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Analytical Method Development and Validation for the Simultaneous Estimation of Brinzolamide and Timolol Maleate by RP-HPLC Method in Bulk and Tablet Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Brinzolamide and Timolol Maleate by using C_{18} Column (150mm x 4.6mm)5 µm, flow rate was 1 ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 4.0 (70:30 v/v), detection wavelength was 260 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector, Empower-software version 2. The retention times were found to be 2.137 min and 2.844 min. The % purity of Brinzolamide and Timolol Maleate was found to be 99.86% and 100.1% respectively. The system suitability parameters for Brinzolamide and Timolol Maleate such as theoretical plates and tailing factor were found to be 5742, 1.4 and 5167 and 1.2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Brinzolamide and Timolol Maleate was found in concentration range of 5µg-25µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 99.86% and 99.96% respectively. %RSD for repeatability and precision was found to be <2.LOD values were 0.021 and 0.025 and LOQ value was 0.025 respectively for Brinzolamide and Timolol Maleate.

Keywords: Brinzolamide, Timolol Maleate, HPLC.

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1. Introduction

Brinzolamide is a highly specific inhibitor of CA-II, which is the main CA iso-enzyme involved in the secretion of aqueous humor. Inhibition of CA in the ciliary process of the eye slows the formation of bicarbonate, and reduces sodium and fluid transport. This results in a reduction in the rate of aqueous humor secretion and the intraocular pressure. Brinzolamide is absorbed systemically following topical ocular administration. Since it has a high affinity for CA-II, Brinzolamide binds extensively to red blood cells, where CA-II is primarily found. As sufficient CA-II activity remains, adverse effects resulting from the systemic inhibition of CA by Brinzolamide are not observed. The metabolite N-desethyl brinzolamide is also formed. This metabolite binds to CA and accumulates in red blood cells as well. In the presence of Brinzolamide, the metabolite binds mainly to carbonic anhydrase I.

Timolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle and beta(2)-receptors in the bronchial and vascular smooth muscle. Beta(1)-receptor blockade results in a decrease in resting and exercise heart rate and cardiac output, a decrease in both systolic and diastolic blood pressure, and, possibly, a reduction in reflex orthostatic hypotension. Beta(2)-blockade results in an increase in peripheral vascular resistance. The exact mechanism whereby Timolol reduces ocular pressure is still not known. The most likely action is by decreasing the secretion of aqueous humor.

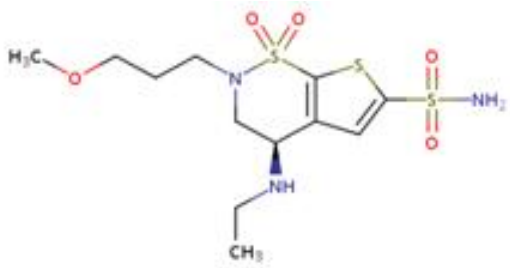

Figure 1: Brinzolamide

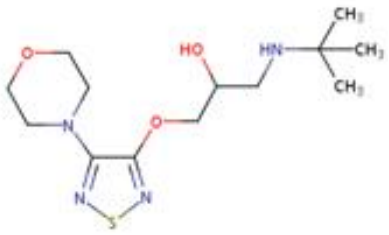

Figure 2: Timolol Maleate

Analytical methods

The technique employed in qualitative and quantitative analysis is based upon the performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods, there can be no "second quality" in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production.

Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence methods of analysis, Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of pharmacopeia
3. The analysis should be economical.
4. The selected method should be precise and selective.

2. Materials and Methods

Apparatus

The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2690, photo diode array detector with Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Ortho phosphoric acid, Potassium dihydrogen ortho phosphate, Tri Ethyl Amine of HPLC Grade and HPLC Water.

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Brinzolamide and Timolol Maleate were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was

recorded. From the overlain spectrum, 260 nm was selected as the detection wavelength for the present study.

Selection of mobile phase:

Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 30:70 v/v respectively at pH 4 adjusted with Orthophosphoric Acid.

Chromatographic trials for Simultaneous Estimation of Brinzolamide and Timolol Maleate by RP- HPLC.

