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ORIGINAL ARTICLE

Spectrophotometric Determination of Allopurinol by Oxidative Coupling Reaction Using 2-Nitrophenol Reagent in the Presence of N-Bromosuccinimide

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ABSTRACT

This research included the development of a fast, easy and sensitive spectrophotometric method for the determination of quantities of allopurinol. The method depends on the oxidative coupling reaction between allopurinol and the reagent 2-nitrophenol in the presence of N-bromosuccinimide, so the product is a yellow color soluble in water that gives the highest absorption at the wavelength 420 nm and it follows Beer's law In a range of concentrations ranged from 24-2.4 µg/ml and the molar absorbance value was 9282.02 liters. ml-1.cm-1, the value of the correlation coefficient is R = 0.9963 and the Sandell index value has 0.0146 µg/cm2, the relative standard deviation value is 1.273 RSD%, and the detection limit is 0.6635 µg/ml. The proposed method has been successfully applied in the determination of allopurinol in its preparation. Pharmacist

keywords: Allopurinol, oxidative conjugation, 2-Nitrophenol

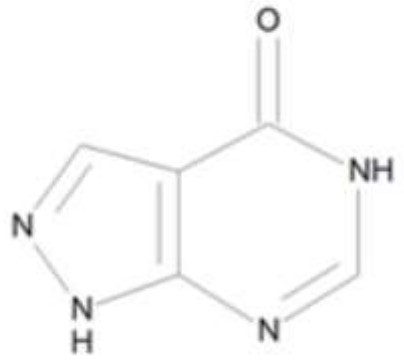
INTRODUCTION

Oxidative Coupling Reactions: The oxidative coupling reaction is one of the most important organic reactions with wide applications, especially in analytical chemistry, and the reaction involves the coupling of two organic substances with the presence of an oxidizing agent under the appropriate reaction conditions, where oxidation of these substances occurs, which leads to the formation of multiple compounds that interact with each other and give a colored product that can be measured spectroscopically. They are used in the quantification of many different organic compounds [1,2] and the oxidative coupling reactions that are electrogenic [4,3] or fluorescent [5] have been widely exploited in the estimation of important compounds, food, environment, clinical and pharmaceutical analyzes by applying different analytical techniques such as Spectroscopic methods [7,6], fluorescence and chromatographic methods [8], and flow injection technique [9]

Allopurinol: It is a white powder, molecular formula $C_5H_4N_4O$ and molecular weight 136.1 g/mol, slightly soluble in water and ethanol.

Scientific name: 1,5-Dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one

It is a drug used to treat gout and kidney stones by reducing the production of uric acid (responsible for causing gout and kidney stones if it increases in the human body and is taken orally only and is available in the form of tablets with commercial names Hyporic, Zyloric [10] and is found in pharmaceutical preparations in the form of tablets and syrup [11]



The practical part

Devices used: The following devices were used for measurements.

• Ultraviolet and visible absorption spectrometer.
Shimadzu UV-visible spectrophotometer UV-1800

• Sensitive balance KERN ACS120-4 .
glass cells.

Chemicals used

Chemicals were used as shown in the table(1)

Chemicals	Chemical formula	Assay
Allopurinol	$C_5H_4N_4O$	Pure
2-Nitrophenol	$C_6H_6NO_3$	Pure
N-Bromosuccinimide	C_4H_4BrNO	Pure

Preparation of solutions of used chemical

Standard solution of allopurinol 1000 µg/mL (7.346 x 10-3 molar): Prepare by dissolving 0.1000 g of pure allopurinol in a small amount of 1 M NaOH and then fill the volume with distilled water to the mark in a 100 ml volumetric vial.

Allopurinol solution 300 µg/ml (2.204 x 10-3 molar): Prepare by withdrawing 30 ml of the prepared standard allopurinol solution at a concentration of 0100 µg/ml, then put it in a 100 ml volumetric vial and fill the volume with distilled water up to the mark.

