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NOVEL SIMULTANEOUS AND SECOND ORDER DERIVATVE METHOD DEVELOPMENT OF DOXOFYLLINE AND SALBUTAMOL SULPHATE IN PURE AND FIXED DOSE COMBINATION BY UV – SPECTROPHOTOMETRY

Dr. G. Abirami* and Dr. T. Vetrichelvan

Department of Pharmaceutical Chemistry Adhiparasakthi College of Pharmacy Melmaruvathur-603306.

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***Corresponding Author**
Dr. G. Abirami
Department of Pharmaceutical Chemistry Adhiparasakthi College of Pharmacy Melmaruvathur-603306.

ABSTRACT

Two novel simple, rapid, precise and reproducible UV spectrophotometric methods has been developed for Simultaneous estimation of two component drug mixture of Doxofylline (DOX) and Salbutamol (SAL) in bulk and combined tablet dosage form. The literature review reveals that there is no derivative method was developed for this combination of drugs, hence this method was developed. First method employs simultaneous equation method using 274nm (λ max of DOX) and 224 (λ max of SAL) as two wavelengths for estimation. The second method involves second order derivative spectroscopic method, the wavelength used were 233nm for DOX (zero crossing for SAL) and 229nm for SAL (zero crossing for DOX). For the two methods distilled water was used as solvent. Linearity was observed in the concentration range of 5- 25 µg/ml or DOX and 5- 25 µg/ml for DOX. The % recovery was found in the range of 99.64-100.07 for DOX and 98.48-100.55 for Salbutamol. The developed method was validated statistically by recovery studies. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of Salbutamol in bulk and combined tablet dosage form.

KEYWORDS: Salbutamol sulphate, Derivative spectrophotometer UV detection.

INTRODUCTION

Doxofylline (DOX) chemically, 7(1, 3 dioxolone-2-yl methyl) theophylline (Fig. 1) is a bronchodilator xanthine drug which has the therapeutic properties of theophylline with lower incidence of side-effects.[7] Doxofylline inhibits Phosphodiesterase (PDE IV) activities with consequent increase of Cyclic AMP that determines relaxation of smooth musculature. Doxofylline does not interfere with calcium influx into the cells or antagonize calcium channel blockers.[9] unlike aminophylline it has low secretagogue activity & suitable for asthmatic patients with peptic ulcer disease.[10] Doxofylline is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD).

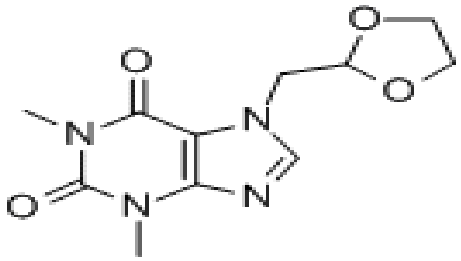


Fig-1 Doxofylline

Salbutamol sulphate (SBS) chemically, bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate (Fig. 2) is a β2-adrenergic receptor agonist. Used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease.[07] Activation of the β-2 adreno-receptors opens ATPase channels and drives potassium from the extra cellular to the intracellular space.[7] This both decreases extracellular hyperkalaemia and increases intracellular potassium, so decreasing the chance of arrhythmia[12]

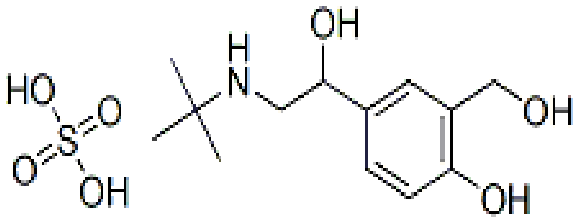


Fig 2: Salbutamol Sulphate.

Salbutamol sulphate (SAL) is official in European Pharmacopoeia, which describes a potentiometric titration in non-aqueous medium. Literature survey reveals that various analytical methods have been reported for the assay of Doxofylline in pure form and in Pharmaceutical formulations. While other methods reported are in British Pharmacopoeia[09]

and Indian Pharmacopoeia.[10] Various methods reported for Salbutamol sulphate alone or in combination with other drugs is reported to be estimated by UV, HPLC in pharmaceutical dosage form[28-32], Titrimetric and Spectrophotometric[33] Doxofylline was reported with other combinations by UV, HPLC & HPTLC.[14-26] Extensive literature survey reveals that no UV method has been reported for simultaneous determination of SAL and DOX in tablet dosage form.

