J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(3): 876-889, 2024
Doi: 10.33483/jfpau.1348367

ORIGINAL ARTICLE / ÖZGÜN MAKALE

SIMPLE AND SENSITIVE SPECTROPHOTOMETRIC ASSAYS FOR THE DETERMINATION OF TERBINAFINE HCL ANTIFUNGAL DRUG IN PHARMACEUTICALS

FARMASÖTİKLERDE TERBİNAFİN HCL ANTİFUNGAL ETKEN MADDESİNİN BELİRLENMESİ İÇİN BASİT VE HASSAS SPEKTROFOTOMETRİK TAYİNLER

Nagib QARAH[1]*, Ezzouhra EL-MAAIDEN[2], Kanakapura BASAVAIAH[3]

[1]Hodeidah University, Faculty of Education- Zabid, Department of Chemistry, 3114, Hodeidah, Yemen
[2]University of Hassan 1[er], Laboratory of Biochemistry and Neurosciences, Department of Chemistry, 26000, Settat, Morocco
[3]University of Mysore, Department of Chemistry, 570 006, Mysuru, India

ABSTRACT

Objective: *Two highly sensitive, accurate, inexpensive, and simple spectrophotometric assays were developed and validated for the determination of an anti-fungal drug, Terbinafine HCl (TBH), in pure drug and tablets using potassium permanganate (PP) and disodium 2-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl)quinoline-6,8-disulfonate (DSOQ).*
Material and Method: *In the present study, Sebifin and Terbiforce 250 mg tablets were used as pharmaceuticals, potassium permanganate $KMnO_4$ and disodium 2-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl)quinoline-6,8-disulfonate in water were used as reagents, and DR 3900 spectrophotometer equipped with 1cm matched quartz cells was used for absorbance measurements.*
Result and Discussion: *The amount of terbinafine hydrochloride reacting with permanganate and disodium 2-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl)quinoline-6,8-disulfonate in an acidic medium has been determined. The colored reaction products in both cases were measured at the maximum absorptions of 540 nm and 440 nm, respectively. The absorbance measured in each assay as a function of TBH concentration was related to TBH concentrations. Different experimental and variable conditions of assays were done carefully, accurately studied, and optimized. The validation of two assays also was done by following the current guidelines of the International Conference on Harmonization (ICH). Beer's law for the two methods is obeyed over the concentration ranges 1-15 µg/ml (Correlation coefficient = 0.9983) and 1-18 µg/ml (Correlation coefficient = 0.9989) for methods PP and DSOQ, respectively. Molar absorptivity, limits of detection, and quantification (LOD & LOQ) values were (1.38×10^4 l/ mol cm, 0.92 & 2.78 µg/ml) for PP assay, and (1.73×10^4 l/ mol cm, 0.09 & 0.27 µg/ml) for DSOQ assay, respectively. The two assays were successfully applied for the determination of TBH in commercial tablets with reliable and satisfactory results, and hence the proposed assays can be applied in pharmaceutical laboratories of quality control.*
Keywords: *Ion-pair, $KMnO_4$, spectrophotometry, terbinafine HCl*

ÖZ

Amaç: *Bir anti-fungal ilaç olan Terbinafin HCl'nin (TBH) saf ilaç ve tabletlerde potasyum permanganat (PP) ve disodyum 2-(1,3-diokso-2,3-dihidro-1H-inden-2-il)kinolin-6,8-izülfonat (DSOQ) kullanılarak tayini için oldukça hassas, doğru, ucuz ve basit iki spektrofotometrik test*

* **Corresponding Author / Sorumlu Yazar:** Nagib Qarah
e-mail / e-posta: nagib2015qarah@gmail.com, **Phone / Tel.:** +967734296789

Submitted / Gönderilme : 24.09.2023
Accepted / Kabul : 13.06.2024
Published / Yayınlanma : 10.09.2024

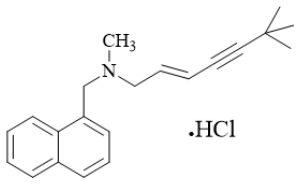
geliştirilmiş ve valide edilmiştir.
Gereç ve Yöntem: *Bu çalışmada, ilaç olarak Sebifin ve Terbiforce 250 mg tabletler, reaktif olarak potasyum permanganat $KMnO_4$ ve disodyum 2-(1,3-diokso-2,3-dihidro-1H-inden-2-il)kinolin-6,8-izülfonat ve absorbans ölçümleri için 1 cm eşleştirilmiş kuvars hücrelerle donatılmış DR 3900 spektrofotometre kullanılmıştır.*
Sonuç ve Tartışma: *Asidik bir ortamda permanganat ve disodyum 2-(1,3-diokso-2,3-dihidro-1H-inden-2-il)kinolin-6,8-disülfonat ile reaksiyona giren terbinafin hidroklorür miktarı belirlenmiştir. Her iki durumda da renkli reaksiyon ürünleri sırasıyla 540 nm ve 440 nm maksimum absorpsiyonlarında ölçülmüştür. TBH konsantrasyonunun bir fonksiyonu olarak her bir deneyde ölçülen absorbans TBH konsantrasyonları ile ilişkilendirilmiştir. Deneylerin farklı deneysel ve değişken koşulları dikkatlice yapıldı, doğru bir şekilde incelendi ve optimize edildi. İki tahlilin validasyonu da Uluslararası Uyumlaştırma Konferansı'nın (ICH) güncel kılavuzları takip edilerek yapılmıştır. İki yöntem için Beer yasasına, PP ve DSOQ yöntemleri için sırasıyla 1-15 µg/ml (Korelasyon katsayısı = 0.9983) ve 1-18 µg/ml (Korelasyon katsayısı = 0.9989) konsantrasyon aralıklarında uyulmuştur. Molar absorptivite, tespit ve miktar belirleme sınırları (LOD ve LOQ) değerleri PP testi için sırasıyla (1.38×104 l/mol cm, 0.92 ve 2.78 µg/ml) ve DSOQ testi için (1.73×104 l/mol cm, 0.09 ve 0.27 µg/ml) idi. Bu iki analiz, ticari tabletlerde TBH tayini için güvenilir ve tatmin edici sonuçlarla başarıyla uygulanmıştır ve bu nedenle önerilen analizler kalite kontrol farmasötik laboratuvarlarında uygulanabilir.*
Anahtar Kelimeler: *İyon çifti, $KMnO_4$, spektrofotometri, terbinafin HCI*

