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Four Spectrophotometric Methods for Determination of Cefradine in Presence of Its Alkaline Degradation Product Depending on Two Wavelengths: A Comparative Study

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ABSTRACT

The manuscript discussed the application of four simple, accurate and precise stability-indicating spectrophotometric methods based upon manipulating two wavelengths for the determination of cefradine in the presence of its alkaline degradation product without preliminary separation. These methods are; dual wavelength, amplitude modulation, absorbance subtraction and Q-analysis. Several variables such as the choice of the divisor, wavelength of measurement and other parameters were optimized to get the most sensitive and reproducible results. The following analytical parameters: limit of detection, limit of quantitation, accuracy, precision and linearity ranges of the methods were determined. The standard addition technique was performed to assess the validity of the proposed methods. The specificity was validated by analyzing laboratory prepared mixtures containing cefradine and its alkaline degradation product. The analysis results of the dosage form were statistically compared to the reported method with no significant difference.

INTRODUCTION

Cefradine(*7R*)-7-(α -D-Cyclohexa-1,4-dienylglycylamino)-3-methyl-3-cephem-4-carboxylic acid **Fig.1** is a first generation cephalosporin antibiotic, beta-lactam and its action is via inhibiting cell wall synthesis [1-3].

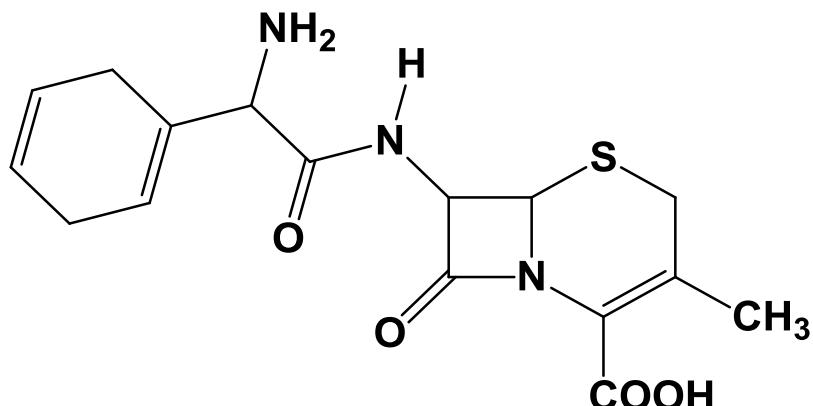


Fig. 1. Structure of Cefradine

In reviewing the literature, a variety of spectrophotometric [4-9], electrochemical [10, 11], chromatographic [12-16] and fluorimetric[17-20]methods have been reported for the assessment of cefradine.

Overlapping spectrum is a great problem in the field of UV spectrophotometry; many methods manipulating two wavelengths have been progressed. In this manuscript, four different methods were applied namely; dual wavelength[21, 22], amplitude modulation[23-25],absorbance subtraction [23, 24] and Q-analysis [26, 27].

MATERIALS AND METHODS

Instruments

- SHIMADZU dual beam UV-visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and aHP1020 laserjet printer. The bundled software, UV-Probe personal spectroscopy software version 2.1 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min and 1 nm data interval.
- Jenway, 3510 pH meter (USA).
- Hotplate (Torrey Pines Scientific, USA).

- UV lamp with short wavelength 254nm (Vilber Lourmat, VL-4LC, France).
- Precoated TLC plate, silica gel 60 GF₂₅₄ (20x20), (Flukachemie, Switzerland).

Software

The Student's t-test, F-value and ANOVA were performed using Microsoft Excel. Error bars were executed using IBM SPSS Statistics software version 21.

Materials and reagents

- (a) Cefradine was kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO), 10th of Ramadan City, Egypt; its purity was found to be 99.79± 0.502.
- (b) Pharmaceutical Preparations: "Velosef" tablets: (batch number N104052) containing 1000 mg of cefradine per tablet bought from the Egyptian market.
- (c) Reagents: All reagents used were of analytical grade:

- Water used throughout the procedure was freshly double distilled.
- Sodium hydroxide (El-Nasr Company, Egypt), prepared as 0.5 M aqueous solution.
- Hydrochloric acid (El-Nasr Company, Egypt), prepared as 0.5 M aqueous solution.
- Methanol (Sigma-Aldrich, Germany).

