# Spectroscopic Study for Determination of Amoxicillin Using Cobalt(II) as Complexing Metal

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## Abstract

This study includes analytical methods for the determination of the drug amoxicillin trihydrate (Amox.) in some pharmaceutical preparations using Cobalt ion (Co(II)) as complexing metal. The best conditions for complexation were: the reaction time was 20 minutes, pH=1.5 and the best temperature of reaction was 70 ˚C. Benzyl alcohol was the best solvent for extraction the complex.

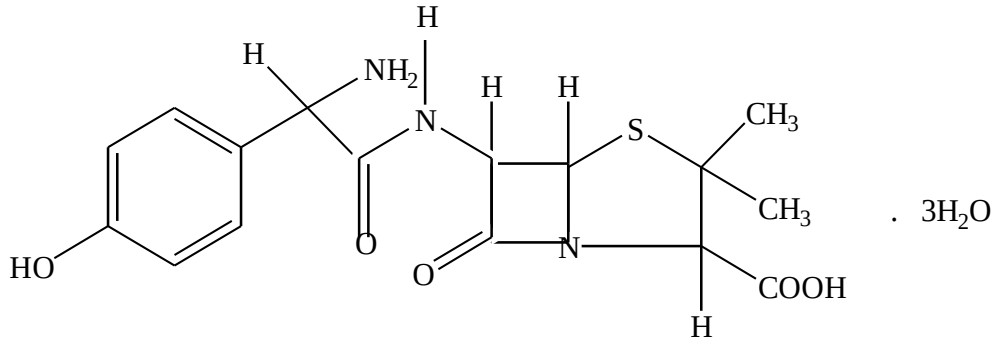
***Keywords: Amoxicillin, Cobalt(II), Complex, Molar ratio.***

**الخلاصة**

يتضمن البحث استحداث طرق تحليلية جديدة في تقدير المركب الدوائي اموكسسلين ثلاثي جزيئات الماء (Amoxicillin Trihydrate) وذلك بتكوين معقد للعقار مع أيون الكوبلت ( Co II) حيث وجد أفضل pH لعملية التعقيد عند دالة حامضية (pH=١,٥) وان أفضل درجة حرارة لعملية التعقيد مابين العقار والايون الفلزي كانت بحدود ٧٠ درجة مئوية, وأفضل زمن للتفاعل فكان بحدود ٢٠ دقيقة,قد جربت عدة مذيبات حيث وجد إن الكحول البنزيلي هو أفضل مذيب لعملية الاستخلاص.

## Introduction

Amoxicillin is one of the important derivatives of semisynthetic penicillin; it is active against Gram positive and to less extent Gram negative bacteria. Its nomenclature according to penicillins is 6-[D(-)-α-Amino-p-hydroxyphenyl acetamido] penicillanic acid or α- amino-p-hydroxy benzyl penicillin[(1)] ,while its systematic (IUPAC) name is 7-[2-Amino-2-(4-hydroxypHenyl)-acetyl]amino-3,3,dimethyl-6-oxo-2-thia-5- azabicyclo [3, 2, 0] heptane-4-carboxylic acid, the chemical structure of the drug is [(1)]




The formula structure of Amoxicillin as trihydrate (drug) is $C_{16}H_{19}N_3O_5S.3H_2O$, its molecular weight = 419.45 gm.mole$^{-1}$. It is off white or almost white crystalline powder, slightly soluble in water and alcohol such as methanol and ethanol [(2)]. It has UV max. (ethanol): 230,274 nm and in (0.1N HCl): 229,272 nm [(1)].Imran et al. prepared complexes of amoxicillin with Zn(II), Cu(II), Ni(II)and Ag(I), they identify these complexes by (C, H, N)elemental analysis and IR Spectra. These complexes have increased the biological activity of the drug [(3)]. Jian et al. determined amoxicillin in tablets, they used a quick and simple method which is (second differential derivative) at $\lambda_{max}$. 282 nm and the standard deviation was less than 1.2% and the standard recovery for the drug was 97-100.5 % [(4)].Denis et al. determined amoxicillin and clavulanic acid in blood plasma by HPLC supplied by UV detection, and they found the linearity was (0.62 – 20 µg.ml$^-$) while the detection limit for amoxicillin was 0.312 µg.ml$^-$ [(5)].