N-bromosuccinimide solution 10^{-2} M: This solution was prepared by dissolving 0.1779 g of N-bromosuccinimide in 5 ml acetone, and the volume was filled to 100 ml of distilled water in a 100 ml volumetric vial and kept in an opaque vial to prevent it from being affected by light.

Nitrophenol solution at a concentration of 10^{-2} molarity: Prepare by dissolving 10.139 g of the pure substance in 5 ml of ethanol, then complete the volume to the mark in a 100 ml volumetric vial with distilled water.

Pharmaceutical solution at a concentration of 300 µg / ml (Allopurinol tablets 100 mg produced by Bristol Company): The contents of 10 tablets 4.30 g were carefully weighed and after crushing and mixing, 0.430, equivalent to 0.1000 g of pure allopurinol, was taken and dissolved in a small amount of ethanol with stirring. The solution was filtered and washed several times. The filtrate was completed with distilled water up to the mark in a volumetric vial. 100 ml. Take 30 mL of the prepared solution and dilute in a 100 mL volumetric vial. Complete with distilled water to the mark to prepare a solution of 300 µg/mL.

RESULTS AND DISCUSSION:

The principle of the method: The principle of the method is the conjugation of allopurinol 2-nitrophenol with the presence of the oxidizing agent N-bromosuccinimide, resulting in a yellow-colored product that gives the highest absorption at the wavelength of 420 nm compared to the mock solution.

Preliminary Tests: 1 ml of 10^{-2}M N-bromosuccinimide was added to 1 ml of allopurinol solution at a concentration of 300 µg/ml, then 1.4 ml of 2-nitrophenol reagent at a concentration of 10^{-2}M was added. A yellow product was observed, then diluted with distilled water to the mark in a 25 ml volumetric vial. The absorption

spectrum of the solution was measured after minutes, as it gave the highest absorption at the wavelength of 420 nm compared to the mock solution.

Study of the optimum reaction conditions: For the purpose of obtaining the highest sensitivity of the method, the optimal conditions that give the highest absorption of the product were studied and selected. Measurements were made using 25 volumetric vials and 1 cm glass cells.

Choosing The Best Reagents: 1.4 ml of each of the used reagent solutions with a concentration of 10^{-2}M was taken and added to 25 ml volumetric vials each containing 1 ml of allopurinol at a concentration of 300 µg/ml and 1 ml of N-bromosuccinimide 10^{-2} M, then the volume was completed. With distilled water up to the mark. The absorption spectrum of each sample was measured against its mock solution after 15 minutes, and the results are shown in Table (2).

Table 2: Effect of reagent type

Reagent 10^{-2} M	Chemical formula	λ_{max} (nm)	Absorbance
2-nitrophenol	$C_6H_6NO_3$	420	0.780
P-Ansidine	C_7H_9NO	447	0.252
2-Aminobenzothiazole	$C_7H_6N_2S$	550	0.0091
1,4- diamine benzene	$C_6H_8N_2$	370	0.013
4-Amino-2 hydroxy benzoic acid	$C_7H_7NO_3$	360	0.342

It was found that the best conjugation reagent is 2-nitrophenol, the wavelength is 420 nm, so it was used in subsequent experiments.

Effect of Reagent Quantity: The effect of the amount of conjugation reagent was studied by taking different volumes (0.2-1.4 ml) of 10^2 M metol solution with different volumes (0.5, 1, 1.4 ml) of 300 µg/ml allopurinol solution in the presence of 1 ml of N-Bromosuccinimide in a final volume of 25 ml and the absorbance was measured at 420 nm wavelength after 15 minutes and the results are shown in Table (3).

Table 3: Effect of reagent quantity

ml of Reagent 10^{-2} M	Allopurinol of 6 µg/ml		Allopurinol of 12 µg/ml		Allopurinol of 16.8 µg/ml	
VmL	SB	SW	SB	SW	SB	SW
0.2	0.301	0.364	0.313	0.318	0.229	0.232
0.4	0.320	0.414	0.435	0.563	0.578	0.621
0.6	0.382	0.420	0.634	0.656	1.142	1.123
0.8	0.408	0.527	0.655	0.762	1.174	1.278
1	0.326	0.446	0.648	0.747	1.329	1.439
1.2	0.358	0.519	0.758	0.902	1.381	1.409
1.4	0.392	0.512	0.780	0.913	1.392	1.430

It was noticed from the results shown in Table (3) above that a volume of 1.4 ml of 2-nitrophenol solution at a concentration of 10^{-2}M gave the highest value for absorption, so it was adopted in subsequent experiments.

