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Journal of Chemical and Pharmaceutical Research, 2014, 6(7):1577-1582

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Simultaneous determination of pseudoephedrine hydrochloride, ambroxol hydrochloride, guaiphenesin and chlorpheniramine maleate in multicomponent pharmaceutical preparations (syrup) by RP-HPLC

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ABSTRACT

An RP-HPLC method for the simultaneous determination of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate in syrup was developed and validated as per ICH & FDA guidelines. The separation was achieved with a Hypersil BDS C18, 5µm, 100 mm x 4.6 mm, by using a isocratic system at 215 nm. The mobile phase was prepared with Buffer (0.007 mM/L of KH_2PO_4 adjusted pH 3.0 with Orthophosphoric acid) and Acetonitrile in ratio (78:22) v/v. The flow rate was 0.8 mL/min and column temperature was maintained at 30^0C. The separation was achieved within 12 minutes. The linearity of the proposed method was investigated in the range 0.02404-0.03606 mg/mL (r= 0.999) for Pseudoephedrine, 0.04002-0.06002mg/mL (r= 1.000) for Guaiphenesin, 0.01202-0.01804mg/mL (r= 1.000) for Ambroxol, and 0.00162-0.00242 mg/mL (r= 0.998) for Chlorpheniramine. Blank and placebo did not disturb the detection of Pseudoephedrine, Guaiphenesin, Ambroxol, and Chlorpheniramine. The developed method has an advantage that all the drugs can be quantified alone or in combination using a single mobile phase.

Key words: Pseudoephedrine, Ambroxol, Guaiphenesin, Chlorpheniramine Maleate, RP-HPLC, ICH, Validation.

INTRODUCTION

Pseudoephedrine is a sympathomimetic drug of the phenethyl amine and amphatamine chemical classes. It may be used as a nasal/sinus decongestant, as a stimulant, or as a wakefulness-promoting agent. Guaiphenesin is an expectorant drug. [1] It is chemically known as (2*RS*)-3-(2-methoxyphenoxy)propane-1,2-diol. Ambroxol is a secretolytic agent and exhibit anti-inflammatory properties, used in the treatment of respiratory. [3-5] chemically it is known as *trans*-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol.Chlorpheniramine Maleate is used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. [6-7] It is chemically known as 2-[p-chloro-(alpha)-[2-(dimethylamino)ethyl]benzyl]pyridine Maleate (1:1).

Literature survey revealed that there are two methods available for simultaneous determination of these active ingredients. [8-9] However, none of the method available has been reported for the simultaneous determination of Ambroxol, Pseudoephedrine, Guaiphenesin, and Chlorpheniramine Maleate in pharmaceutical dosage form - syrup (30 mg of Pseudoephedrine hydrochloride, 50 mg of Guaiphenesin 30 mg of Ambroxol hydrochloride, and 2 mg of Chlorpheniramine maleate).

The method was validated as per the present ICH guideline on validation of analytical procedure Q2A (R1).[10-11] Quantitation was achieved with UV detection at 215 nm based on peak area with linear calibration curves at concentration ranges. The method was linear over wide concentration range of 0.02404-0.03606 mg/mL (r= 0.999)

for Pseudoephedrine, 0.04002-0.06002mg/mL (r= 1.000) for Guaiphenesin, 0.01202-0.01804mg/mL (r= 1.000) for Ambroxol, and 0.00162-0.00242 mg/mL (r= 0.998) for Chlorpheniramine. The accuracy of the method was evaluated in triplicate at five concentration level i.e. 80%, 100% and 120% of target test concentration.

