

Stability indicating methods for the determination of cefpodoxime proxetil in the presence of its acid and alkaline degradation products

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Abstract

Four different, accurate, sensitive and reproducible stability-indicating methods for the determination of cefpodoxime proxetil (CPD) in the presence of its acid and alkaline degradation products are presented. The first method is based on derivative spectrophotometry. Second derivative spectrophotometry was applied where CPD was determined at 261 nm in the presence of its acid degradation product and also third derivative spectrophotometry was applied where CPD was determined at 282 nm in the presence of its alkaline degradation product. The second method is based on the first derivative of ratio spectrophotometry (¹DD) of CPD at 215 nm and 255 nm in the presence of its acid degradation product and at 243 nm in the presence of its alkaline degradation product. The third method is ratio subtraction spectrophotometry where the drug is determined at 232 nm in laboratory prepared mixtures of CPD and its acid or alkaline degradation product. Calibration graphs were established for 4.0-40.0 µg/ml for CPD determination by the three spectrophotometric methods. The fourth method is an isocratic reversed-phase HPLC procedure, a Zorbax C₈ column was used to separate CPD from its acid and alkaline degradation product using acetonitrile: water: triethylamine (60:40:1, v/v/v) as a mobile phase and detection at 232 nm. The suggested procedures were successfully applied for the analysis of CPD in bulk powder and in pharmaceutical preparations. The results obtained by applying the proposed methods were statistically analyzed and compared with those obtained by the official method.

Keywords cefpodoxime proxetil, stability indicating method, degradation, high-performance liquid chromatography

Introduction

Cefpodoxime proxetil (CPD), an orally absorbed, broad spectrum, third-generation cephalosporin, is a prodrug that is de-esterified in vivo to its active metabolite, cefpodoxime. It demonstrates strong activity against pathogens frequently associated with the upper respiratory tract, urinary tract, skin and soft tissue infections [1-2]

Various techniques have been utilized for the determination of CPD in body fluids and pharmaceuticals; these include spectrophotometric [3-7], chromatographic [8-12], electrochemical [13-14] and However, although the selectivity and the detection limits have been improved in these methods, these are time consuming methods and little of them which exposed to stability of CPD [9]. Therefore simple and easy techniques have been investigated for the determination of CPD as intact form in presence of its acid and alkaline induced degradation products. These methods include spectrophotometric methods and HPLC method.

Experimental

Apparatus

- Spectrophotometric studies were carried out with Shimadzu 1651 dual beam UV-Vis spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software.
- High-performance liquid chromatography (HPLC) analysis was performed on a liquid chromatography consisted of:
 - An isocratic pump (Agilent model G1310A)
 - An ultraviolet variable wavelength detector (model G1314 A, Agilent 1100 series).

A Rheodyne injector (model 7725I, Rohnert Park, CA., USA) equipped with 20µl injector loop, Agilent (USA).

Hamilton syringe (50 µl)

Stationary phase is Zorbax C₈ (5µm , 4.6 x 150 mm) Analytical column.

- Infrared (IR) spectrometer, vector 22 (Bruker Optics, Ettlingen, Germany)
- The mass spectra were performed on Shimadzu Qp-2010 operated on EI mode at 70 eV.

Materials

Pure Sample

Cefpodoxime proxetil was kindly supplied by Pharco / Rexcel , Egypt. Its purity was found to be 99.50 ± 0.56 according to HPLC official method ⁽⁸⁾.

Pharmaceutical dosage forms

- Orelox film coated tablets (Sanofi Aventis), batch no 7FH5E labeled to contain cefpodoxime proxetil equal to 100 mg cefpodoxime / tablet.
- Orelox suspension (Sanofi Aventis), batch no 7FE1E labeled to contain cefpodoxime proxetil equal to 40 mg cefpodoxime / 5 ml.

Reagents and solvents

All reagents and solvents used throughout this work were of analytical pure grade.

- Water was obtained in a Milli-Q water purification system (Millipore),
- Acetonitrile and triethylamine are HPLC- grade; E. Merck (Darmstadt, Germany).
- Methanol and dimethyl formamide (DMF); (Prolabo, West Chester, PA, USA)
- HCl (1M HCl), NaOH (1M NaOH), ammonium hydroxide (30%) and ammonium acetate; (ADWIC) – (El Nasr Pharmaceutical & Chemical Co. Egypt)

Degraded samples

Preparation of acid degradation product of CPD; 1mg ml⁻¹

It was prepared by adding 25 ml 1M HCl to 100 mg CPD and reflux for 1.5 hours. The solution was neutralized and quantitatively transferred into 100-ml volumetric flask and the volume was completed with water to the mark.

Preparation of alkaline degradation product of CPD; 1mg ml⁻¹

It was prepared by adding 25 ml 1M NaOH to 100 mg CPD and reflux for 1 hour. The solution was neutralized and quantitatively transferred into 100-ml volumetric flask and the volume was completed with water to the mark.

Elucidation of the structure of degradation products

Complete degradation was achieved, as investigated by thin layer chromatography using methanol: ammonium hydroxide (10: 0.1, v/v) as a developing solvent. The solution was neutralized and evaporated under vacuum to dryness. The degradation products were dissolved using 20 ml dimethyl formamide (to avoid dissolution of NaCl). DMF extract was evaporated again under vacuum to dryness and extracted using 5 ml methanol. The methanolic extract was evaporated at room temperature to give crystals of degradation products. The structure of the isolated degradation product was elucidated using IR, and mass spectrometry.

Standard stocks and working solutions

- Standard stock solutions of CPD, its acid degradation product and its alkaline degradation product (1 mg ml⁻¹) of each.
- Working standard solutions of CPD, its acid degradation product and its alkaline degradation product (100 µg ml⁻¹) of each.
- Working standard solutions of CPD, its acid degradation product and its alkaline degradation product (200 µg ml⁻¹) of each.
- Laboratory prepared mixtures of different ratios of CPD with either its acid or alkaline degradation product from 10 to 90%.

Procedures

Spectrophotometric Methods

Construction of calibration curves:

Aliquots of CPD working standard solution (100 µg ml⁻¹) equivalent to 100-1000 µg of CPD were accurately transferred into series of 25-ml volumetric flasks, volume was completed with water. The absorption spectra were recorded in the range of 200-400 nm.

The same procedures were applied to construct calibration graphs of both the acid and the alkaline degradation products of CPD using the corresponding working standard solution ($100 \mu\text{g ml}^{-1}$) of each.

Recording the absorption spectra of laboratory prepared mixtures

Aliquots from 9 to 1 ml were separately transferred from CPD working standard solution ($100 \mu\text{g ml}^{-1}$) into 25-ml volumetric flasks. To the previous solutions, aliquots of 1 to 9 ml of acid degradation product of CPD working standard solution ($100 \mu\text{g ml}^{-1}$) were added separately and the volume was completed with water. The absorption spectra were recorded in the range of 200-400 nm.

The same procedures were applied to record the absorption spectra of laboratory prepared mixtures of CPD and its alkaline degradation product using the corresponding working standard solution ($100 \mu\text{g ml}^{-1}$) of each.

Derivative spectrophotometric method

Second derivative spectrophotometry was applied to the absorption spectra of CPD and its acid degradation product binary mixtures with $\Delta\lambda = 8 \text{ nm}$. CPD was determined at 261 nm. Third derivative spectrophotometry was applied to the absorption spectra of CPD and its alkaline degradation product binary mixtures with $\Delta\lambda = 8 \text{ nm}$. CPD was determined at 282 nm.

The first derivative ratio spectrophotometric method (1DD)

The absorption spectra of CPD and its acid degradation product binary mixtures were divided by standard spectrum of the acid degradation product ($40 \mu\text{g ml}^{-1}$). From the ratio spectra thus obtained, first derivatives calculated with $\Delta\lambda = 4 \text{ nm}$, were recorded. CPD was determined at 215 and 255 nm.

The absorption spectra of CPD and its alkaline degradation product binary mixtures were divided by standard spectrum of the alkaline degradation product ($40 \mu\text{g ml}^{-1}$). From the ratio spectra thus obtained, first derivatives calculated with $\Delta\lambda = 4 \text{ nm}$, were recorded. CPD was determined at 243 nm.

Ratio subtraction spectrophotometric method

The absorption spectra of CPD and its acid degradation product binary mixtures were divided by standard spectrum of the acid degradation product ($20 \mu\text{g ml}^{-1}$) to obtain a plateau region, subtraction of the absorbance values of the plateau region and multiplication of the obtained spectra by the divisor spectrum of the acid degradation product ($20 \mu\text{g ml}^{-1}$) then the final obtained spectra will correspond to CPD.

The same procedures were applied to the spectra of CPD and its alkaline degradation product binary mixtures using the standard spectrum of alkaline degradation product ($20 \mu\text{g ml}^{-1}$) as divisor.

