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Note

Thermospray high-performance liquid chromatographic–mass spectrometric analysis of the degradation products of piroximone

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Piroximone is a non-adrenergic, non-glycoside cardiotonic agent currently undergoing clinical trials for the treatment of congestive heart failure[1–3]. During development it was necessary to determine the route for formation of potential degradation products in order to develop methodology to control these substances. The rapid development of thermospray (TSP) as a technique for on-line high-performance liquid chromatography–mass spectrometry (LC–MS) has been shown for applicability of mixture analysis[4]. This paper describes an analysis of the degradation products of piroximone by TSP LC–MS and LC–MS–MS.

EXPERIMENTAL

Materials

Piroximone and its N-oxide were prepared at the Merrell Dow Research Institute. 5-Ethylhydantoin was purchased from CTC Organics (Atlanta, GA, U.S.A.). Isonicotinic acid was purchased from Reilly Chemicals (Indianapolis, IN, U.S.A.). Acetonitrile and water were HPLC grade obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Other reagents used were commercial reagent grade.

Sample preparation

Samples were prepared in 0.05 *M* aqueous buffers and 1% aqueous hydrogen peroxide solution at a concentration of 1 mg/ml. The ionic strength of the aqueous buffers was adjusted to 0.13 *M* by adding a suitable amount of sodium chloride. The pH of the aqueous buffers were measured by a Corning Model 130 pH meter (Medfield, MA, U.S.A.). The samples were stored in sealed ampules at ambient temperature.

TSP LC–MS

The HPLC system consisted of two Waters Model 510 pumps (Milford, MA, U.S.A.) controlled by a Waters Model 680 gradient controller, a Waters WISP 710B autoinjector, a Waters Model 481 UV detector and a LiChrosorb RP-18, 10 μm, column (25 cm × 4.0 mm I.D.) (EM Science, Cherry Hill, NJ, U.S.A.). The mobile phase used was composed of water as solvent A and water–acetonitrile (85:15, v/v) as solvent B. The gradient elution consisted of solvent A for 6 min followed by a

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linear gradient from solvent A to solvent B at 20 min. The flow-rate was 1.3 ml/min. The column effluent was mixed in a mixing tee, located between UV detector and TSP interface (Finnigan MAT, San Jose, CA, U.S.A.), with 0.5 *M* ammonium acetate at flow-rate of 0.3 ml/min as delivered by a Waters Model 590 pump. The UV detector was set at 320 nm for assay of piroximone and at 230 nm for assay of degradation products. Volumes injected for assay of piroximone were 20 μl and those for assay of degradation products were 100 μl. Chromatograms were processed by a laboratory data system (Beckman, Waldwick, NJ, U.S.A.).

The TSP interface was connected to a Finnigan MAT TSQ-46 triple quadrupole mass spectrometer. It was operated with a jet temperature of 250°C and a vaporizer temperature of 130°C to 170°C, depending on solvent composition. The repeller voltage was set to −125 V.

For LC–MS work the triple quadrupole was operated as a single stage mass spectrometer using quadrupole 3 scanning from 80 to 500 *m/z* in 2 s. TSP LC–MS–MS experiments were run using argon as the collision gas at an indicated pressure of 1 mTorr and a collision energy of −20 eV. The data were collected and processed using an INCOS data system (Finnigan MAT). Background ions as determined by single ion chromatograms were eliminated in order to simplify the spectra.

