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Research Article

Different spectrophotometric methods applied for simultaneous analysis of binary mixture of formoterol and fluticasone: a comparative study

Abstract

The quantitative analysis is of an interesting issue for the analytical chemistry especially if the compounds of interest have been analyzed in term of sensitivity, low cost, without previous separation and saving analysis time; in this article simultaneous quantitative analysis of binary mixture of formoterol and fluticasone by four different spectrophotometric methods have been discussed and compared these methods namely, simultaneous equation, graphical absorbance ratio, absorbance subtraction and amplitude modulation. The proposed methods were simple, sensitive, accurate and precise. They do not need any sophisticated apparatus and could be easily applied in quality control laboratories. Linearity of the proposed methods was investigated in the range of 2-14µg/ml for formoterol and 5-40µg/ml for fluticasone. All the methods were validated according to the ICH guidelines, statistically compared with a reported method .Another statistical comparison of the obtained results by the proposed methods and the reported method were done using one-way ANOVA test. According to the results obtained by applying these methods there is no significant difference between all of them.

Keywords: formoterol, fluticasone, simultaneous equation, graphical absorbance ratio, absorbance subtraction; amplitude modulation

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Nasr Mohamed A El-Abasawy, Khalid Abdel-Salam M Attia, Ahmed A Abouserie, Ahmed El-Olemy, Ayman Osman Elsayed
Department of Pharmaceutical Analytical Chemistry, Al-Azhar University, Egypt

Correspondence: Ayman Osman Elsayed, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, 11751 Nasr City, Cairo, Egypt, Tel +20 1000785052, Email ayman_phd2015@yahoo.com

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Introduction

Formoterol fumarate dihydrate is N-[2-Hydroxy-5-(1-hydroxy-2-{[2-(4methoxyphenyl)-1-methylethyl] amino} ethyl) phenyl] formamide fumarate, (Figure 1). It's a fine white powder soluble in water and methanol; slightly soluble in ethanol and chloroform with a molecular formula C42H56N4O14 and molecular weight 840.92.[1] Formoterol is a long-acting beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease.[2] Fluticasone propionate is S-(fluoromethyl)-6α,9-difluoro-11β,17-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioate,17 propanoate (Figure 2). It's a white powder practically insoluble in water, freely soluble in dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF); slightly soluble in methanol and 95% ethanol with a molecular formula $C_{25}H_{31}F_3O_5S$ and molecular weight 500.58.[1] It's a synthetic trifluorinated glucocorticoid receptor agonist with anti-allergic, anti-inflammatory used as powder or aerosol inhalation for the prophylaxis of asthma and treatment of allergic rhinitis.[2] Flutiform is an inhaler (a pressurized inhalation, suspension) which contains two active ingredients; Formoterol fumarate dihydrate and fluticasone propionate, together these two active ingredients helps to prevent breathing problems such as asthma and helps to stop breathless and wheezing.[3] There are few reported chromatographic methods for analysis of both components simultaneously; these were high-performance liquid-chromatography[4–6] and thin layer chromatography[7] but to date there is no reported UV spectrophotometric method for simultaneous determination of formoterol and fluticasone.

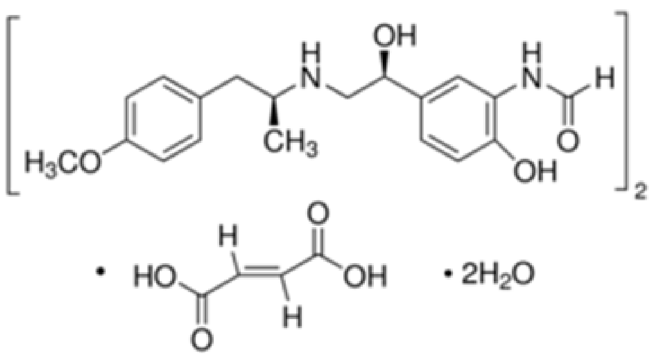

Figure 1 Structural formula of formoterol fumarate dehydrate.