Standard solutions

- (a) A standard solution of cefradine (100µg/mL) was prepared by dissolving 10 mg of the drug powder in 50 mL of water and completed to 100 mL with water.
- (b) Preparation of the degradation product: dissolve 100mg of pure cefradine in 50mL of 0.5M NaOH solution and stand for 90 minutes in boiling water bath [17]. Then, cool the solution, neutralize to pH 7 and evaporated to dryness. Extract the residue three times with 25 mL of methanol, filter into 100-mL measuring flask and complete to volume with distilled water to obtain a stock solution labeled to contain degradate derived from 1mg/mL of cefradine. Complete degradation was confirmed by TLC and HPLC. The stock solution was diluted with water to obtain the working solution of degradate (100 µg/mL).

Methods

Over the range of 200–400 nm record the absorption spectra using water as a blank.

Dual wavelength

Transfer various aliquots of cefradine standard solution (100 μ g/mL) into 10-mL volumetric flasks and complete to volume with water. Record the absorption spectra. Then, measure the difference in absorbance between 261 and 311 nm and plot the obtained amplitude against the corresponding concentrations (μ g/mL) of cefradine.

Amplitude modulation

Transfer precisely, into a series of 10-mL volumetric flasks, aliquots equivalent to 60–260 μ g) cefradine from its stock working solutions (100 μ g/mL) and then complete with water to the volume. The absorbance spectra of the resultant concentrations were calculated in the range of 200–400 nm. The scanned spectra of cefradine were divided by the normalized absorption spectrum of its alkaline degradation product (1 μ g/mL). The calibration curves were constructed via plotting the amplitudes of ratio spectra (cefradine/1 μ g/mL degradate) at 245.5 nm, versus their corresponding concentrations and derive the regression equation.

Absorbance subtraction

Transfer precisely, into a series of 10-mL volumetric flasks, aliquots equivalent to 60–260 μ g) and (60–260 μ g) of both cefradine and its alkaline degradation product from their stock working solutions (100 μ g/mL) and then complete with water to the volume. The absorbance spectra of the resultant concentrations were calculated in the range of 200–400 nm. Then, the scanned spectra of degradate were calculated at 245.5 nm and 310 nm. The absorption factor (ratio of the absorbance at 245.5 nm and 310 nm) for degradate was calculated. The absorption of cefradine at 245.5 nm was calculated after the subtraction of the absorption of degradate using the absorption factor method. Finally, construct calibration graph connecting the absorption of cefradine, zero order spectra, at 245.5 nm vs. the corresponding concentrations and derive the regression equation.

Q-analysis

From the standard solutions of cefradine and its alkaline degradation product (100 µg/mL) transfer different aliquots into two separate series of 10-mL volumetric flasks and complete to volume with water. Record absorption spectra (from 200 to 400 nm) using water as a blank. Measure the absorbance values at 245 nm (λ_{iso}) and 261 nm (λ_{max} of cefradine) and determine the absorptivity values for cefradine and its alkaline degradation product at the selected wavelengths. Determine concentration of cefradine via utilizing absorptivity and absorbance ratio values.

Application to laboratory prepared mixtures

Transfer aliquots of cefradine and its alkaline degradation product from their working solutions into a series of 10-mL volumetric flasks to prepare different mixtures of them. Complete to the volumes with water. Record and store the absorption spectra of the prepared mixtures in the range of 200 to 400 nm. Then, manipulate the obtained spectra as mentioned previously. Finally, calculate concentrations of cefradine as described under each method.

Analysis of Velosef® by the proposed methods

Ten tablets of velosef® 1g were finely powdered. Transfer appropriate weight of powder equivalent to 10mg of cefradine to 100-mL conical flask and adjust the volume to 75 mL with water. Shake the volume vigorously for 15 min and make sonication for 10 minutes. Then, filter into 100-volumetric flask and complete to volume with water. Repeat the general procedure for each method and determine content of the tablets from the corresponding regression equations.

RESULTS AND DISCUSSION

Determination of cefradine in presence of its alkaline degradation product is difficult due to severe overlapping, as shown in **Fig.2**. Through utilizing the proposed methods, cefradine concentrations could be determined without any interference.

