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Research Article

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Determination of Clotrimazole in Creams by Derivative Spectrophotometry

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Abstract Clotrimazole is an antifungal agent widely used in pharmaceutical formulations. Quality control of pharmaceutical products requires accurate, rapid, and selective analytical procedures. In this work, a derivative spectrophotometric method for clotrimazole determination using the first derivative of the absorption spectra was developed. The analytical curve was linear at concentrations between 1.18×10^{-4} and 5.90×10^{-4} mol L^{-1}. The limit of detection and the limit of quantification were 2.0×10^{-5} mol L^{-1} ($3 \times s_{y/x}$) and 6.4×10^{-5} mol L^{-1} ($10 \times s_{y/x}$), respectively. The proposed clotrimazole determination method does not include a liquid extraction step and requires simple sample preparation. Relative standard deviations values lower than 1.5% were obtained for standard clotrimazole solutions. The derivative spectrophotometric method showed no matrix interference in the analysis of the studied commercial cream samples, and results in acceptable recovery values (95–105%).

Keywords derivative spectrophotometry; pharmaceutical analysis; pharmaceutical product; validation

Introduction

Clotrimazole (CTZ) is an imidazole derivative with a broad antifungal spectrum, and its main degradation products are imidazole and (2-chlorophenyl)diphenylmethanol [1]. The development of accurate and reproducible quality control procedures for pharmaceuticals is of great interest.

Several analytical procedures have been proposed for CTZ determination in pharmaceutical preparations, such as HPLC and TLC densitometric methods [2], HPLC-UV [1], micellar electrokinetic chromatography and HPLC [3], differential pulse polarography [4], spectrofluorimetry [5, 6], visible spectrophotometry [5, 7, 8], derivative spectrophotometry [9], and spectrophotometric-assisted chemometric techniques [10]. However, in these procedures, toxic organic solvents such as acetonitrile [5, 7], chloroform [6], tetrachloromethane [8], dichloromethane [7, 8], methanol [4, 5, 10], and supercritical fluid extraction [9] are usually used.

Simpler and less time-consuming laboratory procedures for the quality control of pharmaceuticals are highly desirable. In this work, a direct spectrophotometric procedure for the determination of CTZ is proposed based on the dissolution of pharmaceutical formulations (in particular, creams) without a complex sample preparation step. A severe interference of the sample matrix was observed, which was reduced by using a derivative spectrophotometric method.

Hence, a first-order derivative spectrophotometric procedure for the determination of CTZ in cream formulations without liquid extraction and using user-friendly solvents was developed.

Materials and Methods

All reagents were of analytical grade, and all solutions were prepared with water purified by a Millipore Milli-Q system model UV plus (Bedford, MA, USA). Ethanol was purchased from VETEC (Rio de Janeiro, Brasil).

A 2.95×10^{-3} mol L^{-1} CTZ stock solution was prepared by dissolving 1.0 g of CTZ (Sigma) in pure ethanol in a 100-mL volumetric flask. Reference solutions ranging from 1.18×10^{-4} to 5.90×10^{-4} mol L^{-1}were prepared by dilution of the stock solution with pure ethanol in a 50-mL volumetric flask.

Pharmaceutical formulations containing CTZ were purchased from local drugstores and consisted of creams containing 0.10 g of CTZ per 100 g of cream.

A Cary Varian (model 50 CONC) UV-Vis spectrophotometer was used, and the spectra were recorded at room temperature using 1-cm path length quartz cells in the 240–300 nm range.

The first-order derivative of the spectra of the CTZ solutions were obtained using Savitzky-Golay smoothing with $\Delta\lambda = 8$ nm.

The CTZ concentration in the sample solutions was determined from the equation of the regression curve of the absorbance vs the CTZ concentration in solution, obtained using the least squares method.

General spectrophotometric procedure

Aliquots of CTZ standard or sample solutions were transferred into 25-mL volumetric flasks, and the solutions were brought to volume with ethanol. The CTZ concentrations ranged from 1.18×10^{-4} to 5.90×10^{-4} mol L^{-1}. The absorption spectra were recorded in the range from 240 to 300 nm using pure ethanol as a blank. The analytical curve was obtained by plotting the absorbance at 260 nm vs the CTZ concentration.

