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Simultaneous Estimation of Salbutamol and Theophylline in Bulk Drugs and Marketed Formulation using Simultaneous Equation Method

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Abstract: Salbutamol and Theophylline used for the treatment of respiratory diseases. A Simple, specific, accurate and precise Ultra-Violet spectroscopy method has been developed and validated for simultaneous determination of Salbutamol and Theophylline in bulk drugs and marketed formulation (tablets). The developed method involves solving of simultaneous equations using 0.1N NaOH as solvent where an absorbance maximum for Salbutamol and Theophylline was found to be at 242nm and 268nm respectively. Both the drugs obeyed Beer's law in the concentration range of 5- 25 µg/ ml & 3–19 µg/ ml. The developed method was validated as per ICH guidelines. The method showed good correlation coefficients (r^2) 0.999, indicated good linearity of calibration curve for both the drugs. The recovery of Salbutamol and Theophylline was found to be 100.16% and 98.72% respectively. The developed method was found to be sensitive showing LOD 0.3 µg/ml for Salbutamol and 0.25 µg/ml for Theophylline and LOQ 0.9 µg/ml for Salbutamol and 0.75 µg/ml for Theophylline. The %RSD values for Robustness and Ruggedness was found to be within the acceptable limits indicating the method was robust. The methods were found to be better than previously reported methods because of lack of any extraction procedure, use of cheap solvent, no interferences during method development and time consuming and can be successfully applied for estimation of Salbutamol and Theophylline in Pharmaceutical dosage forms without any interference in Quality control.
Keywords : Simultaneous Estimation method, UV spectroscopy, Salbutamol, Theophylline, ICH Guidelines, Method development and Validation.

Introduction

Salbutamol IUPAC Name is 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol and Molecular formula $C_{13}H_{21}NO_3$ and Molecular weight 239.311 g/mol[1]. It is a white crystalline powder. It is freely soluble in water, practically insoluble or very slightly soluble in alcohol and in methylene [2]. Salbutamol is official in BP, IP, USP and Martindale. It is Anti-asthmatic drug. Salbutamol is a short-acting Beta-adrenergic receptor agonist used for the relief of bronchospam for asthma and chronic obstructive pulmonary disease [3].

Theophylline IUPAC Name is 1,3-Dimethyl-7H -purine-2,6-dione and Molecular formula $C_7H_8N_4O_2$ and Molecular weight 180.164 g/mol[1]. It is a white powder. It is freely soluble in methanol, sparingly soluble in water [2]. Theophylline is official in BP 2010, IP 2010, USP and Martindale. It is used as Anti-asthmatic drug. The structures are shown in Fig:1&2.

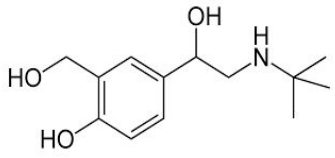

Fig-1: Structure of salbutamol

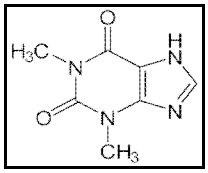

Fig-2: Structure of theophylline

Literature survey reveals information that the methods like UV [4,5] and UV differential [6] and RP-HPLC [7-10] are developed individually or with other combination of drugs. Hence a new analytical method was developed with less economical, precise and accurate which is used for the routine analysis in small scale industries.

Materials and Methods

Chemicals and Reagents:

Salbutamol (99.93%) and Theophylline (99.05%) standard drugs obtained as Gift samples from Koch organics pvt Ltd. The marketed formulation (tablets) obtained from local pharmacy with brand name Theo-Asthaline forte with label claim salbutamol(4mg):theophylline**(**200mg**)** manufacturer Mfg by CIPLA Ltd, Goa. All the chemicals and reagents used are of analytical grade.

Instrumentation:

A Shimadzu UV – 1800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV – probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Analytical balance Shimadzu (220h) was used for weighing purpose, volumetric glassware was used of class A.

