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Colorimetric Analysis of Piroxicam

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Abstract. A simple and accurate spectrophotometric method is proposed for the analysis of piroxicam. A yellowish-green complex was formed between piroxicam and copper sulphate pentahydrate at room temperature, which was determined at 480 nm. A valid Bee-Lambert's plot over the range of 2 to 12 μg/ml and the calculated molar absorptivity is 3.98×10^3 l/mol/cm. Nine (9) out of eleven (11) brands tested passed the test with values ranging from 97 to 103%, while the other two brands having percentage values outside the specified range failed the test. This colorimetric method was successfully applied to the analysis of bulk pharmaceuticals and pharmaceutical dosage preparations and the results have been statistically analyzed. The proposed method is precise, simple, sensitive and fast, enabling the direct determination of piroxicam without previous extraction and use of expensive equipment and toxic/unaffordable reagents.

Keywords: piroxicam, copper sulphate pentahydrate, pharmaceutical preparations, colorimetry

Introduction

Piroxicam [N-(2-pyridyl-2-methyl-4-hydroxyl-2H-1, 2-benzothiazine-3-carboxamide-1,1-dioxide)] is an extensively used non-steroidal anti-inflammatory agent (NSAID) of the oxicam class of enolic acids, normally used as an antipyretic and analgesic drug especially in the treatment of acute gout and rheumatoid arthritis. The drug acts by inhibiting enzymes involved in the biosynthesis of prostaglandins (Steigerwald, 1978). It is better tolerated than indomethacin and patient compliance is better because of its long biological half-life that permits once daily regimen (Hobbs, 1986; Hobbs and Twomey, 1979).

Although numerous analytical methods developed for the quantitative assay of piroxicam, include high performance liquid chromatography (HPLC) (Basan *et al.*, 2001; Troconiz *et al.*, 1993; Richardson *et al.*, 1986), gas liquid chromatography (GLC), (Capitanni *et al.*, 1988), coulometric (Nikolic *et al.*, 1993) and spectrofluorimetry method (Manzoori and Amjadi, 2003; Damiani *et al.*, 1998), spectrophotometric and potentiometric methods (El-Ries *et al.*, 2003), and voltammetric determination of piroxicam (Paniagua *et al.*, 1992). However, the colorimetric method described here is simple, accurate, reproducible and reliable for the fast routine determination of piroxicam in bulk pharmaceuticals and dosage forms.

Materials and Methods

Piroxicam pure powder was obtained from Pfizer Products Plc, Lagos, Nigeria, and piroxicam commercial brands (capsules) were purchased from local pharmacy shops in Benin city, Nigeria. All the chemicals and reagent used were of analytical grade. Spectrophotometer Model 722S, was used for absorbance measurement. Scanning of piroxicam solution from 200-500 nm showed peak absorbances at 250 nm and 360 nm while scanning for the piroxicam-copper complex was from 300-800 nm and peak absorbance was at 480 nm.

Stock solutions of piroxicam (0.1 g%) in methanol and copper sulphate pentahydrate (0.008 M) in deionized water were prepared. Aliquots (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml) of piroxicam stock solution were placed separately in 25 ml volumetric flasks and the volume in each flask was made up to 3 ml with methanol. 1 ml copper sulphate pentahydrate stock solution was then added to each flask, and the contents were swirled before diluting to the 25 ml mark with methanol. 1:10 dilution of the solution was made with methanol in each flask, before taking their absorbance at 480 nm, using the solution without piroxicam as reagent blank.

For the determination of piroxicam capsules, contents of twenty (20) capsules were carefully mixed and weighed. An amount equivalent to 0.1 g (100 mg) of piroxicam was accurately weighed and dissolved in 100 ml of methanol. 2.0 ml aliquot of this solution was transferred to 25 ml volumetric flask and the volume was made up to 3 ml with methanol. 1 ml of the copper sulphate pentahydrate stock solution was added to the flask, swirled and diluted to 25 ml mark with methanol. 1:10 dilution was made with methanol, and the absorbance was taken at 480 nm against the reagent blank.

Determination of the stoichiometric relationship (ratio of reactants in copper-piroxicam complex). The Job's method of continuous variation (Martin, *et al.*, 1983; Job, 1928) was used. Stock solutions of equimolar concentrations (1.0×10^{-4}M) of piroxicam and copper sulphate pentahydrate were

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prepared. Complimentary preparations of the two stock solutions (5 ml) such as (0.25:4.75, 0.5:4.5, 0.75:4.25, 1.00:4.00 .... 4.75:0.25) were made in different volumetric flasks. The absorbances of the resulting complexes were recorded.

