Simultaneous determination of amoxicillin and potassium clavulanate antibiotics in pharmacueutical sample using derivative spectrophotometric method

التحليل المتعاقب للمضادات الحيوية لدواء ألأموكسيسيلين و كلافونيت البوتاسيوم باستخدام المشتقات الطيفية

Khaleda H. Al-Saidi **Sarah S. Abdulameer**
College of Science / Al-Nahrain University
خالدة حميد محمد **سارة ساجد عبد الأمير**
كلية العلوم \ جامعة النهرين

Abstract

Derivatives spectrophotometric techniques were developed for the determination of Amoxicillin Trihydrate (Amox) with Potassium Clavulanate (PC) antibiotic binary mixtures. The simultaneous determination of these compounds was accomplished by derivative (^{1}D, ^{2}D and ^{3}D) spectrophotometric technique and applying zero-crossing technique used for determination of (Amox) and (PC) in tablets. The second order derivative absorption spectra at valley λ=299 nm were used for (Amox) and also the second order derivative spectra at peak λ=239.5 nm were used for (PC). No interferences were found between both determined constituents and those of matrix. A good accuracy and precision of simultaneous determination of (Amox) and (PC) were confirmed by statistical analysis. The recovery of individual constituents under established conditions is very high and ranges for synthetic standards mixture and tablets from 100.11, 99.33 and 96.98, 96.84 respectively. Linearity is maintained within a wide concentration range from 2.0 to 90.0 µg.mL^{-1} and from 10.0 to 90.0 µg.mL^{-1} for (Amox) and (PC) and linearity percentage 99.98 and 99.99 respectively. The detection limit is 0.211 µg.mL^{-1} for (Amox) and 0.259 µg.mL^{-1} for (PC). The corresponding quantitation limits are 0.704 µg.mL^{-1} (Amox) and 0.864 µg.mL^{-1} for (PC).

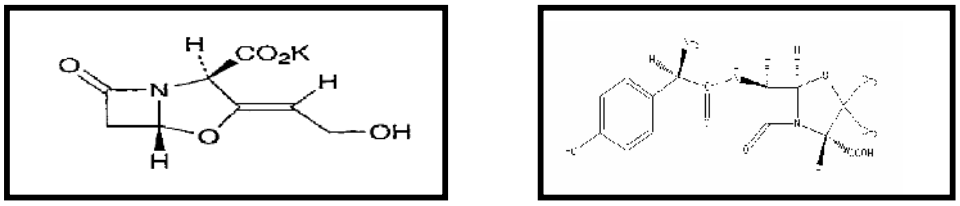
المستخلص

أستخدم في هذا البحث المشتقات الطيفية لتقدير المضادات الحيوية في المزيج المزدوج من الأموكسيسلين كلافونيت البوتاسيوم . التحليل المتعاقب لهذه المركبات تم باشتقاق كل من المشتقة الأولى والثانية و الثالثة مع طريقة التقاطع الصفري لتحديد الأطوال الموجية المناسبة لتقديركل منهما في حبات الأدوية . وجد أن المشتقة الثانية كانت مناسبة لتقدير الأموكسيسلين كلافونيت البوتاسيوم وفي الأطوال الموجية (299 ،239.5)نم على التوالي ، وبدون تداخلات من مكونات الدواء ، ودقة وحدود ثقة جيدة للتقدير المتعاقب لكليهما اعتمادا على التحليل الاحصائي وكمايلي : نسبة استرجاع لكل منهما في النموذج القياس والحبات الدوائية 100.11، 99.33 ، 96.98 ، 96.84 على التوالي . ومدىخطي عريض لتركيزالأموكسيسيلين (2.0- 90.0)ملغم/لتر ونسبة مئوية خطية 99.98 و كلافونيت البوتاسيوم (10.0- 90.0) ملغم/لتر وبنسبة مئوية خطية 99.99. أما حد الكشف 0.211 ملغم/لترللأموكسيسيلين و 0.259 ملغم/لتر كلافونيت البوتاسيوم . وان الحد الكمي 0.704 ملغم/لترللأموكسيسيلين و 0.864 ملغم/لتر كلافونيت البوتاسيوم .

Keywords: Amoxicillin Trihydrate, Potassium Clavulanate antibiotics binary

Introduction

Potassium clavulanate (PC), as shown in figure (1-a) is the salt of clavulanic acid that belongs to the β-lactamase inhibitors. The empirical formula of potassium clavulanate was ($C_8H_8KNO_5$), and the molecular weight 237.3 g/mol. Potassium clavulanate occurs as a white to light yellowish white, crystalline powder, hygroscopic. It is freely soluble in water, slightly soluble in alcohol, very slightly soluble in acetone [1].



a

b

Fig (1): Structure of a- potassium clavulanate, b- Amoxicillin trihydrate

Several different methods have been used for determination of potassium clavulanate including; High-performance liquid chromatographic [2-6]. An electrophoresis method [7] A method using sequential injection analysis [8, 9], potentiometric methods [9-10], A densitometric method [11]. A chemiluminescence (CL) method [12].

