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Determination of Terazosin by MBTH and BPB reagents using Spectrophotometric Technique

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ABSTRACT

A simple, sensitive, selective rapid spectrophotometric method has been developed for the determination of terazosin in pure form, pharmaceutical formulations and blood sample based on the oxidative coupling reaction with MBTH (3-methyl-2-benzothiazolone hydrazone hydrochloride- Method-A), and Ion association reaction with BPB(Bromophenol Blue-Method-B) reagents at P^{H}–4.0 which is extractable at 620 nm (Method-A) and 410nm (Method-B) respectively. Beer's law is obeyed in the concentration ranges 10-60 µg $ml^{-1}$and 5-30 µg ml^{-1} for formulations, 4-24 µg ml^{-1} and 3-18 µg ml^{-1} for blood sample respectively. The developed method was applied directly and easily for the analysis of the pharmaceutical formulations and blood samples. % R.S.D was found between 0.09699%-0.38910% (Method-A), and 0.45101%-0.75718% (Method-B), respectively and recovery was found between 99.85%-99.98% (Method-A), and 98.62%-99.86% (Method-B), respectively. The proposed methods were applied successfully for the analysis of terazosin. No interference was observed from common pharmaceutical excipients.

Keywords: Spectrophotometry, Terazosin, MBTH, BPB, Oxidative coupling reaction & Ion association reaction.

INTRODUCTION

Terazosin, a post-synaptic α1-adrenoceptor antagonist is used to treat hypertension and the symptom of benign prostatic hyperplasia (BPH) [1, 2]. When compared with alfuzosin and tamsulosin, terazosin is the drug with a favorable effect in four areas of interest, including BPH symptoms, blood pressure, platelet aggregation, and endothelial functions in the patients with BPH [3]. Also terazosin was proved effective and safe in the treatment of lower urinary tract symptoms (LUTS) in male or female patients [4, 5]. As an antihypertensive drug, terazosin immunotherapy or in combination with other antihypertensive agents significantly reduces the blood pressure, especially diastolic blood pressure in patients with mild to moderate hypertension [1, 6]. The antihypertensive effect and the dose of terazosin showed a strong dose-response relationship in the dose of 1 to 10 mg, with a maximum response plateau above10 mg. The maximum antihypertensive response to

terazosin is10.7 mm Hg for SBP and 8.0 mmHg for DBP in the patients with mild to moderate essential hypertension. Besides the hypertensive patients, terazosin can also decrease the blood pressure in healthy people. A single 0.5 mg terazosin can decrease the supine and 50°tilt in DBP, while higher doses were required to decrease SBP in healthy Japanese volunteers [7]. It is worth noting that syncope and orthostatic hypotension, as serious adverse events, are prone to occur after the first dose of terazosin, namely "first-dose" effect [8].

Terazosin α-Adrenoreceptor antagonists are frequently used to treat patients with lower urinary tract symptoms (LUTS) and prostatic enlargement because of their significant effect on storage and voiding symptoms, QOL, flow rate, and post void residual urine volume [9]. The α1-blockers reduce smooth muscle tone in the prostate and result in rapid improvements in urinary symptoms and flow [10]. The greatest safety concern associated with the use of these agents is the occurrence of vasodilatory symptoms such as dizziness and orthostatic hypotension resulting from inhibition of α1-ARs in the systemic vasculature [11]. However, rossitto et al recommended the use of alpha one blocker therapy in a patient having both hypertension and BPH [12]. Terazosin hydrochloride dehydrate RS-1-(4-amino-6, 7-dimethoxy-2-quinazolinyl)-4-[(tetra-hydro-2-furanyl)carbonyl]-piperazine monohydrochloride [Fig.1] is a α1-adrenoceptor blocker with a long lasting action; which is official in European Pharmacopoeia, USP and BP [13-15].Indication of in mild to moderate hypertension and benign prostatic hyperplasia [16]. Terazosin is used in the management of hypertension and in benign prostate hyperplasia to relieve symptoms of urinary obstruction [17].

Terazosin could be determined by using several analytical techniques like potentiometry [18], voltammetry [19, 20], spectrophotometry [21, 22], and fluorimetry [23, 24]. Thermal analysis including TGA, DTG, DTA and DSC are also useful techniques that have been successfully applied in the pharmaceutical industry to get important information regarding the physicochemical properties of drug and excipients such as polymorphism, stability and purity [25, 26]. Simultaneous determination of prazosin, terazosin, doxazosin, tamsulosin and alfuzosin in their respective pharmaceutical dosage forms can be determine by using HPLC and HPTLC methods [27]. Fluorescence has allowed liquid chromatography (LC) to expand into a high-performance technique. High-performance liquid chromatography (HPLC) procedures with fluorescence detection were used in routine analysis for assay in the low nanogram per milliliter range and in concentrations as low as picogram per milliliter often can be measured [28]. Fluorescence-based HPLC has been used as a sensitive and less costly alternative approach to LC-MS [29].

