



# Stability Indicating RP-HPLC and Spectrophotometric Methods for Simultaneous Estimation of Sodium Benzoate and Cefdinir in the Presence of its Degradation Products-Application to Blank Subtraction Method

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## ABSTRACT

**Objectives:** Empower 3 software is important in modeling, optimization, and reducing the time of manual calculation of related substance by subtracting the baseline of a blank chromatogram from the unknown sample automatically; so, the major objective of the developed method is to introduce a new, selective, and economical high performance liquid chromatography (HPLC) and spectrophotometric method for simultaneous estimation of sodium benzoate (SDB) and cefdinir (CFR) in the presence of its degradation products.

**Materials and Methods:** Chromatographic separation is optimized and adjusted using two methods; method (I) is characterized for separation of active pharmaceutical ingredient (CFR) in pure and dosage forms using Atlantis dC18 column [4.6 mm x 250 mm (5  $\mu$ m particle size or equivalent)] with a mobile phase consisting of methanol: 0.02 M phosphate buffer solution pH 3.0 (40:60 v/v) at a flow rate of 1.0 mL/minute, injection volume 10  $\mu$ L and wavelength 254 nm. Method (II) is identified for related substances in a Hichrom C18 column (15 x 0.46 cm), 5  $\mu$ m particle size or equivalent, using a binary gradient consisting of solution A [0.1% tetramethylammonium hydroxide solution (pH: 5.5) with 0.1 M EDTA (1000:0.4 v/v)] and solution B (0.1% tetramethylammonium hydroxide solution (pH 5.5): acetonitrile: methanol : 0.1 M EDTA (500:300:200:0.4 v/v) using injection volume 10  $\mu$ L for reversed-phase HPLC with a wavelength equals to 254 nm and flow rate 1.0 mL/min. Two ecofriendly spectrophotometric methods were successfully used to resolve the spectral overlap of drugs.

**Results:** Method A, the first derivative of ratio spectra spectrophotometric method (1<sup>st</sup>DD) where CFR was determined at two wavelengths 283.5 nm, 313.4 nm and SDB was determined at 216.7 nm, 235.5 nm. Method B, ratio subtraction method is performed to overcome the interference between CFR and the preservative SDB. The ultraviolet spectrum of the laboratory mixture is divided by that of CFR (20  $\mu$ g/mL) as a divisor then subtracting the amplitudes in the plateau region at 250-315 nm (the constant) from that of the ratio spectrum. The zero-order spectra of SDB were obtained at 225 nm by multiplying the resulting ratio spectra by the divisor (CFR), zero order of CFR was been estimated at a wavelength value of 283 nm after multiplication of the divisor by the obtained constant.

**Conclusion:** The optimized method was adjusted and validated as *per* International Conference on Harmonization guidelines and could be easily utilized by quality control laboratories and for laboratory-prepared mixtures.

**Key words:** Empower 3 software, RP-HPLC-UV, spectrophotometric methods, blank subtraction method, cefdinir, sodium benzoate

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## INTRODUCTION

The application of blank subtraction method in related substances and degradation products has been widely applied in the quality control lab in the pharmaceutical industries to optimize, achievement and decrease number of experimental trials of a chromatographic system for manual calculations by using the empowered software. Empower 3 software photodiode array (PDA) detector can subtract mobile phase effects of a standard or sample. The possibility of blank subtraction is useful when the application is affected by gradient runs, in which least a solvent contains a ultraviolet (UV)-absorbing compound, system peaks and contaminants in the mobile phase. The blank subtraction removes the chromatographic tool from the dataset, resulting in a 3D chromatographic scheme corrected to the baseline. In fact, this 3D chromatogram is the difference between the blank and the standard or the sample at the specific wavelengths. Blank baseline subtraction procedure improves the chromatogram scheme in these ways; baseline closer to 0 absorbance units means, no additional peaks, less drift and peaks are easier to integrate.<sup>1</sup>

Cefdinir (CFR) in omnicef capsules and powder for granule suspension is referred to for the treating of patients with minor to major infections resulting from micro-sensitive strains. CFR is an extended group of semi-synthetic cephalosporins. CFR compound A is a combination of four isomers called CFR open ring lactones a, b, c, and d. Its molecular formula is  $C_{14}H_{15}N_5O_6S_2$  and its molecular weight is 413.43. The molecular formula for CFR-related compound B is  $C_{14}H_{14}N_4O_4S_2$  and its molecular weight is 366.41 (Figure 1a-m) for CFR and their related substances.<sup>2</sup> Sodium benzoate (SDB) is chemically known as sodium benzenecarboxylate ( $C_7H_5NaO_2$ ), (Figure 1n). The ingredient is used as an excipient; treatment of hyperammonemia due to urea cycle disorders and treatment of non-ketotic hyperglycinemia.<sup>3</sup>

CFR and SDB are formally announced in the European and British Pharmacopeias that illustrated chromatographic method for CFR and a titration one for SDB,<sup>3</sup> while United States Pharmacopeia (USP) prescribed a chromatographic method for each.<sup>4</sup> Some new articles were published for the determination of CFR and its impurities using liquid chromatography-tandem mass spectrometry methods,<sup>5-9</sup> high performance liquid chromatography (HPLC) method.<sup>10,11</sup> Only one HPLC and ultra-high performance liquid chromatography (UPLC) method has been reported for simultaneous quantitation of CFR and SDB in their dosage forms, but this method is not indicated for the determination of impurities and there is no spectrophotometric method for simultaneous optimization of the laboratory mixture of CFR and SDB in their dosage forms,<sup>12</sup> thin layer chromatography,<sup>13,14</sup> and spectrophotometric.<sup>15-21</sup>

The novelty of the proposed method is lying in its ability to detect, identify, and separate all related substances to CFR while the previous published methods cannot separate most related substances in CFR. Besides, it overcomes the overlapping of the binary mixture by using a spectrophotometric method without the need of sophisticated

application. A unique advantage of the proposed method is the reduction of waste time in manual calculation of impurities through the application of the blank subtraction method, in which Empower PDA software can subtract the effects of the mobile phase on a standard or sample and peaks will be easier to integrate. Therefore, the main objective of this method is to identify and separate CFR and SDB in the presence of CFR's degradation products using blank subtraction method and solve the interference of the binary mixture using a simple spectrophotometric method.

## MATERIALS AND METHODS

### *Experimental and reagents*

CFR and SDB were provided by Hikma Pharmaceutical Industries which is located in Beni-Suef governorate, Egypt. Reference standards of related CFR (compound A and B) were purchased from USP store (USA, Rockville, MD). All HPLC- and analytical grades were purchased from (Fisher Scientific, USA).

### *Instrumentation and data processing*

Waters UHPLC system (Waters Corporation, USA), equipped with an LC quaternary pump with PDA detector, autosampler and quaternary solvent management, has the potential for multiple uses and flexibility to move from HPLC and UPLC and is provided with Empower™ 3 Software for processing methods. UV 1900 (Shimadzu-Japan) provided with UV probe (2.7.1) software for processing data.

