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ORIGINAL ARTICLE

New developed spectrophotometric method for simultaneous determination of salmeterol xinafoate and fluticasone propionate in bulk powder and Seritide® diskus inhalation

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Binary mixture

Abstract A new simple, accurate, precise, rapid and economical method was developed for the simultaneous determination of Salmeterol xinafoate and Fluticasone propionate in their binary mixture in bulk powder and Seritide diskus® inhalation. The new method depends on new calculations using the mixture's absorbance at 225 and 256.5 nm where the absorptivity of Salmeterol xinafoate is double the absorptivity of Fluticasone propionate, while the content of Salmeterol xinafoate was determined by measuring the absolute value of the first derivative ultraviolet curves at 352 nm, without interference from Fluticasone propionate. The proposed method was validated and the results obtained by adopting the proposed method were statistically analyzed and compared with those obtained by reported methods.

1. Introduction

Salmeterol xinafoate and Fluticasone propionate are formulated together in an inhalation known as Seritide® Diskus inhalation, used to treat asthma. Salmeterol xinafoate is grouped in a class of medications called β_2 adrenergic receptors agonists. Fluticasone propionate is grouped in a class of medications called corticosteroids. The combination of Salmeterol xinafoate and Fluticasone propionate works by reversing the bronchoconstriction occuring in asthma.

Salmeterol xinafoate was determined by several methods including spectrophotometry[1–3], HPTLC[4] HPLC[5–7] and electrophoresis.[8] Fluticasone propionate was determined by spectrophotometric methods[2,9,10] and by HPLC methods.[6,7,11–13]

The aim of this work, to develop new method that can be applied for the determination of the concentrations of two component of different absorptivities in binary mixture, so this method has advantage of wide application over the classical isoabsorptive point method[14–16] that restricted for the components of similar absorptivities at certain point and to apply this method for the simultaneous determination of salmeterol xinafoate and

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Fluticasone propionate in the mixture in Seritide Diskus inhalation. The absorptivity factor is a novel method and it depends on applying a simple mathematical equation to calculate the concentration of both components in such a binary mixture. In this mixture like many other mixtures there is a large difference in the absorptivity of each drug from the absorptivity of the other so there is no isoabsorptive point occurs. In this case the simultaneous determination of each drug in the presence of the other can't be carried out by applying the isoabsorptivity technique. In this work a new method was developed by modifying the classical isoabsorptive method. The new method called absorptivity factor method can solve this problem and enable the annalists to carry out the simultaneous determination of such mixtures using zero order spectra of the mixture by substitution in a simple equation without the need to use an advanced software or to carry out several trials like those required in aother spectrophotometric techniques. The aim of that work is to use the newly developed method for the simultaneous determination of salmeterol xinafoate and Fluticasone propionate in the mixture in Seritide® Diskus inhalation.

2. Theory

For the two drugs X and Y, in the mixture, where concentration of Y can be determined by using any of the well established spectrophotometric methods.

For the determination the concentration of X, The absorptivity factor method is applied .This method depends on the calculation of the absorptivity factor which is the ratio between the two absorptivities (a_x, a_Y) at intersection point with the same absorbance value. This point is called the absorbtivity factor point (λ_F). This is summarized as follows:

$$A_x = A_y$$
$$a_x b_x C_x = a_y b_y C_y$$
$$\text{Where } b_x = b_y = 1Cm$$
$$a_x C_x = a_y C_y$$
$$a_x/a_y = C_y/C_x$$
$$a_x/a_y = F$$
$$a_x = Fa_y$$

where, F is the absorptivity factor, $\boldsymbol{a_x}$, $\boldsymbol{a_Y}$ are the absorptivities of X and Y' respectively.

For mixture of X and Y the total absorbance of X and Y at absorptivity factor point λ_F can be expressed as follows:

$$A_m = A_x + A_y$$
$$A_m = a_x b_x C_x + a_y b_y C_y \quad (\text{Where } b_x = b_y = 1Cm)$$
$$A_m = a_x C_x + a_y C_y$$

where A_X, A_y and A_m are the absorbance of X, Y and their mixture at λ_F respectively C_x and C_Y are the concentrations of X and Y respectively a_x and a_Y are the absorptivities of X and Y at λ_F respectively. a_x is substituted by Fa_y

$$A_m = a_y F C_x + a_y C_y$$
$$A_m = a_y(FC_x + C_y)$$

So, the total concentration of the mixture($FC_x + C_Y$) can be calculated by using a regression equation representing the linear relationship between the absorbance of Y and its corresponding concentration at the absorptivity factor point.