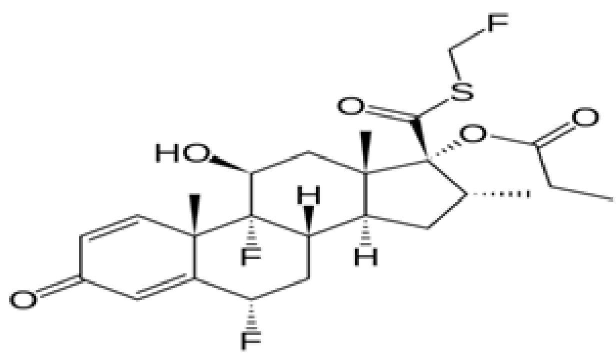

Figure 2 Structural formula of Fluticasone propionate.

In the present work, a comparative study using one-way ANOVA test was done between four methods as first reported spectrophotometric methods for the simultaneous determination of formoterol and fluticasone; namely simultaneous equation,[8–11] graphical absorbance ratio,[12–14] absorbance subtraction[15,16] and amplitude modulation.[17,18]

Experiment

Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/ Japan), model UV-1650PC connected to IBM compatible and a HP1020 LaserJet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (Shimadzu) was used. The spectral band was 2 nm and scanning speed was is 2800nm/min with 0.2nm interval.

Materials

Pure samples

a. Pure formoterol (99.60%) was kindly supplied by GlaxoSmithKline (GSK) Company, Egypt.

b. Pure fluticasone (99.75%) was kindly supplied by GlaxoSmithKline (GSK) Company, Egypt.

Pharmaceutical preparation

Flutiform® inhaler: Pressurized inhalation labeled to contain 120 inhalations each inhalation contains 5µg of formoterol and 50µg of fluticasone (B.No. 6H185FR), manufactured by Fisons company, United Kingdom), purchased from local market.

Chemicals and reagents

Methanol; was procured from El-Nasr Company, Egypt Methanol, analytical grade (El-Nasr Company, Egypt).

Standard solutions

Standard solution of formoterol: A standard solution of formoterol (100µg/mL) was prepared by transferring 10mg of the drug powder to 100ml volumetric flask, dissolving in 50ml of methanol and completed to 100ml with the same solvent.

Standard solution of fluticasone: A standard solution of fluticasone (100µg/mL) was prepared by transferring 10mg of the drug powder to 100-ml volumetric flask, dissolving in 50ml of methanol and completed to 100ml with the same solvent.

Procedures

Linearity and construction of calibration curves

Simultaneous equation method: Different aliquots equivalent to (20–140)µg of formoterol and (50–400)µg of fluticasone were accurately transferred from their standard solutions (100µg/ml) into two separate series of 10ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400nm) of these solutions were recorded using methanol as a blank. The absorptivity is calculated for formoterol and fluticasone at 214nm and 236nm.

Graphical absorbance ratio method: The zero order spectra was obtained as mentioned in simultaneous equation method. The absorbance values at 232nm (λiso) and 214nm (λmax of formoterol) were measured from which the absorptivity values for both drugs at the selected wavelengths were calculated.

Absorbance subtraction method: The zero order spectra was obtained as mentioned in simultaneous equation method. The absorbance values of both drugs at 232nm (isoabsorptive point) and the absorbance values of formoterol at 286nm (λ2) were recorded. , then the absorbance factor of formoterol at 232nm and 286nm [=A232/A286] was calculated.

Amplitude modulation method: The zero order spectra was obtained as mentioned in simultaneous equation method. The amplitude of ratio spectra for fluticasone and formoterol at isoabsorptive point 232nm and 286nm were recorded respectively.

Application to laboratory prepared mixtures: Accurate aliquots of formoterol and fluticasone were transferred from their working solutions into a series of 10ml volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with methanol.with the solvent. The spectra of the prepared series from 200 to 400nm were recorded and stored. The concentrations of both drugs were calculated for each proposed method.

Procedure for pharmaceutical preparation: Contents of one device of Flutiform® inhaler equivalent to 120 inhalations each inhalation contains 5µg of formoterol and 50µg of fluticasone were sprayed into a beaker containing 50ml of methanol. A portion equivalent to 0.5 mg of formoterol and 5mg of fluticasone were transferred to 100ml volumetric flask, the volume was made up to the mark with the same solvent to get a stock solution labeled to containing 5µg/ml of formoterol and 50µg/ml of fluticasone. By using aliquots covering the working concentration ranges. The content of the inhaler were calculated using the mentioned general procedure for each method.

