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FULL PAPER

Ciprofloxacin assessment *via* the formation of precipitate reaction product with ammonium metavanadate as a reagent using ISNAG-fluorimeter

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The assessment of Ciprofloxacin.HCI using continuous flow injection diverging light was investigated. A moder, simple, rapid, and sensitive approach has been developed by using an ISNAG-Fluorimeter analyzer for assessing Ciprofloxacin.HCI pure state as well as pharmaceuticals (tablets); the process was based on the interaction of Ciprofloxacin.HCI with ammonium metavanadate (v) (AMV) as a precipitating agent to yield an ion pair association yellow precipitate rapidly in salt medium. To increase the affectability of the established approach, optimum parameters have been selected. For the instrument response vs Ciprofloxacin-HCl concentrations, the scatter plot range and linear dynamic range were 0.0-50 mmol/L. For linear dynamic range, the correlation coefficient (r) was 0.98623, and the percentage linearity (R^2 percent) was 97.26%. With a concentration of 10, 35 mmol/L, the percentage linearity for repeatability (n=8) was less than 0.34 percent. The detection limit of gradual dilution for the lowest concentration in the scatter plot range of the calibration curve was 0.221 g/sample. Ciprofloxacin.HCI in pharmaceutical tablets was effectively estimated using the described approach. The individual t-test with 95 percent confidence (α=0.05) was used to analyze drugs using the usual incremental technique. The results were also compared to those obtained using traditional UV-Spectrophotometric techniques at a wavelength of 272 nm. The ISNAG-Fluorimeter analyzer and traditional techniques yielded no significant differences in the results.

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KEYWORDS

Ciprofloxacin.HCI; flow injection; diverge light; ISNAG-fluorimeter analyser.

Introduction

Ciprofloxacin hydrochloride is a fluoroquinolone, a broad spectrum second generation microbiological agent activity against Gram-positive and Gram-negative bacteria such as pseudomonas aeruginosa, streptococcus faecalis, staphylococcal aureus, and enterobacter aerogenes. Antibiotic Ciprofloxacin complex exerts its disinfectant effect by inhibiting the synthesis of DNA in bacteria, preventing their growth and the spread of infection. Tablets have been marketed since 1987 for the treatment of a wide variety of infectious diseases in adults [1].

Ciprofloxacin hydrochloride has IUPAC name: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [1] and molecular formula: $C_{17}H_{19}ClFN_3O_3$ [2].

The followings are the chemical and physical properties:

Trade names: Ciloxan, Cipro, Neofloxin, others.
State: Solid (i.e., white powder with bitter taste.)
Molecular weight: 367.802 g/mol.
solubility: soluble in acetic acid, and slightly soluble in water, methanol, ethanol, or acetone, Melting point (°C): 255-257 pKa (Strongly Acidic) 5.76
pKa (Most grounded Basic) 8.68 and biological half-life 3.5 h [3,4].

Ciprofloxacin hydrochloride is used to treat several diseases, such as urinary tract infections, ophthalmic, respiratory, bone and joint, intraabdominal infections, bacterial diarrheal diseases and periodontal microorganisms [5,6]

Its side effects are as follow: CNS nervousness, agitation, anxiety, confusion, depression, orthostatic hypotension, vasculitis, blurred vision, burning, stinging, irritation, nausea, vomiting, diarrhea, constipation, discomfort, albuminuria, candiduria, renal calculi, rashes, exfoliative dermatitis, and erythema [5,7] .

The aim of the present study is establishing a new, accurate, sensitive and simple method by flow injection analysis combined with ISNAG-Fluorimeter analyzer for determination of Ciprofloxacin.HCl as ionic pair precipitated using metavanadate (v) (AMV) as a reagent.

Materials and methods

Analytical reagents, all chemical substances, and distilled water were used in all solutions ($C_{17}H_{18}FN_3O_3$.HCl. 3.8580 g/100 mL (SDI) , Na_2CO_3 5.299 g /100 mL (Fluka), CH_3COONH_4 3.884 g/100 M (BDH), KCl 7.350 g/100 mL (Hopkin&Williams), NaCl 7.313 g/250 mL(BDH), NH_4Cl 2.675 g/100 mL (BDH),, CH_3COOH 57.47 mL/L (BDH), HCl 88.28 mL/L (BDH), Tartaric acid ($C_4H_6O_6$) 7.513 mg/200 mL (T.Homas baker), ascorbic acid ($C_6H_8O_6$) 8.807 gm/200 mL (Himedia) and H_2SO_4 55.52 mL/L (BDH)).

Sample preparation of Ciprofloxacin. hydrochloride (CIP.HCl)

Twenty tablets, containing 500 and 750 mg CIP.HCI, were weighted, then crushed and ground, resulting in 0.9195 g of active ingredient (50 mmol/L) in each batch for (AL-jazeera-750 mg-Iraq, Micro labs limited -500 mg- India, citro pharma Inc-500 mg- Canada and pioneer-500 mg-Iraq). A solution of distilled water was made to dissolve the powder. The volume was increased to 25 mL in order to remove any undissolved materials that may have affected the calibration. A hot distilled water wash was performed on the residue, and volume was increased to 50 mL with the same solvent (distilled water).

Apparatus

The apparatus used in this article are as follows:

- Two-line sophisticated design system for precipitating agent and CIP use. The nuances of the complex used were depicted in HCI (Figure 1).
- Two speed-changing channels in the peristaltic pump (Ismatec, Switzerland).
- 6-Port medium-pressure injection valve with test loop (I D E X corporation, USA) (1 mm i.d. Teflon variable length)
- The yield response was recorded using a potentiometric recorder (Siemens, Germany).
- UV light spectrophotometer (Shimadzu 1800).

Methodology

Ciprofloxacin.HCl was tested in a two-line manifold unit configuration using ammonium metavanadate (v) (AMV) as a precipitating agent. Yellow precipitate is most likely formed as an ion pair (Figure 1). A carrier stream will pass through the injection valve to drain the sample segment (150 mL with open valve mode) with 12 mmol/L CIP.HCl concentration and connect with a second stream at the Y-junction point to create the precipitate (3.20

mL flow rate for each line). This precipitate will be exposed to two primary wavelengths, 184.9 nm and 253.7 nm (prominent). Because of their high frequency, both lines are readily diverged. All types of dispersed light will be detected at 90° using a route of 2 mm optical aperture stretched for 100 mm distance using two sides solar cells, each side containing four solar cells (i.e., 2[4x2.5 cm (long) [8-4]. Sketch 1 depicts the mechanism of reaction.

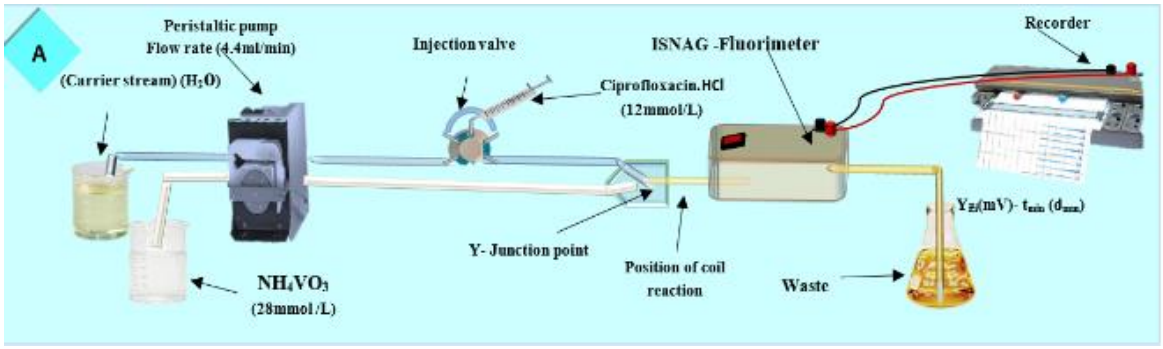
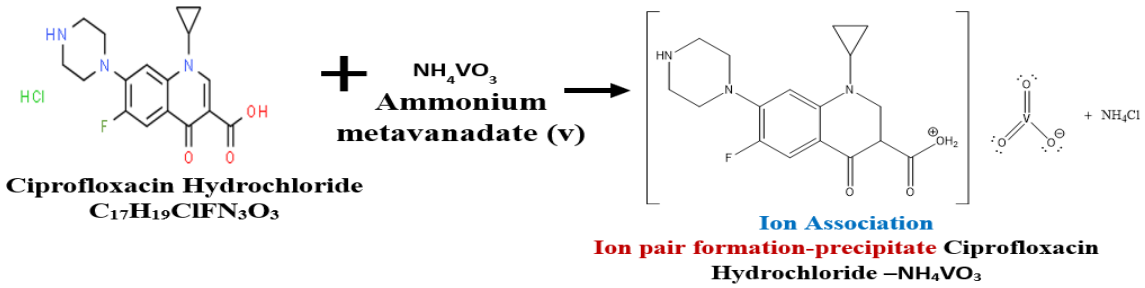


FIGURE 1 The flow gram of the manifold was employed throughout this investigation



SCHEME 1 Proposal mechanism for the reaction between Ciprofloxacin.HCl and ammonium metavanadate (V) [15,16]

Rustle and discussion

Chemical variables

Effect of ammonium metavanadate (V) (AMV) concentration on (S/N) response function

Variables concentrations of precipitator 4-68 mmol/L were synthesized, using 78.5 µL sample volume, distilled water as a carrier stream, 12 mmol/L concentration of CIP.HCl injected at injection valve with 4.4 mL/min flow rate to the carrier stream; moreover, precipitator stream were used. It was observed (Figure 2) that an increase in AMV concentration up to 20 mmol/L causes a rise in diverge light in terms of peak height. It is probable that this will enhance the divergence of light toward the solar cells. An excess of AMV more than 20 mmol/L causes a considerably greater effect of agglomeration development of nuclei in a short period of time, preventing effective diverged light from reaching the detector. The total obtained output were summed up in Table 1A. Slope-intercept method of choice for the optimum concentration that will be used as a reagent will be regarded as the best way for the selection of AMV concentration. Four segmentations were used as shown in Figure 2. A Plot of Y_{Zi}(mV) versus reagent concentration was based on real chart recorded of some selected concentration-profile. Table 1-B shows the r, a and b values. The researcher found that segment No.2 will satisfy the request of high sensitivity and high responses profile. The choice will be in the region of 20-28-40 mmol/L i.e., 20 mmol/L →40 mmol/L region which represent highest

intercept (sensitivity) and 20 mmol/L was the choice which was within the chosen segment. This will give much more segment for much more accurate choice than choosing the apex of prepared solution. The researcher will use moving average instead of the apex point prepared by the chemist.

TABLE 1A AMV concentration affects the evaluation of (S/N) energy transducer responses, which are essential to determining the CIP.HCI.

$[NH_4VO_3]$ mmol/L	$\bar{Y}_{Zi}$ (mV) average (n=3)	RSD %	Confidence interval at 95% $\bar{Y}_{Zi}$ (mV) $\pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
4	64	1.89	64 ± 3.006
12	480	0.44	480 ± 5.217
20	792	0.12	792 ± 2.435
28	744	0.16	744 ± 3.006
40	736	0.16	736 ± 3.006
60	728	0.18	728 ± 3.329
68	672	0.18	672±3.900

TABLE 1B A slope-intercept segmentation pattern is used to select the optimum segment of concentration (i.e., a_3-a_5)

No. of segment	Range of $[NH_4VO_3]$ mmol/L	Segment	Slope mV/mmol.L^{-1}	Intercept mV	r*
1	4-20	$a_1 - a_3$	45	-100	0.9966
2	20-40	$a_3 - a_5$	-2	834	0.8746
3	40-68	a_5-a_7	-1	819	0.7954

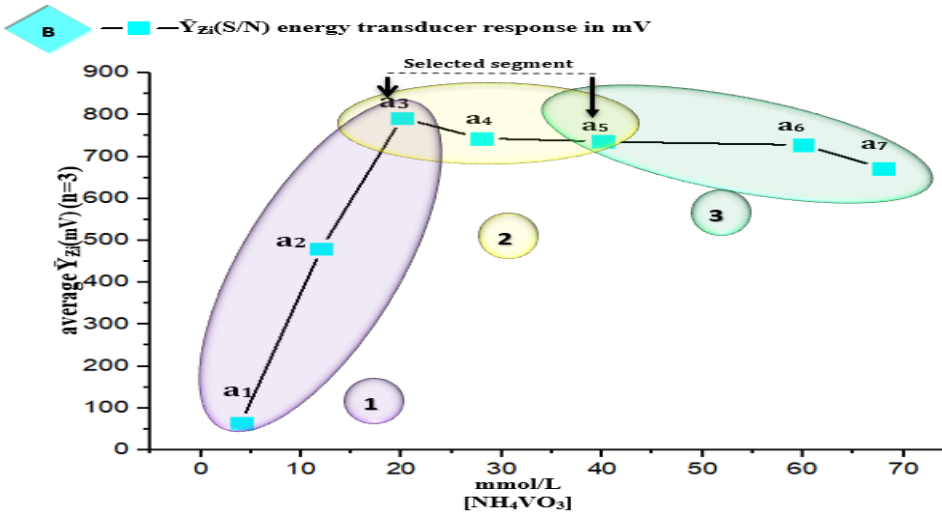


FIGURE 2 The effect of different AMV concentrations on the height of $\bar{Y}_{Zi}$ (mV): (S/N) energy transducer response (n=3) in mV, and three data points as one segment, their interaction and selection

Effect of different salts and acids as a carrier stream on diverge light response±90°

The reaction of CIP.HCI (12 mmol/L) with AMV (20 mmol/L) was studied in different media of salts and acid (50 mmol/L) (Na_2CO_3, CH_3COONH_4, KCl, NaCl, NH_4Cl and Na_2SO_4, Tartaric acids ($C_4H_6O_6$), CH_3COOH, HCl, H_2SO_4 and HNO_3) solution as a carrier stream instead of distilled water. Figure 3 shows that different salt and acid solutions cause a decrease in S/N response when compared to

D.W; there may be more vacant spaces between agglomerates of particulates due to its effect on increasing agglomeration and compactness rather than increasing the intensity of light transmitted through the particulate. On this premise, the salts and acids were cancelled throughout the project, making distilled water the best carrier stream.

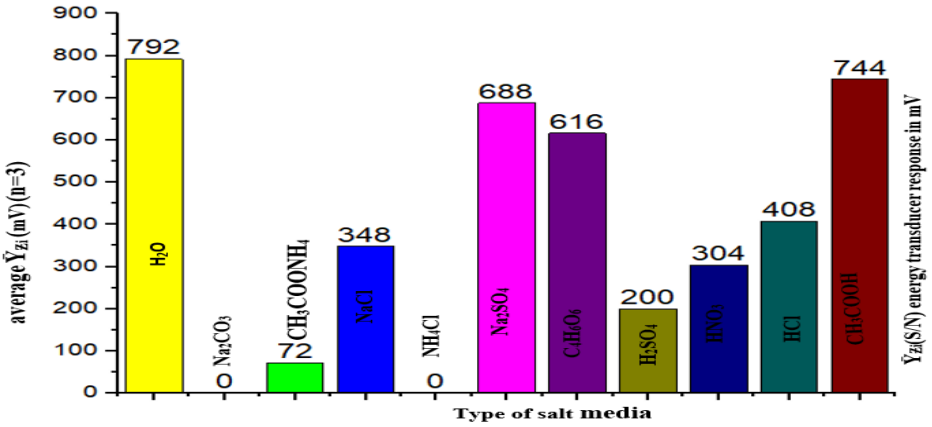


FIGURE 3 TA comparison of the effects of various salts and acids on the height of $\bar{Y}_{zi}$ (mV): (S/N) energy transducer response (n=3) in mV

Effect of physical parameters

Effect of flow rate

Flow rate express amount of fluid that will be pumped out to the manifold system. Variation of flow rates ranging from 1 mL/min to 7.2 mL/min for each line were used for CIP.HCl (12 mmol/L)-AMV(20 mmol/L) system, 78.5µL and open valve mode In Figure 4, dispersion and dilution improve at low flow rates, resulting in an increase in $*t_B$ (base width of the profile) and broadening of response maxima while at higher speed leading to regular profile and very sharp maxima specifically at 3.2 mL/min. Therefore, a flow rate 3.2 mL/min was used for both lines (reagent stream and carrier stream) as a compromise to obtain narrower Δt_B, minimizing the consumption of reactant solution. Table 2 tabulates the slope-intercept method and its segmentation; it shows the segmentation sectors and how to use range of values instead of single point reading. A region between 3.2 up to 5.4 mL/min is the selected segment of optimum working condition and 3.2 mL/min was chosen.

TABLE 2 Calculation of the slope-intercept of a segmentation pattern to identify the optimal segment for flow rate (i.e.; a_4-a_6)

No. of segment	Flow rate range (mL/min)	Segment	Slope mV/mL.min^{-1}	Intercept mv	r
1	1-2.5	$a_1 - a_3$	-261	1389	0.9952
2	3.2-5.4	a_4-a_6	-35	930	0.9265
3	5.4-7.2	a_6-a_8	-98	1273	0.9105

r: Correlation coefficient

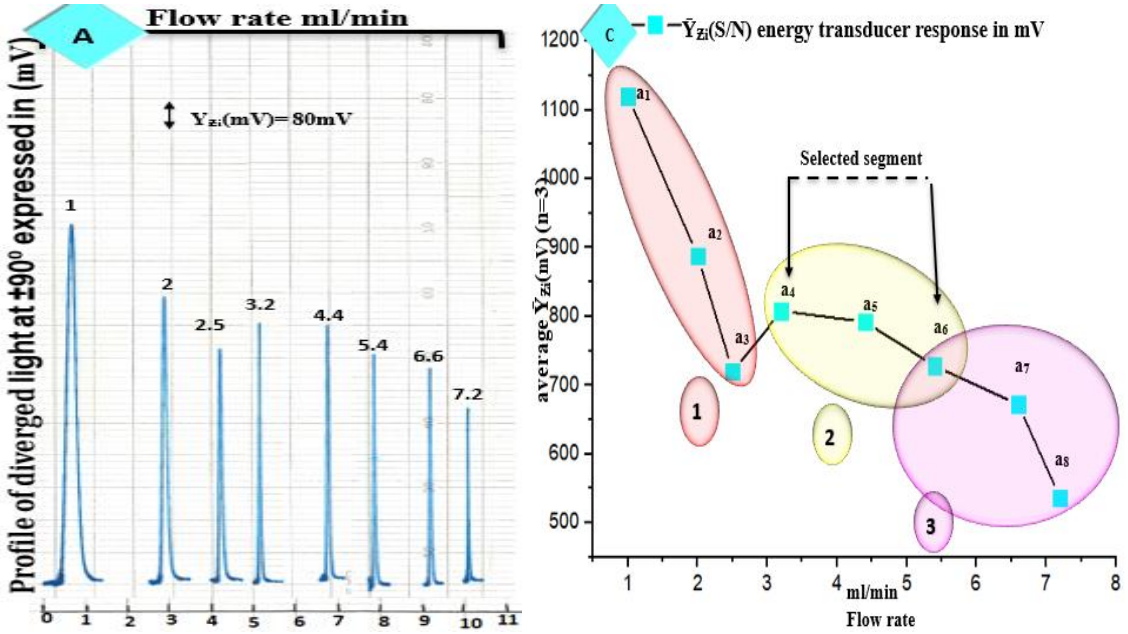


FIGURE 4 Effect of flow rate on the height of $\bar{Y}_{Zi}$ (mV): (S/N) energy transducer response (n=3) in mV and three data point as one segment of their interaction selected

*Effect of sample volume*Using CIP.HCI (12 mmol/L)-AMV(20 mmol/L) system, the sample volume was studied. Six variable lengths of Teflon tube of inside diameter of 1 mm were used. A suitable Teflon tubes were cut to have different volumes ranging 40, 78.5, 135, 150, 200 and 281 µL. The optimum parameter of flow rate at 3.2 mL/min, 60 sec(10mm) scanning rate of chart recorder, and 2V (80 mV)-output recorder were used. Table 3-A tabulates the volume and output voltage of signal. A choice is made on the basis of lower acceptable slope and r due to extended linearity plus high intercept. Because a large sample volume may, in some cases, block a narrow tube diameter, other considerations were taken into account, a choice was made for segment No. 3 i.e., range of 150→281 µL. 150 µL was selected as the ideal sample as summed up in Table 3-B.

TABLE 3A Effect of sampling volume variations and corresponding measurement output with CIP.HCI (12mmol/L) and AMV(20mmol/L) systems

Length cm	Sample volume µl $v = r^2\pi h$ r= 0.5 mm	$\bar{Y}_{Zi}$ (mV) average (n=3)	RSD %	Confidence interval at 95% $\bar{Y}_{Zi}$ (mV) $\pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	Δt_B Sec	V_{add} mL at flow cell	Concentration mmol/L at flow cell	D.F at flow cell	t* sec
5.1	40	616	0.19	616±3.006	18	2.060	0.815	14.71	17.22
10.0	78.5	808	0.13	808±2.708	17.7	2.124	0.803	14.94	17.7
17.2	135	976	0.13	976±3.205	18.1	1.991	0.791	15.17	18
19.12	150	1200	0.09	1200±2.857	20.4	2.316	0.725	16.55	19.2
25.47	200	1072	0.10	1072±2.708	20.8	2.487	0.965	12.43	19.6
35.8	281	1064	0.10	1064±2.559	21	2.38	0.76	17.00	19.8

*: Time taken for the injection valve to reach the measurement cell (in seconds), Δt_B: The width of the base of the peak (Sec), $t_{0.05/2,2=4.303,}$ $\bar{Y}_{Zi}$ (mV): (S/N) The average peak heights of energy transducer responses (n=3) in mV. D.F: Dulition facter

TABLE 3B Segmentation pattern slope-intercept to select the optimum segment of sample volume (i.e.; a_4-a_6)

No. of segment	Range of Sample volume µL	Segment	Slope mV/µL	Intercept mV	r
1	40-135	$a_1 - a_3$	3	484	0.989
2	135--200	a_3- a_5	0.4	1008	0.1394
3	150-281	$a_4 - a_6$	-0.9	1311	0.8213

r: Correlation coefficient

Effect of reaction coil expressed as a volume (in microliter)

Variable delay reaction coils (0-235.5 µL) were used for this study, which was carried out on the CIP.HCI (12 mmol/L)- AMV (20 mmol/L) system. In most cases, adding more length to the flow gram in CFIA will improve dispersion through a variety of processes (e.g. convection or diffusion). Reaction coil was attached after Y-junction point in the manifold design as shown in Figure 1. The profile and effect of delay reaction coil on sensitivity expressed as an S/N energy transducer response which is presented in Figure 5 from the result of each reaction coil with a variable volume reaction coil (varying length of the coil), dilution of the sample segment reaction product will be introduced leading to an even distribution, where with a high volume, precipitate in a highly dispersed pattern causes a weaker signal. Moreover, there was a decrease in sensitivity with an increase in coil length, which might be due to the generation of small particles and distributing them over a larger surface area, making them impossible to detect, i.e., spectrum filtering. The effect of the presence of tiny particles is abolished since the number of particles is dispersed over a vast volume. As a result, delayed reaction coils were not used in this study.

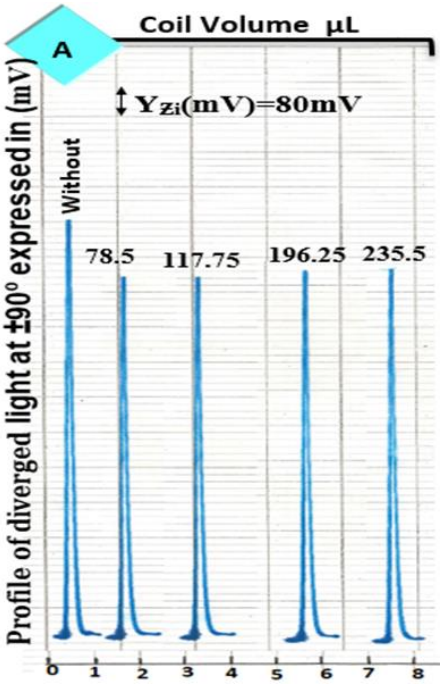


FIGURE 5 Effect of mixing coil length on the response profile Y_{zi} (mV)-t_{min} (d_{mm}). CIP.HCI (12 mmol/L)-AMV(20 mmol/L)system

Estimation of linear dynamic range based on scatter plot variety of Ciprofloxacin.HCl. via (S/N)

Chemical and physical factors were achieved in the previous sections using AMV (20 mmol/L) as a precipitant agent, distilled water as a carrier stream, a 150 L- open valve mode sample volume, 3.2 mL/min flow rate per line and without coil.

A series of solutions for CIP.HCI were prepared in order to study calibration graph representing by linear equation for scatter plot range and linear range $\hat{Y}_i=a\pm s_a t+b\pm s_b t$ [CIP.HCI] mmol/L [17,18]. Additionally, the correlation coefficient r, coefficient of determination r^2, % capital R-squared R^2% and t-value only the new developed methodology but also available classical methods (spectrophotometric). Therefore, a comparison between newly method and literature cited methods was made. A series of solutions for [CIP.HCI] (0.01-50 mmol/L(n=20)) were prepared for the assessment by ISNAG-Fluorimeter. All estimation was reduplicated three times. All of (S/N) response (output) of the peak height (mV) was plotted versus the CIP.HCI concentration. An increase in CIP.HCI concentration leads to an increase in peak height response due to regular increase of precipitated particle plus its convenient movement with the optimum flow rate used which in turn causes the increase of diverged light (as an illustration, deals with the CIP.HCI concentration and the S/N energy transducer response being exactly proportional). The correlation coefficient r =0.98623, coefficient of determination r^2 = 0.97.26264, and percent capital R-squared R^2=97.26 percent were obtained from a scatter plot range for a trial concentration (0.01-50 mmol/L(n=20). As a result, in order to enhance the assessment of the mathematical formulation, a shorter range (0.01-40 mmol/L (n=19) should be used to increase the correlation coefficient r=0.99390 and percent capital R-squared R^2= 98.78 percent should be employed, as shown in Table 4. Similarly, the UV-spectrophotometric approach, which is based on measurements of absorbance in the UV-region for a variable concentration range of 0.001-0.1 mmol/L, is based on observations of absorbance in the UV-Region.at λ_{max}=272 nm (Figure 6) [19,20]. The correlation coefficient for the range (0.001-0.1 mmol/L(n=14) was r =0.98616, the coefficient of determination was r^2 =0.97251, and the percent capital R-squared R^2 percent =97.25. A narrower range was used (0.001-0.09 mmol/L(n=13)). As a result, the best linear range is obtained, with a correlation coefficient of 0.99056 and a percent capital R-squared of 98.12%. In the end, each method's t-calculate exceeds t_{tab} (i.e., t-value > t_{tab}), suggesting that the linearity versus non-linearity is acceptable. In Table 4, all data is summarized.

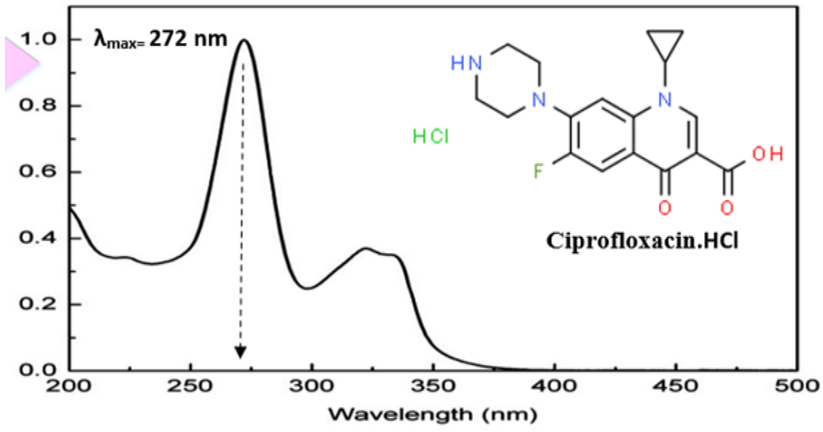


FIGURE 6 Absorbance UV-spectra of CIP.HCI standard solutions dissolved in water against distill water as a reference by UV-spectrophotometer (Shimadzu 1800 (classical method))

TABLE 4 Summarizing the results of the various range regressions using the first degree equations of linear $\hat{Y}$ =a+bx with Ciprofloxacin.HCl concentration at the ideal condition

Type of mode	Range of [CIP.HCl] mmol/L (n)	$\hat{Y}_i = a \pm s_a t + b \pm s_b t$ [CIP.HCl] mmol.L^{-1} at confidence level 95%, n-2	r r^2 R^2%	t_{tab} at 95 % , n-2	Calculated t-value $\frac{\lvert r\rvert\sqrt{2}-2}{\sqrt{1-r^2}}$
ISNAG-Fluorimeter is used for developing the method. UV. Sp Traditional absorbance measurement method at λ_{max}= 272 nm					
Scatter plot	0.01-50 (20)	203.632±127.310+75.058±6.234 [CIP.HCL] mmol/L	0.98623 0.97264 97.26	2.101	**25.298**
	0.001-0.1 (14)	0.096±0.108+21.295±2.252 [CIP.HCL] mmol/L	0.98616 0.97251 97.25	2.179	**20.602**
Linear range or Liner dynamic range	0.01-40 (19)	141.289±81.750+82.068±4.662 [CIP.HCL] mmol/L	0.99390 0.98783 98.78	2.110	**37.142**
	0.001-0.09 (13)	**0.066±0.192+22.895±2.103 [CIP.HCl]mmol/L**	**0.99056 0.98121 98.12**	**2.201**	**23.966**

$\hat{Y}_i$: Estimated response(n=3) in mV for modern technique and without unit for uv-spectrophotometric. r: Correlation coefficient, r^2:Coefficient of determination, R^2:%capital R-squared, R^2= explain variation / total variation, n:no.of measurements, $t_{tab.}$=$t_{0.05/2,n-2.}$

Detection limit (L.D)

A study was achieved to estimate the limit of detection of CIP.HCl by three variation methods under optimum condition, which is tabulated in Table 5.

1. Practical approach: depends on gradual dilution of the lowest concentration in scatter plot (Using Newly developed method ISNAG-Fluorimeter (4 µmol/L) and UV-spectrophotometric method as a classical method (0.8 µmol/L).

2. Theoretical approach:

A. Depends on slope method and based on the dynamic range for newly developed method.

B. Depends on the dynamic range or linear range due to low value of residual ($S_{y/x}$) which equals to S_b of the form $\hat{Y}=Y_b+ 3 S_b$. Y_b [19],[20].

TABLE 5 Detection limit of CIP.HCl using 150 µL and three ways

Practically depended on the progressive dilution for the minimum concentration in calibration curve		Theoretical (slope method) depend on the value of slope X= $3S_B$/slope	Theoretically (linear equation) depend on the value of $\hat{Y}= Y_b +3S_b$
Newly developed method (4) µmol /L ($\tilde{Y}_{zi}$ (mV)	Classical method UV– spectrophotometric (0.8) µmol/L (Absorbance)		
0.221 µg/sample 32 mV	0.294 µg/sample 0.0456	1.371 µg/sample	233.370 µg/sample

S_B: Standard deviation of blank for 13 times, Y_b: average response for blank= intercept (a), S_b: Standard deviation equal to $S_{y/x}$ (residual)

Repeatability

The repeatability and efficiency of ISNAG-Fluorimeter readings, which are equivalent to the relative standard deviation represented as a percentage using a resonant mercury lamp, were investigated at a constant Ciprofloxacin concentration. In optimal parameters, HCl was used at two concentrations (10 and 35 mmol/L). Table 6 summarizes the results of a

repetition of measurements for eight consecutive injections as estimated and the output achieved, with the highest relative standard deviation (RSD percent) less than 0.34 percent.

TABLE 6 Repeatability results of CIP.HCI

[CIP.HCI] mmol/L	$\bar{Y}_{Zi}$ (mV) average (n=8)	RSD %	Confidence interval 95% at $\bar{Y}_{Zi}$ (mV) ± $t_{0.05/2,7}\ \sigma_{n-1}/\sqrt{n}$
10	976	0.33	976±2.717
35	3040	0.13	3040±3.328

$\bar{Y}_{Zi}$ (mV): S/N as an mV energy transducer response expressed, $t_{0.05/2,7}$=2.365, n= number of injections.

Comparison between two using different methods of analysis CIP.HCI i.e., ISNAG-Fluorimeter versus UV-spectrophotometric measurement

An attempt was made to establish a comparison between the new method (ISNAG-Fluorimeter) and traditional method (UV-spectrophotometric) through applying the same series of CIP.HCI concentration in both methods. When making such a comparison, the primary concern will be determined, which is more sensitive than the other and dose the new method give results that are significantly higher or lower than the traditional method. The two methods are to be compared at corresponding analyte concentration whose results were summarized in Table 7. This idea was applied for individual untreated data to find the linear regression of developed method using AMV (20 mmol/L), 150 µL- sample volume open valve mode, 3.2 mL/L flow rate for each line and without coil, and traditional method, which both have a range from 0.01–0.1 mmol.L^{-1}. The linear regression have a slope value 2039.706 mV/mmol.L^{-1}, while the linear regression plot for the UV-spectrophotometric method using the same range of concentration have slope of 19.772 absorbance/mmol.L^{-1}.

If it is assumed that Y_1 is the slope (2039.706 mV /mmol.L^{-1}) of the linear regression plot of the ISNAG-Fluorimeter method. Which is equivalent = Response$_1$/ concentration …1

And if it is assumed that Y_2 is the slope (19.772 absorbance/mmol.L^{-1}) of the linear regression plot of the UV-spectrophotometric method

Which is equivalent = Response$_2$/concentration… 2

Divide equation no.1 by equation no. 2

The result is =R_1/R_2 … 3

=2039.706/19.772 = 103.153

A slope ratio shows that the value of 103.153 fold ISNAG-Fluorimeter is more sensitive than UV-spectrophotometric method.

TABLE 7 Analysis of two methods demonstrating the effects of CPH-HCl concentration on the response of the (S/N) energy transducer (n=3) in mV by ISNAG-Fluorimeter analyzer and absorbance without uinte by UV– spectrophotometric

[CIP.HCI] mmol/L	ISNAG-Fluorimeter analyzer development method	UV– spectrophotometric Classical method
	$\bar{Y}_{Zi}$ (mV) average (n=3)	Absorbance at λ_{max} =272 nm
0.01	32	0.271
0.02	45	0.520
0.03	80	0.768
0.04	108	1.040
0.05	137	1.300
0.06	163	1.560
0.07	170	1.780
0.09	193	1.890
0.10	210	1.990

In addition, when using another idea, one axis of the regression graph is used for the responses obtained by new method while the other axis is used for the results obtained by applying the reference or comparison method to the same concentration. Each point on the graph represents a single sample analyzed by two separate methods. Applying all this to the data in Table 7 assumes that the X-axis is the response of the UV-spectrophotometric method while the Y-axis is for the ISNAG-Fluorimeter method. Figure 7 shows the plot of ISNAG-Fluorimeter method response (R_1) in mV (Y-axis) *vs.* UV-spectrophotometric method response(R_2) (X-axis) that gives a slope 102.350 mV/absorbance. So the ISNAG-Fluorimeter is more sensitive than UV-spectrophotometric method according to slope ratio. It indicates that the response of R_1 (i.e. ISNAG-Fluorimeter) is more sensitive assuming zero intercept. At non zero intercept, one of the methods is more sensitive than the other by constant value of concentration.

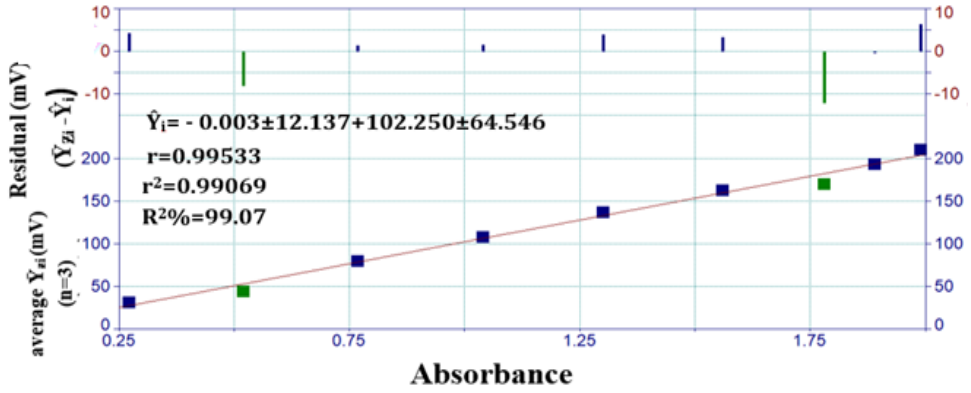


FIGURE 7 Calibration graph for the variation of CIP-HCl concentration represented by (S/N) energy transducer response (n=3) in mV by diverged of incident light vs. Absorbance UV-spectra

Applications of the ISNAG-Fluorimeter for determining different drug concentrations

Four different companies of drugs (AL-jazeera-750 mg-Iraq, Micro labs limited-500mg-India, citro pharma Inc-500mg-Canada and pioneer-500 mg-Iraq) were selected.

Ammonium metavanadate were injected on 20 mmol/L of as precipitation reagent. Distilled water used as a carrier stream through a two-line manifold design system which coupled with a homemade ISNAG-Fluorimeter analyzer for measure of diverged light of low pressure mercury lamp and this was compared with the classical UV-spectrophotometric method using maximum wavelength absorbance at 272 nm [17,18]. Medications were made by transferring 1 mL (50 mmol/L) to five volumetric flasks (10 mL), then adding 0, 2, 3, 4, and 5 mL from a standard solution of CIP.HCI (12 mmol/L) to achieve a concentration range of 0, 2.4, 3.6, 4.8, and 6 mmol/L (i.e.,0-6 mmol.L-1) when CFIA used the identical method of transferring 0.5 mL of 0.1 mmol/L drug concentration (variant manufacturing) to all volumetric flasks, followed by 0, 0.1, 0.2, 0.3, and 0.4 mL of 0.1 mmol/L to get 0, 0.01,0.01, 0.02, 0.03 and 0.04 mmol/L for Uv-spectrophotometric analysis (UV-1800 Shimadzu). The data was mathematically adjusted for standard additions before being compiled into Table 8A at confidence interval 96% in addition to practically content of ciprofloxacin. HCI in each sample of drug using two different methods and efficiency of determination. Moreover, a comparison treatment of date was subjected at two different paths. The results were summed in Table 8B.

Individual t-test between the weight of practically value from newly developed method (ISNAG-Fluorimeter) and claimed value was applied.

A hypothesis can be evaluated in the following way: [21]

There is no substantial difference between the (w_i) and the stated value (μ), according to the null hypothesis.i.e.; H_o: μ = w_i

Against alternative hypothesis, There could be a significant difference in the amount of ciprofloxacin.HCI within the specific corporations with claimed value, i.e.; H_1: μ ≠ w_i Since; the value obtained of CIP.HCI- pioneer - Iraq company t_{cal} /-5.136/>> t_{tab} (4.303) which mean that the alternative hypothesis will be accepted if the null hypothesis is rejected, indicating that there is a substantial discrepancy between the stated and measured values. While, it was inferred that there was no significant difference between the measured value of ISNAG-Fluorimeter and the claimed value due to t_{cal}. (/-3.464/, 4.052 and /-0.648/) less than t_{tab} (4.303) by the manufacturer companies of AL-jazeera -Iraq, Micro labs limited – India and citro pharma Inc- Canada respectively

In the second test, paired t-tests were used with a significance level of α =0.05 to compare the developed method using the ISNAG Fluorimeter analyzer with the classical method employing the Shimadzu (UV-1800 double beam) spectrophotometer.

Assumption

Null hypothesis H_o: $\mu_{ISNAG-Fluorimeter} = \mu_{UV-sp}$ or $\mu_{ISNAG-Fluorimeter} - \mu_{UV-sp}$ = zero where there is no discernible difference in the mean of the two approaches.

An alternate theory is that the mean of the conventional approach and the ISNAG-Fluorimeter analyzer differ significantly i.e. alternative H_1: $\mu_{ISNAG-Fluorimeter} \neq \mu_{UV-SP}$ or $\mu_{ISNAG-Fluorimeter} - \mu_{UV-sp} \neq$ zero

The acquired outcomes suggest virtually that there has been no huge distinction between newly advanced approach and UV-spectrophotometric approach (classical method) at 95% (α=0.05) confidence level as the calculated t_{cal} (/-0.0381/) is less than t_{tab} (3.182) for the determination of Ciprofloxacin.HCl.

In addition, the Two-way ANOVA was used to study the effect of different methods and the active ingredient content in addition to the manufacturing companies for the drug using the following postulate:

1. Difference in methodology
2. Difference in each company and the active ingredient
3. The hypothesis test between rows i.e, different methods as follows:

H_0: There is no difference between rows (i.e., methods)

H_0:$\mu_{B.P} = \mu_{ISNAG-Fluorimeter} = \mu_{UV-spectrophotometer\ at\ \lambda max = 272nm}$

While an alternative by hypothesis is that there are a significant difference between the three methods used for the analysis

H_1:$\mu_{B.P} \neq \mu_{ISNAG-Fluorimeter} \neq \mu_{UV-spectrophotometer\ at\ \lambda max = 272nm}$

4. The hypothesis test between columns i.e, if you found difference between companies of drug or active ingredient, the assumption is as follows:

H_0: $\mu_{(ciprofloxacin,750mg)} = \mu_{(Microflox,500mg)} = \mu_{(Citroflox,500mg)} = \mu_{(Ciproneer,500)}$

There is no significant difference between used company and active in gradient. While H_1: Alternative hypothesis is a significant difference is available i.e., H_1: $\mu_{(ciprofloxacin,750mg)} \neq \mu_{(Microflox,500mg)} \neq \mu_{(Citroflox,500mg)} \neq \mu_{(Ciproneer,500)}$

From the obtained results (shown in Table 8-B), it was found that H_0 is accepted against H_1 due to $F_{cal.}$ (0.175) < $F_{tab.}$ (5.143) (sig > 0.05 i.e. no significant difference), regarding the comparison between methods. i.e, there are no significant difference between the means of assessment used for analysis of Ciprofloxacin.HCI while with regard to the comparison between different companies and content, it was noted that the value of F_{cal} (133.117) >> F_{tab} (4.757 which means that

significant difference exists between four different companies of drugs and content (sig < 0.05 i.e., a significant difference).

TABLE 8A Summary of results by standard additions method for the determination of CIP.HCI from different companies by different methods: - ISNAG- Fluorimeter method using: CIP.HCI - AMV(20mmol/L) system, 150µL sample volume, 3.2mL/L flow rate for each line and without coil and UV-spectrometer method.

No. of sample	*scientific& Commercial Name, Company Content Country*	*Type of method* Newly developed method using ISNAG-Fluorimeter (mV) UV. Sp* Classical method absorbance measurement at λ_{max}= 272 nm									
		Confidence interval For the average Weight of Table $\bar{w}i \pm 1.96\sigma n\text{-}1/\sqrt{n}$ at 95% (g)	Weight of Sample equivalent to 0.9195gm (50mmol/L) Of the active Ingredient (w_i)	Theoretical content for the active ingredient at95% (mg) $Wi\pm1.96\ \sigma_{n-1}/\sqrt{n}$	[Ciprofloxacin.HCl] mmol/L for newly developed and Classical methods					Equation of standard addition at 95% for n-2	r r^2 R^2%
					0	2.4	3.6	4.8	6	$\hat{Y}_i=a\pm s_a t+b\pm s_b t$ [CIP.HCI] mmol/L	
					0	0.01	0.02	0.03	0.04		
1	ciprofloxacin AL-jazeera CIP.HCI 750mg Iraq	0.95568±0.0087	1.17166	750±6.8409	498	718	838	960	1093	489.297±28.358+98.840±7.191 [CIP.HCI] mmol/L	0.99922 0.99843 99.84
					0.108	0.360	0.601	0.805	1.070	0.115±0.0389+23.690±1.587 [CIP.HCI] mmol/L	0.99934 0.99871 99.87
2	ciprofloxacin& Microflox Micro labs limited CIP.HCI 500mg India	0.77807±0.0062	1.43087	500±3.9582	460	670	779	880	1010	455.243±25.422+90.642±6.447 [CIP.HCI] mmol/L	0.99251 0.99850 99.85
					0.140	0.394	0.624	0.945	1.201	0.126±0.061+26.740±2.507 [CIP.HCI] mmol/L	0.99870 0.99740 99.74
3	Ciprofloxacin& Citroflox citro pharma Inc CIP.HCI 500mg Canada	0.66562±0.00324	1.22407	500±2.4321	499	745	867	990	1114	498.856±1.622+102.421±0.411 [CIP.HCI] mmol/L	0.98616 0.97251 97.25
					0.121	0.342	0.581	0.797	1.050	0.116±0.025+23.130±1.00941 [CIP.HCI] mmol/L	0.99972 0.99944 99.94
4	ciproneer & Ciprofloxacin pioneer CIP.HCI 500mg Iraq	0.7556±0.00788	1.38950	500±5.21475	360	520	610	698	796	353.351±2.145+72.455±5.438 [CIP.HCI] mmol/L	0.99917 0.99833 99.83
					0.121	0.341	0.582	0.798	1.023	0.121±0.016+22.610±0.641 [CIP.HCI] mmol/L	0.99988 0.99976 99.98

r: correlation coefficient, r^2: coefficient of determination and R^2:% capital R-square,R^2= explain variation / total variation, $t_{0.025,\infty}$ = 1.96 at 95% , $t_{0.025,2}$ = 4.303 for n-1, $t_{0.025,3}$ = 3.182.

Table 8B Summary of results for two mode of comparison: Individual t-test (between claimed value and practically value of CIP.HCI) and paired t- test (between two methods)

No. of sample	Type of method: Developed method using ISNAG-Fluorimeter (mV); UV. Sp* Classical method Absorbance measurement at λ_{max}= 272 nm							
	Practical concentration (mmol/L) in 10 mL Practical concentration (mmol/L) in 50 mL Practical weight of CIP in (g)	Weight of ADB in tablet $\bar{W}_{i(mg)} \pm 4.303\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Rec.%	Individual t-test For compared between claim value & practical value $(\bar{W}_{i(mg)}-\mu)\sqrt{n}/\sigma_{n-1}$	Paired t- test compared between two methods: $t_{cal}=\bar{W}d\sqrt{n}/\sigma^*_{n-1}$	Paired t- test compared between two methods: tab at 95% confidence level (n-1)	F-test: F_{cal}= MSB / MSE	F-test: F_{tab}
1	4.9504 49.5039 0.7426	742.565±9.235	99.01	/-3.4642/ ≪ 4.303	$\bar{W}d$= -0.6335 σ^*_{n-1} =33.22622 n = 4 /-0.03813/ ≪ 3.182		The comparison between methods 0.174909< 5.143253	
	0.0049 48.5435 0.7282	728.153±8.314	97.09					
2	5.0224 50.224 0.5022	502.245±2.384	100.45	4.0521 << 4.303				
	0.0047 47.1627 0.4716	471.628±11.782	94.33				The comparison between different companies 133.1167>> 4.757063	
3	4.8707 48.707 0.4871	487.073±7.532	97.41	/-0.648/ << 4.303				

μ: claim value (mg) =750 and 500 mg, $\bar{W}_i$: practically weight (n=3),$\bar{W}_d$: average of different between two methods (developed and classical UV-spectrophotometer) method , $t_{tab}=t_{0.025,3}$=3.182 for paired t-test, n(no. of samples) =4 , σ_{n-1}: standard deviation for Individual t-test ,σ^*_{n-1}: standard deviation of difference (paired t-test), $t_{0.025,2}$=4.303 (for Individual t-test)

Conclusion

A decisive conclusion was achieved using advanced data treatments which conclude that there were no significant difference between the quoted well establish manufacturer claims and the practically found values. So the simplicity of this treatment indulges to use the method achieved in this work as alternative successful, reliable, trustful, easy of operation and it is simplicity that doesn't really need or necessitate the presence of an experience persons to do the analysis. All the results obtained in this whole project shows that these simple instruments (i.e. ISNAG- Fluorimeter which use a long distance of 100 mm length for 2 mm path length for flow cell . The source for irradiation is a long extended distance for 100 mm and on both sides of 90° angle, each side have four solar cells. The radiation source is a low pressure mercury lamp which characteristic emitted light: 184.9 nm and predominant at 253.7 nm) can be used as a trustful instrument. Solar cells were used in this whole project which was a new approach for using simple electronic circuit, noise free signal. In comparison between our method and classical method there was no significant difference between them.

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details of this piece of instrument with its simplicity that challenges any available commercial instrument.

References

[1] A.A. Tunde, *World Sci. News*, **2020**, *145*, 62-73. [Pdf], [Google Scholar], [Publisher]
[2] M.P. Chrysouli, C.N. Banti, N. Kourkoumelis, E.E. Moushi, A.J. Tasiopoulos, A. Douvalis, C. Papachristodoulou, A.G. Hatzidimitriou, T. Bakas, S.K. Hadjikakou, *Dalton Trans.,,* **2020**, *49*, 11522-11535. [crossref], [Google Scholar], [Publisher]
[3] X. Wang, R. Yin, L. Zeng, M. Zhu, *Environ. Pollut.*, **2019**, *253*, 100–110. [crossref], [Google Scholar], [Publisher]
[4] N. Hosseini-Ashtiani, A. Tadjarodi, R. Zare-Dorabei, *Int. J. Biol. Macromol.*, **2021**, *176*, 459-467. [crossref], [Google Scholar], [Publisher]
[5] N.S. Turkey, A.F. Khudhair, *J. Kerbala Univ.*, **2013**, *9*, 153–169. [crossref], [Google Scholar], [Publisher]
[6] P. Schacht, G. Arcieri, R, Hullmann, *Am. J. Med.*, **1989**, *87*, S98–S102. [crossref], [Google Scholar], [Publisher]
[7] D. Lu, S. Xu, W. Qiu, Y. Sun, X. Liu, J. Yang, J. Ma, *J. Clean. Prod.*, **2020**, *264*, 121644. [crossref], [Google Scholar], [Publisher]
[8] I.M.A. Shakir, N.S.T. Al-Awadie, ISNAG-Fluorimeter to measure the radiation of fluorine in the visible region of electromagnetic radiation with a mercury tube lamp as an irradiation source for continuous injection analysis and solar cells. IRQ. Patent No.3884, **2014**, International classification H01J61/00.
[9] N.S. Turkie, H.F. Abd Alrazack, *Int. J. Sci. Res.*, **2017**, 6, 201–216. [crossref], [Google Scholar], [Publisher]
[10] P.N.S. Al-Awadie, H.F. Abd Alrazack, *IOSR J. Appl. Chem.*, **2018**, *11*, 25–39. [crossref], [Google Scholar], [Publisher]
[11] N.S. Turkie, H.F. Abd-Alrazack, *J. Glob. Pharma Technol.*, **2019**, *11*, 333–348. [Google Scholar], [Publisher]
[12] N.S. Turkie, H.F. Abd Alrazack, *IOSR J. Pharm. Biol. Sci.*, **2018**, *13*, 60–75. [crossref], [Google Scholar], [Publisher]
[13] N.S. Turkie, H.F. Abd Alrazack, *J. Glob. Pharma Technol.*, **2019**, *11*, 122–136. [Pdf], [Google Scholar], [Publisher]
[14] N.S. Turkie, H.F. Abd Alrazack, *J. Glob. Pharma Technol.*, **2018**, *10*, 447–460. [Google Scholar], [Publisher]
[15] R.T. Morrison, R.N. Boyd, Organic Chemistry, 5th Ed., Allyn & Bacon, Inc., Boston, London, Sydney, Toronto, **1983**.
[16] A.I. Vogel, Text book of Macro and Semimicro qualitative inorganic analysis, 5th Ed., Longman, London, **1979**.
[17] J.C. Miller, Statistics for analytical chemistry, Sixth edition, Pearson education limited, UK., **2010**.
[18] D. Brink, Essentials of statistics, Ventus publishing ASP, **2010**.
[19] E.C.L. Cazedey, H.R.N. Salgado, *Adv. Anal. Chem.*, **2012**, *2*, 74–79. [crossref], [Google Scholar], [Publisher]
[20] M.I. Pascual-Reguera, G.P. Parras, A.M. Díaz, *Microchem. J.*, **2004**, *77*, 79–84. [crossref], [Google Scholar], [Publisher]
[21] P. British, Stationary Office under license from the controller of Her Majesty's Stationary Office for the Department of Health on behalf of the Health Ministers, **2003**, I, 73.

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