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Analytical Method Development and Validation for Estimation of Impurities of Prostaglandin (Lifitegrast) in Bulk Drug

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Goutham Govardhan Paluru*[1], Satyavir [2], S.B.Puranik[3]

[1]Research scholar OPJS University, Churu, Rajasthan, India [2]Research Guide OPJS University, Churu, Rajasthan, India [3]Drishti Institute of Distance learning, Bangalore, India

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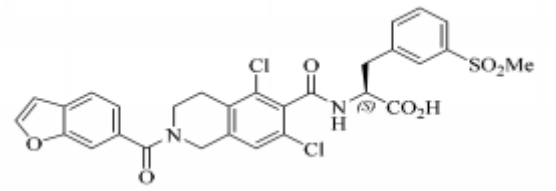
ABSTRACT

A rapid, robust and accurate indicating HPLC methods for impurity estimation were developed and validated for prostaglandin (Lifitegrast) in bulk drug candidate and ophthalmic suspensions. The system consisted of Primesil Cl 8, 3µm, 4.6 X 250mm, 5µ and detection was performed at 215 nm for estimation of Impurities of Lifitegrast API. The mobile phase is gradient with mobile phase A is 100% buffer and mobile phase B consisted 70% Acetonitrile and 30% buffer solution. The flow rate maintained as 1.0 mL/min and column temperature is 25°C and autosampler temperature was maintained at 5°C. The standard concentration for impurities estimation was prepared about 1.0 µg/mL. The calibration curve was linear from LOQ to 150% of standard concentration with r^2> 0.95. Accuracy (mean recovery for Impurity – I: 95.7% and Impurity – II: 96.2%) and precision were found to be satisfactory for Lifitegrast bulk candidate. Specificity with available impurities (Impurity – I and Impurity – II) were studied for bulk candidates. All the impurities peaks were not interfered with each and Lifitegrast, thus the methods can be considered as a specific method. The proposed method is suitable for routine quantification of impurities in Lifitegrastin bulk drug candidate.

INTRODUCTION

The chemical name for Lifitegrast is (S)-2-(2-(benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl)propanoic acid. The molecular formula of Lifitegrast is $C_{29}H_{24}Cl_2N_2O_7S$ and its molecular weight is 615.5. The structural formula of Lifitegrast is as follows [1]:



Molecule Formula: $C_{29}H_{24}Cl_2N_2O_7S$

Lifitegrast is an N-acyl-L-alpha-amino acid obtained by formal condensation of the carboxy group of N-[2-(1benzofuran-6-carbonyl)]-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid with the amino group of 3(methanesulfonyl)-L-phenylalanine. Used for treatment of keratoconjunctivitis sicca (dry eye syndrome). It has a role as an anti-inflammatory drug and a lymphocyte function-associated antigen-1 antagonist. It is a L-phenylalanine derivative, a sulfone, a N-acyl-L-alpha-amino acid, a member of isoquinolines and a member of 1-benzofurans. [2]

Lifitegrast is a FDA approved drug for the treatment of keratoconjunctivitis sicca (dry eye syndrome). It is a tetrahydroisoquinoline derivative and lymphocyte function-associated antigen-1 (LFA-1) antagonist that was discovered through the rational design process. The ophthalmic solution was approved in July 2016 under the trade name Xiidra. It has shown to protect the corneal surface and alleviate the symptoms of dry eye syndrome with fast onset of action and well tolerated profile in both local and systemic setting. [2]

This paper describes a simple, precise, accurate and robust, specific to impurities reversed phase HPLC methods for the determination of impurities of Lifitegrast bulk candidate and Lifitegrast ophthalmic solution in the presence of its degradation impurities and formulation excipients. The proposed HPLC methods utilizes economically available common solvent system and HPLC columns, well separated impurities from each other's and from main Lifitegrast peak, good

retention time, sharp and symmetrical peak shapes. The method was validated as per International Conference on Harmonization (ICH) [3] suggestions.

