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Determination of degradation products of sumatriptan succinate using LC-MS and LC-MS-MS

Xiaohui Xu, Michael G. Bartlett, James T. Stewart *

Department of Pharmaceutical & Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA 30602-2352 USA

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Abstract

Acid, base, heat, oxidation and UV irradiation stress methods were applied to study the stability of the bulk drug form of sumatriptan succinate. Liquid chromatography coupled with mass spectrometry (LC-MS and LC-MS-MS) was used to analyze the degraded samples and tentative structural identifications were assigned based upon known reactivity of the drug, molecular weight measurements and MS-MS fragmentation patterns. Sumatriptan succinate was found to be stable to exposure of acid, base, oxidation and UV irradiation at ambient conditions, but was found to degrade under acidic, basic and oxidative conditions when heated to 90°C.

Keywords: Sumatriptan Succinate; LC-MS; LC-MS-MS; Stability; Degradation pathway; Degradant identification

1. Introduction

Sumatriptan {3-[2-(dimethylamino)-ethyl]-*N*–methyl-1H-indole-5-methane sulfonamide} succinate is a serotonin agonist effective in the acute treatment of migraine headaches. Structurally related to the neurotransmitter serotonin, sumatriptan acts by selectively binding to serotonin type-1D receptors, resulting in vasoconstriction of extensively dilated cranial blood vessels and subsequent relief of migraine-related pain. Sumatriptan administered subcutaneously or orally is effective in relieving acute migraine symptoms and cluster headaches and is well tolerated by patients [1–3].

Stability testing provides evidence of the quality of a bulk drug when exposed to the influence of environmental factors such as pH, temperature, humidity and light. The data from such studies enables storage conditions, re-test periods and shelf lives to be established [4]. Stress testing helps to determine the intrinsic stability of the molecule by establishing the degradation pathways. Establishing a drug stability profile is analogous to establishing a metabolic profile. Stability studies of a bulk drug or drug dosage form are no less important. Degradant and impurity profiles are critical to the safety and potency assessment of a

* Corresponding author. Tel.: +1-706-5424410; fax: +1-706-5425358.

E-mail address: jstewart@rx.uga.edu (J.T. Stewart).

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drug product during clinical trials. and in drug product assessment. Degradants are usually present at low concentrations and their identification may be interfered with by larger amounts of excipients and/or other active drugs in a given formulation. It is difficult to isolate and identify individual degradants using mass spectrometry (MS) or NMR alone. On-line LC-MS and LC-MS-MS are ideal methods for performing these degradation studies.

The stability of sumatriptan succinate in bulk drug and oral liquids using HPLC has been studied by Fish [5] and Nii [6]. Fish found that sumatriptan was stable for at least 21 days when stored in the dark at 4°C as three extemporaneous liquid preparations made from crushed tablets [5]. The stability of sumatriptan in a tablet dosage form has also been performed using HPLC-UV [7,8]. However, these studies did not attempt the identification of the degradant structures.

Assays for sumatriptan and other -triptan drugs in biological fluids have included LC with coulometric [9], electrochemical [10] and MS detection [11–15]. In this study, on-line LC-MS and LC-MS-MS were used to identify or predict sumatriptan degradants after the drug was exposed to various physical and chemical conditions.

2. Experimental

2.1. Reagents and chemicals

HPLC grade methanol and acetonitrile, concentrated sodium hydroxide solution (electrophoresis grade), concentrated hydrochloric acid, glacial acetic acid, formic acid and 3% hydrogen peroxide were obtained from J. T. Baker (Phillipsburgh, NJ). Trifluoroacetic acid (TFA, 99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All chemicals were used without further purification.

2.2. Preparation of standard solution

Sumatriptan succinate bulk drug was supplied by Glaxo Wellcome, Inc. (RTP, NC),. A 0.4 mg/ml sumatriptan succinate standard solution was prepared in deionized water (Picotech Water System, RTP, NC).

2.3. LC-UV analysis

LC-UV analyses were performed using Waters Model 515 pumps set up in the gradient mode (Milford, MA,), with a Rheodyne Model 7125 manual injector, and a Waters Model 996 Photodiode Array detector. The HPLC column was a Brownlee ethylsilane (22 cm × 4.6 mm I.D., 10 μm particle size, Applied Biosystems, Foster City, CA).

The mobile phase was 90:10 (v/v) 0.1% aqueous formic acid: acetonitrile-methanol (6:1 v/v). The injection volume was 50 μl and the flow rate was 0.6 ml/min. The assay was performed at 23°C. Diode-array UV spectra were used to assess HPLC peak purity. Millennium$_{32}$ (Waters Corp, Milford, MA) software was used for data acquisition.

2.4. LC-MS and LC-MS/MS analysis

Chromatographic separations were performed using a Hewlett Packard (Palo Alto, CA) Model 1100 HPLC system consisting of a vacuum degassing module, an autosampler, a quaternary pump and a column heater. The injection volume, flow rate and mobile phase compositions were identical to those used in the LC-UV studies.

LC-MS and LC-MS-MS experiments were performed using a Micromass (Beverly, MA) Quattro II triple quadrupole mass spectrometer equipped with a megaflow electrospray ionization (ESI) interface using nitrogen as a sheath gas. The needle voltage was approximately 3500 V and the cone voltage was held at approximately 40 V. The source temperature was 140°C. Scans were acquired over the mass range from m/z 50–400 at 1 s per scan. MS data acquisition and analysis were performed using MassLynx NT version 2.22 (Beverly, MA). For MS-MS experiments, the positively charged molecular ions of sumatriptan and each degradant were mass selected and focused into a collision cell containing argon gas (99.999% purity) maintained at a pressure of $\sim 13 \times 10^{-3}$ Torr. The precursor and collision induced frag-

ment ions were monitored by the post-collision quadrupole analyzer. Product ion mass spectra at collision energies of 20 and 25 eV were monitored in separate channels for each compound.