There is, however, no UV- Visible spectrophotometric method for the analysis of terazosin in its technical grade and formulations. In this study describes validated UV- visible spectrophotometric methods for the quantitative determination of terazosin. Functional group used for colour development of terazosin is primary amine group. The results obtained in this method were based on the oxidative coupling reaction with MBTH/ Ferric chloride (Method - A). It was also studied ion association complex reaction with BPB/HCl/Chloroform (Method - B).

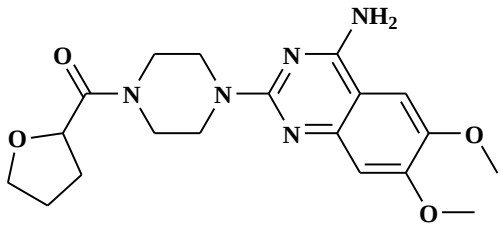


Figure-1 Chemical Structure of Terazosin

EXPERIMENTAL

Chemicals and Instruments

The pure gift samples was collected from Bio-Leo Analytical Labs INDIA pvt ltd, Plot No 135, Oasis Towers 9, IDA, Kukatpally, Prashantinagar, Hyderabad – 500 072. 3-methyl-2-benzothiazolone hydrazone hydrochloride, Bromophenol Blue and acetonitrile all chemicals are analytical grade were purchased in SSR Enterprises, Tirupati-517 501 A.P, India. UV-Vis spectrophotometer (UV-1800 Shimandzu, North America) connected to computer loaded with spectra manager software vision light was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. For scanning, the wavelength range selected was 300 nm to 1000 nm with medium scanning speed. All weights were taken using electronic balance (Denver, Germany). All experiments were performed at room temperature (25 ± 1) °C.

Preparation of standard stock solution

Accurately weighed 100 mg of terazosin was dissolved in 40 ml of acetonitrile in 100 ml volumetric flask and the solution was made up to the mark with acetonitrile which was the stock solution A. 10 ml solution was pipetted out into a 100 ml volumetric flask and the same was made up to the mark with acetonitrile to obtain a final concentration of 100 µg ml^{-1} which was the stock solution B.

Preparation of Calibration curve

Method-A

Fresh aliquots of terazosin ranging from 1.0 to 6.0 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 10 to 60 µg ml^{-1}. 1 ml of (0.5%) MBTH solution, 1 ml of (0.7%) Ferric chloride solutions were added to each flask and resulting solution was heated for 15 min and finally 1 ml (0.5N) HCl solution was added. The solutions were cooled to room temperature and made up to mark with acetonitrile. The absorbance of green colored chromogen was measured at 620 nm against reagent blank. The amount of Terazosin present in the sample solution was computed from its calibration curve.

Method-B

Fresh aliquots of terazosin ranging from 0.5 to 3.0 ml were added to 2 ml of 0.2 % Bromophenol blue (BPB), 1ml (0.5N) Hydrochloric acid and 2ml of chloroform were added to get a final concentration range of 5 to 30 µg ml^{-1}. The sample solutions were transferred into a 50 ml separating funnels and were shaken for 2min.The two phases were allowed to separate and the absorbance of yellow colored chromogen was measured at 410 nm against reagent blank. The amount of terazosin present in the sample solution was computed from its calibration curve.

Procedure for formulations

Twenty tablets containing terazosin were weighed and finely powdered. An accurately weighed portion of the powder equivalent to100 mg of terazosin was dissolved in 100 ml of acetonitrile and mixed for about 5 min and then filtered. The acetonitrile was evaporated to get a dry powder. The powder was again diluted with acetonitrile and made up to 100 ml to get the stock solution A. 10 ml of aliquots was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with acetonitrile to obtain a final concentration of 100 µg/ml^{-1} which was the stock solution B. Subsequent dilutions of this solution were made with acetonitrile to get a concentration range 10 to 60 µg ml^{-1} (method-A) and 5 to 30 µg ml^{-1} (method-B) respectively. These were analyzed at the selected wavelengths, 620 nm (Method-A) and 410 nm (Method-B), the results were statistically validated.

