SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF GATIFLOXACIN AND DEXAMETHASONE IN THEIR BINARY MIXTURE

Sawsan A. Abdel-Razeq¹, Manal M. Fouad¹, Manal K. Darwish², Hala E. Zaazaa³, Zeinab A. Nasr ⁺¹
¹Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
²Pharmaceutics and Industrial Pharmay Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.
³Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

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Six simple, specific, accurate and precise spectrophotometric methods were developed and validated for the simultaneous determination of gatifloxacin (GFC) and dexamethasone (DEX) without preliminary separation. The first method was based on direct spectrophotometric determination of GFC at 291.6 nm as DEX shows nearly zero crossing at this wavelength. The second and third ones, used for simultaneous determination of both drugs were dual wavelength method and a first derivative method. The other methods were manipulating ratio spectra namely first derivative of the ratio spectra, ratio difference and mean centering of ratio spectra and were used for determination of DEX. Quantitation of the two studied drugs using the proposed methods was achieved in the concentration range of 1.5-17.5 and 5-50 µg/ml, respectively. The developed methods were successfully applied for the analysis of of GFC and DEX in their synthetic mixtures and pharmaceutical formulation without any interference from common excipients; recoveries ranging from 98.59- 101.78 % ± 0.44- 2.06. The results obtained were statistically compared to that of a reported method showing no significant difference regarding both accuracy and precision. The proposed methods are very simple with minimum manipulation steps, don't need any sophisticated apparatus or a special program. The RSD for all parameters was found to be within the limits, which indicates the validity of method. Accordingly, these methods could be successfully applied for routine analysis of the studied drugs either in their pure form or combined dosage form in quality control laboratories.

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INTRODUCTION

Gatifloxacin (GFC); 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid is a synthetic broad-spectrum antibiotic belonging to fluoroquinolone family, which eradicate bacteria by interfering with DNA replication [1,2]. Dexamethasone (DEX); 9α-fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1, 4-pregnadiene-3, 20-dione, is a synthetic glucocorticoid class of steroid drugs with anti-inflammatory and immunosuppressive activity [3]. It is widely used in the treatment of rheumatoid arthritis, asthma, ocular diseases, especially related to connective tissue [4]. Gatifloxacin in combination with dexamethasone is used in several anti-infective eye preparations to treat acute and sub acute conjunctivitis caused by susceptible strains of aerobic gram positive and negative bacteria.

Numerous methods have been reported for the analysis of GFC individually or in combinations using several analytical techniques as UV-spectrophotometry [5-8], colorimetry[9-12], spectrofluorimetry [13-16], HPLC [17-21], TLC [22-24] and capillary zone electrophoresis [25-27]. Whereas, DEX was determined individually or with other drugs by UV spectroscopy [28-30], HPLC [31-34], TLC [35, 36], electrochemically [37, 38] and gas chromatography [39]. Meanwhile, few HPLC methods [40, 41] were reported for the simultaneous determination of both drugs.

The main problem of spectrophotometric binary mixtures analysis is the simultaneous determination of two drugs without prior separation. So, the main task of this work is to establish novel methods by using smart mathematical techniques for the quantitative estimation of GFC and DEX as bulk or in the commercially available pharmaceutical formulation containing both drugs.

EXPERIMENTAL

Instrumentation
Spectrophotometric measurements were carried out on Shimadzu 1601-PC spectrophotometer, using 1.00 cm quartz cells. Scans were carried out in the range from 200–400 nm at 0.5 nm intervals. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

Materials and solvents
Pure samples: Gatifluxacin working standard was kindly supplied by EPCI Company, Egypt. Dexamethasone was kindly supplied from EIPICO Company, Egypt.
Market samples: Zigat-D eye drops containing 3 mg/mL of GFC and 1 mg/mL DEX (FDC proxima, India).
Solvents: Methanol (Merck, UK) was selected as the suitable solvent for both GFC and DEX.

Standard solutions
Separate stock solutions of GFC and DEX (1 mg/mL) were prepared by dissolving 100 mg of each standard in methanol then completing to volume in 100- mL measuring flasks. Working solutions were freshly prepared by diluting the stock solutions with the same solvent to obtain a concentration of 0.1 mg/mL of each drug.

Procedures

Construction of calibration curves
Aliquots equivalent to 0.015 – 0.175 mg/mL GFC and 0.05-0.5 mg/mL DEX were accurately transferred from its standard solution (0.1 mg/mL) into a series of 10 -mL volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer.

Zero-Order method -The absorbance of the prepared GFC solution was measured at 291.6 nm against methanol as blank. A calibration curve relating the absorbance to the corresponding drug concentrations in μg/mL was constructed and the regression equation was computed.

