ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 201–208

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Differential pulse polarographic determination of clotrimazole after derivatization with Procion Red HE-3B

F.C. Pereira [a], M.V.B. Zanoni [a,*], C.C.I. Guaratini [a], A.G. Fogg [b]

[a] *Departamento de Química Analítica, Instituto de Química-Universidade Estadual Paulista, Caixa Postal 355, 14800-900, Araraquara SP, Brazil*

[b] *Sensors and Cryobiology Research Group, The Research Centre, University of Luton, 2 Adelaide Street, Luton LU1 5DU, UK*

Received 21 November 2000; received in revised form 5 June 2001; accepted 3 July 2001

Abstract

Clotrimazole was shown to react at room temperature in Britton Robinson buffer pH 2 with the reactive dye Procion Red HE-3B. The product exhibited a differential pulse polarographic peak at −0.38 V, which was well separated from the peaks of the reactive dye at −0.08, −0.80 and −0.95 V, and this allowed the indirect determination of clotrimazole in the presence of excess of the reactive dye. The method has been applied satisfactorily to the determination of clotrimazole in pharmaceutical formulations, calibration graphs are rectilinear up to at least 40 μg ml^{-1}. The detection limit was calculated to be 2.6 μg ml^{-1} (3 σ).

Keywords: Clotrimazole; Differential pulse polarography; Procion Red HE-3B dye

1. Introduction

Clotrimazole 1-{[(2-chlorophenil) diphenil methil]-1 H-imidazole}, is a broad spectrum antimycotic agent which is effective against dermal infections and combats vulvovaginal candidiasis [1,2]. Many analytical methods for the determination of clotrimazole in pharmaceutical preparations and biological fluids have been published [2–11]. Titrimetric methods involving titrants such as perchloric acid [2], picric acid [3,4], and sodium lauryl sulphate [2,5] used to require high clotrimazole concentration. Spectrophotometric analysis [6] has been limited due to its low molar absorptivity and some spectrophotometric methods have been based on the ion-pair complex reaction [7,8] or acid hydrolysis [9] requiring extraction procedures, time consuming and low sensitivity. Chromatographic techniques like thin-layer chromatography [1], gas chromatography [10] and high performance liquid chromatography [10–14] have been reported successfully and need sophisticated equipment or long experimental procedures for the sample clean-up.

A particular advantage of voltammetric analytical methods when applied to pharmaceutical formulations is that excipients often do not interfere to the same extent as they do in the cases of spectrophotometric and chromatographic meth-

* Corresponding author. Fax: +55-16-222-7932.
E-mail address: boldrinv@iq.unesp.br (M.V.B. Zanoni).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00526-X

ods [15]. The only application of polarography to the quantitative determination of clotrimazole uses the ill-defined reduction wave obtained in acidic solution, which occurs near the potential cut off of the electrolyte [16].

Recent studies in these laboratories [17–21] have included polarographic and stripping voltammetric studies of reactive dyes that can be analysed voltammetrically by reduction of the chromophore (azo or anthraquinone groups) and their reactive groups (chlorotriazine or vinylsulphone moieties) present in the dye molecule. The present communication describes the use of Procion Red HE-3B (C.I. Reactive Red 120) a reactive dye bearing a bisazo group as chromophore and a bis-monochlorotriazine as chemically reactive site, in the derivatization of clotrimazole, with the aim to develop a reductive electroanalytical procedure that permits selective determination of clotrimazole in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

Voltammetric experiments were performed with a Metrohm Polarecord E 506 linked to a compatible microcomputer, through a Microquimica interface. The multimode electrode Metrohm stand 663 VA was used in both the hanging mercury electrode (HMDE) and dropping mercury electrode (DME). The three electrode system was completed by means of an Ag/AgCl (3 M KCl) reference electrode and a glassy carbon auxiliary electrode.

2.2. Reagents

Suprapur grade reagents supplied by Merck and desmineralized water from a Milli-Q system (Milli-pore) were used in the preparation of all solutions. Britton–Robinson (B–R) buffer used as supporting electrolyte was prepared by mixing apropriate amounts of 0.2 mol l^{-1} sodium hydroxide to orthophosphoric acid, acetic acid and boric acid (0.04 mol l^{-1} in each) solution.