Therefore, an attempt was made to develop a new, rapid and sensitive UV derivative method for the Simultaneous determination of SAL and DOX in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH Guidelines[1], which is mandatory also.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu-1700 double beam UV-Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and chemicals

Pharmaceutically pure sample of Doxofylline and Salbutamol sulphate were generously gifted by Himalayan Pharmaceuticals Pvt. Ltd, Himachal Pradesh.

Combination product **DOXORIL PLUS** containing 400 mg Doxofylline and 4 mg Salbutamol sulphate. The tablet dosage was purchased from a local Pharmacy.

Selection of solvents

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeial standards. Solubility was carried out from non polar solvents to polar solvents. The common solvent was found to be distilled water for the analysis of Doxofylline and Salbutamol sulphate for proposed method

Method development

UV spectrophotometric method for estimation Doxofylline and Salbutamol sulphate was carried by the simultaneous and second order derivative spectroscopic method.

Preparation of standard stock solution

Accurately weighed drug samples of both DOX and SAL (20 mg each) were transferred into a suitable standard volumetric flask separately, dissolved and diluted to mark with distilled water. Both the drug in solutions was diluted so as to get 10 µg/ml. These solutions were scanned in the UV region of 200 - 400 nm in 1cm cell against distilled water as blank and the overlaid spectra was recorded.

Selection of wavelengths for estimation and stability studies

From the overlaid spectra of DOX (10 µg/ml) and SAL (10 µg/ml) in distilled water, wavelengths 274 nm (λ max of DOX) and 224 nm (λ max of SAL) were selected for the formation of Simultaneous equation method. For Derivative Spectroscopic method, the zero order spectra was derivatized to second order spectra in that 233 nm was selected for the estimation of DOX which is zero crossing for SAL and 229 nm was selected for the estimation of SAL which is zero crossing for DOX.

Preparation of calibration graph

From the primary stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 5-30 µg/ml of DOX and 5-30 µg/ml of SAL. Absorbances of these solutions were recorded in the respective wavelengths. For derivative method, concentration ranges from 10-60 µg/ml of DOX and 10-60 µg/ml of SAL respectively.

Analysis of tablet formulation (Standard addition method)

Twenty tablets (DOXORIL PLUS) were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 25 mg of DOX was transferred into a 25 ml volumetric flask, then added 24.75 mg of Salbutamol sulphate raw material and sufficient quantity of distilled water was added and the solution was sonicated for 15 minutes and diluted to the mark with distilled water. It was filtered through Whatmann filter paper No. 41, filtrate was suitably diluted to get final concentration of 15 µg/ml of DOX and 15 µg/ml of SAL with distilled water. For derivative method, the filtrate was diluted to get the expected concentration 30 µg/ml of DOX and 30 µg/ml of SAL with distilled water. The absorbance of sample solution was measured six times at all selected wavelengths for all the methods.

Recovery studies

The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drug standard solution was 15 µg/mL, 30 µg/mL of DOX and 15 µg/mL,30 µg/mL of SAL for all the methods.

Validation of the Developed Methods

Linearity

Linearity was checked by diluting standard stock solution at five different concentrations. DOX was linear with the concentration range of 5-25 µg/ml and SAL showed linearity in the range of 5-25 µg/ml at 224nm and 274nm for Simultaneous method. In derivative spectroscopy method DOX and SAL was linear in the range of 10-60 µg/ml at 233nm and 229nm.The calibration curves [n=5] were plotted between concentration and absorbance of drugs were measured. Optical parameters were calculated. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained: Y

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated, in accordance with ICH guidelines, LOD = 3.3σ/S and LOQ = 10σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Accuracy

Percent Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies were carried out for all the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated

three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

RESULTS AND DISCUSSION

The selected drugs Doxofylline and Salbutamol sulphate were estimated by using simultaneous estimation by second order derivative spectroscopic method as per ICH guidelines. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Doxofylline and Salbutamol sulphate was 5-30 µg/ml and 10-60 µg/ml with R^2 value of 0.9987 and 0.9983 respectively. The % RSD for intraday and interday precision and interday precision was <2%. The method has been validated in assay of tablet dosage forms. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits.RSD values suggest that the precision of the method was further confirmed. The ruggedness of the method was confirmed by performing the analysis with the different analysts and different instruments. The % average of synthetic mixture was found to be 99.884 for DOX and for SAL 100.106. The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation.

