SPECTROPHOTOMETRIC DETERMINATION OF BINARY MIXTURE OF SOME B-LACTAM ANTIBIOTICS

Khaleda H. Al-Saidi, Nabil S. Nassory and Shahbaz A. Maki
Chemistry Department, College of Science, Al-Nahrain University, Al-Jaderia, Baghdad-Iraq.

Abstract

Derivative spectrophotometric techniques (first, second, third and fourth derivative) were developed for the determination of four β-lactam antibiotic binary mixtures; in combinations containing these compounds. The simultaneous determination of these compounds was accomplished by derivative (^{1}D, ^{2}D, ^{3}D and ^{4}D) spectrophotometric technique and applying zero-crossing technique amoxiclline trihydrate with cephalexin monohydrate (mix I) using ^{1}D spectrum zero crossing at valley 238.2 nm, and ^{2}D spectrum at valley 263.8 nm, respectively, amoxiclline trihydrate with cloxacillin sodium (mix II) using ^{2}D at valley 281 and ^{4}D at valley 230.6, respectively, cephalexin monhydrate with cloxacillin sodium (mix III) using ^{1}D spectrum zero crossing at 272 nm and ^{2}D spectrum at 253.2 nm, respectively and ampicillin trihydrate with cloxacillin sodium (mix IV) using ^{1}D at 282nm and ^{2}D at 271.6 nm, respectively. From this studying it was noticed that in all the applications of the previous methods the correlation coefficient of calibration curves not less than 0.999 and the relative standard deviation not exceed to 0.214. The four described procedures were successfully applied to the determination of these compounds in synthetic mixtures and in preparations with high percentage of recovery, accuracy and precision .The procedures do not require any separation step.

Keywords: β-lactam antibiotics binary mixtures; Derivative spectrophotometry; zero-crossing technique.

Introduction

Amoxicillin

Amoxicillin has the empirical formula ($C_{16}H_{19}N_3O_5$ S. $3H_2O$) and the molecular weight is equal to (419.4 gram/mol), it's prepared as capsules, oral suspension Amoxicillin trihydrate contains not less than 95.0 per cent and not more than the equivalent of 100.5 per cent of (2*S*,5*R*,6*R*)-6-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7 -oxo-4-thia-1- azabicyclo [3.2.0] heptane-2-carboxylic acid, calculated with reference to the anhydrous substance[1].

It is white, or almost white crystalline powder, slightly soluble in water and in alcohol, practically insoluble in ether and in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides. It is better absorbed from the gut, and it is more effective against *Salmonella, Strep. faecalis* and penicillin-resistant pneumococci. It is used in empiric treatment (with or without clavulanate) of bite wound infections, otitis media, sinusitis and urinary tract infections and in prevention of endocarditis in persons at risk with an oral dose of 250 mg 8 hourly[2]. The recent methods for determination of amoxicillin include, spectrometric methods[3-5] and liquid chromatographic[6-9], the method involves solid-phase fluorescence immunoassay[10] and voltammetric determination of amoxicillin[11].

Cephalexin monohydrate

Cephalexin monohydrate contains not less than 95.0 per cent and not more than the equivalent of 101.0 per cent of (6*R*, 7*R*)-7-[(*R*)-2-amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, calculated with reference to the anhydrous substance. The empirical formula of cephalexin was ($C_{16}H_{17}N_3O_4$ S, H_2O), and the molecular weight (365.4g/mol) [1]. Cephalexin occurs as white, or almost white, crystalline monohydrate powder. It is soluble in water, practically insoluble in alcohol and in ether, resistant to acid and well absorbed orally. Several different methods have been used for determination of cephalexin monohydrate including; High-performance

liquid chromatographic[8]. A capillary zone electrophoresis method[12]. Parallux, a solid-phase fluorescence immunoassay[10].And there are several methods used for the determination of cephalexin[13].

Ampicillin

Ampicillin trihydrate contains not less than 96.0 per cent and not more than the equivalent of 100.5 per cent of (2*S*, 5R, 6R)-6-[(*R*)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicylo [3.2.0]heptane-2-carboxylic acid, calculated with reference to the anhydrous

It's a white, crystalline powder, slightly soluble in water, practically insoluble in alcohol, in ether and in fatty oils. It dissolves in dilute solutions of acids and of alkali hydroxides. Which has the empirical formula ($C_{16}H_{19}N_3O_4$ S. $3H_2O$) and the molecular weight is equal to 403.5 gram/mol[1]. It is the semi synthetic penicillin, an antibacterial spectrum broader than that of penicillin G, the α-amino group which plays a significant role in the broader activity, but the mechanism for its action is not known. Ampicillin is not resistant to penicillinase, and it produces the allergic reaction. The protonated α- amino group of ampicillin has a pK_a of 7.3 and it thus extensively protonated in acidic media, which explains ampicillin's stability toward acid hydrolysis and instability toward alkaline hydrolysis. It is administered orally and is absorbed from the intestinal tract to produce peak blood level concentrations in about 2 hours. Oral doses must be repeated about every 6 hours, because it is rapidly excreted, without any change through the kidneys. It is available as anhydrous powder or trihydrate or as sodium salt which is very soluble in water and solutions for injections should be administered within one hour after being made. Ampicillin is one of extended-spectrum penicillins, it is acid stable and moderately well absorbed when taken orally. It has a spectrum broader than that of benzyl penicillin and it is bactericidal against gram-negative bacteria including *H. influenzae*, *Salmonella*, *Shigellae*, *E. Coli*, and some proteus strains [2] . The recent methods for determination of ampicillin include, electrochemical[14], liquid chromatographic[15] and spectrophotometric methods[16, 17].

