INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Method Development And Validation For Simultaneous Estimation Of Lopinavir In Combined Dosage Form By HPLC Chromatography

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ARTICLE INFO

Received: 22 May 2024
Accepted: 26 May 2024
Published: 07 June 2024
Keywords:
Lopinavir, UV-Spectrophotometer, Equation, RP-HPLC, Validation, tablet.
DOI:
10.5281/zenodo.11520468

ABSTRACT

The purpose of this study was to provide an accurate, straightforward, and RP-HPLC method for analyzing lopinavir in tablet form. A UV-Spectrophometric technique was created to estimate the dose of hydrochlorothiazide and fosinopril sodium in tablet .The suggested techniques were utilized to determine the drug's dose in tablet form. The equation approach is used to determine lopinavir. By solving the equation and using the resulting absorbtivity values for the drug's wavelength of 233 nm, the concentration of each medication was determined using this approach. The estimate of Lopinavir in tablet dosage form was developed and verified using a fast and accurate RP-HPLC technique. Using a gradient mode and acetonitrile (90 ml and 10 ml, pH 3.0, 0.05% OPA with TEA) as the mobile phase, the RP-HPLC procedure was carried out on a C18-(250 mm x 4.6 mm) sample with a particle size of 5 µm. The sample was detected at nm. According to the retention time of 3.99 minutes for lopinavir, respectively. The technique was used on tablet formulations that were sold. In compliance with ICH recommendations, the tablet assay was carried out for combination and validated for accuracy, precision, linearity, specificity, and sensitivity. The procedure is repeatable, precise, fast, accurate, and dependable in terms of validation. The calibration plots for Lopinavir showed a linear trend between 12.5 and 62.5 µg/ml, and the recovery rates from tablets ranged from 97% to 101%. The technique is applicable. The method can be used for routine of the quality control in pharmaceuticals. The UV- Spectrophometric method was found to be simple, economical and rapid as compared to RP-HPLC. But, RP-HPLC method was found to be more accurate, precise and robust. Both these methods can be used for routine analysis of Lopinavir in tablet dosage form.

INTRODUCTION

Analytical science involves the systematic study and quantification of components within material systems. The process of component determination is termed analysis, encompassing both physical and chemical methodologies. Chemical analysis, or analytical chemistry, specifically focuses on methods for discerning the chemical composition

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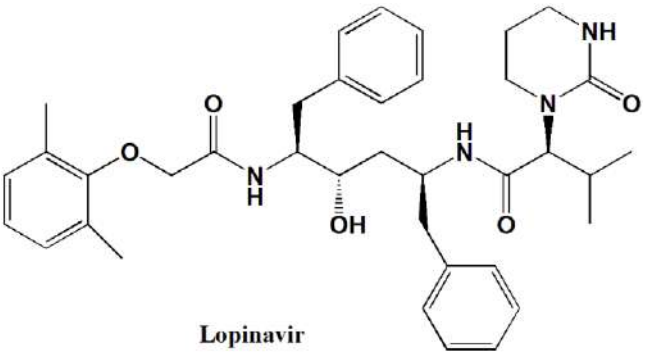
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

of matter. Qualitative approaches reveal details about atomic or molecular species, including functional groups, while quantitative methods provide numerical data on component concentrations .Modern analytical chemistry demands precise measurements at minimal concentrations, often necessitating high-resolution separations using chromatographic techniques before analysis. Proficiency in analytical instrumentation is crucial for advancing scientific endeavors across disciplines such as pharmaceutical chemistry, medicinal chemistry, biochemistry, biotechnology, and environmental sciences. Analytical techniques are integral to ensuring substance quality and form a cornerstone of quality assurance and control. Analytical chemists bear responsibility for the reliability, utility, accuracy, precision, and specificity of measurements. Pharmaceutical analysis encompasses methods essential for determining the identity, potency, quality, and purity of drugs and chemicals.



