

# JMS Letter

Dear Sir,

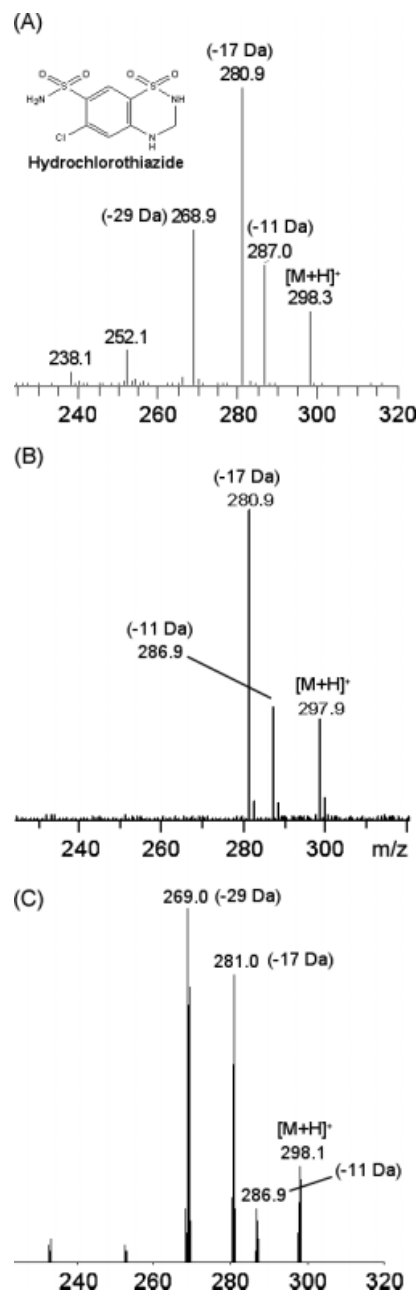
## Formation of the unusual $[M+H-11\text{ Da}]^+$ ion peak in the collision-induced dissociation mass spectrum of $[M+H]^+$ ion of hydrochlorothiazide

Hydrochlorothiazide (HCT) is one of the diuretics that is banned in sport by the World Anti-Doping Agency (WADA). In continuation of our diuretics confirmation work for anti-doping purposes, the tandem mass (MS/MS) spectrum of the HCT under low-energy collision-induced dissociation (CID) mass spectrometry with positive electrospray ionization (ESI) was investigated and interpreted in detail. The CID of the protonated HCT at  $m/z$  298 generated the unexpected product ion at  $m/z$  287 corresponding to the  $[M+H-11\text{ Da}]^+$  ion of HCT on a 3D-ion trap mass spectrometer (MS). Although it is well known that ions undergo gas-phase rearrangements under low-energy CID conditions, the formation of this unexpected product ion could not be easily explained by the conventional mechanism.<sup>[1–8]</sup> Therefore, the aim of the present study is essentially to rationalize the uncommon behavior resulting in the unusual  $[M+H-11\text{ Da}]^+$  ion peak under the low-energy CID.

CID experiments were performed using three MS systems: Agilent 1100 series LC/MSD (3D) ion trap, Thermo hybrid-linear (2D) ion trap/Orbitrap and Sciex API-2000 triple-quadrupole (TQ) mass spectrometers, each equipped with an electrospray interface using nitrogen gas. Samples (10  $\mu\text{g}/\text{ml}$ ) in MeOH/water/formic acid (50:50:0.1, vol:vol:vol) solution were infused into the ESI source in a positive ion mode at a rate of 5  $\mu\text{l}/\text{min}$  via a syringe pump. For ion traps and the TQ mass analyzer, ion spray voltage was adjusted to 4500 and 5500 V, respectively. Helium and nitrogen were used as collision gases for the tandem mass spectrometric experiments. For accurate mass measurement, high-resolution ( $R = 60\,000$ ) and high-accuracy 2D ion trap mass spectrometric analyses were conducted using a Finnigan LTQ Orbitrap mass spectrometry (Thermo Fisher Scientific Inc., MA, USA) operated in a positive-ion electrospray mode, whereas others followed the 2D ion trap mass spectrometer.

