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## Simultaneous Estimation of Salmeterol Xinafoate and Fluticasone Propionate in Bulk and Capsule Dosage Form by Different UV Spectrophotometric Methods



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**Keywords:** Salmeterol xinafoate, Fluticasone propionate, Simultaneous equation method, Absorbance ratio method, Area under the curve, Capsule dosage form.

### ABSTRACT

A simple, precise and accurate three different UV-Spectrophotometric methods have been developed for estimation of Salmeterol xinafoate and Fluticasone propionate. Method A is simultaneous equation method for which estimation of Salmeterol xinafoate and Fluticasone propionate was carried out at 216.5 nm and 236 nm respectively. Method B is the Absorbance ratio method which is based on the ratio of absorbance at two selected wavelengths one which is an absorptive point and other being the  $\lambda$  max of one of the two components. The overlay spectra of two drugs, it is evident that Salmeterol xinafoate and Fluticasone propionate show an isoabsorptive point at 262.5 nm. Another wavelength selected as 236 nm of Fluticasone propionate. For Method C detection of Salmeterol xinafoate and Fluticasone propionate were selected as 214 nm - 218 nm and 234 nm - 238 nm respectively for the area under curve method. Linearity for Salmeterolxinafoateand Fluticasone propionate was between 2-12  $\mu\text{g/mL}$  respectively. These methods were successfully applied for estimation of Salmeterol xinafoate and Fluticasone propionate in routine analysis work.

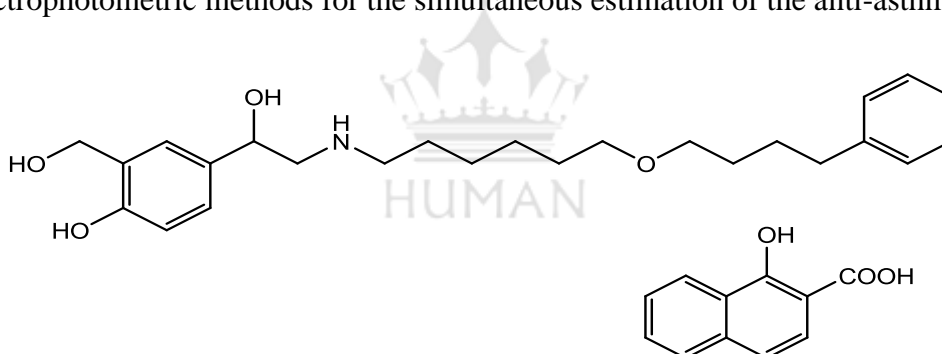


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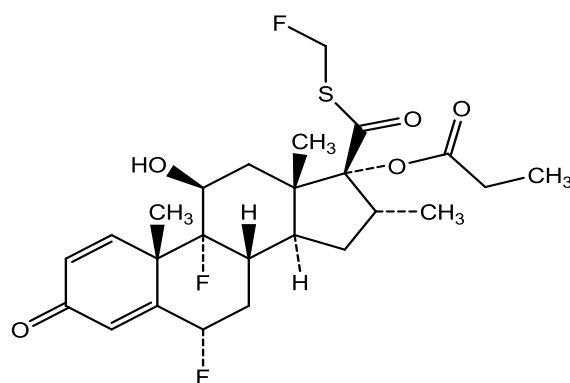
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## INTRODUCTION:

Salmeterol xinafoate is chemically, 2-(hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl]phenol; 1-hydroxynaphthalene-2-carboxylic acid. Salmeterol xinafoate is used in the treatment of asthma and chronic obstructive pulmonary disease [1,4]. Fluticasone propionate is chemically, [(6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-17-(fluoromethylsulfonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] propionate. It is used in the treatment of asthma, allergic rhinitis, and atopic dermatitis [2, 5]. Literature survey reveals that UV spectroscopy, HPLC, LC-MS, have been reported for the determination of Salmeterol xinafoate. Liquid chromatography coupled with APCI-MS, RP- HPLC, tandem mass spectrometers, have been reported for analysis of Fluticasone propionate. [7]. Simultaneous determination of both drugs is reported by HPLC and HPTLC, no different spectrometric method in methanol is reported for both drugs in dosage forms [8, 9]. Hence, the present work was a good attempt to develop and validate simple, precise and accurate UV- Spectrophotometric methods for the simultaneous estimation of the anti-asthmatic drug.



