A New Visible Spectrophotometric Approach for Mutual Determination of Allopurinol drug in Pharmaceuticals after Cloud Point Extraction

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Abstract: A new spectrophotometric methodology for allopurinol (ALLO) drug determination in pharmaceuticals was a proved. The method is based on formation of allopurinol-CoII complex in alkaline medium. A non-ionic surfactant Triton X-114 was used for the extraction of this complex at 586 nm. High extraction for complex was maintained under optimized conditions. The linearity was performed form (5.0-35.0) μg mL⁻¹ with correlation coefficient (R²=0.9995). Limit of detection (LOD) and limit of quantification (LOQ) were 0.393 and 1.308 μg mL⁻¹, respectively. This new method was applied successfully for allopurinol determination in pharmaceuticals and maintained acceptable accuracy and precision.

1. Introduction

Allopurinol (Allo) was developed in 1946 by Elion, et al. at the Burroughs–Welcome Company. It was discovered together with other molecules using spectrographic techniques when other purines were being considered for the treatment of cancer(1). Allopurinol (1H-pyrazolo[3,4-d]pyrimidin-4-ol) (Fig. 1) is a drug which was commonly used in treatment of chronic gout or hyperuricaemia associated with leukaemia, radiotherapy, antineoplastic agents and also in treatment with diuretics conditions(2-3). Allopurinol regarded as a structural isomer of hypoxanthine and effectively used for inhabitation of xanthine oxidase by catalyzes xanthine formation from hypoxanthine and further to uric acid(4-5). AP has been estimated by micelle-stabilized room temperature phosphoresence in urine samples(6). Cloud point extraction (CPE) includes formation of two isotropic phases in optimizing conditions, aqueous poor phase and organic rich phase(7).

Figure (1) : Structure of Allopurinol

This drug can be extraction by used cloud point extraction method(8). Cloud point extraction (CPE) is based on the phase behavior of non-ionic surfactants in aqueous
solution\(^9\), which exhibit phase separation after an increasing in temperature or the addition of a salting out agent \(^{10}\). Separation and pre-concentration based on (CPE) are becoming an important and practical application of surfactant in analytical chemistry\(^{11}\). This method is easy, sensitive, experimental conditions are free as heating and environmental friendly because use a small particular for analysis\(^{12}\).

The aim of present work was to develop simple, economical, rapid, precise, accurate and ecofriendly method for determination of single drug by using cloud point extraction.

2. Materials and Methods
2.1. Instrumentation and apparatus

UV-Visible spectrophotometer SHIMADZU, Double beam, model UV-1800, Japan. Hotplate stirrer (model L-18labincov). Electric Balance, Sartorius, Germany. Oven, Memmert, Germany. Thermostat water bath, Unitemp. Centrifuge, TRIU 800 Centrifuge, Korea. PH-meter, BP 3001.

2.2. Drug and Materials

The chemicals used for this work are of high purity and used as received. Distilled water was used in the preparation of all solutions and for final rinsing of glass wares. A pure grade of allopurinol was obtained from Drug Industries and Medical Appliance (SID) Samarra/ Iraq. A stock solution of ALLO (250\(\mu\)g ml\(^{-1}\)) or (1.836\(\times\) 10\(^{-3}\)M) was prepared by dissolving appropriate amount of ALLO (0.025 g) in 100 mL distilled water. Sodium hydroxide (0.1M) supplied from (BDH, UK) was prepared by dissolving (0.4 g) in 100 mL distilled water. A stock solutions (500\(\mu\)g ml\(^{-1}\)) of cobalt Ion (95.5%, Sigma, USA) were prepared by dissolving (2.0 g) in 1000 mL distilled water. Triton X-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL with distilled water in a 100 mL volumetric flask.

