DEVELOPMENT AND VALIDATION OF A SPECTROFLUORIMETRIC METHOD FOR THE DETERMINATION OF CEFDINIR VIA ITS DEGRADATION PRODUCTS**

S. A. Boltia, S. E. Algmaal*, N. M. Mostafa, Y. S. El Saharty

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, ET-11562 Cairo, Egypt; e-mail: shrouk.elgmaal@pharma.cu.edu.eg

A sensitive and accurate stability-indicating spectrofluorimetric method was created and validated for the determination of Cefdinir (CEF), a third-generation cephalosporin drug, via its acid and alkali-induced degradation products. The drug was determined via its acid-induced degradation products at 408 nm using an excitation wavelength of 292 nm, and via its alkali-induced degradation products at 458 nm after excitation at 330 nm. Linearity was achieved in the ranges 0.7–7.0 and 0.03–0.30 μg/mL for the acid-induced and alkali-induced degradation products, respectively. The investigation of degradation pathways was achieved using IR and LC/MS/MS. The technique was also validated by the International Conference on Harmonization Guidelines. The proposed technique was effectively implemented with excellent accuracy and precision to determine CEF in bulk powder and pharmaceutical dosage forms. The final results obtained by the application of the spectrofluorimetric method excellently agreed with the reported HPLC method.

Keywords: *Cefdinir, stability indicating, spectrofluorimetric method, cephalosporins, degradation, validation.*

СПЕКТРОФЛУОРИМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ ЦЕФДИНИРА ПО ПРОДУКТАМ ЕГО РАЗЛОЖЕНИЯ

S. A. Boltia, S. E. Algmaal*, N. M. Mostafa, Y. S. El Saharty

УДК 543.42.062

Каирский университет, Каир, 11562, Египет; e-mail: shrouk.elgmaal@pharma.cu.edu.eg

(Поступила 3 января 2020)

Разработан и апробирован чувствительный и точный спектрофлуориметрический метод определения цефдинира (CEF) — цефалоспоринового препарата третьего поколения, основанный на обнаружении продуктов его разложения в реакциях с кислотой и щелочью. Лекарство определяли по продуктам его кислотно-индуцированной деградации при 408 нм и возбуждении на 292 нм и по его продуктам разложения, индуцированным щелочью, при 458 нм после возбуждения при 330 нм. Для продуктов деградации при воздействии кислоты и щелочи получена линейная зависимость в диапазонах 0.7–7.0 и 0.03–0.30 мкг/мл. Исследование путей разложения осуществлено с помощью ИК-спектроскопии и методики LC/MS/MS. Данный подход одобрен на Международной конференции по гармонизации, метод апробирован с высокой точностью определения CEF в нефасованных порошках и фармацевтических лекарственных формах. Результаты, полученные с применением спектрофлуориметрического метода, согласуются с полученными методом HPLC.

Ключевые слова: *цефдинир, индикатор стабильности, спектрофлуориметрический метод, цефалоспорины, деградация, валидация.*

** Full text is published in JAS V. 88, No. 2 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 88, No. 2 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

Introduction. Cephalosporins are the second after penicillin most significant β-lactam antibiotics to treat infectious illnesses. [1]. Cefdinir (CEF), chemically known as 7-(2 (2-aminothiazol-4-yl)-2hydroxyiminoacetamido)-3-vinyl-3-cephem-4-carboxylic acid, is an orally active, broad spectrum, third-generation cephalosporin used to treat infections of the respiratory tract, including pneumonia, sinusitis, and bronchitis.

Owing to the therapeutic importance of this drug, a number of techniques have been proposed for CEF determination in biological body fluids and pharmaceuticals, including spectrophotometric [2–7], chromatographic [8–14], electrochemical [15], and spectrofluorimetric methods [6, 16, 17]. However, these methods are time-consuming, tedious, and lacking selectivity.

Therefore, it is necessary to develop a simple, accurate, and rapid procedure that can be applied in QC laboratories for evaluating CEF in the presence of its degradation products in pure powder and pharmaceutical formulations.

Experimental. Cefdinir was kindly delivered by Sigma Pharmaceutical Industries, Cairo, Egypt. Its purity was determined by the reported HPLC method [9] and was found to be 99.49±1.21%.

Dinar® capsule (Batch No. 140641/1320081) was labelled to contain 300 mg of CEF/capsule (Adwia Pharmaceuticals company, Cairo, Egypt). Dinar® suspension (100 mL) (Batch No. 1303103/1320081) was labelled to contain 125 mg of CEF/5 mL (Adwia Pharmaceuticals company, Cairo, Egypt).

