DEVELOPMENT OF A KINETIC SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF CICLOPIROX OLAMINE IN PHARMACEUTICAL SAMPLE

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ABSTRACTS

A simple and sensitive kinetic spectrophotometric method was developed for the determination of ciclopirox olamine in pharmaceutical formulations (cream). The method is based on the oxidation of ciclopirox olamine with $KMnO_4$ in alkaline medium to form K_2MnO_4, a bluish green color compound, at ionic strength controlled and room temperature. The reaction is monitored measuring the rate of change of absorbance at 610 nm. The absorbance-concentration plot corresponding to synthetic pharmaceutical samples (cream) was rectilinear, over the range of 0.32 – 10.0 µg mL^{-1}, with detection and quantification limits of 0.095 µg mL^{-1} and 0.32 µg mL^{-1}, respectively. Different experimental parameters affecting the development and stability of the reaction product were carefully studied via factorial screening and optimized by univariate method. The determination of ciclopirox olamine by the fixed time method is feasible and more advantageous with the calibration equation obtained at 30 min. The proposed method was validated for the application of the determination of the drug in pharmaceutical sample (cream).

Keywords: kinetic, spectrophotometric, ciclopirox olamine.

1. INTRODUCTION

Ciclopirox olamine (CXO), 6-cyclohexyl-1-hydroxy-4methyl-2(1H)-pyridona is a synthetic hydroxypyridone, with fungicide action of broad-spectrum that inhibits the growth of pathogenic dermatophytes (skin fungi, hair and nail) [1,2,3]. This drug has a half-life of 1.7 h and renal excretion. Studies with human skin of corpses showed capillar penetration through the epidermis to the hair follicles and sebaceous glands, while a portion of the drug remains in the stratum corneum [4] and it presents systemic absorption when applied in cream 1% (CXO). The mechanism of action is related to its chelating action on polyvalent metal cations such as Fe (III) and Al (III) essential for two fungus enzymes, catalase and peroxidase [2,5]. The USP has published two standard methods for the determination of CXO in pharmaceutical formulations, by HPLC [6] and spectrophotometry [7]. Other methods reported include, HPLC [8,9], LC[10] in raw material and lotions; micro-liquid chromatography [11] in topical formulations; spectrofluorimetry [12], amperometry in flow and batch injection systems [13], polarography [14,15], micellar electrokinetic capillar chromatography [16] and capillar electrophoresis with capacitively coupled contactless conductivity detection [17] in pharmaceutical formulations; and LC/MS/MS in human nail [18]. The development of new analytical methods requires accurate results and sensitivity in the analytical determination, and therefore are mostly used methods of high cost and technical complexity from this perspective is important to develop inexpensive methods such as kinetic spectrophotometry, these methods has comparable levels of accuracy and sensitivity to the methods above mentioned [19]. Only few works in analytical procedures based on kinetics especially for pharmaceutical in biological fluids has been published in the last decade [20]. Furthermore, these methods present sensitivities selective due to the measurement of the increase in the absorbance in time function instead of the measurement of an only one absorbance value [21]. This work describes the development and validation of kinetic spectrophotometric methods for the quantification of CXO in pharmaceutical formulations (cream), using the monitoring of the reduction of $KMnO_4$ with CXO in alkaline medium. In this reaction, the absorption band between 570 and 650 nm increases, this band corresponds to manganate (K_2MnO_4), the product of reduction of $KMnO_4$ [22]. Consequently, it was found that the generation of this product is in function of the time and concentration of the reactants; this shows its analytical usefulness and develops a reliable and specific kinetic spectrophotometric method for the determination of CXO in pharmaceutical samples.

2. EXPERIMENTAL

2.1. Instruments

A Shimadzu UV-1603 double beams spectrophotometer with 10 mm quartz cells was used for measurement of the absorbance. For all solutions, the spectra were recorded on the range between 190–800 nm against blank, using sampling intervals of 0.2 nm with a scan speed of 480 nm min^{-1}. The spectral data were processed by Shimadzu software kit Ver. 3.7 (P/N 206-60570-04). The pH measurements were realized with Oyster™ pH Meter (Measures pH: 0.00 to 14.00; pH Accurate to 0.02 and pH and resolution of 0.01), before and after each one of the assays carried out. The solid samples were weighted with a ± 0.01 mg of uncertainty using a Sartorius R 200D balance.

