WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 1215-1232. Research Article ISSN 2277– 7105

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF PARACETAMOL, GUAIFENESIN, AMBROXOL HYDROCHLORIDE, PHENYLEPHRINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE IN BULK AND COMBINED DOSAGE FORM

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Article Received on
10 September 2014,

Revised on 04 Oct 2014,
Accepted on 28 Oct 2014

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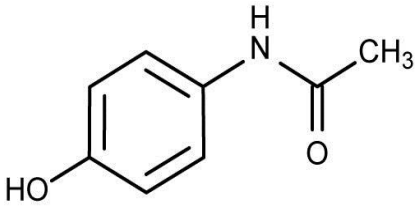
ABSTRACT

An RP-HPLC method for the simultaneous determination of Paracetamol, Guaifenesin, Ambroxol hydrochloride, Phenylephrine hydrochloride and Chlorpheniramine maleate in tablets was developed and validated ® as per ICH and FDA guidelines. The method was carried out on a with Zodiac C18 (50mm x 4.6mm, 5µm) column. Ortho phosphoric acid buffer (1M) and Acetonitrile in the ratio (50:50v/v) used as mobile phase and flow rate of 1.5ml/min. The detection was carried out at 225nm and ambient column temperature was maintained. The linearity of the proposed method was investigated in the range 10-75µg/mL (r2= 0.999) for Phenylephrine, 200-1500 µg/mL (r2= 0.999) for Paracetamol, 20-150µg/mL (r2= 0.999) for Guaifenesin, 6-45µg/mL (r2=0.999) for Ambroxol, and 4-30µg/mL (r2=0.999) for Chlorpheniramine. The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combined dosage form.

KEYWORDS: Paracetamol, Guaifenesin, Ambroxol hydrochloride, Phenylephrine hydrochloride, Chlorpheniramine maleate, RP-HPLC, Validation, Zodiac C18 column.

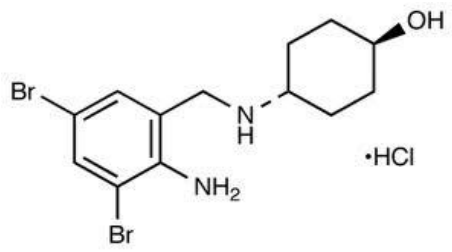
INTRODUCTION

Paracetamol (PARA) (acetaminophen), N-(4-Hydroxyphenil)-acetamide is a widely used analgesic and antipyretic agent for the relief of fever, headaches, minor pains, etc. It is soluble in water [1], slightly soluble in methanol.

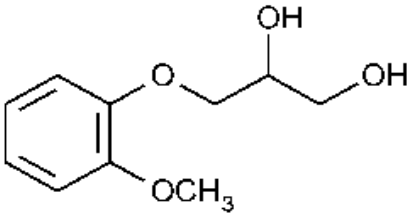


The drug is official in Indian pharmacopeia and British pharmacopoeia and estimated by liquid chromatographic methods [2, 3], it was approved by US-FDA in 2007. Literature survey revealed RP-HPLC method for estimation of PARA in biological samples, RP-HPLC method for the determination of PARA in combination with other pharmaceutical drugs [4] .Potentiometric and HPLC methods for the determination of Paracetamol in Bulk and drug formulation.

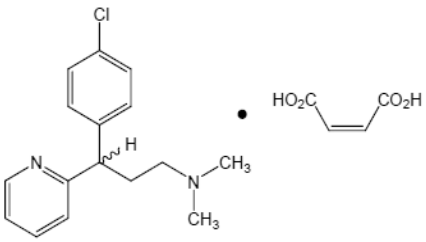
Ambroxol hydrochloride (AMB) is chemically, trans-4-((2-amino-3, 5-dibromobenzyl) amino) cyclohexanol hydrochloride. Levocetirizine dihydrochloride (LEVC) is chemically, (RS)-2-{4-[(R)-p-chloro-α-phenylbenzyl]-1-piperazinyl} ethoxyacetic acid dihydrochloride [5] . AMB reduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. LEVC is usually used in allergic conditions including rhinitis. Combination of AMB and LEVC is used for the treatment of bronchitis. These two drugs are not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and LEVC in formulations. Capillary electrophoresis [7,8,9] spectrometry [9], gas chromatography [10] LC with potentiometric detection, MS detection [11] and UV detection methods have been reported for the estimation of AMB. However, no references have been found for simultaneous determination of AMB and LEVC in pharmaceutical formulations. A successful attempt has been made to estimate these two drugs simultaneously by spectrophotometric analysis. It is freely soluble in water, DMSO, and sparingly soluble in methanol.



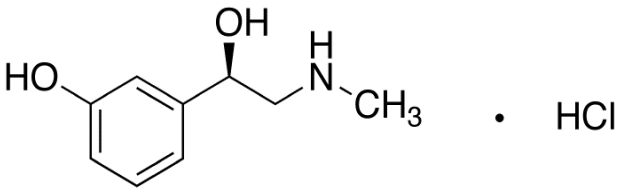
Guaifenesin (GUAI), IUPAC name (RS)-3-(2-methoxyphenoxy) propane-1, 2-diol is an It is is an expectorant drug sold over the counter and usually taken orally to assist the bringing up (expectoration) of phlegm from the airways in acute respiratory tract infections. It is the component of numerous cough cold preparations available worldwide. It is soluble in water, dimethyl formamide and slightly soluble in ethanol. Guaifenesin has not been approved by the FDA for the treatment of fibromyalgia. Based on a small, non-blinded study,[12] guaifenesin has been promoted to facilitate conception, by thinning and increasing cervical mucus, during the few days before ovulation.[13]



Chlorpheniramine maleate (CPM), IUPAC name 2, 2-dichloro-N-[(1R, 2R)-2- hydroxy1 hydroxyl methyl 2 (4 nitro phenyl) ethyl] HCl, is it is effective to relieve symptoms such as watery eyes and runny nose. Chlorpheniramine occurs as an odorless, white crystalline powder. Chlorpheniramine maleate is a H1-receptor antagonist, chemically, it is freely soluble in water, soluble in alcohol &chloroform, slightly soluble in ether & benzene. [14, 15]Literature survey revealed that Chlorpheniramine maleate can be estimated spectrophotometric method, RP-HPLC and HPTLC method.



