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A Stability Indicating RP-HPLC Method for Simultaneous Estimation of Salbutamol, Carbocisteine and Theophylline in Combined Tablet Dosage Forms

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ABSTRACT

A rapid and sensitive stability indicating RP-HPLC method was developed for simultaneous estimation of salbutamol, carbocisteine and theophylline in combined tablet formulations. Chromatography was carried out on a Discovery HS C18 HPLC Column at 35 °C (250 x 4.6 mm; 5µ) by eluting with a mobile phase consisting of a 50:50 v/v mixture of acetonitrile and 0.1 % orthophosphoric acid in water at a flow rate of 1.0 mL/ min. The detection wavelength was set at 215 nm. Accuracy was assessed by using standard addition method. The developed HPLC method was validated with respect to precision, specificity, accuracy, linearity and robustness. Forced degradation studies on the formulation were conducted by adopting the proposed method to assess the stability of the analytes under acid, base, peroxide, thermal and photolytic conditions and suitability of the method to resolve the degradation products.

Keywords: Salbutamol, Carbocisteine, Theophylline, RP-HPLC, Forced degradation, Method validation.

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INTRODUCTION

Salbutamol is a selective β-adrenergic agonist. It is a direct-acting sympathomimetic agent with a selective action on β_2 receptors. It acts as a bronchodilator. Salbutamol is mainly used to treat asthma and in chronic obstructive pulmonary disease. It decreases uterine contractility and thus can be used to stop premature labor. Carbocisteine is an acetyl cysteine derivative used mainly as a mucolytic agent. It acts by reducing the viscosity of the sputum and thus helps to relieve the symptoms in chronic pulmonary disorder and bronchiectasis. Theophylline, a xanthine analog is a respiratory stimulant and relieves bronchospasm and relaxes bronchial smooth muscles. It is mainly used as a bronchodilator in asthma. It is also known to stimulate the myocardium and CNS. It acts by increasing the cyclic AMP by inhibiting the phosphodiesterase enzyme[1-4]**.** Chemical structures of salbutamol, carbocisteine and theophylline are given in Figure 1. Many liquid chromatographic methods for determination of salbutamol, carbocisteine and theophylline either individually or in a combination of any two of them were reported earlier. These methods are based on HPLC[5-10], TLC[11] and LC-MS[12]. However, to my knowledge no stability-indicating HPLC method for simultaneous determination of all these three drugs in combined dosage forms has so far been published.

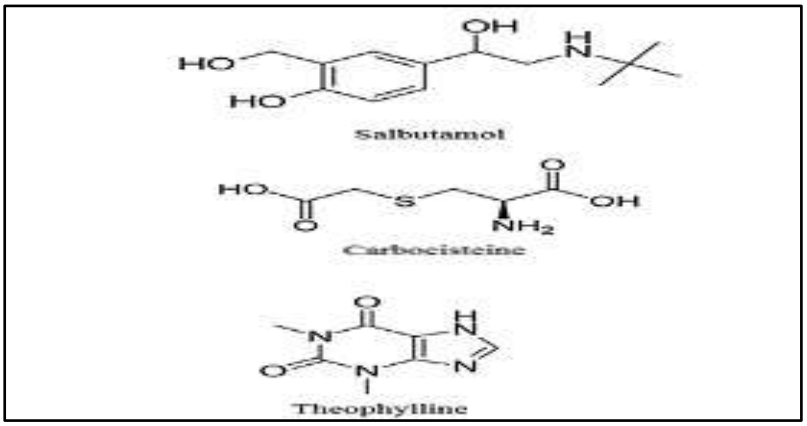


Figure 1: Chemical structures

MATERIALS AND METHOD

The reference samples of salbutamol, carbocisteine and theophylline were procured from Merck, Mumbai, India. The branded tablet formulation CARBASMA (salbutamol 2 mg, carbocisteine 150 mg and theophylline 100 mg) of Grandix Pharmaceuticals, Ltd., Chennai, India was purchased from the local market. All the HPLC solvents and analytical reagent grade chemicals were purchased from S.D. Fine Chemicals, Hyderabad, India.

Instrumentation:

A Waters HPLC system equipped with a 2695 binary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The output signal was monitored and processed with Empower software. Photo stability studies were carried out in a Mack Pharmatech, Model MK*-20PH photo stability chamber. Thermal stability studies were carried out in a dry air oven of Labline, Maharashtra, India.

Chromatographic conditions:

The separation of the drugs was achieved on a Discovery® HS C18 HPLC Column (250 x 4.6 mm; 5µ) by running a mobile phase containing a 50:50 v/v mixture of 0.1% orthophosphoric acid in water and acetonitrile at a flow rate of 1.0 mL/min. The injection volume was 10 µL. The column temperature was maintained at 30 °C and the analytes in the eluates were monitored at 215 nm. The run time was 8.0 min.

Preparation of Solutions:

Diluent:

A 50:50 v/v mixture of water and acetonitrile was used as the diluent.

Mobile phase:

Transferred 1.0 mL of ortho phosphoric acid, 85% solution in to a 1000 mL of volumetric flask. Made up to volume with water and mixed well. This is the buffer solution. Buffer solution and acetonitrile was mixed in 50:50 ratios and degassed.

Stock and working standard drug solutions:

Reference standard samples of 15 mg of carbocisteine and 10 mg of theophylline were weighed and transferred to a 10 mL volumetric flask and the volume was made up with the diluent to prepare the combined stock solution of the two drugs. Similarly, the reference standard of 2.0 mg of salbutamol was weighed into a 100 mL volumetric flask, about 70 mL of the diluent was added to it and the contents sonicated for 10 min. The volume was then made up with the diluent to prepare the stock solution of salbutamol. Then, 1.0 mL each of the above two stock solutions was transferred into another 10 mL volumetric flask and the volume was made up with the diluent to prepare the combined working standard solution of the three drugs.

