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COLORIMETRIC DETERMINATION OF CERTAIN ANTIFUNGALS IN PURE FORMS AND IN THEIR PHARMACEUTICAL FORMULATIONS*

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تم في هذا البحث إستنباط وتقيم طريقه لونية مرئية ، دقيقه وحساسة لخمسة عقاقير مضادة للفطريات هى الفلوكونازول ، الكيتوكونازول ، نترات الميكونازول والطولنفتات. وقد إعتمدت هذه الطريقة على تكثيف أي من العقاقير سالفة الذكر مع محلول مشبع لحامض الستريك في أستيك انهيدريد اللامائى في حمام مائي عند درجة ١٠٠مئوية لمدة تتراوح من ٢ إلى ٣٠ دقيقة ثم قياس اللون الناتج عند ٥٣٥ نانوميتر بعد تخفيفه بالاثياتول اللامائي .وقد تم أيضا ضبط كل العوامل المؤثرة على ظروف التفاعل والحصول على علاقة خطية بين شدة اللون والتركيز بمعامل إرتباط جيد يتراوح بين ٠,٩٩٩٦ – ٠,٩٩٩٩ لتركيز عام لكل العقاقير المستخدمة يتراوح من ٠,٠٨ – ٧ ميكرو جرام لكل مللى لتر مع الحصول على حدود للكشف والتقدير تتراوح بين ٠,٠٣٩ – ٠,٢٦ و٠,١٣ – ٠,٨٧ ميكروجرام لكل مالى لتر على التوالي. كما تم تطبيق الطريقة المقترحة بنجاح لتحليل العقاقير المذكورة في صورها النقية والمستحضرات الصيدلية مثل المحاليل والبودرة المستخدمة من الخارج ، الأقراص المستخدمة عن طريق الفم أو الأقراص المهبلية والكريمات ذات الاستخدام الخارجي . هذا وقد تم الحصول على متوسط عام في حدود ٩٤,١٤ –٩٩,٠٢ % وبدرجة عالية من الدقة بعد تحليل هذه المستحضرات بالطريقة المقترحة . كما لم يلاحظ أي تداخل لكل من مشتقات الكورتيزون مثل البيتاميثازون ، الديكساميثازون والإضافات الصيدلية المستخدمة عامة في تحضير المستحضرات الصيدلية . ولقد تمت مقارنة الطريقة المقترحة بالطرق المنشورة وتم الحصول على نتائج إحصائية مرضية باستخدام بعض الطرق الإحصائية المتعارف عليها (اختبارى ت ، ف) مما يؤكد عدم وجود فرق ذو دلالة واضحة بين نتائج الطريقة المقترحة والمنشورة. هذا وتبرهن النتائج لكل من النسب المئوية والإحصائيه على كفاءة الطريقة المقترحة لتحليل المركبات المقترحة في مستحضراتها الصيدلية بدقه مرضية.

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An accurate and sensitive visible spectrophotometric method was developed and validated for the analysis of five antifungal drugs namely; clotrimazole, fluconazole, ketoconazole, miconazole nitrate and tolnaftate in pure as well as in their pharmaceutical dosage forms. This method was based on condensation of any of the cited drug with saturated solution of citric acid in anhydrous acetic anhydride in boiling water bath for about 20–30 min. The produced color was measured spectrophotometrically at 535 nm, after dilution with absolute ethanol. All variables affecting reaction conditions were optimized and the regression analysis of Beer's *plots showed good correlation coefficients (0.9996 - 0.9999) for the previously mentioned drugs in a general concentration range of 0.8–7 μg ml^{-1} with overall limits of detection and quantitation in the following ranges: 0.039–0.26 μg ml^{-1} and 0.13–0.87 μg ml^{-1}, respectively. The proposed method has been applied successfully for the analysis of bulk drugs and their dosage forms such as topical powder and solution, oral and vaginal tablets, and topical creams. Overall percentage recoveries in the average range: 94.14–99.02% was obtained with considerable accuracy upon analysis of these dosage forms by the proposed method. No interference could be detected from betamethasone or dexamethosane, encountered excipients and additives. The obtained results have been compared with those obtained from reported and / or official method(s) and proper F- and t- values were observed; indicate no significance difference between the results of the proposed and reported methods. The good percentage recoveries and proper statistical data proved the efficiency of the proposed method for the analysis of the cited drugs in their commercial dosage forms with quite satisfactory precision.*

INTRODUCTION

Antifungal drugs are widely used and commercially available in different pharmaceutical dosage forms.[1] Four of the studied drugs namely clotrimazole, fluconazole, ketoconazole, and miconazole nitrate are considered as imidazole derivatives antifungal drugs.[1] Those imidazole antifungal drugs are considered as a drug class for their broad spectrum, oral bioavailability and low toxicity.[1] The fifth one, tolnaftate is a topical antifungal drug belongs to thionoester or sulphur containing group.[1] It has been found widespread use over the past several decades. It is highly recommended for

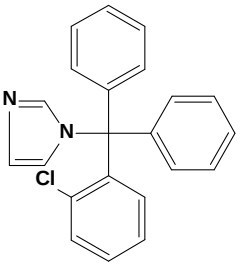
the treatment of various dermatophtes and several forms of microsporon that can cause infection conditions like *Tinea pedis* (athlete's foot), *Tinea cruris* (jock itch) and *Tinea capitis* (body ring worm). However, it is ineffective against *candida albicans* or gram negative and gram positive bacteria.[1]

