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Spectrophotometric determination of oxiconazole in topical lotion using methyl orange

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Abstract

A spectrophotometric method is described for the determination of oxiconazole in raw material and in topical lotion. This method is based on the reaction of the oxiconazole with methyl orange in buffered aqueous solution of citric acid at pH 2.3. The chromogen, being extractable with dichloromethane, could be measured quantitatively with maximum absorption at 427 nm. The Lambert-Beer law was obeyed in the concentration range of 4.0–14.0 μg ml^{-1}. A prospective validation of the method showed that the method was linear ($r = 0.9995$), precise (intra-day: CV = 1.57% and inter-day: CV = 1.50%) and accurate (mean recoveries: 99.69%). The results compared favourably with those of the HPLC method.

Keywords: Oxiconazole determination; Visible light spectrophotometry; Imidazole analysis; Methyl orange; Quality control

1. Introduction

Oxiconazole is a broad-spectrum antifungal imidazole derivative active against infections caused by dermatophytes, yeast like fungi, moulds and mixed infections due to fungi and Gram-positive bacteria [1,2]. The chemical name of oxiconazole is 2′,4′-dichloro-2-(imidazol-1-yl) acetophenone *O*- (2,4-dichlorobenzyl) oxime nitrate (Fig. 1) and is not yet official in any pharmacopoeia. Imidazole derivatives are agents present in numerous pharmaceutical formulations. Several methods have been reported for the determination of these compounds [3–11]. However, for oxiconazole (Ox), few reports about its analytical determination are available in the literature. High performance liquid chromatography of Ox in raw material and pharmaceutical formulations has been described [12]. Nevertheless, the literature has not showed spectrophotometric methods for determination of Ox in pharmaceutical formulations. The aim of this study was to develop a sensitive and accurate spectrophotometric method for determination of Ox in raw material and topical lotion through ion-pair complex formation between the drug and methyl orange (MO). The reaction conditions and the application of the method are presented. The spectrophotometric method can be applied routinely because it does not require high cost reagents and equipment when it is compared with HPLC analysis.

2. Experimental

2.1. Materials

Ox nitrate reference substance (assigned purity 100.4%) was kindly supplied by Roche Laboratories, São Paulo, Brazil. It was tested for purity by performing its melting point, UV, HPLC and NMR spectrum. No impurities were found. The drug was used without further purification. Pharmaceutical topical lotion was obtained commercially and claimed a concentration 10 mg of the drug (as base) per milliliter and benzyl alcohol as a bacteriostatic preservative. All other chemicals and reagents were of analytical grade. The colour reagent used was methyl orange 0.1% (w/v) in

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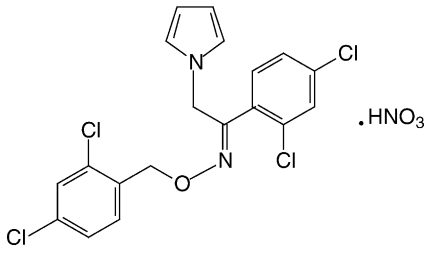

Fig. 1. Chemical structure of oxiconazole nitrate.

40% aqueous methanol (v/v) and as buffer solution 0.1 M citric acid (pH 2.3). All substances, as well as the reagents were kept at room temperature and stored protected from light. The absorbance value of each solution was determined in a 10 mm quartz cell using a Spectronic Genesys 2 UV–vis spectrophotometer.

2.2. Method

2.2.1. Standard solution

A quantity of Ox nitrate reference substance equivalent to 25 mg of Ox was accurately weighed and transferred to a 50-ml volumetric flask; methanol was added to make up the volume in order to give a final concentration of 500 $\mu g\,ml^{-1}$. Ten-milliliter aliquots of this solution were transferred into 50-ml volumetric flasks and brought to volume with the same solvent to give a final concentration of 100 $\mu g\,ml^{-1}$.

2.2.2. Procedure for topical lotion solution

A quantity of the topical lotion containing 100.0 mg of Ox was transferred to 100-ml volumetric flask and methanol added to make up the volume. An aliquot of the 10 ml of this solution was transferred to a 100-ml volumetric flask and methanol added to make up the volume in order to give a final concentration of 100 $\mu g\,ml^{-1}$.

