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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE AND DOXOPHYLLINE IN COMBINED SOLID DOSAGE FORM

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ABSTRACT

Objective: Salbutamol sulphate (SBS) and doxophylline (DOX) was used for the treatment of asthma and bronchitis. In the present study, two simple, accurate, precise, reproducible and economical UV-spectroscopic methods (A and B) for simultaneous estimation of SBS and DOX in tablet dosage form have been developed.

Methods: In the present study the simultaneous estimation of SBS and DOX was carried out by two methods. Method A employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 272 nm and 276 nm which are the λ_{max} values of SBS and DOX respectively in phosphate buffer (pH 7.4). Method B is based on the principle of *Q*-Analysis where in, absorbance was measured at 225 nm (iso-absorptive point, λ_1) and 276 nm (λ_{max} of DOX, λ_2) in phosphate buffer (pH 7.4).

Results: Both SBS and DOX shows linearity at all the selected wavelengths and obeys beer's law in the concentration range of between 0.2-1.6μg/ml and 0.1-3.5µg/ml at 276 nm; 0.2-1.6 µg/ml and 0.1-4.5 µg/ml at 272 nm and 0.2-2.0µg/ml and 0.2-3.5µg/ml at iso-absorptive point 225 nm. Recovery studies for SBS and DOX were performed and the percentage recovery for both the drugs was obtained in the range of 97.45-98.63% (Method A) and 97.49–98.87 % (Method B) confirming the accuracy of the proposed method.

Conclusion: Both the methods showed good reproducibility and recovery with % RSD less than 2. Statistical validation of the data shows that the proposed methods can be successfully applied for the routine analysis of drugs in commercial tablets. Hence, it could be used in the analysis of laboratory samples and marketed formulations containing these two drugs in combined dosage form without the interference of common excipients.

Keywords: Simultaneous equation method, Q-absorbance ratio method, Salbutamol, Doxophylline

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INTRODUCTION

SBS is chemically (RS)-2-(hydroxymethyl)-4-{1-hydroxy-2-[(2-methyl-2-propanyl)-amino] ethyl} phenol sulfate (2:1) (fig. 1). It is β2-adrenergic receptor agonist used for the relief of bronchospasm 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. Activation of the β-2 adrenoreceptors opens ATPase channels and drives potassium from the extra cellular to the intracellular space. This both decreases extracellular hyperkalaemia and increases intracellular potassium, so decreasing the chance of arrhythmia [1-3].

DOX is chemically 7(1,3 dioxolone-2-yl-methyl)-1,3-dimethylpurine-2,6-dione (fig. 2). It is bronchodilator xanthine drug which has the therapeutic properties of theophylline with lower incidence of side-effects [4]. Doxophylline do not affect gastric acid secretion; either *in vivo* or *in vitro*; unlike theophylline. The lack of side effects with DOX indicates that the drug can be used safely and effectively in the treatment of COLD. DOX inhibits phosphodiesterase (PDE IV) activities with consequent increase of cyclic AMP that determines relaxation of smooth musculature. DOX appears to have decreased affinities toward adenosine A1 and A2 receptors which may account for the better safety profile of the drug. DOX does not interfere with calcium influx into the cells or antagonize calcium channel blockers. Unlike aminophylline it has low secretagogue activity and suitable for asthmatic patients with peptic ulcer disease. DOX is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis [5-6].

SBS is official in European Pharmacopoeia [7], which describes a potentiometric titration in non-aqueous medium. SBS alone or in combination with other drugs is reported to be estimated by HPLC in pharmaceutical dosage form [8-11], plasma; titrimetric [12], TLC, microtitrimetric, conductometric, HPLC, UV-spectrophotometry and immunoaffinity-chromatography. Some analytical methods for quantitative determination of doxophylline in pharmaceutical formulations are described in literature are some of reported methods used for analysis. UV-spectrophotometry estimation of doxophylline in biological samples, plasma and serum [13-19].