Amoxicillin trihydrate (Amox) as shown in Figure (1-b) is β-lactam antibiotic that belong to the group of penicillin's. Amox is a 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. The empirical formula of Amox was (C16H19N3O5S.3 (H2O)), and the molecular weight is equal to (419.4 g/mol) [1].

Amox is a commonly used β-lactam antibiotic, which is highly active against a broad spectrum of bacteria. High rate of absorption and stability of Amox under acid conditions are among the most important advantages of this antibiotic. Amox like the other β-lactam antibiotics is usually produced by a semisynthetic route using reactive groups of 6-aminopenicillanic acid (6-APA). Also, various hydrated forms of amoxicillin, including monohydrate, dihydrate, and trihydrate, have been reported, among which, the trihydrate is the most stable hydrated form. Crystallization of Amox, like the other crystalline drugs, plays a critical role in controlling the crystal form, shape, size, and size distribution [13].

The recent methods for determination of amoxicillin include, liquid chromatographic [14 -19], voltammetric determination of amoxicillin [20, 21], Chemiluminescence Flow-Injection Analysis [22 -24] and Chemiluminescence method [25], Flow Injection Analysis using UV-Detection, Potentiometry, and Conductometry [26] and different spectrometric methods [27 -34]

Derivative spectrophotometry is an analytical technique of great utility for resolving some mixtures of compounds with overlapping spectra such determination of some selected antihypertensive combinations [35], determination of binary mixtures of prednisolone with some antibiotics [36], and other applications [37 -39]. 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 CP) SHEMADZU (Japan), interfaced with computer via SHEMADZU UV-probe data system program version 1.10 and Ultra Sonic device (ultrasonicator) for dissolving samples, (SONOREX), (W. Germany).

1. Chemicals

Standard antibiotic drugs: Amoxicillin trihydrate (Amox) ($C_{16}H_{19}N_3O_5S.3(H_2O)$; F.W. 419.4), was a gift from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI-Samara), and potassium clavulanate (PC), which contains the silicon dioxide (SD) 1:1 weight ratio ($C_8H_8KNO_5$; F.W. 237.3) was gift from the pharmacie centrale de tunisie- Tunis. Commercial drugs: Amoxicillin capsule (APMOX-500 mg) made by Ajanta pharmaceutical limited company (India), and the mixture of amoxicillin with potassium clavulanate (Co-amoxiclav-625 mg) made by globalpharma-U.A.E.

Preparation of Standard Solutions

1. Stock standard solutions of 200 $\mu g.mL^{-1}$ standard were prepared by dissolving an accurately weighed amount [20 mg Amox and 40 mg from (PC+SD)] of the studied drugs, 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. Two series of pure single standards drugs prepared by dilution from stock solutions with the deionized water.
3. Solutions for binary mixtures of standard drugs Amox and PC were prepared by two series; first series of mixture solutions were prepared by using a fixed concentration of (30 $\mu g.mL^{-1}$) of drug Amox with different concentrations (5, 10, 20, 30, 40, 50 ,60) $\mu g.mL^{-1}$ of drug PC, second series of mixture contain a fixed concentration (30 $\mu g.mL^{-1}$) of drug PC with different concentration of (5, 10, 20, 30, 40, 50 ,60) $\mu g.mL^{-1}$ of drug Amox.

Preparation of Commercial drugs Solutions

Stock solution of (500 $\mu g.mL^{-1}$ Amox + 125 $\mu g.mL^{-1}$ PC) for Co-amoxiclav was prepared by dissolving average weight of one capsule from the weight of five capsules in 1000 mL distilled water. Other standard solution of (50 $\mu g.mL^{-1}$ Amox + 12.5 $\mu g.mL^{-1}$ PC) was prepared by taking 10 mL from the stock solution diluted to 100 mL volumetric flask.

UV- Measurement

The absorption spectra of the amoxicillin trihydrate, and potassium clavulanate were measured from (200-600)nm against distilled water as blank. The wavelength at absorption maximum (λ_{max}) was identified. The calibration curves were constructed for these drugs at their respective (λ_{max}).

The derivative spectra ^1D, ^2D, ^3D have been taken from normal spectrum for each drug by the computer via a SHIMADZU UV probe data system program and the parameters S and Δλ were optimized. The suitable wavelengths peaks (P) and valley (V) at (λ_{max}) were identified for standard drug. The calibration curves of each derivative were constructed and used to determine the concentrations of each drug.

The derivative spectra ^1D, ^2D and ^3D have been taken from normal spectrum (zero order) for binary drugs of Amox with PC, The suitable derivative and at suitable wavelength using zero crossing techniques were used to construct the calibration curves for these standard drugs, which were used to determine the concentration of each drug present in the mixtures.

Pharmaceutical sample Analyses

The synthetic standard sample and the commercial drug Co-amoxiclav (50 µg.mL^{-1} Amox+12.5 µg.mL^{-1} PC) were measured by using ^1D, ^2D, ^3D spectra depending on the calibration curves of the standard drugs.