2.2.3. General procedure for the assay method

In a separator funnel, aliquots of Ox standard or sample (100 $\mu g\,ml^{-1}$) were added to 3 ml 0.1 M citric acid solution (pH 2.3). Then, 2 ml of 0.1% MO (w/v) in 40% aqueous methanol (v/v) was added. The reaction mixture was extracted by shaking with 10 ml of dichloromethane. This extraction was conducted three times until the solution became clear. The organic extracts were collected in a 50-ml volumetric flask and diluted to volume with dichloromethane. Approximately 0.2 g anhydrous sodium sulphate was added in each volumetric flask, shaken slowly for about 1 min, filtered and the first portion of the filtrate discarded. After approximately 3 min, the absorbance of the resulting solution was measured at 427 nm against a reagent blank prepared in the same manner without the addition of the drug.

2.3. Method validation

The method was validated by determination of linearity, precision and accuracy [16,17].

2.3.1. Linearity

In order to assess the validity of the assay, appropriate amounts of the standard solution (100 $\mu g\,ml^{-1}$) were transferred to a separator funnel and thereafter the general procedure was followed yielding final concentrations of 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 $\mu g\,ml^{-1}$. The linearity of the calibration curves was determined for intra- and inter-day precision in three different days. The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis.

2.3.2. Precision

The precision of analytical procedure was evaluated through the repeatability (intra-assay) and intermediate precision (inter-assay) by assaying six samples of topical lotion. Method repeatability was studied by assaying samples at the same concentration during the same day and under same experimental conditions. The intermediate precision was evaluated by comparing the assays achieved in two different days.

2.3.3. Accuracy

The recoveries were determined at three concentration levels, by adding known amounts of reference substance in the beginning of the process. Ox reference substance solution (100 $\mu g\,ml^{-1}$) was added in 1.0, 2.0 and 3.0 ml aliquots to a separator funnel containing 3 ml 0.1 M citric acid solution (pH 2.3). Then, 2 ml of 0.1% MO (w/v) in 40% aqueous methanol (v/v) and 3.0 ml of Ox topical lotion solution (100 $\mu g\,ml^{-1}$) were added. The reaction mixture was extracted as described at general procedure, yielding a final estimated concentration of 8.0, 10.0 and 12.0 $\mu g\,ml^{-1}$ corresponding at 133.3, 166.6 and 200.0%, respectively, of the sample concentration used in the assay.

3. Results and discussion

UV spectrophotometric analysis of imidazoles has been limited due to its low absorptive and absence of characteristic bands. Therefore, the determination of this group of drugs by spectrophotometric methods has been based on the reaction of formation of an ion-pair complex between the drug and colour reagent [5–7,10]. Ion-pair extraction spectrophotometry has received considerable attention for quantitative estimation of many pharmaceutical compounds [13–15]. In this study MO being an anionic dye, forms with Ox in acidic medium, a yellow-orange coloured ion-pair complex, which is soluble in dichloromethane and can be measured at λ_{max} 427 nm (Fig. 2). The optimum conditions for the quantitative estimation of the associated ion-pair formed were established by a number of preliminary experiments. Different acidic pH

