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RESEARCH ARTICLE

BIO PHARMACEUTICS

ANALYSIS OF COUGH AND ANALGESIC RANGE OF PHARMACEUTICAL ACTIVE INGREDIENTS USING RP-HPLC METHOD

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ABSTRACT

A novel and single RP-HPLC method was developed for the determination of ten active ingredients (Codeine phosphate, Paracetamol, Chloropheniramine Maleate, Theophylline, Pseudoepidrine HCl, Ambroxol, Salbutamol, Guaiphenesin, Dextromethorphan and Diphenhydramine HCl) in all pharmaceutical dosage forms, along with preservative (Sodium benzoate) and validated the method as per ICH and FDA guidelines. The separation was achieved on a X-Terra C18, 15cm x 4.6mm, 3.5 µ in the simple gradient mode using Sol-A: buffer and Sol-B: Acetonitrile (0-5min, sol-A:97-97; 5-10min- sol-A:97-92; 10-15min- sol-A:92-68; 15-23min- sol-A:68-68; 23-25min- sol-A:68-97 and 25-30min- sol-A:97-97) with 0.8 mL per min flow rate. Column oven temperature maintained at 40°C and performed the analysis with 220 nm. Quantification was achieved with 40µg per mL for all ingredients with 100±3.0% recovery. The method was validated by determining its sensitivity, linearity, accuracy and precision. The proposed method is single, shorter runtime, accurate and reproducible. This method can be applied for routine analysis of all ten active ingredients quantification in all pharmaceutical dosage forms.

KEY WORDS

Paracetamol, Salbutamol, Theophylline, Pseudoepidrine HCl, Codeine phosphate, Guaiphenesin, Chloropheniramine Maleate, Dextromethorphan, Diphenhydramine HCl, Ambroxol, Sodium benzoate, analgesic drugs, decongestant, cough and single RP-HPLC method.

INTRODUCTION

Present days chromatographic methods plays a main role in pharmaceutical industry development. Pharmaceutical industry has developed an increasing variety of combination dosage forms (double or multiple actives) for human therapies. Analytical method development for these combination drug products is big challenge to develop the methods for analysis. At this point industry requires chromatographic methods for each active component. An alternative to the commonly used practice of developing multiple reversed phase chromatographic methods for analyzing combination pharmaceutical products. In our present study developed the single RP-HPLC method for multiple combination products of cough and analgesic therapeutic drug products. The developed single RP-HPLC method is applicable for ten active ingredients combination products like Codeine phosphate, Paracetamol, Chloropheniramine Maleate, Theophylline, Pseudoepidrine HCl, Ambroxol, Salbutamol, Guaiphensin, Dextromethorphan, Diphenhydramine HCl in pharmaceutical dosage forms along with preservative (sodium benzoate).

Codeine phosphate is predominant alkaloid in opium [1, 2], it is considered a prodrug, metabolised to active compounds of morphine and codeine-6-glucuronide [3]. It is used for cough, diarrhea and mild to moderate pain. A dose of approximately 200 mg (oral) must be administered to give analgesia approximately equivalent to 30 mg (oral). However, in general, not used in single doses of greater than 60 mg (and no more than 240 mg in 24 hours) [4].

Paracetamol or acetaminophen is used for the treatment of pain and fever reducer [5- 7]. It is a class of aniline analgesics drugs [8, 9]. It has few anti-inflammatory effects in comparison to NSAIDs [10-12].

Dextromethorphan [13-15] is the dextrorotatory enantiomer of the methyl ether of levorphanol and stereoisomer of levomethorphan. Dextromethorphan is antitussive (cough suppressant) drug and used for pain relief, psychological applications [16-18]. The total dose for day is between 10 mg and 45 mg [19].

