Research Article CODEN: IJPRNK IMPACT FACTOR : 4.278 ISSN: 2277-8713
Patel BM, IJPRBS, 2014; Volume 3(4): 1-13 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF TIMOLOL MALEATE AND PILOCARPINE NITRATE IN COMBINED PHARMACEUTICAL DOSAGE FORM

PATEL BM, SOLANKI SD

Quality assurance department, K.B. Raval College of pharmacy, Kasturinagar, Ghandhinagar, Gujrat, India

Accepted Date: 23/06/2014; Published Date: 27/08/2014

Abstract: A simple and precise one UV-spectrophotometric and one RP-HPLC methods have been developed for the simultaneous determination of Timolol Maleate and Pilocarpine Nitrate in combined pharmaceutical dosage form. The UV spectrophotometric method was a determination using the Absorbance Ratio method at 239nm (isoabsortive point) and 217nm (λmax of pilo) over the concentration range 2-10 µg/ml and 8-40 µg/ml for Timolol Maleate and Pilocarpine Nitrate respectively. The mean recoveries obtained for Timolol Maleate and Pilocarpine Nitrate were in the range of 98.25-101.66 % and 99.34-101.25 %. In RP-HPLC analysis is carried out using Phosphate Buffer: ACN (60:40), adjusted pH-4.5 with 1% H_3PO_4 as the mobile phase at a flow rate of 1ml/min and Purospher® RP-C18 (4.6 mm i.d×250 mm) column as stationery phase with detection wavelength of 239 nm. Linearity was obtained in the concentration range of 2-10 and 8-40 µg/ml for Timolol Maleate and Pilocarpine Nitrate respectively. The retention time was found to be 3.467 and 6.807 min for Timolol Maleate and Pilocarpine Nitrate. The mean recoveries obtained for Timolol Maleate and Pilocarpine Nitrate were in the range of 98.60-100.27% and 98.83-100.80%. The developed method has been statistically validated according to ICH guidelines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the determination of Timolol Maleate and Pilocarpine Nitrate in routine quality control analysis in bulk and its formulations.

Keywords: Timolol Maleate, Pilocarpine Nitrate, RP-HPLC, Absorbance Ratio method

PAPER-QR CODE

Corresponding Author: MS. BHOOMIKA M. PATEL

Access Online On:

www.ijprbs.com

How to Cite This Article:

Patel BM, Solanki SD; IJPRBS, 2014; Volume 3(4): 1-13

INTRODUCTION

Timolol Maleate (TIMO), chemically is (S)-1-tert-butylamino-3-(4-morpholino-1,2,5-thiadiazol-3-yloxy}propane-2-ol hydrogen maleate. It is non selective beta-adrenergic antagonist used in open-angle glaucoma. Pilocarpine Nitrate (PILO), chemically is (3S,4R)-3-ethyl-4-[(1-methyl-1H-imidazol-5-yl)methyl]oxolan-2-one. It is non selective muscarinic receptor used in treatment of chronic and acute closure angle glaucoma. TIMO and PILO is official in IP, BP and USP. These two drugs are marketed as combined dose Eye Drop formulation in the ratio of 25:100 mg (TIMO: PILO). Literature survey revealed that a number of methods have been reported for determination of TIMO and PILO individually or in combination with other drugs. but no method has been reported for this combination drugs. Objective of this study is to develop a Simple, fast and precise method for simultaneous determination of Timolol Maleate and Pilocarpine Nitrate by RP-HPLC and UV spectroscopy method.

