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Extractive Spectrophotometric Determinations of Clotrimazole in Formulations

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Four simple sensitive and reproducible visible spectrophotometric methods (A–D) for the determination of clotrimazole (CTZ) in bulk samples and pharmaceutical formulations are described. Mehods A, B and C are based on the formation of ion-association complexes between CTZ and tropaeolen-000 (Tp000, λ_{max} 480 nm), supracen violet 3B (SV3B, λ_{max} 560 nm) or wool fast blue BL (WFB, λ_{max} 580 nm). Method D is based on the formation of a molecular complex between CTZ and cobalt thiocyanate (CTC, λ_{max} 600 nm). Regression analysis of Beer's plots showed good concentration ranges: 1.0–6.0 μg/mL for methods A, B and C and 1.25–7.5 μg/mL for method D. No interference was observed from the usually existing additives in pharmaceutical formulations and the applicability of the methods was examined by analysing cream and powder containing CTZ. Relative standard deviations were typically ≤ 0.33%. Recoveries were ≥ 99.5%.

Key words: Extractive, spectrophotometric, determinations, clotrimazole.

INTRODUCTION

Clotrimazole (CTZ) is a broad spectrum antimycotic agent effective against pathogenic dermatophytes, yeasts and several species of *Candida*, *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia*. Preparations of the drug are used in topical treatment of dermal infections and to combat vulvovaginal candidiasis[1]. It is a chemical known as 1-(*o*-chloro-α,α-diphenyl benzyl) imidazole.

The drug and its formulations are official in USP[2], BP[3] and IP[4]. Few methods have appeared in the literature for the determination of CTZ in pharmaceutical formulations. The techniques used in this connection include HPLC[5–10], polarography[11], titrimety[12–15], UV[16–21] and visible spectrophotometry[22–28]. However, analytically important functional groups have not been exploited thoroughly in developing visible spectrophotometric methods. This paper describes some attempts in this direction, with four visible spectrophotometric procedures by exploiting the basic property of the tertiary nitrogen in the imidazole of CTZ

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(ion-association complex formation with acidic dyes such as Tp000 (method A), SV 3B (method B) and WFB (method C) and molecular complex formation with CTC (method D).

EXPERIMENTAL

Instrumentation: - A Systronics model 117 visible spectrophotometer, Milton Roy UV-visible spectrophotometer and an Elico LI-120 model digital pH meter were used for absorbance and pH measurements.

Reagents: All chemicals were of analytical grade and all solutions were prepared in triply distilled water.

Aqueous solutions of Tp000 (Fluka, 5.70×10^{-3} M), SV 3B (chroma, 4.63×10^{-3} M), WFB (Fluka, 3.26×10^{-3} M), hydrochloric acid (0.1 M, method A) and glycine-HCl buffer solutions of pH (1.3, method B; 1.5, method C) and trisodium citrate-HCl buffer solution of pH 2.0 (method D) were prepared in the usual way[29].

Cobalt thiocyanate (CTC, 2.5×10^{-1} M) solution was prepared by dissolving 3.8 g of ammonium thiocyanate and 7.25 g of cobaltous nitrate in 100 mL of distilled water and the solution was saturated with sodium chloride.

Preparation of standard and formulations: CTZ pharmaceutical grade was used as standard without further treatment. An accurately weighed 100 mg amount of CTZ was dissolved in chloroform and made up to 100 mL with chloroform to give a stock solution of 1 mg/mL. The stock solution was further diluted with chloroform to give the working standard solutions (20 μg/mL) for methods A, B and C. For method D, 25 μg/mL working standard solution was prepared in 1 : 1 aqueous methanol in the usual way.