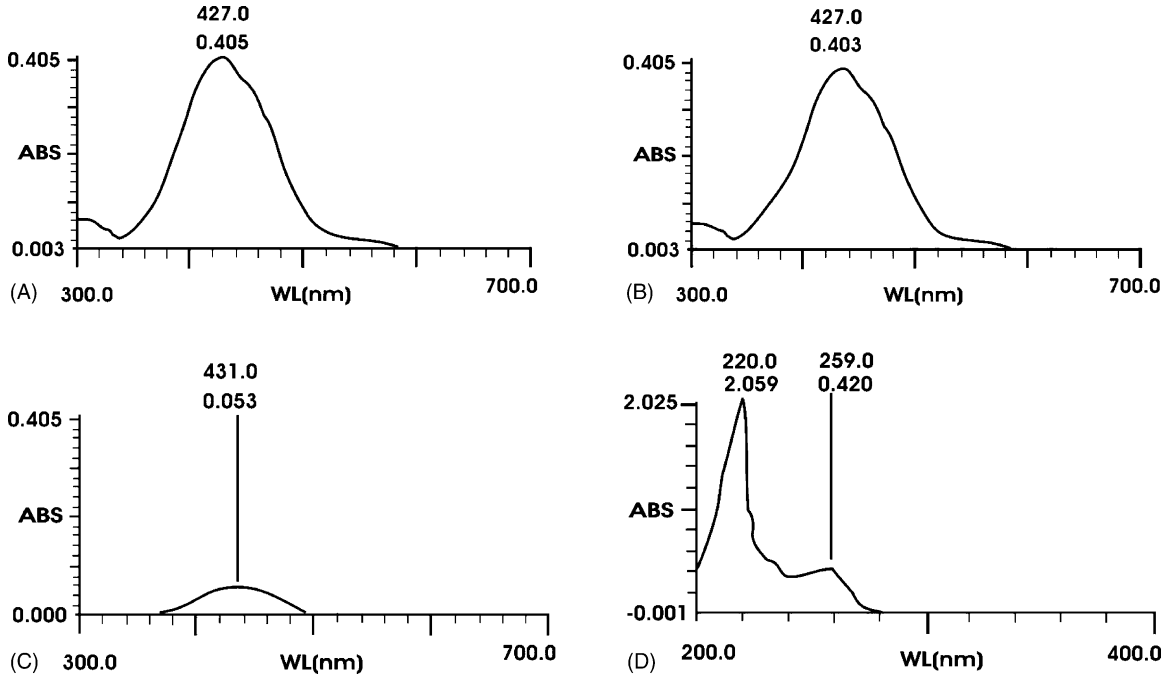

Fig. 2. Absorption spectrum of oxiconazole with methyl orange ion-pair complex (A) reference substance (B) topical solution (C) placebo blank (D) oxiconazole without the ion-pair complex.

values, extractor solvent, optimum volume of the dye used were also studied. The best results were found at pH 2.3 with dichloromethane as extractor solvent and 2 ml as volume of the dye. The ion-pair complex obtained was stable for a period of more than 30 min. No interfering absorbance was found due to the benzyl alcohol, bacteriostatic preservative present in the lotion formulation, and solvents utilized. The blank reagent absorption is practically negligible in all system. Agreement with Beer's law was evident in the concentration range of the final dilution of 4.0–14.0 μg ml^{-1} of Ox. The correlation coefficient obtained for the line was 0.9995 indicating good linearity (Fig. 3). The representative linear equation was: $y = 0.0433x - 0.0199$, where x is concentration and y is absorbance. The analysis of variance of the data indicated no significant difference in slopes of the three calibration curves ($p < 0.01$). The precision is usually expressed as the variance, standard deviation or coefficient of variation (CV %) of series of measurements and may be considered at three levels: repeatability, intermediate precision and reproducibility [16,17]. In the present study the repeatability and intermediate precision were evaluated. The method showed a coefficient of variation of 1.57 and 1.50% for repeatability and intermediate precision respectively, indicating good precision. Results were comparable with declared amounts and with those obtained by HPLC (Table 1). Analysis of variance indicated no significant difference between spectrophotometry and HPLC ($p < 0.01$). Accuracy expresses the agreement between the accepted value (either as conventional true value or an accepted reference value) and the value found [16,17]. The accuracy of the method was determined by recovery studies. These were carried out and the recovery percentage was calculated (Table 2). The mean recovery was found to be 99.69%. From the obtained data, recoveries of the standard drug were accurate.

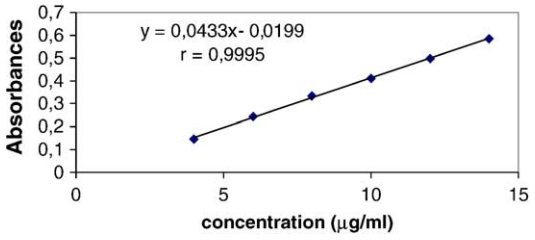

Fig. 3. Calibration curve of oxiconazole by visible spectrophotometry at 427 nm.

Table 1
Analyses of oxiconazole in topical lotion

Method	N	Linear range (μg ml^{-1})	Purity (%)	CV (%)[a]
Spectrophotometry	6	4.0–14.0	99.60	1.57
HPLC	6	40.0–140.0	100.99	1.57

[a] Percent coefficient of variation of the assay.

Table 2
Recovery test of oxiconazole topical lotion using spectrophotometry at 427 nm

	Concentration of standard (μg ml^{-1})		Recovery (%)[a]
	Added	Recovered	
R1	2.0	2.02	101.0
R2	4.0	3.83	95.75
R3	6.0	6.14	102.33

[a] Mean of three replicates analyses.

4. Conclusion

The proposed method is simple, precise, accurate and convenient. Therefore, they can be useful for routine analyses and quality-control assays of Ox in raw material and topical lotion. This method is an acceptable alternative to the HPLC method previously described.

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