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SIMPLE, RAPID UV SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF TOLTERODINE TARTRATE

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ABSTRACT

In the present study simple and rapid UV spectroscopic method was developed to quantify Tolterodine Tartarate (ToT) in bulk and marketed tablets. The λ_{max} of ToT in proposed mediums were 266nm and 284nm respectively and follows linearity in the concentration range 5-30µg/ml with a correlation coefficient 0.9996 and 0.9992. The % recovery of ToT from the marketed tablets were 99.66% and 100.12% with low % RSD values for two proposed mediums and was found to be in good agreement with the labelled claim. The % recovery in the standard addition method was in the range of 100.4 to 101%, with low % RSD indicate the proposed mediums had good reproducibility. The % RSD value < 2 indicate that the method is precise, robust and can be rugged. The above method is a rapid tool for routine quantification of ToT in the bulk and in the pharmaceutical dosage form.

KEYWORDS

Tolterodine Tartrate, UV spectroscopy, Validation, Accuracy and Precision.

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INTRODUCTION

Tolterodine Tartrate (TOT) is[1] used in the treatment of overactive bladder (OAB). It acts by competitively antagonizing muscarinic receptors, inhibiting bladder contractions, and reducing urinary frequency[2]. Chemically TOT is 2-[(1R)-3-[bis (1-methylethyl) amino]-1-phenylpropyl]-4-methyl-phenol, 2R, 3R-dihydroxy butanedioate has CAS-124937-52-6 as shown in Figure No.1. TOT appeared as crystalline solid, soluble in organic solvents viz., ethanol, DMSO and dimethyl

formamide. Literature reveals TOT can be estimated by RP-HPLC[3-6], Potentiometric[7], HPLC[8], LC-MS[9], Colorimetric[10-12] and UV[13]. The aim of the present study was to develop a simple, sensitive, precise and accurate UV spectrophotometric method for the determination of TOT in its pure form and marketed formulations further, validate the developed method as per ICH guidelines.

MATERIAL AND METHODS

Materials

Tolterodine Tartrate obtained as gift sample (Fleming Laboratories Ltd, Hyderabad). Two brands of Marketed tablets Detrol (Intas pharmaceuticals ltd, south Sikkim, India) and Detrol (Sun Pharma Laboratories ltd, Jammu, India) procured from local community pharmacy. Methanol and Acetonitrile purchase from SD Fine-Chemicals, Bangalore, India. All the chemicals of analytical grade were used for the proposed study.

Instruments

A double beam UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan) with UV Probe software was used for the analysis. Quartz cells having 3cm length with 1cm path length were used for spectral measurement.

Methods

Preparation of TOT standard stock solution (TST)

25mg of TOT was transferred into a 2ml volumetric flask to this add 20ml of Methanol: DW 1:3 (Medium 1), shake for 5 min and sonicate for 5 min to dissolve completely, then make the volume with same Medium 1 obtain 1mg/ml concentration. Similarly prepare standard stock solution of TOT in Acetonitrile: DW 1:1 (Medium 2).

Preparation of TOT working standard solution (TWST)

2.5ml of TST was transfer into a 25ml volumetric flask, make the volume with Medium 1 to obtain 0.1mg/ml concentration. Similarly TWST in Medium 2.

Determination of absorption maxima (λ max)

Appropriately dilute TWST with Medium 1 and Medium 2 separately in 10ml volumetric flask to get 10µg/ml solution, scan both the solutions in the range of 200 to 400 nm using double beam UV spectrophotometer, and observe the characteristic peak at standard wavelength (nm).

Preparation of calibration curve

The calibration curve was prepared by diluting TWST in Medium 1 and Medium 2 to get 5-30µg/ml standard solutions. An absorbance of every calibration standard was estimated at λmax 264nm for Medium 1 and 284nm for medium 2 using fixed wavelength measurement mode. The calibration curves representing concentration vs. absorbance was plotted utilizing Graph pad prism V9.

Method Validation

Developed UV method for the estimation of TOT was validated in terms of parameters viz., linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ) and limit of detection (LOD) using predefined calibration standards as per ICH guidelines[14,15].

Linearity and Range

The linearity is the ability of analytical procedure to produce test results, which are proportional to the concentration (amount) of analyte in samples within a given concentration range, linearity should be determined by using a minimum of six standards. Linearity of the proposed UV method was established using six different calibration standards. Based on analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method. Appropriately dilute the TWST with Medium 1 and Medium 2 in a series of 10ml volumetric flask to obtain 5-30µg/ml concentrations and measure the absorbance at 266nm and 284nm keeping respective mediums as blank.

