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DEVELOPMENT OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF DOXOFYLLINE AND SALBUTAMOL SULPHATE IN COMBINED DOSAGE FORM

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ABSTRACT

A simple and sensitive HPLC method has been developed for the simultaneous estimation of doxofylline (DOX) and salbutamol sulphate (SAL) in combined dosage form. Doxofylline is bronchodilator. It is a xanthine derivative and a bronchial muscle relaxant, It also relaxes vascular smooth muscle in pulmonary blood vessels. Salbutamol sulphate is beta-adrenoreceptor agonist. It is chemically related to isoprenaline. It has a prominent brochodialator (ß-2 receptor) and poor cardiac (ß-1) stimulant action as compared to isoprenaline. The HPLC method was developed by using C18 Inertsil column (250 mm length, 4.6 mm internal diameter and 10 µm particle size) using mobile phase methanol : water : glacial acetic acid (80 : 19 : 01% v/v/v) at a flow rate of 1.0 mL/min, on an isocratic HPLC, Monitoring was done by a UV detector at 239 nm. Retention time of DOX and SAL was found to be 3.233 min. and 2.19 min. respectively. The method was statistically validated for its linearity, accuracy and precision. The developed method was simple and accurate. This method can be used for routine quality control of doxofylline and salbutamol sulphate in bulk and in formulations.

Key words: Doxofylline (DOX), salbutamol sulphate (SAL), HPLC Method.

INTRODUCTION

Doxofylline (DOX) is a bronchodilator. It is a xanthine derivative and a bronchial muscle relaxant. It also relaxes vascular smooth muscle in pulmonary blood vessels, Chemically, doxofylline is 7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione and is used as bronchodilator[1]. Spectrometric method[2] has been reported for estimation of DOX. Also HPLC and stability indicating methods for estimation of DOX in

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plasma have been reported[3,4]. Salbutamol sulphate (SAL) is used as beta-adrenoreceptor agonist. Salbutamol is a ß-adrenergic stimulant, which is chemically related to isoprenaline. It has a prominent bronchodilator (ß-2 receptor) and poor cardiac (ß-1) stimulant action as compared to isoprenaline. It is less likely to produce palpitation or rise of blood pressure in therapeutic doses[5]. Chemically, it is (1RS)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-hydroxymethyl) phenyl] ethanol[6]. Many spectrophotometric methods[7-11] have been reported in the literature for estimation of SAL alone and in combination. Estimation of SAL in human serum by HPLC has been reported[12]. However, there is no RP-HPLC method reported for the simultaneous estimation of doxofylline and salbutamol sulphate in combined dosage forms.

EXPERIMENTAL

A high performance liquid chromatography system consisting of JASCO PC 1580, intelligent pump and variable wavelength UV-Visible (JASCO UV) detector and precision loop injector (Rheodyne, 20 l) was used for the analysis. The data were recorded using LC 2010 solutions software. The optimized chromatographic conditions consisted of a column Inertsil 4.6 x 250 mm (id) with a particle size packing 10 µm bonded with a C18 Inertsil stationary phase. Optimized chromatographic conditions are listed in Table 1.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	JASCO PC 1580
Column	C18 Inertsil stationary phase
Mobile phase	*Methanol : Water : Glacial Acetic Acid (80 : 19 : 01 % v/v/v)
Flow rate	1 mL/min.
Detection wavelength	239 nm
Injection volume	20 µL
Temperature	Ambient
Retention time-DOX	3.23 min.
Retention time-SAL	2.19 min.

Materials and chemicals

Pure samples of doxofylline and salbutamol sulphate were obtained from Piramal Healthcare Ltd. For the estimation of doxofylline and salbutamol sulphate in commercial formulations, the marketed preparation ware purchased from the local market having brand name Doxoril plus 2 manufactured by Macleod's Pharma. Ltd., Mumbai, having composition doxofylline (400 mg) and salbutamol sulphate (2.4 mg). HPLC grade sodium dihydrogen phosphate, glacial acetic acid, water HPLC grade and methanol were procured from Merck, India.

Preparation of standard stock solution

Accurately weighed quantity of DOX 100 mg was transferred to 100 mL volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol. Accurately weighed quantity of SBS 10 mg was transferred to 100 mL volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol. The standard solution of DOX and SBS were mixed and diluted with mobile phase properly to obtain laboratory mixtures containing a concentration 400 µg/mL of DOX and 2.4 µg/mL of SBS.

Preparation of sample solution

Twenty tablets **(**Doxoril plus 2 manufactured by Macleod's Pharma. Ltd., Mumbai were taken. Each tablet was labeled to contain DOX (400 mg) and SAL (2.4 mg). The average weight of tablet was determined. The tablets were finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 400 mg of DOX was transferred in a 100 mL volumetric flask and methanol was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with methanol. The solution was filtered through Whatman filter paper No. 42. Further dilution was done with mobile phase to get concentration of 400 µg/mL of DOX and 2.4 µg/mL of SBS. This solution was used for estimation.

Assay procedure

The above prepared samples were used under optimized chromatographic conditions. The prepared solution of each standard and sample solution were injected into HPLC system to obtain chromatogram for standard drug solution (five replicates) and sample solution (five replicates). Concentrations of DOX and SAL in the formulation were calculated by comparing AUC of sample with that of standard. Chromatogram showing the retention time of DOX (3.233 min) and SBS (2.19 min) in tablet formulation is shown in Fig.1.

