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Comparative Study for the Analysis of Cefixime Trihydrate and its Degraded Products by Two RP-HPLC Methods, One its Official and Other Developed Validated Method

Elsadig HK* and Abdalfatah MB

Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Riyadh, Al-kharj, Saudi Arabia

Abstract

The aim of the present work was aimed to carry out comparative study between method (1) and method (2) for separation of cefixime trihydrate and its degraded products by using two different mobile phases, keeping the other parameters such as stationary phase, column condition, wavelength, and device. Mobile phase for method (1) consist of a solution of 0.03 M Tetra butyl ammonium hydroxide (pH 6.5) and acetonitrile with a ratio of 3:1 respectively while Mobile phase for method (2) consist of a mixture of 0.1 M sodium dihydrogen phosphate monohydrate solution (pH 2.5) and methanol with a ratio 3:1 respectively. To study the degraded products sample was subjected to Sun light, UV light, and thermal effects. From data obtained proved the method (2) gave less retention time for the separation of drug with a larger number of decomposed products being detected compared by method (1).

Keywords: Cefixime trihydrate; Comparative study; RP-HPLC method; Cefixime trihydrate; Stability indicating method

Introduction

Cefixime is used to treat a wide variety of bacterial infections. This medication is known as a cephalosporin antibiotic. It works by stopping the growth of bacteria. Cefixime is a broad spectrum cephalosporin antibiotic and is commonly used to treat bacterial infections of the ear, urinary tract, and upper respiratory tract. The bactericidal action of Cefixime is because of the inhibition of cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) which inhibits the final trans peptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death [1-3]. Cefixime is an orally active 3rd generation cephalosporin which exerts its bactericidal action against both gram positive and gram negative organism by in bacterial cell wall synthesis. Chemically, Cefixime trihydrate name is (6R,7R)-7-[[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid trihydrate (Figure 1) and it's molecular formula is $C_{16}H_{15}N_5O_7S_2.3H_2O$, and molecular weight is 507.50 g/mol. It is a white powder that is freely soluble in water (1 g/5 ml) and stable in air, heat and acid solutions, while it is unstable in alkaline medium and light.

Materials and Methods

Materials (Chemicals and reagents)

Cefixime trihydrate was donated from DSM Company, all chemicals and regents used were of a HPLC grade. Tetra butyl ammonium hydroxide 40% aqueous solution, Sodium dihydrogen phosphate monohydrate were obtained from AppliChem, Germany. Methanol and acetonitrile were obtained from fisher scientific UK Limited, UK. Water (HPLC gradient grade) supplied from Panreac, E.U. Orthophasophoric acid 85% was obtained from BDH, England.

Instrument and equipment

a. HPLC instruments a water Breeze 2 system, consisting of binary pump series 1525, UV/VIS detector 2489, and auto sampler series 2707.

b. Sensitive balance, A and D Company limited, Japan.

c. 827 pH lab. metrohm ion analysis, Herisau/ Switzerland.

Experimental

Preparation of mobile phase for method (1) of 0.03 M tetra butyl ammonium hydroxide solution (pH 6.5)

Solution was prepared by weighing 8.2 g Tetra Butyl Ammonium hydroxide (or 20 ml of Tetra Butyl Ammonium hydroxide 40% aqueous solution) and dissolving into 800 ml of distilled water and adjusted to pH 6.5 with 10% ortho phospharic acids and diluted up to 1000 ml with distilled water, and mix with acetonitrile with a ratio of 4:1 respectively and degassed [4].

Preparation of mobile phase for method (2) of 0.1 M sodium dihydrogen phosphate monohydrate solution (pH 2.5)

Solution was prepared by weighing 13.67 g of sodium dihydrogen phosphate monohydrate and dissolving into 900 ml of distilled water and adjusted to pH 2.5 with diluted orthophospharic acid and diluted up to 1000 ml with distilled water, and mix with methanol with a ratio of 3:1 respectively and degassed [5].

