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DEVELOPMENT AND VALIDATION OF SIMPLE UV SPECTROSCOPIC METHOD FOR THE QUANTIFICATION OF OXYBUTYNIN HYDROCHLORIDE IN BULK AND MARKETED TABLETS

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ABSTRACT

Two Simple and precise UV spectroscopic methods developed and validated as per ICH guidelines. The solvent systems such as 0.01M HCl and double distilled water selected for the study. The two proposed solvent systems validated for linearity, accuracy, precision, robustness, ruggedness and solution stability. The percent recovery in the marketed tablet formulation is with god agreement with the label claim. The proposed methods validated statistically by linearity, precision, repeatability, reproducibility, specificity and solution stability. The results suggest these methods can employ for the routine analysis of oxybutynin hydrochloride in bulk as well as marketed tablet formulations. The results suggest these methods can employed for the routine analysis of oxybutynin hydrochloride in bulk as well as marketed tablet formulations.

KEYWORDS: Oxybutynin hydrochloride, UV spectroscopy, Validation, Accuracy, Precision.

INTRODUCTION

Oxybutynin hydrochloride is an anticholinergic drug used in the management of urinary frequency, urinary urgency, urinary incontinence, idiopathic bladder instability, idiopathic detrusor instability and neurogenic bladder instability.[1-4] Chemically it is 4-(diethyl amino) but-2-ynyl (RS)-2-cyclohexyl-2-hydroxy-2- phenyl acetate hydrochloride.[5] White or almost white, crystalline powder, freely soluble in water and in ethanol (96 percent), soluble in acetone, practically insoluble in cyclohexane.[6]

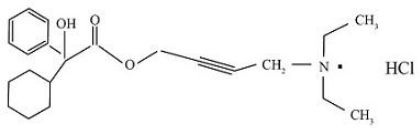

Figure 1: Chemical structure of oxybutynin hydrochloride.

British pharmacopoeia reported HPLC method for the estimation of oxybutynin chloride in oral solution, tablets and prolonged release tablets.[7] Official methods such as non-aqueous titration, potentiometric, colorimetric and HPLC reported in pharmacopoeies.[8-10] From the literature survey, found that, few methods reported for the quantitative estimation of oxybutynin hydrochloride in formulations viz., spectroscopic[11,12], UV and RP-HPLC[13], HPTLC[14-15], HPLC[16-18], RP-HPLC[19-21], colorimetric[22], LC-MS.[23] However, most of these methods were complex, tedious, require expensive experimental setup and skilled personnel, suffer from time-consuming procedures. Keeping all in the present study it is s aimed to develop and validate simple, precise, economical UV spectroscopic method for the quantification of oxybutynin hydrochloride in bulk and available marketed tablets.

MATERIALS

Oxybutynin hydrochloride obtained as gift sample (Unichem Laboratories Ltd, Mumbai, India). Cystran-5mg tablets (Intas Pharmaceuticals Ltd, Ahmadabad, India) and Tropan®5mg (Sun Pharma Laboratories Ltd, Assam, India) tablets procured from local retail pharmacy. All reagents, solvents used were of analytical grade (SD Fine-Chemicals, Bengaluru, India). UV-1900 Shimadzu UV-VIS Spectrophotometer Corp/Japan, UV-1700 Pharma Spec UV-VIS Spectrophotometer-Shimadzu Corp, KYOTO JAPAN. UV-VIS spectrophotometers connected to a compatible computer and supported with UV Probe software used for spectrophotometric measurements.

METHODS

Preparation of 0.01M HCl solution: Place about 0.85 ml of Hydrochloric acid IP[24] (11.5M) in 1000 ml volumetric flask, and then add double distilled water to volume.

Preparation of oxybutynin hydrochloride standard stock solution: Transfer accurately weighed 50 mg of

oxybutynin hydrochloride into a 50ml volumetric flask to this add 40ml of 0.01M HCl solution, shake for 5min and sonicate for 5min to dissolve completely, then make the volume with 0.01M HCl solution to obtain 1mg/ml concentration. Similarly prepare the standard stock solutions in double distilled water.

Preparation of oxybutynin hydrochloride working standard solution: Transfer accurately measured 5ml of oxybutynin hydrochloride standard stock solution into a 50ml volumetric flask to this add 40ml of 0.01M HCl solution, then make the volume with 0.01M HCl solution to obtain 0.1mg/ml concentration. Similarly prepare the working standard solutions in double distilled water.