Phenylephrine HCl (PHE) , IUPAC name (R)-1-(3-hydroxyphenyl) 2methylaminoethanol hydrochloride. It is a selective α1-adrenergic receptor agonist. It acts as an agent to constriction of blood vessels in the nasal mucosa is intended to relieve nasal congestion. It is soluble in Water, DMSO, alcohol and methanol [16]. Literature surveyrevealed that Phenylephrine HCl can be estimated spectrophotometric method, RP-HPLC and HPTLC method.



MATERIALS AND METHODS

Chemicals

Paracetamol was the gift sample from Nirmala College of Pharmacy and Ambroxol hydrochloride, Guaifenesin, Chlorpheniramine maleate and Phenylephrine hydrochloride was purchased from Indian market manufactured by Ranbaxy, Hyderabad. Potassium dihydrogen phosphate, HPLC grade Acetonitrile, Methanol, O-phosphoric acid were purchased from Merck (Mumbai) and HPLC grade water from cystron laboratories.

Instrumentation

Analysis was performed on waters HPLC 717® plus equipped with UV detector, Auto sampler and Zodiac C_{18} column compartment with Empower 2 software. Other equipment used in the study was analytical balance (DENVER®) and P^H meter (EUTECH instrument ®). Ultra sonic bath (UNICROME ASSOCIATES: UCA-701®).

Chromatographic Conditions

Zodiac C_{18} column (250 mm × 4.6 mm i.d, 5µm) was used for chromatographic separation. The mobile phase composed of Ortho-phosphoric acid buffer in HPLC grade water, and Acetonitrile in the ratio of (50:50v/v); at a flow rate of 1.5ml/min with run time 8 min. Mobile phase and sample solutions were filtered through a 0.45 µm Nylon syringe filter and degassed. The detection of all the drugs was carried out at 225 nm.

Method Development

Standard stock solutions of 30mg/ml of Ambroxol HCL, 50mg/ml of Guaifenesin, 1000mg/ml of paracetamol, 50mg/ml of phenylephrine HCL and 20mg/ml of Chlorpheniramine maleate were prepared separately using diluent (Acetonitrile: water-70:30v/v). The Ambroxol HCL stock solution was diluted with diluent to give working standard solution containing 6-45µg/ml concentration, the Guiafenesin stock solution was diluted with diluent to give working standard solution in the range 20-150 µg/ml, the Paracetamol stock solution was diluted with diluent to give working standard solution containing 200-1500µg/ml concentration, the Phenylephrine HCL stock solution was diluted

with diluent to give working standard solution containing 10-75µg/ml concentration, the Chlorpheniramine maleate stock solution was diluted with diluent to give working standard solution containing 4-30µg/ml concentration. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for AMB, GUAI, PARA, PHE and CPM by injecting eight concentrations of both drugs prepared in diluent and calibration curves were constructed by plotting area against the respective concentrations.

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing Meloxicam and Amoxicillin sodium. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of AMB, GUAI, PARA, PHE and CPM. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed samples solution of AMB, GUAI, PARA, PHE and CPM, a known amount of standard drug powder of AMB, GUAI, PARA, PHE and CPM were added at 50, 100, 150% level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is a procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line.

Robustness was evaluated by making deliberate variations such as variation of wavelength, flow rate and change in mobile phase composition. The robustness of the method was studied for AMB, GUAI, PARA, PHE and CPM. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 6µg/ml of AMB, 20 µg/ml of GUAI, 200 µg/ml of PARA, 10 µg/ml of PHE and 4 µg/ml solutions of CPM, respectively. The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at 25^0c for 12hr and 24hrs. Sample solution was reanalysed after 12 hrs and 24 hrs. Time intervals and assay was determined for AMB, GUAI, PARA, PHE and CPM and compared against fresh sample.