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Ashry et al. detected phenolic antibiotic like amoxicillin by its reaction with benzocaine in the presence of triethylamine at $\lambda_{max}$. = 455 nm the linearity was (2 – 16 µg.ml$^{-1}$) while the detection limit was 0.0034 µg.ml$^{-1}$ [6].Co(II) forms blue-colored complex in the organic phase with Cyanex 923, a sensitive analytical reagent, the $\lambda_{max}$. of the complex was 635 nm and the concentration that obeyed Beer's law is (58.9-589.0 µg. ml$^{-}$)[7].Zayed et al. in a new study, prepared different complexes of amoxicillin with Zn (II), Ni (II), Co (II) and Cu (II), these complexes were studied using elemental analysis, IR and mass spectra. The molar ratio of complexes were found to be Metal:Drug =1:1,1:2, and the stability constant $K_f$ of these chelates was($10^7$-$10^{14}$) [8].In a recent study, Alekseev et al. prepared mixed complexes of β-lactam antibiotics (Ampicillin, Amoxicillin and Cephalexin) in solutions containing Co(II) and glycine anions(Gly). These complexes had been investigated using pH-metric titration at 20$^o$C in alkaline medium as mixed ligands complex [Co Gly Ampicillin], [Co Gly Amoxicillin], and [Co Gly Cephalexin] [9].

## Instruments , Materials and Method

***A - Instruments***

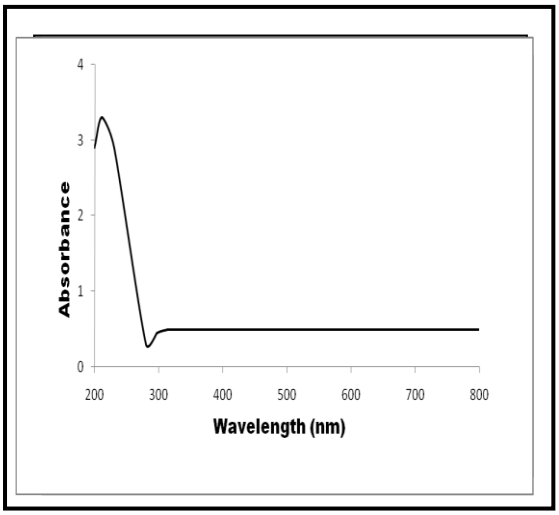
1. UV-Visible Spectrophotometer (CARY 100) wave length 200-1100nm.
2. Shimadzu (AA-670) Flame Atomic Absorption Spectrophotometer (400S).
3. Mettler, Balance Model 210S, ISO 9001.
4. pH-meter type 60A, USA.
5. Water Bath with Thermostat, Memmert.

***B - materials***

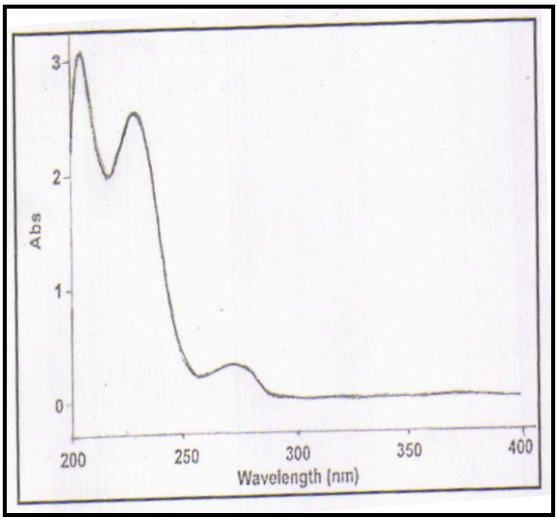
All the chemical stock solutions were prepared from analytical grade BDH, SDI, and India.