Several methods have been reported for the analysis of these drugs either alone or in presence of their degradation products or other drugs or in pharmaceutical formulation or biological fluids. These methods are mostly based on spectrophotometry,[2-12] spectrofluorimetry,[6&13] titrimetry,[14-16] chromatographic procedures,[12,17-22] electrophoresis[23&24] and electrochemical methods.[25-27]

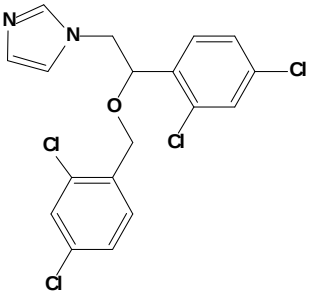
Direct UV absorption for the cited drugs is very limited because they possess either no significant absorption or relatively low absorption intensity in the UV range. The pharmacopeial methods available for the determination of the cited drugs are either HPLC[28] which are expensive, or non aqueous titrimetry[15-16] which are time consuming and they lack the necessary sensitivity. Some of the reported spectrophotometric methods are based on ion pair extraction technique using halogenated solvent[10] which are dangerous to life and environment. Most of the reported methods for analysis of tolnaftate are considered as indirect ones because they depend upon either its hydrolysis or degradation to other intermediate that can be analyzed as reported.[3&7]

Therefore the aim of this work is to develop an accurate, sensitive, inexpensive and non-extractable spectrophotometric method for the quantitative determination of these drugs in pure and in dosage forms either alone or in presence of other active ingredients such as betamethasone dipropionate or dexamethasone acetate, that could be applied for quality control of the cited drugs in its pharmaceutical formulations.

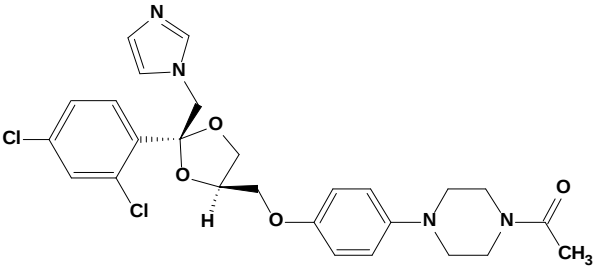
In the present work, the developed method depends on the reaction of the tertiary nitrogen, exists in all the cited drugs (Figure 1), with citric acid /acetic anhydride (CAA) at boiling temperature water bath, with the formation of a colored condensation product. The developed color can be measured spectrophotomerically at 535 nm after completion to volume with absolute ethanol, against reagent blank treated similarly.



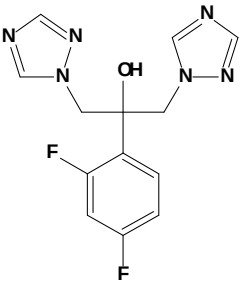
Clotrimazole



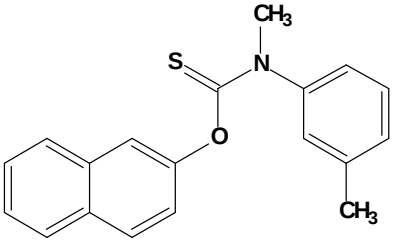
Miconazole (as nitrate)



Ketoconazole



Fluconazole



Tolnaftate

Fig. 1: structures of the studied drugs

EXPERIMENTAL

Apparatus

Spectronic Genesys, connected to an IBM computer loaded with the Winspec application software (Milton Roy, USA) and Jenway 6505 (Jenway LTD., UK) ultraviolet-visible spectrophotometers with matched 1 cm quartz cells were used throughout this study for all measurements. A thermostatically controlled water bath was also used.

Chemicals and reagents

Ketoconazole and miconazole nitrate (Janssen Pharmaceuticals, Beerse, Belgium), clotrimazole (Alexandria Pharmaceutical Co., Alexandria, Egypt), fluconazole (E.I.P.I. Co. Egyptian Intrnational. Pharmaceutical Industries Co. 10th of Ramadan City, Egypt), tolnaftate (Kahira Pharmaceuticals & Chemical industries Co., Cairo, Egypt), betamethasone dipropionate (Memphis Chemical Co, Cairo, Egypt), dexamethasone acetate (Alexandria Pharmaceutical Co., Alexandria, Egypt) were obtained as gifts from their companies as working standards as certified by their local companies and were used as received. Acetic anhydride and citric acid (El-Nasr Pharmaceutical Co., Cairo, Egypt), other chemicals and solvents used in this work were of analytical grade.

Pharmaceutical formulations

The following pharmaceutical preparations were obtained from local market and were subjected to analysis by the proposed analytical procedure. Locasten™ vaginal tablets, Locasten™ powder, Locasten™ topical solution and Locasten™ topical cream (Alexandria Co., Egypt and Bayer, GFR) labeled to contain clotrimazole as 100 mg/tablet, 10 mg/g, 10 mg/ml and 10 mg/g, respectively. Lotriderm™ cream (Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt) each gram labeled to contain 10 mg clotrimazole and 0.64 mg betamethasone dipropionate, equivalent to 0.5 mg betamethasone. Baycuten™ N cream (Memphis Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt) each gram labeled to contain I0 mg clotrimazole and 0.443 mg dexamethasone acetate, equivalent to 0.4 mg dexamethasone. Nizoral™ tablets and Nizoral™ topical cream (Janssen Pharmaceuticals, Beerse, Belgium) labeled to contain ketoconazole as 200 mg/tablet and 20mg/g, respectively. Miconaz™ powder, Miconaz™ cream (Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt) labeled to contain miconazole nitrate as 20 mg/g, 20 mg/g, respectively. Flucoral™ capsules, (Sedico Pharmaceutical Co. 6 October City, Egypt) each capsule labeled to contain 150 mg fluconazole. Tineacure™ cream (kahira Pharmaceuticals and Chemical industries Co., Cairo, Egypt) each gram labeled to contain 10 mg tolnaftate.

