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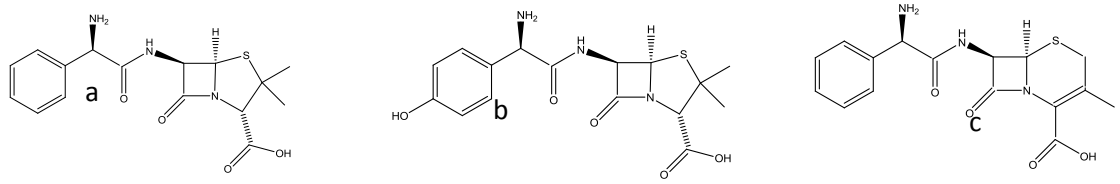
Determination of β-lactam Antibiotics in Pharmaceutical Preparations by Uv-visible Spectrophotometry Atomic Absorption and High Performance Liquid Chromatography

[*]A. J. Abdulghani, [1]H. H. Jasim and [1]A. S. Hassan
[*]Department of chemistry, College of Sciences, University of Baghdad, Jaderiya, Iraq
[1]Department of chemistry, College of Sciences**,** Al-Mustansiria University, Baghdad, Iraq
Email: [*]ahlamjameel@scbaghdad.edu.iq

ABSTRACT
The determination amoxicillin, ampicillin and cephalexin was studied by complexation of the antibiotics with Au(III) and Hg(II) ions in bulk and pharmaceutical preparations using uv-visible spectrophotometry, atomic absorption, and HPLC techniques. Optimum conditions for complex formation were fixed at pH 4 and (2-4) for Au(III) and Hg(II)complexes respectively, heating temperature at (60 °C) and heating time for (10 minute). All complexes were extracted from aqueous solution with benzyl alcohol prior to measurements except in the case of HPLC. The L:M ratios for all complexes were determined and stability constants were calculated using mole ratio method. The Beer's law was obeyed over the concentration range (5-60) and 5-50 µg/ml of antibiotics for Au(III) and Hg(II) complexes using colorimetric method and (1-25 µg/ml) of Au(III) for FAAS. The linearity for HPLC method was (10-110 and 10-120 µg/ml) respectively. The correlation coefficients (r) were (0.9981-0.9997). Generally, the highest sensitivity was recorded by FAAS.

Keywords: β-lactam drugs, solvent extraction, complex formation, correlation coefficient, direct methods

1. INTRODUCTION
A wide range of metal ions have been reported for the determination and assay of antibiotics using different methods [1-9]. In this work the determination of the three antibiotics Ampicillin, Amoxicillin, and Cephalexin (**Scheme (1)**) is investigated by studying their complexation behavior with Au(III) and Hg(II)ions depending on uv–visible molecular spectrophotometry, atomic absorption and HPLC methods.



Scheme (1): Chemical structure of: a-Ampicillin, b-amoxicillin and c-cephalexin.

2. EXPERIMENTAL
UV-Visible spectra were recorded on Varian Gary 100 conc. UV-Visible spectrophotometer. FTIR spectra were measured in KBr disc using 8400S Fourier transforms spectrophotometer (Shimadzu). Determination of Au metal by flame atomic absorption spectrophotometry (FAAS) was carried out using GBC 933 plus flame atomic absorption. HPLC of the drugs and their metal complexes was performed on Shimadzu LC 2010A, with UV-Vis detector. pH values of the prepared solutions were measured by using HANA, HI 98150 GLP PH/ORP-meter calibrated with buffer solutions of pH 4.0 and 9.0.All chemicals used were of analytical reagent grade.

2.1 *Preparation of standard solutions*
Stock solutions (1000 $\mu g.ml^{-1}$) of antibiotics were prepared by dissolving 0.10 g standard powder in 100 ml distilled deionized water (DDW). Working solutions (100 $\mu g.ml^{-1}$) for the present study were prepared by diluting 10 ml of stock solutions to 100 ml with (DDW) in a volumetric flask. The stock solution of Hg^{+2} ion was prepared by dissolving 0.1350g of $HgCl_2$ in 100 ml (DDW) in a volumetric flask, while 100 $\mu g.ml^{-1}$ standard solution of Au^{3+} ion was obtained from 1000 $\mu g.ml^{-1}$ of Au^{+3} solution prepared by dissolving 0.1g of the metal in aqua regia followed by dilution with DDW.

2.2 *UV-Visible spectrophotometry assay method*
Aqueous solutions containing (5-60 $\mu g.ml^{-1}$ for amp., amox., or ceph.) and 10 $\mu g.ml^{-1}$ of metals ion solutions were prepared by pipetting (1 ml) of (100 $\mu g.ml^{-1}$) metal ion solution to 0.5-6 ml of antibiotic solution (100 $\mu g.ml^{-1}$) in (10 ml) volumetric flasks followed by dilution to the mark. Complexes were extracted with (1ml) benzyl alcohol and the absorbance in each case was recorded at the recommended λ_{max}. Optimum values of concentrations, pH, and temperature, heating time and extraction efficiency were tabulated. The absorbance values were plotted against the concentration of the cited antibiotic to obtain the standard calibration curves.

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2.2.1 *Mole ratio method for complex formation*

Complex formation by mole ratio method at optimum conditions was carried out by pipetting (0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 ml) of ligand solution (5.08 X 10^{-3} M) to 1ml aliquots of metal ion solutions (5.08 X 10^{-3} M) in 10ml volumetric flask.

2.2.2 *Determination of antibiotic-complexes in dosage form by direct method*

0.1g of powder obtained from 20 capsules of (ampicillin, amoxicillin and, cephalexin (500 mg) were dissolved in 100 ml distilled deionized water in volumetric flasks. 10 ml of the resulted solution was diluted to 100 ml DDW in volumetric flasks. (1, 2, and 3 ml) of this solution were transferred to (10 ml) volumetric flasks and (1 ml) of metal ion solutions (100 μg.ml^{-1}) were added and the volumes were diluted to the marks. Absorbance of these solutions was recorded against blank solution and the concentration of the studied analyst was calculated depending upon the respective standard calibration curves.

2.2.3 *Determination of antibiotic-complexes in dosage form by standard addition method*

To a solution mixture containing (1 ml) of metal ion solution (100 μg.ml^{-1}) and 0.5-5.5 ml of standard antibiotic solutions (100 μg.ml^{-1}) in 10ml volumetric flasks was added (1 ml) of the dosage antibiotic solution (100 μg.ml^{-1}) . Volumes were completed to the marks. After adjusting the optimum conditions absorbance values were plotted against concentration to construct standard addition curves.