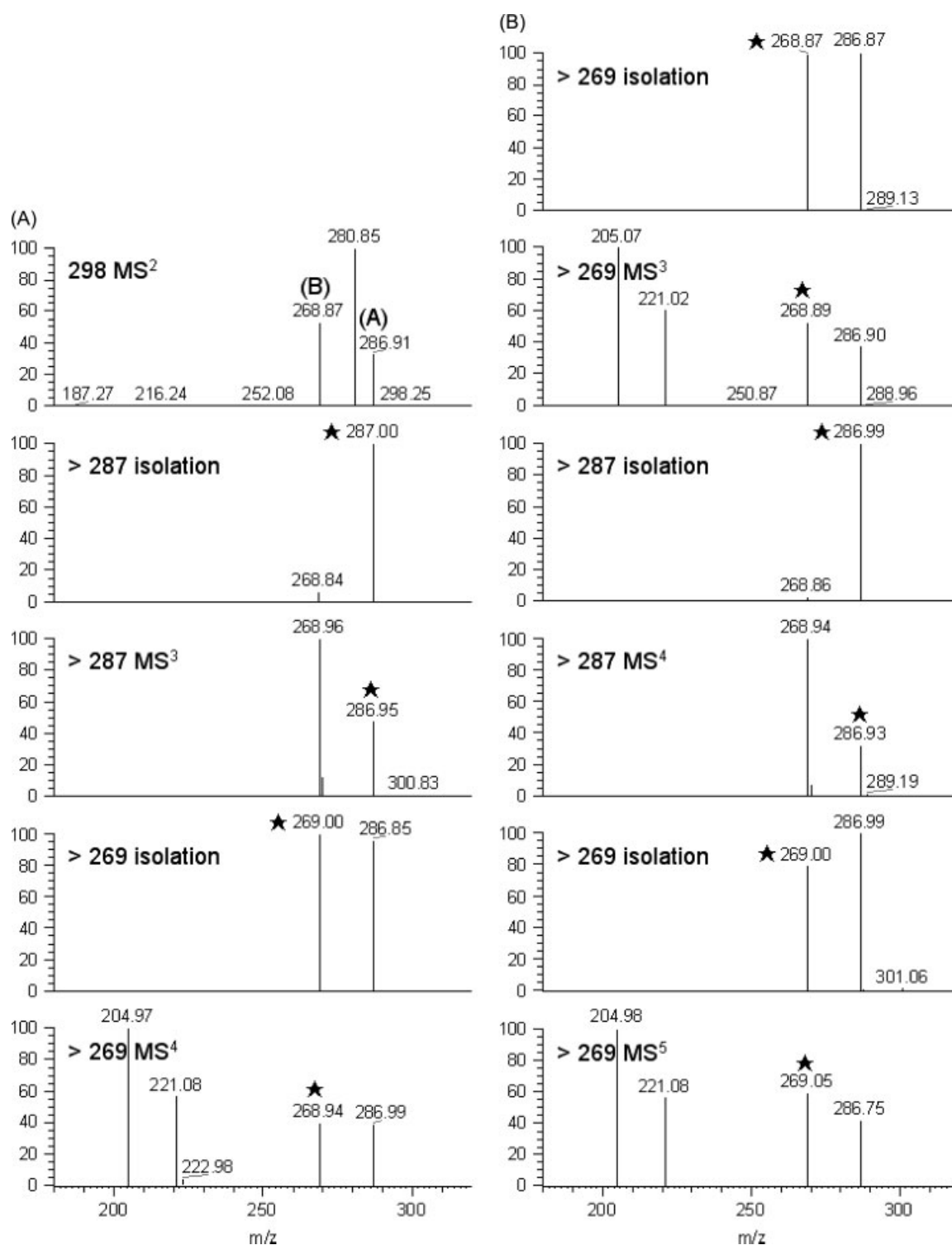
The MS/MS spectrum of the protonated HCT ( $m/z$  298) on the 2D-ion trap MS is depicted in Fig. 1(A). Representative fragment ions of HCT were observed at  $m/z$  287, 281 and 269, and the product ions at  $m/z$  281 and 269 could be rationalized by the neutral loss of  $\text{NH}_3$  (17 Da) and  $\text{NH}=\text{CH}_2$  (29 Da), respectively. As shown in Fig. 1(B) and (C), the product ion at  $m/z$  269 was not observed in the 3D-ion trap MS and it was shown to have a relatively high intensity on TQ MS. Among these product ions, we were especially interested in the ion at  $m/z$  287 corresponding to the unusual  $[M+H-11\text{ Da}]^+$  ion.

