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# Pharmaceutical application of LC–MS. 1 — Characterization of a famotidine degradate in a package screening study by LC–APCI MS

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**Abstract**: The application of LC–MS to characterize low-level degradates in pharmaceutical dosage formulations is a new and challenging field. In a package screening study, a low-level degradate of famotidine (**1**, 3-[[[2-[[aminoiminomethyl]-amino]-4-thiazolyl]methyl]thio]-N-(aminosulphonyl)-propanimid-amide, an $H_2$-receptor antagonist, molecular weight: 337) was detected by HPLC in film-coated tablets packaged in child-resistant (CR) foil pouches which were stressed at 40°C/75% relative humidities (RH) for 4 months. LC–atmospheric pressure chemical ionization (APCI) mass spectrometry using positive ion mode yielded a molecular weight of 349 for the degradate, suggesting that it was formed by the addition of one carbon to the famotidine molecule. A detailed analysis of the positive product ion mass spectrum of the protonated degradate ion in a LC–MS–MS study indicated that the carbon was added to the side of N-(aminosulphonyl)-propanimid-amide of famotidine. The structure of the degradate was determined to be **2**, which was confirmed by LC–APCI MS and HPLC study of the product formed from the reaction of famotidine with formaldehyde — a one-carbon reagent.

**Keywords**: *Famotidine; LC–atmospheric pressure chemical ionization (APCI); degradate; pharmaceutical dosage formulations.*

## Introduction

Famotidine (**1**,3-[[[2-[[aminoiminomethyl]-amino]-4-thiazolyl]methyl]thio]-N-(amino-sulphonyl)-propanimid-amide), an important $H_2$-receptor antagonist, is a highly potent inhibitor of gastric acid secretion in humans [1]. Famotidine can degrade via hydrolysis and oxidation reactions to yield the hydrolysis degradates A1, A2, A3 and the oxidation degradate A6. These degradation products have been carefully studied and reported [2]. Stability-indicating reversed-phase HPLC methods have been reported in the literature [2–6] for the determination of famotidine and its degradates.

In the development of an over-the-counter (OTC) famotidine formulation, a package screening study was conducted in which the stability of film-coated famotidine tablets was evaluated in different packages. In this study, a famotidine degradate, specific to child-resistant (CR) foil pouches made of Archer laminate (92 gauge polyester/primer/7.5 #LDPE/0.00035 aluminum foil/primer/15 #LDPE sealant), was detected. The degradation formation was eliminated by using CR foil pouches made of another Archer laminate (92 gauge polyester/ink/primer/7.5 #LDPE/0.00085 aluminum foil/22 #EAA sealant). However, the detection of the unknown, a package-related famotidine degradate prompted us to carry out a detailed study to identify the degradate. Using the reversed-phase LC conditions described in this report (cf. Experimental section), the degradate was found to elute after famotidine, implying that it is less polar than famotidine. However, no detailed structural information could be obtained from the LC study.

LC–MS with atmospheric pressure chemical ionization (APCI) source was applied to the characterization of this new low-level degradate. LC–MS has been extensively used in the pharmaceutical industry and related laboratories for drug identification, metabolite profiling and identification, as well as screening and quantification [7, 8]. Especially, the interest in LC–APCI MS has been revived 10 years after the work of Horning and co-workers [9].

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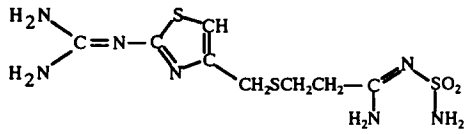

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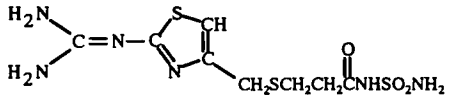

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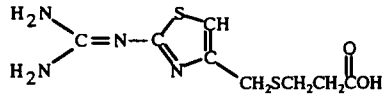

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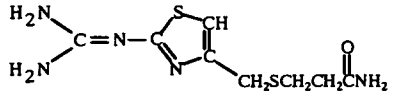

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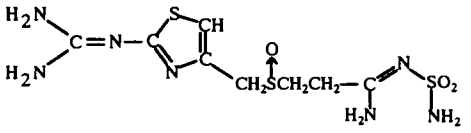

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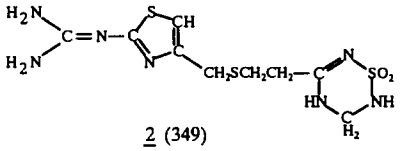

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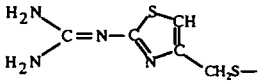

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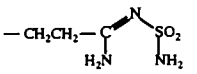

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**Structures**

Because of its capability of achieving extreme low detection limits, LC–APCI MS with a heated nebulizer interface has been applied in the drug quantification [9–12]. But, to the best of our knowledge, there has been no published report on the application of LC–APCI MS to the structural characterization of low-level degradates in pharmaceutical dosage formulations. This study, therefore, will be scientifically interesting and of particular interest in pharmaceutical research.

The system utilized in the LC–MS study was the PE SCIEX API III triple quadrupole mass spectrometer [13], which uses an APCI source with a heated nebulizer. The use of this source enabled the determination of molecular weight (M.W.) of 349 for the degradate, which is 12 daltons (one carbon) higher than that of famotidine (337), suggesting that it was formed by addition of one carbon to the famotidine molecule. The structure of the degradate was determined to be **2**, which was confirmed by LC–APCI MS and LC study of the product formed in the reaction of famotidine with formaldehyde — a one carbon reagent. The instrument also has tandem mass spectrometry (MS–MS) with on-line LC. Using the LC–MS–MS technique, a positive product ion mass spectrum of the protonated degradate ion was obtained, giving detailed structural information for the degradate, which is in good agreement with the structure **2**.

In the course of the drug development, it is important that dosage formulations be studied under different stress conditions such as high temperatures, high humidities, oxidizing conditions and light. These stressing conditions which may cause drugs to degrade are utilized to validate the stability-indicating property of the analytical method. The identification and characterization of low-level degradates have become a daily task, which cannot be fulfilled by LC only. Isolation of very low level degradates for characterization by techniques like

NMR is difficult and time-consuming. This study demonstrates that the combination of HPLC and LC–MS is applicable and most valuable in fulfilling this task.

## Experimental

*Chemicals*

Famotidine was obtained from Merck Research Laboratories (West Point, PA). Potassium phosphate monobasic (certified A.C.S. grade), sodium acetate (HPLC grade), ammonium acetate (certified A.C.S. grade), acetic acid, acetonitrile (Optima grade) and methanol (Optima grade) were purchased from Fisher Scientific (Philadelphia, PA). Paraformaldehyde (95%) was purchased from Aldrich (Milwaukee, WI). They were all used as received without further purification. Deionized water with at least 18 M-Ohm purified by a Milli-Q (Bedford, MA) system was used for mobile phase and sample preparations.

