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DEVELOPMENT OF VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BETAXOLOL HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract: In this present research work we have developed a validated UV spectrometry method for estimation of Betaxolol Hydrochloride in pure and pharmaceutical dosage form. The developed method is accurate, cost efficient, fast and reproducible for the estimation of Betaxolol Hydrochloride in pure and pharmaceutical dosage form. Based on measurement of absorption of UV light, the spectra of Betaxolol Hydrochloride in simulated tear fluid as a solvent showed maximum absorption wavelength (λ max) at 224 nm. The calibration curve was plotted over the concentration range from 2-200 µg/ml of Betaxolol Hydrochloride with correlation coefficient 0.996. Validation was performed as per ICH Q2 guidelines for linearity, accuracy, precision, and recovery. This method has good reproducibility with % RSD less than one. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.2740 and 0.8305 respectively by simple UV spectroscopy. Thus this proposed validated method can successfully apply for estimation of Betaxolol Hydrochloride in quality control, routine analytical work, and in pharmaceutical dosage forms.

Keywords: Betaxolol Hydrochloride, Spectrophotometric method, ICH Q2 guidelines

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INTRODUCTION

Betaxolol hydrochloride is a Cardio selective β1 receptor blocking agent used in the treatment of chronic open angle glaucoma and ocular hypertension.[1] Chemically it is 2-propanol, 1- (4- (2- (cyclopropylmethoxy) ethyl) phenoxy) - 3 - ((1 - methylethyl) amino)- , hydrochloride. [2] It has molecular formula $C_{18}H_{29}NO_3$.HCl and molecular weight is 343.89.[3] Betaxolol hydrochloride is a white, crystalline powder. [4] It is freely soluble in water, ethanol, chloroform, and methanol, has a pKa of 9.4.[5]

The ocular hypertensive effect caused by β-blocker is probably due to suppression of aqueous humor formation by blockage of the β adrenal receptors in the ciliary body. β -blockers decrease aqueous humor production by approximately one-third. To obtain the desired lowering in IOP, large quantity of conventional eye drops of betaxolol hydrochloride are used.[6]

AIM OF PRESENT RESEARCH WORK:

1. Up to present date there is no valid UV spectrophotometric method available for estimation of Betaxolol hydrochloride.
2. The goal of our present research work was to develop a validated UV spectrometry method for estimation in pure and pharmaceutical dosage form.
3. To develop rapid, economical, and reproducible UV spectroscopic method for quality control of pharmaceutical formulations containing betaxolol hydrochloride as the active ingredient.
4. To utilize this method for quality control and analysis of drug in pharmaceuticals containing Betaxolol hydrochloride.

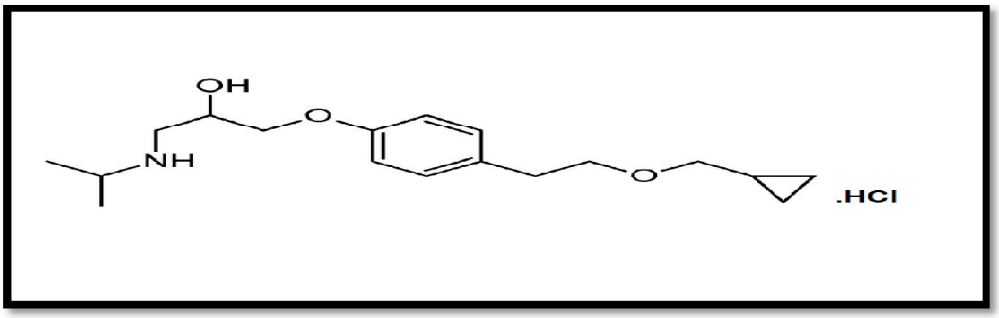

Fig 1: structure of Betaxolol hydrochloride

MATERIAL AND METHODS:

Instrument:

Instruments used were: Electronic balance, Model-AJ- Vibra, Mfg by Essae-Teraoka Ltd. Japan. UV-Visible double beam spectrophotometer Shimadzu UV1800 with one cm. Matched quartz cells. The glass wares used in each procedure were soaked overnight in a mixture of chromic acid and sulfuric acid, rinsed with double distilled water and dried before use. The absorption spectra of reference and test solution were carried out in a one cm quartz cell over the range of 200-400 nm.

