DRUG FORMULATIONS AND CLINICAL METHODS

Stability-Indicating Spectrofluorometric Method for the Determination of Some Cephalosporin Drugs via Their Degradation Products

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A stability-indicating spectrofluorometric method was investigated for the determination of three cephalosporin drugs, namely, cefpodoxime proxetil (CPD), cefixime trihydrate (CFX), and cefepime hydrochloride (CPM), via their acid and alkali degradation products. The three drugs were determined via their acid degradation at 432, 422, and 435 nm using an excitation wavelength of 310, 330, and 307 nm for CPD, CFX, and CPM determination, respectively, and via their alkali degradation at 407, 411, and 405 nm using an excitation wavelength of 310, 305, and 297 nm for CPD, CFX, and CPM determination, respectively. Linearity was achieved in the ranges of 0.35–3.50, 0.4–4.0, and 0.3–3.0 µg/mL for the acid degradation products of CPD, CFX, and CPM, respectively, and in ranges of 0.05–0.5, 0.1–1.0, and 0.08–0.80 µg/mL for the alkali degradation products of CPD, CFX, and CPM, respectively. The method was validated for various parameters according to International Conference on Harmonization guidelines. The method was successfully applied for the determination of these cephalosporin drugs in pharmaceutical dosage forms with good accuracy and precision. The results obtained by the proposed spectrofluorometric method were compared with good agreement to the official HPLC method.

Cephalosporins are the second most important β-lactam antibiotics after penicillin for treating infectious diseases (1). They are classified into four generations (2). Cephalosporins are among the safest and most effective broad spectrum bactericidal antimicrobial agents and are used widely in clinical therapy for the treatment of severe infectious diseases (3). Cefpodoxime proxetil (CPD) and cefixime trihydrate (CFX) are orally active, broad spectrum, third-generation cephalosporins that are extensively used in clinical practice and therapy. Cefepime hydrochloride (CPM) is a semisynthetic, broad spectrum, fourth-generation cephalosporin; it has improved activity over other commercially available cephalosporin drugs against Gram-negative and Gram-positive bacterial infections. It also exhibits increased stability against β-lactamase overproducing bacteria (4, 5).

Several methods have been utilized for their determination in body fluids and dosage forms. Published methods for determining CPD include spectrophotometric (6–8), chromatographic (9–12), electrochemical (13, 14), and chemometric (15). Other methods such as spectrofluorometric (16), spectrophotometric (17), chromatographic (18–20), electrochemical (21, 22), and chemometric (15) have been reported for the determination of CFX. Several methods have been described for the determination of CPM in biological body fluids and pharmaceuticals; these include spectrophotometric (23–25), chromatographic (26–30), and electrochemical (31–33). Unfortunately, the reported spectrophotometric and spectrofluorometric methods for the determination of these drugs in their pharmaceutical formulations have disadvantages such as lack of selectivity and tedious and lengthy procedures. The official method for pharmaceutical preparations utilizes HPLC (9). Therefore, it was desirable to develop a simple, accurate, and fast procedure that could be applied in QC laboratories for evaluation of the drugs in the presence of their degradation products in pure powder and pharmaceutical formulations.

This paper describes a simple, specific, and highly sensitive technique for stability determination of each of the studied drugs via its degradation products. The method is based on monitoring the native fluorescence of the formed acid and alkali degradation products at specified excitation and emission wavelengths.

Experimental

Pure Samples

CPD was kindly supplied by Pharco/Rexcel, Cairo, Egypt. Its purity was 99.50 ± 0.56% using the HPLC official method (9). CFX was kindly supplied by Sigma Pharmaceutical Industries, Cairo, Egypt. Its purity was found to be 99.74 ± 0.59% using the HPLC official method (9). CPM was purchased from Chem-

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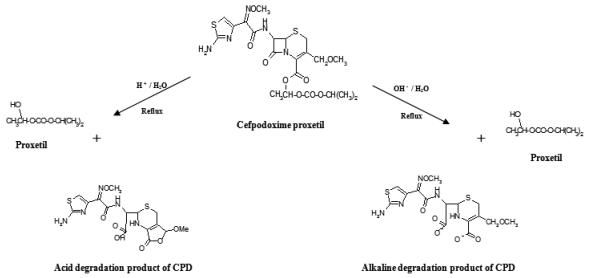

Figure 1. Suggested acid and alkali induced degradation pathway of CPD.

Impex International (Wood Dale, IL). Its purity was found to be 99.93 ± 0.34% using the HPLC official method (9).

Market Samples

(**a**) *Orelox® film coated tablets*.—Batch No. 7FH5E, labeled to contain CPD equal to 100 mg cefpodoxime/tablet (Sanofi Aventis, Cairo, Egypt).

(**b**) *Cepodem® suspension*.—Batch No. 100155, labeled to contain CPD equal to 40 mg cefpodoxime/5 mL (one bottle 60 mL; Pharco/Rexcel).

(**c**) *Ximacef® capsules*.—Batch No. 70921, labeled to contain 400 mg CFX/capsule (Sigma).

(**d**) *Ximacef suspension (30 mL)*.—Batch No. 01633, 81418, 10944, and 11841, labeled to contain 100 mg CFX/5 mL (Sigma).

(**e**) *Maxipime® vial*.—Batch No. G42343, B60321, H02298, and K111361, labeled to contain CPM equal to 1 g cefepime (SmithKline Beecham, Cairo, Egypt).

(**f**) *Maxipime vial*.—Batch No. E106021 and M115324, labeled to contain CPM equal to 500 mg cefepime (SmithKline Beecham).

Apparatus

(**a**) *Shimadzu RF-1501 spectrofluorometer*.—No. 206-62901, using a quartz cell (1 × 1 × 4.5 cm) and slit width 2.5 nm (Tokyo, Japan).

(**b**) *Digital pH meter*.—Jenway, No. 924005-BO3-Q11C (Staffordshire, UK).

Reagents

All chemicals and reagents used were of analytical grade.

(**a**) *Methanol*.—E. Merck (Darmstadt, Germany).

