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Bromatometric Estimation of Cefepime, Cefoperazone, Cefotriaxone and Captopril in Bulk and Dosage Forms

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ABSTRACT

Two spectrophotometric methods are described for determination of Cefepime, Cefoperasone and Cefotriaxone in bulk and pharmaceutical dosage forms using *in situ* generated bromine as oxidizing agent and either methylene blue or methyl orange as chromogenic agents. Drugs are treated with known excess of bromine and residual unreacted bromine is determined by treating with fixed amount of either methylene blue or methyl orange then measuring absorbances at 678 nm and 510 nm, respectively. The amount of bromine reacted corresponds to the amount of each drug. Effect of acidity, bromate - bromide volume and reaction time, on the absorption was studied. Calibration curves were linear over ranges of 1–3 $\mu g.ml^{-1}$ for Cefepime,0.4- 1.0 $\mu g.ml^{-1}$ for Cefoperazone and 0.3-0.8 $\mu g.ml^{-1}$ for Cefotriaxone in case of methylene blue and of 0.05–3.0 $\mu g.ml^{-1}$ for Cefepime, 0.75-2.0$\mu g.ml^{-1}$ for Cefoperazone and 0.2-1.4 $\mu g.ml^{-1}$ for Cefotriaxone in case of methyl orange. The methods were satisfactory applied for the determination of drugs in both bulk and pharmaceutical forms and results were compared statistically with reference methods.

Key words: Cefepime, Cefoperazone, Cefotriaxone, Methylene blue and Methyl orange.

INTRODUCTION

Cephalosporins, like all β-lactam antibiotics, inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis (Katzung, 2001). Cephalosporins consist of a fused β-lactam-A -dihydrothiazine two-ring system, known as 7-ACA, and vary in their side chain substituents at C_3, and C_7 (acylamido) (Van Krimpen *et al.*, 1987).

The later generation agents, with their better spectrum of activity against gram-negative bacteria make them useful for hospital-acquired infections or complicated community-acquired infections. Several methods have been developed for determination of cefepime, including spectrophotometric methds (Rodenas *et al.*, 1995; Raval *et al.*, 2011; Navin *et al.*, 2012; Rabindra *et al.*, 2012; Rambabu *et al.*, 2012; Vimal *et al.*, 2012; Chafle, 2013; Singh *et al.*, 2013), high-performance liquid chromatography (HPLC) (Das Gupta, 1997; Elkhaili *et al.*, 1997; Calahorra *et al.*, 1999; Maddox *et al.*, 1999; Valassis *et al.*, 1999; Chang *et al.*, 2001; Cherti *et al.*, 2001; Palacios *et al.*, 2005; Hurum *et al.*, 2009; Patel *et al.*, 2010; Trivedi *et al.*, 2013), capillary zone electrophoresis (Chen *et al.*, 2005), electro chemical methods (Palacios *et al.*, 2000; Ozkan *et al.*, 2002).

Several methods have been developed for determination of cefoperazone, including spectrophotometric methds (Saleh *et al.*, 2001; Salem and Saleh, 2002; Salem, H.F. Askal, 2002; Salem, 2004; Rageh *et al.*, 2010; Senthilraja and Sanjaypai, 2010), high-performance liquid chromatography (HPLC) (El-Shanawani, 1998; Senthilraja and Sanjaypai, 2006; Hurum *et al.*, 2009), electro chemical methods (Ali *et al.*,1993; El-Maali *et al.*,1993). Several methods have been developed for determination of ceftriaxone, including spectrophotometric methds (Abdel-Hamid, 1998; ; Amin and Ragab, 2004; Zhao *et al.*, 2008; Lin *et al.*, 2010; Rageh *et al.*, 2010; Sultana *et al.*, 2010), spectroflurometry (Liu *et al.*, 2007), high-performance thin layer chromatography (HPTLC) (Agbaba *et al.*, 1998; Eric-Jovanovic *et al.*, 1998; Zarapkar *et al.*, 2004)

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high-performance liquid chromatography (HPLC) (Nahata, 1991; Misztal, 1998; Tsai *et al.*, 1999; Glaria *et al.*, 2005; Gandhimathi *et al.*, 2010; Trivedi *et al.*, 2013), electro chemical methods (El-Maali *et al.*,1993; Reddy, 1997)

Redox reactions are employed in determination of inorganic cations and anions as well as organic substances. They have also been used as indicator reaction for kinetic catalytic methods. In redox reactions, the reaction products include the oxidized (or reduced) form of the analyte and the reduced (or oxidized) form of the reagent. Change in the absorbance of one of the reactants or products, induced by the reaction, can be employed in the determination.

Redox reactions are classified into two main groups; reduction of analyte by reagent and oxidation of analyte by reagent. In both cases, the redox reactions can be classified as follow:

1. The spectrophotometrically active analyte product is formed and evaluated.
2. The spectrophotometrically active reagent product is formed and evaluated.
3. The spectrophotometrically active reagent is used and its concentration (absorbance decrease) is evaluated.
4. The excess reagent or the reagent product is determined using other spctrophotometric reaction.

An example of the last class is the oxidation of the analyte by reagent (bromine) and then excess reagent is determined using other spectrophotometric reaction (such as oxidation of methylene blue or methyl orange by excess bromine followed by determination of residual dye).

This method has been widely employed in determination of pharmaceuticals (as a sensitive and rapid method) such as levofloxacin HCl, lomefloxacin HCl and sparfloxacin (El-Shanawany *et al.*, 2011), Doxcycline (Ramesh *et al.*, 2010), Simvastatin (Tharpa and Basavaiah, 2009), Gatifloxacin (Basavaiah and Tharpa, 2008), Lansoprazole (Basavaiah *et al.*, 2007), Pantoprazole (Basavaiah and Anil Kumar, 2007a), Amlodipine (Basavaiah and Anil Kumar, 2007b), Cyproheptadine (Basavaiah *et al.*, 2006) and Salbutamol sulphate (Somashekar and Basavaiah, 2006).

In this study, cefepime, cefoperasone and cefotriaxone have been determined spectrophotometrically through indirect redox method depending on oxidation of drug by insitu generated bromine and evaluation of excess bromine by using either methylene blue or methyl orange.

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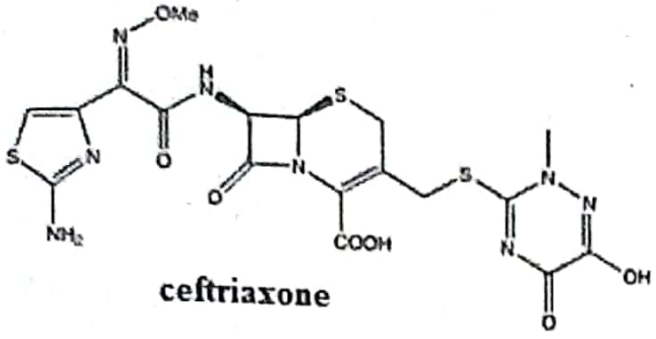
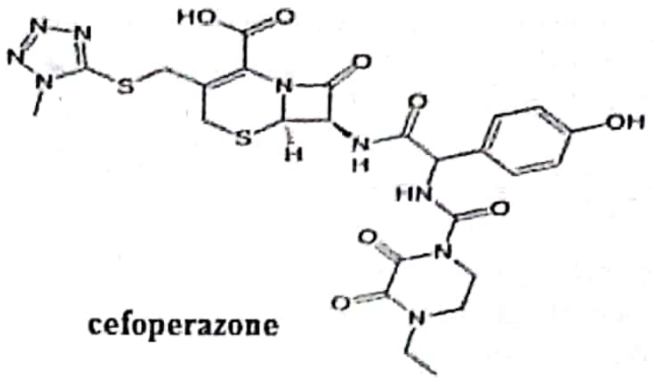
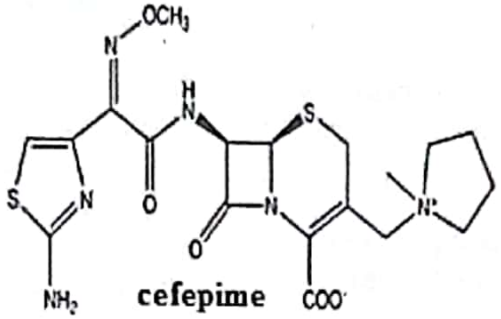


ceftriaxone



cefoperazone



cefepime

MATERIALS and METHODS

Apparatus

Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells connected to windows compatible computer using UV Win 5 Software v5.0.5.