2.3. Recommended CPE Procedure for ALLO Drug

Aliquots 10 mL of a solution containing known amount of allopurinol drug mixed with Co(II) ions, then pH was adjusted by using 0.1M NaOH, followed by addition of 1mL of 10% (v/v) Triton x-114 and the mixture was diluted to 10 mL with distilled water. The mixture was shaken for 1 min and left to stand in a thermo-stated bath at 50 °C, for 10 min. Separation of the phases was achieved by centrifugation at 4000 rpm for 10 min, then putted in 5°C ice bath for 5 minute. The aqueous phase was removed and the remaining of micellar phase was dissolved by ethanol for spectrophotometric measurement at \(\lambda_{\text{max}}\) 586nm for ALLO–Co II complex against blank which was prepared in the same way but without drug.

2.4. Statistical Analysis

Excel 2010 (Microsoft Office R) was employed to carry out all statistical calculations.
3. Results and discussion

3.1. Absorption Spectra

The absorption spectrum of the complex product formed was recorded against the corresponding metal blank from 200 to 1100 nm before obtaining optimum conditions according to the recommended CPE procedure. It was observed that the absorption maximum of the colored product complex of allopurinol in 1.0 mL of 10% TX-114 occurred at 586 nm, giving the molar absorptivity of \((1.203 \times 10^4 \text{ L.mol}^{-1} \cdot \text{cm}^{-1})\) for allopurinol-CoII, thus the wavelength maximum at 586 nm for the complex product was chosen throughout this study.

![Absorption spectrum](image)

Figure 2. The absorption spectrum of [drug-CoII] complex [drug (25 μg.mL\(^{-1}\)) + CoII (50 μg.mL\(^{-1}\)] versus blank.

3.2. Optimization of CPE Methodology

A group of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for drug under study using a classical optimization. The variables such as the concentration of metal ion, best of pH, best of buffer, best of volume buffer, Triton X-114 amount, equilibration temperature and incubation time.

3.2.1 Effect of metal ions concentration

Figure (3) shows the effect of Cobalt ion concentrations upon the absorbance values of the extracted complex using (500 μg mL\(^{-1}\)) of drug solution. The optimum concentration of the metal ions that gave maximum absorbance was (50 μg mL\(^{-1}\) ) of Co(II) for the drug. The absorbance results are shown in table(1). Plotting of the absorbance values versus the concentration of metal ion is shown in figure (3).
### Table 1: Data of Absorbance to Optimum Volume of (0.8 μg ml⁻¹) metal ion.

| Volume of metal /mL | Absorbance at λmax586nm |
|--------------------|-------------------------|
| 0.2                | 0.066                   |
| 0.4                | 0.203                   |
| 0.6                | 0.360                   |
| 0.8                | 1.101                   |
| 1.0                | 0.349                   |
| 1.2                | 0.298                   |
| 1.4                | 0.214                   |

![Figure 3: Effect of Co(II) ion concentration on absorbance of (Allo-CoII) complex.](image1)

#### 3.2.2 Effect of pH

Figure(4) showed the value of absorbance intensity for the (Allo-CoII) complex against the value of pH, the best values of pH recorded for the highest absorbance values, then absorbance values were found to decrease with increasing pH which may be attributed to the formation of metal hydroxides. The absorbance results are illustrated in table (2).

### Table 2. Data of Absorbance to value of pH

| value of pH | Absorbance at λmax 586nm for (Allo-CoII) complex |
|------------|---------------------------------------------------|
| 1          | 0.011                                             |
| 2          | 0.372                                             |
| 3          | 0.456                                             |
| 4          | 0.598                                             |
| 5          | 0.677                                             |
| 6          | 0.934                                             |
| 7          | 1.098                                             |
| 8          | 1.116                                             |
| 9          | 1.178                                             |
| 10         | 1.139                                             |
| 11         | 1.167                                             |
| 12         | 0.834                                             |
| 13         | 0.729                                             |
| 14         | 0.167                                             |

![Figure 4: Effect of pH on the absorbance for (Allo-CoII) complex.](image2)