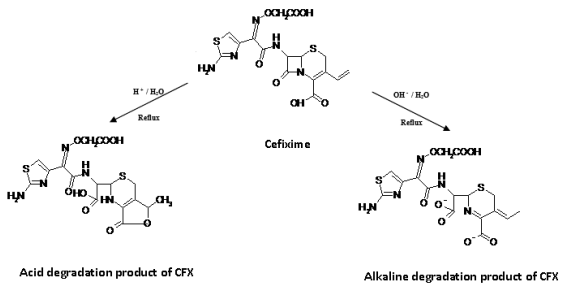

Figure 2. Suggested acid and alkaline induced degradation pathway of CPM.

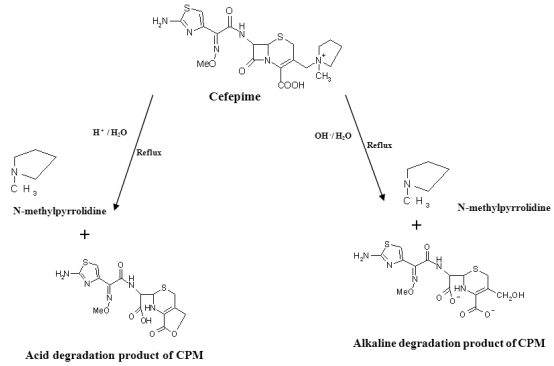

Figure 3. Suggested acid and alkaline induced degradation pathway of CFX.

(**b**) *Dimethyl formamide (DMF)*.—Prolabo (West Chester, PA).

(**c**) *HCl (1 M HCl), NaOH (1 M NaOH), and ammonium hydroxide (30%)*.—ADWIC, El Nasr Pharmaceutical & Chemical Co., Cairo, Egypt).

(**d**) *Water*.—Obtained in a Milli-Q water purification system (EMD Millipore, Billerica, MA).

Standard Solutions

(**a**) *Standard stock solution of intact CPD (0.35 mg/mL), CFX (0.40 mg/mL), and CPM (0.30 mg/mL)*.—Prepared by dissolving 35 mg pure CPD and 40 mg pure CFX in 5 mL methanol and then diluting to 100 mL with water. CPM solution was prepared by dissolving 30 mg pure CPM in 20 mL water, then diluting to 100 mL with water. Working standard solutions of intact CPD (17.5 µg/mL), CFX (20.0 µg/mL), and CPM (15.0 µg/mL) were prepared by diluting 5 mL of the standard stock solution of each drug to 100 mL with water.

(**b**) *Standard stock solution of acid degradate of CPD (0.35 mg/mL), CFX (0.40 mg/mL), and CPM (0.30 mg/mL)*.—Prepared by refluxing 35, 40, and 30 mg pure CPD, CFX, and CPM, respectively, with 25 mL 1 M HCl for 1.5 h, then neutralizing with 2 M NaOH. The solution was quantitatively transferred into a 100 mL volumetric flask, and the volume was completed to the mark with water. Working standard solutions of the acid degradation product of CPD (17.5 µg/mL), CFX (20.0 µg/mL), and CPM (15.0 µg/mL) were prepared by diluting 5 mL standard stock solution of each drug degradation product to 100 mL with water.

(**c**) *Standard stock solution of intact CPD (0.05 mg/mL), CFX (0.1 mg/mL), and CPM (0.08 mg/mL)*.—Prepared by dissolving 5 mg pure CPD and 10 mg pure CFX in 5 mL methanol and then diluting to 100 mL with water. CPM solution was prepared by dissolving 8 mg pure CPM in 20 mL water and then diluting to 100 mL with water. Working standard solutions of intact CPD (2.5 µg/mL), CFX (5.0 µg/mL), and CPM (4.0 µg/mL) were prepared by diluting 5 mL standard stock solution of each drug to 100 mL with water.

(**d**) *Standard stock solution of alkali degradate of CPD (0.05 mg/mL), CFX (0.1 mg/mL), and CPM (0.08 mg/mL)*.—Prepared by refluxing 5, 10, and 8 mg CPD, CFX, and CPM, respectively, with 25 mL 1 M NaOH for 1 h and then neutralizing with 2 M HCl. The solution was quantitatively transferred into a 100 mL volumetric flask, and the volume was completed to

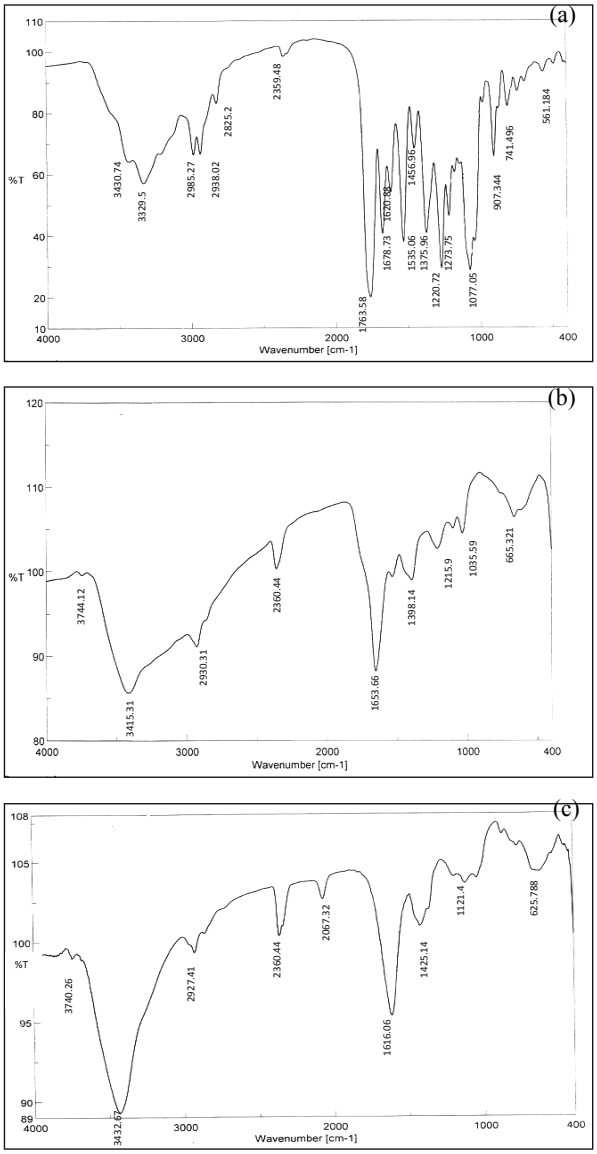

Figure 4. IR spectrum of (a) intact CPD, (b) of acid degradation product of CPD, and (c) of alkali degradation product of CPD.

the mark with water. Working standard solutions of the alkali degradation product of CPD (2.5 µg/mL), CFX (5.0 µg/mL), and CPM (4.0 µg/mL) were prepared by diluting 5 mL standard stock solution of each drug degradation product to 100 mL with water.

