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COLORIMETRIC DETERMINATION OF PIROXICAM IN CAPSULES

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ABSTRACT

A simple colorimetric procedure to quantify piroxicam in capsules has been developed. The method is based on the reaction between piroxicam and 4-aminoantipyrine producing an orange color which can be measured at 490 nm. The method is accurate and precise with a percent relative standard deviation of 0.8 based on 5 readings. The results compared very well with the results obtained using the HPLC procedure. The extraction of piroxicam from the capsule powder is very simple which requires only 4 minutes, versus a 30 minute mechanical shaking recommended by the USP-NF. The results of the decomposed samples were similar to the results obtained using the HPLC method.

INTRODUCTION

Piroxicam is a new non-steroidal antiinflammatory drug which is extensively used in medicine. The USP-NF procedure[1] for the quantitative determination of piroxicam in capsules is based on high-performance liquid chromatography (HPLC). The HPLC methods require expensive equipment and are often time consuming. It is well known that 4-aminoantipyrine couples with some selected drugs with a phenolic group[2-3]. One of the drugs which was assayed using this

reaction was phenylephrine hydrochloride[4]. These studies were undertaken to determine if piroxicam would couple with 4-aminoantipyrine and if it could be used to quantify the drug in capsules.

The purpose of these investigations was to develop an alternate method which would require inexpensive equipment and could be used in those laboratory which do not have HPLC available. The method could also be used to confirm HPLC results because of its simplicity.

METHODOLOGY

Chemicals and Reagents - All the chemicals and reagents were USP-NF or ACS quality and used without further purification. Piroxicam powder was generously supplied by Pfizer Laboratories. The capsules were of the commercial lots.

Equipment - A Bausch & Lomb Spectronic 20 was used to measure the color. The pH values were determined using a Beckmann digital pHmeter (model 4500).

Preparation of Standard Solutions - A 0.5 mg/ml solution of piroxicam in methanol was prepared fresh every week.

Preparation of Assay Solutions from Capsules - A quantity of the powder representing 25.0 mg of piroxicam (based on the label claim) was mixed with enough methanol to make 50.0 ml of the mixture. The mixture was stirred for 3-4 minutes, filtered (Fisher 9-803-5E filter paper was used), first 10 ml of the filtrate was rejected and then some collected for assay. For content uniformity, the contents of one capsule containing 20 mg of piroxicam were mixed with enough methanol to make 40.0 ml of the mixture. The rest of the procedure is the same as given above.

Preparation of Solutions for the Assay Method - The aqueous solutions of potassium ferricyanide (6%), 4-aminoantipyrine (3%) and sodium borate (2%) were prepared fresh every 4 hours.

Assay Procedure - A 1.2 ml quantity of the standard solution of the assay solution was transferred to a 25 ml volumetric flask, a 1.0 ml quantity of potassium ferricyanide added and the mixture brought to approximately 23 ml with sodium borate buffer. It was mixed, a 1.0 ml quantity of 4-aminoantipyrine solution added and brought to volume with water. The orange color so produced was measured at 490 nm (the wavelength of maximum absorbance) against a reagent blank prepared by substituting methanol for the piroxicam solution.

Calculations - Since preliminary investigations indicated that the concentrations were directly related to the absorbance values (range tested $\pm$ 33.3% of the standard concentration), the results were calculated using a simple equation

$$\frac{A_a}{A_s} \times 100 \text{ - Percent of the label claim found}$$

where A_a is absorbance value of the assay solution and A_s that of the standard solution. For comparison purposes, some of the samples were also assayed using a HPLC method similar to the one given in the USP-NF[1]. The mobile phase was prepared by mixing 300 ml of acetonitrile with 700 ml of the citrate-phosphate buffer solution[1]. The final pH of the mobile phase was 4.0 ($\pm$ 0.1). The flow rate was 2.5 ml/min and the sensitivity was set at 0.04 (254 nm). The stationary phase was microbondapak C_{18} column (30 cm x 3.9 mm i.d., Waters Associates). Fluoxymesterone was used as the internal

standard. The injection volume was 20 µl containing 100 µg/ml of piroxicam and 200 µg/ml of fluoxymesterone in methanol. The results were calculated by comparing the ratios of the peak heights (drug/internal standard) since both the assay and standard solutions contained identical concentrations of the drug (based on the label claim) and the internal standard.

Decomposition of Piroxicam Under Drastic Conditions - Ten (10.0) ml of the standard solution of piroxicam was mixed with 10 ml of water (in a 150 ml beaker) and either 1 ml of 1N NaOH or 1 ml of 1N H_2SO_4 solution and the mixtures were heated to boiling on a hot plate. After 10 minutes of boiling, the mixtures were cooled, neutralized (using either 1N H_2SO_4 or 1N NaOH solution) and heated again (gently) to evaporate the solvent. The residues were dissolved in 10.0 ml of methanol and filtered. The clear filtrate were assayed using both the developed colorimetric method and the HPLC method.

