Journal of Chromatography, 367 (1986) 254–259
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 882

Note

Stability studies on piriprost and piriprost potassium using high-performance liquid chromatography

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(Received June 17th, 1986)

Piriprost potassium [potassium salt of 6,9-deepoxy-6,9(phenylimino)-$\Delta^{6,8}$-prostaglandin I_1 (Fig. 1) is a selective inhibitor of leukotriene synthesis and is currently the subject of clinical studies[1–4]. An analytical method was needed to analyze bulk samples of piriprost and potassium piriprost. Our efforts to develop such a method showed rapid degradation of piriprost in solution. Extensive stability studies were necessary to identify conditions where piriprost was stable during the analysis. The effects of pH, light, degree of aeration and presence of metal ions were investigated. Conditions were identified where samples could be analyzed within a 3-h time period.

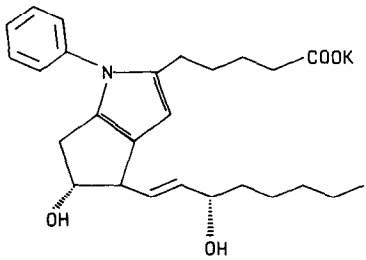


Fig. 1. Structure of piriprost potassium.

EXPERIMENTAL

Chromatographic conditions

A Zorbax ODS (5–6 μm) column (250 mm × 4.6 mm I.D.) was used with a mobile phase of acetonitrile–water–ammonium dihydrogen phosphate–phosphoric acid (550:450:3.0:2.0, v/v/w/v) and a flow-rate of 1–1.5 ml/min. Detection was at 254 nm (LDC UV III, Laboratory Data Control, Riveria Beach, FL, U.S.A.). With a flow-rate of 1 ml/min the retention time of piriprost was 14 min. An internal standard of methylprednisolone acetate was used at a concentration of 0.025 mg/ml and had a retention of 0.7 relative to piriprost.

Solution stability studies

Solutions of piriprost were prepared in acetonitrile–water (50:50) at a concen-

tration of 0.5 m*M*. Metal ions were added as their perchlorate salts at a level of 0.05 m*M*, one-tenth the molar concentration of the piriprost. Control of pH was achieved with the use of buffers appropriate to the pH range being studied, *i.e.* phosphate for pH 7 and 3; acetate for pH 5. Fluorescent light refers to exposure to a 30-W fluorescent light at a distance of 30 cm. These studies were conducted at near room temperature by placing the solution in an autosampler vial and repetitively injecting an aliquot of the solution to determine the kinetics of degradation. The amount of piriprost was determined with the high-performance liquid chromatographic (HPLC) conditions described below. A "blank" was run for each experiment to assure the asbsence of interferences from the reagents used.

Analysis of kinetic data

Piriprost concentrations were fit to a log-linear (concentration, time) model which assumes pseudo-first order degradation. The experiments were usually conducted over a time-frame of 30 days which was usually > three half-lives for all except the most stable conditions. Time to degrade 1% was calculated from the slope of the least-squares fit of the data.

Sample preparation

Bulk powders were prepared at a concentration of 0.06 mg/ml in a solution of acetonitrile–water (55:45) containing the internal standard. Sonication was generally needed to ensure the complete dissolution of piriprost but was not necessary for potassium piriprost. Rapid weighing is needed to prevent water uptake for potassium piriprost and protection from light is needed to prevent degradation. To protect liquid samples, amber vials were used and the autosampler (WISP, Waters Assoc., Milford, MA, U.S.A.) window was covered with aluminum foil. Storage at −20°C is suggested to assure the integrity of the sample.

RESULTS AND DISCUSSION

Initial efforts to develop an HPLC method relied on ion-suppression with acetonitrile–water mixtures. As the pK_a of the carboxylic acid group of piriprost is *ca.* 5, a pH of 3 was used to suppress ionization. Given this pH and a mobile phase containing 45% acetonitrile, a k' of 6 was observed with a C_{18} column and was judged suitable for studies on the stability of the piriprost. Fig. 2 shows a chromatogram of piriprost and the internal standard, methylprednisolone acetate. A chromatogram of a partially degraded sample of piriprost (Fig. 3) shows resolution of piriprost from degradation products. Methylprednisolone acetate was selected as the internal standard as it is eluted at a retention time where there is no significant level of interference from degradation products.

Initial studies on the stability of piriprost in the mobile phase showed decreases in piriprost of approximately 1%/h. This would limit analysis to being done immediately after dissolving the sample. Therefore, an investigation into the factors affecting the rate of degradation was undertaken. Some of the studies were performed over a time period longer than needed for improving the analysis because information on solution stability could also be used in the development of formulations.

