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Original Article

DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF FLORFENICOL IN BULK AND DOSAGE FORMS

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ABSTRACT

Objectives: The objective of this research was to develop simple, sensitive and accurate zero (°D), first (^{1}D) and second (^{2}D) order derivative spectrophotometric methods for the analysis of florfenicol in bulk and dosage forms.

Methods: The original UV spectrum (zero-order) of florfenicol aqueous solution was measured at 267 nm against its blank. This spectrum was then differentiated instrumentally to generate the first and second derivative spectra which were measured at 274 nm and 281 nm, respectively. The developed methods were validated as per ICH guidelines.

Results: Regression data of the developed methods obeyed Beer's law over the concentration range 3-15µg /ml with a good correlation coefficient (not less than 0.998). Limits of detection were found to be 0.68, 1.30, 1.13µg/ml and limits of quantification were 2.05, 3.87, 3.58µg/ml for °D, ^{1}D and ^{2}D order derivative, respectively. The developed methods demonstrated good inter-day and intra-day precision at the three modes. The obtained recovery percentage (98.3±1.8%; n=3) reflected the freedom from interference by the excipients.

Conclusion: The statistical validation at 95% confidence level proves the sensitivity, accuracy and precision of the developed methods.

Keywords: Florfenicol, Spectrophotometry, Derivative spectra, Dosage form

INTRODUCTION

Florfenicol (fig. 1) is a fluorinated synthetic analog of thiamphenicol [1]. 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 [2].

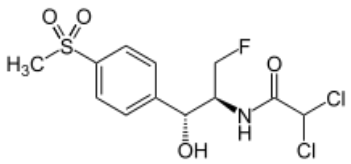

Fig. 1: Chemical structure of florfenicol

Literature review revealed different methods for the analysis of florfenicol [3-6], none of which has been applied for its analysis in pharmaceutical dosage form.

Derivative spectrophotometry is a simple, powerful technique. It is suitable for analysis of turbid solutions [7], and can be used successfully for the assay of pharmaceutical formulations.

In derivative spectrophotometry, the absorbance (A) of a sample is differentiated with respect to wavelength (λ) to generate the first, second or higher order derivative.

In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or °D spectrum [8].

Derivative spectrophotometry also solves the problem of analysis associated with drugs combination, stability studies of drug and degradation products, drug impurities and interference of excipient in drugs. It also solves the problem of analysis of drugs in biological fluids.

A number of reports have been cited for the application of derivative methods for solving analytical problems associated with interferences (9-12)

Therefore, the aim of the present work was to develop simple and accurate spectrophotometric methods (°D, ^{1}D, ^{2}D) for the analysis of florfenicol in bulk and dosage forms.

MATERIALS AND METHODS

Apparatus

UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, double beam, (Kyoto, Japan). The operating conditions were as follows:

-Wavelength range: 250-400 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®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.

Preparation of stock solutions

Distilled water was the diluent solvent used in all the experimental work.

Standard stock solution

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

Sample stock solution

One ml of florfenicol injection solution was accurately pipetted and transferred into 100 ml volumetric flask. The volume was completed to mark with the solvent. 1 ml was further diluted to 100 ml (solution B; 30µg/ml).

Determination of λ_{max}

The standard stock solution of florfenicol was diluted to obtain a concentration of 9µg/ml. The solution was scanned within the range 200-400 nm in °D, ^{1}D and ^{2}D order derivative modes, respectively.

Method validation

Linearity

Serial dilutions were made from solution A by transferring accurately measured volumes (1-5 ml) into a set of 10 ml volumetric flasks. The volumes were then completed to mark with the solvent and the °D, ^{1}D and ^{2}D order derivative spectra were recorded over the range 250-350 nm. The procedure was repeated three times. The mean absorbance values were plotted against concentration to construct the calibration curves.

Limits of detection and quantification were determined from the calibration curve using the adopted formulae [13].

