A novel spectrophotometric for the determination of cephalosporins using 8-hydroxy-1, 3, 6-pyrenetrisulfonic acid trisodium salt (HPTS) as a chromogenic reagent

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Abstract
A simple, accurate and precise spectrophotometric method has been proposed for the determination of three cephalosporins, namely; cefixime (cefi), cephalexin (ceph) and cefotaxime sodium (cefo) in pharmaceutical formulations. The proposed method is based on the derivatization of cephalosporins with 1-hydroxy-3, 6, 8-pyrenetrisulfonic acid trisodium salt, (HPTS). The optimum experimental conditions have been studied carefully. Beer's law is obeyed over the concentration of 0.1-0.6, 0.3-1.8 and 0.5-3 µg/mL for cefi, ceph and cefo, respectively.
The detection limits were found to be 0.03, 0.14 and 0.15 µg/ mL for cefi, ceph and cefo, respectively, with a linear regression correlation coefficient of 0.99902, 0.99728 and 0.99892 for cefi, ceph and cefo, respectively. And recovery in range form 97.57-102.45, 91.61-95.6 and 96.25-103.85 for cefi, ceph and cefo, respectively. Effects of pH, temperature, standing time and HPTS concentration on the determination of cefi, ceph and cefo, have been examined. This method is simple and can be used for the determination of cefi, ceph and cefo in pharmaceutical formulations.

Keywords: *Cephalosporins, 1-hydroxy-3, 6, 8-pyrenetrisulfonic acid trisodium salt, (HPTS), Spectrophotometric analysis, Pharmaceutical analysis.*

1.Introduction

Cephalosporins anti bacterial are commonly used to control gram positive and gram negative activity. Cephalosporins are the second most important blactams after penicillins for treating infectious diseases (Adkinson,1998). Many of these manifestations, such as urticaria and exanthema, are cutaneous, but anaphylactic reactions have also been reported (Pumphrey Davis, 1999).
Chemical structure of cephalosporins drive from the 7-aminocephalosporanic acid (7-ACA) composed of a β-lactam ring fused with a dihydrothaizine ring (Fig. 1), but differ in the nature of substituent at the 3- and/or 7-positions of the cephem ring (Delgad and Wilson; 2004; Dollery,1999).
Many methods have been described for the quantitative determination of cephalosporins included spectrophotometry (Saleh et al., 2001;2003; Ayad et al., 1999; Ahmed et al., 2011, spectrofluorometery (Elbashir et al., 2012; Aly et al., 1996). High performance liquid chromatography (Misztal, 1998; Moore, 1991; Baranowska et al., 2006; Tsai and Chen 2006; De Diego Glaría, et al., 2005; Sørensen and Snor, 2000; Chen et al., 2003) , potentiometry (Lima et al., 1998) and voltammetry (Özkanet al., 2000). These methods were time-consuming, tedious, and dedicated to sophisticated and expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Unfortunately, the spectrophotometric methods that have been reported for determination of cephalosporins in their pharmaceutical formulations were associated with some major disadvantages such as the lack of selectivity, tedious extraction procedures and time-consuming. The official procedures in pharmaceutical preparations utilize high performance liquid chromatography (HPLC) (United States Pharmacopoeia 2008). Therefore, the development of new alternative spectrophotometric method for the determination of cephalosporins that can overcome the disadvantages of the existing methods was very essential.
The pH-sensitive fluorescent dye 1-hydroxy-3, 6, 8-pyrenetrisulfonic acid trisodium salt also known as pyranine (HPTS) has distinct absorption band in the visible light region, and has thus utilized in many aqueous-phase CO2 optical sensors (Weigl and Wolfbeis, 1995; Wolfbeis et al., 1998; Neurauter et al., 1998; Ertekin et al., 2003; Malins , and MacCraith, 1998; Von Bultzingslowen et al., 2002; Nivens et al., 2002; Naga et al., 1998). However this is the first time that HPTS used as chromogenic reagent for determination of cephalosporins in their

pharmaceutical formulations, HPTS contains three sulfonate groups at the positions, 1, 3, 6 which make it suitable for nucleophilic substitution reactions with cephalosporins, therefore in this work a rapid spectrophotometric method for determining the content of cefi, ceph and cefo in pharmaceutical formulations which is based on the reaction of HPTS with cephalosporin was reported.

