

# Mechanisms of Ion Formation for Famotidine and Azithromycin using Hydrogen/Deuterium Exchange and High Resolution Mass Measurements

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## Introduction

Famotidine is in a class of medications called histamine H<sub>2</sub>-receptor antagonists and is being used to treat ulcers and gastroesophageal reflux disease.<sup>[1]</sup> Azithromycin (Zythromax) is a semi-synthetic macrolide antibiotic which is effective against a wide variety of bacteria organisms. Azithromycin has a long half-life allowing for once a day dosing and for shorter treatment courses for most infections.<sup>[2]</sup>

Mass spectrometry has played a significant role in the quantitative analysis of famotidine and azithromycin. However, detailed CID studies of these drugs have not been reported. The present study was conducted to assess the structures and mechanisms of formation of principal fragment ions in the ESI mass spectra of these three drugs. The CID mass spectra of famotidine and azithromycin and their corresponding deuterated analogs have been studied in both 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 measurement. The mass spectra of famotidine and azithromycin can serve as useful models for structural determination of chemically or biologically modified famotidine and azithromycin or related compounds.

## Goals

Demonstrate the utility of the 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). Famotidine and azithromycin (Zythromax) were provided by Pfizer Global Research and Development, Groton, CT. Ammonium-<sup>15</sup>N, d<sub>4</sub> deuterioxide solution, CD<sub>3</sub>OD, D<sub>2</sub>O and Acetic acid-d<sub>4</sub> were purchased Sigma-Aldrich (St. Louis, MO). All chemicals were used as received.

**Sample preparation:** Stock solutions of famotidine and azithromycin 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 (Thermo). 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<sub>4</sub>, and D). 50:50 HPLC grade methanol and 50 mM Ammonium-<sup>15</sup>N, d<sub>4</sub> deuterioxide. 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 (famotidine); 23eV azithromycin
Collision pressure:	1.2 mTorr
Q1/Q3 resolution:	0.1 Da FWHM
Accurate mass mode:	Internal
Micro scans:	2

## Key Words

- TSQ Quantum Ultra AM™
- CID
- ESI
- Ionization mechanisms

## Results

Protonated famotidine dissociates primarily through loss of  $\text{NHSO}_2$  to give rise to the fragment ion at  $m/z$  259 which dissociates through two competitive fragmentation pathways; loss of  $\text{NH}_3(\text{ND}_3)$  and elimination of  $\text{C}_3\text{H}_6\text{N}_2$ . CID mass spectrum of  $[\text{M}-\text{H}]^-$  ion shows the loss of  $\text{H}_2\text{NSO}_2\text{NH}_2$  ( $\text{D}_2\text{NSO}_2\text{ND}_2$ ) and subsequent loss of  $\text{C}_3\text{H}_3\text{N}$  as primary dissociation pathways. The fragment

ion at  $m/z$  95(98) was also detected in the mass spectrum and corresponded to  $\text{H}_2\text{NSO}_2\text{NH}^-$ . Subsequent loss of  $\text{NH}_3(\text{ND}_3)$  gave rise to the fragment ion at  $m/z$  78.

For protonated azithromycin, elimination of  $\text{H}_2\text{O}$  and successive loss of the two sugar moieties were the major fragmentation pathways. The CID mass spectrum of the  $[\text{M}-\text{H}]^-$  of azithromycin was very similar to that of the protonated species and showed successive loss of the two sugar moieties as the major dissociation pathways.