*Sample preparation*

Sample solutions of famotidine at a concentration of 0.1 mg $ml^{-1}$ were prepared by dissolving 10 mg of the famotidine standard or one famotidine tablet (10 mg potency) in 100 ml of the deionized water.

*LC Analysis*

The LC system consisted of a Hewlett–Packard (Avondale, PA) Model 1090 pump module, with a Spectra 100 variable-wavelength detector (Spectra-Physics, San Jose, CA), and a Perkin–Elmer LC-235 Diode Array Detector (Norwalk, CT). The analytical column was a Hypersil $C_{18}$ (150 mm × 4.6 mm i.d., 5 μm particle size) (Keystone Scientific, Bellefonte, PA). The mobile phase was acetonitrile–sodium acetate (pH 6.0, 0.1 M) (7:93, v/v). All chromatography was performed at 40°C. The detection by UV was set at 270 nm.

*LC–MS and LC–MS–MS analysis*

LC–MS and LC–MS–MS were performed on a PE Sciex (Thornhill, Ontario) Model API III triple-quadrupole mass spectrometer interfaced via a Sciex heated nebulizer probe to a LC consisting of a Perkin–Elmer ISS-100 autoinjector equipped with a 200 μl loop.

LC was performed using a Hypersil $C_{18}$ column (150 mm × 4.6 mm i.d., 5 μm particle size). For the LC–MS study, both isocratic and gradient LC methods were developed. Isocratic method used a mobile phase of acetonitrile–ammonium acetate (adjusted to pH 4.0 using acetic acid, 2 mM) (18:82, v/v) and a flow rate of 1 ml $min^{-1}$. Two solvents were used in the gradient method. Solvent A consisted of acetonitrile–ammonium acetate (adjusted to pH 4.0 by acetic acid, 2 mM) (5:95, v/v). Solvent B was acetonitrile–ammonium acetate (adjusted to pH 4.0 by acetic acid, 2 mM) (95:5, v/v). The gradient sequence was: from time 0 to 1 min, 80%A/20%B; from 1 to 20 min, 80%A/20%B to 100%B. The flow rate was 1 ml $min^{-1}$. For the LC–MS–MS study, the isocratic LC method was employed.

The settings of the interface and mass spectrometry were: the nebulizer probe temperature setting was 500°C. The nebulizing gas (air) pressure and auxiliary flow were set at 70 psi and 2.0 l $min^{-1}$, respectively. Gas-phase chemical ionization was done by a Corona discharge needle (+3 μA), and positive ions (protonated famotidine and its degradates) entered into the first quadrupole mass analyzer through a 0.0045 in. pinhole aperture (for MS study). The mass spectrometer was programmed to allow the protonated famotidine (**1**) molecules at $m/z$ = 338 and protonated famotidine degradate (**2**) at $m/z$ = 350 to pass through the first quadrupole filter (Q1) with collision-induced fragmentation in the second quadrupole (Q2) (collision gas, argon, 50 eV, $4 \times 10^{14}$ atoms $cm^{-2}$) and to be analysed in the third quadrupole (Q3) (for MS–MS study). The scan of the MS spectra was from $m/z$ 200 to $m/z$ 900.

*Reaction of famotidine with formaldehyde*

About 20 mg of the powder of the famotidine standard was placed in a capped glass vial together with 5 mg of paraformaldehyde wrapped in a piece of paper so that the famotidine powder was not in direct contact with paraformaldehyde. The vial was placed in an 80°C oven. At 80°C, paraformaldehyde molecules dissociated to form the vapour of the formaldehyde molecules, which reacted with famotidine in the closed capped vial. After 24 h, the famotidine standard was taken out and analysed by the reversed-phase HPLC method. Famotidine standard stressed at 80°C for 24 h in a capped glass vial in the absence of paraformaldehyde was analysed as a control. A famotidine film-coated tablet (10 mg, stressed at 40°C/75% relative humidity (RH) for 4

months in a CR foil pouch) was also analysed for comparison.

## Results and Discussion

*LC Study*

In the package screening study, the stability of famotidine tablets was examined in different packages under various stress conditions. In the study of film-coated famotidine tablets packaged in the CR foil pouches, HPLC (cf. Experimental section) detected an unknown peak eluting at a retention time of about 9 min. Since the unknown eluted after the famotidine (5.5–5.8 min) and the A3 degradate (6.8–7.2 min), it was less polar than famotidine and the A3 degradate. The unknown was not found in the stressed placebo tablets, indicating that it was famotidine-related. Using a diode array detector, an HPLC–UV scan of the unknown peak showed similar UV absorption of the unknown and famotidine, as illustrated in Fig. 1. Both had a maximum absorption at around 270 nm in the mobile phase (pH 6). This strongly supported that it is a famotidine degradate. But, the structure of the unknown could not be determined without a detailed LC–MS study.

*Determination of the molecular weight of the degradate by LC–APCI MS*

An important piece of information about the structure of an unknown is its molecular weight (M.W.), which was achieved by LC–APCI MS. A film-coated tablet (10 mg) in a CR foil pouch was stressed at 40°C/75% RH for 4 months. It contained about 1.1% (area) the unknown degradate as determined by the reversed-phase HPLC method with a UV detector. To determine its molecular weight, the tablet was examined by the isocratic LC–MS method (cf. Experimental section). Figure 2 shows the Total Ion Current (TIC) chromatogram of the tablet, which illustrates resolution of chromatographic peaks of famotidine and its degradates. This paper focuses on the unknown degradates which was detected at about 8.4 min as indicated in the TIC chromatogram.

Figure 3(a) shows the LC–MS spectrum of the unknown degradate under positive ion mode. The spectrum consists of an intense peak with *m/z* 350, which is the protonated species (unknown + $H^+$). The M.W. of the unknown was derived to be 349. In APCI using positive ion mode, the ionization of the analyte can be achieved by proton transfer, adduct formation and charge exchange reactions [14].