HPLC method

Chromatographic conditions

Chromatograms of CPD with either its acid or alkaline degradation products were recorded under the following parameters:

- Flow rate was 1ml/min. at ambient temperature.
- Detector was adjusted at 232 nm.
- Column was Zorbax C₈ ($5\mu\text{m}$, $4.6 \times 150 \text{ mm.}$), Analytical column
- Mobile phase was acetonitrile: water: triethylamine (60:40:1, v/v). (The mobile phase was filtered using $0.45 \mu\text{m}$ membrane filter and degassed by ultrasonic vibrations for 15 min.)

Construction of calibration curve

Aliquots of CPD working standard solution ($200 \mu\text{g ml}^{-1}$) equivalent to 30-1000 μg were accurately transferred into series of 10-ml volumetric flasks, volume was completed with mobile phase.

The prepared samples were analyzed using the previously mentioned chromatographic conditions using $50 \mu\text{g ml}^{-1}$ of CPD as an external standard.

Determination of CPD in the presence of either its acid or alkaline degradation products

Aliquots from 4.5 to 0.5 ml were separately transferred from CPD working standard solution ($200 \mu\text{g ml}^{-1}$) into 10-ml volumetric flasks. To the previous solutions, aliquots of 0.5 to 4.5 ml of acid degradation product of CPD working standard solution ($200 \mu\text{g ml}^{-1}$) were added separately and the volume was completed with mobile phase. The prepared samples were analyzed using the previously mentioned chromatographic conditions using $50 \mu\text{g ml}^{-1}$ of CPD as an external standard.

The same procedures were applied to analyze the laboratory prepared mixtures of CPD and its alkaline degradation product using the corresponding working standard solution ($200 \mu\text{g ml}^{-1}$) of each.

Analysis of pharmaceuticals

Analysis of tablets

A total of 10 tablets were weighed and finely powdered. An amount equivalent to 100 mg was weighed, transferred to 100-ml volumetric flask, stirred with 10 ml methanol then the volume was completed with water. The solution was filtered. Further dilution was made using either water (for spectrophotometric procedures) or mobile phase (for HPLC procedure).

Analysis of suspension

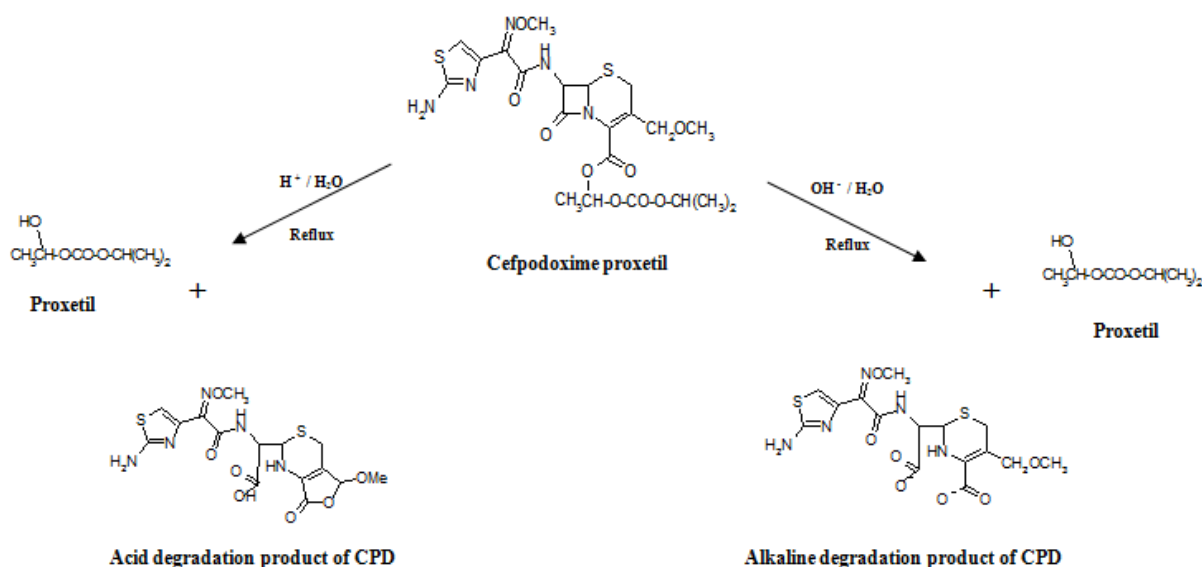
The contents of 3 bottles were extracted using 50 ml methanol then filtration was performed into 100-ml volumetric flask, the volume was completed with water. Further dilution was made using either water (for spectrophotometric procedures) or mobile phase (for HPLC procedure).

Results and Discussion

Degradation of CPD

CPD was degraded by refluxing with 1M HCl or 1M NaOH and the degradation process were monitored by spotting on TLC plates using methanol: ammonium hydroxide (10: 0.1, v/v) as developing system. Only one spot different than that of CPD was observed, for either acid or alkaline degradation products. It was found that complete degradation of CPD occurred after 1.5 hrs and 1 hr for acid and alkaline degradation process, respectively. According to Koda *et al* ^(15, 16) study for the degradation of cefixime drug, the suggested acid and alkaline degradation pathways of CPD were illustrated in scheme 1. The acid and alkaline degradation products were separated and their structures confirmed by IR and mass spectrometry. The IR spectrum of intact CPD figure (1a) shows that the characteristic band at 1763.58 cm^{-1} originating from the lactam carbonyl group was not observed in IR spectra of both acid and alkaline degradation products figures (1b & 1c), respectively which indicates the opening of β -lactam ring upon degradation. The absorption band at 1678.73 cm^{-1} corresponding to carbonyl group of ester in intact CPD was disappeared in both acid and alkaline degradation products IR spectra which refer to the ester hydrolysis of cefpodoxime proxetil prodrug releasing cefpodoxime. The proposed structures of acid and alkaline degradation products of CPD were further confirmed by mass spectrometry where ion peaks at m/z 444 and 443 which are equivalent to molecular weights of acid and alkaline degradation products were showed at figures (2a & 2b), respectively.