2. Experimental

2.1. Apparatus

All of the spectrophotometric measurements were made with a Double beam UV1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU Japan) also temperature controller was used for the spectrophotometer measurements. pH meter model pH 211(HANNA Italy) was used for adjusting pH.

2.2. Reagents and solutions

All reagents were of analytical reagent grade. Double distilled water was used in all experiments.
The standards of cefi, ceph and cefo were supplied by (Orchid Chemicals and Pharms LTD).
HPTS was supplied by (Aldrich chemical Co., St. Louis, USA)

2.2.1. Pharmaceutical formulation.

The following available commercial preparations were analyzed:
(1) cefi capsules (AMIPHARMA laboratories, Sudan), labeled to contain 200 mg cefi per capsule.
(2) ceph monohydrate capsules(AMIPHARMA laboratories, Sudan), labeled to contain 500 mg ceph per capsule.
(3) cefo for injection (KILITCH drugs, India) labeled to contain 1000 mg cefo per injection.

2.2.2. Stock standard solution of cefi,ceph and cefo (1000µg/mL)

An accurately weighed 0.1000 g standard sample of the three drugs was dissolved in methanol for cefi and in double distilled water for ceph and cefo, transferred into a 100 mL standard flask and diluted to the mark with methanol for cefi and with double distilled water for ceph and cefo and mixed well. This stock solution was further diluted to obtain working solutions in the ranges of 0.1-0.6, 0.3-1.8 and 0.5-3 µg/ mL for cefi,ceph and cefo, respectively.

2.2.3. 1-hydroxy-3, 6, 8-pyrenetrisulfonic acid (0.02%, 0.025% w/v)

An accurately weighed 0.02 and 0.025 g of HPTS was dissolved in double distilled water, transferred into a 100 mL standard flask and diluted to the mark with double distilled water and mixed well to prepare(0.02% and 0.025% w/v), respectively. The solution was freshly prepared and protected from light during use.

2.2.4. Buffer solutions

Buffer solution of pH 12.0 was prepared by mixing 25 mL of 0.2 M KCl with 12 mL of 0.2 M NaOH, and buffer of pH 13.0 was prepared by mixing 25 mL of 0.20 M KCl solution with 65 mL of 0.20 M NaOH solution, in 100 mL volumetric flask and adjusted by a pH meter. Buffer solutions of different pH value were also prepared.

2.2.5. Sample Solutions

The contents of 20 capsules or the contents of 20 injection powder were evacuated and well mixed. Then an accurately weighed amount equivalent to 100 mg was transferred into a 100 mL calibrated flask, and dissolved in about 40mL in methanol for cefi and in double distilled water for ceph and cefo. The contents of the flask were swirled, sonicated for 5 minutes, and then completed to volume with methanol for cefi and with double distilled water for ceph and cefo. The contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with methanol for cefi and with double distilled water for ceph and cefo to obtain a suitable concentration for the analysis.

2.2.6. General recommended procedure

About 1.00 mL of (1-6, 3-18 and 5-30 µg/mL) for cefi, ceph and cefo, respectively, were transfer in to 10mL volumetric flask subsequently, 3mL of pH 12.0 for cefi,3 and 2mL of pH 13.0 for ceph and cefo respectively were added and 1 mL of 0.025% HPTS were added for cefi and 1 mLof 0.02% HPTS solution was added for ceph and cefo the solution was heated in a thermostat at 80℃ for 15 minutes, at 85℃ for 10 minutes and at 60 for 15 minutes for cefi ,ceph and cefo respectively, the mixture was diluted with methanol for cefi or double distilled water for ceph and cefo. The absorbance of the solution was measured at 470,480 and 479 nm for cefi, ceph and cefo respectively against a reagent blank prepared in the same manner but containing no drugs.

2.2.7. Determination of the stoichiometric ratio of the reaction (Job's method)
The Job's method of continuous variation was employed (Job, 1928). Equimolar (5×10−3 M) methanolic solutions of cefi and aqueous solution for ceph, cefo and HPTS were prepared. Series of 10-mL portions of the master solutions of cefi, ceph and cefo and HPTS were made up comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3,8:2, 9:1, 10:0), The solution was further treated as described under the general recommended procedures.