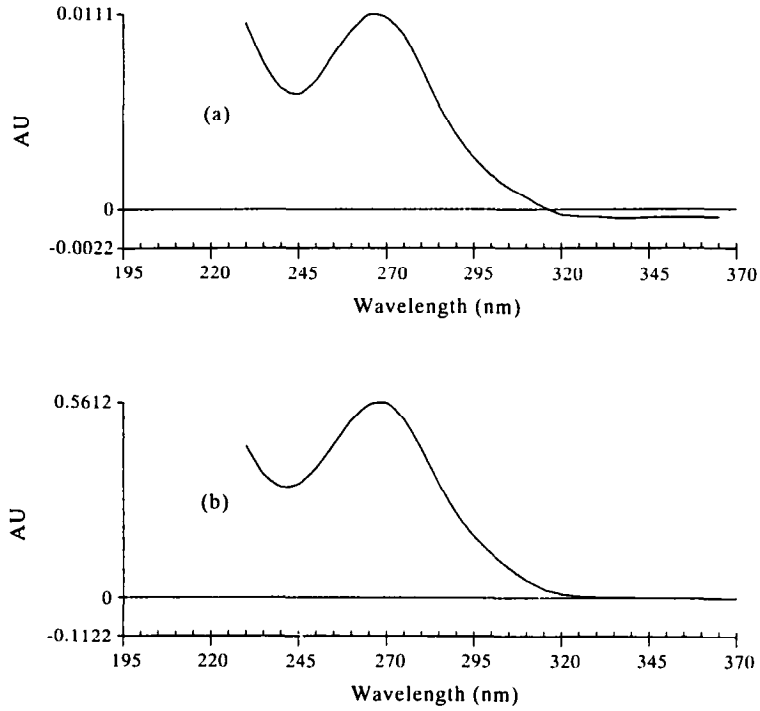



**Figure 1**
UV absorption spectra of (a) the unknown degradate and (b) famotidine observed for a film-coated famotidine tablet after stress at 40°C/75% RH for 4 months in a CR foil pouch by a diode array detector.

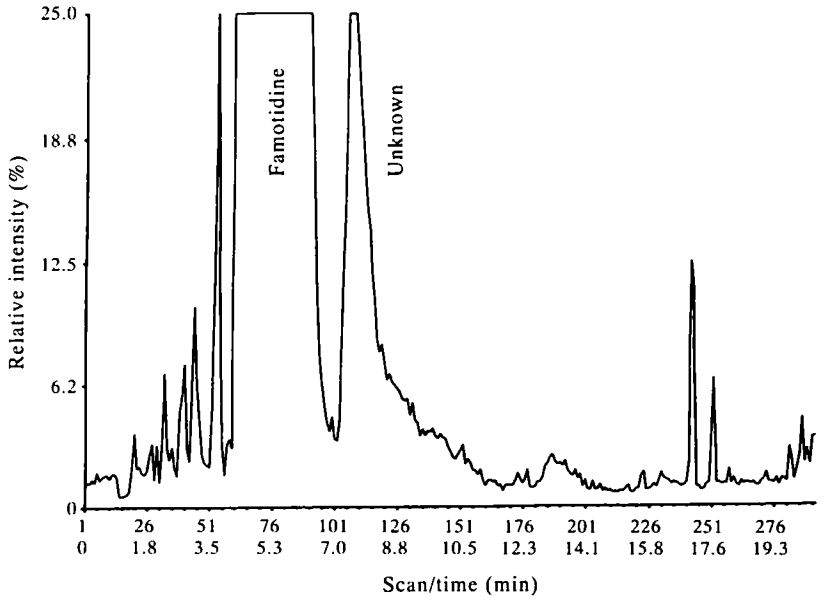



**Figure 2**
A TIC chromatogram of a film-coated famotidine tablet after stress at 40°C/75% RH for 4 months in a CR foil pouch, showing famotidine (marked) and its degradates including the unknown degradate (marked).

However, ammonium adduct ions of the unknown were not observed although 82% 2 mM ammonium acetate was used in mobile phase. Figure 4(a) shows the LC–MS spectrum of famotidine obtained under the same conditions. The spectrum consists of an intense peak with *m/z* 338 clearly assigned to the protonated famotidine (famotidine + $H^+$). Ammonium adduct ions of famotidine was also not observed. The similar observation that ammonium adduct ions were not observed for both famotidine and the unknown was consistent with that the degradate was famotidine-related [9]. A small peak at *m/z* 259 in Fig. 4(a) was assigned to the fragment ion ((famotidine + $H^+$)-$SO_2NH$). The LC–APCI MS correctly indicated the M.W. of famotidine — 337, demonstrating the reliability of the technique. The M.W. of the unknown is 12 daltons higher than that of famotidine, indicating that it was formed by the addition of one carbon (12) to the famotidine molecule. The elucidated structure of the degradate is **2**. The formation pathway of this degradate appeared to be very different from those of the hydrolysis or oxidation degradates [2–6].

That the degradate is **2** formed by addition of one carbon to the famotidine molecule was further confirmed by two experimental observations. First, a careful examination of Fig. 3(a) reveals that the molecular peak of the protonated degradate ion (relative intensity of 100%) has isotope peaks at *m/z* 351 and 352 (both have relative intensities of about 17%), which is similar to the molecular peak of the protonated famotidine ion possessing isotope peaks at *m/z* 339 and 340 (both have relative intensities of about 17%) (see Fig. 4a). The unusual, large (M(moleclar ion) + 2) peaks were mainly attributed to the fact that $^{34}S$ has natural abundance of 4.22% as compared to $^{32}S$. The observed isotopic distributions of the famotidine and the degradate ions agreed fairly well with the calculated distributions (the M + 1 and M + 2 ions of famotidine gave relative intensities of 14 and 15, respectively; and the M + 1 and M + 2 ions of **2** gave relative intensities of 15 and 15, respectively). This comparison strongly supported the assignment of the structure **2**. Secondly, and perhaps more decisively, the positive product ion spectra of the protonated degradate and famotidine ions (LC–MS–MS experiments, cf. Experimental section), shown in Figs 3(b) and 4(b), respectively, demonstrate that famotidine and the degradate have the same fragment ions below 189 (both have peaks of *m/z* 189, 155, 138 and 113), indicating that the moiety having *m/z* 187 in famotidine (moiety **3**) remained intact when famotidine was reacted to form the degradate.