Dual Wavelength method –From the stored data, calibration curves were constructed by plotting difference in absorbance (A_{220} and A_{260}) for GFC and (A_{245} and A_{270}) for DEX versus drugs concentrations. The regression equations for both drugs were formulated.
First derivative (1D) method - 1D spectra of both drugs were recorded against methanol as blank, where the zero crossing wavelengths at 301 nm 345 nm and at 224.8 nm, 261.7 nm were selected for the analysis of GFC and DEX, respectively. The calibration curves were constructed and the concentration of individual drug present in the mixture was determined. The regression equations for both drugs were calculated.

Derivative ratio (1DR) method - For the determination of DEX in presence of GFC, the stored spectra of DEX was divided by the spectrum of 10µg/mL GFC. Then the first derivative of the ratio spectra (1DR) with scaling factor 1 is obtained and smoothed with Δλ = 8 nm. The first derivative signals at 267.4 nm was measured and then plotted against its corresponding concentration from which regression equation was computed.

Ratio difference (RD) method - The above procedure detailed under derivative ratio (1DR) method was followed and after the division of the stored spectra of DEX by the spectrum of GFC (10 µg/mL), the amplitude difference between 244 nm and 270 nm for each spectra was plotted against its corresponding drug concentration and the regression equation was evaluated.

Mean centering (MC) method – The above procedure detailed under derivative ratio (1DR) method was followed and the obtained spectra were then mean centered. The amplitude of the mean centered peaks of (DEX/GFC) were measured at 248.2 nm and plotted against corresponding drug concentrations. The regression equations were computed.

Application to laboratory prepared mixtures
Into a series of 10-mL volumetric flask, accurate aliquots of GFC and DEX were transferred from their working solutions to prepare five mixtures containing different ratios of the cited drugs. The volumes were completed with methanol and the spectra of the prepared solutions were recorded at 200–400 nm. The concentration of each drug was calculated by substitution in the corresponding regression equation after applying the corresponding manipulating steps for each method.

Application to pharmaceutical formulation
Sample solutions of GFC and DEX were prepared by transferring 5.0 mL of the eye drops into a 50-mL volumetric flask. Volume was made up to the mark with methanol to give a concentration of 0.3 mg/mL and 0.1 mg/mL for each drug, respectively. Further dilution was done into 10-mL volumetric flask using the same solvent for the quantitative determination of GFC and DEX. The concentration of each drug was calculated using the corresponding regression equation. When carrying out the standard addition technique, different known concentrations of pure standard of each drug were added to the pharmaceutical dosage form before proceeding in the previously mentioned methods.

RESULTS AND DISCUSSION
Gatifloxacin (GFC) and dexamethasone (DEX) are co-formulated in Zigat- D® eye drops which is labeled to contain 0.3% w/v GFC and 0.1% w/v DEX (in ratio 3: 1). The main problem in spectrophotometric analysis of GFC and DEX binary mixture was the spectral overlap of the two cited drugs. Thus, the aim of this work is to develop novel methods for the quantitative estimation of each drug in their mixture by using smart original mathematical techniques.

Zero-order method
The zero-order absorption spectra (D0) of GFC and DEX showed marked overlapping; Figure (2), however it allows the analysis of GFC in presence of DEX at 291.6 nm as DEX show nearly zero crossing at this wavelength.

![Absorption spectra of GFC and DEX](image)

Figure 2: Absorption spectra of GFC (—–) and DEX (….) in methanol.
Dual Wavelength method

The principle for dual wavelength method is “the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest” [42]. The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of GFC and DEX. In Dual wavelength method, the diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra shown in Figure (2), two wavelengths 220 and 260 nm were selected for the estimation of GFC in presence of DEX as DEX shows the same absorbance at these wavelengths. Also, the two wavelengths 245 and 270 nm were selected for the estimation of DEX in presence of GFC as GFC shows the same absorbance at these wavelengths.

First derivative (1D) method

For the simultaneous determination of the studied drugs, a simple one-step correction procedure based on generation of first derivative spectra (1D) was developed, where GFC can be determined at 301 and 345 nm; a zero crossing point for DEX. Similarly, DEX can be determined by measuring its first derivative amplitudes at the zero crossing of GFC at 224.8 and 261.7 nm; Figure (3).

