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Thermally induced intramolecular oxygen migration of *N*-oxides in atmospheric pressure chemical ionization mass spectrometry

Xin Wang, Min Li* and Abu M. Rustum

ACDS – Supply Analytical Sciences, Merck & Co., Inc., 1011 Morris Avenue, Union, NJ 07083, USA

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***N*-Oxides are known to undergo three main thermal degradation reactions, namely deoxygenation, Cope elimination (for *N*-oxides containing a β-hydrogen) and Meisenheimer rearrangement, in atmospheric pressure chemical ionization mass spectrometry (APCI-MS). The ions corresponding to these thermal degradants observed in the ensuing APCI mass spectra have been used to identify *N*-oxides as well as to determine the *N*-oxidation site when the analyte contains multiple tertiary amine groups. In this paper, we report a thermally induced oxygen migration from one *N*-oxide amine to another *tert*-amine group present in the same molecule through a six-membered ring transition state during APCI-MS analysis. The observed intramolecular oxygen migration resulted in the formation of a new isomeric *N*-oxide, rendering the results of the APCI-MS analysis more difficult to interpret and potentially misleading. In addition, we observed novel degradation behavior that happened after the Meisenheimer rearrangement of the newly formed *N*-oxide: a homolytic cleavage of the N−O bond instead of elimination of an aldehyde or a ketone that usually follows the rearrangement. Understanding of these unusual degradation pathways, which have not been reported previously, should facilitate structural elucidation of *N*-oxides using APCI-MS analysis. Copyright © 2010 John Wiley & Sons, Ltd.**

Liquid chromatography/mass spectrometry (LC/MS) has become the most effective and widely used technique in the pharmaceutical industry for unknown impurity, degradant and metabolite identification.[1] LC/MS analysis can not only provide the molecular weight or chemical formula of an unknown analyte if accurate mass information or isotope distribution is available, but it can also reveal structural information on the unknown analyte via tandem mass spectrometry (MS/MS). For LC/MS analyses, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) provide efficient ionization for the vast majority of molecules with diverse structural features. In positive ion mode both ionization processes can generate intact gas-phase $[M+H]^+$ ions, which facilitates the determination of the masses of unknown analytes. Compared with ESI, APCI is a less 'soft' ionization technique due to the higher temperature used in the vaporizer tube. In some cases fragment ions formed by thermal decomposition are observed along with the protonated molecules during the APCI process. The occurrence of these fragment ions may present some challenges when interpreting the APCI mass spectra. On the other hand, additional information may be gained by studying these fragment ions in relation to the structure of the precursor ion.

**Correspondence to*: M. Li, ACDS – Supply Analytical Sciences, Merck & Co., Inc., 1011 Morris Avenue, Union, NJ 07083, USA.
E-mail: min.li2@merck.com

Tertiary amine functional groups are present in a large number of active pharmaceutical ingredients (APIs) and they are susceptible to oxidation, resulting in the formation of the corresponding *N*-oxides. Hence, *N*-oxides are a class of commonly observed degradants as well as metabolites for many drugs.[2] An *N*-oxide degradant has the same elemental composition as those compounds resulting from hydroxylation of the same API. The hydroxylated compounds are also potential degradants and metabolites. It is impossible to differentiate these two types of isomeric degradants solely based on the measurement of their molecular formulae by mass spectrometry because their formulae are identical. In addition, their product ion spectra could be very similar if hydroxylation or *N*-oxidation had occurred on the same moiety of the molecule. It has been shown that *N*-oxides show some unique fragment ions which are only present in APCI mass spectra. These unique fragment ions are generated by three major thermal degradation reactions taking place during the APCI of *N*-oxide compounds: (1) deoxygenation;[3,4] (2) Cope elimination leading to the formation of an olefin and a hydroxylamine for *N*-oxides containing a β-hydrogen;[4,5] and (3) Meisenheimer rearrangement which is followed by loss of an aldehyde or ketone via an internal hydrogen transfer for *N*-oxides containing an alkyl or benzyl group on the *N*-oxide nitrogen.[4,6] Therefore, these thermal degradation pathways can be used as a diagnostic tool to differentiate the *N*-oxide degradants from hydroxylated degradants and to localize the *N*-oxidation site when multiple *tert*-amine groups are present in the

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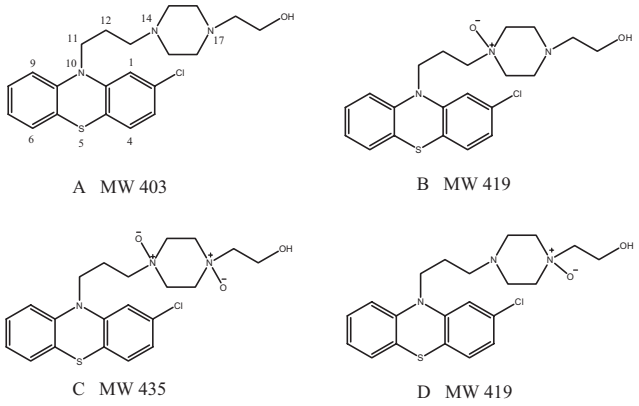

Figure 1. Structures of perphenazine (A) with the numbering of key positions, perphenazine 14-*N*-oxide (B), perphenazine 14,17-*N*,*N*-dioxide (C), and perphenazine 17-*N*-oxide (D).