Cloxacillin Sodium

Cloxacillin sodium contains not less than 95.0 per cent and not more than the equivalent of 101.0 per cent of sodium (2*S*,5*R*,6*R*)-6-[[[3-(2-chlorophenyl)-5-methylisoxazol-4-yl] carbonyl]amino] -3,3- dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, calculated with reference to the anhydrous substance. It is a white or almost white, crystalline powder, hygroscopic, freely soluble in water, in methanol, and in alcohol. It has the empirical formula ($C_{19}H_{17}ClN_3NaO_5S$. H_2O) and the molecular weight is equal to 475.9 gram/mol[1]. The recent methods for determination of cloxacillin include, liquid chromatographic[17] and spectrophotometric methods[10]. All studied combinations are broad-spectrum antibiotic combination widely used in the treatment of wide range of gram-negative and gram-positive organisms. They are acid stable and well absorbed producing good serum and urine concentration[2]. Derivative spectrophotometry is an analytical technique of great utility for resolving some mixtures of compounds with overlapping spectra[18-22]. In this work, new methods were used to develop spectrophotometric methods for the simultaneous determination of the components of these binary mixtures without prior separation.

Experimental

Instruments and Equipments:

Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZ (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10), using 1.00 cm quartz cells, Ultra sonic devise (ultrasonicator) for dissolving samples, (SONOREX), (W. Germany), Ultra pure water manufacturing devise, (TORAYPURE), model LV-08 (Japan).

Chemicals:

Standard antibiotic drugs: Amoxicillin trihydrate (amox), cephalexin monohydrate (ceph), cloxacillin sodium (clox) and ampicillin trihydrate (amp) were gift from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI- Samara) and the mixture of ampicillin sodium with

cloxacillin sodium injection (AMPCLOX-500) were marketed by (Ajanta pharmaceutical limited company, India). All drugs were used as working standards without further purification and analyzed to one of the official methods or reported methods to determine their purity and compliance with the requirements.

Preparation of Stock and working Standard Solutions

1-Stock solutions of standard were prepared by dissolving an accurately weighed amount (50 or 25 mg) of the studied drugs in about 80 ml of the deionized water 100 ml volumetric flask .using ultra sonic devise (ultrasonicator) for dissolving samples, The solutions are then made up to the volume with deionized water, to obtain the suitable working standard solutions according to the linear calibration range for each drug.

2-Four series of pure single standards drugs prepared by dilution from stock solutions with the deionized water.

3-Solutions for binary mixtures of standard drugs Amoxicillin trihydrate (A) and cephalexin monohydrate (B) solutions were prepared by two series.

First series of mixture solutions were prepared by using a fixed concentration of (30 mg/L) for drug (A) with different concentrations (10, 20, 30, 40, and 50 mg/L) of drug (B), second series of mixture contain a fixed concentration (30 mg/L) of drug (B) with different concentration of (10, 20, 30, 40, and 50mg/L) of drug (A).

4- The solutions of other binary mixture were prepared by the same procedure used for Amoxicillin trihydrate and cephalexin monohydrate binary mixture, other binary mixtures, which are:

1- Amoxicillin trihydrate and cloxacillin sodium.

2- Cephalexin monohydrate and cloxacillin sodium.

3- Ampicillin trihydrate and cloxacillin sodium.

4- (AMPCLOX 500) all contents of vial diluted to 1L the resultant solution may be contained 250 mg/L ampicillin sodium + 250 mg/L cloxacillin sodium, the other diluted solutions were prepared by the serial dilution.

Results and Discussion

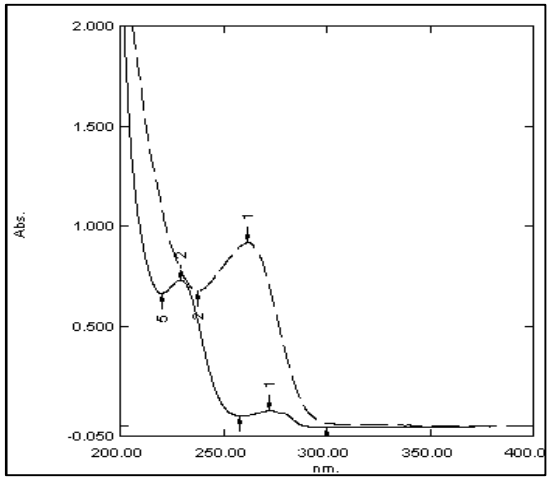
***Amoxicillin trihydrate with Cephalexin Monohydrate Mixture (mix I)*:**

Normal spectrum can not be used to determine each of amox and ceph present in mixture, due to interference between the spectra, as shown in Fig.1a. Therefore, UV derivative can be used in this case**.** As shown in Fig.(1) and (2), first derivative may be used to determine ceph in the presence amox by using zero crossing method at valley 264 nm. The calibration curve of ^{1}D for standard solutions (8-80 mg/L) ceph was constructed gave linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (1).

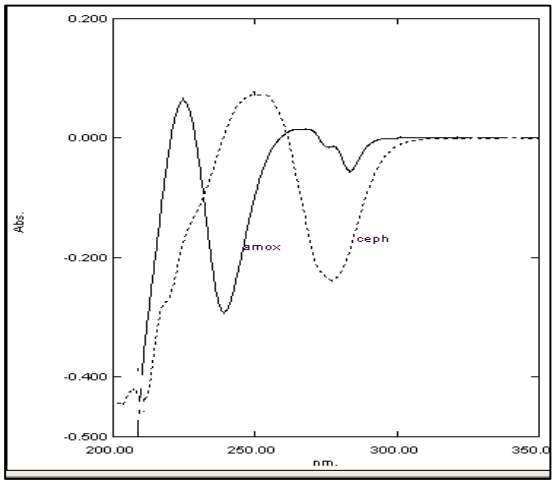
And the relative standard deviation for each concentration represents an average of at least three measurements is between 0.587-1.272. This method can not be used, when the mixture contain more than 25% amox therefore the second derivative may be taken to obtain more accurate results to determine ceph. Also ^{1}D spectrum can be used to determine for amox in the presence of ceph in the mixtures as shown in Fig.(2) and Fig.3 by using zero crossing method[22] at valley 238.2 nm. The calibration curve was constructed for standard amox solutions (10-50 mg/L amox) gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (1). And the relative standard deviation for each concentration represents an average of at least three measurements is between 0.271-0.542. The results in Table (1) that the zero crossing method at valley 238.2 nm can be used for determination amox in presence of ceph with good result, but at valley 264 nm for determining ceph in the mixture contain less than 25% amox.