2.3 *Flame atomic absorption spectrometry assay method (FAAS)*

This method was based on measuring the absorbance of standard solutions containing a mixture of (1-25 μg.ml^{-1}) (ampi. amox. ceph.) and 8 μg.ml^{-1} gold ion after adjusting all optimum conditions. Absorbance values were plotted against concentration to produce standard calibration curves. For drug determination by direct method, solutions containing (0.6 , 0.8 , and 1.0 ml) of each dosage antibiotic (500 mg capsules) solutions (100 μg.ml^{-1}) and (0.8 ml) of (100 μg.ml^{-1}) gold (III) ion solution in (10 ml) volumetric flasks were prepared . The volumes were completed to the mark and the absorbance values of these solutions at optimum conditions were measured against blank solution. The concentrations of solutions were then calculated depending on standard calibration curves. For standard addition curves, aliquots (0.8 ml) of (100 μg.ml^{-1}) of dosage drug solution were added to a series of 10 ml aqueous solution mixtures containing Au(III) ion solutions (100 μg.ml^{-1}, 0.8ml) and standard antibiotic solutions (100 μg.ml^{-1}, 0.1-2ml). The absorbance values were plotted against concentrations to produce standard addition curves.

2.4 *Direct method for determination of antibiotic and Au (III) complexes in dosage form by high performance liquid chromatography (HPLC)*

Chromatographic optimum conditions for determination of antibiotics and their metal complexes were studied simultaneously in aqueous solution mixture without extraction with benzyl alcohol**.** Standard calibration curves were obtained using solution mixtures of 10-120 μg.ml^{-1} of each antibiotic and 10 μg.ml^{-1} of metal ions. A series of aqueous solutions containing 30, 50 and 100 μg.ml^{-1} of dosage antibiotics and10 μg.ml^{-1} of metal ion solution were prepared and all optimum conditions were adjusted. Peak area of complexes was measured and the concentrations of solutions were calculated.

3. RESULTS AND DISCUSSION

The FTIR spectra of the Au (III) complexes exhibited the disappearance of the OH stretching vibration of the COOH group and shifts of lactam and carboxylate carbonyl groups to lower frequencies. This refers to the coordination of the two carbonyls with the metal ion[1, 10a]. Complexes of the Hg(II) ion exhibited the shift of both the OH stretching vibration of COOH groups and lactam carbonyl groups to lower frequencies as a result of coordination of these groups to Hg(II)ion[10]. No significant changes were observed on the vibrational modes of amide or amine groups. Conductivity measurements showed no electrolytic nature of the complexes[10b].

3.1 *Uv-visible spectrophotometry*

3.1.1 *The optimum absorbance conditions of metal complexes:*

Maximum absorption peaks of the metal free ligand solutions ampicillin, amoxicillin and cephalexin) were observed at λ (230,325), (230,295) and (240) nm respectively (Figure 1) and were assigned to $\boldsymbol{\pi}\ \pi^*$ transitions[11]. Complexation with metal ions caused hypsochromic shifts of the $\pi \rightarrow \pi^*$ transition bands with a sharp decrease in the intensity of the lower energy band in case of ampicillin and amoxicillin. New absorption bands were observed in the near visible region at λmax 395 nm and 350nm for Au(III) and Hg(II) ions respectively for the three drugs (Figure1).These bands were assigned to ligand to metal charge transfer and $^1A_1g \rightarrow {}^1Eg$ transitions for square planar Au(III) complexes[10b,12] and to ligand to metal charge transfer transitons of tetrahedral geometries of Hg(II) complexes [10b,12,13] as illustrated in scheme 2.

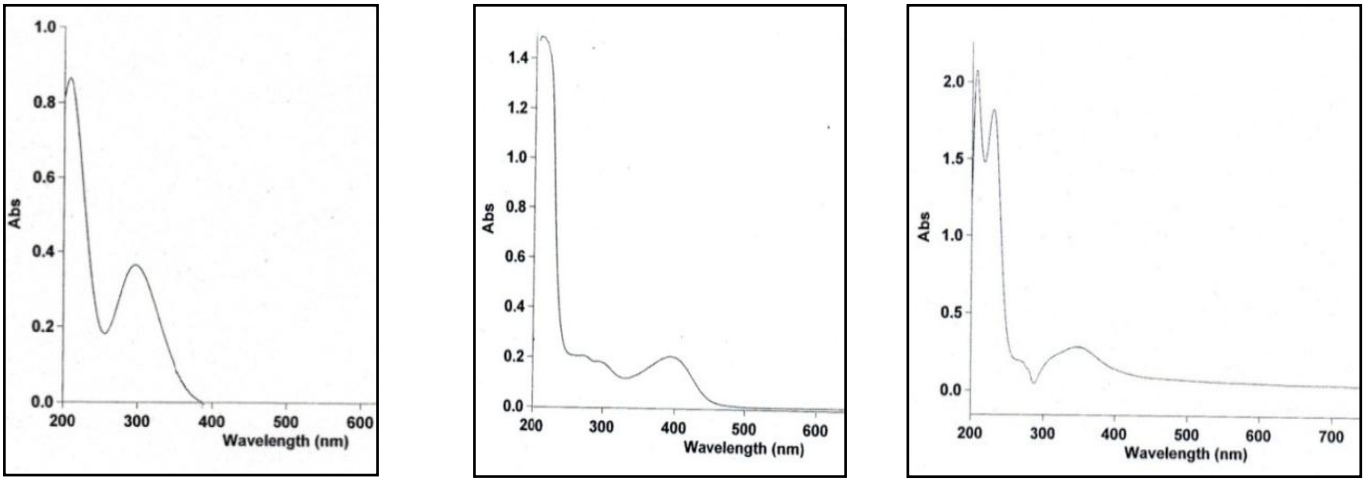
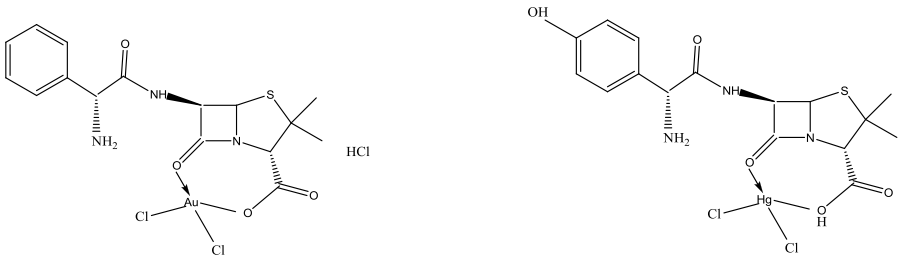