Procedure for Blood sample

Blood sample collected was centrifuged. Terazosin was isolated from plasma using acetonitrile. This was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M sodium hydroxide, followed by extraction with 30% dichloromethane in hexane. The upper organic layer was evaporated to dryness. The dry residue 100 mg was dissolved in 100 ml of acetonitrile (1000 µg ml^{-1}). 10 ml of aliquot was taken into a 100 ml of volumetric flask and made up to the mark with acetonitrile (100 µg ml^{-1}) samples of aforesaid solutions ranging from 0.4 -2.4 ml (4- 24 µg ml^{-1}) were transferred in to 10 ml volumetric flasks and to each flask 1 ml of (0.5%) MBTH

solution (Method-A) was added followed by 1ml of (0.7%) Ferric chloride solution and finally were made up to the mark with acetonitrile. The resulting solutions were heated and finally 1ml (0.5N) hydrochloric acid solution was added. The solutions were cooled to room temperature and made up to the mark with acetonitrile. The absorbance of green colored chromogen was measured at 620 nm against the reagent blank.

The aforesaid sample solutions that had a range of 0.3.-1.8 ml were added 2 ml of 0.2% Bromophenol blue (BPB) (Method-B, 1ml (0.5N) hydrochloric acid and to 2ml of chloroform. The final concentration range was 3- 18µg ml^{-1}. These were transferred in to 50 ml. separating funnels and were shaken for 2min.The two phases were allowed to separate and the absorbance of yellow colored chromogen was measured at 410 nm against reagent blank. The amount of terazosin present in the sample solutions was computed from its calibration curves.

MBTH – (Method-A)