3. Results and discussion

3.1. Absorption spectra

As shown in (Fig. 2) the absorption spectrum of HPTS in aqueous solution shows a maximum absorption at 400nm. The investigated drugs has no absorption in the range 400-800 nm, taking cefo as a representative example it gives absorption maximum at 237 nm, the interaction colored product of cefo with HPTS shows absorption maximum at 479 nm.

3.2. Determination of stoichiometric ratio

Under the optimum conditions Table.2. The stoichiometric ratio between HPTS and each of investigated cephalosporins was found to be 1: 1 (Fig.3). Based on this ratio, the reaction pathways were postulated to be proceeded as shown in scheme 1. cefi, ceph and cefo were found to be susceptible for reaction with HPTS producing agreen color products.

3.3. Optimization of Derivatization reaction and Spectrophotometric procedure

3.3.1. Effect of pH

The effects of pH on the reaction of cefi, ceph and cefo with HPTS were examined by varying the pH from 4.0 to 13.0, The results revealed that cefi, ceph and cefo have difficulty to react with HPTS in acidic media (Fig. 4).This was possibly due to the existence of the amino group of cefi, ceph and cefo in the form of hydrochloride salt, thus it loses its nucleophilic substitution capability. As the pH increased, the readings increased rapidly, as the amino group of cefi, ceph and cefo (in the hydrochloride salt) turns into the free amino group, thus facilitating the nucleophilic substitution (Darwish et al., 2005). The maximum readings were attained at pH values of 12.0 for cefi and 13.0 for ceph and cefo. At pH values more than 12.0 for cefi and more than 13.0 fore ceph and cefo a decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ions that hold back the reaction of cefi, ceph and cefo with HPTS.

3.3.2. Effects of reaction temperature and time

The effect of temperature on the reaction was also studied by varying the temperature from 25 °C to 90 °C for cefi ,ceph and cefo. The reaction does not go in room temperature and the highest absorbance is obtained at 80 °C for 15 minutes for cefi and at 85°C for 10 minutes for ceph and at 60 for 15 minutes for cefo °C. (Figs. 5 and 6).

3.3.3. Effect of HPTS concentration

The studying of HPTS concentrations revealed that the reaction was dependent on HPTS reagent. The highest absorption was attained when the concentration of HPTS was 0.025% for cefi and 0.02% for ceph and cefo. (Fig. 7). From the previously described experiments the optimum conditions for the reaction of HPTS with cefi, ceph and cefo were summarized in Table 2.

3.4. Validation of the Method

3.4.1. Linearity and Limits of Detection.

In the proposed methods, linear plots (n = 6) with good correlation coefficients were obtained in the concentration ranges of 0.1-0.6, 0.3-1.8 and 0.5-3 µg/mL for cefi, ceph and cefo, respectively Table 3. The limits of detection (LOD) and quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ =3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope (ICH, 1996). The LOD values were 0.03, 0.14 and 0.15 µg / mL for cefi, ceph and cefo, respectively.

3.4.2. Accuracy and precision

The accuracy and precision of the proposed spectrophotometric method were determined at three concentration levels of cefi, ceph and cefo by analyzing three replicate samples of each concentration. The relative standard deviations (R.S.D.) for the results did not exceed 3% Table 4, proving the high reproducibility of the results and the

precision of the method. This good level of precision was suitable for quality control analysis of cefi, ceph and cefo in their pharmaceutical formulations.

3.4.3. Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were shown in Table 5.

3.5. Application of the Method

The proposed method was applied to some pharmaceutical formulations containing cefi, ceph and cefo. The results in Table 6 indicate the high accuracy of the proposed method for the determination of the studied drugs. The proposed method has the advantage of being virtually free from interferences by excipients. The percentages were 102.40 ± 0.439, 101.81± 1.24 and 97.60 ± 1.15 for cefi, ceph and cefo, respectively Table 6. This results were compared with that obtained by reported spectrophotometric method (Saleh et al., 2003) by statistical analysis with respect to the accuracy (by t-test) and precision (by F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the determination of cephalosporins by both methods.