Results and discussion

Amoxicillin Trihydrate with Potassium Clavulanate Mixture

Figure (1-a) shows the normal spectrum cannot be used to determine each of Amox and PC present in their mixture, due to interfering between the spectra, therefore; derivative spectrophotomeric methods ^1D, ^2D, ^3D can be used in this case Figure (1-b,c, d).

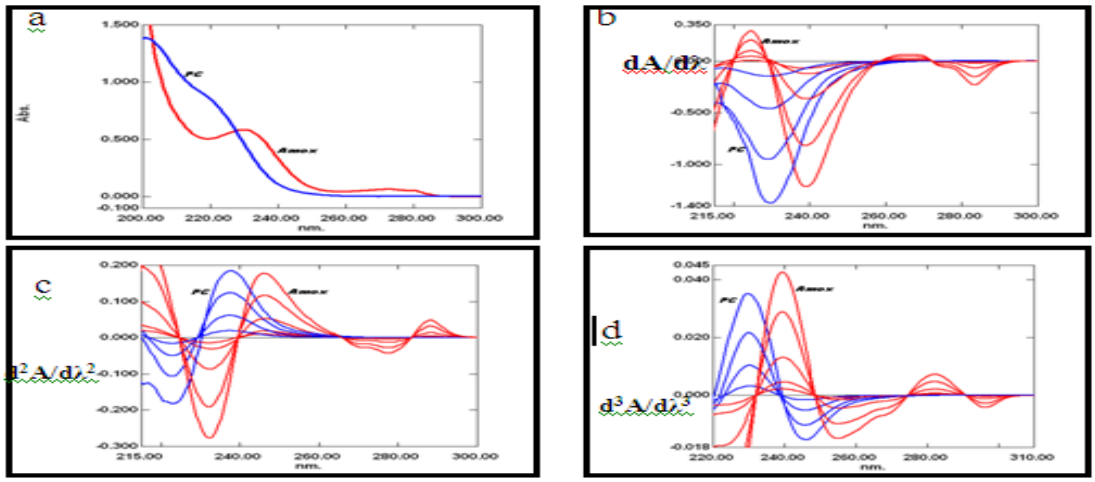


Fig (1)a: The zero order spectra of 30 µg.mL^{-1} for each Amox and PC. b- First derivative spectra.c- Second derivative spectra .d- Third derivative spectra for 5–90 µg.mL^{-1} Amox and 10–90 µg.mL^{-1} PC.

First derivative (^1D) can be used to determine each of Amox and PC in their mixture, as shown in figure (2). Amox can be determined at P = 267.0, V = 283.5 nm, on the other hand, PC can be determined at V = 229.0 nm. The results and the relative errors for the determination Amox and PC in their mixture are listed in Tables (1, 2) respectively.

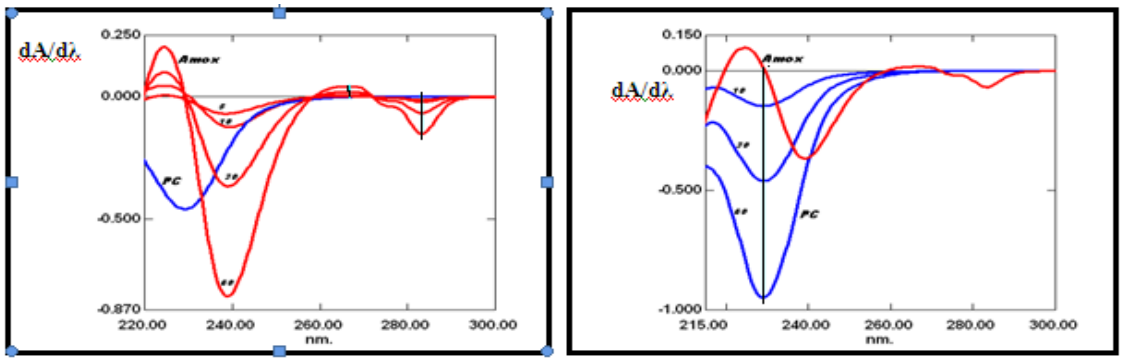


Fig (2): a-^{1}D spectra for 5–60 µg.mL^{-1} Amox and 30 µg.mL^{-1} PC at 267 and 283.5 nm, b- ^{1}D spectra for 10–60 µg.mL^{-1} PC and 30 µg.mL^{-1} Amox (zero crossing) at 229 nm.

Table (1): The relative error and recovery for the determination of Amox in the presence of PC at 267 and 283.5 nm using ^{1}D method.

