UPI Journal of Pharmaceutical Medical, and Health Sciences
Content Available at www.uniquepubinternational.com ISSN: 2581-4532

Open Access Research Article

SIMULTANEOUS ESTIMATION OF PSEUDOEPHEDRINE, AMBROXOL AND DESLORATIDINE IN COMBINED TABLET DOSAGE FORM BY USING RP-HPLC

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DOI: https://doi.org/10.37022/jpmhs.v9i2.228

Article History
Received: 19-03-2026
Revised: 14-04-2026
Accepted: 03-06-2026

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Keywords: RP-HPLC, Pseudoephedrine, Ambroxol and Desloratidine.

ABSTRACT

A practical, sensitive, and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of Pseudoephedrine, Ambroxol, and Desloratadine in combined tablet dosage forms by optimizing suitable chromatographic conditions. Chromatographic separation was achieved on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm) using an isocratic mobile phase consisting of Buffer:Acetonitrile (45:25:30, v/v/v). The flow rate was maintained at 1.0 mL/min, the detection wavelength was set at 274 nm, the injection volume was 10 µL, and the total run time was 9 minutes. The developed method was validated according to ICH Q2(R2) guidelines with respect to specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The retention times of Pseudoephedrine, Ambroxol, and Desloratadine were found to be 2.37, 3.97, and 5.45 minutes, respectively. The method exhibited excellent linearity over the concentration ranges of 15–90 µg/mL for Pseudoephedrine, 30–180 µg/mL for Ambroxol, and 2.5–15 µg/mL for Desloratadine. The percentage recoveries were in the ranges of 99.57–100.48% for Pseudoephedrine, 99.38–100.39% for Ambroxol, and 100.14–100.57% for Desloratadine, demonstrating the accuracy of the method. The validated RP-HPLC method was found to be simple, precise, accurate, robust, and suitable for the routine quantitative analysis of Pseudoephedrine, Ambroxol, and Desloratadine in combined tablet dosage forms.

1. INTRODUCTION

Pseudoephedrine HCl is (1S, 2S)-2-(methylamino)-1-phenylpropan-1-ol hydro-chloride, it is a stereoisomer of ephedrine and has similar action. Pseudoephedrine and its salts are orally used for the symptomatic relief of nasal congestion and are commonly used in combination with other ingredients in preparations intended for the relief of cough and cold symptoms.[1] It has the function of constricting the blood vessel, eliminating mucous membrane congesting and tumefying of nasopharynx, alleviating symptom of the nasal congestion[2].

Ambroxol hydrochloride ([Trans-4-[(2-amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride]) is a semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica*. It is a metabolic product of bromhexine and possesses mucokinetic (improvement in mucus transport) and secretolytic (liquefies secretions) properties. It promotes the removal of tenacious secretions in the respiratory tract and reduces mucus stasis (arresting the secretion of mucus).[3] It is a secretolytic agent used in the treatment of trachea bronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma.

Desloratadine is chemically 8-chloro-6, 11-dihydro-11-(4-piperdinylidene)-5H benzo [5, 6] cyclohepta [1, 2-b] pyridine is a second generation antihistaminic drug. It is an orally administered non sedative, long acting antihistaminic with selective H1-receptor antagonistic

activity. Desloratadine is an active metabolite of loratadine. It is used for the relief of symptoms of seasonal allergic rhinitis, perennial (non-seasonal) allergic rhinitis and for the symptomatic treatment of pruritus and urticaria (hives) associated with chronic idiopathic urticarial.[4, 5]

Detailed survey of literature revealed that only few methods have been described in the literature for the determination of Pseudoephedrine in combination with other drugs by HPLC.[6, 7]

Literature for Ambroxol revealed Spectrophotometric[8-9], HPLC[10-13], HPTLC[14] methods reported for its determination in combination with other drugs.

Literature survey reveals few analytical methods[15-21], for the determination of Desloratidine.