4. Conclusion

The present paper described for the first time the application of HPTS as analytical reagent in the development of simple, sensitive, and accurate spectrophotometric method, for the determination of cefi, ceph and cefo in pharmaceutical formulations. The described method is superior to the previously reported spectrophotometric methods in terms of the simplicity and sensitivity. The proposed method has comparable analytical performances and devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of cefi, ceph and cefo in quality control laboratories.

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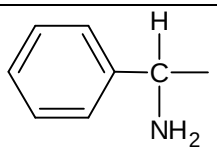
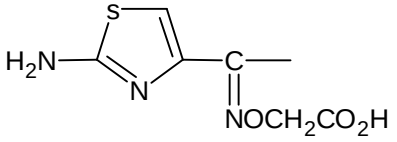
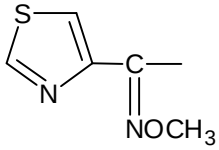
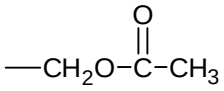
Table 1 chemical structure of the investigated cephalosporin antibiotics.

name	R1	R2	R3	generation
cephalexine		-CH_3	-H	first
cefixime		-$CH{=}CH_2$	-H	third
cefotaxime sodium			-Na	third

Condition	cefi	ceph	cefo
pH	12.0	13.0	13.0
Volume of buffer	3 ml	3 ml	2 ml
Temperature	80 °C	85 °C	60 °C
Reaction time	15 minutes	10 minutes	15 minutes
HPTS concentration	0.025%	0.02%	0.02%

Table 2 Optimum conditions for the reaction of cefi, ceph and cefo with HPTS

Table 3 Summary of quantitative parameters and statistical data using the proposed procedure

parameter	drug HPTS derivatives		
	Cefi	ceph	cefo
Linear range(µg/ml)	0.1-0.6	0.3-1.8	0.5-3
LOD(µg/ml)	0.03	0.14	0.15

LOQ (µg/ml)	0.09	0.43	0.45
Slope	0.29971	0.1219	0.16086
Intercept	0.07327	0.21533	0.00967
Correlation coefficient(r)	0.99902	0.99728	0.99892
Molar absorptivity, ε (L mol^{-1} cm^{-1})	1.5×10^{6}	1.08×10^{6}	4.9×10^{5}

Table 4 Recovery of the proposed methods

Drug	sample content (µg/mL)	added (µg/mL)	found (µg/mL)	recovery (% ± RSD)*
Cefi	0.1	0.2	0.29	97.57 ± 1.24
	0.1	0.3	0.407	101.82 ± 0.769
	0.1	0.4	0.512	102.45 ± 0.335
Ceph	0.2	0.2	0.366	91.61 ± 0.384
	0.2	0.8	0.94	94.33 ± 0.45
	0.2	1.4	1.53	95.60 ± 0.37
Cefo	0.3	0.3	0.61	101.67 ± 0.463
	0.3	1.3	1.54	96.25 ± 1.93
	0.3	2.3	2.7	103.85 ± 1.12

* Recovery was calculated as the amount found/amount taken×100. Values are mean ± R.S.D. for 3 determinations

Table 5 Robustness of the proposed spectrophotometric method.

Recommended condition	cefi	Recovery% ± SD*	ceph	Recovery% ± SD*	cefo*	Recovery% ± SD*
Standard		98.90 ± 1.08		99.28 ± 1.60		99.60 ±1.00
pH	11.8	99.17 ± 0.26	12.8	105.19 ± 2.04	12.8	95.36 ± 2.88
	12.2	103.61 ± 0.21	13.2	95.60 ± 0.80	13.2	96.81 ± 1.09
HPTS concentration (wt/v %)	.02	96.11 ± 0.61	0.015	95.20 ± 1.00	0.015	97.54 ± 1.01
	.03	103.06 ± 1.26	0..025	99.70 ± 0.89	0.025	98.05 ± 0.90
temperature℃	75	102.78 ± 0.32	80	98.33 ± 1.25	55	97.64 ± 0.78
	85	99.44 ± 0.31	90	96.54 ± 1.29	65	101.15 ± 0.35
reaction time(min)	13	98.33 ± 0.36	8	95.63 ± 1.40	23	101.71 ± 1.54
	17	97.78 ± 0.50	12	96.99 ± 1.27	28	99.98 ± 1.32