MATERIALS AND METHODS

Chemicals, samples and reference standards

Analytical reagent grade of Perchloric Acid (70%), HPLC grade of Methanol and Acetonitrile. Perchloric Acid (70%), Methanol and Acetonitrile were obtained from Merck KGaA, Germany. A Milli-Q purification system (Millipore, Bedford, MA, USA) was used to further purify demineralized water.

A Small amount of Lifitegrast API, Impurity – I and Impurity - II were got as gift materials. Impurity – I stock solution 1.0mg/mL solution was prepared in methanol and Impurity – II stock 1.0 mg/mL was prepared with chilled mixture of Acetonitrile: Methanol (1:1). 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, Vial sampler injects from up to 132 standard 2 mL vials and has a pressure rating of 600 or 800 bar and time-programmable wavelength switching provides optimum sensitivity and selectivity for your applications. Analysis was done using a high pH-resistant column, the Primesil C18, 3µm, 4.6 X 250 mm from WESLEY Technologies Inc 7052 S. Eagle Valley Road, Port Matilda, PA 16870 for impurity estimation from Lifitegrast bulk drug. These columns were designed to resist a pH range from 1.0 to 10.0. Column temperature was adjusted to 25°C and maintained constant using inbuilt column thermostat. Several trials were carried out to separate all the possible degradation impurities from main peak. At finally a simple gradient method with mobile phase A 100% buffer and mobile phase B consists of 70% Acetonitrile and 30% buffer solution in bulk drugs.

Buffer solution: Transfer accurately 2.0 mL of Perchloric acid (70%) in 1000 mL of Milli-Q water. Filter the solution through 0.22 µ PVDF filter and degas it.

The gradient program is follows.

Gradient Program

Time (in min)	Mobile phase A (%)	Mobile Phase B (%)
0.01	55	45
3	55	45
18	40	60
37	08	92
50	08	92
51	55	45
60	55	45

The flow rate maintained 1.0 mL/min and injection volume is 5 µL for bulk drug. Before use, the mobile phase was degassed by sonication for about 10.0 min.

Method validation

The stated study is aimed to develop an analytical method for the estimation of impurities of Lifitegrast API. We have focused validation efforts toward necessary test parameters for estimation of impurities, 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. The analytical method validation was performed as per the recommended guidelines provided by the International Conference on Harmonization (ICH) guidelines [3]. Accuracy parameter was done as this method was developed for estimation of impurities for bulk drugs. The developed and validated methods are well suitable for estimation of impurities in Lifitegrast bulk drug.

Precision

Repeatability and intermediate precision should be evaluated for assessment of precision. Repeatability was determined by six repetitive sample preparations of Lifitegrast bulk drug with known concentrations of Impurity – I and Impurity -II. The relative standard deviations (RSD) would be calculated for impurities [4]. For intermediate precision, impurities estimation was

done on different day by using same HPLC equipment using the respective standard. Samples were prepared in such a way to obtain 1000 µg/mL solutions for bulk drug.

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 impurity estimation method, it is crucial to be able to selectively determine the concentration of the impurity compound without interferences from the expected related impurity substances. Therefore, resolution between the impurity peaks 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. [3]

The accuracy of the impurity method for bulk drug was evaluated by spiking the available impurities in triplicate at five concentration levels, i.e., LOQ, 50%, 100% and 150%, by considering impurity levels in bulk drugs at 0.15% of sample concentration.

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 Impurity – I and Impurity – II of Lifitegrast, the stock solutions were diluted with diluent to impurity samples solution of LOQ to 2.25 µg/mL (i.e LOQ to 150% of Impurity level 0.15%). This range was sufficiently large as ICH guidelines usually prescribe a range for impurities. 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 method of impurities, the optimized HPLC conditions set for this method have been slightly modified for samples of Lifitegrast with 0.15% of impurity levels to evaluate the method robustness. The small changes include the effect of column temperature at 25° ± 5Ci.e. 20°C and 30°C instead of 30°C. The effect of flow rate was at 0.9 and 1.1mL/min instead of 1.0mL/min.

