

DETERMINATION OF MEFENAMIC ACID USING CE(IV)SULFATE AS AN OXIDANT REAGENT VIA THE USE OF THE 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 via turbidimetric measurement (0-180°) by Ayah 6SX1-ST-2D Solar cell CFI Analyser. The method was based on the reaction of Ce(IV) Sulfate with mefenamic acid in aqueous medium to form bluish green color precipitate for the ion-pair complex. Turbidity was measured via the reflection of incident light that collides on the surface precipitated particles at 0-180°. Chemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 0.3-7 mMol.L⁻¹, with correlation coefficient $r = 0.9954$. The limit of detection 7.35 µg/sample from the step wise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.2% for 1,5 mMol.L⁻¹ (n=6,10 respectively) concentration of mefenamic acid. The method was successfully applied to the determination of mefenamic acid in four pharmaceutical drugs. A comparison was made between the newly developed method analysis with the classical method in addition to between four different pharmaceutical preparations (UV- Vis spectrophotometry at wave length 465nm) using the standard addition method via the use of t-test. It was noticed that there was no significant difference between two methods at 95 % confidence level & no significant difference for drugs (Ponstidin - SDI- Iraq and Ponamec-Mvc - India) and significant difference for drugs (piostan- pioner- Iraq and ponstan - Pfizer-USA).

Keywords: Mefenamic acid, Flow injection analysis, Turbidity.

INTRODUCTION

Mefenamic acid (MFA) [2-(2,3-dimethyl phenyl)amino] benzoic acid, is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic and anti-pyretic properties¹. And it is used in the treatment of rheumatoid arthritis and osteoarthritis and other musculoskeletal diseases. The structure of mefenamic acid is shown in Figure 1.

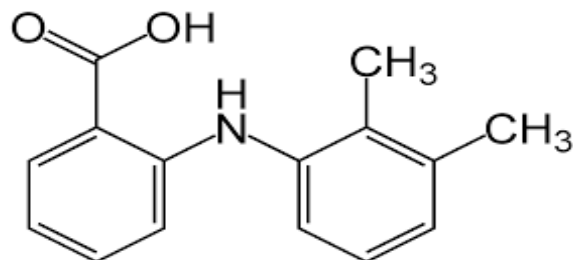


Fig. 1: Chemical structure of mefenamic acid

MFA is a NSAID used to treat pain, including menstrual pain. MFA decreases inflammation (swelling) and uterine contractions and is mainly due to the inhibition of prostaglandin synthesis. This medication may interact with other blood pressure medications or other anti inflammatory drugs. It may also affect lithium, methotrexate, rifampin and anticoagulants. MFA is available as white to off-white, crystalline powder that darkens on prolonged expose to light , it melts at 227 – 232 C⁰ , which is insoluble in water; sparingly soluble in chloroform and ether , and soluble in 0.1M NaOH ^{2,3}.

The side effect of mefenamic acid is known to cause an upset stomach, therefore it is recommended to take prescribed doses together with food or milk. Instances of drowsiness may also occur. As such, it is recommended to avoid driving or consuming alcohol while taking this medication. Other known mild side effects of mefenamic acid include, nervousness, head ache and vomiting. Serious side effects may include bloody vomit, diarrhoea, blurred vision, skin rash, itching and swelling, sore throat and fever. A number of analytical methods have been developed for the quantitative determination of mefenamic acid in dosage forms and in biological samples. Among those are spectrophotometry⁴⁻⁷, chromatography⁸⁻¹⁰, titration methods¹¹, chemiluminescence¹², and electrochemical sensors¹³⁻¹⁵.

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 determination of mefenamic acid in pharmaceutical formulation. The method based on the formation of bluish green color precipitate as an ion-pair compound by Ce(IV)Sulfate with mefenamic acid in aqueous medium. The turbidimetry is measured via reflection of incident light from the surfaces of precipitate particles at 0-180°. The positive signal from reflection recorded by Ayah 6SX1-ST-2D Solar cell supplier with linear array of six snow-white light emitting diode as a source & two solar cells as a detector.

EXPERIMENTAL

REAGENTS AND CHEMICALS

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions . A standard solution (0.1 Mol.L⁻¹) of Mefenamic acid C₁₅H₁₅NO₂ (241 g. mol⁻¹) was prepared by dissolving 2.41 g in 100 ml 0.1M NaOH. A stock solution (0.1 Mol.L⁻¹) of Ce(IV)SulfateCe(SO₄)₂.(332.298 g.mol⁻¹ , Hopkin & Williams) was prepared by dissolving 8.3075g in 250 ml of H₂SO₄ (1Mol.L⁻¹), a 1Mol.L⁻¹ of sulfuric acid solution (98% w/w, 1.84 g.ml⁻¹,BDH) was prepared by pipetting 13.6 ml of concentrated sulfuric acid and dilute to250 ml volumetric flask ,which standardized against standard solution of 1Mol.L⁻¹from Na₂CO₃(BDH,105.99 g.mol⁻¹); which prepared by dissolving10.60 g in 250 ml distilled water Sodium hydroxide solution (NaOH, 40, BDH,0.1Mol.L⁻¹) was prepared by dissolving 0.4g 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) 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 M 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 M 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 uses a six snow-white light emitting diode LEDs for irradiation of the flow cell at 2 mm path length . Two solar cell used as a detector for collecting signals via sample travel for 60 mm length . The readout of the system composed of x-t potentiometric recorder (Kompensio Graph C-1032) Siemens (Germany), this

recorder measured by (1-500) 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.

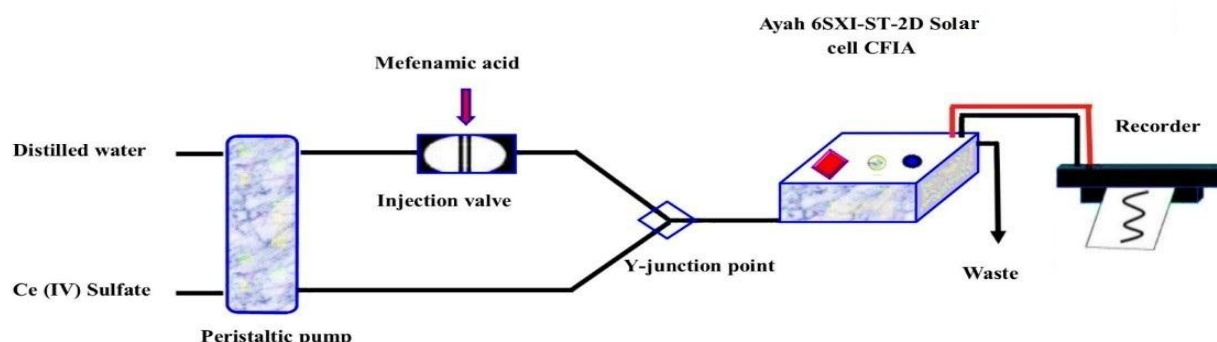
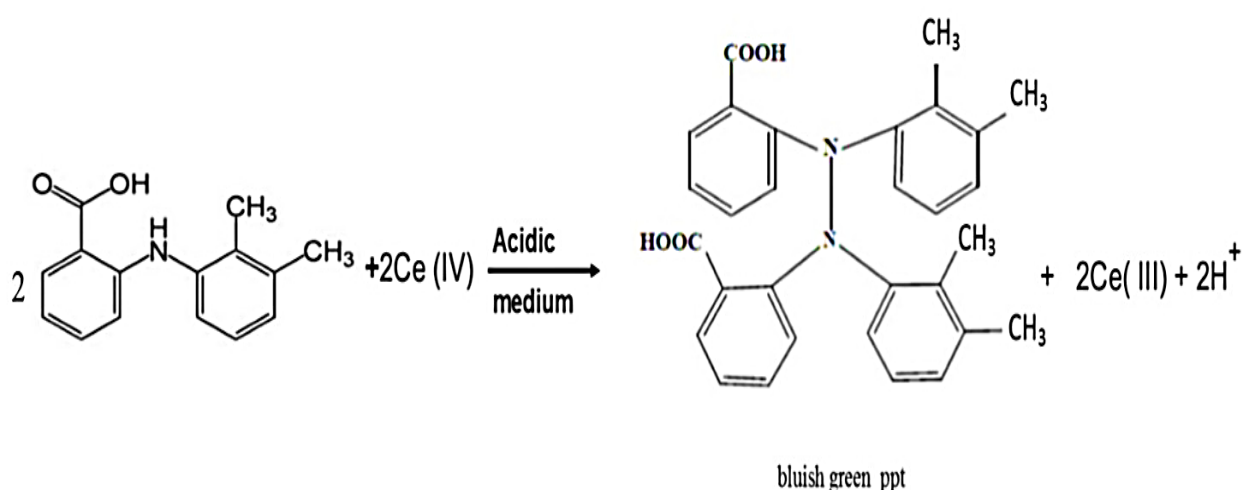


Fig. 2: Flow diagram manifold system used for the determination of MFA

Methodology

The flow system consisting of two lines was used for the determination of MFA by the reaction between MFA with Ce(IV) Sulfate (0.1 mMol.L^{-1}) in aqueous medium to form a bluish green color precipitate as an ion pair complex form. The first line represent the carrier stream (Distilled water) at 1.3 ml.min^{-1} flow rate which lead to the injection valve to carry MFA, sample volume $122 \mu\text{l}$; while the second line supplies Ce(IV) Sulfate solution at 1.7 ml.min^{-1} . Both lines meet at a Y-junction, with an outlet for reactants product from complex, which passes through a homemade Ayah 6SXI-ST-2D solar cell CFI Analyser that work with a six snow white light emitting diodes LEDs will be used as a source. Each solution injected was assayed in three time. 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 $0-180^\circ$. A probable mechanism of ion pair formation for MFA - Ce(IV) Sulfate system is represented in scheme 1.



