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STABILITY-INDICATING SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF CEFDINIR IN PURE FORM AND PHARMACEUTICAL PREPARATION

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ABSTRACT: Three Simple, rapid, sensitive, accurate and precise stability-indicating spectrophotometric methods was developed for the determination of cefdinir in bulk powder and in pharmaceutical preparation. Method (A) Ratio derivative method(^{1}DD); is used for the determination of intact cefdinir in presence of its degradation product at 307 nm in the range of $2 - 14$ µg ml^{-1} with LOD of 0.049 µg ml^{-1} and LOQ of 0.162 µg ml^{-1}. Method (B) Ratio difference method ;is based on measuring the difference in the amplitude of intact cefdinir in presence of its degradation product at two different wavelengths ,this is done at 219 nm and 250 nm in the range of $2 - 14$ µg ml^{-1} with LOD of 0.050 µg ml^{-1} and LOQ of 0.165 µg ml^{-1}. Method (C) First derivative method(^{1}D); is used for the determination of intact cefdinir in presence of its degradation product at 304 nm in the range of $2 - 14$ µg ml^{-1} with LOD of 0.034 µg ml^{-1} and LOQ of 0.114 µg ml^{-1}. The methods was validated and successfully applied to the determination of cefdinir tablets with an average percent recovery $\pm$ RSD% of 100.02 ± 0.643 for method (A), 100.32 ± 0.21 for method (B) and 100.43 ± 0.86. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

INTRODUCTION: Cefdinir (**Fig. 1**) is ;(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 72-(Z)-oxime; 7-{(2-Amino-1,3-thiazol-4-yl)-2-[(Z)-hydroxyimino]acetamido}-3-vinyl cephem-4-carboxylic acid [1]. Cefdinir is the member of what is generally termed the third generation orally active cephalosporin antibiotics [2].

Literature survey shows that several spectrophotometric [3-6] and chromatographic [7-13] methods for determination of cefdinir in pure form, pharmaceutical preparations and/or biological fluids have been reported.
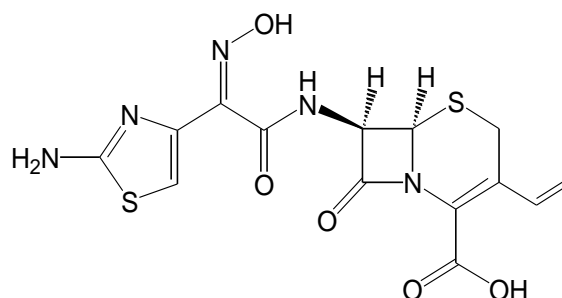
FIGURE 1: STRUCTURAL FORMULA OF CEFDINIR

MATERIALS AND METHODS:

Apparatus:

- Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).

- Hot plate (Torrey pines Scientific, USA).

- Jenway, 3510 pH meter (Jenway, USA).

Materials and Reagents: All chemicals and reagents used throughout the work were of analytical grade.

- Cefdinir powder was kindly supplied by Novartis Pharmaceutical Industries Company. (B. NO. A200707).

- Cefdin® 300mg capsule. The product of Novartis Pharmaceutical Industries Company. (B. NO.CDRM090002).

- Hydrochloric acid, Sodium hydroxide and Methanol (El-Nasr Co., Egypt).

Standard Solution: A stock solution of cefdinir (100 µg/ml) was prepared by dissolving 10 mg of cefdinir in 50 ml of methanol and complete to 100 ml with methanol and was further diluted with the same solvent as appropriate. The standard solution was stable for 2 weeks when kept in the refrigerator.

Degraded Sample [8]: Base-induced forced degradation was performed by adding 10 mg of cefdinir to 100 mL 0.1 M NaOH and refluxing at 60°C for approximately six hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.1 M HCl, evaporated to dryness, the residue was extracted three times with 25 ml methanol, filtered into 100 ml volumetric flask then the volume was adjusted by the same solvent. The obtained solution was claimed to contain (0.1 mg ml^{-1}).

Procedure:

Construction of the Calibration Curves (General Procedures):

Method A: Aliquots equivalent to (0.02 – 0.14 mg ml^{-1}) cefdinir and (0.02–0.14mgml^{-1}) cefdinir degradate are accurately transferred from their standard working solutions (20 µg ml^{-1}) into two separate 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.

