RESEARCH ARTICLE

Determination of Cefotaxime Sodium in Drugs via the use of Six Source of White LEDs Coupled with One Solar Cell as a Detector using CFI Analyser

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Abstract

A direct method is described for the determination of cefotaxime sodium in drugs and pure formulations using six LEDs for turbidimtric measurements and one solar cell as a detector. The method is based on formation of a red-brown precipitate produce via the reaction between cefotaxime sodium with Ce(IV) sulfate at acidic medium. Under the optimum established conditions, the linear range of 0.001-0.3mmol/L along with r of 0.9975, limit of detection (LOD) 35.81ng/sample and precision expressed as relative standard deviation for eight replication measurements at 0.07 and 0.25mmol/L less than 1% were obtained for cefotaxime sodium. The method was successfully applied for the estimation of cefotaxime sodium in three drugs. The newly method using the standard additions procedure and the results have shown that no significant difference between the two methods using ANOVA one way test.

Keywords: *Cefotaxime sodium, Flow injection analysis, Turbidity.*

Introduction

Cefotaxime sodium (Claforan), its chemically; sodium (6R,7R)-3-(acetyloxymethyl)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2 carboxylate (Fig.1) is a 3rd generation cephalosporins (cephalosporins are the most significant antibiotics having ß-lactam ring and obtained from an organism Acremonium, also named cephalosporium) with broad band activity beside gram positive and Gram negative bacteria [1]. It is reflected to be comparable to ceftriaxone in terms of safety and efficiency but cefotaxime does not cause a significant incidence of coagulopathies, as observed with some cephalosporins (e.g.,

cefamandol and cefoperazone) [2]. May increase blood dyscrasias and gastrointestinal as potential toxicities by accumulation of cefotaxime, beta-lactam antibiotic that related to dose usage [3]. A several analytical methods have been reported for the determination of cefotaxime sodium in (pure and pharmaceutical) preparations. These methods include spectrophotometric [4-8], liquid chromatography mass spectrophotometry [9], HPLC with solid-phase extraction [10], chemiluminescence coupled with flow injection analysis [11], fluorometric-flow injection analysis [12], turbidimetric-flow injection analysis [13].
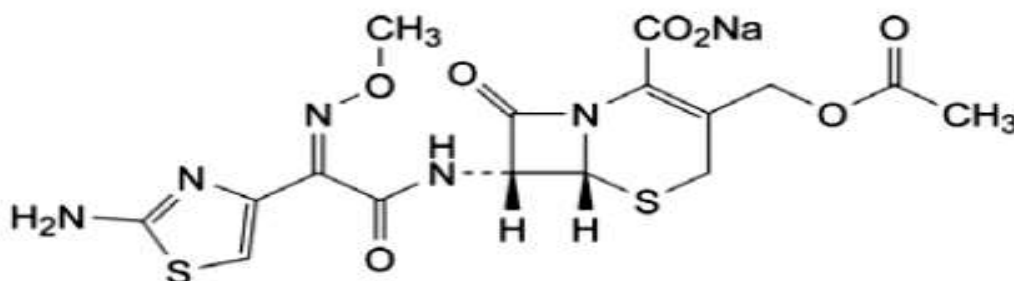

Figure 1: Chemical structure of cefotaxime sodium

In the present study, turbidimetric flow injection analyser was used as a sensitive and simple method for the determination of cefotaxime via the recorded of the signal from the attenuation of incident light by the formation of the solid particulate using of Ayah 6SX1-T-1D-CFI analyser[14].

Experimental

Chemicals

- Cefotaxime sodium 0.05 mol/L (477.447 g/mol, SDI, Iraq).Dissolve 2.3872g of $C_{16}H_{16}N_5NaO_7S_2$ in distilled water (100mL volumetric flask).

- Ceric sulfate 0.1 mol/L (332.298 g/mol, Hopkin & Williams).Dissolve 3.3229g of $Ce(SO_4)_2$ in H_2SO_4(1mol/L) (100mL volumetric flask).

- -Sulfuric acid 1mol/L (98%, 1.84g/mL, 98g/mol, BDH). Pipetting 0.05mL of concentrated H_2SO_4 and completed the volume with distilled water to 1L capacity of volumetric flask (Standardization with Na_2CO_3).