EXPERIMENTAL SECTION

Materials and methods

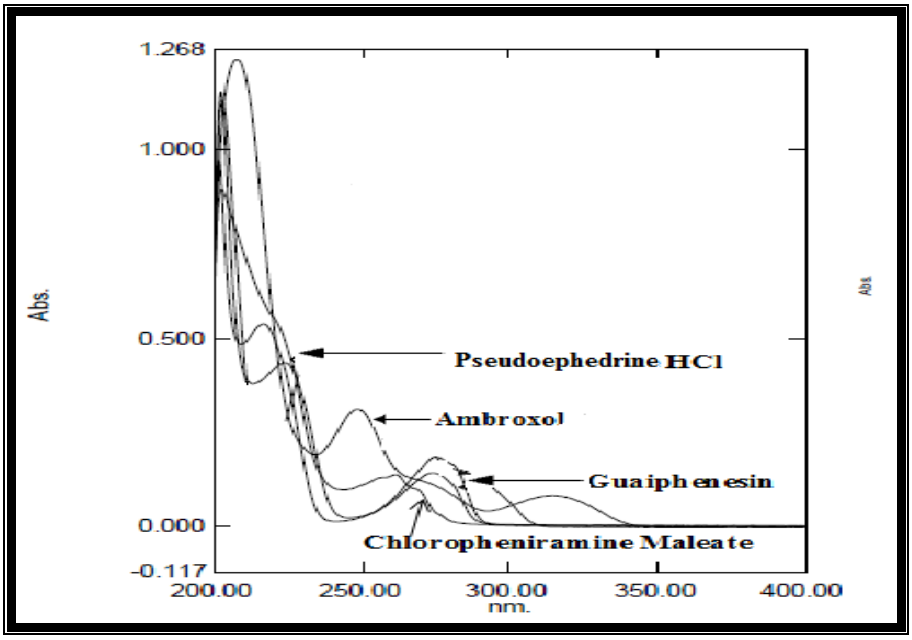
Solvin syrup (containing 30 mg of Pseudoephedrine hydrochloride (PSE), 50 mg Guaiphenesin (GPN), 15 mg of Ambroxol hydrochloride (ABX), and 2 mg of Chlorpheniramine maleate (CPM)) were purchased from Centaur pharmaceutical industries Ltd, India. Standards for Pseudoephedrine hydrochloride, Ambroxol hydrochloride, Guaiphenesin and Chlorpheniramine Maleate, were provided by Medley Pharmaceutical, Mumbai, India.

HPLC grade, methanol, Acetonitrile, Orthophosphoric acid (88%) and sodium perchlorate were purchased from Merck chemicals. Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore.

Selection of UV wavelength:

10ppm solution of each Pseudoephedrine, Paracetamol, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate was prepared separately in methanol. UV scan of the above solutions were carried out over a wavelength range of 200–400 nm by using the Shimadzu UV spectrophotometer, Model- UV-1800. The detection wavelength was set at 215 nm because all the components had higher responses. An overlaid UV absorption spectrum is shown in Figure-1.

Figure 1: Overlaid UV absorption spectrum of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate



HPLC instruments and analytical conditions

Chromatographic separation was achieved using HPLC System (Waters Alliance 2695 Separation Module) containing binary solvent manager, an autosampler and UV detector. The output signal was monitored and processed using Empower software.

A Hypersil C18 column (100 mm X 4.6 mm id and 5 µm particle size) was used as the stationary phase. Mobile phase consisting of Buffer (0.007mM/L of KH_2PO_4, adjusted to pH 3.0 with OPA) and Acetonitrile in ratio of 78:22 v/v, with simple isocratic elution. Flow rate of 0.8 mL/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15 min. The column temperature was kept at 30°C. The detector was set at the wavelength of 215 nm. Injection volume kept was 10 µL.

Solutions and sample preparation

For the system suitability test, the solution containing 0.001 mg/mL PSE, 0.01 mg/mL of GPN, 0.0015 mg/mL of ABX and 0.0002 mg/mL of CPM was prepared in diluent containing Acetonitrile and water (5:5 v/v).

For the linearity studies, a standard stock solution containing 0.1 mg/mL PSE, 1.0 mg/mL of GPN, 0.15 mg/mL of ABX and 0.02 mg/mL of CPM was prepared by diluent and diluted with the same solvent to yield solutions at different concentration.

For test sample solution, Weighed 5ml of syrup then transferred to a 100 mL volumetric flask and added 75 mL of diluent. The solution was sonicated for 10 minutes with intermittent shaking. Removed the flask and kept at room temperature. Cooled to room temperature and diluted to the mark with diluent. The solution was filtered through Nylon 0.45 µm membrane filter, discarding first 2 mL of the filtrate. 1 mL of this filtrate was further diluted to 10 mL with diluent to obtain test solution of Pseudoephedrine hydrochloride (0.030 mg/mL), Guaiphenesin (0.050 mg/mL), Ambroxol hydrochloride (0.015 mg/mL) and Chlorpheniramine maleate (0.002 mg/mL). 10 µL of this test solution was injected in HPLC system.

