Application Note: 375

Collisionally-Induced Dissociation of Methimazole and Acetylsalicylic Acid: Proposed Mechanisms of Ion Formation using Hydrogen/Deuterium Exchange

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Key Words

- TSQ Quantum Ultra AM™
- ESI
- High-resolution Mass Measurements
- Ionization Mechanisms

Introduction

Acetylsalicylic acid (Aspirin) belongs to a class of medications known as non-steroidal anti-inflammatory drugs (**NSAIDs**) and has been among the most popular medications for treating mild to moderate pain. It also reduces inflammation, clotting of blood, fever, redness, swelling and discomfort caused by medical disorders such as headaches, infections and arthritis.[1] Methimazole is used to treat hyperthyroidism, a condition that occurs when the thyroid gland produces too much thyroid hormone.[2]

Mass spectrometry has played a significant role in the characterization and analysis of small molecules such as Methimazole and Aspirin.[3,4] The present study was undertaken to assess the structures and mechanisms of formation of the principal fragment ions in the ESI mass spectra of these two drugs. The CID mass spectra of Methimazole and Aspirin and their corresponding deuterated analogs have been studied in both the positive and negative ion modes.

Decomposition mechanisms are proposed for the principal fragment ions using H/D exchange and the combination of multiple-stage CID at low collision energy with high-resolution mass measurements. The mass spectra of Methimazole and Aspirin can serve as useful models for structural determination of chemically or biologically modified Methimazole/Aspirin or related compounds.

Goals

Thermo Scientific TSQ Quantum mass spectrometer systems feature HyperQuad quadrupoles. The hyperbolic shape of the electrodes yield higher ion transmission, minimizing false positives, while maximizing sensitivity. This work demonstrates the utility of the Thermo Scientific TSQ Quantum Ultra AM for assessing the structures and mechanisms of formation of principal fragment ions in the high-resolution CID mass spectra of famotidine and azithromycin in both negative and positive ion modes.

Experimental Conditions

Chemicals and Reagents

Acetic acid and ammonium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade methanol was acquired from Burdick and Jackson (Muskegon, MI), and HPLC grade water was purchased from J.T. Baker (Phillipsburg, NJ). Methimazole and Acetylsalicylic acid were provided by Pfizer Global Research and Development (Groton, CT). Ammonium-^{15}N, d_4 deuteroxide solution, CD_3OD, D_2O and Acetic acid-d_4 were purchased Sigma-Aldrich (St. Louis, MO). All chemicals were used as received.

Sample preparation: Stock solutions of Methimazole and Aspirin were prepared in HPLC grade methanol at 1.0 mg/mL. The stock solutions were then diluted with mobile-phase from each solvent system to give a final concentration of 20 µg/mL. No further sample preparation was required.

Sample analysis: Infusion analyses were performed on a TSQ Quantum Ultra AM triple-quadrupole mass spectrometer (San Jose, CA). The solvent systems used in this study were: A). 50:50 HPLC grade methanol and 0.1 acetic acid, B). 50:50 HPLC grade methanol and 50 mM ammonium hydroxide, C). 50:50 HPLC grade methanol and 0.1% Acetic acid-d_4, and D). 50:50 HPLC grade methanol and 50 mM Ammonium-^{15}N, d_4 deuteroxide. All infusion studies were conducted using the instruments' integrated syringe pump at a flow rate of 2.0 µL/min for total of four minutes.

MS conditions: The TSQ Quantum Ultra AM was calibrated in normal and high resolution modes with a solution of 1,3,6-Polytyrosine. Accurate mass calibration of the instrument was performed with a 50 pmol/µL solution of ammoniated polyethylene glycols (PEGs).

