

# QUANTITATIVE DETERMINATION OF KETOTIFEN FUMARATE IN THE PURE FORM AND TABLETS VIA THE USE OF TURBIDITY BY REACTION WITH CERIUM(IV) SULFATE USING AYAH 6SX1-T-1D CFI ANALYSER

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## ABSTRACT

Newly developed analytical method characterized by its speed and sensitivity has been developed for the determination of ketotifen fumarate (KTF) in pure and pharmaceutical preparation is established via turbidimetric measurement (0-180°) by Ayah 6SX1-T-1D Solar cell CFI Analyser. The method was based on the reaction of Ce(IV)sulfate with ketotifen fumarate in sodium chloride medium to form greenish blue precipitate as an ion-pair complex. Turbidity was measured via the attenuation of incident light that collides on the surface precipitated particles at 0-180°. The chemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 0.1-50 mMol.L<sup>-1</sup>, with correlation coefficient  $r=0.9975$ . The limit of detection 1.136 µg/sample from the step wise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.8% for 5 and 10 mMol.L<sup>-1</sup> (n=8,6 respectively) concentration of ketotifen fumarate. The method was successfully applied to the determination of ketotifen fumarate in two pharmaceuticals. A comparison was made between the newly developed method analysis and the classical method, in addition to between two different pharmaceutical preparations (UV- spectrophotometry at wave length 298 nm) using the standard additions method via the use of F-test. It was noticed that there was no significant difference between two methods at 95 % confidence level and no significant difference between two drugs.

**Keywords:** Ketotifen fumarate, Flow injection analysis , Turbidity.

## INTRODUCTION

Ketotifen is a second-generation H1-antihistamine and mast cell stabilizer. The chemical name of Ketotifen fumarate is 4-(1-Methyl-4-piperidylidene)-4H-benzo[4,5]cyclohepta[1,2-b]thiophen-10(9H)-one hydrogen fumarate. Ketotifen fumarate is a finely crystalline powder with an empirical formula of C<sub>23</sub>H<sub>23</sub>NO<sub>5</sub>S and a molecular weight of 425.50 g.mol<sup>-1</sup>. The structure of Ketotifen fumarate was shown in **Figure 1** [1].

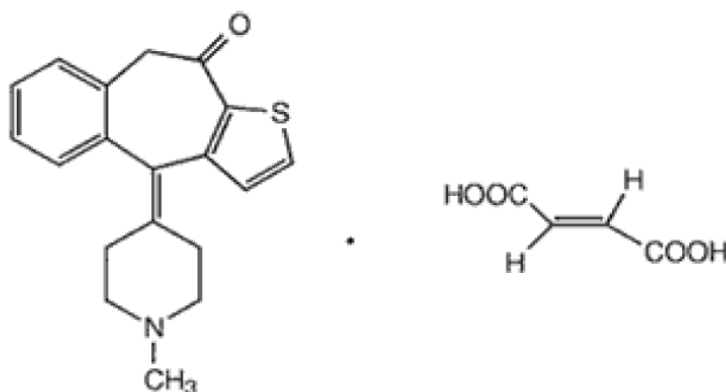


Fig. 1: Structure of Ketotifen fumarate

Ketotifen fumarate is able to inhibit the release of histamine, other relay basophils 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 is most commonly available as a salt of Fumaric Acid, Ketotifen fumarate, 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, it also works antianaphylactically. In its oral form, it is used to prevent asthma attacks. Side effects include drowsiness, weight gain, dry mouth, irritability, and increased nosebleeds [2-3]. 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 [4-5].

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 and H2 receptors. It annihilates effects of histamine as a mediator of inflammation and immune response. Ketotifen fumarate 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 Ketotifen fumarate in pharmaceutical formulations. The method based on the formation of greenish blue color precipitate as an ion-pair compound by Ce(IV) with Ketotifen fumarate in sodium chloride medium.

## EXPERIMENTAL

### REAGENTS AND CHEMICALS

All chemicals were used of analytical-reagent grade and distilled water used to prepare the solutions. A standard solution ( $0.05 \text{ Mol.L}^{-1}$ ) of Ketotifen fumarate  $\text{C}_{23}\text{H}_{23}\text{NO}_5\text{S}$  ( $425.497 \text{ g.mol}^{-1}$ ) was prepared by dissolving 5.3187 g in 250 ml distilled water. A stock solution ( $0.1 \text{ Mol.L}^{-1}$ ) of Ceric sulfate  $\text{Ce}(\text{SO}_4)_2$  ( $332.298 \text{ g.mol}^{-1}$ ) was prepared by dissolving 8.3075g in 250 ml sulfuric acid  $\text{H}_2\text{SO}_4$  ( $1 \text{ Mol.L}^{-1}$ ). A stock solution of acids Hydrochloric acid HCl (35% w/w,  $1.18 \text{ g.mL}^{-1}$ , BDH,  $1 \text{ Mol.L}^{-1}$ ), Sulfuric acid  $\text{H}_2\text{SO}_4$  (96% w/w,  $1.84 \text{ g.mL}^{-1}$ , BDH,  $1 \text{ Mol.L}^{-1}$ ), Nitric acid  $\text{HNO}_3$  (70% w/w,  $1.42 \text{ g.mL}^{-1}$ , BDH,  $1 \text{ Mol.L}^{-1}$ ), Acetic acid  $\text{CH}_3\text{COOH}$  (99.5% w/w,  $1.05 \text{ g.mL}^{-1}$ , BDH,  $1 \text{ Mol.L}^{-1}$ ) all were prepared by pipetting 88.28 ml, 55.52 ml, 63.39 ml and 57.47 ml respectively of concentrated acids and complete the volume with distilled water to 1000 ml volumetric flasks. Each acid was standardized against standard solution from  $\text{Na}_2\text{CO}_3$  (BDH,  $105.99 \text{ g/mol}$ ,  $0.1 \text{ Mol.L}^{-1}$ ); which prepared by Dried in an oven at  $115^\circ\text{C}$  for overnight before weighting. Sodium chloride NaCl ( $0.5 \text{ Mol.L}^{-1}$ ) was prepared by dissolving 0.146 g in 50 ml distilled water. Potassium bromide KBr ( $0.5 \text{ Mol.L}^{-1}$ ) was prepared by dissolving 2.975 g in 50 ml distilled water. Potassium nitrate  $\text{KNO}_3$  ( $0.5 \text{ Mol.L}^{-1}$ ) 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 Ketotifen fumarate for (Julphar, Micro companies) were weight (6.1978, 6.9958 g) equivalent to 0.0532 g of active ingredient respectively to obtain  $5 \text{ mMol.L}^{-1}$  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 (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 KTF using 1 cm quartz cell.