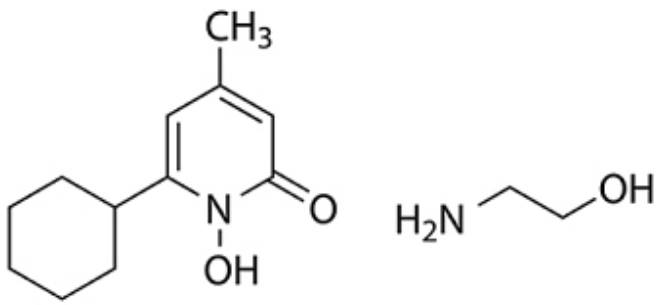

Figure 1. Ciclopirox olamine (CXO).

2.2. Reagents

CXO was purchased from Sigma-Aldrich®, all reagents were of analytical reagent grade and all solutions were prepared with Milli-Q water. Stock solutions of 1.0×10^{-3} mol L^{-1} of CXO was prepared by dissolving 15.0 mg and diluted to 50 mL in NaOH 0.01 mol L^{-1}. The same solution was used to prepare other ranges of concentrations by appropriate dilution using the same NaOH solution. $KMnO_4$ 0.02 mol L^{-1} aqueous solution was used. NaOH 2.0 mol L^{-1} aqueous solution was prepared.

3. PROCEDURES

All assays were realized by triplicate and amber calibrated flasks were used.

3.1. Preliminary studies

The studies of CXO-$KMnO_4$-system were realized using: pH 12.0 -13.5 (With adequate NaOH solution); Na_2SO_4 concentration: 0.0 - 0.7 mol L^{-1}; $KMnO_4$ concentration: $5.0x10^{-4}$ - 0.001 mol L^{-1}; reaction time: 0 -120 min and CXO $1.0x10^{-5}$ mol L^{-1}. The absorption spectra were realized between 190 and 800 nm at room T°.

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3.2. Kinetic procedure

The complete kinetic measurements were performed under pseudo first order conditions. The $KMnO_4$ was used in excess over CXO, at pH and Na_2SO_4 concentration (ionic strength), of 13.2 and 0.6 mol L^{-1}, respectively. The temperature was maintained at 20 ± 0.1°C. The course of the reaction was followed by monitoring the increase in the absorbance at 610 nm (K_2MnO_4). To a series of 5 mL volumetric amber flasks, were added adequate aliquots of 2.0 mol L^{-1} NaOH solution, 0.02 mol L^{-1} $KMnO_4$ solution and 2.0 mol L^{-1} Na_2SO_4 solution and aliquots of CXO $1.0x10^{-3}$ mol L^{-1} over the concentration range $1.0x10^{-6}$ to $13.0x10^{-6}$ mol L^{-1} and dilute to the mark with Milli-Q water; then mix all flasks for 30 min and measure the absorbance at 610 nm against an appropriate blank prepared simultaneously. Plot absorbance values v/s drug concentrations to prepare calibration graph, measured at a fixed time of 30 min the corresponding regression equation was derived. Furthermore log v v/s log [CXO] was plotted to get the order of the reaction at 30 min.

3.3. Factorial study parameters

It was performed using a factorial design, with a Statistical software (Statgraphics Centurion XV for Windows, Rockville, MD). A factorial screening design 2^4, with 6 centers and 1 replica was used, at $1.0x10^{-5}$ mol L^{-1} CXO concentration, the parameters studied were: pH (13 to 13.2 with adequate volume of NaOH solution), Na_2SO_4 concentration (0.1 - 0.5 mol L^{-1}), reaction time (15 - 30 min) and $KMnO_4$ concentration ($8.0x10^{-4}$ -$1.0x10^{-3}$ mol L^{-1}). The analyte solutions were prepared in a volume of 5 mL and reaction monitoring at 610 nm.

