RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2004; **18**: 1645–1654
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1534

RCM

Synthesis and identification of two potential oxidation degradants of oxymetazoline

Ian P. Leader*, John Halket and Paul Finch
Royal Holloway, Egham TW20 0EX, UK

Received 16 December 2003; Revised 23 May 2004; Accepted 23 May 2004

Two potential oxidation degradants of oxymetazoline have been isolated by liquid chromatography and monitored by electrospray single quadrupole mass spectrometry. The structures of the products are shown to be oxymetazoline *N*-oxide and hydroxyamine by multiple-stage fragmentation ion trap mass spectrometry. The product ion spectra were installed in a library database and the library was used to examine an aged commercial product; one of the degradants was detected, but at a level of less than 0.1% of the parent. Copyright © 2004 John Wiley & Sons, Ltd.

For a commercial over-the-counter (OTC) health care product to be viable it needs to undergo a rigorous programme of stability testing prior to market launch.[1] From the point of product manufacture, the acceptable lead-time to pack and ship product to the point of sale, together with shelf life for sales and consumer usage, is typically assessed as being 3 years. A full-scale stability programme to mimic the proposed shelf life of the product in real time must be performed. If any changes are found (such as degradation of the active constituent, formation of toxic degradants), over this stability programme, causing the product to be unmarketable, significant time and money have effectively been lost. The ability to predict what may happen over this extended time period would be of considerable benefit in assessing the viability of a potential new product. Also, being able to identify any degradation products in advance would enable a predictive study of possible effects of ingredient interactions once the product is in the market place.

To this end, a currently available commercial product containing the well-studied active oxymetazoline,[2,3] and a common cause of product degradation, oxidation, were chosen for investigation. It was decided that exposing the raw material to a harsh oxidative environment would provide suitable conditions for generating degradants. Electrospray ionisation[4] (ESI) mass spectrometry was chosen as the preferred technique to identify, and where necessary quantify, any products of degradation.[5] ESI is a soft ionisation technique ideally suited to sampling polar compounds[6,7] from the liquid phase for mass spectrometry. The ionisation process allows optimum ion formation without pre-fragmenting the species.

Oxymetazoline is a commonly used decongestant available in various OTC cold treatment formulations. This was deemed a suitable candidate for such a study; it has two nitrogen centres that may undergo degradation via an oxidation mechanism to form *N*-oxide species (Schemes 1(a) and 1(b) using meta-chloroperoxybenzoic acid, MCPBA). A possible alternative degradation mechanism is hydrolysis of oxymetazoline (Scheme 1(c)). This reaction has not been reported in the literature, but it is analogous to hydrolysis of naphazoline[8] (Fig. 1).

The spectra generated by any degradants will be stored in a searchable reference library.[9] By analysing a physically stressed, commercial product containing oxymetazoline, it will then be possible to match any degradants formed in the product with that of the oxidised raw material.

Previous work on dextromethorphan[10] (Fig. 2), an antitussive, showed almost 100% *N*-oxide (Fig. 2) formation using MCPBA as an oxidant. MCPBA is a commonly used oxidising agent for reactions in organic media.[11] The oxidation of tertiary amines to *N*-oxides by peroxy compounds is a general reaction; in a mixed chemical environment, a possible source of (hydro)peroxide is addition of molecular oxygen to a double bond followed by abstraction of a hydrogen atom (cf. lipid peroxidation).

Distinguishing the *N*-oxide (Scheme 1(a)) from the hydrolysed (hydroxyamine) species (Scheme 1(b)) formed proved difficult[12] using traditional techniques such as HPLC-UV. Nuclear magnetic resonance (NMR) would require milligram amounts of sample material to differentiate the two forms, and might be problematic owing to exchange reactions of the two species. In this study, an ion trap mass spectrometer was used to perform multiple-stage fragmentations of the oxidised degradant product(s). The spectra generated have been compared with those produced by analogues of the degradant species formed (oxymetazoline, deuterium-labelled oxymetazoline, a hydroxylated degradant). Based on this information the structures of the major degradation products have been determined.

**Correspondence to*: I. P. Leader, Royal Holloway, Egham TW20 0EX, UK.
E-mail: leader.i@pg.com

RCM

EXPERIMENTAL

Instrumentation

Single-stage mass spectrometry experiments were performed on a Finnigan Navigator mass spectrometer (ThermoFinnigan, Hemel Hempstead, UK). Structural identification was performed on an LCQ Classic quadrupole ion trap (ThermoFinnigan). Chromatography was performed using a Surveyor autosampler and MS pump (ThermoFinnigan), coupled to a HP1050 UV detector (Agilent Technologies, Bracknell, UK) when using the Navigator mass spectrometer, and a Thermo Separation Products pump and autosampler (ThermoFinnigan) when using the LCQ Classic. Infusion experiments were performed using the integral infusion pump on the LCQ Classic.

Where necessary, separation was achieved using an Xterra reversed-phase C_{18} column (250 × 2.1 mm i.d.) maintained at 25°C (Waters Ltd, Elstree, UK).

Fractions were collected using an electronic valve actuator column-switching device (Jones Chromatography, Hengoed, UK). Using the high/low output controls from the Surveyor autosampler, the device was triggered to divert the flow at the appropriate time to collect different fractions.

The mass spectrometric and chromatographic conditions are presented in Table 1.

Chemicals and reagents

All reagents and chemicals were of AnalaR grade unless stated otherwise.

