IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
June 2021 Vol.:18, Issue:4

Robust Analytical Method Development and Validation for Estimation of Anti-Inflammatory Corticosteroid (Loteprednol Etabonate) in Combination Ophthalmic Dosage Form

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Pramod kumar*[1], Rohit Saraswat[2], Soumen Pattanayek[3]

[1]Research scholar OPJS University, Churu, Rajasthan, India

[2]Research Guide OPJS University, Churu, Rajasthan, India

[3]Teva Pharmaceuticals 1776 Aspen Ln Weston, FL, 33327

Submitted: 22 May 2021
Accepted: 28 May 2021
Published: 30 June 2021

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Loteprednol etabonate, HPLC Method, ophthalmic suspensions

ABSTRACT

A robust and stability indicating HPLC assay methods were developed and validated for quantitative determination of loteprednol etabonate and Tobramycin separately in ophthalmic suspensions in the presence of degradation products generated from forced degradation studies. The system consisted of GL Sciences Inc, Japan. Inertsil C8, 150 x 4.6 mm, 5µ and detection was performed at 245 nm for Loteprednol Etabonate. The mobile phase consisted of 2 mL of formic acid in 2000 mL water as Mobile phase A and 2 Ml of formic acid in methanol as Mobile phase B with gradient program. The flow rate maintained as 1.0 mL/min and column temperature is 35°C. The standard concentration was prepared about 200 µg/mL. The calibration curve was linear from 50 to 400µg /mL with r > 0.999. Accuracy (mean recovery 101.3%) and precision were found to be satisfactory. Specificity with available impurities was studied. All the impurities were not interfered with loteprednol etabonate, thus the method can be considered as a stability-indicating method. The proposed method can be used for quality control assay of loteprednol etabonate in ophthalmic suspensions and for stability studies as a result of the ability of the method to separate loteprednol etabonate from its degradation products and excipients.

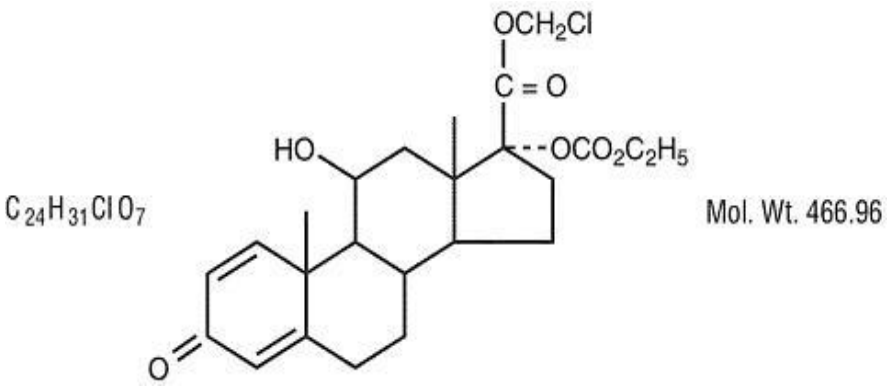
INTRODUCTION:

Loteprednol etabonate is an etabonate ester, chloromethyl (8S,9S,10R,11S,13S,14S,17R)-17-ethoxycarbonyloxy-11-hydroxy-10,13-dimethyl-3-oxo-7,8,9,11,12,14,15,16octahydro-6H-cyclopenta[a]phenanthrene-17-carboxylat. It has a role as an anti-inflammatory drug. It derives from a loteprednol. Loteprednol Etabonate is the etabonate salt form of loteprednol, an ophthalmic analog of the corticosteroid prednisolone with anti-inflammatory activity. Loteprednol etabonate exerts its effect by interacting with specific intracellular receptors and subsequently binds to DNA to modify gene expression. This results in an induction of the synthesis of certain anti-inflammatory proteins while inhibiting the synthesis of certain inflammatory mediators. Loteprednol etabonate specifically induces phospholipase A2 inhibitory proteins (collectively called lipocortin's), which inhibit the release of arachidonic acid, thereby inhibiting the biosynthesis of potent mediators of inflammation, such as prostaglandins and leukotrienes. [1].

