



Development and validation of RP-HPLC Method for the Simultaneous Estimation of Brimonidinetartrate and Timolol maleate in Combined Dosage form

Nagaraju P*, Kiran Kumar S, Poornima Ch

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, (A.P.) India.

Received: 11 November 2013, Accepted: 27 March 2014, Published Online: 12 April 2014

Abstract

A simple, efficient, and reproducible RP-HPLC method for the simultaneous determination of Brimonidine tartrate and Timolol maleate in bulk and in pharmaceutical formulations has been developed and validated. The chromatographic analysis was performed on a Inertsil ODS hypersilC₁₈ (150 x 4.6 mm i.d, 5 μ) column in isocratic mode using Acetonitrile:0.02 M 1-octane sulfonic acid buffer (adjusted to pH 3.35 with orthophosphoric acid) in the ratio of 50:50v/v as eluent. The flow rate was 1 ml/min and eluent was detected at 285 nm. The retention time of Brimonidine tartrate and Timolol maleate were 3.017 and 5.943 min, respectively. The linear dynamic range was 2-12 μ g/ml and 5-30 μ g/ml for Brimonidine tartrate and Timolol maleate, respectively. Percentage recoveries for Brimonidine tartrate and Timolol maleate were 100.05 and 100.48 %, respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise and robust for the simultaneous determination of Brimonidine tartrate and Timolol maleate in tablet dosage forms.

Keywords: Brimonidine tartrate, Timolol maleate and Simultaneous determination

Contents

1. Introduction	58
2. Experimental	59
3. Results and discussion	60
4. Conclusion	63
5. Acknowledgement	63
6. References	63

*Corresponding author

Nagaraju P
E-mail: pappulanagaraju@gmail.com
Manuscript ID: AJCPR1983



PAPER-QR CODE

© 2014, AJCPR All Rights Reserved

1. Introduction

Brimonidine is an alpha adrenergic receptor agonist (primarily alpha-2). It is used to treat glaucoma. It acts via decreasing aqueous humor synthesis. It has a dual mechanism of action by reducing aqueous humor production and increasing uveoscleral outflow. It is used to treat raised pressure inside the eye due to open-angle glaucoma or ocular hypertension. It is used to lower the pressure inside the eye by reducing the amount of watery fluid in the eye (aqueous humour). It does this by constricting blood vessels, which reduces the amount of fluid that filters out of the vessels to form aqueous humour (Figure.1). Timolol maleate is a non-selective beta-adrenergic receptor antagonist which has been proposed as an antihypertensive, antiarrhythmic, antiangina, and antiglaucoma agent. The levo-isomer is the more active. It is used to treat open-angle and occasionally secondary glaucoma by reducing aqueous

humour production through blockage of the beta receptors on the ciliary epithelium. 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. It is also used in the treatment of migraine disorders and tremor.(Figure 2). Literature survey reveals a few spectrophotometric and bioanalytical methods for the estimation of both drugs as a single component and in combination with other drugs¹⁻²⁶. However no method has been reported for analysis of these drugs in combined dosage form. The objective of present communication is to develop simple, rapid, and precise RP-HPLC method for the estimation of Brimonidine tartrate and Timololmaleatein combined tablet dosage form.

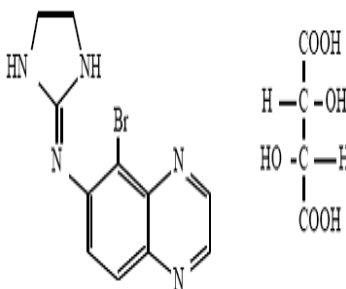


Figure 1: Structure of Brimonidine tartrate

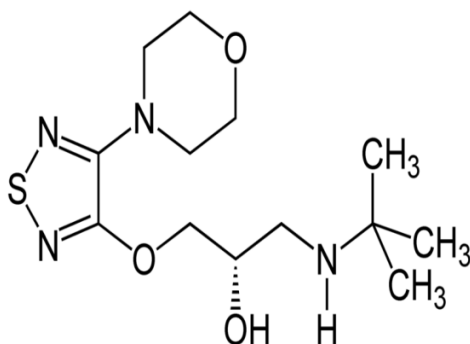


Figure 2: Structure of Timolol maleate

2. Materials and methods

Reagents and chemicals:

Brimonidine tartrate and Timolol maleate reference substances are gifted Dr. Reddy's laboratories limited, Hyderabad, India. Ophthalmic dosage forms (COMBIGAN, Allergan Pharmaceutica Pvt Ltd.) were procured from the local market, each dosage form containing 0.2 % w/v of Brimonidine tartrate and Timolol maleate of 0.5% w/v. HPLC grade water and Acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Ortho-phosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Instrumentation:

The Instrument is equipped with LC-20 AT Vp Series binary pump & variable wavelength UV-VISIBLE detector, SPD-20A. A 20µL Rheodynesyringe was used for injecting the samples. Data was analysed by using spinchrom21 CFR software. Shimadzu Prominence HPLC was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Chromatographic conditions:

Inertsil ODS hypersil C₁₈ column (150 x 4.6 mm i.d, 5µ) was used for separation. The mobile phase containing Acetonitrile:0.02 M 1-octane sulfonic acid buffer (adjusted to pH 3.35 with ortho-phosphoric acid) in the ratio of 50:50v/v was delivered at a flow rate of 1.0 ml/min with detection at wavelength 285 nm. The Injection volume was 20 µl and the analysis was performed at ambient temperature.

Standard stock solution:

Stock solutions of Brimonidine tartrate and Timolol maleate (1 mg/ml) were prepared separately using mobile phase as solvent. From the standard stock solutions, mixed standard solutions of different concentrations ranging from 2-12µg/ml Brimonidine tartrate and 5-30µg/ml of Timolol maleate were prepared by diluting with mobile phase. With

the optimized chromatographic conditions, a steady base line was recorded. Twenty micro litres of each mixed standard solution was injected six times and chromatograms were recorded. The retention time of Brimonidine tartrate and Timolol maleate were found to be 3.017 and 5.943 min, respectively. Calibration curves were constructed by plotting the average peak areas against the respective concentrations and found to be linear in the above range with the correlation coefficients (r^2) 0.999 and 0.999 for Brimonidine tartrate and Timolol maleate, respectively.

Analysis of Brimonidine tartrate and Timolol maleate in combined dosage form:

Locally available ophthalmic dosage form (COMBIGAN) contains 0.2 %w/v of Brimonidine tartrate and Timololmaleate of 0.5% w/v were taken. About 10ml of sample solution was transferred to a 100ml volumetric flask containing 50 ml of the mobile phase and the volume was made up to 100 ml with mobile phase. Then the mixture was filtered through 0.45 μ membrane filter. Above solution was further diluted and 20 μ l was then injected six times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated.

Validation^{27,28}

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Linearity:

The linearity of the method was determined at six concentration levels ranging from 2-12 μ g/ml Brimonidine tartrate and 5-30 μ g/ml of Timolol maleate. The regression equation of calibration curves were $Y = 41.35x - 4.038$ for Brimonidine tartrate and $Y = 47.28x - 15.57$ for Timolol maleate.

Accuracy:

The accuracy of the method was determined by recovery experiment. To carry out recovery studies, fixed amount of sample was taken and standard drug was added at three different levels (50, 100, 150 %). Each level was repeated three times.

Precision:

Precision was studied to find out intra and inter day variation in the proposed method at three different levels on the same day and on three different days, respectively. The %RSD was calculated for intra-day and inter-day precision was found to be less than 1%.

Limit of detection (LOD) and limit of quantitation (LOQ):

Calibration curves were prepared using concentrations in the range of 2-12 μ g/ml Brimonidine tartrate and 5-30 μ g/ml of Timolol maleate (expected detection limit range). The standard deviation of Y intercepts of regression lines were determined and kept in the following equation for the determination of LOD and LOQ. Detection limit = $3.3\sigma/S$; Quantitation limit = $10\sigma/S$; where, σ is the Standard deviation of Y intercept of regression lines and S is the slope of calibration curve. The LOD was found to be 0.43 μ g/ml for Brimonidine tartrate and 1.24 μ g/ml for Timolol maleate. Limit of quantitation was found to be 1.30 μ g/ml for Brimonidine tartrate and 3.77 μ g/ml for Timolol maleate, respectively.