In order to examine the origin of the unexpected ion at  $m/z$  287, the respective ions at  $m/z$  287 and 269 were isolated and then subjected to collisional activation in 2D-ion trap MS. As shown in Fig. 2, the attempt to isolate the ion at  $m/z$  269 was unsuccessful, and the ion at  $m/z$  287 was constantly present. When the ion at  $m/z$  269 regenerated by the CID of the ion at  $m/z$  287 was isolated, the same result was observed. Consequently, 'ping-pong' MS<sup>n</sup> experiments between the ions at  $m/z$  269 and  $m/z$  287 were allowed.<sup>[9]</sup> These results strongly suggest that there is a correlation between the ions at  $m/z$  287 and 269, and the interesting ion at  $m/z$  287 was generated by an 18 Da increase from the ion at  $m/z$  269. The fact that the ion at  $m/z$  287 was 18 Da greater in mass than the ion at  $m/z$  269 allowed us to speculate that the  $m/z$  287 ion might have one more  $\text{H}_2\text{O}$  molecule than the  $m/z$  269 ion. The accurate mass of the ions at  $m/z$  269 and 287 was measured as 268.94529 and 286.95586 Da using LTQ Orbitrap mass spectrometry and was identical to the calculated mass of the even electron cation  $\text{C}_6\text{H}_6\text{O}_4\text{N}_2^{35}\text{Cl}^{32}\text{S}_2^+$  (268.94520 Da) and  $\text{C}_6\text{H}_8\text{O}_5\text{N}_2^{35}\text{Cl}^{32}\text{S}_2^+$  (286.95576 Da) with errors of 0.317 and 0.316 ppm, respectively.



**Figure 1.** MS/MS spectra of protonated HCT obtained from 2D ion trap (A), 3D ion trap (B) and triple-quadrupole (C) mass spectrometry.

\* Correspondence to: Jaeick Lee, Doping Control Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul, South Korea. E-mail: jaeicklee@kist.re.kr

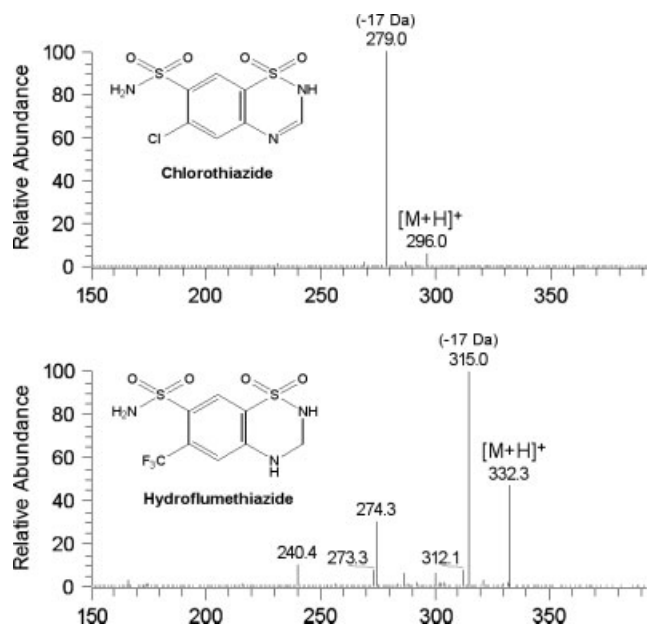


**Figure 2.** Isolation and MS<sup>n</sup> spectra of the ions at *m/z* 287 (A) and 269 (B) generated from MS<sup>2</sup> of protonated HCT (*m/z* 298).

The MS/MS spectra of chlorothiazide (CT) and hydroflumethiazide (HFT) as structural analogs on the 2D-ion trap MS are depicted in Fig. 3. Surprisingly, the unusual fragment ion as well as the aminobenzenedisulfone ion corresponding to the neutral loss of 29 Da of the HCT was not observed in the CID of protonated CT and HFT. Furthermore, the CID of bendroflumethiazide, benzthiazide and polythiazide showed the same results (data not shown). When considering the structural similarity, these results revealed that the generation of the unusual fragment ion was essentially initiated from the aminobenzenedisulfone

ion, and the proton affinity to the 4-position of thiadiazinane moiety played a significant role in the formation of the aminobenzenedisulfone ion.

A mechanism for the formation of the ions at *m/z* 287, 281 and 269 is proposed in Fig. 4. We suggest two possible protonation sites for the HCT. The formation of protonated HCT, in which the protonation occurred on -NH<sub>2</sub> of benzenesulfonamide could dissociate to the even-electron cation (EE<sup>+</sup>) at *m/z* 281 and the neutral molecule of 17 Da (NH<sub>3</sub>) via heterolytic cleavage of an S–N bond. On the other hand,



**Figure 3.** MS/MS spectra of protonated CT and HFT obtained from 2D ion trap mass spectrometry.

the protonation on -NH- at the 4-position of thiazidiazine moiety could give rise to the even-electron cation at  $m/z$  269 and the neutral molecule of 29 Da ( $\text{NH}=\text{CH}_2$ ) via heterolytic cleavage of the N-C and N-S bond, subsequently leading to the generation of the even-electron cation at  $m/z$  287 by ion-molecule reaction with the water molecule.