Table (1)
The Methods using for determination binary mixture ceph. and amox (mix.I).

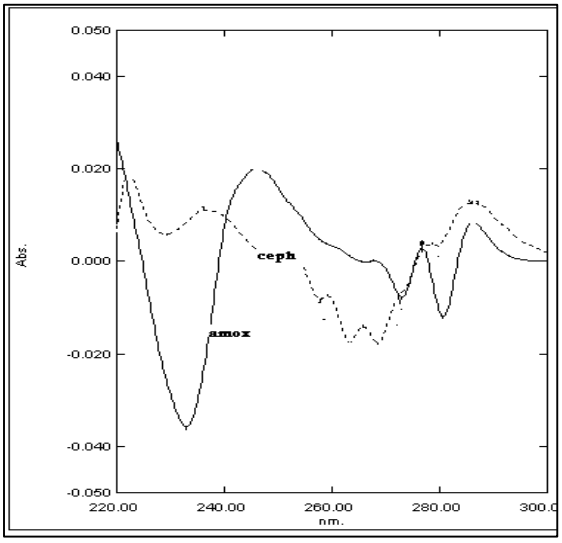
Drug	Concentration range mg/L	Method		Equation	Relative error for 50% mixture	r
Ceph.	8-80	^{1}D	Valley=264.0	Y=0.00157X + 0.00193	-4.303	0.99927
Amox.	10-50	^{1}D	Valley= 238.2	Y=0.00931X + 0.00710	+0.223	0.99990
Ceph.	8-80	^{2}D	Valley=263.8	Y=0.00066X + 000000	0.757	0.99992
Amox.	5-50	^{2}D	Peak=250.50	Y=0.00057X + 000000	1.207	0.99996



a-Normal spectrum of 30 mg/l of amox and 30 mg/Lof ceph.



b- D1of each 30 mg/l of amox and 30 mg/L of ceph.



c- D2 of 30mg/lamox and 30mg/L ceph.

Fig.(1): The spectra: a- normal spectra of amox and ceph, b- D1 of each amox and ceph, c- D2 of each amox and ceph.

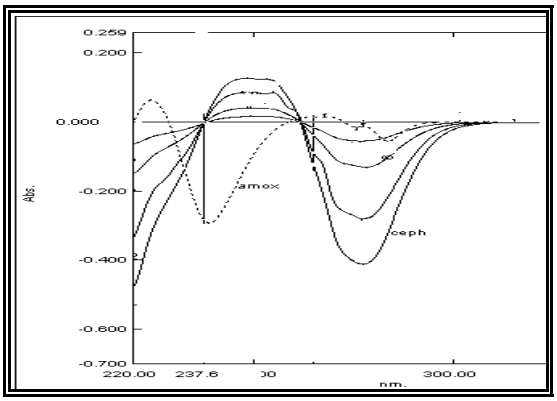


Fig. (2) : ^{1}D spectrum for 30mg/L amox with zero crossing at valley 238.2 nm for amox and ^{1}D spectra for 8- 40 mg/L ceph near zero crossing at valley 264 nm for ceph.

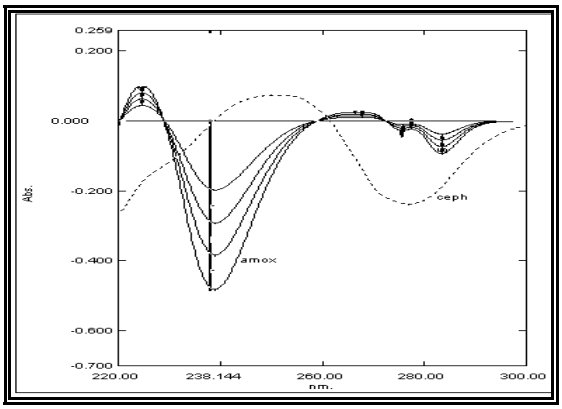


Fig.(3) : ^{1}D spectra of solutions 20-50 mg/Lamox using zero crossing method at valley 238.2 nm with ^{1}D spectrum 30mg/L of ceph.

Second Derivative (^{2}D) spectra of 30 mg/L amox and 30 mg/L ceph show the valley at 263.8 nm for ceph and at 250.5 nm for amox, which were crossing zero and they are suitable for measuring ceph and amox respectively as shown in Fig.(4). Cephalexin can be determined in the presence of amox using ^{2}D spectrum at valley 263.8 nm. The calibration curve of ^{2}D with the range of concentrations (8-80 mg/L ceph) at 263.8 gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (1). And the relative standard deviation for each concentration represents an average of at least three measurements is between 0.881- 1.163. Amoxicillin can be determined in the presence of ceph using ^{2}D spectrum at 250.5 nm. The calibration curve of ^{2}D for standard amox solutions was ranged from 5 to 50 mg/L at 250.5 nm gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (1). And the relative standard deviation for each concentration represents an average of at least three measurements. is between 0.283- 0.663.