Amox and PC mix	Amox found µg.mL^{-1} at 267nm	RE %	RC %	Amox found µg.mL^{-1} at 283.5nm	RE %	RC %
30PC+5Amox	5.335	+6.700	106.700	5.250	+5.000	105.000
30PC+10Amox	9.609	-3.910	96.090	10.434	+4.340	104.340
30PC+20Amox	19.580	-2.100	97.900	20.404	+2.020	102.020
30PC+30Amox	29.551	-1.496	98.503	30.374	+1.246	101.246
30PC+40Amox	39.522	-1.194	98.805	40.344	+0.860	100.860
30PC+50Amox	49.493	-1.014	98.986	50.314	+0.628	100.628
30PC+60Amox	59.464	-0.893	99.106	60.284	+0.473	100.473
10PC+30Amox	29.551	-1.496	98.503	29.975	-0.083	99.916

The results of Tables (1,2) show that Amox and PC can be determined by ^{1}D when the mixture contain more than 25% of each drug. Therefore second derivative (^{2}D) may be used to determine Amox and PC to obtain more accurate results, because it has higher resolution than the ^{1}D.

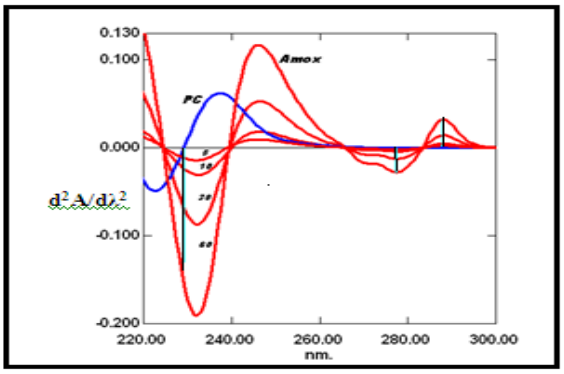
Table (2): The relative error and recovery for the determination of PC in the presence of Amox at 229 nm using ^{1}D method.

Amox and PC mix.	PC found µg.mL^{-1} at 229nm	RE %	RC %
30Amox+10PC	10.250	+2.500	102.500
30Amox+20PC	20.397	+1.985	101.985
30Amox+30PC	30.358	+1.193	101.193
30Amox+40PC	40.368	+0.920	100.920
30Amox+50PC	49.610	-0.780	99.220
30Amox+60PC	59.563	-0.728	99.270
10Amox+30PC	29.833	-0.556	99.443

The second derivative spectra used to determine Amox and PC in their mixture, Figure (3) show that Amox can be determined at P = 288.0 nm, V = 277.5, 229.0 nm, while PC can be determined at P = 239.5, V = 224.5 nm. The results and the relative errors for the determination of Amox and PC in their mixtures using ^{2}D method are listed in Tables (3,4) respectively.

The results of Table (3) show that Amox can be determined by ^{2}D method at V = 229.0 nm,

The results of Table (4) show that PC can be determined by ^{2}D method at P = 239.5, V = 224.5 nm. The suitable wavelength to determine PC is 239.5 nm because it gives more accurate results than the 224.5 nm.



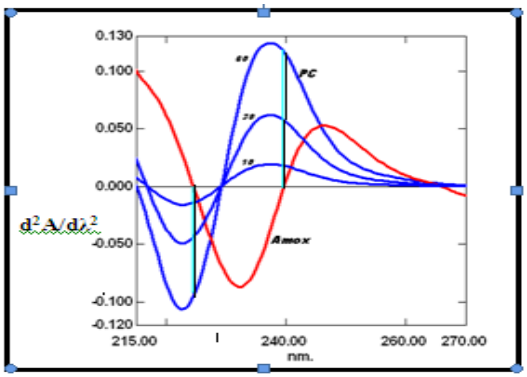


Fig (3): ^{2}D spectra for 5–60 µg.mL^{-1} Amox and 30 µg.mL^{-1} PC at 288, 277.5 and 229 nm. b-Figure (3-88) ^{2}D spectra for 10–60 µg.mL^{-1} PC and 30 µg.mL^{-1} Amox (zero crossing) at 239.5 and 224.5 nm.

Table (3): The relative error and recovery for the determination of Amox in the presence of PC at 288, 277.5 and 229 nm using ^{2}D method.

Amox and PC mix	λ (nm)	Amox found µg.mL^{-1}	RE %	RC %
	P=288.0	4.088	-18.240	81.760
30PC+5Amox	V=277.5	4.830	-3.400	96.600
	V=229.0	5.010	+0.200	100.200
	P=288.0	9.743	-2.570	97.430
30PC+10Amox	V=277.5	9.106	-8.940	91.060
	V=229.0	9.932	-0.680	99.320
	P=288.0	19.169	-4.155	95.845
30PC+20Amox	V=277.5	19.795	-1.025	98.975
	V=229.0	20.187	+0.935	100.935
	P=288.0	30.480	+1.600	101.600
30PC+30Amox	V=277.5	30.483	+1.610	101.610
	V=229.0	30.032	+0.106	100.106
	P=288.0	39.906	-0.235	99.765
30PC+40Amox	V=277.5	39.034	-2.415	97.585
	V=229.0	39.877	-0.307	99.692
	P=288.0	49.332	-1.336	98.664
30PC+50Amox	V=277.5	49.723	-0.554	99.446
	V=229.0	50.132	+0.264	100.264
	P=288.0	60.644	+1.073	101.073
30PC+60Amox	V=277.5	60.412	+0.686	100.686
	V=229.0	59.977	-0.038	99.961
	P=288.0	30.480	+1.600	101.600
10PC+30Amox	V=277.5	30.483	+1.610	101.610
	V=229.0	30.032	+0.106	100.106

Table (4): The relative error and recovery for the determination of PC in the presence of Amox at 239.5 and 224.5 nm using ^{2}D method.