Apparatus and Manifold

The manifold used in this work (Fig.2) is consisting of:

- Two channel of peristaltic pump (ISMATEC , Switzerland)

- Rotery six port injection valve (IDEX corporation, with a variables loop sample: 0.7mm i.d. Teflon, USA, different length).

- Ayah 6SX1-T-1D solar cell CFI analyser (homemade) [14] which is made to measure the turbidity at 0-180° angle via the irradiation with a white snow LED that is repeated for six locations in adjacent successive positions.

- X-t plotter (potentiometric recorder, Siemens, Germany, 1-500 volt or 1-500 mV).
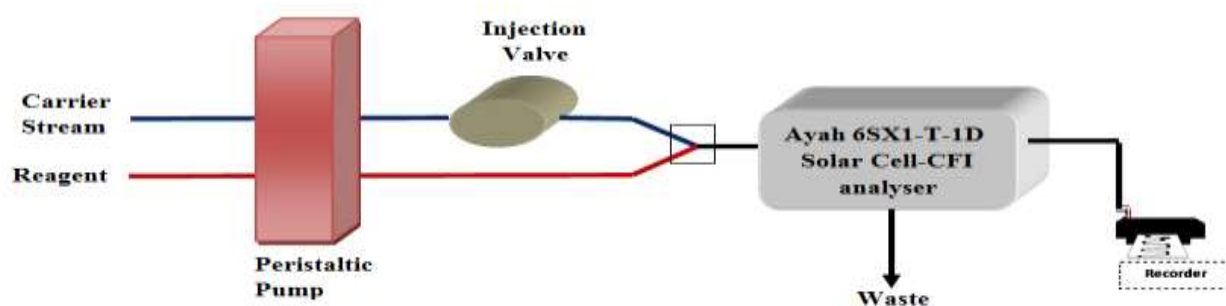

Figure2: Flow gram for the determination of cefotaxime sodium

Methodology

The manifold reaction (Fig.2) used in this paper for the reaction of cefotaxime sodium with Ce(IV) sulfate at acidic medium is consist of two lines : first line supplied the distilled water (carrier stream) transfer to the injection valve , which permits the use of 150μL (0.25 mmol/L cefotaxime sodium) and 1.9mL/min (flow rate) while the another line supply the $Ce(SO_4)$ (0.7 mmol/L were prepared in 0.3mol/L H_2SO_4) at 2.3 mL/min. Both lines meet together at Y-junction before it enters to the turbidimetric CFI analyser to form red brown precipitate. A proposed mechanism [15, 16] shown in the following Scheme.

(Red-brown precipitate)
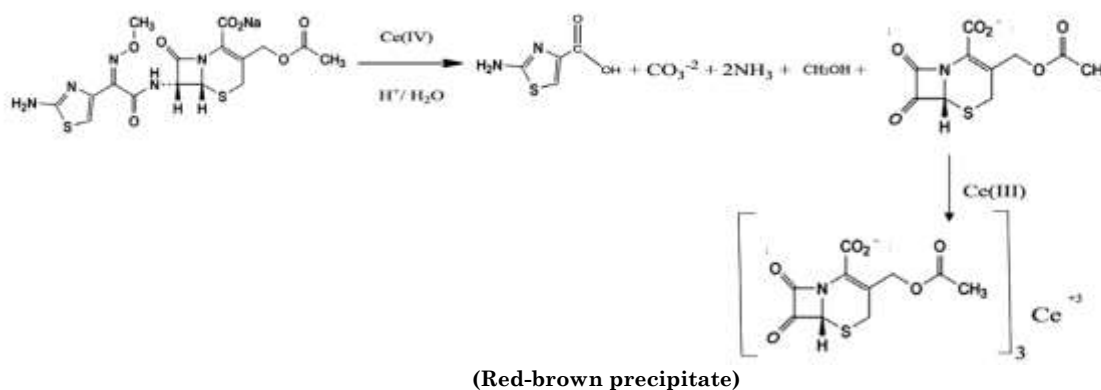
Scheme 1: Schematic representation the mechanism for the reaction between Ce (SO₄) with cefotaxime sodium in acidic medium

Results and Discussion

Chemical Variables

Ce (IV) Sulfate Concentration

Series of precipitating reagent ranging from 0.05-1mmol/L were prepared in 0.5mol/L H_2SO_4.0.25mmol/L cefotaxime sodium (125μL, open valve mode) was used at 1.5 and 2mL/min flow rate for line no.1 and line no.2 respectively with intensity of light emitting diode (6LEDs) was 1.652V DC. It was found that 0.7mmol/L of Ce(IV) sulfate was necessary to obtained the apex of S/N response as shown in table 1 and fig.3. Above of 0.7mmol/L lead to decrease of S/N response which it most probably attributed to increase density of particles of red-brown precipitate. Therefore 0.7mmol/L of Ce(IV) sulfate was used for the next studies.