### *Chromatographic method*

Chromatographic separation of CFR and its preservative SDB was accomplished using an Atlantis dC18 column [4.6 mm x 250 mm (5  $\mu$ m particle size or equivalent)] with a mobile phase consisting of methanol: 0.02 M phosphate buffer solution pH 3.0 (40:60 v/v) at a flow rate of 1.0 mL/minute, injection volume 10  $\mu$ L and wavelength 254 nm. Also, a binary gradient consisting of solution A [0.1% tetramethyl ammonium hydroxide solution (pH 5.5) with 0.1M EDTA (1000:0.4 v/v)] and solution B [0.1% tetramethyl ammonium hydroxide solution (pH 5.5): acetonitrile: methanol: 0.1M EDTA (500:300:200:0.4 v/v) with Hichrom C18 column (15 x 0.46 cm, 5  $\mu$ m or equivalent, 150 Å pore size) at flow rate 1.0 mL/min, using injection volume 10  $\mu$ L for reversed-phase-HPLC and wavelength at 254 nm with auto sampler temperature 4.0°C, column temperature 40.0°C and gradient program following the scheme: (i) 0-8 min: 95% (A), 5% (B) isocratic; (ii) 8-28 min: 75% (A), 25% (B); (iii) 28-43 min: 50% (A), 50% (B)] and (iv) 43-64 min: 95% (A), 5% (B) for separation of related substances, no statistical analysis data was used in the chromatographic method.

### *Solution preparations*

#### *A) For analysis of laboratory-prepared mixture*

Solvent preparation: methanol: 0.02 M phosphate buffer solution pH 3.0 (50:50 v/v).

CFR solution: Accurately weighed about 10 mg CFR working standard, transferred to a 100 mL volumetric flask, added some

solvent, dissolved by sonication, allowed to cool and made up to the mark with solvent.

Sodium benzoate solution: Accurately weigh about 10 mg SDB, transfer to 100 mL volumetric flask and proceed similarly.

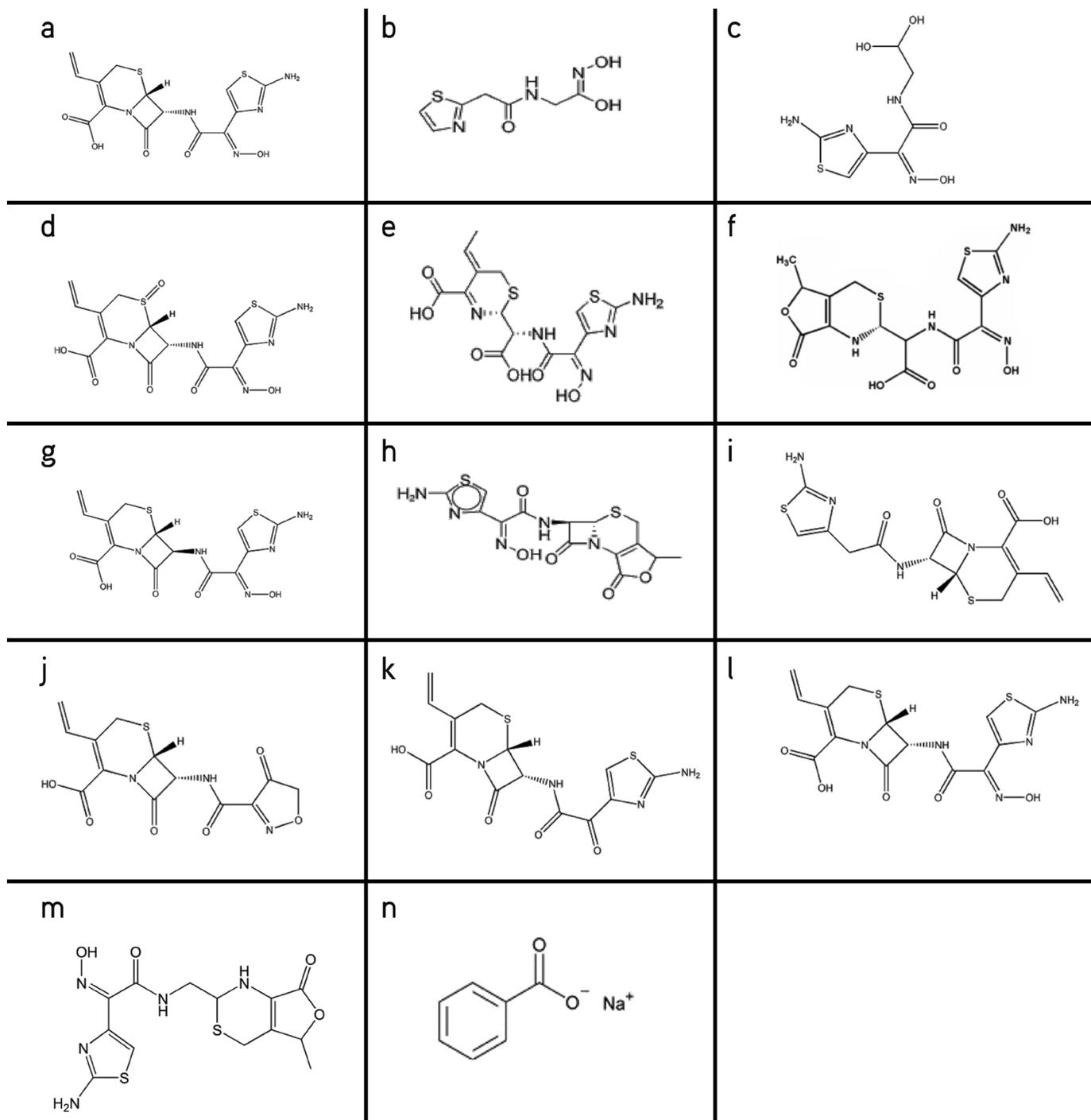
Resolution solution: 10.0 mL of both stock solutions of the above prepared drugs were pipetted into 100 mL volumetric flask, completed to the mark with solvent and the chromatogram is drawn as displayed in Figure 2a.

### B) For related substances

Solution (1): Weigh about 14.2 g of anhydrous dibasic sodium phosphate transfer it to 1000 mL flask and dissolve in deionized water.

Solution (2): Weigh about 27.2 g of monobasic potassium phosphate and dissolve in 2.0 L of deionized water.

Buffer preparation: Combine the suitable quantities of solutions 1 and 2 (about 2:1) to get a solution having the pH value of  $7.0 \pm 0.1$ .



