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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF PSEUDOEPHEDRINE, AMBROXOL AND DESLORATIDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:
A new simple, precise, selective, accurate and rapid reverse phase high performance liquid chromatographic stability indicating method had been developed and validated for simultaneous quantitative determination of Pseudoephedrine, Ambroxol and Desloratidine in bulk and pharmaceutical dosage form. The chromatographic separation was achieved with InertisilODS 3V, (250×4.6 mm) and 5 µm particle size column. The optimized mobile phase consisting of phosphate buffer: Acetonitrile: Methanol (50:20:30 %v/v). The flow rate was 1.0 mL/min and eluents were detected at 225 nm using PDA detector. The retention time of Pseudoephedrine, Ambroxol and Desloratidine were found to be 2.379, 3.971 and 5.450 respectively. The percentage recoveries for three molecules were found to be in the range of 99-102%. The calibration curve was constructed between peak area vs concentration and demonstrated good linearity in the range of 2.5 -15 µg/ml for Pseudoephedrine, 30-180 µg/ml for Ambroxol and 2.5-15 µg/ml for Desloratidine. Degradation studies were studied for Pseudoephedrine, Ambroxol and Desloratidine under various stress conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal, photochemical and UV. All the degradation peaks were resolved effectively using developed method with different retention times. The developed method was validated according to ICH Q2-R1 guidelines. As the method could effectively separates the degradation products from active ingredients, it can be used for routine analysis of drug both in bulk and pharmaceutical dosage form.

Key words: Pseudoephedrine, Ambroxol, Desloratidine, Acetonitrile, Methanol, Buffer, RP-HPLC.

INTRODUCTION:

Pseudoephedrine HCl (PSH) is (1*S*, 2S)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride, it is a stereoisomer of ephedrine and has similar action. PSH 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] PSH as the function of constringing the blood vessel, eliminating mucous membrane congesting and tumefying of nasopharynx, alleviating symptom of the nasal congestion.[2] Only few methods have been described in the literature for the determination of Pseudoephedrine in combination with other drugs by HPLC.[1-2] The chemical structure of Pseudoephedrine HCl is given in Fig. 1.

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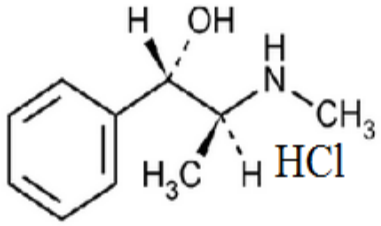

Fig. 1: Pseudoephedrine HCl

Ambroxol hydrochloride (AB) [*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 (liquifies 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 tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma. [4-7] It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP describes High Performance Liquid Chromatography (HPLC) [8] method and BP describes HPLC [9], Spectrophotometric and High Performance Thin Layer Chromatography (HPTLC) methods. Literature survey also reveals HPLC [10-13], Spectrophotometric [14] and HPTLC methods for determination of Ambroxol with other drugs. The chemical structure of Ambroxol is given in Fig. 2.

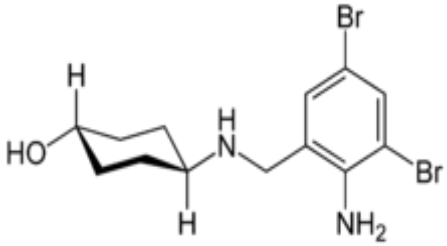

Fig. 2: Ambroxol

Desloratadine (DES) is chemically 8-chloro-6, 11-dihydro-11-(4-piperdinylidene)-5*H* benzo [5, 6] cyclohepta [1, 2-b] pyridine is a second generation antihistaminic drug.

Desloratadine 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. [5] Desloratadine is not official in any Pharmacopoeia [5]. Literature survey reveals that RP-HPLC [10, 11, 15, 16, 17] methods for determination of DES individually and in combination with other drugs. Literature survey also reveals spectrophotometric methods [18] for the determination of DES. It is available in the Merck index [19] and Martindale [20], the complete drug reference. The chemical structure of Desloratadine is given in Fig. 3.

Therefore, the main aim of the present work is to develop and validate RP HPLC method for simultaneous estimation of Pseudoephedrine, Ambroxol and Desloratadine in bulk and pharmaceutical dosage forms according to ICH Q2-R1 guidelines. [21] Hence, it has driven the authors to develop a method which is new, simple, precise, and accurate for the simultaneous determination of all the three drugs in their pharmaceutical dosage forms.

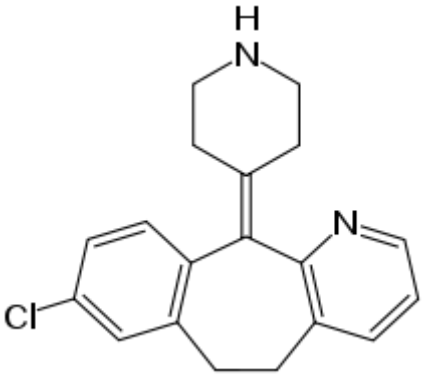

Fig. 3: Desloratadine

The present RP-HPLC method was validated following ICH Q2-R1 guidelines. [21]

MATERIALS AND METHOD:

Chemicals and solvents:

Pseudoephedrine, Ambroxol and Desloratidine were obtained as gift sample from Spectrum Pharma Research Solutions,

Hyderabad, India. The commercial Pharmaceutical Preparation NUCOPE-AD tablets containing 30 mg, 60 mg and 5 mg of Pseudoephedrine, Ambroxol and Desloratidine respectively (Marketed by Mankind Pharmaceuticals Private Limited) were procured from local pharmacy. Acetonitrile, Methanol and water used are of HPLC grade.

Instrumentation:

The chromatographic separations were performed using HPLC-Waters alliance (Model-2695) consisting of an in-built auto sampler, a column oven and 2996 PDA detector. The data was acquired through Empower-2-software. The column used was Inertisil ODS 3V, (250×4.6 mm i.d, 5 µm particle size). Meltronics sonicator was used for enhancing dissolution of the compounds. Elico pH meter was used for adjusting the pH of buffer solution. All weighing was done on Sartorius balance (Model AE-160).

Chromatographic conditions:

The mobile phase consists of Buffer: Acetonitrile: Methanol in the ratio of 50:20:30 %v/v. The mobile phase was pumped from solvent reservoir in the ratio of 50:20:30 %v/v to the column in the flow rate of 1.0 ml/min whereas run time set was 9 min. The separation was performed on Inertisil ODS 3V column and the column was maintained the temperature of 30°C and the volume of each injection was 10 µL, prior to injection, the column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 225 nm. The optimized chromatographic conditions were shown in Table-1.

