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UV Spectrophotometric Method for Simultaneous estimation of Salmeterol xinafoate and Fluticasone propionate in Bulk and Dosage form

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Abstract: Fixed dose combination containing salmeterol xinafoate and fluticasone propionate is widely used in the treatment of bronchial asthma and chronic obstructive pulmonary diseases. In this research we aimed to develop a simple, accurate, precise, sensitive, selective and economical spectrophotometric method that requires no prior separation for the simultaneous estimation of salmeterol xinafoate and fluticasone propionate in capsule dosage form. The estimation was based upon the simultaneous equation method which was carried out at the wavelength of 214 nm and 246 nm for salmeterol xinafoate and fluticasone propionate respectively. Phosphate buffer (pH 7.4): Ethanol (95%) (90:10) was used as solvent. The linearity lies between 5 to 14 µg/ml for salmeterol xinafoate and 2 to 14 µg/ml for fluticasone propionate. The mean results of estimation in capsule were 99.82±0.11% & 99.89±0.05% of the label claim for salmeterol and fluticasone respectively. The accuracy and precision of the method were determined and validated according to ICH guidelines. The method had good reproducibility and recovery with % RSD less than 1. Thus the proposed method can be successfully applied for simultaneous determination of salmeterol xinafoate and fluticasone propionate in routine analysis work.
Key words: Salmeterol xinafoate, Fluticasone propionate, Simultaneous Equation method.

INTRODUCTION

Salmeterol xinafoate (SX) is, (RS)-2-(hydroxymethyl)-4-{1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino] ethyl} phenol[1]. It is a long acting and highly selective β2 agonist formulated as its 1-hydroxy-2-napthoate (xinafoate) salt used in the treatment of asthma and chronic obstructive pulmonary disease. Inhaled salmeterol works like other beta 2-agonists, causing bronchodilation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. The long duration of action occurs by the molecules initially diffusing into the plasma membrane of the lung cells, and then slowly being released back outside the cell where they can come into contact with the beta-2 adrenoceptors, with the long carbon chain forming an anchor in the membrane[2].

Fluticasone propionate (FP), is 5-fluromethyl-6α,9α-difluro-11β-hydroxy-16α-methyl-17α-propionyloxy-3-oxoandrosta-1, 4-diene-17β-carbothio -nate, 17 propanoate. It is a neutral, highly potent trifluorinated corticosteroid based on the androstane nucleus. It is effective in treatments of asthma and allergic rhinitis because of its anti-inflammatory

activity. It is also used in the treatment of eosinophilic esophagitis. Fluticasone mimics the naturally-occurring hormone produced by the adrenal glands, cortisol or hydrocortisone[2].

These two drugs are formulated as dry powder inhalers or pressurized metered dose inhalers individually or in combined formulation[3]. Spectrophotometric techniques have been reported for the determination of salmeterol xinafoate in its dosage forms[4-5]. , Liquid chromatography with MS, MS/MS and fluorescence detection; GC with MS are reported for analysis of salmeterol xinafoate from body matrices[6-9]. Also, analysis by capillary zone electrophoresis is reported[10.]

Liquid chromatography coupled with APCI-MS and tandem mass spectrometers have been reported for the determination of fluticasone propionate in human plasma[11-12]. Also, validated assays have been reported for each drug individually and concurrently by HPLC, CE, and HPTLC[13-15]. As no validated assay is reported for both drugs concurrently by UV spectroscopy using phosphate buffer; there is a need for an assay method that permits simultaneous quantification of salmeterol xinafoate and fluticasone propionate.

Therefore the aim of this work is to develop and validate a simple, rapid, selective and quite sensitive UV assay method in phosphate buffer (pH 7.4): ethanol (90:10) for simultaneous estimation of salmeterol xinafoate and fluticasone propionate in the bulk powders.

EXPERIMENTAL

INSTRUMENTATION

UV-visible double beam spectrophotometer, Make: JASCO spectrophotometer, model V-550 with a pair of 10 mm matched quartz cells was used for experiments. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-400 nm.

