Development and Validation of HPTLC Method for the Simultaneous Estimation of Loteprednol Etabonate in Drug Dosage Form

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***Abstract*- A simple, precise and accurate high-performance thin-layer chromatographic method for the simultaneous determination of Loteprednol Etabonate LE in an ophthalmic formulation was developed and validated. The method used TLC aluminum plates pre-coated with silica gel 60F254 as the stationary phase. The solvent system consisted of methanol:ethyl acetate:triethylamine (7:3:0.2 by volume). Densitometric analysis was performed at 272 nm for LE. The system was found to provide compact spots for LE at an Rf value of 0.70. Linear regression analysis data showed a good linear relationship in the concentration range of 1-5 µg/point for LE. % recovery was found to be 99-102% for LE. For LE, the correlation coefficient was found to be 0.999. The % RSD values indicated that the proposed method was accurate. The specificity of the method was established by a peak purity profiling study and the developed method was specific. The method was successfully applied in the analysis of the combined dosage form.**

***Keywords*: Loteprednol Etabonate, HPTLC and method validation.**

INTRODUCTION

Loteprednol Etabonate (LE) is a commonly used steroidal anti-inflammatory drug that is not an official compound in any pharmacopoeia. HPLC methods for the determination of LE in bile, blood, and urine can be found in the existing literature as well as in the ophthalmic formulation. The drug combination is not official in any pharmacopoeia and therefore no official method is available for simultaneous estimation of these drugs. However, the above drugs are prescription drugs; there is no spectrophotometric method for simultaneous estimation of LE in combined dosage forms.

MATERIALS AND METHODS

Materials Methanol, ethyl acetate, triethylamine, and chloroform were obtained from Merck Chemicals. Loteprednol Etabonate was collected as a gift sample from Lupine Ltd, Mandideep, India. Moxiblu-LP Eye Drops (brand claim: Loteprednol Etabonate (0.5% w/v) + moxifloxacin (0.5% w/v)) was purchased from the local market by Lupine Ltd.

furniture

Camag Linomat V Sampler, Camag TLC Scanner 3, Camag Plate Heater, Camag Double Glass Camera, 200µm Coated Plate (20cm X 20cm), UV Lamp (190-400nm) Camag HPTLC System (Swiss). Camag winCATS software, a 100 µl Hamilton syringe and Sartorius Analytical balances were used for analysis.

Chromatographic conditions

The experiment used methanol:ethyl acetate:triethylamine in a ratio of 7:3:0.2 v/v/v. as the stationary phase on an aluminum strip of silica gel 60F254 (20 x 20 cm). This solution was applied to a TLC plate as a 6 mm wide strip under a stream of nitrogen gas using a Camag Linomat V autosampler. A continuous application rate of 0.1 ml/s was used and the gap between the two strips was set to 5 mm. Growth to 80 mm was performed in a double 10 cm x 10 cm Camag glass chamber saturated with mobile phase for 30 min at room temperature. The developed TLC plates were air dried and then scanned between 200 and 400 nm using a Camag TLC 3 scanner using WinCATS software. The LE component shows a significant response at 272 nm.
Preparation of standard solution LE (100 mg) was accurately measured, transferred to a 100 ml volumetric flask, dissolved and diluted with methanol to obtain 1000 µg/ml (1 µg/µl). Different volumes of mixed solutions (1 µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl, 7 µl and 8 µl) were applied to TLC plates to obtain concentrations of 1 µg, 2 µg, 4 µg, 5 µg , 6 µg, 8 µg, 8 LE.

Prepare a sample solution of the Moxiblu-LP ophthalmic formulation (0.5% w/v LE). 3.3 mL was taken into a 10 mL volumetric flask and the volume was made up to the mark with methanol. 1 µl of the sample solution was spotted onto a TLC plate to obtain a concentration of 1.66 µg/spot for LE.

Validation of the proposed method

The proposed method was validated according to the guidelines of the International Conference on Harmonization (ICH).

Linear (calibration curve)
The calibration curve is plotted in the concentration range of 1-5µg/point for LE, resp. Accurately measured LE mixed standard solutions were applied to TLC plates. TLC plates were developed and analyzed photometrically as described in Chromatographic Separation. A calibration curve was prepared by plotting the peak area against the corresponding concentration (µg/point) for each point. Each reading is the average of five determinations.

