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Analysis of some antifungal drugs by spectrophotometric and spectrofluorimetric methods in different pharmaceutical dosage forms

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Abstract

Simple spectrophotometric and spectrofluorimetric methods are suggested for the determination of antifungal drugs; clotrimazole, econazole nitrate, ketoconazole, miconazole and tolnaftate. Spectrophotometric one depends on the interaction between imidazole antifungal drugs as n-electron donor with the π acceptor 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol or with *p*-chloranilic acid (*p*-CA) in acetonitrile. The produced chromogens obey Beer's law at λ_{max} 460, and 520 nm in the concentration range 22.5–200 and 7.9–280 μg ml^{-1} for DDQ, and *p*-CA, respectively. Spectrofluorimetric method is based on the measurement of the native fluorescence of ketoconazole at 375 nm with excitation at 288 nm and or the induced fluorescence after alkaline hydrolysis of tolnaftate with 5 M NaOH solution at 420 nm with excitation at 344 nm. Fluorescence intensity versus concentration is linear for ketoconazole at 49.7–800 ng ml^{-1} while for tolnaftate, it is in the range of 20.4–400 ng ml^{-1}. The proposed methods were applied successfully for the determination of all the studied drugs in their pharmaceutical formulations. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Imidazole antifungal drugs; π-Acceptors; Charge transfer complex; Ion-pair complex; Spectrofluorimetry; Thiono ester; Sodium β-naphtholate

1. Introduction

Antifungal drugs are widely used and commercially available in different pharmaceutical dosage forms [1] Four of the investigated drugs namely clotrimazole, econazole nitrate, ketoconazole and miconazole; possess imidazole ring to which almost all chemical and physical properties are attributed [2] while the fifth one, tolnaftate, contains thiono ester nucleus. The studied drugs possess either no significant absorption or relatively low absorption in the UV range. Reported methods for their analysis are mostly titrimetry [3], or based on ion-pair complex reaction [4,5], derivative spectrophotomerty [6,7], chromatographic methods [8–10], and electrochemical method [11]. All pharmacopeial methods for their determinations are either chromatographic [12,13] that are

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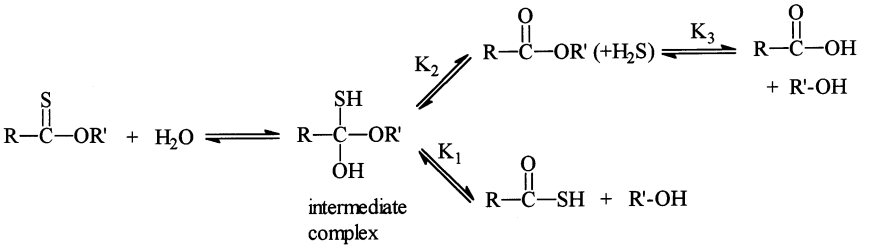


Scheme 1.

expensive or non-aqueous titrimetry [12–14] which are less sensitive methods. Recently CT complexation reactions of iodine as σ acceptor and the studied imidazole antifungal drugs have been performed in our laboratory [15]. π-Acceptor as DDQ reagent has been applied for the analysis of clotrimazole and ketoconazole by measurement of the coloured chromogenic product formed at λ_{max} 588 nm after heating at ~50°C [16] also it has been used successfully for the assay of other drugs of pharmaceutical importance [17–20]. On the other hand, *p*-chloranilic acid (*p*-CA) reagent has been widely used for the spectrophotometric analysis of many basic nitrogenous compounds [17,21–24]. Consequently, the present work describes additional two simple, rapid, and reliable spectrophotometric methods using these two reagents. The suggested methods depend mainly on the interaction of the tertiary amine moiety of the cited drugs with DDQ in methanol in the first method or with *p*-CA in acetonitrile in the second method. Concerning the structures of the studied drugs, only ketoconazole exhibits native fluorescent properties. At the same time one can expect in analogous to hydrolysis of thiono esters [25] (Scheme 1) that tolnaftate upon alkaline hydrolysis produces a β-naphthol moiety which is reported to have fluorescent properties [26]. However the hydrolysis of the other investigated drugs gives non-fluorescent products.

Therefore in attempt to increase sensitivity, two highly sensitive spectrofluorimetric methods were also developed for the assay of ketoconazole and tolnaftate. The first depends on the measurement of the native fluorescence of ketoconazole and the second depends on the measurement of the induced fluorescence after the alkaline hydrolysis of tolnaftate. All these suggested methods have been applied successfully to the analysis of the cited drugs in their pure forms as well as in their pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

A Jasco (Tokyo, Japan) Uvidec model 320 Spectrophotometer; Perkin-Elmer (USA) Model Lambda 3B UV–VIS Spectrophotometer, Kontron Spectrofluorometer SFM 23/B and W + W recorder Series 1100 (Swizierland).

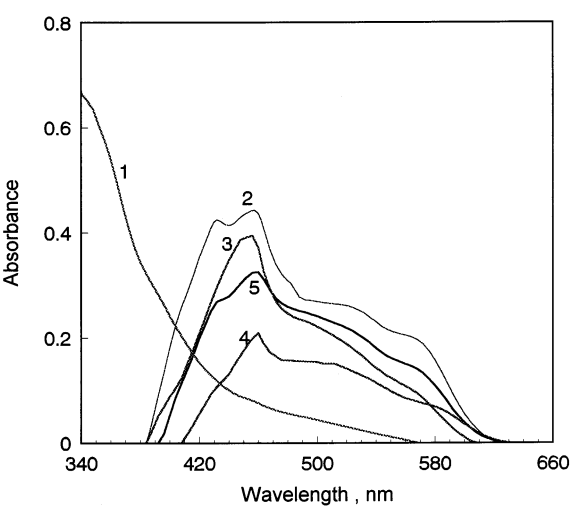


Fig. 1. Absorption spectra of DDQ reagent in methanol (1) and its coloured reaction product with clortimazole, 80 μg ml^{-1} (2), econazole, 80 μg ml^{-1} (3), ketoconazole, 50 μg ml^{-1} (4), and micronazole 65 μg ml^{-1} (5). Reference for the coloured reaction product is DDQ reagent in methanol.

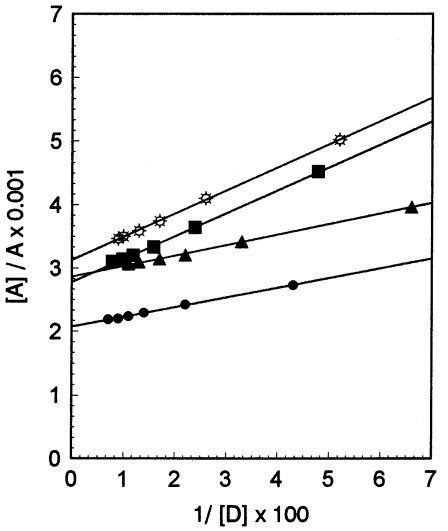


Fig. 2. Benesi-Hildebrand plot for the reaction product of DDQ with the studied drugs. (●) Clotrimazole; (■) econazole; (▲) ketoconazole (✡) Miconazole.

