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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF DOXOFYLLINE AND SALBUTAMOL SULPHATE IN PHARMACEUTICAL DOSAGE FORM

Arun Kumar Dhote*, Alok Pal Jain, Harikishor Barange and Gaurav Gupta

RKDF College of Pharmacy, Sarvepalli Radhakrishnan University, Bhopal.

***Corresponding Author: Arun Kumar Dhote**

RKDF College of Pharmacy, Sarvepalli Radhakrishnan University, Bhopal.

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ABSTRACT

A new, simple, sensitive, precise, accurate and stability indicating Method has been developed and validated for simultaneous estimation of Doxofylline and Salbutamol Sulphate in Pharmaceutical oral solid tablet dosage form by RP-HPLC. The successful analysis was carried out of the marketed product by using C18, Hypersil BDS, 250mm× 4.6 mm, 5µm or equivalent at ambient temperature using Buffer: Acetonitrile (80:20v/v) as mobile phase composition. The flow rate was adjusted to 1.2mL/minute and the absorption maxima were observed on UV detector at 225 nm. Retention Time for Doxofylline 4.7±0.1min and Salbutamol 7.3±0.1min. The linearity was obtained in the concentration range of 64-96µg/mL and 16-24µg/mL for Doxofylline and Salbutamol respectively. Mean percentage recoveries were 98.87% Doxofylline and 99.14% for Salbutamol. Percentage relative standard deviation of percent assay values for replicate sample preparation was 0.73% for Doxofylline and 0.72% for Salbutamol. The method was robust with respect to change in flow rate, temperature and composition of mobile phase. The method was validated statistically and applied successfully for the determination of Doxofylline and Salbutamol. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for the routine determination of Doxofylline and Salbutamol in pharmaceutical oral solid tablet dosage form.

KEYWORDS: Doxofylline, Salbutamol, RP - HPLC, Analytical Method, Method Validation.

INTRODUCTION

Doxofylline (Chemical Name: 7-(1,3-Dioxolan-2-ylmetyl)theophylline) is used in the treatment of asthma and other obstructive lung disorders. It is used to manage the symptoms like wheezing, chest tightness and shortness of breath associated with lung disorders. Doxofylline is a bronchodilator, that helps relax the smooth muscles of the airways in your lungs.[1-3] This medicine is available on prescription as a tablet, suspension, syrup and injection forms. Doxofylline may show some side effects like vomiting, headache, nausea, and an upset stomach. It is recommended not to consume alcohol and caffeine containing substances like coffee, tea and dark chocolates because it may increase the risk of its side effects. Doxofylline mechanism of action is related to the inhibition of phosphodiesterase activities within the smooth muscle cells and thereby causes smooth muscle relaxation and thus suppressing asthma.[4]

The Chemical structure of the drug is given in Fig.1

Chemical Structure

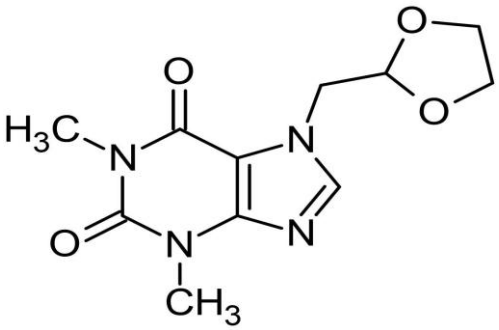


Fig. 1: Chemical structure of Doxofylline

Chemical Formula: $C_{11}H_{14}N_4O_4$ and Molecular Weight: 266.25g/mol

IUPAC Name of Doxofylline is 7-[(1,3-dioxolan-2-yl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione. Synonyms for Doxofylline- Doxofilina, Doxofylline, Doxophylline Ansimar, Maxivent and Ventax. Doxofylline is Soluble in water, acetone, ethyl acetate, hot methanol, hot ethanol, benzene or chloroform.[5-8]

Salbutamol sulphate is a β2-adrenergic receptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. Selective β2-adrenoceptor stimulant that causes the relaxation of the smooth muscles through the increase of the intracellular cyclic adenosine monophosphate (cAMP) due to this, bronchial and uterine muscles get relaxed, the peripheral vessels are dilated and heart rate increases.[9-13]