Fig-1: Electronic spectra of a-ampicillin, (50 µg/ml) and its complexes with b-Au(III) and c-Hg(II) ions (50µg/ml) in aqueous solutions



Scheme-2: The suggested structures of: a-ampicillin-Au (III) and b- amoxicillin-Hg (II) complexes

The complexation behavior of the three antibiotics (50µg/ml) with various concentrations of Au (III) and Hg (II) ions by uv-visible spectrophotometry was studied at λmax 395 and 350 nm respectively. Optimum conditions of absorbance were fixed at metal ion was 10 µg/ml at pH= 4 for Au(III)complexes and pH= 2-4 for Hg(II) complexes at heating temperature of 60 ^{O}C and heating time of 10 minutes (except the Ceph-Hg complex, 15 minutes) . At higher pH values the decrease in absorbance resulted from the formation of metal hydroxides [14, 15]. Table 1 describes the molar extinction coefficients for Au (III) and Hg (II) complexes of the studied drugs extracted in benzyl alcohol[16] at optimum conditions.

Table -1: Optimum conditions for the complex formation of antibiotics (50µg/ml) with Au (III) and Hg (II) ions (10µg/ml each), molar extinction coefficient (ε_{max}) in benzyl alcohol at λmax 395 and 350nm respectively and the stability constants of complexes

Complex	pH	Temp. (°C)	Heating Time (min.)	Phase ratio (org: aqu.)	Extraction time (min.)	Extraction% E. %)	of ε_{max} complexes ($l.mol^{-1}\ cm^{-1}$)	Stability constant K (M^{-1})
Ampi-Au(III)	4	60	10	1:5	1	96.819	$3.482x10^3$	$8.4950x10^5$
Amox-Au(III)	4	60	10	1:5	1	95.714	$4.494x10^3$	$6.1611x10^5$
Ceph-Au(III)	4	60	10	1:5	1	93.626	$5.735x10^3$	$1.1630x10^4$
Ampi-Hg(II)	2-4	60	10	1:5	1	97.465	$6.2789x10^3$	$4.3464x10^5$
Amox-Hg(II)	2-4	60	10	1:5	1-2	94.696	$6.9227x10^3$	$6.3937x10^5$
Ceph-Hg(II)	2-4	60	15	1:5	1-2	95.959	$5.7972x10^3$	$4.2387x10^6$

3.1.2 *Stability constants (K) of complexes by mole ratio*

The study of complex formation by mole ratio method [16, 17] showed that the drug :metal ion ratios in all complexes were 1:1 using a constant volume of metal ion solution($5.08x10^{-3}$ M) with different volumes of ligand solutions ($5.08x10^{-3}$ M) (figure 2). The stability constant values calculated in optimum conditions by mole ratio method[17] are described in table1.

3.1.3 *Determination of dosage Drug-Au complexes by direct calibration and standard addition UV-Vis method*

The results obtained from the uv-visible spectrophotometric determination of dosage antibiotic by complexation with Au(III) and Hg(II) ions are described in tables 2 and 3 respectively depending on direct and standard addition calibration curves curves shown in figures 3and 4 respectively. Tables (2,3) show that t-tabulated are more than t-calculated which indicates that the results of applied method were acceptable. The slopes of standard addition method were parallel to slopes of direct method which means that no matrix interference exists in these methods and that there

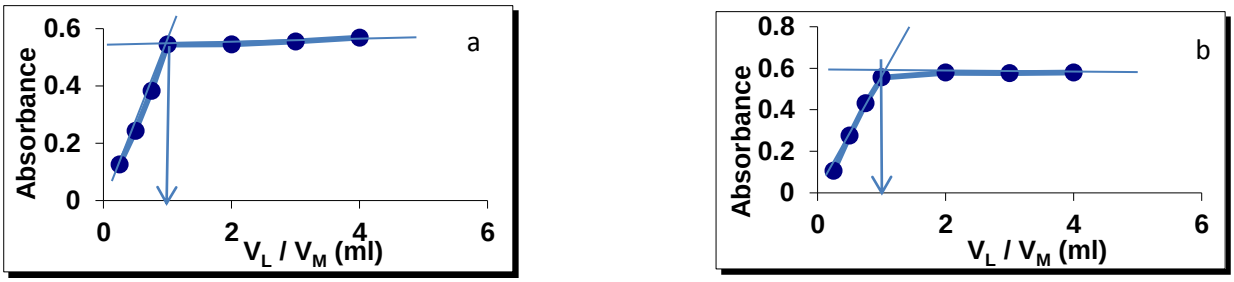


Fig-2: Mole ratio plots for the complex formation of ampicillin ($5.08x10^{-3}$M) with a- Au (III) and b- Hg (II) ions ($5.08x10^{-3}$M each)

is a linear relationship between concentration and absorbance[18,19]. The Hg(II) complex of cephalexin recorded the highest DL and RSD values .Percentage relative errors for determination of dosage Drug -Au and Hg(II) complexes by direct and standard addition UV-Vis method using 500mg /unit capsules were quite acceptable as is described in table-4

