



New Methodology Colorimetric-SFI Analysis for the Determination of Doxycycline Hyclate in Pharmaceutical Preparations

Nawras M. Hassouni^{1*}  and Mohammad K. Hammood² 

^{1,2}Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.

*Corresponding Author.

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Abstract

Doxycycline is one of the tetracycline-class antibiotics used to treat bacteria and protozoa infections. The development method aimed to determine the Doxycycline hyclate in pharmaceutical drugs using a novel, rapid, and sensitive colorimetric-flow injection approach. The adopted method depended on forming a purplish violet colour complex for the doxycycline hyclate-ammonium chloroaurate reagent in an alkali medium, which a homemade SFI Analyzer measured. Both the physical and chemical variables were analyzed and improved. The calibration plot was linear in the 5-70 $\mu\text{g.mL}^{-1}$ for the green light source, with correlation coefficient $r = 0.9976$ using the peak height of green light. The limit of detection was 0.5 $\mu\text{g.mL}^{-1}$ for dilution of the lowest concentration in the linear area of the calibration plot with RSD % less than 1 % of $\mu\text{g.mL}^{-1}$ ($n = 6$) concentration of doxycycline hyclate of the green light source. The technique was effectively used to determine the presence of doxycycline hyclate in pharmaceuticals. The devised approach was tested against the conventional method (ultraviolet (UV) spectrophotometry at 553 nm) and was shown to be more sensitive. Moreover, a t-test was used to contrast "true value" with "practical value". At the 95% confidence level, it was found that the values were not significantly different.

Keywords: Ammonium chloroaurate, Doxycycline hyclate, Flow injection analysis, Pharmaceutical preparations.

1. Introduction

Doxycycline hyclate is a tetracycline antibiotic that is water-soluble and effective against many different types of bacteria, both gram-positive and gram-negative. It is used for the control and treatment of Lyme disease, chlamydia, syphilis, gonorrhea, and other sexually transmitted diseases, as well as the prevention and treatment of malaria, skin infections, and acne (1,2). Additionally, doxycycline hyclate is an excellent medication for treating infectious diseases such as cholera, mycoplasma, tularemia, typhus, and rickettsia (3,4). Tetracyclines,



and doxycycline in particular, have been shown in several trials to possess immunomodulatory effects and help manage inflammation in conditions like rheumatoid arthritis (5,6). Acne vulgaris, rosacea, bullous dermatoses, granulomatous disease, and lupus vasculitis all respond well to treatment with tetracyclines like doxycycline (7,8). The anti-collagenase and anti-matrix metalloproteinase activity of doxycycline hyclate in the gingival crevicular fluid provides a narrow therapeutic window for treating adult periodontal disease. There is no proof that the natural periodontal flora or opportunistic pathogens have changed or are more sensitive to antibiotics (9). The literature provides several analytical methods for the spectrophotometric determination of doxycycline hyclate (10-12), High-performance thin layer chromatography (HPTLC) (13), RP-HPLC, UHPLC (14-18), voltammetric (19), potentiometric sensor (20,21), flow injection techniques (22-25), molecularly imprinted electrochemical sensor (26). This work uses the flow injection-colorimetric method to form a purplish violet colour complex, which is formed by the reaction of doxycycline hyclate with ammonium chloroaurate reagent. This work uses the proposed FIA approach to monitoring doxycycline hyclate in pure and pharmaceutical preparations. The measuring instrument includes a new design for the flow cell and an RGB light source, in addition to using an electronic detector.

2. Materials and Methods

All solutions were prepared using purified water and chemicals of analytical reagent grade. 0.1 g of doxycycline hyclate was dissolved in 100 mL of distilled water to make a typical solution ($0.001835 \text{ mol.L}^{-1}$) of $\text{C}_{24}\text{H}_{33}\text{ClN}_2\text{O}_{10}$ (545 g.mol^{-1} , SDI). 356.8 g of ammonium chloroaurate NH_4AuCl_4 was dissolved in 100 mL distilled water to prepare a stock solution (0.1 mol.L^{-1}). HCl (35%, w/w, 1.19 g.mL^{-1} , BDH, 0.1 mol.L^{-1}), and H_2SO_4 (98%, w/w, 1.84 g.mL^{-1} , BDH, 0.1 mol.L^{-1}) in a stock solution. Pipetting 4.4 mL, 2.724 mL, and 2.88 mL of concentrated acids and completing the volume with distilled water to 500 mL volumetric flasks resulted in the preparation of acetic acid CH_3COOH (99.5% w/w, 1.05 g.mL^{-1} , BDH, 0.1 mol.L^{-1}). Na_2CO_3 (BDH, $105.99 \text{ g.mol}^{-1}$, 0.1 mol.L^{-1}) was used as the standard against which all acids were measured and weighed after being dried in an oven at 115°C for a whole night. Sodium hydroxide NaOH (40 g.mol^{-1} , BDH) was dissolved in distilled water to prepare a 2 mol.L^{-1} stock base solution. Sodium bicarbonate NaHCO_3 ($84.007 \text{ g.mol}^{-1}$, BDH) was dissolved in 2.1 g of distilled water to prepare a 0.1 mol.L^{-1} stock solution.

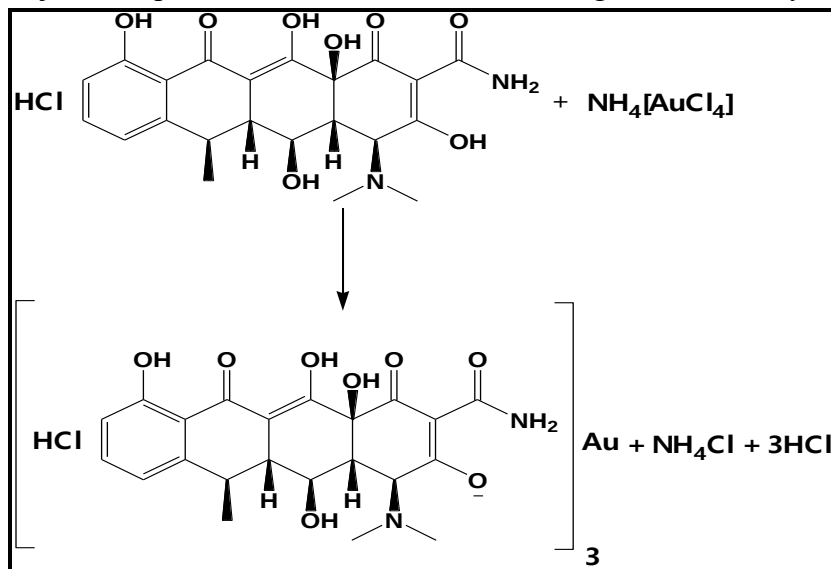
2.1. Apparatus

The peristaltic pump used to determine doxycycline hyclate has two channels with adjustable speeds (Standard peristaltic pump-lone; Zhangqiu district; Jinan; Shandong, China). Medium-pressure injection valve with six ports (IDEX company, USA) and a sample loop of varying lengths (0.7 mm i.d. Teflon). The response was measured. The readout of the system is composed of (Laptop-computers, Dell). The spectrum of doxy was scanned using a 1 cm quartz cell and a digital double-beam UV spectrophotometer made by Shimadzu in Japan (model UV-1800).