Salbutamol is generally used for acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic bronchopulmonary disorders such as chronic obstructive pulmonary disorder (COPD). It is also used prophylactically for exercise-induced asthma.[14]

Activation of the β-2 adreno-receptors opens ATPase channels and drives potassium from the extracellular to the intracellular space. This both decreases extracellular hyperkalaemia and increases intracellular potassium, so decreasing the chance of arrhythmia.[15-16]

The Chemical structure of the drug is given in Fig.2

Chemical Structure

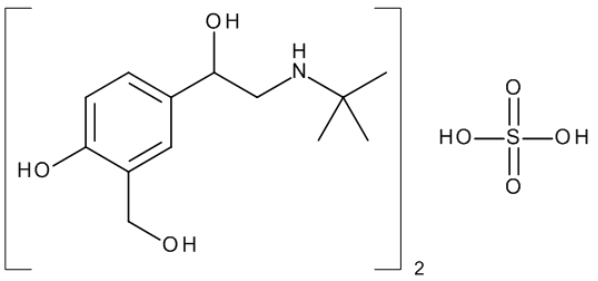


Fig. 2: Chemical structure of Salbutamol sulphate.

Chemical Formula: $C_{13}H_{21}NO_3.H_2SO_4$ and Molecular Weight: 239.31 g/mol, IUPAC Name for Salbutamol sulphate is bis(4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol); sulfuric acid. Synonyms for Salbutamol- Albuterol sulfate, Salbutamol sulfate, Ventolin, Asthavent, Ventorlin, Asthalin. Solubility of Salbutamol, Sparingly soluble in water; soluble in ethanol (96%); slightly soluble in ether.[17-20]

LITERATURE SURVEY

From Literature survey it has been concluded that many methods have been done for Doxofylline and Salbutamol sulphate in combination with other drugs like RP-HPLC, HPTLC and UV Spectrophotometry.[21-26] However, no method is reported for simultaneous estimation of Doxofylline and Salbutamol sulphate by RP-HPLC in any literature which is simple, sensitive, precise, accurate and stability indicating. In the present investigation, a specific RP-HPLC method is described for the simultaneous estimation of Doxofylline and Salbutamol sulphate in pharmaceutical tablet formulation.

The present study describes the development of a new rapid, simple, sensitive and reproducible RP-HPLC method for the analysis of Doxofylline and Salbutamol Sulphate that offer certain advantages in its simplicity and sensitivity and applicable in routine analysis. It also describes the development of validation work as per ICH guidelines recommended by the Food and Drug Administration (FDA) of the United States.[28-29]

MATERIALS AND METHODS

Chemicals and Reagents

Potassium Dihydrogen Orthophosphate (ACS grade or equivalent), Heptane-1-sulfonic acid sodium salt (AR grade or equivalent), Acetonitrile (HPLC grade or equivalent), Orthophosphoric acid (AR grade or equivalent) Purchased from SD Fine Chemicals Hyderabad, India. Doxofylline Reference standard and Salbutamol sulphate Reference standard (purchased from Spectrum Labs, Hyderabad. Water (Milli Q).

Instrumentation: Chromatographic separation was performed on a Shimadzu LC-2010CHT instrument (Software: LC Solution) equipped with UV-VIS/PDA detector. HPLC column use C18, hypersil BDS, 250mm× 4.6 mm, 5 μ (particle size) or equivalent. Other instruments are used Balance (Mettle Toledo), pH meter (Lab India), Sonicator (RT-60C Stainless Steel), Oven (RBE), UV-Chamber (Thermo lab). All the glass wares used were 'A' grade.

EXPERIMENTAL DESIGN

Selection of Detection Wavelength: Sensitivity of HPLC method with UV detection depends on proper selection of detection wavelength. An ideal wavelength is the one, at which both drugs gives good response. In the present study, standard solution of Doxofylline and Salbutamol Sulphate were scanned over the range of 200-400 nm. Wavelength of Doxofylline and Salbutamol Sulphate, 225nm was selected for analysis because of Salbutamol Sulphate is low dose concentration in this oral solid dosages formulation.

Method Development and Validation: Several numbers of trails takes for method development of Doxofylline and Salbutamol Sulphate. During the method development many changes done for make method easy and cost effective according to useful for routine analysis in laboratory. Finally select a method which is simple, accurate and precise.