*values are mean of 3 determinations

Table 6 Determination of the studied drugs in their pharmaceutical dosage forms.

drug	Pharmaceutical product	Proposed method ± SD*	Reported method[5] ± SD	t value	f value
cefi	200 mg of cefi /capsule	102.46% ± 0.40	102.10 ± 1.00	2.01	6.25
ceph	500 mg of ceph monohydrate/capsule	98.20% ± 0.40	98.50 ± 0.80	1.68	4.00
Cefo	1000 mg of cefo/injection	97.60% ±0 .2	97.70 ± 0.50	1.12	6.25

*Five determinations were used for the proposed and reported methods the tabulated t and f at 95%confidence limit are t= 2.26 and f= 6.39

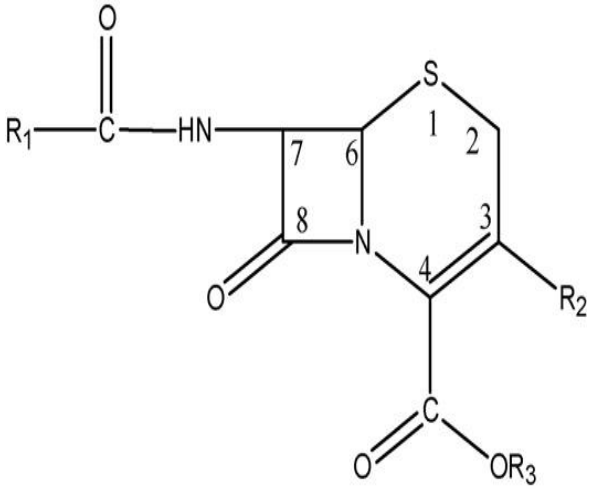


Figure (1) Chemical struture of cephalosporin.

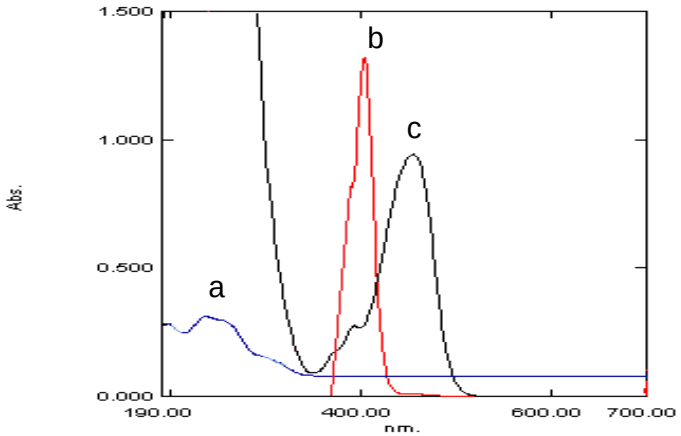


Figure 2 Absorption spectra of (a) cefo (3µg/mL), (b) Absorption spectra of HPTS (0.02%) , (c) Absorption spectra of cefo (3µg/mL) with HPTS 0.02%