Choosing the best oxidizing agent: Several oxidizing agents were used at a concentration of 10^{-2} M, added to 1 ml of allopurinol at a concentration of 300 µg/ml, then 1.4 ml of 2-nitrophenol at a concentration of 10^{-2} M was added, and the volume was filled with distilled water up to the mark in a volumetric vial of 25 ml. The absorption spectrum was measured after 15 minutes for each sample against its mock solution, and the results are shown in Table (4).

Table 4: The effect of the oxidizing agent on absorption

Oxidizing agent 5×10^{-3}	Chemical formula	λ_{max} (nm)	Absorbance
Potassium periodate	KIO_4	363	0.089
Potassium iodate	KIO_3	421	0.678
Ammonium ferric sulphate	$NH_4Fe(SO_4)_2.H_2O$	416	0.362
N-Bromosuccinimide	C_4H_4BrNO	420	0.780
Ammonium Sulfate	NH_4HSO_4	365	0.008

It was found that the best oxidizing agent is N-bromosuccinimide, so it was used in subsequent experiments.

Effect of the amount of oxidizing agent: The effect of the oxidizing agent amount of 10-2 molar N-bromosuccinimide solution was studied by adding different volumes of it (0.5 – 2.5 ml) to a 25 ml volumetric vial containing 1 ml of allopurinol with a concentration of 300 µg/ml, and then Add 1.4 ml of 2-nitrophenol reagent solution and complete the volume to the mark with distilled water After 15 minutes, the absorption spectrum of the solutions was measured at 420 nm wavelength, and the results are shown in Table (5).

Table 5: Effect of the amount of oxidizing agent

ml, of NBS 10^{-2} M	Absorbance
0.5	0.614
1	0.775
1.5	0.765
2	0.748
2.5	0.713

It was noted from the results that the best volume that gives the highest absorption of the stained product is 1 ml of N-bromosuccinimide.

Effect of the PH function: 1-6-3-3 Effect of the acid: This study was carried out using different volumes of both strong and weak acids (hydrochloric acid, sulfuric acid and acetic acid) with different concentrations, all of which work on the disappearance of color, so the use of acid was excluded.

Effect of the base :

The effect of the base was studied using different concentrations and sizes of each of sodium hydroxide and ammonia, each of which works to turbid the solution, so it was excluded.

The sequence of additions: The effect of changing the sequence of adding the reaction materials on the absorption of the colored product was studied by conducting a number of experiments. It was found that changing the addition sequence affects the absorption of the colored product and the sequence No. 1 was adopted as shown in Table (6).

Table 6: the sequence of additions

Order number	Order of addition	Absorbance
1	D+O+R	0.780
2	D+R+O	0.620
3	R+O+D	0.579
4	O+D+R	0.775

So, D: the substance to be determined, R: the reagent, O: the oxidizing agent.

The effect of temperature: The effect of temperature on the absorption of the colored product was studied according to the reaction conditions using temperatures from 15-50 ºC at a wavelength of 420 nm and the results are shown in Table (7).

Table 7: Effect of temperature

Temperature C°	Absorbance
15	0.750
20	0.763
25	0.775
30	0.780
35	0.789
40	0.746
45	0.739
50	0.732

From the above table it can be seen that changing the temperature does not affect the absorption of the colored product, so the work was done at the laboratory temperature.

Effect of solvents: The effect of some solvents on the absorption of the colored product from the reaction of allopurinol with 2-nitrophenol in the presence of N-bromosuccinimide was studied. After completing the additions, the volume was completed to 25 ml

with different solvents. The absorption spectrum was recorded after 15 minutes for each solution versus its sham solution. The results are recorded in Table (8) and Figure (1).