Selection and Optimization of Chromatographic Conditions: Selection of proper HPLC method depends upon nature of drugs (ionic or neutral molecule), its molecular weight and its solubility. RP-HPLC method was selected initial for separation because of its simplicity, efficiency, reproducibility and easy to use for daily routine analysis in quality control department. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate, column temperature and solvent ratio were

studied. The condition that gave best resolution, symmetry, theoretical plates and capacity factor was selected for estimation.

ANALYTICAL METHODOLOGY

Preparation of Buffer Solution: Weigh and dissolve 2.8 gm of Heptane-1-sulfonic acid sodium salt and 2.5 gm of Potassium Dihydrogen Orthophosphate in 1000 mL of water. Adjust pH to 3.60 ± 0.05 with dilute Orthophosphoric acid solution.

Mobile Phase: Mix buffer solution and Acetonitrile in the ratio of 80:20 v/v. Filter through 0.45 µm membrane filter and sonicate.

Preparation of Diluent: Use Mobile phase as diluent without heptane salt.

Chromatographic Conditions for Developed RP-HPLC method

Stationary Phase/Column: C18, Hypersil BDS, 250mm× 4.6 mm, 5µm or equivalent, Flow rate: 1.2ml/min, Column Temperature: Ambient Temperature, Detection wavelength: 225 nm, Injection Volume: 20 mL.

Preparation of Standard solution

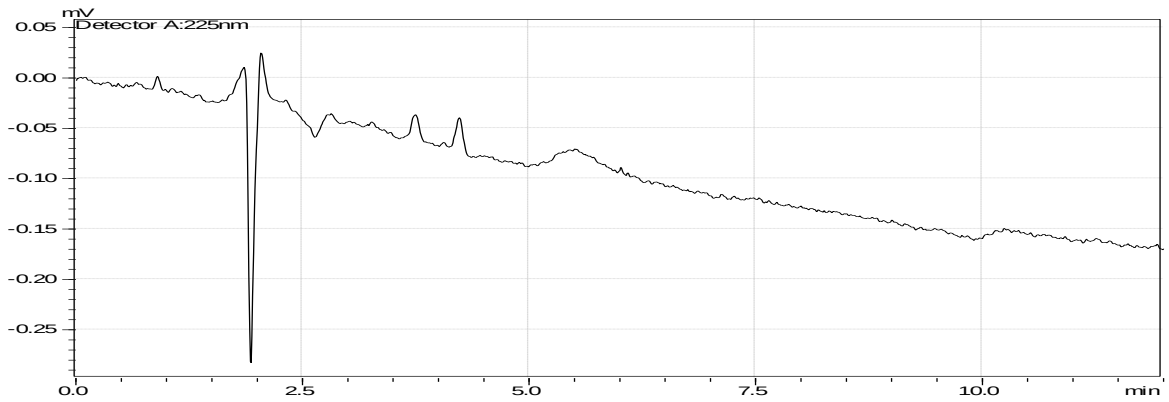
Weigh accurately and transfer equivalent to about 80 mg of Doxofylline reference/working standard and about 20 mg of Salbutamol sulphate reference/working standard as salbutamol into a 100 ml clean and dry volumetric flask, add about 60 ml of diluent, sonicate to dissolve and make up to volume with diluent. Filter the solution through 0.45 µm PVDF syringe filter. Further dilute 5 ml clear solution into a 50 ml clean and dry volumetric flask and make up to volume with diluent (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml).