3.4. Procedure for Determination of CXO in pharmaceutical formulation (cream)

Preparation of synthetic pharmaceutical formulation (cream) sample. A simulated sample was prepared by weighing the following reagents: 0.98 mg of benzoic acid, 30.5 mg of cetyl alcohol, liquid petrolatum 152.5 mg, 87.5 mg of distilled water, 30.5 mg of monostearate of glycerol and 3.05 mg CXO. The preparation consisted initially in to heat the liquid petrolatum at 70±3°C, then the drug is added and was stirred for 30 minutes, then the sample was allowed to stand in refrigerator until pulping. The same procedure was performed for a blank.

CXO extraction procedure. The cream contains analyte (1%) as the salt, ciclopirox olamine (Micopirox™ Lab. Cassara Chile). The drug extraction was conducted by adding 50 mL of acetonitrile to 0.300 g of sample and was stirred for 1 h at 5°C ± 0.5°C, the content was vacuum filtered (0.45 mm PVDF filter). The solution was left for 1 h 30 min in a freezer at a temperature close to 0°C, then filtered through syringe filter with Nylon membrane (pore diameter of 0.20 mm). The supernatant was dried under N_2 flow and was reconstituted in 0.01 mol L^{-1} NaOH solution to a final volume of 100 mL.

4. RESULTS AND DISSCUSIONS

For the determination of CXO, through the development of a kinetic method, $KMnO_4$ was using as the oxidant. Taking into account the redox properties of $KMnO_4$, it chose to work in the alkaline pH range because the $KMnO_4$ reduction produces K_2MnO_4, which has a bluish green color, this reaction is mono-electronic and has moderate oxidizing character, these factors favor the development of analytical kinetic methods. In this context and in order to study the stability of the drug, their spectra were evaluated in a range of pH from 12.0 to 13.5 and after 2 h stirring, finding a defined band at 310 nm. The spectrum of oxidant presented the absorption bands in the following ranges: 400 to 470 nm and 570 to 650, the latter shown defined and with a maximum centered at 610 nm, these bands grow the same time with the pH. However, values of pH > 13.5, produce displacement and increase of bands at 430 and 610 nm over than expected. This can attributable to absorption of K_2MnO_4. This phenomenon is favored at these pH values, because of self-destruction of $KMnO_4$, increasing the concentration of K_2MnO_4 formed through the mechanism proposed by Jezowska [23]. In this context, the development of a kinetic method is restricted to the pH range, 12.0 to 13.3, which was studied. In first instance, pH 13.2 was selected and the reaction time was studied between 0 and 120 min (Figure 2). The equilibrium is not reached even in the maximum reaction time studied, this result does not provide adequate conditions for analytical purposes, for this reason 30 min was selected as reaction time for the following studies. Spectrograms of the reaction between CXO and $KMnO_4$ (pH 13.2), in function of the reaction time and the concentration of Na_2SO_4, individually, showed changes in the spectral signals of $KMnO_4$, demonstrating that the analyte oxidation is performed by $KMnO_4$ action. From the temperature (T°) effect studies at 20°C and 80°C, it was established that the appropriate value of T° for monitoring the reaction was 20°C (room T°), because the higher T° can produce the precipitation of MnO_2. The effect of stirring also was studied, it found that increases the course of the oxidation of CXO with $KMnO_4$. Therefore, the stirring was chosen for all work. The light favors the autodecomposition of the $KMnO_4$, for this reason amber calibrated flasks were used.

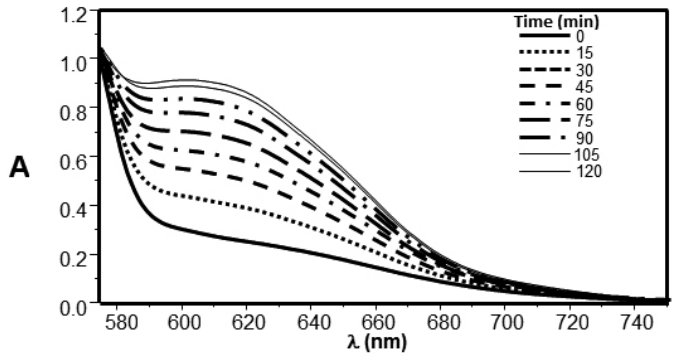

Figure 2. Absorption spectra of the following CXO-$KMnO_4$-system. Experimental conditions, CXO $1.0x10^{-5}$ mol L^{-1}, $KMnO_4$ $1.0x10^{-3}$ mol L^{-1}, pH 13.2, room T° and reaction time between 0 and 120 min.

