RESEARCH PAPER

Development and Optimization of a Reverse Phase HPLC Method for Simultaneous Estimation of Loratadine and Pseudoephedrine in Combined Dosage Form

Krati Dhakad[1*], Dr. Ramakant Sharma[2], Dr. Sudha Vengurlekar[3]

[1]Research Scholar, University Institute of Pharmacy, Oriental University, Indore M. P.

[2]Associate Professor, University Institute of Pharmacy, Oriental University, Indore M. P.

[3]Dean and Principal, University Institute of Pharmacy, Oriental University, Indore M. P.

ABSTRACT

A simple, rapid, and efficient reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and optimized for the simultaneous estimation of Loratadine and Pseudoephedrine in combined pharmaceutical dosage form. Chromatographic separation was achieved using an Agilent C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of methanol and 0.1% orthophosphoric acid in water (37:63, v/v, pH 3.2) at a flow rate of 1.0 mL/min. Detection was carried out at 232 nm. The method was optimized through systematic variation of mobile phase composition and flow rate to achieve satisfactory resolution and peak symmetry. The retention times for Pseudoephedrine and Loratadine were found to be 3.47 min and 5.61 min, respectively. The method exhibited excellent linearity in the concentration range of 24–120 µg/mL for Pseudoephedrine and 1–5 µg/mL for Loratadine, with correlation coefficients (R^2) of 0.999. The developed method demonstrated good chromatographic performance and can be successfully applied for routine quality control analysis of combined dosage forms.

Keywords: RP-HPLC, Method Development, Loratadine, Pseudoephedrine, Chromatographic Optimization, Simultaneous Estimation

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INTRODUCTION

Loratadine is a widely used second-generation antihistaminic agent indicated for the treatment of allergic conditions such as allergic rhinitis and urticaria, whereas Pseudoephedrine is a sympathomimetic drug commonly used as a nasal decongestant. The combination of these drugs is frequently prescribed to provide enhanced therapeutic efficacy in respiratory allergic disorders (Sweetman, 2009). Due to the increasing use of such combination therapies, the development of reliable and efficient analytical methods for their simultaneous estimation is essential for ensuring quality, safety, and efficacy of pharmaceutical products.

High-performance liquid chromatography (HPLC) is one of the most widely employed analytical techniques in pharmaceutical analysis due to its high sensitivity, accuracy, and reproducibility (Snyder, Kirkland, & Dolan, 2010). Several analytical methods have been reported for the determination of individual drugs; however, simultaneous estimation of combination drugs often presents challenges such as peak overlapping, poor resolution, and longer retention times (Blessy et al., 2014).

Method development in HPLC involves careful optimization of chromatographic parameters such as mobile phase composition, pH, flow rate, and detection wavelength to achieve adequate separation with good peak symmetry and resolution. The selection of appropriate chromatographic conditions is critical to ensure reproducibility and robustness of the analytical method (Kazakevich & Lobrutto, 2007).

Therefore, the present study aims to develop and optimize a simple, rapid, and reliable RP-HPLC method for the simultaneous estimation of Loratadine and Pseudoephedrine in combined dosage forms by systematically optimizing chromatographic conditions to achieve efficient separation and accurate quantification.

MATERIALS AND METHODS

Chemicals and Reagents

Pseudoephedrine and Loratadine used in the study were procured from SM Pharma & Chemicals, Mumbai. All reagents and chemicals employed were of analytical or HPLC grade, including methanol, acetonitrile, orthophosphoric acid buffer, and triethylamine, which were obtained from Merck Ltd., India. The pharmaceutical formulation selected for analysis was a marketed preparation containing a combination of Pseudoephedrine and Loratadine, commercially available under the brand name Loratadine-D 12 hr. The formulation was procured from the local market and used as such for the study.

Chromatographic conditions

The chromatographic conditions for HPLC during method development were carried out using an Agilent Technologies gradient system equipped with an auto-injector, with DAD Detector and controlled by Chemstation software. Separation was achieved on an Agilent C18 column (4.6 mm × 250 mm) with a particle size of 5 µm, serving as the stationary phase. The mobile phase consisted of methanol and water containing 0.1% orthophosphoric acid in the ratio of 37:63 (v/v), with the pH adjusted to 3.2. Detection was performed at 232 nm with a flow rate of 1.0 mL/min under ambient temperature conditions. A sample injection volume of 20 µL was used, and the total run time was set to 10 minutes. All solutions were filtered through a 0.45 µm membrane filter prior to analysis to ensure clarity and reproducibility.

Study of Pseudoephedrine and Loratadine on the chromatographic conditions used in method development of HPLC for the Following Mobile phase were tried

During method development, various chromatographic conditions were investigated to achieve optimal separation of Pseudoephedrine and Loratadine. Different mobile phase compositions were systematically evaluated using combinations of acetonitrile and methanol with water, both with and without 0.1% orthophosphoric acid (OPA), at varying ratios and flow rates. Initial trials included acetonitrile:OPA (90:10, v/v) and methanol:water systems ranging from 90:10 to 60:40 (v/v) at a flow rate of 0.7–0.8 mL/min, which did not yield satisfactory peak resolution. Further optimization involved the use of methanol:water containing 0.1% OPA at different ratios (50:50, 40:60, and 35:65, v/v) at pH 3.0, but these conditions also resulted in inadequate separation or prolonged retention times. Finally, an optimized mobile phase consisting of methanol and 0.1% OPA in water (37:63, v/v) at pH 3.0 and a flow rate of 1.0 mL/min provided sharp, well-resolved peaks with acceptable retention times, and was selected for further analysis.

Analysis of standard drugs

Analysis of standard drugs was done by Melting point, Solubility, UV spectra and λ_{max} and HPLC chromatogram and retention time

Selection of wavelength by UV-Visible Spectrophotometry:-

Preparation of standard stock solution:-

The standard stock solutions were prepared by accurately weighing 120 mg of Pseudoephedrine (PDE) and dissolving it in methanol in a 50 mL volumetric flask, making up the volume to obtain a concentration of 2400 µg/mL (Stock I). Similarly, 5 mg of Loratadine (LTD) was accurately weighed, dissolved in methanol, and diluted to obtain a concentration of 100 µg/mL (Stock II). For the preparation of the combined standard stock solution (Stock III), 120 mg of Pseudoephedrine and 5 mg of Loratadine were transferred into a 50 mL volumetric flask, dissolved in methanol, and sonicated for 15 minutes to ensure complete dissolution and removal of dissolved gases, resulting in concentrations of 2400 µg/mL and 100 µg/mL, respectively. Further, appropriate aliquots of this solution were mixed in a ratio of 37:63, diluted to volume with mobile phase, and subsequently 0.2 mL of this mixture was transferred into a 10 mL volumetric flask and diluted to the mark with methanol and 0.1% OPA (pH 3.2) to obtain the final working standard solution.