Amox and PC mix	PC found µg.mL^{-1} at 239.5nm	RE %	RC %	PC found µg.mL^{-1} at 224.5nm	RE %	RC %
30Amox+10PC	9.933	-0.670	99.330	10.788	+7.880	107.880
30Amox+20PC	19.992	-0.040	99.960	20.246	+1.230	101.230
30Amox+30PC	30.050	+0.166	100.166	29.704	-0.986	99.013
30Amox+40PC	40.109	+0.272	100.272	39.754	-0.615	99.385
30Amox+50PC	50.168	+0.336	100.336	49.803	-0.394	99.606
30Amox+60PC	60.226	+0.376	100.377	59.852	-0.246	99.753
10Amox+30PC	30.050	+0.166	100.166	30.296	+0.986	100.986

Third Derivative

The third derivative (^{3}D) was used to determine each of Amox and PC in their mixtures, as in Figure (4), Amox can be determined at P = 239.0 nm, as shown on the other hand, PC can be determined at P = 232.0, V = 248.5 nm, The results of recovery and the relative errors for the determination of Amox and PC in their mixtures using ^{3}D method are listed in Tables (5,6) respectively.

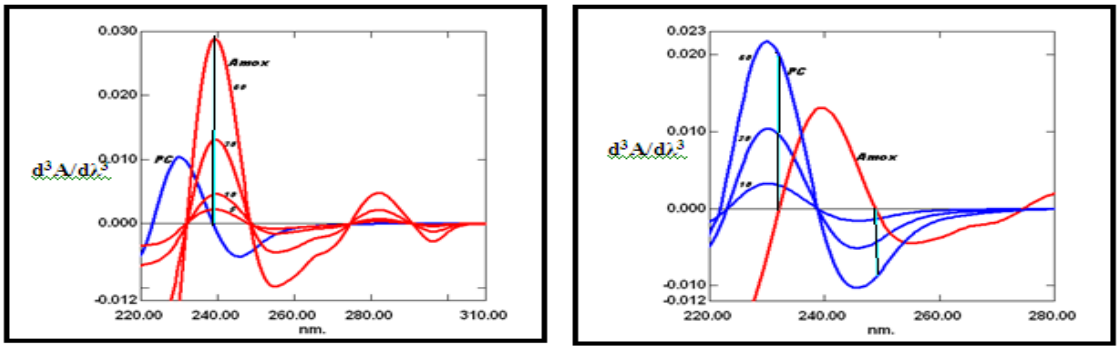


Fig (4): Third derivative spectra-a- for 5–60 µg.mL^{-1} Amox and 30 µg.mL^{-1} PC (zero crossing) at 239 nm. b- ^{3}D spectra for 10–60 µg.mL^{-1} PC and 30 µg.mL^{-1} Amox (zero crossing) at 232 and 248.5.

Table (5): The relative error and recovery for the determination of Amox in the presence of PC at 239 nm using ^{3}D method.

Amox and PC mix	Amox found µg.mL^{-1} at 239nm	RE %	RC %
30PC+5Amox	3.939	-21.220	78.780
30PC+10Amox	8.125	-18.750	81.250
30PC+20Amox	18.589	-7.055	92.945
30PC+30Amox	29.054	-3.151	96.846
30PC+40Amox	39.518	-1.205	98.795
30PC+50Amox	49.983	-0.034	99.966
30PC+60Amox	60.448	+0.746	100.746
10PC+30Amox	29.054	-3.151	96.846

The results of Table (5) show that Amox can be determined by ^{3}D method at P = 239.0 nm, when the mixture contain more than 50% Amox and the low concentration

for Amox (5–30 µg.mL^{-1}) have weak ^{3}D value; therefore, the determination at low concentration is not accurate; therefore, not used to determine Amox.

Table (6): The relative error and recovery for the determination of PC in the presence of Amox at 232 and 248.5 nm using ^{3}D method.

Amox and PC mix	PC found µg.mL^{-1} at 232nm	RE %	RC %	PC found µg.mL^{-1} at 248.5nm	RE %	RC %
30Amox+10PC	9.474	-5.26	94.740	9.308	-6.920	93.080
30Amox+20PC	20.000	+0.000	100.000	22.000	+10.000	110.000
30Amox+30PC	30.526	+0.853	100.853	28.346	-5.513	94.486
30Amox+40PC	41.053	+2.632	102.632	41.038	+2.595	102.595
30Amox+50PC	48.947	-2.106	97.894	47.385	-5.230	94.770
30Amox+60PC	59.474	-0.876	99.123	53.731	-10.448	89.551
10Amox+30PC	30.526	+0.853	100.853	28.346	-5.513	94.486

The results of Table (6) show that PC may be determined by ^{3}D method at P = 232.0, but the V = 248.5 nm cannot be used to determine PC, because it has weak ^{3}D value comparing with 232.0 nm. In general ^{3}D spectra have weak ^{3}D value and gave not accurate results.