A factorial "screening" study was designed to determine the principal experimental factors in the oxidation of CXO with $KMnO_4$. The factors considered were: reaction time, oxidant concentration, pH, and Na_2SO_4 concentration (ionic strength). According to the respective procedure, these factors were evaluated by an experimental design of 2^4 with six centers and one replica, monitoring at 610 nm (Figure 3). All variables were statistically significant ($p < 0.05$), but the Na_2SO_4 concentration present greater effect on the reaction. The influence of the factors set out as significant, do not fit a normal distribution (Figure 3). Therefore, according to the "screening" factorial analysis, all factors should be considered in the optimization of the reaction by the univariate method.

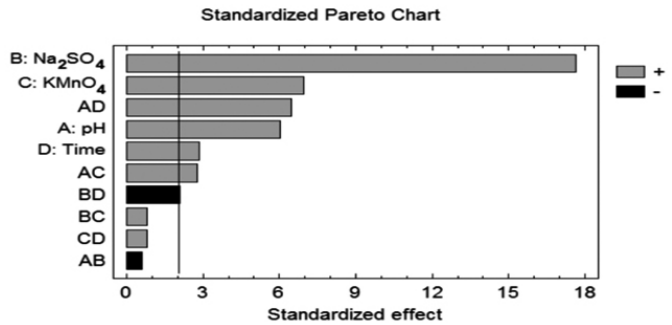

Figure 3. Selection of factors in the oxidation of CXO with alkaline $KMnO_4$. Standardized Pareto chart.

4.1. Optimization of Variables

According to the results of preliminary and factorial "screening" studies the experimental parameters were carefully studied and optimized. These include, reaction time, oxidant concentration, pH, and Na_2SO_4 concentration (ionic strength). Such factors were changed individually while the others were kept constant, and the constant values used were, 30 min, $8.0x10^{-4}$ molL^{-1}, 13.2 and 0.6 molL^{-1}, respectively. The reaction monitoring was carried out at 610 nm using CXO $1.0x10^{-5}$ molL^{-1} and room temperature.

Time effect. Using the experimental conditions detailed above, this study was carried out between 0 and 120 min and in this interval the absorbance at 610 nm increased, indicating that the equilibrium is not reached even in the maximum reaction time, this result does not provide adequate conditions for analytical purposes, however t = 30 min represents a convenient value, because the corresponding absorbance values are significant and coherent, being proportional to the analyte concentration changes. Therefore, 30 min was selected for further studies.

pH effect. The influence of pH in alkaline range on the reaction was studied between 12.0 and 13.5. It was found that increasing of the pH value

resulted in a corresponding increase in the absorbance of the reaction product. It was observed that the most significant variation begins at pH = 13.0. Thus, 13.2 was established as the most suitable pH value for this study (Figure 4).

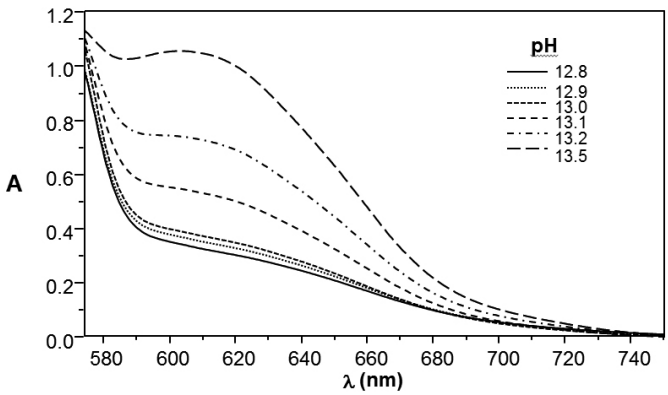

Figure 4. Absorption spectra of pH effect on the CXO-$KMnO_4$-system. Experimental conditions: The pH values were maintained with adequate volume of NaOH solutions, CXO $1x10^{-5}$ mol L^{-1}, $KMnO_4$ $8.0x10^{-4}$ mol L^{-1}, room T°, Na_2SO_4 0.6 mol L^{-1}, 30 min of reaction time at 610 nm.