Proper wavelength selection of method depends upon the nature of sample and its solubility. 1mg of standard drug sample was taken and its solubility was checked in various solvents like Distilled water, Acetonitrile, 0.1N NaOH, 0.1NHCl, Phosphate Buffer and Acetone. These studies are carried out at 25 ± 20 °C. The drugs are freely soluble in 0.1N NaOH and it is selected as solvent for the development of new method.

Preparation of Standard Stock Solutions

Preparation of Salbutamol standard stock solution (1000 µg/ml):

Accurately weighed 10mg of pure Salbutamol (API) was taken in a 10ml of volumetric flask, dissolved in 0.1N NaOH and made up to the mark to get a concentration of 1000 µg/ml. From above stock solution 1mlwas transferred into 10 ml volumetric flask and diluted to10ml with 0.1N NaOH to get a concentration of 100 µg/ml. It was taken as working standard concentration.

Preparation of Theophylline standard stock solution (100µg/ml):

Accurately weighed 10mg of Pure Theophylline (API) was taken and dissolved in 10ml of diluent in a 10ml of volumetric flask to get a concentration of 1000µ g/ml. From above stock solution 1ml was transferred into 10ml volumetric flask and diluted to 10ml with 0.1N NaOH to get a concentration of 100 µg/ml it was taken as working standard concentration.

Procedure for Selection of Wave Length:

From working standard solution 1ml of Salbutamol and Theophylline was transferred into two 10ml volumetric flasks, diluted separately and make up to mark with 0.1N NaOH.

These two solutions were taken and scanned between 200nm to 400nm on scan/spectrum mode using 0.1N NaOH as blank. As per spectra recorded, Salbutamol shows λ max at 242nm (λ_1) and Theophylline shows λ max at 268nm (λ_2) and respectively (Fig: 3&4)

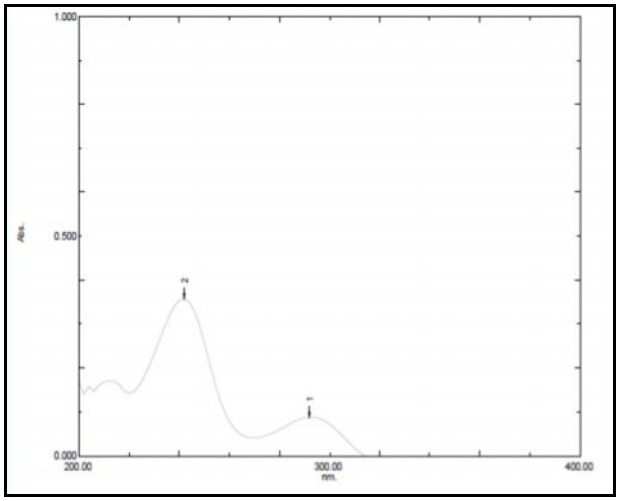

Fig-3: UV Spectrum of Salbutamol

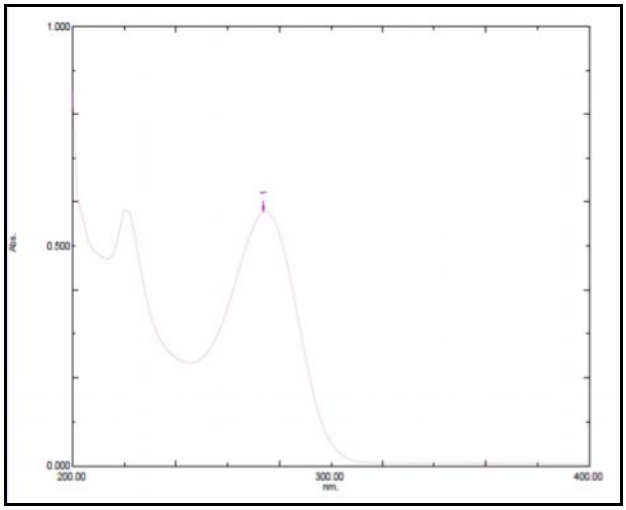

Fig-4: UV Spectrum of Theophylline

Plotting of calibration curve:

The calibration curves were plotted over a concentration range of 5-25 µg/ml for Salbutamol and 3-19 µg/ml for Theophylline. Accurately measured standard solutions of Salbutamol and Theophylline were transferred to a series of 10 ml of volumetric flask and diluted to the mark with 0.1N NaOH. The absorbances of the both solutions (Salbutamol and Theophylline) were measured at both wavelengths i.e. 242 nm and 268 nm against 0.1N NaOH as blank. Calibration curves was plotted .At both wavelengths, two equations were solved using the absorptivity values. The concentration of unknown sample were prepared from the tablet dosage form and calculated using the following simultaneous equations:

$Cx = (A_2\ ay_1 - A_1\ ay_2)\ /(ax_2\ ay_1 - ax_1\ ay_2)$

$Cy=(A_1\ ax_2 - A_2\ ax_1)/(ax2\ ay1 - ax1\ ay2)$

Where A_1, A_2 are absorbance of formulation at 242 nm and 268nm , ax_1 and ax_2 are absorptivity of Salbutamol at 242 nm and 268 nm , ay_1 and ay_2 are absorptivity of Theophylline at 242 nm and 268 nm , Cx and Cy are concentrations of Salbutamol (0.56µ/ml) and Theophylline (9.80µ/ml) respectively (Fig:5&6).

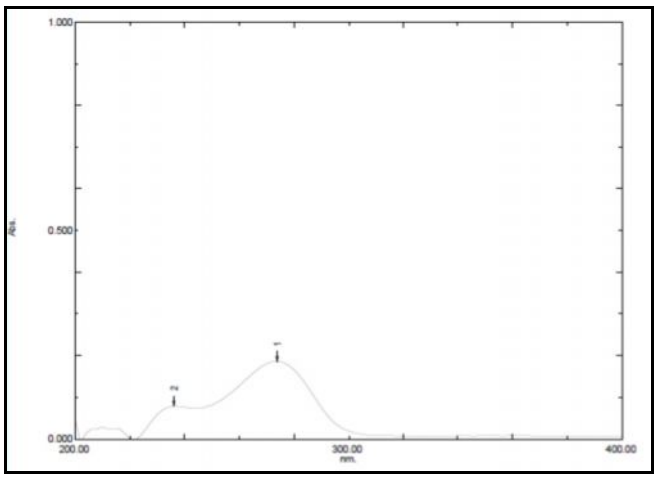

Fig-5: UV Spectrum of Theo-Asthaline forte

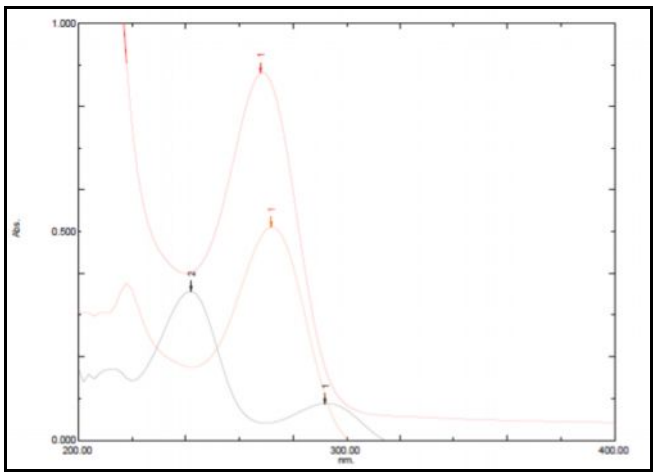

Fig-6: Overlay spectrum of Sabutamol, Theophylline & Theo-Asthaline forte

Analytical Method Validation

Validation of the developed method was carried out as per ICH guidelines. Parameters such as specificity, linearity, accuracy, precision, LOD and LOQ were taken up as tests for method validation.