molecule.[3,4] These unique fragmentation pathways occurring in the thermal degradation processes are due to thermal energy activation in the vaporizer of the APCI source and not to collision-induced dissociation (CID).[3,4,7]

Perphenazine (Fig. 1) is one of the first generation of antipsychotic drugs; it has three tertiary amine groups. During a recent oxidative stress study of perphenazine performed in our laboratory, six *N*-oxide degradants were generated. In this paper we report the observation of several unique fragment ions observed in the APCI mass spectra of two *N*-oxide degradants; the occurrence of these unique fragments has not been previously reported. These novel fragmentation pathways were observed only in APCI mode but not in ESI and CID, suggesting they are thermally induced degradation products in the APCI process. The structures of the two *N*-oxide compounds (perphenazine 14-*N*-oxide and perphenazine 14,17-*N*,*N*-dioxide) are shown in Fig. 1.

EXPERIMENTAL

Chemicals

Perphenazine is an in-house reference standard. Acetonitrile, methanol and ammonium acetate (all HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid (TFA), 30% (wt%) H_2O_2 solution and ~2–3% $H_2^{18}O_2$ solution were purchased from Sigma (St. Louis, MO, USA).

Sample preparations

An aliquot of 20 μL of 30% (wt%) H_2O_2 solution was added to 1 mL of 1 mg/mL perphenazine methanol solution. The mixture was heated in a heating block (Fisher Scientific) at 50°C for up to 2 h and then analyzed by LC/ESI-MS and LC/APCI-MS.

To prepare the ^{18}O-isotope-labeled perphenazine *N*-oxide compounds, 40 μL of a ~2–3% (wt%) $H_2^{18}O_2$ solution was added to 1 mL of 1 mg/mL perphenazine methanol solution. The resulting mixture was heated in a heating block (Fisher Scientific) at 60°C for up to 3 h and then analyzed by LC/APCI-MS.

LC-PDA (photo-diode array)-MS^n analyses

A Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer was used in positive ion mode for all the LC-PDA-MS and LC-PDA-MS/MS experiments. An ACE3 C8 column (150 × 4.6 mm, 3 μm; Mac Mod, Chadds Ford, PA, USA) was used with the temperature controlled at 40°C. Mobile phase A consisted of 10 mM ammonium acetate and mobile phase B consisted of acetonitrile. A linear gradient was started from 25% to 60% B in 20 min, followed by equilibration at 25% B for 5 min at a flow rate of 1.5 mL/min. For all the LC/ESI-MS experiments, the LC flow was split 10:1 prior to the mass spectrometer, and ~150 μL/min of the flow was directed into the ESI source of the mass spectrometer. The operating conditions for the ESI ion source were as follows: spray voltage 4.5 kV, capillary voltage 36 V, tube lens voltage 85 V, capillary temperature 275°C, sheath gas (N_2) pressure 45 (arbitrary units), and auxiliary gas (N_2) pressure 5 (arbitrary units). For all the LC/APCI-MS experiments, the entire LC flow was directed into the APCI source of the mass detector. The operating conditions for the APCI source were as follows: vaporizer temperature 450°C, capillary temperature 300°C, capillary voltage 26 V, tube lens 85 V, sheath gas (N_2) 45 (arbitrary units of pressure), and auxiliary gas (N_2) 5 (arbitrary units of pressure). The mass scale was calibrated at the beginning of a working day using a solution of a polytyrosine mixture. Full scan mass spectra were acquired in the range *m*/*z* 100–800 with a resolving power of 60 000 at *m*/*z* 400. CID experiments were conducted using helium as the collision gas. Low-resolution MS/MS experiments were performed in the LTQ mass spectrometer, with a precursor ion isolation width of 2.0 *m*/*z* units and normalized collision energy of 28%.

RESULTS AND DISCUSSION

Deoxygenation and dehydration of *N*-oxides

The UV chromatogram at 254 nm of the hydrogen peroxide-stressed perphenazine sample is shown in Fig. 2. Multiple oxidative degradants were observed and they can be divided into two categories: three mono-oxidized ones (peaks 3, 5 and 6) and three di-oxidized ones (peaks 1, 2 and 4). The mono-oxidized 17-*N*-oxide of perphenazine is the most abundant degradant under these liquid-phase stressing conditions. It is interesting to note that this degradant is also the most abundant oxidative degradant in a solid formulation of perphenazine under stability testing (data not shown). The identities of these degradants were confirmed by various 1D and 2D ^{1}H, ^{13}C and ^{15}N NMR analyses (The ^{1}H NMR spectra of perphenazine and perphenazine 17-*N*-oxide are shown in the Supporting Information). The full details of the structural characterization will be published elsewhere. In this paper, we focus on the unique fragment ions observed during the LC/APCI-MS analysis of two of the *N*-oxide degradants: perphenazine 14-*N*-oxide and perphenazine 14,17-*N*,*N*-dioxide (Fig. 1). The ESI and APCI mass spectra of these two degradants are shown in Figs. 3 and 4, respectively. The ESI mass spectra displayed only the protonated molecules, while the APCI mass spectra displayed a number of fragment ions in addition to the protonated molecules. The fragment ion at *m*/*z* 404 in the APCI mass spectrum of perphenazine 14-