Scheme. 1: Proposed mechanism of reaction between of MFA & Ce(IV) Sulfate

RESULTS AND DISCUSSION

Study of the Optimum Parameters

The chemical parameters such as (concentration of reagents used for the precipitation reaction and pH of the reaction medium), while the physical parameters (intensity of incident light, flow rate, sample volume, purge time) were investigated.

Chemical Variables

Ceric(IV) Sulfate Concentration

A study was conducted to optimize concentration of Ceric(IV) Sulfate. A series of reagent solution (Ceric(IV) Sulfate) having the concentrations ranging from 0.02 to 1 mMol.L⁻¹, 5 mMol.L⁻¹ constant concentration of mefenamic acid was prepared, intensity of incident light of LEDs 2.1 V, flow rate of carrier stream line and reagent line 1.7 ml.min⁻¹ and 2.1 ml.min⁻¹ respectively with 100 μl sample volume. Each measurement was repeated for three times. It was found that 0.1 and 0.3 mMol.L⁻¹ of Ceric(IV) Sulfate was the most suitable for a maximum reflection of incident light, more than 0.3 mMol.L⁻¹ leads to decrease in the response. This might be due to increase in amount of precipitate particle in front of the solar cell, which prevent light arrival to the detector ; this causes a decrease in peak height as shown in **figure 3 A,B**. Therefore, 0.1 mMol.L⁻¹ (Ceric(IV) Sulfate) was selected as an optimum concentration than 0.3 mMol.L⁻¹ to reduce reagent consumption. The data obtained were summarized in **table 1**.

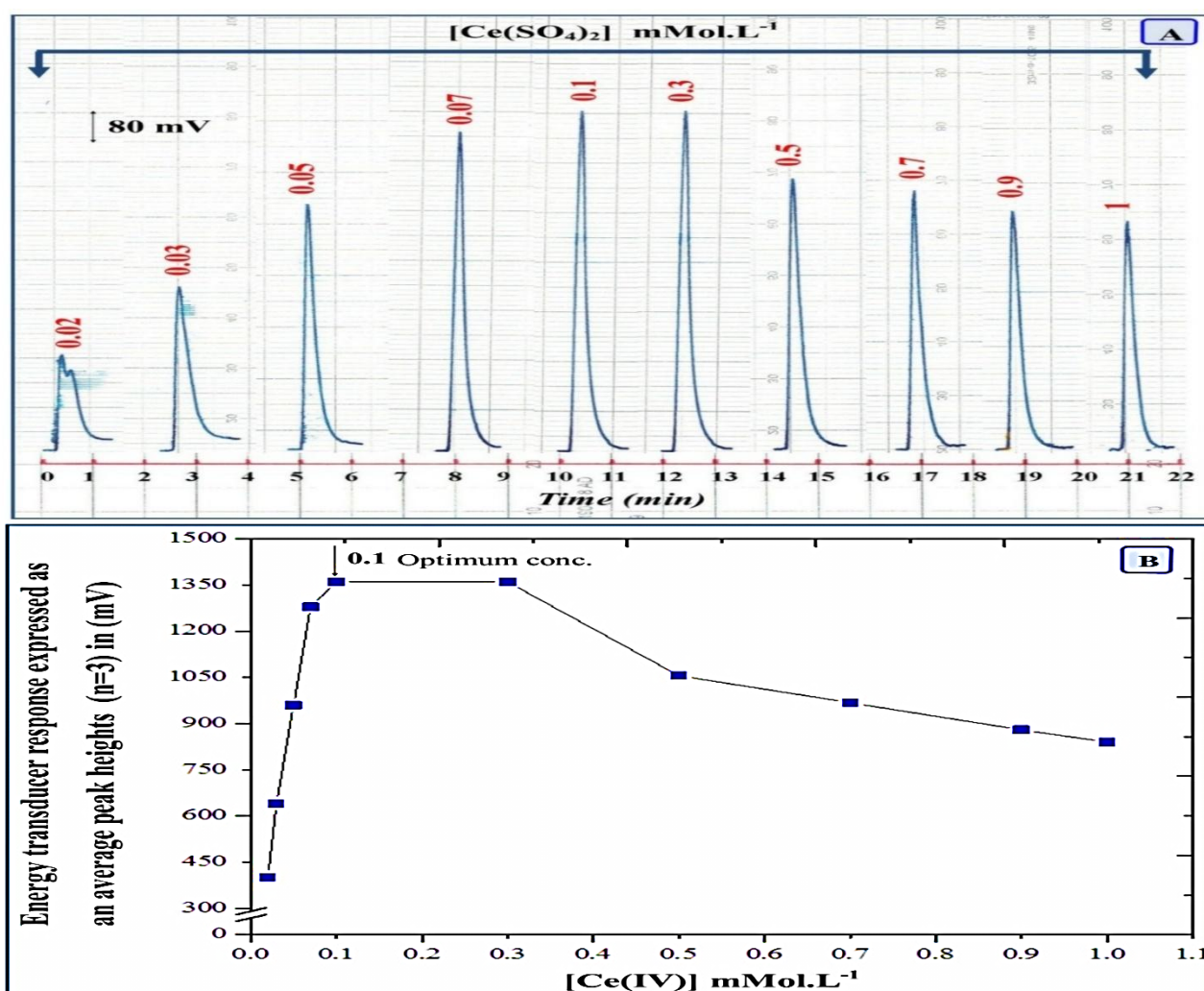


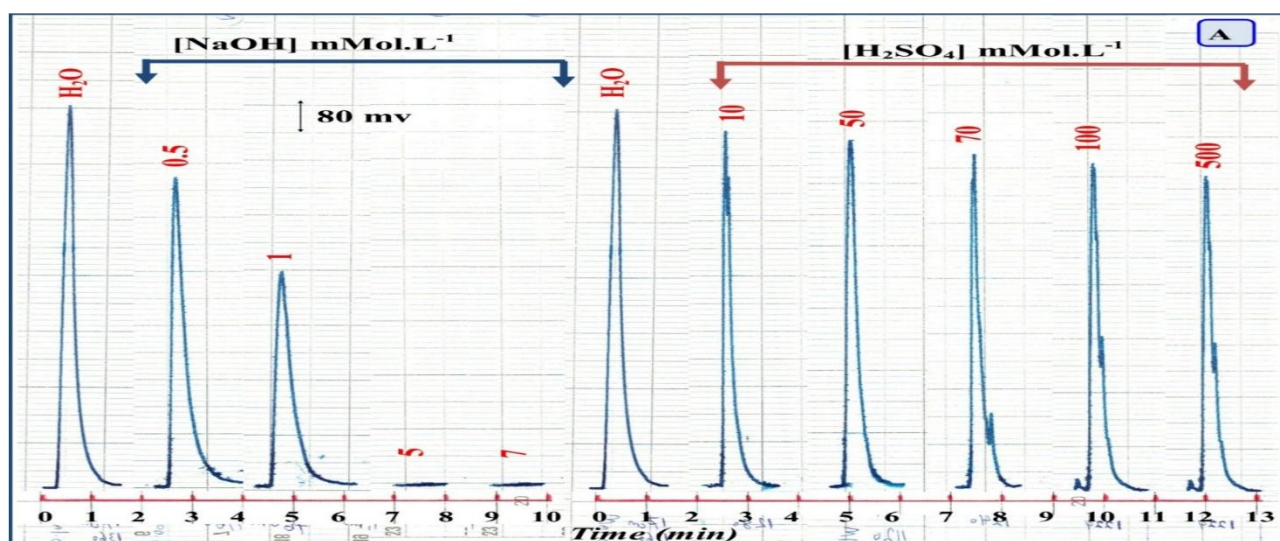
Fig. 3: Effect of the [Ceric(IV) Sulfate] on:
(A): Peak height of the energy transducer response by reflection of incident light
(B): Profile versus time using variation of [Ceric(IV)]

Table 1: Effect of (Ceric(IV) Sulfate) concentration on the measurement of energy transducer response for the determination of MFA.

[Ce(SO ₄) ₂] mMol.L ⁻¹	Energy transducer response expressed as an average peak heights (n=3) \bar{y}_i in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1}/\sqrt{n}$
0.02	400	0.05	400 ± 0.497
0.03	640	0.03	640 ± 0.477
0.05	960	0.03	960 ± 0.715
0.07	1280	0.02	1280 ± 0.636
0.1	1360	0.02	1360 ± 0.676
0.3	1360	0.03	1360 ± 1.014
0.5	1056	0.03	1056 ± 0.787
0.7	968	0.02	968 ± 0.481
0.9	880	0.02	880 ± 0.437
1	840	0.02	840 ± 0.417

Effect of acidic & basic media

The ion pair of MFA (5 mMol.L⁻¹)- Ceric (IV)Sulfate(0.1 mMol.L⁻¹) system was studied in different concentration of NaOH (0.5,1,5,7) mMol.L⁻¹, H₂SO₄(10,50,70,100,500) mMol.L⁻¹ in addition to the aqueous medium as a carrier stream, 1.7ml.min⁻¹ flow rate for the carrier stream and 2.1 ml.min⁻¹ flow rate for the reagent, 100µl sample volume. All responses profile were shown in figure 4A. The data obtained were plotted in figure 4B. It can be seen that an increase in sensitivity of response in aqueous medium as a carrier stream. In spite of the use of acidic medium to dissolve Ce(IV) sulphate, but, any increase in acidic medium i.e: pH< 6.53 might lead to dissolution of precipitate that is resulted from the oxidation of the drug due to cerium(IV) and on this basis, it was noticed that a decrease in the height of the profile from the reflection of light vs time was distorted. While the use of increase basicity (pH>6.53) might lead to neutralization of acidic medium and the return of Ce(IV) to original form (salt form). Therefore a return to the aqueous media i.e: the use of distilled water as a carrier stream is the most suitable for completion reaction between MFA -Ce (IV)Sulphate. Table 2 summarized the results obtained.