Elucidation of the Structures of Degradation Products

Complete degradation was achieved as shown by silica gel TLC using methanol–ammonium hydroxide (10:0.1, v/v) mobile phase. The solution was neutralized and evaporated under vacuum to dryness. The degradation product was extracted using 30 mL DMF 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 product. The

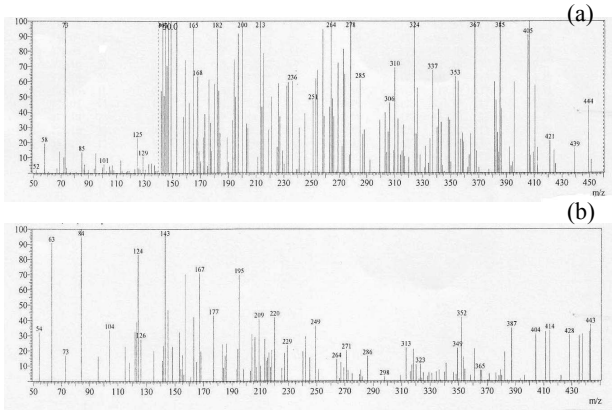

Figure 5. Mass spectrum of (a) acid degradation product of CPD, and (b) alkali degradation product of CPD.

structure of the isolated degradation product was elucidated using IR spectrometry and MS.

Linearity Determination

(**a**) *For acid degradation.*—Aliquots equivalent to 8.75–87.5, 10–100, and 7.5–75 µg intact CPD, CFX, and CPM from working standard solutions of 17.5, 20.0, and 15.0 µg/mL, respectively, were transferred accurately into a series of stoppered 20 mL test tubes; 10 mL 1 M HCl was added, and the tubes were put in an oven at 150°C for 1.5 h. The test tubes were cooled and solutions then neutralized with 2 M NaOH. The solutions were quantitatively transferred into a series of 25 mL volumetric flasks, and the volume was completed to the mark with water.

Aliquots equivalent to 8.75–87.5, 10–100, and 7.5–75 µg intact CPD, CFX, and CPM from working standard solutions of 17.5, 20.0, and 15.0 µg/mL, respectively, were transferred accurately into a series of 25 mL volumetric flasks, and the volume was completed to the mark with water (used as a blank).

The fluorescence intensity of each concentration was recorded and the fluorescence intensity of its corresponding concentration of blank was manually subtracted at excitation wavelengths of 310, 330, and 307 nm, and at emission wavelengths of 433, 422, and 435 nm for CPD, CFX, and CPM, respectively.

(**b**) *For alkali degradation*.—Aliquots equivalent to 1.25–12.5, 2.5–25, and 2.0–20 µg intact CPD, CFX, and CPM from working standard solutions of 2.5, 5, and 4 µg/mL, respectively, were transferred accurately into a series of stoppered test tubes; 10 mL 1 M NaOH was added and the tubes were put in an oven at 150°C for 1 h. The test tubes were cooled and solutions then neutralized with 2 M HCl. The solutions were quantitatively transferred into a series of 25 mL volumetric flasks, and the volume was completed to the mark with water.

Aliquots equivalent to 1.25–12.5, 2.5–25, and 2.0–20 µg intact CPD, CFX, and CPM from working standard solutions of 2.5, 5, and 4 µg/mL, respectively, were transferred accurately into a series of 25 mL volumetric flasks, and the volume was completed to the mark with water (used as a blank).

The fluorescence intensity of each concentration was recorded, and the fluorescence intensity of its corresponding concentration of blank was manually subtracted at excitation wavelengths of 310, 305, and 297 nm and at emission wavelengths of 407, 411, and 405 nm for CPD, CFX, and CPM, respectively.