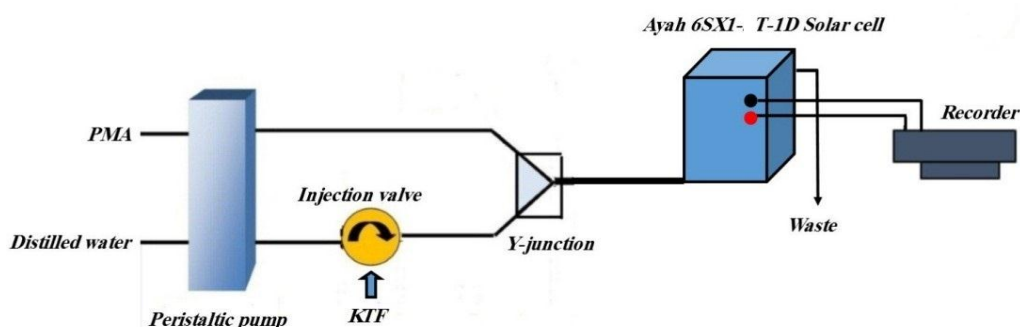
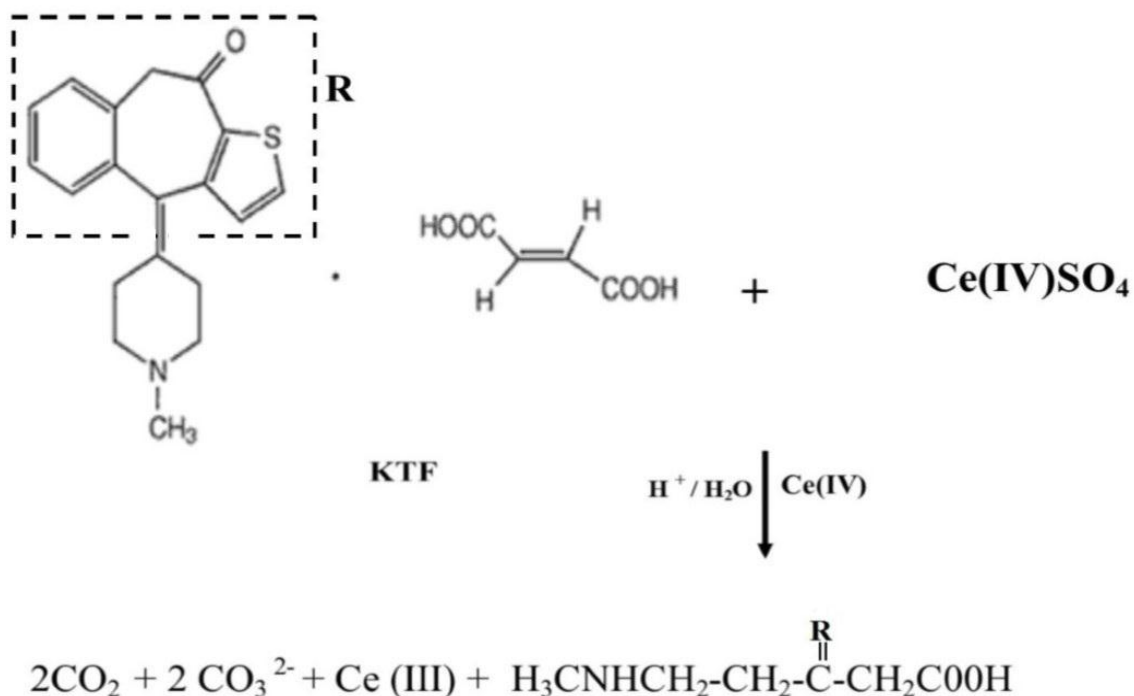


Fig. 2: Flow diagram manifold system used for the determination of KTF

### Methodology

The flow system consisting of two lines was used for the determination of KTF by the reaction between KTF and Ce(IV) Sulfate ( $7 \text{ mMol.L}^{-1}$ ) in sodium chloride medium to form a greenish blue color precipitate as an ion pair complex form. The first line represent the carrier stream (sodium chloride) at  $1.6 \text{ ml.min}^{-1}$  flow rate which lead to the injection valve to carry KTF, sample volume  $267 \mu\text{l}$ ; while the second line supplies solution at  $1.2 \text{ ml.min}^{-1}$  Ce (IV) Sulfate. 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  $0-180^\circ$ . A probable mechanism of ion pair formation for KTF–Ce (IV) system is represented in **scheme 1**. [20-22].



**Scheme-1: Proposed mechanism of reaction between of KTF & Ce(IV) Sulfate**

## RESULTS AND DISCUSSION

### Variable optimization

The flow injection manifold design as shown in **Fig.2** was employed for the study of chemical and physical variables in order to obtain the optimum conditions for determination of KTF using KTF-Ce(IV) sulfate system.

