ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 471–485

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Identification of oxidative degradates of the thrombin inhibitor, 3-(2-phenethylamino)-6-methyl-1-(2-amino-6-methyl-5-methylenecarboxamidomethylpyridinyl)pyrazinone, using liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry

Yunhui Wu *, Xin Chen, Lynn Gier, Örn Almarsson, Drazen Ostovic, Alice E. Loper

Pharmaceutical Research and Development, Merck Research Laboratories, P.O. Box 4, West Point, PA 19486, USA

Received 3 September 1998; received in revised form 28 January 1999; accepted 28 January 1999

Abstract

Liquid chromatography/mass spectrometry was used to identify reaction products from a solution of 3-(2-phenethylamino)-6-methyl-1-(2-amino-6-methyl-5-methylenecarboxamidomethylpyridinyl)pyrazinone (L-375,378) and hydrogen peroxide, a system that generates high levels of the oxidative degradates which form in the tablets and intravenous (i.v.) solutions of L-375,378. Two major hydrogen peroxide reaction products of L-375,378 (m/z 407) with m/z values of 369 and 370 were separated and identified. Both compounds were products of ring opening with elimination of three carbon atoms from the center pyrazinone ring. The structural assignments for these two products were α-amidinoamide and α-diamide compounds, respectively. In addition, five products (m/z 423) with a molecular weight 16 Da greater than that for L-375,378 were separated. Further liquid chromatography/tandem mass spectrometry experiments indicated that three of these M + 16 products were phenolic derivatives of L-375,378. Among them, the *para*-hydroxy compound has been verified using an authentic standard. The other two phenolic compounds were believed to be the *meta*- and *ortho*-hydroxy derivatives of L-375,378. The fourth M + 16 product was derived from hydroxylation of the methyl group on the center pyrazinone ring. The fifth M + 16 product was derived from oxidation on the aminopyridine moiety, most likely *N*-oxide of the pyridine ring. Other minor hydrogen peroxide reaction products were not studied in detail because they did not appear in tablets or i.v. formulations.

Keywords: L-375,378; Degradates; Liquid chromatography/mass spectrometry; Tandem mass spectrometry; Oxidation; Isomers

* Corresponding author. Fax: + 1-215-652-5299.
E-mail address: yunhui_wu@merck.com (Y. Wu)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00035-7

1. Introduction

3-(2-Phenethylamino)-6-methyl-1-(2-amino-6-methyl-5-methylenecarboxamidomethylpyridinyl) pyrazinone, L-375,378 (**I**), is a rapid-binding, reversible, competitive inhibitor of thrombin that reacts stoichiometrically with the protease [1]. It is a potent and specific inhibitor of coagulation via the intrinsic or extrinsic pathway which permits oral therapy of thromboembolic disorders. L-375,378 is a crystalline di-hydrochloride salt which exists as a monohydrate. No evidence of degradation for L-375,378 was observed in the solid state following heat stress for 6 weeks. However, oxidative degradates were detected in the presence of formulation excipients in both tablets and intravenous (i.v.) solutions. Preliminary profiling of the degradates indicated that the major degradates in the formulations were the same species as those observed from treatment of L-375,378 with hydrogen peroxide [2]. The latter mixture was then used as a system that could generate high levels of the degradates seen in the tablets and i.v. solutions of L-375,378.

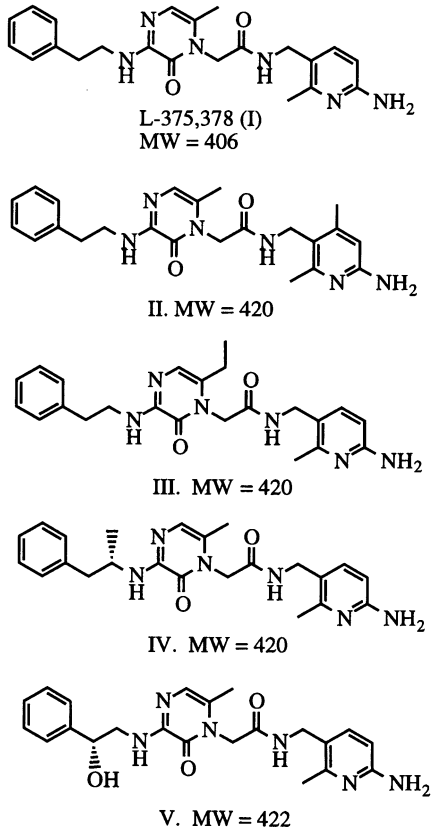


Fig. 1. Chemical structures of L-375,378 and its analogs.

To obtain structural information on the major oxidative degradates of L-375,378, liquid chromatography combined with simultaneous mass spectrometry (LC/MS) and UV detection was used for rapid and accurate elucidation of the degradate structures. In the last few years, LC/MS has become one of the most powerful tools for the identification of impurities and degradates in the pharmaceutical industry [3–14]. The soft ionization characteristics of the electrospray ionization technique have shown broad applications in the studies of reaction products [15–19]. The coupling of high-performance liquid chromatography (HPLC) separation and soft ionization tandem mass spectrometry (MS/MS) provides not only molecular weight information, but also detailed structural information of each individual chromatographic peak. LC/MS/MS becomes extremely powerful when analysis of a mixture of isomeric compounds is the objective of a study [20–23].

In this work, the reaction products of L-375,378 and hydrogen peroxide were studied by LC/MS and LC/MS/MS techniques. The fragmentation pathway of L-375,378 was thoroughly studied using L-375,378 and its analogs. With understanding of the gas phase chemistry of this class of compounds, the structural elucidation of the unknown degradates became much more straightforward.

2. Experimental

2.1. Chemicals and reagents

L-375,378 and its structural analogs were obtained from the Process Chemistry and Medicinal Chemistry Departments of Merck Research Laboratories (Rahway, NJ, and West Point,

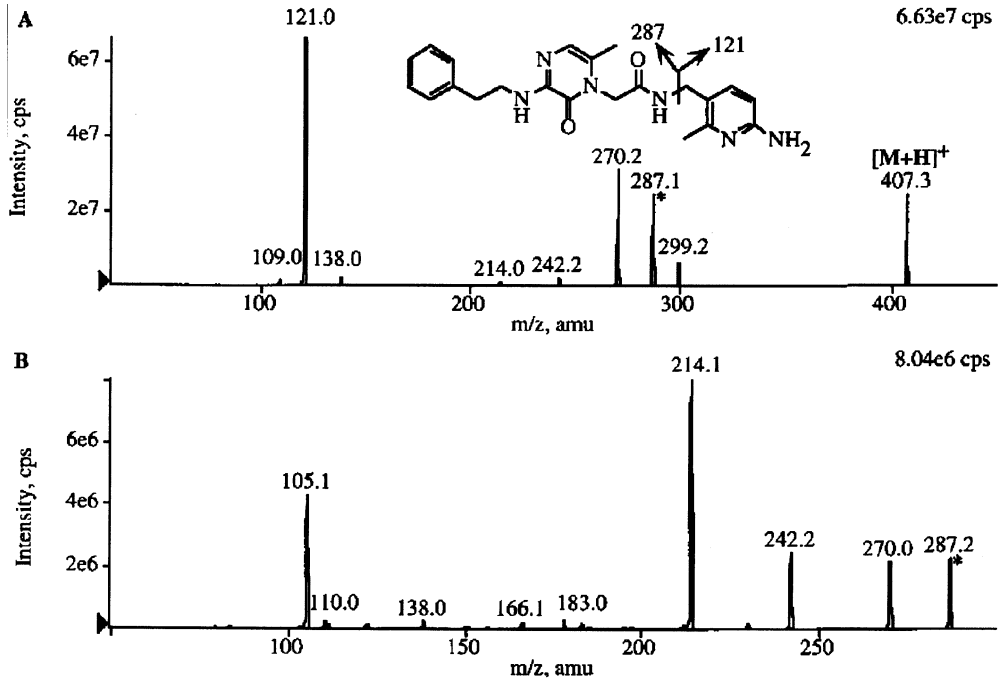


Fig. 2. (A) Full-scan product ion spectrum of L-375,378. (B) Full-scan quasi-MS/MS/MS spectrum of L-375,378 with precursor ion at m/z 287.

