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Determination of Mefenamic Acid Using a New Mode of Irradiation (Array of Six Identical LEDs) and Detection(Twin Solar Cells) Through Turbidity Measurement by CFIA

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Abstract:

A newly developed analytical method characterized by its speed and sensitivity for the determination of mefenamic acid (MFA) in pure and pharmaceutical preparation is established via turbidimetric measurement (0-180^o) by Ayah 6SX1-ST-2D Solar cell CFI Analyser . The method was based on the reaction of phosphomolybdic acid with mefenamic acid in aqueous medium to form blue color precipitate as an ion-pair complex . Turbidity was measured via the reflection of incident light that collides on the surface precipitated particles at 0-180^o . The chemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 0.3-7 or 0.3-10 mMol.L^{-1}, with correlation coefficient r = 0.9907 or 0.9556 respectively. The limit of detection 4.92 µg/sample from the step wise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.3% for 3 and 5 mMol.L^{-1} (n= 6,10 respectively) concentration of mefenamic acid. The method was successfully applied to the determination of mefenamic acid in four pharmaceuticals . A comparison was made between the newly developed method analysis and the classical method , in addition to between four different pharmaceutical preparations (UV- spectrophotometry at wave length 288 nm) using the standard additions method via the use of t-test. It was noticed that there was no significant difference between two methods at 95 % confidence level and significant difference between four drugs.

Keywords: Mefenamic acid , Flow injection analysis , Turbidity.

تقدير حامض الميفيناميك باستخدام نمط جديد من التشعيع (مصفوفة متشابهة لستة ثنائيات وصلة باعثة) والتحسس (اثنان من الخلايا الشمسية) خلال قياس التعكرية بوساطة التحليل بالحقن الجرياني المستمر

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الخلاصة:

طورت طريقة تحليلية جديدة ،تميزت بالسرعة والحساسية لتقدير حامض الميفيناميك بشكله النقي او على هيئة مستحضرات صيدلانية عن طريق قياس التعكرية (0–180°) بواسطة محلل الحقن الجرياني المستمر 6SX1–ST–2D Solar cell Ayah. استندت الطريقة على تكوين راسب ازرق اللون لمزدوج ايوني بين حامض الميفيناميك وحامض مولبدات الفسفوريك في الوسط المائي. تم قياس التعكرية عن طريق انعكاس الضوء المسلط والمصطدم بسطوح دقائق الراسب بزاوية0–180°. تم دراسة كافة المتغيرات الكيميائية والفيزيائية. مدى الخطية لمنحني المعايرة لحامض الميفيناميك يمتد 0.3–7 أو 0.3–

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10 مللي مول.لتر$^{-1}$ بمعامل ارتباط=0.9907 و0.9556 على التوالي. كان حد الكشف 4.92 مايكروغرام/انموذج من التخفيف التدريجي لأقل تركيز في منحني المعايرة مع انحراف قياسي نسبي معنوي اقل من 0.3% للتراكيز 3 و5 مللي مول.لتر$^{-1}$ (n=10,6 على التوالي) من حامض الميفيناميك. طبقت الطريقة بنجاح لتعيين حامض الميفيناميك في اربعة مستحضرات صيدلانية. اجريت المقارنة بين الطريقة المستحدثة للتحليل والطريقه التقليديه للقياس الطيفي عند λmax=288 نانومتر بأستخدام منحني الأضافات القياسية بالاضافة الى المقارنه بين اربعة مستحضرات صيدلانية بوساطة اختبار t المزدوج ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعة 95 % مع فرق جوهري بين المستحضرات الصيدلانيه الاربعة.

Introduction:

Mefenamic acid (MFA) N-[(2,3-dimethyl phenyl)amino]benzoic acid , Figure-1 is the structure of mefenamic acid, is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-pyretic properties [1]. Chemically it belongs to the anthranilic acid derivatives class. Mefenamic acid inhibits the enzyme cyclooxygenase (COX) to exert its anti-inflammatory effect and inhibits the synthesis of prostaglandin to produce analgesic action. Mefenamic acid produces both central and peripheral analgesic action. It is a nonselective COX inhibitor, which inhibits both the COX-1 enzyme and COX-2 enzyme [2]. It was invented in 1961 by Claude Winder from parke-davis and is marketed as Ponstel , Ponstan , Ponstal , Parkemed , Mafepain , Mephadolor , Meftal , Dyfenamic and Potarlon. MFA is available as white to off-white, crystalline powder that darkens on prolonged exposure to light , it melts at 227 – 232 C^{o} , insoluble in water; sparingly soluble in chloroform and ether , and soluble in 0.1M NaOH [3,4].

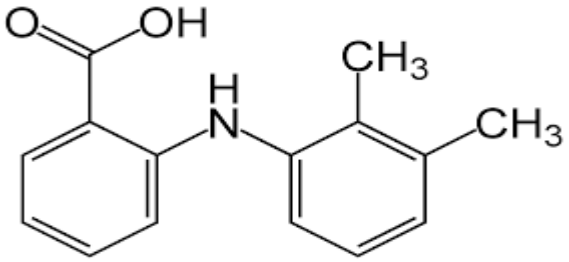


Figure 1-Chemical structure of mefenamic acid

MFA is a type of analgesics or painkiller. NSAID are drugs with analgesic and antipyretic (fever-reducing) effects and which have, in higher doses, anti-inflammatory effects [5]. MFA is indicated for the treatment of mild to moderate pain, primary dysmenorrhea, dental extractions, menstrual cramps, muscle ache and athletic injuries. Diarrhea is the most important dose related side effect and haemolytic anaemia is a rare but serious complication. Other untoward effects include thromobocytopenia purpura, bone-marrow hypoplasia, leucopenia, pancytopenia agranulocytosis, gastrointestinal discomfort, dizziness, headache, vomiting, urticarial and rashes. A number of analytical methods have been developed for the quantitative determination of mefenamic acid in dosage forms and biological samples. Among those are spectrophotometry [6–9], chromatography [10–12], titration methods [13], chemiluminescence [14], and electrochemical sensors [15–17].

The purpose of this work is to describe a simple, precise and sensitive flow injection turbidimetric method with the use of Ayah 6SX1-ST-2D Solar cell CFI Analyser for the determination of mefenamic acid in pharmaceutical formulations. The method based on the formation of blue color precipitate as an ion-pair compound by phosphomolybdic acid with mefenamic acid in aqueous medium. The turbidimetry is measured via reflection of incident light from the surfaces of precipitate particles at 0-180º. The positive signal from reflection recorded by Ayah 6SX1-ST-2D Solar cell supplied with linear array of six snow-white light emitting diode as a source and two solar cells as a detector.

Experimental

Reagents and Chemicals

All chemicals used were of analytical-reagent grade and distilled water used to prepare the solutions. A standard solution (0.1 Mol.L^{-1}) of Mefenamic acid $C_{15}H_{15}NO_2$ (241 g. mol^{-1}) was prepared

by dissolving 2.41 g in 100 mL of 0.1 Mol.L^{-1} NaOH. A stock solution (0.012 Mol.L^{-1}) of phosphomolybdic acid $H_3PMo_{12}O_{40}$,(1825.25 g.mol^{-1} ,Fluka) was prepared by dissolving 10.9515g in water, filter and dilute to 500 mL. Sodium hydroxide solution (NaOH, 40, BDH,0.1Mol.L^{-1}) was prepared by dissolving 0.4 g in100 mL distilled water (Standardized with HCl solution).

Sample Preparation

Twenty tablets weight, crushed and grinded. Tablets containing 500 mg of mefenamic acid for (SDI- Pioner , Pfizer, MVC companies) were weight (2.8688, 3.4501, 3.3908, 4.3780 g) equivalent to 2.41 g of active ingredient respectively to obtain 100 mMol.L^{-1} conc. of MFA for each drug . The powder was dissolved in 0.1 Mol.L^{-1} NaOH followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 100 mL with the same solvent (0.1 Mol.L^{-1} NaOH).

Apparatus

The flow system used for the determination of MFA is shown schematically in Figure-2 , Peristaltic pump – 2 channels variables speed (Ismatec , Switzerland), Injection valve with valve 6-port medium pressure (IDEX corporation, USA) with sample loop(0.7mm i.d.Teflon ,different length) . The response was measured by a homemade Ayah 6SX1-ST-2D Solar cell CFI Analyser, which used a six snow-white light emitting diode LEDs for irradiation of the flow cell at 2 mm path length . Two solar cells are used as detector for collecting signals via sample travel for 60 mm length . The readout of the system composed of x-t potentiometric recorder (Kompenso Graph C-1032) Siemens (Germany), this recorder measured by(1-200) mV or voltage and digital AVO-meter (auto range) (0-2volt) (China). UV spectrophotometer digital double beam type UV-1800, Shimadzu, Japan was used to scan the spectrum of MFA using 1 cm quartz cell.