### Chemical variables

#### [Ce(IV) Sulfate] Concentration

Using variable concentration of Ceric (IV) Sulfate  $0.5\text{-}9\text{ mMol.L}^{-1}$ , a constant concentration of KTF ( $7\text{ mMol.L}^{-1}$ ) with sample volume  $200\mu\text{L}$  at  $1.6\text{ mL.min}^{-1}$  flow rate for carrier stream. It was noticed that incident light is attenuated with increasing Ce(IV) ion concentration up to  $7\text{ mMol.L}^{-1}$ ; followed by a steady constant profile response describing the availability and the requirement of formation of optimum precipitate amount at  $7\text{ mMol.L}^{-1}$  which then in turn regarded as the optimum concentration of Ce(IV) sulfate. **Fig. 3 A,B** shows that  $7\text{ mMol.L}^{-1}$  of [Ce(IV) sulfate] is the optimum concentration and **Table 1** summarizes the total results obtained.

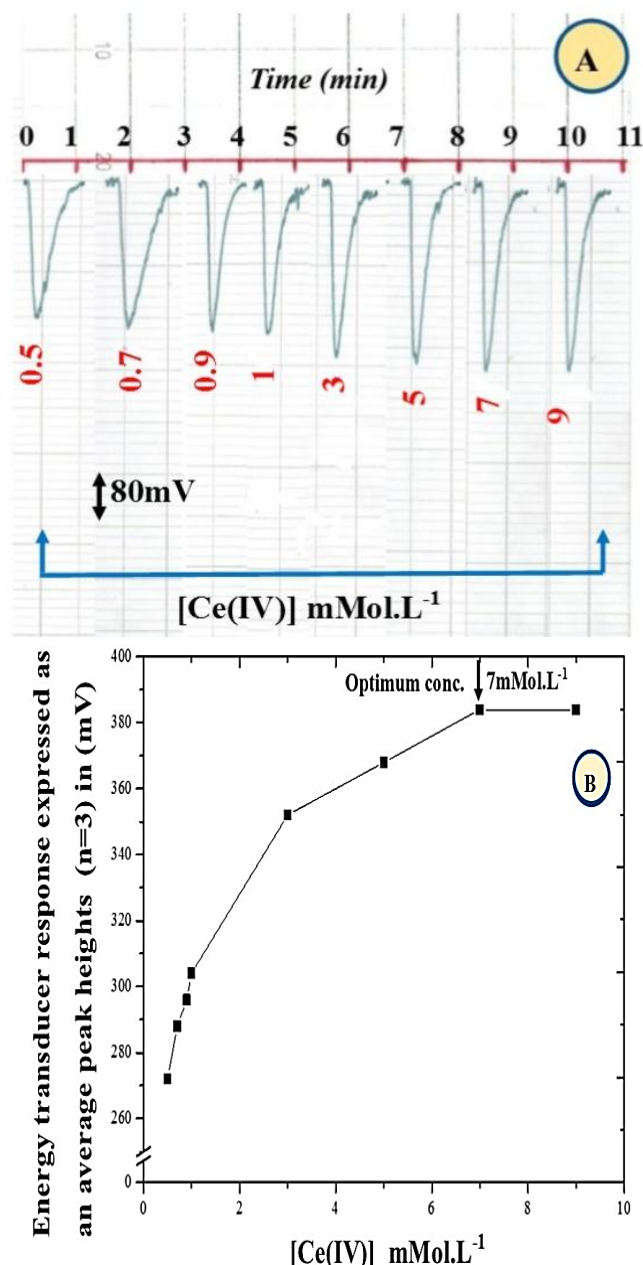


Fig. 3: Variation of [Ce(IV) sulfate] on:  
 (A): response profile for KTF-Ce(IV) Sulfate system .  
 (B):transducer energy response(mV)expressed as an attenuation of incident light

Table 1: Variation of Ce(IV) sulfate concentration on the transducer energy response of KTF-Ce(IV)Sulfate system

[Ce(SO <sub>4</sub> ) <sub>2</sub> ] mMol.L <sup>-1</sup>	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
0.5	272	0.36	272±2.433
0.7	288	0.27	288±1.932
0.9	296	0.11	296±0.809
1	304	0.16	304±1.208
3	352	0.11	352±0.962
5	368	0.29	368±2.651
7	384	0.32	384±3.053
9	384	0.34	384±3.244

### Effect of acidic media

The precipitation of KTF by Ce(IV)Sulfate as a reagent was studied in the different acidic media (Sulfuric acid, hydrochloric acid, Acetic acid and nitric acid) at  $0.1\text{Mol.L}^{-1}$  concentration in addition to the aqueous medium. Fig.4 A,B shows the variation of transducer energy response expressed as average peak height ( $n=3$ ) in mV with different media; which shows that when used different acids as a carrier streams gave in general distorted or deformed unstable profile; in addition to the formation of precipitates at the injection valve in which KTF sample solution is injected. This might possibly due to negative radical anion resulted from the dissociation of used acids which might lead to the precipitation of KTF previous to the reacting with oxidant Ce(IV) sulfate and on this basis a return to the use of distilled water was stuck to as the most suitable carrier for the studied in hand reaction system. Table 2 summed up the results which obtained from this studied.

