International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LOTEPREDNOL ETABONATE AND LEVOFLOXACIN IN COMBINED DOSAGE FORM

Vijay Kumar Singh, Prem Prasad, Dr. Sanjay Kumar Kushwaha*
Bhavdiya Institute of Pharmaceutical Sciences and Research, Ayodhya (U.P.)

***Correspondence Info:**
Sanjay Kumar Kushwaha
Bhavdiya Institute of Pharmaceutical Sciences and Research, Ayodhya (U.P.)
Email:
sanjaykushwaha78927@rediffmail.com

***Article History:**

Received: 12/04/2025
Revised: 08/05/2025
Accepted: 25/05/2025

ABSTRACT

A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Loteprednol Etabonate (LPE) and Levofloxacin (LFC) in combined ophthalmic dosage form. The chromatographic separation was achieved using a C18 column with a mobile phase composed of acetonitrile and phosphate buffer (pH 3.5) in a suitable ratio, at a flow rate of 1.0 mL/min and detection wavelength set at 254 nm. The retention times for Loteprednol Etabonate and Levofloxacin were found to be approximately 3.452 min and 5.614 min, respectively. The method was linear in the concentration range of 2–10 µg/mL for both drugs, with correlation coefficients (r^2) greater than 0.999. Accuracy was confirmed by recovery studies, with mean recoveries ranging from 97.56% to 99.14%. The method demonstrated excellent precision with %RSD values less than 1% for intra- and inter-day studies. LOD and LOQ were found to be 0.20 µg/mL and 0.45 µg/mL for LPE, and 0.25 µg/mL and 0.75 µg/mL for LFC, respectively. The method was also found to be robust and specific, and successfully applied to assay analysis of marketed formulations. These results suggest that the developed method is suitable for routine quality control of LPE and LFC in combined dosage forms.

Keywords: Loteprednol Etabonate; Levofloxacin; RP-HPLC; Method Validation; Simultaneous Estimation; Ophthalmic Formulation; Linearity; Precision; Accuracy; Assay.

INTRODUCTION

The development of reliable, accurate, and validated analytical methods for pharmaceutical formulations is essential to ensure the safety, efficacy, and quality of drug products. In the field of ophthalmology, fixed-dose combinations are increasingly used to enhance therapeutic outcomes and patient compliance. Among these, Loteprednol Etabonate (LPE) and Levofloxacin (LFC) represent a potent therapeutic combination with anti-inflammatory and antibacterial properties, respectively.

Loteprednol Etabonate is a C-20 ester corticosteroid, classified as a "soft drug" due to its rapid metabolic deactivation to inactive carboxylic acid derivatives after exerting its therapeutic action. This property significantly reduces the risk of adverse effects such as increased intraocular pressure, commonly associated with corticosteroids (Kaplan *et al.*, 2001; Bodor *et al.,* 1991). LPE is prescribed for a variety of ocular inflammatory conditions, including postoperative inflammation, allergic conjunctivitis, and uveitis (Rajpal *et al.,* 2013).

Levofloxacin, a third-generation fluoroquinolone, acts by inhibiting bacterial DNA gyrase and topoisomerase IV enzymes, which are critical for bacterial replication and repair. Its broad-spectrum antimicrobial activity encompasses Gram-positive and Gram-negative bacteria, making it highly effective in treating ocular infections (Fish *et al.,* 1995; Asbell *et al.,* 2008). The combination of LPE and LFC is particularly beneficial post-surgery or in conjunctivitis where both inflammation and infection coexist.

Given the rising demand for combination therapies in ophthalmology, it becomes imperative to develop analytical methods that can accurately and simultaneously quantify multiple active pharmaceutical ingredients (APIs). High-Performance Liquid Chromatography (HPLC) is widely employed for this purpose due to its superior sensitivity, selectivity, reproducibility, and capability for simultaneous multi-drug analysis (Snyder *et al.,* 2012; Dong *et al.,* 2006).

Although several methods have been reported for the individual estimation of LPE and LFC, there is limited literature available for their simultaneous estimation in a single dosage form using a validated HPLC method. Previously reported methods include spectrophotometry, capillary electrophoresis, and single-analyte HPLC, but they often lack robustness or are not validated as per International Council for Harmonisation (ICH) guidelines Q2(R1), which emphasize critical validation parameters like specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) (ICH, 2005).

