Research Paper

Development and Validation of Stability-indicating RP-HPLC Method for the Estimation of Pseudoephedrine, Ambroxol and Desloratadine in Bulk and Tablet Dosage Forms

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A simple, accurate, selective and stability-indicating RP-HPLC method was developed for the simultaneous estimation of pseudoephedrine, ambroxol and desloratadine in bulk and their tablet formulations. Effective chromatographic separations were achieved on a Hypersil BDS C8, 250×4.6 mm, 5 µ column with reverse phase elution. The mobile phase composed of 0.01 M potassium dihydrogen phosphate and acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml/min. The detection was carried out at 220 nm. The retention times were 2.2 min for pseudoephedrine, 3.4 min for ambroxol and 5.5 min for desloratadine. The linearity ranges for pseudoephedrine, ambroxol and desloratadine were 15 to 90 µg/ml, 30 to 180 µg/ml and 2.5 to 15 µg/ml, respectively with correlation coefficient 0.999. The developed method was validated statistically with respect to linearity, range, precision, accuracy, specificity, robustness, ruggedness, detection and quantification limits and also subjected to stress conditions like acidic and alkaline hydrolysis, oxidation, photolysis and thermal degradation. The method was accurate, precise, specific, rapid and found to be suitable for the analysis of commercial samples.

Key words: Stability-indicating, pseudoephedrine, ambroxol, desloratadine, RP-HPLC

Pseudoephedrine hydrochloride (PSD) is (1S,2S)-2-methylamino-1-phenylpropan-1-ol hydrochloride and official in European Pharmacopoeia and U.S. Pharmacopoeia. Ambroxol hydrochloride (AMB) is trans-4-[(2-amino-3,5-dibromobenzyl)amino] cyclohexanol hydrochloride and official in British Pharmacopoeia. Desloratadine (DLT) is 8-chloro-6,11-dihydro-11-(4-piperdinylidene)-5H-benzo[5,6] cyclohepta[1,2-b] pyridine is non pharmacopeial drug. Fig. 1 shows chemical structures of three analytes. The three drug combination in the ratio of 30:60:5 mg PSD, AMB and DLT, respectively in tablet dosage form is available under the brand name Nucope-AD. A few methods have been reported individually or in combination with other drugs including simultaneous estimation of AMB and loratadine by spectrophotometric method[1], high performance liquid chromatographic (HPLC) method[2-3], simultaneous estimation of AMB and DLT by spectrophotometric method[4-6], HPLC[7,8], HPTLC[9]. Since no methods were reported for the simultaneous estimation of PSD, AMB and DLT in bulk and pharmaceutical dosage forms, an attempt was made to develop a simple, accurate, precise and rugged method was developed for the estimation of these drugs simultaneously in bulk and tablet formulations.

MATERIALS AND METHODS

HPLC grade acetonitrile, potassium dihydrogen phosphate AR Grade and potassium hydroxide AR grade were purchased from SD Fine-Chem, Mumbai, India were used in the study. Reference samples were obtained from M/s. Richer Pharmaceuticals, Hyderabad, India.

Chromatographic conditions:

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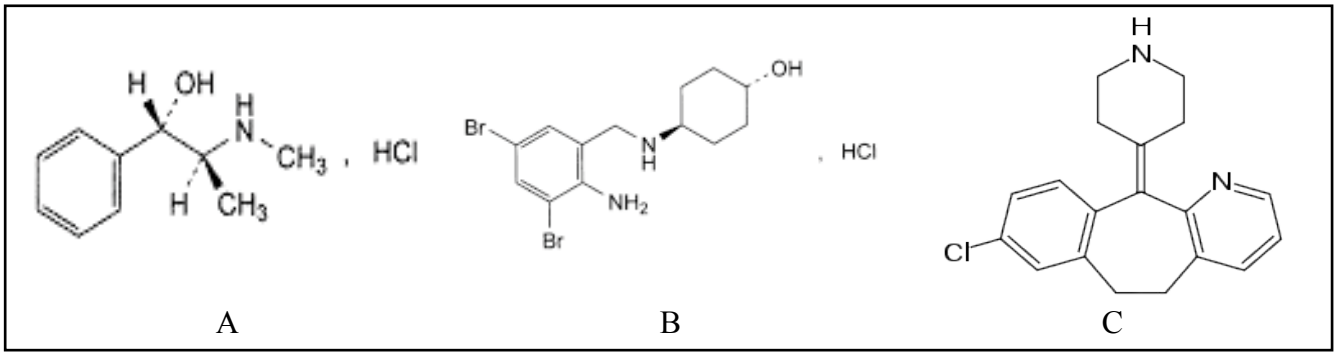

Fig. 1: Chemical structures of analytes.
Chemical structures of A. pseudoephedrine HCl, B. ambroxol HCl and C. desloratadine.