2.2. Procedure of flow injection

The determination of doxycycline hyclate in an alkaline medium using the absorbance of light during the reaction of doxycycline hyclate with Ammonium chloroaurate reagent (NH_4AuCl_4 and NaHCO_3 ($20 \text{ }\mu\text{g.mL}^{-1}$)) is described in detail using a flow gram system. **Scheme 1** depicts a possible working mechanism for this process (27). Meanwhile, the manifold design consists of

two distinct lines. The sample segment (doxycycline hyclate, 100 μL , which is carried by the first line at a flow rate of 3 $\text{mL}\cdot\text{min}^{-1}$, meets the ammonium chloroaurate (NH_4AuCl_4 , 20 $\mu\text{g}\cdot\text{mL}^{-1}$), which is loaded by the second line at a flow rate of 3 $\text{mL}\cdot\text{min}^{-1}$, where they meet at a Y-junction point before it enters the measuring cell CFI Analyzer.



Scheme 1. A proposed mechanism for a purplish violet colour complex for the doxycycline hyclate–ammonium chloroaurate (27).

3. Results and Discussion

The physical characteristics (flow rate, sample volume, and reaction coil volume, if necessary) and chemical characteristics (mainly the effect of variable reagents, the concentration of reagent, and the type of carrier stream for the system) of doxycycline hyclate with ammonium chloroaurate reagent were investigated using a two-lines manifold system.

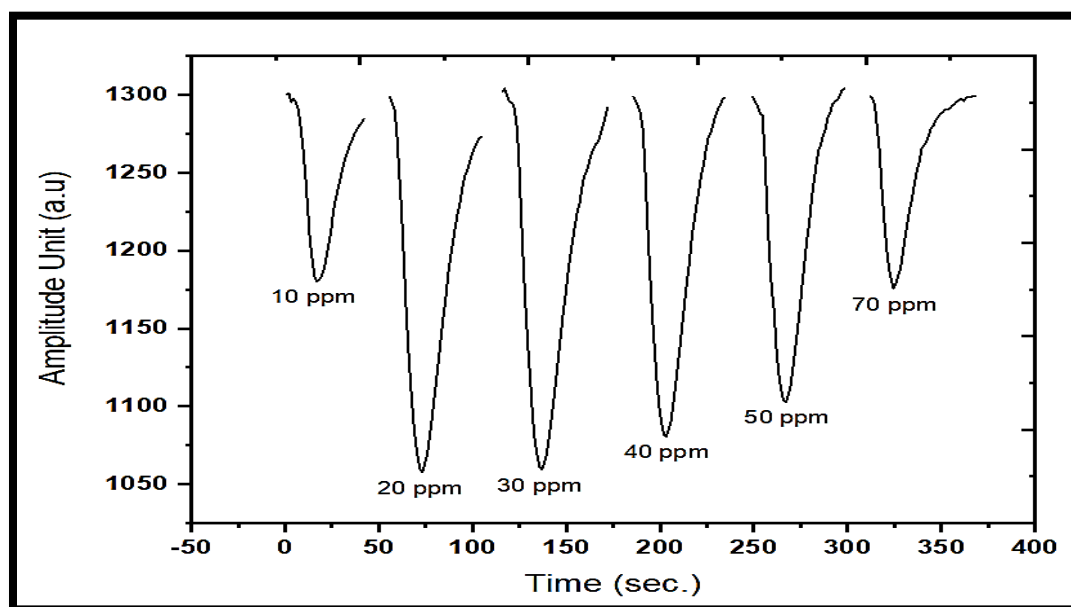
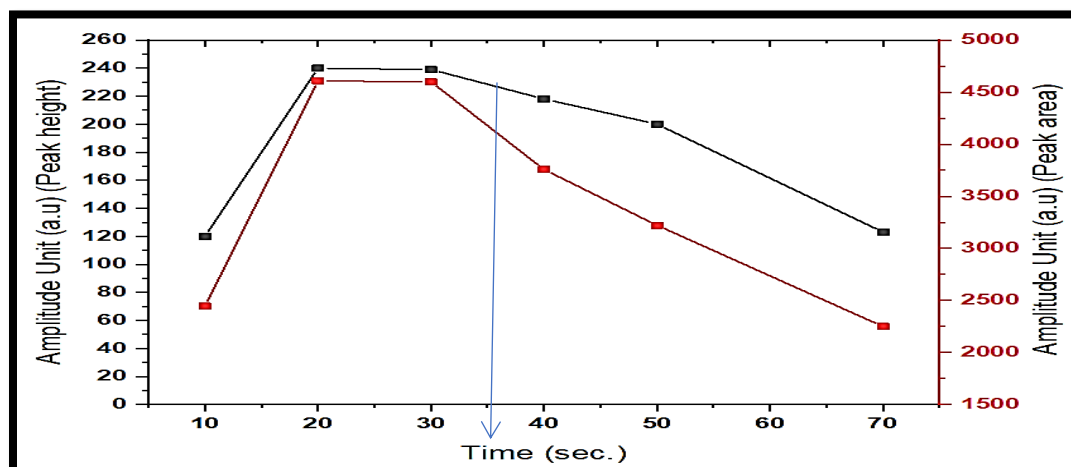
3.1. Chemical variables

3.1.1. The effect of ammonium chloroaurate reagent concentration

The effects of different concentrations of ammonium chloroaurate were tested by a series of solutions ranging from 10- 70 $\mu\text{g}\cdot\text{mL}^{-1}$. Reagents were prepared with a sample volume of 100 μL , and a two-line manifold system was used at 3 mL per min for the carrier stream (NaHCO_3) and ammonium chloroaurate line. Three successive measurements were averaged, and the average, standard deviation, and 95% confidence interval for the average response are summarized in **Table 1**. **Figure 1 (A, B)** shows the plot of the results obtained from the CFIA microphotometer. It was observed that when ammonium chloroaurate concentration increased up to 20 $\mu\text{g}\cdot\text{mL}^{-1}$, the response of the colourful species increased; more than 20 $\mu\text{g}\cdot\text{mL}^{-1}$ decreased absorbance due to the interfilter effect of the colour species (28). Therefore, (20 $\mu\text{g}\cdot\text{mL}^{-1}$) was selected as the ideal concentration of ammonium chloroaurate in the following investigations for each light source.