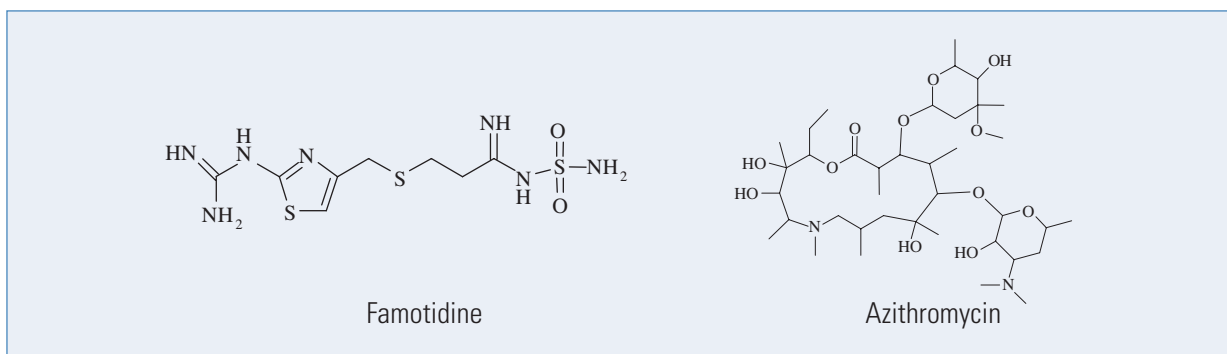


Figure 1: Structures of famotidine and azithromycin (Zythromax)