Analysis of formulations: A quantity equivalent to 100 mg of CTZ cream or powder was mxed with 30 mL of a mixture of 1 volume of 1 M H_2SO_4 and 4 volumes of methanol and shaken with two quantities, each of 30 mL of carbon tetrachloride, discarded the organic layers. The aqueous phase was made alkaline with dilute ammonia solution, added a further 10 mL of dilute ammonia solution and extracted with two quantities, each of 30 mL of chloroform. Combined the chloroform extracts, shaken with 5 g of anhydrous sodium sulphate, filtered and added sufficient chloroform to bring the total volume to 100 mL. 20 mL of this stock solution was further diluted stepwise with chloroform to 20 μg mL^{-1} for methods A, B and C. The chloroform was completely evaporated from another 20 mL aliquot of chloroform and the residue was dissolved and diluted with 1 : 1 aqueous methanol to get 25 μg mL^{-1} working sample solution in method D. They were analysed as under procedures described for bulk samples.

Procedures

Methods A, B and C: Drug aliquots of CTZ (0.5–3.0 mL, 20 μg mL^{-1} for methods A, B and C) were placed in a series of 125 mL separating funnels. Then 6.0 mL of 0.1 M HCl (method A) or buffer (pH 1.3, method B; pH 1.5, method C) and 2.0 mL of dye solution (Tp000, method A; SV 3B, method B; WFB, method C) were added to each separating funnel. The total volumes of aqueous and chloroform layers in each separating funnel were brought to 15 mL and 10 mL respectively with appropriate solvent and the contents were shaken for 2 min. The two phases were

allowed to separate and the absorbance of the separated chloroform layer was measured at appropriate λ_{max} (480 nm, method A; 560 nm, method B; 580 nm, method C) against a reagent blank similarly prepared within the stability period (1 min–6 h) at laboratory temperature (28 ± 5°C). The amounts of CTZ in methods A, B and C were computed from their respective calibration graphs.

Method D: Into a series of 125 mL separating funnels, aliquots of standard CTZ solution (0.5–3.0 mL, 25 μg mL^{-1}) were taken. Then 2.0 mL of buffer (pH 2.0) and 5.0 mL of cobalt thiocyanate solutions were added. The total volume of aqueous layer in each one was brought to 15 mL with distilled water. A 10 mL portion of nitrobenzene was added to each funnel and the contents were shaken for 2 min. The absorbance of the separated nitrobenzene layer was measured after 5 min at 600 nm against a reagent blank. The amount of CTZ present was computed from a calibration graph.

RESULTS AND DISCUSSION

Conditions under which the reaction of CTZ with each dye fulfills the essential analytical requirements were investigated. All the experimental conditions studied were optimised at room temperature (25 ± 3°C) and were established by varying one parameter at a time and observing the effect on the absorbance of the coloured species.

In the preliminary experiments, in view of developing methods of analysis suitable for assaying small quantities of CTZ, several acidic dyes (other than methyl orange[30] which was reported[31], RP) such as alizarin red S, supracen violet 3B, tropaeolin-00, tropaeolin-000, naphthalene blue 12BR, wool fast blue BL and naphthol blue black were tested at various pH ranges as the colour producing agents by a dye-salt partition technique. Different organic solvents such as chloroform, carbon tetrachloride, benzene, toluene were tested for the extraction of ion association complex formed between CTZ and each dye. The criterion of the best dye[31] was the highest absorbance value of the complex in the organic phase at the wavelength of maximum absorbances. The above studies reveal that three dyes namely Tp000 (CI No. 14600, azo dye), SV 3B (CI No. 60730, anthraquinone dye) and WFB (CI No. 50315, phenazine dye) gave better results than the other dyes. These dyes also gave low absorbance for the reagent blank. Chloroform was suggested as a solvent of choice for the extraction of coloured complex with respect to maximum stability.