This compound was designed based on prednisolone and to reduce side effects, carboxylic ester functionality was introduced at 17b-position, in the expectation that hydrolysis by non-specific esterases would transform it into the indicative steroid carboxylic acid metabolite. Loteprednol etabonate is used in the topical management of inflammatory and allergic disorders of the eye. It is usually employed as eye drops containing 0.2 or 0.5%. Coffey and co-workers describe a novel ophthalmic gel formulation of loteprednol etabonate in the treatment of ocular inflammatory conditions. Prolonged application to the eye of preparations containing corticosteroids has caused raised intra-ocular pressure and reduced visual function. A literature survey revealed two high-performance liquid chromatographic methods for loteprednol etabonate for estimation of Loteprednol etabonate and one literature for combination of Loteprednol and tobramycin dosage form [2].

This paper describes a simple, precise, accurate and robust, specific to impurities reversed-phase HPLC method for the determination of Loteprednol etabonate in combination eye drops in the presence of its degradation impurities and formulation excipients. The proposed HPLC method utilizes economically available common solvent system, well separated impurities from main Loteprednol Etabonate peak, good retention time, sharp and symmetrical peak shapes. The method was validated as per International Conference on Harmonization (ICH) [3] suggestions.

Loteprednol etabonate:



Chemical name: Chloromethyl 17α-[(ethoxycarbonyl)oxy]-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate

MATERIALS AND METHODS:

Chemicals, samples and reference standards

Analytical reagent grade of Formic acid, HPLC grade of Methanol and Acetonitrile were obtained from MerckKGaA, Germany. A Milli-Q purification system (Millipore, Bedford, MA, USA) was used to further purify demineralized water.

Loteprednol Etabonate was purchased from Sigma-Aldrich. All available impurities 1,2-Dihydro Diethyl carbonate impurity, Prednisolone, 1,2-Dihydro Loteprednol Etabonate impurity, Prednisolone 17-acid 17-ethyl carbonate, Loteprednol Etabonate 11-keto, Prednisolone 17-Beta Hydroxy acid and Methyl ester small quantities were got as gift materials. Loteprednol etabonate and tobramycin ophthalmic suspension was purchased from market. All the individual impurity stock solution 0.1 mg/mL solution was prepared. Loteprednol Etabonate standard solution 0.2 mg/mL was prepared in 50:50 water and acetonitrile mixture. All the stock solutions were stored at 2-8°C and used for all the research work.

HPLC system and chromatographic conditions

The HPLC Agilent(Agilent Technologies, USA) 1260 Infinity II LC System, consisted of quaternary pump which can be operates at pressures up to 400 bar and flow rates up to 10 mL/min, High performance degasser is designed for low-flow and analytical LC up to 5 mL/min,

reducing baseline noise and quenching effects, Vialsampler injects from up to 132 standard 2 mL vials and has a pressure rating of 600 or 800 brand time-programmable wavelength switching provides optimum sensitivity and selectivity for your applications. Analysis was done using a high pH-resistant column, the Inertsil, C8 C18 (150 x 4.6 mm, 5µ) from GL Sciences Inc, Japan). This column was designed to resist a pH up to 7.5. Column temperature was adjusted to 35°C and maintained constant using inbuilt column thermostat. Several trials were carried out to separate all the possible degradation impurities from main peak as well from placebo peaks. At finally gradient method was optimised with mobile phase A is 2.0 mL of formic acid in water and Mobile phase B is 2.0 mL of formic acid in methanol. The gradient program is follows. Initially, mobile phase A is 45% and gradually decreased to 20% at 25 min. The same hold for 5 min up to 30.0 min. After 30.0 min mobile phase was increased to 45% at 30.01 min. The same ratio was holds for 10 min up to 40 min. The flow rate maintained 1.0 mL/min and the injection volume is 10µL. Before use, the mobile phase was degassed by sonication followed by sonication for about 10.0 min.

Method validation:

The stated study is aimed at assay method development for Loteprednol Etabonate from a combination of anti-inflammatory corticosteroid and anti-infective combination ophthalmic dosage form. We have focused validation efforts toward necessary tests for assays, such as precision, accuracy, linearity, and selectivity. Sensitivity have been determined with crucial importance since impurity profiling was a goal to well separated from each other's and from placebo peaks. The analytical method validation was performed as per the recommended guidelines provided by the International Conference on Harmonization (ICH) guidelines [3]. Accuracy was also validated as this method was developed for estimation of assay for a combination product. The developed and validated method is well suitable for assay estimation of Loteprednol Etabonate liquid pharmaceutical formulations. A standard addition test will give indication of the accuracy of this method.