We used a Shimadzu RF-1501 spectrofluorimeter (No. 206-62901) with a quartz cell (1×1×4.5 cm) and a slit width of 2.5 nm (Tokyo, Japan). IR spectra were obtained using a Nicolet 6700 FT-IR Spectrometer, (Thermo Scientific, USA). Moreover, for our investigation we used a digital pH meter, Jenway, No. 924005-BO3-Q11C (Staffordshire, UK); a UPLC MS/MS ―Waters‖3100‖USA‖, TQ detector (Acquity ultra performance LC), a binary solvent manager pump, and an autosampler using Mass lynx V 4.1 software.

The analytical grade was used for all chemicals and reagents: methanol: E. Merck (Darmstadt, Germany); dimethyl formamide (DMF): Prolabo (West Chester, PA); sodium hydroxide: LOBA Chemie (India); ammonium hydroxide solution (30%); chloroform, acetonitrile, and hydrochloric acid, obtained from Sigma-Aldrich (Germany); purified water, obtained by a Milli-Q water purification system (USA).

Standard solutions. A stock standard solution of intact CEF (0.7 mg/mL) was used. It was prepared by dissolving 70 mg of pure CEF in 100 mL methanol. Working standard solutions of intact CEF (35 μg/mL) were prepared by diluting 5 mL of the standard stock solution of each drug to 100 mL with water. A stock standard solution of acid-induced degradation products was obtained from 0.7mg/mL CEF. It was prepared by refluxing 70 mg of pure CEF with 25 mL of 1 M HCl for 2 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. A working standard solution of acid-induced degradation products was obtained from 35.0 μg/mL CEF. It was obtained by diluting 5 mL of the stock standard solution of the CEF acid-induced degradation product to 100 mL with water. The stock standard solution of CEF was 0.03 mg/mL. It was prepared by dissolving 3 mg of pure CEF in 100 mL of methanol. The working standard solution of CEF was 1.5 μg/mL. It was obtained by transferring 5 mL of the standard stock solution of intact CEF (0.03 mg/mL) into a 100-mL volumetric flask, and the volume was completed to the mark with water. A stock standard solution of alkali-induced degradation products was obtained from 0.03 mg/mL CEF. It was prepared by refluxing 3 mg of CEF with 25 mL of 1 M NaOH for 1 h and then neutralizing it with 2 M HCl. 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 alkali-induced degradation products were obtained from 1.5 μg/mL CEF. They were obtained by diluting 5 mL of the stock standard solution of the CEF alkali-induced degradation product to 100 mL with water.

Structure elucidation of the degradation products. Complete degradation was checked by TLC silica gel using methanol–chloroform and ammonium hydroxide (8:2:0.2, v/v) as a mobile phase. The solution was neutralized and evaporated to dryness using a water bath. The degradation product was extracted using 30 mL of DMF to avoid the dissolution of NaCl. Furthermore, the DMF extract was evaporated again to dryness and extracted using 5 mL of methanol. The methanolic extract was evaporated at room temperature to give crystals of the degradation product. The isolated degradation product structure was elucidated using FT-IR spectrometry and the UPLC-MS/MS method.

Procedures. *Linearity. Acid-induced degradation.* Aliquots, equivalent to 17.5–175.0 μg of intact CEF (from working standard solutions of 35.0 μg/mL), were transferred accurately into a series of stoppered 20-mL test tubes; 10 mL of 1M HCl was added, and the tubes were put in an oven at 150°C for 2 h. The test tubes were cooled and then the solutions were neutralized with 2M NaOH. The solutions were quantitatively transferred into a series of 25-mL volumetric flasks, and the volume was completed to the mark with water.

New aliquots equivalent to 17.5–175.0 μg intact CEF were transferred accurately into a series of 25-mL volumetric flasks (from working standard solutions of 35.0 μg/mL), and the volume was completed to the mark with water (used as a blank). The fluorescence intensity of each concentration was recorded against its corresponding blank concentration at excitation and emission wavelengths of 292 and 408 nm, respectively, for the acid-induced degradation products of CEF.

Alkali-induced degradation. Aliquots, equivalent to 0.75–7.50 μg of intact CEF, were transferred accurately (from working standard solutions of 1.50 μg/mL) into a series of stoppered 20-mL test tubes; 10 mL of 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 then the solutions were 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. New aliquots, equivalent to 0.75–7.5 μg of intact CEF, were transferred accurately (from working standard solutions of 1.5 μg/mL) 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 against its corresponding blank concentration at excitation and emission wavelengths of 330 and 458 nm, respectively, for the alkali-induced degradation products of CEF.