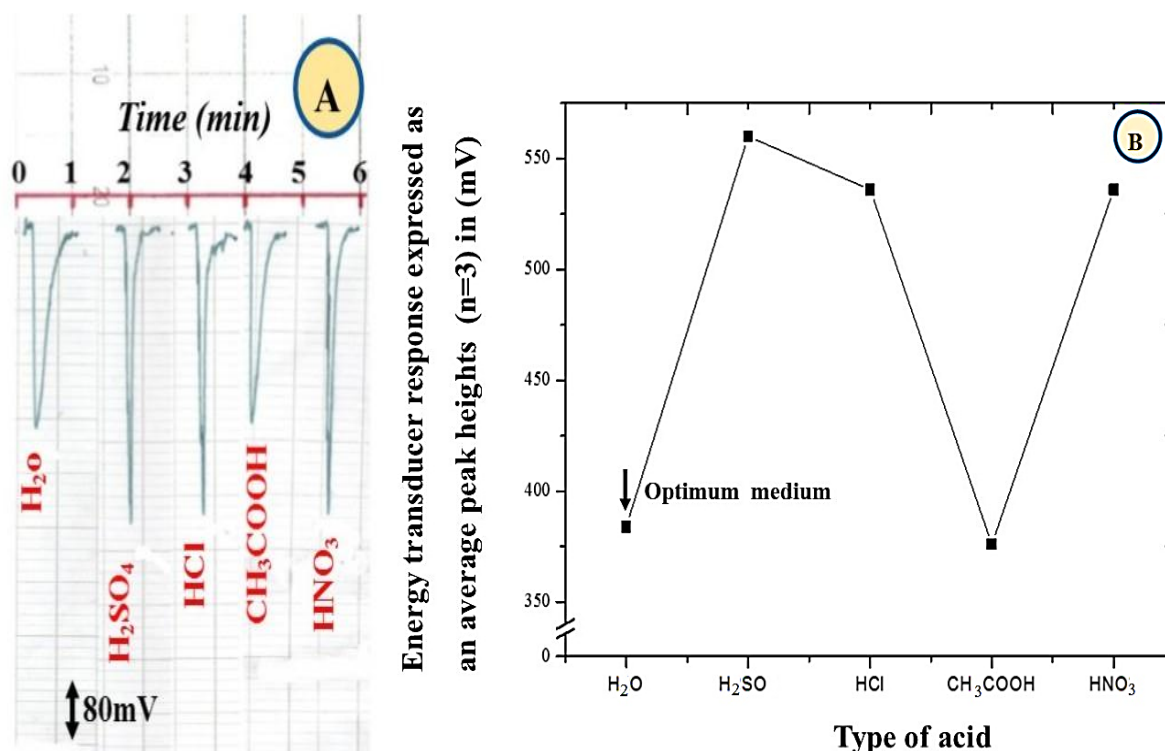


Fig. 4: Effect of different acids concentration on :  
 (A): Response profile versus time,  
 (B): Energy transducer response expressed as an average peak heights

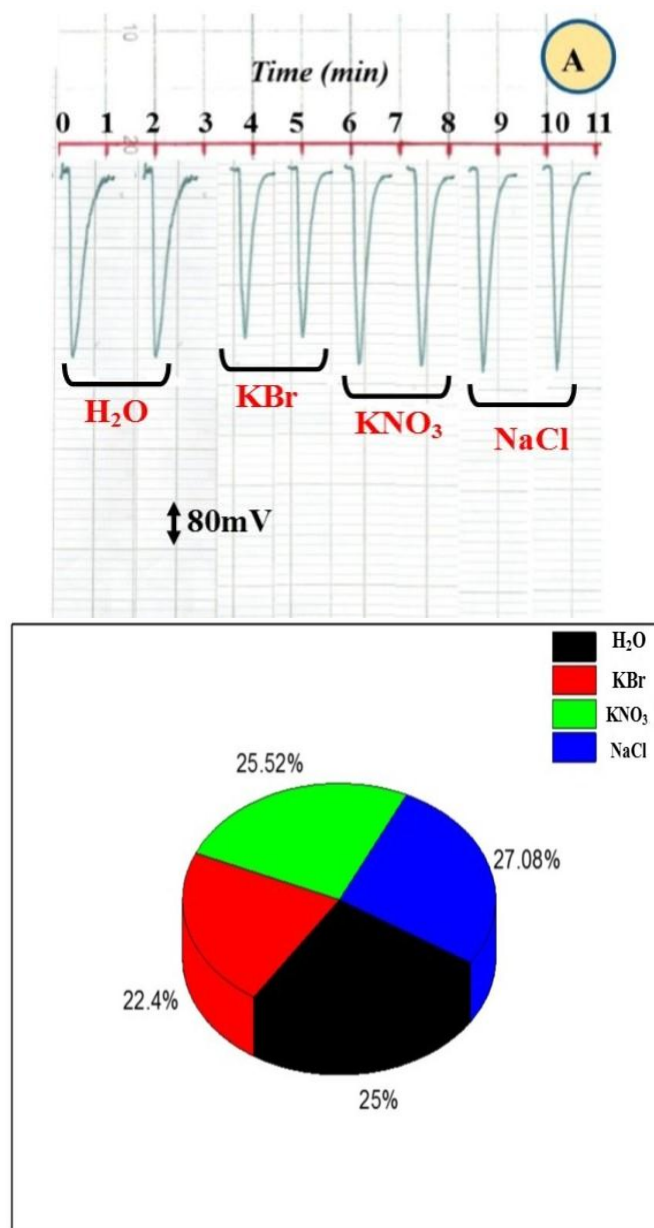
Table 2: Effect of different acid concentration as a carrier stream on the transducer energy response for determination of KTF

Type of medium	Energy transducer response expressed as an average peak heights ( $n=3$ ) $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
H <sub>2</sub> O	384	0.32	$384 \pm 3.053$
H <sub>2</sub> SO <sub>4</sub>	560	0.42	$560 \pm 5.843$
HCl	536	0.56	$536 \pm 7.457$
CH <sub>3</sub> COOH	376	0.54	$376 \pm 5.044$
HNO <sub>3</sub>	536	0.56	$536 \pm 7.457$

### Effect of variable media

The oxidation of KTF ( $7\text{mMol.L}^{-1}$ ) by [Ce(IV)sulfate] ( $7\text{mMol.L}^{-1}$ ) was studied in different salts media (potassium bromide, potassium nitrate & sodium chloride) at  $0.3\text{Mol.L}^{-1}$  concentration in addition to aqueous medium as a carrier stream; due to expect that these salt solutions increase the formation of a dense precipitate. From this study observed that the effect of using different salt concentration as a carrier stream did not indeed improve the obtained signal except that NaCl solution gave higher response profile. This might be probably due to increased agglomeration of precipitate formed. Which

explain that the profile obtained is more sharper than the rest as shown in **Fig 5**. **Table 3** summed up the obtained results for the effect of different salts media on the attenuation of incident light response expressed as an energy transducer response.



