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Spectrophotometric Method Development and Validation for the Estimation of Loteprednoletabonate in Marketed Formulation

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ABSTRACT
Postoperative inflammation after cataract surgery can prolong visual recovery time, increase the treatment burden and elevate the risk of poor visual outcomes. Corticosteroids are potent anti-inflammatory agents that have been used successfully for decades to treat ocular inflammation. However, the side effects associated with topical corticosteroids including increased intraocular pressure, risk of cataract formation after long-term use, and decreased resistance to infection are of major concerns. Amongst the available topical corticosteroids Loteprednol Etabonate (LE), an esterbased corticosteroid has been selected for studies. The aim of the present study was to develop Loteprednol etabonate loaded nanodispersion for treatment of steroid responsive inflammatory conditions of eye with objectives of reducing the frequency of administration and increasing the solubility of drug. Non-solvent method was used to formulate nanodispersion. The formulation was assessed for particle size and polydispersity index, drug content, surface morphology, isotonicity, test of sterility, in-vitro & ex-vivo release. Mean particle size and PI of optimized batch was found to be 50±10nm and PI 0.2±0.1. No microbial growth was observed in optimized formulation during sterility testing and the formulation was isotonic when compared with marketed formulation and plain blood cells. The percent drug release at end of 30mins for optimized batch and marketed formulation was found to be 102±1% and 13±1%, respectively at the end of 30 mins. Overall results of present investigation suggest that Loteprednol Etabonate Nanodispersion formulation would reduce dosing frequency, improve therapeutic 9574 efficacy and show greater potential for the treatment of post-operative inflammation after cataract surgery.

Keywords - Loteprednol Etabonate (LE), HPLC, UV-VisibleSpectrophotometer and Validation

I. INTRODUCTION

Spectrophotometric method development and validation is an important part of the drug development process. This process involves the development of an analytical method capable of accurately measuring the concentration of an active pharmaceutical ingredient (API) in a drug product. The method typically involves the use of a spectrophotometer, which is an instrument that measures the intensity of light of a specified wavelength. The method must be validated to ensure it is accurate and reliable. Validation includes verifying the accuracy, precision, specificity, linearity, range, and robustness of the method. It also includes establishing appropriate quality control measures. The successful development and validation of a spectrophotometric method can help to ensure the efficacy and safety of a drug product.

To develop and validate a spectrophotometric method for the determination of loteprednol etabonate in marketed formulation.

Loteprednol Etabonate is for ophthalmic use only. Eye drops: Lie down and tilt your head backwards. Pull your lower eyelid gently with your index finger to form a pocket. In still the number of drops advised by the doctor into the pocket of the lower eyelid. Close your eyes for 1-2 minutes. Eye gel/Ointment: Lie down and tilt your head backwards. Pull your lower eyelid gently with your index finger to form a pocket. Squeeze a tiny amount of the medicine into the pocket of the lower eyelid. Close your eyes for 1-2 minutes.

Storage
Store in a cool and dry place away from sunlight

Side Effects of Loteprednol Etabonate
- Watery eyes
- Irritation in the eyes
- Eye itching
- Foreign body sensation

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Uses of Loteprednol Etabonate (LE)
Post-operative inflammation and pain following ocular surgery.

Medicinal Benefits
Loteprednol Etabonate is prescribed to treat eye inflammation and pain after eye surgery. It is a corticosteroid that works by blocking prostaglandins (a chemical messenger) in the brain that cause inflammation and swelling. As a result, inflammation and pain are reduced after using Loteprednol Etabonate.