**Figure 1.** Chemical structures of (a-m) CFR and their related substances, and (n) SDB  
CFR: Cefdinir, SDB: Sodium benzoate

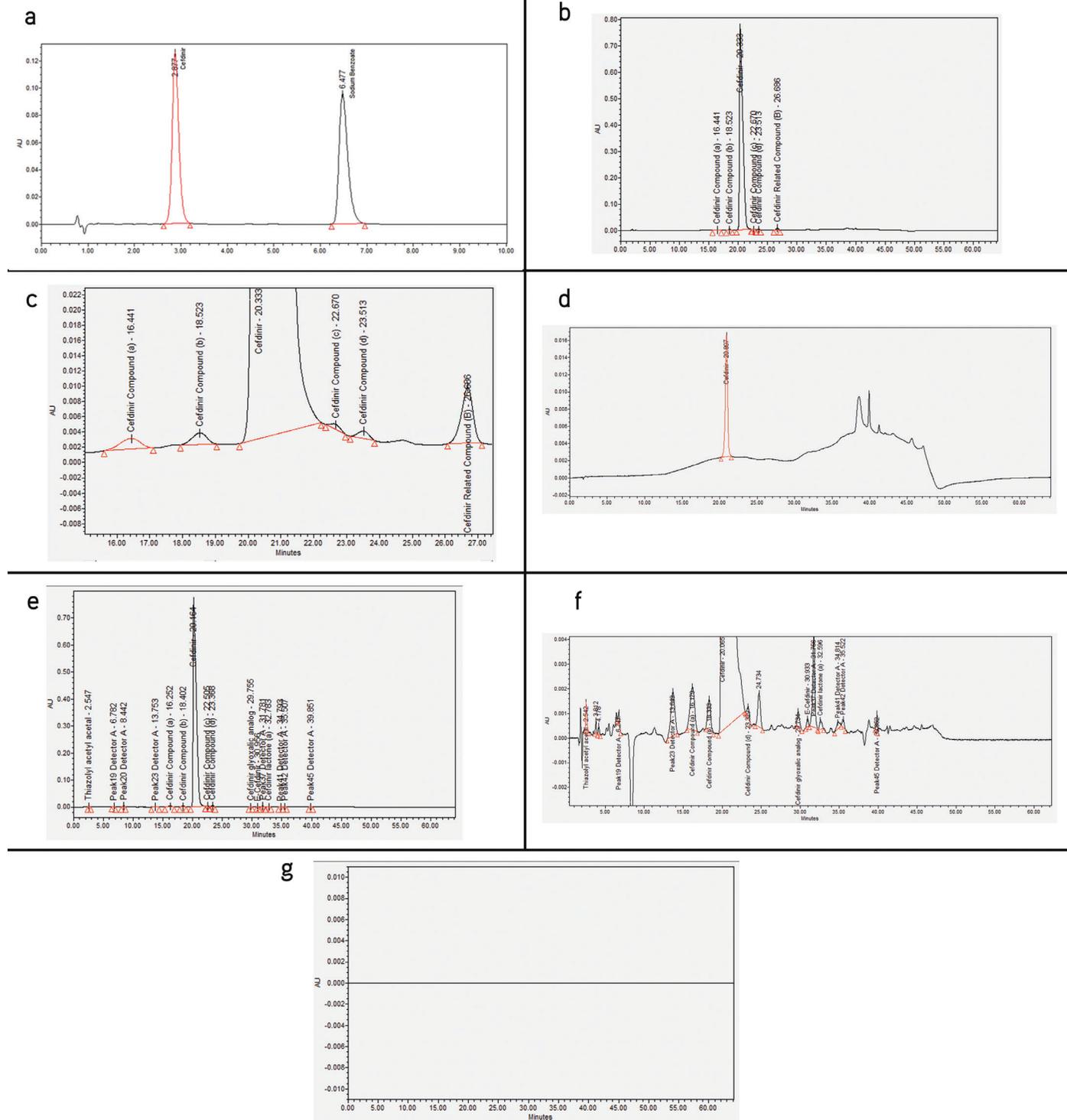
Solvent preparation: Dilute 8 mL of tetramethylammonium hydroxide (25% in water) to 2000 mL with deionized water; adjust pH to  $5.5 \pm 0.1$  using diluted appropriate acid.

#### Resolution solution preparation

Stock solution (A): Weigh about 10.0 mg of USP related CFR compound A transfer it quantitatively to a 250 mL volumetric

flask using an appropriate volume of solvent, sonicate to complete dissolution, then complete to volume with a solvent to get a concentration of 0.04 mg/mL.

Stock solution (B): Weigh about 10.0 mg of USP related CFR compound B and proceed similarly to stock solution (B) to get a concentration of 0.04 mg/mL.



**Figure 2.** HPLC chromatograms of (a) 10  $\mu\text{g/mL}$  of laboratory prepared mixture of CFR and SDB, (b, c) 1500  $\mu\text{g/mL}$  of system suitability solution of [CFR and related compound (A, B)], (d) 15  $\mu\text{g/mL}$  of standard solution of (CFR), (e, f) sample solution of omnicef, and (g) blank subtraction

CFR: Cefdinir, SDB: Sodium benzoate, HPLC: High performance liquid chromatography

Working resolution solution: Weigh about 37.5 mg of CFR convey quantitatively to 25 mL volumetric flask using 10 mL buffer. Sonicate until dissolution, complete to volume using buffer. Posteriorly, add 5.0 mL from each solution of related compounds (A and B) and makes up for the marks with solvent. Mix well and filter through 0.45  $\mu\text{m}$  nylon membrane, then inject in the HPLC. The HPLC chromatogram is presented in Figure 2b, c.

CFR standard solution preparation: Weigh about 37.5 mg of CFR in 50 mL volumetric flask and complete to volume with buffer. Of the resulting solution, an aliquot of 2 mL was diluted to 100 mL with solvent, mix, and filter through the nylon membrane, then inject into the HPLC. The HPLC chromatogram is shown in Figure 2d.

Sample solution preparation: Constitute omnicef as directed in the product label and leaflet. Transfer accurately about the equivalent to 150 mg CFR in 100 mL volumetric flask using 30 mL buffer and completely to the mark with solvent. Similarly, mix, filter, and inject Figure 2e, f.

#### Calculations

Identify any peak for solvent and placebo by the processing method to be excluded.

Consider any peak that present in the chromatogram other than solvent, placebo, known impurities, and CFR as unknown impurities.

The percentage of each impurity in CFR portion of the oral suspension powder can be calculated by the equation given below:

$$\text{Result} = (\text{Pt}/\text{PS}) \times (\text{Ds}/\text{Dt}) \times (100/\text{F}) \text{ where}$$

Pt= Peak area of individual impurity in the test solution

PS= Peak area of CFR standard solution

Ds= Actual concentration of CFR (mg/mL)

Dt= Nominal concentration of CFR in the test solution (mg/mL)

F= FRR (factor of relative response)

#### C) For UV spectroscopic methods

Solvent: Use methanol HPLC grade.

Standard solutions of CFR and SDB: Precisely weigh 10 mg of each CFR and SDB in two 100 mL volumetric flasks, add 70 mL of the solvent and sonicate to dissolve and complete to the mark with the same solvent and mix well.