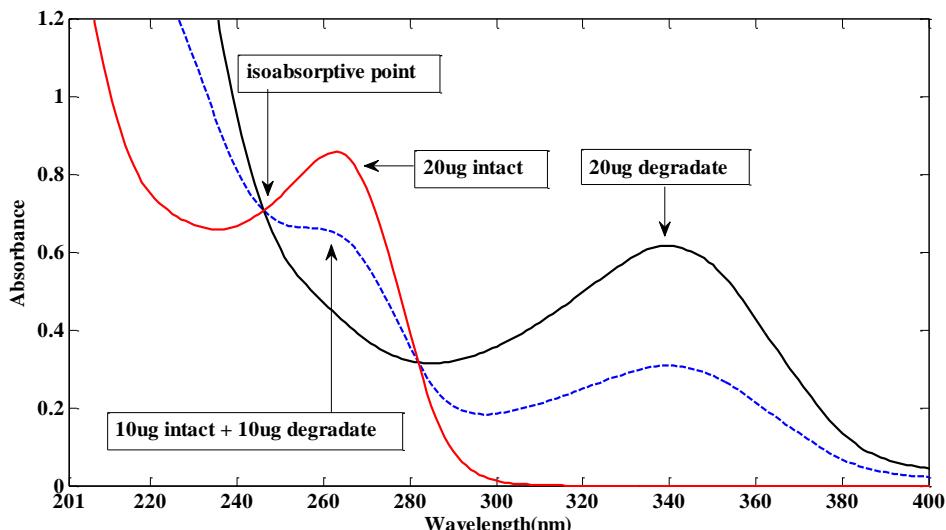


Fig. 2. Overlain zero order absorption spectra of cefradine (20 µg/mL), its alkaline degradate (20 µg/mL) and 10 µg/mL of each in methanol using distilled water as a blank

Dual wavelength

Construct the calibration graph via plotting the differences in absorbance at 261 and 311 nm versus drug concentrations in µg/mL. The calibration graph was linear over the range of 6-26 µg/mL. The only requirement of this method is the existence of two points having the same absorbance at two different wavelengths for the component that must be removed, while the component of interest must have different absorbances at the two wavelengths.

Absorbance subtraction method

This method is derived from the absorption factor method [28, 29]. The isosbestic point for different components is called isoabsorptive point (where the components at this point have equal absorptivity). For the determination of cefradine in presence of its alkaline degradation product, we will utilize their isoabsorptive point at 245.5 nm, **Fig. 2**.

The absorption spectra of different concentrations of the degradate were recorded in the wavelength range of 200–400 nm in order to calculate the absorption factor which was found to be 1.138 (the ratio between the absorbance of degradate at 245.5 nm and 310 nm). Absorbance of cefradine in mixture (cefradine + degradate) can be calculated from the following equation:

Absorbance of cefradine in the mixture at $\lambda_{iso} = \text{abs } \lambda_{iso} (X+Y) - \frac{\text{abs}_1}{\text{abs}_2} \times \text{abs}_2 (\lambda_2)(X+Y)$

Where; abs_1 , abs_2 is the absorbance of pure degradate at λ_{iso} and λ_2 ; $\frac{\text{abs}_1}{\text{abs}_2}$ is called the absorbance factor and $\text{abs } \lambda_{iso}$ (cefradine + degradate) and $\text{abs } \lambda_2(X + Y)$ are the absorbances of the mixture at these wavelengths (λ_{iso} , λ_2).

The concentration of cefradine is calculated using the regression equation obtained by plotting the absorbance values of the zero order curves of cefradine at isoabsorptive point (λ_{iso}) against their corresponding concentrations.

This method requires first, the presence of isoabsorptive point for both constituents and second, the extension of the spectra of one constituent. The advantage of this method over the methods based on isoabsorptive point, there is no complementary spectrophotometric method is required to calculate the concentration of one of the two constituents. The disadvantage of this method there may be an error in estimating the absorbance factor in case of low concentrations of the extended constituent or the extended constituent may have a low absorbance value in the extension region.

