Mechanism of $[M + H]^+$ Formation in Atmospheric Pressure Photoionization Mass Spectrometry: Identification of Propionitrile in Acetonitrile with High Mass Accuracy Measurement and Tandem Mass Spectrometry and Evidence for Its Involvement in the Protonation Phenomenon

Amin Kamel,[a] Patrick Jeanville,[b] Kevin Colizza,[a] and Lauren Elizabeth J-Rivera[c]

[a] Department of Exploratory Medicinal Sciences, Pfizer Global Research and Development, Groton Laboratories, Groton, Connecticut, USA
[b] ThermoFisher Scientific, Drug Metabolism, West Palm Beach, Florida, USA
[c] Biomedical Sciences Department, Barry University, Miami Shores, Florida, USA

The role of propionitrile in the production of $[M + H]^+$ under atmospheric pressure photoionization (APPI) was investigated. In dopant-assisted APPI using acetone and anisole, protonated acetone and anisole radical cations were the most prominent ions observed. In dopant-free or direct APPI in acetonitrile, however, a major ion in acetonitrile was detected and identified as propionitrile, using high accuracy mass measurement and collision induced dissociation studies. Vaporizing a 10^{-5} M althiazide and bendroflumethazide under direct APPI in acetonitrile produced their corresponding protonated species $[M + H]^+$. In addition to protonated acetonitrile, its dimers, and acetonitrile/water clusters, protonated propionitrile, propionitrile dimer, and propionitrile/water clusters were also observed. The role of propionitrile, an impurity in acetonitrile and/or a possible product of ion–molecule reaction, in the production of $[M + H]^+$ of althiazide and bendroflumethazide was further investigated in the absence of dopant using propionitrile-d_5. The formation of $[M + D]^+$ species was observed, suggesting a possible role of propionitrile in the protonation process. Additionally, an increase in the $[M + H]^+$ signal of althiazide and bendroflumethazide was observed as a function of propionitrile concentration in acetonitrile. Theoretical data from the literature supported the assumption that one possible mechanism, among others, for the formation of $[M + H]^+$ could be attributed to photo-initiated isomerization of propionitrile. The most stable isomers of propionitrile, based on their calculated ionization energy (IE) and relative energy (ΔE), were assumed to undergo proton transfer to the analytes, and mechanisms were proposed. (J Am Soc Mass Spectrom 2008, 19, 1579–1589) © 2008 American Society for Mass Spectrometry

Atmospheric pressure photoionization (APPI) is a novel ionization technique recently introduced by Robb et al. [1] and Syage and Evans [2] to broaden the range of ionizable analytes to include molecules with weaker polarity. The ionization process in APPI is initiated by 10 eV photons, emitted by a krypton (Kr) discharge lamp. The photons can ionize compounds that possess ionization energies (IEs) below their energy (10 eV), which may include analytes, but leaves out most LC solvents that have relatively higher IEs compared with that of the Kr lamp. Thus, the analytes can be ionized selectively, with less susceptibility to matrix-induced ion suppression and minimum background interference [3–7].

Two approaches of APPI have emerged: dopant-assisted APPI and dopant-free or direct APPI and the ionization mechanisms for the formation of $[M^+]$, $[M^{-\cdot}]$, $[M + H]^+$, and $[M - H]^-$ have been thoroughly investigated and reported [8–19]. The initial reaction in dopant-assisted positive ion APPI is the formation of a radical cation of the dopant if the IE of the dopant is lower than that of the photon energies of the Kr lamp

Address reprint requests to Dr. A. Kamel, Department of Exploratory Medicinal Sciences, Pfizer Global Research and Development, Groton Laboratories, Groton, CT 06340, USA. E-mail: amin.m.kamel@pfizer.com

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(Reaction 1). The radical cation of the dopant may ionize the analyte through a charge exchange reaction if the IE of the radical cation of the dopant is higher than that of the neutral analyte (Reaction 2). Alternatively, the dopant radical cation can ionize the analyte (Reaction 3), and solvent molecules (Reaction 4) by proton transfer if the proton affinity (PA) of the analyte and solvent is higher than that of the deprotonated dopant radical cation. Similarly, the analyte radical cation can be deprotonated and ionize solvent molecules by proton transfer if the PA of the solvent is higher than that of the deprotonated analyte radical cation (Reaction 5). The protonated solvent molecules can then protonate the analyte if the PA of the analyte is higher than that of the solvent (Reaction 6).

$$D\ (\text{dopant}) + h\nu \rightarrow D^{\cdot+} + e^- \text{ if IE (D)} < h\nu \quad (1)$$

$$D^{\cdot+} + M\ (\text{analyte}) \rightarrow M^{\cdot+} + D \text{ if IE (M)} < \text{IE (D)} \quad (2)$$

$$D^{\cdot+} + M \rightarrow [M + H]^+ + [D - H]^{\cdot} \text{ if PA (M)} > [D - H]^{\cdot} \quad (3)$$

$$D^{\cdot+} + S\ (\text{solvent}) \rightarrow [S + H]^+ + [D - H]^{\cdot} \text{ if PA (S)} > \text{PA } [D - H]^{\cdot} \quad (4)$$

$$M^{\cdot+} + S\ (\text{solvent}) \rightarrow [S + H]^+ + [M - H]^{\cdot} \text{ if PA (S)} > \text{PA } [M - H]^{\cdot} \quad (5)$$

$$[S + H]^+ + M \rightarrow [M + H]^+ + S \text{ if PA (M)} > \text{PA (S)} \quad (6)$$

In dopant-free or direct APPI, the formation of $M^{\cdot+}$ can be easily produced, in principle, by direct photon irradiation (Reaction 7) and the formation of $[M + H]^+$ could be attributed to the reaction between $M^{\cdot+}$ and neutral molecules (Reaction 8). Syage has examined the formation of $[M + H]^+$ by direct photoionization and, based on the overall agreement of the experimental results with a simple thermodynamic model, concluded that the molecular radical ion formation $M^{\cdot+}$ (Reaction 7) followed by hydrogen atom abstraction from protic Solvent S (Reaction 9) is the principal mechanism for the formation of $[M + H]^+$ under direct photoionization [10]. However, other possible proton donors such as water, water/solvent clusters, and analyte itself could also contribute to the formation of $[M + H]^+$ under direct APPI.

$$M + h\nu \rightarrow M^{\cdot+} + e^- \quad (7)$$

$$M^{\cdot+} + M \rightarrow [M + H]^+ + [M - H]^{\cdot} \quad (8)$$

$$M^{\cdot+} + S \rightarrow [M + H]^+ + [S - H]^{\cdot} \quad (9)$$

The role of solvents, including acetonitrile, in the mechanism of ion formation under APPI was investigated by several research groups. Under direct APPI, Marotta and Traldi provided explanation for the unexpected formation of $[M + H]^+$ of some furocoumarins in acetonitrile and attributed their findings to photo-initiated isomerization of acetonitrile [20]. These findings were further supported by semiempirical calculations and the most stable isomer of acetonitrile, ketene imine, was assumed to undergo photoionization by the Kr radiation based on its low IE compared with that of the Kr lamp (Reaction 10). Once ionized, the radical cation of ketene imine can easily react with a neutral molecule of acetonitrile to form the protonated ketene imine (Reaction 11), which can then transfer a proton to form the protonated molecules of furocoumarins.