Preparation of reagent and sample solutions

1- Preparation of saturated solution of citric acid in acetic anhydride (CAA) reagent[29]

About 100 ml of acetic anhydride was added to excess solid citric acid in a stoppered conical flask and the mixture was shaken for about 2 hr. The flask was left to stand for about additional 15 min to allow sedimentation of the excess solid, and then the supernatant solution was decanted. This solution must be freshly prepared.

2- Preparation of standard stock solutions

Stock solutions of clotrimazole, ketoconazole and miconazole nitrate were prepared in absolute methanol to obtain 0.08 mg ml^{-1}. For tolnaftate and fluconazole stock solutions were also prepared in the same solvent to obtain 1.6 mg ml^{-1}. From these solutions working standard solutions were prepared by appropriate dilution, with methanol, in order to obtain 8–24 µg ml^{-1} for clotrimazole, ketoconazole and miconazole nitrate, and 30–70 µg ml^{-1} for fluconazole and tolnaftate.

3- Preparation of samples for Locasten™ vaginal tablets, Locasten™ topical powder and Nizoral™ oral tablets

Twenty tablets were weighed, finely powdered and mixed thoroughly. An accurate amount from powdered tablets or topical powder preparation, equivalent to 25 mg of drug, was transferred into a 50 ml volumetric flask followed by addition of about 30 ml methanol, and the mixture was shaken well for about 5 min and then completed to volume with the same solvent. The resulting solution was filtered where the first portion of the filtrate was rejected. Then the filtrate was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range where suitable aliquot(s) was/or were subjected to analysis as explained under general procedure.

4- Preparation of sample for Locasten™ topical solution

Into a 25 ml volumetric flask, an aliquot of the commercially available solution, equivalent to 25 mg of the drug, was accurately measured and diluted to volume with methanol. The resulting solution was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range where suitable aliquot(s) was/ or were subjected to analysis as explained under the general procedure.

5- Preparation of samples for Locasten™, Lotriderm™, Baycuten™ N and Nizoral™ topical creams

An accurately weighed portion cream, equivalent to 20 mg of the drug, was placed in a 100 ml beaker. 20 ml of a mixture of 1 M sulfuric acid and methanol (1:4) were added. The base was melted in a water bath at about 50° for 5 min, and then sonicated for another 5 min. The resulting mixture was transferred

quantitatively to a 125 ml separating funnel, then was shaken with 3x10 ml portions of carbon tetrachloride and the combined extracts were discarded.[16] The aqueous layer was rendered alkaline with 20% aqueous ammonia solution, followed by addition of further 5 ml of ammonia solution (20%), and then extracted with 3x10 ml portions of chloroform. The chloroform extract was filtered through anhydrous sodium sulfate, which is further washed with additional 10 ml chloroform. The chloroform extract as well as washing were collected and evaporated under vacuum to dryness. The residue remained was dissolved in about 20 ml methanol, transferred quantitatively into 50 ml volumetric flask and completed to volume with the same solvent. The resulting solution was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range where suitable aliquot(s) was/ or were subjected to analysis as explained under the general procedure.

6- Preparation of sample for Miconaz™ cream

An accurately weighed portion of cream, equivalent to 20 mg of miconazole nitrate was placed in a 100 ml beaker. Aqueous methanol (20 ml, 80% v/v, methanol in water) was added. The base was melted in a water bath at about 50° for 5 min, and then sonicated for about 5 min. The resulting mixture was transferred quantitatively to a 125 ml separating funnel, then shaken with 3x10 ml portions of carbon tetrachloride and the combined extracts were discarded.[6] The aqueous layer was drained into 50 ml volumetric flask and completed to volume with methanol. The resulting solution was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range where suitable aliquot(s) was/ or were subjected for analysis as explained under the general procedure.

7- Preparation of sample for Tineacure™ cream

An accurately weighed portion of the cream, equivalent to 10 mg of the drug was transferred into 250 ml separating funnel and 75 ml of chloroform was added. The chloroform solution was washed with two portions; each of 25 ml of 0.1 M sodium hydroxide; two portions; each of 25 ml 0.1 M hydrochloric acid; and 25 ml of distilled water. The chloroform extract was filtered through chloroform washed cotton pledget. The filter bed was washed thoroughly with additional 10 ml chloroform. The combined filtrate and washing were evaporated to dryness. The residue was then dissolved in about 20 ml methanol and the resultant solution was transferred quantitatively into 25 ml volumetric flask and diluted to volume with the same solvent. The resulting solution was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range for this drug and then suitable aliquot(s) was/or were subjected to analysis as

explained under the general procedure.

8- Preparation of sample for Flucoral™ capsules

The contents of 10 capsules were emptied, weighed, mixed well and finely powdered. An accurately weighed amount of the powder, equivalent to 50 mg fluconazole was transferred into 50 ml volumetric flask and was diluted to about 30 ml with methanol. The mixture was shaken well for about 5 min and finally completed to volume with the same solvent. The resulting solution was filtered where the first portion of the filtrate was rejected. Then the resulting solution was diluted quantitatively, with methanol, to obtain concentration within the working standard solutions range for this drug and then suitable aliquot(s) was/ or were subjected to analysis as explained under the general procedure.