2.5. Sample stress conditions

Solutions of 0.1N HCl, 0.1 N NaOH, and 3% H_2O_2 were prepared and used in the degradation studies. In each case, a 0.4 mg/ml sumatriptan stock solution was combined with acid, base or peroxide solution and allowed to stand at 23°C or heated at 90°C for 30 min to 10 h according to the stability testing procedures described by Weiser [16]. For comparison, a 0.4 mg/ml sumatriptan solution was heated at 90°C for 10 h without the addition of acid, base or peroxide. Sumitriptan was also degraded with UV irradiation at 254 nm.

For acid degradation studies, 1 ml of 0.1 N HCl was added to 1 ml of 0.4 mg/ml sumatriptan succinate stock solution in a sealed scintillation vial. This solution was allowed to stand at 23°C or heated at 90°C for 10 h. The acidified solutions were neutralized with a corresponding volume of 0.1 N NaOH and diluted with methanol–water (1:1, v/v) to 0.1 mg/ml prior to assay.

For base degradation studies, 1 ml of 0.1 N NaOH was added to 1 ml of the 0.4 mg/ml sumatriptan succinate stock solution in a sealed scintillation vial. It was allowed to stand at 23°C or heated at 90°C for 10 h. The solution was neutralized with a corresponding volume of 0.1 N HCl and diluted with methanol–water (1:1, v/v) to 0.1 mg/ml prior to assay.

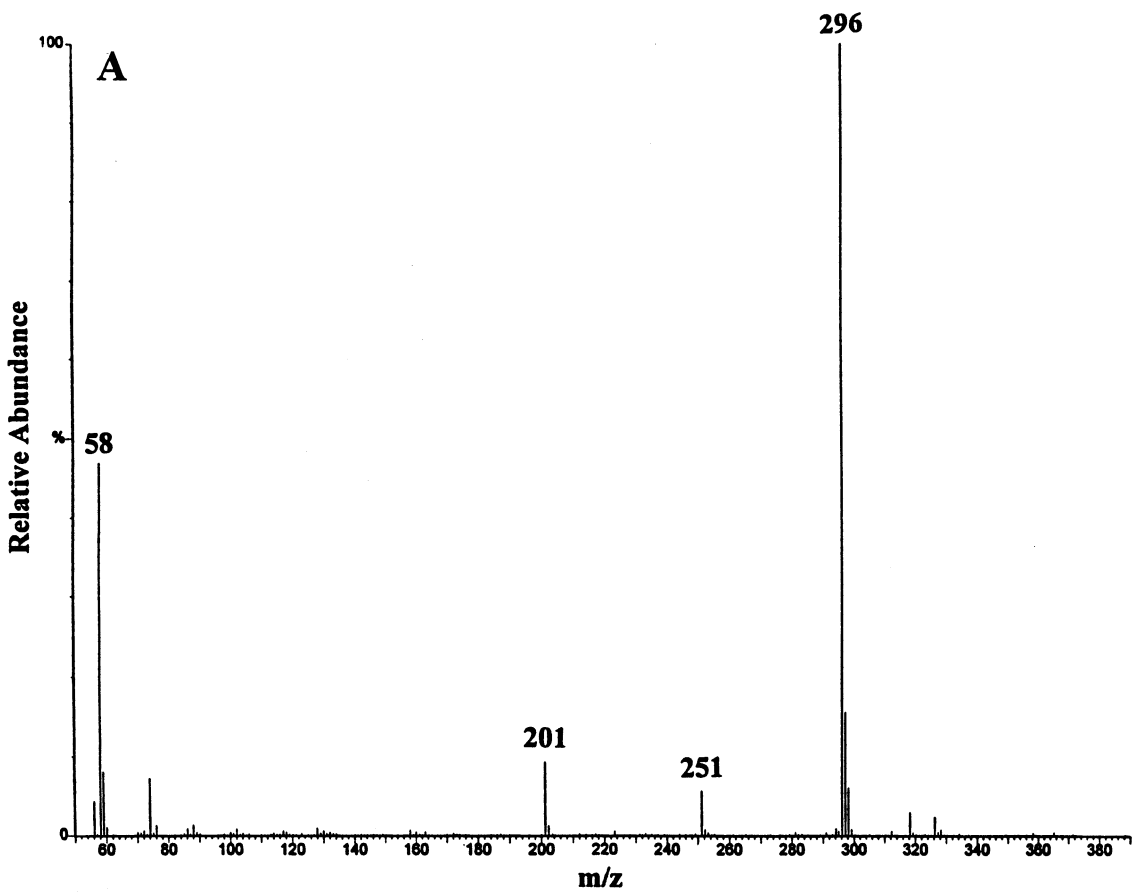


Fig. 1. Positive-ion electrospray MS (A), and collision-induced dissociation tandem MS (B) of sumatriptan.