Tablet sample solution:

20 tablets of 'Carbasma' (salbutamol: 2 mg, carbocisteine: 150 mg and theophylline: 100 mg) were weighed and the average weight of the tablet was calculated. The tablets were finely powdered and a quantity of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated for 10 minutes. Then the volume was made up with the diluent and mixed well to prepare the sample stock solution. This solution was filtered

through a 0.45 µm nylon filter. 1.0 mL of the filtrate was transferred to a 10 mL volumetric flask and the volume made up to give final theoretical concentrations of 2 µg/mL, 150 µg/mL and 100 µg/mL of salbutamol, carbocisteine and theophylline respectively.

Method Development

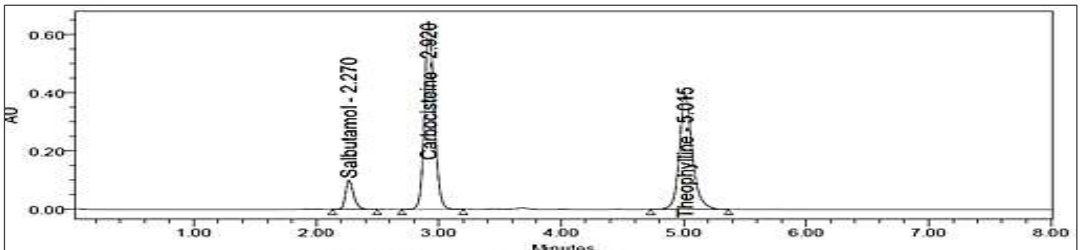
Different mobile phases were considered for simultaneous separation of the three drugs on a Discovery® HS C18 HPLC Column. Selection of the mobile phase was done on the basis of ideal resolution among salbutamol, carbocisteine and theophylline and also their impurities formed during forced degradation studies. The required chromatographic conditions were optimized.

METHOD VALIDATION

The developed method was validated for precision, specificity, accuracy (recovery), linearity and robustness as per the ICH guidelines[14].

System Suitability

System suitability was established for initial evaluation of the method before running the sample for the validation parameters. The test was performed according to the USP [13]. The standard solutions prepared as per the proposed method. The standard solution was injected six times. The results of the system suitability study are presented in Figure 2. The acceptance criterion is % RSD ≤ 2.0. A percent RSD of 0.8 indicates good system precision of the method.



Peak Name: Carbocisteine

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Carbocisteine	2.914	3485332	6523	1.14	4.8
2	Carbocisteine	2.918	3504671	6548	1.11	4.8
3	Carbocisteine	2.919	3519645	6369	1.13	4.8
4	Carbocisteine	2.919	3512941	6709	1.12	4.9
5	Carbocisteine	2.920	3505305	6633	1.13	4.8
6	Carbocisteine	2.923	3507178	6200	1.11	4.8
Mean			3505845			
Std. Dev.			11532.8			
% RSD			0.3			

Peak Name: Salbutamol

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Salbutamol	2.263	202374	5635	1.25
2	Salbutamol	2.268	202666	5915	1.20
3	Salbutamol	2.269	204782	5545	1.27
4	Salbutamol	2.269	201253	6036	1.22
5	Salbutamol	2.269	205312	5822	1.21
6	Salbutamol	2.270	205006	5739	1.23
Mean			203566		
Std. Dev.			1684.1		
% RSD			0.8		

Peak Name: Theophylline

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Theophylline	5.007	2828773	12176	1.19	12.6
2	Theophylline	5.008	2837557	12475	1.20	12.5
3	Theophylline	5.009	2847566	11908	1.20	12.6
4	Theophylline	5.015	2866441	12342	1.20	12.3
5	Theophylline	5.018	2800653	11942	1.21	12.3
6	Theophylline	5.072	2823227	11865	1.19	12.5
Mean			2834036			
Std. Dev.			22380.9			
% RSD			0.8			

Figure 2: A representative chromatogram and results of system suitability study

Method Precision

The precision of the proposed method was evaluated by performing six independent assays of the test sample preparation and calculating the % RSD. The intermediate precision of the proposed method was checked by performing the same procedure on a different day under the same experimental conditions. The % RSD was found to be below 2.0 % which indicates that the proposed method is precise. Data obtained from precision experiments are given in Table 1.

Table 1: Precision results of the drugs

Sample Number	% Assay					
	Salbutamol		Carbocisteine		Theophylline	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	99.132	102.905	100.089	99.131	99.315	100.310
2	99.275	99.089	100.645	99.590	99.624	100.377
3	100.312	99.530	101.075	99.425	99.975	100.397

4	98.583	98.102	100.882	99.858	100.638	100.990
5	100.571	98.290	100.663	99.711	98.328	100.198
6	100.421	100.277	100.717	100.046	99.121	100.414
Average	99.716	99.699	100.679	99.627	99.500	100.448
Standard deviation (SD)	0.825	1.763	0.331	0.324	0.786	0.277
%RSD	0.83	1.77	0.33	0.32	0.79	0.28

Specificity

Interference with the diluent was evaluated by injecting the diluent into HPLC as per proposed method. No peaks were found except for the solvent front peaks. Specificity was also evaluated by forced degradation studies as per the ICH guidelines[14].

'Carbasma' tablets were subjected to different stress conditions as per the procedures described below. Solutions from each stressed sample were then injected into the HPLC system and analyzed as per the proposed method. Peak purity was established in 200–400 nm regions by using the Empower Software. The percentage degradation and the peak purity data obtained from each degradation study are given in the Tables 2-4. In acid and alkali degradation the drug product samples showed degradation. In the chromatogram of the acid degradation sample, the peak corresponding to salbutamol is completely absent. In these case two peaks i.e. peak1 and peak2 were found at retention times of 2.370 and 2.640 minutes respectively. Carbocisteine and theophylline also showed some degradation in acid and alkali conditions. In the alkali degradation sample peak1 was found at a retention time of 2.387 minutes. The peaks 1 & 2 Peaks obtained from both acid and alkali degradation samples have purity angles lesser than their purity threshold indicating that the peaks are homogeneous and they are not interfering with the other drug peaks. In the heat, light, peroxide and control (water) stress studies detectable degradation of the drugs was not observed. Forced degradation results were given in tables 2-4. The relevant chromatograms are presented in Figures 3-8.