LOD = 3.3 SB/Slope LOQ = 10SB/Slope

Where SB is the standard deviation $s_{y/x}$ calculated from the regression analysis data

Content uniformity

The procedure under linearity was repeated using solution B instead of solution A. The content uniformity of the injection solution was evaluated by the direct comparison of sample/standard absorbance values.

Precision

Serial dilutions were made from solution A to obtain concentrations of 6µg/ml, 9µg/ml, and 12µg/ml. These solutions were scanned at the three modes (°D, ^{1}D and ^{2}D) three times within the same day (inter-day) and at three consecutive days (intra-day). The results obtained were used to evaluate the precision of the developed method in terms of relative standard deviation values (RSD %).

Recovery percentage

The freedom of interference by the injection excipients was confirmed by the results obtained for recovery testing of added amount of authentic florfenicol to the sample solution in the ratio of 1:1. 2 ml of each solution A and B were transferred to separate stoppered glass tubes. Another 2 ml of solution B was mixed with 2 ml of solution A in a third tube. The above solutions were then treated as under linearity. The above solutions were scanned in the three modes. The recovery percentage was determined using the following equation [14]:

$$\text{Percent Recovery} = [(A_{mix} - A_{sam})/A_{std}] \times 100$$

Where A_{mix} is the absorbance of mixture; A_{sam} is the absorbance of sample; A_{std} is the absorbance of the standard.

RESULTS AND DISCUSSION

Florfenicol is a drug used in veterinary medicine, and all the previous reported methods were mainly directed towards its assay in biological fluids [4]. In the point of the pharmaceutical analysis view, any drug in its pharmaceutical formulations needs to be evaluated chemically (content %). Therefore, we deemed it useful to develop simple, direct methods utilizing the easily available, easy-to-use, cheap and robust spectrophotometric methods that can offer good precision for the quantification of florfenicol in its dosage form. These methods are also meant to be used for quality control analysis within a short time using the safe and available water as a diluent solvent.

Determination of λ_{max}

The zero-order derivative spectrum of florfenicol shows some vibrational bands with absorption maxima at 267 nm (fig. 2). First and second derivatization of the resultant spectrum showed sharper and better-resolved bands at 274 nm and 281 nm, respectively (fig. 3 and 4).

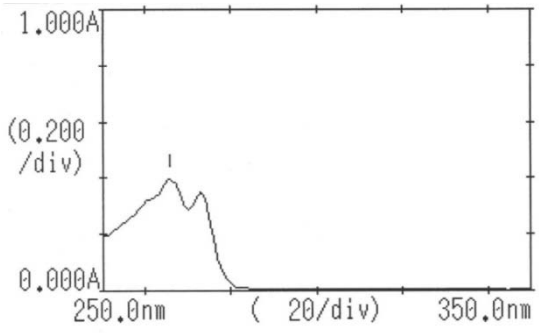

Fig. 2: UV spectrum of florfenicol solution (6µg/ml; 267 nm)

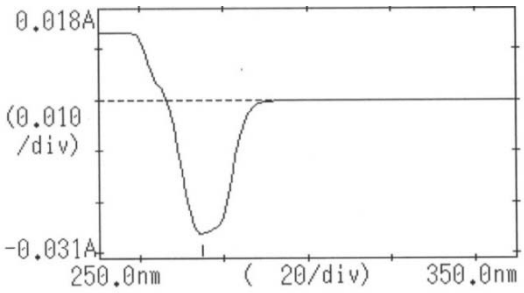

Fig. 3: First derivative spectrum of florfenicol solution (6µg/ml; 274 nm)