Plotting of the absorbance values versus the value of pH is shown in figure (4). Cloud point extraction yield plays unique role on metal a set of similar experiments in the pH range of 1.0 the described procedure The maximum sensitivity for CPE was obtained at pH 11. In more acidic solutions, deteriorate ion of the signal occurs due to the ligand protonation,
3.2.3 Effect of buffer solutions

The best values of buffer pH 11 were recorded for the highest absorbance values. Results of the measured absorbance for (CoII- allopurinol) complex are shown in table (3).

| Type of buffer solution | Absorbance |
|-------------------------|------------|
| Sodium bicarbonate buffer solutions | 1.227 |
| Sodium hydrogen ortho phosphate | 1.004 |

3.2.4 Effect of Volumes buffer solutions

Figure(5) showed the value of absorbance intensity for the (Allo- CoII) complex against the value of buffer solutions, the best values of sodium bicarbonate buffer and potassium dihydrogen phosphate solutions were recorded for the highest absorbance values. Plotting of the absorbance values versus the concentration of volume of pH is shown in figure (5).

Table 4. Data of Absorbance to value of buffer solutions

| value of pH | Absorbance at $\lambda_{max}$ 586nm for (Allo- CoII) complex to value of sodium bicarbonate buffersolutions |
|-------------|--------------------------------------------------------------------------------------------------|
| 0.2         | 0.480                                                                                           |
| 0.4         | 0.780                                                                                           |
| 0.6         | 1.277                                                                                           |
| 0.8         | 0.977                                                                                           |
| 1           | 0.954                                                                                           |
| 1.2         | 0.651                                                                                           |
| 1.4         | 0.469                                                                                           |

3.2.5 Effect Type of Surfactant

The type of surfactant plays very substantial role in cloud point extraction process where each surface owns spectral properties depend on practical basis of micelles. A liquid of 10ml solution contains[1mL allopurinol, 0.8ml Co(II) ion, 0.6 mL buffer pH 11 and Triton X-114] in 10ml volumetric flask and use different surfactant for drug [Tween 20, Tween80, CTAB, SDS, Triton X-100, Triton X-114] at 50°C for 10 min for metal incubation, then it centrifugated at 4000 rpm for 10min, separated the surfactant-rich phase and dissolved in 1mL ethanol and measured at $\lambda_{max}$ 586 nm. Results were illustrated in table (5).
### Table 5. Data of Absorbance to Type of Surfactant with (Allo-CoII) complex

| No | Surfactant     | Absorbance at $\lambda_{\text{max}}$ 586 nm for (Allo-CoII) complex |
|----|----------------|---------------------------------------------------------------------|
| 1  | Tween 20       | 1.132                                                               |
| 2  | Tween80        | 0.934                                                               |
| 3  | SDS            | 0.152                                                               |
| 4  | CTAP           | 0.049                                                               |
| 5  | Triton X-100   | 0.227                                                               |
| 6  | Triton X-114   | 1.461                                                               |

Plotting the absorbance values of the cloud point versus the type of surfactant is shown in Figure (6).

![Figure 6. Type of Surfactant for (Allo-CoII) complex](image)

From the results obtained, the non-ionic surfactant Triton X-114 is of high absorbance and this surfactant increases the efficiency of the extraction process in cloud point extraction.

### 3.2.6 Effect of Triton X-114 Volume

Amount of 10ml solution are prepared [0.5ml allopurinol, 0.8ml CoII, 0.6 ml buffer pH 11 and 0.1 ml Triton X-114] in 10ml volumetric flask and uses varying volumes of 10%(v/v) Triton X-114 (0.2-2.0)ml for complex then it is completed to the mark by distilled water, are mixed, heated at 50°C for 10 min for (Allo-CoII) complex to form cloud point then centrifugation at 4000 rpm for 10min, 1ml ethanol is added to the surfactant-rich phase to dissolve it then it is measured by UV-Vis at $\lambda_{\text{max}}$ 586 nm for (Allo-CoII) complex results shown in table (6). Plotting the absorbance values of the cloud point versus the volume of Triton X-114 is shown in Figure (7).
It is clear from the result that the absorbance increases with the increase volume of Triton X-114 but suddenly decreases at higher amount. Effect the amount of surfactant on the efficiency of extraction and improve the enrichment factor (98). These represent the optimum volume to reach equilibrium extraction process that give highest efficiency with smaller size and higher density in cloud layer. The decrease in absorbance below the optimum volume is due to insufficient micelles to entrap the hydrophobic product quantitatively. Therefore the optimum volume of Triton X-114 (0.2-2) ml for (Allo-CoII) complex fixed in subsequent experiments to achieve high extraction efficiency.