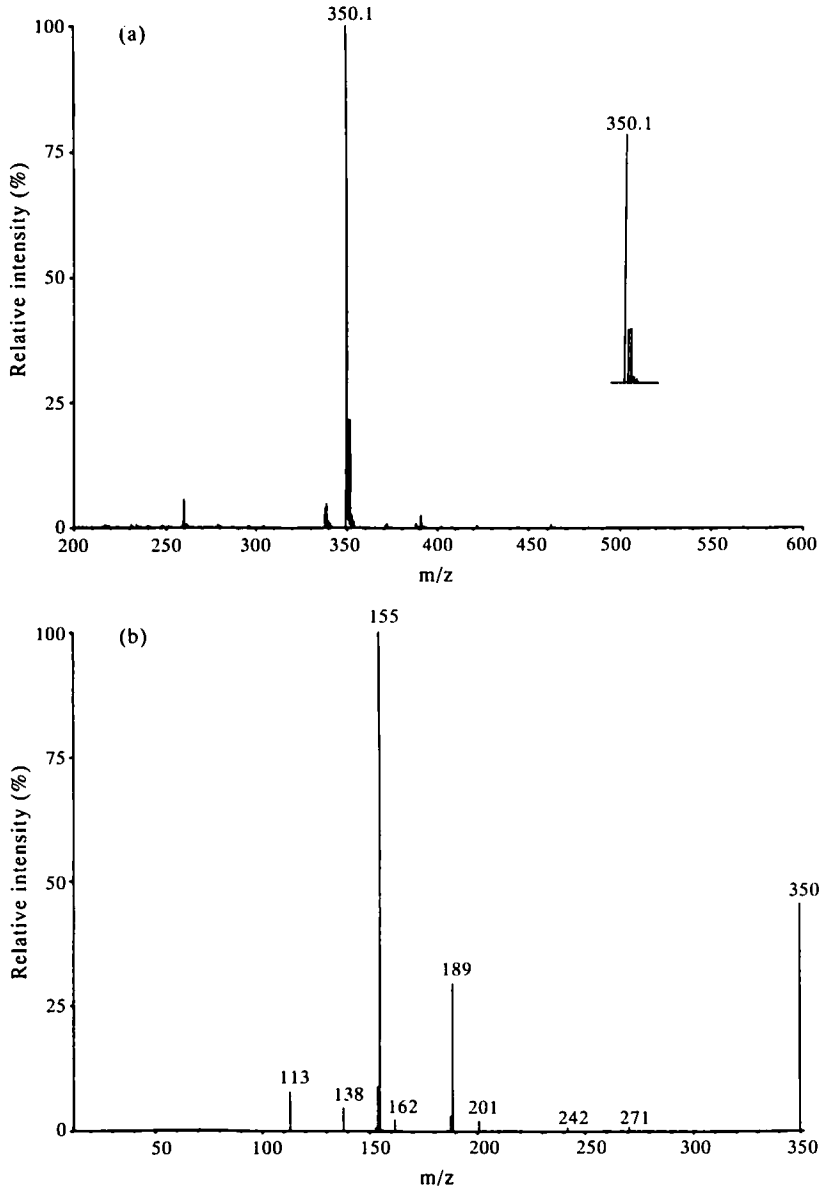



**Figure 3**
(a) LC–MS spectrum of the protonated degradate ion **2** and (b) positive product ion mass spectrum of the protonated degradate ion **2**.

Above *m/z* 189, the degradate shows weak fragment ions at *m/z* 201, 242 and 271 while famotidine shows only a strong fragment ion at *m/z* 259, indicating that the moiety having *m/z* of 150 (**4**) of famotidine has undergone reaction(s) to form the degradate. The largest fragment ion of the degradate (*m/z* 271, ((**2** + $H^+$)-$SO_2NH$)) was 12 daltons higher than that of famotidine (*m/z* 259, ((famotidine + $H^+$)-$SO_2NH$)), which had the same difference as their molecular weights, in good agreement with the structure **2** (for further analysis of the product ion spectra, see below).

The determination of the M.W. for famotidine and the degradate demonstrates the advantage of the LC–APCI MS over the electron impact ionization mass spectrometry for the study of highly polar and/or thermally labile compounds such as famotidine. The molecular ion peak of famotidine could not be observed by mass spectrometry using electron impact ionization (studied on an LKB Model 9000 mass spectrometer and a Varian MAT Model 731 mass spectrometer, Merck Research Laboratories, unpublished results). The most intense peak was at *m/z* 188 correspond-

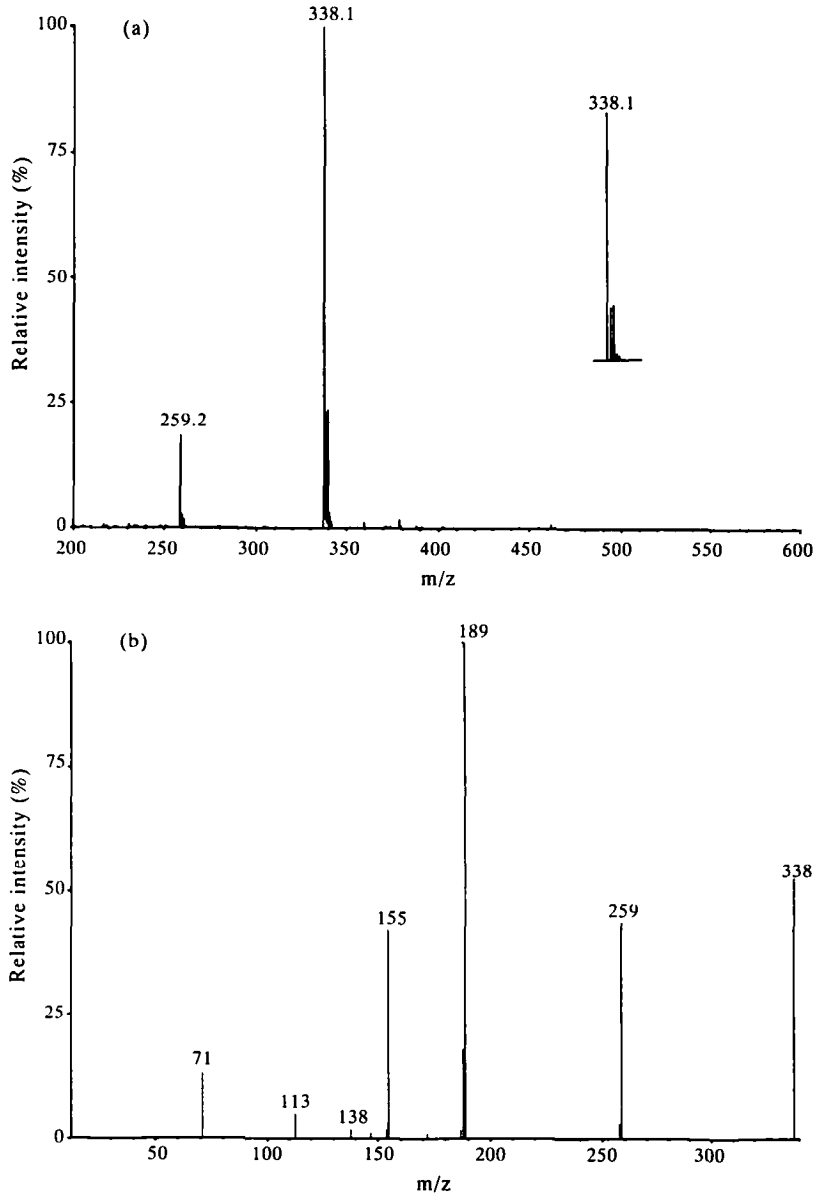
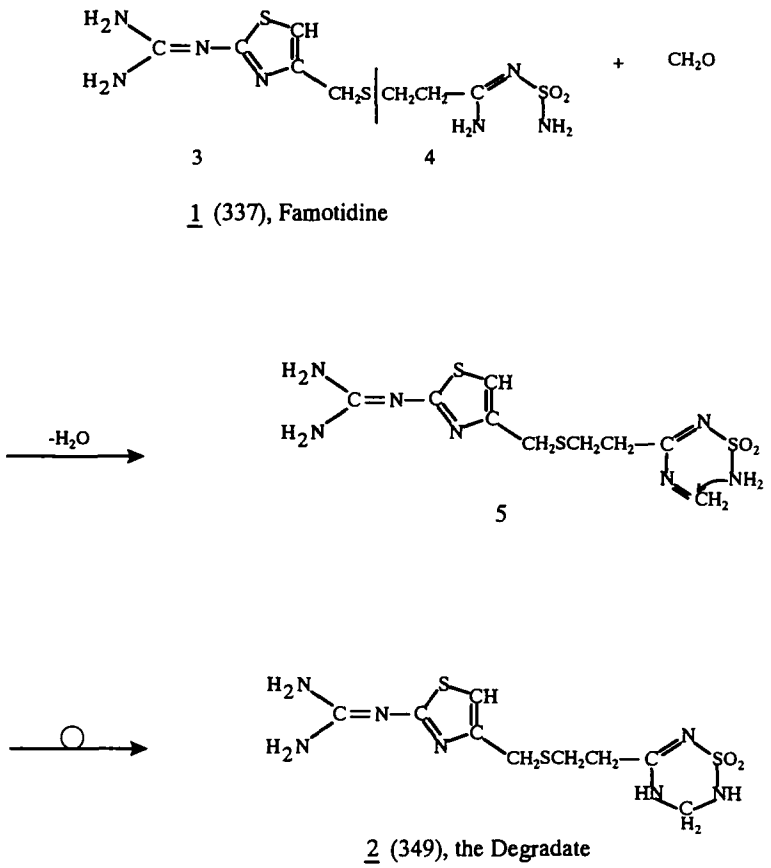