Determination of piroxicam in the presence of lactose Interferant. Stock solutions of piroxicam (0.005 g%) and lactose (0.1 g%) were prepared in methanol. 1 ml, 2.5 ml and 5 ml of the piroxicam stock solution was transferred into two series of 25 ml volumetric flasks. To the flasks containing 1 ml piroxicam, was added 0.25 ml and 2.5 ml of stock lactose solution. The flasks containing 2.5 ml piroxicam 0.5 ml and 5 ml of stock lactose solution was added; and the flasks containing 5 ml piroxicam was added 1.25 ml and 12.5 ml of stock lactose solution, respectively. The mixtures were thoroughly shaken and made up to the 25 ml mark with methanol. The mixture was further shaken vigorously for about 10 minutes and filtered. Aliquots of the filtrate were then analyzed colorimetrically to determine the amount of piroxicam.

Results and Discussion

The method was based on the instantaneous formation of a stable yellowish-green coloured complex with copper sulphate pentahydrate at room temperature. Sordelli *et al.* (1993) reported that piroxicam spontaneously formed complexes with copper sulphate and the complexes formed provided antiinflammatory and superoxide dismutaselike activities. The complex formed was stable for over 72 h as the absorbance readings taken remained constant. The complex showed an absorption peak at λ_{max} 480 nm; it was believed that the spectral interferences were minimal (Connors, 1982). Methanol was used as a solvent for the analysis because piroxicam is soluble in it and the colour of the complex formed was not discharged when the solution was left to stand. Using the continuous variation method, the stoichiometry of the stable complex formed is 1:1 copper-piroxicam complex (Fig. 1). Since previous studies on the metal complexes of piroxicam showed that the piroxicam ligand is coordinated to metal ions through the pyridyl-N and carbonyl-O of the amide moiety (Christofis *et al.*, 2005; Zayed *et al.*, 2004; Cini, 1996). The possible structure of the complexation between piroxicam and copper (II) is shown in Fig. 2.

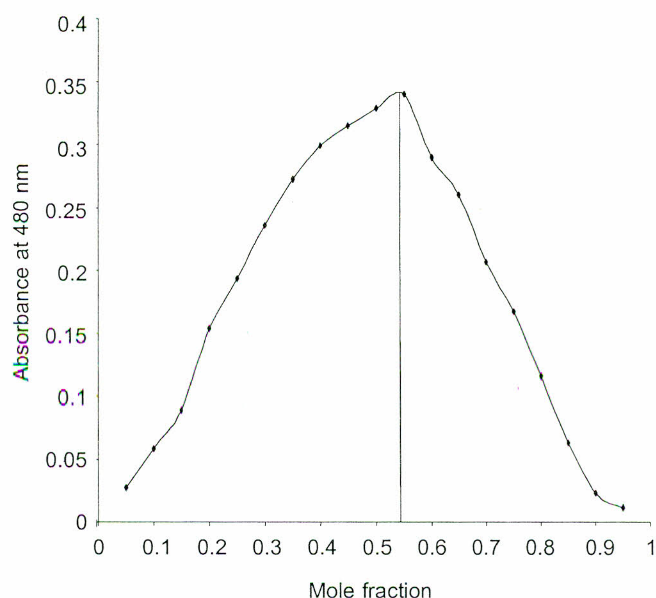

Fig. 1. Stoichiometry of piroxicam-copper complex.

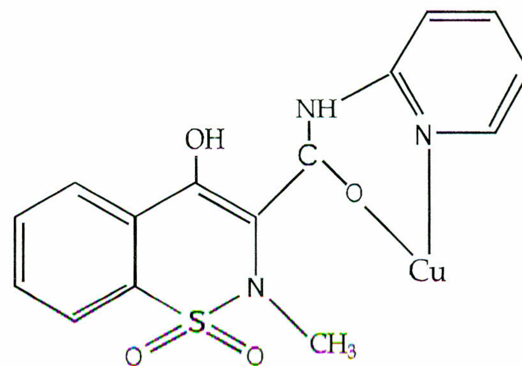

Fig. 2. Proposed structure of the copper-piroxicam complex.

For the quantitative determination of piroxicam, the absorbances were found to increase linearly with increase in the concentration of piroxicam, which were corroborated by the correlation coefficient of 0.9983 and the fact that the two-tailed p value < 0.0001 ($F = 1428.5$) considered extremely significant. A valid Beer-Lambert's plot over the range of 0.0002 - 0.0012 g% (i.e. 2 to 12 μg/ml) was prepared (Fig. 3) and the linear correlation which was calculated using Graphpad Instat (version 10) statistical package is given by $A = -0.0046 + 121.79\,C$ (where, A is the absorbance and C is the concentration). The calculated absorption $A^{1\%}_{1cm} = 120$ and the molar absorptivity is 3.98×10^3 l/mol/cm. The method was then applied to the determination of piroxicam in a number of pharmaceutical preparations in the absence and presence of excipients. The results obtained were presented in Table 1 and Table 2.

