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UV-SPECTROSCOPIC METHOD FOR ESTIMATION OF TIEMONIUM METHYLSULFATE 50 MG TABLET IN BULK AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT: Tiemonium Methylsulfate is an antispasmodic drug. It strengthens calcium bonding with phospholipids and proteins. A simple, sensitive and highly accurate UV spectroscopic method has been developed for the determination of Tiemonium Methylsulfate in bulk and its tablet dosage form. Tiemonium Methylsulfate in its distilled water solutions was determined at the wavelength range of 200-400 nm by the spectroscopic method. Solution of Tiemonium Methylsulfate in distilled water shows a maximum absorbance at 234 nm. Beer's law was obeyed in the concentration of 10-30µgmL^{-1}, correlation coefficient, detection and quantification limit were also calculated. The proposed method has been applied successfully to assay of Tiemonium Methylsulfate in pure and tablet dosage form. Result of percentage recovery and placebo interference shows that the method was not affected by the presence of common excipients. The percentage of assay of Tiemonium Methylsulfate in tablet was 99.90-100.12% of the label claimed 50 mg per tablet. The method was then validated statistically as per ICH guidelines which yielded good results concerning range, precision, accuracy, reproducibility, specificity and ruggedness.

INTRODUCTION: Tiemonium Methylsulfate **(Figure 1)** is chemically described as 4-(3-hydroxy-3-phenyl-3-(2-thienyl) propyl)-4-methyl morpholinium methylsulfate (salt). It is a quaternary ammonium antimuscarinic agent with peripheral effect similar to those of atropine and is used in the relief of visceral spasms [1].

It prevents the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle of GI tract [2].

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The literature survey reveals that there is no suitable UV method for the estimation of Tiemonium Methylsulfate (TMS) in bulk and tablet dosage forms reported till to date. Since, the drug is not included in any official pharmacopeia, i.e. INN, till date, an economical, accurate, simple method has been developed for estimation of TMS in bulk as well as in pharmaceutical preparation.

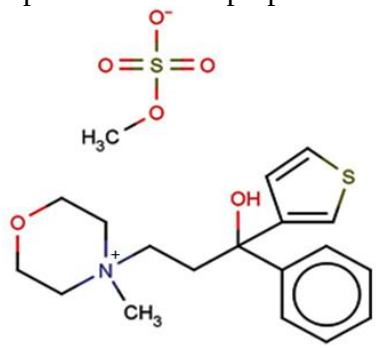

FIG. 1: CHEMICAL STRUCTURE OF TIEMONIUM METHYLSULFATE

MATERIALS AND METHODS:

Apparatus: A UV-1650PC UV-Visible Spectrophotometer, Shimadzu, Japan, double beam spectrophotometer with 1 cm matched quartz cell was used for spectral measurement. Sartorius CPA224S analytical balance was used for weighing purpose.

Reagents and solutions: Pharmaceutical grade of Tiemonium Methylsulfate INN was gifted by Srikrishna Drugs Ltd., Hyderabad, India and certified to contain 99.9% w/w of Tiemonium Methylsulfate. It was used without further purification. Excipients used in tablet formulation were Microcrystalline Cellulose (PH 101 and 102), Colloidal Silicon Dioxide, Magnesium Stearate, Hydroxypropyl Methylcellulose (5 Cps and 15 Cps), PEG-6000, Talcum Purified and Titanium Dioxide. The distilled water used was analytical grade produced by Biopharma Ltd., Bangladesh.

Wavelength selection: Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200 – 400nm. The λ_{max} was found at the wavelength 234nm. The absorption spectra thus obtained was derivatized for zero order. This zero order spectrum was selected for analysis of the drug.

Tiemonium Methylsulfate stock solution preparation: Standard Tiemonium Methylsulfate 50mg was weighed and transferred to 100ml volumetric flask and dissolved in distilled water. The flask was then shaken and volume was made up to the mark with water to a solution containing 500µgmL^{-1}. From this stock solution, 2 ml was pipetted out and placed into 50ml volumetric flask. The volume was made up to the mark with water to give a solution containing 20µgmL^{-1}.

