Global Scientific JOURNALS

GSJ: Volume 9, Issue 3, March 2021, Online: ISSN 2320-9186

www.globalscientificjournal.com

Evaluation by analytical method of content uniformity of marketed anti-glaucoma eye drops

Ashish Dubey*[1], Thirumurthy Velpandian[2], Anil kumar[3], Km Uma[3], Sachin Kumar*[1]

[*1] N. K. B. R College of Pharmacy and Research Center, Meerut-250002, Uttar Pradesh, India,

[2]Ocular Pharmacology and Pharmacy division, Dr. R. P. Center, AIIMS, New Delhi 110029,

[3]Assistant Professor, Department of Pharmacy, Monad University, N.H. 9, Delhi Hapur Road, Village & Post Kastla, Kasmabad, P.O Pilkhuwa - 245304, Dist. Hapur (U.P.), India.

***Address for correspondence:** N. K. B. R College of Pharmacy and Research Center, Uttar Pradesh, India,
Contact No: 08527206709
E-mail:- ashishdubey775@gmail.com

ABSTRACT

Evaluation of content uniformity of marketed anti-glaucoma eye drops by analytical method.

The research work emphasis the concentration of active ingredients of anti-glaucoma drug in generic and non-generic formulation. The model drugs are brimonidine tartrate and timolol used in this study.

The concentration of brimonidine tartrate and timolol as active ingredient were compared in different generic formulation using liquid chromatography with mass spectrometry (LC/MS-MS).

Brand name formulations like brimonidine tartrate and timolol contained active ingredients and Concentrations that were generally in claim with their package inserts. A maximum generic eye drops contained brimonidine tartrate and timolol were in the range as mentioned in the labeled values. There were no significant differences of active ingredients contents in the intraday and interlay studies.

Conclusion: Different marketed eye drops explored the specified ranges of active ingredients as that of the labeled value in generic formulations that may not lead to influence of clinical efficacy.

Key words: Marketed Anti-glaucoma, analytical method, chromatographic, Formulation.

INTRODUCTION:

In early civilization, vision and eye had a unique place in the growth. The significance of ophthalmic eye paint and ointments was specially signified in the Egyptian cultureto frighten mosquitoes and insects that have been attributed to a number of eye problems. Egyptian practitioner brought a unique kit featuring black kohl. The actual composition of kohl is burnt almonds, lead, chrysocolla crushed stibnite, oxidized ochre, copper, and ash malachite. This was reported by French researchers in 2010. Siddha and Ayurveda medical system arise from distinct part of India, reported in various medicine for ophthalmic and in early Indian Ayurvedic literature, Shalakya Tantra was alluded to as Ashtang Aurveda primarily for the treatment, treatment and recovery of all disorders of the eyes, nose, ear and mouth. Global eye drops and lubricant industry value is $15,587 million in the year 2017 and is expected to hit $22,625 million by the year 2015. Increasing at the CAGR by 4.7% from year 2018 to 2025. The global market for eye drops is divided by type, use, area, by type of market is classified into antibiotics, artificial tears, and others are dependent on usage, The industry is categorized into eye disorders, vision care, eye disorders divisions identified as dry eye, Glaucoma, conjunctivitis, etc. Incisional surgery, Laser therapy includes selective laser Trabeculoplasty and Argon laser Trabeculoplasty, both procedures helping to decreases the IOP through enhancing the output of humor, across the Trabecular meshwork. The motive of the studyistoassess the uniformity of content of marketed anti-glaucoma eye drops via using LC-MS/MS. Method Through this we can find out the actual percentage of drug content present in theformulationsorclaimed percentage by the manufacturer company. We can also prevent substandard of drugs to patient safety and healthy medicines, which are available in market. So Ipurchased randomly these Anti-glaucoma eyes drops from market for quantification or content analysis. Followed byTheUnited State Pharmacopoeia USP<797> guidelines 2012 and Indian Pharmacopeia (IP), standard Quality control guidelines of ophthalmic preparation, such as pH, Osmolarity, Viscosity, content uniformity,

Materials and Methods:

Materials:

Chemicals and Reagents–

Pure reference standard of Brimonidine Tartrate (BRT) was procured as gift samples from Enaltec, (Navi Mumbai) India. and Timolol Maleate was purchased from Nanjing Chemlin Chemical Industry Co, Ltd, Nanjing, China. Sulfadimethoxine (SDM) was procured from Sigma Aldrich, st Louis, Missouri, USA. LC-MSgrade actonitrile (ACN) formic acid (FA) and methanol were procured from Merck (Darmstadt, Germany). Ultra purified water (18.2 Ω) was purified using a Milli-Q purification system (Millipore corp, Bedford, MA, USA).All other chemicals and solvent were of the highest analytical grades available and marketed anti-glaucoma eye drops were procured from local pharmacies (New Delhi).

Instrumentation and software–

LC-ESI-MS/MS experiments were performed using a triple quadruple Tandem mass spectrometer (4000 Q-Trap, AB Sciex, Foster City, CA, USA) coupled with HPLC system (Agilent Technologies, 1260 infinity, Santa Clara, CA, USA) consisted of quaternary pump (G1311C), multisampler (G7167A),thermos tatted column compartment (G1316A) with variable wavelength UV detector (G1314F) and online degasser. All the parameters of tandem mass spectrometer and HPLC were controlled by Analyst software, version 1.5.2 (AB Sciex, Foster City, CA, USA) and open AB control panel software (Agilent Technologies, 1260 infinity, Santa Clara, CA,USA) respectively.

Optimization of liquid chromatographic conditions-

Analytical separation of Brimonidine Tartrate (BRT), Timolol Maleate, and Sulfadimethoxine (SDM) as internal standard was optimized using reverse phase chromatographic conditions. Liquid Chromatography was performed using Reprospher100, Phenyl-Hexyl column (50 × 2 mm), 3 µm, Dr. Maisch HPLC Gmbh, Ammerbuch, Germany) using mobile phases combination consisting of 18.2MΩ ultrapure water with 0.1% FA(A), Pure Methanol (B),Acetonitrile with 0.1% FA (C),10mM Ammonium formate with 0.1% FA (D).pumped at the rate of 0.2 mL/min. Analytes were eluted using isocratic conditions at ratio 45:10:30:15 of A:B:C:D (0-5min). Total

run time of the method of 5 min. The temperature of auto sampler try and the column oven were maintained at 25º C. Samples were injected at the volume of 5µL for analysis.