The present research work is aimed to develop a simple, rapid, accurate, sensitive and stability indicating method for simultaneous estimation of the above mentioned three drugs for routine analysis with shorter run time. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[22]

EXPERIMENTAL METHODS

1.1 Chemicals and Reagents Used

Potassium dihydrogen phosphate was procured from Merck Life Science Pvt. Ltd. HPLC-grade methanol and HPLC-grade acetonitrile were obtained from Rankem, while HPLC-grade water was procured from Merck Life Science Pvt. Ltd. Reference standards of Pseudoephedrine, Ambroxol, and Desloratadine were received as gift samples from Zydus Lifesciences Ltd., Ankleshwar.

HPLC Instrumentation

- **Make:** Shimadzu
- **Model:** LC-2010
- **Injector:** 10 µL fixed loop
- **Detector:** UV detector
- **Software:** LC Solution

1.2 Preparation of Mobile Phase

The mobile phase consisted of phosphate buffer, acetonitrile, and methanol in the ratio of 45:25:30 (% v/v/v). The prepared mobile phase was filtered through a 0.45 µm membrane filter under vacuum and degassed before use.

1.3 Preparation of Standard Solution

A standard stock solution containing Pseudoephedrine and Ambroxol was prepared by accurately weighing 6 mg of Pseudoephedrine and 12 mg of Ambroxol into a 10 mL volumetric flask containing approximately 7 mL of diluent. Separately, 5 mg of Desloratadine was accurately weighed and transferred into a 50 mL volumetric flask containing approximately 35 mL of diluent. The solutions were sonicated for 30 minutes to ensure complete dissolution of the drugs, and the volumes were made up to the mark with the diluent. The resulting standard stock solutions contained 600 µg/mL of Pseudoephedrine, 1200 µg/mL of Ambroxol, and 100 µg/mL of Desloratadine.

1.4 Preparation of Sample Solution

Five NUCOPE-AD tablets, each containing 6 mg of Pseudoephedrine, 12 mg of Ambroxol, and 5 mg of Desloratadine, were accurately weighed and finely powdered. An amount of powder equivalent to the average weight of one tablet was transferred into a 50 mL volumetric flask containing 30 mL of diluent. The mixture was sonicated for 30 minutes to ensure complete extraction of the analytes, and the volume was made up to the mark with the diluent. Subsequently, 1 mL of this solution was transferred into a 10 mL volumetric flask and diluted to volume with the diluent. The resulting sample solution contained 600 µg/mL of Pseudoephedrine, 1200 µg/mL of Ambroxol, and 100 µg/mL of Desloratadine. The prepared solution was filtered through a 0.45 µm membrane filter before chromatographic analysis to remove insoluble excipients and particulate matter.

1.5 Validation of the RP-HPLC Method

The developed RP-HPLC method was validated according to ICH Q2(R2) guidelines with respect to linearity, precision, accuracy, robustness, system suitability, detection limit (DL), and quantitation limit (QL).

1.5.1 Linearity

Linearity was evaluated by preparing standard solutions at six different concentration levels. Each solution was injected into the HPLC system, and the corresponding peak areas were recorded. Calibration curves were constructed by plotting peak area against concentration, and the correlation coefficient (R^2) was determined.

1.5.2 Precision

a) Repeatability

Repeatability was assessed by injecting the standard solution six consecutive times into the HPLC system. The peak areas obtained were used to calculate the percentage relative standard deviation (%RSD). The %RSD values were found to be within the acceptable limits.

b) Intraday Precision

Intraday precision was evaluated by analyzing three different concentrations of the standard solution, each in triplicate (n = 3), on the same day. The %RSD values obtained demonstrated good repeatability of the developed method.

c) Interday Precision

Interday precision was determined by analyzing three different concentrations of the standard solution in triplicate (n = 3) on three different days. The %RSD values obtained confirmed the reproducibility of the developed analytical method.