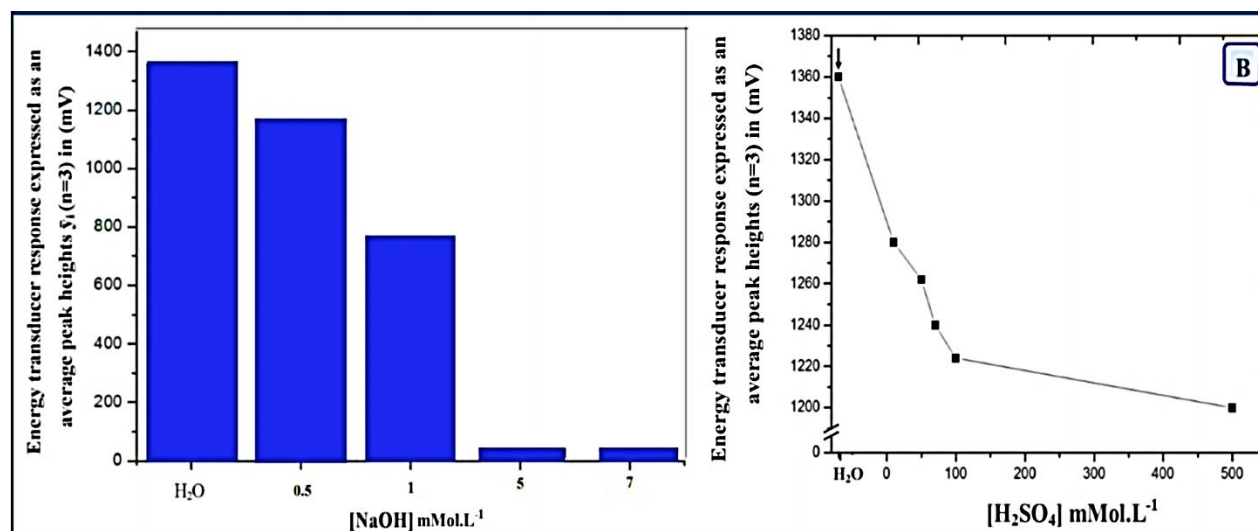


Fig. 4: Effect of the acidic & basic media on the:
 (A): Profile versus time for variation of [NaOH] & [H₂SO₄].
 (B): Peak height of the energy transducer response at MFA (5mMol.L⁻¹)-Ce(IV) Sulphate (0.1mMol.L⁻¹) system.

Table 2: Effect of variation of acidic & basic media on the measurement of energy transducer response for determination of MFA

Type of medium	PH	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 ₂ O	6.53	1360	0.02	1360 ± 0.676
[NaOH] mMol.L ⁻¹	0.5	1160	0.05	1160 ± 1.441
	1	760	0.03	760 ± 0.566
	5	0	0	0 ± 0
	7	0	0	0 ± 0
	[H ₂ SO ₄] mMol.L ⁻¹	10	1280	0.04
50		1262	0.03	1262 ± 0.941
70		1240	0.03	1240 ± 0.924
100		1224	0.02	1224 ± 0.608
500		1200	0.03	1200 ± 0.894

Physical Variables

Flow rate

Using optimum concentration of MFA (5mMol.L⁻¹)-Ceric(IV) Sulfate(0.1mMol.L⁻¹) system and sample volume of 100 µl was used at a variable flow rate (0.3-2.6 mL.min⁻¹), (0.4-3.4 mL.min⁻¹) for carrier stream and reagent line respectively, in addition to 2.1 V is the intensity of light emitting diodes (LEDs). In this study observed that at slow flow rate a base width (Δt_B) of the response was increased as shown in figure 5 A,B. This might be attributed to the increase in the dispersion (due to diffusion) of sample segment, which in turn leads to the relatively longer duration of the precipitate particle segment in front of the detector. While at high speed of flow rate more than 1.3 mL.min⁻¹, peak heights decrease but its sharp maxima and regular response, this probably due to increase in physical variable mostly dilution and dispersion due to convection that will affect on precipitate particles segment. Therefore 1.3 mL.min⁻¹ & 1.7 mL.min⁻¹ for carrier stream and reagent respectively, the optimum flow rate was used as a compromise to obtain a regular response, narrower (Δt_B), minimize the consumption of reactions solutions and to complete precipitation of MFA -Ceric(IV) Sulfate as ion pair. Results were summarized in table 3.

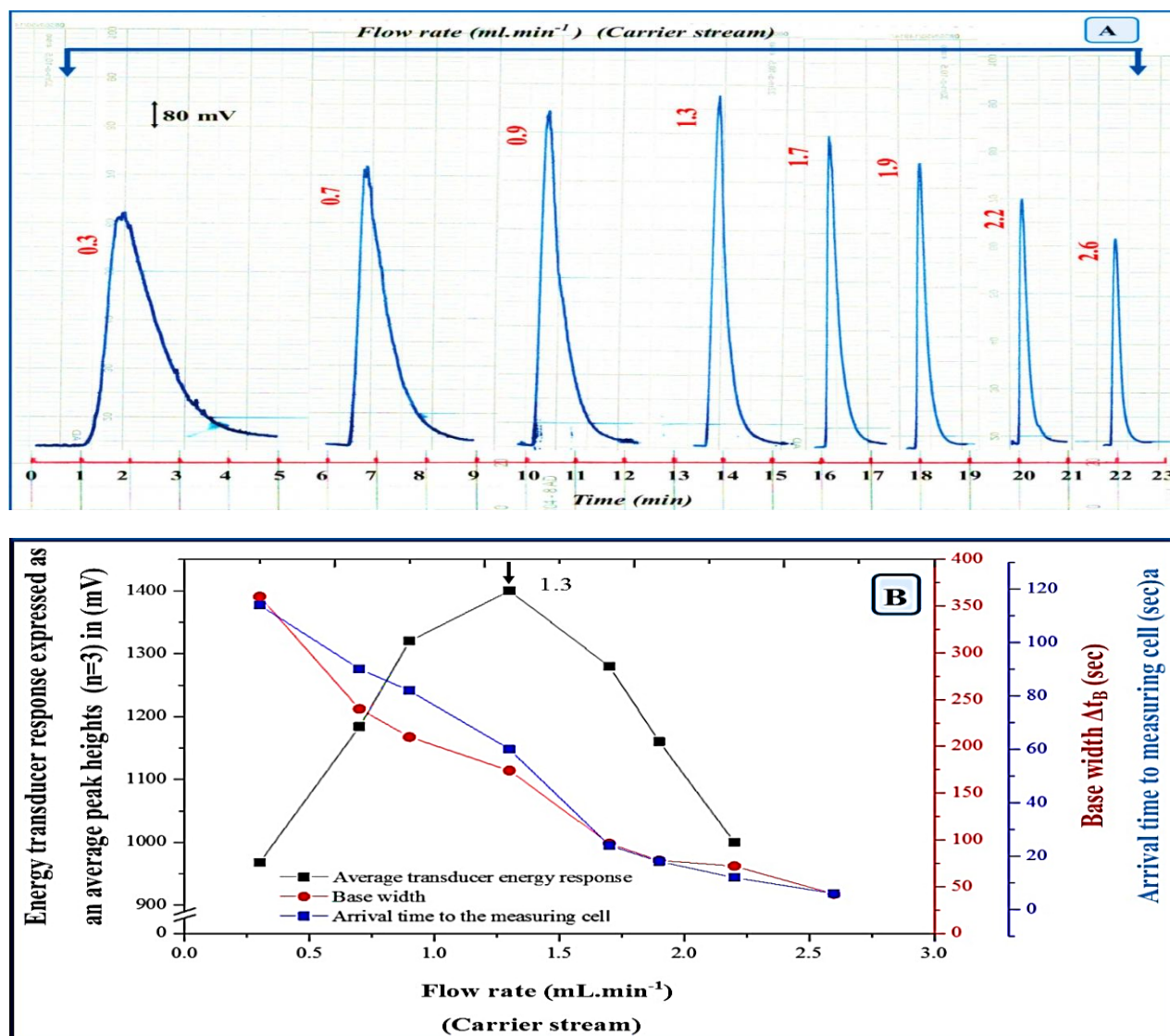


Fig. 5: Effect of the variation of flow rate on:
 (A): profile versus time
 (B): Variation of energy transducer response , Base width and Departure time for sample segment from injection valve to the measuring cell.

Table 3: Effect of the variation of flow rate on the measurement of energy transducer response via reflection of incident light for determination of MFA using (5 mMol.L⁻¹)-Ceric(IV) Sulfate(0.1 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	968	0.05	968 ± 1.202	360	114	4.300	0.116
10	0.7	0.8	1184	0.05	1184 ± 1.471	240	90	6.100	0.082
15	0.9	1.3	1320	0.06	1320 ± 1.968	210	82	7.800	0.064
20	1.3	1.7	1400	0.07	1400 ± 2.435	174	60	8.800	0.057
25	1.7	2.1	1280	0.03	1280 ± 0.954	96	24	6.180	0.081
30	1.9	2.6	1160	0.03	1160 ± 0.865	78	18	5.950	0.084
35	2.2	2.8	1000	0.02	1000 ± 0.497	72	12	6.100	0.082
40	2.6	3.4	872	0.05	872 ± 1.083	42	6	4.300	0.116

Δt_B (sec): Base width of response

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

Sample volume

Using MFA (5mMol.L^{-1})-Ce(IV) sulfate (0.1mMol.L^{-1}) system and variable sample volumes ($77\text{-}122\ \mu\text{l}$) were used, flow rate ($1.3, 1.7\ \text{ml.min}^{-1}$) for carrier stream and reagent respectively, $2.1\ \text{V}$ applied voltage to the LEDs. The plot of change in sample volume vs. reflection of incident light and Δt_B is shown in **figure 6 A,B**. It was found that an increase in sample volume led to a significant increase in sensitivity, more perceptible than low sample volume. Therefore, $122\ \mu\text{l}$ was selected as an optimum sample volume. The results were tabulated in **table 4**, which shows an increase in the injection volume leading to a significant increase in sensitivity (increase in peak height), which might be attributed to decrease effect of physical parameters on precipitate particles segment.