3.2.7. Effect of the incubation Time

Amount of 10ml solution is prepared in volumetric flask containing [1mL allopurinol, 0.8mL Co(II),0.6 mL buffer pH 11 and 1.2mL Triton X-11410%(v/v)] then it is completed to the mark by distilled water, are mixed and the temperature is set at 50°C for CoII-ALLO complex and the incubation time varies from (5.0-35.0) min. to form cloud point extraction, then centrifugation at 3000 rpm for 10min, 1mL ethanol is added to the surfactant-rich phase and measured at $\lambda_{\text{max}}$ 586 nm for Allo- CoII complex. Plotting the absorbance values of the cloud point versus the time (min.) is shown in Figure (8)

| Time of heating (min) | Absorbance at $\lambda_{\text{max}}$ 589 nm for Allo- CoII complex |
|-----------------------|---------------------------------------------------------------|
| 5                     | 0.760                                                         |
| 10                    | 1.579                                                         |
| 15                    | 1.654                                                         |
| 20                    | 1.279                                                         |
| 25                    | 1.164                                                         |
| 30                    | 1.102                                                         |
| 35                    | 1.020                                                         |
| 40                    | 0.921                                                         |

Figure 7. Volume of Triton X-114 for (Allo- CoII) complex

Figure 8. Absorbance Versus Time for (Allo- CoII) complex
Results obtained in figure 8 showed the effect of reaction time on the complex formation, by following the variation of absorbance values. Maximum absorbance for all extracted (Allo-CoII) complex were observed after 15 min. Heating for longer time resulted in decreased absorbance values results shown in table (7). Cloud point extraction requires enough time to get equilibrium between aqueous phase and surfactant-rich phase by more aggregation the micelles. This time represents the amount of heat accumulated in the solution that allows Micelles lose water molecules in order to give small size hydrophobic with high viscosity easily entrap the product in it. It is clear that the optimum incubation time is 15 min for (Allo-CoII) complex

### 3.2.8. Effect of Temperature

The complexation reactions of the studied (Allo-CoII) complex were very slow at room temperature, while the highest absorbance of complex (Allo-CoII) was maintained at 50°C as explained in Figure (9). Heating the solutions to higher temperatures decreases the absorbance and this may be due to the decomposition of complexes (table 8). Plotting the absorbance values of the cloud point versus the temperature is shown in Figure (9).

#### Table 8. Data of Absorbance to Temperature / °C

| Temperature / °C | Absorbance at $\lambda_{\text{max}}$, 586 nm for (Allo-CoII) complex |
|------------------|-------------------------------------------------|
| 30               | 0.732                                           |
| 35               | 0.901                                           |
| 40               | 1.239                                           |
| 45               | 1.491                                           |
| 50               | 1.655                                           |
| 55               | 1.524                                           |
| 60               | 1.123                                           |
| 65               | 1.067                                           |
| 70               | 0.967                                           |

**Figure 9:** Absorbance Versus Temperature for (Allo-CoII) complex

We need to heat the aqueous solution at a certain temperature to form a layer of the cloud point that smaller size and high viscosity due to aggregation the micelles this called (Cloud point temperature - CPT). The results show that the highest absorbency and extraction efficiency of the drug at temperature 50°C for (Allo-CoII) complex then decreases in absorbance at higher temperature due to decomposition of product which reduces the extraction efficiency. This temperature is fixed in subsequent experiments.