1.5.3 Accuracy

Accuracy was determined using the recovery method at three concentration levels corresponding to 50%, 100%, and 150% of the target concentration. Standard solutions containing known amounts of

Pseudoephedrine, Ambroxol, and Desloratadine were analyzed, and the percentage recoveries were calculated for each level. The individual and mean recovery values were determined to evaluate the accuracy of the method.

1.5.4 Robustness

Robustness was evaluated by introducing deliberate variations in chromatographic conditions, including flow rate, detection wavelength, and column temperature. Standard and sample solutions were analyzed under the modified conditions. No significant changes were observed in chromatographic parameters such as resolution, tailing factor, asymmetry, or theoretical plate count, indicating that the method was robust.

1.5.5 System Suitability

System suitability was evaluated by injecting five replicate standard preparations of Pseudoephedrine, Ambroxol, and Desloratadine. The acceptance criteria included a tailing factor not greater than 1.5 and a theoretical plate count greater than 2000 for each analyte. All system suitability parameters complied with the specified acceptance criteria, confirming the suitability of the chromatographic system.

1.5.6 Detection Limit (DL) and Quantitation Limit (QL)

The detection limit (DL) and quantitation limit (QL) were estimated from the calibration curves obtained during the linearity study.

The detection limit was calculated using the following equation:

DL = 3.3 × (SD / Slope)

where **SD** is the standard deviation of the y-intercepts of six calibration curves, and **Slope** is the mean slope of the six calibration curves.

The quantitation limit was calculated using the following equation:

QL = 10 × (SD / Slope)

where **SD** is the standard deviation of the y-intercepts of six calibration curves, and **Slope** is the mean slope of the six calibration curves.

2. RESULTS AND DISCUSSION

2.1 Validation

The developed RP-HPLC method was validated according to the International Council for Harmonisation (ICH) Q2(R2) guidelines for analytical method validation. The validation parameters included linearity, precision, accuracy, robustness, system suitability, detection limit, and quantitation limit. The results obtained demonstrated that the developed method was suitable for the simultaneous estimation of Pseudoephedrine, Ambroxol, and Desloratadine in pharmaceutical dosage forms.

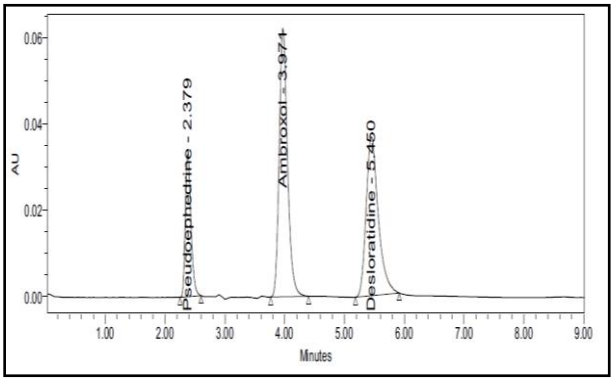
2.1.1 Linearity

Linearity was established by analyzing six concentration levels for each analyte. The concentration ranges were 15–90 µg/mL for Pseudoephedrine, 30–180 µg/mL for Ambroxol, and 2.5–15 µg/mL for Desloratadine (Table 1). Each solution was injected into the HPLC system, and calibration curves were constructed by plotting peak area against concentration (Figures 1–9). Excellent linearity was observed for all three analytes, with correlation coefficients (R^2) of 0.999, satisfying the acceptance criterion of not less than 0.999.