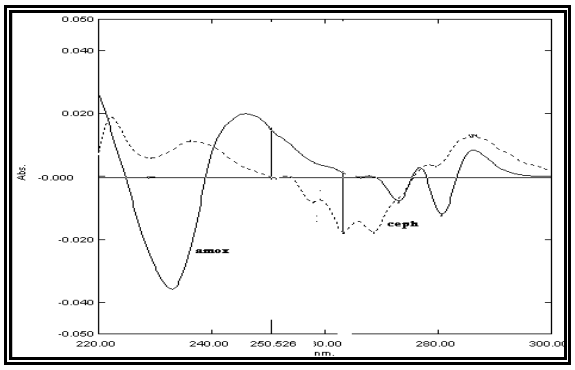


Fig.(4) : ^{2}D spectra for 30mg/L amox with zero crossing at 250.5 nm and ^{2}D spectra for 30mg/L ceph with zero crossing at valley 263.8 nm .

Amoxicillin Trihydrate with Cloxacillin Sodium Mixture (mix.II):

Normal spectrum can not be used to determine each of amox and clox present in mixtures due to the high interfering between their normal spectra. Also standard clox alone can not be determined because there is no district wavelength. Fig.(5a) shows the normal spectra of 30 mg/L of amox and 30 mg/L clox. Therefore, UV derivative can be used to solve this problem.

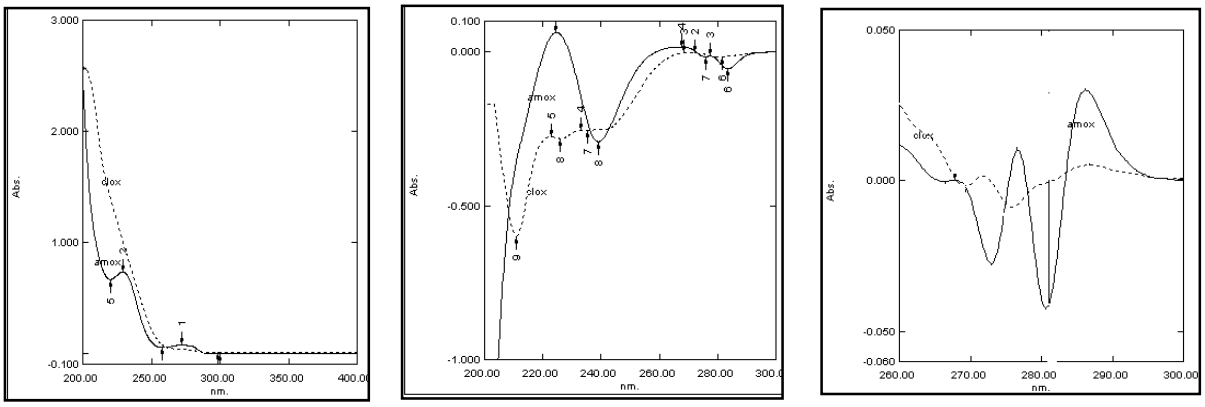
First derivatives for 30 mg/L amox and 30 mg/L clox also can not be used to determine each amox and clox present in the mixture, because there is no certain wavelength suitable to determine each of the drugs. (Fig.5b) shows the interference between their ^{1}D spectra. Second derivatives for 30 mg/L amox and 30 mg/L clox can be used to determine amox only because there is only one wavelength suitable to determine amox without interfering with clox at valley 280.6 nm, the absorbance of clox equal to –0.001. Therefore, the valley at 281 nm which was crossing zero of clox was

used to determine amox as shown in Fig.(5c). The calibration curve of ^{2}D for standard amox solutions was ranged from 5 to 50 mg/L at 281 nm gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (2). And the relative standard deviation for each concentration represents an average of at least three measurements. is between 0.447- 0.829. Third derivative was taken for clox and amox because clox can not determine by normal, first and second derivatives, due to the interfering between the spectra of the drugs. Fig.(5 a, b, and c) show that interference. In the presence of amox, a third derivative must be taken and tried to determine clox at valley 266 nm, which is crossing zero with spectrum of amox, as shown in Fig.(5d). This wavelength is suitable to determine the standard clox, but in the mixture the error was exceeding to 10% for mixtures containing more than 50% amox.

Fourth Derivative***:*** Fig.(5e) shows ^{4}D spectra for 30 mg/L clox and 30 mg/L amox and the suitable wavelength for determine clox, which is the valley at 230.6 nm. In order to obtain a better result for determining clox in the presence of amoxicillin fourth derivative is used. The calibration curve of ^{4}D for standard clox. solutions was ranged from 10 to 50 mg/L at 230.6 nm gave a linear equation with slope and the correlation coefficient and the relative errors for the mixtures containing 50% for each drug were listed in Table (2). And the relative standard deviation for each concentration represents an average of at least three measurements. is between 0.365- 1.325.

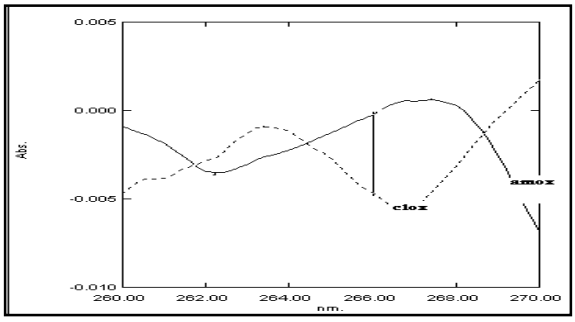
Table (2)
The Methods using for determination binary mixture amox and clox (mix.II).