$$H_2C{=}C{=}NH + h\nu \rightarrow H_2C{=}C{=}NH^{\cdot+} + e^- \quad (10)$$

$$H_2C{=}C{=}NH^{\cdot+} + H_3C - C{\equiv}N \rightarrow H_2C{=}NH_2^+ + H_2C^{\cdot} - C{\equiv}N \quad (11)$$

Syage et al. further investigated the photoabsorption characteristics of acetonitrile and studied solvent ions generated from acetonitrile under direct APPI. In addition to protonated acetonitrile and its dimmer, propionitrile was detected and its formation was attributed to ion–molecule reaction [17]. Propionitrile could also be attributed to an impurity in acetonitrile [22], and trace amount of solvent impurities were suggested to be involved in the ionization process of analytes under APPI [1, 8, 11].

The present study was therefore undertaken to investigate the role of propionitrile in analyte ionization under APPI. We have identified propionitrile, found in acetonitrile either as a result of ion–molecule reaction and/or an impurity, and studied its role as a possible proton donor for the formation of $[M + H]^+$ of althiazide and bendroflumethazide under direct APPI assuming photo-initiated isomerization of propionitrile. Calculated values of the ionization energy (IE) and the relative energy (ΔE) from the literature were used to study the possible role of the most stable isomers of propionitrile to undergo proton transfer to the analyte. These data and further studies may help understand different mechanisms involved in the formation of $[M + H]^+$ in the APPI process.

Experimental

Chemicals and Materials

Althiazide and bendroflumethazide (Figure 1) were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile, propionitrile, anisole, acetone, methanol, and deionized water (HPLC-grade) were obtained from J. T. Baker (Phillipsburg, NJ). Propionitrile-d_5 (99% deuterium content) was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada).

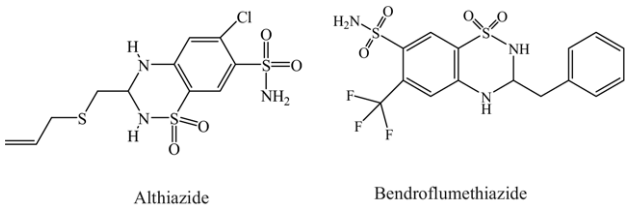


Figure 1. Structures of althiazide and bendroflumethiazide.

Sample Preparation and Introduction

Working standards of althiazide and bendroflumethazide were prepared at a concentration of 1.0 ng/μL in acetonitrile and propionitrile-d_5 for use in this study. For direct APPI, loop injections (5.0 μL) of althiazide and bendroflumethazide were made into a continuous solvent stream of acetonitrile at a flow rate of 200 μL/min. Althiazide and bendroflumethazide in propionitrile-d_5 were infused via a Harvard syringe pump (South Natick, MA) at a flow rate of 20 μL/min. For dopant-assisted APPI, dopant solutions (anisole and acetone) were infused via a Harvard syringe pump as a sheath liquid at a flow rate of 20 μL/min. Propionitrile (from acetonitrile) and propionitrile standard were infused via syringe pump as a sheath liquid at a flow rate of 20 μL/min, and collision induced dissociation (CID) mass spectra were obtained for $[M + H]^+$. To study the effect of propionitrile concentration on $[M + H]^+$ signal of althiazide and bendroflumethazide, a 100 μL aliquot of working standards of althiazide and bendroflumethazide (1.0 ng/μL in acetonitrile) was transferred to separate tubes and dried down. Each residue was redissolved in a 100 μL aliquot of acetonitrile containing varying amounts of propionitrile (0%, 0.01%, 0.02%, 0.05%, 0.1%, 0.5%, 1%, and 2%, vol/vol) and vortex-mixed. Loop injections (3 $\times$ 5.0 μL) of each mixture were made into a continuous solvent stream of acetonitrile at a flow rate of 200 μL/min.

Mass Spectrometry

All experiments were performed using a Finnigan TSQ Quantum Ultra AM and Finnigan Surveyor LC system (Thermo Electron Corp., San Jose, CA). For