Stability of stock solutions

This study was performed on Lifitegrast sample preparation with impurities. The prepared solution was kept at 5°C for 5 days. The Lifitegrast sample solution with impurities at 0.15% level was prepared and stored at 5°C. At scheduled time intervals, this solution was injected into the HPLC system and chromatogram was recorded. Calculated % of impurity level and difference was estimated from initial result.

RESULTS

Method development

As part of method development, literatures were referred for Lifitegrast impurities estimation wavelength where it will give the maximum absorbance of impurities. All the literatures were stating that the maxima wavelength for Lifitegrast would be about 215 nm [5]. To confirm wavelength maxima, 50 µg/mL of Lifitegrast API was prepared in Methanol: Water (50:50v/v) mixture and scanned for maximum wavelength. From the above solution spectrum, the maximum was found to 215 nm and same was utilized for the further development and validation actives for bulk drug. The spectrum was presented as figure 1.

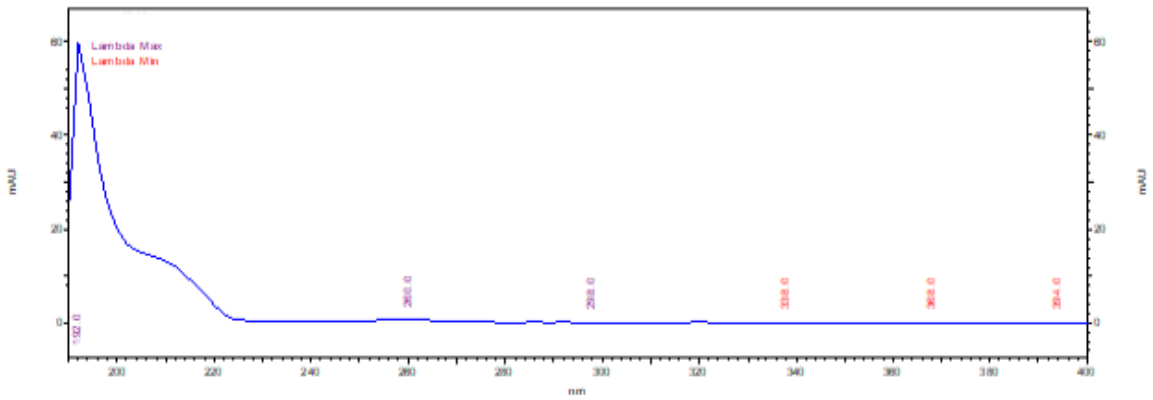


Figure No. 1: UV spectrum of 50 µg/mL of Lifitegrast API in mixture of Methanol: Water (50:50 v/v)

Initially, the assay method development was initiated with a simple mobile phase n-Hexane and dehydrated alcohol 50:50 v/v and the HPLC column Inertsil SIL100A, 250X4.6 mm, 5.0µ. The flow rate used as 1.0 mL /min. The impurity peaks was merged and not resolving properly. The mobile phase was modified to n-Hexane and dehydrated alcohol 94:06 v/v with same column. With the proposed mobile phase composition and column, all the impurity peaks were resolving in bulk drug product.