$KMnO_4$ concentration effect. This effect was studied over the range of $5.0x10^{-4}$ to$10.0x10^{-4}$ mol L^{-1} of $KMnO_4$. It was found that, increasing of oxidant concentration causes a gradual increase without reaching a constant value of absorbance between 590 and 650 nm. Because, the concentration $8.0x10^{-4}$ mol L^{-1} presented greater separation of signals and less deviation between the data, was selected as the oxidant concentration for this work.

Na_2SO_4 concentration effect (ionic strength). For investigating the influence of this parameter on reaction performance, several experiments were performed by adding or not of Na_2SO_4 solution using the following concentration range of 0.1 to 0.7 mol L^{-1}. The results show that the salt addition had significant impact in the spectral signal. An increase in absorbance of the spectral signals was observed for Na_2SO_4 concentrations from 0.5 to 0.7 mol L^{-1}. Only this study was carried out with 3 concentrations values of CXO ($7x10^{-6}$, $1x10^{-5}$ and $4x10^{-5}$ mol L^{-1}) because the experimental answer was unclear when the use only one value. The better linearity and low spread between absorbance data corresponding to 0.6 mol L^{-1}, this value was selected.

4.2. Stoichiometry Determination

The limit logarithmic method [24] was used for study of the reaction stoichiometry with the experimental conditions selected above and monitoring the reaction at 610 nm. The plots corresponding to logA v/s log[$KMnO_4$] at a constant value of [CXO] and logA v/s log[CXO] at constant value of [$KMnO_4$], gave straight lines with slopes 1.194 and 0.681, respectively. Thus, the molar ratio of the reaction is (1.194:0.681) = (2:1). Taking into account the stoichiometric ratio and the chemical behavior of both reactants in alkaline medium. Is possible infer that the reaction proceeds in two steps, where the second is the rate-determining step. This process seems produce the formation of carboxylic acid from the alcoholic group of the analyte.

4.3. Kinetic of the reaction

Using the optimal physicochemical values and pseudo-first order conditions where $KMnO_4$ used was at least 10 fold over the CXO concentration, the absorbance-time signals were monitoring at 610 nm, for different CXO solutions in the range $7.0x10^{-6}$ to $7.0x10^{-5}$ mol L^{-1}. Therefore, the kinetics equation for reaction can correspond to: $\mathbf{v = k'[CXO]^n}$, where **k`** is the pseudo-rate constant and **n** is the reaction order. The logarithmic form of this equation is: $\mathbf{\log v = \log k' + n \log[CXO]}$. The plot of the log v v/s log[CXO], allows evaluate the reaction order, the equation obtained was, log v = 0.7038 log[CXO] – 0.7274; R^2 = 0.9973. Then, k'= 5.34 s^{-1} and the reaction is pseudo-first order (n = 0.704 ~1) with respect to CXO.

4.4. Evaluation of the kinetic methods

The quantification of CXO was carried out according to the pseudo-first order rate equation: v = 5.34 s^{-1} $[CXO]^{0.704}$. Many assays were carried out to determine of the CXO concentration using the rate data (rate law). Initial rate, constant rate and fixed time methods [25,26] were tried and the selection of the kinetic method is based in the applicability, sensitivity, intercept and R^2.

Initial rate. The initial rate kinetic method was evaluated by plotting the rate (at the beginning of the reaction at time 250 s) v/s [CXO]. As it mentioned above the first step of the reaction was not rate determining (fast) and the tangents to the curves at previously defined value of time are not easy to draw. Eq. 1 shows the results obtained, these correspond to behavior of pseudo-zero order, and the value of R^2 indicates a low linearity, this method was discarded.

$$\mathbf{V_0 = 17.6C + 1.10x10^{-4}\ (R^2 = 0.9641)} \qquad \textbf{Eq. 1}$$

Rate constant. Graphs of logA v/s time (0-1800s) were plotted, for CXO concentration ([CXO]) in the range of $1.0x10^{-6}$ to $13x10^{-6}$ molL^{-1} and presented linear behavior. To each of CXO concentrations the pseudo-first order rate constants (K) were determined, multiplying the respective slopes by -2.303. The equation obtained with the linear regression of K v/s [CXO] was:

$$\mathbf{K = 8.57C - 6.98x10^{-4}\ (R^2 = 0.9853).} \qquad \textbf{Eq.2}$$

From the linear regression, an unsatisfactory R^2 value was obtained. The inconsistency in the rate values was due to that the reaction does not reach equilibrium in a short reaction time, so the dynamic range used influences obtaining data by this method, for this reason was discarded.