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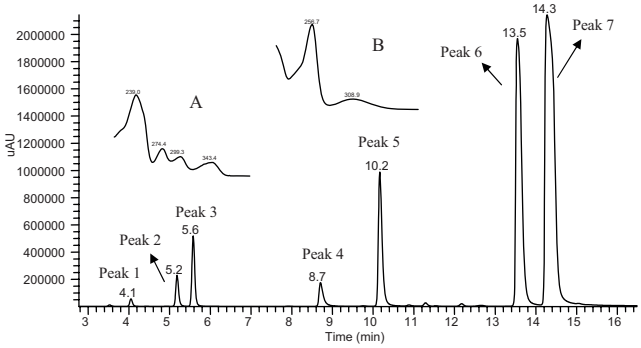

Figure 2. UV chromatogram of perphenazine oxidative degradants generated by stressing with H_2O_2 at 50°C for 2 h. Peak 1, perphenazine sulfoxide 14-*N*-oxide; Peak 2, perphenazine sulfoxide 17-*N*-oxide; Peak 3, perphenazine sulfoxide; Peak 4, perphenazine 14,17-*N*,*N*-dioxide; Peak 5, perphenazine 14-*N*-oxide; Peak 6, perphenazine 17-*N*-oxide; Peak 7, perphenazine. Peaks 1, 2 and 3 displayed the same UV spectrum as shown in insert (A). Peaks 4, 5, 6 and 7 displayed the same UV spectrum as shown in insert (B).

N-oxide (Fig. 3(B)) should correspond to loss of one oxygen from the $[M+H]^+$ ion at *m/z* 420 due to thermal activation in the vaporizer of the APCI source, according to the reported deoxygenation mechanism of *N*-oxides.[3,4,7] These distinctive $[M+H-O]^+$ ions resulting from thermally induced *N*-oxide deoxygenation can be used to differentiate an *N*-oxide from its hydroxylated isomers since the latter compounds do not undergo deoxygenation in APCI (and hence no $[M+H-O]^+$ ion is generated). The hydroxylated isomers typically

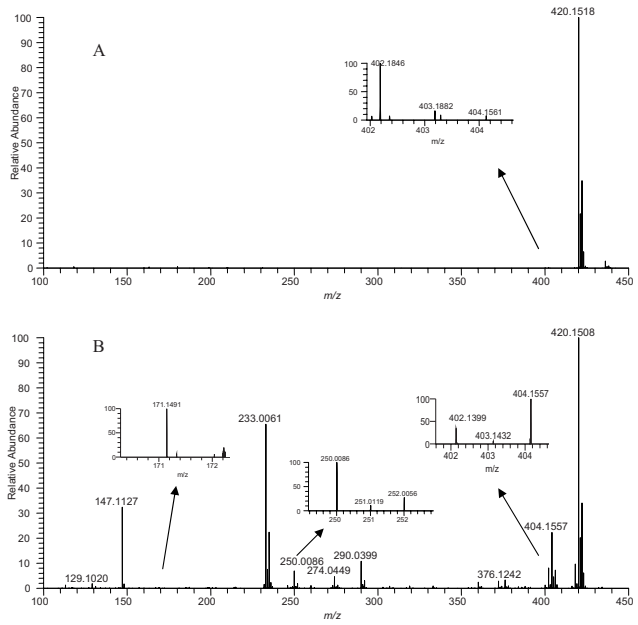

Figure 3. (A) High-resolution ESI mass spectrum of perphenazine 14-*N*-oxide. (B) High-resolution APCI mass spectrum of perphenazine 14-*N*-oxide. The occurrence of a significant M+2 peak at *m/z* 422 (~30%) in both the ESI and APCI mass spectrum is consistent with the molecular formula that contains a chlorine atom.

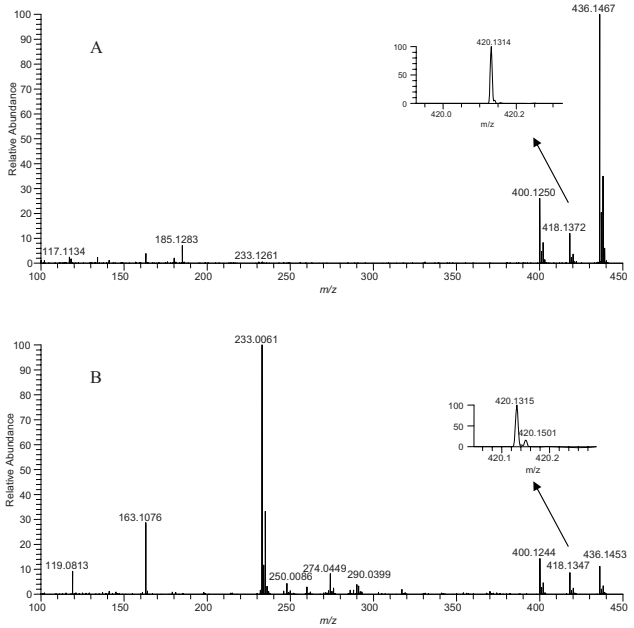

Figure 4. (A) High-resolution ESI mass spectrum of perphenazine 14,17-*N*,*N*-dioxide. (B) High-resolution APCI mass spectrum of perphenazine 14,17-*N*,*N*-dioxide. The occurrence of a significant M+2 peak at *m/z* 438 (~30%) in both the ESI and APCI mass spectrum is consistent with the molecular formula that contains a chlorine atom.

