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RESEARCH PAPER

Piroxicam Benzoate—Synthesis, HPLC Determination, and Hydrolysis

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ABSTRACT

An improved method of piroxicam benzoate synthesis was described, and an isocratic reversed-phase high-performance liquid chromatography method for its determination was developed and fully validated. The method was found to be specific, precise (relative standard deviation 0.3%), accurate (mean recovery 99.9%), and robust. Limit of detection was estimated at $0.055\,\mu g\,mL^{-1}$ and limit of quantification at $0.185\,\mu g\,mL^{-1}$. The kinetics of piroxicam benzoate hydrolysis in aqueous buffer solutions (pH 1.1 and 10), simulated gastric and intestinal fluids was studied. The hydrolysis followed first-order kinetics. The following rate constants were obtained at pH 10: $k = 1.8 \times 10^{-3}\,hr^{-1}$ at 37°C and $k = 3.4 \times 10^{-2}\,hr^{-1}$ at 60°C. In acidic media, no significant hydrolysis was observed after 24 hr. During the 24-hr period in simulated intestinal fluid, only 10.9% of the starting ester was hydrolyzed.

Key Words: Piroxicam benzoate; Piroxicam; HPLC; Validation; Hydrolysis; Kinetics.

INTRODUCTION

Piroxicam is a well-known analgesic and nonsteroidal anti-inflammatory drug (NSAID), indicated for acute or long-term treatment of inflammation and associated with musculoskeletal and joint disorders, such as osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.[1] However, prolonged therapy with piroxicam and other NSAIDs causes unwanted side effects, which may lead to gastric irritation and ulceration.[2] In the continuing search for improved anti-inflammatory agents, a series of

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acyl piroxicam derivatives had been synthesized and pharmacologically evaluated.[3] These compounds proved to be useful in alleviating inflammatory conditions after oral, parenteral, or topical administration. Piroxicam blood levels recorded after topical administration of these compounds were much higher than with piroxicam itself when administered under the same conditions at a higher concentration. One compound from the series, piroxicam benzoate [*N*-(2-pyridyl)-2-methyl-4-benzoyloxy-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide], an ester prodrug of increased lipophilicity, showed significant anti-inflammatory activity, especially after topical administration.[3] In this article, an improved method for piroxicam benzoate synthesis and the kinetic study of its hydrolysis are reported. In addition, an analytical method for piroxicam benzoate determination by high-performance liquid chromatography has been developed and fully validated.

MATERIALS AND METHODS

Apparatus and Chemicals

The high-performance liquid chromatography (HPLC) system consisted of a Thermo Separation spectraSYSTEM AS 3000 autosampler with a variable volume loop injector, a spectraSYSTEM P1000 isocratic pump and a spectraSYSTEM UV 1000 programmable variable wavelength detector with a 10-mm analytical flow cell, a spectraSYSTEM SN 4000 signal converter (Thermo Separations, Riviera Beach, USA), and a Pentium MMX 166 MHz computer, TSP PC 1000 software package with an IBM OS/2 Warp version 3 operating system. A Phenomenex Luna C_{18} (Thermo Separations) (150×4.6 mm) packed with 5.00 ± 0.30 μm particles with 100 ± 10 Å pore diameter was used. The mobile phase was acetonitrile/water/acetic acid (45/47/8, v/v). The eluant was flushed at a flow rate of 1.5 mL min^{-1} at room temperature (approximately 25°C), and the injection volume was 20 μL. The analyte was detected at 254 nm.

Peak areas were used to quantify piroxicam benzoate in the various samples. Limit of detection (LOD) and limit of quantification (LOQ) values were estimated using an average standard deviation (SD) obtained by repetitive measurements ($n = 6$) of piroxicam benzoate in the concentration range of 0.2–0.5 μg mL^{-1}. This mean SD was divided by the slope of the standard curve and multiplied by a factor of 3 to obtain the LOD and a factor of 10 to obtain the LOQ.

Infrared (IR) spectra were recorded on a Fourier transform IR Paragon 500 spectrometer (Perkin–Elmer, Beaconsfield, UK) and ultraviolet (UV) spectra on a Hewlett Packard 8452A Diode Array spectrophotometer (Hewlett Packard, Avondale, PA) and a Shimadzu UV-2100 spectrophotometer (Shimadzu, Kyoto, Japan). Dissolution tests were performed in a Vankel VK 7000 apparatus (Vankel Industries, Edison, USA) using the paddle dissolution method of the USP. Piroxicam was purchased from Rolab, Isando, South Africa. Simulated gastric fluid (SGF) and simulated intestinal fluids (SIF) were prepared according to USP,[4] and glycine buffer (pH 1.1) or boric acid buffer (pH 10) are prepared according to BP.[5]