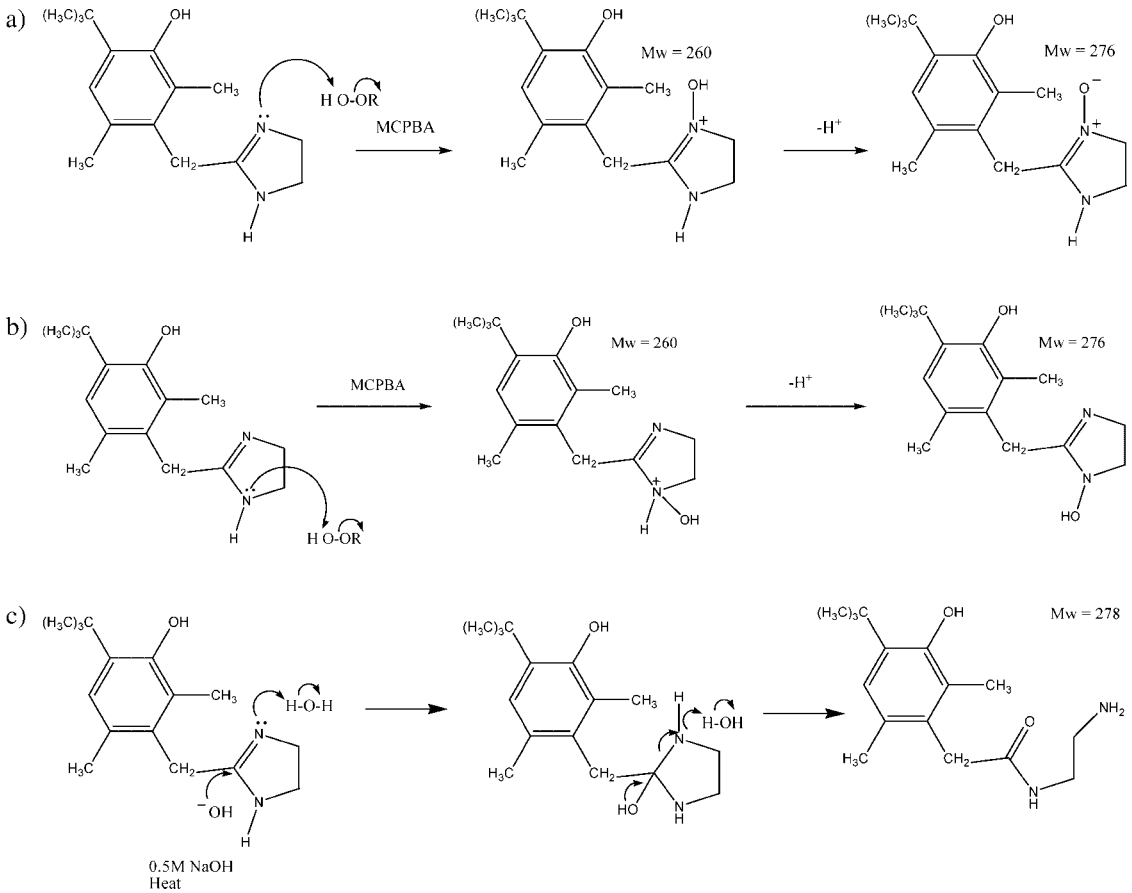
Oxymetazoline hydrochloride was purchased from Promochem (Welwyn Garden City, UK). Deuterated (D_9) oxymetazoline was supplied by Procter & Gamble (Mason, OH, USA). A potential hydrolysed degradant of oxymetazoline, 3-hydroxy-2,6-dimethyl-*tert*-butylphenylacetylethylenediamine, was synthesised and supplied by Procter & Gamble (Egham, UK). 3-Chloroperoxybenzoic acid (*meta*-chloroperoxybenzoic acid, MCPBA), ammonium formate, and sodium bicarbonate were purchased from Sigma-Aldrich Chemicals (Gillingham, UK). Acetonitrile, formic acid, sodium hydroxide, dichloromethane and sodium sulphate were purchased from Fisher Scientific (Loughborough, UK).

Where applicable, all solutions were prepared using 18 MΩ deionised water (Milli-Q, Millipore, Harrow, UK).

Procedures

(a) Preparation of oxymetazoline free base

Oxymetazoline hydrochloride (0.2834 g, 0.9558 mmol) was dissolved in sodium hydroxide (50 mL, 1 M). The resultant solution was extracted with dichloromethane (3 × 10 mL) using a separating funnel, dried over anhydrous sodium



Scheme 1. Mechanisms of formation of (a) *N*-oxide degradant species from oxidation of oxymetazoline; (b) hydroxyamine degradant species from oxidation of oxymetazoline; and (c) hydrolysed product.

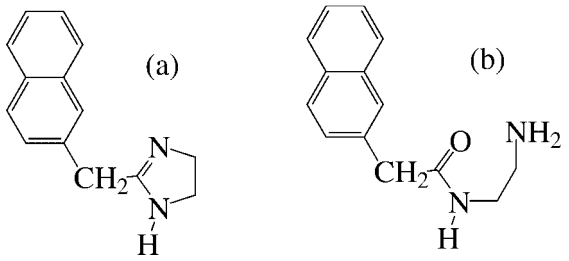


Figure 1. Structures of (a) naphthazoline and (b) its hydrolysed degradant.

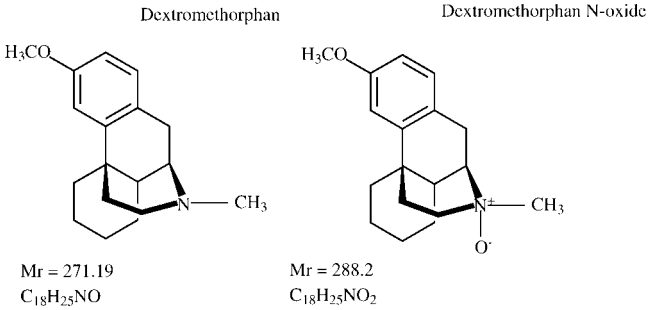


Figure 2. Dextromethorphan and its oxidised degradant.

sulphate, and concentrated to dryness under a constant flow of nitrogen.

(b) Oxidation of oxymetazoline

Oxymetazoline free base (10.86 mg, 0.0418 mmol) and the oxidant MCPBA (19.76 mg, 0.1146 mmol) were dissolved in dichloromethane (5 mL). The resulting mixture was stirred for 120 h, protected from sunlight. After 120 h the reaction was quenched with saturated sodium bicarbonate solution (20 mL). The organic phase was washed three times with sodium bicarbonate (saturated) and the organic phase was separated and dried over anhydrous sodium sulphate. The organic phase, which contained the oxidised degradants and oxymetazoline, was transferred to a pre-weighed glass vessel and concentrated to dryness under nitrogen.

(c) Preparation of hydrolysis product

Oxymetazoline hydrochloride (0.2 g) was refluxed with sodium hydroxide (25 mL, 0.1 M) until all the solids were dissolved (ca. 2 h). On cooling, the solution was neutralised to pH 7 with hydrochloric acid (1 M), and made up to a volume of 200 mL with deionised water.

(d) Sample separation

The resultant organic residue containing the oxidised/degraded form of oxymetazoline was dissolved in diluent (10 mmol ammonium formate and acetonitrile, 50:50) and analysed using the Navigator mass spectrometer under the conditions described in Table 1. The resulting total ion current (TIC) chromatogram (Fig. 3) indicated the presence of at least two oxidised species at the expected mass-to-charge ratio, *m/z* 277. Previous work on oxymetazoline in rat blood had allowed for direct analysis of the analyte without purification.[3] However, in order to perform structural analysis of the degradant species, it was necessary to separate and collect enough of each species to perform infusion experiments using an ion trap mass spectrometer. The fractions were collected using the electronic valve actuator as described above.

(e) Mass spectrometric analysis

Experiments were performed by infusing a known concentration (5 ng μL^{-1}) of the analyte at a rate of 5 $\mu L\,min^{-1}$ directly into the ion trap mass spectrometer. The Autotune programme (Xcalibur Software version 1.2; ThermoFinnigan, UK) was used to optimise the ion trap operating conditions for the analysis of protonated oxymetazoline. The mass spectrometer was operated with the automatic gain control (AGC) turned off, and the ion injection time value previously determined from the Autotune program was used (this gave consistent levels of ions being injected into the trap and enabled comparison of ion abundance between MS stages). After tuning, the activation energy (in Xcalibur an arbitrary unit) was increased in 5% steps until the precursor ion had been reduced to 10% of its abundance (isolation width used was 1.5 Da). In the next step, product ions of interest were isolated and subjected to resonant excitation until they reached the 10% level, when the product ion spectra were recorded (isolation width used was 5 Da). Multiple-stage mass spectrometric experiments (i.e. MS^2, MS^3, etc.)