undergo dehydration either during in-source fragmentation or by CID, producing the dehydration ion, $[M+H-H_2O]^+$. Therefore, the hydroxylated isomers should show only the dehydration ion, $[M+H-H_2O]^+$, while the *N*-oxide should show the deoxygenation ion, $[M+H-O]^+$. In other words, the occurrence of the deoxygenation ion, $[M+H-O]^+$, in APCI mass spectra is a fingerprint of *N*-oxide compounds. As shown in the inserts of Figs. 3(B) and 4(B), the deoxygenation $[M+H-O]^+$ ion and the dehydration $[M+H-H_2O]^+$ ions are both present in the APCI mass spectra of perphenazine 14-*N*-oxide and perphenazine 14,17-*N*,*N*-dioxide. The two $[M+H-H_2O]^+$ ions observed at *m/z* 402 and 418, respectively, should result from the in-source fragmentation pathway due to the hydroxyl groups present in these two compounds. This view is supported by the fact that these two dehydration ions were also observed in the CID spectra of the $[M+H]^+$ ions of the two compounds (Fig. 5).

It was interesting to note that the relative abundances of the deoxygenation $[M+H-O]^+$ and dehydration $[M+H-H_2O]^+$ ions in the APCI mass spectra varied between the two compounds as shown in the inserts of Figs. 3(B) and 4(B). For perphenazine 14-*N*-oxide (Fig. 3(B)), the abundance of the deoxygenation ion (*m/z* 404) is higher than that of the dehydration ion (*m/z* 402). On the contrary, for perphenazine 14,17-*N*,*N*-dioxide (Fig. 4(B)), the abundance of the dehydration ion (*m/z* 418) is much higher than that of the deoxygenation ion (*m/z* 420). The much enhanced dehydration for perphenazine 14,17-*N*,*N*-dioxide may be facilitated by the oxygen of the 17-*N*-oxide attacking the neighboring hydroxylated carbon atom (Scheme 1). Since perphenazine contains both Cl and S, it would be difficult to

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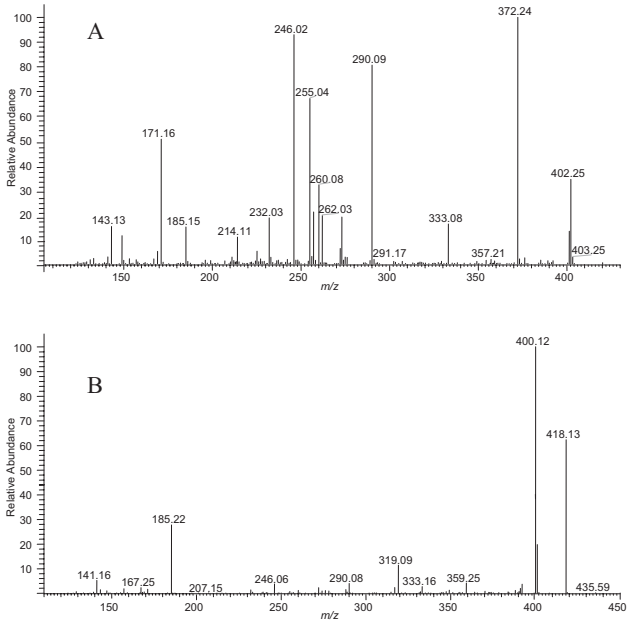
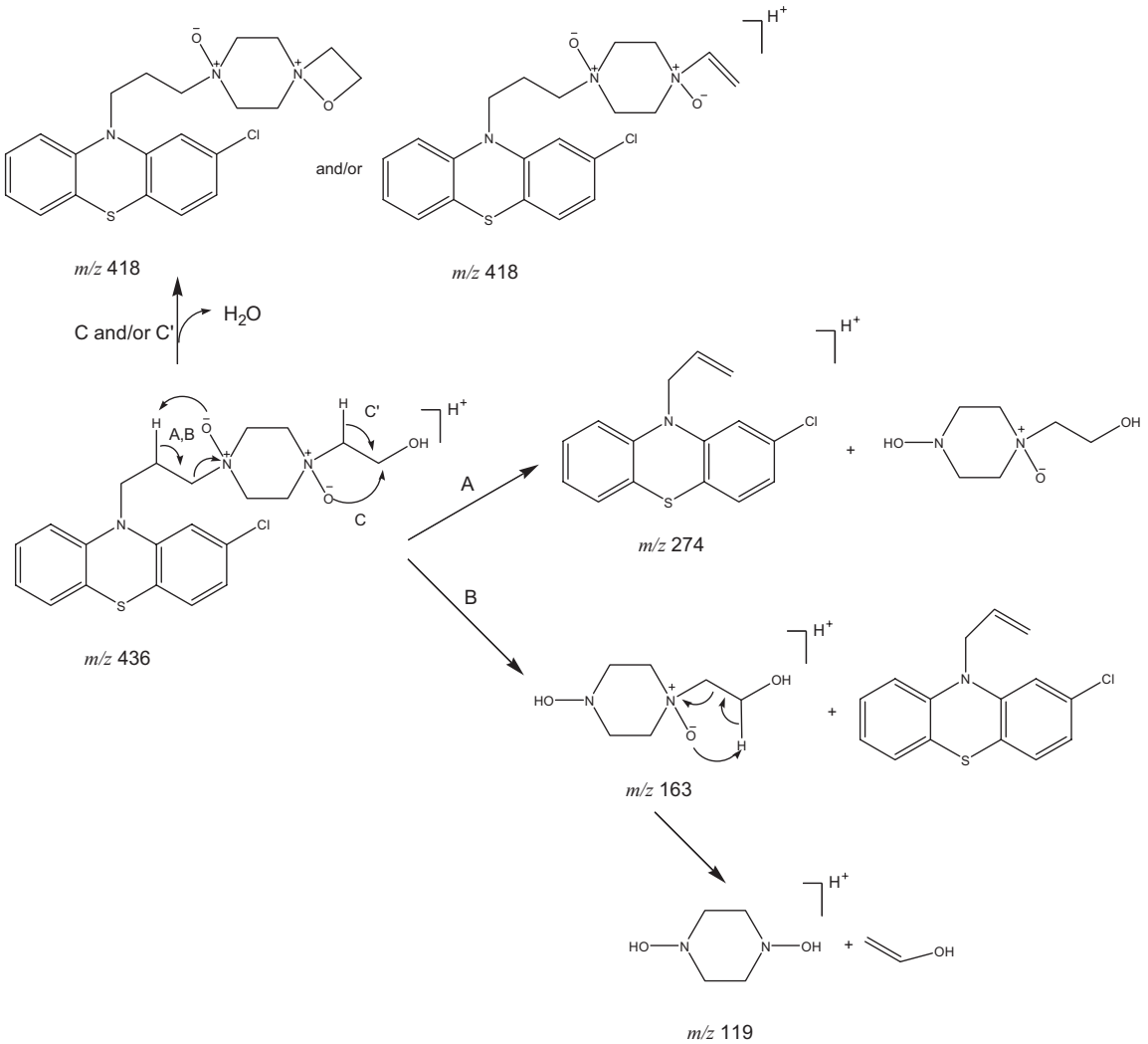