Table 1. Variation of ammonium chloroaurate concentration on absorbance of color species.

[Reagent] $\mu\text{g.mL}^{-1}$	Average peak H*	S.D. peak H*	RSD% peak H*	Average peak A*	S.D. peak A*	RSD% peak A*
		$\bar{y} \pm t.s.d/\sqrt{3}$ 95%			$\bar{y} \pm t.s.d/\sqrt{3}$ 95%	
10	120.33	$\frac{2.51}{120.33 \pm 6.24}$	2.09	2444.33	$\frac{32.00}{2444.33 \pm 79.49}$	1.31
20	240.00	$\frac{3.60}{240.00 \pm 8.94}$	1.50	4609.66	$\frac{30.00}{4609.66 \pm 74.53}$	0.65
30	239.33	$\frac{3.51}{239.33 \pm 8.72}$	1.47	4599.00	$\frac{27.00}{4599.00 \pm 67.07}$	0.59
40	218.33	$\frac{3.51}{218.33 \pm 8.72}$	1.61	3757.00	$\frac{31.51}{3757.00 \pm 78.28}$	0.84
50	200.33	$\frac{4.04}{200.33 \pm 10.04}$	2.02	3221.33	$\frac{26.02}{3221.33 \pm 64.64}$	0.81
70	123.66	$\frac{3.05}{123.66 \pm 7.58}$	2.47	2249.00	$\frac{22.00}{2249.00 \pm 54.66}$	0.98

**Figure 1A.** Response of profile versus time for determination of doxycycline hyclate.**Figure 1 B.** Variation effect of ammonium chloroaurate concentration on the peak height and peak area.

3.1.2. The effect of different acid-base media carrier stream

The reaction of the Doxycycline hyclate ($25 \mu\text{g.mL}^{-1}$) ammonium chloroaurate ($20 \mu\text{g.mL}^{-1}$) system was tested in different media (acids, bases) at (0.02 mol.L^{-1}) concentration in addition to aqueous media (distilled water) flow rate 3 mL per minute for each line and specimen volume of $100 \mu\text{L}$. The data's findings were plotted, as shown in **Figure 2**, where the variance in transducer energy response is represented as an average peak height ($n=3$) with various mediums.

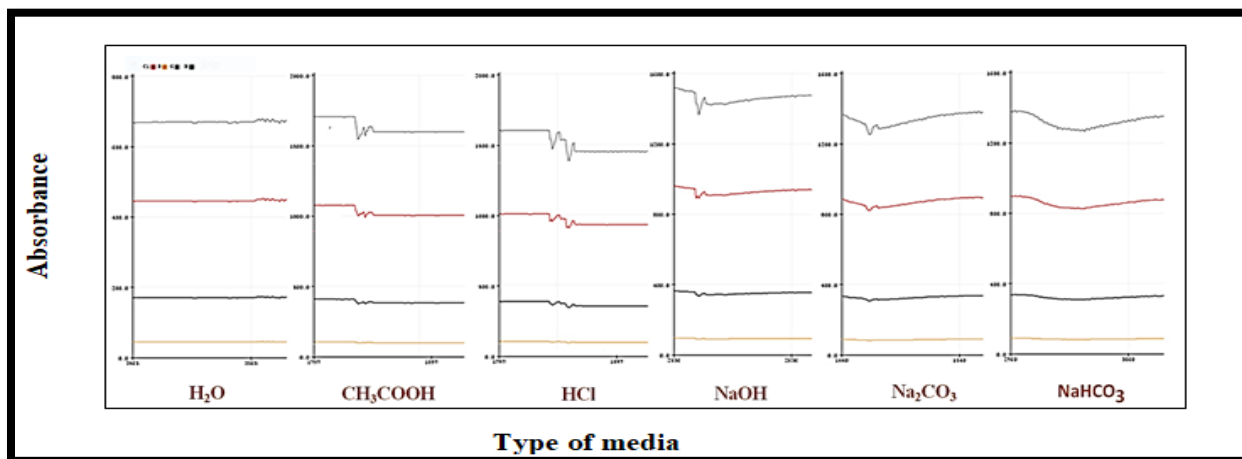


Figure 2. Variation effect of acidic& base media on the response of microphotometer using doxycycline hyclate ($25 \mu\text{g.mL}^{-1}$) -Ammonium chloroaurate reagent ($20 \mu\text{g.mL}^{-1}$).

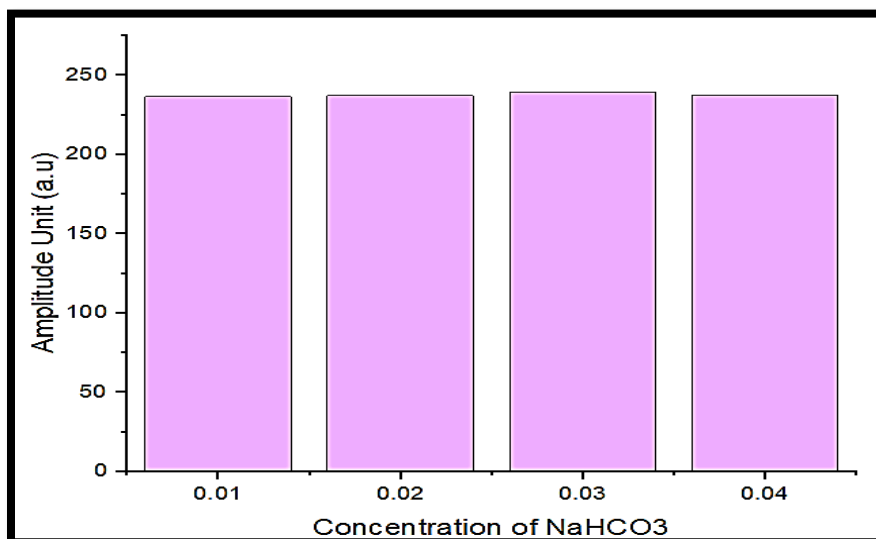
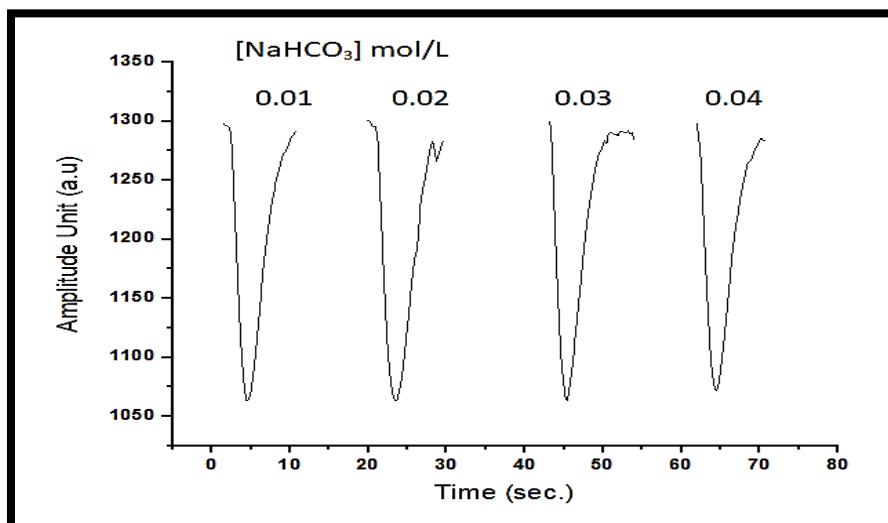
All used acids caused a decrease in the responses. This may be attributed to disassembling and breaking the complex colour. At the same time, the bases (NaOH , Na_2CO_3) worked to dissolve the complex, except sodium bicarbonate, which worked to complete the reaction, giving the most pronounced response compared to distilled water. This might be due to the ions, which may help form a dense precipitate and decrease intermolecular spaces before reaching the granulation state, forming compact crystals that act as reflection surfaces. Therefore, after testing several acids, bases, and distilled water, sodium bicarbonate was discovered to be optimal as a carrier stream for optimum attenuation of the incoming light.