Table-1: Optimized Chromatographic conditions for proposed HPLC method for Pseudoephedrine, Ambroxol and Desloratidine

S.No.	Parameter		Chromatographic conditions
1.	Instrument	:	Waters 2695, High performance Liquid Chromatography
2.	Flow rate	:	1 ml/min
3.	Column	:	Inertisil ODS3V, 250 x 4.6 mm, 5 µ
4.	Detector wave length	:	225 nm
5.	Column temperature	:	30°C
6.	Injection volume	:	10 L
7.	Run time	:	9 min
8.	Diluent	:	Water: Methanol (30:70)
9.	Mode of separation	:	Isocratic mode

Preparation of buffer solution:

Accurately weighed 1.36 gm of Potassium dihyrogen ortho phosphate in a 1000 ml of Volumetric flask to this about 900 ml of milli-Q water added and degassed to sonicate and finally made up the volume with water and adjusted the pH to 3.0 with dilute ortho phosphoric acid.

Preparation of mobile phase:

Buffer, Acetonitrile and Methanol were taken in the ratio of 50:20:30. Filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution:

Accurately weighed and transferred 6 mg of Pseudoephedrine, 5 mg of Desloratidine and 12 mg of Ambroxol working standards into 10 ml, 50 ml and 10 ml clean dry volumetric flasks respectively, to this added 7 ml of diluent, sonicated for 30 minutes and finally made up the volume up to the mark with diluents. From the above stock solution, 1 ml was pipette out in to a 10 ml volumetric flask and then make up to the final volume with diluent. (60 µg/ml Pseudoephedrine, 120 µg/ml Ambroxol and 10 µg/ml of Desloratidine)

Preparation of sample solution:

5 tablets were weighed and calculated the average weight of each tablet, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 30 ml of diluent was

added and sonicated for 30 min, further volume made up to the mark with diluent and filtered.

From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and volume made up to 10 ml with diluent. (60 µg/ml Pseudoephedrine, 120 µg/ml Ambroxol and 10 µg/ml of Desloratidine)
Label Claim: 30 mg of Pseudoephedrine + 60 mg of Ambroxol+ 5 mg of Desloratidine
HPLC Chromatograms of Standard, Sample and Placebo were shown in Fig. 4-6.

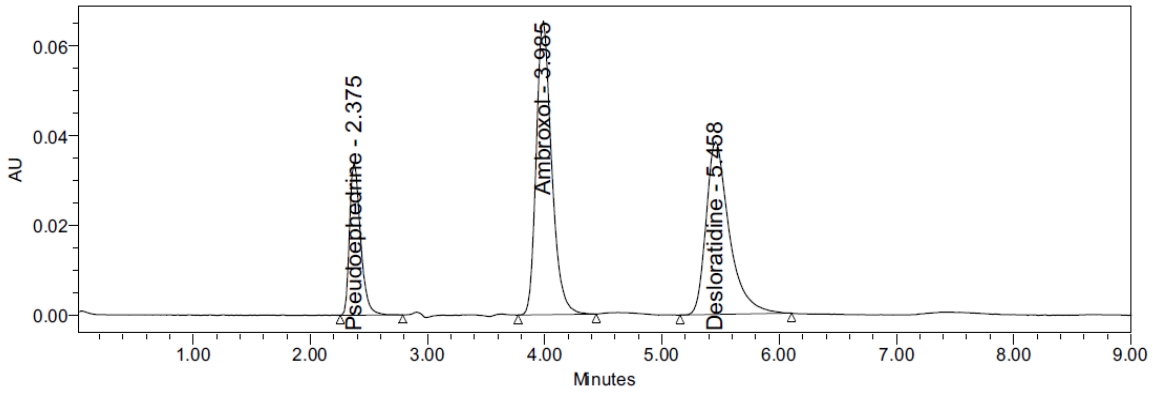

Fig. 4: HPLC Chromatogram of Standard

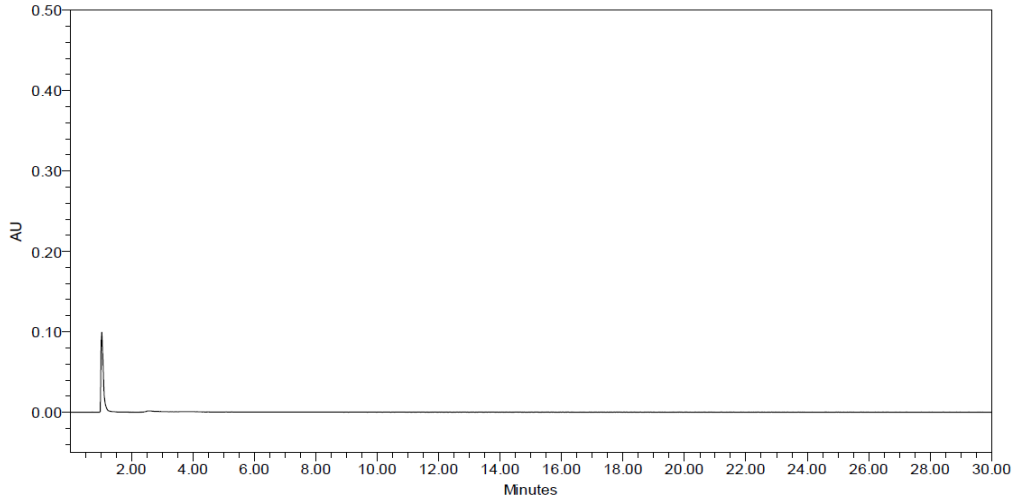

Fig. 5: HPLC Chromatogram of Placebo

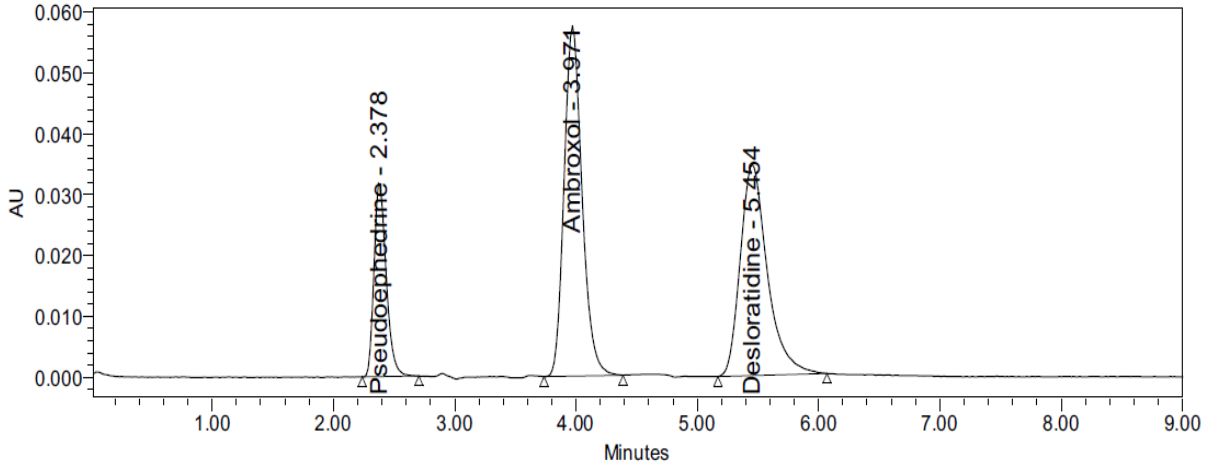

Fig. 6: HPLC Chromatogram of Sample

Validation of proposed method:

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Forced degradation studies, Linearity, Accuracy, Limit of detection and Limit of quantification.