***C- Method***

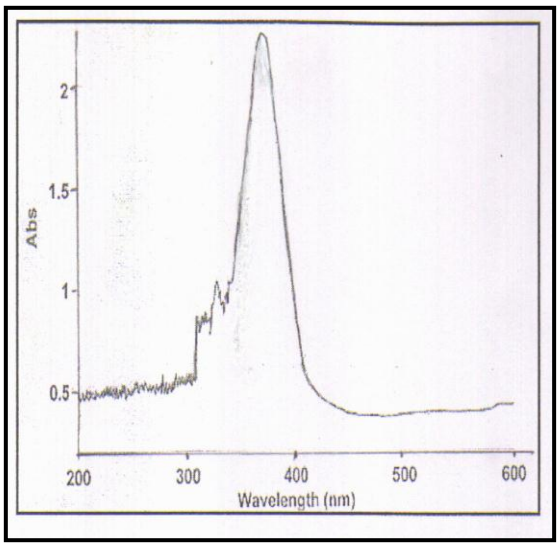
1. Stock solution of Co(II) 1000 ppm is prepared by dissolving 0.2010 gm hydrated cobalt chloride ($CoCl_2.6H_2O$) in distilled water and complete the volume to 50 ml.
2. Stock solution of Amoxicillin 1000 ppm is prepared by dissolving 0.1 gm amoxicillin in 5 ml. of 1M HCl then complete to 100 ml with distilled water.
3. Choosing the optimum conditions for complex formation: The experimental work showed that the reaction did not proceed at room temperature; heating was needed, media must be acidic for this reason. We studied the effect of pH, temperature, reaction time, extraction time, and suitable solvent for the extraction process.
4. Spectral study:
   - **a)** Amoxicillin spectrum: transfer 1 ml from stock solution of amoxicillin to 5 ml volumetric flask then dilute with distilled water the absorbance is measured at (200-1100 nm) using acidic water as blank. Table (2) shows the proper pH of the solution and Figure (1) shows UV spectrum for Amoxicillin.
   - **b)** Cobalt spectrum: transfer 1 ml of Co (II) stock solution to 5 ml volumetric flask then dilute with distilled water the absorbance is measured at (200-1100 nm) using distilled water as blank. Figure (2) shows UV spectrum for Cobalt.
   - **c)** Amoxicillin-Cobalt(II) [Amox.-Co(II)] Complex Spectrum: transfer (2-5 ml) from the standard of Amoxicillin stock solution to 5 ml volumetric flask then 1 ml of Cobalt stock solution is added. The chelating complex was extracted by 5 ml benzyl alcohol then measures the absorbance at (200-1100 nm) using benzyl alcohol as blank. Figure (3) Shows UV spectrum for [Amox.-Co (II)] Complex.




**Figure(1): UV Spectrum of amoxicillin**




**Figure(2) :UV Spectrum of the element Co(II)**




**Figure(3): UV Spectrum of [Amox-Co(II) ] Complex**

## Results and Discussion

Amoxicillin spectrum, illustrated at Figure (1), consist of 2 bands at $\lambda_{max.}$ (272 nm) and (228 nm), we depend on the band at $\lambda_{max.}$ (228 nm) because the other peak will disappear in some experiments also it may interfere with benzyl alcohol peak.

- Co (II) Spectrum gives peak at $\lambda_{max.}$ (211 nm) using distilled water as a blank, (Figure 2).
- Chelating complex [Amox.-Co (II)], a new peak at $\lambda_{max.}$ (375 nm) as shown in Figure (3) which indicate the formation of the complex that extracted by benzyl alcohol. Table (1) shows the color and $\lambda_{max.}$ for the amoxicillin, Co (II), and the complex.

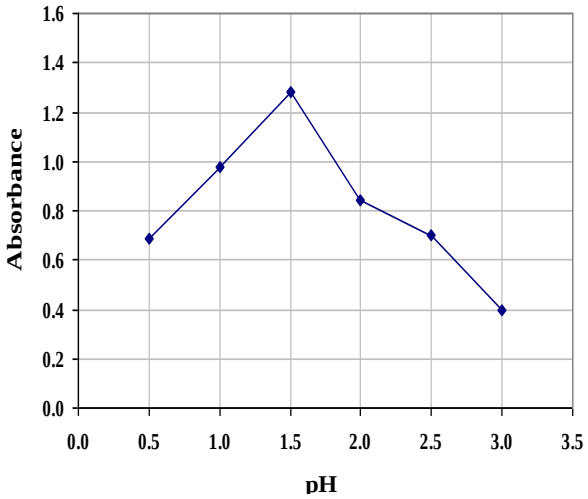
| Compound | $\lambda_{max.}$ ( nm) | Color |
|---|---|---|
| Amoxicillin | 228<br>272 | Off- white |
| Co(II) | 211 | Bluish- white |
| [Amox.-Co(II)] | 375 | White |