Analysis of Formulation

To determine the content of AMB, GUAI, PARA, PHE and CPM in injection formulation (PARA 325mg, GUAI 100mg, AMB 30mg, PHE 10mg and CPM 2mg) an accurately weighed drug powder equivalent to 10 mg of MEL and 200mg of AMXS were transferred into 200mL volumetric flask, dissolved in 150mL of diluent and sonicated for 5 min. After achieving complete solubility of the drug, the volume was made up to the mark using diluent .The solution was filtered through the 0.45 µm nylon syringe filter. From the filtrate a 1mL solution was transferred into 50 mL volumetric flask and volume was made up to the mark with diluent to obtain a concentration of 30µg/ml of AMB, 100mg/ml of GUAI, 325mg/ml of PARA, 10mg/ml of PHE and 2µg/mL of CPM which was then subjected to proposed method and the amounts of AMB, GUAI, PARA, PHE and CPM were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantitation of AMB (RT 6.000min), PARA (RT 3.993min), GUAI (RT 6.869min), PHE (RT 4.450min) and CPM (RT 7.319min) with good resolution, peak shapes and minimal tailing. The overlay UV spectra and typical chromatogram were shown in Figures 1 and 2. The individual chromatograms for AMB, PARA, GUAI, PHE and CPM -API were shown in Figure 3, 4, 5, 6, 7. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.9990 for AMB, PARA, GUAI, PHE and CPM. The AMB, PARA, GUAI, PHE and CPM have followed linearity in the concentration range of 6-45µg/mL, 200-1500µg/mL, 20-150µg/mL, 10-75µg/mL and 4-30µg/mL respectively Figure 8, 9,10,11,12. Percent recoveries for AMB, PARA, GUAI, PHE and CPM were 99.9-100.8 and 99.4-101.2%. %RSD for injectable dosage form analysis, recovery studies and intra and inter-day precision studies was less than 2. LOD and LOQ were found to be 0.5047µg/mL and 1.529µg/mL for AMB, 0.0474µg/mL and 0.1431µg/mL for PARA, 1.7623µg/mL and 5.4303µg/mL for GUAI, 0.266µg/mL and 0.806µg/mL for PHE and 0.0567µg/mL and 0.1720µg/mL for CPM. The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 0.236-0.0.699, 0.397-0.146, 1.440-0.988, 0.960-0.753 and 0.677-0.753%. As the RSD values were < 2%, the developed method was found to be precise (Table 1). The accuracy of the method studied at three different concentration levels i.e. 50, 100, 150% showed acceptable recoveries in the range of 99.3-100.8%for AMB, 99.2-100.9% for PARA, 99.8-101.1% for

GUAI, 99.4-100.7% for PHE and 99.2-101.2% for CPM (Table2). The LOD for AMB, PARA, GUAI, PHE and CPM was found to be 0.5047µg/mL, 0.0474µg/mL, 1.7623µg/mL, 0.266µg/mL and 0.0567µg/mL respectively. Further the LOQ for AMB, PARA, GUAI, PHE and CPM was found to be 1.529, 0.1431, 5.4303, 0.806 and 0.1720µg/mL respectively. Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 mL/min), wave length (± 5nm) and mobile phase composition (± 2%). The validation parameters were summarized in (Table 3).

The results of robustness study of the developed method was validated by change in flow rate, change in wave length and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4).

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method. The system suitability parameters such as number of theoretical plates and tailing factor were studied and shown in (Table 3).

Stability of sample solution was established by the storage of sample solution at 25^0c for 12hr and sample was reanalysed after 24hr and assay was determined for the compounds (AMB, PARA, GUAI, PHE and CPM) and compared against fresh sample. Sample solution did not show any appreciable change in assay value (% RSD<2) when stored at ambient temperature up to 24 hrs. The results were shown in (Table 6).The results of forced degradation studies were shown in (Table 7, 8, 9, 10, and 11).

Six replicates of sample solutions containing 6µg/ml for AMB, 200µg/ml for PARA, 20µg/ml for GUAI, 10µg/ml for PHE and 4µg/ml for CPM were injected for quantitative analysis. The amounts of AMB, PARA, GUAI, PHE and CPM estimated were found to be 100.1, 100.2, 100.1, 100.7 and 99.9% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of AMB, PARA, GUAI, PHE and CPM with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance

indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges for the above drugs. The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for AMB, PARA, GUAI, PHE and CPM were 99.3-100.8, 99.2-100.9, 99.8-101.1, 99.4-100.7 and 99.2-101.2% respectively, indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate, wave length and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of AMB, PARA, GUAI, PHE and CPM from the commercial combined tablet dosage form were in good agreement with respective to label claim indicating that there were no interferences from the commonly used tablet excipients and buffer used in analysis.

Table 1: Precision of Developed Method.

S. NO	METHOD PRECISION									
	AMB		PARA		GUA		CPM		PHE	
	RT	Area	RT	Area	RT	Area	RT	Area	RT	Area
1	6.705	1864194	3.978	6254481	7.482	5642048	7.851	942040	5.144	798582
2	6.707	1869965	3.971	6280521	7.484	5615301	7.855	946014	5.147	798428
3	6.713	1867697	3.987	6218061	7.487	5684597	7.860	954317	5.149	784446
4	6.706	1862856	4.002	6286167	7.482	5621043	7.853	944839	5.145	799324
5	6.704	1873478	3.868	6244879	7.482	5825334	7.849	958584	5.131	807246
6	6.707	1862370	3.982	6259805	7.462	5597451	7.834	952932	5.146	792493
MEAN	6.707	1866760	3.964	6257319	7.480	5674296	7.849	949787	5.143	796753
±SD		0.804		0.387		0.459		1.118		0.214
% RSD		0.236		0.397		1.440		0.677		0.960

S. NO	INTER-DAY PRECISION									
	AMB		PARA		GUA		CPM		PHE	
	RT	Area	RT	Area	RT	Area	RT	Area	RT	Area
1	6.734	1728165	3.668	6093418	7.519	5399933	7.863	832497	5.138	757170
2	6.711	1745680	3.719	6087594	7.514	5430201	7.884	843374	5.061	766002
3	6.751	1726945	3.743	6077201	7.532	5510775	7.880	831411	5.144	770603
4	6.715	1737865	3.584	6095212	7.503	5398652	7.847	842767	5.104	761001

5	6.724	1723551	3.546	6075528	7.511	5357266	7.837	825027	5.134	755074
6	6.698	1754443	3.539	6077087	7.507	5382201	7.855	835559	5.048	763703
MEAN	6.722	1736108	3.633	6084340	7.514	5413173	7.861	835106	5.104	732259
±SD		0.701		0.997		0.671		0.627		0.855
% RSD		0.699		0.146		0.988		0.846		0.753

Table 2: Accuracy Data.