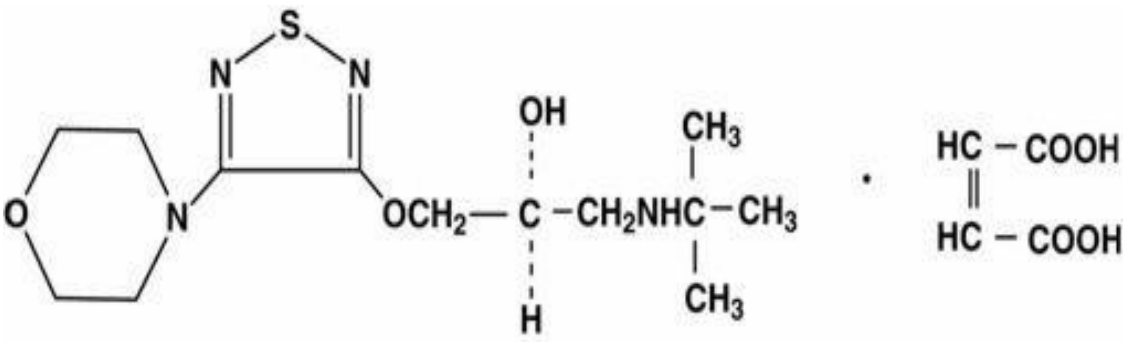

Fig1: Structure of Timolol Maleate

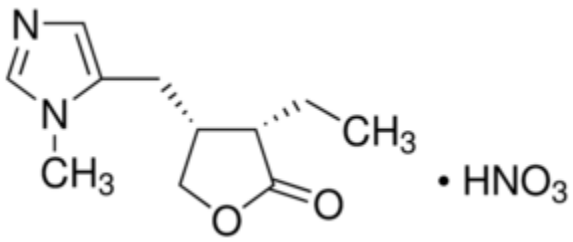

Fig2: Structure of Pilocarpine Nitrate

Materials and Methods

Instrumentation:

For UV-spectrophotometric method Double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path was used. For RP-HPLC method Young Lin, YL 9100 HPLC systems, YL9110 Quaternary solvent delivery Pump, YL9160

Photodiode array (PDA) detector, Purospher® RP-C18 Column (5µm) with Young Lin software for data processing was used.

Materials

Standard gift sample of TIMO was received from Zydus cadila healthcare Ltd. Ahmedabad, PILO as gift sample as from Intas Pharmaceuticals Ltd, Ahmedabad. Combined dose Eye Drop formulation, Sun Pharmaceutical ltd containing TIMO (25mg), PILO (100mg) was purchased from a local pharmacy Store. Methanol used for UV-spectrophotometric method was of AR grade. TEA (Merck Chemicals, India), Water (Rankem Ltd. Ahmedabad, India) used in RP-HPLC were of HPLC grade.

Procedure

Preparation of standard stock solution

Accurately weighed 10mg TIMO and 10mg PILO was transferred in to different two 10ml volumetric flask and dissolved in methanol and dilute upto the mark with methanol to give a stock solution having concentration of 1 mg/ml (1000µg/ml). Accurately measured 1ml of above two Stock solutions was transferred in to different two 10 ml volumetric flask and diluted to the mark with methanol to obtain a working standard solution (100µg/ml) of Timolol Maleate and Pilocarpine Nitrate.

Absorbance Ratio method (Method A)

In absorbance ratio method (method A), for the selection of analytical wavelength, solutions of TIMO (4 µg/ml) and PILO (16 µg/ml) were prepared separately by appropriate dilution of above standard stock solution and scanned in the spectrum mode from 200 to 400 nm. From the overlay spectra of these drugs [Figure 3], wavelengths 239 nm (isoabsorbtive point) and 217 nm (λ_{max} of PILO) were selected for analysis. The calibration curves for TIMO and PILO were prepared in the concentration range of 2-10 µg/ml and 8-40 µg/ml, respectively at the selected wavelengths. The absorbance's were measured at the selected wavelengths.. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations:

CX = [(QM – Qy) / (QX –QY)] × A1/ax1..... (3) CY = (A1/ax1) – CX ..... (4) Where,

CX and CY were the concentration of Atenolol and Chlorthalidone in sample solution respectively.

QM = (absorbance of sample solution at 217 nm) / (absorbance of sample solution at 239 nm)

QX = (absorptivity value of Timolol at 217 nm) / (absorptivity value of Timolo at 239 nm)

Qy = (absorptivity value of Pilocarpine at 217 nm) / (absorptivity value of Pilocarpine at 239 nm)

A1 was the absorbance of sample solution at 239 nm.

ax1 = absorptivity value of Timolo at 239 nm.

Validation parameter [1]

The proposed methods were validated as per ICH guidelines.