Table 01: Linearity results of Pseudoephedrine, Ambroxol and Desloratidine

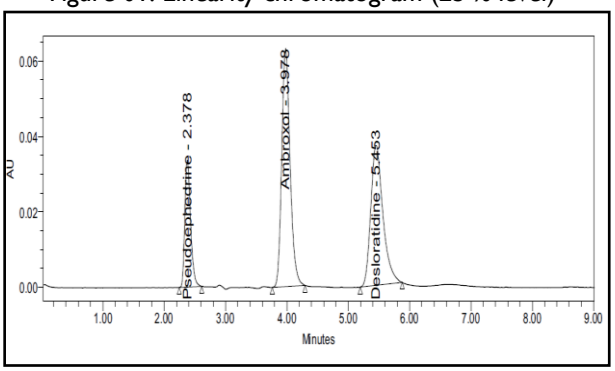
Pseudoephedrine		Ambroxol		Desloratidine	
Concentration in µg/mL	Peak area*	Concentration in µg/mL	Peak area*	Concentration in µg/mL	Peak area*
15	65282	30	171465	2.5	134771
30	126557	60	331741	5	267448
45	179865	90	487978	7.5	414170
60	250286	120	675989	10	534666
75	314103	150	847462	12.5	670044
90	367965	180	988345	15	809195

*mean of three determinations



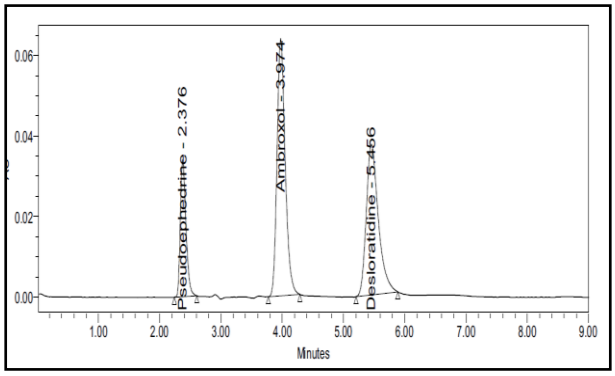
25% Working Standard Solution: A working standard solution containing 15 µg/mL of Pseudoephedrine, 30 µg/mL of Ambroxol, and 2.5 µg/mL of Desloratadine was prepared by appropriate dilution of the respective standard stock solutions with the diluent.

Figure 01: Linearity chromatogram (25 % level)



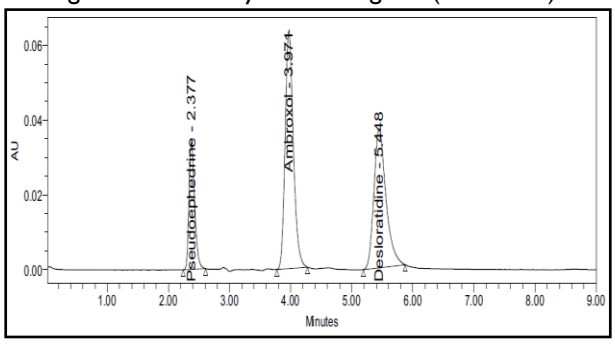
50% Working Standard Solution containing 30 µg/mL of Pseudoephedrine, 60µg/mL of Ambroxol and 5.0µg/mL of Desloratidine

Figure 02: Linearity chromatogram (50 % level)



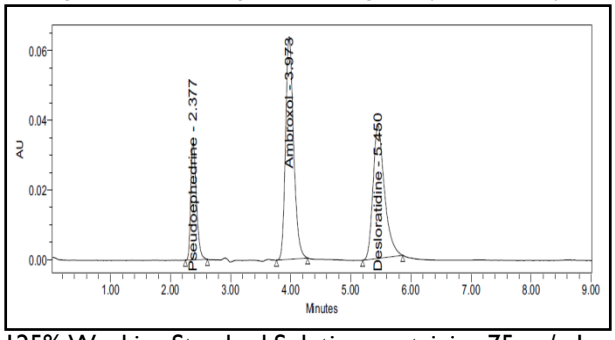
75% Working Standard Solution containing 45µg/mL of Pseudoephedrine, 90µg/mL of Ambroxol and 7.5µg/mL of Desloratidine.