**Fig.5: Effect of the different media on:**  
**(A): Response profile versus time using 200 $\mu$ l sample volume.**  
**(B):Energy transducer response in (mV).**

**Table 3: Effect of different media on the measurement of energy transducer response for determination of ketotifen fumarate using 200 $\mu$ l sample volume and Ce(IV) Sulfate as an oxidant agent**

Type of medium	Energy transducer response expressed as an average peak heights(n=3) $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
H <sub>2</sub> O	384	0.32	384 $\pm$ 3.053
KBr	344	0.37	344 $\pm$ 3.162
KNO <sub>3</sub>	392	0.33	392 $\pm$ 3.214
NaCl	416	0.44	416 $\pm$ 4.547

### Effect of variable concentration of sodium chloride

A series of solutions were prepared at ranging (0.005-0.7)Mol.L<sup>-1</sup> of sodium chloride as a carrier stream, and using KTF (7 mMol.L<sup>-1</sup>)-Ce(IV)Sulfate (7mMol.L<sup>-1</sup>) system with 200 $\mu$ l sample volume at 1.6 ml.min<sup>-1</sup> and 1.2 ml.min<sup>-1</sup> flow rate for carrier stream and reagent solution respectively. It was realized that with increase the concentration of NaCl; it causes an increased attenuation effect (via the increased negative profile response ) up to 0.5 Mol.L<sup>-1</sup>(Fig 6 A,B) ; followed by a little increase in the obtained response .This slight increase might be due to the increased effect of coagulation. In order to maintain the most possible life time of the injection valve due to the high concentration of salt solution ; 0.5 Mol.L<sup>-1</sup> was chosen as the best possible choice.The results from this study tabulated in Table 4.

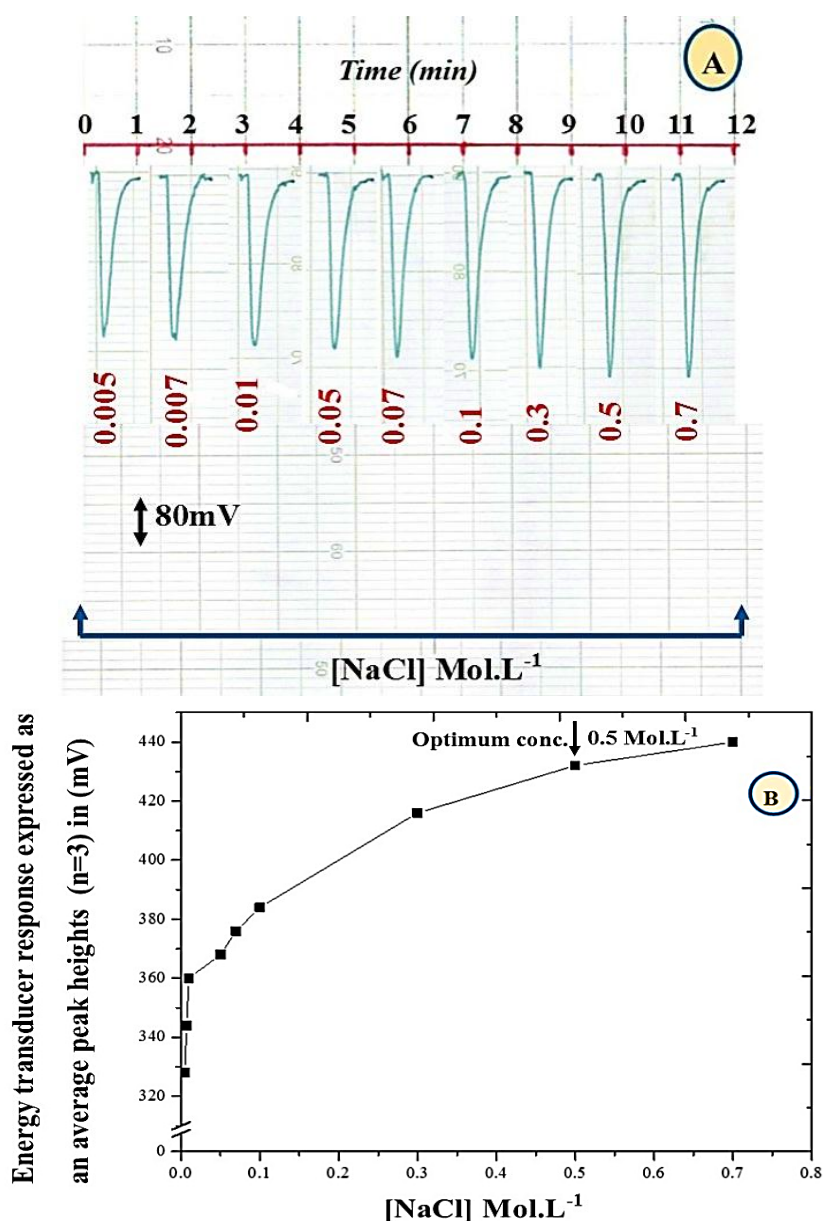


Fig. 6 : Effect of NaCl concentration as a carrier stream on :  
 (A): Response profile versus time,  
 (B): Energy transducer response expressed as an average peak heights in(mV)