Chromatographic conditions used for the analysis of cefixime trihydrate and its degraded

Mobile phase: 1 and 2, Flow Rate, 1.0 ml/min, Injection volume=50 µl, Column=Waters Spherisorb® 5.0 µm ODS2 250 mm × 4.6 mm ID, Temperature=Room temperature (Ambient), Detection wave length at 254 nm.

Preparation of standard stock solution

Stock standard solution having concentration 100 µg/ml was prepared by dissolving pure drug of cefixime trihydrate in water, injected into the chromatographic column (Figures 2-4; Table 1).

***Corresponding author:** Elsadig HK, Department of pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Riyadh, Al-kharj, Saudi Arabia, Tel: +966115886094; E-mail: elsadigk@yahoo.com

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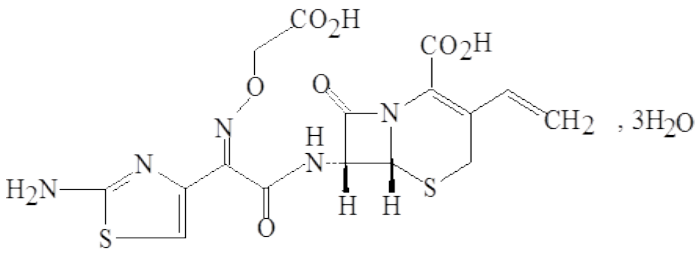

Figure 1: Structural formula of Cefixime Trihydrate.

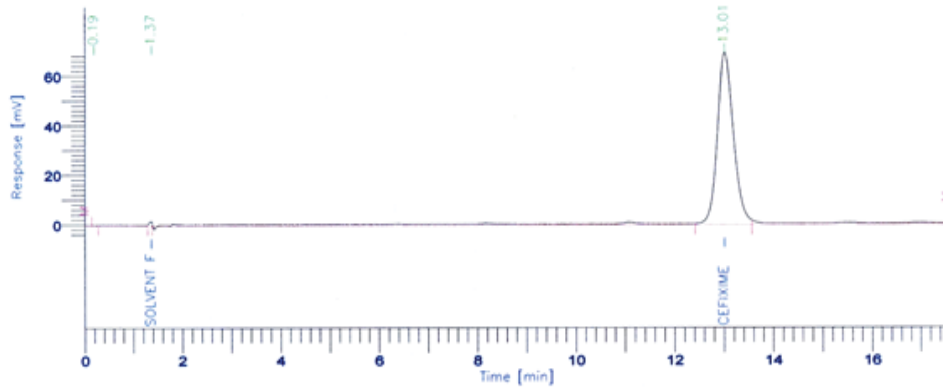

Figure 2: HPLC chromatogram test for the analysis of cefixime trihydrate reference standard by method 1.

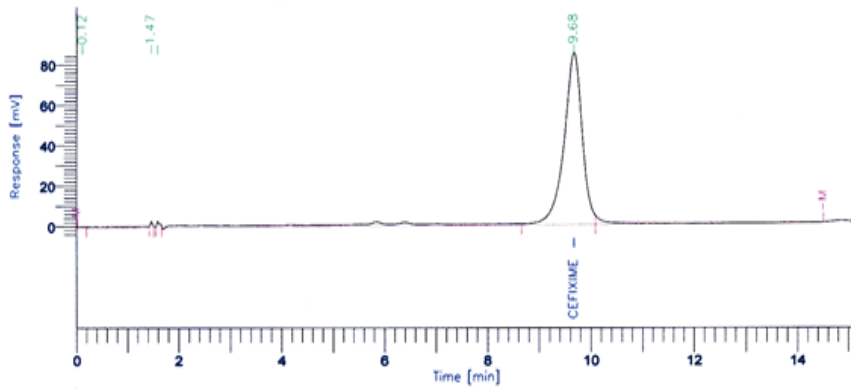

Figure 3: HPLC chromatogram test for the analysis of cefixime trihydrate reference standard by method.

Preparation of degradation product of cefixime

Preparation of cefixime trihydrate solid sun decomposition product: About 5.0 grams of cefixime trihydrate solid were placed between two glass plates (20 ×20 cm), sealed with gum tape and directly exposed to sunlight for six months (March to August). Samples were taken every month and tested for degradation by HPLC (Table 2 and Figure 5).