**Figure 4**
(a) LC–MS spectrum of the protonated famotidine ion and (b) positive product ion mass spectrum of the protonated famotidine ion.

ing to the fragment ion of (famotidine − **4** + H)$^+$. Although trimethylsilylation could produce molecular ions for tri-, tetra- and penta-trimethylsilyl derivatives of famotidine, derivatization is usually time-consuming. The advantage of APCI MS could be attributed to the high efficiency of the ion-molecule reactions under APCI conditions that facilitated the longer lifetime of the protonated famotidine and the unknown degradate ions [14].

*Reaction of famotidine and formaldehyde — HPLC and LC–APCI MS study*

To further confirm the assignment of the structure **2** to the degradate, the reaction of famotidine with formaldehyde (a one-carbon reagent) was investigated by HPLC and LC–APCI MS (cf. Experimental section). It was expected that formaldehyde can undergo nucleophilic addition reactions with one of the two primary amine groups in **4** of the famotidine molecule, as shown in Scheme 1. The intermediate formed from the addition loses a water molecule to form an imine group ($CH_2$=N—). The other primary amine group of **4** then can add to the carbon side of the imine group to form the degradate (**2**) after a 1,3-proton migration in the intermediate.




**Scheme 1**
Proposed reactions between famotidine and formaldehyde to form the degradate 2.

The HPLC results are shown in Fig. 5(a)–(c). Figure 5(a) shows the HPLC chromatogram of the stressed tablet. As marked, famotidine eluted at 5.46 min and the degradate eluted at 9.14 min. Figure 5(b) shows the HPLC chromatogram of the famotidine standard stressed at 80°C in a capped vial in the presence of paraformaldehyde, which clearly consisted of the degradate peak at 9.20 min in addition to the famotidine peak at 5.65 min. Since the retention time of this peak in Fig. 5(b) was the same as that of the degradate observed for the stressed film-coated tablet (Fig. 5(a)), the same degradate was believed to be observed in the reaction of famotidine with formaldehyde. Figure 5(c) shows the HPLC chromatogram of the famotidine standard stressed at 80°C in a capped vial in the absence of paraformaldehyde. It consists of the famotidine peak at 5.35 min and several very low level peaks (<0.1%). Within the detection limit, no peak at about 9–10 min attributable to the degradate is seen, indicating that famotidine itself (in contact with air) did not degrade to the degradate at 80°C.

The above samples were further examined by LC–APCI MS employing the gradient LC–MS method and a positive ion mode (cf. Experimental section). The expectation was that if the species formed from the reaction of famotidine with formaldehyde was indeed the same as the degradate detected in the stressed film-coated tablet, the species and the degradate should have the same retention time under the gradient LC conditions as observed under the isocratic LC conditions described above. More critically, the species should have the same M.W. of 349 as the degradate. Figure 6(a) shows a TIC chromatogram of the famotidine standard after stressed at 80°C with formaldehyde. A chromatographic peak at 8.4 min, which is overlapping with the strong peak of famotidine in the TIC chromatogram, can be clearly seen in the extracted chromatogram