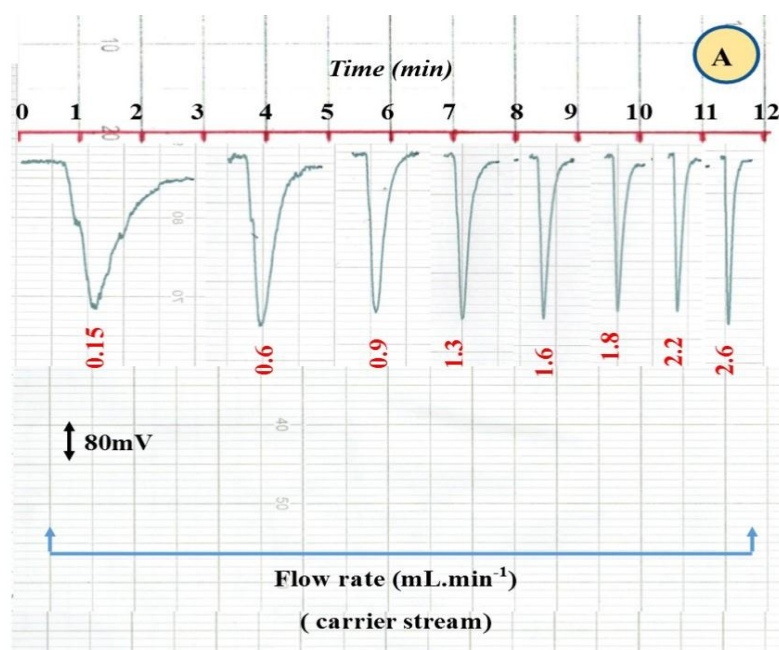
**Table 4: Effect of [NaCl] as a carrier stream on the transducer energy response for determination of KTF using KTF-Ce(IV)Sulfate reaction**

[NaCl] Mol.L <sup>-1</sup>	Energy transducer response expressed as an average peak heights(n=3) $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
0.005	328	0.30	328±2.445
0.007	344	0.30	344±2.564
0.01	360	0.30	360±2.683
0.05	368	0.34	368±3.108
0.07	376	0.32	376±2.989
0.1	384	0.34	384±3.244
0.3	416	0.30	416±3.100
0.5	432	0.29	432±3.112
0.7	440	0.29	440±3.170

### Physical variables

#### Flow rate

Using KTF (7 mMol.L<sup>-1</sup>)-Ce(IV)Sulfate (7 mMol.L<sup>-1</sup>) system in 0.5 Mol.L<sup>-1</sup>NaCl medium with variable flow rates (0.15-2.6 mL.min<sup>-1</sup>) for carrier stream & reagent respectively controlled by the peristaltic pump. **Fig 7** shows that at low flow rate there is an increase in turbidity and attenuation of incident light but wider base width (increase of analysis time), this might be attributed to the increase of dispersion due to diffusion which lead to increase of area of precipitate particle segment in flow cell up to 1.6 & 1.2 mL.min<sup>-1</sup> for carrier stream and reagent respectively (**Table 5**). While at higher pump speed; although the profile was characterized a regular & sharp maxima but it is not very high due to departure of precipitate particle from measuring cell at a short time. On this basis a flow rate of 1.6 & 1.2 mL.min<sup>-1</sup> for carrier stream & Ce(IV)Sulfate line respectively was chosen optimum flow rate.



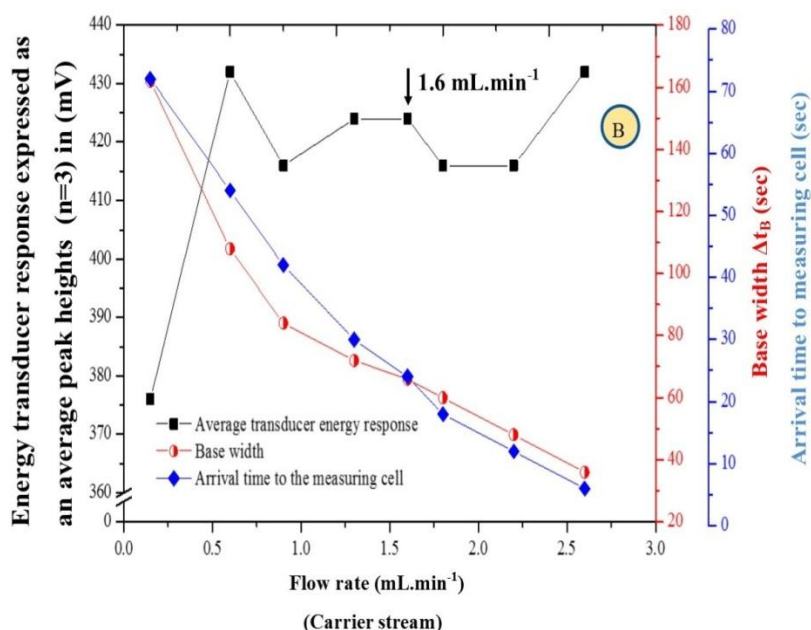


Fig.7: variation of flow rate on:

- (A): response profile using Ayah 6SX1-T-1D solar cell CFIA, for determination of KTF.  
 (B): transducer energy response for KTF, Peak base width ( $\Delta t_B$ ) and time for the departure of sample segment from injection valve reaching to the measuring flow Cell