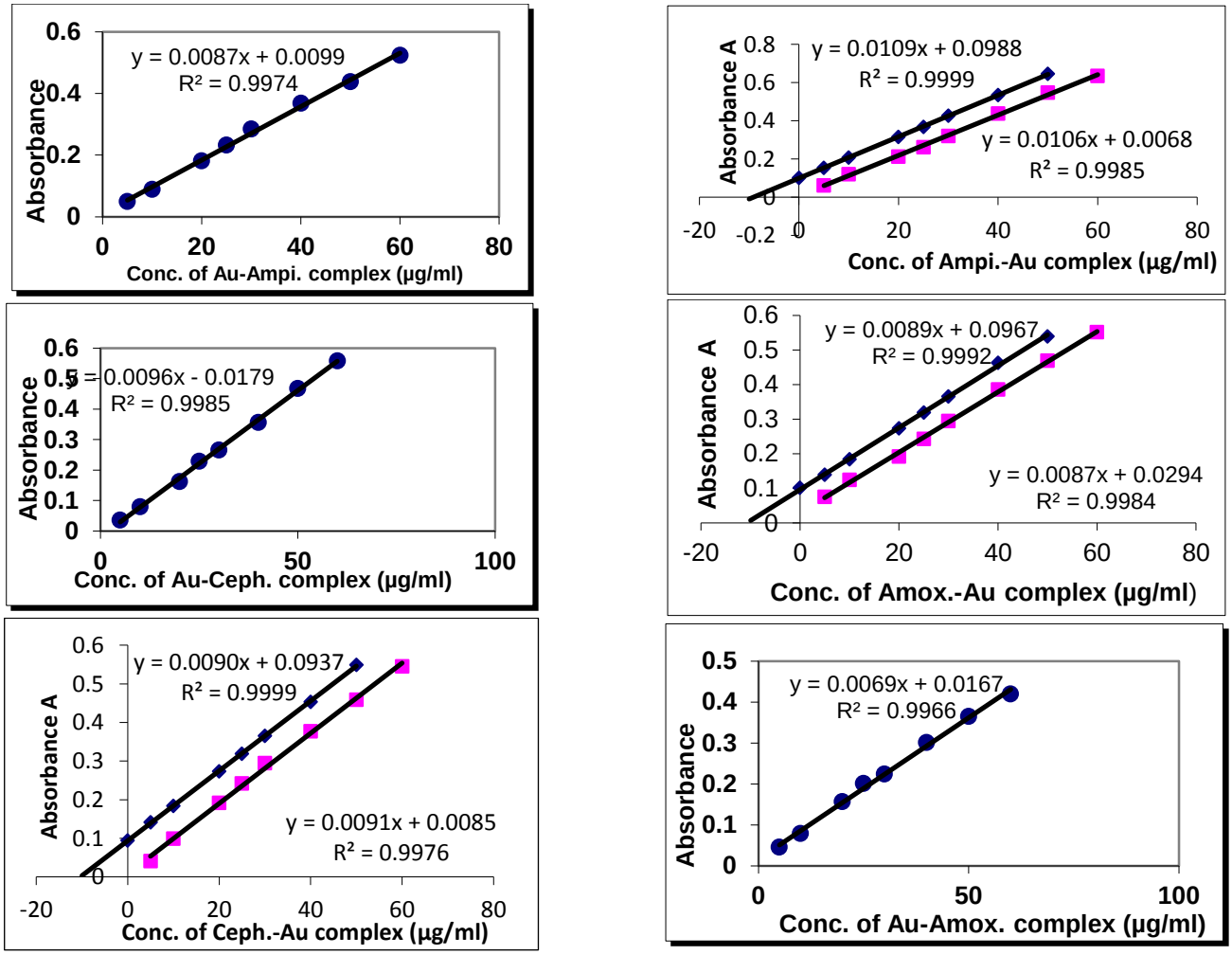


Fig-3: Direct and standard addition calibration curves for determination ampicillin, amoxiciline and cephlexine -Au complexes.by colorimetric method

3.2 *Determination of Au (III)-antibiotics complexes by flame atomic absorption spectrophotometry FAAS*

The flame atomic absorption spectroscopy was applied for the determination of drugs indirectly by determination gold ions in their complexes following the direct and standard addition curves shown in figure 5. As in the case of uv-visible method the data described in table 5 show that t-tabulated are more than t-calculated, and the slope of standard addition method was parallel to slope of direct method which means that the results of applied method are accepted[18] .However the detection limits by FAAS method are much lower than uv-visible spectrophotometry especially in the case of cephalexin -Au(III) complex where both the RSD and DL are the lowest . Percentage relative error for determination Drug-Au complexes by direct and standard addition FAAS method is described in table-6. The correction coefficient factors support the studied method.