Analytical concentration Range selection: The spectrum for Tiemonium Methylsulfate is sufficiently separated from other spectrum at a specific wavelength (λ_{max}). From the standard stock solution of Tiemonium Methylsulfate, appropriate aliquots were pipetted out into 50ml volumetric flasks and dilutions were made with water to obtain working standard solutions of concentration from 10 – 30µgmL^{-1}. Absorbance for these solutions was measured at 234nm. For the standard solution analytical concentration range was found to be 10 – 30µgmL^{-1}.

Calibration Curve for the Tiemonium Methylsulfate: Appropriate value of aliquots from standard Tiemonium Methylsulfate stock solutions were transferred to different volumetric flask of 50 ml capacity. The volume was adjusted to the mark with water to obtain concentration of 10, 15, 20, 25 and 30µgmL^{-1}. Absorbance spectra of each solution against water as blank were measured at 234nm and the graphs of zero order overlain spectra in **Figure 2.** The Regression equation and correlation coefficient (r^2) were determined and presented in **Table 5**.

Analysis of Tablet: Twenty tablets were weighed and finely powdered. The powder equivalent to 50mg of Tiemonium Methylsulfate was accurately weighed and transferred to volumetric flask of 100ml capacity containing 50ml of distilled water and sonicated for 30 minutes. The flask was shaken and volume was made up to the mark with water to give a solution of 500µgmL^{-1}. The above solution was filtered through Whatman Filter Paper (No. 41). From this solution, 2 ml was taken and diluted to 50ml with distilled water to give a solution of 20µgmL^{-1} and was used for the estimation of Tiemonium Methylsulfate.

Method validation: The developed method was validated for its linearity, accuracy, precision, range, specificity and robustness. System suitability is the performance of an analytic system on a given day. Spectrums automatically integrated and visually inspected for an acceptable integration. The relative standard deviations (RSD) of the absorbance for 10 times for system suitability were calculated. The linearity of an analytical procedure is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of the analyte in the samples within a given range [3].

Linearity of analytical method can be determined by performing the three studies: Linearity of response with different concentration of Active Ingredient, Linearity with different concentration of active ingredient and fixed concentration of formulation placebo and Linearity with fixed concentration of active ingredient and different concentration of formulation placebo.

The specified range is normally derived from linearity studies. Range is the interval between the upper and the lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The optical characteristics were summarized in **Table 5**.

Specificity of a procedure is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the test sample[4]. From the spectrum at specific wavelength (λ_{max}) obtained with both working standard and sample we can conclude that the method is sufficiently specific to discriminate Tiemonium Methylsulfate from the excipients. The spectrum for Tiemonium Methylsulfate is sufficiently separate from other spectrum at a specific wavelength (λ_{max}).

Placebo effect was studied by using blank, placebo and active solution to ensure that the absorption for active solution is not present in blank and placebo solution. The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree [5].

The accuracy of an analytical method is established across its range. In case of assay of a drug in a formulated product, accuracy is determined into a solution of blank matrix (Placebo) for the product (containing all ingredients except for the drug substance); spike the drug substance at levels 50, 100 and 150 of the target level in the product. The procedure has to be performed at least three times using separately prepared blank matrix and drug substance and preferably over two or more days. The result of analysis by the UV method should be compared to the known amount added for each spike.

Average recovery of the analyte is 98 to 102% at each level. Accuracy is assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., 3 concentration and 3 replicates of each concentration). The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous samples under the prescribed conditions [6].

Repeatability precision was carried out by six determinations at different test concentration of a homogeneous solution. Intermediate precision expresses within-laboratory variation as on different days, different analysts and different equipment within the same laboratory. Reproducibility expresses the precision between laboratories as in a collaborative study. It is generally applied for standardization of methodology.

The determinations were carried out by another 3rd analyst 6 (six) determinations immediately one after the other under different conditions (Laboratory). Robustness (or Ruggedness) is the ability of the procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions[7]. It was carried out to observe the stability of sample solution at ambient temperature (20-25°C) for different time interval (0hr, 12hr and 24 hr).

RESULTS AND DISCUSSIONS: The proposed method is precise, simple, rapid and free from interference of excipients. Performance of the analytical system was confirmed by system suitability, and %RSD of absorbance was 0.768 that complies the recommended range (NMT 2%). The data is presented in **Table 1**.