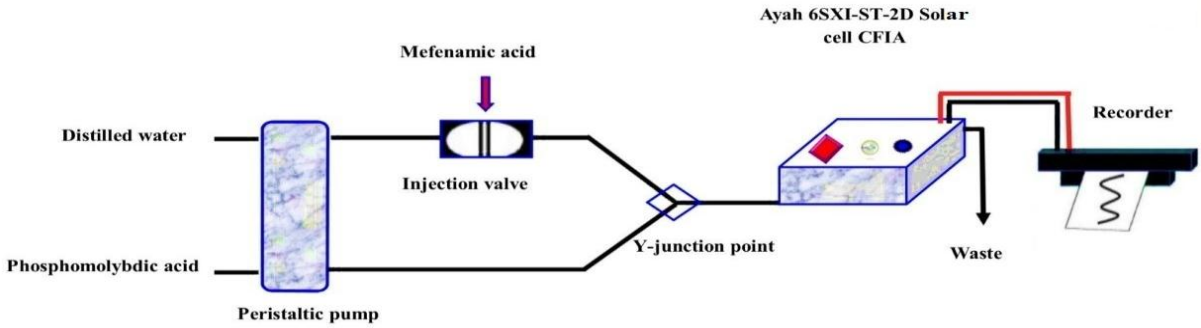
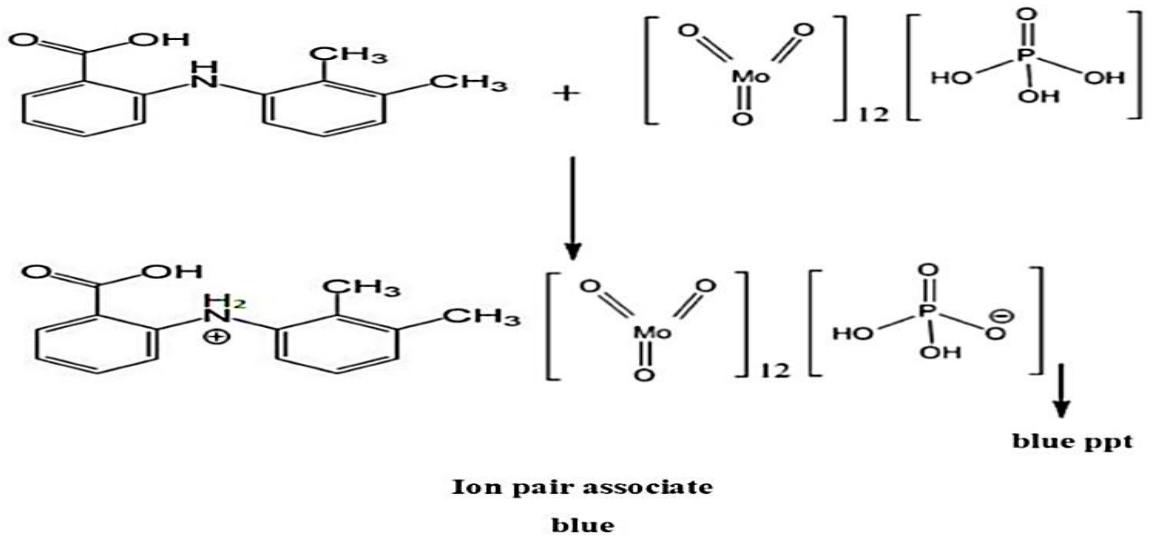


Figure 2-Flow diagram manifold system used for the determination of MFA.

Method

The flow system consisting of two lines was used for the determination of MFA by the reaction between MFA and phosphomolybdic acid (0.5 mMol.L^{-1}) in aqueous medium to form a blue color precipitate as an ion pair complex form. The first line represent the carrier stream (Distilled water) at 1.7mL.min^{-1} flow rate which lead to the injection valve to carry MFA, sample volume 102µL; while the second line supplies phosphomolybdic acid solution at 2.1mL.min^{-1} .Both lines meet at a Y-junction ,with an out let for reactants product from complex , which passes through a homemade Ayah 6SX1-ST-2D solar cell CFI Analyser that work with a six snow white light emitting diodes LEDs used as a source . Each solution injected was assayed in three times. The response profile of which was recorded on x-t potentiometric recorder to measure energy transducer response expressed as average peak height in mV by reflection of incident light at 0-180º .A probable mechanism of ion pair formation for MFA -PMA system is represented in scheme-1.



Scheme 1-Proposed mechanism of reaction between MFA and PMA

Results and Discussion:

Study of the Optimum Parameters:

The flow injection manifold system as shown in Figure-2 was used to investigate in the relation of chemical & physical variables, in order to obtain optimum conditions for the system. They were optimized by making all variable constant and varying one at a time , i.e fixed variable optimization .

Chemical Variables

Phosphomolybdic Acid (PMA) Concentration

A series of the precipitating reagent (PMA) solutions (0.1-12) mMol.L^{-1} were prepared, at constant concentration of mefenamic acid (5 mMol.L^{-1}) ,100 µL sample volume at 1.7 mL.min^{-1} flow rate for carrier stream line and 2.1 mL.min^{-1} flow rate for reagent line and the intensity of incident light of LEDs (1.68) mV were used. It can be shown that an increase in PMA concentration leads to increase in the reflection of incident light on the precipitate particles surfaces up to 0.5 mMol.L^{-1}, following this concentration; there was a decrease in the reflected light intensity, which was might be attributed to an increase in particles density which might lead to accumulation effect of precipitate particles in front of the detector as shown in Figure-3 A, B. Therefore; 0.5 mMol.L^{-1} PMA concentration was chosen as the optimum concentration that used for further experiment. All results tabulated in Table-1.

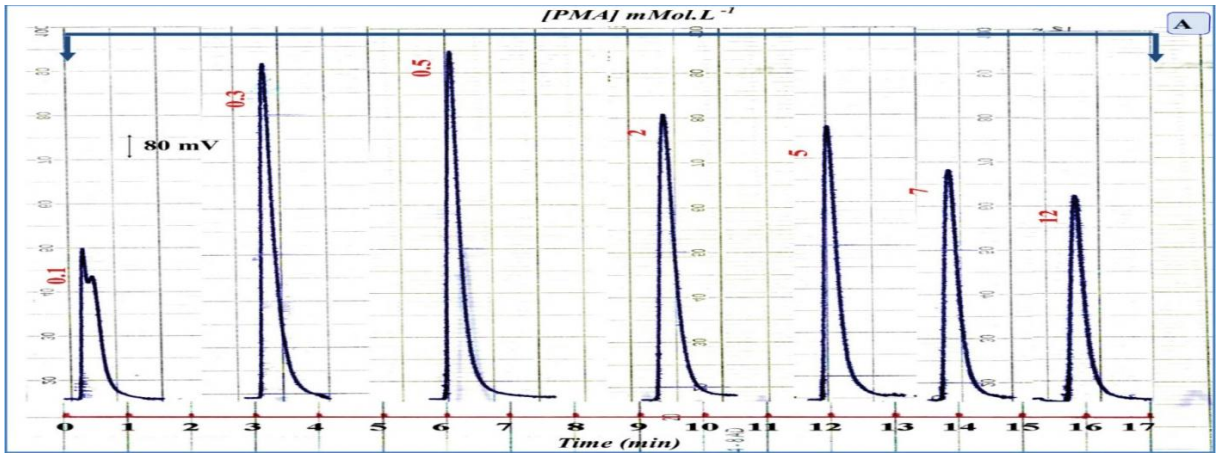


Figure 3-Effect of [PMA] on:

(A) Response profile versus time

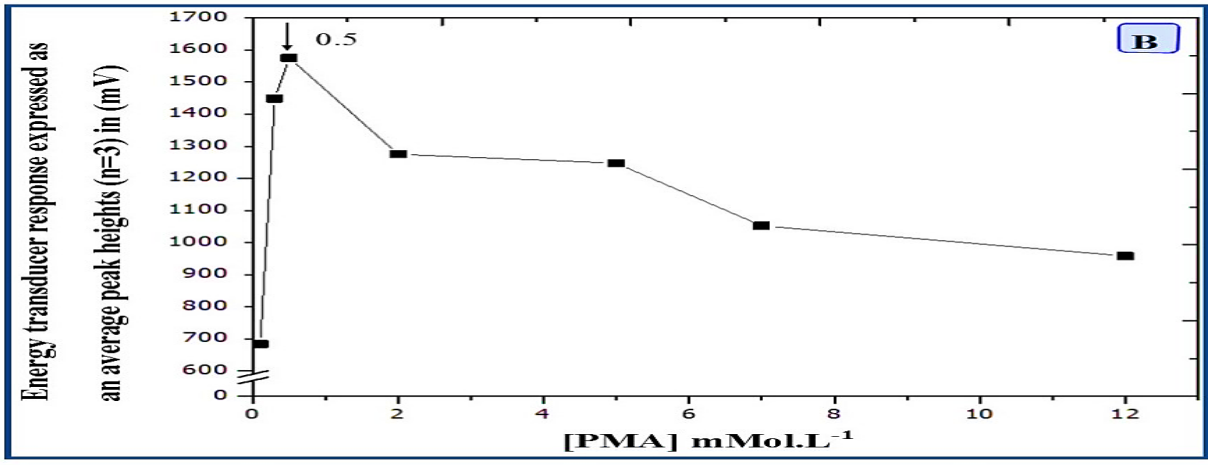


Figure 3-Effect of [PMA] on:
(B) Energy transducer response expressed as an average peak heights

Table 1-Effect of PMA concentration on the measurement of energy transducer response via reflection of incident light for the determination of MFA

[PMA] mMol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
0.1	683.73	0.55	683.73±9.342
0.3	1448.00	0.00	1448.00±0.000
0.5	1575.00	0.22	1575.00±8.608
2	1275.20	0.25	1275.20±7.920
5	1246.66	0.38	1246.66±11.769
7	1052.26	0.67	1052.26±17.515
12	957.86	0.92	957.86±21.893

Effect of basic media

The determination of mefenamic acid was studied in different concentrations of NaOH at the range (0.01- 0.1) Mol.L^{-1} in addition to the aqueous medium as a carrier stream. Using the optimum concentration of Phosphomolybdic acid (0.5 mMol.L^{-1}), mefenamic acid (5 mMol.L^{-1}), flow rate 1.7 mL.min^{-1} for carrier stream line and 2.1 mL.min^{-1} for reagent line with 100 µL sample volume. All responses profile are shown in Figure 4-A. The data obtained are plotted in Figure-4 B. It can be seen that the distilled water is chosen as the best medium due to the most suitable medium for completing reaction of phosphomolybdic acid with mefenamic acid , while the basic medium caused to decrease the output of transducer energy response due to solubility of precipitate particles of ion pair (PMA-MFA) in the presence of basic medium. Table-2 summarized the results obtained.