Calibration curves relating the fluorescence intensity at

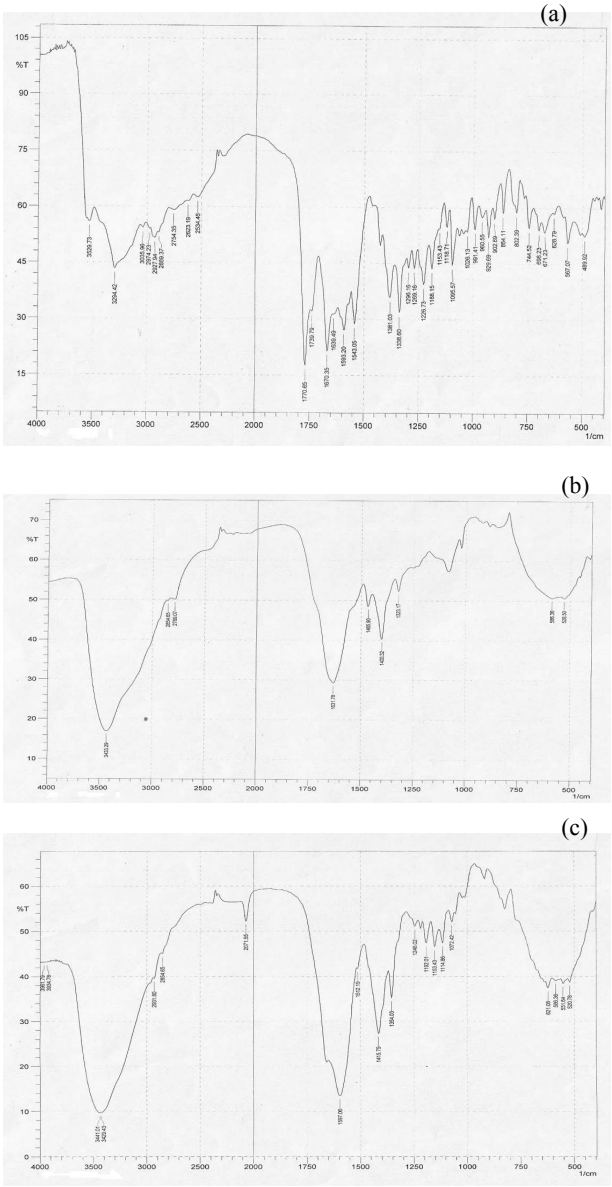

Figure 6. IR spectrum of (a) intact CFX, (b) acid degradation product of CFX, and (c) alkali degradation product of CFX.

emission wavelengths of 432, 407 nm; 422, 411 nm; and 435, 405 nm for acid and alkali degradates of CPD, CFX, and CPM, respectively, to the corresponding concentrations of CPD, CFX, and CPM were constructed, and then the regression equations were computed.

Analysis of Laboratory Prepared Mixtures

(**a**) *For acid degradation*.—Aliquots from 4.5 to 0.5 mL were separately transferred from working standard solutions of intact CPD, CFX, and CPM of concentration 17.5, 20.0, and 15.0 μg/mL, respectively. Aliquots of 0.5 to 4.5 mL acid degradate of CPD, CFX, and CPM of concentration 17.5, 20.0, and 15.0 μg/mL, respectively, were added to the previous solutions separately. To each mixture 10 mL 1 M HCl was added, and procedures were completed as under *Linearity Determination* above.

(**b**) *For alkali degradation*.—Aliquots from 4.5 to 0.5 mL

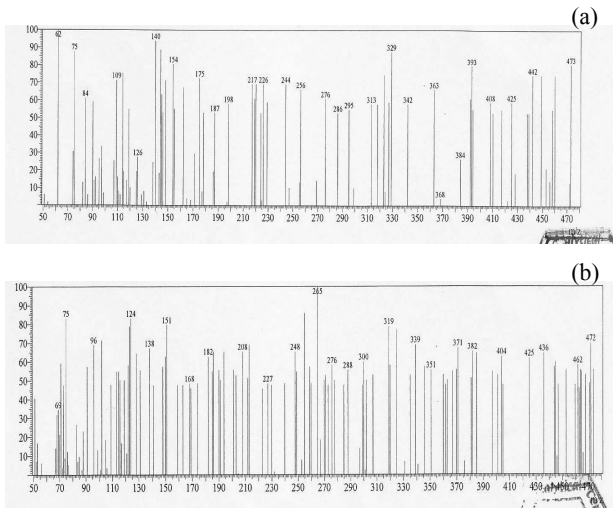

Figure 7. Mass spectrum of (a) acid degradation product of CFX and (b) alkali degradation product of CFX.

were separately transferred from working standard solutions of intact CPD, CFX, and CPM of concentration 2.5, 5, and 4.0 μg/mL, respectively. Aliquots of 0.5 to 4.5 mL alkali degradate of CPD, CFX, and CPM of concentration 2.5, 5, and 4.0 μg/mL, respectively, were added to the previous solutions separately. To each mixture 10 mL 1 M NaOH was added, and procedures were completed as under *Linearity Determination* above.

Analysis of CPD in Pharmaceutical Dosage Forms

(**a**) *Analysis of tablets.*

(*1*) *For acid degradation*.—A total of 10 tablets were weighed and finely powdered. An amount equivalent to 40 mg was weighed, transferred into a 100 mL volumetric flask, and stirred with 20 mL methanol, and the volume was completed with water. The solution was filtered through No. 1 Whatman filter paper, and further dilution was made using water to obtain a concentration of 17.5 μg/mL; 2 mL of this solution was transferred to a test tube, and 10 mL 1 M HCl was added and procedures were completed as under *Linearity Determination* above. The concentration of CPD was calculated from the corresponding regression equation.

(*2*) *For alkali degradation*.—Procedures were performed as mentioned under (*1*) for CPD tablets until the solution was filtered, and further dilution was made using water to obtain a concentration of 2.5 μg/mL; 2 mL of this solution was transferred to a test tube, and 10 mL 1 M NaOH was added and procedures were completed as under *Linearity Determination* above. The concentration of CPD was calculated from the corresponding regression equation.

(**b**) *Analysis of suspension.*

(*1*) *For acid degradation*.—The contents of two bottles were extracted using 50 mL methanol, and then filtered into a 100 mL volumetric flask. The residue was washed several times with water, and the volume was completed with water. Further dilution was made using water to obtain a concentration of 17.5 μg/mL; 2 mL of this solution was transferred to a test tube, 10 mL 1 M HCl was added, and procedures were completed as under *Linearity Determination* above. The concentration of CPD was calculated from the corresponding regression equation.