Amplitude modulation

This method depends on two facts; first, if there is an isosbestic point in the absorption spectra, it will be maintained at the same position in the ratio spectra even after division by a one constituent. Second, the resulting ratio spectra are significantly influenced by the chosen divisor. Therefore, to get rid of the influence of the divisor, we will utilize the normalized spectrum of alkaline degradation product (1 µg/mL) (normalized spectrum is mathematically prepared by utilizing summation of different spectra of degradate and divided by total number). Via dividing the spectra of cefradine by the normalized curve of its alkaline degradation spectrum (1 µg/mL), we get the ratio spectra as shown in **Fig. 3**. The amplitude value of ratio spectra was modulated to concentration at the isoabsorptive point. At the plateau region (at 310 nm) determine the amplitude value of the constant, which is equal to the degradate concentration. At the isoabsorptive point ($\lambda_{iso}=245.5$ nm), the amplitudes of the ratio spectra will be equal to the summation of amplitudes of cefradine and its alkaline degradation product. Subtract the recorded amplitude at 310 nm from the formerly attained constant at 245.5nm, we obtain the amplitude of cefradine, which is equal to the concentration of cefradine in the mixture ($C_{Recorded}$ of cefradine).

$$C_{\text{Recorded}} = 0.9899 C + 0.0404$$

Where, C_{Recorded} is the amplitude of ratio spectra at 245.5 nm and C is the concentration of cefradine.

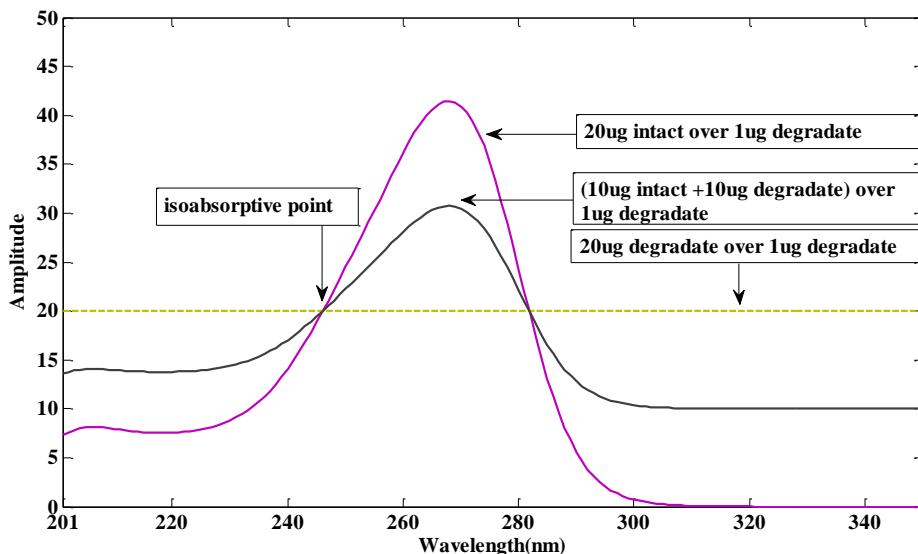


Fig. 3. Overlaid ratio spectra of cefradine (20 µg/mL), its alkaline degradate (20 µg/mL) and 10 µg/mL of each using normalized spectrum of cefradine (1µg/mL) as a divisor