Calibration curves relating the fluorescence intensity of the acid- and the alkali-induced degradation products of CEF at emission wavelengths of 408 and 458 nm, respectively, to the corresponding concentrations of CEF were constructed, and the regression equations were computed.

Analysis of the laboratory prepared mixtures. In case of the acid-induced degradation into two series of stoppered 20-mL test tubes, aliquots from 4.5 to 0.5 mL were separately transferred from the intact CEF working standard solution (35.0 μg/mL) to the previous solutions; aliquots of 0.5 to 4.5 mL of the acid-induced degradation product of the CEF working standard solution (35.0 μg/mL) were added separately. To each test tube, 10 mL of 1 M HCl was added, and the fluorescence intensity of each mixture was recorded against its corresponding blank concentration at excitation and emission wavelengths of 292 and 408 nm, respectively.

In case of the alkali-induced degradation into two series of 20-mL stoppered test tubes, aliquots from 4.5 to 0.5 mL were separately transferred from the intact CEF working standard solution (1.5 μg/mL) to the previous solutions; aliquots of 0.5 to 4.5 mL of the alkali-induced degradation product of the CEF working standard solution (1.5 μg/mL) were added separately. To each test tube, 10 mL of 1 M NaOH was added, then the fluorescence intensity of each mixture was recorded against its corresponding blank concentration at excitation and emission wavelengths of 330 and 458 nm, respectivelly.

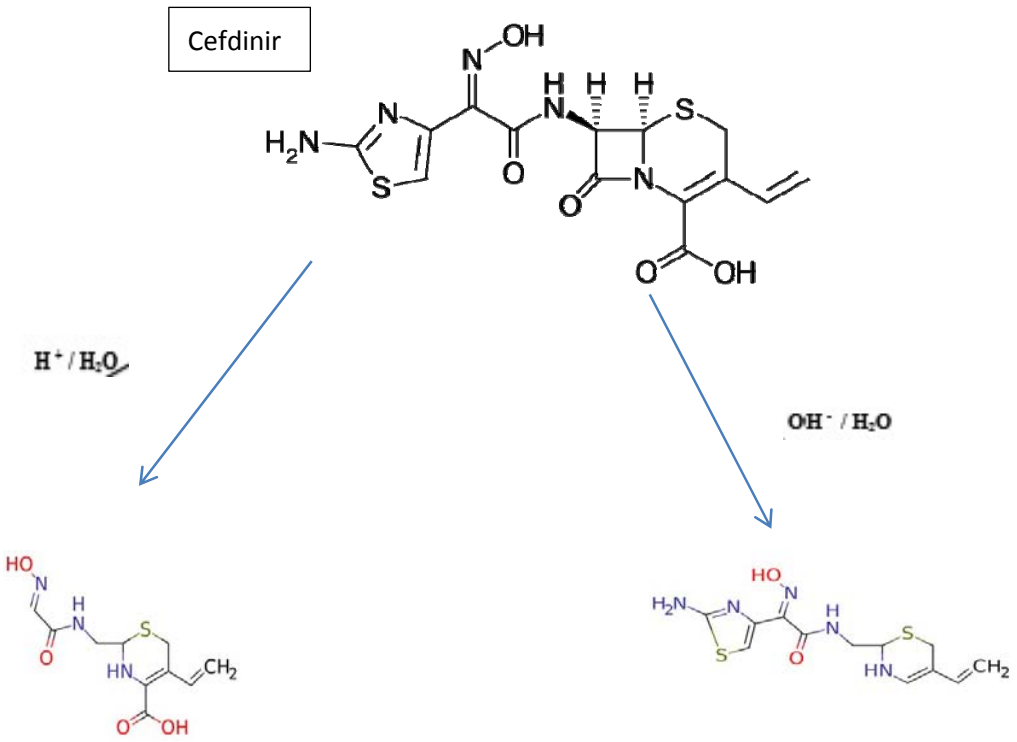
Analysis of CEF in pharmaceutical dosage forms. *Analysis of capsules. For* the *acid-induced degradation.* A total of 10 capsules was weighed and finely powdered. An amount equivalent to 40 mg was weighed, transferred into a 100-mL volumetric flask, and stirred with 20 mL of methanol, and the volume was completed with methanol. The solution was filtered, and further dilution was made using water to obtain a concentration of 35.0 μg/mL; 2 mL of this solution was transferred to a 20-mL test tube, and 10 mL of 1 M HCl was added. The concentration of CEF was calculated from the corresponding regression equation.