Materials and reagents

All solvents and reagents were of analytical grade and double distilled water was used throughout the work. Cefepime (Adwia), Cefoperazone (EPICO) and Cefotriaxone (EPICO) Standard solutions 25 ug.ml-1 of cefepime and 10 $\mu g.ml^{-1}$ of others were prepared by dissolving each pure drug in 100 ml bidistilled water in case of methylene blue. Cefepime (Adwia), Cefoperasone (EPICO) and Cefotriaxone (EPICO) Standard solutions 10 ug.ml-1 of cefotriaxone and 25 $\mu g.ml^{-1}$ of others was prepared by dissolving each pure drug in 100 ml bidistilled water in case of methyl orange. 5 M HCl (El-Nasr Chemicals, Egypt) was prepared by diluting 225 ml of concentrated HCl (36%) to 500 ml with bidistilled water. Methylene Blue & Methyl Orange 60 µg/ml (Universal Fine Chemicals, India) 60 mg were dissolved in 20 ml methanol then completed to 100 ml with bidistilled water (stable for 2 weeks at least). Bromate / Bromide stock solution was prepared by dissolving 0.1 gm of potassium bromate (Winlab, England) and 1.0 gm of potassium bromide (Winlab, England) in 100 ml bidistilled water (stable for 10 days at least). Working solution was freshly prepared daily by diluting 2.5 ml of stock solution to 100 ml with bidistilled water (25 µg/ml in case of methylene blue), 1.25 ml of stock solution (12.5 µg/ml in case of methyl orange).

Pharmaceutical preparations

The following available vial preparations were analyzed: Wincef® vials labeled to contain 1000 mg cefepime per vial. Batch No. 090235\9869 (Adwia, Egypt), cefosone® vials labeled to contain 1000 mg cefoperazone per vial. Batch No.1005019 (Eipico, Egypt) and ceftriaxone® vials labeled to contain 200 mg cefotriaxone per vial. Batch No.1280325 (Kahira, Egypt).

General spectrophotometric procedures and construction of calibration curves using Methylene Blue method

To 1 ml (in case of cefepime and cefoperazone) or 1.2 ml (in case of cefotriaxone) bromate - bromide working solution in 10 - ml volumetric flasks, add 0.4 – 1.2 ml (in case of cefepime), 0.4 - 1 ml (in case of cefoperazone), 0.3 – 0.8 ml

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(in case of cefotriaxone) drug solution then acidify using 0.2ml (in case of cefepime and cefotriaxone) or 0.4 ml (in case of cefoperazone) 5 M HCl, close flasks and stand for15 minutes (in case of cefotriaxone) or 10 minutes(in case of others) , add 1 ml dye working solution then stand for another 10 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 678 nm.

General spectrophotometric procedures and construction of calibration curves using Methyl Orange method

To 1 ml (in case of cefoperazone), 0.8 ml (in case of cefepime) or 0.6 ml (in case of cefotriaxone) bromate - bromide working solution in 10 - ml volumetric flasks, add 0.2 – 1.2 ml (in case of cefepime),0.3 – 0.8 ml(in case of cefoperazone) or 0.2 -1.4 ml (in case of cefotriaxone) drug solution then acidify using 0.2 ml 5 M HCl, close flasks and stand for 10 minutes, add 1 ml dye working solution then stand for 2 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 510 nm.

Procedures for pharmaceutical preparations (vials)

Wincef: The contents of two vials were weighed. An accurately amounts of the powder equivalent to 250 mg of cefepime were dissolved in bidistilled water, filtered into 100-ml measuring flask and completed to volume with bidistilled water to give a final concentration of 2500 $\mu g.ml^{-1}$ then 1 ml transferred to 100 ml measuring flask aand completed to give a final concentration of 25 $\mu g.ml^{-1}$. The procedures were then completed as previously mentioned under the general procedures (2.4.1.and 2.4.2.).

Cefosone: The contents of two vials were weighed. An accurately amounts of the powder equivalent to 100 mg of cefoperasone were dissolved in bidistilled water, filtered into 100-ml measuring flask and completed to volume with bidistilled water to give a final concentration of 1000 $\mu g.ml^{-1}$ then 1 ml transferred to 100 ml measuring flask and completed to give a final concentration of 25 $\mu g.ml^{-1}$(in case of methyl orange) or 10 $\mu g.ml^{-1}$(in case of methylene blue) The procedures were then completed as previously mentioned under the general procedures(2.4.1.and 2.4.2.).

Cefotriaxone: The contents of two vials were weighed. An accurately amounts of the powder equivalent to 100 mg of cefotriaxone were dissolved in bidistilled water, filtered into 100-ml measuring flask and completed to volume with bidistilled water to give a final concentration of 1000 $\mu g.ml^{-1}$ then1 ml transferred to 100 ml measuring flask and completed to give a final concentration of 10 $\mu g.ml^{-1}$ The procedures were then completed as previously mentioned under the general procedures(2.4.1.and 2.4.2.).

RESULTS and DISCUSSION

The proposed spectrophotometric methods are indirect and based on the oxidation of the mentioned drugs by bromate solution followed by determination of the residual bromine (insitu generated) after allowing the reaction between each drug and a measured amount of excess bromine to be complete. The surplus bromate was determined by reacting it with a fixed amount of either methylene blue or methyl orange dye. The methods rely on the bleaching action of bromine on the dyes due to oxidation of these dyes (in case of methylene blue).

Cefepime, Cefoperazone and Cefotriaxone when added in increasing amounts to a fixed amount of *in situ* generated bromine, consume the latter proportionately with a concomitant fall in the concentaration of bromine. When a fixed amount of dye is added to the decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently,

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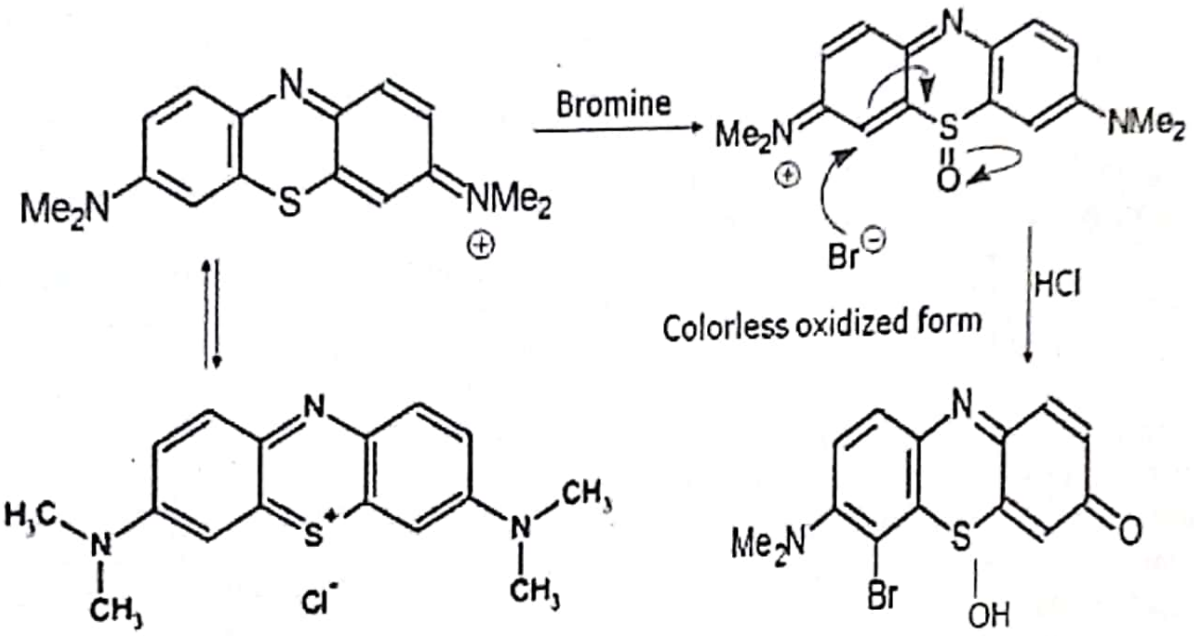
the increase in the absorbance of the residual dye at the respective λ_{max} is proportional with increasing concentration of each drug. In studying the molar ratios of the reaction by job's method (Mendham *et al.*, 2000) it was found that bromine and methylene blue react in the ratio 1: 1 (Figure 11).