Trial-1 Chromatographic conditions

Parameters	Description
Flow rate	1ml min^{-1}
Column	YMC C_{18} Column (250mm x 4.6 mm)5µ
Mobile Phase	Water: Methanol (30:70 v/v)
Detector	PDA
Column temperature	Ambient
Wavelength	260 nm
Type of elution	Isocratic
Injection volume	10 µl
Run time	10 min

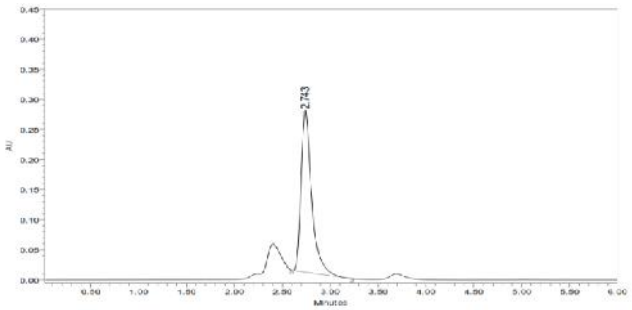

Figure 3: Chromatogram of Trial-1

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-2 Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Agilent C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Buffer: Methanol P^H 3.0 (40:60 v/v)
Buffer	Potassium dihydrogen orthophosphate ph2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260nm
Injection volume	20µl
Run time	10min

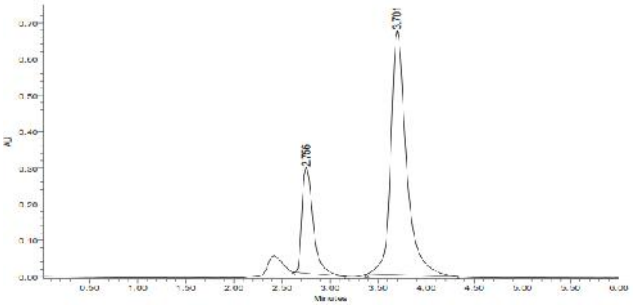

Figure 4: Chromatogram of Trial-2

Observation: The separation of two analytical peaks was not proper, So the mobile phase ratio has been changed for next trial.

Trial-3 Chromatographic condition

Parameters	**Description**
Flow rate	1 ml min^{-1}
Column	Agilent C_{18} Column (250mm x 4.6 mm) 5µg.
Mobile Phase	Buffer P^H 4.0: ACN (60:40 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with OPA
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260 nm
Injection volume	20µl
Run time	10min

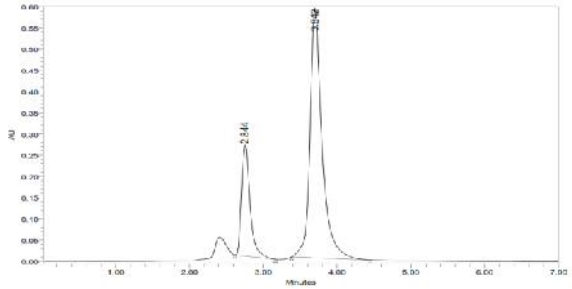

Figure 5: Chromatogram of Trial-3

Observation: The separation of two analytical peaks was not proper, so the mobile phase ratio has been changed for next trial.

Trial-4 Chromatographic condition

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Inertsil C_{18} Column (150mm x 4.6mm) 5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 4.0 (40:60 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjust with orthophosphoric acid
Detector	PDA

Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260 nm
Injection volume	20µl
Run time	10min

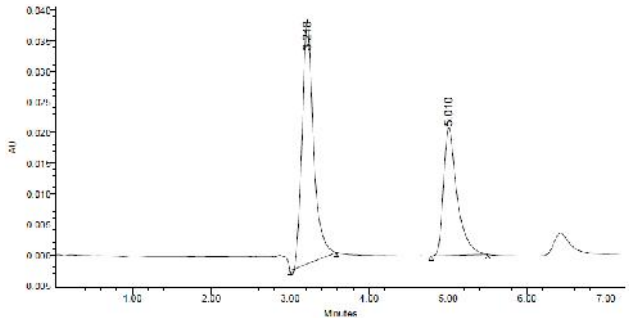

Figure 6: Chromatogram of Trial-4

Observation: The separation of two analytical peaks is occurred but fronting occurs in Brinzolamide peak.