Drug	Concentration range mg/L	Method		Equation	Relative error for 50% mixture	r
amox.	10-50	^{2}D	Valley=281.0	Y=0.00139X + 0.00002	-4.303	0.99927
Clox	10-50	^{4}D	Valley=230.6	Y=0.00075X + 00290	+1.297	0.99938

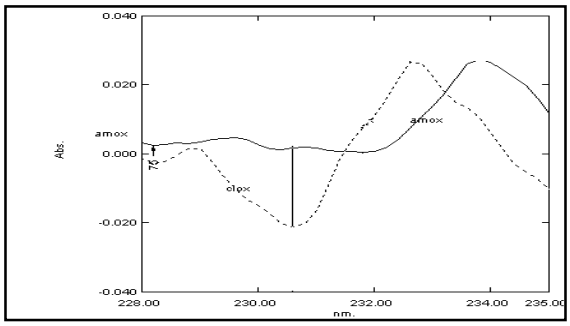


a- Normal spectra of amox b-2-D1 spectra of amox and clox c- ^{2}D spectrum at valley 281 nm and clox for determining amox.

Fig.(5) : The spectra of 30 mg/L of amox and 30 mg/L of clox: a- The normal spectra, b- ^{1}D spectra. c- The spectra of 30 mg/L amox and 30 mg/L clox, ^{2}D spectrum at valley 281 nm for determining amox.



a-[3]D clox and amox spectra, at 266 nm for determining clox.



b-[2]D clox and amox spectra, at valley 230.6 nm for determining clox.

Fig.(6) : a- Third derivatives for 30 mg/L clox and 30 mg/L amox spectra, at 266 nm for determining clox., b- Fourth derivatives for 30 mg/L clox and 30 mg/L amox spectra, at valley 230.6 nm for determining clox.

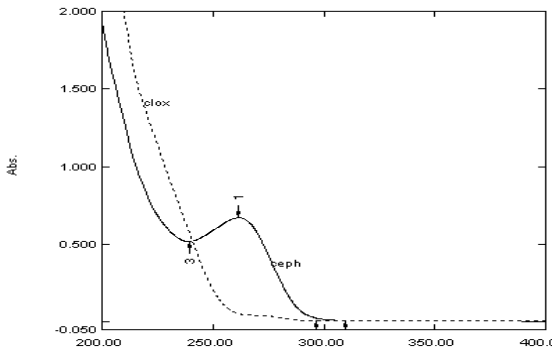
Table (3)
The Methods using for determination binary mixture ceph and clox.

Drug	Concentration range mg/L	Method		Equation	Relative error for 50% mixture	r
Ceph.	10-50	^{1}D	Valley =272	Y=0.02212X + 0.01380	+0.207	0.99928
Clox	10-50	^{2}D	Peak=253.2	Y=0.00161X + 0.00170	+0.323	0.99961

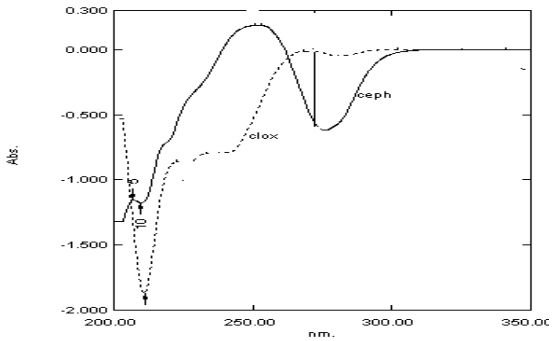
Cephalexin Monohydrate with Cloxacillin Sodium Mixture (mix.III):

Normal spectrum can not be used to determine each of clox and ceph present in the mixture, due to the interfering between their normal spectra of the drugs. Fig.(9a) shows the spectra of 30 mg/L ceph and 30 mg/L clox, therefore, UV derivative (^{1}D, ^{2}D, ^{3}D and ^{4}D) for 30 mg/L ceph and 30 mg/L clox spectra have been taken as shown in Fig.(7). First derivative shows the suitable wavelength used to determine standard ceph at valley 275 nm but in the mixture the valley at 272 nm near zero crossing with cloxacillin was used. But ^{1}D spectrum can not be used to determine clox, due to the interfering of spectra of clox with spectra of ceph. The second derivative spectrum may be using to determine ceph spectrum at 298 nm near zero crossing but with error about 8% for mixture contains 50% clox. Also ^{2}D spectra can be used to determine cloxacillin at peak 253.2 nm which used to determine clox with excellent results.

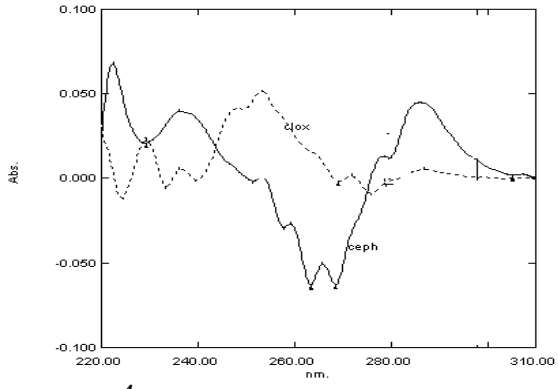
Fig.(7c and d) shows ^{2}D spectra shows the wavelengths for determining ceph at 298 nm and for clox at 253.2 nm respectively. A calibration curve for standards ceph and clox ranged 10 to 50 mg/L gave a linear equations with slope and the correlation coefficient and the relative errors for Ceph and clox at 272 and 253.2 nm respectively for the mixtures containing 50% for each drug were listed in Table(3). And the relative standard deviation for each concentration represents an average of at least three measurements. is between 0.765-1.821. Fig.(7) shows third derivative spectrum may be used to determine of ceph at 293.4 nm where clox absorbance equal to zero and clox at peak 251.2 nm. Also fourth derivative spectra can be use to determine clox in the presence of ceph at 232.8 nm and for ceph in the presence of clox at 268 nm. But the results which obtained from ^{3}D and ^{4}D for determination of both ceph and clox are not accurate, due to the noise[22,23].