Linearity

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10µg/ml for Timolol Maleate and 8-40µg/ml for Pilocarpine Nitrate at 239nm and 217 nm for absorbance ratio method. The calibration curve of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for Timolol Maleate and Pilocarpine Nitrate were determined. (Table 3)

Precision (Repeatability)

For Repeatability, it was carried out by preparing 6 replicates of 6 same concentrations, within the linearity range and measuring the absorbance of each solution on the same day. % RSD (% relative standard deviation) was calculated. The %RSD values were found to be below 2% which indicate that the proposed methods are repeatable (Table 3).

Intermediate precision (Reproducibility)

The intermediate precision for the proposed method was determined by estimating standard solution of TIMO (4, 6, 8 µg/ml) and PILO (16, 24, 32 µg/ml) for three times on the same day (intraday) and on three different days (interday). The results are reported in terms of relative standard deviation (RSD). The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 3)

Accuracy

The accuracy of the method was determined by calculating recoveries of TIMO and PILO by the standard addition method. Known amount of standard of TIMO and PILO (80%, 100%, and 120%) were added to the sample solutions of eye drop forms. The amounts of TIMO and PILO were estimated by regression equation. The results are shown in (Table 9). The values prove that the method is accurate. (Table 1)

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of TIMO and PILO were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ data are presented in (Table 3).

Analysis of eye drop formulation

It was tested by analysis of commercially available marketed formulation. To Take a 1 ml of liquid containg 5 mg Timolol Maleate and 25 mg of Pilocarpine Nitratet was transferred to 10 ml volumetric Flask then the volume was made up to the mark with methanol to get 100 µg/ml concentration. Shaking was carried out for 5 min. then solution was filtered through whatman filter paper. From the 100µg/ml of sample solution take 1.6ml of solution and further diluted up to the mark in 10ml volumetric flask to get 16µg/ml. So the final solution was made which contains 16µg/ml Pilocarpine Nitrate and 4µg/ml Timolol Maleate both. The solution was scanned from 400-200 nm. The concentration of both TIMO and PILO were determined by measuring absorbance of sample solution at 239 nm & 217 nm and using equations (1) and (2). Amount of TIMO and PILO in mg/drop was then calculated. Results of eye drop analysis are shown in (Table 2).

RP-HPLC (Method B)

Chromatographic conditions: Preliminary studies were conducted and trails are made for the method development. Separation and analysis was carried out on Purospher® RP-C18 column (4.6 x 250mm), 5µ particle size. The optimized mobile phase consisting of Phosphate Buffer: ACN (60:40 v/v/), adjusted pH 4.5 with 1% $H_3PO_4$and filtered through 0.45 µm membrane filter using vacuum pump. Flow rate was maintained at 1 ml/min and run time for 10 min, prior to sample injection, column was saturated with mobile phase for 40 min and injection volume was 20 µl injected by auto sampler. The detection response was measured at 239 nm and maintained at ambient temperature.

Preparation of optimized mobile phase: Take one 500 ml volumetric flask, washed with distilled water and then methanol (AR Grade) then dried it in oven at 60˚C for 20-25 min. ACN and Buffer pH-4.5 filtered through 0.45µm Chrom Tech Nylon-66 filter paper. After filteration it was sonicated for 20min on ultrasonicator. pH- 4.5 adjusted with 1% H_3PO_4 in 500 ml volumetric flask.

Preparation of standard stock solution: same as UV spectroscopy method.

Validation Parameter [1]

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of Calibration curve in the range of 2-10 µg/ml and 8-40 µg/ml for TIMO and PILO Respectively. Plot the calibration curve of Area versus respective concentration and Find out correlation co-efficient and regression line equation for TIMO and PILO. (Table 7)

Precision (Repeatability)

For Repeatability, it was carried out by preparing 6 replicates of 6 same concentrations, within the linearity range and measuring the Peak area of each solution on the same day. % RSD (% relative standard deviation) was calculated. The %RSD values were found to be below 2% which indicate that the proposed methods are repeatable (Table 7).

Intermediate precision (Reproducibility)

The intermediate precision for the proposed method was determined by estimating standard solution of TIMO (4, 6, 8 µg/ml) and PILO (16, 24, 32 µg/ml) for three times on the same day (intraday) and on three different days (interday). The results are reported in terms of relative standard deviation (RSD). The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 7).