A HPLC system equipped with Waters e2695 separation module with HPLC instrument provided with photo diode array (PDA) detector, autosampler with Empower 2 software from Waters corporation, Milford, USA was employed in the study. The chromatographic separations were achieved on a Hypersil BDS C8, 250×4.6 mm, 5 µ column.

Preparation of mobile phase:

Weighed and dissolved 1.36 g of potassium dihydrogen phosphate in 1000 ml of water (adjusted pH to 6.5±0.05 with dilute potassium hydroxide) and acetonitrile in the ratio of 50:50 v/v was filtered through 0.22 µ membrane filter and degassed. Mobile phase was used as diluent. The mobile phase was filtered and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min. The column temperature was maintained at 30° and the detection of the drug was carried out at 220 nm.

Preparation of standard solution:

Weighed accurately 30.0 mg of PSD, 60.0 mg of AMB and 5.0 mg of DLT on a Sartorius semi-micro balance model-CPA225D and transferred in to a 50 ml volumetric flask. The solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution containing 600 µg/ml PSD, 1200 µg/ml AMB and 100 µg/ml DLT. Working solutions were prepared by diluting the working standard solution in the concentration ranges 15-90 µg/ml, 30-180 µg/ml, and 2.5-15.0 µg/ml for PSD, AMB and DLT, respectively. Each concentration was injected thrice under described chromatographic conditions. The calibration curve constructed peak area against concentration.

Sample preparation:

Weighed accurately previously weighed and crushed 20 tablet powder equivalent to one tablet weight containing 30 mg of PSD, 60 mg of AMB and 5 mg of DLT. This powder was transferred to a 50 ml volumetric flask, and volume is made up to the mark in mobile phase, sonicated to dissolve and filtered through Whatman filter paper. It was further diluted 10 ml of the stock solution to 100 ml with mobile phase.

Forced degradation and stability-indicating tests[10]:

Weighed accurately 30.0 mg of PSD, 60.0 mg of AMB and 5.0 mg of DLT, and transferred to 50 ml volumetric flask and dissolved in mobile phase with sonication and the resulting solution was diluted with the mobile phase to get a working standard solution containing 600 µg/ml PSD, 1200 µg/ml AMB and 100 µg/ml DLT.

To determine acid degradation, 10 ml of 1 N HCl was added to 10 ml of stock solution, kept at 80° for about 6 h in a water bath, cooled volume was made up to 100 ml with the mobile phase and filtered through 0.22 µ membrane filter.

To determine alkali-induced degradation, 10 ml of 1 N NaOH was added to 10 ml of stock solution, heated at 80° at 6 h in a water bath, allowed to cool, volume made up to 100 ml with mobile phase and filtered through a 0.22 µ membrane filter.

To determine oxidative degradation 5 ml of 3% H_2O_2 was added to 10 ml of stock solution, heated at 80° for 3 h in a water bath, allowed to cool, volume made up to 100 ml with mobile phase and filtered through a 0.22 µ membrane filter.

Thermal degradation was determined by keeping 10 ml of stock solution at 80° for 10 days, allowed to cool, volume made up to 100 ml with mobile phase and filtered through a 0.22 µ membrane filter.

RESULTS AND DISCUSSION

Method validation was performed following ICH Q2 guideline specifications[11]. System suitability is an integral part of the method validation and performed to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections. The results were within the limits and were presented in Table 1 while the fig. 2 displayed the system suitability chromatogram.