**Fig.1: Structure of Salmeterol xinafoate**



**Fig. 2: Structure of Fluticasone Propionate**

## 1. MATERIALS AND METHODS:

### 2.1 Apparatus

A Jasco V-730 double beam UV spectrophotometer was used for all the spectrophotometric measurements with 1cm quartz cells. The samples were weighed on electronic analytical balance (Digital weighing balance Shimadzu).

### 2.2 Materials

All chemicals and reagent used during the project work were of AR grade. Salmeterol Xinafoate and Fluticasone Propionate were provided by Glenmark pharmaceutical Ltd, Sinnar, Nashik.

### 2.3 Method Development

#### 2.3.1 Preparation of stock solution

The accurately weighed quantity of 10 mg of Salmeterol xinafoate was transferred to 10 ml volumetric flask. It was dissolved in mobile phase and volume was adjusted to the mark. The accurately weighed quantity of 10 mg of Fluticasone propionate was transferred to 10 ml This solution is sonicated for 10 minutes to get dissolve and then the volume was adjusted to mark

Methanol was added into the volumetric flasks to dissolve the standards and finally, volume was made up to the mark of the volumetric flask with Methanol to obtain standard solutions of Salmeterol xinafoate (1000 $\mu$ g/mL) and Fluticasone propionate (1000 $\mu$ g/mL) respectively.

#### 2.4 Determination of wavelength of maximum absorption

A standard stock solution of salmeterol xinafoate and Fluticasone Propionate were scanned in the spectrum mode from 400 nm to 200 nm separately. From the spectra of drug  $\lambda$  max of Salmeterol Xinafoate, 216.5 nm [Fig.3] and  $\lambda$  max of Fluticasone Propionate 236nm [Fig.4] were selected for the analysis.

#### 2.4 Solvent selection

After several trials of various solvents like Water, Ethanol, and Acetonitrile, both the drugs were found to be soluble in Methanol AR grade. Therefore Methanol is selected as a solvent.

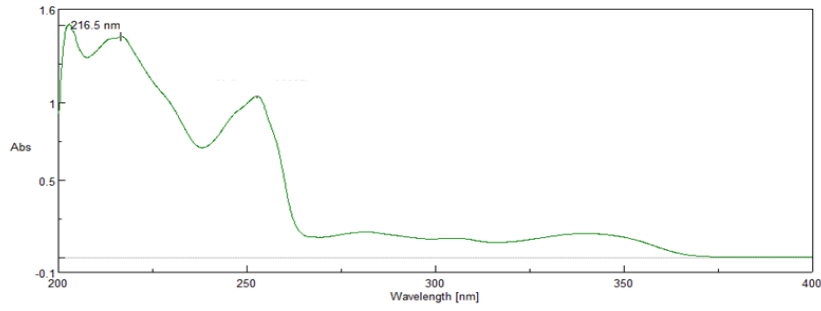
## Calibration curve

### 3.1 Preparation of calibration curve of standard Salmeterol xinafoate and Fluticasone propionate:

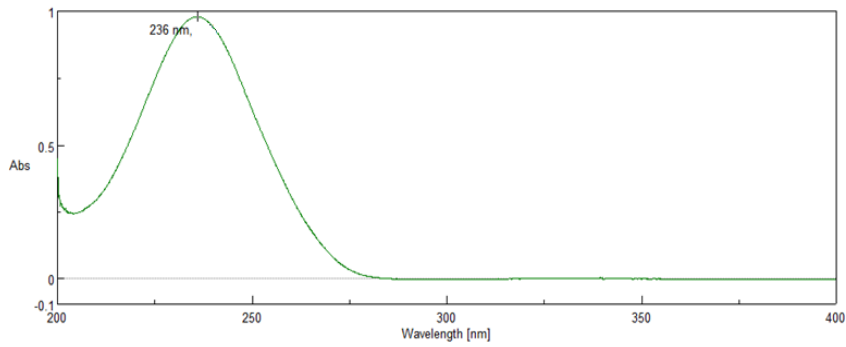
From standard stock solution of Salmeterol xinafoate (1000 $\mu$ g/mL), aliquots of 0.2mL, 0.4mL, 0.6mL, 0.8mL 1.00mL and 1.2mL were withdrawn and transferred to 10mL volumetric flasks. Volume was made up to the mark with Methanol to produce 2 $\mu$ g/mL, 4 $\mu$ g/mL, 6 $\mu$ g/mL, 8 $\mu$ g/mL, 10 $\mu$ g/mL and 12 $\mu$ g/mL of Salmeterol xinafoate respectively. From the working standard solution of Fluticasone propionate (100 $\mu$ g/mL), aliquots of 0.2mL, 0.4mL 0.6mL, 0.8mL, 1.0mL and 1.2mL were transferred to 10mL volumetric flasks and volume was made up to the mark with Methanol to produce 2 $\mu$ g/mL, 4 $\mu$ g/mL 6 $\mu$ g/mL, 8 $\mu$ g/mL, 10 $\mu$ g/mL and 12 $\mu$ g/mL of Fluticasone propionate respectively. Mixed standard solutions of salmeterol xinafoate and Fluticasone propionate were prepared in a ratio of 1:2 as present in the marketed formulation.