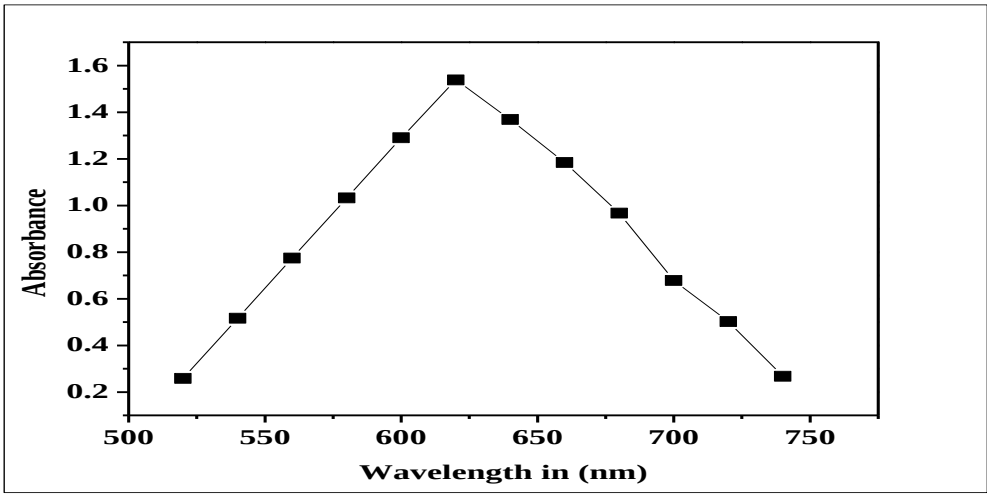


Fig-2: Absorption spectrum of Terazosin with MBTH / $FeCl_3$

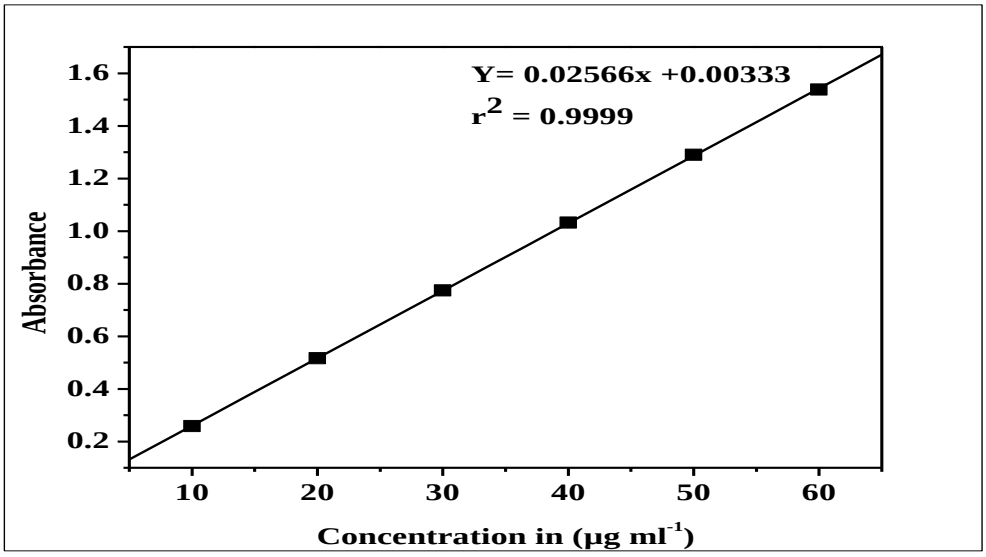


Fig-3: Beer's law plot of Terazosin with MBTH / $FeCl_3$

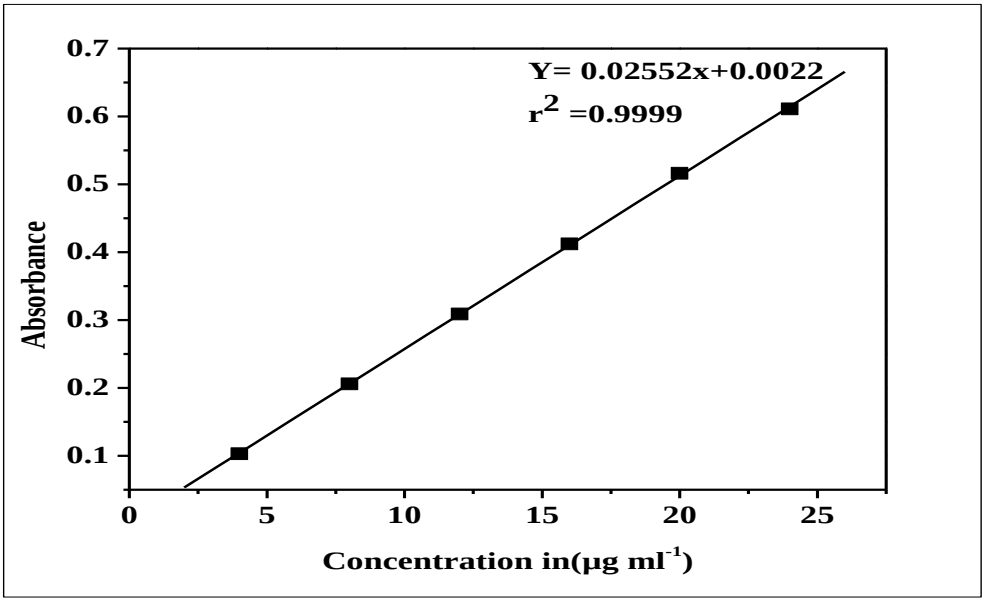
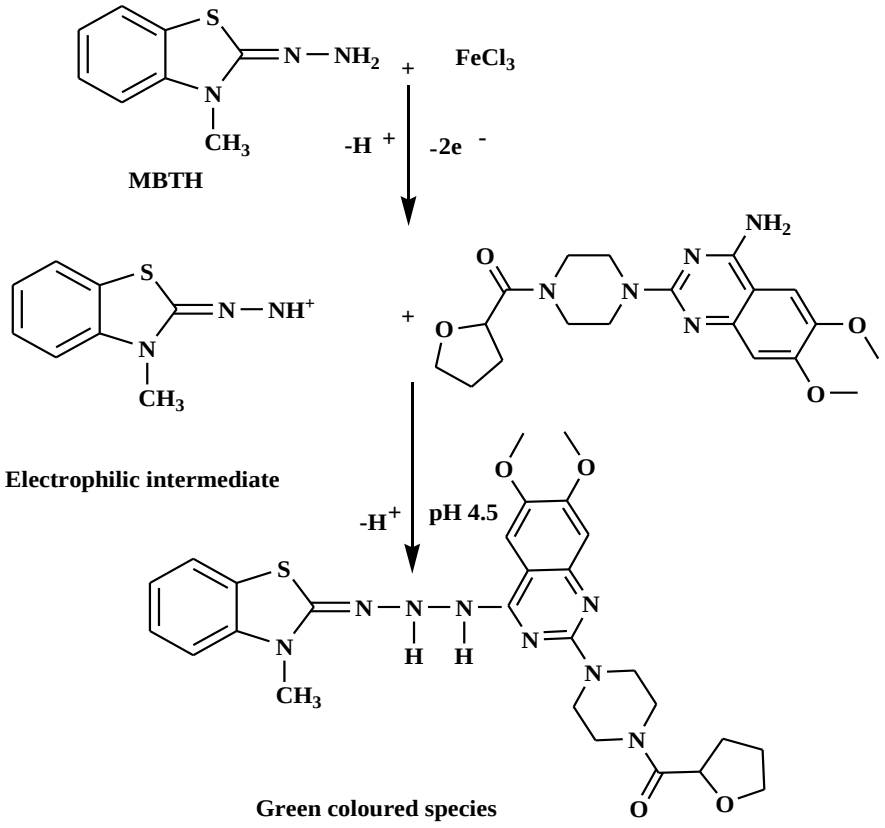