![Figure (3): First order spectra of GFC (—) and DEX (…) in methanol.](image)

Derivative ratio (1DR) method

In this method the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph [43]. Practically, it was found that the spectra of GFC can not accept any concentration of DEX as divisor, whereas DEX spectra can be divided by certain concentrations of GFC, allowing only for the determination of DEX in presence of GFC and not the reverse. At wavelengths 222, 237 and 267.4 nm, good linearity was observed but the recovery percent at 267.4 nm was the best, which may be attributed to its higher signal to noise ratio; Figures (4 and 5). Measuring the amplitude between 237 and 267.4 nm did not show significant improvement in the recovery percent. Consequently, the peak amplitudes of the first derivative of ratio spectra are then recorded at 267.4 nm. It was also found that at high speed noisy spectra are obtained while at low scanning speed, the noise is decreased but a longer time is needed for the measurements, so medium scanning speed is chosen to perform measurements. Effect of delta lambda is studied; using $\Delta \lambda = 8$ nm gave best results. Different concentrations of divisor were used (1.5, 2.5, 5 and 10 $\mu$g/ml and normalized spectrum) of GFC and (5, 15, 20, 40 and 50 $\mu$g/ml and normalized spectrum) of DEX and the divisor concentrations 10 $\mu$g/ml of GFC was the best regarding average recovery percent when they are used for the prediction of DEX concentrations, in bulk powder as well as in laboratory prepared mixtures.
Ratio difference (RD) method

This method comprises two critical steps. The first is the choice of the divisor and the selected divisor should compromise between minimal noise and maximum sensitivity. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and a good linearity is present at each wavelength individually [44]. Linear correlation was obtained between the differences in amplitudes at 244 and 270 nm, against the corresponding concentration of dexamethasone.

Mean centering (MC) method

For further improvement of the selectivity to resolve the spectral overlap present between GFC and DEX, a simple method is applied; this method is based on the mean centering of ratio spectra. It eliminates the derivative step and therefore the signal-to-noise ratio is enhanced [43].

In this method the ratio spectra of the binary mixture are obtained after which the constant is removed by mean centering of the ratio spectra; Figure (6), the amplitude of the mean centered peak of (DEX / GFC) is measured at 248.2 nm.
Figure (6): Mean centered ratio spectra of DEX (5-50 µg/mL) using 10 µg/mL of GFC as a divisor and methanol as blank.

Method validation
The proposed methods were validated according to ICH guidelines [45].

Linearity
The proposed methods were found to be valid over the concentration range of 1.5–17.5 µg/mL and 5- 50 µg/mL for GFC and DEX, respectively as shown by the small intercept and correlation coefficient approaching unity (0.9997-0.9999). Regression parameters were calculated and summarized in Table (1).

Accuracy
To study the accuracy of the proposed methods, procedures under construction of the calibration curves, for both drugs using the proposed methods, were repeated three times for the determination of six different concentrations of pure GFC and DEX within the linearity range. The accuracy expressed as percentage recoveries (mean) and standard deviation was shown in Table (1). Good accuracy of the developed methods was indicated by the results obtained.

Precision
Repeatability and intermediate precision were determined using three concentrations of each of GFC (1.5, 8, 17.5 µg/mL) and DEX (10, 35, 50 µg/mL) which were analyzed three times intra-daily and inter-daily on three different days using the proposed methods and results are presented in Table (1). RSD values found were well within the acceptable range indicating that these methods have excellent repeatability under the specified conditions.

Selectivity
Selectivity was ascertained by analyzing different laboratory prepared mixtures of GFC and DEX in different ratios within the linearity range. Satisfactory results are shown in Table (2). Good recovery percentages with accepted standard deviations were obtained in all cases.

