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New Mode for Long Path Irradiation and Two Sided Detection for the Determination of Ibuprofen in Pure and Pharmaceutical Drugs using Continuous Flow Feed via the use of ISNAG Fluorometer Instrument

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Abstract: ***A new, simple, accurate, fast and sensitive method has been developed for the determination of Ibuprofenin pure form and drugs (tablets) by continuous flow injection diverged light. The method was based on the reaction of the Ibuprofen with potassium chromate to form a precipitate, using homemade ASNAG- fluorimeter. Optimum parameter has been studied to increase the sensitivity for developed method. The linear dynamic range for the instrument response versus Ibuprofen concentration was 5- 30mmol/L while the L.O.D was 1.630µg/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 0.9886while percentage linearity (R^2%) was 97.74%. RSD% for the repeatability (n=8) was lower than 0.5%for the determination of Ibuprofen, with concentration of 5, 30 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 (UV-Vis spectrophotometry at wavelength 220nm, and 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 value of each individual company with calculated t-value at 95% confidence interval from developed method.***

Keywords: Ibuprofen, flow injection diverged light, homemade instrument

1. 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].

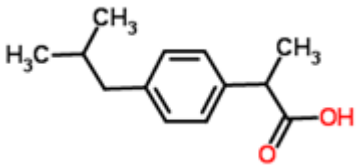


Figure 1: structure of Ibuprofen IUPAC name [**2-(4-Isobutylphenyl) propanoic acid].**

There are Different analytical methods for determination of Ibuprofen and pseudoephedrines 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 [19-27], 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 via diverged (all kind of scattered light) of incident beam, since it is lies on the 0-90° 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.

2. 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 500mmol/L of potassium chromate molecular formula K_2CrO_4molar mass 194.21 g/mole and Merck-USA was prepared by dissolving 48.5525 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) for Apifen 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 –UAE 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 was measured by a homemade ASNAG-fluorimeter. Low-pressure 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. UV-Vis Spectrophotometer digital double beam type (UV- Vis spectrophotometer, UV-1800, shimadzu, Japan) was also used to scan the spectrum of Ibuprofen, 4cm quartz cell, Turbidemtric readings by Turbidity-meter, HANNA Company (Hungary).

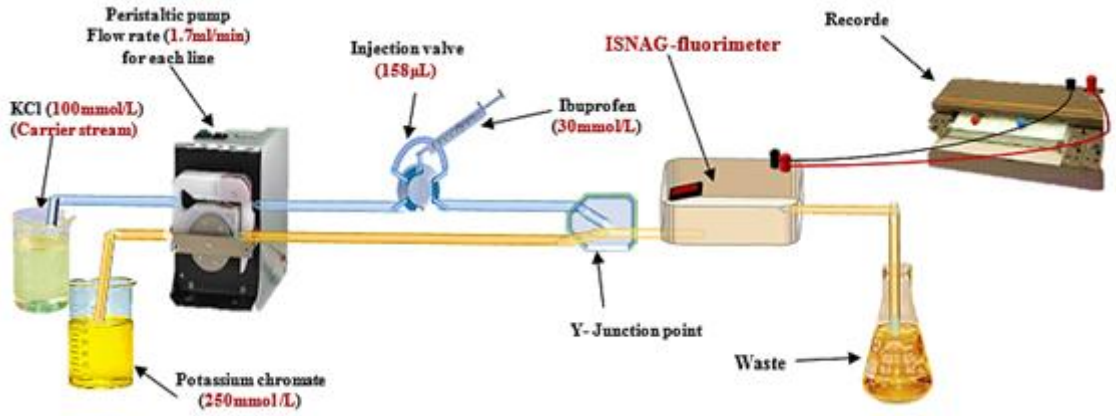
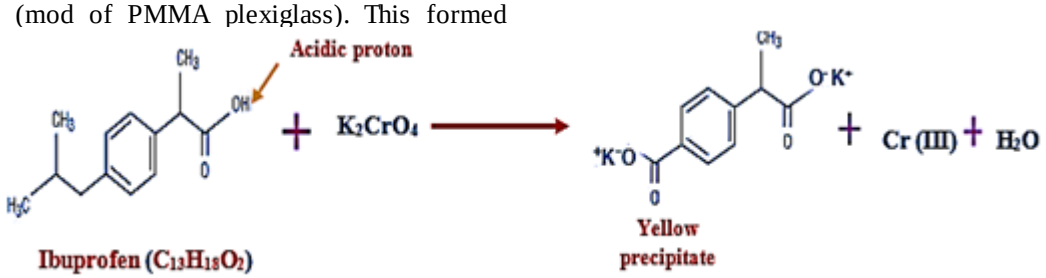


Figure 2: schematic diagram of flow injection analysis for determination of Ibuprofen via using ASNAG- fluorimeter at 1.7ml/min flow rate, 158µL, and open valve mode

3. Methodology

Tow lines design system (Fig. no. 2) was used for Ibuprofen determination. First line is the carrier stream(1.7ml/min) from potassium chloride(100mmol/L) that will take and introduce the sample loop segment (158µL, 30mmol/L) from Ibuprofen into the reaction stream by combining with the second line (1.7ml/min) that carry the reagent (potassium chromate 250mmol/L) 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 lines 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 in scheme 1. [28, 30]



Scheme 1: proposal mechanism for the reaction between Ibuprofen and potassium chromate

4. Result and Discussion

Optimization of reaction pattern parameter

All variable will be based upon that the data obtained after subjecting the raw data that were obtained experimentally taken from ISNAG to Savitzky- Golay smoothed filter that is expected to smooth the data trying avoiding sharp noise(if any random noise a rising from different sources whether instrumental or kind of precipitate formed or any fluctuation in electricity main supply. These entire factors will effect on the signal-to-noise ratio which in turn will affect the response profile leading to miss calculate peak heights in which all assessment of Ibuprofen will relay upon. Therefore, it is going to be in use for Savitzky-Golay smoothed filter on same of the variable at hand for studying to obtain best range of the independent(x) variable to be at its most beneficial use in the research work conducted here in the recent advance in predicting the optimum variable that will be used throughout this research work.

The following steps are followed:-

1) Plot of the laboratory measurement values at the Y-axis versus the variable that will be used throughout this study and presented have, as the Y-predicted values according to best fit mathematical model which based on the laboratory measurement values.
2) Plot Savitzky-Golay smoothed filtering data which is based on the Y-predicted values which obtained from mathematical model

Chemical variation

Potassium chromate concentration

The study was carried out using a series of solutions by different concentration of potassium chromate 50-400 mmol/L as a precipitate reagent at flow rate 1.3mL/min, the water is a carrier stream and sample volume79µL from Ibuprofen 30 mmol/L. Figure no. 3-A shows the response profile. The results obtained were summarized in table 1. In which that, the increase of potassium chromate concentration leads to the formation of solid particulate a mixture of a time gradient particulate growth which might gives different forms of rigid, crystalline or colloidal miniature precipitated reaction product. In all above trends of reaction pattern in a flow cell of 2mm path length that extend for 100mm distance will furnish a good medium for refraction or reflection of incident light that will allow as a result a diverged beam of light causing scattering of the incident light (λ = 184.9nm and 253.7nm) which also a positive stock shift might happen. This effect increase with increasing potassium chromate concentration reaching up to 250mmol/L. On the above explanatory reason 250mmol/L was found to satisfy research work needed in the assessment of ibuprofen (Fig. 3-B). As part of the different variables will be dealt with here.

Table 1: Effect of potassium chromate concentration on response function expressed as an average peak height $\bar{y}_i$ (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 $[K_2CrO_4]$ 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 $\hat{y}_{i\,(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,\,2}\ \sigma_{n-1}/\sqrt{n}$		
50	280	0.29	280 ± 2.04	291.159	285.799
100	304	0.31	304 ± 2.31	292.45	394.463
150	368	0.26	368 ± 2.34	368.43	562.757
200	958	0.10	958 ± 2.43	957.806	743.4791
250	986	0.10	986± 2.41	986.38	862.547
300	920	0.14	920 ± 3.23	919.706	909.534
400	776	0.18	776 ± 3.53	776.057	904.0529

$t_{0.025,\,2}$=4.303, $\hat{y}j = \sum_{i=-\frac{m-1}{2}}^{\frac{m-1}{2}} (Ci\,\bar{y}j+i)$, m= convolution coefficient, m+1/2≤ j ≥ - m-1/2

Note: As it can be seen there is no differences between the practical obtained values and the mathematical model.

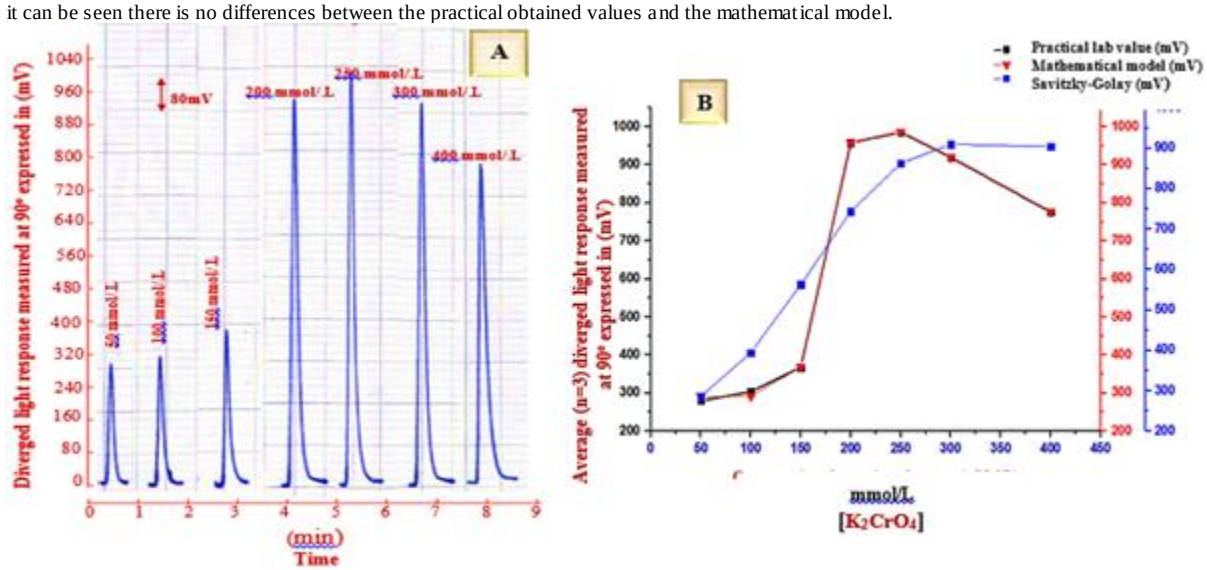


Figure 3:

a) Response –time profile of Ibuprofen with variable concentration of potassium chromate solution (clear unobstructed peak with no deformation at different level of potassium chromate solution.
b) Plot of averaged peak height responses vs. potassium chromate solution concentration.

Carrier stream effect

The reaction between Ibuprofen (30mmol/L) with potassium chromate (250mmol/L) to form a yellow color precipitate was study in different solution media (NaCl, KCl, KI, KNO_3, 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 79µL (using open valve mode). Since, it is a fact that at normal condition of precipitate formation in a dynamic system (as it is the case here) gives a various shapes of formed geometry of precipitate particulate formation, most of it will be in the form of small sized particulate mainly it could be in the form of a nucleis this eventually will not give a huge diverged light because of its uncompleted growth form of particles that will collect in its peaked blocked form a good reflecting surface for divergence and detection at 0-90° in which ISNAG does. Therefore any salt that will help in agglomeration and nucleation condensation might give the above described behaviour is the best salt. Table no. 2 sum up some salt solution that were used as a carrier stream in the manifold (shown in fig. no 4-A). It was noticed that KCl was the most favorable salt solution to be used (Fig. no. 4-B).

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

Independent type of salt as a carrier stream	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	984	0.1	984 ± 2.41
NaCl	624	0.17	624 ± 2.61
KBr	864	0.15	864 ± 3.28
KNO_3	496	0.24	496 ± 3.01
KCl	1015	0.09	1015 ± 2.31
NH_4Cl	776	0.16	776 ± 3.03
CH_3COONa	308	0.35	308 ± 2.71
CH_3COONH_4	370	0.43	370 ± 3.93

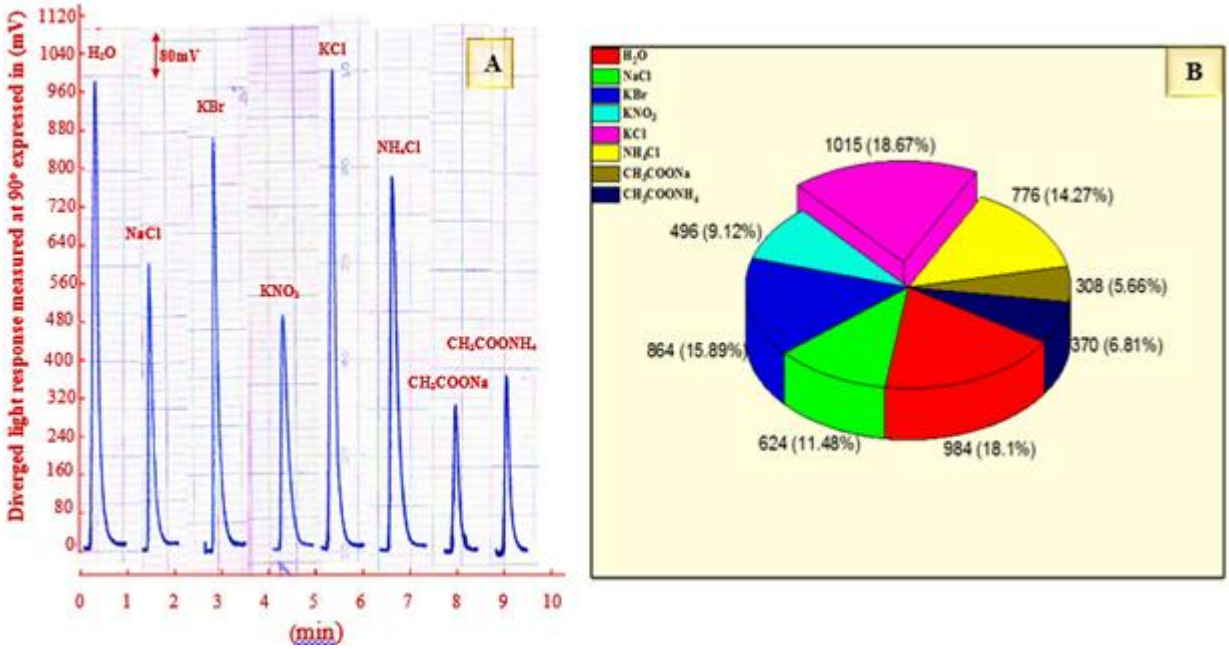


Figure 4: Effect of salt solution used as a carrier stream on profile (**A**), and pie percentage Representation of the contribution of each salt solution (B)

Potassium chloride concentration effect

Under the same experimental condition, a set of variable concentration ranging 30- 300 mmol/L of potassium chloride solutions were prepped in order to decide the most favourable concentration that will fit the methodology that will be adopted in this conducted research work. Figure no.5 shows clearly that 100 mmol/L is the most optimal concentration. An excess of KCl concentration (i.e.> 100mmol/L) leads to a weakening S/N which might be attributed to peptization effect (i.e.: refers to the process by which a coagulated particles reverts to its original dispersed state).The obtained results are shown in table3.

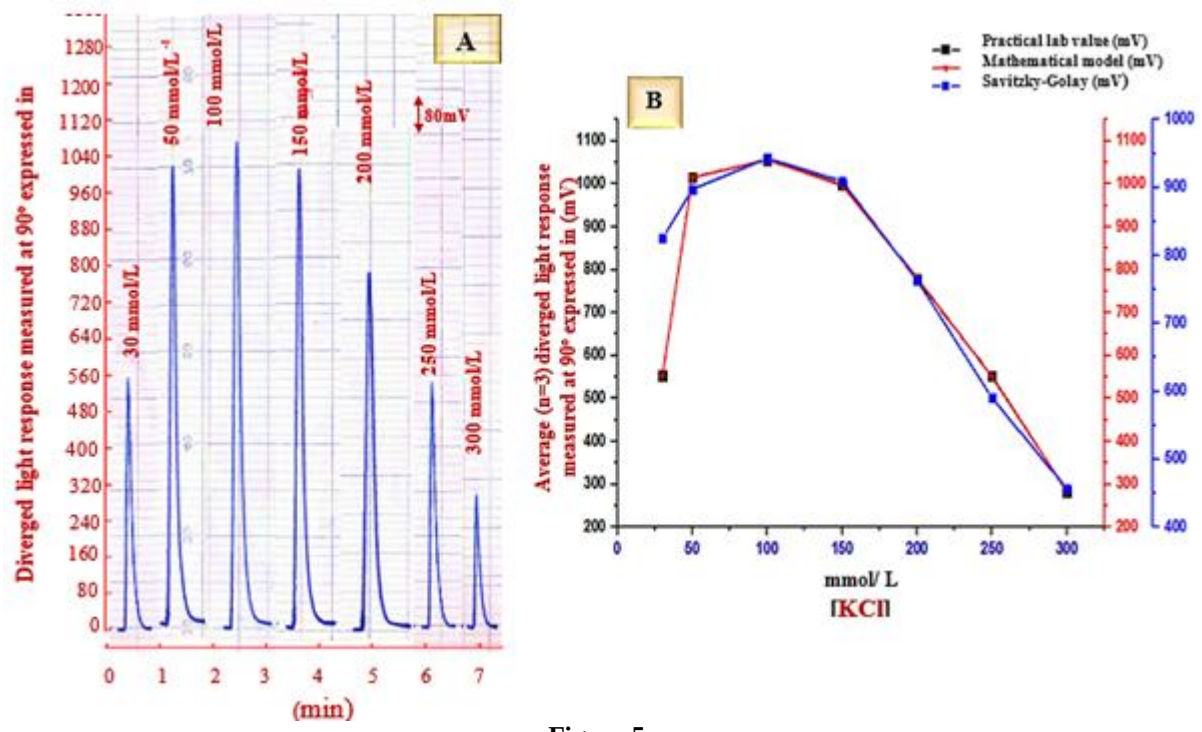


Figure 5:
a. Variable of KCl concentration on: A. Diverged light profile vs. time
b. Average diverged light measured at laboratory values (practically) which represented by best fit mathematical model and smooth digital filtering using Savitzky- Golay filter.

Table 3: Effect of potassium chloride on diverged light response by reflection, refection, dispersed light 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 [KCl] 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 $\hat{y}_{i\,(S\text{-}G)}$
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i(mV) \pm t_{0.025,\,2}\,\sigma_{n-1}/\sqrt{n}$		
30	554	0.26	554 ± 3.60	554	825.189
50	1015	0.12	1015 ± 3.06	1014.958	897.3915
100	1056	0.08	1056 ± 2.21	1056.467	943.0878
150	998	0.11	998 ± 2.61	996.305	909.1594
200	778	0.16	778 ± 3.01	780.605	763.594
250	552	0.22	552 ± 2.96	550.44	590.349
300	283	0.52	283 ± 3.68	283.225	457.192

$t_{0.025,\,2}$ =4.303, $\hat{y}j = \sum_{i=-m-\frac{1}{2}}^{m-\frac{1}{2}} {}_i(Ci\,\bar{y}j + i)$, m= convolution coefficient, m+1/2 ≤ j ≥ - m-1/2

Note: As it can be seen there is no differences between the practical obtained values and the mathematical model

Physical Parameters

Electronic filter effect

The results obtained throughout this research work were subjected to two kind of smoothing of:-

a) Response via the use of electronic low band pass filter (RC-filter).
b) Obtained data smoothing via Savitzky- Golay filter.

This study was carried out for the determination of preferred low band pass electronic filter using 79µL sample volume of Ibuprofen (30mmol/L) at 1.3ml/min flow rate at each line and 100mmol/L concentration of potassium chloride 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. Figure no. 6 shows that there were no improvements at this stage that necessitate the use of RC-low band pass filter. While data smoothing can not 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 were the choice of this part of research work. Table no. 4 tabulates all the results obtained.

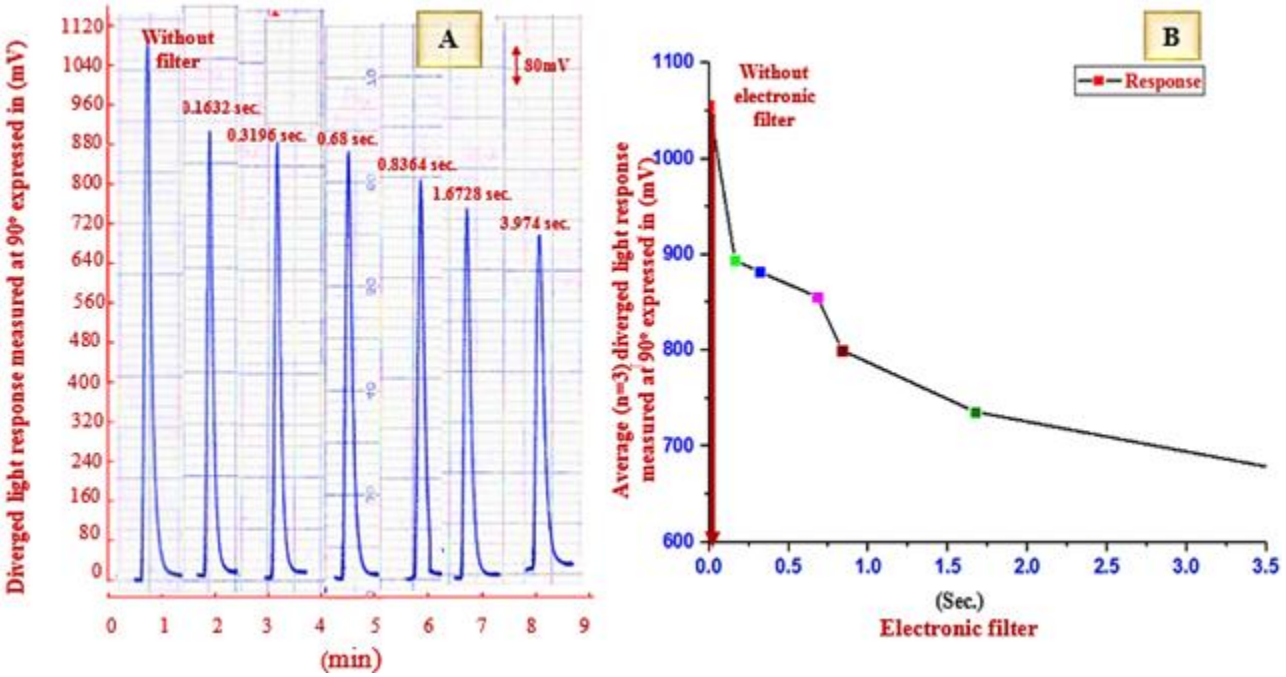


Figure 6:
a. Diverged beam of incident light vs. time profile using RC- low band electronic filter
b. Graphical representation of the effect of using RC- low band pass filter.

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 (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	1056	0.09	1056 ± 2.41
0.1632	894	0.1	894 ± 2.11
0.3196	882	0.14	882 ± 3.06
0.68	856	0.11	856 ± 2.36
0.8364	800	0.14	800 ± 2.78
1.6728	736	0.14	736 ± 2.61
3.974	664	0.16	664 ± 2.71

Flow rate effect

Variable flow rate (0.4- 3.6) ml/min was used at Ibuprofen(30mmol/L)- potassium chromate (250mmol/L)- potassium chloride (100mmol/L) system and 79µL 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 figure 7 that at slow flow rate (0.4-1ml/min) a wide broad response profile is obtained which might cause 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.

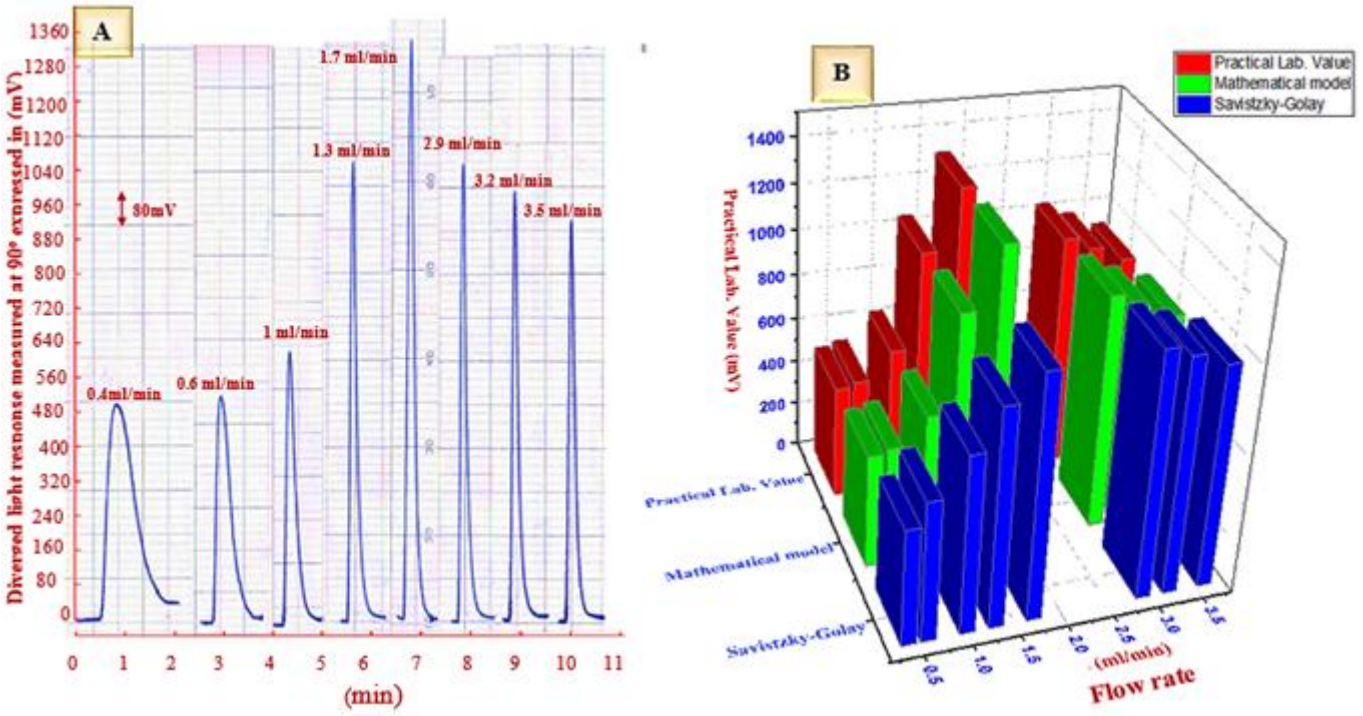


Figure 7: Effect of flow rate on:-
a. Diverged beam of light vs. time profile
b. Laboratory measurements values, mathematical response values, and Savitzky- Golay smoothed filtering date as the Y-axis.

Therefore, a 1.7 ml/min for each line was the optimum choice to compromise between sensitivity, response profile and consumption of chemicals since a response is a function of physical and chemical variable. All results tabulated in table no. 5.

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 (mL/min)	Dependent variable Average (n=3) diverged light response measured at 90°expressed in mV							
	Practical lab. value						Mathematical model $\hat{y}_i$	Savitzky-Golay $\hat{y}_{i(S-G)}$
	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	496	0.31	496 ± 3.80	82	495.999	508.6103
10	0.6	0.6	516	0.26	516 ± 3.28	48	516	611.837
15	1	1	632	0.22	632 ± 3.53	38	631.955	777.545
20	1.3	1.3	1052	0.10	1052 ± 2.71	34	1052.23	954.7721
25	1.7	1.7	1312	0.07	1312 ± 2.36	31	1311.56	1069.902
30	2.9	3	1040	0.10	1040 ± 2.68	29	1043.34	1089.643
35	3.2	3.3	984	0.12	984 ± 3.01	26	979.152	1045.384
40	3.5	3.6	920	0.17	920 ± 3.78	24	921.763	988.168

Δt_b (sec) : Time lapse for the preciptate response within measuring cell or peak base width

$t_{0.025,2}$ =4.303, $\bar{y}j = \sum_{i=-m-\frac{1}{2}}^{m-\frac{1}{2}} (Ci\,\bar{y}j + i)$**, m= convolution coefficient, m+1/2 ≤ j ≥ - m-1/2**

Sample loop volume

Using the optimum parameters achieved in previous sections. The effect of sample volume (Ibuprofen 30 mmol/L) as an analyte was used. Variable sample volume (79-329µL) were injected in the valve mode. The obtained results are shown in figure 8 and the data tabulated in table no. 6. It was noticed that, the use of sample loop volume of less than 158µL (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 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 definitely 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, 158µL sample loop volume is the most satisfactory sample plug.

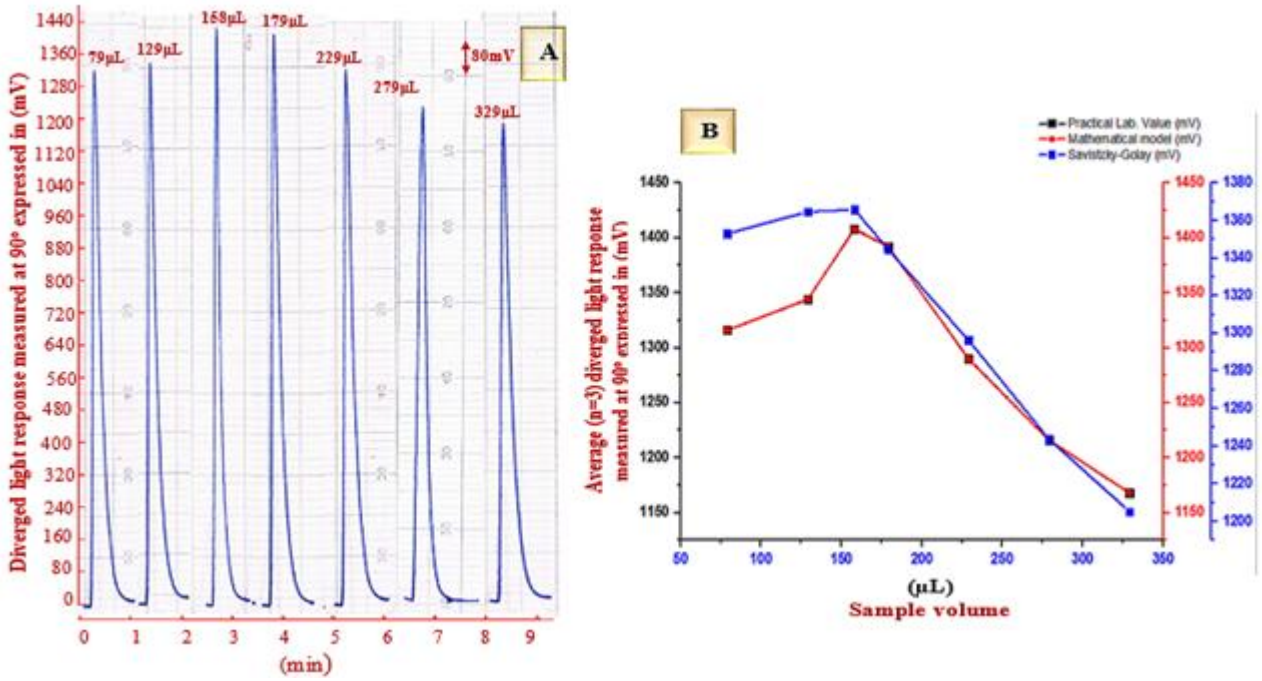


Figure 8: Effect of sample loop volume on:

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.

Table 6: variation of injection sample volume 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.

length of sample loop(cm) Diameter (0.5mm)	Independent variable sample loop volume ml/min	Dependent variable Average (n=3) diverged light response measured at 90°expressed in mV					
		Practical lab. value				Mathematical model $\hat{y}_i$	Savitzky-Golay $\hat{y}_{i\ (S\text{-}G)}$
		Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,\ 2}\ \sigma_{n-1}/\sqrt{n}$	Δt_b(Sec)		
10	79	1316	0.09	1316 ± 3.06	31	1316.02	1353.001
16.33	129	1344	0, 08	1344 ± 2.61	34	1344.093	1364.677
20	158	1408	0.07	1408 ± 2.51	36	1407.63	1365.813
22.66	179	1392	0.09	1392 ± 3.01	38	1392.45	1344.694
29	229	1290	0.17	1290 ± 5.49	39	1289.75	1296.366
35.32	279	1216	0.10	1216 ± 3.01	43	1216.09	1243.735
41.65	329	1168	0.12	1168 ± 3.53	47	1167.96	1205.194

Δt_b (sec) : Time lapse for the preciptate response within measuring cell or peak base width

$t_{0.025,\ 2}$ = 4.303, $\bar{y}j = \sum_{i=-m-\frac{1}{2}}^{m-\frac{1}{2}} {}_1(Ci\ \bar{y}j + i)$, m= convolution coefficient, m+1/2 ≤ j ≥ - m-1/2

Purge time

Using optimum parameters that were achieved in the previous sections, purge time of the sample volume to be injected via the carrier stream (KCl 100mmol/L) 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 tables 7, 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. no. 9). a vice versa will insure a complete discharge and a full purge of the sample plug from injection valve. 45 second was found a time that compromise a suitable purge time and through output with a good response profile a voiding any irregularity.

Table 7: 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	Savitzky-Golay
	Average peak height($\bar{y}_i$)	RSD%	Reliability(two tailed) $\bar{y}_i$(mV)± $t_{0.025,2}$ $\sigma_{n-1}/\sqrt{n}$	$\hat{y}_i$	$\hat{y}_{i(S-G)}$
5	104	0.95	104 ± 2.46	103.90	173.1719
10	280	0.36	280 ± 2.51	280.77	307.42
15	552	0.22	552 ± 3.06	549.298	528.655
20	776	0.16	776 ± 3.08	781.404	763.359
25	992	0.11	992 ± 2.61	985.245	937.213
30	1042	0.13	1042 ± 3.28	1047.404	1064.058
35	1072	0.10	1072 ± 2.78	1069.298	1185.363
40	1392	0.09	1392 ± 3.06	1392.772	1299.172
Open valves(45)	1410	0.07	1410 ± 2.53	1409.904	1376.394

$t_{0.025,2}$ = 4.303, $\bar{y}j = \sum_{i=-m-\frac{1}{2}}^{m-\frac{1}{2}} (Ci\ \bar{y}j + i)$, m= convolution coefficient, m+1/2 ≤ j ≥ - m-1/2

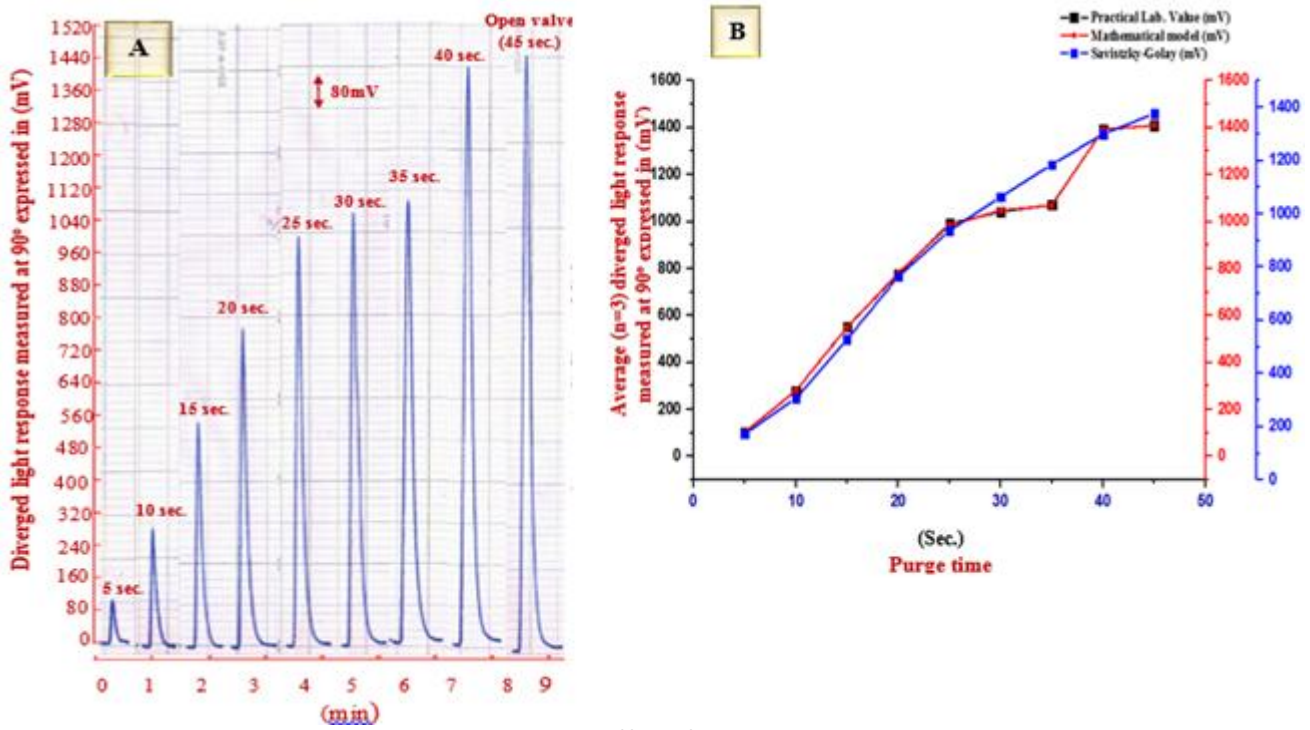


Figure 9: Effect of purge time on:-

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.

5. 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 (250mmol/L concentration of potassium chromate, 100mmol/L for potassium chloride, 158µL sample volume, and 1.7 ml/min flow rate for each line). Set of series (0.5-50mmol/L) solutions were prepared an output came was depicted in fig. no. 10

All prepared concentration was used. An increase in Ibuprofen concentration causes an increase number of nuclei formed up to 30mmol/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 - 90o. An increase in Ibuprofen concentration more than 30mmol/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 sixteen points (fig. no. 10-A) that were measured trying to fit a linear equation of the form y= a+ b x in which a correlation coefficient of r = 0.9365 while capital squared- R gave 87.71% for the whole chosen range (0.5- 50 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 (0.5-30mmol/L) (fig. no. 10- B, C) has a correlation coefficient of r = 0.9886 with a capital squared- R of 97.74% (≈ 98%). This indicate that the linear equation. chosen:

RDL(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 8.

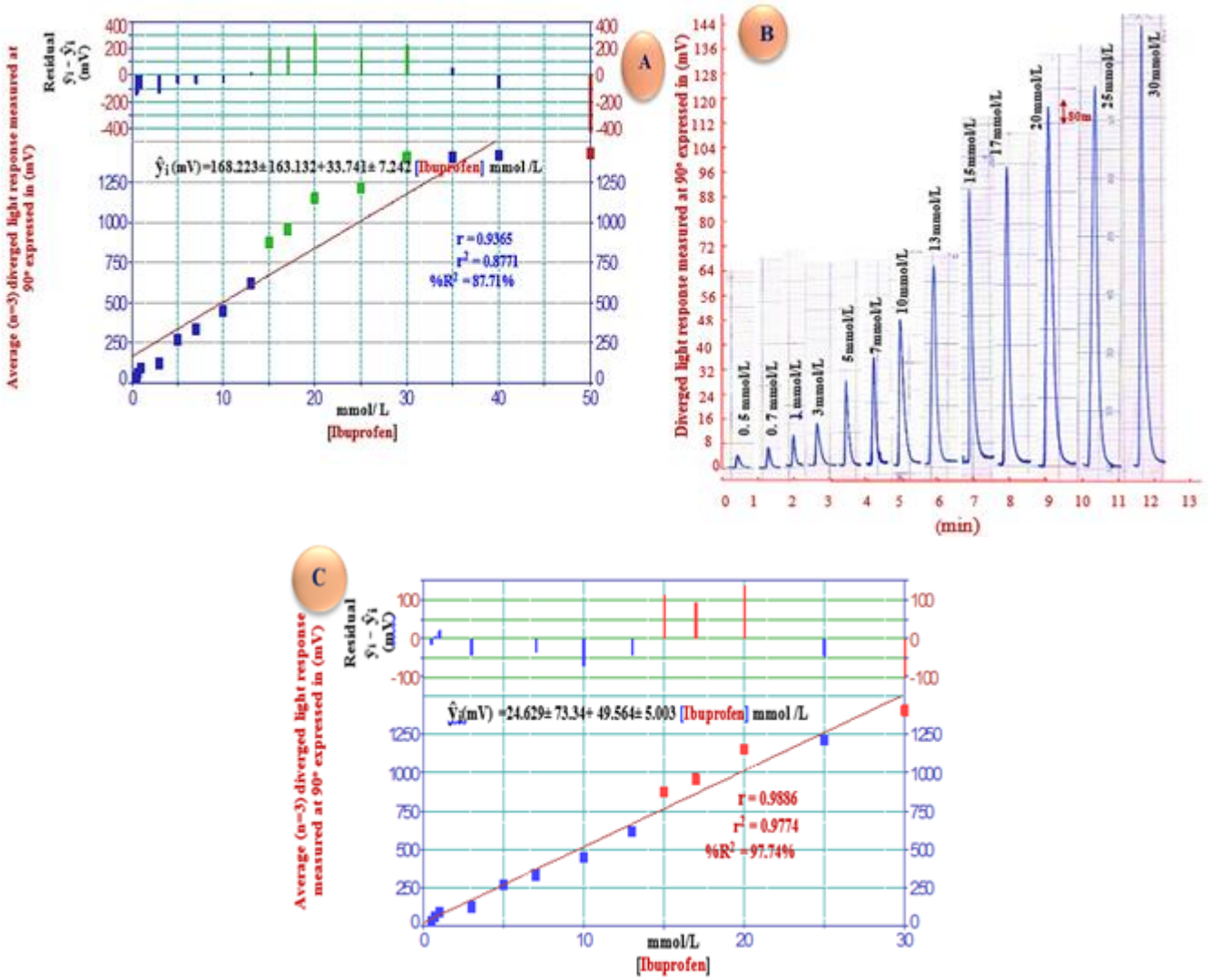


Figure 10:

A- variation of scattered diverged light at range (0.5-50mmol/L), n=16 against Ibuprofen concentration
B- Diverged beam of light Vs. time profile.
C- Calibration graph deduced from scatter point plot at range (0.5-30mmol/L), n= 13.
Residual = ($\bar{y}_i$-$\hat{y}_i$) in mV, $\bar{y}_i$ = practical value, $\hat{y}_i$ =estimated value

The assessment evolution of the new developed methodology for the determination of Ibuprofen was compared with the available literature cited methods, namely turbidemetry and spectrophotometric methods. Here a description of the used methods:

1- Turbidemtric measurement, which is based on the reaction of potassium chromate (0.35 mol/L, which already it used after established as can be seen in fig. no. 11-A. With the drug for a suitable ranged of concentration (0.5-50mmol/L) that the instrument is capable of handling it. A scatter plot shows that a calibration graph of having capital square-R of **99.08%** with correlation coefficient of **0.9954** for a linear regression equation of the form of

Response (NTU)=a+slope(Ibuprofen]mmol/L(figure11-B).

2- Spectrophotometric method based on the measurements of absorbance for the range of concentration (0.5- 50 mmol/L) at max wavelength (λ_{max} **=220nm**)[29], (fig. no. 11-C) using quartz cell.From fig. no. 11-D, the best linear range extend from 0.01- 0.4 mmol/L with correlation coefficient of **0.9782** and capital square-R = **95.69%**, n= 12 (no. ofmeasurement). Table 8 shows the variable data treatments. 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 (fig.no.12).

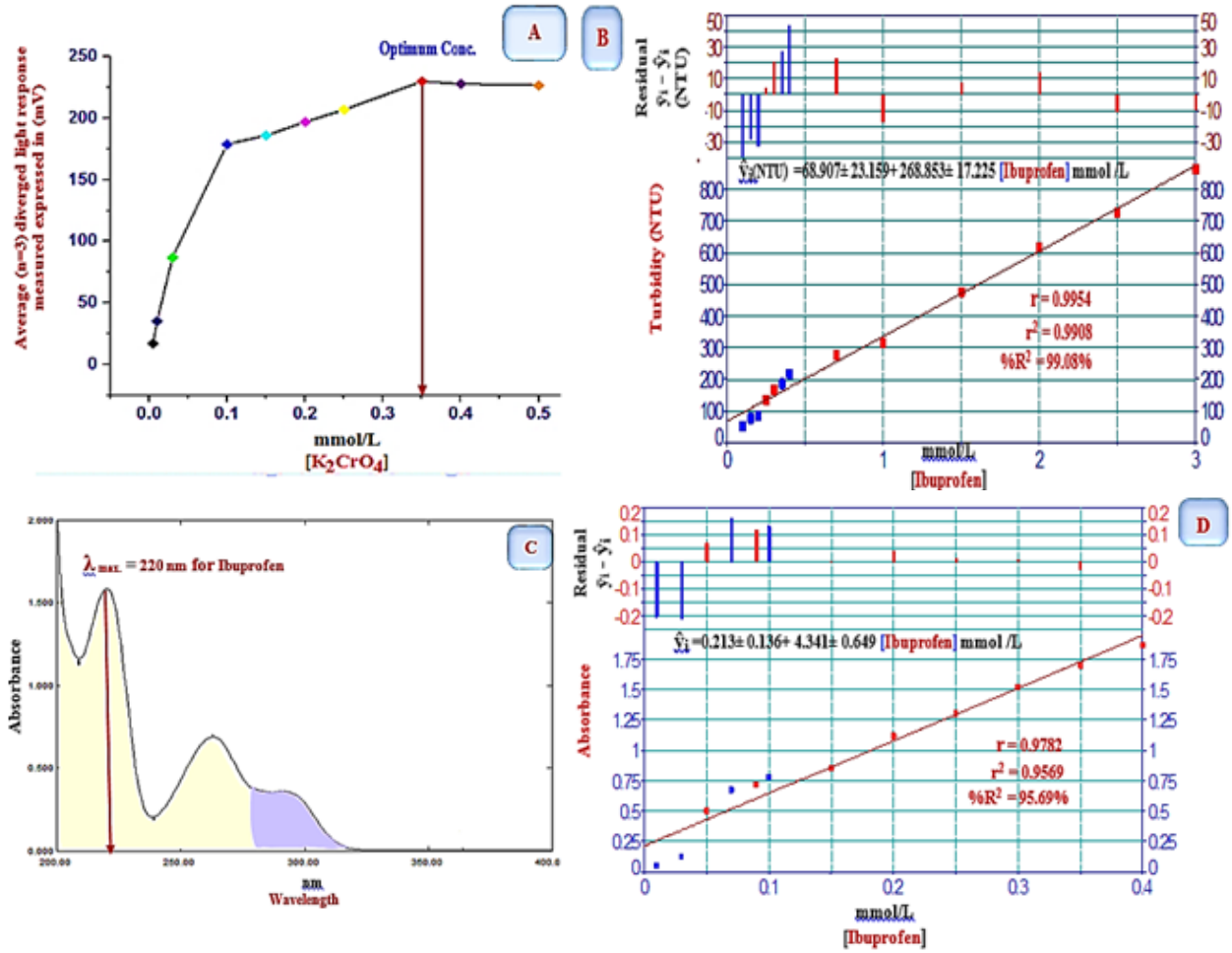


Figure no. 11:

A-Graphical representation shows the optimum concentration of K_2CrO_4 reacted with Ibuprofen and gave best-scattered measurement in Turbidemtric method

B-Calibration graph deduced from scatter point plot of Turbidemtric method (classical method) at range (0.5-50mmol/L), n=13 against Ibuprofen concentration

C-Figure of Absorbance UV-spectra of Ibuprofen standard solutions (1mmol/L) dissolved in water.

D-Calibration graph deduced from scatter point plot of spectrophotometry method (classical method) at range (0.5-50mmol/L), n=12 against Ibuprofen concentration.

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

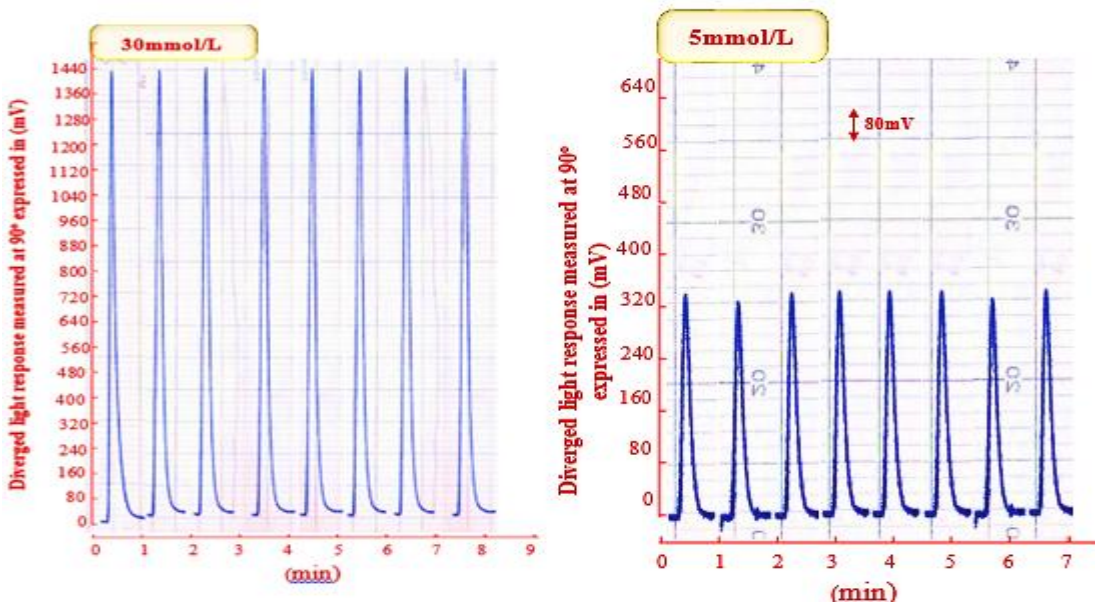


Figure 12

Effect of flow rate on: -Response profile of repeatability of Ibuprofen (5mmol/L and 30mmol/L).

Table 8: Comparison of different assessment method of Ibuprofen.

Method		No. of measurements	[Ibuprofen] mmol/L		Linear regression equation at 95%	r r^2 R^2%	t- value at 95%, n-2		Detection limit		Repeatability at 95% confidence level, n-1		%RSD
			Measured	Linear dynamic range	New development $\hat{y}_i(mV)=(a\pm S_a t)+(b\pm S_b t)$ [x] UV- spectrophotometry $\hat{y}_i=(a\pm S_a t)+(b\pm S_b t)$ [x] Turbidemetry 0- 90° $\hat{y}_i(NTU)=(a\pm S_a t)+(b\pm S_b t)$ [x]		t_{tab}	t_{cal}	Practically based on the gradual dilution of the minimum concentration	Theoretical based on slope	[Ibuprofen] mmol/L	Reliability of average diverged light $\bar{y}_i \pm t_{0.025,\,n-1}\,\sigma_{n-1}/\sqrt{n}$ n=8	
Newly developed methodology	ISNAG fluorimeter	16	0.5- 50	0.5- 50	168.223± 163.132+ 33.741± 7.242 [Ibuprofen]mmol/L	0.9365 0.8771 87.71%	2.145<< 9.99		(0.05mmol/L) 1.630µg	(6.052µmol/L) 0.197 µg	5	340 ±1.2124	**0.426**
		13		0.5- 30	24.629± 73.34+ 49.564± 5.003 [Ibuprofen]mmol /L	0.9886 0.9774 97.74%	2.201<<21.80				30	1408 ±1.957	**0.166**
Using method based on	UV- absorbance at λ_{max} =220nm (Shimadzu)	12		0.01- 0.4	0.213± 0.136+ 4.341± 0.649 [Ibuprofen]mmol /L	0.9782 0.9569 95.69%	2.228<<14.90		(0.05mmol/L) 41.257µg	(0.207mmol/L) 170.080 µg	0.15	0.860 ±1.0285	**143.02**
	Turbidmetry (NTU) Using Hanna instrument	13		0.1- 3	68.907± 23.159+ 268.853± 17.225 [Ibuprofen]mmol /L	0.9954 0.9908 99.08%	2.201<<34.35		(0.08mmol/L) 132.022µg	(5.579µmol/L) 9.207 µg	1	320 ±1.1288	**0.422**

$t_{tab} = t_{0.025,\,n-2}$, $t_{cal} = r/\sqrt{\frac{n-2}{1-r^2}}\,t_{tab} = t_{0.025,\,n-1} = t_{0.025,\,7} = 2.365$, R^2 = R- square, R^2= explain variation / total variation

[x] = [Ibuprofen] mmol/L, r =rootcorrelation coefficient, r^2 =correlation coefficient, R^2 % = linearity percentage, %RSD = Percent relative standard deviation $\bar{y}i$ = practical value, $\hat{y}i$ =estimated value

6. 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 two methods which in dudes UV-spectrophotometric via the measurement of absorbance at λ_{max} = 220nm by UV-1800, UV-spectrophotometer- Shimadzu, [(2)] and turbidemetry via Turbidity-meter, HANNA, (Hungary). the measurement of scattered light at 0- 90° for yellow precipitate particles of Ibuprofen- potassium chromate (0.35mol/L) system. A series of solutions were prepared of each drug (50mmol/L) (1.0314g of active ingredient in 100ml) (C.F. section 2) by transferring 1ml to each five volumetric flask (10ml), followed by the addition of gradual volumes of standard solution of Ibuprofen (0, 1, 2, 3, and 4 ml) of 50mmol/L to obtain (0, 5, 10, 15, and 20mmol/L) when use ISNAG fluorimeter (newly developed methodology), while transferring 0.02ml to each five volumetric flask, followed by the addition of gradual volume of standard solution of Ibuprofen (50mmol/L) (0, 0.01, 0.02, 0.03, and 0.04ml) to obtain (0, 0.05, 0.1, 0.15, and 0.2 mmol/L) concentration, in addition to Turbidemtric method that depend on the measurement at 90°. the series of solutions were prepared by transferring 0.1ml of 50 mmol/L concentration of each sample, followed by the gradual addition of 0, 0.1, 0.2, 0.3, and 0.4 ml from standard solution of Ibuprofen (50mmol/L) to obtain 0, 0.5, 1, 1.5, and 2 mmol/L. the measurements were conducted by three methods. Figure 13-A, B, C and D shows standard addition calibration graphs using newly developed methodology. The results were summed in table no. 9 at confidence level 95% (2-tailed), showing practically content of Ibuprofen in each sample of drug using three different methods and efficiency of determination.

7. 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), turbidemetry, and UV-spectrophotometric (scheme2), 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 assumptions statistically is made [31-32]

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_0= Nullhypothesis= No significant difference between

$\mu_{ASNAG\ fluorimeter} = \mu_{B.P} = \mu_{turbidmetry} = \mu_{UV\text{-}spectrophotometry}$

OR

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

$\mu_{ISNAG\ fluorimeter} - \mu_{turbidmetry} = zero$

$\mu_{ISNAG\ fluorimeter} - \mu_{UV\text{-}spectrophotometry} = zero$

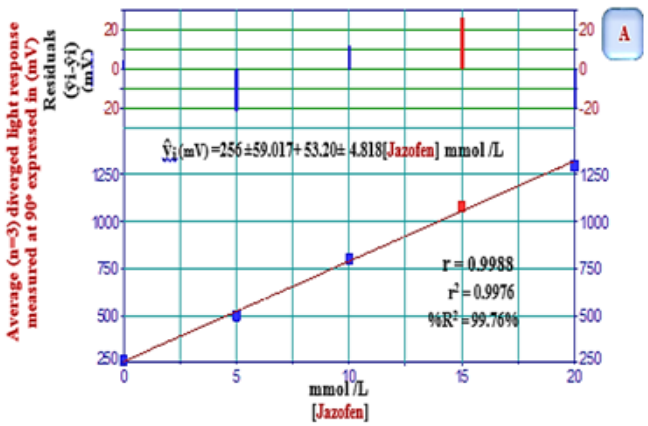
The alternative hypothesis **H_1**:-

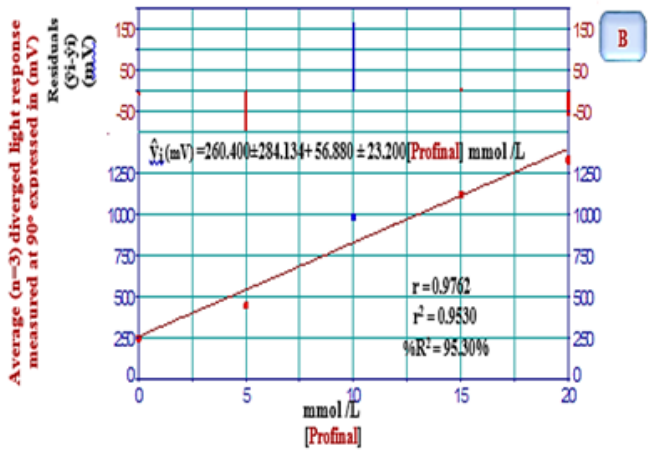
$\mu_{ISNAG\ fluorimeter} \neq \mu_{B.P}$

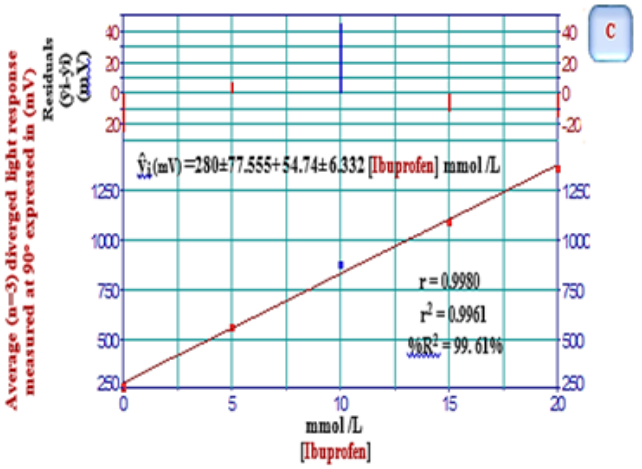
$\mu_{ISNAG\ fluorimeter} \neq \mu_{turbidmetry}$

$\mu_{ISNAG\ fluorimeter} \neq \mu_{UV\text{-}spectrophotometry}$

Conducting paired t- test will all possible pairs (i.e.; 6-pairs). The necessary comparison of the paired t- test are three which are as follows: ISNAG **Vs**. British Pharmacopoeia, ISNAG **Vs**. Turbidemetry, and ISNAG **Vs**. UV- spectrophotometry. 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, so 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 no.10, it can be found that there is six comparisons, three what were mentioned above and another extra three. These extra three is the evidence and prove of the validity of the comparison methods shows from table no. 10 that the all four methods possible combination are strongly correlated as can be seen from the value tabulated in above table no. 10. As it compare, ISNAG method with the other there standard method as shown above. Which significance test indicate that at 95% confidence ($\alpha = 0.05/2$ two tailed) there is no significant difference between the newly developed method and the other three standard method. Therefore, the analyst should be able to choose any method for analysis i.e.; ISNAG or the other three. Thus accepting null hypothesis. This indicate that the high efficiency of ISNAG as a reliable instrument for analysis of Ibuprofen.







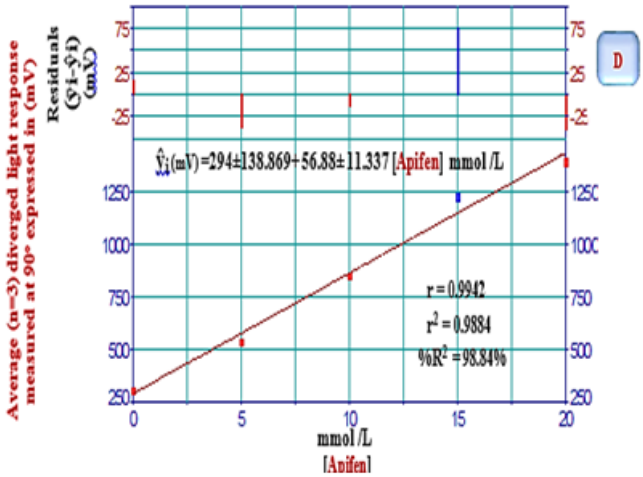


Figure13: Standard addition calibration graph using ISNAG fluorimeter for:

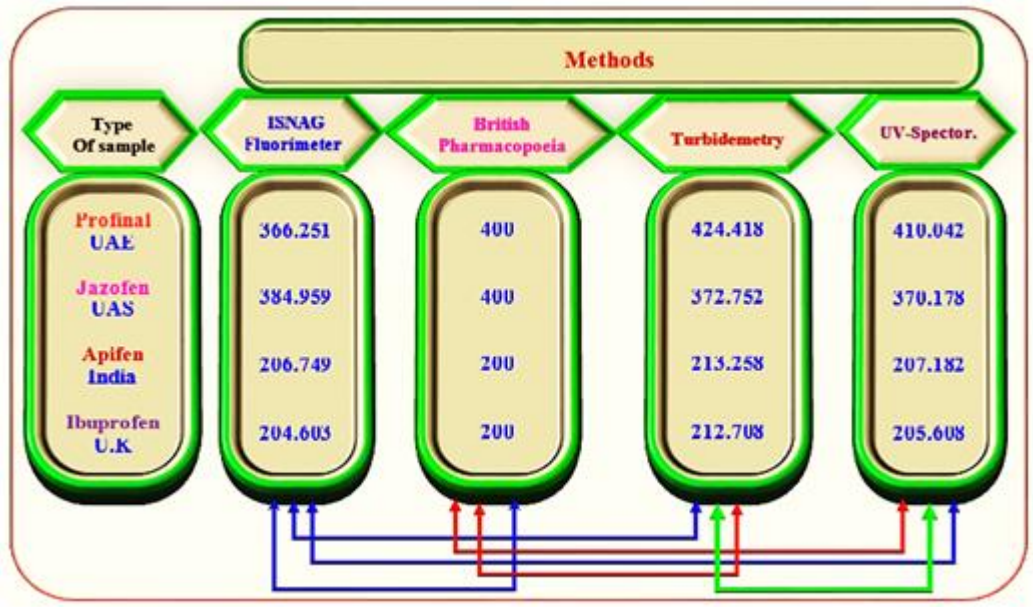
A -Jazofen,
B -Profinal,
C- Ibuprofen,
D- Apofin,

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

Table 9: Summary of results by standard additions method for the determination of Ibuprofen by system using ISNAG fluorimeter method, UV-spectrometer method, and Turbidmeter method

Number of sample		Confidence interval for the average weight of	Theoretical content for the active ingredient $\ddot{W}_i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (mg)	ample weight equivalent to 1.03143 g (50mmol/L of th active ingredient. w_i (g)	ISNAG fluorimeter / UV-spectrometer / Turbidmeter										
					[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		Weight in 100ml $\ddot{W}i(g) \pm t0.025\ \sigma n-1/\sqrt{n}$ at 95%, n-1	In tablets $\ddot{W}i(mg) \pm t0.025\sigma n-1/\sqrt{n}$
					0	5	10	15	20			10ml	100ml In prepared sample (50 mmol/L)		
					0	0.05	0.1	0.15	0.2			$X_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$			
					0	0.5	1	1.5	2						
1	Profinal 400mg Julphar -UAE	0.6228 ± 0.0058	400± 3.725	1.6059	[Profinal] mmol/L										
					250	448	992	1120	1336	260.40±284.134 +56.88±23.20 [Profinal] mmol/L	0.9762 0.9530 95.53%	4.5781	45.781	0.9444 ± 0.0153	366.251 ± 5.934
					0.693	0.868	1.639	1.802	1.94						Efficiency of determination REC% 91.56%
										0.703±0.487+ 6.856±3.981 [Profinal] mmol/L	0.9535 0.9092 90.92%	0.1025	51.254	1.0572 ± 0.0212	410.042± 8.222
															Efficiency of determination REC% 102.51%
					20.23	61.41	77.8	93.27	129	26.462±20.769+ 49.880±16.960 [Profinal] mmol/L	0.9833 0.9669 96.69%	0.5305	53.051	1.0944 ± 0.0212	424.418 ± 8.222
															Efficiency of determination REC% 106.10%
2	Jazofen 400mg Jenapharm -UAS	0.6286 ± 0.0017	400± 1.082	1.6209	[Jazofen] mmol/L					**256** ±59.017+ **53.20**± 4.818 [Jazofen] mmol/L	0.**99**88 0.**9976** **99.76**%	4.8120	48.120	0.9927 ± 0.0183	384.959 ± 7.097
					260	500	800	1080	1300						Efficiency of determination REC% 96.24%
					0.588	0.914	1.512	1.749	1.901	0.641±0.325+ 6.9220±2.857 [Jazofen] mmol/L	0.9757 0.9520 95.20%	0.0926	46.273	0.9545 ± 0.0259	370.178 ± 10.045
															Efficiency of determination REC% 92.55%
					32	59.8	70.99	112.8	151	27.118±24.670+ 58.20±20.142 [Jazofen] mmol/L	0.9827 0.9657 96.57%	0.4659	46.595	0.9612 ± 0.0194	372.752 ± 7.523
															Efficiency of determination REC% 93.19%
3	Apifen 200mg Zauba -India	0.2899 ± 0.00197	200± 1.359	1.49506	[Apifen] mmol/L					**294**±138.869+ **56**.88± 11.337 [Apifen] mmol /L	0.9942 0.9884 98.84%	5.1688	51.688	1.0662 ± 0.0173	206.749 ± 3.355
					310	540	850	1224	1390						Efficiency of determination REC% 103.38%
					0.697	0.959	1.626	1.859	2.001	0.727±0.395+ 7.016± 3.223 [Apifen] mmol /L	0.9701 0.9411 94.11%	0.1036	51.796	1.0685 ± 0.0292	207.182± 5.662
															Efficiency of determination REC% 103.59%
					38.52	50.45	81.09	127	144	30.710±24.508+ 57.502± 20.012 [Apifen] mmol /L	0.9825 0.9654 96.54%	0.5340	53.407	1.1017 ± 0.0234	213.258 ± 4.530
															Efficiency of determination REC% 106.63%
4	Ibuprofen 200mg DHP Co. - U.K	0.5748± 0.0167	200± 5.811	2.82508	[Ibuprofen] mmol/L					**280**±77.555+ **54**.74± 6.332 [Ibuprofen] mmol /L	0.9980 0.9961 99.61%	5.1151	51.151	1.055 ± 0.0132	204.603 ± 2.560
					256	560	872	1089	1360						Efficiency of determination REC% 102.30%
					0.612	0.932	1.232	1.555	1.816	0.623±0.048+ 6.062±0.370 [Ibuprofen] mmol/L	0.9994 0.9989 99.98%	0.1028	51.402	1.0604 ± 0.0285	205.608 ± 5.526
															Efficiency of determination REC% 102.80%
					26	67.8	84.91	108	147.7	30.162±18.907+ 56.720±15.439 [Ibuprofen] mmol/L	0.9892 0.9785 97.85%	0.5317	53.177	1.0970 ± 0.0193	212.708± 3.742
															Efficiency of determination REC% 106.35%

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



Scheme no. 2:

Summed up the path for comparison between four different methods using paired t-test

Table 10: Paired t-test for the comparison between four different methods of four samples for the analysis of Ibuprofen in drugs for n= 4 at 95% confidence level (α = 0.05) and DF = 3

Paired	Correlation coefficient r	Paired differences				Significant (2 tailed)
		$\bar{X}d$	Standard Deviation (σ_{n-1})	t_{cal}	t_{tab}	
Pair- 1 ISNAG- B.P	0.9970	9.3595	18.987	0.986 < 3.182		0.397 > 0.05 Not significant
Pair- 2 ISNAG- Turbidemetry	0.9630	|-14.9665|	30.186	|-0.992| < 3.182		0.394 > 0.05 Not significant
Pair- 3 ISNAG- UV-Spector.	0.9740	|-7.612|	25.203	|-0.604| < 3.182		0.588 > 0.05 Not significant
Pair- 4 B.P- Turbidemetry	0.9810	|-5.607|	22.603	|-0.496| < 3.182		0.654 > 0.05 Not significant
Pair- 5 B.P- UV-Spector	0.9880	1.7475	18.806	0.186 < 3.182		0.864 > 0.05 Not significant
Pair- 6 Turbidemetry-UV-Spector	0.999	7.3545	4.991	2.947 < 3.182		0.06 > 0.05 Not significant

DF: Degree of freedom (n-1) = 3, $\bar{X}d$: average of difference between two methods, $t_{tab} = t_{0.025, 3} = 3.182$

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