The concentration of X can be determined after subtraction of concentration of Y and multiplication by the inverse of F

$$(FC_x + C_y) - C_y$$
$$C_x = FC_x \cdot \mathbf{1/F}$$

3. Experimental

3.1. Apparatus

UV–Visible spectrophotometer, Shimadzu UV-1800. connected to an IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The absorption spectra of the reference and test solutions were carried out in 1 cm quartz cells.

3.2. Chemicals and pharmaceutical preparation

- Salmeterol xinafoate pure sample was kindly provided by ADCO Co., Cairo, Egypt while Fluticasone propionate pure sample was kindly provided by Galaxo Wellcome, Cairo, Egypt. their percentage purity was certified to be 99.87 ± 0.342 and 100.31 ± 0.52, respectively.
- Seritide® diskus inhaler batch No. R467369 (Galaxo-SmithKline group) purchased from Egyptian market labeled to contain 50 μg Salmeterol xinafoate and 100 μg Fluticasone propionate/dose.

3.3. Standard stock and working solutions

Stock standard solutions of Salmeterol xinafoate and Fluticasone propionate were prepared separately by dissolving 40 mg of each drug in methanol and completing the volume to 100 mL using the same solvent. Working stock solutions were prepared by diluting 20 mL of each stock solution to 100 mL in a volumetric flask using the same solvent to get working solutions containing 80 μg/mL of Salmeterol xinafoate or Fluticasone propionate. Series of concentration of each drug is prepared by diluting this working solutions in a series of 10 mL volumetric flasks to obtain a concentration range of 4–60 μg/mL for Salmeterol xinafoate and 4–28 μg/mL for Fluticasone propionate.

3.4. Analysis of laboratory prepared mixtures

From the first derivative spectra of the laboratory prepared mixtures the concentration of Salmeterol xinafoate obtained at 352 nm by substituting in regression equation, the corresponding concentration of Fluticasone propionate is obtained by subtracting the concentration of Salmeterol xinafoate from the results obtained by substitution in regression equations at absorptivity factor points (225 nm and 256.5 nm) then multiplying by two.

4. Results and discussion

4.1. Method of analysis

The zero order absorption spectra of 8 μg mL^{-1} of Salmeterol xinafoate and 16 μg mL^{-1} of Fluticasone propionate were

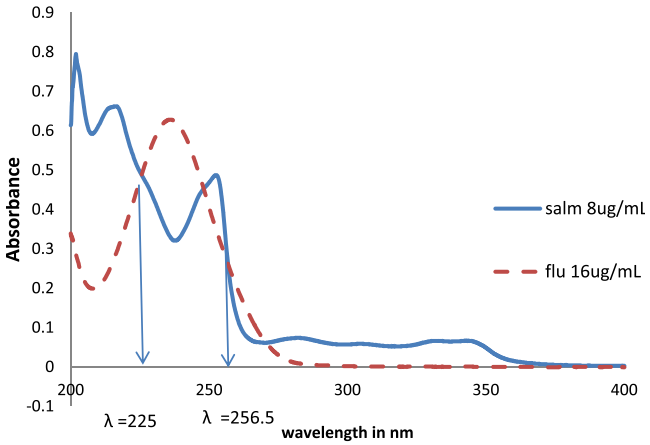

Figure 1 The Zero order spectra of 8 μg/mL Salmeterol xinafoate and 16 μg/mL Fluticasone propionate showing the absorptivity factor points.

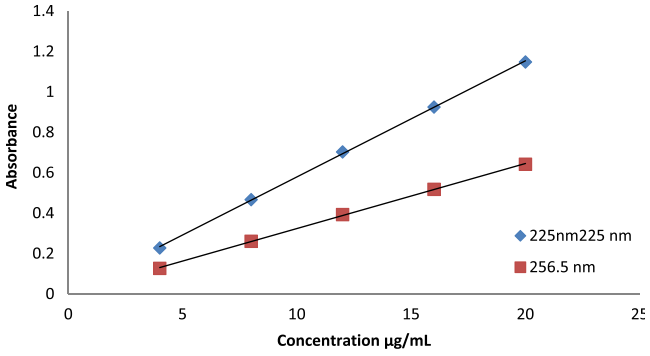

Figure 2 Calibration curves of Salmeterol Xinafoate at 225, 256.5.