Table (7): Statistical data for the calibration curves that used to determine Amox and PC in their mixture.

Drug	Amox		PC	
Method	^{1}D	^{2}D	^{1}D	^{2}D
λ (nm)	V=283.5	V=229.0	V=229.0	P=239.5
Linearity range (µg.mL^{-1})	2–100	2–90	10–80	10–90
r	0.99939	0.99951	0.99955	0.99993
Slope	-0.00251	-0.00244	-0.01547	0.00199
*RSD % (slop)	0.396	1.214	1.242	0.772
Intercept	0.00117	0.00079	0.00260	0.00175
*RSD% (intercept)	1.290	1.282	2.197	1.520
LOD (µg.mL^{-1})	0.205	0.211	0.033	0.259
LOQ (µg.mL^{-1})	0.685	0.704	0.111	0.864
*RSD (concentration)**	0.281	0.174	0.386	0.119
*SD	0.141	0.087	0.191	0.059
†F experimental	2.626		10.480	
††F theoretical	19.000		19.000	

*n = 3. ** Concentration = 50 µg.mL^{-1}. †F = SD_1^2/ SD_2^2, where SD_1>SD_2.

††F theoretical = theoretical value at 95% confidence limit.

The results of Table (7) show that Amox and PC can be determined in their mixtures by using ^{1}D and ^{2}D methods, but ^{2}D method gave higher precision than ^{1}D method and the F test shows that no difference between ^{1}D and ^{2}D methods because the value of the F experimental is less than the value of the F tabulated at 95% confidence limit.

Analysis of Pharmaceutical Samples

Simultaneous determination of amoxicillin and potassium clavulanate antibiotics in pharmaceutical sample using derivative spectrophotometric method with the best derivative and best wavelengths for Co-amoxiclav sample (50 µg.mL^{-1} Amox + 12.5 µg.mL^{-1} PC) was measured by using ^{1}D and ^{2}D methods, as shown in Table (8).

Table (8): The relative error and recovery for the determination of Co-amoxiclav sample (50 µg.mL^{-1} Amox + 12.5 µg.mL^{-1} PC) by using DS methods.

Drugs	Co-amoxiclav (Amox)		Co-amoxiclav (PC)	
Method	^{1}D	^{2}D	^{1}D	^{2}D
λ (nm)	283.5	229.0	229.0	239.5
Conc. found (µg.mL^{-1})	47.123	48.492	13.288	12.105
RE %	-5.754	-3.016	+6.304	-3.160
RC %	94.246	96.984	106.304	96.840

Table (8) shows the results for the determination of Co-amoxiclav (Amox +PC mixture) by ^{1}D and ^{2}D methods. The suitable method that gave more accurate result was the ^{2}D method at (229.0, 239.5) nm for Amox and PC respectively. Table (9) shows the comparison between standard and commercial drug and statistical data for the determination of (Amox + PC) in their mixture by using ^{2}D method. The parameters obtained from the calibration curve for ^{2}D method of Amox at 229 nm., PC at 239.5 nm Table (10) show the concentration range and the linear equations and the linearity percentage R^2%

Table (9): Statistical data for the determination of (Amox + PC) in their mixture in pure standard and pharmaceutical form by ^{2}D method.

			Standard					
Amox+PC mixture	Amox found µg.mL^{-1} at 229nm	RE %	RC %	*RSD %	PC found µg.mL^{-1} at 239.5nm	RE %	RC %	*RSD %
30Amox+10PC	30.032	+0.106	100.106	0.448	9.933	-0.67	99.330	2.117
			Co-amoxiclav					
Amox+PC mixture	Amox found µg.mL^{-1} at 229nm	RE %	RC %	*RSD %	PC found µg.mL^{-1} at 239.5nm	RE %	RC %	*RSD %
50Amox+12.5PC	48.492	-3.016	96.984	0.856	12.105	-3.16	96.840	1.655

*n = 3

Table (10): The parameters obtained from the calibration curve for ^{2}D method of Amox at 229 nm. and PC at 239.5 nm.

Method	Conc. range µg.mL^{-1}	λ (nm)	Equation	r	R^2
^{2}D	2–90	V=229.0	Y=-0.00244x-0.00079	0.99951	99.98
^{2}D	10–90	P=239.5	Y=0.00199x-0.00175	0.99993	99.996

Conclusions

A quick and accurate method for determining amoxicillin and potassium clavulanate antibiotics in pharmaceutical sample was carried out using derivative spectrophotometric method. The advantage of this method is that both constituents can be determined directly in a single sample without the need to be separated. It was also found that auxiliary drug components had no effect on the results of determination obtained under established conditions.