Laboratory prepared mixture: Prepare mixtures of CFR and SDB containing different ratios from their working standard solutions (100  $\mu\text{g}/\text{mL}$ ) into a series of 10 mL volumetric flasks.

#### Procedures

##### Derivative ratio method (DD<sup>1</sup>)

The absorption spectra of CFR were divided by a divisor of SDB (5  $\mu\text{g}/\text{mL}$ ), and the first derivative values caused by ratio spectra were registered. The linear curves for maximum and minimum amplitudes at 283.5 nm and 313.4 nm are established versus the congruous concentrations of CFR to calculate the

regression equation. Alternatively, the scanned absorption spectra of SDB were divided by a CFR divisor (10  $\mu\text{g}/\text{mL}$ ) and the first derivative (D<sup>1</sup>) was stored. As well, the linear curve for maximum and minimum amplitudes at 216.7 nm and 235.5 nm were dotted against the congruous concentrations of SDB to construct the regression equation.

##### Ratio subtraction method

The spectra of the bilateral mixture were divided by the advisor of CFR (10  $\mu\text{g}/\text{mL}$ ), then the amplitudes were subtracted in the plateau region at  $\lambda$  250-315 nm (the constant) from that ratio spectrum. The zero order spectra of SDB were resolved by multiplying the resulting ratio spectra by the divisor (CFR). The concentration of SDB was computed through the congruous regression equation at 225 nm.

## RESULTS AND DISCUSSION

### Methods development and optimization

#### Blank subtraction

Before it is decided to proceed 3D blank baseline subtraction in empower 3 software, it should be considered whether these types of issues with your chromatography exist: Incapable of properly integrating the standard or sample due to small-noise peaks or a drifting noisy baseline. The blank chromatogram includes characteristics that are worth subtracting (for example; small noise peaks). The blank chromatogram does not change from run to run and 3D blank baseline subtraction does not improve the signal-to-noise ratio of the signal. Blank baseline subtraction removes only the background signal and may increase the noise. After that, select alter sample in the sequence or sample set, then labeling the blank injection with a special mark as "B", open the method set used to obtain the data, then press the top of derived channels and select to create a new derived channel. In the first tab, "first (only) channel", press the channel drop-down list and select "DAD", in the second tab, choose the operator "-" and "DAD" from the channel drop-down list and check box form injection labeled and write down "B", write a name for the new-derived channel "Blank Subtraction" press ok, edit the processing method and change channel from "DAD" to "Blank Subtraction," then save method set and process the data with the method set, the blank chromatogram will subtracted from the sample automatically,<sup>22</sup> as clarified in Figure 2g.

#### Detection of wavelengths

Various wavelengths are checked and scanned at (200-400 nm) for 20  $\mu\text{g}/\text{mL}$  of each mixture member of both pure CFR and SDB drugs and in their dosage forms to accomplish the best selectivity wavelength at 254 nm with minimum noise (Figure 3).

#### Optimization of temperature and flow rates

To achieve the best resolution and separation, many trials were performed at column temperatures of (35, 40, and 45°C), in addition to changes in flow rates (0.7, 1.0, and 1.3 mL/min); the flow rates of 1.0 mL/min and 40°C were the best couple for a system with good selectivity.

### Stationary phase

Preparatory experiments had been performed by trying various columns with different lengths and particle sizes, including Thermo® C18 column (15 x 0.46 cm, 5.0 µm), Agilent ZORBAX -C18 column (15 x 0.46 cm, 5.0 µm) and Hichrom C18 column (0.46 x 15 cm, 5.0 µm, 150 Å pore size) and the last column is the best better selectivity and resolution for peaks of all impurities.

### Optimization of gradient programs

The binary gradient program is experimented using various systems: (1) (i) 0-2 min: 20% (B), 80% (A); (ii) 2-20 min: 30% (B), 70% (A); (iii) 20-35 min: 50% (B: A), (iv) 35-55 min: 80% (A), 20% (B). (2) (i) 0-2 min: 10% (B), 90% (A); (ii) 2-20 min: 30% (B), 70% (A); (iii) 20-35 min: 50% (A, B), (iv) 35-55 min: 90% (A), 10% (B). The followed binary gradient proved to be the best system for selectivity and resolution: (i) 0-8 min: 95% (A), 5% (B) isocratic; (ii) 8-28 min: 75% (A), 25% (B); (iii) 28-43 min: 50% (A), 50% (B); and (iv) 43-64 min: 95% (A), 5% (B).

### Derivative ratio method (DD<sup>1</sup>)

The method was verified for simultaneous estimation of the compounds to resolve the interference in binary mixtures. DD<sup>1</sup> spectrophotometric method was established to increase the selectivity of the analysis of CFR without interference from SDB. To adjust DD<sup>1</sup> method, many concentrations of the SDB as a divisor were tried including 1, 2, 4, and 5 µg/mL of SDB and optimum results were achieved by applying 5 µg/mL of SDB as a divisor. The obtained ratio spectra are distinguished as *per* the used wavelength, and DD<sup>1</sup> values showed good selectivity at the maximum 283.5 nm and a minimum of 313.4 nm (Figure 4). For the estimation of SDB in the presence of CFR, many concentrations of CFR are tried including, 2, 5, 10, and 15 µg/mL of CFR, and the best results were achieved when using CFR as a divisor with a concentration of 10 µg/mL. The obtained ratio spectra were recorded for maximum and minimum amplitudes at 216.7 nm and 235.5 nm, respectively (Figure 5).

### Ratio subtraction method

This method was selected for estimating binary mixtures in which the spectrum of one component is more extended than

that of the other one. It was applied to solve the overlapping spectra of the mixture of CFR and SDB to get the extended (SDB) in zero order. The method involves dividing of the spectrum of the mixture in the zero-order by divisor of CFR (10 µg/mL). The resulted ratio spectrum is a new graph representing the plateau region. By subtracting this constant (plateau in 250-315 nm), after that multiplying the new graph with the divisor, the original spectrum of SDB in the mixture can be obtained at 225 nm. Thus, the interference of the CFR was removed (Figure 6). Also, the same procedure is repeated to gain the extended (CFR) in the zero order by subtracting the constant which is found in the plateau region (205-230 nm) and multiplying the new graph with the divisor of SDB (5 µg/mL) consequently, the zero-order spectrum of CFR is obtained at  $\lambda_{max}$  283 nm. These data are represented in (Figure 7).

### Method validation

The proposed methods have been achieved and fully validated by following the guidelines of International Conference on Harmonization (ICH) recommended for method validation.<sup>23</sup> Then the applied USP pharmacopeia method was verified regarding system suitability testing, limit of quantification, and precision.<sup>4</sup>

### 1) Method (A) for active pharmaceutical ingredient (API)

#### Linearity and range

Linearity ranges, *e.g.* (0.003-0.075 mg/mL) and (0.002-0.050 mg/mL) were evaluated for CFR and SDB, respectively, with a correlation coefficient of regression >0.9999, Y-intercept of level 100% response of CFR equal to 0.4%. After running each preparation in triplicate, the relative standard deviations (RSD) percentage of the peak area of 3 injections for each level  $\leq 2.0\%$ . All the parameters of the regression analysis of the developed methods are presented in Table 1.

