SPECRTOPHTOMETRIC DETERMINATION OF CEFOTAXIME AND CEFTRIAXONE IN VIALS BY OXIDATIVE COUPLING

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Abstract

A simple, sensitive, accurate and rapid specrtophtometric method has been developed for the determination of cefotaxime and ceftriaxone . It is based on the oxidation coupling reaction of cefotaxime and ceftriaxone with o-aminophenol reagent in presence of potassium ferricyanide to from red color product with maximum absorption at 510 nm of cefotaxime and 514 nm ceftriaxone . Beer's law is obeyed over the concentration range 50-600µg of cefotaxime and 25-450 µg of ceftriaxone in final volume of 25 ml with molar absorptivities 2.8x104 and 4.4x104 l.mol-1.cm-1 and a relative standard deviation of 0.358-1.121% .The method can also be applied successfully for the analysis of pharmaceutical vials.

Introduction :

Chemically , Cefotaxime sodium (CFT) is (6R,7R)-3-[Cacetyloxy] methyl)-7-{[(2Z)-2-(2-aminotheiazol-4-yl)-2-(methoxyimino) acetyl] amino }-8-oxo-5-thia – 1- azabicyclo (4.2.0) – oct – 2- ene-2-Carboxyl ate [(1)].Ceftriaxone sodium (CFX) is (6R,7R)-7-[2-(2-Aminothiazol –4-yl)-2-(methoxyamino)acetamido]-8-oxo-3-[(2,5-Dihydro –2-methyl –6-oxido -5-oxo-1,2,4-trazin-3-yl) thiorethyl]-5-thia-1-azabicyclo (4,2.0) oct-2-ene-2-carboxylate [(1)]. Cefotaximea and ceftriaxone are third generation broad spectrum cephalosporins for perenteral administration and are bactericidal and mainly used in the treatment of various bacterial infections caused by Gram– positive and Gram-negative micro-organism [(2,3)] .Cefotaxime and ceftriaxone are official in BP[(1)] and USP[(4)].The BP and USP describe HPLC method for estimation of cetotaxime and ceftriaxone . literature revealed that determination of CFT and CFX by liquid chromatographic in haman and rat plasma[(5,6)] , HPLC with different detectors in pharmaceuticals[(7,8)],
Serum[(9)] and plasma[(10)] . Polarography[(11)] , voltammetry[(12,13)] , flow injection Specrtophtometric[(14)], spectrophotmetry[(15-19)]. In this paper specrtophtometric method for determination CFT and CFX is presented .The method is sample, rapid, sensitive and doesn't require temperature control or solvent extraction step and can be applied successflly to pharmaceutical vials.

Experimental :-

Apparatus :-

Double beam Unico, U.V -2100 spectrophotometer UV/Vis spectrophotometer with two matched quartz cells 1 cm light path was employed for the spectral measurement .

Standard and reagents:

All chemicals used were of analytical reagent and cefotaxine sodium , ceftriaxone sodium (pharmacare Int . Mfg . Co ., Sana,'a Yemen).

- Stock solution of CFT and CFX (1000 μg .ml^{-1}) was prepared by dissolving 100 mg of CFT or CFX in 100 ml volumetric flask separately using distilled water .

Working solution of CFT and CFX (100 μg .ml^{-1}) was prepared by diluting 10 ml of stock solution to 100 ml volumetric flask separately using distilled water .

O- Aminophenol reagent (5x10^{-3} M) was prepared by dissolving 0,0545g of O-aminophenol in 10 ml ethanol and mixed and made up to 100ml volumetric flask with the same solvent .

Potassium ferricyanide (1x10^{-2} M) was prepared by dissolving 0.8212g of potassium ferricyanide in distilled water and made up 250 ml volumetric flask with distilled water.

Procedure :-

Into a series of 25 ml volumetric flask , cefotaxime or ceftriaxone (100 μg .ml^{-1}) to cover the rang of the calibration curve (50 to 600 μg .ml^{-1}) of cefotaxime and (25 to 450 μg .ml^{-1}) of ceftriaxone in find volume of 25 ml, add 3ml of potassium ferricyanide (1x10^{-2} M) and 2.5ml of o-aminophenol (5x10^{-3}M) and shake well , followed by 2ml of ammonuim buffer solution. Dilute the solution to mark with distilled water and allow the reaction mixture to stand for 15 min at room temperature measure the absorbance at 510 nm of cefotaxime and 514 nm of ceftriaxone, against a reagent blank .

Analysis of pharmaceutical vials:-

Weight and finally powder an enough of vials followed by extraction of an cutely weight portion of the powder equivalent to 100mg each cetotaxime or ceftriaxone was transferred into 100 ml volumetric flask, dissolved in 2 ml of methanol for 2 min. The solution were mixed well by skaking for 15 min and then completed to volume with distilled water.The solution were filtrated.The first potion of the filtrate was rejected. The filtrated were quantitatively diluted with distilled water to yield concentrations (1000 μg .ml^{-1}), dilute 10 ml this solutions to 100 ml volumetric flask by distilled water to prepare (100 μg .ml^{-1}) of cefotaxime and ceftriaxone.