Lopinavir

MATERIAL AND METHOD

Reagents and materials

Lopinavir were kindly supplied as a gift sample by J. P. Npharma. (Mumbai, India) And Auspi Life pharma private Ltd. (Hyderabad, Telangana). These drugs were used as working standard. All the chemicals used were of HPLC grade (Merck Chem. Ltd., Mumbai) used without further purification. Double distilled water was used for mobile phase preparation.

Selection of chromatographic Mode

RP-HPLC was used for separation of both these drugs.

Selection of stationary phase

RP- Agilent C18(250 mm ×4.6 mm I.D.) with particle size 5 µm was selected.

Selection of mobile Phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey; acetonitrile and phosphate buffer was selected.

Preparation of stock standard solution

Mixed stock standard solution of Lopinavir (1000 µg/mL) were prepared by dissolving 10 mg of Lopinavirin 10 mL methanol.

Selection of Detector and Detection wavelength

From the spectra 260 nm was selected for the estimation of both these drugs simultaneously .

Optimization of Chromatographic Parameters

Optimization in HPLC is the process of indentifying a set of conditions that effectively separate and allow the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity,specificity, cost, ease and speed.

Optimization of mobile phase strength

With a view to separate out both the drugs simultaneously, various mobile phases consisting of methanol and water were tried, but tailing and low resolution of the chromatogram was observed . Therefore, mobile phase consisting of methanol and potassium dihydrogenphosphate (65:35v/v) was tried and both these drugs were resolved properly. Well defined chromatograms were observed when the pH of the buffer was adjusted to 3.5 with OPA at flow rate of 0.7 mL/min ; the retention time for Lopinavir was found to be 4.94 ± 0.02 min, respectively. The total time of analysis was less than 10min.

Linearity studies

From the stock standard solution, aliquots portions (5-25mL) were transferred into a series of 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of5-25µg/mL for Lopinavir. A constant volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Analysis of Bulk Material

In order to see the feasibility of the method in the marketed formulation, it was first tried in physical laboratory mixture. Accurately weighed quantity of 10 mg (Lopinavir) were transferred to 10 mL volumetric flask containing 10 mL methanol and volume was adjusted up to mark. It was further diluted to get concentration 40 µg/mL of Lopinavir. Constant volume 20 µL was injected into column and peak area was recorded. The concentration of both these drugs were determined from their respective linearity curves. The procedure was repeated for two times.

Analysis of tablet formulation

To determine the content of Lopinavirin capsule formulation; twenty tablets (Label claim: Lopinavir10 mg) were weighed accurately, their content removed and finely powdered. A quantity of powder equivalent to 10 mg of Lopinavir was weighed and transferred into 10 mL volumetric flask containing about 10mL methanol. The solution was filtered through 0.45 µm membrane filter paper. The solution was further diluted with mobile phase to obtain concentration 15µg/mL (Lopinavir). The sample solutions were injected into column for six times. The concentrations of both these drugs were calculated from their linearity curve. Validation, the proposed method was validated as per ICH guidelines.

Accuracy

It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard Lopinavir were added to pre-analyzed sample (10±g/mL of Lopinavir) and subjected them to the proposed HPLC method.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Intra-day precision was studied by analyzing 10,15 and 20µg/mL of Lopinavir for three times on the same day. Inter-day precision was checked analyzing the same concentration for three different days over a period of week. Repeatability was measured by analyzing 10□g/mL of Lopinavir for six times.

Sensitivity

The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae. LOD= 3.3(SD)/S; LOQ = 10 (SD)/S; Where SD = Standard Deviation of response, S = the slope of the calibration curve. LOD and LOQ were found to be µg and respectively.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of both the drugs; also the base line did not show any significant noise. The specificity of the HPLC method was determined by complete separation of lopinavir along with other parameters like retention time (tR), tailing factor etc.