In order to establish the optimum acid strength (for method A), or pH range (for methods B and C), the CTZ was allowed to react with the respective dye in aqueous solution in dil. HCl ranging from 0.05–1.5 M (method A) or solutions buffered between pH 1.0–6.0 (methods B and C) and the complex formed was extracted into chloroform for absorbance measurement. The results show that a quantitative extraction was produced with an acid strength of 0.08-0.12 M HCl (method A) or pH 1.1–1.5 (method B) or pH 1.4–1.8 (method C). All subsequent studies were carried out with 0.1 M HCl (for method A) or pH 1.3 (for method B) or pH 1.5 (for method C). The pH was adjusted using glycine-HCl buffer solution (this buffer was chosen on account of its elevated complexing ability, which could be of use in overcoming interferences). The volume of this buffer

added (4–10 mL) had no effect in methods B and C. A 6.0 mL portion of 0.1 M HCl solution was found to be optimal in method A. The minimum shaking time was determined by varying the shaking time from 1–10 min; although 1 min was sufficient, prolonged shaking has no adverse effect on the extraction and 2 min was selected for this study. A ratio of 1 : 1.5 (methods A, B and C) of organic to aqueous phases were required for efficient extraction of the coloured species and lowest reagent blank reading. It was found that better reproducibility and a lowest reagent blank was achieved if the dye was purified by extraction with chloroform initially. In order to establish the optimum experimental conditions for method D, the drug was allowed to react with CTC in aqueous solution buffered (trisodium citrate-HCl) to pH 1.5–4.0 and the molecular complex formed was extracted into nitrobenzene for measurement. Constant absorbance was obtained over the pH range 1.7–2.5, hence pH 2.0 was used. A 2 mL portion of CTC solution was found to be optimal. Constant absorbance was obtained for shaking periods between 1–4 min, hence 2 min was selected. Nitrobenzene was preferred for the selective extraction of drug-CTC complex among the water-immiscible solvents. A ratio of 1.5 :1 aqueous to nitrobenzene phases was required for efficient extraction of the coloured species.

Analytical data

The optical characteristics such as λ_{max}, Beer's law units, molar extinction coefficient, Sandell's sensitivity, regression equation and correlation coefficient obtained by linear least square treatment[32] of the results for the systems involving CTZ with the mentioned dyes or CTC are presented in Table-1. The precision of each method was tested by estimating six replicates of CTZ within Beer's law limits. The per cent standard deviation and the per cent range of error at 95% confidence limit are given in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR CTZ

	Method A Tp000	Method B SV 3B	Method C WFB	Method D CTC
λ_{max} (nm)	480	560	580	600
Beer's law limits ($\mu g\ mL^{-1}$, C)	1.0–6.0	1.0–6.0	1.0–6.0	1.25–7.50
Molar absorptivity ($L\ mole^{-1}\ cm^{-1}$)	3.79×10^4	3.69×10^4	2.96×10^4	1.39×10^4
Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001 absorbance unit)	9.17×10^{-3}	9.35×10^{-3}	1.17×10^{-2}	2.49×10^{-2}
Regression equation (Y)*:				
Slope (b)	1.1×10^{-1}	1.07×10^{-1}	1.14×10^{-1}	4.0×10^{-2}
Intercept (a)	-1.33×10^{-4}	-1.33×10^{-4}	1.33×10^{-4}	6.66×10^{-5}
Relative standard deviation (%)**	0.145	0.245	0.317	0.257
% range of error (confidence limits)**				
0.05 level	0.152	0.257	0.333	0.270
0.01 level	0.239	0.424	0.548	0.424
% error in bulk samples†	0.110	0.230	0.230	0.250

*Y = a + bC , where C is concentration.
†Six replicate samples.

The effects of various substances that often accompany in various pharmaceutical formulations were studied. None of them interfered in proposed methods (A–D) even when added in excess fold than anticipated in formulations.

In order to confirm the utility of the proposed methods, they were applied to the estimation of CTZ in various creams and powders and the results are presented in Table-2. The rsults obtained by the proposed and UV reference (R)[1] methods for the formulations were compared statistically[33] by means of F- and t-tests and were found not to differ significantly. As an additional check of accuracy of proposed methods, recovery experiments were performed by adding a fixed amount of the CTZ to the preanalysed formulation and the results are also summarized in Table-2. The results indicate that the proposed procedures do not differ significantly from reference method.