Optimization of mass spectrometric detection-

Mass spectrometer was operated with electro spray ionization (ESI) source in positive ion mode using turbo ion spray source (AB Sciex, Foster City, CA, USA), Full scan mass spectra (Q1 MS parent ion),enhanced resolution (ER) and fragment ion scan spectra (MS2 product ion) were acquired by flow infusion analysis (FIA), Compound dependent and source dependent parameters were manually optimized by infusing individual standard solution at 100 µg/ml into the ion source of the mass spectrometer at a flow rate of 10µL using a Harvard pump (Harvard Company, Reno, NV, USA) connected with a Hamilton syringe (Holliston, MA, USA). Multiple reactions monitoring (MRM) mode was used for quantification of Brimonidine Tartrate and Timolol Maleate using SDM as an internal standard. Quantification of Brimonidine Tartrate and Timolol maleate was performed using precursor to product ion transition of m/z 292.1/212.2 (MS2) and 317.2/261.3, the precursor to product ion transition of m/z 311.0/156.6 was used for internal standard (SDM). Compound dependent parameters such as Declustering potential (DP), Entrance potential (EP), Collision energy (CE) and Cell exit potential (CXP) for mass transitions of Brimonidine Tartrate were optimized as 107,10, 33, 5 volts and 107, 10, 42, 5 volts respectively. Compound dependent parameters (DP, EP, CE, CXP) for SDM (IS) was optimized as 70, 10, 28, 7 volts respectively. Source dependent parameters were optimized and maintained as curtain gas 30 psi, Collision ally activated dissociation CAD gas- 10 psi, ion spray voltages- 5000 eV, temperature- 500º C, gas 1- 30 psi, and gas2- 60 psi. Dwell time for Brimonidine, Timolol, and SDM were kept at 100 ms, respectively.

Preparation of standard stock solution-

Standard stock solutions of Brimonidine Tartrate, Timolol Maleate, and SDM (IS) were prepared at concentration of 1mg/ml by dissolving accurately weighed in 18.2MΩ resistant Milli-Q water. Working standard drugs solutions were prepared by taking equivalent weight and dissolved in Milli-Q water at concentration of 100 µg /ml in 10mL volumetric flask. These stock solutions were kept at -20 º C and used for further experiments

Methods:

Sample preparation protocol-

For the analysis of marketed eye drops sample which are already procured. firstly prepared Extraction solvent (ES) 50% ACN with 0.1%FA+10ng/mL SDM for dilution. I was taken 12 marketed eye drops formulationswhichbelongs to different -2 Mfg Company with claimed percentage, there are two type of marketed formulation – individual formulation and in combination-

Formulation no **– F1, F2, F3**- **Timolol maleate** (0.5%) 5mg/mL

↓ **(**10µl+990 µL Extraction solvent)

50µg/mL

↓ **(**10µl+990µL Extraction solvent)

500ng/mL

↓ **(**10µl+990µL Extraction solvent)

5ng/mL (Final concentration)

- Formulation no **– F4, F5, F6, F7- Brimonidine tartrate-** (0.1%),1mg/mL (Stock vials)
  (0.15%), 1.5mg/mL
  (0.2%), 2mg/mL

↓ **(**10µl+990µl Extraction solvent)

1µg/mL, 15µg/mL, 20µg/mL

↓ **(**10µl+990µlExtraction solvent)

100ng/mL, 150ng/mL, 200ng/mL

↓ **(**10µl+990µl Extraction solvent**)**

10ng/mL, 15 µg /mL, 20ng/mL (final concentration)

Formulation no **– F8, F9, F10, F11, F12-(Dilutions)**

Timolol maleate -5mg**,**5 mg, 5mg, 5mg, 5%w/v

Brimonidine tartrate- 2mg,1.5 mg, 2mg, 2mg, 0.2%w/v

(10µl+990µl(Extraction solvent)

Timolol maleate -50µg, 50µg, 50µg, 50µg, 50µg

Brimonidine tartrate-20µg, 15 µg, 20µg, 20µg, 20µg

(10µl+990µl Extraction solvent)

Timolol maleate -500µg, 500µg, 500µg, 500µg, 500µg

Brimonidine tartrate-200µg, 150 µg, 200µg, 200µg, 200µg

(10µl+990µl Extraction solvent)

Timolol maleate – 5ng, 5ng, 5ng, 5ng, 5ng

Brimonidine tartrate- 20ng, 15 ng, 20ng, 20mg, 20ng

(Final concentration)

Table 4 Compound related parameters

S.N0	Q1 Mass (Da)	Q2 Mass(Da)	Dwell time (msec)	ID	DP (volts)	CE (volts)	CXP (volts)
1	292	212	100	Brimonidine -1	107	33	5
2	292	249	100	Brimonidine-2	107	42	5
3	317	261	100	Timolol-1	69	23	5
4	317	244	100	Timolol-2	69	30	5
5	311	156	100	SDM-IS	70	28	7

Table-5: Source related parameters

S.No.	Mass Parameter	Values
1.	Curtain Gas (CUR)	30
2.	Collision Gas (CAD)	10
3.	Ion Spray voltage(IS)	5000
4.	Temperature (TEM)	500
5.	Ion Source Gas 1(GS1)	30
6.	Ion Source Gas 2(GS 2)	60
7.	Interface heater	ON

Optimized Liquid Chromatography related parameters

Table-6: Optimized conditions for the developed method

Time (min)	A%	B%	C%	D%	Flow
0	**45**	**10**	**30**	**15**	**0.2 ml/min**
5	**45**	**10**	**30**	**15**	**0.2 ml/min**

Where,

Solvent A- Milli-Q+0.1%FA **Solvent C**- ACN+0.1%FA

Solvent B- Methanol (Pure) **Solvent D**- 10mM Ammonium Format+0.1%FA

Extraction solvent (ES) - 50%ACNwith 0.1%FA+10ng/mL (SDM)

Column- Phenyl-Hexyl column (50 × 2 mm), particle size 3 µm

Method – Gradient

Flow rate – 0.2 mL/min

Injection volume – 5µl

Run time – 5 min.

Results and Discussion:

Fourier transforms infrared Spectroscopy-

Infrared (IR) spectroscopy study was performed for identification of drug or drug present in the drug product, identify test should be specific for drug substance. The structural analysis of the samples of Brimonidine Tartrate (BRT) and Timolol maleate by using Fourier transformed infrared spectrophotometer, (Perkin Elmer, USA). The potassium bromide (spectrometric grade dried KBr) taken 98% and drug sample 2%. Mixture was ground into a fine powder using mortar pestle and compressed into KBr disc under a hydraulic press at 10,000 psi. and released pressure gently after 10 sec hold time. Firstly, scanned blank background before the scanning of drug sample. Each Kbr disc was scanned 32 times at 4 mm s^{-1} at a resolution of 2 cm^{-1} over a wave number region of 4000-400 cm^{-1} and characteristic bands were recorded.