(*2*) *For alkali degradation*.—Procedures were performed as

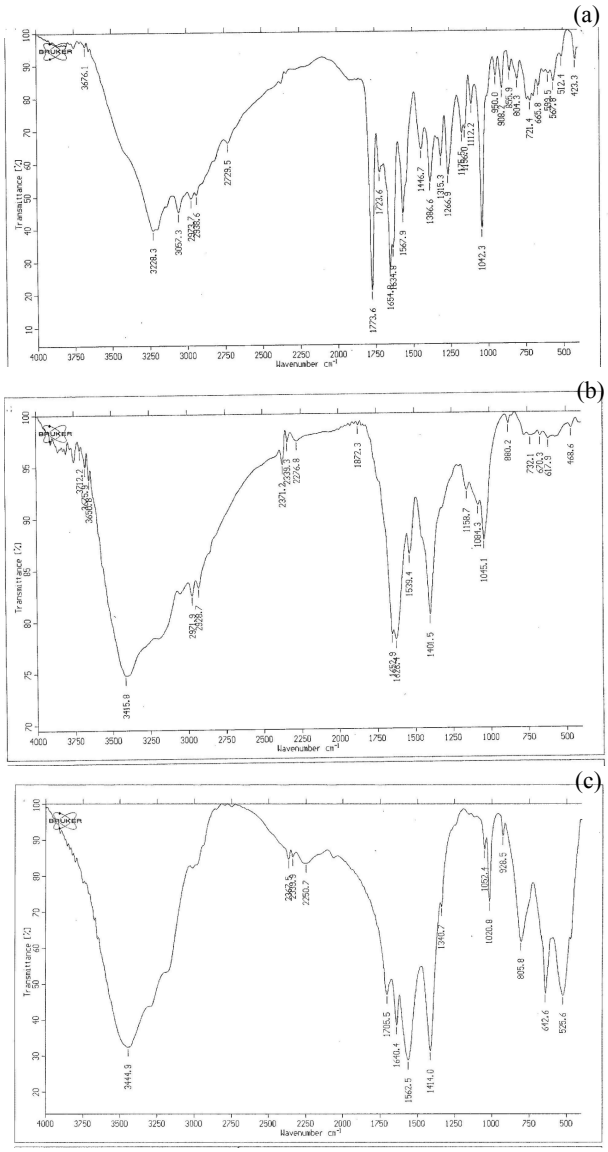

Figure 8. IR spectrum of (a) intact CPM, (b) acid degradation product of CPM, and (c) alkali degradation product of CPM.

mentioned under (*1*) for CPD suspension until the solution was filtered, and further dilution was made using water to obtain a concentration of 2.5 μg/mL; 2 mL of this solution was transferred to a test tube, 10 mL 1 M NaOH was added, and procedures were completed as under *Linearity Determination* above. The concentration of CPD was calculated from the corresponding regression equation.

Analysis of CFX in Pharmaceutical Dosage Forms

(**a**) *Analysis of capsules.*

(*1*) *For acid degradation*.—The contents of 10 capsules were emptied and weighed. An amount equivalent to 25 mg CFX was weighed, transferred into a 100 mL volumetric flask, stirred with 10 mL methanol, and the volume was completed with water. The solution was filtered, and further dilution was made using water to obtain a concentration of 20.0 μg/mL; 2 mL of this solution was transferred to a test tube, 10 mL 1 M HCl was added, and procedures were completed as under *Linearity Determination*

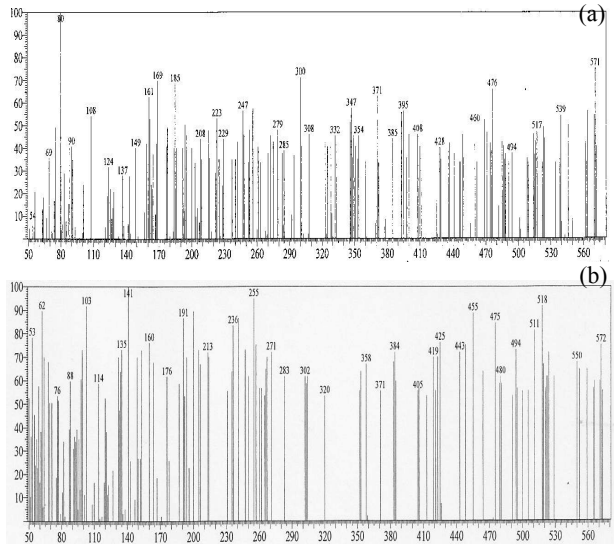

Figure 9. Mass spectrum of (a) acid degradate of CPM, and (b) alkali degradate of CPM.

above. The concentration of CFX was calculated from the corresponding regression equation.

(*2*) *For alkali degradation*.—Procedures were performed as mentioned under (*1*) for CFX capsules until the solution was filtered, and further dilution was made using water to obtain a concentration of 5 μg/mL; 2 mL of this solution was transferred to a test tube, 10 mL 1 M NaOH was added, and procedures were completed as under *Linearity Determination* above. The concentration of CFX was calculated from the corresponding regression equation.

(**b**) *Analysis of suspension.*

(*1*) *For acid degradation*.—The contents of two bottles were extracted using 50 mL methanol, and then filtered into a 100 mL volumetric flask. The residue was washed several times with water, and the volume was completed with water. Further dilution was made using water to obtain a concentration of 20.0 μg/mL; 2 mL volume of this solution was transferred to a test tube, 10 mL 1 M HCl was added, and procedures were completed as under *Linearity Determination* above. The concentration of CFX was calculated from the corresponding regression equation.

(*2*) *For alkali degradation*.—Procedures were performed as mentioned under (*1*) for CFX suspension until the solution was filtered, and further dilution was made using water to obtain a concentration of 5 μg/mL; 2 mL volume of this solution was transferred to a test tube, 10 mL 1 M NaOH was added, and procedures were completed as under *Linearity Determination* above. The concentration of CFX was calculated from the corresponding regression equation.

Analysis of CPM in Pharmaceutical Dosage Forms

(*1*) *For acid degradation*.—The contents of three vials were extracted using 30 mL water into a 100 mL volumetric flask. The vials were washed several times with water, and the volume was completed with water. Further dilution was made using water to obtain a concentration of 15.0 μg/mL; 1.5 mL volume of this solution was transferred to a test tube, 10 mL 1 M HCl was added, and procedures under *Linearity Determination* above