Robustness:

Robustness of the method was determined by making slight changes in the composition of mobile phase ± 2 %, flow rate by ± 0.2 ml, detection wavelength by ± 2 nm and temp by $\pm 2^\circ\text{C}$. It was observed that there were no marked changes in the retention time and area of the chromatograms and the % RSD was less than 1 %, which demonstrated that the RP-HPLC method developed was robust.

Specificity:

Commonly used excipients (starch, lactose, magnesium stearate) were spiked into a pre weighed quantity of drug mixture. The chromatogram was taken by appropriate dilutions and the amount of each drug present in the sample mixture was determined.

Stability:

In order to demonstrate the stability of the both the standard and sample solutions during analysis, both the solutions were analyzed over a period of 5 hours at room temperature. The peak areas and retention time of both the drugs remained almost unchanged and no significant degradation within the indicated period.

3. Results and Discussion

The goal of this study was to develop a rapid HPLC method for analysis Brimonidine tartrate and Timolol maleate in its bulk and pharmaceutical formulations using a commonly used reverse phase C_{18} column. To develop an effective method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solution were studied. The mobile phase containing Acetonitrile:0.02 M 1-octane sulfonic acid buffer (adjusted to pH 3.35 with ortho-phosphoric acid) in the ratio of 50:50v/v with a flow rate of 1.0 ml/min was selected for analysis after preliminary tests. The retention time of Brimonidine tartrate and Timolol maleate were found to be 3.017 and 5.943 min, respectively, respectively. Resolution between Brimonidine tartrate and Timolol maleate was found to be 14.983, which indicate good separation of both the compounds.

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in Table 1. The values obtained demonstrated the suitability of the system for analysis of these drugs in combined dosage form. Typical chromatogram of Brimonidine tartrate and Timolol maleate is shown in Figure 3. The calibration curves of Brimonidine tartrate and Timolol maleate were constructed by plotting the peak area and the drug (Y-axis) to the concentration (x-axis). It was found to be linear with a correlation coefficient of 0.999 and 0.999 which shows that good correlation exists between area of the peak and the concentration (Figure 5 and 6). This method is validated for its intra-day and inter-day precision. The results obtained were in the acceptable limit (Table 1). Robustness of the method was studied by changing the chromatographic conditions slightly and the results were presented in the Table 2. Detection limit for Brimonidine tartrate and Timolol maleate was 0.43 $\mu\text{g/ml}$ and 1.23 $\mu\text{g/ml}$ and quantitation limit was 1.30 $\mu\text{g/ml}$ and 3.77 $\mu\text{g/ml}$, which suggest that a nanogram quantity of both the compounds can be estimated accurately. The RP-HPLC method developed in present study was used to quantify Brimonidine tartrate and Timolol maleate in combined dosage form and the results of assay were comparable with the corresponding labeled amounts (Table 3). High recovery values and no additional peaks, in the chromatogram indicate that the proposed procedure is free from interference of the commonly used excipients in the formulation. So the proposed method is accurate and specific and can be used for routine analysis of Brimonidine tartrate and Timolol maleate in their combined dosage form.