3.1.3. The effect of sodium bicarbonate

Using doxycycline hyclate ($25 \mu\text{g.mL}^{-1}$), ammonium chloroaurate ($20 \mu\text{g.mL}^{-1}$), and an NaHCO_3 system, a variable concentration of sodium bicarbonate base ranging from (0.01 - 0.04 mol.L^{-1}) was prepared. $100 \mu\text{L}$ as a specimen volume and a flow rate of 3 mL per minute for carrier stream and reagent stream were used in this study. **Figure 3 A and B** shows that an increase in peak height is expressed as an attenuation of incident light with an increase of sodium bicarbonate base concentration, which may be linked to the production of small solid particles that led to an increase in attenuation of incident light up to 0.02 mol.L^{-1} concentration of NaHCO_3 as shown in **Table 2**. While more than 0.02 mol.L^{-1} , there was a gradual decrease of S/N energy transducer response probably due to the dispersion of precipitation particulate. Therefore, and based on the slop-intercept method, which is shown in **Table 2** and **Figure 3**, segment number three which extends from (0.01 - 0.04 mol.L^{-1}) in which that 0.02 mol.L^{-1} complete within it is the optimum concentration of sodium bicarbonate as a carrier stream and it will be used for further experiments.

Table 2. Variation of sodium bicarbonate concentration on the absorbance of colour species.

[NaHCO ₃] mol.L ⁻¹	Average of H*	S.D of H*	RSD % of H*	Average A*	S.D of A*	RSD % of A*
		$\bar{y} \pm t.sd/\sqrt{3}$ 95%			$\bar{y} \pm t.sd/\sqrt{3}$ 95%	
0.01	236.33	1.52	0.65	4284.00	17.34	0.40
		236.33 ± 3.78			4284.00 ± 43.08	
0.02	237	2.64	1.12	4295.66	17.61	0.41
		237 ± 6.56			4295.66 ± 43.75	
0.03	239	3.60	1.51	4299.66	19.13	0.45
		239 ± 8.94			4299.66 ± 47.53	
0.04	237	2.00	0.84	4292.00	29.46	0.69
		237 ± 4.97			4292.00 ± 73.19	

Figure 3A. Response of profile versus time for determination of sodium bicarbonate.**Figure 3B.** Variation of sodium bicarbonate on peak height and peak area.

3.2. Physical variables

3.2.1. The effect of flow rate

The flow rate ranged from two to six milliliters per minute for every line, for example, the carrier stream and the reagent ammonium chloroaurate. The flow rate for the determination was investigated to determine the optimum preferred flow rate used throughout the work. The optimum concentration was $20 \mu\text{g.mL}^{-1}$ of ammonium chloroaurate and using $25 \mu\text{g.mL}^{-1}$ of doxycycline hyclate, with $100 \mu\text{L}$ as a sample volume, while NaHCO_3 (0.02 mol.L^{-1}) was employed as a carrier stream. **Table 3** shows the results obtained, **Figures 4 A and B** exhibits the increase of absorbance with an increase of flow rate up to 4 mL/min for each line and each light source. On the other hand, when the flow rate was increased to 4 mL/min , the absorption of the coloured species into the measured flow cell decreased. The final flow rate was selected as the optimum flow rate for the carrier stream and ammonium chloroaurate for a green light source.

Table 3. difference of flow rate (mL.min^{-1}) on the absorbance of colour species.

Flow rate mL.min^{-1}	Average of H^*	S.D of H^*	RSD % of H^*	average A^*	S.D of A^*	RSD % of A^*
		$\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}$ 95%			$\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}$ 95%	
2	251.00	$\frac{3.00}{251.00 \pm 7.45}$	1.20	7742.67	$\frac{93.40}{7742.67 \pm 232.04}$	1.21
3	252.67	$\frac{4.04}{252.67 \pm 10.04}$	1.60	7705.33	$\frac{96.04}{7705.33 \pm 238.59}$	1.25
4	296.33	$\frac{5.13}{296.33 \pm 12.74}$	1.73	4864.33	$\frac{80.75}{4864.33 \pm 200.61}$	1.66
6	245.00	$\frac{4.58}{245.00 \pm 11.38}$	1.87	2125.00	$\frac{40.15}{2125.00 \pm 99.75}$	1.89

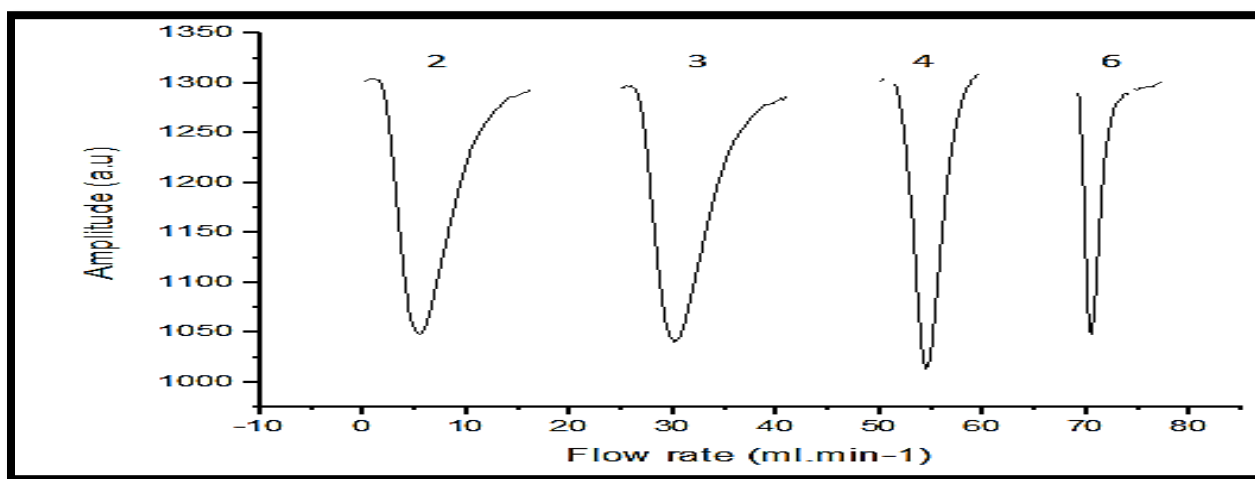


Figure 4A. Response of variation effect of flow rate for Doxycycline hyclate-ammonium chloroaurate system.