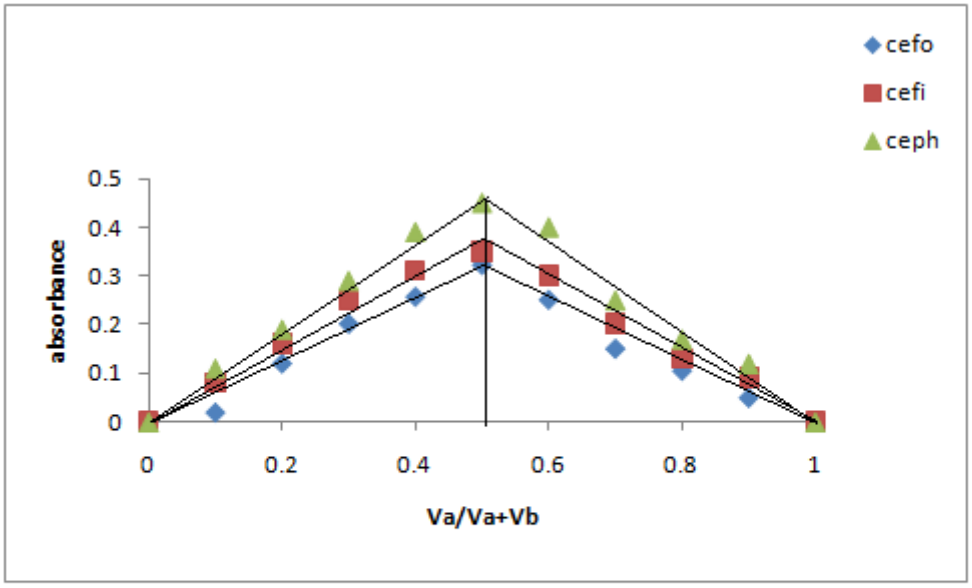
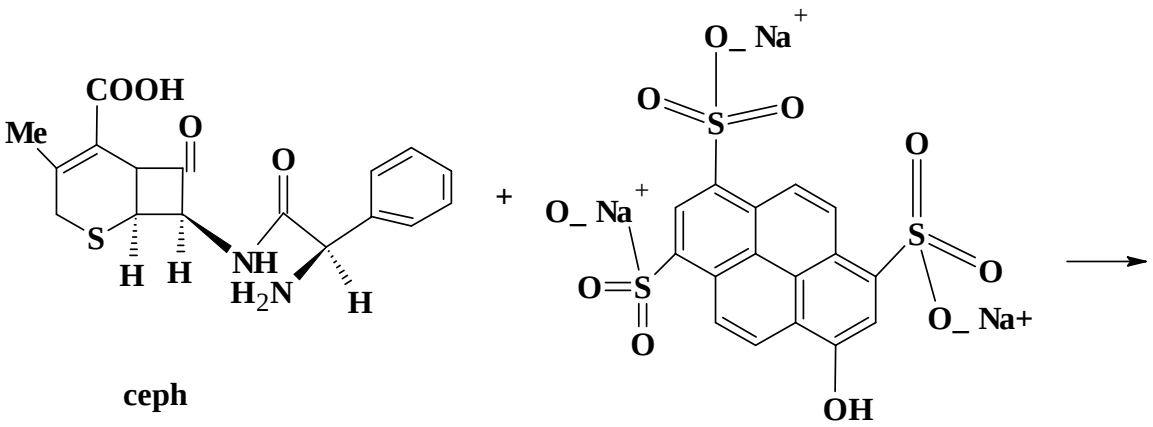
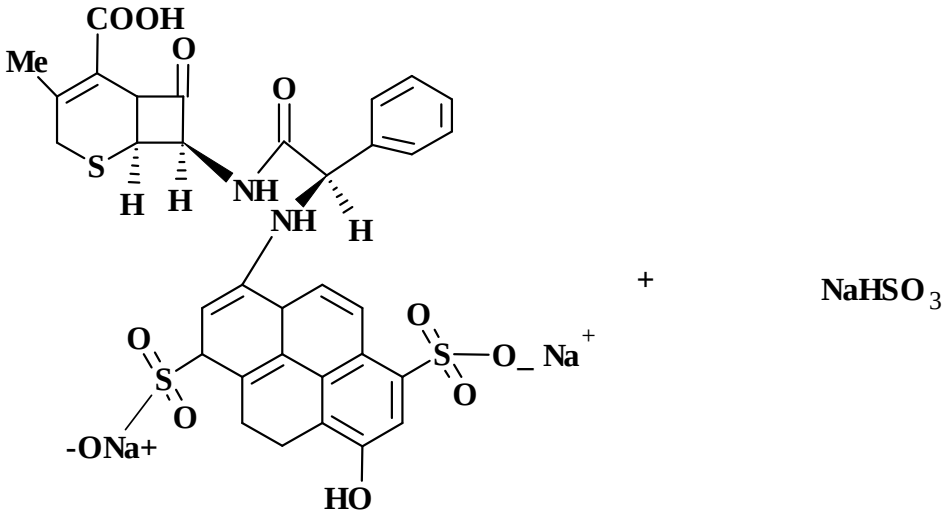


