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Assay of Tolterodine Tartrate Using MBTH Reagent in Bulk and Its Pharmaceutical Formulations

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ABSTRACT

A simple and sensitive visible spectrophotometric method for the determination of tolterodine tartrate using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) reagent has been developed in bulk and tablet dosage forms. It is based on the formation of intense blue colored species by treating the drug with MBTH reagent in the presence of ferric chloride with an absorption maximum of 650nm. The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 5-25µg/ml. The proposed method is validated with respect to accuracy, precision, linearity and limit of detection. The suggested procedure is successfully applied to the determination of the drug in pharmaceutical preparation, with high percentage of recovery, good accuracy and precision. The results of analysis have been validated statistically by repeatability and recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of tolterodine tartrate in capsule form without the interference of excipients.

Keywords: Anti-muscarinic agent, Beer's Law, Ferric chloride, MBTH, oxidative coupling reaction, Visible Spectrophotometric method.

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INTRODUCTION

Tolterodine tartrate (TT), chemically, (R)-N,N-diisopropyl-3-(2-hydroxy-5-methyl phenyl)-3-phenyl-propanamine L-hydrogen tartrate **(Figure.1)** is a potent and competitive muscarinic receptor antagonist used for the treatment of urinary incontinence (incontinence in detrusor instability) and other overactive bladder symptoms, such as urgency and high micturition frequency. The drug also increases functional bladder volume. The drug blocks muscarinic receptors, which can be found on the muscle cell of the bladder wall. Stimulation of these receptors causes the bladder to contract and empty when these receptors are blocked the muscle of the bladder wall contracts less. Tolterodine (TLD) acts on M1, M2, M3, M4 and M5 subtypes of muscarinic receptors whereas modern anti muscarinic treatments for overactive bladder only act on M3 receptors making them more selective. The drug exists in two isomeric forms (R) and (S) and its empirical formula and molecular weight are $C_{26}H_{37}NO_7$ and 475.6 respectively. TT is a white crystalline powder, soluble in water, methanol, slightly soluble in ethanol and practically insoluble in toluene. The drug is listed in the Merck Index [1] but the drug is not yet official in any pharmacopoeia.

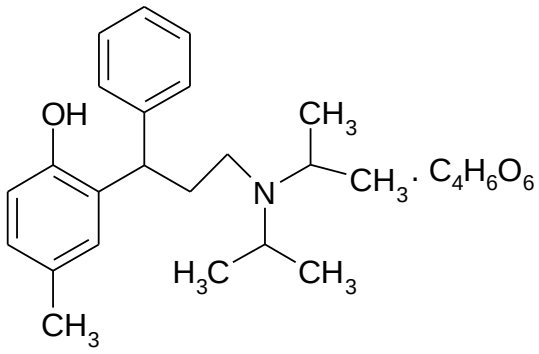

Figure 1: Showing the Chemical structure of TT

Tolterodine has a high affinity and specificity for muscarinic receptors in vitro and exhibits the selectivity for the urinary bladder over salivary glands in vivo, so it has the advantageous tolerability profile in terms of the low frequency of bothersome dry mouth. The drug undergoes immediate and extensive first-pass hepatic metabolism, mainly by way of CYP 2D6-mediated oxidation and CYP 3A4-mediated N-dealkylation After oral administration, TLD is metabolized in liver by way of cytochrome P450 2D6 (CYP 2D6)-mediated oxidation, resulting in the formation of the 5- hydroxymethyl derivative, a major pharmacologically active metabolite. It is the product of the predominating CYP 3A4 pathway and is pharmacologically equipotent with TLD.

Some analytical methods which include HPLC[2-9], HPLC-ESI-MS[10], UPLC[11], GC-MS[12], LC-MS-MS [13-16], UV [17] and visible spectrophotometric [18-19] have been reported in the literature for the determination of TT in biological fluids and in pharmaceutical preparations. The main purpose of the present study was to establish a relatively simple, sensitive and validated visible spectrophotometric method for the determination of TT in pure form and in pharmaceutical dosage forms, since most of the previous methods involve sophisticated equipments which are costly and pose problems of maintenance.

Honing and Fritsch [20] described oxidative coupling of MBTH with aromatic amines or phenols in the presence of an oxidant under acidic conditions to form an intense colored oxidative coupling products. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and MBTH-Fe (III) [21]. The method can be extended for the routine assay of TT formulations.

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. MBTH (Fluka, 0.2%, $8.56x10^{-3}$M, solution prepared by dissolving 200mg of MBTH in 100ml distilled water), Ferric chloride (Qualigens, 0.5%, $1.65x10^{-2}$M solution prepared by dissolving 500mg of ferric chloride hexahydrate in 100ml of 0.1N HCl) were prepared.