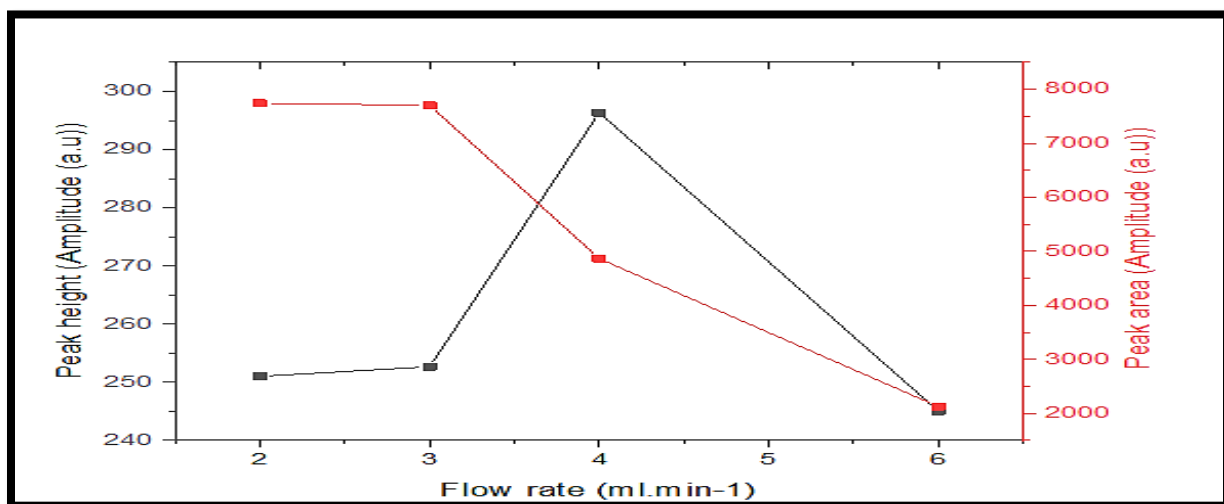


Figure 4B. Variation of flow rate on peak height and peak area.

3.2.2. Sample volume

The study used 4 mL.min⁻¹ as the ideal flow rate of the carrier stream and ammonium chloroaurate (20 µg.mL⁻¹), respectively, with 25 µg.mL⁻¹ of doxycycline hyclate. Variable sample volumes (5, 10, 20, 30, 40, 50) mL were used, using open valve mode. **Table 4** summarizes the findings, which show that a sample volume of 20 mL is ideal for doxycycline hyclate analysis. **Figure 5 A and B** demonstrate that, up to a sample volume of 20 mL, an increase in sample volume increased the height of the response. A higher sample volume (>20 mL) resulted in a drop in the height of the reaction, which was most likely caused by a continuous, long-lasting colour segment in front of the detector (29). Therefore, the 20 cm³ was the optimum sample segment for determining the green light source.

Table 4. The difference of injected sample volume on transducer energy response.

Sample volume (mL)	Average of H*	S.D of H* $\frac{\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}}{95\%}$	RSD% of H*	Average A*	S.D of A* $\frac{\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}}{95\%}$	RSD% of A*
5	256.67	$\frac{3.06}{256.67 \pm 7.60}$	1.19	5011.33	$\frac{115.52}{5011.33 \pm 286.99}$	2.31
10	314.67	$\frac{2.52}{314.67 \pm 6.26}$	0.80	5135.00	$\frac{81.30}{5135.00 \pm 201.98}$	1.58
20	390.67	$\frac{4.51}{390.67 \pm 11.20}$	1.15	7313.67	$\frac{181.15}{7313.67 \pm 450.04}$	2.48
30	387.00	$\frac{5.57}{387.00 \pm 13.84}$	1.44	7493.33	$\frac{155.93}{7493.33 \pm 387.38}$	1.84
40	298.67	$\frac{5.69}{298.67 \pm 14.13}$	1.90	7230.67	$\frac{136.76}{7230.67 \pm 339.76}$	1.66
50	300.67	$\frac{4.16}{300.67 \pm 10.33}$	1.38	7510.67	$\frac{140.91}{7510.67 \pm 350.07}$	1.76

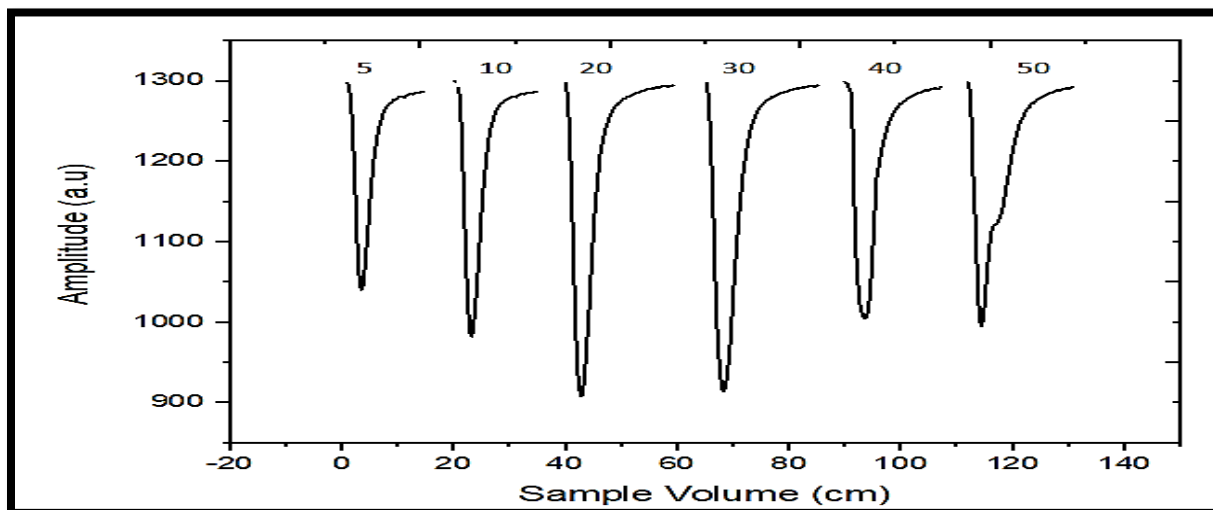


Figure 5A. Response of variation effect of sample volume for doxycycline hyclate-ammonium chloroaurate system.