% Level of recovery	Area	Amount of sample added (µg/ml)	Amount of API added (µg/ml)	Amount found (µg/ml±SD)	Recovery %±SD	%RSD
AMBROXOL HYDROCHLORIDE						
50%	1016065	60	30	59.8	99.9	0.250
	929799	60	31	59.1	99.6	
	1062123	60	33	60.1	100.1	
100%	2115447	60	60	59	99.3	0.600
	1826402	60	61	60.5	100.4	
	2633266	60	59	60.3	100.4	
150%	2611848	60	90	61	100.8	0.440
	2674682	60	89	60.4	100.6	
	3061273	60	90	60	100.0	
PARACETAMOL						
50%	3500770	200.2	100.1	100.1	100.0	0.820
	3184424	200.2	100.3	99.8	99.2	
	3681832	200.2	100.1	100.3	100.9	
100%	7371544	200.2	200.2	200	99.8	0.650
	6350792	200.2	200.1	200	99.4	
	9236634	200.2	200	200.3	100.7	
150%	9146524	200.2	300.1	299.9	99.2	0.460
	9506432	200.2	300.3	300.4	100.1	
	10779324	200.2	300.2	300	99.7	
GUAIPHENESIN						
50%	3340671	105.4	52.5	53	101.1	0.620
	3043955	105.4	53	53.1	100.1	
	3435621	105.4	52.7	52.7	100.0	
100%	6598814	105.4	105.4	105.7	100.8	0.250
	5727540	105.4	105	105.4	100.6	
	7861747	105.4	105.7	106	101.1	
150%	7833844	105.4	153	153.1	100.3	0.480
	7902378	105.4	153.7	154	100.8	
	8477862	105.4	153.4	153	99.8	

CHLORPHENIRAMINE MALEATE						
50%	491881	12	6	6.2	100.3	0.080
	449986	12	5.9	6.1	100.4	
	517454	12	6	6.2	100.5	
100%	1046816	12	12	12.3	100.8	0.660
	910831	12	11.9	11.2	99.6	
	1341907	12	12.1	11.9	99.7	
150%	1335407	12	18	17.6	99.2	1.000
	1370102	12	18	18.3	101.2	
	1570022	12	18.2	18.5	100.5	
PHENYLEPHRINE HYDROCHLORIDE						
50%	447851	10.6	5.3	5.5	100.2	0.400
	405022	10.6	5.5	5.2	99.4	
	471581	10.6	5.4	5.1	99.7	
100%	933229	10.6	10.6	11	100.6	0.640
	808373	10.6	10.7	10.3	99.5	
	1173431	10.6	10.6	10	99.5	
150%	1167785	10.6	15.9	15.5	99.4	0.650
	1210501	10.6	16	15.9	99.9	
	1376408	10.6	16	16.2	100.7	

Table 3: Validation and System Suitability Parameters.

Parameter	PARA	AMB	GUAI	PHE	CPM
Range (µg/ml)	200-1500	6-45	20-150	10-75	4-30
Slope	6499.80	59940.19	56213.84	16402.07	46535.99
Intercept	-171617.38	-14829.07	135142.97	-20846.26	-65488.11
Correlation coefficient (R^2)	0.99892	0.99968	0.99295	0.99909	0.99755
Retention time	3.964	6.707	7.479	5.143	7.850
Precision (intra and inter day)% RSD	<2	<2	<2	<2	<2
Accuracy	99.2-100.9	99.3-100.8	99.8-101.1	99.4-100.7	99.2-101.2
LOD(µg/ml)	0.0474	0.5047	1.7623	0.0567	0.266
LOQ(µg/ml)	0.1431	1.529	5.04303	0.806	0.1720
Tailing factor	1.00	1.09	1.05	1.09	1.00
Theoretical plates	2572	60579	73730	36450	747043
Resolution	-	14.24	6.91	5.49	3.21

Table 4: Influence of Flow Rate, Wavelength And Mobile Phase Composition On Analytical Parameters.

Parameter	PARA			AMB			GUAI		
	RT	Area	Tailing	RT	Area	Tailing	RT	Area	Tailing
Flow rate(±0.2ml/min)									
1.3	4.209	11431191	1.08	7.393	31219415	1.10	8.188	9036815	1.10
1.5	3.978	6254481	0.99	6.704	1873478	1.03	7.482	5642048	1.05
1.7	3.602	8657362	0.99	6.217	2472747	1.05	6.986	7476245	1.03

Wave length(±5nm)									
220	3.986	1361570	1.05	5.998	741161	1.58	6.854	2088967	1.25
225	3.791	6280521	1.01	6.707	1869965	1.08	7.484	5615301	1.01
230	3.986	971210	1.05	5.986	488685	1.05	6.852	1952995	1.25
Mobile phase composition (±5%v/v)									
45:55	4.420	9947047	1.06	6.849	2966709	1.08	7.630	8332130	1.05
50:50	3.987	6218061	1.01	6.713	1867697	1.06	7.487	5684597	1.07
55:45	2.879	10175125	1.17	6.589	2963067	1.10	7.372	8208501	1.08

Parameter	PHE			CPM		
	RT	Area	Tailing	RT	Area	Tailing
Flow rate(±0.2ml/min)						
1.3	5.759	1565834	1.15	8.555	1694123	1.11
1.5	5.145	799324	1.07	7.851	942040	1.02
1.7	4.669	1099400	1.08	7.338	1189287	0.96
Wave length(±5nm)						
220	4.438	189702	1.10	7.299	281251	1.34
225	5.147	798428	1.08	7.855	946014	1.05
230	4.438	160958	1.08	7.300	257061	1.35
Mobile phase composition (±5%v/v)						
45:55	5.317	1318112	1.126	7.985	1550299	1.03
50:50	5.149	784446	1.10	7.860	954317	1.00
55:45	4.921	1332222	1.14	7.718	1522542	1.03

Table 5: Assay of Commercial Formulation.