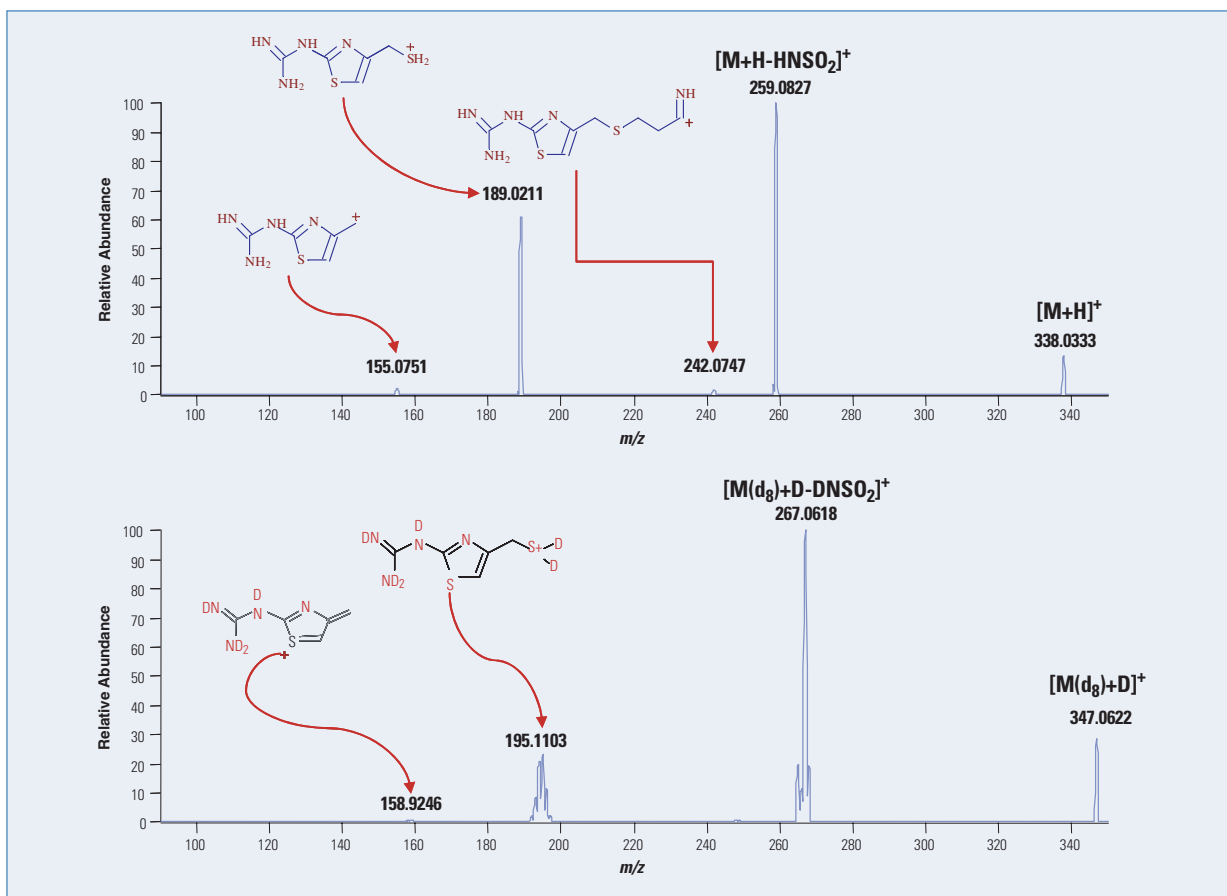


Figure 2: Positive ion ESI mass spectra of famotidine (MW= 337): CID product ion spectra (MS/MS) of  $[\text{M}+\text{H}]^+$  at  $m/z$  338 and the fully exchanged  $[\text{M}(\text{d}_8)+\text{D}]^+$  at  $m/z$  347. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

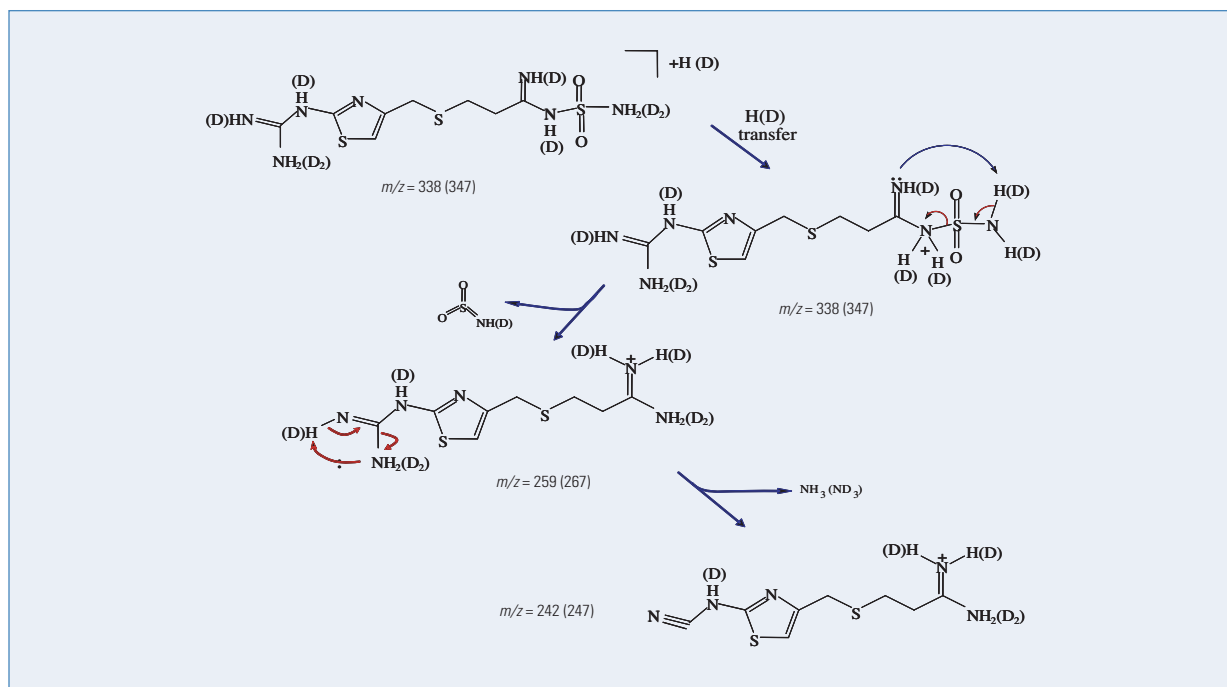


Figure 3: Proposed CID fragmentation mechanisms for the major fragment ions from protonated famotidine at  $m/z$  338 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments. Numbers in parentheses refer to deuterated fragment ions.