Extensive literature survey has revealed that no UV spectroscopic method is reported for simultaneous determination of SBS and DOX in combine dosage form. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. Therefore, in the present work successful attempt has been made to estimate both the drugs simultaneously by two simple UV spectrophotometric methods i. e simultaneous equation method and Q-absorbance ratio method in combine dosage form. The proposed methods were optimized and validated as per ICH guidelines [20-26].

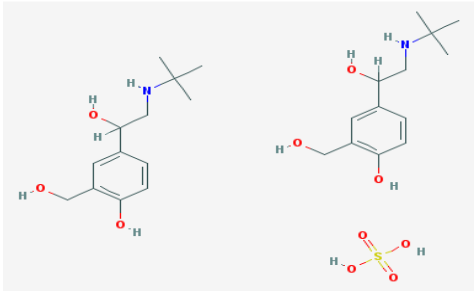

Fig. 1: Structure of SBS [27]

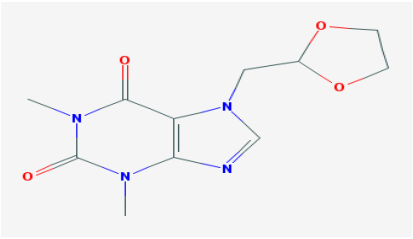

Fig. 2: Structure of DOX [28]

MATERIALS AND METHODS

Instruments

Absorbance measurements were made on double beam UV-Visible spectrophotometer, model 1800, Shimadzu, Japan, with software UV Probe 2.10 and 1 cm matched quartz cells.

Chemicals

Gift samples of salbutamol sulphate and doxophylline were provided by Key Pharmaceuticals Limited, Ambala, Haryana, India. The pharmaceutical dosage form used in the study was Doxoril plus 4 (Macleods Pharmaceuticals Pvt Ltd). Each uncoated tablet contains 4 mg SBS and 400 mg DOX. All chemicals were of analytical reagent grade and solutions were prepared with double distilled water.

Preparation of standard stock solution

Standard stock solutions (20 μg/ml) of both of SBS and DOX were prepared separately by dissolving accurately weighed (2.0 mg) quantity of pure SBS and DOX in 100 ml volumetric flask and diluting up to the mark with phosphate buffer (pH 7.4) to get working standard solution of each containing 20µg/ml of both SBS and DOX.

Preparation of working standard solutions

From the above stock solution desired concentrations were prepared by transferring specific volume to separate 10 ml volumetric flasks and volume was made up to 10 ml with phosphate buffer.

Determination of isoabsorptive point and absorption maxima

By appropriate dilution of standard solutions of SBS and DOX with phosphate buffer (pH 7.4), solutions containing 10 μg/ml of both drugs were scanned separately in the range of 200-400 nm against phosphate buffer (pH 7.4) as blank. The overlaying spectrum was also obtained to determine isoabsorptive point and wavelength of maximum absorbance λmax of both the drugs.

Methods

Simultaneous equation method (Method A)

1µg/ml solutions of SBS and DOX were prepared separately in phosphate buffer (pH 7.4) and the solutions were scanned against blank in the entire UV range to determine the λmax values. Clear peaks were observed at 272 nm for SBS and 276 nm for DOX. Hence these wavelengths were chosen as λmax values for each drug respectively (fig. 1). Standard solutions of SBS and DOX in the concentration range 0.1-5μg/ml were prepared in the phosphate buffer (pH 7.4) and the absorbance of these solutions was measured at 272 nm and 276 nm. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm) [21-24].

$$\text{At } \lambda_1\ A_1 = ax_1 bC_x + ay_1 bC_y \ldots. (1)$$

$$\text{At } \lambda_2 A_2 = ax_2 bC_x + ay_2 bC_y \ldots\ldots. (2)$$

For measurements in 1 cm cells b=1

Rearrange eq. (2)

$$Cy = \frac{A2 - ax2Cx}{ay2}$$

Substituting for Cy in eq (1) and rearranging

$$Cx = \frac{A2ay1 - A1\ ay2}{ax2\ ay1 - ax1\ ay2} \ldots\ldots\ldots (3)$$

$$Cy = \frac{A1ax2 - A2\ ax1}{ax2\ ay1 - ax1\ ay2} \ldots\ldots\ldots (4)$$

Where, Cx and Cy are the concentrations of SBS and DOX measured in gm/100 ml in sample solutions, A1 and A2 are absorbance of mixture at selected wavelengths 272 nm and 276 nm respectively.