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Table 1: Final Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
HPLC System	Agilent Technologies 1100 series (Gradient System)
Pump	G 1311 A solvent delivery system (Quaternary pump)
Detector	G1315D Diode array detector
Data processor	Chemstation
Stationary phase	Agilent C18 column (250 m×4.6mm,5µ)
Mobile phase	Acetonitrile :0.05 % OPA Water (65:35,*v/v*)
Detection wavelength	260 nm
Flow rate	0.7mL/min
Sample size	20 µl

Table 2: Linearity Study of Lopinavir

Sr. No	Concentration of Lopinavir [±g/mL]	Peak area [Mean ± SD; n= 5]	% RSD
1	5	367.16 ± 2.17	0.59
2	10	655.02 ± 3.37	0.51
3	15	922.17 ± 6.41	0.69
4	20	1242.73 ± 1.58	0.13
5	25	1498.98 ± 0.57	0.04

Table 3 Analysis of bulk material

Component	Amount Taken [mg]	Amount Found [mg] ±SD	%RSD [n=6]
Lopinavir	15	937.93±12.17	1.30

Brand Name: MONOPRIL HCT Mfg. By: Bristol-Myers

Batch No.: NDC 0087-1492-01Average Weight: 10/12.5 mg

Table 4: Analysis of Capsule formulation

Component	Amount Taken [mg]	Amount Found [mg] ±SD	% Label Claim	%RSD [n=6]
Lopinavir	15	937.93±12.17	100.07	1.30

Table 5: Recovery studies

Drugs	Initial amount [±g/mL]	Excess drug added to the analyte [%]	Amount recovered ± S.D.[±g/mL]	Excess drug Recovery[%]	%RSD [n = 3]
Lopinavir	10	80	18.05 ±0.08	100.28	0.44
	10	100	20.11± 0.03	101.14	1.07
	10	120	22.23± 0.11	101.97	0.23

Table 6: Precision studies

Drug	Concentration [±g/mL]	Peak Area Mean ± SD, [n=6]	% RSD
Lopinavir	25	24.54± 0.23	0.92

Table 7: Repeatability studies

	HCZ	
Parameters	**Tailing Factor**	**Theoretical Plates**
Change in Detector signal 259 261	 0.68 0.67	 6376 6375
Change in mobile phase composition (acetonitrile:OPA Water 64 : 36*v/v*) (acetonitrile:OPA Water 66 : 34*v/v*)	 0.68 0.69	 6730 6957
Change in flow rate 0.6 0.8	 0.67 0.68	 7181 6377

Table 8: Robustness Studies

System suitability Parameters	Lopinavir
Retention time (tR)	3.66 min
Theoretical plate (N)	6938
Tailing factor (T)	0.73

Figure 1. Calibration curve for Lopinavir
Y = 14.51x + 10.18

Slope = 14.51, Intercept = 10.18, Correlation coefficient = 0.999

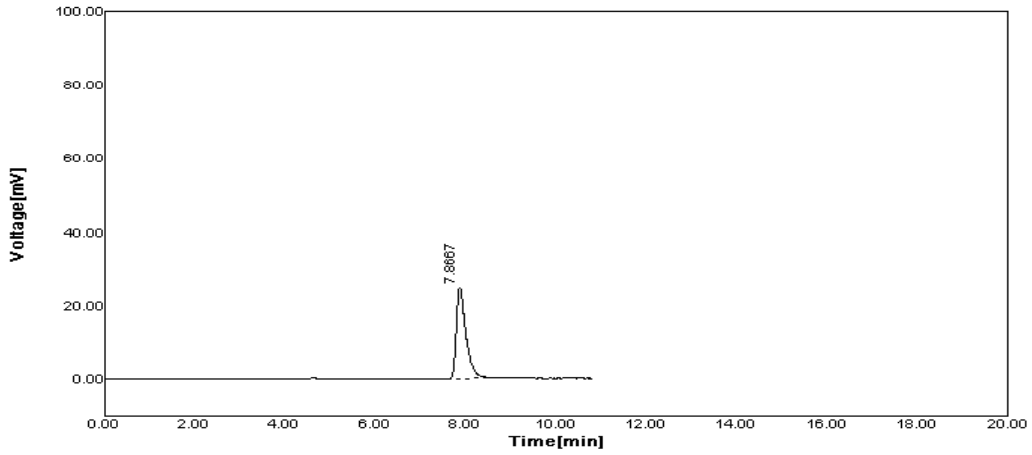


Figure 1: Chromatogram of Lopinavirin acetonitrile: Water (65: 35v/v) as mobile phase