**Table (1): Color and $\lambda_{max}$ for the drug, metal, complex.**

- Detection the optimum conditions for complexation using UV-Visible Spectrophotometer:
  1. pH effect: Table (2) shows the absorbance of the [Amox.-Co (II)] complex using different pH (0.1-3), the optimum pH for the complexation was (1.5) where the absorbance is gradually increased from 0.5 to the maximum peak at pH 1.5 and then it decreases. Figure (4) shows the effect of pH on the absorbance of [Amox.-Co (II)].

**Table (2): Complex absorption values at different pH.**

| pH | Absorbance |
|---|---|
| 0.5 | 0.69 |
| 1.0 | 0.98 |
| 1.5 | 1.28 |
| 2 | 0.84 |
| 2.5 | 0.7 |
| 3.0 | 0.4 |

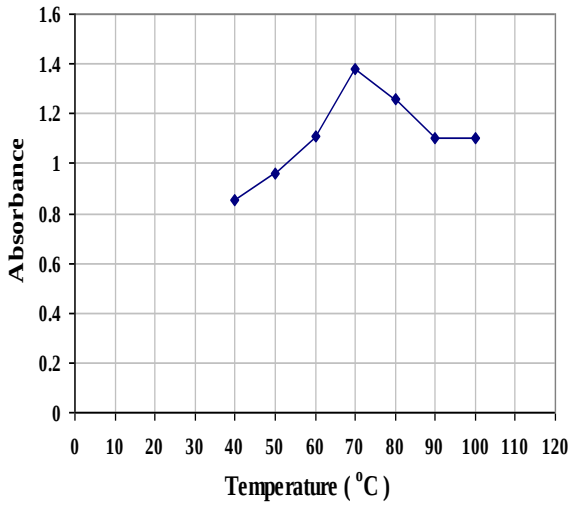



**Figure(4): The effect of pH on the absorbance of the [Amox-Co(II) ] Complex**

**2.** Temperature effect: the reaction of the metal and Amoxicillin proceeded slowly, to increase the reaction velocity we intend to increase the temperature of the reaction from 40 to100 ˚C, then the complex is extracted and measured the absorbance using UV Spectrophotometer. Table (3) gives the absorbance of the complex at different temperatures at pH 1.5 and Figure (5) shows the $\lambda_{max}$ for the complex is at 70 ˚C.

**Table (3): Complex absorption values at different temperatures at (pH 1.5).**

| Temp. $^{o}$C | Absorbance |
|---|---|
| 40 | 0.85 |
| 50 | 0..96 |
| 60 | 1.11 |
| 70 | 1.38 |
| 80 | 1.26 |
| 90 | 1.1 |
| 100 | 1.1 |




**Figure(5) The effect of temperature on the [Amox-co(II) ] formation**

**3.** Reaction time: the complex formation increased as well as the absorbance when the reaction time is increased, before the extraction process takes place, we used different times (5-30 min.), Table (4) and Figure (6) showed that 20 min. is the best time for the reaction and give maximum absorbance for the [Amox.-Co (II)] at (70 ˚C and pH 1.5).

**Table (4): Complex absorbance value at different reaction time at 70 $^{o}$C**

| Time(min.) | Absorbance |
|---|---|
| 5 | 0.75 |
| 10 | 0.99 |
| 15 | 1.16 |
| 20 | 1.30 |
| 25 | 1.22 |
| 30 | 1.13 |