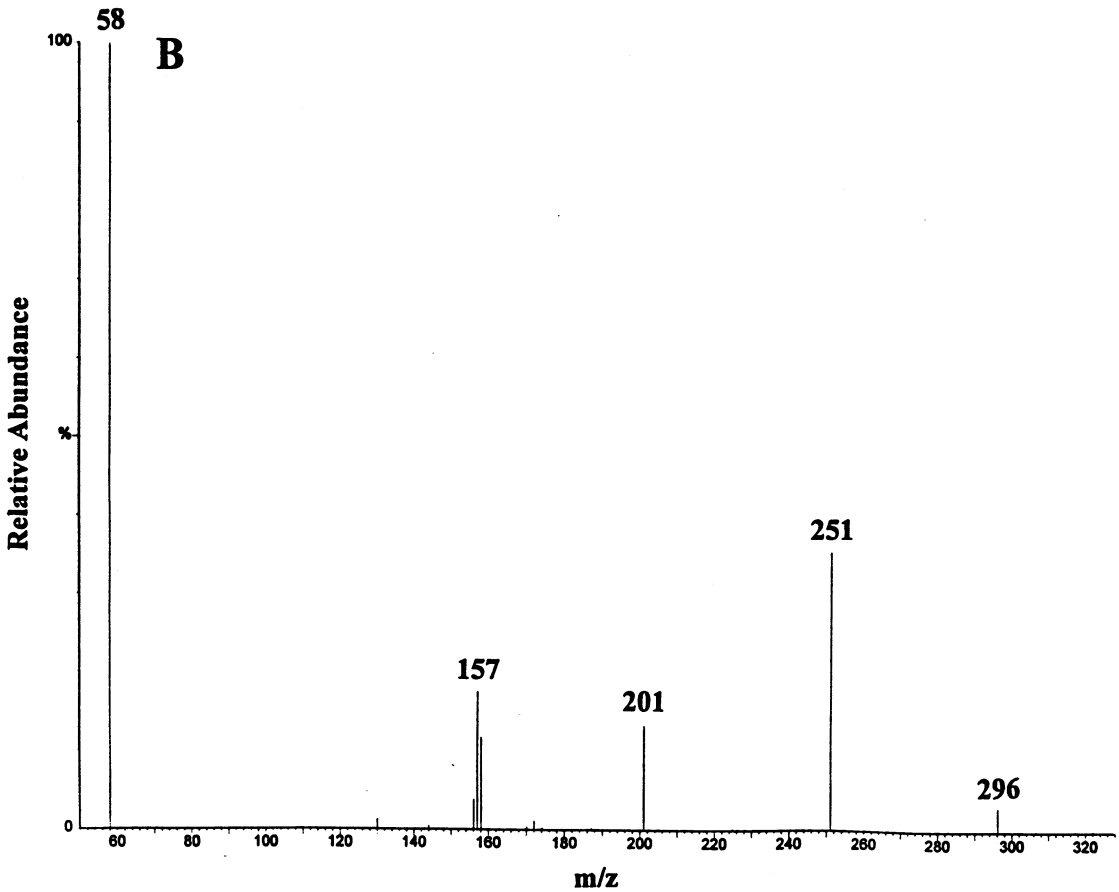


Fig. 1. (*Continued*)

For oxidative degradation studies, 1 ml of a 3% hydrogen peroxide solution was added to 1 ml of the 0.4 mg/ml sumatriptan succinate stock standard in a sealed scintillation vial. This solution was allowed to stand at 23°C or heated at 90°C for 30 min. Prior to analysis, the solution was diluted to 0.1 mg/ml using methanol–water (1:1, v/v).

For UV irradiation studies, 1 ml of the 0.4 mg/ml of sumatriptan succinate stock standard was diluted with methanol to 0.2 mg/ml and placed in a sealed quartz cuvette. The cuvette was placed inside a cabinet equipped with a 254 nm UV lamp and the solution was irradiated at 23°C for up to 24 h. Prior to analysis, the solution was further diluted with methanol–water (1:1, v/v) to a final concentration of 0.1 mg/ml.