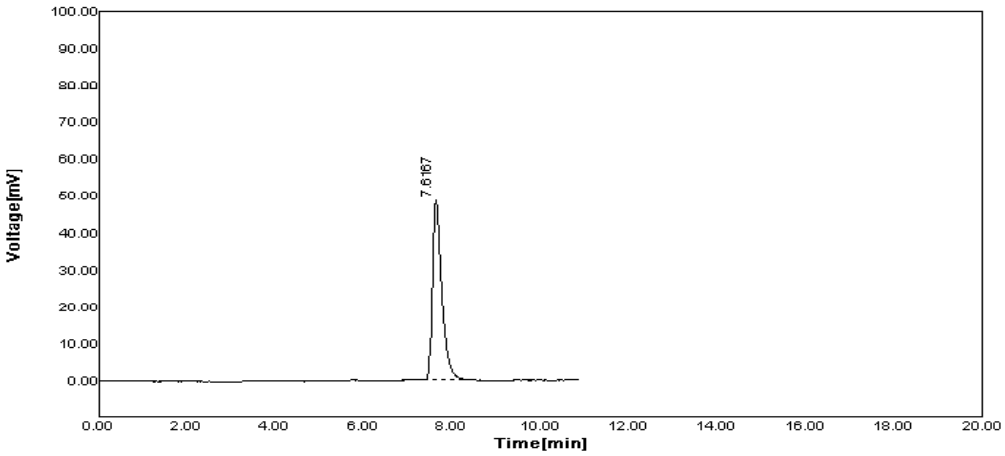


Figure 2: Chromatogram of Lopinavir (10µg) mixed stock standard solution

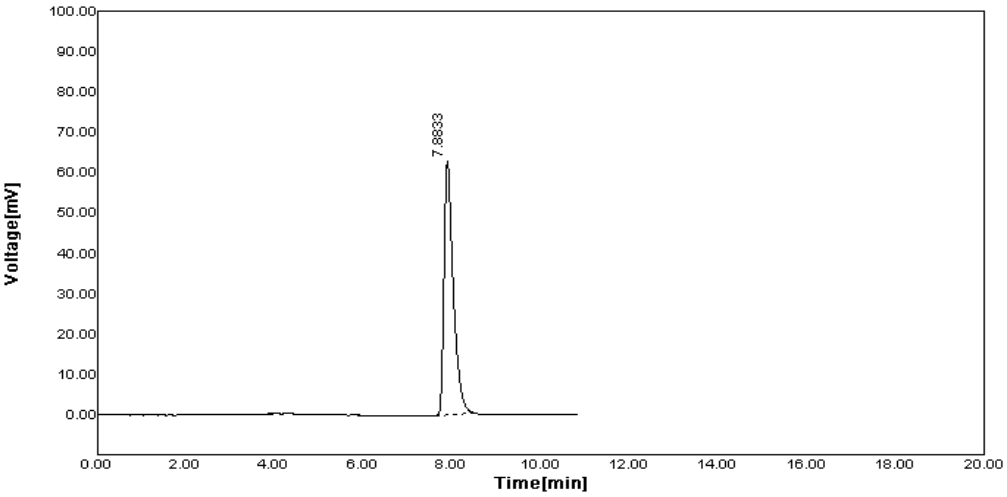


Figure 3: Chromatogram of Lopinavir (Extracted from tablets)

