INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

KETOTIFENFUMARATE DETERMINATION VIA THE NEW APPROACH OF TURBIDITY FORMED BY THE REACTION WITH PHOSPHOMOLYBDIC ACID USING AYAH 6SX1-T-1D CFI ANALYSER

Nagam S. Turkie Al-Awadie* and Manhl H. Ibraheem

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.

ABSTRACT

A newly developed analytical method characterized by its speed and sensitivity for the determinationof ketotifenfumarate (KTF) in pure and pharmaceutical preparation is established viaturbidimetricmeasurement (0-180^{o}) by Ayah 6SX1-T-1D Solar cell CFI Analyser. The method was based on the reaction ofphosphomolybdic acid with ketotifenfumarate in sodium chloride medium to form yellowish green precipitate as an ion-pair complex. Turbidity was measured via theattenuation of incident light that collides on the surface precipitated particles at 0-180^{o} . Thechemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 0.5-50 mMol.L^{-1} , with correlation coefficient r = 0.9976. The limit ofdetection 4.255 µg/sample fromthe step wise dilution forthe minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.7% for 9 and 20 mMol.L^{-1} (n=6,8 respectively) concentration of ketotifenfumarate. The method was successfully applied to the determination of ketotifenfumarate in two pharmaceuticals . A comparison was made between the newly developed method analysis and the classical method ,in addition to between twodifferentpharmaceutical preparations (UV- spectrophotometry at wave length 298 nm) usingthe standard additions method via the use of t-test. It was noticed that there was no significant difference between two methods at 95 % confidence levelandnosignificant difference between two drugs.

Keywords: Ketotifenfumarate, Flow injection analysis, Turbidity.

INTRODUCTION

Ketotifen is a second-generation H1-antihistamine and mast cell stabilizer. The chemical name of Ketotifenfumarate is 4-(1-Methyl-4-piperidylidene)-4H-benzo [4, 5]cyclohepta[1,2-b]thiophen-10(9H)-one hydrogen fumarate . Ketotifenfumarate is a finely crystalline powder with an empirical formula of C23H23NO5S and a molecular weight of 425.50 g.mol^{-1}. The structure of Ketotifenfumarate was shown in **Figure 1**[1]. It is most commonly available as a salt of Fumaric Acid, Ketotifenfumarate, and is accessible in two forms. In its ophthalmic form, it is used to treat allergic conjunctivitis, or the itchy red eyes caused by allergies. In its oral form, it is used to prevent asthma attacks. Side effects include drowsiness, weight gain, dry mouth, irritability, and increased nosebleeds[2].

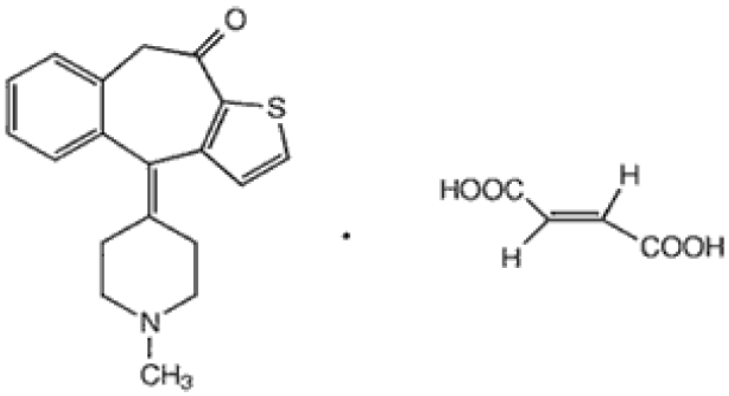


Fig. 1: Structure of Ketotifen fumarate

It is widely accepted as an antiasthmatic/antianaphylactic drug and also alleviates allergic disorders via a combination of several actions. For example, ketotifen is a relatively selective, noncompetitive antagonist of histamine H1 receptors and is a mast cell stabilizer, inhibiting the release of inflammatory mediators from mast cells[3,4,5].

Ketotifen fumarate is able to inhibit the release of histamine, other relaybasophils and mast cells. It causes long-lasting inhibition of histamine reactions. It is effective in the treatment of allergic diseases as well as asthma. It is used to prevent asthma attacks caused by allergy, it also works antianafilactically.

Allergy means all excessive reactions of healthy people to neutral substances. Histamine is a physiologically active, endogenous substance that activates H1 and H2 receptors. It is responsible for allergic reaction in our body. The mechanism of action of antihistamines is based on their competitive and reversible connection between H1 andH2 receptors. It annihilates effects of histamine as a mediator of inflammation and immune response . Ketotifenfumarate was determined using spectrophotometric[6–10], chromatographic[11–15], and electroanalysis methods[16–19].The purpose of this work is to describe a simple, precise and sensitive flow injection turbidimetric method with the use of Ayah 6SX1-T-1D Solar cell CFI Analyser for the determination of Ketotifenfumarate in pharmaceutical formulations. The method based on the formation of yellowish green color precipitate as an ion-pair compound by phosphomolybdic acid with Ketotifenfumaratein sodium chloride medium .

EXPERIMENTAL

REAGENTSAND CHEMICALS

All chemicals were used of analytical-reagent grade and distilled water used to prepare the solutions . A standard solution (0.05 Mol.L^{-1}) of Ketotifenfumarate$C_{23}H_{23}NO_5S$ (425.497 g. mol^{-1}) was prepared by dissolving 5.3187 g in 250 ml distilled water. 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 distilled water , filter and dilute to 500 ml. Sodium chloride NaCl (0.5 Mol.L^{-1}) was prepared by dissolving 0.146 g in 50ml distilled water. potassium bromide KBr (0.5 Mol.L^{-1}) was prepared by dissolving 2.975 g in 50ml distilled water. Potassium nitrate KNO_3 (0.5 Mol.L^{-1}) was prepared by dissolving 2.525 g in 50 ml distilled water.

Sample Preparation

Sixty tablets weight, crushed and grinded. Tablets containing 1 mg of Ketotifenfumarate for (Julphar, Micro companies) were weight (6.1978, 6.9958 g) equivalent to 0.0532 g of active ingredient respectively to obtain 5 mMol.L^{-1} conc. of KTF for each drug . The powder was dissolved in distilled water followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 25 ml .

APPARATUS

The flow system used for the determination of KTF 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-T-1D 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 . One 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 (Kompenso 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 KTF using 1 cm quartz cell.

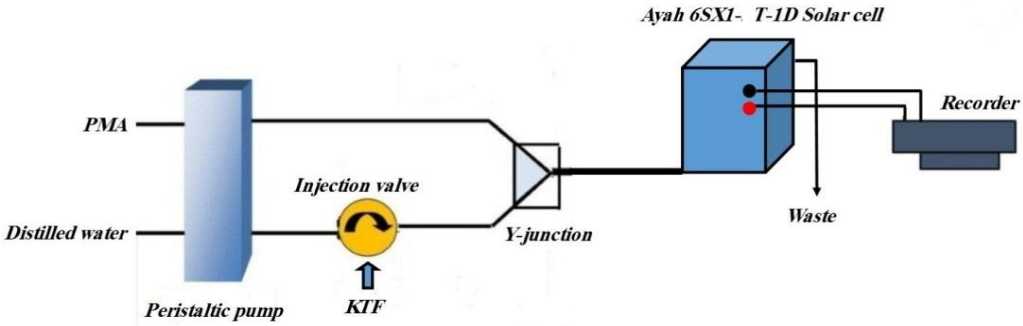
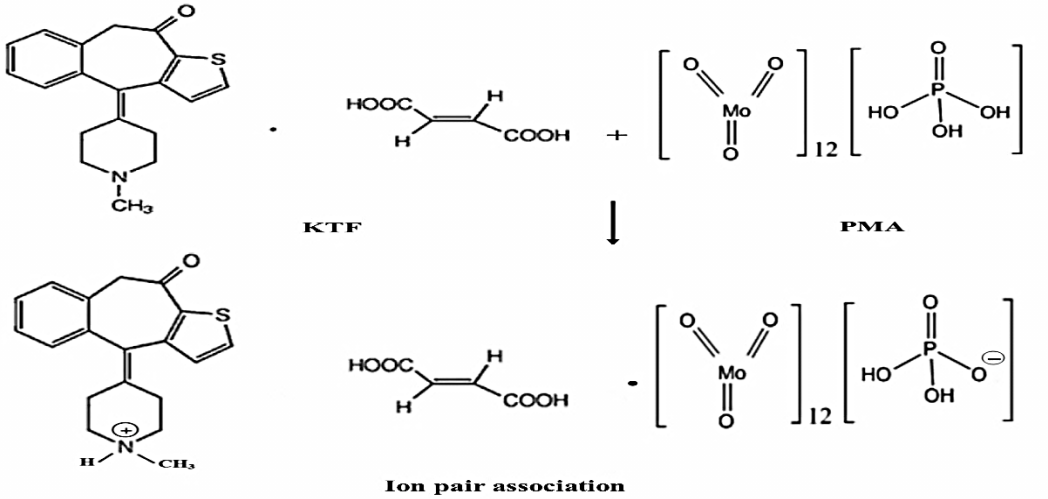


Fig. 2: Flow diagram manifold system used for the determination of KTF

Methodology

The flow system consisting of two lines was used for the determination of KTF by the reaction between KTF andphosphomolybdic acid (7 mMol.L^{-1}) in sodium chloride medium to form a yellowish green color precipitate as an ion pair complex form. The first line represent the carrier stream (sodium chloride) at 0.9ml.min^{-1} flow rate which lead to the injection valve to carry KTF , sample volume 200 µl;while the second line supplies phosphomolybdic acid solution at 0.8 ml.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-T-1D 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 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 attenuation of incident light at 0-180º . A probable mechanism of ion pair formation for KTF -PMA system is represented in scheme 1.