Figure 5. Low-resolution product ion spectra of protonated perphenazine 14-*N*-oxide at *m/z* 420 (A) and protonated perphenazine 14,17-*N*,*N*-dioxide at *m/z* 436 (B) in the ESI mode.

tell, without the use of high-resolution mass spectrometry, if the *m/z* 420 ion is a deoxygenated species from *m/z* 436 or is due to the contribution of the ^{37}Cl or ^{34}S isotopes of the dehydration ion at *m/z* 418. As shown in the insert of Fig. 4(B), when using the Orbitrap mass spectrometer with the mass resolution set at 60 000 for *m/z* 400, two ions were detected at nominal mass *m/z* 420 – *m/z* 420.1315 and 420.1501. Based on the accurate mass measurement and isotope simulation, the ion at *m/z* 420.1315 was found to correspond to either $C_{21}H_{25}N_3O_2^{35}Cl^{34}S$ (mass error of 1.3 ppm) or $C_{21}H_{25}N_3O_2^{37}Cl^{32}S$ (mass error of 1.7 ppm), suggesting that the *m/z* 420.1315 ion is due to the contribution of either the ^{37}Cl or ^{34}S isotope of the dehydration ion at *m/z* 418. On the other hand, the *m/z* 420.1501 ion was found to correspond to $C_{21}H_{27}N_3O_2ClS$ (mass error of 1.4 ppm), suggesting that this ion is indeed due to loss of an oxygen from the $[M+H]^+$ ion at *m/z* 436.

In order to separate all the *N*-oxide compounds generated in the oxidative stress of perphenazine, different mobile phases were used during method development. It was observed that the relative abundances of the dehydration ion $[M+H-H_2O]^+$ and the deoxygenation ion $[M+H-O]^+$ in the APCI mass spectra also varied with different mobile phases



Scheme 1. Fragmentation pathways for the Cope elimination of perphenazine 14,17-*N*,*N*-dioxide (pathways A and B) in APCI-MS analysis. A significant dehydration pathway (C and/or C') was also observed (refer to Fig. 4(B)).

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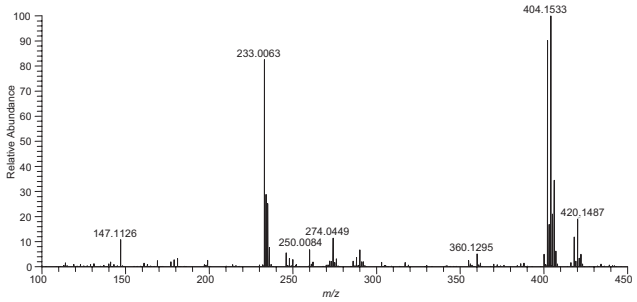

Figure 6. High-resolution APCI mass spectrum of perphenazine 14-*N*-oxide using mobile phase A consisting of 0.1% trifluoroacetic acid in water and mobile phase B consisting of acetonitrile. A linear gradient was started from 40% B for 5 min, then increased to 70% B in 20 min.

