INTRODUCTION

Cefdinir (CFD), third generation cephalosporin, is chemically known as [6R-[6a,7β (Z)]-7-[(2-aminoo-4-thiazoyl) hydroxyimino] acetyl]-3-ethyl-8-oxo-5-Thia-1-azabicyclo(4.2.0.)-oct-2-one-2-carboxylic acid (Merck, 1996). Of several oral cephalosporins, CFD is recommended as an alternative therapy for children with acute otitis media who have type I hypersensitivity to betalactamase (Bowlware et al, 2006). It is usually a well-tolerated antibiotic, with most adverse effects being mild and self-limiting (Perry et al, 2004; Arguedas, 2006). CFD contains a β-lactam ring which is very labile to acid and base (Helaleh and Abu-Nameh, 1998; El-Obeid et al, 1999) making it very important to develop specific methods for estimation of this drug in presence of possible degradation products. Detailed survey of literature of CFD revealed several methods for its determination in pharmaceutical formulations and biological matrices like, spectrophotometry (Shah and Pandarikakshud, 2006; Singh et al, 2010), polarographic technique (Rajeev et al, 2007), HPLC (Hadad et al, 2009; Khan et al, 2011), and LC/MS/MS methods (Chen, 2006). However, most of these analytical methods do not appear to have widespread utility, especially at the industrial level, where simple, cost-effective, and specific methods are needed. Moreover, to our present knowledge, no stability indicating spectrophotometric nor HPTLC methods has been published in the literature. Therefore, we attempted to develop rapid, sensitive, accurate, and specific spectrophotometric methods (derivative, derivative ratio and mean centering of ratio spectra) and HPTLC-densitometric method for determination of cefdinir in bulk powder and oral dosage forms, and in presence of its acid and alkaline induced hydrolytic degradation products. The results were validated in accordance with International Conference on Harmonization guidelines (ICH, 2005).
MATERIALS AND METHODS

Instrumentation
- Double-beam Shimadzu (Japan) 1601 PC UV-Visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu).
- Desaga densitometer model CD 60 (Germany). AS 30 Desaga applicator. Desaga UV lamp with short wavelength (254nm). HPTLC plates precoated with silica gel 60 F254 (20.0 x 10.0 cm) from E. Merck, Germany.

Materials and reagents
CFD pure sample was obtained from (Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt), 99.71%. CFD capsules (Novartis Pharma S.A.E, Cairo, under licence from Novartis Pharma AG., Basle, Switzerland, Egypt), labelled to contain 300 mg CFD per capsule. CFD suspension (Novartis Pharma S.A.E, Cairo, under licence from Novartis Pharma AG., Basle, Switzerland), labelled to contain 125 mg CFD per 5.0 mL. A diluent (50 mM potassium dihydrogen phosphate, adjusted to pH 6.8 with 1 M NaOH) was used for the preparation of stock solutions of the standard and test samples. Methanol and water (HPLC grade) were purchased from (Riedel-de Hien, Sigma-Aldrich, Germany). Diethylether and glacial acetic acid were purchased from (E. Merck, Darmstadt, Germany); sodium hydroxide and hydrochloric acid (BDH).

Preparation of standard solution of cefdinir
For spectrophotometric methods, stock standard solution of CFD (1.0 mg/mL) was prepared by accurately weighing 100.0 mg of CFD into 100-mL volumetric flask, dissolved in 50 mL of the specified diluent solution and the volume was completed with diluent. Then, working standard solution (100 µg/mL) was prepared by transferring 10.0 mL of the stock standard solution into 100-mL volumetric flask, then volume was completed with diluent. For HPTLC, stock standard solution of CFD (2.0 mg/mL) was prepared by accurately weighing 200.0 mg of CFD into 100-mL volumetric flask, dissolved in 50 mL of the specified diluent and the volume was completed with methanol.

Preparation of standard solutions of hydrolytic degradation products
- Stock standard solution of acid degradation products (1.0 mg/mL) was prepared by accurately weighing 50.00 mg of CFD, dissolving in 20.0 mL 1 M HCl, heating in water-bath at 80°C for 3.5 hrs, then cooling, neutralizing the media with 1 M NaOH (to give pH 7.0) and then completing volume to 50.0 mL with the diluent (for spectrophotometry) or methanol (for HPTLC).
- Working standard solutions (100 µg/mL) of the acid- and alkaline-degradation products were prepared separately for spectrophotometry by transferring 10.0 mL of their stock standard solutions into100-mL volumetric flasks, and then volumes were completed with the diluent.
- Complete hydrolytic degradation of CFD in acid and alkaline media was confirmed by applying the proposed HPTLC method.