LOD and LOQ

Limit of detection (LOD) is the lowest amount of an analyte detected in a sample and Limit of quantitation (LOQ) is the lowest amount of an analyte quantified in a sample with a suitable

precision and accuracy. Appropriately TWST was diluted with Medium 1 and Medium 2 in a series of 10 ml volumetric flasks, measure the absorbance at fixed wave lengths viz., 266nm and 284nm respectively. LOD and LOQ for both mediums were determined based on standard deviation (SD) of response and slope (S) by using the following equations

(LOD=3.3xSD/S) (LOQ=10xSD/S)

Precision

Precision of proposed solvent systems was carried out at different concentrations prepared by diluting appropriately the TWST in Medium 1 and Medium 2 under the study and express the results in terms of % RSD, similarly inter-day and intra-day precision were performed.

Robustness

A robustness study performs to check the influence of method parameters varied intentionally on the proposed solvent systems results. Robustness of Medium 1 and Medium 2 were carried out at by diluting appropriately the TWST in Medium 1 and Medium 2 in a series of 10ml volumetric flask to obtain 15µg/ml, 20µg/ml (n=3) concentrations and measure the absorbance at actual wavelength i.e., 266nm and 284 nm and small variated wavelength i.e., ± 5nm keeping Medium 1 and Medium 2 as blank. The results were expressed in terms of % RSD. Similarly inter-day and intra-day precision were performed.

Ruggedness

A ruggedness study was performs to check the influence of parameters varied intentionally on the proposed solvent systems. During study TWST was appropriately diluted in Medium 1 and Medium 2 in a series of 10ml volumetric flask to obtain 15µg/ml, 20µg/ml (n=3) concentrations and measure the absorbance at actual wavelength i.e., 266nm and 284nm and by two different analyst and two different UV spectrophotometer. The results were expressed in terms of % RSD.

Accuracy

The test working standard was prepared by triturating accurately weighed 5 marketed tablets (Detrol), further weigh the triturated powder equivalent to 25mg of TOT was extracted with 50ml of Medium 1 and Medium 2 separately for 1 hr and sonicate for 15 min. Filter the content and dilute appropriately with Medium 1 and Medium 2 to obtain test working standard solutions. These solutions were used for accuracy and drug content studies. The most common technique for determining accuracy in analytical method development studies is the recovery method, recovery defined as the ratio of the observed result to the expected result expressed as a percentage. Standard addition method applied for recovery studied, in which a sample assayed with known amount of TOT (40%, 80% and 120%) added to the test working standard solvent systems under the study, and the sample assayed as percent recovered.

Drug content

The TOT content in marketed tablets were analyzed by preparing samples of TOT marketed tablets and were analyzed using prevalidated UV-method and results were reported in terms of average percent assay.

Solution stability

The stability of stock solutions of TOT in Medium 1 and Medium 2 studied at room (25°C) and refrigerate temperature (2-8°C). The samples were stored in tightly sealed glass containers protected from light. Appropriately dilute the standard stock solutions of proposed solvent systems in a series of 10ml volumetric flask and the absorbance measured at 0 hr and 24 hr time interval.

RESULTS AND DISCUSSION

Absorption maxima development

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for TOT in Medium 1 and Medium 2 (10µg/ml) were carried out using full scan mode of UV-Visible spectrophotometer (Figure No.2). Full scan was processed using UV software and the λmax with characteristic peak was identified and it was

found to be 266nm and 284nm for TOT respectively for Medium 1 and Medium 2.

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration and the response. Considering the utility of quantitative analysis calibration curve for TOT was developed using six different calibration standards. The absorbance of different calibration standards at 266nm and 284nm were recorded using UV-Visible spectrophotometer. Calibration curve was repeated three times and reported as shown in Table No.1. The calibration parameter, best fit values and regression equations for Medium 1 and Medium 2 were given in Table No.2 and linearity curve in Figure No.3.

Linearity

Linear relationship was found in the concentration of 5-30µg/ml for both solvent systems. The goodness of fit study suggests good correlation coefficient (R^2 -0.9998 and 0.9997 for proposed mediums) shows the validity of Beer's law with intercept response < 2% calculated by the least square method indicating functional linearity between the concentration of analyte and the absorbance.