### 3.2 The method I: Simultaneous equation method:

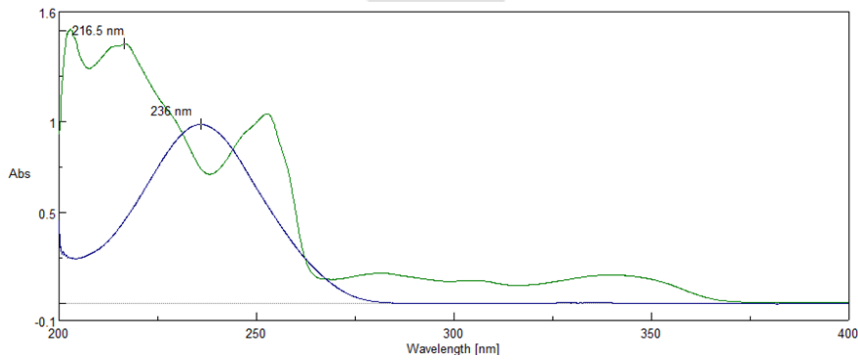
For the simultaneous equation method development, the wavelength absorbance maxima ( $\lambda_{max}$ ) of both drugs are required. The working standard solutions containing 10 $\mu$ g/mL of Salmeterol xinafoate and 10 $\mu$ g/mL of Fluticasone propionate were scanned separately in the range of 200-400 nm for absorbance maxima ( $\lambda_{max}$ ) against Methanol as blank solution. Salmeterol xinafoate shows maximum absorption at 216.5 nm while Fluticasone propionate at 236nm. From the overlain spectra of both, the drugs wavelength selected for quantification were 216.5 nm for Salmeterol xinafoate and 236 nm for Fluticasone propionate. The absorptivity coefficients of these two drugs were determined at a selected wavelength and the concentrations of both drugs are calculated by using the equations (1) and (2). The absorption spectrum was obtained for Salmeterol xinafoate, Fluticasone propionate, and their overlay is shown in fig.5.



**Fig. 3: Absorption spectrum of Salmeterol xinafoate**



**Fig. 4: Absorption spectrum of Fluticasone propionate**



**Fig. 5: Overlay spectrum of SX and FP**

$$c_x = \left( \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \right) \text{----- (1)}$$

$$c_y = \left( \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \right) \text{----- (2)}$$

Where, C<sub>x</sub> and C<sub>y</sub> = the concentrations of Salmeterol xinafoate and Fluticasone propionate respectively in gm/100 mL; A<sub>1</sub> and A<sub>2</sub> = the absorbance of mixture at λ<sub>1</sub> (216.5 nm) and λ<sub>2</sub> (236 nm) respectively a<sub>x1</sub> and a<sub>x2</sub> = the absorptivities of Salmeterol xinafoate at λ<sub>1</sub> (216.5 nm) and λ<sub>2</sub> (236 nm) respectively a<sub>y1</sub> and a<sub>y2</sub> = the absorptivities of the Fluticasone propionate at the λ<sub>1</sub> (216.5nm) and λ<sub>2</sub> (236 nm) respectively.