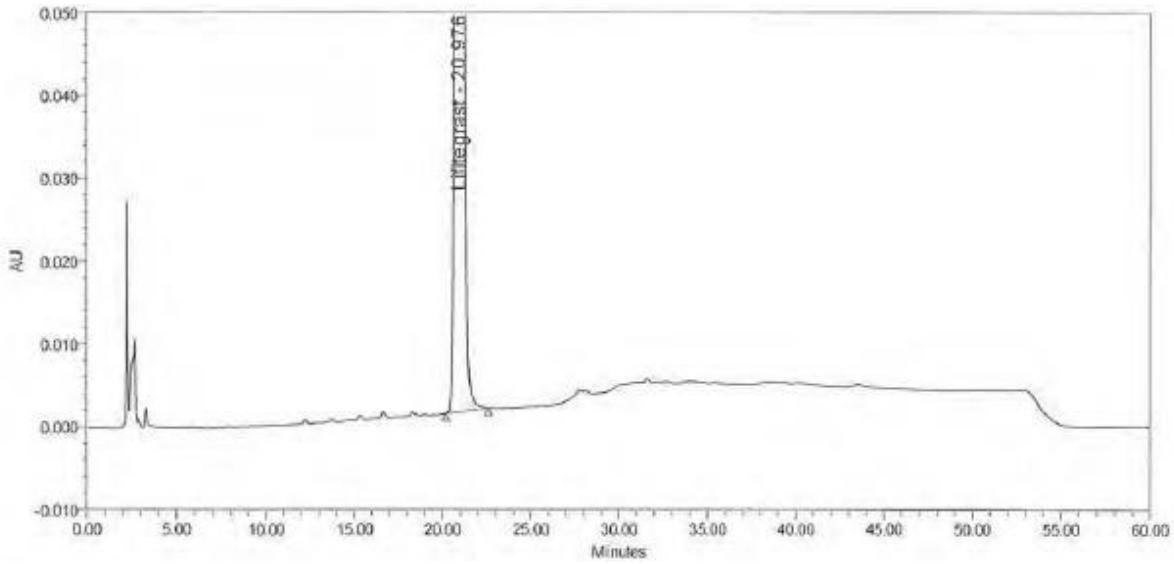


Figure No. 2: Typical chromatogram of Lifitegrast bulk drug

Validation

Precision

As discussed, repeatability and intermediate precision have been evaluated. Six consecutive sample preparation of 1000 µg/mL of Lifitegrast spiked with impurities at 0.15% level of bulk drugs analysis have been performed. The % RSD of impurities were calculated against diluted standard peak were found below 15.0% for both bulk drug. This analysis was performed during two different days on same HPLC systems and the RSD calculated on the obtained averages for each day was below 15.0%. These results show a sufficient intermediate precision of the impurity estimation method. The results were tabulated below table. (Table 1)

Table No. 1: Precision and Intermediate Precision data for Lifitegrast bulk drug

S. No	Sample Description	% of Impurities w/w			
		Precision (Day-1)		Intermediate Precision (Day-2)	
		Imp-I (RRT 1.10)	Imp-II (RRT 1.70)	Imp-I (RRT 1.10)	Imp-II (RRT 1.70)
1	Preparation - 1	0.1438	0.1469	0.1448	0.1485
2	Preparation – 2	0.1453	0.1487	0.1473	0.1431
3	Preparation – 3	0.1409	0.1403	0.1439	0.1408
4	Preparation – 4	0.1456	0.1546	0.1466	0.1519
5	Preparation – 5	0.1510	0.1506	0.1523	0.1540
6	Preparation - 6	0.1456	0.1511	0.1414	0.1517
Average:		**0.1454**	**0.1487**	**0.1461**	**0.1483**
% RSD		**2.3**	**3.3**	**2.5**	**3.6**
Cumulative % RSD				**2.3**	**3.3**

Accuracy:

The accuracy of the analytical method was performed by spiking the impurities to Lifitegrast API at LOQ, 50%, 100% and 150% levels. i.e. 0.1µg/mL (LOQ level) , 0.75 µg/mL (50% level), 1.5 µg/mL (100%)to 2.25 µg/mL (150% level). The accuracy results were tabulated below. From

the recovery data, it can be concluded that the analytical method is found accurate from 0.1µg/mL to 2.25 µg/mL (i.e. LOQ to 150% level).