Fixed time. In this method, the absorbance (A) of the reactions corresponding to different CXO concentration ($1.0x10^{-6}$ to $13x10^{-6}$ mol L^{-1}) were measured at fixed times of 5, 10, 15, 20, 25 and 30 min. Calibration plots of A v/s [CXO] at each fixed time value were realized. For each fixed time value, the linear regression equations, the coefficients of determination and respective statistical parameters were obtained, such as variance, standard deviation, confidence limits. The slopes and intercepts are increased with time. Moreover, a t-student was performed considering a two-tailed t-test and n-2 degrees of freedom as a function of r. The values obtained are shown in Table 1. Considering a statistical null hypothesis H_0, of no correlation between the CXO concentration and absorbance, all values of t-student obtained proved to be greater than the t_{crit} ($t_{exp} > t_{crit}$) therefore the null hypothesis is rejected, concluding that there is linear correlation for each fixed time method. The results showed that the slopes and intercepts increase with time. The fixed time 30 min has: high slope value, lower variance implying lowest dispersion expected values in distribution, therefore based on the parameters studied 30 min was selected as the most suitable time for measurements and to develop the analytical method and its subsequent application in pharmaceutical formulations.

4.5. Calibration curve and analytical parameters using CXO standards solutions

After performing the optimization of experimental conditions, the fixed time (30 min) method was applied to the CXO determination. Linear regression analysis of the calibration data and measurements of 11 independent blanks (samples without analyte) were carried out, in order to obtain the detection and quantitation limits, LOD and LOQ, respectively (Table 2).

Using the proposed method in the range studied of drug concentrations and under experimental conditions optimized, was possible to obtain good sensitivity and lows LOD and LOQ, which would allow of the CXO determination in pharmaceutical samples. With three different concentrations of CXO (0.27, 1.88 and 3.49 mg mL^{-1}) the accuracy was evaluated. For the accuracy of the assays "intra-day" and "inter-day", were carried out for five consecutive days (Table 3). On the other hand the precision of the procedure was expressed as the relative standard deviation (RSD) and considering the same concentration levels were also obtained (Table 3).

Table 1. Calibration curves and statistical parameters for different fixed times at room T° and monitoring at 610nm.

	Fixed time [min]					
Parameters	**5**	**10**	**15**	**20**	**25**	**30**
Concentration Range [µg mL^{-1}]	0.27 - 3.49	0.27 - 3.49	0.27 - 3.49	0.27 - 3.49	0.27 - 3.49	0.27 - 3.49
Linear Regression	A=0.0245C +0.0212	A=0.0327C +0.0532	A=0.0348C+ 0.0657	A=0.0415C + 0.0559	A=0.0522C +0.0315	A=0.0606C +0.0583
S_n	4.45 x10^{-3}	3.80 x10^{-3}	3.38 x10^{-3}	3.04 x10^{-3}	2.67 x10^{-3}	1.89 x10^{-3}
$\pm tS_n$~	1.42 x10^{-2}	1.21 x10^{-2}	1.08 x10^{-2}	9.67 x10^{-3}	8.49 x10^{-3}	6.02 x10^{-3}
S_m	2.03 x10^{-3}	1.73x10^{-3}	1.54 x10^{-3}	1.38 x10^{-3}	1.22 x10^{-3}	8.61 x10^{-4}
$\pm tS_m$~	6.45 x10^{-3}	5.51 x10^{-3}	4.89 x10^{-3}	4.40 x10^{-3}	3.88 x10^{-3}	2.74 x10^{-3}
R^2	0.9732	0.9889	0.9922	0.9956	0.9978	0.9992
Variance (S_o^2) [µg mL^{-1}]^2	2.66 x10^{-5}	1.94 x10^{-5}	1.53 x10^{-5}	1.24 x10^{-5}	9.61 x10^{-6}	4.80 x10^{-6}
S_o^* [µg mL^{-1}]	5.16 x10^{-3}	4.41 x10^{-3}	3.91 x10^{-3}	3.52 x10^{-3}	3.10 x10^{-3}	2.19 x10^{-3}
t_{exp}	10.4	16.3	19.5	26.1	36.9	61.2

