Research Article

RP-HPLC Method for the Simultaneous Determination of Paracetamol, Guiafenesin, Ambroxol, Phenylephrine Hydrochloride, and Chlorpheniramine Maleate in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

An RP-HPLC method for the simultaneous determination of Paracetamol, Guaifenesin, Ambroxol, Phenylephrine Hydrochloride, and Chlorpheniramine Maleate in tablets was developed and validated as per ICH & FDA guidelines. The separation was achieved with a 228 mm x 4.6 mm, 5 µm C18 column, by using a simple linear gradient. The mobile phase A was 0.01M sodium perchlorate, monohydrate, pH 3.0 and mobile phase B was Acetonitrile. The flow rate was 1.5 mL/min and column temperature was maintained at 25°C. The gradient separation was achieved within 15 minutes. The linearity of the proposed method was investigated in the range 0.0008-0.0012 mg/mL (r2= 0.999) for Phenylephrine, 0.04-0.06 mg/mL (r2= 0.999) for Paracetamol, 0.008-0.012 mg/mL (r2= 1.000) for Guaifenesin, 0.0024-0.0036 mg/mL (r2= 1.000) for Ambroxol, and 0.00016-0.00024 mg/mL (r2= 1.000) for Chlorpheniramine. Blank and placebo did not disturb the detection of Paracetamol, Guaifenesin, Ambroxol, Phenylephrine, and Chlorpheniramine and the assay can thus be considered specific. The developed method has an advantage that all the drugs can be quantified alone or in combination using a single mobile phase.

Keywords: Ambroxol, Chlorpheniramine Maleate, Guaifenesin, Paracetamol, Phenylephrine Hydrochloride, RP-HPLC, Validation.

INTRODUCTION

Paracetamol or Acetaminophen is a widely used as an analgesic (pain reliever) and antipyretic (fever reducer).[1,2] It is chemically known as *N*-(4-hydroxyphenyl) acetamide. Phenylephrine Hydrochloride is a selective α_1-adrenergic receptor agonist used primarily as a decongestant.[1,2] It is chemically known as (1R)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol Hydrochloride. Guaifenesin 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 is only one method available for simultaneous determination of these active ingredients with 30 minutes runtime and the method is linear in the concentration range 10-80 µg per mL for these drugs.[8] However, none of the method available has been reported for the simultaneous determination of Ambroxol, Phenylephrine Hydrochloride, Guaifenesin, Chlorpheniramine Maleate and Paracetamol in pharmaceutical dosage form with great difference in label claim (Paracetamol 500 mg, Guaifenesin 100 mg, Ambroxol 30 mg, Phenylephrine Hydrochloride 10 mg, Chlorpheniramine Maleate 2 mg).

The method was validated as per the present ICH guideline on validation of analytical procedure Q2A (R1).[9,10] Quantitation was achieved with UV detection at 228 nm based on peak area with linear calibration curves at concentration ranges. The method was linear over wide concentration range of 0.0008-0.0012 mg/mL for Phenylephrine, 0.04-0.06 mg/mL for Paracetamol, 0.008-0.0012 mg/mL for Guaifenesin, 0.0024-0.0036 mg/mL for Ambroxol and 0.00016-0.00024 mg/mL 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.

MATERIALS AND METHODS

Chemicals and reagents

Ambroxol (ABX), Guaifenesin (GPN), Phenylephrine Hydrochloride (PEP), Chlorpheniramine Maleate (CPM) and Paracetamol (PRCT) were obtained from Medley Pharma, Mumbai, India. Excipients for preparation of placebo were obtained from Medley Pharma, Mumbai, India. Branded formulation of Phenylephrine Hydrochloride, Paracetamol, Ambroxol, Guaifenesin, and Chlorpheniramine Maleate were procured from local market.

HPLC grade, methanol and acetonitrile were obtained from Merck Chemicals (Mumbai, India). HPLC grade Orthophosphoric acid (88%) was from Merck (Mumbai, India). Sodium perchlorate was obtained from Merck Chemicals (Mumbai, India), Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore (Mumbai, India). All the other used reagents were of analytical grade.

Selection of UV wavelength

10ppm solution of each Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, 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 228 nm because all the components had higher responses. An overlaid UV absorption spectrum is shown in Figure 1.

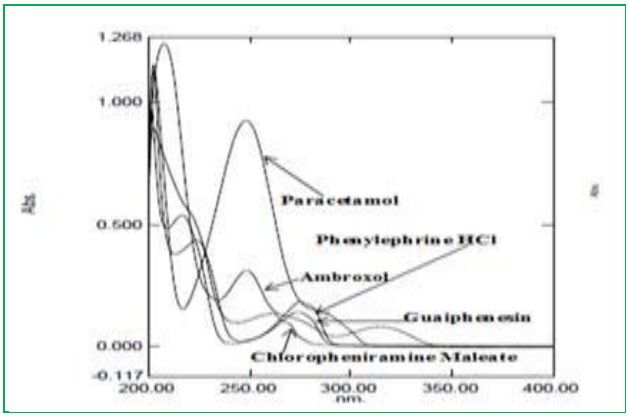

Figure 1: Overlaid UV absorption spectrum of Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate.