Linearity:

Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml were taken from stock solution of concentration 0.5 mg/ml Pseudoephedrine, 1.2 mg/ml Ambroxol, and 0.5 mg/ml Desloratidine and then diluted up to mark with diluent, such that the final concentrations were in the range 2.5 ppm-15 ppm for Pseudoephedrine, 30 ppm-180 ppm for Ambroxol and 2.5 ppm-15 ppm for Desloratidine. Volume of 10 µl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak area vs. concentration was observed in the range of study. The observations and calibration curve were shown in Table-2 and Fig. 7A-7C.

Table-2: Linearity

S.No.	Conc. of Pseudoephedrine in ppm	Pseudoephedrine Area	Conc. of Ambroxol in ppm	Ambroxol area	Conc. of Desloratidine in ppm	Desloratidine area
1	2.5	65282	30	171465	2.5	134771
2	5	126557	60	331741	5	267448
3	7.5	179865	90	487978	7.5	414170
4	10	250286	120	675989	10	534666
5	12.5	314103	150	847462	12.5	670044
6	15	367965	180	988345	15	809195

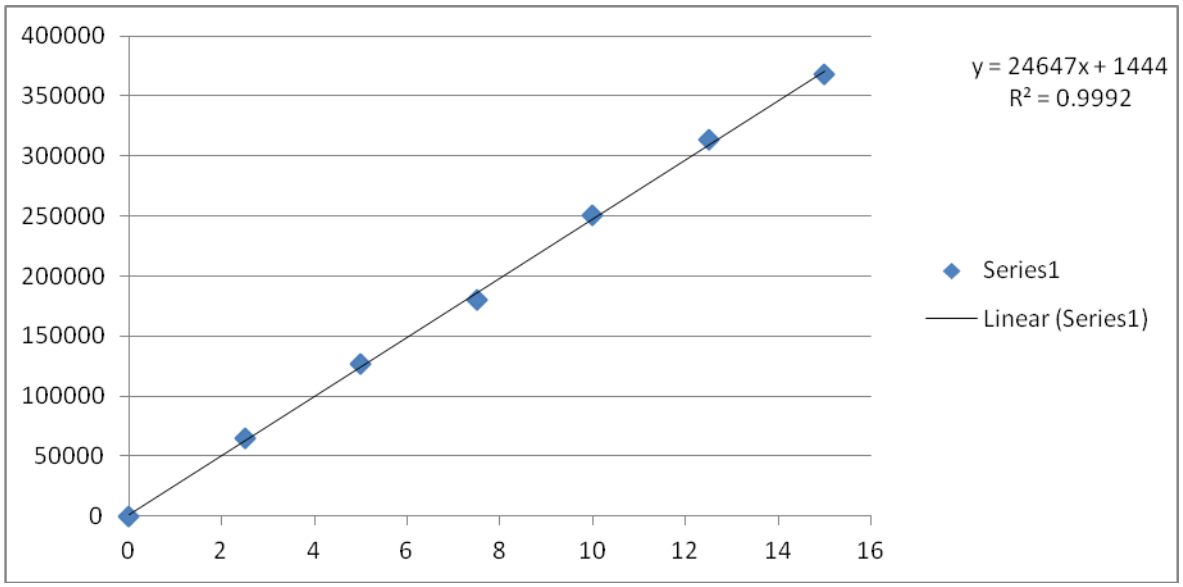

Fig. 7A: Linearity of Pseudoephedrine

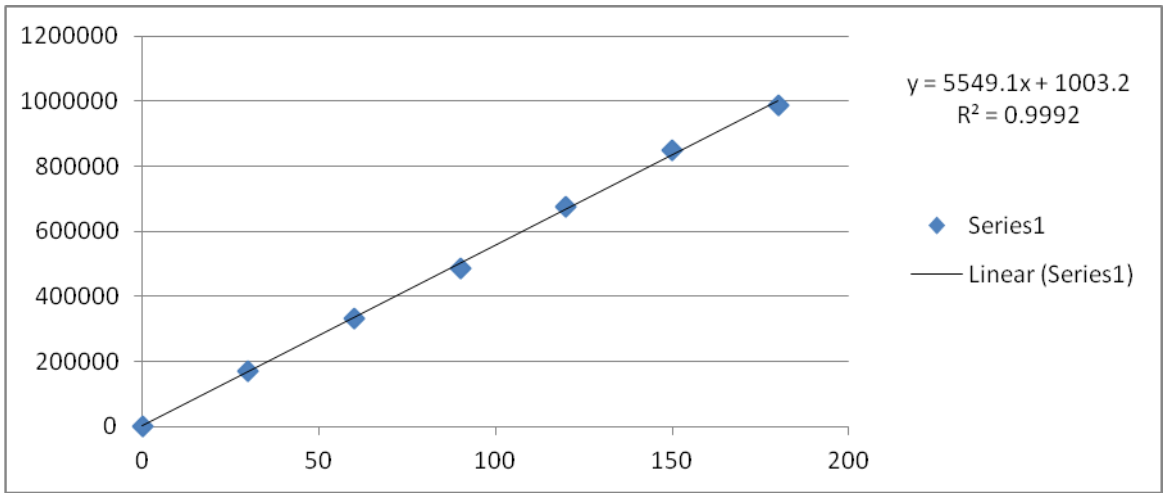

Fig. 7B: Linearity of Ambroxol

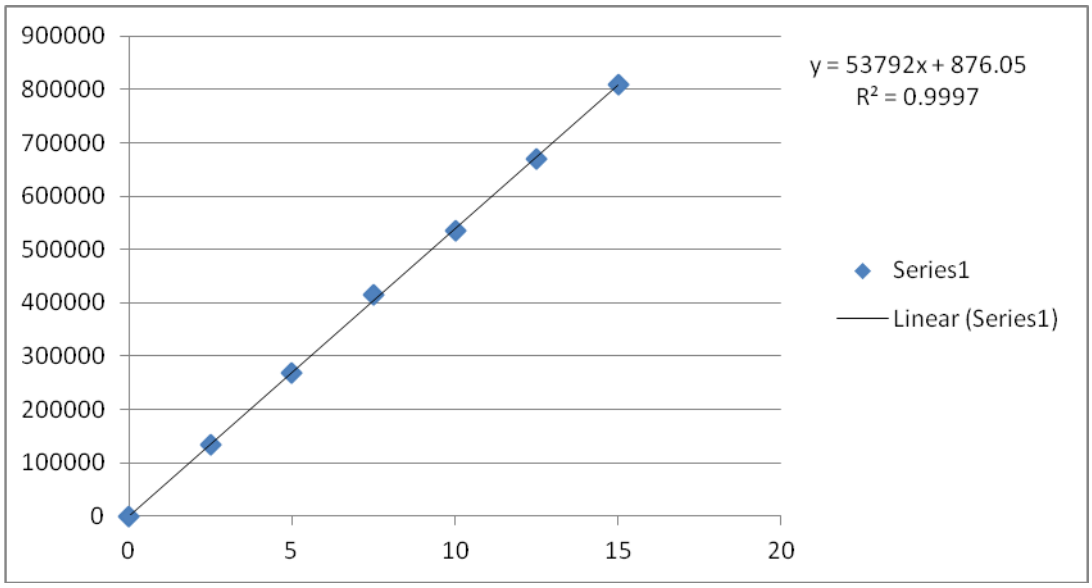

Fig. 7C: Linearity of Desloratidine

System precision:

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Standard solution of Pseudo-ephedrine (10 µg/ml), Ambroxol (120 µg/ml) and Desloratidine (10 µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table-3.