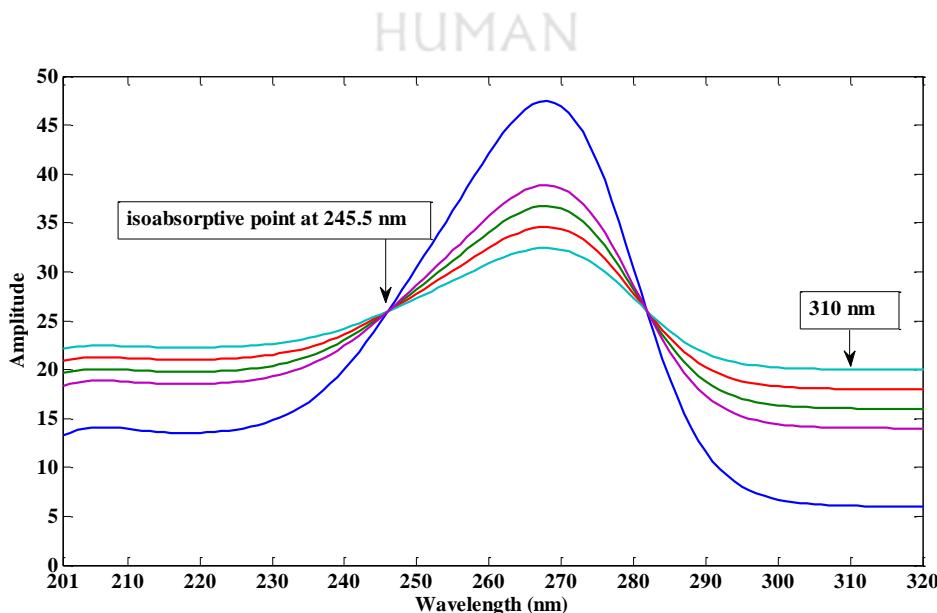


Fig. 4: Division spectra of laboratory prepared mixtures (6µg/mL, 14µg/ml, 16µg/mL, 18µg/mL and 20µg/mL cefradine with various concentrations of its alkaline degradation

product using normalized spectrum of degradate (1 μ g/ml) as a divisor showing two wavelengths 245.5 nm and 310 nm

This method requires first, the existence of isoabsorptive point for both constituents at zero order spectra and accordingly in the ratio spectra, and second, the extension of the spectra of one constituent. The advantages of amplitude modulation method over other methods using the constant are the reduction in the manipulation steps and only one divisor is required. Via utilizing the normalized divisor, the choice of divisor does not affect the results. This method has an advantage over the isoabsorptive based methods that it does not require complementary method to measure the concentration of both components. Additionally, this method has two advantages over absorbance subtraction method first, via using the normalized divisor, the obtained amplitude will directly give the concentration of each constituent and second, eliminating the absorbance factor calculation step will reduce the risk of error. That is reason behind that, the sensitivity of amplitude modulation method is better than absorbance subtraction method, as shown in **Table 1**.

Q analysis

The calibration graphs were constructed by plotting the absorbance values at 245.5 and 262 nm (for both components) versus drug concentrations in μ g/mL. The calibration graphs are linear over the range of (6-26) μ g/mL for both components. The linear regression equations are:

$$\text{Absorbance} = 0.0427 x + 0.0013 \quad (\text{for cefradine at } 261 \text{ nm})$$

$$\text{Absorbance} = 0.0355 x - 0.0022 \quad (\text{for cefradine at } 245.5 \text{ nm})$$

$$\text{Absorbance} = 0.0313 x - 0.0098 \quad (\text{for cefradine degradate at } 261 \text{ nm})$$

$$\text{Absorbance} = 0.0356 x - 0.0027 \quad (\text{for cefradine degradate at } 245.5 \text{ nm})$$

Table 1: Linearity studies and regression equations of the proposed methods

Parameter	Dual wavelength	Amplitude modulation	Absorbance subtraction	Q-analysis	
Wavelength	311 -261 nm	245.5 nm	245.5 nm	245.5 nm	261 nm
Calibration range	(6-26µg/mL)	(6-26µg/mL)	(6-26µg/mL)	(6-26µg/mL)	(6-26µg/mL)
Slope	0.0437	0.9899	0.0353	0.0355	0.0427
Intercept	- 0.0084	0.0404	-0.0004	- 0.0022	0.0013
The determination	0.9996	0.9999	0.9996	0.9998	0.9998
LOD	0.519	1.147	1.370	1.380	1.294
LOQ	1.572	3.475	4.151	4.181	3.921

Table 2: Accuracy and precision obtained by applying the proposed methods

Methods	Accuracy* (%R) ^a	Precision*	
		Repeatability (RSD) ^b	Intermediate precision (RSD) ^c
Dual wavelength	99.19	1.035	1.382
Amplitude modulation	100.38	1.186	0.941
absorbance subtraction	100.07	1.115	1.124
Q-analysis	100.48	1.135	1.059

* Each result is the average of three separate determinations

^a Average of % recoveries (10, 18 and 24 µg/mL) for cefradine within the day

^b The intraday (n = 3), average of three concentrations (10, 18 and 24 µg/mL) for cefradine within the day

^c The interday (n = 3), average of three concentrations (10, 18 and 24 µg/mL) for cefradine in three consecutive days

Methods Validation

In accordance with ICH recommendations, validation was run[30].

Linearity

The linearity was evaluated via determining six concentrations of cefradine and each determination was repeated three times. In accordance with the previously mentioned experimental conditions, the assay was executed. Summarization of the linear equations was presented in **Table 1**.