Trial-5 Chromatographic condition (Optimized Method)

Parameters	**Description**
Flow rate	1ml min^{-1}
Column	Inertsil C_{18} Column (150mm x 4.6mm)5µm.
Mobile Phase	Phosphate buffer: Methanol P^H 4.0 (30:70 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 4.0 adjust with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260 nm
Injection volume	10µl
Run time	10min

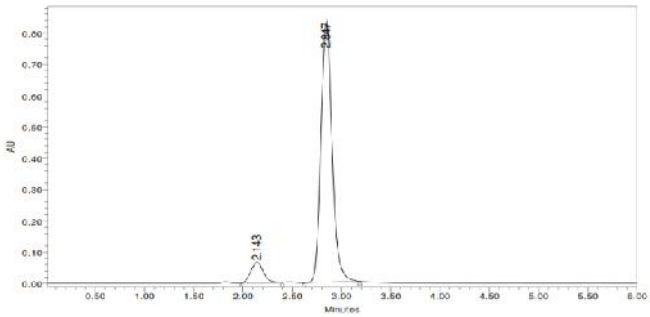

Figure 6: Chromatogram of Trial-5(Optimized)

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Procedure

Preparation of Buffer:

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000 ml of HPLC grade water and pH 4.0 was adjusted with Orthophosphoric acid. It was filtered through 0.45 µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase:

Mobile phase consist of buffer: Methanol of P^H 4.0 (30:70) was taken sonicated and degassed for 10 min and filtered through 0.45 µm nylon membrane filter

Standard Preparation:

Weigh accurately 10 mg Brinzolamide Working Reference Standard and 15 mg of Timolol Maleate working Reference Standard is taken in to 100 ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that, 50 ml of the above solution was taken into 100 ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents

3. Results and discussions

Method Validation Parameters

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

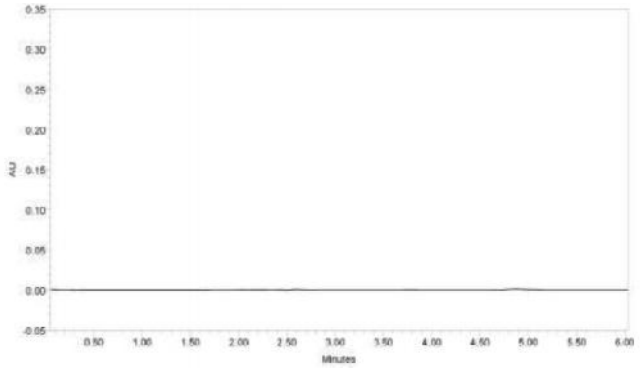

Figure 7:.Chromatogram of Blank

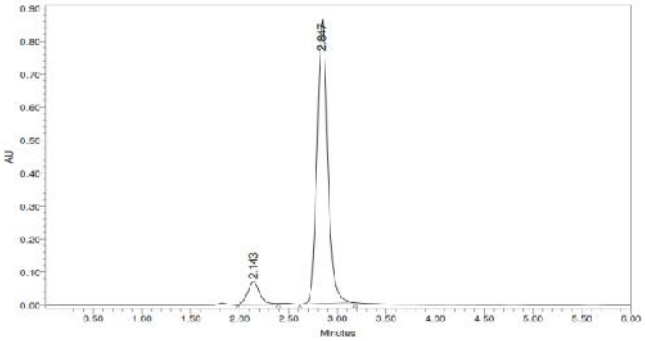

Figure 8:.Chromatogram of Sample

2. Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Serial dilutions of Brinzolamide and Timolol Maleate (5-25µg/ml and 20-100 µg/ml) were injected into the column and detected at a wavelength set at 260 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 5-25µg/ml and 20-100 µg/ml for Brinzolamide and Timolol Maleate respectively.

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2 types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Brinzolamide and Timolol Maleate at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

20µL of the standard and sample solutions of Brinzolamide and Timolol Maleate were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

5. Precision: Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Brinzolamide and Timolol Maleate formulation.

Acceptance criteria: The % RSD for the area of six sample injections results should not be more than 2.

Selection of solvent: Solutions of Brinzolamide and Timolol Maleate were prepared in different solvents like methanol, ethanol, acetonitrile and UV spectrum of each were recorded by scanning between 200-400 nm.

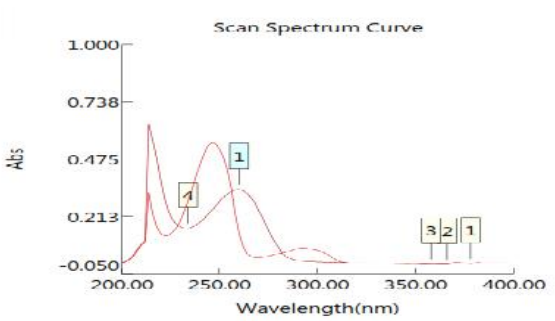

Figure 9:.Overlain Spectra of Brinzolamide and Timolol Maleate in Methanol

Validation of the method

Linearity

Brinzolamide and Timolol Maleate: Serial dilutions of Brinzolamide and Timolol Maleate (5-25µg/ml and 20-100 µg/ml) were injected into the column and detected at a wavelength set at 260 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.999 and 0.999 respectively.