Scheme. 1: Proposed mechanism of reaction between of KTF &PMA

RESULTS AND DISCUSSION

Variable optimization

Chemicals parameters (mainly concentration of reagent and type of carrier stream for the KTF-PMA system) as well as physical parameters (sample volume , flow rate , purge time &volume of coil) were studied using two lines manifold system (**Fig.2**)

Chemical Variables

Phosphomolybdic Acid (PMA) Concentration

Variables concentration of precipitating reagent (1-9) mMol.L^{-1} were prepared. 200µl sample volume was injected through the carrier stream (distilled water) . 7 mMol.L^{-1} concentration of ketotifenfumarate was injected with 1.8 & 1.4 ml.min $^{-1}$ flow rate for carrier stream and reagent respectively in addition to 1.19 V applied voltage to the source (6 LEDs). From **Fig.3-A,B** ,it was found that an increase in peak height expressed as an attenuation of incident light (negative response) with increase of PMA concentration which might be due to increase of precipitate particulate and the colour of precipitate plays an important role in deciding absorption & attenuation of incident light up to 7mMol.L^{-1}.While at higher concentration (>7mMol.L^{-1})lead to decrease in the height of negative response , it might be due to an increase in the concentration of PMA does not give any significant increase in the precipitate reaction as there is a constant concentration of KTF which mean a fixed amount of formed precipitated whatever the concentration of PMA . Therefore 7mMol.L^{-1} was selected as optimum concentration of PMA . The results were summarized in **Table1.**

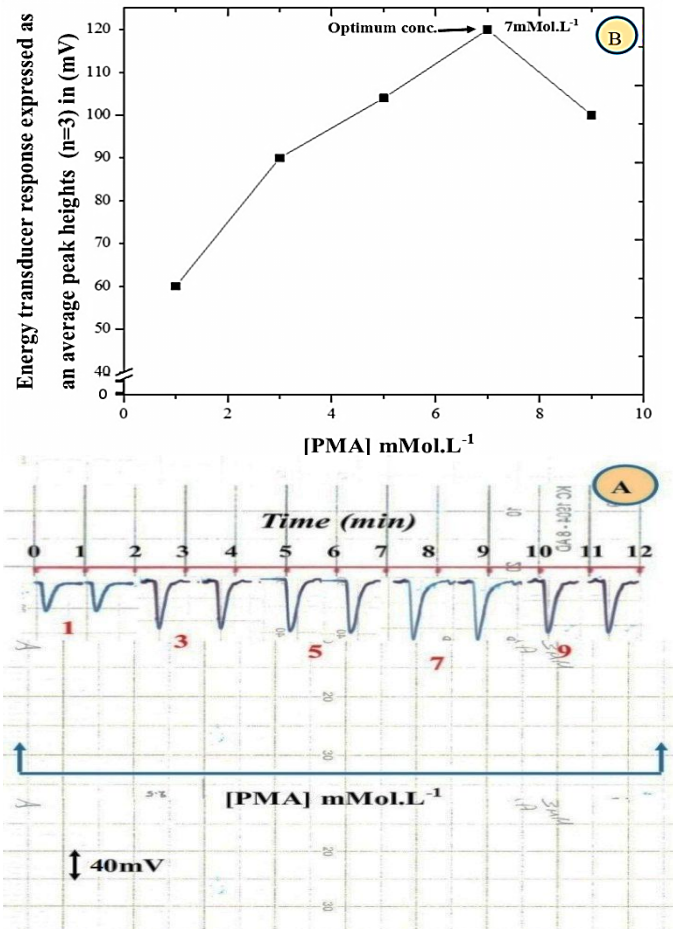


Fig. 3: Effect of PMA concentration on :
(A): Response profile versus time, (B) : Energy transducer response expressed as an average peak heights versus PMA concentration.

Table 1: Effect of variable concentration of PMA on transducer energy response of KTF– PMA system

[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%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
1	60	0.83	60± 1.237
3	90	0.33	90± 0.738
5	104	0.49	104± 1.266
7	120	0.43	120 ± 1.282
9	100	0	100± 0

Effect of Different Medium on the KTF - PMA System

The ion pair of KTF (7 mMol.L^{-1})-PMA(7 mMol.L^{-1}) system was studied in different solution media (potassium nitrate, sodium chloride , potassium bromide) at 0.5 Mol.L^{-1} concentration in addition to aqueous medium as a carrier stream . it is expected that these salt solutions increase the formation of a dense precipitate ; which in reflect increase sharpness of obtained response at an added sensitivity . It was found and supported by what is shown in **fig.4-A** and **B**,that NaCl solution was the choice .**Table 2** tabulate the results obtained.

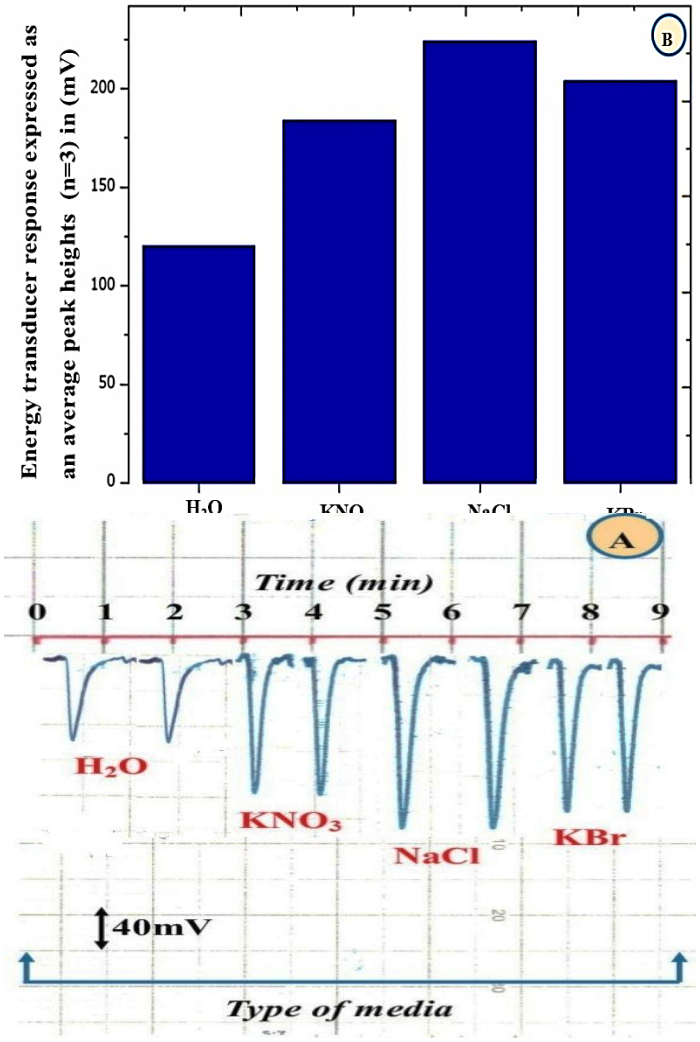


Fig. 4: Effect of the different media on:
(A) : Response profile versus time using 200µl sample volume, flow rate 1.8,1.4 ml.min^{-1} of carrier stream and reagent ,applied voltage to the LEDs source 1.19 volt DC &open valve mode.
(B) :Energy transducer response .

Table 2: Effect of different media on the measurement of energy transducer response fordetermination of ketotifenfumarate

Type of medium	Energy transducer response expressed as an average peak heights(n=3) $\bar{y}i$(mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
H2O	120	0.01	120±0.030
KNO_3	184	0	184±0
NaCl	224	0.02	224±0.111
KBr	204	0.01	204±0.051

Effect of variable concentration of sodium chloride

A series of solutions were prepared at ranging (0.01-0.9)mMol.L^{-1}of sodium chloride , using KTF(7 mMol.L^{-1})-PMA(7 mMol.L^{-1})system. 200µl sample volume at 1.8 ml.min^{-1} and 1.4 ml.min^{-1} flow rate for carrier stream and reagent solution respectively.The results obtained were summarized in. **Fig.5-A,B** . It was noticed that an increase in NaCl concentration ; a decrease in transmitted light due to the attenuation of incident light up to 0.6 Mol.L^{-1}. It is expected that NaCl causes coagulation of precipitated particles which leads to attenuated light . The results summed up in **table3**.