Precision

Repeatability and intermediate precision should be evaluated for assessment of precision. Repeatability was determined by six repetitive sample preparations of Loteprednol and

tobramycin formulation product. The relative standard deviations (RSD) would be calculated for obtained assay of Loteprednol Etabonate, should be below 1% [4]. For intermediate precision, a commercial sample of Loteprednol and tobramycin ophthalmic suspension was analyzed on different day on different HPLC equipment using the respective standard to make daily one-point calibration. Samples were prepared in such a way to obtain 200 µg/mL solutions calculated as per label claim. The averages of different days were compared and the RSD on those averages was expected to be <1%.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

In an assay, it is crucial to be able to selectively determine the concentration of the main compound without interferences from the expected related impurity substances. Therefore, resolution between the peak of the main compound and those of the impurities was investigated. Separation of impurities from each other and from main peak was our goal.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e., 100, 200 and 300µg/mL of a commercial formulation of Loteprednol etabonate. The percentage recoveries were calculated with against known concentration of standard solution.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample preparation. For the evaluation of linearity for Loteprednol Etabonate assays, the stock solutions

were diluted with diluent to samples of 50, 100, 150, 200, 300 and 400 µg/mL. This range was sufficiently large as ICH guidelines usually prescribe a range of 80–120% for an assay. Linearity for the main compounds was determined in this range.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

For the robustness evaluation of the analytical assay method, the optimized HPLC conditions set for this method have been slightly modified for samples of Loteprednol Standard as a means to evaluate the method robustness. The small changes include: The effect of flow rate was at 0.9 and 1.1mL/min instead of 1.0mL/min. The effect of column temperature at 30°C and 40°C instead of 35°C.

Stability of stock solutions

This study was performed on Bench top for 1 week for standard solution. This solution was injected into the HPLC system and chromatogram was recorded. Sample solution was injected at room temperature and stored samples solution at refrigerator at periodic time interval.

RESULTS:

Method development:

As part of method development, literatures were referred for Loteprednol Etabonate maximum wavelength where it will give the maximum absorbance. All the literatures were stating that the maxima wavelength for Loteprednol Etabonate would be about 245 nm. To confirm wavelength maxima, 200 µg/mL of Loteprednol Etabonate was prepared in Acetonitrile: Water (50:50v/v) mixture and scanned for maximum wavelength. From the above solution spectrum, the maxima was found to 245 nm and same was utilised for the further development and validation actives. The spectrum was presented as figure 1.

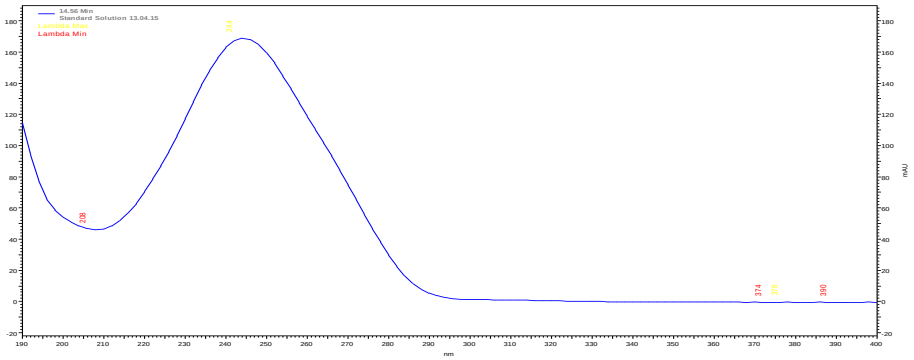


Figure No. 1: UV spectrum of 200 µg/mL solution in Acetonitrile: Water (50:50 v/v)

Initially, the assay method development was initiated with simple mobile phase water and methanol 50:50 v/v and the HPLC column C18, 150X4.6 mm, 5.0µ. The flow rate used as 1.0 mL /min. The main peak shape was not symmetrical. The HPLC column was changed to C8, 150X 4.6, 5.0µ. Reaming chromatographic conditions were used same as mentioned above. The Loteprednol Etabonate peak was symmetrical and the Retention Time is about 15.0 min. Refer Figure 2.