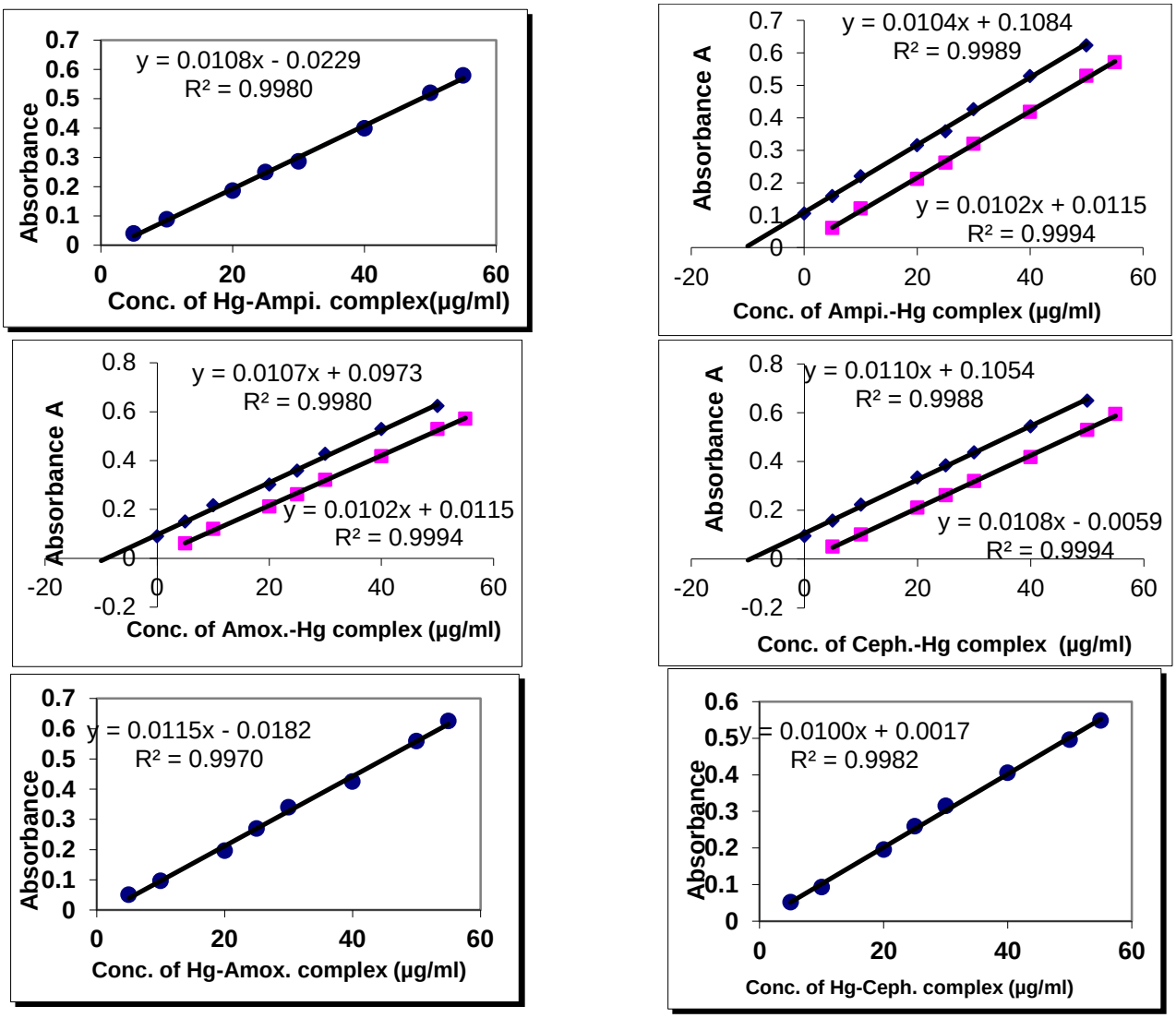


Fig-4: Direct and standard addition calibration curves for determination of ampicillin, amoxicillin and cephalexin-Hg (II) complexes by uv-visible spectrophotometric method

Table-2: Regression Equation (Regr.eq.**)** ,Correlation coefficient **(**Corr.Coef.,r) , linear range of antibiotic concentration , detection limit(D.L), t-test, confidence limit, RSD% and mean recovery% (Rec.%) , for determination of Drug-Au complexes by direct calibration and standard addition uv-visible spectrophotometric methods.

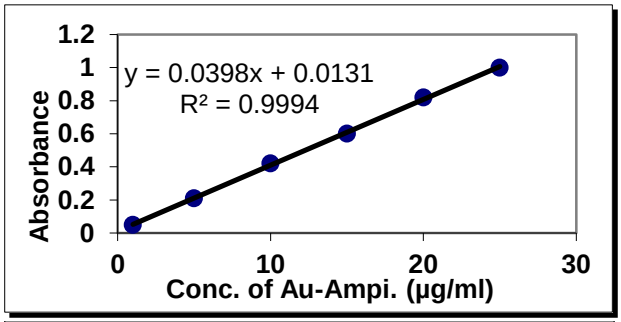
Name of drug	Regr.eq. y=Bx±A	Corr. Coef (r)	Linear Range (µg/ml)	D.L. (µg/ml)	t-test calc.	Tabulated t-test two tailed %95 C.I.	Mean RSD% (n=4)	Mean Rec.%
Direct Method								
Amp.	y=0.0087x-0.0099	0.9987	5-60	0.2362	0.4320	2.365	1.7789	101.05
Amox.	y=0.0069x-0.0167	0.9983	5-60	0.3056	0.5022	2.365	0.9309	101.02
Ceph.	y=0.0096x-0.0179	0.9992	5-60	0.2044	0.2757	2.365	1.7049	101.06
Standard Addition Method								
Amp.	y=0.0109x+0.0988	0.9992	5-55		1.134	2.365	1.046	95.940
Amox.	y=0.0089x+0.09	0.9988	5-55		1.870	2.365	0.6673	103.45
Ceph.	y=0.0090x+0.0937	0.9992	5-55		1.547	2.365	1.0062	94.450

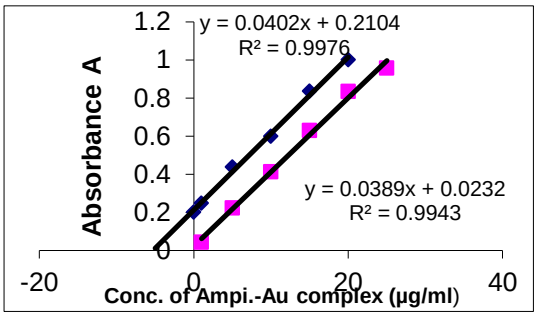
Table-3 : Regression Equation, Correlation coefficient, t-test ,the concentration ranges, detection limits, RSD% and mean recovery values using direct calibration and standard addition curves for Drug-Hg complexes by uv-visible spectrophotometric methods

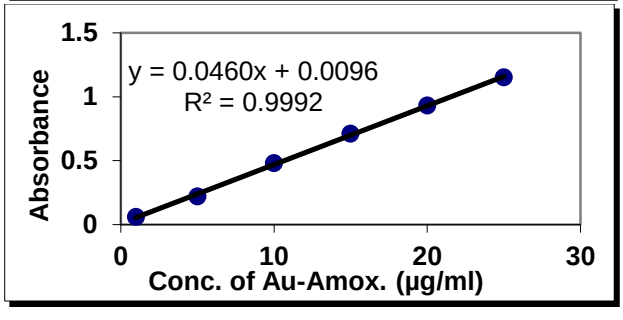
Name of drug	Regr.eq. Y=Bx±A	Corr. Coef. (r)	Linearity (µg/ml)	D.L. (µg/ml)	t-test statisticalc.	Tabulated t-test two tailed %95 C.I.	Mean RSD% (n=4)	Mean Rec.%
Direct Method								
Amp.	y=0.0108x-0.0229	0.9990	5-55	0.2667	0.3562	2.365	1.3083	100.970
Amox.	y=0.0115x-0.0182	0.9985	5-55	0.2007	0.4488	2.365	0.9547	100.996
Ceph.	y=0.0100x+0.0017	0.9990	3-55	0.4333	0.1500	2.365	1.9567	100.983
Standard Addition Method								
Amp.	y=0.0104x+0.1084	0.9994	5-50		2.1909	2.365	1.973	108.150
Amox.	y=0.0107x+0.0973	0.9990	5-50		1.8721	2.365	1.044	97. 050
Ceph.	y=0.0110x+0.1054	0.9994	5-50		1.760	2.365	1.9567	95.450