2.2. Chemicals and reagents

Clotrimazole (Alexandria Pharmaceutical Co., Alexandria, Egypt), Econazole nitrate (Amoun Pharmaceutical industries Co., APIC, Cairo, Egypt), Ketoconazole and miconazole (Janseen Pharmaceutical, Beerse, Belgium) and tolnaftate (Kahira Pharmaceuticals and Chemical industries Co., Cairo, Egypt) were obtained as gifts from their companies and were used as working standards. The purity of clotrimazole, econazole nitrate, ketoconazole or miconazole was 98.5% and of tolnaftate was 99.1%, as determined by $A_{1\,cm}^{1\%}$ [27]. DDQ (Sigma Chemical Co., USA) stock solutions, 4 mg ml^{-1} in methanol; *p*-CA (Sigma Chemical Co., USA) solution, 3.5 mg ml^{-1} in acetonitrile. Teorrell and Stenhagen buffer, pH range 7–12; Clark and Lubs buffer, pH 10; Kolthoff and Vleeschhouwer buffer, pH 10 [28]. All other chemicals and solvents used were of analytical grade.

2.3. Commercial formulations

Clotrimazole as canesten vaginal tablets, canesten powder, canesten cream and canesten topical solution (Alexandria Co., Egypt and Bayer, GFR); ketoconazole as nizoral tablets and nizoral cream (Janseen Pharmaceutica, Beerese, Belgium); econazole nitrate as gynoryl vaginal suppositories (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt); miconazole nitrate as daktarin powder and daktarin cream, (Janssen Pharmaceutica, Beerse, Belgium), gynodaktarin vaginal cream (Advanced Biochemical Industries, ABI, Cairo, Egypt); miconazole base as daktarin oral gel (Janssen Pharmaceutica Beerse, Belgium); tolnaftate as tineacure powder and Tineacure cream (Kahira Pharmaceuticals and chemical Industries Co., Cairo, Egypt). Tolnaftate as multicomponent cream; Quadriderm cream; (Memphis Pharmaceuticals and chemical Industries Co., Cairo, Egypt) labelled to contain tolnaftate as 10 mg g^{-1}, betamethazone as 0.5 mg g^{-1}, gentamycin sulphate as 1 mg g^{-1}, and iodochlorohydroxyquin as 10 mg g^{-1}.

2.4. Preparation of standard solutions

2.4.1. Clotrimazole, ketoconazole, miconazole and tolnaftate

Accurately weighed 100 mg of each of clotrimazole, ketoconazole, and miconazole and 25 mg of

Table 1
Association constant and correlation coefficient obtained from Benesi–Hildebrand equation and the standard free energy change of drug–DDQ reaction product at 460 nm[a]

Drug	Correlation coefficient (r)	ΔG^0 (kcal)	$K_c^{AD} \times 10^{-3}$ (l mol^{-1})
Clotrimazole	0.9992	−4.28	1.38
Econazole	0.9993	−3.94	0.77
Ketoconazole	0.9991	−4.44	1.79
Miconazole	0.9998	−4.00	0.86

[a] ΔG^0, standard free energy change; K_c^{AD}, association constant. Negative sign indicates endothermic reaction.

Table 2
Assay of dosage forms of clotrimazole and ketoconazole by the proposed DDQ, *p*-CA methods and reported methods[a]

Drug	Dosage form	Claimed (mg)	Proposed methods % found ± S.D.[b]		Reported methods
			DDQ	*p*-CA	% Found ± S.D.[b]
Clotrimazole	Vaginal tablets	100/tab	96.30 ± 0.52 $F = 2.799$ $t = 0.997$	96.49 ± 0.50 $F = 2.999$ $t = 1.408$	95.82 ± 0.87[c]
	Powder	10 g^{-1}	97.21 ± 0.67 $F = 1.535$ $t = 1.260$	97.13 ± 0.58 $F = 2.048$ $t = 1.135$	96.58 ± 0.83[c]
	Cream	10 g^{-1}	97.06 ± 0.64 $F = 2.250$ $t = 0.387$	97.72 ± 0.79 $F = 1.447$ $t = 1.592$	96.86 ± 0.96[c]
	Solution	10/ml	101.66 ± 0.30 $F = 3.610$ $t = 0.184$	101.28 ± 0.31 $F = 3.381$ $t = 1.175$	101.73 ± 0.57[c]
Ketoconazole	Tablets	200/tab	99.16 ± 0.50 $F = 2.074$ $t = 0.798$	99.29 ± 0.49 $F = 2.159$ $t = 1.091$	98.80 ± 0.72[d]
	Cream	20 g^{-1}	98.45 ± 0.71 $F = 2.858$ $t = 0.991$	99.57 ± 0.50 $F = 1.417$ $t = 1.762$	98.88 ± 0.42[d]

[a] Theoretical value for $t = 1.812$ and $F = 5.05$ (at $P = 0.05$).
[b] Mean of six determinations.
[c] Ref. [4].
[d] Ref. [35].

tolnaftate were transferred into separate standard flasks, dissolved and diluted quantitatively in the suitable solvent. For standard calibration curves, series of dilution were prepared in methanol (for DDQ) to obtain a range 292–1800, 344–2000, 225–1750 μg ml^{-1}, or in acetonitrile (for *p*-CA) to obtain a range 79–2400, 116–2800, and 149–2800 μg ml^{-1} for clotrimazole, ketoconazole and miconazole, respectively, and in distilled water to obtain a range of 0.49–8 μg ml^{-1} and 0.20–4 μg ml^{-1} for ketoconazole and tolnaftate, respectively (for fluorimetric method).

2.4.2. Econazole nitrate

An accurately weighed 100 mg of econazole base was dissolved in about 20 ml ethanol: water (1:1) mixture v/v and transferred into a 125-ml separating funnel, then 3 ml of 20% v/v ammonium hydroxide solution was added and shaken for 3 min. The alkaline aqueous layer was extracted successfully with four portions of chloroform each of 15 ml. The chloroform extract was filtered through anhydrous sodium sulphate, collected and evaporated under vacuum to dryness. The base (residue) was dissolved in 25 ml methanol or acetonitrile then diluted with the respective solvent to obtain series of dilution in the range 239–1750 μg ml^{-1} methanol and 128–2800 μg ml^{-1} acetonitrile for DDQ and *p*-CA method, respectively.