Table 2: Forced degradation results of salbutamol

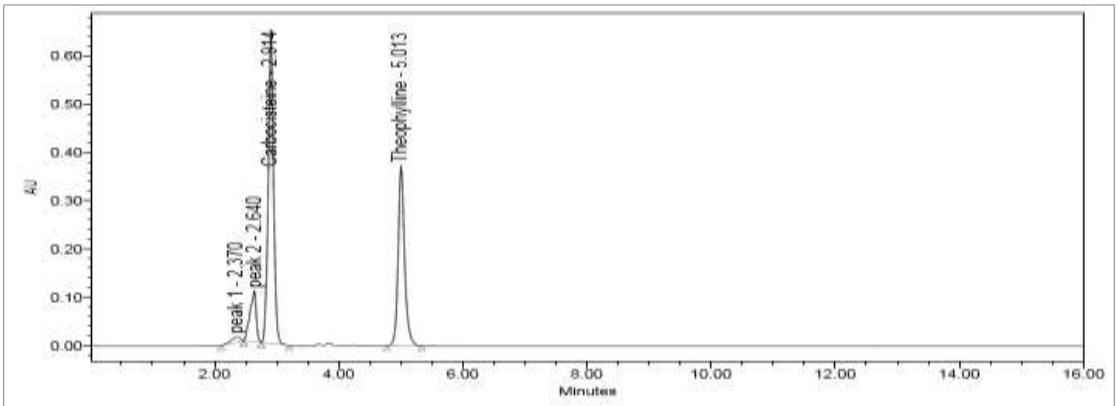
Nature of degradation	**Stress conditions**	**Salbutamol**		**Percent Assay**
		Purity Angle	**Purity Threshold**	
Acid	1N HCl at room temperature for 60	Degraded	Degraded	Degraded
Base/Alkali	1N NaOH at room temperature for 60	0.252	0.458	97.72809
Peroxide	30 % H2O2 at room temperature for 60	0.348	0.493	99.46583
Dry heat	Drug substance heated at 105°C for 6	0.329	0.479	100.06283
Photolytic	UV chamber at 250 Watts hours/m^2 for	0.326	0.482	100.16083
Neutral	Water at room temperature for 60 minutes	0.350	0.509	100.46530

Table 3: Forced degradation results of carbocisteine

Nature of degradation	Stress conditions	Carbocisteine Purity Angle	Purity Threshold	Percent Assay
Acid	1N HCl at room temperature for 60 minutes	0.128	0.306	96.86634
Base/Alkali	1N NaOH at room temperature for 60	0.129	0.301	97.14213
Peroxide	30 % H2O2 at room temperature for 60	0.130	0.304	98.84734
Dry heat	Drug substance heated at 105°C for 6 hours	0.149	0.306	99.76897
Photolytic	UV chamber at 250 Watts hours/m^2 for 21.8	0.137	0.306	100.00132
Neutral	Water at room temperature for 60 minutes	0.129	0.306	100.14531

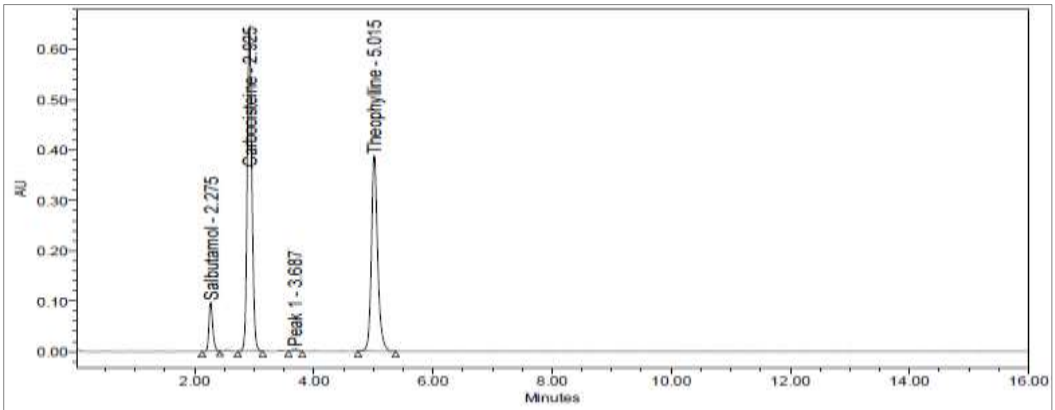
Table 4: Forced degradation results of theophylline

Nature of degradation	Stress conditions	Theophylline Purity Angle	Purity Threshold	Percent Assay
Acid	1N HCl at room temperature for 60 minutes	0.069	0.287	94.71505
Base/Alkali	1N NaOH at room temperature for 60 minutes	0.099	0.281	97.29277
Peroxide	30 % H2O2 at room temperature for 60 minutes	0.068	0.284	97.50943
Dry heat	Drug substance heated at 105°C for 6 hours	0.094	0.282	98.78646
Photolytic	UV chamber at 250 Watts hours/m^2 for 21.8	0.099	0.281	98.96789
Neutral	Water at room temperature for 60 minutes	0.080	0.287	99.50937



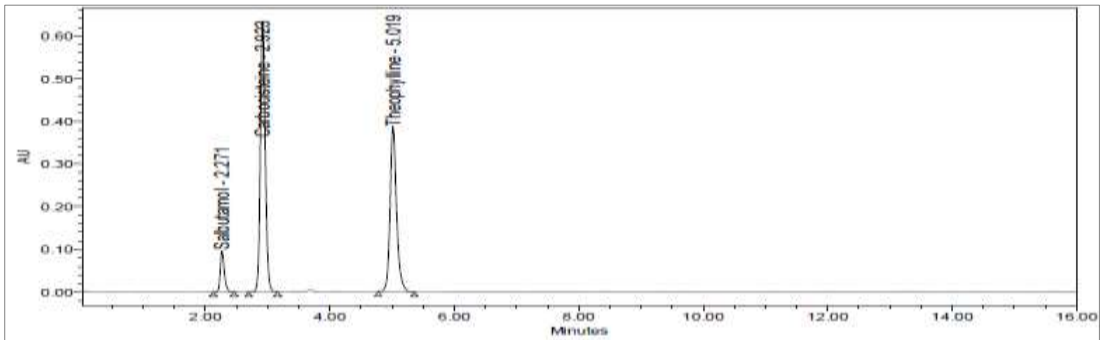
	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	peak 1	2.370	115101	1.56	2.757	2.938	963	0.7
2	peak 2	2.640	195691	10.01	0.804	1.405	2852	0.8
3	Carbocisteine	2.914	3342261	52.14	0.128	0.306	5827	1.0
4	Theophylline	5.013	2674505	36.29	0.069	0.287	12502	1.2