Table No. 2: Accuracy data for Lifitegrast Impurity – I

S. No	Level	Recovery results		
		Amount Add (in µg/mL)	**Amount found (in µg/mL)**	**Recovery (%)**
1	LOQ	0.09	0.0945	105.0
2	50%	0.75	0.7461	99.5
3	100%	1.51	1.4632	96.9
4	150%	2.26	2.1569	95.4
Acceptance Criteria: Recovery should be 85% to 115%				

Table No. 3: Accuracy data for Lifitegrast Impurity – II

S. No	Level	Recovery results		
		Amount Add (in µg/mL)	**Amount found (in µg/mL)**	**Recovery (%)**
1	LOQ	0.11	0.1061	96.5
2	50%	0.76	0.7542	99.2
3	100%	1.48	1.4962	101.1
4	150%	2.25	2.2156	98.5
Acceptance Criteria: Recovery should be 85% to 115%				

Linearity

The adjusted method yielded a linear calibration curve over the chosen range for Lifitegrast in both methods. The regression equation obtained was y = 37088x-115.11, with the correlation coefficient (r^2) being 0.9999 for Lifitegrast Impurity – I and y = 27785x+318.52, with the correlation coefficient (r^2) being 0.9996 for Lifitegrast Impurity – II. Since the correlation coefficients (r^2)of curves is greater than 0.95, a good linear relationship between the detector response and the concentration of analyte could be concluded [7]. For the Lifitegrast 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. 4: Linearity data of Lifitegrast Impurity I

S. No	% of Nominal Concentration	Concentration (µg/mL)	Avg. Peak Area
1.	LOQ	0.09	3526
2.	50	0.75	27456
3.	80	1.21	44692
4.	100	1.51	55964
5.	120	1.81	66754
6.	150	2.26	84153
Correlation coefficient (r^2)			0.9999
Slop			37088
Intercept			-115.11

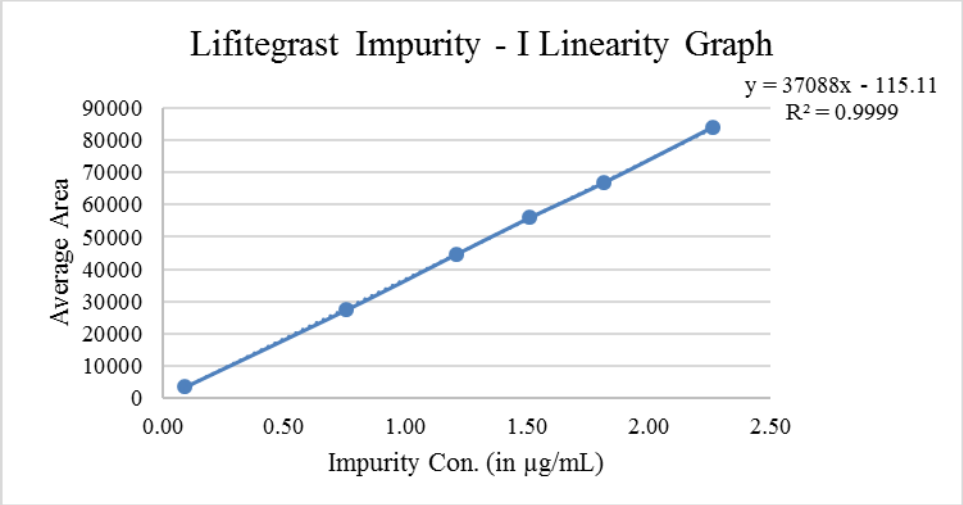


Figure No. 3: Lifitegrast Impurity – I Linearity Graph

Table No. 5: Linearity data of Lifitegrast Impurity - II:

S. No	% of Nominal Concentration	Concentration (µg/mL)	Avg. Peak Area
1.	LOQ	0.11	3425
2.	50	0.76	21263
3.	80	1.18	33245
4.	100	1.48	41856
5.	120	1.82	50145
6.	150	2.25	63146
Correlation coefficient (r2)			0.9996
Slop			27785
Intercept			318.52

