

QUANTITATION OF TOLNAFTATE BY SPECTROPHOTOMETRIC METHODS IN PHARMACEUTICAL FORMULATIONS

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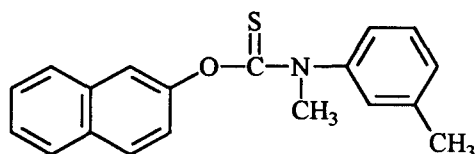
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يصف هذا البحث طريقتين طيفيتين لتعيين التولنافتيت في مستحضراته الصيدلانية إما منفردا أو مع مركبات أخرى. تعتمد الطريقة الأولى على تكوين صبغة الميثيلين الزرقاء (عند طول موجة ٦٦٥ ن.م) وذلك نتيجة لتفاعل ثنائي ميثيل-بارا-فينيلين ثنائي أمين الهيدروكلوريد في وجود سلفات الأمونيوم للسريك مع كبريتيد الصوديوم الناتج من التحليل القلوي للتولنافتيت. وقد تم دراسة جميع المتغيرات التي تؤثر على تحليل التولنافتيت. كذلك تم ضبط جميع العوامل المؤثرة على تكوين اللون مثل تركيز حمض الكبريتيك - تركيز الكاشف - زمن التفاعل - درجة الحرارة والمذيبات المخففة. وتعتمد الطريقة الثانية على تعيين كلا من التولنافتيت والكلويكينول في نفس الوقت في وجود كبريتات الجنتاميسين والبيتاميثازون وذلك بقياس الشق التفاضلي الأول في الميثانول لمنحنى الامتصاص الضوئي عند ٢٣٣,٩٧ ن.م. للتولنافتيت وعند ٢٤٥,٠٥ ن.م. وقد تبعت كلا من الطريقتين لقانون بيير للتركيز ١٠,٠٥-١٠,٠٥، ١٢-١٠، ١٠-٠,٥، ١٠-٠,٥، ٠,٠١٦ و ٠,٠٠٦-٠,٠٠٦ للاكتشاف مع حد ادنى للاكتشاف ٠,٠٠٦ و ٠,٠٠٦. وقد تم بنجاح تطبيق الطرق المقترحة على تحليل التولنافتيت في مستحضراته الصيدلانية اما منفردا او مع مركبات اخرى وكانت نتائج الارتجاع جيدة وتتراوح بين ٩٧,٥-١٠٢,٦ ± ١,٦-١,٠٨ كذلك كانت نتائج الطرق المقترحة متوافقة مع مثيلتها من الطرق المرجعية. وقد تم عمل دراسة دقيقة للتداخل في الكريم المركب الناتج من وجود البيتاميثازون - الكلويكينول - وكبريتات الجنتاميسين باستخدام كلا الطريقتين - واثبتت الدراسة عدم وجود اي تداخل لهذه المركبات.

*Two selective spectrophotometric methods are described for the assay of tolnaftate in single and/or combined pharmaceutical formulations. The first one depends on the formation of a methylene blue dye (λ_{max} 665 nm) by the reaction of *N,N*-dimethyl *p*-phenylene diamine dihydrochloride in the presence of ceric ammonium sulphate with sodium sulphide resulting from the alkaline hydrolysis of the thiono ester-tolnaftate. All variables affecting hydrolysis of tolnaftate were studied. Also other parameters affecting the colour development as sulphuric acid and reagent concentrations, reaction time, temperature and dilution solvents were optimized. The second method depends on the simultaneous estimation of tolnaftate and clioquinol in the presence of gentamycin sulphate and betamethasone by the measurement of the first derivative spectrum in methanol at $\lambda = 233.87$ nm and 245.05 nm for tolnaftate and clioquinol respectively. Both methods obey Beer's law in concentration ranges of 2-10, 0.5-10, and 1-12 $\mu\text{g.ml}^{-1}$ with a good correlation coefficient, and limit of detection of 0.06, 0.06 and 0.16 $\mu\text{g.ml}^{-1}$ for tolnaftate (by methylene blue method), tolnaftate and clioquinol respectively (by derivative spectrophotometric method). The proposed procedures have been applied successfully for the assay of tolnaftate in single or in multicomponent cream with good recovery percentage ranged from 97.5-102.6 \pm 1.08-1.6. Results of both suggested methods are comparable and agree well with those obtained with reported method. Interference study in combined cream due to the presence of betamethasone, clioquinol, and gentamycin sulphate has been investigated carefully by both methods and proves the absence of interference due to these drugs.*

INTRODUCTION

Tolnaftate (I) (TF) (O-2-Naphthyl m, N-dimethylthiocarbamate) is a topical antifungal agent that has found widespread use over the past several decades.