Specificity: The UV graphs obtained depicts there is no interference of excipients, solvent with the absorbance of analyte which indicate that the method is specific for the analysis of analytes in the dosage form.

Linearity and range:

The linear response of samples was determined over a concentration range of 5-25 µg/ml for Salbutamol and 3-19 µg/ml for Theophylline. Accurately measured working standard solutions of Salbutamol and Theophylline were transferred to a series of 10 ml of volumetric flask and diluted up to the mark with 0.1N NaOH. The absorbance of the solutions was measured at 242 nm and 268 nm against 0.1N NaOH as blank. The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient (r^2) and regression line equations for Salbutamol and Theophyllin were calculated. The results are shown in the table-1&2, Fig:7&8.

Table.1: Linearity Results of Salbutamol

S.No	Concentration	Absorbance
1	5µg/mL	0.191
2	10µg/mL	0.372
3	15µg/mL	0.527
4	20µg/mL	0.701
5	25µg/mL	0.859
Regression equation		y = 0.034x + 0.014
Slope		0.034
Intercept		0.014
R^2		0.998

Table-2: Linearity Results of Theophylline

S.No	Concentration	Absorbance
1.	3µg/mL	0.176
2	5µg/mL	0.294
3	7µg/mL	0.369
4	9µg/mL	0.478
5	11µg/mL	0.559
6	13µg/mL	0.650
7	15µg/mL	0.767
8	17µg/mL	0.887
9	19µg/mL	0.947
Regression equation		y = 0.049.x + 0.022
Slope		0.049
Intercept		0.022
R^2		0.997

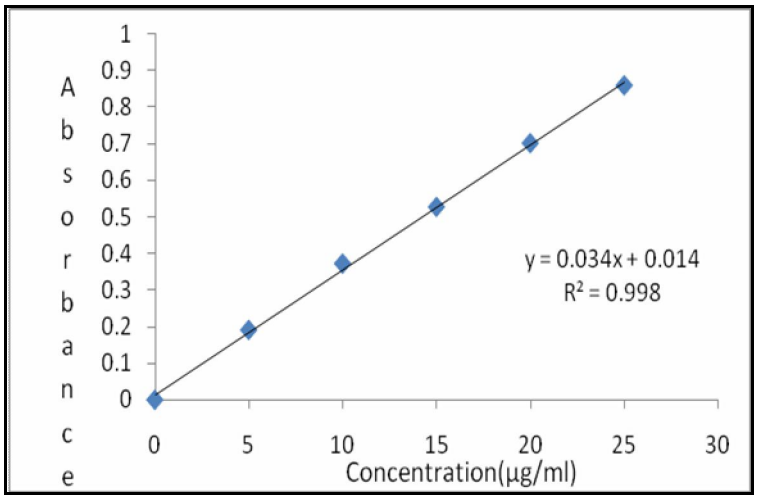

Fig-7: Calibration Curve of Salbutamol

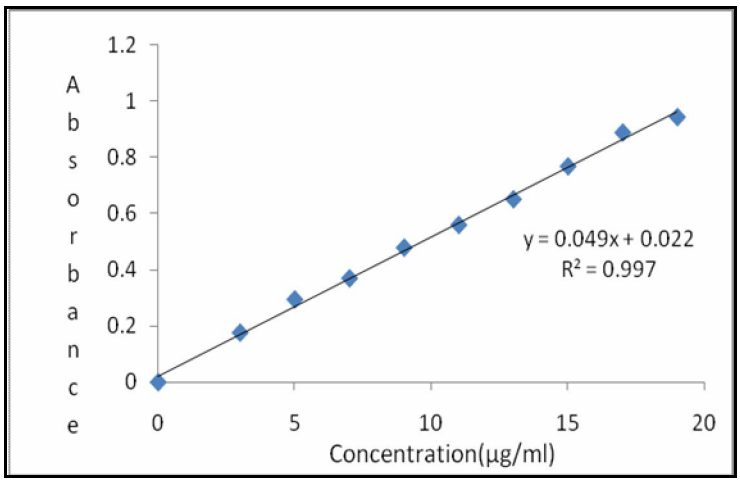

Fig-8: Calibration curve of Theophylline

Accuracy:

Accuracy is determined by calculating percentage recovery. Recovery studies was carried by standard addition method, where to the formulation (pre analyzed sample), the standard of the Salbutamol and Theophylline were added at three concentration level of 50%, 100%, 50% of assay concentration and recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug. The results are shown in the table-3&4.