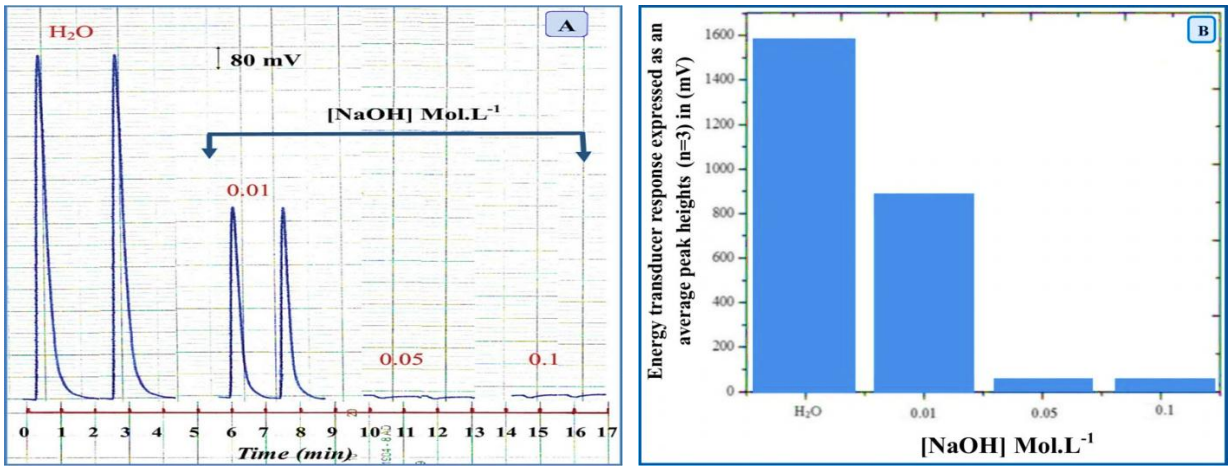


Figure 4-Effect of the NaOH concentration & Distilled water on the: **(A):** Response profile versus time **(B):** Energy transducer response expressed as an average peak heights using: mefenamic acid (5 mMol.L^{-1}), 100µL sample volume.

Table 2- Effect of basic media as a carrier stream on the transducer energy response for determination of mefenamic acid

[NaOH] Mol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
H_2O	1575	0.22	1575± 8.608
0.01	880	0	880 ± 0
0.05	0	0	0 ± 0
0.1	0	0	0 ± 0

Physical Variables

Flow rate

Using MFA (5 mMol.L^{-1})-PMA (0.5 mMol.L^{-1}) system with variable flow rates (0.3-2.6 mL.min^{-1}) for carrier stream (first line) and (0.4-3.4 mL.min^{-1}) for reagent (second line) controlled by the peristaltic pump. It was noticed that an increase in peak base width (Δt_B) at slow flow rates as shown in Figure-5 A which caused an irregular response due to the dispersion of precipitate species in a large area , while at higher flow rate (>1.7 mL.min^{-1}) although the effect of physical parameter was not very crucial on the height of response ; obtaining regular response and sharp maxima but it is a decrease in peak height due to the speed of departure of precipitate particles from flow cell (measuring cell) at a short time. Therefore, a flow rate of 1.7 & 2.1 mL.min^{-1} for carrier stream and reagent respectively were chosen as optimum flow rate in out this work **(**Figure -5B**)**. The results are tabulated in Table-3.

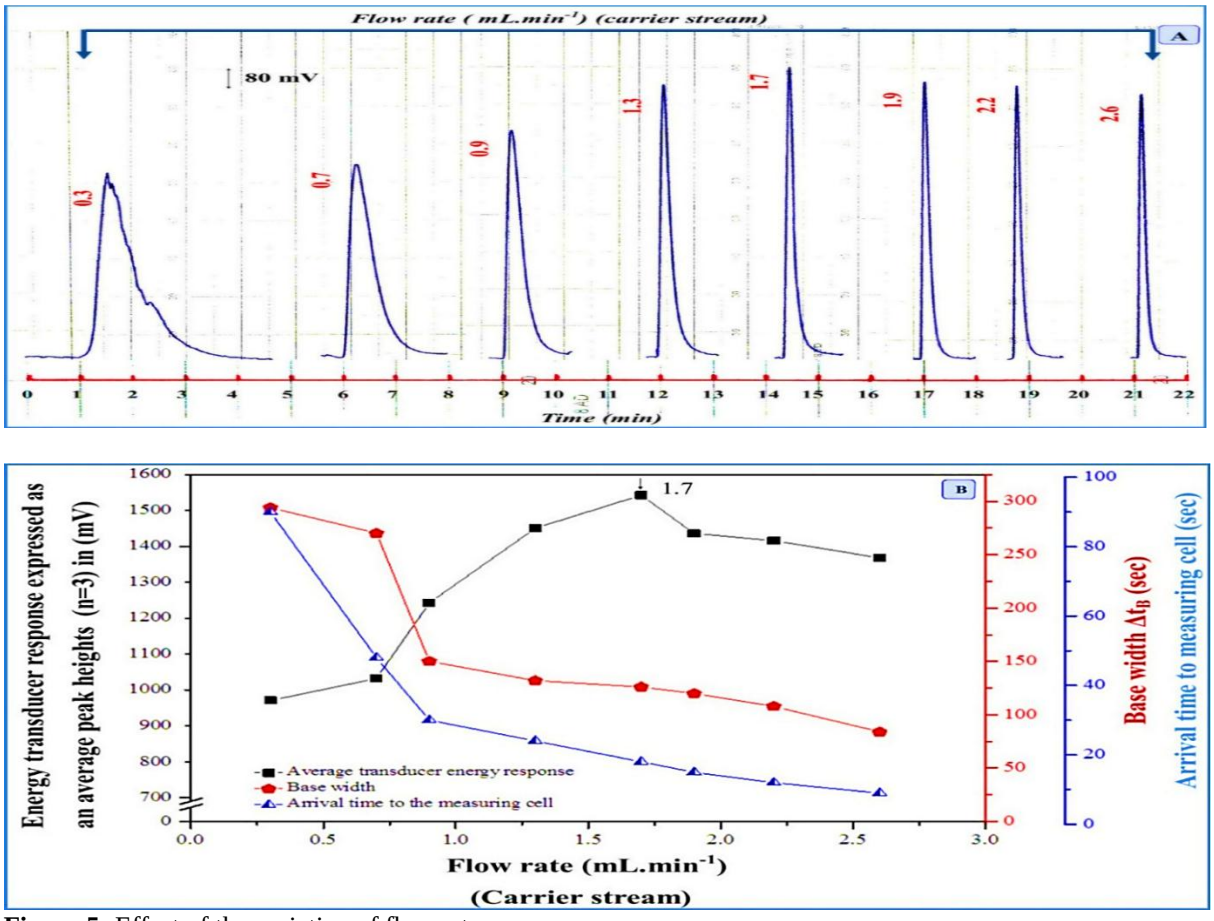


Figure 5- Effect of the variation of flow rate on:

(A): Response profile versus time

(B): Energy transducer response expressed as peak heights in mV , base width and departure time for sample segment from injection valve to the measuring cell

Table 3-Effect of the variation of flow rate on the measurement of energy transducer response via the reflection of incident light for determination of MFA using MFA (5 mMol.L^{-1}) –PMA (0.5 mMol.L^{-1}) system using 100µL sample volume.

Peristaltic Pump speed	flow rate mL.min^{-1}		Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD %	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)	t* (sec)	V* (mL)	C* (mMol.L^{-1})
	Line 1	Line 2							
5	0.3	0.4	970.66	0.47	970.66±11.334	294	90	3.530	0.142
10	0.7	0.8	1032.00	0.42	1032.00±10.768	270	48	6.850	0.073
15	0.9	1.3	1242.66	0.37	1242.66±11.423	150	30	5.600	0.089
20	1.3	1.7	1450.66	0.28	1450.66±10.091	132	24	6.700	0.075
25	1.7	2.1	1550.00	0.21	1550.00±8.087	126	18	8.080	0.062
30	1.9	2.6	1436.00	0.28	1436.00±9.989	120	15	9.100	0.055
35	2.2	2.8	1416.00	0.20	1416.00±7.036	108	12	9.100	0.055
40	2.6	3.4	1368.00	0	1368.00±0	84	9	8.500	0.059

t* = Departure time for sample segment from injection valve to the measuring cell
V*= Volume of segment at flow cell
C* = Concentration of segment at flow cell
Line 1: Carrier stream, Line 2: reagent.