3. Results and discussion

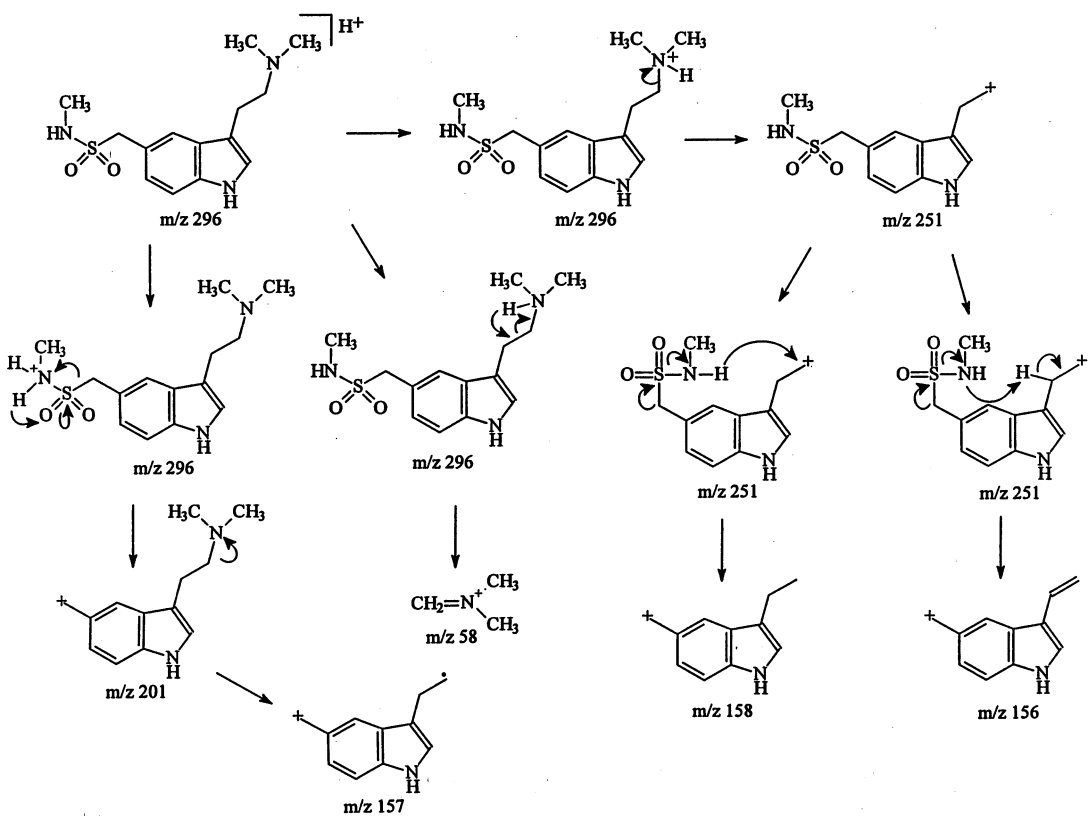
3.1. LC-MS Studies

Mass spectrometry source conditions were optimized by flow injection analysis (FIA) of the sumatriptan standard solution. Initially, positive-ion electrospray MS was performed using 90:10 (v/v) 0.1% aqueous acetic acid:acetonitrile–methanol (6:1v/v) as mobile phase. However, acetic acid did not provide adequate chromatographic separation. Thus, formic acid was substituted for acetic acid as a mobile phase additive since it promoted the selective and highly sensitive formation of protonated analytes and gave improved chromatographic performance.

In a typical degradation study, 10–30% degradation of the active drug is sufficient, but not so severe as to generate secondary products. The degradation can be accelerated by increasing the temperature to 90 ± 5°C, above which significant loss of the degradation solution due to evaporation may occur. At 23°C, sumatriptan succinate was stable in water and in acid, base, 3% H_2O_2 and UV irradiation conditions. Also, a sumitriptan succinate solution in water was stable when heated at 90°C for 10 h. The irradiation conditions used in this study do not match those recommended by ICH guidelines. In this study, we have used the more energetic 254 nm wavelength rather than broadband irradiation from 300–800 nm. While the absence of degradation products when using the 254 nm irradiation is suggestive of high photostability, it can not be considered conclusive.

3.2. Degradant structure characterization by LC-MS and LC-MS-MS

A key step in elucidating degradant structures is to understand the fragmentation pattern of the active drug substance. The positive-ion electrospray mass spectrum (Fig. 1A), tandem mass spectrum (Fig. 1B) and proposed fragmentation mechanism (Scheme 1) for sumatriptan are shown. The base peak was observed predominately as the $[M + H]^+$ of sumatriptan at m/z 296. A solution adduct $[M + Na]^+$ at m/z 318 was generally observed at a lower abundance. Peaks at m/z 297 and 298 are related to carbon and sulfur isotopes. ^{34}S is a very important isotope which can be used to track the presence of the methylsulfonamide group in the mass spectrum (relative abundance of approximately 4%). In addition to the molecular species observed from sumatriptan, there are two diagnostic fragment ions at m/z 201



Scheme 1. Proposed collison-induced dissociation fragmentation mechanism for sumatriptan.

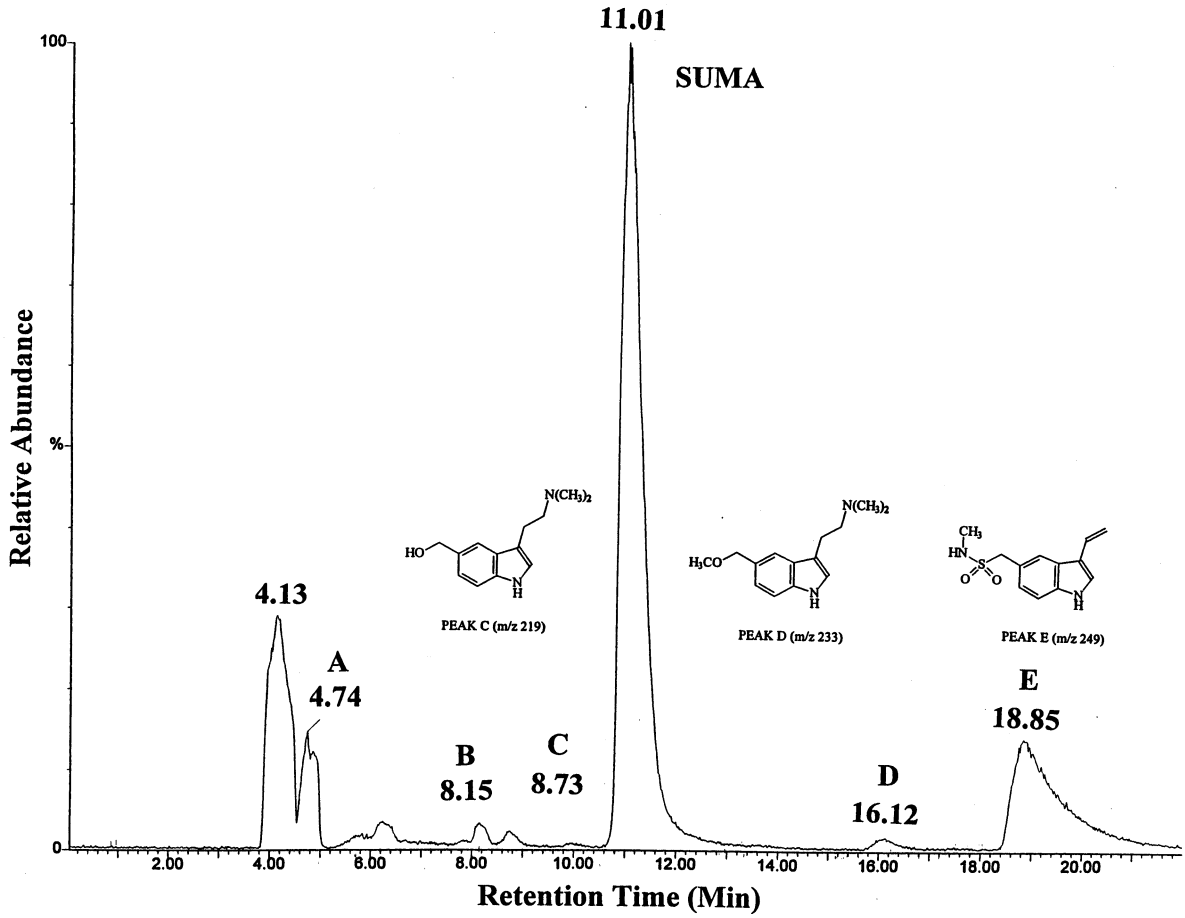


Fig. 2. LC/MS total ion chromatogram of sumatriptan succinate with 0.1 N hydrochloric acid for 10 h at 90°C.