Table-3: Recovery Studies of Proposed Method for Salbutamol

S.no	Level of recovery	Pre analyzed conc.(µg/ml)	Amount added(µg/ml)	Amount* found(µg/ml)	% Recovery
1	50	15	7.5	22.55	100.22%
2	100	15	15	30.23	100.06%
3	150	15	22.5	37.32	99.52%

*Mean of three determinations

Table-4: Recovery Studies of Proposed Method for Theophylline

S.no	Level of recovery	Pre analyzed conc. (µg/ml)	Amount added(µg/ml)	Amount* found(µg/ml)	% Recovery
1	50	15	5.5	20.12	99.14%
2	100	15	11	25.64	98.61%
3	150	15	16.5	31.32	99.42%

*Mean of three determinations

Precision:

a) **Method precision:** Variation of results on same day analysed by actual determination of absorbance fixed concentration of the sample preparation consisting of 10µg/ml for Salbutamol and 11µg/ml of Theophylline for six preparations on same day within the Beer's range and finding out the absorbance at two wave lengths. The results are shown in the table-5.

Table-5: Method Precision Studies of Proposed Method for Salbutamol And Theophylline

S.No	Salbutamol			Theophylline		
	Concentration (µg/mL)	Mean±SD	%RSD	Concentration (µg/mL)	Mean±SD	%RSD
1	10	0.370±0.037		11µg/ml	0.564±0.004	
2	10	0.370±0.024		11µg/ml	0.564±0.0036	
3	10	0.370±0.022	1.16%	11µg/ml	0.564±0.0004	0.36%
4	10	0.370±0.019		11µg/ml	0.564±0.005	
5	10	0.370±0.024		11µg/ml	0.564±0.006	
6	10	0.370±0.027		11µg/ml	0.564±0.006	

b) System precision: The variation of results on same day analysed by actual determination of absorbance of fixed concentration of the standard preparation (API) consisting of 10µg/ml for Salbutamol and 11µg/ml of Theophylline for six times on the same day within the Beer's range and finding out the absorbance at two wave lengths for the two drugs. The results are shown in the table-6.

Table-6: System Precision Studies of Proposed Method for Salbutamol and Theophylline

S.No	Salbutamol			Theophylline		
	Concentration (µg/mL)	Mean±SD	%RSD	Concentration (µg/mL)	Mean±SD	%RSD
1	10	0.370±0.001		11µg/ml	0.574±0.0004	
2	10	0.370±0.0008		11µg/ml	0.574±0.0004	
3	10	0.370±0.004	0.54%	11µg/ml	0.574±0.0008	0.5%
4	10	0.370±0.001		11µg/ml	0.574±0.0012	
5	10	0.370±0.004		11µg/ml	0.574±0.008	
6	10	0.370±0.002		11µg/ml	0.574±0.008	

Table 7: Limit of Detection and Limit of Quantification

S.No	Parameter	Salbutamol	Theophylline
1	LOD	0.3µg/mL	0.25 µg/mL
2	LOQ	0.9µg/mL	0.75 µg/mL

Limit of detection (LOD) & Limit of quantification (LOQ):

The LOD and LOQ of developed method were studied as per ICH guidelines. LOD and LOQ are calculated from the calibration curves. The results are shown in the table-7.

Robustness and Ruggedness

Ruggedness was carried out by two analysts and Robustness was carried out by changing the maximum wavelength. The results were indicated by low values of the % RSD. The results are shown in the table-8.