Preparation of standard stock solution:

100mg TT was dissolved initially in 10ml of methanol and then followed by dilution to 100 ml with distilled water to get 1mg/ml stock solution. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of 100µg/ml.

Sample solution:

About 20 tablets were weighed to get the average tablet weight and pulverized and the powder equivalent to 100mg of TT was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solution in the same way as under working standard solution.

Determination of wavelength maximum (λ_{max}):

The 2.5 ml of working standard solution of TT (100µg/ml) was taken in 10ml calibrated tube. To this, 1.5ml MBTH and 1.0ml of ferric chloride was added successively, kept for 10min.at room temperature for complete color development. The volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-760 nm UV-Visible spectrophotometers against a reagent blank. From the

absorption spectra (**Figure.2),** it was concluded that 650nm is the most appropriate wavelength for analyzing TT with suitable sensitivity.

RESULTS AND DISCUSSION

Aliquots of working standard TT solution (100µg/ml) such as 0.5, 1.0, 1.5, 2.0, 2.5 ml were taken separately in a series of 10ml graduated test tubes, to get a concentration of 5, 10, 15, 20 and 25 µg/ml respectively. A 1.5 ml portion of MBTH ($8.56x10^{-3}$M) solution was added to each test tube and allowed to stand for 2 minutes at room temperature. Then 1.0ml of ferric chloride ($1.65x10^{-2}$M) solution was added, kept for 10 minutes and diluted to the mark with distilled water.

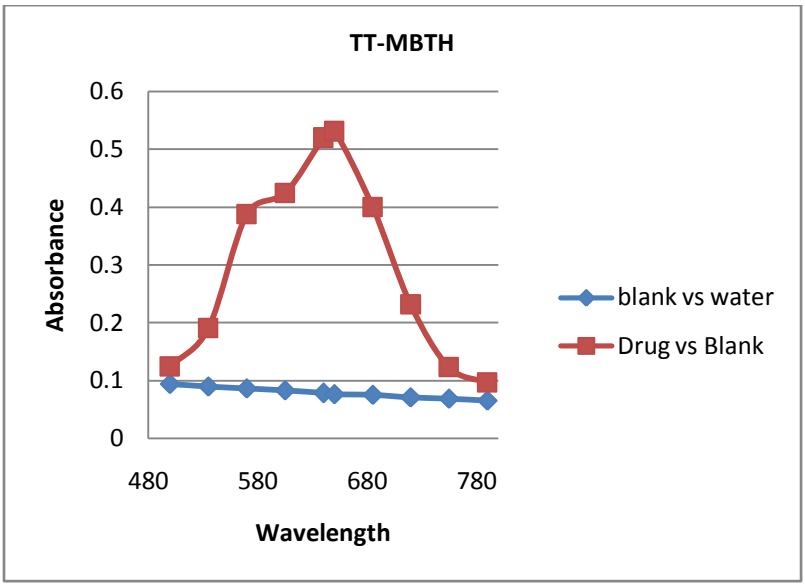

Figure 2: Absorption spectra of TT-MBTH-Fe (III) system

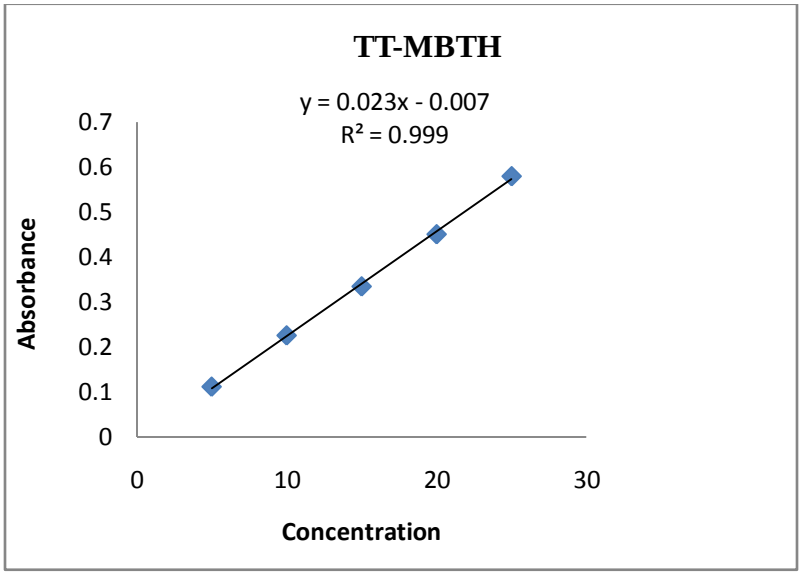

Figure 3: Beer's Law plot of TT-MBTH-Fe (III) system

The absorbance was measured at 650 nm against a similar reagent blank within 30 min. The calibration graph was constructed by plotting the drug concentration versus absorbance **(Figure.3)**

