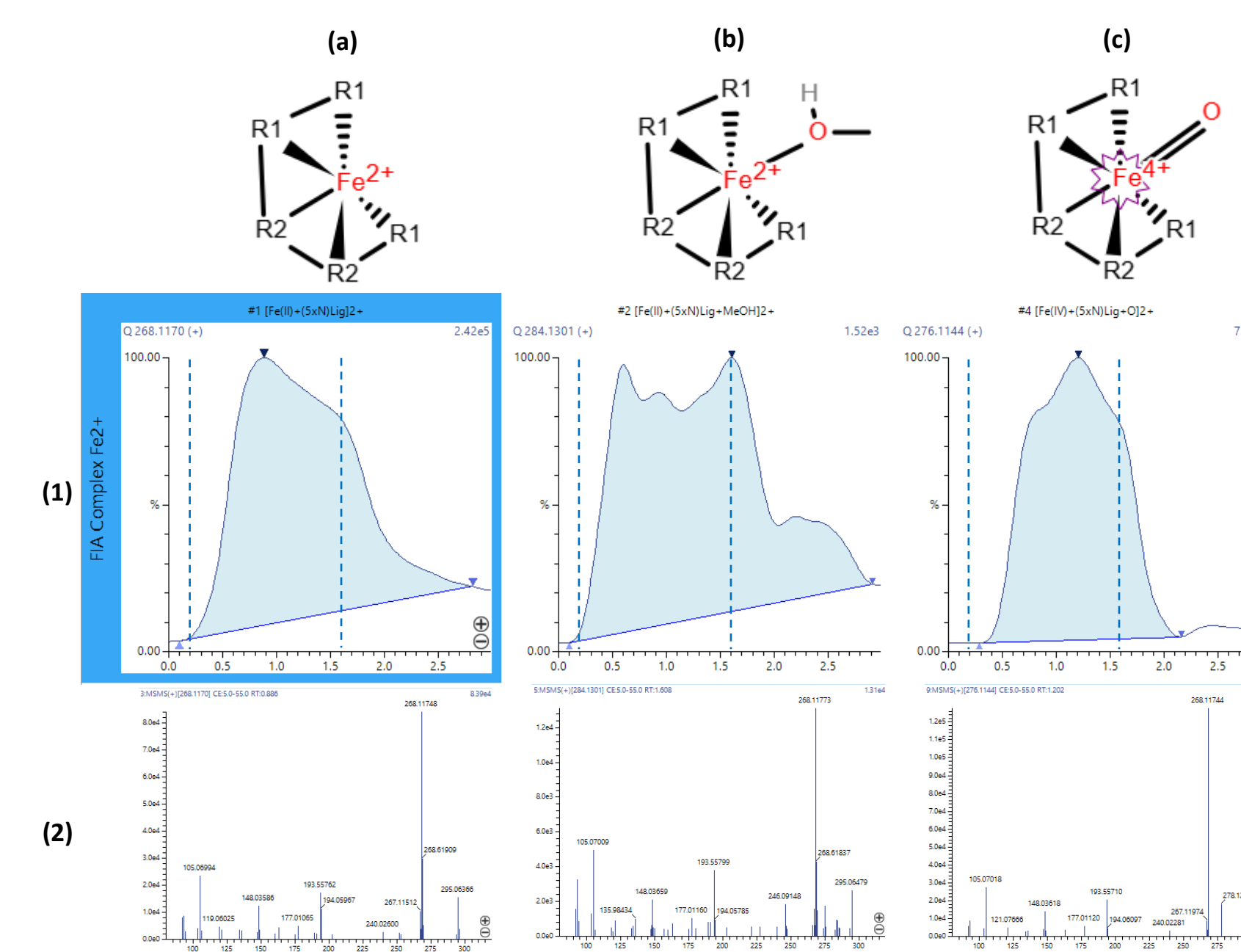


Figure 5. (1) Extracted ion chromatograms at 5 ppm of (a) doubly charged precursor of chelated Fe^{II} injected at 0.1 min, (b) MeOH adduct of the doubly charged chelated Fe^{II}, and (c) doubly charged oxidized chelated Fe^{IV}. (2) Respective MS² were acquired with a spread collision energy ranging from 5 to 55 V.

- The fragmentation patterns are similar for each isolated parent ions.
- Even if the chelated iron precursor was injected at the 0.1 min, the shape of the chromatographic peak indicates non-specific interactions. These interactions could occur either prior to the ESI nebulization or inside the stainless steel desolvation line located at the interface of atmospheric pressure and the primary vacuum.
- Interestingly, the production of the chelated Fe^{IV} appears to correspond to the consumption of the chelated Fe^{II} MeOH adduct.
- While the intensity decrease of the chelated Fe^{IV} at 1.6 minutes aligns with the bottle switch infusion from hydrogen peroxide to MeOH, the initial intensity increase was delayed by a few seconds. This observation can be explained by the time required for the reaction to occur. In this case, it would suggest that the reaction takes place in the desolvation line.
- Numerous potential reaction intermediates were detected. To fully identify these extremely complex spectra, the development of an algorithm based on intelligent isotopic pattern comparison has begun.