9- Recovery experiments

An accurately weighed amount of each of the studied drug was added to an accurately weighed quantity of its corresponding formulation, and then the procedure was continued as mentioned under the analysis of these dosage forms.

General analytical procedure

Into a set of clean and dry test tubes, aliquots of the working standard solution or sample preparation of the drug within the working concentration range were pipetted and evaporated to dryness in a boiling water bath. Then about 2.5 ml of CAA reagent were added to each tube and the set was allowed to stand in a boiling water bath for about 20 min for clotrimazole, ketoconazole and miconazole nitrate; for about 25 min for fluconazole and for about 30 min for tolnaftate. After cooling the resultant solution to room temperature, the content of each tube was transferred quantitatively into 10 ml volumetric flask using absolute ethanol. The resulting solution was completed to volume with the same solvent. The intensity of the developed violet color was measured at 535 nm against reagent blank solution treated similarly.

RESULTS AND DISCUSSION

Citric acid – acetic anhydride reagent has been used for determination of some pharmaceuticals containing tertiary nitrogen.[29&30] This reagent was selected for determination of the cited drugs either in pure or in their pharmaceutical dosage forms because it has several advantages over other reported reagent(s) commonly used for the spectrophotometric determination of the cited drugs. These advantages are; it is commercially available at low cost relative to those used as charge transfer complex reaction,[6] its preparation does not require any specific solvent. In addition, no need to release free base, where good results were observed with free bases such as clotrimazole, ketoconazole and with salt such as

miconazole nitrate as well. Finally, compared to ion pair spectrophotometric method,[10] the intensity of condensed colored product was measured directly, after proper dilution with absolute ethanol without prior extraction step. Thus under the established experimental conditions, the cited reagent was condensed with each of the studied drugs to give violet colored condensation product, which upon proper dilution with absolute ethanol gave maximum absorbance intensities at about 535 nm.

Optimization of reaction parameters

In order to establish the optimum volume of reagent, temperature, time of reaction and proper solvent used for dilution as well as stabilities of the product formed, recommended for maximum absorbance intensities, a series of experiments were conducted using fixed amount of drug and the parameter to be studied is varied, then the general suggested procedure was followed.

1- Effect of reagent's volume

The effect of reagent's volume on the color intensities of the products formed is summarized in Figure 2. Maximum color intensities were achieved upon using 2–3 ml of reagent.Thus 2.5 ml was used withall the studied drugs and chosen for subsequent investigation.

2- Effect of temperature and heating time

The effect of temperature and heating time at the chosen reagent's volume on the color intensities of the reaction product(s) was also studied (Figures 3, 4). Maximum color intensities were achieved at boiling water bath temperature. The optimum heating time was about 15–30 min for clotrimazole, ketoconazole and miconazole nitrate; about 20–30 min for fluconazole, and about 25–35 min for tolnaftate. With all the studied drugs shorter or longer periods of time resulted in lower color intensities. Thus heating time about 30, 25, 20 min were chosen for tolnaftate, fluconazole and the rest of drugs respectively and were used for subsequent investigation.

3- Effect of diluting solvent

Many types of solvents were tested, which cover wide range of dielectric constant, such as water, methanol, absolute ethanol, acetonitrile, dimethylformamide and acetone. Better sensitivity, stability and sharp λ_{max} was observed upon using absolute ethanol as diluting solvent. Accordingly; this solvent was selected along this work. The absorption spectrum of clotrimazole, as representative example, is shown in Figure 5.

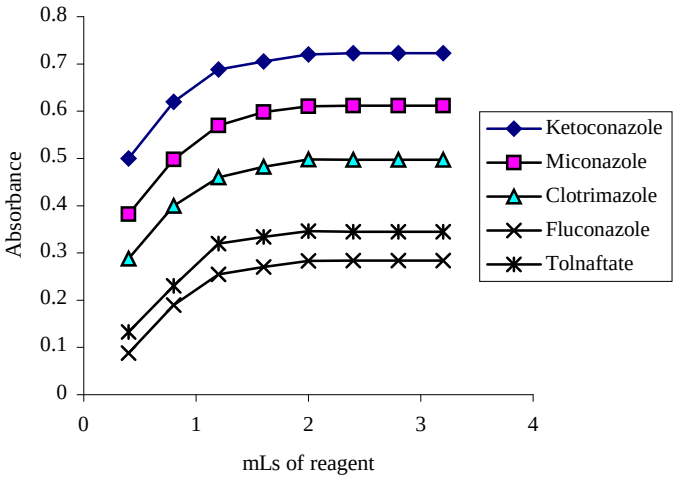


Fig. 2: Effect of reagent's volume on the absorption intensities of the studied drugs: clotrimazole, ketoconazole and miconazole nitrate (2 µg ml^{-1}). Fluconazole and tolnaftate (4 µg ml^{-1}).