TSQ Quantum Ultra AM Conditions

Ionization mode and source:	Positive and Negative ESI
Electrospray voltage:	(+) 3.5kV; (-) -2.5kV
Sheath gas:	1
Auxiliary gas:	0
Ion transfer tube temperature:	270 °C
Ion Transfer Tube offset:	35 V
Tube lens offset:	77V
Collision energy:	25eV (Methimazole); 15eV (Aspirin)
Collision pressure:	1.2 mTorr
Q1/Q3 resolution:	0.1 Da FWHM
Accurate mass mode:	Internal
Micro scans:	2

Results

CID of protonated methimazole (Figure 1) exhibits primary dissociations via eliminations of HCN and H_2S (Figure 2). Supplementary losses of CH_3, S and C_2H_3N are minor dissociation pathways from $[M+H]^+$ ion. Proposed mechanistic decompositions from the $[M+H]^+$ ion are given in Figure 3 and corroborate well with the empirical data. The CID mass spectrum of $[M-H]^-$ ion (Figure 4) shows loss of methyl group as the initial dissociation step, followed by the elimination of HCN (Figure 5). The fragment ion NCS^- at *m/z* 58 is also observed.

For protonated aspirin (Figure 1), elimination of H_2O (Figure 6) and the acetyl group (CH_2CO) were the major decomposition pathways (Figure 7). The CID mass spectrum of $[M-H]^-$ of aspirin (Figure 8) was much simpler and showed the loss of CH2CO at *m/z* 137 as the primary dissociation pathway (Figure 9). The ensuing elimination of CO_2 from the fragment ion at *m/z* 137 was a minor dissociation pathway.

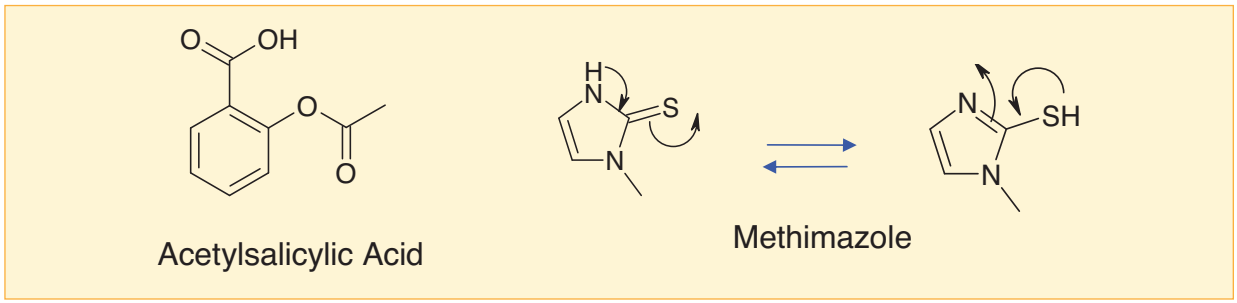

Figure 1: Structures of Methimazole and Acetylsalicylic acid (aspirin)