Directions for Use

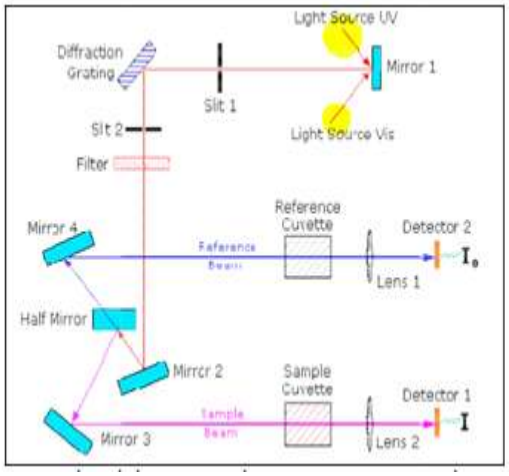

Fig 1.6: Ultraviolet spectrophotometry Instrumental components

Sources of UV radiation
It is significant that the power of the radiation resource doesnot modify abruptly in excess of its wave length range.

The electrical excitation of deuterium or hydrogen at low pressure produces a constant UV spectrum. The machinery for this involves pattern of a thrilled molecular species, which breaks upto provide two atomic species and an ultra violet photon. Together deuterium and hydrogen lamps release radiation in the range160-375nm. Quartz windows have to be used in these lamps, and quartzcuvettes have to be used, since glass absorbs radiation of wavelengths less than 350nm.

Sources of visible radiation

The tungsten filament lampis usually employed as a basis of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted via a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the vigor output to be steady, the voltage to the lamp have to bevery steady indeed. Electronic voltage regulators or constant-voltage transformers are used to make sure this constancy.

Tungsten/halogen lamps hold a small quantity of iodine in a quartz "envelope" which also has the tungsten filament. The iodine reacts with gaseous tungsten, shaped by sublimation, producing the unstable compound WI_2. When molecules ofWI2hit the filament they decompose, redepositing tungsten back on the filament. The lifetime of a tungsten/halogen lamp is roughly double that of an ordinary tungsten filament lamp. Tungsten/halogen lamps are extremely efficient, and their output extends well into the ultra-violet. They are used in a lot of modern spectrophotometers.

II. METHODS AND MATERIALS

Instruments

The HPLC instrument used that was gradient SHIMADZUHPLCLC-2010 CHT with software LC solution and UV Detector with variable wave length programme was used for the method development. Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm) column was used for the separation. SHIMADZU double beam UV/Visible Spectrophotometer model UV 1900i, software- Lab solution. REPTECH Electronic balance model and Ultra Sonicator (Athena Technology)were also used during the analysis

Reagents and chemicals
Loteprednol Etabonate (LE) were kindly supplied as a gift samples from Aarti Industry, Vapi, Gujarat (India). Methanol used as solvent and all calibrated glass wares were used through out the work.

Marketed formulation
Eye drop formulation LEPRED-T was purchased from the local market.

Chromatographic condition:
Stationary phase : Shim-pack solarC18(250 mm× 4.6 mm,5 µm)
Mobilephase: Acetonitrile: Methanol: Water (pH4.5 adjusted with or thophosphoric acid) (60:10:30%v/v/v)
Flow rate :1 ml/min Wavelength:205nm.
Wavelength:205nm.

Wavelength Selection:

0.2 ml from working solution of LE (100

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µg/ml) were pipette out into three separate 10 ml of volumetric flask and volume was made upto the mark with methanol to get 2 µg/ml of LE Each Solutions of LE were scanned between 200-400 nm using UV-Visible Spectrophotometer. Wavelength was selected from the overlay spectra of above solutions. Fig.5.

Preparation of standard stock solution

The standard stock solution of LE was prepared by dissolving 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 1000 µg/ml of each solution. The standard stock solution of formulation was prepared by dissolving equivalent to 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 1000 µg/ml of each solution.

Preparation of second stock solution

The second stock solution of LE was prepared by dissolving 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 100µg/ml of each solution. The second stock solution of formulation was prepared by dissolving equivalent to 10 mg of each API in 10 ml of different volumetric flask in methanol and volume make up with Methanol to produce 100 µg/ml of each solution.

