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RESEARCH ARTICLE

A New RP-HPLC Method Development and Validation for Simultaneous Estimation of Salbutamol Sulphate and Theophylline in Pharmaceutical Syrup Dosage Form

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ABSTRACT

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of salbutamol sulphate and theophylline in a cough syrup formulation marketed as Theoasthalin. Chromatographic separation was done using Phenomenex LunaC18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of acetonitrile and water (40:60 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 230nm. The retention times of salbutamol sulphate and theophylline was found to be 2.1 and 3.5mins. The Proposed method has been validated for linearity, range, precision, accuracy and robustness were within the acceptance limit according to ICH Q2B guidelines. Quantification of the components in actual syrup formulations was calculated against the responses of freshly prepared external standard solutions. Linearity for salbutamol sulphate and theophylline was found in range of 0.25ppm-1.5ppm & 12.5ppm-75ppm and correlation coefficient was found to be 0.999 and 0.999, %RSD for intermediate precision was found to be 0.67 and 0.49 and for system precision 0.58 and 0.57 and for repeatability was 0.67 and 0.49.The percentage purity of salbutamol sulphate and theophylline was found to be 99.70and 99.54%v/v respectively.The method was found to be robust even by change in the mobile phase ±5% and in less flow condition.

KEYWORDS

Salbutamol sulphate and Theophylline, RP-HPLC, Method validation

INTRODUCTION

Salbutamol sulphate (SS) chemically,4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxyl-methyl) phenol; sulfuric acid is a short acting β2-adrenergic receptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease (Figure 1).

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Its empirical formula is $(C_{13}H_{21}NO_3)_2H_2SO_4$ and its molecular weight is 576.70g/mol. Theophylline (TP) chemically,1,3-dimethyl-7H-purine-2,6-dione also known as dimethyl-xanthine, is a methylxanthine drug used in respiratory diseases such as COPD and asthma (Figure 2). Its empirical formula is $C_7H_8N_4O_2$ and its molecular weight is 180.164g/mol.

Literature survey reveals that few UV, HPLC, GC and HPTLC based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in

pharmaceutical dosage forms. But there are no validated analytical methods for the simultaneous determination of SS and TP. Therefore, the present work was aimed to develop a simple, rapid, precise, and accurate isocratic reversed-phase HPLC method for simultaneous determination of SS and TP in the syrup dosage form. Validation of developed analytical method was carried out as per ICH guidelines.

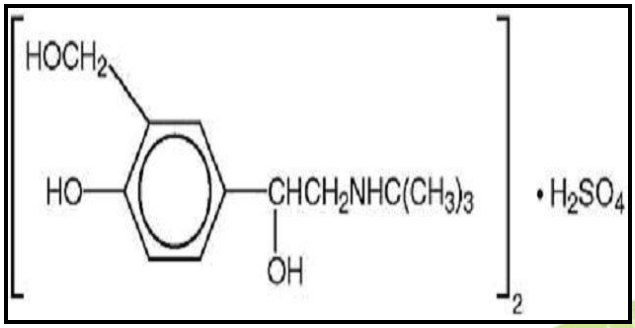

Figure: 1 Structure of Salbutamol sulphate

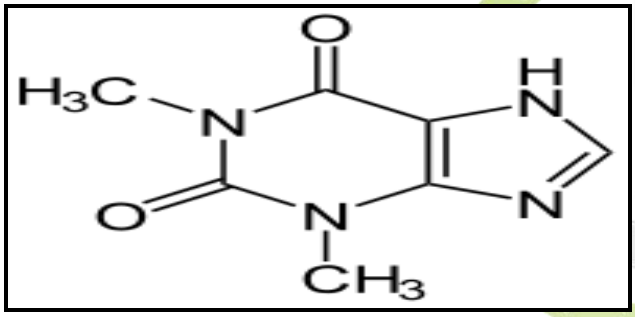

Figure: 2 Structure of Theophylline

MATERIALS AND METHOD

Reagents and Chemicals

Reference standards of SS and TP were procured as gift samples from Torrent Pharmaceutical, Gandhinagar, India. Theoasthalin1 (SS)/50(TP) mg syrup was purchased from local market. All reagents used, were at least of analytical grade except acetonitrile which was HPLC grade obtained from Rankem, RFCL Limited, New Delhi, India. HPLC grade water Milli-Q system (Millipore, Milford, MA, USA.