### 3.2.9. Effect of extraction time

Results in Figure 10 explained that the centrifugation time does not have a considerable effect on the analytical characteristics of the CPE method. This parameter was examined in the range of 5.0–25.0 min at 4000 rpm. A time of 10 min was selected as optimum, since complete phase separation occurs in this time and no appreciable improvements were results shown in table (9).
Table 9. Data of Absorbance to extraction time

| centrifugation | Absorbance at $\lambda_{max}$ for ( Allo- CoII ) complex |
|---------------|--------------------------------------------------------|
| time(min)     | 586nm for ( Allo- CoII ) complex                        |
| 5             | 1.589                                                  |
| 10            | 1.655                                                  |
| 15            | 1.652                                                  |
| 20            | 1.642                                                  |
| 25            | 1.640                                                  |

Figure 11. Extraction time effect on absorbance values of ( Allo- CoII ) complex

3.2.10. Effect of Order Additions

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Fig (12) shows that the best order of addition is the number 1 for target analytes due to giving a highest absorption signal among the others. The absorbance is measured and the absorbance results are shown in table (10).

Table 10. Data of Absorbance to Order Additions

| No | Order Additions | Absorbance at $\lambda_{max}$ 586nm for ( Allo- CoII ) complex |
|----|----------------|---------------------------------------------------------------|
| 1  | D+ M+B+T       | 1.650                                                          |
| 2  | M+D+B+T        | 1.236                                                          |
| 3  | D+B+M+T        | 0.253                                                          |
| 4  | M+B+D+T        | 0.056                                                          |

Plotting of the absorbance values versus the order additions is shown in figure (12).

Figure-12: Effect of Order Additions For ( Allo- CoII ) complex

It is noted that the best addition is the first order of ( Allo- CoII ) complex and the best addition is the four order of ( Allo- CoII ) complex because if it's another order gets lost in the intensity of color and this order fixed in subsequent experiment.
### 3.2.11. Effect of Solvents

The absorbance is measured and the absorbance results are shown in table (11).

| No | Solvents     | Absorbance at $\lambda$ max 586 nm for (Allo-CoII) complex |
|----|--------------|-------------------------------------------------------------|
| 1  | Water        | 1.657                                                       |
| 2  | Ethanol      | 0.986                                                       |
| 3  | Methanol     | 1.523                                                       |
| 4  | Acetonitril  | 0.786                                                       |
| 6  | Chloroform   | 0.416                                                       |
| 7  | Acetyl aceton| 0.134                                                       |
| 8  | Dimethylformamide| 0.178                                                 |
| 9  | Dimethy phthalate | 0.246                                               |
| 10 | Dimethy malonate | 0.787                                               |

Plotting of the absorbance values versus the solvent is shown in figure (13)

**Figure 13:** Effect of Solvents  For (Allo-CoII) complex

It has been shown that water is the optimum solvent, economically, sensitivity method, cheap price, to provide and nontoxic. This solvent is fixed in subsequent experiment

### 3.2.12. The Effect of Interference

The effect of interference expected present in (Allo-CoII) complex has been studied to know method selectivity under study by added 1ml (100 ppm) from each interference [Lactose, Starch, Arabic Gum, Glucose, Talc, Ca$_3$(PO$_4$)$_2$, CaCO$_3$] with 1ml(10 ppm) from each drug and the rest of addition are optimal conditions then diluted with distilled water in 10ml volumetric flask then measured. The interference experiment is performed to estimate the systematic error caused by other materials that may be present in the specimen being analyzed. It must be the size of interference is small for a sample to limit the dilution of sample and use the maximum concentration expected in the sample. The results are shown in Table (12)
Table 12. Data of Absorbance for Interference

| 100ppm interference | Absorbance at $\lambda_{\text{max}}$ 586nm |
|---------------------|------------------------------------------|
| With out            | 1.656                                    |
| Lactose             | 0.791                                    |
| Starch              | 0.018                                    |
| Arabic Gum          | 0.032                                    |
| Talc                | 0.930                                    |
| Glucose             | 0.979                                    |
| $\text{Ca}_3(\text{PO}_4)_2$ | 0.343                      |
| $\text{CaCO}_3$     | 0.856                                    |

From the previous tables we notice that there is no expected interference to be present with drug in pharmaceuticals.