Absorbance ratio method/Q-analysis (method B)

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obey Beer's law at all wavelength, the ratio of absorbance at any two wavelengths in constant value independent of concentration or path length. E. g. two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance are measured at two wavelengths, one being the λ_{max} of one of the component (λ_2) and the other being wavelength of equal absorptivity of two components (λ_1) i.e. an isoabsorptive point.

A series of standard solutions of SBS and DOX in the concentration range of 0.1-5 μg/ml were prepared in phosphate buffer and the absorbance of these solutions was measured at 225 nm (isoabsorptive point) and 276 nm (λ_{max} of DOX) (fig. 1). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelength for both the drugs. The absorptivity values were reported in table 1. [21-24]

The concentration of two drugs in mixture was calculated by using the following equations:

$$\mathbf{Cx} = \frac{Qm - Qy}{Qx - Qy} X \frac{\mathbf{A1}}{ax\mathbf{1}} \ldots\ldots (5)$$

$$\mathbf{Cy} = \frac{Qm - Qx}{Qy - Qx} X \frac{\mathbf{A1}}{ay\mathbf{1}} \ldots\ldots\ldots (6)$$

Where Qm = A_2/A_1, Qx=ax_2/ax_1, Qy=ay_2/ay_1

A_1 is absorbance of mixture at isosbestic point i. e 225 nm

A_2 is absorbance of mixture at 276 nm λ_{max} of DOX

ax_1 and ax_2 represent absorptivities of SBS at 225 nm and 276 nm

$ay_1$and ay_2 denotes absorptivities of DOX at 225 nm and 276 nm.

Cx and Cy are the concentration of SBS and DOX.

Validation of proposed method (Method A and B)

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine linearity, sensitivity, accuracy and precision for each analyte [20].

Linearity

Appropriate dilutions of working standard solutions for SBS and DOX were prepared in the concentration range of 0.1-5µg/ml and 0.1-3µg/ml, respectively and analyzed as per the developed methods A and B. Calibration curves were generated and the linearity was evaluated by the least square regression method. The results are reported in table 1, 5.

Accuracy (Recovery studies)

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of SBS and DOX at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at λ_{max} of 272 nm and 276 nm (Method A) and 225 nm and 276 nm (method B) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated. The result of the analysis is given in table 2, 3.

Precision

Precision is the degree of repeatability of analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate (interday) and reported as %RSD for a statistically significant number of replicate measurement. The intermediate precision was studied by comparing the assays on three different days and the results documented as standard deviation and %RSD.

Precision studies were performed in triplicate at three different concentration levels covering the entire linearity range for SBS and DOX. The result of the analysis is given in table 4.

Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quntitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve: (table 5)

$$LOD = \frac{3.3\ \sigma}{s}$$

$$LOQ = \frac{10\ \sigma}{s}$$

Where, σ is mean standard deviation of y-intercepts of regression lines, s is slope of the standard curve.

Assay of tablets formulation

For estimation of drugs in the commercial formulations, twenty tablets containing 400 mg DOX and 4 mg of SBS were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 1 mg of SBS and100 mg of DOX was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of phosphate buffer. It was sonicated for 30 min and volume was made upto obtain a stock solution 10µg/ml of SBS and1000µg/ml of DOX. This solution was then filtered through watmann filter paper #42. Further dilutions were made from this stock solution to get required concentration. In method A, the concentration of SBS and DOX was determined by measuring absorbance of sample solutions at 272 nm (λ_{max} of SBS) and276 nm (λ_{max} of DOX) using simultaneous equation. In method B, the concentration of both SBS and DOX was determined by measuring absorbance of sample solutions at 276 nm (λ_{max} of DOX; λ_2) and 225 nm (isosbestic point of both drugs; λ_1). The results of analysis and statistical validation for the marketed tablet formulation are reported in table 2-4. The results of recovery studies conducted by the addition of different amount of pure drugs at different levels to a tablet solution were found to be satisfactory.