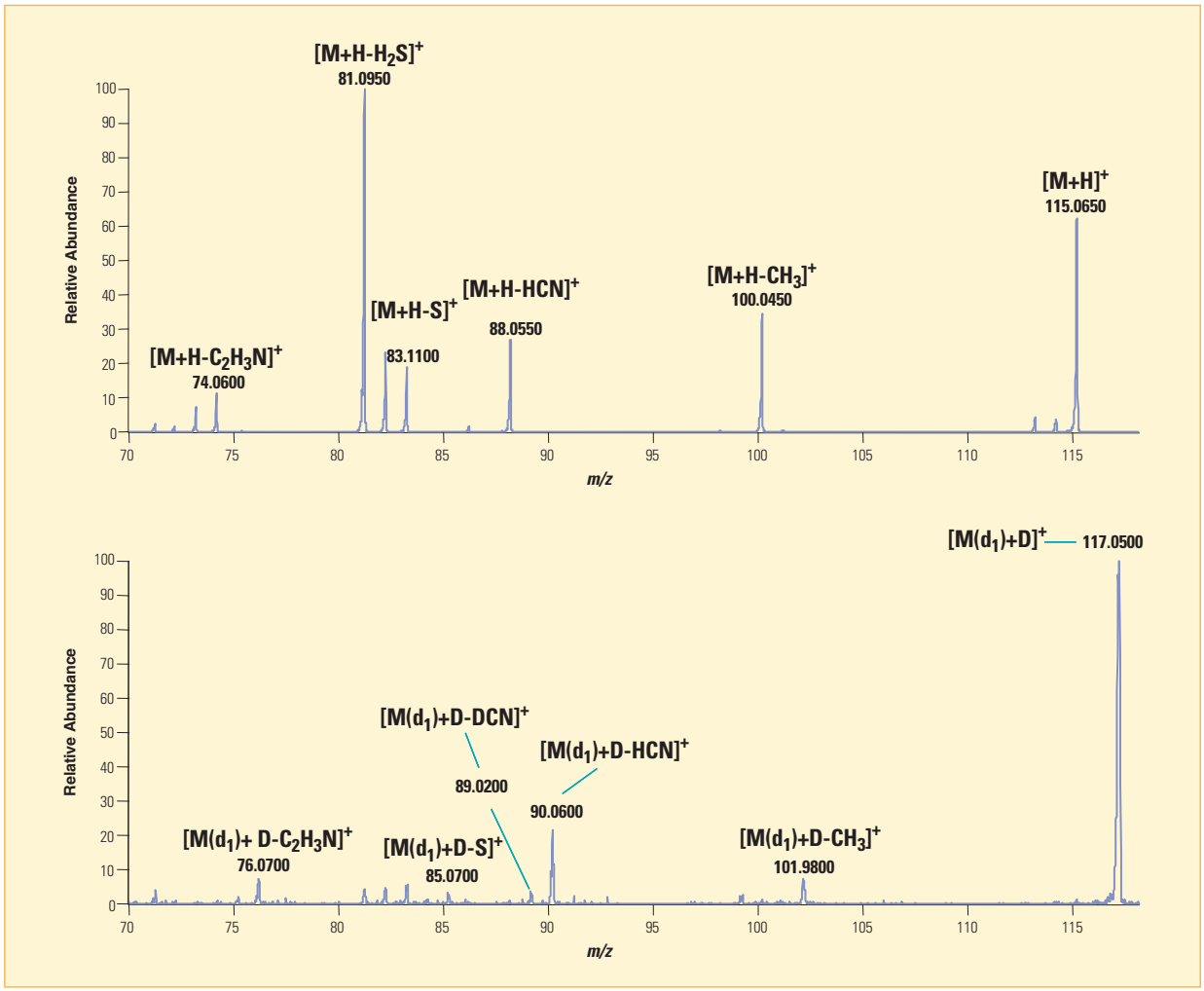

Figure 2: Positive ion ESI mass spectra of Methimazole (MW= 114): CID product ion spectra (MS/MS) of $[M+H]^+$ at *m/z* 115 and the fully exchanged $[M(d_1)+D]^+$ at *m/z* 117. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