For the alkali-induced degradation. The procedures were performed as mentioned above for the acid-induced degradation for CEF tablets until the solution was filtered, and further dilution was made using water to obtain a concentration of 1.5 μg/mL; 2 mL of this solution was transferred to a 20-mL test tube, and 10 mL of 1 M NaOH was added. The concentration of CEF was calculated from the corresponding regression equation.

Analysis of suspension. *For the acid-induced degradation.* The powder contents of two bottles were extracted using 50 mL of methanol and then filtered into a 100-mL volumetric flask. The residue was washed several times with methanol, and the volume was completed with methanol. Further dilution was made using water to obtain a concentration of 35.0 μg/mL; 2 mL of this solution was transferred to a 20-mL test tube, and 10 mL of 1 M HCl was added. The concentration of CEF was calculated from the corresponding regression equation.

For the alkali-induced degradation. The procedures were performed as mentioned above for the acid-induced degradation of the CEF suspension until the solution was filtered, and further dilution was made using water to obtain a concentration of 1.5 μg/mL; 2 mL of this solution was transferred to a 20-mL test tube, and 10 mL of 1 M NaOH was added. The concentration of CEF was calculated from the corresponding regression equation.

Results and discussion. To study the degradation behavior of CEF and to determine the specificity of the proposed spectrofluorimetric method, CEF was subjected to different stress circumstances of hydrolysis. The complete degradation of CEF was achieved after 2 and 1 h at 150°C for the acid and for the alkali-induced degradation processes, respectively. The suggested acid and alkali-induced degradation pathways of CEF.



Acid-induced degradation product of CEF Alkali-induced degradation product of CEF

The acid and alkali-induced degradation products were separated, and their structures were confirmed by IR and MS/MS spectrometry (Figs. 1, 2). The IR spectra of intact CEF show that the characteristic bands at 1768.8 cm^{-1} originating from the lactam carbonyl group are not observed in the IR spectra of both acid and alkali-induced degradation products, indicating the opening of the β-lactam ring upon degradation, which increases the fluorescence intensities of these compounds. For the acid-induced degradation product, acidic OH is still present at 3358–2900 cm^{-1} and acidic carbonyl at 1766.8 cm^{-1}, but both disappear in the alkali-induced degradation product, which indicates decarboxylation of the two carboxylic groups in the alkali-induced degradation product. The mass spectra of the acid-induced degradation product show a peak at 271 *m*/*z*, while the alkali-induced degradation product shows a peak at 325 *m*/*z*.

The fluorescence spectra of CEF compared to their acid or alkali-induced degradation products show that the intact drug has very weak native spectrofluorimetric characteristics, but after acid or alkali-induced degradation, strong fluorescent species are produced. The proposed method depends on measuring the difference in fluorescence intensities between the acid or alkali-induced degradation product and intact drug; hence, any amount of degradation found in the drug sample in both experiment and blank samples will be cancelled, and the fluorescence intensities before and after hydrolysis will correspond only to the intact drug, so the method is considered as stability-indicating. Different wavelengths were tried as the excitation wavelength (including these of Lambda max of each compound) either for cefdinir and its acid or alkali-induced degradation products. The best results were obtained using 292 and 330 nm as excitation wavelengths for the acid and alkali-induced degradation products, respectively, in terms of sensitivity, accuracy, and specificity.

Emission spectra were observed at 408 and 458 nm for the acid and alkali-induced degradation products, respectively (Fig. 3).