Scheme 1: Suggested acid and alkaline induced degradation pathways of CPD



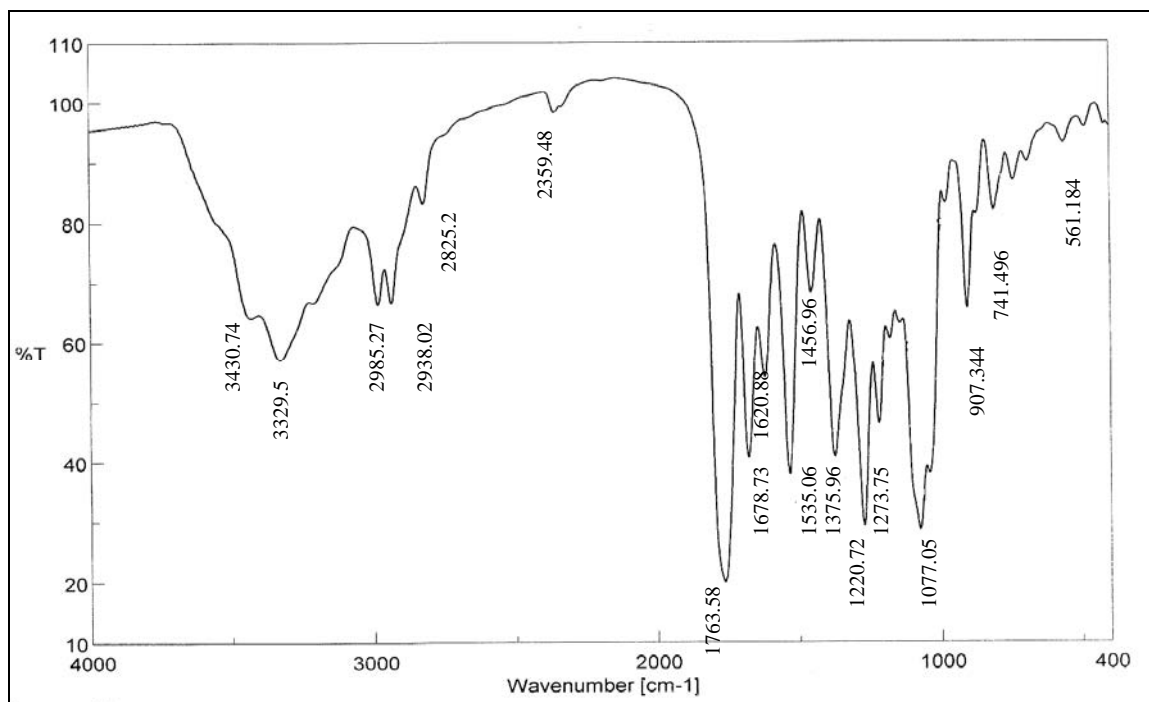


Figure (1a): IR spectrum of intact CPD

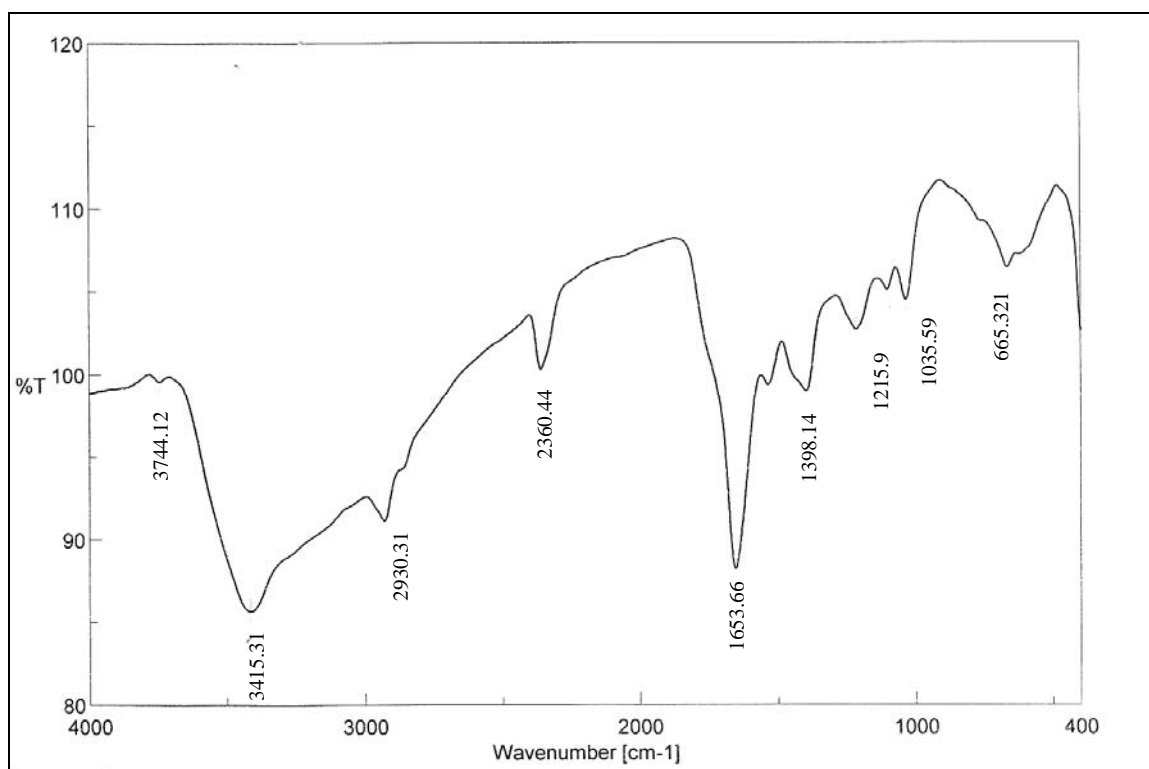


Figure (1b): IR-spectra of acid degradation product of CPD

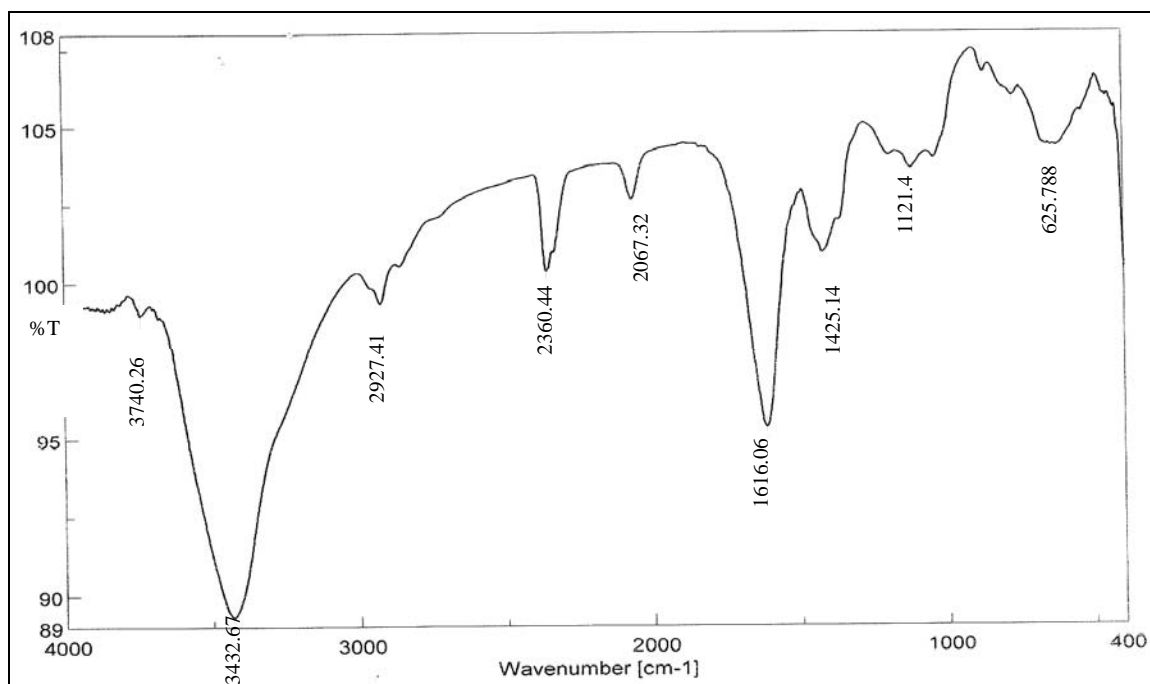


Figure (1c): IR-spectra of alkaline degradation product of CPD

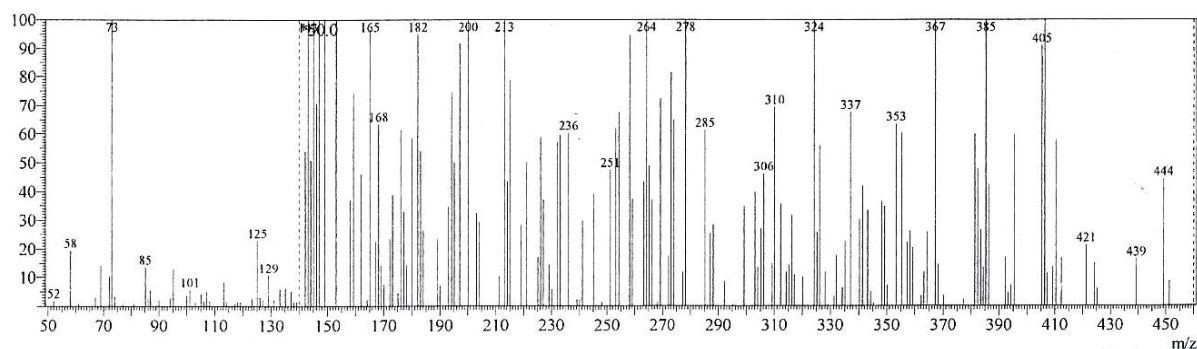


Figure (2a): Mass spectrum of acid degradation product of CPD

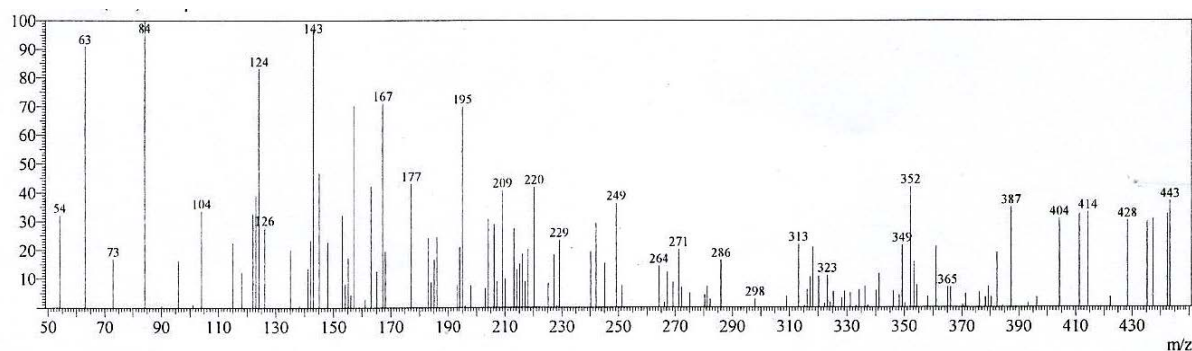


Figure (2b): Mass spectrum of alkaline degradation product of CPD

Spectrophotometric methods

Derivative spectrophotometry

Fig. 3 shows the absorption spectra corresponding to CPD with its acid and alkaline degradation products ($20 \mu\text{g ml}^{-1}$) of each with sufficiently overlapped spectra. This overlap could be resolved by applying second derivative spectrophotometry by which CPD could be determined in presence of its acid degradation product, fig. (4a). Peak amplitudes could be measured at 261 nm (at which acid degradation product give zero value) to determine

CPD in laboratory prepared mixtures of CPD and its acid degradation product. While CPD could be analyzed in presence of its alkaline degradation product by applying third derivative at 282 nm, fig. (4b). CPD could be determined in the presence of up to 80% of acid or alkaline degradation products by derivative spectrophotometry, table 1. The effect of $\Delta\lambda$ was tested and it was found to be suitable to use $\Delta\lambda = 8$ for both second and third derivative.