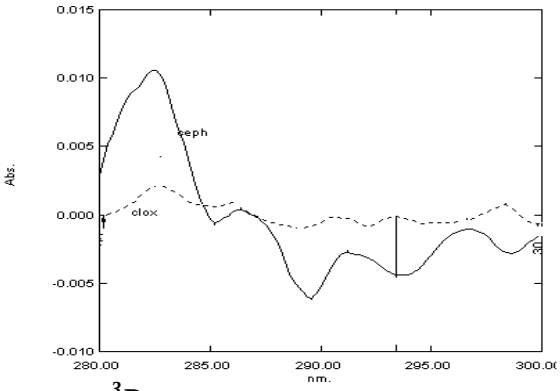
a-Normal spectra of ceph and clox.



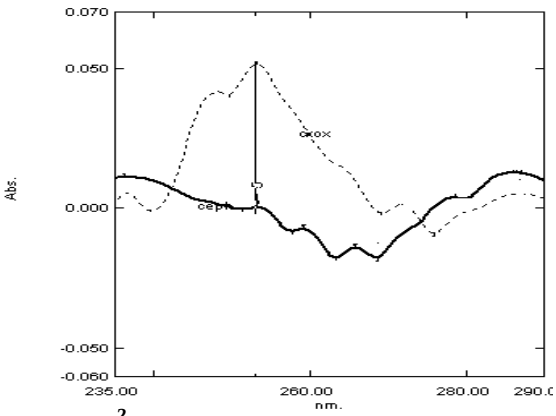
b- ^{1}D spectra of ceph and clox at valley 272 nm.



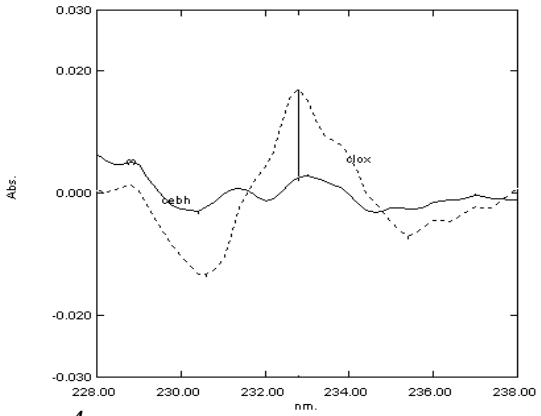
c- ^{4}D of ceph and clox. at 298 nm.



e- ^{3}D of ceph and clox. at 293.4 nm.



d- ^{2}D of ceph and clox at 253.2 nm.



f- ^{4}D spectra of ceph and clox. 232.8 nm.

Fig.(7) : The spectra for 30 mg/L of ceph and for 30 mg/L of clox: a- normal spectra, b- ^{1}D spectra of ceph and clox at 272 nm, c- ^{2}D spectra of ceph and clox at 298 nm, d- ^{2}D spectra of ceph and clox at 253.2 nm, e- ^{3}D spectra of ceph and clox at 293.4 nm , f- ^{4}D spectra of ceph and clox 232.8 nm.

Ampicilln with Cloxacillin Sodium Mixture (mix.IV):

Ampicillin and Cloxacillin (Ampeclox):

Normal spectrum can not be used to determine for each cloxacillin and ampicillin (sodium or trihydrate form) because there is no specific wavelength, the derivative method was studied to solve this problem. Fig.8 shows. The first derivative spectrum for cloxacillin sodium, ampicillin trihydrate and ampicillin sodium. There is only one wavelength, which is suitable for determination cloxacillin in the presence of

ampicillin trihydrate or ampicillin sodium or both at valley 282 nm. The calibration curve was constructed using standard clox solutions ranged (5–50 mg/L).

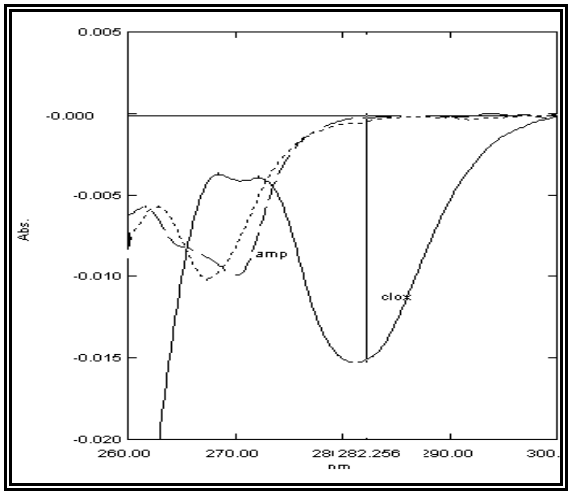


Fig.(8) : ^{1}D spectrum of 30 mg/L amp.Na and ^{1}D spectrum of 30 mg/L amp.3H$_2$O and ^{2}D spectrum of 30 mg/L clox and shows the wavelength for determination clox at 282 nm.

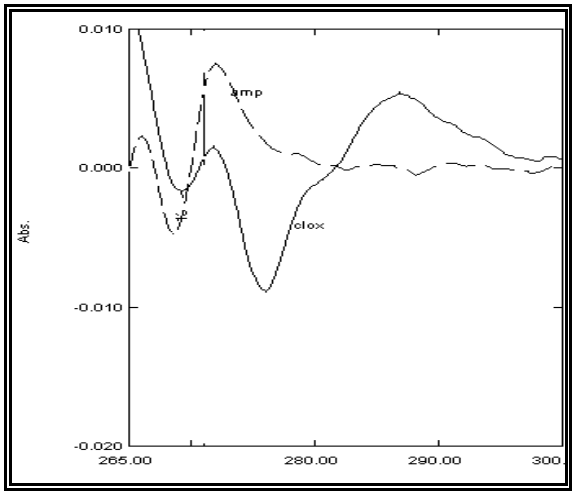


Fig.(9): ^{2}D spectra 30 mg/L amp.3H$_2$O and ^{2}D 30 mg/L clox. and the suitable wavelength for measuring ampicillin trihydrate at 271.6 nm.