Calculation

All active ingredients were quantified with the following calculation:

$$\% \text{ Assay} = \frac{\text{Sample Area} \times \text{Standard dilution factor} \times 100}{\text{Standard area} \times \text{Sample dilution factor}}$$

RESULTS AND DISCUSION

Literature survey revealed that, no method is available in the official compendia using HPLC for analyzing Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate in syrup. The present proposed method was compared with the reported method in the literature and comparison is shown in Table-1. The complete separation of the analytes was accomplished in less than 12 min and the method can be successfully applicable to perform routine analysis of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate in bulk and in commercially available dosage forms mainly syrups.

Table 1 Comparison of the performance characteristics of the present method with the published methods

Sr. No.	Method	Reagents	Detection Wavelength/ Runtime	Method applicable to	Reference
1	HPLC	Ammonium acetate, Acetonitrile	220 nm /30 min	Pharmaceutical dosage forms like tablets	[8]
2	HPLC	Sodium perchlorate, Acetonitrile	228 nm /15 min	Pharmaceutical dosage form like tablets	[9]
3	HPLC	Potassium phosphate, Acetonitrile	215 nm /12 min	Pharmaceutical dosage form mainly syrups	Present work

Method Validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guideline, VALIDATION OF ANALYTICAL PROCEDURES: Q2 (R1), for the parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness.

System suitability

The system suitability test performed according to USP36. [12] The standard solution was injected six times and results were recorded to find the adequate peak separation (resolution), percentage relative standard deviation for area and retention time, peak asymmetry and theoretical plates. The results obtained were compiled in Table-2.

Table 2: System suitability

Reference solution Peak Area, for n=6				
	Phenylephrine	**Guaiphenesin**	**Ambroxol**	**Chlorpheniramine**
%RSD	0.73	0.71	0.70	0.71
Acceptance Criteria	Not more than 2.0%			
Reference solution Peak retention time (min,) for n=6				
%RSD	0.04	0.03	0.03	0.06
Acceptance Criteria	Not more than 1.0%			
Reference solution Peak resolution, for n=6				
Resolution	-	4.34	6.34	10.73
Acceptance Criteria	Not less than 2.0			
Reference solution Peak Symmetry factor, for n=6				
Symmetry Factor	0.98	1.08	1.19	1.27
Acceptance Criteria	Should be between 0.8 – 1.2			
Reference solution Peak Theoretical plates, for n=6				
Theoretical plates	3465	3878	4212	5675
Acceptance Criteria	Not less than 2000			

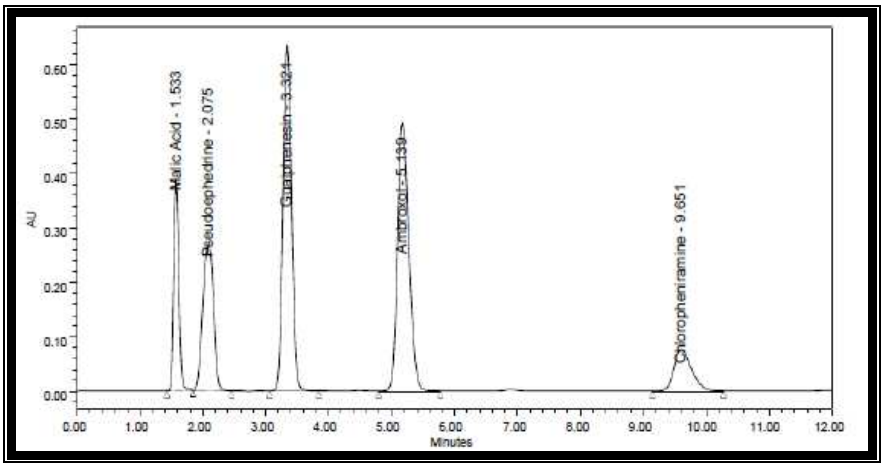
Results: It was observed that limits for percentage standard deviation for peak area`s and retention time for individual analyte, as well as resolution, symmetry factor and theoretical plates for all individual analytes are within the limit, which shows that the method have good system suitability.

