Indirect Spectrophotometric Determination of Tolnaftate in Pharmaceutical Preparations

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الخلاصة

تم تطوير طريقة طيفية غير مباشرة وذات حساسية عالية لتقدير التولنافتيت في بعض المستحضرات الدوائية . تعتمد الطريقة على أكسدة التولنافتيت بواسطة الحديد الثلاثي في الوسط الحامضي حيث يختزل الحديد الثلاثي إلى الحديد الثنائي والذي بدوره يقترن مع فيروسيانيد البوتاسيوم ليكون صبغة بروسيا الزرقاء والتي لها أقصى امتصاص عند 785 نانوميتر وقد لوحظ أن قانون بير يسري على الكميات التي تتراوح بين (0.02-0.16) جزء بالمليون وبامتصاصية مولارية 1.7×10^6 لتر .مول$^{-1}$.سم$^{-1}$. ان الانحراف القياسي النسبي للطريقة اقل من 2.5% وبمعدل استرجاعية 100.3% ولقد تم دراسة الظروف المثلى لتكوين المعقد مثل درجة الحرارة وفترة التسخين وتراكيز الكواشف والمتداخلات وطبقت الطريقة بنجاح في تقدير التولنافتيت في بعض المستحضرات الدوائية واختبر نجاح الطريقة بمقارنتها مع الطريقة الدستورية القياسية المعتمدة باستخدام اختباري (t وF) وعند حدود ثقة 95% مما يدل على صلاحية التطبيق التحليلي للطريقة.

Abstract

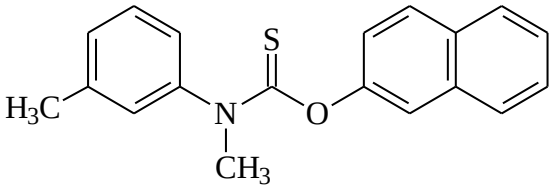
A highly, sensitive, indirect spectrophotometric method has been develop for the determination of tolnaftate in pharmaceutical preparations. The method is based on the oxidation of tolnaftate with Fe(III) in acidic medium. Fe (III) subsequently reduces to Fe(II),which is coupled with potassium ferricyanide after heating for 10 minutes at 70 ^{0}C to form prussian blue and the absorbance of the product was measured at 785 nm against a

reagent blank. Beer's law was obeyed in the range of 0.02-0.16 ppm with molar absorptivity of 1.7 $x10^6$ $L.mol^{-1}.cm^{-1}$, relative standard deviation of the method was less than 2.5% and accuracy (average recovery %) was 100.3%. The effect of various factors such as temperature, heating time,concentration of reagents, and interferences were investigated to optimize the procedure. The proposed method has been applied for the determination of tolnaftate in pharmaceutical preparations (quadrim cream and topical solution). A statistical compartsion of these results with those of official method shows good agreement using "t" and "F" test at 95% confidence level. the results indicated that there is no systematic error and the present method has good validity.

Keywords: Tolnaftate, spectrophotometric, pharmaceutical preparations

Introduction:

Tolnaftate: (o-naphthalen -2-yl methyl (3-methyl phenyl) thiocarbamate.(I)



$C_{19}H_{17}NOS = 307.4$

(I)

Has been widely used as a kind of topical antifungal in the treatment of cutaneous disease such as Jock itch, athlete's foot (1,2) and other skin infections due to ,Epidermophyton floccosum, microsporum and uinii, M. canis and T. verrucosum(3,4).Tolnaftate has been shown tobe an inhibitor of squalene epoxidase in susceptible fungi, it is therefore classified with allylamines(5) Very few methods have been reported for the determination of tolnaftate in pharmaceutical formulations, these methods include HPLC, UV spectrophotometry (6-8), a survey of literature revealed that only two visible spectrophotometric methods are reported (9,10), the oxidative coupling reaction of tolnaftate with 2,6-dichloroquinone-4-chloromide to produce a chromophoric acid with λmax at 490 nm or coupling reaction with the N-chloroquinone diimine to give a colored product with λmax at 530 nm and spectofluorimetry (11), however these methods lack sensitivity, and simplicity needed for routine analysis . in this paper we described a precise

and accurate method for spectrophotometric determination of tolnaftate in pharmaceutical preparation by using iron (III) with ferricyanide.