2.3. Procedure

Clotrimazole stock solution (1×10^{-2} mol l^{-1}) were prepared from the dried pure substance (kindly supplied by Bayer S.A.) in methanol. An aliquot of the clotrimazole standard solution to be investigated was added by micropipette to 20 ml of deaerated phosphate buffer (or B–R buffer) at the appropriate pH containing 5×10^{-4} mol $l^{-1}$of RR120 dye. The differential-pulse mode was used with a pulse amplitude of 50 mV, a drop time of 0.8 s, unless stated otherwise.

Analysis of dosage forms were carried out using a commercial spray of Canesten and Dermobene 1% (nominally 10 mg ml^{-1} in clotrimazole). An aliquot of 10 ml of these formulations after evaporation of the organic solvent under a stream of nitrogen, were diluted with 10 ml of methanol. Aliquots of 200 μl of these stock solutions were transferred directly into the voltammetric cell containing 20 ml of B–R buffer pH 2.0 and 5×10^{-4} mol l^{-1} RR120 dye. The voltammetric curves were recorded as above procedure. The method was also applied in Canesten vaginal tablets (labeled to contain clotrimazole as 100 mg per tablet as dosage forms). One tablet were weighed, finely powdered and it was shaken with 10 ml of methanol. After transferred to a centrifuge tube, an aliquot of 200 μl of the supernatant solution were analysed as above discussed.

3. Results and discussion

3.1. Differential pulse polarographic behavior

The polarographic reduction of 5×10^{-4} mol l^{-1} clotrimazole in B–R buffer pH 2.0 occurs at a very negative potential (peak I, at about -1.1 V) and it is ill-defined due to be very close to the cut-off potential for the electrolyte, as shown Curve *X*, Fig. 1. Therefore, the peak is not suitable for analytical purposes.

On the other hand, the electrochemical behaviour of Procion Red HE-3B (RR120) in the same experimental conditions have shown three well defined reduction peaks (peaks *A*, *B* and *C*) as shown Curve *Y*, Fig. 1. The electrochemical

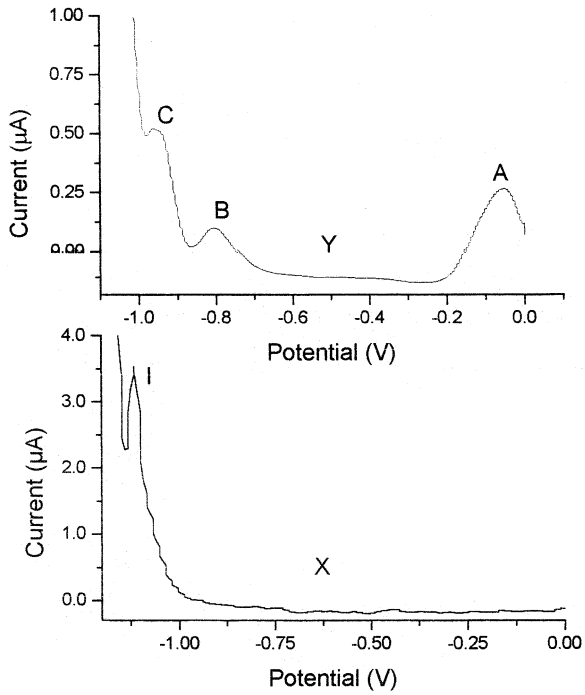

Fig. 1. Differential pulse polarograms of 5×10^{-4} mol l^{-1} clotrimazole in B–R buffer pH 2.0 (Curve *X*) and differential pulse polarograms of 5×10^{-4} mol l^{-1} RR120 dye in B–R buffer pH 2.0 (Curve *Y*).

behaviour of the RR120 dye has been investigated previously [21] and the peak at -0.08 V (peak A) is attributed to the simultaneous electrochemical reduction of the bis-azo moiety to the bis-hydrazo derivative. The pair of peaks at -0.80 and -0.95 V (peaks *B* and *C*) are due to the electrochemical reduction of the bis-monochlorotriazine groups, which involves elimination of chloride from the molecule [21]. Both peaks *A* or *B* can be used to follow the dye concentration in acidic solution from 1×10^{-6} to 1×10^{-4} mol l^{-1} with good reproducibility.