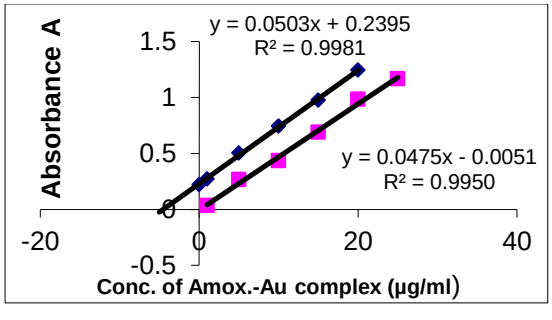
Table-4: Percentage relative error for determination of dosage Drug -Au and Hg(II) complexes by direct and standard addition UV-Vis method using 500mg /unit capsules

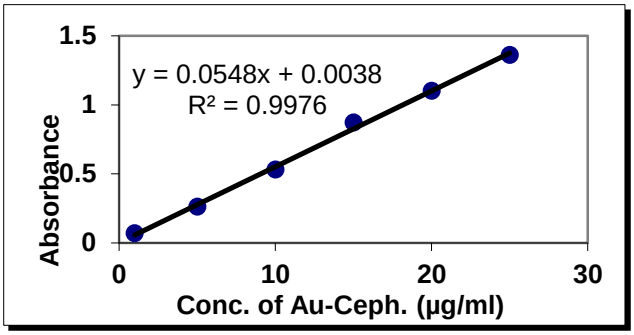
Drugs	Stated conc. (mg per unit)	Found direct calb. (mg per unit)	Erel. %	Found st.add. calb. (mg per unit)	%Erel
Au(III) complexes					
Ampi.	500	505.250	+1.050	479.705	-4.060
Amox.	500	505.100	+1.020	517.265	+3.453
Ceph.	500	505.300	+1.060	472.255	-5.550
Hg(II) complexes					
Amp.	500	505.500	+1.100	477.980	-4.404
Amox.	500	504.830	+0.966	516.750	+3.550
Ceph.	500	504.915	+0.983	478.650	-4.270











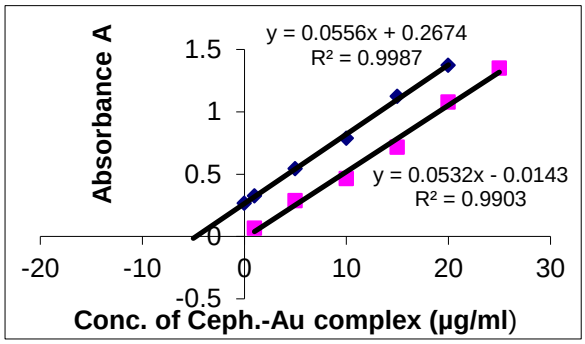


Fig-5: Direct and standard addition calibration curves for determination of Ampi- , Amox- and Ceph.-Au complexes by FAAS

Table- 5: Regression Equation, Correlation coefficient, t-test, RSD% and Percentage recovery for determination Drug-Au complexes by direct and standard addition FAAS

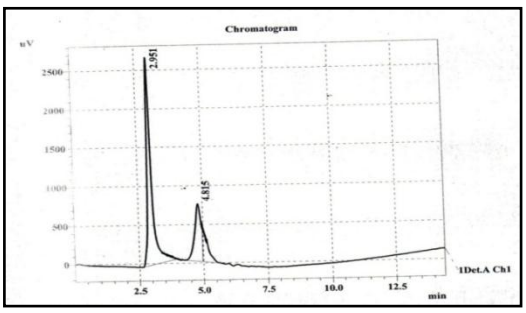
drug	Regr.eq. y=Bx±A	Corr. coef. (r)	Linearity (µg/ml)	D.L. (µg/ml)	t-test calc.	Tabulated t-test %95 C.I.	RSD% (n=4)	Mean Rec.%
Direct method								
Ampi.	y=0.0398x+ 0.0131	0.9997	1-25	0.0766	2.5201	2.571	1.199	101.425
Amox.	y=0.0460x+ 0.0096	0.9996	1-25	0.0770	0.8626	2.571	1.209	101.540
Ceph.	y=0.0548x+ 0.0038	0.9988	1-25	0.0194	2.4175	2.571	0.9363	104.229
Standard Addition Method								
Ampi.	y=0.0402x+ 0.2104	0.9934	1-22		0.8680	2.571	2.024	105.00
Amox.	y=0.0503x+ 0.2395	0.9990	1-22		0.8619	2.571	3.504	95.600
Ceph.	y=0.0556x+ 0.2674	0.9993	1-22		0.8538	2.571	3.389	97.090

Table-6: Percentage relative error for determination Drug-Au complexes by direct and standard addition FAAS method

Name of drug	Type of prepart-ion	Stated concentration (mg per unit)	Found direct-calb.(mg per unit)	Eer. %	Found st. ads. cal.(mg per unit)	Erel. %
Ampi.	Capsule	500	507.125	+1.425	525.00	+5.000
Amox.	Capsule	500	507.700	+1.440	478.00	-4.400
Ceph.	Capsule	500	521.145	+4.229	485.454	-2.909

3.3 Determination of antibiotics by High Performance Liquid chromatography (HPLC) method

The determination of each metal complex was studied by plotting the area under the curve against concentrations at detection wavelengths 254nm for all complexes except Amox-Hg complex at 276nm. HPLC chromatograms and calibration curves are shown in figures 6 and 7.



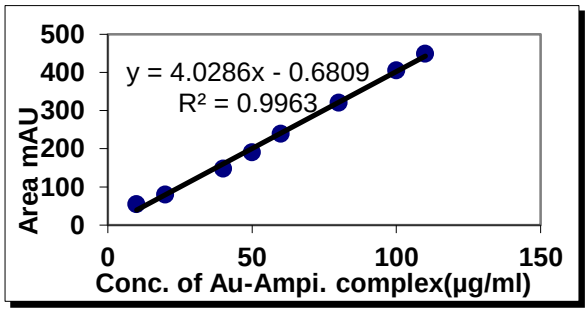


Fig-6a: HPLC chromatogram of ampicillin and its Au(III)complex with retention times (2.951and 4.815min)respectively at λmax.254nm (left) and calibration curve of peak area of Amp-Au(III) complex against concentration(right)