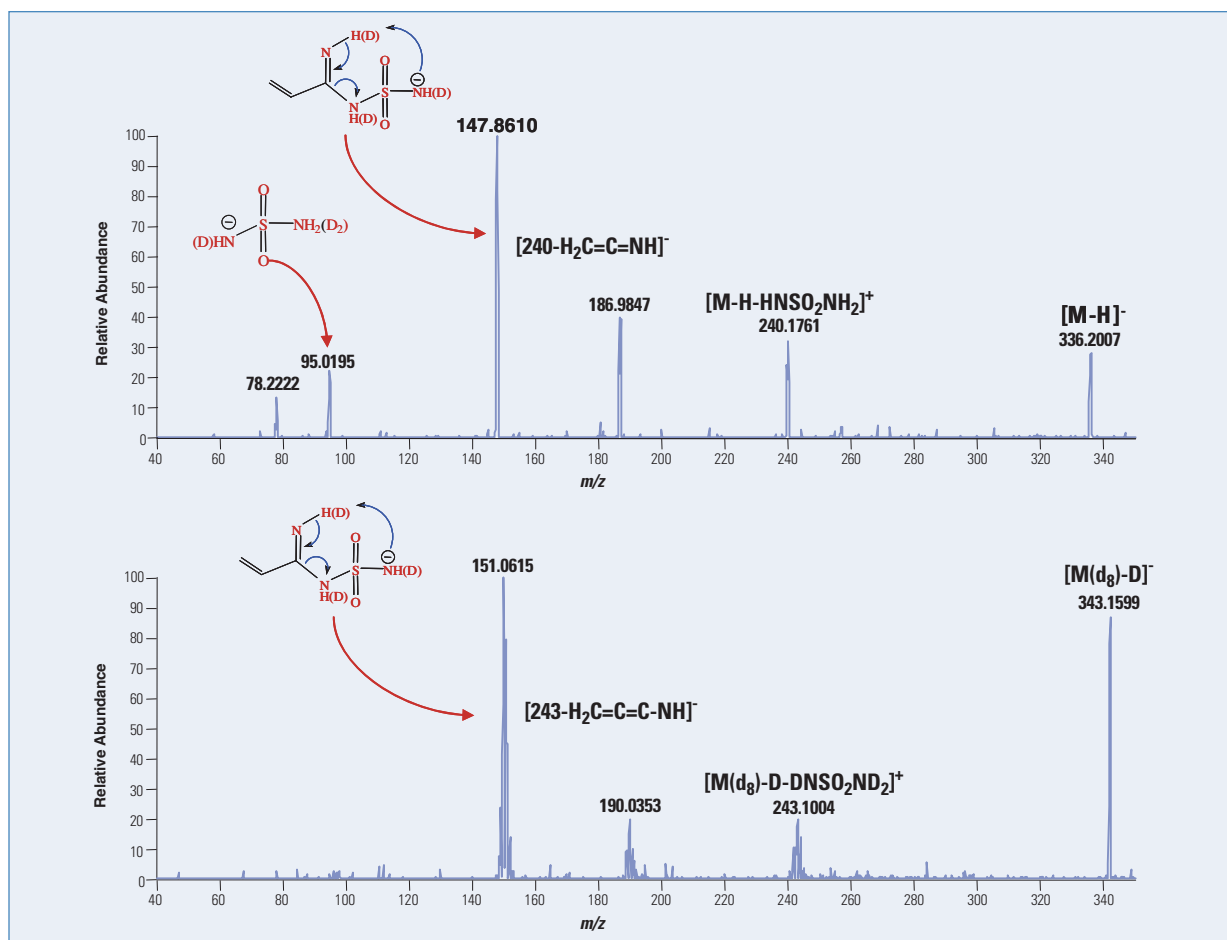


Figure 4: Negative ion ESI mass spectra of famotidine (MW=337): CID product ion spectra (MS/MS) of  $[M-H]^-$  at  $m/z$  336 and the fully exchanged  $[M(d_8)-D]^-$  at  $m/z$  343. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