Preparation of sample solution

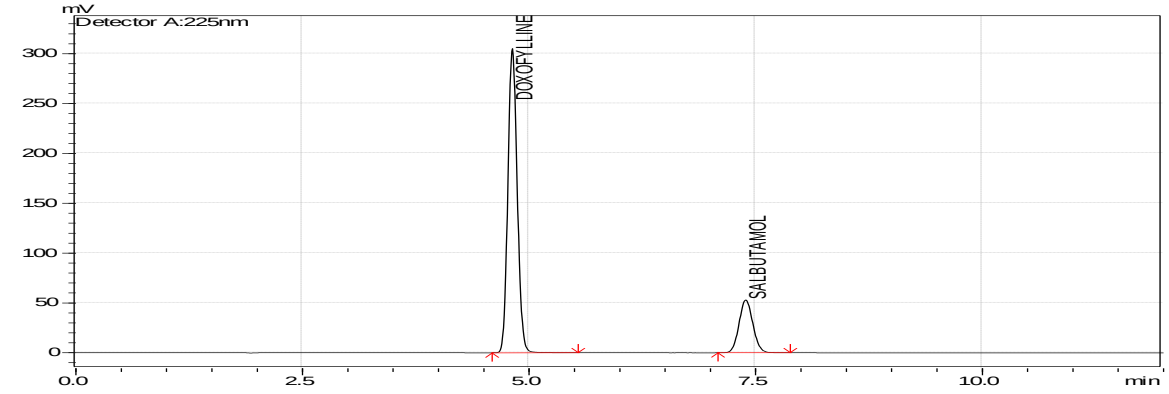
Weigh and crush 20 tablets in mortar and pestle to a fine powder. Weigh accurately sample equivalent to 400 mg of Doxofylline and 2 mg of Salbutamol into a 100 mL clean and dry volumetric flask. Add about 60 ml of diluent, sonicate about 15 minutes with intermittent shaking. Cool the solution to room temperature and make up to volume with diluent. Filter the solution through 0.45 µm PVDF syringe filter (For Salbutamol). Further dilute 2 ml into a 100 ml clean and dry volumetric flask and make up the volume with diluent (For Doxofylline). (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml)

Procedure: Inject the blank, standard solution and sample solution in to the chromatograph and record the chromatograms. Examine the chromatogram of blank solution and discard the corresponding peak in sample chromatograms. Calculate average area RSD, tailing factor and theoretical plates for standard solution. Record the chromatograms for sample solutions and calculate average area and calculate the assay of sample.

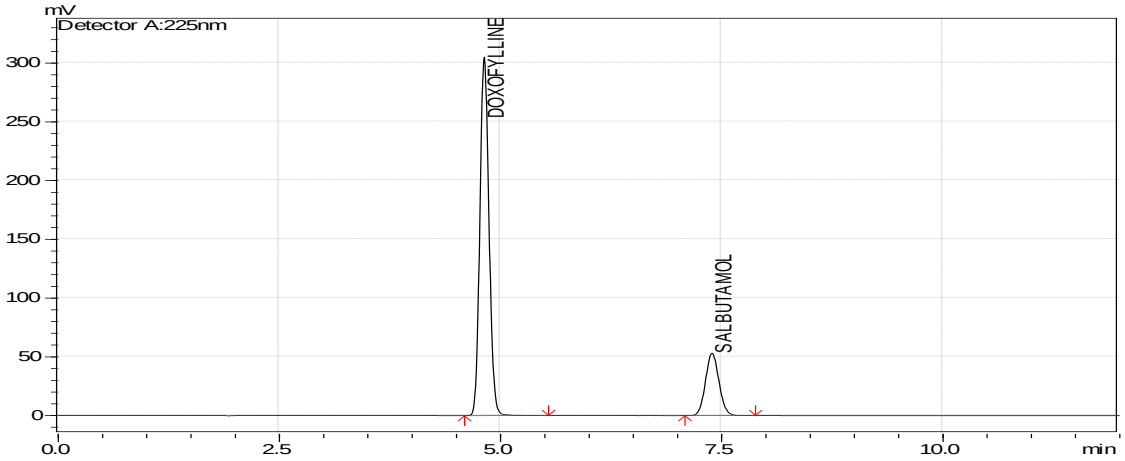
HPLC Chromatogram for Blank solution



HPLC Chromatogram for Standard solution



HPLC Chromatogram for Sample solution



VALIDATION OF PROPOSED ANALYTICAL METHOD

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines.[30-34]

Analysis of sample was carried out using the above method and the result are show in table No.1.

Table 1: Initial Assay results of sample.

Contents	Label claim	Assay % of label amount
Doxofylline IP	400 mg	99.52%
Salbutamol Sulphate IP equivalent Salbutanol	2 mg	99.87%

System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. In that the Theoretical Plates, tailing factor and Peak Retention time were calculated for the standard solutions table 2. The values obtained demonstrated the suitability of the system for the analysis of this drug combination.