For the determination of cefdinir in presence of its degradation product, the stored spectra of cefdinir are divided by the spectrum of 4 µg ml^{-1} degradate, smoothed with $\Delta\lambda = 16$ nm and scaling factor 10, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda = 4$ nm is obtained. The amplitude of the first derivative trough of (cefdinir / degradate) is measured at 307.0 nm.

A calibration graph relating the trough amplitude at 307.0 nm to the corresponding concentrations in µg ml^{-1} of cefdinir is constructed alternatively, the regression equation was derived.

Method B: Aliquots equivalent to (0.02 – 0.14 mg) were accurately transferred from cefdinir standard stock solution (0.1 mg ml^{-1}) 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. For the determination of cefdinir in presence of its degradation product, the stored spectra of cefdinir are divided by the spectrum of (4 µg ml^{-1}) of the alkaline degradate. The amplitude difference at 219.0 and 250.0 nm (ΔP219.0 – 250.0) was plotted against the corresponding cefdinir concentration in µg ml-1 and the regression equation was computed.

Method C: Aliquots of standard cefdinir solution in methanol (0.1 mg ml^{-1}) containing (0.02 – 0.14) mg of the drug were added to a series of 10 ml volumetric flasks and then diluted to the mark with methanol. First - derivative (^{1}D) spectra of the drug were recorded against methanol as blank. The height of the trough at 304 nm was measured in cm for each drug concentration.

Calibration curve relating troughs height in cm to drug concentration in µg ml^{-1} was constructed, the regression equation was derived.

Analysis of Pharmaceutical Preparation: Ten Cefdin® 300 mg capsules were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of cefdinir was shaken three times with 25 ml methanol 15 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with water to obtain a concentration of (0.1 mg ml^{-1}). The solution was analyzed using the procedure described under method A, method B and method C.

RESULTS AND DISCUSSION:

Spectral Characteristics: The zero order (D_0) absorption spectra of cefdinir (10 µg ml^{-1}) and its alkaline degradation product (10 µg ml^{-1}) were recorded against methanol as blank over the range of $200 – 400$ nm (**Figure 2**)*.*

For method A*:* Salinas et al.[14] designed a spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum) [15-17]. 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 in the presence of other compounds and excipients which possibly interferes the assay. 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.

The main parameters that affect the shape of the ratio spectra are wavelength, scanning speed, the concentration of the standard solution used as a divisor, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing function are carefully tested. The ratio spectra presented in **Figure (3)** and the first derivative of the ratio spectra presented in **Figure (4)** may provide a good proof for this understanding.

The effect of wavelength scanning speed is studied. It is 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 divisor concentration is also tested, different concentrations of divisor are used ($10, 8, 4$ µg ml^{-1}) of cefdinir degradate and the divisor concentration 4 µg ml^{-1} of cefdinir degradate is found the best regarding average recovery percent when it is used for the prediction of cefdinir concentrations in bulk powder as well as in laboratory prepared mixtures.

The absorption spectra of cefdinir are divided by the absorption spectrum of 4 µg ml^{-1} cefdinir degradate and smoothed **Figure (3)** for determination of cefdinir in the presence of its degradate. This gave the best compromise in terms of sensitivity, repeatability and signals to noise ratio. The choice of wavelength for the measurement was carefully studied. The trough amplitude at 307.0 and 230 nm and peak amplitude at 279 nm of the first derivative of ratio spectra are then recorded respectively. Good linearity was observed but the recovery percent at 307 nm was better, which may be attributed to its higher signal to noise ratio.

For method B : Ratio difference [18] is a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and wider range of application. The aim of the present work was to develop a new simple, rapid, selective method for simultaneous determination of components having overlapping spectra in a binary mixture. The binary mixture of cefdinir and its alkaline degradation product was chosen as an example for the application of the new ratio difference method.

The absorption spectra of cefdinir and its degradate show a degree of interference as shown in **figure (2)**, that the application of direct spectrophotometry failed to determine cefdinir in the presence of its degradate. Several approaches have been developed to remove the overlapping constant in the ratio spectrum, either using certain order derivative [14] or through a sophisticated subtraction followed by

multiplication procedure [19]; the later was capable of determining only the component with the less extended spectrum in the mixture. The ratio difference method is a simple innovative method was capable of determining cefdinir in presence of its alkaline degradate with minimal data processing, high selectivity regardless which component has more extended spectrum.