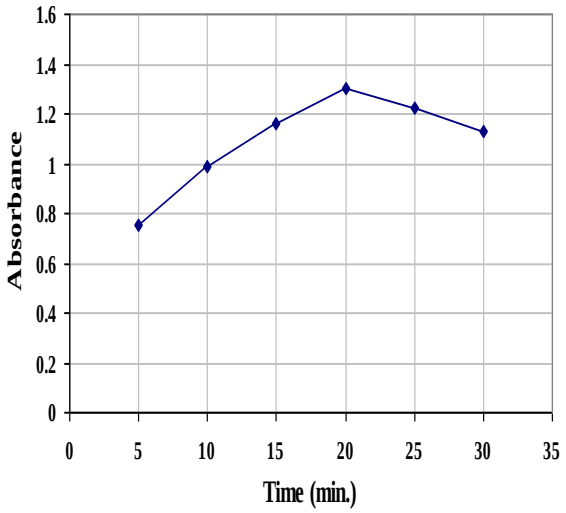



**Figure (6) The effect of reaction time on complex formation**

**4.** Suitable extraction solvent: different organic solvents were used like; methanol, ethanol, chloroform, benzyl alcohol, and ethyl acetate to choose the proper solvent that dissolves the complex, but can not dissolve the metal (Cobalt) and amoxicillin as well give the highest absorbance for the complex. Table (5) shows the solubility of the amoxicillin, metal and the complex in different organic solvents.

**Table (5): The solubility of amoxicillin, metal and the complex in different solvents.**

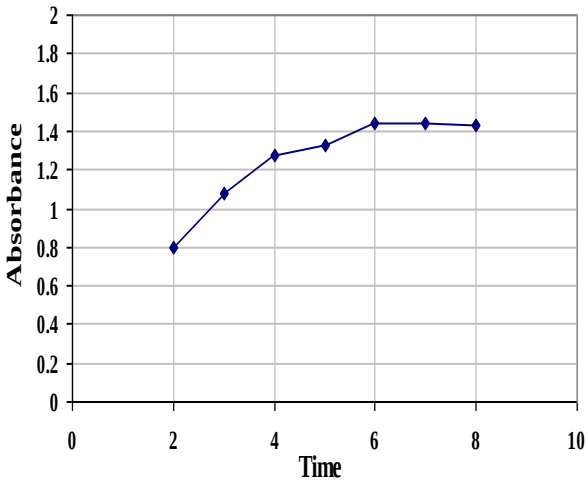
| Solvent | Amoxicillin solubility | Metal solubility | Complex solubility |
|---|---|---|---|
| Methanol | + | + | – |
| Ethanol | + | + | – |
| Chloroform | – | – | – |
| Benzyl alcohol | – | – | + |
| Ethyl acetate | + | – | + |

(+): Soluble.
(–) :Very slightly soluble or insoluble

5. Extraction time: the optimum time for shaking during the extraction of the complex was 6 min. which gives maximum absorbance as shown in Table (6) and Figure (7).

**Table (6): Effect of shaking time on the extraction process against the absorbance.**

| Extraction time (min) | Absorbance |
|---|---|
| 2 | 0.8 |
| 3 | 1.08 |
| 4 | 1.27 |
| 5 | 1.33 |
| 6 | 1.44 |
| 7 | 1.44 |
| 8 | 1.43 |

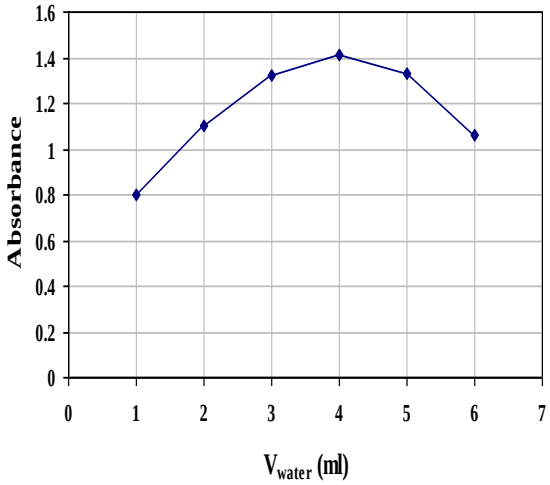



**Figure(7) The effect of the extraction time on the absorbance of the complex**

6. Solvent phase ratio: it was found that 4 ml of aqueous phase and 4 ml of organic phase is enough to give maximum absorption for the complex as shown in Figure (8). Table (7) showed that the increase in the aqueous phase to 6 ml with 4 ml of organic phase will decrease the absorbance of the complex.