PA). HPLC-grade water, methanol and acetonitrile were obtained from EM Science (Gibbstown, NJ). Hydrogen peroxide and formic acid were purchased from Aldrich Chemical (St. Louis, MO). Ammonium hydroxide was obtained from Fisher Scientific (Malvern, PA). All solid and liquid reagents were reagent grade.

2.2. Sample preparation

The stock solution of the bulk drug of L-375,378 (di-hydrochloride salt) was prepared in water at a concentration of 1 mg ml^{-1}. For the oxidation reaction, L-375,378 (0.5 mg ml^{-1} in water) was treated with 25% molar equivalent of hydrogen peroxide at 80°C for 44 h. The reaction mixture was diluted with an equal volume of methanol, and subsequently injected onto the LC column.

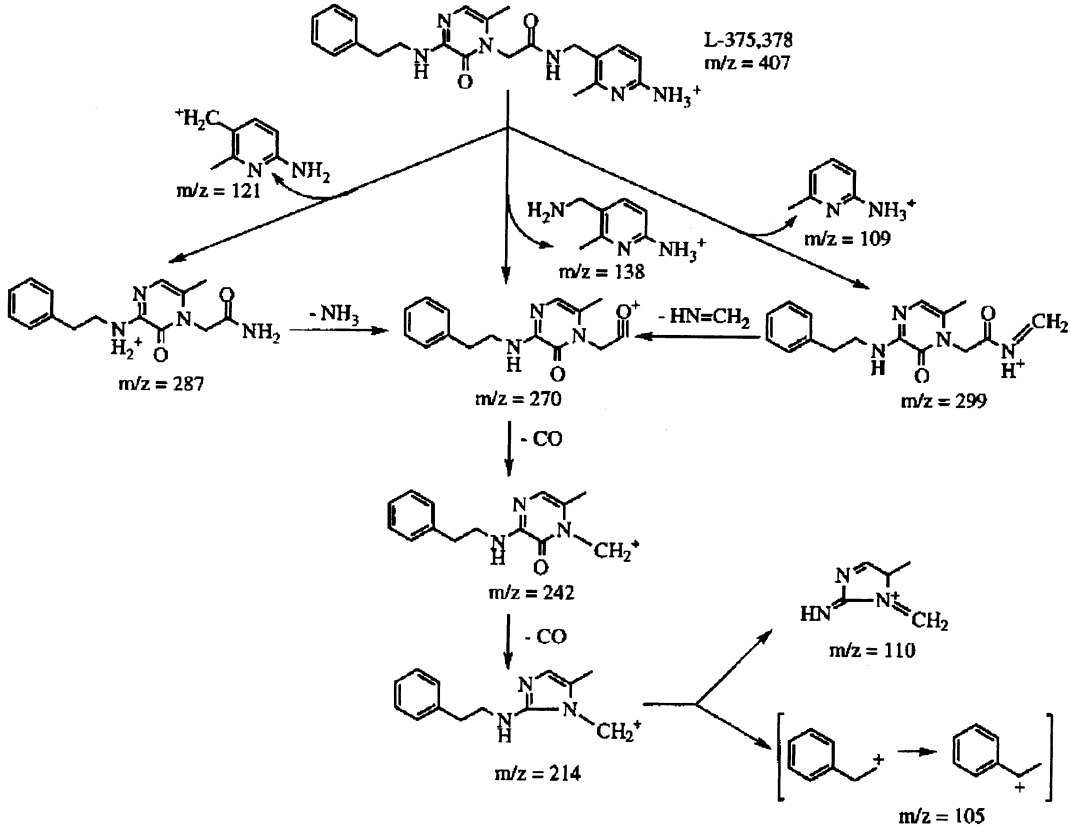
2.3. Liquid chromatography

A Shimadzu HPLC system (Columbia, MD) composed of two LC-10AD pumps with a SCL-10A system controller and a CTO-10A column oven was used. A Zorbax Rx-C8 column (250 × 4.6 mm, 5 μm; MAC-MOD Analytical, Chadds Ford, PA) was eluted with mobile phase A, 0.1% formic acid (adjusted to pH 6.0 with concentrated ammonium hydroxide), and mobile phase B, acetonitrile using a linear gradient program ($t = 0$ min, A/B 94:6; $t = 20$ min, A/B 70:30; $t = 40$ min, A/B 30:70; $t = 41$ min, A/B 94:6). The flow rate was at 1 ml min^{-1}. The column temperature was maintained at 35°C. One-hundred microliters of the sample solution were injected onto the LC column with a Perkin Elmer series 200 autosampler (Norwalk, CT). The needle wash solvent for the autosampler was a mixture of acetonitrile–

0.1% formic acid (50:50 v/v). The Waters 486MS UV detector (Milford, MA) used in this study was operated at 315 nm.

2.4. Electrospray mass spectrometry

A PE-SCIEX (Concord, Ontario, Canada) API 300 tandem quadrupole mass spectrometer equipped with a TurboIonSpray interface was used for the on-line LC/MS and LC/MS/MS experiments. The LC effluent was split approximately 1:3 for mass spectrometer and UV detection, respectively. The mass axis calibration for the mass range used was performed using a PPG calibration solution provided by PE-SCIEX. Unit mass resolution was maintained through the whole analysis. For infusion experiments, a Harvard Model 22 syringe pump (Harvard Apparatus, South Natick, MA) was used to infuse the test solutions at 10–100 ml min^{-1}. For LC/MS experiments, the first quadrupole (Q1) was scanned from 250 to 500 amu at a scan rate of 1.2 s per scan. For LC/MS/MS and LC/quasi-MS/MS/MS experiments, the precursor ions were selected by the Q1 followed by fragmentation in the collision cell (Q2) with nitrogen gas, and then full-scan detection by the third quadrupole (Q3). The scan rate for LC/MS/MS was 1 s per scan. The collision energy was optimized through infusion experiments for each individual precursor ion.



Scheme 1. Proposed fragmentation pathway for L-375,378.

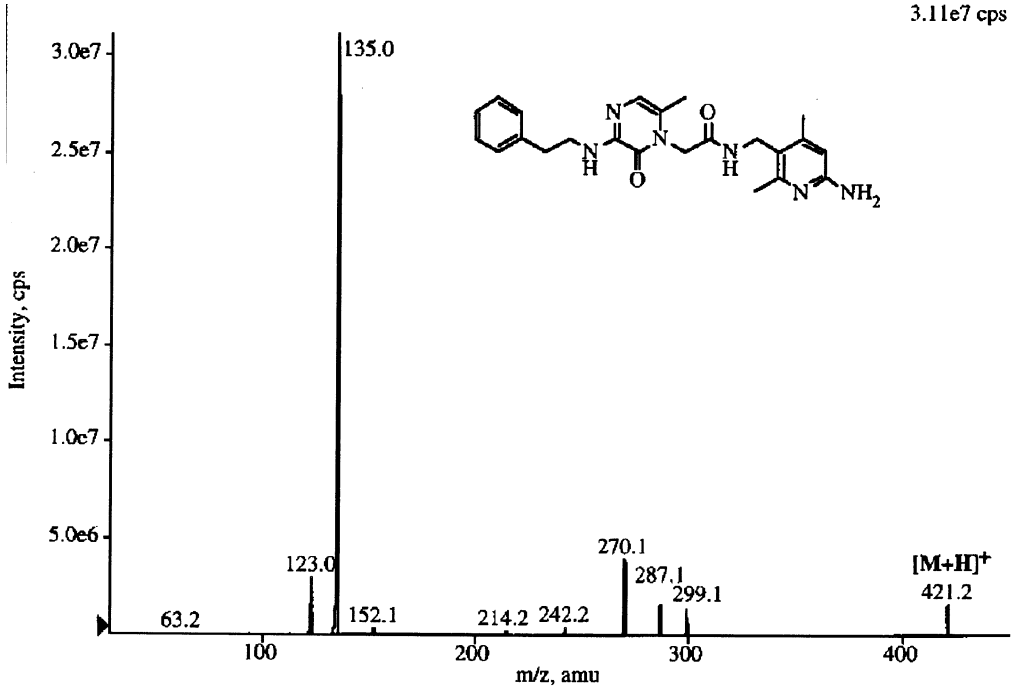


Fig. 3. Full-scan product ion spectrum of compound **II**.