Accuracy

The accuracy of the method was determined by calculating recoveries of TIMO and PILO by the standard addition method. Known amount of standard of TIMO and PILO (80%, 100%, and 120%) were added to the sample solutions of eye drop forms. The amounts of TIMO and PILO were estimated by regression equation. The results are shown in (Table 5).

System suitability

Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated. (Table 4)

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of TIMO and PILO were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ data are presented in (Table 7).

Analysis of eye drop formulation

Same as UV Spectroscopy method. (Table 6)

RESULTS AND DISCUSSION

In **Absorbance Ratio method**, the linearity range was found to be 2-10µg/ml for TIMO and 8-40µg/ml for PILO. % Assay was found to be 99.68% and 99.87% for TIMO and PILO respectively. Percentage recovery for TIMO was 98.25-101.66%**,** while for PILO it was found to be in range of 99.64-101.25%. LOD and LOQ values were found to be and 0.09 & 0.28 for TIMO and 0.80 & 2.43 for PILO.

In **RP-HPLC** method was developed and validated. The mobile phase used was Phosphate Buffer: ACN (60:40 v/v/), adjusted pH 4.5 with 1% H_3PO_4. The retention time of TIMO and PILO was found to be 3.467 min and 6.807 min respectively. Linearity range was found to be 2-10 µg/ml for TIMO and 8-40 µg/ml for PILO. % Assay was found to be 99.50% and 98.25% for TIMO and PILO respectively. Percentage recovery for TIMO was 98.60-100.27%**,** while for PILO, it was found to be in range of 98.83-100.80 %. LOD and LOQ values were found to be for 0.084 & 0.25 TIMO and 0.70 & 2.12 for PILO.

TABLES AND FIGURES

For method A:

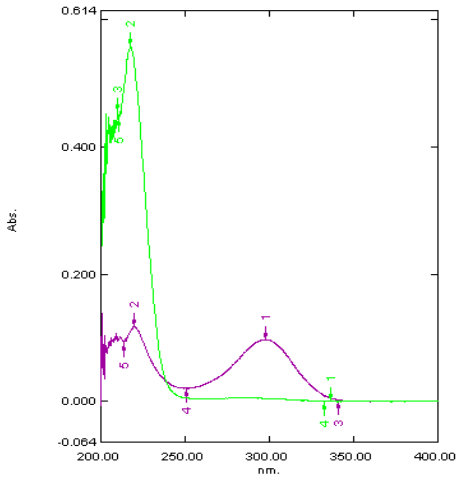

Fig3: Overlay spectra of TIMO (4µg/ml) and PILO (16 µg/ml) for Absorbance Ratio method

