SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF FORMOTEROL FUMARATE AND MOMETASONE FUROATE IN RESPICAPS

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ABSTRACT

The present work represents a UV-Spectrophotometric method for the simultaneous estimation of Formoterol Fumarate (FF) and Mometasone Furoate (MF) in respicaps dosage form. This method is based on area under curve (AUC) in the wavelength range of 210-220nm and 242-252nm. Linearity was found in the concentration ranges of 3-18µg/ml and 6-21µg/ml, for Formoterol Fumarate and Mometasone Furoate, respectively. This method was validated as per International Conference on Harmonization (ICH) guidelines. Validation results suggest that the proposed method is simple, rapid, accurate, sensitive and precise that can be applied for routine estimation of FF and MF in bulk drug and combined dosage form.

Keywords: Simultaneous estimation, Formoterol Fumarate, Mometasone Furoate, Area under curve, Ultraviolet spectrophotometry, Respicaps.

INTRODUCTION

Formoterol Fumarate dihydrate (FF) is chemically known as N-[2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl] amino] ethyl] phenyl] formamide (E)-butanedioate dihydrate¹ (Figure 1). It is a selective beta-2 (β_2) adrenergic receptor agonist which acts as bronchodilator for the treatment of asthma by relaxing muscle spasms in the air passages of lungs²⁻⁴. This drug is official in USP⁵, EP⁶, IP⁷ and BP⁸. Mometasone Furoate (MF) is chemically known as 9, 21-dichloro-11 β -hydroxy-16 α -methyl-3, 20-dioxopregna-1, 4-diene-17-yl furan-2-carboxylate⁹ (Figure 2). It is a corticosteroid which helps to decrease inflammation in the lungs and controls various asthmatic symptoms^{6,10}. This drug is official in BP⁸, USP⁹ and EP¹¹. The combination of FF and MF is used to control asthma effectively, which cannot be control by single drug alone^{4,6}. Literature survey reveals that several analytical methods have been published for the estimation of Formoterol Fumarate alone or in combination with other drugs. Some of these methods include HPLC¹²⁻¹⁵, GC¹⁶ and UV spectrophotometry¹⁷. Various methods have been reported for estimation of Mometasone Furoate alone or in combination with other drugs including UV spectrophotometry¹⁸ and supercritical fluid chromatography¹⁹. However, no UV method has been reported for simultaneous estimation of FF and MF by area calculation technique. Therefore, attempts were made to develop a UV spectrophotometric method by area under curve (AUC) for simultaneous estimation of Formoterol Fumarate Dihydrate and Mometasone Furoate in respicaps.

MATERIALS AND METHODS

Instruments

A Shimadzu UV 1800 double beam spectrophotometer with spectral band width of 1nm and a pair of matched quartz cell and UV probe software, was used for this study. Shimadzu AX200 (Japan) digital balance and Spectrolab UCB40 (Germany) ultrasonicator were also used in the work.

Reagents and Materials

The active pharmaceutical ingredients of Formoterol Fumarate Dihydrate and Mometasone Furoate were supplied

as gift sample by Zydus Cadilla Pvt. Ltd., Ahmedabad, India. Respicaps (Formost 200 contains 6µg of Formoterol Fumarate and 200µg of Mometasone Furoate) were procured from the market, AR grade methanol was obtained from Merck specialties Pvt. Ltd., Mumbai, India and Acrodisc PSF GHP 0.45µm nylon filters were purchased from Pall Life Science limited, Mumbai, India.

Preparation of Standard Solutions

Formoterol Fumarate (100mg) was accurately weighed and transferred to a 100ml volumetric flask containing small amount of methanol. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark. This standard stock solution was further diluted with same solvent to get 12µg/ml. Similarly, solution of Mometasone Furoate in methanol was also prepared to get a concentration of 12µg/ml.

Preparation of sample solution

Twenty capsules were accurately weighed and average weight was calculated. Capsule shells were removed; powder equivalent to one capsule was weighed accurately and transferred into a 10ml volumetric flask. Then, Methanol (5ml) was added to dissolve the content present in this flask and 0.94ml of standard stock solution of Formoterol Fumarate was added. This solution was sonicated for 30minutes with occasional swirling and diluted up to mark with same diluent. This solution was filtered using Acrodisc PSF GHP 0.45µm nylon filters and further subjected for UV analysis.

Area under curve method

The AUC method^{20,21} involves area calculation of integrated value of absorbance with respect to wavelength in indicated region of wavelengths. Area calculation processing item calculates the area bounded by the curve and horizontal axis²². Here horizontal axis represents baseline.

$$\text{area calculation } (\alpha + \beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region. The selection of wavelength range was done on the basis of linearity between area under curve and concentration. Working standards were prepared by dilutions of standard stock solutions of these drugs and these solutions were scanned in the spectrum mode (200-400 nm), an overlain spectrum was recorded (Figure 3). The selected sampling wavelength ranges for calculation of area under curve were 220-230 nm and 242.0-252.0 nm. Similarly, Area under curve of sample solutions was also measured at these selected wavelength ranges.

Method Validation

Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application²³. The proposed method was validated for various parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines.