Chemistry of coloured species

In methods A, B and C, the formation of the coloured complex is based on the basic nature of the drug (CTZ), which under specified experimental conditions forms ion-association complexes with certain acidic dyes (Tp000, SV 3B or WFB) which are extractable into chloroform. The stoichiometric ratio of CTZ to Tp000, SV 3B or WFB was determined with the slope ratio method[34] and found to be 1 : 1. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The drug CTZ (1 mole) and the oppositely charged form of the dye (1 mole) behave as a single unit, being held together by electrostatic attraction.

In method D, the drug (CTZ) forms a coordination complex with CTC reagent. The coloured complex formed between the drug and CTC was quantitatively extractable into nitrobenzene from the aqueous phase and it was observed that CTZ, cobalt and thiocyanate were in the ratio of 2 : 1 : 4.

Conclusions

The order of λ_{max} values among the proposed (A–D), reported (RP) and a reference method (R) in the determination of CTZ is: method D > method C > Method B > Method A > RP > R. The higher λ_{max} of the proposed methods is a decisive advantage since the interference from the associated ingredients should be far less at these higher wavelengths. The sensitivity order of the methods is method A > method B > method C > method D > RP.

The proposed methods are simple, selective and sensitive for the determination of CTZ and provide a wide choice depending upon the needs of the specific situation.

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TABLE-2
ASSAY OF CTZ IN DOSAGE FORMS

Samples*	Labelled amount (g)	Amount found by proposed methods†				Reference method	% recovery by proposed methods‡			
		Method A	Method B	Method C	Method D		Method A	Mehtod B	Method C	Method D
$Cream_1$	30	29.90 ± 0.10	29.94 ± 0.052	29.92 ± 0.07	29.92 ± 0.07	29.92 ± 0.075	99.66 ± 0.35	99.81 ± 0.16	99.75 ± 0.22	99.75 ± 0.22
		F = 1.77	F = 2.08	F = 1.14	F = 1.14					
		t = 1.38	t = 1.33	t = 0.99	t = 0.99					
$Powder_1$	75	74.88 ± 0.20	74.84 ± 0.27	74.78 ± 0.38	74.84 ± 0.27	74.88 ± 0.20	99.85 ± 0.25	99.79 ± 0.36	99.70 ± 0.50	99.79 ± 0.36
		F = 1.10	F = 1.82	F = 3.61	F = 1.82					
		t = 1.0	t = 1.0	t = 1.0	t = 1.0					
$Cream_2$	15	14.98 ± 0.023	14.98 ± 0.028	14.98 ± 0.025	14.98 ± 0.025	14.08 ± 0.028	99.91 ± 0.15	99.92 ± 0.13	99.90 ± 0.17	99.90 ± 0.17
		F = 1.48	F = 1.0	F = 1.25	F = 1.25					
		t = 1.0	t = 0.99	t = 0.99	t = 0.99					
$Powder_2$	100	99.90 ± 0.16	99.84 ± 0.27	99.90 ± 0.17	99.81 ± 0.30	99.90 ± 0.16	99.90 ± 0.16	99.84 ± 0.27	99.90 ± 0.17	99.81 ± 0.30
		F = 1.0	F = 2.84	F = 1.12	F = 3.50					
		t = 0.99	t = 1.0	t = 0.99	t = 0.99					

*Different batches of creams and powders from different pharmaceutical companies.

†Average ± standard deviation of six determinations, the t- and F-test values refer to comparison of the proposed method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57

‡Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations)