Fig-4: Beer's law plot for Terazosin in Blood sample



Schem : 1. A Schematic Reaction Mechanism of Terazosin with MBTH

Table-1: Assay results of Terazosin in formulations by visible method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method[30-32] (mg)	% Recovery
HYTRIN	250	249.96 t=0.00296 F=1.2053	247.22	99.96
HYTRINEX	250	249.89 t=0.002967 F=1.2073	246.86	99.89

- T and F- values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits t= 0.0019 and F= 0.9681

Table-2: Determination of accuracy of Terazosin

Amount of TRZ in formulation (mg)	Amount of Standard TRZ added (mg)	Total amount found (mg)	% Recovery
249.89	200	449.80	99.80
249.77	200	449.58	99.58
249.65	200	449.37	99.37
249.98	250	499.96	99.96
249.92	250	499.84	99.84
249.89	250	499.78	99.78
249.96	300	549.92	99.91
249.73	300	549.40	99.40
249.48	300	549.85	98.85

Table-3: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
449.58	0.21502	0.04782
499.86	0.09165	0.02037
549.39	0.28219	0.05136

The results are the mean of six readings at each level of recovery.

Table-4: Repeatability data for Terazosin at 620 nm

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std.deviation	(%)RSD
10	0.258	0.256	0.257	0.257	0.001	0.38910
20	0.516	0.514	0.515	0.515	0.001	0.19417
30	0.774	0.772	0.773	0.773	0.001	0.12936
40	1.032	1.030	1.031	1.031	0.001	0.09699
50	1.290	1.289	1.290	1.2896	0.00058	0.04497
60	1.538	1.536	1.535	1.5363	0.00153	0.09958

Average of Six determinations.

Table-5: Assay results of Terazosin in Blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method[30,31] (mg)	% of Recovery
HYTRIN	2	1.99 t=0.002966 F=1.2401	1.89	99.99
HYTRINEX	2	1.97 t=0.002965 F=1.2365	1.90	99.97

- T and F values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits t=0.00196 and F=1.024

Table-6: Determination of accuracy of Terazosin

Name of the Formulation in (mg)	Amount of Drug in Blood sample (mg)	Amount of Standard Drug added in (mg)	Total amount found (mg)	% Recovery
HYTRIN (2mg)	1.99	2	3.98	99.98
HYTRINEX(2mg)	1.97	2	3.94	99.94

The results are the mean of two readings at each level of recovery.

Table-7: Repeatability data for Terazosin at 620nm

Concentration in (µg)	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
4	0.1032	0.1031	0.103	0.1031	0.0001	0.09699
8	0.206	0.205	0.206	0.20567	0.00058	0.28200
12	0.309	0.308	0.307	0.308	0.001	0.32467
16	0.412	0.411	0.412	0.41167	0.00058	0.14088
20	0.5162	0.516	0.516	0.51607	0.00012	0.02325
24	0.619	0.618	0.617	0.618	0.001	0.16181

Average of six determinations

BPB – (Method-B)