Results and discussion

The zero-order absorption spectra of formoterol and fluticasone show a certain degree of overlapping, with isoabsorptive point at 232nm and formoterol is more extended in plateau region from 280-308nm in which fluticasone has no absorbance, as shown in (Figure 3). The overlapping also does not permit direct determination of fluticasone in presence of formoterol which can be overcome using different manipulation methods for determination of both components without previous separation.

Simultaneous equation method

The λ max of both formoterol and fluticasone were determined and it was found to be 214 and 236nm respectively (Figure 4). Absorption value at these two wavelengths (λ1, λ2) of formoterol and fluticasone in the concentration ranges of 2-14µg/mL and 5–40µg/mL were calculated, respectively. The absorptivity of formoterol and fluticasone were calculated at each wavelength. The concentrations of formoterol and fluticasone can be obtained by applying Cramer's rule and matrices in Equations. (1) and (2). Concentration of formoterol and fluticasone were calculated according to the following equations:

$$\boldsymbol{A}_1 = 0.0969\boldsymbol{C}_{\boldsymbol{form}} + 0.0155\boldsymbol{C}_{\boldsymbol{flut}} \text{ at 214nm } (\lambda_1) \text{ equation (1)}$$

$$\boldsymbol{A}_2 = 0.0267\boldsymbol{C}_{\boldsymbol{form}} + 0.0411\boldsymbol{C}_{\boldsymbol{flut}} \text{ at 236 nm } (\lambda_2) \text{ equation (2)}$$

Where C_{form} and C_{flut} are the concentrations of formoterol and fluticasone in µg/mL, respectively. 0.0969 and 0.0267 are the absorptivity values of formoterol at (λ_1) and (λ_2), respectively. 0.0155 and 0.0411 are absorptivity values of fluticasone at (λ_1) and (λ_2), respectively. A_1 and A_2 are the absorption values of sample solutions at the wavelength range (λ_1, λ_2), respectively. The proposed simultaneous equation method was successfully applied for the simultaneous determination of formoterol and fluticasone in their laboratory prepared mixtures with a high degree of accuracy. Therefore, the method is considered selective for determination of the studied drugs.

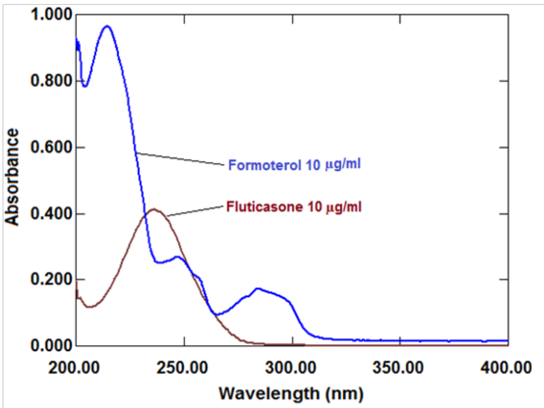

Figure 3 Zero-order absorption spectra of formoterol (10µg/ml), fluticasone propionate (10µg/ml).

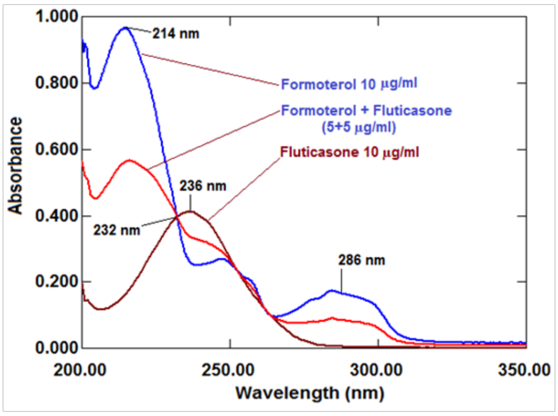

Figure 4 Zero-order absorption spectra of formoterol (10µg/ml), fluticasone propionate (10µg/ml) and their mixture (5+5µg/ml) of each.