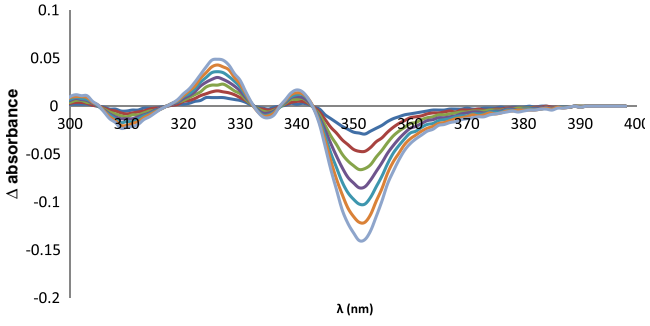

Figure 3 First derivative spectra of Salmeterol xinafoate (12–60 μg/mL), $\Delta\lambda = 4$ nm showing a peak at 352 nm.

recorded (Fig. 1). Two crossing points were obtained where the absorptivity of Salmeterol xinafoate is double that of the Fluticasone propionate ($F = ½$) to construct the calibration curve of Salmeterol xinafoate at chosen absorptivity factor points 225 and 256.5 nm (Fig. 2). For this mixture absorptivity of salmeterol xinafoate can be determined without the interference of Fluticasone propionate by first derivative spectra at 352 nm (Fig. 3) so the calibration curve of the first derivative spectra of salmeterol xinafoate is also constructed at 352 nm taking $\Delta\lambda$ equals 4 nm (Fig. 4).the concentration of Fluticasone propionate is then calculated by subtraction and multiplying by $1/F$. This method is considered as a modification of isoabsorptive technique[14–16] but in the isoabsorptive technique the spectra of the same concentration of the two studied drugs should cross at a point called isoabsorptivity point at which they have equal absorptivities while in absorpitivity factor method the crossing point did not occur at equal concentration so the crossing point is obtained between different concentrations of the two drugs. At this point the absorptivities of the two drugs are not equal but they are equal to the inverse of the ratio of the used concentrations.

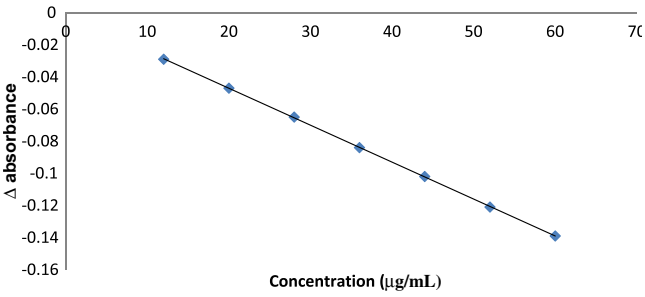

Figure 4 Calibration curve of first derivative spectra of Salmeterol xinafoate at 352 nm, $\Delta\lambda = 4$ nm.

4.2. Mixture analysis

The first derivative spectra of the mixture spectra are found and the concentration of salmeterol xinafoate is found by substitution in the first derivative linearity equation at 352 nm. Then by using the zero order values at 225 and 256.5 nm the sum of salmeterol xinafoate and half the concentration of Fluticasone propionate can be found by substitution in zero order linearity equations of salmeterol xinafoate at those points, the concentration of Fluticasone propionate can be calculated by subtraction then multiplying by two

4.3. Analysis of pharmaceutical products

The proposed Absorptivity factor method was applied for the simultaneous determination of Salmeterol xinafoate and Fluticasone propionate in Seritide® Diskus inhaler. The performance of the method was statistically compared with that of a reported method[6] by Student's *t*-test and *F*-values at 95% confidence level. The calculated *t* and *F*-values did not exceed the tabulated values (Table 6), indicating that there was no significant difference between the results of the developed and those of the reported method.

4.4. Validation of the methods

4.4.1. Linearity

The linearity ranges for the determination of Salmeterol xinafoate and Fluticasone propionate by the proposed method was evaluated by analyzing a series of different concentrations of each drug. Fluticasone propionate gave a linearity range 4–28 μg/mL at chosen points while Salmeterol xinafoate gave a linearity range 4–20 μg/mL at chosen points and 12–60 μg/mL

Table 1 Validation parameters of constructed calibration curves of Salmeterol xinafoate zero order spectra at 225 nm and 256.5 and first derivative at 352 nm.