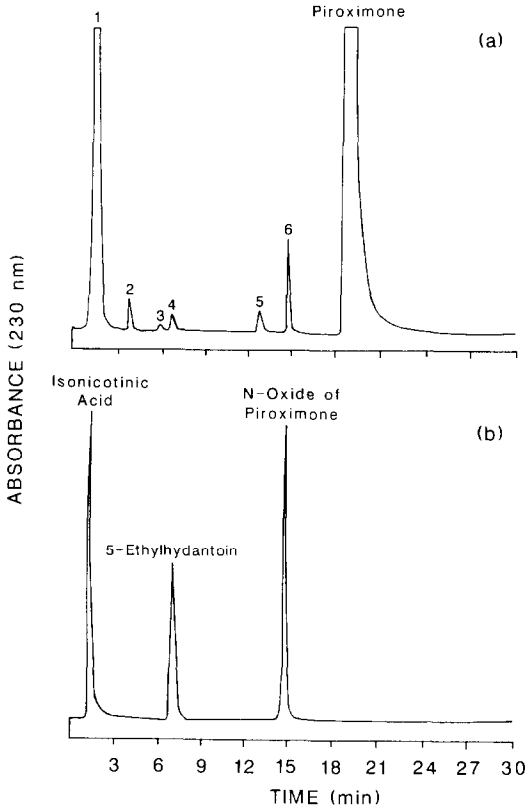


Fig. 1. Chromatograms of hydrogen peroxide degraded sample (a) and authentic materials (b). For chromatographic conditions, see Experimental.

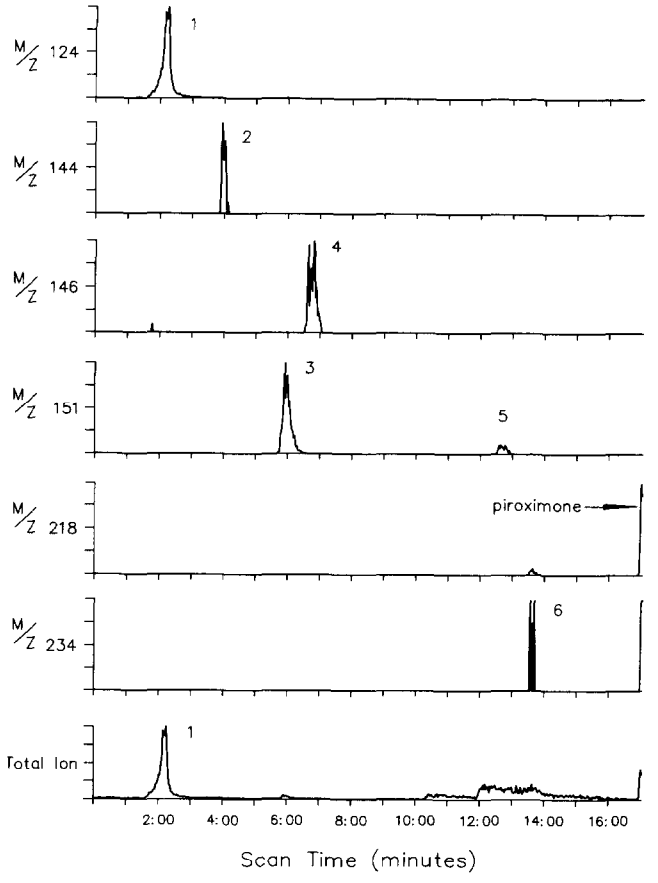


Fig. 2. Total ion and single ion chromatograms of hydrogen peroxide degraded sample.

RESULTS AND DISCUSSION

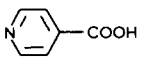
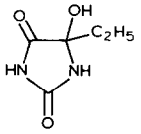
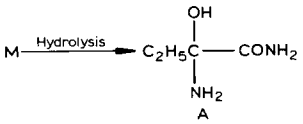
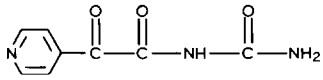
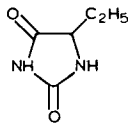
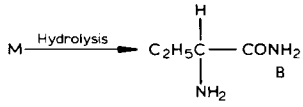
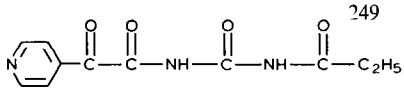
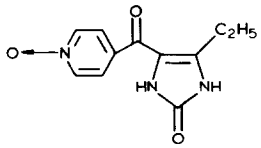
HPLC analysis of piroximone dissolved in aqueous buffers and 1% aqueous hydrogen peroxide indicated that the compound was quite stable in acidic and neutral media. However, in basic and hydrogen peroxide solutions, degradation was observed.

In the base degraded sample, only one degradation peak was observed. Based upon comparison of retention time to an authentic sample, this peak has been identified as isonicotinic acid. However, the pattern is more complex in the hydrogen peroxide degraded sample (Fig. 1). The retention times of peaks 1, 4 and 6 corresponded to those of isonicotinic acid, 5-ethylhydantoin and N-oxide of piroximone, respectively. Peaks 1–6 represented 9.1, 0.1, 0.03, 0.1, 0.1 and 0.3%, respectively, of the total peak areas.