Table 1: Results of the analysis of laboratory prepared mixtures containing different ratios of CPD with either the acid degradate or alkaline degradate by the proposed spectrophotometric methods

% Degradation	Concentration Of intact CPD $\mu\text{g/ml}$	Recovery % of intact CPD						
		Derivative spectrophotometry		First derivative of ratio spectrophotometry			Ratio subtraction spectrophotometry	
		In presence of its acid degradate	In presence of its alkaline degradate	In presence of its acid degradate		In presence of its alkaline degradate	In presence of its acid degradate	In presence of its alkaline degradate
		$^2D_{261}$	$^3D_{282}$	$^1DD_{215}$	$^1DD_{255}$	$^1DD_{243}$		
10	36.00	100.22	97.68	100.68	98.12	100.22	98.99	100.34
20	32.00	100.20	99.34	100.19	97.89	100.69	99.73	99.48
30	28.00	98.57	100.63	100.38	98.4	100.2	98.34	99.33
40	24.00	98.25	100.23	100.64	98.61	100.27	100.36	100.36
50	20.00	100.04	100.88	99.84	98.63	99.56	99.74	100.23
60	16.00	99.94	101.40	100.09	99.36	99.81	100.97	99.52
70	12.00	99.78	101.27	100.31	101.50	99.15	100.14	100.97
80	8.00	99.44	99.19	100.76	105.81*	97.5	100.65	100.33
90	4.00	114.73*	122.47*	121.28 *	122.93*	100.84	98.71	100.08
Mean		99.56	100.03	100.36	98.93	99.8	99.74	100.07
S.D.		0.75	1.27	0.32	1.23	1.00	0.90	0.53
R.S.D.%		0.76	1.27	0.32	1.24	1.00	0.90	0.53

* Rejected values

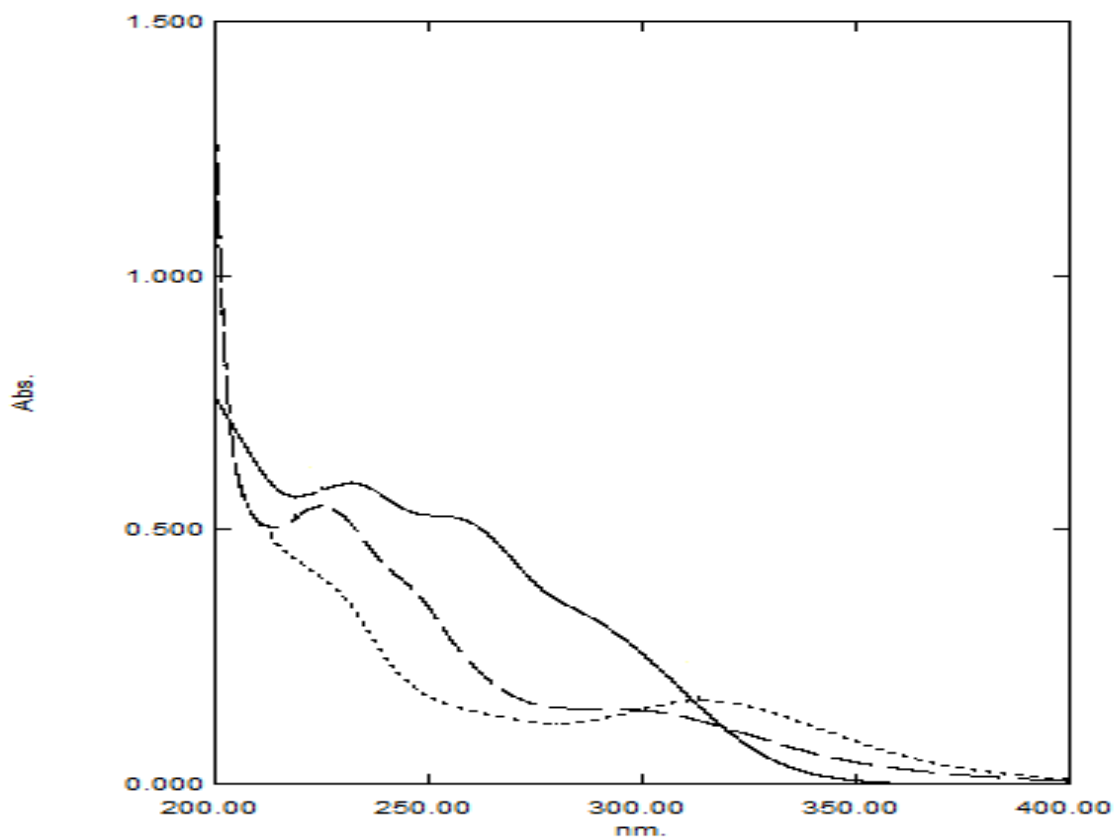


Figure 3: Absorption spectra of CPD (—), acid degradate (---) and alkaline degradate (....), $20 \mu\text{g ml}^{-1}$ for each.

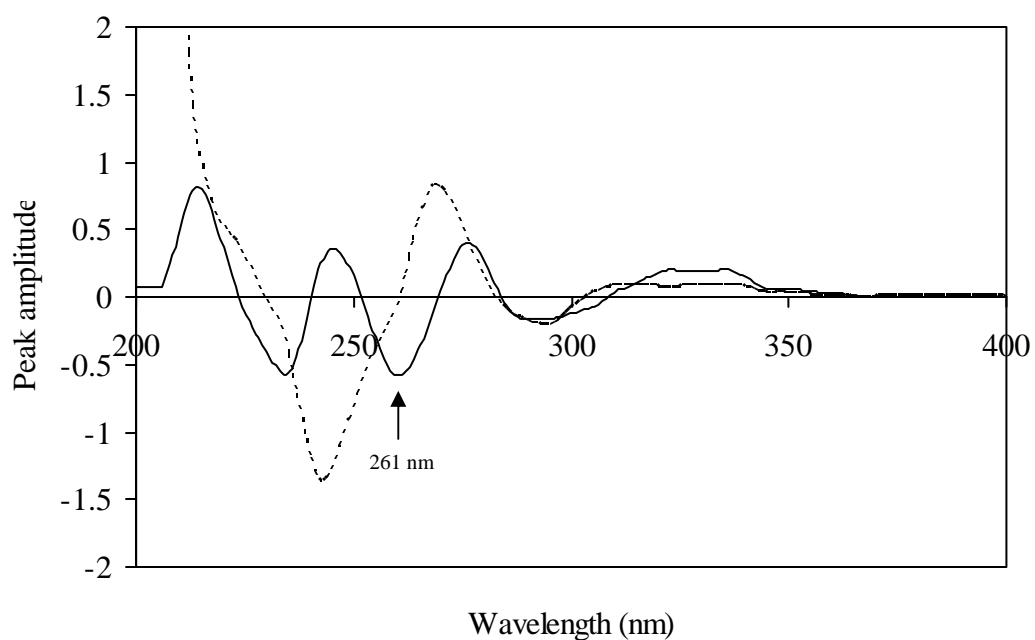


Figure 4a: Second derivative of CPD (—) and acid degradate (.....) spectra, $20 \mu\text{g ml}^{-1}$ for each

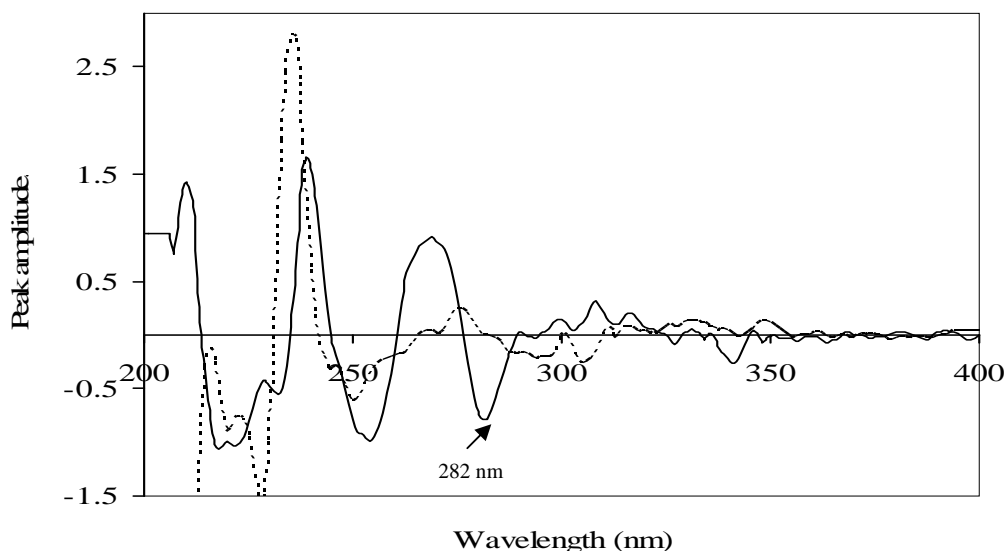


Figure 4b: Third derivative of CPD (—) and alkaline degradate (.....) spectra, $20 \mu\text{g ml}^{-1}$ for each.