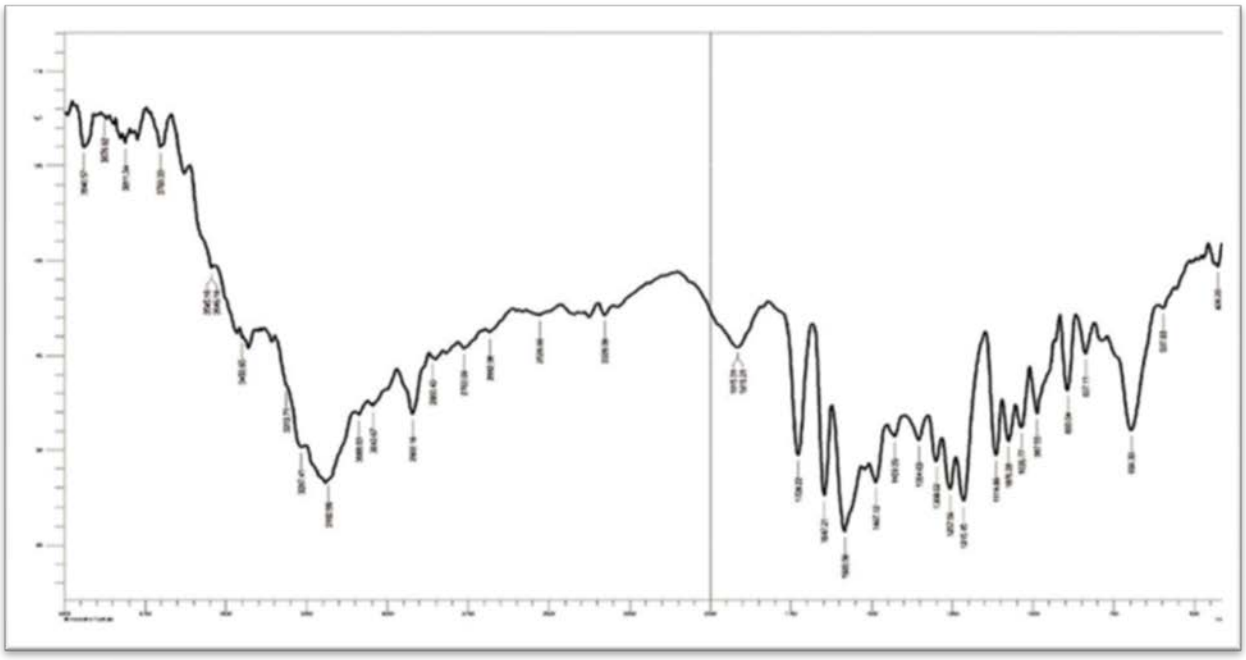

Figure 17: FTIR Spectra of Brimonidine Tartarte of pure powder

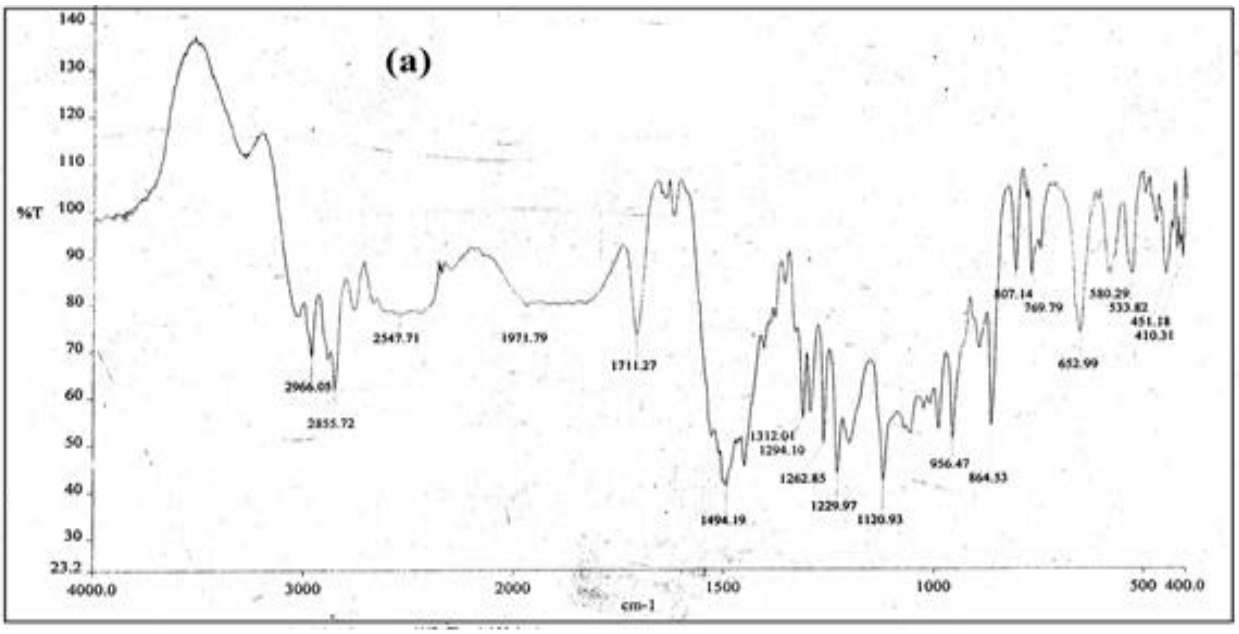

Figure 18: FTIR Spectra of Timolol Maleate pure powder

Ultraviolet (UV) Spectrophotometrically Analysis-

UV-visible spectrophotometer was used for confirmation of purity and absorption maxima of the drug. The concentration of standard stock solution of Brimonidine Tartrate (BRT) and Timolol Maleate in Ultra purified water (18.2 Ω) (100 µg/mL) 10 mL in volumetric flask individually. From the stock solution (100µg/mL) taken 100 µl then add 900µl (50% ACN + 0.1%FA) 10µg/mL individual concentration of both compound for the purpose of scanned from the range of (λ),max 200-400 nm against blank using the UV-visible spectrophotometer.