Tolnaftate (I)

It is recommended for the treatment of various dermatophytes and several forms of microsporon that can cause infectious condition like *Tinea pedis* (athlete's foot), *Tinea cruris* (jock itch) and *Tinea capitis* (body ring worm). Tolnaftate is ineffective against candida albicans or gram negative and gram positive bacteria.¹ It is usually formulated as single or in combination with other antibacterial and antifungal agent as clioquinol and gentamycin sulphate and steroid drugs as betamethasone. Current compendial methods for the assay of TF and related dosage forms as cream, gels and solution are based on a chloroform extraction step and UV measurement at λ_{\max} 258 nm.^{2,3} While assay of powder and aerosol powder is based on methanol extraction followed by liquid Chromatography using progesterone as an internal standard.² A survey of the literature revealed that one of the two reported UV-VIS spectrophotometric methods⁴ involve the coupling of TF through naphthyl moiety at position 1 with 2,6-dichloroquinone-4-chlorimide; (Gibb's reagent) to yield a colored coupling product at λ_{\max} 490 nm. The second method involves the reaction of N',N'-dimethyl p-phenylene diamine with chloramine T to yield N-chloro-N',N'-dimethyl quinone diiminium salt then subsequent reaction of the latter with TF to yield a similar colored coupling product at λ_{\max} 530 nm. As a thiono ester, alkaline hydrolysis of TF yields the highly fluorescent sodium β -naphtholate, which has been used for the assay of the drug spectrofluorimetrically.⁵ Other methods based on chromatographic techniques have been reported.⁶⁻⁹ The aim of the present work is to provide selective spectrophotometric methods for the assay of TF in either single and/or combined dosage forms allowing the

increase in sensitivity of the reported method⁴ as well as to develop a new UV spectrophotometric method which is more selective than official UV methods^{2,3} and could be applied for the assay of TF in combination with other drugs.

EXPERIMENTAL

Apparatus

A Jasco Uvidec model 320 spectrophotometer (Tokyo, Japan) and a Shimadzu, UV-1601 PC spectrophotometer (Kyoto, Japan) both with 1 cm quartz cells, were used for the measurement of the absorbance and derivative spectra. For derivative curves, the spectra of solutions were recorded over a range of 200-400 nm versus methanol. Derivative mode were set at D₁ (dA/d λ) with a fast scan speed, a delta λ = 2 nm and a scaling factor =10. The derivative UV spectra were recorded against methanol using D₁ mode by measuring the zero crossing amplitude (D₁) at λ 233.87nm (for TF) and at λ 245.05 nm (for CL).

Materials and reagents

All solvents used were of analytical-reagent grade. Tolnaftate (Kahira Pharmaceuticals and Chemical industries Co., Cairo, Egypt), betamethasone (Amrya, Alexandria, Egypt) clioquinol and gentamycin sulphate ((Memphis Pharmaceuticals and Chemical Industries Co., Cairo, Egypt) were obtained as gifts and were used as working standards.

N,N-dimethyl p-phenylenediamine dihydrochloride (DPD) (BDH, Chemicals Ltd, Poole England) 0.3 g % w/v was prepared by dissolving 0.3 g in 100 ml of 0.08 M H₂SO₄. The solution was found to be stable for one week at 4°C. Ceric ammonium sulphate (Sigma Chemie, GmbH, West Germany) 0.08g % w/v was prepared by dissolving 0.08 g in 100 ml of 1M H₂SO₄. All other chemicals used were of analytical grade. Double distilled water was used throughout the work.

Dosage forms

Tinea cure cream (Kahira Pharmaceuticals and Chemical Industries Co., Cairo, Egypt) labelled to contain tolnaftate as 10 mg.g⁻¹. Quadriderm cream (Memphis Pharmaceuticals and Chemical Industries Co., Cairo, Egypt)

labelled to contain tolnaftate as 10 mg.g⁻¹, betamethasone as 0.5 mg.g⁻¹, gentamycin sulphate as 1 mg.g⁻¹, clioquinol as 10 mg.g⁻¹.

Preparation of standard solutions

For methylene blue method (MB method)

Twenty-five milligrams of TF was weighed accurately, transferred into a 25-ml volumetric flask and dissolved in methanol to provide a standard solution containing 1 mg.ml⁻¹. From this solution a series of separate working standard solutions were diluted with methanol to obtain concentration ranges of 50-250 µg.ml⁻¹ in methanol.

For derivative spectra method (DS method)

Twenty-five milligrams of TF and or CL was weighed accurately, transferred each into a 25-ml volumetric flask and dissolved in methanol to provide each a standard solution containing 1 mg.ml⁻¹. From these solutions a series of separate working standard solutions were diluted with methanol to obtain concentration ranges of 5-100 and 10-120 µg.ml⁻¹ of TF and CL respectively.

Preparation of sample solutions

Cream:² A portion of the cream equivalent to about 10 mg TF accurately weighed, was transferred to a 250-ml separating funnel containing 75 ml of chloroform. The chloroform solution was washed with two 25-ml portions of 0.1N sodium hydroxide, two 25-ml portions of 0.1N hydrochloric acid and 25-ml of distilled water. The chloroform extract was filtered, evaporated to dryness. The residue was then transferred into 100-ml volumetric flask, dissolved in methanol, and completed to volume with same solvent. The resulting solution was diluted with methanol to obtain concentrations assumed to be 175 µg.ml⁻¹ TF (for MB), 50 µg.ml⁻¹ TF and 50 µg.ml⁻¹ CL (for DS).