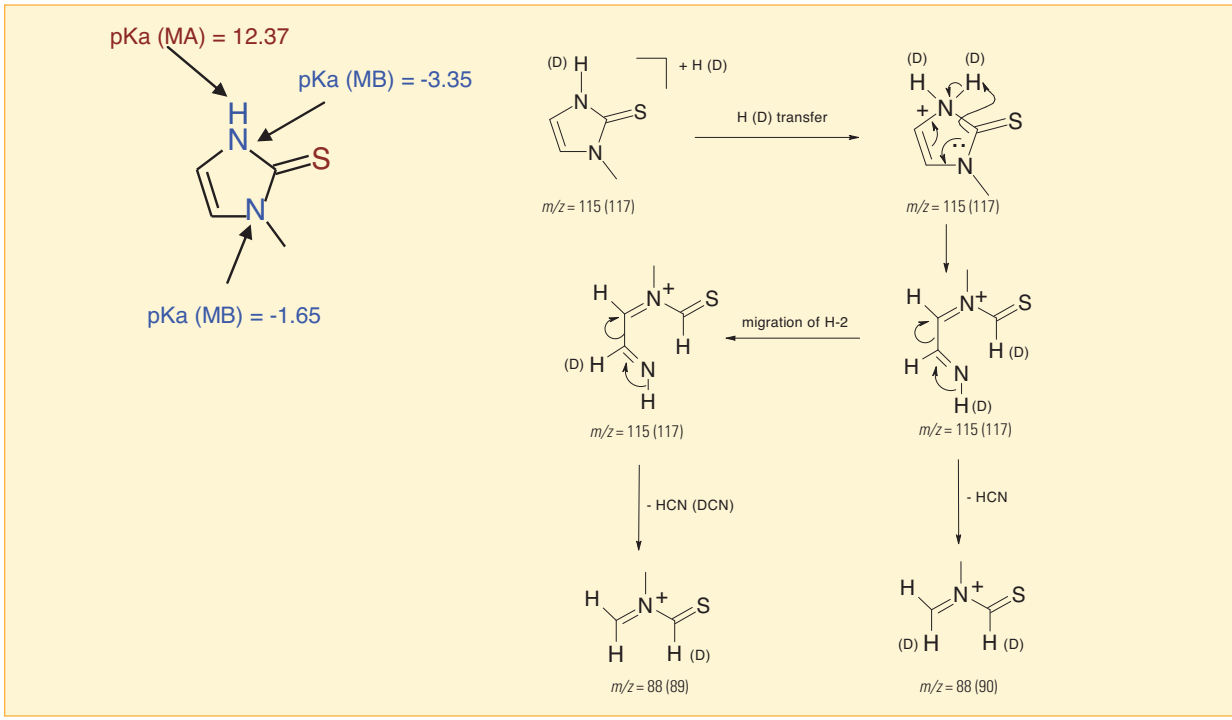

Figure 3: Proposed CID fragmentation mechanisms for the major fragment ions from protonated Methimazole at *m/z* 115 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments. Numbers in parentheses refer to deuterated fragment ions