RESULTS AND DISCUSSION

The simultaneous equation method is generally used to estimate two absorbing substances (SBS and DOX) each of which absorbs at the wavelength of the other drug. By constructing and placing values in simultaneous equations 3 and 4 the concentration of two drugs was determined. The absorption ratio method generally used to estimate two absorbing substances (SBS and DOX) each of which absorbs at the wavelength of the other by constructing and placing values in absorption ratio equation 5 and 6 to determine the concentration of SBS and DOX.

SBS and DOX exhibited maximum absorption at 272 nm and 276 nm (Method A), so using these wavelengths simultaneous equation method for analysis of SBS and DOX in combine form was developed. For Q-absorption method (Method B) of simultaneous analysis of SBS and DOX in combine form, 225 nm (iso-absorptive point) and 276 nm (λ_{max} of DOX) was used. Beer's law were found to be obeyed in the concentration range between 0.2-1.6µg/ml and 0.1 to 3.5µg/ml at 276 nm; 0.2-1.6 µg/ml and 0.1-4.5 µg/ml at 272 nm and 0.2 to 2.0µg/ml and 0.2 to 3.5µg/ml at iso-absorptive point 225 nm for SBS and DOX respectively (Method A and B). Calibration curves were prepared for both the drugs at 276 nm, 272 nm and 225 nm (fig. 4-6, table 1, 5). The overlain UV-absorption spectra of SBS (272 nm) and DOX (276 nm) showed isoabsorptive point (225 nm) in ethanol is shown in fig. 3. All calibration curve obtained was linear with coorelation coefficient (r^2) greater than 0.998. Hence the relationship between the concentrations and absorbances of SBS and DOX showed linearity (table 5).