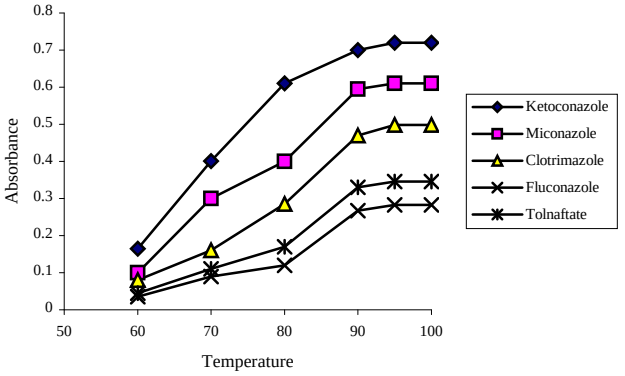


Fig. 3: Effect of temperature on the absorption intensities of the studied drugs : clotrimazole, ketoconazole and miconazole nitrate (2 µg ml^{-1}). Fluconazole and tolnaftate (4 µg ml^{-1}).

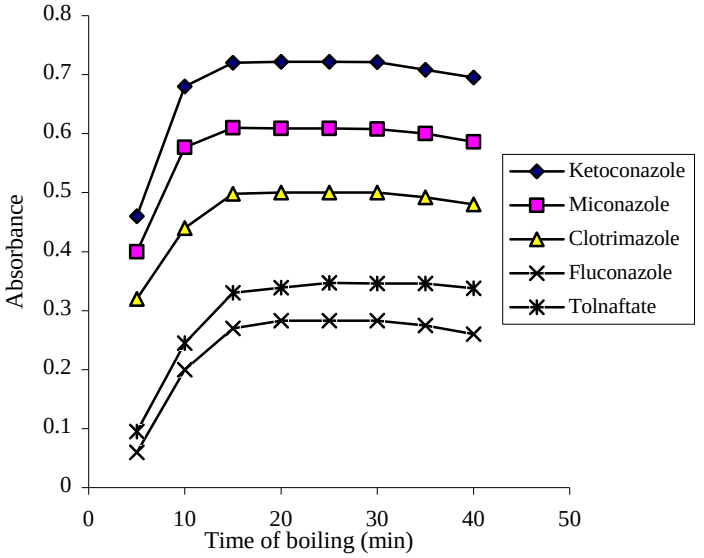


Fig. 4: Effect of boiling time on the absorption intensities of the studied drugs : clotrimazole, ketoconazole and miconazole nitrate (2 µg ml^{-1}). Fluconazole and tolnaftate (4 µg ml^{-1}).

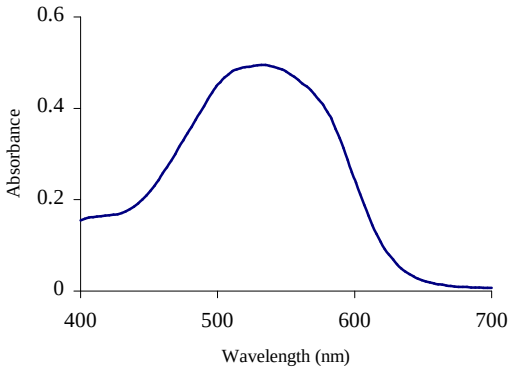


Fig. 5: Absorption spectrum of the condensed colored obtained with clotrimazole (2 µg ml^{-1}).

4- Stability of the colored products

Under the previously established experimental conditions, the stability of the color formed was also studied with all the studied drugs. With clotrimazole, ketoconazole and miconazole nitrate, the produced color was found to be stable for at least 35 min, while with fluconazole and tolnaftate, the product was only stable for about 25 min.

Validation of the proposed method[28]

1- Linearity, detection and quantitation limits

Under the optimized reaction conditions, the calibration curves for the investigated drugs with the target reagent used in this work were constructed by analyzing a series of the working standard solutions of the drugs. The assay was performed according to the general suggested analytical procedure previously described under the experimental section. The regression equations for the results were derived by using the least square method. In all cases absorbencies (n = 3) were linear with good correlation coefficients in the general concentration range of 0.8–7 µg ml^{-1}. The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the following equation:[31] LOD or LOQ = k SD_a/b; where k = 3 for LOD and 10 for LOQ. SD_a is the standard deviation of the intercept, and b is the slope. On the basis of 3 replicate measurements the overall LOD and LOQ values were 0.039–0.26 µg ml^{-1} and 0.13-87 µg ml^{-1} respectively. Ketoconazole gave relatively the highest sensitivity, followed by miconazole nitrate, clotrimazole, tolnaftate and finally fluconazole. The reason of such higher sensitivity observed with ketoconazole can be accounted on the presence of piperazine ring in which the tertiary amine group, para-substituted to phenoxy group is more basic than imidazole nitrogen. In case of tolnaftate, the tertiary nitrogen exists in its structure being a substituted thionocabamate ester, thus it is considered a weaker base than imidazole derivatives. Accordingly; its catalytic effect is much less than them. Fluconazole being a triazole derivative, since it is very weak base, indicated by its pKa value,[32] therefore its catalytic effect should be much less than all the studied drugs. Data of such analysis are summarized in Table 1.

2- Repeatability

The precision of the proposed method was checked by replicate analysis for six separate samples solutions of each of the cited drugs. The relative standard deviations (RSD) were 0.89, 1.03, 1.73, 1.94 and 1.91 for a concentration of 2 µg ml^{-1} for clotrimazole, ketoconazole, miconazole nitrate and 4 µg ml^{-1} for fluconazole, tolnaftate, respectively. The bias values (% deviation from the regression line values) were -2.4, -0.5, -2.1, -1.75 and -1.83 for clotrimazole, ketoconazole, miconazole nitrate, fluconazole and tolnaftate respectively. These levels

of precision and accuracy of the proposed method was adequate for routine analysis of any of the cited drugs in their pharmaceutical dosage forms.