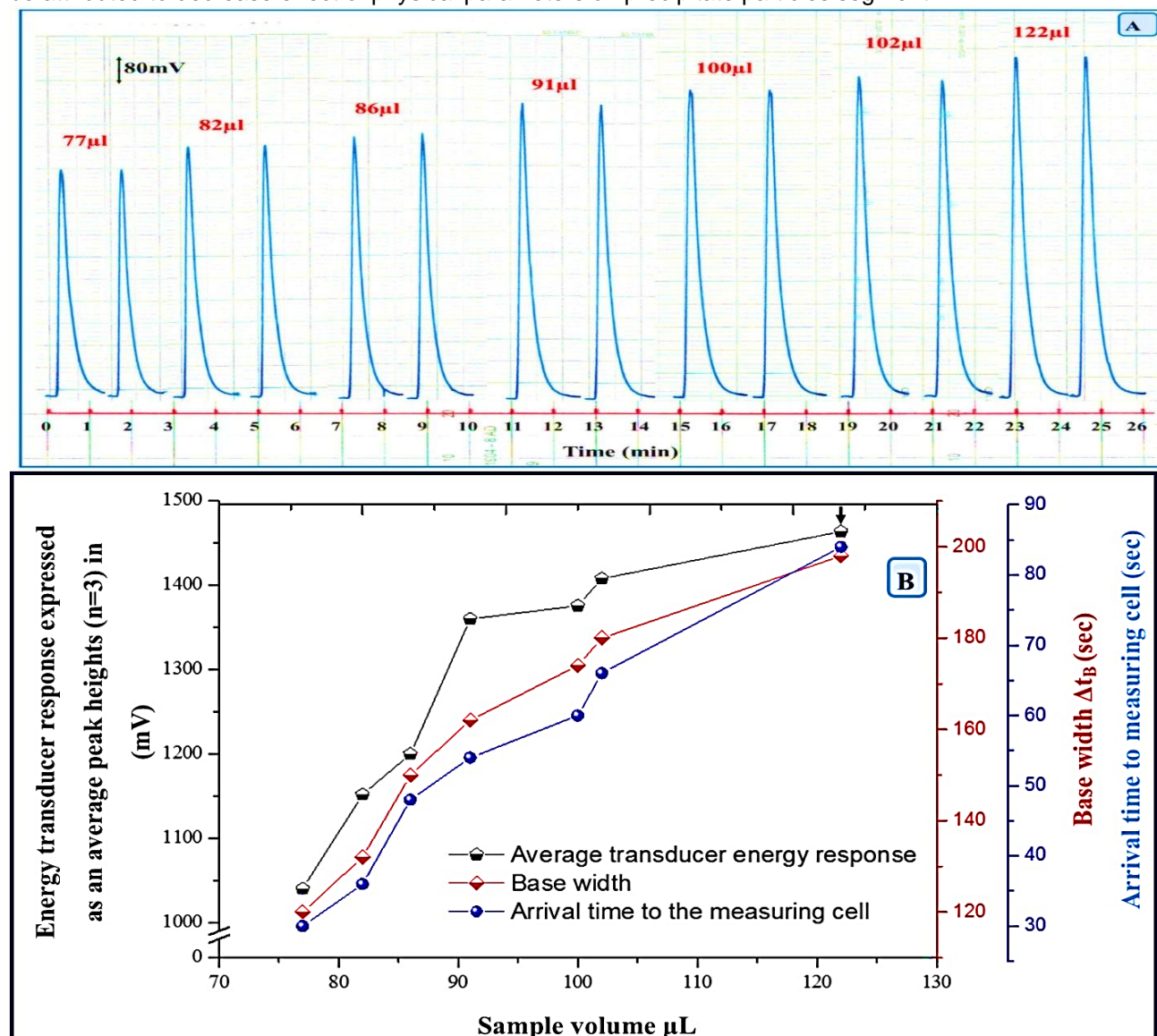


Fig. 6: Effect of the variation of sample volume on:
(A): Profile versus time

(B): Variation of energy transducer response using optimum parameters

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 (5 mMol.L⁻¹)- Ceric(IV)sulfate (0.1 mMol.L⁻¹) system.

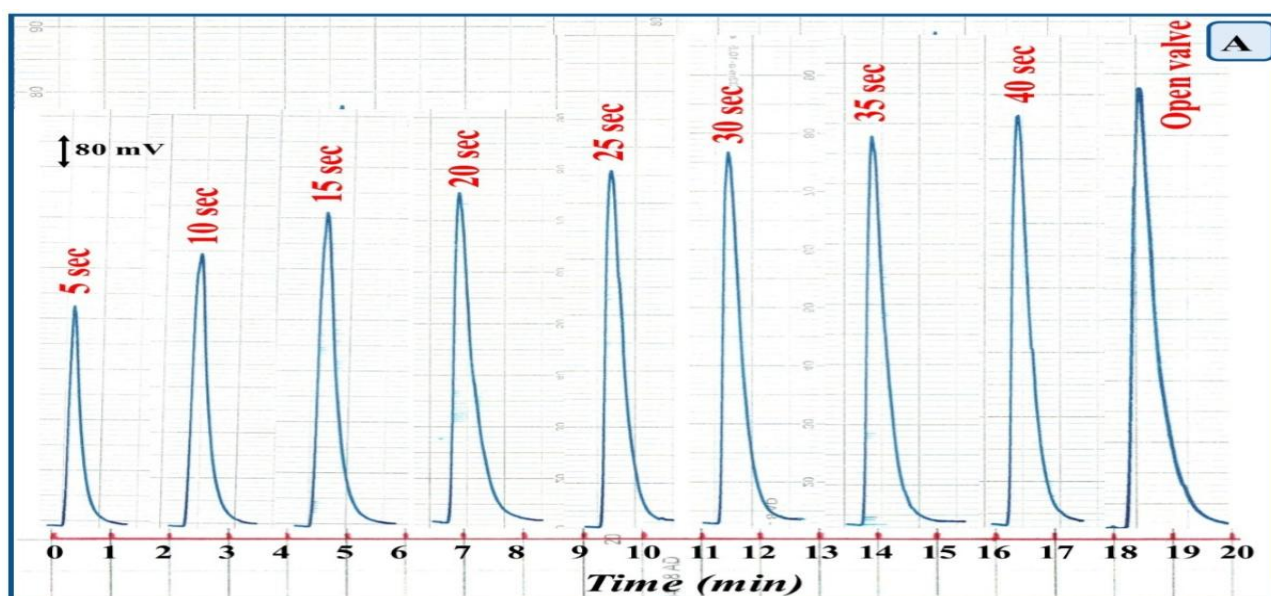
Sample volume μl	Energy transducer response expressed as an average peak heights (n=3) \bar{y}_i in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$	Base width Δt_B (sec)	t^* (sec)
77	1040	0.02	1040 ± 0.517	120	30
82	1152	0.03	1152 ± 0.859	132	36
86	1200	0.03	1200 ± 0.894	150	48
91	1360	0.04	1360 ± 1.351	162	54
100	1376	0.04	1376 ± 1.367	174	60
102	1408	0.02	1408 ± 0.700	180	66
122	1464	0.01	1464 ± 0.364	198	84

Δt_B : Base width of response

t^* = Departure time for sample segment from injection valve to the measuring cell

Purge time

Using optimal parameter that were achieved in the previous sections, MFA (5mMol.L⁻¹)-Ceric(IV)Sulfate (0.1mMol.L⁻¹)system , flow rate (1.3,1.7) ml.min⁻¹ for carrier stream and reagent respectively and 122 μl sample volume. Using variable purge time from (5 – 40) sec in addition to open valve as an allowed permissible time for the carrier stream to continuous passing through the injection valve to carry the sample segment(inject mode) through the injection valve reaching to the measuring cell, It can be seen from **figure 7A,B**there is an increase in the response with increasing the purge time .While the decrease in response when using less than open valve, this might be attributed to the incomplete purge of the sample from sample loop in the injection valve .All results were tabulated in **table 5**.



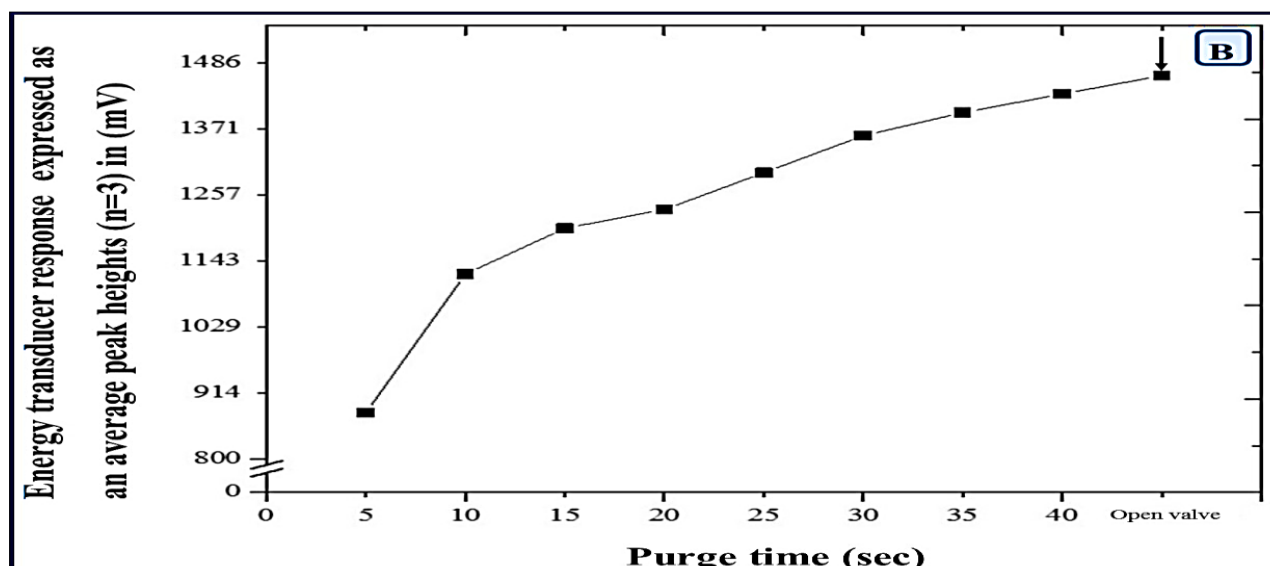


Fig. 7: Effect of the variation of purge time on:

(A): Response profile versus time

(B): Energy transducer response using 122 μ l & 2.1 volt applied voltage

Table 5: Effect of the variation of purge time on the energy transducer response at 95% confidence level

Pu 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	880	0.02	880 \pm 0.437
10	1120	0.03	1120 \pm 0.835
15	1200	0.01	1200 \pm 0.298
20	1232	0.06	1232 \pm 1.836
25	1296	0.02	1296 \pm 0.644
30	1360	0.05	1360 \pm 1.689
35	1400	0.04	1400 \pm 1.391
40	1432	0.05	1432 \pm 1.779
Open valve	1464	0.02	1464 \pm 0.727

Intensity of light

Intensity of light source was studied using 0.1 mMol.L⁻¹ of Ceric (IV)Sulfate; while 122 μ l sample volume of 5 mMol.L⁻¹ mefenamic acid, flow rate for carrier stream line 1.3 mL.min⁻¹ and 1.7 mL.min⁻¹ for reagent line. Variable intensity of incident light source was used ranging 0.53 – 2.1 V by variation of light intensity knob in front of the panel of Linear Array Ayah 6SX1-ST-2D Solar CFI Analyser whereread by AVO-meter. **Figure 8 A,B** shows that an increase in peak height with increase of intensity; Therefore 1.9 volt selected as the optimum voltage for six snow white light emitting diodes compared with 2.1v to preserved the life of LEDs. All results tabulated in **table 6**.

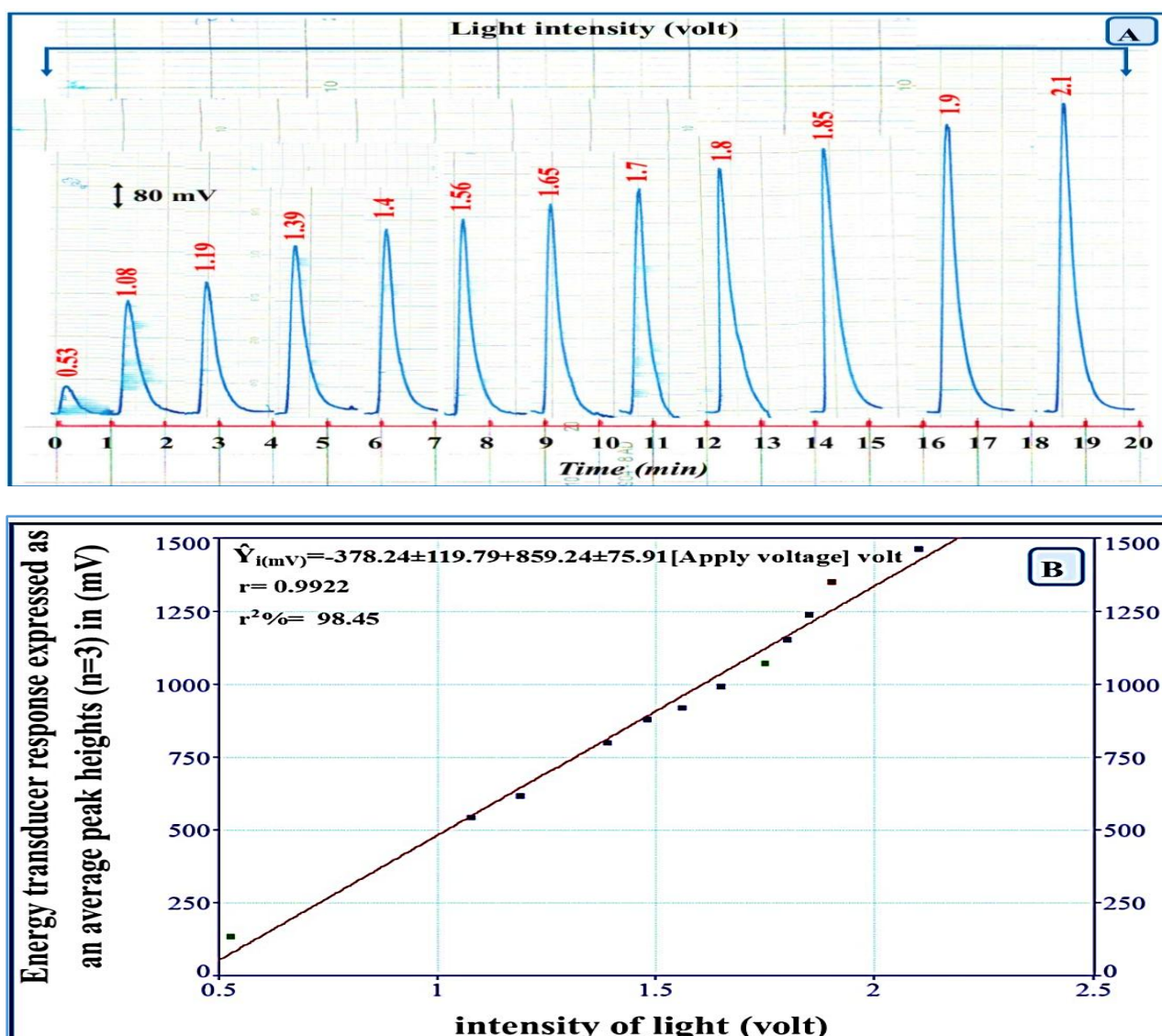


Fig. 8: Effect of the variation of light intensity on :
(A): Profile versus time

(B): Energy transducer response by reflection of incident light using optimum parameters.

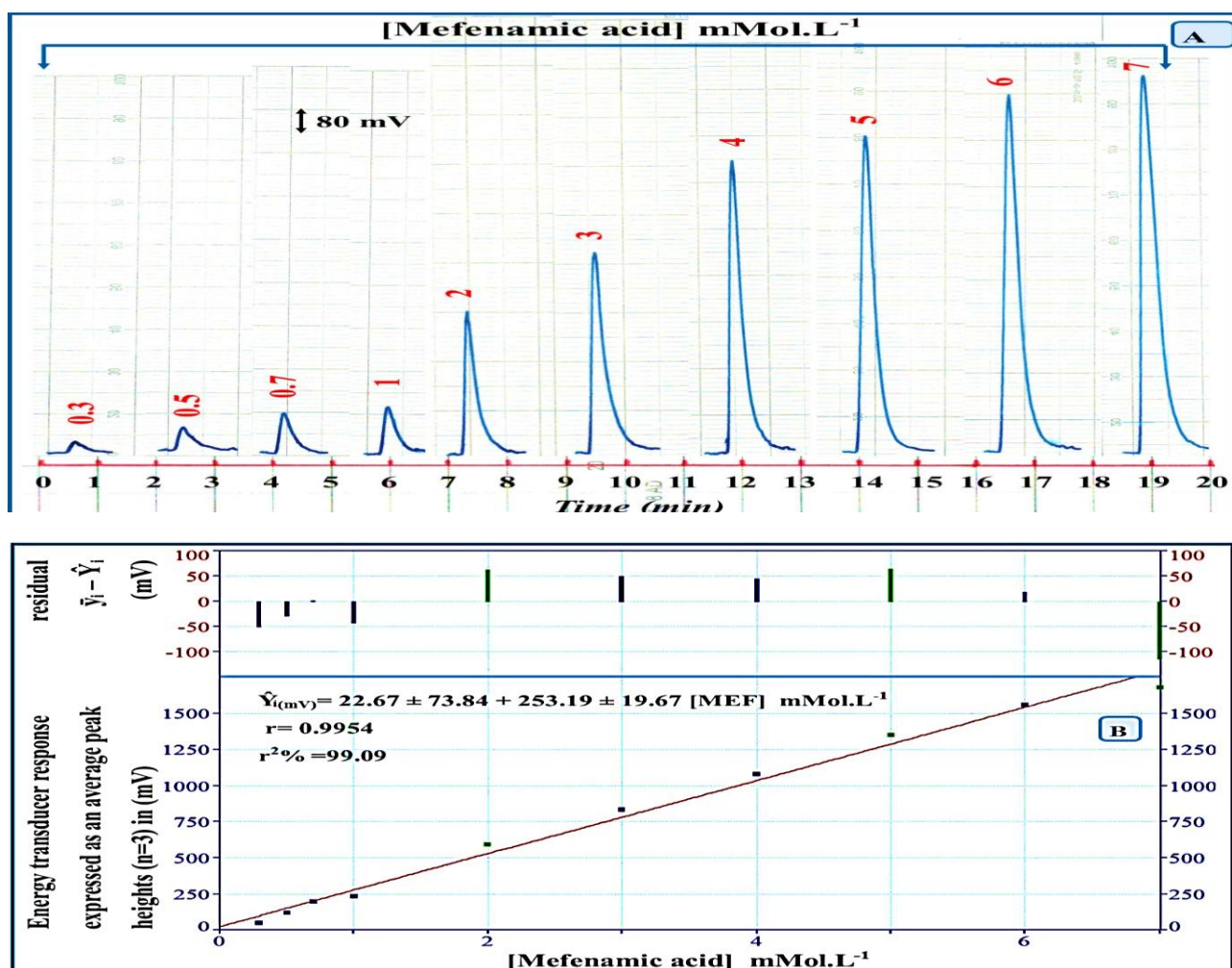
Table 6: Effect of intensity of light on the measurement of energy transducer response via reflection of incident light using 122 μ l, MFA (5 mMol.L⁻¹)-Ceric(IV)sulfate (0.1mMol.L⁻¹) system