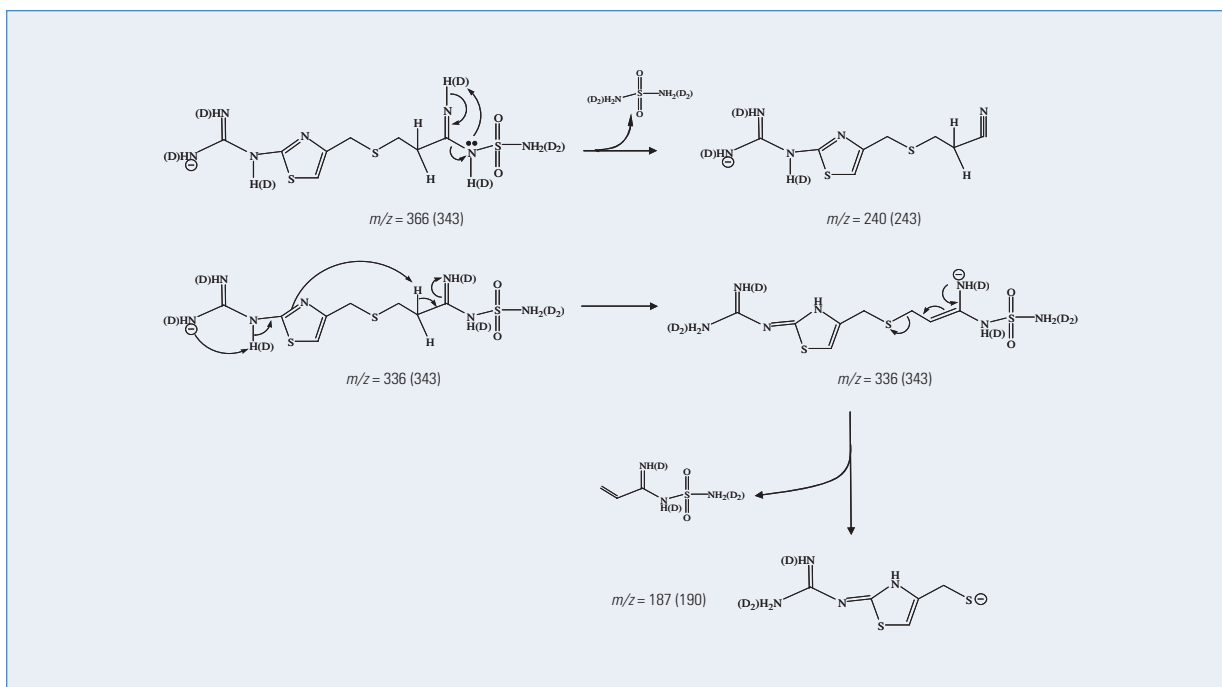


Figure 5: Proposed CID fragmentation mechanisms for the major fragment ions from deprotonated famotidine at  $m/z$  336 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments.