Table 1. Conditions used for LC/MS and MS^n experiments

Surveyor MS pump	
Mobile phase:	
Acetonitrile	Initial: 80% gradient to end 20%
Ammonium formate, 10 mmol, pH 3	Initial: 60% gradient to end 40%
Run time	30 min
Flow rate	210 $\mu L\,mL^{-1}$
Surveyor autosampler	
Injection volume	10 μL
HP 1050 UV detector	
Wavelength	280 nm
Navigator MS	
Ionisation mode	Positive electrospray
Nitrogen flow rate	400 L h^{-1}
Source temperature	200°C
Cone voltage	40 V
LCQ Classic ion trap	
Ionisation mode	Positive electrospray
Sheath gas	Set to 40
Auxiliary gas	Off
Capillary temperature	200°C
Capillary voltage	20 V
Spray voltage	3.5 kV
Wideband activation	Off
AGC	Off
Activation energy	This was adjusted according to the fragmentation step required
Syringe pump	5 $\mu L\,min^{-1}$
TSP LC pump	
Mobile phase:	
Acetonitrile	Initial: 80% gradient to end 20%
Ammonium formate, 10 mmol, pH 3	Initial: 60% gradient to end 40%
Run time	30 min
Flow rate	210 $\mu L\,min^{-1}$
TSP autosampler	
Injection volume	10 μL

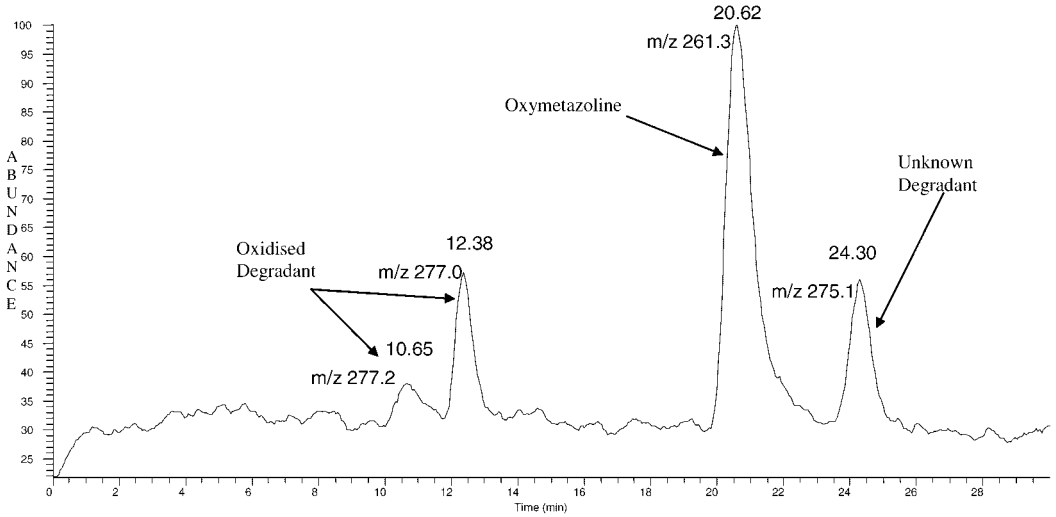


Figure 3. Full scan chromatogram of oxymetazoline and oxidation degradants.

were conducted on the product ions until the concentration of the ions in the trap had decreased to less than 0.1% abundance of the starting $[M+H]^+$ species.

When performing multiple-stage mass spectrometric experiments on unknown compounds, the mass spectrometer conditions used were as for the protonated oxymetazoline species.

RESULTS AND DISCUSSION

Analysis of oxymetazoline

Oxymetazoline is ionised using electrospray[13] in positive mode to form the protonated molecule $[M+H]^+$ at *m/z* 261. This ion fragments to yield product ions at *m/z* 205, 191 and 177. The postulated fragmentation pathway is given in Scheme 2. The *m/z* 205, 191 and 177 product ions yielded no further information when selected for fragmentation analysis.

Deuterated (D_9) oxymetazoline (confirmation of oxymetazoline fragmentation using the deuterated analogue)

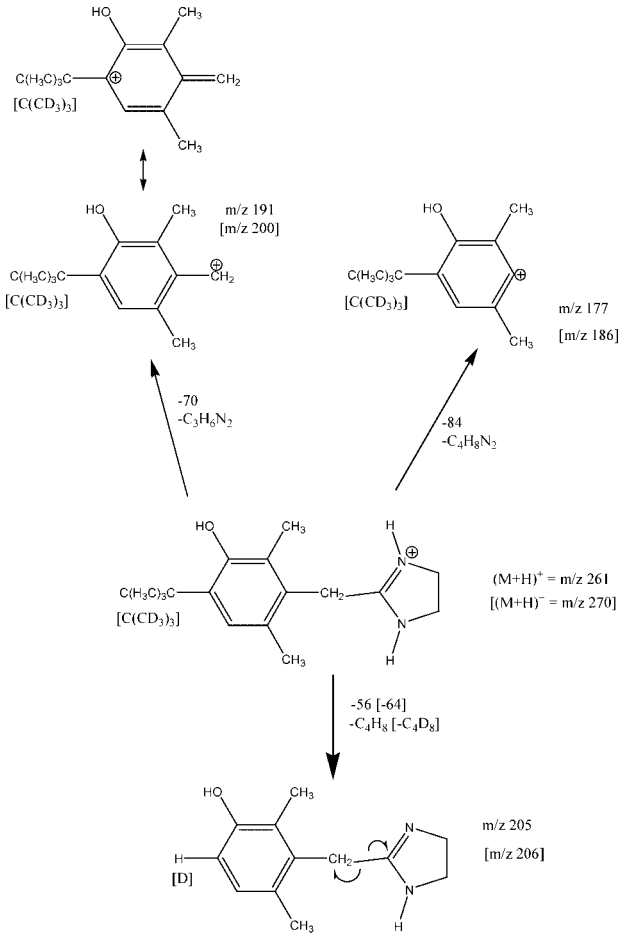
To confirm that the most abundant fragmentation process seen for oxymetazoline was the loss of the *tert*-butyl group (*iso*-butene), an experiment using a stable isotope labelled analogue was devised. Oxymetazoline with a completely deuterated *tert*-butyl group was analysed under the same conditions as oxymetazoline. The fragmentation pattern exhibited by the labelled analogue is similar to that seen with oxymetazoline, with the protonated molecule being nine Thomsons heavier (*m/z* 270). The loss of 64 Da to form a product ion with *m/z* 206 confirms that the first fragmentation seen from these compounds was the loss of the *tert*-butyl group (in the form of *iso*-butene), with one hydrogen, or in this case deuterium, being substituted back into the phenyl group (*ipso* substitution). This process is marked in parentheses in Scheme 2.