under an identical tune method. In the APCI mass spectrum of perphenazine 14-*N*-oxide when 0.1% TFA and acetonitrile were used as the mobile phases (Fig. 6), the abundance of the $[M+H-O]^+$ ion was higher than that of the $[M+H-H_2O]^+$ ion. However, the abundance of the $[M+H-O]^+$ ion turned out to be much lower than that of the $[M+H-H_2O]^+$ ion when 20 mM ammonium acetate and methanol were used as the mobile phases (Fig. 7). However, under the final LC conditions where 10 mM ammonium acetate and acetonitrile were used as the mobile phases, the abundance of the $[M+H-O]^+$ ion again became higher than that of the $[M+H-H_2O]^+$ ion (Fig. 3(B)). On the other hand, the different mobile phases used had no effect on the APCI mass spectra of perphenazine 14,17-*N*,*N*-dioxide. The abundance of the $[M+H-H_2O]^+$ ion was always higher than that of $[M+H-O]^+$ ion (data not shown), which is probably due to the attack by the neighboring 17-*N*-oxide oxygen as discussed above (pathway C), although a 1,2-elimination mechanism (pathway C′) cannot be completely ruled out. It has been reported that ESI and APCI spectra are subject to instrumental conditions such as source design, source temperature and mobile phase composition.[8] Such a change in the relative abundances of the deoxygenation fragment ion and dehydration fragment ion observed in the low-resolution APCI mass spectra may potentially impede efforts to differentiate between an *N*-oxide compound and its

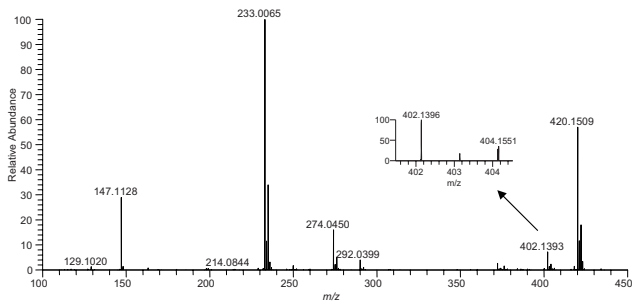

Figure 7. High-resolution APCI mass spectrum of perphenazine 14-*N*-oxide using a mobile phase containing 20 mM ammonium acetate and methanol. Details are described in the Experimental section.

hydroxylated isomers when the analyte contains a significant M+2 isotope. To overcome this ambiguity, a simple on-line hydrogen/deuterium (H/D) exchange experiment should be performed in ESI mode when a high-resolution mass spectrometer is not available.[9]

Oxygen migration from one *N*-oxide amine to another *tert*-amine group in the same molecule

Another prominent fragment ion observed in the APCI mass spectra of perphenazine 14-*N*-oxide and perphenazine 14,17-*N*,*N*-dioxide is that at *m*/*z* 233. Based on the accurate mass measurement, it corresponds to the 2-chlorophenothiazine radical cation ($C_{12}H_8ClNS^+$, **d**, Scheme 2) with a mass error of 0.3 ppm from its theoretical value. The occurrence of this significant ion cannot be explained by the three existing fragmentation pathways of *N*-oxides mentioned above. We consider that this fragment ion could result from thermally induced oxygen migration from the *N*-14 to the *N*-10 position via a six-membered ring transition state (**a**, Scheme 2). The newly formed perphenazine 10-*N*-oxide (**b**) could then undergo a Meisenheimer rearrangement forming an *N*-alkoxylamine (**c**). The *N*-alkoxylamine could then undergo homolytic cleavage of the N−O bond, to produce the presumed 2-chlorophenothiazine radical cation (**d**). Strong supporting evidence for the proposed oxygen migration is the presence of another fragment ion at *m*/*z* 250.0086 in the high-resolution APCI mass spectra of both perphenazine 14-*N*-oxide and perphenazine 14,17-*N*,*N*-dioxide (Figs. 3(B) and 4(B)). The accurate mass of this common ion matches the formula of $[C_{12}H_9NOClS]^+$ with a mass error of 0.7 ppm from its theoretical value. The formation of this particular fragment ion should be due to the Cope elimination of the newly formed perphenazine 10-*N*-oxide after oxygen migration (pathway A2). Another fragment ion at *m*/*z* 171 resulting from the Cope elimination was also observed in perphenazine 14-*N*-oxide although the abundance was quite low (insert of Fig. 3(B)). Cope elimination is one of the three thermally activated reactions for *N*-oxide compounds containing a β-hydrogen, which results in the formation of an olefin and a hydroxylamine.[4,5] On the other hand, two fragment ions at *m*/*z* 147 and 274 were also observed in the APCI mass spectrum of perphenazine 14-*N*-oxide (Fig. 3(B)), which apparently resulted from direct Cope elimination of perphenazine 14-*N*-oxide (pathway B). This observation indicates that only a portion of the perphenazine 14-*N*-oxide isomerized to the 10-*N*-oxide via oxygen migration during APCI-MS analysis (pathway A). The abundances of these fragment ions increased with increasing vaporizer temperature in the APCI mode (data not shown). These ions were not, however, observed in the ESI mode even when the temperature of the ion transfer tube (capillary) was increased from 300 to 375°C (data not shown). The temperature of the ion transfer tube may not be high enough for the thermal activation process to take place in the ESI mode. In the APCI mode, ions/droplets are already activated in the vaporizer prior to entering the ion transfer tube, which is not the case in the ESI mode.