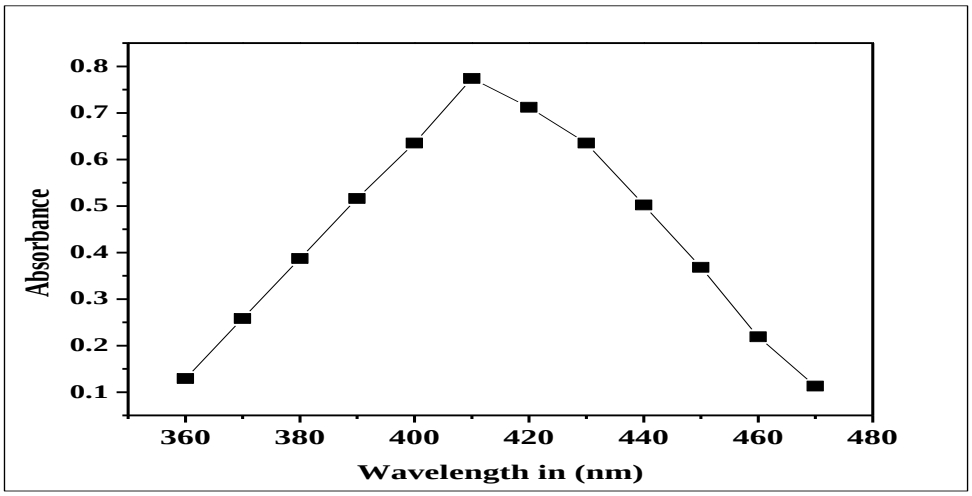


Fig-5: Absorption spectrum of Terazosin with BPB/ $CHCl_3$ / HCl

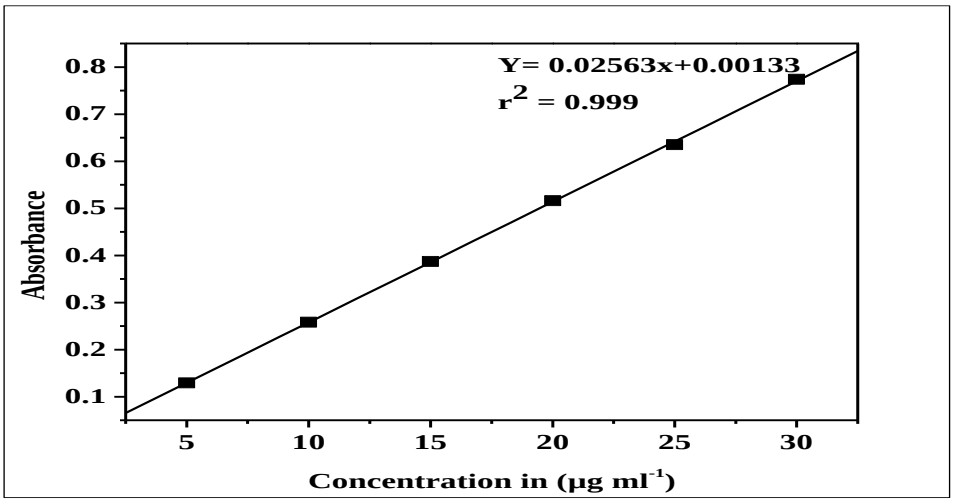


Fig-6: Beer's law plot of Terazosin with BPB / $CHCl_3$ /HCl

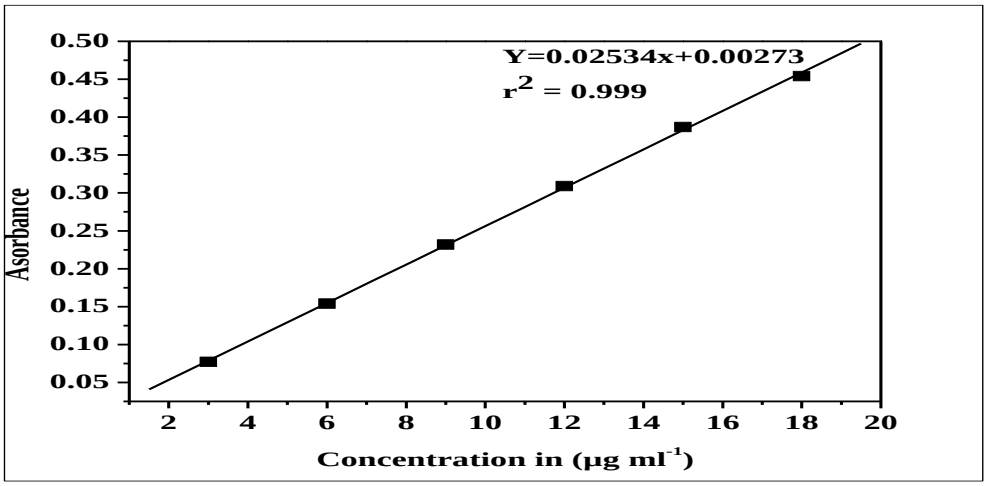


Fig-7: Beer's law plot for Terazosin in Blood sample

Scheme -2: A Schematic Reaction Mechanism of Terazosin with BPB

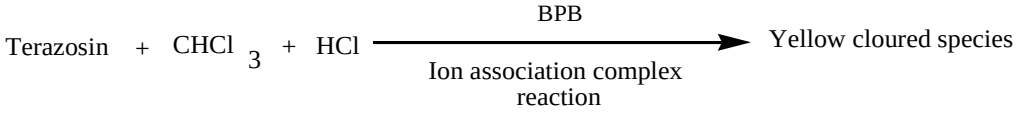


Table-8: Assay results of Terazosin in formulations by visible method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method[30,31] (mg)	% Recovery
HYTRIN	250	249.81 t=0.002966 F=1.23866	247.22	99.81
HYTRINEX	250	249.73 t=0.00296 F=1.20256	247.86	99.73