	SAM		
λ (nm)	352 (first derivative)	225	256.5
Linearity range (μg/mL)	12–60	4–20	4–20
LOD (μg/mL)	0.022	0.101	0.104
Slope	−0.00229	0.05745	0.03218
SE of slope	−7.7E-06	0.000582	0.000335
Intercept	−0.00109	0.0048	0.0021
SE of intercept	0.000304	0.007717	0.00444
r^2	0.9999	0.9997	0.9997
r	−1.000	0.9998	0.9998

Table 2 Precision of determination of Salmeterol xinafoate and Fluticasone propionate by the proposed and absorptivity factor method.

		Salmeterol xinafoate			Fluticasone propionate[a]					
		352 nm (first derivative)			At 225 nm			At 256.5 nm		
		8 μg/mL	12 μg/mL	20 μg/mL	8 μg/mL	12 μg/mL	20 μg/mL	8 μg/mL	12 μg/mL	20 μg/mL
Intraday	1	101.02	100.26	101.23	98.51	100.22	101.46	99.92	100.29	100.85
	2	100.36	100.89	100.33	101.34	102.08	100.08	98.06	99.85	101.92
	3	100.88	98.91	100.76	100.19	100.68	99.42	98.93	102.22	100.06
	X	100.75	100.02	100.77	100.01	100.99	100.32	98.97	100.79	101.28
	±SD	0.348	1.012	0.45	1.423	0.969	1.041	0.93	1.261	0.567
	%RSD	0.351	1.012	0.453	1.423	0.979	1.044	0.920	1.271	0.574
Interday	1	100.72	100.69	99.52	100.22	100.89	99.65	100.82	99.85	98.93
	2	100.96	100.85	101.35	99.07	101.35	99.65	101.42	102.04	100.25
	3	98.42	101.68	101.02	99.57	100.66	101.06	100.74	100.26	99.77
	X	100.03	101.07	100.63	99.62	100.97	100.12	100.99	100.72	99.65
	±SD	1.402	0.531	0.975	0.577	0.351	0.814	0.372	1.164	0.668
	%RSD	1.402	0.531	0.975	0.577	0.351	0.814	0.372	1.164	0.668

[a] After subtraction and multiplication by 2.

Table 3 Determination of Salmeterol xinafoate and Fluticasone propionate by the proposed absorptivity factor method.

Flu:Sam ratio	Salmeterol xinafoate			Fluticasone propionate				
	Taken (μg/mL)	Found (μg/mL)	% recovery	Taken (μg/mL)	Found[a] at 225 (μg/mL)	% recovery	Found[a] at 256.5 (μg/mL)	% recovery
2.5:3	12	12.12	101.00	10	10.07	100.75	10.20	101.99
2:2	8	7.86	98.19	8	7.87	98.43	7.99	99.91
2:3	12	12.09	100.75	8	7.87	98.38	7.89	98.59
2:4	16	16.04	100.88	8	8.36	102.07	8.28	101.11
3:3	12	12.13	100.25	12	11.55	97.94	11.74	99.50
3:4	16	15.89	99.33	12	11.90	99.18	12.21	101.71
3:5	20	19.96	99.78	12	11.65	97.05	11.94	99.51
4:2	8	7.98	99.76	16	15.67	97.94	15.98	99.88
4:3	12	11.90	99.13	16	15.96	99.74	16.33	102.06
4:4	16	15.78	98.64	16	15.81	98.84	16.23	101.46
4:5	20	19.96	99.78	16	15.76	98.48	16.19	101.17
5:2	8	7.78	97.28	20	19.93	99.67	20.18	100.92
5:3	12	11.70	97.46	20	19.81	99.03	20.04	100.18
5:4	16	16.04	100.27	20	19.40	97.01	19.71	98.53
X			99.46			98.89		100.46
SD			1.202			1.369		1.189
%RSD			1.209			1.384		1.184
N			14.00			14.00		14.00

[a] After subtraction and multiplication by 2.

Table 4 Application of the standard addition technique for the determination of Salmeterol xinafoate and Fluticasone propionate in their pharmaceutical dosage form by the proposed absorptivity factor method.