The *in situ* generation of bromine is carried out using a mixture of potassium bromate and potassium bromide in presence of 5 M HCl according to the following equation:

$$5Br^- + BrO_3^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

The proposed pathway is suggested as follows (Tharpa and Basavaiah, 2009) :



Two tautomeric forms of methylene blue

Colorless brominated form

Absorption spectra

Absorption spectra for determination of Cefepime, Cefoperasone, and Cefotriaxone were studied over range of 200-800 nm. After oxidation of both drugs and portions of dyes with bromine, residual unoxidized methylene blue and methyl orange are absorbed at 678 and 510 respectively (Figures 1 and 2).

Effect of Acidity

5 M HCl was used throughout experiments and it was found that for 0.2ml or 0.4 ml (in case of cefoperasone with methylene blue) of 5 M HCl (accurately measured) is the appropriate acid volume and increasing HCl volume results in a decrease in absorption (Figures 3 and 4).

Effect of bromate - bromide volume

Bromate - bromide volume was studied by varying the reagent volume while other factors were held constant. It was found that for methylene blue 1 ml (in case of cefepime and cefoperasone) or 1.2 (in case of cefotriaxone) and for methyl orange 1 ml (in case of cefoperasone), 0.8 ml (in case of cefepime) or 0.6 ml (in case of cefotriaxone) of bromine is sufficient for the reaction using these stated concentrations (25, 12.5 µg/ml for methylene blue and, methyl orange respectively) (Figures 5 and 6).

Effect of time

Time required for bromination and subsequent oxidation of the drug before addition of dye and time required to irreversibly oxidize dye after its addition was studied. The bromination reaction was found to be complete in 10 minutes or 15 minutes (in case of cefotriaxone with

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methylene blue) while contact times up to 25 minutes had been examined and no further bromination was detected using TLC technique (Figures 7 and 8).

A contact time of 10 minutes (in case of methylene blue) (Figures 9 and 10) was necessary for the bleaching of the dye colour by the residual bromine and the colour of residual the two dyes remains stable for at least two hours after mixing with the reaction mixture.

Method of validation

The developed methods were validated according to international conference on harmonization guidelines (Basavaiah and Tharpa, 2008). The linearity range of absorbance as a function of drug concentration (Tables 1, 2, 3 and 4) provides good indication about sensitivity of reagents used. Calibration curves have correlation coefficients (r) around 0.999 indicating good linearity. The accuracy of the methods was determined by investigating the recovery of drugs at concentration levels covering the specified range (three replicates of each concentration). The results showed good recoveries (tables 5, 6, 7 and 8). Also, the Limit of detection (L.D.), Limit of quantitation (L.Q.), Sandell's sensitivity (S.S.) and Molar absorbitivity were calculated. Intra - day precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug (tables 13 and 14). The SD values revealed the high precision of the methods F or inter-day reproducibility standard drug solutions were analyzed each for five days (tables 13and 14) and the results were reproducible. The robustness of the methods was evaluated by making small changes in the volume of acid, bromated bromine mixture and dye solution and the effect of the changes was studied on the percent recovery of drugs (tables 15 and 16). The changes had negligible influence on the results as revealed by small SD values (≤ 1.93).

Applications

Some Pharmaceutical formulations (vials) containing stated drugs have been successfully analyzed by the proposed methods. Excipients did not show interference indicating high specificity. Results obtained were compared to those obtained by applying reported reference methods using aqoues NaOH by ultraviolet spectroscopy in case of cefepime (Singh *et al.*, 2013), and the reaction of hydrolysate with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) in the presence of HCl in case of cefotriaxone and cefoperazone (Rageh *et al.*, 2010). Student's t-test and F-test were performed for comparison. Results are shown in tables 5, 6 and 7 where the calculated t and F values were less than tabulated values which in turn indicate that there is no significant difference between proposed methods and reference ones relative to precision and accuracy.

In conclusion, the proposed indirect spectrophotometric method is simple, fast, accurate, adequately sensitive and inexpensive. It is suitable for routine quality control analysis. The amounts obtained by the proposed methods are between 98.3% and 98.9%, within the acceptance level of 95% to 105%. The present methods are superior to the reference method with respect to both sensitivity and selectivity. The methods have been successfully applied for the analysis of marketed vials.

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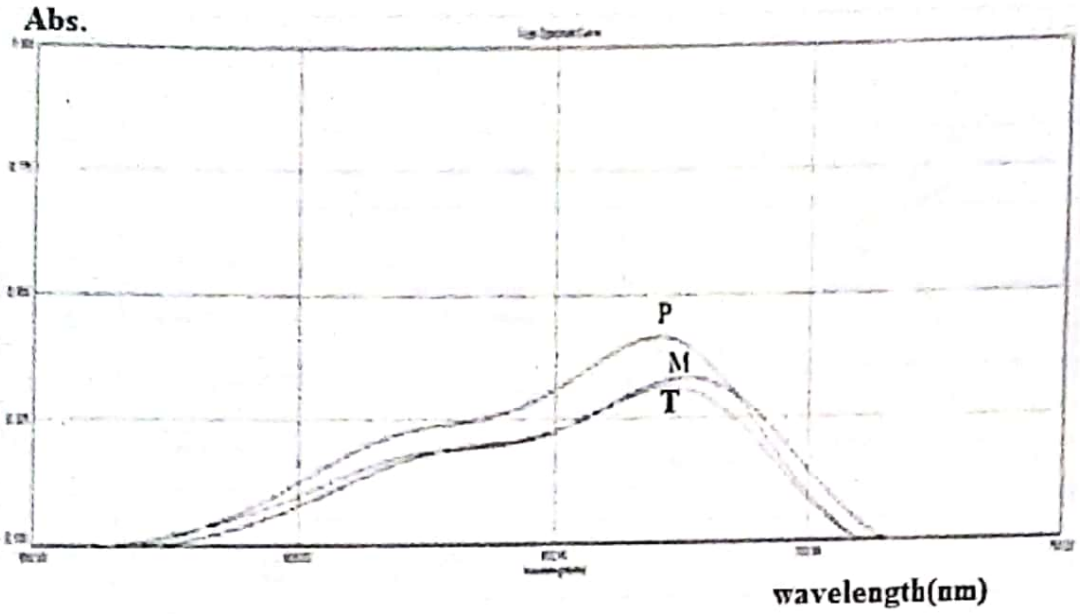

Figure 1. Absorption spectra of 60µg/ml methylene blue using 1 µg/ml cefotriaxone (T), cefoperazone (P), and cefepime (M) after bromine oxidation at 678 nm.

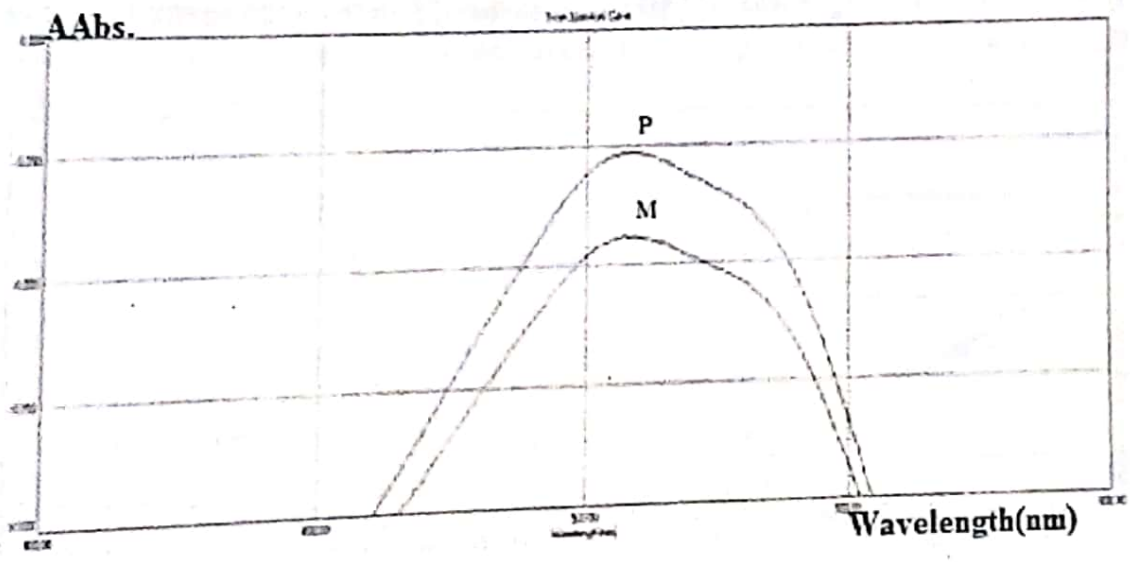

Figure 2. Absorption spectra of 60µg/ml methyl orange using 1 µg/ml cefoperazone (P), and cefepime (M) after bromine oxidation at 510 nm.