Graphical absorbance ratio method

In this method, the absorption spectra of 10µg/ml formoterol, 10µg/ml fluticasone, and of a mixture containing equal concentration of them (5µg/ml of each) showed isoabsorptive point at 232nm, as shown in (Figure 4). The spectra show also two isoabsorptive point at 252 and 263nm which not involved in the proposed methods due to the low sensitivity of the drugs at these wavelengths. The absorbance values were measured at 214 nm (λmax for formoterol) and 232 nm (λiso) in the range of 2-14µg/ml for formoterol and 5-40µg/ml for fluticasone.

Absorptivity values were determined for each drug at two selected wavelengths and the average values were taken. The absorptivity and the absorbance ratio values were used to develop the sets of equations from which the concentration of both drugs in the sample can be calculated:

$$C_{Formoterol} = \left\{\left(Q_M - 0.3953\right) / \left(2.3780 - 0.3953\right)\right\} \times \left(A_1 / 0.0408\right)$$

$$C_{fluticasone} = \left\{\left(Q_M - 2.3780\right) / \left(0.3953 - 2.3780\right)\right\} \times \left(A_1 / 0.0393\right)$$

Where,

$C_{formoterol}$ and $C_{fluticasone}$ are the concentrations of formoterol and fluticasone in µg/ml, respectively.

The value 2.3780 is the division of the mean absorptivity of formoterol at 214nm over the mean absorptivity of the formoterol at 232nm.

The value 0.39535 is the division of the mean absorptivity of fluticasone at 214nm over the mean absorptivity of fluticasone at 232nm.

The value 0.0408 is the mean absorptivity of formoterol at 232nm.

The value 0.0393 is the mean absorptivity of fluticasone at 232nm.

Absorbance subtraction method

In this method; absorbance factor of formoterol which is the average of the absorbance of different concentrations of formoterol using isoabsorptive point at 232nm to that at 286nm which shows no contribution of fluticasone [*abs232 /abs286*] was used for calculating the absorbance values of formoterol and fluticasone at isoabsorptive point (232nm) in their mixture as follow:

$$\textit{Absorbance of formolterol in the mixture at}_{237} = \frac{abs_{232}}{abs_{286}} \times abs_{286(formoterol+fluticasone)}$$

$$\textit{Absorbance of fluticasone in the mixture at}_{232} = abs_{232(formoterol+fluticasone)} - \frac{abs_{232}}{abs_{286}} \times abs_{286\left(salmeterol+fluticasone\right)}$$

Where, $abs\lambda$ $\left(\textbf{formoterol} + \textbf{fluticasone}\right)$ is the absorbance of the binary mixture at 232nm or 286nm and abs232/abs286 is the absorbance factor of pure formoterol at 232nm to 286nm and it was calculated and found to be 2.3993. The calculated absorbance value corresponding to formoterol and fluticasone can be separately used to identify each of their concentration using the corresponding regression equations at isoabsorptive point 232nm.

Amplitude modulation method

In this method; by dividing the spectrum of the binary mixture by the normalized formoterol divisor spectrum, we obtain the ratio spectra as shown in (Figure 5). At the isoabsorptive point of ratio spectra the amplitude value was modulated to concentration. The amplitude value of the constant can be determined at the plateau region at 286nm, which is equal to the amplitude constant value of formoterol along the whole spectrum. At the isoabsorptive point (λ_{iso}) 232nm, the amplitude of the ratio spectra at this point will be equal to the sum of the amplitudes of formoterol and fluticasone. After the subtracting recorded amplitude at 232nm from the previously obtained constant at 286nm, we get the corresponding recorded amplitude of formoterol, which is equivalent to recorded concentration of formoterol in the mixture, while the recorded amplitude of constant value will be directly equal to the recorded concentration of fluticasone in the mixture. To eliminate any error due to signal to noise ratio, the actual concentration of formoterol and fluticasone could be calculated by using their corresponding regression equations at 286nm and isosbestic point 232nm respectively.