INTRODUCTION

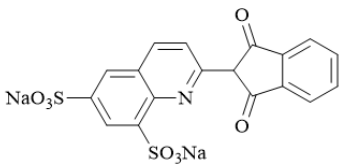
Terbinafine hydrochloride (TBH) (Figure 1) antifungals drug belong to the allylamine class. According to IUPAC, terbinafine hydrochloride TBH drug is known as [(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)[(naphthalene-1-yl)methyl]amine hydrochloride [1]. The drug has a molecular weight corresponding to its molecular formula of $C_{21}H_{26}ClN$ is 327.9 g mol^{-1}. With good efficacy, terbinafine hydrochloride is used as a short-term treatment for various skin infections and fungi, especially, for the treatment of onychomycosis [2]. Onychomycosis is the most prevalent nail disorder attributed to yeasts; non-dermatophytes molds, and dermatophytes. Terbinafine hydrochloride has been approved medication for onychomycosis treatment by the Food and Drug Administration (FDA) of the US [3]. The drug is used in different therapeutic doses; as tablet formulations for fingernail and toenail fungus treatment, cream formulations for jock itch, athlete's foot, and other similar skin infections treatment, and other formulations of the drug as needed with therapeutic doses ranging from 1 to 5% [4].

The anti-fungal drug is official in United States Pharmacopeia (USP) [5], British Pharmacopeia (BP) [6], and European Pharmacopeia (EP) [7]. The USP recommended an HPLC method with UV detection at 280 nm. The BP and EP recommended a titrimetric assay in which the TBH drug is dissolved in ethanol (96%), and 5 ml of HCl (0.01M) is added followed by potentiometric titration with 0.1M NaOH.

Apart from the pharmacopoeial methods, in pharmaceuticals, the TBH drug has been determined by different methods, including titrimetry using non-aqueous solvent [8], Electrochemical [9-12], UV-spectrophotometry [8,13-16], spectrofluorimetry [17], capillary electrophoresis [18,19], and HPLC [20-32].



Terbinafine hydrochloride (TBH)



disodium 2-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl) quinoline-6,8-disulfonate (DSOQ)

Figure 1. Chemical structures of TBH and DSOQ

Many of the reported methods [8-32] need expensive and sophisticated instruments, which are

not available in most quality control laboratories, as well as, involve tedious multiple extraction steps and are time-consuming. They also pose the problem of maintenance. Hence, they are not within the reach of most quality control laboratories and small-scale pharmaceutical industries, especially in the Asian sub-continent.

The official titrimetric methods [6,7] are cumbersome and require a large quantity of drug (250 mg) for each trial. The non-aqueous titrimetric method reported by Cardoso et al. [8] requires a perfect anhydrous medium and a large quantity of mercuric acetate. The presence of the amine group in the molecule has led to the development of a few visible spectrophotometric assays based on ion-pair complex, and redox reactions. Methyl orange [33], orange-G, molybdenum(V) thiocyanate, and alizarin red S [34], bromophenol blue bromothymol blue, and bromocresol green [35], alizarin red S [36], bromocresol purple [37], have been used as ion-pair reagents where the ion-pair complexes with the drug were formed, extracted into an organic solvent and measured, but few reported methods [33-37] suffer from elevated temperature for reactions to occur and poor sensitivity, with a narrow dynamic of linear ranges. Also, permanganate in alkaline medium [38], cerium (IV)-Chromotrope 2R [39] and $KBrO_3$-KBr mixture-methylene blue/methyl red [40], have been used as redox reagents, but also that reported methods suffer from some disadvantages such as elevated temperature for reactions to occur [38-40], yields considerable blank absorbance [38], employ multiple reagents and time-consuming [39,40].

To avoid one or more disadvantage in the reported spectrophotometric methods, the proposed methods, which are inexpensive, mild experimental conditions, simple, rapid, sensitive, and accurate assays were developed and validated for the terbinafine HCl antifungal drug in bulk form of drug as well as in tablets using PP and DSOQ reagents. The first proposed assays relied on the oxidation of TBH by PP in an H_2SO_4 medium, which results in the fading of PP solution. The Beer's law is obeyed between the concentration of TBH and the decrease value of absorbance, and the content of TBH can be indirectly measured by measuring the decrease value of absorbance at 540 nm (PP method). The second assay relied on the reaction of opposite charges ions, TBH as a cation and DSOQ as an anion, to form an ion-pair complex in acidic buffer conditions and measured at 440 nm. The proposed methods' results, under the optimum conditions, were found to be sensitive and simple compared to the most currently available methods for TBH.

MATERIAL AND METHOD

Pure TBH Drug, Dosage Forms, and Reagents Used

All reagents and other chemicals used in the present assays were of analytical reagent grade. The aqueous solutions required were prepared in double distilled water. Pure Terbinafine hydrochloride (TBH, 99.9%), was obtained from Cipla Ltd. (Maharashtra India). Permanganate PP (Merck, Mumbai, India), and DSOQ (Loba Chemie, Mumbai, India), the concentration of PP and DSOQ reagents were for each 0.06% (w/v) in water, respectively. A solution of PP (0.1%) was prepared and standardized according to a described procedure in a textbook of quantitative inorganic analysis, the standard solution was then diluted appropriately with water to a concentration of 0.06% and kept in a dark bottle [41], preparing all solutions freshly each day. Sebifin and Terbiforce tablets used (Glaxo Smith/ Lifestar Pharmaceuticals Ltd., India) contain 250 mg TBH of each tablet purchased from the local market.

Potassium biphthalate buffer (KHP) solution at pH 3.4, which was used in the present study was prepared by placing 50 ml of KHP solution (0.2 M) in a 200 ml volumetric flask and adding the specified volume of the hydrochloric acid (0.2 M) solution, 15.7 ml, then dilute to 200 ml with distilled water [42]. Buffer solutions covering a wide range of pH (1.4 to 5.4) were freshly prepared using (0.2M KCl – 0.2M HCl)) system for buffers in the pH range of 1.4 – 2.2, (0.2M potassium biphthalate, 0.2 M HCl) system for buffers in the pH range of 2.6 – 3.8, and (0.2M biphthalate , 0.2M NaOH) system for buffers in the pH range 4.2 – 5.4. all buffer solutions systems were prepared according to procedures described by the official methods [42].