Preparation of working standard solution

Accurately measured second stock solutions of LE and transferred to a series of volumetric flask separately and prepare 15,20,25,30,35ppm of LE. Accurately measured second stock solution of formulation was transferred to a series of volumetric flask separately and prepare 25ppm of LE.

Selection of analytical wavelength

Standard solutions of LE (15µg/ml) and scanned in the range of 200 to 800 nm than convert into derivative spectra. From the overlain spectra, Zero Crossing Point was found at 223.62nm for LE. For the first Order Derivative method 223.62nm and selected as analytical wavelengths.

Method :

First Order Derivative Method

The overlain spectra were converted to first order derivative spectra and from these overlain Derivative Spectra (Fig 3) Zero Crossing Point was found at 223.62nm for LE and selected for the First Order Derivative method of two drugs. The absorbance at 223.62nm for LE was measured

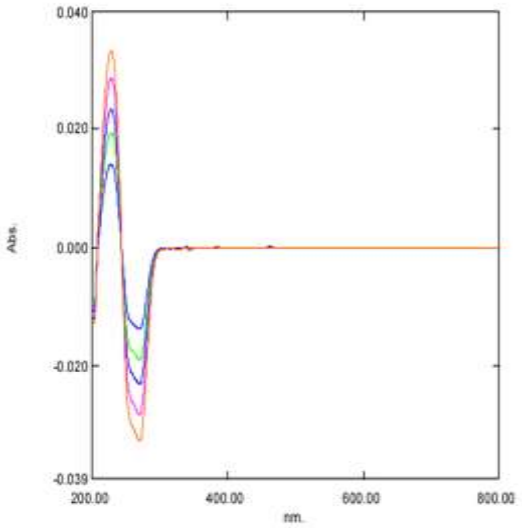

Figure 1.8 : Derivative spectra of Loteprednol Etabonate

Loteprednol Etabonate

Validation of the proposed method

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 15-35µg/ml for LE. Accurately measured standard stock solutions of LE and transferred to a series of volumetric flask separately and prepare 15,20,25,30,35ppm of LE. The absorbance of solution was measured. These spectra were converted to first order derivative spectra and Absorbance at 223.62nm for LE and measured for First Order Derivative method. The calibration curves were constructed by plotting $dA/d\lambda$ versus concentration for First Order Derivative method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of LE (25 µg/ml), without changing the parameters of derivative method.

Intermediate precision (reproducibility)

The intraday and inter day precisions of the proposed method was determined by estimating the Corresponding responses of the drug 3 different concentrations three times on the same day and on 3 Different days over a period of one week for LE (20, 25 and 30µg/ml)). The results were reported in terms of relative standard deviation.

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Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of LE by the standard addition method. Known amount of standard solutions of LE and added to formulation having LE (15µg/ml) .The amounts of LE were obtained by applying regression line equations.

It was performed at three levels 80%, 100%, 120% by Standard addition method. Each concentration was analyzed 3 times and average recoveries were measured n =number of runs, S.D.=Standard Deviation showed in Figure 3 and 5 respectively indicate that the response is linear over the concentration range studied with correlation coefficient (r2) value 0.998 for LE.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal to- noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of Y- intercept of
6 calibration curves and
S = the mean slope of the 6 calibration curves.

III. RESULT AND DISCCUSION

The proposed technique became demonstrated as in step with ICH guiding principle. Technique mentioned inside the gift work provide a handy and correct way for evaluation of LE . In First Order derivative method, wavelengths selected had been 220.62 nm for LE. The plot of absorbance versus respective concentrations of LE were discovered to be linear in the awareness variety of 15- 35µg/ml for LE with correlation coefficient zero.9986 at 220.62nm for LE as proven in table 3 and figures four-five. Precision became calculated in terms of repeatability, intraday and interday precision turned into observed to be in acceptance range (table 3). The accuracy of method turned into decided via fashionable addition method. The % recovery stages from ninety eight.52-a hundred seventy three % for LE (Table 4).

