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Quantitative analysis of cefadroxil in presence of its degradation product by various spectrophotometric techniques

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ABSTRACT

Simple, Accurate, selective and sensitive spectrophotometric methods have been developed and validated for determination of cefadroxil in presence of its alkaline degradation product without preliminary separation. These methods include area under the curve method (AUC), Q-Analysis method (QA), ratio derivative method, Ratio difference method and Mean centering method. These methods were validated and successfully applied to the determination of Duricef® 500mg capsule. 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.

KEYWORDS

Cefadroxil;
Stability;
Area under curve;
Q analysis;
Ratio derivative;
Ratio difference and mean centering.

INTRODUCTION

Cefadroxil (Figure 1) is (7R)-7-(á-D-4-Hydroxyphenylglycylamino)-3-methyl-3cephem 4-carboxylic acid monohydrate[1]. it is indicated for the treatment of patients with infection caused by susceptible strains of the designated organisms in the following diseases: Urinary tract infections, Skin and skin structure infections, Pharyngitis and/or tonsillitis[2]. A wide variety of analytical methods have been reported for the determination of cefadroxil isn pure form, in pharmaceutical preparations and in biological fluids. These methods mainly involve spectrophotometry[3-9], fluorimetry[10-13], electrochemically[14], HPTLC[15,16] and (HPLC)[17-20].

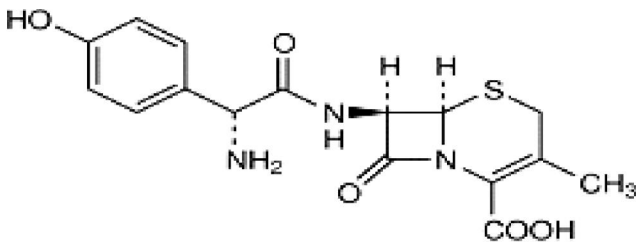

Figure 1 : Chemical structure of cefadroxil

MATERIALS AND METHODS

Apparatus

1. Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).
2. Hot plate (Torrey pines Scientific, USA).
3. Jenway, 3510 pH meter (Jenway, USA).
4. Rotatory evaporator (scilogex, USA)

Materials and reagents

1. Cefadroxil powder was kindly supplied by Glaxo Smith Kline Egypt. Haram Giza, Egypt. (B. NO.B339313).

2. Duricef® 500mg capsule. The product of Glaxo Smith Kline Egypt. Haram Giza, Egypt. (B. NO.N104546). which labeled to contain 500 mg Cefadroxil per capsule.
3. Hydrochloric acid, Sodium hydroxide and Methanol (El-Nasr Co., Egypt).
4. Distilled water

Standard solutions

A stock solution of cefadroxil (100 μg ml^{-1}) was prepared by dissolving 10 mg of cefadroxil in 50 ml of distilled water and complete to 100 ml with distilled water and was further diluted with distilled water as appropriate.

Degraded sample[7]

Alkaline-induced forced degradation was performed by adding 100 mg of cefadroxil to 100 ml of 1 N sodium hydroxide and leaving for thirty minutes at room temperature. The solution was then neutralized to pH 7 by addition of 1 N hydrochloric acid solution, evaporated to dryness, the residue was extracted three times with 25 ml methanol, filtered into 100 ml volumetric flask then the volume was adjusted to the mark by the same solvent. The obtained solution was claimed to contain (1 mg ml^{-1}).

GENERAL PROCEDURES

Methods

- **Area under the curve method**

Aliquots from cefadroxil and its degradate working solutions (100 μg ml^{-1}) equivalent to (50–500μg ml^{-1}) were accurately transferred into two separate sets of 10-mL volumetric flasks and completed to the mark with distilled Water. The zero order absorbance of each set was scanned in the range of 200–400 nm. Area under the curve for the wavelength ranges selected for determination of cefadroxil and its degradate are 223–233 nm (λ_1– λ_2) and 265–275 nm (λ_3– λ_4), the absorptivity 'Y' values of each of the cefadroxil and its degradate were determined at the selected wavelength ranges. The absorptivity 'Y' values were determined as, Y = area under curve of component (from 223 to 233nm or 265 to 275 nm)/concentration of the component (in μg ml^{-1}). Mixed standard were prepared and their area under the curve were measured at the selected wavelength ranges. Concentration of cefadroxil in mixed standard solution were calculated using the corresponding equations.