Salmeterol xinafoate			Fluticasone propionate				
Standard added (μg/mL)	Found at 352[a] (μg/mL)	% recovery	Standard added (μg/mL)	Found[b] at 225[a] (μg/mL)	% recovery	Found[b] at 256.5[a] (μg/mL)	% recovery
4	4.02	100.5	4	4.05	101.25	4.03	100.75
6	5.98	99.67	6	5.88	98	6.07	101.17
8	7.99	99.88	8	7.92	99	7.95	99.38
X		100.01			99.42		100.43
±SD		0.438			1.665		0.938
%RSD		0.438			1.675		0.934
N		9			9		9

[a] Average of three determinations.
[b] After subtraction and multiplication by 2.

Table 5 Statistical analysis of the results obtained by applying the proposed absorptivity factor method and the reported method for the analysis of Salmeterol xinafoate and Fluticasone propionate pure samples.

Method	Salmeterol xinafoate		Fluticasone propionate		
	Reported method[6]	absorpativity Factor method	Reported method[6]	absorpativity Factor method	
λ (nm)		352 (1st derivative)		225	256.5
X ± SD[a]	100.44 ± 0.502	100.29 ± 1.117	100.31 ± 0.719	99.83 ± 1.405	100.24 ± 0.605
N	5	5	5	5	5
t (2.306)		0.270		0.683	0.162
F (6.338)		4.951		3.818	1.412

[**]Eight degrees of freedom.
[a] Average of five determinations.

Table 6 Statistical analysis of the results obtained by applying the proposed absorptivity factor method and the reported method for the analysis of Salmeterol xinafoate and Fluticasone propionate in their pharmaceutical dosage form.

Method	Salmeterol xinafoate		Fluticasone propionate		
	Reported method[6]	absorpativity Factor method	Reported method[6]	absorpativity Factor method	
λ (nm)		352 (1st derivative)		225	256.5
X ± SD[a]	100.29 ± 1.226	100.73 ± 1.298	100.58 ± 0.805	101.16 ± 1.313	100.57 ± 1.510
N	5	5	5	5	5
t (2.306)[b]		0.549		0.76	0.096
F (6.338)		1.120		2.660	3.519

[a] Average of five determinations.
[b] Eight degrees of freedom.

in the first derivative technique. Each concentration was repeated three times; in order to provide information on spectrophotometric values between samples of same concentration. The linearity of the calculated graphs was validated by the high value of the correlation coefficient and law intercept value (Table 1). Characteristic parameters for regression equations of the studied methods obtained by least squares treatment of the results are given in Table 1. The obtained calibration curves of Salmeterol xinafoate at the absorptivity factor points (225 and 256.5 nm) are shown in Fig. 2 and the calibration curve of the first derivative spectra of salmeterol xinafoate at 352 nm is shown in Fig. 4.

4.4.2. Detection limits

According to the International Conference on Harmonization (ICH) recommendations, the approach based on the standard deviation (SD) of the response and the slope was used for determining the detection limits. The theoretical values for the proposed methods were assessed practically and given in Table 1.

4.4.3. Precision

The precision of the methods was evaluated by calculating the relative standard deviation of the interday and intraday assay

results. The mean relative standard deviations are presented in Table 2 and can be considered to be satisfactory.

4.4.4. Selectivity

Method selectivity was achieved by preparing several laboratory-prepared mixtures of the studied compounds at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed according to the previous procedure described under the proposed method. Satisfactory results were obtained (Table 3) indicating the high selectivity of the proposed method for the simultaneous determination of Salmeterol xinafoate and Fluticasone propionate binary mixture.

4.4.5. Accuracy

Accuracy of the method was determined by the analysis of some salmeterol xinafoate and Fluticasone propionate pure samples by the proposed absorptivity factor method and the results were compared with the results obtained by a reported method (Table 5). The interference of excipients in the pharmaceutical formulations was studied by the absorptivity factor method. For this reason, standard addition method was applied to the pharmaceutical formulation containing these compounds. In the application of standard addition method to the pharmaceutical formulation, the mean percentage recoveries and their standard deviation for the proposed method for nine replicates were calculated (Table 4). According to the obtained results the excipients in pharmaceutical formulation do not interfere in the analysis of these compounds in the pharmaceutical formulation.

5. Conclusion

For routine analytical purposes, it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with high accuracy and precision. Spectrophotometric techniques can generate large amounts of data within a short period of analysis, the developed absorptivity factor method has many advantages as it is a novel spectrophotometric method and it depends on applying a simple mathematical equation to calculate the concentration of both components in the binary mixture with no need for a special expensive software or an advanced expensive device, also it doesnot need any special reagents or high grade solvents which make it a simple, economic, and time saving method as well as an accurate, precise, and problem solving method.

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