The method comprises two critical steps [18], the first is the choice of the divisor, and the selected divisor should compromise between minimal noise and maximum sensitivity. Different concentrations of divisors are used (10, 8, 6 and 4 µg ml^{-1}) of the degradate and the divisor concentration 4 µg ml^{-1} was found the best regarding average recovery percent when it was used for the prediction of cefdinir concentration in bulk powder as well as in laboratory prepared mixtures.

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.

Linear correlation was obtained between the differences in amplitudes at 219.0 and 250.0nm, against the corresponding concentration of cefdinir. Good linearity is obtained in the concentration range of 2 - 14 µg ml^{-1} cefdinir.

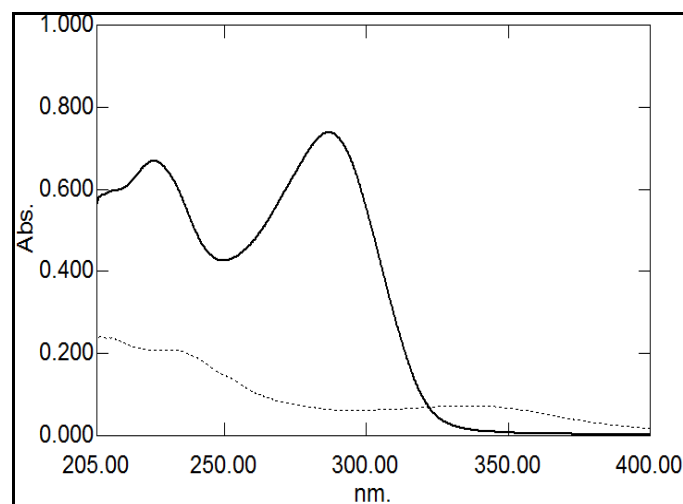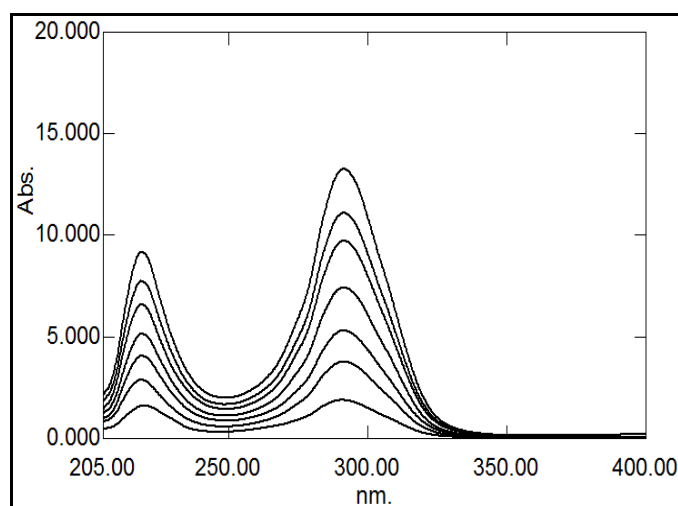FIGURE 3: Smoothed Ratio Spectra of Cefdinir (2 - 14µgml^{-1}) using (4 µgml^{-1}) Cefdinir Degradate as Divisor and Methanol as Blank.

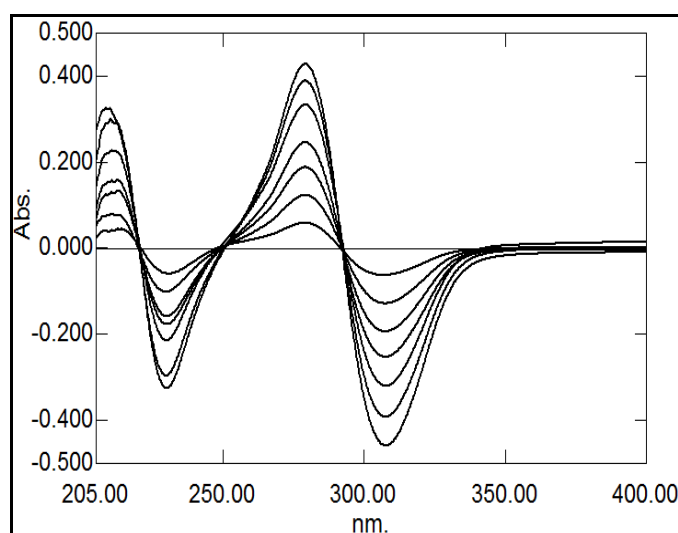FIGURE 4: First Derivative of Smoothed Ratio Spectra of Cefdinir (2– 14 µgml^{-1}) Using (4 µgml^{-1}) Cefdinir Degradate as Divisor and Methanol as Blank.