Sample volume

Using the optimum parameter of flow rate (1.7 mL.min^{-1} for carrier stream line, 2.1 mL.min^{-1} for reagent line) and PMA (0.5 mMol.L^{-1}) for the determination of mefenamic acid. Variable sample volume (77-122) µL were used using open valve mode. It was noticed that any increase in volume of sample up to 102 µL lead to an increase in the energy transducer response as shown in Figure-6 A, B. while above 102 µL volume of sample, even though it gave a slightly higher response but there is an increase in base width (Δt_B) (i.e. increase analysis time). This might be attributed to the long duration of reacted sample segment (i.e. precipitate species) in front of a detector; in addition to increase of the particles size causing a slow movement of particles. Therefore; 102 µL was chosen as an optimum sample volume. The results were tabulated in Table-4.

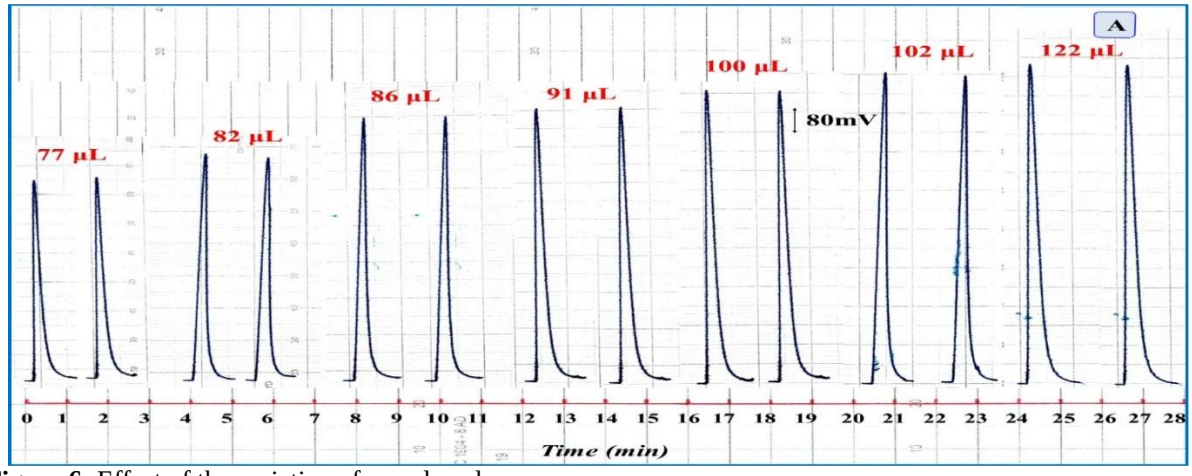


Figure 6- Effect of the variation of sample volume on:
(A): Response profile versus time

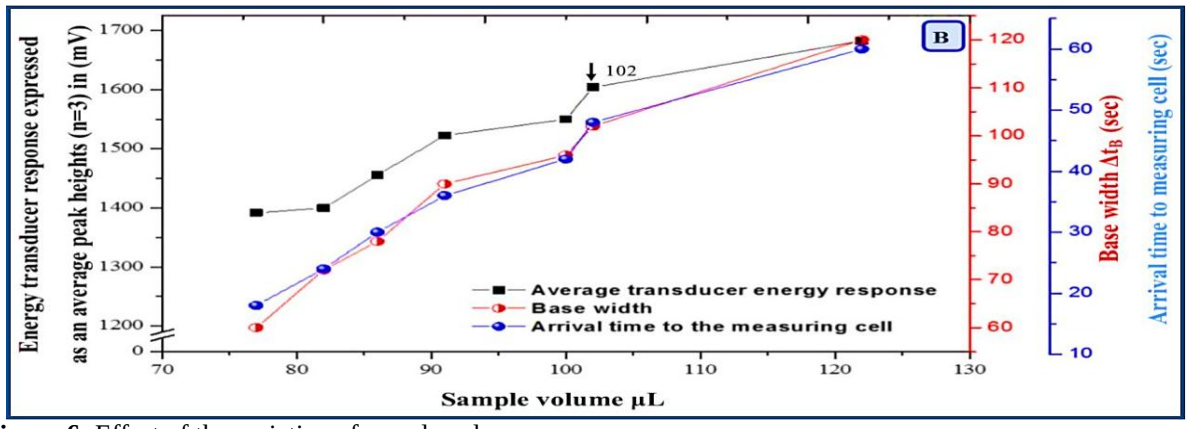


Figure 6- Effect of the variation of sample volume on:
(B): Energy transducer response expressed as an average peak heights in (mV)

Table 4- Effect of the variation of sample volume on the measurement of energy transducer response via reflection of incident light for determination of MFA

Sample volume µL	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)	t* (sec)
77	1392.00	0.02	1392.00 ± 0.692	80	7
82	1400.00	0.02	1400.00 ± 0.696	96	9
86	1456.00	0.03	1456.00 ± 1.085	102	13
91	1522.66	0.03	1522.66 ± 1.135	110	15
100	1550.00	0.03	1550.00 ± 1.155	126	18
102	1604.00	0.02	1604.00 ± 0.797	128	19
122	1682.66	0.01	1682.66 ± 0.418	133	31

t* = Departure time for sample segment from injection valve to the measuring cell
Δt_B= Base width of response

Purge time

This study was carried out to establish the optimum allowed permissible time for the sample segment to be injected from the injection valve at ranging (5-40) sec in addition to allow the injection valve in the open mode. Sample volume of 102 µL and MFA (5 mMol.L^{-1})- PMA (0.5 mMol.L^{-1}) system were used. It can be seen from Figure-7A, B, there is an increase in the response with increasing the allowed permissible time for the sample injection up to 20 sec. while above 20 sec there were a decrease in the response height; which might be attributed to the resistance of flow due to the continouse passage of carrier stream through the injection valve which in turn to slow movement of reflecting surface. Therefore; 20 sec was chosen as an optimum allowed permissible time for the sample segment (MFA) to be injected. The obtained results were tabulated in Table-5.

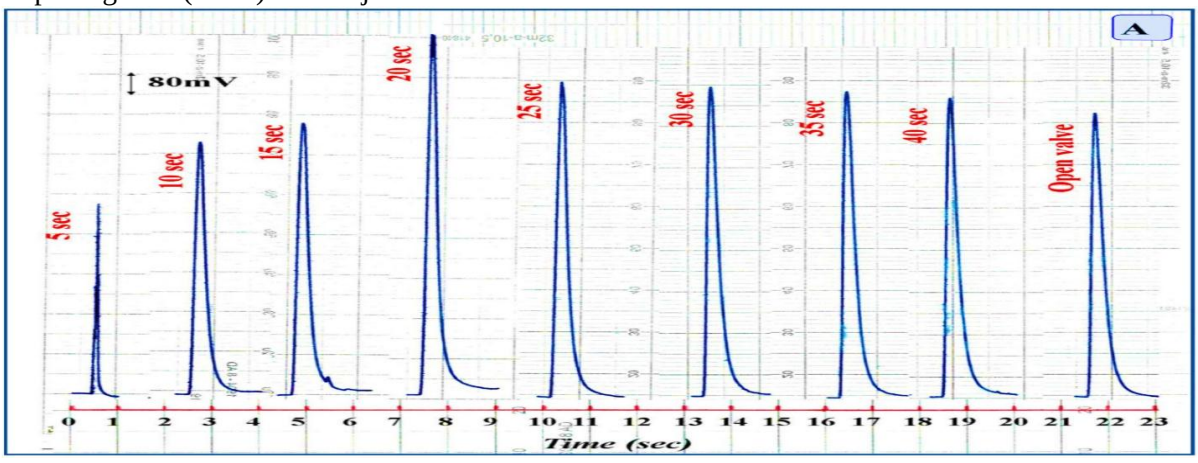


Figure 7- Effect of the variation of purge time on:
(A): Response profile versus time

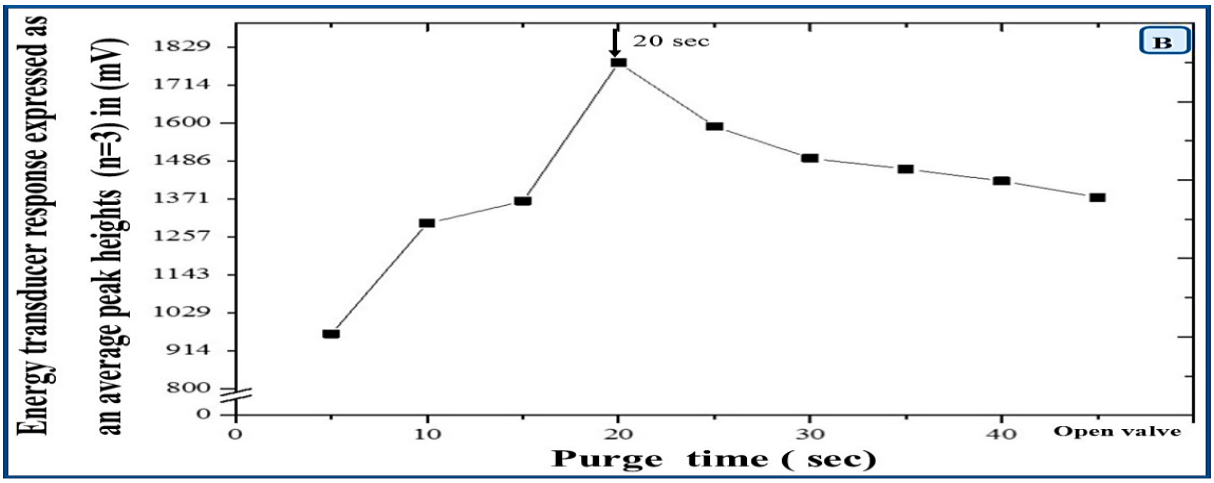


Figure 7- Effect of the variation of purge time on:

(B): Energy transducer response expressed as average peak heights in (mV)

Table 5- Effect of the variation of purge time on the energy transducer response.