Linearity of analytical method was determined by performing three studies: linearity of response with different concentration (10, 15, 20, 25 and 30µgmL^{-1}) of active ingredient, linearity with different concentration (50%-150%) of active ingredients and fixed concentration of active ingredient and different concentration of formulation placebo. The linear regression equations were calculated as: y= 0.0205x + 0.0064 (R^2=0.9998) for few different concentration of TMS and respective absorbance (**Table 2, Figure 2 and 3**), y=0.0226x + 0.0024 (R^2=0.9996) for study of different concentration of TMS plus fixed concentration of formulation placebo (**Table 3, Figure 4**).

Moreover, absorbance of fixed concentration of TMS and different concentration of formulation placebo were checked and found a plot of absorbance against added amount of placebo was almost constant and there was no interference of placebo in the response of Active Ingredient.

Data are represented in **Table 4, Figure 5**. The proposed spectroscopic method for Tiemonium Methylsulfate was found to be linear in the range of 10-30µgmL^{-1} at 234nm and obeyed Beer's law. Linear regression of absorbance vs concentration is shown in **Table 5**. From the Linearity data in Table 2, it is found that the lower limit of quantitation (LLOQ) is defined as the lowest concentration within the linear range (10µgmL^{-1}). The upper limit of quantitation (ULOQ) is defined as the highest concentration within the linear range (30µgmL^{-1}). So the measured concentration was 50% to 150% of active ingredient. The specificity of the method was checked by monitoring a standard solution of TMS, formulated tablet sample, blank and placebo. No absorbance was found in blank and placebo whereas maximum absorbance (λ_{max}) was found at 234 nm in formulated tablet sample. The spectrum of TMS was sufficiently separated from other spectrum at a specific wavelength (**Table 6**). Hence, the determination of TMS in the tablet is considered to be free from interference due to excipients.

TABLE 1: RESULT OF SYSTEM SUITABILITY TEST

No of Sample (Replicate)	Absorbance	RSD (%) of Absorbance		Pass/Fail
		Limit	Result	
01	0.464	NMT 2.0	0.768	Passed
02	0.468			
03	0.460			
04	0.458			
05	0.462			
06	0.461			
07	0.456			
08	0.459			
09	0.457			
10	0.460			

TABLE 2: RESULTS OF CALIBRATION CURVE AT 234NM FOR TIEMONIUM METHYLSULFATE BY ZERO ORDER SPECTROSCOPIC METHOD

Tiemonium Methylsulfate (µgmL^{-1})	% of nominal concentration	Absorbance	Regression coefficient (R^2)		Pass/ Fail
			Limit	Result	
10	50	0.213	NLT 0.995	0.9998	**Passed**
15	75	0.311			
20	100	0.416			
25	125	0.522			
30	150	0.620			
Lower Limit of quantitation (LLOQ)			**10 µgmL^{-1}**		
Upper Limit of quantitation (LLOQ)			**30 µgmL^{-1}**		

TABLE 3: ABSORBANCE WITH DIFFERENCE CONCENTRATION OF TIEMONIUM METHYLSULFATE AND FIXED CONCENTRATION OF FORMULATION PLACEBO

Tiemonium Methylsulfate (µgmL^{-1})	% of nominal concentration	Formulation placebo concentration (µgmL^{-1})	Absorbance
10	50	53.6	0.226
15	75	53.6	0.345
20	100	53.6	0.455
25	125	53.6	0.562
30	150	53.6	0.682

TABLE 4: ABSORBANCE OF TIEMONIUM METHYLSULFATE WITH FIXED CONCENTRATION AND DIFFERENT CONCENTRATION OF FORMULATION PLACEBO

Placebo (µgmL^{-1})	% of nominal concentration	Tiemonium Methylsulfate (µgmL^{-1})	Absorbance
26.8	50	20	0.468
40.2	75	20	0.467
53.6	100	20	0.468
67.0	125	20	0.467
80.4	150	20	0.469

TABLE 5: OPTIMUM CONDITIONS, OPTICAL CHARACTERISTICS AND STATISTICAL DATA OF THE REGRESSION EQUATION IN ZERO ORDER SPECTROSCOPIC METHOD

Parameters	UV Method
λ_{max} (nm)	234
Beer's law limit (μgmL^{-1})	10 – 30
Molar extinction coefficient	8.934×10^3
Regression equation (Y*)	Y = 0.0205X + 0.0064
Slope (b)	0.0205
Intercept (a)	0.0064
Correlation Coefficient (r^2)	0.9998
LOD (μgmL^{-1})	0.2
LOQ (μgmL^{-1})	5.0

Y* = mX + c where X is the concentration of Tiemonium Methylsulfate in μgmL^{-1} and Y is the Absorbance at the respective λ_{max}.