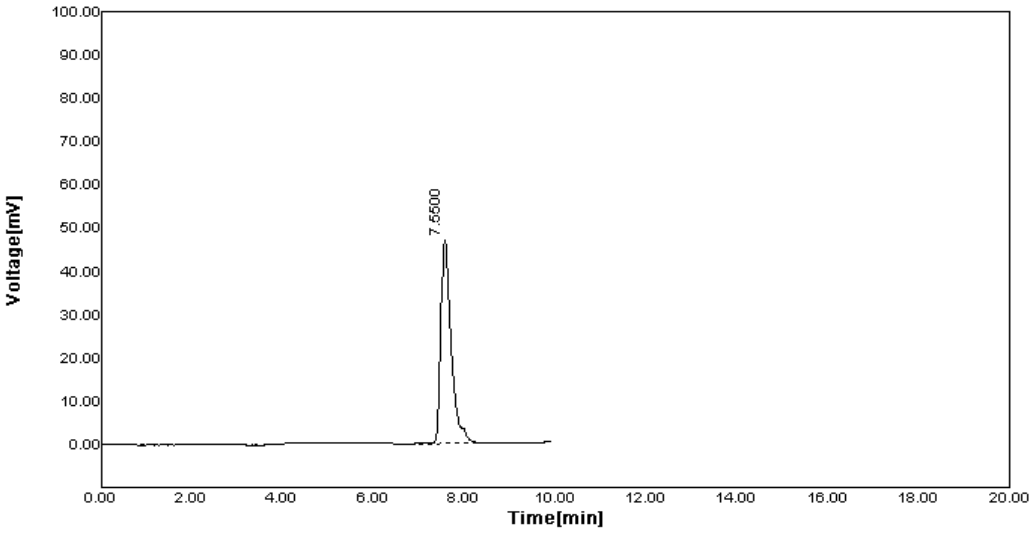


Figure 4: Chromatogram of Lopinavir at ACN: OPA Water64:36v/v

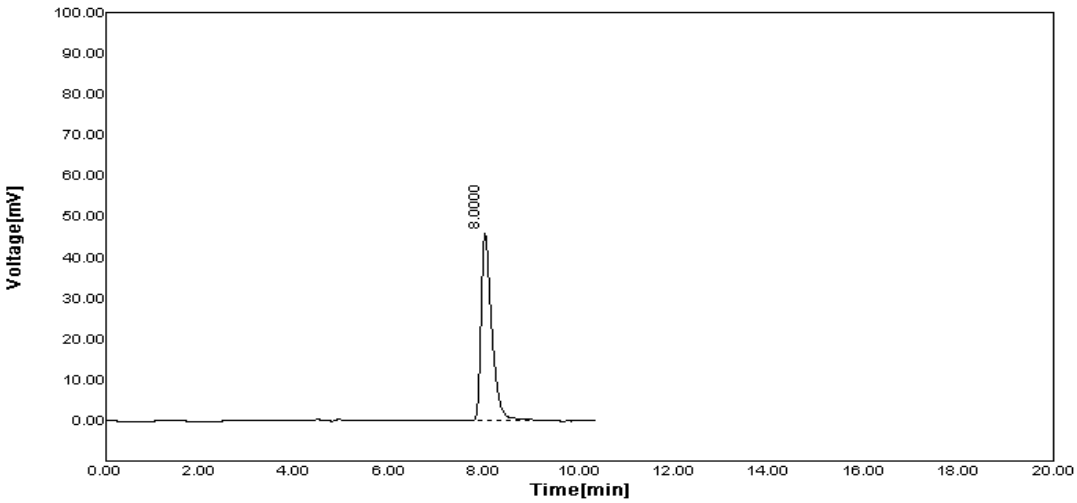


Figure 5 Chromatogram of Lopinavirin ACN: OPA Water (61:34v/v) pH 3.3 as mobile phase

