Spectrophotometric Determination of Allopurinol in Tablet Formulation

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Abstract: *A new spectrophotometric method for the determination of allopurinol drug was investigated. The proposed method was based on the reaction of the intended drug with catechol and Fe(II) to form a blue soluble complex which was measured at λ_{max} 580 nm. A graph of absorbance versus concentration shown that Beer's law was obeyed over the concentration range of 2–10 μg ml^{-1} with molar absorptivity of 9.4 x 10^3 l mol^{-1} cm^{-1} and Sandell sensitivity of 1.4 x 10^{-2} μg cm^{-2}. A recovery percentage of 100% with RSD of 1.0%–1.3% was obtained. The proposed method was applied successfully for the determination of allopurinol drug in tablets with a good accuracy and precision. The optimum condition for the color development has also been investigated.*

Keywords: allopurinol, spectrophotometric methods, tablet

1. INTRODUCTION

Allopurinol (1H-pyrazolo[3,4-d]pyrimidin-4-ol) (Fig. 1) is a commonly used drug in the treatment of chronic gout or hyperuricaemia associated with leukaemia, radiotherapy, antineoplastic agents and treatment with diureticsconditions.[1] Allopurinol is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and acts to inhibit xanthine oxidase. In the presence of xanthine oxidase, allopurinol will be converted to alloxanthine (Fig. 2), after that the formation of uric acid from xanthine and hypoxanthine will be inhibited.

This enzyme is responsible for the successive oxidation of hypoxanthine and xanthine resulting in the production of uric acid, the product of human purine metabolism.[2] In addition to block uric acid production, inhibition of xanthine oxidase causes an increase in hypoxanthine and xanthine, which are converted to closely related purine ribotides adenosine and guanosine monophosphates. Increased levels of these ribotides causes feedback inhibition of

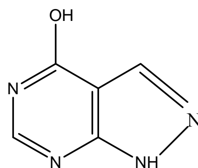

Figure 1: Chemical structure of allopurinol ($C_5H_4N_4O$).

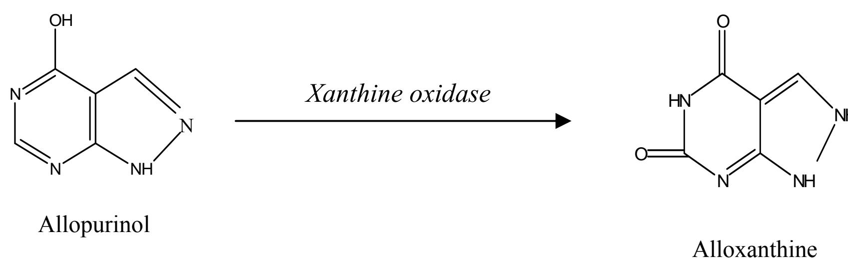

Figure 2: Inhibition of uric acid production.

amidophosphoribosyl transferase, the first and rate-limiting enzyme of purine biosynthesis. Allopurinol therefore decreases both uric acid formation and purine synthesis.[3]

Many methods had been carried out for the quantitive analysis of allopurinol such a titration method[4] (with linearity 2–16 μg ml^{-1}), spectrophotometric method[5] by using dithizone and mercuric nitrate, and measure the absorbance at 494 nm, the linearity was 0.2–1.2 μg ml^{-1}, but this method needs large amounts of chemicals and many steps to prepare standard solutions before measurement. Several chromatographic techniques, such as HPLC using UV detection[6,7] and capillary electrophoresis.[8,9] However, the published methods are not always ideal for practical purposes, because they are either unsuitable in the presence of some compounds such as oxypurinol or time-consuming, i.e. they contain a derivatization step, involve arduous sample preparation and need long chromatographic run times. Flow injection technique was also developed for the allopurinol analysis.[10] Allopurinol was determined in pharmaceutical preparations by using Differential Pulse Polarography but this method cannot get good recovery when applied in real samples.[11]

Very few spectrophotometric assays were reported for allopurinol detection so far. The proposed method is based on the reaction of allopurinol with catechol reagent and Fe(II) to form a blue soluble complex which was measured at λ_{max} 580 nm. The method was applied successfully for the determination of allopurinol in pharmaceutical preparations.

2. EXPERIMENTAL

2.1 Apparatus and Chemical Materials

The spectrophotometer model was Double Beam UV-Visible Recording Spectrophotometer (Shimadzu, UV-260). Ammonium ferrous sulphate was supplied by BDH Company (UK), catechol reagent from SCL Company (UK), potassium hydroxide from Merck (Germany) and allopurinol standard was purchased from Samara Drug Company (Iraq).