and 251 associated with losses of the methylsulfonamide and dimethylamine groups, respectively. The presence of these fragment ions are important indicators of these functional groups in the various degradation products.

LC-MS total ion chromatograms were obtained for sumatriptan at each stress condition (i.e. acid, base and oxidation). The peaks corresponded to the parent drug and its degradants. For each peak, a signal representing a distinct $[M+H]^+$ value was observed. Definitive mass-to-charge value assignments were then made for each component based on molecular adduct ions observed under the corresponding ESI conditions. This data led to tentative structural assignments. LC-MS-MS was then used to obtain further structural information. Sumatriptan and its degradation products showed several characteristic fragmentation pathways and these helped to identify the degradation products. When MS-MS fragmentation data was less definitive, more than one possible degradation structure was proposed.

Fig. 2 shows the LC-MS total ion chromatogram from acid degradation of sumatriptan after 10 h at 90°C. Four reaction products were proposed with molecular ions found at m/z 312, 219, 233 and 249. The peak at retention time 11 min (m/z 296 in Fig. 2) labeled Suma is a single component peak consistent with the UV spectrum of authentic sumatriptan. Peak A is a mixture of low molecular weight species which were not identifiable as sumatriptan degradation products.

Peak B at m/z 312 was likely formed by addition of an oxygen atom. Using data from the peroxide degradation study discussed later in this paper, peak B was proposed to be hydroxylated on the indole ring of sumatriptan. Peak C at m/z 219 involved the loss of the methylsulfonamide group following nucleophilic attack by water. Peak D at m/z 233 was involved with the loss of the methylsulfonamide and the attack of methoxide based on both mass-to-charge values and MS-MS fragmentation data. Peak E at m/z 249 involved the loss of the dimethylamine moiety.

The formation of peak D at m/z 233 is consistent with the inclusion of a methoxide moiety (see Fig. 2). The source of this group is thought to arise from the methanol used to prepare the sumatriptan stock solution. To better understand the formation of this degradant, studies in both methanol and water were performed under acidic and basic conditions. There was no m/z 233 observed with sumatriptan solution prepared only in water. Fig. 3 shows a product ion mass spectrum of m/z 233. The proposed solution phase mechanism for the formation of this degradation product under acidic conditions in a methanolic stock solution is shown in Scheme 2. From the product ion mass spectrum of m/z 233, the peak at m/z 58 showed the retention of the dimethylamine group on the sumatriptan side chain. Also, the loss of 45 amu (the difference between m/z 233 and 188) provided further evidence that the fragmentation pathway of m/z 233 included the