General procedure

1- For MB method

Into a Pyrex test tube, one milliliter of standard or sample solution was measured accurately. A volume of 2 ml methanol and 2 ml of 5M sodium hydroxide were added and the solution was heated in a water bath at 90°C for 30 minutes then allowed to cool in ice cold bath. To this solution, 1.5 ml of 5M sulphuric acid

was added followed immediately with 1 ml of 0.3% DPD (in 1 M sulphuric acid) and 1.5 ml of 0.08 g% ceric ammonium sulphate (in 0.08M sulphuric acid). The mixture was shaken for few seconds then completed to 25 ml with water. After 10 minutes the resulting chromogen was measured spectrophotometrically at λ_{max} 665 nm against a blank experiment treated similarly.

2- For DS method

One milliliter of standard or sample solution of TF and or CL was diluted with methanol in 10 ml volumetric flask. The derivative UV spectra were measured against methanol by recording simultaneously the zero crossing amplitude (D_1) at λ 233.87 nm and 245.05 for TF and CL respectively and compared with the corresponding calibration graphs. Values of the first derivative spectra were multiplied by 10.

RESULTS AND DISCUSSION

1- For MB method

The formation of methylene blue-like dyes were reported for the determination of cephalosporins after their alkaline degradation to hydrogen sulphide^{10,11} and for a variety of pharmaceutical compounds as phenothiazines,¹² sulphonamides¹³ p-aminophenol and acetaminophen.¹⁴ In the present study, all variables affecting the reaction product of hydrolyzed tolnaftate with DPD and Ce(IV) to form a methylene blue dye were studied carefully.

Absorption spectrum

Tolnaftate was hydrolyzed by heating in a water bath at 90°C for 30 minutes in the presence of 2M sodium hydroxide producing sulphide, which was allowed to react with DPD and Ce(IV) as an oxidant. The resulting blue chromogen (λ_{max} 665 nm) is shown in Fig. 1.

Hydrolysis of tolnaftate as a sulphide producing compound

Various concentration of sodium hydroxide was tested for the hydrolysis of tolnaftate. Fig. 2 shows that 5 M sodium hydroxide in a volume of 2 ml is suitable to produce sufficient sulphide for maximum color intensity. To avoid

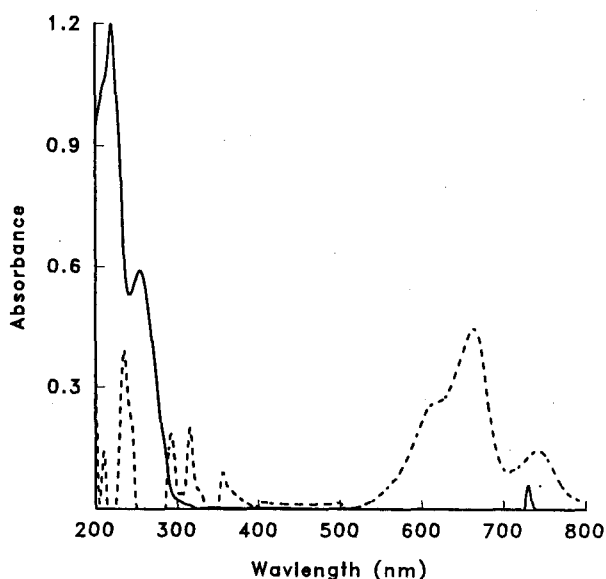


Fig. 1: Absorption spectra of (—) $7 \mu\text{g.ml}^{-1}$ tolnaftate in methanol and (----) MB reaction product of hydrolyzed tolnaftate with 0.3% DPD and 0.08% Ce(IV) in sulphuric acid.

precipitation of tolnaftate due to its insolubility in aqueous medium, 2 ml of methanol was added before addition of sodium hydroxide. Complete hydrolysis was attained in a period of 30 minutes as shown in Fig. 2C. Since reaction condition for methylene blue dye formation needs to be more or less acidic, different

concentration of sulphuric acid was tested so as to neutralize excess sodium hydroxide after hydrolysis. Fig. 2D indicates that 1.5 ml of 5M sulphuric acid is quite enough, however it should be added while keeping reaction in ice cold bath so as to avoid loss of hydrogen sulphide.

Effect of DPD concentration

Figure 3 indicates that maximum absorption intensity was obtained using a volume of 1 ml of 0.3 % DPD. Higher concentrations show decrease in absorbance intensity.