2.5. Preparation of sample solutions

2.5.1. Spectrophotometric methods

2.5.1.1. Tablets, vaginal tablets and powder

For clotrimazole and ketoconazole. Twenty tablets were weighed, finely powdered and mixed thoroughly. An accurate amount from powdered tablets or powder equivalent to 100 and 75 mg of each of clotrimazole or ketoconazole was shaken with 150 ml methanol (for DDQ method) or with

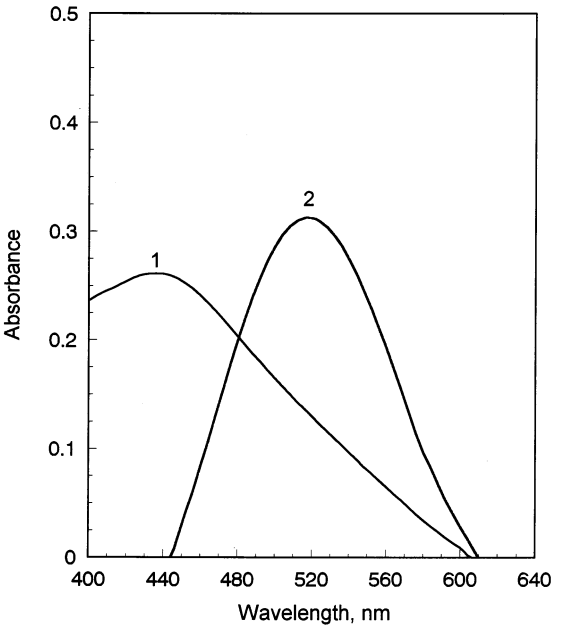


Fig. 3. Absorption spectra of *p*-chloranilic acid (1) and its coloured reaction product with econazole, 80 μg ml^{-1} (2) Reference for the coloured reaction product is *p*-CA reagent in acetonitrile.

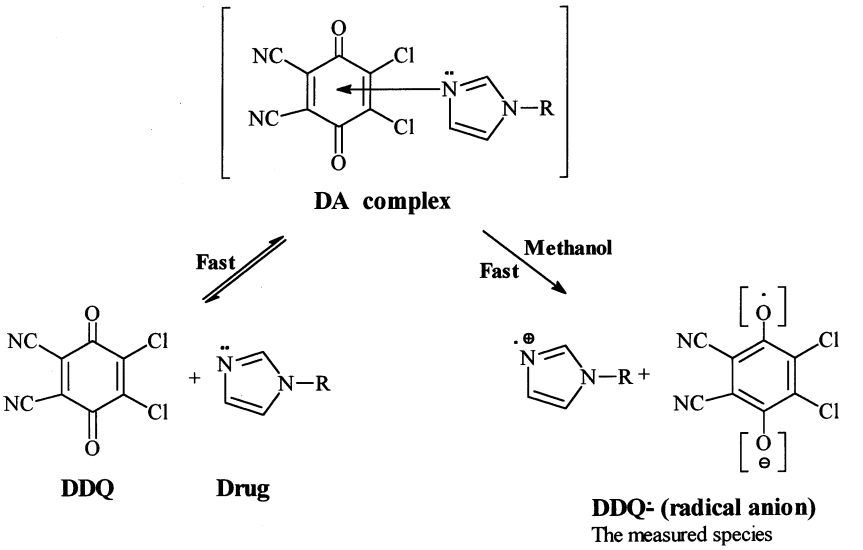
30 ml acetonitrile (for *p*-CA method) for about 2 min. The resulting solution was filtered into a 50-ml standard flask and insoluble residue was washed twice with 5–10 ml of the respective solvents. Filtrates were transferred to the standard flask and diluted with the same solvent so as to obtain sample solutions of clotrimazole or ketoconazole as 1 mg ml^{-1} in acetonitrile (for DDQ method) and 1.5 mg ml^{-1} in methanol (for *p*-CA).

For miconazole nitrate. An accurately weighed quantity of the powder equivalent to 250 mg of miconazole base was dissolved in 100 ml of methanol and filtered into a 250-ml round bottomed flask. The insoluble residue was washed twice with 10 ml methanol and filtrates were collected and concentrated by evaporation under vacuum. The concentrated solution was transferred quantitatively into a 25-ml standard flask and completed to volume with methanol. An aliquot of the methanolic solution equivalent to 100 or 75 mg of miconazole base (for DDQ and *p*-CA, respectively) was accurately transferred into a 125-ml separating funnel then 20 ml of distilled water and 5 ml of 20% v/v ammonium

Table 3
Assay of dosage forms of econazole nitrate and miconazole by the proposed DDQ, *p*-CA methods and standard addition method

Drug	Dosage form	Claimed (mg)	Proposed methods % found ± S.D.[a]		Standard addition method[b]		
			DDQ	*p*-CA	Added (mg)	% Recovery ± S.D.[a]	
						DDQ	*p*-CA
Econazole nitrate	Vaginal suppositories	50	97.88 ± 0.55	98.08 ± 0.40	50	99.81 ± 0.69	100.19 ± 0.83
						99.06 ± 0.53	99.57 ± 0.69
Miconazole	Oral gel	20 g^{-1}	97.89 ± 0.81	98.01 ± 0.60	20	99.62 ± 0.71	100.26 ± 0.54
					30	99.49 ± 0.63	99.67 ± 0.36
Miconazole nitrate	Vaginal cream	20 g^{-1}	97.16 ± 0.49	97.68 ± 0.84	20	98.93 ± 0.66	99.36 ± 0.86
					30	99.53 ± 0.59	98.90 ± 0.70
	Cream	20 g^{-1}	96.97 ± 0.57	97.35 ± 0.43	20	99.31 ± 0.88	99.39 ± 0.59
					30	99.50 ± 0.75	100.27 ± 0.62
	Powder	20 g^{-1}	97.73 ± 0.94	97.52 ± 0.37	20	98.61 ± 0.92	98.97 ± 0.77
					30	99.57 ± 0.54	99.95 ± 0.49

[a] Mean of six determinations.
[b] Ref. [31] page 132.



Scheme 2.

hydroxide solution were added and shaken for 5 min. The procedure was then completed as under econazole nitrate standard solution starting with: The alkaline aqueous layer was extracted … to dryness. The base residue was dissolved in the suitable solvent and diluted quantitatively so as to obtain 1 mg ml^{-1} miconazole in methanol for DDQ method or 1.5 mg ml^{-1} miconazole in acetonitrile for *p*-CA method.

2.5.1.2. Topical solution

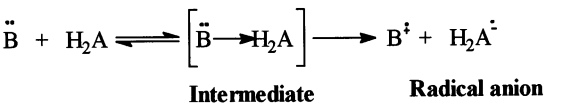
For clotrimazole. An aliquot of the solution, equivalent to 75 or 50 mg of clotrimazole was accurately transferred into a 50-ml standard flask and diluted to volume with acetonitrile or with methanol for DDQ or *p*-CA, respectively. Then solutions were diluted quantitatively as directed under tablets and vaginal tablets.