General derivative spectrophotometric procedure

Aliquots of CTZ standard or sample solutions were transferred into 25-mL volumetric flasks as described in the previous section. The CTZ concentrations ranged from 1.18×10^{-4} to 5.90×10^{-4} mol L^{-1}. The absorption spectra were recorded in the range from 240 to 300 nm using pure ethanol as a blank. The analytical curve was obtained using the first-order derivative (1D) absorption spectra of each solution at $\lambda = 272$ nm, obtained in triplicate.

Sample preparation

A 1.0 g sample was transferred into a 100-mL volumetric flask, and the solution was brought to volume with ethanol. An aliquot of solution was transferred to 10-mL volumetric flask to provide the sample solutions with concentrations ranging from 1.18×10^{-4} to 5.90×10^{-4} mol L^{-1}.

Results & Discussion

The spectrum of Clotrimazole (CTZ) is characterized by a strong absorption band between 200 and 250 nm, and a shoulder at 260 nm. Because of the close vicinity of these bands, sample matrix interference can occur due to substances (excipients) that absorb in this range.

The determination of CTZ in cream formulations was attempted by direct UV analysis. However, for most samples, pronounced interference from other excipients was observed.

In our investigation of a direct method for CTZ determination based on the intrinsic UV absorption of CTZ (at λ = 260 nm), strong interferences were observed in the spectrophotometric analysis of commercial samples, and the recovery values ranged from 91 to 98%. Thus, to improve the accuracy in CTZ determination in pharmaceutical samples, we envisioned the use of a first-order derivative spectrophotometric method.

The zero-order spectra (0D) of CTZ standard solutions in ethanol were transformed into first-order derivative (1D) spectra, with $\Delta\lambda$ ranging from 1 to 10 nm. The first-order derivative spectrum of CTZ could be obtained with $\Delta\lambda = 8$ nm. The absorption spectra and derivative spectra of CTZ standard solutions are shown in Figure 1.

A linear regression was observed between 1D and a CTZ concentration ranging between 1.18×10^{-4} and 5.90×10^{-4} mol L^{-1}, which can be described by the following equation: $1D = 1.783 \times 10^{-4} (\pm 1.671 \times 10^{-4}) - 50.78 (\pm 0.56) \times [CTZ]$; r =-0.999, where 1D is the first derivative at 272 nm, and [CTZ] is the concentration of CTZ in mol L^{-1}.