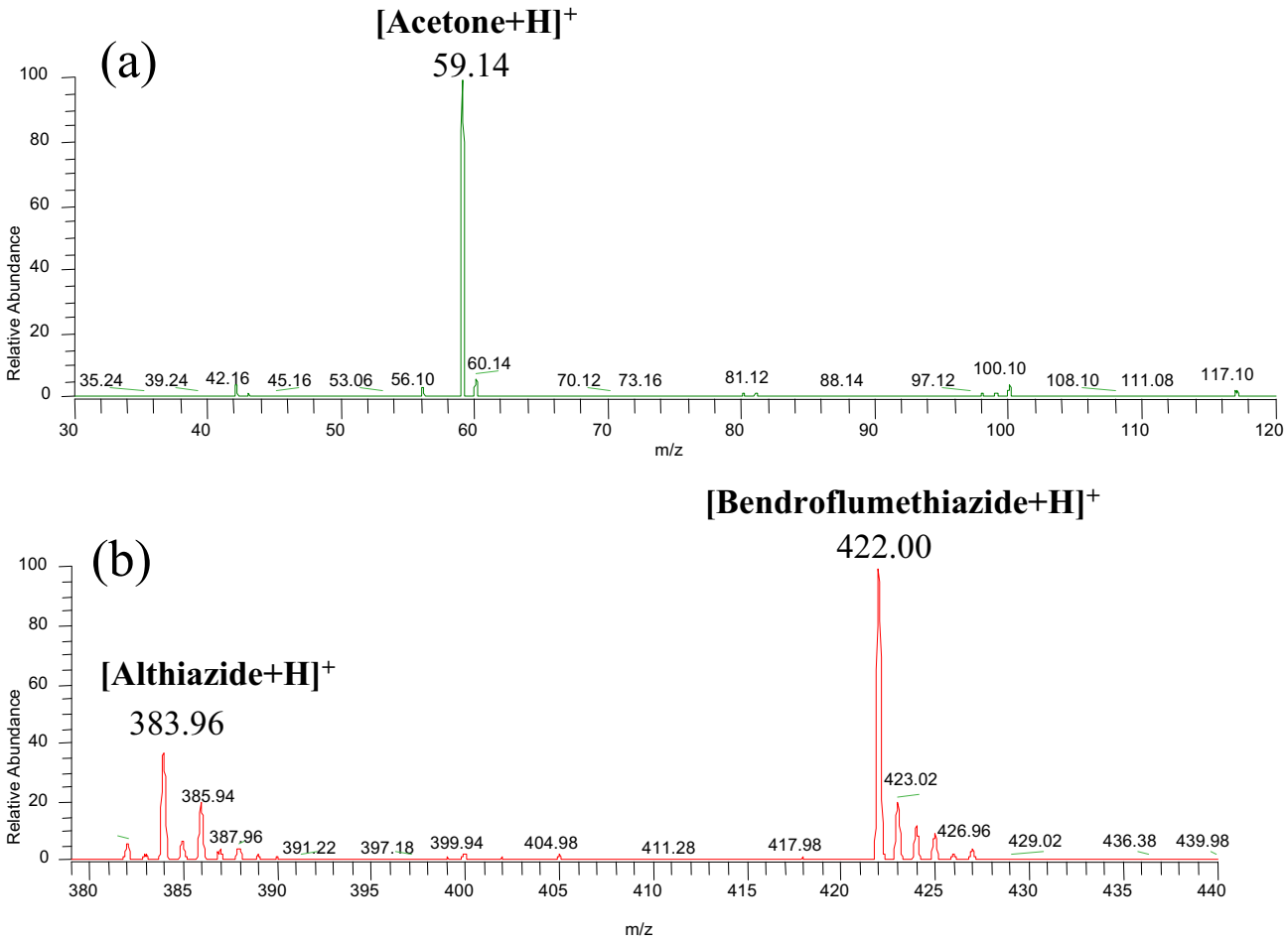


Figure 2. APPI positive ion mass spectra of althiazide and bendroflumethiazide in acetonitrile with acetone as a dopant in the low mass range region (**a**) and in the parent ion region (**b**).

experiments involving accurate mass measurement, a Finnigan, LTQ Orbitrap mass spectrometer operated in the positive ion mode was used. The instrument was calibrated using 1,3,6-polytyrosine and 50 pmol/μL ammoniated polyethylene glycol in 50/50 methanol/water. For accurate mass determinations, solvent ions/clusters ($[ACN + H]^+$ and $[ACN + H_2O + H]^+$) were employed as internal standard references. Mass spectral analyses were acquired over the scan range 30–500 Da (Q1MS) and 30–70 Da (Q3MS for accurate mass determinations). The scan time was 0.25 s [30–500 Da (Q1MS)] and 25 Da/s [30–70 Da (Q3MS for accurate mass determinations)]. For collision induced dissociation study of propionitrile, the mass range of the TSQ was lowered to 15 Da to examine some of the low mass fragment ions. The mass spectrometer was equipped with an APPI source with a 10 eV Krypton discharge lamp. The sheath and auxiliary gas pressure (N_2) were 47 and 25 (arbitrary units), respectively. The vaporizer temperature was 475 °C and the ion transfer tube temperature was set at 200 °C. The capillary and tube lens offsets were 35 and 112 V, respectively.

Results and Discussion

Positive Ion APPI Mass Spectra of Althiazide and Bendroflumethazide with Acetone as a Dopant

Figure 2 shows the positive ion mass spectra of althiazide and bendroflumethazide in acetonitrile with acetone as a dopant in the low mass region (Figure 2a) and in the parent ion region (Figure 2b). Protonated acetone at *m/z* 59 was almost exclusively the main ion observed in the low mass range spectrum and acetone radical cation was not observed (Figure 2a). No major impurities or background ions were observed. In the parent ion region, high abundance of protonated althiazide at *m/z* 383 and protonated bendroflumethazide at *m/z* 422 were observed, and their corresponding radical cation species were absent and/or not detected (Figure 2b). As mentioned above, charge exchange and proton transfer are two possible routes of ionization in positive ion APPI. Ionization energies and proton affinities of solvent, analyte, and dopant influence the route of ionization. The ease and the abundance of the formation of protonated acetone at *m/z* 59 is consistent with the higher proton affinity of acetone (812 kJ/mol) com-