Preparation of working test standard solution for tablets: Triturate accurately weighed 20 tablets to get fine powder. Weigh accurately triturated powder equivalent to 50 mg of oxybutynin hydrochloride and transfer into 50ml volumetric flask, add 50ml of methanol, extract the content by shaking for 60 min and sonicated for 10min. Filter the content through whatmann filter paper No.44. Appropriately dilute this working standard solution with 0.01M HCl and double distilled water separately to obtain working standard solution and these solutions used for further studies. Similarly prepare working standard solution for other branded tablets.

Determination of absorption maxima (λ max): Appropriately dilute the working standard solution with 0.01M HCl and double distilled water separately in 10ml volumetric flask to get 10µg/ml solution, scan this solution in the range of 200 to 400 nm using double beam UV spectrophotometer, and observe the characteristic peak at standard wavelength (nm).

Validation

The validation of proposed methods carried out as per ICH guideline.[25,26]

Range: Appropriately dilute the oxybutynin hydrochloride working standard solution with 0.01M HCl in a series of 10ml volumetric flask to obtain 2-40µg/ml concentrations and measure the absorbance at 223 nm keeping 0.01M HCl as blank**.** Similarly prepare series of oxybutynin hydrochloride working standard solution i.e. 2-40µg/ml concentrations in double distilled water, measure the absorbance at 222 nm, keeping double distilled water as blank. Determine the linearity range by plotting concentration vs absorbance curve.

Linearity: 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. Appropriately dilute the oxybutynin hydrochloride working standard solution with 0.01M HCl in a series of 10ml volumetric flask to obtain 4,8,12,16,18,20 and 24µg/ml concentrations and measure the absorbance at 223 nm keeping 0.01M HCl as blank**.** Similarly prepare series of oxybutynin hydrochloride working standard solution i.e. 4,8,12,16,18,20 and 24µg/ml concentrations in double distilled water, measure the absorbance at 222 nm, keeping double distilled water as blank, plot the concentration vs absorbance curve and regression equation was computed.

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. Both are determined based on standard deviation (SD) of response and slope by using the following equations.

Precision: Precision of proposed analytical method was carried out at different concentrations prepared by diluting appropriately the oxybutynin hydrochloride working standard solution in medium under the study and express the results in terms of % RSD, similarly inter-day and intra-day precision were performed.

Robustness: Robustness studies perform to check the influence of method parameters varied intentionally on the proposed method results. Dilute the oxybutynin hydrochloride working standard solution separately with 0.01M HCl and double distilled water in a series of 10ml volumetric flask to obtain 12 µg/ml (n=5) concentrations and measure the absorbance at actual wavelength i.e., 223 nm / 222 nm and small variated wavelength i.e., ±1-5 nm keeping 0.01M HCl/double distilled water as blank. Interpret the results interms of percentage RSD.

Ruggedness: Ruggedness studies perform to check the influence of parameters varied intentionally on the proposed method results. Dilute the oxybutynin hydrochloride working standard solution separately with 0.01M HCl and double distilled water in a series of 10ml volumetric flask to obtain 10µg/ml, 12 µg/ml (n=5) concentrations and measure the absorbance at 223 nm / 222 nm by two different analyst and two different UV spectrophotometer. Interpret the results interms of percentage RSD.

Accuracy: 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 oxybutynin hydrochloride (40%, 80% and 120%) added to the test working standard mediums under the study, and the sample assayed as percent recovered.

Solution stability*:* The stability of stock solutions of oxybutynin hydrochloride in proposed methods studied

at room (°C) and refrigerated temperature (2-8°C). The samples were stored in tightly sealed glass containers protected from light. Appropriately dilute the standard stock solutions of proposed methods in a series of 10ml volumetric flask and the absorbencies measured at 0hr-, 4hr and 24hr time interval.

RESULTS AND DISCUSSION

The optimum wavelength of maximum absorption of the proposed methods were found to be 223 nm for 0.01M HCl and 222 nm for double distilled water with characteristic peak Figure 1.

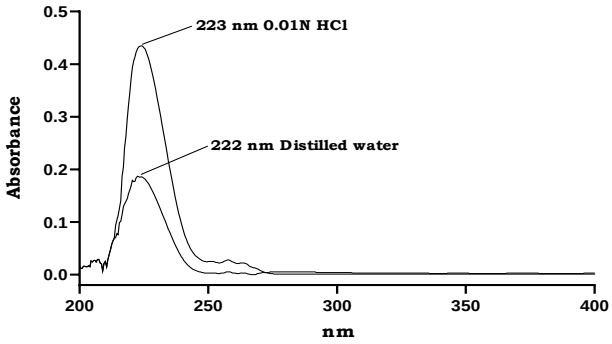

Figure 1: Absorption maxima of oxybutynin hydrochloride in 0.01N HCl and double distilled water.