HPLC used for chromatographic condition applies on the Preparation of standard solution

For HPLC analysis, standard working solutions were prepared from freshly prepared stock solutions. An aliquot of 0.2 mL from the Pseudoephedrine stock solution (2400 µg/mL) was transferred into a 10 mL volumetric flask and diluted up to the mark with mobile phase to obtain a final concentration of 48 µg/mL (Stock I). Similarly, 0.2 mL of the Loratadine stock solution (100 µg/mL) was pipetted into a 10 mL volumetric flask and diluted with mobile phase to obtain a concentration of 2 µg/mL (Stock II). For the combined standard solution (Stock III), appropriate aliquots from the mixed stock solution (2400 µg/mL of Pseudoephedrine and 100 µg/mL of Loratadine) were taken (0.1–0.5 mL), transferred into a 10 mL volumetric flask, and diluted to volume with mobile phase to obtain final concentrations of 3.7 µg/mL for Pseudoephedrine and 6.3 µg/mL for Loratadine.

Selection of mobile phase:

Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until steady baseline was obtained. The standard solution containing mixture of Pseudoephedrine and Loratadine was run with different

individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing Methanol &OPA was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Pseudoephedrine and Loratadine. Chromatograms of Pseudoephedrine and Loratadine are shown in **(Table No:)** respectively.

Studies of Calibration plot

Optimization of Chromatographic condition:

The chromatographic conditions were optimized through a series of trial-and-error experiments and subsequently maintained constant throughout the analysis. Separation was achieved using an Agilent C18 column (250 mm × 4.6 mm) packed with 5 µm particle size. The mobile phase consisted of methanol and 0.1% orthophosphoric acid in water in the ratio of 37:63 (v/v), delivered at a flow rate of 1.0 mL/min. The detection was carried out at a wavelength of 232 nm, while the column was maintained at ambient temperature. A sample injection volume of 20 µL was used for all chromatographic runs, ensuring consistent and reproducible results.

Procedure for calibration curve of Pseudoephedrine and Loratadine:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution, pipette out 120 mg Pseudoephedrine and 5 mg Loratadine in 50 ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5 ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 24,48,72,96,120 µg/ml of Pseudoephedrine and 1,2,3,4,5 µg/ml of Loratadine. Sample were injected and peaks were recorded at 232 nm as the graph plotted as concentration of drug verses peak area is depicted in **(fig. no.)** respectively.

Study of system suitability parameters:

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution.

Calibration Experiment

RP-HPLC Method

Calibration experiments were performed using both RP-HPLC and UV spectrophotometric methods. For RP-HPLC, the standard stock solution (2400 µg/mL of Pseudoephedrine and 100 µg/mL of Loratadine) was suitably diluted with mobile phase to prepare five calibration standards in the concentration range of 24–120 µg/mL for Pseudoephedrine and 1–5 µg/mL for Loratadine. For UV spectrophotometric analysis, standard solutions were scanned between 200–400 nm, showing λmax at 220 nm for Pseudoephedrine and 239 nm for Loratadine, with an isosbestic point observed at 232 nm, which was selected as the detection wavelength for HPLC analysis. Calibration curves were constructed by plotting concentration versus peak area, and regression equations were determined. All calibration standards were analyzed in triplicate under optimized chromatographic conditions using an Agilent C18 column (250 mm × 4.6 mm, 5 µm), with a mobile phase of methanol and 0.1% OPA (37:63, v/v), a flow rate of 1 mL/min, injection volume of 20 µL, and detection at 232 nm.

RESULT AND DISCUSSION

Preliminary studies on Pseudoephedrine and Loratadine

Melting point

The procured reference standard of Pseudoephedrine and Loratadine were found to melt in the range of 182-188^{0}C and 134-139^{0}C respectively.

Solubility

Solubility studies revealed that Pseudoephedrine is highly soluble in water and freely soluble in alcohols, whereas Loratadine is poorly soluble in water but exhibits good solubility in organic solvents such as dimethyl sulfoxide (DMSO), ethanol, and methanol.

UV Spectroscopy

UV absorption of 10 µg/mL solution of Pseudoephedrine and Loratadine in methanol was generated and absorbance was taken in the range of 200-400 nm. λmax of Pseudoephedrine and Loratadine in Methanol was found to be 220 nm and 239 nm respectively.