Effect of Ce(IV) concentration

Different oxidants as Fe(III), N-Bromosuccinimide (NBS) and Ce(IV) were tested. Preliminary investigations revealed that reaction was not quantitative with Fe(III) despite its better sensitivity. Low absorption intensity was obtained with NBS. However Ce(IV) was found to be the most suitable oxidant, therefore the effect of different concentration of Ce(IV) was studied. Results in Table 1 indicates that methylene blue reaction product is incomplete using lower concentration of Ce(IV), however 0.08 g % was quite enough for maximum color intensity. By using different volume of the same concentration, it was found that a volume of 1.5 ml was chosen as optimal.

Table 1: Effect of Ce(IV)^{*} concentration on the methylene blue reaction product of hydrolysed tolnaftate ($7 \mu\text{g.ml}^{-1}$).

Conc. of Ce(IV) g%	Absorbance ^o
0.02 [*]	— [*]
0.04 [*]	— [*]
0.06	0.320
0.08	0.435
0.1	0.426
0.15	0.400
0.2 [#]	— [#]

^{*} Volume used is 1.5 ml

^o Average of three experiments.

^{*} Reaction is incomplete (faint yellow colour)

[#] The solution is slightly turbid.

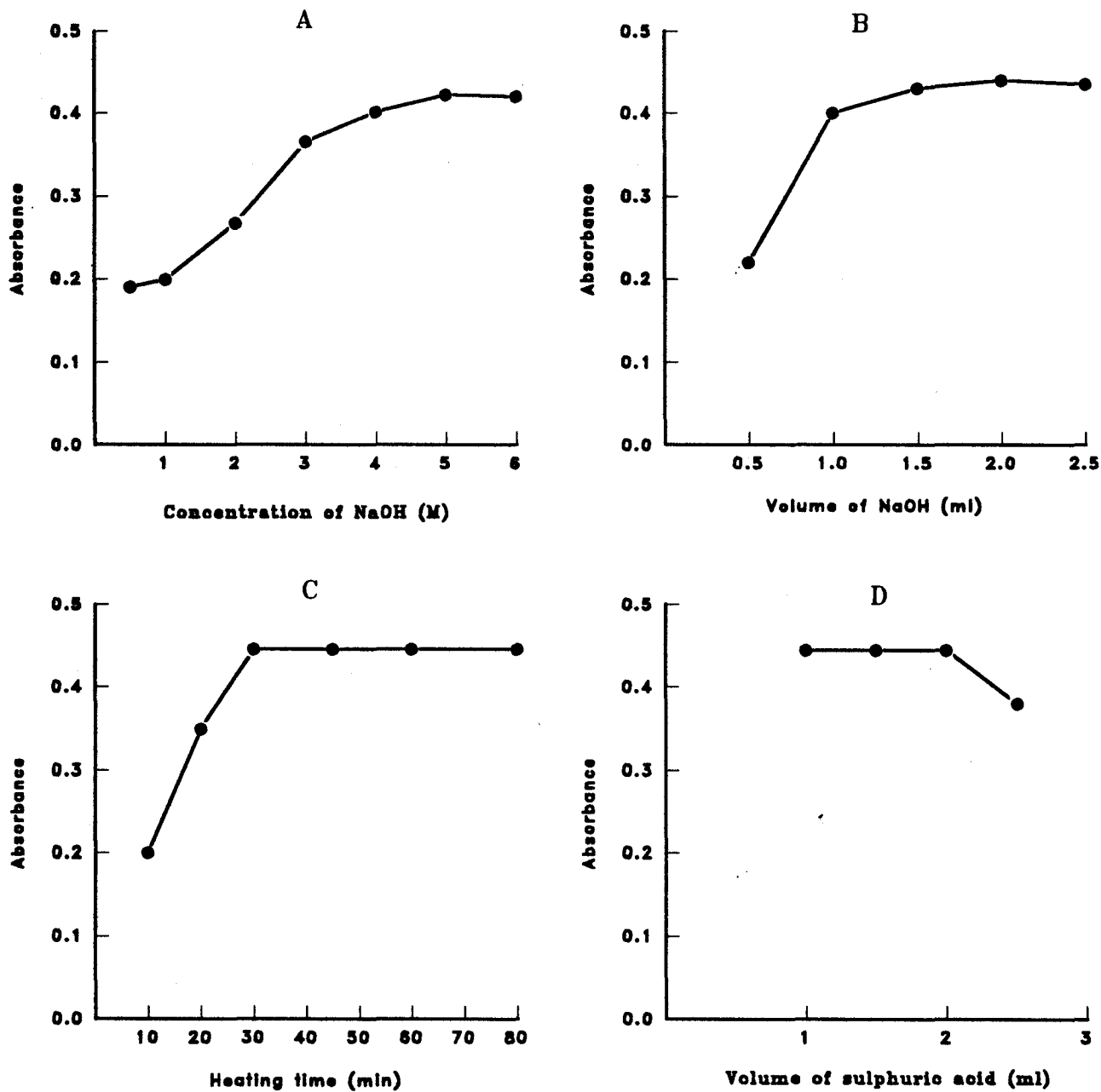


Fig. 2: Influence of different variables on the hydrolysis of tolinafate as a sulphide producing compound used for the proposed methylene blue dye method.