General Procedures
Spectrophotometric methods
- For derivative spectrophotometry, second derivative (′DD) was applied to the zero-order absorption spectra of CFD and its acid degradation product binary mixtures with ∆λ = 8 nm and scaling factor 100, and CFD was determined at 298.2 nm. First derivative spectrophotometry (′D) was applied to the absorption spectra of CFD and its alkaline degradation product binary mixtures with ∆λ = 8 nm and scaling factor 10, and CFD was determined at 313.4 nm.
  - For first derivative ratio spectrophotometry (′DD), Zero order spectra of CFD & its acid degradation product binary mixtures were divided by the spectrum of the acid degradation product (40 µg/mL) then first derivative was applied for these ratio spectra (′DD) using scaling factor 10 and ∆λ = 4 nm. Peak amplitudes were measured at 312 nm. While Zero order spectra of CFD and its alkaline degradation product binary mixtures were divided by the spectrum of the alkaline degradation product (40 µg/mL) then first derivative was applied for these ratio spectra (′DD) using scaling factor 10 and ∆λ = 4 nm. Peak amplitudes were measured at 310.2 nm.
  - For mean centering of ratio spectra, the previously obtained ratio spectra for CFD, and its acid and alkaline degradation products binary mixtures were mean centered and peak amplitudes were measured at 288.4 nm and 284.8 nm, respectively.

HPTLC-densiometric method
Analysis was performed on 20 x 10 cm HPTLC plates pre-coated with silica gel 60 F254 (E. Merck). The plates were spotted 2 cm apart from each other and 1cm apart from the bottom edge.

The chromatographic tank was pre-saturated with the developing system for 15 min, then the plates were developed by ascending chromatography using diethylether-methanol-water-glacial acetic acid (6: 3: 1: 0.05, v/v) as a developing system to a distance of 9.5 cm. The plates were air dried, detected under UV-lamp and then, scanned at 285 nm under the following experimental conditions of measurements: photo mode=reflectance, scan mode=linear slit scanning, slit width = 0.4mm, slit height = 0.02mm, result output= densitogram and peak list.
Method validation

Linearity
In case of spectrophotometric methods, accurately measured volumes of CFD working standard solution (100.00 µg/mL) were transferred into a series of 10-mL volumetric flasks, diluted to volume with the diluent to obtain suitable concentration range for each proposed spectrophotometric method. Zero order spectra were recorded using the diluent as blank. The previously mentioned general procedures were then followed and the values of peak amplitudes were plotted against the corresponding concentrations; the regression equations were then computed.

In HPTLC, accurately measured volumes (0.5–6.0 mL) of CFD stock standard solution (2.0 mg/mL) were transferred into a series of 10-mL volumetric flasks, diluted to volume with methanol to obtain a concentration range of 0.10–1.20 mg/mL. A 5 µL volume of each solution was applied to the plates in triplicates, the chromatographic conditions were adjusted, the plates were developed and the peak areas were measured. The calibration curve representing the relationship between the integrated peak area and its corresponding concentration was constructed and the regression equation was recorded.

Accuracy
The previously mentioned procedures under linearity were repeated for determination of different concentrations of CFD. The concentrations were calculated from the regression equations and the percentage recoveries were then calculated.

Precision
Three concentrations of CFD were analyzed five times intra-daily and on five successive days using the previously mentioned procedures under linearity. The mean percentage recovery and the relative standard deviation were calculated.

Specificity
The specificity of the proposed methods was established by the analysis of laboratory mixtures, consisting of the intact drug with its acid and alkaline degradation products, in triplicate. The peak amplitudes (for spectrophotometry) and peak areas (for HPTLC) were measured and the concentrations of CFD were then calculated from the regression equations. The mean percentage recovery and the relative standard deviation were calculated.

Application to the pharmaceutical formulations
In case of capsules, the content of ten capsules were weighed, finely powdered, and mixed thoroughly. An accurately weighed amount of the powder obtained from capsules equivalent to 300 mg of CFD was transferred into a 100-mL volumetric flask containing 50 mL the specified diluent. For oral suspension, 5 mL of reconstituted suspension was accurately transferred (after vigorous shaking) into a 100-mL volumetric flask containing 50 mL of the diluent. The content of each flask was shaken well for 15 min, completed to the mark with the diluent (in case of spectrophotometry) or methanol (in case of HPTLC) and then filtered; the first portion of the filtrate was rejected. A suitable aliquot of each solution was diluted quantitatively with the diluent (in case of spectrophotometry) or methanol (in case of HPTLC) to obtain a concentration within the linearity range. The suggested procedures stated under linearity were followed for CFD assay. For standard addition technique, known amounts of CFD were added to the drug product, the suggested procedures stated under linearity were carried out. The concentrations, the mean percentage recovery and the relative standard deviation were then calculated.