Table 1, showed, that the presence of lactose and then a tenfold increase in the concentration of lactose, did not interfere with the complexation of piroxicam with copper ion (Cu^{2+}) significantly. Therefore, the developed method is useful for

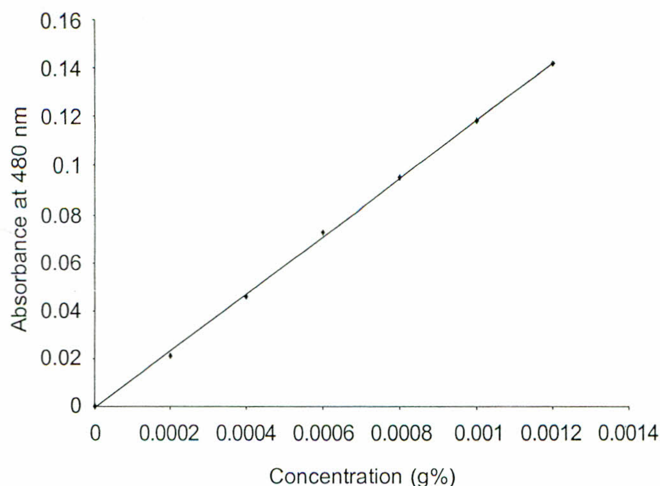

Fig. 3. Calibration plot for the copper-piroxicam complex.

Table 1. Results of the determination of piroxicam in presence of lactose interferant

Concentration of piroxicam (g%)	Concentration of lactose (g%)	Recovery of piroxicam (%)
0.0002	0.001	100.15
0.0002	0.010	101.10
0.0005	0.002	98.98
0.0005	0.020	99.24
0.0010	0.005	100.78
0.0010	0.050	98.77

average percentage recovery of piroxicam = 99.84 ± 0.98; measurements were taken at 480 nm

the determination of piroxicam in the capsules investigated (Table 2). The interference of lactose with the developed method was studied because it is the main excipient in the dosage forms analyzed by this method.

Considering the United States Pharmacopoeia (USP, 2000), which specifies that piroxicam capsules must contain not less than 92.5% and not more than 107.5% of the label claim i.e. 100 ± 7.5% piroxicam, nine (9) out of the eleven (11) brands tested passed the test. Only Feldene® capsules manufactured by Neimeth, Nigeria and Nkoyo® piroxicam capsules manufactured by Maxheal Pharmaceuticals, India failed the test. (where ® stands for the trade name of particular brand). This means that the trade brands of Feldon® capsules and Nkoyo® piroxicam capsules are substandard products and as such should be withdrawn from the market. It is rather unfortunate

Table 2. Results of the determination of piroxicam in pure and in pharmaceutical preparations

Drug proprietary name (Supplier)	Composition	Recovery ± standard deviation*
Piroxicam (pure form)	99%	100.80 ± 0.22
Felvin-20 capsules (Rajat Pharm., India)	20 mg	97.50 ± 0.18
Feldene capsules (Neimeth, Nigeria)	20 mg	86.30 ± 0.17
Pixicam capsules (V.S. Int. Pvt. Ltd., India)	20 mg	100.60 ± 0.25
Feloxin capsules (Emil Pharm, India)	20 mg	125.50 ± 0.32
Nkoyo piroxicam capsules (Maxheal Pharm, India)	20 mg	88.60 ± 0.27
Felxicam capsules (Hovid, Malaysia)	20 mg	102.50 ± 0.62
Roxiden capsules (Medopharm, India)	20 mg	98.50 ± 0.14
Neoxicam capsules Greenlife Pharm, Nigeria)	20 mg	97.80 ± 0.41
Uphaxicam capsules (UPHA Pharm., Malaysia)	20 mg	99.05 ± 0.36
Maxvin capsules (Maxheal Pharm, India)	20 mg	102.40 ± 0.28
Laxidene capsules (Clarion Med. Ltd, Nigeria)	20 mg	97.20 ± 0.36

* = average of five determinations, assay as a percentage of label claim

that Feldene capsules which are acknowledged as the best piroxicam capsule failed the test. On close examination of the package, it was observed that the drug was relabelled by miscreants to wrongly reflect a valid expiry date. The advantage of this method over spectrofluorimetric and HPLC methods is that it is simple and the colorimeters are readily available in virtually all laboratories in the developing countries.

Conclusion

The proposed method is precise, simple, sensitive, rapid, reproducible and enables the direct determination of piroxicam without previous extraction. It is propose that colorimetric method can be used for the routine analysis of piroxicam. In addition, the method does not require the use of expensive equipment and toxic/unaffordable reagents.

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