For method C: It is clear from the spectra in figure (2) that, there is a band overlapping between the drug and its degradation product. Such overlapping was eliminated by the first derivative (^{1}D) scanning of cefdinir and its degradation product in methanol, cefdinir has a trough at 304 nm which shows no interference from the degradation product.

Thus, it would be possible to adopt the (^{1}D) spectrophotometry at 304 nm for direct determination of cefdinir in presence of its degradation product as seen in Figure (5).
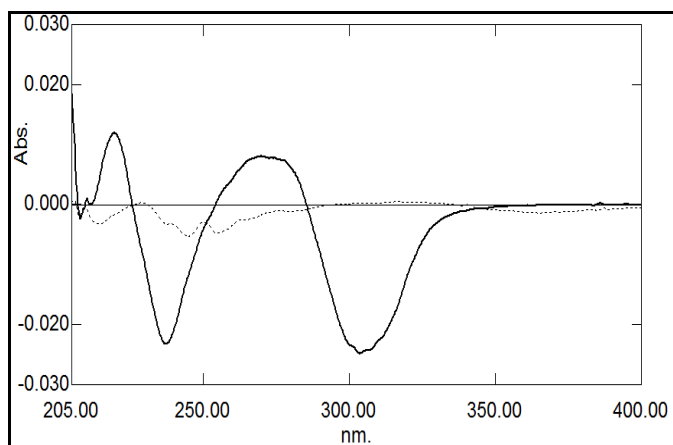
FIGURE 2: Zero-order Spectra of Intact Cefdinir (10µg ml^{-1}) (—) and its Degradation Product (10 µg ml^{-1}) (…..) in Methanol.

FIGURE 5: First-order Spectra of Intact Cefdinir (10 μg ml^{-1}) (—) and its Degradation Product (10 μg ml^{-1}) (…..) in Methanol.

Validation of the Methods:

(i) Linearity:

Method A: Under the described experimental conditions, the calibration graph for the method was constructed by plotting trough height versus concentration in μg/ml. The regression plot was found to be linear over the range of 2-14 μg/ml. The linear regression equation for the graph is:

$$P_{307\ nm} = 0.7446C - 0.1714$$

$$r^2 = 0.9999$$

Where C is the concentration of cefdinir in μg ml^{-1}, P is the trough height of the first derivative of the ratio spectrum curve at 307 nm and r^2 is the correlation coefficient.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table 1**.

Method B: Linear correlation was obtained between the differences in amplitudes at 219.0 and 250.0nm, against the corresponding concentration of cefdinir. Good linearity is obtained in the concentration range of 2 - 14 μg ml^{-1}. The corresponding regression equation was computed to be:

$$\Delta P_{219.0-250.0} = 0.486\ C + 0.286$$

$$(r^2 = 0.9998)$$

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration in μg ml^{-1} and r^2 = the correlation coefficient.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table 1**.

Method C: At the described wavelength linear relationship was obtained between the trough in cm and the cefdinir concentration in the range (2 - 14 μg ml^{-1}). The linear regression equation of the trough was:

$$A_{304} = 0.76071\ C + 0.05714$$

$$(r^2 = 0.9999)$$

Where **A** is a trough height at 304 in cm and **C** is the drug concentration in μg ml^{-1}.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table 1**.

(ii) Sensitivity: The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH Q_2 Recommendation [20] from the following equations:

$$LOD = 3.3\ S_a\ /\ slope$$

$$LOQ = 10\ S_a\ /\ slope$$

Where S_a is the standard deviation of the intercept of regression line.

LOD was found to be 0.049, 0.05 and 0.034 μg/ml, while LOQ was found to be 0.162, 0.165 and 0.114 μg/ml for method A, B and C respectively. The small values of LOD and LOQ indicate good sensitivity.