EXPERIMENTAL

Apparatus :

A Spectro scan 50 UV visible spectrophotometer with 1.0 cm quartz cells and CFL1083 water bath were used.

Reagents :

All chemicals used were of analytical grade and the tolnaftate standard material was provided from state company for drug industries and medical appliance (NDI) Ninavah-Iraq.

Tolnaftate stock solution (100 ppm).

This solution was prepared by dissolving 0.1gm of Tolnaftate in 200 ml methanol and diluting to 1L with distilled water.

Tolnaftate standard solution (1ppm).

This solution was prepared by diluting 1ml of stock solution into 100ml by distilled water in a volumetric flask.

Ferric chloride solution 0.1%

This solution was prepared by dissolving 0.1 gm of ferric chloride in 100 ml of water containing 2 ml of concentrated HNO_3.

Potassium ferricyanide solution 0.1%

Acetic acid solution 1N

Recommended procedure:

A liquots of standard solution of tolnaftate (0.5-4.0µg) were transferred into a series of 25ml calibrated flasks, added 2ml of 1N acetic acid, 6ml of 0.1% ferric chloride solution and 6ml of 0.1% potassium ferricyanide solution the flasks was immersed in water bath (70 ^{0}C) for 10 mint, cool and make up to 25ml in volumetric flasks with water. The absorbance was measured at 785 nm against a reagent blank.

Procedure for pharmaceutical preparations:

Cream:

1gm of cream, equivalent to about 10mg of tolnaftate was transfered to a 250ml separator containing about 75ml of chloroform. The chloroform solution successively washed with two 25ml portions of 0.1N NaOH, two 25ml portions of 0.1N HCl and 25ml of water. The chloroform layer was

filtered through a chloroform-washed cotton pledget into a 100ml volumetric flask. Chloroform was added to volume, and mix. 1ml of chloroform solution was evaporated on a steam bath just to dryness and the residue was dissolved in 20 ml of methanol and diluted up to 100 ml with distilled water (7). 3 ml of this solution was treated as mentioned under recommended procedure.

Topical solution

0.1 ml of solution containing 1mg of tolnaftate was transferred into 1L volumetric flask and diluted up to mark with distilled water, a 3 ml of this solution was treated as mentioned under the recommended procedure.

Results and discussion

Tolnaftate is oxidized in acidic medium by ferric chloride which subsequently reduced to Fe (Π) and immediately reacts with Potassim-ferricyanide. This way formerly termed Turubull's blue, it is now considered to be identical with Prussian blue (12,13), which absorbs maximally at 785 nm as shown in Fig [1], the colorless reagent blank has practically negligible absorbance at this wavelength. And this wavelength was recommended for determination.