#### Limit of detection and composition

The quantitation limit refers to the lowest quantity of analytical material in a sample that can be quantified with appropriate accuracy. The obtained results for the limit of detection and limit quantitation are shown in Table 1.

#### Precision

##### System precision (repeatability)

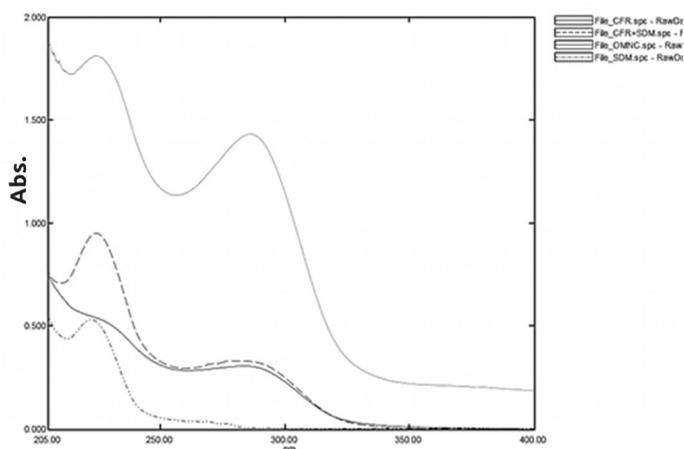
The obtained results for six preparations were tabulated with RSD% <2.0%, as listed in Table 1.

##### Method precision

Method precision was evaluated by analyzing three different concentrations of the drugs being studied, each in triplicate on different days, performed by different analysts and equipment and RSD% was calculated, see Table 2 for ruggedness-related substance results.

##### Stability of analytical solutions

This method was been carried out by analyzing the assay of standard solution during three consecutive days at room temperature and in a fridge and comparing them with corresponding fresh results. The recovery results of the stability of the analytical solution are displayed in Table 2 with



**Figure 3.** Zero order absorption spectra of 20 µg/mL of each of CFR, SDB, mix of CFR and SDB and omnicef 100 mg/5 mL using solvent as blank  
CFR: Cefdinir, SDB: Sodium benzoate

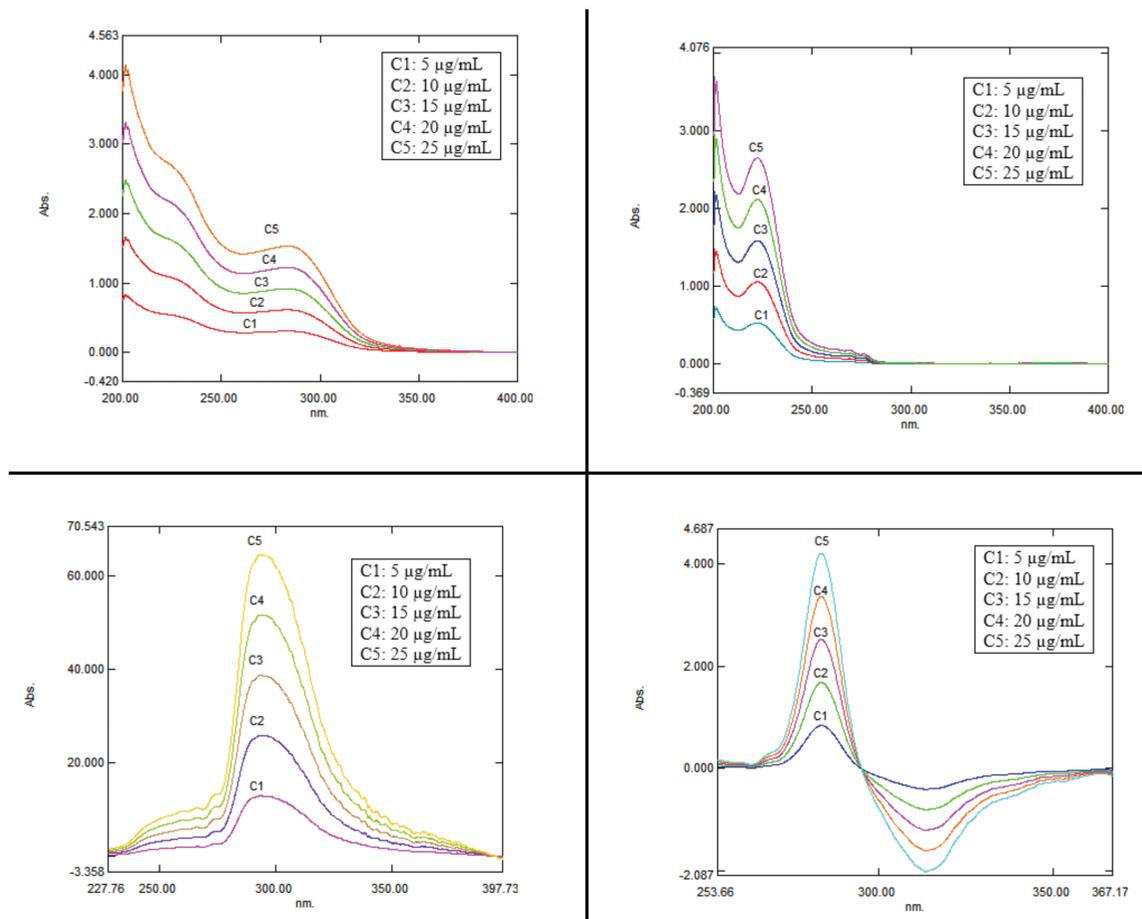
RSD <2.0%.

#### Accuracy and recovery

The accuracy is estimated using three different concentrations (50%, 100% and 150%) with replicates and RSD between six injections from the same concentration <2.0% and recovery results between 98 and 102% as shown in Table 3.

#### Specificity and selectivity

If interference is observed (due to placebo, blank, diluent, etc.), it must not exceed 2.0% of the main peak target concentration limit. Placebo preparation was proceeded as under test preparation and (Figure 8a) confirmed that the API does not interfere with the placebo and solvent.