- **The Graphical Absorption Ratio (Q-Analysis) method**

Aliquots from cefadroxil and its degradate working solutions (100 μg ml^{-1}) equivalent (50–500μg ml^{-1}) were accurately transferred into two separate sets of 10-mL volumetric flasks and completed to the mark with distilled Water. The zero order absorbance of each set was scanned in the range of 200–400 nm and stored in the computer. The absorbance were measured at 250 nm (λ max of cefadroxil) and 275.2 nm (iso-absorptive point). the absorptivity values for cefadroxil and its alkaline degradate at the selected wavelengths were calculated. The method employs Q values and the concentrations of the studied drug in the prepared mixed solutions were determined by using the following equations:

$$C_x = [(Q_m - Q_y)/(Q_x - Q_y)] \times A_{iso}/a_{iso}.$$

- **Ratio derivative method**

Aliquots from cefadroxil and its degradate working solutions (100 μg ml^{-1}) equivalent (50–500μg ml^{-1}) were accurately transferred into two separate sets of 10-mL volumetric flasks and completed to the mark with distilled water. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer. For the determination of cefadroxil in presence of its degradation product, the stored spectra of cefadroxil are divided by the spectrum of 20 μg ml^{-1} degradate, to obtain the ratio spectra, then The first derivative of the obtained ratio spectra was employed. The calibration curve was constructed relating the amplitudes of the first derivative values to the corresponding concentrations in μg ml^{-1} of Cefadroxil at 234 nm, the regression equation was derived.

- **Ratio difference**

To the ratio spectra obtained as in ratio derivative, The amplitude difference at 251 and 233nm (ΔP 251 - 233) was plotted against the corresponding cefadroxil concentration in μg ml^{-1} and the regression equation was computed.

- **Mean centering method**

The ratio spectra obtained as before in ratio

derivative method were mean centered using MATLAB. The calibration curve was constructed relating the amplitudes of the mean centered values to the corresponding concentrations of cefadroxil at 243 nm. the regression equation was derived.

Analysis of pharmaceutical preparation

five Duricef® 500mg capsule were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of cefadroxil was shaken three times with 25 ml methanol 10 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with distilled water to obtain a concentration of (100 µg ml^{-1}). The solution was analyzed using the procedure described under previous methods.

RESULTS AND DISCUSSION

Spectral characteristics

The zero order (D^0) absorption spectra of cefadroxil (50 µg ml^{-1}), its alkaline degradation product (50 µg ml^{-1}) and their mixture containing equal concentration of them (25µg ml^{-1} of each) were recorded against distilled Water as blank over the range of 200 – 400 nm. (Figure 2)

Area under the curve method[21]

The proposed area under the curve method has the advantage of being simple and selective for determination of cefadroxil in presence of its alkaline degradation with minimal sample and data manipulation. Selection of the wavelength region to construct AUC method has a great effect on the analytical parameters such as slope, intercept and correlation coefficient. Different wavelength regions were tested where the wavelength ranges 223–233 nm and 265–275 nm were selected which showed good selectivity and percentage recovery (Figure 3).

Area under curve of the absorption spectra in the wavelength ranges 223–233 nm ($\lambda_1-\lambda_2$) and 265–275 nm ($\lambda_3-\lambda_4$) were calculated for both cefadroxil and its alkaline degradate in the concentration range of (5–50 µg ml^{-1}) The absorptivity 'Y' values of cefadroxil and its alkaline degradate were calculated at each wavelength range. The concentrations of cefadroxil in presnce of its alkaline degradate can be obtained by applying Cramer's rule and matrices in Eqs. (1) and (2).

A_1 =0.3000 C_{cf} + 0.238 C_D —at 223–233 nm ($\lambda_1-\lambda_2$)

A_2 = 0.164 C_{cf} + 0.115C_D —at 265–275 nm ($\lambda_3-\lambda_4$)

where C_{cf} and C_D are the concentrations of cefadroxil and its alkaline degradation in µg ml^{-1}, respectively. 0.3000 and 0.164 are the absorptivity (Y value) of cefadroxil at ($\lambda_1-\lambda_2$) and ($\lambda_3-\lambda_4$), respectively. 0.238 and 0.115 are absorptivity (Y value) of cefadroxil degradate at ($\lambda_1-\lambda_2$) and ($\lambda_3-\lambda_4$), respectively. A_1 and A_2 are the area under curve of sample solutions at the