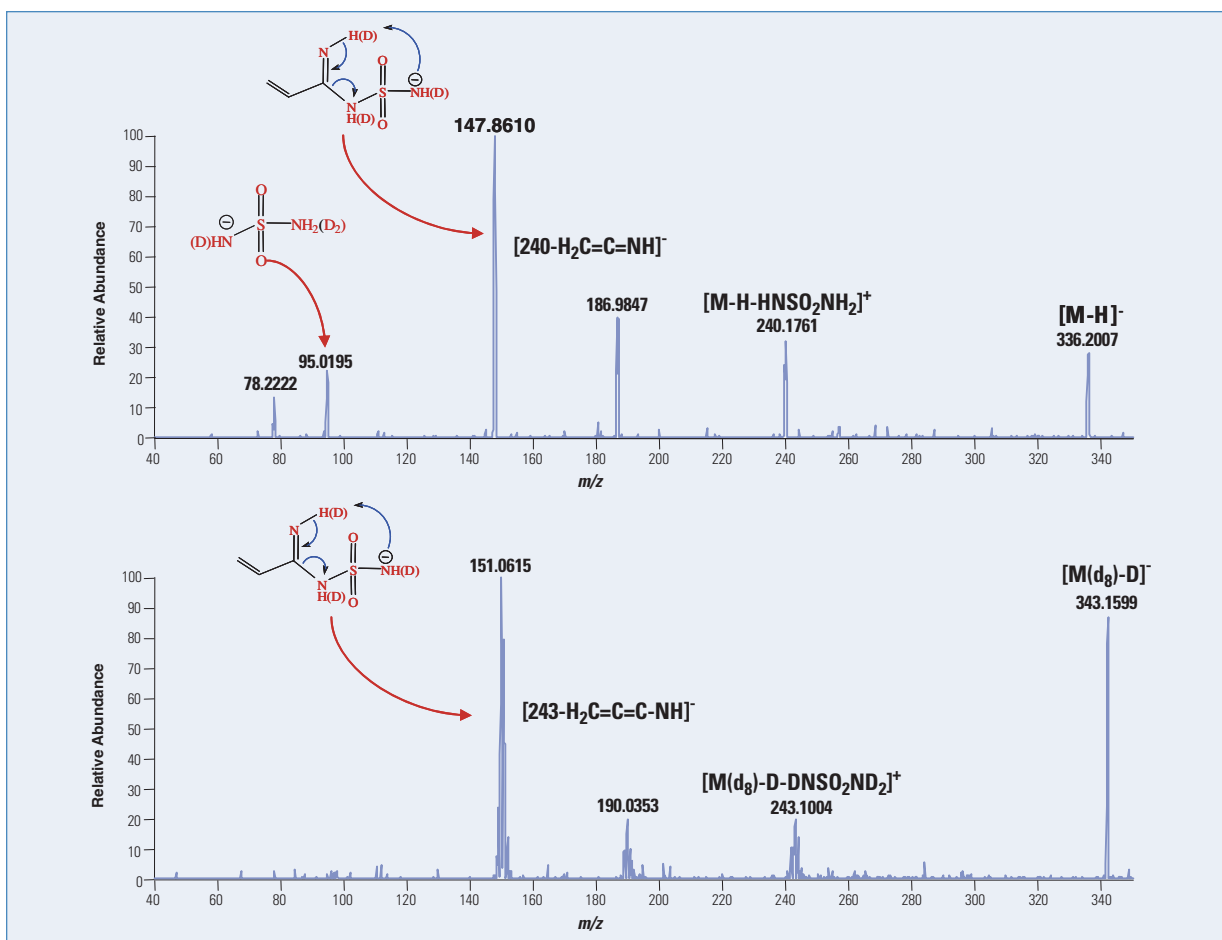


Figure 6: Positive ion ESI mass spectra of azithromycin (MW=748): CID product ion spectra (MS/MS) of  $[\text{M}+\text{H}]^+$  at  $m/z$  749 and the fully deuterated  $[\text{M}(\text{d}_8)+\text{D}]^+$  at  $m/z$  755. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