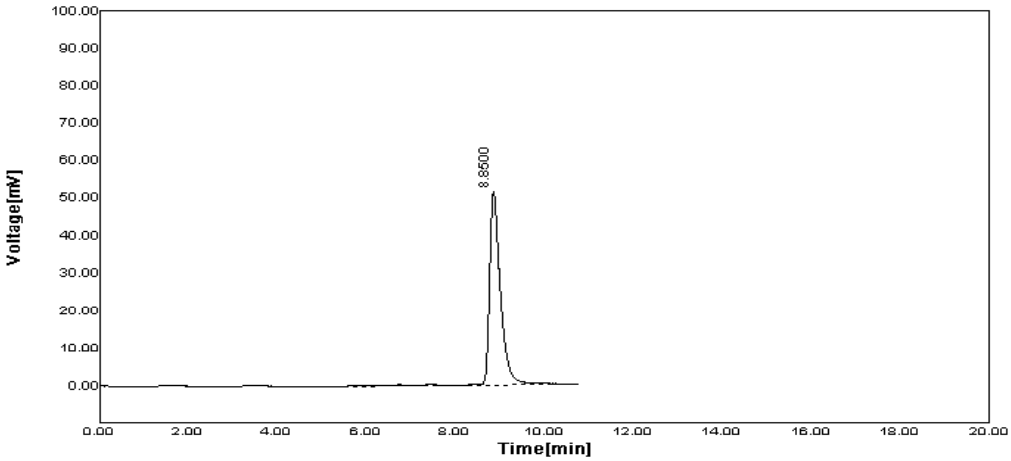


Figure 6 Chromatogram of Lopinavir at Flow rate 0.8mL/min

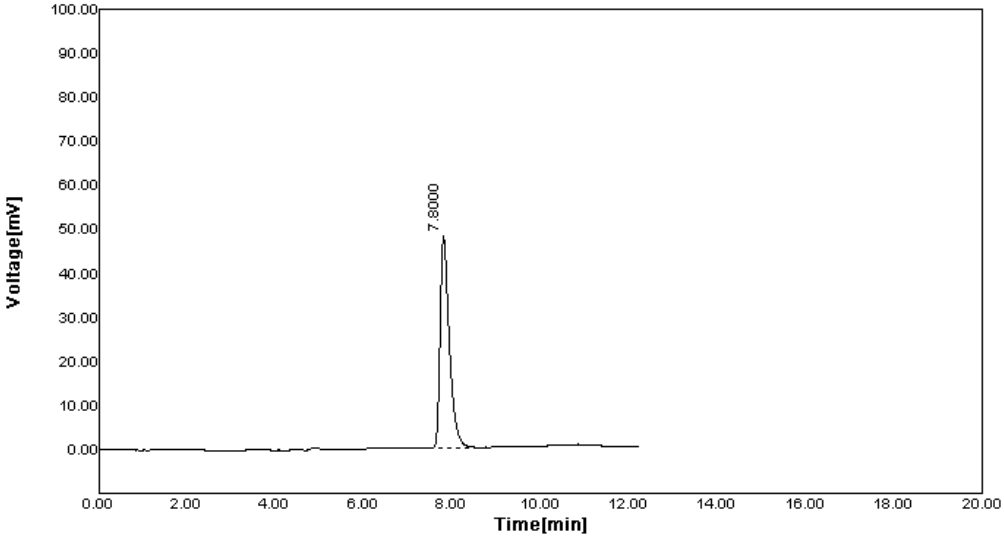


Figure 7 Chromatogram of Lopinavir at Wavelength change 259 nm

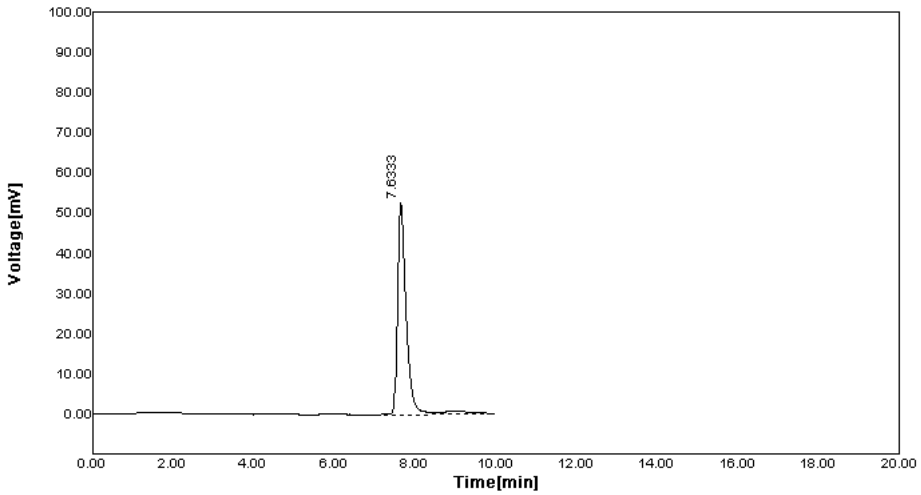


Figure 8 Chromatogram of Lopinavir at Wavelength change 261 nm

CONCLUSION

A RP-HPLC method has been developed and validated for the determination of Lopinavirin bulk and in capsule formulation. The HPLC analysis was performed on the Agilent C18 column (250 mm ×4.6mm) 5µm particle size ingredient mode, at ambient temperature using acetonitrile :OPA water(65:35v/v) as mobile phase; flow rate was set at 0.7mL/min. The detection was carried out at 260 nm. The retention time for Lopinavir was found to be found to be 3.66± 0.02 min and 4.94± 0.02 min, respectively. Lopinavir followed linearity in the concentration range of 12.5–62.5 µg/mL (r2 = 0.999) and 10-50µg/mL(r2 = 0.999), respectively. The method has successively been applied for the determination of Lopinavirin marketed formulation. There was no Interference from the excipients routinely present in the capsule. The drug contents for HCZ and FNZ were found to be 99.90 ± 0.48 % and 99.54 ± 0.36%, respectively. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within Range of 99.02 – 99.83%.The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The results were examined as %RSD values of concentration of drugs determined. The low value of %RSD(less than 2) indicates high precision of the method. The method proved to be adequately sensitivity as indicated by low values of LOD and LOQ. The robustness of the method was studied by making deliberate variations in chromatographic conditions and the effects on the results were examined as %RSD, (less than 2). The low values of % RSD indicate robustness of the method. The result did not show any statistical difference between operators suggesting that method developed was rugged. Methods (RP-HPLC multi-component mode of analysis) have been developed for simultaneous determination of Lopinavir. RP-HPLC methods are found to be accurate, precise, rugged and robust.