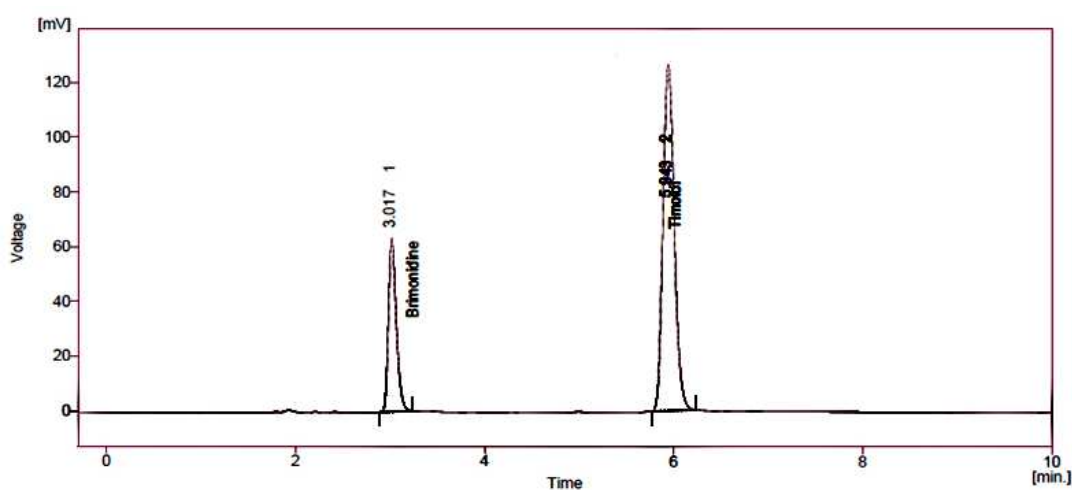


Figure 3: Typical chromatogram of Brimonidine tartrate (8 $\mu\text{g/ml}$) and Timolol maleate (20 $\mu\text{g/ml}$) in combined dosage formulation