3. Results and discussion

Oxidative degradates were detected when L-375,378 was formulated as both tablets and i.v. solutions. Preliminary HPLC/UV studies showed that the major degradates present in the formulations were also observed when L-375,378 was treated with hydrogen peroxide. Therefore, the reaction of L-375,378 and hydrogen peroxide was used as a model for studying accelerated degradation.

3.1. Fragmentation mechanism for L-375,378

During the course of this study, the fragmentation mechanism of L-375,378 was extensively investigated using L-375,378 and its structural analogs (Fig. 1). Infusion of a bulk drug solution of L-375,378 (di-hydrochloride salt) in methanol–water (50:50 v/v) provided the full-scan product ion spectrum as shown in Fig. 2A. Major fragment ions were observed at m/z 299, 287, 270, and a base peak at m/z 121. Minor fragments include ions at m/z 242, 214, 138, and 109. Under high orifice voltage, the fragment ion at m/z 287 was produced in the source region prior to the Q1, and subsequent MS/MS of this ion in the triple quadrupole provided a quasi-MS/MS/MS spectrum as shown in Fig. 2B. Major fragment ions at m/z 270, 242, 214, and 105 were detected. Fragmentation of the ion at m/z 214 produced intense product ions at m/z 110 and 105 (data not shown). A proposed fragmentation pathway for L-375,378 is shown in Scheme 1. The results from neutral loss of 17 (for NH_3), 28 (for CO_2), and 29 amu (for $CH_2{=}NH$) also strongly supported this mechanism.

In order to fully understand the fragmentation mechanism, three analogs of L-375,378 were also studied under the same infusion conditions. Compound **II**, an analog with an extra methyl group on the aminopyridine ring, provided a similar MS/MS spectrum (Fig. 3) to that of L-375,378, except that ions at m/z 138, 121 and 109 were replaced by ions at m/z 152, 135 and 123. From Scheme 1, it is easy to understand that this result

reflects only the structural change on the pyridine ring. Compound **III**, an ethyl analog of L-375,378, underwent the same fragmentation pathway as that for L-375,378 (Fig. 4). These results suggested that the ion at m/z 121 was indeed derived from the aminopyridine moiety, not from the center portion of the molecule. Interesting results were obtained when compound **IV**, a β-methyl analog of L-375,378, was studied under the same conditions. The MS/MS spectrum (Fig. 5A) of the protonated molecular ion at m/z 421 looked very similar to that of L-375,378 (Fig. 2A). However, the MS/MS spectrum of the fragment ion at m/z 301 (Fig. 5B) showed very different product distribution from that of ion at m/z 287 for L-375,378 (Fig. 2B). The fragmentation pathway shown in Scheme 1 became much less significant for compound **IV**. Instead, the formation of ions at m/z *183*, 166, 138, 119, and 91 from the ion at m/z 301 dominated the spectrum (Fig. 5B). A proposed fragmentation mechanism for compound **IV** is shown in Scheme 2. The ion formation at m/z 183, 166, and 138 was driven by the formation of a secondary carbocation, 1-methylphenylethyl cation, comparing to the less favorable process of forming a primary carbocation for L-375,378.

3.2. Oxidative degradates of L-375,378

As described earlier, hydrogen peroxide was used to generate high levels of the oxidative degradates observed in the formulations. Under the LC/MS conditions used, L-375,378 was well-separated from all major reaction products. Fig. 6A shows the sum of extracted ion chromatograms (XIC) for the reaction mixture at 44 h. Fig. 6B is the UV chromatogram for the same sample. Two known synthetic impurities (m/z 287 and 288) were detected from the LC/MS trace.

Two major hydrogen peroxide reaction products (Fig. 7) with m/z values of 369 (**VI**) and 370 (**VII**) were identified by LC/MS. For compound **VI**, its molecular weight of 368 indicates that it is

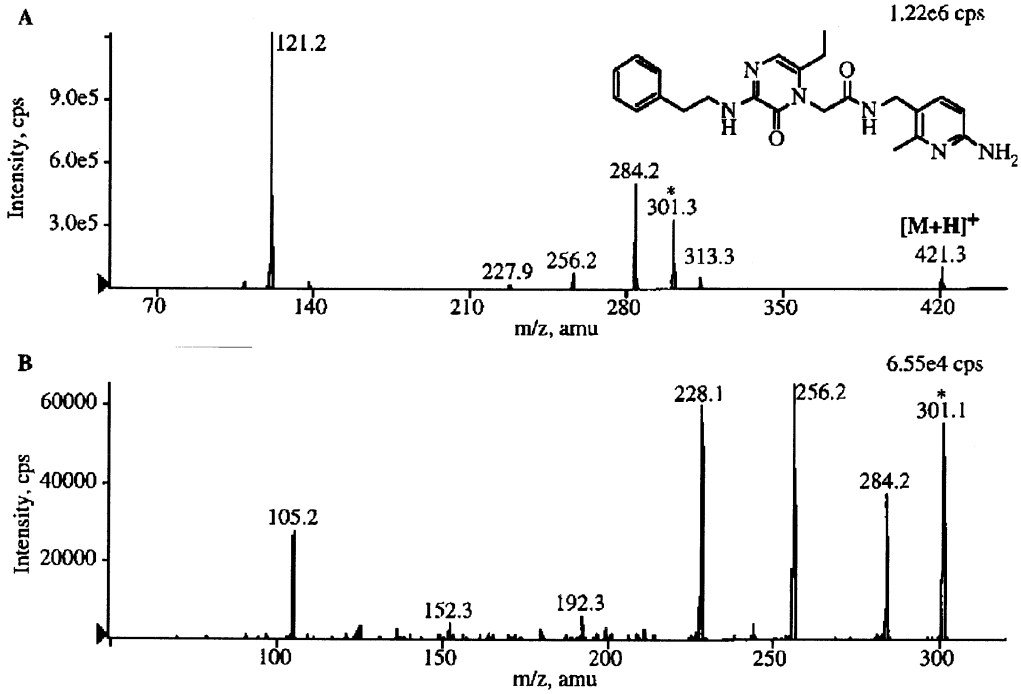


Fig. 4. (A) Full-scan product ion spectrum of compound **III**. (B) Full-scan quasi-MS/MS/MS spectrum of compound **III** with precursor ion at m/z 301.