The results of studies on the influence of pH, light, oxidizing agents, degree of

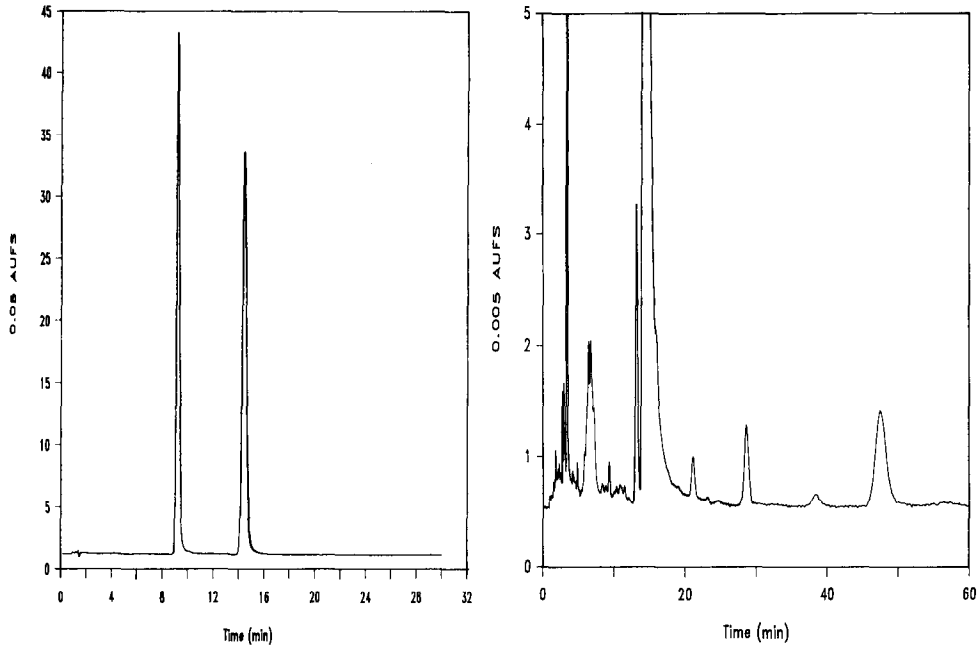


Fig. 2. Chromatogram of piriprost and the internal standard methylprednisolone acetate.
Fig. 3. Chromatogram of a partially degraded sample of piriprost.

aeration and trace metal ions on the stability of aqueous solutions of piriprost are shown in Table I and Fig. 4. Pseudo-first order degradation was observed for the experiments with the metal ions, hydrogen peroxide and for solutions with a pH < 3. At higher pH values, a bi-exponential decrease in piriprost was observed with the rate of degradation becoming slower at long times. For the purposes of determining the effect on the analytical method, the initial rate of degradation is most

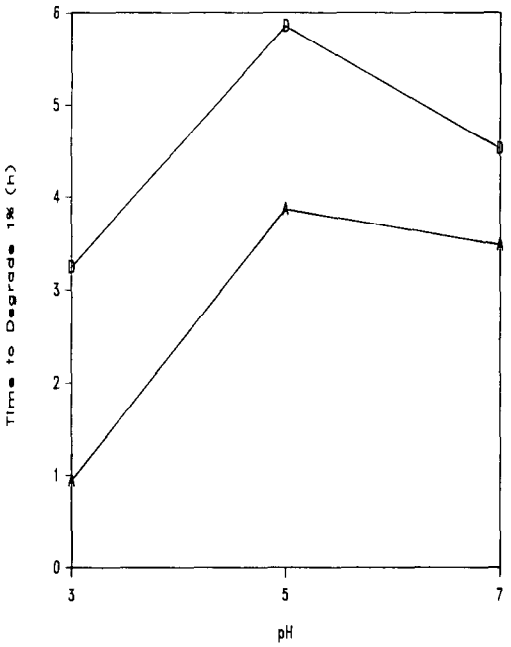
TABLE I

EFFECT OF METAL IONS, OXIDIZING AGENTS AND LIGHT ON PIRIPROST DEGRADATION

Piriprost at 0.5 m*M* concentration.

Condition	*Time (h) to degrade 1%*
Acetonitrile–water (50:50)*	3.2
0.1 *M* Sodium hydroxide	3.3
0.05 m*M* Zn(II)	2.6
0.05 m*M* Cr(II)	2.2
0.1 *M* Hydrochloric acid	1.6
0.05 m*M* Fe(III)	0.8
0.05 m*M* Cu(II)	0.6
1% Hydrogen peroxide	0.2
Fluorescent light	0.2

* Solvent for other experiments.



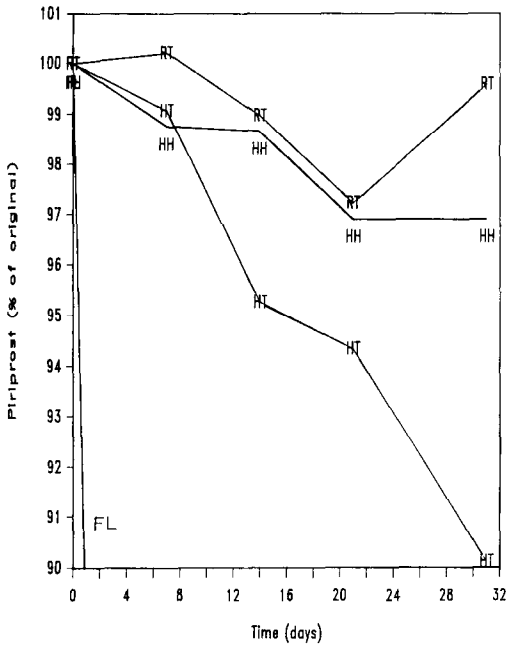


Fig. 4. Rate of degradation of piriprost in solution as a function of pH and degree of aeration. D = solutions purged with nitrogen; A = solutions saturated with air by shaking.