Table 8: Ruggedness and Robustness Results of Salbutamol and Theophylline

Parameters		% RSD	
		Salbutamol	Theophylline
Robustness	Change in λmax (± 2nm)	0.354	0.656
Ruggedness	1st analyst	0.290	0.552
	2nd analyst	0.302	0.469

Table 9: Results of Marketed Formulation Analysis

Brand name	Drugs	Label claim(mg)	Test conc(µg/mL)	Amount found (µg/mL)	%Assay	%RSD
Theo-Asthaline forte	Theophylline	200	10	9.80	98.00	0.5
	Salbutamol	4	10	0.56	99.8	0.8

Estimation of Salbutamol and Theophylline in tablet formulation (Assay):

From calibration curve the concentration 100% is selected to perform assay.20 tablets were weighed and powdered tablet equivalent to 100mg Theophylline (0.2mg Salbutamol) was weighed and taken into 100ml volumetric flask then 50ml 0.1N NaOH was added and shaken well to dissolve tablet powder completely and volume was made up to mark with diluent then solution as sonicated for about 20min and filtered with 0.45 µ whattman filter paper to remove particles if any. From the above stock solution 1ml of solution was withdrawn and taken in 10ml volumetric flask and volume was made up to mark with diluent. From this again the further dilution were prepared to obtain concentration of 10µg/ml solution. The concentration of Salbutamol and Theophylline was obtained from simultaneous equation. The results are shown in the table-9.

Results And Discussion:

The conditions tested for method development indicates that all the Validation parameters are according to ICH guidelines were achieved by using Shimadzu UV – 1800 with detection wavelengths of 242 nm for Salbutamol and 268nm for Theophylline. The optical characteristics of the proposed method results were shown in the table-10.

Table 10: Optical Characteristics of The Proposed Method

S.NO.	Parameter	Salbutamol	Theophylline
1	λmax	242nm	268nm
2	Linearity(µg/mL)	5-25	3-19
3	Regression equation	y=0.034x+0.014	Y=0.049x+0.022
4	Slope	0.034	0.049
5	Intercept	0.014	0.022
6	Correlation coefficient	0.998	0.997

Table 11: Summary of Validation Parameters

S.NO.	Parameter	Salbutamol	Theophylline
1	Linearity(µg/ml)	5-25	3-19
2	Precision indicated by		
	%RSD	1.16%	0.36%
	Method precision	0.54%	0.50%
3	System precision	99.93%	99.04%
	Accuracy indicated by		
4	%recovery	0.3	0.25
5	LOD(µg/mL)	0.9	0.75
6	LOQ(µg/mL)	0.354	0.656
7	Robustness	0.290, 0.302	0.552, 0.469
	Ruggedness		

To validate the UV method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range of 5-25µg/mL for Salbutamol and 3-19µg/mL for Theophylline and correlation coefficient was found to be 0.998 for Salbutamol

and 0.997 for Theophylline respectively. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding different amounts to preanalysed concentration and summarized in Table-3&4. The precision (expressed as the % RSD) was determined for Salbutamol and Theophylline repeated analysis and the values are presented in Table-5&6.The %RSD values for Robustness and Ruggedness was found to be within the acceptable limits indicating the methods were robust and the results were presented in Table-8. The summary of the all the validation parameter were shown in the Table-11.The methods were found to be better than previously reported methods because of lack of any extraction procedure, use of cheap solvent, no interferences during method development and time consuming and can be successfully applied for estimation of Salbutamol and Theophylline in Pharmaceutical dosage forms without any interference in Quality control.

Conclusion

The developed UV spectroscopy method for the determination of Salbutamol and Theophylline was validated as per ICH guidelines. All the validation parameters like Specificity, Accuracy, Precision, Linearity, Robustness and Ruggedness obtained results were within the limits. Hence from obtained data it is concluded that the developed method is simple, accurate, reliable and economic and it can be employed for routine quality control analysis of Salbutamol and Theophylline tablets in drug testing laboratories and pharmaceutical industries without any interference from excipients.

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