Diphenhydramine hydrochloride is mainly used to treat allergies, antihistamines. It has a powerful hypnotic effect and often it is used as a nonprescription sleep aid and a mild anxiolytic and antipsychotics [20- 22]. The maximum dose for adults is 50 mg for every four to six hours [23, 24]. The main side effects include motor impairment, flushed skin, short-term memory loss, visual disturbances, irregular breathing, dizziness, irritability, itchy skin, confusion, decreased body temperature, erectile dysfunction and excitability [25].

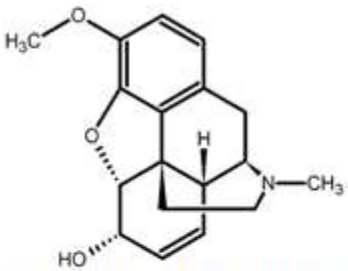
Guaifenesin is also called as guaiphenesin [26- 28]. It is used for the treatment of cough and primary dysmenorrhea [29, 30]. Salbutamol [31] is a β_2-adrenergic receptor agonist and used for the relief of asthma and chronic obstructive pulmonary disease [32]. The common side effects are fine tremor, nervousness, headache, muscle cramps, dry mouth and palpitation, arrhythmias, flushing and myocardial ischemia [33, 34].

Ambroxol is an active mucolytic agent. Ambroxol is used in the treatment of respiratory diseases, pain relief in acute sore throat [35, 36]. Ambroxol is a very potent inhibitor of the neuronal Na+ channels [37].

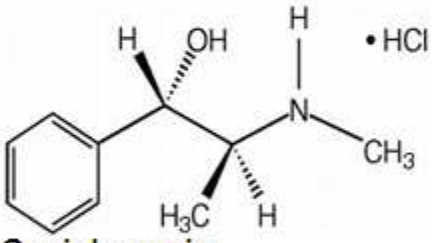
Sodium benzoate [38-40] is the sodium salt of benzoic acid, it acts as bacteriostatic and fungistatic under acidic conditions. It is used as preservative for the preparation of acidic foods such as salad dressings, carbonated drinks, jams, fruit juices and cough syrups [41-43].

Pseudoephedrine [44-46] reduces tissue hyperemia, edema and nasal congestion, colds or allergies. Adverse effects are hypertension, sweating and anxiety. Pseudoephedrine is useful for suppressing of cough [47]. Chlorpheniramine maleate [48-50] is used for cough and analgesia, allergy, hay fever, the common cold, vasomotor rhinitis and allergic conjunctivitis. Theophylline [51-54] is a methylxanthine drug used in therapy for respiratory diseases such as COPD and asthma. All active ingredients structures were represented in figure-1.

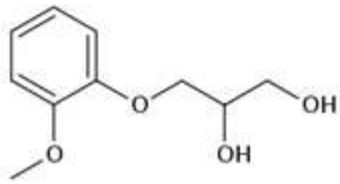
Codeine phosphate:



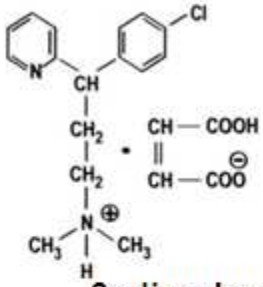
Pseudoepidrine hydrochloride



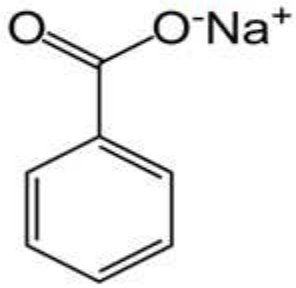
Guaiphenesin



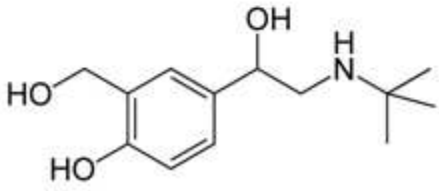
Chloropheniramine maleate



Sodium benzoate



Salbutamol



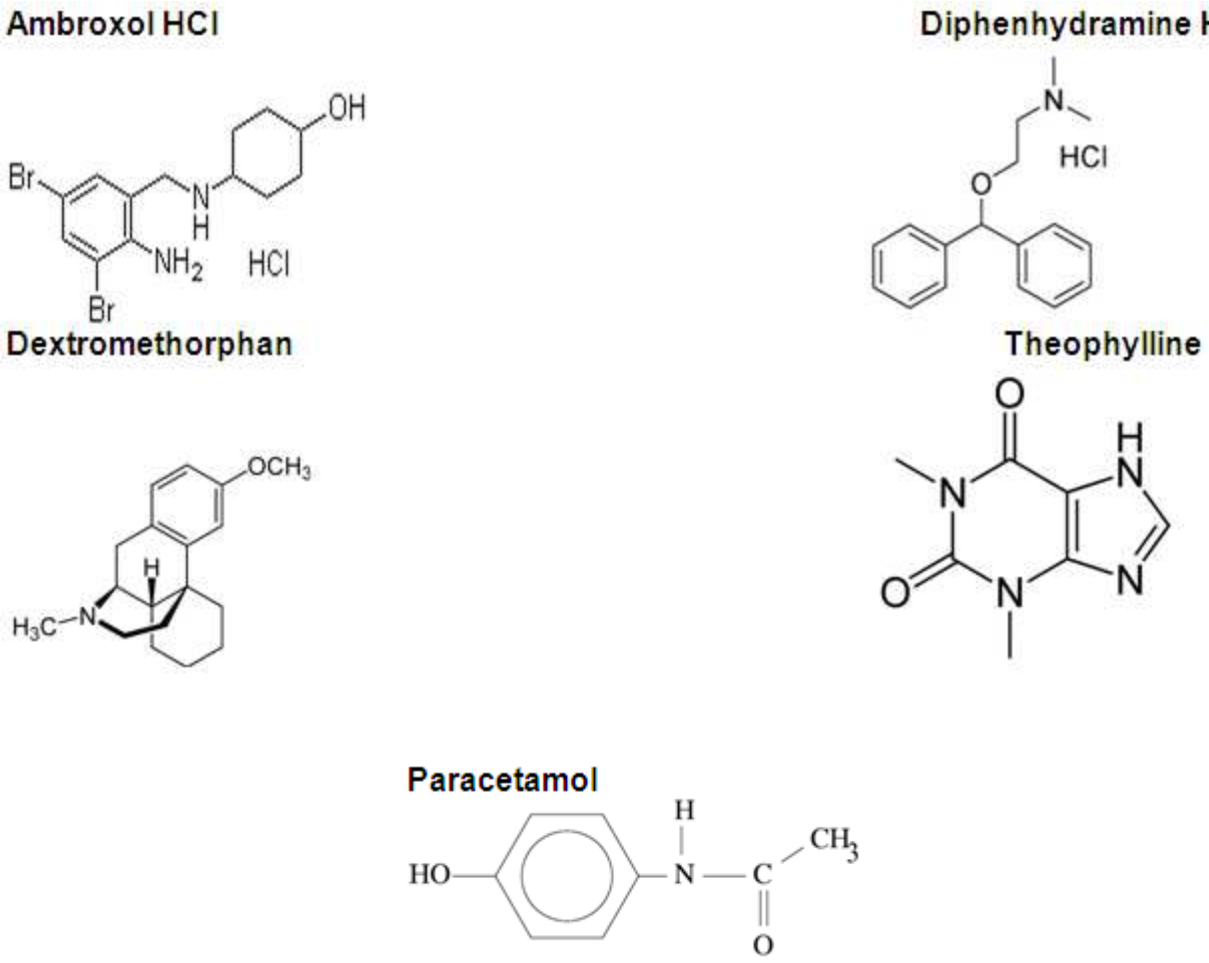


Figure – 1
Chemical structure of active ingredients

All ten active ingredients codeine phosphate, pseudoepidrine hydrochloride, paracetamol, dextromethorphan hydrobromide, diphenhydramine hydrochloride, guaiphenesin, salbutamol, ambroxol, chloropheniramine maleate and theophylline are available in individual and combination dosage forms. All combination products have individual methods [55 to 67]. The available products in market are tabulated in the table-1.