In order to investigated the application of the RR120 dye as an analytical reagent for the derivatization of clotrimazole, differential pulse polarograms of mixture of RR120 dye and clotrimazole were recorded. Differential pulse polarograms obtained after direct mixture of 5×10^{-4} mol l^{-1} of RR120 dye in B–R buffer pH 2 in the presence of variable concentration of clotrimazole from 7.5×10^{-5} to 2.5×10^{-4} mol l^{-1} are shown in the Fig. 2. There is no significant change in the potential of the differential pulse peak of the azo groups of RR120 (peak *A*) after derivatization of clotrimazole except for occurrence of a small shoulder that do not increases in function of clotrimazole concentration, but the peaks due to the reactive

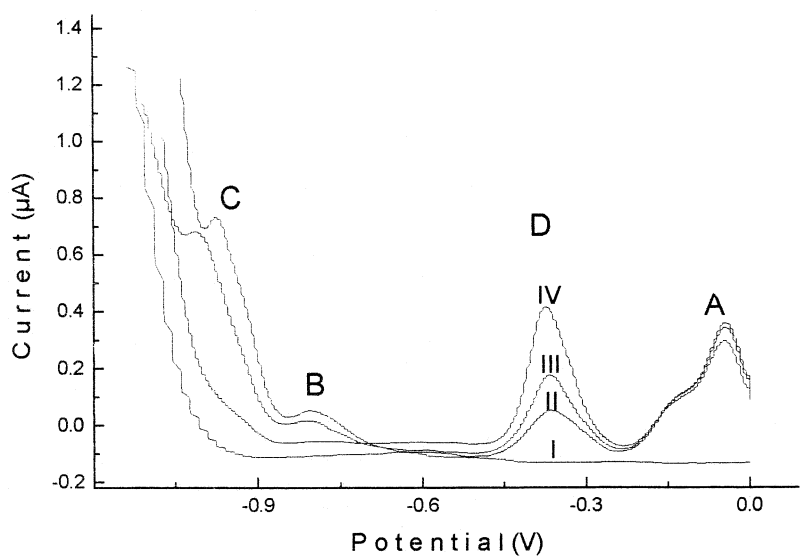

Fig. 2. Effect of clotrimazole on differential pulse polarograms of 5×10^{-4} mol l^{-1} of RR120 in B–R buffer pH 2.0. Clotrimazole concentration, II, 7.5×10^{-5} mol l^{-1}, III, 9.5×10^{-5} mol l^{-1}, and IV, 2.5×10^{-4} mol l^{-1}. I, Supporting electrolyte.

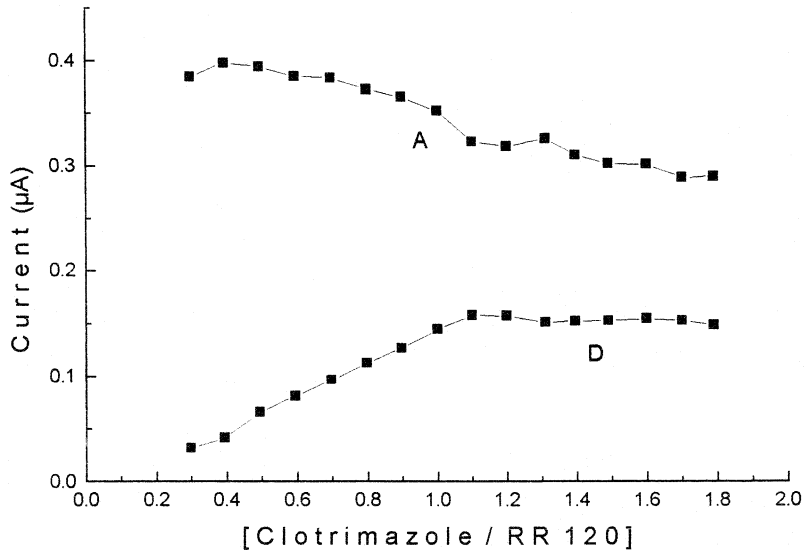

Fig. 3. The effect of the mole ratio of clotrimazole to RR120 on the current obtained. RR 120 concentration, 1×10^{-4} mol l^{-1}.

chlorotriazine groups (peaks *B* and *C*) are significantly reduced in size. In addition, a new peak at -0.38 V (peak *D*) appears on addition of clotrimazole and increases in height with further addition of the drug. The loss of the peaks owing to the reactive groups of the azo dye after derivatization of clotrimazole indicates that the derivatization involves reaction of the chlorotriazine groups of RR120 with the clotrimazole and presents great analytical potentiality.

Further information about the derivatization reaction was investigated by comparative spectrophotometric study carried out in B–R buffer pH 2.0. The UV-visible absorption spectrum of 1×10^{-5} mol l^{-1} of clotrimazole B–R buffer pH 2.0 presents characteristic absorption band at $\lambda =$ 213 nm [1]. The absorption spectra of the dye solution at pH 2 is characterised by three main bands, two in the visible range, $\lambda = 542$ and 514 nm, due to the long conjugated π system of aromatic ring connected by two azo groups and one in the UV region at $\lambda = 294$ nm, characteristic of the two adjacent rings [22]. The another in the UV region at 233 nm is due to the chlorotriazine groups absorption [22]. Solutions containing 1×10^{-5} mol l^{-1} of clotrimazole in the presence of 1×10^{-5} mol l^{-1} of the dye, presents no change in the bands attributable to the azo group (maximum absorption bands at 542, 514 and 294 nm), but the band at 233 nm is eliminated on the addition of clotrimazole. These results indicates that the interaction between dye and clotrimazole occurs only in the chlorotriazine group, while no modification is seen in the azo groups absorption bands.

The effect of dye concentration on the derivatization reaction was studied by obtaining polarograms with various amounts of clotrimazole (3×10^{-5}–1.5×10^{-4} mol l^{-1}) and a constant concentration of 1.0×10^{-4} mol l^{-1} of RR 120 dye in B–R buffer pH 2. The resulting peak currents of peak *A* (azo group) and peak *D* (triazine derivative) are shown in Fig. 3 as a function of the drug to dye ratio. The first peak current (*A*) is only slightly decreased when the drug concentration is increasing, despite higher half-width values are observed at higher clotrimazole concentrations. This behaviour is indicative that the reduction potential of the azo groups is not greatly affected by the derivatization, but possibly it is influencing the diffusion coefficients and/or reversibility. The peak current corresponding to reduction of the derivative, however, increases linearly up to 1×10^{-4} mol l^{-1} of the RR

120 and then reaches a limiting value. A similar behavior is observed for constant concentration of 5.0×10^{-4} mol l^{-1} of RR 120 dye that increases the linear relationship between i_p (peak D) versus clotrimazole concentration up to 4.7×10^{-4} mol l^{-1}. Thus, as observed by spectrophotometry the reaction ratio is 1:1, and this indicates that only one triazine group is taking part in the derivatization reaction. So, concentration of 5.0×10^{-4} mol l^{-1} of RR 120 dye was chosen as best experimental conditions.

In order to obtain the optimum derivatization condition for the clotrimazole, several parameters including pH influence, reaction temperature and reaction time were studied.

3.2. Effect of pH

The effect of pH and the composition of the supporting electrolyte on the voltammetric measurement of the RR120/clotrimazole derivative was examined by comparing the response in various electrolytes, such as B–R and acetate buffers, as well as in HCl/KCl solutions, with the aim of obtaining the best conditions in which to determine clotrimazole using the polarographic peak at -0.38 V. The most satisfactory supporting electrolyte was B–R buffer, as it allowed the best discrimination between the dye peak and that of the derivative. In addition, it was observed that the new compound can be identified only at pH < 4.5, although the reduction of the azo group in the RR120 dye is present in over the whole pH range investigated of 2–12.

Table 1
Polarographic peak potentials and currents obtained from differential pulse polarograms of 5×10^{-4} mol l^{-1} of RED120 and 1×10^{-4} mol l^{-1} of clotrimazole in B-R buffer of different pH values

pH	Peak A (dye)		Peak D (derivatized clotrimazole)	
	$-E_{pc}$ (V)	i_{pc} (μA)	$-E_{pc}$ (V)	i_{pc} (μA)
1.0[a]	0.021	0.408	0.352	0.275
1.5[a]	0.096	0.390	0.383	0.218
2.0	0.085	0.357	0.379	0.214
2.5	0.160	0.260	0.408	0.170
3.0	0.244	0.263	0.421	0.177
3.5	0.282	0.233	0.442	0.113
4.0	0.348	0.273	0.474	0.017
4.5	0.388	0.247	–	–

[a] System HCl/KCl.