3.3 Supercharging of single-stranded oligonucleotides

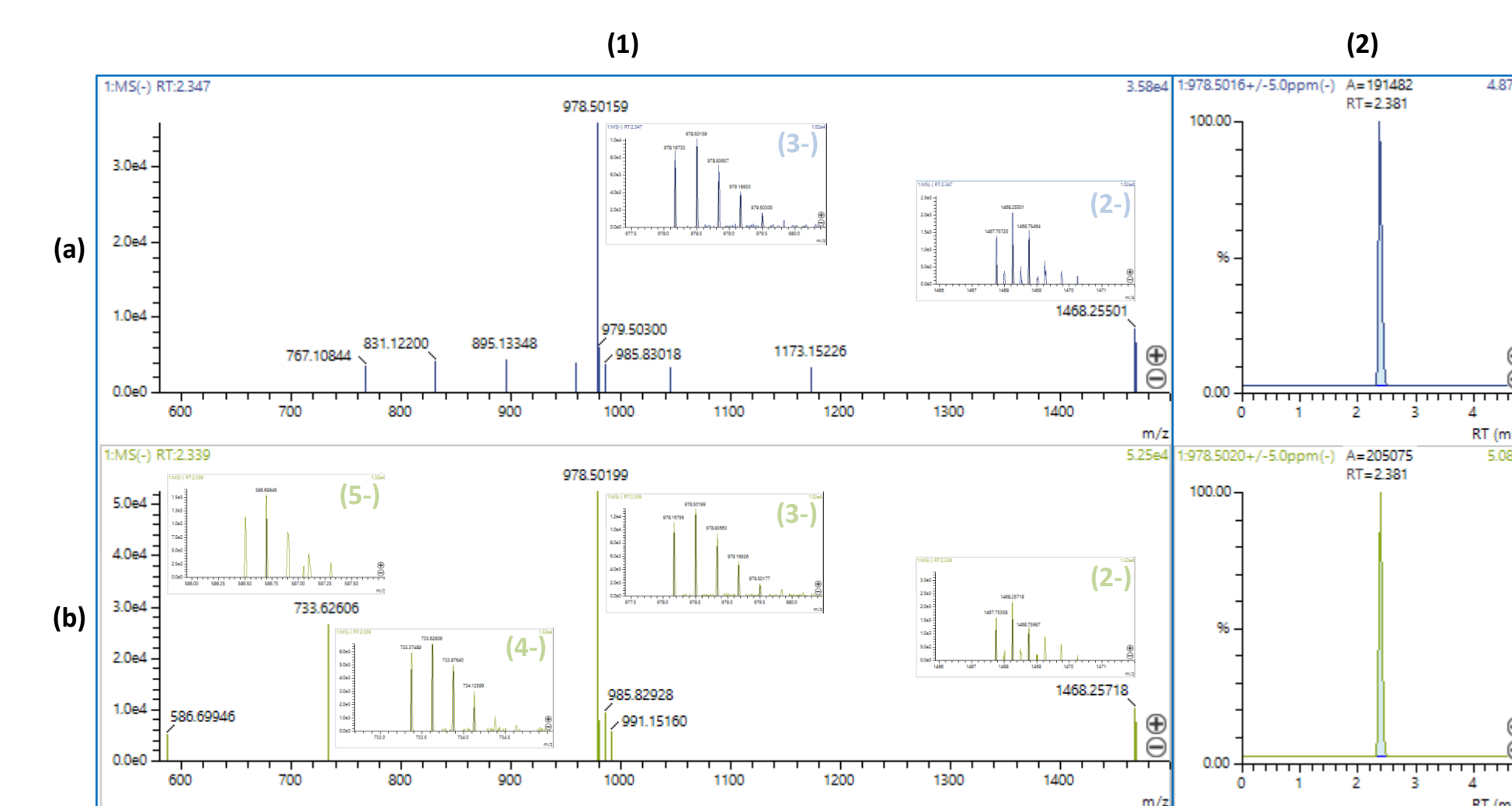


Figure 6. (1) HRMS spectra obtained in negative ionization mode for the injection of the single-stranded oligonucleotide CCTTCGCTAC through a Shim-pack Scepter Claris C18-120 (50 x 2.1 mm, 1.9µm) in isocratic mode with a mixture of solvents (100 mM of NH₄OAc in H₂O / MeOH, 50/50 v/v). (2) Extracted ion chromatograms at 5 ppm of the triply charged oligonucleotide. The infusion through the sub-interface of (a) MeOH and (b) mNBA (0.4% in MeOH) was configured to encompass the expected peaks.

- The "in-source" addition of mNBA was found to improve total signal intensity, increase the highest observed charge states, raise the average charge states, and limit the uncontrolled dissociations occurring prior to the detection.
- This efficient "in-analysis" reagent addition unlocks vast possibilities for the charge-controlled analysis of multicharged biopolymeric ions.

4. Conclusions

- The dual electrospray ionization source, equipped on the time-of-flight mass spectrometer LCMS-9050, facilitated the exploration of chemical reactions at the interface between the solution and gas phase.
- The efficient merging of both sprays in the early stages of microdroplet evaporation ensures the combination of both reagents within the same microdroplet. The increased concentration of the reagent in the later stages of evaporation can enhance and possibly expedite many reactions, thereby facilitating their study.
- In some well-designed experiments, the desolvation line can be utilized as a chemical reactor for slower reactions, thereby enabling the identification of chemical intermediates.
- The combination of five reagent bottles with the complete control of each parameter of both ESI probes at any given moment during the analysis opens limitless application possibilities, enhanced by the exceptional precision provided by high-resolution mass spectrometry.