Table 1: Summarize of results indicating: [Ce (IV) sulfate], S/N response, RSD% and confidence interval at 95%

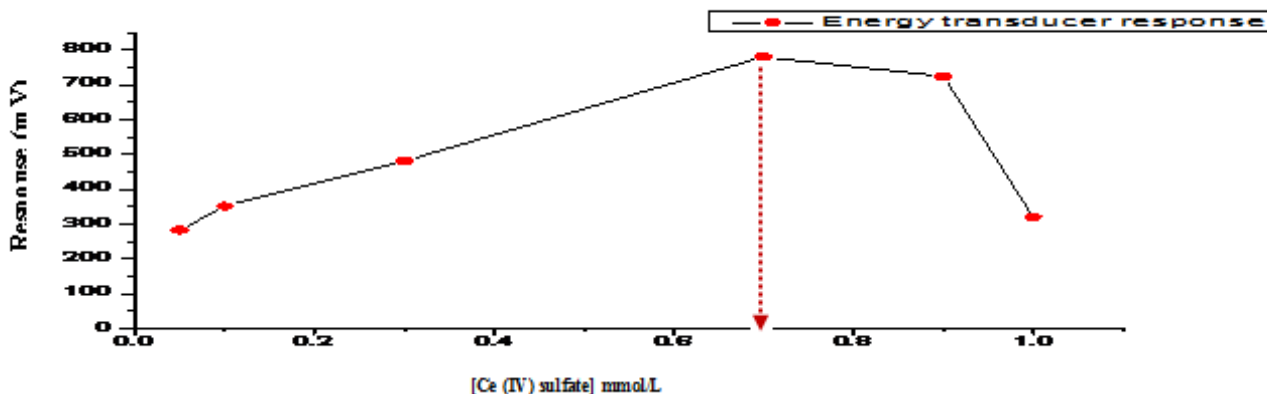| [Ce (IV) sulfate] mmol/L	Height of response output (n=3) ȳi (mV)	RSD%	$\bar{y}i \pm t_{0.05/2,n-1} , \sigma_{n-1}/\sqrt{n}$
0.05	283	0.45	283±3.18
0.1	352	0.38	352 ± 3.33
0.3	482	0.20	482± 2.43
0.7	781	0.16	781± 3.03
0.9	725	0.17	725±3.08
1	321	1.07	321±8.50

Figure 3: Variation of [Ce(IV) sulfate] versus S/N response output

H_2SO_4 Concentration

Variable concentration of H_2SO_4 ranging from 0.05-0.5mol/L was prepared at constant Ce(IV) sulfate concentration (0.7mmol/L) using 0.25mmol/L cefotaxime sodium(125μL sample segment). A flow rate of distilled water and Ce (IV) sulfate stream were 1.5 and 2mL/min respectively. It can be seen from table 2 and fig.4 that 0.3mol/L of H_2SO_4 was the most suitable concentration for all subsequent experiments because at higher concentration more than 0.3mol/L mostly causing increase solubility of some of the precipitate particles and minimize the sensitivity.

Table 2: Summarize of results indicating: [H₂SO₄], S/N response, RSD% and confidence interval at 95%

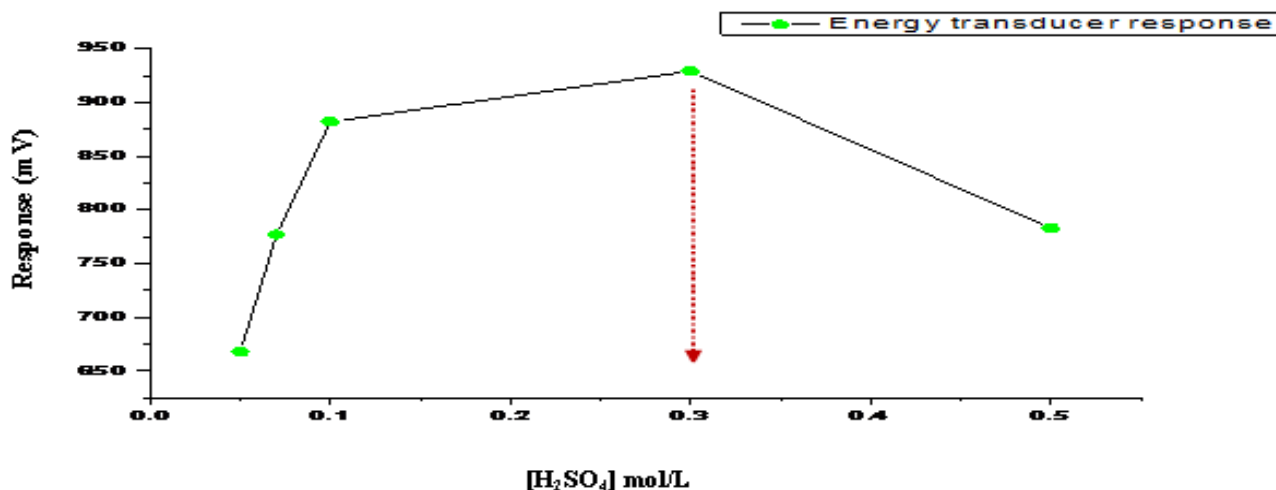| [H₂SO₄] mol/L	Height of response output (n=3) ȳi (mV)	RSD%	$\bar{y}i \pm t_{0.05/2,n-1} , \sigma_{n-1}/\sqrt{n}$
0.05	668	0.23	668±3.83
0.07	777	0.26	777 ± 4.97
0.1	882	0.14	882± 3.06
0.3	929	0.13	929± 3.11
0.5	783	0.33	783±6.38

Figure 4: Effect of the [H₂SO₄] on S/N response

Physical Variables

Flow Rate

The study dealing with the effect of variation of flow rates 0.9-2.3mL/min for distilled water line and 1.0-3.0 mL/min for Ce(IV) sulfate line (0.7mmol/L) for determination of cefotaxime sodium at 0.25mmol/L (125µL,open valve mode). Table 3 shows at low flow rate increase dispersion and more dilution with expanding of time spent in front of one solar cell as a detector (distribution of precipitate segment at flow cell). While at >1.9 mL/min and 2.3mL/min of line no.1 and line no.2 respectively decrease in peak height due to decrease of time reaction (analysis time for formation of precipitate) is very short (decrease in base width). Therefore the best flow rate that give a regular and sharp peak height with best time for formation of red-brown precipitate and distribution at flow cell in front of detector (t=30sec) is 1.9 mL/min and 2.3mL/min.

Table 3: Effect of flow rate on responses of Cefotaxime sodium (0.25mmol/L)-Ce(IV)sulfate(0.7mmol/L)-H₃O⁺(0.3mol/L)

Flow rate (mL.min⁻¹)		Height of response output (n=3)	RSD%	$\bar{y}i \pm t_{0.05/2,n-1}, \sigma_{n-1}/\sqrt{n}$	Response base width Δt_B (sec)
Carrier stream	Reagent line	$\bar{y}i$ (mV)			
0.9	1.0	900	0.22	900±4.99	180
1.2	1.1	880	0.19	880±4.32	100
1.3	1.5	920	0.18	920±4.01	60
1.5	2.0	930	0.16	930±3.75	40
1.9	2.3	1080	0.12	1080±3.13	30
2.0	2.5	1000	0.14	1000±3.35	28
2.3	3.0	998	0.15	998±3.75	27

Sample Volume

Using cefotaxime sodium (0.25mmol/L)-Ce(IV) sulfate (0.7mmol/L) system ,the parameters optimum in previous sections with variable sample volume extended from 75-250µL were studied. tabulated all results in table 4 which represent that the higher recorder reading at 150µL that give the best time duration of red-brown segment particles in front of one solar cell(detector) (optimum Δt_B=35sec,fig.5) comparing with 200 and 250µL (base width of response =45,50sec respectively) which causing slow movement thus 150µL is the best.