Table 2: System Suitability Parameter.

Parameter	Doxofylline	Salbutamol
Precision of the method (n = 6) % RSD	0.78%	0.47%
Theoretical Plates	5620	8726
Tailing factor	1.10	1.12
Retention time	4.7 min	7.2 min
Peak purity	Pass	Pass

LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.

Preparation of stock solution: Weigh accurately and transfer equivalent to about 80 mg of Doxofylline reference/working standard and about 20 mg of Salbutamol sulphate reference/working standard as Salbutamol into a clean and dry 100 mL volumetric flask, add about 60 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and make up to volume with diluent. (The solution contains about 800 ppm of Doxofylline and 200 ppm of Salbutamol).

Table 3: Linearity Concentration.

Level (%)	Volume of stock to be taken (ml)	Dilute to volume (ml)
120	6.0	50
110	5.5	50
100	5.0	50
90	4.5	50
80	4.0	50

Procedure: Inject each linearity solution and measure the peak response of Doxofylline and Salbutamol. Plot a graph by taking respective concentration on the x-axis and the area count of each concentration on the y-axis.

The correlation coefficient, y-intercept, slope of the regression line shall be reported.

Acceptance criteria

The correlation coefficient should be not less than 0.998.

For Doxofylline

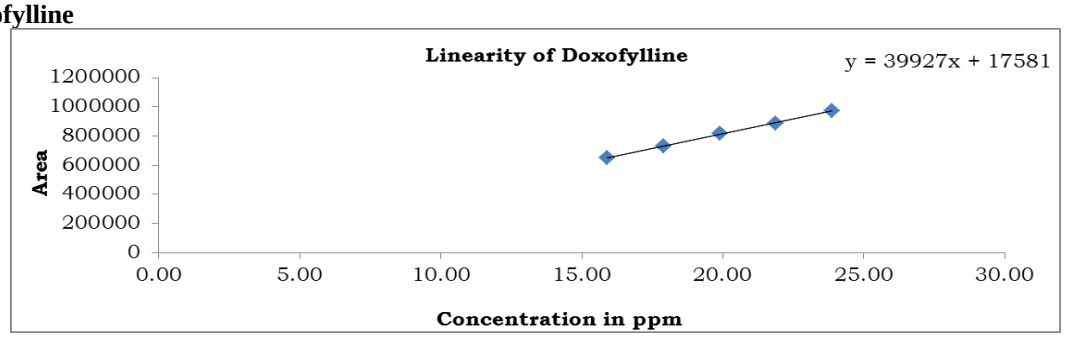


Fig.3 Linearity plot for Doxofylline.

Table 4: Linearity Level for Doxofylline.

Linearity Level (in %)	Concentration (in ppm)	Average Area (mV)
80	64.40	1211036
90	72.82	1361999
100	80.24	1516016
110	88.67	1651258
120	96.09	1816331
Slope		**6392.4**
Intercept		**17581.3**
Correlation coefficient		**0.99974**
Residual sum of squares		**32434309.3**

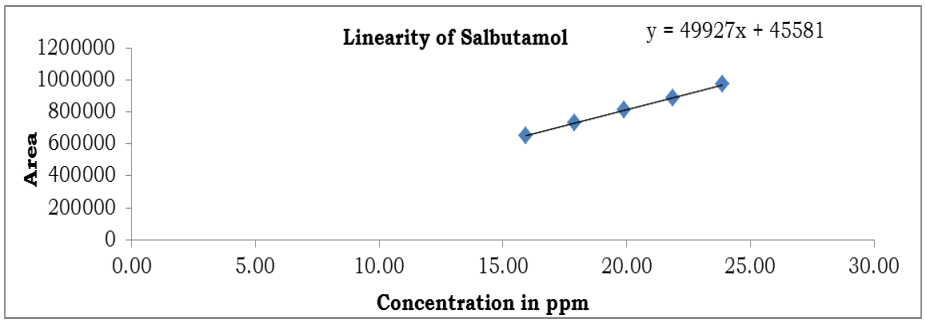


Fig. 4: Linearity plot for Salbutamol.

Table 5: Linearity Level for Doxofylline.