Oxymetazoline hydrolysis product

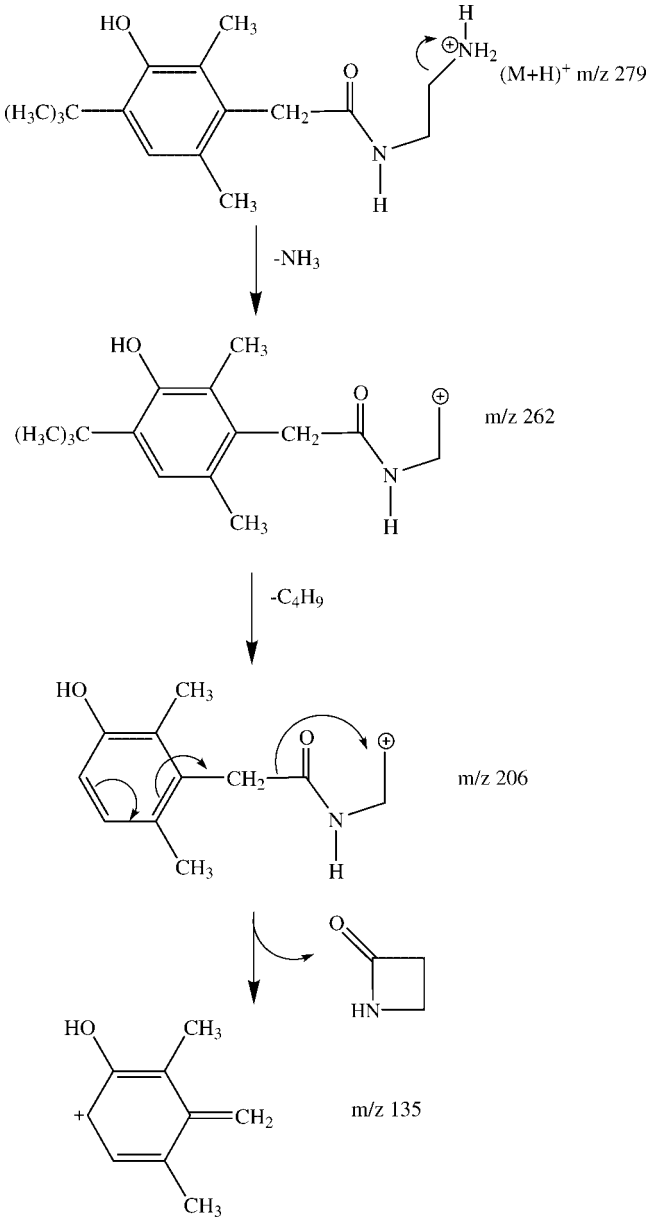
A proposed mechanism of formation of the hydrolysis product is shown in Scheme 1(c). For reasons of consistency of analysis, a sample of this product was synthesised and characterised using the ion trap mass spectrometer. The proposed identities of the product ions are shown in Scheme 3.

Analysis of the newly formed oxidation degradants

The degradant components of each collected fraction were studied to determine the structures from the product ion



Scheme 2. Proposed fragmentation paths of oxymetazoline. [Analogous fragmentation paths for d9-oxymetazoline.]



Scheme 3. Proposed mechanism for fragmentation path of hydrolysed oxymetazoline.

fragments using ion trap mass spectrometer infusion experiments. The fractions will be referred to as fraction 1, retention time of 10.65 min, and fraction 2, retention time of 12.38 min, from hereon, relating to the order in which they eluted from the HPLC column (Fig. 3).

Fraction 1

The proposed structure of this species is the *N*-oxide. The proposed fragmentation pattern is shown in Scheme 4 with associated spectra in Fig. 4. There are two abundant fragmentations that occur for this protonated molecule. The first transition (Scheme 4(a)), the loss of the *tert*-butyl group (m/z 277 − 56 = 221), was followed by a dehydration of the imidazoline group (m/z 221 − 18 = 203). This was only seen after the initial fragmentation, at a high collisionally induced dissociation (CID) energy and generated a relatively low ion abundance (about 1% of the starting protonated molecule). No evidence for dehydration of the protonated molecule as the initial step was found (formation of m/z 259). This is indicative of the stabilising effect that the oxide has on the imidazoline group and the protonated molecule as a whole. In the second transition (Scheme 4(b)), losses of 42/43 Da are present. These were tentatively assigned to losses of C_3H_6 and C_3H_7 from the *tert*-butyl group, leaving a methyl group in its position. However, MS^3 experiments revealed such a loss after the *tert*-butyl group had already left the molecule. In addition, experiments with the deuterated oxymetazoline demonstrated that the *tert*-butyl group left the molecule as a whole through an *ipso* substitution process. Given the relatively low abundances of the species, it was difficult to attribute a fragmentation pattern. The two sections of the molecule appeared thermodynamically stable, but the likelihood was that the imidazoline part underwent a rearrangement. Based on the evidence available it was postulated that the loss would be that of ethylenimine. No further significant fragments were observed for this protonated molecule, again supporting the fact that the protonated molecule is stable.

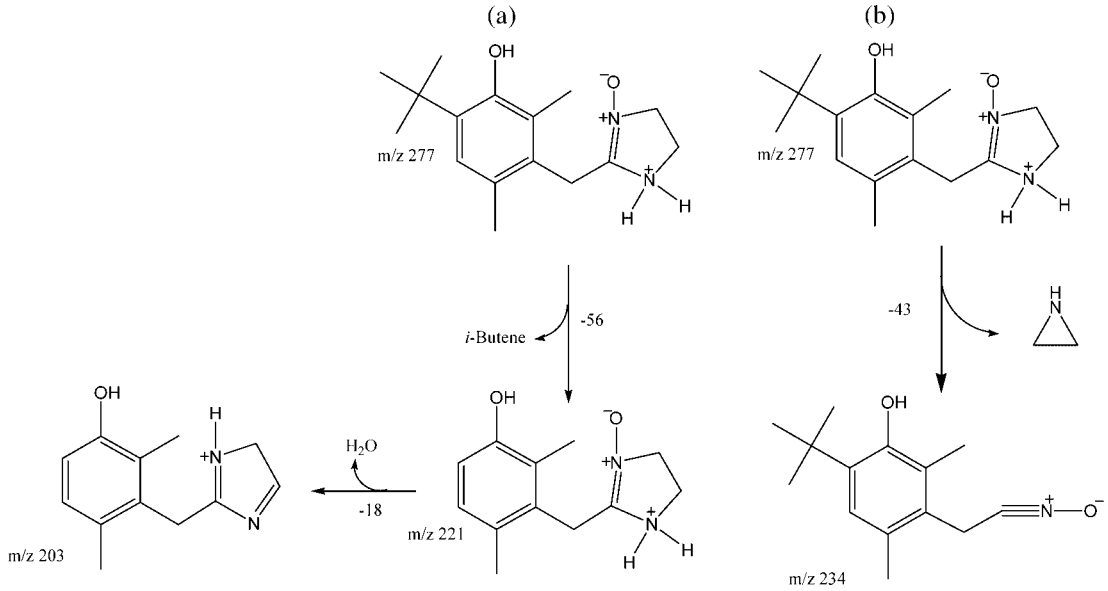
The proposed mechanism of formation for this *N*-oxide degradant species is shown in Scheme 1(a). The double-bond electron density provides a stabilising influence for the species[14] formed as evidenced by the inability of the protonated molecule to readily fragment in the ion trap.