Range

According to Beer's law, the calibration range was established as shown in **Table 1**.

Accuracy

The accuracy of the proposed methods was examined in the form of percentage recoveries as shown in **Table 2**.

Repeatability

The repeatability of the proposed methods was examined in the form of relative standard deviation as shown in **Table 2**.

Reproducibility (intermediate precision)

On three different days, the reproducibility of the proposed methods was examined in the form of relative standard deviation as shown in **Table 2**.

Specificity

Via the analysis of different laboratory prepared mixtures of cefradine and its alkaline degradation product inside the linearity range, **Fig. 4**. Acceptable results were displayed in **Table 3**.

Via applying the standard addition technique, the results showing that the excipients had no effect, as shown in **Table 4**.

Table 3: Determination of intact cefradine in laboratory prepared mixtures with its alkaline degradate by the proposed methods

Conc. of cefradine ($\mu\text{g/mL}$)	Conc. of degradate ($\mu\text{g/mL}$)	% of degradate	Dual wavelength ^a	Amplitude modulation ^a	Absorbance subtraction ^a	Q-analysis ^a
20	6	23.08	100.55	99.40	100.71	99.80
16	10	38.46	99.31	100.79	99.61	99.55
12	14	53.85	100.75	101.99	98.95	99.78
8	18	69.23	98.50	101.59	101.77	100.13
6	20	76.92	99.83	100.57	101.94	99.81
		Mean	99.79	100.87	100.60	99.81
		% RSD	0.920	0.995	1.311	0.206

^a % Recovery

^b Underlined values are out of accepted range and not considered in the calculation of Mean or SD

Table 4: Application of standard addition technique to the analysis of cefradine tablets by applying the proposed methods

Pharmaceutical Conc ($\mu\text{g/mL}$)	Added standard ($\mu\text{g/mL}$)	Dual wavelength ^a	Amplitude modulation ^a	Absorbance subtraction ^a	Q-analysis ^a
10	10	101.50	101.10	98.15	99.72
	12	100.67	100.08	98.17	99.70
	14	100.64	98.43	101.34	100.30
Mean		100.94	99.87	99.22	99.91
SD		0.4881	1.3484	1.8372	0.344
RSD%		0.4836	1.3501	1.8516	0.344

^a % Recovery

Statistical Analysis

Statistical comparison between the results obtained from the proposed methods and reported method was shown in **Table 5**. There were no significant differences between the proposed methods and the reported first derivative method [9] because the calculated Student's t-test and F value were less than the theoretical values as shown in **Table 5**. Using one-way ANOVA test, the obtained results by applying these methods showed no significant differences among all of them as shown in **Table 6**. Besides, overlapping the standard error (SE) bars for means of the proposed methods indicating that the differences among the means are not statistically significant as shown in **Fig.5**.

Table 5: Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and reported method [9] for determination of Velosef® in tablets

Parameter	Dual wavelength	Amplitude modulation	Absorbance subtraction	Q-analysis	Reported method ^b
Mean	99.08	99.87	99.87	99.80	99.70
S.D.	1.312	0.735	0.428	0.216	1.060
N	5	5	5	5	5
Student's t-test ^a	0.831 (2.306)	0.293 (2.306)	0.325 (2.306)	0.190 (2.306)	
F-value ^a	1.534 (6.388)	2.080 (6.388)	6.141 (6.388)	3.614 (6.388)	

^aThe values between the parenthesis are the tabulated values of t and F at (P = 0.05)

^b Spectrophotometric determination of cefradine in presence of its alkali-induced degradation product, by measuring the values of the first derivative spectra at 267nm in aqueous medium

Table 6: One-way ANOVA test for the different proposed methods used for the determination of cefradinein Velosef® tablets

Drug	Source of variation	DF	Sum of squares	Mean of squares	F value
Cefradine	Between groups	4	2.141	0.535	0.690 (2.866)
	Within groups	20	15.512	0.776	