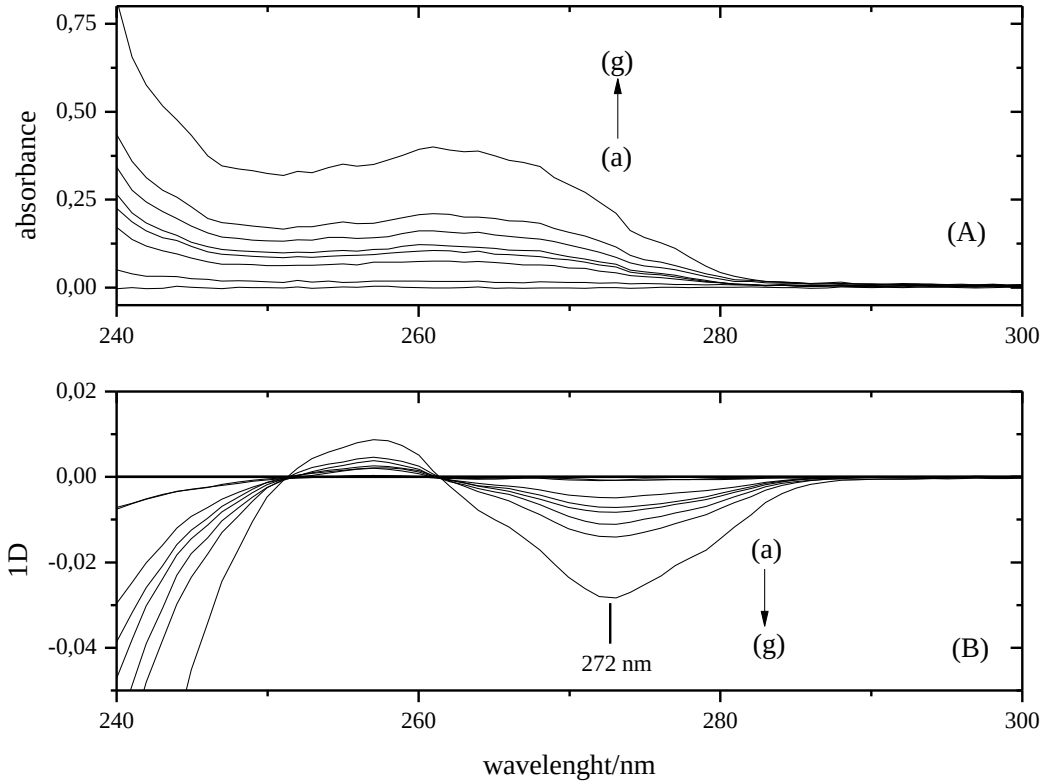

Figure 1: (a) Absorption spectra of clotrimazole standard solutions in ethanol. (b) First-order derivative spectra (1D) of clotrimazole standard solutions (Δλ = 8 nm). Clotrimazole concentrations: (a) blank, (b) 1.18×10^{-4}, (c) 1.48×10^{-4}, (d) 1.77×10^{-4}, (e) 2.36×10^{-4}, (f) 2.95×10^{-4}, (g) 5.90×10^{-4} mol L^{-1}.

The analytical curve and the residual plot are presented in Figure 2. The statistical significance of the regression was evaluated from the $MS_{regression}/MS_{residuals}$. The $F_{calculated}$ value of 8,592 showed that the regression was statistically significant, with ($F_{1,12,\ 95\%}$) = 4.75. The standard deviation of the analytical curve ($s_{y/x}$) was calculated to be 3.0×10^{-4} [11].

As can be seen from the residual plot of the linear regression of the curve in Figure 2b, the residuals were randomly scattered around a horizontal central line, showing no deviation from normality and no outliers [12]. The obtained residuals are less than 2 times the standard deviation of the analytical curve ($2 \times s_{y/x}$) [13].

The lack-of-fit test ($F_{calculated}$ = 13.629) reveals that the regression adequately fits the analytical curve at a significant confidence level of 99%. The normality of the obtained analytical signals (1D) was verified by the Shapiro-Wilk test, which gave a *p*-value of 0.1971.A limit of detection of 2.0×10^{-5} mol L^{-1} ($3 \times s_{y/x}$) and a limit of quantification of 6.4×10^{-5} mol L^{-1} ($10 \times s_{y/x}$) were obtained [11].

The precision of the proposed procedure was evaluated from the relative standard deviation (RSD) of CTZ standard solutions at concentrations of 1.18×10^{-4}, 2.95×10^{-4}, and 5.90×10^{-4} mol L^{-1}. RSD values lower than 1.5% were obtained, indicating the precision of the procedure.

The RSD of the sensitivity of the analytical curves obtained in five different days was 3.5%, demonstrating that the procedure is robust.