The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of Doxofylline and were found to be 0.178946 and 1.118034 for SAL respectively. The low % RSD values suggest that the method has good precision.

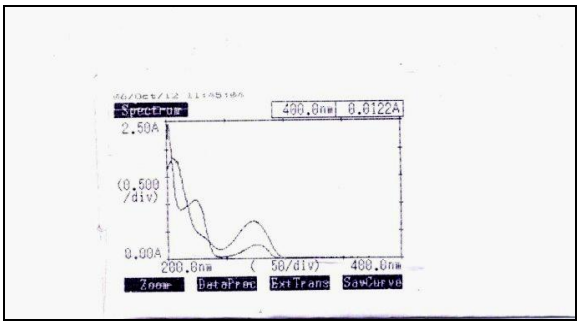


Fig-1-Overlaid spectrum of Doxofylline and Salbutamol

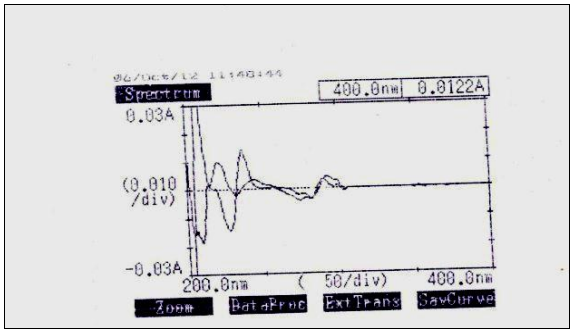


Fig 2-Second order derivative spectrum

(SIMULTANEOUS EQUATION METHOD)