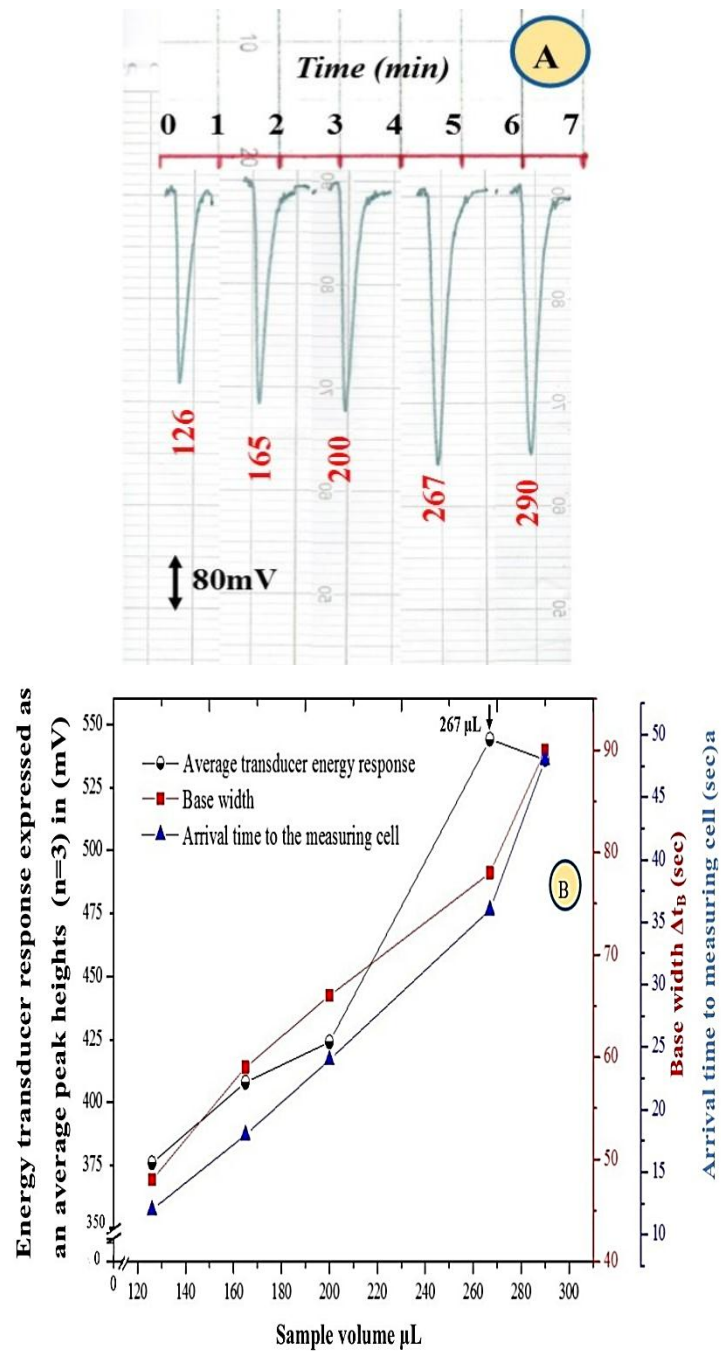
Table 5: Variation of flow rate (ml.min<sup>-1</sup>) on the transducer energy response (mV)

Pump speed (approximate)	Flow rate ml .min <sup>-1</sup>		Energy transducer response expressed as an average peak heights (n=3) $\bar{y}$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y} \pm t_{0.05/2, n-1} \sigma_{n-1}/\sqrt{n}$	Base width $\Delta t_B$ (sec)	t sec	V ml	C mMol.L <sup>-1</sup>
	Carrier stream	Reagent							
5	0.15	0.15	376	0.06	376±0.560	162	72	1.010	1.386
10	0.6	0.5	432	0.28	432±3.005	108	54	2.180	0.642
15	0.9	0.8	416	0.30	416±3.100	84	42	2.580	0.543
20	1.3	1	424	0.29	424±3.055	72	30	2.960	0.473
25	1.6	1.2	424	0.29	424±3.055	66	24	3.280	0.427
30	1.8	1.4	416	0.24	416±2.480	60	18	3.400	0.412
35	2.2	1.6	416	0.07	416±0.723	48	12	3.240	0.432
40	2.6	2	432	0.44	432±4.722	36	6	2.960	0.473

V: volume at flow cell. C: Concentration at flow cell.  $\Delta t_B$ : base width of response  
 t: Arrival time from injection valve reaching to the measuring cell.

### Sample volume

Variation sample volumes (126, 165, 200, 267 & 290  $\mu$ L) with open valve mode were studied at optimum flow rate 1.6 mL.min<sup>-1</sup> for the carrier stream and 1.2 mL.min<sup>-1</sup> for the Ce(IV) sulfate solution (reagent line). A selected concentration 7 mMol.L<sup>-1</sup> of KTF and optimum concentration of Ce(IV) sulfate (7 mMol.L<sup>-1</sup>) were used. It was found that an increase in the sample volume led to an increase in the attenuation of incident light (increase of peak height) than low sample volume as shown in Fig.8 up to 267  $\mu$ L gave regular response. While when using sample volume > 267  $\mu$ L it gave a slightly decrease in height of response due to continuous passage of carrier stream through the injection valve which in turn to cause a slow movement of particles in front of flow cell. There for 267  $\mu$ L was found to the best sample volume that gave a sharp regular response. All results were tabulate in Table 6.