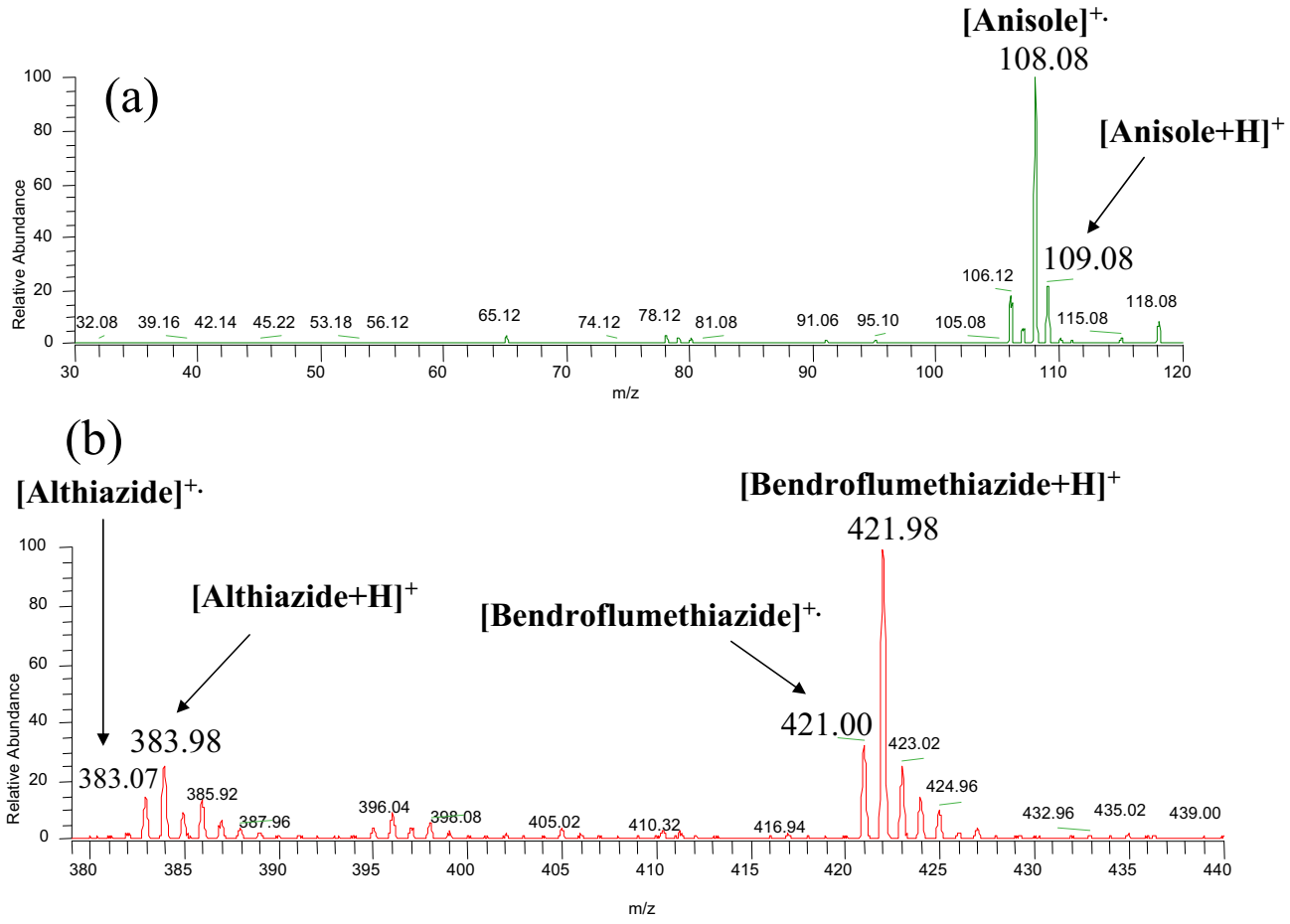


Figure 3. APPI positive ion mass spectra of althiazide and bendroflumethiazide in acetonitrile with anisole as a dopant in the low mass range region (**a**) and in the parent ion region (**b**).

pared with that of acetonitrile solvent (779.2 kJ/mol) [21]. The absence of acetone radical cation could be attributed to its neutralization as a result of proton transfer between acetone radical cation and the solvent (Reaction 4) and thus promotes proton transfer rather than charge exchange as the dominant route of ionization. Additionally, the IE of acetone (9.7 eV) [21] is slightly lower than that of the photon energies of Kr lamp (~10 eV), which makes the formation of acetone radical cation less efficient. This is consistent with the formation of $[M + H]^+$ of althiazide and bendroflumethazide and the absence of their corresponding radical cation species.

Positive Ion APPI Mass Spectra of Althiazide and Bendroflumethazide with Anisole as a Dopant

The positive ion mass spectra of althiazide and bendroflumethazide in acetonitrile with anisole as a dopant are shown in Figure 3. In addition to some minor background/impurity ions, the most prominent ion observed in the low mass range region (Figure 3a) was anisole radical cation at *m/z* 108, which is consistent with the low IE of anisole (8.2 eV) [21] compared with that of the Kr lamp (~10 eV). Protonated anisole at *m/z* 109 was also observed as a minor peak. In the parent ion region, both protonated analytes and their corresponding radical cation species were observed (Figure 3b). These data suggest that both charge exchange and proton transfer influence the ionization of althiazide and bendroflumethazide in the positive ion APPI with anisole as a dopant. The relative abundance of $[M + H]^+$ and $M^{\cdot+}$ may indicate the dominant mechanism by which althiazide and bendroflumethazide are ionized. However, Syage reported that the equilibrium ratio $[M + H]^+/M^{\cdot+}$ depends on the properties of the solvent, analyte and correlates with the reaction length in the photoionization source [10]. Overall, the use of anisole as a dopant promoted the formation of $M^{\cdot+}$ via charge exchange mechanism. However, proton transfer ionization pathway is more effective and represents the dominant route of ionization as indicated by the higher ratio $[M + H]^+/M^{\cdot+}$, and would suggest the possible involvement of acetonitrile and other ionic species in the protonation process.

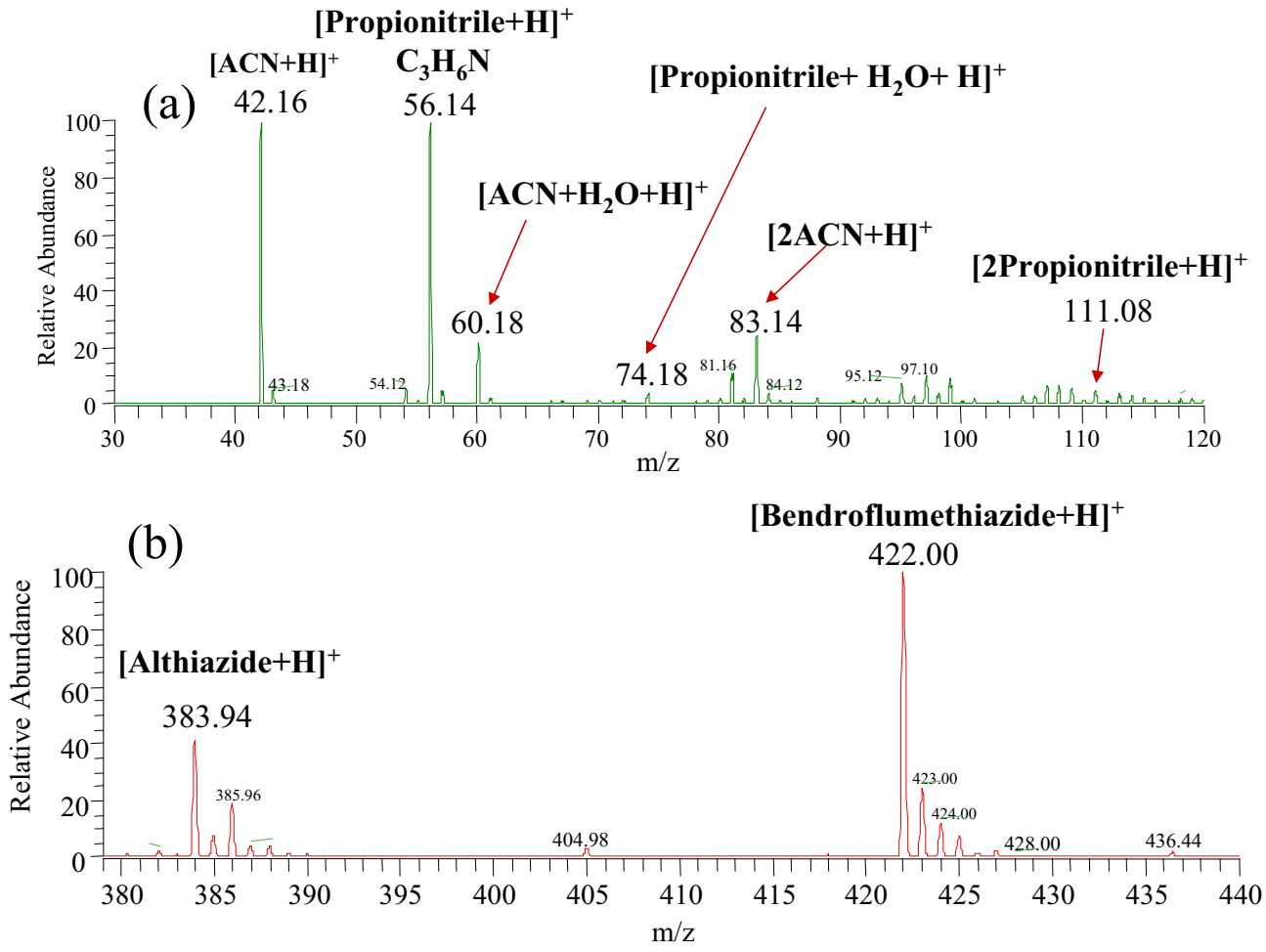


Figure 4. APPI positive ion mass spectra of althiazide and bendroflumethiazide in acetonitrile (no dopant) in the low mass range region (**a**) and in the parent ion region (**b**).