![Figure 14: Effect of Interference](image)

**Selected Optimum Conditions**

The optimization conditions method that was used above to study the effect of variables on the absorbance intensity. After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product, that gave the optimum conditions shown in Table (13).
Table 13: The optimum conditions for the determination of Complex

| Optimum                              | Concentrations | Range selected | Optimum quantities of complex (allopurinol-CoII) |
|--------------------------------------|----------------|----------------|-----------------------------------------------|
| \( \lambda_{\text{max}}(\text{nm}) \) | ---            | 190-1100       | 586                                           |
| Effect of volume of metal ion required | 500 ppm       | 0.2 - 2 mL     | 0.8mL                                          |
| Buffer pH                            | 0.1M(NaOH)    | 1-14           | 11                                            |
| Effect of volume of triton x114 required | 10%(v/v)      | 0.2 - 2.0mL    | 1.2 mL                                        |
| Allopurinol solution required        | 250ppm         | 5-35 ppm       | 0.5mL                                         |
| Effect of volume of required         | M              | 0.5-10mL       |                                               |
| Bath temperature                     |                | 30-70°C        | 15 min on 50°C                                |

The optimum conditions for the proposed procedure were summarized in Table (13) and were used in all subsequent experiments.

3.2.13. Preparation of Calibration Curve in CPE

Amount of 10ml solution is prepared containing increasing concentration of drug allopurinol by taking \((5.0-35.0) \mu g \text{ mL}^{-1}\) allopurinol, 0.8 mL Co(II), 0.6 mL buffer pH 11, and 1.2mL10%(v/v)Triton X-114] then it is completed to the mark by distilled water. Solutions were mixed and heated at optimum temperature in the thermostat water bath at optimum incubation time to maintain cloud point. The aqueous phase is separated from the rich phase layer by centrifuging at 4000 rpm for 10min, 1mL ethanol is added to the surfactant-rich phase and measured at \(\lambda_{\text{max}}\) 586 nm for ALL-CoII complex. Plotting the absorbance values of the cloud point versus the concentration of ALL-CoII complex is explained in Figure (15)
Figure 15: (allopurinol-CoII) Calibration Curve

Linear calibration graph is established by plotting absorbance versus concentration of allopurinol in Figure (15) to determine the Complex allopurinol with cobalt by CPE technique and it is found that the extent (5.0-35.0) μg ml⁻¹ obeys the Beer law and then gets a negative deviation from the law, the molar absorption coefficient of product equals (1.203×10⁻⁴ L.mol⁻¹.cm⁻¹) and Sandall's sensitivity (1.1314×10⁻⁸ μg.cm²) from equations that mentioned in clause (3.1.5) by taking ten replication of blank solution against distilled water with drop of ethanol. LOD in (0.392191 μg ml⁻¹) and LOQ in (1.307305 μg ml⁻¹) for (Allo-CoII) complex. The results of statistical data analyses of the CPE determination of Complex by the proposed method are tabulated in table (14).

Table (14) Analytical Data Analysis of the CPE of allopurinol

| Parameter                          | Complex (allopurinol-Co II) |
|------------------------------------|-----------------------------|
| Color of product                   | Mauve                       |
| Wave length λ_max (nm)             | 586                         |
| Concentration rang (μg ml⁻¹)       | (5.0-35.0 μg ml⁻¹)          |
| Regression equation                | y=0.0657 x +0.0141          |
| Correlation coefficient(r)         | 0.9997                      |
| Correlation coefficient (r²)       | 0.9995                      |
| Variation coefficient (%)          | 99.95                       |
| Limit of Detection (μg ml⁻¹)       | 0.392191                    |
| Limit of Quantitation(μg ml⁻¹)     | 1.307305                    |
| Sandell's sensitivity (μg cm⁻²)    | 1.1314×10⁻⁸                 |
| Slope (m)                          | 0.0657                      |
| Intercept (C)                      | 0.0141                      |
| Molar absorptivity(L.mol⁻¹.cm⁻¹)   | 1.203×10⁻⁴                  |