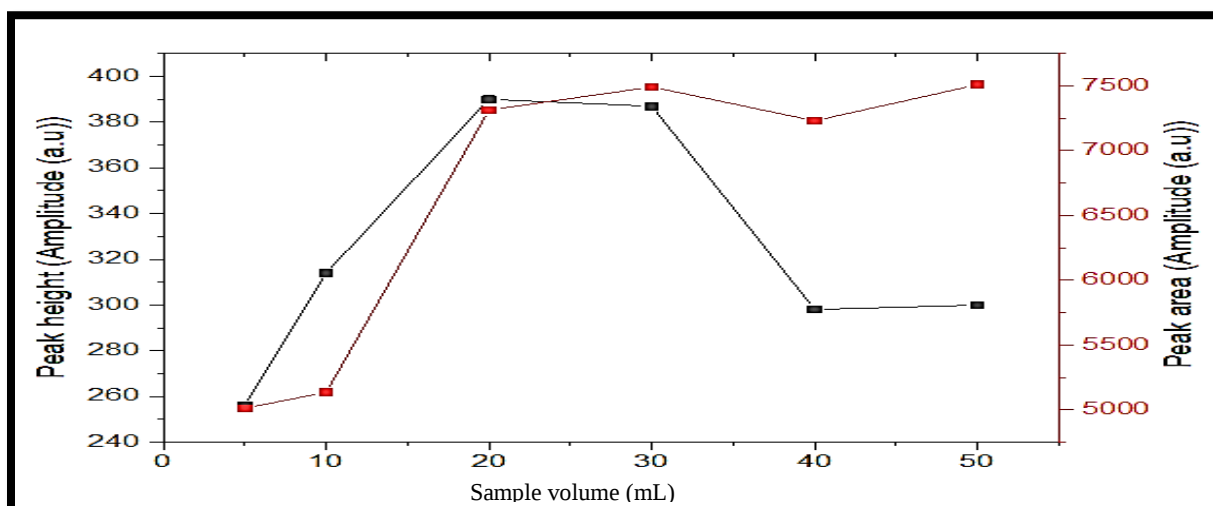


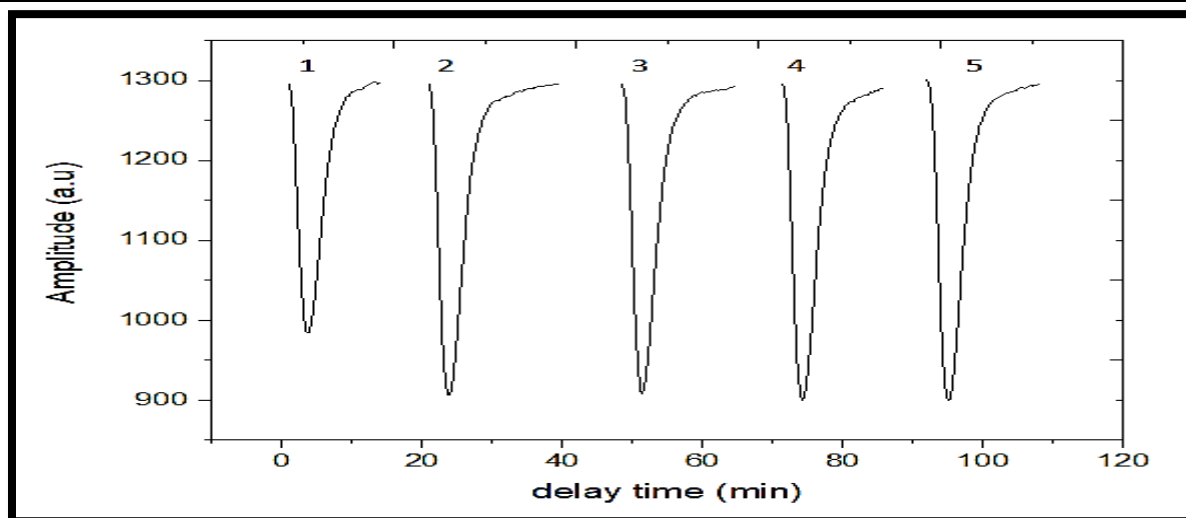
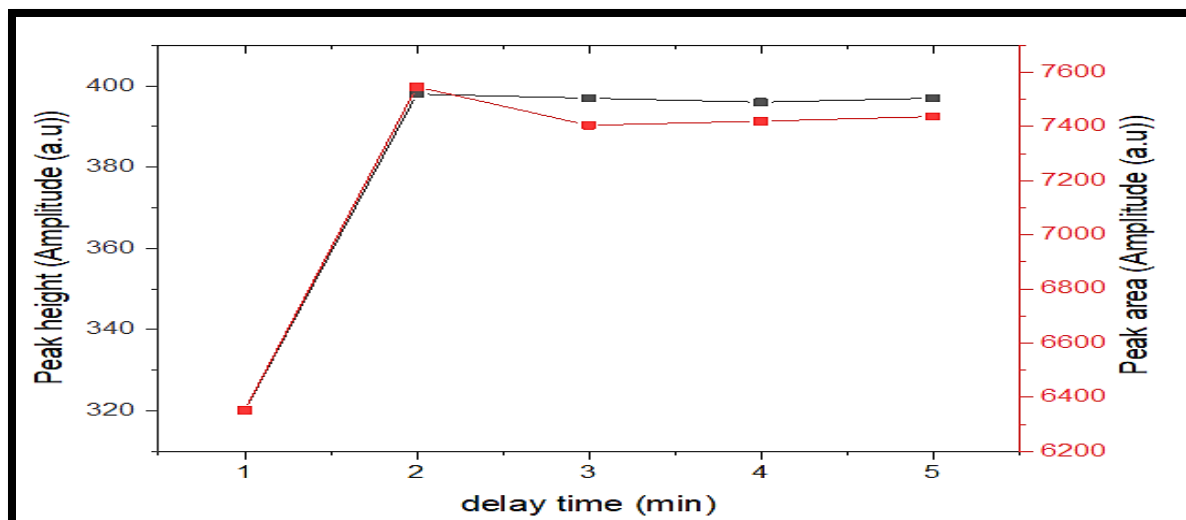
Figure 5B. Variation of sample volume on peak height and peak area.