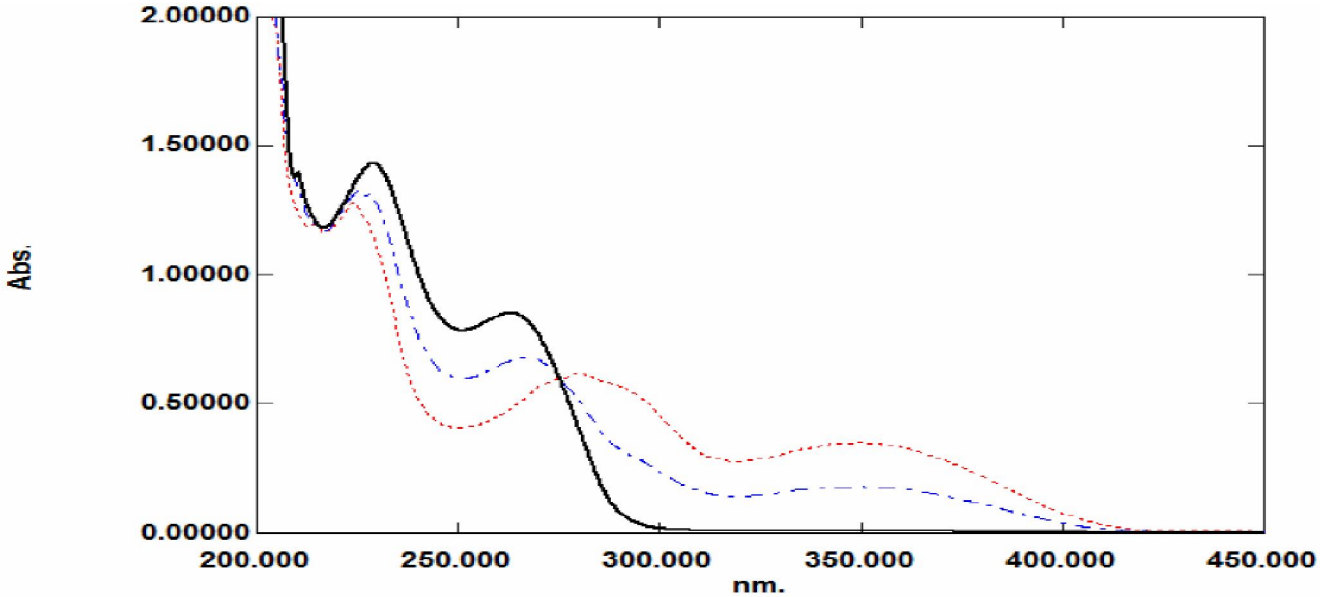

Figure 2 : Zero - Order Spectra of Intact cefadroxil (50µg ml^{-1})(—), its alkaline degradate (50 µg ml^{-1}) (…..) and Their Mixture (25 µg ml^{-1} of each) (– –).

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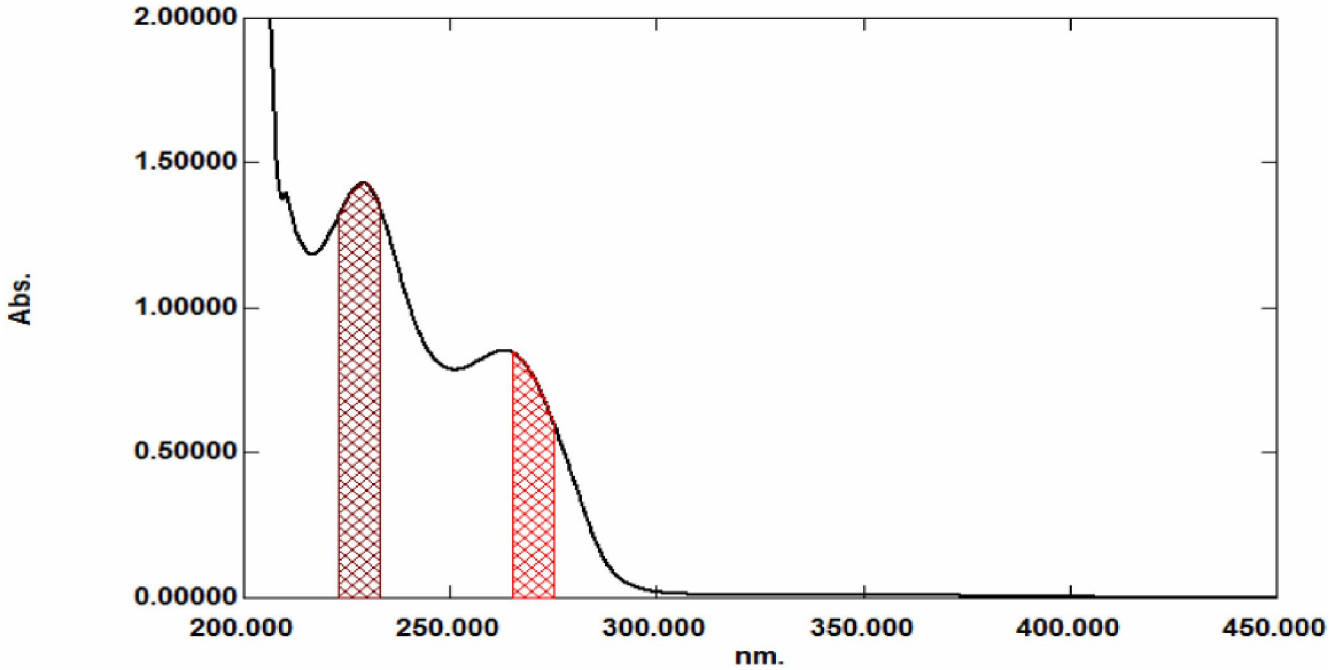