Preparation of Standard Terbinafine HCl Drug Solutions

A 1 mg/ml (1000 μg/ml) stock standard drug solution was prepared accurately. Stock TBH

Solutions were prepared in H_2SO_4 (0.1 M) For reaction with PP, and in HCl (0.1 M) for reaction with DSOQ. A working concentration of assays using PP (20 µg/ml) and DSOQ (40 µg/ml), were prepared using a stock solution by accurately stepwise diluted with the corresponding solvents.

General Procedures

PP Method

One ml each of H_2SO_4 (1 M) followed by PP solution (0.06% w/v) was transferred into the two comparison flasks (10 ml). Then different volumes of 20 µg/ml TBH solution (0.0-7.5) ml equivalent to (0.0–15) µg/ml were added into one of the two a set of 10 ml comparison flasks. The flasks were diluted with distilled water to the mark, and standing for 5 min at room temperature (30±1 ºC). After the standing time for 5 min, the absorbance (A1) of the blank solution ($KMnO_4$+H_2SO_4) and the absorbance (A2) of the determination solution ($KMnO_4$+H_2SO_4+TBH) are measured at 540 nm against water. The △A(A1-A2) is calculated.

DSOQ Method

In the DSOQ method, aliquots (0.25-4.5 ml) of 40 µg/ml TBH solution were accurately transferred, into a set of 125 ml separating funnels, and the volume of each funnel was brought to 4.5 ml using (0.1 M) HCl. Six milliliters each of KHP buffer pH 3.4, and DSOQ (0.06% w/v) solutions to each funnel separately were added and the volume of each funnel was brought to 20 ml with distilled water. After the shaking time for 3 min, the organic layers were separated using dichloromethane and dried using anhydrous Na_2SO_4. The resulting absorbance of solutions was measured at 440 nm vs. the reagent blank prepared simultaneously.

The calibration graph in each case was plotted, and using the regression equation, the concentration of unknown was computed.

The Procedure Applied for Formulations

Twenty tablets (250 mg for each) of TBH were weighed and powdered and mixed well. A portion of the tablet powder equivalent to a hundred mg of TBH was transferred to a volumetric flask (100 ml). The quantity was treated with solvent and shaken for 20 min. solvents were sulphuric acid for the PP assay and hydrochloric acid for the DSOQ assay. The volume of resulting solutions was brought to mark with the corresponding solvent and then filtered. The resulting tablet extracts were stepwise diluted with the corresponding solvent to get 20 µg/ml for the PP assay and 40 µg/ml for the DSOQ assay. Five milliliters and a 2.5-milliliter aliquot of that concentrations respectively, were subjected accurately to analysis in five replicates (n=5) as described in PP and DSOQ procedures. Also, the fine powder of the tablet was subjected to analysis by reference method [5] for comparison.

Procedure for Excipients and Additives

Ten mg of placebo blank, which consists of magnesium stearate (15 mg), sodium citrate (15 mg), starch (20 mg), acacia (15 mg), talc (20 mg), and methylcellulose (10 mg), was extracted uniformly as described under procedure for the assay of TBH in formulations. After that, 2 milliliters of extract were analyzed by following the general procedures. To 10 milligrams of the placebo, 10 milligrams of a pure drug (synthetic mixture) were added and mixed well. The content was transferred accurately to a 100 ml volumetric flask and followed the procedure steps for formulations.

RESULT AND DISCUSSION

Chemistry and Pathway of Reactions / Absorption Spectrum

Here (Figure 2), in the first, TBH was treated with an excess known concentration of PP in an acid condition, and after appropriate standing time, the unreacted PP was determined by measuring its decreasing absorbance value at 540 nm. The amount of permanganate reacted corresponds to the drug amount which served as the basis of assay. Based on the proposed assay, in the range of 420-720 nm the absorption spectrums of the determination solutions ($KMnO_4$+H_2SO_4+TBH) and the permanganate

solutions ($KMnO_4$+H_2SO_4) were plotted, and the maximum absorption wavelength of each was found at 540 nm as shown in the Figure 3(a). So, 540 nm was chosen for all measurements.

In the second method, TBH reacted with the DSOQ reagent solution in an acidic condition (pH = 3.40), to form an ion-pair complex (Figure 2). The ion-pair product was extracted using dichloromethane and then measured at 440 nm. The absorption spectrum of blank and determination solutions were also plotted, and the maximum absorption wavelength of 440 nm was selected for all measurements. The absorbance of the reagent blank was not high as shown in Figure 3(b).