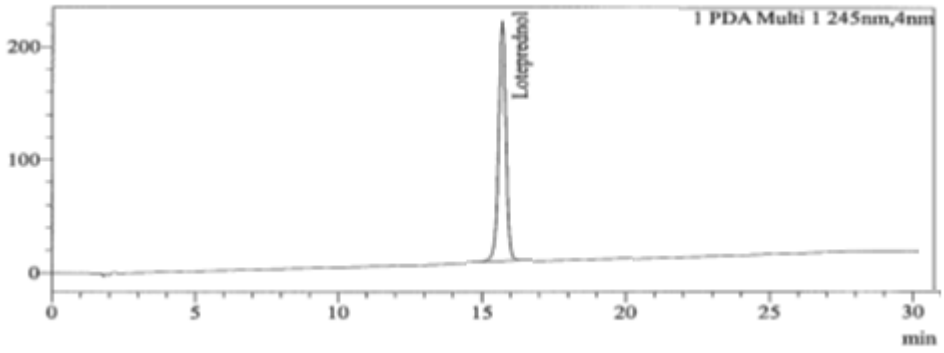


Figure No. 2: Typical chromatographs with C8, 150 X 4.6, 5 µ

As part of specificity verification, all the available impurities were injected and verified for separation.1, 2 Dihydro Loteprednol Etabonate impurity was coeluting with main Loteprednol Etabonate peak. To optimize the chromatographic conditions, Formic acid was added to mobile phase. Elution mode was changed to gradient, column temperature introduced to 35°C. The final chromatographic conditions were as follows.

Column: Inertsil C8, 150 x 4.6 mm, 5µ

Wavelength: 245 nm

Flow Rate: 1.0 mL/min

Column Temperature: 35°C

Injection volume: 10 µL.

Gradient program

Initially, mobile phase A is 45% and gradually decreased to 20% at 25 min. The same hold for 5 min up to 30.0 min. After 30.0 min mobile phase was increased to 45% at 30.01 min. The same ratio was holds for 10 min up to 40 min. The final typical chromatographs were given as figure 3.

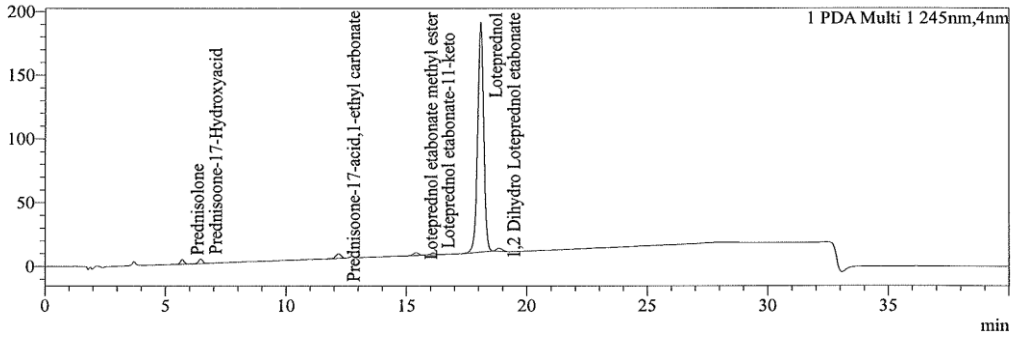


Figure No. 3: Final typical chromatograph

Validation

Precision

As indicated earlier, repeatability and intermediate precision have been evaluated. Six consecutive sample preparation of 200 µg/mL of Loteprednol Etabonate prepared and analysis have been performed. The RSD of Loteprednol Etabonate assay against standard peak was 0.8%. The precision of the method thus complied with specifications in terms of assay content as well as retention time. This analysis was performed during two different days on various HPLC systems and the RSD calculated on the obtained averages for each day was 0.5%. These results show a sufficient intermediate precision of the assays. The results were tabulated below table. (Table 1)

Table No. 1: Precision and Intermediate Precision data

S. No	Sample Description	% Assay	
		Precision (Day-1)	Intermediate Precision (Day-2)
1	Preparation - 1	98.4	97.5
2	Preparation – 2	99.0	98.9
3	Preparation – 3	98.0	98.3
4	Preparation – 4	99.7	97.6
5	Preparation – 5	97.5	99.1
6	Preparation - 6	98.3	97.8
Average:		98.5	98.2
%RSD		0.8	0.7
Cumulative % RSD			0.7

Linearity

The adjusted method yielded a linear calibration curve over the chosen range for Loteprednol Etabonate. The regression equation obtained was y = 20740x+58999, with the correlation coefficient being 0.9993. The % bias is 1.4. Since the correlation coefficients of curves is greater than 0.995, a good linear relationship between the detector response and the concentration of analyte could be concluded [5]. For the Loteprednol Etabonate compound, zero is included in the 95% confidence interval of the intercept allowing one-point calibration. A residual plot was produced to assess the appropriateness of linear regression to fit the data. Since the points were distributed randomly around the horizontal axis, it was concluded that linear regression is suitable for these data. The data were tabulated below in Table 2.