Figure 3 : Zero order absorption spectra of (50 µgml^{-1}) cefadroxil showing wavelength ranges for area under curve method using distilled Water as a Blank.

wavelength range at ($\lambda_1 - \lambda_2$) and ($\lambda_3 - \lambda_4$) respectively.

The graphical absorption ratio (Q-Analysis) method[22]

This method depends on the property that for the substance that obeys Beer's Lambert's law at all wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent of the concentration or path length.

This ratio is referred as Q-ratio[23]. One of the two selected wavelengths is an isoabsorptive point and the other is the wavelength of maximum absorption of the drug. the overlain spectra of cefadroxil, its alkaline degradate and their mixture,(Figure 2), show isoabsorptive points at 275.2 nm. The absorbance values at 275.2nm (λ_{iso}) and 250 nm (λ_{max}) for cefadroxil and its alkaline degradate in the range of 5–50 µg ml^{-1} were measured, absorptivity coefficients were determined for both and the average values were taken. The values and the absorbance ratio were used to develop the following equation from which the concentration of cefadroxil in the sample mixture can be calculated:

$$C_x = [(Q_m - Q_y)/(Q_x - Q_y)] \times A_{iso}/a_{iso}$$

where C_x is the concentrations of cefadroxil in µg ml^{-1}; Q_m is the absorbance of sample at λ_{250}/absorbance of sample at $\lambda_{275.2}$; Q_x is the mean of absorptivity of cefadroxil at λ_{250}/mean of absorptivity of cefadroxil at $\lambda_{275.2}$; Q_y is the mean of absorptivity of cefadroxil degradate at λ_{250}/mean of absorptivity of cefadroxil degradate at $\lambda_{275.2}$; A_{iso} is the absorbance of the sample at $\lambda_{275.2}$ and a_{iso} is the mean of absorptivity of cefadroxil at λ_{250}.

Ratio derivative method[24]

Upon dividing the absorption spectrum of a compound by a spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. However, upon dividing the absorption spectrum of a compound (X) by the absorption spectrum of another compound (Y), a new spectrum (ratio spectrum) will result. The amplitude of the first or second derivative of the ratio spectrum at a maximum or a minimum is proportional to concentration of X without interference from Y. In this method, the absorption spectra of cefadroxil were divided by the absorption spectrum of the degradate (20 µg/ml) as a divisor to get the ratio spectra, as shown in Figure (4). The amplitudes of the first derivative of the ratio spectra at 234 nm are proportional to the concentrations of the drug without interference from its degradate. as shown in Figure(5).

Ratio difference[25]