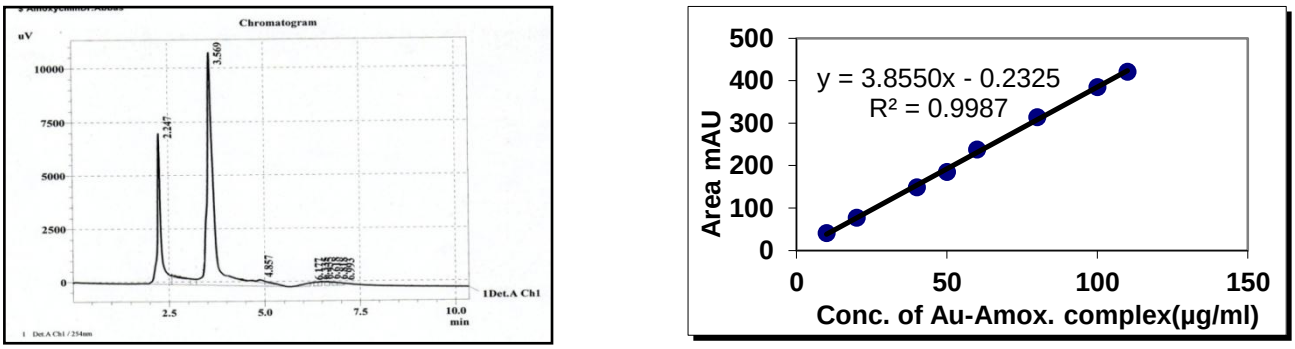


Figure-6b: HPLC chromatogram of amoxicillin and its Au (III) complex, retention time for each (2.247, 3.569min respectively) at λmax.254nm (left) and calibration curve of peak area of amoxicillin-Au (III) complex against concentration (right)

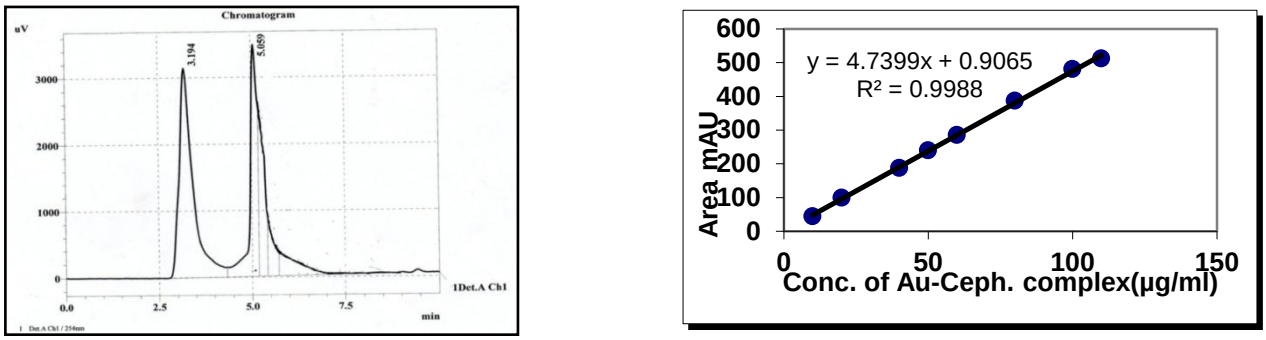


Fig-6c: HPLC chromatogram of cephalexin and its Au(III) complex , retention time(3.194, and 5.059min)respectively at λmax.254nm(left) and calibration curve of peak area of of Ceph.-Au complex against concentration(right)

The peaks corresponding to the complexes appeared at higher retention times than the original drug. The linearity of peak area with concentrations was 10-110 and 10-120 for Au (III) and Hg (II) complexes respectively. From the calibration curves t-statistic were found less than t-tabulated (table-7) which indicates that there is a linear relationship between concentration and peak area and that the results of applied method acceptable [18,19].

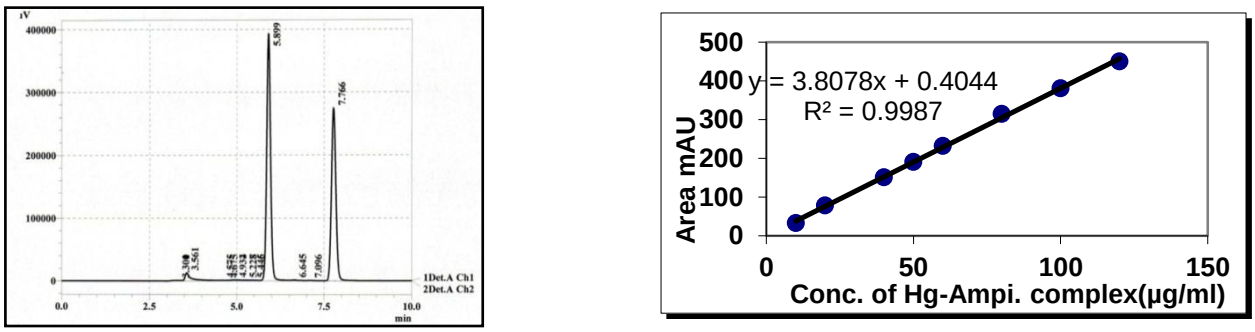


Fig-7a: HPLC chromatogram of ampicillin and its Hg(II) complex with retention times (5.899,7.766 min) respectively at λmax.254 nm(left) and direct calibration curve of Ampi.-Hg(II) complex(right)