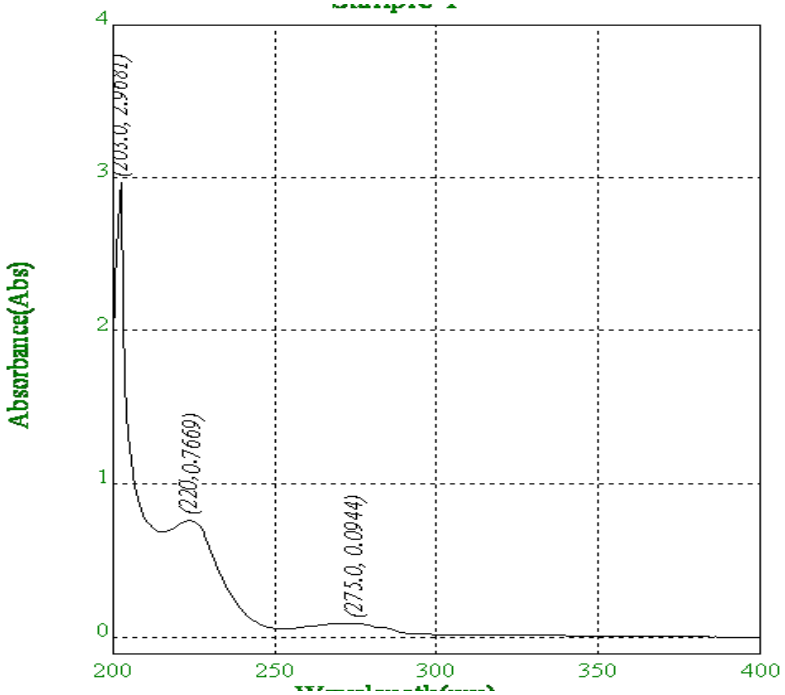

Figure 1: UV Spectrum of Pseudoephedrine

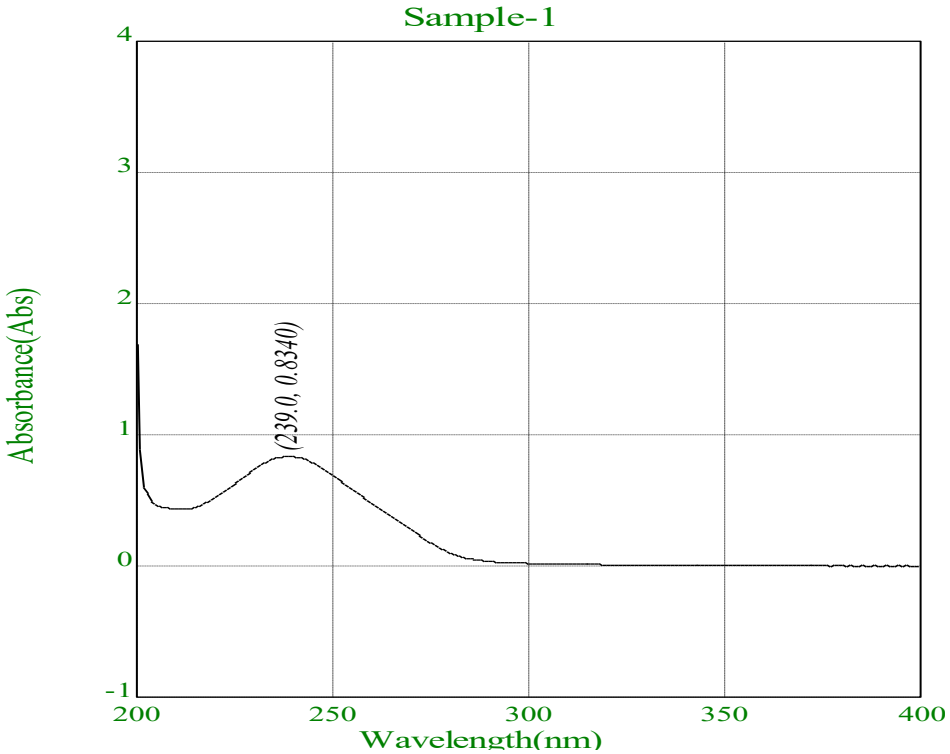

Figure 2.: UV Spectrum of Loratadine

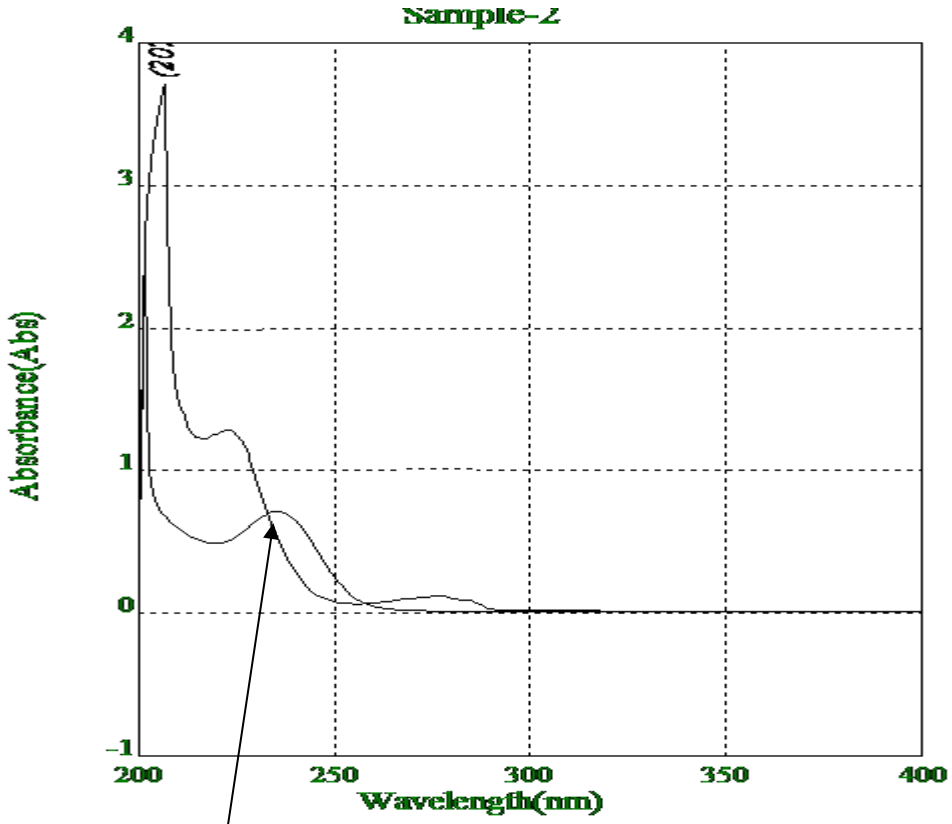

Figure 3: Iso-absorptive point of Pseudoephedrine and Loratadine

Studies on the chromatographic behavior of Pseudoephedrine and Loratadine

TABLE 1: Chromatographic behavior of Pseudoephedrine and Loratadine mobile phase of various compositions.

Sr No.	Mobile Phase	Remark
1.	ACN : (0.1%OPA) (90:10 % v/v) 232 nm, 0.7 ml/min.	Sharp Peak was not obtained
2	Methanol : Water 0.1 %OPA (80 : 20%v/v),232 nm flow 0.7 ml/min.	Broad and single peak was obtained
3	Methanol : Water 0.1 %OPA (80 : 20%v/v),232 nm flow 0.7 ml/min.	No Sharp peak
4	Methanol : water 0.1 %OPA (70 : 30%v/v),232 nm, flow 0.7ml/min.	Resolve peak was not obtained
5	Methanol : water 0.1 %OPA (65 : 35%v/v),232 nm, flow 0.8ml/min.	Resolve peak was not obtained

6	Methanol : water 0.1 %OPA (60 : 40%v/v),232 nm, flow 0.7 ml/min.	Resolve peak was not obtained
7	Methanol: water 0.1 %OPA (50:50% v/v) PH 3. 232 nm,0.7 ml/min.	Resolve peak was not obtained
8	Methanol: water 0.1 %OPA (40:60% v/v) PH 3 with. 232 nm,0.7 ml/min	Resolve peak was not obtained
9	Methanol: water 0.1 %OPA (35:65% v/v) PH 3 with. 232 nm,0.7 ml/min	Larger RT
10	Methanol: water 0.1 %OPA (37:63% v/v) PH 3 with. 232 nm,1 ml/min	Sharp peak was obtained

Thus, from the above, it has been observed that, using mobile phase of Methanol + OPA (0.1%OPA) PH 3.2 (37+63 % v/v) 232 nm, 1ml, gave adequate retention time at 3.479 min and 5.615 min. with good peak shape (Theoretical plates of 4907 of Pseudoephedrine& 2925 of Loratadine.

Chromatogram of Trial 1:

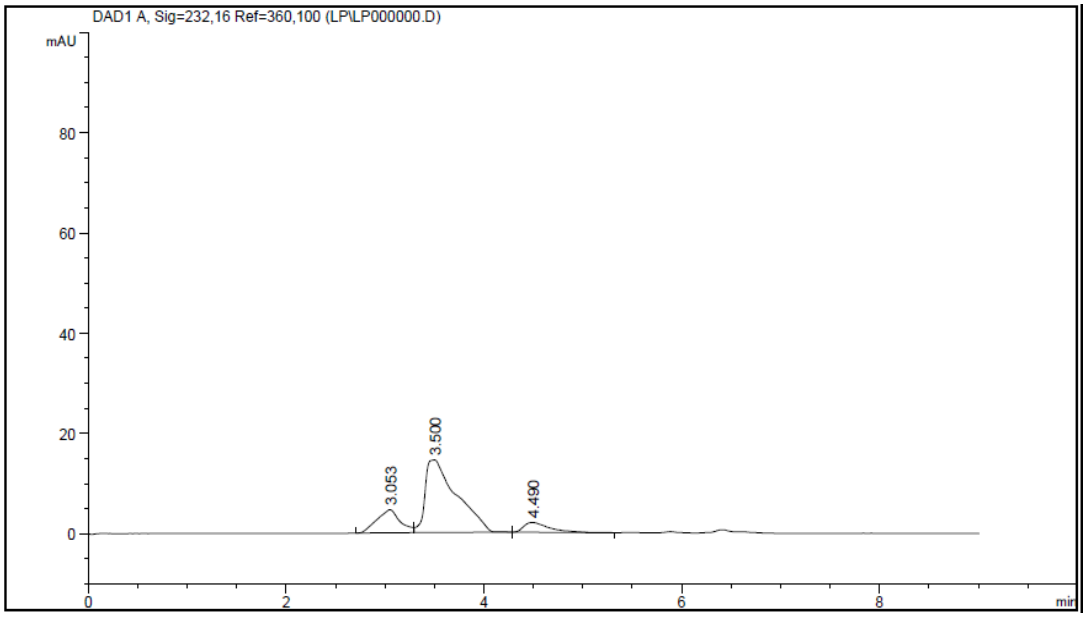

Figure 4: Representative Chromatogram of Pseudoephedrine and Loratadine using ACN: Water (90:10 % v/v), 0.7ml/min.