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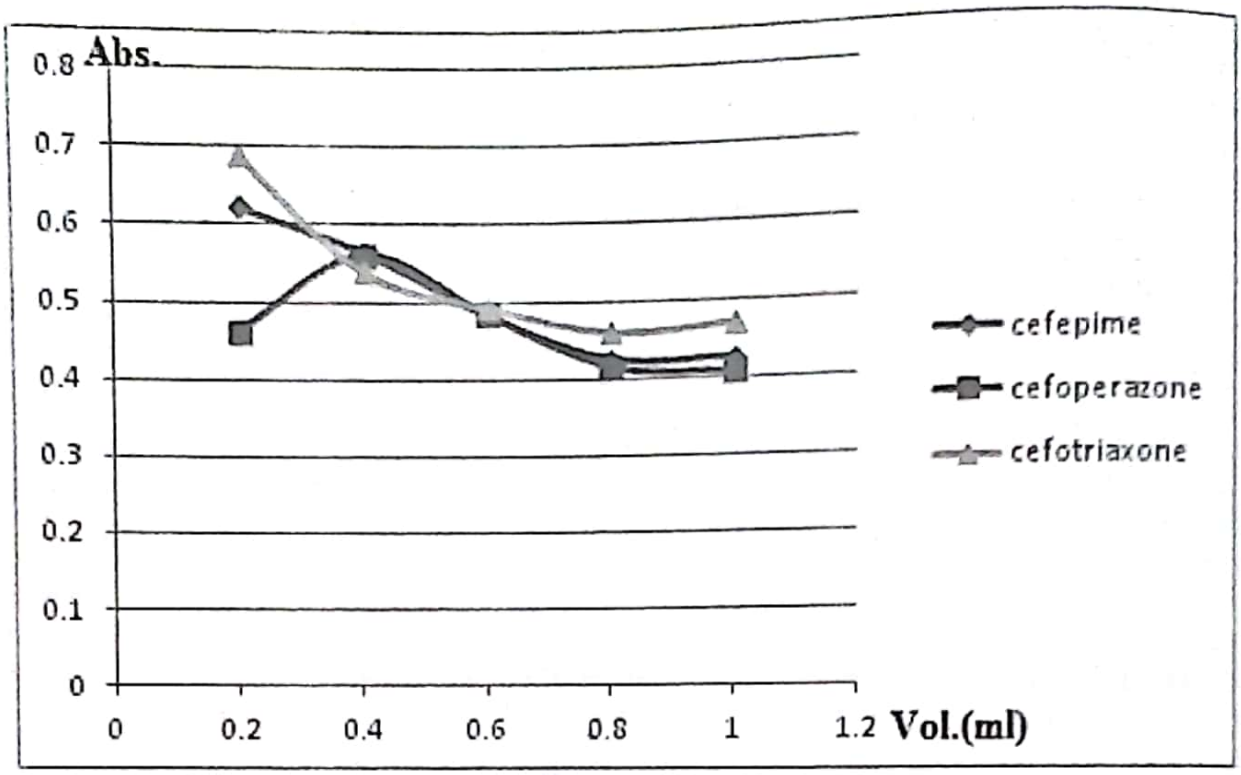

Figure 3. Effect of volume of 5M HCL on absorbance in case of methylene blue (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 678 nm.

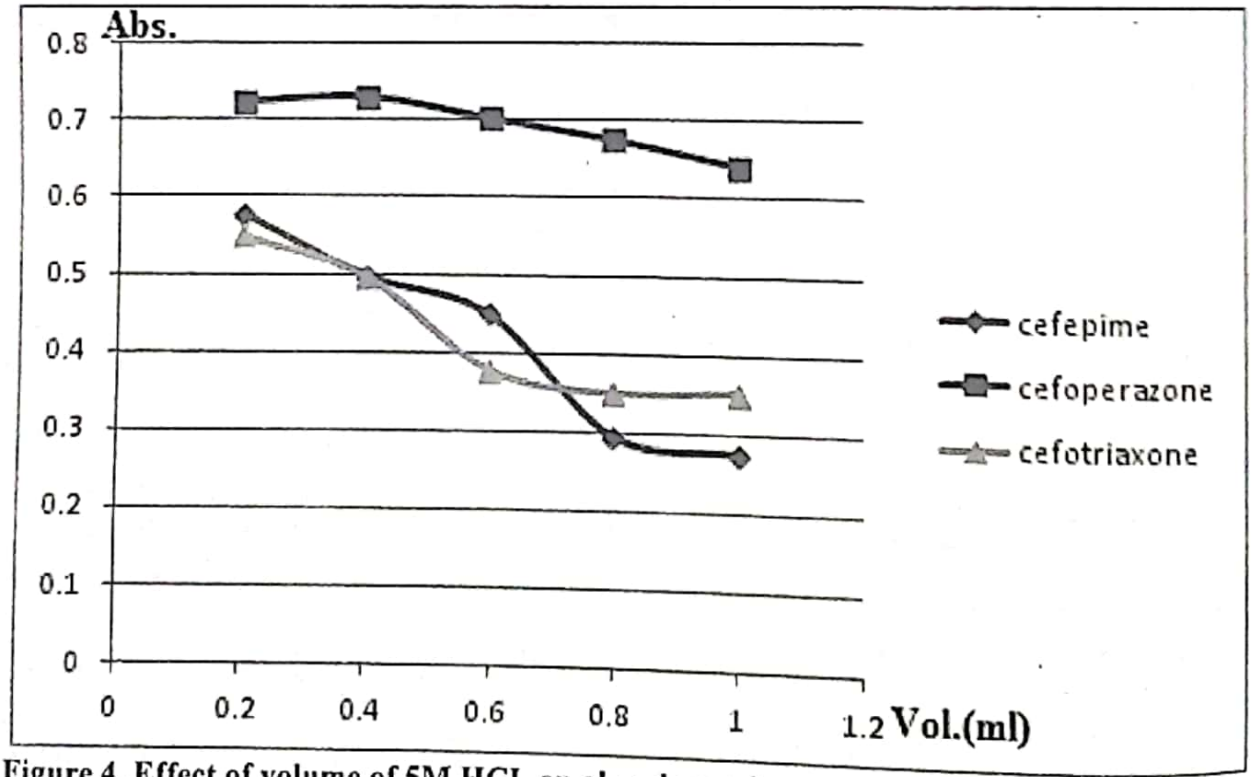

Figure 4. Effect of volume of 5M HCL on absorbance in case of methyl orange (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone, and cefotriaxone at 510 nm.