TABLE 6: RESULT OF SPECIFICITY

Sample Information	Active Ingredient	Measured Absorbance At ----- (max)	Observation	Pass/Fail
Blank	-------	-----	NAF	Passed
Placebo	-------	------	NAF	
Standard	Tiemonium METHYLSULFATE INN	234	MAF	
Sample	Tiemonium METHYLSULFATE INN	234	MAF	

NAF: No absorbance, MAF: Maximum absorbance found

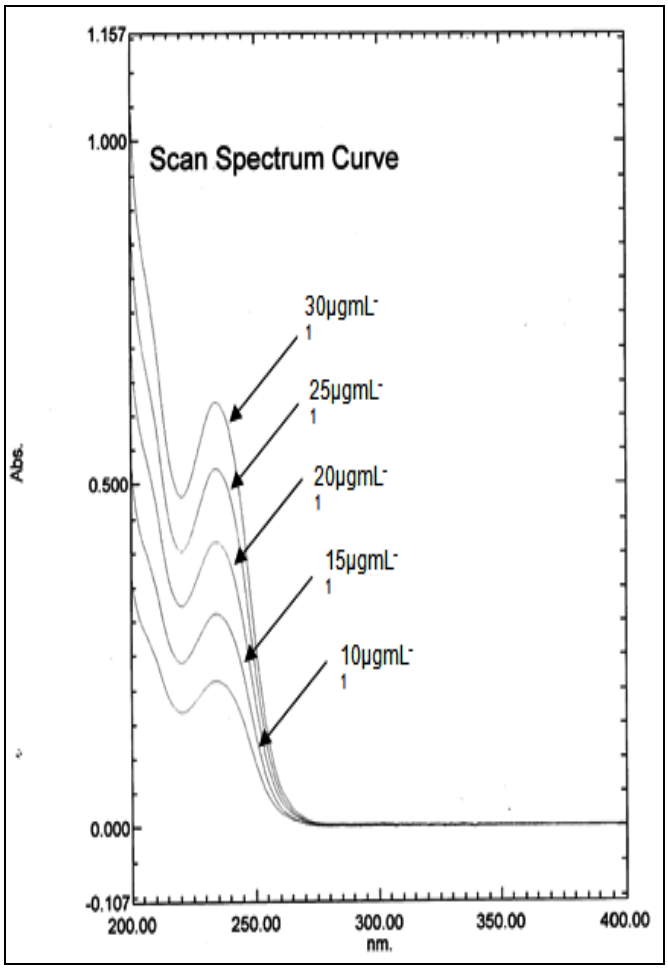

FIGURE 2: ZERO ORDER SPECTRA OF TIEMONIUM METHYLSULFTE AT FIVE CONCENTRATIONS (30µGML^{-1}, 25µGML^{-1}, 20µGML^{-1}, 15µGML^{-1} AND 10µGML^{-1}) AT 234NM