Synthesis of Piroxicam Benzoate

To a suspension of piroxicam (9.94 g, 0.030 mol) in 90 mL dry CCl_4, a solution of triethylamine (4.55 g, 0.045 mol) in 50 mL CCl_4 and a solution of benzoyl chloride (4.22 g, 0.030 mol) in 50 mL CCl_4 were simultaneously added dropwise. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 24 hr. The solvent was evaporated under reduced pressure and replaced with 150 mL CH_2Cl_2 and 100 mL water. The organic layer was separated, extracted three times with water, dried (Na_2SO_4), and evaporated under reduced pressure. The crude residue was treated with hot ethanol. The insoluble pure product was filtered off (5.23 g, 40%), and the mother liquor was evaporated. The residue was dissolved in CH_2Cl_2 and extracted with a cold solution of $NaHCO_3$. The organic layer was washed with water, dried, and evaporated. Thus the obtained product was recrystallized from toluene to give 2.09 g (16%) of pure piroxicam benzoate. [Melting point 147°–150°C. Melting point[3] 145°–148°C. IR (KBr) ν_{max}: 3330, 1752, 1715, 1690, 1516, 1435, 1359, 1304, 1233, 1185, 1143, 1063, 1014, 772, 727, and 706 cm^{-1}.]

Standard Preparation

Piroxicam benzoate standard solutions for HPLC analyses were prepared in a water/acetonitrile 1:1 (v/v) mixture in the concentration range of 400–1,000 μg mL^{-1}. Standard solutions for UV analyses were prepared in a methanol/0.1 mol L^{-1}

HCl 3:97 (v/v) mixture. $A^{1\%}_{1cm}$ value of 514.8 and molar absorptivity (ε) of 22,408 at 320 nm were determined. Piroxicam standard solutions were prepared in a water/acetonitrile 1:1 (v/v) mixture in the concentration range of 5–500 $\mu g\,mL^{-1}$.

Solubility Test

The solubility of piroxicam benzoate in a specific solvent was determined by weighing 1 g of the compound in a test tube and adding 10 mL of solvent, where the test tube was rotated at 45 rpm (25°C) for 24 and 48 hr (different time intervals were applied to determine if the solubility differed). Each of the solvents saturated at the different time intervals was then diluted to fit into the analytical range, where a UV spectrum was recorded (Table 1).

Hydrolysis in Buffer Solutions

Two samples of piroxicam benzoate (25 mg) were dissolved in the respective buffer solutions (glycine buffer pH 1.1 or boric acid buffer pH 10) in 50 mL of volumetric flasks in a temperature-controlled water bath at 37°C or 60°C. Six samples were withdrawn at specific intervals, cooled for 10 min to room temperature, and neutralized to prevent further hydrolysis. Each of the samples was injected three times into the HPLC column, and chromatograms were recorded. The reaction rate constants for the hydrolyses were then determined.

Table 1. Solubility profile of piroxicam benzoate.

Solvent	Solubility ($g\,mL^{-1}$)	UV λ_{max} (nm)
Acetonitrile	1/23	312
Chloroform	1/4	313
Dimethylformamide	1/3	313
Diethyl ether	1/560	313
Ethanol	1/410	312
Methanol	1/230	312
Tetrahydrofuran	1/15	313

Powder Dissolution Test in Simulated Biological Fluids

Six individual samples of 0.180 g of piroxicam benzoate were vortexed in 10 mL of dissolution medium (SGF or SIF) for 2 min and then introduced into the dissolution vessel containing 900 mL of dissolution medium. The solution in the vessels was stirred at 100 rpm. Samples taken at different time intervals (5, 10, 15, 30, 45, 90, 120, 180, and 1,440 min) were analyzed by HPLC.

RESULTS AND DISCUSSION

Piroxicam benzoate was synthesized according to a modified literature procedure[3] from piroxicam and benzoyl chloride (Fig. 1). Although the synthetic procedure was essentially the same as the reported one, prolongation of the reaction time, change of solvent, and workup of the product resulted in a significantly better yield (56% compared with the reported 19.5%). It is worth mentioning that analogous acylation of piroxicam with benzyloxycarbonyl (Cbz) glycine or β-alanine chlorides failed in our hands: so did the attempts to synthesize piroxicam Cbz-glycine or β-alanine esters by DCC (1,3-dicyclohexylcarbodiimide) or CDI (1,1′-carbonyldiimidazole) methods. Reaction of piroxicam with succinic acid anhydride was also unsuccessful. Similar problems with maleic anhydride have been reported.[6]

A solubility test was performed to better characterize piroxicam benzoate. Results are presented in Table 1.