In this method, the absorption spectra of the drug

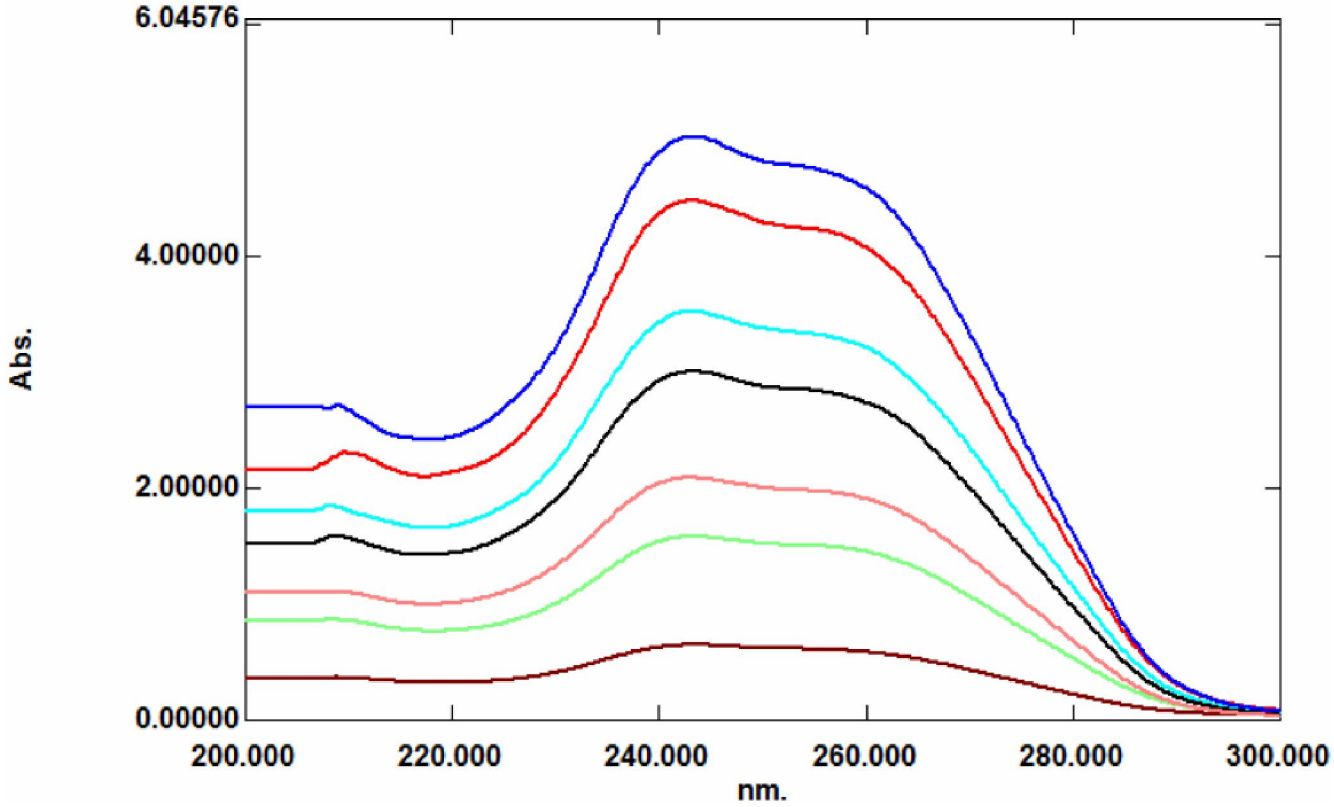

Figure 4 : Ratio Spectra of cefadroxil (5-50 µg ml^{-1}) using (20 µg ml^{-1}) of cefadroxil Degradate as a Divisor and distilled Water as a Blank.