LOD and LOQ

Based on standard deviation of the response and slope, the LOD values of tolterodine Tartrate for the proposed methods found to be 0.001868 ± 0.0198µg/ml, 0.001868 ± 0.06µg/ml and limit of quantitation LOQ values found to be 0.002205 ± 0.03135µg/ml, 0.002205 ± 0.0102µg/ml with % RSD values less than 2.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the Medium 1 and Medium 2 were justified from the absorbance values obtained viz., six replicates in repeatability studies, three concentrations and three replicates in intra and inter day studies of a fixed amount of TOT in proposed mediums. The SD and % RSD calculated for the proposed solvent systems and are given in Table No.3. The percentage RSD values for repeatability studies, intraday and inter day studies is less than 2% indicate proposed solvent systems were precise and reproducible.

Accuracy

The proposed mediums analysed for assay in two marketed tablet formulations and data given in Table No.4. The percentage recovery was within the permissible limit with RSD values less than 2%. The accuracy performed for the proposed solvent systems by standard addition method and the percentage recovery found within the permissible limits with RSD values less than 2% indicate non-interference of the excipients in the formulations. The Tolterodine Tartrate content of two marketed products determined by the proposed solvent systems was in good agreement with the label claim with % RSD values less than 2 and data given in Table No.5.

Robustness and Ruggedness

Change in λ max of ± 5 nm to the actual λ max in robust analysis results significant different in the percentage recovery in both proposed solvent systems indicates the methods were not robust. In ruggedness, analysis by different analyst and change of instrument indicates the proposed solvent systems were significantly rugged. The robustness and ruggedness data given in Table No.6.

Table No.1: Calibration curve of TOT in Medium 1 and Medium 2

S.No	Concentration µg/ml	Mean ± SD (n=3)	
		Medium 1	Medium 2
1	5	0.0306 ±0.0005774	0.048 ±0.0005773
2	10	0.0613 ±0.0005774	0.096 ±0.0005774
3	15	0.0900 ±0.0010000	0.144 ±0.0005774
4	20	0.1193 ± 0.001155	0.191 ±0.0015280
5	25	0.1507 ±0.0005774	0.239 ±0.0005774
6	30	0.1800 ± 0.001000	0.288 ±0.0010000

Table No.2: Statistical data of calibration curve of TOT in Medium 1 and Medium 2

S.No	Parameters	Medium 1	Medium 2
1	Absorption maxima	266nm	284nm
2	Beer's range	5-30µg/ml	5-30µg/ml
3	Molar absorptivity	0.006mol/cm^{-1}	0.0096mol/cm^{-1}
Best-fit values			
4	Slope	0.006071	0.009552
5	y-intercept	-0.0002600	0.0006800
6	x-intercept	0.04283	-0.07119
7	1/Slope	164.7	104.7
95% Confidence Intervals			
8	Slope	0.005977 to 0.006165	0.009485 to 0.009619
9	X-intercept	-0.05441 to 0.1374	-0.1893 to 0.04546
10	Y-intercept	-0.0008458 to 0.0003258	-0.0004370 to 0.001797
		Goodness of fit	
11	R Square	0.9998	0.9997
12	Sy.x	0.0003719	0.0003347
13	Equation	Y=0.006071*X-0.000260	Y= 0.01930*X-0.002867

Table No.3: Repeatability, Intra-day and Inter-day precision data

S.No	Solvent blends	Concentration µg	Amount recovered µg	% Recovered Mean ±SD (n=6)	% RSD
1	Medium 1	2	1.997	99.99±1.181	1.081
		4	3.995	99.87±0.920	0.922
2	Medium 2	5	4.92	98.43±1.895	1.125
		10	9.96	99.63±0.0568	0.570
3	Medium 2	**Intra-day precision**			
4	Medium 1	15	14.98	99.94±0.712	0.712
		20	19.94	99.85±0.595	0.595
5	Medium 2	5	4.87	99.70±1.48	1.48
		10	9.99	99.96±1.322	1.32
6	Medium 2	**Inter-day precision**			
7	Day 1	15	14.88	99.26±1.287	1.29
		20	20.06	100.5±0.462	0.46
8	Day 2	15	14.92	99.46±1.491	1.49

		20	20.06	100.3±0.462	0.46
9	Day 3	15	15.06	100.4±0.346	0.34
		20	19.94	99.72±0.490	0.49
10	Medium 2	**Inter-day precision**			
11	Day 1	4	4.88	98.48±1.71	1.73
		10	9.90	99.02±1.03	1.04
12	Day 2	4	4.84	98.57±1.70	1.72
		10	9.86	99.22±1.02	1.02
13	Day 3	4	4.90	99.15±1.72	1.73
		10	9.89	100.63±1.06	1.05