In the blank chromatogram, there were no peaks observed at the retention times of PSD, AMB and DLT, and also the degradation studies showed that there was no interference with degradants, purity angle was less than the purity threshold for the sample solution indicating that the method is specific. To determine the accuracy of the proposed method, recovery experiments were conducted; known concentration of pure drug was spiked at three different levels, 50, 100 and 150% and was calculated. Accuracy was calculated as the percentage of recovery and the results were tabulated in Table 2.

The precision was evaluated at three levels, repeatability, reproducibility and intermediate precision each level

TABLE 1: SYSTEM SUITABILITY RESULTS

Parameter	Results		
	Pseudoephedrine	Ambroxol	Desloratadine
% RSD of peak area	0.17	0.32	049
% RSD of retention time	0.19	0.19	0.13
Tailing factor (T)	1.15	1.16	1.38
Theoretical plate (N)	3239	3278	3116
Resolution (R)	-	5.35	6.48

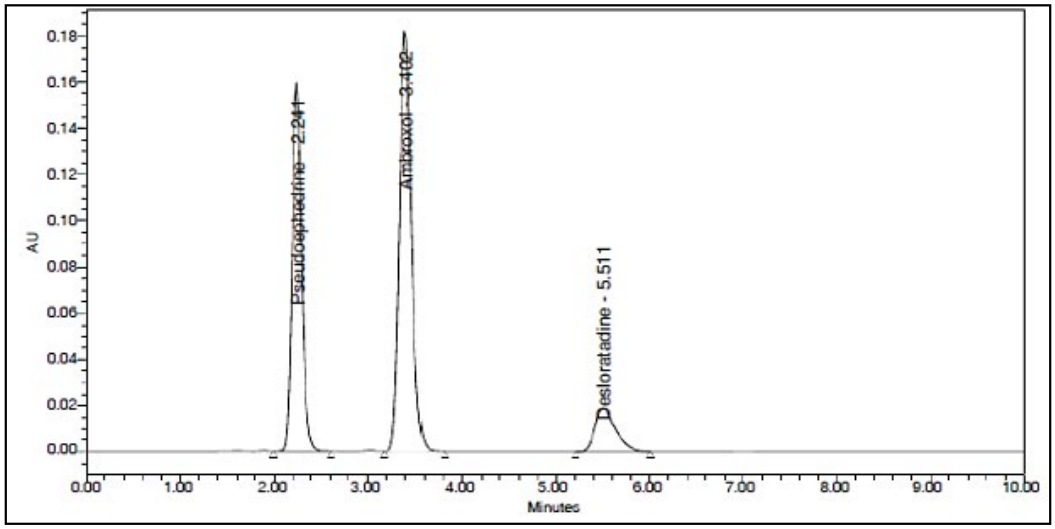

Fig. 2: Typical HPLC chromatogram of mixture containing three components.

TABLE 2: ACCURACY DATA

Parameter	Amount added (µg)	Amount recovered (µg)	% recovery	Mean % recovery
Pseudoephedrine				
50% level	30.0	29.79	99.31	99.54
100%level	60.0	59.89	99.81	
150%level	90.0	89.54	99.49	
Ambroxol				
50% level	60.0	59.5	99.17	99.85
100%level	120.0	120.42	100.35	
150%level	180.0	180.18	100.1	
Desloratadine				
50% level	5.0	4.94	98.71	99.68
100% level	10.0	10.005	100.05	
150% level	15.0	15.04	100.27	

Values are mean of triplicate analysis at 50,100 and 150% levels

of precision was investigated by six replicate injections of concentrations 120, 100 and 10 µg/ml PSD, AMB and DLT, respectively. The result of precision was expressed as percentage relative standard deviation (% RSD) and was tabulated in Table 3.

The linearity of the measurement was evaluated by analyzing different concentrations (25% to 150%) of the standard solutions of PSD, AMB and DLT. Calibration curve was constructed by plotting concentration against mean peak area and the regression equation was computed. The summary of the parameters is shown in Table 4. Estimation of limit of detection (LOD) and limit of quantification (LOQ) considered the acceptable signal-to-noise ratios 3:1 and 10:1, respectively. LOD and LOQ determined were 0.3235 µg/ml and 0.98 µg/ml for PSD, 1.2344 and 3.741 µg/ml for AMB and 0.1575 µg/ml and 0.4772 µg/ml for DLT, respectively.