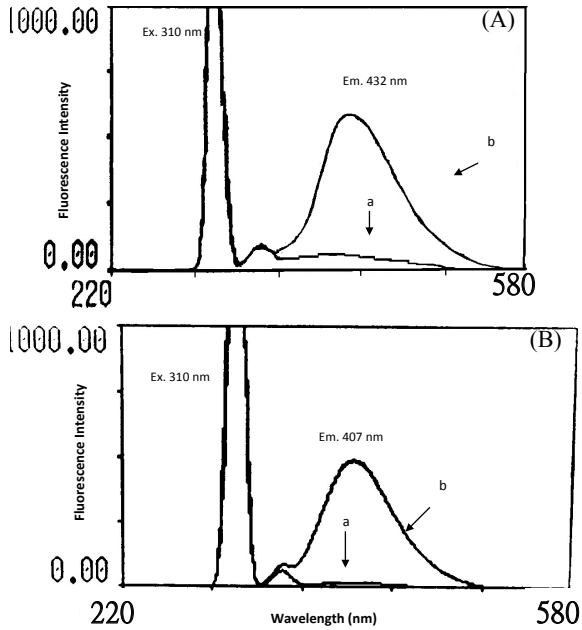

Figure 10. (A) Excitation (Ex.) and emission (Em.) spectra of (a) intact CPD (a) and (b) acid degradation product, 1.75 µg/mL of each. (B) Ex. and Em. spectra of (a) intact CPD, and (b) alkali degradation product, 0.25 µg/mL of each.

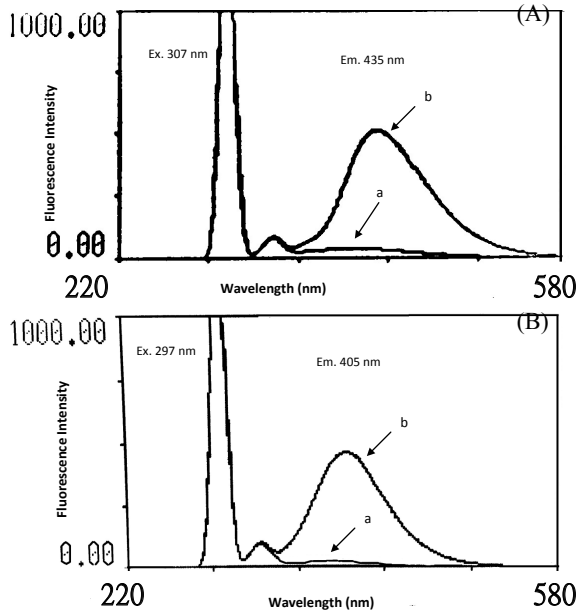

Figure 12. (A) Excitation (Ex.) and emission (Em.) spectra of (a) intact CPM and (b) acid degradation product, 1.5 µg/mL of each. (B) Ex. and Em. spectra of (a) intact CPM, and (b) alkali degradation product, of 0.4 µg/mL of each.

were followed. The concentration of CPM was calculated from the corresponding regression equation.

(*2*) *For alkali degradation*.—Contents of vials were extracted as mentioned under (*1*) for CPM vials until the solution was filtered. Further dilution was made using water to obtain a concentration of 4.0 µg/mL; 2 mL volume of this solution was transferred to a test tube, 10 mL 1 M NaOH was added, and procedures under *Linearity Determination* above were followed. The concentration of CPM was calculated from the corresponding regression equation.

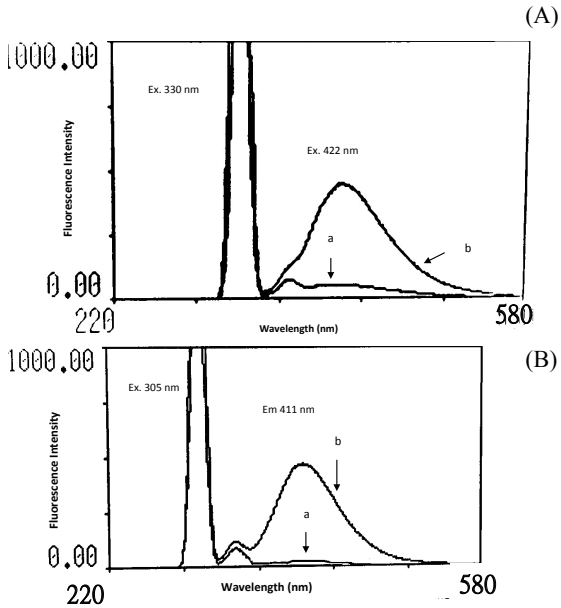

Figure 11. Excitation (Ex.) and emission (Em.) spectra of (a) intact CFX and (b) acid degradate, 2 µg/mL of each. (B) Ex. and Em. spectra of (a) intact CFX and (b) alkali degradate 0.5 µg/mL of each.

Results and Discussion

The suggested acid and alkaline degradation pathways of CPD, CFX, and CPM are illustrated in Figures 1–3. It was found that complete degradation of CPD, CFX, and CPM occurred after 1.5 and 1 h at 350°C for the acid and alkaline degradation process, respectively. The acid and alkali degradation products were separated and their structures confirmed by IR spectrometry and MS (Figures 4–9). The IR spectra of intact CPD, CFX, and CPM show that the characteristic bands at 1763.58, 1770.65, and 1773.6 cm^{-1} originating from the lactam carbonyl group were not observed in IR spectra of both acid and alkaline degradation products, indicating the opening of the β-lactam ring upon degradation that increases the fluorescence intensities of these compounds. The fluorescence spectra of CPD, CFX, and CPM compared to their acid or alkali degradates showed that the intact drugs did not have native spectrofluorometric characteristics, but after acid or alkaline degradation the resulting degradates had strong native fluorescence. The proposed method depends on measuring the difference in fluorescence intensities between the acid or the alkali degradates and their intact drugs; hence, any amount of degradation found in the drug sample will be subtracted from the reading of experiments, and the fluorescence intensities before and after hydrolysis will correspond only to the intact drug.

The fluorescence spectra of each of the studied drugs (CPD, CFX, and CPM) and its acid degradate showed that the resulting degradate has fluorescence at excitation wavelengths of 310, 330, and 307 nm, respectively, and emission wavelengths of 432, 422, and 435 nm, respectively, while the alkali degradate showed intensive fluorescence at excitation wavelengths of 310, 305, and 297 nm, respectively, and emission wavelengths of 407, 411, and 405 nm, respectively (Figures 10–12).