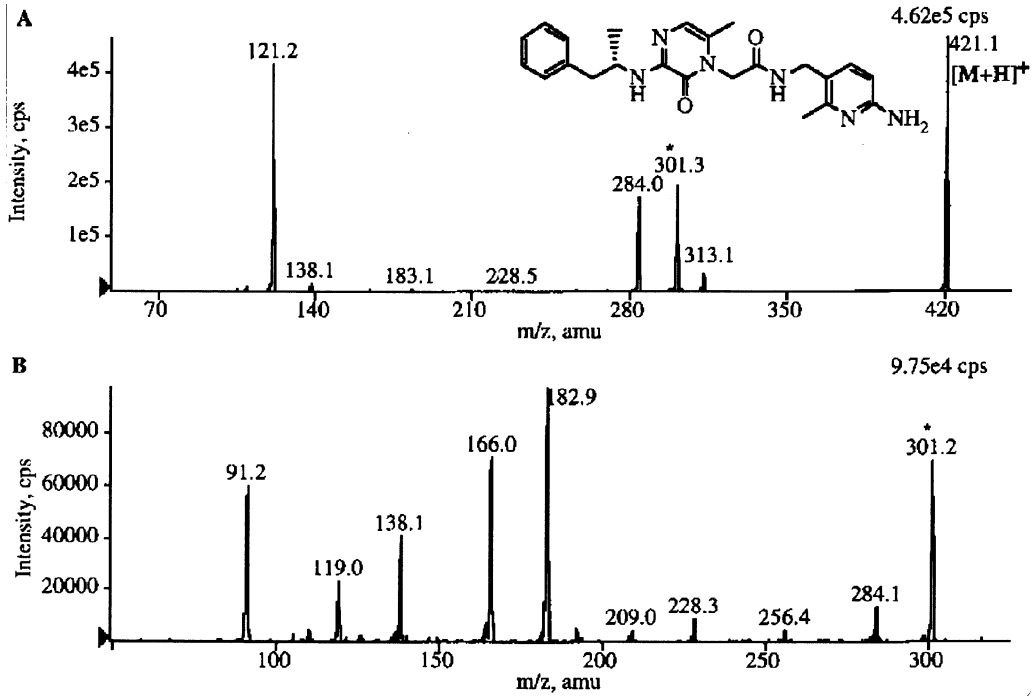
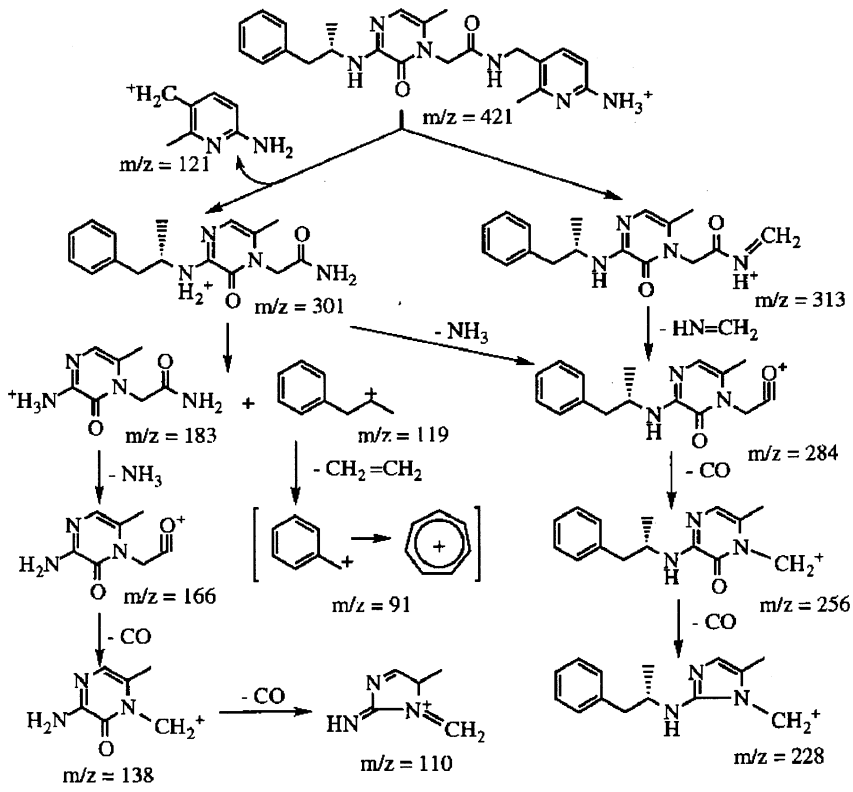


Fig. 5. (A) Full-scan product ion spectrum of compound **IV**. (B) Full-scan quasi-MS/MS/MS spectrum of compound **IV** with precursor ion at m/z 301.

a molecule containing an even number of nitrogen atoms. The ion at m/z 121, from the fragmentation of the protonated molecular ion at m/z 369 (Fig. 8A), indicated the presence of the aminopridine moiety. The fragmentation pattern for the ion at m/z 249 (Fig. 8B), which was the remaining portion of the ion at m/z 369 after the loss of the methylaminopyridine portion (two nitrogen atoms), was very similar to that of ion at m/z 287 for L-375,378, suggesting that no structural change occurred at the phenylethyl group and the amide bond (one nitrogen atom) next to the aminopyridine ring. Therefore, the three nitrogen atoms in the center portion of L-375,378 remained in the ion at m/z 369. These results led to a possible ring opening product with elimination of three carbon atoms from the center pyrazinone ring. Compound **VII**, however, has a molecular weight of 369, suggesting that it contains an odd number of nitrogen atoms. The MS/MS spectrum for compound **VII** (Fig. 9A) was quite different from that for compound **VI** (Fig. 8A), but not very informative. The only piece of information derived from Fig. 9A was that the aminopyridine moiety remained unchanged. However, the similarity for the quasi-MS/MS/MS spectra of the ions at m/z 249 (Fig. 8B) and 250 (Fig. 9B) implied that the two compounds have very similar structures. The difference in mass by 1 Da could come from replacing the =NH with =O (Fig. 7). This will also lead to a molecule with an odd-number of nitrogen atoms. A proposed fragmentation mechanism is shown in Scheme 3 for both compounds; note the common product ions at m/z 105, 121, and 187. Both compounds **VI** and **VII** have been isolated, and the nuclear magnetic resonance results verified the structural assignments.

Also found in the reaction mixture were five products with the same molecular weight of 16 Da greater than that for L-375,378 (labeled as peaks # 1– # 5 in Fig. 6A). LC/MS/MS was employed



Scheme 2. Proposed fragmentation pathway for compound **IV**.

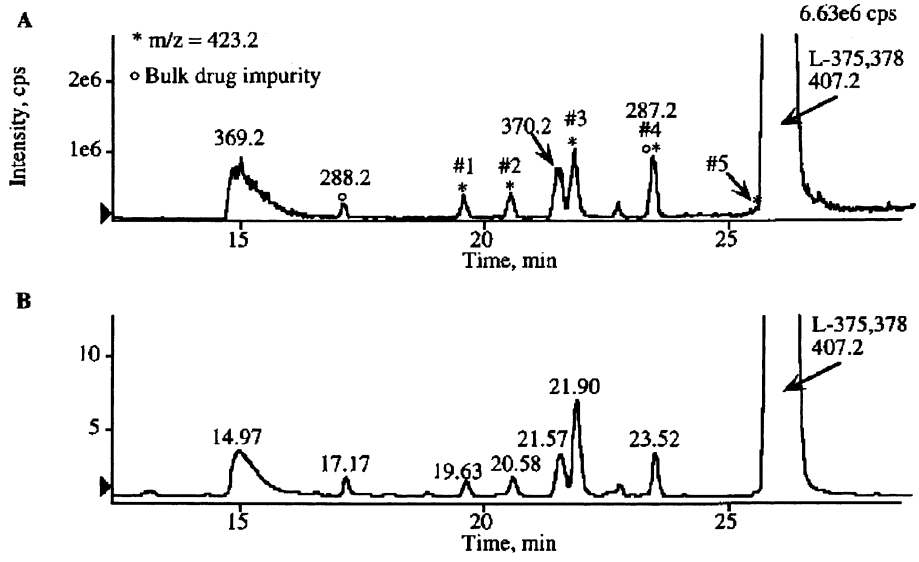


Fig. 6. LC/MS and LC/UV chromatograms for the reaction mixture of L-375,378 with hydrogen peroxide. (A) Sum of XICs from LC/MS trace. Numbers are m/z values. (B) UV profile obtained at 315 nm. Numbers are retention times.