For quantitative determination of piroxicam benzoate in different matrices, it is important to

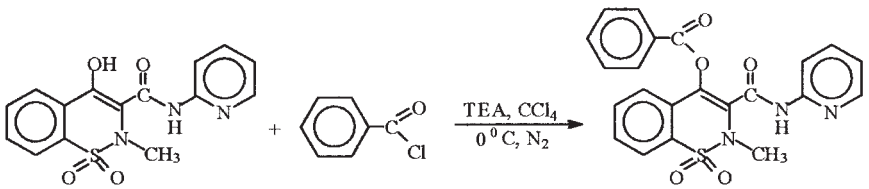

Figure 1. Synthesis of piroxicam benzoate. TEA = triethylamine.

utilize a robust HPLC method that can be qualified by parameters such as precision, accuracy, and selectivity. Validation was performed according to the protocol suggested by the U.S. Food and Drug Administration guidelines for chromatographic method validation.[7]

Chromatographic parameters, such as the capacity factor, peak symmetry, and theoretical plate count, were used to evaluate the chromatographic system performance. To establish the performance criteria for the piroxicam benzoate chromatographic method, the most suitable chromatographic system was chosen (see Materials and Methods) and extensively validated.

Precision

Injection repeatability of 0.2–0.4% ($n = 5$) and intra-assay precision of 0.4% ($n = 6$) were estimated using a piroxicam benzoate sample of $c = 500\,\mu g\,mL^{-1}$. The obtained value was within the recommended precision criteria.[7–9]

Accuracy

Accuracy data set was obtained by spiking five different concentration levels of piroxicam benzoate (50, 75, 100, 125, and 150%) with known concentration of the same compound. The average recovery ranged from 99.1% to 100.5%, when the mobile phase of pH 2.45 was used. The method can thus be regarded as accurate.

Specificity

Piroxicam benzoate was spiked with a $2\,mg\,mL^{-1}$ solution of possible interfering compounds (piroxicam and benzoic acid) at five concentration levels, ranging from 400 to $600\,\mu g\,mL^{-1}$. Piroxicam or benzoic acid/piroxicam benzoate mass ratio ranged from 5:1 to 3.3:1. Agreement of the results of $99.9 \pm 0.4\%$ (range 99.7–100.4%) indicates the absence of piroxicam or benzoic acid interference (e.g., the method was found specific for piroxicam benzoate in the presence of these two compounds). A chromatogram representing the spiked piroxicam benzoate solution is shown in Fig. 2.

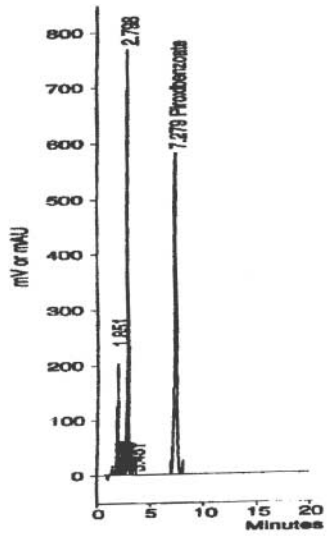

Figure 2. Chromatogram of piroxicam benzoate (7.3 min), piroxicam (2.8 min), and benzoic acid (1.9 min).

Linearity and Sensitivity

Linearity was determined using a series of five piroxicam benzoate concentrations ranging from 80 to 120% of the expected concentration (e.g., in the concentration range of 400–$600\,\mu g\,mL^{-1}$). Peak area (mV sec) vs. concentration (c) ($\mu g\,mL^{-1}$) data fit the regression line: $y = 16{,}370c - 245{,}315$ (multiple $R = 0.999$, $R^2 = 0.999$, adjusted $R^2 = 0.999$; 19 observations).

Ruggedness and Robustness

To determine the ruggedness of the method, analysis was performed on two different instruments (H1 and H2) and on different days. Both instruments performed well, with a precision of 0.4 and 0.3%, respectively; overall recovery of 99.9 and 98.9%; and the linearity correlation coefficient of 0.999 for both instruments. Intermediate precision of 1.0% was calculated as a measure of overall precision on different days.

Robustness was tested by deliberately changing the pH of the mobile phase from 2.45 to 2.15 and 2.75. Recoveries obtained at pH 2.15, 2.45, and 2.75 were estimated at 99.9 ± 0.5, 99.9 ± 0.5, and 99.8 ± 0.6, respectively. It is evident that favorable recovery values were achieved at all the pH values tested. The correlation coefficients for all three mobile phases were 0.999, indicating that the pH change

had little effect on linearity; however, the slope did increase slightly.

To evaluate the influence of the organic phase ratio, the results obtained with the mobile phase containing 25% and 65% acetonitrile were compared with the 45% acetonitrile ratio used in this investigation (average retention time was 7.3 min). A decreased acetonitrile ratio increased the tailing and retention time (average value was 17.4 min), whereas an increased ratio had no influence on the tailing factor, but decreased the retention time as expected (average value was 4.7 min). These results suggest that the method is robust and will accommodate small changes in the mobile phase composition and pH.