The method depends on measuring the difference in

Table 1. Determination of CPD, CFX, and CPM in laboratory-prepared mixtures by the proposed method

Degradation product, %	Concn of intact CPD added, µg/mL			Concn of intact CFX added, µg/mL			Concn of intact CPM added, µg/mL		
	In the presence of acid degradate	Recovery, %	In the presence of alkaline degradate	In the presence of acid degradate	Recovery, %	In the presence of alkaline degradate	In the presence of acid degradate	Recovery, %	In the presence of acid degradate	Recovery, %
10	3.15	99.24	0.45	3.60	100.23	0.90	2.70	100.28	0.72	101.03
20	2.80	100.45	0.40	3.20	98.77	0.80	2.40	98.65	0.64	100.72
30	2.45	99.87	0.35	2.80	100.81	0.70	2.10	100.44	0.56	100.32
40	2.10	100.12	0.30	2.40	101.61	0.60	1.80	99.09	0.48	101.36
50	1.75	100.94	0.25	2.00	99.69	0.50	1.50	100.50	0.40	99.97
60	1.40	100.72	0.20	1.60	100.78	0.40	1.20	100.85	0.32	100.86
70	1.05	101.50	0.15	1.20	101.10	0.30	0.90	99.86	0.24	100.36
80	0.70	100.77	0.10	0.80	100.49	0.20	0.60	99.64	0.16	101.15
90	0.35	100.86	0.05	0.40	105.01[a]	0.10	0.30	98.99	0.08	101.13
Mean, %		100.50			100.40			99.81		100.77
SD		0.67			0.88			0.77		0.46
RSD, %		0.67			0.88			0.77		0.46

[a] Rejected value.

Table 2. Determination of CPD, CFX, and CPM in pharmaceutical dosage forms by the proposed method

Pharmaceutical preparations	Recovery, % ± SD[a]	
	By acidic degradation	By alkaline degradation
	CPD	
Orelox tablet 7FH5E	101.36 ± 0.59	102.37 ± 1.02
Cepodem suspension 100155	100.75 ± 1.14	103.99 ± 1.15
	CFX	
Ximacef capsule 70921	102.68 ± 0.77	105.28 ± 0.83
Ximacef suspension 10944	101.15 ± 1.17	101.50 ± 1.62
	CPM	
Maxipime vial 1 g G42343	103.66 ± 0.38	105.44 ± 1.26
Maxipime vial 500 mg E108021	106.24 ± 0.34	106.61 ± 1.19

[a] Average of three different determinations.

fluorescence intensities at 432 or 407 nm for CPD, 422 or 411 nm for CFX, and 435 or 405 nm for CPM of two solutions with the same concentration of the analyzed drug before and after complete acid or alkali degradation. Hence, any amount of the degradation products found in the analyzed samples will be subtracted from the readings of the experiments at the corresponding wavelength after degradation, and the difference in fluorescence intensities before and after hydrolysis will correspond only to the intact drugs, so the method is stability-indicating.

By applying the suggested procedure, linear correlation was obtained between the difference in fluorescence intensities at 432 and 407 nm before and after complete acid and alkali degradation, respectively, and the corresponding concentration of pure CPD over the ranges 0.35–3.50 and 0.05–0.50 µg/mL for acid and alkaline degradation, respectively. The regression equations were found to be:

$$Y = 246.92C - 4.1333 \text{ (for acid degradation) } r = 1$$

$$Y = 1638.1C + 2.5333 \text{ (for alkali degradation) } r = 0.9999$$

where Y is the difference in fluorescence intensity, C is the concentration of CPD in µg/mL, and r is the correlation coefficient.

Linear correlation was obtained between the difference in fluorescence intensities at 422 or 411 nm before and after complete acid and alkali degradation, respectively, and the corresponding concentration of pure CFX over the ranges 0.40–4.0 and 0.1–1.0 µg/mL for acid and alkali degradation, respectively. The regression equations were found to be:

$$Y = 196.89\ C + 0.9 \text{ (for acid degradation) } r = 0.9999$$

$$Y = 847.52C + 0.1056 \text{ (for alkali degradation) } r = 0.9999$$

where C is the concentration of CFX in µg/mL.

Also, linear correlation was obtained between the difference in fluorescence intensities at 435 or 405 nm before and after complete acid and alkali degradation, respectively, and the corresponding concentration of pure CPM over the ranges

Table 3. Application of the standard addition technique for the determination of CPD, CFX, and CPM in pharmaceutical dosage forms by the proposed method

Pharmaceutical preparations	By acidic degradation			By alkaline degradation		
	Taken, µg/mL	Added, µg/mL	Recovery, %[a]	Taken, µg/mL	Added, µg/mL	Recovery, %[a]
CPD						
Orelox tablet 7FH5E	1.40	0.04	100.03	0.15	0.05	101.34
	1.40	0.70	99.36	0.15	0.10	100.73
	1.40	1.05	99.36	0.15	0.15	100.93
	1.40	1.40	99.51	0.15	0.20	99.51
	1.40	1.75	98.18	0.15	0.25	99.63
Mean, %			99.29			100.43
SD			0.68			0.81
RSD, %			0.68			0.81
Cepodem suspension 100155	1.40	0.04	99.63	0.15	0.05	100.12
	1.40	0.70	99.22	0.15	0.10	99.51
	1.40	1.05	99.09	0.15	0.15	100.93
	1.40	1.40	100.74	0.15	0.20	100.73
	1.40	1.75	100.47	0.15	0.25	99.63
Mean, %			99.83			100.18
SD			0.74			0.64
RSD, %			0.74			0.64
CFX						
Ximacef capsule 70921	1.20	0.50	100.68	0.40	0.10	100.29
	1.20	0.10	99.66	0.40	0.20	101.47
	1.20	1.50	101.35	0.40	0.30	99.90
	1.20	2.00	99.66	0.40	0.40	100.44
	1.20	2.50	100.68	0.40	0.50	99.82
Mean, %			100.41			100.39
SD			0.73			0.66
RSD, %			0.73			0.66
Ximacef suspension 10944	1.20	0.50	100.68	0.40	0.10	100.94
	1.20	1.00	99.41	0.40	0.20	101.53
	1.20	1.50	100.17	0.40	0.30	100.75
	1.20	2.00	100.68	0.40	0.40	101.24
	1.20	2.50	99.86	0.40	0.50	99.41
Mean, %			100.16			100.78
SD			0.55			0.82
RSD, %			0.54			0.81
CPM						
Maxipime vial 1 g G42343	0.90	0.30	100.06	0.32	0.16	100.00
	0.90	0.60	100.06	0.32	0.24	100.06
	0.90	0.90	99.72	0.32	0.32	100.78
	0.90	1.20	99.55	0.32	0.40	101.34
	0.90	1.50	100.06	0.32	0.48	101.71
Mean, %			99.89			100.78
SD			0.24			0.76
RSD, %			0.24			0.75
Maxipime| vial 500 mg E108021	0.90	0.30	99.04	0.32	0.16	101.63
	0.90	0.60	100.82	0.32	0.24	99.97
	0.90	0.90	99.72	0.32	0.32	101.40
	0.90	1.20	100.44	0.32	0.40	101.59
	0.90	1.50	100.56	0.32	0.48	101.32
Mean, %			100.12			101.18
SD			0.73			0.69
RSD, %			0.73			0.68

