Indirect Spectrophotometric Determination of Tolnaftate in Pharmaceutical Preparations

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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 °C to form prussian blue and the absorbance of the product was measured at 785 nm against a
Indirect Spectrophotometric Determination of Tolnaftate in Pharmaceutical ...

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 comparison 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.\(^{(I)}\)

\[
\begin{align*}
\text{C}_{19}\text{H}_{17}\text{NOS} &= 307.4 \\
\text{(I)}
\end{align*}
\]

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 unii, M. canis and T. verrucosum(3,4). Tolnaftate has been shown to be 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 \(\lambda_{\text{max}}\) at 490 nm or coupling reaction with the N-chloroquinone diimine to give a colored product with \(\lambda_{\text{max}}\) 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 HNO₃.

**Potassium ferricyanide solution 0.1%**

**Acetic acid solution 1N**

**Recommended procedure:**
A liquorots 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 °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 transferred 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 (II) 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.

![Scan Spectrum Curve](image)

**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 various 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.

| Acids  | Acids (1N) | Absorbance/ml of acid added |
|--------|------------|-----------------------------|
|        |            | 0.5 | 1    | 2    | 3    | 5    |
| HCl    | Turbid     | Turbid | Turbid | Turbid | Turbid |
| H₂SO₄  | Turbid     | Turbid | Turbid | Turbid | Turbid |
| HNO₃   | 0.11       | 0.101 | 0.070  | 0.056  | 0.042  |
| CH₃COOH| 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.

| MI 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.

| MI 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°C, table[4]. The absorbance was then stable for at least 6h.

| Temp °C | 50 | 70 | 90 |
|---------|----|----|----|
| Time (mint) | 10 | 20 | 30 |
| Absorbance | 0.205 | 0.210 | 0.220 |

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

**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 Tolnaftate, 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 $1.7 \times 10^6$ 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).

$$\text{LOD}=3.3 \frac{S_o}{b}$$

Where $b$ is the slope and $S_o$ 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.](image)

**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].

| 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-FeCl₃- K₃Fe(CN)₆ at 785nm. Thus the suggested reaction might be written as (13,16)

\[
\text{FeCl}_2 + K_3\text{Fe(CN)}_6 \rightarrow K\text{Fe}[\text{Fe(CN)}_6] + 2\text{KCl}
\]

Prussian blue.

**Fig. [3] : Molar ratio method of toluaftate-FeCl₃-K₃Fe(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 3\mu g\ 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 alone (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.

| Excipients          | Amount taken µg | Average recovery* % |
|---------------------|-----------------|---------------------|
| 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.

| 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 n2=10, at 95% confidence level tabulated value 3.18

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