Application to pharmaceutical formulation
The proposed methods were applied for the determination of GFC and DEX in their combined pharmaceutical formulation; Zigat D® eye drops and the results are shown in Table (2). Good percentage recovery confirms suitability of these methods for the routine determination of these components in their combined formulation. The validity of the proposed procedures is further assessed by applying the standard addition technique. The results obtained are shown in Table (2). Table (3) showed statistical comparisons of the results obtained by the proposed methods and a reported one [41]. The calculated t- and F- values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.
Table 1: Regression parameters for the determination of gatifloxacin and dexamethasone by the proposed methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GFC</th>
<th></th>
<th></th>
<th></th>
<th>DEX</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Zero-order method</td>
<td>DW method</td>
<td>'D method</td>
<td>DW method</td>
<td>'D method</td>
<td>'DR method</td>
<td>RD method</td>
<td>MC method</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>291.6</td>
<td>220-260</td>
<td>301</td>
<td>345</td>
<td>245-270</td>
<td>224.8</td>
<td>261.7</td>
<td>267.4</td>
</tr>
<tr>
<td>Linearity range</td>
<td>1.5-17.5</td>
<td>1.5-17.5</td>
<td>1.5-17.5</td>
<td>5-50</td>
<td>5-50</td>
<td>5-50</td>
<td>5-50</td>
<td>5-50</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1169</td>
<td>0.0306</td>
<td>0.0048</td>
<td>0.0016</td>
<td>0.0166</td>
<td>0.0009</td>
<td>0.0007</td>
<td>0.0040</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0280</td>
<td>-0.0077</td>
<td>-0.0003</td>
<td>-0.0009</td>
<td>-0.0066</td>
<td>0.0006</td>
<td>0.0003</td>
<td>-0.0010</td>
</tr>
<tr>
<td>Correlation coefficient ( r^2 )</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9997</td>
<td>0.9999</td>
<td>0.9997</td>
</tr>
<tr>
<td>Accuracy (mean±SD)</td>
<td>98.67±0.61</td>
<td>100.38±0.68</td>
<td>100.03±0.72</td>
<td>100.13±1.16</td>
<td>100.30±1.31</td>
<td>99.46±0.23</td>
<td>100.62±0.47</td>
<td>100.60±1.26</td>
</tr>
<tr>
<td>Precision (RSD%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Interday</td>
<td>0.91</td>
<td>1.29</td>
<td>1.77</td>
<td>1.36</td>
<td>0.92</td>
<td>1.30</td>
<td>1.58</td>
<td>1.35</td>
</tr>
<tr>
<td>Intraday</td>
<td>1.62</td>
<td>1.58</td>
<td>1.72</td>
<td>1.54</td>
<td>1.33</td>
<td>1.63</td>
<td>1.27</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Table 2: Determination of GFC and DEX in their laboratory prepared mixtures, in eye drops and by applying standard addition technique by the proposed methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GFC</th>
<th></th>
<th></th>
<th></th>
<th>DEX</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero-order method</td>
<td>DW method</td>
<td>'D method</td>
<td>DW method</td>
<td>'D method</td>
<td>'D method</td>
<td>RD method</td>
<td>MC method</td>
</tr>
<tr>
<td>Lab-prepared mixtures (n=5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.85±0.78</td>
<td>100.60±1.44</td>
<td>100.54±0.85</td>
<td>100.54±0.88</td>
<td>99.90±1.42</td>
<td>100.77±1.26</td>
<td>101.47±1.67</td>
<td>99.71±1.61</td>
</tr>
<tr>
<td>301 nm</td>
<td>345 nm</td>
<td>224.8 nm</td>
<td>261.7 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zigat D® eye drops&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.59±0.44</td>
<td>99.70±0.74</td>
<td>100.23±1.02</td>
<td>100.66±1.60</td>
<td>100.41±0.97</td>
<td>101.65±1.87</td>
<td>101.78±1.68</td>
<td>101.12±2.06</td>
</tr>
<tr>
<td>Standard addition</td>
<td>98.80±1.90</td>
<td>99.34±1.00</td>
<td>102.06±0.50</td>
<td>100.17±1.34</td>
<td>100.46±0.70</td>
<td>101.42±2.07</td>
<td>100.17±1.34</td>
<td>100.73±1.26</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five sets each of three replicates
<sup>b</sup> Recovery ± RSD %
Table 3: Statistical comparison between the results obtained by the proposed methods and reported method for determination of GFC and DEX in Zigt D® eye drops.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GFC</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero-order method</td>
<td>DW method</td>
</tr>
<tr>
<td></td>
<td>301 nm</td>
<td>345 nm</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>98.59 ±0.44</td>
<td>99.70 ±0.74</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Variance</td>
<td>0.19</td>
<td>0.55</td>
</tr>
<tr>
<td>F-test</td>
<td>1.52 (2.31)</td>
<td>0.96 (2.31)</td>
</tr>
<tr>
<td>F-value</td>
<td>3.53 (6.39)</td>
<td>1.22 (6.39)</td>
</tr>
</tbody>
</table>

-Figures in parenthesis are the corresponding theoretical values at P = 0.05.
-Reported method [41] depends on HPLC method for simultaneous quantitative determination of DEX and GFC in ophthalmic solution using C₁₈ column with UV detection at 254 nm and a mobile phase consisting of phosphate buffer (pH 3): acetonitrile (70: 30 v/v), at flow rate of 1.0 ml/min.

CONCLUSION

This work describes six simple, specific, accurate and precise spectrophotometric methods for the simultaneous determination of gatifloxacin and dexamethasone in bulk powder, in laboratory prepared mixture and in dosage form. All developed methods do not involve several steps to analyze each drug alone, or other sophisticated calculation. As a final conclusion, the proposed methods could be easily applied in quality control laboratories without any preliminary separation step as they showed good accuracy and precision as indicated from the obtained results.

Competing Interests

The authors declare no conflict of interest.

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45. ICH, Q2B In proceedings of The International Conference on Harmonization, Geneva, 1993.