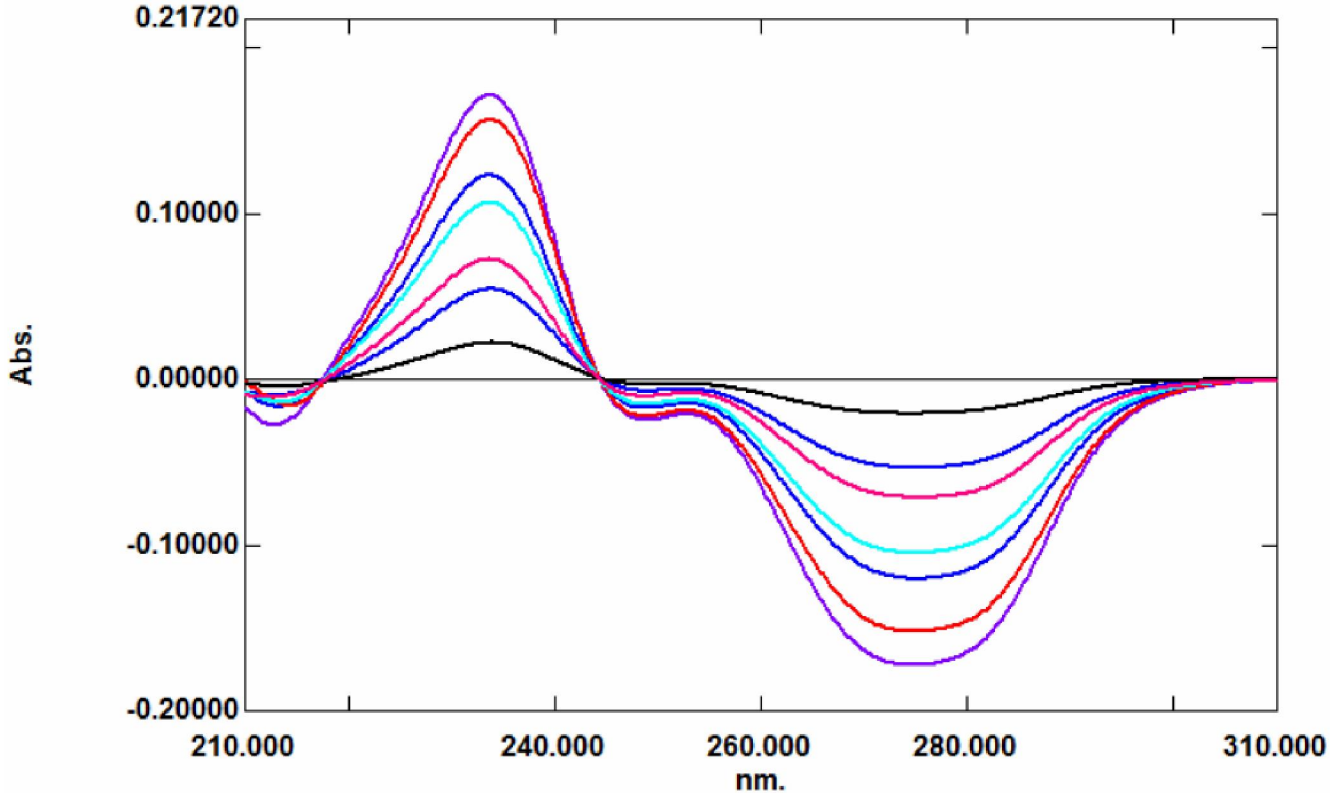

Figure 5 : First Derivative of Ratio Spectra of cefadroxil (5-50 µg ml-1) Using (20 µgml^{-1}) cefadroxil Degradate as a Divisor and distilled water as a Blank

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were divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra. Different concentrations of divisor (cefadroxil degradate) are used (10,20,25,30 and40 µg ml^{-1}) and the divisor concentration 20 µg $ml^{-1}$of cefadroxil degradate is found the best regarding average recovery percent. The difference in peak amplitudes between two selected wavelengths in the ratio spectra is proportional to the concentration of the cefadroxil without interference from its degradate (Figure 4). The method comprises two critical steps, the first is the choice of the divisor. The selected divisor should compromise between minimal noise and maximum sensitivity. 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

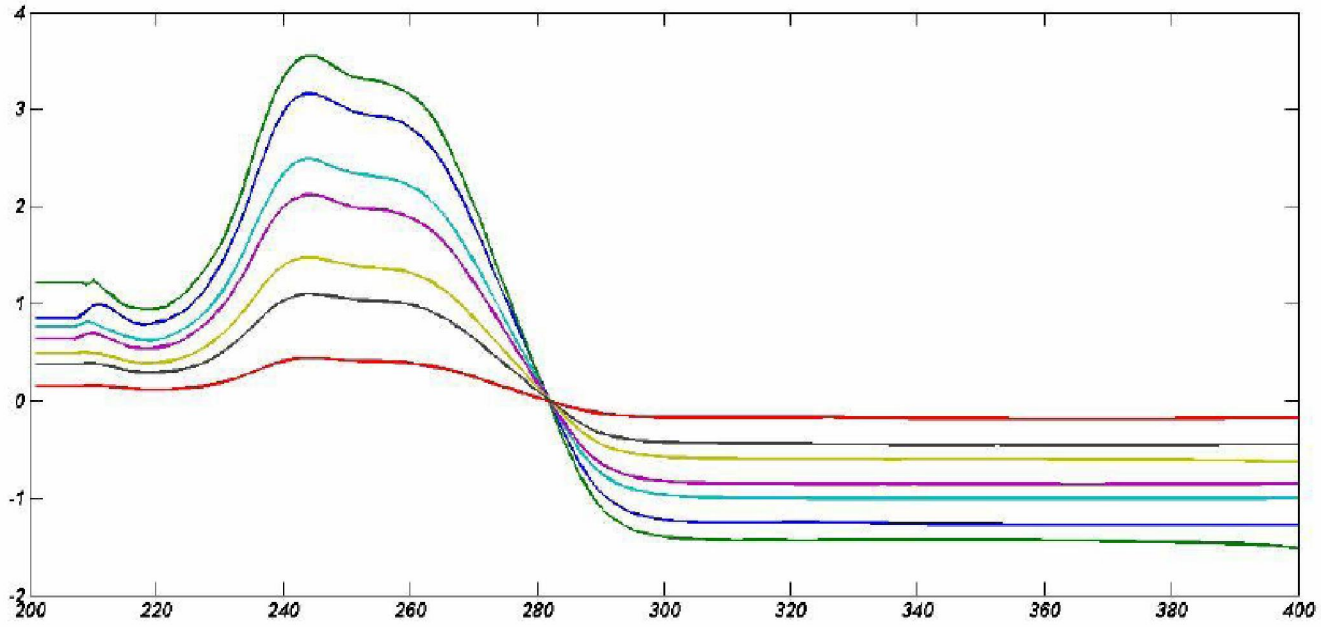

Figure 6 : Mean Centered Ratio Spectra of cefadroxil (5-50 µg ml^{-1}) Using (20 µg ml^{-1})of its Degradate as a Divisor and distilled Water as a Blank