2.5.1.3. Oral gel

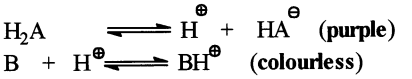
For miconazole. An accurately weighed quantity of the gel equivalent to 100 or 75 mg of miconazole was dissolved by sonication for 20 min in 30 ml of methanol or in acetonitrile for DDQ or *p*-CA, respectively. The resulting solution was filtered into a 50-ml standard flask. The insoluble residue was washed twice with 5 ml of the respective solvent and filtrates were transferred to the standard flask then completed to volume with the same solvent. The resulting solutions were diluted as directed under miconazole nitrate.

2.5.1.4. Cream

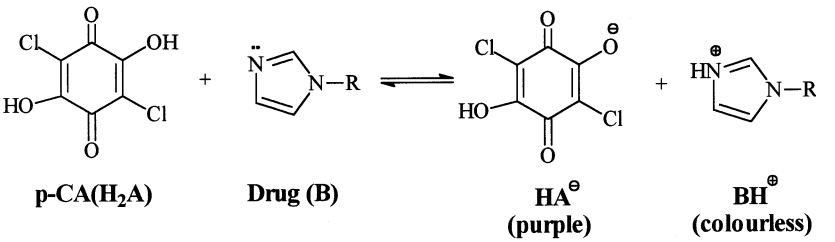
For clotrimazole, ketoconazole and miconazole nitrate. An accurately weighed portion of the cream equivalent to 100 mg (for DDQ method) or 75 mg (for *p*-CA method) of the base of each drug was placed in a 50-ml beaker. Aqueous methanol (30 ml, 70% v/v) (for miconazole nitrate) or 30 ml of a mixture of 1 M sulphuric acid and methanol, 1:4, (for clotrimazole and ketoconazole base) were added. The base was melted in a water bath at ~50°C and sonicated for 5 min. The mixture was transferred quantitatively



Scheme 3.



Scheme 4.



Scheme 5.

to a 125-ml separating funnel, shaken with three portions of carbon tetrachloride and organic layer was discarded. Ammonium hydroxide (5 ml, 20% v/v) was added and the mixture was shaken for 5 min. Then procedure was completed as mentioned under econazole nitrate standard solution starting with: The alkaline aqueous layer was extracted … to dryness. The base (residue) was transferred into standard flask and dissolved in the suitable solvents and resulting solution was diluted quantitatively as directed under tablets and vaginal tablets.

2.5.1.5. Vaginal suppository

For econazole nitrate. An accurately weighed portion of econazole nitrate suppository equivalent to 100 mg (for DDQ method) or 75 mg (for *p*-CA method) of econazole base was placed in a 50-ml beaker and melted in a water bath at ~ 50–60°C. The procedure was then completed as under cream starting with: The mixture was transferred quantitatively 125-ml separating funnel …

2.5.2. Spectrofluorimetric method

2.5.2.1. For ketoconazole and tolnaftate powder. An accurately weighed quantity of the powder equivalent to 25 mg of ketoconazole or tolnaftate was shaken with about 30 ml methanol for 3 min and filtered into 100 ml standard flask. The insoluble residue was washed twice with 10 ml methanol and the filtrates were transferred into the standard flask and completed to volume with methanol. The methanolic solution was diluted with water or with methanol so as to obtain 5 and 2 μg ml^{-1} ketoconazole and tolnaftate, respectively.

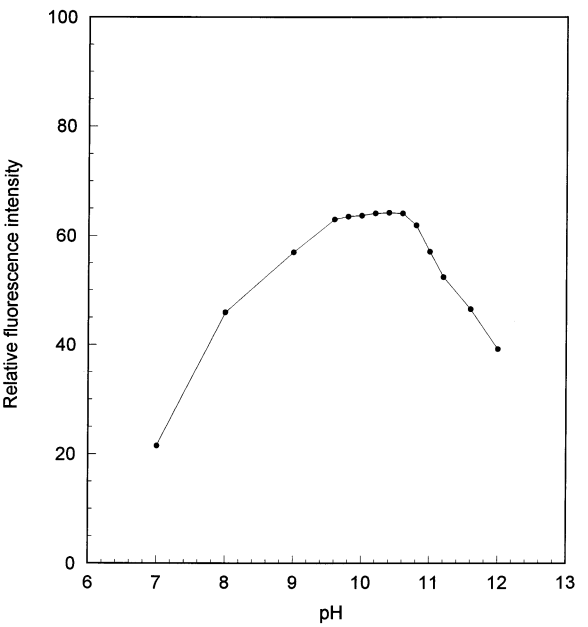


Fig. 4. Effect of Teorell and Stenhagen buffer pHs on the fluorescence of ketoconazole (0.5 μg ml^{-1}).

Table 4
Effect of solvent on the fluorescence of ketoconazole (0.5 μg ml^{-1})

Solvent	λ_{Exc}	λ_{Em}	Relative fluorescence intensity[a]
Water	288	375	69.8
DMSO	–	–	–
DMF	269	365	28.4
Acetonitrile	288	376	71.3
Acetone	326	432	20.6
Methanol	292	368	54.7
Ethanol	288	368	53.9
Isopropanol	–	–	–

[a] Mean of three determinations.

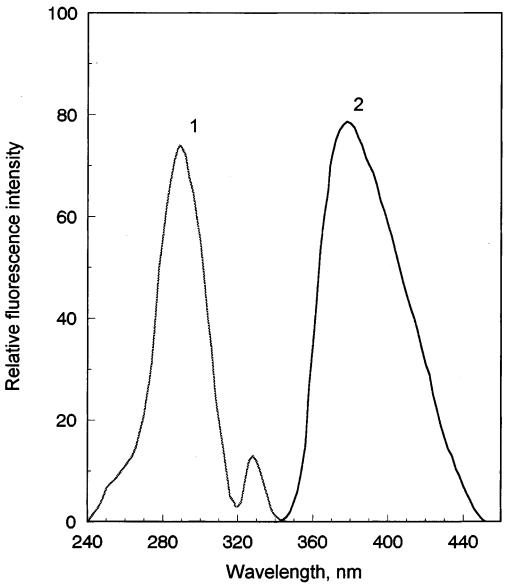
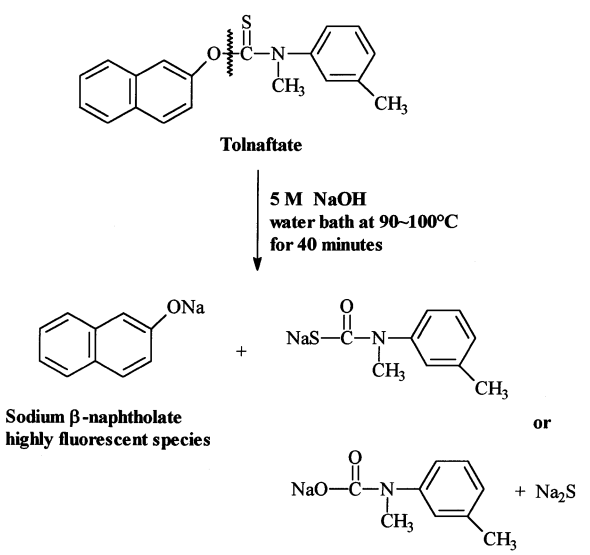


Fig. 5. Excitation (1) and emission (2) spectra of ketoconazole, 0.6 μg ml^{-1}, in 0.5 M Teorell and Stenhagen buffer, pH 10.