REAGENTS AND CHEMICALS

Pure drug samples of salmeterol xinafoate (SX) and fluticasone propionate (FP) were obtained as gift sample from Vamsi Labs Ltd., Solapur and Sun Pharmaceutical Industries Ltd., Mumbai. Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH), was obtained from Finar Chemicals Ltd. Ahmedabad, India. Ethanol (95%) and double distilled water were used throughout the study. Combined dose capsule formulation (Seroflo rotacap 100) 50:100 µg was procured from retail out lets.

SELECTION OF DISSOLUTION MEDIUM

Various dissolution medias were tested for the development of an assay method. For the sake of solubility of both drugs in phosphate buffer (pH-7.4); ethanol (95%) was used according to the solubility characteristics of drugs, in 90:10 proportions to dissolve the drugs completely. The use of ethanol was considered to enhance the solubility of both drugs in phosphate buffer pH 7.4.[4]

PREPARATION OF WORKING STANDARD STOCK

Stock standard solutions were prepared by dissolving 5 mg of SX and 5 mg of FP in 30 ml of phosphate buffer pH 7.4: ethanol (90:10) in two separate 50 ml volumetric flask. The contents were dissolved with the aid of shaking and sonication for about 15 minutes, and then diluted to volume with the same solvent. The resultant individual stock solution was of concentration 100µg/ml.

PREPARATION OF SAMPLE SOLUTIONS

From the above stock solution of concentration of 100 µg/ml, serial dilutions were done so as to get sample solution of concentration range from 1 µg/ml to 15 µg/ml for both drugs individually.

DETERMINATION OF ABSORPTION MAXIMA

From the standard stock solutions of SX and FP (100 µg/ml) pipette out 1 ml of each in two separate 10 ml volumetric flask and make up the volume to get a concentration of 10 µg/ml each. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. SX showed an absorbance peaks at 214 nm and 249 nm, whereas FP indicated at 246 nm. The absorbance maxima 214 nm and 246 nm were selected for analysis of SX and FP respectively. (Figure 1)

PREPARATION OF STANDARD CALIBRATION CURVE

The absorbance of serial dilutions was recorded at 214 nm and 246 nm for SX and FP respectively and calibration curve was plotted. (Figure 2 and 3)

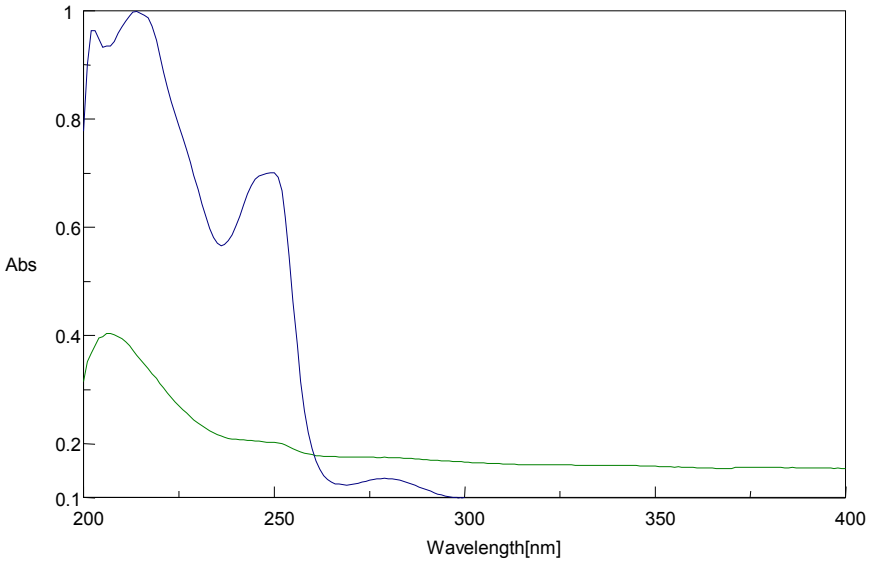

Figure 1: UV overlain spectra for Salmeterol xinafoate and Fluticasone propionate