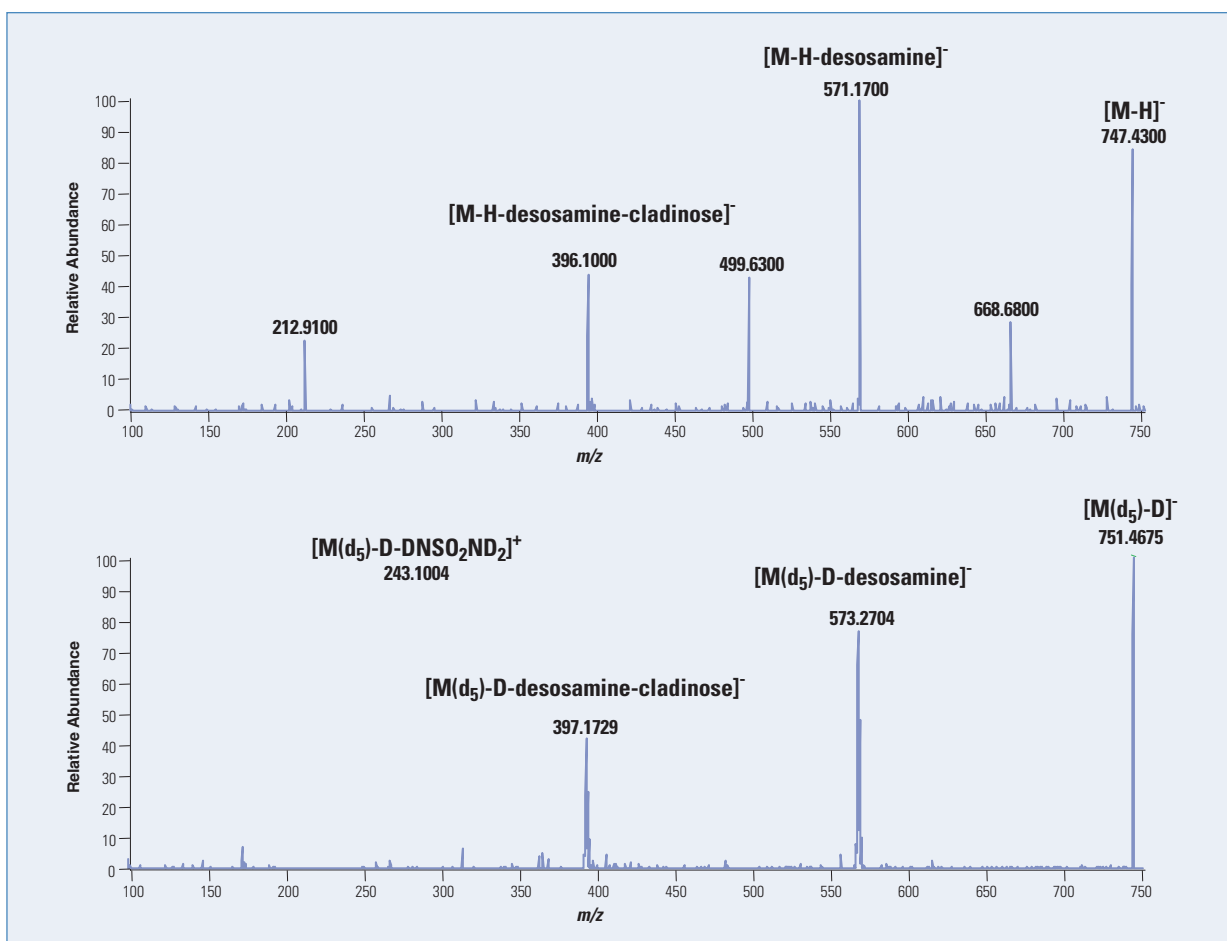


Figure 7: Negative ion ESI mass spectra of azithromycin (MW=748): CID product ion spectra (MS/MS) of [M-H]<sup>-</sup> at *m/z* 747 and the fully exchanged [M(d<sub>5</sub>-D)]<sup>-</sup> at *m/z* 751. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