Figure (3) Job's plots of continuous variation of product: cefi; ceph; cefo; with HPTS. Va: HPTS (5×10^{-3} M), Vb:(cefi,ceph and cefo) (5×10^{-3} M);Va+Vb=10mL.



ceph **HPTS**



green product

Scheme (1) Proposed reaction pathway of ceph with HPTS

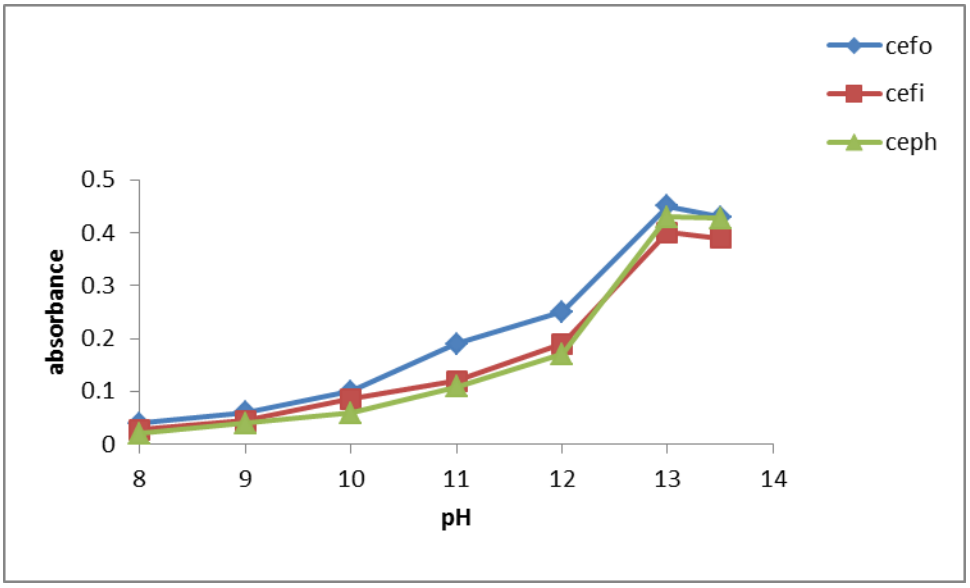


Figure 4. Effect of pH on absorbance of product cefi; ceph; cefo; with HPTS.

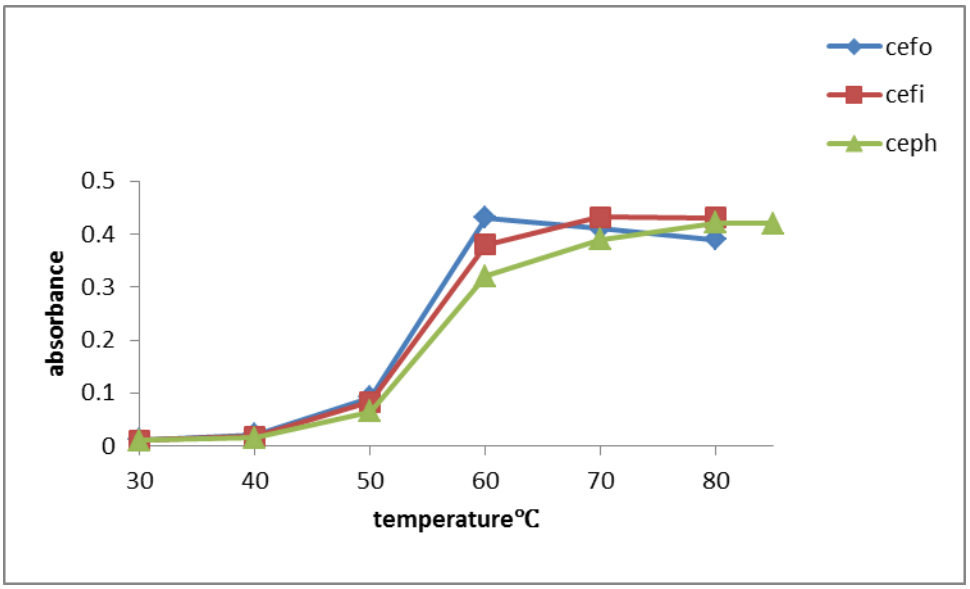


Figure 5. Effect of temperature on absorbance of product cefi; ceph; cefo with HPTS.