The value of student "t" for n-2 degrees of freedom with a confidence level of 95% with n = 5 (t_{crit} = 3.182).
~tS_n = confidence limit for the intercept; ~tS_m = confidence limit for the slope. * So is the standard deviation of the curve.

Table 2. Analytical parameters for the CXO determination.

Parameters	**Values**
Linear Regression	A = 6.06 x10^{-2} C+ 5.83 x10^{-2}
R^2	0.9992
LOD *(µgmL^{-1})	0.07
LOQ *(µgmL^{-1})	0.23
Concentration Range (µg mL^{-1})	0.23 – 10.7
σ (Blanks)	1.42 x10^{-3}

*** LOD: 3σ/S ; LOQ: 10σ/S**

Table 3. Results of the evaluation of precision and accuracy, by studies "intra-day" and "inter-day", using CXO standards solutions.

CXO (µg mL^{-1})		**"Intra-day"**				
Added	**Found**	**σ**	**Recovery (%)**	**RSD (%)**	**ES***	**Confidence Limit†**
0.27	0.27	8.13x10^{-3}	101.3	3.0	4.06x10^{-3}	1.29x10^{-2}
1.88	1.87	4.76x10^{-2}	99.6	2.5	2.38x10^{-2}	7.58x10^{-2}
3.49	3.49	3.44x10^{-2}	100.0	1.0	1.72x10^{-2}	5.47x10^{-2}
"Inter-day"						
Added	**Found**	**σ**	**Recovery (%)**	**RSD (%)**	**ES***	**Confidence Limit†**
0.27	0.26	1.35x10^{-2}	98.4	5.1	6.74x10^{-3}	2.14x10^{-2}
1.88	1.91	4.26x10^{-2}	101.9	2.2	2.13x10^{-2}	6.78x10^{-2}
3.49	3.54	5.64x10^{-2}	101.5	1.6	2.82x10^{-2}	8.97x10^{-2}

***Error standard deviation**
† Limits by 95% and 3 degrees of freedom (t = 3.182)

The recoveries were between 98 and 102%, and show a satisfactory accuracy for kinetic-analytical methods evaluated. The precision for the different concentrations studied was RSD < 5.0% (0.27 except for the "inter-day" precision), these results indicated a satisfactory repeatability and intermediate precision, for the proposed quantitation.

4.6. Validation

To validate the proposed method (fixed time kinetic method at 30 min), a synthetic sample of cream was prepared. For obtain standard deviation of the blanks, a portion of sample was used. Others portions were spiked with different analyte concentrations in order to obtain the calibration curves considering excipients and active ingredient. Then the assays "intra-day" and "inter-day" of a group of 30 blanks were realized, the measurements were carried out for five consecutive days, with these results the standard deviation was determined (Table 4). The analytical parameters were obtained through the respective linear regression and the measurements of the above blanks of the pharmaceutical formulation (only excipients), the results obtained are shown in Table 4. In order to assess the accuracy of the method, the recoveries were obtained using different drug concentrations (1.07, 2.68 y 3.49 ug mL^{-1}). The precision of the method was evaluated by the relative standard deviation, considering the same analyte concentration levels and the respective blanks, for the accuracy of the assays "intra-day" and "inter-day", (Measured for five consecutive days). The results obtained of the analysis to assess the precision and accuracy of the method, "intra-day" and "inter-day" in synthetic samples

are shown in Table 5. The maximum allowed for commercial use in cream CXO is 1%, for commercial pharmaceutical products the excipients commonly used are: cetyl alcohol, monostearate of glycerol, liquid petrolatum and benzoic acid (preservative). The acceptable ranges used for each of these compounds are: 2-30%, 1-10%, without requirement and 0.1 - 0.2% (w/w), respectively [27]. The interfering studies were realized in the range of maximum and minimum allowed, finding that only benzoic acid (AB) interfere in proportion, AB: CXO = 1: 2. The amounts of benzoic acid utilized in the synthetic samples (0.33%) were above the recommended value in cream, but the results prove the suitability of proposed method to be applied in pharmaceutical formulations with other benzoic acid proportions.