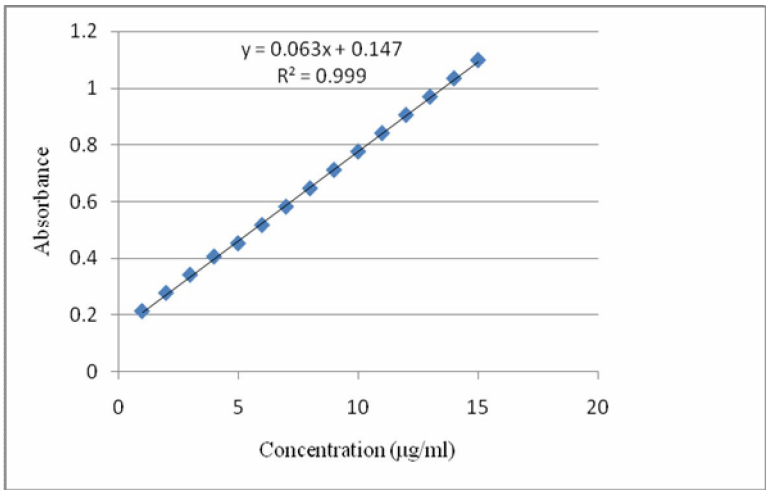

Figure 2: Calibration curve for Salmeterol xinafoate

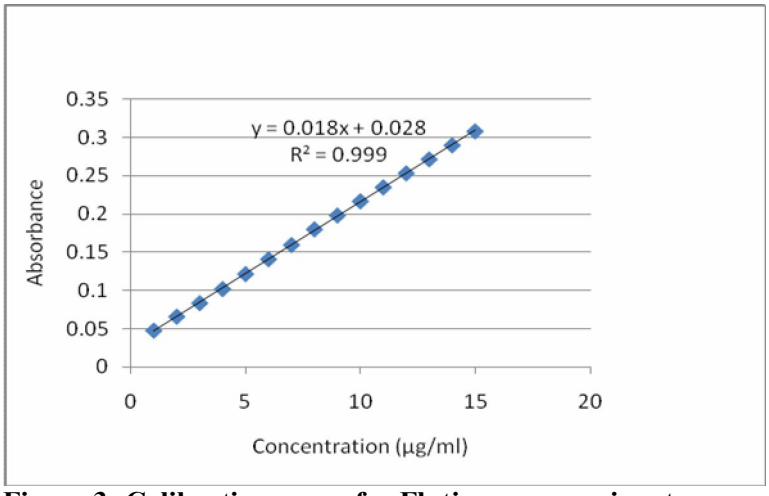

Figure 3: Calibration curve for Fluticasone propionate

ASSAY PROCEDURE FOR CAPSULE FORMULATION

20 capsules of marketed formulation of SX and FP corresponding to 50 µg and 100 µg (Seroflo 100) respectively were weighed; their average weights determined. The correct amount of drug powder equivalent to label claim was weighed and transferred to 10 ml volumetric flask, dissolved in phosphate buffer pH 7.4: ethanol (90:10) and sonicated for 15 min. The volume was then made up to the mark using same solvent. The resultant solution was filtered through 0.45 µ membrane filter. The filtrate was having concentration 5 µg/ml for SX and 10 µg/ml for FP. Absorbance of this sample solutions was recorded at 214nm ((λmax of SX) and 246 nm (λmax of FP) and concentrations of two drugs in the sample were determined by using simultaneous equations[16] (Table 1).

METHOD VALIDATION

The method was validated as per ICH guidelines[17].

SPECIFICITY

The specificity of the method was investigated by observing any interference encountered from any excipients of the capsule. It was found that these excipients did not interfere with the proposed method.

LINEARITY AND RANGE

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law, were found to be 5-14 µg/ml for SX (r^2 = 0.99) and 2-14 µg/ml for FP (r^2 = 0.99). The standard calibration curve is given in figure 2 and 3.

PRECISION

Precision was studied to find out intra and inter-day variation in the test method of SX and FP. Calibration curves prepared in medium were run in triplicate in same day for three days.