Scheme 6.

2.5.2.2. For ketoconazole cream. An accurately weighed portion of the cream equivalent to 25 mg of ketoconazole base was placed in a 50-ml beaker and then treated as described under ketoconazole cream in spectrophotometric method. The base (residue) was dissolved in 100 ml methanol. The methanolic solution was then diluted quantitatively with distilled water so as to obtain 5 μg ml^{-1} of ketoconazole.

2.5.2.3. For tolnaftate cream. An accurately weighed portion of the cream equivalent to 25 mg of tolnaftate was transferred into a 250-ml separating funnel and 75 ml of chloroform were added. The chloroform solution was washed with two portions; each of 25 ml of 0.1 N NaOH; two portions; each of 25 ml 0.1 N HCl; and 25 ml of distilled water. The chloroform extract was filtered and evaporated to dryness. The residue was then dissolved in methanol and the volume was diluted to 100 ml with the same solvent. The resulting solution was diluted quantitatively with methanol so as to obtain 2 μg ml^{-1} tolnaftate.

2.5.3. General procedure for spectrophotometric method

Into 10-ml standard flask, 1 ml of standard or sample solution of the studied drugs was transferred followed by 1 ml of DDQ solution (4 mg ml^{-1} for clotrimazole and econazole and 2.75 and 3.5 mg ml^{-1} in methanol for ketoconazole and miconazole, respectively) in case of DDQ method, or 1 ml of *p*-CA reagent solution (3.5 mg ml^{-1} in acetonitrile) for *p*-CA method. The solutions were allowed to stand for 7 min at room temperature for DDQ method then diluted with methanol to the mark and mixed well or diluted immediately with acetonitrile in case of *p*-CA method and mixed well. The absorbance was then measured at 460 and 520 nm (for DDQ and *p*-CA methods, respectively) against a reagent blank treated similarly.

2.5.4. General procedure for spectrofluorimetric method

2.5.4.1. Ketoconazole. An accurately measured quantity of 1 ml was transferred into different series of standard flasks. Teorell and Stenhagen buffer (4 ml, 0.5 M, pH 10) were added and mixed well. The solutions were allowed to stand for 15 min at 25 ± 1°C and diluted with water to the mark. The fluorescence intensity was measured at 375 nm with excitation at 288 nm against a reagent blank treated similarly.

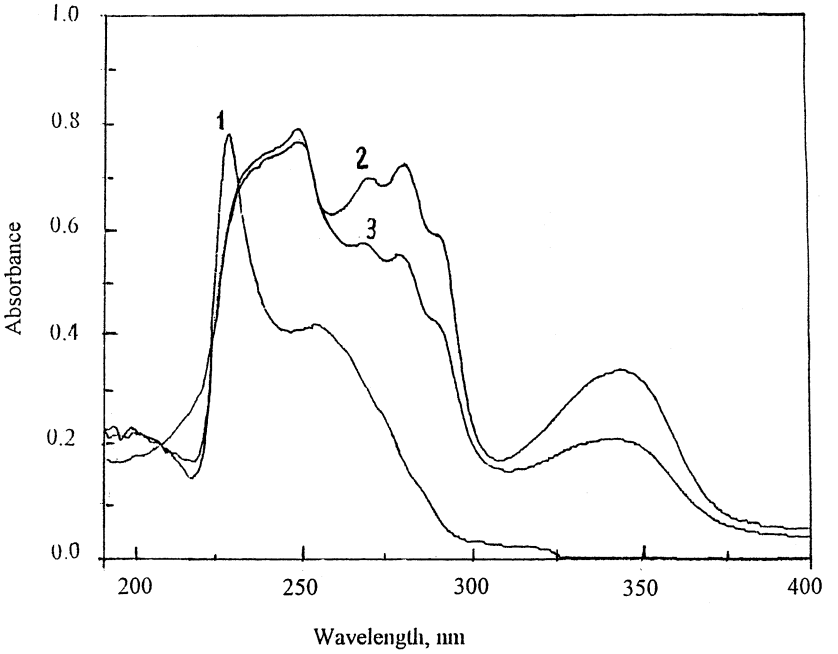


Fig. 6. Absorption spectra of tolnaftate (1), standard β-naphtol (2), and the hydrolytic product of tolnaftate (3), all in 5 M NaOH.

2.5.4.2. Tolnaftate. The standard or sample solution (2 ml) of tolnaftate was measured, transferred accurately into series of 10-ml test tubes, then 1 ml 5 M NaOH solution was added and mixed well. The solutions were allowed to stand for 40 min in a water bath at 95 ± 5°C, cooled to room temperature, transferred to 10-ml standard flasks and then diluted with 66% v/v aqueous methanol solution to the mark. The fluorescence intensity was measured at λ_{em} at 420 nm with λ_{ex} at 344 nm against a reagent blank prepared similarly.

2.5.5. Stoichiometric relationship

Job's method of continuous variation was used [29].

2.5.6. Association constant and free energy change for DDQ method

Series of drug solutions in methanol was prepared (2.32, 4.64, 6.96, 9.28. 11.6 and 13.92×10^{-3} M) for clotrimazole, (2.10, 4.19, 6.29, 8.38, 10.48 and 12.58×10^{-3} M) for econazole, (1.51, 3.01, 4.52, 6.02, 7.53 and 9.03×10^{-3} M) for

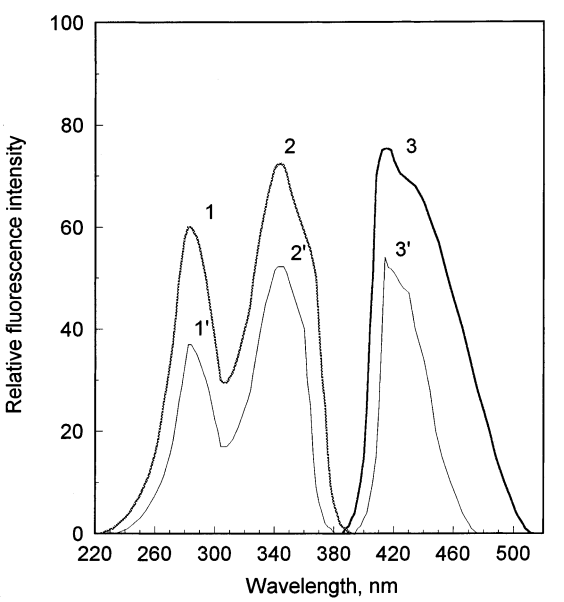


Fig. 7. Excitation (1, 2) and emission (3) spectra of the hydrolytic product of tolnaftate and excitation (1′, 2′) and emission (3′) spectra of the standard sodium B-naphtholate (B-naphtol in 20% N NaOH, 20% methanol and 60% water).

