SPECTROSCOPIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF MOMETASONE FUROATE AND FORMOTEROL FUMARATE IN ROTACAPS.

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ABSTRACT

Two spectrophotometric methods Zero crossing derivative and ratio- spectra derivative spectrophotometric methods were developed for the determination of Mometasone furoate (MF) and Formoterol fumarate (FF) in the pharmaceutical dosage form. Zero crossing derivative spectrophotometry involves amplitudes measurement of the first derivative spectra of the standard and sample solution at 267.56 nm for MF and 306 nm for FF. Ratio spectra derivative spectrophotometry involves amplitudes measurement of the ratio first derivative spectra at 263nm and 267.30 nm for MF and 293.08 and 302.60 nm for FF. The calibration graph follows beer’s law in the range of 30-200 µg /ml of MF and 2-6 µg /ml for FF. The accuracy and precision of the method were validated statistically. So it can be a preferable method for routine analysis due to its simplicity and economical advantages.

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INTRODUCTION

Nowadays combinational therapy of mometasone fumarate and formoterol furoate has been used in the treatment of COPD and asthma as it produces the additive effect for improving the symptom, lung function and reduces exacerbation in patient.[1] Mometasone furoate (MF) (Fig.1) is a highly potent synthetic chlorinated glucocorticosteriod. It is used in the treatment of glucocorticoid responsive disorders of dermatology, seasonal and perennial allergic rhinitis and asthma. [2]. Formoterol fumarate (Fig 2) appear to be more effective than shorter acting β₂-agonists in the treatment of nocturnal and exercise induced asthma. It acts locally in the lung as a bronchodilator [3]. Literature survey reveals that serval analytical method have been published for the estimation of MF alone or in combination with other drugs like fucidic acid, terbinafine HCl, miconazole, eberconazole nitrate etc. Some of these methods include HPLC, GC, supercritical fluid chromatography and UV spectrophotometry[4-11]. Various method has also been reported for the estimation of FF alone and in combination with other drugs including HPLC, GC, and UV spectrophotometry [12-14]. However, no UV method has been reported for the simultaneous estimation of MF and FF in rotacaps by zero crossing derivative and ratio derivative spectrophotometry method. So, the aim of the present work was to develop simple, sensitive and validated two spectrophotometric methods for the simultaneous determination of MF and FF in their pharmaceutical preparations.

Experimental work

Instrumentation:
Shimadzu UV-1800 spectrophotometer connected to computer loaded with Shimadzu UV probe 2.10 software was used for all the spectrophotometric measurements. All weighing were done on electronic balance( model Ohaus –adventurer Pro). Ultrasonicicator was used for sample solution preparation.

Reagents and chemicals:
Analytical pure sample of MF and FF were obtained as a gift sample from Sun Pharma, Vadodara. These sample were used without further purification. The formulation Evocort manufactured by Cipla was purchased from the local market containing MF (200 µg) and FF (6 µg) per rotacap. Analytical grade methanol was purchased from Merck Mumbai was used throughout the study.

Preparation of standard solution and calibration curve:
Individual standard stock solution of MF (100 µg/ ml) and FF (100 µg/ ml) were prepared by dissolving 10 mg of MF and 10 mg of FF in 100 ml methanol in separate volumetric flask. The std stock solution were further diluted separately to obtained working standard solution of the conc. 30-200 µg/ ml of MF and 2-6 µg/ ml of FF.

First derivative zero crossing spectrophotometry:
The working standard solutions of both the drug were scanned between the range 200-400 nm in 1 cm cell against blank. The zero order spectrum of 10 µg/ml of both the drug were tried for the first- fourth order derivative absorption spectra. And then first order derivative method was selected. The recorded spectrums were converted into first order derivative spectra with Δλ= 8 and scaling factor 32. For the estimation of MF amplitude 267.56 nm was selected which was the zero crossing of formoterol fumarate and for the estimation of FF amplitude 306 nm was selected which was the zero crossing of mometasone furoate as shown in fig 4.
Fig. 3 Overlain zero order spectra of the standard solution of MF and FF.