Fig. 5. Degradation of piriprost in the solid state. RT = 25°C in the dark; HH = 25°C and 75% relative humidity; HT = 37°C; FL = fluorescent light.

important and was used for the data shown in Fig. 4 and Table II. The change in rate of degradation occurred at 20, 30 and 40 days for pH 5, 7 and 0.1 *M* sodium hydroxide, respectively.

Decreasing pH, the presence of oxidizing cations such as Fe(III), Cu(II), Cr(III) and exposure to fluorescent light all negatively affected the stability of piriprost. The effect of fluoresscent light had the largest effect on stability. The rate of degradation was also dependent on the degree of aeration as seen in Fig. 4. These

TABLE II

WEIGHT GAIN FOR PIRIPROST POTASSIUM AFTER 3 h AT DIFFERENT RELATIVE HUMIDITIES

Relative humidity (%)	*Weight gain (%)*
12	<0.1
31	<0.1
51	<0.1
66	7.4
81	19
90	>25
100	>25

results are consistent with oxidation being an important route of degradation.

The solid-state stability of piriprost was also investigated as samples stored at room temperature discolored over a period of a few weeks. Fig. 5 shows the changes in piriprost content when stored at 25°C, at 25°C and 75% relative humidity, at 37°C and under fluorescent light. Again, exposure to fluorescent light caused the largest rate of degradation. Also, measurable rates of degradation occurred at room temperature even in the dark. Hence, samples were subsequently stored at freezer temperatures. Piriprost potassium in comparison to piriprost showed slower rates of degradation at high temperature but a similar susceptibility to fluorescent light.

Another concern in handling solid samples is the hydroscopicity of the bulk drug. Table II shows the amount of water up-take as a function of relative humidity.

Based on the solution and solid-state stability studies, several precautions were needed in order to perform accurate assays of samples. Solid samples have to be protected from light and weighed rapidly to avoid water uptake. Optimum solution stability depends on: (i) protection from light, (ii) pH, (iii) degree of aeration of solutions and (iv) the presence of oxidizers. Protection from light was done by using amber autosampler vials. The optimum pH for stability is 5. The influence of pH on stability is shown in Fig. 6 where the stability in the mobile phase (pH 3) is significantly less than in unbuffered mobile phase (45% acetonitrile). As it would be too inconvenient to deaerate the samples, the principal precautions are to use deaerated mobile phase and to avoid excess shaking. By preparing the samples in 45% acetonitrile and taking the precautions mentioned above, the relative standard deviation of the method is 0.5–1% over a 3-h time frame for each sample. Use of a refrigerated autosampler would also increase the stability of the samples.

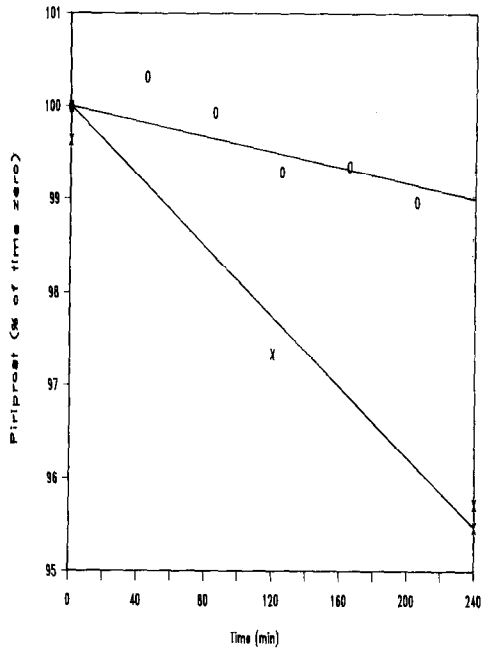


Fig. 6. Stability of piriprost in mobile phase, pH 3, (×) and in mobile phase without acidic buffer (0).

Validation

Linearity of peak heights has been determined over the range of 20 to 150 μg/ml with a correlation coefficient of 0.9994 and an intercept not significantly different from zero. The reproducibility of the method depends on the length of time between sample preparation and injection of the samples. When this length of time was restricted to less than 3 h, relative standard deviations of 0.52 and 1.1% for peak heights and peak areas, respectively, were obtained. No significant decrease in the concentration of the methylprednisolone acetate was observed over a six-day period. During the analysis of degraded solutions of piriprost no changes in the piriprost peaks were detectable that would indicate any interferences.

CONCLUSIONS

Analytical methods were developed for bulk samples of piriprost and piriprost potassium and were optimized to prevent degradation. Piriprost is susceptible to degradation from exposure to light, low pH, aerated solutions and oxidizing cations in solution. In the solid state, light and heat increase the rate of degradation of piriprost.

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