Table 8: Effect of solvent

Solvent	λ_{max} (nm)	Absorbance
Ethanol	419	0.176
Methanol	434	0.104
Acetone	426	0.989
Water	420	0.595

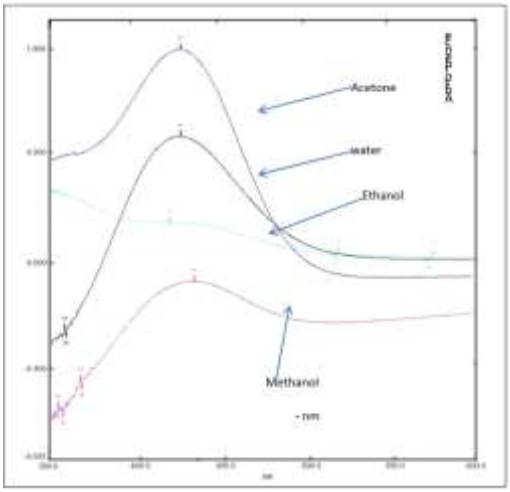


Figure 1: Effect of solvents

It is clear from the results in the above table that acetone gives the highest absorption of the resulting solution compared to other solvents, but it is preferable to use water for its abundance and cheapness, and for this reason, distilled water was used in subsequent experiments.

Stability of the reaction product: This study was carried out by taking three different volumes (1.4, 1, and 0.5 ml) of a solution of allopurinol with a concentration of 300 µg/ml, their final concentrations being 16, 12 and 6 µg/ml, respectively, adding 1 ml of N-bromosuccinimide with a concentration of 10^{-2}M, and adding 1.4 mL of 2-Nitrophenol 10^{-2}M solution into a 25 mL volumetric vial and fill the volume to the mark with distilled water. The absorbance of each sample was measured against its mock solution, and the results are shown in Table (9). It was found that the formation of the product is completed after 15 minutes and remains stable for at least 75 minutes, which is a sufficient period for measurements.

Table 9: stability of product

Time (min.)	Absorbance of Allopurinol		
	µg/ml 6	12 µg/ml	16 µg/ml
0	0.267	0.632	0.822
5	0.329	0.741	0.861
10	0.346	0.765	0.880
15	0.353	0.778	0.879
20	0.352	0.780	0.876
25	0.351	0.783	0.873
30	0.352	0.779	0.882
35	0.350	0.775	0.880
40	0.356	0.770	0.884
45	0.362	0.773	0.854
50	0.362	0.763	0.850
55	0.357	0.760	0.853
60	0.352	0.741	0.861
65	0.350	0.739	0.860
70	0.343	0.730	0.859
75	0.341	0.729	0.853

Final absorption spectrum: After stabilization of optimal conditions, use 1 mL of 10^{-2}M N-bromosuccinimide and 1.4 mL of a 2-nitrophenol solution of 10^{-2} M and complete the volume to the mark in a 25 mL volumetric vial containing 1mL 300 allopurinol.µg/ml, the absorption spectrum of the colored product was measured after 15 minutes, and it was found that the wavelength of the highest absorption is 420 nm, as shown in Figure (2). Table (10) Optimum conditions for the reaction

Table 10: Optimum conditions for the reaction

2-nitrophenol	10^{-2} M, 1.4 ml
N-bromosuccinimide	10^{-2} M , 1 ml
wave length	420 nm
Reaction completion time	15min
add sequence	R+O+D

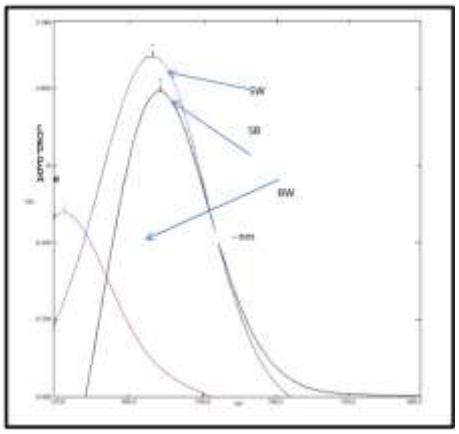


Figure 2: final absorption spectrum of allopurinol as that

SW represents the absorption spectrum of allopurinol solution versus distilled water