A: Effect of different concentration of sodium hydroxide

B: Effect of different volume of 5 M sodium hydroxide

C: Effect of heating time

D: Effect of sulphuric acid concentration used for neutralization of excess sodium hydroxide.

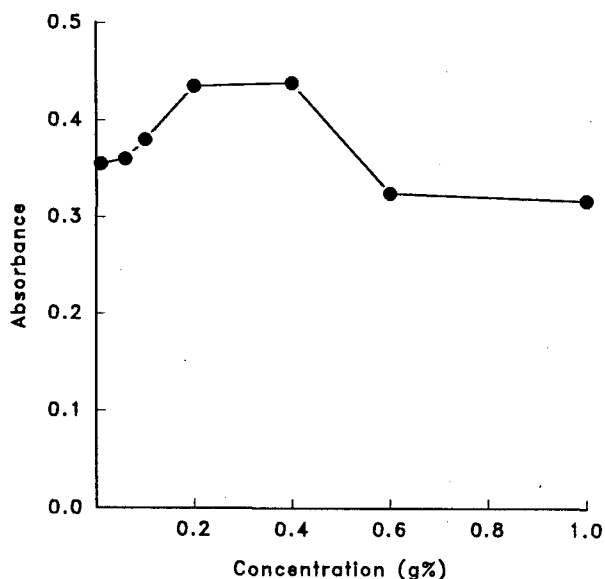


Fig. 3: Effect of DPD concentration (1 ml) on the methylene blue reaction product of hydrolysed tolanaftate ($7 \mu\text{g}.\text{ml}^{-1}$).

Effect of sulphuric acid concentration

It is well known that acid concentration is very important for methylene blue dye reaction.¹⁵ Therefore different concentrations of sulphuric acid ranging from 0.04 M - 3 M were tested to dissolve each of Ce(IV) and DPD reagent. Figure 4 indicates that for maximum absorbance, it is necessary to use 0.08 M and 1 M sulphuric acid as solvent for Ce(IV) and DPD respectively.

Order of reagent addition

It has been found that the order of reagent addition is very important. DPD must be added first to the sulphide followed by the oxidant (Ce(IV)), otherwise the blue chromogen is not formed.

Effect of reaction time

The reaction time was studied by monitoring the color development at different time intervals at room temperature as well as in a thermostatic water bath of different temperature. Results indicate that at room temperature maximum color intensity was attained within 10 minutes. While at higher temperature colour intensity was obtained immediately then decreased with increasing heating time due to mainly loss of hydrogen sulphide. Therefore, ten minutes reaction time at room temperature was selected.

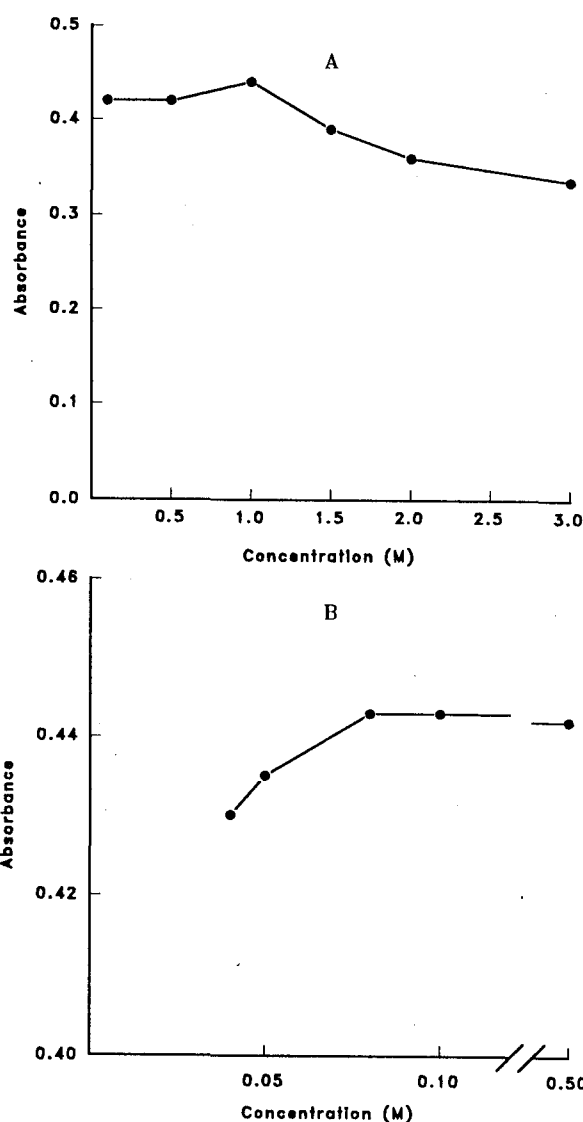


Fig. 4: Effect of sulphuric acid concentration as a solvent for: (A) DPD and (B) Ce(IV) reagents used for the methylene blue dye method (conc. of tolanaftate = $7 \mu\text{g}.\text{ml}^{-1}$).