The peak potentials and currents obtained for 5×10^{-4} mol l^{-1} of RR120 dye in the presence of 1×10^{-4} mol l^{-1} clotrimazole in acidic medium are shown in Table 1. The peak potentials for both the azo and the derivative peaks can be seen to shift towards more negative values as the pH is increased. The equations are as follows: $Ep_I(V) = -91.8 + 107.2$ pH; $R = 0.988$ (peak A) and $Ep_{II}(V) = 314 + 37.5$ pH; $R = 0.990$ (peak D). Higher currents and better separation from the background electrolyte reduction is obtained at pH 2.0, and this pH is the best value for the determination of clotrimazole. The pK_a value of clotrimazole is reported to be 4.7 [1]. This indicates that derivatization occurs when the imidazole moiety is in the protonated form. Therefore, taking into consideration the high reactivity of the bis-monochlorotriazine groups in the dye molecule, it is possible to suggest that the interaction between the dye and the drug could involve the displacing of the leaving group (chloride) of the chlorotriazine group, which would replace it, of the pharmaceutical compound. This reaction would be similar to other processes observed in the dyeing of fibres [22], and the substituted triazine group could be easier to reduced bearing the polarographic peak at less negative potential than the chlorotriazine group.

3.3. Effect of temperature and reaction time

The reaction between clotrimazole and RR 120 to form the reductive product was studied in the range 20–95 °C using a heating time of 30 min. A significant decrease in the peak current and a clear shifting of the peak potential of the compound formed occurred on heating above 70 °C. Room temperature was chosen as the best condition for analysis.

The effect of reaction time on the formation of the clotrimazole derivative was studied at room temperature using 5×10^{-4} mol l^{-1} RR 120 dye and 2×10^{-4} mol l^{-1} clotrimazole in B–R pH

2.0. The reaction occurred instantaneously. The signals obtained immediately on mixing for 19 determinations had a relative standard deviation of 3.5%. In this study the differential pulse polarograms were recorded at time intervals of 5 min for up to 4 h, and they were found not to change. This indicates the stability of the derivative under these conditions and its suitability for the determination of clotrimazole.

The stability of clotrimazole in solution is pH dependent [1]. In alkaline media it is stable, but it is hydrolysed in strongly acidic solution to (*o*-chlorophenyl)-diphenylmethanol plus imidazole. In order to determine whether it might be possible to monitor the hydrolytic degradation of clotrimazole using the derivatization reaction with RR 120, the effect of addition of imidazole and (*o*-chlorophenyl)-diphenylmethanol on the differential pulse polarograms obtained for reduction of RR 120 in B–R buffer pH 2.0 were investigated. There were no significant differences between the polarograms obtained before and after addition of these two compounds. These results indicate that the derivatization reaction must involve the imidazole group of the original molecule and not the released imidazole. Clearly (*o*-chlorophenyl)-diphenylmethanol does not interfere with the determination of clotrimazole, and the hydrolysis of clotrimazole to (*o*-chlorophenyl)-diphenylmethanol can be monitored using this derivatization method.

3.4. Polarographic determination of clotrimazole and analytical characteristics of the method

Calibration graphs obtained using the above optimum parameters and a concentration of 1×10^{-4} mol l^{-1} RR 120 were shown to be linear between 8 and 40 μg ml^{-1} of clotrimazole, but a plateau is observed at the higher concentrations. Nevertheless, using a higher dye concentration of 5×10^{-4} mol l^{-1}, the linear relationship can be extended from 10 to 69 μg ml^{-1}, equation, i_p (μA) $= -0.0233 + 0.053$ C (C = μg ml^{-1} and $r = 0.997$; $n = 12$). The reproducibility of the polarographic response was evaluated for 14 determinations of 69 mg ml^{-1} of clotrimazole in B–R buffer pH 2 and the relative standard deviations (R.D.S.) were calculated to be 4.1%. The detection limit based on a signal-to-noise ratio of 3 was calculated to be 2.6 ± 0.0025 μg ml^{-1}. Samples of 25.9 μg ml^{-1} clotrimazole in B–R buffer pH 2.0 containing 5×10^{-4} mol l^{-1} of RR 120 have shown a mean recovery ($n = 7$) of 99.4 ± 1.81% when submitted to standard additions of the pure drug.

3.5. Interferences

The method was tested for other imidazole derivatives that are used extensively as antifungal and antibacterial agents, tinidazole 1-{[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole} and miconazole 1-{[2-(2,4-dichlorophenyl)-2-(2,4-dichlorophenyl) methoxy} ethyl} -1H-imidazole.