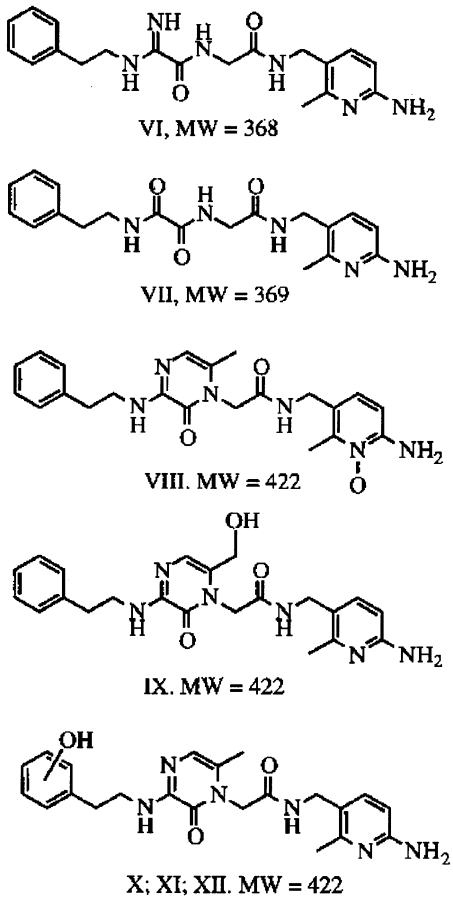


Fig. 7. Chemical structures of the oxidative degradates of L-375,378.

to chromatographically separate and identify the five M + 16 structural isomeric products. Fig. 10 shows the on-line LC/MS/MS results with the ion at m/z 423 as the precursor ion. All five compounds provided abundant fragments. Peaks # 1, # 2 and # 4 had nearly identical MS/MS spectra, while peaks # 3 and # 5 showed very different fragmentation patterns. By comparing the MS/MS spectrum for peak # 5 to those for L-375,378 (Fig. 2A) and compound **II** (Fig. 3), it was clear that peak # 5 was derived from oxidation of the aminopyridine moiety. The formation of an abundant ion at m/z 120 from the fragmentation of the ion at m/z 137 (data not shown) indicated that the oxygen was located on the nitrogen atom in the pyridine ring (compound **VIII** in Fig. 7) [24].

The MS/MS spectrum of peak # 3 indicated that this molecule easily lost a water molecule. However, both *N*-oxide and hydroxylated compounds may provide this spectrum. To eliminate the possibility that peak # 3 was the hydroxylation product at the benzylic position, compound **V** was analyzed for comparison. The MS/MS spectrum for compound **V** (Fig. 11) obtained under the same collision-induced dissociation (CID) conditions showed a very different product ion distribution from that for peak # 3. Compound **V** was also spiked into the reaction mixture. On-line LC/MS/MS results indicated that none of the five M + 16 degradates was compound **V**. The formation of ion at m/z 121 indicated that there was no structural change on the aminopyridine moiety. Therefore, the oxygen atom might reside in the center portion of the molecule. An authentic standard of the hydroxylated L-375,378, compound **IX**, was tested under the same LC/MS conditions. It indeed had an identical retention time to peak # 3. Under the same CID conditions as those in Fig. 10, the MS/MS spectrum of compound **IX** (data not shown) showed the same product ion distribution as that for peak # 3. This evidence strongly suggested that compound **IX** and peak # 3 were the same species.

For peaks # 1, # 2 and # 4, the observation of ion formation at m/z 121 plus the conservation of ions at m/z 303 and 286 from the protonated molecular ion at m/z 423 indicated that the oxidation was not occurring at the aminopyridine moiety. The strong similarities among the MS/MS spectra for peaks # 1, # 2, and # 4 indicated that the CID experiments were not isomer-specific. To overcome this limitation, an LC/quasi-MS/MS/MS experiment was attempted to differentiate peaks # 1, # 2 and # 4 using in-source CID to generate the fragment ion at m/z 303 for each chromatographic peak, and subsequent MS/MS of this ion in the triple quadrupole (Fig. 12). Unfortunately, the fragments of the ion at m/z 303 from the quasi-MS/MS/MS data were still similar. By comparing Fig. 12 to Fig. 2B, two ions at m/z 183 and 166 seemed much more intense for the oxidation products than L-375,378.

In addition to the formation of fragment ion at m/z 121 from the aminopyridine moiety for the ion at m/z 423 (Fig. 10), a second ion at m/z 121 was detected from the fragmentation of the ion at m/z 303 (Fig. 12). The phenylethylamine radical cation has a m/z value of 121; however, the ion at m/z 287 from L-375,378 did not produce this ion (Fig. 2B). Instead, an ion at m/z 105 was detected for L-375,378 for the formation of phenylethyl cation. This result led to the possibility that the oxygen atom was on the phenyl ring, the ethylene portion, or the center portion of the molecule.

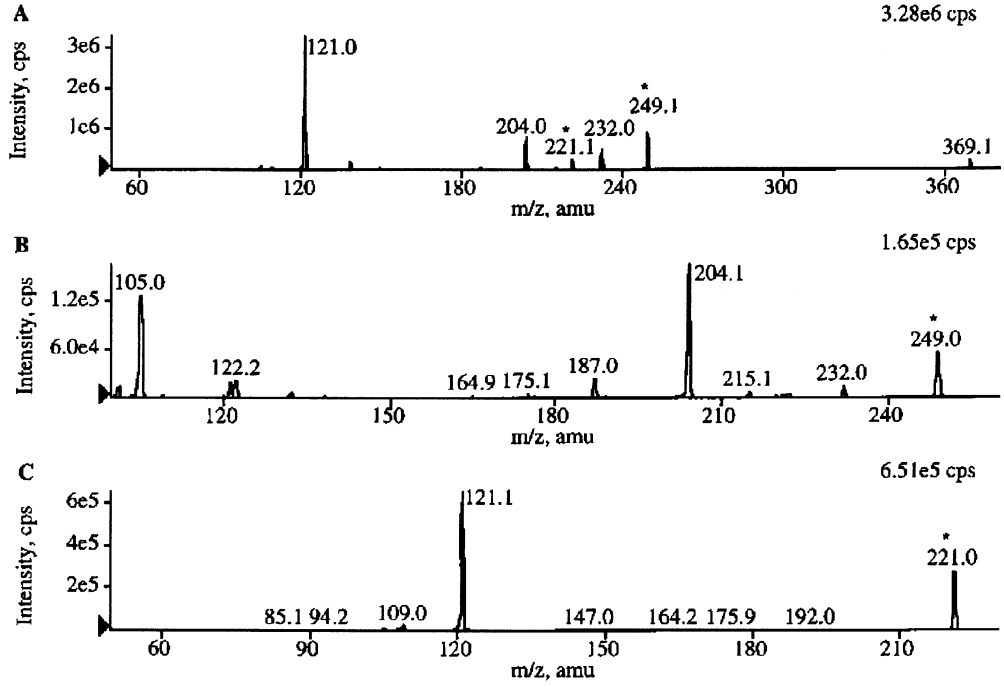


Fig. 8. (A) Full-scan product ion spectrum of compound **VI**. (B) Full-scan quasi-MS/MS/MS spectrum of compound **VI** with precursor ion at m/z 249. (C) Full-scan quasi-MS/MS/MS spectrum of compound **VI** with precursor ion at m/z 221.