Table – 1
Available dosage forms in market.

Dosage form	Active ingredients
Tablets 	Ambroxol HCl -30mg
	Chloropheniramine maleate-1mg/4mg
	Psedoepidrine HCl-15mg/60mg
	Paracetamol-125mg/500mg
	Paracetamol-500mg
	Codeine phosphate-10mg

Syrup (each 5mL contains) 	Ambroxol HCl -30mg/5mL
	Ambroxol-30mg Guaphenesin-50mg Salbutamol-2mg
	Ambroxol-30mg Salbutamol-2mg
	Ambroxol-15mg/30mg Theophylline-50mg/100mg Salbutamol-1mg/2mg
	Ambroxol-15mg Chloropheniramine maleate-2mg Guaiphenesin-50mg Pseudoepidrine HCl-30mg
	Dextromethorphan HCl-5mg Chloropheniramine meleate-2.5mg Guaiphenesin-50mg
	Codeine phosphate-15mg
	Codeine phosphate-10mg Chloropheniramine meleate-4mg
	Salbutamol-1mg Guaiphenesin-50mg
	Paracetamol-125mg/150mg
Injection (1mL contains) 	Paracetamol-7mg Paracetamol-125mg Paracetamol-150mg

The developed single RP-HPLC method has applicable for the above all ingredients and validated the method as per ICH and FDA guidelines [68-72].

MATERIALS AND METHODS

Reagents and Materials:

Pure standard of all active ingredients (with minimum of 98.5% purity) were used for this research work. HPLC grade acetonitrile (Merck India Ltd), HPLC grade water, ammonium acetate (S. D. Fine Chemicals) and all market samples were used for analysis. Electronic analytical balance (Mettler Tolledo), Micro pipette (In labs, 10-100µl) were employed in the study. All glassware employed in the work is cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-22°C.

HPLC instrument and chromatographic conditions:

The HPLC system (Waters India Pvt Ltd) consisted of an alliance model 2695, a waters dual lambda variable wavelength detector. Gradient elution of mobile phase with flow rate of 0.8 ml per min was performed on C18 analytical column (Waters X-Terrra MS C18, 3.5µm; 150mmx4.6mm). The run time

was set for 30 min and column temperature maintained at 40°C. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was measured at 220 nm.

Preparation of mobile phase and elution:

Mobile phase elution was gradient elution and sol-A: buffer and Sol-B: acetonitrile. Both solvent-A and B were filtered through 0.45 µm membrane filter and degassed by sonication before use. The gradient program was 0-5min, sol-A: 97-97; 5-10min- sol-A: 97-92; 10-15min- sol-A: 92-68; 15-23min- sol-A: 68-68; 23-25min- sol-A: 68-97 and 25-30min-sol-A: 97-97 used for separation.

Preparation of Standard Solution

A stock solution was prepared by dissolving 40mg of each active ingredient standard in to 100ml volumetric flask and 50ml of diluent added, sonicated for 10-15min and diluted to 100ml with diluent. The resulting solution diluted to get the known concentration of 40microgram per ml (40ppm) of each ingredient with diluent.

Analysis of pharmaceutical dosage forms:

Individually prepared, all pharmaceutical dosage forms to get the known concentration of each ingredient are equal to 40microgram per ml (40ppm).

RESULTS AND DISCUSSIONS

Method development:

Initial method development trials were performed with ammonium acetate buffer pH 6.8 and Acetonitrile in the ratio of 30:70 v/v as a mobile phase but the elution of diphenhydramine and ambroxol elution was poor and elution was observed after 60min. The finalized RP–HPLC method is revealed as simple, accurate and precise with significant shorter retention time of 30min with simple gradient program. The typical chromatogram of diluent and standard solution was shown in figure-2 and 3.