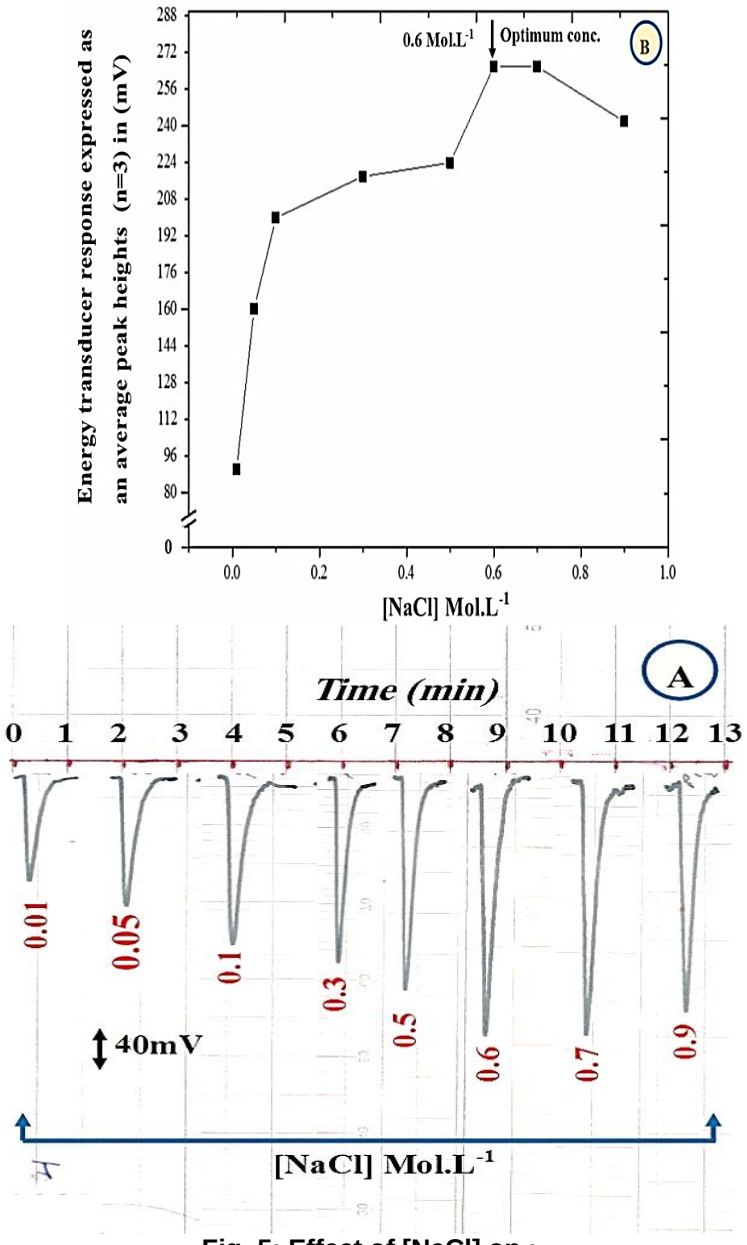


Fig. 5: Effect of [NaCl] on :
(A): Response profile of attenuation of incident beam versus time.
(B):Energy transducer response expressed as an peak height in mV versus concentration of NaCl solution

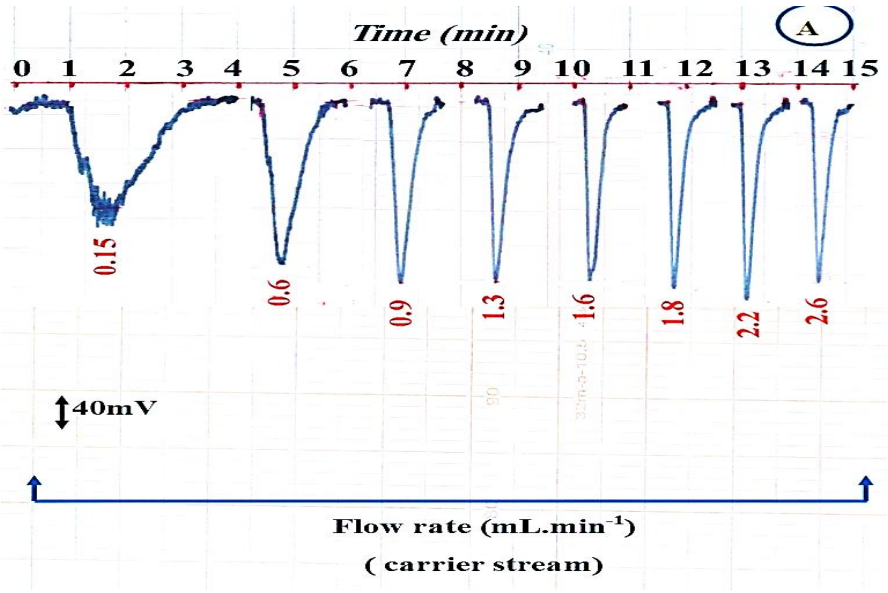
Table 3: Effect of [NaCl] on the measurement of energy transducer response via attenuation of incident light for determination of KTF

[Nacl] Mol.L^{-1}	Energy transducer response expressed as an average peak heights(n=3) $\bar{y}i$(mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
0.01	90	0.13	90±0.291
0.05	160	0.20	160 ±0.795
0.1	200	0.46	200±2.286
0.3	218	0.53	218±2.870
0.5	224	0.59	224±3.283
0.6	266	0.76	266±5.022
0.7	266	1.12	266±7.401
0.9	242	1.24	242±7.455

Physical variables

Flow rate

Using the settling shown in **Fig.2-A** which shows that a two line manifold system were used . Variable flow rates were used . Different variable responses were obtained even different profile were described .**Fig. 6-A** shows the different kinds of responses described by response of attenuated signal versus flow rate. While **Fig. 6-B** shows average peak heights in mV versus flow rate of the carrier stream . 0.9 ml.min^{-1} (carrier stream) uses the optimum choice even it is at the edge of the curve . This was chosen due to the stability and acceptable range of material consumption . While **table 4** tabulate all the obtained results.



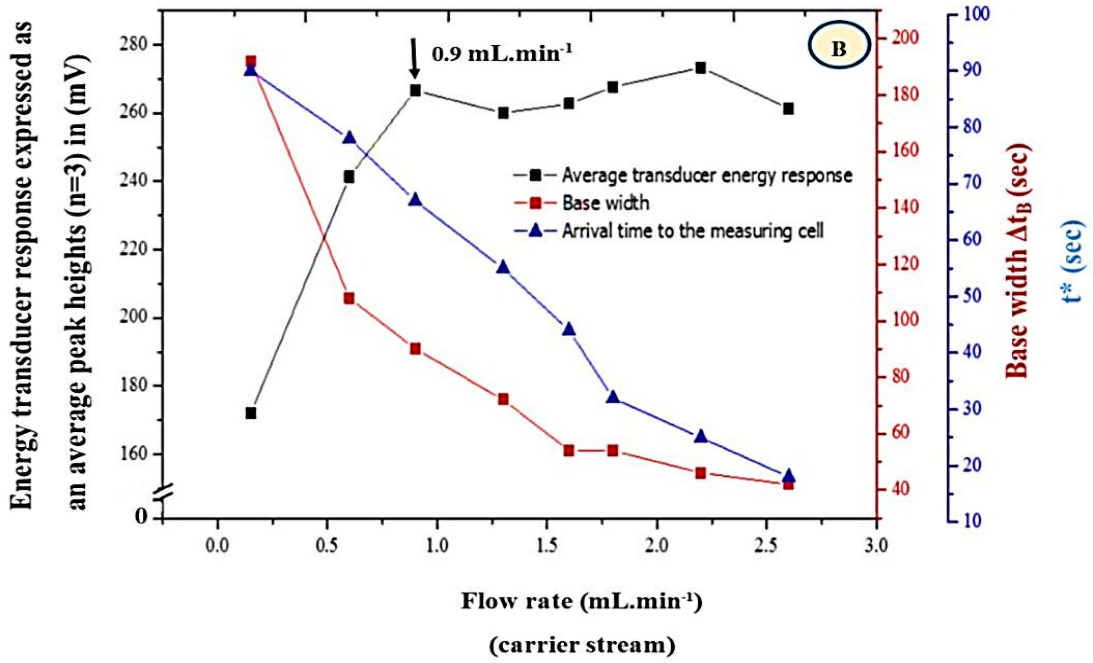


Fig. 6: Effect of the variation of flow rate on :
(A): Response profile versus time, (B):Energy transducer response expressed as an average peak heights in mV , Base width and Departure time for sample segment from injection valve to the measuring cell.