Figure 3: Acid degradation chromatogram of the drugs



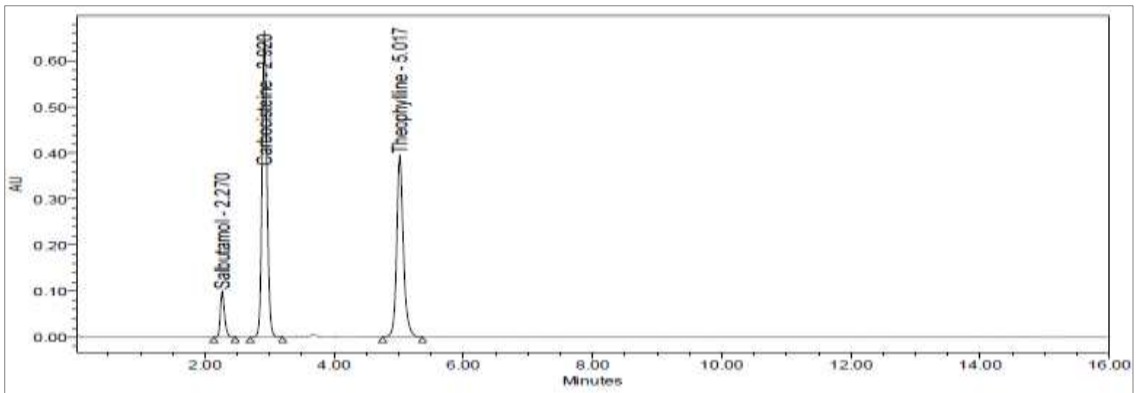
	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Salbutamol	2.275	196441	6.45	0.252	0.458	5809	1.2
2	Carbocisteine	2.925	335177	51.57	0.129	0.301	6734	1.1
3	Peak 1	3.687	724654	0.36	2.619	2.938	10457	1.0
4	Theophylline	5.015	2747293	41.62	0.099	0.281	11922	1.2

Figure 4: Alkali degradation chromatogram of the drugs



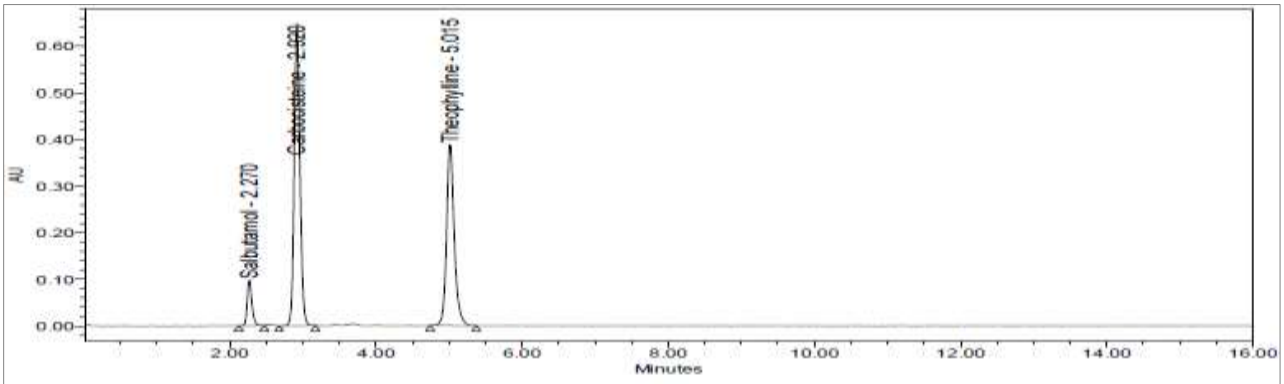
	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Salbutamol	2.271	199934	6.54	0.348	0.493	5619	1.2
2	Carbocisteine	2.923	3410613	51.88	0.130	0.304	6891	1.1
3	Theophylline	5.019	2753411	41.57	0.068	0.284	12003	1.2

Figure 5: Peroxide degradation chromatogram of the drugs



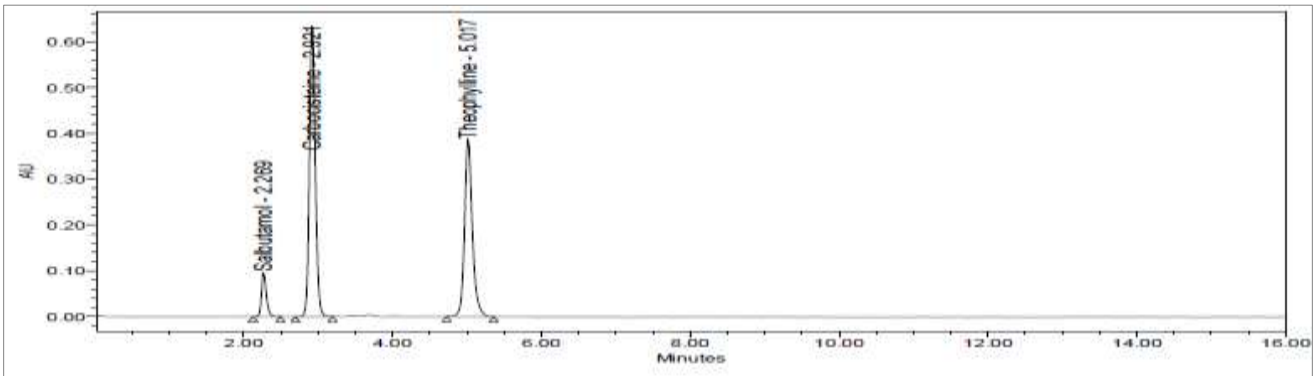
	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Salbutamol	2.270	201134	6.54	0.329	0.479	6262	1.2
2	Carbocisteine	2.920	3442413	51.83	0.149	0.306	6726	1.1
3	Theophylline	5.017	2789471	41.63	0.094	0.282	12672	1.2