LIMIT OF DETECTION AND QUANTIFICATION

Determination of the detection and quantification limits was performed based on the standard deviations of y-intercept and the slope of the least square line parameters.

RECOVERY STUDY

To study the accuracy of the proposed method, recovery study was carried out by standard addition method at three different levels. A known amount of drug was added to preanalyzed capsule powder and percentage recoveries were calculated. The recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical additives and excipients (Table-2).

Table-1: Result of UV analysis for marketed capsule formulation

Capsule content	Label claim (µg/cap)	Label claim*(%)	±SD*	RSD* (%)
SX	50	99.82	0.1160	0.1161
FP	100	99.89	0.0549	0.0005

SX: Salmeterol xinafoate, FP: Fluticasone propionate, SD: standard deviation, RSD: Relative standard deviation, *: Mean of six estimations.

Table-2: Result of recovery study

Drug	Level of Recovery (%)	Amount present (µg/ml)	Amount found	% Recovery	±SD	%RSD
SX	80	9	8.9982	99.94	0.1120	0.1120
	100	10	10.0602	100.59	0.0354	0.0352
	120	11	11.0090	100.08	0.2364	0.2362
FP	80	18	17.9939	99.96	0.0797	0.0797
	100	20	20.0023	100.01	0.0266	0.0266
	120	22	22.0098	100.02	0.0958	0.0956

SX: Salmeterol xinafoate, FP: Fluticasone propionate, SD: standard deviation, RSD: Relative standard deviation, *: Mean of six estimations.

Table no.3- Precision study of Salmeterol xinafoate:

Conc. (µg/ml)	Intra-day Absorbance			Inter-day Absorbance		
	Mean absorbance	± SD	%RSD	Mean absorbance	± SD	%RSD
3	0.3426	0.0002	0.0583	0.3427	0.0003	0.0875
5	0.4531	0.00015	0.0331	0.4534	0.0004	0.0882
7	0.5820	0.00011	0.0189	0.5822	0.0005	0.0858

SD: standard deviation, RSD: Relative standard deviation

Table no.4- Precision study of Fluticasone propionate:

Conc. (µg/ml)	Intra-day Absorbance			Inter-day Absorbance		
	Mean absorbance	± SD	%RSD	Mean absorbance	± SD	%RSD
3	0.0830	0.00020	0.2409	0.0834	0.00035	0.4196
5	0.1211	0.00011	0.0908	0.1212	0.00050	0.4125
7	0.1592	0.00015	0.0942	0.1594	0.00030	0.1882

SD: standard deviation, RSD: Relative standard deviation

RESULTS AND DISCUSSION

From the dissolution point of view, attempt was made to dissolve both drugs in phosphate buffer pH 7.4 by using ethanol. The proposed method for determination of SX and FP showed molar absorptivity 6147.28068 L/mol.cm and 1391.61794 L/mol.cm respectively. The calibration curve of SX and FP plotted at 214 nm and 265 nm respectively (Figure 1 and 2) a linear relationship was obtained between 5 to 14 µg/ml for salmeterol xinafoate and 2 to 14 µg/ml for fluticasone propionate.

Further the simultaneous estimation of marketed capsule formulation was carried out and found to be in the range of 99.82 to 99.89% w/w (Table 1). The accuracy of method was determined by calculating mean percentage recovery. It was found to be within range of 99.94-100.59% for both the drugs (Table 2). Precision was calculated as repeatability, inter and intraday variations and % RSD was less than 1 for both drugs (Table 3 and 4). The LOD value was found to be 0.2386 and 0.42 while LOQ value was found to be 0.1057 and 0.3205 for SX and FP respectively.

CONCLUSION

The proposed spectrophotometric method is accurate, precise, economic and reliable for the simultaneous measurement of SX and FP in combined dosage form. The % RSD for all parameters were found to be less than one, which revealed the validation of new method and assay results obtained by this method are fairly satisfactory. Hence, it can be concluded that the developed UV spectrophotometric method can be employed successfully as an alternative for HPLC and HPTLC methods for the quantitative estimation of SX and FP in combined dosage form.

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