TABLE 1 : Spectral data for determination of the studied drug by the proposed methods

Parameters	AUC	Q analysis	Ratio derivative	Ratio difference	Mean centering
Wavelength (nm)	223-233 265-275	250&275.2	234	251&233	243
Linearity range (µgml^{-1})	5-50	5-50	5-50	5-50	5-50
LOD (µgml^{-1})	0.720	0.406	0.256	0.355	0.259
LOQ (µgml^{-1})	2.184	1.230	0.778	1.078	0.784
Regression equation*					
Slope (b)	0.1463	0.0115	0.0034	0.0205	0.068
Intercept (a)	0.2697	0.0245	0.0045	0.0368	0.0897
Correlation coefficient (r^2)	0.9999	0.9998	0.9997	0.9999	0.9998

*** y= bx + a where y is the response and x is the concentration**

TABLE 2 : Intra-day and inter-day accuracy and precision for the determination of the cefadroxil by the proposed methods

Method	Conc. μg.ml^{-1}	Intra-day			Inter-day		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
A UC	15	15.08±0.007	100.55	0.045	15.17±0.079	101.11	0.518
	25	25.09±0.236	100.36	0.943	25.13±0.197	100.52	0.783
	35	35.35±0.044	101.01	0126	35.51±0.134	101.46	0.378
Q A	15	15.02±0.181	100.19	1.204	15.00±0.080	100	0.579
	25	24.82±0.173	99.30	0.700	24.85±0.050	99.42	0.201
	35	35.28±0.351	101.82	0.995	35.28±0.181	100.82	0.512
Ratio derivative	25	24.85± 0.294	99.41	1.183	25.14± 0.29	100.58	1.169
	35	34.85± 0.294	99.57	0.843	35.34± 0.16	100.98	0.480
	45	45.04± 0.612	100.10	1.359	45.04± 0.74	100.10	1.643
Ratio difference	15	14.92± 0.122	99.46	0.822	15.01± 0.028	100.11	0.187
	25	24.83± 0.341	99.35	1.374	24.98± 0.212	99.94	0.851
	35	35.11± 0.43	100.32	1.252	35.24± 0.149	100.70	0. 422
Mean centering	25	24.91± 0.233	99.66	0.938	25.18± 0.352	100.72	1.401
	35	34.76± 0.324	99.33	0.933	35.35± 0.331	101.00	0.938
	45	44.76± 0.200	99.47	0.447	44.97± 0.389	99.93	0.865

ratio spectrum and good linearity is present at each wavelength individually. The selected wavelengths are 251and 233 nm ($\Delta P_{251\text{-}233\ nm}$) which gave the best results.

Mean centering method[26]

This method depend on the fact that mean centering of constant equal zero.

If the spectrum of a mixture of two compounds X and Y is divided by a standard spectrum of Y as a divisor (Y'), a ratio spectrum will result and therefore:

$$P = AX/AY' + AY/AY' \quad (1)$$

Where, P is the amplitude of the mixture in the ratio spectrum. AX, AY and AY' are the absorbance values of X, Y and divisor (Y'), respectively. Mean centering of equation (1) will lead to:

$$MC(P) = MC(AX/AY') + MC(AY/AY') \quad (2)$$

Since AY /AY' is a constant value, so its mean centering is equal to zero and therefore:

$$MC(P) = MC(AX/AY') \quad (3)$$

From this equation we note that the mean centering value will related to the X component only. so for determination of cefadroxil in a mixture with its degradation product the mixture was divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra (Figure 4) then the obtained ratio spectra were mean centered using MATLAB and the concentration of cefadroxil was determined by measuring the amplitude at 243 nm (Figure 6).

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TABLE 3 : Determination of cefadroxil in presence of its alkaline degradate in their laboratory mixtures by the proposed methods

Method	Intact in (µg ml^{-1})	Degradate In (µg ml^{-1})	Percent of degradate	Intact found in (µ ml^{-1})	Recovery % of intact
AUC	45	5	10 %	44.82	99.61
	35	15	30 %	34.64	98.99
	25	25	50 %	24.77	99.10
	10	40	80 %	9.88	98.86
	Mean ± SD%				99.14±0.33
QA	45	5	10	44.78	99.51
	35	15	30	34.84	99.55
	25	25	50	25.15	100.61
	15	35	70	15.29	101.98
	Mean ± SD%				100.41±1.157
Ratio derivative	45	5	10	45.14	100.32
	35	15	30	35.14	100.42
	25	25	50	25.44	101.76
	15	35	70	15.14	100.98
	Mean ± SD%				100.87±0.655
Ratio difference	45	5	10 %	45.03	100.07
	35	15	30 %	34.88	99.67
	25	25	50 %	25.13	100.52
	10	40	80 %	10.05	100.58
	Mean ± SD%				100.21±0.423
Mean centering	45	5	10	45.73	101.62
	35	15	30	34.68	99.09
	25	25	50	24.59	98.39
	15	40	70	14.99	99.99
	Mean ± SD%				99.77±1.399