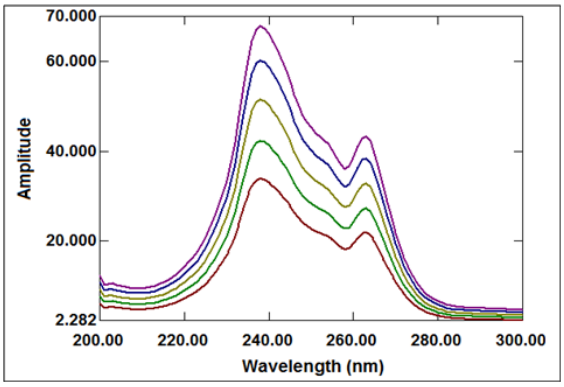

Figure 5 Division spectra of laboratory prepared mixtures (2-4μg/ml) formoterol with (20-40μg/ml) fluticasone using normalized spectrum of formoterol (1μg/ml) as a divisor.

Validation of the methods[19,20]

Validation of the proposed methods was done according to ICH recommendations[4] as shown in Table 1**.** In which the limit of detection(LOD) and limit of quantitation (LOQ) were calculated from the following equations : LOD=3.3σ/S & LOQ=10σ/S ,Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve . The accuracy of the method was calculated by measuring the mean percent recovery of three determinations for three concentration of the studied drugs . The precision of the developed method was checked by measuring percent relative standard deviation (% RSD) of both drugs for three concentrations in the same day (repeatability) and in three days (intermediate precision). The obtained values confirming high precision of the methods. The selectivity of the proposed procedures was assessed by the analysis of laboratory prepared mixtures of both drugs as shown in Table 2. The validity of the proposed procedures is further assessed by applying the standard addition technique showing no interference from excipients. The results obtained were shown in Table 3**.**

Table 1 Assay validation of the proposed method

Parameter	Simultaneous equation		Graphical absorbance ratio		Absorbance subtraction		Amplitude modulation	
	Formoterol	Fluticasone	Formoterol	Fluticasone	Formoterol	Fluticasone	Formoterol	Fluticasone
Accuracy (%R)[a]	100.02	100.17	99.8	100.04	100.02	99.99	100.07	99.98
Precision								
Repeatability (%RSD)[b]	0.805	0.451	0.443	0.646	0.509	0.615	0.545	0.38
Intermediate precision (%RSD)[c]	0.892	0.729	0.61	0.889	1.201	0.824	0.662	0.52
Linearity range (μg/mL	(2-14μg /mL)	(5-40μg / mL)	(2-14μg /mL)	(5-40μg /mL)	(2-14μg /mL)	(5-40μg /mL)	(2-14μg / mL)	(5-40μg / mL)
Regression Equation	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*	*y= b x+ a*
Slope(a)	0.0972	0.0409	0.0409	0.0392	0.0171	0.0391	0.9948	0.9538
Intercept(b)	-0.0015	0.0012	-0.0007	0.0007	-0.0004	0.0011	-0.0155	0.014
Regression coefficient (r^2)	0.9998	0.9997	0.9998	0.9997	0.9998	0.9998	0.9998	0.9997
LOD(μg/mL)	0.186	0.665	0.198	0.666	0.201	0.643	0.193	0.662
LOQ(μg/mL)	0.562	2.016	0.599	2.018	0.608	1.948	0.585	2.005

[a] Average of three determinations for three concentrations (4, 8, and 12μg/mL) and (15, 25, and 35μg/mL) for formoterol and fluticasone respectively.

[b] The intraday (n = 3), average of three concentrations (4, 8, and 12μg/mL) and (15, 25, and 35μg/mL) for formoterol and fluticasone respectively.

[c] The interday (n = 3), average of three concentrations (4, 8, and 12μg/mL) and (15, 25, and 35μg/mL) for formoterol and fluticasone respectively.