The present investigation aims to develop a simple, sensitive, accurate, and validated reverse-phase HPLC (RP-HPLC) method for the simultaneous estimation of Loteprednol Etabonate and Levofloxacin in their combined ophthalmic dosage form. The method will be validated in accordance with ICH guidelines to ensure suitability for routine quality control and regulatory compliance.

MATERIALS AND METHODS

Materials

Loteprednol Etabonate (LPE) and Levofloxacin (LFC) were obtained as gift samples from reputed pharmaceutical sources. HPLC-grade solvents including acetonitrile and methanol were procured from Merck, India. Orthophosphoric acid (OPA) was used for mobile phase preparation, and all other chemicals and reagents were of analytical grade. A validated High-Performance Liquid Chromatography (HPLC) system equipped with UV detector and C18 column (250 mm × 4.6 mm, 5 µm) was utilized for chromatographic analysis. Double-distilled water was used throughout the study for solution preparation and system cleaning.

Methods

Selection of mobile phase

Initially to estimate Loteprednol Etabonate and Levofloxacin in fix dosage form number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of separation variable

Preparation of Stock Solution:

Accurately weighed 10mg API of LPE and LFC was transferred into 10 ml volumetric flask separately and added 5ml of mobile phase as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50ml with diluent (mobile phase) to give concentration of 100µg/ml of LPE and LFC respectively (Stock-B).

Preparation of Different Solution

0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (mobile phase). This gives the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml, for LPE. In same manner 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml of LFC also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 2-10µg/ml for LPE and 2-10µg/ml for LFC were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of LPE 10µg/ml for LPE and 10µg/ml LFC was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10µg/ml for LPE) and (2 to 10µg/ml for (LFC) and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be

present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 2, 4, 6, 8 and 10µg/ml for LPE and 2, 4, 6, 8 and 10µg/ml for LFC indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table no. 6 respectively.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for LPE and LFC reported in table no. 6.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Methanol: Acetonitrile (50:50% v/v) to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in formulation

Determined the content of LPE and LFC in eye drop (label claim 0.5% and 1.5%) cream was weighed and weight equivalent to 10mg LFC was calculated and dissolved in 10ml mobile phase and the extraction was sonicated for 15 min and centrifuge at 300rpm. Then 1ml solution from it was diluted with 10 ml mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. The peak area regression equation and amount of both the drug in sample was calculated. Analysis procedure was repeated six times with formulation. Results of eye drop analysis are reported in table no. 8.

RESULTS AND DISCUSSION

The validation of the developed RP-HPLC method for the simultaneous estimation of Loteprednol Etabonate (LPE) and Levofloxacin (LFC) was carried out according to ICH Q2(R1) guidelines. Linearity studies demonstrated an excellent correlation over the concentration range of 2–10 µg/mL for both drugs, with a high correlation coefficient (r^2 = 0.999) indicating a strong linear relationship between peak area and concentration. The slope and intercept for LPE and LFC were 899.61, 41.552 and 861.03, 49.711, respectively, confirming the method's suitability for quantitative estimation.

System suitability parameters were within acceptable limits. The retention times were found to be 3.452 minutes for LPE and 5.614 minutes for LFC, with minimal standard deviations (±0.003), demonstrating the reproducibility of the chromatographic system. Theoretical plate counts were more than 2000 for both analytes, indicating

efficient column performance. The tailing factor values were 1.127 for LPE and 1.157 for LFC, suggesting symmetric peak shapes. Additionally, the area under the curve (AUC) values showed negligible variation, indicating consistent detector response.

The response ratio summary for linearity further validated these results, with mean response ratios of 912.990 for LPE and 839.137 for LFC. The %RSD values for both drugs were below 1.5%, which is well within acceptable criteria, confirming the method's precision in linear response. Chromatographic analysis of the drug mixture (Figure 1) showed well-resolved, sharp peaks with no significant baseline drift. The blank chromatogram (Figure 2) confirmed the specificity of the method by showing no interfering peaks at the retention times of the analytes.