Figure 03: Linearity chromatogram (75 % level)



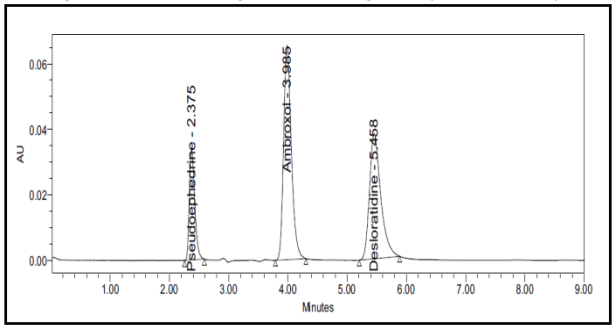
100% Working Standard Solution containing 60µg/mL of Pseudoephedrine, 120µg/mL of Ambroxol and 10µg/mL of Desloratidine.

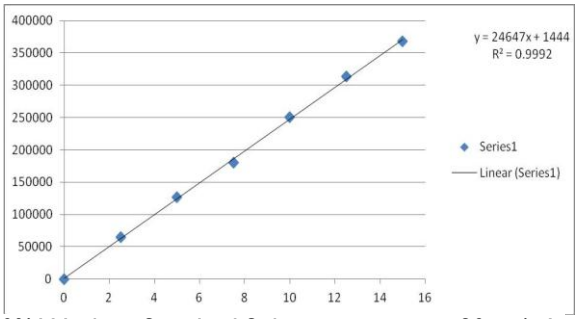
Figure 04: Linearity chromatogram (100 % level)



125% Working Standard Solution containing 75 µg/mL of Pseudoephedrine, 150 µg/mL of Ambroxol and 12.5 µg/mL of Desloratidine.

Figure 05: Linearity chromatogram (125 % level)





150% Working Standard Solution containing 90 µg/mL of Pseudoephedrine, 180 µg/mL of Ambroxol and 15 µg/mL of Desloratidine.

Figure 06: Linearity chromatogram (150 % level)

Figure 07: Standard Calibration Curve of Pseudoephedrine

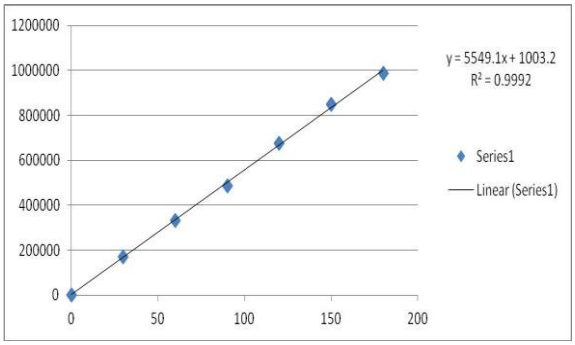


Figure 08: Standard Calibration Curve of Ambroxol

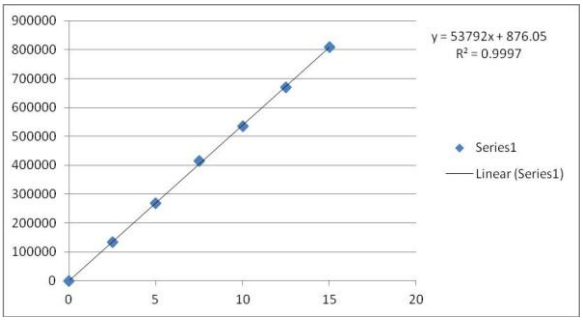


Figure 9: Standard Calibration Curve of Desloratidine

2.1.2 Precision

Repeatability: The data of repeatability for Pseudoephedrine, Ambroxol and Desloratidine are shown in (Table 02).