- T and F- values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits t= 0.0019 and F= 0.7961

Table-9: Determination of accuracy of Terazosin

Amount of TRZ in formulation (mg)	Amount of Standard TRZ added (mg)	Total amount found (mg)	% Recovery
249.52	200	449.13	99.13
249.40	200	448.92	98.92
249.28	200	448.74	98.70
249.69	250	499.38	99.38
249.50	250	499.00	99.00
249.31	250	498.62	98.62
249.89	300	549.75	99.75
249.56	300	549.03	99.03
249.40	300	548.68	98.68

Table-10: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
448.91	0.19519	0.043480
499.00	0.38	0.076152
549.15	0.5455	0.099335

The results are the mean of six readings at each level of recovery.

Table-11: Repeatability data for Terazosin at 410 nm

Conc. ($\mu g\ ml^{-1}$)	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%) RSD
5	0.129	0.128	0.129	0.1286	0.00058	0.45101
10	0.2581	0.258	0.256	0.2573	0.00115	0.44694
15	0.387	0.384	0.386	0.3856	0.00152	0.39419
20	0.516	0.515	0.513	0.5146	0.00153	0.29731
25	0.645	0.642	0.643	0.6433	0.00153	0.23783
30	0.774	0.769	0.772	0.7716	0.00252	0.32659

Average of Six determinations.

Table-12: Assay results of Terazosin in Blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method[30,31] (mg)	% of Recovery
HYTRIN	2	1.93 t=0.00297 F=1.1346	1.89	99.93
HYTRINEX	2	1.89 t=0.00296 F=1.1450	1.91	99.89

- T and F values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits t=0.00196 and F=1.1024

Table-:13 Determination of accuracy of Terazosin

Name of the Formulation in (mg)	Amount of Drug in Blood sample (mg)	Amount of Standard Drug added in (mg)	Total amount found (mg)	% Recovery
HYTRIN (2mg)	1.93	2	3.86	99.86
HYTRINEX(2mg)	1.89	2	3.78	99.78

The results are the mean of two readings at each level of recovery.

Table-14: Repeatability data for Terazosin at 410 nm

Concentration in (µg)	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
3	0.077	0.076	0.077	0.0766	0.00058	0.75718
6	0.154	0.153	0.152	0.153	0.001	0.65359
9	0.232	0.231	0.232	0.2316	0.00058	0.25043
12	0.309	0.308	0.309	0.3086	0.00058	0.18794
15	0.387	0.386	0.387	0.3866	0.00058	0.15002
18	0.454	0.454	0.453	0.4536	0.00058	0.12786

Average of six determinations

Table-15: Optical characteristics, Comparisons and precision by two methods

Parameter	MBTH- Method	BPB-Method
Colour	Green	Yellow
Absorption maxima(nm)	620	410
Beer's law limits (µg ml^{-1})	10 – 60	5-30
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.1538x10^4$	$0.0774x10^4$
Sandell's Sensitivity (µg cm^{-2})	0.65019	1.29198
Regression equation (Y*)	Y=mx+c	Y=mx+c
Slope (b)	0.02566	0.02563
Intercept(a)	0.00333	0.00133
Standard deviation(SD)	0.00345	0.0042
Correlation coefficient (r^2)	0.9999	0.999
%RSD (Relative standard deviation)	0.09958	0.44694
Limits of detection (LOD)(µg ml^{-1})	0.40335	0.49161
Limits of quantification (LOQ) (µg ml^{-1})	1.34450	1.63870

RSD of six independent determinations

RESULTS AND DISCUSSION

Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV Spectrophotometric method and the colored species formed in each two visible Spectrophotometric methods, specified amount of terazosin in solution which contained 10 µg ml^{-1} (method-A) and 5 µg ml^{-1} (method-B) was taken and the colours were developed following the procedures described earlier. The absorption spectra were scanned on spectrophotometer in the wavelength region of 300-

1000 nm against corresponding reagent blank. The regent blank absorption spectrum of each method was also recorded against distilled water / acetonitrile. The results are graphically represented in (fig- 2&5).

Parameters fixation

In developing these methods, a systematic study of the effects of various parameters encountered in these methods were under taken by verifying one parameter at a time to get the maximum colour development (methods A&B), reproducibility and reasonable period of stability of colored species. The following studies were conducted.