Table 5
Assay of dosage forms of ketoconazole and tolnaftate by using the proposed spectrofluorimetric method and reported or offical methods[a]

Drug	Dosage Form	Claimed (mg)	Found % ± S.D.[b]	
			Proposed method	Reported or official method
Ketoconazole	Powder		98.30 ± 1.06 $F = 2.571$ $t = 0.043$	98.32 ± 0.66[c]
	Cream		98.34 ± 1.27 $F = 1.267$ $t = 1.786$	99.73 ± 1.43[c]
Tolnaftate	Powder	10 g^{-1}	98.06 ± 0.79 $F = 3.680$ $t = 0.945$	97.72 ± 0.41[d]
	Cream[e]	10 g^{-1}	97.64 ± 1.07 $F = 2.510$ $t = 0.615$	97.95 ± 0.68[d]
	Cream[f]	10 g^{-1}	102.34 ± 0.94 $F = 2.510$ $t = 0.615$	135.42 ± 1.25[d]

[a] Theoretical values for $t = 1.812$ and $F = 5.05$ at ($P = 0.05$).
[b] Mean of six determinations.
[c] Ref. [35].
[d] Ref. [39] USP method.
[e] Single component cream contains tolnaftate.
[f] Multi component cream contains tolnaftate.

miconazole. These solutions and a DDQ solution in methanol (1.5×10^{-3} M) were placed in a thermostatically water bath at room temperature for 30 min. Each drug solution (5 ml) was mixed rapidly with 5 ml of DDQ solution. The absorbance of each solution was measured immediately at 460 nm against a reagent blank treated similarly. Association constant and free energy change were then calculated [30].

3. Results and discussion

3.1. Spectrophotometric method

The interaction of all the studied imidazole antifungal drugs with DDQ in methanol at room temperature gave an orange red coloured chromogen at 460 nm (Fig. 1). Maximum absorption was obtained at room temperature using 1 ml of DDQ solution containing 4.0, 4.0, 2.8 and 3.5 mg ml^{-1} methanol for clotrimazole, econazole, ketoconazole, and miconazole, respectively. Optimum reaction time was attained within 2.5–10 min for clotrimazole and 5–12.5 min for the other studied imidazole drugs. Among a variety of 11 different diluting solvents have been tested, methanol was found to be the best solvent. The developed chromogen attained its maximum stability after dilution within a period of 10 min. Under these optimum reaction conditions, regression analysis by the least square method [31] indicated excellent conformity with Beer's law in the concentration range 29.2–170, 23.9–170, 34.4–190, and 22.5–160 μg ml^{-1} for clotrimazole, econazole, ketoconazole and miconazole, respectively. Regression equations are:

$$A(\text{clotrimazole}) = 0.0051 + 0.0053c \quad (r = 0.9989).$$

$$A(\text{econazole}) = 0.0124 + 0.0052c \quad (r = 0.9992).$$

$$A(\text{ketoconazole}) = 0.0277 + 0.0049c \quad (r = 0.9985).$$

A(miconazole)

$= -0.0535 + 0.0058c \quad (r = 0.9994).$

where A is the absorbance, c is the concentration in μg ml^{-1}, and r is the correlation coefficient. The closely related slopes in the above regression equations prove that structural variation between the drugs did not affect the degree of formation of DDQ-radical anion which depends only on the presence of imidazole ring. Molar absorptivity ($\varepsilon \times 10^{-3}$) is 1.85, 1.96, 2.51, and 2.23 $dm^3\ mol^{-1}\ cm^{-1}$ as well as limit of detection (LOD) [32] (the lowest concentration of analyte in a sample that can be detected) is 8.76, 7.16, 10.31, and 6.75 μg ml^{-1} for clotrimazole, econazole, ketoconazole and miconazole, respectively. Using Job's method, the ratio of DDQ to each of the tested drugs was found to be 2:1 which is unexpected for clotrimazole, econazole, and miconazole possessing only one center; pyridine nitrogen of the imidazole ring; available for CT reaction. This could be attributed to the consuming of one molecule of DDQ in electrophilic substitution reaction on the 2-position of the imidazole ring by a chlorine atom from the DDQ and another molecule was consumed in CT complex reaction [2]. For ketoconazole, there is another center for the reaction; which is the tertiary amine moiety of piperazine ring; beside the imidazole ring. However, the first amido nitrogen of piperazine is not basic enough to participate as n-electron donor and the second one is blocked by the steric hindrance effect of DDQ as a bulky molecule. The association constant was evaluated at 460 nm for each drug–DDQ complex using the Benesi–Hildebrand equation [30]:

$$\frac{[A_0]}{A^{AD}} = \frac{1}{\varepsilon^{AD}} + \frac{1}{K_c^{AD}} \times \frac{1}{[D_0]}$$

where $[A_0]$, the concentration of the acceptor; $[D_0]$, the concentration of the donor; A^{AD}, the absorbance of the complex formed at 290 nm; ε^{AD}, the molar absorptivity of the complex formed at 290 nm; K_c^{AD}; the association constant of the complex (l mol^{-1}).

On plotting the values $[A_0]/A^{AD}$ versus $1/[D_0]$, straight lines were obtained (Fig. 2), from which the association constant, correlation coefficient and ΔG^0 [33] of all drug–DDQ reaction products were calculated (Tables 1 and 2). The standard free energy change of complexation (ΔG^0) is related to the association constant by the following equation [33]

$$\Delta G^0 = -2.303RT \log K_c$$

where ΔG^0, the free energy change of complex; R, the gas constant (1.987 cal mol^{-1}°C); T, the temperature in Kelvin degrees (273°C); K_c, the association constant of drug–iodine complex (l mol^{-1}).

The low values obtained for the association constants are common in these complexes due to the dissociation of the original donor–acceptor complex to the radical anion [34].