Intraday precision: The data for intraday precision for Pseudoephedrine, Ambroxol and Desloratidine are depicted in the (Table 03)

Interday precision: The data for interday precision for Pseudoephedrine, Ambroxol and Desloratidine are depicted in the (Table 04)

Table 02: Precision of recommended procedure using working standard solution

Injection	Pseudoephedrine		Ambroxol		Desloratidine	
	Retention Time in Min	Peak Area	Retention Time in Min	Peak Area	Retention Time in Min	Peak Area
1	2.37	221092	3.97	597184	5.44	540160
2	2.37	220671	3.97	600606	5.45	537956
3	2.37	220894	3.97	601288	5.45	537867
4	2.37	221361	3.97	598214	5.45	536842
5	2.37	222545	3.97	599337	5.45	535587
6	2.37	221357	3.98	599080	5.45	530241
Mean	2.37	221320	3.97	599285	5.45	536442
Std. dev	0.00	657.0	0.00	1507.5	0.00	3390.9
%RSD	0.05	0.30	0.13	0.3	0.07	0.6

Table 03: Intraday Precision of working sample solution

Sample Preparations	%Assay		
	Pseudoephedrine	Ambroxol	Desloratidine
Sample-1	99.84	100.30	100.07
Sample-2	99.80	100.98	99.67
Sample-3	99.20	99.22	99.45
Sample-4	99.72	100.29	99.67
Sample-5	99.10	99.31	99.85
Sample-6	100.33	99.34	100.19
Mean	99.66	99.91	99.82
Std. dev	0.454	0.72	0.275
%RSD	0.46	0.72	0.28

Table 04: Interday Precision of working sample solution

Sample Preparations	%Assay		
	Pseudoephedrine	Ambroxol	Desloratidine
Sample-1	98.27	99.25	98.05
Sample-2	98.89	99.82	98.09
Sample-3	98.82	99.93	98.35
Sample-4	99.49	99.42	98.30
Sample-5	99.87	99.61	98.13
Sample-6	98.53	99.57	98.40
Mean	98.98	99.60	98.22
Std. dev	0.599	0.25	0.148
%RSD	0.60	0.25	0.15

2.1.3 Accuracy

The % Recovery study was conducted using the Standard Addition Method. Known quantities of Pseudoephedrine, Ambroxol and Desloratidine standard solutions were added at 50%, 100%, and 150% levels to pre-measured sample solutions containing Pseudoephedrine, Ambroxol and Desloratidine. The amounts of Pseudoephedrine, Ambroxol and Desloratidine were determined using the regression equation from the calibration curve. The low standard deviation values indicate the accuracy of the proposed method. The recovery study results are presented in (Table 05).

Table 05: Recovery studies of Pseudoephedrine, Ambroxol and Desloratidine in tablet dosage form

Drug/ Parameters	Pseudoephedrine			Ambroxol			Desloratidine		
Concentration in %	50	100	150	50	100	150	50	100	150
Concentration of sample in µg/mL	30	60	90	60	120	180	5	10	15
Concentration of standard in µg/mL	60	60	60	120	120	120	10	10	10
Total Amount after spiking in µg/mL	90	120	150	180	240	300	15	20	25
Total amount recovered in µg/mL*	90.14	120.16	149.61	179.62	239.93	300.69	15.00	20.01	25.08
% recovery*	100.48	100.28	99.57	99.38	99.95	100.39	100.16	100.14	100.57
% RSD of Recovery	0.47			0.50			0.24		

* Mean of three determinations

2.1.4 Robustness

Some deliberate modifications were made to the test parameters to carry out the robustness investigation. typical variations are Wavelength: ±1 nm, Flow rate: ± 0.1 mL/min, Column temperature: ±2 °C (Table 6 to 8).