The first derivative ratio spectrophotometric method (1DD)

Fig. (5a) shows the ratio spectra of laboratory prepared mixtures of CPD and its acid degradation product (spectra divided by the standard spectrum of a $40 \mu\text{g ml}^{-1}$ of acid degradation product then first derivative was applied). Peak amplitudes at 215 nm and 255 nm were proportional to CPD concentration and it could be determined in the presence of its acid degradation product in binary mixture up to 80% and 70% of acid degradation product at 215nm and 255 nm, respectively, table 1. Peak amplitudes at 300 nm did not give satisfactory results.

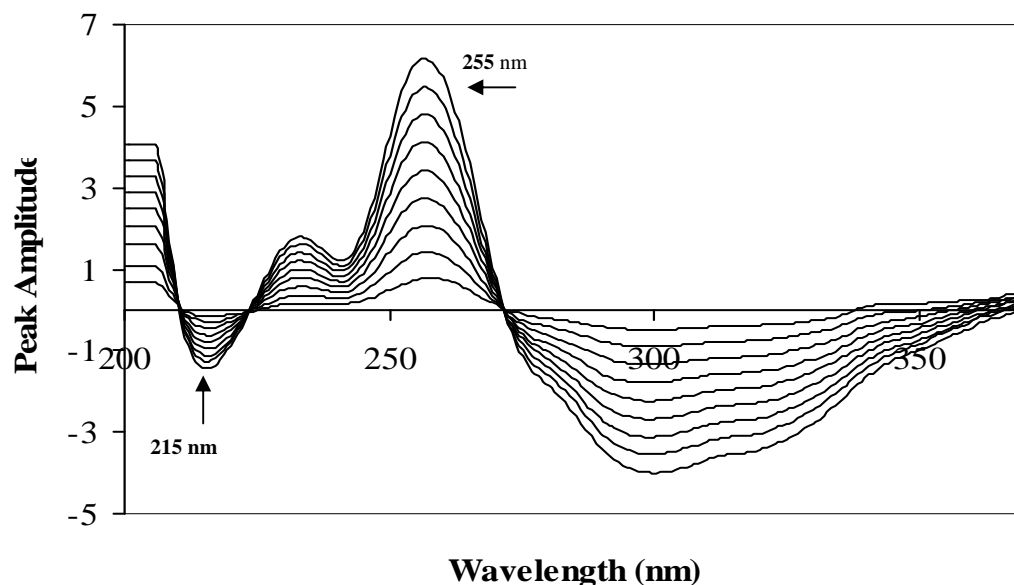


Figure 5a: First derivative of ratio spectra of laboratory prepared mixtures of CPD ($4\text{-}36 \mu\text{g ml}^{-1}$) and its acid degradate ($36\text{-}4 \mu\text{g ml}^{-1}$) using $40 \mu\text{g ml}^{-1}$ of acid degradate as divisor.

Fig. (5b) shows the first derivative of ratio spectra of laboratory prepared mixtures of CPD and its alkaline degradation product (using $40 \mu\text{g ml}^{-1}$ of alkaline degradation product as divisor). Peak amplitudes at 243 nm were proportional to CPD concentration and it could be determined in the presence of alkaline degradation product in binary mixture up to 90% of alkaline degradation product, table 1. Peak amplitude at 295 nm did not give satisfactory results.

The ratio spectra derivative method permits the use of different concentrations of degradation products as divisor to obtain different calibration graphs. For selecting the suitable divisor, concentrations of both

degradation products in range $4\text{--}40\ \mu\text{g ml}^{-1}$ were tested. A concentration of $40\ \mu\text{g ml}^{-1}$ of acid and alkaline degradation products as divisors gave highest correlation coefficient values, being an indication of quality of fitting the data to straight line. The influence of $\Delta\lambda$ for the first derivative spectra was also tested. It was found to be suitable to use the value of $\Delta\lambda = 4\ \text{nm}$.

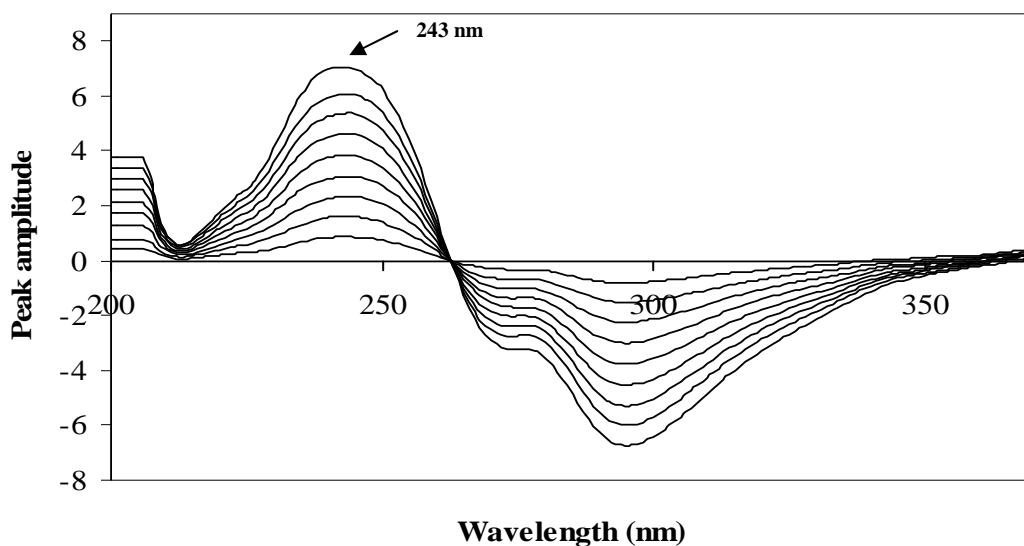


Figure 5b: First derivative of ratio spectra of laboratory prepared mixtures of CPD ($4\text{--}36\ \mu\text{g ml}^{-1}$) and its alkaline degradate ($36\text{--}4\ \mu\text{g ml}^{-1}$) using $40\ \mu\text{g ml}^{-1}$ of alkaline degradate as divisor.

Ratio subtraction spectrophotometry

The theory of ratio subtraction method^[17] depends on that the method was applied for determination of mixtures of drug (X) and its degradate (Y) where the spectrum of the degradate is extended than the other, as shown in (Fig. 3). The determination of CPD could be achieved by scanning the zero order absorption spectra of the laboratory-prepared mixtures, then dividing them by a carefully chosen concentration of standard degradation product to produce a new ratio spectra that represents $X/Y + \text{constant}$, as shown in Fig. (6a, 6d); then, subtraction of the absorbance values of these constants in plateau as shown in Fig. (6b, 6e) followed by multiplication of the obtained spectra by the divisor as shown in Fig.(6c, 6f); finally, the original spectra of CPD, which are used for direct determination of CPD at 232 nm, could be obtained and the concentration from the corresponding regression equation could be calculated. This can be summarized as follows:

$$\frac{X+Y}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + \text{constant} \quad (1)$$

$$\frac{X}{Y'} + \text{constant} - \text{constant} = \frac{X}{Y'} \quad (2)$$

$$\frac{X}{Y'} \times Y' = X \quad (3)$$

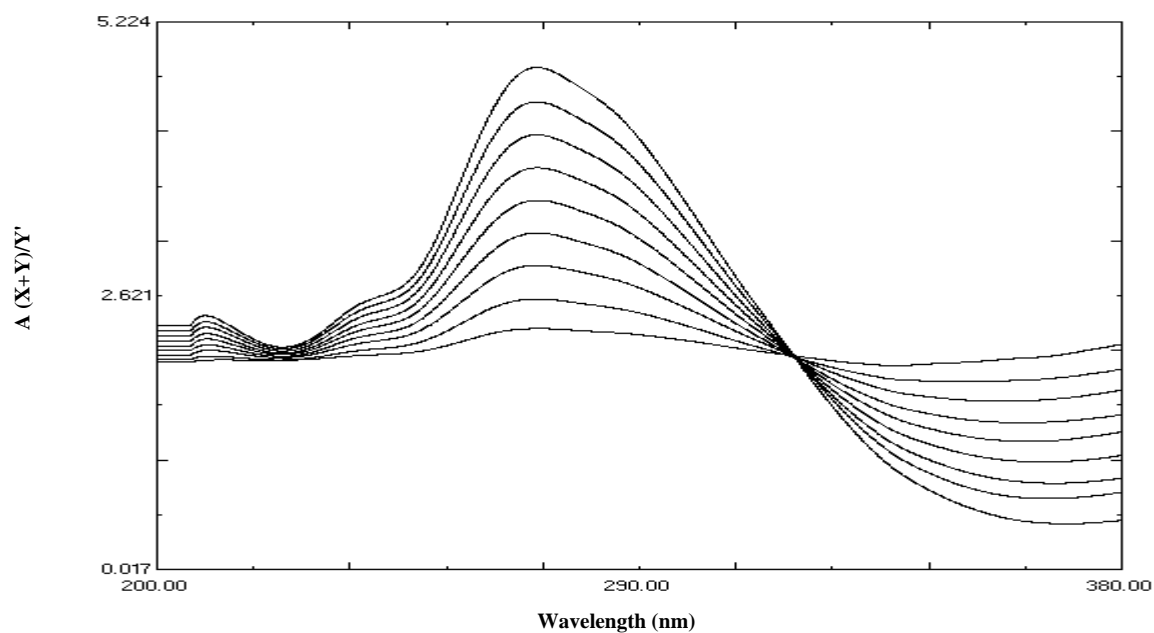


Figure 6a: Division spectra of laboratory prepared mixtures of CPD (X) and its acid degradate (Y), using $20 \mu\text{g ml}^{-1}$ of acid degraded (Y') as divisor.