3.3. Applications of the Cloud Point Extraction on Pharmaceuticals

CPE has been applied on pharmaceutical allopurinol, the manufacture company [GlaxoSmithKline pharmaceuticals S.A., Poznan, Poland] that contains (300mg) from
allopurinol. Acceptable results with high reliability in the analysis of samples were obtained in the pharmaceutical preparation. The results are summarized in the table (16).

**Table 15. Data for Determination (Allo-CoII) complex in the Pharmaceutical Preparation**

| Amount of allopurinol (ng ml⁻¹) | *Found* | Recovery% | Average Recovery% | Erel% | Average Erel% |
|---|---|---|---|---|---|
| 5 | 4.59 | 91.9 | | -8.2 | |
| 10 | 8.78 | 87.8 | 91.65 | -12.2 | |
| 15 | 14.29 | 95.27 | | -4.73 | |

[*]= Average of three determinations

The proposed method is also applied on table allopurinol 100mg the manufacture company is [Novartis]. As (Allo-CoII) complex from drug contains (100mg) allopurinol we get good and high reliability results that are summarized in the table (16) for allopurinol by CPE.

**Table 16. Data for Determination (Allo-CoII) complex in the Pharmaceutical Preparation**

| Amount of allopurinol (ng ml⁻¹) | *Found* | Recovery% | Average Recovery% | Erel% | Average Erel% |
|---|---|---|---|---|---|
| 5 | 4.22 | 84.59 | 85.88 | -15.6 | |
| 10 | 8.30 | 83.08 | | -17 | |
| 15 | 13.49 | 89.99 | | -10.0 | |

[*]= Average of three determinations

3.4. **Calculation of the stability constant (K) of complex**

The conditional or apparent stability constant of the 1:1 (Drug and metal) product was evaluated and described as shown. Complete founding the stability constant [K] colored product Formed imputation of (metal:drug) as followed: A series of solution were prepared containing three different concentration of metal and allopurinol (1:1) and the concentration (1.2 ×10⁻⁵) molL⁻¹ for (Cobalt - allopurinol) when Formed imputation under this Condition easily to Hydrolysis and the Intensity Absorption was very low. Another series of solution was prepared containing three deferent concentration of metal and allopurinol but with abundance of the metal (the best concentration) The complex was prepared with no decomposition express of the intensity absorption Aₘₙ and application the relationship the value degree of decomposition can be calculated as follows (α):

\[ \alpha = \frac{A_m}{A_m} \]
Stability constant \([K]\) as follows; and calculated the

\[
S + R \rightarrow SR
\]

\[
\alpha c \quad \alpha c \quad (1 - \alpha)c
\]

\[
K = \frac{[SR]}{[S][R]}
\]

\[
K = \frac{(1 - \alpha)c}{(\alpha c)(\alpha c)} = \frac{1 - \alpha}{\alpha^2 c}
\]

Where: \(K\); stability constant

\(C\); the concentration of the product complex .and it equivalence the concentration of allopurinol .are shown in Table (17)

| Vol. of allopurinol | Absorbance at \(\lambda_{\text{max}}\) 586nm | \(K\) (Average) |
|---------------------|------------------------------------------|-----------------|
|                     | \(A_s\) | \(A_m\) | \(\alpha\) | \(\text{L.mol}^{-2}\) |
| 0.3                 | 0.230  | 0.296  | 0.2229 | 4.4939          |
| 0.5                 | 0.581  | 0.625  | 0.0704 | 1.42×10^{-5}    |
| 0.7                 | 0.790  | 0.864  | 0.0856 | 1.170×10^{-5}   |

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