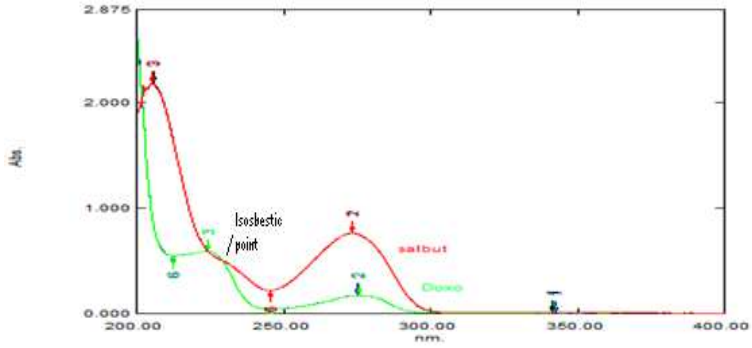

Fig. 3: UV overlay spectrum of SBS and DOX showing isoabsorptive point

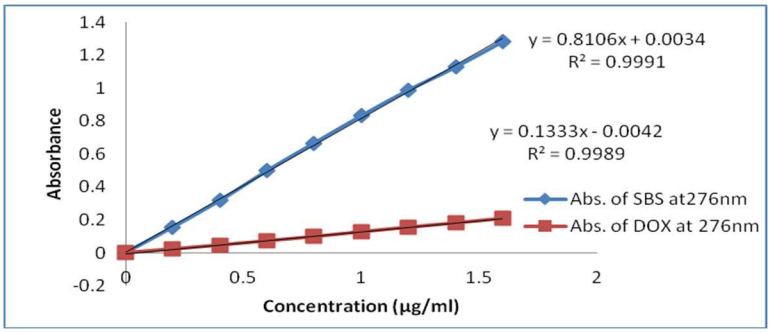

Fig. 4: Calibration curve of SBS and DOX at 276 nm

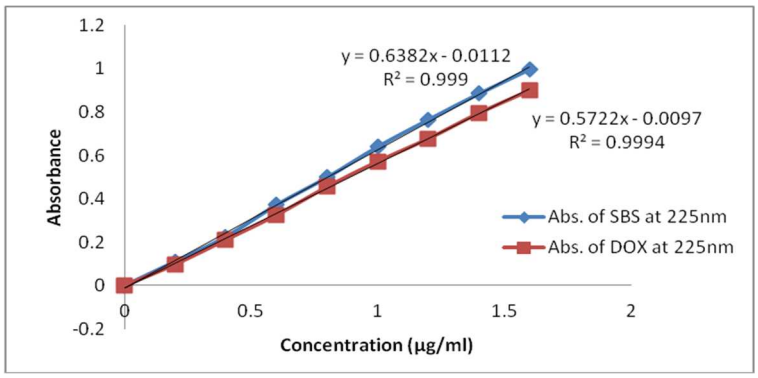

Fig. 5: Calibration curve of SBS and DOX at 225 nm

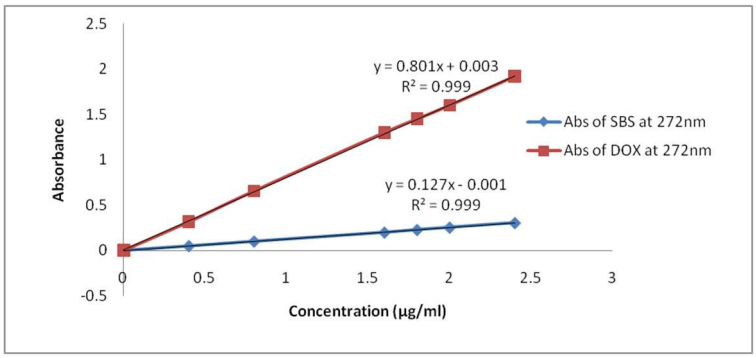

Fig. 6: Calibration curve of SBS and DOX at 272 nm

Table 1: Absorptivity values (A1%, 1 cm) of SBS and DOX for methods A and B i. e at all three (225 nm, 276 nm and 272 nm) wavelengths

Concentration of the solution (µg/ml)	Absorptivity, A(1%, 1 cm)					
	SBS mean absorptivity (n=3)			DOX mean absorptivity (n=3)		
	225 nm	276 nm	272 nm	225 nm	276 nm	272 nm
0.4	6400	7925	7900	5625	1175	1225
0.6	6200	8300	8200	5750	1216	1250
0.8	6237	8325	8000	5637	1237	1265
1	6400	8320	7444	5600	1210	1261.9
1.2	6375	8233	8175	5666	1216	1270
1.4	6321	8057	8007	5678	1214	1243.75
1.6	6231	8018	7500	5618	1231	1268
Mean	6309.311	8116.153	7889.429	5665.811	1225.171	1254.809

Each value represents mean value (n=3)

The validation parameters were studied on marketed formulation at all the wavelengths for the proposed methods. As per IP, tablets should contain not less than 95.0% and not more than 105.0% of active ingredients of the stated amount. The average % drug content was found to be of 97-98% and 96.89-98.16% for SBS and DOX by Method A and Method B respectively, which was found to be within the acceptance limit with %RSD values less than the limit of 2%. Accuracy was determined by calculating the recovery by standard addition method. Results revealed percentage recovery more than 97.50% with % RSD value within the accepted limit for both the components by both methods at all the three levels of recovery analysis. Hence both the proposed methods were found to be accurate for estimation of SBS and DOX in tablet formulation. Both the methods were subjected for study of repeatability, intraday and interday precision for both the drugs. % RSD values for repeatability, intraday and interday precision were calculated and found to be well below the specified limit of 2% (%RSD<2) indicating good precision in the specified range (table 4). The sensitivity of the proposed methods was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD values for SBS and DOX were found to be 0.048 and 0.064 µg/ml at 276 nm; 0.048 and 0.013 at 272 nm and 0.015 and 0.057 µg/ml at 225 nm. LOQ values for SBS and DOX were found to be 0.148 and 0.195 µg/ml at 276 nm; 0.158 and 0.042 µg/ml at 272 nm and 0.047 and 0.173 µg/ml at 225 nm. (table 5)