Table-3: System precision

Injections	Areas		
	Pseudoephedrine	**Ambroxol**	**Desloratidine**
1	221092	597184	540160
2	220671	600606	537956
3	220894	601288	537867
4	221361	598214	536842
5	222545	599337	535587
6	221357	599080	530241
AVG	221320	599285	536442
SD	657.0	1507.5	3390.9
%RSD	0.30	0.3	0.6

Precision:

Intraday and interday precision study of Pseudoephedrine, Ambroxol and Desloratidine were carried out by estimating corresponding responses for 6 times on the same day and on consecutive days for the concentration of 10 µg/ml for Pseudo-ephedrine, 120 µg/ml for Ambroxol and 10 µg/ml for Desloratidine. The percent relative standard deviation (%RSD) was calculated which was within the acceptable criteria of not more than 2. The results were shown in Table-4.

Table-4: Precision

Table-4A: Intra-day precision

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
AVG	99.66	99.91	99.82
SD	0.454	0.72	0.275
%RSD	0.46	0.72	0.28

Table-4B: Inter-day precision

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
AVG	98.98	99.60	98.22
S.D	0.599	0.25	0.148
%RSD	0.60	0.25	0.15

Accuracy (Recovery studies):

To determine the accuracy in sample preparation, method of standard additions was made for measuring the recovery of the drugs. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were found to be within the limits. The accuracy was expressed as the percentage of the analyte recovery. The results were shown in Table-5.

Table-5: Accuracy

%Conc.	Pseudoephedrine			Ambroxol			Desloratidine		
	Amount Added (µg/ml)	Amount Found (µg/ml)	%Recovery	Amount Added (µg/ml)	Amount Found (µg/ml)	%Recovery	Amount Added (µg/ml)	Amount Found (µg/ml)	%Recovery
50	5	4.98	99.68	60	59.75	99.58	5	4.97	99.41
50	5	5.00	99.94	60	59.66	99.43	5	5.01	100.10
50	5	5.08	101.62	60	59.48	99.13	5	5.05	100.98
100	10	10.19	101.89	120	120.73	100.61	10	10.08	100.81
100	10	9.91	99.10	120	119.33	99.44	10	10.06	100.60
100	10	9.97	99.72	120	119.75	99.79	10	9.90	99.00
150	15	14.92	99.47	180	180.69	100.38	15	15.12	100.81
150	15	14.93	99.50	180	179.38	99.65	15	14.94	99.62
150	15	14.94	99.63	180	182.02	101.12	15	15.19	101.27
	Average		100.06	Average		99.90	Average		100.29
	SD		0.99	SD		0.65	SD		0.79
	RSD		0.99	RSD		0.65	RSD		0.79

SD = Standard Deviation; RSD = Relative Standard Deviation.

Specificity:

The specificity of the method was performed by injecting blank solution (without any sample) and then a drug solution of 10 µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of three molecules Pseudoephedrine, Ambroxol and Desloratidine from any of the impurities, if present. As there was no interference of impurities, the method was found to be specific.

Limit of Detection and Limit of Quantification:

LOD and LOQ were calculated using the following formula LOD = 3.3(SD)/S and LOQ= 10 (SD)/S, where SD = standard deviation of response (peak area) and S= slope of the calibration curve. Limit of Detection and Limit of Quantification were found to be 0.041 µg/ml and 0.125 µg/ml respectively for Pseudoephedrine, 0.143 µg/ml and 0.435 µg/ml respectively for Ambroxol and 0.034 µg/ml and 0.103 µg/ml respectively for Desloratidine as per ICH guidelines. The results were shown in Table-6 and chromatograms were shown in Fig. 8A & 8B.

Table-6: Characteristics of HPLC method

Drug	Parameters defined	Obtained value
Pseudoephedrine	Linearity range (ppm)	2.5-15 ppm
	Regression coefficient(r^2)	0.999
	Intercept	1444
	Slope	24647
	LOD (ppm)	0.041
	LOQ (ppm)	0.125
	Tailing factor	1.37
	Plate count	3120
Ambroxol	Linearity range (ppm)	30-180 ppm
	Regression coefficient(r^2)	0.999
	Intercept	1003
	Slope	5549
	LOD (ppm)	0.143
	LOQ (ppm)	0.435
	Tailing factor	1.23
	Plate count	4016
Desloratidine	Linearity range (ppm)	2.5-15ppm
	Regression coefficient(r^2)	0.999
	Intercept	876
	Slope	53792
	LOD (ppm)	0.034
	LOQ (ppm)	0.103
	Tailing factor	1.45
	Plate count	3651