Fraction 2

The proposed structure of this species is a hydroxyamine. The protonated species underwent more extensive fragmentation than the degradant component of fraction 1; the proposed fragmentation patterns are presented in Schemes 5(a), 5(b) and 5(c), and the associated spectra in Figs. 5–7. The first stage was always a water loss (step 1, m/z 277 − 18 = 259), indicating a dehydration across the carbon–nitrogen bond of the imidazoline ring. The instability of the protonated molecule was shown by the formation of multiple-stage (MS^3 and MS^4) fragments at relatively high abundances. One interesting observation was the recurrent formation of ions one or two mass units apart. This could be indicative of the loss of one or two hydrogen atoms, often associated with a double-bond formation; however, in this case, it would most likely be the result of ring closure to form a more stable five- or six-membered ring adjoining the original phenyl and imidazoline groups.

The proposed fragmentation pathways have been broken into individual stages. Step 1, as previously stated, was the dehydration step. Following the formation of the dehydrated form, multiple-stage experiments, MS^3, produced peaks at m/z 244, 231 and 203. Each fragment was examined in order of their relative abundance. The first fragment examined was product ion m/z 203 (Scheme 5(a)), formed by the loss of isobutene (56 Da), *ipso* substitution of the *tert*-butyl group as previously discussed for oxymetazoline. The multiple-stage, MS^4, studies of this species produced two subsequent product ions by losses of 2 and 15 Da. Given the proposed structure of the ion with m/z 203, the most likely cause of the −2 Da loss is a ring closure to give a tricyclic system. The most likely source of ion m/z 188 was the loss of a methyl group from the phenol ring. Whether this occurs at the *ortho* or *para* position is unknown. The next fragment studied was m/z 231

RCM



Scheme 4. Proposed fragmentation paths for the component of fraction 1 (*N*-oxide).

(Scheme 5(b)), formed by a loss of 28 Da. This can likely be attributed to the well-known[15] loss of carbon monoxide from phenols. Once again, the characteristic loss of 2 Da was observed to form *m/z* 201, and was attributed to the loss of H_2 in a ring-closure process between a methyl group and the imidazoline ring. A product ion at *m/z* 175 was also present, formed from the *m/z* 231 product ion by loss of isobutene as previously described. Product ion *m/z* 244 (Scheme 5(c)) was attributed to the loss of a methyl group. The source of this methyl was first thought to be from the *tert*-butyl group, followed by the loss of a hydrogen radical to leave a propylene side chain. However, given that we also saw the loss of 15 Da after the *tert*-butyl group had already been cleaved (Scheme 5(a)), it would seem likely that a similar process occurred here to give an ion at *m/z* 244. The sequence of the subsequent MS^4 steps to form *m/z* 243, 216 and 215 was unclear but it seemed likely that a ring-closure process accompanied by loss of carbon monoxide occurred (as for Scheme 5(b)).

No fragmentation of the imidazoline group was seen during the analysis of fraction 2 (unlike fraction 1), consistent with the formation of multiple ring systems in fraction 2. The proposed mechanism of formation of the hydroxylated oxymetazoline is shown in Scheme 1(b). The protonated molecule was shown to be inherently unstable by its readiness to fragment in the ion trap.