Preparation of cefixime trihydrate solution UV decomposition product:100 µg / ml of cefixime trihydrate solution in water were prepared and transferred to a stoppered tube. The solutions were placed under UV radiation at λ 254 nm. Samples were taken at 30, 60, 90, 120, and 150 minutes and tested for degradation by HPLC (Table 3 and Figures 6 and 7).

Preparation of cefixime trihydrate solid thermal decomposition at 100°C: Few grams of CEF-3H_2O solid were placed in a petridish and put it in oven at 100°C. Samples were taken every hour and tested for degradation by HPLC (Table 4 and Figures 8 and 9).

Preparation of cefixime trihydrate solution thermal decomposition at 100°C for 45 minutes: Solution of cefixime trihydrate (10 mg / 100 ml water) was prepared. The flask was placed into a water-path thermostatic at 100°C for 45-minute (Table 5; Figure 10).

Results and Discussion

Under optimization condition for the RP-HPLC method 1 and method 2 with keeping others fixed and changeable mobile phase, the separation chromatogram obtained of cefixime trihydrate

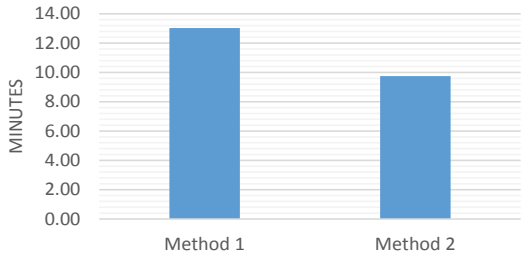

Figure 4: Time vs. method 1 and 2 for the separation of cefixime trihydrate reference standard.

Parameters	Retention Time RT
Method 1	13:03
Method 2	10:15

Table 1: Analysis of cefixime trihydrate reference standard by the method 1 and method 2.

Interval time/month	Method 1% Remaining content	Method 2% Remaining content
1 Month	79.72	78.56
2 Months	64.71	64.51
3 Months	47.45	48.05
4 Months	22.99	22.57
5 Months	11.62	11.65
6 Months	8.11	8.15

Table 2: Analysis of decomposed cefixime trihydrate solid form by sunlight using method 1 and 2.