**Table (7): Absorbance value of the [Amox.-Co(II)] as the volume of water phase increased.**
**Note: organic phase volume = 4 ml.**

| Water phase volume (ml.) | Absorbance |
|---|---|
| 1 | 0.8 |
| 2 | 1.1 |
| 3 | 1.32 |
| 4 | 1.41 |
| 5 | 1.33 |
| 6 | 1.06 |

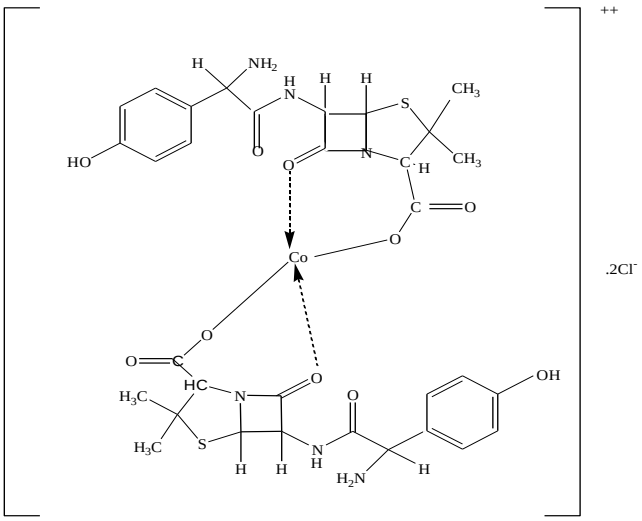



**Figure(8): The effect of water volume on the absorbance of the [Amox.-Co(II)] Complex**

7. Times of extraction: the first extraction process is enough to extract the major concentration of the complex because the second extraction process for the complex that remained in aqueous phase gives a very weak absorbance less than 0.1.

***Calculation of the ligand (Amoxicillin) to metal (Cobalt) ratio in the complex [Amox.-Co (II)]:***

***Molar ratio:*** to detect the ratio of complexation i.e. the molar ratio of Ligand (L) to the Metal (M) by taking different volumes of the Ligand ($V_L$) with constant volume of Metal ($V_m$) at the same concentration for each ($1.2 \times 10^{-3}$ M) at the optimum conditions for complexation, then drawing the relation between absorbance and $V_L/V_m$ as in Figure (9), and Table (8) shows the absorbance against $V_L/ V_m$. The molar ratio about (2:1) for the (L: M) and the suggested chemical structure for the [Amox.- Co(II)] complex is:

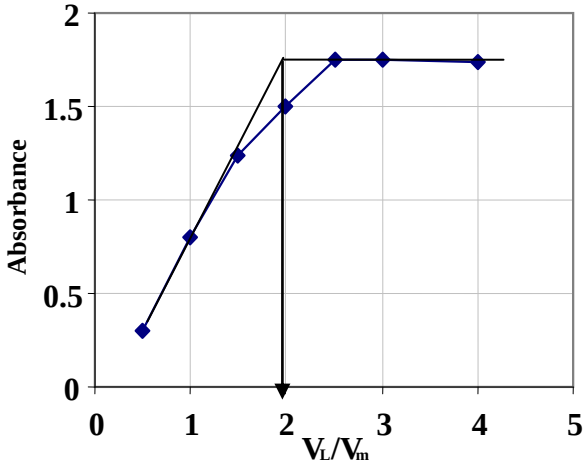


$$Co(II)+2Amox. \xrightarrow{pH = 1.5} Co(II) - 2Amox.$$

Imran [3] and his partners defined the Amoxicillin complex form with transition elements in M:L is 1:2 ratio, and this was identical with proportion of metal with the ligand (drug) that we have reached by molar ratio.

**Table (8): The values of the absorbance of the complex against $V_L/V_m$**

| $V_L/V_m$ | Molar ratio | Absorbance |
|---|---|---|
| 1/2 | 0.5 | 0.3 |
| 2/2 | 1 | 0.8 |
| 3/2 | 1.5 | 1.24 |
| 4/2 | 2 | 1.5 |
| 5/2 | 2.5 | 1.75 |
| 6/2 | 3 | 1.75 |
| 8/2 | 4 | 1.74 |