Material:

Pure sample:

Betaxolol hydrochloride was kindly supplied by Hikal labs Ltd. Bangalore, India.

Reagents and Chemicals

Sodium chloride (Thermo Fischer scientific India Pvt. Ltd), Sodium bicarbonate, Calcium chloride (Loba Chemie Pvt. Ltd Mumbai, India), other chemicals and reagents used of analytical grade.

Preparation of Simulated Tear Fluid:[7]

Simulated Tear Fluid **(STF)** is a composition of accurately weighed sodium chloride 0.67gm, sodium bicarbonate 0.20 gm, calcium chloride 0.008 gm dissolved in 100 ml distilled water .

Preparation of standard stock solution:

I Stock solution

Standard drug solution of Betaxolol hydrochloride was prepared by dissolving 100 mg of pure Betaxolol hydrochloride in a small amount of Simulated Tear Fluid in 100ml volumetric flask and then the volume was adjusted with Simulated Tear Fluid as a solvent. The resultant solution gives the concentration of 1mg/ml. ie.1000µg/ml.

II Stock solution

From I stock solution: 10 ml solution was taken and then diluted up to100 ml with the same solvent in a volumetric flask and then concentration of this stock was 100µg/ml.

Determination of Lambda Max:

The 10µg / ml solution was prepared by withdrawing 10 ml of solution from II stock solution and further diluted with Simulated Tear Fluid to get concentration. This solution was then scanned at a wavelength of 200-400 nm against the blank. The lambda max was found to be at 224 wavelengths where observance was maximum at this wavelength. Hence this is considered as absorption maxima which are used for preparation of calibration curve. **(Figure 2)**

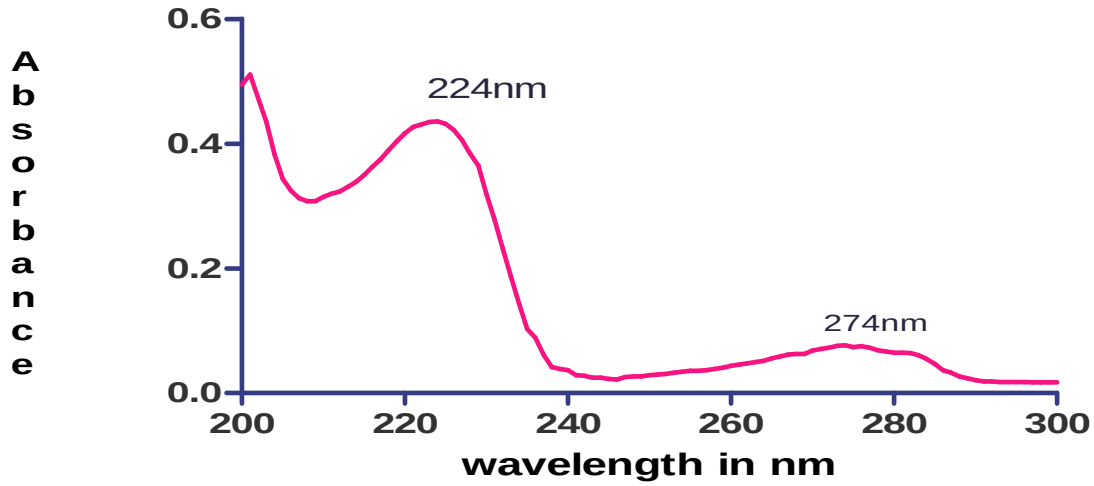

Figure 2: Determination of lambda max of Betaxolol hydrochloride.

Preparation of Calibration Curve:

I Stock solution

Standard drug solution of Betaxolol HCl was prepared by dissolving 100mg of pure Betaxolol HCl in small amount of STF in 100 ml volumetric flask and then the volume was adjusted with STF then the volume was adjusted with STF the resultant solution gives a concentration of 1mg/ml ie. 1000µg/ml.

II stock solution

From I stock solution 10 ml solution was taken and then diluted up 100 ml with the same solvent in a volumetric flask and then the concentration of this stock was 100µg/ml. From this II stock solution 02,04,06,08,10,12,14,16,18,20,30,40,50,60, 70,80,90 and100 ml solutions were pipette volume was made to 100 ml using STF as a solvent to get concentrations of 02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml respectively. The absorbance of these solutions was measured at 224 nm (lambda max of Betaxolol HCl). The standard calibration curve was obtained from data of concentration v/s absorbance; standard calibration curve data reported in **(Table no. 1, Figure3)**

Table no. 1: Calibration data for the method development for Betaxolol HCl.