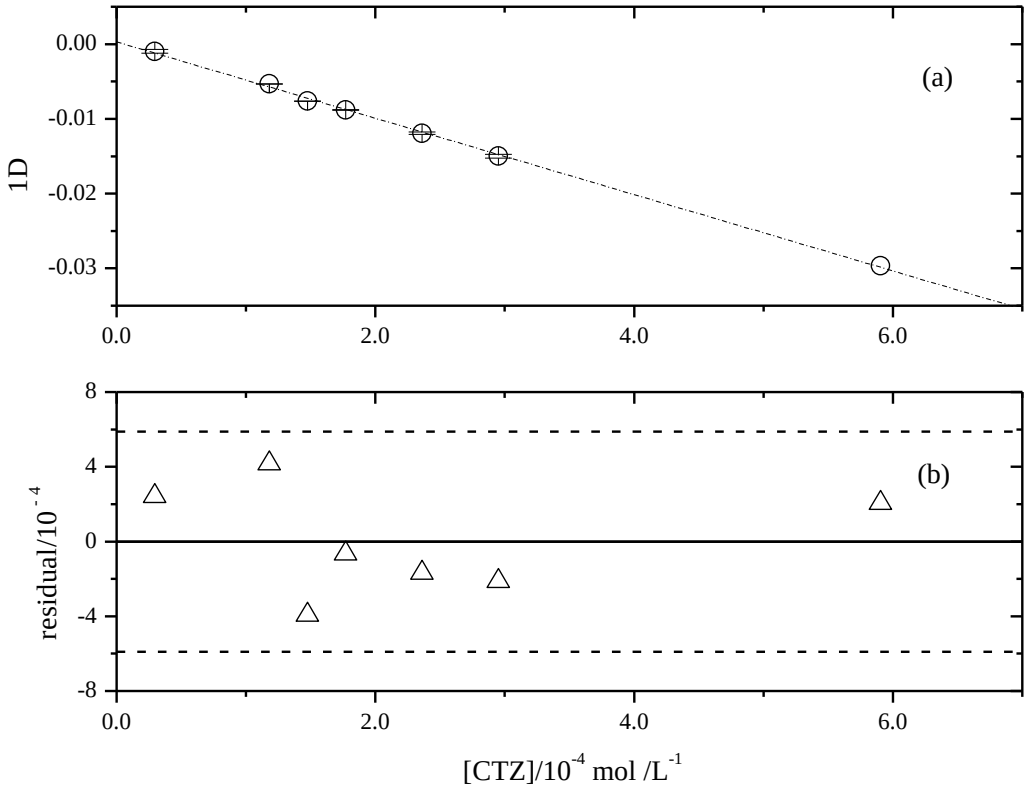

Figure 2: (a) Analytical curve for clotrimazole determination; (b) Residual plot obtained from the regression of the analytical curve. The dashed lines correspond to 2 × $s_{y/x}$.

The recovery of CTZ in cream samples was evaluated determining the CTZ concentration added to samples by zero-order and first-derivative (1D) spectrophotometric methods. The average recovery was tested for significance by using the Student's *t*-test, the null hypothesis being that the recovery was 100% (no matrix effect) [13]. As shown in Table 1, the recoveries obtained by the derivative spectrophotometric method ranged from 94.8 to 105%, and were better than those obtained using the zero-order spectrophotometric procedure, which ranged from 91.8 to 98.1%. The derivative method gave $t_{calculated}$ values lower than $t_{critical}$ at 95% significance level, accepting the null hypothesis and indicating the accuracy of the procedure. The same conclusion was reached considering analyte concentrations (>0.1%) that give a recovery in the range of 95–105%. A matrix effect was not observed in the determination of CTZ in cream formulations by the derivative spectrophotometric method.

Table 1: Recovery of clotrimazole in cream samples obtained by direct (0D) and first-derivative (1D) spectrophotometric methods

Samples	**Found[a] / mol L^{-1}**	**HORRAT[b]**	**Recovery 0D/%**	**Recovery 1D/ %**	***p* (0.05)[c]**
01	$1.52 \times 10^{-4} \pm 1.16 \times 10^{-5}$	1.72	97.5	104	
	$2.82 \times 10^{-4} \pm 3.58 \times 10^{-6}$	0.31	91.8	95.2	0.441
	$4.64 \times 10^{-4} \pm 1.52 \times 10^{-5}$	0.87	97.9	105	
02	$1.53 \times 10^{-4} \pm 1.16 \times 10^{-5}$	1.86	94.7	104	
	$2.80 \times 10^{-4} \pm 3.92 \times 10^{-6}$	0.75	94.5	94.8	0.477
	$4.53 \times 10^{-4} \pm 1.52 \times 10^{-5}$	0.65	98.1	102	
03	$1.33 \times 10^{-4} \pm 1.18 \times 10^{-5}$	2.00	91.7	90.2	

	$2.98 \times 10^{-4} \pm 1.86 \times 10^{-6}$	1.56	93.4	101	0.349
	$4.58 \times 10^{-4} \pm 2.45 \times 10^{-5}$	1.42	97.4	103	
04	$1.48 \times 10^{-4} \pm 8.00 \times 10^{-6}$	1.31	100	100	
	$2.96 \times 10^{-4} \pm 1.03 \times 10^{-5}$	0.87	100	100	0.250
	$4.47 \times 10^{-4} \pm 1.05 \times 10^{-5}$	0.63	101	101	

[a] n = 3; mean ± standard deviation. [CTZ] added: 1.47×10^{-4}, 2.95×10^{-4}, and 4.42×10^{-4} mol L^{-1}.
[b] HORRAT = $RSD_{derivative}/RSD_{HORRAT}$; $RSD_{HORRAT} = 2^{(1 - 0.5 \times \log[CTZ])}$.
[c] values found comparing recovery obtained with derivative procedure (1D). $\mathbf{H_0}$: recovery = 100%.

The precision of CTZ determination was evaluated comparing the $RSD_{procedure}$ obtained in the recovery analysis with the values calculated by the Horwitz equation [14]. The HORRAT values were ≤2, suggesting that the method showed satisfactory reproducibility values.

Conclusion

A simple,fast, cost-effective, and robust first-derivative spectrophotometric method was developed. This procedure did not include a liquid extraction step nor the use of toxic solvents for sample preparation. The method showed no matrix interference in the analysis of the studied commercial samples, and the use of ethanol as a solvent makes the procedure user-friendly.

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