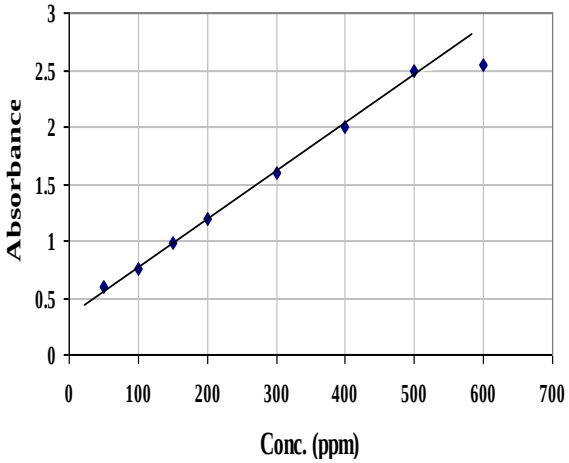

**Figure(9) : Detection the molar ratio for the complex formation**

***Calibration curve of the UV spectrum:***

It was carried out through taking different concentrations for the complex (ppm) and measuring the absorbance, as shown in Table (9). Figure (10) shows the calibration curve of [Amox.-Co(II)] complex that obeys Beer's law for the concentrations (50-500 µg. ml $^{-}$) at 375 nm.

**Table (9): The value of maximum absorbance for the [Amox.-Co(II)] at different concentrations.**

| Conc. ppm | Absorbance |
|---|---|
| 50 | 0.6 |
| 100 | 0.75 |
| 150 | 0.98 |
| 200 | 1.2 |
| 300 | 1.6 |
| 400 | 2.0 |
| 500 | 2.49 |
| 600 | 2.55 |




**Figure(10): The calibration curve for detecting of [Amox.-Co(II)] at 375 nm**

***Determination of the drug concentration in different pharmaceutical preparations:***

This was done by taking an average weight of one capsule from six capsules that have been mixed previously then the absorbance of the active ingredient (Amoxicillin) was measured from calibration curve (Figure 10), after complexation process takes place at the optimum condition mentioned above.

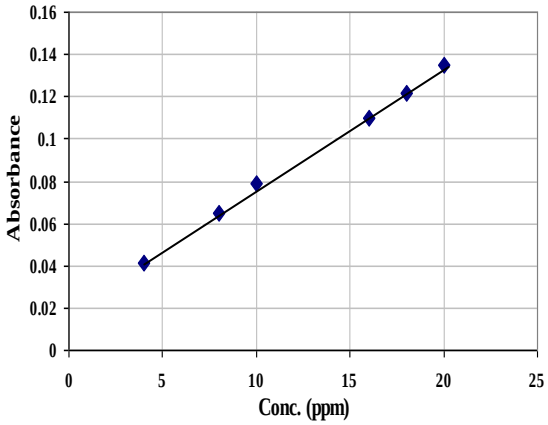
Note: we carried out the same procedure for 500 mg. amoxicillin capsules of different trademarks (SDI, Julphar and Ajanta). Table (10) shows the results of these preparations.

**Table (10): The concentration of amoxicillin in 500 mg amoxicillin capsule of different trademarks.**

| Capsule | Absorbance | Conc. ppm |
|---|---|---|
| SDI | 2.5 | 501 |
| JulpHar | 2.48 | 499 |
| Ajanta | 2.42 | 487 |

***Determination of the [Amox.-Co(II)] by Flame Atomic Absorption Spectrophotometer (FAAS):***

The complex was prepared under the optimum conditions of pH, temperature, proper solvent etc.. and we used the FAAS to detect the amoxicillin concentration by indirect measurement the absorbance of the Co(II) in the complex as shown in Figure (11), also we can measure the concentration of the amoxicillin in these pharmaceutical preparations using the calibration curve of indirect (FAAS).




**Figure(11): The calibration curve for detecting amoxicillin using FAAS**

## Conclusion

The developments of new analytical methods to determine the amount of amoxicillin, such methods are UV-Visible and flame atomic absorption spectrophotometrics, are very sensitive and precise. Amoxicillin forms chelated complex with Cobalt ions at 70$^{o}$C and pH: 1.5 and the molar ratio for complex formation, Amoxicillin:Cobalt, is (1:2). The results of analysis of amoxicillin capsules of different trademarks show the amount of amoxicillin in SDI and Julphar capsules are almost as presented by the package, on the other hand the Ajanta capsules show some differences.

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