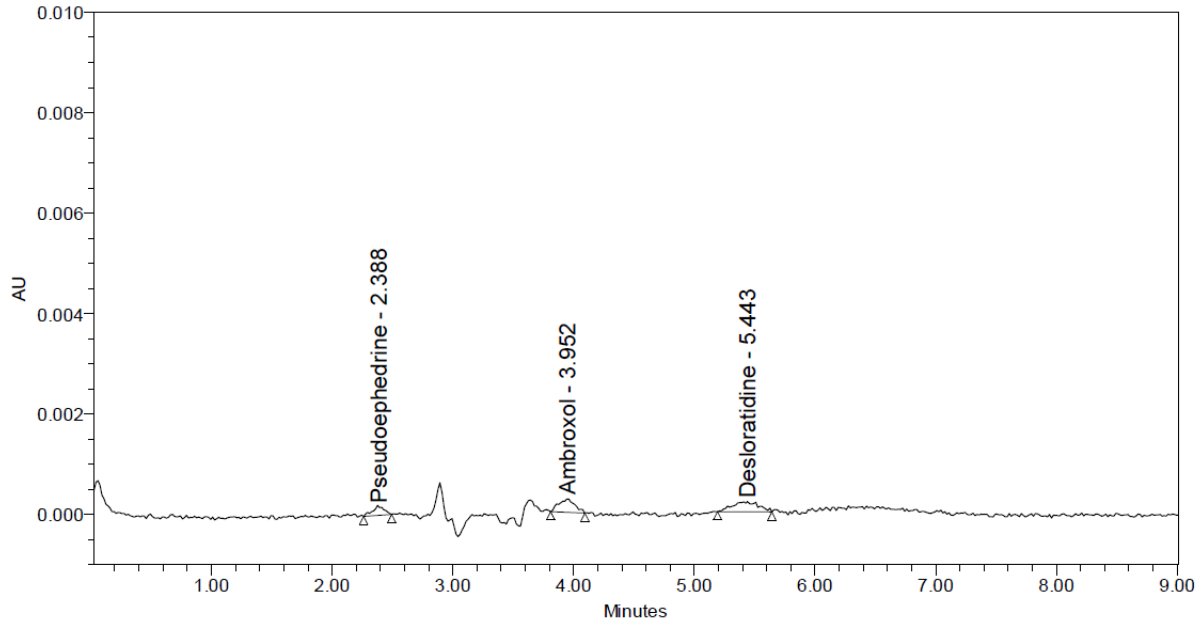

Fig. 8A: HPLC Chromatogram of LOD

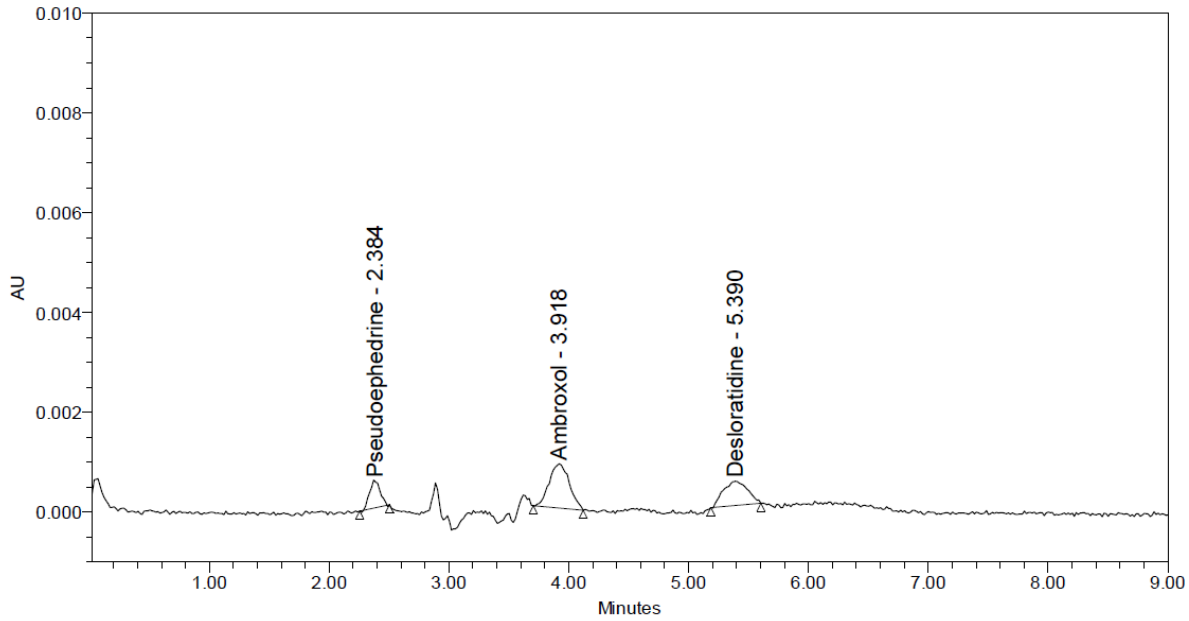

Fig. 8B: HPLC Chromatogram of LOQ

Robustness:

Robustness was carried by varying three parameters from the optimized chromatographic conditions such as making small changes in flow rate (±0.1 ml/min), mobile phase composition (±5%) and column temperature (±5ºC). It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak of interest and the %RSD was not more than 2.0. The degree of reproducibility of the results proven that the method is robust. The results were shown in Table-7A-7C.

Table-7A: Robustness of Pseudoephedrine

S.No.	Parameter	Optimized	Used	Peak Area	Retention Time	Plate count	Tailing Factor
1.	Flow Rate (±0.1 ml/min)	1.0 ml/min	0.9 ml/min	245494	2.665	2578	1.30
			1.0 ml/min	221092	2.375	3120	1.37
			1.1 ml/min	200865	2.146	2044	1.24
2.	Column Temperature (±5ºC)	30ºC	25ºC	243382	2.665	2589	1.29
			30ºC	221092	2.375	3120	1.37
			35 ºC	198528	2.146	2055	1.22
3.	Mobile phase composition	Buffer : ACN : Methanol 50:20:30 v/v	55:20:25	246518	2.377	2365	1.27
			50:20:30	221092	2.375	3120	1.37
			45:20:35	230506	2.375	2386	1.27

Table-7B: Robustness of Ambroxol

S.No.	Parameter	Optimized	Used	Peak Area	Retention Time	Plate count	Tailing Factor
1.	Flow Rate (±0.1 ml/min)	1.0 ml/min	0.9 ml/min	609374	4.391	3050	1.17
			1.0 ml/min	597184	3.971	4016	1.23
			1.1 ml/min	506876	3.534	2457	1.14
2.	Column Temperature (±5ºC)	30ºC	25ºC	608241	4.391	3053	1.17
			30ºC	597184	3.971	4016	1.23
			35 ºC	500539	3.534	2467	1.15
3.	Mobile phase composition	Buffer : ACN : Methanol 50:20:30 v/v	55:20:25	668908	3.911	2785	1.14
			50:20:30	597184	3.971	4016	1.23
			45:20:35	596848	3.918	2780	1.14

Table-7C: Robustness of Desloratidine

S.No.	Parameter	Optimized	Used	Peak Area	Retention Time	Plate count	Tailing Factor
1.	Flow Rate (±0.1 ml/min)	1.0 ml/min	0.9 ml/min	587083	6.029	2719	1.32
			1.0 ml/min	540160	5.448	3651	1.45
			1.1 ml/min	485894	4.863	2161	1.29
2.	Column Temperature (±5ºC)	30ºC	25ºC	578794	6.029	2737	1.30
			30ºC	540160	5.448	3651	1.45
			35 ºC	470597	4.863	2192	1.22
3.	Mobile phase composition	Buffer : ACN : Methanol 50:20:30 v/v	55:20:25	604648	5.372	2510	1.32
			50:20:30	540160	5.448	3651	1.45
			45:20:35	556599	5.382	2491	1.31

System suitability & System precision:

The system suitability was determined by making six replicate injections from freshly prepared standard solutions. The observed RSD values were well within usually accepted limits (≤2%). Theoretical plates, tailing factor of Pseudoephedrine, Ambroxol and Desloratidinewere determined. The results are all within acceptable limits summarized in Table-3 & 6.