Specificity

Specificity was performed to detect the presence of interference peak (blank and placebo peaks) at the retention time of the analyte peak. The specificity of the method was checked by comparison of chromatograms obtained from test sample solution and the corresponding placebo. The interference of placebo was detected by preparing placebo solution equivalent to about the weight in proportion of tablet preparation as per the test method and was injected into the HPLC system. The interference of blank was detected by injecting diluent as per the test method.

The representative chromatogram obtained for Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate is shown in Figure-2.

Figure 2: Typical Chromatograms of Standard Solution containing Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine



Results: No interference from diluent, excipients or any other peak was found at the retention time of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine.

Precision and Ruggedness (Intermediate precision)

Method precision was evaluated by carrying out six different test sample solution preparation. Different analyst from the same laboratory evaluated the intermediate precision of the method.

The assay of these samples was determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD. The values were given in Table-3.

Table 3: Precision and Intermediate Precision results

	PSE	GPN	ABX	CPM
Precision (Day 1) –Assay %				
Average Assay (%)	98.30	97.61	99.43	99.76
%RSD	0.37	0.54	0.33	0.24
Intermediate Precision (Day 2) – Assay %				
Average	98.48	97.14	98.42	98.72
%RSD	0.66	0.32	0.21	0.24
Average for Precision and Intermediate Precision	98.39	97.37	98.91	99.01
% RSD for Precision and Intermediate Precision	0.52	0.49	0.58	0.82
Acceptance Criteria	%RSD should not be more than 2.0% for day-1 and day-2.			

Results: Percentage Relative standard deviation (%RSD) obtained was found to be less than 2% for day – 1 and day -2

Linearity and range

The linearity of detector response was determined by preparing a series of solution of the working standards (mixture of all active ingredients) over the range of 80% to 120% of targeted concentration. These solutions were injected into the chromatographic system and response area was recorded.

Calibration curve was constructed by plotting area against concentration and regression equation was computed. The

linearity plots with values were shown in Table-4.

Table 4: Summary of Linearity and range results of Pseudoephedrine hydrochloride, Guaiphenesin, Ambroxol hydrochloride and Chlorpheniramine maleate in assay method

Active Ingredient	Concentration Range (mg/mL)	correlation coefficient	%Y Intercept	Slope
PSE	0.02404-0.03606	0.999	0.54	117815659.3
GPN	0.04002-0.06002	1.000	-0.50	136958797.2
ABX	0.01202-0.01804	1.000	1.06	467620897.5
CPM	0.00162-0.00242	0.998	-1.16	845090099
Acceptance criteria		**NLT 0.99**	**NMT ± 3 %**	**For information only**

Results: The correlation coefficient values were within the limit 0.99 and Y-intercept values were within ± 3 %.

Accuracy (Recovery)

To study the accuracy of the method recovery experiments were carried out. The accuracy of the test method was determined by varying weights of crushed test sample at the level of 80%, 100% and 120% of targeted concentration. The recovery samples were prepared in triplicate at each level. The contents were determined from the respective chromatograms. The samples at different levels were chromatographed and the percentage recovery for the amount added was calculated. The values were given in Table-5.

Table 5: Accuracy (Recovery)

Active Ingredient Name	Concentra- tion (%)	Amount Added (mg/mL)	Amount found (mg/mL)*	Mean Recovery (%)**	Average Recovery (%)
PSE	80	0.02404	0.02406	99.7	99.6
	100	0.03005	0.02995	99.7	
	120	0.03606	0.03581	99.4	
GPM	80	0.04002	0.03974	99.6	99.7
	100	0.05002	0.04992	99.8	
	120	0.06002	0.05988	99.8	
ABX	80	0.01202	0.01199	99.8	99.4
	100	0.01503	0.01493	99.3	
	120	0.01804	0.01790	99.2	
CPM	80	0.00162	0.00160	99.0	99.2
	100	0.00202	0.00200	99.1	
	120	0.00242	0.00242	99.6	
Acceptance criteria	**The mean and individual recoveries should be within 95.0 – 105.0%**				

** mean of 3 readings for individual level*
*** Average recovery for all levels*

Results: Accuracy results obtained shows that the mean and individual recoveries were in range of 95.0 – 105.0%

Robustness - Effect of variation in Temperature and variation in flow rate

To study robustness of the test method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the temperature and flow rate. Standard solution prepared as per the test method and was injected into the HPLC system at 25°C and 35°C temperature. Flow rate change was done by varying flow rate at from 0.8 mL/min to 0.72 mL/min and 0.88 mL/min.