Results and Discussion :-

Absorption spectra :-

When a dilute solution of cefotaxime or ceftriaxone was mixed with potassium ferricyanide and o-aminophenol reagent and ammonium buffer solution , an intense red coulor forms after 2 min , which become stable after 30 min .The red product has a maximum absorption at 510 nm of cefotaxime and 514 nm of ceftriaxone [fig(1)] shows the spectra of the red complex formed and of the reagent blank , the maximum absorption was used in all subsequent experiments .

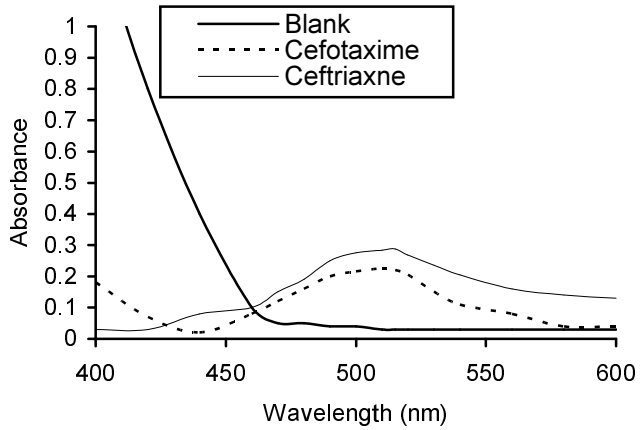


Fig :1. Absorption spectra of 4 μg.ml^{-1} Cefotaxime sodium Ceftriaxone Sodium ,treated as described under procedure and measured agaiast a reagent blank .

Optimization of reaction conditions:-

The effects of various parameters on the absorption intensity of the formed product were studied and the reaction conditions were optimized .

Effect ion of reagent :-

When various concentration of o-aminophenol solution added to a fixed amount of cefotaxime or ceftriaxone solution 2.5 ml of ($5x10^{-3}$M) solution was found enough to develop the color to its full intensity and give a minimum blank value.

Effect of oxidant concentration :-

The product formation reached maximum with about 5ml at ($1x10^{-2}$M) potassium ferricyanide solution and remained at this maximum when 0.5 to 8 ml of the oxidant concentration was added to cofotaxime or ceftriaxone ,5 ml of oxidizing agent solution was therefore used in procedure since it gave high sensitivity.

Effect of pH :-

The effect of pH range (3-12) was examined and only pH = 9 (ammonium buffer) was found to be optimum . The effect of the amount of the buffer used was also investigated and 2 ml was found to be optimal.

Effect of reaction time :-

The dye intensity reached a maximum after cefotaxime or ceftriaxone solution had been reacted immediately with potassium ferricyanide and o-aminophenol in alkaline medium and become stable after 15 min , therefore 15 min development time was selected as optimum in the general procedure . The coulor obtained was stable for 2 hour .

Effect of temperature :-

The effect of temperature on the coulor intensity of the dye was studied . In practice the same absorbance was obtained when the coulor was developer at room temperature ($25C^0$) , or when the volumetric flask was placed in an ice –bath at ($0C^0$) or in a water –bath at ($50C^0$), therefore it is recommended that the coulor reaction should be carried out at room temperature ($25C^0$) .

Effect of order addition :-

To obtain optimum results , the order of the addition of reagents should be followed as given under the procedure , otherwise a loss in color intensity stability were observed.

Calibration curve :-

Employing the conditions described under procedure , a linear calibration curve for cefotaxime and ceftriaxone were obtained which shows that is beer's law were obeyed over the concentration range (50 to 600 µg /25ml) or (2 to 24 $\mu g.ml^{-1}$) of cefotaxime and (25 to 450 µg/25 ml) or (0.8 to 18 $\mu g\ ml^{-1}$) of ceftriaxone with correlation coefficient of 0.9987 and .9974 , an intercept of -0.0344 and 0.0204 ,slope 0.064 and 0.0647 [fig:(2)] and [fig:(3)] respectively. Conditional molar absorptivity of the red product formed with cefotaxime and ceftriaxone were found to be $2.8x10^4$ $l.mol^{-1}.cm^{-1}$ and $4.4x\ 10^4$ l.mol-1.cm-1 respectively.

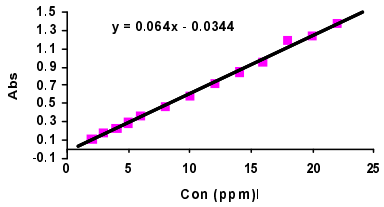


Fig:(2) Calibration curve of cefotaxime

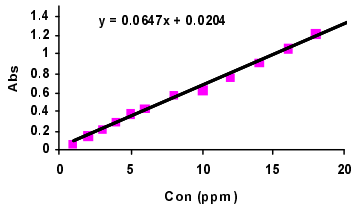


Fig:(3) Calibration curve of ceftriaxone

Accuracy and precision :-

To determine the accuracy and precision of the method cefotaxime and ceftriaxone were determined at three different concentrations . The results obtained are shown in [(Table (1)] and [(Table(2)].A satisfactory precision and accuracy obtained proposed method.