Linearity and Range

The linearity of response for Formoterol Fumarate and Mometasone Furoate was determined by measuring the area under curve in the concentration range of 3-18 $\mu\text{g/ml}$ of Formoterol Fumarate and 6-21 $\mu\text{g/ml}$ of Mometasone Furoate. The calibration curves were plotted between concentration range and area under curve, as shown in Figure 4 and 5 for FF and MF, respectively.

Precision

Precision of the method was studied by intra and inter-day variations in the test method of FF and MF. Method repeatability (intra-day precision) was evaluated by assaying six samples, prepared as described under sample preparation. Inter day precision was performed by assaying six samples in different days as described in the sample preparation. The results were expressed as percentage relative standard deviation (%RSD) in Table 2 and 3.

Accuracy

Accuracy of the method was determined by calculating recovery of FF and MF at 80%, 100% and 120% level of sample solutions of FF and MF. The accuracy of the analytical method was established in triplicate across its range. The results of recovery experiments are given in Table 4.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

Six sets of known concentrations were prepared for both drugs. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

$$LOD = 3.3 \frac{SD}{S}$$

$$LOQ = 10 \frac{SD}{S}$$

Where, S is value of slopes of calibration plot and SD is calculated using values of y intercepts of regression equations. The results of LOD and LOQ are presented in Table 5.

Table 1: Results of Analysis of Respicaps Formulation

Name of drug	Amount of drug added (μg)	Amount of drug found (μg)	Mean % drug recovered $\pm$ SD*	% RSD*
FF	100	98.29	98.18 $\pm$ 0.105	0.1069
		98.08		
		98.17		
MF	200	197.66	98.33 $\pm$ 0.884	0.8990
		196.34		
		195.98		

*n=3

Table 2: Results of Intraday Precision

Drug	Concentration Added ($\mu\text{g/ml}$)	Mean % assay $\pm$ SD	% RSD*
FF	10	97.84 $\pm$ 1.08	1.36
MF	20	98.37 $\pm$ 1.24	1.27

*n=6

Table 3: Results of Inter-Day Precision

Drug	Concentration($\mu\text{g/ml}$)	Mean % assay $\pm$ SD	% RSD*
FF	10	98.12 $\pm$ 1.66	1.54
MF	20	98.67 $\pm$ 1.05	1.39

* n=6

Table 4: Results of Recovery Studies

components	Amount added($\mu\text{g/ml}$)	Amount recovered($\mu\text{g/ml}$)	% Recovery $\pm$ SD	% RSD*
FF	80%(8 $\mu\text{g/ml}$)	7.82	97.75% $\pm$ 0.86	1.11
	100%(10 $\mu\text{g/ml}$)	9.75	97.5% $\pm$ 0.353	0.87
	120%(12 $\mu\text{g/ml}$)	11.87	98.45% $\pm$ 1.20	1.32
MF	80%(16 $\mu\text{g/ml}$)	15.62	97.6% $\pm$ 1.63	1.56
	100%(20 $\mu\text{g/ml}$)	19.77	98.85% $\pm$ 0.43	0.95
	120%(24 $\mu\text{g/ml}$)	23.74	98.9% $\pm$ 0.31	0.98

*n=3

Table 5: Summary of Results of Analytical Method Validation

Parameters	FF	MF
λ max (nm)	210-220	242-252
Linearity range ($\mu\text{g/ml}$)	3-18	6-21
Slope	0.0393	0.0102
y-intercept	0.003	0.0017
Correlation Coefficient (R^2)	0.999	0.9991
Intraday	1.36	1.27
Inter-day	1.54	1.39
Accuracy (Mean % Recovery)	97.5-98.45	97.6-98.9
LOD ($\mu\text{g/ml}$)	0.0491	0.278
LOQ ($\mu\text{g/ml}$)	0.016	0.0195

Figure 1: Chemical structure of Formoterol Fumarate dehydrate

Figure 2: Chemical structure of Mometasone Furoate

Figure 3: Overlaid spectrum of Formoterol Fumarate and Mometasone Furoate in Methanol

Figure 4: Calibration curve of Formoterol Fumarate at 210-220nm

Figure 5: Calibration curve of Mometasone Furoate at 242-252nm

RESULTS AND DISCUSSION

The percentage assay of marketed formulation was 98.18 ± 0.105 and 98.33 ± 0.884 , respectively for FF and MF (Table 1), which suggest no interference from the excipients of formulation. The summary of validation parameters for the proposed analytical spectrophotometric method is given in

Table 5. Here, correlation coefficients were very close to 1, suggest that the developed method is following linearity. Results of Table 2 and 3 indicate that the proposed method is precise as % R.S.D values for intraday as well as inter-day precision were less than 2%. Satisfactory results of % recovery study (Table 4) proved that the developed method is

accurate. Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantification (LOQ). Limit of detection for FF and MF was 0.0491 µg/ml and 0.278 µg/ml, respectively. Limit of quantitation with desired accuracy as well precision for FF and MF was found 0.016 µg/ml and 0.0195 µg/ml, respectively.

CONCLUSION

It can be concluded from the results that the proposed spectroscopic method was found to be simple, sensitive, accurate and precise for the analysis of FF and MF in respicaps dosage form. This method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of FF and MF in bulk and combined dosage form.

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