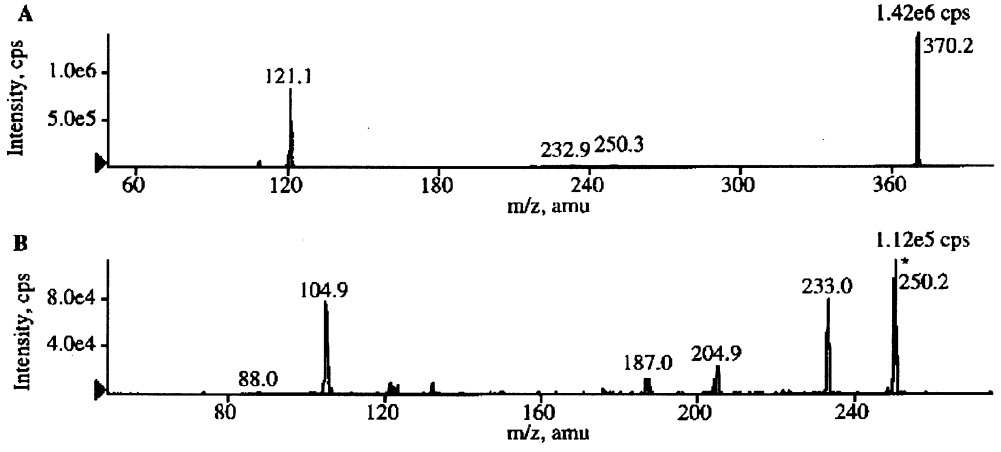
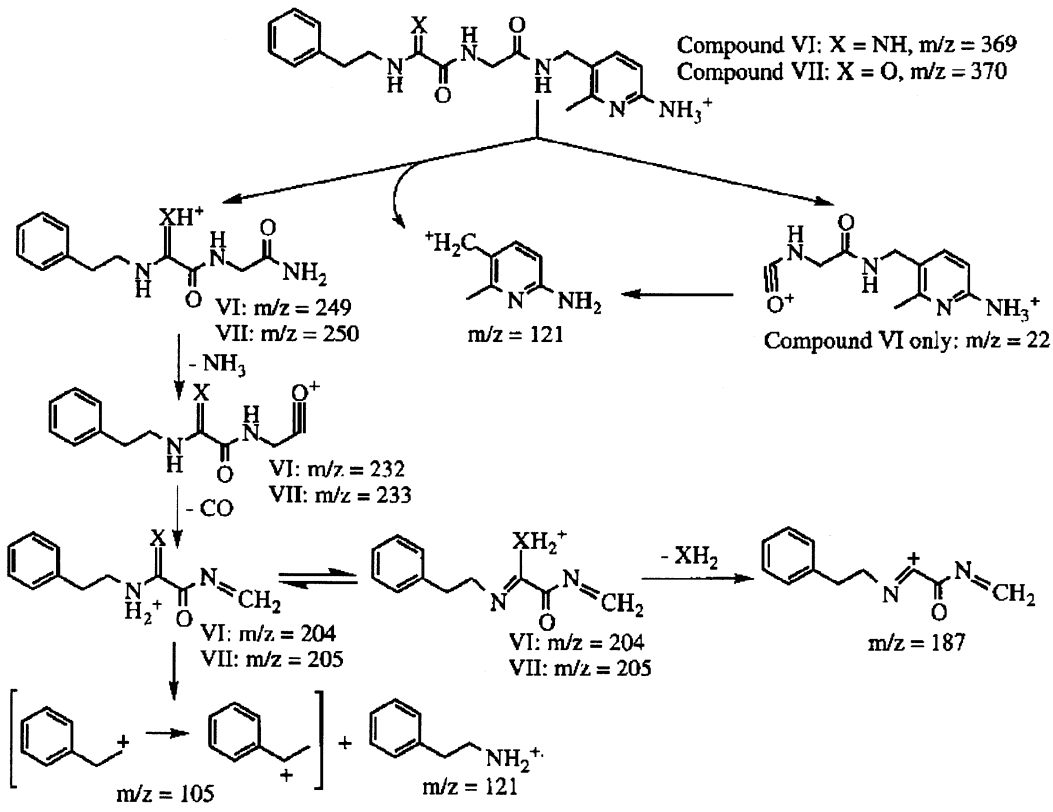


Fig. 9. (A) Full-scan product ion spectrum of compound **VII**. (B) Full-scan quasi-MS/MS/MS spectrum of compound **VII** with precursor ion at m/z 250.



Scheme 3. Proposed fragmentation pathway for compounds **VI** and **VII**.

The MS/MS data for peaks #1, #2 and #4 showed no loss of a water molecule suggesting that none of these compounds has a hydroxy group on the methylene portion or the methyl group on the pyrazinone ring. The fact that compound **V** was not one of the M + 16 products also supports this conclusion.

Valuable information was obtained from the fragmentation of compound **IV** (Fig. 5B), i.e. stabilization of the phenylethyl cation would facilitate the formation of ions at *m*/*z* 183, 166, and 138. Therefore, it became clear that peaks #1, #2, and #4 were three structural isomers of phenolic derivatives of L-375,378 (Fig. 7). To verify this assignment, an authentic standard of the *para*-hydroxy analog of L-375,378 (X) was studied under both infusion and LC/MS conditions. Its MS/MS and quasi-MS/MS/MS (Fig. 13) spectra were virtually identical to those for peak #1. A proposed fragmentation pathway is shown in Scheme 4 for the *para*-hydroxy analog of L-375,378. Neutral loss experiments for NH_3, CO, and $HN{=}CH_2$ provided support for the proposed mechanism. LC/MS analysis also proved that the *para*-hydroxy derivative of L-375,378 and peak #1 had the same retention time. Peak #2 was believed to be the *meta*-hydroxy derivative (**XI**) because of the relatively low abundance of ion at *m*/*z* 121 from the fragmentation of ion at *m*/*z* 303. Unlike the *para*- and *ortho*-hydroxy groups, the *meta*-hydroxy functionality could not stabilize the benzylic cation (*m*/*z* 121) by resonance. Peak #4, therefore, was the remaining *ortho*-hydroxy derivative of L-375,378 (**XII**).

Our immediate attention was devoted to the identification of the degradates that also appeared