Table No. 2: Linearity data of Loteprednol Etabonate

S. No	% of Nominal Concentration	Concentration (ug/mL)	Avg. Peak Area
1.	25	50.38	1051946
2.	50	100.76	2127552
3.	75	151.14	3231227
4.	100	201.52	4259539
5.	150	302.28	6437850
6.	200	403.04	8323150
Correlation coefficient			**0.9996**
Slop			**20740**
Intercept			**58999.1765**
% bias			**1.4**

Selectivity

This study focuses on the assay of the main peak of Loteprednol Etabonate and therefore also needs to investigate the separation from relevant impurities. All the available seven loteprednol Etabonate impurities are were spiked with in Loteprednol Etabonate and Tobramycin Ophthalmic suspension combination formulation and evaluated for specificity study. All the impurities were well separated from each other and there is no interference with main peak. It is evident that the proposed method is specific to estimation of Loteprednol Etabonate assay. The specimen chromatograph is given as figure 4.

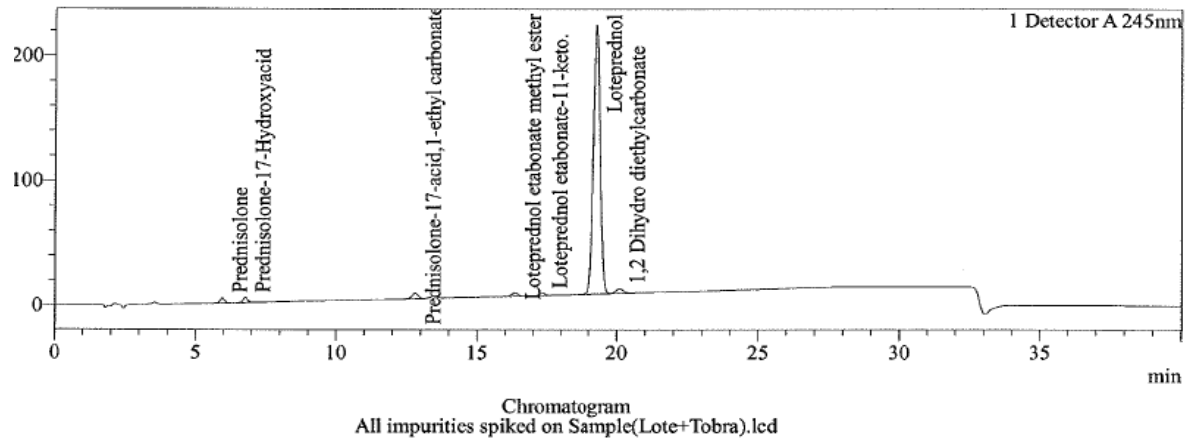


Figure No. 4: Loteprednol Etabonate Impurity Mixture

Accuracy

Accuracy of analytical method was evaluated by spiking the known concentration of Loteprednol Etabonate to placebo of Loteprednol Etabonate. Placebo containing Tobramycin and all other excipients except Loteprednol Etabonate. The results were tabulated below in Table No. 3.

Table No. 3: Accuracy of Loteprednol Etabonate

Accuracy Level	(mg) added of Loteprednol Etabonate	(mg) found of Loteprednol Etabonate	% Accuracy	Mean Accuracy
50%	0.0998	0.1010	101.2	100.9
	0.0986	0.0991	100.5	
	0.1012	0.1023	101.1	
100%	0.1995	0.2014	101.0	100.7
	0.2003	0.2009	100.3	
	0.2005	0.2021	100.8	
150%	0.2993	0.3042	101.6	100.8
	0.3012	0.3032	100.7	
	0.3008	0.3011	100.1	

Robustness

As per ICH guidelines [3], the robustness of an analytical method is its ability to withstand small but deliberate changes in the experimental variables. In this study, the robustness was evaluated by an experimental design examining the simultaneous influence of flow rate variation and column temperature variation on the peak area as the response variable as well peak RT. It was found that the peak area is significantly influenced by the column temperature variation from the optimum temperature conditions and there is no effect on the assay determination. There is no significant effect on the RT and peak response with flow rate alteration. This means that the method was found to be robust with respective to flow rate and column temperature.