3- Interference studies

The possible interference that may be existed is due to the presence of some drugs in some binary combination with clotrimazole such as betamethasone dipropionate and dexamethasone acetate was studied. Those active ingredients are tested separately by the suggested procedure, in the general concentration range 0.08–1.0 µg ml^{-1} (after final dilution) based on non ester form, where no color could be detected. This is expected because they do not have tertiary amino nitrogen in their structures. Accordingly; analysis of laboratory prepared mixtures of clotrimazole with either betamethasone dipropionate or dexamethasone acetate, in different proportion by the proposed method, are summarized in Table 2. The results of such studies indicate the accuracy as well as the precision of the suggested procedure for analysis of clotrimazole in presence of these cortisone derivatives.

4- Application

Data obtained from the analysis of vaginal or oral tablets, topical cream, powder and solution as well as oral capsule dosage forms are summarized in Table 3. The current method was also judged by comparing it with other reported and/or official method(s).[6&28] According to the *F*- and *t*– tests, there is no significant difference between the proposed and reported method(s) used for comparison.

The accuracy of the proposed method was also confirmed through recovery studies using standard addition method. Results obtained indicate good recoveries which reflect selectivity of the extraction procedures, using methanol with tablets, capsules, topical powder or solution. Use of aqueous methanol for miconazole cream, or aqueous mixture of methanol – sulfuric acid mixture for clotrimazole and ketoconazole, or selective extraction by chloroform for tolnaftate cream preparation. In fact the methods developed for extraction for cream preparations are adapted from pharmacopeial methods,[16&28] reported for the analysis of either clotrimazole or tolnaftate in their cream formulation. Thus no interference could be expected from frequently encountered common excipients additives and other active ingredients. Therefore, the proposed method can be considered specific for determination of the cited drugs in commercially available dosage forms. Although the proposed method can be considered as nonselective for all the studied drugs, however this nonselectivity, in fact, does not represent any problem. This can be accounted on the fact that almost all the studied drugs are actually formulated as a single component in their pharmaceutical preparation.

5- Robustness and ruggedness

A study of small variation of some of the operational conditions such as reagent volume ±0.2 ml, heating time (±2 min) used on the recoveries and standard deviations of bulk drugs was carried out. The results obtained were not significantly affected within the studied ranges of variations in the procedural operational conditions Table 4), thus the proposed procedure can be considered robust. The ruggedness of the suggested procedure was also examined at varying time, analyst and two different instruments (listed in the experimental section). The results obtained were found to be reproducible, since no significant difference in the recoveries or the standard deviation values were obtained.

Table 1: Calibration parameters of the studied drugs by the proposed method (n = 3).

Drug	Calibration Range $\mu g\,ml^{-1}$	Intercept	Slope	r	r^2	LOD $\mu g\,ml^{-1}$	LOQ $\mu g\,ml^{-1}$	ε* $L\,mol^{-1}cm^{-1}$
Clotrimazole	0.8–2.8	-0.0062	0.2508	0.9998	0.9996	0.057	0.19	$8.27x10^4$
Fluconazole	3-7	-0.0035	0.0705	0.9996	0.9992	0.26	0.87	$21.37x10^3$
Ketoconazole	0.8–2.4	-0.0056	0.3615	0.9998	0.9996	0.045	0.15	$18.98x10^4$
Miconazole nitrate	0.8–2.4	-0.0048	0.3090	0.9999	0.9998	0.039	0.13	$14.64x10^4$
Tolnaftate	3-7	0.002	0.0856	0.9997	0.9994	0.23	0.77	$26.45x10^3$

* Molar absorptivity

Table 2: Analysis of laboratory prepared mixtures of clotrimazole with either betamethasone dipropionate or dexamethasone acetate (n = 3).

Prepared mixtures	$\mu g\,ml^{-1}$	% Recoveries ± SD
Clotrimazole	2.00	98.40 ± 1.15
Betamethasone*	0.10	----------
Clotrimazole	2.00	99.37 ± 1.65
Betamethasone*	1.00	----------
Clotrimazole	2.00	98.07 ± 1.72
Dexamethasone*	0.08	----------
Clotrimazole	2.00	99.30 ± 1.61
Dexamethasone*	0.80	----------

* calculated based on the non ester form

Table 3: Analysis of the commercial dosage forms of the studied drugs by the proposed and reported methods.