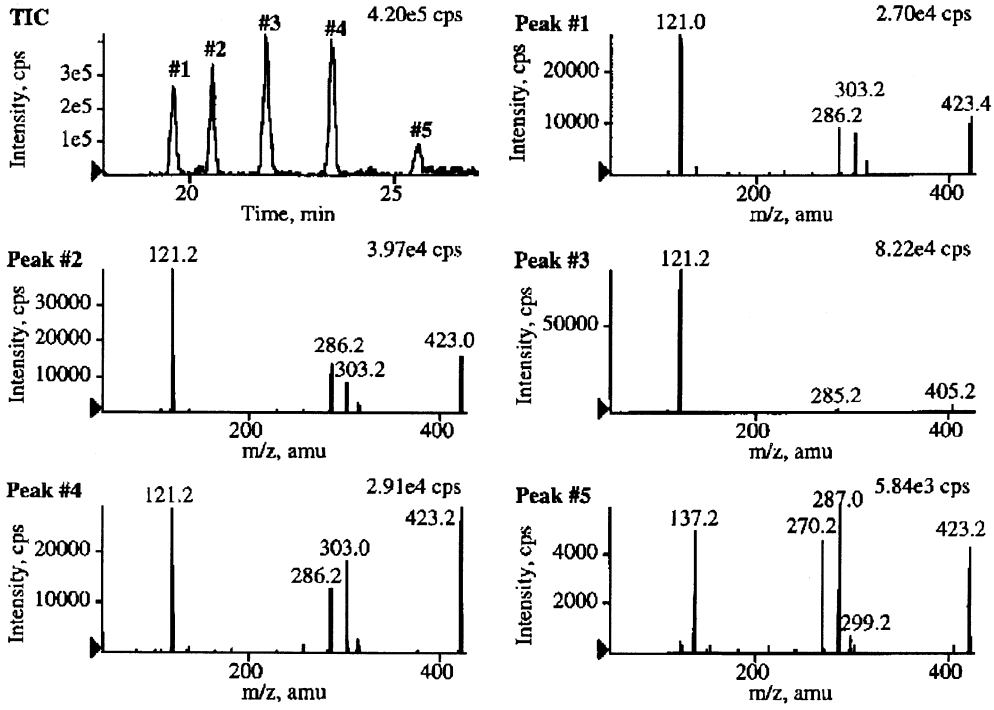


Fig. 10. Total ion chromatogram (TIC) (top left panel) and product ion spectra for each individual chromatographic peak from the LC/MS/MS analysis of the reaction mixture of L-375,378 with hydrogen peroxide. The precursor ion selected by the Q1 was ion at m/z 423.

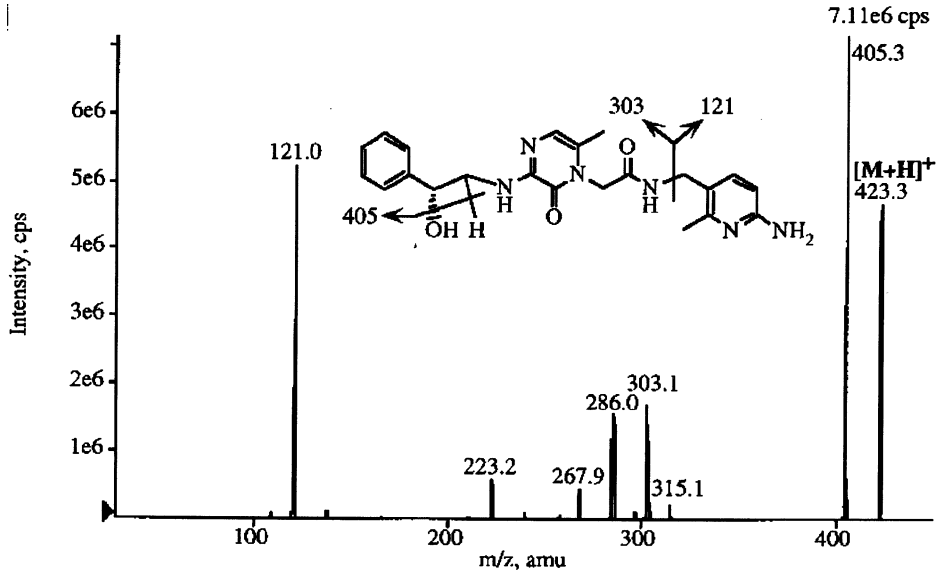


Fig. 11. Full-scan product ion spectrum for compound **V**.

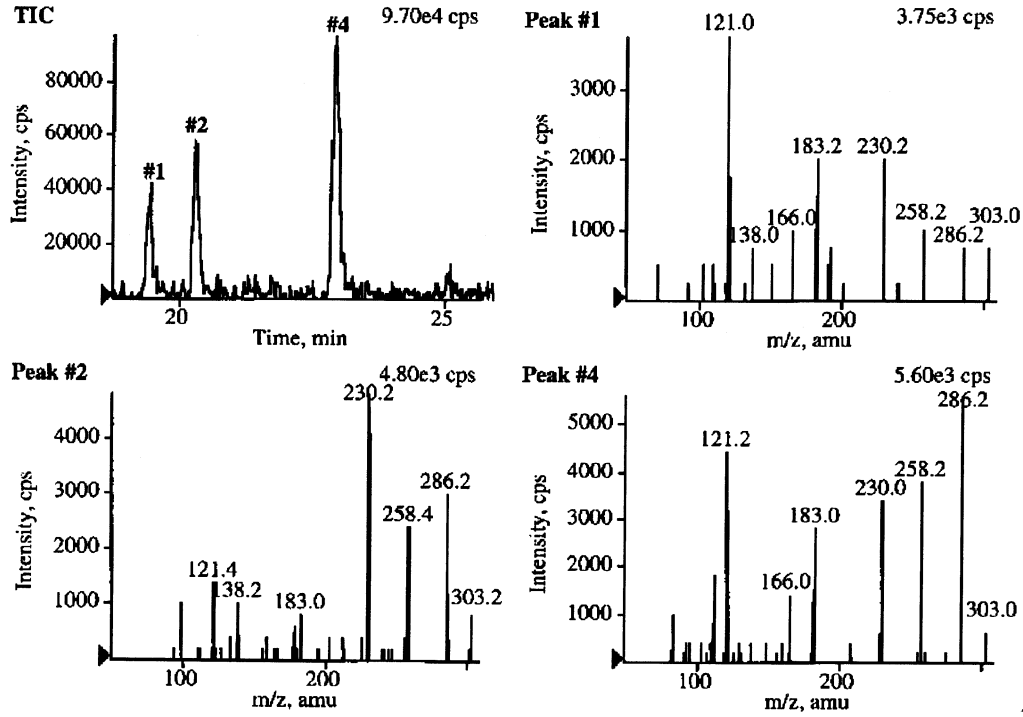


Fig. 12. Total ion chromatogram (TIC) (top left panel) and product ion spectra for each individual chromatographic peak from the LC/MS/MS analysis of the reaction mixture of L-375,378 with hydrogen peroxide. The precursor ion selected by the Q1 was ion at m/z 303.

in the tablets and i.v. formulations. Other reaction products observed in the LC/MS experiments were not studied in detail.

4. Conclusions

From our work, it was clearly demonstrated that the chromatographic and mass resolving power of an LC/MS system can greatly facilitate the degradation product identification process. The methodology of using a quasi-MS/MS/MS approach to extend the ability of a triple quadrupole mass spectrometer is very useful when MS/MS data are not sufficient for the deduction of unknown degradate structures. Mass spectrometric studies of the analogs of the target molecule were extremely important for understanding the gas-phase chemistry of the parent molecule as well as for the structure assignments of the degradates. Neutral loss scan was also found to be a valuable tool for the confirmation of fragmentation mechanism. In addition, hydrogen peroxide oxidation of the target molecule to produce high levels of oxidative degradates was a useful model for fast profiling of oxidative degradates of L-375,378.

Acknowledgements

The authors gratefully thank Dr Philip E. Sanderson, Dr Margaret R. Davis, Kellie Cutrona, and Matthew Stanton for providing L-375,378 and its structural analogs.