Sr. No.	Concentration(µg/ml)	Absorbance at 224nm ± standard deviation
1	2	0.090 ± 0.0015
2	4	0.177 ± 0.0029
3	6	0.255 ± 0.0025
4	8	0.318 ± 0.0031
5	10	0.389 ± 0.0017
6	12	0.468 ± 0.0030
7	14	0.562 ± 0.0022
8	16	0.602 ± 0.0036
9	18	0.633 ± 0.0042
10	20	0.997 ± 0.0030
11	30	1.070 ± 0.0017
12	40	1.280 ± 0.0017
13	50	1.704 ± 0.0026
14	60	1.868 ± 0.0036
15	70	2.222 ± 0.0033
16	80	2.571 ± 0.0036
17	90	2.855 ± 0.0026
18	100	2.914 ± 0.0038

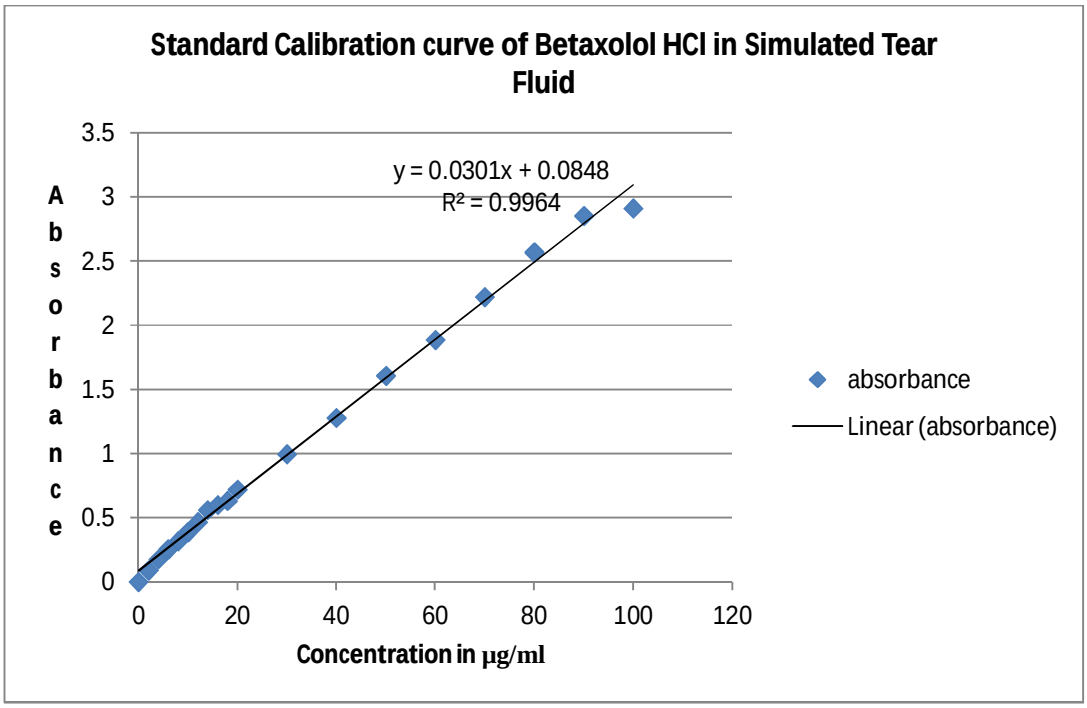

Figure 3: Calibration Curve of Betaxolol HCl in Simulated Tear Fluid.

METHOD VALIDATION:

Linearity and Range:

The linearity of the response of the drug was obtained at 2 to 100 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance v/s concentration data and was treated by linear regression analysis (Table no.3). The equation of the calibration curve for Betaxolol HCl obtained was y = 0.0301x+ 0.0848, the calibration curve was found to be linear in the for ementioned concentrations (The correlation co-efficient (R^2) of determination was 0.996).