Purge time (Sec)	Energy transducer expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
5	965.33	0.02	965.33 ± 0.480
10	1298.66	0.02	1298.66 ± 0.645
15	1365.00	0.01	1365.00 ± 0.339
20	1781.33	0.04	1781.33 ± 1.770
25	1590.00	0.02	1590.00 ± 0.790
30	1494.13	0.05	1494.13 ± 1.856
35	1461.33	0.03	1461.33 ± 1.089
40	1425.86	0.05	1425.86 ± 1.771
Open valve	1376.00	0.02	1376.00 ± 0.684

Intensity of light

Intensity of light source was studied at optimum condition, 0.5 mMol.L^{-1} of phosphomolybidic acid concentration, 102 µL sample volume of 5 mMol.L^{-1} mefenamic acid, flow rate for carrier stream 1.7 mL.min^{-1} and 2.1 mL.min^{-1} for reagent . Variable intensity of light source was used 0.15-1.9 volt by changing of light intensity knob operation in Ayah 6SX1-ST-2D-solar cell CFI Analyser , reading by AVO-meter. Figure 8 A showed that an increase of applied voltage to the LEDs led to the increase of incident light reflection. Therefore, the intensity of 1.7 V was chosen as the optimum voltage output that can be supplied to give a better response and reproducible outcome. Figure-8B showed the linearity calibration curve between applied voltage and energy transducer response, with linearity percentage r^2%= 0.9542 .The results obtained are summarized in Table-6.

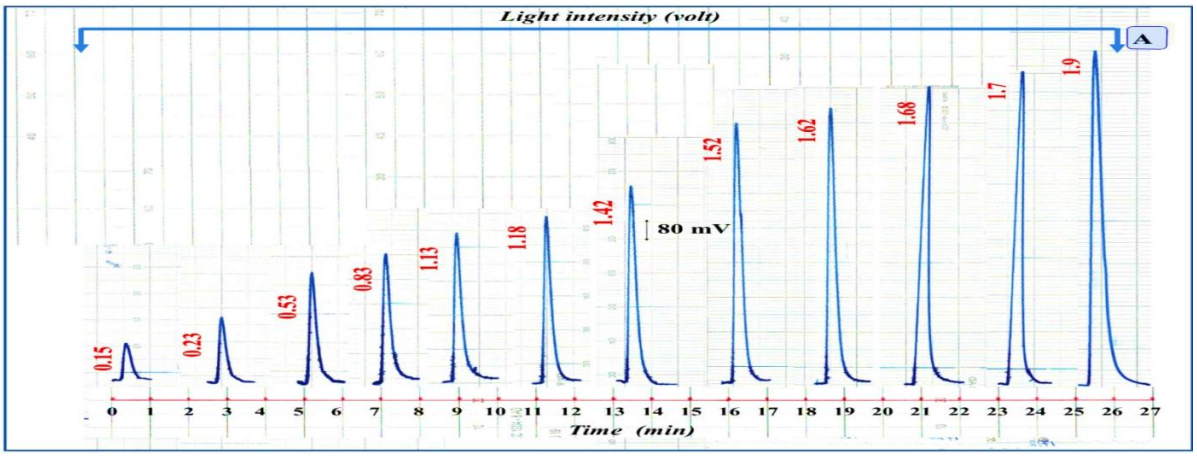


Figure 8- Effect of light intensity on:

(A): Response profile versus time

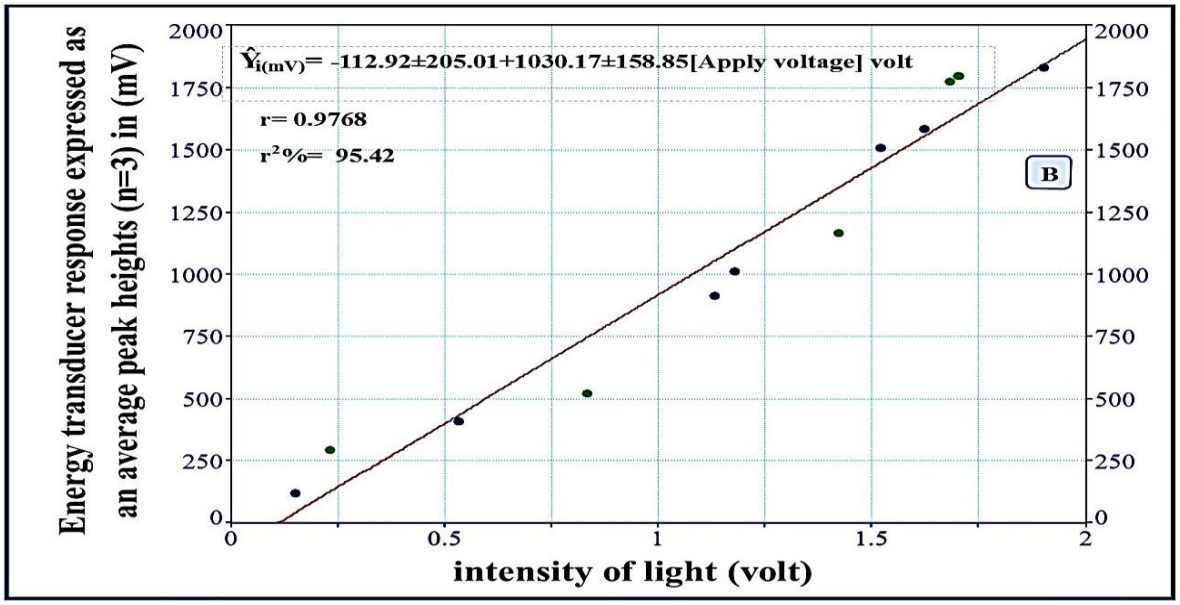


Figure 8- Effect of light intensity on:

(B): Calibration graph for the variation of applied voltages to the LEDs on the energy transducer response

Table 6- Effect of intensity of light on the measurement of energy transducer response via reflection of incident light

Intensity of light (Volt)	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
0.15	121.86	0.28	121.86 ± 0.848
0.23	292.80	0.18	292.80 ± 1.309
0.53	410.66	0.15	410.66 ± 1.530
0.83	522.66	0.09	522.66 ± 1.169
1.13	917.33	0.10	917.33 ± 2.279
1.18	1013.33	0.01	1013.33 ± 0.252
1.42	1168.00	0.02	1168.00 ± 0.580
1.52	1512.00	0.04	1512.00 ± 1.503
1.62	1584.00	0.05	1584.00 ± 1.968
1.68	1778.00	0.04	1778.00 ± 1.767
1.7	1800.00	0.05	1800.00 ± 2.236
1.9	1833.33	0.05	1833.33 ± 2.277

Scatter plot calibration curve for the variation of mefenamic acid concentration

Under the established optimum conditions, the variation of mefenamic acid (0.01- 20) $mMol.L^{-1}$ solutions were prepared. Each measurement was repeated for three successive measurements. Figure -9A showed responses profile for this study. Energy transducer response of the average peak heights (mV) of Ayah 6SXI-ST-2D solar cell CFI Analyser was plotted against the concentration of mefenamic acid. A straight-line ranging from (0.3-7) $mMol.L^{-1}$ or (0.3-10) $mMol.L^{-1}$ with correlation coefficient (r): 0.9907 &0.9556 respectively as shown in Figure-9B,C . It was noticed, that above 10 $mMol.L^{-1}$ the value for correlation coefficient will decrease and deviate from linearity. This is most probably due to the high intensity of the precipitate species opposite the detector which might probably leads to minimize the transmitted light. While the UV-spectrophotometric (classical method) at λ_{max}=288nm [18], the calibration graph was made for determination of mefenamic acid from (0.01-0.2) $mMol.L^{-1}$ with correlation coefficient (r): 0.9989 (Figure-9D) .The results were tabulated in Table 7 at confidence level 95% and limit of detection was 5µ$Mol.L^{-1}$ using first degree equation $\hat{y}$=a+bx [19,20] at optimum conditions. In addition to observe that the t-calculate of each method more than t_{tab} (t-value >> t_{tab}) which indicating that the linearity against non linearity is accepted.