[a] Average of three different determinations.

0.3–3.0 and 0.08–0.8 µg/mL for acid and alkali degradation, respectively. The regression equations were found to be:

$$Y = 328.15\ C + 0.2222 \text{ (for acid degradation) } r = 0.9999$$

$$Y = 1050.2\ C + 0.0333 \text{ (for alkali degradation) } r = 0.9999$$

where C is the concentration of CPM in µg/mL.

It was found that the sensitivity of the method was increased by the determination of the studied cephalosporin drugs via their alkali degradation products.

The specificity of the proposed procedure was assessed by the analysis of laboratory-prepared mixtures. Several mixtures of CPD, CFX, and CPM each with its acid and alkali degradates were prepared and analyzed by the proposed method. The results proved that the method is highly selective for stability testing of the studied drugs as it can determine CPD in the presence of up to 90% of its acid and alkali degradate; CFX in the presence of up to 80 and 90% of its acid and alkali degradate, respectively; and CPM in the presence of up to 90% of its acid or alkali degradate, as shown in Table 1.

The proposed procedure was also successfully applied for the determination of CPD in Orelox tablets and Cepodem suspension, CFX in Ximacef capsules, and Ximacef suspension, and CPM in Maxipime vials 1 g and 500 mg with no interference from the excipients, as shown in Table 2. The validity of the method was

Table 4. Assay validation parameters obtained by applying the proposed method

	CPD		CFX		CPM	
Parameter	By acidic degradation	By alkaline degradation	By acidic degradation	By alkaline degradation	By acidic degradation	By alkaline degradation
Accuracy						
Mean ± RSD, %	100.00 ± 0.11	99.99 ± 0.54	100.00 ± 0.69	99.95 ± 0.77	99.97 ± 0.53	99.93 ± 0.59
Precision						
Intraday[a]	0.19	0.31	0.71	0.36	0.58	0.63
Interday[a]	0.23	0.25	1.01	0.82	0.2	0.38
Linearity						
Slope	246.92	1638.1	196.89	847.52	328.15	1050.2
SE of the slope	0.17889	6.81905	0.7363	1.5124	2.1107	3.2123
Intercept	4.1333	2.5333	0.9	0.1056	0.2222	0.0333
SE of the intercept	0.38849	2.11555	1.8275	2.4374	1.1339	1.5946
Correlation coefficient, r	1	0.9999	0.9999	0.9999	0.9999	0.9999
Range, μg/mL	0.35–3.5	0.05–0.5	0.4–4.0	0.1–1.0	0.3–3.0	0.08–0.80
LOD, μg/mL[b]	0.007	0.006	0.043	0.008	0.03	0.01
LOQ, μg/mL[b]	0.022	0.018	0.128	0.025	0.09	0.02
Specificity[c]	100.50 ± 0.67	100.45 ± 0.81	100.44 ± 0.88	99.81 ± 0.77	100.50 ± 0.67	100.77 ± 0.46

[a] $n = 3$.

[b] LOD and LOQ are calculated according to International Conference on Harmonization recommendations, 3.3 and 10 SD of the response/slope, respectively.

[c] Specificity was calculated from the analysis of laboratory-prepared mixtures.

Table 5. Statistical analysis of the results obtained by proposed method and the official method for the determination of CPD, CFX, and CPM in pure powder form

	CPD			CFX			CPM		
Parameter	By acidic degradation	By alkaline degradation	Official method[a]	By acidic degradation	By alkaline degradation	Official method[a]	By acidic degradation	By alkaline degradation	Official method[a]
Mean, %	99.97	99.87	100.22	100	99.95	99.74	99.97	99.93	99.9
SD	0.8	0.76	1.19	0.69	0.77	0.59	0.53	0.59	0.52
n	10	10	5	10	10	5	10	10	8
Variance	0.64	0.58	1.42	0.48	0.59	0.35	0.28	0.35	0.27
F-value[b]	2.21 (3.63)	2.45 (3.63)		1.37 (6.00)	1.70 (6.00)		1.04 (3.68)	1.29 (3.68)	
Student's *t*-test[b]	0.424 (2.160)	0.599 (2.160)		0.759 (2.160)	0.585 (2.160)		0.281 (2.120)	0.115 (2.120)	

[a] Official HPLC method (Ref. 9).

[b] The values between parenthesis are the corresponding theoretical values of *t* and *F* at $P = 0.05$.

further assessed by applying the standard addition technique as shown in Table 3.

Assay validation parameters are presented in Table 4. Statistical analysis of the results obtained for the analysis of CPD, CFX, and CPM in pure powder form by the suggested method were compared with those obtained by applying the official method (9), and there were no significant differences between the results with respect to accuracy and precision (Table 5).

Conclusions

The proposed difference spectrofluorometric method is selective, sensitive, accurate, and precise for stability-indicating studies and purity testing of CPD, CFX, and CPM in the presence of their acid and alkaline degradation products.

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