Equipment

A Waters e2695 gradient system with EMPOWER-2 software and 2489 UV/Vis detector is the most sensitive and versatile dual wave length absorbance detector was used. It was manufactured by the company Waters, Alliance, Japan. Intelligent LC pump with sampler programmed at 20µL capacity per injection was used.

Chromatographic Conditions

The column used was Phenomenex Luna C18 column (250 X4.6 mm ID), 5µm particle size was used for analytical separation. The mobile phase consisted of Acetonitrile and Water in the ratio of (40:60 v/v). The flow was adjusted to 1.0 mL/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 230nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL capacity. The mobile phase was filtered through 0.45 µm membrane filter prior to use.

Preparation of Analytical Solutions

Preparation of Mobile Phase

Mix a mixture of above 400 ml of Acetonitrile, 600ml Water (HPLC grade-70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 1mg of standard drug of Salbutamol Sulphate and 50mg Theophylline taken in to 100ml volumetric flask to which add 40ml of mobile phase [Acetonitrile-Water (40:60 v/v)] and sonicated for about 10 min then the final volume was made upto100 ml with the mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration.

Preparation of Standard Solution

Pipette out 10ml from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Preparation of Sample Solution (Marketed Formulation)

Accurately measured volumes of the cough syrup equivalent to 1 mg and 50mg each of SS,

and TP (5ml) was taken in a 100 ml volumetric flask and made up the volume with diluting solvent. Final solution had a concentration of 100 mg/ml of each component. Further filter the solution through 0.45µ membrane filter.

Quantification of Formulation (Assay)

1mg of standard drug of Salbutamol Sulphate and 50mg Theophylline was taken into 100ml volumetric flask to which add 40ml of mobile phase [Acetonitrile-Water (40:60,v/v)] and sonicated for about 10 min then the final volume was made upto100 ml with the mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration (Standard Solution). Accurately measured volumes of the cough syrup equivalent to 1 mg and 50mg each of SS, and TP (5ml) was taken in a 100 ml volumetric flask and made up the volume with solvent. Final solution had a concentration of 100 mg/ml of each component. The resulting solution was filtered through 0.45 µm membrane filter (Sample Solution). 20 µl of each solution was injected and chromatograms were recorded.

Validation of HPLC

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, robustness and system suitability.

Specificity

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced degradation studies are carried out by using 0.1M HCl, 0.1M NaOH, heat and U.V light.

Linearity

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of Salbutamol Sulphate and Theophylline. The solutions of six different concentration levels 0.25-1.5µg/ml (SS) and 12.5-75 µg/ml (TP) are injected in to the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-r^2 and standard error (Er) were determined.

Precision

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments). Precision was investigated using one batch of freshly manufactured cough cold syrup formulation. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage R.S.D. values. Injecting a freshly prepared standard solution six times and calculating mean and percentage R.S.D. values evaluated system repeatability.

Ruggedness

Ruggedness of the method was studied by using different sources of analysts, instruments and columns with same experimental conditions.

Accuracy

Accuracy of the method was studied by recovery investigation. This also provided the working range for the method. Placebo syrup solution containing all components apart from salbutamol sulphate and theophylline was used. Known amounts of each of these two were then ‘spiked’ in to separate 25 ml aliquots of placebo to give pseudo sample solutions of approximately 50, 100 and 150 % of stated label strength values. These samples were then

analyzed according to procedure and percentage recoveries calculated.

Robustness

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±5%.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of salbutamol sulphate and theophylline and it was calculated by injecting standards in six replicates at 6 minutes interval and the values were recorded. The system suitability parameters, theoretical plates (N) and asymmetry factor (As), were calculated, as reported by European pharmacopoeia. System suitability was performed daily during entire validation of this method.

RESULTS AND DISCUSSION

In the present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Salbutamol Sulphate and Theophylline. The method developed was proceeding with wavelength selection. The optimized wavelength was 230nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. The mobile phase consisted of Acetonitrile and Water in the ratio of (40:60 v/v) and the column used was Phenomenex Luna C18 column (250 X 4.6 mm ID), 5µm particle size. The flow rate was adjusted to 1ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 230nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL. (Figure 3) show example of the standard chromatogram obtained using the optimized chromatographic conditions. The retention times of Salbutamol Sulphate and Theophylline were found to be 2.152 and 3.555 min, respectively.