Table 2: Details of chromatogram of Trials 01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.053	75.3739	828	1.20	-
2	3.500	319.34854	588	0.36	0.89
3	4.165	34.96914	1680	0.45	195

Observation: Sharp Peak was not observed, so unsatisfactory result was found, method was rejected.

Chromatogram of Trial 2:

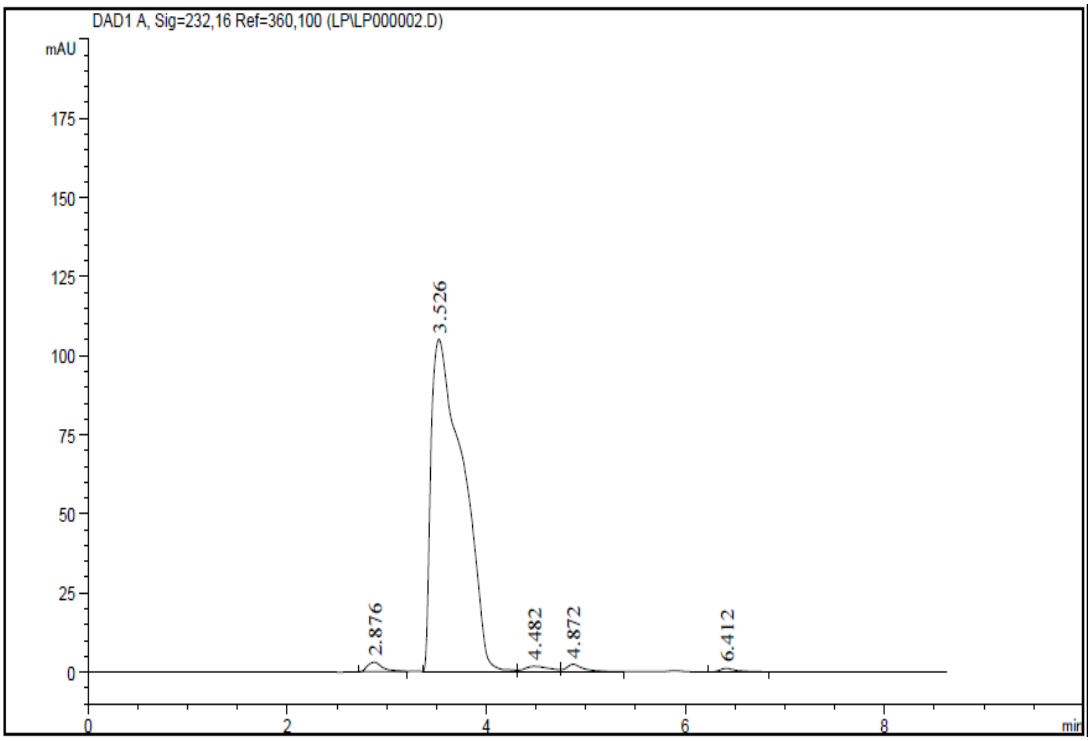

Figure 5: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water (90: 10%v/v) 0.7ml/min.

Table 3: Details of chromatogram of Trials 02

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	2.876	31.0164	1905	0.77	-
2	3.526	2342.81177	416	0.30	1.36
3	4.482	27.36665	1430	0.58	1.64
4	4.872	25.62663	5317	0.62	1.05
5	6.412	11.81525	9487	0.58	5.79

Observation: Broad and single peak was obtained, it's not Sharp peak so method was rejected.

Chromatogram of Trial 3:

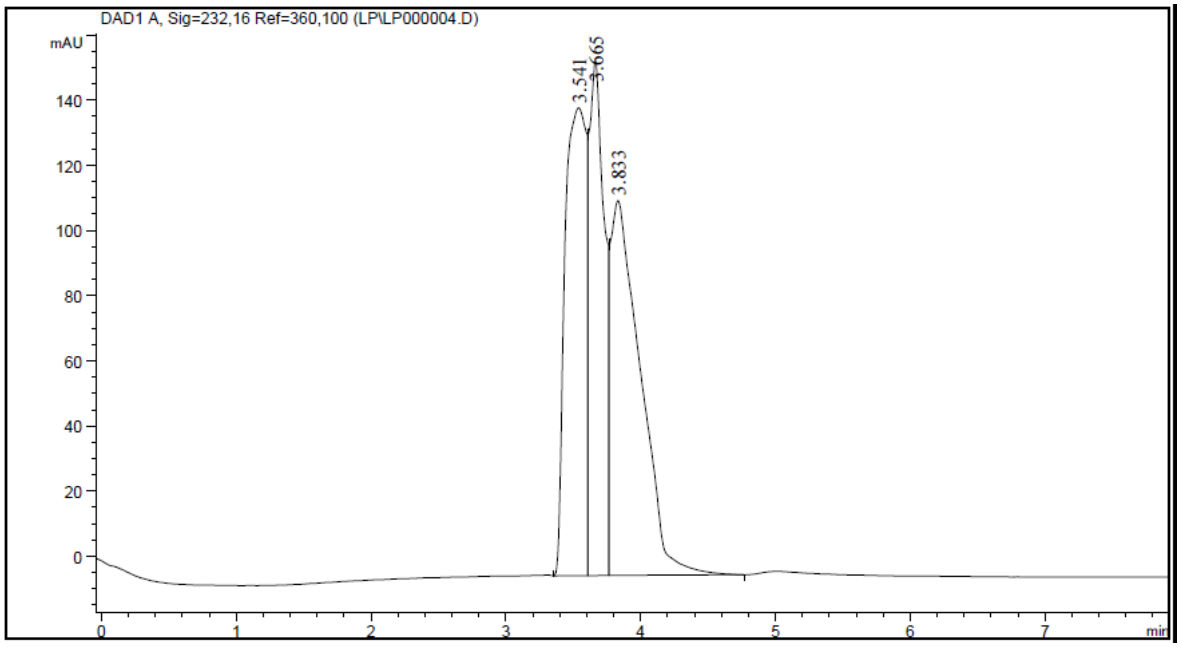

Figure 6: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water (70: 30%v/v).