Recovery studies were conducted to assess the accuracy of the method at three concentration levels (80%, 100%, and 120%). The percent recovery for LPE ranged from 98.33% to 99.14%, while for LFC it ranged from 97.56% to 98.91%. The low standard deviation and %RSD values (all below 0.3%) reflect the high accuracy and consistency of the method. These results demonstrate that the method can accurately quantify the drugs in the presence of formulation excipients without interference.

Precision was evaluated through repeatability, intermediate precision (day-to-day and analyst-to-analyst variability), and robustness. In all cases, the %RSD values were found to be extremely low (≤ 0.073%), indicating the method's high reproducibility and robustness under slightly altered conditions. The consistency of results despite changes in experimental parameters supports the method's reliability for routine use.

The method’s sensitivity was confirmed through LOD and LOQ studies. The LOD values for LPE and LFC were 0.20 µg/mL and 0.25 µg/mL, respectively, while the LOQ values were 0.45 µg/mL and 0.75 µg/mL. These low values demonstrate the method's capability to detect and quantify even trace amounts of the drugs, making it suitable for stability studies and trace-level analysis.

Finally, the assay of the marketed formulation revealed drug content very close to the label claim. The percent assay values were 98.00% for LPE and 98.67% for LFC, with %RSD values of 0.115 and 0.185, respectively. These results further affirm the method’s accuracy, reliability, and applicability for quality control in pharmaceutical formulations.

The developed and validated HPLC method for the simultaneous estimation of Loteprednol Etabonate and Levofloxacin is precise, accurate, robust, and sensitive. It can be successfully applied for routine analysis, formulation assay, and quality control, adhering to international validation guidelines.

Table 1: Linearity Parameters of Loteprednol Etabonate (LPE) and Levofloxacin (LFC)

Drug	Linearity Range (µg/ml)	Correlation Coefficient (r^2)	Slope (m)	Intercept (c)
Loteprednol Etabonate (LPE)	2–10	0.999	899.61	41.552
Levofloxacin (LFC)	2–10	0.999	861.03	49.711

Table 2: System Suitability Parameters of LPE and LFC

Parameter	Drug	Mean	S.D.
Retention Time (RT) (min)	LPE	3.452	0.003
	LFC	5.614	0.003
Area Under Curve (AUC)	LPE	9020.555	7.170
	LFC	8539.260	4.590
Theoretical Plates (N)	LPE	3558.333	26.227
	LFC	2654.000	28.657
Tailing Factor	LPE	1.127	0.043
	LFC	1.157	0.034

Table 3: Response Ratio Summary for Linearity of LPE and LFC

Parameter	Drug	Value
Mean Response Ratio	LPE	912.990
	LFC	839.137
Standard Deviation	LPE	11.823
	LFC	11.823
%RSD	LPE	1.295
	LFC	1.409