(iii) Accuracy and Precision: Three replicate determinations of three different concentrations of cefdinir in pure form within linearity range were performed in the same day (intra-day) and in three successive days (inter-day). Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were

calculated and results are listed in **table 2**. The small values of RSD% indicate high precision of the method. Moreover, the good R% confirms excellent accuracy.

Specificity: The specificity of the proposed methods was assured by applying the laboratory prepared mixtures of the intact drug together with its degradation product.

The proposed method was adopted for the specific determination of intact cefdinir in presence of up to 57.14 % of its corresponding degradates for the three methods.

The percentage recovery±SD% was 99.66 ± 0.915%, 100.43±0.96 and 100.14±0.899 for method A, B and C respectively, as seen in **table 3.**

Pharmaceutical Applications: The proposed method was applied to the determination of the studied drug in its capsule preparation. The results were validated by comparison to a previously reported method [4].

No significant difference was found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as seen in **table 4**.

TABLE 1: SPECTRAL DATA FOR DETERMINATION OF CEFDINIR BY THE PROPOSED METHODS:

Parameters	Ratio derivative	Ratio difference	First derivative
λ_{max} (nm)	307	219.0-250.0	304
Linearity range (μgml^{-1})	2-14	2-14	2-14
LOD (μgml^{-1})	0.049	0.05	0.034
LOQ (μgml^{-1})	0.162	0.165	0.114
Regression equation[*]			
Slope (*b*)	0.745	0.486	0.76
Intercept (*a*)	0.171	0.286	0.057
Correlation Coefficient (r^2)	0.9999	0.9998	0.9999

TABLE (2): INTRADAY AND INTERDAYS ACCURACY AND PRECISION FOR THE DETERMINATION OF CEFDINIR BY THE PROPOSED METHODS:

	Conc. $\mu g.ml^{-1}$	Intra-day			Inter-day		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
Method A	6	5.99 ± 0.028	99.85	0.645	6.037 ± 0.025	100.61	0.58
	8	8.059 ± 0.039	100.47	0.684	8.02 ± 0.017	100.25	0.285
	10	10.08± 0.064	100.838	0.873	10.11 ± 0.085	101.129	1.154
Method B	6	5.907± 0.03	98.46	0.969	5.97 ± 0.03	99.44	0.95
	8	7.876±0.01	98.45	0.243	7.87±0.034	98.43	0.84
	10	10.018±0.05	100.18	0.974	10.107±0.049	101.07	0.94
Method C	6	5.995± 0.032	99.92	0.696	5.99± 0.046	99.84	1.004
	8	7.96±0.01	99.52	0.173	7.94±0.038	99.194	0.631
	10	10.04±0.033	100.399	0.428	10.032±0.052	100.32	0.672

TABLE 3: DETERMINATION OF CEFDINIR IN MIXTURES WITH ITS DEGRADATION PRODUCT BY THE PROPOSED METHODS:

Intact ($\mu g\ ml^{-1}$)	Degradate ($\mu g\ ml^{-1}$)	Degradate %	Recovery% of Intact		
			Ratio derivative	Ratio difference	First Derivative
12	2	14.29	99.28	100.09	101.25
10	4	28.57	101.01	101.4	100.47
8	6	42.86	99.4	99.25	99.3
6	8	57.14	98.96	100.99	99.53
Mean ± SD			99.66±0.916	100.43±0.959	100.14+0.899

TABLE 4: DETERMINATION OF CEFDINIR IN CEFDIN® CAPSULES (300 mg) BY THE PROPOSED AND REPORTED METHODS

Parameters	Proposed Methods			Reported method [4]
	Ratio Derivative	Ratio Difference	First derivative	
N*	7	7	7	5
$\bar{X}$	100.02	100.32	100.43	99.86
SD	0.64	0.21	0.864	0.71
RSD%	0.64	0.21	0.86	0.71
t**	0.401 (1.8125)	1.405 (1.8125)	1.251 (1.5125)	____
F**	1.23 (6.16)	0.087 (6.16)	0.675 (6.16)	____

* No. of experimental. ** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

CONCLUSION: The proposed method is simple, rapid and inexpensive. So, it is good alternative to the other few reported methods and to the high cost HPLC methods.

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