2.2 Preparation of Standard Solutions

A quantity of 400 ppm allopurinol standard solutions was prepared by dissolving 0.2 g in 0.1 M potassium hydroxide and completed the volume to 500 ml by deionized water. Catechol and ammonium ferrous sulphate solutions (7×10^{-3} M) were prepared directly by dissolving suitable amounts of reagents in deionized water.

2.3 Determination of Allopurinol in Pharmaceutical Drug

Ten tablets were weighed, ground and mixed in a mortar then this powder was sieved. A quantity of 0.25 g of the powder was taken and dissolved in 10 ml of 0.1 M potassium hydroxide and diluted to 250 ml with deionized water. The sample was filtered through a Whatman filter paper (No. 4). The concentration was calculated in-terms of ppm. Then a suitable volume was transferred to 25 ml volumetric flask and an appropriate volume of catechol and Fe(II) were added. The volume was completed with deionized water and after 10 min, the sample was introduced to the spectrophotometer.

2.4 Mechanism of the Reaction

The mechanism of the reaction is described as follow: First step, catechol reacts with allopurinol ($C_5H_4N_4O$) to produce catechol anion [Fig. 3(a)] then Fe(II) ion will be oxidized to ferric ion (in the presence of allopurinol cation) which reacts with catechol anion to form a blue color complex [Fig. 3(b)].

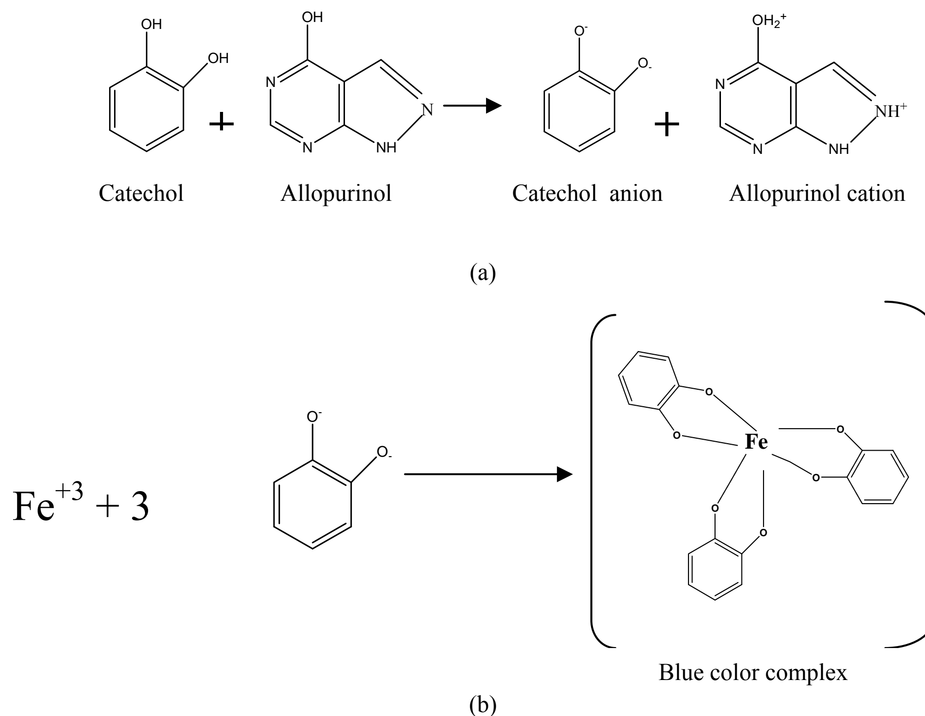

Figure 3: (a) Ionization of catechol in the presence of allopurinol and (b) blue color complex formation.

3. RESULTS AND DISCUSSION

3.1 Optimization of Experimental Conditions

3.1.1 Effect of the order of addition

The effect of the order of addition was studied by preparing three solutions with different addition orders (Table 1). The order No. III was selected because it gave the highest sensitivity.

Table 1: Effect of order of addition [A = 10 ppm allopurinol; C = 7×10^{-3} M catechol; F = 3×10^{-3} M Fe(II)].

Order No.	Reaction Components	Abs.
I	F+C+A	0.700
II	F+A+C	0.379
III	C+A+F	0.750

3.1.2 Effect of the amount of Fe(II) and catechol

In order to select the best volume of Fe(II) and catechol for a good sensitivity, the effect of different volumes of catechol (7 x 10^{-3} M) and Fe(II) (3 x 10^{-3} M) was studied. Figure 4 shows that 2 ml of Fe(II) and 3 ml of catechol gave a good intensity for the complex formation.

3.1.3 Effect of the time on the stability of complex

The effect of time on the reaction and stability of the allopurinol complex were also studied. Figure 5 shows that the high intensity can be obtained after 10 min from the beginning of reaction and the complex color was stable up to 2 h. Thus, 10 min was selected as a waiting time in this study.