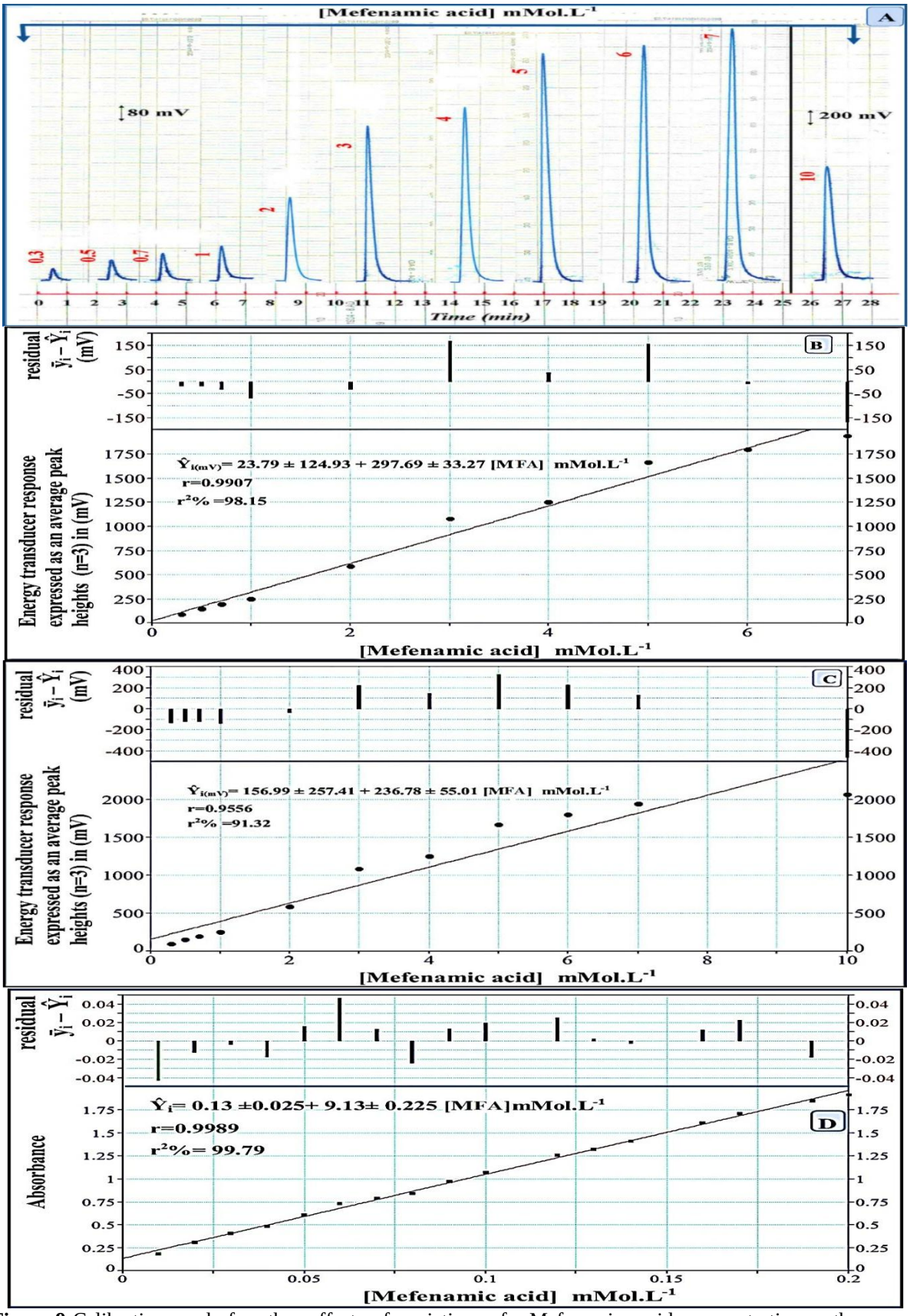


Figure 9-Calibration graph for the effect of variation of Mefenamic acid concentration on the energy transducer response by reflection of incident light using Ayah 6SX1-ST- 2D solar cell –CFI Analyser on **(A):** Response profile versus time **,(B):** Range (0.3-7) mMol.L ^{1}, **(C):** Range (0.3-10) mMol.L^{-1} , **(D):** Calibration graph using uv-sp. For measurement of Abs, residual ($\bar{y}_i - \hat{Y}_i$), $\bar{y}_i$: practical value, $\hat{Y}_i$: estimate value.

Table 7-Summary of linear regression for the variation of energy transducer response with Mefenamicacid concentration using first degree equation of the form $\hat{Y}$= a+bx at optimum conditions using two method

Type of method	Measured [MFA] $mMol.L^{-1}$	n	Range of [MFA] $mMol.L^{-1}$	$\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[MFA] $mMol.L^{-1}$ At confidence interval 95%, n-2 $\hat{Y}_i^*=a\pm s_a t+b\pm s_b t$[MFA] $mMol.L^{-1}$ At confidence interval 95%, n-2	r r^2 r^2%	t_{tab} at 95 % , n-2	Calculated t-value $/r/\sqrt{n-2}/\sqrt{1-r^2}$	LOD from gradual dilution
Ayah 6SX1-ST-2D solar cell CFI Analyzer	0.01-20	10	0.3- 7	23.79±124.93 + 297.69 ± 33.27 [MFA] $mMol.L^{-1}$	0.9907 0.9815 98.15	2.306 << 20.619		200 $\mu Mol.L^{-1}$
		11	0.3-10	156.99±257.41+236.78±55.01 [MFA] $mMol.L^{-1}$	0.9556 0.9132 91.32	2.262 << 9.735		
UV-Spectr ophoto metric	0.01-0.2	17	0.01-0.2	0.13±0.025+9.13±0.225[MFA] $mMol.L^{-1}$	0.9989 0.9979 99.79	2.131 << 85.51		5 $\mu Mol.L^{-1}$

$\hat{Y}_i^*$ =estimate value, r = correlation coefficient , r^2= coefficient of determination (C.O.D), r^2% = Linearity percentage.

Limit of detection (L. O. D):

A study was carried out to determine the L.O.D of mefenamic acid by three different methods at injected sample volume of 102 µL; that include:

1. (Gradual dilution).

Practically based on successive dilution of the lowest concentration used in calibration graph, this should be regarded as the real, and trustable value of D.L. (i.e. reliable D.L. for the proposed method).

2. Theoretically (slope method)

L.O.D =$3S_B$ / slope

$S_B = \sigma_{(n-1)B}$ (standard deviation of blank, n=13)

3. Theoretically (Linear equation) method

$\hat{Y} = Y_B + 3S_B$

Y_B (average response for the blank solution, this is equivalent to intercept (a) in straight line equation y=a+bx)

A study was carried out to calculate the limit of detection of mefenamic acid through three methods as tabulated in Table-8.

Table 8-Limit of detection for MFA at optimum parameters depend on different approach

Practically based on the gradual dilution for the minimum concentration 0.2 $mMol.L^{-1}$	Theoretical based on the value of slope $x=3S_B/slope$ for n=13	Theoretical based on the linear equation $\hat{Y}=Y_B+3S_B$
4.92 µg/sample	7.43 ng/sample	26.22 µg/sample

X= value of L.O.D based on slope, S_B=standard deviation of blank repeated for 13 times, Y_B=Average response for blank= intercept, L.O.D=limit of detection

Repeatability

The repeatability of the proposed method was studied at two concentrations of mefenamic acid (3,5)$mMol.L^{-1}$. Six and ten successive measurements were studied for this experiment. Figure-10A,B showed the response profile versus time .The obtained results were tabulated in Table-9 which showed that the percentage relative standard deviation was less than 0.3%, indicating clearly that the proposed method and the instrument was most suitable for the determination of MFA.

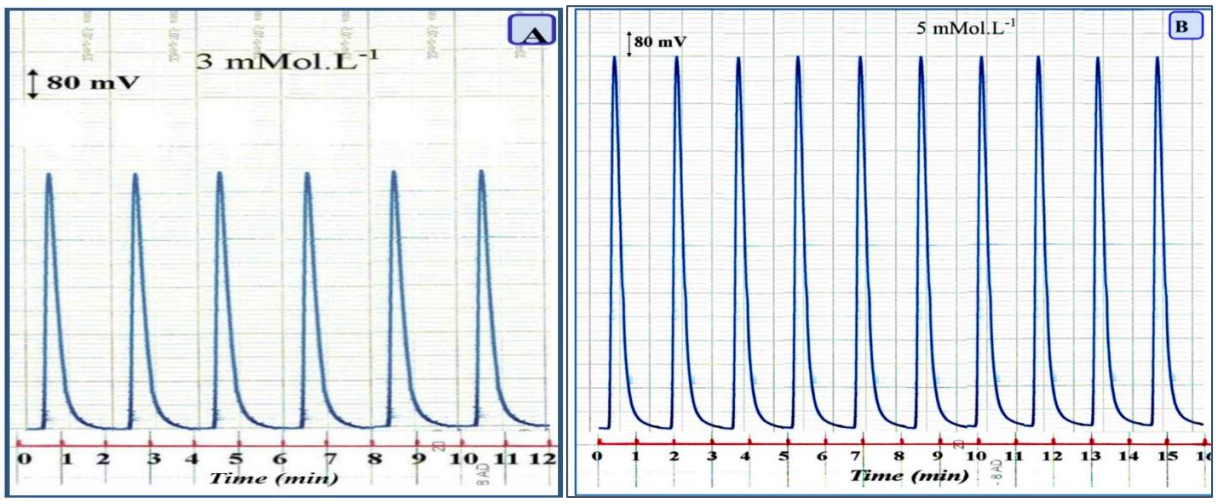


Figure 10- Response profile -time of:
(A): six successive repeatable measurements of Mefenamic acid concentration (3mMol.L^{-1})
(B): ten successive repeatable measurements of Mefenamic acid concentration (5mMol.L^{-1})

Table 9- Repeatability of energy transducer response for MFA.