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.

An Inertsil C18 column (250 mm X 4.6 mm id and 5 µm particle size) was used as the stationary phase. Mobile phase consisting of Mobile phase A: buffer (0.01 M Sodium perchlorate. Monohydrate pH 3.0 with OPA) and Mobile Phase B: Acetonitrile with simple gradient program (0-10 min:: MP-A : 95-40; 10-12 min :: MP-A : 40-95; 12-15min :: MP-A : 95-95) was delivered at a flow rate of 1.5 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 25°C. The detector was set at the wavelength of 228 nm. Injection volume kept was 10 µL.

Solutions and sample preparation

For the system suitability test, the solution containing 0.001 mg/mL PEP, 0.05 mg/mL of PRCT, 0.01 mg/mL of GPN, 0.003 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 PEP, 5.0 mg/mL of PRCT, 1.0 mg/mL of GPN, 0.3 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, 10 tablets were weighed and crushed to a fine powder. Powder equivalent to one tablet (containing Paracetamol 500 mg, Guaifenesin 100 mg, Ambroxol 30 mg, Phenylephrine Hydrochloride 10 mg, Chlorpheniramine Maleate 2 mg) was accurately weighed and transferred into a 100 mL volumetric flask. Added 70mL diluent, and then the contents of the volumetric flask were sonicated for 15 min to enable complete dissolution of analytes. Then volume was made up to 100 mL with diluent. The solution was filtered through 0.45 µm filters and the 1 mL of this filtrate was further diluted to 100 mL with diluent. 10 µL of these solutions were injected into the HPLC system and the peak area was recorded from the respective chromatogram.

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 DISCUSSION

Literature survey revealed that, no method is available in the official compendia using HPLC for analyzing Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate in dosage forms. 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 15 min and the method can be successfully applicable to perform routine analysis of Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate in bulk and in commercially available dosage forms.

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.[11] 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 1: Comparison of the performance characteristics of the present method with the published methods

Method	Reagents	Detection Wavelength/ Runtime	Linearity (mg/mL)	Remark	Reference
HPLC	Ammonium acetate, Acetonitrile	220 nm / 30 min	0.01 to 0.08 mg/mL for all components.	Very narrow linearity range	[8]
HPLC	Sodium perchlorate, Acetonitrile	228 nm / 15 min	0.0008-0.0012 mg/mL for PEP, 0.04-0.06 mg/mL for PRCT, 0.008- 0.0012 mg/mL for GPN, 0.0024-0.0036 mg/mL for ABX and 0.00016-0.00024 mg/mL for CPM	Wide linearity range	Present work

Table 2: System suitability

Reference solution Peak Area, for n=6					
	Phenylephrine	**Paracetamol**	**Guaifenesin**	**Ambroxol**	**Chlorpheniramine**
% RSD	1.33	0.65	0.82	1.15	0.78
Acceptance Criteria	Not more than 2.0%				
Reference solution Peak retention time (min,) for n=6					
% RSD	0.11	0.11	0.12	0.20	0.23
Acceptance Criteria	Not more than 1.0%				
Reference solution Peak resolution, for n=6					
Resolution	-	2.20	2.33	7.48	2.86
Acceptance Criteria	Not less than 2.0				
Reference solution Peak Symmetry factor, for n=6					
Symmetry Factor	1.07	1.06	1.07	1.11	1.30
Acceptance Criteria	Should be between 0.8 – 1.2				
Reference solution Peak Theoretical plates, for n=6					
Theoretical plates	5383	4939	5974	6405	5829
Acceptance Criteria	Not less than 2000				

Table 3: Precision and Intermediate Precision results

	Phenylephrine	**Paracetamol**	**Guaifenesin**	**Ambroxol**	**Chlorpheniramine**
Precision (Day 1) –Assay %					
Average Assay (%)	101.61	99.58	98.41	100.81	100.97
% RSD	0.13	0.29	0.33	0.44	0.57
Intermediate Precision (Day 2) – Assay %					
Average	99.77	99.27	99.93	99.99	99.15
% RSD	0.69	0.27	0.41	0.85	0.85
Average for Precision and Intermediate Precision	100.69	99.43	99.17	100.40	100.06
% RSD for Precision and Intermediate Precision	1.07	0.31	0.88	0.77	1.17
Acceptance Criteria	% RSD should not be more than 2.0% for day-1 and day-2.				