Precision:

Precision of the method was analysed by repeatability and determined by analyzing 10µg/ml of Betaxolol HCl for 6 times the results are reported in **(Table no. 3)**

Table no.2: Data showing Repeatability of absorbance's

Sr. No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance	Mean± S.D.	%R.S.D
1	10	224	0.392	0.389±0.003742	0.356
2		224	0.387		
3		224	0.391		
4		224	0.388		
5		224	0.386		
6		224	0.390		

S.D- Standard Deviation, R.S.D- Relative Standard Deviation

Precision:

The precision of the method was studied as intra-day and inter-day variations. Intraday precision was determined by analyzing µg/ml of Betaxolol for three times within the day. Inter-day precision was determined by analyzing the same concentration of solutions daily for three days, the results are reported in **(Table no.3)**

Table 3: Results for Intra-day and Inter-day precision of Betaxolol.

Drug	Conc. (µg/ml)	Intra-day Mean Absorbance ± S.D.	%RSD	Inter-day Mean Absorbance ± S.D.	%RSD
Betaxolol Hydrochloride	10	0.390±0.001732	0.443	0.388±0.001927	0.451
	30	1.070±0.003082	0.324	1.102±0.002741	0.238
	90	2.799±0.002645	0.121	2.783±0.00456	0.255
Mean %RSD			0.296		0.314

S.D. = Standard Deviation, R.S.D. = Relative Standard Deviation

Limit of Detection and Quantification (LOD and LOQ):

Determination of the detection and quantification limits was performed based on the standard deviations of y-intercept and the slope of the least square line parameters as defined in the International Conference on Harmonization (ICH) guidelines. The LOD and LOQ were and respectively and data is reported in **(Table no.4)**

Table no.4: Validation parameters for Betaxolol Hydrochloride

Sr. No.	Parameters	Results
1	Absorption maxima (nm)	224nm
2	Linearity range (µg/ml)	2-100µg/ml
3	Standard Regression Equation	y = 0.0301x +0.0848
4	Correlation coefficient (R^2)	R^2 = 0.996
5	Specificity	A 10 µg /ml solution of candidate drug in solvent (simulated tear fluid) at UV detection lambda max of 224 nm will show an absorbance value of 0.389 ± 0.003742
6	Accuracy (% Recovery)	99.78%
7	Precision RSD Repeatability (n=6) Intra-day(n=3) Inter-day(n=3)	0.356 0.296 0.314
8	Molar Absorptivity	$1.2560*10^4$ L/Mol. cm.
9	LOD	0.274µg/ml
10	LOQ	0.8305µg/ml

n=no. Of determinations, LOD=Limit of Detection, LOQ =Limit of Quantification, RSD= Relative Standard Deviation.

Recovery Study:

To analyse the accuracy of developed method, it was applied to analyse commercially available Betaxolol HCl eye drop (IOBET 5ml- FDC). The amount of eye solution equivalent to 10 mg of Betaxolol accurately measured and transferred to 10 ml volumetric flask. Then sufficient quantity of Simulated Tear Fluid added and kept for 10-20 min with frequent shaking and volume made up to mark with given solvent. This solution utilized to get 10µg/ml concentration. Then absorbance was measured against the blank solution. The recovery was performed at three different levels which are 80%, 120%, and180%. To the preanalysed sample solution a known amount of standard drug solution was added at three different levels and absorbance was recorded. The drug content of preparation was calculated using the standard calibration curve and amount of drug estimated by this method is given in **(Table no.5)**

Table no.5: Determination of Accuracy by Percentage Recovery Method.

Drug	Eye Drop amount (µg/ml)	Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average% Recovery
Betaxolol hydrochloride	10	80	8	17.96	99.78	99.78
	10	120	12	21.96	99.83	
	10	180	18	27.92	99.74	

RESULTS AND DISCUSSION:

The proposed method showed molar absorptivity 1.2560 *10^4 L/mol.cm. The calibration curve of Betaxolol hydrochloride plotted at 224 nm **(Figure no.3)** a linear relationship was obtained between 2-100 µg/ml. The accuracy of the method was determined by calculating mean percentage recovery it was found to be 99.78% **(Table no.5)**. Further precision was calculated as repeatability, inter and intra-day variations and % RSD was less than one **(Table no.4)**. The LOD value was found to be 0.2740 and LOQ value was found to be 0.8305.

CONCLUSION:

The developed method was found to be simple, accurate, sensitive, reproducible, and can be used for routine quality control analysis of Betaxolol HCl.

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