[MFA] mMol.L^{-1}	Average response $\bar{y}_i$(mV)	RSD %	$\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$ At confidence interval 95%	Number Of injection
3	1082.66	0.23	1082.66 ± 2.614	6
5	1778.00	0.26	1778 ± 3.307	10

$t_{0.025,5}$=2.571 , $t_{0.025,9}$=2.262

Evaluation of the use of Ayah 6SX1-ST-2D- Solar cell CFI Analyser for the determination of mefenamic acid in pharmaceutical preparations as an application

Two methods were used for determination of mefenamic acid in the four different manufactured drugs (Ponstidin - 500 mg/SDI - Iraq, Piostan- 500 mg/Pioner- Iraq, Ponstan- 500 mg/pfizer- USA and Ponamec - 500 mg/MVC – India). The first method was employed with Ayah 6SX1-ST-2D Solar CFI Analyser while the second method was employed with the classical UV-spectrophotometric method through the measurement at λ_{max} (288 nm). A series of solutions were prepared of each pharmaceutical drug 0.1Mol.L^{-1} by transferring 0.1 mL to each of the five volumetric flask (25 mL), followed by the addition of gradual volumes of 0.1 Mol.L^{-1} standard mefenamic acid (0,0.1,0.15,0.2,0.25) mL which equivalent to (0,0.4,0.6,0.8,1) mMol.L^{-1} in the case of use Ayah 6SX1-ST-2D Solar cell –CFI Analyzer, while transferring 1ml from 1 mMol.L^{-1} pharmaceutical drug to each of the five volumetric flask (25 mL) , followed by the addition of gradual volumes of 1mMol.L^{-1} standard mefenamic acid (0,1,2,2.5,3.5) mL in order to have (0,0.04,0.08, 0.1,0.14) mMol.L^{-1} in the case of use classical UV- spectrophotometric method . Flask no.1 is the sample. Figure-11 A showed the responses profile for this study. Figure-11B,C,D,E showed standard addition calibration graphs using Ayah 6SX1-ST-2D solar cell –CFI Analyser. Table-10 sum up the summary of standard additions method results from the four samples with the amount of mefenamic acid in pharmaceutical drug. While the data in Table-11 sum up the results for two methods showing practical content of active ingredient at 95% confidence level, efficiency of determination and paired t-test for comparison at two different paths :

♦ Individual paired t-test; for comparison between practical weight content with quoted value (500 mg) by calculated t-value of each individual company .The following hypothesis should be used : H_o (Null hypothesis) : for sample$_1$: $\mu_o=\overline{w}_1$ for SDI-Iraq
for sample$_2$: $\mu_o=\overline{w}_2$ for Pioner- Iraq
for sample$_3$: $\mu_o=\overline{w}_3$ for pfizer-USA
for sample$_4$: $\mu_o=\overline{w}_4$ for MVC-India

i.e.: There is no significant difference between the means for four different companies ($\overline{w}_i$) and quoted value (μ_o=500 mg)

Against
Alternative hypothesis: There is a significant difference between the quoted value and means for four different companies
i.e:

$$H_1 : \mu_o \neq \overline{w}_i \text{ for four different companies}$$

-The obtained values subjected that there is a significant different between the quoted value (500mg) and calculated t-value for four different companies , must be attributed to interferences effect.
♦ Secondary paired t-test: was used to compare between two different methods (i.e: Ayah 6SX1-ST-2D
Solar cell CFI Analyzer & UV- spectrophotometric)
Taking into consideration a neglecting individual difference between one manufacturer and another.
A hypothesis can be estimated as a follow:
Null hypothesis $H_o : \mu_{\text{Ayah 6SX1-ST-2D Solar cell CFI Analyzer}} = \mu_{\text{uv.sp}}$
Against
Alternative hypothesis $H_1 : \mu_{\text{Ayah 6SX1-ST-2D Solar cell CFI Analyzer}} \neq \mu_{\text{uv.sp}}$
-Since calculation t_{value} of $|-0.733| < t_{tab}(3.182)$ at 95% confidence level. Therefore; H_o is accepted against H_1,
i.e.: that there is no significant difference between two methods.

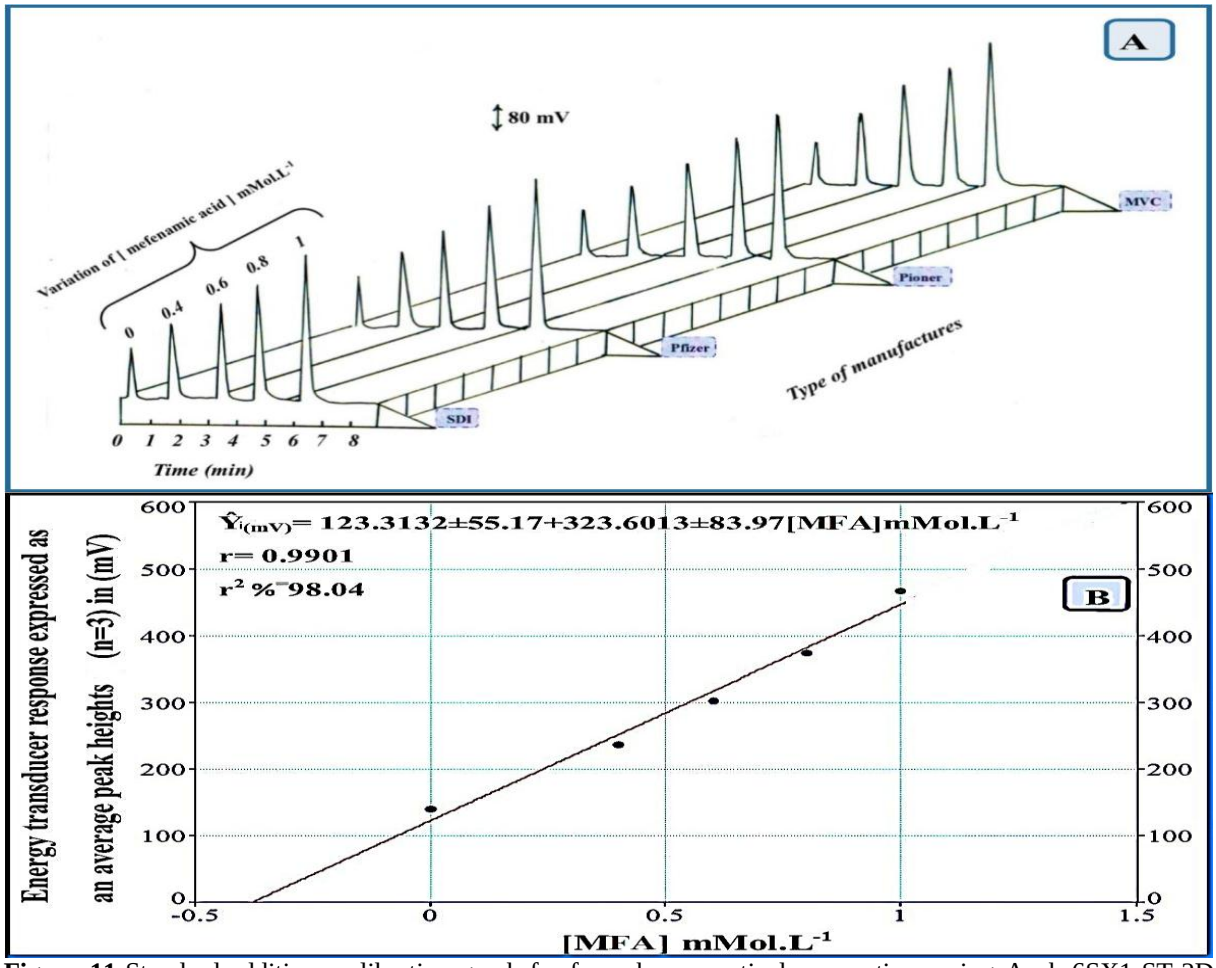


Figure 11-Standard additions calibration graph for four pharmaceutical preparations using Ayah 6SX1-ST-2D solar cell –CFI Analyser.
(A): Response profile verses time
(B): SDI company