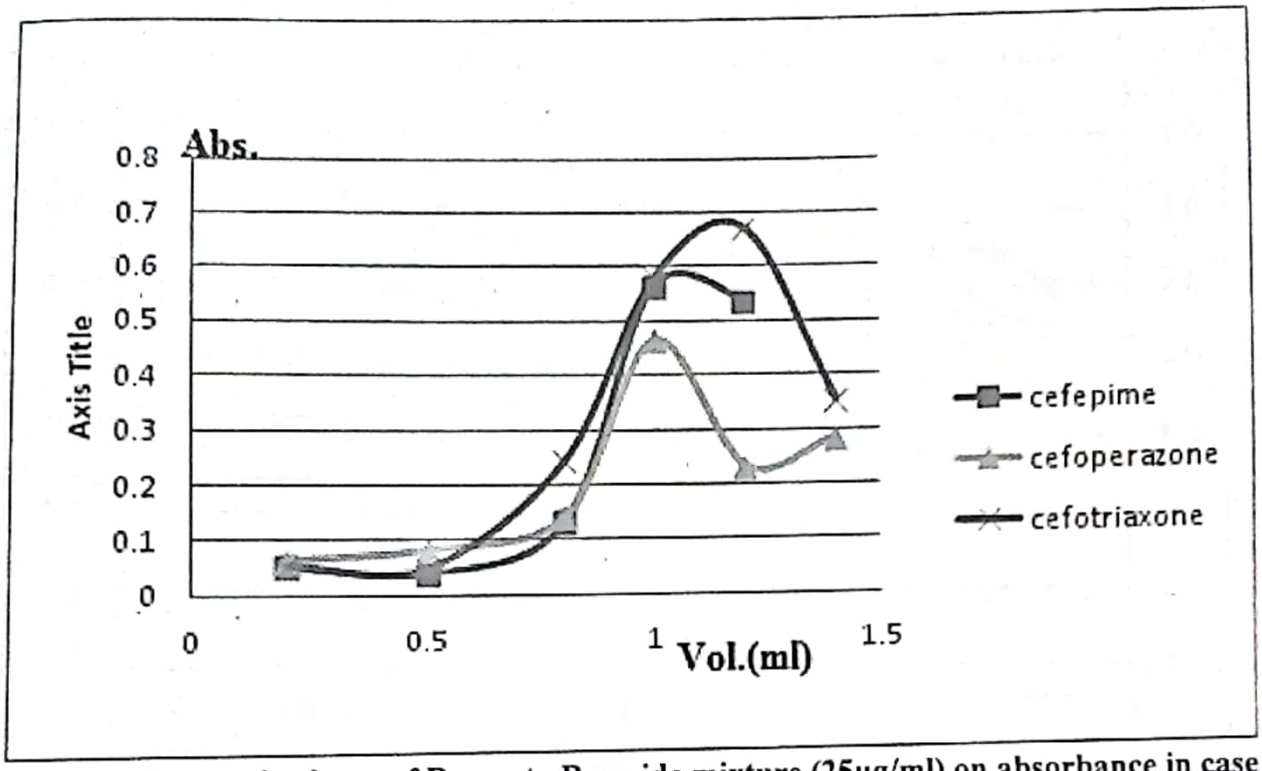

Figure 5. Effect of volume of Bromate-Bromide mixture (25µg/ml) on absorbance in case of methylene blue (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 678nm.

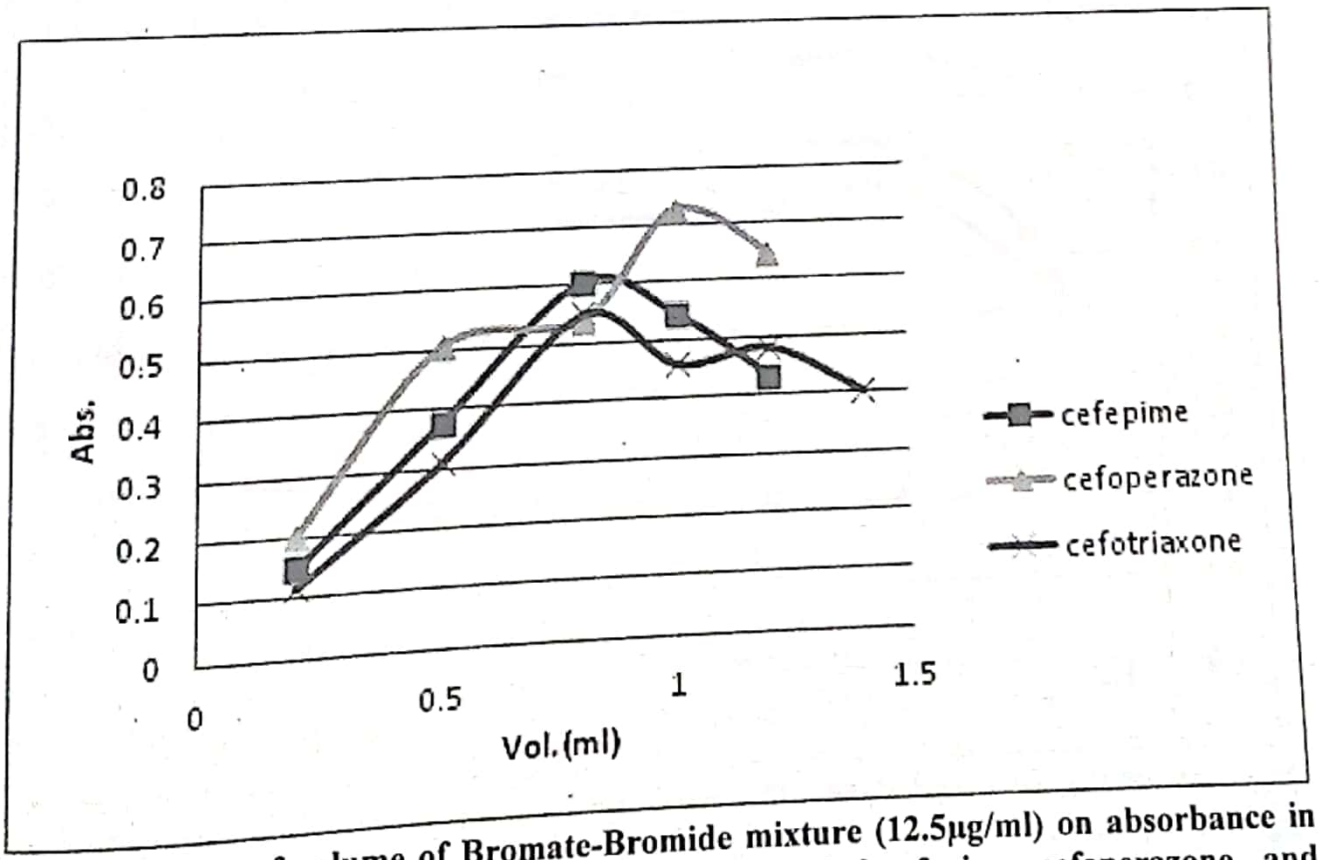

Figure 6. Effect of volume of Bromate-Bromide mixture (12.5µg/ml) on absorbance in case of methyl orange (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 510 nm.

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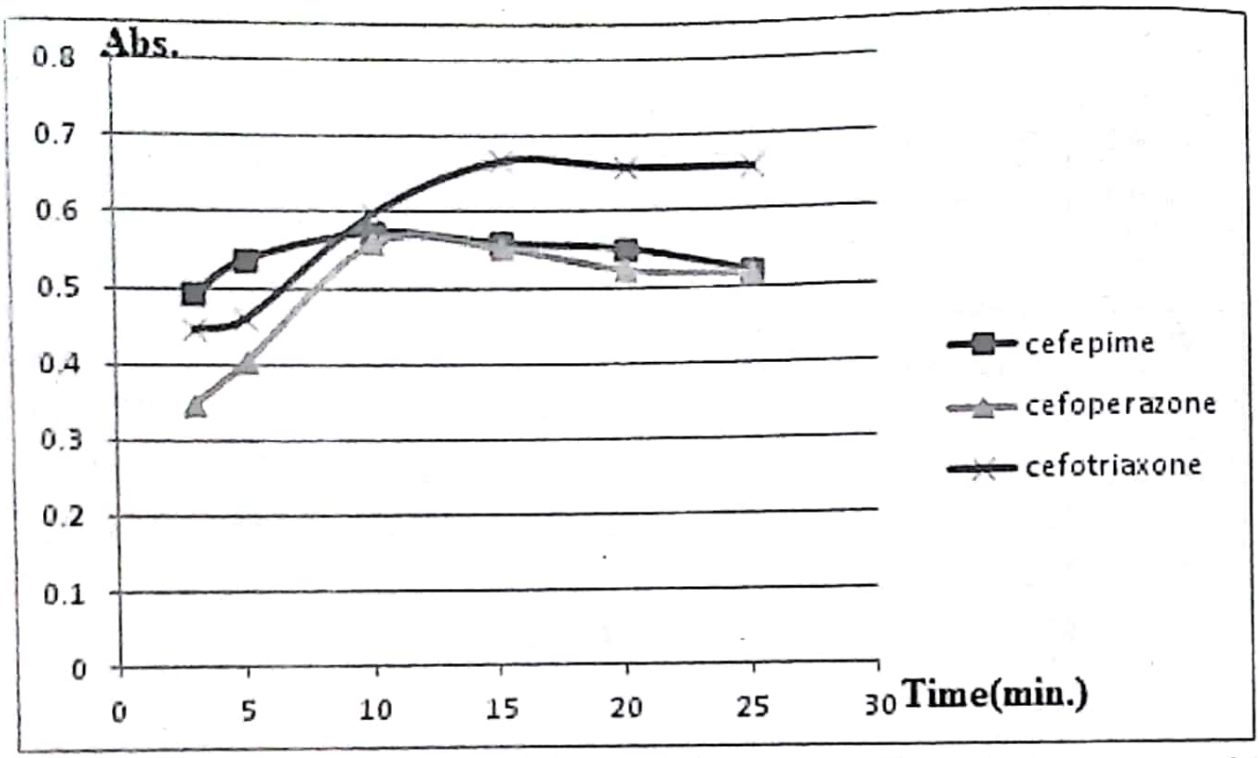

Figure 7. Effect of time before addition of methylene blue (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 678 nm.

