Differential Spectrophotometric Method for Determination of Florfenicol

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Abstract: Simple, selective and accurate differential first derivative spectrophotometric method ($\Delta D_1$) was developed for the determination of florfenicol in bulk and pharmaceutical formulation. The developed method was based on measuring the $\Delta D_1$ of florfenicol in alkaline solutions against its aqueous solutions as blank. Beer’s law was found to be linear over the concentration range 3-15 µg/mL with good correlation coefficient (0.999). The selectivity of the method was proved through freedom from interferences.

Keywords: Differential, First derivative, Florfenicol, Alkaline solutions.

Introduction

Florfenicol (Figure 1) is a fluorinated synthetic analog of thiamphenicol. It is currently indicated for the treatment of bovine respiratory disease (BRD). It is also used in aquaculture and is licensed for use in the United States for the control of enteric septicemia in catfish.

Figure 1. Chemical structure of florfenicol

Literature review revealed different methods for the analysis of florfenicol, which have been applied mainly for its analysis in biological fluids. Recently, we developed simple and accurate methods for its analysis and stability studies in pharmaceutical dosage form.

Differential spectrophotometry is an analytical technique which has been used to improve the selectivity and accuracy of the measurement. It is based on measuring the difference absorbance of two equimolar solution of the analyte in different chemical forms. It is not only eliminates matrix interference due to excipients, but also it resolves spectral overlap of other accompanying drugs.
The accuracy and selectivity of conventional UV absorption methods can be increased by conversion of normal zero-order or differential UV spectra into higher order\textsuperscript{11, 12}. The stability-indicating property, coupled with the selectivity and simplicity of application of the derivative spectrophotometry (first, second... etc) and $\Delta D_1$ make these methods more preferable to be used for drug analysis than the costly HPLC methods, specially in developing countries.

Therefore, the aim of the present work was to develop simple and accurate differential spectrophotometric method for the analysis of florfenicol

**Experimental**

UV spectrophotometric studies were carried out on Shimadzu UV1800ENG240V, double beam, (Kyoto, Japan). The operating conditions were; wavelength range: 250-350 nm, scan speed: medium, 0.2 nm/s, sensitive balance: Kern ALS 120-4, Germany.

**Chemicals and reagents**

Florfenicol reference standard was kindly provided by colleagues in the Central Lab, Riyadh, King Saudi Arabia. Florfenicol sample (Norflor\textsuperscript{®} injection solution, 300 mg/mL) was obtained from Schering-Plough Sante Animale, La Grindoliere, Serge-France. All solutions were prepared using distilled water as a solvent. Distilled water was the pipette solvent used in all the experimental work

**Standard stock solution**

An accurately weighed quantity of florfenicol standard (0.15 g) was dissolved in 20 mL distilled water and transferred into 100 mL volumetric flask. The volume was then completed to mark with the pipette. 1 mL of the resultant solution was further diluted to 50 mL (solution A; 30 $\mu$g/mL).

**Sample stock solution**

One mL of florfenicol injection solution was accurately pipetted out and transferred into 100 mL volumetric flask. The volume was completed to mark with the solvent. 1 mL of the resultant solution was further diluted to 100 mL (solution B; 30 $\mu$g/mL).

**Procedure**

**Calibration curves**

Different accurately measured volumes (1-5 mL) from solution A were transferred into five stoppered glass tubes. 1 mL of 1M NaOH was added to each tube. The tubes were heated in a boiling water bath for 30 minutes. The reaction was then quenched by cooling and the volumes were completed to 10 mL using distilled water. $\Delta D_1$ spectrum was then recorded over the range 250-350 nm by measuring the absorbance of the florphenicol alkaline solutions against the corresponding aqueous florphenicol solutions as blanks. The obtained $\Delta D_1$ values were then plotted against the corresponding concentrations.

**Sample content**

The procedure under calibration curve was repeated using 3 mL of solution B instead of solution A. The content of the injection solution was then evaluated from the calibration curves or by the direct comparison of sample/standard absorbance values.

**Method validation**

The developed method was validated in terms of linearity, accuracy and precision according to ICH guidelines\textsuperscript{13}. 
Results and Discussion

Florfenicol is an amide containing drug which is liable to chemical degradation. Referring to the stability studies conducted on florfenicol, it was found to undergo chemical degradation depending on [OH\(^-\)]. As shown in Figures 2 & 3, the first derivative spectra exhibited different absorption bands for florfenicol aqueous and alkaline solutions.

![Figure 2](image1.png)

**Figure 2.** First derivative spectrum of florfenicol aqueous solution (9 µg/mL; 274 nm)

![Figure 3](image2.png)

**Figure 3.** First derivative spectrum of florfenicol solution treated with 1M NaOH (heating time 20 minutes)

This bathochromic shift (from 274 nm to 325 nm) forms the basis of the quantitative determination of florfenicol by measuring \(\Delta D_1\) at 281 nm (Figure 4)

![Figure 4](image3.png)

**Figure 4.** Differential first derivative spectrum of florfenicol (9 µg/mL; 281 nm)

Method validation

A calibration curve was constructed relating the concentration of florphenicol in a concentration range 3-15 µg/mL to \(\Delta D_1\) at 281 nm (Figure 5).
Figure 5. Calibration curve for Florphenicol sample (ΔD₁, mean absorbance)

The results obtained for linearity data of the proposed method are summarized in Table 1. The correlation coefficient (not less than 0.999) along with the low values of standard errors of slope and intercept reflected the consistency of the calibration curve.

Table 1. Linearity data for the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ΔD₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope ± t₁b</td>
<td>0.0057±0.00038</td>
</tr>
<tr>
<td>Intercept ± t₁a</td>
<td>0.0005±0.0038</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9991</td>
</tr>
<tr>
<td>Range</td>
<td>3-15 µg/mL</td>
</tr>
<tr>
<td>LOD</td>
<td>0.67 µg/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.00 µg/mL</td>
</tr>
</tbody>
</table>

*SStandard error of slope calculated at 95% confidence limit for n-2 degrees of freedom

Sample content, accuracy and precision

The %± SD data for florphenicol assay and the added recovery using the proposed method were 102.55±0.35% and 100.00±0.00% (n=3), respectively. Both results reflected the accuracy of the method and the freedom of interference by the injection excipients.

The validity of the method was assessed by the statistical evaluation of results obtained. As the calculated t-value (6.1) at 95% confidence limit was less than tabulated one (12.78), the developed method proved to be accurate.

Reproducibility and repeatability of the developed method were obtained by the follow-up of within-day and between-day data for three concentrations within the linearity range. The results obtained indicate the precision of the method which was reflected by the low RSD values (Table 2).

Table 2. Within-day and between-days results for the developed method

<table>
<thead>
<tr>
<th>Concentration, µg/mL</th>
<th>Within-day RSD%, n=3</th>
<th>Between-days RSD%, n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>12</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>15</td>
<td>1.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Conclusion

The developed method proved to be simple, accurate and selective for the determination of florfenicol in bulk and pharmaceutical form. The application of differential spectrophotometry
is expected to have the totality of advantages of both derivative spectrophotometry (first, second, etc.,) combined with delta spectrophotometry. The selectivity and simplicity of the method permits the determination of florfenicol in the presence of its degradation product (alkaline hydrolysis). This encourages its application for the routine quality control analysis of the drug.

References