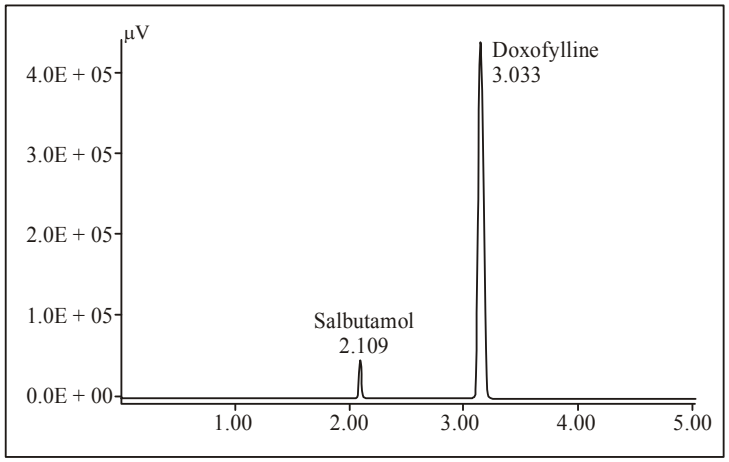

Fig. 1: Chromatogram obtained by tablet formulation of DOX and SBS showing retention time for SBS– 2.19 min. and DOX – 3.23 min

RESULTS AND DISCUSSION

After several systematic trials, the mobile phase consisting of mixture of methanol : water : glacial acetic acid (80 : 19 : 01 % v/v/v) was selected, since it gave sharp reproducible retention time for DOX and SBS and hence, it was fixed as mobile phase. The UV absorption spectrum of DOX and SAL was recorded in mobile phase. The λ_{max} was determined on Shimadzu UV-Visible spectrophotometer (Model UV-2409) in the range 200-400 nm using mobile phase as blank. The solution of mixture exhibited maxima at about 239 nm (Fig. 2).

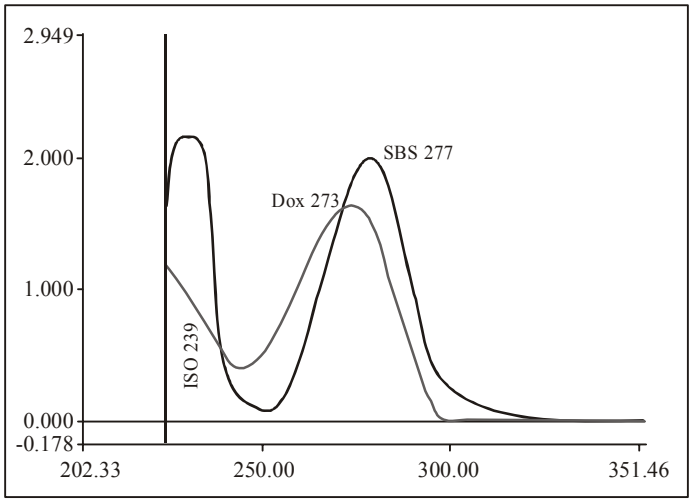

Fig. 2: UV Absorption spectrum of DOX and SBS laboratory mixture

This system gave good resolution and optimum retention time with appropriate tailing factor (< 2). The mean values of system suitability test result are depicted in Table 2.

Table 2: Summary of system suitability test results

Parameter	DOX	SAL
Peak area	1162942	346997
No. of theoretical plates	9877.6	5868.5
Retention time (min)	3.233	2.19
Asymmetry	0.113	0.090
Capacity factor	3.196	4.92
Selectivity	2.878	
Resolution	12.49	

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analyzed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation. The summary of results of laboratory mixture and marketed formulation are given in the Table 3.

Table 3: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method

Sample	Statistical data	% Estimation		% Recovery	
		DOX	SAL	DOX	SAL
Standard laboratory mixture	Mean	99.52	100.54	-	-
	S.D.	0.2272	0.2966	-	-
	C.V.	0.2282	0.6132	-	-
DOXORIL PLUS 2	Mean	99.97	99.802	99.793	100.08
	S.D.	0.05176	0.61310	0.273	0.606
	C.V.	0.051775	0.61122	0.273	0.605

These results clearly indicate that developed RP-HPLC technique can be successfully applied for the estimation of these drugs in their combined dosage formulation without prior separation. The method was validated as per the ICH guidelines for various parameters like accuracy, which is ascertained from the recovery studies by standard addition method. Results are shown in the Table 3. The proposed method was precise, showing ± S.D. < 2. Specificity studies show that there is no interference of peak from the component of matrix showing retention time for DOX (3.65 min.) and SAL (6.56 min.) Ruggedness studies were also carried out for different parameters like different time, different day and different analyst. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the ruggedness of the method under varying condition of its performance. Summary of results of ruggedness studies are shown in Table 4.

Table 4: Summary of results of ruggedness by RP-HPLC method

Parameters	Statistical data	% Estimation by RP-HPLC method	
		DOX	SAL
Inter-day	Mean	99.993	99.79
	S.D	0.0321	0.0854
	C.V.	0.0321	0.0855
Intra-day	Mean	99.61	99.70
	S.D	0.1442	0.5220
	C.V.	0.1447	0.5235
Different analyst	Mean	99.815	99.83
	S.D	0.23334	0.16971
	C.V.	0.23373	0.16998

The developed RP-HPLC technique for estimation of DOX and SBS marketed formulation was found to be linear in the range of 80 % to 120 % of test concentration with $R^2 \approx 1$ at selected wavelength for both the methods. Same procedure as described in USP was followed. From the studies, it can be concluded that the developed RP-HPLC technique can be successfully used for the estimation of the doxofylline and salbutamol sulphate in their combined dosage tablet formulations.

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