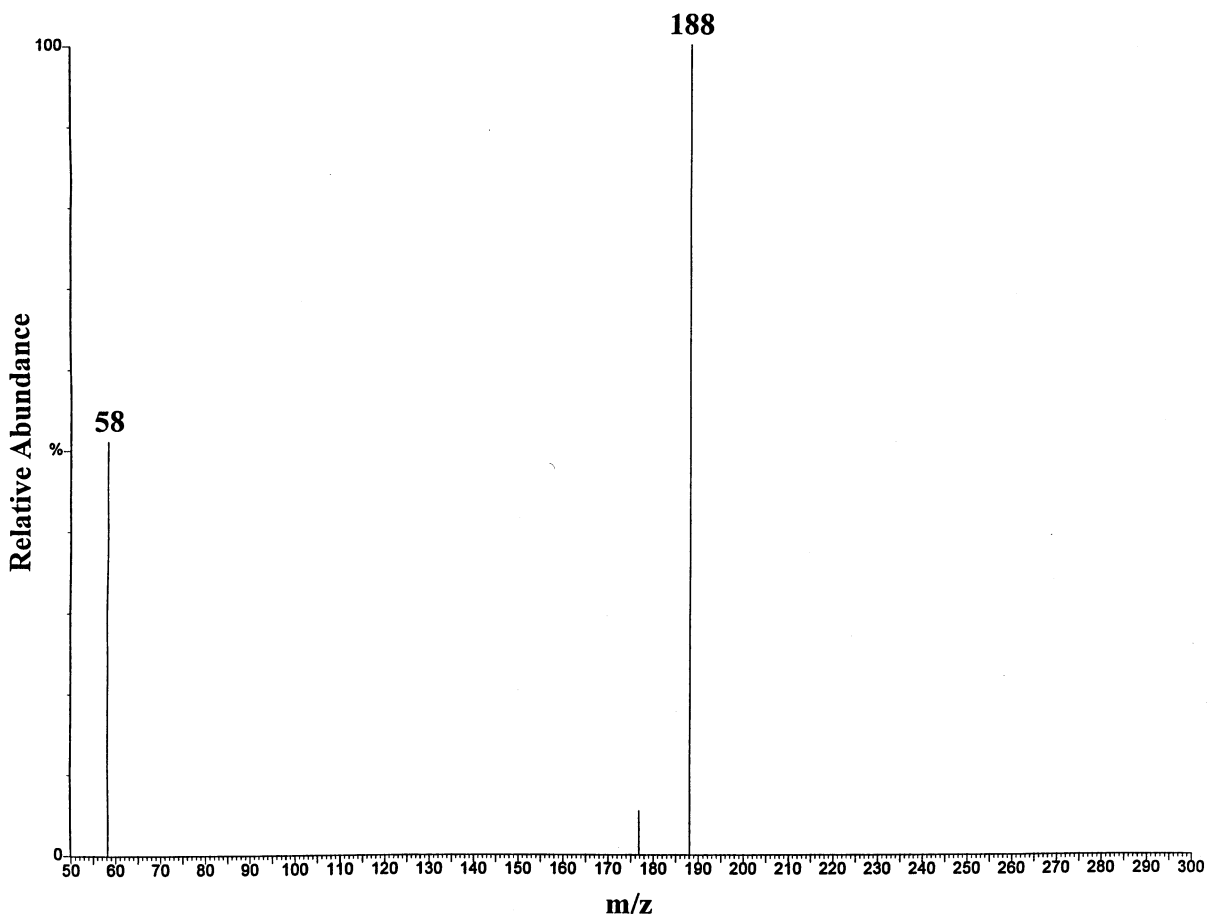
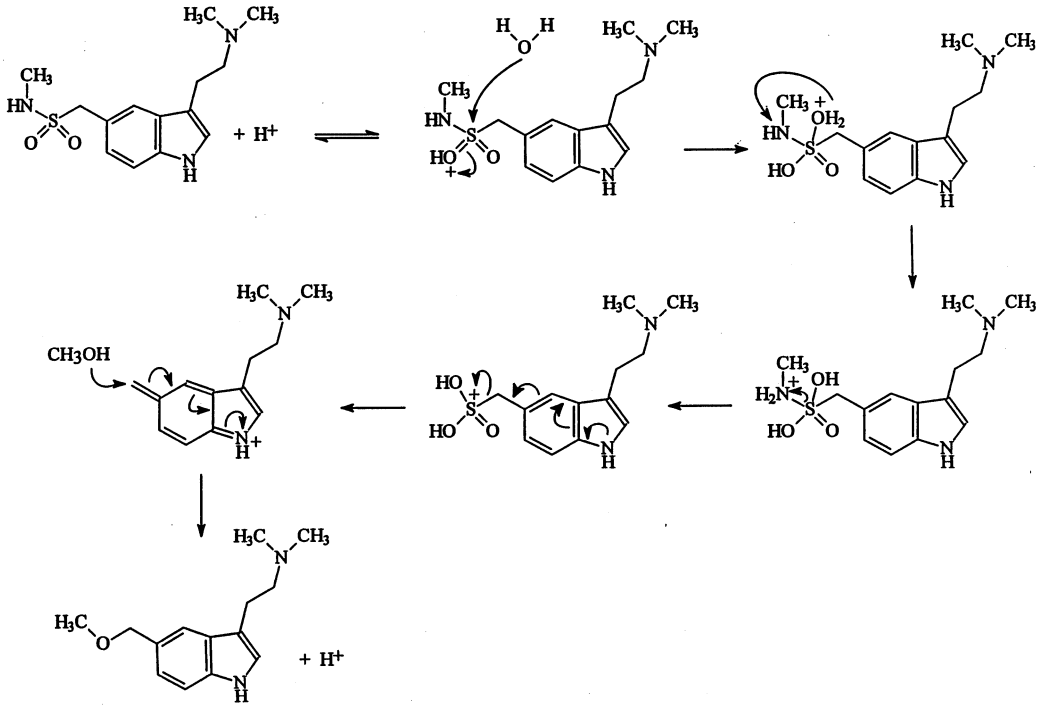


Fig. 3. Positive ion electrospray collision-induced dissociation tandem mass spectrum from protonated molecular ion at m/z 233.



Scheme 2. Proposed solution phase reaction mechanism of degradation product of sumatriptan.

loss of the dimethylamine group. The loss of the methylsulfonamide group following attack of a methoxide on the alpha-carbon of the sumatriptan side chain is the best explanation for the loss of 63 amu observed between sumatriptan and this degradation product.

Under acid conditions, sumatriptan is protonated on the oxygen atom of the methylsulfonamide group. The first steps involve a typical ester hydrolysis during which water attacks the sulfur atom. Since sulfate is a good leaving group, this results in the release of a sulfuric acid molecule. Concurrently, nucleophilic attack by a methoxy anion causes the regeneration of aromaticity resulting in the formation of the degradation product at m/z 233. An identical mechanism can be employed to explain the formation of peak C(m/z 219).

Fig. 4 shows the LC-MS total ion chromatogram obtained from the base degradation of sumatriptan after 10 h at 90°C. Three degradation products were detected at m/z 219, 282 and 233. Peak F is a mixture of low molecular weight species, which were not identified as specific degradation products. Peak G at m/z 219 involved the loss of the methylsulfonamide group following attack by a hydroxide anion as observed in the acid degradation. Peak H at m/z 282 occurred following loss of a methyl group from the sulfonamide moiety. Peak I at m/z 233 was formed by the loss of the methylsulfonamide moiety following attack by a methoxide anion as previously observed under acid degradation.