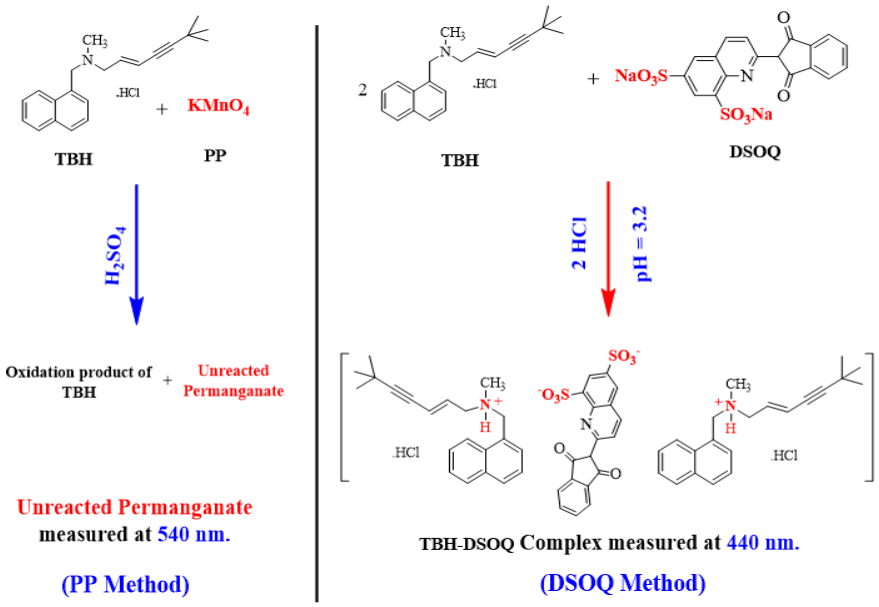


Figure 2. The probable reaction pathways

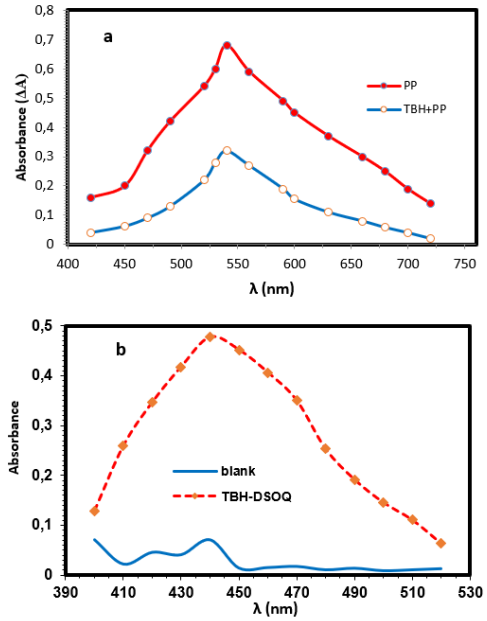


Figure 3. Absorption spectra of **(a)** (—●—) PP [PP= 0.06% (w/v), H_2SO_4=1 M] against water, (—○—) the reaction product of TBH and PP against water [TBH= 9 µg/ml, PP= 0.06% (w/v), H_2SO_4=1 M]. **(b)** the reaction product of TBH and DSOQ [TBH= 9 µg/ml, DSOQ = 0.06% (w/v), pH=3.4] against the blank

Optimization and Method Development of Experimental Variables

Several variables were investigated for spectrophotometric methods, such as PP, DSOQ, acid concentrations, reaction time, pH, and volumes of buffer. The investigated variables were optimized by varying one experimental variable at a time and keeping the other variables constant.

PP Method

Effect of Volume of Sulfuric Acid

The reaction between fixed concentrations of TBH and permanganate was carried out with used different volumes of 1 M H_2SO_4 in the range (0.5-2.5 ml). It was found to be optimum, complete, and quantitative with corresponds to the highest absorbance reading when using one ml of sulfuric acid (Figure 4).

Effect of PP Reagent

To study this effect, Different volumes of PP were examined to determine the optimum volume of permanganate that gives the highest absorbance at wavelength 540 nm in acidic conditions while keeping the other reagent concentrations constant. Various volumes of PP were token in the range of 0.5 to 3.5 ml as shown in Figure 4. A 1.5 ml of PP (0.06% (w/v)) gives the maximum absorbance. However, 1.5 ml of PP was selected in the final solution as the optimum reagent volume.

Effect of Reaction Time and Stability

Here, the reaction mixture between TBH drug and PP was subjected to different standing times in the range from 5 to 25 min (Figure 4). It was found to be completed in 5 min, and the absorbance was found to be constant up to 25 min thereafter.

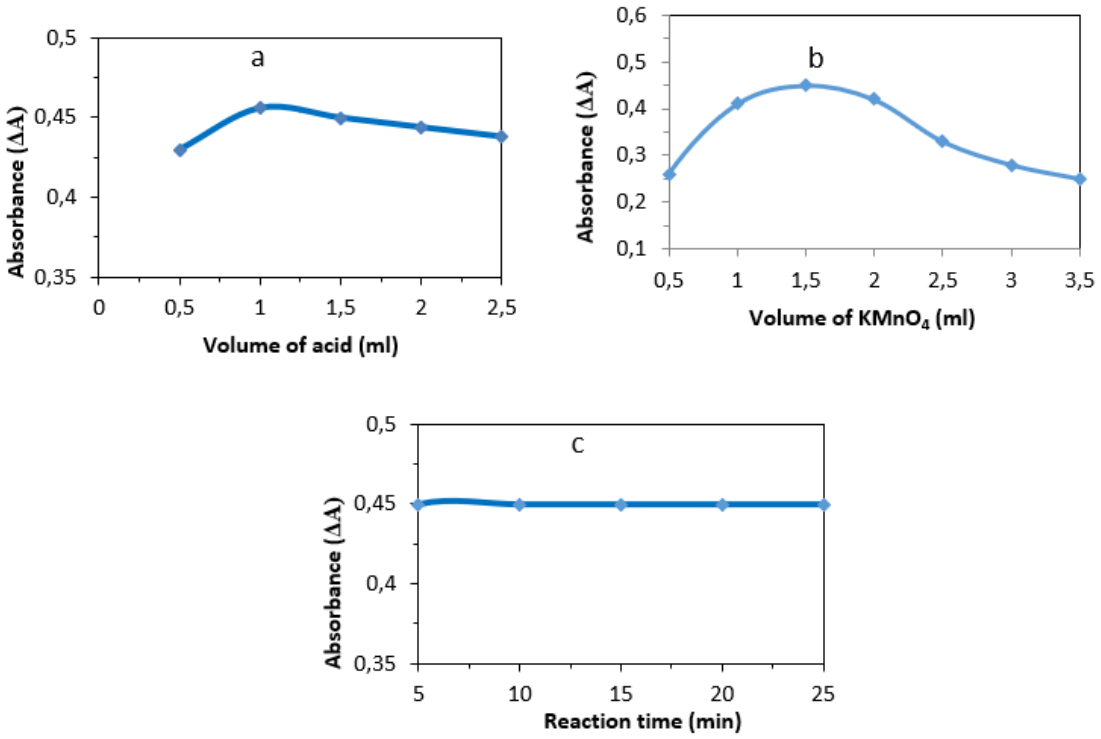


Figure 4. The effects of different parameters on the developed methods: volume of H_2SO_4 (TBH: 9 µg/ml) **(a)**, reaction time and stability (TBH: 9 µg/ml) **(b)**, volume of PP (TBH: 9 µg/ml) (**c**), pH of buffer (TBH: 10 µg/ml)

DSOQ Method

Effect of Buffer Solutions

The pKa value of TBH is 8.86 and says the TBH drug is the strongest basic, this means, the reaction at acidic pH has proceeded because the amino group of TBH easily becomes in the protonated form as well as the acid dye DSOQ becomes an anionic form. So, the reaction of TBH with DSOQ is only performed in an acidic medium.

To investigate the effect of pH on the absorbance of ion-pair formed in the aqueous layer, the procedure using DSOQ was adjusted to the pH in the range of 1.4 – 5.4. The resulting absorbance of that ion-pair increases with increases in the pH from 1.4 to 2.9, then the absorbance remains almost constant at pH 3.9, after that the absorbance was decreased. So, the pH value of 3.4 was chosen as to optimum value (Figure 5). The volume of biphthalate buffer at pH 3.4 was investigated in the range of 2 to 10 ml (Figure 5), and 6 ml of buffer solution was optimum.