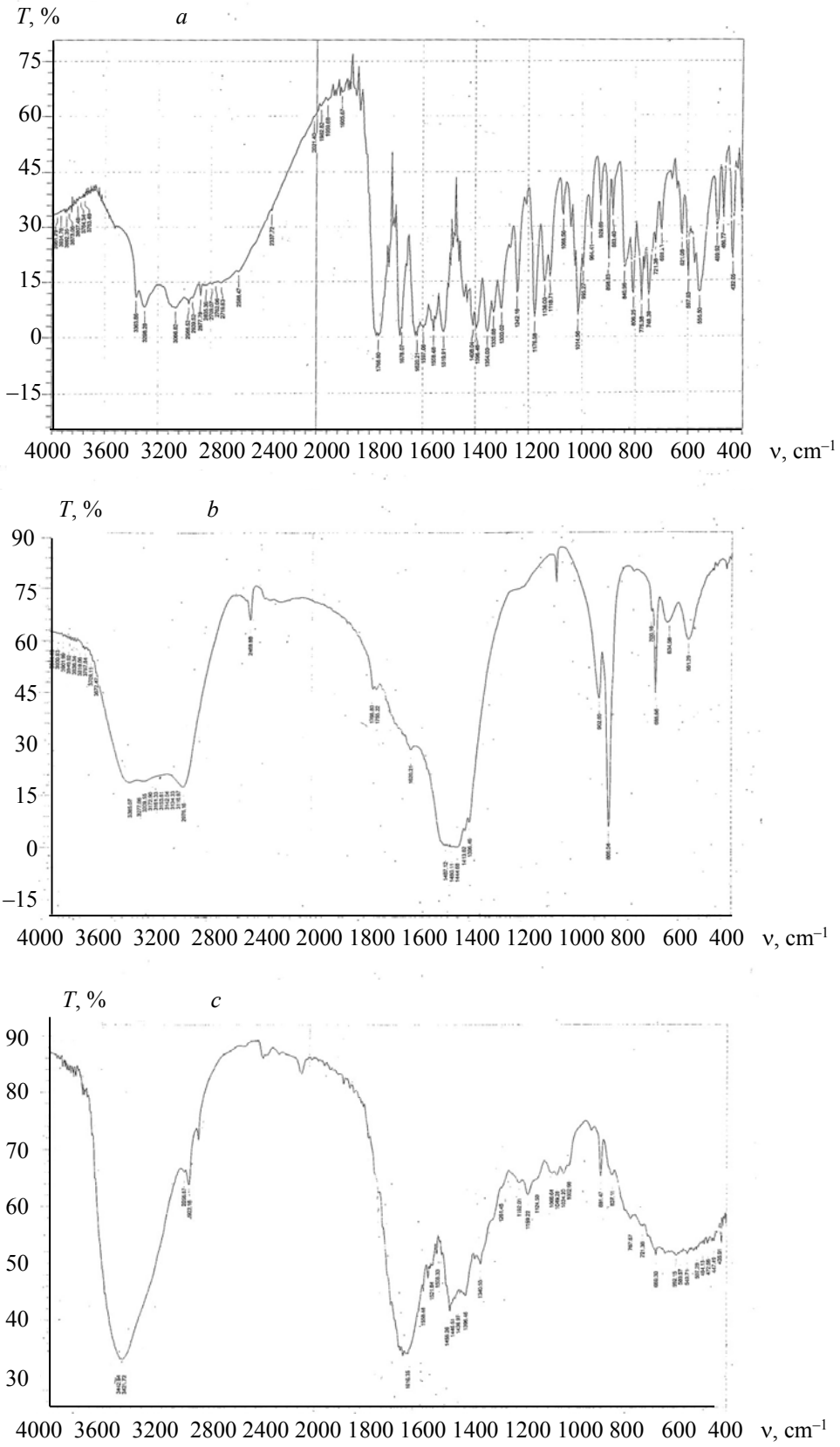


Fig. 1. IR spectrum of a) intact CEF, b) acid-induced degradation product of CEF, and c) alkali-induced degradation product of CEF.