The robustness of the method was evaluated by subjecting the 100% test concentration to small but deliberate changes in the chromatographic conditions like, flow rate, mobile phase composition and column temperature and the results were shown in Table 5. The ruggedness of the proposed method was determined

TABLE 3: PRECISION STUDIES

Parameter	Pseudoephedrine	Ambroxol	Desloratadine
Repeatability			
RSD of Retention time	0.19	0.19	0.13
RSD of Peak Area	0.17	0.32	0.49
Reproducibility			
RSD of Retention time	0.13	0.11	0.11
RSD of Peak Area	0.59	0.56	0.49
Intermediate Precision			
RSD of Retention time	0.20	0.13	0.09
RSD of Peak Area	0.17	0.29	0.49

TABLE 4: REGRESSION EQUATION PARAMETERS

Parameter	Pseudoephedrine	Ambroxol	Desloratadine
Linearity range(µg/ml)	15 to 90	30 to 180	2.5 to 15
Correlation co-efficient	0.999	0.999	0.999
Slope	19380	13786	28705
Y-intercept	-12178	-16085	-3795

TABLE 5: ROBUSTNESS STUDY

Parameter	Variation	Chromatographic Conditions											
		Retention time			Area			Theoretical Plates			Tailing Factor		
		PSD	AMB	DLT	PSD	AMB	DLT	PSD	AMB	DLT	PSD	AMB	DLT
Flow change	0.9 ml/min	2.553	3.868	6.274	1327568	1891871	324566	3474	3601	3230	1.18	1.17	1.39
	1 ml/min	2.241	3.402	5.511	1140569	1621587	281024	3239	3278	3116	1.15	1.16	1.38
	1.1 ml/min	2.005	3.038	4.908	1035509	1474678	254981	3982	2991	2897	1.14	1.15	1.38
Temperature	25°	2.257	3.512	6.450	1158053	1647565	284220	2212	3102	2527	1.17	1.16	1.41
	30°	2.241	3.402	5.511	1140569	1621587	281024	3239	3278	3116	1.15	1.16	1.38
	35°	2.029	3.299	4.792	1161505	1654521	283817	2239	3525	4172	1.16	1.15	1.29
Wavelength	218 nm	2.227	3.496	5.55	1163102	1659076	283013	2223	3554	4154	1.16	1.15	1.28
	220 nm	2.241	3.402	5.511	1140569	1621587	281024	3239	3278	3116	1.15	1.16	1.38
	222 nm	2.223	3.495	5.448	1161021	1654354	284565	2246	3564	4176	1.15	1.15	1.28

TABLE 6: ASSAY RESULTS

Drug	Labeled amount (mg/tab)	Amount found (mg/tab)	% of Assay
Pseudoephedrine	30	29.76	99.21
Ambroxol	60	59.522	99.20
Desloratadine	5	4.969	99.37

by analysing the same sample using different columns, different analysts, and on different instruments.

The stability studies of the standard solution were conducted at intervals of 24 h and 48 h at room temperature. There were no significant changes observed in system suitability parameters like theoretical plates, tailing factor, retention time and resolution. Hence the standard solution was found to be stable up to 48 h on the bench top. The stability study of the mobile phase was conducted at intervals of 24 h and 48 h at room temperature. There were no significant changes

TABLE 7: DEGRADATION STUDIES

Stress condition	% Assay of active ingredient			Purity angle	Purity threshold
	PSD	AMB	DLT		
Acid degradation (1N HCl for 6 h)	88.1	84.7	89.4	0.829	1.071
Alkali degradation (1M NaoH for 6 h)	89.3	89.0	87.1	0.757	0.989
Thermal degradation (80° for 10 days)	90.9	85.2	88.8	0.981	1.082
Photolytic (UV 200 Watt hours)	90.0	89.1	88.8	0.763	0.921