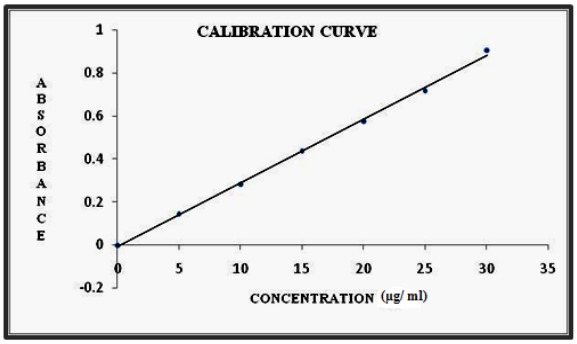


Fig-3: CALIBRATION CURVE OF DOXOFYLLINE AT 224nm

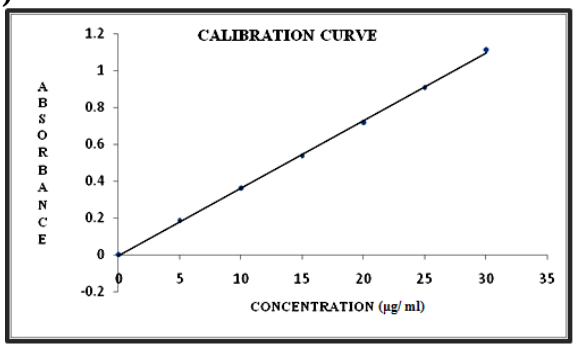


Fig-4: CALIBRATION CURVE OF DOXOFYLLINE AT 274nm

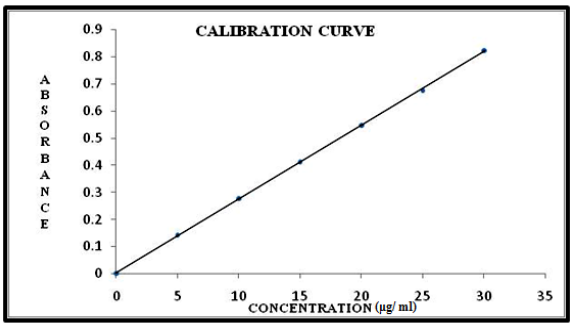


Fig-5: CALIBRATION CURVE OF SALBUTAMOL SULPHATE AT 224nm

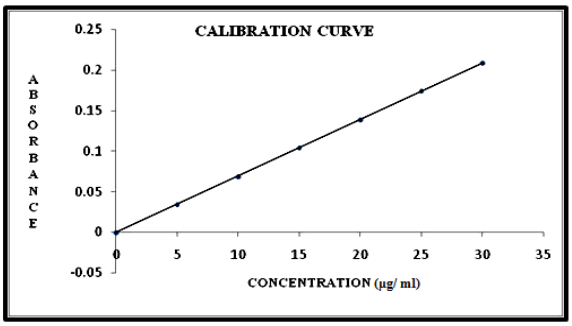
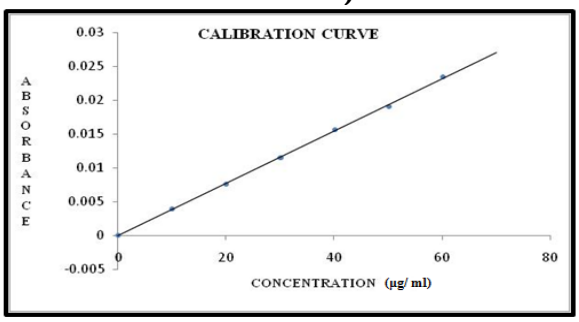
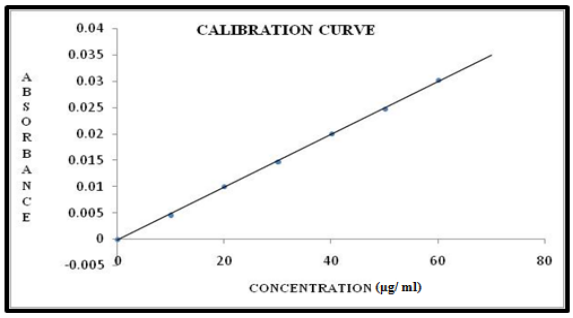


Fig-6: CALIBRATION CURVE OF SALBUTAMOL SULPHATE AT 274nm

(DERIVATIVE METHOD)



Salbutamol Sulphate



Doxofylline at 229nm

Fig-07: CALIBRATION CURVE FOR

TABLE 1: OPTICAL CHARACTERISTICS OF SALBUTAMOL SULPHATE (SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 224 nm	AT 274 nm
Beer's law limit (µg/ ml)	5 – 25	5 - 25
Molar absorptivity (L mol^{-1} $cm^{-1)}$	6705.49422	165.60321
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.03702058	0.144628099
Correlation coefficient (r)	0.99992957	0.9996607
Régression équation (Y = mx+c)	Y = 0.027012x + 0.0038333	Y = 0.0069142x + (-0.00019523)
Slope (m)	0 .027012	0.0069142x
Intercept (c)	0.0038333	-0.00010952
LOD (µg/ ml)	0.009498	0.000927
LOQ (µg/ ml)	0.287871	0.002811
Standard error	0.0033528	0.0005956

TABLE 2: QUANTIFICATION FOR FORMULATION (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	S.D	% R.S.D.	S.E.
DOX	1 2	400 400	399.99 400.08	99.99 100.02	0.178878	0.178	0.004969
SAL	1 2	4 4	3.99 4.07	99.75 101.75	1.118034	1.11803	0.031056

TABLE 3: INTER DAY AND INTRADAY ANALYSIS (SIMULTANEOUS EQUATION METHOD)

Drug	Percentage obtained*		S.D		% R.S.D.	
	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
DOX	100.43 99.65 100.34	99.98 100.04 100.76	0.426732	0.434051	0.426135	0.432925
SAL	99.41 100.02 100.01	98.98 99.53 99.76	0.349333	0.400791	0.349896	0.044532