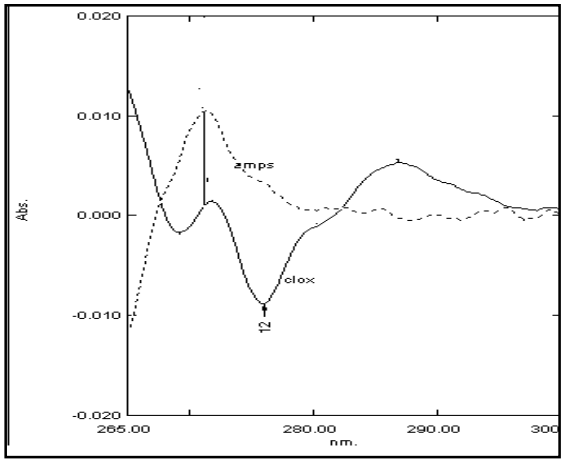


Fig.(10) : ^{2}D spectra 30 mg/L amp.Na and D2 30 mg/L clox and suitable wavelength for measuring ampicillin sodium at 271.2 nm.

Table (4)
The methods for determination each of cloxacillin, Ampicillin trihydrate and Ampicillin sodium binary mixture in the drug.

Drug	method	wavelength	equation	r
cloxacillin sodium	D1	Valley=282nm	Y=-0.00155x-0.00100	0.99964
Amoxicillin trihydrate	D2	271.6 nm	Y=0.00053x-0.00022	o.99912
Amoxicillin sodium	D2	271.2	Y=0.00035x-0.00070	0.99878

Determination of Ampicillin and Cloxacillin in the Pharmaceutical Sample

Second derivative spectrum of 30 mg/L cloxacillin and the spectrum of 30 mg/L ampicillin trihydrate which contain a suitable wavelength for measuring ampicillin trihydrate at 271.6 nm as shown in Fig.(9), the suitable wavelength for measuring ampicillin sodium at 271.2 nm when the absorbance of cloxacillin equal zero as shown in Fig.(10). The calibration curves of ^{1}D and ^{2}D for standards cloxacillin, ampicillin trihydrate and ampicillin sodium solutions (5–50 mg/L) gave a linear equation and the correlation coefficient and were listed in Table (4).

Analysis of Pharmaceutical Sample:

AMPECLOX samples with different concentration (10, 20, 30 and 40 mg/L) were measured using the methods, which listed in Table (5) for determination each of cloxacillin, ampicillin trihydrate and ampicillin sodium.. ^{1}D at 282, ^{2}D at 271.6 and ^{2}D at 271.2 nm, respectively were used. The results are listed in Table (5). From the results in the Table (5), the average percentage , standard deviation and relative standard deviation were calculated and listed in Table (6).

Table (5)
Determination of cloxacillin, Ampicillin trihydrate and Ampicillin sodium in the drug.

Clox (475.9)		**Amp.3H_2O (419.4)***			**Amp.Na (388.4)***		
mg/L	**% clox**	**mg/L**	**%amp.3H_2O**	**%amp**	**mg/L**	**%amp.Na**	**%amp**
5.158	51.580	5.287	52.870	46.063	…	….	…
10.334	51.67	10.741	53.705	46.79	9.870	49.35	46.428
15.270	50.900	15.542	51.807	45.137	15.163	50.540	47.547
20.667	51.668	21.125	52.813	46.013	19.857	49.643	46.703
30.354	50.59	30.542	50.903	44.349	29.429	49.048	46.143
40.686	50.858	41.724	52.155	45.44	40.161	50.201	47.228

Listed increment in 50% clox and 50% amp.Na, *the molecular weigh of drug.

Table (6)
Statistical calculations.

drug	**Average %**	**Standard deveation**	**RSD%**
clox	51.211%	0.482	0.941
amp.3H_2O	52.376	0.974	1.859
amp.Na	49.756	0.611	1.228

The pharmaceutical sample AMPECLOX analysis was in range of the certificate.

References

[1] British Pharmacopoeia 2000, CD, ROM.

[2] D. A. Williams, and T. L. Lemke," Foye's Principles of Medicinal Chemistry" 5th ed. Lippincott Williams & Wilkins, 2002.

[3] H. Salem, and G. A. Saleh, "Selective spectrophotometric determination of phenolic betalactam antibiotics. *J. Pharmaceut. Biomed.*, Vol. 28, 2002, pp. 1205-1213.

[4] B. S. Nagaralli, J. Seetharamappa, and M. B. Melwank,." Sensitive and Accurate Spectrophotometric Methods for Determination Amoxicillin, ciprofloxacin and Piroxicam in pure and pharmaceutical." *J. Pharm-Biomed-Anal.*, Vol.29, 2002, pp. 859-864.

[5] Fernadez-Gonzalez, A, Badia, R. and Diaz-Garcia, M. E. "Comparative study of the micellar enhaced spectrophotometric determination of beta-lactam antibiotic by batch and flow injection analysis using multisimplex design." *J. Pharm-Biomed-Anal.*, Vol. 29, 2002, pp. 669-679.

[6] S. Husain, V. Ghoulipour, and H. Sepahrian,."Chromatographic determination of some betalactam antibiotics". *Acta Chromatogr.*, Vol. 14:, 2004, pp. 102-109.