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 Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate is shown in Figure 2.

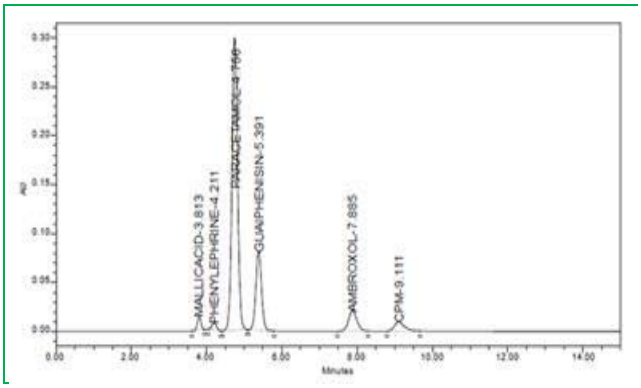

Figure 2: Typical Chromatograms of Standard Solution containing Phenylephrine, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine

Results

No interference from diluent, excipients or any other peak was found at the retention time of Phenylephrine, Paracetamol, Guaifenesin, 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.

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 Figure 3.

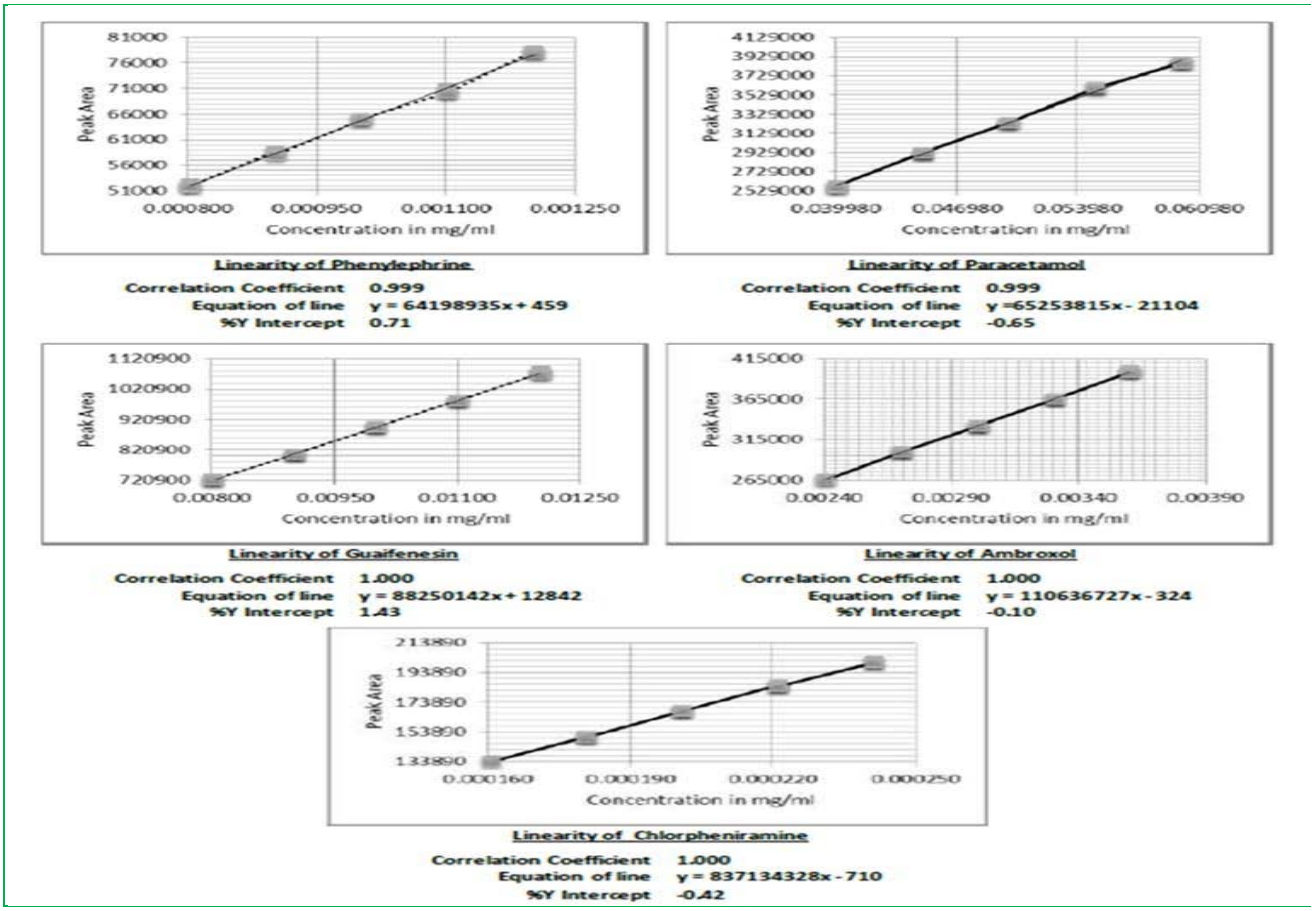

Figure 3: Calibration curves of Phenylephrine, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine showing linearity

Results: The correlation coefficient values were within the limit 0.998 and Y-intercept values were within ± 2 %.