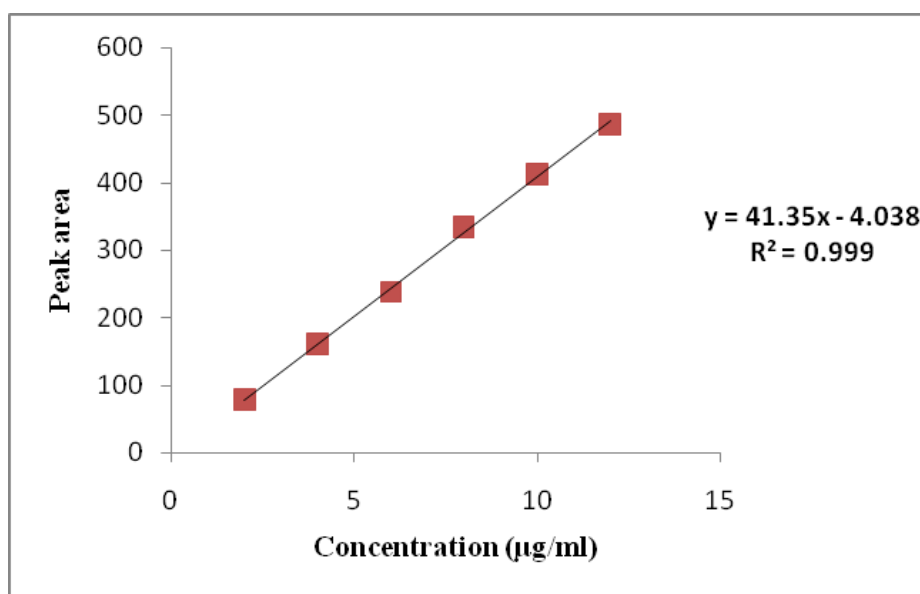


Figure 4: Calibration curve of Brionidine tartrate

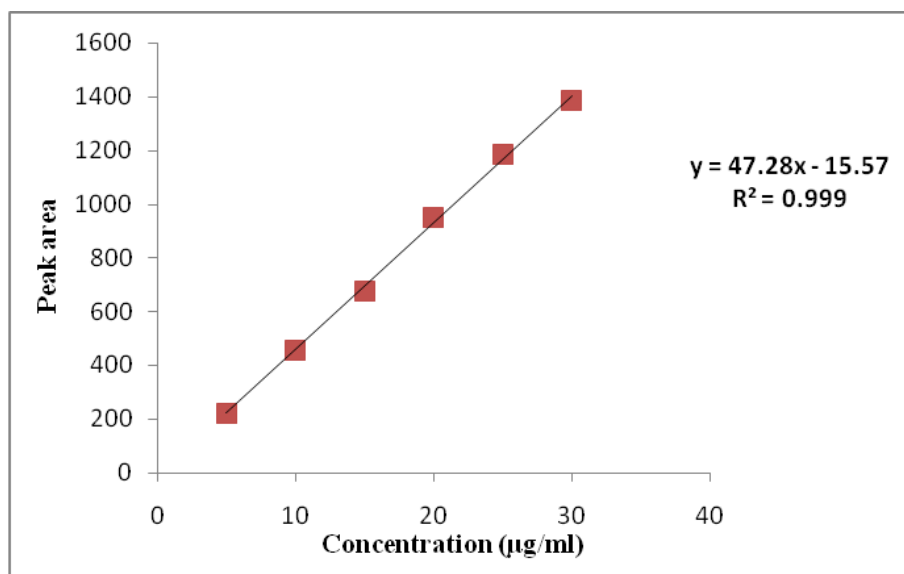


Figure 5: Calibration curve of Timolol maleate

Table 1: System Suitability and Validation Parameters

Parameters	Brimonidine tartrate	Timolol maleate
Theoretical plates	5673	11142
Resolution	-----	14.983
Tailing factor	1.32	1.25
Retention Time(min)	3.01	5.94
Linearity range(µg/ml)	2-12	5-30
Regression Equation Y=mx+c	Y = 41.35x - 4.038	Y = 47.28 x - 15.57
Slope (m)	41.35	47.28
Intercept (c)	4.038	15.57
Correlation coefficient	0.999	0.999
Percent RSD		
Intra day (n=3)	0.16	0.16
Inter day (n=3)	0.09	0.04
LOD (µg/ml)	0.43	1.24
LOQ(µg/ml)	1.30	3.77

*Average of six determinations, Y-Area under the curve and x is the concentration, RSD-Relative standard deviation, LOD-Limit of detection, LOQ-Limit of quantitation

Table 2: Robustness data

Parameter	Proposed	Variation	Brimonidine tartrate	Timolol maleate
			% RSD*	
Wavelength (nm)	285	287	0.087	0.171
		283	0.068	0.165
Mobile phase	50:50v/v	48:52	0.129	0.175
		52:48	0.183	0.260
Flow rate (ml/min)	1 ml/min	1.2	0.164	0.101
		0.8	0.154	0.239
Temperature	30°C	28	0.100	0.078
		32	0.120	0.053

*%RSD –Percent Relative Standard Deviation (Six determinations)

Table 3: Results of the recovery studies

Drug	Recovery Level	Amount added (µg/ml)	Amount recovered* (µg/ml)	%Recovery±SD*	%RSD*
Brimonidine tartrate	50%	2	2.04	102.16±0.01	0.74
	100%	4	4.01	100.33±0.01	0.14
	150%	6	5.97	99.50±0.02	0.44
Timolol maleate	50%	5	4.94	98.86±0.04	0.81
	100%	10	10.01	100.06±0.01	0.15
	150%	15	14.96	99.75±0.03	0.25

* Mean of three replicates, SD-Standard deviation, %RSD- Relative standard deviation

Table 4: Results of analysis of commercial formulation

Drug	Amount mg/tablet		%RSD	% Drug estimated
	Label claim	Amount found±SD		
Brimonidine tartrate	2	2.01±0.38	0.11	100.50
Timolol maleate	5	5.02±0.17	0.16	100.42

*Average of six determinations, SD-Standard deviation

Table and figure titles and legends:

Table 1: System Suitability and Validation Parameters

*Average of six determinations, Y-Area under the curve and x is the concentration, RSD-Relative standard deviation, LOD-Limit of detection, LOQ-Limit of quantitation

Table 2: Robustness data

*%RSD –Percent Relative Standard Deviation (Six determinations)

Table 3: Results of analysis of formulation and recovery study

*Average of six determinations, SD-Standard deviation

Figure 1: Structure of Brimonidine tartrate

Figure 2: Structure of Timolol maleate

Figure 3: Typical chromatogram of Brimonidine tartrate (8 µg/ml) and Timolol maleate (20 µg/ml) in combined dosage formulation

Figure 4: Calibration curve of Brimonidine tartrate

Figure 5: Calibration curve of Timolol maleate

4. Conclusion

. Proposed study describes a new RP-HPLC method for the estimation Brimonidine tartrate and Timololmaleate in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise.

5. Acknowledgement

Authors wish to thank Dr. Reddy's laboratories limited, Hyderabad, for providing the gift samples of Brimonidine tartrate and Timololmaleate for this work. We are also thankful to the management of Hindu College of Pharmacy for providing the required facilities and for their constant encouragement.

6. Reference

1. PrakashBhagav, PandurangDeshpande, SaurabhPandey, SajeevChandran. Development and validation of stability indicating UV spectrophotometric method for the estimation of brimonidine tartrate in pure form, formulations and preformulation studies. Der Pharmacia Lettre. 2010; 2(3):106-122.
2. Pritamjain, Rahul khatal, Sanjay surana. Development and validation of first order derivative UV-spectrophotometric method for determination of brimonidine tartrate in bulk and in formulation. Asian J Phar Biol Res.2011; 1(3):323-329.
3. Shirke RR, Pai N.RP-HPLC determination of brimonidine tartrate in brimonidine tartrate eye drops. Indian drugs. 2002; 39(9):484-486.
4. MahajanAnand, Athensia Fonseca, Gandhi Santosh V, DeshpandePadmanabh B. Development and validation of High Performance Thin Layer Chromatographic method for estimation of brimonidine tartrate as bulk drug and in ophthalmic solutions. International Journal of PharmTech Research. 2010; 2(3):1376-1379.