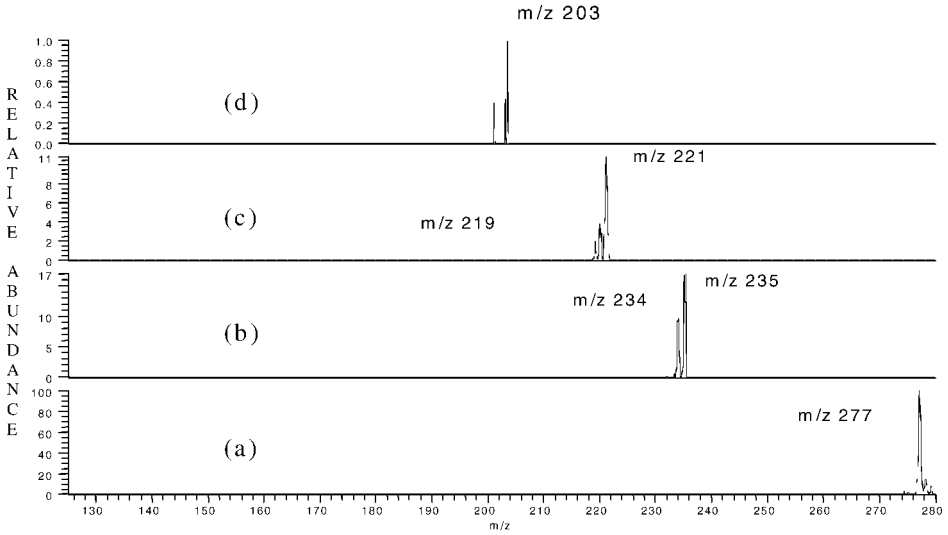
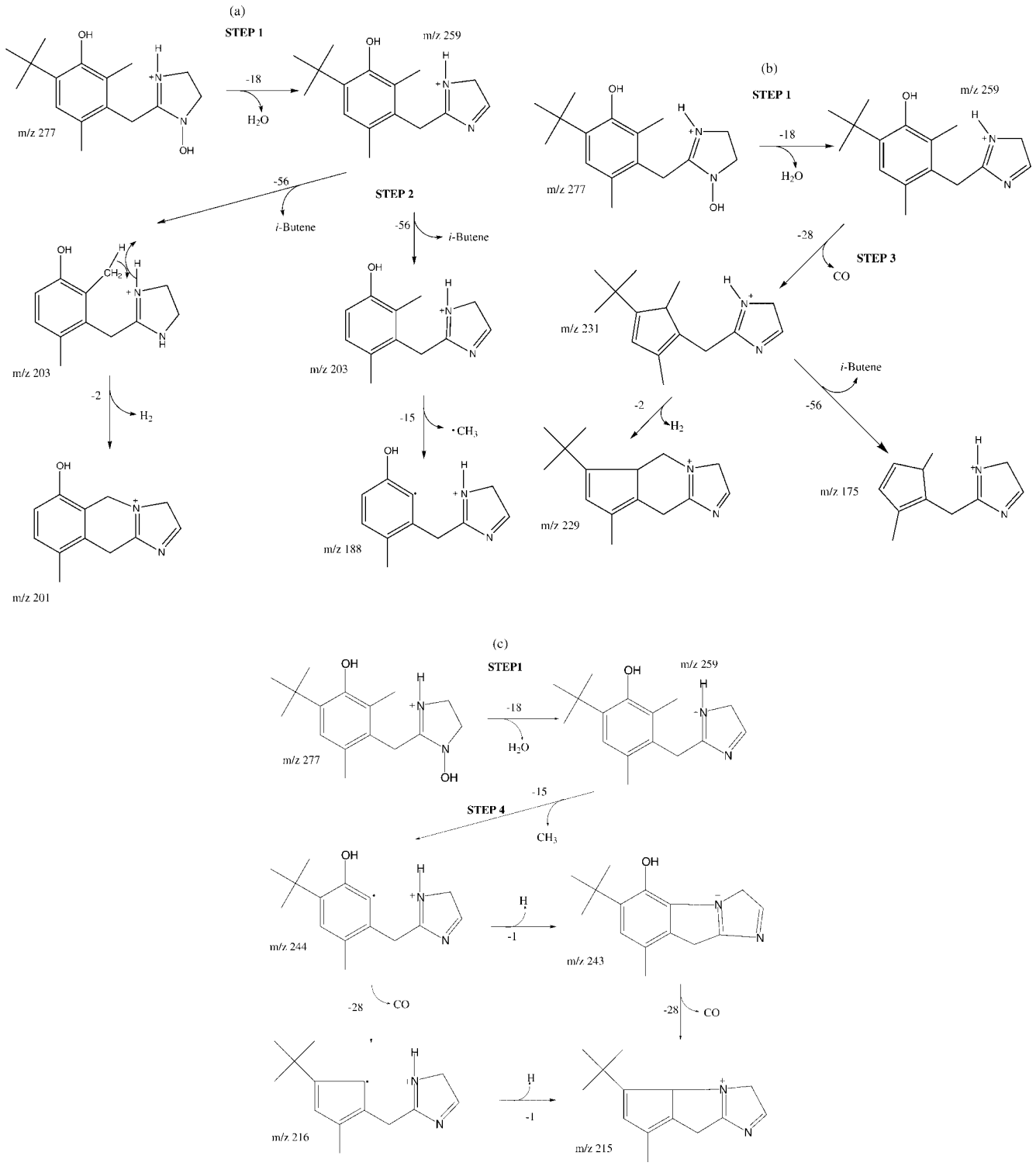


Figure 4. Product ion spectra of degradant from fraction 1: (a) ion trap spectrum of precursor ion $[M+H]^+$; (b) ion trap MS^2 spectrum ($277^+ \Longrightarrow$ product ions) of precursor ion; (c) ion trap MS^2 spectrum ($277^+ \Longrightarrow$ product ions) of precursor ion; and (d) ion trap MS^3 spectrum ($277^+ \Longrightarrow 221^+ \Longrightarrow$ product ion) of precursor ion.



Scheme 5. (a) Proposed primary fragmentation paths for component of fraction 2 (hydroxyamine); (b) proposed secondary fragmentation paths for component of fraction 2 (hydroxyamine); and (c) proposed tertiary fragmentation paths for component of fraction 2 (hydroxyamine).

Comparison of a stressed commercial product containing oxymetazoline

The spectra generated for each oxidation product were entered into a mass spectrometry database which utilises the high degree of reproducibility of the LCQ ion trap product ion mass spectra (normalised collision energy).[16] A commercial product at the end of its shelf life was prepared and analysed using HPLC/MS and the same conditions as for the analysis of the degraded raw material were used to detect the components present. The chromatogram and spectra generated were searched using the created library[9] and no matches were found.

RCM

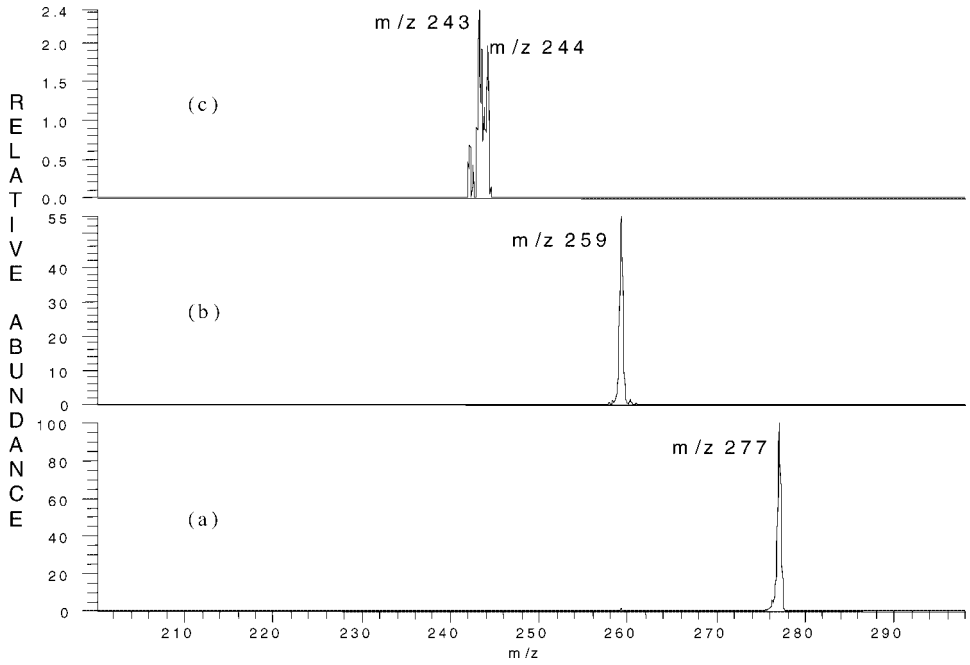


Figure 5. Product ion spectra of degradant from fraction 2: (a) ion trap spectrum of precursor ion $[M+H]^+$; (b) ion trap MS^2 spectrum ($277^+ \Longrightarrow$ product ion) of precursor ion; and (c) ion trap MS^3 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow$ product ions) of precursor ion.

After overloading the mass spectrometer with the aged, oxymetazoline-containing product, a small amount of fraction 2 (the hydroxyamine form) was observed, as shown in Fig. 8. This was confirmed by a library search of this species. Levels of the oxidised form were determined to be present at 0.1% of parent. However, as the system was considerably overloaded with the oxymetazoline species, the quantitation was well outside the linear range of the analysis so it may be concluded to be present at a level significantly less than the measured 0.1% of the parent peak.