Figure – 2
Diluent chromatogram

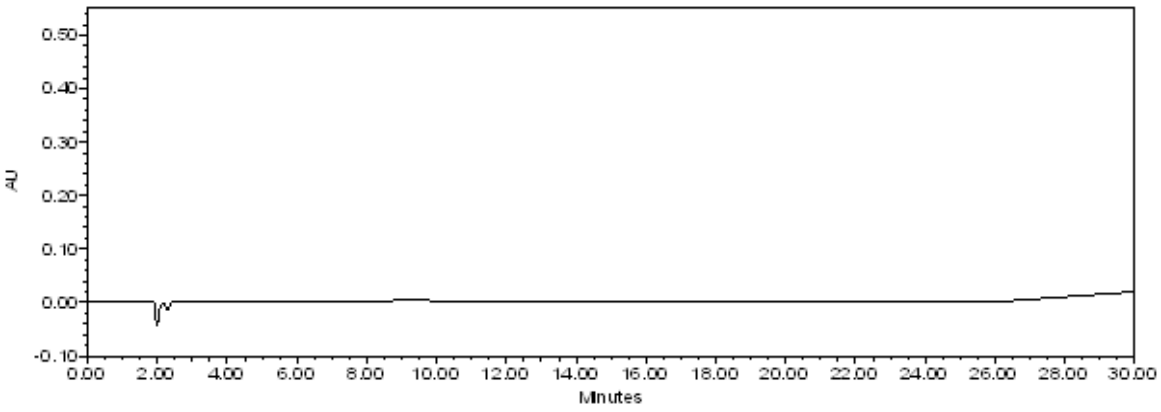
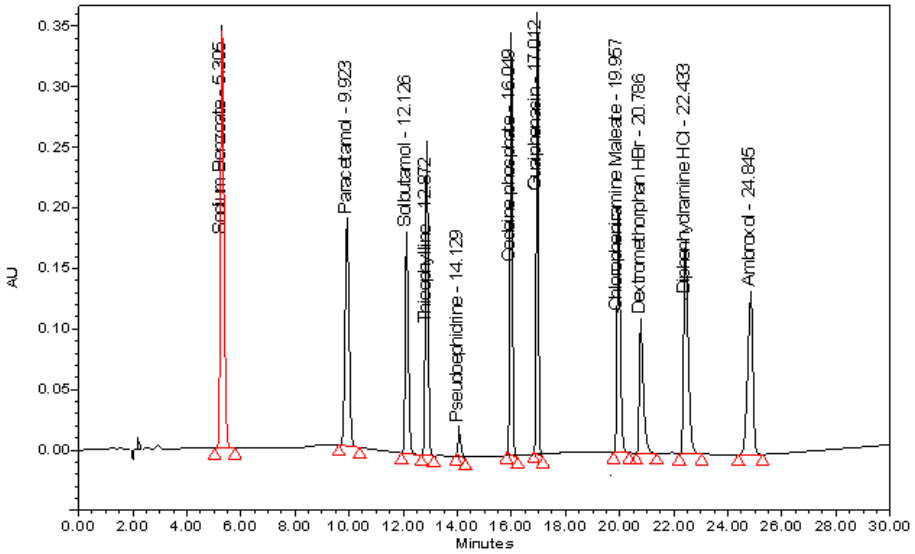


Figure – 3
Standard solution



Standard solution was injected in to the system for five replicate injections and system suitability parameters (tailing factor, resolution-NLT 2.0 and theoritical plates) are cross checked and found to be good. Relative standard deviation for area and retention time of all ingredients was cross checked and found to be satisfactory results and meeting the ICH and FDA specifications (%RSD= not more than 2.0%). Five replicate standard solution injections were represented in figure-4 and system suitability results were tabulated in table-2 and 3.