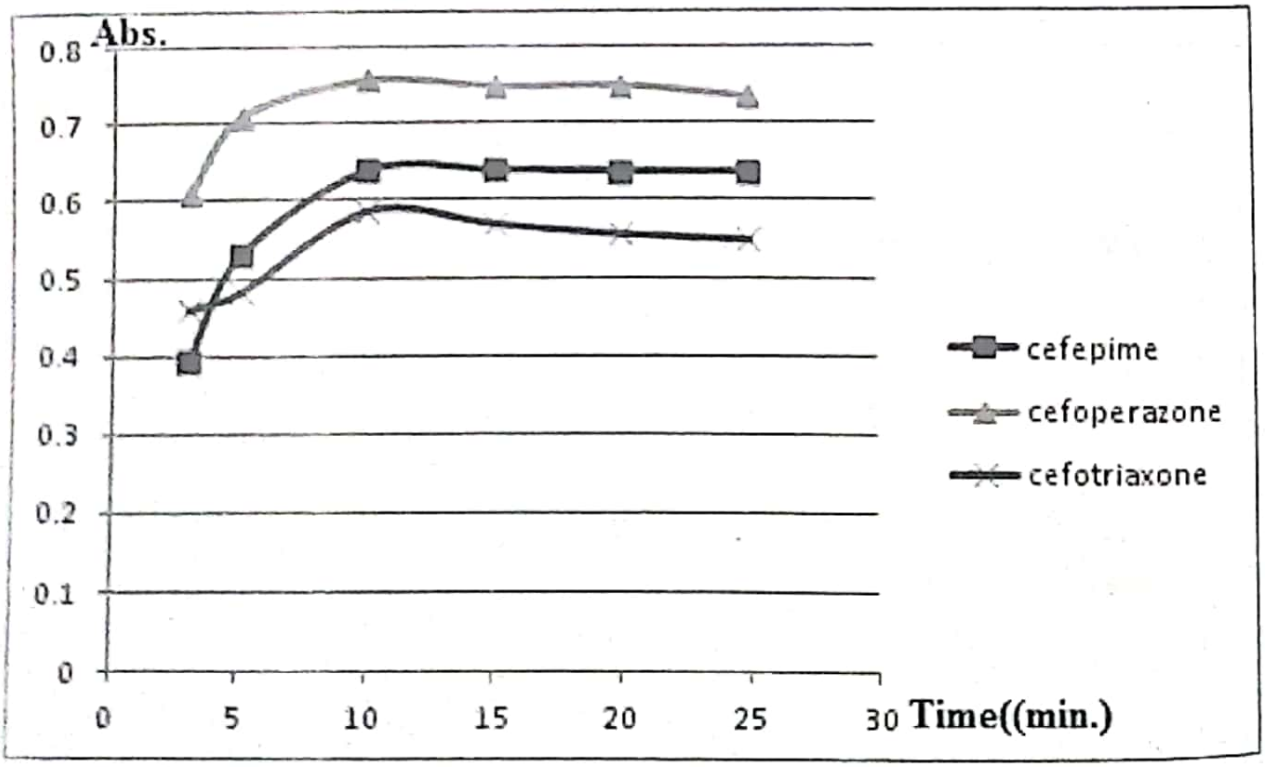

Figure 8. Effect of time before addition of methyl orange (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 510 nm.

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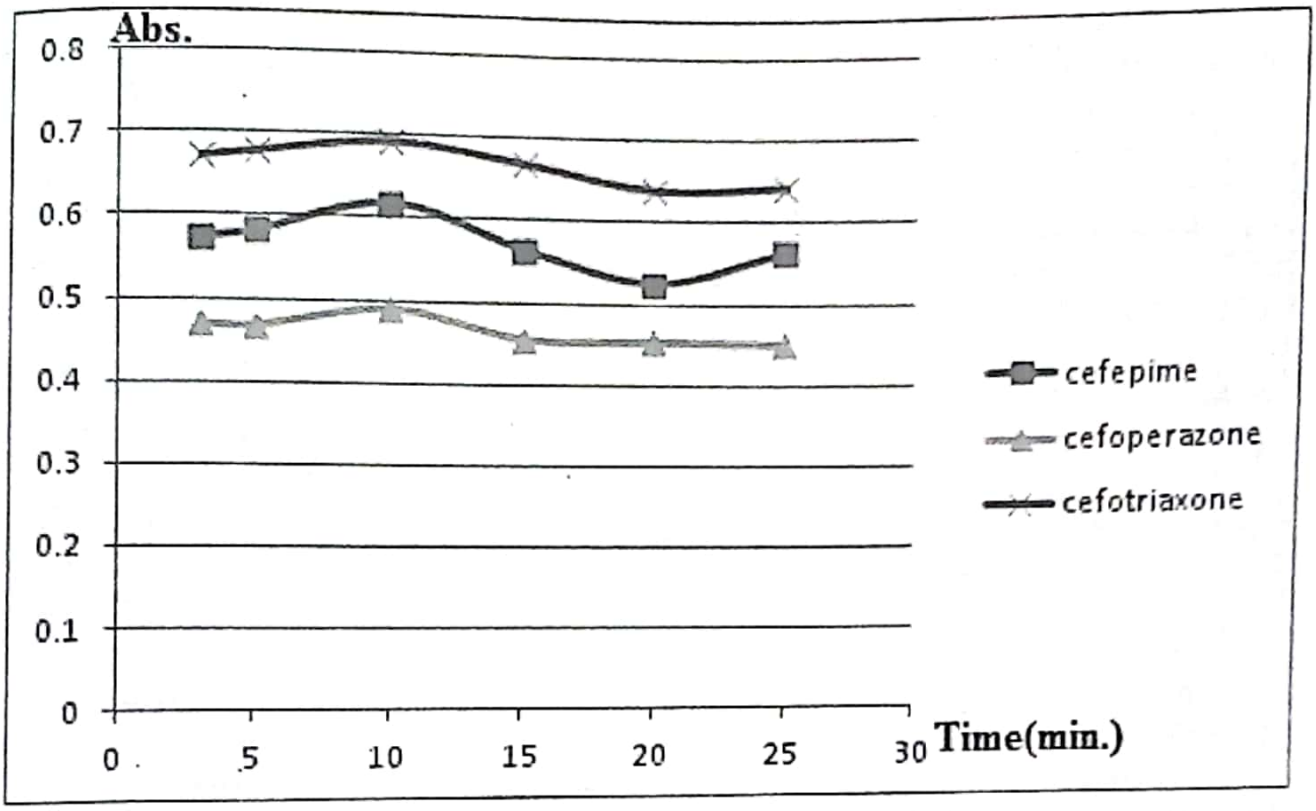

Figure 9. Effect of time after addition of methylene blue (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 678 nm.