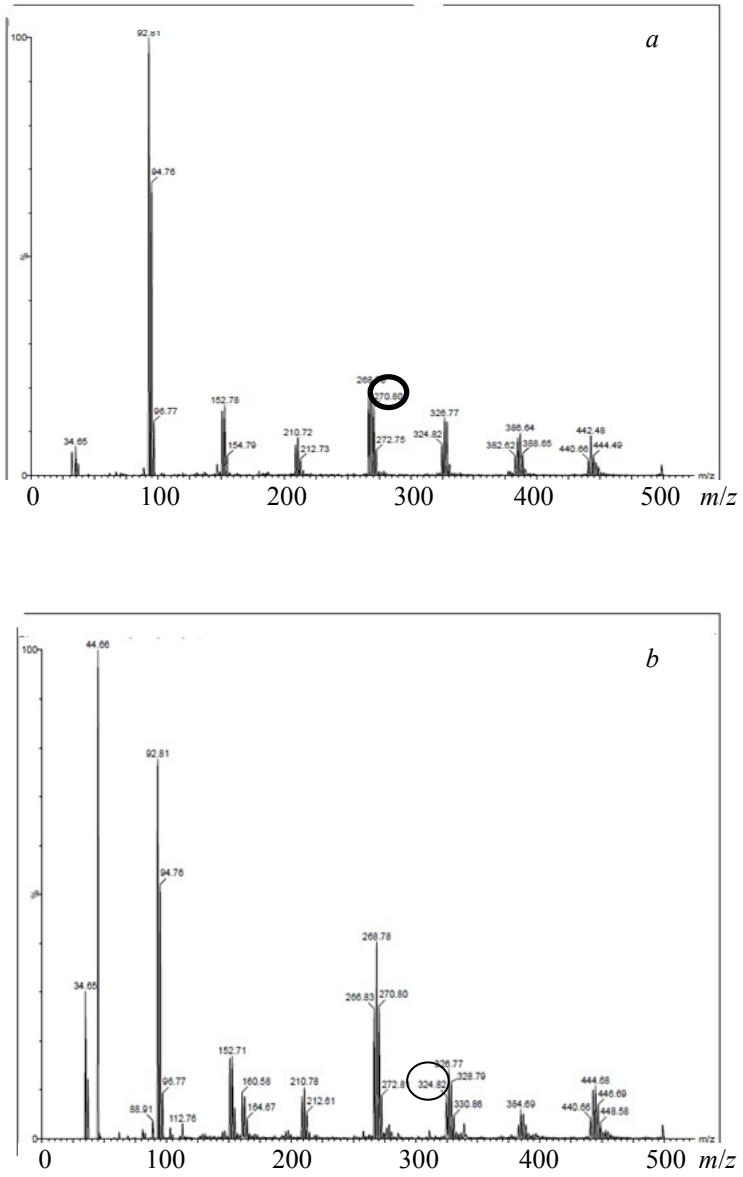


Fig. 2. Mass spectrum of a) acid degradation product of CEF and b) alkali degradation product of CEF.

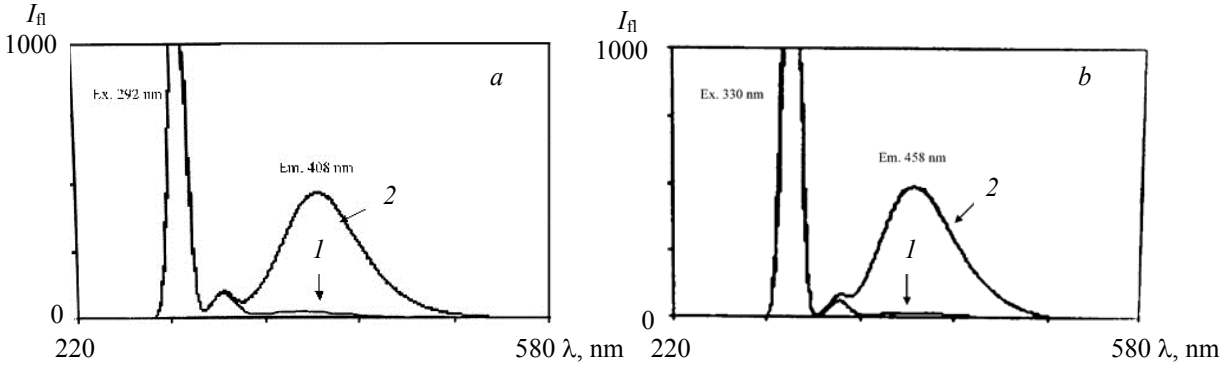


Fig. 3. Excitation and emission spectra of a) intact CEF (1) and its acid-induced degradation product (2), 4.2 μg /mL of each; b) intact CEF (1) and its alkali-induced degradation product (2), 0.24 μg/mL of each.

TABLE 1. Determination of CEF in Laboratory-Prepared Mixtures by the Proposed Spectrofluorimetric Method

Degradation product, %	Concentration		Recovery, %	Concentration		Recovery, %
	of intact drug added, µg/mL	of intact drug, found, µg/mL		of intact drug added, µg/mL	of intact drug, found, µg/mL	
	Via acid degradation			Via alkali degradation		
10	6.30	6.319	100.32	0.27	0.270	100.17
20	5.60	5.654	100.89	0.24	0.241	100.42
30	4.90	4.917	100.41	0.21	0.212	101.04
40	4.20	4.245	100.95	0.18	0.182	101.49
50	3.50	3.516	100.57	0.15	0.149	99.47
60	2.80	2.779	99.29	0.12	0.119	99.38
70	2.10	2.136	101.90	0.09	0.089	98.80
80	1.40	1.403	100.00	0.05	0.058	97.31
90	0.70	0.690	98.57	0.03	0.031	103.31
Mean			100.32			100.15
S.D.			0.91			1.65
R.S.D. %			0.91			1.65