Table 2 Determination of formoterol and fluticasone in laboratory prepared mixtures by the proposed methods

Conc. of formoterol (μg/mL)	Conc. of fluticasone (μg/mL)	Simultaneous equation		Graphical absorbance ratio		Absorbance subtraction		Amplitude modulation	
		%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone
2	20	100.63	99.13	98.43	99.28	100.34	99.48	99.06	101.61
2.5	25	99.19	99.24	99.09	99.37	100.22	99.44	100.55	98.57
3	30	100.04	100.56	98.85	100.56	99.16	100.71	98.69	100.21
3.5	35	99.33	100.75	98.11	100.78	100.41	100.97	99.45	99.85
4	40	100.63	99.15	99.36	99.16	99.3	99.46	99.83	99.92
Mean ± %RSD		99.96 ±0.688	99.77 ±0.813	98.77 ±0.496	99.83 ±0.773	99.89 ±0.604	100.01 ±0.762	99.52 ±0.715	100.03±1.085

Table 3 Recovery study of formoterol and fluticasone by adopting standard addition technique via the proposed methods

Pure added (µg/ml)		Simultaneous equation		Graphical absorbance ratio		Absorbance subtraction		Amplitude modulation	
Formoterol	Fluticasone	%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone	%Recovery of formoterol	%Recovery of fluticasone
2	5	98.86	97.42	99.32	97.43	99.63	99.34	99.32	99.5
3	10	99.75	99.16	99.2	99.18	99.02	99.16	100.83	101.02
4	15	98.25	98.25	100.2	98.39	98.11	100.29	99.74	101.48
Mean±%RSD		98.95±0.746	98.28±0.855	99.57±0.544	98.33±0.862	98.92±0.757	99.60±0.605	99.96 ±0.779	100.67±1.043

Statistical analysis[21]

In order to compare the ability of the proposed methods for the simultaneous determination of formoterol and fluticasone in their pharmaceutical preparation, the results obtained by applying each of the proposed methods and the reported method[13] were subjected to statistical analysis Table 4. The calculated t and F values were less than the theoretical ones indicating that there were no significant differences between the proposed and the reported methods. Another statistical comparison of the results obtained by the proposed methods and the reported method for determination of formoterol and fluticasone in pharmaceutical product using one-way ANOVA test was shown in Table 5. The results obtained by applying these methods show no significant differences between all of them.

Table 4 Determination of formoterol and fluticasone by the proposed and reported methods

Parameters	Simultaneous equation		Graphical absorbance ratio		Absorbance subtraction		Amplitude modulation		Reported method (4)	
Drug	formoterol	Fluticasone	formoterol	Fluticasone	formoterol	Fluticasone	formoterol	Fluticasone	formoterol	Fluticasone
n*	5	5	5	5	5	5	5	5	5	5
Average(% Recovery)	99.87	99.83	100.11	99.93	99.45	100.01	100.03	100.05	99.71	99.37
%RSD	0.833	0.969	0.945	0.921	0.645	0.857	0.625	0.953	1.029	1.014
t** (2.31)	0.28	0.75	0.64	0.93	0.47	1.09	0.6	1.1	--	--
F** (6.39)	1.52	1.08	1.18	1.2	2.25	1.38	2.69	1.12	--	--

*Number of samples.

**The values in parenthesis are tabulated values of "t "and "F" at (P=0.05).

(4) HPLC using C_{18} column, mobile phase was phosphate buffer: acetonitrile [80:20 v/v], pH 3.5) at a flow rate (1mL/min) and UV detection at 215nm.

Table 5 One-way ANOVA test for the different proposed methods used for the determination of binary mixture of formoterol and fluticasone propionate in Flutiform® inhaler:

Drug	Source of variation	Sum of squares	Degree of freedom	Mean of squares	F*
Formoterol	Between groups	1.377	4	0.344	0.5
	Within groups	13.77	20	0.689	-2.87
Fluticasone	Between groups	1.526	4	0.381	0.43
	Within groups	17.777	20	0.889	-2.87

*The value in parenthesis is the critical value of "F" at (P = 0.05)

Conclusion

Four methods (simultaneous equation, graphical absorbance ratio, absorbance subtraction and amplitude modulation.) were presented as first reported spectrophotometric powerful methods for simultaneous determination and to resolve the overlapping of formoterol and fluticasone. The proposed methods were very simple with minimum manipulation steps, very sensitive, accurate and precise. They do not need any sophisticated apparatus and could be easily applied in quality control laboratories.

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Conflict of interest

The author declares that there is no conflict of interest.

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