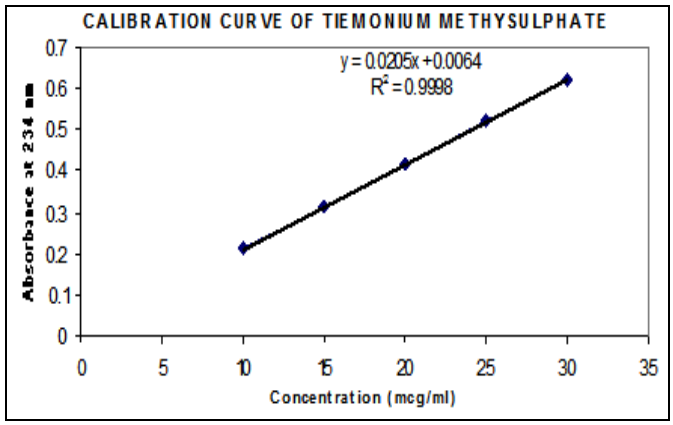

FIGURE 3: LINEARITY CURVE OF TIEMONIUM METHYLSULFATE AT 234 NM BY ZERO ORDER SPECTROSCOPIC METHOD

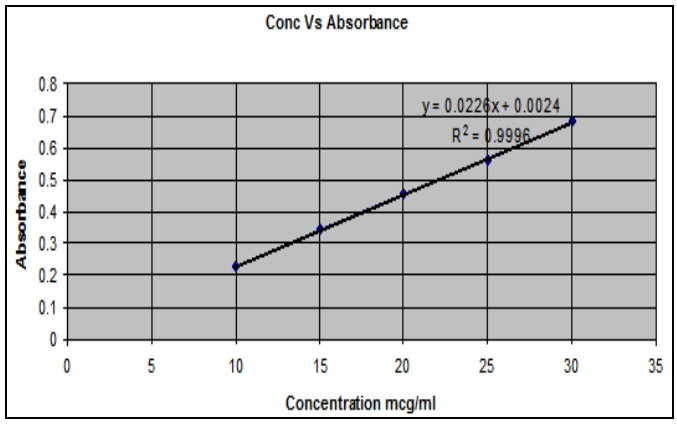

FIGURE 4: LINEARITY GRAPH FOR DIFFERENT CONCENTRATION OF TMS AND FIXED CONCENTRATION OF FORMULATION PLACEBO

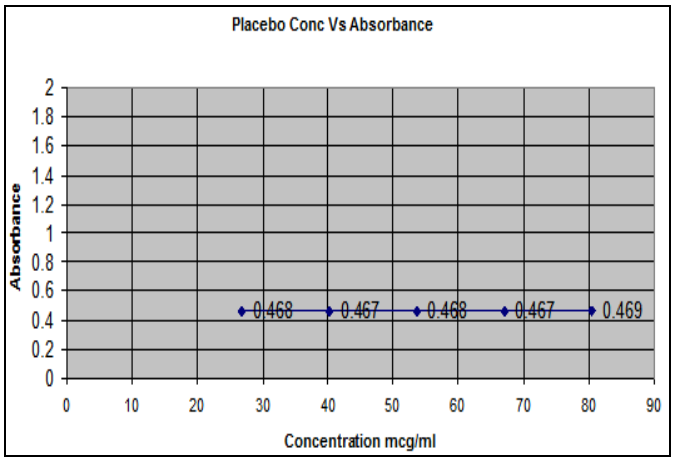

FIGURE 5: LINEARITY GRAPH FOR FIXED CONCENTRATION OF TMS AND DIFFERENT CONCENTRATION OF FORMULATION PLACEBO

Accuracy was assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range i.e. three concentration and three replicates of each concentration (**Table 7**). Values of recovery was 100.14% (99.45-101.50) % that complied the limit (98%-102%); thus indicated the proposed method was accurate for the analysis of the drug. Repeatability precision was carried out by six determinations at different test concentration of a homogeneous solution (**Table 8**).

Precision of the proposed method was established in terms of repeatability and intermediate precision wherein the method was repeated on two different days, different analysts and different equipment with the same laboratory. Percent recoveries of the analyst 2 were combined with that of the analyst 1 (n=12) and the combined relative standard deviation was called the intermediate precision. The precision of the method was found to be 0.756% (limit NMT 2%).

The data represented through **Table 9** reveals that the proposed method is precise enough for the analysis of the drug. The reproducibility of the method was confirmed by performing the proposed method by three different analysts in different laboratories. Combined RSD of the tests found 0.757% which complied the limit (NMT 2%) (**Table-10**).

Moreover, the sample solution was allowed to stand at ambient temperature (20-25°C) for different time intervals (0, 12, 24hrs). The relative standard deviation obtained as a measure of the stability of sample solution over a period of 24 hours is 0.329% (Limit: NMT 2%) which indicates reliability during normal usage (**Table-11**).