Linearity Level (in %)	Concentration (in ppm)	Average Area (mV)
80	16.25	652450
90	18.12	732694
100	20.02	814608
110	22.42	886692
120	24.35	972561
Slope		**29727.0**
Intercept		**11479.7**
Correlation coefficient		**0.99967**
Residual sum of squares		**148949843.9**

ACCURACY (AS RECOVERY)

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. Accuracy of the method shall be demonstrated by recovery studies.

Preparation of standard stock solution for Salbutamol: Weigh and dissolve accurately equivalent to about 40 mg of Salbutamol sulphate API as Salbutamol into a clean and dry 100 ml volumetric flask, add about 60 ml of diluent, sonicate to dissolve and make up to volume with diluent.

Preparation of 80% recovery solution: Weigh accurately and transfer equivalent to about 320 mg of Doxofylline API and 3.5 ml of standard stock solution of Salbutamol into a 100 mL clean & dry volumetric flask containing about 228 mg of placebo, add about 60 mL of diluent and sonicate about 15 minutes with intermittent shaking. Cool the solution to room temperature and make up to volume with diluent. Filter the solution through 0.45 µm PVDF syringe filter and collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains about 0.056 mg/mL of Doxofylline and 0.014 mg/mL of Salbutamol) (**Prepare the samples in triplicate).**

Preparation of 100% recovery solution: Weigh accurately and transfer equivalent to about 400 mg of Doxofylline API and 5 ml of standard stock solution of Salbutamol into a 100 mL clean & dry volumetric flask containing about 228 mg of placebo, add about 60 mL of diluent and sonicate about 15 minutes with intermittent shaking. Cool the solution to room temperature and make up to volume with diluent. Filter the solution through 0.45 µm PVDF syringe filter and collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains about 0.08 mg/mL of Doxofylline and 0.020 mg/mL of Salbutamol) (**Prepare the samples in triplicate).**

Preparation of 120% recovery solution: Weigh accurately and transfer equivalent to about 480 mg of Doxofylline API and 6.5 ml of standard stock solution of Salbutamol into a 100 mL clean & dry volumetric flask containing about 228 mg of placebo, add about 60 mL of diluent and sonicate about 15 minutes with intermittent shaking. Cool the solution to room temperature and make up to volume with diluent. Filter the solution through 0.45 µm PVDF syringe filter and collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains about 0.104 mg/mL of Doxofylline and 0.026 mg/mL of Salbutamol) (**Prepare the samples in triplicate).**

Acceptance Criteria

1. The % recovery should be between 98.0 % 102.0%.
2. The relative standard deviation at each level of recovery solution should not be more than 2.0%
3. The relative standard deviation of all recovery solution should not be more than 2.0%.

Recovery of Doxofylline

Table 6: Linearity Level for Doxofylline.

Level	Sample	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Average % recovery at each level	% RSD
80%	1	256.66	255.01	99.36	99.62	0.25
	2	255.92	254.12	99.29		
	3	256.13	256.72	100.23		
100%	1	320.15	318.15	99.38	99.79	
	2	321.02	322.02	100.31		
	3	320.36	319.36	99.69		
120%	1	384.79	385.79	100.26	99.82	
	2	383.21	381.21	99.48		
	3	384.55	383.55	99.74		

Recovery of Salbutamol

Table 7: Linearity Level for Salbutamol.

Level	Sample	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Average % recovery at each level	% RSD
80%	1	80.12	80.79	100.83	100.49	0.85%
	2	81.29	81.67	100.46		
	3	80.85	81.00	100.18		
100%	1	100.77	101.95	101.17	100.21	
	2	100.54	101.13	100.58		
	3	101.33	100.21	98.89		
120%	1	120.67	119.96	99.41	99.48	
	2	121.89	120.72	99.04		
	3	120.65	120.66	100.00		

PRECISION

The precision of an analytical procedure expresses the closeness of agreement between series of measurements obtained from multiple sampling of same homogeneous sample under prescribed condition. Precision of the analytical method shall be demonstrated by showing system precision, method precision and intermediate precision.

System precision

Prepare the standard solution as described under specificity and inject the solution six times calculate the mean area and percent relative standard deviation (% RSD) of area counts of Doxofylline and Salbutamol peak.

Table 8: System precision.