Table 06: Results of robustness by variations in flow rate, column temperature Mobile phase composition of Pseudoephedrine

S. No.	Parameter	Used	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile: Methanol (45:25:30 %v/v/v) 1.0 mL/min; 30 ^{0}C;	221092	2.374	3120	1.37
1	Flow Rate (±0.1 mL/min)	0.9	245494	2.665	2578	1.30
		1.1	200865	2.146	2044	1.24
2	Column Temperature (±5 ^{0}C)	25	243382	2.665	2589	1.29
		35	198528	2.146	2055	1.22
3	Mobile phase composition	50:25:25 %v/v/v	246518	2.377	2365	1.27
		40:25:35 %v/v/v	230506	2.375	2386	1.27

* Mean of three determinations, **Retention time in min

Table 07: Results of robustness by ariations n flow rate, column temperature, Mobile phase composition of Ambroxol

S. No.	Parameter	Used	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile: Methanol (50:20:30 %v/v/v) 1.0 mL/min; 30 ^{0}C;	597184	3.971	4016	1.23
1	Flow Rate (±0.1 mL/min)	0.9	609374	4.391	3050	1.17
		1.1	506876	30534	2457	1.14
2	Column Temperature (±5 ^{0}C)	25	608241	4.391	3053	1.17
		35	500539	30534	2467	1.15
3	Mobile phase composition	50:25:25 %v/v/v	668908	3.911	2785	1.14
		40:25:35 %v/v/v	596848	3.918	2780	1.14

* Mean of three determinations, **Retention time in min

Table 08: Results of robustness by variations in flow rate, column temperature, Mobile phase composition of Desloratidine

S. No.	Parameter	Used	Peak Area*	Retention Time**	Plate count	Tailing Factor
Optimized Conditions		Buffer : Acetonitrile: Methanol (50:20:30 %v/v/v) 1.0 mL/min; 30 ^{0}C;	540160	5.448	3651	1.45
1	Flow Rate (±0.1 mL/min)	0.9	587083	6.029	2719	1.32
		1.1	485894	4.863	3161	1.29
2	Column Temperature (±5 ^{0}C)	25	578794	6.029	2737	1.30
		35	470597	4.863	2192	1.22
3	Mobile phase composition	50:25:25 %v/v/v	604648	5.372	2510	1.32
		40:25:35 %v/v/v	556599	5.382	2491	1.31

* Mean of three determinations, **Retention time in min

2.1.5 DL and QL

The DL and QL for Pseudoephedrine was found to be 0.25 µg/mL and 0.75 µg/mL respectively. The DL and QL for Ambroxol was found to be 0.14 µg/mL and 0.43 µg/mL respectively. The DL and QL for Desloratidine was found to be 0.03 µg/mL and 0.10 µg/mL respectively.

CONCLUSION

The availability of literature on the simultaneous determination of Pseudoephedrine, Ambroxol and Desloratidine in bulk and combined tablet dosage form is scarce prior to commencement of this work. Hence, the author has developed a sensitive, accurate and precise RP-HPLC for the estimation of these three drugs in bulk and combined tablet dosage form. A mixture of Potassium dihydrogen ortho phosphate buffer, Acetonitrile and methanol are taken in the ratio 45:25:30 as mobile phase was found to be most suitable to obtain a good chromatographic separation with well-defined peaks, free from tailing. From the typical chromatogram, it was found that the retention times were 2.37, 3.97, 5.45 minutes for Pseudoephedrine, Ambroxol and Desloratidine respectively. In the present developed RP-HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship of r^2=0.999 for all the three drugs was observed between the concentration range of 15-90µg/mL, 30-180µg/mL and 2.5-15µg/mL for Pseudoephedrine, Ambroxol and Desloratidine respectively. Low values of standard deviation for retention time and peak areas are indicative of the high precision of the method. The assay of Pseudoephedrine, Ambroxol and Desloratidine was found to be 99.66, 99.91 and 99.82 respectively. From the recovery studies, it was found that the % RSD of the three drugs was within the limits, which indicate high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation.

This demonstrates that the developed HPLC method is simple, linear, precise, reproducible and sensitive. Thus, the developed method can be easily used for the routine quality control of bulk and combined tablet dosage form in a short analysis time with virtually no interference of the usual additives present in the pharmaceutical formulation.

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