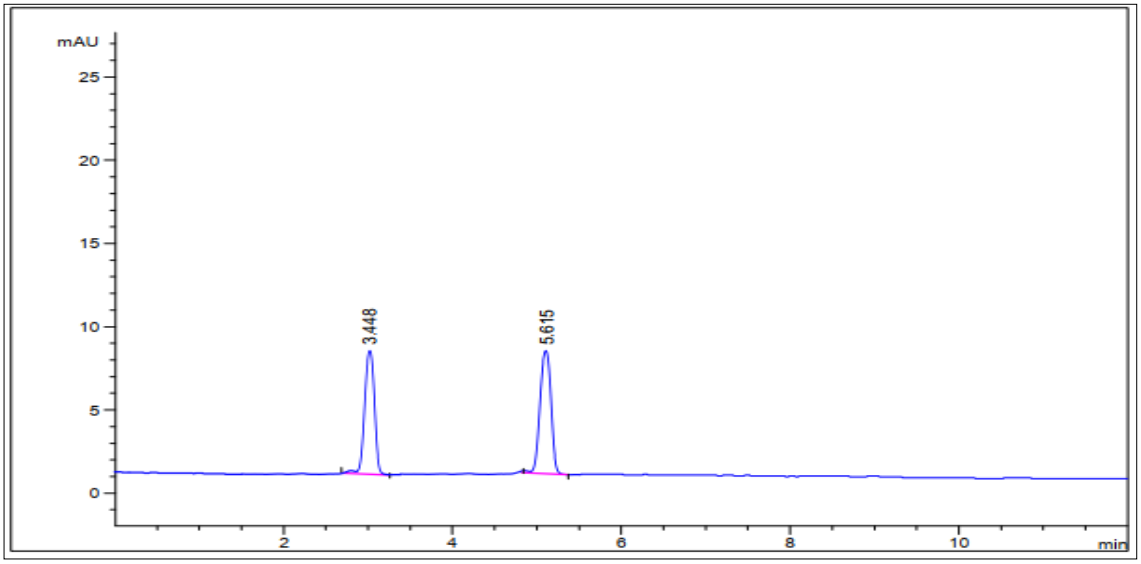

Figure 1: Chromatogram of Both the drug

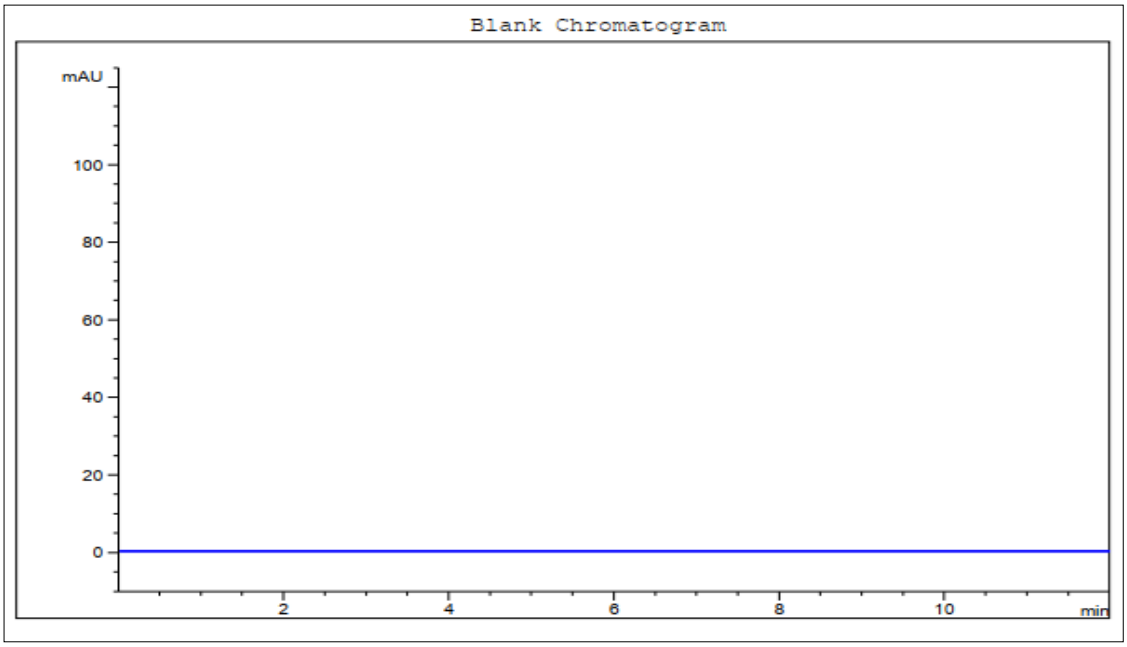

Figure 2: Chromatogram of Blank

Table 4: Summary of Recovery Study of LPE

Recovery Level	Mean % Recovery	Standard Deviation (SD)	%RSD
80%	98.98%	0.263	0.266%
100%	98.33%	0.263	0.267%
120%	99.14%	0.263	0.265%

Table 5: Summary of Recovery Study of LFC

Recovery Level	Mean % Recovery	Standard Deviation (SD)	%RSD
80%	98.47%	0.263	0.267%
100%	97.56%	0.263	0.270%
120%	98.91%	0.263	0.266%