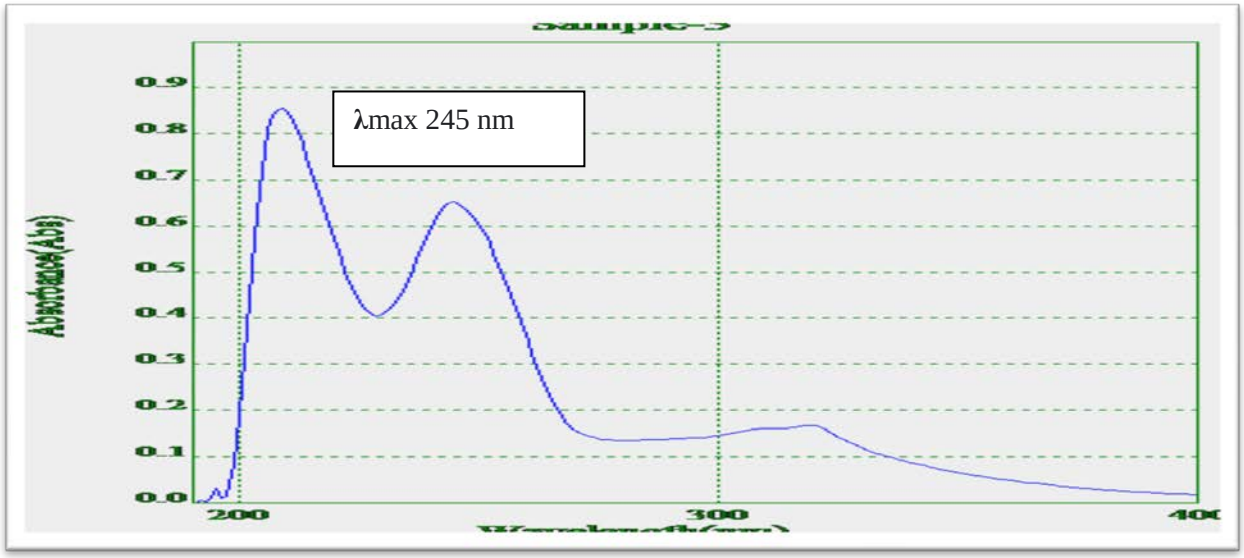

Figure 19: The peak of Brimonidine Tartrate is found to be 245 nm

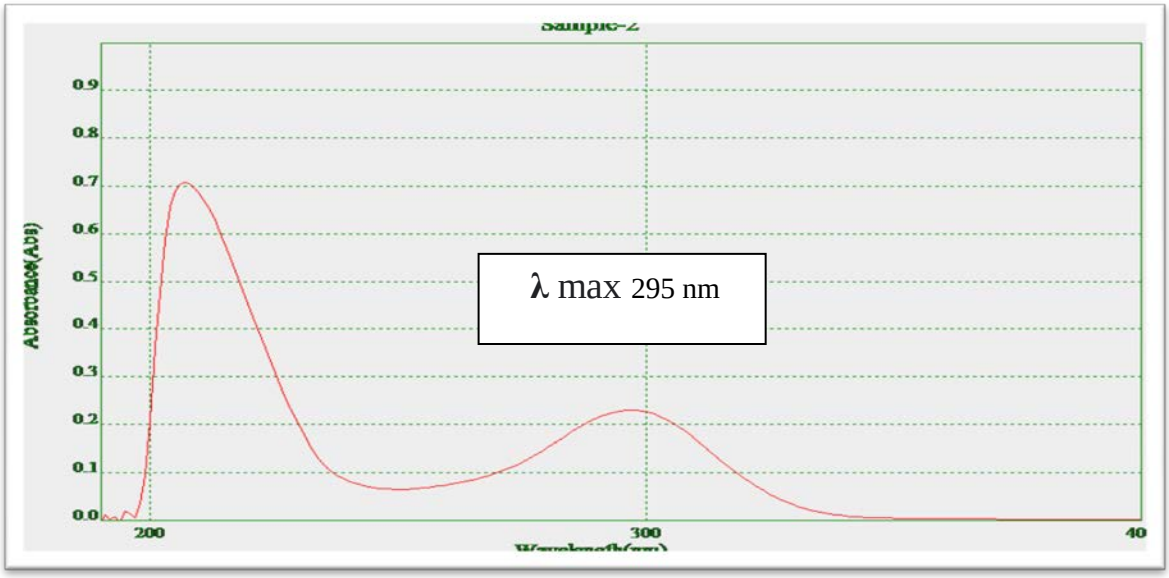

Figure 20: The peak of Timolol Maleate is found to be 295 nm

2OPTIMIZATION OF MASS SPECTROMETRY DETECTION-

Mass spectrometry parameters namely compound related parameters (DP, EP, CE, CXP) and source related parameters (Curtain gas, Collision gas, Ion spray voltage, Temperature, Ion source gas-1, Ion source gas-2, Interface heater) were carefully optimized to attain adequate abundance of Brimonidine Tartarte and Timolol Maleate using flow infusion analysis .ESI ionization in positive ion mode was chosen due to maximum signal intensity was found than negative ion mode. Due to higher specificity and fragmentation pattern obtained, ESI in positive ion mode was used for the method development.

Optimized Mass Parameters of Brimonidine Tartrate and Timolol Maleate

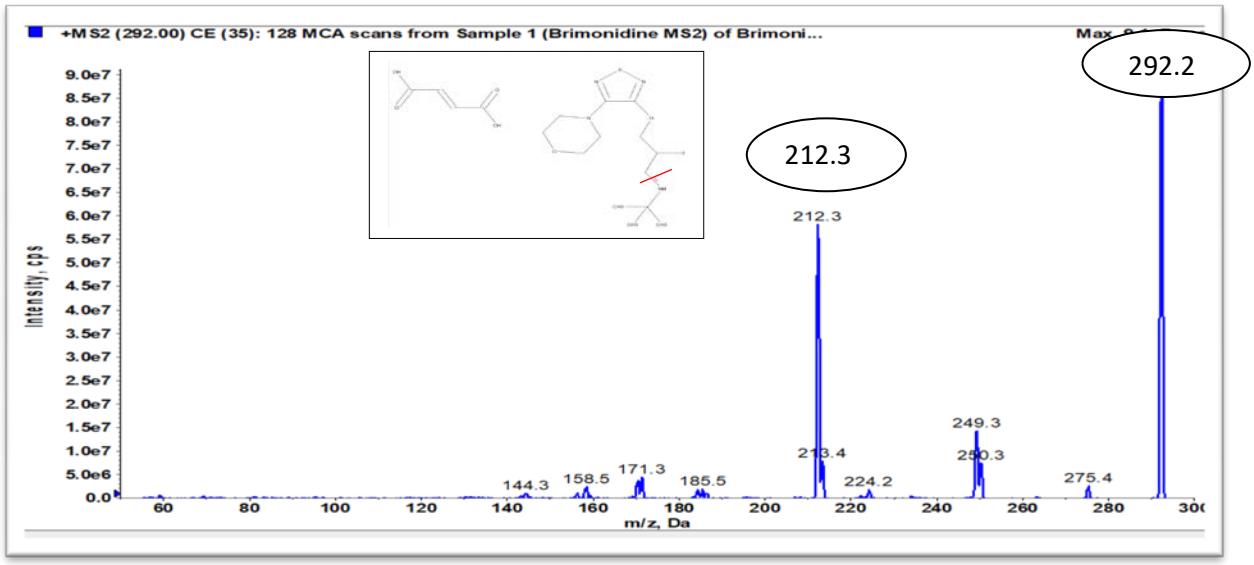

Figure 21: MS-2 Scan of brimonidine tartrate (daughter ions)