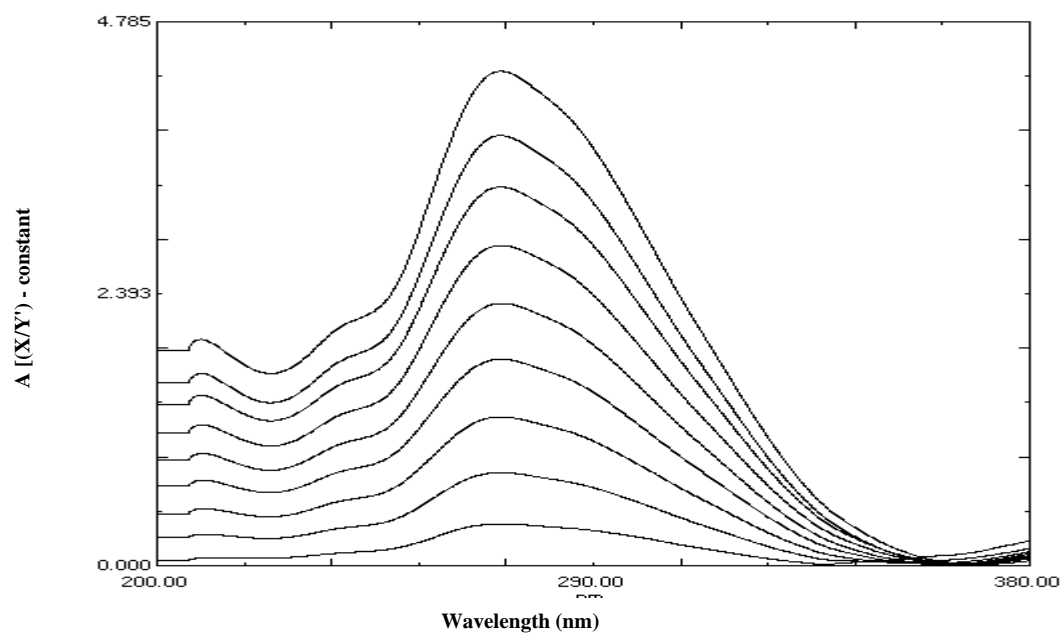


Figure 6b: Division spectra of laboratory prepared mixtures of CPD (X) and its acid degradate (Y), using $20 \mu\text{g ml}^{-1}$ of acid degraded (Y') as divisor, after subtraction of the constant.

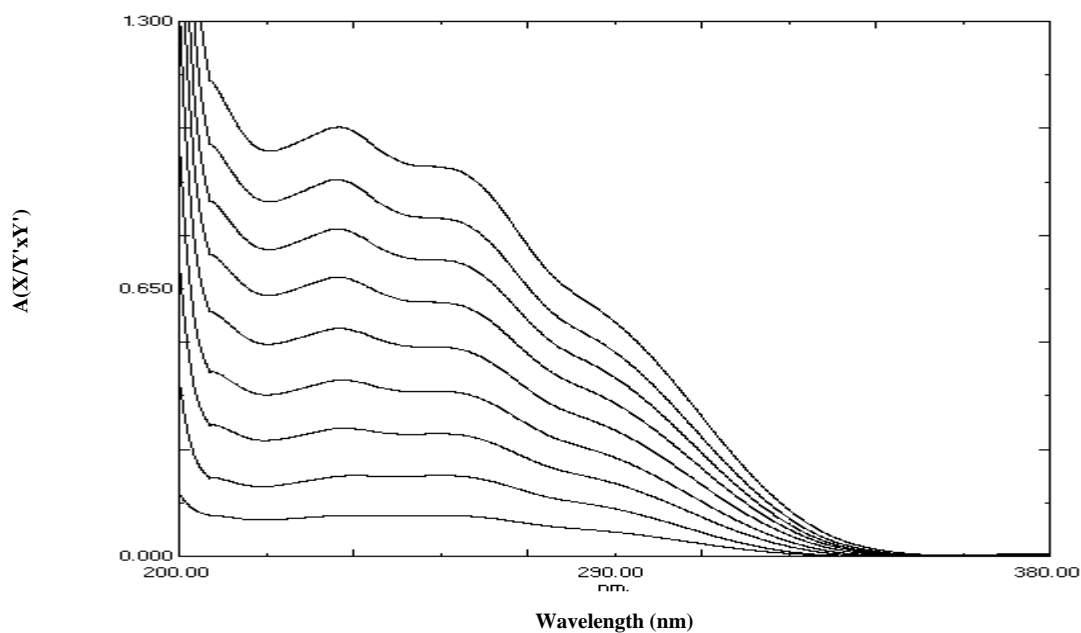


Figure 6c: Zero order absorption spectra of CPD obtained by the proposed method for the analysis of laboratory prepared mixtures of CPD and its acid degradate after multiplication by the divisor Y' ($20 \mu\text{g ml}^{-1}$ of acid degraded).

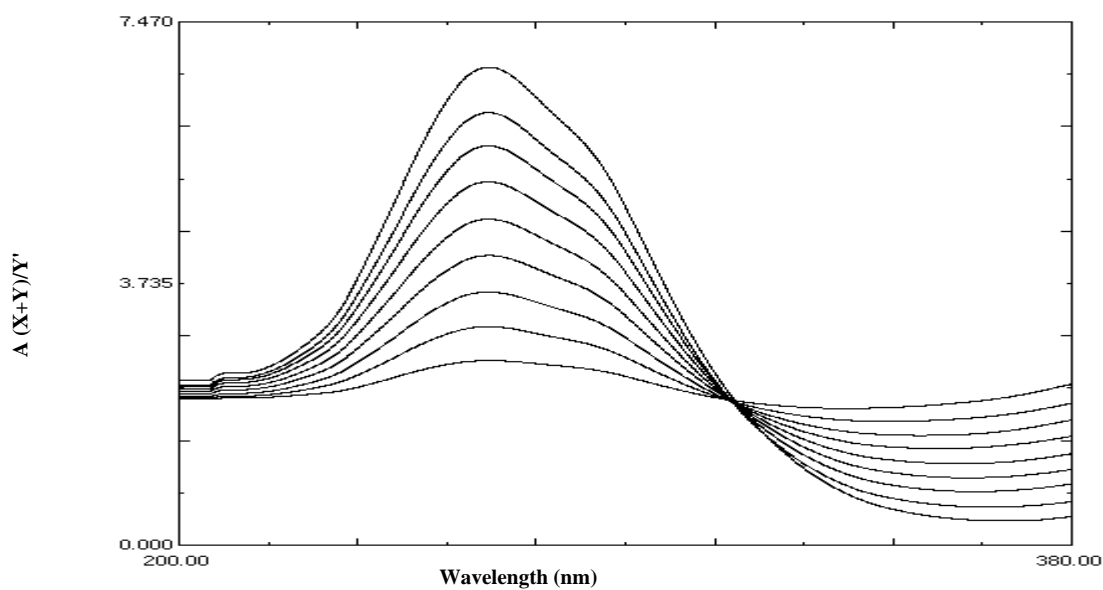


Figure 6d: Division spectra of laboratory prepared mixtures of CPD (X) and its alkaline degradate (Y), using $20 \mu\text{g ml}^{-1}$ of alkaline degradate (Y') as divisor.