Dosage Forms	Drug	Label claimed (mg)	Found (% ± SD)*		Reported or official method
			Dosage Form	Add method	
Locasten vaginal tablet	Clotrimazole	100 mg/tab.	97.66±1.22	98.66 ± 1.41	98.08±1.17[a]
			F =1.09, *t* = 0.56		
Locasten topical powder	Clotrimazole	10 mg/g	97.32±1.00	98.12 ± 1.72	96.60±1.52[a]
			F =2.31; *t* = 0.88		
Locasten Topical solution	Clotrimazole	10 mg/ 1 ml	96.5±1.37	99.36±1.74	96.40±1.21[a]
			F =1.28; *t* = 0.122		
Locasten topical cream	Clotrimazole	10 mg/g	96.38±1.76	99.14 ± 1.98	97.24±1.38[a]
			F =1.63; *t* = 0.86		
Lotriderm cream	Clotrimazole & Betamethasone	10 mg/g	96.6±1.62	98.98 ± 1.51	97.32±1.41[a]
			F =1.32; *t* = 0.75		
Baycuten N cream	Clotrimazole & Dexamethasone	10 mg/g	95.64±1.6	99.28±1.90	96.70±1.68[a]
			F =1.10; *t* = 1.02		
Nizoral oral tablet	Ketoconazole	200 mg/tab.	100.18±1.62	99.56±1.3	99.84±1.06[a]
			F =2.34; *t* = 0.39		
Nizoral cream	Ketoconazole	20 mg/g	94.88±1.01	99.52 ± 1.31	95.36±1.16[a]
			F =1.32; *t* = 0.70		
Miconaz topical powder	Miconazole nitrate	20 mg/g	98.02±1.30	100.02±1.61	97.48±1.12[a]
			F =1.35; *t* = 0.71		
Miconaz topical cream	Miconazole nitrate	20 mg/g	98.14 ± 1.00	99.00 ± 1.46	99.52±1.52[a]
			F =2.3; *t* = 1.7		
Tineacure topical cream	Tolnaftate	10 mg/g	96.28±1.38	99.36±1.58	96.80±1.14[b]
			F =1.47; *t* = 0.65		
Flucoral capsules	Fluconazole	50 mg/cap	98.28±1.37	99.34±1.25	----------

*Average of 5 determinations ± SD.
[a] Ref. (6) using DDQ method.
[b] Ref. (28) using USP method.
Theoretical values at 95% confidence limit are $t = 2.78$ and $F = 6.39$.

Table 4: Influence of small variations in the assay conditions on the analytical performance of the proposed method for the determination of antifungals.

Variation	Recovery % ± SD (n = 3)				
	Clotrimazole (2.4 µg ml^{-1})	Fluconazole (5 µg ml^{-1})	Ketoconazole (1.6 µg ml^{-1})	Miconazole nitrate (1.6 µg ml^{-1})	Tolnaftate (5 µg ml^{-1})
No variation	98.77 ± 1.03	98.20 ± 0.89	98.00 ± 1.55	98.23 ± 0.87	99.50 ± 0.87
Volume of reagent ± 0.2 ml	98.20 ± 1.25	97.07 ± 1.01	97.07 ± 1.66	99.07 ± 1.42	100.37 ± 1.29
Heating time ± 2 min	99.30 ± 1.08	99.10 ± 1.35	98.7 ± 1.36	100.67 ± 1.17	98.00 ± 1.42

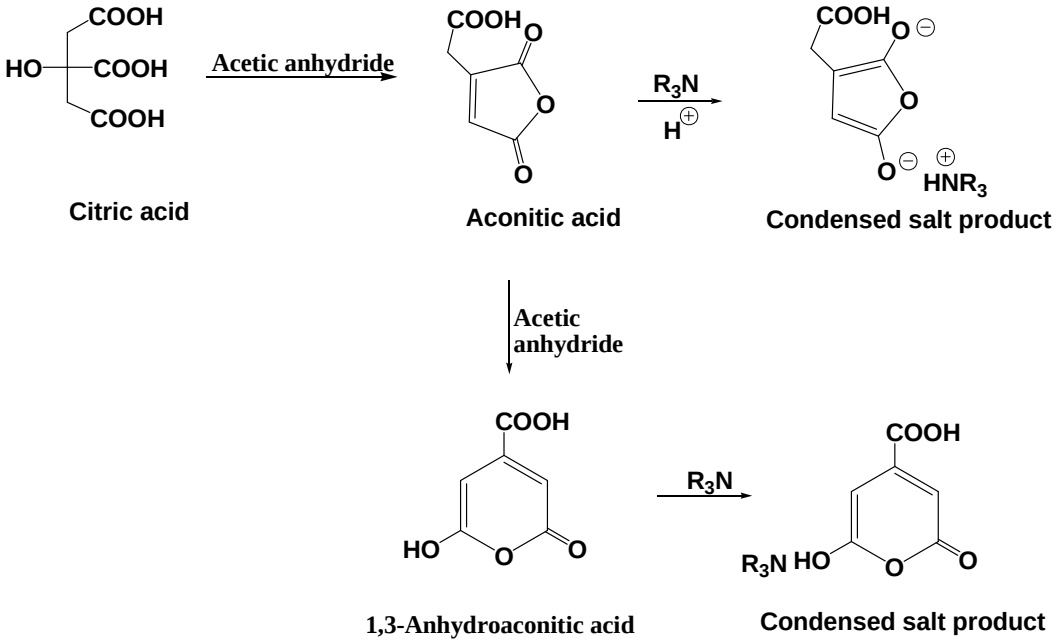


Fig. 6: Suggested reaction mechanism between the studied drug (s) and reagent (Reference 36).

6- Suggested reaction mechanism

Reactions of tertiary amines with mixed anhydrides are well known since long time ago.[33-36] Similar colors tests were observed with either citric acid or malonic acid or ascorbic acid or aconitic acid, each in combination with acetic anhydride.[35] It was clear, from the reported literature, that thin layer chromatography of the reaction mixtures showed that the color formed in these reactions consists of many components.[35] Accordingly, based on the possibility of dehydration of citric acid upon action of acetic anhydride, a suggested mechanism is outlined in Figure 6. Thus, this suggested mechanism indicates the possibility of formation of two products which are likely to be existing together. These products are described before in case of using aconitic acid / acetic anhydride mixture with some tertiary amines compounds.[36]

Conclusion

A validated, sensitive, precise and specific spectrophotometric method is described for the determination of antifungal drugs in bulk drug, tablets, capsules and some topical preparation such as cream, powder as well as solution. No need for releasing free miconazle base which required extensive and long extraction step before analysis. The good percentage recoveries and proper statistical data prove that the proposed method is of great value in quality control determination of the studied drugs because of its adequate accuracy; reliability for determination of the cited drugs in their pharmaceutical dosage forms without interference from common encountered excipients, other active ingredients such as betamethasone dipropionate and dexamethasone acetate. The suggested procedure has also other advantages such as low cost, no need for extraction of the colored product or expensive and/or sophisticated instruments and critical analytical reagent(s).