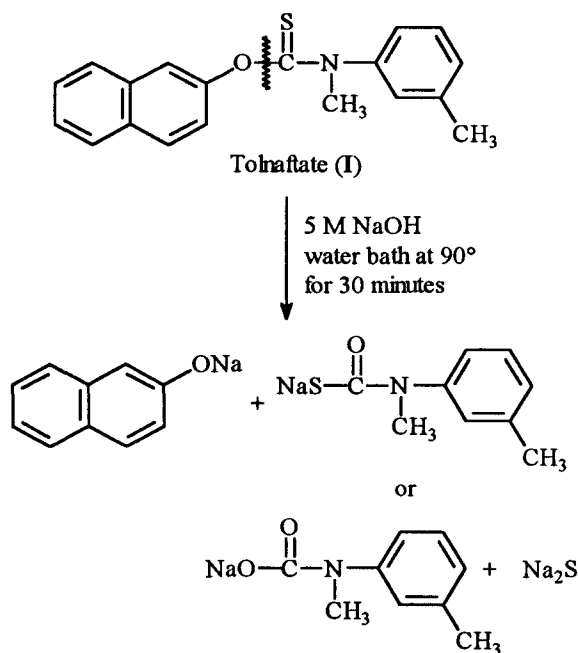
Effect of dilution solvent and colour stability

Dilution of MB reaction product of TF with a variety of solvents as ethyl alcohol, methyl alcohol and acetonitrile resulted in the appearance of slight turbidity. However dilution of the blue chromogen with water gave a clear solution, which remained stable for up to two hours. Therefore water was selected as the diluting solvent throughout the work.

Suggested mechanism for the MB method

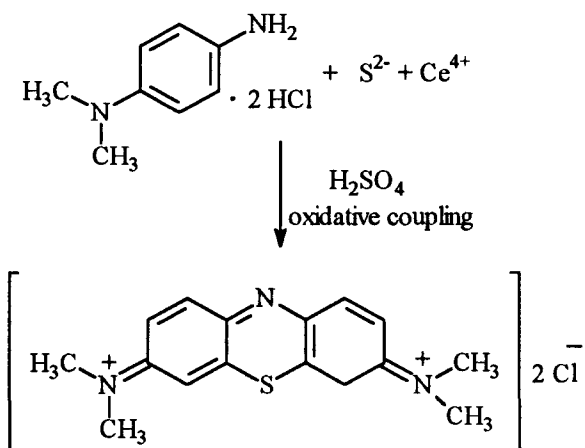
Although similar reactions have been reported for methylene blue^{11,16} and methylene blue-like structure, the present work describes

for the first time the use of DPD and ceric sulphate in the quantitative estimation of the cited drug through the formation of methylene blue dye. The suggested mechanism could be explained first by the alkaline hydrolysis of TF as a thiono ester with sodium hydroxide producing sodium sulphide¹⁷ (Scheme 1) which could be utilized for the dye formation by the reaction with DPD as the dihydrochloride salt and ceric sulphate in the presence of sulphuric acid (Scheme 2). The overall reactions carried out may be represented as follow:



Scheme 1

Hydrolysis of tolinaftate in alkaline medium



Scheme 2

Suggested mechanism

2- For DS method

Extreme overlapping of the absorption maxima of the multicomponent mixtures of tolinaftate, clioquinol, betamethazone and gentamycin sulphate did not allow TF to be determined by zero order spectrophotometry (Fig. 5). However the first derivative spectra offers an extremely valuable means for the simultaneous estimation of TF as well as CL by measuring the zero crossing amplitude D_1 ($dA/d\lambda$) at 233.87 and 245.05 for TF and CL respectively with nil contribution from betamethazone and gentamycin sulphate (Fig. 6). Recording the first derivative spectra using other solvents such as chloroform was not recommended as only CL could be estimated at 247.8 nm but TF derivative spectrum was not separated. Therefore recording the first order mode in methanol at the selected wavelengths 233.87 nm for TF and 245.05 for CL was the ideal conditions for DS method.

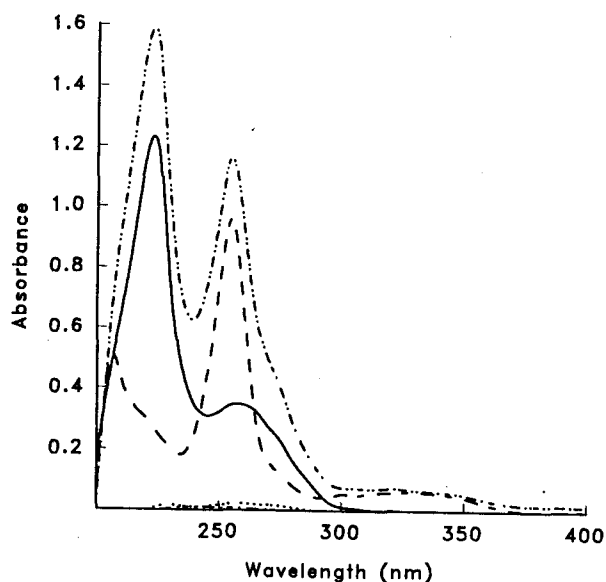


Fig. 5: Absorption spectra of : (—) 5 $\mu\text{g}.\text{ml}^{-1}$ tolinaftate, (---) 5 $\mu\text{g}.\text{ml}^{-1}$ clioquinol, (.....) 0.25 $\mu\text{g}.\text{ml}^{-1}$ betamethazone, (-.-.-) 5 $\mu\text{g}.\text{ml}^{-1}$ gentamycin sulphate, and (-.-.-.-) mixture of all four drugs. All drugs are dissolved in methanol.