Injection	Peak Area of Doxofylline	Peak Area of Salbutamol
1	1489336	802202
2	1488063	802235
3	1489040	802485
4	1491112	805112
5	1491228	804950
6	1489957	804132
Mean	**1489789**	**803519**
SD	**1232.441**	**1372.114**
% RSD	**0.08**	**0.17**

Method precision

Prepare sample solution as described under specificity six times and analyze as per methodology. Calculate the % assay of Doxofylline and Salbutamol and % RSD for all six results.

Table 9: Method precision.

Sample Solution	Results of Doxofylline (% w/w)	Results of Salbutamol (% w/w)
1	98.9	99.5
2	99.1	99.3
3	99.8	100.9
4	100.1	100.6
5	99.3	100.4
6	98.0	99.2
Mean	**99.2**	**100.0**
SD	**0.73**	**0.72**
% RSD	**0.73**	**0.72**

Intermediate precision

Six sample solutions of Tablets shall be prepared as described under specificity on a different day, on a different instrument, by a different analyst and using the column of different serial number. Calculate the % assay of Doxofylline and Salbutamol and % RSD for all six results. Compare the results of this analysis with the result of method precision and calculate the overall % RSD.

Table 10: Intermediate precision.

Sample solution	Results of Doxofylline (% w/w)	Results of Salbutamol (% w/w)
1	98.0	98.9
2	100.0	99.2
3	98.3	99.4
4	98.4	98.3
5	98.3	99.2
6	99.4	99.8
Mean	**98.7**	**99.1**
SD	**0.80**	**0.50**
% RSD	**0.81**	**0.50**

Acceptance Criteria

System precision: The % RSD of area counts of Doxofylline and Salbutamol peak from the six injections of standard solution should not be more than 2.0%.

Method Precision: The % RSD of assay results of Doxofylline and Salbutamol should be less than 2.0%

Intermediate Precision: The overall % RSD of twelve assay results of Doxofylline and Salbutamol should be less than 2.0%.

ROBUSTNESS

Robustness is a measure of its capacity to remain unaffected by small but deliberate variations in the chromatographic method parameters and provides an

indication of its reliability. This was done by small deliberate changes in the chromatographic conditions at 3 different levels and retention time of Doxofylline and Salbutamol was noted. The factor selected were flow rate, pH and % Acetonitrile in the mobile phase. It was observed that there were no deliberate changes in the chromatogram, which demonstrated that the RP-HPLC method developed, are robust. Results describe in Table 8.

For Doxofylline

Table 11: Robustness parameter for Doxofylline.

Robustness Conditions	Asymmetry	Theoretical plates	% RSD	Results
As per method	1.12	5019	0.17	Passes
Column oven temperature 20°C	1.23	4845	0.27	Passes
Column oven temperature 30°C	1.24	5345	0.26	Passes
Flow rate 1.1 mL/minute	1.25	5373	0.26	Passes
Flow rate 1.3 mL/minute	1.23	4816	0.23	Passes
-2% Absolute	1.24	5027	0.17	Passes
+2% Absolute	1.23	5111	0.18	Passes
Mobile phase buffer pH 3.6	1.23	5108	0.06	Passes
Mobile phase buffer pH 4.0	1.23	5107	0.04	Passes

For Salbutamol

Table 12: Robustness parameter Salbutamol.

Robustness conditions	Asymmetry	Theoretical plates	% RSD	Results
As per method	1.12	8164	0.08	Passes
Column oven temperature 20°C	1.23	7951	0.27	Passes
Column oven temperature 30°C	1.25	8499	0.26	Passes
Flow rate 1.1 mL/minute	1.26	8699	0.26	Passes
Flow rate 1.3 mL/minute	1.23	7877	0.23	Passes
-2% Absolute	1.23	8445	0.17	Passes
+2% Absolute	1.23	7907	0.18	Passes
Mobile phase buffer pH 3.6	1.24	8103	0.06	Passes
Mobile phase buffer pH 4.0	1.22	8065	0.04	Passes

* Data taken from Method precision studies

**Data taken from Intermediate precision studies

FILTER COMPATIBILITY

Preparation of Standard solution

Prepared as mention in initial Standard solution Preparation.