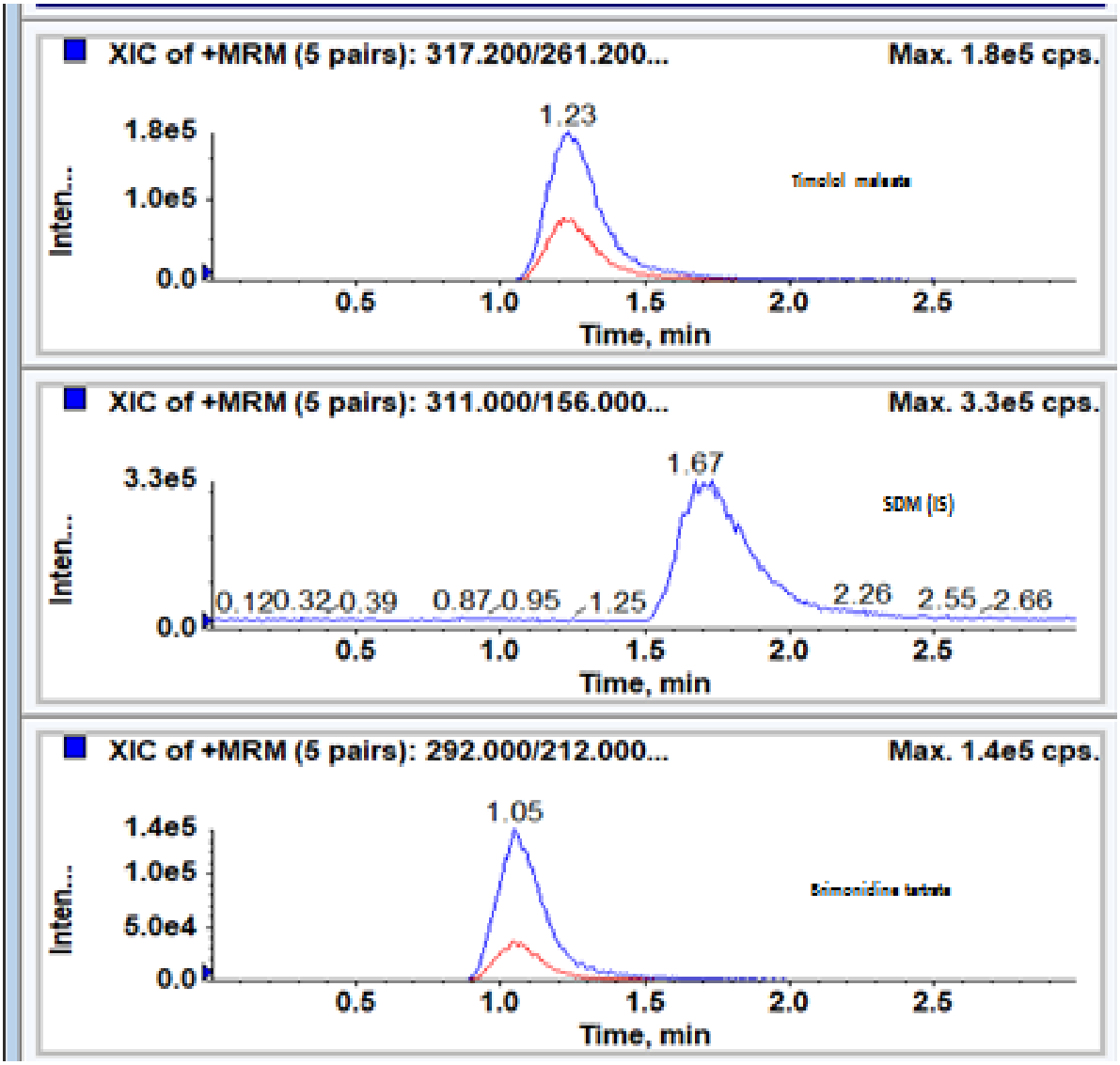

Figure 24: Separated peaks of compounds at different time points

Calibration curve and linearity-

Analyte peak area (y –axis) to IS was plotted against spiked concentration (x-axis) of the analyte to build the standard plot for Brimonidine tartrate and timolol maleate. Six point standard curves for brimonidine tartrate and timolol maleate was plotted. The standard curve was found to be linear over the range of 2.5-80 µg/mL with correlation coefficients (R^2) value of 0.9994. and 0.625-20 µg/mL. With correlation coefficient (R^2) value of 0.9996. Calibration plot of Brimonidine Tartrate shows below

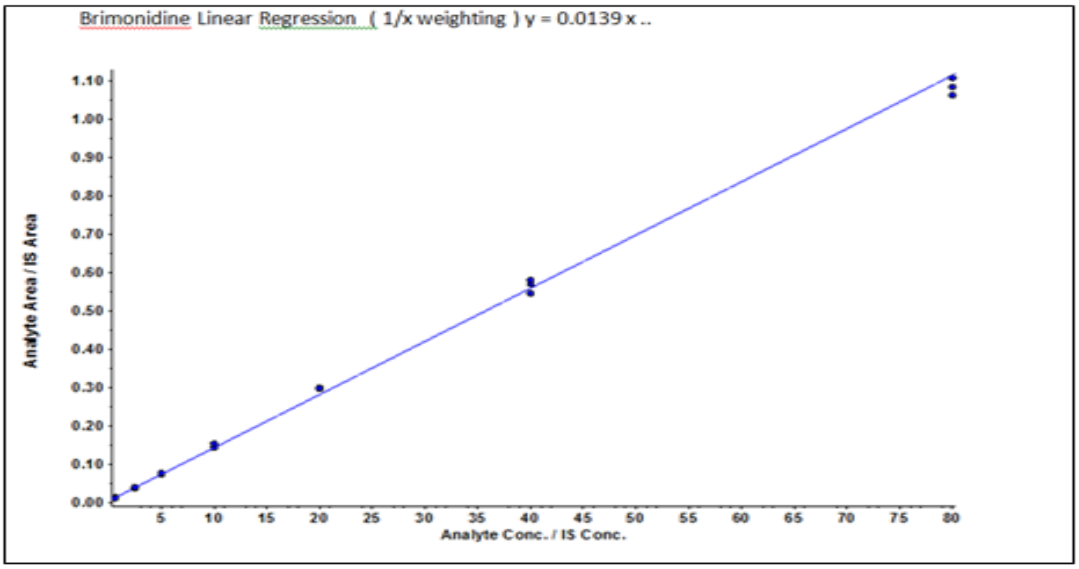

Figure 25: Representative standard calibration curve obtained after analysis of eye drops standards of Brimonidine Tartarte in the range of 2.5-80 ng/mL.

Table -7 Metric; Concentration Analyte, Brimonidine sample type Standard-

Expected Conc. (µg/mL)	Sample ID	No. of values	Mean	Std. Dev.	%CV	Accuracy
2.5	2.5ng-1,2.5ng-2,2.5ng-3	3 of 3	2.47	0.10	4.25	99.12
5.00	5ng-1,5ng-2,5ng-3	3 of 3	5.10	0.14	2.78	102.06
10.00	10ng-1,10ng-2,10ng-3	3 of 3	10.51	0.40	3.80	105.13
20.00	20ng-1,20ng-2,20ng-3	3 of 3	21.15	0.08	0.38	105.76
40.00	40ng-1,40ng-2,40ng-3	3 of 3	40.45	1.27	3.15	101.13
80.00	80ng-1,80ng-2,80ng-3	3 of 3	77.86	1.60	2.06	97.32

Table8: Metric; Concentration Analyte, Timolol sample type Standard

Expected Conc (µg/mL)	Sample Name	No. of values	Mean	Std. Dev.	%CV	Accuracy
0.625	0.625ng-1,0.625ng-2,0.625ng-3	3 of 3	0.61	0.06	10.05	98.26
1.25	1.25ng-1,1.25ng-2,1.25ng-3	3 of 3	1.18	0.06	5.32	94.58
2.50	2.5ng-1,2.5ng-2,2.5ng-3	3 of 3	2.53	0.08	3.45	101.48
5.00	5ng-1,5ng-2,5ng-3	3 of 3	5.37	0.088	1.64	107.45
10.00	10ng-1,10ng-2,10ng-3	3 of 3	9.96	0.140	1.40	99.68
20.00	20ng-1,20ng-2,20ng-3	3 of 3	19.70	0.26	1.36	98.50

Accuracy and Precision-

The Intra–day and Inter-day accuracy precision of the developed method were determined for Brimonidine tartrate and timolol maleate in marketed ophthalmic formulation at 3 QC concentrations, The describes intra-day and inter-day accuracy and precision data

The intra-day accuracy (%) and precision (% CV) of Brimonidine tartrate found in marketed ophthalmic formulation were ranged from **82.53 –89.13 % and 1.95-2.31%** respectively andinter-day accuracy and precision of Brimonidine tartrate found in marketed ophthalmic formulation were ranged from 83.91–93.11 % and 1.33-5.02%.

Table 9: Intra-day and Inter-day accuracy and precision data of the Brimonidine tartrate marketed formulation sample, accuracy and precision data are expressed as % (n=3)

Brimonidine Tartare (µg/ml)	Accuracy (%)		Precision (% CV)	
	Intra-day	Intra-day	Inter-day	Inter-day
3.75 (LQC)	82.53	83.91	2.141.33	
15 (MQC)	89.13 93.11		2.31	2.71
60 (HQC)	87.58 88.94		1.955.02	

The intra-day accuracy (%) and precision (% CV) Timolol maleate found in marketed ophthalmic formulation were ranged from 96.83–102.32 % and 1.12- 3.14% respectively and inter-day accuracy and precision of Timolol maleate found in marketed ophthalmic formulation were ranged from 92.50-105.30% and 0.59-2.25%

Table 10: Intra-day and Inter-day accuracy and precision data of the Timolol maleate marketed formulation sample, accuracy and precision data are expressed as % (n=3)

Timolol maleate Conc. (µg/ml)	Accuracy (%)		Precision (% CV)	
	Intra-day	Intra-day	Inter-day	Inter-day
1.12 (LQC)	96.83	92.50	3.14	2.25
4.5 (MQC)	102.32	105.30	2.88	0.59
18 (HQC)	99.78	101.27	1.12	1.93

Analyze Brimonidine Tartrate and Timolol Maleate marketed anti-glaucoma eye drops formulation through LC-MS/MS. We found the content percentage (%) of both drug compound according to Indian Pharmacopoeia (IP) standards limits (Eye drops contains an amount of equivalent to not less than 90.0 % and not more than 110.0 % of stated amount of eye drops).

Table 11: Timolol maleate contain equivalent content percentage (%) was found in the marketed formulations

Trade Name	Sample Code	Actual conc. (mg/mL)	Calculated/observed Conc. (mg/mL)	% Content equivalent
Timolet- T-0.5% w/v	F1	5	5.02	100.4
Glucomal- T-0.5% w/v	F2	5	4.94	98.8
Iotim-T- 0.5% w/v	F3	5	4.53	90.6
Brimocom T-5mg,-2mg	F8	5	6	120.0
Brimolol T-5mg,B-1.5mg	F9	5	5.3	106..0
Combigan T-5mg ,B-2mg	F10	5	5.32	106.4
Iotim-B T-5mg, B-2mg	F11	5	5.50	110

Bidin-T, T-0.5%, B-0.2%	F12	5	4.94	98.8

Table 12: Brimonidine tartrate contain equivalent content percentage (%) was found in the marketed formulations

Trade Name	Sample Code	Actual Conc. (mg/mL)	Calculated/observed Conc. (mg/mL)	%Content equivalent
Alphagan 0.2%	F4	20	22.04	110.23
IoBrim 0.2%	F5	20	20.38	101.93
Alphagan P 0.15%	F6	15	15.1	100.67
Bidin-LS 0.1%	F7	10	8.78	87.88
Brimocom T-5mg,-2mg	F8	20	23.55	117.78
Brimolol T-5mg, B-1.5mg	F9	15	14.37	95.83
Combigan T-5mg,B-2mg	F10	20	21.29	106.46
Iotim-B T-5mg, B-2mg	F11	20	21.89	109.48
Bidin-T, T-0.5%, B-0.2%	F12	20	18.57	92.87