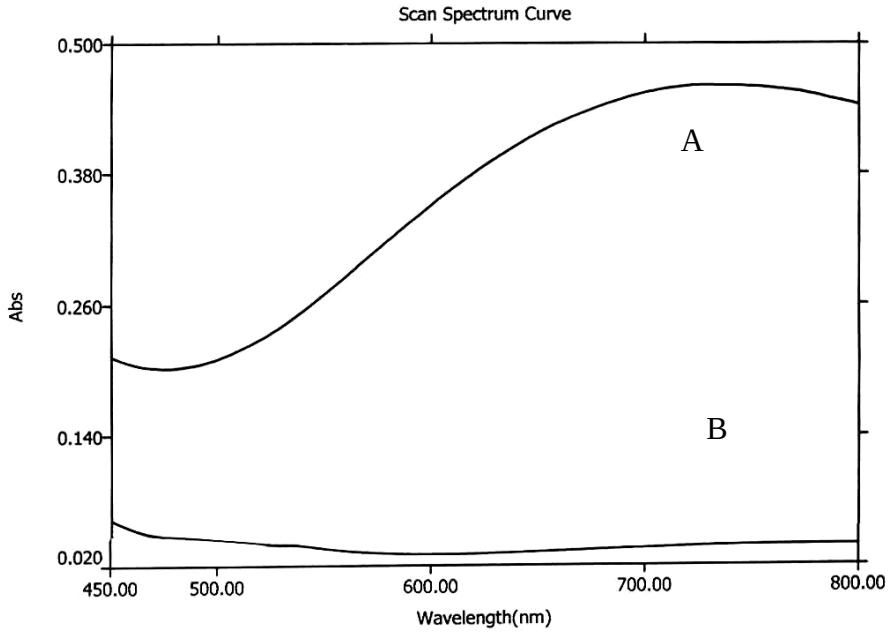


Fig. [1]: Absorption spectra of tolnaftate product against blank (A), with reagent blank against water (B) (tolnaftate taken 2 µg/25 ml)

Study of the optimum reaction conditions

The varios experimental parameters affecting the formation of colored product were optimized and used throughout the experiment. (Tolnaftate taken 2µg/25ml).

Effect of acids

The effect of different acids on the absorbance of the colored product is shown in table [1], which shows that maximum intensity was reached when using 2ml of acetic acid. This amount was selected for the subsequent experiments.

Table [1]: Effect of different acids on the absorbance of colored product.

Acids (1N)	Absorbance\ml of acid added 0.5	1	2	3	5
HCl	Turbid	Turbid	Turbid	Turbid	Turbid
H_2SO_4	Turbid	Turbid	Turbid	Turbid	Turbid
HNO_3	0.11	0.101	0.070	0.056	0.042
CH_3COOH	0.070	0.180	0.428	0.428	0.430

Effect of the amount of ferric chloride reagent

The amount of ferric chloride solution for maximal color intensity was examined. It was observed that the addition of 6ml of 0.1% ferric chloride solution was required to obtain a maximum absorbance table [2]. This amount was selected for subsequent work.

Table [2]: Effect of the amount of ferric chloride reagent.

Ml of 0.1% ferric chloride	2	4	6	8	10
Absorbance	0.327	0.428	0.430	0.430	0.431

Effect of the amount of Potassium ferricyanide solution.

The amount of Potassium ferricyanide solution for maximal color intensity was examined. It was observed that the addition of 6 ml of 0.1% Potassium ferricyanide solution was required to obtain a maximum absorbance table [3]. This amount was selected for subsequent work.

Table [3]:-Effect of the amount of potassium ferricynide solution.

Ml of 0.1% potassium ferricyanide	2	4	6	8	10
Absorbance	0.376	0.419	0.430	0.430	0.432

Effect of temperature and heating time:

The effect of temperature and heating time on the color intensity were studied in practice the absorbance of the color reached maximum after 10

min at 70^0C, table[4]. The absorbance was then stable for at least 6h.

Table [4]: Effect of temperature and heating time.

Temp ^{0}C	50			70			90		
Time (mint)	10	20	30	10	20	30	10	20	30
Absorbance	0.205	0.210	0.220	0.432	0.431	0.432	0.431	turbid	turbid

Effect of order of addition

To test the effect of order of addition of the reagents on absorbance of the product, different orders were tested. The selected order was Tolanaftate, acetic acid, ferric chloride, followed by potassium ferricyanide solution.