Figure 6 : Thermal degradation chromatogram of the drugs



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Salbutamol	2.270	201331	6.54	0.326	0.482	5781	1.2
2	Carbocisteine	2.920	3450430	51.77	0.137	0.306	6728	1.1
3	Theophylline	5.015	2794594	41.69	0.099	0.281	11922	1.2

Figure 7 : Photolytic degradation chromatogram of the drugs



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Salbutamol	2.269	201943	6.54	0.350	0.509	5614	1.2
2	Carbocisteine	2.921	3455398	51.81	0.129	0.306	6889	1.1
3	Theophylline	5.017	2809884	41.65	0.080	0.287	11987	1.2

Figure 8 : Control chromatogram of the drugs

Accuracy

As the validation is performed using the commercial drug product and active pharmaceutical ingredients, it is not possible to obtain the placebo components to perform accuracy study. Hence, it was done by adding known quantities of the analyte (standard solution) to a pre qualified commercial tablet composite. As per the ICH Q2 (R1)[14] "in cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well characterized procedure".

An amount of tablet powder equivalent to the weight of one pre qualified commercial tablet was transferred into each of nine volumetric flasks. The amount present in each sample was calculated from the weight of the powder transferred into the individual flasks. The mixed standard solution

containing known concentrations of the three drugs was used to spike the above volumetric flasks containing pre qualified commercial tablet sample. Three sets of accuracy samples were prepared at approximately 50%, 100% and 150% drug levels (n=9, three at each level) and injected into the column. The recovery data is given in Tables 5-7.

Table 5: Percent recovery results of salbutamol

Accuracy	Amount added (µg/mL)	Amount Recovered (µg/mL)	Percentage Recovered	Mean % Recovery	SD	%RSD
50%	3.162	3.22327	101.932	101.391	0.647	0.64
	3.164	3.21389	101.567			
	3.199	3.22009	100.674			
100%	4.259	4.31044	101.210	100.797	0.362	0.36
	4.260	4.28713	100.642			
	4.263	4.28581	100.537			
150%	5.312	5.33996	100.523	100.050	0.625	0.62
	5.356	5.32087	99.341			
	5.357	5.37258	100.287			
Overall Mean			100.746			
Overall Standard deviation			0.757			
Over all % RSD			0.75			

Accuracy	Amount added (µg/mL)	Amount recovered (µg/mL)	Percentage Recovered	Mean % Recovery	SD	%RSD
50%	153.245	154.74893	100.981	101.047	0.162	0.16
	153.529	154.95409	100.928			
	153.350	155.23896	101.232			
100%	202.247	206.22050	101.965	101.414	0.507	0.50
	204.198	206.86894	101.308			
	204.428	206.40751	100.968			
150%	254.656	258.58069	101.545	101.128	0.537	0.53
	254.845	258.20002	101.317			
	256.893	258.23368	100.522			
Overall Mean			101.196			
Overall Standard deviation			0.413			
Over all % RSD			0.41			

Table 6 : Percent recovery results of carbocisteine

Accuracy	Amount added (µg/mL)	Amount Recovered (µg/mL)	Percentage Recovered	Mean % Recovery	SD	%RSD
50%	227.489	228.73605	100.548	100.367	0.158	0.16
	227.920	228.51800	100.262			
	227.649	228.30744	100.289			
100%	299.777	302.90276	101.043	101.003	0.328	0.32
	302.738	304.72837	100.657			
	300.073	304.00021	101.309			
150%	377.523	379.48406	100.520	100.654	0.923	0.92
	380.538	379.80028	99.806			
	374.601	380.73331	101.637			
Overall Mean			100.675			
Overall Standard deviation			0.568			
Over all % RSD			0.56			

Table 7 : Percent recovery results of theophylline

Linearity

Linearity of the method was demonstrated by preparing different concentrations of drug substance and analyzing as per the proposed method. A plot of the area of the peak as a function of analyte concentration was prepared and its regression equation computed. The linearity data of the three drugs are given in Table 8. The relevant graphs are presented in Figures 9-11.

Table 8: Linearity results of the drugs

Salbutamol **Concentration (µg/mL)**	Average Area	Carbocisteine Concentration (µg/mL)	Average Area	Theophylline Concentration (µg/mL)	Average Area
0.534	51892	38.222	947566	25.101	751277
1.068	104225	76.443	1861750	50.203	1498011
1.602	148421	114.665	2677927	75.304	2152368
2.136	199238	152.887	3505647	100.406	2864336
2.670	253666	191.109	4365577	125.507	3681294
3.204	296134	229.330	5314131	150.609	4360825
4.272	399102	305.774	7054440	200.812	5821661
5.340	496684	382.217	8821753	251.015	7283121
slope	92497.547	slope	22853.2788	slope	28938.306
y-intercept	2891.609	y-intercept	60325.150	y-intercept	10440.998
correlation coefficient	0.9999	correlation coefficient	0.9999	correlation coefficient	0.9999