RESULTS AND DISCUSSION

The results indicate (Table 1) that the developed method can be used to assay piroxicam in capsules. The results were similar to the results obtained using the HPLC method (Table 1). The recovery from the synthetic mixtures was quantitative (Table 1). The method is accurate and precise with a percent relative standard deviation of 0.8 based on 5 readings. The method can also be used to determine the content uniformity of the capsules as required by the USP-NF[1]. The results obtained indicated (Table 2) that piroxicam was very well mixed with the excipients. The percent relative standard deviation was only 1.3 (Table 2). Furthermore, it was determined

Table I - Assay Results

Sample	Claim per Capsule	Percent of the Label Claim Found Using the Developed Method	HPLC Method
Capsules	20.0 mg	99.8	99.3
Capsules (Different lot)	20.0 mg	98.2	97.8
Capsules (Different lot)	20.0 mg	99.9	100.2
Synthetic Mixture #1	25.0 mg piroxicam and 250 mg of lactose	100.0	99.7
Synthetic Mixture #2	25.0 mg piroxicam and 250 mg of dextrose	100.8	100.5
Decomposed Sample Using Sodium Hydroxide		98.5	98.2
Decomposed Sample Using Sulfuric Acid		97.6	97.2

that mechanical shaking for 30 minutes to extract piroxicam (as recommended by the USP-NF) from the capsule powder is not necessary. A 3-4 minutes occasional stirring with methanol gave the same results as 30 minute shaking using a mechanical shaker. Also in content uniformity experiments, the empty capsule (the shells) were also added to the methanol powder mixture in order to extract piroxicam completely. These shells did not interfere with the developed assay procedure or the HPLC method.

The samples decomposed using drastic conditions (see text) indicated (Table 1) that piroxicam is a very stable drug. The

Table II - Content Uniformity Results Using the The Developed Colorimetric Method

Capsule	Weight with Capsule (mg)	Percent of the Label Claim Found
1	455	100.0
2	455	100.2
3	450	98.9
4	450	99.1
5	455	100.0
6	450	98.7
7	455	100.0
8	460	101.1
9	440	96.7
10	460	101.3
Average	453	99.6
Percent RSD	1.3%	1.3%

results using HPLC method were similar and there were no new peaks in the chromatogram (Figure 1).

When developing the method, a number of other conditions were tried. For example, it was found that the quantity of methanol can effect the absorbance value. Therefore, it is very important to keep the quantity of methanol same. In these studies, 1.2 ml was

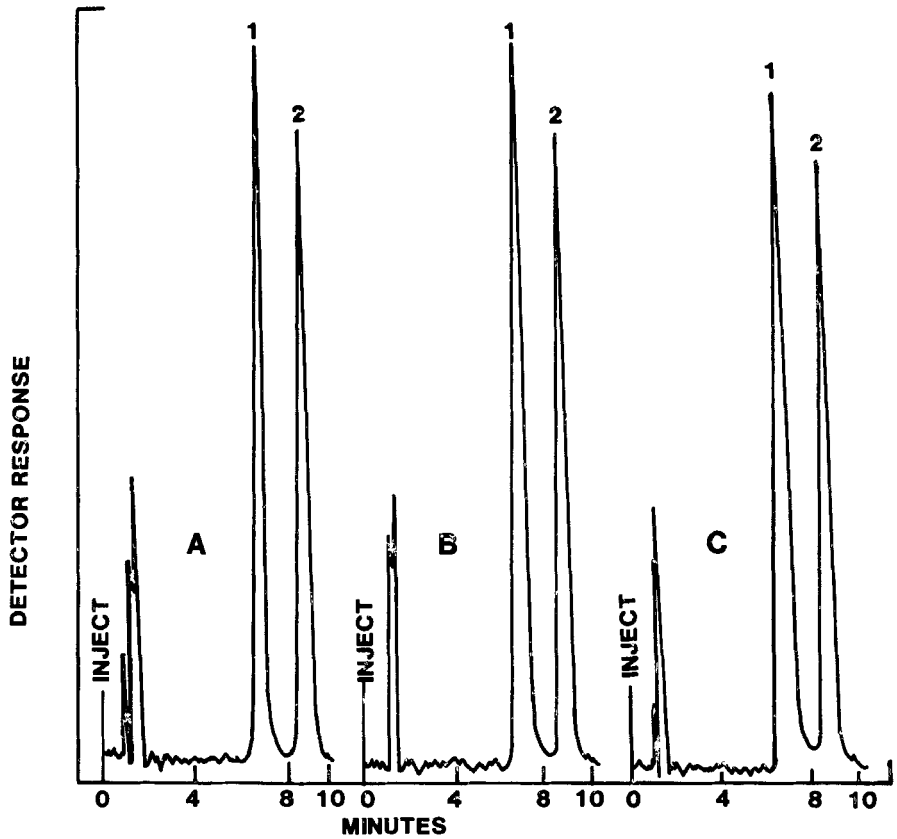

Figure 1

Sample chromatograms. Peaks 1-2 are from piroxicam and fluoxymesterone (the internal standard), respectively. Chromatogram A is from a standard solution; B, from the capsules and C from a sample decomposed by using sodium hydroxide (see text). For chromatographic conditions, see text.

used per 25 ml of the mixture. When testing for the Beer's law, the methanol quantity in the final mixture must be kept constant.

The high concentrations of potassium ferricyanide gave higher absorbance values. However, higher than 6% concentration started precipitating in both the blank and the assay samples. The higher concentration of 4-aminoantipyrine did not make the method more sensitive. The higher concentration of sodium borate (3%) did not change the absorbance value. However, if water was replaced for borate buffer, there was a reaction even in the blank, forming a deep red color. It has been reported to be due to the formation of antipyrine red[2]. A similar reaction occured if the borate buffer was replaced with a phosphate buffer of pH 7.2. It is therefore, clear that the reaction between potassium ferricyanide and 4-amino-antipyrine does not proceed in the presence of borate buffer which made it possible to develop this method. Koshy and Mitchner[4] used the same buffer (2% sodium borate) to develop the assay method for phenylephrine hydrochloride.

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