Beer's law

The method obeyed Beer's law in the concentration range of 0.5-4 µg/25ml with molar absorptivity of 1.7×10^6 $L.mol^{-1}.cm^{-1}$. a regression analysis of Beer's law plot at 785nm revealed a good correlation (r= 0.9888, n= 8) the graph of the absorbance versus the concentration of tolnaftate showed a low intercept (0.029) and slope (0.09), and is described by a regression equation. Y= ax + b (where x is the concentration of tolnaftate in µg/ml, Y is the absorbance, a is the slope and b is the intercept) and the limit of detection was evaluated as (14).

$$LOD=3.3\frac{So}{b}$$

Where b is the slop and So is the standard deviation of the regression line. The limit of detection was 0.0039µg.ml^{-1} (n= 8)

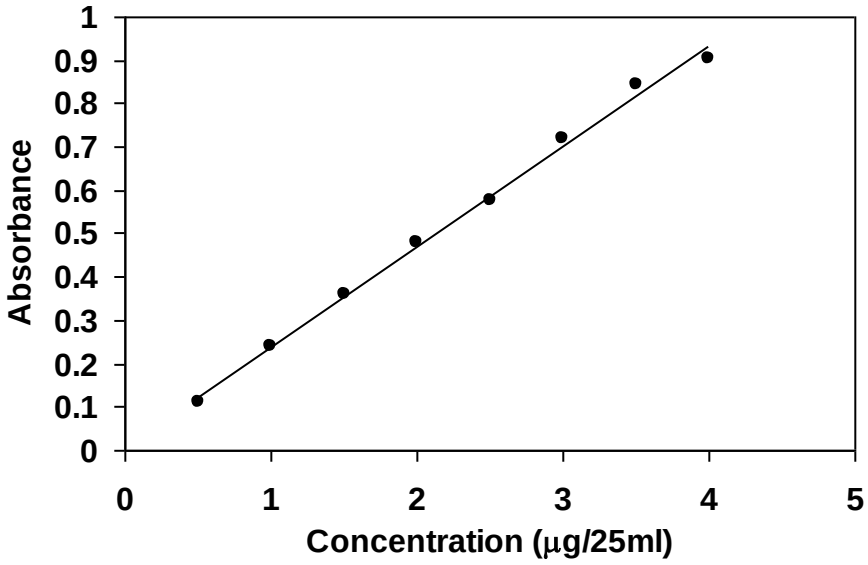


Fig [2]: calibration graph of tolnaftate.

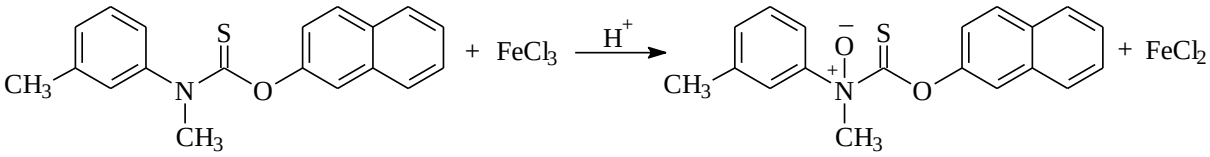
Accuracy and precision:

The accuracy and precision of the method were established by analysing the pure drug at three different concentrations and seven determinations. The average recovery which is a measure of accuracy is 100.3% revealing high accuracy of the method. The relative standard deviation (RSD), which is an indicator of precision is less than 2.5% the results are cited in table [5].

Table [5]:- Accuracy and precision of the method

Amount of tolnaftate (µg/25ml)		Recovery (%)	RSD(%)
Taken	Found		
1	1.008	100.8	±2.33
2	2.009	100.45	±1.15
3	2.99	99.66	±0.84

Stoichiometry of reaction:- The stoichiometry of reactants was investigated by the mole ratio method (15). The results obtained (fig 3) indicated the existence of 1:1:1 Tolnaftate-$FeCl_3$- $K_3Fe(CN)_6$ at 785nm. Thus the suggested reaction might be written as (13,16)



$$FeCl_2 + K_3Fe(CN)_6 \rightarrow KFe[Fe(CN)_6] + 2KCl$$

Prussian blue.