Effect of DSOQ Reagent

The ion-pair complex formation of drug and DSOQ solutions was examined using 2-10 ml volumes of DSOQ dye (0.06% (w/v)), which was added to a constant concentration of TBH (10 µg/ml). Six ml of DSOQ solution in the aqueous phase (20 ml) adequately for optimum complex formation (Figure 5).

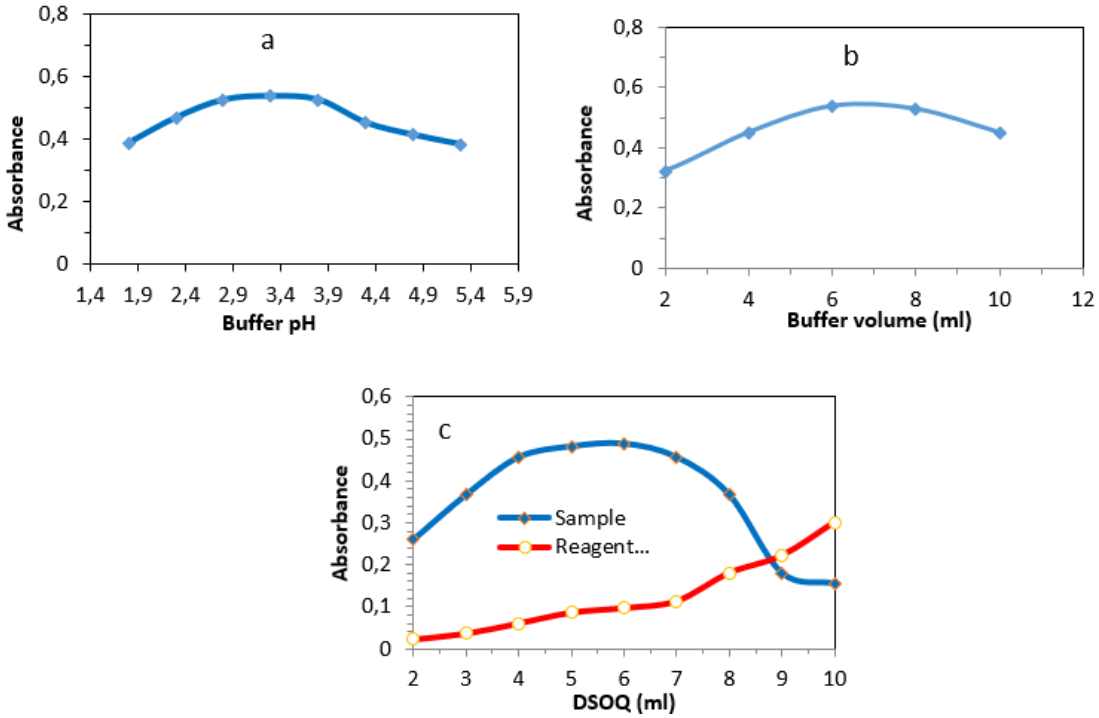


Figure 5. pH of buffer (TBH: 10 µg/ml) **(a)**, the volume of buffer (TBH: 10 µg/ml) **(b)**, the volume of DSOQ (TBH: 10 µg/ml) **(c)**

Method Validation

Range and Sensitivity

According to the optimum conditions for spectrophotometric methods using PP and DSOQ reagents, a linear resulting range between concentration and absorbance at λ_{max} was observed in 1-15 µg/ml for PP assay and 1-18 µg/ml for DSOQ assay, and Beer's law is obeyed inversely (Figure 6). Regression parameters values: intercept (a), slope (b), and the correlation coefficient (r) of each assay were calculated by linear regression (method of the least squares), and the other parameters of sensitivity

such as linear ranges, Sandell's sensitivity, molar absorptivity, LOD and LOQ values for each method are compiled in Table 1, which all generally indicating highly sensitive of the proposed methods.

The LOD and LOQ values were calculated using the formulae:

$$\text{LOD } (\mu g\ ml^{-1}) = \frac{3s}{b} \quad \text{and} \quad \text{LOQ } (\mu g\ ml^{-1}) = \frac{10s}{b}$$

where 's' is the standard deviation of the replicate absorbance values of the reagent blank, and 'b' is the slope of the calibration curve.

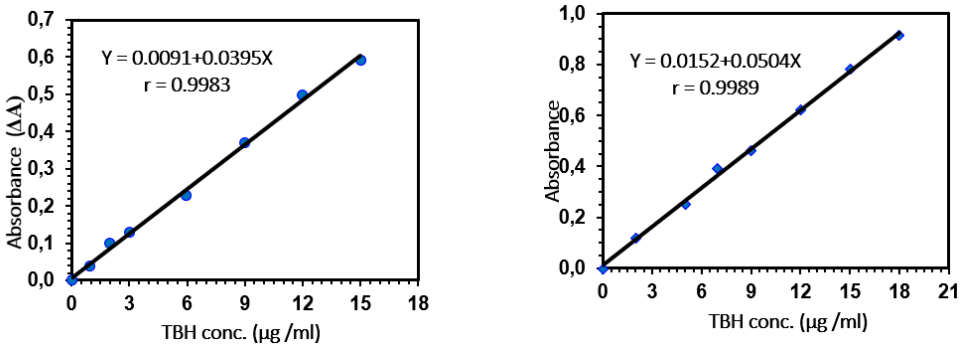


Figure 6. The calibration curves for PP (●) and DSOQ (■) assays

Table 1. Sensitivity and regression parameters were obtained from calibration curves for the determination of the TBH drug by spectrophotometric assays

Parameters	PP method	DSOQ method
λ_{max}, nm	540	440
Color stability	25 min	45 min
Linear range (µg/ml)	1–15	1–18
Molar absorptivity (l/mol cm)	1.38×10^4	1.73×10^4
Sandell sensitivity (µg/cm^2)	0.0238	0.0189
Intercept (a) ± (STD_a)*	0.0091 ± 0.0989	0.0152 ± 0.0998
Slope (b) ± (STD_b)*	0.0395 ± 0.00757	0.0504 ± 0.0052
LOD/LOQ (µg/ml)	0.13/0.39	0.09/0.27
Correlation coefficient (r)	0.9983	0.9989