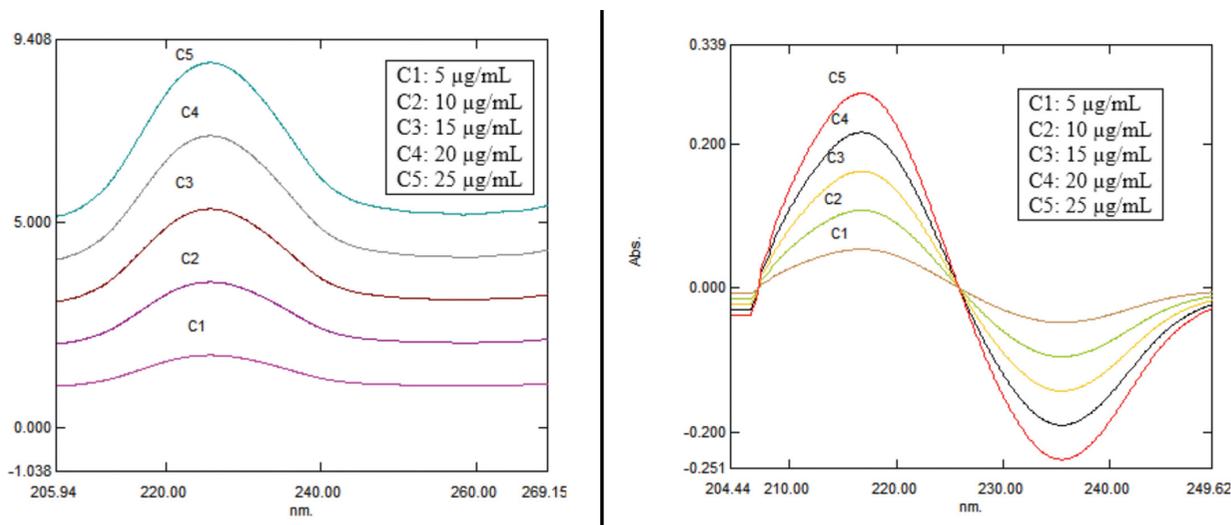
**Figure 4.** Ratio spectra and first derivative of the ratio spectra of standard solution of CFR using 5 µg/mL of SDB as a divisor and solvent as a blank  
CFR: Cefdinir, SDB: Sodium benzoate

**Table 1.** Regression and validation parameters

Parameter	HPLC		DD <sup>1</sup>		RSM	
	CFR	SDB	CFR	SDB	CFR	SDB
Wavelength	254 nm	254 nm	283.5 nm	216.7 nm	283 nm	225 nm
Range (µg/mL)	3-75	2-50	5-25	5-25	5-25	5-25
Slope	18651283	1850.2	0.0705	0.3150	0.0563	0.3234
Intercept	988.5	1093.6	0.028	0.400	0.0063	0.0250
Correlation coefficient	0.9999	0.9999	0.9996	0.9997	0.9995	0.9996
Repeatability	0.2	0.1	0.3	0.2	0.4	0.2
LOD <sup>a</sup> (µg/mL)	0.41	0.42	0.73	0.36	0.33	0.30
LOQ <sup>a</sup> (µg/mL)	1.22	1.29	2.20	1.11	1.03	0.92

<sup>a</sup>LOD: Limit of detection ( $3.3 \times \sigma/\text{slope}$ ) and LOQ: Limit of quantitation ( $10 \times \sigma/\text{slope}$ )

HPLC: High performance liquid chromatography, RSM: Ratio subtraction method, CFR: Cefdinir, SDB: Sodium benzoate



**Figure 5.** Ratio spectra and first derivative of the ratio spectra of standard solution of SDB using 10 µg/mL of CFR as a divisor and solvent as a blank  
CFR: Cefdinir, SDB: Sodium benzoate

**Table 2. Ruggedness, robustness, and stability of analytical solution of the proposed methods**

Parameter	HPLC		UV		Limit %
	CFR	SDB	CFR	SDB	
Day to day	0.80	0.75	0.70	0.64	
Analyst to analyst	1.22	1.13	0.90	0.74	
Column to column	0.77	0.79	-	-	
Flow rate change ( $\pm 0.1$ mL/min)	0.71	0.85	-	-	
pH changes of mobile phase ( $\pm 0.2$ )	0.88	0.79	-	-	RSD $\leq 2.0\%$
Wavelength change ( $254 \pm 2.0$ nm)	0.80	0.78	0.76	0.57	
Column temperature change (30, 25°C)	0.93	0.82	0.89	0.59	
Fresh sample	0.12	0.14	0.19	0.22	
Stored sample in fridge	0.66	0.47	0.54	0.49	
Stored sample at room temperature	0.89	0.94	0.83	0.77	

HPLC: High performance liquid chromatography, UV: Ultraviolet, CFR: Cefdinir, SDB: Sodium benzoate, RSD: Relative standard deviation

### Forced degradation

The forced degradation of CFR was performed under different acid, base, oxidative, thermal, photolytic and neutral conditions. To establish the stability indicating capability of the related substance test method for omnicef, standard solution of CFR was separately subjected to the above-mentioned conditions. All degradants of CFR are well resolved and do show any interference with CFR peaks. CFR peak was found to be pure under all forced degradation conditions since that the peak purity angle match for CFR under all conditions was found to be less than the purity threshold as displayed in Table 4 and Figure 8.

### II) Method (B) for UV spectroscopic method

#### Linearity and range

Linearity range (0.005-0.025 mg/mL) was evaluated for CFR and SDB for spectrophotometric methods with a correlation coefficient of regression  $>0.999$ , RSD percentage for each level  $\leq 2.0\%$ . All the parameters of the regression analysis of the developed methods are presented in Table 1.

#### Limit of detection and composition

The quantitation limit refers to the lowest quantity of analytical material in a sample that can be quantified with appropriate accuracy. The obtained results for limit of detection and limit quantitation are shown in Table 1.

## i) Acid hydrolysis

Transfer 10 mL of the standard stock solution	3 mL of 1 N HCl, sonicate for 20 min and mix well, store at room temperature for 2 h ----- > Add 50 mL solvent	Complete to 100 mL volumetric flask with solvent
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## ii) Basic hydrolysis

Applied to 10 mL standard stock solution	3 mL of 1 N NaOH, sonicate for 20 min and mix well, store at room temperature for 2 h ----- > Add 50 mL solvent	Complete to 100 mL volumetric flask with solvent
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## iii) Oxidation

Transfer 10 mL of the standard stock solution	3 mL of 30% H <sub>2</sub> O <sub>2</sub> , sonicate for 20 min and mix well, store at room temperature for 2 h ----- > Add 50 mL solvent	Complete with solvent in 100 mL volumetric flask
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## iv) Thermal decomposition

Applied to 10 mL of the standard stock solution	Heat for 2 h at 85°C in water bath ----- > Add 50 mL solvent	Complete to 100 mL volumetric flask with solvent
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## v) Light decomposition

Applied to 10 mL of the standard stock solution	Keep in light for 2 h ----- > Add 50 mL solvent	Complete to 100 mL with a solvent
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*Precision**System precision (repeatability)*

The obtained results for six preparations were tabulated with RSD% <2.0%, as listed in Table 1.

*Method precision*

Method precision was evaluated by analyzing three different concentrations of the drugs under study, each in triplicate on different days, performed by different analysts and equipment and RSD% was calculated, see Table 2 for ruggedness-related substance results.