Fig. 5 shows the total ion chromatogram of sumatriptan from peroxide degradation following 0.5 h at 90°C. From the chromatogram, six degradation products were identified. Peaks L, M and O showing protonated molecular ions at m/z 312 are formed from the addition of a single oxygen atom to sumatriptan. Peaks, J, K and N were formed by the addition of two oxygen atoms (molecular ion m/z 328). Peaks J and K each also contain smaller amounts of species with m/z 344 resulting from the addition of a third oxygen atom to the sumatriptan molecule

The LC-MS-MS product ion mass spectra for peaks L and M were similar with each containing the m/z 58 peak representing the dimethylamine group. In addition, there are peaks representing the loss of the dimethylamine group (m/z 267), loss of the methylsulfonamide group (m/z 217), and the loss of both groups (m/z 172). Therefore, the additions of oxygen observed in these two degradation products (L and M) must be on the indole ring and not on either side chain.

For peak O, there was no fragment ion observed at m/z 58. In addition, the peak representing the loss of the dimethylamine group at m/z 267 was absent and was replaced by a new signal at m/z 251. This demonstrated that the oxidation site was the nitrogen atom in the dimethylamine group. The difference between m/z 251 and m/z 312 is 61, which equals 45 amu (dimethylamine) plus 16 amu (oxygen). Thus, it can be concluded that peak O is the dimethylamine N-oxide product.

Peaks J, K and N originate from m/z 328. The product ion mass spectra of peaks, J, K and N all contained the fragment ion at m/z 58. Therefore, these three compounds represent three positional isomers where all of the oxidation sites are found on the indole ring. The product ion mass spectra of the two minor components of peaks J and K at m/z 344 are almost identical Since these two compounds also contain a strong signal at m/z 58, they must be positional isomers where the three oxygen atoms have been added to the indole ring.

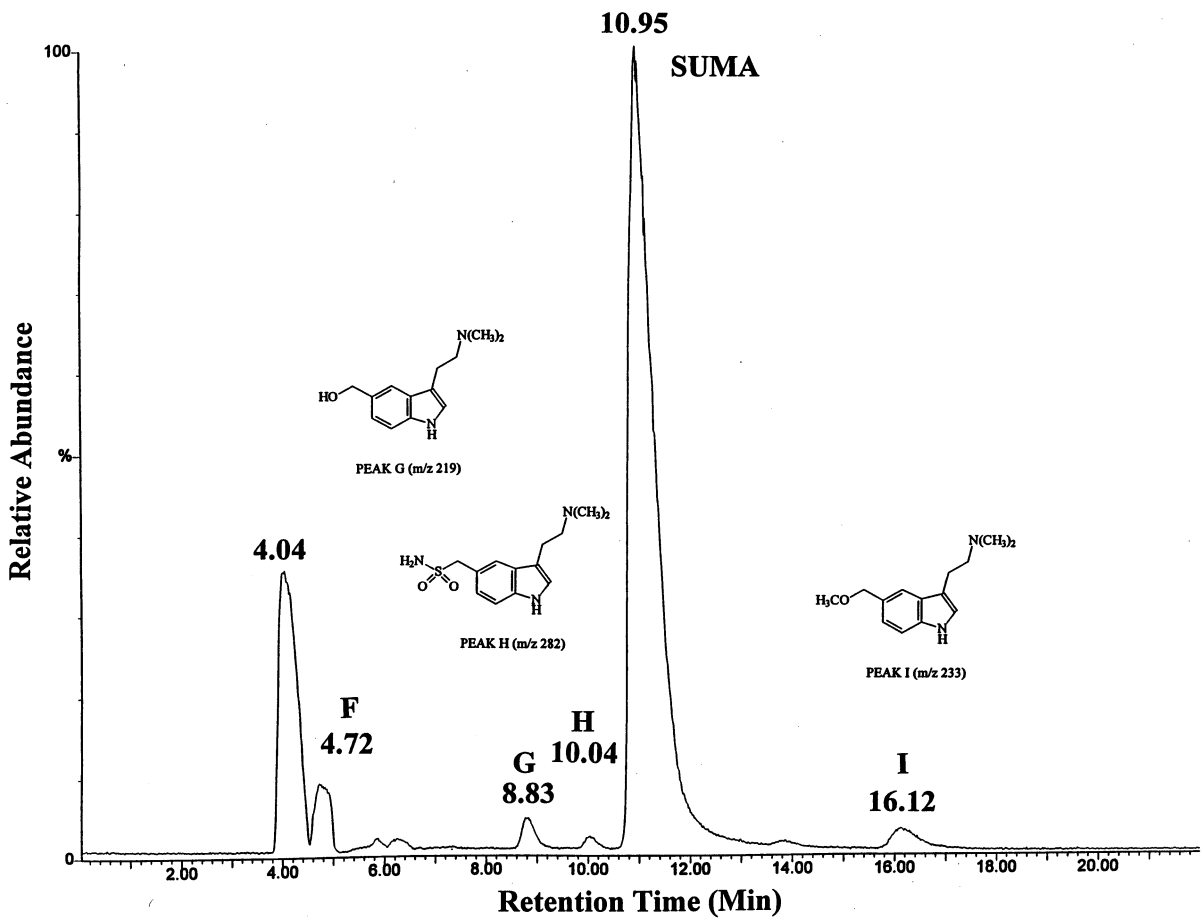


Fig. 4. LC/MS total ion chromatogram of sumatriptan succinate with 0.1 N sodium hydroxide for 10 h at 90°C.