Table 4: Effect of sample volume on responses of Cefotaxime sodium (0.25mmol/L)-Ce(IV)sulfate(0.7mmol/L)-H₃O⁺(0.3mol/L)system

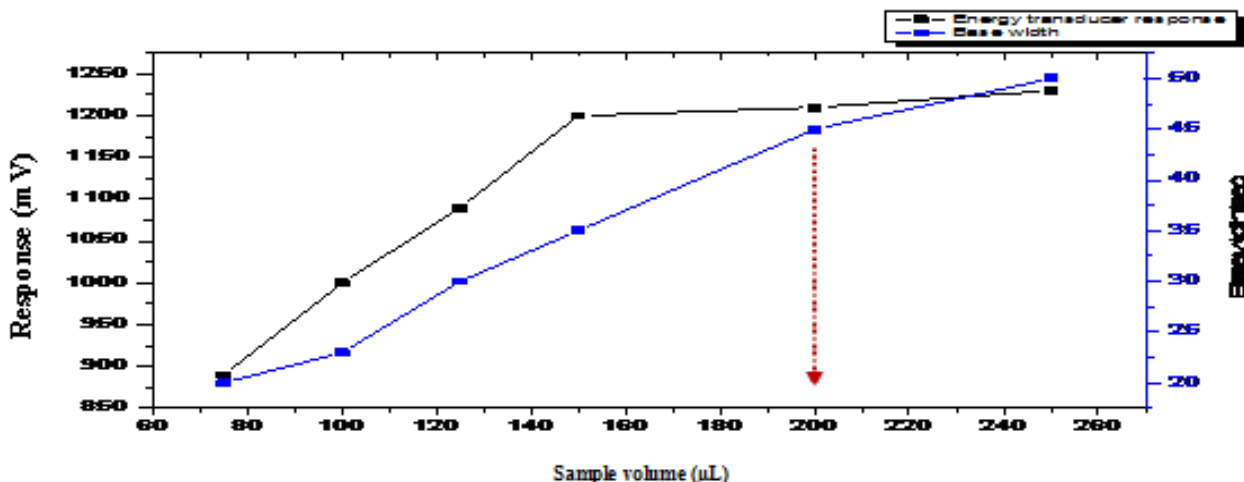| Sample volume µL	Height of response output (n=3) $\bar{y}i$ (mV)	RSD%	$\bar{y}i \pm t_{0.05/2,n-1}, \sigma_{n-1}/\sqrt{n}$	Response base width Δt_B (sec)
75	890	0.29	890±6.36	20
100	1000	0.26	1000±6.51	23
125	1090	0.23	1090±6.24	30
150	1200	0.18	1200±5.44	35
200	1210	0.14	1210±4.35	45
250	1230	0.14	1230±4.20	50

Figure 5: Variation of sample volume versus S/N response output

Purge Time

A set of experimental was carried out for select of the optimum purge time (variable 10-30sec). It was noticed as shown in table 5 and fig.6 that increases in peak height with increase the time for sample segment evacuation from sample loop that will be attributed to giving enough time for reaction between cefotaxime sodium with ceric sulfate to formation of red brown precipitate so that the open valve mode is most, favorable choice to give the highest response and will induce sharp and smooth apex responses.

Table 5: Effect of purge time on responses of Cefotaxime sodium (0.25mmol/L)-Ce(IV)sulfate(0.7mmol/L)-H_3O^+(0.3mol/L) system

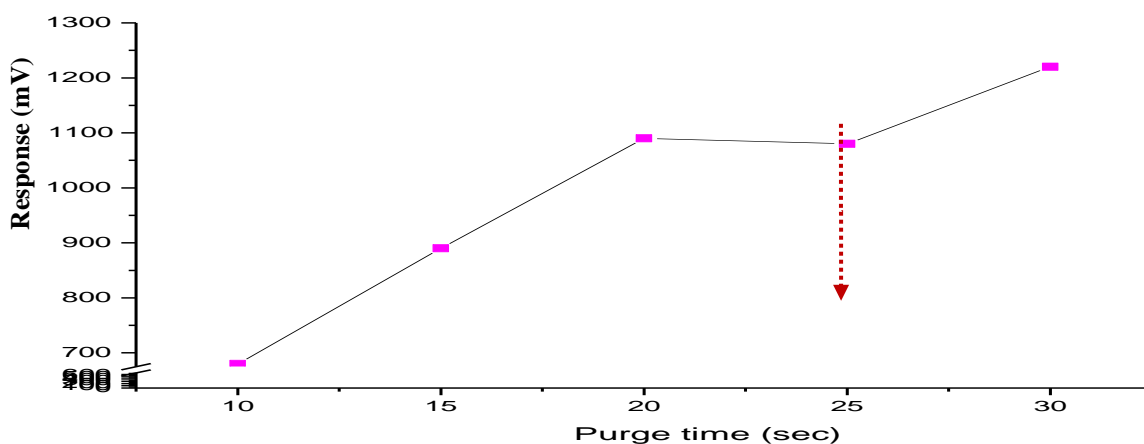| Purge time (sec)	Height of response output (n=3) $\bar{y}i$ (mV)	RSD%	$\bar{y}i \pm t_{0.05/2, n-1}, \sigma_{n-1}/\sqrt{n}$
10	680	0.21	680±3.60
15	890	0.16	890±3.53
20	1090	0.14	1090±3.75
25	1080	0.31	1080±8.25
30(open valve)	1220	0.10	1220±3.03

Figure 6: Variation of purge time Effect of purge time on responses of Cefotaxime sodium (0.25mmol/L)-Ce(IV)sulfate(0.7mmol/L)- H_3O^+(0.3mol/L) system

Calibration Curve

Various concentrations 0.001-0.3mmol/L of cefotaxime sodium were prepared by using the parameters achieved above. Table 6 summarized all results of the linear regression analysis (Fig.7) including: coefficient of determination of 0.9951 and correlation coefficient of 0.9975 with linearity percentage r^2%= 99.51 and tabulates all the data for the classical method [13]

(spectrophotometric method via measurement of λ_{max} at 260nm).

Table 6: Summary of calibration graph results using developed method and classical method

Range of cefotaxime sodium mmol/L	$\hat{Y}=a\pm s_a t+b\pm s_b t$ cefotaxime sodium mMol.L^{-1} at confidence level 95%,n-2	r r^2 $r^2\%$	t_{tab} at 95%,n-2	$t_{cal} = \dfrac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$
0.001-0.3 (n= 13)	107.05±28.79+4613.69±214.79[cefotaxime sodium]mmol/L	0.9975 0.9951 99.51	2.201<<47.27	
0.03-0.35 (n=10)	0.448±0.039+3.836±0.203 [cefotaime sodium]mmol/L	0.9979 0.9958 99.58%	2.306 << 34.618	

[X]=Cefotaxime sodium mMol.L^{-1}, $\hat{Y}$=estimate value , r = correlation coefficient

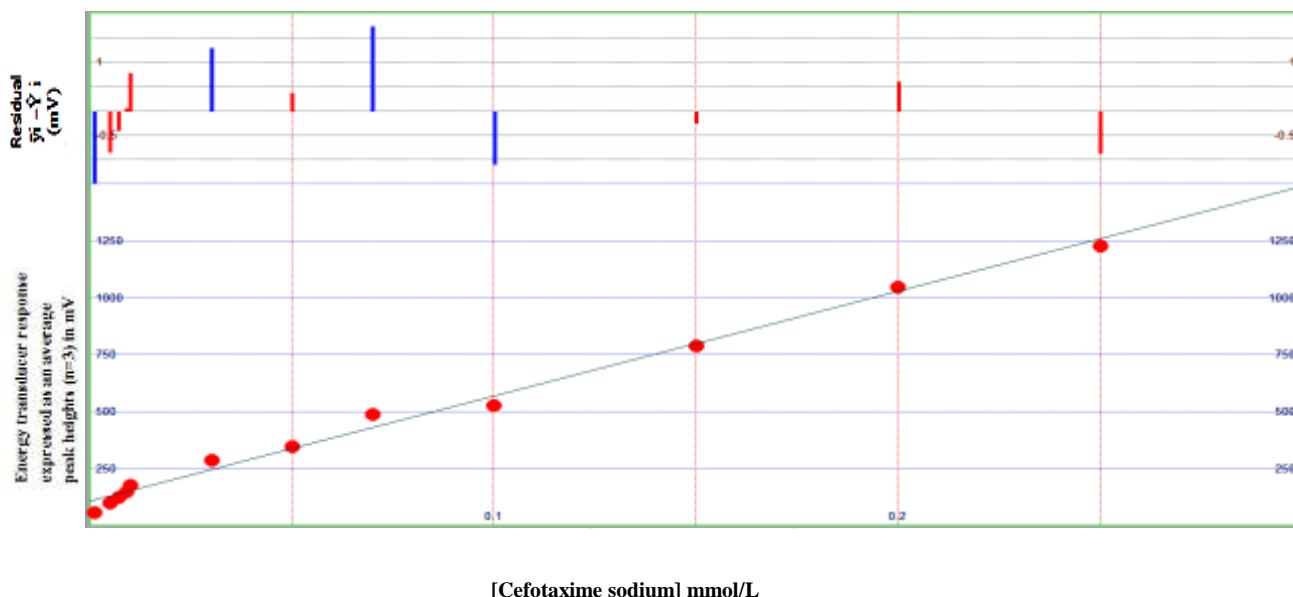$r^2\%$ = Linearity percentage, r^2= coefficient of determination (C.O.D)