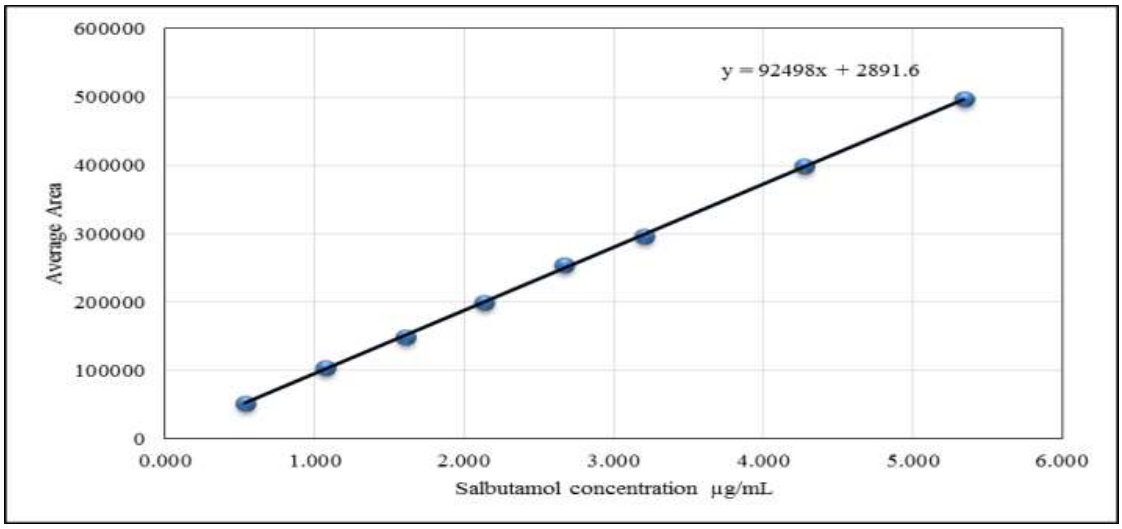


Figure 9: Linearity plot of salbutamol

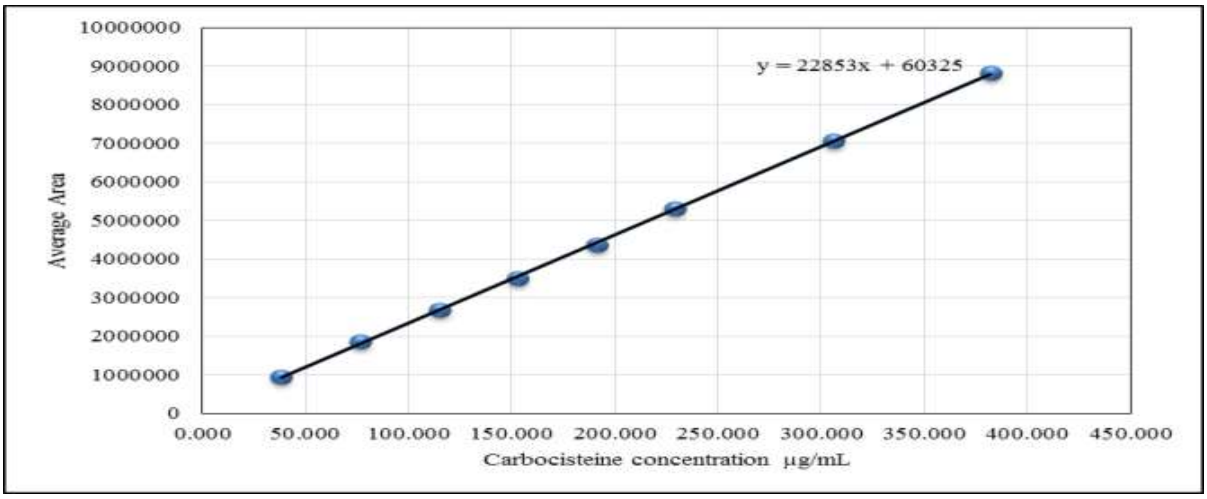


Figure 10: Linearity plot of carbocisteine

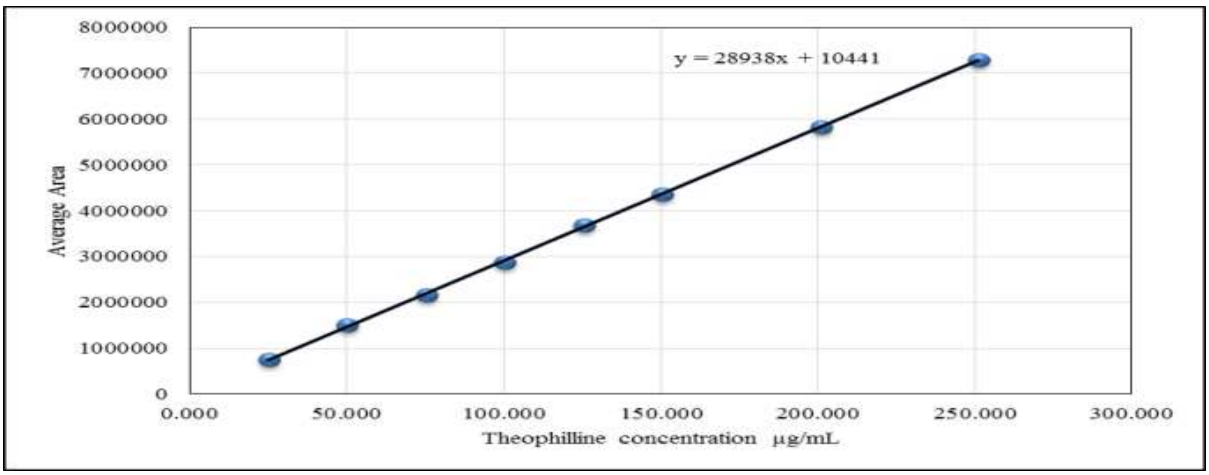


Figure 11 Linearity plot of theophylline

Robustness

The robustness of the proposed analytical method was established by studying the effect of small deliberate changes in the flow rate, column temperature and mobile phase composition. This test was carried out by injecting the working standard solution six times under the changed condition. The chromatograms showed that all the system suitability parameters such as peak area, theoretical plates, tailing factor and retention time of the analytes were not affected much and were within limits. Hence the developed stability indicating method was robust. The relevant results are presented in tables 9-11. The relevant chromatograms are shown in Figures 12-17.

Table 9 Robustness results of salbutamol

Parameters	Salbutamol		
	Rt (min)	USP Plate Count	USP Tailing
Flow rate 0.8 mL	2.493	6159	1.21
Flow rate 1.2 mL	2.171	5618	1.23
Temperature 25ºC	2.289	5948	1.22
Temperature 35ºC	2.298	6039	1.22
Mobile phase*	2.254	5682	1.21
Mobile phase**	2.286	5714	1.24

Table 10 : Robustness results of carbocisteine

Parameters	Carbocisteine			
	Rt (min)	USP Plate Count	USP Tailing	USP Resolution
Flow rate 0.8 mL	3.209	6811	1.14	4.9
Flow rate 1.2 mL	2.793	6552	1.12	4.8
Temperature 25 ºC	2.920	6682	1.13	4.8
Temperature 35 ºC	2.920	6673	1.13	4.6
Mobile phase*	2.916	6868	1.16	5.0
Mobile phase**	2.927	6384	1.11	4.7

Table 11: Robustness results of theophylline

Parameters	**Theophylline**			
	Rt (min)	**USP Plate Count**	**USP Tailing**	**USP Resolution**
Flow rate 0.8 mL	5.496	12775	1.22	12.7
Flow rate 1.2 mL	4.780	12099	1.19	12.3
Temperature 25 ºC	5.005	12357	1.20	12.5
Temperature 35 ºC	5.007	12389	1.20	12.4
Mobile phase*	4.684	11817	1.22	11.0
Mobile phase**	5.395	12510	1.20	14.2

* Buffer solution and acetonitrile in 45: 55 ratio.