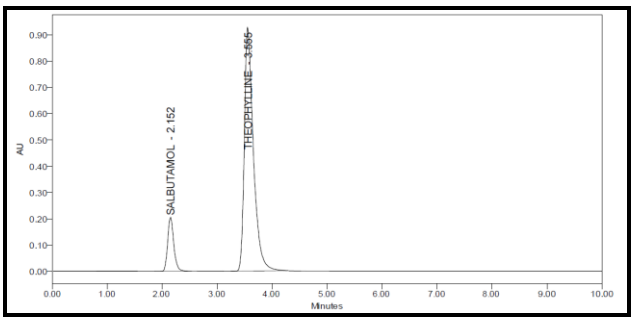

Figure: 3 Optimized HPLC Chromatogram for Salbutamol sulphate and Theophylline

These retention times did not vary to any considerable degree during and in between analyses (% R.S.D. less than 2% for the retention time of each peak) Resolution of the Salbutamol Sulphate from the Theophylline. The number of theoretical chromatographic plates for Salbutamol Sulphate and Theophylline were 2778.17 and 2227.58 respectively. The percentage purity of marketed formulation for Salbutamol sulphate and Theophylline was found to be 99.70% and 99.54%.

The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak.

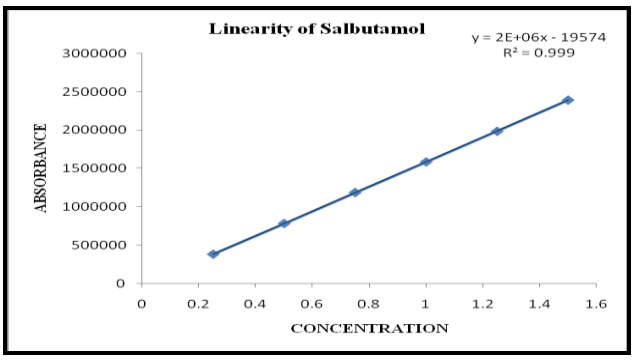

Figure: 4 Linearity curve for Salbutamol sulphate

The linearity was determined as linearity regression of the claimed analyte concentration of the range 0.25-1.5µg/ml (Salbutamol Sulphate) and 12.5-75 µg/ml (Theophylline).

Table: 1 Linearity results for Salbutomol sulphate and Theophylline

Sr. No	Salbutamol Sulphate		Theophylline	
	Conc^{n} (µg/ml)	Area	Conc^{n} (µg/ml)	Area
1	0.25	382569	12.5	2608322
2	0.5	783707	25	5311863
3	0.75	1187374	37.5	8017850
4	1.0	1586767	50	10688015
5	1.25	1986652	62.5	13316724
6	1.5	2393643	75	15986130

The calibration curves (Figure 4) and (Figure 5) obtained by plotting peak area versus concentration and the results presented in (Table 2) was linear and the correlation coefficient was found to be 0.999 and 0.999 for Salbutamol sulphate and Theophylline respectively

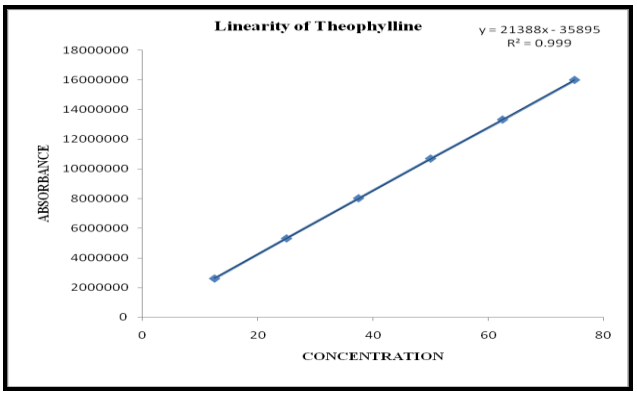

Figure: 5 Linearity curve for Theophylline

Accuracy of the method was studied by recovery investigation as described in section. The results of this investigation are shown in (Table 3) and (Table 4).