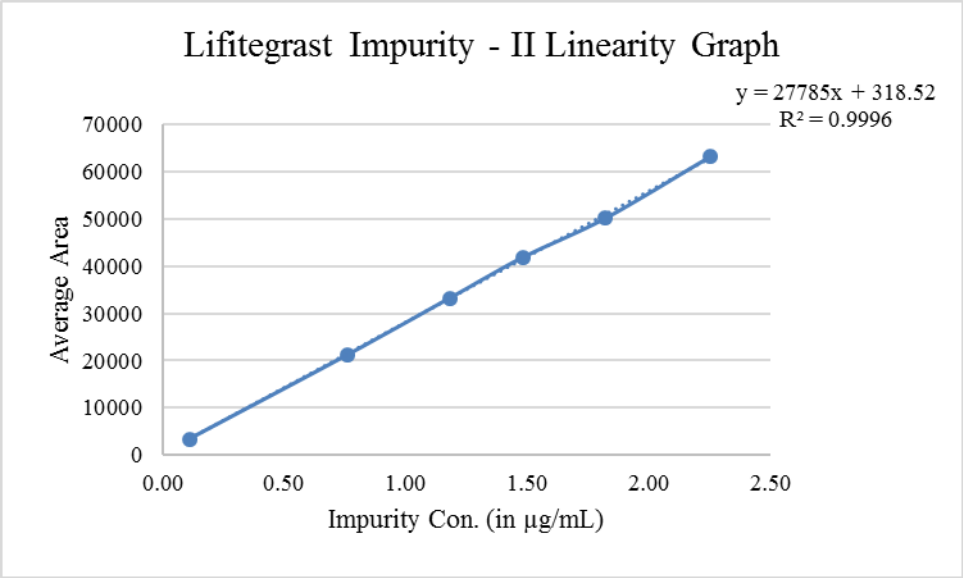


Figure No. 4: Lifitegrast Impurity – I Linearity Graph

Selectivity

This study focuses on possible impurity separation from each other's and from the main peak of Lifitegrast and therefore also needs to investigate the separation from relevant impurities. Lifitegrast was thermally degraded and analysed for impurity separation. About 5.0 mg of Lifitegrast was heated at 60°C for 24.0 hrs. The typical chromatograph is given below. Finally,

the sample preparation was done as per methodology and chromatographed for impurity separation. All the degraded impurities were well separated from each other's and from main peak from bulk drugs. The typical chromatograph is given below.