Table 4: Effect of the variation of flow rate on the energy transducer response using 200 µland7mMol.L^{-1} concentration of KTF

Pump speed (appro	Flow rate ml .min^{-1} Carr	Flow rate ml .min^{-1} Rea	Energy transducer response expressed as an avera	RSD%	Confidence interval at (95%) ȳi±t0.05/2,n-1 σn-1/√n	Base width Δt_B (sec)	t* sec	V* ml	C* mMol. l-1
5	0.15	0.15	172.00	0.11	172.00±0.470	192	90	1.160	1.207
10	0.6	0.5	241.33	0.09	241.33±0.540	108	78	2.180	0.642
15	0.9	0.8	266.67	0.11	266.67±0.729	90	67	2.550	0.549
20	1.3	1	260.00	0.12	260.00±0.775	72	55	2.760	0.507
25	1.6	1.2	262.67	0.46	262.67±3.00	54	44	2.720	0.515
30	1.8	1.4	267.67	0.55	267.67±3.657	54	32	3.080	0.455
35	2.2	1.6	273.33	0.63	273.33 ±4.278	46	25	3.113	0.450
40	2.6	2	261.33	0.69	261.33±0.242	42	18	3.420	0.409

V*: addition volume
C*:Concentration at flow cell
t*: Arrival time from injection valve reaching to the measuring cell.
Δt_B: base width of response

Sample volume

Using previously obtained parameters for optimum single variable optimization to describe well the methodology adopted in the determination of KTF .Variable length of teflon tube (16-37) cm of diameter = 1mm was used in decission of optimum sample volume that will be used throughout the procedure . A 200 µl Sample volume was chosen as the most suitable sample volume. **Fig.7-A,B** shows various aspects of obtained results including profile of energy transducer response versus time also arrival time , Δt_B, and average peak heights in mV for all various aspects of used sample volume. All these obtained data were tabulated in **table 5.**

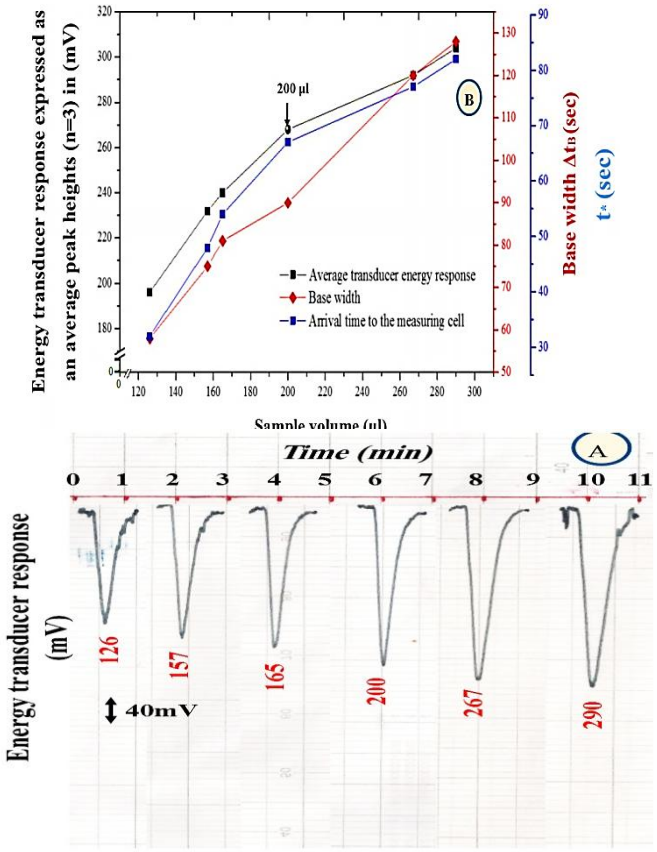


Fig. 7: Effect of the variation of sample volume on:
(A): Response profile versus time, (B): Energy transducer response expressed as an average peak heights in (mV) using KTF (7 mMol.L^{-1}) –PMA (7 mMol.L^{-1}) system , flow rate 0.9, 0.8 ml.min^{-1} for carrier stream , reagent respectively

Table 5: Effect of the variation of sample volume on the transducer energy response fordetermination of KTF (7mMol.L^{-1})

Loop length Cm r=0.5 mm	Sample volume µl	Energy transducer response expressed as an average peak height (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
16	126	196.00	0.67	196.00±3.262	58	32
20	157	232.00	0.53	232.00±3.055	75	48
21	165	240.00	1.34	240.00±7.990	81	54
25	200	268.00	1.12	268.00±7.457	90	67
34	267	292.00	0.80	292.00±5.803	120	77
37	290	304.00	0.65	304.00±4.909	128	82

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

Purge time

Studying the time required to purge the sample from sample loop to the rest ofthe manifold reaction setting shows , clearly that an open valve mode was most suitable allowable permissible time as no flactuation can occur as can be seen from **Fig8-A**. while **Fig 8-B.** shows a maximum response profile at open valve mode.Therefor an interupted valve mode of action was ignored . Therefor at this stage a flow rate of 0.9 ml.min^{-1}and valve mode was used . **Table6**tabulate the results obtained.

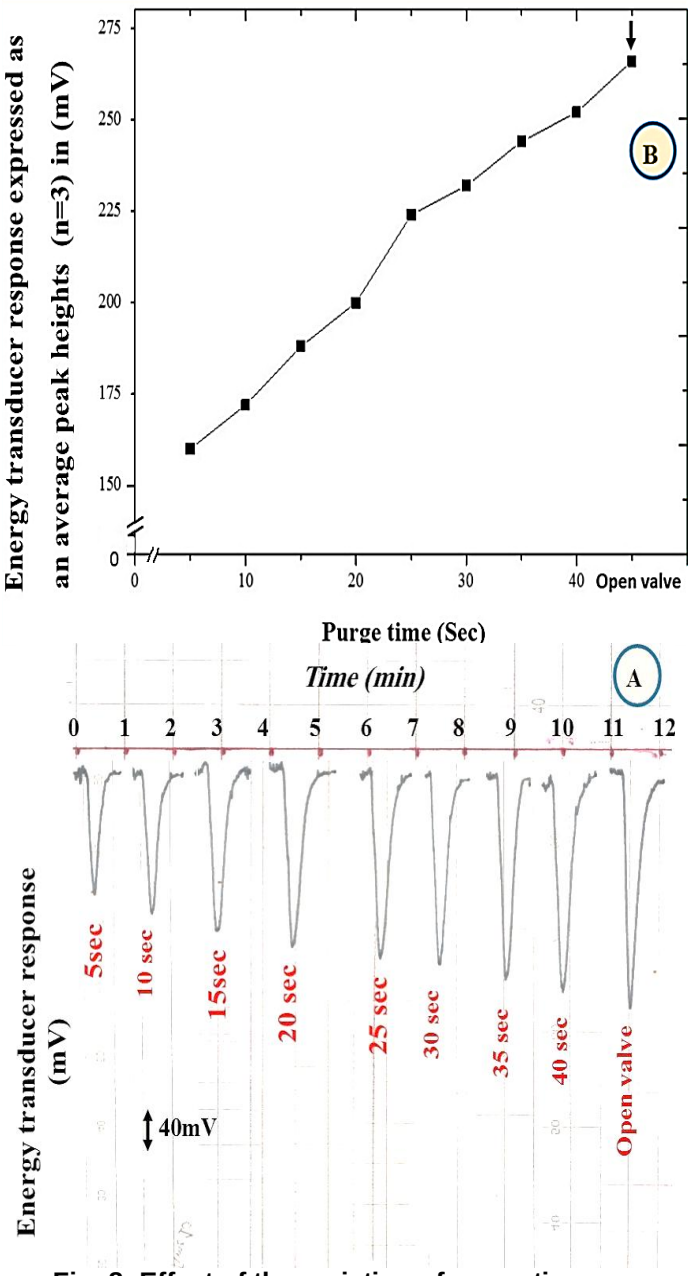


Fig. 8: Effect of the variation of purge time on:
(A): Response profile versus time.
(B): Energy transducer response expressed as average peak heights in (mV), 200 µl& KTF(7mMol.L^{-1}).