Forced Degradation Studies:

Forced degradation studies were performed to demonstrate the optimized method is stability indicating. And to prove the method which can be able to measure accurately active pharmaceutical ingredient in presence of degradants which are expected to be formed during different types of degradations applied to the drug sample. Forced degradation conditions were optimized during method development. Final optimized forced degradation conditions are as follows

For forced degradation analysis, aliquots of stock (0.1 mg/ml, 1.2 mg/ml and 0.1 mg/ml) were prepared separately and treated with 1 ml of 2N HCl (Acid stability), 1 ml of 2N NaOH (Alkaline stability), 1 ml of 30% H_2O_2 (Oxidative degradation), exposure of standard drug solution at 105ºC for 5 h (dry heat degradation), photo stability degradation (exposure of drug at 200 watt/m^2) and neutral degradation (refluxing with water at 60ºC for 8 h. Stability of these samples was compared with fresh sample on the day of analysis. The HPLC chromatograms of degraded products show no interference at the analyte peaks, hence the method was

specific and stability indicating. The results were shown in Table-8 and the chromatograms were shown in Fig. 9A-9F. The detailed degradation for each condition is as follows:

Table-8: Forced Degradation Studies

Table-8A: Forced Degradation Studies for Pseudoephedrine

S.No	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1.	Controlled sample	99.66	--	0.987	1.456	No
2.	Acid Degradation	94.59	5.09	0.834	1.170	No
3.	Base Degradation	95.81	3.86	0.135	0.215	No
4.	Peroxide Degradation	92.61	7.07	1.565	5.926	No
5.	Thermal Degradation	97.45	2.22	1.253	4.345	No
6.	UV Degradation	98.55	1.11	1.134	3.546	No
7.	Water Degradation	99.00	0.66	0.934	5.324	No

Table-8B: Forced Degradation Studies for Ambroxol

S.No.	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1.	Controlled sample	99.91	--	0.556	1.211	No
2.	Acid Degradation	93.64	6.28	1.102	1.402	No
3.	Base Degradation	96.01	3.90	0.154	0.256	No
4.	Peroxide Degradation	94.03	5.89	1.093	2.854	No
5.	Thermal Degradation	96.89	3.02	1.345	2.835	No
6.	UV Degradation	98.77	1.14	1.123	2.456	No
7.	Water Degradation	99.41	0.50	0.789	1.435	No

Table-8C: Forced Degradation Studies for Desloratidine

S.No.	Injection	%Assay	%Degradation	Purity Angle	Purity Threshold	Purity Flag
1.	Controlled Sample	99.82	--	0.867	1.231	No
2.	Acid Degradation	93.47	6.36	0.192	0.323	No
3.	Base Degradation	93.22	6.61	0.185	0.308	No
4.	Peroxide Degradation	92.59	7.24	0.190	0.334	No
5.	Thermal Degradation	95.37	4.46	0.343	1.324	No
6.	UV Degradation	97.36	2.46	0.176	0.338	No
7.	Water Degradation	98.37	1.45	0.214	0.421	No