Positive Ion Mass Spectra of Althiazide and Bendroflumethazide in Acetonitrile Under Direct APPI

Vaporizing ca. 10^{-5} M of althiazide and bendroflumethazide under direct APPI in acetonitrile (no dopant) only produced their corresponding protonated species at *m/z* 384 and 422, respectively (Figure 4b). In different experiments (data not shown), the ion intensity of $[M + H]^+$ of althiazide and bendroflumethazide was relatively lower in acetonitrile compared with that when methanol was used as a solvent. The effect of acetonitrile on the relative signal intensity of analyte was also investigated by Syage and coworkers and the lower analyte response using acetonitrile compared with that of methanol was attributed to the higher IE and PA of acetonitrile. The authors reported that the higher IE of acetonitrile results in less ionization by ion–analyte reactions whereas the higher PA of acetonitrile results in acetonitrile monomer and cluster ions that are strongly bound to act as efficient protonating agents to chemically ionize the analyte [3, 17]. The APPI mass spectra of althiazide and bendroflumethazide in acetonitrile at the low mass range (Figure 4a) showed the ions at *m/z* 42 and 83, which correspond to protonated acetonitrile and its protonated dimer, respectively. The ion at *m/z* 60 can be attributed to the protonated acetonitrile/water cluster with water present at trace level inside the source. Additionally, an intense ion at *m/z* 56 was observed along with its apparent protonated water cluster and dimer at *m/z* 74 and 111, respectively.

Identification of the Ionic Species at m/z 56 in Acetonitrile Mass Spectrum

Accurate mass measurement of the intense ion at *m/z* 56.0543 in acetonitrile mass spectrum utilizing the ele-

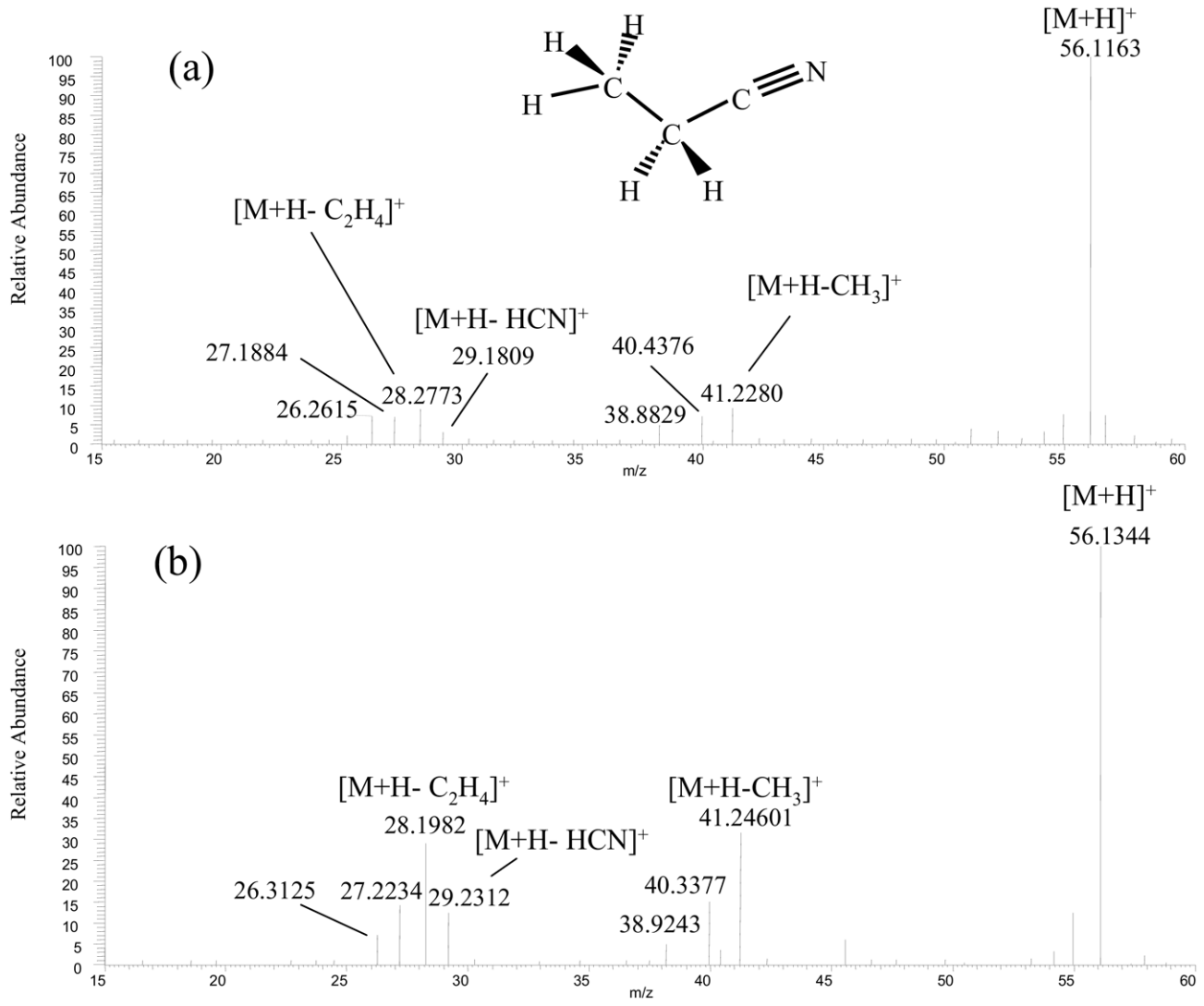


Figure 5. CID product ion spectra (MS^2 of $[M + H]^+$ of propionitrile at *m/z* 56 from (**a**) propionitrile from acetonitrile and (**b**) propionitrile standard.

mental composition calculator produced three elemental formulas of $C_3H_6N_1$, $C_1H_2N_3$, and $C_2H_2O_1N_1$ with a corresponding mass error of 4.854, 30.006, and 41.240 mmu, respectively, relative to the theoretical accurate mass of propionitrile at *m/z* 56.0495. Based on the accurate mass measurements and mass errors, the elemental formula of $C_3H_6N_1$ was assigned to the ion at *m/z* 56 and tentatively identified as protonated propionitrile. The propionitrile ion could originate from impurities or be the product of gas-phase ion molecule reactions. Acetonitrile is mostly prepared by purifying crude acetonitrile, which contains impurities, including propionitrile. Although excellent highly pure acetonitrile can be obtained by different processes, it still suffers from problems that propionitrile is not sufficiently reduced [22]. Syage et al. suggested that the formation of propionitrile is possibly due to an ion–molecule reaction, similar to that of methanol, which favors the formation of propionitrile at high cone voltage [17].