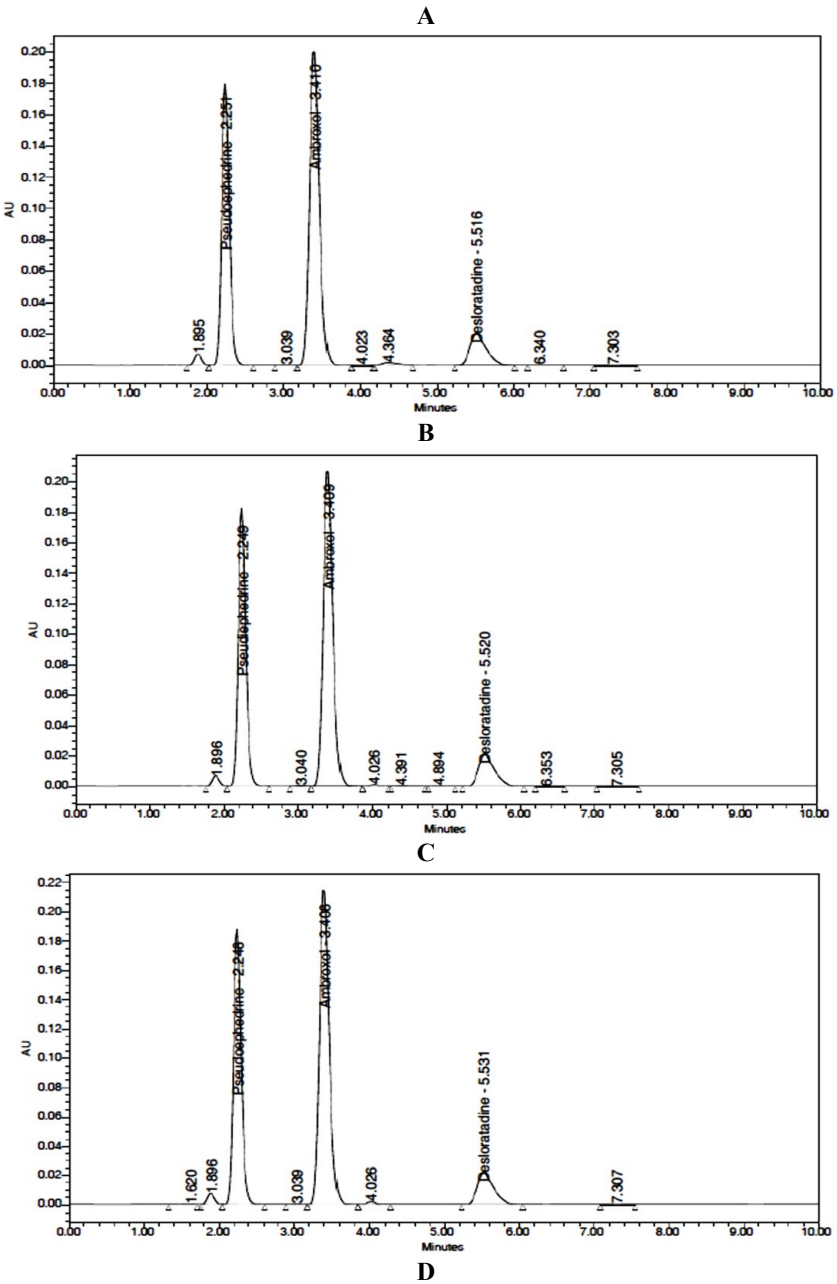

Fig. 3: Chromatograms of test sample subjected to different forced degradation conditions.
A. acid stress degradation; B. alkali stress degradation; C. oxidative stress degradation; D. thermal stress degradation.

observed in system suitability parameters like peak area, theoretical plates, tailing factor, retention time and resolution. Hence the mobile phase is stable up to 48 h at room temperature. The proposed method was applied successfully for the analysis of PSD, AMB and DLT in tablet dosage forms, satisfactory results were obtained and the results were summarized in Table 6.

Since no interference of blank and degradants, the HPLC results showed that the three active ingredients PSD, AMB and DLT. Purity angle was less than the purity threshold and hence the proposed method was the specific and revealed its stability-indicating power. The results were summarized in Table 7. Fig. 3 displays chromatograms of samples subjected to different stress degradations.

A simple, specific and reliable reverse phase HPLC-DAD method was developed for the estimation of PSD, AMB and DLT in their pharmaceutical formulation. The three compounds were subjected to forced degradation applying several stress conditions. The proposed method successfully separated all the three compounds with degradants, the active contents were estimated. The proposed method is specific and stability-indicating. Hence the developed method can be adapted to regular quality control analysis.

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Conflicts of interest:

There are no conflicts of interest.

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