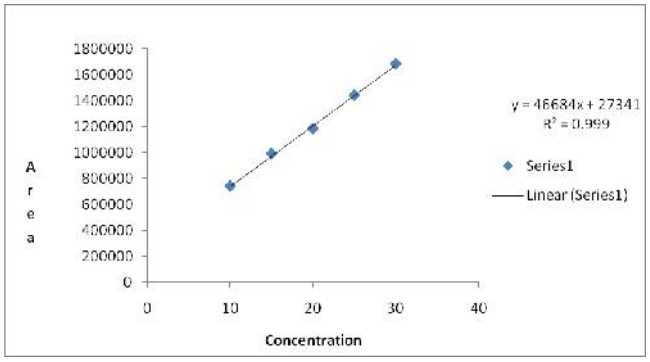

Figure 10:.Calibration graph of Timolol Maleate

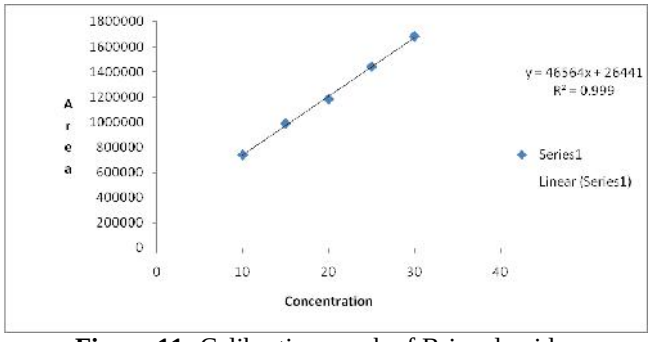

Figure 11:.Calibration graph of Brinzolamide

Table 1: Calibration data of Brinzolamide and Timolol Maleate

Sample ID	Brinzolamide		Timolol maleate	
	Conc (mcg/ml)	Area	Conc (mcg/ml)	Area
20% of operating conc	5	1324140	20	940046
40% of operating conc	10	1395681	40	990204
60% of operating conc	15	1392966	60	1083023
80% of operating conc	20	1356546	80	1139886
100% of operatingconc	25	1397214	100	1082302
Correlation Coefficient		0.999	0.999	

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Brinzolamide and Timolol Maleate were added at 50%, 100% and 150% level and the contents were re-analyzed by the proposed method.

Table 2: Showing accuracy results for Brinzolamide

Sample Id	Conc. found (µg/ml)	Conc. Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2		
50%	5	4.86	98.2	99.86	**%RSD= 0.506**
50%	5	4.89	98.8		
100%	10	10.0	100		
100%	10	9.82	98.4	99.8	**%RSD=0.64**
100%	10	9.86	98.4		
150%	15	14.84	97.8		
150%	15	14.74	98.2	99.4	**%RSD=1.42**
150%	15	15.02	100.1		

Table 3: Showing accuracy results for Timolol Maleate

Sample Id	Conc. Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
50%	4.94	98.2		
50%	4.92	99.4	**100.1**	**1.4**
50%	5.01	100.5		
100%	9.94	99.6		
100%	9.92	99.2	**99.6**	**0.3**
100%	9.96	99.4		
150%	14.79	98.2		
150%	14.96	99.4	**99.2**	**0.520**
150%	14.86	98.9		

Table 4: Robustness

	Name	Retention Time	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Brinzolamide	2.433	651049	56878		1.1	2430.1
2	Temolol	3.237	7526136	766177	2.9	1.2	3543.0

System Suitability Results at flow rate 0.8 ml/min

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Brinzolamide	1.902	536403	69867		1.1	2369.7
2	Temolol	2.533	5870230	876719	3.3	1.2	3226.9

System Suitability Results at flow rate 1.2 ml/min

Table 5: LOD and LOQ

Brinzolamide			Timolol Maleate		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
5	1296	S = 38092 c = 608048 LOD: 0.021 µg/ml LOQ: 0.024 µg/ml	20	1641	S = 38092 c =359381 LOD:0.025 µg/ml LOQ: 0.025 µg/ml
5	4126		20	5568	