Table 4: Details of chromatogram of Trials 03

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.541	1543.47998	2038	1.78	-
2	3.665	1235.2189	-	0.64	-
3	3.833	1785.86926	1289	0.30	-

Observation: sharp peak were not obtained, so method was rejected

Chromatogram of Trial 4:

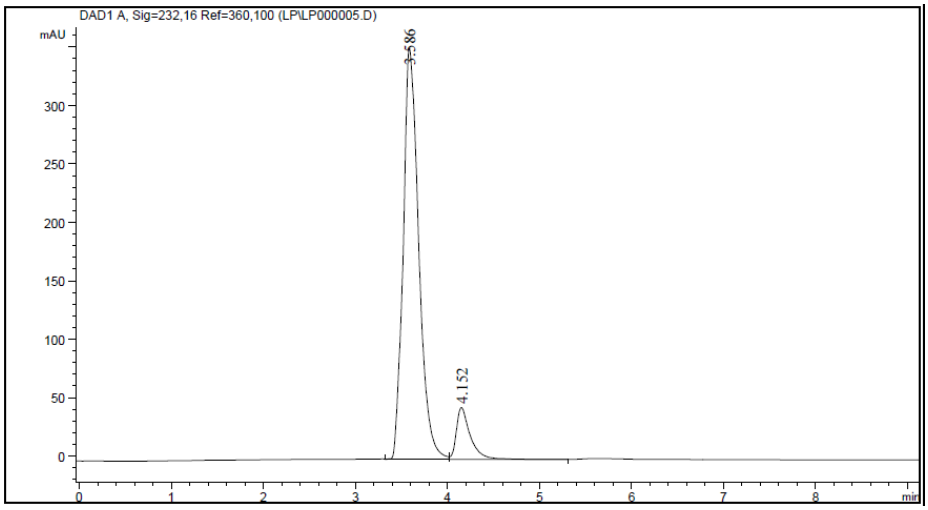

Figure 7: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water 0.1 % OPA (70: 30%v/v) 0.7 ml/ min.

Table 5: Details of chromatogram of Trials 04

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.586	4130.2221	2417	0.64	-
2	4.152	464.02457	4572	0.51	2.10

Observation: Resolve peaks were not obtained, so method was rejected

Chromatogram of Trial 5:

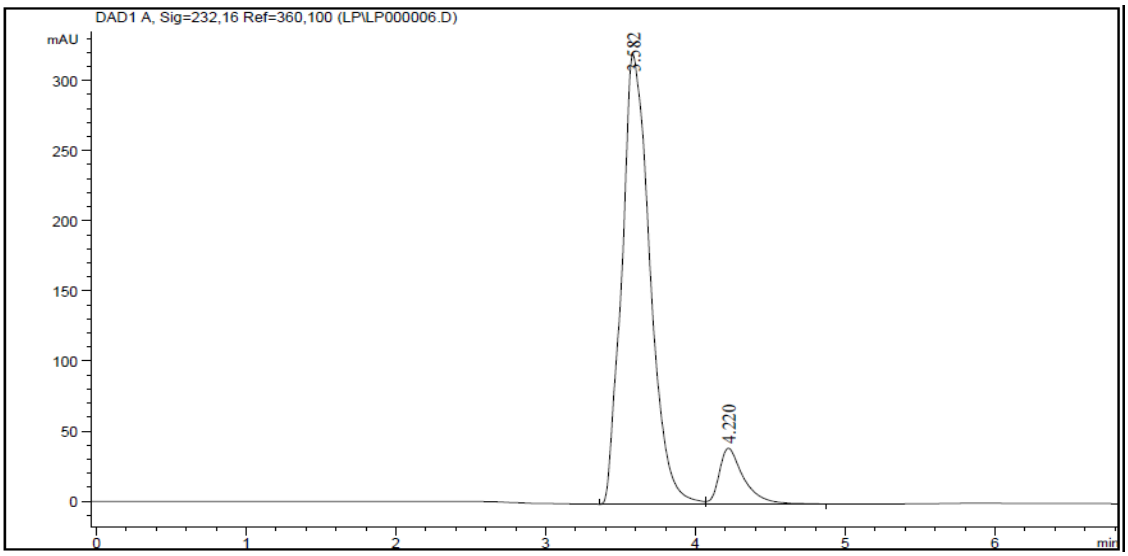

Figure 8: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water 0.1 % OPA (65: 35%v/v) 0.7 ml/ min.

Table 6: Details of chromatogram of Trials 05

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.582	4189.1377	1921	0.64	-
2	4.220	449.6123	3780	0.55	2.12

Observation: Resolve peaks were not obtained, so method was rejected

Chromatogram of Trial 6:

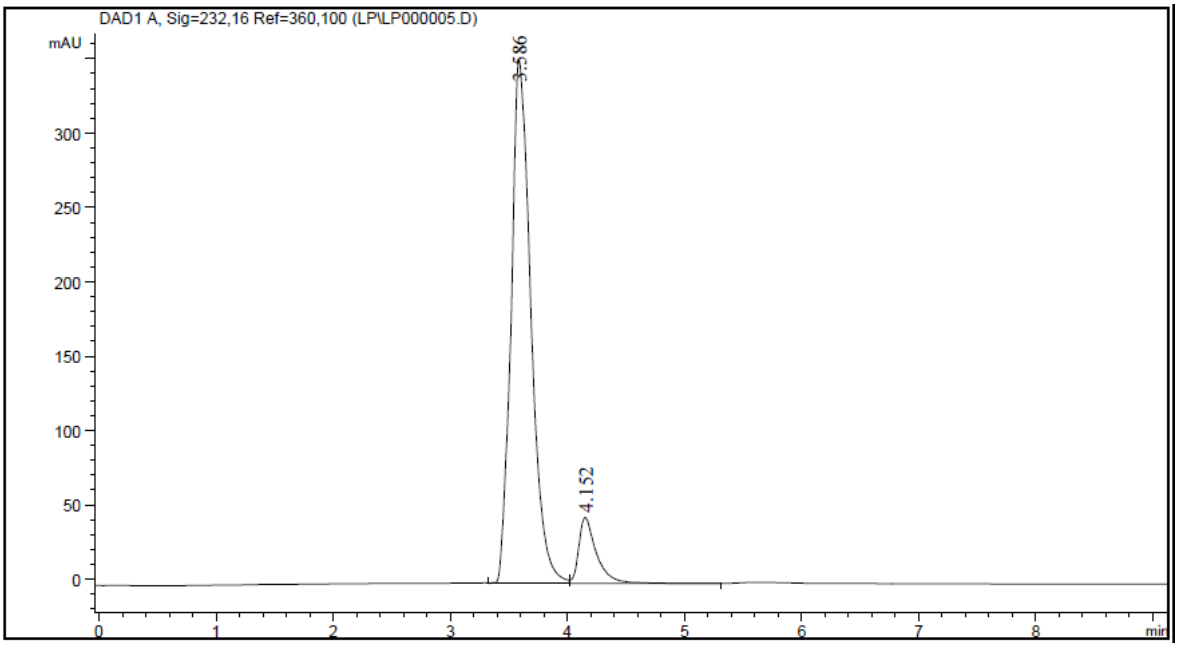

Figure 9: Representative Chromatogram of Pseudoephedrine and Loratadine usingMethanol: Water 0.1 % OPA (60: 40%v/v) 0.7 ml/ min.