References

1. British Pharmacopoeia 2000, CD, ROM
2. Aghazadeh, A. and Kazemifard, G.; "Determination of Amoxicillin and Clavulanic Acid in Pharmaceutical Dosage Forms by HPLC with Amperometric Detection", *J. of Sci. I. R. Iran*, 12(2), 127-131, (2001).
3. Kyung-Hwan, Y.; So-Young, L.; Won, K.; Jong-Sei, P. and Hie-Joon, K.; "Simultaneous Determination of Amoxicillin and Clavulanic Acid in Human Plasma by HPLC–ESI Mass Spectrometry", J. of Chromatography B, 318(1-2), 121-127, (2004).
4. Hoizey, G.; Lamiable, D.; Frances, C.; Trenque, T.; Kaltenbach, M.; Denis, J. and Millart, H.; "Simultaneous Determination of Amoxicillin and Clavulanic Acid in Human Plasma by HPLC with UV Detection", J. of Pharm. Biomed. Anal., 30(3), 661-666, (2002).
5. Tsou, L.; Wu, R.; Young, D. and Wang, M.; "Simultaneous Determination of Amoxycillin and Clavulanic Acid in Pharmaceutical Products by HPLC with Beta-Cyclodextrin Stationary Phase", J. of Pharm. Biomed. Anal., 15(8), 1197-1205, (1997).
6. Islam Ullah, K.; Shahzad, S.; Muhammad, A. and Muhammad, N.; "Simultaneous Determination of Potassium Clavulanate and Cefixime in Synthetic Mixture by HPLC", *J. of AOAC Int.*, 91(4), 744-749, (2008).
7. Jian-Jun, X.; Hai-Yun, Z.; Zuan-Guang, C.; Xue-Cai, T.; Pei-Xiang, C. and Jin-Yuan, M.; "Simultaneous Determination of Amoxicillin and Potassium Clavulanate by Capillary Electrophoresis Combined with High Frequency Conductivity Detection", J. of C.E.P.S., 22(6), 581-585, (2005).
8. Pasamontes, A. and Callao, M.; "Sequential Injection Analysis for the Simultaneous Determination of Clavulanic acid and Amoxicillin in Pharmaceuticals Using Second-Order Calibration", J. of Anal. Sci., 22(1), 131-135, (2006).
9. Pimenta, A.; Araujo, A. and Montenegro, M.; "A Sequential Injection Analysis System for Potassium Clavulanate Determination Using Two Potentiometric Detectors", J. of Pharm. Biomed. Anal., 30(4), 931-937, (2002).
10. Adriana, M.; Cristina, M.; Alberto, N. and Conceição, M.; "Clavulanate-Selective Electrodes - Application to Pharmaceutical Formulations", Fresenius' J. of Anal. Chem., 371(3), 400-403, (2001).
11. Gunawan, I.; Tan, K. and Sonja, W.; "Simultaneous Determination of Amoxycillin Trihydrate and Clavulanate Potassium in Pharmaceutical Preparations by Thin-Layer Chromatography/Densitometry", J. of AOAC Int., 83(6), 1493-1496, (2000).
12. Fatma, A.; Nawal, A. and Abdulrahman, A.; "Sensitive Assay for Clavulanic Acid and Sulbactam in Pharmaceuticals and Blood Serum Using a Flow-Injection Chemiluminometric Method", J. of Anal. Chem. Acta., 414, 15-23, (2000).
13. Ghassempour, A.; Rafati, H.; Adlnasab, L.; Bashour, Y.; Ebrahimzadeh, H. and Erfan, M.; "Investigation of the Solid State Properties of Amoxicillin Trihydrate and the Effect of Powder pH", J. of AAPS Pharm. Sci. Tech., 8(4), (2007).

14. Foroutan, S.; Zarghi, A.; Shafaati, A.; Khoddam, A. and Movahed, H.; "Simultaneous Determination of Amoxicillin and Clavulanic Acid in Human Plasma by Isocratic Reversed-Phase HPLC using UV Detection", J. of Pharm. Biomed. Anal., 45(3), 531-534, (2007).

15. Nelis, H.; Vandenbranden, J.; De Kruif, A. and De Leenheer, A.; "Liquid Chromatographic Determination of Amoxicillin Concentrations in Bovine Plasma by Using a Tandem Solid-Phase Extraction Method", J. of AAC, 36(9), 1859-1863, (1992).

16. Lucius, D. and Marvin, A.; "High-Pressure Liquid Chromatographic Determination of Amoxicillin in Urine", J. of Pharm. Sci., 68(4), 454-458, (2006).

17. Mei-Chich, H. and Pei-Wen, H.; "High-Performance Liquid Chromatographic Method for Potency Determination of Amoxicillin in Commercial Preparations and for Stability Studies", J. of AAC, 36(6), 1276-1279, (1992).