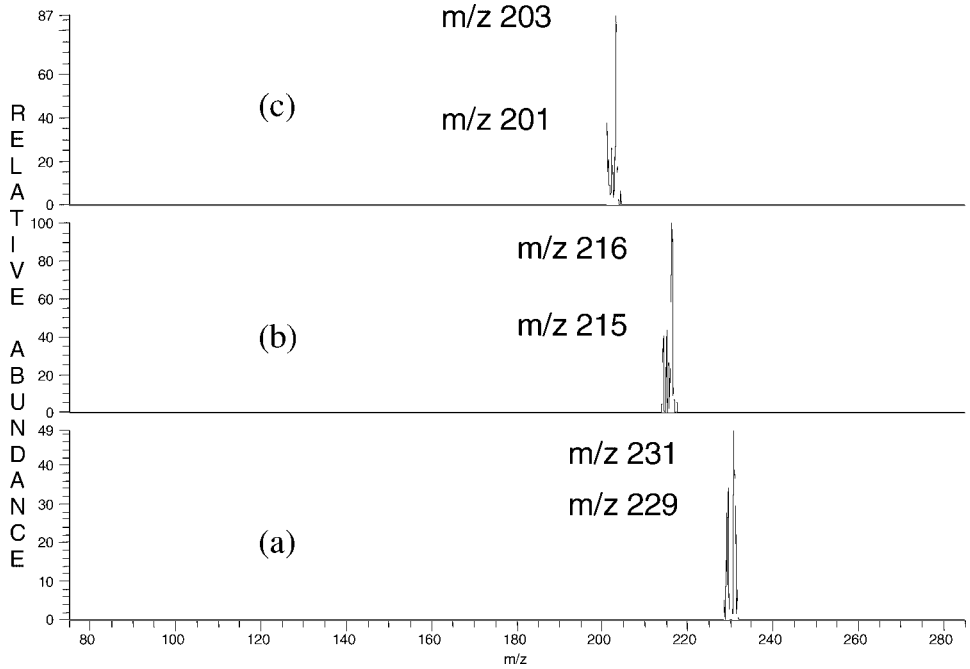


Figure 6. Product ion spectra of degradant from fraction 2: (a) ion trap MS^3 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow$ product ions) of precursor ion; (b) ion trap MS^4 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow 244^+ \Longrightarrow$ product ions) of precursor ion; and (c) ion trap MS^3 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow$ product ions) of precursor ion.

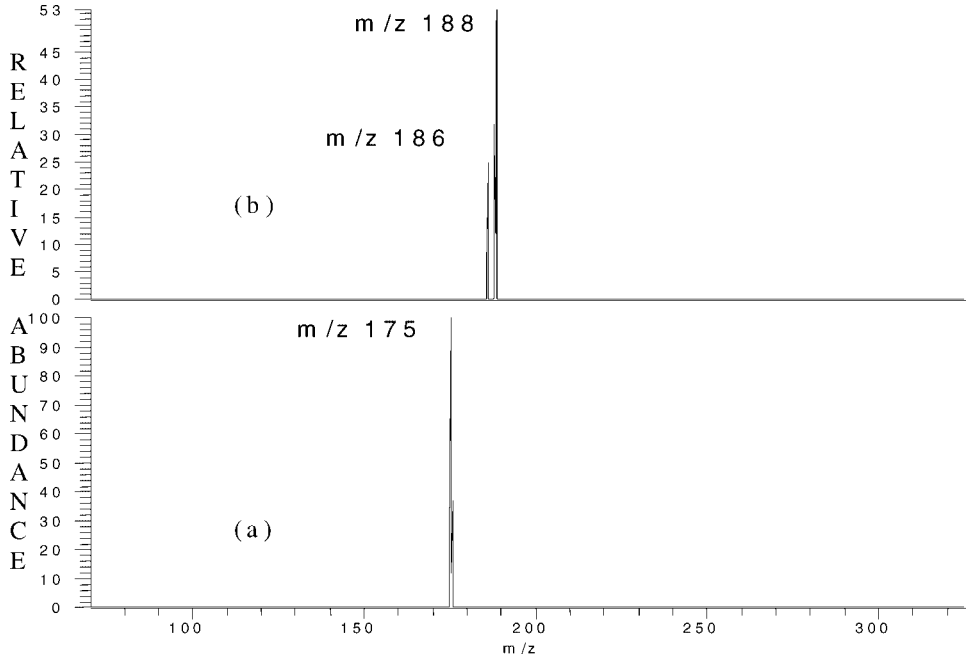


Figure 7. Product ion spectra of degradant from fraction 2: (a) ion trap MS^4 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow 231^+ \Longrightarrow$ product ion) of precursor ion and (b) ion trap MS^4 spectrum ($277^+ \Longrightarrow 259^+ \Longrightarrow 203^+ \Longrightarrow$ product ions) of precursor ion.