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%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
0.53	136	0.24	136 \pm 0.811
1.08	544	0.08	544 \pm 1.081
1.19	616	0.08	616 \pm 1.224
1.39	800	0.03	800 \pm 0.596
1.48	880	0.03	880 \pm 0.656
1.56	920	0.07	920 \pm 1.600
1.65	992	0.02	992 \pm 0.493
1.75	1072	0.01	1072 \pm 0.266
1.8	1152	0.07	1152 \pm 2.003
1.85	1240	0.04	1240 \pm 1.232
1.9	1352	0.05	1352 \pm 1.679
2.1	1464	0.03	1464 \pm 1.091

Scatter plot calibration curve for variation of mefenamic acid concentration versus transducer energy response

Using optimum physical as well as chemical parameters achieved in previous section. A series of solutions of mefenamic acid ($0.3\text{--}13\text{ mMol.L}^{-1}$) were prepared. Each measurement was repeated three successive measurements. The responses profile for this study as shown in **Figure 9 A**. Energy transducer response of the average peak height (mV) was plotted against the concentration of mefenamic acid. A straight-line graph (**Figure 9 B**) from $0.3\text{--}7\text{ mMol.L}^{-1}$ with correlation coefficient (r): 0.9954 . Above 7 mMol.L^{-1} the value for correlation coefficient will decrease and deviate from linearity most probably due to accumulation of precipitate particulate which in turn leads to loss of some of the reflecting surface in addition to prevent the reflection of incident light pass to the solar cell detector.

While the UV-Vis spectrophotometric (classical method) at $\lambda_{\text{max}} = 465\text{ nm}$ [16], uses 5 mMol.L^{-1} of Potassium hexacyanoferrate (III) ($\text{K}_3[\text{Fe}(\text{CN})_6]$) which reacts with variable concentration of mefenamic acid to obtain colour species (orange). Calibration graph (**Figure 9 C**) was made to determine the concentration of mefenamic acid from $(0.6\text{--}10)\text{ mMol.L}^{-1}$ with correlation coefficient (r): 0.9786 at confidence level 95% and limit of detection was $7\text{ }\mu\text{Mol.L}^{-1}$. In addition to observe that the t -value of each method more than t_{tab} ($t\text{-value} \gg t_{\text{tab}}$) which indicates that the linearity against non-linearity is accepted. The obtained data are summed up in **Table 7** using first degree equation $\hat{y} = a + bx$ [17,18].



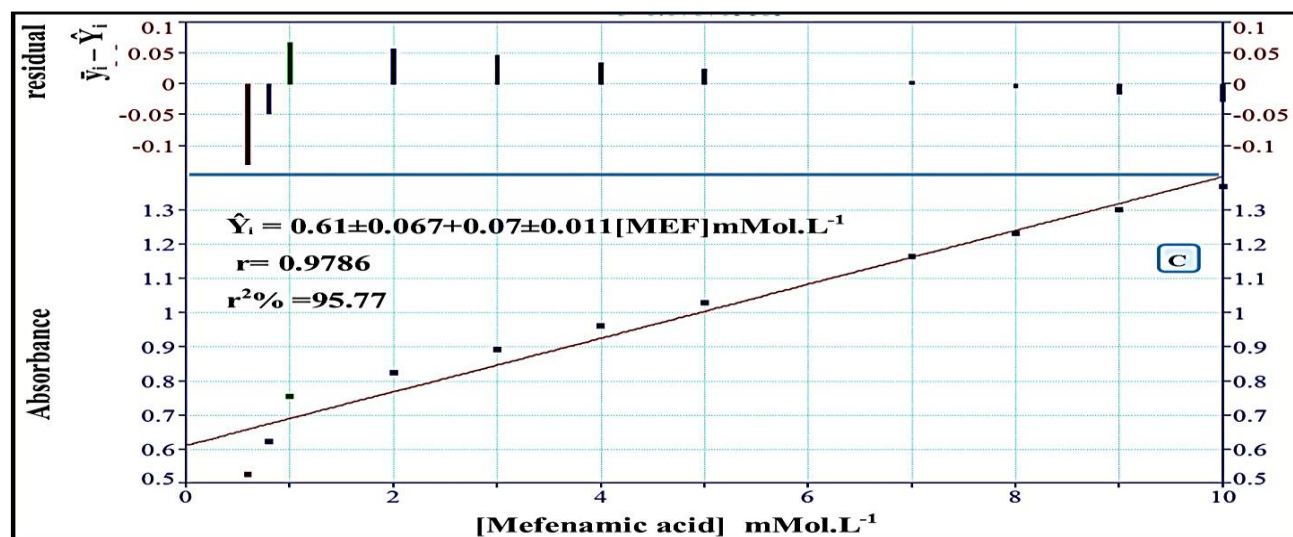


Fig. 9: Calibration graph for the variation of MFA concentration on:

(A): Profile verses time, (B): Energy transducer response expressed by linear equation using Ayah 6SX1-ST-2D Solar-CFI Analyser .

(C): Abs by linear equation using UV-Vis spectrophotometric using MFA-[Fe(CN)₆]³⁻ system, residual ($\bar{y}_i - \hat{Y}_i$), \bar{y}_i :practical value, \hat{Y}_i : estimate value.

Table 7: Summary of results for linearity regression analysis using Ayah 6SX1-ST-2D-Solar cell-CFIA and MFA-Ce(IV) Sulphate system and classical method depend on MFA-[Fe(CN)₆]³⁻ system using first degree equation

Type of method	Measured [MFA] mMol.L ⁻¹	n	Range of [MFA] mMol.L ⁻¹	$\hat{Y}_{i(mv)} = a \pm s_a + b \pm s_b t$ [MFA] mMol.L ⁻¹ At confidence level 95%, n-2	r r^2 $r^2\%$	t_{tab} at 95 % , n-2	Calculated t-value $\frac{r/\sqrt{n-2}}{\sqrt{1-r^2}}$	L.O.D from gradual dilution
				$\hat{Y}_i = a \pm s_a + b \pm s_b t$ [MFA] mMol.L ⁻¹ At confidence level 95%, n-2				
Ayah 6SX1-ST-2D solar cell CFI Analyzer	0.3-13	10	0.3-7	$22.67 \pm 73.84 + 253.19 \pm 19.67$ [MFA] mMol.L ⁻¹	0.9954 0.9909 99.09	2.306 << 29.672		250 μ Mol.L ⁻¹
UV-VIS Spectrophotometric	0.6-10	11	0.6-10	$0.61 \pm 0.067 + 0.07 \pm 0.011$ [MFA] mMol.L ⁻¹	0.9786 0.9577 95.77	2.262 << 14.283		7 μ Mol.L ⁻¹

\hat{Y}_i =estimate value, r = correlation coefficient, $r^2\%$ = Linearity percentage, r^2 = coefficient of determination (C.O.D),n: no. of measurement

Limit of detection (L. O. D)

Limit of detection for mefenamic acid calculated through three approaches :gradual dilution of lowest concentration in the calibration graph, detection based on the numerical value of slope and from the linear regression plot. All calculation value of detection limit for 122 μ L sample volume are tabulated in table 8

Table 8: Summary of limit of detection based on different approaches.

Practically based on the gradual dilution for the minimum concentration 0.25 mMol.L ⁻¹	Theoretical based on the value of slope $x = 3S_B / \text{slope}$ for n=13	Theoretical based on the linear equation $\hat{Y} = Y_B + 3S_B$
7.35 μ g/sample	34.84 ng/sample	21.81 μ g/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, \hat{Y} = estimated value

Repeatability

The relative standard deviation expressed as a percentage which is equally to the repeatability of the measurement. A repeated measurements for six and ten successive injections were measured at two concentrations of MFA (1,5) mMol.L⁻¹. Figure 10A,B is shown response profile of

repeatability at 1 and 5 mMol.L^{-1} respectively . The results obtained were tabulated in **table 9** which shows that the percentage relative standard deviation was less than 0.2%.