*STD: standard deviation

Precision and Accuracy

The proposed PP and DSOQ assays were validated for each intra-day (within-day) and inter-day (between-day) precision. The precision and accuracy were evaluated by subjecting the pure TBH standard solution at three concentration levels (2,4,6 µg/ml using PP and 4,8,12 µg/ml using DSOQ) to within a day for intra-day variation analyzed, and on five successive days for inter-day variation analyzed, on seven replicates for each, taking into account the preparation of all chemical solutions daily to ensure accurate results. The precision was expressed as relative standard deviation (RSD). The %RSD (precision) for the intra-day assay ranged from 0.54–1.73 %, and 0.41–1.66 % for the inter-day assay, which should be satisfactory to the determination of the TBH in the different sample matrices used. The accuracy was calculated using the formula, Bias% = (found-taken/found x 100) and expressed as relative error percent (%RE). For three levels of drug, the accuracy varied from 0.67 to 1.5 % for intra-day and

0.84% to 2.25% for inter-day assays. These results are summarized in Table 2 and meet the requirement for precision, and the results indicate that the proposed methods are sufficiently precise and accurate.

Table 2. Intra-day and inter-day accuracy and precision (Reproducibility and intermediate precision)

Assay		Intra-day precision (n=7)			Inter-day precision (n=5)		
	Taken µg/ml	[a]Found µg/ml	RSD%	RE%	[a]Found µg/ml	RSD%	RE %
PP	2	2.02	0.91	1.0	2.03	0.83	1.50
	4	3.97	1.61	0.75	4.04	1.37	1.00
	6	6.04	0.54	0.67	6.05	0.41	0.84
DSOQ	4	4.06	1.23	1.50	3.99	1.53	2.00
	8	8.07	1.73	0.88	7.93	0.81	2.25
	12	12.08	1.07	0.67	12.19	1.66	1.58

[a] Mean value of 7 determinations

Robustness and Ruggedness

The optimized experimental variables were slightly altered in each method and the impact of these alterations was investigated on the performance of the proposed assays as a part of a robustness study. Experimental variables slightly altered were: the volume of acid, reaction time (PP method), buffer pH, and dye volume (DSOQ method). Assays were repeated and performed by only one analyst on the three different instruments in the laboratory and also by three analysts using the same instrument to understand the ruggedness of the methods. The results expressed as intermediate precision in RSD%, were encouraging assuring the utility of the proposed methods in routine use. The results are shown in Table 3.

Table 3. Evaluation of robustness and ruggedness for proposed methods

Method	TBH taken µg/ml	Robustness (RSD%)[a]				Ruggedness (RSD%)[a]	
		Parameters altered				Inter-analysts	Inter-instruments
		Acid volume (ml)	Reaction time (min)	Buffer pH	DSOQ volume (ml)		
PP	3	0.63	1.18	-	-	1.01	1.27
	6	0.75	0.92	-	-	1.53	1.19
	9	1.21	1.06	-	-	0.74	1.47
DSOQ	4	-	-	1.33	0.87	1.84	1.23
	8	-	-	1.55	1.35	1.45	1.65
	12	-	-	1.21	1.46	1.77	1.78

[a] Mean value of three determinations

Selectivity

This was investigated by studying and evaluating the impact of the additives and excipients present in the tablets by applying the proposed methods on the placebo blank and synthetic mixture. The results of the measurements showed that they were equal to those obtained from the blank. When applied the proposed methods to synthetic mixture analysis yielded recoveries of row TBH drug ranging from 98.46±0.76 to 103.6±1.85. The results saying non-interference from the additives and excipients of tablets such as sodium citrate, talc, starch, stearate, methylcellulose, etc. in the assays.

Application to Tablets Analysis

The developed methods were applied to the determination of terbiforce and sebifin tablet brands, each containing terbinafine HCl. The results for both assays presented in Table 4 say the excellent agreement with its label claim and with those obtained using the reference assay (USP) [5]. Statistically, for accuracy using the student's t-test, and for precision using F-test (variance ratio), the results revealed no significance when performing the developed methods and the reference method.

Table 4. Statistical analysis of obtained results by the proposed methods in tablets and comparison of it with the official method

Tablets		PP method	DSOQ method	Official method
Terbiforce 250 mg	Found[a] (% of label claim ± SD)	99.54 ± 0.95	99.89 ± 0.7	100.5±0.54
	t-test	1.97	1.54	
	F-test	3.09	1.68	
Sebifin 250 mg	Found[a] (% of label claim ± SD)	99.17 ± 1.63	99.77 ± 1.44	98.92 ± 1.29
	t-test	0.27	0.98	
	F-test	1.60	1.25	

[*]Mean found concentrations of n=5 determinations, tabulated t-value= 2.77 and F-value= 6.39, with 95% confidence level

Accuracy

To realize the accuracy of the present assays using PP and DSOQ reagents, the standard addition procedure (recovery study), was followed. A pure TBH drug was spiked well with tablet brands powder at three different levels, then the total amount was determined by proposed methods in the triplicate determinations. The results of the standard addition procedure, that present in Table 5 reveal that the co-formulated substances in the tablets do not affect the proposed methods.

Table 5. Recovery experiment of proposed methods via standard-addition method

Methodology	Formulations	Taken (μg/ml)	Added (μg/ml)	Found (μg/ml)	[a]Recovered % ± SD
PP	Terbiforce	2.99	1.5	4.550	101.3 ± 1.63
		2.99	3.0	5.960	99.51 ± 0.81
		2.99	4.5	7.440	99.37 ± 0.71
	Sebifin	2.98	1.5	4.440	99.15 ± 1.07
		2.98	3.0	6.110	102.1±1.150
		2.98	4.5	7.410	99.02 ± 0.63
DSOQ	Terbiforce	5.95	3.0	3.890	98.29 ± 0.61
		5.95	5.0	12.07	101.12 ± 1.07
		5.95	9.0	15.12	100.99 ± 1.06
	Sebifin	5.15	3.0	9.090	99.05 ± 1.42
		5.15	6.0	12.25	100.93 ± 1.52
		5.15	9.0	15.20	100.33 ± 1.18