SB represents the absorption spectrum of the allopurinol solution versus the mock solution

BW represents the absorption spectrum of the mock solution versus the distilled water

Approved working method and calibration curve: The additions were completed according to the optimal conditions shown in Table (10), as a series of 25-ml volumetric bottles was taken containing volumes of 2-0.2ml of allopurinol solution at a concentration of 300 µg/ml (final concentrations 24-2.4 µg/ml) and the absorption spectrum of the solutions was measured Compared to the mock solution after 15 minutes, and Figures (3) and (4) represent the calibration curve and absorption spectrum, as it was found that the concentrations of Beer's Law (24-2.4)) µg/ml of allopurinol solution, with a correlation coefficient of 0.9806, and the value of the molar absorptivity was calculated and its value was 9282.02 L/mol.cm and Sandell's index value is 0.0146 µg/cm2.

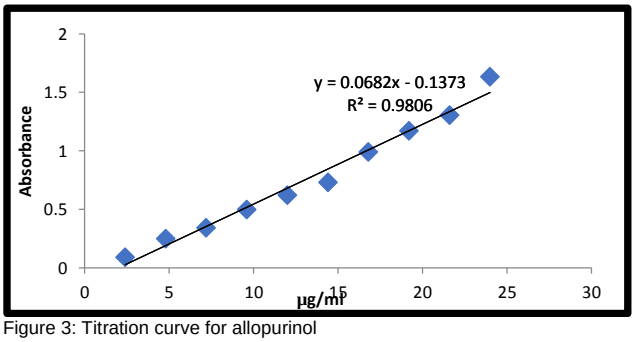


Figure 3: Titration curve for allopurinol

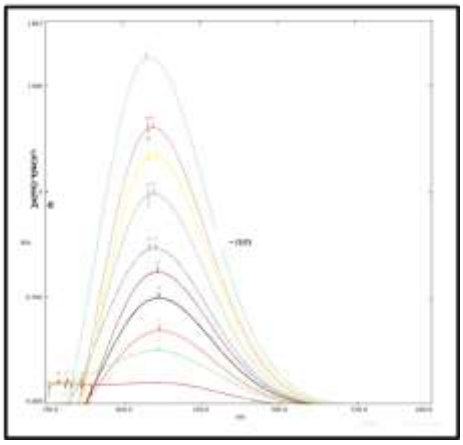


Figure (4) Absorption spectrum of 24-4.2 µg/mL

Accuracy and compatibility: Optimum conditions were used in the working method to test the accuracy and compatibility of the method. Six readings were taken for three different concentrations (19.2, 14.4, 7.2 µg/ml) of allopurinol solution within the limits of Beer's law in the calibration curve, as the recall rate and relative standard deviation were calculated [12] . it was found that the method has high accuracy and concordance as the results shown in Table (11)

Table 11: Accuracy and compatibility

Conc. of Allopurinol (taken) µg/ml	Conc. of Allopurinol (found) µg/ml	RE %	Recovery, %	Average of recovery, %	RSD,%
7.2	7.04	-2.22	97.77	95.35	1.614
14.4	12.73	-11.95	88.40		1.393
19.2	19.18	-0.104	99.89		0.813

Detection limit and quantitative limit: The detection limit and the quantitative limit [13] were calculated by measuring the lowest concentration taken from the calibration curve of 2.4 µg/ml six times and under optimal conditions. The results are shown in Table (12), as the detection limit was 0.6635 µg/ml and the quantitative limit was 2.211 µg/ml.

Table 12: detection limit and quantitative limit

Conc. of Allopurinol	$\overline{X}$	S	D.L µ/ml	LOQ µ/ml
2.4	0.102	0.0094	0. 6635	2.211

So, **D.L:** Detection limit , **LOQ**: Limit of Quantity, and **Conc. Of Allopurinol**: the lowest concentration of allopurinol in the titration curve and $\mathbf{X}^{-}$: the average concentration of six readings.