REFERENCES

1- S. C. Sweetman, Ed., "Martindale The complete Drug Reference", 33ed, Pharmaceutical Press, Press, London, UK, 2002, pp. 382–385, 389-391, 395.
2- K. Fardadi and R. Maleki, J. Pharm. Biomed. Anal. 30, 1023 (2002).
3- P. Y. Khashaba, Bull. Pharm. Sci., Assiut University, 25, 31 (2002).
4- H. K. Li, G. Z. Zhao, Y.Q. Zhao and Z. Li, Fenxi-Huaxue, 30, 334 (2002).
5- N. A. El-Ragehy and Y.S. El-Saharty, J. Assoc. Offi. Anal. Chem. Int., 84, 563 (2001).
6- P.Y. Khashaba, S. R. El-Shabouri, K. M. Emara and A. M. Mohamed, J. Pharm. Biomed. Anal., 22, 363 (2000).
7- S. R. El-Shabouri, K. M. Emara, P. Y. Khashaba and A. M. Mohamed, Anal. Lett., 31, 1367 (1998).

8- D. Agarwal, D. K. Jain and P. Trivedi, Indian Drugs, 35, 499 (1998).
9- K. Kelani and L. I. Bebawy, Anal. Lett., 30, 1843 (1997).
10- Abdelmageed and P. Y. Khashaba, Talanta, 40, 1289 (1993).
11- Bedair, M. A. Korany, M. A. Elsayed and O. T. Fahmy, J. Assoc. Offi. Anal. Chem. Int., 72, 432 (1989).
12- E. M. Abdel-Moety, K. O. Kelani and A. M. Abou Al-Alamin, Saudi Pharm. J. 40, 931 (2002)
13- A. El-Bayoumi, A. A. El-Shanawany, M. E. El-Sadek and A. Abdel-El Satter, Spectrosc. Lett., 30, 25 (1997).
14- M. Shasmsipur and F. Jalali, Chem. Anal., 47, 905 (2002).
15- The European Pharmacopoeia, 4th Ed., Council of Europe, 2002, pp. 949–950, 1430–1431, 1587–1588.
16- The British Pharmacopoeia, Her Majestry's Stationary Office, London, UK, 1998, pp 369, 764, 898–900, 1586-1587.
17- R. Hamoudova, M. Pospisilova, A. Kavalirova, P. Solich and J. Sicha, J. Pharm. Biomed. Anal., 40, 215 (2006).
18- I. Baranowska, P. Markowski and J. Baranowski, Analytica Chimica Acta., 570, 46 (2006).
19- E. M. Abdel-Moety, F. I. Khattab, K. M. Kelani and Abou Al-Alamein, IL Farmaco, 57, 931 (2003).
20- L. Gagliardi, D. de-Orsi, P. Chiment, R. Porra and D. Tonlli, Anal. Sci., 19, 1195 (2003).
21- P. A. Majcherczyk, P. Moreillon, L. A. Decosterd, D. Sanglard, J. Bille, M. P. Glauser and O. Marchetti, J. Pharm. Biomed. Anal., 28, 645 (2002).
22- H. Y. Aboul-Enein and I. Ali, ibid., 27, 441 (2002).
23- A. L. Crego, J. Gomez, M. L. Marina and J.L. Lavandera, Electrophoresis, 22, 2503 (2001).
24- Y. C. Guillaume and E. Peyrin, Talanta, 50, 533 (1999).
25- M. Shamsipur and K. Farhadi, Chem. Anal., 46, 387 (2001).
26- M. Shamsipur and K. Farhadi, Analyst, 125, 1639 (2000).
27- M. Shamsipur and F. Jalali, Anal. Sci., 16, 549 (2000).
28- The United States Pharmacopeia *24*, "The National Formulary" 19th Ed., US Pharmacopoeial Convention, Rockville, MD., 2000, pp. 451–454, 945, 1112–1115, 1679–1680, 2149–2152.
29- F. A. El-Yazbi, A. A. Gazy, H. Mahgoub, M. A. El-Sayed and R. M. Youssef, J. Pharm. Biomed. Anal., 31, 1027 (2003).
30- A. A. Gazy, H. Mahgoub, F. A. El-Yazbi, M. A. El-Sayed and R. M. Youssef, ibid., 30, 859 (2002).
31- J. N. Miller, Analyst., 116, 3 (1991).
32- G. K. McEvoy, Ed. "Drug Information" The Board of the American Society of Health-System Pharmacists, Inc.,

Bethesda, MD, USA, 2000, p. 90.
33- S. Ohkuma, J. Pharm. Soc. Japan, 75, 1124 (1955).
34- W. D. Langley, Anal. Chem. 39, 199 (1967).
35- B. A. Groth and G. Wallerbhrg, Acta Chem. Scend., 20, 2628 (1966).
36- M. Pesez and J. Bartos (Senior Eds) “Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs”, Marcel Dekker, Inc., New York, USA, 1974, pp. 166–181.