[7] G. Hoizey; D. Lamiable, T. Trenque, M Kaltenbach,. J. Denis, and H Millart, "Simultaneous Determination of amoxicillin and Clavulanic acid in Human Plasma by HPLC with UV Detaction." *J. Pharm-Biomed-Anal.*, Vol. 30, 2002, pp. 661-666.

[8] S. Ghidini, E. Zanardi, G. Varisco, and R Chizzolini,." Residues of beta-lactam antibiotic in bovine milk,: confirmatory analysis by liquid Chromatography Tandem Mass spectrometry after microbial assay screening." *Food-Addit-Contm.*, Vol.20, 2003, pp. 528-534.

[9] M. Dousa,.and R. Hosmanova, ,"Rapid determination of amoxicillin in premixes by HPLC." *J. Pharm-Biomed-Anal.*, Vol.37, 2005 , pp.373-377.

[10] L Okerman, K. De-Wasch, J. Van-Hoof, and W. Smedts, "Simultaneous determination of different antibiotic residues in bovine and in porcine kidneys by solid-phase fluorescence immunoassay." *J. AOAC-Int.*, Vol. 86, 2003, pp. 236-240.

[11] B. Uslu, and I. Biryol,," Voltammetric determination of amoxicillin using using a poly (N-vinyl imidazole) modified carbon paste electrode". *J-Pharm-Biomed-Anal.*, Vol.20, No 3, 1999, pp. 591-598.

[12] C. E. Lin. H. W. Chen E. C. Lin, K. S. Lin, and H. C.Huang, "Optimization of separation and migration behavior of cephalosporins in capillary zone electrophoresis.", *J – Chromatogr – A.*, Vol. 879, 2000, pp.197-210.

[13] Gallo-Martinez, L Campins-Falco, P. and Sevillano-Cabeza, A.,"Comparison of several methods for determination of cephalosporine analysis of cephalexin in pharmaceutical sample. "*J. Pharm-Biomed-Anal.*, Vol.29, 2002, PP.405-423.

[14] B. Prasad, and B. Arora, "Potentiometric determination of ampicillin", *Electroanalysis*, Vol. 15, 2003, pp.1212-1218.

[15] I. Palabiyik, and F. Onur," Analytical study of Ampicillin sodium" *Anal. Lett.* Vol. 37, 2004, pp. 2125-2150.

[16] G. G. Mohamed, ," Spectrophotometric Determination of some Antibiotics Binary Mixtures" *J-Pharm-Biomed-Anal*. Vol. 24, 2001, pp. 561-567.

[17] T.L Tsou, J. R Wu, C.D. Young, and T. M.Wang, , *J-Pharm-Biomed-Anal*., Vol. 15, No.8, 1997 , pp. 1197.

[18] M. M. Mabrouk; H. Salem: M. F. Radwan and T. S. Kaood." Derivative Spectrophotometric Determination of some Selected Antihypertensive combinations.' Egypt, *J. Biomed. Sci.*, Vol. 12, 2003, pp. 141-173.

[19] H. Salem." Atomic absorption Spectrometric, spectrophotometric and derivative spectrophotometric determination Sildenafil citrate (Viagra) in Bulk Powder and in Pharmaceutical Dosage form." *J. Appl. Sci*.,Vol. 8, 2006, pp.28-43.

[20] I. Abd El-Maboud : H. Salem and E. Maher." Spectrophotometric Determination of Binary Mixtures of Prednisolone with some Antibiotices."*Thai. J. Pharm. Sci.*, Vol. 30, 2006, pp.63-81.

[21] A. Mohamed and H. Salem," Determination of certain Antihypertensive mixtures using Chemometrics – assisted Spectrophotometric Method.", *Anal.*

Bioanal.Chem., Vol. 383, 2005, pp. 1066-1072.
[22] I. Abd El-Maboud : H. Salem and E. Maher." Chemometric-assisted Spectro-photometric Determination of certain β-lactam Antibiotices Combinations."*Thai. J. Pharm. Sci.*, Vol. 31, 2007, pp. 1-24.
[23] T. C. O'Haver and T. Begley," Signal-to-Noise Ratio in Derivative Spectroscopy", Anal.Chem.., Vol. 53, 1981, pp. 1876-1878.
[24] T. C. O'Haver," Derivative Spectroscopy: Theoretical Aspects", *Anal. Proc*., Vol. 19, 1982, pp. 22-28.

الخلاصة

في هذا البحث تم تقدير هذه المركبات أنيا باستخدام المشتقات الطيفية الأولى والثانية والثالثة والرابعة للأدوية. على شكل أمزجة ثنائية للأدوية فقد تم تعين الأطوال الموجية بتطبيق التقاطع الصفري لطيف المشتقات لتعين كل دواء بوجود الأخر وكما يلي:

مزيج الأموكسيسيلين والسيفليكسين باستخدام المشتقة الأولى (238.6 nm) والمشتقة الثانية في (263.8 nm) على التوالي.

مزيج الأموكسيسيلين الكلوكساسلين باستخدام المشتقة الثانية (281 nm) والمشتقة الرابعة في (230.6 nm) على التوالي.

مزيج السيفليكسين و الكلوكساسلين باستخدام المشتقة الأولى (272 nm) والمشتقة الثانية في (253.2 nm) على التوالي.

مزيج الكلوكساسلين و الأمبيسلين باستخدام المشتقة الأولى (282 nm) والمشتقة الثانية في (271.6 nm) على التوالي.

لوحظ من هذه الدراسة ان جميع التطبيقات ذات منحنيات معايرة بمعامل ارتباط r لا يقل عن 0.999 ومعدل انحراف نسبي لا يزيد عن241. وكانت طرق ناجحة تطبيقية لتعين هذه المركبات في امزجة دوائية أو صناعية. هذه الطرق سهلة لا تحتاج الى فصل أو أي معاملة وهي ذات دقة عالية.