VALIDATION OF THE METHODS

Linearity and rang

- **Area under the curve method**

Under the described experimental conditions, the calibration graph for the method was constructed by plotting area under curve versus concentration of cefadroxil in μg ml^{-1}. The regression plot was found to be linear over the range of 5-50 μg ml^{-1}. The linear regression equation for the graph is:

P_{AUC} = 0.1463 C + 0.2555 ……(r^2 = 0.9999)

Where P_{AUC} is area under curve at the selected wavelength, C is the concentration of cefadroxil in μg ml^{-1} and r^2 is the correlation coefficient, as shown in TABLE 1.

- **The graphical absorption ratio (Q-Analysis) method**

Linear correlation was obtained between the absorbance at 275.2 nm, versus concentration of cefadroxil in μg ml^{-1} Good linearity is obtained in the concentration range of 5 - 50 μg ml^{-1}. The linear regression equation for the graph is:

A = 0.0115 C + 0.0245…….(r^2 = 0.9998)

Where A is the bsorbance at the selected wavelength, C is the concentration of cefadroxil in μg ml^{-1} and r^2 = the correlation coefficient as shown in TABLE 1.

- **Ratio derivative method**

Linear correlation was obtained between the absorbance values at 234 nm, against the corresponding concentration of cefadroxil Good linearity is obtained in the concentration range of (5 - 50 μg ml^{-1}). The corresponding regression equation was computed to be:

A = 0.0034 C - 0.0045…….(r^2 = 0.9997)

Where A is the absorbance at the selected wavelength, C is the concentration of cefadroxil in μg ml^{-1} and r^2 = the correlation coefficient as shown in TABLE 1.

- **Ratio difference method**

Linear correlation was obtained between the differences in amplitudes at 251 and 233 nm, against the corresponding concentration of cefadroxil Good linearity is obtained in the concentration range of 5 - 50 μg ml^{-1}. The corresponding regression equation was computed to be:

$\Delta P_{251-233}$ = 0.0205C + 0.0368……(r^2 = 0.9999)

Where ΔP is the amplitude difference at the selected wavelengths (251 & 233), C is the concentration in μg ml^{-1} and r^2 is the correlation coefficient as shown in TABLE 1.

- **Mean centering method**

Linear correlation was obtained between the mean centered values at 243 nm, against the corresponding concentration of cefadroxil. Good linearity is obtained in the concentration range of (5 - 50 μg ml^{-1}). The corresponding regression equation was computed to be:

MCN_{243} = 0.068 C + 0.0897…….(r^2 = 0.9998)

Where MCN is the peak amplitude of the mean centered ratio spectrum curve at 243 nm, C is the concentration of cefadroxil in μg ml^{-1} and r^2 is the correlation coefficient, as shown in TABLE 1.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines[27] from the following equations:

LOD = 3.3 S_a / slope

LOQ = 10 S_a / slope

Where S_a is the standard deviation of y-intercepts of regression lines.

LOD and LOQ values of cefadroxil for each method were listed in TABLE 1.

Accuracy and precision

According to the ICH guidelines[24], three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in TABLE 2.

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drug and its degradate. The results are

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TABLE 4 : Determination of Cefadroxil in Duricef ® 500 Mg capsule by the proposed and reported methods

	AUC	QA	Ratio derivative	Ratio difference	Mean centering	Reported method[7]
N*	5	5	5	5	5	5
X^-	99.65	100.45	98.99	100.22	99.88	99.83
SD	1.502	0.861	1.326	1.162	0.783	1.490
RSD%	1.507	0.857	1.340	1.160	0.784	1.493
t**	0.1827 (2.3060)	0.8063 (2.4469)	0.9386 (2.3060)	0.4630 (2.3060)	0.0709 (2.4469)	——
F**	1.0162 (6.388)	2.991 (6.388)	1.2622 (6.388)	1.6434 (6.388)	3.616 (6.388)	——

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

listed in TABLE 3.

Pharmaceutical applications

The proposed methods were applied to the determination of the studied drug in (Duricef® 500) capsule The results were validated by comparison to a previously reported method[7]. No significant differences were found by applying t-test and F-test at 95% confidence level[12],indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form (TABLE 5).

CONCLUSION

The proposed methods are simple, rapid, accurate and precise and can be used for the determination of Cefadroxil in pure form and in pharmaceutical dosage form as well as in presence of its degradation product.

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