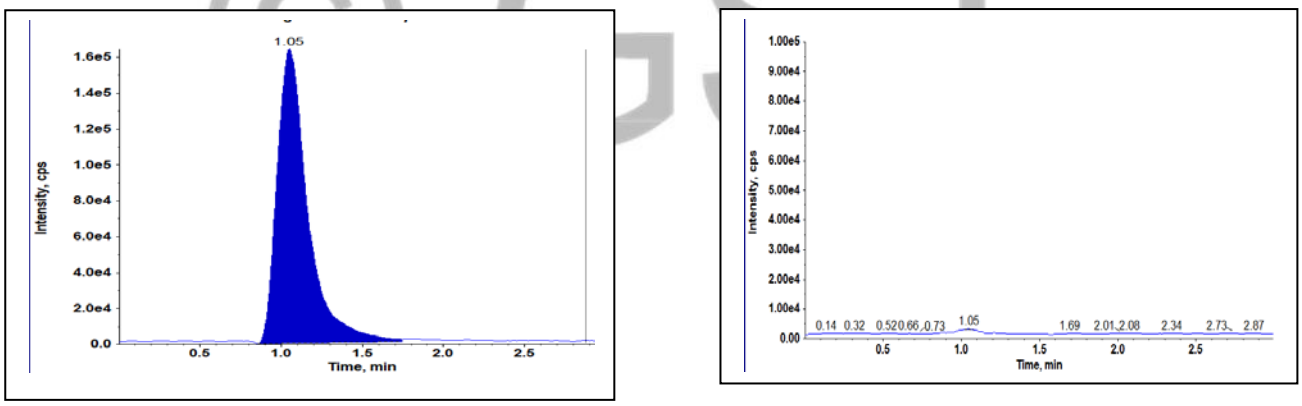

Figure 27: Quantification of Brimonidine tartrate with reference to blank (Mass Spectra)

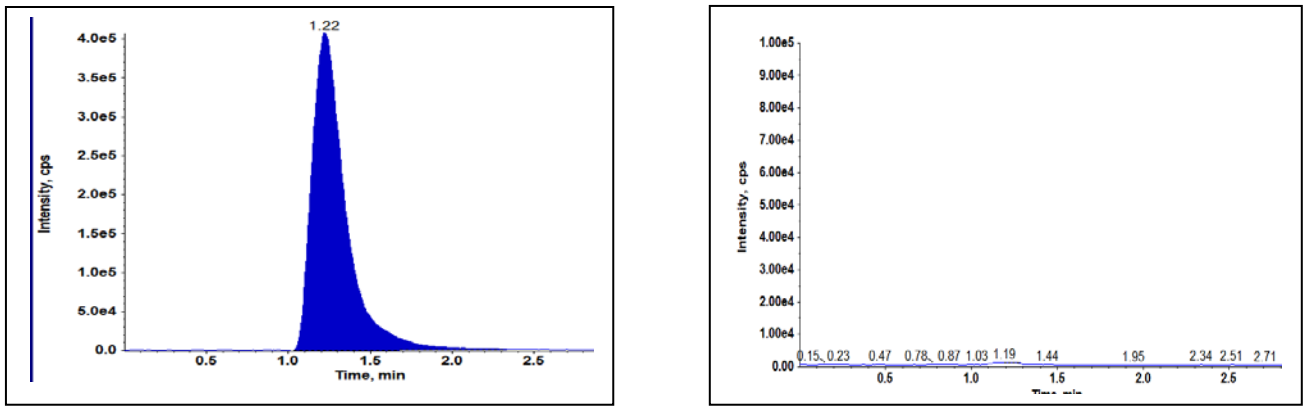

Figure 28: Quantification of Timolol maleate with reference to blank (Mass Spectra)

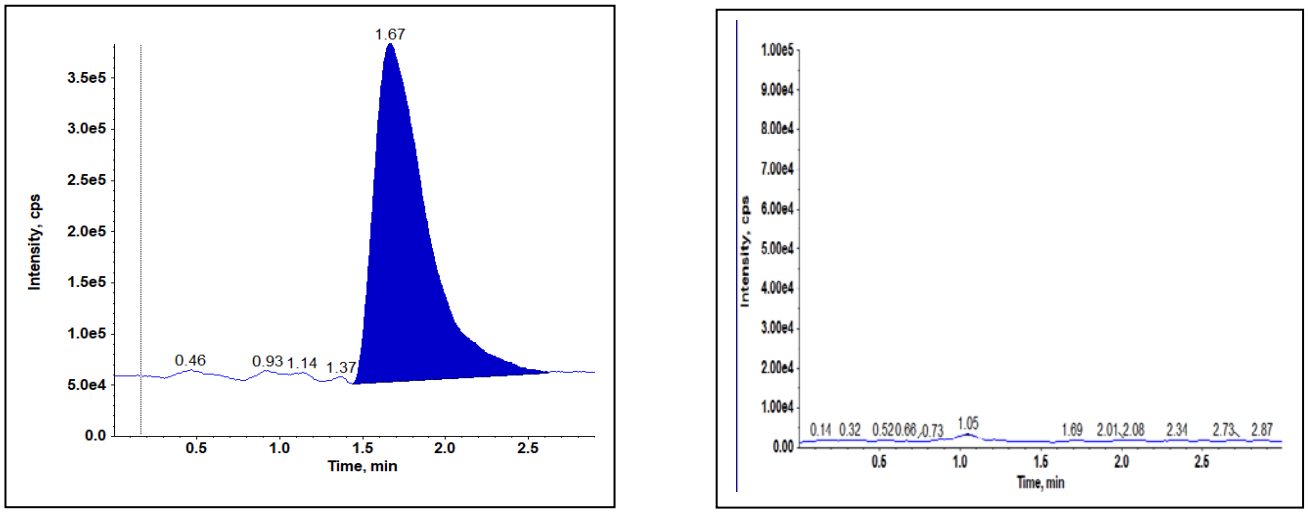

Figure 29: Sulfadimethoxine (SDM) Quantitation with reference to blank

Table13: Other Quality Control parameters of eye drops formulations

Formulation Code	pH	Osmolarity (mosm/Lt)	Viscosity (Cp)
F-1	±7.51	±241	10.5
F-2	±7.05	±163	7.85
F-3	±6.97	±274	9.83
F-4	±6.31	±280	10.3
F-5	±6.62	±261	9.24
F-6	±7.25	±277	9.87
F-7	±6.39	±244	8.65
F-8	±6.42	±292	9.85
F-9	±6.28	±220	10.04
F-10	±7.14	±261	10.2
F-11	±7.08	±272	7.55
F-12	±6.44	±273	6.72

Conclusion

Method Development and its validation using LC-MS/MS were performed for quantification of anti-glaucoma drugs i.e. Brimonidine tartrate and timolol maleate. It found that LC-MS/MS

method is more reliable and short method for drug content analysis. The percentage of content uniformity in marketed formulations under the range specified. In addition, quality control parameters of anti-glaucoma formulation were performed as per pharmacopoeia guidelines. It found that all quality control parameters meet the standards with existing formulations. Bio analytical assay were performed and found that the drug content of each eye drops or formulations was under the range that claimed by companies.

