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Original Research Article

Development of a new methodology for Lincomycin.HCI (LINO.HCI) determination using phosphomolybdic acid (PMA) as a reagent and ISNAG-Fluorimeter Instrument via CFIA

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ABSTRACT

The reaction is based on the Lincomycin that has been responded. In an acidic solution, combine HCl and phosphomolybdic acid to produce a white color precipitate that may be measured using an ISNAG-Fluorimeter (homemade instrument). Some chemical and physical variables have been evaluated. The correlation coefficient was r=0.9983. The calibration curve was linear (the range was 0.0051 mmol/L). The minimum detection limit concentration in the linear dynamic range of the calibration curve was 19.935 ng. The RSD percent of lincomycin HCl at 0.3 and 0.8 mmol/L was less than 0.25% (n=8). The approach was used to determine lincomycin HCl in three pharmaceutical tablets. Comparisons were made using a standard expansion approach and a matched t-test between the recently created method and the traditional strategy (UV-Vis spectrophotometry and turbidimetry with a turbidimeter). At the 95% confidence level, there was no significant difference between the two techniques.

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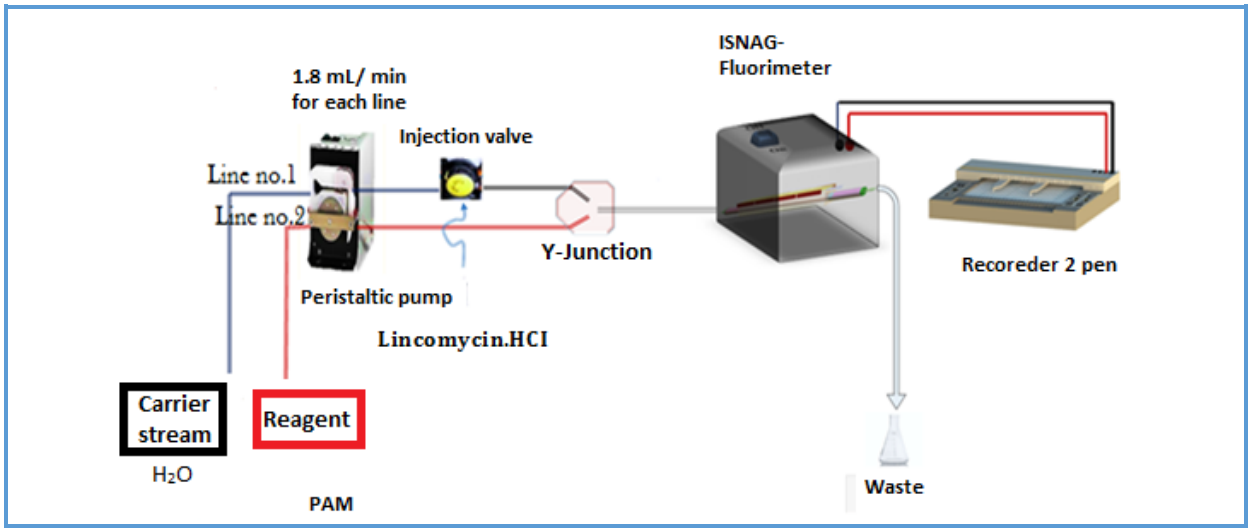
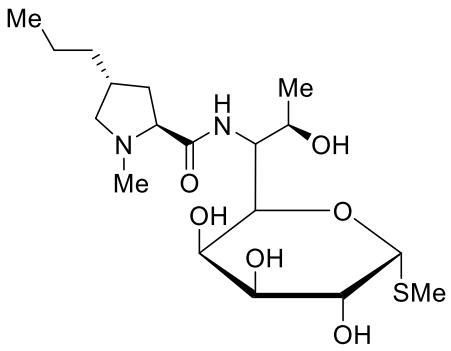
Graphical Abstract



Introduction

Lincomycin hydrochloride (LMH), a widely used systemic antibiotic, is a member of the lincosamide family of antibiotics. It is effective against the majority of gram-positive bacteria. By binding forcefully and especially with the 50 S ribosomal subunit at the same time appropriate locations (Scheme 1), lincomycin hydrochloride limits mobileular proliferation and microbial protein synthesis [1]. Chemical name of LMH is Methyl 6, 8-dideoxy-6-[(2S, 4R)-1-methyl-4-propyl-2-pyrrolidinyl] carbonyl] amino]-1-thio-L-threo-α-D-galacto-octopyranoside hydrochloride, streptomyces lincolnensis var, and lincocin [2, 3]. It is recognized by the Indian Pharmacopoeia [4, 5], the British Pharmacopoeia [6], the United States Pharmacopeia, and the National Pharmacopoeia [7]. Lincomycin hydrochloride is a crystalline powder that is white or nearly white and has a mild odor. The right-handed solution is acidic [8]. Diarrhoea, stomach ache; nausea, vomiting; swollen or sore tongue; vaginal itching or discharge; mild itching or rash; ringing in the ears, or dizziness are the most common side effects [9]. A few analytical methods have been stated for their quantitative determination in pharmaceutical formulations like GC liquid chromatography with pulsed electrochemical detection [10], UV spectrophotometry [11], colorimetry [12], and atomic absorption spectroscopy [13].



Scheme 1. Molecular structure of Lincomycin hydrochloride.

Apparatus: A homemade ISANG fluorimeter was used with multichannel more than one-line feed (In this part of the research work, only two lines) were used, a four-channel peristaltic pump (Ismatec, Switzerland) and a six-port medium pressure injection valve. The output of measurement i.e.; $\bar{Y}_{zi}$(mV)-t_{min}(d_{mm})was plotted by a potentiometric recorder was used to determine the output signals (Siemens,

Germany (1-5 V)). HANNA instrument for turbidity measurement was used as classical method.

Experimental

Materials and methods

All chemicals were utilized as explanatory reagents and distilled H_2O was utilized to get ready all solutions. 443 g lincomycin HCl (LINO.HCl) ($C_{18}H_{35}ClN_2O_6S.HCl$, M.W=443 g/mol, 0.1 mol.L^{-1}) was dissolved in 100 mL distilled H_2O for prepared standard solution. The solution provides phosphomolybdic acid $H_3PMo_{12}O_{40}$ (molecular weight 1825.25 g/mol, 9.1263 g/500 mL), Na_2CO_3 (5.299 g/100 mL), CH_3COONH_4 (3.884 g/100 mL), potassium chloride (7.350 g/100 mL), sodium chloride (7.313 g/250 mL), NH_4Cl (2.675 g/100 mL), CH_3COOH (57.47 mL/L), hydrochloric acid (88.28 mL/L) and H_2SO_4 (55.52 mL/L).

Sample preparation

For Dar Aldawa-Jordan, Dar al-Hikma-Iraq, and SDI-Iraq, a batch of twenty tablets containing 500 mg of lincomycin hydrochloride were weight 0.1346 g, 0.13341 g, and 0.1401 g (equal to 0.11075 g of the active component, 10 mmol/L) correspondingly. The powder was dispersed in distilled H_2O. Filtered the solution to eliminate any undissolved residue that might have influenced the reaction, and then distilled water was added to bring the volume up to 100 mL.

Methodology

Lincomycin.HCl

The use of phosphomolybdic corrosive (PMA) as a precipitating reagent was used to test HCl. As illustrated in Figure 1, the manifold reaction system comprises two lines. The first line is a distilled (D.W) carrier stream with a 1.8 mL/min flow rate that will carry the 140 Lincomycin sample segment through the injection valve. Before being introduced to the ISNAG-fluorimeter analyzer, HCl (0.7 mmol/L) will encounter PMA (2 mmol/L) in the second line at the Y-junction point [14–21]. The signals produced for Lincomycin as a result of 90° diverging light on the particle surface of the ion pair. System of HCl and phosphomolybdic acid. The repeated successive measurements of ISNAG-fluorimeter instrument output Y_{Zi}(mV) versus $t_{min}(d_{mm})$ for Lincomycin.HCl (0.7 mmol/L)-phosphomolybdic acid (2 mmol/L) system. Scheme 1 reveals the ion pair's expected reaction pattern (Scheme 2).

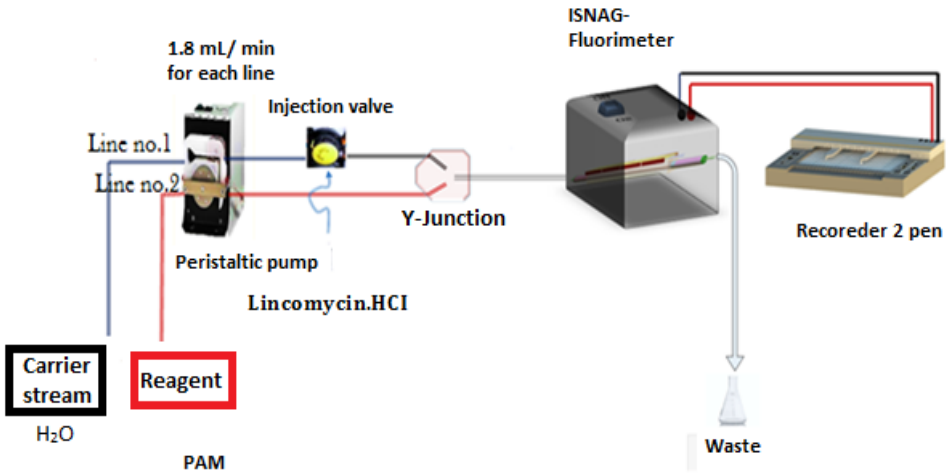
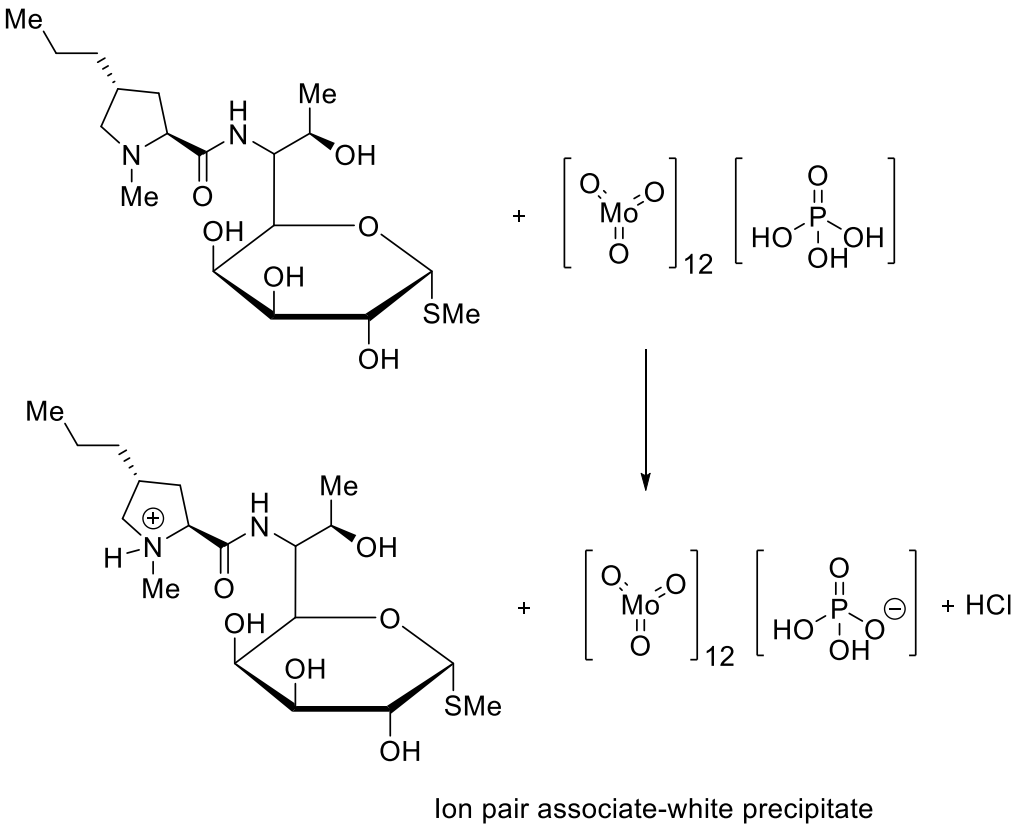


Figure 1. Flow diagram of used manifold throughout, this part of research work



Scheme 2. The proposed reaction between Lincomycin HCl with phosphomolybdic acid to form ion pair

Results and Discussion

Optimization of reaction pattern parameters

Phosphomolybdic acid concentration

A chain of phosphomolybdic acid range of solutions from 0.5-5 mmol/L concentration was used as a precipitating agent at a flow rate of 1.8 mL/min for each line; and 140-liter sample volume segment contained 0.7 mmol/L Lincomycin.HCl as an injected sample. All types of reactions vs. time of profile (YZi(mV)-tmin (dmm) 1(10), as shown in Figure 2**a**. When the concentration of PMA is increased to 3 mmol/L, the peak height response increases. This rise could be ascribed to an increase in small nuclei density, which leads to the production of granules, which reflect light more intensely and at a higher frequency toward the detector, resulting in a shorter wavelength and falling under the solar cell sensing limit. Above 3 mmol/L, the reaction is less sensitive due to the formation of vacancies or pockets to accommodate unwanted particles. For example, causing spread or dispersed particulate impurities relative to the nature of the precipitate resulting in a decrease in diverged light toward the solar cell. By the slope-intercept approach to pick the ideal segment with the highest intercept and lowest or acceptable slope value, the selected segment with the highest intercept value is $\mathbf{a_3}$-$\mathbf{a_5}$ (segment no. 3) (Figure 2**b**), which has 3 mmol/L inside its limits. Any chosen PMA concentration within this section can also be employed as an alternative in the newly designed optimization process developed optimization methodology.

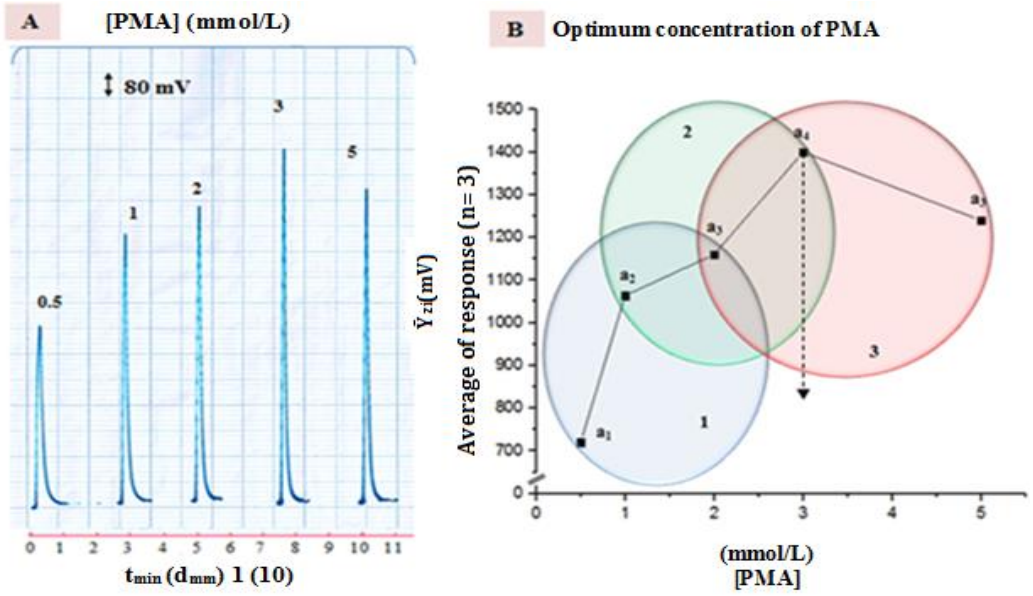


Figure 2. Variation of PMA concentration on a) profile of LINO.HCl-PMA system, b) Height of Y_{zi}(mV) expressed as an energy transducer response in Mv with three data points as one segment their interaction and choice

Media effect (salts and acids) as a carrier stream on diverged of light

The effect of different media was used as a carrier stream to study its effect on Lincomycin reaction HCl (0.7 mmol/L) with PMA (3 mmol/L). Different medium at CH_3COOH (50 mmol/L of concentration), tartaric acid ($C_4H_6O_6$), ascorbic acid ($C_6H_8O_6$), HCl, H_2SO_4 and HNO_3 as an acid media, as well as the use of 50 mmol/L concentration of CH_3COONH_4, NaCl, Na_2SO_4, $NaNO_3$, NH_4Cl and KCl as a salt media in addition to distilled water (D.W) used as a carrier stream at 1.8 mL/min flow rate for each line. It was noticed that an increase in S/N-response using H_2SO_4 compared with the use of (D.W) H_2O. This might be due to an increase of peptization effect, which leads to coagulating particles reverting to their original dispersed state. Therefore, H_2SO_4 was selected as the best carrier for further work.

Investigation of effect of sulfuric acid concentration

The reaction between Lincomycin HCl (0.7 mmol/L) with PMA (3 mmol/L) to form a white color precipitate was studied at a variable concentration of H_2SO_4 range (10-70 mmol/L) as a carrier stream, 140 µL sample volume at 1.8 mL/min flow rate for each line (Figure 3**a** and **b**). When working with acids (sulfuric acid), it was discovered that increasing the sulfuric acid concentration above 50 mmol/L causes the peak height of the reaction to drop (Table 1).

This could be due to sulfuric acid penetrating to the depths of the precipitate during crystal development, generating a soft particle that reduces light scattering and deviates toward the sensor (detector in ISNAG-Fluorimeter instrument). As a result, 50 mmol/L sulfuric acid was chosen as the best carrier stream for this study. When using the slope-intercept method, keep in mind that it was noticed that the chosen segment of S_3 (i.e., 30-70 mmol/L) is the most acceptable region, and 50 mmol/L within the chosen segment, in addition to any other concentration, can be chosen within this segment (Figure 3**b**).

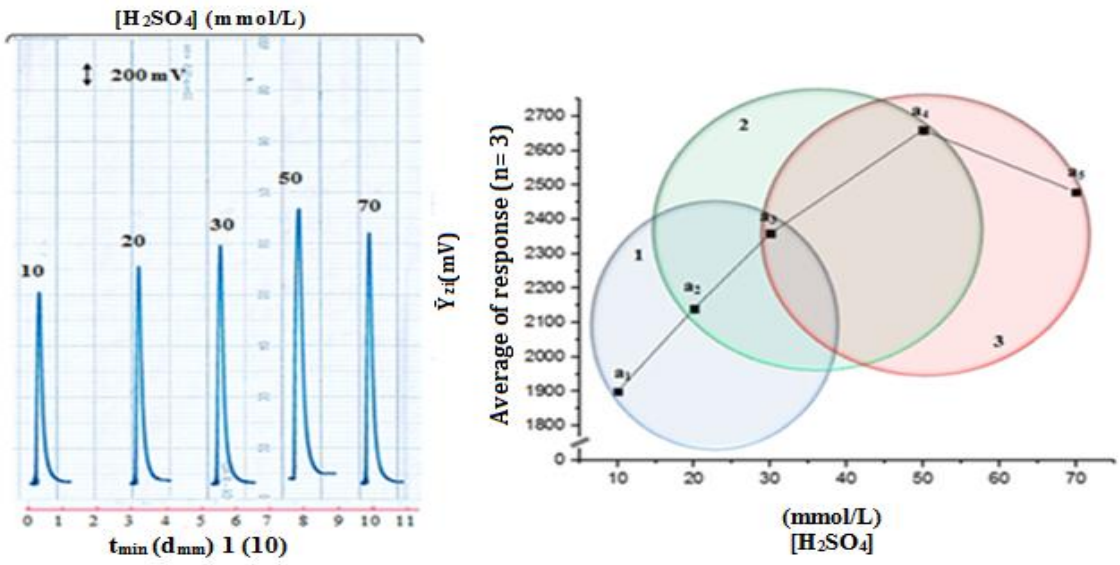


Figure 3. Effect of variable concentration of H_2SO_4 solution on: A-Response profile Y_{Zi}-(mV)-$t_{min}(d_{mm})$.B-output of (S/N) energy transducer response in mV with chosen segment

Table 1. Data set point obtained for the variation of H_2SO_4 concentration in the determination of LINO.HCl using LINO.HCl (0.7 mmol/L)–PMA (3 mmol/L)–H_2SO_4 system

$[H_2SO_4]$ mmol/L	$\bar{Y}_{zi}$(mV) average of response (n=3)	RSD %	Confidence interval at 95% $\bar{Y}_{zi}(mV)\pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
10	1900	0.125	1900±5.912
20	2140	0.120	2140±6.384
30	2360	0.132	2360±7.751
50	2660	0.120	2660±7.974
70	2480	0.141	2480±8.745

$t_{0.05/2,2}$=4.303
$\bar{Y}_{Zi}$ (mV)
(S/N) energy transducer response

Physical variables

Variation effect of flow rate

Fixing all past experimental parameters of chemical variety for Lincomycin HCl (0.7 mmol/L)-PMA (3 mmol/L)-H_2SO_4 (50 mmol/L) framework, a variable flow rates from (0.7-2.8 mL/min) for each lines (carrier streamLine & reagent line) were utilized for this work. From Figure 4, it can be noticed that at a slow flow rate, a wide broad profile is obtained, which might be caused by enhancing densification, thus increasing the effect of gravity factor of precipitated particulate movements, and irregularly shaped grain particles might cause an irregularity of flow, which in turn to deformed or broad of response-time. Above 1.8 mL/min, the peak height of the reaction was decreased, which was due to the fact that the crystal did not have time to grow into larger particles that act as reflectors of incident light. As a result, a flow rate of 1.8 mL/min for each line was the best compromise between sensitivity, profile, and chemical consumption.

Using the slope-intercept approach, the selected segment with the greatest intercept value (high sensitivity) is (a_3-a_5), with 1.8 mL/min falling inside its bounds. Any specified flow rate inside this section can also be used as an option in the newly created optimization process.

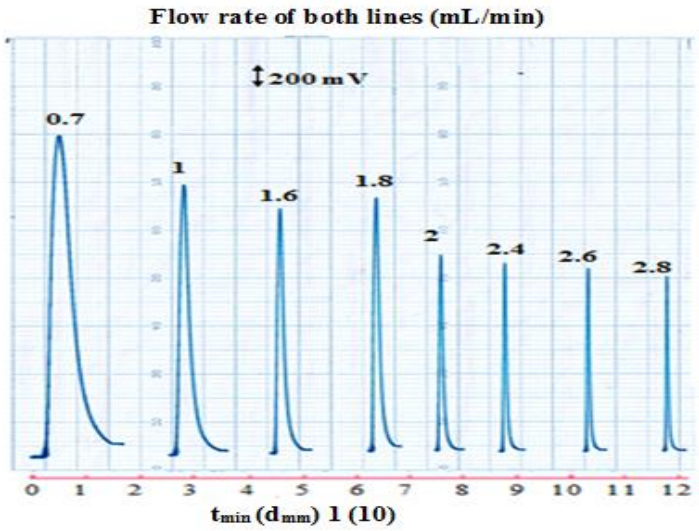


Figure 4. Effect of flow rate on A-Response profile $\bar{Y}_{Zi}$(mV)-t_{min} (d_{mm})

Investigation of Variation effect of sample volume

At Lincomycin HCl of 0.7 mmol/L, PMA of 3 mmol/L, and 1.8 mL/min flow rate for each line, the effect of variable sample volumes ranging 40, 90, 110, 140, 150, and 175 µL were studied. The increase in sample volume led to a significant increase in sensitivity (S/N profiles) up to 150 µL, giving a uniform profile of the space incident of light with base width (Δt_B). While when using sample volume more than 150 µL, it gave a slight increase in the height of the peak, possibly due to a long duration of carrier stream to passing through injection valve causing limitation of the flow, which lead to an increase of dispersion of the precipitate particles segment and increase of base width (Δt_B). Therefore, 150 µL is the most satisfactory sample plug. So to choose the best sample volume that gave good sharp and regular responses and based on slope-intercept calculation, the sample volume 150 µL, which is within the chosen segment no.4 (i.e., a_4-a_6) was chosen as the optimum sample volume.

Investigation of Effect of delay reaction coil on S/N response profile

This study aimed to decide whether a reaction or delay coil is necessary for maximum reaction rate or precipitated particulate formation or growing to form a dense precipitated increased response and high sensitivity. A compromise should be made between reaction completion and avoiding excessive dilution. Using optimum chemical and physical parameters, variable reaction coil from 0 µL (without) to 236 µL which connected after Y- junction point directly in a manifold design system. It can be noticed that increasing the coil volume enhanced the sensitivity and peak height of response up to 196 µL. In contrast, at more than 196 µL it reduced the diverged light with an increase of base width and departure time of sample segment from injection valve

reaching to the measuring cell. This might be attributed to of the increased diffusion and dispersion of precipitate particulate segment causing loss of some of the reflecting surface. Therefore, delayed reaction coils were used in this research. This result corresponds with slope-intercept method, which shows that the selected segment that given the highest value of intercept is a_3-a_5 (i.e., segment no.3), in which any sample segment within it can be used to obtain acceptable results and high sensitivity (Figure 5).

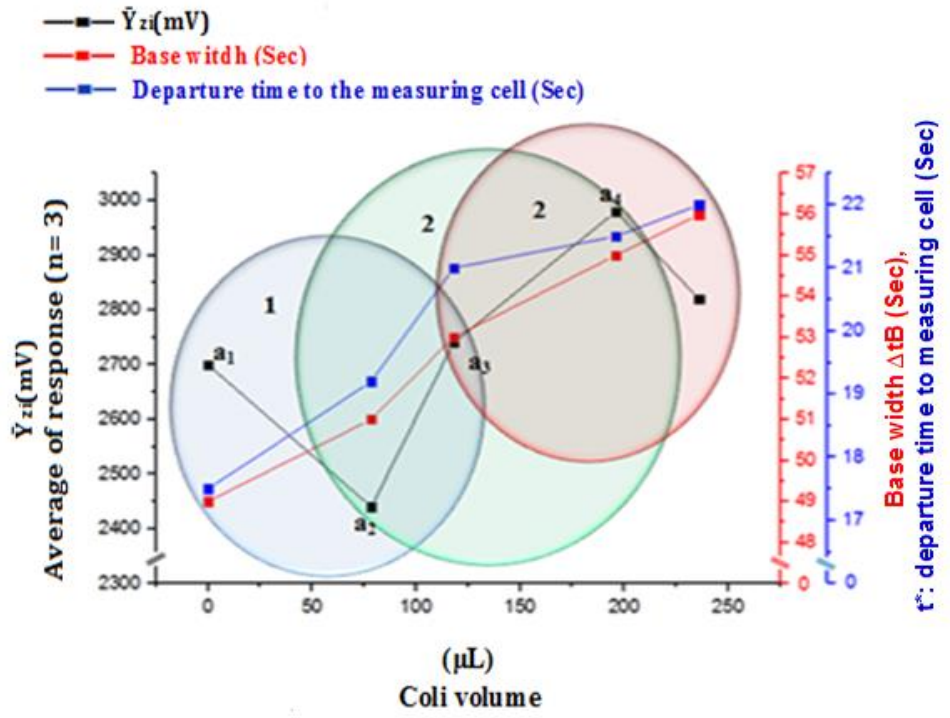


Figure 5. Variation effect of coil volume on: Height of $\bar{Y}_{Zi}$ (mV):(S/N) energy transducer response in mV, two segments (three-point data), and chosen segments

Study for the variation of energy transducer output of diverged light versus Lyncomycin.HCl concentration

Using the optimum chemical & physical parameters, a set of LINO.HCl solution (0.005-2 mmol/L) for (LINO.HCl-PMA (3 mmol/L)-H_2SO_4 (50 mmol/L) system were prepare and this represented the independent variable of the x-axis. in which directly proportional up to 2 mmol/L between the variation of precipitate particulate formation and concentration; might be attributed to an increase of many factors such as refraction, absorbance, reflection, and diverged light from within the precipitated particles when the beam of light diffused inside of particles, the measurement that is recorder at 0-90° only. is represented scatter plot at range 0.005-2 mmol/L; and in which gave a correlation of Y_{Zi} (mV) versus t_{min} (d_{mm})1(10) of 0.9584 with coefficient of determination of 0.9186; and linear dynamic range of 0.005-1 mmol/L. A short range that should be used to improve the assessment of mathematical formulation and the best Linear equation with the correlation between Lincomycin.HCl concentration against diverged light as a dependent variable with has r=0.9983 and % capital R-squared of 99.66% (Table 2) on the form:

$\hat{Y}_{Zi}$ = 12.494 ± 53.343 + 4138.335 ± 115.214 [LINO.HCl] mmol/L [22–24] explained much of obtained results from n=22 were the outcome of the scatter plot. The assessment evaluation of the newly developed methodology for the determination of Lincomycin HCl was compared with available references two methods which includes UV-Spectrophotometric method and turbidimetric method, which was based on:

1- UV.Spectrophotometric method; which is based on the measurements of absorption for the range of concentration 0.005-0.1 mmol/L at λ_{max}=196 nm [25], using quartz cell the best linear range extended from 0.005-0.09 mmol/L with coefficient of 0.9933 and % capital square =98.66%, n=11 (no.of measurements). Table 2 shows the variable data treatments.

2- Turbidometry method, which is based on the reaction between PMA as a precipitating agent with optimum concentration 0.07 mmol/L in aqueous medium and the drug of Lincomycin HCl for a variable range of concentration 0.005-1.4 mmol/L. The results were tabulated in table 2 at confidence level 95% using first-degree equation of the form of:

$$\hat{Y}_i(NTU)=a + b\ [LINO]\ mmol/L$$

The best linear range extend from 0.005-1 mmol/L of n=16 with correlation coefficient of 0.9757 and % capital R-squared=95.20% (Table 2).

Table 2. Summary of different ranges using linear regression for the variation of S/N energy transducer response and using a spectrophotometer and Turbidimeter method (classical methods) with Lincomycin HCl concentration using first-degree equation of linear $\hat{Y}$=a+bx at optimum condition for both methods

Type of mode	Range of [LINO] mmol/L (n)	$\hat{Y}_i$=a±s_at+b±s_bt[LINO.HCl] mmol/L at confidence interval 95%, n-2	$_{r, r^2, R^2\%}$	t_{tab} at 95%, n-2	Calculated t-value $\frac{/r/}{\sqrt{1-r^2}}\sqrt{n-2}$
Developed method using ISNAG-fluorimeter					
UV-Spectrophotometer at λ_{max}=196 nm					
Turbidometry method (NTU) Using Hanna Instrument					
Linear range or Liner dynamic range	0.005-1(21)	12.494±53.343+4138.335±115.214 [LINO.HCl] mmol/L	0.9983, 0.9966, 99.66	2.093<75.177	
	0.005-0.09(11)	0.074±0.098+22.014±1.928 [LINO.HCl] mmol/L	0.9933, 0.9866, 98.66	2.26<25.827	
	0.005-1(16)	27.242±11.734+215.194±27.692 [LINO.HCl] mmol/L	0.9757, 0.9520, 95.20	2.145<16.669	
Scatter plot	0.005-2(22)	287.635±269.710+3124.976±433.869 [LINO.HCl]mmol/L	0.9584, 0.9186, 91.86	2.086<15.024	
	0.005-0.1(12)	0.108±0.123+20.778±2.175 [LINO.HCl] mmol/L	0.9892, 0.9786, 97.86	2.228<21.384	
	0.005-1.4(18)	34.797±16.203+173.732±27.447 [LINO.HCl] mmol/L	0.9583, 0.9184, 91.84	2.120<13.419	

Ŷi: Estimated response(n=3) in mV for newly developed and without unite for spectrophotometric method or in NTU for Turbidometric method (classical method) for n=3 expressed as an average peaks heights or turbidometric value of linear equation of the form $\hat{Y}$=a+bx, r: Correlation coefficient, r^2:Coefficient of determination, R^2:% capital R-squared, R^2=explain variation/total variation, [LINO.HCl] concentration of Lincomycin.HCl, Developed method: using ISNAG-fluorimeter, n:no.of measurements, $t_{tab.}=t_{0.05/2,n-2}$, volume of measuring cell 1 mL for UV-Sp. and 10 mL for turbidimetric

Limit of detection (LOD)

The detection limit of Lincomycin HCl using PMA (3 mmol/L), the sample volume of 150 µL, depends on the actual research needs, using three different approaches, as shown in Table 3.

Repeatability

The repeatability was studied for the determination of Lincomycin HCl via the measurements of the diverged of incident light using ISNAG-fluoremeter formed by the reaction of Lincomycin HCl with PMA in the presence of H_2SO_4. Relative standard deviation expressed as percentage (RSD%<0.25%) which is equal to reproducibility of the measurement eight successive injections were repeated at a fixed concentration of LINO.HCl at two concentrations (0.3 and 0.8 mmol/L), each with optimal parameters (Table 4).

Table 3. Detection Limit of LINO.HCl using 150 µL as an injection sample and optimum parameters using LINO.HCl -PMA (3 mmol/L)-H_2SO_4 (50 mmol/L) system

Practically based on the gradual dilution for the minimum concentration in scatter plot			Theoretical based on the value of slope $x=3S_B/slope$	Theoretical based on the linear equation $\hat{Y}=Y_b+3Sb$	Limit of quantitative L. O. Q $\hat{Y}=Y_b+10S_b$
Newly developed method (0.0003)mmo/L	UV-Spectrophotometer (0.001) mmol/L	Turbidimetry method (0.0025) mmol/L			
19.935 ng/sample	0.443 µg/sample	11.075 µg/sample	21.677 ng/ sample	3.903 µg/ sample	13.005 µg/ sample

Table 4. Repeatability of LINO.HCl at optimum parameters with 150 µL sample volume

[LINO.HCl] mmol/L	$\bar{Y}_{Zi}$ (mV) average of responses (n=8)	RSD %	Confidence intervalat 95% $\bar{Y}_{zi}$ (mV)$_{0.05/2,\, n-1}\sigma_{\, n-1}/\sqrt{n}$
0.3	1248	0.207	1248±2.158
0.8	3220	0.043	3220±1.154

$\bar{Y}_{Zi}$ (mV): Energy transducer response expressed, $t_{0.05/2,7}$=2.365, n=number of injection

Determination of Lincomycin.HCl in drugs using ISNAG-fluoremiter analyzer and different classical methods

The newly developed method (ISNAG-fluoremiter) was used to determine Lincomycin HCl in three different samples of drugs from three different companies (Lincodar-500 mg-Dar Al Dawa-Jordan, LINCOMYCIN-500 mg-Hikma-Jordan, and Lincomycin-500 mg-S.D.I-Iraq). Using LINO.HCl-PMA (3 mmol/L)-H_2SO_4 (50 mmol/L) system; 150 µL sample volume and with 196 µL reaction coil that represent optimum conditions parameters (Chemical &physical parameters). The continuous flow injection analysis coupled with homemade ISNAG-fluorimeter analyzer, in which mercury tube lamp as a source and four solar cells at every two sides (0-90°) as a detector; and this compared with two classical methods which include UV-Spectrophotometric via the measurements of absorbance at λ_{max}=196 nm and turbidometry-method via turbidity-meter-HANNA-Taiuan. The measurements of scattering light at 0-90° for a white precipitate LINO.HCl-PMA (3 mmol/L)-H_2SO_4 (50 mmol/L) system were conducted by three methods. A series of solutions were prepared from each pharmaceutical drug via transferring 0.5 mL of each sample (5 mmol/L) to five volumetric

flasks (10 mL) followed by the addition of gradual volumes of standard solution (10 mmol/L) of Lincomycin.HCl (0, 0.1, 0.2, 0.3 and 0.4 mL) to obtained (0, 0.1, 0.2, 0.3 and 0.4 mmol/L) concentration for developed method. While UV-spectrophotometric method (classical method) by transferring 0.05 mL from 5 mmol/L of each sample to five volumetric flasks (10 mL) followed by the addition of 0, 0.01, 0.02, 0.03 and 0.04 mL from 10 mmol/L of a standard solution of Lincomycin HCl to obtain 0, 0.01, 0.02, 0.03 and 0.04 mmol/L; As for the turbidimetric method by transferring 0.5 mL of each sample (5 mmol/L) to five volumetric flasks (10 mL) followed by the addition of gradual volumes of standard solution (10 mmol/L) of Lincomycin HCl (0, 0.1, 0.2, 0.3 and 0.4 mL) to obtained (0, 0.1, 0.2, 0.3 and 0.4 mmol/L).

Table 5**a** and **b** were shown the results and mathematically treated using three different methods (developed method, UV-Spectrophotometric method, and turbidometry method), and in which a practical content of active ingredient at 95% confidence level and efficiency of determination; in addition to t-test.

First test: Using individual t-test comparison, the mean of practically of active ingredient (using Newly developed methodology) with claimed value (i.e., μ=500 mg) as shown in Table 5**b** (Column 5). The hypothesis depends on:

Null hypothesis: There is no significant difference between the means obtained from three different companies ($\underline{w}_i$) and claim value (μ = 500 mg), (Dar Al Dawa, 0.1346 g, Jordan), (Hikma, 0.13341 g, Jordan), (Iraq, 0.1401 g, India) obtained from three different companies

Against, Alternative hypothesis, there is a significant difference between the means and claim value [26]. According to the obtained results, there was significant difference between the means of practically value obtained from three different companies ($\underline{W}$i) compared to the Quoted value (μ=500 mg) due to some of t_{cal} more than t_{tab}. Therefore, the null hypothesis will be rejected, and the alternative hypothesis will be accepted.

Second test: Using paired t-test at α=0.05 of three drugs from different manufacturers. The comparison was made between the developed method using ISNAG-fluorimeter analyzer (depends on scattered light±90°) and turbidimetry via turbidity-meter, HANA, (Taiuan) as well as UV-spectrophotometer at λ_{max}=196 nm. The comparison results are summed up in Table 5**b** (Column 6). The Assumption as follows:

Null hypothesis:

H_0: $\mu_{\text{ISNAG-fluorimeter}} = \mu_{\text{UV-spectrophotometry}} = \mu_{\text{turbidimetry}}$

There is no significant different between the mean of turbidity method and ISNAG-fluorimeter analyzer against alternative hypothesis

i.e.; **H_1:** $\mu_{\text{ISNAG-fluorimeter}} \neq \mu_{\text{UV-spectrophotometry}} \neq \mu_{\text{turbidimetry}}$

The obtained results indicate no significant differences between the developed method and two classical methods (UV-spectrophotometric and turbidimetric methods).

i.e; $H_1 : \underline{w}_i \neq \mu$ (500 mg) for each different company

All value obtained $t_{cal} < t_{tab}$ (4.303) at α = 0.05 (at confidence level 95 %).

Based on the obtained results and 2 as a degree of freedom, the null hypothesis (H_0) will be accepted, and the alternative hypothesis will be rejected, which means there is no significant difference between the three methods.

Table 5a. Standard addition results for the determination of Lincmycin.HCl in three samples of drug using ISNAG-fluorimeter analyzer for developed method and two classical methods

No. of sample	Commercial Name, Company Content Country	Confidence interval For the average Weight of Tablet $\bar{w}i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Weight of Sample equivalent to 0.11075 gm (5 mmol/L) of the active ingredient W_i (g)	Theoretical content for the active ingredient at 95% (mg) $Wi \pm 1.96\ \sigma_{n-1}/\sqrt{n}$	Type of method Developed method using ISNAG Fluorimeter (mV) UV- Spectophotometru Classical method Absorbance measurement at λ_{max}=196 nm Turbidiometry method (Classical method) NTU Lincomycin-HCL mmol/L for Developed method Lincomycin-HCL mmol/L for Absorbance method Lincomycin-HCL mmol/L for Turbidiometry method					Equation of standard addition at 95% for n-2 $\hat{Y}Zi(mV)=a\pm sat+b\pm sbt$ [LINO.HCl]mmol/L $\hat{Y}_{Zi}=a\pm s_at+b\pm s_bt$ [LINO.HCl]mmol/L $\hat{Y}_{Zi}(NTU)=a\pm s_at+b\pm s_bt$ [LINO.HCl]mmol/L	r r^2 R^2%
					0	0.1	0.2	0.3	0.4		
					0	0.1	0.2	0.3	0.4		
					0	0.0 1	0.02	0.03	0.04		
					0	0. 1	0. 2	0. 3	0. 4		
1	Lincodar Dar Al Dawa LINCO=500 mg Jordan	0.6078±0.0131	0.1346	500±10.8152	525	766	980	1180	1400	537.400±30.227+2194.000±123.401 [LINO]mmol/L	0.9995, 0.9990, 99.90
					0.655	0.852	1.153	1.423	1.621	0.640±0.081+25.030±3.328 [LINO]mmol/L	0.9974, 0.9948, 99.48
					52	90	105	130	150	85.200±15.167+236.000±61.920 LINO]mmol/L	0.9899, 0.9800, 98.00
2	LINCOMYCIN Hikma LINO=500 mg Jordan	0.6023±0.0039	0.1334	500±3.2380	650	960	1200	1480	1720	670.000±51.701+2660.000±211.070 [LINO]	0.9990, 0.9981, 99.81
					0.47	0.691	0.851	1.023	1.241	0.480±0.049+18.740±1.986 [LINO]mmol/L	0.9983, 0.9967, 99.67
					75	101	135	160	190	74.400±5.456+289.000±22.274 [LINO]mmol/L	0.9991, 0.9982, 99.82
3	Lincomycin S.D.I LINO=500 mg Iraq	0.6325±0.0028	0.1401	500±2.1890	560	720	920	1130	1380	532.000±72.978+2050.000±297.932 [LINO]mmol/L	0.9969, 0.9938, 99.38
					0.580	0.750	1.020	1.230	1.480	0.556±0.064+22.800±2.625 [LINO]mmol/L	0.9980, 0.9961, 99.61
					78	108	133	163	198	77.000±7.462+295.000±30.465 [LINO]mmol/L	0.9984, 0.9968, 99.68

$\bar{Y}_i$: Energy transducer in mV for developed method, without unite for UV-Spectrophotometric method and Turbidimtric method in NTU, for (n=3), r:correlation coefficient, r^2 coefficient of determination, R^2%:Percentage capital R-squared:R^2= explain variation as a percentage / total variation , $t_{0.05/2,\infty}$ =1.96 at 95 %, $t_{0.05/2,3}$ =3.182 for n=5

Table 5b. Summar of results for practical content, (Rec%) efficiency for determination of Lincomycin.HCl in three samples of drugs and t-test for comparison between two methods

No. of sample	Type of method Developed method using ISNAG Fluorimeter (mV) UV- Spectophotometru Classical method Absorbance measurement at λ_{max}=196 nm Turbidiometry method (Classical method) NTU Practical concentration (mmol.L^{-1}) in 10 mL Practical concentration (mmol.L^{-1}) in 50 mL Practical weight of LINO.HCl in (g)	Weight of LINO.HCl in each sample (g) $\overline{W}_{i(g)} \pm 4.303\ \sigma_{n-1}/\sqrt{n}$ Weight of LINO.HCl in tablet $\overline{W}_{i(mg)} \pm 4.303\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Rec.%	Individual t-test For compared between claim value & practical value $(\overline{W}_{i(mg)}-\mu)\ \sqrt{n}\ /\sigma_{n-1}$	Paired t–test Compared between two methods $t_{cal}=\overline{w}d\ \sqrt{n}\ /\sigma^{*}_{n-1}$	t_{tab} at 95%confidence level (n-1)
1	0.2483 4.9667 0.1100	0.1100±0.0007 496.816 mg±3.283	99.36	/- 4.173 / <4.303	Paired t-test for development with UV-Sp. $\overline{X}$ d= 2.576 ,σ_{n-1}= 25.04 0.178 < 4.303 $t_{cal} < t_{tab}$	
	0.0256 5.1150 0.1133	0.1133±0.0004 511.467 mg±1.7820	102.29			
	0.2466 4.9322 0.1093	0.1093±0.0006 493.362 mg±2.9700	98.67			
	0.2519 5.0375 0.1116	0.1116±0.0004 503.766 mg±1.9830	100.75			
2	0.0256 5.1270 0.5127	0.5127±0.0030 512.692 mg±2.9820	102.54	8.172>>4.303		
	0.2574 5.1488 0.1140	0.114±0.0009 514.885 mg±4.2500	102.98		Paired t-test for development with turb. $\overline{X}$ d= - 3.558 ,σ_{n-1}= 7.3019 / - 0.844/ < 4.303 $t_{cal} < t_{tab}$	
	0.2595 5.1902 0.1150	0.11450±0.0006 519.019 mg±2.9820	103.80			
3	0.0244 4.8772 0.1080	0.1080±0.0003 487.715 mg±1.3570	97.54	27.445>>4.303		
	0.2610 5.2203 0.1156	0.1156±0.0011 522.029 mg±4.8900	104.41			

μ: claim value=500 mg, $\overline{W}$i:mean of practical weight (n=3), $\overline{W}$d: average of different between two methods (developed method &*Turbidimetric(turb.) (NTU)Using Hanna Instrument (classical method), for n (No.of samples)=3, σ_{n-1}: standard deviation, σ^{*}_{n-1}:standard deviation of difference (paired t-test), $t_{0.025,2}$= 4.303. sp: spectrophotometric

Conclusions

In this research study, a new turbidimetric, simple, sensitive, accurate, and fast method was used to determine the lincomycin HCI in pharmaceutical drugs using a newly developed homemade ISNAG-fluorimeter-CFIA. The comparison between this work with classical turbidometry and UV spectrophotometer method via the t-test (as the comparison tools) was shown that the newly developed method (ISNAG-fluorimeter procedure) is as good as the classical method.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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References

[1]. British Pharmacopoeia, Ministry of Health and Social Services for Northern Ireland, United Kingdom: The Stationary Office, **1988**, p 334
[2]. Egutkin N.L., Maidano V.V., Nikitin Y.N. *Pharmaceutical Chemistry Journal*, 1984, **18**:128
[3]. Xue-zhong F., Guang-Huil W., Bing-Huz F., huapengi L. Pan yuan-zhil and Wen zi-ming I.: Determination of Lincomycin hydrochloride Injection by UVSpectrophotometry.
[4]. Goodman and Gilman's. The pharmacological basis of the rapeutics,10th edition, Mc-Graw Hill medical publish division, 2001, p 1256
[5]. Indian Pharmacopoeia, Ghaziabad; The Indian Pharmacopoeia Commission, Delhi. Manager of Publication, 2010, p 1588
[6]. Szúnyog J., Adams E., Liekens K., Roets E., Hoogmartens J. Journal of Pharmaceutical and Biomedical Analysis, 2002, **29**:213
[7]. El Ries, M.A. *Analytical Letters*, 1994, **27**:1517
[8]. Sweetman, S.C. editor. Martindale: The Complete Drug Reference, 33rd edition, London, Royal Pharmaceutical Society of Great Britain, The Pharmaceutical Press, 2002, p 219.2
[9]. Birkenmey R.D., Kagan F. *Journal of Medicinal Chemistry*, 1970, **12**:465
[10]. Jszunyog. *J Pharm Biomed*., 2002, **29**:13
[11]. United States Pharmacopoeia: The National Formulary, Rockville, MD: United States Pharmacopoeia Convention, Inc.: 1990, XXII, XVII, p 771
[12]. Egutkin N.L., Maidano V.V., Nikitin Yu E. *Pharm Chem J*., 1984, **18**:128
[13]. EL Ries M.A. *Anal Lett*., 1994, **27**:1517
[14]. Al-Awadie N.S.T., Ahmed F.K. *Journal of Kerbala University*, 2013, **11**:153
[15]. Al-Awadie N.S.T., Hussein F.A. *Journal of Global Pharma Technology*, 2018, **10**:447
[16]. Al-Awadi N.S.T., Munshid H.Q. *Journal of Pharmaceutical Sciences and Research*, 2019, **11**:1563
[17]. Nagham S. T., Mustafa K.K., *International Journal of Research in Pharmacy and Chemistry*, 2016, **6**:759
[18]. Nagham S.T., Manhl H.I. *International Journal of Research in Pharmacy and Chemistry*, 2016, **6**:230
[19]. Al-Awadie N.S.T., Khudhair A.F. *Iraqi Journal Science*, 2014, **55**:12
[20]. Al-Awadie N.S.T., Ibraheem M.H. *Int J Res Pharm Chem*., 2016, **6**:230
[21]. Al-Awadie N.S.T., Hussein F.A. *Journal of Applied Chemistry*, 2018, **11**:25

[22]. Al-Awadie N.S.T., Hussein F.A. *Journal of Global Pharma Technology*, 2018, **10**:447
[23]. Hussein G.F., Turkey N.S. *Eurasian Chem. Commun*., 2021, **11**:763
[24]. Rajalakshmi S., Subbiramaniyan K., Shen-Ming C. *Ultrasonic–Sonochemistry,* 2019, **54**:68
[25]. Sabri Najim S. *International Journal of Development Research*, 2017, **7**:11813
[26]. Hussein G.F., Turkey N.S. *Chem. Methodol*., 2021, **5**:498

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