Table 6: Effect of the variation of purge time on the energy transducer response

Purge time (sec)	Energy transducer response expressed an average peak heights (n=3)$\bar{y}_i$(mV)	RSD %	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
5	160	0.58	160±2.305
10	172	0.57	172±2.436
15	188	0.59	188±2.756
20	200	0.75	200±3.727
25	224	0.74	224±4.118
30	232	0.78	232±4.496
35	244	0.76	244±4.607
40	252	0.75	252±4.695
Open valve	266	0.71	266±4.692

Reaction coil

Variable reaction coils length (30,60,100) cm were used to verify a better reaction pattern or even decideing the speed of reaction.It was noticed that no reaction coil or a delay reaction coil was required . **Fig. 9-A** shows kind of obtained profile ,when the reaction coil was placed after junction point .While **Fig.9-B**shows the variation of peak heights and Δt_B with coil volume . **Table 7** describe all the obtained results. The manifold design as followed in **Fig.9-C .**

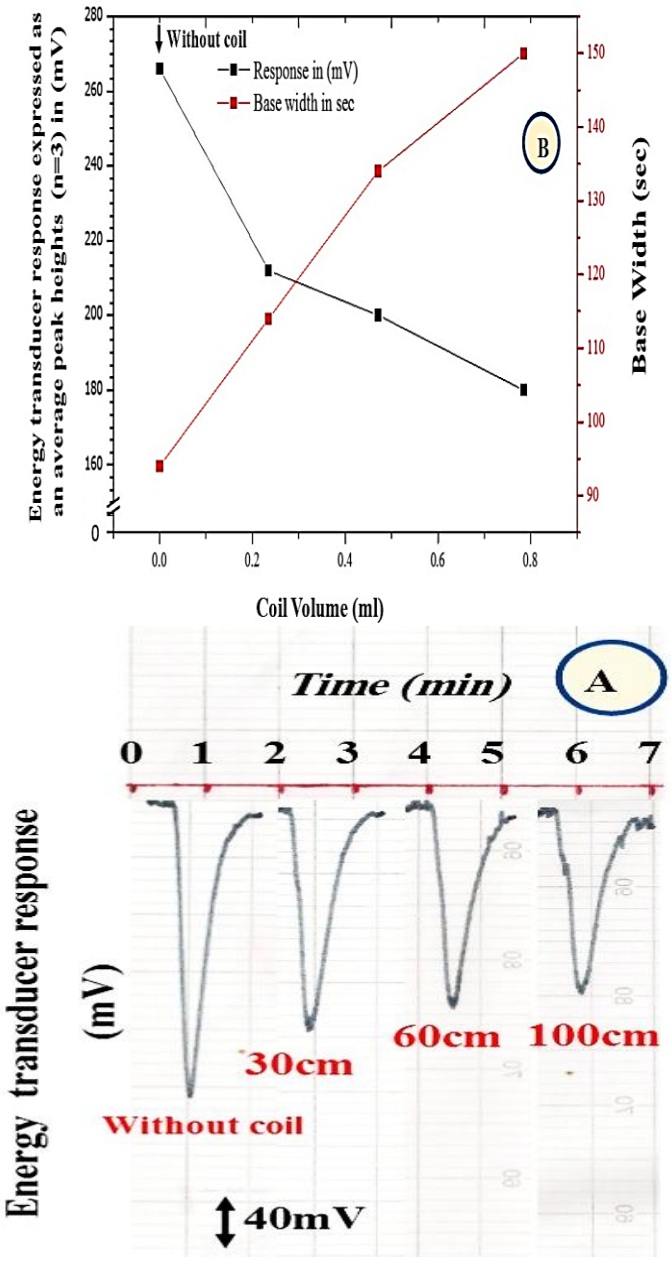


Fig. 9: Effect of reaction coil on :
(A) : Response profile vs. time, (B): Energy transducer response expressed as an average peak heights in mV,using KTF (7mMol.L^{-1}) –PMA (7 mMol.L^{-1}) system , flow rate 0.9,0.8 ml.min^{-1} for carrier stream, reagent respectively

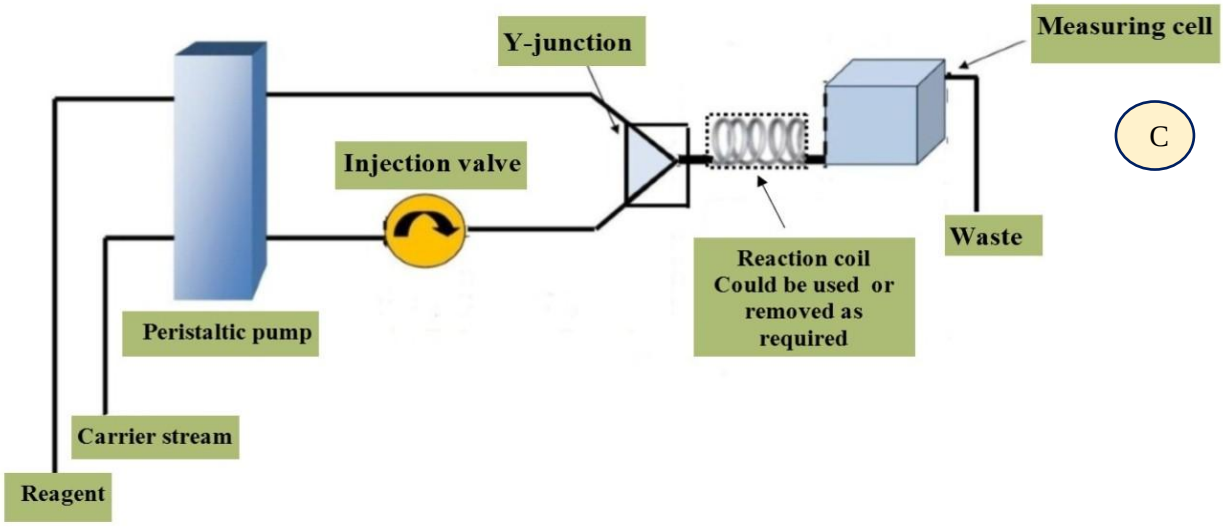


Fig. 9C: Manifold design system for determination of KTF in the brescence of reaction coil

Table 7: Effect of coil length on energy transducer response expressed as an average peak heights (mV) for determination of ketotifen fumarate

Coil length (cm)	Coil volume (ml) $r^2\pi$ h, r =0.5 mm	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$(mV)	RSD%	Confidence interval at (95%) $\bar{y}i \pm t0.05/2,n-1\ \sigma n-1/\sqrt{n}$	t* (sec)	Base width Δt_B (sec)	V* (ml)	Concentration in mMol.L^{-1} at flow cell
0	0	266	0.42	266±2.776	60	94	2.863	0.489
30	0.235	212	0.48	212±2.528	77	114	3.430	0.408
60	0.471	200	0.49	200±2.435	95	134	3.997	0.350
100	0.785	180	0.55	180±2.459	120	150	4.450	0.315

t* : Departure time for sample segment from injection valve to the measuring cell
V*: Addition volume (ml) at flow cell
Δt_B: base width of response

Study of the variation of KTF concentration on the response of The precipitation reaction

Two procedure were adopted first the use of conventional spectrophotometric method and secondly using the newly developed instrument based on the formation of precipitation reaction .**Fig 10-A**shows the kind of response obtained using the newly developed method of analysis for various concentration of KTF expressed in mMol.L^{-1} . While **Fig.10- B**shows the calibration graph range (0.5-50) mMol.L^{-1} obtained from the scatter points which shows a good correlation between the response and concentration in the linear representation and the extended range of linearity . While **Fig.10-C**present the calibration graph of KTF using conventional spectrophotometric method . Low level concentration (0.01-0.3)mMol.L^{-1} can be used using the conventional method .

While an extent up to approximately 125 time range. A conclusion can be drawn that both method can be used depending on the range of concentration to be used. All results were summarized in **table 8**.

