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RESEARCH ARTICLE

COMPARATIVE STUDY FOR THE ANALYSIS OF CEFIXIME TRIHYDRATE AND ITS DEGRADED PRODUCTS BY TWO RP-HPLC METHODS, ONE ITS OFFICIAL AND THE OTHER DEVELOPED VALIDATED METHOD

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ABSTRACT

The aim of the present work was aimed to carry out comparative study between method (1) [official method (BP, 2017)] and method (2) [developed validated analytical method (Adam *et al*., 2012)] for the 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 constant. 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 3:1respectively while Mobile phase for method (2) consist of a mixture of 0.1M 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).

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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 (Martindale, 2009; The United States pharmacopeia 33-National formulary, 2010; The United States Pharmacopeia 34-National Formulary, 2011). Cefixime is a broad spectrum cephalosporin antibiotic and is commonly used to treat bacterial infections of the ear, urinary tract, and upper respiratory tract (British Pharmacopoeia Commission, 2017; Brogden and Richards, 1989; Martindale, 2009). 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 transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death (Adam, Elsadig *et al*., 2012; Amin and Bryan, 1973; Analytical Chemistry, 2003). Cefixime is an orally active 3 rd. generation cephalosporin which exerts its bactericidal action against both gram positive & gram negative organism by in bacterial cell wall synthesis (Brogden and Richards, 1989; Martindale, 2009). 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, chemical structure (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 (1g/5ml) and stable in air, heat and acid solutions, while it is unstable in alkaline medium and light (British Pharmacopoeia Commission, 2017; Martindale, 2009; The United States pharmacopeia 33-National formulary, 2010; The United States Pharmacopeia 34- National Formulary, 2011).

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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.

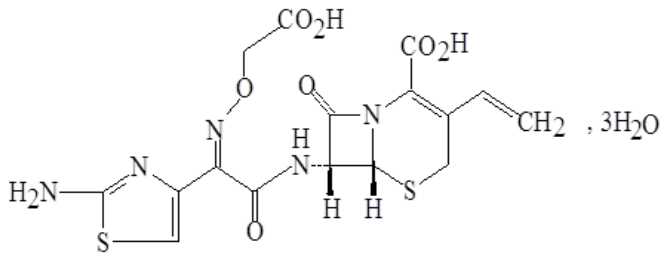


Fig.1. Chemical Structure of Cefixime Trihydrate

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&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) (British Pharmacopoeia Commission, 2017; The United States pharmacopeia 33-National formulary, 2010; The United States Pharmacopeia 34- National Formulary, 2011)

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 orthophospharic acid and diluted up to 1000 ml with deionized water, and mixed with acetonitrile with a ratio of 4:1 respectively and degassed (BP., 2017).

Preparation of mobile phase for method (2) of 0.1M sodium dihydrogen phosphate monohydrate solution (pH 2.5) (Adam, Elsadig *et al*., 2012)

Solution was prepared by weighing 13.67 g of sodium dihydrogen phosphate monohydrate and dissolving into 900 ml of deionized water and adjusted to pH 2.5 with diluted orthophospharic acid and diluted up to 1000 ml with distilled water, and mixed with methanol with a ratio of 3:1 respectively and degassed (Adam *et al.*, 2012).

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

- Mobile phase: (1) & (2)
- Flow Rate: 1.0 ml/min
- Injection volume = 50 µl
- Column = Waters Spherisorb®5.0µm ODS2 250 mm x4.6 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, (Figure (2) & (3) and (Table 1), (Figure 4).

Preparation of degradation products of cefixime

Preparation of the decomposed product cefixime trihydrate solid by sun-light

About 5.0 grams of cefixime trihydrate solid were placed between two glass plates (20 x 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), Figure 5.

Preparation of the decomposed product cefixime trihydrate solution by UV-light

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), Figure 6 & 7.