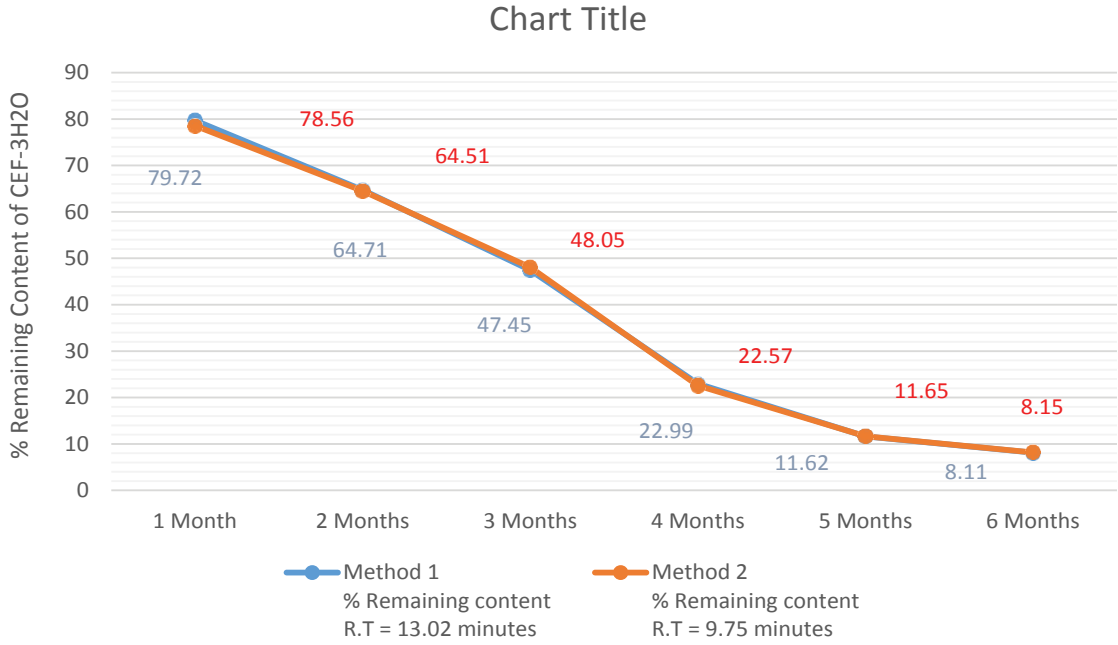

Figure 5: Remaining content of cefixime trihydrate subjected to sunlight analysis by method 1 and 2 vs. Intervals time.

reference standard was appear in Figures 2 and 3, it was found that cefixime trihydrate separation at 9.75 minute by using method 2 while separation at 13.03 minute by using method 1 [6-9]. Discrimination developed validated method 2 versus method 1 illustrated that from the assay test, the retention time of CEF-3H_2O less than that time obtained by method 1 were 9.75, 13.03 minutes respectively (Table 1 and Figure 4). There's very little noticeable change when testing for degradation of cefixime trihydrate solid under the influence of sunlight by using the analysis methods 1 and 2 (Table 2 and Figure 5). From the results obtained for the tested degraded products under stress condition of UV for cefixime trihydrate solution, two methods were given two degradation products but better resolution by the method 2 (Table 3 and Figures 6 and 7). The analysis of cefixime trihyrate solid thermal decomposed at 100°C by using method 1 and 2, revealed that method

Results	Method (1)		Method (2)	
	Content %	RT	Content %	RT
Decomposed (1)	17.46%	4.62	18.83%	2.97
Decomposed (2)	23.34%	11.05	22.88%	15.04
Remaining CEF-3H_2O	26.89%	13.03	27.19%	9.75

Table 3: Analysis of decomposed cefixime trihydrate solution by UV-light using method 1 and 2.

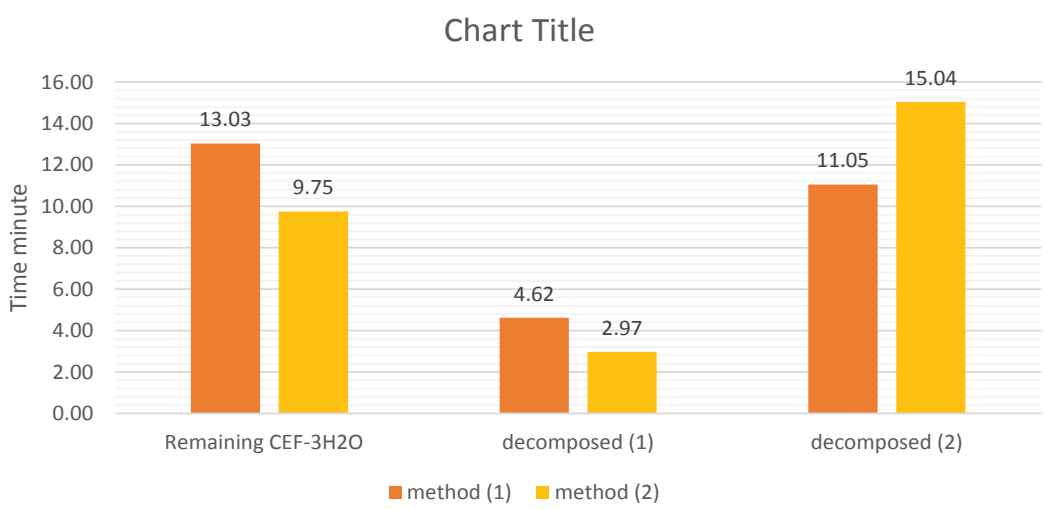

Figure 6: Time vs. degraded products obtained from the analysis of decomposed cefixime trihydrate solution by UV-light using method 1 and 2.