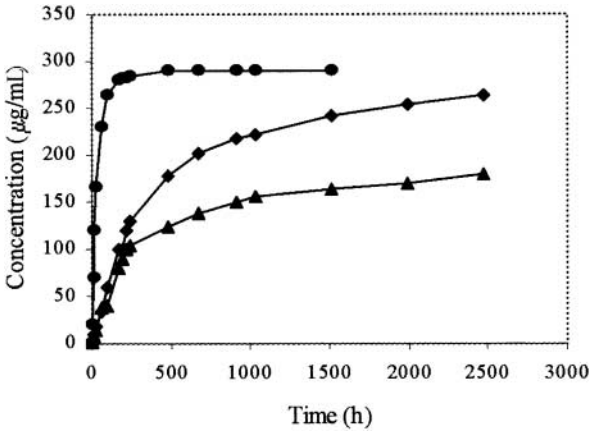

Figure 3. Piroxicam released vs. time at pH 10, 37°C (◆), pH 10, 60°C (●); and pH 1.1, 60°C (▲).

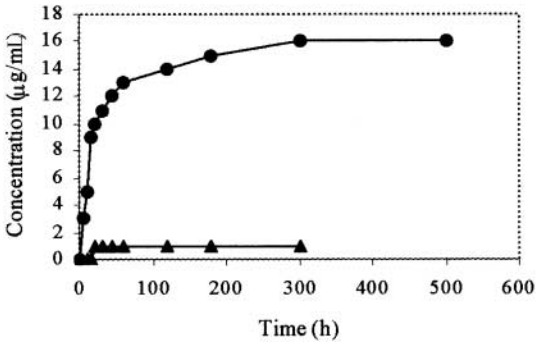

Figure 4. Piroxicam released vs. time in SGF (▲) and SIF (●) at 37°C.

Limiting Values

Using the described HPLC system, the LOD for piroxicam benzoate was $0.055\,\mu g\,mL^{-1}$. The concentration that could be quantitatively determined with acceptable accuracy and precision was $0.185\,\mu g\,mL^{-1}$. These LOD and LOQ values will be very important if the future in vivo studies of piroxicam benzoate are to be undertaken using this HPLC method.

Hydrolysis of piroxicam benzoate in SGF and SIF at 37°C and in aqueous buffer solutions of pH 1.1 (glycine/NaCl/HCl) and pH 10 (H_3BO_3/KCl/NaOH) at 37°C and 60°C was studied. Hydrolysis occurred to a significant extent only in basic conditions and followed first-order kinetics. The following rate constants were obtained at pH 10: $k = 1.8 \times 10^{-3}\,hr^{-1}$ at 37°C ($t_{1/2} = 385$ hr) and $k = 3.4 \times 10^{-2}\,hr^{-1}$ at 60°C ($t_{1/2} = 20.4$ hr). In the buffer solution of pH 1.1 and SGF, no significant hydrolysis was observed after 24 hr. During the same period in SIF, only 10.9% of the starting ester was hydrolyzed.

The concentration of released piroxicam was determined by HPLC using the chromatographic system described in the Experimental section. The average piroxicam concentration is plotted against time to show the elapse of hydrolysis in the depicted experimental conditions (Figs. 3 and 4).

To determine the reaction rate constant k, $\ln(c_\infty - c_t)$ was plotted against time, where c_∞ represents the concentration in which the graph of concentration vs. time levels off, and c_t represents the concentration at each time interval.

The energy needed to activate the reaction (E_a) was determined from the equation:

$$\ln\frac{k_2}{k_1} = \frac{E_a}{R}\frac{T_2 - T_1}{T_1 T_2}$$

where $T_1 = 310$ K, $T_2 = 333$ K, $R = 8.31434\,J\,K^{-1}\,mol^{-1}$, k_1 is the rate constant at T_1, and k_2 is the rate constant at T_2. The values of activation energy were determined as $95.3\,kJ\,mol^{-1}$ (pH 1.1) and $109.6\,kJ\,mol^{-1}$ (pH 10), and were typical of a sigma bond.[10]

CONCLUSIONS

An HPLC method for piroxicam benzoate determination was developed. The most suitable chromatographic system (including C_{18} stationary phase, acetonitrile/water/acetic acid 45/47/8, and flow rate $1.5\,mL\,min^{-1}$) was subjected to extensive validation

to ensure that all data obtained using the HPLC method were reliable. Even under deliberate variations in the method parameters—such as spiked contaminants, mobile phase pH changes and solvent composition, different instruments, and time intervals—the method still proved to be highly precise, accurate, and specific. It might be expected that the method will form the basis for piroxicam benzoate determination in biological fluids as well.

Hydrolysis studies have shown that piroxicam benzoate is a very stable ester in acidic medium, but it can be hydrolyzed in basic solutions.

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