Table 7: Details of chromatogram of Trials 06

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.779	4180.81055	5599	0.53	-
2	4.487	474.9083	4548	0.43	3.02

Observation: Resolved peaks were not obtained, so method was rejected

Chromatogram of Trial 7:

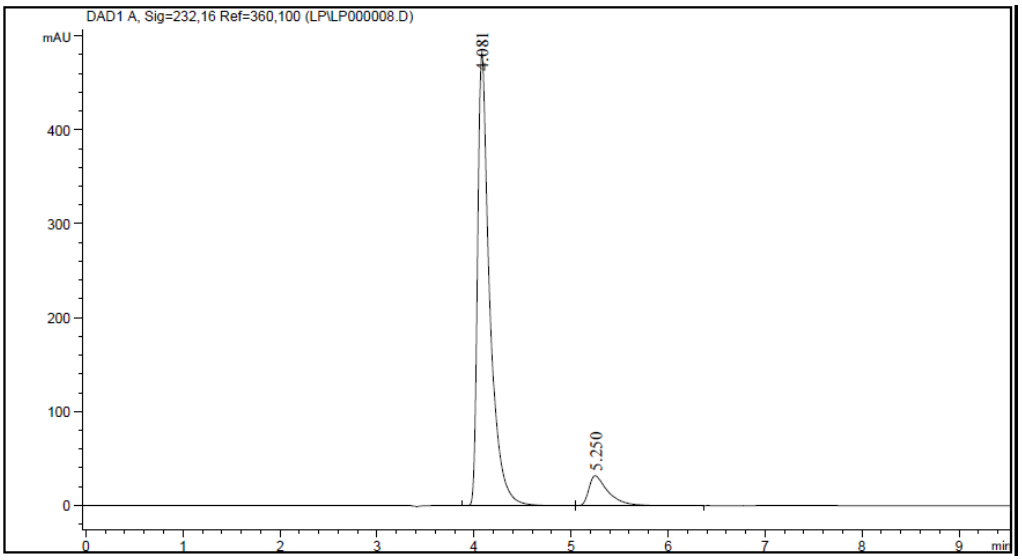

Figure 10: Representative Chromatogram of Pseudoephedrine and Loratadine usingMethanol: Water 0.1 % OPA (50: 50%v/v) 0.7 ml/ min.

Table 8: Details of chromatogram of Trials 07 (50 methanol +50% water), 0.7 ml

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.081	4167.02197	6375	0.48	-
2	5.250	439.24551	4020	0.44	4.36

Observation: Resolve peaks were not obtained, so method was rejected.

Chromatogram of Trial 8:

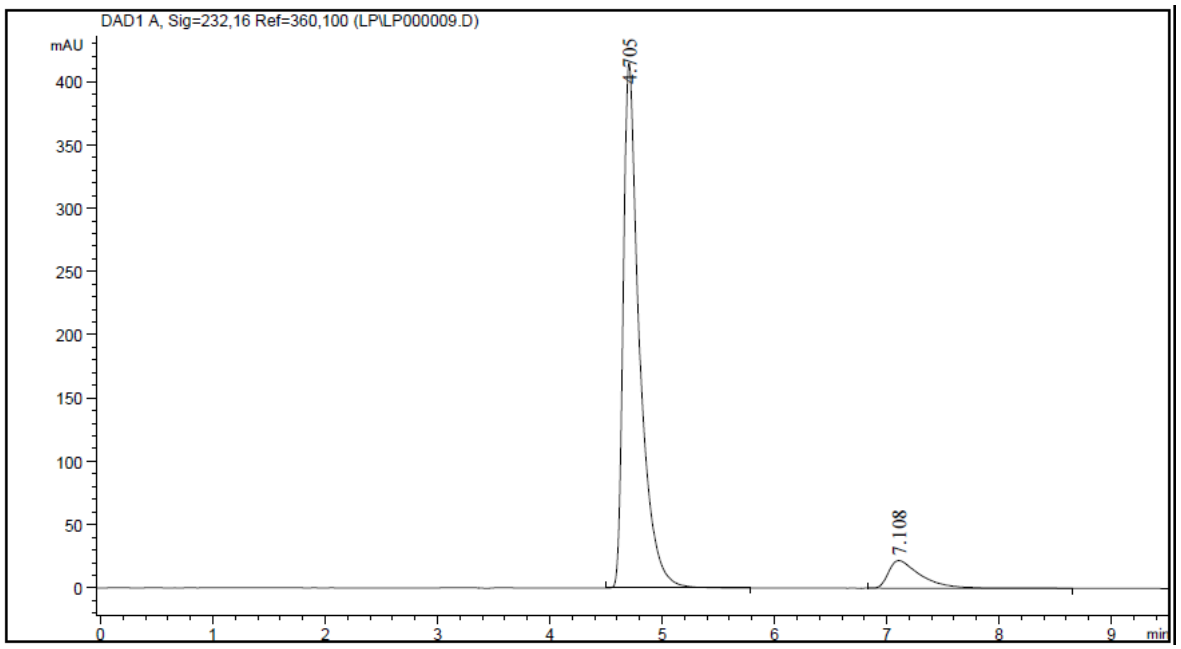

Figure 11: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water 0.1 % OPA (40: 60%v/v) 0.7 ml/ min.

Table 9: Details of chromatogram of Trials 08 (40 methanol +60% water), 0.7 ml

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.705	4172.2636	6145	0.98	-
2	7.108	439.69156	3487	0.92	6.65

Observation: Resolve peaks were not obtained, so method was rejected.

Chromatogram of Trial 9:

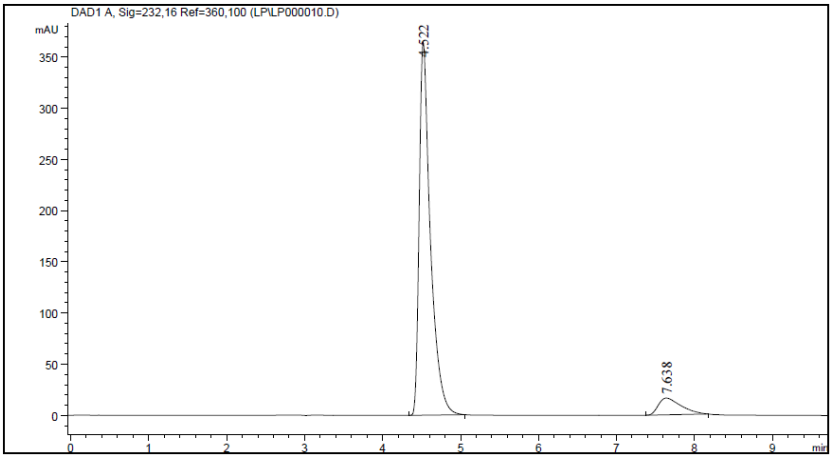

Figure 12: Representative Chromatogram of Pseudoephedrine and Loratadine using Methanol: Water 0.1 % OPA (35: 65%v/v) 0.7 ml/ min.