TABLE 4: RUGGEDNESS STUDY (SIMULTANEOUS EQUATION METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
DOX	Analyst 1	101.94	0.4419	0.4463	0.0128
	Instrument 2	99.96	1.5147	1.5153	0.0420
SAL	Analyst 1	99.00	1.5795	1.5954	0.0438
	Instrument 2	101.94	0.2943	0.2887	0.0081

TABLE 5: RECOVERY ANALYSIS OF FORMULATION (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
DOX	1	15.002	12	27.0135	12.0115	100.09	0.11898	0.119007	0.01322
	2	15.002	15	29.980	14.978	99.853			
	3	15.002	18	33.0012	17.9992	99.99			
					Mean	**99.9776**			
SAL	1	15.032	12	26.9980	11.966	99.71	0.11874	0.118939	0.01319
	2	15.032	15	30.0234	14.9914	99.942			
	3	15.032	18	33.0100	17.978	99.87			
					Mean	**99.840**			

TABLE 6: OPTICAL CHARACTERISTICS (DERIVATIVE METHOD)

PARAMETERS	DOX AT 233 nm	SAL AT 229 nm
Beer's law limit (µg/ ml)	10 - 60	10 – 60
Sand ell's sensitivity (µg/cm^2/0.001A.U)	1.1836	1.30597
Correlation coefficient (r)	0.9998851	0.999794
Régression équation (Y = mx+c)	Y = 0.00084485x + 0.00010476	Y = 0. 000765714x + 0.0000285
Slope (m)	0. 0.00084485	0. 000765714
Intercept (c)	0.00010476	(0.0000285)
LOD (µg/ ml)	0.9930918	0.008375
LOQ (µg/ ml)	3.00936	0.02537
Standard error	0.00026788	0.0001625

TABLE 7: QUANTIFICATION FOR FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DOX	1	400	399.56	99.89	99.9858	0.16936	0.16938	0.00470
	2	400	399.65	99.91				
SAL	1	4	3.98	99.5	99.7083	0.79713	0.79946	0.02214
	2	4	4.04	101.0				

TABLE 8: INTER DAY AND INTRADAY ANALYSI (DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
DOX	1	400	99.65	99.76	0.892501	0.1253	0.887693	0.12545
	2	400	101.435	100.01				
	3	400	100.54	99.87				
Mean			**100.5417**	**99.88**				
SAL	1	4	99.84	99.67	0.1789	0.325013	0.179188	0.3261
	2	4	99.73	99.99				
	3	4	100.08	99.34				
Mean			**99.883**	**99.666**				

TABLE 9: RUGGEDNESS STUDY (DERIVATIVE METHOD)

Drug	Condition	Average*% Obtained	S.D	% R.S.D	S.E.
DOX	Analyst 1	98.37	0.847703	0.861749	0.023547
	Instrument 1	98.42	0.424001	0.430771	0.011778
SAL	Analyst 2	98.67	0.380443	0.385564	0.010568
	Instrument 2	99.92	0.500586	0.507317	0.013905

TABLE 10: RECOVERY ANALYSIS OF FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
DOX	1	15.002	12	27.004	12.002	100.01	0.12741	0.127317	0.014157
	2	15.002	15	29.987	14.985	99.99			
	3	15.002	18	33.042	18.04	100.22			
					Mean	**100.0733**			
SAL	1	15.032	12	26.987	11.955	99.80	0.01914	0.1917	0.002127
	2	15.032	15	29.897	14.865	99.773			
	3	15.032	18	32.998	17.966	99.81			
					Mean	**99.7943**			

* Mean of Three Observations.

CONCLUSION

The results indicate that the proposed UV spectrophotometeric methods are simple, rapid, precise and accurate. The developed UV spectrophotometeric methods were found suitable for determination of DOX and SAL as bulk drug and in marketed tablet dosage formulation without any interference from the excipients. The validated methods produce results within known uncertainties that are helpful to continuing drug development and provide emerging knowledge supporting the product. The time and effort that is devoted into developing

scientifically sound and robust analytical methods should be aligned with the drug development stage. Statistical analysis proves that, these methods are repeatable and selective for the analysis of DOX and SAL.

Thus the above study's findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms.

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