Differential pulse polarograms recorded for 5×10^{-4} mol l^{-1} of RR 120 dye in B–R buffer pH 2.0 exhibit an extra wave at -0.21 V in the presence of miconazole, in addition to the azo reduction wave of the RR 120 dye at -0.09 V. Although, the discrimination between derivatizing reagent and the derivatized miconazole is not good enough to allow the use of the reaction for the determination of miconazole, miconazole can be a potential interferent in the proposed method for the determination of clotrimazole. Tinidazole has a nitro group in the molecule, which is reduced at the same potential of the reactive dye. No new wave corresponding to derivatized tinidazole was observed.

3.6. Application

The method described was applied to the determination of clotrimazole in bulk drug as pharmaceutical formulations. A Canesten commercial spray and Canesten vaginal tablets labeled to contain clotrimazole as 10 mg ml^{-1} and 100 mg per tablet, respectively, and a topic solution commercialised as Dermobene containing 10 mg ml^{-1} were tested. These were analysed using the method developed above.

An aliquot of the test solution prepared as described in the experimental section was diluted to 20 ml with B–R buffer pH 2.0, the final solution containing 5×10^{-4} mol l^{-1} of RR 120

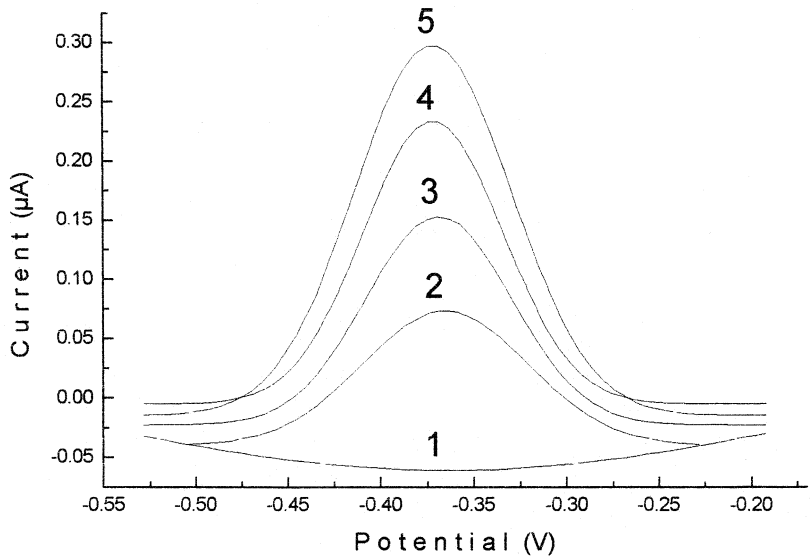

Fig. 4. Differential pulse polarograms obtained in the determination of clotrimazole in a Canesten sample using the standard additions method. RR120 concentration = 5×10^{-4} mol l^{-1}. B–R buffer pH 2.0. Plot 1 RR120 only, plots 2–5 in presence of 29.3 μg ml^{-1} of Canesten sample with standard additions of 10 to 34 μg ml^{-1} of clotrimazole solution (plots 3–5).

Table 2
Analysis of clotrimazole in pharmaceutical formulations by polarographic determination and spectrophotometric methods [7]

Formulation	Nominal content (μg ml^{-1})[a]	Reported method (μg ml^{-1})[b]	Polarographic method (μg ml^{-1})[c]	Recovery (%) ± S.D.
Canesten	43.1	–	42.8 ± 0.023	99.5 ± 0.001
solution	80.0	79.6 ± 0.070	–	96.5 ± 0.075
Dermobene	43.1	–	44.4 ± 0.007	103 ± 0.100
solution	80.0	83.2 ± 0.098	–	104 ± 0.120
Canesten	56.9	–	54.8 ± 0.053	96.3 ± 0.018
tablets	80.0	79.1 ± 0.380	–	98.9 ± 0.210

S.D., Standard deviation.
[a] Concentration based on information supplier by the manufacturer.
[b] Label claim.
[c] Median of three determinations.

dye. Typical differential pulse polarograms before and after standard additions of clotrimazole to commercial spray of Canesten are shown in Fig. 4. The results show that the excipients contained in these formulations do not interfere in the polarographic method. The average recoveries ($n = 7$) was tested using add method, which high percentage obtained from 96.48 to 102.2% indicates the efficiency and reproducibility of the method.