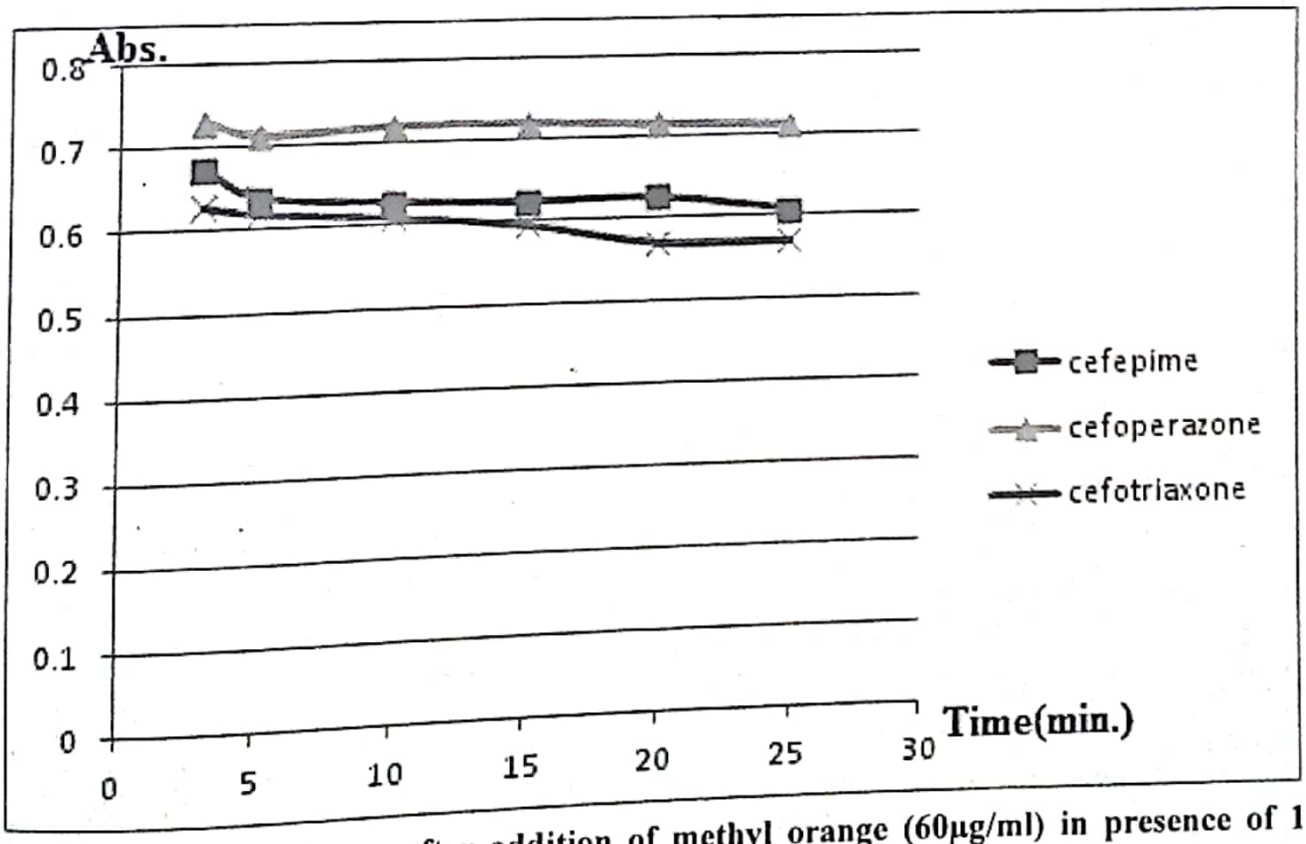

Figure 10. Effect of time after addition of methyl orange (60µg/ml) in presence of 1 µg/ml cefepime, cefoperazone and cefotriaxone at 510 nm.

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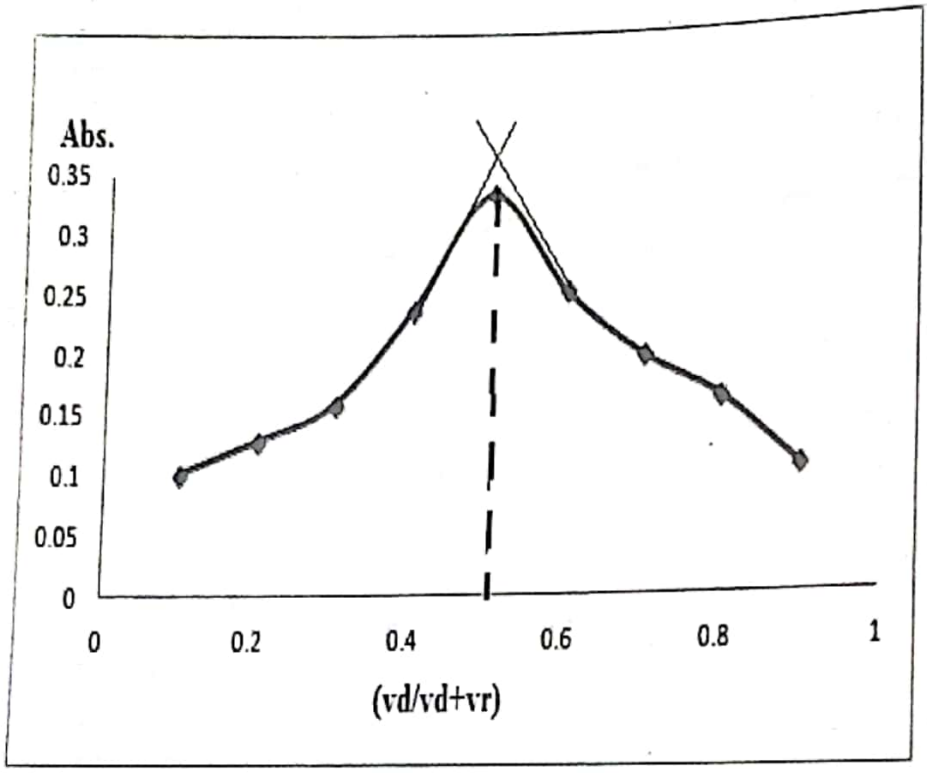

Figure 11. Job's method for molar ratio estimation of $1.5x10^{-4}$M bromine with $1.5x10^{-4}$M methylene blue) in presence of 1 µg/ml cefepime at 278 nm.

Table (1): Analytical parameters for the determination of cefepime, cefoperazone and cefotriaxone using methylene blue method.

Parameters	Methylene Blue (60µg/ml)		
	cefepime	cefoperazone	cefotriaxone
ëmax, nm	678	678	678
Volume of dye, ml	1	1	1
Volume of 5M HCL, ml	0.2	0.4	0.2
Volume of Bromate - Bromide mixture (25µg/ml) , ml	1	1	1.2
Time before dye addition, min	10	10	15
Time after dye addition, min	10	10	10
Beer's law limits, µg/ml	1-3	0.4-1.0	0. 3-0.8
Regression equation	y=0.233x - 0.037	y=0.666x - 0.050	y=0.931 x - 0.070
Correlation Coefficient	0.9994	0.9983	0.9994

y = a + bx, where y is the absorbance, a is the intercept, b is the slope and x is the concentration in µg/ml.

Table (2): Analytical parameters for the determination of cefepime, cefoperazone and cefotriaxone using methyl orange method.

Parameters	Methyl orange(60µg/ml)		
	cefepime	cefoperazone	cefotriaxone
ëmax, nm	510	510	510
Volume of dye, ml	1	1	1
Volume of 5M HCL, ml	0.2	0.4	0.2
Volume of Bromate - Bromide mixture (25µg/ml) , ml	0.8	1	0.8
Time before dye addition, min	10	10	10
Time after dye addition, min	2	2	2
Beer's law limits, µg/ml	0. 5-3.0	0.75-2.0	0.2-1.4
Regression equation	y=0.222x+0.012	y=0.431x - 0.129	y=0.537x+0.018
Correlation Coefficient	0.9993	0.9993	0.9995

y = a + bx, where y is the absorbance, a is the intercept, b is the slope and x is the concentration in µg/ml.

Table(3). Results of the analysis for determination of cefepime, cefoperazone and cefotriaxone using methylene blue method.

parameters	Methylene Blue								
	cefepime			cefoperazone			cefotriaxone		
	Taken µg/ml	Found µg/ml	Taken µg/ml	Taken µg/ml	Taken µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	1	1.0128	101.2875	0.3	0.3	98.597	0.3	0.295	98.4604
	1.5	1.4721	98.140	0.4	0.4	98.3498	0.4	0.402	100.698
	2	2.004	100.214	0.5	0.5	101.760	0.5	0.504	100.9667
	2.5	2.5193	100.772	0.6	0.6	101.367	0.6	0.591	98.6394
	3	2.9914	99.714	0.7	0.7	101.07	0.7	0.708	101.27
				0.8	0.8	99.0099	0.8	0.794	99.3555
			100.02			100.026			100.007
Mean			1.20810			1.5352			1.34768
±SD			1.20779			1.5348			1.34758
±RSD			0.54029			0.6268			0.6027
±SE			1.4595			2.3569			1.816
Variance			0.2332			0.6068			0.946
Slope			0.2850			0.1095			0.093
L.D.			0.9500			0.365			0.3132
L.Q.			0.00314			0.000914			0.000621
S.S.			101688.1			349899.2			514772.06
Apparent Molar Absortivity $L.Mol^{-1}.cm^{-1}$									

* Average of three independent procedures.