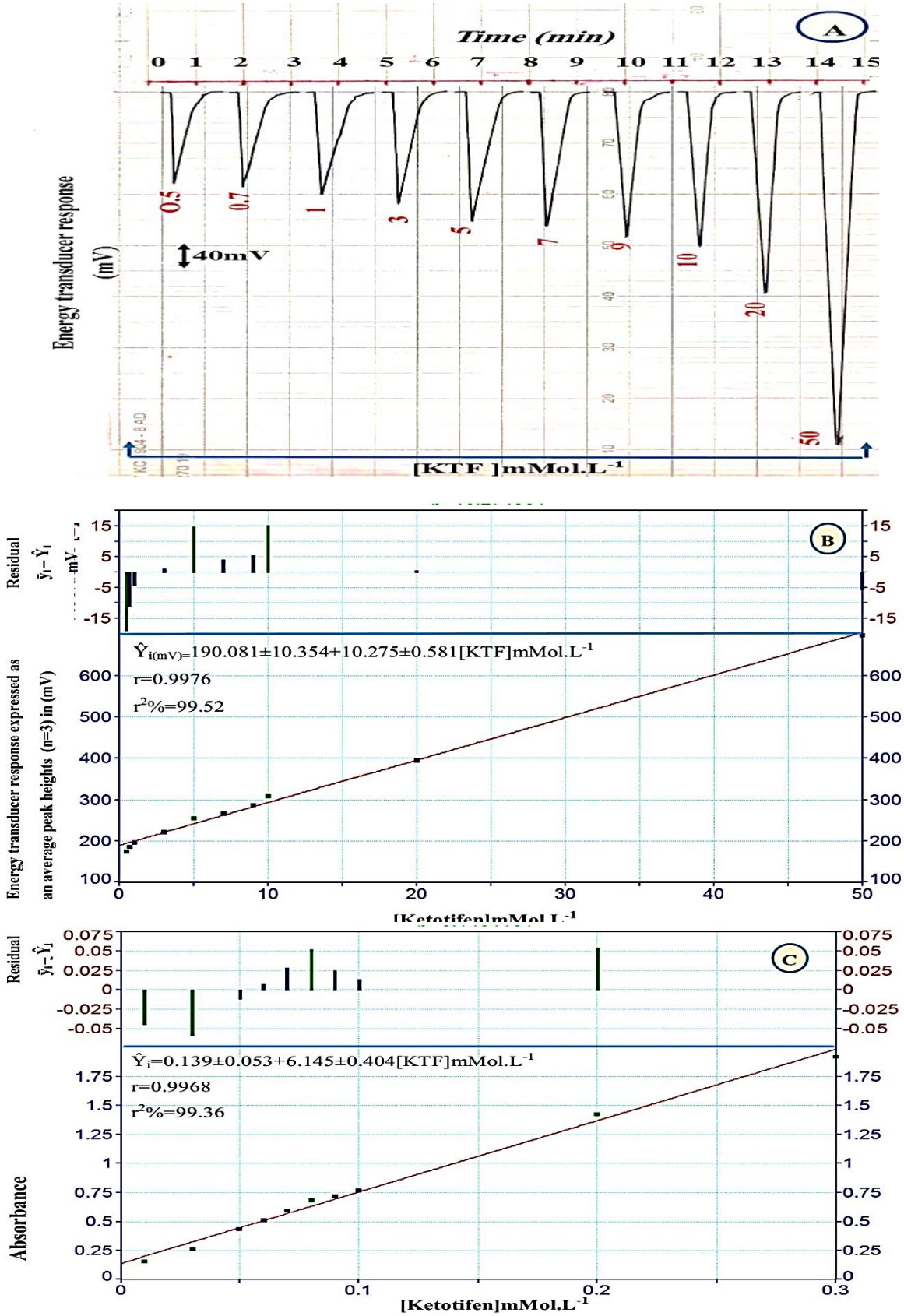


Fig. 10: (A): Selected peak profile each out of three responses for any single concentration was chosen using KTF-PMA (7mMol.L^{-1}),200µl.
(B):Linear presentation by ŷ=a+bx of the result of variation of response with concentration
(C):Calibration graph using UV-Spectrophotometric method,residual ($\bar{y}_i - \hat{Y}_i$), $\bar{y}_i$: practical value, $\hat{Y}_i$: estimate value.

Table 8: Summary of result for linear regression for the variation of energy transducer response with KTF concentration using first degree equation .

Type of method	Measured [KTF] $mMol.L^{-1}$	Range of [KTF] $mMol.L^{-1}$ (n)	$\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[KTF]$mMol.L^{-1}$ at confidence level 95%,n-2 $\hat{Y}_i=a\pm s_a t+b\pm s_b t$[KTF]$mMol.L^{-1}$ at confidence level 95%,n-2	r r^2 r^2%	t_{tab} at 95% ,n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r2}}$
Ayah6x s1-T-	0.1-50	0.5-50 10)(	190.081±10.354+10.275±0.581[KTF]$mMol.L^{-1}$	0.9976 0.9952 99.52%	2.306<< 40.721	
UV-Spectrophotometric	0.01-0.4	0.01-0.3 (10)	0.139±0.053+6.145±0.404 [KTF]$mMol.L^{-1}$	0.9968 0.9936 99.36%	2.306 << 35.161	

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

The limit of detection (L.O.D) of analyte in general may be described as that concentration which gives an instrument signal (y) significantly different from the blank or back ground signal (y_B). A commonly, the limit of detection is the analyte concentration giving a signal equal to the blank signal (y_B) plus three standard deviation of the blank (S_B) $Y=Y_B+3S_B$ or$Y-Y_B=3S_B$ Or using successive gradual dilution of the minimum concentration of KTF drug that was used in the calibration graph and the three method, which depend on the slope value.

Table 9: summarizes L.O.D of KTF conducted through three methods

Type of method	Practically based on gradual dilution for the minimum concentration ($mMol.L^{-1}$)or weight/sample	Theoretical based on the value of slope $X=3S_B$/Slope	Theoretical based on the linear equation $\hat{Y}=YB+3SB$
Attenuation of incident light using Ayah6SX1-T-1D Solar cell	($0.05\ mMol.L^{-1}$) 4.255 µg/sample	12.423µg/sample	0.283 mg/sample
Absorbance using UV-spectrophotometric	($0.01\ mMol.L^{-1}$) 0.581 µg/sample	16.618 µg/sample	1.911 µg/sample

Sample volume for Ayah 6SX1-T-ID Solar cell 200µl. & (4ml) for classical method .

Repeatability is a measure of performance of the analyst ,the method and instrument used . Successive measurements were carried out for 9 & $20mMol.L^{-1}$ of KTF using the optimum parameters used in this research work. **Table 10**shows the arithmetic's , which shows that the percentage relative standard deviation was less than . This low percentage of relative standard error indicate a reliable measurement can be achieved using this method .While the kind of the response profile is shown in **Fig. 11**.

Table 10: Repeatability for the response obtained for the formation of precipitation reaction from KTF-PMA ($7mMol.L^{-1}$)-Nacl ($0.6\ Mol.L^{-1}$) system with 200µl sample volume

[KTF] $mMol.L^{-1}$	Number of injection (N)	Average response $\bar{y}_{i(mV)}$	σ_{n-1}	RSD %	Confidence interval at (95%) $\bar{y}_{i(mV)}\pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
9	6	288	1.83	0.64	288 ± 1.921
20	8	396	2.54	0.64	396 ± 2.124

$t_{0.025,n-1}$

$t_{0.025,5}$=2.571, $t_{0.025,7}$= 2.365

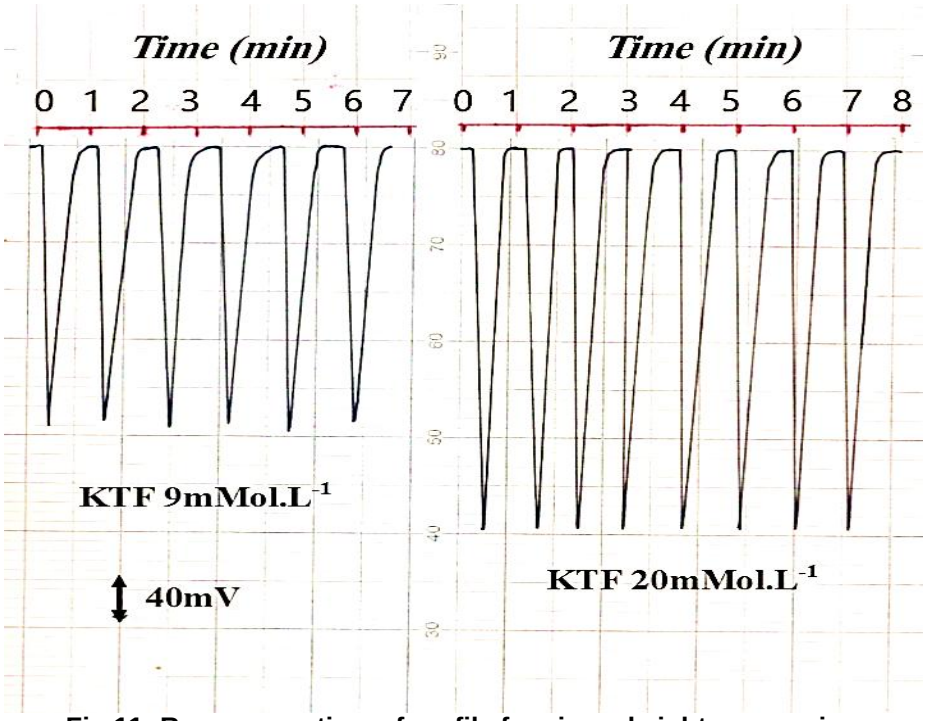


Fig.11: Response – time of profile for six and eight successive repeatable measurement of 9& 20mMol.L^{-1} of KTF

Application of the use of Ayah 6SX1-T-1D solar cell CFIAnalyser for determination of KTF in the pharmaceutical preparation & treatment of data

The aim and the target of any analytical method and it`sapplicability to do analysis for the determination of analyte in any specific given sample. The success of any analytical method is judged by it is reality to give repeatability and correct content of analyte .Two different companies of pharmaceutical preparations (Asmafort, Julphar , UAE , Privent , Micro, INDIA) having a constituent of 1 mgketotifenfumarate as an active ingredient were chosen for the applicability using two methods (Ayah 6SX1-T-ID solar cell CFI Analyser with six snow white light emitting diode as a source for measuring turbidity via attenuation of incident light . A series of solutions were prepared of each pharmaceutical drag (5mMol.L^{-1}) (53.187mg,$C_{23}H_{23}NO_5S$,M.wt: 425.497 g.mol^{-1},SDI of active ingredient in 25 ml) (C.F. in section 2.1.) by transferring 1.2 ml to each five volumetric flask (10ml), followed by the addition of gradual volumes of standard KTF (0,0.2,0.3,0.4&0.5) ml of 0.05 Mol.L^{-1} to obtain (0,1,1.5,2&2.5) mMol.L^{-1} using developed method (Ayah 6SX1-T-1D solar cell CFI Analser , while transferring 0.1ml to each five volumetric flask (10)ml, followed by the addition of gradual volumes of standard soluationof KTF (0.05mMol.L^{-1}) (0,0.01,0.015,0.02&0.025) ml to obtain (0,0.05,0.07,0.1&0.125) mMol.L^{-1} for the classical method (UV_ spectrophotometric at λmax=298nm) [19].