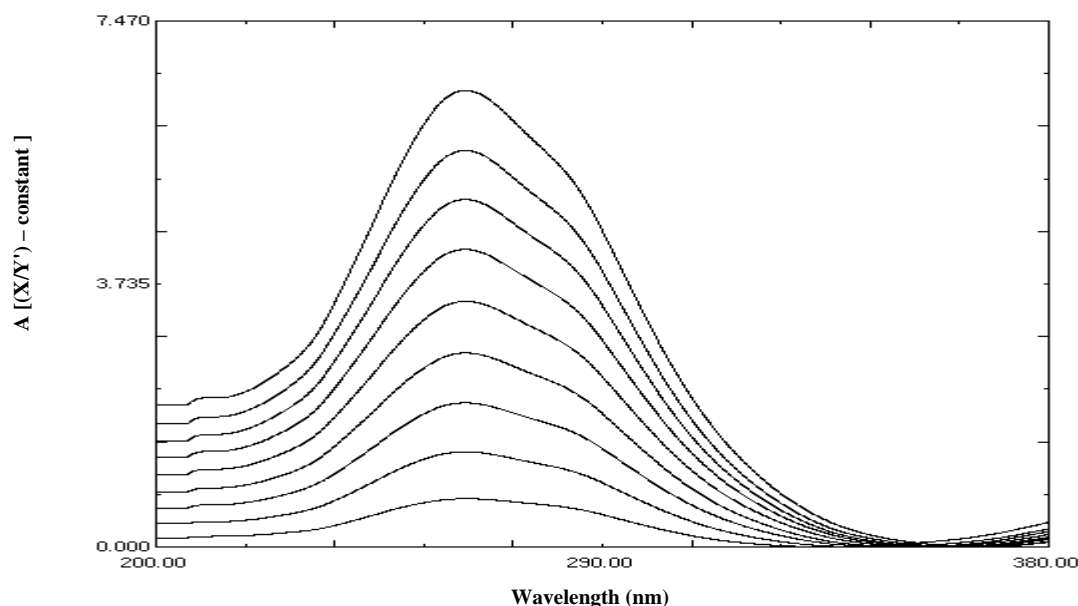


Figure 6e: Division spectra of laboratory prepared mixtures of CPD (X) and its alkaline degradate (Y), using $20 \mu\text{g ml}^{-1}$ of alkaline degradate (Y') as divisor, after subtraction of the constant.

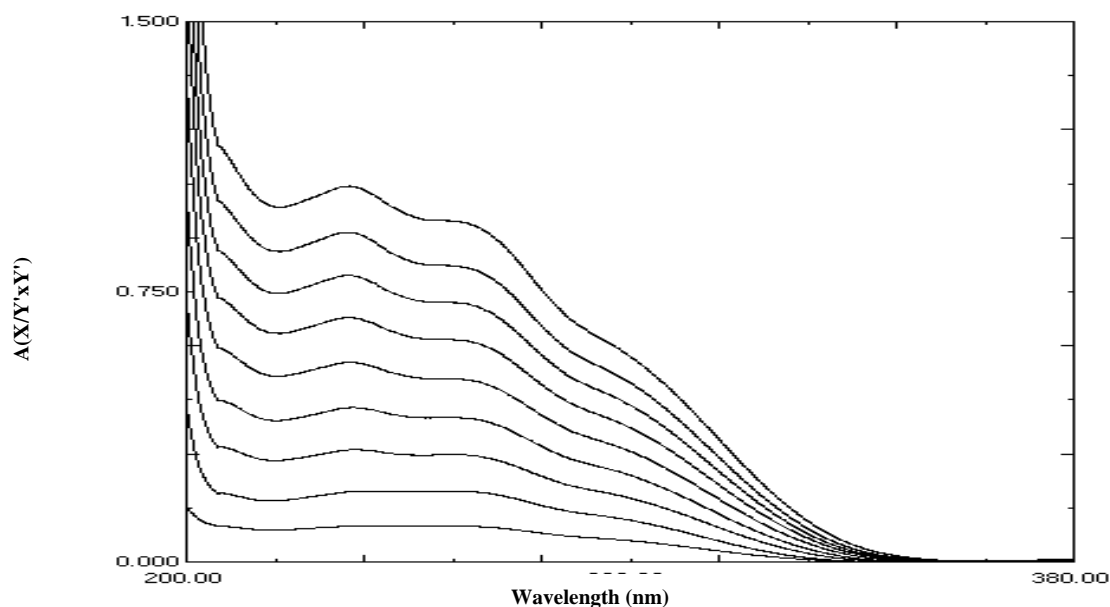


Figure 6f: Zero order absorption spectra of CPD obtained by the proposed method for the analysis of laboratory prepared mixtures of CPD and its alkaline degradate after multiplication by the divisor Y' ($20 \mu\text{g ml}^{-1}$ of alkaline degradate).

The constant can be determined directly from the curve by the straight line which is parallel to the wavelength axis in the region where the degradate is extended. The correct choice of the divisor is fundamental, as, if the concentration of the divisor increases or decreases, the resulting constant value will be proportionally decreased or increased. A linear correlation was obtained between the absorbance and the corresponding concentration of CPD at its corresponding wavelength.

Fig. (6a) shows ratio spectra of laboratory prepared mixtures of CPD and its acid degradation product (spectra divided by carefully chosen the standard spectrum of a $20 \mu\text{g ml}^{-1}$ of acid degradation product). These spectra gave plateau at region 355-370 nm, the values at this region is subtracted figure (6b), the obtained spectra were then multiplied by the spectrum of divisor, figure (6c). CPD could be determined at 232 nm in the presence of acid degradation product in binary mixture up to 90% of acid degradation product, table 1

Also spectra of laboratory prepared mixtures of CPD and its alkaline degradation product (spectra divided by carefully chosen standard spectrum of $20 \mu\text{g ml}^{-1}$ of alkaline degradation product) were shown in figure (6d). These spectra gave plateau at region 345-368 nm, the values at this region is subtracted, figure (6e). The

obtained spectra were then multiplied by the spectrum of divisor, figure (6f). CPD could be determined at 232 nm in the presence of alkaline degradation product in binary mixture up to 90% of alkaline degradation product, table 1

HPLC method

The reversed phase HPLC method was developed to determine CPD in presence of its acid and alkaline degradation products. Different mobile phases were tried for chromatographic separation of CPD from its acid or alkaline degradation products. Finding a mobile phase for the separation of the three components could not be achieved. The chromatograms of acid and alkaline degradation products could not be separated, so laboratory mixtures were prepared for CPD with either its acid or its alkaline degradation products. The best resolution was achieved using a mobile phase acetonitrile: water: triethylamine (60:40:1, v/v/v). Using the specific chromatographic conditions, retention times were found to be 1.23, 1.19, and 2.52 for acid degradation product, alkaline degradation product, and CPD, respectively (figures 7a, 7b). CPD could be determined in the presence of up to 90 % of acid degradation product and up to 80% of its alkaline degradation product, table 2. Statistical analysis of the parameters required for system suitability test of HPLC method was shown in table 3 giving good resolution of CPD from its two degradation products.

Table 2: Results of the analysis of laboratory prepared mixtures containing different ratios of CPD with either the acid degradate or alkaline degradate by the proposed HPLC method ⁽⁸⁾

% Degradation	Concentration of intact CPD $\mu\text{g/ml}$	Recovery % of intact CPD	
		In presence of its acid degradate	In presence of its alkaline degradate
10	90.00	98.47	100.12
20	80.00	99.89	101.98
30	70.00	99.37	101.65
40	60.00	98.06	101.55
50	50.00	98.08	101.26
60	40.00	98.37	98.63
70	30.00	98.41	99.83
80	20.00	98.11	97.18
90	10.00	99.57	93.73*
Mean		98.70	100.28
S.D.		0.71	1.69
R.S.D. %		0.72	1.68

* Rejected value

Table 3: Parameters required for system suitability test of HPLC method

parameter	Obtained value				Reference value
	CPD	Acid degradate	CPD	Alkaline degradate	
Resolution	3.8		4.77		$R > 0.8$
T (tailing factor)	1	1.16	1	1.02	$T = 1$ (for a typical symmetric peak)
α (relative retention time)	3.40		3.66		> 1
K (column capacity)	3.21	1.05	3.20	1	1 – 10 (acceptable)
N (column efficiency)	878	213	770	637	Increases with efficiency of separation
HETP	0.017	0.070	0.019	0.024	The smaller the value the higher the column efficiency

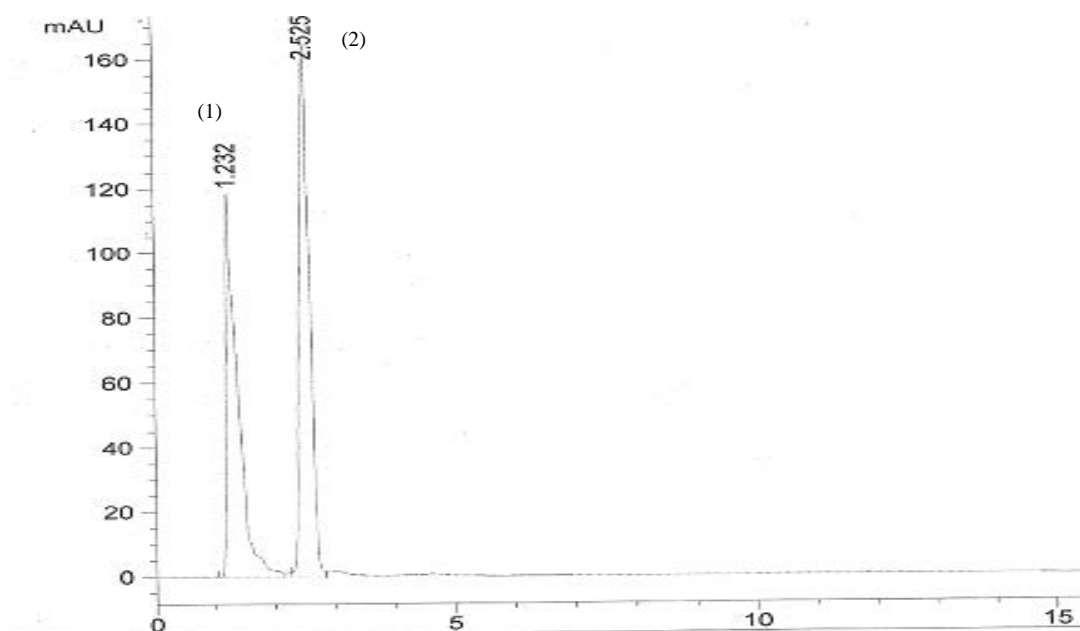


Figure 7a: HPLC chromatograms of a mixture of acid degradate of CPD (1) and CPD (2), [50 $\mu\text{g ml}^{-1}$] for each.