Similar phenomena that in the ion trap certain ions undergo ion-molecule reactions with background-solvent molecules such as water under positive ESI conditions were reported in the CID of isoquinolines, ilicicolin H, perlatolinic acid, amiloride and guanosine.<sup>[9-12]</sup> The CID of these compounds generated common arylacylium-like fragment ions, and it was revealed that arylacylium-like fragment ions undergo ion-molecule reactions with the water molecule. To our knowledge, it may be the first report that the arylsulfonium ions of HCT can undergo ion-molecule reactions with the water molecule.

To date, there have been two possibilities to the source of the water molecule in the collision cell.<sup>[10,12]</sup> One is the contamination from ion source and the other is the origin from collision gas. In order to

examine the source of the water molecule, we performed an infusion experiment with modified solvents (methanol, ethanol, etc). The formation of other adducts was not observed (data not shown). As a result, based on the evidence by Esmans *et al.*<sup>[12]</sup>, we indirectly suggest that this reaction of arylsulfonium ion results from the residual water molecule of collision gas, but alternative suggestions for this reaction cannot be excluded.

In conclusion, these data collectively suggest that the arylsulfonium ion can undergo the ion-molecule reaction with the water molecule under the low-energy CID condition. Additionally, the reaction was observed only for HCT. This reaction was rationalized by the reaction with the water molecule originating from collision gas via the formation of the aminobenzendisulfone ion, but alternative suggestions for this reaction cannot be excluded.

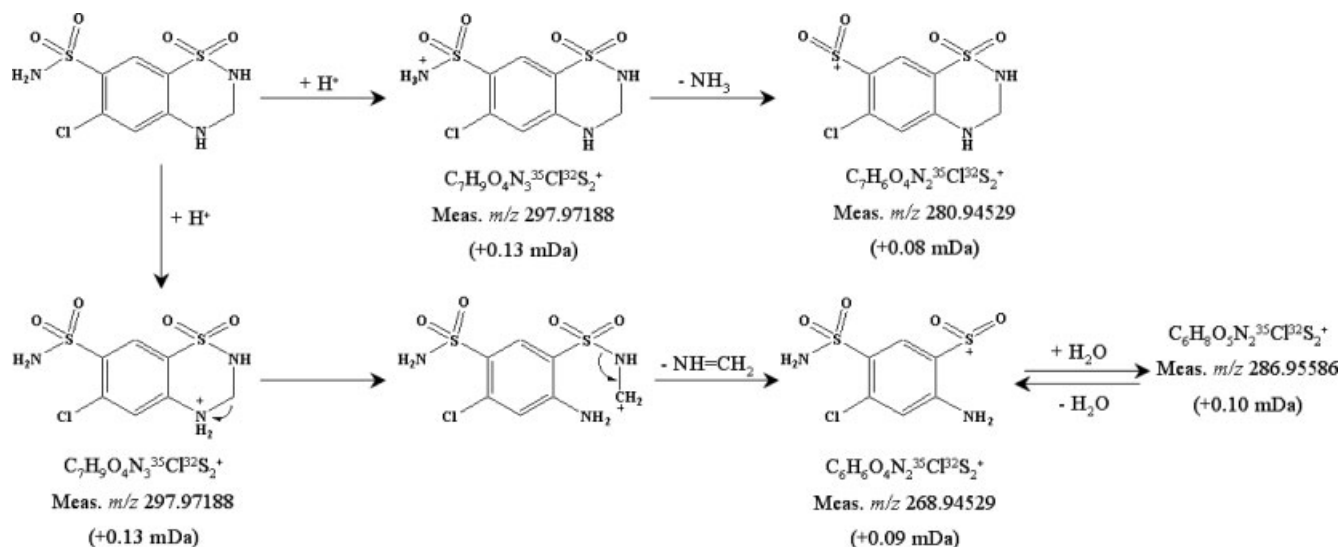
Yours,

SANG KYU LEE, HO JUN KIM, CHANGBAE JIN AND JAEICK LEE\*

Doping Control Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul, South Korea

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**Figure 4.** Proposed fragmentation mechanism for the formation of product ions of the  $[\text{M}+\text{H}]^+$  ion ( $m/z$  287) of HCT.

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