The measurements were conducted by both methods. **Fig 12-A,B**, showsprofile versus time and standard addition calibration graphs using developed method .While **Fig 12-C** standard addition calibration graphs using classical method .The results were summed up in **table 11 -A**, at confidence level 95%, showing practical concentration in each pharmaceutical preparations using two method of analysis .

Table 11-B. was shown a practical content of active ingredient expressed as a weight in mg and efficiency of determination in addition to paired t-test at two different comparison:-

First - Individual t-test

Comparing individual between mean ($\overline{w}_i$) which represented the practically content of KTF with quoted value (μ) [British pharmacopeia] as described by the manufacturer.**Table 11-B**Column 8 was shown individual dependent t-test . Two sources of two different companies and manufacturer were used : Asmafort, UAE , Privent , INDIA.

Assuming the following assumption :

Null hypothesis : H$_\circ$Asmafort, UAE $\bar{w}_i$ **= μ (1mg)**

Privent , INDIA $\bar{w}_i$ **= μ (1mg)**

Against

Alternative hypothesis: H_1

$\bar{w}_i \neq$ **μ (1mg)**

A calculated t value shows that at 0.05 probability a value of calculated t-value of |-0.7962|was obtained while at D_f (n-1) of the critical t-value was 4.303 this clearly indicate that the H$_\circ$ Should be accepted, that mean no significance difference is found between the quoted active ingredient and the measured value. On the same base of calculation for KTF in another companies and manufacturer .

Secondary

A paired t-test was conducted between the sample from two different manufacturer by either method of analysis i.e :using Ayah 6SX1-T-1D solar cell-CFI Analyser with classical method as shown in **table 11-B** column 11 follows:

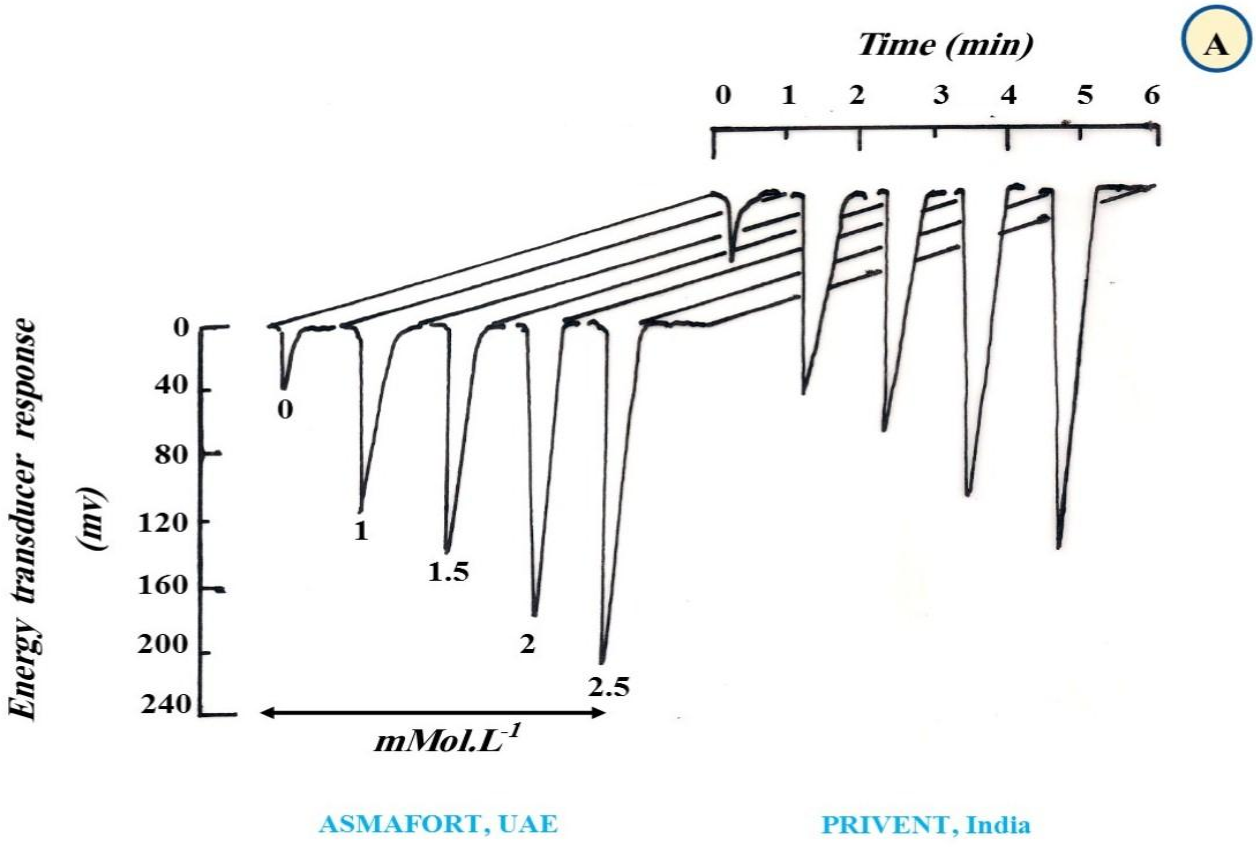
Null hypothesis: H$_\circ$: $\mu_{\text{Ayah 6SX1-T-1D solar cell-CFI}} = \mu_{\text{UV-spectrophotometric}}$

against

Alternative hypothesis :

$H_1 = \mu_{\text{Ayah 6SX1-T-1D solar cell-CFI}} \neq \mu_{\text{UV-spectrophotometric}}$

Since $t_{calculate}$= 0.351<t_{tab}=(12.71),therefore , H$_\circ$ is accepted against H_1 .These indicated , there is no significant different between two method .



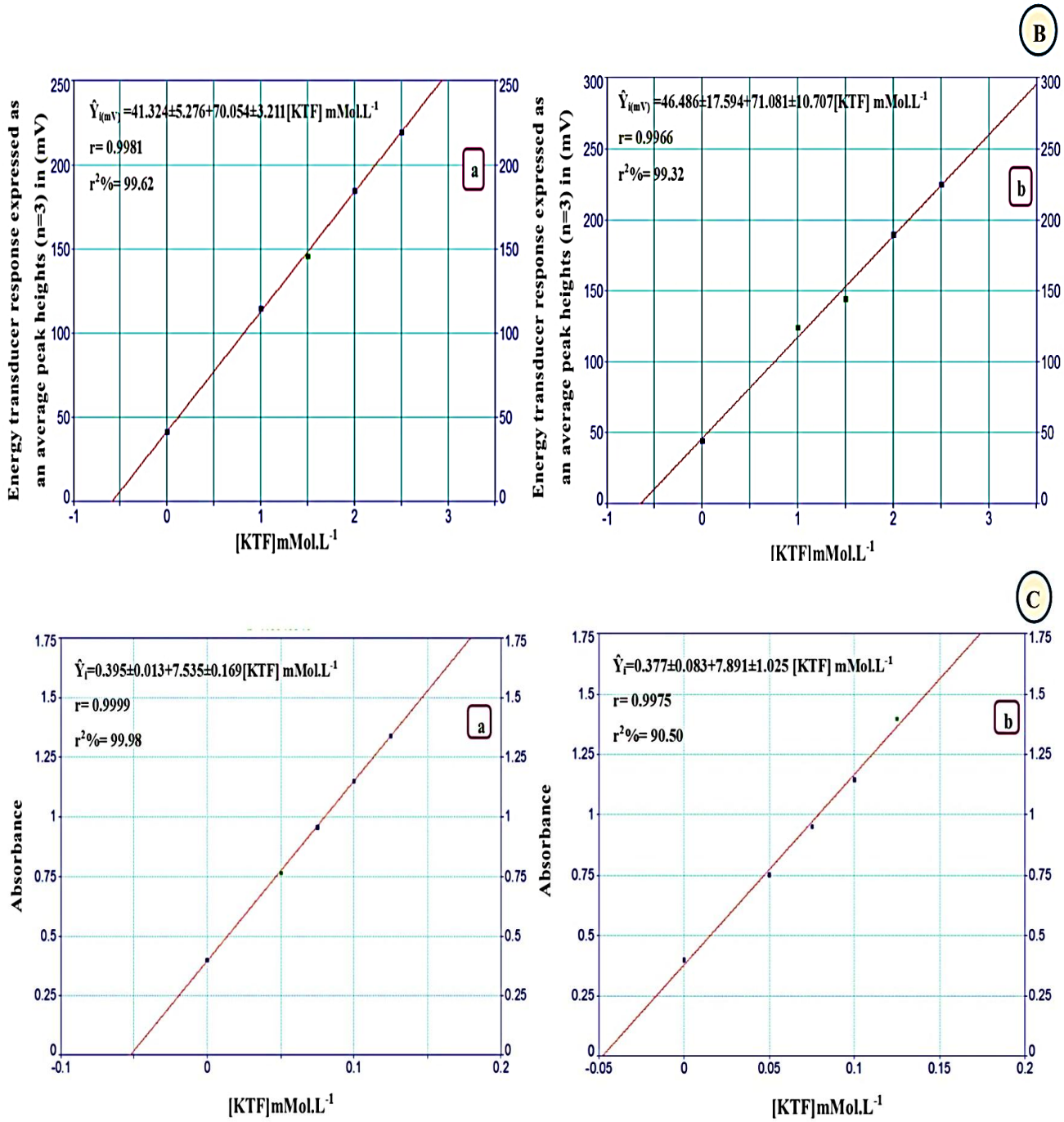


Fig. 12: Standard addition calibration graph and sample of response profile versus time for two pharmaceutical preparations (A): profile versus time.
(B): standard addition calibration graphs using developed method
(C): standard addition calibration graphs using classical method, a:Asmafort, UAE , b:Privent, INDIA .