Table 6: Precision

Name: Brinzolamide

	Name	RT	Area	Height (µV)
1	Brinzo	2.138	596886	63755
2	Brinzo	2.137	597766	63808
3	Brinzo	2.135	600318	61988
4	Brinzo	2.136	600832	65724
5	Brinzo	2.138	600884	64272
Mean			599337	
Std. Dev.			1875.2	
% RSD			0.31	

Name: Temolol

	Name	RT	Area	Height (µV)
1	Temolol	2.860	6423669	779071
2	Temolol	2.860	6418299	791461
3	Temolol	2.860	6435957	781924
4	Temolol	2.852	6426016	810297
5	Temolol	2.846	6425928	799359
Mean			6425974	
Std. Dev.			6400.9	
% RSD			0.10	

4. Conclusion

A new method was established for simultaneous estimation of Brinzolamide and Timolol Maleate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Brinzolamide and Timolol Maleate by using Agilent C18 column (4.6×150 mm)5µ, flow rate was 1 ml/min, mobile phase ratio was (70:30 v/v) methanol: Buffer, detection wavelength was 260 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Brinzolamide and Timolol Maleate in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

5. References

[1] Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, **1986**:1-15.

[2] Douglas A. Skoog, F. James Holler, Timothy A**.** Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2nd edition, **1980**:725-760.

[3] United States of Pharmacopeia, USP30-NF25 and The official compendia of standards, official May 1, **2007**.

[4] Khatun, Rahima; Islam, S. M. Ashraful. Development and Validation of Analytical Method for Simultaneous Estimation of Brinzolamide and Timolol by HPLC from Ophthalmic Preparation. International Journal of Pharmacy & Life Sciences; Mar **2014**, Vol. 5 Issue 3, 1001-8.

[5] Purvi A Shah, Ankita S Kadikar, Niketa R Gevariya, and Kalpana G Patel, Simultaneous Estimation of Brinzolamide and Timolol Maleate Using Chromatographic Methods. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(5), 1010-14.

[6] Rajashree Mashru, Bharat Senta, Development And Validation of Spectrophotometric Method For Simultaneous Estimation Of Brinzolamide And Brimonidine Tartrate. Asian Journal of Pharmacy and Life Science, Vol.4 (2), April-June, **2014**.124-7.

[7] Heta H. Desai and Anandkumari D. Captain, Three Simple Validated UV Spectrophotometric Methods For The Simultaneous Estimation Of Timolol Maleate And Brimonidine Tartrate And Their Comparison Using Anova. International Journal of Pharmaceutical Research & Analysis, 4(3), **2014**, 168-177.

[8] B. P. Nagoria , Amit Maru b, Pankaj Muysunic and Subhash Guptad, Method Development and Its Validation for Simultaneous Estimation of Timolol Maleate and Dorzolamide Hydrochloride in as API and In Ophthalmic Solution Dosage Form by RPHPLC. Journal of Chemical and Pharmaceutical Research, **2011**, 3(4): 866-874.

[9] RS Wanare, AO Kabra, a Deshmukh, N Aher. Simultaneous Estimation of Dorzolamide Hydrochloride And Timolol Maleate In Eye Drops by A RP-HPLC Method. International Journal of Comprehensive Pharmacy, **2012**,4(3):11-5

[10] Abdullah A Elshanawane, Lobna M Abdelaziz, Mustafa S Mohram and Hany M Hafez**,** Development and Validation of HPLC Method for Simultaneous Estimation of Brimonidine Tartrate and Timolol Maleate in Bulk and Pharmaceutical Dosage Form. Journal of Chromatography & Separation Techniques. **2011**, 3(4):125-7

[11] Nagaraju P, Kiran Kumar S, Poornima Ch, Development and validation of RP-HPLC Method for the Simultaneous Estimation of Brimonidine tartrate and Timolol maleate in Combined Dosage form. Pharma research library. **2011**, 3(4):114-17

[12] Boiero C, Allemandi D, Longhi M, Llabot JM. RP-HPLC method development for the simultaneous determination of timolol maleate and human serum albumin in albumin nanoparticles. J Pharm Biomed Anal. **2015** Jul 10,111:186-9.

[13] Umesh D. Laddha, Rohan K. Barse, Rasika V. Zilpelwar, Amol A. Tagalpallewar. Development and validation of stability indicating reverse phase high performance liquid chromatography method for Timolol Maleate. Int J Pharm Tech Res, Vol.6, No. 5, pp 1429-1435.