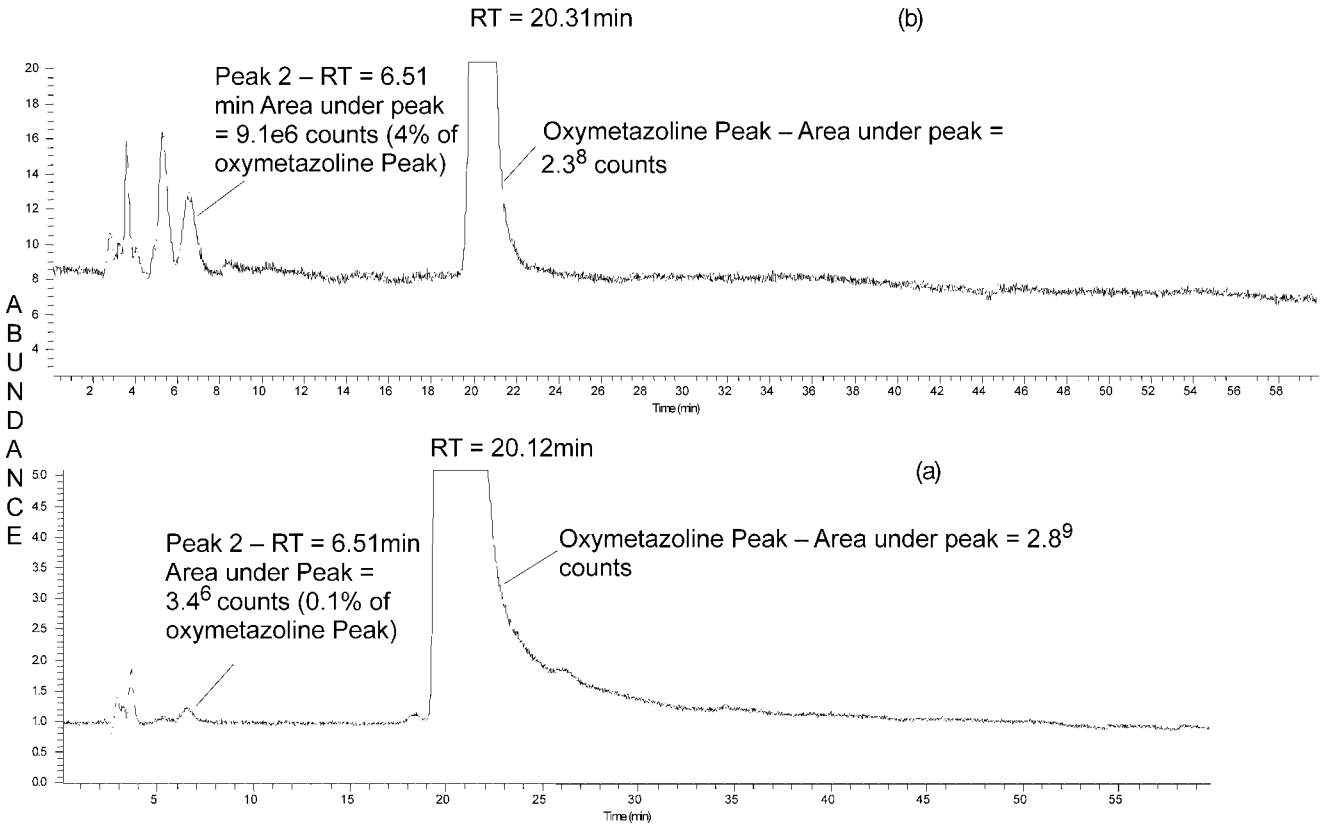


Figure 8. Comparison of oxidised oxymetazoline with a commercial product containing oxymetazoline: (a) oxymetazoline containing commercial product after storage at 40°C for 3 years and (b) *m*-CPBA-oxidised oxymetazoline raw material.

RCM

Table 2. Energies of formation of degradant species. (kcal mol^{-1})

	Preferred	Non-preferred
N-Oxide	−25.1976	−18.6215
Hydroxyamine	−35.4579	65.8997

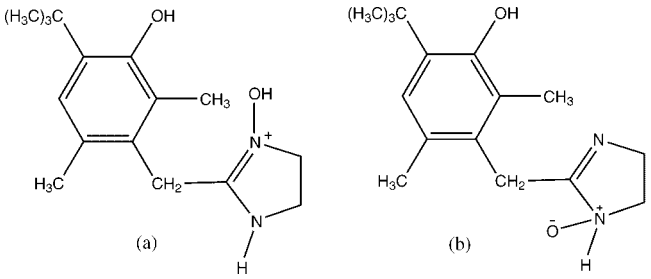


Figure 9. Non-preferred structures of (a) hydroxyamine degradant and (b) *N*-oxide degradant.

CONCLUSIONS

Oxidative stress of oxymetazoline with *m*-chloroperoxybenzoic acid produced three degradant species, which were isolated by liquid chromatography. Two of the products, a hydroxylamine and an *N*-oxide, have been identified and studied by electrospray ionisation mass spectrometry.

The formation reaction of the oxidised degradants may be similar to the Katada reaction,[14] where the products are *N*-oxides, one undergoing a Meisenheimer rearrangement[11] to form an hydroxyamine product. In order to assess the likelihood of formation of each species, a basic calculation of formation energies has been performed using the CAChe software (version 6.1) modelling package. The results of this experiment are shown in Table 2. The predicted structures of each species are shown to be energetically favourable versus the proposed thermodynamically unfavourable structural conformation (Fig. 9). In addition, the calculations suggest that the hydroxyamine would be more favourable than the *N*-oxide, which is supported by the apparent greater abundance seen in the TIC chromatogram (Fig. 3).

The procedure and results described in this work can be used to predict and study the degradation of active compounds in commercial products. This can be performed by comparing the conditions of storage of a product with those used in the accelerated oxidation reactions. In this particular case it can be concluded that none of the species identified here are likely to be formed to significant extents during the typical shelf life of this product.

The identification of potential degradants of the individual constituents can be used to inform the analysis of a commercial product during its shelf life. The normal practice of monitoring total active concentration may not elucidate product degradation until it is well under way, whereas tracking low-level degradants by mass spectrometry will reveal degradation at an early stage of the product shelf life and enable remedial action such as the addition of radical scavengers, chelants, anti-oxidants and stabilisers to the product. This procedure is not limited to drug activities, but may be broadened to all commercial product formulations, such as oral care cosmetics, household cleaning products, etc.

For further confirmation of structure, infrared (IR) spectroscopy of isolated fractions would give a clear indication of functionality change in the imidazoline group. Nitrogen NMR may also indicate changes in functionalities that have occurred. The structures of the fragments formed on electrospray ionisation could be confirmed by accurate mass measurements.

Acknowledgements

The authors would like to thank Dr. Bart Verhaeghe for helping to initiate the project and Dr. Bob Strife for guidance and instruction in the use of the ion trap. They are also grateful for Dr. Karen Sutton for valuable comments and suggestions.

REFERENCES

1. ICH Steering Committee, *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, 2003.
2. Hoffman T, Thompson R, Seifert J. *Drug Develop. Ind. Pharm.* 1989; **15**: 743.
3. Baker T, Hayes F, Dobson R, Tsueda M. *J. Chromatogr. A* 1995; **1/2**: 73.
4. Bruins AP. *J. Chromatogr. A* 1998; **1/2**: 345.
5. Wu Y. *Biomed. Chromatogr.* 2000; 384.
6. Bruins AP. *Trends Anal. Chem.* 1994; **1**: 37.
7. Bruins AP. *Trends Anal. Chem.* 1994; **2**: 81.
8. Ruckmick SC, Marsh DF, Doung ST. *J. Pharm. Sci.* 1995; **84**: 502.
9. Baumann C, Cintora MA, Eichler M, Lifante E, Cooke M, Halket J. *Rapid Commun. Mass Spectrom.* 2000; **14**: 349.
10. Strife RJ, Robosky LC, Garrett G, Ketcha MM, Shaffer JD, Zhang N. *Rapid Commun. Mass Spectrom.* 2000; **14**: 250.
11. Jones CW. *Applications of Hydrogen Peroxide and Derivatives*. Royal Society of Chemistry: Cambridge, 1999; chap. 3.
12. Ramanathan R, Su AD, Alvarez N, Blumenkrantz N, Chowdhury SK, Alton K, Patrick J. *Anal. Chem.* 2000; **72**: 1352.
13. Halket J, Cooke M. *Chromatogr. Separation Technol.* 1998; 8.
14. Ochiai E, Ishikawa M, Katada M. *Yakugaku Zasshi* 1943; 307.
15. Mclafferty FW. *Interpretation of Mass Spectra* (3rd edn). University Science Books: 1980; chap. 9.
16. Lopez LL, Tiller PR, Senko MW, Schwartz JC. *Rapid Commun. Mass Spectrom.* 1999; **13**: 663.