Table 4. Analytical parameters for the CXO determination.

Parameters	CXO
Linear Regression	$A=9.50 \times 10^{-2} C+2.88 \times 10^{-2}$
R^2	0.9957
LOD *(μgmL^{-1})	0.095
LOQ *(μgmL^{-1})	0.32
Concentration Range (μg mL^{-1})	0.32 – 10.0
σ (Blanks)	3.0×10^{-3}

*** LOD: 3σ/S ; LOQ: 10σ/S**

Table 5. Analysis to assess the precision and accuracy of the method, "intra-day" and "inter-day" in synthetic samples.

CXO (μgmL^{-1})		"Intra-day"			
Added	**Found**	**Recovery (%)**	**RSD (%)**	**ES***	**Confidence Limit†**
1.07	1.08	100.6	3.70	1.97×10^{-2}	6.27×10^{-2}
2.68	2.69	100.5	0.59	7.90×10^{-3}	2.51×10^{-2}
3.49	3.45	98.9	1.07	1.84×10^{-2}	5.86×10^{-2}
"Inter-day"					
Added	**Found**	**Recovery (%)**	**RSD (%)**	**ES***	**Confidence Limit†**
1.07	1.06	99.8	4.4	2.37×10^{-2}	7.54×10^{-2}
2.68	2.71	100.9	2.5	3.37×10^{-2}	0.107
3.49	3.45	98.8	2.6	4.41×10^{-2}	0.140

***Error standard deviation**
† Limits by 95% and 3 degrees of freedom (t = 3,182)

The differences in RSD values, between Tables 3 and 5 is inherent to the extraction process, which could affect, since all complex matrix can modify the final concentration of the analyte, for that reason the calibration curves must be carried in the matrix used, which also ensures adequate robustness to obtain the method developed.

4.7. Application of the proposed method

The developed kinetic spectrophotometric method for the CXO determination in commercial pharmaceutical formulation was applied (cream at 1% CXO). In all cases, the samples were prepared and analyzed according to respective procedure. The drug concentrations using the linear regression equation obtained in synthetic samples were calculated. For real samples an average value of 0.993 g of CXO, with RSD = 3.0, were found. These results prove that the common excipients do not interfere in the CXO determination, evidencing a high selectivity, although the method used the oxidation reaction with $KMnO_4$, which is not selective. The values of recoveries support the suitability of the method for the CXO analysis in the proposed sample.

5. CONCLUSIONS

According to the optimization of kinetic of the oxidation reaction of CXO-$KMnO_4$ system by factorial screening and univariate method a pH = 13.2, 8×10^{-4} mol L^{-1} $KMnO_4$, 0.6 mol L^{-1} Na_2SO_4, were found for 30 min of stirring and room T°. The molar relation CXO:$KMnO_4$ was 1:2. The concentration of CXO was studied kinetically using different approaches: the reaction rate, initial rate, constant rate and fixed time methods. This last method at 30 min prove to be the most suitable, which was confirmed by satisfactory values of the correlation coefficients (R^2) and slopes obtained of calibration plots and can be easily applied in the determination of investigated fungicide in pure and pharmaceutical forms (cream), obtaining the results with RSD < 5 and < 4, respectively. Furthermore provide the improving on selectivity, avoiding interference of colored or turbidity background of samples because it measures the increase in absorbance with time against the blank treated similarly. The proposed method is simple, sensitive and the results prove the suitability of method to be applied in pharmaceutical formulations with other benzoic acid proportions. Also do not require an elaborate extraction of the chromophore produced, expensive instruments and /or critical analytical reagents. This supports its use in routine quality control of drugs in pharmaceutical and industrial laboratories.

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