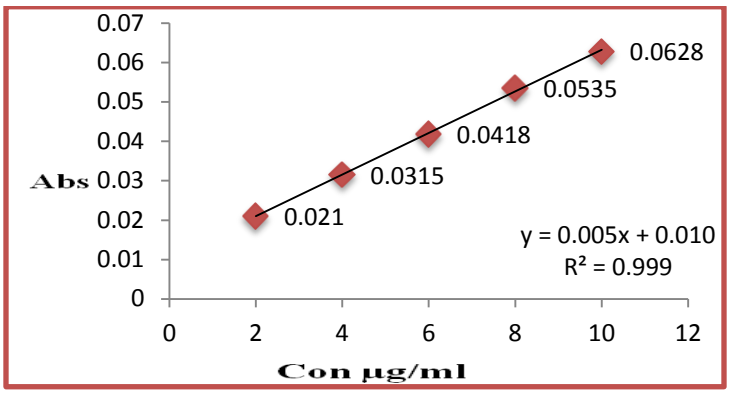

Fig4: Calibration curve of standard TIMO at 239nm

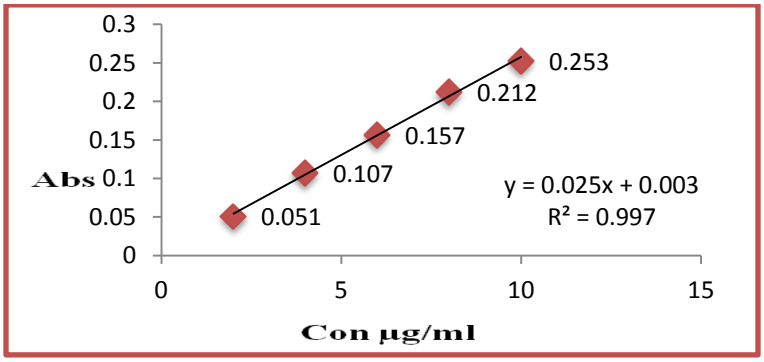

Fig5: Calibration curve of standard TIMO at 217nm

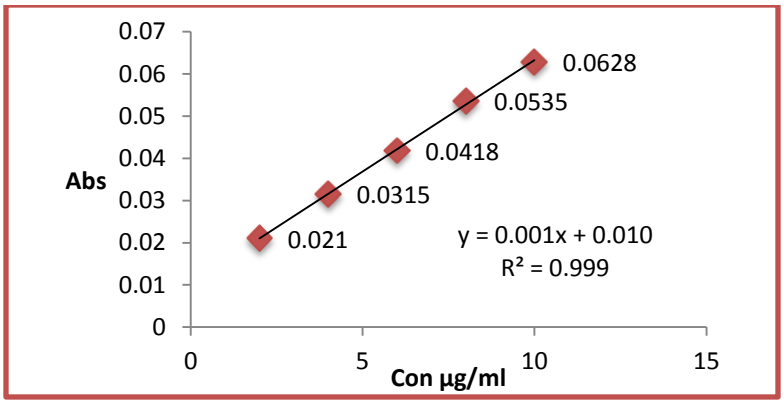

Fig6: Calibration curve of standard PILO at 239nm

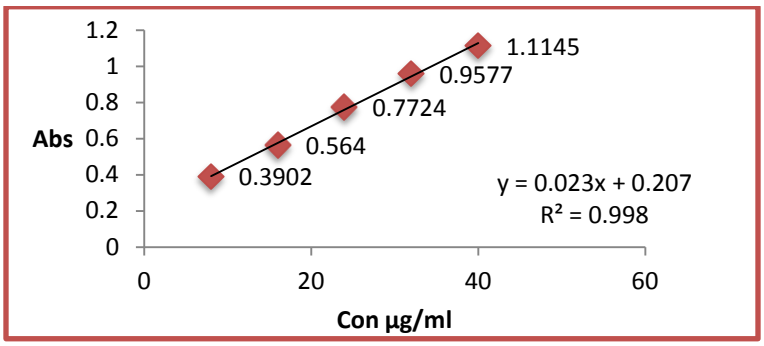

Fig7: Calibration curve of standard PILO at 217nm

Table 1: Recovery studies data.

Drugs	% Level	Amount of drug taken (µg/ml)	Amount of std. drug added (µg/ml)	Mean amount found (µg/ml).	% Recovery
	0	4	0	3.93	98.25
TIMO	80	4	3.2	7.32	101.66
	100	4	4	7.98	99.75
	120	4	4.8	8.72	99.09
	0	16	0	16.20	101.25
PILO	80	16	12.8	28.61	99.34
	100	16	16	32.27	100.84
	120	16	19.2	35.11	99.74

Table 2: Assay of Eye Drop formulation.

Tablet	Formulation	Drug	Label claim (mg)	Amount found (mg)	% Amount found
Timolet plus	Eye Drop 5 ml	Timolol Maleate	25	24.92	99.68
		Pilocarpine Nitrate	100	99.87	99.87

Table 3: Summary of validation parameter of Absorbance Ratio method.