Fig. 4 Overlain first derivative spectra of MF and FF, for the estimation of MF when FF spectra is showing zero crossing at 267.56 nm and for FF when MF is showing zero crossing at 306 nm.

Fig. 5 First order derivative spectra for the estimation of MF at 267.56 nm for linearity.

Fig. 6 First order derivative spectra for the estimation of FF at 306 nm for linearity.

Ratio spectra derivative method:

According to the theory of the ratio spectra derivative method, for the estimation of MF the stored UV absorption spectra of the standard solution of the MF were divided wavelength by wavelength by a standard spectrum of FF (6 µg/ml). The first derivative was calculated for the obtained spectra with Δλ= 2 and scaling factor 4. The amplitudes at 263 nm and 267.30 nm were measured. For the FF, the stored spectrum UV absorption spectra of the std solution of FF were divided wavelength by wavelength by a standard spectrum of MF (30 µg/ml). The amplitudes at 293.08 nm and 302.60 nm were measured.

Fig. 7 Ratio derivative spectra of mometasone furoate at different concentration using a standard spectrum of FF (6 µg/ml) as a divisor.

Fig. 8 Ratio derivative spectra for formoterol fumarate at different concentration using a standard spectrum of MF (30 µg/ml) as a divisor.
Analysis of Sample formulation:

The content powder of 20 rotacaps were emptied, accurately weighed and mixed. A portion of powder equivalent to 2 mg of mometasone furoate which will also contain 0.06 mg of formoterol fumarate was transferred to 25 ml of volumetric flask containing 5 ml of methanol. This solution was sonicated for 30 minutes and diluted up to mark with methanol. The sample was filtered. Further dilution was carried out with methanol to obtain the conc. of both the drug within the calibration range. The procedures describe above for zero crossing derivatives and ratio derivative were followed and the concentrations of MF and FF were calculated from the calibration curved of respective method.

Method Validation:

Both the method was validated as per ICH Q2 (R1) guideline[15]. The standard calibration curve was plotted for MF and FF in both the method at their respective peak amplitude and correlation coefficient was calculated. The limit of detection (LOD= 3.3 σ/s, where σ is the standard deviation of the response and s is the slope) and limit of quantitation (LOQ=10 σ/s) of MF and FF was calculated. Intraday and interday precision was studied by the analyzing three replicates of the standard solution at three concentration levels. To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, adding know amount of each drug to the preanalysed capsule powder at three levels 80%, 100%, 120% of the label claim.

RESULTS AND DISCUSSION

The zero-crossing first order derivative and ratio- spectra first derivative spectrophotometry method permits a more selective identification and determination of the two drugs in mixture. The zero crossing method involves measurements of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero crossing wavelengths of the derivative spectra of the individual component. Fig (3-5) shows the D1 spectra of MF and FF. The selection of the optimum wavelength has the best linear response to the analyte concentration, it is not affected by the conc. of any other component and gives a near zero intercept on the ordinate axis of the calibration curve. Therefore 267.56 nm (zero crossing wavelength point of FF) and 306 nm (zero crossing wavelength point of MF) were chosen as optimum working wavelength for the simultaneous determination of MF and FF in a binary mixture. Measurements of the absolute value of the derivative spectrum taken at these wavelengths gave the best linear response to the analyte concentration.

Table 1. Regression parameter of the proposed zero-crossing first order derivative (Method D1) and ratio- spectra first derivative.