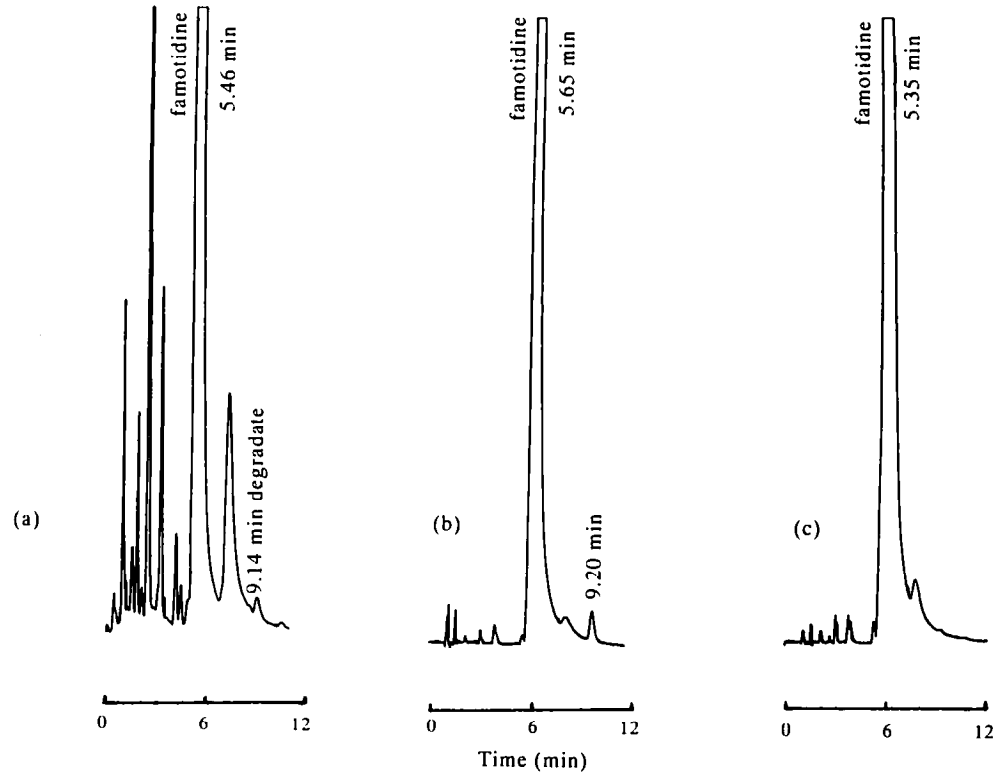



**Figure 5**
HPLC chromatograms of (a) a film-coated famotidine tablet after stress at 40°C/75% RH for 4 months in a CR foil pouch, (b) famotidine standard after stress at 80°C in a capped glass vial with paraformaldehyde for 24 h, and (c) famotidine standard after stress at 80°C in a capped glass vial without paraformaldehyde for 24 h.

(a chromatogram displaying peak(s) with a specified *m/z* ratio) in Fig. 6(b). The peak has *m/z* 350 as shown in the LC–MS spectrum in Fig. 6(c). For comparison, Fig. 7(a) shows the TIC chromatogram of the stressed famotidine film-coated tablet obtained under the same LC–MS conditions. Similarly, a chromatographic peak eluting at 8.6 min is shown in the extracted chromatogram in Fig. 7(b). The peak has *m/z* 350 as shown in the LC–MS spectrum of Fig. 7(c). These indicated that the species formed from the reaction of famotidine with formaldehyde has the same retention time of about 8.5 min and the same M.W. of 349 as those of the degradate formed in the stressed film-coated tablets. In addition, the *m/z* 350 peaks shown in Figs 6(c) and 7(c) also have the same isotopic distributions. As shown in the inserted, expanded spectra in Figs 6(c) and 7(c), both peaks have isotopic ions with the relative intensities about 15% for M + 1 and M + 2 ions, respectively.

In summary, the same retention times observed under two different LC conditions — isocratic and gradient, and the same molecular weight and isotopic distributions clearly demonstrated that the species formed from the reaction of famotidine with formaldehyde was the same as the degradate detected in the stressed film-coated famotidine tablet, which, in turn, confirmed the assignment of the structure **2** to the degradate.

It should be pointed out that, in this study, the reaction of famotidine with formaldehyde was used as a synthetic route of the degradate **2** in order to confirm its structure, not to demonstrate the pathway it was formed in the stressed film-coated tablets. Although it is possible that the degradate in the stressed tablets was indeed formed from such a reaction considering that trace amount of formaldehyde could be produced from thermal or photodegradation of certain material(s) in the package, other pathways in which the degradate **2** could be produced should also be investigated. This paper deals with the structure of the degradate. A detailed discussion of the pathways in which the degradate was formed in the

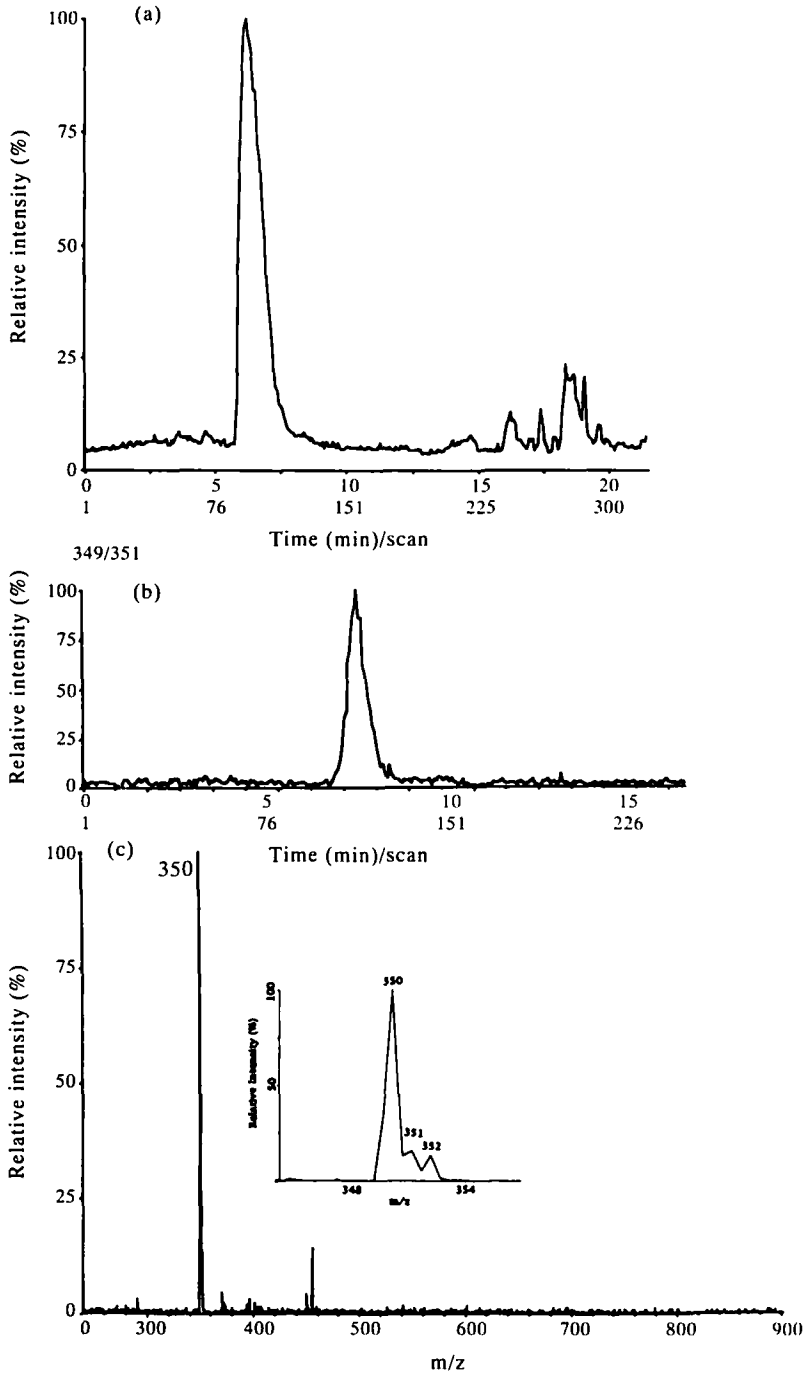



**Figure 6**
(a) TIC chromatogram of the famotidine standard after stress at 80°C in a capped glass vial containing paraformaldehyde for 24 h, (b) extracted chromatogram for *m/z* 350, and (c) LC–MS spectrum of the peak of *m/z* 350.

formulations will be addressed in a separate publication.