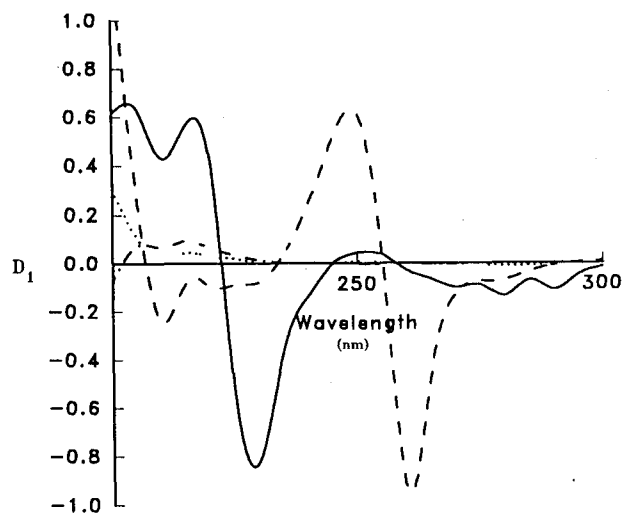


Fig. 6: First order spectra of : (—) $5 \mu\text{g.ml}^{-1}$ tolnaftate, (---) $5 \mu\text{g.ml}^{-1}$ clioquinol, (.....) $0.25 \mu\text{g.ml}^{-1}$ betamethazone and (-.-.-) $5 \mu\text{g.ml}^{-1}$ gentamycin sulphate. All drugs are dissolved in methanol.

Validation of the proposed methods¹⁸

Linearity and sensitivity

The blue chromogen obtained by the MB-method and the total derivative spectrum taken at the selected optimum wavelengths by DS-method afforded the best linear response to the analyte concentration. Parameters of the regression curves evaluated by the least square method are represented in Table 2. Beer's plots were linear over concentration ranges listed in Table 2 with good correlation coefficients (0.9996 – 0.9998) and a RSD of 0.01-0.02% as well as lower values of LOD ($0.06\text{-}0.16 \mu\text{g.ml}^{-1}$) and LOQ ($0.19\text{-}0.55 \mu\text{g.ml}^{-1}$)¹⁸ indicating higher sensitivity of the proposed procedures as compared to other reported method.⁴

Accuracy

In order to demonstrate accuracy and applicability of the proposed procedures, both methods were applied successfully for the assay of TF (by MB method) either in single or multicomponent creams or simultaneously with CL (by DS method) in multicomponent cream and in synthetic mixture that represents the same composition of commercial cream. Results in Table 3 show good recovery percentage of TF (97.5-101.4%) and CL (97.9 %) and good

agreement with those obtained by official² and reported methods⁵ as indicated by the values of F and t test.¹⁹ It must be noted that the official USP method² is useful only in case of single component cream. In case of the investigated multicomponent cream the UV absorption spectra of clioquinol, betamethazone and gentamycin sulphate do interfere (Fig. 5). Therefore for multicomponent cream a spectrofluorimetric method⁵ was used for comparison since the interfering drugs were not fluorescent under the condition of the assay.

Precision

Precision of the developed procedure was confirmed by analyzing five replicate samples, each containing $7 \mu\text{g.ml}^{-1}$ of TF (MB method) and $5 \mu\text{g.ml}^{-1}$ of TF and CL (DS method) and the percent recoveries \pm SD were found to be: 100.7 ± 1.12 for TF (MB method) and 99.2 ± 0.84 for TF and 99.8 ± 1.18 for CL (DS method).

Selectivity

Analyzing each drug separately or in a synthetic mixture that represents the composition of commercial cream tested selectivity of the suggested MB method. Results prove the absence of interference of CL, betamethasone and gentamycin sulphate upon analysis by MB method, which is mainly due to the absence of sulphide after alkaline hydrolysis of these drugs. In addition, analysis of synthetic mixture of the same composition ratio of the multicomponent cream by the DS method proves also the absence of interference of betamethazone and gentamycin sulphate as measurement of zero crossing derivative spectra of both TF and CL were recorded with nil contribution of these drugs at the selected wavelengths. Besides a part of the validation of the DS method was to calculate simultaneously the possible interference due to different ratios of TF and CL. Results of these studies prove an excellent recovery percentage of both TF and CL with small RSD (Table 4) and hence confirm also the selectivity of the proposed DS method. However interference was observed when using higher ratios of one drug to the other as indicated in the ratios 4:1 and 1:5 of TF: CL and low percentage recoveries was obtained for CL (mixt.4) and for TF (mixt. 8).