Figure – 4
Five replicate standard solution

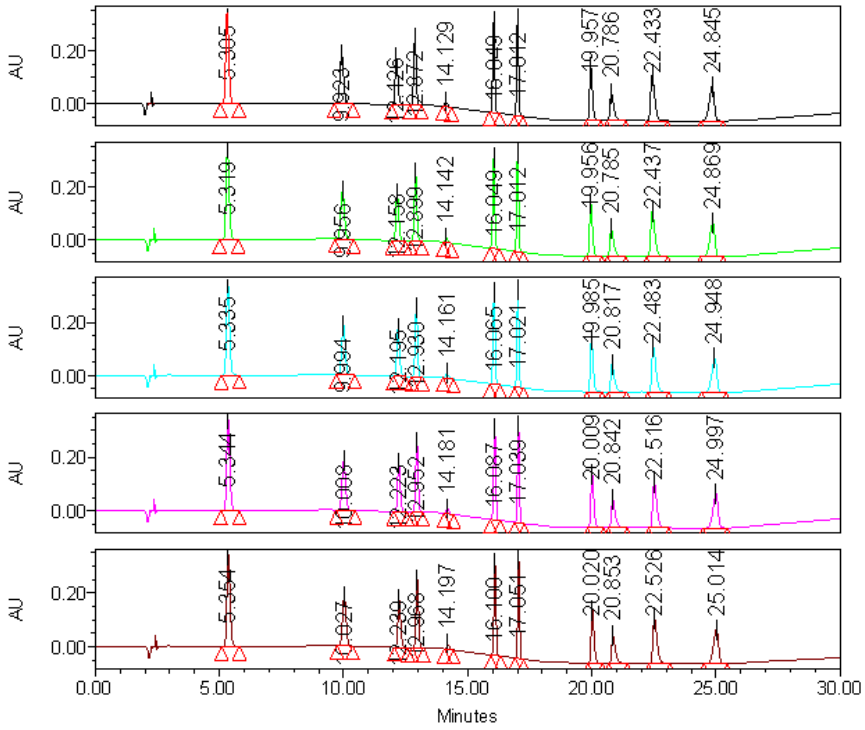


Table – 2
System suitability (Area %RSD)

Active Ingredient Name	Standard solution Area						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Sodium benzoate	3086465	3090421	3083702	3070420	3072936	3080789	0.28
Paracetamol	1927184	1931305	1923467	1916415	1914495	1922573	0.37
Salbutamol	1457031	1449109	1442294	1439627	1439635	1445539	0.52
Theophylline	1849494	1852342	1852496	1847846	1850343	1850504	0.11
Pseudoepidrine	142550	143300	143717	143918	142573	143612	0.44
Codeine phosate	2052397	2052203	2053160	2048237	2048062	2050812	0.12
Guaiphenesin	1990546	1993966	1987531	1980359	197008	1976282	0.33
Chloropheniramine maleate	1655192	1661644	1657411	1652896	1648013	1655031	0.31
Dextromethorphan HBr	1122370	1123688	1119390	1118522	1114428	1119480	0.33
Diphenhydramine HCl	2000971	2005997	2001406	1990268	1990399	1997808	0.36
Ambroxol HCl	1786260	1811047	1805576	1799687	1798262	1800166	0.52

Table – 3
System suitability (Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Sodium benzoate	5.30	5.31	5333	5.34	5.35	5.33	0.39
Paracetamol	9.92	9.95	9.99	10.00	10.02	9.98	0.40
Salbutamol	12.12	12.15	12.15	12.22	12.23	12.33	0.40
Theophylline	12.87	12.89	12.93	12.95	12.96	12.92	0.30
Pseudoepidrine	14.12	14.14	14.16	14.18	14.19	14.16	0.20
Codeine phosate	16.04	16.04	16.06	16.07	16.00	16.04	0.18
Guaiphensin	17.01	17.01	17.12	17.02	17.05	17.02	0.10
Chloropheniramine Maleate	19.95	19.95	19.98	20.00	20.01	19.98	0.15
Dextromethorphan HBr	20.78	20.78	20.81	2.84	20.85	20.81	0.16
Diphenhydramine HCl	22.43	22.43	22.48	22.51	22.52	22.47	0.19
Ambroxol HCl	24.84	24.86	24.94	24.99	25.01	24.93	0.30

METHOD VALIDATION

Specificity

The specificity for the proposed method demonstrated that the placebo and diluent have no interference with all active ingredients. Furthermore, well shaped peaks indicate the specificity of the method.