RESULTS AND DISCUSSION

Spectrophotometric methods

Derivative spectrophotometry
Acid and alkaline degradation products of CFD have absorption spectra which overlap with that of intact CFD (Figure 1). Upon examining their first derivative spectra (1D), the severe overlap could be resolved for CFD and its alkaline degradation product and so CFD could be determined at 313.4 nm (Figure 2). By applying second derivative spectrophotometry (2D), CFD could be determined at 298.2 nm, where its acid degradation product has no contribution (zero crossing) (Figure 3). Δλ was optimized to give a well resolved peak and thus high selectivity was obtained.

First derivative ratio spectrophotometry (1DR)
In this method, careful choice of the divisor is of great importance (Nevado, 1992; Gracia, 1995), so different concentrations of the acid and alkaline degradation products were tried as a constant divisor, the best one for each degradation product was 40.00 µg/mL as it gave the best linear response, i.e. wider linearity range, good correlation coefficient and a near zero intercept on the ordinate of the graph. The first derivative of the ratio spectra were obtained using scaling factor 10 and Δλ=8 nm, (Figures 4 and 5). Peak amplitudes at 312nm using acid degradation product spectrum as divisor and at 310.2 nm using alkaline degradation product spectrum as divisor were proportional to CFD concentration in the range of 2-30 µg/mL.

Mean centering of ratio spectra
This method eliminates the derivative steps and therefore signal-to-noise ratio is enhanced (Afkhami and Bahram, 2004) and it has been applied for resolving binary and ternary mixtures in complex samples with unknown matrices (Afkhami and Bahram, 2005). The mathematical explanation of the method was illustrated by Afkhami and Bahram. Beer’s Lambert’s law has been obeyed at 288.4 nm using acid degradation products spectrum as divisor and at 284.8 nm using alkaline degradation products spectrum as divisor (Figures 6 and 7). Assay validation parameters for the spectrophotometric methods are presented in Table 1. The specificity of these methods was tested by analyzing laboratory prepared mixtures (Table 2). The data obtained shows that CFD can be quantitatively determined in presence of up to 50% of its acid and alkaline degradation products.
Table 1: Assay validation parameters of the proposed methods for the determination of cefdinir

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ±RSD%</th>
<th>Mean±RSD%</th>
<th>Mean±RSD%</th>
<th>Mean±RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9998</td>
<td>99.73±0.552</td>
<td>99.97±0.546</td>
<td>99.54±0.618</td>
</tr>
<tr>
<td>slope</td>
<td>0.0161</td>
<td>100.19±0.378</td>
<td>100.10±0.769</td>
<td>99.76±0.905</td>
</tr>
<tr>
<td>Coefficient1 (b1)</td>
<td>0.0231</td>
<td>99.84±0.571</td>
<td>99.45±0.676</td>
<td>99.76±0.599</td>
</tr>
<tr>
<td>Coefficient2 (b2)</td>
<td>0.26</td>
<td>100.45±0.76</td>
<td>100.30±0.90</td>
<td>100.31±0.87</td>
</tr>
<tr>
<td>Intercept</td>
<td>99.15±0.552</td>
<td>100.77±0.734</td>
<td>100.37±1.258</td>
<td>100.54±1.258</td>
</tr>
</tbody>
</table>

Table 2: Determination of cefdinir in laboratory prepared mixtures with its acid and alkaline degradation products by the proposed spectrophotometric methods.

<table>
<thead>
<tr>
<th>Degradation products</th>
<th>Cefdinir products</th>
<th>% Recovery of cefdinir*</th>
<th>Mean ±RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/mL</td>
<td>µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>101.85</td>
<td>96.99</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>101.27</td>
<td>100.32</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>99.98</td>
<td>101.03</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>98.90</td>
<td>100.62</td>
</tr>
<tr>
<td>16</td>
<td>20</td>
<td>99.82</td>
<td>101.71</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>99.89</td>
<td>100.27</td>
</tr>
<tr>
<td>Mean ±RSD%</td>
<td>100.28±1.077</td>
<td>99.87±0.895</td>
<td>100.77±0.734</td>
</tr>
</tbody>
</table>

Table 3: Determination of cefdinir in laboratory prepared mixtures with its acid and alkaline degradation products by the proposed HPTLC method.

<table>
<thead>
<tr>
<th>Degradation products</th>
<th>Cefdinir products</th>
<th>% Recovery* of cefdinir in presence of</th>
<th>Mean ±RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/mL</td>
<td>µg/mL</td>
<td>Acid degradation products</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>99.44</td>
<td>99.32</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>101.91</td>
<td>98.65</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>100.26</td>
<td>100.66</td>
</tr>
<tr>
<td>Mean ±RSD%</td>
<td>100.54±1.258</td>
<td>99.54±1.023</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Determination of cefdinir in pharmaceutical formulations and application of standard addition technique.