Preparation of calibration graph:

In developing a method, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of MBTH reagent, order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. Among the various oxidants ($NaIO_4$, $K_2Cr_2O_7$, Chloramine-T, potassium hexacyanaferrate (III), Ce (IV) and Fe (III) tried in combination with MBTH for oxidative coupling reaction. Ce (IV) and Fe (III) were responded for color development with MBTH. But MBTH-Fe (III) was found to be the best by virtue of high ϵ_{max} values and stability considerations. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is selected as a solvent for final dilution of the colored species. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in **Table-1.**

Table 1: Optical Characteristics, Precision and Accuracy Of Proposed Analytical Method

Parameter	Values
λ_{max} (nm)	650nm
Beer's law limit(µg/ml)	5-25
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.004477612
Molar absorptivity (Litre/mole/cm)	106217.3333
Regression equation (Y)*	
Intercept (a)	-0.007
Slope(b)	0.023
Correlation Coefficient (R^2)	0.999
%RSD	1.14
% Range of errors(95% Confidence limits)	
0.05 significance level	1.19
0.01significance level	1.87

*Y = a+ b x, where Y is the absorbance and x is the concentration of Tolterodine tartrate in µg/ml

Commercial formulations containing TT were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV method in distilled water λ_{max} 281.5nm (**Figure.4 & 5)** for formulations were compared statistically by the t-and F-test and found not to differ significantly.

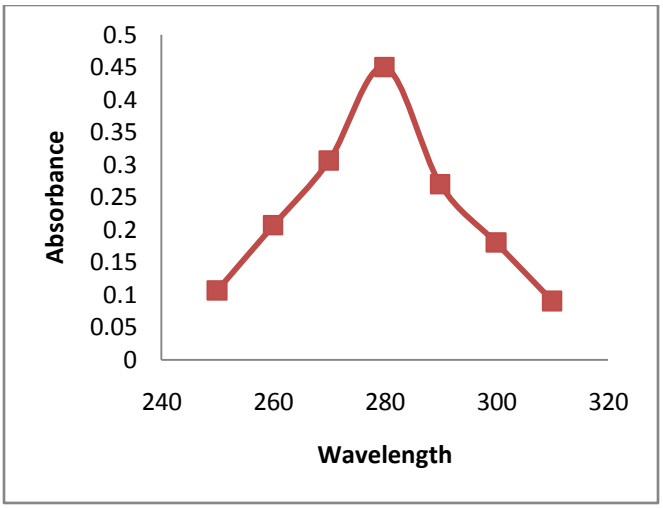

Figure.4: Absorption spectra of UV Ref. method

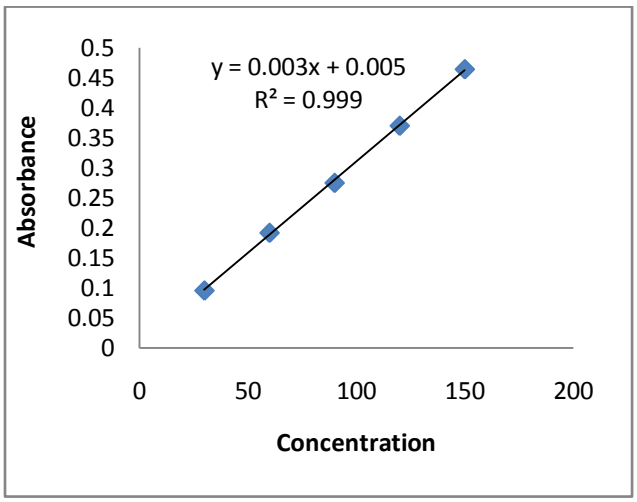

Figure.5: Beer's law plot of UV Ref. method

As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in **Table-2**. The ingredients usually present in formulations of TT did not interfere with the proposed analytical method.

In the present investigation the reactive electrophillic intermediate formed in-situ from MBTH upon treatment with an oxidant Fe (III), was found to oxidative couple with TT which possesses p-substituted phenol group. Based on the analogy, the probable sequence of reactions is presented in scheme **(Figure.6).**

Table-2 Analysis of Tolterodine Tartrate in Pharmaceutical Formulations

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	f		
TT-MBTH-Fe(III)	Batch-1	2	1.98 ±0.011	2.15	5.59	1.99 ±0.005	98.78± 0.549
	Batch -2	4	3.98 ± 0.012	1.57	2.73	3.99 ± 0.0073	99.37± 0.302

* Batch-1 and Batch- 2 extended release capsules of two different companies (TEROL LA-2 of Cipla Ltd and TORQ SR 4 of Dr Reddy's)

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits t =2.57 and f = 5.05.