References:

1. Abdelrahman AM (2015) Noninvasive glaucoma procedures: current options and future innovations. Middle East Afr J Ophthalmol 22: 2–9
2. Bachu RD, Chowdhury P, Al-Saedi ZH, Karla PK, Boddu SH (2018) Ocular drug delivery barriers—role of nanocarriers in the treatment of anterior segment ocular diseases. Pharm dev and tech 16: 627-636
3. Sutradhar KB, Amin ML (2013) Nanoemulsions: increasing possibilities in drug delivery. Eur J Nanomed 5:97-110
4. Baranowski P, Karolewicz B, Gajda M, Pluta J (2014) Ophthalmic drug dosage forms: characterisation and research methods. The Sci World J 1-14
5. Agarwal R, Iezhitsa I, Agarwal P, Abdul Nasir NA, Razali N, Alyautdin R, Ismail NM (2016) Liposomes in topical ophthalmic drug delivery: an update. Drug deliv 23:1075-1091
6. Fetih G (2016) Fluconazole-loaded niosomal gels as a topical ocular drug delivery system for corneal fungal infections. J drug deliv sci tech 35:8-15
7. Das S, Suresh PK, Desmukh R (2010) Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine 6:318-23
8. Ding D, Kundukad B, Somasundar A, Vijayan S, Khan SA, Doyle PS (2018) Design of mucoadhesive PLGA microparticles for ocular drug delivery. ACS Appl Bio Mater 16:561-571
9. Mahmoud AA, El-Feky GS, Kamel R, Awad GE (2011) Chitosan/sulfobutylether-β-cyclodextrin nanoparticles as a potential approach for ocular drug delivery. Int J Pharm 413:229-236
10. Chiang B, Kim YC, Doty AC, Grossniklaus HE, Schwendeman SP, Prausnitz MR (2016) Sustained reduction of intraocular pressure by supraciliary delivery of brimonidine-loaded poly (lactic acid) microspheres for the treatment of glaucoma. J Cont Release 228:48-57

11. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Euro J Pharm and Biopharm 75:186-93
12. Gajra* B, Pandya SS, Vidyasagar G, Rabari H, Dedania RR, Rao S (2012) Poly vinyl alcohol hydrogel and its pharmaceutical and biomedical applications: a review. Int J of Pharm Res 4:20-26
13. Makadia HK, Siegel SJ (2011) Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers 3:1377-1397
14. Reed K, Berger N (2018) The Effect of Polyvinylpyrrolidone (PVP) on Ocular Gel Forming Solutions Composed of Gellan and Calcium Gluconate. Int J Pharm Sci and Res 9:20-28
15. Mandal S, Thimmasetty MK, Prabhushankar GL, Geetha MS (2012) Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. Intl J Pharm Invest 2:78-82
16. Edsman K, Carlfors J, Petersson R (1998) Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Euro J Pharm Sci 6:105-112
17. Duan Y, Cai X, Du H, Zhai G (2015) Novel in situ gel systems based on P123/TPGS mixed micelles and gellan gum for ophthalmic delivery of curcumin. Colloid Surface B 128:322-330
18. Morsi N, Ibrahim M, Refai H, El Sorogy H (2017) Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide. Euro J Pharm Sci 104:302-314
19. Pehlivan SB, Yavuz B, Çalamak S, Ulubayram K, Kaffashi A, Vural I, Çakmak HB, Durgun ME, Denkbaş EB, Ünlü N (2015) Preparation and in vitro/in vivo evaluation of cyclosporin a-loaded nanodecorated ocular implants for subconjunctival application. J Pharm Sci 104:1709-1720
20. Bernards DA, Bhisitkul RB, Wynn P, Steedman MR, Lee OT, Wong F, Thoongsuwan S, Desai TA (2013) Ocular biocompatibility and structural integrity of micro-and nanostructured poly (caprolactone) films. J Ocul Pharmacol and Ther 29:249-257
21. Da Silva GR, Lima TH, Oréfice RL, Fernandes-Cunha GM, Silva-Cunha A, Zhao M, Behar-Cohen F (2015) In vitro and in vivo ocular biocompatibility of electrospun poly (ε-caprolactone) nanofibers. Euro J Pharm Sci 73:9-19

22. Nasr FH, Khoee S, Dehghan MM, Chaleshtori SS, Shafiee A (2016) Preparation and evaluation of contact lenses embedded with polycaprolactone-based nanoparticles for ocular drug delivery. Biomacromol17:485-495
23. Kong LY, Su BG, Bao ZB, Xing HB, Yang YW, Ren QL (2011) Direct quantification of mono-and di-d-α-tocopherol polyethylene glycol 1000 succinate by high performance liquid chromatography. J Chromatogr A 48:8664-8671
24. Alkholief M, Albasit H, Alhowyan A, Alshehri S, Raish M, Kalam MA, Alshamsan A (2019) Employing a PLGA-TPGS based nanoparticle to improve the ocular delivery of Acyclovir. Saudi Pharm J 27:293-302
25. Ren F, Jing Q, Cui J, Shen Y (2009) Synthesis and Characterization of D-α-Tocopheryl Polyethylene Glycol 1000 Succinate-Block-Poly (ε-caprolactone) Copolymer Used as Carriers for Microparticles. J Disper Sci and Tech 30:1129-1134
26. Thu HP, Nam NH, Quang BT, Son HA, Toan NL, Quang DT (2015) In vitro and in vivo targeting effect of folate decorated paclitaxel loaded PLA–TPGS nanoparticles. Saudi Pharma J 23:683-688
27. Bernabeu E, Helguera G, Legaspi MJ, Gonzalez L, Hocht C, Taira C, Chiappetta DA (2014) Paclitaxel-loaded PCL–TPGS nanoparticles: In vitro and in vivo performance compared with Abraxane®. Colloid Surface B 113:43-50
28. Zhen L, Wei Q, Wang Q, Zhang H, Adu-Frimpong M, Kesse Firempong C, Xu X, Yu J (2020) Preparation and in vitro/in vivo evaluation of 6-Gingerol TPGS/PEG-PCL polymeric micelles. Pharm Dev and Tech 25:1-8
29. Koulouktsi C, Nanaki S, Barmpalexis P, Kostoglou M, Bikiaris D (2019) Preparation and characterization of Alendronate depot microspheres based on novel poly (-ε-caprolactone)/Vitamin E TPGS copolymers. Int J pharm 100014