The results obtained using the proposed method and the comparative determination of clotrimazole in different commercial pharmaceutical preparation using a spectrophotometric method [7] is also shown in Table 2. The values are in agreement with the values provided by the manufacturers. No significant differences were found between the two methods, at a confidence level of 95% values of 42.8 ± 0.023 and 44.4 ± 0.007 μg ml^{-1} was obtained for clotrimazole in canesten and dermobene, respectively. Nevertheless, the method have shown low efficiency when applied to Canesten cream, using usual extraction

procedures described in the literature [7,11]. Low percentage recovery obtained indicates that the method is influenced by common excipients and additives encountered in this matrix and requires specific clean up sample.

4. Conclusions

A polarographic method has been developed for the determination of clotrimazole based on its derivatization with a bis-diazo bis-chlorotriazine reactive dye. The reaction appears to involve the elimination of one chloride ion with the addition of the imidazole moiety of the clotrimazole. A new polarographic peak is obtained which is distinct from the peaks of the excess of dye. The results have shown that this technique could be used as a convenient method for the analysis of clotrimazole in bulk material and pharmaceutical preparations namely solution form.

Acknowledgements

The authors thank FAPESP, CNPq and The British Council/CAPES for financial support.

References

[1] J.G. Hoogerheide, B.E. Wyka, Analytical Profiles of Drug Substances, vol. II, Academic Press, New York, 1982, pp. 225–255.
[2] United States Pharmacopeia-USP XXII-NF XVII-United States Pharmacopeial, pp. 334–337. Convention Inc., Rockville, 1990.
[3] D. Volkmann, Fachz. Lab. 27 (6) (1983) 524–526.
[4] D. Volkmann, Chem. Abstr. 99 (1983) 58985k.
[5] F. Pellerin, J.A. Gautier, D. Demay, Talanta 12 (1965) 847–851.
[6] A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop, in: J. Clarke (Ed.), Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986, p. 487.
[7] O.H. Abdelmageed, P. Khashaba, Talanta 40 (8) (1993) 1289–1294.
[8] M.K. Ibrahim, M.W. Nassar, N.M. El-Abssawy, S.G. Hamid, J. Pharm. Sci. 14 (1994) 184–192.
[9] M.M. Bedair, M.A. Korany, M.A.E. Elsayed, T. Fahny, J. Assoc. Off. Anal. Chem. 72 (3) (1989) 432–435.
[10] S.M. Wallace, V.P. Shah, S. Riegelman, W.L. Epstein, Anal. Lett. 11 (6) (1978) 461–468.
[11] J.G. Hoogerheid, S.H. Strusiak, C.R. Taddei, E.R. Townley, B.E. Wyka, J.Assoc. Off. Anal. Chem. 64 (4) (1981) 864–869.
[12] A.M. Di Pietra, V. Cavrini, V. Andrisano, R. Gotti, J. Pharm. Biomed. Anal. 10 (10–12) (1992) 873–879.
[13] C Valenta, A. Lexer, P. Spiegl, Pharmazie 47 (8) (1992) 641–642.
[14] A.M. Di Pietra, V. Andrisano, R. Gotti, V. Cavrini, J. Pharm. Biomed. Anal. 14 (8–10) (1996) 1191–1198.
[15] P.M. Bersier, J. Bersier, in: M.R. Smyth, J.G. Vos (Eds.), Anaytical Voltammetry, vol. 27, Elsevier, Amsterdam, 1992.
[16] Z. Fijalek, J. Chodkowski, M. Warowna, Acta Pol. Pharm. 49 (1–2) (1992) 1–5.
[17] M.V.B. Zanoni, A.G. Fogg, J. Barek, J. Zima, Anal. Chim. Acta 315 (1995) 41–54.
[18] J. Barek, A.G. Fogg, J.C. Moreira, M.V.B. Zanoni, J. Zima, Anal. Chim. Acta 320 (1996) 31–42.
[19] M.V.B. Zanoni, A.G. Fogg, J. Barek, J. Zima, Anal. Chim. Acta 349 (1997) 101–109.
[20] M.V.B. Zanoni, P.A. Carneiro, M. Furlan, E.S. Duarte, C.C.I. Guaratini, A.G. Fogg, Anal. Chim. Acta 385 (1999) 385–392.
[21] C.C.I. Guaratini, M.V.B. Zanoni, A.G. Fogg, Electroc. Soc. Proc. 97 (19) (1997) 467–476.
[22] H. Zollinger, Color Chemistry, second ed., VCH Publishers, New York, 1991.