*Stability of analytical solutions*

This method will be determined by analyzing the assay of standard solution during three consecutive days at room temperature and in a fridge and calculated it corresponding to fresh results. The recovery results of stability of the analytical solution are displayed in Table 2 with RSD <2.0%.

*III) Method (C) for related substances**System suitability testing*

System suitability of the analytical method is determined by preparing a standard solution of impurities and analyzed

at least 6 times with RSD ≤2.0%, resolution not less than 1.5 between CFR and the third peak of USP CFR related compound A, tailing factor not more than 1.5 for CFR related compound B. All system suitability parameters are tabulated in Table 5.

*Limit of detection and composition*

The quantitation limit refers to the lowest quantity of analytical material in a sample that can be quantified with appropriate accuracy. The obtained results for limit of detection and limit quantitation are shown in Table 1.

*System precision (repeatability)*

The related substances obtained results for six preparations were tabulated and the average of the 12 preparations with RSD% <2.0% was calculated as listed in Table 1.

*Specificity and selectivity*

If interference is observed (due to placebo, blank, diluent, etc.), it must not exceed 10.0% of the impurity peak at the specification limit.

**CONCLUSION**

Efficient and novel stability indicated that HPLC and spectrophotometric methods have been validated and

**Table 3. Accuracy and recovery of CFR in the proposed method**

Concentration	Test	Result (%)	Average result (%)	RSD (%)
50%	T1 inj-1	99.98%	99.88%	0.18%
	T1 inj-2	99.76%		
	T2 inj-1	99.72%		
	T2 inj-2	99.68%		
	T3 inj-1	100.12%		
	T3 inj-2	99.98%		
100%	T1 inj-1	99.66%	99.49%	0.10%
	T1 inj-2	99.53%		
	T2 inj-1	99.48%		
	T2 inj-2	99.49%		
	T3 inj-1	99.40%		
	T3 inj-2	99.36%		
150%	T1 inj-1	99.93%	99.75%	0.13%
	T1 inj-2	99.88%		
	T2 inj-1	99.63%		
	T2 inj-2	99.73%		
	T3 inj-1	99.74%		
	T3 inj-2	99.60%		

CFR: Cefdinir, RSD: Relative standard deviation

**Table 4. Stability indicating capability of the related substances**

Cefdinir			
Condition	Peak area	% Degradation	Peak purity match
Normal	290534	-	Pass
Thermal	283365	2.47%	Pass
Light	283087	2.56%	Pass
Acidic	279986	3.63%	Pass
Basic	283603	2.39%	Pass
Oxidative	19087	93.43%	Pass

developed for simultaneous quantification of the CFR and SDB in the presence of its degradants. As for the chromatographic methods, it was developed and validated as *per* ICH guidelines using the blank subtraction method on empower PDA software that reduces waste time in manual calculation of impurities. Two spectrophotometric methods were developed, the first derivative of ratio spectra spectrophotometric method (1<sup>st</sup>DD) and the second is ratio subtraction method, which were used to resolve the interference between CFR and SDB. Based on peak purity results, which have obtained from selectivity and

**Table 5. System suitability testing parameters of the developed methods**

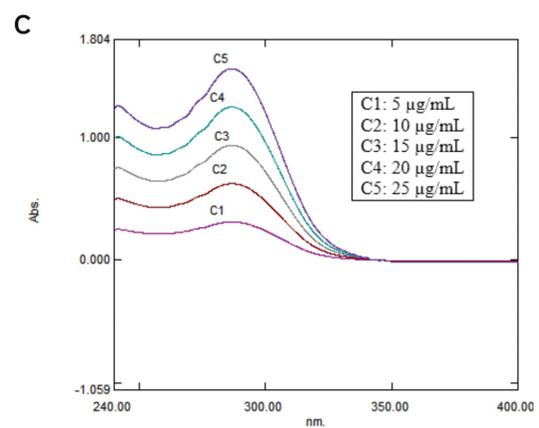
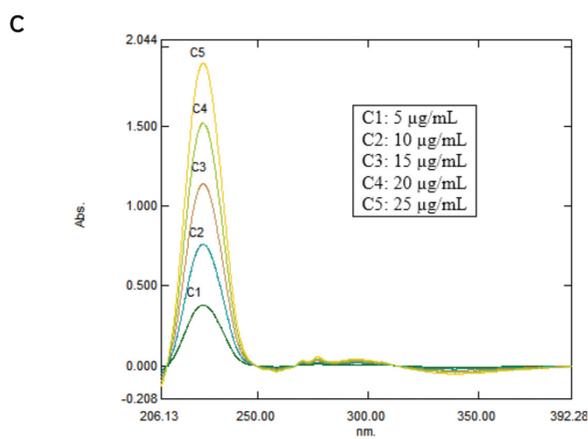
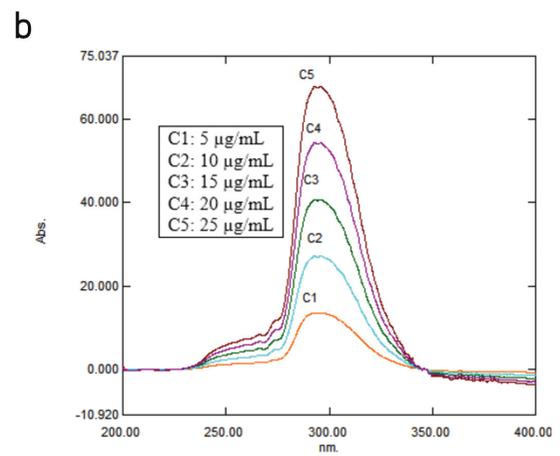
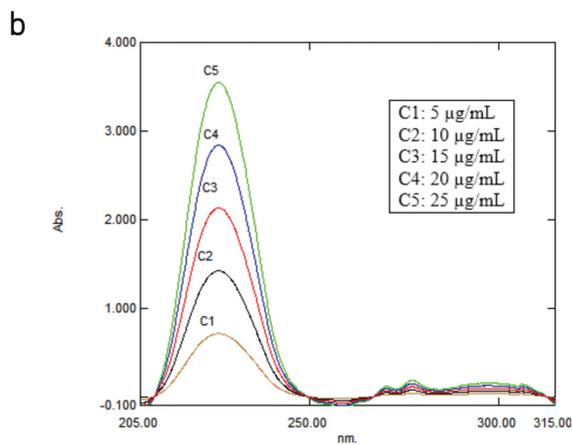
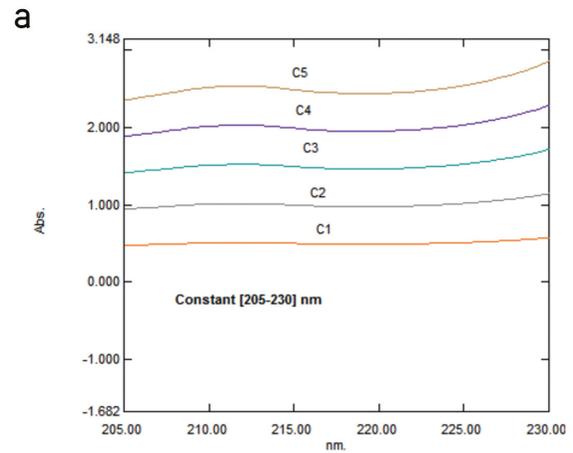
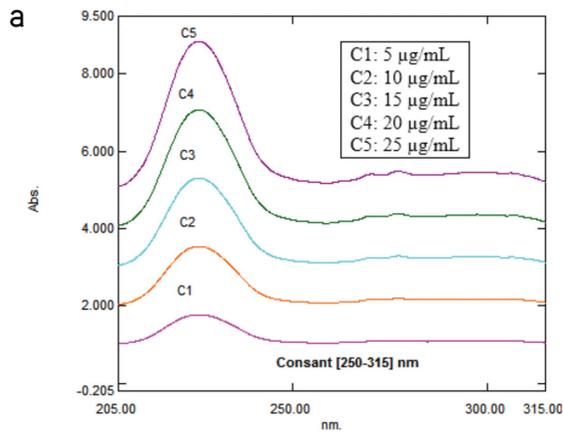
Item	HPLC	Reference values
	CFR	-
Tailing factor	0.92	T ≤1.5
Injection precision	0.17	RSD ≤1%
Number of theoretical plates (N)	4850	N >2000
Resolution	3.0	R <sub>s</sub> >1.5
Retention time (R <sub>t</sub> )	0.10	RSD ≤1%