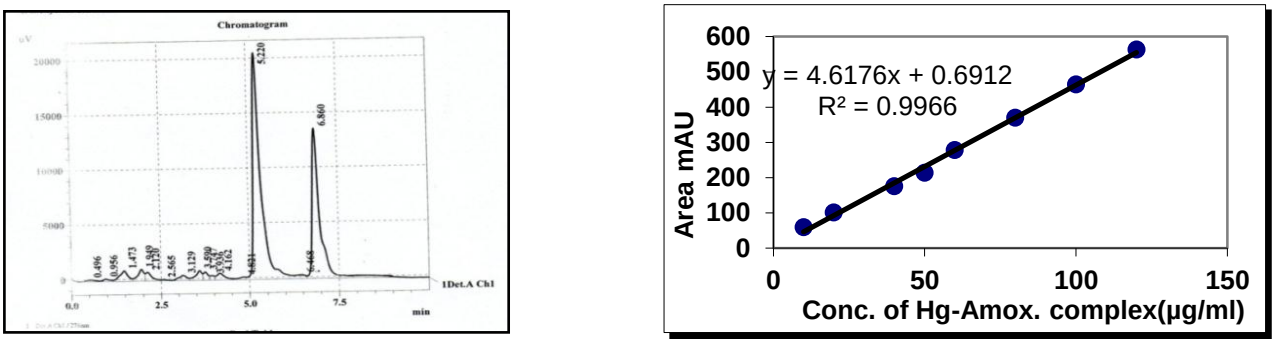
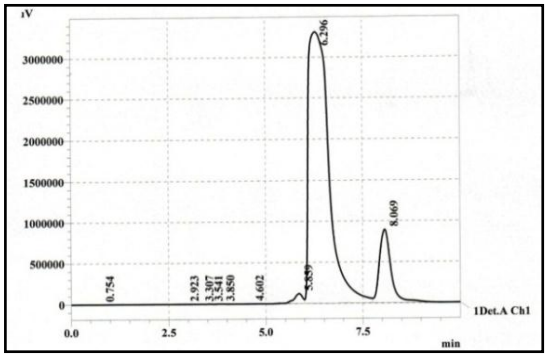


Fig-7b: HPLC chromatogram of amoxicillin and its Hg (II) complex with retention times (5.220, 6.860 min) respectively at λmax.276 nm (left) and direct calibration curve of Amox.-Hg complex (right)



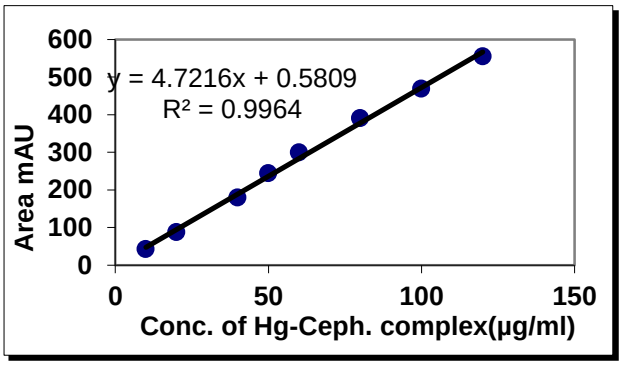


Fig-7c: HPLC chromatogram of cephalexin and its Hg (II) complex with retention times (6.296, 8.0690min) respectively at λmax.254 nm (left) and direct calibration curve of Ceph.-Hg (II) complex (right)

Table-7: Regression Equation, Correlation coefficient, linearity, detection limits , t-test , RSD%, and mean recovery % for Drug complexes with Au(III) and Hg(II) ions by HPLC using direct method

Name of drug	Regr.eq. y=Bx±A	Corr. Coef. (r)	Linearity µg/ml	D.L. (µg/ml)	t-test statistic	Tabulated t-test two tailed %95 C.I.	RSD% (n=4)	Mean Rec.%
Au(III) complexes								
Ampi.	y=4.0286x-0.6809	0.9981	10-110	2.192	1.1782	2.365	0.9387	101.208
Amox.	y=3.8550x-0.2325	0.9993	10-110	2.398	1.5625	2.365	1.0412	101.611
Ceph.	y=4.7399x+0.9065	0.9994	10-110	2.236	0.5571	2.365	0.8771	101.388
Hg(II) complexes								
Ampi.	y=3.8078x+0.4044	0.9993	10-120	2.450	1.6481	2.365	1.013	101.696
Amox.	y=4.6176x+0.6912	0.9983	10-120	3.667	1.4940	2.365	1.09999	102.666
Ceph.	y=4.7216x+0.5809	0.9982	10-120	2.090	0.4292	2.365	1.2599	101.533

The detection limits in this method are higher than the two other methods. However these cannot be considered as final results because HPLC depends mainly on the selected mobile phase which has a large effect on the sensitivity, and resolution[19].

3.4 *Comparison of the three assay methods*

Table-9 shows a summary of the determination of the three drugs by complexation with Au (III) ion using direct methods where the FAAS method is more sensitive. Although the HPLC methods took lower period of time and no extraction process was required for analysis compared with the two spectrophotometric methods, the results obtained by HPLC showed higher detection limits. The selection of a better mobile phase and columns may lead to better separation result.

4. CONCLUSIONS

In this study uv-visible absorption spectrophotometry, flame atomic absorption spectrophotometry FAAS, and high performance liquid chromatography HPLC were adopted to determine three β- lactam antibiotics in bulk and in pharmaceutical preparations by studying their complexation behavior with gold(III) and mercury(II) ions . The mole ratio of drugs: metal ions L: M was 1:1 in all complexes. The FTIR spectra suggest the bonding of metal ions with carbonyl groups of lactam ring and oxygen atoms of carboxyl group.

Depending on direct and standard addition calibration curves of concentrations, the FAAS method, showed higher sensitivity, lower detection limits and linear ranges compared with the other two methods. The HPLC method showed lower period of time for analysis compared with the other methods. In all the applied methods, the slopes of standard addition plots were parallel to slopes of direct plots which means that no matrix interference exists in these methods and that there is a linear relationship between concentration and absorbance. The calculated t-test values were lower than tabulated and correlation coefficients were within the accepted assay limits.

Table-8: Relative percentage error for determination of Au (III) and Hg(II) complexes of drugs in pharmaceutical dosage (capsule) by direct method using HPLC

Drugs	Stated dose (mg per unit)	Found (mg per unit)	Mean %Eer
Au(III) complexes			
Amp.	500	506.041	+1.208
Amox.	500	508.055	+1.611
Ceph.	500	506.944	+1.388
Hg(II)complexes			
Amp.	500	509.833	+1.96666
Amox.	500	511.388	+2.277
Ceph.	500	507.6665	+1.5333

Table-9: Comparison of analytical assay data for Au(III)-drug complexes by direct methods

Name of Drug	UV-Vis. Method	FAAS method	HPLC method
Amp. LDR(µg/ml) r D.L.(µg/ml)	5-60 0.9987 0.2362	1-25 0.9997 0.0766	10-110 0.9981 2.192
Amox. LDR(µg/ml) r D.L.(µg/ml)	5-60 0.9983 0.3056	1-25 0.9996 0.0770	10-110 0.9993 2.3988
Ceph. LDR(µg/ml) r D.L.(µg/ml)	5-60 0.9992 0.2044	1-25 0.9988 0. 1940	10-110 0.9994 2.2360

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