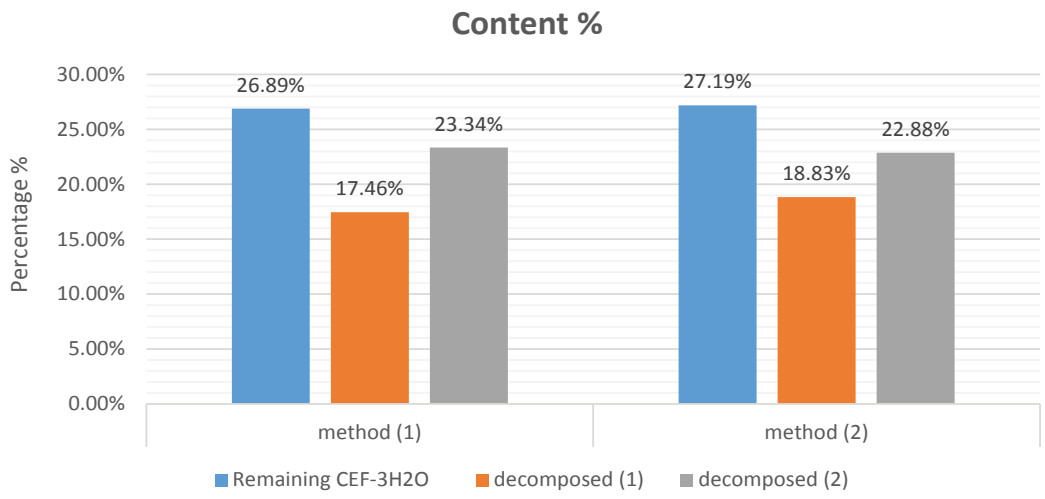

Figure 7: Percentage content of degraded products obtained from the analysis of decomposed cefixime trihydrate solution by UV-light vs. method 1 and 2.

Results	Method (1)		Method (2)	
	Content %	RT	Content %	RT
Decomposed (1)	8.50%	4.51 min	7.89%	3.91 min
Decomposed (2)	12.71%	6.38 min	14.86%	4.216 min
Decomposed (3)	-	-	8.20%	5.899 min
Remaining CEF-3H_2O	71.06%	13.014 min	66.34%	9.750 min

Table 4: Analysis of decomposed cefixime trihydrate solid by thermal effect at 100°C using method 1 and 2.

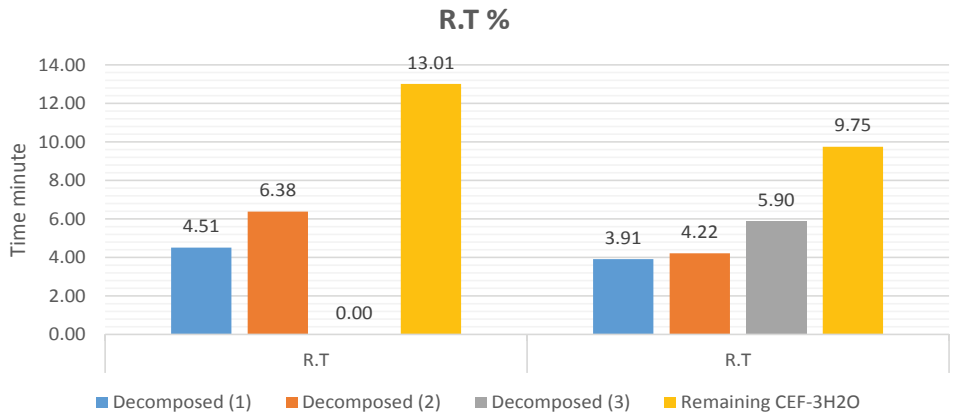

Figure 8: Time vs. degraded products obtained from the analysis of decomposed cefixime trihydrate solid by thermal effect at 100°C using method 1 and 2.