The Beer's law range, molar absorptivity, sandell's sensitivity, best fit values for two proposed methods viz., 0.01M HCl and double distilled water are given in table 1, 2 and linearity curve in figures 2,3. A linear relationship found in the concentration range of 4-24µg/ml for both methods. The goodness of fit study suggest good correlation coefficient (R square- 0.9999 and 0.9998 for proposed methods) 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. From the standard deviation of the linearity curve and the slope the limit of detection values for oxybutynin hydrochloride for the proposed methods were determined and found to be 0.3447 ± 0.002380µg/ml, 0.2725 ± 0.001401µg/ml and limit of quantitation values found to be 1.046 ± 0.002458µg/ml, 0.8226 ± 0.0006506µg/ml with % RSD values less than 2.

Table 1: Linearity curve data.

Concentration µg/ml	Absorbance mean ± SD (n=5)	
	0.01M HCl	Double distilled water
4	0.03167±0.000577	0.067±0.002646
8	0.06333±0.002082	0.1313±0.004041
12	0.09433±0.001528	0.1957±0.003055
16	0.1257±0.002082	0.2573±0.004041
18	0.1417±0.001528	0.2903±0.005033
20	0.159±0.001000	0.323±0.002646
24	0.188±0.002000	0.383±0.005568

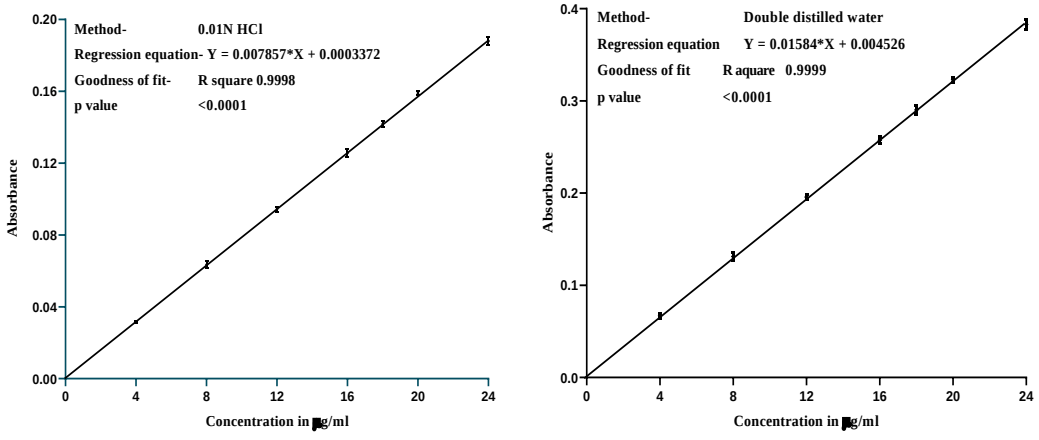

Figure: Linearity curve of oxybutynin hydrochloride in 0.01M HCl and Double distilled water.

Table 2: Statistical data of linearity curve for proposed methods.

Parameters	0.01M HCl	Double distilled water
Absorption maxima (λ_{max})	223 nm	222 nm
Beer's range (µg/ml)	4-30 (µg/ml)	4-30 (µg/ml)
Molar absorptivity(ε),	$7.872x10^3$ 1/(m-cm)	$1.6127x10^4$ 1/(m-cm)
Sandell's sensitivity(л)	0.1259 µg/cm^2/0.001	0.0256 µg/cm^2/0.001
Best-fit values		
Slope	0.007857	0.01584
Y-intercept	0.0003372	0.004526
X-intercept	-0.04292	-0.2857
1/slope	127.3	63.12
95% Confidence Intervals		
Slope	0.007733 to 0.007980	0.01565 to 0.01604
Y-intercept	-0.001628 to 0.002303	0.001409 to 0.007643
X-intercept	-0.2974 to 0.2043	-0.4880 to -0.08797

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 proposed methods were justified from the absorbance values obtained viz., six replicates in repeatability studies, two concentrations and three replicates in intra and inter day studies of a fixed amount of oxybutynin hydrochloride in proposed mediums. The SD and % RSD calculated for the proposed methods and are given in table 3, 4. The percentage RSD values for repeatability studies, intraday and interday studies is less than 2% indicate proposed methods were precise and reproducible.

Table 3: Repeatability precision data.

Concentration (µg/ml)	Absorbance (n=6)	
	0.01M HCl	Double distilled water
12	0.0943	0.196
12	0.0938	0.198
12	0.0941	0.193
12	0.0946	0.195
12	0.0939	0.196
12	0.0940	0.197
Mean ± SD	0.09412 ± 0.0002927	0.1958 ± 0.001722
% RSD	0.3110	0.8795

The proposed methods analyzed for assay in two marketed tablet formulations and data given in table 5. The percentage recovery was within the permissible limit with RSD values less than 2%. The accuracy performed for the proposed methods 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 oxybutynin hydrochloride content of two marketed products determined by the proposed methods was in god agreement with the label claim with % RSD values less than 2 and data given in table 6.