Method: A

The results obtained in this method were based on oxidation followed by coupling reaction of terazosin with MBTH, Ferric chloride and HCl to get green colored chromogen that exhibited maximum absorption at 620 nm against the corresponding reagent blank. The functional group used for the colour development in this method was primary amine group present in terazosin. A schematic reaction mechanism of terazosin with MBTH reagent is shown in (scheme-1). The effect of various parameters like concentration and volume of MBTH and strength of acid was studied by means of control experiments varying one parameter at a time.

Method: B

The results obtained in this method were based on ion association complex reaction of terazosin with BPB. Chloroform and HCl yielded an yellow colored chromogen that exhibited maximum absorption at 410 nm against the corresponding reagent blank. The functional group used for the colour development in this method was amino group. A schematic reaction mechanism of terazosin with BPB reagent is shown in (scheme-2). The effect of various parameters such as concentration and volume of BPB and strength of acid were studied by means of control experiments varying one parameter at a time.

Optical characteristics

The reference method which followed Beer's law was studied at appropriate wave length using a set of solutions that contained different amounts of terazosin using specified amount of reagents. The Beers law plot of the system is illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Beer's law limits, molar absorptivity & sandells sensitivity for terazosin with each reagent were calculated. In order to test whether the colored species formed in the methods follow the Beer's law, the absorbance at appropriate wavelength of a set of solutions that contained different amounts of terazosin and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks. The beers law plots of the system are illustrated graphically (fig-3, 4, 6&7). Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Beer's law limits molar absorptivity, Sandells sensitivity for terazosin with each of mentioned reagents were calculated. The optical characteristics were presented in the table-15.

Precision

The precision of each one amongst the three proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of terazosin 10 µg ml^{-1} and 5 µg ml^{-1} respectively- methods A,B&C in final solution. The percent relative standard deviations were calculated for the proposed methods and are given table-15.

Analysis of formulations

Commercial formulations of terazosin were successfully analysed by using the aforesaid methods. The values obtained from the proposed and reference methods were compared statistically by the T and F tests. It is found that the proposed methods do not differ significantly from those reported in the literature. The result are presented in tables-1&8 the proposed methods were also applied to biological samples (Blood). Good recoveries are obtained which are given in tables-5&12.

Accuracy

Recovery studies were carried by applying the method to drug sample present in formulations which contained specific amounts of terazosin. The recovery studies were carried by applying the same method to biological samples (Blood) to which a known amount of terazosin correspond to 2

mg of a formulation taken by the patient. By following the Standard addition method, 2 mg of the label claim was added. And the contents were transferred to 100 ml volumetric flask and dissolved in the solvent. Finally the volume was made up to the mark with the solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analysed and their absorbance values were determined. At each level of recovery five determinations were performed and are presented in Tables-2&9. The results obtained were compared with expected results and were statistically validated in Tables-3&10.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyse in the sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyse that have been demonstrated within a suitable level of precision, accuracy and linearity.

Repeatability

Standard solutions of terazosin were prepared and absorbance was measured against the solvent as the blank. The Absorbance of the same concentration solution was measured five times and standard deviation was calculated and the results are presented in tables-4, 7, 11&14.

Interferences Studies

The effect of wide range of inactive ingredients usually present in the formulations for the assay of terazosin under optimum conditions was investigated. None of them interfered in the proposed methods even when they were present in excess.

APPLICATION OF THE PROPOSED METHODS

The proposed methods (A&B) were successfully applied to the determination of terazosin in tablet dosage forms of two different brands. The results of the proposed methods were compared statistically to those of the reference method [32]. The calculated t- and F-values at 95 % confidence level, shown in Tables-1,5,8&12, did not exceed the tabulated values of 2.77 and 6.39, respectively, thus confirming no significant differences between accuracy and precision of the methods compared.

CONCLUSION

In this study, two UV- Vis-spectrophotometric methods were developed and validated for the determination of terazosin in bulk and tablet dosage forms. The spectrophotometer instrument is simple and not of high cost, on the other hand in terms of simplicity and expense. The apparatus and reagents used are easily accessible even for the simple laboratories and the procedures do not involve any critical reaction conditions or tedious sample preparation. As seen from the molar absorptivity values, the order of sensitivity of the proposed methods were MBTH>BPB. MBTH method compared to BPB method, MBTH method is more sensitive than BPB method. The methods show no interference from the ingredients usually found in the tablet dosage forms. The statistical parameters and recovery data reveal the good accuracy and precision of the proposed methods. Therefore, it is concluded that the proposed methods are simple, sensitive, reproducible, accurate and precise and can be recommended for routine and quality control analysis of terazosin.

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