Preparation of sample stock solution: Weigh accurately sample equivalent to 400 mg of Doxofylline and 2 mg of Salbutamol into a 100 mL clean and dry volumetric flask, add about 60 mL of diluent and sonicate about 15 minutes with intermittent shaking. Cool the solution to room temperature and make up to volume with diluent. (The solution contains Doxofylline 4.0 mg/ml and Salbutamol 0.02 mg/ml).

Preparation of sample solution using 0.45 µm PVDF syringe filter: Filter a portion of sample prepared from stock solution, through 0.45 µm PVDF syringe filter, collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml).

Preparation of sample solution using 0.45 µm Nylon syringe filter: Filter a portion of sample prepared from stock solution, through 0.45 µm Nylon syringe filter, collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml).

Preparation of sample solution using 0.45 µm PTFE syringe filter: Filter a portion of sample prepared from stock solution, through 0.45 µm PTFE syringe filter, collect the filtrate after discarding first few mL of filtrate (For Salbutamol). Transfer 2 mL of this filtrate into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml).

Sample preparation unfiltered (Centrifuged): Centrifuge a portion of sample stock solution at 3000 rpm for 5 minutes. Transfer 2 mL of this centrifuged sample into a 100 mL clean and dry volumetric flask and dilute to volume with diluent. (The solution contains Doxofylline 0.08 mg/ml and Salbutamol 0.02 mg/ml).

Table 13: Filter Compatibility Results.

Type of Filter	Doxofylline Assay (in %)	Salbutamol Acid Assay (in %)
0.45 µm PVDF syringe filter	100.1	100.6
0.45 µm NYLON syringe filter	99.8	100.1
0.45 µm PTFE syringe filter	99.6	100.3
Centrifuge (3000 rpm for 5 minutes)	98.3	98.9

Acceptance criteria: % Variation in assay results of 0.45 µm PVDF syringe filter, NYLON syringe filter, PTFE syringe filter and Centrifuge should not be more than 2% from assay results of PVDF syringe filter sample.

STABILITY OF ANALYTICAL SOLUTION

Stability of standard solution and sample solution at room temperature.

Standard preparation

Prepare the standard solution as described under specificity, keep the standard solution at room temperature and inject at different time interval.

Sample preparation

Prepare the sample solution as described under specificity, keep the sample solution at room temperature and inject at different time interval.

Stability of standard solution stored at 25^0C

Table 14: Solution stability for Standard solution.

Time (hrs)	Standard area		% Cumulative RSD	
	Doxofylline	Salbutamol	Doxofylline	Salbutamol
Initial	1489336	802202	-	-
After 4 Hrs	1492656	804999	0.16	0.25
After 8 Hrs	1496237	807192	0.23	0.31
After 12 Hrs	1508142	810715	0.55	0.45
After 16 Hrs	1511865	811003	0.66	0.47
After 20 Hrs	1510104	812281	0.65	0.49
After 24 Hrs	1511070	816392	0.64	0.59

Stability of sample solution stored at 25^0C

Table 14: Solution stability for sample solution.

Time (hrs)	Sample area		% Cumulative RSD	
	Doxofylline	Salbutamol	Doxofylline	Salbutamol
Initial	1525919	830611	-	-
After 4 Hrs	1529490	831923	0.17	0.11
After 8 Hrs	1532557	835624	0.22	0.31
After 12 Hrs	1559225	859012	0.99	1.59
After 16 Hrs	1534995	838178	0.86	1.38
After 20 Hrs	1537063	837256	0.77	1.23
After 24 Hrs	1535697	835907	0.70	1.13

Acceptance criteria

The cumulative RSD of area counts of Doxofylline and Salbutamol peak should be not more than 2.0 %.

CONCLUSION

Based on the results, this study is a typical example of the development of an assay method following ICH guidelines. A new isocratic RP-HPLC method has been developed and validated for determination of Doxofylline and Salbutamol in the pharmaceutical oral solid formulation. The results of the validation studies showed that the RP-HPLC method possesses significant linearity, precision, accuracy, specificity, sensitivity, high efficiency and resolution, and no interference from the excipients, as were demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of Doxofylline and Salbutamol in combined pharmaceutical oral solid formulations for QC, where method is economy and time are essential and to assure therapeutic efficacy.

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