Table 10: Details of chromatogram of Trials 09 (35 methanol +65% water), 0.7 ml

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.522	3618.01636	5631	0.50	-
2	7.638	331.3358	3093	0.51	7.87

Observation: Larger Retention time was obtained so method was rejected.

Chromatogram of Final Trial 10 :

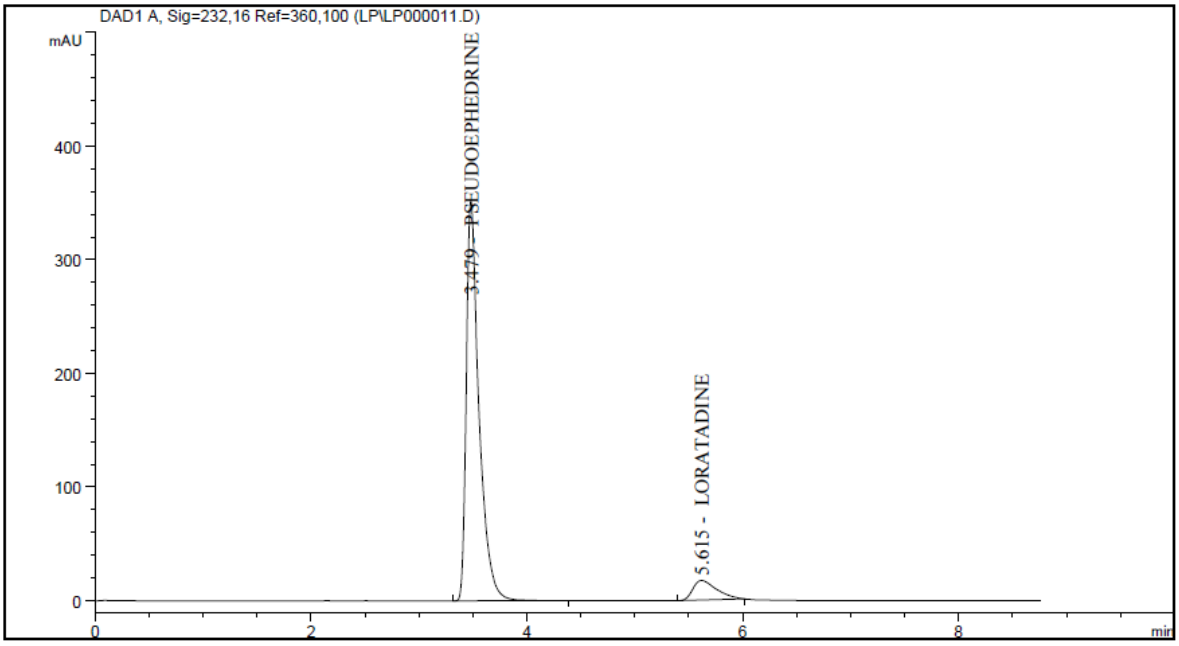

Figure 13: Representative Chromatogram of Pseudoephedrine and Loratadine usingMethanol: Water 0.1 % OPA (37: 63 %v/v) 1 ml/ min.

Table 11: Details of chromatogram of Final Trials 10

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.479	2872.63525	4907	0.91	-
2	5.615	263.4451	2925	0.93	6.95

The final optimized chromatographic conditions consisted of an Agilent C18 analytical column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase comprising methanol and 0.1% orthophosphoric acid in water (37:63, v/v, pH 3.2). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 232 nm. A sample injection volume of 20 µL was used, and the total run time for each analysis was set at 10 minutes, ensuring efficient and reproducible chromatographic separation.

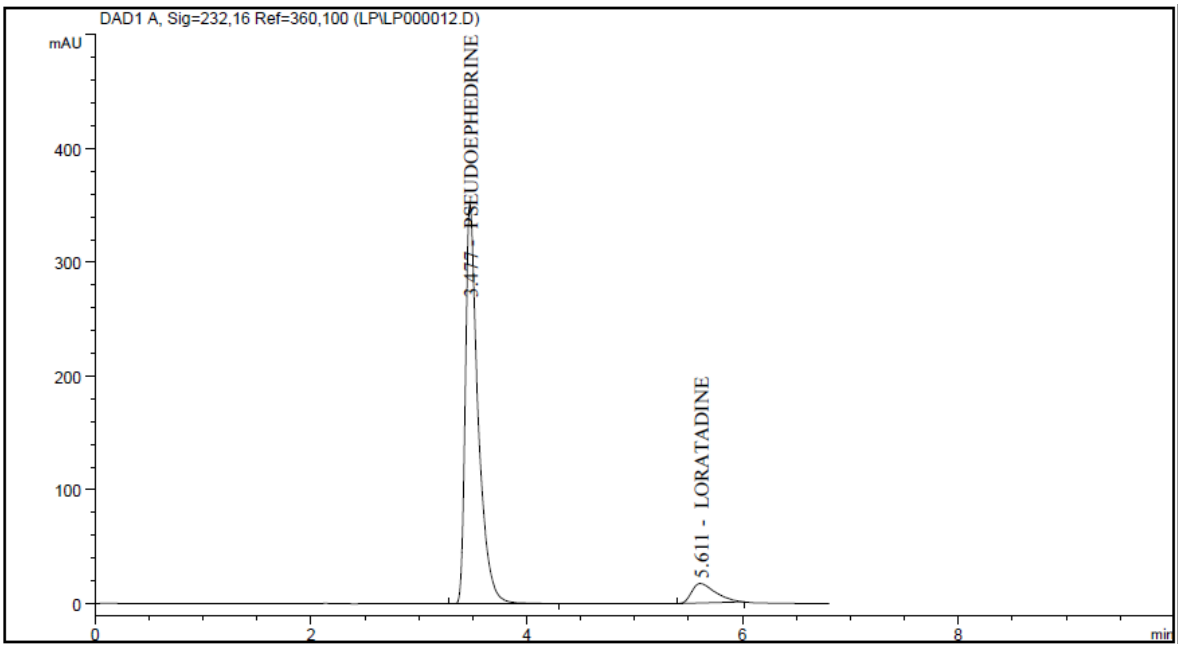

Figure 14: Chromatogram of standard Combination of Pseudoephedrine and Loratadine

Table 12: Details of chromatogram of standard Combination containing Pseudoephedrine and Loratadine

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.477	2873.2033	4874	0.91	-
2	5.611	263.50766	2917	0.93	6.93

In the standard mixture of Pseudoephedrine and Loratadine theoretical plates were found above 2000 i.e. for Pseudoephedrine and Loratadine 4874 and 2917 at minimum RT 3.477 and5.611 respectively**.**

Calibration experiment

RP-HPLC Method :

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 24-120 µg/mL for Pseudoephedrine and 1-5 µg/mL for Loratadine (**Table No:,and Table No:)**depict the calibration data of Pseudoephedrine and Loratadine The respective linear equation for Pseudoephedrine was y = 114.29 X+ 369.92 and Loratadine equation y = 310.93 X-19.682 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Pseudoephedrine and Loratadine is depicted in **(Fig No. and Fig No.).**