Table. 4 Linearity data of LE at 220.62nm

Sr. No	Conc.(µg\ml)	Absorbance at 220.62nm Mean ± S.D (n=3)
1	15	0.0113±0.000212
2	20	0.0158±0.000234
3	25	0.0202±0.000236
4	30	0.0244±0.0002
5	35	0.0302±0.000203

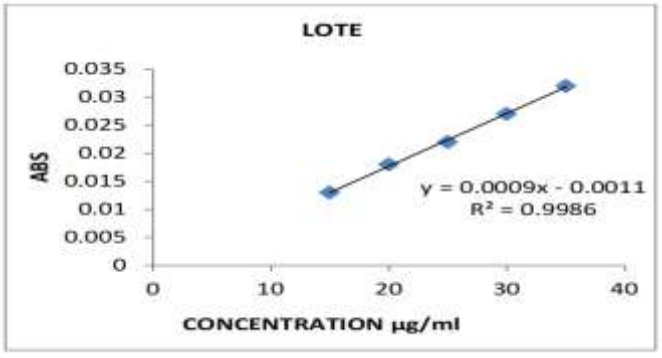

Figure1.9 :Calibration curve of standard LE at 220.62nm

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Table 5 : Recovery study

Concentration (µg/ml) (formulation)	Spiked level (µg/ml		% recovery ±SD (n=3)
LE	LE		LE
15	0%	0	-
15	80%	13	99.93 % ± 0.9806
15	100%	17	100.94% ± 0.3065
15	120%	21	98.52% ± 0.3119

Table6 :Results of estimation of LE in marketed formulation

Marketed Formulation(Tab)	Labeled claim		Amount Obtained		% Assay (n=3)	
LEPRED-T	35µg/ml	21µg/ml	34.55µg/ml	20.66µg/ml	99.12%	98.96%
					±0.3879	±0.5720

This method can be successfully used for simultaneous estimation of LE in their combined dosage form. Marketed Formulation was analyzed and results obtained were within the range of 98- 102

Validation of Proposed Method

Absorbance correction Method

Result of UV analysis has been shown in Table 7. The standard deviation and %RSD calculated for the method is low, indicating high degree of precision. The %RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98- 102% indicating high degree of accuracy and specificity of the proposed method. The results of the recovery study are shown in Table 7. The developed absorbance correction method was validated for simultaneous estimation of Vildagliptin, LEogliflozin Etabonate and Metformin using linearity, range, accuracy and precision and the results were interpreted in Table 7. The % RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

Table No. 7 Result of validation parameter

Parameter	LE
Selected WavelengthRange	226.76-238.65nm
Linearity (µg/ml)(n=5)	2-10(µg/ml)
Regression Equation(y=mx+ c)	Y=0.0238x+0.0152
Regression coefficient(R^2)	0.9973
Correlation coefficient(r)	0.9981
Repeatability (%R.S.D.)(n=6)	0.2786
IntradayPrecision (%R.S.D.)(n=3)	0.1009-0.1813
InterdayPrecision (%R.S.D.)(n=3)	0.1203-0.7289
LOD(µg/ml)(n=5)	0.101576
LOQ(µg/ml)(n=5)	0.307806
%Recovery (n=3)	98.25-99.72
Assay(%)±S.D.(n=3)	98.66

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High Performance Liquid Chromatography (HPLC)
HPLC Data of Optimization of Chromatographic Conditions of LE in shown Table.8

Table No.8 Data of Optimization of Chromatographic Conditions of LE

Parameter	Condition
Mobile Phase	Methanol: Water (60:10:30%v/v/v)(pH-4.5adjustedwith 1% OPA)
Flowrate	1.0 mL/min
Run time	20 min
Volume of Injection	10µL
Detectionof Wavelength	205nm
Diluent	Methanol
RetentionTime	LE-4.497min
Tailing Factor	LE-1.5943
TheoreticalPlate	LE-14436
Resolution	4.741and 3.921

System Suitability studies Evaluation of system suitability was done by analyzing six replicates of LE in a mixture at concentration of 2 µg/ml of LE. The column efficiency, peak asymmetry and resolution were calculated for each replicate and data are shows in Table 9.