### 3.3 Method II: Absorbance ratio method (Q-Analysis)

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths one which is an isoabsorptive point and other being the λ max of one of the two components. From the overlay spectra of two drugs, it is evident that Salmeterol xinafoate and Fluticasone propionate shows an isoabsorptive point at 262.5 nm. The second wavelength used is 236 nm which is the λ max of Fluticasone propionate. Six working standard solutions having concentration 2, 4, 6, 8, 10, 12 μg/mL for Salmeterol xinafoate and 2, 4, 6, 8, 10, 12 μg/mL for Fluticasone propionate were prepared in Methanol and the absorbances at 262.5 nm (isoabsorptive point) and 236 nm (λ max of Fluticasone propionate) were measured and absorptivity coefficients were calculated using the calibration curve. The concentration of two drugs in the mixture can be calculated using equations:

$$CX = [(QM - QY) / (QX - QY)] \times A1/ax_1 \dots\dots\dots (3)$$

$$CY = [(QM - QY) / (QX - QY)] \times A2/ay_1 \dots\dots\dots (4)$$

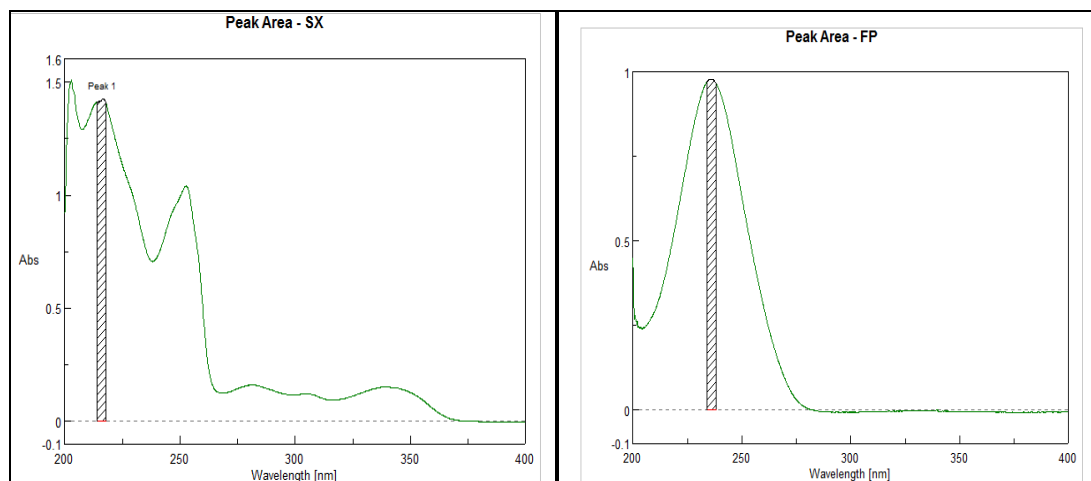
Where, A<sub>1</sub> and A<sub>2</sub> are absorbances of mixture at 262.5 nm and 236 nm, and a<sub>x1</sub> and a<sub>y1</sub> are absorptivities of Salmeterol xinafoate and Fluticasone propionate at 262.5 nm, a<sub>x1</sub> and a<sub>y1</sub> are absorptivities Salmeterol xinafoate and Fluticasone propionate of respectively at 236 nm and

$$QM = A2 / A1, QX = a_{x2} / a_{x1} \text{ and } QY = a_{y2} / a_{y1}.$$

### 3.4 Method III: Area under the curve

For the simultaneous estimation using the area under curve method, the working standard solution containing 10 μg/mL of salmeterol xinafoate and 10 μg/mL Fluticasone propionate were scanned separately in the range of 200-400nm for absorbance maxima (λ<sub>max</sub>) against Methanol as blank solution. Salmeterol xinafoate shows maximum absorption at 216.5 nm while Fluticasone propionate at 236 nm. The area under the curve of both the drugs was determined at the selected wavelengths in the range of 214 nm – 218 nm (SX) and 234 nm -

238 nm (FP). The absorptivity coefficients of these two drugs were determined at the selected area under a curve and the concentrations of both drugs are calculated by using the equations (5) and (6). The area under the curve spectrum was obtained for Salmeterol xinafoate and Fluticasone propionate is shown in Fig.6.



**Figure.6 AUC for salmeterol xinafoate and Fluticasone propionate**

$$CA = \frac{(XB_2).(AUC M_1) - (XB_1).(AUC M_2)}{(XB_2).(XA_1) - (XB_1).(XB_2)} \quad \text{----- (5)}$$

$$CB = \frac{(XA_2).(AUC M_2) - (XA_1).(AUC M_1)}{(XB_2).(XA_1) - (XB_1).(XB_2)} \quad \text{----- (6)}$$

#### 4. Method validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

##### 4.1 Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-14 µg/ml for each SX and FP. Accurately measured standard stock solutions of each SX and FP (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of the solution were then measured at 216.5 nm

and 236 nm. The calibration curves were by plotted by absorbances versus concentration and the regression equations were calculated.