To further support the proposed oxygen migration mechanism, perphenazine was oxidized using an isotope-labeled $H_2^{18}O_2$ solution. The APCI mass spectrum of the

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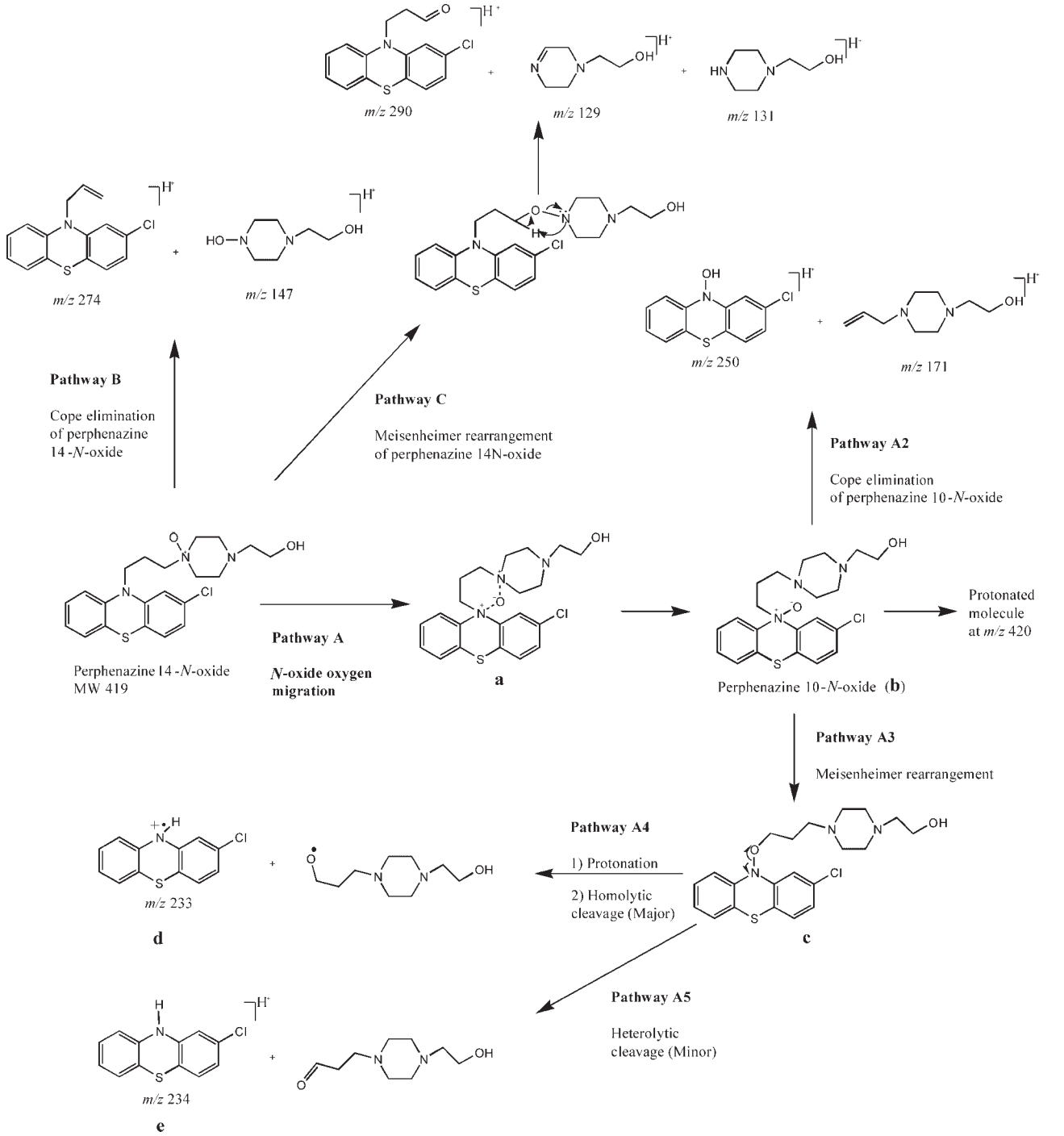

Scheme 2. Proposed fragmentation pathways for perphenazine 14-*N*-oxide in APCI-MS analysis. A significant portion of the analyte underwent oxygen migration from the *N*-14 to the *N*-10 position (pathway A).

isotope-labeled perphenazine 14-*N*-oxide is shown in Fig. 8. As expected, the protonated molecule shifted 2 *m*/*z* units higher to *m*/*z* 422. The fragment ion at *m*/*z* 404 should correspond to loss of an ^{18}O radical from *m*/*z* 422. The characteristic fragment ion, $[C_{12}H_9NOClS]^+$, which is attributed to the Cope elimination of perphenazine 10-*N*-oxide, also shifted 2 *m*/*z* units higher to *m*/*z* 252 (pathway A2, Scheme 2). For the expected Cope elimination of perphenazine 14-*N*-oxide, the hydroxylamine fragment shifted 2 *m*/*z* units higher to *m*/*z* 149 as expected (pathway B). The mass for the olefin fragment ion (*m*/*z* 274) did not shift because that ion does not contain any oxygen atoms. The fragment ions due to the Cope elimination of perphenazine 14,17-*N*,*N*-dioxide

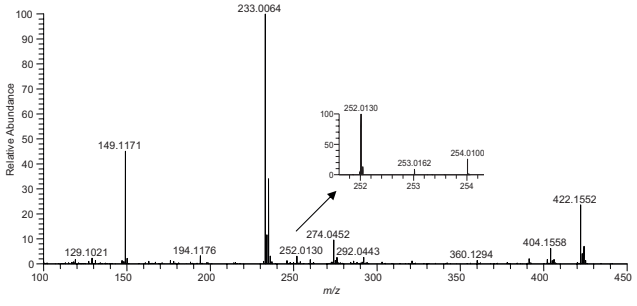

Figure 8. High-resolution APCI mass spectrum of ^{18}O-labeled perphenazine 14-*N*-oxide.