For p-CA method, the studied drugs gave an orange chromogen in acetonitrile at 520 nm (Fig. 3) using 1 ml of p-CA solution containing 3.5 mg ml^{-1} acetonitrile. The coloured chromogen was developed immediately and attained its maximum stability after dilution with acetonitrile for 1.5 h. Under these conditions, Beer's law is valid over the concentration range 7.9–240, 12.8–280, 11.6–280, and 14.9–280 μg ml^{-1} for clotrimazole, econazole, ketoconazole and miconazole, respectively. Regression equations are

A(clotrimazole) $= 0.0053 + 0.0039c \quad (r = 0.9999).$

A(econazole) $= 0.0023 + 0.0034c \quad (r = 0.9999).$

A(ketoconazole) $= 0.0221 + 0.0029c \quad (r = 0.9998).$

A(miconazole)

$= -0.0010 + 0.0032c \quad (r = 0.9997).$

The higher slope of clotrimazole (0.0039) reflects its low basicity comparing to other drugs. The LOD, 2.39, 3.83, 3.48 and 4.47 as well as molar absorptivity ($\varepsilon \times 10^{-3}$), 1.36, 1.30 1.56, and 1.34 $dm^3\ mol^{-1}$ cm for clotrimazole, econazole ketoconazole and miconazole, respectively, indicate that sensitivity of p-CA method is relatively lower than that in DDQ method. Using Job's method the ratio of p-CA to all the studied drugs is 1:1. Again due to the low basicity and the steric effect of the tertiary nitrogen of piperazine the ratio of p-CA to ketoconazole was found to be 1:1 instead of 2:1.

The two proposed spectrophotometric methods were applied successfully to the analysis of commercial dosage forms of the investigated imidazole drugs. The results in Table 2 are in good agreement with that obtained by the reported colorimetric method for clotrimazole [4] and reported UV method for ketoconazole [35]. The calculated t and F values do not exceed the theoretical values so there is no significant difference between the proposed and reported method. The method accuracy was confirmed by analysing samples of each of econazole nitrate and miconazole base added to known quantities of the drug. The difference between the analytical results for sample with and without the added amount gives the recovery of the amount of drug added [31]. Results of the recovery studies indicate accuracy of the proposed methods as well as absence of interference from common excipients and additives (Table 3). Comparing to reported method [15,17], both proposed methods offer the advantage of time saving, however they are less sensitive than iodine CT reported method [15]. Generally non-selectivity is the main disadvantage of the present study and of iodine method [15]. But fortunately this actually does not represent any problem because almost all studied drugs are usually formulated as single component in their pharmaceutical preparations.

3.2. Suggested mechanism for the reaction of DDQ and p-CA with imidazole drugs

3.2.1. For DDQ method

The mechanism of the reaction produced by the proposed method (DDQ method) depends on the formation of an original donor–acceptor (DA) complex through the interaction between tertiary amine moiety of the selected drugs as n-electron donor and DDQ as π-acceptor. The dissociation of DA complex was promoted by the high ionising power of the solvent methanol where complete electron transfer from the donor to the acceptor moiety takes place. This is followed by formation of the DDQ radical anions as a predominant chromogen [20,36] (Scheme 2).

3.2.2. For p-CA method

Some of the literature reveal that the reaction of p-CA (H_2A) with certain basic nitrogenous compounds (B) is probably due to CT complexation reaction according to Scheme 3[24].

Also other literature explains the reaction to be first a proton transfer from p-CA to the basic center of the drug (Scheme 4). Dissociation of the obtained ion pair salt was enhanced in the highly polar solvent acetonitrile to give the purple anion form of p-CA (HA^-). This was confirmed by IR spectrum, electron spin resonance and NMR[37,38].

Preliminary studies on the CT complex reaction between the studied iomidazole drugs and π-acceptors such as e.g. p-chloranil results in a very weak and slow reaction product. In spite, reaction of p-CA with the studied imidazole drugs is so fast and the sensitivity as well as stability of the purple chromogenic product is relatively high. Therefore we suggest that p-CA reaction could be an ion pair salt rather than a CT complex (Scheme 5). However, this suggestion is far from being conclusive.

3.3. Spectrofluorimetric method

To enhance the native fluorescence of ketoconazole, Teorell and Stenhagen buffer pHs ranged from 7 to 12 were tested and results prove that pH 10 gave the highest fluorescence by using 0.5 M solution in a volume of 4 ml of the same buffer (Fig. 4). Study the effect of other different buffer components; Clark and Lubs, Kolthoff and Vleesch-Houwer [28]; but of the same pH (pH 10) indicates that Teorell and Stenhagen buffer is the buffer of choice. Moreover by using different diluting solvents, acetonitrile and water were found to be the best solvents and almost gave equal readings (Table 4). Instead water was selected, as it is cheaper and safe. Other solvents resulted in a bathochromic shift as in acetone or a hypsochromic shift as in DMF. The effect of temperature revealed that the highest fluorescence was obtained at room temperature (25 ± 1°C). Further increase in temperature until 60°C shows a gradual decrease in FI about 1.4% for each degree. Also the time required to pass through

maximum is 10 min and fluorescence remained stable for 40 min then decreased sharply till 80 min. Consequently, all measurements were recorded after 20 min. Under the above mentioned conditions ketoconazole exhibited an excitation and emission spectra at 288 and 375 nm, respectively (Fig. 5).

For the thiono ester tolnaftate, preliminary study in various buffers of different pHs, or with 2–10 M hydrochloric acid solution at room temperature or in boiling water bath as well as with 2–10 M sodium hydroxide solution at room temperature did not yield any fluorescent properties. However, treatment of the drug with 2 M sodium hydroxide in a boiling water bath for about 20 min produced a fluorescent solution with two excitation maxima at 280 and 344 nm and one maximum emission at 420 nm. This highly fluorescent product could be due to the hydrolysis of tolnaftate at the thiono ester bond producing sodium β-naphtholate (Scheme 6) which is responsible for the strong fluorescent properties of the hydrolytic product.

This assumption was confirmed successfully by UV (Fig. 6) and fluorimetric (Fig. 7) as well as TLC techniques in comparison with pure sodium β-naphtholate (β-naphthol in 20% ethanol, 20% 1 N NaOH and 60% water [22]. By using chloroform as the mobile phase for TLC plate, the R_f values for both hydrolytic product of tolnaftate and standard β-naphthol is found to be the same (0.43). All variables affecting the hydrolysis of tolnaftate were studied; consequently complete hydrolysis was obtained by heating the drug solution in a water bath at 90–100°C with 5 M NaOH solution in a volume of 1 ml for 25 min. To enhance the FI of the hydrolytic product, different polar solvents /water mixtures was tested and methanol water (2:1) was found to be the optimum solvent. However pure solvents like dimethylsulphoxide, acetone, and acetonitrile were excluded due to insufficient mixing in presence of high concentration of sodium hydroxide. By applying the least-square method, Beer's law is valid in the concentration range of 49.7–800 ng ml^{-1} for ketoconazole and 20.4–400 ng ml^{-1} for tolnaftate. Regression equations are

$$A(\text{ketoconazole}) = 0.6966 + 0.13906c \quad (r = 0.9998).$$

$$A(\text{tolnaftate}) = -3.121 + 1.1588c \quad (r = 0.9970).$$

Limits of detection — 14.89 and 6.11 ng ml^{-1} for ketoconazole and tolnaftate, respectively, confirm the higher sensitivity of the proposed methods compared to other reported methods [35,39]. The developed methods are quite applicable to the analysis of ketoconazole and tolnaftate in their commercial formulations (Table 5). Results obtained are in good agreement with the reported method for ketoconazole [35] and the official USP method for tolnaftate [39] in single component dosage forms. For multicomponent drugs, no interference was observed by applying the proposed method for the assay tolnaftate in presence of other drugs (cream b) as betamethazone, gentamycin sulphate, and iodochlorohydroxyquin (Table 5).