The system suitability parameters were evaluated. The values were given in Table-6.

Table 6: Robustness results

Summary of system suitability Parameters												
Variations	**Resolution**				**Symmetry Factor**				**Theoretical plates**			
	PSE	**GPN**	**ABX**	**CPM**	**PSE**	**GPN**	**ABX**	**CPM**	**PSE**	**GPN**	**ABX**	**CPM**
0.80 mL/min 30°C	-	4.31	6.32	10.71	0.97	1.08	1.19	1.28	3233	3872	4232	5642
25°C		4.11	6.73	10.57	0.95	1.08	1.20	1.29	3596	3953	4097	6052
35°C	-	4.40	6.11	11.00	0.99	1.09	1.21	1.30	2834	3044	4890	5524
0.72 mL/min	-	4.38	6.47	10.83	0.97	1.07	1.18	1.27	3701	3758	4749	5558
0.88mL/min	-	4.38	6.47	10.83	1.05	1.07	1.18	1.27	3701	3758	4749	5585
Acceptance Criteria	**Not less than 2.0**				**Not more than 2.0**				**Not less than 2000**			

Results: From variation in Temperature and flow rate, it was observed that there were no marked changes in the chromatograms, which demonstrated that the method developed is robust. Resolution, symmetry factor and

Theoretical plate limits for flow rate variation and temperature variation are within the acceptance criteria, which show that the method exhibit good system suitability under given set of conditions.

Solution Stability

To assess the solution stability, reference standard and test solutions were kept at 25 °C (laboratory temperature) for 24 hours, and injected in HPLC system at predetermined time interval.

The percentage change with respect to initial of test and reference standard solutions were evaluated. The values were given in Table-7.

Table 7: Solution Stability results

Test Solution - Solution stability								
Time (Hrs)	**% Assay of PSE**	**% Change w.r.t. Initial**	**% Assay of GPN**	**% Change w.r.t. Initial**	**% Assay of ABX**	**% Change w.r.t. Initial**	**% Assay of CPM**	**% Change w.r.t. Initial**
Initial	98.76	**0.00**	97.48	**0.00**	99.18	**0.00**	99.75	**0.00**
12	98.38	**0.38**	97.09	**0.39**	99.54	**0.36**	99.54	**0.21**
24	98.62	**0.14**	97.35	**0.13**	99.33	**0.15**	99.54	**0.20**
Acceptance Criteria :	**% Change w.r.t. initial for Test solution should NMT 2% of initial assay results.**							
Reference Solution - Solution stability								
Time (Hours)	**Area of PSE**	**% Change w.r.t. Initial**	**Area of GPN**	**% Change w.r.t. Initial**	**Area of ABX**	**% Change w.r.t. Initial**	**Area of CPM**	**% Change w.r.t. Initial**
Initial	3125467	**0.00**	5721546	**0.00**	6070506	**0.00**	1444547	**0.00**
12	3131131	**0.18**	5736892	**0.27**	6077848	**0.12**	1436526	**0.56**
24	3109998	**0.49**	5718585	**0.05**	6045125	**0.42**	1429899	**1.01**
Acceptance Criteria :	**% Change w.r.t. initial for reference solution should NMT 2%.**							

Results: Both Test and reference solution was found to be stable upto 24hours, at 25 °C (laboratory temperature).

CONCLUSION

An isocratic RP-HPLC method has been developed and validated for the analysis of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate by RP-HPLC in syrup dosage forms. The results of the method validation revealed that the assay method is specific, selective, linear, accurate and robust. The validation performed further gives documented evidence, that the analytical method for the simultaneous estimation of Pseudoephedrine, Guaiphenesin, Ambroxol and Chlorpheniramine Maleate by RP-HPLC in syrup dosage forms will consistently analyze these drugs quantitatively in combination and single dosage form and can be used for routine analysis in quality control and R&D laboratory.

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