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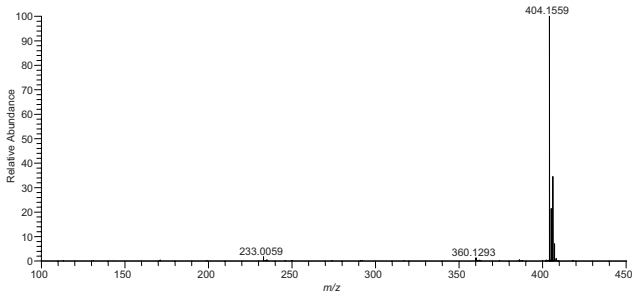

Figure 9. High-resolution APCI mass spectrum of perphenazine.

also shifted 2 *m/z* units higher for the ^{18}O-labeled perphenazine 14,17-*N*,*N*-dioxide (data not shown). These results further support the proposed mechanism (pathway A) in which the *N*-oxide oxygen migrates from *N*-14 to *N*-10 through a six-membered ring transition state during the APCI process.

Deviation from the Meisenheimer rearrangement mechanism

Another unique aspect of the proposed fragmentation mechanism is the formation of the 2-chlorophenothiazine radical cation (**d**, Scheme 2). The presence of radical cations in ESI and APCI mass spectra is not new.[10–12] In ESI, radical cations are formed because of the electrochemical oxidation of analytes at the metal/solution interface of the electrospray needle.[10,11] The occurrence of radical cations has also been shown in the APCI of heterocyclic compounds.[12] In the APCI mass spectrum of perphenazine, the 2-chlorophenothiazine radical cation has a relative abundance of approximately 1% (Fig. 9). However, the relative abundance of this radical cation is approximately 70% in the APCI mass spectrum of perphenazine 14-*N*-oxide (Fig. 3(B)). We propose that the 2-chlorophenothiazine radical cation in the APCI mass spectrum of perphenazine 14-*N*-oxide was formed after Meisenheimer rearrangement of the perphenazine 10-*N*-oxide ion (pathway A4). This fragmentation pathway deviates from the previously reported Meisenheimer rearrangement.[4] In the previous report, *N*-oxide compounds containing an alkyl or benzyl group on the *N*-oxide nitrogen underwent Meisenheimer rearrangement, followed by elimination of an aldehyde or a ketone through an internal hydrogen transfer under thermal activation in the APCI and APPI ion sources.[4] According to the reported pathways, the newly formed perphenazine 10-*N*-oxide would be expected to produce the hydroxylamine fragment ion at *m/z* 234 with loss of an aldehyde (pathway A5). Nevertheless, the relative abundance of this fragment ion was about 1% in this particular case. Instead, a fragment ion at *m/z* 233 (Fig. 3(B)) was observed at a relative abundance of 70%. It appears that the majority of the *N*-alkoxylamine (**c**) formed during the initial step of the Meisenheimer rearrangement proceeded with a homolytic cleavage (pathway A4) to produce the fragment ion at *m/z* 233 (**d**), while only a very small potion proceeded to a heterolytic cleavage (pathway A5). The stable conjugated system in the 2-chlorophenothiazine radical cation (**d**) may be attributed to the preference for homolytic cleavage.

Three additional ions at *m/z* 119.0813, 163.1076 and 274.0449 were observed in the APCI mass spectrum but not in the ESI mass spectrum of perphenazine 14,17-*N*,*N*-dioxide (Fig. 4). They were not detected during CID as shown in the product ion spectrum of *m/z* 436 (Fig. 5(B)). Their accurate mass values indicate that they correspond to $C_4H_{11}N_2O_2$ (mass error 2 ppm), $C_6H_{15}N_2O_3$ (mass error 0.6 ppm) and $C_{15}H_{13}NClS$ (mass error 1 ppm). Based on these formulae, it is apparent that their formation is due to thermally induced Cope elimination, as illustrated in Scheme 1. Since there are two *N*-oxidation sites, the *m/z* 163 ion could undergo further Cope elimination to give the ion at *m/z* 119.

CONCLUSIONS

We have reported a thermally induced oxygen migration from one *N*-oxide amine to another *tert*-amine group present in the same molecule during an APCI-MS analysis. This phenomenon has not been previously reported. A mechanism is proposed to account for the observed oxygen migration through a six-membered ring transition state in the APCI process, which is supported by an ^{18}O-labeling experiment. We also observed a novel homolytic N–O cleavage after the Meisenheimer rearrangement of the newly formed *N*-oxide. A clear understanding of these unusual degradation pathways should facilitate structural elucidation of *N*-oxides using APCI-MS.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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