Drug	Label claim (mg/tablet)	Calculated value (ml±SD/tablet)	% of Assay
PARA	325	341.91	101.2
AMB	30	35.18	100.1
GUAI	100	105.21	101.7
PHE	10	13.5	100.7
CPM	2	2.24	101.8

Table 6: Stability Studies.

S. NO	Drug	Stability	RT	Area	USP Tailing	USP Plate count
1	PARA	24hrs	3.979	1494283	1.036	18173
2	AMB		5.979	457856	1.055	40940
3	GUAI		6.846	1981470	1.252	28167
4	PHE		4.432	196163	1.059	25516
5	CPM		7.284	267890	1.442	28626
S. NO	**Drug**	12 hrs	**RT**	**Area**	**USP Tailing**	**USP Plate count**
1	PARA		3.98	1486450	1.021	18173
2	AMB		5.984	497237	1.194	38800
3	GUAI		6.851	1980230	1.263	28683
4	PHE		4.438	197974	1.092	25659
5	CPM		7.288	253873	1.319	30560
S. NO	**Drug**	0 hrs	**RT**	**Area**	**USP Tailing**	**USP Plate count**

1	PARA		3.996	1505985	1.05	18010
2	AMB		5.996	441373	1.04	40856
3	GUAI		6.867	1997986	1.22	33427
4	PHE		4.446	201261	1.09	24787
5	CPM		7.327	253467	1.23	26098

Table 7: Forced Degradation Study for Chlorpheniramine Maleate.

S. No	Stress conditions	SAMPLE WT	Peak area	% degraded	%label claim
1	STD	4015.6	253467	-0.6	100.6
2	Acid	4192.8	206216	21.6	78.4
3	Base	4264.2	215702	19.4	80.6
4	Peroxide	4268.8	115264	27.1	72.9
5	Reduction	4258.5	212827	26.8	73.2
6	Thermal	4251.5	209126	21.8	78.2
7	Photo	4221.8	210492	21.1	78.9
8	Hydrolysis	4235.6	205311	22.5	77.5

Table 8: Forced Degradation Study for Ambroxol Hydrochloride.

S. No	Stress conditions	SAMPLE WT	Peak area	% degraded	%label claim
1	STD	4015.2	481107	0.1	99.9
2	Acid	4192.8	402400	20	80
3	Base	4264.2	406037	20.6	79.4
4	Peroxide	4268.1	391042	23.6	76.4
5	Reduction	3628.8	328507	24.5	75.5
6	Thermal	4258.8	408975	20.1	80
7	Photo	4251.5	407432	20.1	79.9
8	Hydrolysis	4221.8	406305	19.8	80.2

Table 9: Forced Degradation Study for Paracetamol.

S. No	Stress conditions	SAMPLE WT	Peak area	% degraded	%label claim
1	STD	4015.6	1479028	-0.2	100.8
2	Acid	1892.8	1376946	23	77
3	Base	4866.2	1360551	23.4	76.6
4	Peroxide	4268.1	1123938	27.8	72.1
5	Reduction	4028.8	1066969	27.5	72.5
6	Thermal	4288.5	1171676	25.2	74.8
7	Photo	4251.5	1176158	24.5	75.5
8	Hydrolysis	4221.5	1170968	24.1	75.9

Table 10: Forced Degradation Study for Guaiphenesin.

S. No	Stress conditions	SAMPLE WT	Peak area	% degraded	%label claim
1	STD	4015.6	1994471	-0.6	100.4
2	Acid	4892.8	1904041	21.2	78.8
3	Base	4864.2	1830799	23.8	76.2
4	Peroxide	3268.4	1272842	21.1	78.9
5	Reduction	4028.8	1548734	22.2	77.8
6	Thermal	4288.5	1695234	19.9	80.1
7	Photo	4251.5	1679509	20	80
8	Hydrolysis	4221.8	1644379	21.1	78.9

Table 11: forced degradation study for phenylephrine hydrochloride.

S. No	Stress conditions	SAMPLE WT	Peak area	% degraded	%label claim
1	STD	4015.2	197982	-0.4	100.6
2	Acid	4892.5	183307	23.5	76.5
3	Base	4864.2	178645	25	75
4	Peroxide	4268.1	159909	23.5	76.5
5	Reduction	4028.8	141401	28.4	71.6
6	Thermal	4288.5	155995	25.8	74.2
7	Photo	4251.5	156803	24.7	75.3
8	Hydrolysis	4221.3	156845	24.2	75.8
S. No	**Stress conditions**	**SAMPLE WT**	**Peak area**	**% degraded**	**%label claim**
1	STD	4015.2	197982	-0.4	100.6
2	Acid	4892.5	183307	23.5	76.5
3	Base	4864.2	178645	25	75
4	Peroxide	4268.1	159909	23.5	76.5
5	Reduction	4028.8	141401	28.4	71.6
6	Thermal	4288.5	155995	25.8	74.2
7	Photo	4251.5	156803	24.7	75.3
8	Hydrolysis	4221.3	156845	24.2	75.8