HPLC: High performance liquid chromatography, CFR: Cefdinir, RSD: Relative standard deviation, Rs: Resolution

forced degradation analysis, we can confirm that the proposed method is selective and sensitive, and it can be used as stability indicating one for assay and related substances for CFR in QC labs. The proposed method was demonstrated to achieve a shorter time, high sensitivity, and cost effective of analysis and consumable reagents.

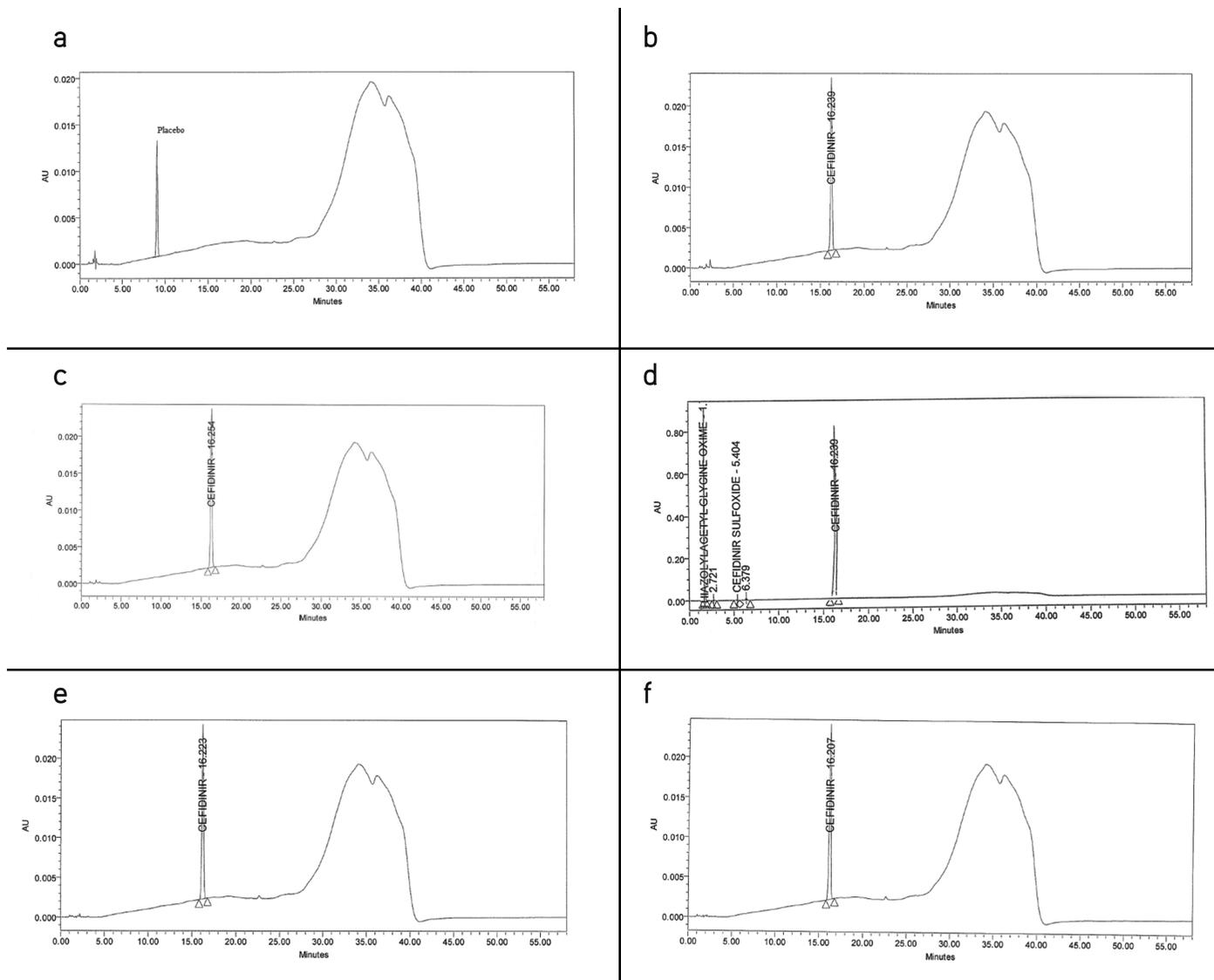
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**Figure 6.** (a) Ratio spectra of a mixture of CFR and SDB using CFR (10 µg/mL) as a divisor. (b) Subtracting the value of the constant from the ratio spectra (c) the obtained SDB spectrum in zero order  
CFR: Cefdinir, SDB: Sodium benzoate

**Figure 7.** (a) Ratio spectra of a mixture of CFR and SDB using SDB (5 µg/mL) as a divisor. (b) Subtracting the value of the constant from the ratio spectra (c) the obtained CFR spectrum in zero order  
CFR: Cefdinir, SDB: Sodium benzoate



**Figure 8.** Chromatograms of (a) placebo, (b) acid hydrolyzed degraded sample, (c) base hydrolyzed-degraded sample, (d) oxidative-degraded sample, (e) thermal degraded sample, (f) sun light degraded sample

### Ethics

**Ethics Committee Approval:** Not applicable.

**Informed Consent:** Not applicable.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: M.A.M., M.E.K.M.H., Design: M.A.M., M.E.K.M.H., Data Collection or Processing: M.A.M., Analysis or Interpretation: M.E.K.M.H., Literature Search: M.A.M., M.E.K.M.H., Writing: M.A.M., M.E.K.M.H.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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