The value between parentheses is the tabulated F value

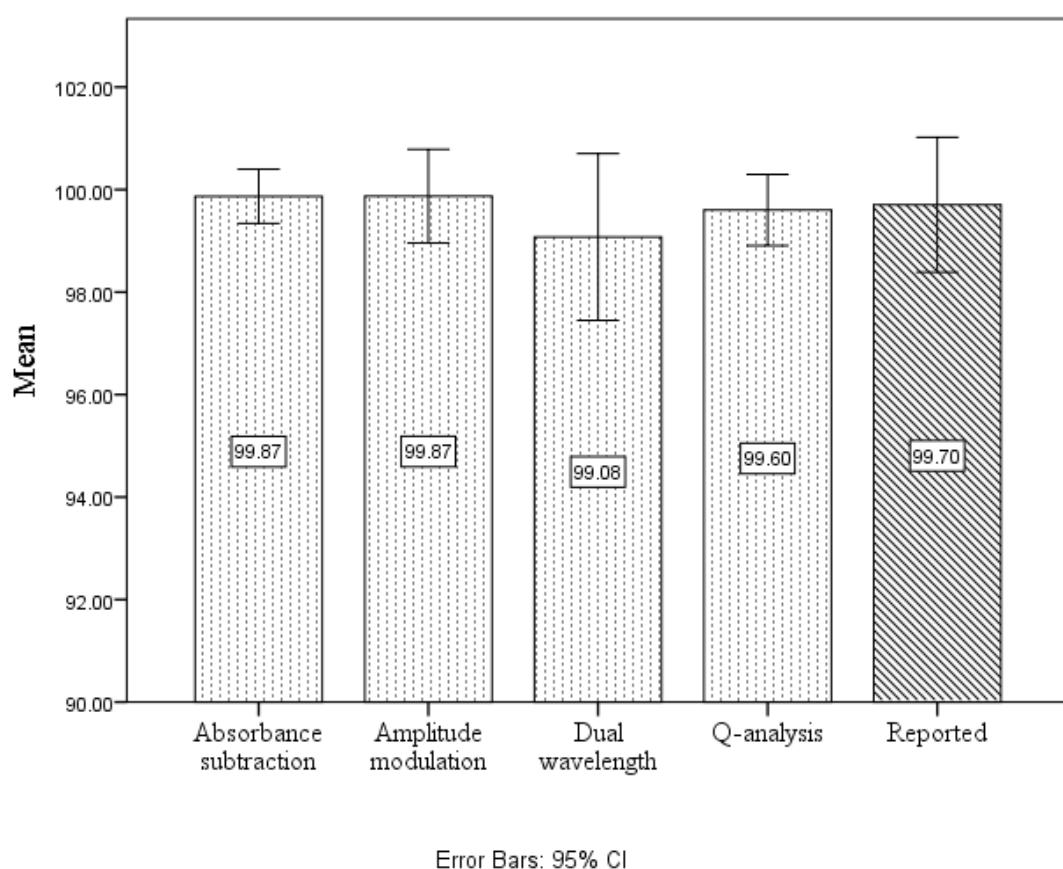


Fig.5. Comparison among the means of the proposed methods via using error bars at confidence interval (CI) = 95%

Comparative study

The dual wavelength method gives the impression to be the simplest one with regard to data manipulation. Absorbance subtraction and amplitude modulation require first, the existence of isoabsorptive point of both components in zero order and ratio spectra second, the extension of the spectra of one constituent. Amplitude modulation has two advantages over other mathematical techniques using the constant first, reducing the manipulation steps second, for determining two components in their mixture only one divisor is required. The choice of the divisor does not affect the results because the divisor is normalized. Also, amplitude modulation method has an advantage over the isoabsorptive point at zero order that determines the two components in the mixture and does not require complementary method to determine one of the components in the mixture. Additionally, utilizing the normalized divisor leads to that the resulted amplitudes at the ratio spectrum are representative for the concentrations and the danger of error due to determination of absorbance factor of lower absorbance will be decreased by removal of the absorbance factor calculation step. As a result, the sensitivity of amplitude modulation method is better than absorbance subtraction method, as shown in **Table 1**. Q-analysis requires only the existence of isoabsorptive point for two components at their zero order spectra.

CONCLUSION

In brief, cefradine can be determined by the proposed methods in pure and pharmaceutical dosage form without preliminary separation. Furthermore, these methods do not require sophisticated techniques, instruments or high priced solvents. Subsequently, these methods can be used for the routine analysis of cefradine in laboratories lacking liquid chromatographic instruments. Further research is needed to determine cefradine in the biological fluids and environment via using the previously mentioned methods.

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