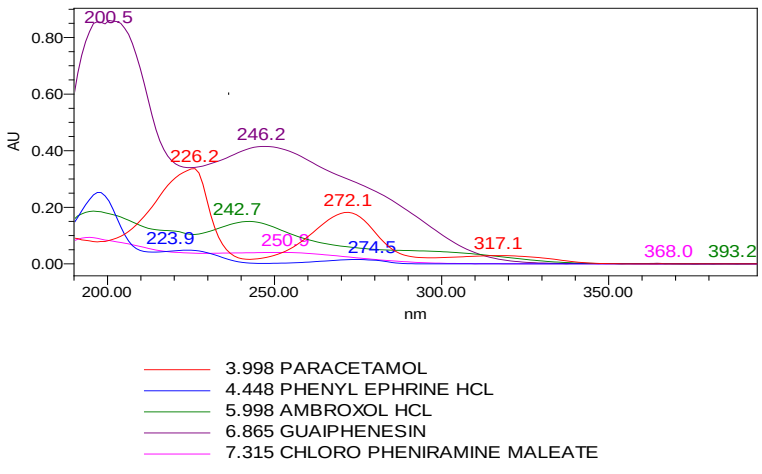


Figure 1: Overlay UV Spectra of Standard of AMB, PARA, GUAI, PHE and CPM.

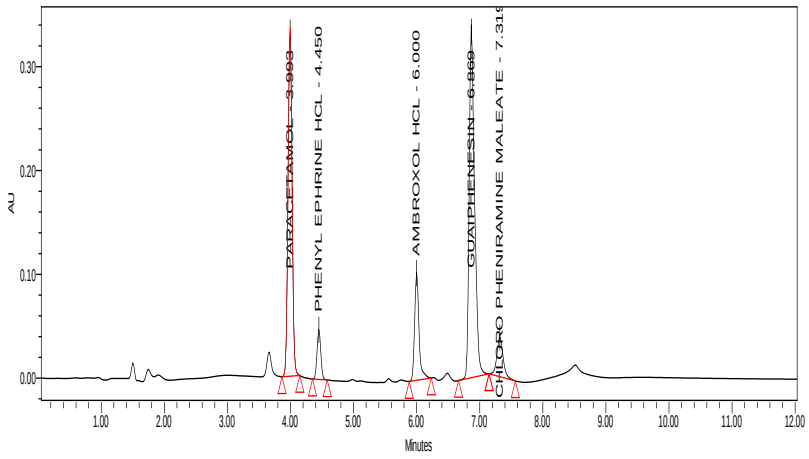


Figure 2: Typical HPLC chromatogram of AMB, PARA, GUAI, PHE and CPM.

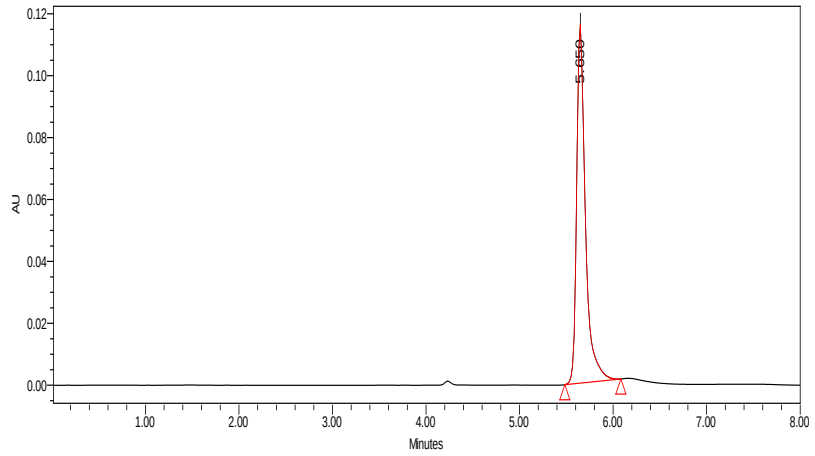


Figure 3: Individual Chromatogram of Ambroxol Hydrochloride

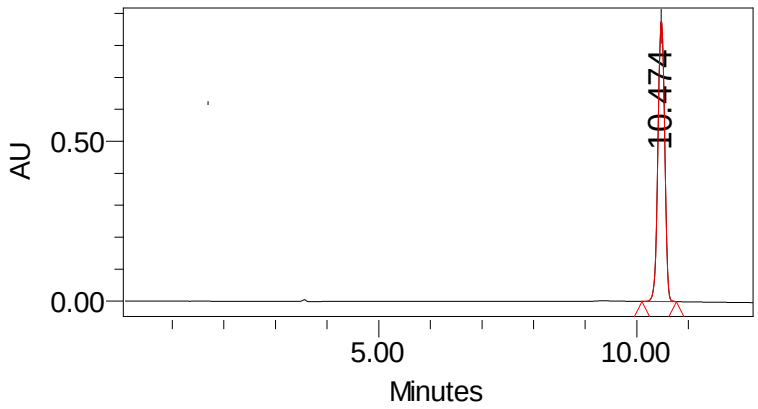


Figure 4: Individual chromatogram of Paracetamol.

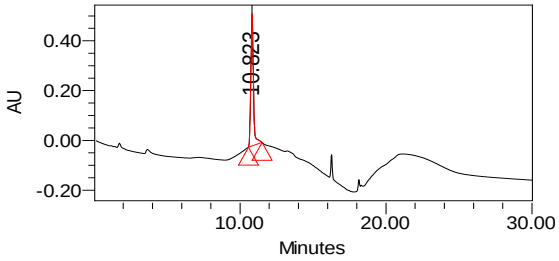


Figure 5: Individual Chromatogram of Guaiphenesin.