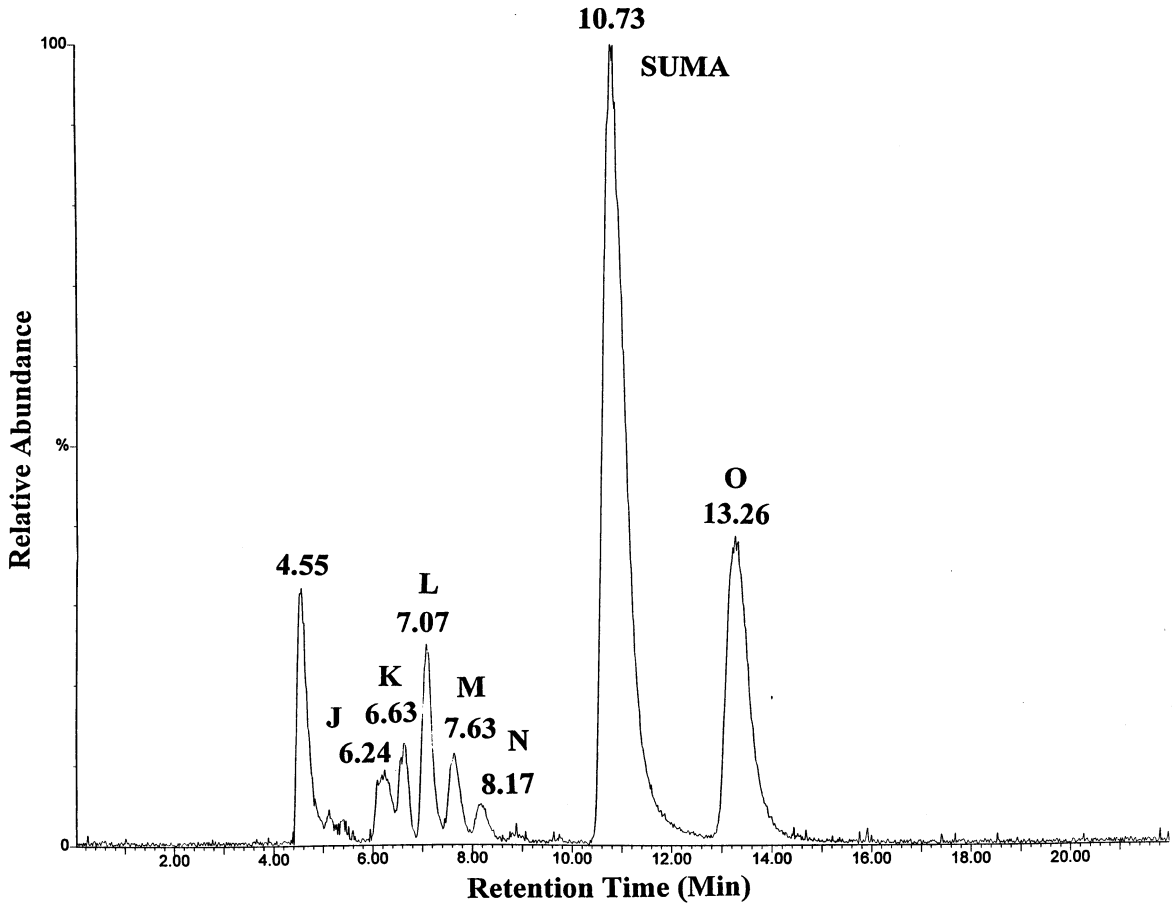
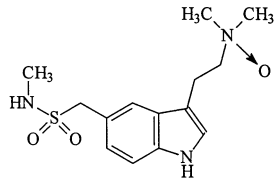


Fig. 5. LC/MS total ion chromatogram of sumatriptan succinate with 3% hydrogen peroxide for 0.5 hr at 90°C.

Studies of indole chemistry have shown that there are several preferred sites of oxidation on the ring [17]. The formation of an N-oxide at the N-1 position would be a favored process. Normally, oxidation at the C-3 position would be favored, however, in the case of sumatriptan, there is already an R group present at this position (dimethylamino ethyl group). In cases where an indole has an R group at position 3, the C-2 position becomes activated for oxidation as has been demonstrated through oxidation using benzoyl peroxide. The C-4 position of the indole ring has also been shown to be reactive toward hydroxyl radical attack. We also speculate that attack at the C-7 position may occur following attack at C-4 since the addition of a hydroxyl group to C-4 would activate the positions both ortho and para to C-4. The ortho position (C-5) is already occupied by another R group leaving the C-7 position as another possible oxidation site. The presence of oxidations at both C-4 and C-7 would allow for the formation of a highly favored quinone-type resonance structure.

Oxidation of sumatriptan seems to proceed through two distinct pathways. The first is formation of the N-oxide on the dimethylamino group. Since all of the compounds containing two or three oxygen atoms possess an unmodified dimethylamino group, it appears that once this N-oxide is formed, no further oxidations occur. However, if the first oxidation occurs on the indole ring at N-1, C-2, C-4 or C-7, then additional oxidations will occur within the ring system leading to a mixture of products containing either



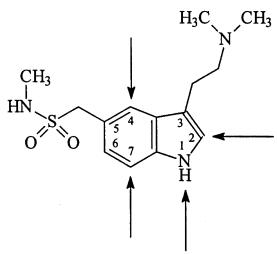


Fig. 6. Proposed structures for peroxide-induced degradation products of sumatriptan succinate.

two or three oxygen atoms at a combination of these sites. Fig. 6 shows the possible structures for the oxidation products of sumatriptan succinate. The actual site of oxidation for the peroxide degradants might be determined using NMR or LC/NMR.

4. Conclusions

The degradation of sumatriptan succinate under acid, base, heat, oxidation and UV irradiation conditions was studied using LC-MS and LC-MS-MS. Structures of the degradation products were tentatively assigned based on the known reactivity of the drug and MS-MS fragmentation patterns. It was found that the drug was stable to exposure of acid, base, oxidation and UV irradiation at ambient temperature, but unstable under acidic and basic conditions when heated to 90°C. Under oxidative conditions, a number of oxygenated products were identified. This work has shown that on-line LC-MS and LC-MS-MS are efficient techniques for the identification of drug degradants. None of the degradants identified in this study could be correlated with known metabolites.

Acknowledgements

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