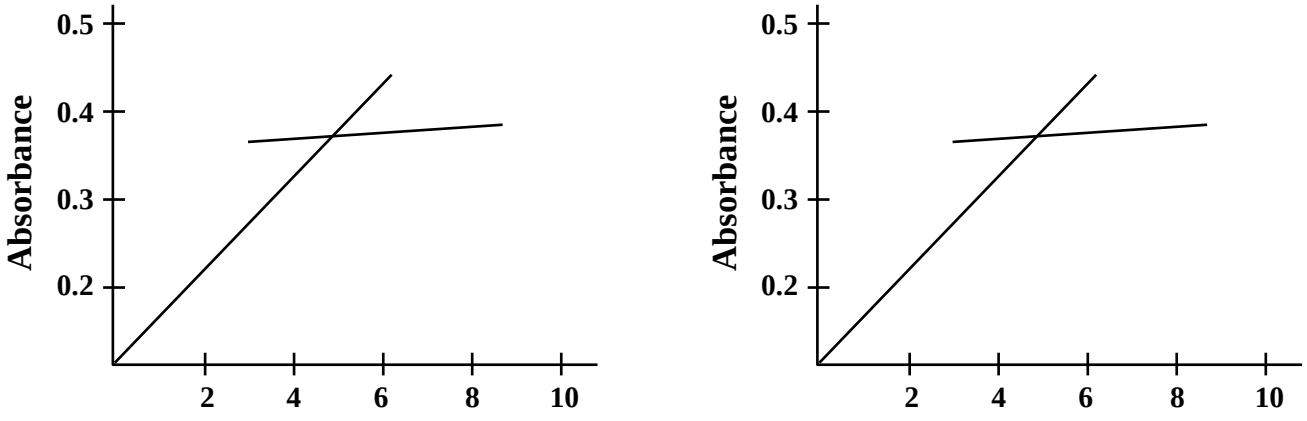


Fig. [3] : Molar ratio method of toluaftate-$FeCl_3$-$K_3Fe(CN)_6$

Effect of interferences:

The interfering effect of foreign species often accompanied with tolnaftate in the pharmaceutical preparations were studied by adding different amounts of foreign species to 3µg\ 25ml of tolnaftate in solution and the recommended procedure for the determination of tolnaftate was followed. The species are considered to interfere seriously if they cause a change of more than 2% in the absorbance obtained for tolnaftate a lone (17). It was observed that the Betamethazone 17-valerate, gentamycine sulphate and clioquinol don't interfere with determination method at levels found in the dosage form cited in table[6] so that the selectivity of method is very good.

Table [6]: determination of tolnaftate in presence of excipients.

Excipients	Amount taken µg	Averagerecovery* %
Betamethazon 17-valerate	0.15	100.05
Gentamycine sulphate	3	100.0
clioquinol	3	100.08

* Average of seven replicate analyses.

Analytical application:

Two types of drugs containing tolnaftate (cream and solution) were analyzed. The results of analysis of pharmaceutical formulations. Table[7] were compared statistically by student t-test and by the variance ratio F-test with those obtained by official method (7) at 95% confidence level. The calculated t and F values did not exceed the theoretical values indicating that there was no significant difference between the precision of the proposed and official methods.

Table [7]: Assay of Tolnaftate in pharmaceutical formulations.

Pharmaceutical preparations supplied by NDI	Amount of tolnaftate•		
	Present method	B.P (official method)	Certified value
Quadrim cream	10.02 mg\gm t=1.43,F=1.23	9.96 mg\gm	10mg\gm
Topical solution	9.98 mg\ml t=1.88,F=1.08	10.06mg\ml	10mg\ml

• Mean of six determinations.

t values (n=10,at 95% confidence level tabulated value 2.262

F values (n1 and n**2**=10,at 95% confidence level tabulated value 3.18

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