Table 2: Statistical parameters for marketed formulation: Doxoril plus 4 (SBS and DOX) by methods A and B

S. No.	Drug	Label claim (mg)	Amount found	% Recovery	% R. S. D
Method A	SBS	4	3.92	98	1.012
	DOX	400	392.65	98.162	1.315
Method B	SBS	4	3.88	97	0.915
	DOX	400	378.56	96.89	1.541

Each value is given in mean+% RSD

Table 3: Recovery studies on marketed formulations of SBS and DOX by methods A and B

Recovery Level %	% recovery (mean±S. D)			
	Method A		Method B	
	SBS	DOX	SBS	DOX
80	98.39±1.113	98.36±1.223	98.05±0.121	97.59±0.911
100	97.45±0.916	98.59±1.412	97.49±0.610	98.36±1.061
120	98.63±1.018	97.82±1.524	98.87±0.912	98.68±1.105
Mean	98.156±1.017	98.257±1.386	97.976±0.547	98.21±1.026

Each value represents mean±SD (n=3)

Table 4: Validation parameters analysis of SBS and DOX by methods A and B

Parameter	Method A				Method B			
Precision (mean±SD)	SBS	%RSD	DOX	%RSD	SBS	%RSD	DOX	%RSD
Repeatability	98.12±1.09	1.113	97.36±0.69	0.934	98.02±1.21	1.052	98.62±0.94	0.953
Day to Day	98.35±0.85	0.864	98.09±1.53	0.654	98.01±1.08	0.618	98.43±0.88	0.804
Analyst to Analyst	97.63±0.94	0.954	98.56±1.05	1.140	98.24±0.81	0.466	99.25±0.67	1.04
Reproducibility	97.62±1.08	1.046	97.06±0.85	1.039	97.06±1.06	0.647	97.86±0.76	0.982

Each value represents mean±SD (n=3)

Table 5: Regression analysis data

Parameters	276 nm		225 nm		272 nm	
	SBS	DOX	SBS	DOX	SBS	DOX
Linearity (µg/ml)	0.2-1.6	0.2-3.5	0.2-2.0	0.2-3.5	0.2-1.6	0.1-4.5
Molar Absorptivity ($1\ mol^{-1}\ cm^{-1}$)	194459	32615	150785	15068	188547	33356
Regression Equation (y = mx+c)	Y=0.810x+0.003	Y=0.133x-0.004	Y=0.638x-0.011	Y=0.572x-0.009	Y=0.801x+0.03	Y=0.127x-0.001
Slope (m)	0.810	0.133	0.638	0.572	0.801	.127
Intercept (c)	0.003	-0.004	-0.011	-0.009	0.03	0.001
Correlation coefficient (r^2)	0.998	0.999	0.998	0.999	0.999	0.999
LOD (µg/ml)	0.048	0.064	0.015	0.057	0.048	0.013
LOQ (µg/ml)	0.148	0.195	0.047	0.173	0.158	0.042

Therefore the result shows that both the proposed methods are specific, accurate and precise as indicated by good recovery results and within acceptance limit relative standard deviation (RSD) values for simultaneous quantitation of SBS and DOX in bulk drug and combined dosage form. Overall proposed methods were found to be suitable for simultaneous quantitative estimation of both the drugs in pharmaceutical dosage form.

CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of SBS and DOX in bulk and in pharmaceutical formulations. The developed methods were validated and from the statistical data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients.

The UV spectrophotometric simultaneous equation method and *Q*-absorption ratio method was developed and validated for the simultaneous analysis of SBS and DOX. The results together established that the methods are simple, accurate, precise, reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of SBS and DOX in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

CONFLICT OF INTERESTS

Declared none

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