Table 11A: A results for the determination of KTF in pharmaceutical preparation by standard addition method

sample no	commerical name ,content company,country	*Ayah 6SX1-T-1D solar CFIA (mV)* *UV- Spectrophotometric at λ_{max}=298nm (classical method for absorbance measurement)* [KTF]mMol.L^{-1}					Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[KTF] mMol.L^{-1} $\hat{Y}_i^*=a\pm s_a t+b\pm s_b t$[KTF]mMol.L^{-1}	r r^2 r^2%	Practical conc. mMol.L^{-1}
		0	1	1.5	2	2.5			in 10 ml
		0	0.05	0.075	0.1	0.125			In 25ml
1	ASMAFOR T,1mg, Julphar, UAE	42	115	140	185	220	41.324±5.276+70.054±3.211[KTF] mMol.L^{-1}	0.9981 0.9962 99.62%	0.590
									4.916
		0.400	0.766	0.958	1.149	1.341	0.395±0.013+7.535±0.169[KTF] mMol.L^{-1}	0.9999 0.9998 99.98%	0.052
									5.242
2	PRIVENT ,1mg, MICRO ,India	45	125	145	190	225	46.486±17.594+71.081±10.707[KTF] mMol.L^{-1}	0.9966 0.9932 99.32%	0.654
									5.450
		0.400	0.753	0.952	1.145	1.399	0.377±0.083+7.891±1.025 [KTF] mMol.L^{-1}	0.9975 0.9950 99.50%	0.048
									4.778

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

Table 11B: Summary of data for paired t-test, practical content and efficiency for determination of KTF in two samples of pharmaceutical preparation

Sample no	Confidence interval for the average weight of tablets $\overline{w}_i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Theoretical content for the active ingredient $\overline{w}_i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (mg)	Sample weight equivalent to 0.0532 g (5 mMol.L^{-1})of the active ingredient w_i(g)	Practical content of active ingredient		Efficiency of determination (Rec%)	Paired t-test			
				In 25 ml of sample $\overline{w}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95%, (mg)	in tablets $\overline{w}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95% ,(mg)		Individual comparison ($\overline{w}_i$ - μ_o)$\sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-T-1D Solar cell-CFI Analyser with Quoted value $t_{0.05/2,2}$=4.303	Comparison between two method		
				Ayah 6SX1-T-1D solar CFIA (mV)				Xd	$\overline{Xd}$ (σ_{n-1})	$t_{cal}=\overline{Xd}\sqrt{n}/\sigma_{n-1}$ at 95 %
				UV- SP. (classical method for absorbance measurement)						
1	0.1165±7.414× 10^{-5}	1 ±6.364× 10^{-4}	6.1978	52.2914 ± 4.9191	0.9829 ± 0.0925	98.29%	|-0.7962| ≪ 4.303	-0.065	0.035 0.141	0.351 ≪ 12.71
				55.7635 ± 9.8879	1.0482 ± 0.1859	104.82%				
2	0.1315 ± 5.668×10^{-5}	1 ± 4.310× 10^{-4}	6.9958	57.9727±6.3352	1.0897 ± 0.1191	108.97%	3.2435 ≪ 4.303	0.134		
				50.8213 ± 10.2212	0.9553± 0.1921	95.53%				

Xd: Difference between two method , $\overline{Xd}$: difference mean , σ_{n-1}:Difference standard deviation , n=3 for individual & n=2 for comparison between two method, μ(quoted value =1mg),
$t_{tab}=t_{0.05/2,n-1}=t_{0.025,2}$=4.303 for individual paired t-test , $t_{0.025,1}$=12.71 for comparison between two method .

CONCLUSION

The suggested method is simple, sensitivities and rapid. Application of the proposed methods to the analysis of ketotifen fumarate in pure and pharmaceutical preparation based on formation yellowish green color precipitate as an ion- pair compound for the reaction of ketotifen fumarate with phosphomolybdic acid in sodium chloride . 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.

REFERENCES

1. British Pharmacopoeia, Stationery Office, UK, 3rd edition, 2009.
2. Klooker K, Braak B, Koopman, Welting K, Wouters O, Schemann M. The mast cellstabiliserketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patientswith irritable bowel syndrome. Gut 59. 2010;9:1213–1220.
3. Abounassif M, Obeid H and Gadkariem E. Stability studies on some benzocycloheptaneantihistaminic agents. Journal of Pharmaceutical and Biomedical Analysis. 2005;36(5):1011–1018 .
4. Miki I, Kusano and Ohta. Histamine enhanced the TNF-α-induced expression of E selectin and ICAM-1 on vascular endothelial cells. Cellular Immunology. 1996;171(2):285–288.
5. Greiner J, Mundorf T and Dubiner H. Efficacy and safety of ketotifenfumarate 0.025% in theconjunctival antigen challenge model of ocular allergic conjunctivitis. American Journal ofOphthalmology. 2003;136(6):1097–1105.
6. Fei N and Jiuru L. Determination of ketotifen by using calcein as chemiluminescence reagent.AnalyticaChimicaActa. 2007;592(2):168–172,.
7. El-Kousy N and Bebawy L. Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods. Journal of Pharmaceutical and Biomedical Analysis. 1999;20(4):671–679.
8. Sastry C and Naidu Y. pectrophotometric estimation of ketotifenfumarate in pharmaceuticalformulations. MikrochimicaActa. 1977;127(3):219–223.
9. Amanlou M, Nazlou H, Azizian E, Souri M and Farsam H. Determination of Ketotifenfumarate in raw material and pharmaceutical products using ion-pair formation. Analytical Letters. 2007;40(17):3267–3279.
10. Singhvi I and Sachdeva D. Spectrophotometric estimation of ketotifenfumarate from tabletformulations .Indian Journalof Pharmaceutical Sciences. 2009;71(1):66–68.
11. Alali Q, Tashtoush M and Najib M. Determination of ketotifen in human plasma by LC-MS.Journal of Pharmaceuticaland Biomedical Analysis. 2004;34(1):87–94.
12. Nnane L, Damani A and Hutt Y. Development and validation of stability indicating high-performance liquid chromatographic assays for ketotifen in aqueous and silicon oil formulations. Chromatographia. 1998;48(11):797–802.
13. Daneshgar P, Norouzi M and Ganjali N. Application of a continuous square-wave potential program for sub nano molar determination of ketotifen .Chemical and Pharmaceutical Bulletin. 2009;57(2):117–121.
14. Julien L, Guerret D, Lavene K and Kiechel R. Quantification of ketotifen and its metabolites in human plasma by gas chromatography mass spectrometry. Biomedical Mass Spectrometry. 1983;10(3):136–142.
15. Chen D, Zhong D and Liu Y. Determination of ketotifen and its conjugated metabolite in human plasma by liquid chromatography/tandem mass spectrometry: Application to a pharmacokineticstudy.RapidCommunications in Mass Spectrometry. 2003;17(22):2459–2463.
16. Ghoreishi S, Behpour M, Zahrani H and Golestaneh M. Preparation and optimization of a ketotifen sensor and its pharmaceutical applications. Analytical &Bioanalytical Electrochemistry. 2010;2(3):112–124.
17. Khater Y, Issa M and Mohammed S. Flow injection determination of ketotifenfumarate using PVC membrane selective electrodes. Bioelectrochemistry. 2009;77(1):53–59.
18. Tabrizivand R, Sabzi M and Farhadi K. Preparation and characterization of a new carbon pasteelectrode based on ketotifen-hexacyanoferrate. Journal of Solid State Electrochemistry. 2006;11(1):103–108.
19. Selvadurai M, Lim H, John Y, Sailishni J and Sokkalingam A. Development of a spectrophotometry method for the estimation of Ketotifenfumarate in bulk and the pharmaceutical tablet dosage form. Scholar Research Library. 2012;4(5):1339-1343.