### *LC–MS–MS Study of the degradate*

The positive product ion mass spectra of **2** and famotidine shown in Figs 3(b) and 4(b) obtained in LC–MS–MS experiments has been analysed earlier. The analysis demonstrated that **2** and famotidine have the same moiety **3**. The fragmentation patterns of protonated ions of **2** and famotidine were thus similar below *m/z* 189. A more detailed analysis showed that the main difference below *m/z* 189 is that there was an extra peak of *m/z* 71 in the famotidine spectrum of Fig. 4(b). This is reasonable since

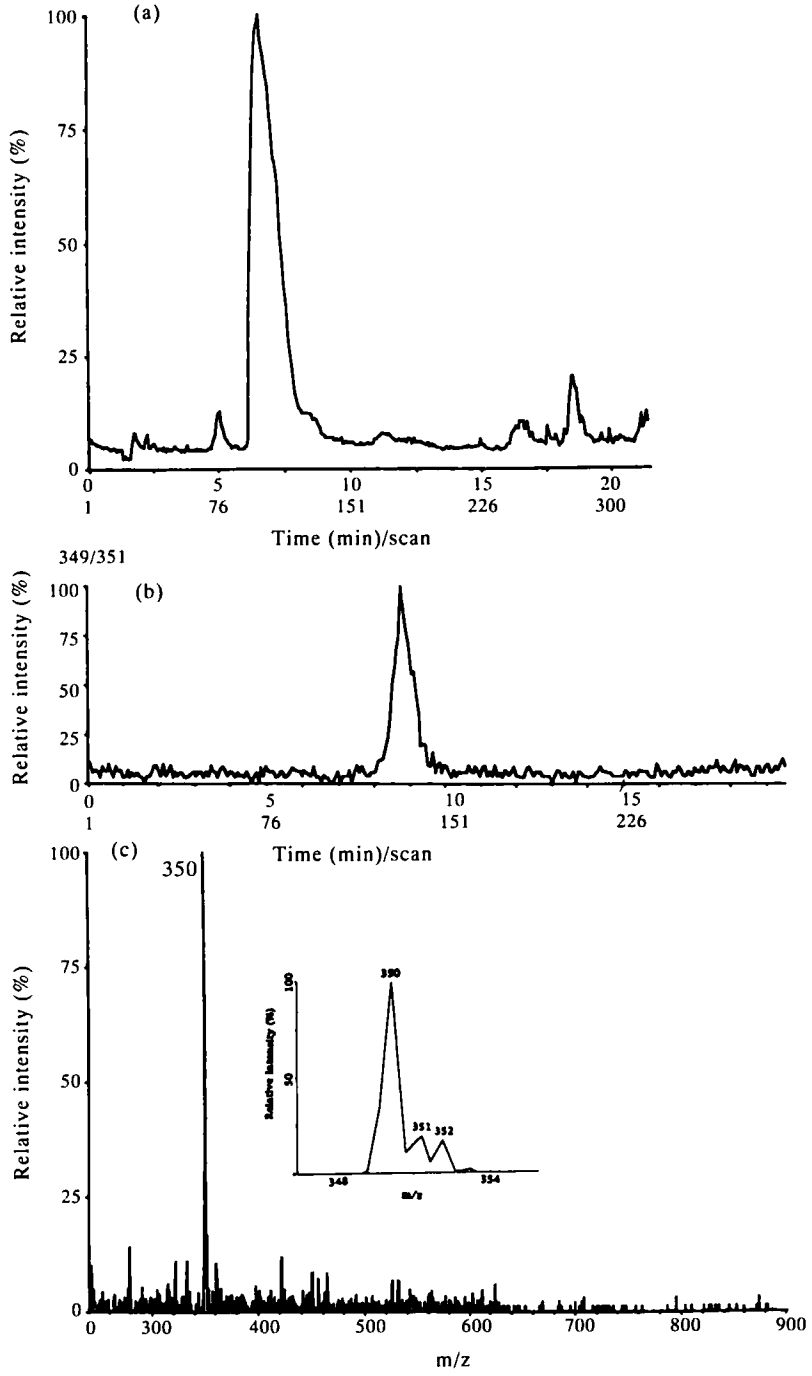
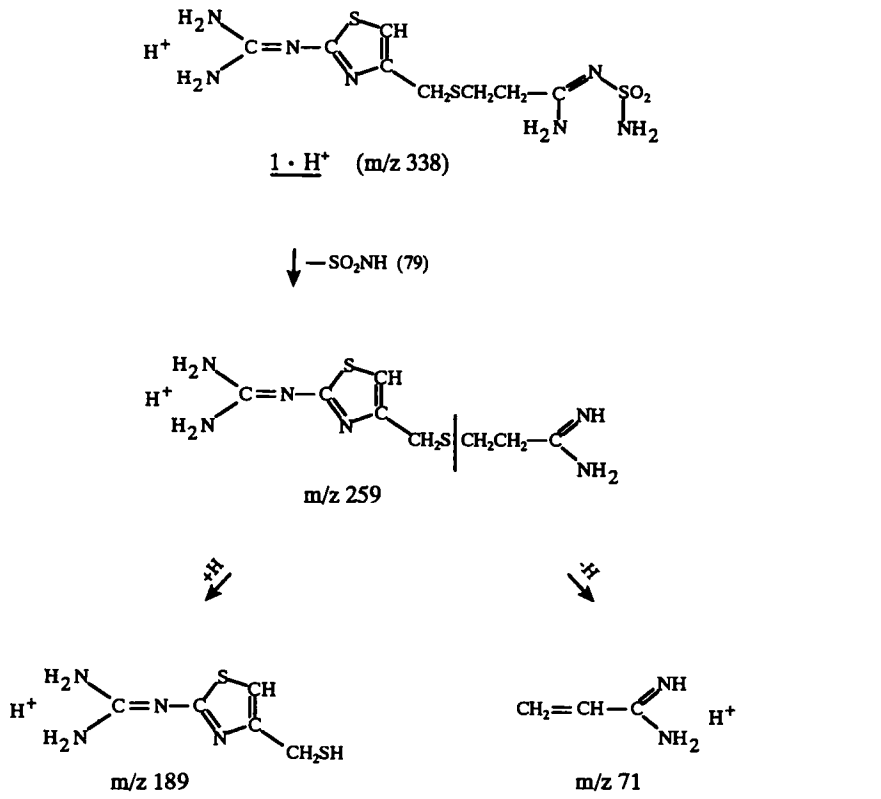
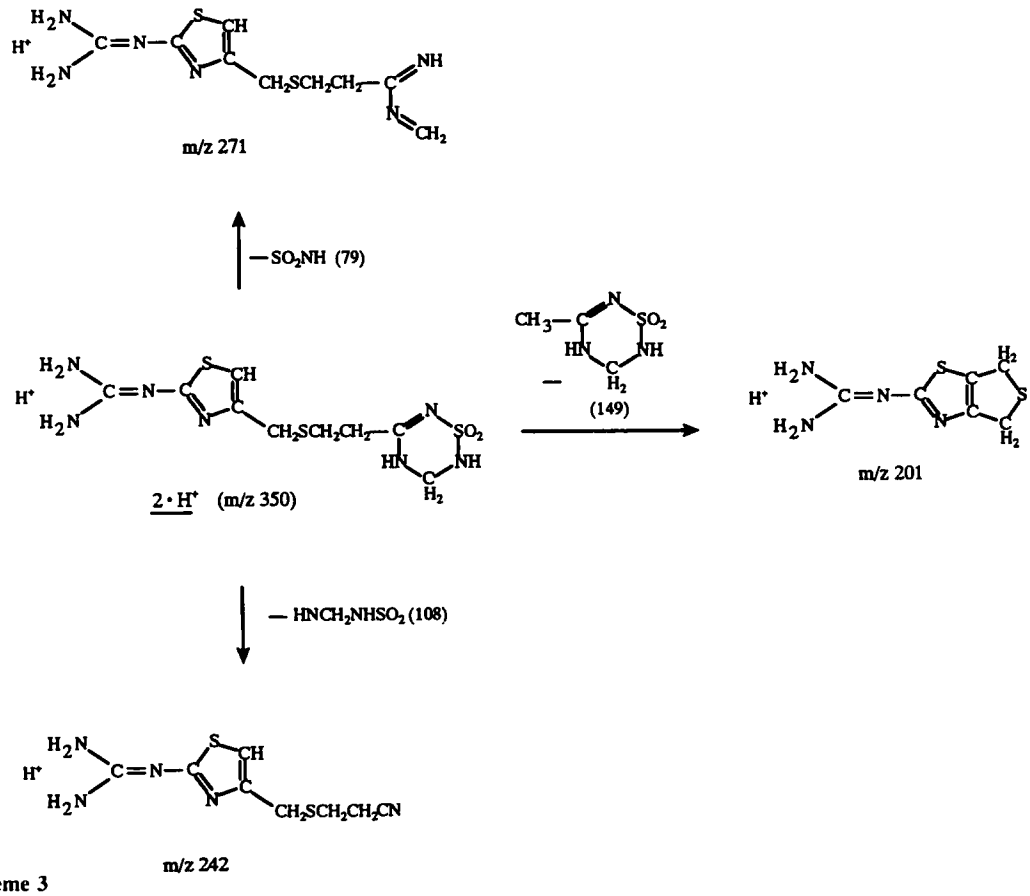