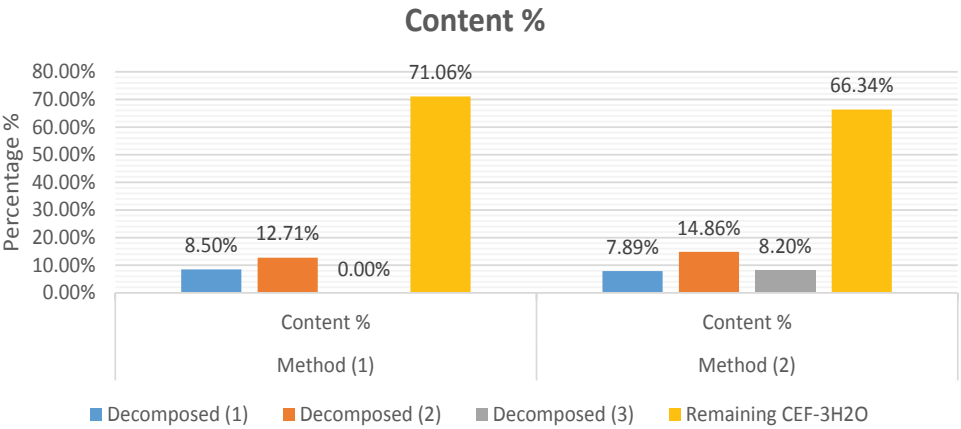

Figure 9: Percentage content of degraded products obtained from the analysis of decomposed cefixime trihydrate solid by thermal effect at 100°C vs. method 1 and 2.

Results	Method (1)		Method (2)	
	Content %	RT	Content %	RT
Decomposed (1)	8.90%	1.78 min	7.86%	14.07 min
Decomposed (2)	37.61%	11.05 min	36.97%	14.99 min
Remaining CEF-3H_2O	51.43%	13.02 min	51.69%	9.70 min

Table 5: Analysis of decomposed cefixime trihydrate solution by thermal effect at 100°C using method 1 and 2.

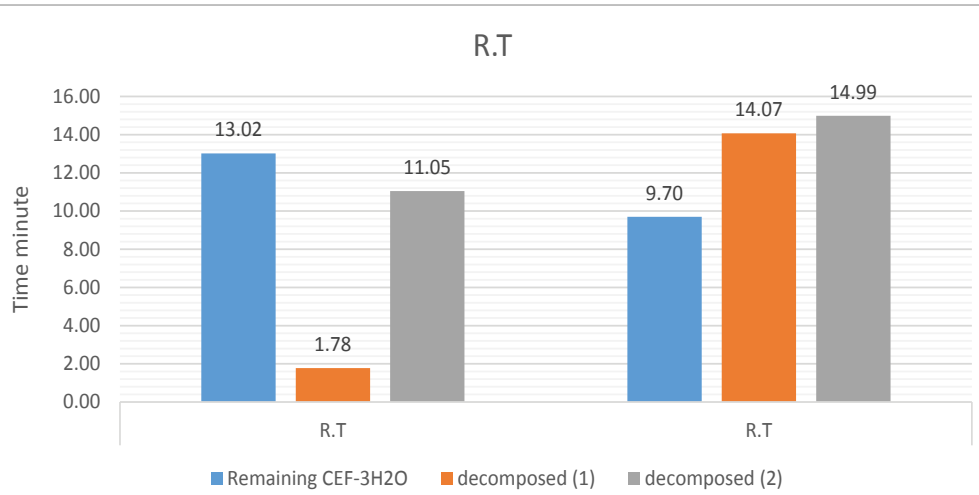

Figure 10: Time vs. degraded products obtained from the analysis of decomposed cefixime trihydrate solution by thermal effect at 100°C using method 1 and 2.

2 detected three decomposed products while method 1 was detect only two decomposed (Table 4 and Figures 8 and 9). There is no noticeable quantitatively and qualitatively change for the analysis of cefixime trihydrate solution thermal decomposed by using method 1 and 2.

Conclusion

This work described the evaluation of analytical method 1 and method 2. The method 2 described enables the quantification and qualification of cefixime trihydrate and its degraded products compared to the method 1. The data obtained demonstrate good precision proves the reliability of the method 2. Hence, the method 2 can be used routinely for qualitative and quantitative estimation of cefixime trihydrate and it can also be use as stability indicating method.

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