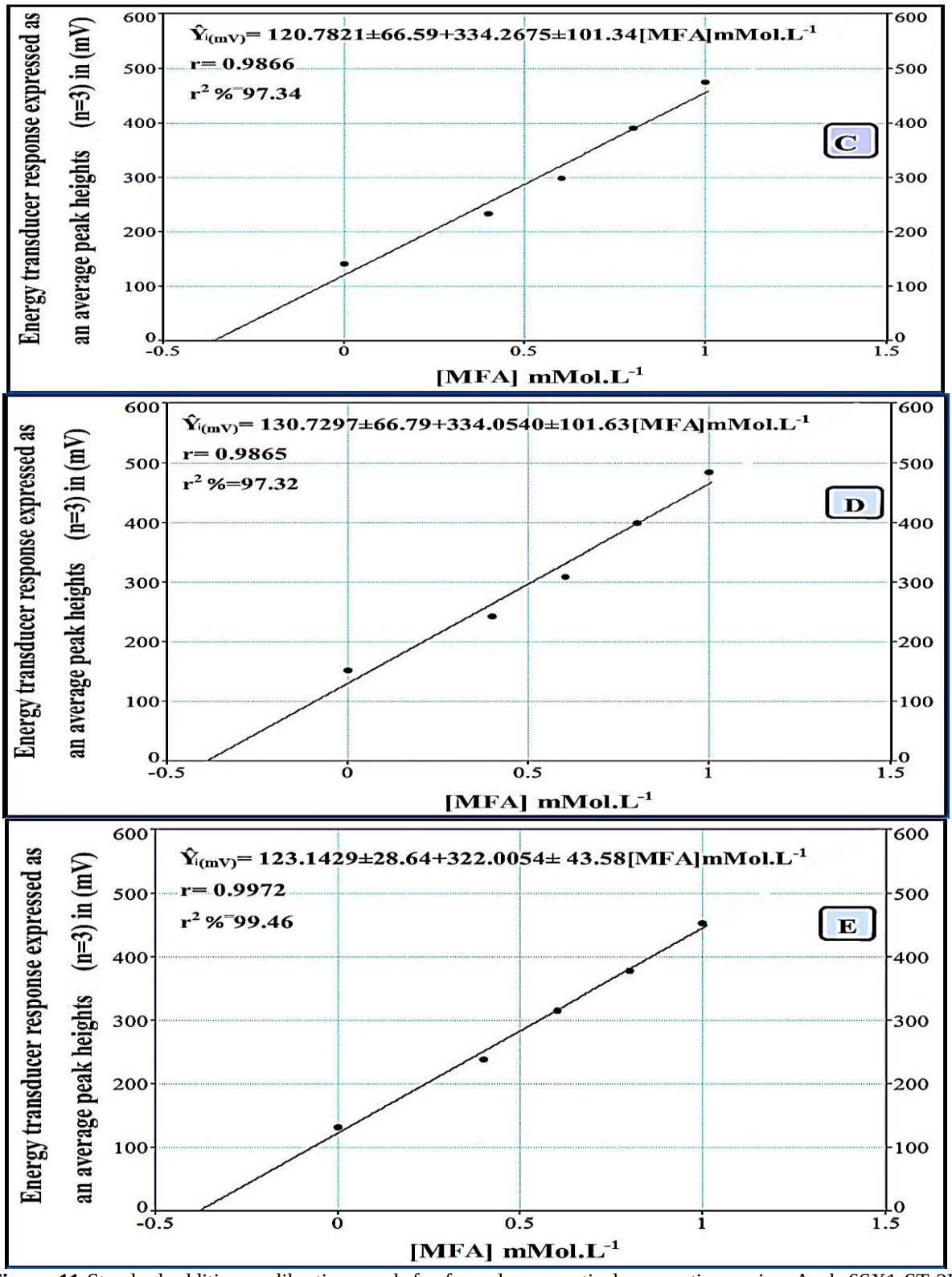


Figure 11-Standard additions calibration graph for four pharmaceutical preparations using Ayah 6SX1-ST-2D solar cell –CFI Analyser.
(C): Pioner company
(D): Pfizer company
(E): MVC company

Table 10- Results for the determination of Mefenamic acid in pharmaceutical preparations by standard additions method using Ayah 6SX1-ST-2D Solar cell CFI Analyser & Classical methods (UV Spectrophotometric).

sample no	commerical name ,content company,country	*Ayah 6SX1-ST-2D solar CFIA (mV)* / *UV SP. (classical method for absorbance measurement)* [Mefenamic acid] mMol.L^{-1}					Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[MFA] mMol.L^{-1} $\hat{Y}_i^*=a\pm s_a t+b\pm s_b t$[MFA] mMol.L^{-1}	r r^2 r^2%	*Ayah 6SX1-ST-2D solar CFIA* Practical mMol.L^{-1} conc. in 25 ml		*UV SP.* conc. in 25 ml	
		0	0.4	0.6	0.8	1			in 100ml	in 25 ml	In 100ml (1mMol.L^{-1})	In 100ml (0.1 Mol.L^{-1})
1	Ponstidin, 500 mg, SDI ,Iraq	140	237.33	302.66	374.66	468	123.3132±55.17+323.6013±83.97[MFA]mMol.L^{-1}	0.9901 0.9804 98.04%	0.381		0.947	0.038
		0.365	0.710	1.010	1.260	1.642	0.3438±0.1027+9.0767±1.1872[MFA]mMol.L^{-1}	0.9974 0.9949 99.49%	95.266		94.693	
2	Piostan, 500 mg, pioner , Iraq	142	233.2	299.33	390	475.33	120.7821±66.59+334.2675±101.34[MFA]mMol.L^{-1}	0.9866 0.9734 97.34%	0.361		1.097	0.044
		0.409	0.762	1.140	1.270	1.700	0.4000±0.0735+9.1136±0.0485[MFA]mMol.L^{-1}	0.9986 0.9974 99.74%	90.333		109.726	
3	Ponstan, 500 mg, Pfizer, USA	152	243	309	400	485	130.7297±66.79+334.0540±101.63[MFA]mMol.L^{-1}	0.9865 0.9732 97.32%	0.391		0.980	0.039
		0.385	0.972	1.390	1.630	2.000	0.4497±0.1673+11.467±1.931[MFA]mMol.L^{-1}	0.9958 0.9917 99.17%	97.836		98.042	
4	Ponamec, 500mg, MVC , India	132	238.33	316	378	453	123.1429±28.64+322.0054±43.58[MFA]mMol.L^{-1}	0.9972 0.9946 99.46%	0.382		0.920	0.037
		0.365	0.775	1.110	1.357	1.755	0.3626±0.07+9.8575±0.8075[MFA]mMol.L^{-1}	0.9989 0.9980 99.80%	95.606		91.960	

$\hat{Y}_i^*$= estimated value for absorbance, r= Correlation coefficient , r^2= coefficient of determination (C.O.D), r^2%= Linearity percentage

Table 11-Summary of data for paired t-test, practical content and efficiency of determination of MFA in four samples of pharmaceutical preparation.

Sample no	Confidence interval for the average weight of tablets $\bar{w}_i \pm 1.96\ \sigma n\text{-}1/\sqrt{n}$ at 95% (g)	Theoretical content for the active ingredient $w_t \pm 1.96\ \sigma n\text{-}1/\sqrt{n}$ at 95% (mg)	Sample weight equivalent to 2.41g (100 mMol.L^{-1})of the active ingredient at 95% w_i (g)	Practical content of active ingredient		Efficiency of determination (Rec%)		Paired t-test		
				In 100 ml of sample $\bar{W} i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	in tablets $\bar{W} i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95% (mg)			Individual comparison ($\bar{W}_i - \mu_0)\sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-ST-2D Solar cell-CFI Analyser with Quoted value $t_{0.05/2,2}$=4.303	Comparison between two method	
				Ayah 6SX1-ST-2D solar CFIA (mV)					Xd	$\bar{Xd}$ (σ_{n-1})	$t_{cal} = \bar{Xd}\sqrt{n}/\sigma_{n-1}$ at 95 %
				UV SP. (classical method for absorbance measurement)							
1	0.5952±0.0012	500±1.0081	2.8688	2.296±0.045	476.342±9.336	95.27%		-10.904 ≪ 4.303	2.87	-19.23 52.493	| -0.733 | ≪ 3.182
				2.282±0.057	473.476±11.827	94.70%					
2	0.7158±0.0024	500±1.6764	3.4501	2.177±0.030	451.674±6.224	90.33%		-33.410 ≪ 4.303	-96.97		
				2.644±0.052	548.640±10.790	109.73%					
3	0.7035±0.0063	500±4.4776	3.3908	2.358±0.042	489.189±8.713	97.84%		-5.339 ≪ 4.303	-1.03		
				2.363±0.047	490.221±9.750	98.04%					
4	0.9083±0.0061	500±3.3579	4.3780	2.304±0.055	478.032±11.411	95.61%		-8.284 ≪ 4.303	18.23		
				2.216±0.206	459.803±42.743	91.96%					

Xd: Difference between two method , $\bar{Xd}$: difference mean , σ_{n-1}:Difference standard deviation , n=3 for individual & n=4 for comparison between two methods.

Conclusion

The suggested method is simple, sensitive and rapid. Application of the proposed method to the analysis of mefenamic acid in pure and pharmaceutical preparation based on formation of blue color precipitate as an ion- pair compound for the reaction of mefenamic acid with phosphomolybdic acid in aqueous medium . It was shown that with no doubt that newly developed method is good as the classical method. An alternative analytical method is found through this research work, which based on simple parameter conditions.

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