TABLE 2. Determination of CEF in Pharmaceutical Dosage Forms by the Proposed Spectrofluorimetric Method

Pharmaceutical		Via acid degradation		Via alkali degradation	
Preparations	Claimed, mg/tab	Found, mg/tab	Recovery% ± SD*	Found, mg/tab	Recovery% ± SD*
Dinar® capsule	300	304.52	101.51 ± 0.28	294.45	98.15± 1.24
B.N.: 140641/1320081	mg/5 mL	mg/5 mL		mg/5 mL	
Dinar® suspension B.N: 1303103/1320081	125	126.49	101.19 ± 0.27	124.27	99.41 ± 0.40

*Average of three different determinations.

By applying the suggested procedure, a linear correlation was obtained between the difference in fluorescence intensities at 408 and 458 nm before and after complete acid and alkali-induced degradation, respectively, and the corresponding concentration of pure CEF over the ranges 0.7–7.0 and 0.03–0.30 µg/mL for the acid and alkali induced degradation products, respectively. Analysis of the laboratory-prepared mixtures indicated the specificity of the suggested operation. Several mixtures of CEF with its acid and alkali-induced degradation products were prepared and analyzed by the proposed method, and the results proved that the method is highly specific for the stability testing of the studied drugs as it can determine CEF in the presence of up to 90% of its acid and alkali-induced degradation products, as shown in Table 1. The proposed procedure was successfully applied for the determination of CEF in Dinar® tablets and Dinar® suspension, with no interference from the excipients, as shown in Table 2. To assure the accuracy of our proposed method, the standard addition technique was used as another mean of verification, which revealed acceptable results, as shown in Table 3.

The results obtained show the high specificity of the proposed method in determining the drug only without the interference of any other absorbing species found in the same medium. On the other hand, the accuracy of this method was also examined by application of the proposed method on different CEF standard solutions with different concentrations other than used in the calibration. The results obtained, expressed as recovery ±SD, were acceptable, as shown in Table 4.

TABLE 3. Application of the Standard Addition Technique for the Determination of CEF in Pharmaceutical Dosage Forms by the Proposed Spectrofluorimetric Method

Pharmaceutical preparations		By acidic degradation				By alkali degradation		
	Taken, µg/mL	Added, µg/mL	Found*, µg/mL	Recovery, %	Taken, µg/mL	Added, µg/mL	Found*, µg/mL	Recovery, %
Dinar capsule® B.N. 140641/1430201	2.80	2.10 3.50 4.20	2.055 3.534 4.129	97.84 100.97 98.32	0.12	0.09 0.15 0.18	0.090 0.153 0.181	100.17 102.12 100.44
Mean				99.64				100.01
S.D.				1.32				0.86
RSD, %				1.33				0.85
Dinar® Suspension B.N. 1303103/1320081	2.80	2.10 3.50 4.20	2.071 3.503 4.088	98.60 100.08 97.33	0.12	0.09 0.15 0.18	0.089 0.152 0.183	98.87 101.03 101.73
Mean				98.70				100.54
S.D.				1.37				1.22
RSD, %				1.39				1.21

* Average of three different determinations.

TABLE 4. Assay Validation Parameters Obtained by Applying the Proposed Fluorimetric Method

Parameter	Spectrofluorimetric Method	
	Via acidic degradation	Via alkali degradation
Accuracy (Mean ± R.S.D.)	99.93±0.19	99.63±0.33
Precision: Repeatability[a] Intermediate precision[b]	 100.05±0.95 100.30±1.72	 99.91±0.12 99.87±0.22
Linearity: Slope	106.14	2635.8
SE of the Slope	0.84	4.17
Intercept SE of the Intercept	31.484 3.69	1.7124 0.84
Correlation coefficient (*r*)	0.9998	1
Range, µg/mL	0.7–7	0.03–0.3
LOD [c], µg/mL	0.083	0.004
LOQ [c], µg/mL	0.25	0.011
Specificity [d]	100.32±0.91	100.15±1.62

[a] $n = 3$ (for acid 2.8, 4.9, 5.6 µg/mL and for alkali 0.15, 0.18, 0.3 µg/mL);
[b] $n = 3$ (for acid 0.7, 1.4, 2.1 µg/mL and for alkali 0.09, 0.24, 0.27 µg/mL);
[c] LOD and LOQ are calculated according to ICH, 3.3×SD of the response/slope and 10×SD of the response/slope, respectively;
[d] Specificity was calculated from the analysis of the laboratory-prepared mixtures.