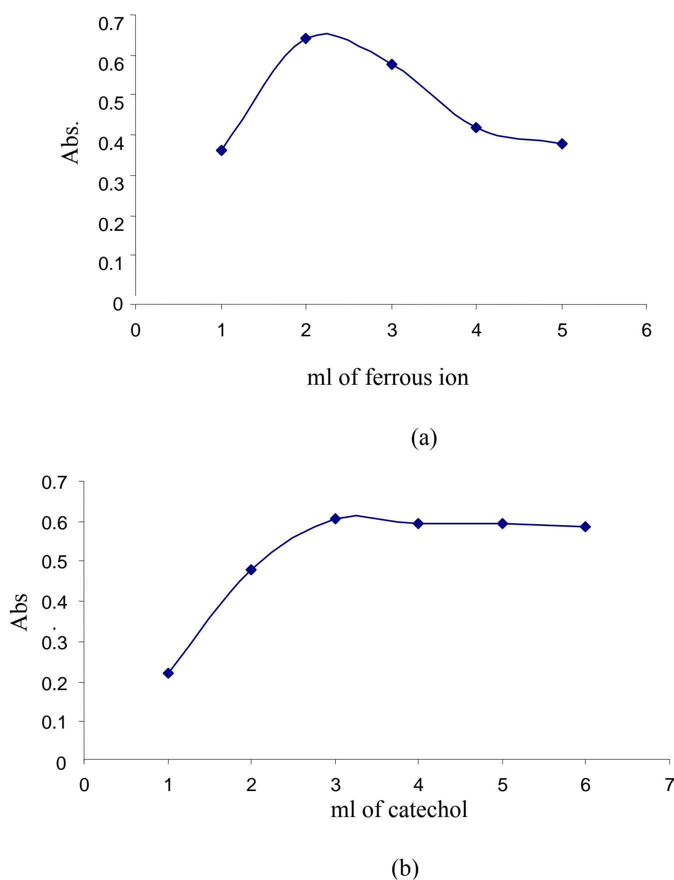

Figure 4: Effect of volume of (a) ferrous ion (3 x 10^{-3} M) and (b) catechol (7 x 10^{-3} M).

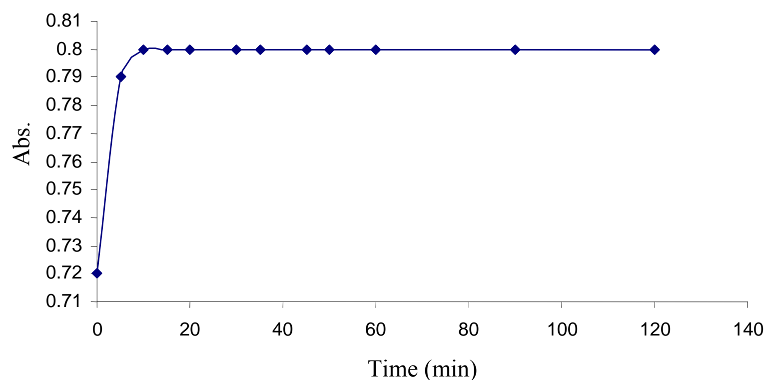

Figure 5: Effect of the time on the stability of complex.

3.1.4 Calibration graph for allopurinol

The calibration curve was prepared by transferring aliquot volume of allopurinol standard solutions (2–10 ppm) to 25 ml volumetric flask, 3 ml of catechol reagent (7 x 10^{-3} M) and 2 ml of 3 x 10^{-3} M Fe(II) solution were added. The volume was completed by deionized water. A good linearity was obtained between absorbance and analyte concentration with good correlation coefficient of 0.9991 as shown in Figure 6. Table 2 shows the results of recovery and RSD % for the two concentrations selected. The results showed that spectrophotometry method produced a high precision, good recovery (~100%) with low RSD (1.0%–1.3%) in determintaion of allopurinol.

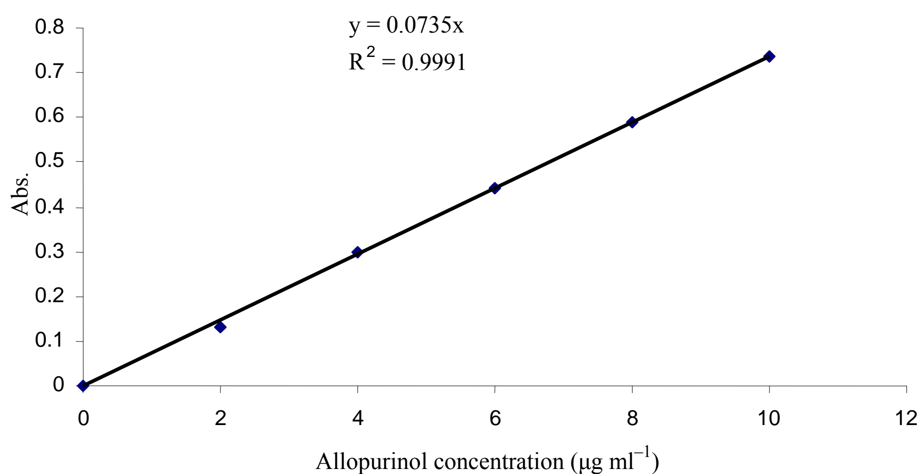

Figure 6: Calibration graph for allopurinol (2–10 μg ml^{-1}).