Collision Induced Dissociation of Propionitrile

The CID mass spectra of $[M + H]^+$ of propionitrile from both acetonitrile and pure standard are shown in Figure 5. The experimental conditions were essentially the same for both species and their corresponding mass spectra are identical and further confirmed that the ion at *m/z* 56 is indeed protonated propionitrile. Protonated propionitrile at *m/z* 56 dissociates primarily through the loss of methyl radical ($\cdot CH_3$), hydrogen cyanide (HCN) and ethylene molecule (C_2H_4) to give rise to the fragment ions at *m/z* 41, 29, and 28, respectively, and agree with previously reported studies [23–25].

Effect of Propionitrile Concentration on the $[M + H]^+$ Signal of Althiazide and Bendroflumethazide Under Direct APPI

The intensity of the ion signal of althiazide and bendroflumethazide in acetonitrile as a function of propionitrile concentration was studied. Introducing varying amounts of propionitrile (0.01% to 2% vol/vol) in the acetonitrile solution of althiazide and bendroflumethazide resulted in a relative increase in the ion intensity of $[M + H]^+$ of the two analytes under direct APPI. The increase in the ion intensity of $[M + H]^+$ of althiazide and bendroflumethazide seems to reach a maxima at ~1% to 2% propionitrile in acetonitrile and further suggests the involvement of propionitrile in the protonation process of althiazide and bendroflumethazide.

Propionitrile as a Possible Proton Donor for the Formation of $[M + H]^+$ of Althiazide and Bendroflumethazide Under Direct APPI

Since the Kr lamp employed under these experimental conditions produces ca. 10 eV photons and the ionization energies (IE) of althiazide and bendroflumethazide are expected to be ≤10 eV (most organic molecules have IE in the range 7–10 eV [1]), as well as considering the

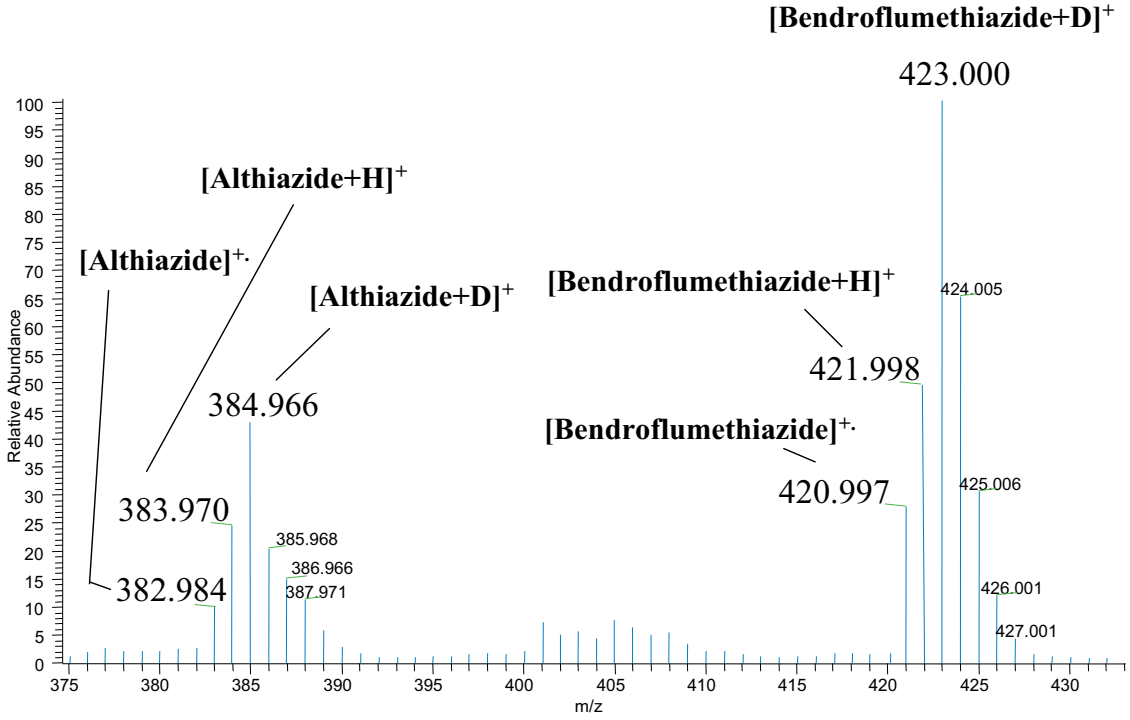
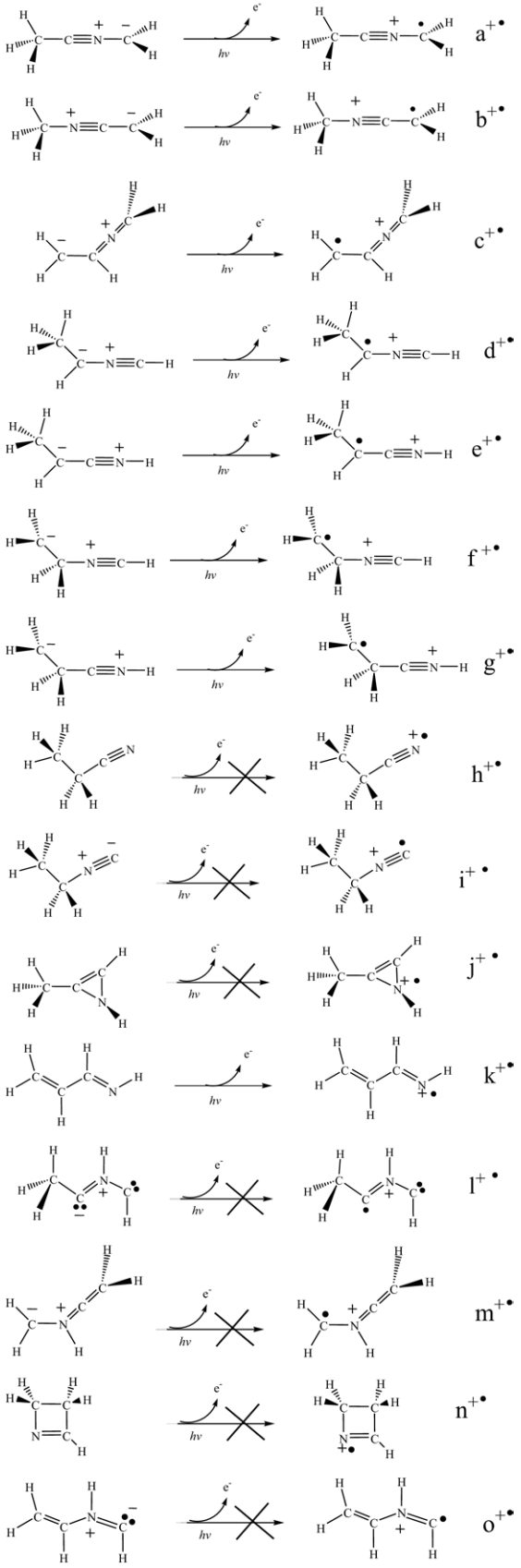