** Buffer solution and acetonitrile in 55: 45 ratio.

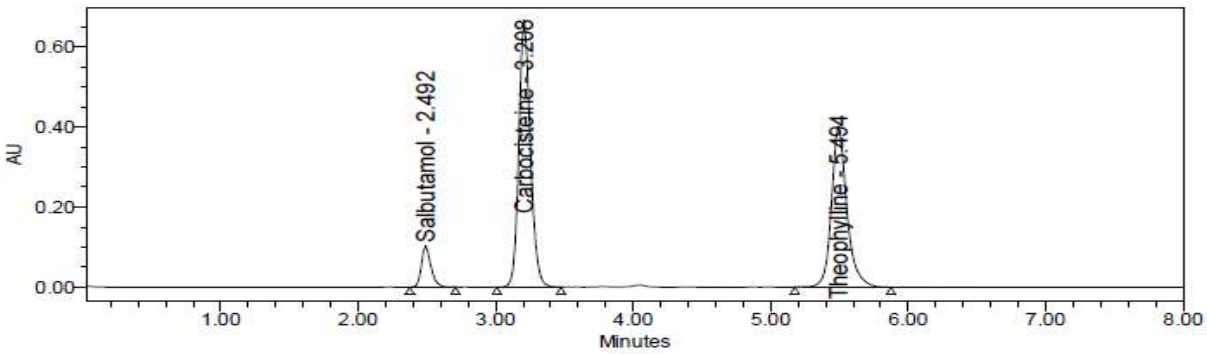


Figure 12 : Robustness chromatograms of the drugs at a flow rate 0.8 mL/min

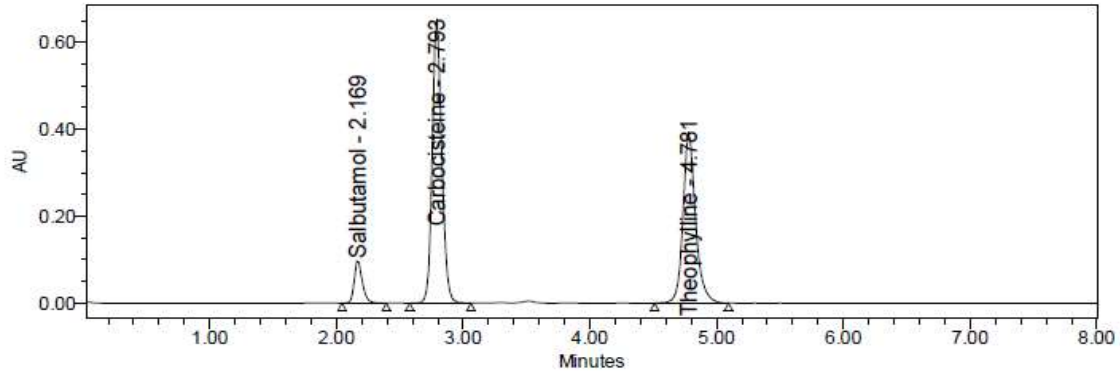


Figure 13: robustness chromatograms of the drugs at flow rate 1.2 mL/min

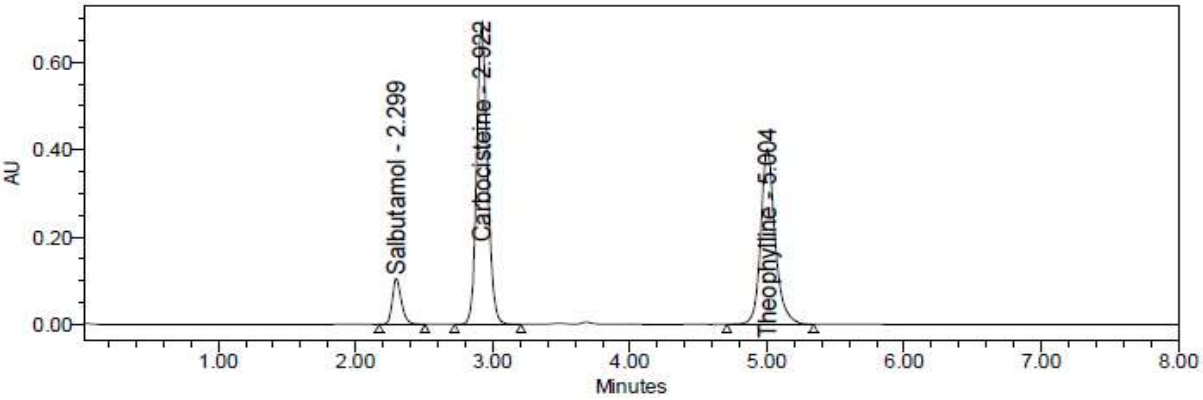


Figure 14 : Robustness chromatograms of the drugs at temperature 25ºC

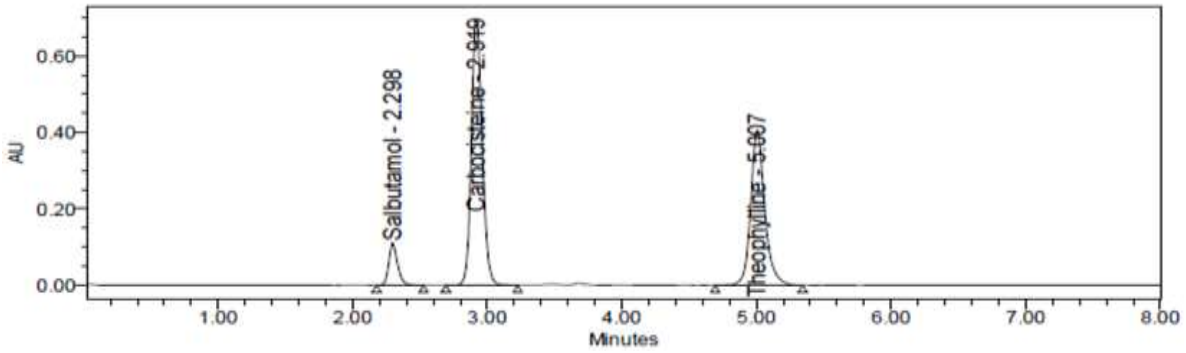


Figure 15 : Robustness chromatograms of the drugs at temperature 35ºC

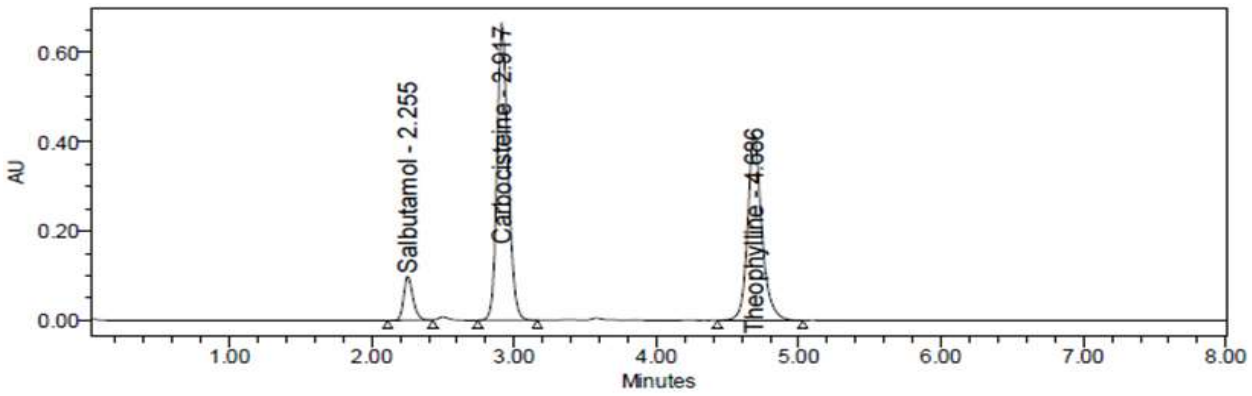


Figure 16 : Robustness chromatograms of the drugs at buffer solution and acetonitrile in 45:55 ratio.

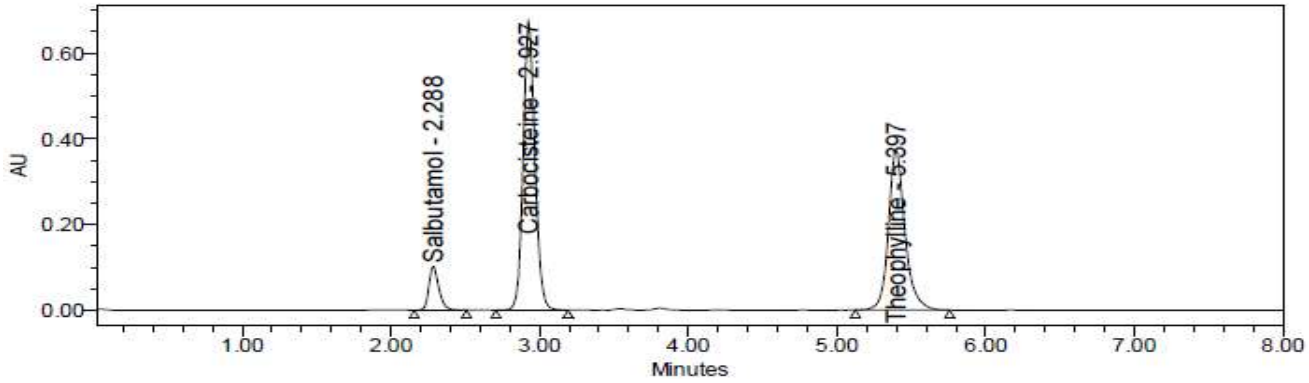


Figure 17 : Robustness chromatograms of the drugs at buffer solution and acetonitrile in 55: 45 ratio.

Stability of the Sample Solution

A study was conducted to establish the stability of the tablet sample solution. The sample solutions were stored in tightly closed Pyrex flasks at 25ºC. Aliquots from the sample solutions were taken at different time intervals and analyzed as per the method. The percent assay values of the drugs at different time points were calculated. Sample solutions are stable for 48 hours when stored at 25 ºC. The stability results are given in Table 12.

Table 12 Stability of the tablet sample solution

Time	Salbutamol		Carbocisteine		Theophylline	
	% Assay	Difference	% Assay	Difference	% Assay	Difference
Initial	99.8	- 0.9	100.1	+ 0.2	100.6	- 0.8
48 hours	98.9		100.3		99.8	

CONCLUSION

A novel stability indicating RP-HPLC method was developed and validated for simultaneous estimation of salbutamol, carbocisteine and theophylline in tablet dosage form. The proposed

method is capable of giving faster elution of the analytes with good resolution. Forced degradation studies were conducted to know the stability of the analytes under specified conditions. During forced degradation study salbutamol was completely degraded and two small peaks (Rt 2.370 minutes and Rt 2.640 minutes) appeared in the chromatogram. An extra peak was found during alkali degradation (Rt 2.387 minutes). Peaks found during acid and alkali degradations were homogeneous and not interfering with any other peaks. During heat, light, peroxide and control (water) degradation conditions the drugs were stable. The percentage recovery and precision studies showed that the method is accurate and precise. Thus, the present stability indicating RP-HPLC method was shown to be simple, specific, accurate, precise and robust and this method is suitable for simultaneous determination of salbutamol, carbocisteine and theophylline in tablet formulations and for monitoring the degradation pattern of these drugs.

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