The nature of the formed product: To find out the nature of the formed product and the ratio of the drug's bonding with the reagent, the two continuous changes methods (Job's method) and the molar ratio method [12] were applied, and in both methods the allopurinol solution and 2-nitrophenol solution are in the same concentration (10^{-2} molar).

In the Job method, different volumes of the drug solution were placed, ranging from 0.5-4.5 ml, and the supplements of these volumes were added to a volume of 5 ml of the reagent solution in a series of 25 ml volumetric vials, then 1 ml of the N-bromosuccinimide solution was added and diluted to the mark. With distilled water, the absorbance of these solutions was measured after 15 minutes at the wavelength of 420 nm. Figure (5) shows that the ratio is 1:1.

As for the molar ratio method, 1 ml of the drug solution was placed in a series of 25 ml volumetric bottles, and the reagent solution was added with volumes ranging from 0.2-1.8 ml, then 1 ml of the N-bromosuccinimide solution of 10^{-2} M concentration was added and the volume was completed to Limit the mark with distilled water and after 15 minutes. The absorption spectrum of these solutions was measured at 420 nm wavelength against the mock solution for each of them, and it was found that the molar ratio agrees with the continuous changes method and achieves the ratio (1:1) as shown in Figure (6).

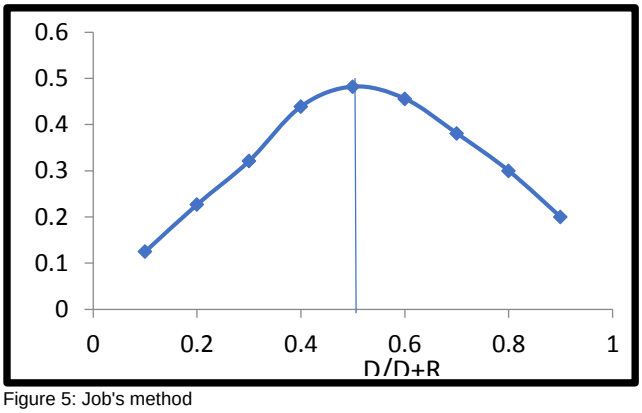


Figure 5: Job's method

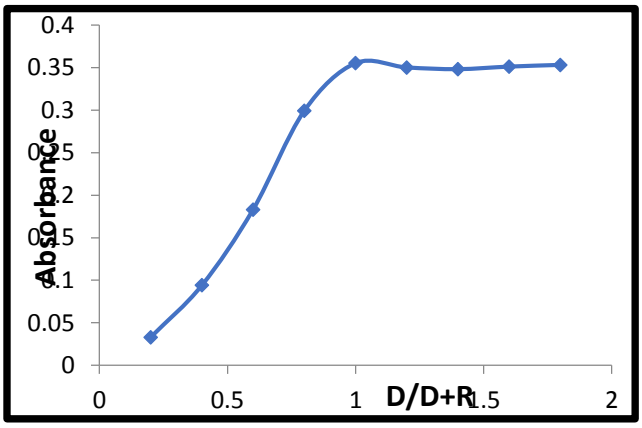
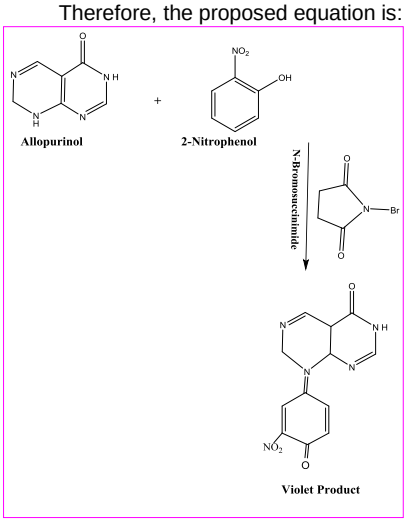


Figure 6: Molar ratio

Therefore, the proposed equation is:



Applications: The method could be applied to pharmaceutical preparations containing allopurinol, which is the pharmaceutical preparation in the form of tablets (Allopurinol tablets 100 mg produced by Bristol Company).