<table>
<thead>
<tr>
<th>Parameter assessed</th>
<th>Method D1</th>
<th>Method RD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>267.56</td>
<td>263</td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>307</td>
</tr>
<tr>
<td>Beers law range (µg/ml)</td>
<td>30-200</td>
<td>2-6</td>
</tr>
<tr>
<td>Correlation Coefficient(R²)</td>
<td>0.9992</td>
<td>0.9991</td>
</tr>
<tr>
<td>Slope</td>
<td>0.042</td>
<td>-0.031</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.009</td>
<td>0.016</td>
</tr>
<tr>
<td>SD of slope*</td>
<td>0.00571</td>
<td>0.00570</td>
</tr>
<tr>
<td>SD of intercept*</td>
<td>0.004359</td>
<td>0.008025</td>
</tr>
<tr>
<td>LOD</td>
<td>0.342</td>
<td>0.8542</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.037</td>
<td>2.654</td>
</tr>
<tr>
<td>Intraday precision (% RSD)**</td>
<td>0.87</td>
<td>0.98</td>
</tr>
<tr>
<td>Interday precision (% RSD)**</td>
<td>1.15</td>
<td>1.26</td>
</tr>
</tbody>
</table>

* for five replicate and ** for three replicate

Table 2: Recovery analysis for the proposed spectrometric method D1 and RD1 applied to the Rotacaps.

<table>
<thead>
<tr>
<th>Spike level (%)</th>
<th>% Recovery of MF</th>
<th>% Recovery of FF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method D1</td>
<td>Method RD1</td>
</tr>
<tr>
<td>267.56nm</td>
<td>263nm</td>
<td>267.3nm</td>
</tr>
<tr>
<td>306nm</td>
<td>293.08nm</td>
<td>302.60nm</td>
</tr>
<tr>
<td>80</td>
<td>99.24±0.32</td>
<td>99.73±0.56</td>
</tr>
<tr>
<td>100</td>
<td>100.18±0.47</td>
<td>99.54±0.68</td>
</tr>
<tr>
<td>120</td>
<td>99.17±0.52</td>
<td>100.10±0.86</td>
</tr>
</tbody>
</table>

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The main advantage of the ratio derivative method is the chance of easy measurement in corresponding to peak so it permits the use of the wavelength of the highest value of analytical signal. Moreover, the presence of a lot of maxima and minima is another advantage by the fact that these wavelengths give an opportunity for the determination of active compounds or excipients which possibly interfere with the analysis. The influence of the Δλ and scaling factor for the obtaining the first derivative of the ratio spectra as well as the effect of divisor concentration on the calibration graphs for the proposed mixture was studied in order to select the best factors affecting the determination of the analytes. Results indicated that Δλ=2 and scaling factor 4 was the most suitable one. Determination of both drugs was done by dividing the absorption spectra of MF by that of standard spectra of FF (6 µg/ml) while the absorption spectra of FF were divided by that of the standard spectra of MF (30 µg/ml).

The proposed methods for the simultaneous estimation of MF and FF in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in table (1) & (2). Linearity was observed by linear regression equation method for MF and FF in different conc. range. The correlation coefficient of these drugs was found to be close to 1.00 indicating good linearity. Percentage RSD for intraday and inter day was found to be less than 2 and the % recovery for both the drug was found within the range(98.10%–100.18%) as mention in table (2), which indicates the validity of both method. The analysis results of the rotacaps are given in the table (3).

### CONCLUSION
Zero crossing derivative and ratio- spectra derivative spectrophotometric methods were applied successfully for the simultaneous analysis of MF and FF in the rotacaps. The proposed methods were simple, cost effective, accurate and precise which can be used for the routine quality control analysis of MF and FF in combined dosage forms.

### ACKNOWLEDGMENT
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### REFERENCES

### Table 3: Analysis results of Rotacaps.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled claim (µg per rotacap)</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>200</td>
<td>100.40±0.736</td>
<td>101.20±0.6377</td>
</tr>
<tr>
<td>FF</td>
<td>6</td>
<td>98.91±0.562</td>
<td>99.20±0.812</td>
</tr>
</tbody>
</table>

Table 3: Analysis results of Rotacaps.