REFERENCES

1. John G. Hoogerheide and Bruce E. Wyka, Analytical Profiles of Drug Substances, Vol.11, p. 225.
2. United States Pharmacopoeia, Vol. XXIV, U.S.P. Convention Inc., Rockville, p. 452 (2000).
3. British Pharmacopoeia, HMSO, London, p. 395 (1999).
4. Pharmacopoeia of India, 3rd Edn., Ministry of Health and Family Welfare, Government of India, New Delhi, p.197 (1996).
5. J. Zhu and C. Coscolluela, *J. Chromatogr. Biomed. Appl.*, **741**, 55 (2000).
6. M. Lin and N. Wu, *J. Pharm. Biomed. Anal.*, **19**, 945 (1999).
7. N.M. Tendolkar, B.S. Desai and V.M. Shinde, *Indian Drugs*, **31**, 551 (1994).
8. J.G. Hoogerheide, S.H. Strusiak, C.R. Taddei, E.R. Townley and B.E. Wyka, *J. Assoc. Off. Anal. Chem.*, **64**, 864 (1981).
9. V. Cavrini, A.M. Dipietra and M.A. Raggi, *Ins. J. Pharm.*, **10**, 119 (1982).
10. Guifang Wang, Xia Shen, Shouyao Zhang and Linong Zhang, *Zhongguo Yiyuan Yaoxue Zazhi*, **16**, 66 (1996).
11. Z. Fijalek, J. Chodkowski and M. Warowna, *Acta Pol. Pharm.*, **49**, 1 (1992).
12. M. Massaccesi, *Analyst (London)*, **111**, 987 (1986).
13. D. Volkmann, *Git. Fachz. Lab.*, **27**, 524 (1983).
14. D. Volkmann, *Git. Fachz. Lab.*, **25**, 375 (1981).
15. Adbel-Khalek and M. Mangoli, *Alexandria J. Pharm. Sci.*, **7**, 151 (1993).
16. C.V.N. Prasad, C. Parihar, K. Sunil and P. Parimoo, *Pharm. Sci.*, **33**, 337 (1997).
17. Q. Zheng and H. Xu, Zhongguo Yiyao, *Gongya. Zazhi*, **20**, 217 (1989).
18. M.M. Bedair, M.A. Korany, Ma. H. Elsayed and O.T. Fahmy, *J. Assoc. Off. Anal. Chem.*, **72**, 432 (1989).
19. D. Bonazzi, V. Cavrini, R. Gatti, E. Boselli and M. Caboni, *J. Pharm. Biomed. Anal.*, **18**, 235 (1998).
20. D. Agarwal, D.K. Jain and P. Trivedi, *Indian Drugs*, **35**, 499 (1998).
21. Y. Zhou and Yao Wu, *Fenxi. Zazhi*, **6**, 359 (1986).
22. Py. Khashaba, S.R. El. Shabouri, K.M. Emara and A.M. Mohamed, *J. Pharm. Biomed. Anal.*, **20**, 363 (2000).
23. S.R. El. Shabouri, K.M. Emara Py. Khashaba and A.M. Mohamed, *Anal. Lett.*, **31**, 1367 (1998).
24. Khadiga Kelani, I. Loris Bebawy, Laila Abdel-Fatiah and S. Abdel-Khader Ahmed, *Anal. Lett.*, **30**, 1843 (1997).
25. D.M. Shingbal and H.S. Kudchadker, *Indian Drugs*, **24**, 408 (1987).
26. Abdel Mageed, H. Osama and Y. Khashaba Pakinaz, *Talanta*, **40**, 1289 (1993).
27. M.L. Chianani, L.S. Rao and S.J. Chavda, *Indian Drugs*, **18**, 405 (1981).
28. C. Zhao, Li. Zhu, Z. Zhang, F. Jin and X. Wang, *Shenyang Yaoxueyuan Xuebao*, **10**, 27 (1993).
29. Ju. Lurie, Hand Book of Analytical Chemistry, Mir Publishers, Moscow, p. 253 (1975).
30. H. Osama, Abdul Mageed and Y. Khashaba Pakinaz, *Talanta*, **40**, 1289 (1993).
31. V. Das Gupta, *Ind. J. Pharma*, **35**, 77 (1973).
32. M.D. Palter Gill and D.E. Sands, *J. Chem. Educ.*, **58**, 244 (1979).
33. D.L. Massart, B.G.M. Vandeginste, S.N. Demung, Y. Micholte and L. Kaufman, Chemometrics: A Text Book, Elsevier, Amsterdam (1988).
34. H. Irwing, F.T.C. Rossotti and R.J.P. Williams, *J. Chem. Soc.*, **II**, 1906 (1958).

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