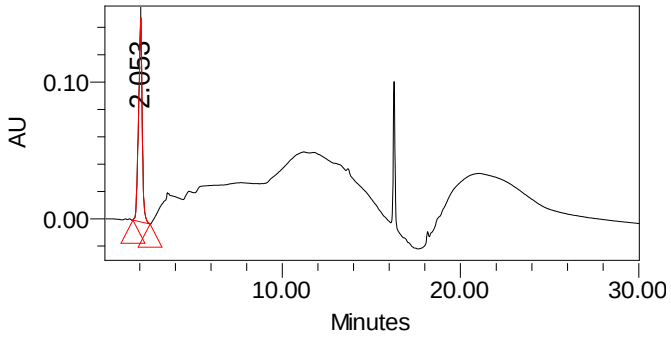


Figure 6: Individual Chromatogram of Phenylephrine Hydrochloride.

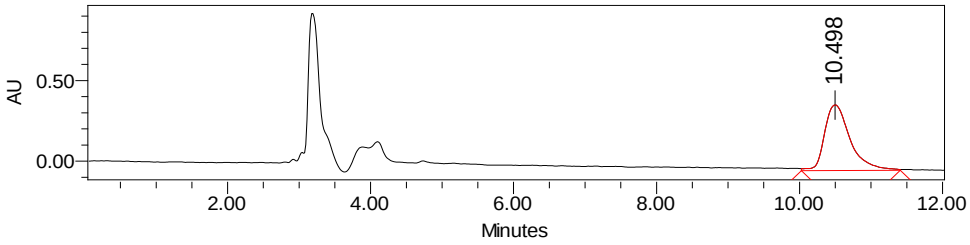


Figure 7: Individual Chromatogram of Chlorpheniramine Maleate.

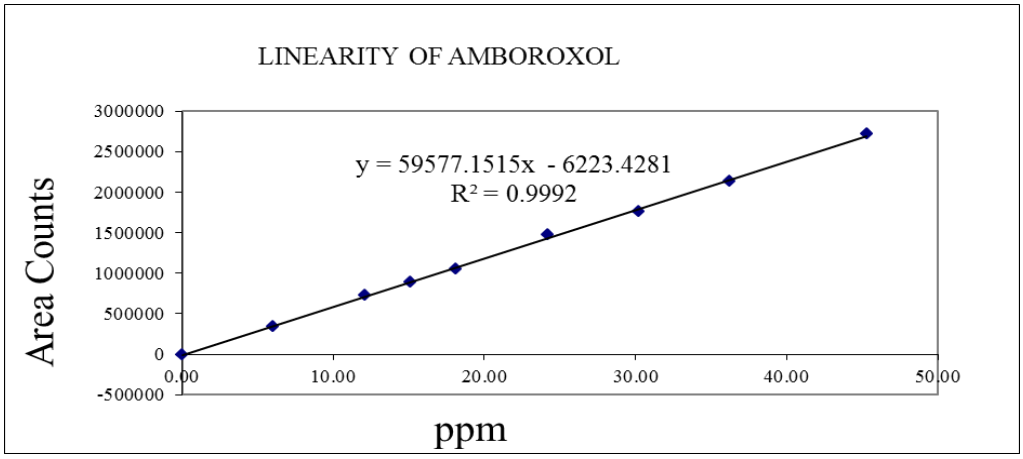


Figure 8: Calibration Curve For Ambroxol Hydrochloride.

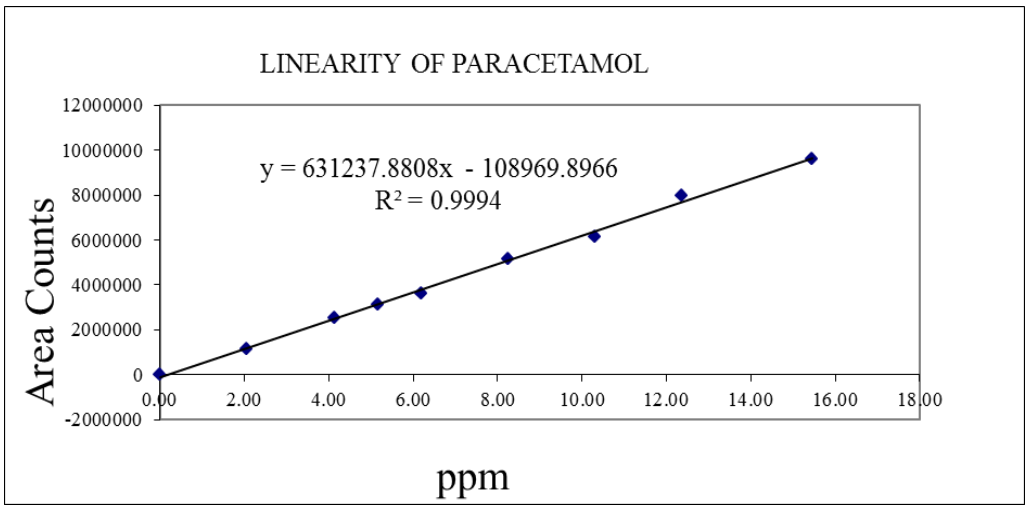


Figure 9: Calibration Curve For Paracetamol.