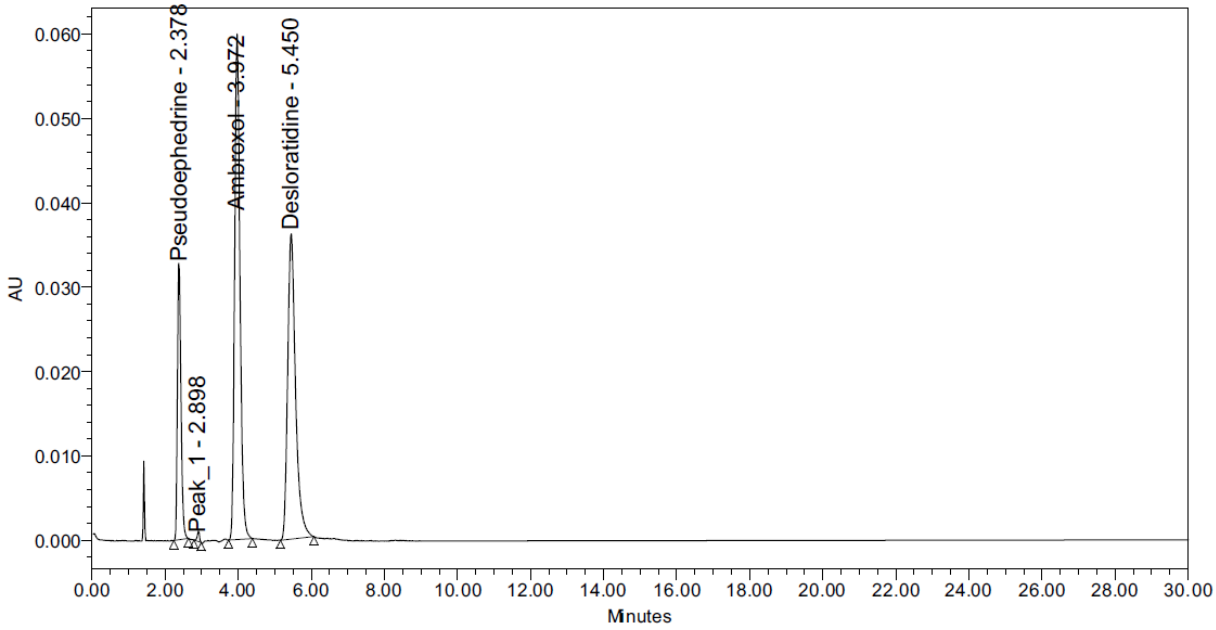

Fig. 9A: Acid Degradation

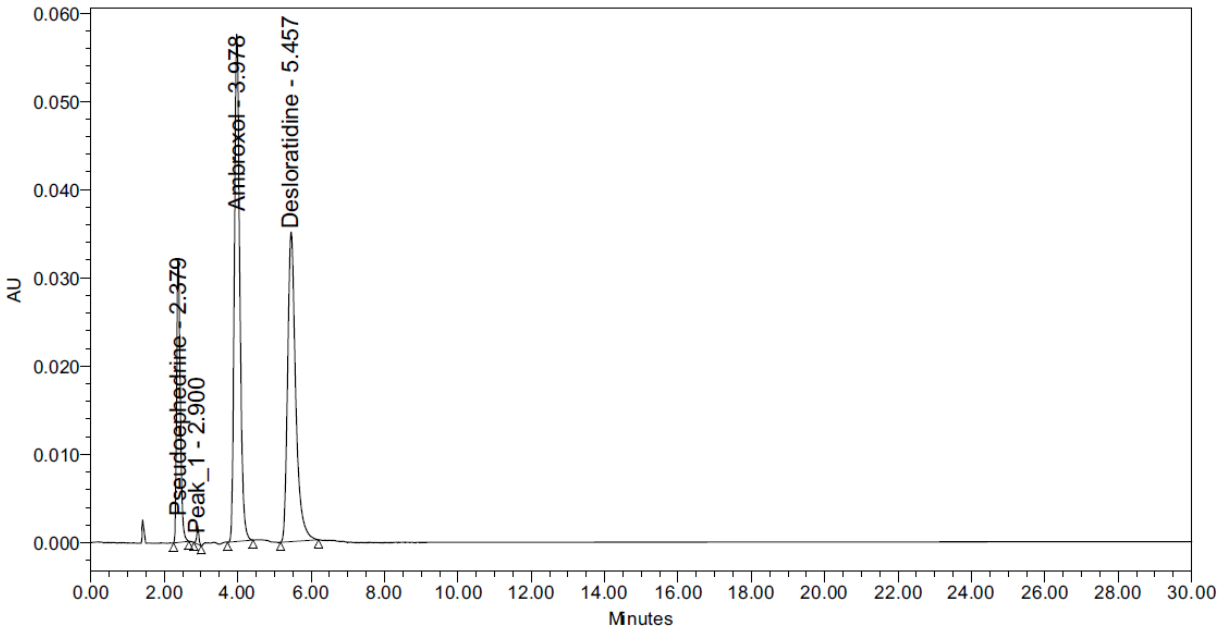

Fig. 9B: Base Degradation

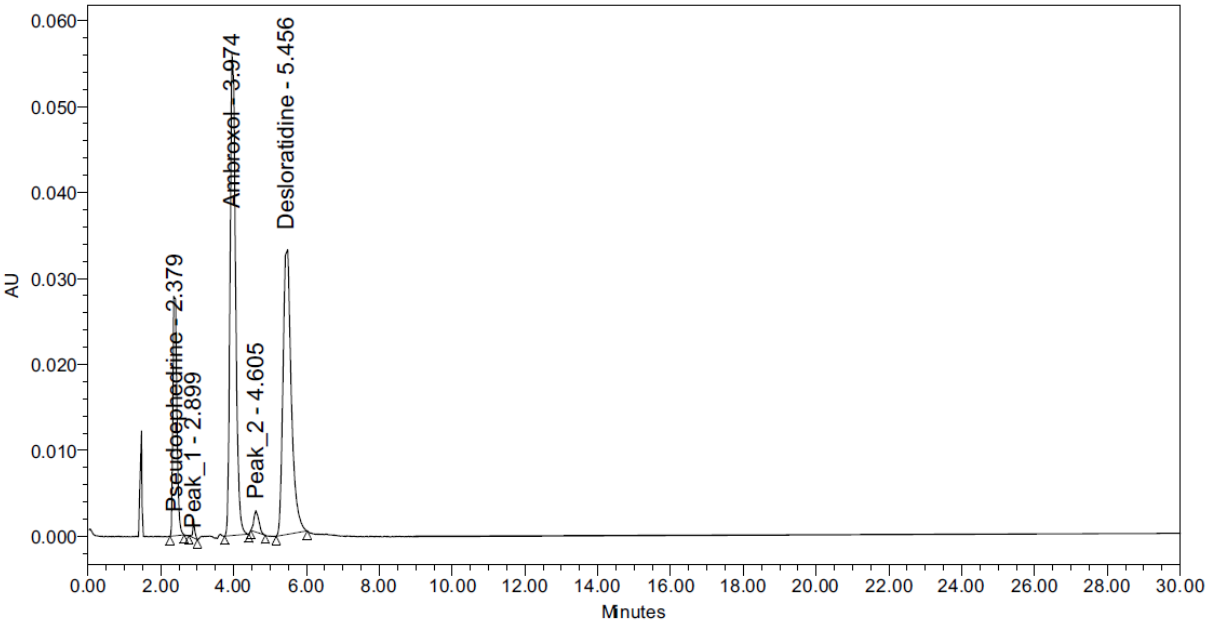

Fig. 9C: Peroxide Degradation

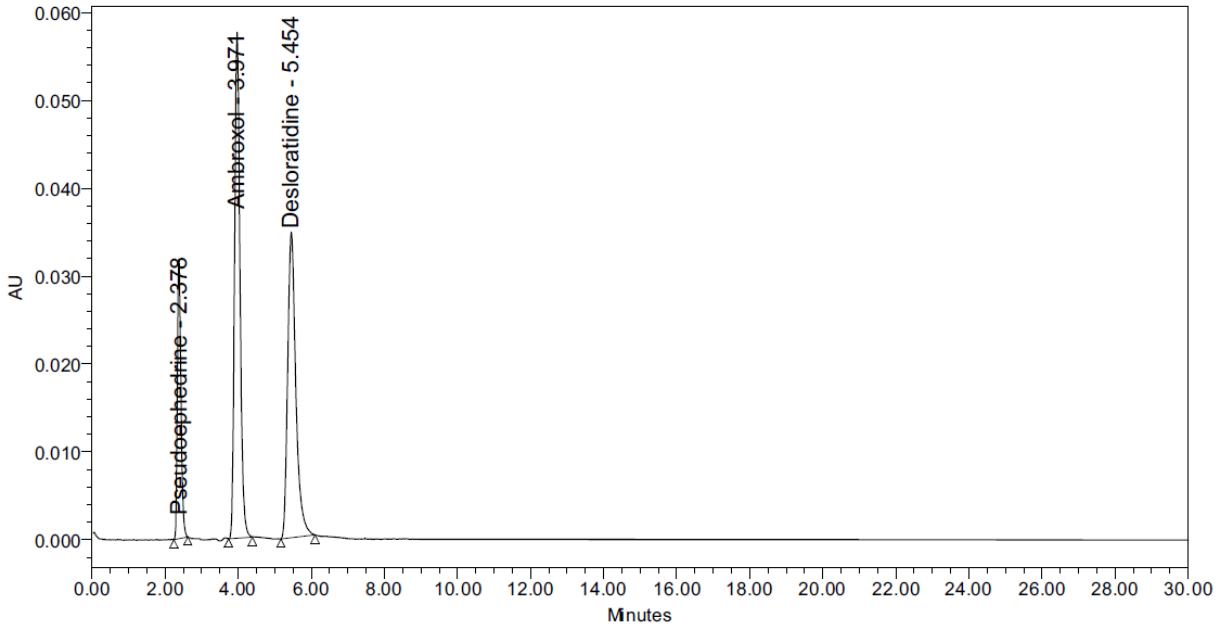

Fig. 9D: Thermal Degradation

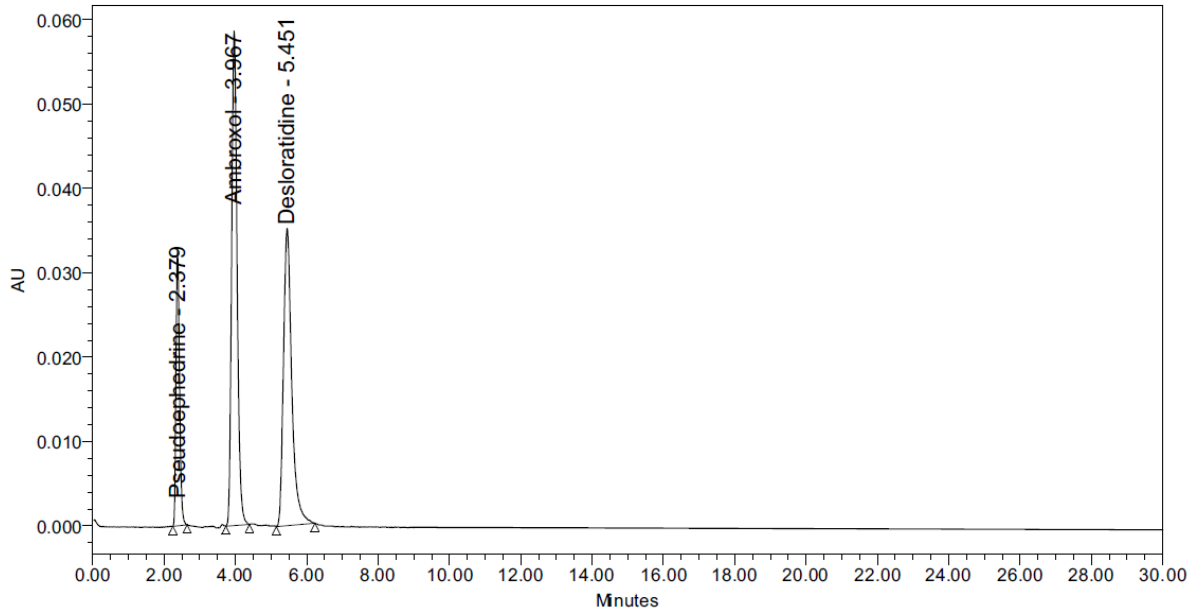

Fig. 9E: UV Degradation

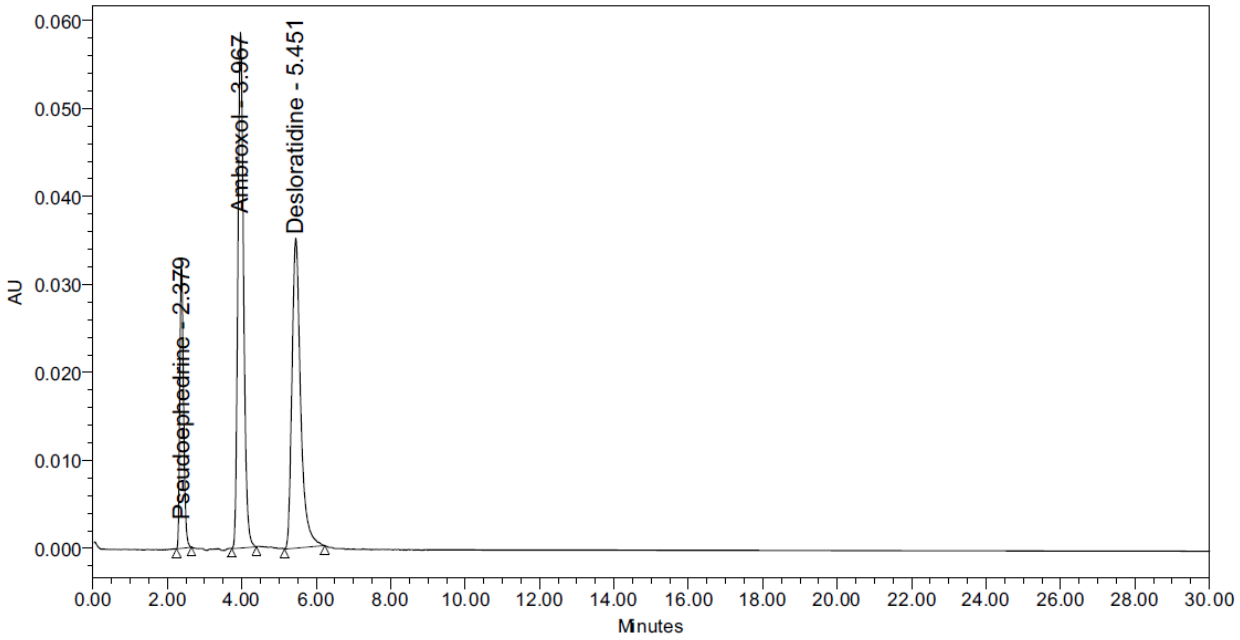

Fig. 9F: Water degradation

Oxidation:

To 1 ml of stock solution of Pseudoephedrine, Ambroxol and Desloratidine, 1 ml of 30% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^{0}C. For HPLC study, the resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solution for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Pseudoephedrine, Ambroxol and Desloratidine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60^{0}C.The resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solution for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution of Pseudoephedrine, Ambroxol and Desloratidine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}C. The resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solution for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105^{0}C for 6hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solutions for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies:

The photochemical stability of the drug was also studied by exposing the 0.1 mg/ml, 1.2 mg/ml and 0.1 mg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solutions for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10 µl were injected into the system and the

chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60^0C. For HPLC study, the resultant solution was diluted to obtain 60 µg/ml, 120 µg/ml and 10 µg/ml solutions for Pseudoephedrine, Ambroxol and Desloratidine respectively and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION:

The main aim for development of chromatographic method was to get reliable method for quantification of Pseudoephedrine, Ambroxol and Desloratidine from bulk and pharmaceutical dosage form and which will be applicable for the degradation products also. Different chromatographic conditions were employed for the analysis of the Pseudoephedrine, Ambroxol and Desloratidine in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using Phosphate Buffer: Acetonitrile: Methanol in the ratio of 50:20:30 %v/v at a flow rate 1.0 ml/min. Samples were analyzed at 225 nm at an injection volume of 10 µL and separation was carried by using Inertisil ODS 3V, (250 x 4.6 