5. Manohar C Sonanis, Rajput AP. Development and validation of a new stability indicating analytical method for the determination of related components of brimonidine tartrate in drug substances and drug product using UPLC. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011; 3(1):145-150.
6. HafsaMohammedi, SomashekarShyale, Shanta Kumar SM. Physico-chemical characterization, UV spectrophotometric method development and validation studies of timolol maleate. *International Journal of Pharmaceutical Sciences Review and Research*. 2011; 6(2):163-166.
7. Maria Ines RM Santoro, Hyun Sun Cho, Erika RM Kedor-Hackmann. First-Derivative Spectrophotometric assay of timolol maleate in ophthalmic solutions. *Analytical Letters*.1996; 29(5):755-762.
8. QIAN Hou-Hai, LIANG Gui-Jian, ZHU Li, LIU Guo-Qi, SUN Shu-Juan, GU Xin. HPLC determination of timolol maleate eye drops. *Chinese Journal of Pharmaceuticals*. 1995; 05:56-58.
9. Kulkarni SP, Amin PD. Stability indicating HPTLC determination of timolol maleate as bulk drug and in pharmaceutical preparations. *J Pharm Biomed Anal*. 2000;23(6):983-987.
10. Ali F. Al-Ghamdi. Stripping Voltammetric determination of timolol drug in pharmaceuticals and biological fluids. *American Journal of Analytical Chemistry*. 2011; 2(2):174-181.
11. Pritam S Jain, Rahul N Khatal, Hardik N Jivani, Sanjay J Surana. Development and validation of TLC-densitometry method for simultaneous estimation of brimonidine tartrate and timolol maleate in bulk and pharmaceutical dosage form. *Chromatography Separation Techniques*. 2011; 2(3):2-5.
12. ArunPhogat, MurugesanSenthil Kumar, NanjaianMahadevan. Simultaneous estimation of brimonidine tartrate and timolol maleate in nanoparticle suspension by RP-HPLC. *International Journal of Recent Advances in Pharmaceutical Research*. 2011; 3:31-36.
13. NevinErk. Simultaneous determination of dorzolamide HCL and timolol maleate in eye drops by two different spectroscopic methods. *Journal of Pharmaceutical and Biomedical Analysis*. 2002; 28(2):391-397.
14. Manjunatha KM, Kulkarni GT, Mruthyunjaya JH. Spectrophotometric determination of combined dosage form of matrix ocular inserts containing dorzolamide hydrochloride and timolol maleate. *Inventi Rapid: Pharm Analysis & Quality Assurance [Inventi:ppaqa/219/11]*. 2011 Aug [cited 2013 Sep 10]; 2011:48-48. Available From <http://www.inventi.in/Article/ppaqa/219/11.aspx>
15. Mathrusri Annapurna M, Narendra A, Deepika D. Development and validation of RP-HPLC method for simultaneous determination of dorzolamide and timolol maleate in pharmaceutical dosage forms. *Journal of Drug Delivery and Therapeutics*. 2012; 2(2):81-87.
16. Wanare RS, Kabra AO, Deshmukh A, Aher N. Simultaneous estimation of dorzolamide hydrochloride and timolol maleate in eye drops by a RP-HPLC method. *International Journal of Comprehensive Pharmacy*. 2012; 3(02):1-4.
17. Nagori BP, AmitMaru, PankajMuysuni, Subhash Gupta. Method development and its validation for simultaneous estimation of timolol maleate and dorzolamidehydrochloride in as API and in ophthalmic solution dosage form by RP-HPLC. *J. Chem. Pharm. Res*. 2011; 3(4):866-874.
18. Shadoul WA, Gad Kariem EA, Adam ME, Ibrahim KEE. Simultaneous determination of dorzolamide hydrochloride and timolol maleate in ophthalmic solutions using HPLC. *Elixir Pharmacy*. 2011; 38:4060-4063.
19. Erk N. Rapid and sensitive HPLC method for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in eye drops with diode-array and UV detection. *Pharmazie*. 2003; 58(7):491-3.
20. Nitish Sharma, Surendra Singh Rao, Malleswara Reddy A. A novel and rapid validated stability-indicating UPLC method of related substances for dorzolamide hydrochloride and timolol maleate in ophthalmic dosage form. *Journal of Chromatographic Science*. 2011; 50(9):745-755.
21. Nejadi R, Kazemipour M, Ansari M, Mehdipour M, Ahmadi R. Simultaneous determination of timolol maleate and latanoprost tartrate in ophthalmic preparations by derivative spectrophotometry. *Research in Pharmaceutical Sciences*, 2012;7(5):667-670.
22. Rele RV, Mhatre VV, Parab JM, Warkar CB. Simultaneous RP-HPLC determination of latanoprost and timolol maleate in combined pharmaceutical dosage form. *J. Chem. Pharm. Res*. 2011; 3(1):138-144.
23. AgarwalAnkit, Tiwari Sunil, NagariyaKashyap. Method development and its validation for quantitative simultaneous determination of latanoprost, timolol and benzalkonium chloride in ophthalmic solution by RP-HPLC. *Journal of Drug Delivery and Therapeutics*. 2013; 3(2):26-30.
24. Maria L Satuf, Juan C Robles, Hector C Goicoechea, Alejandro C Olivieri. Simultaneous determination of timolol maleate and pilocarpine hydrochloride in ophthalmic solutions by First Derivative UV Spectrophotometry and PLS-1 Multivariate Calibration. *Analytical Letters*. 1999; 32(10):2019-2033.
25. Ayad, Magda M, Shalaby, et al. Spectrophotometric methods for determination of enalapril and timolol in bulk and in drug formulations. *Analytical and Bioanalytical Chemistry*. 2003; 375(4):556-559.
26. Jigar Mehta, Vipul Patel, NayanKshatri, NiranjanVyas. A versatile LC method for the simultaneous quantification of latanoprost, timolol and benzalkonium chloride and related substances in the presence of their degradation products in ophthalmic solution. *Analytical Methods*. 2010; 2(10):1737-1744.
27. ICH, Q2A. Text on validation of analytical procedures, International Conference on Harmonization 1994.
28. ICH, Q3B. Validation of analytical procedures: methodology, International Conference on Harmonization 1996.