In order to identify these degradation peaks the hydrogen peroxide degraded sample was subjected to TSP LC–MS. The total ion chromatogram and single ion

TABLE I

TABULAR LISTING OF THE MASS SPECTRA FOR THE DEGRADATION PRODUCTS OF PIROXIMONE

Peak	*Structure*	*Mol. wt.*	*m/z*	*RI (%)*	*Ion*
1		123	124	100	$[M + H]^+$
2		144	162 145 144	82 35 89	$[M + NH_4]^+$ $[M + H]^+$ $[M + NH_4 - H_2O]^+$
		118	119 101	100 13	$[A + H]^+$ $[A + H - H_2O]^+$
3		193	194 178 151 124	2 10 100 27	$[M + H]^+$ $[M + H - NH_2]^+$ $[M + 2H - CONH_2]^+$ $[M + H + OH - CONHCONH_2]^+$
4		128	146 129	100 3	$[M + NH_4]^+$ $[M + H]^+$
		102	103	18	$[B + H]^+$
5		249	250 194 151 124	89 58 100 83	$[M + H]^+$ $[M + 2H - COC_2H_5]^+$ $[M + 2H - CONHCOC_2H_5]^+$ $[M + H + OH - CONHCONHCOC_2H_5]^+$
6		233	234 218 216	17 100 37	$[M + H]^+$ $[M + H - O]^+$ $[M + H - H_2O]^+$

chromatograms characteristic of the degradation products are shown in Fig. 2. Only small peaks were noted for the degradation products except peak 1. This is due to high intensity of the background ion generated in the thermospray process and to the small amount and low mass of the degradation products present in the sample.

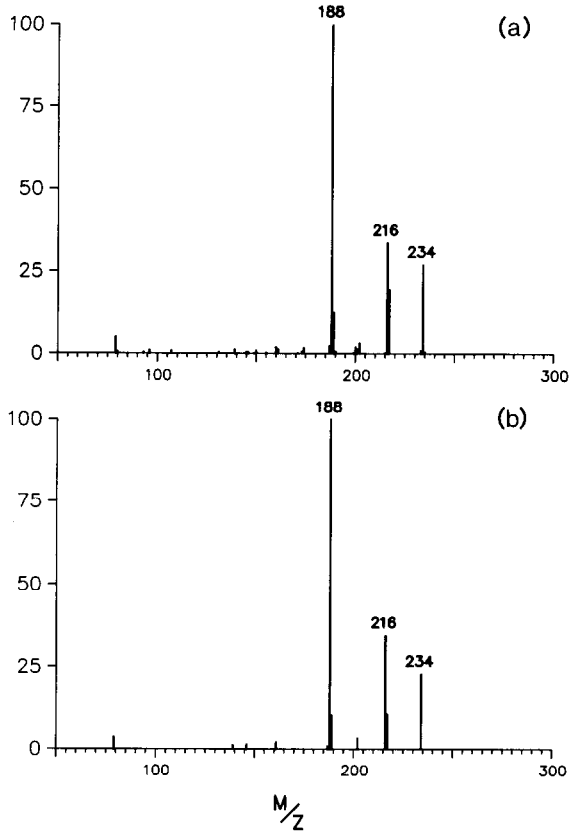


Fig. 3. TSP MS–MS daughter ion mass spectra of m/z 234 from peak 6 (a) and N-oxide of piroximone (b).

Peaks 1–6 corresponded to those peaks as previously seen by the UV detection.

The mass spectra of degradation peaks 1–6 together with proposed structures are shown in Table I. Some of the fragmentation indicate hydrolytic steps which can be seen in the TSP process.