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Table (4): Results of the analysis for determination of cefepime, cefoperazone and cefotriaxone using methyl orange method.

Parameters	Methylene Blue								
	cefepime			cefoperazone			cefotriaxone		
	Taken µg/ml	Taken µg/ml	Recovery %	Taken µg/ml	Taken µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	0.5	0.502	100.448	0.75	0.763	101.7788	0.2	0.197	98.513
	1	1.008	100.89	1	0.995	99.5359	0.4	0.403	100.83
	1.5	1.4798	98.654	1.25	1.2389	99.11832	0.6	0.591	98.513
	2	2.0627	103.139	1.5	1.5058	100.3866	0.8	0.814	101.76
	2.5	2.5112	100.448	1.75	1.7378	99.3039	1	1.005	100.55
	3	2.9820	99.402	2	2.0162	100.812	1.2	1.210	100.83
							1.4	1.386	99.044
Mean			100.498			100.1559			100.009
±SD			1.5321			1.02998			1.3015
±RSD			1.5245			1.02838			1.3013
±SE			0.6256			0.420574			0.4920
Variance			2.3475			1.060871			1.6939
Slope			0.2228			0.431428			0.5376
L.D.			0.1427			0.206947			0.059
L.Q.			0.4759			0.68982			0.1972
S.S.			0.0029			0.001499			0.000866
Apparent Molar absorbitivity $L.Mol^{-1}.cm^{-1}$			111625.3			218262.8			378030.1

* Average of three independent procedures.

Table (5): Statistical analysis of results obtained by the proposed methods applied on pimfast® vials compared with reported method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method[6]
N	5	5	5
Mean Recovery	100.426	99.958	98.655
±SD	1.121	1.548	1.221
±RSD	1.1158	1.548	1.237
±SE	0.5011	0.692	0.4316
Variance	1.2556	2.396	1.490
Student-t	2.389(2.57)a	1.48(2.57)a	
F-test	1.18(6.256)b	1.61(6.256)b	

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table (6): Statistical analysis of results obtained by the proposed methods applied on cefozone® vials compared with reported method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method[29]
N	5	5	5
Mean Recovery	99.856	100.038	98.369
±SD	0.886	0.94267	1.5999
±RSD	0.8875	0.942	1.626
±SE	0.396	0.42159	0.482
Variance	0.785	0.8886	2.559
Student-t	1.82(2.57)a	2.01(2.57)a	
F-test	3.25(6.256)b	2.88(6.256)b	

a and b are the Theoretical Student t-values and F-ratios at p=0.05

Table (7): Statistical analysis of results obtained by the proposed methods applied on cefotriaxone® vials compared with reported method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method[29]
N	5	5	5
Mean Recovery	100.160	100.10	98.86
±SD	1.0684	1.3008	1.332
±RSD	1.0667	1.299	1.347
±SE	0.4778	0.582	0.471
Variance	1.1414	1.692	1.7756
Student-t	1.7(2.57)a	1.49(2.57)a	
F-test	1.56(6.256)b	1.05(6.256)b	

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table (8): Results of the intraday and interday precision for the determination cefepime, cefoperazone and cefotriaxone using methylene blue method.

		intraday		interday	
drug	conc.ug/ml	mean + SD	RSD	mean SD	RSD
Cefepime	2.5	101.8 ± 0.86	0.84	101.3 ± 0.76	0.76
cefoperazone	0.8	101.3 ± 0.52	0.5	100.9 ± 0.71	0.71
cefotriaxone	0.8	99.8 ± 0.53	0.53	99.7 ± 0.37	0.37

Table (9): Results of the intraday and interday precision for the determination cefepime, cefoperazone and cefotriaxone using methyl orange method.

		intraday		interday	
drug	conc.ug/ml	mean + SD	RSD	mean SD	RSD
Cefepime	2.5	100.6 ± 0.74	0.74	100.9 ± 1.04	1.03
cefoperazone	0.8	100.9 ± 0.27	0.27	99.9 ± 0.68	0.68
cefotriaxone	0.8	98.6 ± 0.39	0.39	99.03 ± 0.82	0.83

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Table (10): Results of the robustness for the determination of cefepime, cefoperazone and cefotriaxone using methylene blue method.

Parameters	methylene blue		
	% of recovery ± SD		
	cefoperazone	Cefepime	cefotriaxone
HCl 0.18	98.3 ± 1.6	98.5 ± 1.1	97.7 ±1.4
HCl 0.22	101.8 ± 0.71	101.6 ± 0.55	100.99 ± 0.55
Br_2 0.95	98 ±1.9	98.03 ±1.4	99.3±0.46
Br_2 1.05	100.8 ± 0.35	102 ± 0.45	101.8± 0.80
dye 0.95	98.7 ± 1.4	98.03 ± 1.4	98.1 ± 1.14
dye 1.05	101.8 ± 0.51	100.9 ± 0.86	100.9 ± 0.46

Table (11): Results of the robustness for the determination of cefepime, cefoperazone and cefotriaxone using methylene blue method.

Parameters	Methyl orange		
	% of recovery ± SD		
	cefoperazone	Cefepime	cefotriaxone
HCl 0.18	98.5 ± 1.47	98.1 ± 1.5	99.2 ±0.84
HCl 0.22	101.3± 0.13	101.9 ± 1.09	101.8 ± 0.71
Br_2 0.95	99.8 ±0.66	98.7 ±1.3	98.9±0.96
Br_2 1.05	101.9 ± 0.53	101.7 ±1.01	101.92± 0.77
dye 0.95	99.6 ± 0.80	100.4 ± 0.71	98.6 ± 1.16
dye 1.05	101.8 ± 0.46	101.3 ± 0.88	101.47± 0.52

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استخدام البرومين فى تعيين كل من السيفيبيم، السيفوبيرازون ،السيفوترايكسون فى صورهم النقية ومستحضراتهم الصيدلية

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يصف هذا الجزء طريقتين لتحليل كل من السيفيبيم، السيفوبيرازون ،السيفوترايكسون و الكابتوبريل فى صورهم النقية ومستحضراتهم الصيدلية. وتعتمد الطريقتين على الانتاج اللحظى للبرومين كعامل مؤكسد واستخدام اما الميثلين الازرق أو الميثيل البرتقالى ككاشف طيفى. فتتم أكسدة تلك الادوية باستخدام البرومين المنتج لحظيا حيث تستهلك جزء من ذلك العامل المؤكسد والجزء المتبقى يؤكسد جزء من الكاشف (الميثلين الازرق أو الميثيل البرتقالى) تاركا جزءاً اخر يتم قياسه طيفياً عند طول موجى ٦٧٨و ٥١٠ نانومتر على التوالى حيث ان الزيادة فى الامتصاص للكاشف المتبقى تتناسب طرديا مع تركيز الدواء المؤكسد. وقد تمت دراسة العوامل المختلفة التى تؤثر على التفاعل كالحامضية، تركيز العامل المؤكسد والوقت. وقد أتبع قانون بيير على مدى تركيز قدره (١-٣) ميكروجرام/ملليلتر لمادة السيفيبيم، (١.٠-٠.٤) ميكروجرام/ملليلتر لمادة السيفوبيرازون(٠.٨-٠.٣) ميكروجرام/ملليلتر لمادة السيفوترايكسون(١.٤-٠.٦) ميكروجرام/ملليلتر لمادة الكابتوبريل فى حالة الميثلين الازرق و قدره (٣-٠.٥) ميكروجرام/ملليلتر لمادة السيفيبيم، (٢.٠-٠.٧٥) ميكروجرام/ملليلتر لمادة السيفوبيرازون(١.٤-٠.٢) ميكروجرام/ملليلتر لمادة السيفوترايكسون(١.٧٥-٠.٥) ميكروجرام/ملليلتر لمادة الكابتوبريل فى حالة الميثيل البرتقالى. وقد أستخدمت الطرق فى تعيين هذه الادوية فى بعض مستحضراتهم الصيدلية وتمت مقارنة النتائج إحصائياً مع الطرق المرجعية.