3.2.3. The effect of delay time (stop-flow FIA)

This research aimed to establish the best time for injecting the sample segment (20 mL sample loop), i.e., the maximum time permitted for purging the sample from the injection valve and using the variable of purge time for the segment in the range 1-5 min. The experiment was performed. **Figure 6 A and B** shows that all responses profile with variable peak height which increased with an increasing of injection time up to open valve mode (2 min) of doxycycline hyclate ($25 \mu\text{g.mL}^{-1}$) ammonium chloroaurate ($20 \mu\text{g.mL}^{-1}$) NaHCO_3 (0.02 mol.L^{-1}) system at 4 mL per min flow rate for carrier stream and reagent respectively (30) Therefore, continuous valve mode was selected as the optimum purge time as shown in **Figures 9, 10** and **Table 5**.

Table 5. The effect of delay time on the absorbance of the colour species.

Delay time (min)	Average of H*	S.D of H*	RSD% of H*	Average A*	S.D of A*	RSD% of A*
		$\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}$ 95%			$\bar{y}_i(\text{mV}) \pm \text{t.sd}/\sqrt{3}$ 95%	
1 min	320.00	$\frac{4.58}{320.00 \pm 11.38}$	1.43	6352.00	$\frac{101.68}{6352.00 \pm 252.61}$	1.60
2 min	398.67	$\frac{6.03}{398.67 \pm 14.98}$	1.51	7547.00	$\frac{128.00}{7547.00 \pm 317.99}$	1.70
3 min	397.67	$\frac{6.66}{397.67 \pm 16.55}$	1.67	7405.67	$\frac{151.57}{7405.67 \pm 376.55}$	2.05
4 min	396.33	$\frac{2.52}{396.33 \pm 6.26}$	0.63	7420.33	$\frac{219.30}{7420.33 \pm 544.82}$	2.96
5 min	397.00	$\frac{3.61}{397.00 \pm 8.97}$	0.91	7437.00	$\frac{152.35}{7437.00 \pm 378.49}$	2.05

**Figure 6A.** Response of variation effect of delay time for doxycycline hyclate-ammonium chloroaurate system.**Figure 6B.** Variation of delay time on peak height and peak area.

3.3. Calibration graph

Applying the best possible values for the physical and chemical parameters established in the preceding section. Various solutions for determining drug ranging ($5\text{--}75 \mu\text{g.mL}^{-1}$) were

prepared. Three times every measurement was done. The average absorbance of the three measurements has been plotted versus the concentration of doxycycline hyclate, with peak height and peak area serving as the variables of interest. A straight-line graph (**Figures 7 and 8**) from 5-70 $\mu\text{g.mL}^{-1}$ of doxycycline hyclate was obtained for each green light source. Above 70 $\mu\text{g.mL}^{-1}$, the correlation coefficient value will decrease and deviate from linearity. This is most likely due to an increase in the coloured species in front of the detector, as well as an inner filter that reduces the high intensity of coloured species that are presented in the solution. This reduces the amount of light that is transmitted through the solution. The obtained data are summed up in the linear equation of the range 5-70 $\mu\text{g.mL}^{-1}$ of each light source, green light source, as a form of:

$$y^{(a.u)} = 88.81267 + 12.82475 [\text{doxycycline hyclate}] \mu\text{g.mL}^{-1} \text{ using peak height with } r^2 = 0.99514.$$

$$y^{(a.u)} = 919.01 + 319.8 [\text{doxycycline hyclate}] \mu\text{g.mL}^{-1} \text{ using peak area with } r^2 = 0.9926.$$

The developed method was compared with the classical method by preparing a calibration graph using (measurement of absorbance by spectronic 20 D⁺, Japan), which was made to determine doxycycline hyclate from 5-35 $\mu\text{g.mL}^{-1}$. Figure 13, after fixing the optimum of [ammonium chloroaurate], which was 5 $\mu\text{g.mL}^{-1}$. In the form, an equation of the first degree was used to express the findings.

$$y^{(a.u)} = 0.0679 + 0.0035 [\text{doxycycline hyclate}] \mu\text{g.mL}^{-1} \text{ with } r^2 = 0.9878$$

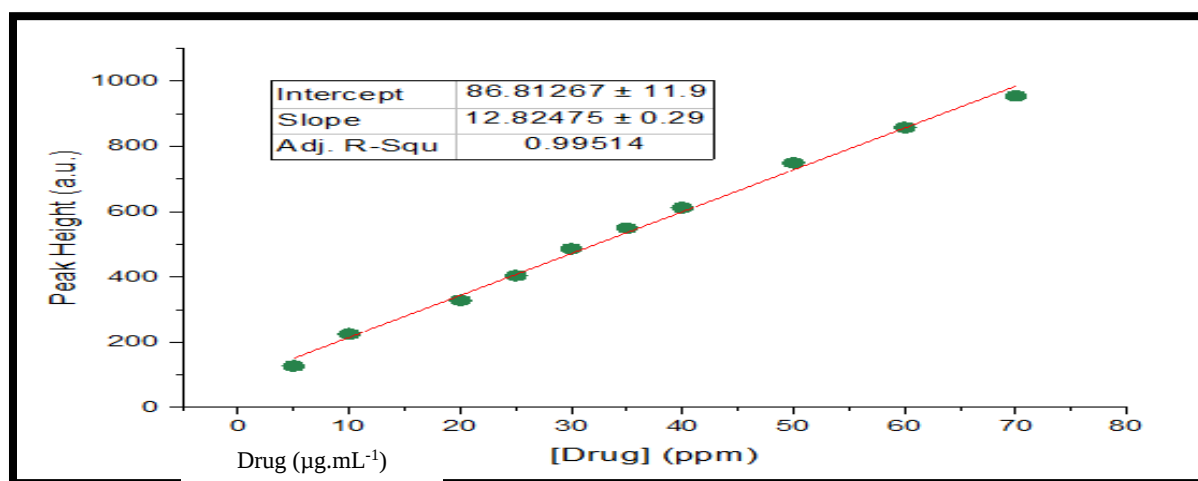


Figure 7. Variation effect of doxycycline hyclate concentration on peak height using green light as a source for doxycycline hyclate-ammonium chloroaurate (20 $\mu\text{g.mL}^{-1}$)- NaHCO_3 (0.02 mol.L^{-1}).