Table 2: The accuracy and precision of the proposed method.

Allopurinol *µg ml^{-1}				
Added	Obtained	SD	%RSD	%Rec.
4	4.01	0.0052	1.3	100.25
8	8.00	0.008	1.0	100.00

* mean value of n = 5

3.2 Determination of Allopurinol in the Pharmaceutical Formulation (Tablet)

The present study has a great potential for a sensitive and rapid determination of allopurinol in pharmaceutical formulation. Thus, the developed method was applied for the analysis of allopurinol in tablet (obtained from Highnoon Company, Iraq) containing 100 mg of allopurinol. The determination was carried out for five times with different concentrations. The calculation was done by using two methods, i.e. matching method and linear equation of calibration graph, and the results obtained are summarized in Table 3. The results obtained from matching method gave a good recovery (98.2%–102.5%) compared to the graph method (94.3%–105.3%). Therefore, matching method for the determination of allopurinol is more successful which exhibit a good sensitivity and high efficiency.

4. CONCLUSION

A rapid, cheap, reliable and simple spectrophotometric method for the quantitive determination of allopurinol in tablets formulation was developed. The proposed method can be carried out at room temperature without solvent extraction step or pH control, and can be achieved within 10 min at λ_{max} 580 nm with a good accuracy and precision.

Table 3: Determination of allopurinol in tablet.

µg of allopurinol* present	Matching method drug content		Calibration curve drug content		%RSD
	% added	% Rec.	% added	% Rec.	
50.4	49.5	98.2	47.5	94.3	2.00
75.6	77.5	102.5	79.06	104.5	0.82
100.8	101.1	100.2	106.15	105.3	1.00

* mean value of n = 5

5. REFERENCES

1. Ruiz, T.P., Lozano, C.M., Tomas, V. & Martin, J. (2003). Determination of allopurinol by micelle-stabilised room temperature phosphorescence in real samples. *Journal of Pharmaceutical and Biomedical Analysis*, 32, 225–231.
2. Nuki, G. (2006). Metabolic and genetic arthropathies. *Medicine*, 34(10), 417–423.
3. Ahmed, M.Z., Azab, K.S. & Abbady, M.I. (2006). Egypt. *J. Rad. Sci. Applic*., 19(2), 373–388.
4. Hassib, S.T., Safwat, H.M. & Elbagry, R.I. (1986). Spectrophotometeric determination of some anti-inflammatory agents using N-bromosuccinimide. *Analyst*, 111(1), 45–481.
5. Guven, K.C. & Ozol, T. (1980). Spectrophotometeric determination of allopurinol. *Sci. Pharm*., 48(1), 80–82.
6. Reinders, M.K., Nijdam, L.C., Roon, E.N., Movig, K.L.L., Jansen, T.L.A., Laar, M.A.F.J. & Brouwers, J.R.B.J. (2007). A simple method for quantification of allopurinol and oxipurinol in human serum by high-performance liquid chromatography with UV-detection. *Journal of Pharmaceutical and Biomedical Analysis,* 45, 312–317.
7. Tada, H., Fujisaki, A., Itoh, K. & Suzuki, T. (2003). Facile and rapid high-performance liquid chromatography method for simultaneous determination of allopurinol and oxypurinol in human serum. *Journal of Clinical Pharmacy and Therapeutics,* 8, 229–234.
8. Sun, X., Cao, W., Bai, X., Yang, X. & Wang, E. (2001). Determination of allopurinol and its active metabolite oxypurinol by capillary electrophoresis with end-column amperometric detection. *Analytica Chimica Acta*, 442, 121–128.
9. Ruiz, T.P., Lozano, C.M., Tomás, V. & Galera, R. (2003). Development of
a capillary electrophoresis method for the determination of allopurinol and its active metabolite oxypurinol. *Journal of Chromatography B*, 798, 303–308.
10. Zen, J.M., Chen, P.Y. & Kumar, A.S. (2002). Flow injection analysis of allopurinol by enzymeless approach at glassy carbon electrodes. *Electroanalysis*,14(10), 645–649.
11. Ghatten, L.G., Pons, B. & Madan, D.K. (1981). Determination of allopurinol in tablets by Differential-pulse Polarography. *Analyst*, 106(1260),365–368.