Table 2: Recovery data for Salbutamol sulphate

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	781054	0.5	0.4978	99.99%	
100%	1556391.67	1.0	0.9919	99.19%	99.52%
150%	2339173	1.5	1.4907	99.38%	

Table 3: Recovery data for Theophylline

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	5309951.7	25	24.98	99.55%	
100%	10626102	50	49.96	99.60%	99.38%
150%	15842121	75	74.9	99.00%	

Table: 4 Method precision results for Salbutamol and Theophylline

Sr. No	**Salbutamol**		**Theophylline**	
	Rt	Area	Rt	Area
1	2.152	1610760	3.555	10819019
2	2.151	1620592	3.554	10797218
3	2.151	1614178	3.555	10791450
4	2.152	1613891	3.555	10782336
5	2.151	1626981	3.555	10827893
6	2.151	1639682	3.557	10925505
Avg	2.15133333	1621014	3.555	10823903.5
SD	0.0005164	10834.11222	0.000983	52641.888
%RSD	**0.02**	**0.67**	**0.03**	**0.49**

Table: 5 System precision results for Salbutamol sulphate and Theophylline

Sr. No	**Salbutamol**		**Theophylline**	
	Rt	Area	Rt	Area
1	2.152	1603022	3.555	10770152
2	2.151	1620975	3.555	10759473
3	2.152	1608505	3.555	10740804
4	2.151	1603679	3.555	10734218
5	2.151	1611235	3.554	10723831
6	2.151	1626207	3.557	10890388
Avg	2.15133333	1612270.5	3.555167	10769811
SD	0.0005164	9429.678971	0.000983	61430.0115
%RSD	**0.02**	**0.58**	**0.03**	**0.57**

Table: 6 Ruggedness results for Salbutamol and Theophylline

Sr. No	**Salbutamol**		**Theophylline**	
	Rt	Area	Rt	Area
1	2.151	1620592	3.554	10797218
2	2.151	1639682	3.557	10925505
3	2.151	1626981	3.555	10827893
4	2.152	1613891	3.555	10782336
5	2.151	1614178	3.555	10791450
6	2.152	1610760	3.555	10819019
Avg	2.15133	1621014	3.555	10823904
St. dev	0.00052	10834.11	0.00098	52641.888
%RSD	0.02	0.67	0.03	0.49

Table 7: List of Robustness values for Salbutamol sulphate and Theophylline

Parameters	**Adjusted to**	**Average area**		**Rt**	
		SS	**TP**	**SS**	**TP**
Flow rate	0.8ml/min	1957000	13085184	2.619	4.323
	1.0ml/min	1608505	10740804	2.152	3.555
	1.2ml/min	1350100	9154651	1.848	3.075
Mobile phase Composition	ACN: Water (45:55)	1584724	10642381	2.081	2.948
	ACN: Water (40:60)	1608505	10740804	2.152	3.555
	ACN: Water (35:65)	3184474	20688988	2.22	3.765

Table 8: System suitability parameters for Salbutamol sulphate and Theophylline

Sr. No	**Parameters**	**Salbutamol Sulphate**	**Theophylline**
1	Average area	1608505	10740804
2	Retention time(min)	2.152	3.555
3	Tailing factor	1.21	1.16
4	USP Plate Count	2778.17	2227.58

For both the analytes at the different concentration levels evaluated the recovery values meet the acceptance criteria of 100±2%.

Method precision was investigated by the analysis of six separately prepared samples of the same batch of syrup. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage R.S.D. values. The results obtained are shown in (Table 5). In all instances the accepted criteria of % R.S.D. of less than 2% was met. Precision of the system was evaluated by injecting a freshly prepared standard solution six times. The results obtained are shown in (Table 6). The %R.S.D. results obtained 0.58 and 0.57 for Salbutamol Sulphate and Theophylline respectively, all well below the accepted maximum of 1%.

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The results obtained are shown in (Table 7).The value of percentage R.S.D. was below 2%, exhibits the ruggedness of developed analytical method.

The robustness was carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in (Table 8). Theoretical plates and tailing factor were observed and were found to be 2778.17 and 2227.58 (theoretical plates) and 1.21 and 1.16(tailing factor) for Salbutamol Sulphate and Theophylline respectively. The content of the analytes was not adversely affected by these changes as evident. From the low value of relative standard deviation indicating that the method is robust.

The system suitability parameters like theoretical plates(N), tailing factor(T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in (Table 8).

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Salbutamol Sulphate and Theophylline from API and pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The advantages lie in the simplicity of sample preparation and the cost economic reagents were used. In addition two compounds were eluted within 6min. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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