#### *4.2 Precision*

##### *4.2.1 Intraday precision*

The intraday precision of the proposed method was determined by estimating the corresponding responses 3 times on the same day for 3 different concentrations of standard solutions of SX and FP (4, 8 and 12 $\mu$ g/ml). The results were reported in terms of relative standard deviation (% RSD).

##### *4.2.2 Interday precision*

The interday precisions of the proposed method were determined by estimating the corresponding responses on 3 different days over a period of one week for 3 different concentrations of standard solutions of SX and FP (4, 8 and 12 $\mu$ g/ml). The results were reported in terms of relative standard deviation (% RSD).

#### *4.3 Accuracy*

The accuracy of the method was determined by preparing solutions of different concentration i.e. 80%, 100%, 120%. Three samples at each concentration were prepared and scanned in UV-Spectrophotometer.

#### *4.4 Assay of marketed formulation*

20 capsules of marketed capsule formulation of SX and FP50/100 (Esiflo 100) were weighed; the correct amount of drugs powder equivalent to label claim of "Esiflo 100" was weighed and transferred to 50 ml volumetric flask, dissolved in 30 ml of dissolution media and sonicated for 15 min. The volume was then made up to the mark and the resultant solution was filtered through a 0.41 $\mu$ m membrane filter. The filtrate was having concentration 5 $\mu$ g/ml for SX and 10 $\mu$ g/ml for FP.

#### *4.5 Robustness*

Robustness of the method was determined by carrying out analysis of different wavelength for SX (214.5 nm and 216.5 nm) and FP (234 nm and 236 nm).



#### 4.6 Ruggedness

Ruggedness was carried out by two analyst and results was indicated by % RSD.

### 5. RESULTS AND DISCUSSION:

The present methods for simultaneous estimation of Salmeterol xinafoate and Fluticasone propionate in combined dosage form were found to be accurate, simple, and precise. The developed methods can be used for analysis of two drugs in combined dosage forms. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the  $\lambda$  max of one of the two components. From the overlay spectra of two drugs, it is evident that Salmeterol xinafoate and Fluticasone propionate shows an isoabsorptive point at 262.5 nm. The area under curve method involves formation and solving of simultaneous equation. Once the equations are formed, then the only measurement of the area of sample solution at two wavelength ranges and simple calculations are required. It can be easily and conveniently adopted for routine quality control analysis.