Accuracy (% recovery)
The accuracy of the method is determined by calculating the LE recovery using the standard addition method. Known LE solutions were added at 50, 100, 150, 200, and 250%, respectively, to predetermined LE sample solutions. The amount of LE was estimated by applying the obtained values to the corresponding regression equation.

Accuracy
Accuracy of the method (reproducible %) The accuracy of the device was checked by repeatedly injecting the LE solution without changing the parameters of the proposed method. Results are reported as relative standard deviation (% RSD).

Mean precision (reproducibility %)
The intra- and inter-day precision of the proposed method was determined by determining each response 3 times on the same day and on 3 different days of the week for different concentrations of the standard solution. designed by LE. method. Results are reported as relative standard deviation (RSD%).

Limit of Detection (LOD) and Limits of Quantification (LOQ)
The LOD and LOQ of a drug are calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.
LOD = 3.3 x a/S
LOQ = 10 x a/S
Where = Standard deviation of the response S = Slope of the calibration curve

Specificity
The specificity of the method was confirmed by the analysis of drugs and standard samples. The LE spot in the sample was confirmed by comparing the Rf and spectrum of the spot with the standard spectrum.

Analysis of Commercial Formulations
Five microliters of sample solutions from the formulations were individually applied to a TLC plate, developed and scanned as described for chromatographic separation. The amount of LE in the sample solution was determined by fitting the peak area values associated with LE to the corresponding calibration curve.

RESULTS AND DISCUSSION
The TLC procedure was optimized to develop a test method for simultaneous estimation of LE. Standard solutions of both drugs were spotted onto TLC plates and tested in different solvent systems. A mobile phase consisting of methanol : ethyl acetate : triethylamine (7:3:0.2, v/v/v) gave sharp and symmetrical peaks with Rf values of 0.30 ± 0.002 and 0.70 ± 0.006 for LE, in given order. Well-defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature (26 ± 30 °C). The combined densitogram of the mixed standards and the 3-D chromatogram showing the LE peaks of indifferent concentrations at 272 nm are shown in Figure 1, respectively. The proposed HPTLC method was validated for linearity, accuracy, precision, LOD, LOQ and specificity. The calibration plot was found to be linear over a concentration range of 1-8 µg/spot for LE, with a correlation coefficient of 0.999 for LE, respectively. The LOD for LE was found to be 0.0112 µg/spot and 0.0634 µg/spot. The LOQ for LE was found to be 0.1921 µg/spot, respectively showing the sensitivity of the method. The low intra-day (0.760 for LE) and inter-day (0.749 for LE) precision %RSD values show that the proposed method is accurate. Recovery studies were performed to study the accuracy of the method. The average yield percentage obtained was 99-102% for LE, respectively, indicating that the proposed HPTLC method is highly accurate (Table 1). The proposed validated method was successfully used for the determination of LE in tablet dosage forms. The percentage test mean was found to be 99.98 ± 1.14 for LE, respectively (Table 2). Low values of standard deviation indicate the suitability of this method for routine analysis of LE in pharmaceutical dosage forms. To confirm the specificity of the proposed method, the formulation solution was spotted on a TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. The regression analysis data and validation parameters are shown in Table 3.

Table 1: Recovery data of proposed method

S.No	Conc. Of Standard added (µg/spot)	Amount Recovered	Recovery % ± SD
1	2	3.6863	101.5865 ± 0.6326
2	3	4.6531	99.5897 ± 0.1975
3	4	5.6635	100.2130 ± 0.1975

SD is Standard deviation and n is number of determinations

Table 2: Assay of GAT and LOTE

Drug	Label Claim (mg)	Amount found (mg)	%Label Claim ±SD
Moxiblu-LP	6	5.999	99.99± 0.1638

Table 3: Validation Parameters of Loteprednol Etabonate

Validation Parameters	Loteprednol Etabonate
Linearity	1-5µg/spot
Correlation Coefficient	0.999
Accuracy	99-102%
LOD	0.0634
LOQ	0.1921
Specificity	Specific

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