Linearity

The concentrations of all active ingredients were prepared from the standard stock solution by taking suitable volume (ml) and diluted up to 50 ml to get the desired concentrations for linearity in the range of 10-80 µg per ml (standard concentration is 40 µg per ml) with eight different concentration levels. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilution was injected into the system. The calibration curve for each ingredient was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 10-80 µg per ml with good correlation in

between concentration and mean peak area. All linearity chromatograms and co-relation coefficient of peak area was found to be linear and overlaid chromatogram was shown in figure-5 and results were tabulated in table-4.

Figure – 5
Linearity solutions

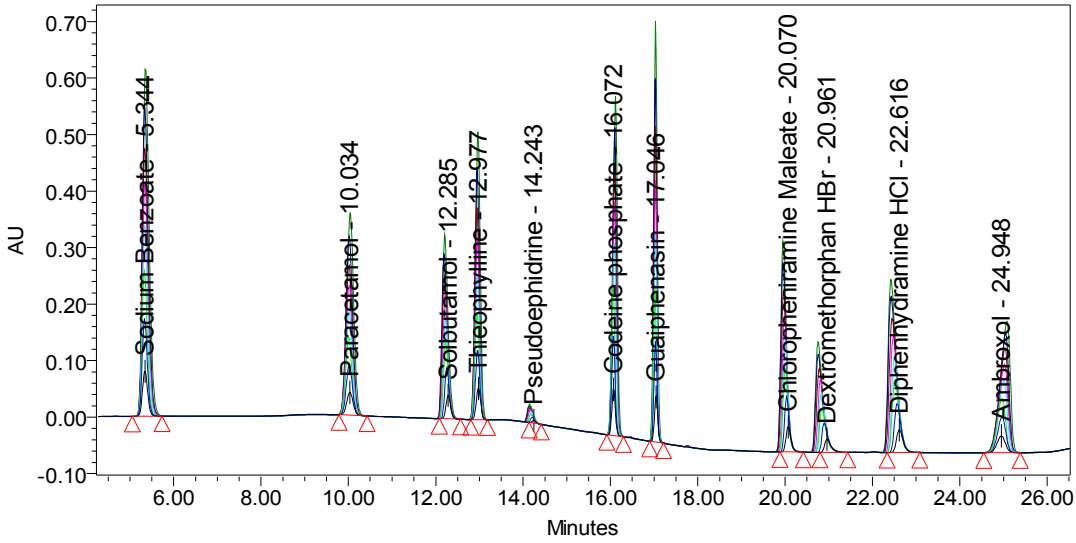


Table – 4
Linearity Results.

Active Ingredient Name	Linearity solutions area								
	Level-1 (10ppm)	Level-2 (20ppm)	Level-3 (30ppm)	Level-4 (40ppm)	Level-5 (50ppm)	Level-6 (60ppm)	Level-7 (70ppm)	Level-8 (80ppm)	Co-relation Coefficient
Sodium benzoate	664999	1463410	2264472	3061839	3858444	4642241	5416614	6145904	0.99991
Paracetamol	416705	916399	1416073	1920976	2417217	2920210	3419706	3916063	0.99999
Salbutamol	312464	686227	1065145	1442294	1819819	2205269	2580239	2959421	0.99999
Theophylline	399230	883877	1368700	1852447	2336187	2820904	3302706	3780084	0.99999
Pseudoepidrine	31580	67642	107028	146243	183793	221748	255901	289623	0.99968
Codeine phosate	467623	995801	1526719	2056562	2580520	3103417	3629255	4148017	0.99999
Guaiphenesin	436243	950543	1467107	1972134	2494540	3021478	3535966	4040456	0.99998
Chloropheniramine Maleate	357033	785126	1218844	1648673	2080007	2515498	2947503	3377792	0.99999
Dextromethorphan HBr	237743	525803	823044	1114536	1409184	1704029	2000320	2293743	0.99999
Diphenhydramine HCl	442942	955757	1474876	1988646	2503855	3027001	3540609	4056054	0.99999
Ambroxol HCl	409586	853829	1325875	1791514	2260696	2730427	3197671	3659961	0.99998