TABLE 7: RESULT OF ACCURACY

Tiemonium Methylsulfate (μgmL^{-1})	% of nominal conc.	Absorbance	Recovery from sample (μgmL^{-1})	Recovery (%)	Average Recovery (%)	Limit	Pass /Fail
10		0.220	10.12	101.20			
10	50	0.222	9.98	99.80			
10		0.224	10.15	101.50			
20		0.466	19.89	99.45			
20	100	0.460	19.97	99.85	100.14	98 to 102%	Passed
20		0.462	19.95	99.75			
30		0.690	30.21	100.70			
30	150	0.692	29.87	99.56			
30		0.695	29.86	99.53			

TABLE 8: RESULT OF REPEATABILITY

Sample	Concentration (μgmL^{-1})	Absorbance	Result (%)	RSD (%)	Limit of RSD (%)	Pass/ Fail
01	20	0.468	99.89			
02	22	0.520	98.36			
03	24	0.558	99.19	0.608	≤ 2.0	Passed
04	26	0.615	99.15			
05	28	0.655	98.97			
06	30	0.712	99.98			

TABLE 9: RESULT OF INTERMEDIATE PRECISION

	Sample	Concentration (μgmL^{-1})	Absorbance	Result (%)	RSD (%)	RSD of 12 (%)	Limit of RSD (%)	Pass/ Fail
Analyst 1 (QC) EQP-QC-002 01.10.2011	01	20	0.468	100.17	0.858	0.756	≤ 2.0	Passed
	02	22	0.520	101.36				
	03	24	0.558	99.97				
	04	26	0.615	99.71				
	05	28	0.655	98.96				
	06	30	0.712	99.16				
Analyst 2 (QC) EQP-QC-003 03.10.2011	01	20	0.470	99.98	0.530			
	02	22	0.522	98.89				
	03	24	0.562	99.58				
	04	26	0.610	98.98				
	05	28	0.660	98.58				
	06	30	0.720	99.56				

TABLE 10: RESULT OF REPRODUCIBILITY

	Sample	Concentration (μgmL^{-1})	Absorbance	Result (%)	RSD (%)	RSD of 18 (%)	Limit of RSD (%)	Pass/ Fail
Analyst 1 (QC) EQP-QC-002 01.10.2011	01	20	0.468	100.17	0.858	0.757	≤ 2.0	Passed
	02	22	0.520	101.36				
	03	24	0.558	99.97				
	04	26	0.615	99.71				
	05	28	0.655	98.96				
	06	30	0.712	99.16				
Analyst 2 (QC) EQP-QC-003 03.10.2011	01	20	0.470	99.98	0.530			
	02	22	0.522	98.89				
	03	24	0.562	99.58				
	04	26	0.610	98.98				
	05	28	0.660	98.58				
	06	30	0.720	99.56				
Analyst 3 (R&D) EQP-R&D-001 06.10.2011	01	20	0.461	101.12	0.821			
	02	22	0.530	99.95				
	03	24	0.569	99.15				
	04	26	0.625	99.92				
	05	28	0.658	98.85				
	06	30	0.709	99.25				

TABLE 11: RESULT OF SAMPLE STABILITY AT AMBIENT TEMPERATURE (20-25ºC)

Trial condition	Sample Absorbance	Sample Potency (%)	Mean value (%)	Total RSD (%)	Limit RSD (%)	Pass/Fail
Initial at ambient temperature (Operating condition)	0.464	99.96	99.45	0.329	NMT 2.0%	Passed
	0.460	99.10				
	0.462	99.52				
	0.461	99.31				
	0.460	99.10				
	0.463	99.74				
Stand at ambient temperature for 12 Hours	0.462	99.52	99.49			
	0.460	99.10				
	0.463	99.74				
	0.464	99.96				
	0.462	99.52				
	0.460	99.10				
Stand at ambient temperature for 24 Hours	0.464	99.96	99.56			
	0.462	99.52				
	0.460	99.10				
	0.462	99.52				
	0.461	99.31				
	0.464	99.96				

CONCLUSION: The method was developed for the analysis of TMS in bulk and formulated tablet. The developed method was validated as per ICH guidelines and can be applied to the pharmaceutical formulations without interference of excipients.

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