mm, 5µ), column. The retention time and tailing factor were calculated. The retention time of Pseudoephedrine, Ambroxol and Desloratidine were found to be 2.375, 3.985 and 5.458 respectively. The proposed column was selected which gave a sharp and symmetrical peak with 1.37 tailing factor and theoretical plates of 3120 for Pseudoephedrine, 1.23 tailing factor and theoretical plates of 4016 for Ambroxol and 1.45 tailing factor and theoretical plates of 3651 for Desloratidine.

The calibration curve was linear over the concentration range of 2.5-15 (ppm) (Pseudoephedrine), 30-180 (ppm) (Ambroxol) and 2.5-15 (ppm) (Desloratidine). Six different concentrations of Pseudoephedrine, Ambroxol and Desloratidine in the given range were prepared and injected into HPLC. The linearity of the method was statistically confirmed. RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters were given in table-6.

Forced degradation studies concluded that the all the degradant peaks obtained during degradation were well resolved from the main drugs i.e. Pseudoephedrine, Ambroxol and Desloratidine. And the peak purity was passed i.e. purity angle was less than purity threshold as per Empower-2 software. Hence the method is found to be stability indicating. The slope (m) and intercept (c) obtained were shown in the table-6.

Therefore proposed developed and validated stability indicating method was successfully applied to determine Pseudoephedrine, Ambroxol and Desloratidine in bulk and pharmaceutical dosage form.

CONCLUSION:

The developed method is accurate, simple, rapid and selective and proved to be stability indicating for the simultaneous estimation of Pseudoephedrine, Ambroxol and Desloratidine in bulk and pharmaceutical dosage form. The sample preparation is simple, the analysis time is short and the elution is by isocratic method. The retention time of Pseudoephedrine, Ambroxol and Desloratidine were found to be 2.375, 3.985 and 5.485 min respectively. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. Forced degradation studies of different conditions shows that all the degradants were well resolved from these main drug peaks and able to quantify the Pseudoephedrine, Ambroxol and Desloratidine in presence of degradants and excipients which proved that the method is found to be stability indicating. Hence the proposed method can be conveniently adopted for the routine quality control analysis in the bulk and combined pharmaceutical formulations.

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