Table 4: Accuracy (Recovery)

Active Ingredient Name	Concentration (%)	Amount Added (mg/mL)	Amount found (mg/mL)*	Mean Recovery (%)**	Average Recovery (%)
Phenylephrine	80	0.0008022	0.0008018	100.1	99.7
	100	0.0010027	0.0010154	98.8	
	120	0.0012033	0.0012008	100.2	
Paracetamol	80	0.0401120	0.0394969	101.6	100.5
	100	0.0502835	0.0496947	100.9	
	120	0.0602325	0.0607597	99.0	
Guaifenesin	80	0.0080224	0.0081401	98.6	99.9
	100	0.0100567	0.0098431	101.9	
	120	0.0120465	0.0121247	99.2	
Ambroxol	80	0.0024059	0.0024205	99.5	99.3
	100	0.0030033	0.0030250	99.5	
	120	0.0036140	0.0036487	98.9	
Chlorpheniramine	80	0.0001604	0.0001631	98.4	98.4
	100	0.0002011	0.0002033	98.7	
	120	0.0002409	0.0002452	98.1	

* 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 98.0 – 102.0%

Table 5: Robustness results

Summary of system suitability Parameters															
Variations	Resolution					Symmetry Factor					Theoretical plates				
	PEP	PRCT	GPN	ABX	CPM	PEP	PRCT	GPN	ABX	CPM	PEP	PRCT	GPN	ABX	CPM
1.5 mL/min 25°C	-	2.23	2.35	7.49	2.83	1.08	1.04	1.06	1.09	1.37	5585	5055	6055	6349	5558
1.7 mL/min	-	2.26	2.39	7.62	2.91	1.15	1.07	1.07	1.12	1.35	5881	5179	6276	6714	5961
1.3 mL/min	-	2.14	2.24	7.16	2.76	1.05	1.04	1.06	1.08	1.3	5260	4571	5572	6012	5467
20°C	-	2.00	2.22	6.96	3.06	0.98	1.06	1.07	1.08	1.22	4213	4801	5928	6403	5809
30°C	-	1.99	2.13	6.58	3.36	0.99	1.06	1.08	1.09	1.34	2619	4720	5812	6633	6498
Acceptance Criteria	Not less than 2.0					Should be between 0.8 – 1.2					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.

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 4.

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 20°C and 30°C temperature. Flow rate change was done by varying flow rate at from 1.5 mL/min to 1.3 mL/min and 1.7 mL/min.

The system suitability parameters were evaluated. The values were given in Table 5.

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 6.

Table 6: Solution Stability results

Test Solution - Solution stability										
Time (Hours)	% Assay of PEP	% Change w.r.t. Initial	% Assay of PRCT	% 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	101.40	0.00	99.31	0.00	98.93	0.00	101.26	0.00	100.97	0.00
6	101.87	0.46	99.23	0.08	99.24	0.32	101.55	0.29	101.14	0.17
12	101.58	0.18	99.28	0.03	99.12	0.19	101.01	0.25	101.09	0.11
18	101.28	0.12	99.13	0.18	98.92	0.01	100.25	1.00	100.31	0.66
24	101.40	0.00	99.18	0.13	98.81	0.12	100.30	0.95	100.36	0.60
Acceptance Criteria	% Change w.r.t. initial for Test solution should NMT 1% of initial assay results.									
Reference Solution - Solution stability										
Time (Hours)	Area of PEP	% Change w.r.t. Initial	Area of PRCT	% 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	64259	0.00	3192645	0.00	881074	0.00	318691	0.00	164956	0.00
6	64223	0.06	3216003	0.73	885145	0.46	320078	0.44	165101	0.09
12	63924	0.52	3214691	0.69	885445	0.50	321093	0.75	165094	0.08
18	64535	0.43	3205983	0.42	884967	0.44	320193	0.47	165124	0.10
24	63720	0.84	3205725	0.41	883926	0.32	320034	0.42	164717	0.14
Acceptance Criteria	% Change w.r.t. initial for reference solution should NMT 1% of initial.									

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

CONCLUSION

A gradient RP-HPLC method has been developed and validated for the analysis of Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate by RP-HPLC in tablet 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 Phenylephrine Hydrochloride, Paracetamol, Guaifenesin, Ambroxol and Chlorpheniramine Maleate by RP-HPLC in tablet 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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