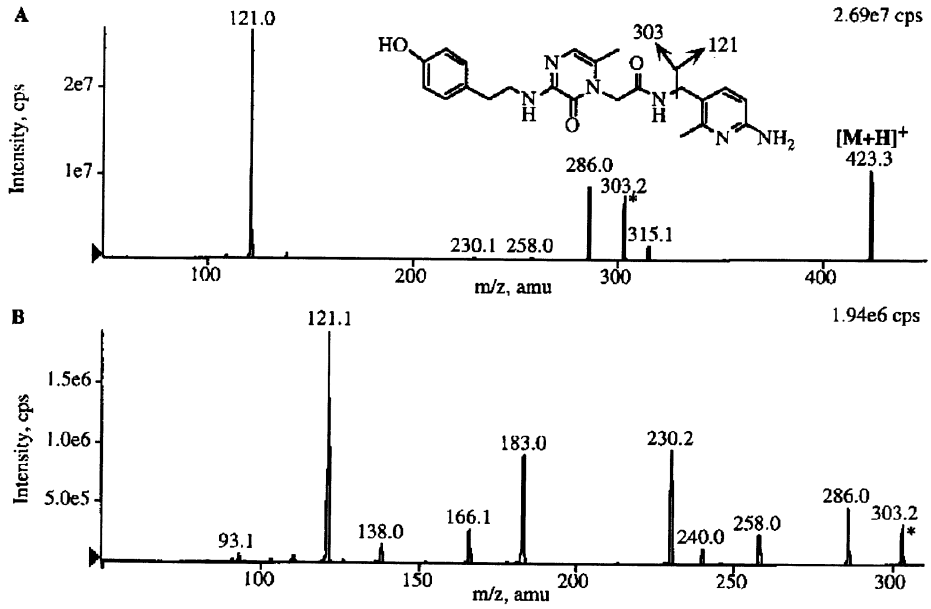
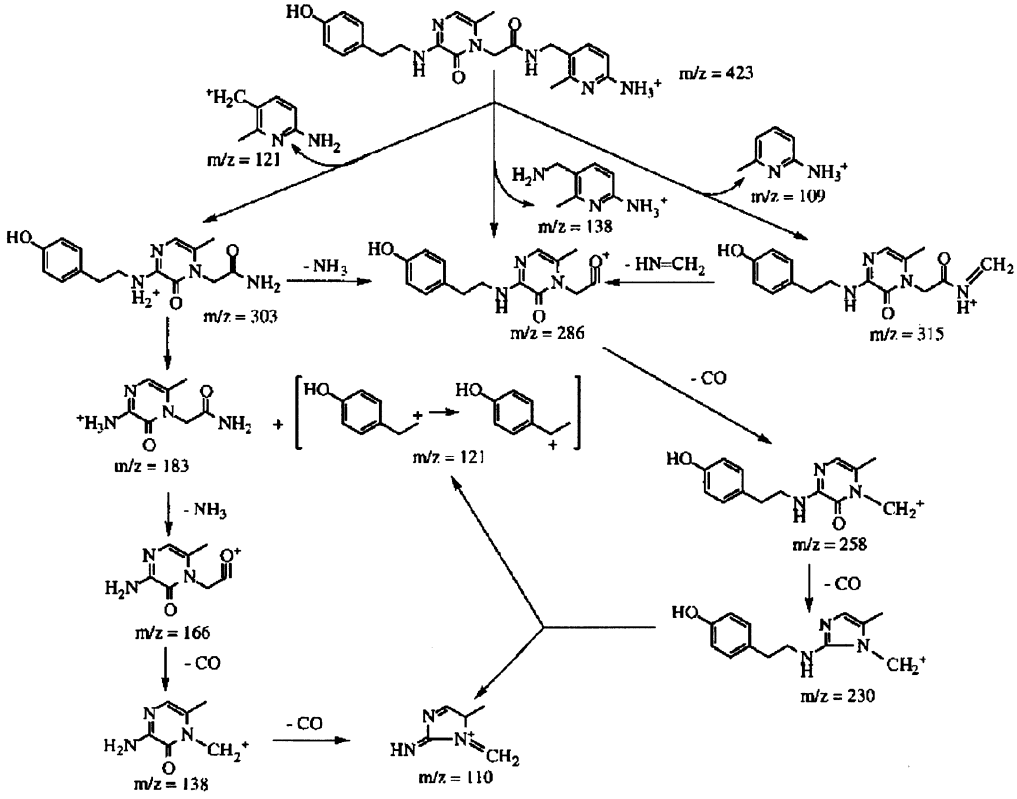


Fig. 13. (A) Full-scan product ion spectrum of an authentic standard of the *para*-hydroxy derivative of L-375,378 (compound **X**). (B) Full-scan quasi-MS/MS/MS spectrum of compound **X** with precursor ion at *m*/*z* 303.



Scheme 4. Proposed fragmentation mechanism for the *para*-hydroxy derivative of L-375,378.

References

[1] P.E.J. Sanderson, The design of orally active pyridinone and pyrazinone acetamide thrombin inhibitors, Presented at the 215th ACS National Meeting, Dallas, TX, March 1998.

[2] L. Gier, Ö. Almarsson, D. Ostovic, The stressed formulation samples and the reaction mixture of L-375,378 with hydrogen peroxide were analyzed by HPLC with a diode array detector using various elution conditions. The retention time and UV spectra of the degradates observed in the stressed formulations were matched with those for the reaction products, unpublished results.

[3] R.A. Rourick, K.J. Volk, S.E. Klohr, T. Spears, E.H. Kerns, M.S. Lee, J. Pharm. Biomed. Anal. 14 (1996) 1743–1752.

[4] X.-Z. Qin, D.P. Ip, K.H.-C. Chang, P.M. Dradransky, M.A. Brooks, T. Sakuma, J. Pharm. Biomed. Anal. 12 (1994) 221–233.

[5] X.-Z. Qin, E.W. Tsai, T. Sakuma, D.P. Ip, J. Chromatogr. A 686 (1994) 205–212.

[6] M. Andre, R. Domanig, E. Riemer, H. Moser, A. Groeppelin, J. Chromatogr. A 741 (1996) 146.

[7] K.J. Volk, S.E. Klohr, R.A. Rourick, E.H. Kerns, M.S. Lee, J. Pharm. Biomed. Anal. 14 (1996) 1663–1674.

[8] E.D. Ramsey, S.D. Lawrence, D.E. Games, M.J.E. Hewlins, M.A. McDowall, Anal. Commun. 33 (1996) 79–83.

[9] E.C. Goosen, K.H. Stegman, D. de Jong, G.J. de Jong, U.A.T. Brinkman, Analyst 121 (1996) 61–66.

[10] L. Silvestro, S.R. Savu, Rapid Commun. Mass Spectrom. 10 (1996) 151–156.

[11] A. Almudaris, D.S. Ashton, A. Ray, K. Valko, J. Chromatogr. A 689 (1995) 31–38.

[12] L. Malkki-Laine, A.P. Bruins, J. Pharm. Biomed. Anal. 12 (1994) 543–550.

[13] J. Ogorka, G. Schwinger, G. Bruat, V. Seidel, J. Chromatogr. 626 (1992) 87–96.

[14] M.S. Lee, E.H. Kerns, M.E. Hail, J. Liu, K.J. Volk, Liq. Chromatogr. Gas Chromatogr. 15 (1997) 542–558.

[15] S.R. Wilson, Y. Wu, Org. Mass Spectrom. 29 (1994) 186–191.

[16] S.R. Wilson, Y. Wu, J. Am. Soc. Mass Spectrom. 4 (1993) 596–603.

[17] S.R. Wilson, Y. Wu, Organometallics 12 (1993) 1478–1480.

[18] S.R. Wilson, Y. Wu, J. Am. Chem. Soc. 115 (1993) 10334–10337.

[19] S.R. Wilson, N. Kaprinidis, Y. Wu, D.J. Schuster, J. Am. Chem. Soc. 115 (1993) 8495–8496.

[20] K.P. Bateman, S.J. Locke, D.A. Volmer, J. Mass Spectrom. 32 (1997) 297–304.

[21] G.J. Feistner, J. Mass Spectrom. 30 (1995) 1546–1552.

[22] J.L. Josephs, Rapid Commun. Mass Spectrom. 10 (1996) 1333–1344.

[23] T.A. Gillespie, T.J. Lindsay, J.D. Cornpropst, P.L. Bonate, T.G. Skaggs, A.F. DeLong, L.A. Shipley, ACS Symp. Ser. 619 (1996) 315–329.

[24] Q.N. Porter, Mass Spectrometry of Heterocyclic Compounds, 2nd ed, Wiley, New York, 1985.