Preparation of the decomposed product cefixime trihydrate solid by thermal at 100 ^{0}C

Five grams of CEF-3H_2O solid were placed in a petri dish and was put in oven at 100 0C. Samples were taken every hour and tested for degradation by HPLC (Table 4), (Figure 8 & 9).

Preparation of the decomposed cefixime trihydrate solution by thermal at 100 ^{0}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 ^{0}C for 45 minute (Table 5), (Figure 10 & 11).

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 reference standard was appear in figure (2 & 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). Discrimination developed validated method (2) (Adam *et al.,* 2012) versus method (1) (BP., 2017), 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).

Table 1. Analysis of cefixime trihydrate reference standard by method (1) & (2)

Parameters	Retention Time R.T/minutes
Method 1	13:01
Method 2	9.75

Table 2. Analysis of decomposed cefixime trihydrate solid form by sun- light using method (1) & (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 3. Analysis of decomposed cefixime trihydrate solution by UV-light using method (1) & (2)

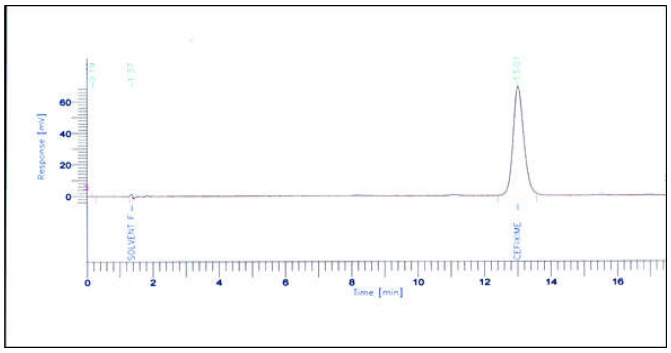
Results	Method (1)		Method (2)	
	Content %	R.T	Content %	R.T
Decomposed (1)	17.46%	4.62	18.83%	2.97
Decomposed (2)	23.34%	11.05	22.88%	15.04
Remaining CEF-3H2O	26.89%	13.03	27.19%	9.75

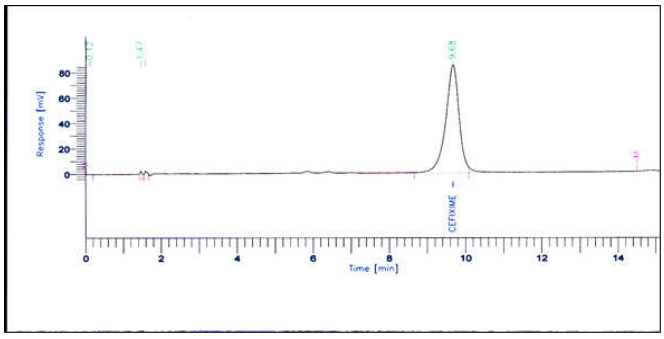
Table 4. Analysis of decomposed cefixime trihydrate solid by thermal effect at 100 ^{0}C using method (1) & (2)

Results	Method (1)		Method (2)	
	Content %	R.T	Content %	R.T
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-3H2O	71.06%	13.014 min	66.34%	9.750 min

Table 5. Analysis of decomposed cefixime trihydrate solution by thermal effect at100 ^{0}C using method (1) & (2)

Results	method (1)		method (2)	
	Content %	R.T	Content %	R.T
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-3H2O	51.43%	13.02 min	51.69%	9.70 min





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) & (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 (Figure 6 & 7). The analysis of cefixime trihydrate solid thermal decomposed at 100 ^{0}c by using method (1) & (2), revealed that method (2) detected three decomposed products while method (1) was detect only two decomposed, (Table 4), and (Figures 8 & 9). There is no noticeable quantitatively and qualitatively change for the analysis of cefixime trihydrate solution thermal decomposed by using method (1) & (2).

Conclusion

This work described the evaluation of analytical method (1) and (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) and separation of the degradation products did not interfere with the active ingredient cefixime trihydrate. 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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