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Continuous Flow Injection Analysis- Precipitation Reaction Of Ibuprofen With Sodium Nitro Pros Side Using Low Pressure Mercury Lamp Tube (UV-Light) And Detection Of Diverged Scattered Lights (Visible Light) At 2 X 90^{o} Using Multi Solar Cells That Covers 2 X 100mm Distance Of 2mm Path Length.

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Abstract: *A new, simple, fast and sensitive method has been developed for the determination of Ibuprofenin pure form and drugs (tablets)by continuous flow injection scatter light. The method was based on the reaction of the Ibuprofen with sodium nitro prosside to form a precipitate, using homemade ASNAG- fluorimeter. Optimum parameter have been studied to increase the sensitivity for developed method. The linear dynamic range for the instrument response versus Ibuprofen concentration was 0.5- 15mmol/L while the L.O.D was 33.934µg/sample from the step wise dilution for the minimum concentration of lowest concentration in the linear dynamic range of the calibration graph. The correlation coefficient (r) was 0.9804 while percentage linearity (R^2%) was 96.13%. RSD% for the repeatability (n=8) was lower than 0.5% for the determination of Ibuprofen, with concentration of 4, 9 mmol/L respectively. The methods: developed method was applied successfully for the determination of Ibuprofen in pharmaceutical tablets. A comparison was made between the newly developed method with the classical method (Turbidemtric method) of analysis using the standard addition method via the use of paired t-test. It shows that there was no significant difference between the quoted values of each individual company with calculated t-value at 95% confidence interval from developed method.*
Keyword: *Ibuprofen, flow injection scatter light, homemade instrument.*

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I. Introduction

The chemical name of Ibuprofen [**2-(4-Isobutylphenyl) propanoic acid]** is a nonsteroidal anti-inflammatory drug (NSAID) widely marketed under various trademarks including Act-3, Advil, Brufen, Motrin, Nuprin, and Nurofen[1-5].. Ibuprofen tablets are sold under the trade names Advil and Motrin. Ibuprofen's molecular formula (figure 1) is $C_{13}H_{18}O_2$. Ibuprofen is 75.69% Carbon, 15.51% Oxygen, and 8.80% Hydrogen.Ibuprofen is only very slightly soluble in water [6-15]. Less than 1 mg of ibuprofen dissolves in 1 ml water (< 1 mg/mL). However, it is much more soluble in alcohol/water mixtures as well as carbonated water [4, 11-18].

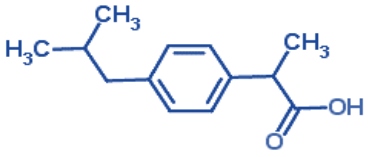


Figure 1: structure of Ibuprofen IUPAC name [**2-(4-Isobutylphenyl) propanoic acid].**

There are Different analytical methods for determination of Ibuprofen and pseudoephedrine in combined Dosage forms are developed. High-performance liquid chromatography is one of the most popular and Sensitive method, which can separate ibuprofen and other active substances in tablets [14-16], granules[17], Soft capsules[18], creams[19] and syrup preparation. For the HPLC determination in ibuprofen tablets [9]. For the

analysis of multicomponent ibuprofen preparations, many UV spectrophotometric methods have been propose[9,17]. Because of near absorption maximums of ibuprofen (264 nm and 272 nm) these methods are based on first or second derivative spectrophotometric assay [19-26], ratio spectra derivative spectrophotometry and chemometric techniques [16-19] or formation of color. In this work using flow injection scattering method, the scattering light is measured at 0-90^{o} angle will be detected by homemade ASNAG fluorimeter via low pressure mercury lamp as a source and using 2[4 x 2.5cm] solar cell.

II. Experimental

Reagent and chemical

All chemicals were use of analytical-reagent and distilled water was use to prepare all the solutions. A standard solution 50mmol/L of Ibuprofen molecular formula $C_{13}H_{18}O_2$, molecular weight 206.285 g/mole and SDI-Iraq was prepared by dissolving 1.0314 g in 100 ml of distilled water. A stock solution 200mmol/L of sodium nitro prosside molecular formula(**Na_2 [Fe(CN)$_5$ NO].2H_2O**)molar mass 297.95 g/mol and Merck-USA was prepared by dissolving 14.8975 g in 500 ml of distilled water.

Sample preparation

Twenty tablets were weight then crushed and grinded. Tablets containing 200mg of Ibuprofen were weighted 1.49506g, 2.82508g (equivalent to 1.0314g of active ingredient, 50mmol/L) forApifen Zauba -India and Ibuprofen DHP Co. - U.K, respectively, and tablets containing 400mg of Ibuprofen were weighted 1.6059g, 1.6209g (equivalent to 1.0314g of active ingredient, 50mmol/L) for Profinal Julphar –UEA and Jazofen Jenapharm –UAS, respectively. Each one from The four kinds of sample dissolved in distilled water. The solution was filtered to get rid of undissolved materials, the residue was washed with distilled water and completed the volume to 100ml with the same solvent(distilled water).

Apparatus

The response wasmeasured by a homemade ASNAG fluorimeter. Lowpressure mercury lamp is used in ASNAG- fluorimeter, which is characterized by two lambdas (184.9 & 253.7) nm. While the detector that is been used a 2[4 x 2.5cm] solar cell. The flow system used to determination of Ibuprofen is shown schematically in figure 2. Peristaltic pump two channels variable speed (Ismatec, Switzerland). Valve 6 – port medium pressure injection valve (I D E X corporation , USA) with sample loop (1 mm i.d. Teflon ,variable length).2[4 x 2.5cm] solar cells are used as detector for collecting signal via sample travel through a line of 2mm optical openture extended for 100mm distance.The output signals were recorded by potentiometric recorder (Siemens, Germany)(1- 5 Volt,1000-5000 mV). Peak height was measured for each signal. Turbidemtric readings by Turbidity-meter , HANNA Company (Hungary).

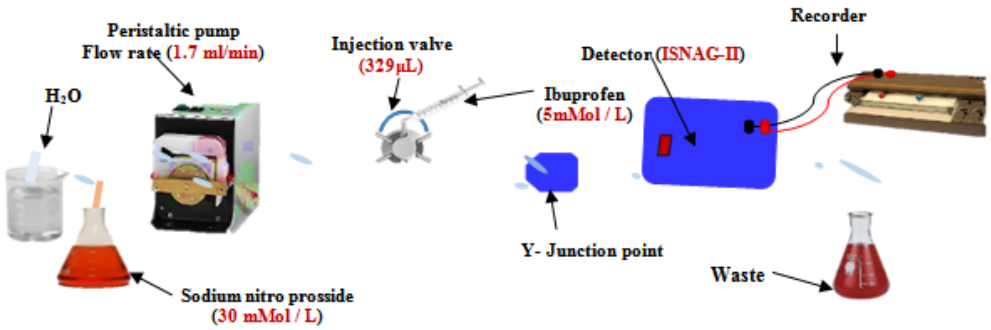
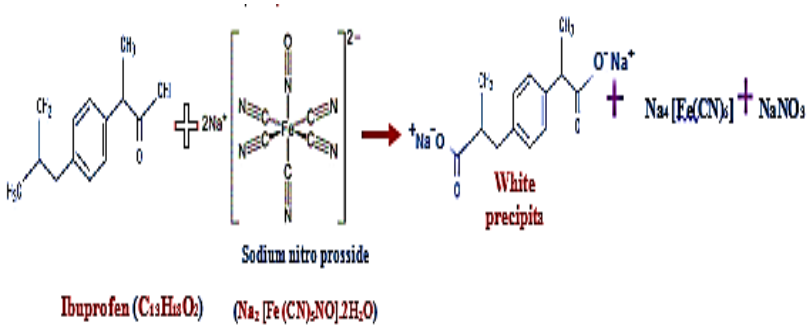


Figure 2: A-Flowgram of manifold system consist of two line.

III. Methodology

Tow lines designsystem (Fig. no. 2) was used for Ibuprofen determination. First line is the carrier stream(1.7ml/min) from aqueous medium (H_2O) that will take and introduce the sample loop segment (329µL, 30mmol/L) from Ibuprofen into the reaction stream by combining with the second line (1.7ml/min) that will form the precipitate in Y-junction zone (mod of PMMA plexiglass). This formed precipitate will be successive measurements were used ASNAG- fluorimeter via low pressure mercury lamp, it's give two main wavelengths

namely 184.9nm and 253.7nm. These both two line are easily diverged due to its high frequency. The divergence of this beam of incident light will be detected at 90° through a flow cell of 2mm path length that extend for 100mm distance by using 2[4 x 2.5cm] solar cell. While the proposed probable reaction pattern is expressed inscheme 1. [26-27]



Scheme 1: Proposed mechanism of the reaction between Ibuprofen and sodium nitro prosside.

IV. Result and Discussion

Study of the optimum parameters

The flow injection manifold system as shown in fig.1.A was investigated in the relation of chemical and physical variables, in order to obtain optimum conditions for the react sodium nitro prosside with Ibuprofen and formed white precipitate. They were optimized by making all variables constant and varying one at a time i.e. fixed variable optimization.

Variation of chemical parameters

Sodium nitro prosside ($Na_2[Fe(CN)_5NO].2H_2O$) concentration

The study was carried out using a series of solutions by different concentration sodium nitro prosside (5-100mMol/L) as appreciate reagent at flow rate 1.3mL/min, the water is a carrier stream, sample volume from Ibuprofen (5 mMol/L) is (158µL). Table (1) and Fig.no. (2) Summarizes the obtain results; from (5-30mMol/L) can be shows that an increase in the response with increase concentration, but at the concentration >30 mMol/L a it was noticed a decrease in response height, Therefore; 30 mMol/Lit is give high and Sharpe response compared with the other concentration used. 30 mMol/Lfrom sodium nitro prosside concentration was chosen as the optimum concentration that used for future experiments.

Table 1: Effect of sodium nitro prosside concentration on response function expressed as an average peak heights ȳi (n=3) and tabulation of all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable $[Na_2[Fe(CN)_5NO].2H_2O]$ mmol/L	**Dependent variable Average (n=3) diverged light response measured at 90°expressed in mV**				
	Practical lab. value			**Mathematical model $\hat{y}_i$**	**Savitzky-Golay filter $\hat{y}_{i\,(S\text{-}G)}$**
	Average peak height($\bar{y}_i$)	**RSD%**	**Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$**		
5	32	1.81	32 ± 1.44	19.577	57.577
10	144	0.69	144 ± 2.48	171.755	193.293
25	360	0.37	360 ± 3.30	335.031	408.384
30	736	0.13	736 ± 2.31	736.987	519.882
50	344	0.41	344 ± 3.50	353.718	455.230
100	304	0.47	304 ± 3.58	302.933	346.352

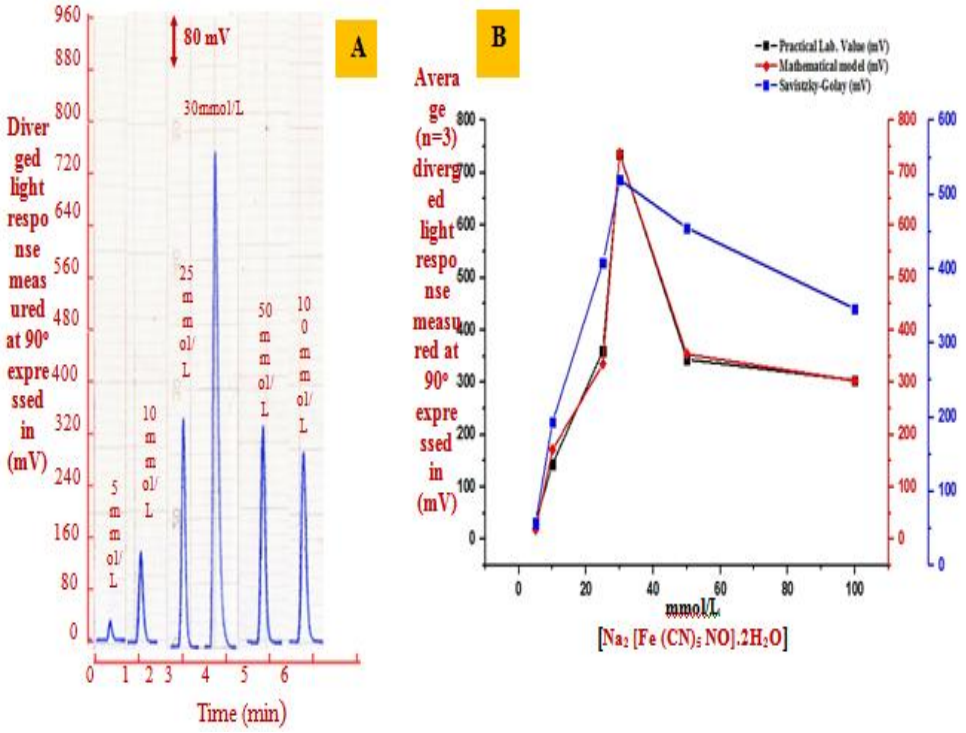


Figure 3:

A- Response –time profile of Ibuprofen with variable concentration of sodium nitro prosside solution (clear unobstructed peak with no deformation at different level of sodium nitro prosside solution.

B- Plot of averaged peak height responses Vs. sodium nitro prosside solution concentration. Carrier stream effect

Salts effect

The reaction between Ibuprofen (5mmol/L) with sodium nitro prosside (30mmol/L) to form a white color precipitate was study in different solution media (KNO_3, KCl, KBr NaCl, NH_4Cl, CH_3COONa, and CH_3COONH_4) at 50mmol/L concentration in addition to aqueous medium as a carrier stream at flow rat 1.3ml/min for each line with sample volume 158µL (using open valve mode).Table 2 Tabulate the average of responses recorded for every one of carrier using in this system. figure 4 show the pest response recorded to distal water (H_2O) because it is give high and Sharpe response compared with the other carrier stream used

Table 2: Effect of different salt as a carrier stream on diverged light response at 90^o.

Independent type of salt as a carrier stream	**Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 90^oexpressed in mV**	**RSD%**	**Reliability of average response(two tailed) at 95% confidence level $\bar{y}_i$ (mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$**
H_2O	736	0.127	376 ± 2.31
KNO_3	136	0.92	136 ± 3.11
KCl	344	0.33	344 ± 2.86
KBr	308	0.40	308 ± 3.08
NaCl	370	0.29	370 ± 2.66
NH_4Cl	144	0.82	144 ± 2.93
CH_3COONa	168	0.65	168 ± 2.71
CH_3COONH_4	240	0.58	240 ± 3.45

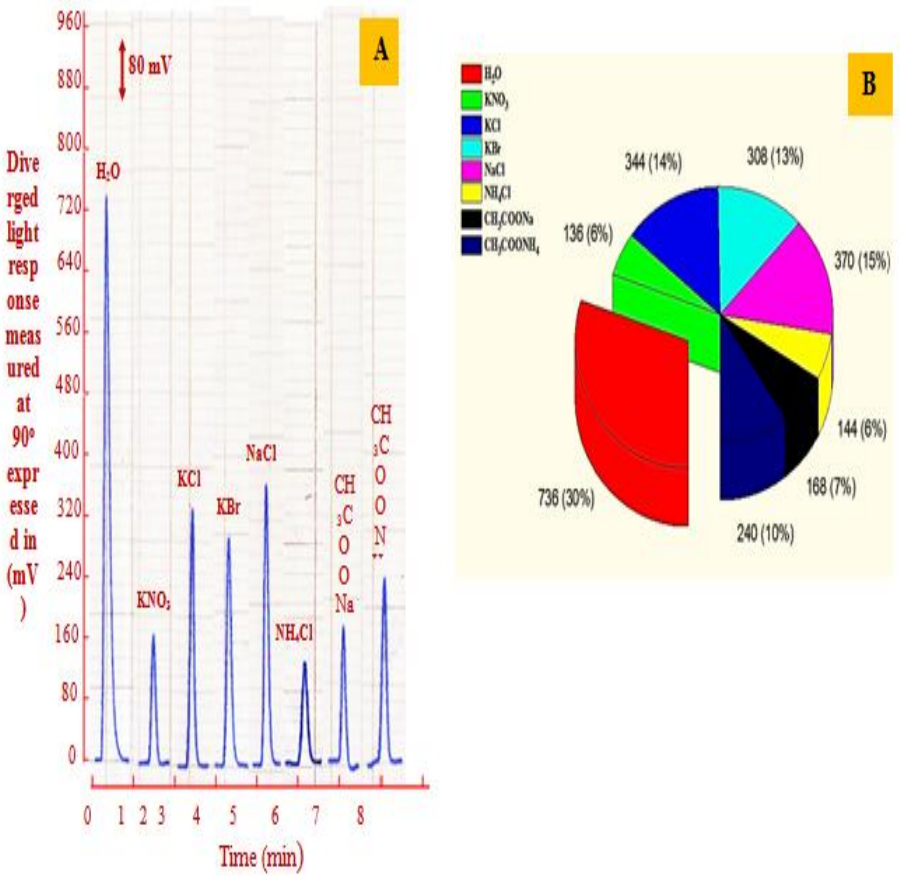


Figure 4: Effect of salt solution used as a carrier stream on profile (A), and pie percentage Representation of the contribution of each salt solution (B).

Acidity Effect

At a sample volume 158µL from Ibuprofen (5mmol/L) reacted with sodium nitro prosside (30mmol/L), study different types of acids medium used a carrier stream (HCl, CH_3COOH, H_2SO_4, and HNO_3) at 50mmol/L concentration in addition to aqueous medium as a carrier stream at flow rat 1.3ml/min for each line with sample volume 158µL (using open valve mode). Study the effect of every one of these acids on reaction and compared with (H_2O) response recorded. Table 3 Tabulate the average of responses recorded for every one of carrier using in this system. Figure5 show the pest response recorded to aqueous medium (H_2O) because it was gave high and Sharpe response compared with the other acids carrier stream used.

Table3: Effect of carrier stream type on precipitate response Expressed as an average peak heights $\bar{y}_i$ (n=3)

t type of acid	Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 90°expressed in mV	RSD%	Reliability of average response(two tailed) at 95% confidence level $\bar{y}_i$ (mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$
H_2O	738	0.126	738 ± 2.41
HCl	112	0.47	112 ± 1.32
CH_3COOH	72	0.99	72 ± 1.79
H_2SO_4	360	0.35	360 ± 3.16
HNO_3	160	0.56	160 ± 2.21

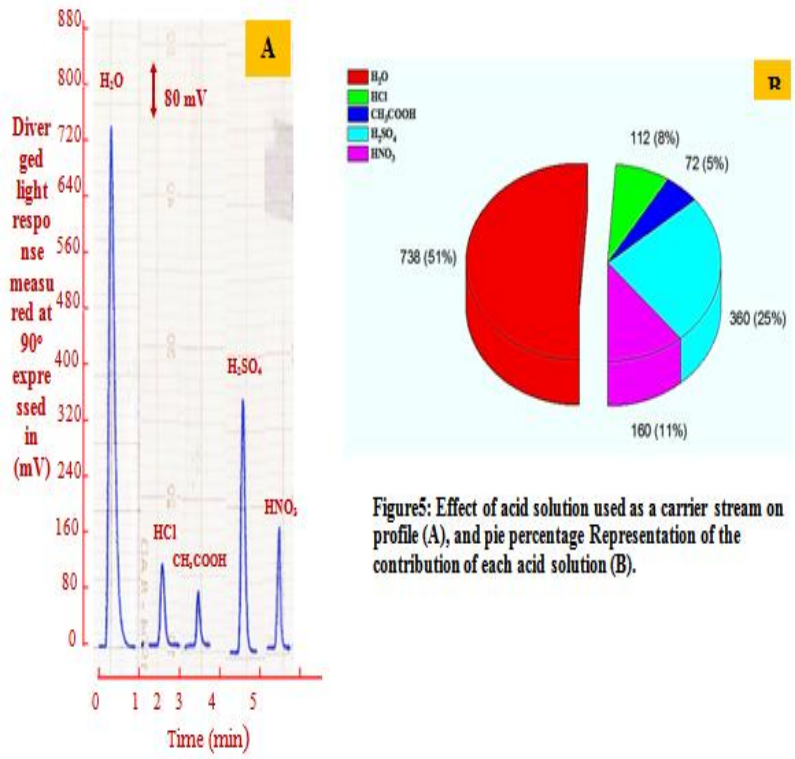


Figure5: Effect of acid solution used as a carrier stream on profile (A), and pie percentage Representation of the contribution of each acid solution (B).

Physical parameters

Electronic filter effect.

This study was carried out for the determination of preferred low band pass electronic filter using 158µL sample volume of Ibuprofen (5mmol/L) at 1.3ml/min flow rate at each line and H_2O as a carrier stream. Variable RC- filters were used to establish optimum response sensitivity and response profile with the sake for increased S/N ratio.. While data smoothing cannot really gave an improved data profile to choose from. As there were no large fluctuation in the measurements. Therefore, no digital filtering was used on RC- response filter. On the above based on measurement and profile response of S/N signals. Direct measurements was the choice of this part of research work.

Table 4 tabulate all the results obtained

Table 4: Effect of electronic filters on precipitate response expressed as an average peak heights $\bar{y}_i$ (n=3)

Independent variable of electronic filter response (Sec.)	Dependent variable ($\bar{y}_i$) Average (n=3) diverged light response measured at 90°expressed in mV	RSD%	Reliability(two tailed) at 95% confidence level $\bar{y}_i$(mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$
Without filter	736	0.27	1056 ± 2.31
0.1632	280	0.47	894 ± 3.28
0.3196	248	0.52	882 ± 3.20
0.68	224	0.50	856 ± 2.81
0.8364	208	0.52	800 ± 2.71
1.6728	184	0.55	736 ± 2.53
3.974	144	0.59	664 ± 2.11

Flow rate effect

Variable flow rate (0.4- 3.6) ml/min was used at Ibuprofen (5mmol/L)- sodium nitro prosside (30mmol/L)- and 158µL sample volume and open valve mode (i.e. allowed permissible time for sample segment to be injected from injection valve). It can be noted from fig. 6-A, B that at slow flow rate (0.4-1ml/min) caused an irregularity of flow, which in turn causes the deformed or broad of response- time profile due to irregular passage of precipitated plug of sample to be dealt with the detector for 100mm distance of 2mm path length. Therefore, a 1.7 ml/min for each line was the optimum choice to compromize between sensitivity, response profile and consuption of chemicals since a response is a function of physical and chemical variable. All results tabulated in table 5.

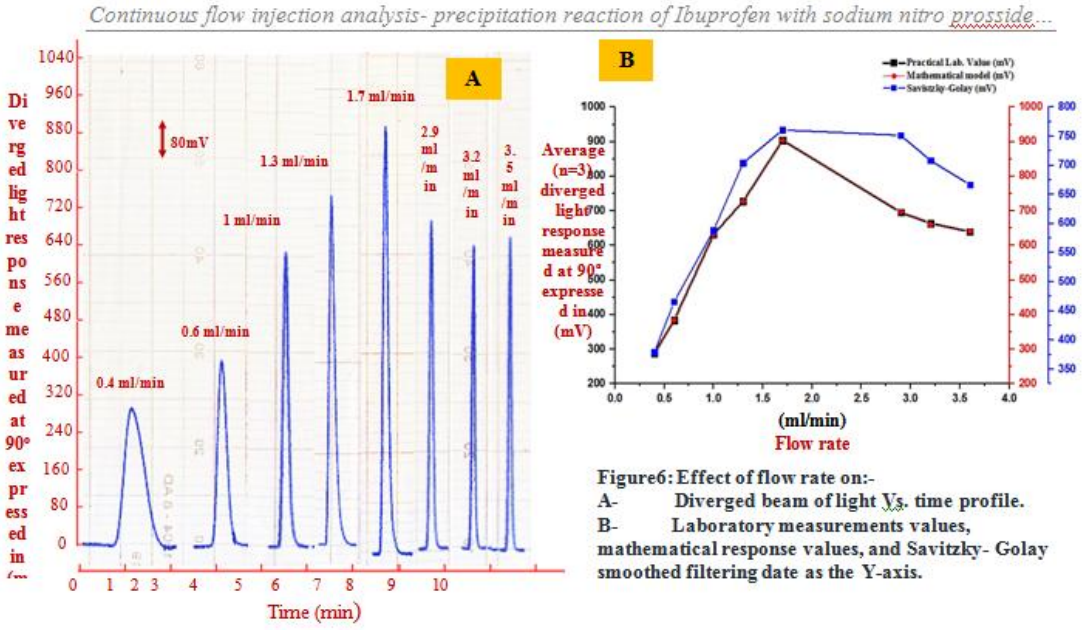


Figure6: Effect of flow rate on:-
A- Diverged beam of light Vs. time profile.
B- Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis.

Table 5: Effect of flow rate on the variation of diverged light response and tabulate all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable of pump Speed	Dependent variable Average (n=3) diverged light response measured at 90°expressed in mV							
	Practical lab. value						Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i(S-G)}$
	Flow rate (mL/min)		Average peak height($\bar{y}_i$)	RSD %	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$	Δt_b (Sec)		
	Line no. 1	Line no. 2						
5	**0.4**	**0.4**	288	0.38	288 ± 2.68	120	287.999	379.365
10	**0.6**	**0.6**	384	0.30	384 ± 2.91	68	384.000	465.661
15	**1**	**1**	632	0.21	632 ± 3.30	52	631.971	588.330
20	**1.3**	**1.3**	728	0.17	728 ± 3.08	49	728.145	704.278
25	**1.7**	**1.7**	904	0.11	904 ± 2.41	41	903.720	760.867
30	**2.9**	**3**	696	0.18	696 ± 3.18	34	698.148	751.615
35	**3.2**	**3.3**	664	0.19	664 ± 3.12	26	660.882	708.336
40	**3.5**	**3.6**	640	0.21	640 ± 3.35	22	641.134	666.806

Δt_b (sec) : Time lapse for the preciptate response within measuring cell or peak base width
ine no.1 = carrier stream (H_2O), Line no. 2= sodium nitro prosside (30mmol/L)

Effect of sample volume

Using the optimum parameters achieved in previous sections. The effect of sample volume (Ibuprofen 5 mmol/L) as an analyte was used. Variable sample volume (79-329µL) were injected in the open valve mode. The obtained results are shown in fig. 7-A,B. It was noticed that, the use of sample loop volume of less than 329µL (calculated). High output response profile was obtained indicating most probably the formation a lots of small nuclei due to the dynamic system property of flow injection. Avoiding static condition that might contribute at this small time interval to the formation of larger precipitated particles which might act a solid barrier preventing the penetration and diffusion giving rise to a **stock shift** that the ISNAG-florimeter arrangement of detectors and long distance 100mm flow cell at 2mm path length. In the case of small particles, even though they are of different size and dimension but they definitly move at a faster rate. Large amount of precipitate formed due to larger and more concentration solution will enhance the formation of precipitate filter affecting incident light intensity as well as diverged light intensity, this mean a dual effect of this filter on the output response. So, 329µL sample loop volume is the most satisfactory sample plug.

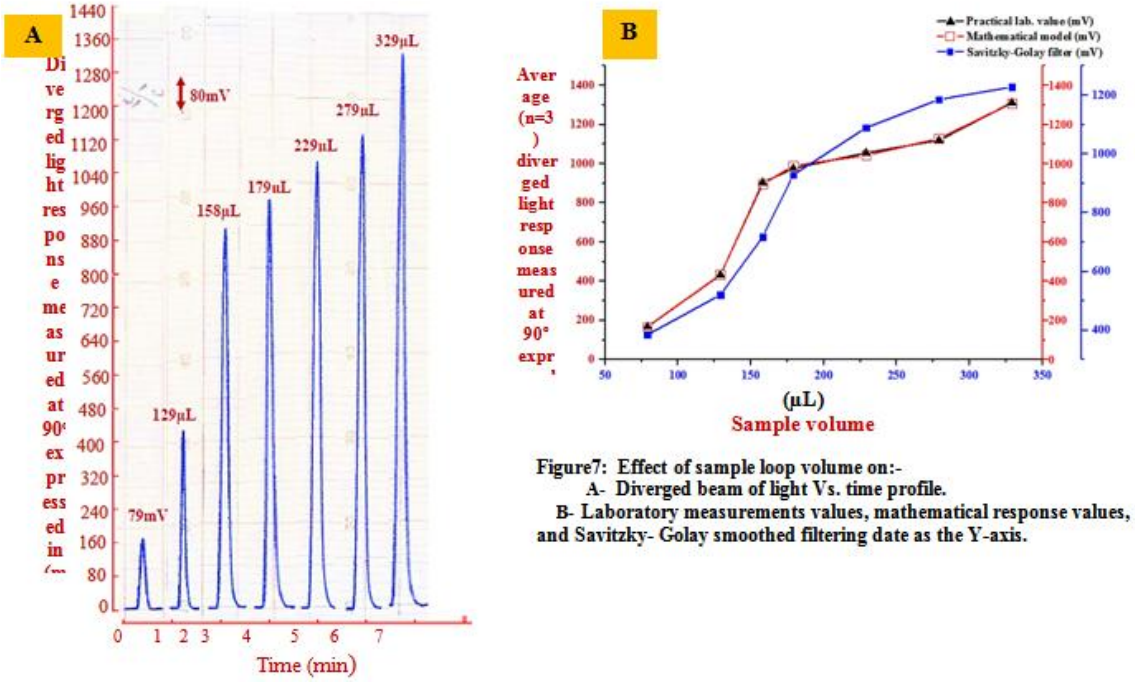


Figure7: Effect of sample loop volume on:-
A- Diverged beam of light Vs. time profile.
B- Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis.

Purge time effect

Using optimum parameters that were achieved in the previous sections, purge time of the sample volume to be injected via the carrier stream (H_2O) was studied. Using different purge time (5-45 sec) for the sample segment to pass through injection valve at pre- selected time interval as shown in table 6, it can be noticed that an evacuation of sample segment from injection valve of less than 40 sec. gave weak response. This is caused by not achieving complete purge of sample. In complete precipitation of reactant was accomplished by incomplete introduction of sample segment. Therefore, a disturbed response- time profile can be noticed or a weakening response might happen (fig. 8-A, B). A vice versa will insure a complete discharge and a full purge of the sample plug from injection valve. So, 45 second was found a time that compromize a suitable purge time and through output with a good response profile avoiding any irregularty.

Table 6: variation of purge time on diverged light response and tabulate all available data obtained practically, calculated as obtained by best fit mathematical model, and smoothed digital filtering using Savitzky-Golay data treatment

Independent variable of Purge time (Sec.)	Dependent variable Average (n=3) diverged light response measured at 90°expressed in mV				
	Practical lab. value			Mathematical model $\hat{y}_i$	Savitzky-Golay filter $\hat{y}_{i(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i(mV) \pm t_{0.025,2}\ \sigma_{n-1}/\sqrt{n}$		
5	120	0.44	120 ± 1.32	120.050	161.133
10	280	0.39	280 ± 2.71	279.597	247.205
15	392	0.30	392 ± 2.96	393.410	429.239
20	664	0.19	664 ± 3.11	661.180	679.675
25	960	0.13	960 ± 2.78	963.524	925.103
30	1144	0.10	1144 ± 3.06	1141.180	1109.742
35	1184	0.10	1184 ± 2.96	1185.410	1216.592
40	1240	0.09	1240 ± 2.86	1239.597	1264.065
Open valves(45)	1312	0.07	1312 ± 1.42	1312.050	1279.756

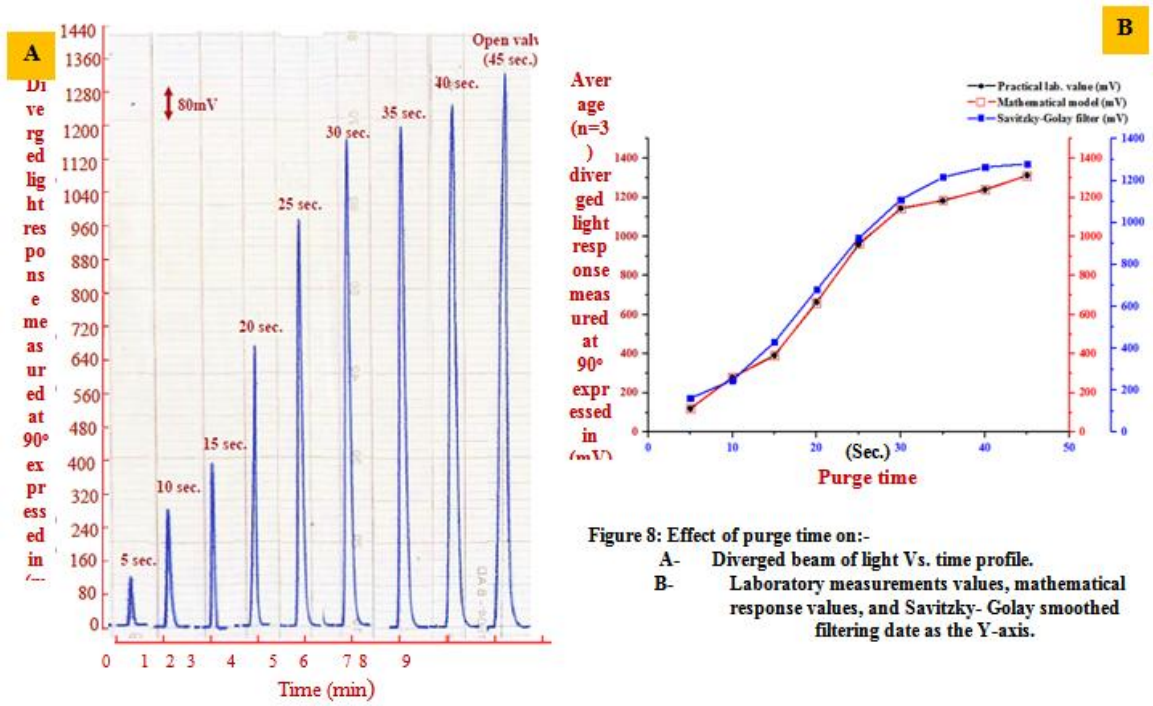


Figure 8: Effect of purge time on:-
A- Diverged beam of light Vs. time profile.
B- Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis.

V. Calibration graph

Selling all achieved experimental parameters that at the end will lead to establish a new methodology regarding the assessment and determination of this crucial drug. In previous section physical as well as chemical variable were set at their optimum values (30mmol/L concentration of sodium nitro prosside, 329µL sample volume, and 1.7 ml/min flow rate for each line). Set of series (0.5-20mmol/L) solutions were prepared an output came was depicted in fig. 9-A. All prepared concentration were used. An increase in Ibuprofen concentration causes an increase number of nuclei formed up to 15mmol/L. In which it will lineup and densification with entraped water molecule, which might cause a diverged beam of light. All what is received by the ISNAG detector is 0 - 90°. An increase in Ibuprofen concentration more than 15mmol/L cause a much more intensification caused by the effect of agglomerate formation which form in this short period of time a relatively more intensified massive precipitate. Which in turn prevent the penetration of light only affecting the reflection of light at a certain extend. Therefore, a shift from linearity is un avoidable affecting the correlation coefficient. Choosing all fourteen points (fig. 9-A) that were measured trying to fit a linear equation of the form **y= a+ b x**in which a correlation coefficient of **r** = 0.9422while capital squared- **R**gave **88.77%** for the whole chosen range (0.5- 20 mmol/L). Searching for better

representation, a shorter range should be used to improve the assessment mathematical formulation. The best fit linear equation representing the diverged response light as dependent variable against concentration of Ibuprofen (0.5-15mmol/L) (fig. 9- B,C) has a correlation coefficient of **r =0.9886**with a capital squared- **R** of **96.13%**. This indicate that the linear equation. chosen :

R_{DL}(mV) = a + slope [Ibuprofen] mmol/L

Was able to explain this much of the obtained results, this chosen thirteen points were the outcome of scatter plot. All results summed up in table 7.

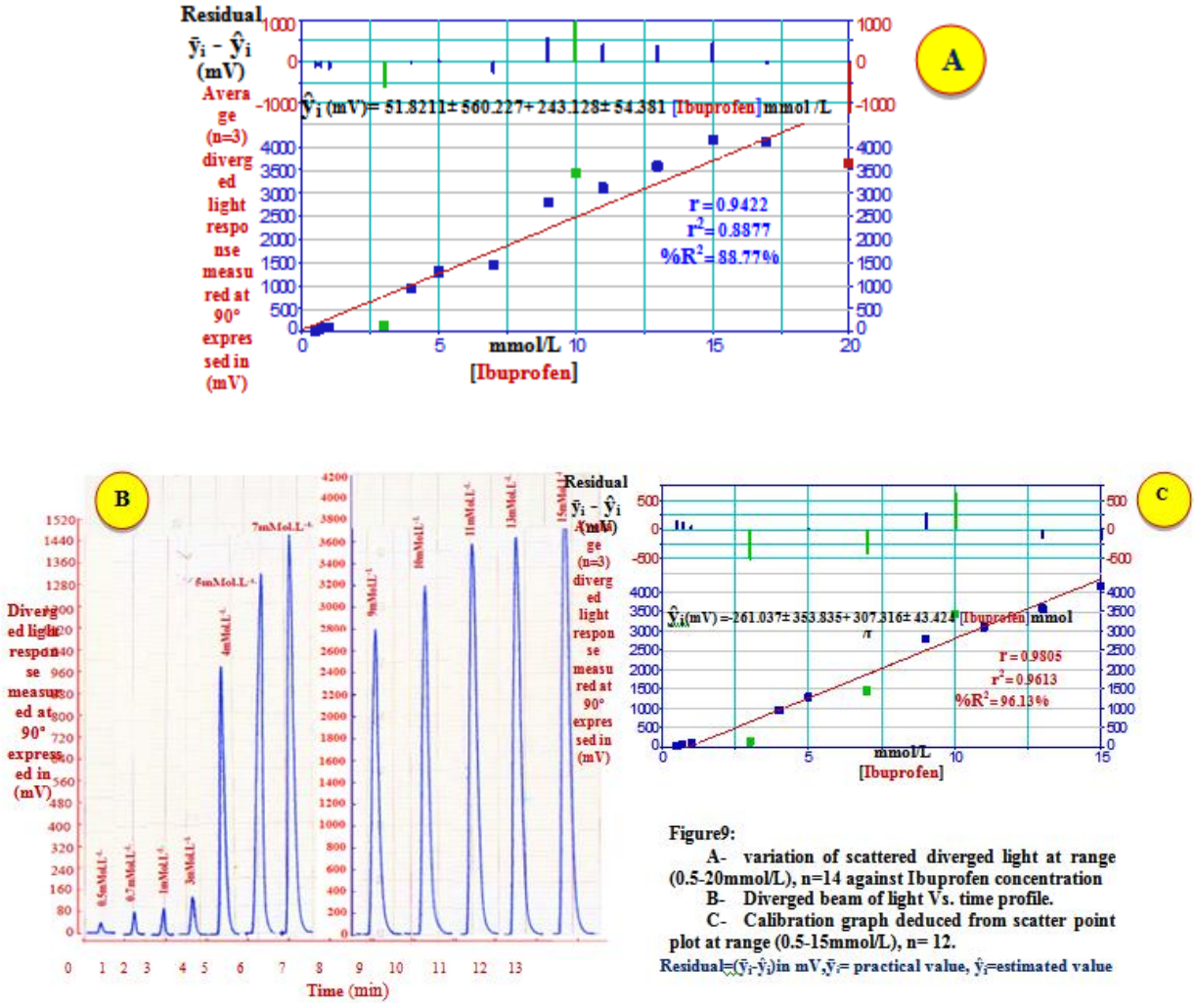


Figure9:

A- variation of scattered diverged light at range (0.5-20mmol/L), n=14 against Ibuprofen concentration

B- Diverged beam of light Vs. time profile.

C- Calibration graph deduced from scatter point plot at range (0.5-15mmol/L), n= 12.

Residual=($\bar{y}_i$-$\hat{y}_i$)in mV,$\bar{y}_i$= practical value, $\hat{y}_i$=estimated value

The assessment evaluation of the new developed methodology for the determination of Ibuprofen was compared with the available literature cited methods, namely turbidometry method. Here a description of the used method:

Turbidometry measurement, which is based on the reaction of sodium nitro prosside (0.1 mol/L, which already it used after established as can be seen in fig. 10-A.) with the drug for a suitable ranged of concentration (0.1- 4mmol/L) that the instrument is capable of handling it. A scatter plot shows that a calibration graph of having capital squared-R of **96.70%** with correlation coefficient of **0.9834** for a linear regression equation of the form of **Response (FTU)=a+slope (Ibuprofen] mmol/L** (fig. 10-B) at range of concentration 0.1-3mmol/L.

It can be clearly noticed that the new adopted methodology satisfies both the use of low as well as high concentration with high precision and repeatability with minimum of the relative standard deviation (RSD% < 0.5% as shown in fig. 11).

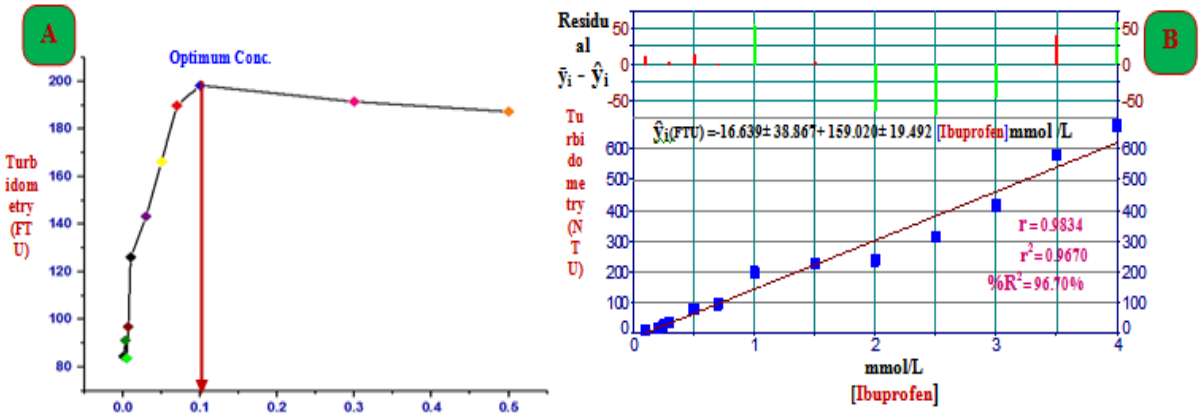


Figure 10:

A- Graphical representation shows the optimum concentration of sodium nitro prosside reacted with Ibuprofen in Turbidomtric method

B- Calibration graph deduced from scatter point plot of Turbidemtric method(classical method) at range (0.1-4mmol/L), n=13 against Ibuprofen concentration

Residual = ($\bar{y}_i$-$\hat{y}_i$) in FTU for turbidometric, $\bar{y}_i$ = practical value in FTU for turbidometric, $\hat{y}_i$=estimated value in FTU for turbidometric

Table 7: Comparison of different assessment method of Ibuprofen.

Method		No. of measurements	[Ibuprofen] mmol/L		Linear regression equation at 95%, n-2	r r^2 R^2%	t- value at 95%, n-2		Detection limit µg/ sample		Repeatability at 95% confidence level, n-1		%RSD
			Measured	Linear dynamic range	New developed $\hat{y}_i$(mV)= a± S_at+ b± S_bt [x] Turbidometry 0- 90° $\hat{y}_i$(FTU)= a± S_at+ b± S_bt [x]		t_{tab}	t_{cal}	Practically based on the gradual dilation of the minimum concentration	Theoretical based on slope	[Ibuprofen] mmol/L	Reliability of average diverged light Average of turbidometry $\bar{y}_i \pm t_{0.025,\,n-1}\ \sigma_{n-1}/\sqrt{n}$ n=8	
Newly developed methodology	ENAG fluorimeter	14	0.5- 20	0.5- 20	51.8211± 560.227+ 243.128± 54.381 [Ibuprofen]mmol /L	0.9422 0.8877 88.77%	2.179<<9.74		(0.5mmol/L) 33.934µg	(66.252µmol/L) 0.197 µg	4	968 ±1.254	0.155
		12		0.5- 15	-261.037± 35.384+ 307.316± 43.424 [Ibuprofen]mmol /L	0.9804 0.9613 96.13%	2.228<<20.80				9	2800 ±1.965	0.084
	Turbidometry (NTU) Using Hanna instrument	13		0.1- 4	-16.639± 38.867+ 159.020± 19.492 [Ibuprofen]mmol /L	0.9834 0.9670 96.70%	2.201<<17.95		(0.08mmol/L) 132.022µg	(9.433 µmol/L) 15.568 µg	1	196.89 ±1.1706	0.711

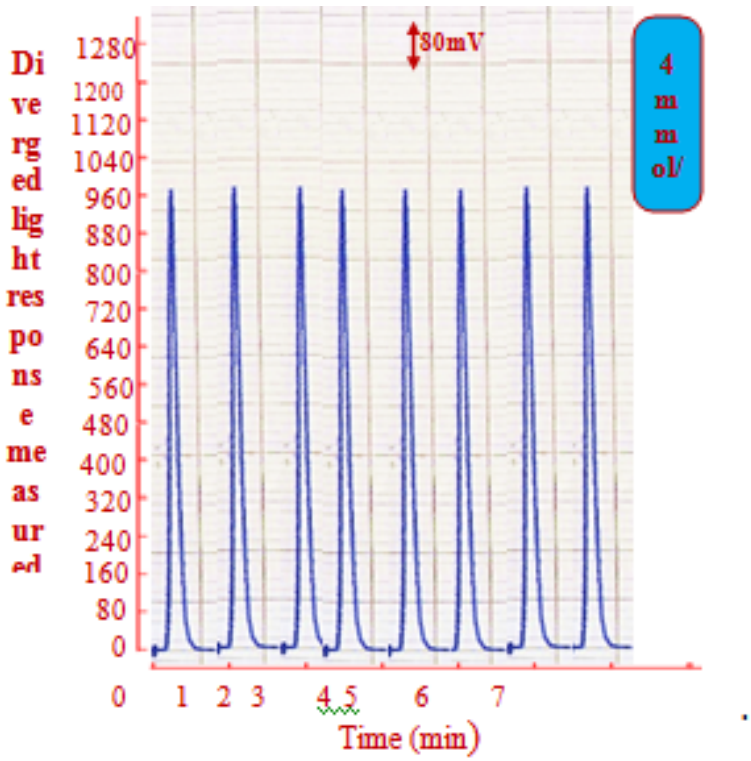


Figure11: Response profile of repeatability of Ibuprofen (4mmol/L and 9mmol/L).

VI. Application

The continuous flow injection analysis via diverged light response using low pressure mercury lamp that used in ISNAG fluorimeter achieved in this work was used for the analysis of Ibuprofen in the four different drug manufactures (Profinal-UAE-400mg, jazofen-UAS-400mg, Apifen- India-200mg, and Ibuprofen- U.K- 200mg) and was compared with turbidometry(classical method) viaTurbidity-meter, HANNA, (Hungary). the measurement of scattered light at 0- 180^{o} for yellow precipitate particles of Ibuprofen- sodium nitro prosside (0.1mol/L) system. A series of solutions were prepared of each drug (50mmol/L) (1.0314g of active ingredient in 100ml) by transferring 0.8ml to each five volumetric flask (10ml), followed by the addition of gradual volumes of standard solution of Ibuprofen (0, 0.1, 0.2, 0.3, and 0.4 ml) of 50mmol/L to obtain (0, 0.5, 1, 1.5, and 2mmol/L) when use ISNAG fluorimeter (newly developed methodology), while transferring 0.1ml to each five volumetric flask, followed by the addition of gradual volume of standard solution of Ibuprofen (50mmol/L) (0, 0.1, 0.2, 0.3, and 0.4ml) to obtain (0, 0.5, 1, 1.5, and 2 mmol/L) concentration, in addition to Turbidomtric method that depend on the measurement at 180^{o}. Figure 12-A, B, C and D shows standard addition calibration graphs using newly developed methodology. The results were summed in table 8 at confidence level 95% (2-tailed), showing practically content of Ibuprofen in each sample of drug using two different methods and efficiency of determination.

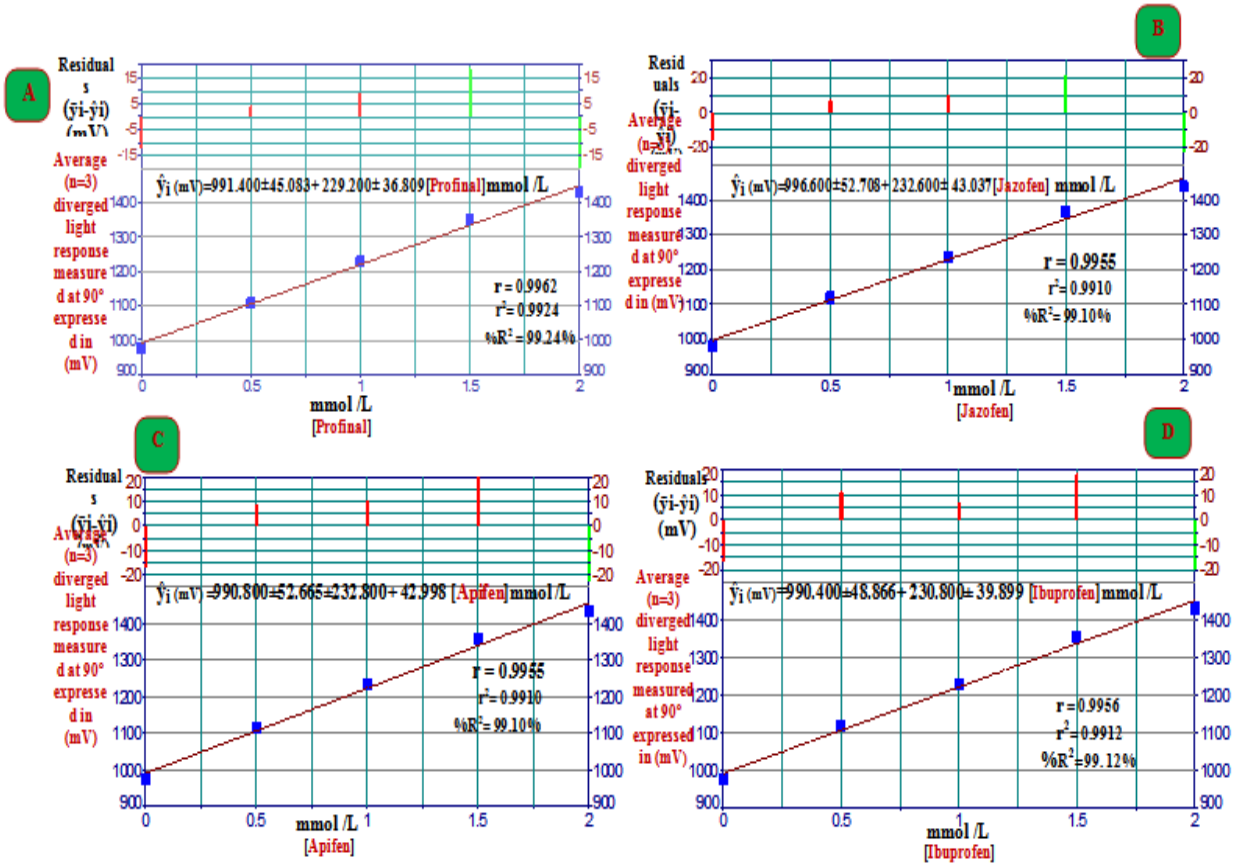


Figure 12: Standard addition calibration graph using ISNAG- fluorimeter for: A - Profinal,B - Jazofen, C- Apifen, D- Ibuprofen

Residual = (ȳi-ŷi) in mV, ȳi = practical value, ŷi =estimated value.

VII. Conclusion

The developed newly adopted methodology in this research work was put into a paired t-test (the tool comparison) for the sake of accepting it as an alternative method for analysis and assessment of Ibuprofen with standard used method. Mainly British Pharmacopoeia (B.P),turbidometry, or rejecting it as an alternative method. The assessment is made on how much they are correlated as a methods and if there is any significant difference that will work against the developed method. On this basis two assumption statisticallyis made [28,29]:-

There is no significant difference between the means of all used four methods (i.e.; undistinguishable differences between the method) and if **μ** indicates the mean then it will annotated with specified term representing the method used as such

H_o= Nullhypothesis= No significant difference between

$\mu_{ISNAG\text{- fluorimeter}} = \mu_{B.P} = \mu_{turbidometry}$

OR

$\mu_{ISNAG\text{ -fluorimeter}} - \mu_{B.P} = zero$, $\mu_{ISNAG\text{- fluorimeter}} - \mu_{turbidometry} = zero$

The alternative hypothesis **H_1:-**

$\mu_{ISNAG\ fluorimeter} \neq \mu_{B.P}$,$\mu_{ISNAG\ fluorimeter} \neq \mu_{turbidometry}$,

Conducting paired t- test will all possible pairs (i.e.; 3- pairs). The necessary comparison of the paired t-test are two which are as follows: ISNAG **Vs**. British Pharmacopoeia, ISNAG **Vs**. Turbidometry, as ISNAG being the suggested alternative or equivalent method of assessment of the drug which challenges the available official method as ISNAG as an instrument is new in its whole properties of working and presenting results for

determination, therefore it is the one whose its capability is under question and its approval as a method with the existing method and the used ones. Following table 9, it can be found that there is three comparison. As it compare, ISNAG method with the other there standard method as shown above. In which a significance test indicate that at 95% confidence (α = 0.05/2 two tailed) there is no significant difference between the newly developed method and the other two standard method. Therefore, the analyst should be able to choose any method for analysis i.e.; ISNAG or the other two. Thus accepting null hypothesis. This indicate that the high efficiency of ISNAG as a reliable instrument for analysis of Ibuprofen.

Table 8: Summary of results by standard additions method for the determination of Ibuprofen by different methods:- ISNAG fluorimeter method, and Turbidometer method

Number of sample	Name, cated value, company And country	Confidence interval for the average weight of tablets $\overline{W_i}(g) \pm t_{0.05}\sigma_{n-1}/\sqrt{n}$ at 95%	Theoretical content for the active ingredient W_i (mg)±t.96 $\sigma_{n-1}/\sqrt{n}$ at 95%	Sample weight equivalent to 1.03143 g (50mmol/L of the active ingredient. w_i (g)	ISNAG fluorimeter / Turbidometer	[Sample]mmol/L					$\hat{y}_i = a \pm S_a t + b \pm S_b t$ [Sample]mmol/L at confidence level 95%, n-2	r / r^2 / R^2%	Practical content of active ingredient: Concentration mmol/L prepared sample in 10ml	100ml	Weight in 100ml $\overline{Wi}(g) \pm t_{0.025}\sigma_{n-1}/\sqrt{n}$ at 95%, n-1	In tablets $\overline{Wi}(mg) \pm t_{0.025}\sigma_{n-1}/\sqrt{n}$	Efficiency of determination REC%
						0	0.5	1	1.5	2					$\bar{x}_i \pm t_{0.05/2,\ n-1}\sigma_{n-1}/\sqrt{n}$			
1	Profinal 400mg Julphar - UEA	0.6228 ± 0.0058	400±3.725	1.6059	[Profinal] mmol/L	979	1110	1230	1354	1430	991.400±45.083+ 229.200± 36.809 [Profinal]mmol /L	0.9962 0.9924 99.24%	4.3254	54.067	1.11533 ± 0.0153	432.547 ± 5.934	108.14%
						112	185.88	222.92	255.90	310.40	27.056±30.926+55.364±25.251 [Profinal]mmol/L	0.9833 0.9669 96.69%	0.4876	48.767	1.0060 ± 0.0196	390.152 ± 7.6013	97.54%
2	Jazofen 400mg Jenapharm - UAS	0.6286± 0.0017	400±1.082	1.6209	[Jazofen] mmol/L	981	1120	1239	1367	1439	996.600±52.708+ 232.600± 43.037 [Jazofen] mmol /L	0.9955 0.9910 99.10%	4.2846	53.558	1.1048 ± 0.0163	428.457 ± 6.3214	106.11%
						114.89	187.65	229.31	262.19	299.66	29.924±34.222+58.816±27.944 [Jazofen] mmol/L	0.9827 0.9657 96.57%	0.5087	50.877	1.0495 ±0.0187	407.006 ± 7.252	101.75%
3	Apifen 200mg Zauba - India	0.2899± 0.00197	200±1.359	1.49506	[Apifen] mmol/L	974	1116	1234	1360	1434	990.800±52.665±232.800+ 42.998 [Apifen] mmol /L	0.9955 0.9910 99.10%	4.2560	53.200	1.09744 ±0.0173	212.7994 ± 3.3546	106.40%
						116	190.59	228.31	265.14	302.66	30.966±33.678+61.574±27.499 [Apifen] mmol /L	0.9825 0.9654 96.54%	0.5029	50.291	1.0374 ± 0.0223	201.042 ± 4.322	100.52%
4	Ibuprofen 200mg DHP Co. - U.K	0.5748± 0.0167	200± 5.811	2.82508	[Ibuprofen] mmol/L	974	1117	1228	1355	1432	990.400±48.866+ 230.800± 39.899 [Ibuprofen] mmol /L	0.9956 0.9912 99.12%	4.2911	53.640	1.0550 ± 0.0152	204.603 ± 2.560	112.57%
						118.45	191.77	223.98	264.16	301.09	32.356±31.670+62.534±25.860 [Ibuprofen] mmol/L	0.9892 0.9785 97.85%	0.5174	51.741	1.0673 ± 0.0193	217.165± 3.927	108.58%

$\hat{y}_i$: Estimated response value (mV) for ISNAG fluorimeter, and Turbidomtric method (FTU)) for (n=3), [sample]: drug concentration (mmol/L), r: correlation coefficient, r^2:coefficient of determination & R^2%: percentage capital R square, $t_{0.025,\ \infty} = 1.96$ at 95% $t_{0.025,\ 2} = 3.182$. For n-2

Using paired t-test, which shows a comparison at two difference paths:

First test : Comparison of newly developed method (ISNAG fluorimeter) with official quoted Value (400 mg or 200 mg) [3] as shown in Table 4.20 (column 4) by calculated t- values of each individual company and these comparison with tabulated t- value hypothesis can be estimated as follow :

Null hypothesis: There is no significant difference between the means obtained from four source of four different companies ($\bar{X}$) and quoted value (μ).

i.e.: $\mathbf{H_o}$: $\bar{X} = \mu$

For: **Profinal** (400mg Julphar) **UEA**, and **Jazofen** (400mg Jenapharm) **UAS** companies. and no significant difference between the means obtained from four source of four different companies ($\bar{X}$) and quoted value (μ).

i.e.: $\mathbf{H_o}$: $\bar{X} = \mu$

For: **Apifen** (200mgZauba) **India**, and **Ibuprofen (**200mg DHP Co**.) U.K** companies.

Against:

Alternative hypothesis: there is a significant difference between the means and Quoted value i.e.: $\mathbf{H_1}$: $\bar{X} \neq \mu$ for: four different companies.

Since all values obtained (t calc.) are > t tab (4.303) at confidence level 95 % and DF(degree of freedom) = n-1 ; Null hypothesis will be reject accepting the alternative hypothesis ;these mean that there is a significant difference between the quoted active ingredient value and the measured value.

Secondary test : Using paired t-test at α= 0.05 (2-tailed) for the comparison of developed method using ISNAG fluorimeter and classical method using HANNA instrument (turbidity measurement) as shown in Table 9 (column 7) . The summary of results are tabulation in Table 9.

Taking in to consideration that all pharmaceutical drug samples from different companies are of the same population standard; i.e. neglecting individual differences between one manufacturer and another, as they all quoted value the same (400 mg for Profinal, Jazofen and 200 mg for Apifen and Ibuprofen).

Assumption:**Null hypothesis H_o: $\mu_{ISNAG\text{- fluorimeter}} = \mu_{turbidometry}$**

Against:

Analternative hypothesis: There is a significant difference between the mean of Classical method and ISNAG fluorimeter,

i.e.; **AlternativeH_1:$\mu_{ISNAG\ fluorimeter} \neq \mu_{turbidometry}$**

The obtained results indicate clearly that there was no significant differences between newly developed method (ISNAG fluorimeter) and turbidemetry method (classical method) at 95% (α = 0.05) confidence level as the calculated $t_{calc.}$ (1.382) is less than $t_{tab.}$ (3.182) for the determination of Ibuprofen in pharmaceutical drugs as shown inTable 9 (column 11).

Table 9: Summary of results for paired t-test, practical content and efficiency, using Ibuprofen – sodium nitro prosside system two different methods of samples for the analysis of Ibuprofen in drugs for n= 4 at 95% confidence level (α = 0.05) and DF = 3

Type of sample	Practical concentration (mmol/L) and what is equivalent of active ingredient (mg) 10 ml 50ml	Practical content of active ingredient In tablets $\overline{Wi}$ (mg) $\pm t_{0.025}\ \sigma_{n-1}/\sqrt{n}$ For (n=4) ,at 95%	Paired differences			
			Individual comparison $(\bar{X}-\mu)\sqrt{n}/\sigma$ n-1 ISNAG with quoted value t 0.25 , 2= 4.303	Comparison between two method		
				Xd	$\bar{X}$d (σn-1)	tcal= $\bar{X}$ d $\sqrt{n}$ / σ n-1 at 95 %
	ISNAG-fluorometer					
	HANNA instrument (classical method) Turbidity measurement					
Profinal 400mg Julphar -UEA	4.3254 54.067	432.547 ± 5.934	23.597 >> 4.303	42.395	15.760 (22. 803)	1.382 << 3.182
	0.4876 48.767	390.152 ± 7.6013				
Jazofen 400mg Jenapharm - UAS	4.2846 53.558	428.457 ± 6.3214	19.367 >> 4.303	21.451		
	0.5087 50.877	407.006 ± 7.252				
Apifen 200mg Zauba -India	4.2560 53.200	212.7994 ± 3.3546	16.422 >> 4.303	11.757		
	0.5029 50.291	201.042 ± 4.322				
Ibuprofen 200mg DHP Co. - U.K	4.2911 53.640	204.603 ± 2.560	7.740 >> 4.303	-12.562		
	0.5174 51.741	217.165± 3.927				

Xd: Difference between two method, **$\bar{X}$d**: difference mean, **σn-1:** Difference standard deviation **n**= no. of sample = 4 $t_{0.025,\ 2}$= 4.303 for individual $t_{tab.}$ at 95 % , $t_{0.025,\ 3}$= 3.182 for paired t- test at 95 %, **μ**: quoted value

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