TableNo.9 System Suitability data for LE

Drugs	Parameters	Mean±S.D(n=6)	%RSD
LE	Retention Time	7.144±0.0083	0.1171
	Theoretical Plate	2913.2±4.0198	0.1379
	Tailing Plate	1.164±0.0020	0.1752

Specificity

Specificity involves quantitative detection of analyte in the presence of those components that may be expected to be part of sample matrix. Specificity of developed method was established by spiking of LE in hypothetical placebo (i.e., might be expected to be present) and expressing that analytes peak were not interfered from excipients.

Linearity

The linearity response was determined by analyzing 5 independent levels of concentration in the range of 1-5 µg/ml, 2-10 µg/ml and 10-50 µg/ml for LE respectively given

Precision

a) Repeatability

Repeatability of the developed method was assessed by analyzing samples from the same batch 6 times with standard solutions containing concentrations 6 µg/ml for LE for R.S.D. was calculated. The results were shown in Table. 11

a) Intradayprecision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 4, 6 and 8 µg/ml for LE. Solutions were analyzed thrice (n=5) on the same day within short interval of time and % R.S.D. was calculated. The results were shown

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a) Interdayprecision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 8 µg/ml for LE.Solutionswereanalyzedthrice(n=5)onthethreedif ferentdayand %R.S.D. wascalculated.Theresults wereshown

2.1. Accuracy

Foraccuracystudydatafromninedeterminati onoverthreeconcentrationsat80%,100%and 120% of expected sample concentration covering the specified range was determined and expressed as recovery values. The results were shown

LOD and LOQ

TheLOD(LimitofDetection)wasassessedfromtheset of5calibrationcurvesthatwere used to determine linearity of the method. The LOD was calculated by using the formula:

LOD=3.3 ×S.D./Slope

Where,S.D.=StandarddeviationoftheY–interceptsof5calibrationcurves Slope = Mean slope of 5 calibration curves

The LOQ (Limit of Quantitation) was assessed from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula:

LOQ=10 ×S.D./Slope

Where,S.D.=StandarddeviationoftheY–interceptsof5calibrationcurves

Slope = Mean slope of 5 calibration curves

The LOD for LE were found to be 0.199752 µg/ml respectively. The LOQ for LE were found 0.605308 µg/ml respectively.

Table16 :Summary of validation parameters

Parameters	LE
ZCP	223.62nm
Conc. Range (µg/ml)	15-35
Regression Equation	Y=0.0009x-0.00011
Slope (m)	0.0009
Intercept (C)	-0.00011
Regression Coefficient(r2)	0.9986
Repeatability (n = 6) % R.S.D.	0.75664
Intraday Precision (n = 3) % R.S.D.	0.84274
Interday Precision (n = 3) % R.S.D.	1.28451
LOD (µg/ml)(n=6)	2.55617
LOQ (µg/ml)(n=6)	7.74597

IV. SUMMARY AND CONCLUSION

The lower value of relative standard deviation for repeated measurement indicates that the method is precise. The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage form without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the estimation of these two drugs in combined dosage form. Retro-metabolic drug design principles have led to the development of LE, a C-20 ester corticosteroid. LE appears to achieve the necessary balance between solubility/lipophilicity, tissue distribution, GR receptor binding, and metabolic deactivation to be effective as a topical ophthalmic steroid. LE is safe and effective in treating a wide variety of ocular inflammatory conditions including giant papillary conjunctivitis, seasonal allergic conjunctivitis, and uveitis as well as in the treatment of ocular inflammation and pain following cataract surgery. ADRs such as cataract formation and IOP elevation were minimized with LE owing to its retro-metabolic design and their absence confirmed in clinical studies

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