#### Linearity

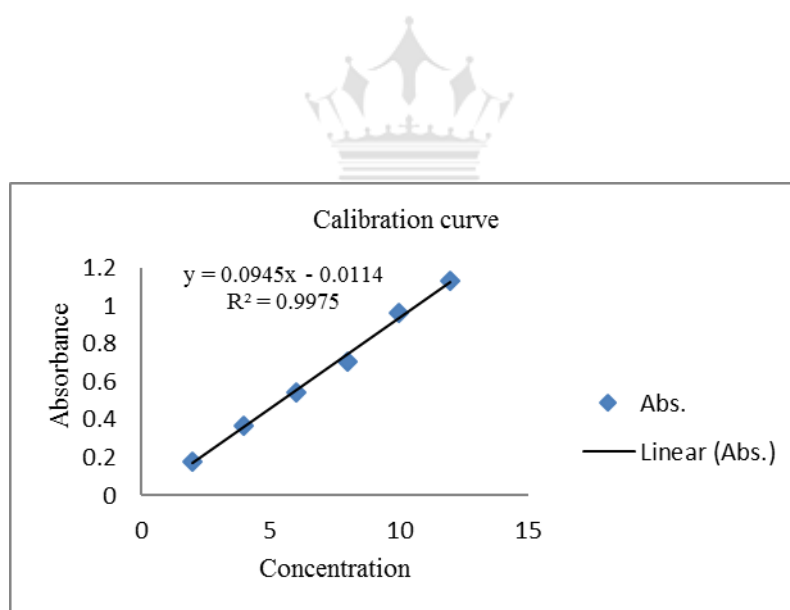


Figure no. 7 Linearity curve for Salmeterol xinafoate

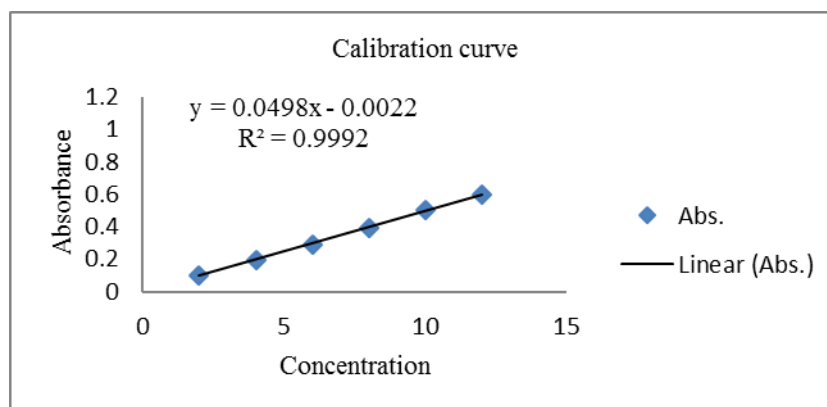


Figure no. 8 Linearity curve for fluticasone propionate

### Precision

Table 1: Results of Precision

Dosage form	Concentration (µg/ml)	Interday precision*(n=3)			Intraday precision*(n=3)		
		Conc. found	SD	% RSD	Conc. found	SD	% RSD
Salmeterol xinafoate	4	3.9	0.0016	0.4391	3.9	0.0020	0.5735
	8	7.9	0.0003	0.0417	7.9	0.0004	0.0584
	12	11.8	0.0017	0.1524	11.9	0.0012	0.1118
Fluticasone propionate	4	4	0.0005	0.2752	4	0.0004	0.2301
	8	7.9	0.0013	0.3319	7.9	0.0012	0.3213
	12	11.9	0.0014	0.2408	11.9	0.0009	0.1623

SD: Standard deviation, RSD: Relative standard deviation, \*: Mean of three estimations.

### Accuracy (Recovery study)

Table 2: Recovery study (Accuracy)

Dosage form	Level of % recovery	Amt. of drug*	Amt. of drug found	% Recovery	SD	% RSD
Salmeterol Xinafoate	80	9	8.99	100.01	0.0051	0.6171
	100	10	10.07	100.7	0.0016	0.1792
	120	11	11.06	101	0.0114	1.1035
Fluticasone propionate	80	18	18	100.1	0.0034	0.3876
	100	20	19.9	99.85	0.0055	0.5637
	120	22	22.2	101.4	0.0016	0.1471

Amt: Amount, \*: Mean of three estimations.

**Assay of marketed formulation**

**Table 3: Results of UV analysis for marketed capsule formulation**

Capsule content	Label claim (µg/ml)	% label claim	SD*	% RSD*
Salmeterol xinafoate	50	98.86	0.0084	0.9085
Fluticasone propionate	100	98.83	0.0021	0.4352

\*: Mean of six estimations.

**Robustness (Different wavelength)**

**Table 4: Results for robustness from different wavelength)**

Dosage form	Conc.taken* (µg/ml)	Conc. found (µg/ml)		% purity		SD	% RSD	
		Wavelength						
		214.5	216.5	214.5	216.5		214.5	216.5
Salmeterol xinafoate	10	9.9	9.9	99.8	99.9	0.0017	0.0150	0.1820
		234	236	234	236	234	236	234
Fluticasone propionate	10	9.9	10	99.9	100.4	0.0058	0.0043	1.1767

\*: Mean of six estimations, Conc: Concentration.

**Ruggedness (Different analyst)**

**Table 5: Results for ruggedness from different analyst**

Dosage form	Conc.taken* (µg/ml)	Conc. found (µg/ml)		% purity		SD	% RSD	
		Wavelength						
		A-I	A-II	A-I	A-II		A-I	A-II
Salmeterol xinafoate	10	10	10	101	100.9	0.0055	0.0069	0.5842
		A-I	A-II	A-I	A-II	A-I	A-II	A-I
Fluticasone propionate	10	10.1	10	100.5	101.4	0.0058	0.0049	1.1621

\*:Mean of six estimations, A-I: Analyst first, A-II: Analyst second.

## 6. CONCLUSION:

The present work for simultaneous estimation of Salmeterol xinafoate and Fluticasone propionate in combined dosage form was found to be accurate, simple, and precise. Since not a single method was reported for simultaneous analysis of the two drugs earlier, the developed methods can be used for routine analysis of two drugs in combined dosage forms. This study is useful because these two drugs are commonly used for the anti-asthmatic drug.

## 7. ACKNOWLEDGMENT:

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