Figure 7: Straight line for the variation of cefotaxime sodium concentration versus output response using turbidity measurements

Limit of Detection (LOD)

Detection limit is calculated from the gradual dilution of the minimum concentration of the used calibration graph and depend on the values of slope. The volume of the sample used was 150µL. Table 7 summarized all calculations obtained.

Table 7: LOD for the determination of cefotaxime sodium

Practically based on the gradual dilution for the minimum concentration (0.0005 mMol.L^{-1})	Theoretical based on the value of slope x=3S$_B$/slope for n=13
35.81ng/sample	9.52ng/sample

X= value of L.O.D. based on slope , S$_B$= standard deviation of blank repeated for 13 times .

Repeatability

The reality and repeatability of continuous flow injection method (Ayah 6SX1-T-1D solar cell CFI analyser) that using for the determination of cefotaxime sodium was studied at 0.07 and 0.25mmol/L concentrations for eight replication measurements. All results obtained are

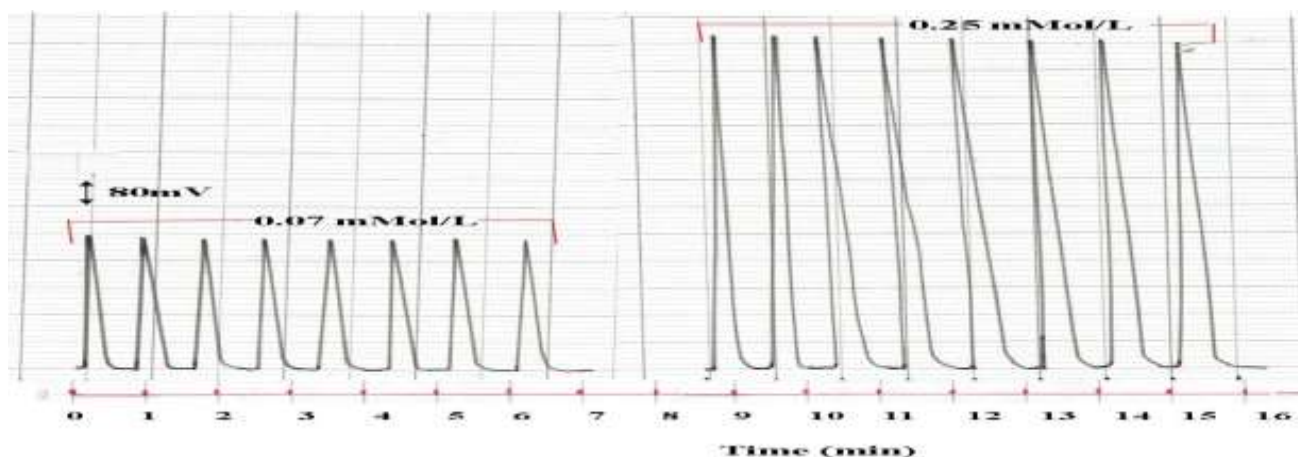summed up in Table 8 while Fig.8 shows a   kind of plotted response.