RESULT

A robust and reliable RP-HPLC method has been successfully developed and validated for the determination of Lopinavir in bulk form and within capsule formulations. The analysis was conducted using an Agilent C18 column with specific chromatographic conditions, demonstrating good sensitivity and precision. The method exhibited excellent linearity within defined concentration ranges for both bulk and capsule formulations. Additionally, the method's accuracy was confirmed through recovery studies,

showing consistent drug content results within acceptance criteria. The precision of the method was evaluated through repeatability, intra-day, and inter-day precision studies, with %RSD values indicating high precision (less than 2%). Robustness was demonstrated by deliberate variations in chromatographic conditions, which did not significantly affect the results. Furthermore, the method's ruggedness was confirmed by consistent outcomes across different operators, highlighting its reliability and suitability for routine use in pharmaceutical analysis. Overall, this RP-HPLC method proves to be accurate, precise, rugged, and robust, making it a valuable tool for the simultaneous determination of Lopinavir in pharmaceutical formulations.

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HOW TO CITE: Priti S. Sonawane, Poonam Varadhe, P. R. Patil , Method Development And Validation For Simultaneous Estimation Of Lopinavir In Combined Dosage Form By HPLC Chromatography , Int. J. of Pharm. Sci., 2024, Vol 2, Issue 6, 442-451. https://doi.org/10.5281/zenodo.11520468