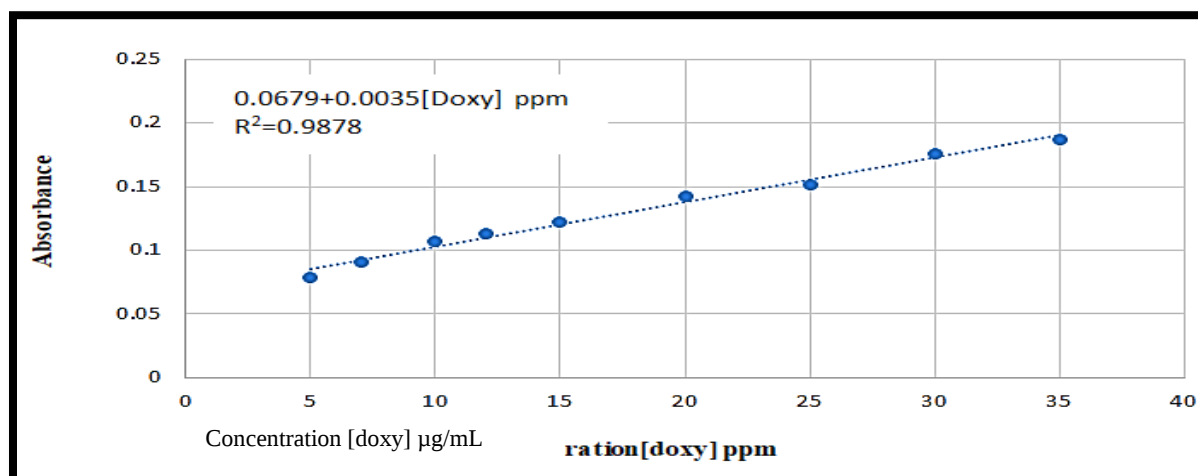


Figure 8. Variation effect of calibration graph using method (UV-spectrophotometric).

3.4. Limit of detection (LOD)

Spectrophotometry is a technique for evaluating how much light a chemical substance absorbs by measuring the intensity of light passing through a sample test. According to the basic principle and flow injection analysis, each substance absorbs or transmits light over a specified wavelength range. The sample zone is injected into a carrier stream of reagent, then the sample solution disperses into it while a product forms interference between the sample zone and the reagent. The detector records the desired physical conditions that change due to the passage of the sample through the flow cell. Two methods were employed to determine the L.O.D., both of which included doxycycline hyclate ($2 \mu\text{g.mL}^{-1}$)-ammonium chloroaurate ($20 \mu\text{g.mL}^{-1}$)- NaHCO_3 (0.02 mol.L^{-1}) and the stepwise dilution of the lowest concentration in the calibration curve ($0.5 \mu\text{g.mL}^{-1}$).

3.5. Repeatability

Constant concentrations of doxycycline hyclate were employed to examine the repeatability of measurements and the effectiveness of the SFI microphotometer, with 20 g.mL^{-1} being the most common concentration utilized. The response-time profile for the employed concentrations is shown in **Figure 9** after being measured six times in a row with an RSD% of less than 1%.

One pharma company's medicine (DoxiN, NCPC, India-100 mg) was injected on NaHCO_3 as a carrier stream using a two-line manifold design system utilizing a microphotometer-SFIA. The results of a t-test comparing the practical value and the quoted value for the doxycycline hyclate-ammonium chloroaurate- NaHCO_3 system are shown in **Table 6** and **Figure 10**. These results show no significant difference between the quoted value and the calculated t-value at the 95% confidence level, as the computed t-value is less than the critical tabulated t-value, such as ($t_{\text{cal.}} (0.5084) < t_{\text{tab}} (4.303)$).

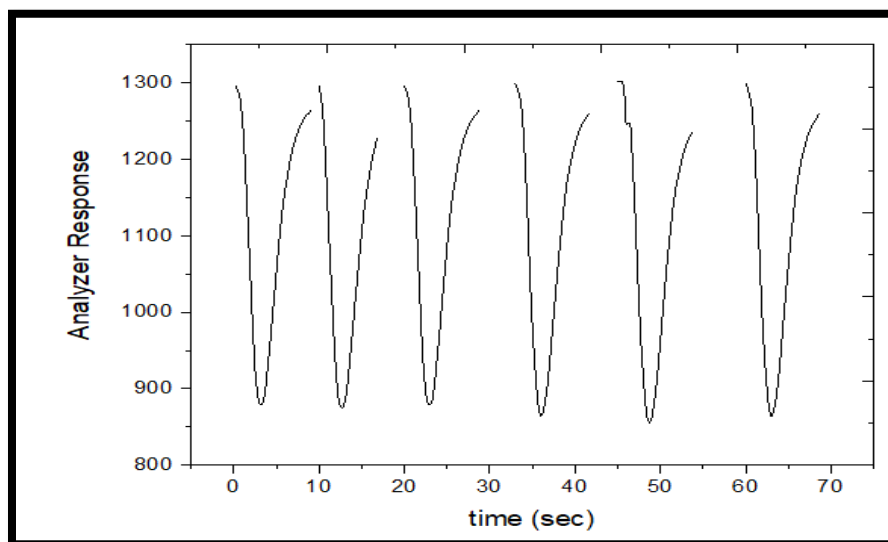


Figure 9. Variation effect of repeatability on absorbance using doxycycline hyclate.

Table 6. Comparison of the result for the determination of Doxycycline hyclate in the drug using t-test.

Type of sample	Concentration of Doxy in preparation sample ($\mu\text{g/mL}$)	$\mu\text{g/mL}$ pure of Doxy (present)	Recovery	Individual t-test compared between t-value and quoted value
DoxiN, NCPC, India-100mg	5	5.6841	113.6 8	0.5084

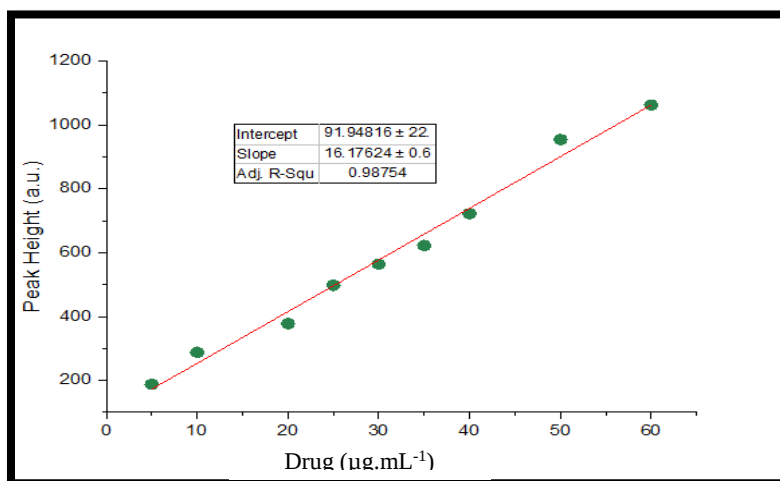


Figure 10. Variation effect of DoxiN concentration with peak height (a.u).

4. Conclusion

The suggested FIA technique for doxycycline hyclate determination in pure and pharmaceutical preparations is straightforward, quick, and sensitive. Using t-tests (as comparison tools) proved that the newly devised approach is just as excellent as the conventional spectrophotometer method. In addition, this investigation has led to the discovery of a new analytical approach, which differs from the traditional one in that it uses just a few straightforward parameters.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

This study was approved by the Scientific Committee at the Department of Chemistry, College of Science, University of Baghdad.

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