Recovery of 10mg added to the pre analyzed sample (average of three determinations).

Reference method (reported UV method) using distilled water (λ_{max}=281.5nm).

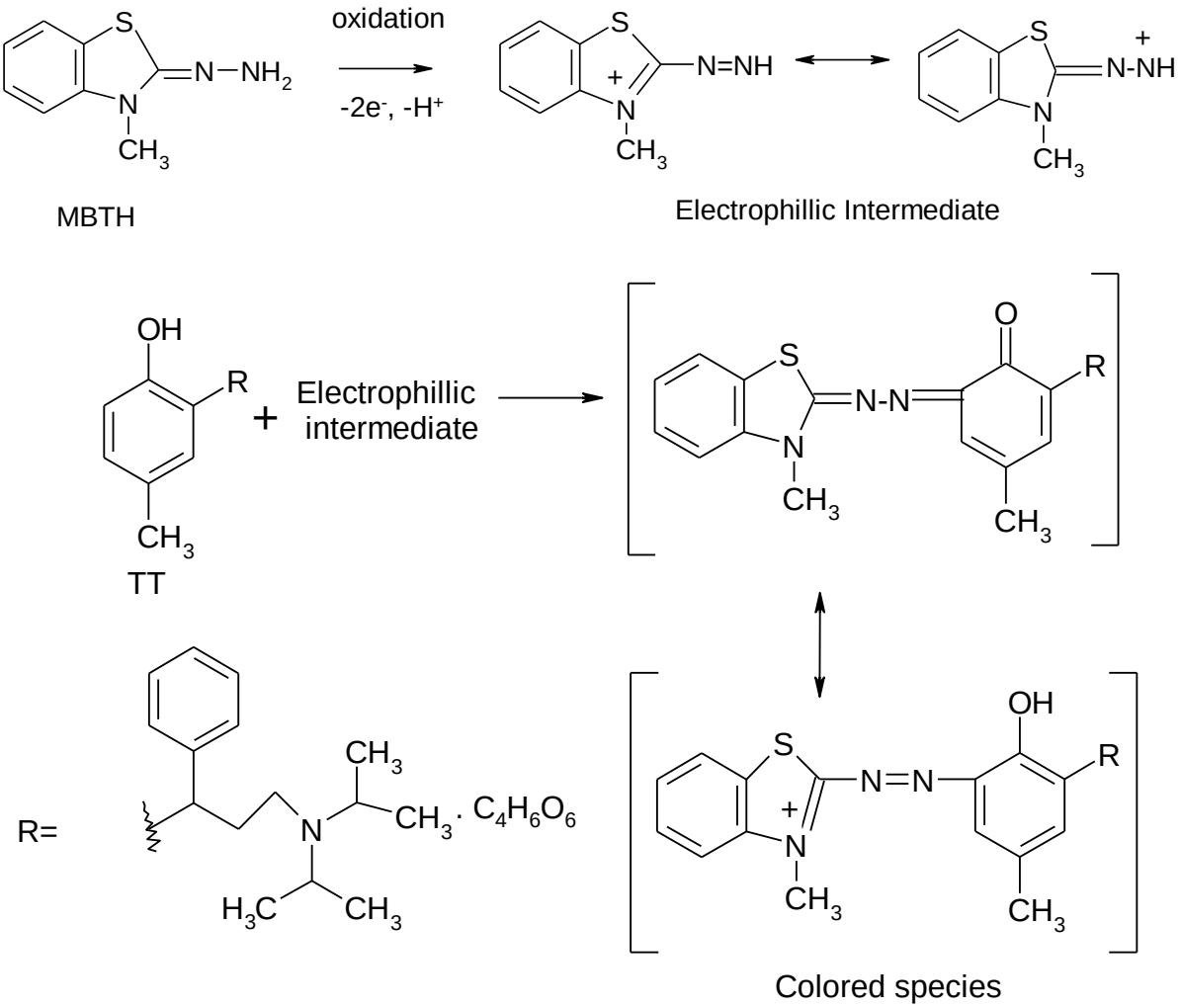

Figure 6: Scheme for the colored reaction of the TT with MBTH

CONCLUSIONS

The reagents utilized in the proposed method are readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy. The method offers the advantages of rapidity, simplicity, sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents and can be used as an alternative method to the reported ones for the routine determination of TT depending on the need and situation.

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