Figure 6. Positive ion APPI mass spectrum of althiazide and bendroflumethiazide in propionitrile-d_5 (no dopant).



high IE of acetonitrile and propionitrile compared with that of Kr lamp (IE of acetonitrile and propionitrile are 12.2 and 11.8 eV, respectively [21]) one possible mechanism for the formation of protonated althiazide and bendroflumethazide under direct APPI could be attributed to the reaction between their $M^{\cdot+}$ ions (produced by direct photon ionization) and neutral molecules as follows:

$$M + h\nu \rightarrow M^{\cdot+} + e^-$$

$$M^{\cdot+} + M \rightarrow [M + H]^+ + [M - H]^{\cdot}$$

Other possible mechanisms, as reported by Marotta and Traldi [20], include proton transfer in the absence of dopant by assuming photo-initiated isomerization of acetonitrile. Similarly, the role of propionitrile in the production of $[M + H]^+$ was investigated in the absence of dopant using propionitrile-d_5 (CD_3CD_2CN). The formation of $[M + D]^+$ species for althiazide and bendroflumethiazide at *m/z* 385 and 423, respectively, were observed (Figure 6), suggesting a possible role of propionitrile in the protonation process. Also shown in Figure 6 the protonated and radical cation species for both analytes and their formation could be attributed to water and anisole present at trace levels inside the source from previous run. Hydrogen–deuterium back exchange could also take place in the gas-phase and lead to the formation of the protonated species.

From the findings of Marotta and Traldi [20], direct photoionization of propionitrile and/or water and subsequent formation of their corresponding protonated species can be excluded due to their high IEs (11.8 and 12.6 eV, respectively). Furthermore, the production of protonated propionitrile under direct photoionization could be attributed to photo-initiated isomerization of propionitrile. Similar to the photo-initiated isomerization of acetonitrile proposed by Marotta and Traldi [20], initial photon-propionitrile interaction is assumed to produce an electronically excited state of propionitrile that is able to trigger isomerization reactions and thus the formation of several propionitrile isomeric structures. Nguyen and coworkers have studied the possible isomeric structures of C_3H_5N, including propionitrile, and the most stable species among the 15 isomers considered have been identified using ab initio molecular orbital calculations [26]. Matsumura and cowork-

Scheme 1. Possible isomeric structures (neutral and radical cation) of C_3H_5N, including propionitrile. Structures are drawn based on bond angles and bond lengths reported by Nguyen et al. (*J. Phys. Chem. A* **1999**, *103*, 938–948, reference [26]). Only isomers a, b, c, d, e, f, g, and k are assumed to undergo direct photoionization by the Kr radiation based on their calculated/assumed ionization energy (IE) and relative energy (ΔE). Initial photon–propionitrile interaction is assumed to produce an electronically excited state of propionitrile that is able to trigger isomerization reactions and the formation of these isomeric structures.

ers have also studied the molecular structures and the stabilities of 21 molecules represented by C_3H_5N using ab initio molecular orbital calculations [27].

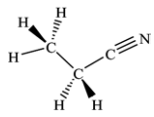
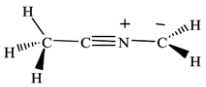
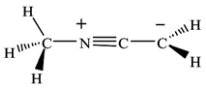
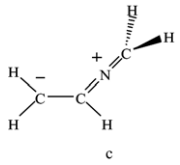
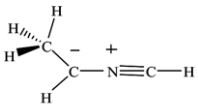
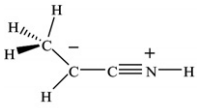
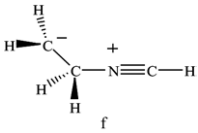
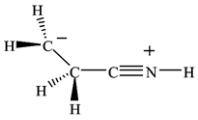
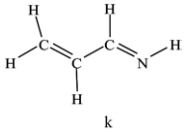
Scheme **1** shows all possible isomeric structures of C_3H_5N, including propionitrile, with structures drawn based on bond angles and bond lengths reported by Nguyen et al. [26]. The neutral form of each isomer and the production of its corresponding radical cation species under direct photoionization by the Kr radiation are based on absorption of a photon by each isomer followed by ejection of an electron (Scheme **1**). Only isomers a, b, c, d, e, f, g, and k with IE less than that of the Kr lamp are assumed to undergo direct photoionization. The relative energies (ΔE) of these isomeric structures have been reported based on ab initio molecular orbital calculations and are summarized in Table 1. These calculations were carried out by second-order unrestricted Møller–Plesset perturbation theory (UMP2) to optimize geometries and unrestricted quadratic configuration interaction theory with all single and double substitutions (UQCISD(T)) with the dp-polarized 6-31G(d,p) basis set [26]. The radical cations $a^{+\cdot}$, $b^{+\cdot}$, $c^{+\cdot}$, $d^{+\cdot}$, $e^{+\cdot}$, $f^{+\cdot}$, $g^{+\cdot}$, and $k^{+\cdot}$ have low energies of 0, −1, 27, 43, 6, 81, 50, and 96 kJ/mol, respectively, and are the most stable forms among the possible isomers (Table 1). The ionization energies of propionitrile and some of the most stable isomers of C_3H_5N have been also reported [26] and are summarized in Table 2. The IEs of isomers a, b, and k are 7.1, 8.0, and 9.6 eV, respectively (Table 2). The IEs of isomers c, d, e, f, and g, however, could not be found in the literature and are reasonably assumed to be <10 eV based on the calculated ΔE of these isomers (Table 1) as well as by taking the reported IEs of isomers a, b, and k as references.

Table 1. Calculated relative energy (ΔE, kJ · mol^{-1}) of possible $[C_3H_5N]^{+\cdot}$ isomers with respect to the energy of structures a, b, c, d, e, f, g, and k representing the lowest determined values (Nguyen et al., *J. Phys. Chem. A* 1999, *103*, 938–948), reference [26])

Structure radical cation	ΔE (UQCISD(T)/6-31G(d,p)[a] kJ · mol^{-1}
$a^{+\cdot}$	0
$b^{+\cdot}$	−1
$c^{+\cdot}$	27
$d^{+\cdot}$	43
$e^{+\cdot}$	6
$f^{+\cdot}$	81
$g^{+\cdot}$	50
$h^{+\cdot}$	228
$i^{+\cdot}$	247
$j^{+\cdot}$	147
$k^{+\cdot}$	96
$l^{+\cdot}$	447
$m^{+\cdot}$	99
$n^{+\cdot}$	134
$o^{+\cdot}$	131

[a] (UQCISD(T)/6-31G(d,p) = unrestricted quadratic configuration interaction theory with single and double substitutions with the dp-polarized 6-31G(d,p) basis set.