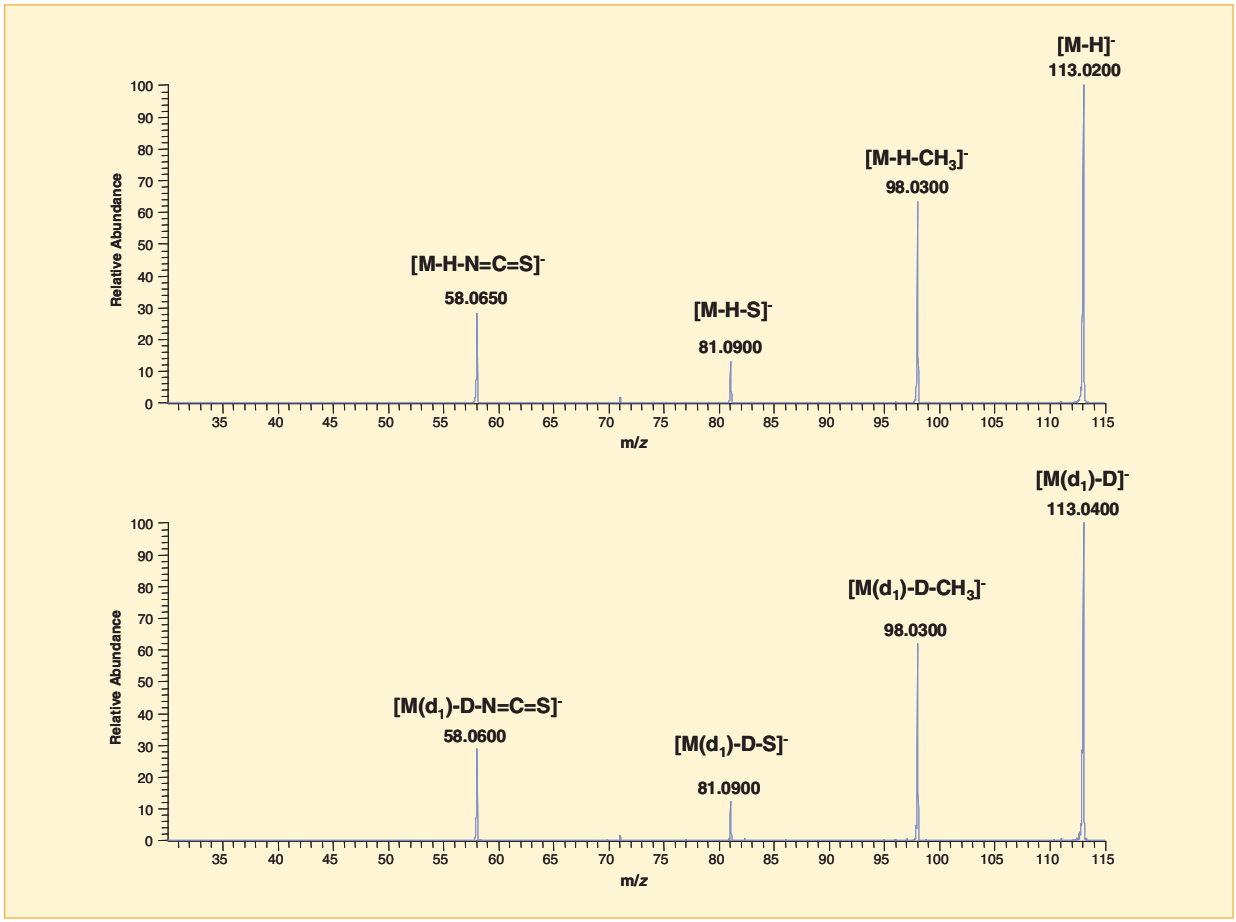

Figure 4: Negative ion ESI mass spectra of Methimazole (MW= 114): CID product ion spectra (MS/MS) of $[M-H]^-$ at *m/z* 113 and the fully exchanged $[M(d1)-D]^-$ at *m/z* 113. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

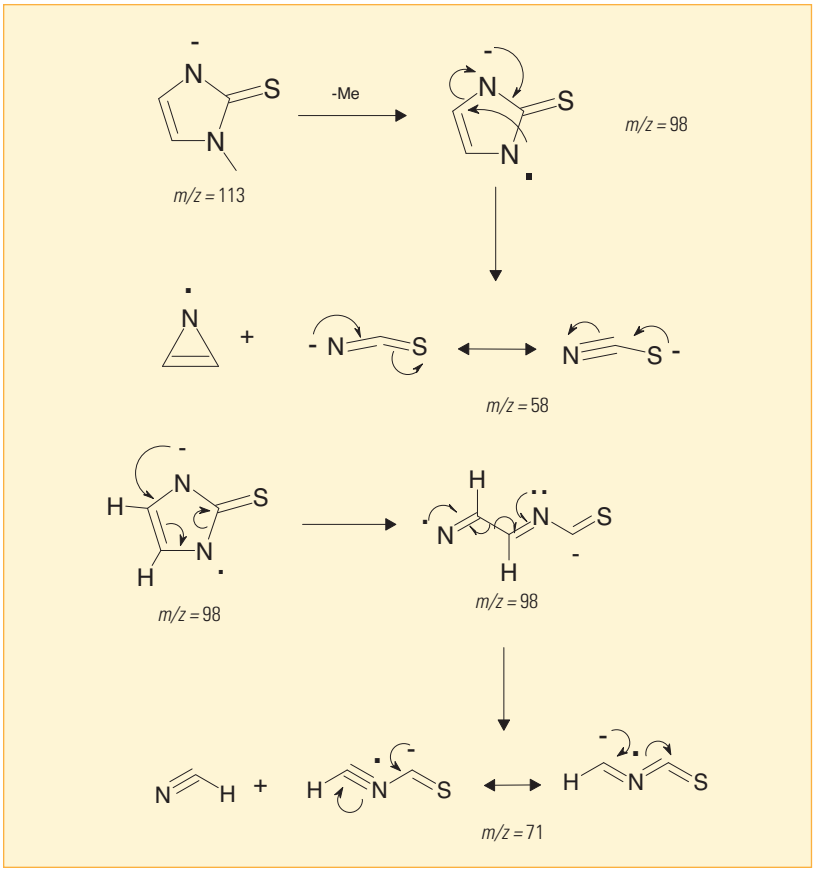

Figure 5: Proposed CID fragmentation mechanisms for the major fragment ions from deprotonated Methimazole at *m/z* 113 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments

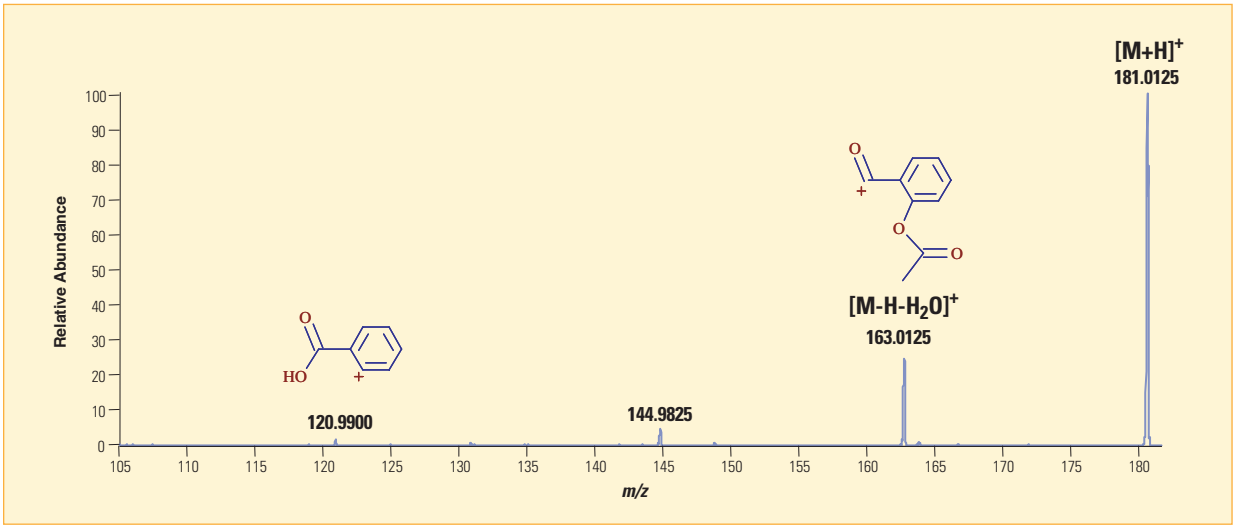

Figure 6: Positive ion ESI mass spectra of acetylsalicylic acid (MW= 180): CID product ion spectrum (MS/MS) of $[M+H]^+$ at *m/z* 181. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer

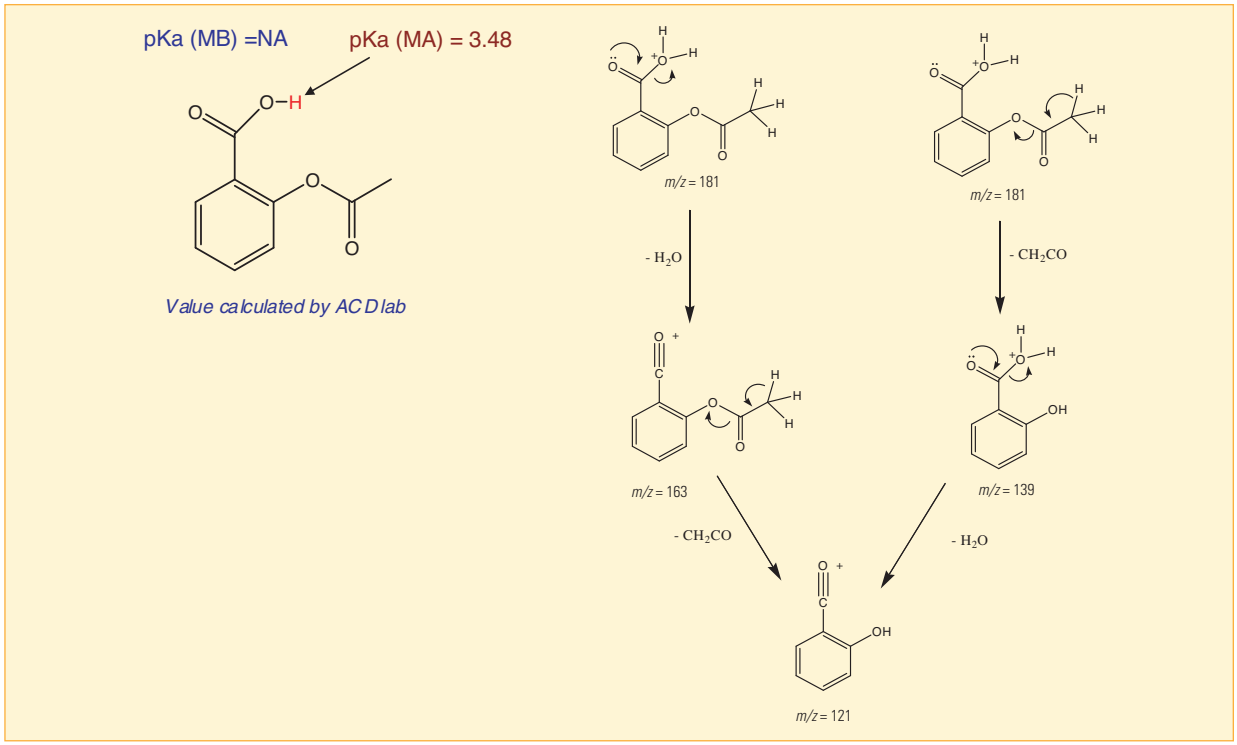

Figure 7: Proposed CID fragmentation mechanisms for the major fragment ions from protonated Acetylsalicylic Acid at *m/z* 181 determined from high-resolution mass measurements and MS/MS experiments