Figure 8: Response profile for eight replication measurements of cefotaxime sodium using 0.07 and 0.25mmol/L

Table 8: Repeatability of the determination of cefotaxime sodium using Cefotaxime sodium (0.07 and 0.25mmol/L)-Ce(IV)sulfate(0.7mmol/L)- H_3O^+(0.3mol/L) system

Concentration mmol/L n=8	Height of response output (n=3) $\bar{y}i$ (mV)	RSD%	$\bar{y}i \pm t_{0.05/2,n-1} , \sigma_{n-1}/\sqrt{n}$
0.07	490	0.27	490±1.10
0.25	1230	0.16	1230±1.66

$t_{0.05/2, 7=2.365}$, **Number of injection = 8**

Analysis of Pharmaceutical Preparation

Using the newly method that is capable to detect any attenuated or reflected or scattered light from the formed precipitate particulate and classical method (estimation of cefotaxime sodium by UV-spectrophotometer) achieved in this work were used for the analysis of cefotaxime sodium in three different pharmaceutical preparations (Table 9) . A series of solution were prepared of each pharmaceutical drug (0.05mmol/L, 2.378mg) by transferring 2.5mL to each volumetric flask(25mL,n=5) followed by addition of 0.0,2.0,2.5,3.5and 4.5mL from 0.05mmol/L of cefotaxime sodium(standard solution) in order to obtain the concentration range 0-0.009mmol/L to constructed the standard additions calibration curve. Table 9 tabulates all results obtained were mathematically treated for additions method by developed and classical method.

Table 9: Summary of results obtained by standard addition procedure using classical and developed method

Sample No.	Commercial name content and company country	Reliability of W_i (g)	Weight of sample (g)	Standard addition equation $\hat{Y}_i = a\pm s_a t + b \pm s_b t[x]$ r,r²,r²%	Content (Theoretical) (g)	Practical content(g) W_i(mg)	Recovery %
1	CEFOTAXIME-LD P- Spain	1.0234±0.044	0.0024	49.23±17.66+9434.78±3022.39[cefotaxime sodium]mmol/L 0.9851,0.9705,97.05%	1±0.099	1.0430±3.528 2.497	104.6
				0.584 ±0.146 + 12.228±2.062 [cefotaxime sodium] mmol/L 0.9958,0.9917,99.17%		0.9601±2.123 2.2918	96.01
2	CETAXAUROBINDO-India	0.9986±0.055	0.0024	67.42±8.18+13956.52±1399.95[cefotaxime sodium]mmol/L 0.9985,0.9970,99.70%	1±0.125	0.9986±3.826 2.2917	96
				0.539± 0.051+12.678 ± 0.694 [cefotaxime sodium] mmol/L 0.9995,0.9991,99.91%		0.8599±2.081 2.0530	85.99
3	Claforan-Sanofi aventis-Franc	1.2345±0.1026	0.0029	62.88±7.03+12543.48±1199.58[cefotaxime sodium]mmol/L 0.9986,0.9973,99.73%	1±0.189	1.2345±1.732 2.392	100.2
				0.625±0.130 + 11.820± 1.817 [cefotaxime sodium] mmol/L 0.9965,0.9930,99.30%		1.0600±1.821 2.5305	106

Treatment of data were subjected using ANOVA one way test for comparing two methods of analysis: newly developed method with classical method as shown in Table 10.

Table 10: ANOVA results for comparison between different methods fo determination of cefotaxime sodium

Source	Sum of Squares (SSq)	df	Mean Square (MSq)	F_{cal}	F_{tab}	Sig.
Between group	$SS_B = 0.028$	2	$MS_B = 0.014$			
Within groups	$SS_W = 0.051$	6	$MS_W = 0.009$	1.604<<5.14		0.277
Total	0.079	8				

The statistical analysis of results shows the value of sig(0.277)>>(0.05) (F_{cal}=1.604<F_{tab}=5.14) therefore Null hypothesis(there is no significant difference between newly and classical method) will be accepted and will rejected the alternative hypothesis (there is a significant difference between two methods).

Conclusion

The newly method is sensitive, rapid and simple for the analysis of cefotaxime sodium in different formulations based on formation red brown precipitate form the reaction of cefotaxime sodium with Ce(IV) sulfate in acidic medium. The statistical test (ANOVA one way) showed no significant difference between developed method and classical method for the analysis of cefotaxime sodium with RSD% less than 1%.

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