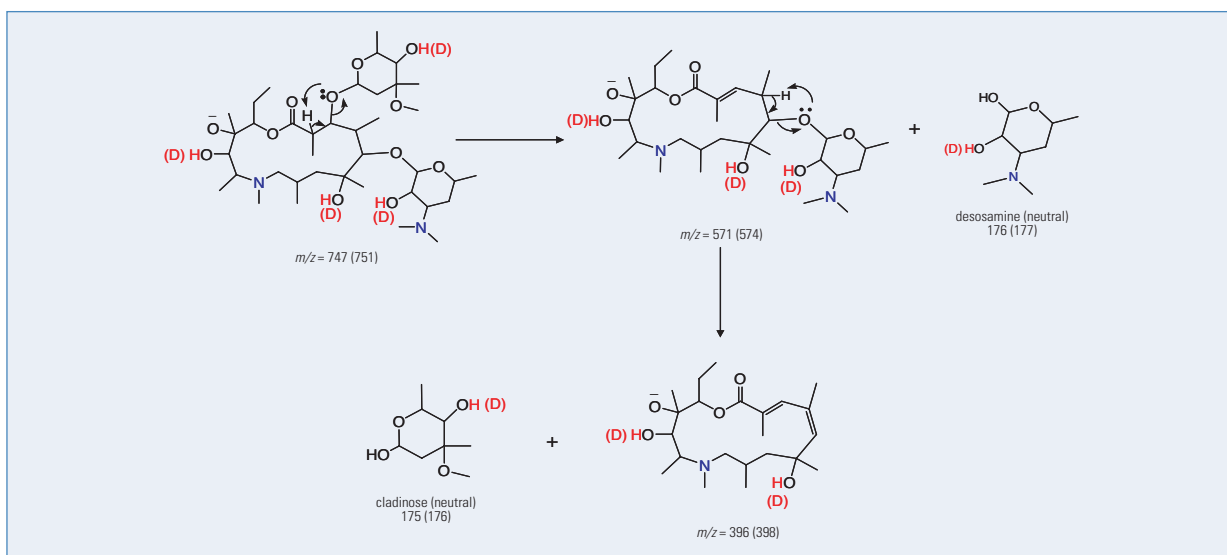


Figure 8: Proposed CID fragmentation mechanisms for the major fragment ions from deprotonated azithromycin at *m/z* 747 determined from H/D exchange patterns, high-resolution mass measurements and MS/MS experiments. Numbers in parentheses refer to deuterated fragment ions. The proposed site of deprotonation is based on the most acidic proton of the lactone ring.

## Conclusions

Protonated famotidine dissociates primarily through loss of  $\text{NHSO}_2$  to give rise to the fragment ion at  $m/z$  259 which dissociates through two competitive fragmentation pathways; loss of  $\text{NH}_3(\text{ND}_3)$  and elimination of  $\text{C}_3\text{H}_6\text{N}_2$ . CID mass spectrum of  $[\text{M}-\text{H}]^-$  ion show the loss of  $\text{H}_2\text{NSO}_2\text{NH}_2$  ( $\text{D}_2\text{NSO}_2\text{ND}_2$ ) and subsequent loss of  $\text{C}_3\text{H}_3\text{N}$  as primary dissociate pathways. The fragment ion at  $m/z$  95(98) was also detected in the mass spectrum and corresponded to  $\text{H}_2\text{NSO}_2\text{NH}^-$ . Subsequent loss of  $\text{NH}_3(\text{ND}_3)$  gave rise to the fragment ion at  $m/z$  78.

For protonated azithromycin, elimination of  $\text{H}_2\text{O}$  and successive loss of the two sugar moieties were the major fragmentation pathways. The CID mass spectrum of the  $[\text{M}-\text{H}]^-$  of azithromycin was very similar to that of the protonated species and showed successive loss of the two sugar moieties as the major dissociation pathways.

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

- HyperQuad™ quadrupole mass analyzers 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 helped determine structural assignments of fragment ions.
- The CID mass spectra of the fully exchanged species and high resolution mass measurements helped determine structural assignments of all fragment ions. The mass spectra of famotidine and azithromycin can serve as useful models for structure determination of chemically or biologically modified famotidine and azithromycin or related compounds.

## References

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