Table 2: Quantitative parameters for the proposed methods of analysis of tolnaftate and clioquinol.

	MB method	DS method	
		TF	CL
Linearity range ($\mu\text{g.ml}^{-1}$)	2-10	0.5-10	1-12
Intercept (a) $\pm\text{SD}^*$	0.02 $\pm 1.17 \times 10^{-3}$	0.02 $\pm 2.08 \times 10^{-3}$	-0.05 $\pm 6.0 \times 10^{-3}$
Slope (b) $\pm\text{SD}^*$	0.06 $\pm 5.19 \times 10^{-4}$	0.10 $\pm 1.0 \times 10^{-4}$	0.11 $\pm 5.0 \times 10^{-4}$
r $\pm\text{SD}^*$	0.9996 $\pm 1.53 \times 10^{-4}$	0.9998 5.8×10^{-5}	0.9997 2.0×10^{-4}
RSD (%)	0.02	0.01	0.02
LOD ($\mu\text{g.ml}^{-1}$)	0.06	0.06	0.16
LOQ ($\mu\text{g.ml}^{-1}$)	0.19	0.21	0.55

*Average of three determinations.

Table 3: Assay of tolnaftate and clioquinol in pharmaceutical formulations by the proposed and reported methods.

Sample	Drug	Claimed (mg)	Found ^o by proposed method $\% \pm \text{SD}^*$		Found ^o by official [^] and reported [*] methods $\% \pm \text{SD}^*$
			M.B.	DS	
Tinea-cure Cream	TF	10/g	97.5 ± 1.6	98.2 ± 1.30	$97.6 \pm 0.77^*$
			F= 4.34	F= 2.86	
			t= 0.82	t= 0.16	
Quadriderm Cream	TF	10/g	102.6 ± 1.3	101.4 ± 1.08	$102.3 \pm 0.94^*$
			F= 1.92	F= 1.32	
	CL	10/g	-	97.9 ± 1.38	
Synthetic mixture [#]	TF	1/ml	99.6 ± 1.1	98.9 ± 0.89	-
	CL	1/ml	-	99.0 ± 0.99	-

^oTheoretical value of 95% confidence limit ($n_1=5, \& n_2= 6$) for F is 5.19 and t is 2.26.

[^]Reference [2] for TF in single component cream.

^{*}Reference [5] for TF in multicomponent cream

^{*}Average of five determinations

[#]Synthetic mixture was prepared in the same ratio of commercial cream (Quadriderm cream).

Table 4: Assay of tolinaftate and clioquinol in laboratory prepared mixtures by the proposed DS method.

Mix.	TF : CL	Conc. ($\mu\text{g.ml}^{-1}$)				% Recovery \pm SD*	
		Taken		Recovered ^o		TF	CL
		TF	CL	TF	CL		
1	3 2	3	2	3.02	2.03	100.7 \pm 0.78	101.7 \pm 0.58
2	2 1	4	2	3.63	2.00	99.1 \pm 0.84	100.0 \pm 1.06
3	3 1	6	2	6.04	2.05	100.7 \pm 0.94	102.4 \pm 0.58
4	4 1	8	2	8.11	1.25	101.1 \pm 0.92	62.6 \pm 2.08
5	1 2	2	4	1.99	4.00	99.7 \pm 0.53	100.2 \pm 0.21
6	1 3	2	6	1.96	5.89	98.2 \pm 0.91	98.1 \pm 0.56
7	1 4	2	8	2.00	8.12	100.0 \pm 0.91	101.5 \pm 0.81
8	1 5	2	10	1.88	10.1	94.1 \pm 1.66	101.3 \pm 0.15
9	1 1	2	2	1.96	1.97	98.0 \pm 1.34	98.4 \pm 1.06
10	1 1	4	4	3.95	3.93	98.7 \pm 0.84	98.4 \pm 0.65
11	1 1	5	5	4.94	4.95	98.9 \pm 0.89	99.0 \pm 0.99
12	1 1	6	6	5.90	5.87	98.2 \pm 1.25	97.8 \pm 0.80
13	1 1	8	8	7.85	7.55	98.1 \pm 0.40	98.4 \pm 0.58

^oConcentrations were compared with those of corresponding calibration curves.

*Average of three determinations

Conclusion

The proposed methods were found to be more sensitive than the reported UV method⁴ and so could be applied successfully for the determination of TF (by MB) and TF and CL (by DS) simultaneously in single and/or multicomponent creams without interference from other drugs. The overall versatility of the two proposed methods suggests possible adaptation to quality control laboratories. Besides, MB method could be recommended as a good qualitative method for the detection of TF - as a thiono ester- in the presence of other drugs.

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