Stability of stock solutions

Loteprednol Etabonate stock solutions was prepared and kept at room temperature and in the refrigerator for 1 week. The stock solution remained stable in the refrigerator during the whole period of test procedure and remained stable at room temperature for 2 days. At room temperature, stability is guaranteed for 2 days and 1 week at refrigerator.

DISCUSSION

During method development, different options were evaluated to optimize sample preparations, peak detection parameters, Impurity interference from main peak and chromatography. A mobile phase containing formic acid in water and formic acid containing in methanol different combinations was tried during the initial development stages. The sensitivity and peak shape were also checked. The best signal and peak shape for Loteprednol etabonate were achieved using a stationary phase Inertsil, C8, 150 X 4.6 mm, 5 µ. The elution mode was finalised as gradient for Loteprednol Etabonate with mobile phase A consisted of 0.1 % v/v formic acid in water and mobile phase B 0.1% v/v formic acid in methanol and ran at a flow rate of 1 mL/min. The proposed method was validated as per the ICH guidelines for its specificity, precision, linearity accuracy, and robustness. No impurity peaks were observed in the chromatogram of placebo solution at the retention time of Loteprednol etabonate. The method is very robust, simple and specific, as all the impurity peak were well separated from each other and excipient peaks which makes it especially suitable for routine quality control analysis.

CONCLUSION

The present method is specific, rapid, robust, precise, linear and accurate. The mobile phase was easy to prepare. The recovery from formulations was in good agreement and suggested no interference in the estimation. Application of this method for the analysis of ophthalmic formulations reveals that neither the degradation products nor the excipients interfere with the analytical determination. This indicates that the proposed method could be used as a stability-indicating method for the determination of loteprednol etabonate either in bulk or in pharmaceutical formulations (ophthalmic suspensions). Therefore; this method could easily be used in a wide range of analytical laboratories.

REFERENCES:

1. Loteprednol Etabonate. National Centre for Biotechnology Information. PubChem Compound Database. [Last accessed on 2020 March 30]. p. CID= 444025. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Loteprednol-etabonate
2. A Validated Specific Stability-Indicating RP-HPLC Assay Method for the Determination of Loteprednol Etabonate in Eye Drops by Yong K. Han and Adriana I. Segall. Journal of Chromatographic Science 2015;53:761–766 doi:10.1093/chromsci/bmu121 Advance Access publication September 18, 2014.
3. International Conference on Harmonisation Guidelines on Validation of Analytical Procedures: Text And Methodology Q2(R1), November 2005.
4. Harris, D.C.; Quantitative and Chemical Analysis, W. H. Freeman and Company, New York, (2007).
5. Yasueda, S., Higashiyama, M., Shirasaki, Y., Inada, K., Ohtori, A.; An HPLC method to evaluate purity of a steroidal drug, loteprednol etabonate; Journal of Pharmaceutical and Biomedical Analysis, (2004); 36: 309–316.
6. DETERMINATION OF LOTEPREDNOL ETABONATE AND TOBRAMYCIN IN COMBINED DOSAGE FORM USING RP-HPLC METHOD, by Sneha A Vashi*, Megha Shah, Zarna Patel, Bhoomi Mistry, Foram Desai, Nupur Mistry.
7. H Russ, D. McCleary, R. Katimy, J.L Montana, R.B Miller, R. Krishnamoorthy & C. W. Davis (1998). Development and Validation of a Stability-Indicating HPLC Method for the Determination of Tobramycin and Its Related Substances in an Ophthalmic Suspension, Journal of Liquid Chromatography & Related Technologies, 21:14, 2165-2181.
8. A review on analytical method development, P. Ravisankar, S. Gowthami1, G. Devlala Rao. Indian Journal of Research in Pharmacy and Biotechnology ISSN: 2321-5674 (Print) ISSN: 2320 – 3471(Online).
9. Novel stability indicating RP-HPLC method for the simultaneous estimation of tobramycin and loteprednol in pharmaceutical dosage forms. By Nagaraju Pappula, Kiran Kumar Palaparthi, Aparna Govindu and Suneetha M. GSC Biological and Pharmaceutical Sciences, 2020, 10(01), 073–080. Article DOI: https://doi.org/10.30574/gscbps.2020.10.1.0226.
10. Analytical Method Development and Validation for the Determination of Loteprednol Etabonate and Tobramycin in Combined Dosage Form. By Sneha A Vashi, Megha Shah, P. Malairajan, Alisha Patel, Zarna Patel. JPSBR: Volume 5, Issue 4: 2015 (379-384).