<table>
<thead>
<tr>
<th>Pharmaceutical formulation</th>
<th>% Recovery of cefdinir</th>
<th>Mean ±RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefdin Capsules 300mg BN: A200707</td>
<td>99.15±0.512</td>
<td>99.16±0.306</td>
</tr>
<tr>
<td>Standard addition technique (mean±RSD%) Cefdin suspension 125mg/5mL BN: N100431</td>
<td>99.63±0.994</td>
<td>99.80±0.981</td>
</tr>
<tr>
<td>Standard addition technique (mean±RSD%)</td>
<td>101.13±0.387</td>
<td>101.61±0.681</td>
</tr>
</tbody>
</table>

Fig 1: Zero order absorption spectra of CFD (—), its acid (- -) and alkaline degradation products (…) (24.00 µg/mL each).
**Fig. 2:** First derivative (1D) absorption spectra of CFD (—) and its alkaline degradation products (…) (24.00 µg/mL each).

**Fig. 3:** Second derivative (2D) absorption spectra of CFD (—) and its acid degradation products (- -) (24.00 µg/mL each).

**Fig. 4:** First derivative of ratio spectra (1DD) for different concentrations (2.00 - 30.00 µg/mL) of CFD at 312 nm using acid degradation products (40.00 µg/mL) as divisor.
Fig. 5: First derivative of ratio spectra (‘DD) for different concentrations (2.00 - 30.00 µg/mL) of CFD at 310.2 nm using alkaline degradation products (40.00 µg/mL) as divisor.

Fig. 6: Mean centering of ratio spectra for different concentrations (2.00 - 24.00 µg/mL) of CFD at 288.4 nm using acid degradation products (40.00 µg/mL) as divisor.

Fig. 7: Mean centering of ratio spectra for different concentrations (2.00 - 26.00 µg/mL) of CFD at 284.8 nm using alkaline degradation products (40.00 µg/mL) as divisor.
HPTLC - densitometric method

For HPTLC, experimental conditions such as developing system and wavelength of detection were optimized to provide accurate, precise and reproducible results. Different developing systems were tried such as diethylether: methanol (7.0: 3.0, v/v). This developing system gave poor resolution between the intact drug and its acid degradation product. Thus, the polarity of this system was changed by adding different volumes of water and glacial acetic acid. The best resolution with minimum tailing of CFD peak from its hydrolytic degradation products was achieved by using diethylether-methanol-water-glacial acetic acid (6: 3: 1: 0.05, v/v). The separated drug spots were determined densitometrically on the plates at 285 nm. The tailing factor of CFD peak was 1.4 and Rf values were 0.50 for CFD and 0.71 and 0.05 for its acid and alkaline degradation products, respectively (Figures 8a and 8b). For quantitative application using HPTLC, the calibration curves are generally inherently non-linear due to scattering of light. They generally comprise a pseudo-linear region at low sample concentration and then departure from linearity begins at higher sample concentrations (Poole and Poole, 1989). Moreover, the ICH guidelines mentioned that for some analytical procedures which do not demonstrate linearity, the analytical response should be described by an appropriate function of the concentration of an analyte sample.

The relationship between the integrated peak area and the concentration was evaluated with linear and polynomial regression functions. Fitting with linear function gave correlation value, $r = 0.9864$ while fitting with polynomial function gave better correlation ($r = 0.9995$) and lower standard deviation values and was therefore used for quantitative analysis. Calibration curve was constructed in the range of 0.50-6.00 µg/spot.

Assay validation parameters for HPTLC method are presented in Table 1. The specificity of this method is illustrated in Figure 6 where complete separation of CFD from its acid and alkaline degradation products was noticed, and also tested by analyzing the laboratory prepared mixtures.

The results are presented in Table 3. From the data obtained, one can conclude that CFD can be determined without any interference from its different hydrolytic degradation products. CFD containing dosage forms were analyzed by the
proposed spectrophotometric and HPTLC methods and satisfactory results were obtained and were in a good agreement with the label claims (Table 4). Standard addition technique was also applied and the results obtained are shown in Table 4. The results of analysis of the pharmaceutical formulation and the standard addition method suggest that there is no interference from any excipients.

CONCLUSION

The proposed spectrophotometric and HPTLC methods are simple, rapid, accurate, precise and specific over the specified ranges. The proposed methods are considered as stability indicating methods for the determination of CFD in presence of its acid and alkaline induced hydrolytic degradation products without prior extraction. Hence, these methods are suitable for stability testing of CFD and for routine quality control analysis in bulk material and in pharmaceutical formulations where economy and time are essential.

REFERENCES


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