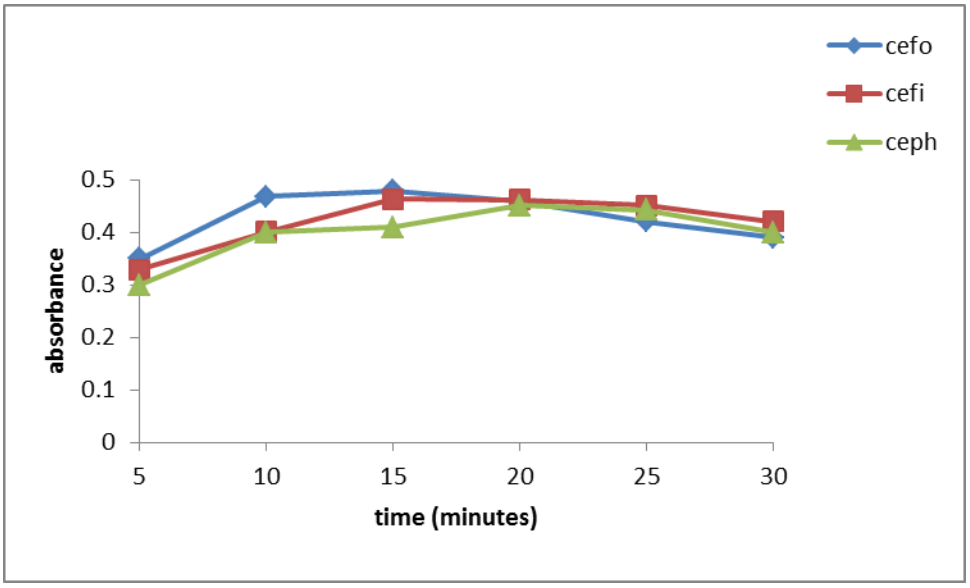


Figure 6. Effect of reaction time on absorbance of product cefi; ceph; cefo with HPTS.

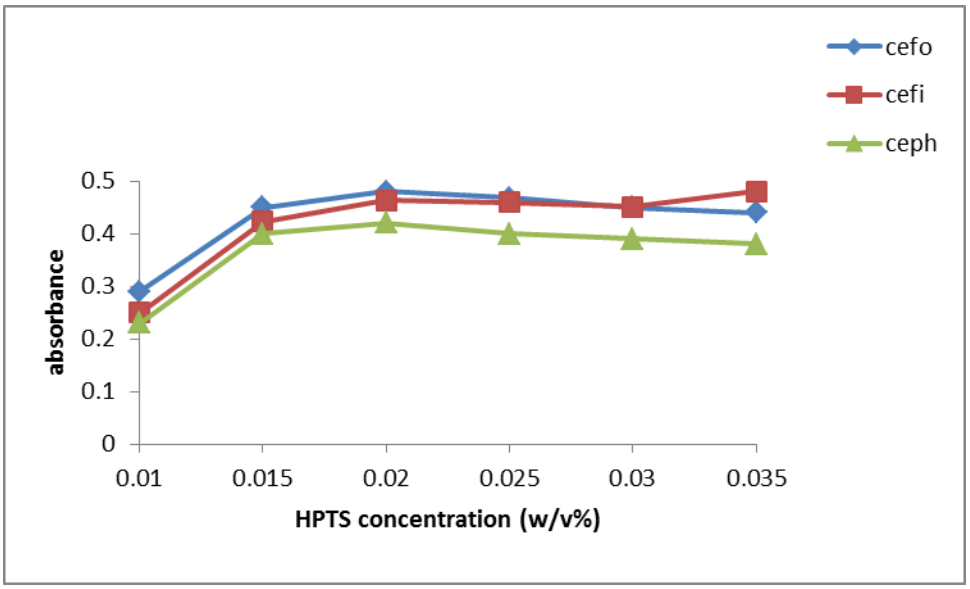


Figure 7. Effect of HPTS concentration on absorbance of product cefi; ceph; cefo with HPTS.