Table 2. Ionization energy (IE, eV) of propionitrile and the most stable isomers of C_3H_5N (Nguyen et al., *J. Phys. Chem. A* **1999,** *103*, 938–948, reference [26])

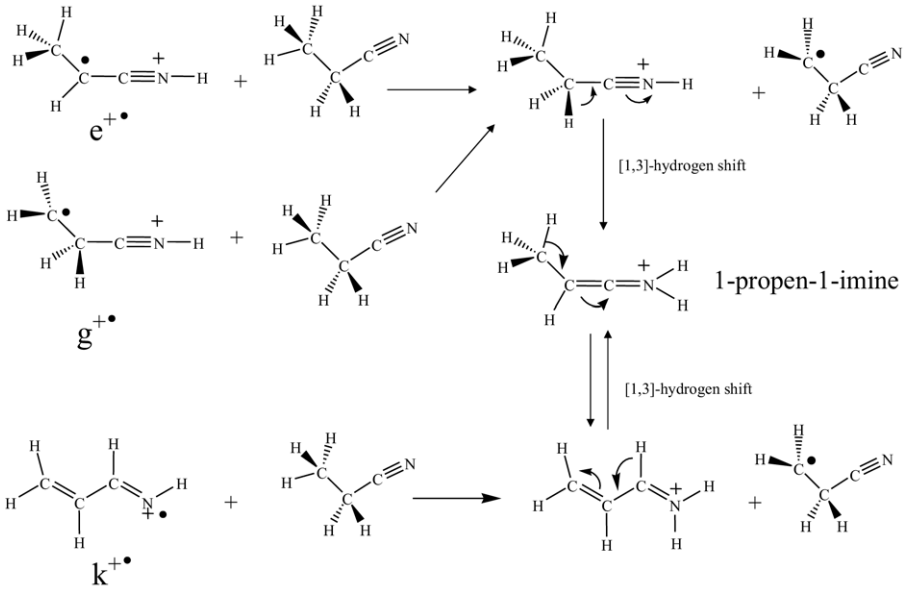
Structure	IE (eV)
	11.8
 a	7.1
 b	8.0
 c	NA
 d	NA
 e	NA
 f	NA
 g	NA
 k	9.6

NA = not applicable; assumed to be <10 eV based on its calculated ΔE; see Table 1.

Isomerization reactions among the possible ionic species shown in Scheme **1** were studied by considering various reversible 1,*n*-hydrogen shifts (n = 2, 3, 4) and their corresponding calculated ΔEs at the transition states [26]. These isomeric reactions included isocyanides–cyanides isomerization. It should be noted that other important resonance structures could be drawn from the neutral isomeric structures shown in Scheme **1** to represent molecular structures similar to those reported by Matsumura et al. [27]. Isomerization of isocyanides RNC, R = CH_3, C_2H_5, *i*-C_3H_7, *t*-C_4H_9, and C_6H_5 to those of the more stable corresponding cyanides RCN has been studied and the PAs of all the isocyanides were reported to be higher than those of the corresponding cyanides, by a constant of 11.5 ± 1 kcal/mol, regardless of the identity of R [28]. The larger PAs of RNC versus RCN is attributed to the larger charge-transfer and electrostatic interactions in the isocyanides. The increase of the PAs of both RNC and RCN as R gets bigger is due primarily to the increasing polarizabilities of the substituents [28]. Kinetic parameters of the isomerization reactions from ethyl isocyanide (CH_3CH_2NC) to propionitrile (CH_3CH_2CN) and from methyl isocyanide (CH_3NC) to acetonitrile (CH_3CN) have been determined, and a mechanism for the isomerization of RNC to RCN was reported to be a type of Wagner–Meerwein 1,2 shift [29–33]. Based on the ion chemistry and the relative stability of isocyanides and cyanides, the isomerization reactions d → e and f → g are expected to occur and be consistent with the lower ΔE of e and g (cyanide isomers) compared with that of their corresponding d and f (isocyanide isomers), respectively (Table 1). The low IEs of isomers a, b, c, d, e, f, g, and k make them susceptible to direct photoionization by the Kr radiation. Once ionized, these radical cation species can react with a neutral molecule of propionitrile to form the corresponding protonated species which act as a proton donor for the formation of $[M + H]^+$ of althiazide and bendroflumethazide. In addition to other possible mechanisms, Scheme **2** shows proposed mechanisms for the formation of the ionic species at *m/z* 56 from the reaction of some of the principal forms of propionitrile isomers (C-C-C-N skeleton, radical cation isomers e, g, and k) with neutral molecule of propionitrile followed by [1, 3]-hydrogen shift to form the protonated 1-propen-1-imine isomer. The stable protonated 1-propen-1-imine isomer can then act as a proton donor for the formation of $[M + H]^+$ of althiazide and bendroflumethazide.

Conclusions

We have identified an ionic species in acetonitrile and provided evidence for its possible involvement in the formation of $[M + H]^+$ of althiazide and bendroflumethazide under direct APPI. High accuracy mass measurement with a corresponding mass error <5 mmu suggested propionitrile as a possible structure for this ionic species. Additionally, the CID mass spectrum of the propionitrile in acetonitrile was identical to that of propionitrile standard and thus confirmed its structure. The formation of $[M + D]^+$ of althiazide and bendroflumethazide using propionitrile-d_5 and the increase in the $[M + H]^+$ signal of both analytes as the concentration of propionitrile in acetonitrile increased further suggested the involvement of propionitrile in the protonation process. Semiempirical calculations from the literature supported the assumption that the formation of [M +



Scheme 2. Proposed mechanisms for the formation of the ionic species at *m/z* 56 under direct APPI from the reaction of the radical cation isomeric structures e, g, and k with neutral molecule of propionitrile followed by 1,3-hydrogen shift to form the protonated 1-propen-1-imine isomer.

$H]^+$ could be attributed to photo-initiated isomerization of propionitrile in acetonitrile. The most stable isomers of propionitrile (based on their calculated IE and ΔE) were assumed to undergo direct photoionization by the Kr radiation to form the radical cation species. The radical cation can react with a neutral molecule of propionitrile to form the corresponding protonated species, which can then act as a proton donor for the formation of $[M + H]^+$ of althiazide and bendroflumethazide. In addition to the immense data in the literature regarding APPI, these data and further studies may help understand different mechanisms involved in the formation of $[M + H]^+$ in the APPI process.

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