[a]Mean value of three determinations

Table 6. Comparison of the proposed methods with the reported methods

Reported methods	Reagents	Range (μg/ml)	LOD/LOQ (μg/ml)	Remarks
Method reported 33	MO	6-17	Not available	Narrow range
Method reported 34	ARS	5-55	Not available	Narrow linear ranges, less sensitive
	Mo (v)/SCN	5-75		
	OG	10-80		
Method reported 35	BPB	2-25	0.23/0.71	Higher LOD and LOQ values
	BTB	2-25	0.28/0.84	
	BCG	2-25	0.54/1.62	
Method 36	ARS	2.5-60	0.22/0.66	Narrow linear ranges, less sensitive
Method 37	BCP	1-10	0.25/0.75	Higher LOD and LOQ values
Method 38	PP	2-16	0.65/1.96	heated at 85°c for 30minutes is required, High blank value
Method 39	Ce(IV) Ce(IV)-C2R	1-7 1-7	1.06/3.53 0.93/3.11	Heating step is required
Method 40	$KBrO_3$-KBr-MEB $KBrO_3$-KBr-MER	1-3 2.5-5	0.3/0.95 0.15/0.46	Heating step is required, high standing time used
Presented method	PP	1-15	0.92/2.78	**Highly sensitive, uses aqueous medium to critical pH adjustment**
Presented method	DSOQ	1-18	0.09/0.27	**Widest linear dynamic range (18-fold), lower LOD and LOQ values**

Conclusion

Terbinafine HCl despite its therapeutic importance in the treatment of fungi, the spectrophotometric methods that dealt with this drug are very few, including two methods by the author. The author presents this new research as a continuation of previous work to develop simple, high-accuracy, and rapid methods to cover the need for such low-cost methods for use in pharmaceutical quality control and clinical laboratories. The proposed spectrophotometric methods are simple without requiring any stringent experimental variable encountered in the published methods. The proposed methods are reasonably sensitive with a wide linear dynamic range as shown in Table 6. They can be useful in routine analysis and quality control assays of TBH in formulations. However, when compared to the HLPC method, which is widely used in pharmaceutical quantity control laboratories, the proposed methods lack selectivity, sensitivity, and speed.

ACKNOWLEDGEMENTS

The author (NASQ) wishes to express his thanks to Hodeidah University, Republic of Yemen for supporting research.

AUTHOR CONTRIBUTIONS

Concept: N.Q., E.E.; Design: N.Q., E.E.; Control: N.Q., E.E.; Sources: N.Q., K.B.; Materials: N.Q., K.B.; Data Collection and/or Processing: N.Q.; Analysis and/or Interpretation: N.Q.; Literature Review: N.Q., K.B.; Manuscript Writing: N.Q., E.E.; Critical Review: N.Q., E.E., K.B.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Drug Bank web site (2023) from https://go.drugbank.com/salts/DBSALT000470. Accessed date: 19.08.2023.
2. Kanakapura, B., Penmatsa, V.K. (2016). Analytical methods for determination of terbinafine hydrochloride in pharmaceuticals and biological materials. Journal of Pharmaceutical Analysis, 6(3), 137-149. [CrossRef]
3. Gupta, I., Adin, S.N., Aqil, M., Mujeeb, M., Sultana, Y. (2022). Investigation on utility of some novel terpenes on transungual delivery of terbinafine for the management of onychomycosis. Dermatology Practical Conceptual, e2022202. [CrossRef]
4. Yuen, M.V., Gianturco, S.L., Pavlech, L.L., Storm, K.D., Mattingly, A.N. (2021). Terbinafine hydrochloride: Summary Report. University of Maryland School of Pharmacy, USA, p. 5.
5. United States Pharmacopeia (2023). USP Monographs, Terbinafine hydrochloride. USP-NF. Rockville, MD: United States Pharmacopeia. [CrossRef]
6. British Pharmacopoeia Commission Office. (2022). Medicines and Healthcare products Regulatory Agency, volume II, London, UK. p. 1095.
7. The European Pharmacopeia (EP). (2011). European Directorate for the Quality of Medicines & Health Care (EDQM). 7th ed. Strasbourg, France: Council of Europe; pp. 3047-3048.
8. Cardoso, S.G., Schapoval, E.E.S. (1999). UV spectrophotometry and nonaqueous determination of terbinafine hydrochloride in dosage forms. Journal of AOAC International, 82(4), 830-833. [CrossRef]
9. Faridbod, F., Ganjali, M.R., Norouzi, P. (2013). Potentiometric PVC membrane sensor for the determination of Terbinafine. International Journal of Electrochemical Science, 8(5), 6107-6117. [CrossRef]
10. Ali, T., Mohamed, G., Abd El-Hamid, N. (2021). Potentiometric determination of mevacaine hydrochloride local anesthetic drug in pharmaceutical and biological fluids using ion selective electrode. Egyptian Journal of Chemistry, 5, 80-92. [CrossRef]
11. Samy, A.I., Sayed, S.A., Haroon, A.A. (2005). Conductimetric determination of cyproheptade, cetirizie, and terbinafine hydrochloride through the formation of ion-associates with manganese and zinc thiocyanate complexes. Journal of Drug Research of Egypt, 26(1-2), 139-143.
12. Mielech-Łukasiewicz, K., Dąbrowska, A. (2014). Comparison of boron-doped diamond and glassy carbon electrodes for determination of terbinafine in pharmaceuticals using differential pulse and square wave voltammetry. Analytical Letters, 47(10), 1697-1711. [CrossRef]
13. Jain, P.S., Chaudhari, A.J., Patel, S.A., Patel, Z.N., Patel, D.T. (2011). Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. Pharmaceutical Methods, 2(3), 198-202. [CrossRef]
14. Vamsi, K.P., Basavaiah, K. (2013). Stability indicating UV-spectrophotometric assay of terbinafine hydrochloride in dosage forms. International Journal of ChemTech Research, 5(5), 2645-2655.
15. Goswami, P.D. (2013). Validated spectrophotometric method for the estimation of terbinafine hydrochloride in bulk and in tablet dosage form using inorganic solvent. Der Pharmacia Lettre, 5(3), 386-390.
16. Patel, K.K., Marya, B.H., Kakhanis, V.V. (2012). Spectrophotometric determination and validation for terbinafine hydrochloride in pure and in tablet dosage form. Der Pharmacia Lettre, 4(4),1119-1122.
17. Belal, F., Sharaf El-Din, M.K., Eid, M.I., El-Gamal, R.M. (2013). Spectrofluorimetric determination of terbinafine hydrochloride and linezolid in their dosage forms and human plasma. Journal of Fluorescence, 23(5), 1077-1087. [CrossRef]