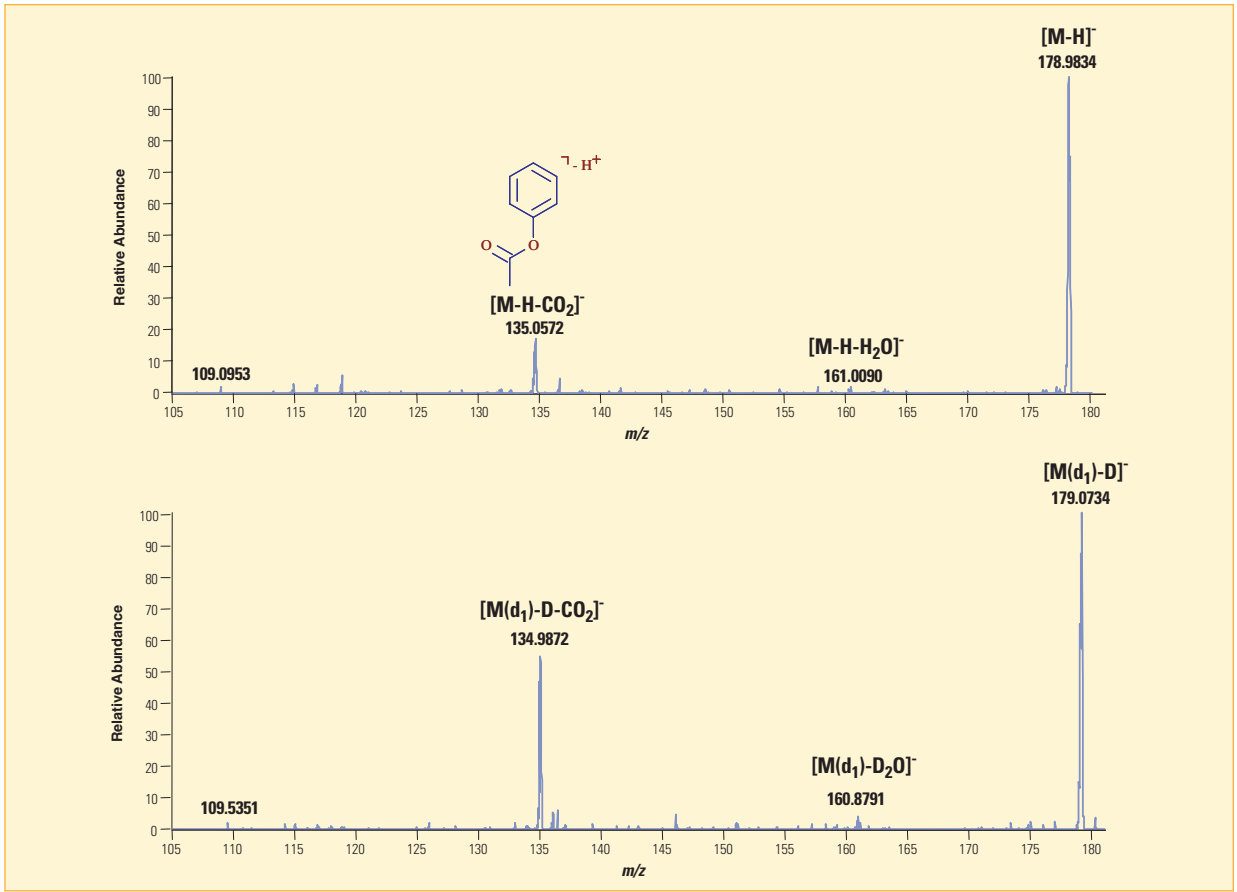

Figure 8: Negative ion ESI mass spectra of acetylsalicylic acid (MW= 180): CID product ion spectra (MS/MS) of $[M-H]^-$ at *m/z* 179 and the fully exchanged $[M(d1)-D]^-$ at *m/z* 179. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

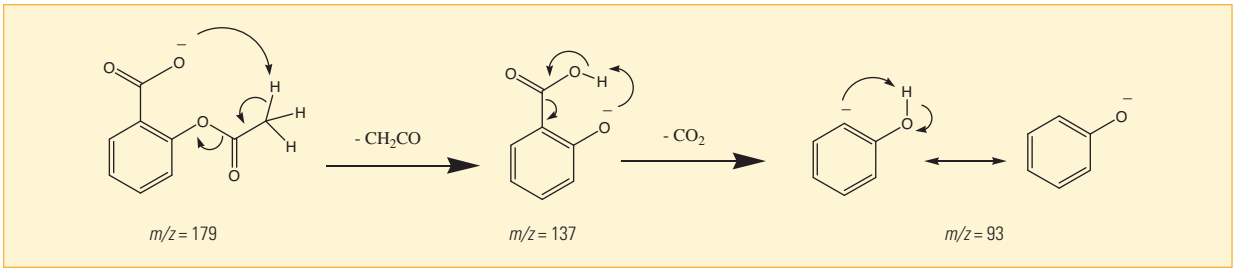

Figure 9: Proposed CID fragmentation mechanisms for the major fragment ions from deprotonated acetylsalicylic acid at *m/z* 179 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments

Conclusions

Protonated methimazole dissociates primarily through elimination of HCN. Loss of CH_3, S, and C_2H_3N are minor dissociation pathways from $[M+H]^+$ ion. The CID mass spectrum of $[M-H]^-$ ion show loss of methyl group as the initial dissociation step followed by the loss of HCN. The fragment ion NCS at *m/z* 58 is also observed.

For protonated aspirin, elimination of H_2O and acetyl group (CH_2CO) were the two major competitive decomposition pathways. The CID mass spectrum of the [M-H]- of aspirin was much simpler and showed the loss of CH_2CO at *m/z* 137 as the primary dissociation pathway. Subsequent elimination of CO_2 from the fragment ion at *m/z* 137 was a minor fragmentation pathway.

The TSQ Quantum Ultra AM Mass Spectrometer system was designed to provide superior performance, while maintaining a level of flexibility not available on similar platforms.

- HyperQuad electrodes enabled acquisition of mass spectra at resolutions below 0.2 Da FWHM, without a significant reduction in signal response.
- High-resolution CID mass spectra were acquired using the TSQ Quantum Ultra AM mass spectrometer system of the fully-exchanged species. The high-resolution mass measurements aided greatly in structural assignments of fragment ions.
- The mass spectra of Methimazole and aspirin can serve as useful models for structure determination of chemically or biologically modified Methimazole and aspirin or related compounds.

References

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