Table No.4: Recovery studies of proposed methods for marketed formulations

S.No	Brand name	Labelled claim	Amount recovered	% Recovered Mean ±SD	% RSD
Medium 1					
1	**Roliten-2**	6	5.98	99.66	1.23
		8	8.01	100.12	1.09
Medium 2					
2	**Roliten-2**	6	6.01	100.16	1.11
		8	7.99	99.87	1.02

Table No.5: Accuracy data for proposed mediums by standard addition method

S.No	Medium 1						
	Amount of Mktd tablet added µg	% Pure drug	Amount of pure drug µg	Total amount claimed µg/ml	Amount recovered µg	% Recovered Mean ±SD	% RSD
1	Roliten	40	6	2.4	2.36	99.53±4.0	1.12
		80	6	4.8	4.71	98.20±2.07	1.10
		120	6	7.2	7.27	101±1.36	1.34
		40	8	3.2	3.05	95.42±2.88	1.01
		80	8	6.4	6.38	99.79±1.53	1.53
		120	8	9.6	9.55	99.50±0.95	0.95
Medium 2							
2	Roliten	40	6	2.4	2.39	99.83±2.45	1.45
		80	6	4.8	4.79	99.86±1.32	1.32
		120	6	7.2	7.15	99.32±0.76	0.76
		40	8	3.2	3.12	100.4±1.98	1.97
		80	8	6.4	6.26	97.92±1.63	1.66
		120	8	9.6	9.5	103.6±1.35	1.30

Table No.6: Robustness data for proposed method

S.No	Robustness				
	Medium 1				
		Concentration (µg/ml)	Amount Recovered (µg)	%Recovery Mean±SD	% RSD
1	Actual (266nm)	15	15	99.95±1.07	1.07
		20	19.89	99.43±0.98	0.98
2	271 (+5nm)	15	16.33	108.9±1.135	0.73
		20	21.50	107.5±0.825	1.42
3	261 (-5nm)	15	12.83	85.51±1.10	1.42
		20	18.27	91.37±1.72	0.94
Medium 2					
4	Actual (284nm)	5	5	99.2±0.63	0.64
		10	10.1	101±0.50	0.49
5	289 (+5nm)	5	5.10	102.3±2.00	1.95
		10	10.18	102.3±1.53	1.49
6	279 (-5nm)	5	4.68	93.67±2.10	2.24
		10	9.89	98.73±1.05	1.06
Ruggedness					
Medium 1					
7	Analyst 1	15	14.94	99.60±0.69	0.69
		20	19.87	99.38±0.54	0.54
8	Analyst 2	15	15.03	100.4±0.61	0.61
		20	19.94	99.72±0.49	0.49
Medium 2					
9	Analyst 1	5	5	100.7±1.15	1.14
		10	10	100.3±0.57	0.57
10	Analyst 2	5	4.99	99.93±2.10	2.10
		10	9.99	99.97±1.05	1.05