Parameters	TIMO		PILO	
	239nm	217nm	239nm	217nm
Linearity and range(µg/ml)	2-10	2-10	8-40	8-40
Accuracy	98.25-101.66%		99.34-101.25%	

(Recovery %) (n=3)				
Precision (%RSD)				
Intra-day (n=3)	0.23-0.37	0.47-1.28	0.23-0.37	0.12-0.35
Inter-day (n=3)	0.47-0.63	0.94-1.92	0.47-0.63	0.26-0.53
Repeatability (n=6)	0.51	0.98	0.78	0.23
LOD (µg/ml)	0.09		0.80	
LOQ (µg/ml)	0.28		2.43	
Assay % (n=3)	99.68		99.87	

For method B

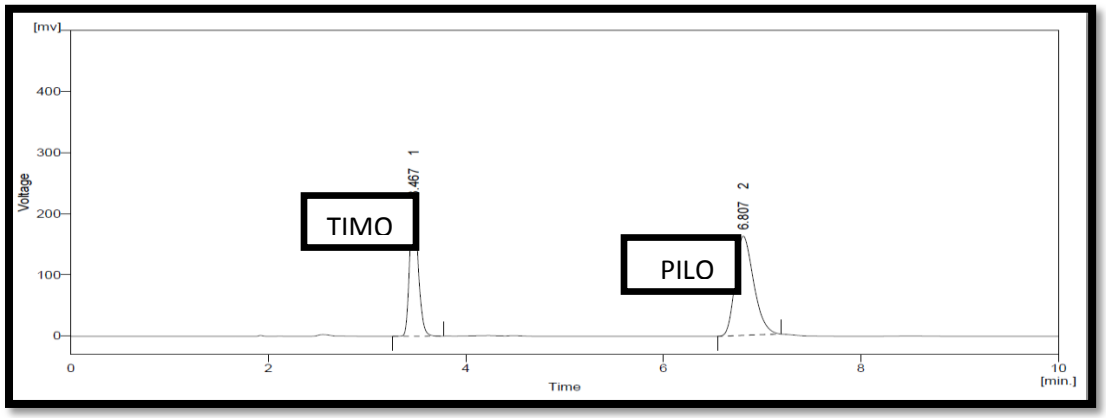

Fig 8: HPLC chromatogram of TIMO (4µg/ml) and PILO(16 µg/ml)

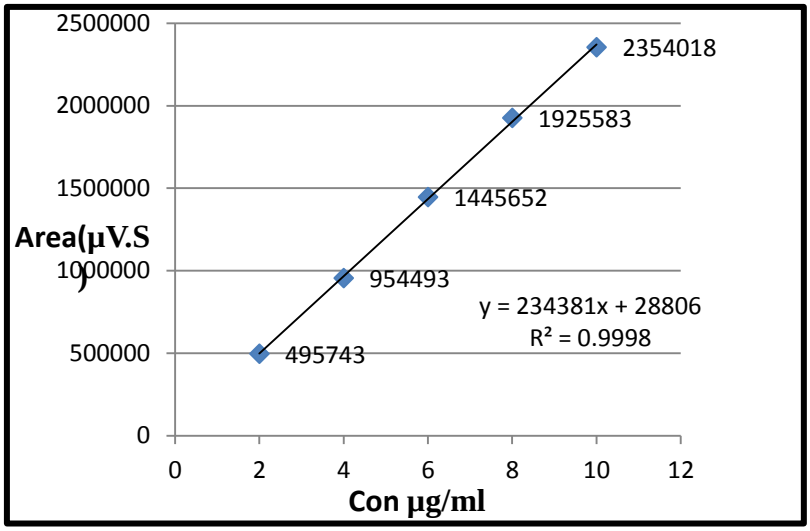

Fig 9: Calibration curve of standard TIMO

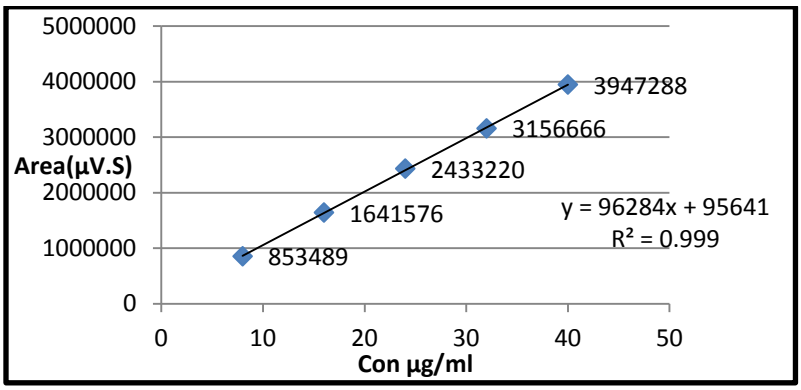

Fig 10: Calibration curve of standard PILO

Table 4: System suitability parameters of RP-HPLC method.