18. Li, W.; Tan, F. and Zhao, K.; "Simultaneous Determination of Amoxicillin and Ranitidine in Rat Plasma by High-Performance Liquid Chromatography", J. of Pharm. Biomed. Anal., 41(2), 594-598, (2006).

19. Özdemir, A. and Korkmaz, A.; "Comparative Study of Electrospray Mass Spectrometry and First Derivative Method and Validation by HPLC Method", J. of Food and Drug Anal., 15(2), 118-125, (2007).

20. Marcio, F.; Marcos, F.; Edward, R.; Nerilso, B. and Eder, T.; "Evaluation of Different Voltametric Techniques in the Determination of Amoxicillin Using a Carbon Paste Electrode Modified with [N, N-ethylene*bis* (salicylideneaminato)] oxovanadium (IV)", J. of ECS, 153(5), E94-E98, (2006).

21. Daniela, P.; Márcio, F. and Maria Valnice, B.; "Voltammetric Sensor for Amoxicillin Determination in Human Urine Using Polyglutamic Acid/Glutaraldehyde Film", J. of Sensors and Actuators B: Chemical, 133(2), 398-403, (2008).

22. Li, Y. and Lu, J.; "Chemiluminescence Flow-Injection Analysis of β-Lactam Antibiotics Using the Luminol-Permanganate Reaction", J. of Luminescence, 21(4), 251-255, (2006).

23. Xiaofeng, X. and Zhenghua, S.; "Ultrasensitive Determination of Amoxicillin Using Chemiluminescence with Flow Injection Analysis", J. of Spectroscopy, 20(1), 37-43, (2006).

24. Yuanyuan, S.; Yuhai, T.; Hong, Y. and Yinhuan, L.; "Flow-Injection Chemiluminescence Analysis of Some Penicillins by Their Sensitizing Effect on the Potassium Permanganate-Glyoxal Reaction", J. of Anal. Sci., 21(4), 457-460, (2005).

25. Sorouraddin, M.; Iranifam, M. and Imani-Nabiyyi, A.; "Study of the Enhancement of a New Chemiluminescence Reaction and its Application to Determination of Beta-Lactam Antibiotics" J. of Luminescence, 11 Sep. (2008).

26. Göksel, A.; Nafiz Öncü, C. and Hassan, Y.; "Determination of Amoxicillin by Flow Injection Analysis using UV-Detection, Potentiometry, and Conductometry in Pharmaceutical Preparations", J. of Liquid Chromatography& Related Technologies, 30(9-10), 1333-1341, (2007).

27. Gamal A.; "Two selective spectrophotometric methods for the determination of amoxicillin and cefadroxil", J. of Analyst, 212, 641-645, (1996).

28. Revanasiddappa, H.; Manju, B. and Ramappa, P.; "Spectrophotometric Method for the Determination of Ritodrine Hydrochloride and Amoxicillin", J. of Anal. Sci., 15(7), 661, (1999).

29. Quanmin, L. and Zhanjun, Y.; "Study of Spectrophotometric Determination of Amoxicillin Using Sodium 1,2-Naphthoquinone-4-Sulfonate as the Chemical Derivative Chromogenic Reagent" J. of Anal. Letters, 39(4), 763-775, (2006).

30. Khalil, F.; Sedigheh, G.; Ramin, M. and Feridoun, S.; "Spectrophotometric Determination of Selected Antibiotics Using Prussian Blue Reaction", J. of Chin. Chem. Soc., 49(6), 993-997, (2002).

31. Al-Abachi, M. Q.; Haddi, H. and Al-Abachi, A. M.; "Spectrophotometric Determination of Amoxicillin by Reaction with N,N-Dimethyl-P-Phenylenediamine and Potassium Hexacyanoferrate(III) ", J. of Analytica Chimica Acta, 554(1-2), 184-189, (2005).

32. Belal, F.; El-Kerdawy, M.; El-Ashry, S. and El-Waseef, D.; "Kinetic Spectrophotometric Determination of Ampicillin and Amoxicillin in Dosage Forms", J. of Farmaco, 55(11-12), 680-686, (2000).

33. Graciele, P.; Marco Flôres, F.; João, C. and Rolf, F.; "Determination of Amoxicillin Content in Powdered Pharmaceutical Formulations Using DRIFTS and PLS", Brazilian J. of Pharm. Sci., 43(1), 89-96, (2007).

34. Hesham, S.; "Selective spectrophotometric determination of phenolic β-lactam antibiotics in pure forms and in their pharmaceutical formulations", J. of Analytica Chimica Acta, 515(2), 333-341, (2004).

35. 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.

36. 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.

37. 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.

38. Mohamed and H. Salem," Determination of certain Antihypertensive mixtures using Chemometrics – assisted Spectrophotometric Method.", Anal. Bioanal.Chem., Vol. 383, 2005, pp. 1066-1072 .

39. I. Abd El-Maboud: H. Salem and E. Maher." Chemometric-assisted Spectrophotometric Determination of certain β-lactam Antibiotices Combinations."Thai. J. Pharm. Sci., Vol. 31, pp. 1-24, 2007.