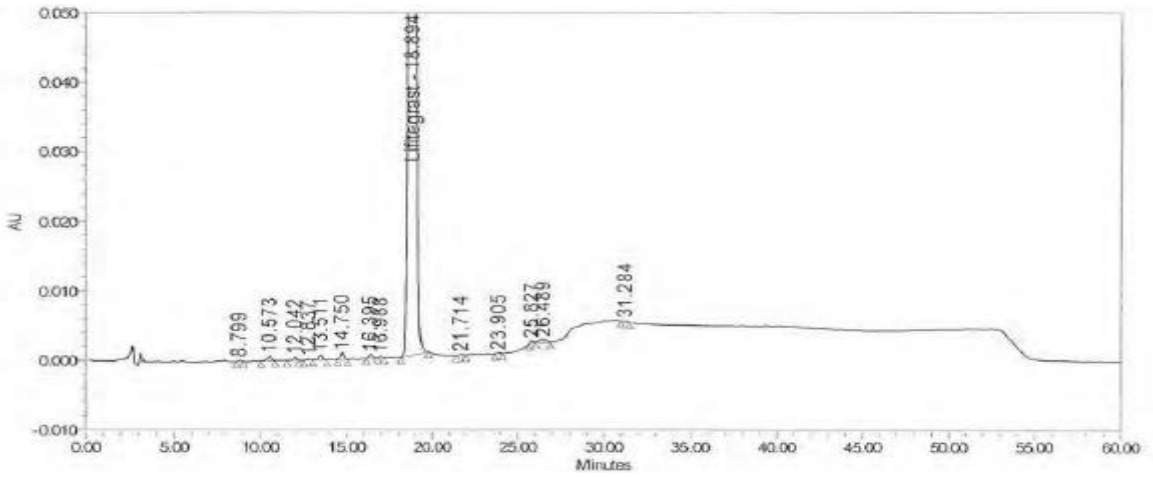


Figure No. 5: Thermally degraded Lifitegrast bulk drug chromatograph

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. It was found that the peak area and retention time of impurity as well as main peaks are slightly influenced by the flow rate variation from the optimum conditions and there is no effect on the quantification of impurity estimation. The results were tabulated below. This means that the method was found to be robust with respective to flow rate.

Table No. 6: Robustness Column Temperature 25°C to 20°C results:

S. No	Sample Description	Robustness recovery results		
		Amount Add (in µg/mL)	Amount found (in µg/mL)	Recovery (%)
1	Lifitegrast Impurity - I	1.5052	1.4985	99.6
2	Lifitegrast Impurity - II	1.5147	1.4663	96.8
Acceptance Criteria: Recovery should be 85% to 115%				

Table No. 7: Robustness Column Temperature 25°C to 30°C results

S. No	Sample Description	Robustness recovery results		
		Amount Add (in µg/mL)	**Amount found (in µg/mL)**	**Recovery (%)**
1	Lifitegrast Impurity - I	1.5052	1.4865	98.8
2	Lifitegrast Impurity - II	1.5147	1.5013	99.1
Acceptance Criteria: Recovery should be 85% to 115%				

Table No. 8: Robustness Flow rate 1.0 mL to 0.9 mL results

S. No	Sample Description	Robustness recovery results		
		Amount Add (in µg/mL)	**Amount found (in µg/mL)**	**Recovery (%)**
1	Lifitegrast Impurity - I	1.5052	1.4887	98.9
2	Lifitegrast Impurity - II	1.5147	1.4975	98.9
Acceptance Criteria: Recovery should be 85% to 115%				

Table No. 9: Robustness Flow rate 1.0 mL to 1.1 mL results

S. No	Sample Description	Robustness recovery results		
		Amount Add (in µg/mL)	**Amount found (in µg/mL)**	**Recovery (%)**
1	Lifitegrast Impurity - I	1.5052	1.4796	98.3
2	Lifitegrast Impurity - II	1.5147	1.4823	97.9
Acceptance Criteria: Recovery should be 85% to 115%				

Stability of stock solutions

Lifitegrast sample solution was prepared by spiking with impurities at 0.15% level and kept at 5°C for 5 days. The sample solution remained stable at 5°C during the whole period of test procedure for 5 days. At 5°C, the solution stability is guaranteed for 5 days.

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 Perchloric acid in water and acetonitrile in buffer solution different combinations was tried during the initial development stages. The sensitivity and peak shape were also checked. The best signal and peak shapes for Lifitegrast and its impurities were achieved using a stationary phase Primesil Cl 8, 3µm, 4.6 X 250mm, 5µ for bulk drug product. The mobile phase is gradient with mobile phase A is 100% buffer and mobile phase B consisted 70% Acetonitrile and 30% buffer solution. The flow rate maintained as 1.0 mL/min and column temperature is 25°C and autosampler temperature was maintained at 5°C. The proposed method was validated as per the ICH guidelines for its precision, linearity, specificity, and robustness. No interference peaks were observed in the chromatogram of blank solution at the retention time of Lifitegrast. The method is very robust, simple and specific, as all the impurity peak were well separated from each other which makes it especially suitable for routine quality control analysis.

CONCLUSION

The present method is specific, rapid, precise, accurate, linear and robust with respective to flow rate. The mobile phase was easy to prepare. Application of these methods for the analysis of impurity estimation in both bulk drugs reveals that the degradation products interfere with the analytical determination. This indicates that the proposed methods could be used as a stability-indicating method for the determination of impurities either in bulk drug product. Therefore, this method could easily be used in a wide range of analytical laboratories.

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