Sr. no.	System suitability Parameter	Observed value Specification		Specification
		TIMO	PILO	
1	Retention time (Rt)	3.467	6.807	
2	Resolution (Rs)	12.805		> 1.5
3	Theoretical plates(N)	7111	5809	> 2000
4	Asymmetric factor (A_f)	1.409	1.553	Not greater than 2.0

Table 5: Recovery studies data.

Drugs	% Level	Amount of drug taken (µg/ml)	Amount of std. drug added (µg/ml)	Mean amount found (µg/ml)	% Recovery
TIMO	80	4	3.2	7.22	100.27
	100	4	4	7.96	99.62
	120	4	4.8	8.67	98.60
PILO	80	16	12.8	28.46	98.83
	100	16	16	31.99	99.96
	120	16	19.2	35.48	100.80

Table 6: Assay of Eye Drop formulation

Tablet	Formulation	Drug	Label claim (mg)	Amount found (mg)	% Amount found
Timolet Plus 5 ml	Eye Drop	Timolol Maleate	25	24.87	99.50
		Pilocarpine Nitrate	100	98.25	98.25

Table 7: Summary of validation parameter of RP-HPLC method.

Parameters	TIMO	PILO
Linearity and Range (µg/ml)	2-10	8-40
Accuracy (Recovery %) (n=3)	98.60-100.27	98.83-100.80
Precision (%RSD)		
Intra-day (n=3)	0.16-0.48	0.46-0.74
Inter-day (n=3)	0.27-0.61	0.70-0.85
Repeatability (n=6)	0.58	0.66
LOD (µg/ml)	0.084	0.70
LOQ (µg/ml)	0.25	2.12
Assay % (n=3)	99.50	98.25

LOD = Limit of detction

LOQ = Limit of quantitation

R.S.D = Relative standard deviation

n = Number of determination

REFERENCES

1. ICH Harmonized Tripartite Guideline, "Validation of Analytical Procedure: Text and Methodology Q2 (R1)." International conference on harmonization, IFPMA Geneva, Switzerland. 2005.

2. Tripathi KD. Essential of Medical Pharmacology; 9th Edn; Jaypee Brothers medical publishers Pvt. Ltd., New Delhi, 2001, pp 113.

3. Gilman AG., Hardman JG., and Limbard LE. Goodman and Gilman's The Pharmacological Basis of Therapeutics; 10th edition; New York: McGraw Hill, 2001, pp 254, 1824, 1834.

4. Rang HP., Dale MM., and Ritter JM. Pharmacology; 6th edition; New York: Churchill Livingston, 2007, pp 324-28.

5. Goyal RK. Elements of pharmacology; 5thedition; CBS publishers, pp 123.

6. Satoskar RS. and Bhandarkar SD. Pharmacology and Pharmacotherapeutics; 20th edition; 2007, pp 280,272.

7. Richard AH. and Pamela CC. Lippincott's Itiustrated Review; 3th edition; 2008, pp 512.

8. ICH Harmonized Tripartite Guideline, Validation of analytical procedure: text and methodology Q2 (R1), Nov 2005.

9. Indian Pharmacopoeia, Government of India, ministry of health and family welfare, Ghaziabad: The Indian Pharmacopoeial commission, 2010, volume 3, pp 2224- 2226.

10. British Pharmacopoeia, London: The Department of health, British Pharmacopoeial Commission, London; 2013, volume 3, pp 2838, 3459, 3460.

11. United Pharmacopoeia 36, NF 31, United States Pharmacopoeial Convention. Inc. Rockville, 2013, volume 3, pp 5400, 5402, 5403.

12. "Timolol maleate Drug Profile", December 2013. www.drugbank.ca/Timolol maleate/DB00373.

13. "Pilocarpine nitrate Drug Profile", December 2013. www.drugbank.ca/Pilocarpine nitrate/DB01085.