18. Felix, F.S., Ferreira, L.M.C., Rossini, P., do Lago, C.L., Angnes, L. (2012). Quantification of terbinafine in pharmaceutical tablets using capillary electrophoresis with contactless conductivity detection and batch injection analysis with amperometric detection. Talanta, 101, 220-225. [CrossRef]
19. Mikus, P., Valaskova, I., Havranek, E. (2005). Determination of terbinafine in pharmaceuticals and dialyzates by capillary electrophoresis. Talanta, 65(4), 1031-1037. [CrossRef]
20. Ramesh, R.R., Bujji, B.N. (2011). Simultaneous analysis of RP-HPLC method development and validation of terbinafine and bezafibrate drugs in pharmaceutical dosage form. Pharmacophore, 2(4), 232-238.
21. Penmatsa, V.K., Basavaiah, K. (2014). Simple, sensitive and stability indicating high performance liquid chromatographic assay of terbinafine hydrochloride in dosage forms. American Journal of PharmTech Research, 4(2), 899-916.
22. Patel, K.K. (2012). A validated RP-HPLC method for determination of terbinafine hydrochloride in pharmaceutical solid dosage form. International Journal of Pharmacy and Technology, 4(3), 4663-4669.
23. Domadiya, V., Singh, R., Kumar Jat, R., Chokshi, R. (2012). Method development and validation for assay of terbinafine HCl in cream by RP-HPLC method. Inventi Rapid: Pharm Analysis & Quality Assurance, 3, 131-145.
24. Kassem, H., Almardini, M.A. (2013). High performance liquid chromatography method for the determination of terbinafine hydrochloride in semi solids dosage form. International Journal of Pharmaceutical Sciences Review and Research, 21, 58-61.
25. Goswami, P.D. (2013). Stability-indicating RP-HPLC method for analysis of terbinafine hydrochloride in bulk and in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 5, 536-540.
26. Tagliari, M.P., Kuminek, G., Borgmann, S.H.M., Bertol, C.D., Cardoso, S.G., Stulzer, H.K. (2010). Terbinafine: Optimization of a LC method for quantitative analysis in pharmaceutical formulations and its application for a tablet dissolution test. Química Nova, 33(8), 1790-1793. [CrossRef]
27. Florea, M., Arama, C.C., Monciu, C.M. (2009). Determination of terbinafine hydrochloride by ion-pair reversed phase liquid chromatography. Farmacia, 57, 82-88.
28. Gopal, P.N., Hemakumar, A.V., Padma, S.V.N. (2008). Reversed-phase HPLC method for the analysis of terbinafine in pharmaceutical dosage forms. Asian Journal of Chemistry, 20(1), 551-555.
29. El-Gendy, H., Zarad, W., Bazan, L., Ali, A., Aboulella, Y., Kamal, M., Emara, S., Shawky, A. (2022). Rapid back flushed direct sample injection bio-analytical HPLC-UV method for therapeutic drug monitoring of terbinafine. Analytical Biochemistry, 659, 114951. [CrossRef]
30. Belal, F., Sharaf El-Din, M.K., Eid, M.I., El-Gamal, R.M. (2016). Micellar liquid chromatography coupled with fluorometric detection for the determination of terbinafine in dosage forms and plasma. Journal of Chromatography & Separation Techniques, 08(06). [CrossRef]
31. Grishma, H.P., Faiz, S.B., Dhananjay, B.M. (2021). RP-HPLC method development and validation for simultaneous estimation of terbinafine hydrochloride and fluticasone propionate in their formulation. Annals of Pharma Research, 9(11), 579-587.
32. Manjusha, V., Sreenivasa, P., Thejomoorthy, K. (2021). Stability indicating method development and validation for the estimation of terbinafine and itraconazole in api and tablet dosage form by RP-HPLC. World Journal of Pharmaceutical and Medical Research, 7(6), 322-332.
33. Florea, M., Monciu, C.M. (2008). Spectrophotometric determination of terbinafine through ion-pair complex formation with methyl orange. Farmacia, 56, 393-401.
34. Elazazy, M.S., El-Mammli, M.Y., Shalaby, A., Ayad, M.M. (2008). Application of certain ion-pairing reagents for extractive spectrophotometric determination of flunarizine hydrochloride, ramipril, and terbinafine hydrochloride. Biosciences Biotechnology Research Asia, 5(1), 107-114.
35. Chennaiah, M., Veeraiah, T., Kumar, T.V., Venkareshwarlu, G. (2012). Extractive spectrophotometric methods for determination of terbinafine hydrochloride in pharmaceutical formulation using some acidic triphenylmethane dyes. Indian Journal of Chemical Technology, 19(3), 218-221.
36. Qarah, N.A.S., Basavaiah, K., Swamy, N., Chandrashekar, U. (2015). Assay of terbinafine hydrochloride by extractive-spectrophotometry with alizarin red S - a modified approach. Eurasian Journal of Analytical Chemistry, 10(1), 34-45.
37. Qarah, N.A.S., Basavaiah, K., Swamy, N. (2016). Ion-pair extractive spectrophotometric assay of terbinafine hydrochloride in pharmaceuticals and spiked urine using bromocresol purple. Journal of Applied Spectroscopy, 83(4), 694-702. [CrossRef]
38. Afaf, A.E., Hanaa, M.S., Magda, M.E., Basma, E.G. (2015) Spectrophotometric determination of terbinafine HCl and telmisartan using potassium permanganate. International Journal of Pharmaceutical Chemical and Biological Sciences, 5(1), 361-371.
39. Afaf, A.E., Hanaa, M.S., Magda, M.E., Basma, E.G. (2014) Spectrophotometric determination of

terbinafine HCl, telmisartan and ramipril through redox reactions using ceric sulphate and ceric sulphate-chromattrope 2R. International Journal of Pharmaceutical Chemical and Biological Sciences, 5(1), 931-943.

40. Afaf, A.E., Hanaa, M.S., Magda, M.E., Basma, E.G. (2015) Spectrophotometric determination of terbinafine HCl, telmisartan and ramipril through redox reactions using bromate-bromide mixture. International Journal of Pharmaceutical Chemical and Biological Sciences, 5(1), 328-346.
41. Vogel, A.I. (1989). Vogel's Text-book of Quantitative Inorganic Analysis, Longman Group UK Limited, London, England, p. 370-377.
42. The United States Pharmacopeial Convention web site. (2020). From https://onlineuspnf.nf.com/. Accessed date: 10.08.2023.