Direct measurement method: Three different concentrations of the preparation solution were taken (7.2, 14.4, and 19.2 µg/ml) and the solutions were treated with the same steps followed when preparing the calibration curve and then the absorbance was measured for it at wavelength 420 nm versus the mock solution, and the average readings were calculated for each concentration in addition to calculating Retrospective results are shown in Table (13).

Table 13: Direct additives for preparation

Type of Pharmaceutical	Conc. of Allopurinol (taken) mg/ml	Conc. of Allopurinol (found) mg/ml	RSD ,%	Recovery ,%	Average Recovery
Tablet	7.2	7.287	2.18	101.2	98.71
	14.4	13.656	2.68	94.83	
	19.2	19.234	1.48	100.1	

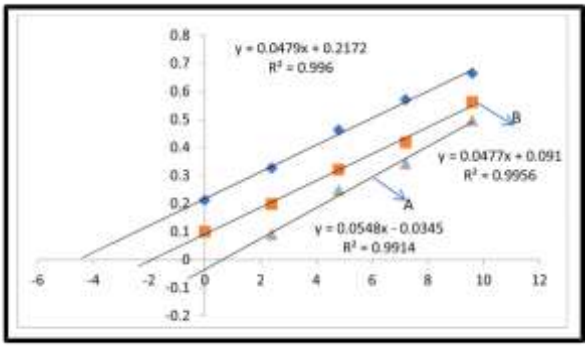
It was found from the above table that the value of the retrospective rate was 98.71 % in the tablets . This indicates the success of the proposed method for the determination of allopurinol in the pharmaceutical preparations containing it.

Standard Addition Method: In order to show that the proposed method above is free from interference, the standard additive method (143) was applied in the determination of allopurinol in its pharmaceutical preparations. The method included adding fixed quantities (0.5, 1 ml) of the solutions of the previously prepared pharmaceutical preparations at a concentration of 300 µg/ml, in two series From 25 ml volumetric bottles, then adding increasing volumes (0.2, 0.4, 0.6, 0.8 ml) of the standard solution of pure allopurinol with a concentration of 300 µg/ml, and the above solutions were treated in the same way as in the calibration curve, where the absorption (rate of Six readings) for each

solution versus its mock solution at the wavelength of 420 nm, and the results are shown in Table (14) and Figure (7)

Table 14: standard additives for the preparation

Type of Pharmaceutical	Conc. of Allopurinol (taken) mg/ml	Conc. of Allopurinol (found) mg/ml	RE,%	Average Recovery,%
Tablet	6	7.2	20	98
	12	9.2	-23	



Figur 7: Standard addition curve for determination of Allopurinol in taplet

The results of the above table show that the standard addition method is in good agreement with the direct method within the acceptable range of error, which indicates that the method is satisfactory.

Comparing the method with other methods: The analytical variables of the current method for the determination of allopurinol were compared with other spectroscopic estimation methods, and Table (15) showed the results of that comparison.

Table 15: comparison of the method with other methods

Analytical Parmeters	Present method	Literature Method [14]	Literature Method[10]
λ_{max} (nm)	420	523	534
Temperature(ºC)	Room temperature	Room temperature	Room temperature
Solvent	Water	Water	Water
Reagent	2-Nitrophenol	Metol	p-aminophenol
Beers Law rang(ppm)	2.4-24	0.8-7.2	8 - 0.8
$L.mol^{-1}.cm^{-1}$)£(	9282.02	1.7×10^4	$\times10^4$1.4
RSD,%	1.27	1.15	0.94- 0.35
Recovery,%	99.998	99.9-100.29	100.09
D.L µ/ml	0. 6635	0.0277	0.050
LOQ µ/ml	2.211	0.09923	0.168

Through the results shown in the above table, it was noted that the proposed method does not need organic solvents in addition to being economical and does not need an extraction process and does not require temperature stabilization and the time period is sufficient to make many measurements, and that its linearity range is wide, with high accuracy and compatibility and with advantages not less than The other methods mentioned above

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