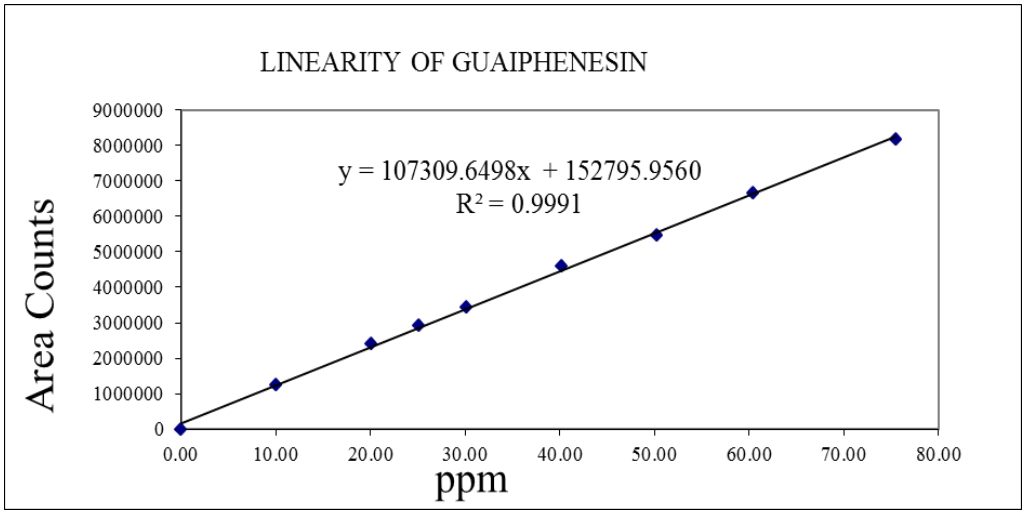


Figure 10: Calibration Curve For Guaiphenesin.

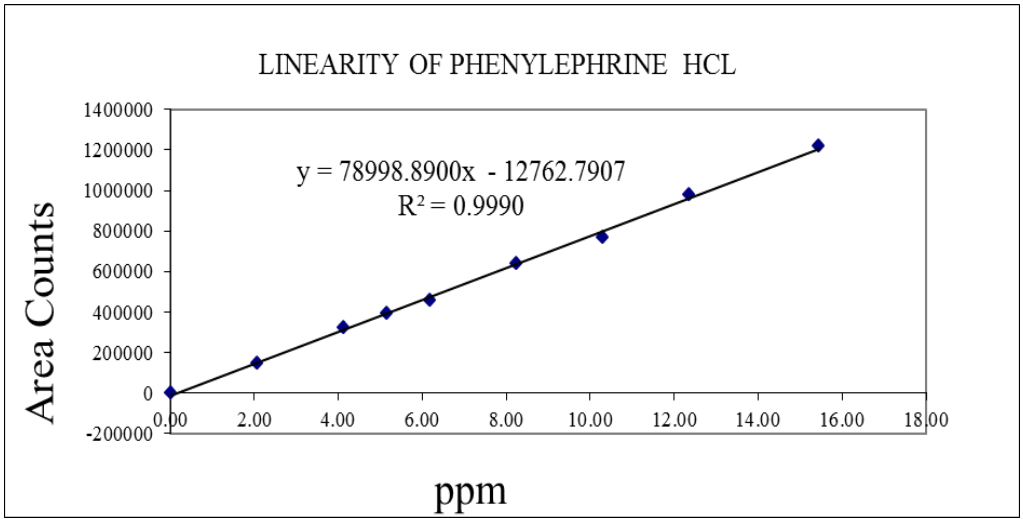


Figure 11: Calibration Curve For Phenyl Ephrine Hcl.

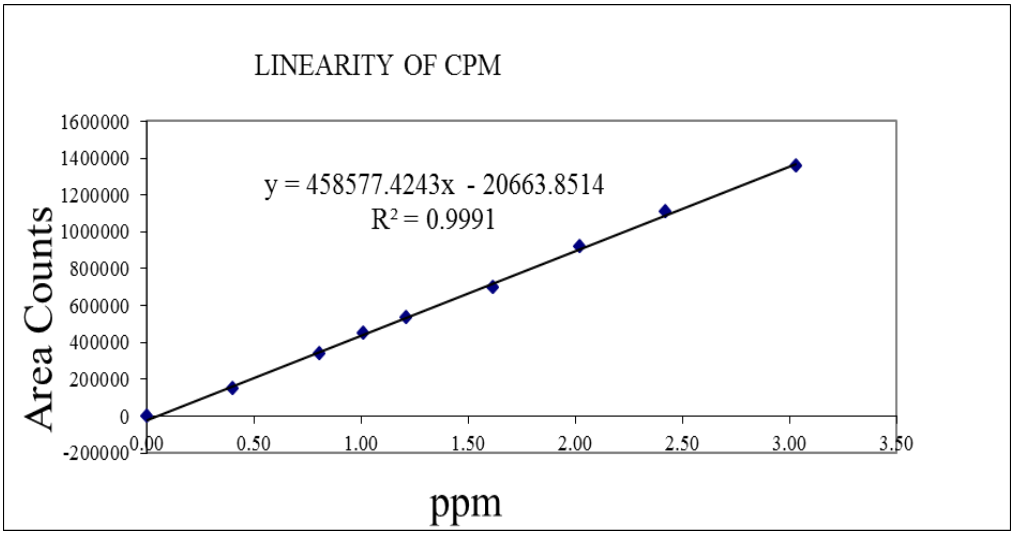


Figure 12: Calibration Curve For Chlorpheniramine Maleate.

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of AMB, PARA, GUAI, PHE and CPM in combined tablet dosage form for routine quality control analysis.

ACKNOWLEDGEMENT

The authors are thankful to Nirmala College of Pharmacy Mangalagiri, Guntur, and Andhra Pradesh, India for providing necessary facilities to carry out the research work.

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