In order to provide additional fragmentation data, the run was repeated using TSP LC–MS–MS. TSP ions characteristic of the degradation products were subjected to collisonally activated decomposition (CAD) by argon to provide fragmentation data on these ions. A comparison of the MS and MS–MS data, as well as HPLC retention times, for peaks 1, 4 and 6 with those of authentic materials provided a confirmation of their identities. An example of MS–MS spectra of peak 6 and N-oxide of piroximone is shown in Fig. 3. The proposed structure for peaks 2, 3 and 5 have not been confirmed by comparison with authentic materials. However, the bis-acylurea structure proposed for peak 5 is analogous to photooxidation product of an imidazolone derivative as reported by Tsuge *et al.*[5]. Its MS–MS data are also consistent with the structure proposed (Fig. 4).

TSP LC–MS has been successfully used to identify the degradation products

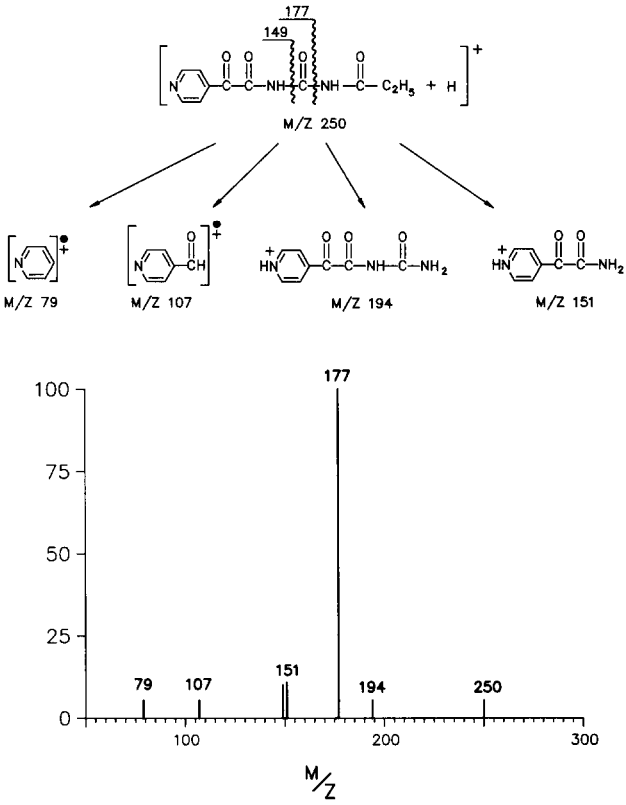


Fig. 4. TSP MS–MS daughter ion mass spectrum of m/z 250 from peak 5.

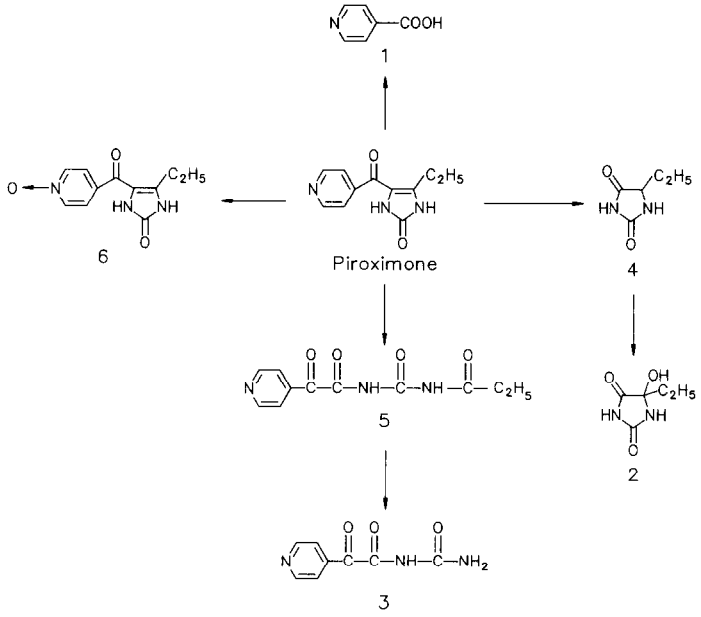


Fig. 5. Degradation scheme of piroximone in hydrogen peroxide solution.

of piroximone in hydrogen peroxide solution. The scheme to form those degradation products is presented in Fig. 5. Except for isonicotinic acid, those degradation products represent only a small proportion (based on the peak area of UV detection and the intensity of their TSP ions) of the degradation that has taken place we believe that this is due to the instability of the degradation products themselves, which presumably degrade rapidly to other non-detected products.

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