Table 13: Linearity data for Pseudoephedrine

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	24	3085.715	3082.68	3084.20	2.14	0.07
	48	5929.86	5919.78	5924.82	7.13	0.12
	72	8607.36	8586.27	8596.81	14.92	0.17
	96	11252.30	11258.0	11255.15	4.03	0.04
	120	14112.00	14156.5	14134.25	31.47	0.22
	Equation		y = 114.29x + 369.92			
	R^2		0.999			

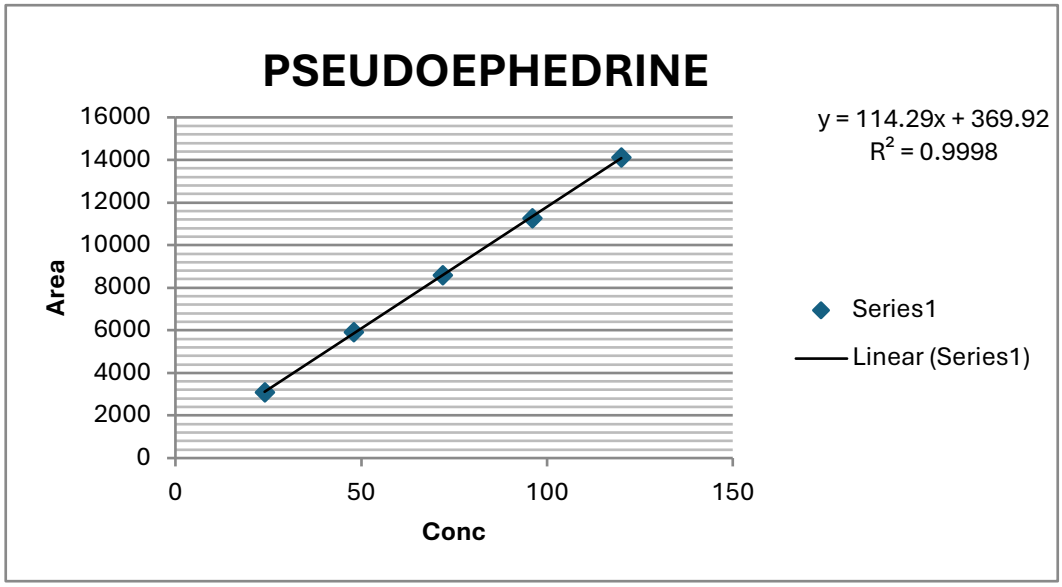

Figure 15: Calibration curve of Pseudoephedrine

The RP-HPLC Method for respective linear equation forPseudoephedrine was y = 114.29 x+369.92 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Pseudoephedrine is depicted in **Fig. 15.**

Table No 14: Linearity data for Loratadine

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	1	290.8701	289.2277	290.05	1.16	0.40
	2	607.302	606.2478	606.77	0.75	0.12
	3	916.5285	913.668	915.10	2.02	0.22
	4	1210.267	1211.711	1210.99	1.02	0.08
	5	1540.928	1544.209	1542.57	2.32	0.15
	Equation		y = 310.93x-19.682			
	R^2		0.999			

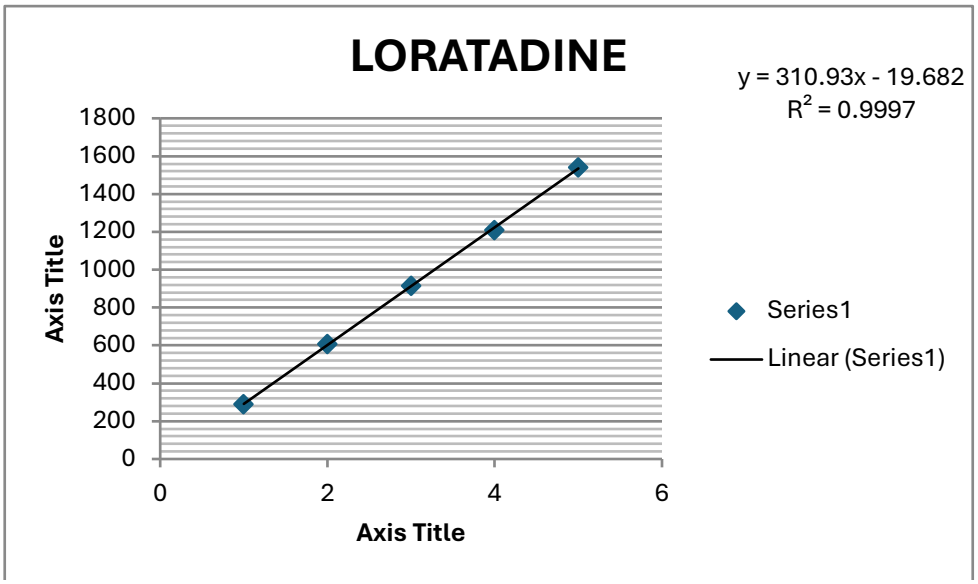

Figure 16: Calibration curve OfLoratadine

The RP-HPLC method for respective linear equation for Loratadine was y = 310.93 X+19.682 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Loratadine is depicted in **Fig. 1.**

CONCLUSION

A simple, rapid, and efficient RP-HPLC method was successfully developed and optimized for the simultaneous estimation of Loratadine and Pseudoephedrine in combined pharmaceutical dosage form. The method demonstrated excellent chromatographic performance with well-resolved peaks, acceptable retention times, and good system suitability parameters. The linearity of the method was established over a suitable concentration range with high correlation coefficients, indicating reliable quantification. The optimized method is precise, reproducible, and suitable for routine quality control analysis of combined dosage forms. Its simplicity and effectiveness make it advantageous for use in pharmaceutical industries and analytical laboratories.

REFERENCES

1. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs—A review.

Journal of Pharmaceutical Analysis, 4(3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003
2. Kazakevich, Y., & Lobrutto, R. (2007). *HPLC for pharmaceutical scientists*. John Wiley & Sons.
3. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to modern liquid chromatography* (3rd ed.). John Wiley & Sons.
4. Sweetman, S. C. (2009). *Martindale: The complete drug reference* (36th ed.). Pharmaceutical Press.
5. Sharma, B. K. (1981). *Instrumental methods of chemical analysis*. Krishna Prakashan Media.
6. Remington, J. P. (2006). *Remington: the science and practice of pharmacy* (Vol. 1). Lippincott Williams & Wilkins.
7. Satoskar, R. S., Rege, N., & Bhandarkar, S. D. (2017). *Pharmacology and pharmacotherapeutics*. Elsevier Health Sciences.
8. ME, W. (1981). Burger's Medicinal Chemistry. *New York: A Willey Interscience Publication*.
9. Davidson, A. G., Beckett, A. H., & Stenlake, J. B. (1997). Practical Pharmaceutical Chemistry.
10. Pharmacopoeia, I. (2007). The Indian pharmacopoeia commission. *Central Indian Pharmacopoeia Laboratory, Ministry of Health and Family Welfare, Govt of India, Sector*, *23*.
11. Pharmacopoeia, B., & British Pharmacopoeia Commission. (2010). Medicines and healthcare products regulatory agency. *London*, *3*(6), 1844.
12. IFPMA, G. (1995). ICH, Text on Validation of Analytical Procedures, ICH–Q2A. In *International Conference on Harmonisation* (pp. 2-3).