Table 4: Inter day and intraday precision data.

Intraday precision*						
Amount tested	0.01M HCl			Double distilled water		
	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD
12 (µg/ml)	11.92	99.30±0.5021	0.5058	11.99	99.88±0.4119	0.4123
16 (µg/ml)	15.93	99.54±0.5361	0.5413	15.94	99.60±0.4029	0.4045
20 (µg/ml)	19.86	99.32±0.9005	0.9067	19.92	99.60±0.5268	0.5289
Interday precision#						
Amount tested	0.01M HCl			Double distilled water		
	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD
12 (µg/ml)	11.74	97.83±1.181	1.2080	11.94	99.47±0.6361	0.6395
16 (µg/ml)	15.75	98.41±1.069	1.0820	15.86	99.12±0.2848	0.2873
20 (µg/ml)	19.49	97.47±0.7006	0.7188	19.80	99.02±0.4311	0.4354

***** Three time intervals in a day # Three day intervals

Table 5: Accuracy data of proposed methods for two marketed formulations.

Brand name Labelled claim	Amount Added (Pure drug)	% Added (Pure drug)	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD
0.01M HCl					
Tropan®5mg	2	40	1.960	98.00±1.000	1.020
	4	80	3.927	98.17±0.8036	0.8186
	5	100	4.967	99.33±0.3055	0.3076
Cystran-5mg	2	40	1.973	98.67±1.041	1.055
	4	80	3.967	99.17±0.6292	0.6344
	5	100	4.987	99.73±0.4163	0.4174
Double distilled water					
Tropan®5mg	2	40	1.973	98.67±0.2887	0.2926
	4	80	3.963	99.08±1.607	1.622
	5	100	4.947	98.93±0.9866	0.9972
Cystran-5mg	2	40	1.990	99.50±0.8660	0.8704
	4	80	3.937	98.42±1.041	1.058
	5	100	4.990	99.80±0.5292	0.5302

Table 6: Drug content data in marketed tablet formulations.

Brand name	Labelled claim	0.01M HCl			Double distilled water		
		Amount recovered	% Recovery Mean ± SD (n=3)	% RSD	Amount recovered	% Recovery Mean ± SD (n=3)	% RSD
Tropan®5mg	**5mg**	4.963	99.27±1.617	1.629	4.940	98.80±1.249	1.264
Cystran-5mg	**5mg**	4.930	98.60±0.9165	0.9295	4.903	98.07±1.222	1.246

Change in λmax of ± 5nm to the actual λmax in robust analysis results significant different in the percentage recovery in both proposed methods indicates the methods were not robust. In ruggedness, analysis by different analyst and change of instrument indicates the proposed methods were significantly rugged. The robustness and ruggedness data given in tables 7,8.

Table 7: Robustness data for proposed methods.

λmax	Concentration (µg)	Amount recovered (µg)	% Recovery Mean ± SD (n=3)	% RSD
0.01M HCl				
Actual 223nm	12µg	11.97	99.75±0.08505	0.08527
228nm (+nm)	12µg	10.84	90.66±2.291	2.527
218nm (-5nm)	12µg	10.75	89.55±1.875	2.094
Double distilled water				
Actual 222nm	12µg	11.99	99.94±0.1277	0.1277
226nm (+nm)	12µg	10.58	89.19±3.358	3.765
217nm (-5nm)	12µg	10.76	89.63±2.001	2.232

Table 8: Ruggedness data for proposed methods.

Parameter	Concentration (µg)	Amount Recovered (µg)	% Recovery Mean ± SD (n=3)	% RSD
0.01M HCl				
Analyst-1	12	11.97	99.75±0.08505	0.08527%
Analyst-2	12	11.98	99.80±0.2425	0.2430%
UV-1700	12	11.96	99.52±0.3932	0.3951%
UV-1900	12	11.93	99.61±0.5405	0.5426%
Double distilled water				
Analyst-1	12	12.01	100.1±0.1277	0.1276%
Analyst-2	12	11.96	99.66±0.08505	0.08534%
UV-1700	12	11.98	99.80±0.2542	0.2547%
UV-1900	12	11.95	99.61±0.4579	0.4597%

The results of stability study of oxybutynin hydrochloride in proposed methods were within the acceptable limit and indicate solutions in proposed methods stable over the period of 24hr.

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric methods are simple, rapid, specific, accurate and precise. Therefore, this method can used for the quantification of oxybutynin hydrochloride in bulk and marketed tablet formulations without interference with commonly used excipients and related substances.

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