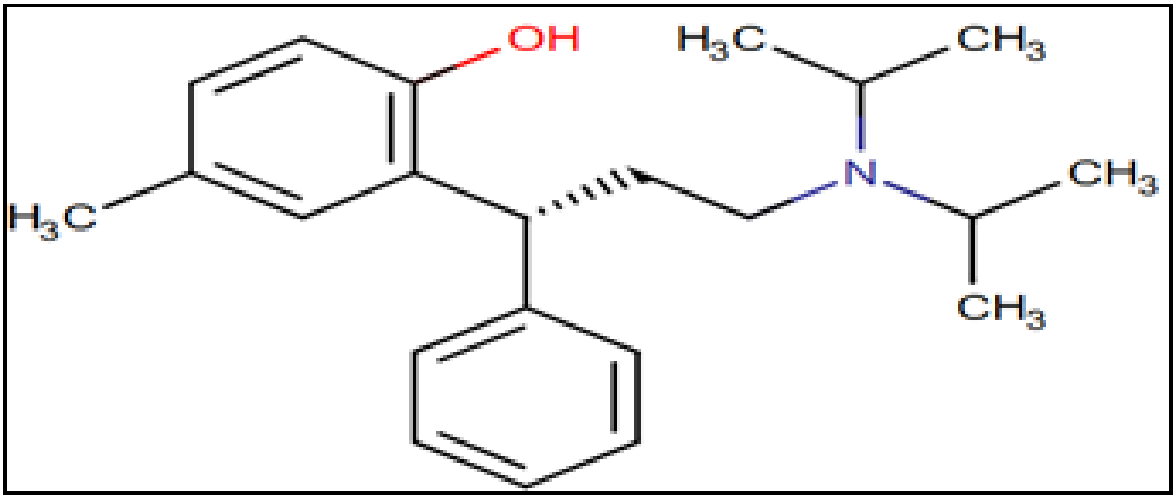

Figure No.1: Chemical structure of Tolterodine Tartrate

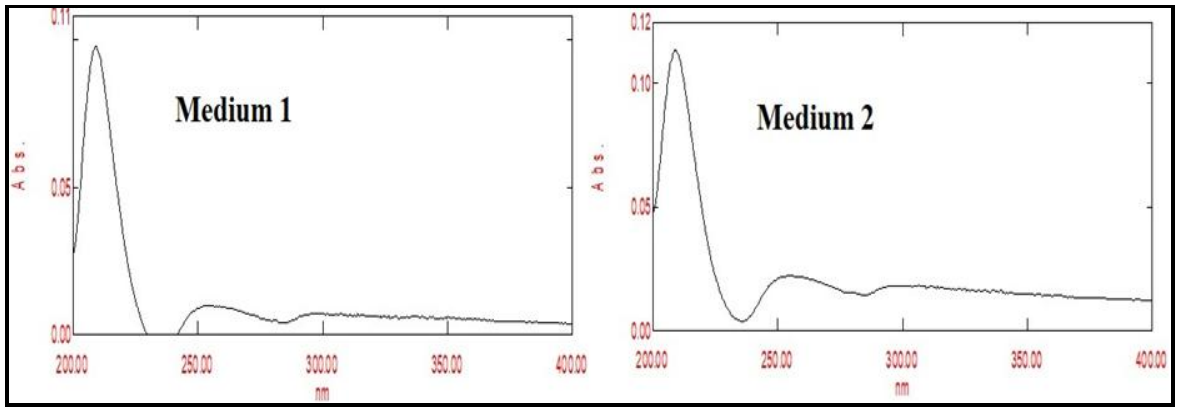

Figure No.2: Absorption maxima of TOT in Medium 1 and Medium 2

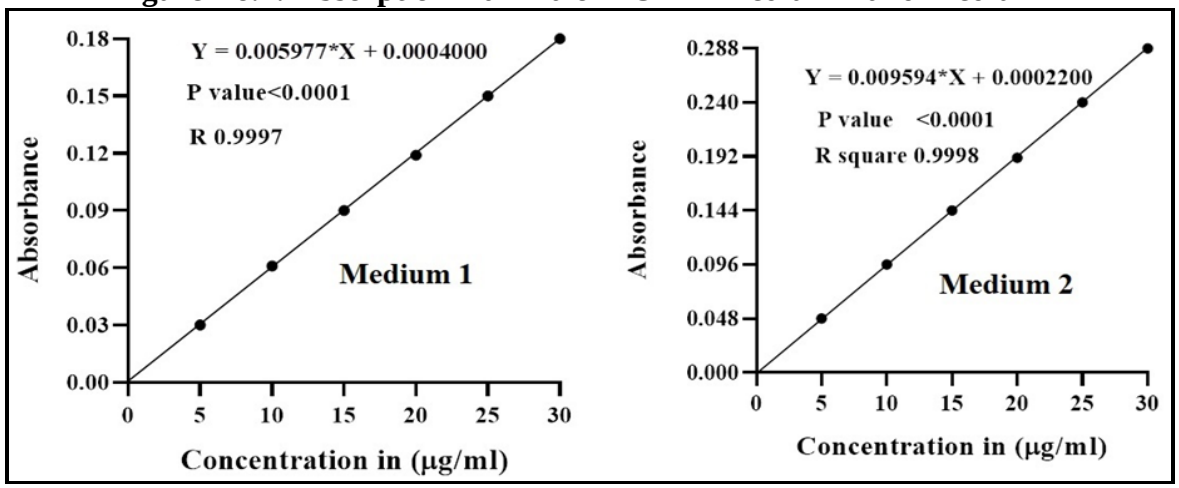

Figure No.3: Calibration curve of TOT in Medium 1 and Medium 2

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric solvent systems are simple, rapid, specific, accurate and precise. Therefore, these solvent systems can use for the quantification of bempedoic acid in bulk and marketed tablet formulations without interference with commonly used excipients and related substances.

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CONFLICT OF INTEREST

No conflict of interest.

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