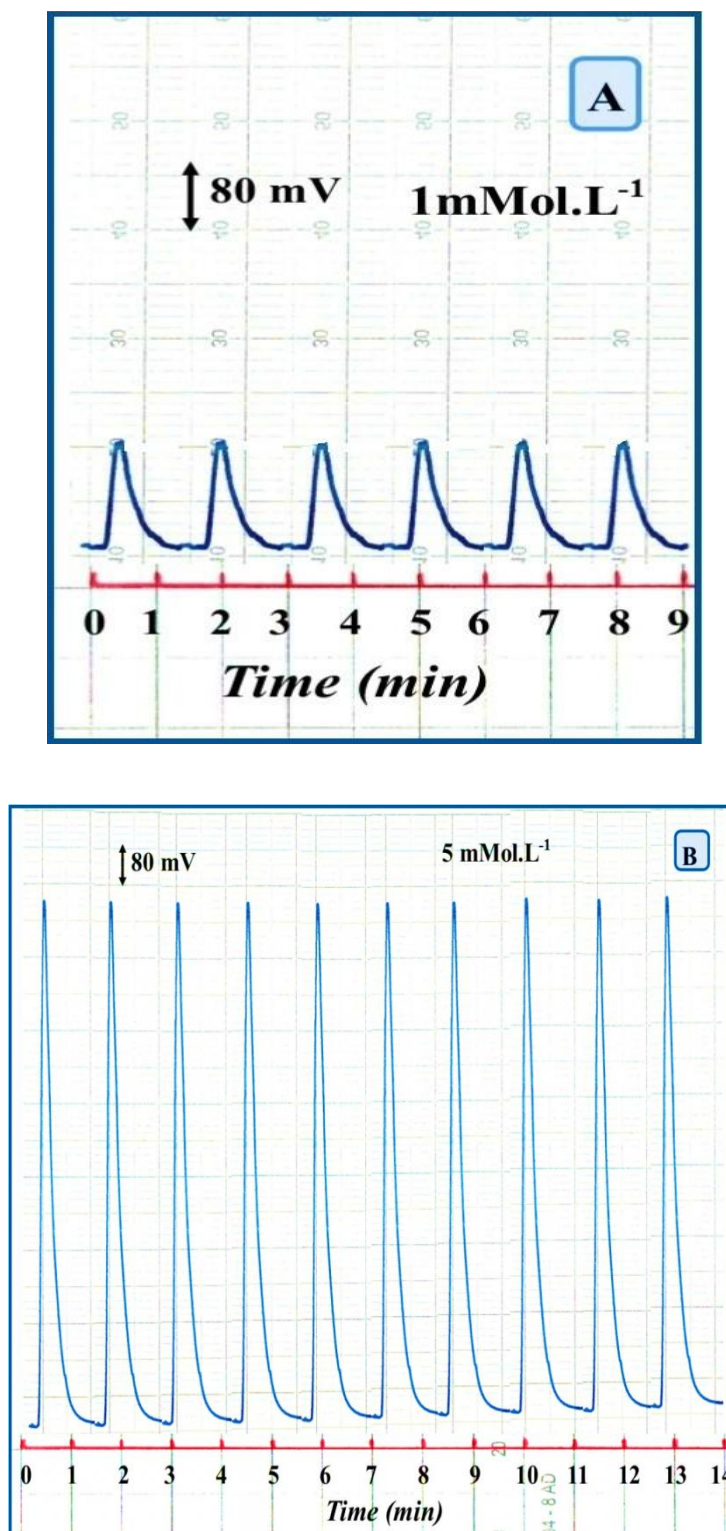


Fig. 10: Response profile -time of :
(A): six successive repeatable measurements of Mefenamic acid concentration (1mMol.L^{-1})
(B): ten successive repeatable measurements of Mefenamic acid concentration (5mMol.L^{-1})

Table 9: Repeatability of MFA at optimum parameters with 122µl sample volume

[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
1	232	0.15	232 ± 0.365	6
5	1400	0.04	1400 ± 0.401	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 preparation as an application

The established method was used for the determination of mefenamic acid in the four different drug manufactures (Ponstidin - 500 mg/SDI- Iraq, Piostan-500 mg/Pioner- Iraq, Ponstan- 500 mg/pfizer- USA and Ponamec-500 mg/Mvc – India). Using Ayah 6SX1-ST-2D Solar CFI Analyser and was compared with classical UV-Vis spectrophotometric method via the measurement of λ_{max} at 465 nm. A series of solutions were prepared of each pharmaceutical drug 0.1 Mol.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 Analyser , while transferring 0.2 ml from 0.1 Mol.L⁻¹ pharmaceutical drug 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 (0,0.2,0.25,0.5,1) ml in order to have (0,0.8,1, 2,4) mMol.L⁻¹ in the case of use classical UV-Vis spectrophotometric method . Flask no.1 is the sample . **Figure 11 A** shows the responses profile for this study .**Figure 11 B,C,D,E** shows standard addition calibration graphs using Ayah 6SX1-ST-2D solar cell –CFI Analyser. **Table 10** shows 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 :

First test

Comparison between quoted value (500 mg) with developed method (Ayah 6SX1-ST-2D Solar cell –CFI Analyser)

The assumption $H_0: \mu_0 (500) = \mu_i$ (i.e: the mean of any drug from different companies) that is accepted against $H_1: \mu_0 \neq \mu_i$ due to $t_{tab} (4.303)$ is more than t-value for drugs

(Ponstidin - SDI- Iraq and Ponamec-Mvc – India)

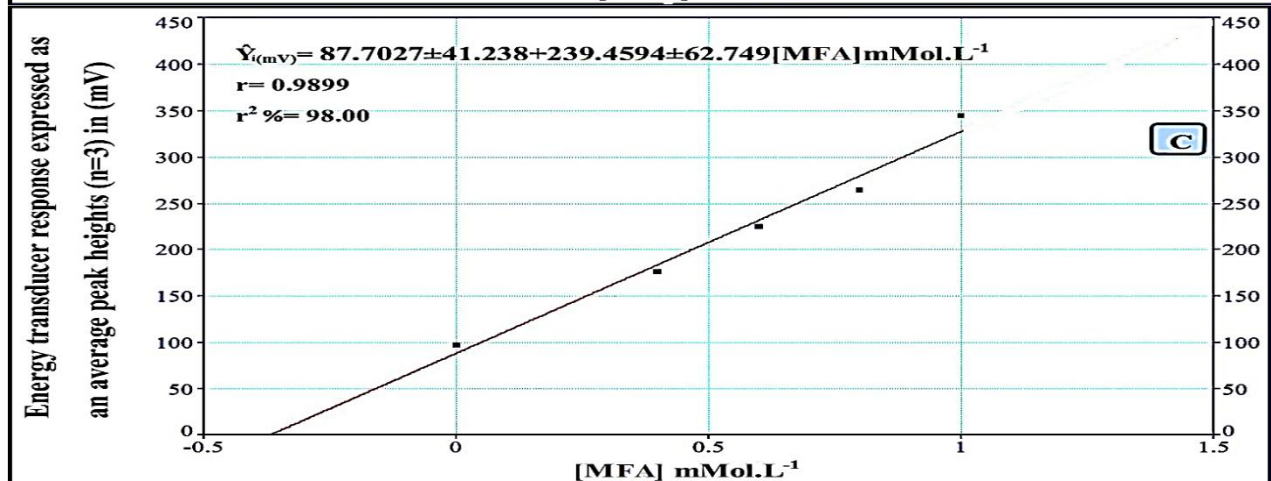
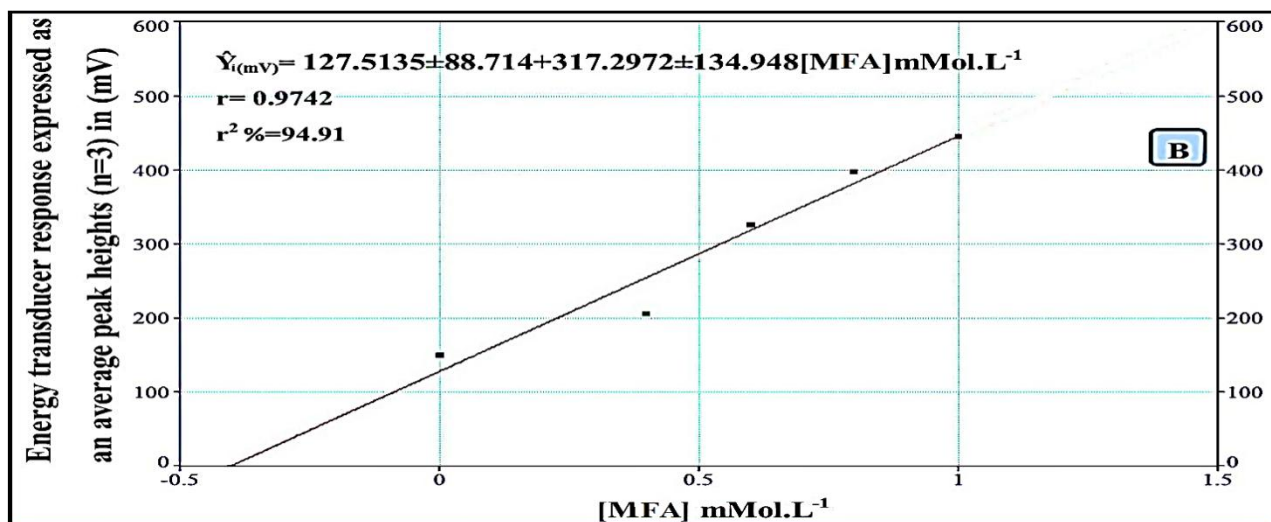
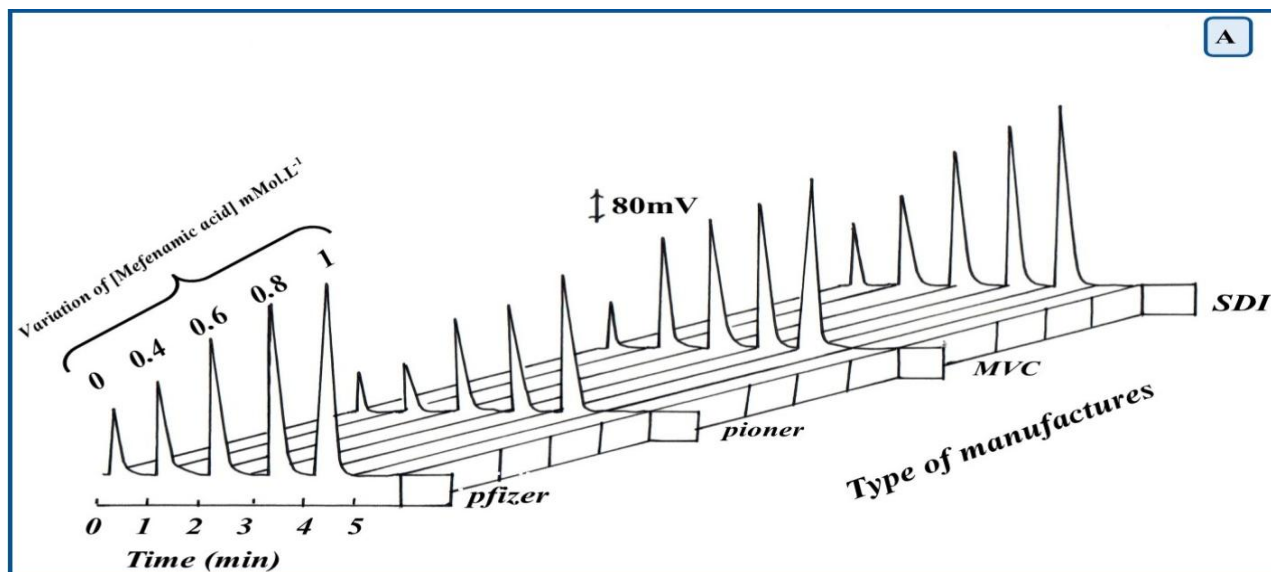
but the alternative hypothesis (H_1) is accepted against null hypothesis for drugs from piostan- pioner- Iraq and ponstan - Pfizer-USA, i.e: there was a significant difference between the quoted value with calculated t-value at 95% confidence level as the calculated t-value is more than critical tabulated t-value