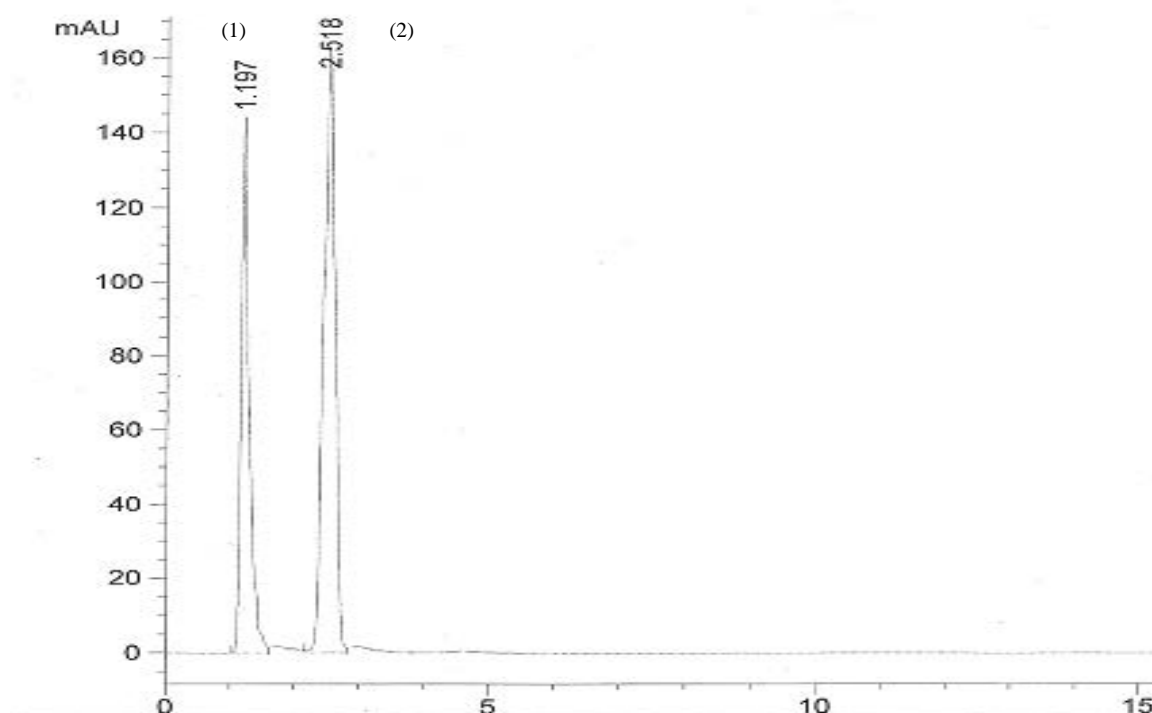


Figure 7b: HPLC chromatograms of a mixture of alkaline degradate of CPD (1) and CPD (2), [50 $\mu\text{g ml}^{-1}$] for each.

Table 4. Assay validation of the proposed methods for the determination of CPD

Parameter	Derivative spectrophotometry		First derivative of ratio spectrophotometry			Ratio subtraction spectrophotometry	HPLC
	² D ₂₆₁	³ D ₂₈₂	¹ DD ₂₁₅	¹ DD ₂₅₅	¹ DD ₂₄₃		
Accuracy (Mean ± S.D.)	99.97 ± 0.34	99.93 ± 0.46	99.96 ± 0.54	99.91 ± 0.76	100.83 ± 0.38	99.89 ± 0.88	100.16 ± 0.83
Precision Repeatability ^a	0.27	0.16	0.23	1.02	0.32	1.11	0.18
Intermediate precision ^b	0.54	0.75	0.57	0.86	0.51	0.85	1.05
Linearity range µg/ml	4.00 - 40.00 µg/ml						3.00 – 100.00 µg/ml
Regression equation	A = 0.0224C + 0.0118	A = 0.0415C - 0.0007	A = 0.043C - 0.0586	A = 0.1776 C - 0.0933	A = 0.2177 C + 0.0073	A = 0.029 C + 0.0015	A = 0.0188 C + 0.0439
Correlation coefficient	1	0.9999	0.9999	0.9999	0.9999	0.9999	1
SE of intercept	0.0011	0.0027	0.0042	0.023	0.0168	0.0031	0.0028
SD of intercept	0.0035	0.0085	0.0133	0.073	0.0532	0.0098	0.0188
LOD µg/ml	0.23	0.3	0.45	0.59	0.36	0.49	0.49
LOQ µg/ml	0.68	0.89	1.35	1.79	1.07	1.48	1.46

^a The intraday (n = 3), average of (8, 20, 32 µg ml⁻¹) of CPD by the proposed methods.^b The interday (n = 3), average of (16, 24, 36 µg ml⁻¹) of CPD by the proposed methods.

Assay validation of the four proposed methods and statistical analysis of regression equations were reported in table 4. The proposed methods were also applied for the determination of CPD in its dosage forms and the results obtained were shown in table 5. The proposed methods were compared statistically with the official method ^[8] (HPLC method using 0.02 M ammonium acetate: acetonitrile (6: 4, v/v) as mobile phase) and no significant difference was obtained as shown in table 6

Table 5: Determination of CPD in pharmaceutical formulations and application of standard addition technique

	Derivative spectrophotometry		First derivative of ratio spectrophotometry			Ratio subtraction spectrophotometry	HPLC
	² D ₂₆₁	³ D ₂₈₂	¹ DD ₂₁₅	¹ DD ₂₅₅	¹ DD ₂₄₃		
Orelox tablet	104.97 ± 0.98	103.66 ± 1.66	103.43 ± 0.21	100.06 ± 0.89	103.87 ± 0.66	102.05 ± 1.74	101.82 ± 1.68
Standard addition technique mean ± SD	100.25 ± 0.67	99.88 ± 0.57	100.71 ± 0.30	100.72 ± 0.78	100.55 ± 0.71	100.49 ± 0.38	100.06 ± 0.96
Orelox suspension	107.20 ± 0.16	108.48 ± 0.11	107.19 ± 0.88	108.86 ± 0.63	107.55 ± 0.57	108.51 ± 0.66	101.69 ± 0.15
Standard addition technique mean ± SD	99.95 ± 0.66	100.06 ± 0.39	100.25 ± 0.32	100.17 ± 0.84	100.68 ± 0.42	99.88 ± 0.94	100.05 ± 0.79

Table 6: Statistical analysis of the results obtained by the proposed methods and the official method ⁽⁸⁾

Parameter	Derivative spectrophotometry		First derivative of ratio spectrophotometry			Ratio subtraction spectrophotometry	HPLC	Official method ⁽⁸⁾ *
	² D ₂₆₁	³ D ₂₈₂	¹ DD ₂₁₅	¹ DD ₂₅₅	¹ DD ₂₄₃			
Mean	100.12	100.26	100.66	99.56	100.54	99.93	99.80	100.22
S.D.	1.06	0.73	0.91	0.97	0.79	0.76	0.77	1.19
n	6	6	6	6	6	6	8	5
Variance	1.13	0.53	0.83	1.13	0.65	0.58	0.59	1.43
F value	1.26 (5.05)	2.66 (5.05)	1.71 (5.05)	1.50 (5.05)	2.27 (5.05)	2.45 (5.05)	2.39 (3.97)	
Student's t-test	0.148 (2.262)	0.069 (2.262)	0.696 (2.262)	1.016 (2.262)	0.618 (2.262)	0.095 (2.262)	0.910 (2.179)	

The values between parenthesis are the corresponding theoretical values of t and F at (P = 0.05)

* Official method is HPLC method using 0.02 M ammonium acetate: acetonitrile (6: 4, v/v) as mobile phase

Conclusion

Four methods were presented for the analysis of CPD in pure powder and in pharmaceutical formulations and could determine CPD in the presence of its acid and alkaline degradation products. The suggested methods are simple, fast and can be used in routine and quality control analysis of intact CPD in raw material and pharmaceutical formulations without interference of degradation products or excipients.

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