Table (1) :-Accuracy and precision of cefotaxime sodium

Concentration of Cefotaxime µg .ml^{-1}		Recovery % *	R S D %*
Present	Found		
2	2.02	101.00	0.973
10	9.87	98.70	1.080
20	20.35	101.75	0.792

*Average of five determinations .

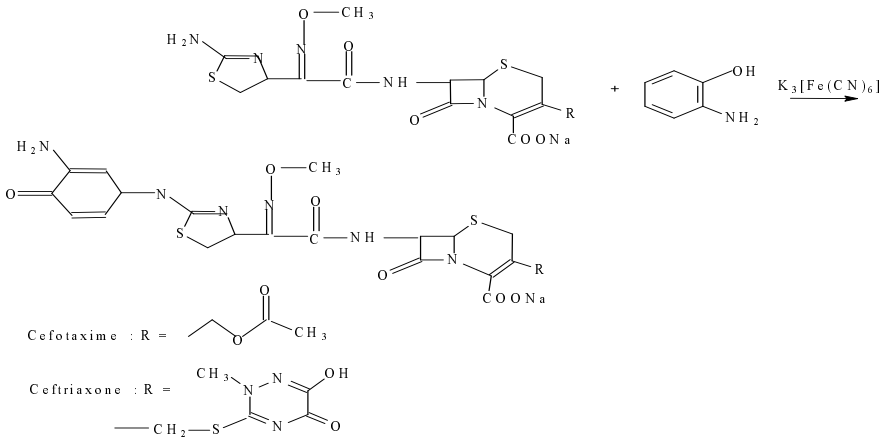
Table (2) :- Accuracy . and precision of ceftriaxone sodium .

Concentration of **C**eftriaxone mg.ml^{-1}		Recovery %*	R S D % *
Present	Found		
1	0.985	98.5	1.121
5	5.08	101.60	0.870
15	15.19	101.27	0.358

*Average of five determinations.

Mechanism of the coupling reaction :-

The stoichiometry of the reaction between cefotaxime or ceftriaxone and o-aminophenol were investigated using job's method . The results obtained (1:1) product were formed between cefotaxime or ceftriaxone and o-aminophenol reagent at 510 nm and 514 nm respectively. The proposed product can be as follows :-



The products formed were soluble in water . The apparent stability constant were calculated by comparing the absorbance of containing stiochimetric amount of cefotaxime or ceftriaxone and o-aminophenol with that of a solution containing a five–fold excess of o- aminophenol reagent . The stability constant of the product in water under the described experimented of condition were ($4.9x10^4 l.mol^{-1}$) and ($6.1x10^4$ $l.mol^{-1}$) respectively

Analytical applications :-

Three types of vials containing cefotaxime and three types of vials containing ceftriaxone have been analyzed and they gave a good accuracy and precision [Table(3)] . The proposed method and official methods [1] were applied to the determination of vials . In the t-test and f-test no significant difference were found between the calculated and theoretical values of the 95% confidence limit of the proposed and official methods [Table (4)] .The investigated method is simpler , faster and more sensitive than of official one .

Table (3):Application of the proposed method for cefotaxime and ceftriaxone in pharma cetitical vials

Drug	Produlct and manufacture	Claimed (mg)	Amount taken ($mg.ml^{-1}$)	R.S.D %*
Cefotaxime	Fotaran (Korea .United Pharm .In). Korea	1000mg	2 6 10	1.98
Cefotaxime	Rametax (Rameda Pharm In). Egypt	500mg	2 6 10	0.86
Cefotaxime	Clphorma (Alpha.pharma).Syria Fosel (Bilim , pharma).	500mg	2 6 10	0.99
Ceftriaxone	Turkey Bioriaxone	1000mg	1 5 10	1.24
Ceftriaxone	(Pharma.Iint.Mfg.Co).Yemen	250mg	1 5 10	1.31
Ceftriaxone	Rozifax (Asia.pharma . Ind).Syria	1000mg	1 5 10	0.65

*average of five determination.

Table (4): Analysis of cefotaxime and ceftriaxone dosage forms using proposed and official method .

Dosage forms(a)	Recovery % (b)		T.tast(c)	F.test(c)
	Proposed method	Official method		
Foltaran	99.85 ±0.77	99.12 ± 0.80	0.84	1.33
Rametax	100.75 ± 0.56	100.81 ± 0.79	0.91	1.59
Clphorama	98.94 ±0.49	98.11 ± 0.68	1.19	1.37
Fosel	100.50 ±1.30	99.37 ± 0.51	1.03	2.17
Bioriaxone	99.08 ±0.95	99.45 ±0.80	1.24	2.53
Rozifax	100.32 ± 0.82	99.51 ±0.73	0.98	1.60

(a) See table (3)
(b) Average of five determinations
(c) The tabled values of t and f-test at 95% confidence limit 2.776 and 6.39.

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