Second test

Comparison of obtained results from both methods with neglecting the difference of origin and Companies. It was noticed that there were no significant between two methods (Ayah 6SX1-ST-2D Solar cell –CFI Analyser & UV-Vis spectrophotometric) since calculated t-value of $0.584 \ll t_{tab} (3.182)$ i.e:

$H_0 (\mu_{\text{Ayah 6SX1-ST-2D Solar cell –CFI Analyser}} = \mu_{\text{UV-Vis spectrophotometric}})$
is accepted against

$H_1 (\mu_{\text{Ayah 6SX1-ST-2D Solar cell –CFI Analyser}} \neq \mu_{\text{UV-Vis spectrophotometric}})$



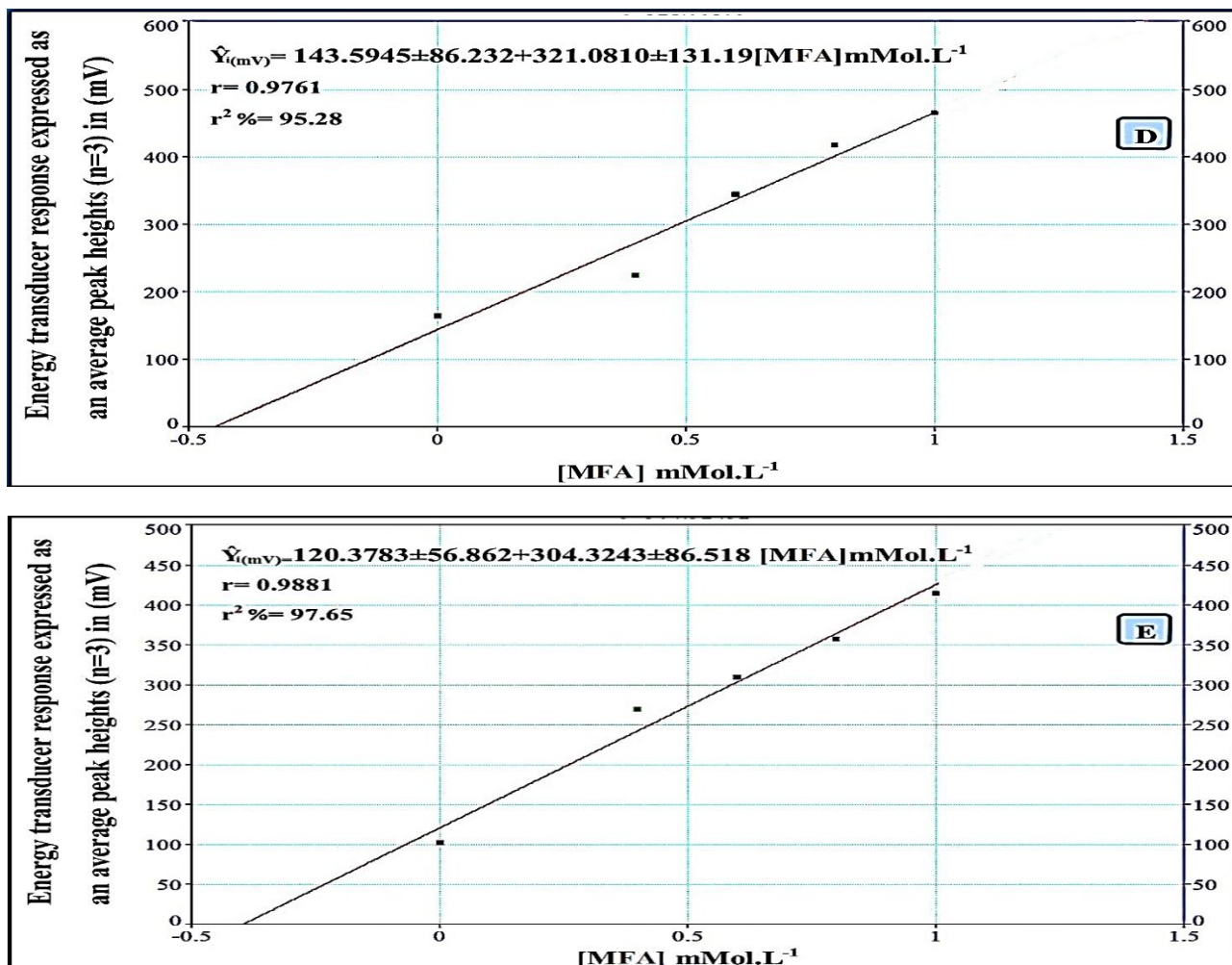


Fig: 11: Standard addition calibration graph for four pharmaceutical preparations using Ayah 6SX1-ST-2D Solar cell –CFI Analyser, 122µl ,open valve mode.

(A):profile versus time, (B): SDI company, (C): Pioner company, (D): pfizer company, (E):MVC company

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

sample no	commercial name ,content ,company,country	Ayah 6SX1-ST-2D solar CFIA (mV)					Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t$ [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	UV-Vis SP.
		UV-Vis SP. (classical method for absorbnace measurement)							Practical conc. mMol.L ⁻¹	
		[Mefenamic acid] mMol.L ⁻¹							in 25 ml	in 25 ml
		0	0.4	0.6	0.8	1			In 100ml	In 100ml
1	Ponstidin, 500 mg, SDI ,Iraq	150	206	326	398	446	127.5135±88.714+317.2972±134.948[MFA] mMol.L ⁻¹	0.9742 0.9491 94.91 %	0.402	0.795
		0.455	0.782	0.958	1.764	2.598	0.4426±0.286+0.5568 ±0.127 [MFA]mMol.L ⁻¹	0.9896 0.9795 97.95 %	100.469	99.362
2	Plostan, 500 mg, pioner , Iraq	97	177	225	265	345	87.7027±41.238+239.4594±62.749[MFA]m Mol.L ⁻¹	0.9899 0.9800 98.00 %	0.366	0.758
		0.435	0.788	0.957	1.743	2.621	0.4278±0.033+0.5646±0.022[MFA]mMol.L ⁻¹	0.9920 0.9841 98.41 %	91.563	94.713
3	Ponstan, 500 mg, Pfizer, USA	165	225	345	417	465	143.5945±86.232+321.0810±131.19[MFA] mMol.L ⁻¹	0.9761 0.9528 95.28 %	0.447	0.856
		0.445	0.782	0.958	1.745	2.500	0.4554±0.318+0.5323±0.127[MFA]mMol.L ⁻¹	0.9874 0.9750 97.50 %	111.806	106.94
4	Ponamec, 500mg, MVC , India	102	270	310	358	414	120.3783±56.862+304.3243±86.518[MFA] mMol.L ⁻¹	0.9881 0.9765 97.65 %	0.396	0.783
		0.443	0.789	0.959	1.744	2.600	0.4368±0.254+0.5578±0.127[MFA]mMol.L ⁻¹	0.9913 0.9828 98.28 %	98.890	97.885

\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 $\bar{w} \pm 1.96 \sigma_{n-1} / \sqrt{n}$ at 95% (g)	Theoretical content for the active ingredient $\bar{w} \pm 1.96 \sigma_{n-1} / \sqrt{n}$ at 95% (mg)	Sample weight equivalent to 2.41g (100 mMol.L ⁻¹) of the active ingredient w_i (g)	Practical content of active ingredient			Efficiency of determination (Rec%)	Paired t-test			
				In 100 ml of sample $\bar{w} \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$ at 95% (g)	in tablets $\bar{w} \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$ at 95% (mg)	Individual comparison $(\bar{w}_i - \mu_0) \sqrt{n} / \sigma_{n-1}$ Ayah 6SX1-ST-2 D Solar cell-CFI Analyser with Quoted value $t_{0.05/2, 2} = 4.303$		Comparison between two method	Xd	\bar{X}_d (σ_{n-1})	$t_{cal} = \frac{\bar{X}_d \sqrt{n}}{\sigma_{n-1}}$ at 95%
				UV-Vis SP. (classical method for absorbance measurement)							
1	0.5952±0.0012	500±1.0165	2.8688	2.421±0.047	502.354±9.752	100.47%	1.039 << 4.303	5.53	4.782	16.370	0.584 << 3.182
				2.395±0.052	496.823±10.787	99.36%					
2	0.7158±0.0024	500±1.6415	3.4501	2.207±0.025	457.824±5.186	91.56%	-34.995 >> 4.303	-15.75	4.782	16.370	0.584 << 3.182
				2.283±0.129	473.573±26.759	94.71%					
3	0.7035±0.0063	500±4.4634	3.3908	2.695±0.050	559.039±10.372	111.81%	24.493 >> 4.303	24.32	4.782	16.370	0.584 << 3.182
				2.577±0.032	534.719±6.640	106.94%					
4	0.9083±0.0061	500±3.3689	4.3780	2.383±0.060	494.450±12.449	98.89%	-1.918 << 4.303	5.03	4.782	16.370	0.584 << 3.182
				2.359±0.127	489.423±26.349	97.88%					

Xd: Difference between two method, \bar{X}_d : difference mean, σ_{n-1} : Difference standard deviation, n=3 for individual & n=4 for comparison between two method, $t_{0.025, 3} = 3.182$, $t_{0.025, 2} = 4.303$

CONCLUSION

The suggested methods is simple, sensitivities and rapid. Application of the proposed methods to the analysis of mefenamic acid in pure and pharmaceutical preparation based on formation bluish green color precipitate as an ion- pair compound for the reaction of mefenamic acid with Ce(IV) Sulfate in aqueous medium. It was shown that with no doubt that newly developed method is a 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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