Linear relationships were obtained between the fluorescence intensities and the concentration in the ranges 0.7–7.0 and 0.03–0.30 µg/mL for the acid and alkali-induced degradation products, respectively, as shown in Table 4. The results obtained for the analysis of CEF in the pure powder form by the suggested method were compared with those obtained by applying the reported method [9], and there was no significant difference between the results related to accuracy and precision, as shown in Table 5.

TABLE 5. Statistical Analysis of the Results Obtained by the Proposed Method and the Reported Method for the Determination of CEF in the Pure Powder Form

Parameter	Via acidic degradation	Via alkali degradation	Reported method [9][a]
Mean	100.24	100.18	99.49
S.D.	1.35	0.72	1.21
n	7	7	5
Variance	1.82	0.51	1.46
F value[b]	1.25 (6.16)	2.86 (4.53)	–
Student's *t*-test[b]	0.90 (2.23)	1.12 (2.23)	–

[a] The reported HPLC method RP-C18 column (250×4.6 mm, 5 μm particle size), with mobile phase consists of [water (pH adjusted to 3.0 by orthophosphoric acid):acetonitrile:methanol 13:5:2 v/v].
[b] The values between parethesis are the corresponding theoretical values of *t* and *F* at $P = 0.05$.

Conclusions. The proposed difference spectrofluorimetric method is specific, sensitive, accurate, and precise for stability-indicating studies and purity testing of CEF in the presence of their acid and alkali-induced degradation products.

REFERENCES

1. D. R. Guay, *Clin. Terap*., **24**, No. 4, 473–489 (2002).
2. O. Abdel-Aziz, M. Farouk, R. Nagi, L. Abdel-Fattah, *J. Appl. Pharm. Sci*., **4**, No. 7, 129 (2014).
3. Erhan Bas, S. Özdemir, M. G. Çağlayan, İ. Murat, F. O. Palabiyik, *Turk. J. Pharm. Sci*., **10**, No. 3, 321–328 (2013).
4. M. El-Ashry, *Am. J. Pharm. Res*., **4**, No. 4, 239–258 (2014).
5. A. A. Elbashir, A. A. Ahmed, S. M. Ali Ahmed, H. Y. Aboul-Enein, *Appl. Spectrosc. Rev*., **47**, No. 3, 219–232 (2012).
6. F. Ibrahim, M. Wahba, G. Magdy, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **188**, 525–536 (2018).
7. B. K. Singh, D. V. Parwate, S. Srivastava, S. Shukla, *Quím. Nova*, **33**, No. 7, 1471–1475 (2010).
8. Z.-j. Chen, J. Zhang, J.-C. Yu, G.-Y. Cao, X.-J. Wu, Y.-G. Shi, *J. Chromatogr. B*, **834**, No. 1-2, 163–169 (2006)
9. P. Hamrapurkar, P. Patil, M. Phale, M. Gandhi, S. Pawar, *Pharm. Methods*, **2**, No. 1, 15–20 (2011).
10. H. Hashem, A. A. Gouda, W. Hassan, *J. Liq. Chromatogr. Relat. Technol.*, **35**, No. 12, 1638–1648 (2012).
11. S. R. Narala, K. Saraswathi, *J. Pharm. Sci. Res*., **3**, No. 1, 1002 (2011).
12. Y. Okamoto, K. Itoh, Y. Namiki, J. Matsushita, M. Fujioka, T. Yasuda, *J. Pharm. Biomed. Anal*., **14**, No. 6, 739–748 (1996).
13. G. M. Hadad, S. Emara, W. M. Mahmoud, *Chromatographia*, **70**, No. 11-12, 1593 (2009).
14. U. Mashelkar, S. D. Renapurkar, *Int. J. Chem. Technol. Res*., **2**, 114–121 (2010).
15. R. Jain, K. Radhapyari, N. Jadon, *J. Electrochem. Soc*., **154**, No. 11, 199–204 (2007)
16. A. Abdollahi, A. B. Tabrizi, *Pharm. Sci*., **22**, No. 1, 28 (2016).
17. A. Suganthi, S. Shrikumar, M. B. Pattesseril, M. Umamaheswari, T. Ravi, *Indian J. Pharm. Sci*., **66**, No. 5, 689 (2004).