**Figure 7**
(a) TIC chromatogram of the film-coated tablet after stress at 40°C/75% RH for 4 months in a CR foil pouch, (b) extracted chromatogram for *m/z* 350, and (c) LC–MS spectrum of the peak of *m/z* 350.

the peak of *m/z* 71 was produced from the fragmentation of the *m/z* 259 ion. As can be seen in Scheme 2, after the homolytic cleavage of the sulphur–carbon bond in the *m/z* 259 ion, two fragments were formed. One with the sulphur atom could abstract a hydrogen from the other with the carbon atom. The resulted species could both be protonated to form either the ion of *m/z* 189 or the ion of *m/z* 71. Since there was no fragment ion of *m/z* 259 in **2**, the *m/z* 71 ion was not observed for **2**. In the *m/z* range above *m/z* 189, the fragmentation patterns of **2** and famotidine were different. Famotidine showed one peak with *m/z* 259, while **2** had three fragment ions at *m/z* 201, 242 and 271. All these three ions can be easily and




**Scheme 2**
Fragmentation of the protonated famotidine ion to form the ions of *m/z* 189 and *m/z* 71.




**Scheme 3**
Fragmentation pathways of the protonated degradate ion **2** above *m/z* 189.

uniquely accounted for by the structure **2** as shown in Scheme 3, giving further strong support to the structure **2**. It should be pointed out that the $—N{=}CH_2$ intermediate **5** (a methyleneimine compound) shown in Scheme 1 also has mass 349, which, therefore, was also a possible structure for the degradate based on the molecular weight alone. However, compounds with $—N{=}CH_2$ function are very unstable [15]. They were generally considered to be highly reactive intermediates which could not be isolated [15]. For example, when 5-chloro-2,4-disulphamoylaniline, which has a similar reaction centre as that of **2**, reacts with paraformaldehyde in non aqueous media, the only product is hydrochlorothiazide. No methyleneimine intermediate could be isolated [16]. In addition, the observation that there was a fragment ion of *m/z* 242 (the protonated ion of **2** — $HNCH_2NHSO_2$) but no fragment ion of *m/z* 309 (the protonated ion of **5** — $CH_3CN$) in the positive proudct ion mass spectrum shown in Fig. 3(b) also excluded **5**.

## Conclusion

A package-related degradate of famotidine was detected by HPLC for stressed film-coated tablets. The degradate has a M.W. of 349 as determined by LC–APCI MS, 12 daltons (one carbon) higher than that of famotidine (337). Therefore, it was assumed that the degradate was formed by the addition of one carbon to the famotidine molecule, which was confirmed by the study of the product formed from the reaction of famotidine with formaldehyde by both LC and LC–APCI MS. The elucidated structure of the degradate is **2**, which was confirmed by LC–MS–MS study.

A significant result in the LC–APCI MS study is that the excipients of the tablet did not interfere with the observation of famotidine and its degradates after a proper method was developed to separate them. This study demonstrates that the combination of HPLC and LC–MS is invaluable in the identification and characterization of low-level degradates (0.1% or above) in pharmaceutical dosage formulations.

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