Precision:

Precision of the method was determined for all active ingredients of all type of dosage forms (solids and liquids). The intraday and inter-day variations were determined using five replicate injections of sample preparation and analyzed on the same day and three different days over a period of three days. Sample assay was determined with six different preparations and assay found to be within the limits (between 98.0% to 102.0%) and %RSD found to be satisfactory (0.36% and 0.16%).

Accuracy:

To ensure the accuracy of the method, the recovery studies were studied by adding a known quantity of drug with preanalysed sample and contents were reanalyzed with the proposed method. Accuracy was evaluated by injecting five times at six different concentrations equivalent to 25%, 50%, 75%, 100%, 125% and 150% of the active ingredient, the recovery of each ingredient % recovery for each concentration. The mean % recoveries were in between 97.0-103.0% and tabulated the results in table -5.

Table – 5
Accuracy Results.

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Sodium benzoate	97.8	99.8	100.2	98.7	98.85	99.21	99.09
Paracetamol	98.27	97.9	101.52	97.98	100.1	99.3	99.18
Salbutamol	98.21	100.5	100.58	100.4	99.81	97.98	99.58
Theophylline	99.1	101.52	100.5	99.78	99.24	99.7	99.97
Pseudoepidrine	98.31	99.63	101.63	100.85	99.86	99.21	99.92
Codeine phosate	99.9	99.27	100.51	99.81	99.7	99.25	99.74
Guaiphensin	100.2	98.7	101.23	98.89	98.12	99.01	99.36
Chloropheniramine Maleate	101.52	97.98	102.01	101.2	98.85	100.5	100.34
Dextromethorphan HBr	100.58	100.4	100.87	99.1	99.13	102.01	100.35
Diphenhydramine HCl	98.9	99.9	99.24	100.52	99.61	99.8	99.66
Ambroxol HCl	98.85	99.21	99.63	101.65	98.16	99.52	99.50

Ruggedness:

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the test sample (for all ingredients) as per the proposed method and each dilution injected in duplicate using different column and analyst on different days.

Robustness:

The proposed method was validated by changing chromatographic parameters and system suitability parameters were found to be within acceptable limits, results were tabulated in table-6. The results indicating that the method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table – 6
Robustness Results.

Parameter	System suitability		
	Variation	Tailing factor	Percent (%) RSD
Standard solution	-----	1.1-1.3	1.2-1.5
Flow Rate	+0.1mL per min	0.8-1.2	1.0-1.5
	-0.1mL per min	0.9-1.1	0.9-1.2
Column Oven Temperature	+5°C	1.0-1.2	0.8-1.0
	-5°C	1.2-1.4	1.1-1.3

CONCLUSION

The complete results of the present study reveal that the proposed RP-HPLC method for the estimation of all ten ingredients is simple, reproducible and accurate. The proposed method is applicable for all type of pharmaceutical dosage forms (individual and combination) of Codeine phosphate, Paracetamol, Chloropheniramine Maleate, Theophylline, Ambroxol, Pseudoepidrine hydrochloride, Salbutamol, Guaiphenesin, Dextromethorphan, Diphenhydramine hydrochloride and preservative (Sodium benzoate). The developed method has potential application in quality, quantity evaluation and applicable for routine quality control analysis.

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