Unlike reported HPLC methods for ketoconazole [10] and tolnaftate [12], both proposed methods are very simple, cheap and need neither expensive solvents nor sophisticated apparatus. In conclusion the described spectrofluorimetric could be applied successfully in quality control laboratories, in addition it could be recommended as a topic of study for the analysis of ketoconazole and or tolnaftate in biological fluids.

References

[1] J.N. Delgado, W.A. Remers, Wilson and Gisfold Textbook of Organic Medicinal and Pharmaceutical Chemistry, 10th ed., Raven Publishers, Philadelphia, NY, 1998, pp. 186–191.
[2] M.R. Grimmett, Comprehensive Heterocyclic Chemistry, vol. 5, first ed., Pergamon Press, Oxford, 1984, pp. 345–346.
[3] M. Massaccesi, Analyst 111 (8) (1986) 987–989.
[4] O.H. Abdelmageed, P.Y. Khashaba, Talanta 40 (8) (1993) 1289–1294.
[5] S.S. Zarapkar, U.P. Halkar, Indian Drugs 28 (6) (1991) 265–269.
[6] M.M. Bedair, M.A. Korany, M.A. Elsayed, O.T. Fahmy, J. Assoc. Offi. Anal. Chem. 72 (3) (1989) 432–435.
[7] N.A. El Ragehy, R.I. El Bagary, Bull. Fac. Pharm. Cairo Univ. 31 (3) (1993) 327–330.

[8] U. Roychowdhury, S.K. Das, J. Assoc. Offi. Anal. Chem. 79 (3) (1996) 656–659.
[9] A.K. Dash, J. Pharm. Biomed. Anal. 11 (9) (1993) 847–853.
[10] A.M. Di Pietra, V. Carvini, V. Andrisano, R. Gatti, J. Pharm. Biomed. Anal. 10 (10) (1992) 873–879.
[11] Z. Fijalek, J. Chodkowski, M. Warowna, Acta Pol. Pharm. 49 (1-2) (1992) 1-5(Pol). Through Anal. Abstr. 55 (1993) 11 G160.
[12] The United States Pharmacopeia XXIII, third revision, The National Formularly, 18th ed., US Pharmacopoeia Convension, Rockville, MD, 1995, pp. 407–409, 569, 570, 864, 865, 1026, 1027, 1569, 1570.
[13] British Pharmacopoeia, HM Stationery Office, London, 1998, pp. 842, 843, 893, 894, 1014.
[14] The European Pharmacopoeia, third ed., Council of Europe 1997, pp. 667, 668, 793, 794, 1201–1204, 1072, 1073.
[15] S.R. El-Shabouri, K.M. Emara, P.Y. Khashaba, A.M. Mohamed, Anal. Lett. 31 (8) (1998) 1367–1385.
[16] K. Kelani, L.I. Bebawy, Anal. Lett. 30 (10) (1997) 1843–1863.
[17] A.S. Amin, G.O. El-Sayed, Y.M. Issa, Analyst 120 (4) (1995) 1189–1193.
[18] H.F. Askal, Talanta 44 (1997) 1749–1755.
[19] G.A. Saleh, H.F. Askal, N. Omar, Analyst 116 (1991) 387–390.
[20] M.E. Abdel Hamid, M. Abdel Salam, M.S. Mahrous, M.M. Abdel-Khalek, Talanta 32 (10) (1985) 1002–1004.
[21] M. Abdel-Salam, M. Abdel-Hamid, M. Bedair, Egypt J. Pharm. Sci. 25 (1-4) (1984) 303–308.
[22] N.A. Zakhari, M.I. Rizk, M.I. Walash, Talanta 33 (2) (1986) 111–114.
[23] M.S. Mahrous, M.A. Abdel-Salam, A.S. Issa, M. Abdel-Hamid, Talanta 33 (2) (1986) 185–186.
[24] S.P. Agarwal, M.A.-H. ElSayed, Analyst 106 (1981) 1157–1162.
[25] S. Patai, The Chemistry of Carboxylic Acids and Esters, Wiley, London, NY, 1969, pp. 741–746.
[26] G.G. Guilbault, Fluorescence, Marcel Dekker, New York, 1967, p. 325.
[27] A.C. Moffat, J.V. Jackson, M. Moss, B. Widdop, E.S. Greefield, Clarke's Isolation and Identification of Drugs, second ed., The Pharmaceutical Press, London, 1986, pp. 487, 488, 579, 696, 697, 784, 785, 1032, 1033.
[28] M. Pesez, J. Bartos, Colourimetric and Fluorimetric Analysis of Organic Compounds and Drugs, Marcel Dekker, New York, 1974, pp. 623–630.
[29] A. Martin, Physical Pharmacy, fourth ed., Lea & Febiger, Philadelphia, London, 1993, pp. 260–261.
[30] H.A. Benesi, J. Hildebrand, J. Am. Chem. Soc. 71 (1949) 2703 through Physical Pharmacy, fourth ed., Lea & Febiger, Philadelphia, London, 1993, pp. 266–267.
[31] G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denny, Vogels Textbook of Quantitative Chemical Analysis, fifth ed., The Bath Press, Great Britain, 1989, pp. 127–148.
[32] The United States Pharmacopeia XXIII, third revision, The National Formularly, 18th ed. U.S. Pharmacopeial Convension, Rockville, MD, 1995, p. 1983.
[33] A.N. Martin, J. Swarbrick, A. Cammarata, Physical Pharmacy, third ed., Lee and Febiger, Philadelphia, 1983, pp. 344–346.
[34] R. Foster, OrganicCharge-transfer Complexes, Academic Press, London, 1969, pp. 190–205.
[35] E.R.M. Kedor-Hackmann, M.M.F. Nery, M.I.R.M. Santoro, Anal. Lett. 27 (2) (1994) 363–376.
[36] J. Dickmann, C.J. Pederson, J. Org. Chem. 28 (1963) 2874–2880.
[37] M.E. Abdel-Hamid, M.S. Mahrous, M.M. Abdel-Khalek, M.A. Abdel-Salam, Egypt J. Pharm. Sci. 25 (1–4) (1984) 291–301.
[38] M.A. Elsayed, M.E. Abdel-Hamid, M.A. Korany, M.H. Abdel-Hay, S.M. Galal, Spectrosc. Lett. 17 (12) (1984) 803–818.
[39] The United States Pharmacopeia XXIII, third revision, The National Formularly, 18th edn. U.S. Pharmacopeial Convension, Rockville, MD, 1995, pp. 1568, 1569.