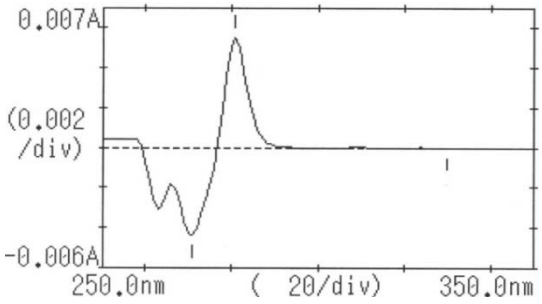

Fig. 4: Second derivative spectrum of florfenicol solution (6µg/ml; 281 nm)

Linearity

The calibration curves, relating the florfenicol concentration in a range 3-15µg/ml to the mean absorbance values, were constructed for the three modes. Linearity was found to obey Beer's law with a good correlation coefficient (not less than 0.998). The regression analysis data was calculated at 95% confidence level for the developed methods using the following formula [15]:

$$y = (b \pm tS_b)\, x + (a \pm tS_a)$$

Where b is the slope, a is the intercept, S_b is the standard deviation of the slope, s_a is the standard deviation of intercept, t is the t-value at 95% confidence level for (n–2) degrees of freedom.

The results obtained for linearity data of the developed methods are summarized in table 1, which reflected the accuracy and consistency of these curves.

Table 1: Linearity data of the developed methods (n=3)

Parameter	°D	^{1}D	^{2}D
λ_{max}	267 nm	274 nm	281 nm
Concentration range	3-15µg/ml	3-15µg/ml	3-15µg/ml
Slope±ts_b*	0.062±0.009	0.0039±0.0011	0.0015±0.0004
Intercept±ts_a**	0.004±0.041	0.0015±0.005	0.0001±0.0018
LOD	0.68 µg/ml	1.30 µg/ml	1.18 µg/ml
LOQ	2.05 µg/ml	3.87 µg/ml	3.58 µg/ml
R	0.998	0.998	0.999

*Slope±standard deviation of slope at 95% confidence level for (n-2) degrees of freedom, **Intercept±standard deviation of intercept at 95% confidence level for (n–2) degrees of freedom,

Table 2: Inter-day and intra-day precision data for °D, ^{1}D and ^{2}D methods

Conc. µg/ml	Inter-day results; RSD%; n=3			Intra-day results; RSD%, n=3		
	°D	^{1}D	^{2}D	°D	^{1}D	^{2}D
6	0.30	1.40	0.58	0.36	1.67	0.87
9	1.56	1.00	1.23	1.97	1.91	0.58
12	0.55	1.48	1.82	0.00	0.00	0.00

The low SB values of response, LOD and LOQ values indicate the sensitivity of the developed methods.

Assay and validation

The developed methods were applied for the drug uniformity testing in Norflor injection. Good assay results ranged 100.70±1.84%; n=3 were obtained. The validity of the methods was assessed by statistical evaluation of the results obtained [15]. As the calculated *t*-value (0.65) at 95% confidence limit was less than tabulated one (4.3), the developed UV methods proved to be accurate.

Precision

The inter-day and intra-day precision was studied for all the modes. RSD% values were found to be within 0.30-1.56% (inter-day) and 0.00-1.97% (intra-day). These results indicate the precision of the developed methods as RSD% values were within the acceptance criteria (<2%). The results are summarized in table 2.

Recovery percentage

The accuracy of the developed methods at the three modes and freedom of interference by the injection excipients were confirmed by the good results of recovery testing (98.3±1.8%; n=3).

CONCLUSION

The developed spectrophotometric methods proved to be simple, sensitive, rapid, accurate and precise for the determination of florfenicol in bulk and dosage forms. In addition, the procedure of the developed method does not require neither extraction step nor chemicals and, thus can be used for the routine analysis of the drug.

The results of the zero-order method (°D) reflected good precision, however, its direct application is expected to be limited for samples free from irrelevant absorption; On the other hand ^{1}D and ^{2}D methods are expected to prove their stability-indicating properties that can allow their use in the presence of irrelevant absorption.

The stability-indicating properties of the developed methods are under investigation for the analysis of florfenicol in the presence of its degradation products (possible amide hydrolysis).

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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