Table 6: Method Validation Summary for LPE and LFC

Parameter	Drug	Mean (µg/mL)	SD	% RSD
Repeatability	LPE	5.898	0.057	0.058
	LFC	5.906	0.054	0.055
Day-to-Day Variation	LPE	5.904	0.044	0.045
	LFC	5.911	0.050	0.051
Analyst-to-Analyst	LPE	5.920	0.042	0.043
	LFC	5.941	0.041	0.042
Robustness	LPE	5.901	0.062	0.064
	LFC	5.888	0.071	0.073

Table 7: LOD and LOQ of LPE and LFC

Name	LOD (µg/ml)	LOQ (µg/ml)
LPE	0.20	0.45
LFC	0.25	0.75

Table 8: Result of assay of formulation

	LPE*	LFC*
Label Claim (mg)	**0.5%**	**1.5%**
% Found (mg)	0.49	1.48
% Assay	98.00	98.67
% RSD	0.115	0.185

*Average of three determination

CONCLUSION

The present study successfully developed and validated a simple, accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Loteprednol Etabonate (LPE) and Levofloxacin (LFC) in combined ophthalmic dosage forms. The method offered excellent linearity over the concentration range of 2–10 µg/mL for both drugs, with high correlation coefficients (r^2 = 0.999), indicating its reliability for quantitative analysis. Recovery studies demonstrated high accuracy with mean recoveries ranging from 97.56% to 99.14%, while precision studies yielded %RSD values well within the acceptable limits (<2%), affirming the method's repeatability and reproducibility. Sensitivity was evident from low LOD and LOQ values, and the method proved robust under small deliberate variations in analytical parameters. The assay results of the marketed formulation showed that the method is suitable for routine quality control applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Kaplan, M. A., Schertzer, R. M., & Aakalu, V. K. (2001). Loteprednol etabonate: A review of ocular anti-inflammatory properties and therapeutic efficacy. *Drugs & Aging*, *18*(4), 305–321. https://doi.org/10.2165/00002512-200118040-00005
- Bodor, N., Loftsson, T., Barabas, E., & Harpak, L. (1991). Design of soft drugs: Loteprednol etabonate as a model compound. *Journal of Medicinal Chemistry*, *34*(2), 791–801. https://doi.org/10.1021/jm00106a057
- Rajpal, R. K., Digby, D., D'Aversa, G., Mah, F. S., Hovanesian, J. A., & Morris, M. (2013). Loteprednol etabonate for the treatment of ocular inflammation and pain. *Clinical Ophthalmology*, *7*, 453–464. https://doi.org/10.2147/OPTH.S40095

- Fish, D. N., Singletary, S. B., & Danziger, L. H. (1995). Antibacterial activity of levofloxacin compared to other fluoroquinolones. *Chemotherapy*, *41*(1), 41–47. https://doi.org/10.1159/000239292
- Asbell, P. A., Sanfilippo, C. M., Sahm, D. F., & DeCory, H. H. (2008). Ocular TRUST: Nationwide antimicrobial susceptibility patterns in ocular isolates. *American Journal of Ophthalmology*, *145*(6), 951–958. https://doi.org/10.1016/j.ajo.2008.01.023
- Snyder, L. R., Kirkland, J. J., & Glajch, J. L. (2012). *Practical HPLC method development* (2nd ed.). Wiley-Interscience.
- Dong, M. W. (2006). *Modern HPLC for practicing scientists*. Wiley-Interscience.
- Patel, N. R., Patel, M. R., Patel, N. M., & Patel, K. P. (2012). Development and validation of RP-HPLC method for estimation of levofloxacin in bulk and dosage form. *International Journal of Pharmaceutical Sciences and Research*, *3*(7), 2101–2105.
- Rao, M., Yadav, S. R., & Singla, A. (2013). Analytical method development and validation of loteprednol etabonate by RP-HPLC. *Journal of Pharmaceutical Analysis and Research*, *2*(3), 118–123.
- Sharma, P., Sharma, S., & Khatri, K. (2016). Simultaneous estimation of fluoroquinolone antibiotics by RP-HPLC in pharmaceutical formulations. *Asian Journal of Chemistry*, *28*(5), 1103–1108.
- International Conference on Harmonisation. (2005). *ICH Q2(R1): Validation of analytical procedures: Text and methodology*. ICH Harmonised Tripartite Guideline. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf