

Determination of Mefenamic Acid using 8-hydroxy quinoline as a Precipitating Agent and Low Pressure Mercury Lamp (184.9 & 253.7 nm) as a Source of Irradiation using of ISNAG Continue Flow Fluorimeter

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Abstract

A new, simple, sensitive and fast method for the determination of Mefenamic acid in pure form and drugs (tablets) by continuous flow feed analysis via measurement of diverged beam of light by ISNAG -fluorimeter (homemade instrument). The method based on the reaction of the Mefenamic acid with 8-HQ to form yellow precipitate. Optimum parameters have been studied to increase the sensitivity for the developed method. The linear dynamic range for the instrument response versus Mefenamic acid concentration was 0.005-7 mmol/L while the L.O.D was 0.216 $\mu\text{g}/\text{sample}$ 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 0.9956 while percentage linearity ($R^2\%$) was 99.12%. RSD % for the repeatability ($n=8$) was lower than 0.2% for the determination of Mefenamic acid, with concentration of 2, 5 mmol/L respectively. The developed method was applied successfully for the determination of Mefenamic acid in pharmaceutical tablets. A comparison was made between the newly developed method with the classical method (UV-Vis spectrophotometry at wavelength 285nm, and Turbidimetric 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.

Keywords: Mefenamic acid, Flow injection analysis, Homemade instrument.

Introduction

Mefenamic acid (MFNC) is 2- [(2, 3-dimethylphenyl) amino] benzoic acid and is used as an analgesic and anti-inflammatory agent (Fig. 1) [1, 4]. It was discovered and brought to market by Parke-Davis in the 1960s. It became generic in the 1980s and is available worldwide under many brand names [5-7]. Scientists led by Claude Winder from Parke-Davis invented Mefenamic acid in 1961, along with fellow members of the class of anthracitic acid derivatives, flufenamic acid in 1963

and meclofenamate sodium in 1964. U.S. Patent 3,138,636 on the drug was issued in 1964[8-11]. It was approved in the UK in 1963 as "Ponstan", in West Germany in 1964 as "Ponalar", and in France as "Ponstyl" and the US in 1967 as "Ponstel"[12]. Ponstan (Mefenamic acid) is a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs). Each blue-banded, ivory capsule contains 250 mg of Mefenamic acid for oral administration [13-16].

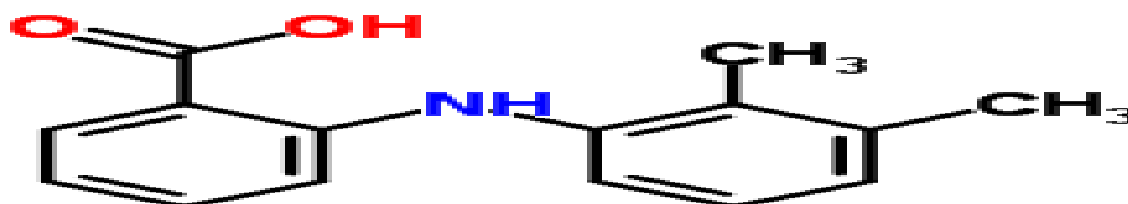


Fig. 1: Chemical structure of Mefenamic acid

Numerous analytical methods have been developed for the determination of Mefenamic acid in pure form, dosage forms and biological fluids. Mefenamic acid is official in both United States Pharmacopoeia and British Pharmacopoeia [3, 5, 9]. The USP recommends a high performance liquid chromatographic (HPLC) method for the determination of Mefenamic acid both in raw materials and dosage forms while the BP recommends an acid-base titration method for the analysis of Mefenamic acid in raw materials and its dosage forms [2,8].

Mefenamic acid is a white to greyish-white, odorless, microcrystalline powder with a melting point of 230°-231°C and water solubility of 0.004% at pH 7.1. The chemical name is N-2, 3-xylylanthranilic acid. The molecular weight is 241.29. Its molecular formula is $C_{15}H_{15}NO_2$ and the structural formula of Mefenamic acid is: Each capsule also contains lactose, NF. The capsule shell and/or band contains citric acid, USP; D&C yellow No. 10; FD&C blue No. 1; FD&C red No. 3; FD&C yellow No. 6; gelatin, NF; glycerol monolete; silicon dioxide, NF; sodium benzoate, NF; sodium lauryl sulfate, NF; titanium dioxide, USP [17,19].

Several spectrophotometric methods have been reported for the estimation of Mefenamic acid using different reagents such as diazotized 4-amino-3,5- dinitrobenzoic acid [20], methyl-2-benzo-thiazolinone hydrazon hydrochloride after oxidation with Ce^{+4} or Fe^{+3} [21], sodium nitroprusside in the presence of hydroxyl ammonium chloride [22], p-dimethyl amino benzaldehyde [23], p-dimethyl amino cinnamaldehyde [24], triton X-114 [25], Fe^{+3} to form coloured complex [26]. Many methods have been used in simultaneous determination of Mefenamic acid in the presence of another drug such as paracetamol [27], ethamsylate [28].

However some of these procedures suffer from one or another disadvantage such as: the product may be extracted to organic solvent [20], or require nonaqueous medium [24] and other required control of temperature [23, 25]. In this work using continue flow injection scattering method, the diverged beam of light is measured at 0-90° angle will be detected by homemade ISNAG-fluorimeter via low-pressure mercury lamp as a source and using 2 sides [4 x 2.5cm] solar cells.

Experimental

Reagent and Chemical

All chemicals were use of analytical-reagent and distilled water was use to prepare all the solutions. A standard solution 5mmol/L of Mefenamic acid molecular formula $C_{15}H_{15}NO_2$, molecular weight 241.29g/mole was prepared by dissolving 0.120645g in 100 ml of NaOH. A stock solution 0.2Mol/L of 8-hydroxyquinoline molecular formula (C_9H_7NO) molar mass 145.16g/mole and Merck-USA was prepared by dissolving 9.0725g in 250 ml of 0.1Mol/L acetic acid.

Sample Preparation

Twenty tablets were weight then crushed and mixed. tablet containing 500mg of Mefenamic acid were weighted 0.17723g, 0.17610g, 0.20288, 0.14445g (equivalent to 0.120645g of active ingredient, 5mmol/L) for Ponstan-forte (German)- 500mg, Ponstan-forte (Egypt)-500mg, Merfile-500(India)-500mg, and ponstidin-500(Iraq)-500mg respectively. Each one from the four kinds of sample dissolved in NaOH. The solution was filtered to get rid of undissolved materials, the residue was washed with NaOH and completed the volume to 100ml with the same solvent (NaOH).

Apparatus

Two line manifold design to figure out the use of precipitating agent and Mefenamic acid. Figure 2 shows the details of the manifold feed used. ISNAG- fluorimeter was used as a fluorimetric instrument for measurement of total diverged light, which is composed of 100mm distance flow cell at 2mm path length with 2sides [4 x 2.5cm] solar cells. Four on each side i.e.; measure fluorescence at +90° and -90°, which is a novel fluorimetric measurement.

Low-pressure mercury lamp as the excitation source characterized by two strong bands: 184.9nm and 253.7nm, which cannot be detected by ISNAG- fluorimeter unless the fluorescence excitation emission is beyond 410 nm (characteristic of solar cell). Peristaltic pump two channels variable speed (Ismatec, Switzerland).Valve 6-port medium pressure injection valve (I D E X corporation , USA) with sample loop (1mm i.d. Teflon ,variable length). The output signals were recorded by potentiometric recorder (Siemens, Germany) (1- 5 Volt, 1000-5000 mV).

Peak height was measured for each signal. Absorbance readings by uv-spectrometer, UV-spectrophotometer (UV-1800 Shimadzu)

(Japan).The scattered beam of light for precipitate measured by turbidometry via Turbidity-meter, HANNA- Hungary.

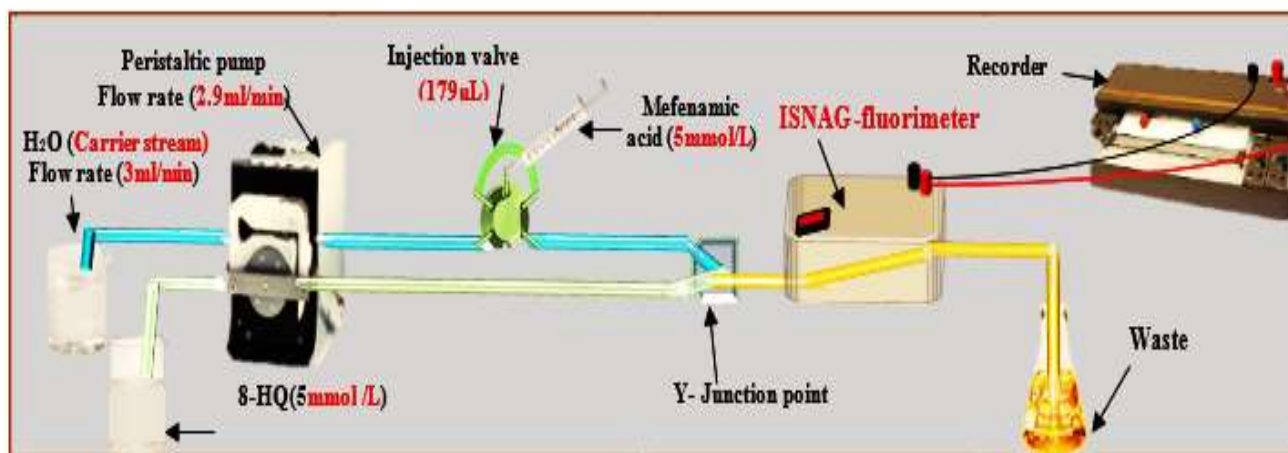
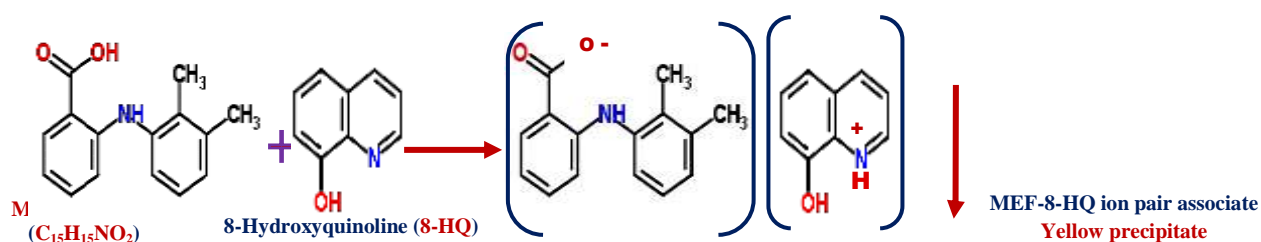


Figure 2: Schematic diagram of flow injection instrument analysis system for determination of Mefenamic acid
Fig. 2: Two line flow feed unit

Methodology

Using two line system (fig. 2) for the determination of Mefenamic acid with expected parameters of Mefenamic acid (5mmol/L)- 8-HQ (5mmol/L) system, 179µL sample size which will be injected on a

carrier stream line (distilled water) at 3 ml/min while the second line 8-HQ at 2.9ml/min.. The precipitate is expected to be probably ion paired compound as the suggested reaction below shows that in scheme 1.



Scheme 1: Proposed mechanism for the reaction between Mefenamic acid and 8-HQ

Result and Discussion

Study of the Optimum Parameters

The flow injection manifold system as shown in Fig.2 was investigated in the relation of chemical and physical variables, in order to obtain optimum conditions for the reaction of 8-HQ with Mefenamic acid and formation of yellow precipitate. They were optimized by making all variables constant and varying one at a time i.e. fixed variable optimization.

Variation of Chemical Parameters

Through this section, all variables that contribute in the reaction for determination of Mefenamic acid will be studying for given

a best diverged light response measured at 0-90° using four solar cells at each side.

8-hydroxy Quinolone Concentration

At a selected concentration of Mefenamic acid (5mmol/L) was chosen for a set of variable 8-HQ concentration (3-20) mmol/L. No electronic filter was introduced through the measurement of variable concentration. Fig. 3-A shows a kind of responses emission versus time profile; the results of which are tabulated in table no. 1.It was noticed that 5mmol/L of 8-HQ concentration is the most favored concentration to use above this concentration (fig. 3-B); a decrease in diverged light occur; as lower responses were

obtained which might be attributed to the formation of tiny minute particulates or a decrease in the interfacial distances i.e.;

prevention of the passage of dispersed and diverged light to the detector.

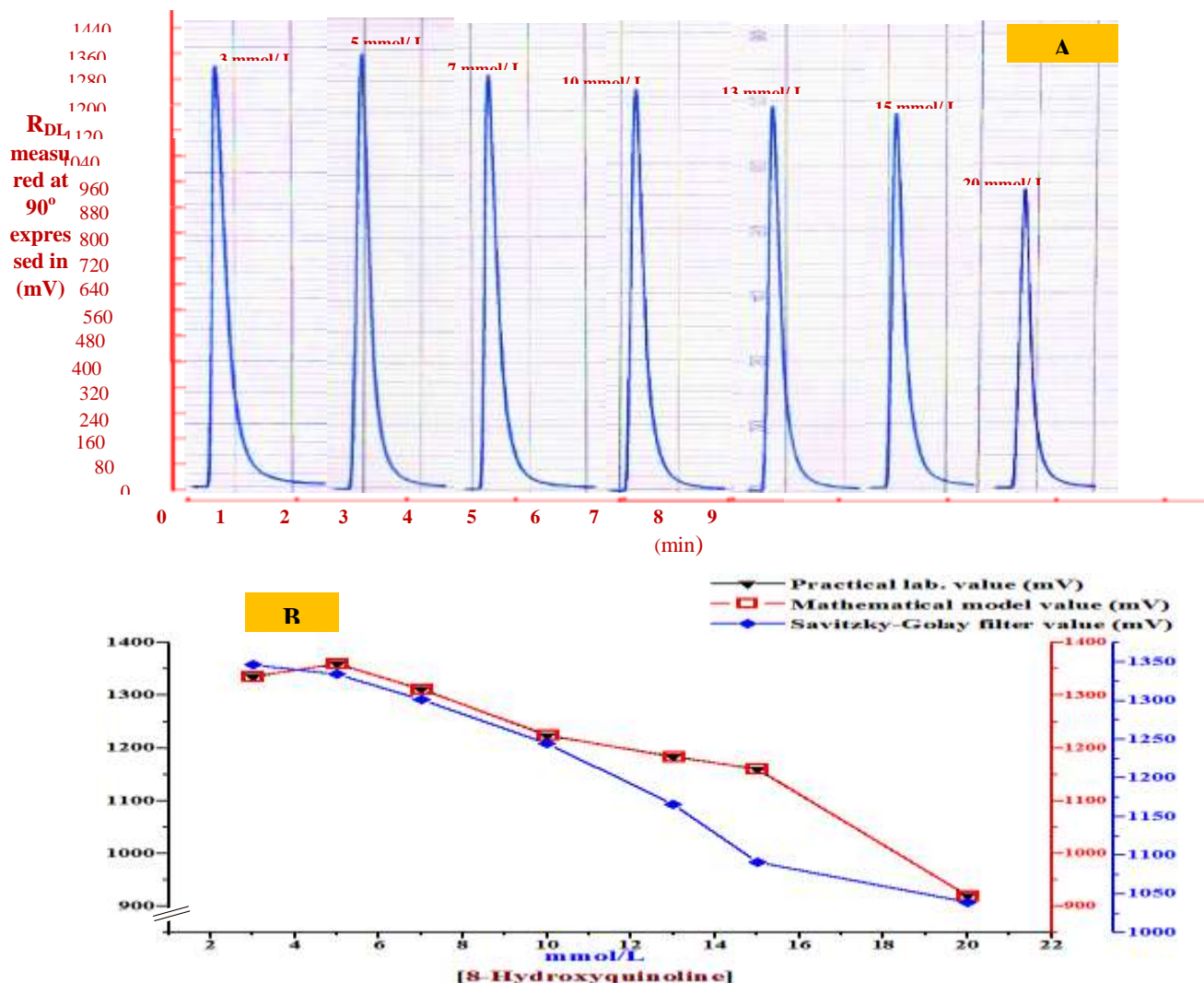


Figure 3:

- Response –time profile of Mefenamic acid with variable concentration of 8-HQ solution (clear un obstructed peak) with no deformation at variable level of 8-HQe solution at (3-20) mmol/L.
- Plot of averaged peak height responses vs. 8-HQ concentration.

Table 1: Effect of 8-HQ concentration on response function expressed as an average peak heights \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 [8-HQ] mmol/L	Dependent variable				
	Average (n=3) diverged light response measured at 0- 90° expressed in mV				
	Practical lab. value			Mathematical model	Savitzky-Golay filter
	Average peak height (\bar{y}_i)	RSD%	Reliability(two tailed) $\bar{y}_i(\text{mV}) \pm t_{0.025,2} \sigma_{n-1}/\sqrt{n}$	\hat{y}_i	\hat{y}_i (S-G)
3	1336	0.07	1336 ± 2.36	1335.992	1346.388
5	1360	0.07	1360 ± 2.44	1360.108	1334.175
7	1312	0.08	1312 ± 2.71	1311.661	1301.644
10	1224	0.09	1224 ± 2.78	1224.658	1244.455
13	1184	0.07	1184 ± 3.28	1183.132	1165.632
15	1160	0.11	1160 ± 3.03	1160.485	1091.126
20	920	0.16	920 ± 3.60	919.964	1039.338

$$t_{0.025,2} = 4.303, \bar{Y}_j = \sum_{i=-\frac{m-1}{2}}^{\frac{m-1}{2}} (C_i \bar{y}_j + i), m = \text{convolution coefficient}, \frac{m+1}{2} \leq j \leq n - \frac{m-1}{2}$$

Carrier Stream Effect

Acidity Effect

Fixing Mefenamic acid at 5mmol/L. A series of different acids (H₂SO₄, HCl, HNO₃, and

CH₃COOH (50mmol/L)) were prepared. Also distilled water was used as a reference. It was noticed a decrease in response profile which might be attributed to the dissociation and dispersed precipitated particles and

minimum large precipitated particles that causes the diverged light beam due to increased reflecting surface area. Therefore,

distilled water was chosen as the most satisfactory medium. The overall results are tabulated in Table 2.

Table 2: Effect of acidity medium as a carrier stream on height of responses expressed as an average peak heights \bar{y}_i (n=3) using 79 μ L from MFE (5mmol/L) and 8-HQ (5mmol/L)

Independent type of acid as a carrier stream [acid] 50mmol/L	Dependent variable (\bar{y}_i) Average (n=3) diverged light response measured at 0- 90° expressed in mV	RSD%	Reliability of average response(two tailed) at 95% confidence level \bar{y}_i (mV) \pm $t_{0.025,2} \sigma_{n-1}/\sqrt{n}$
H ₂ O	1368	0.08	1368 \pm 2.71
H ₂ SO ₄	344	0.36	344 \pm 3.06
HCl	744	0.33	744 \pm 6.09
HNO ₃	808	0.32	808 \pm 6.36
CH ₃ COOH	424	0.76	424 \pm 7.98

Salts Effect

Fixing all previous attained experimental parameters whither chemical or physical. The affect of salts solutions as a carrier streams on accumulation of precipitated particles or its dispersed action. In general; it

was noticed that (refer is mode in Fig. 4) a decrease in response profile which might be attributed; that the precipitated particles is dissociated or due to increased solubility. On this basis, distilled water was used as the most suitable solvent for the reaction.

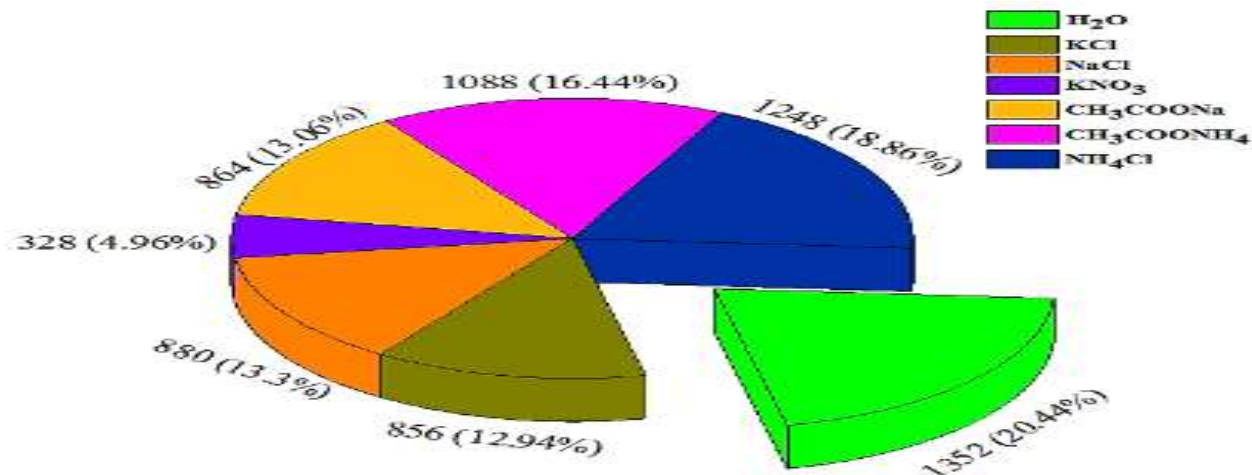


Figure 4: 3D pie percentage representation of the contribution of each salt solution (B) using Mefimanic acid (5mmol/L) - 8-HQ (7mmol/L) system

Physical Parameters

Electronic Filter Effect

Using all optimum conditions concerning chemical concentration for two line manifold design system. Studying the effect of RC- low band pass electronic filter variables through the use of 5mmol/L of Mefenamic acid at 79 μ L sample segment.

Electronically noises filter of low band pass with a time constant of 0.1632- 3.974 sec. to overcome the little bit tiny noise developed by the pulse effect of the peristaltic pump. Using this kind of low band pass electronic filter affect on the measured sensitivity, therefore electronic filter was not used and table 3 in which shows the summary of results from this study.

Table 3: 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 0-90° expressed in mV	RSD%	Reliability(two tailed) at 95% confidence level \bar{y}_i (mV) \pm $t_{0.025,2} \sigma_{n-1}/\sqrt{n}$
Without filter	1364	0.07	1364 \pm 2.73
0.1632	1346	0.08	1346 \pm 2.78
0.3196	1339	0.08	1339 \pm 2.61
0.68	1268	0.10	1268 \pm 3.28
0.8364	1210	0.12	1210 \pm 3.53
1.6728	1187	0.11	1187 \pm 3.33
3.974	944	0.16	944 \pm 3.78

Flow Rate Effect

At the same of optimum parameters in the two line of manifold design for the determination of Mefenamic acid using 8-HQ as a precipitating agent, the variable flow rates ranging (0.4- 3.5)ml/min for carrier stream and (0.4-3.6)ml/min for 8-HQ (5mmol/L) line were used. It can be noticed that at low flow rate there is an increase in dispersion and dilution due to the increase of area of precipitate section in flow cell and an increase of Δt_B (base width of response).

While an increase of flow rate results in a decrease of sample section; in addition to compact of merged layer due to rashness of successive travelled sample segment zones producing an effect similar to Doppler effect causing an increased of diverged beam of incident light as illustrated in figure 5, and which the optimum flow rat 2.9 & 3 for carrier stream and precipitating agent line respectively which is chosen on the basis of better sensitivity. The results were tabulated in Table 4.

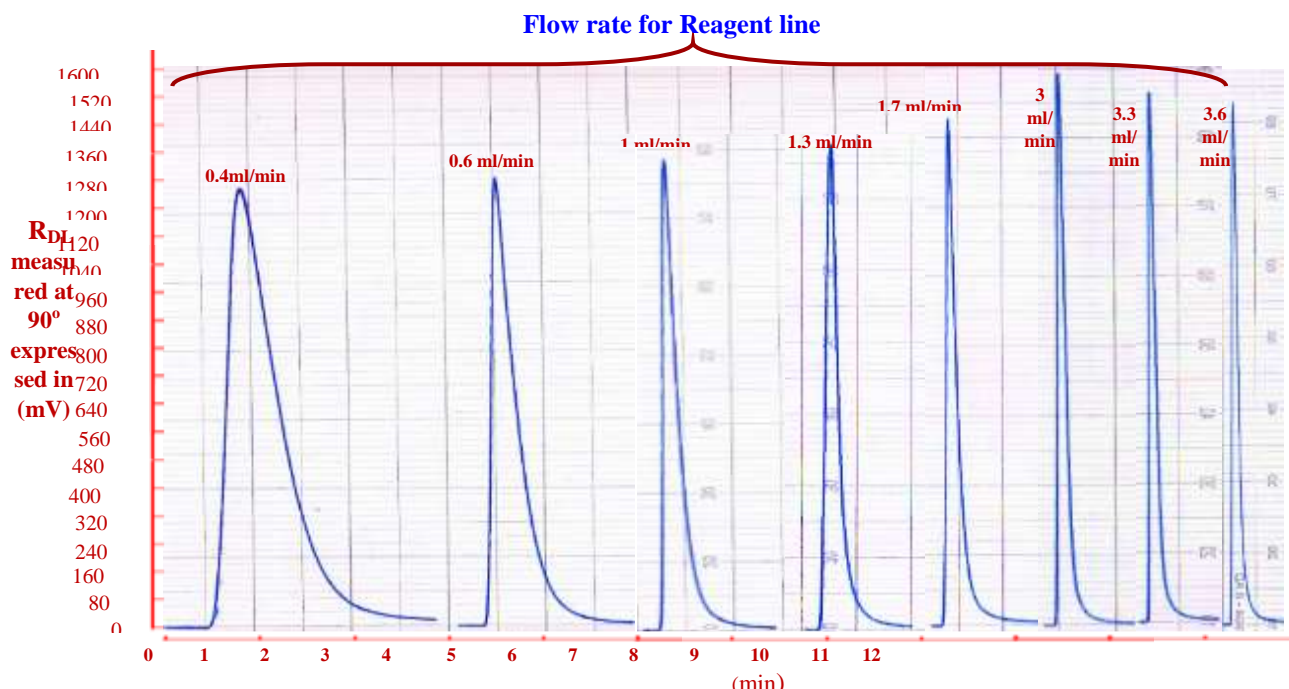


Fig. 5: Effect of flow rate on diverged beam of light vs. time profile

Table 4: 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)	RS D%	Reliability (two tailed) $\bar{y}_i(mV) \pm t_{0.025,2} \sigma_{n-1}/\sqrt{n}$				Δt_b (Sec)
Line no. 1	Line no. 2								
5	0.4	0.4	1272	0.11	1272 ± 3.60		162	2.239	0.176
10	0.6	0.6	1304	0.10	1304 ± 3.38		114	2.359	0.167
15	1	1	1344	0.10	1344 ± 3.28		72	2.479	0.159
20	1.3	1.3	1368	1.09	1368 ± 3.18		46	2.072	0.191
25	1.7	1.7	1448	0.08	1448 ± 2.93		34	2.006	0.197
30	2.9	3	1592	0.07	1592 ± 2.81		30	3.029	0.130
35	3.2	3.3	1576	0.08	1576 ± 3.11		28	3.112	0.127
40	3.5	3.6	1520	0.09	1520 ± 3.33		26	3.156	0.125

Δt_b (sec) : Time lapse for the precipitate response within measuring cell or peak base width
 Line no.1 = carrier stream (H₂O), Line no. 2= 8-HQ (5mmol/L)

Effect of Sample Volume

The study was carried out using variable sample volume extended from 79- 329 μ L.

All other variable were kept unchanged. It was

noticed the increment in responses as the sample volume is increased reaching 179 μ L, more than; the value an increase of Δt_B with non- returned pen recorder to zero base line due to different particulate formed and difficulty of re-evacuation of whole system

from large molecules formed during commencing the precipitation. Open valve model for discharging the sample plug from injection valve. Table 5 summed up the obtained results.

Table 5: Variation of injected sample volume on diverged light response tabulation 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) r=0.5mm	Independent variable sample loop volume μ L	Dependent variable. Average (n=3) diverged light response measured at 0- 90o expressed in mV					
		Practical lab. Value				Mathematical model \hat{y}_i	Savitzky-Golay filter \hat{y}_i (S-G)
		Average peak height(\bar{y}_i)	RSD %	Reliability(two tailed) \bar{y}_i (mV) \pm t0.025,2 σ_{n-1}/\sqrt{n}	Δt_b (Sec)		
10	79	1590	0.06	1590 \pm 2.53	30	1589.997	1944.635
16.43	129	2320	0.05	2320 \pm 2.78	39	2320.469	2118.625
20.13	158	2500	0.05	2500 \pm 3.01	44	2496.024	2380.675
22.80	179	2560	0.05	2560 \pm 2.96	49	2566.660	2677.419
29.17	229	2920	0.04	2920 \pm 3.03	53	2913.249	2924.722
35.54	279	3180	0.04	3180 \pm 3.28	55	3185.317	3091.160
41.91	329	3220	0.04	3220 \pm 3.53	58	3218.284	3175.043

Purge Time Effect

This study was conducted using different variable allowed under control time (5- 45 sec) used to purge the plug from injection valve. It was found that increase of peak height with increase of departure time of

sample segment from injection valve up to 40 sec. it was found that no significant difference of using 40 sec to 45 sec could be followed. Therefore, 40 sec was the optimum that can be used to conduct the research at hand. The summary of results tabulated in Table 6.

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 90o expressed in mV				
	Practical lab. value			Mathematical model \hat{y}_i	Savitzky-Golay filter \hat{y}_i (S-G)
	Average peak height(\bar{y}_i)	RSD%	Reliability(two tailed) \bar{y}_i (mV) \pm t0.025,2 σ_{n-1}/\sqrt{n}		
5	520	0.45	520 \pm 5.84	520.351	620.478
10	840	0.26	840 \pm 5.49	837.190	831.159
15	1200	0.21	1200 \pm 6.31	1209.833	1226.538
20	1740	0.11	1740 \pm 4.92	1720.333	1717.018
25	2160	0.07	2160 \pm 4.41	2184.584	2141.649
30	2460	0.05	2460 \pm 3.06	2440.333	2418.707
35	2500	0.05	2500 \pm 2.96	2509.834	2540.082
40	2560	0.04	2560 \pm 2.58	2557.190	2566.709
Open valve (45)	2560	0.04	2560 \pm 2.68	2560.351	2559.582

Reaction Coils Effect

Variable sample loop sizes (157-1301 μ L) were studied which was attached after Y-junction (refer is made to Fig. 6-A).The reason in why conducting this study is because of having the rearrangement of precipitated particulate or re-crystallization. It was found that; the increase of coil length causes a decreased

sensitivity in general (refer is made to Fig. 6-A&B). This might be explained, that the formation of larger particles is probably the reason by forming a saturation of signal to noise ratio (S/N) output and the difficult associated in the departure of reacted sample too plug due to the enlargement of particulate sample. The condensation of

precipitated particles causes an increase particulate weight and spreading it on a wider surface area and the decrease of particles that reflect and diverge the light. It was formed also in using glass coils; a difficulty is associated with its use while in

using teflon tubing an improved signal responses was at hand, due to hydrophobicity the lack of attraction between. So delayed reaction coils were avoided for use.

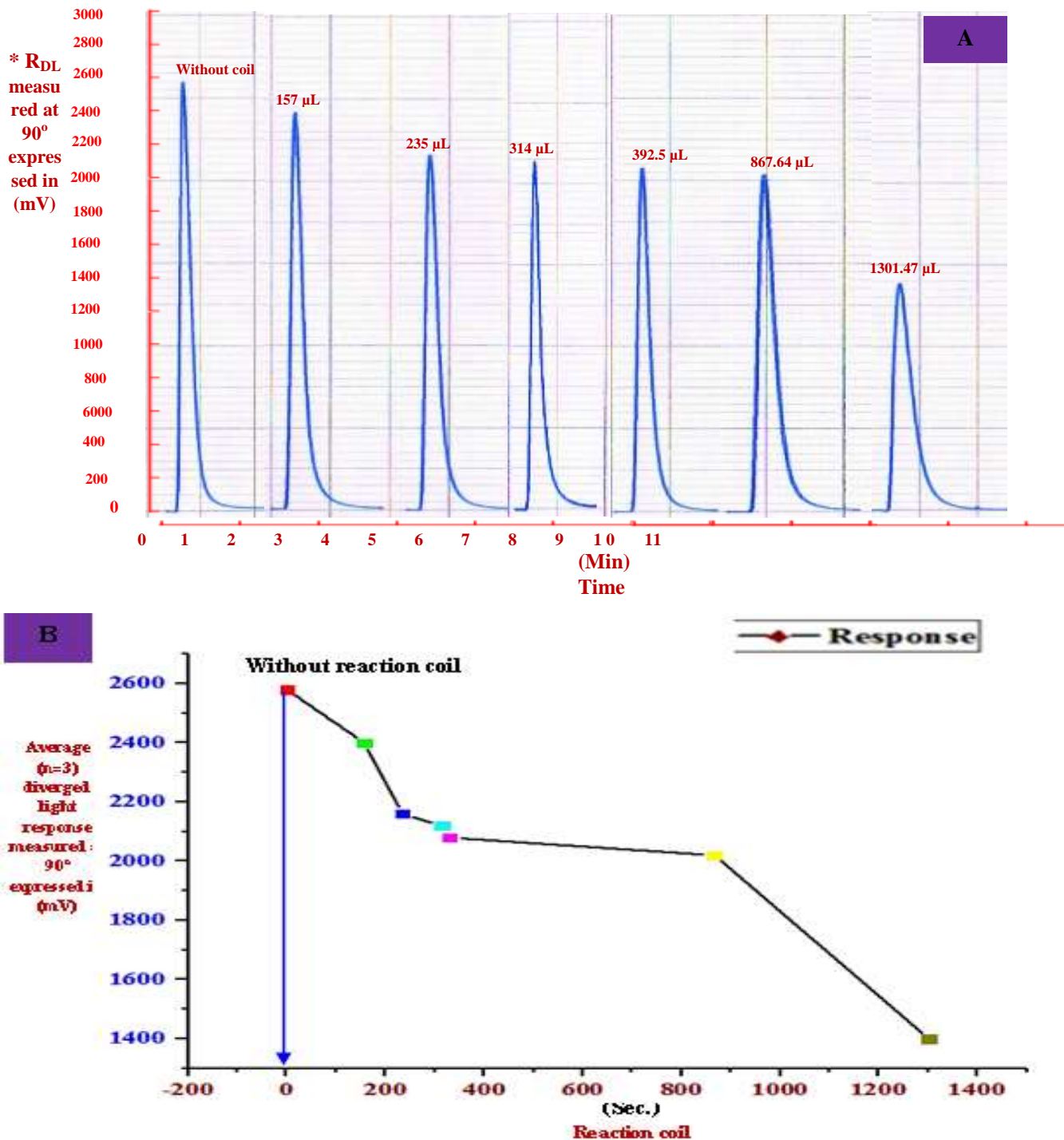


Fig. 6: Effect of different length and material of reaction coil on:-
 •Segment precipitate plug that will affect on the whole measurements and profile.
 • Laboratory measurements values produced from diverged beam of light using Mefenamic acid (5mmol/L)- 8-HQ (5mmol/L) system

Calibration Graph

Fixing all physical as well as chemical parameters that were studied previously. A series of standard solutions of Mefenamic acid (0.005-7) mmol/L were prepared. The

measurements were conducted using ISNAG-fluorimeter, it was noticed that from the obtained peak height and scatter points plot(fig. 7-A) ; that best fit extend from 0.005-6 mmol/L. Correlation coefficient of $r =$

0.9956 and percentage capital R- square gave 99.12% (fig. 7-B) was obtained. While dealing with high concentration i.e.; > 7mmol/L decreases of correlation coefficient might be due to accumulation and agglomerate of precipitated particles causes an increase particulate weight which in turn to lead difficulty discharging the particles from flow cell, causes a broadening in peak height and decrease in correlation coefficient value. All results summed up in table 7. The new development methodologies were compared with classical methods of determination using 8-Hydroxyguinoline as a precipitating agent with optimum concentration of

5mmol/L. The extent of linear plot was 0.001-0.09 mmol/L, and in additional classical method (uv-spectrophotometer at 285nm [3] was compared. The linear plot was from 0.001-0.09mmol/L. All the results tabulated in Table 7. Limit of detections were studied for the two used methods. The results are tabulated of the table 7. It can be found that the newly developed methodology was 5µmol/L using successive dilution while the other classical methods was 0.8µmol/L while the repeatability and the trustability for 2 &5 mmol/L having RSD% < 0.2% while classical method < 4% (Table 7).

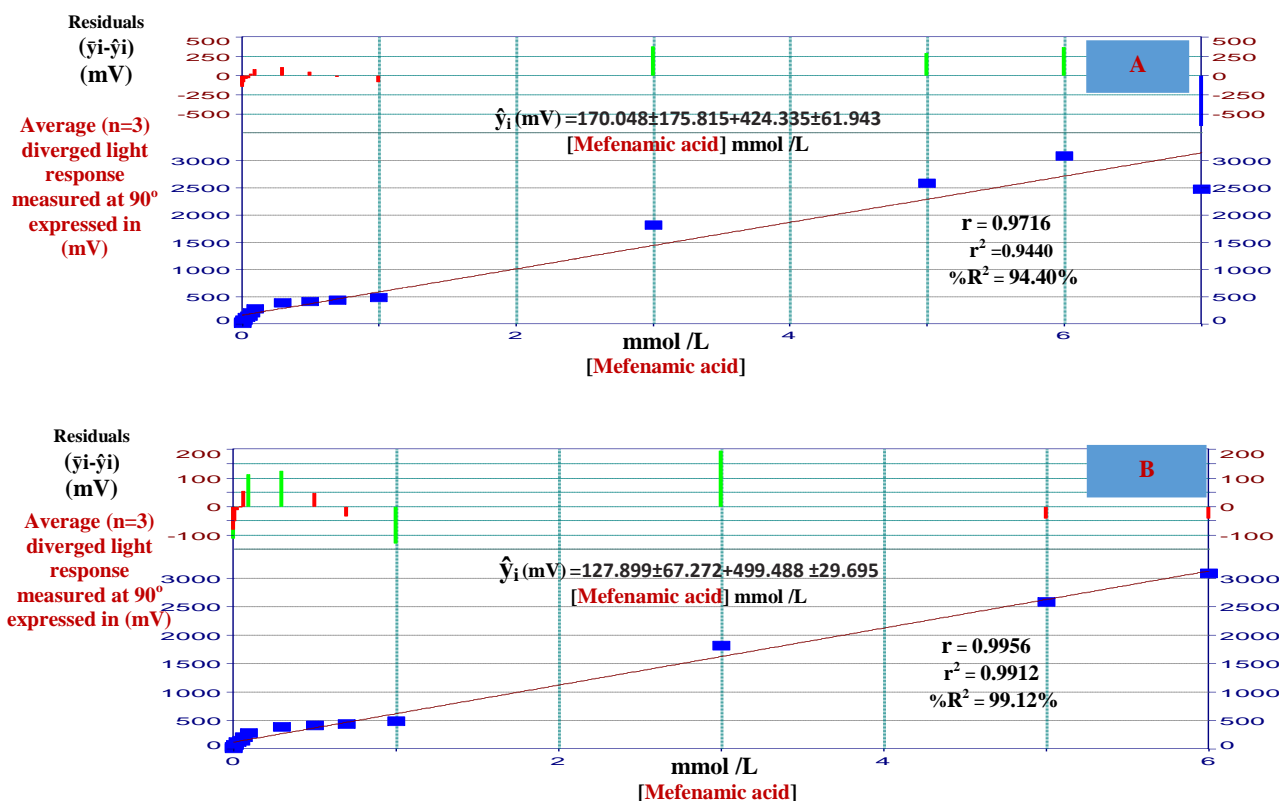


Figure 7: (A) Variation of scattered diverged light at range (0.005-7mmol/L), n=15 against Mefenamic acid concentration (B) Calibration graph deduced from scatter point plot at range (0.005-6mmol/L), n= 14 against Mefenamic acid concentration

Table 7: Summary results of different assessment methods for determination of Mefenamic acid.

Method	No. of measurements (n)	[Mefenamic acid] mmol/L		Linear regression equation at 95%, n-2	r r2 R2 %	t- value at 95%, n- 2		Detection limit		Repeatability at 95% confidence level, n-1		%RS D
		Measured	Linear dynamic range			ttab	tca 1	Practically based on the gradual dilution of the minimum concentration	Theoretical based on slope X = 3SB /slope	[Mefenamic acid] mmol/L	Reliability of average diverged light	
				New developed $\hat{y}_i \text{ (mV)} = a \pm Sat + b \pm Sbt [x] \text{ mmol/L}$							$\bar{y}_i \text{ (mV)}$	
				UV-spectrophotome try $\hat{y}_i = a \pm Sat + b \pm Sbt [x] \text{ mmol/L}$							Average of absorbance \bar{y}_i	
											Average of turbidomet \bar{r}_y	
											$\bar{y}_i \pm t0.025,$	

					Turbidometry 0-180° \hat{y}_i (FTU)= a± Sat+b± Sbt [x] mmol /L						$\frac{n-1}{\sqrt{n}}$ n=8	
Newly developed methodology	ISNAG- fluorimeter	15	0.005- 7	0.005- 7	170.048± 175.815± 424.335± 61.943 [Mefenamic acid]mmol /L	0.9716 0.9440 94.40%	2.160<< 14.797	(5 µmol/L) 0.216 µg/sample	(0.601 µmol/L) 25.941 ng/sample	2	1364 ±1.321	0.116
		14		0.005- 6	127.899± 67.272+ 499.488 ± 29.695 [Mefenamic acid]mmol /L	0.9956 0.9912 99.12%	2.179<< 36.652			5	2580 ±1.957	0.076
Used method based on	UV- absorbance(3) at λmax =285nm	11	0.001-0.09	0.001- 0.09	0.343± 0.074+ 25.395 ± 1.873 [Mefenamic acid]mmol /L	0.9952 0.9905 99.05%	2.262<< 30.671	(0.8µmol/L) 0.772 µg/sample	(3.544 µmol/L) 3.421 µg/sample	0.03	0.999 ±0.026	3.103
	Turbidometry (NTU)	11		0.001- 0.09	9.098± 16.260+ 8730.313± 412.815 [Mefenamic acid]mmol /L	0.9981 0.9961 99.61%	2.262<< 47.838	((0.8µmol/L) 1.544 µg/sample	(0.103 µmol/L) 0.199 µg/sample	0.05	436 ±0.953	0.262

$$t_{tab} \text{ (calibration graph)} = t_{0.025, n-2}, \quad t_{cal} = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}, \quad t_{tab} = t_{0.025, n-1} = t_{0.025, 7} = 2.365, \quad r: \text{correlation coefficient, } r^2: \text{coefficient of}$$

determination & R²%; percentage capital R- square, %RSD = Percentage relative standard deviation. \bar{y}_i = practical value for (n=8) in mV for newly developed method, FTU for turbidometry method, and without unite for spectrophotometry method, \hat{y}_i =estimated value in mV for newly developed method (volume 179µL) FTU for turbidometry method (volume of 8ml), and without unite for spectrophotometry method (volume of 4ml),

Application

The continuous flow injection analysis via diverged light response using ISNAG-fluorimeter achieved in this work was used for the analysis of Mefenamic acid in the four different drug manufactures (Ponstan-forte (German)- 500 mg, Ponstan-forte (Egypt)-500 mg, Merfile-500 (India)-500 mg, and ponstidin- 500 (Iraq)- 500 mg) and was compared with two methods which includes UV-spectrophotometric via the measurement of absorbance at λ_{max} = 285nm by UV-spectrophotometer,[3] (UV-1800 Shimadzu), and turbidometry via Turbidity-meter, HANNA, (Hungary).

The measurement of scattered light at 0- 180° for yellow precipitate particles of Mefenamic acid- 8-HQ (5mmol/L) system. A series of solutions were prepared of each drug (5mmol/L) by transferring 1ml to each five

volumetric flask (10ml), followed by the addition of gradual volumes of standard solution of Mefenamic acid (0, 0.8, 1, 3, and 5ml) of 5 mmol/L to obtain (0, 0.4, 0.5, 1.5, and 2.5 mmol/L) when use ISNAG-fluorimeter (newly developed methodology), while in UV-spectrophotometer method transferring 0.06 ml from 5mmol/L sample solution to each five volumetric flask, followed by the addition of gradual volume of standard solution of Mefenamic acid (5 mmol/L) (0, 0.01, 0.02, 0.03, and 0.04ml) to obtain (0, 0.005, 0.01, 0.015, and 0.02 mmol/L), in addition to Turbidometric method that depend on the measurement at 0-180°. The series of solutions were prepared by transferring 0.06 ml of 5 mmol /L concentration of each sample, followed by the gradual addition of 0, 0.01, 0.02, 0.03, and 0.04 ml from standard solution of Mefenamic

acid (5mmol/L) to obtain 0, 0.005, 0.01, 0.015, and 0.02 mmol/L.

The measurements were conducted by three methods. Fig. 8-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 Mefenamic acid in each sample of drug using three different methods and efficiency of determination.

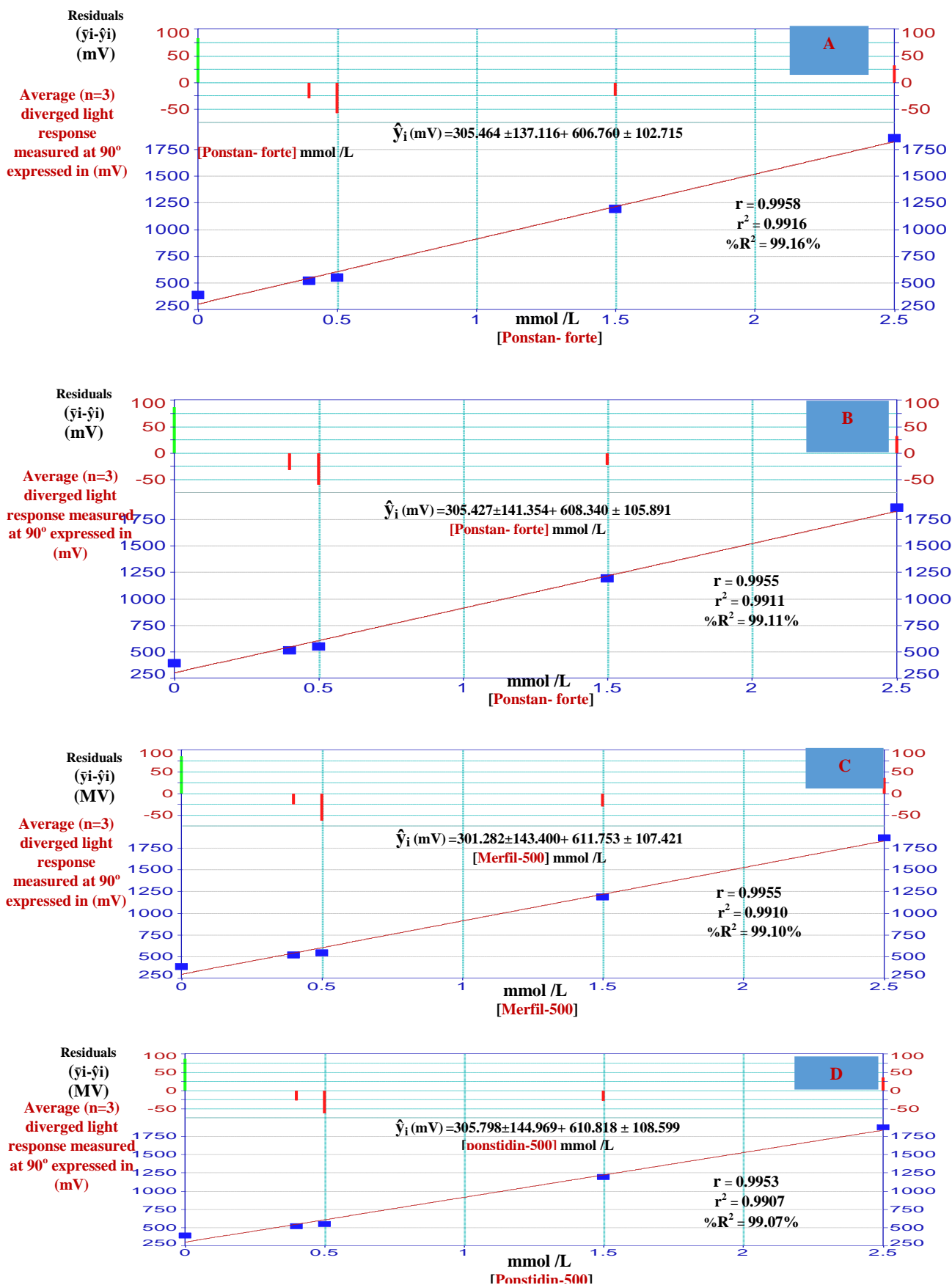


Fig. 8: Standard addition calibration graph using ISNAG- fluorimeter for: A - Ponstan-forte (German), B - Ponstan-

forte (Egypt), C- Merfil-500 (India), D- ponstidin-500 (Iraq) Residual = $(\hat{y}_i - \bar{y}_i)$ in mV, \bar{y}_i = practical value, \hat{y}_i = estimated value

Table 8: Summary of results by standard additions method for the determination of Mefenamic acid by different methods

Number of sample	Name, coted value, company And country	Confidenc e interval for the average weight of tablets $W_i (g) \pm 1.96 \sigma_{n-1}/n$	Theoretical content for the active ingredient $W_T (mg) \pm 1.96 \sigma_{n-1}/n$ at 95%	Sample weight equivalent to 0.120645 g (\hat{w}_i) of the active ingredient. $w_i (g)$	ISNAG fluorimeter $\bar{y}_i (n=3)$ in mV										Efficiency of determination REC%	
					Volume of Mefenamic acid (ml)					$\hat{y}_i = a \pm S_{a+t} + b \pm S_{b+t}$ [Sample] mmol/L at confidence level 95%, n-2	r r ² R ² %	Practical content of active ingredient				In tablets $\bar{W}_i (mg) \pm t_{0.025, n-1} \sigma_{n-1}/\sqrt{n}$
[Mefenamic acid]mmol/L					Concentration prepared sample in		Weight in 100ml $W_i (g) \pm t_{0.025, n-1} \sigma_{n-1}/\sqrt{n}$ at 95%	Efficiency of determination REC%								
\bar{y}_i for n= 3					0.0	0.8			1	3	5	10 ml	100 ml	Efficiency of determination REC%		
1	Ponstan-forte 500mg- German	0.7345 ± 0.0038	500 ± 2.587	0.17723	388	518	550	1190	1854	305.464 ± 137.116	0.9958	0.5035	5.0351		0.1219 ± 0.00199	503.496 ± 8.247
					1.028	1.144	1.311	1.481	1.691	0.998 ± 0.073	0.9951	0.0301	5.0030	0.12072 ± 0.00209	500.287 ± 8.662	100.6%
					258.46	304.86	348.18	400.87	429.11	260.834 ± 16.107	0.9967	0.0298	4.9704	0.11993 ± 0.00184	497.029 ± 7.626	99.41%
2	Ponstan-forte 500mg- Egypt	0.7296 ± 0.0014	500 ± 0.959	0.17605	391	516	549	1194	1858	305.427 ± 14.1354	0.9955	0.5021	5.0201	0.12114 ± 0.0016	502.066 ± 6.631	100.41%
					1.021	1.150	1.319	1.490	1.694	0.998 ± 0.061	0.9971	0.0296	4.9308	0.11898 ± 0.00181	493.080 ± 7.501	98.2%
					256.93	308.11	346.29	408.31	428.44	260.972 ± 18.631	0.9955	0.0299	4.9855	0.12030 ± 0.00169	498.548 ± 7.004	99.71%
3	Merfil-500				386	520	544	1188	1866	301.282 ± 14.3400	0.9955	0.4925	4.9249	0.11883 ± 0.00149	492.481 ± 6.175	8.50%

		0.840 8± 0.00 48	500 ± 2.67 6	0.2028 8						[Merfil] mmol /L	9.09 %					
					1.0 13	1.16 5	1.31 4	1.48 7	1.69 1	0.998±0.05 1+ 33.560 ± 4.175	.997 7	0.02 96	4. 958 3	0.119 64 ± 0.001 67	495.8 20± 6.921	99.1 6%
					25 8.0 8	306. 47	346. 19	402. 81	431. 06	260.462±17 .402+ 8846.00 ± 1420..868	.996 2	0.02 94	4. 907 3	0.118 41 ± 0.001 99	490.7 26 ± 8.247	8.15%
										[Merfil] mmol /L	9.54 %					
					39 2	522	548	119 2	186 8	305.798±14 4.969+ 610.818 ± 108.599	.995 3	0.50 06	5. 006 4	0.120 80 ± 0.001 84	500.6 30 ± 7.626	100.1 3%
4		0.598 7± 0.00 23	500 ± 1.92 1	0.1444 6						[ponstidin] mmol /L	9.07 %					
					1.0 19	1.16 0	1.31 8	1.48 2	1.69 8	0.999±0.06 4+ 33.600 ± 5.075	.996 6	0.02 97	4. 957 3	0.119 62 ± 0.019 6	495.7 27 ± 8.123	9.15%
					25 4.8 8	310. 08	344. 89	401. 44	430. 07	259.924±19 .945+ 8834.800 ± 1628.567	.995 0	0.02 94	4. 903 4	0.118 31 ± 0.001 69	490.3 34± 7.004	8.07%
	Ponstidin-500 500mg- Iraq									[ponstidin] mmol /L	9.00 %					

ŷ: Estimated response value (mV) for ISNAG- fluorimeter, without unite for UV-spectrometric method, and Turbidometric method (FTU) for (n=3), [sample]: drug concentration (mmol/L), r: correlation coefficient, r²: coefficient of determination & R²o: percentage capital R- square, t_{0.025, ∞} = 1.96 at 95%, t_{0.025, 2} = 4.303 for n-1 t_{0.025, 3} = 3.182. For n-2 drawing volume of 1ml for ISNAG-fluorimeter and 0.06 for two classical methods

Conclusion

The newly developed adopted methodology in this research work was put into a paired t-test for the sake of accepting it as an alternative method for analysis and assessment of Mefemanic acid with standard used method. Mainly British Pharmacopoeia (B.P) [31], and turbidometry method (Scheme 2), 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 three assumptions statistically is made [32, 33]. 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

H₀ = Null hypothesis = No significant difference between

H₀: μ_{B.P} = μ_{ISNAG-fluorimeter} = μ_{Turbidometry} = μ_{uv-spectrophotometry}

H₁ (alternative hypothesis): μ_{B.P} ≠ μ_{ISNAG-fluorimeter} ≠ μ_{Turbidometry} ≠ μ_{uv-spectrophotometry}.

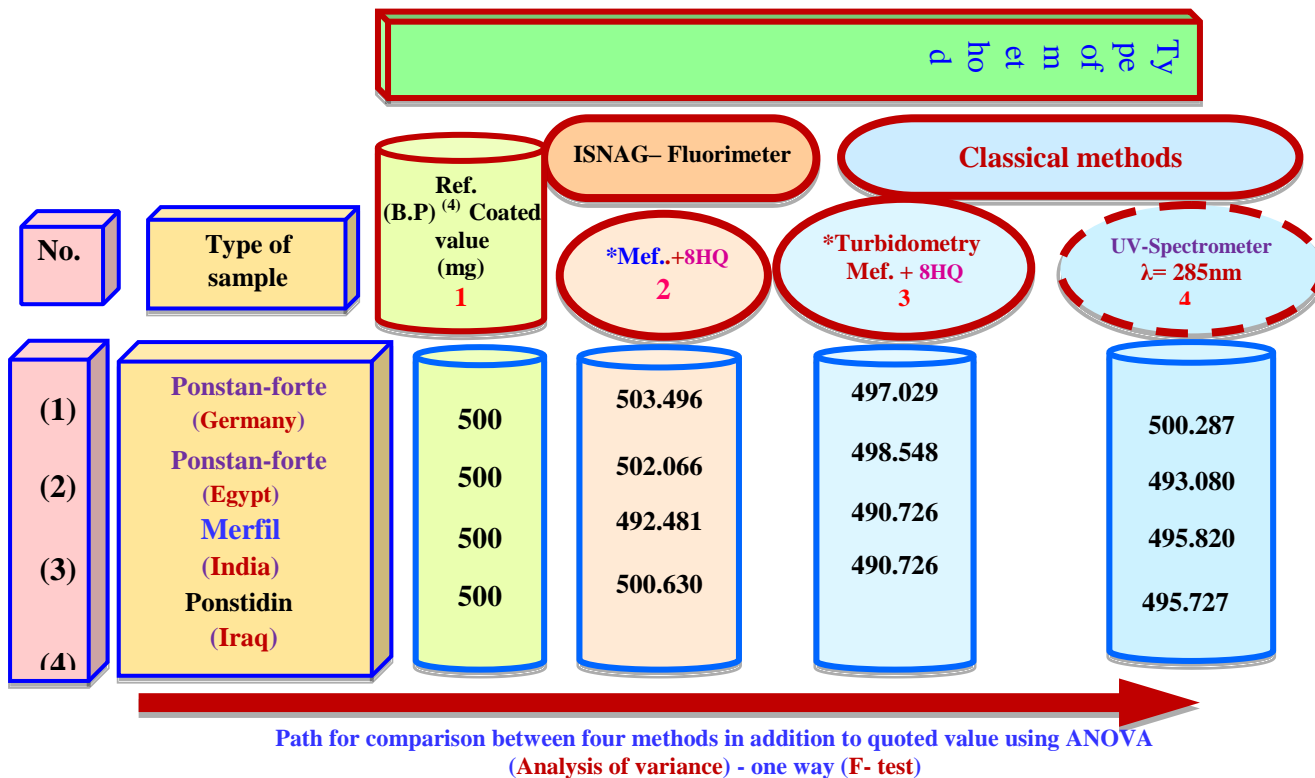
Conducting paired t- test will all possible pairs (i.e.; 5- pairs) five that are as follows: ISNAG- fluorimeter Vs. British Pharmacopoeia (BP), ISNAG Vs. turbidometry, ISNAG Vs. spectrophotometry, spectrophotometry Vs BP and turbidometry Vs BP. As ISNAG- fluorimeter being the suggested alternative or equivalent method of assessment of the drug which challenges the available official method as ISNAG-fluorimeter as an instrument is new in its whole properties of working and presenting results for determination.

So therefore, it is the one who is 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 are five comparisons. As it compare, ISNAG- fluorimeter method with the other there standard method as shown above. Which significance test indicate that at 95% confidence (α = 0.05/2 two tailed) there is no significant difference between the

newly developed method and standard methods.

Therefore, the analyst should be able to choose any method for analysis i.e.; ISNAG-

fluorimeter or spectrophotometry or turbidometry. Thus accepting null hypothesis. This indicate that the high efficiency of ISNAG- fluorimeter as a reliable instrument for analysis of Mefenamic acid.



Scheme 2: Summed up the results for three different methods in addition to quoted value and four different samples for ANOVA * ISNAG- fluorimeter (8HQ: ISNAG-fluorimeter using Mefenamic acid- 8HQ (5mmol/L) system. *Turbidometry: Mefenamic acid -8HQ (0.005mmol/L) system

Table 9: Paired t-test for the comparison between four different methods of four samples for the analysis of Mefenamic acid in drugs for n= 4 at 95% confidence level (α = 0.05) and DF = 3

Paired	Paired differences				Significant (2 tailed)
	Xd	Standard Deviation (σ _{n-1})	t _{cal}	t _{tab}	
Pair- 1 ISNAG fluorimeter - BP	- 0.332	4.932	- 0.135 < 3.182		0.902 > 0.05 Not significant
Pair- 2 ISNAG fluorimeter- spectrophotometry.	3.440	5.129	1.341 < 3.182		0.272 > 0.05 Not significant
Pair- 3 ISNAG fluorimeter- Turbidometry.	5.411	3.571	3.0307 < 3.182		0.0563 > 0.05 Not significant
Pair- 4 BP - spectrophotometry	3.772	2.989	2.524 < 3.182		0.0859 > 0.05 Not significant
Pair-5 BP - Turbidometry	5.743	4.124	2.785 < 3.182		0.069 > 0.05 Not significant

DF: Degree of freedom (n-1) = 3, Xd: average of difference between two methods, t_{tab} = t_{0.025, 3} = 3.182, t_{cal} = Xd √n /σ_{n-1} at 95 %

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