**Fig.8 : Effect of the variation of sample volume on:**  
**(A): Response profile by attenuation light using Ayah 6SX1-T-1D -Solar cell CFI Analyser versus time.**  
**(B): Energy transducer response expressed as an average peak heights in (mV)**

**Table 6: Effect of the variation of sample volume on the energy transducer response for determination of KTF using KTF –Ce(IV) Sulfate system in 0.5 Mol.L<sup>-1</sup> NaCl medium**

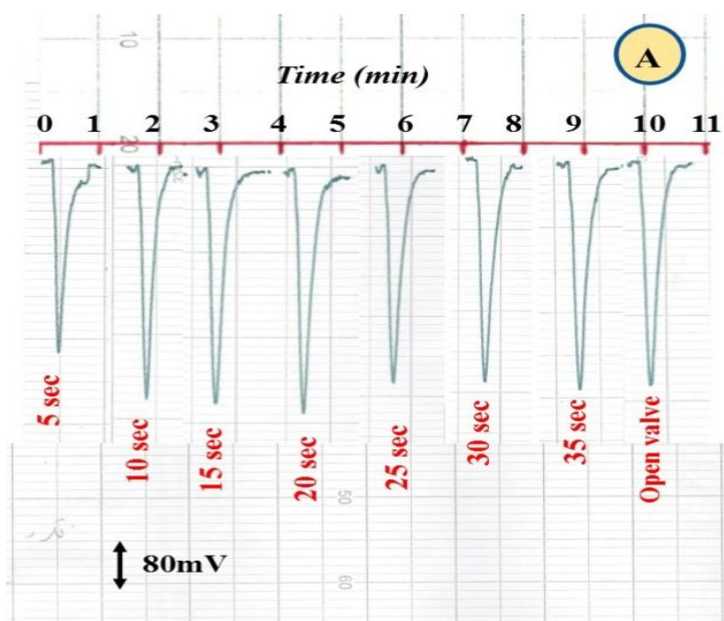
Loop length Cm r=0.5 mm	Sample volume $\mu$ l	Energy transducer response expressed as an average peak height (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1}/\sqrt{n}$	Base width $\Delta t_b$ (sec)	t sec
16	126	376	0.39	376 $\pm$ 3.643	48	12
21	165	408	0.34	408 $\pm$ 3.446	59	18
25	200	424	0.35	424 $\pm$ 3.687	66	24
34	267	544	0.28	544 $\pm$ 3.784	78	36
37	290	536	0.29	536 $\pm$ 3.862	90	48

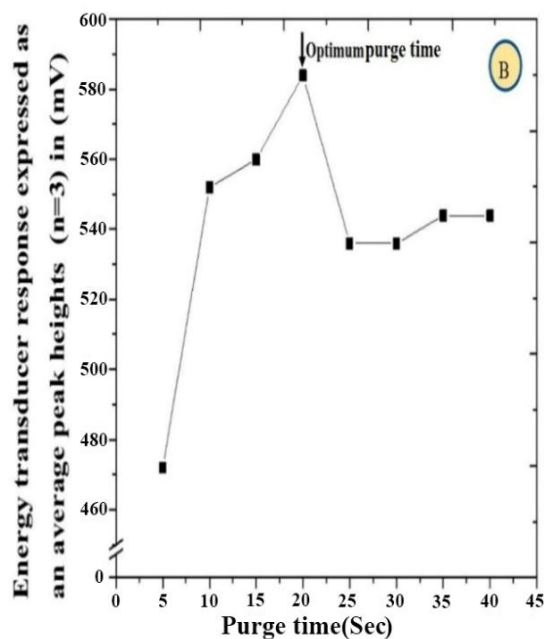
t: Arrival time from injection valve reaching to the measuring cell.

$\Delta t_b$ : base width of response.

### Purge time

Using different purge time for the injected KTF as a sample, ranging 5–35 sec as an allowed permissible time for the KTF sample to passing through the injection valve (injection mode) followed by turning the injection valve to the load position. in this study in addition to allowing the injection valve to be in the open mode. Optimum sample volume of 267  $\mu$ L was used , KTF (7 mMol.L<sup>-1</sup>)-Ce(IV) Sulfate (7 mMol.L<sup>-1</sup>) in 0.5 Mol.L<sup>-1</sup> NaCl medium were used . **Fig.9** shows continuation of the increase the height of response with increase of purge time up to 20 sec , after than there was a decrease in peak height which might be attributed to the increase of physical variable , i.e : dispersion & dilution due to a longer time passage of carrier stream through the valve. On this basis; the permissible time for sample segment departure from the injection valve was 20 sec. The results obtained were tabulated in **Table 7**.





**Fig. 9: Variation of purge time on:**  
**(A): response profile using Ayah 6XS1-T-1D solar cell CFIA Analyser**  
**(B): transducer energy response using optimum condition**

**Table 7: Variation of purge time on the transducer energy response using 267  $\mu\text{L}$**

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	472	0.21	472 $\pm$ 2.462
10	552	0.15	552 $\pm$ 2.057
15	560	0.05	560 $\pm$ 0.696
20	584	0.07	584 $\pm$ 1.016
25	536	0.05	536 $\pm$ 0.666
30	536	0.07	536 $\pm$ 0.932
35	544	0.09	544 $\pm$ 1.216
Open valve	544	0.04	544 $\pm$ 0.541

### Effect of coil length

Variable coil length (0-100) cm was studied, this range of length comprises a volume of 0–0.785 ml which is connected after Y-junction directly in flow system (**Fig.2**). Optimum concentration of Ce(IV) sulfate (7 mMol.L<sup>-1</sup>) and 7 mMol.L<sup>-1</sup> of KTF with sample volume 267  $\mu\text{L}$  were used. **Fig.10 A,B** shows type of response profile and **Table 8** tabulated all the obtain results of coil length that effect on the precipitate particles formation of KTF with Ce(IV) as an oxidant agent which in turn to affect on the attenuation of incident light due to increase of distribution of particles on a larger area that might give an increase in the  $\Delta t_B$  (i.e : base width) of the response. Therefore, delay coil was not used due to it is decreasing effect on the obtained response profile.

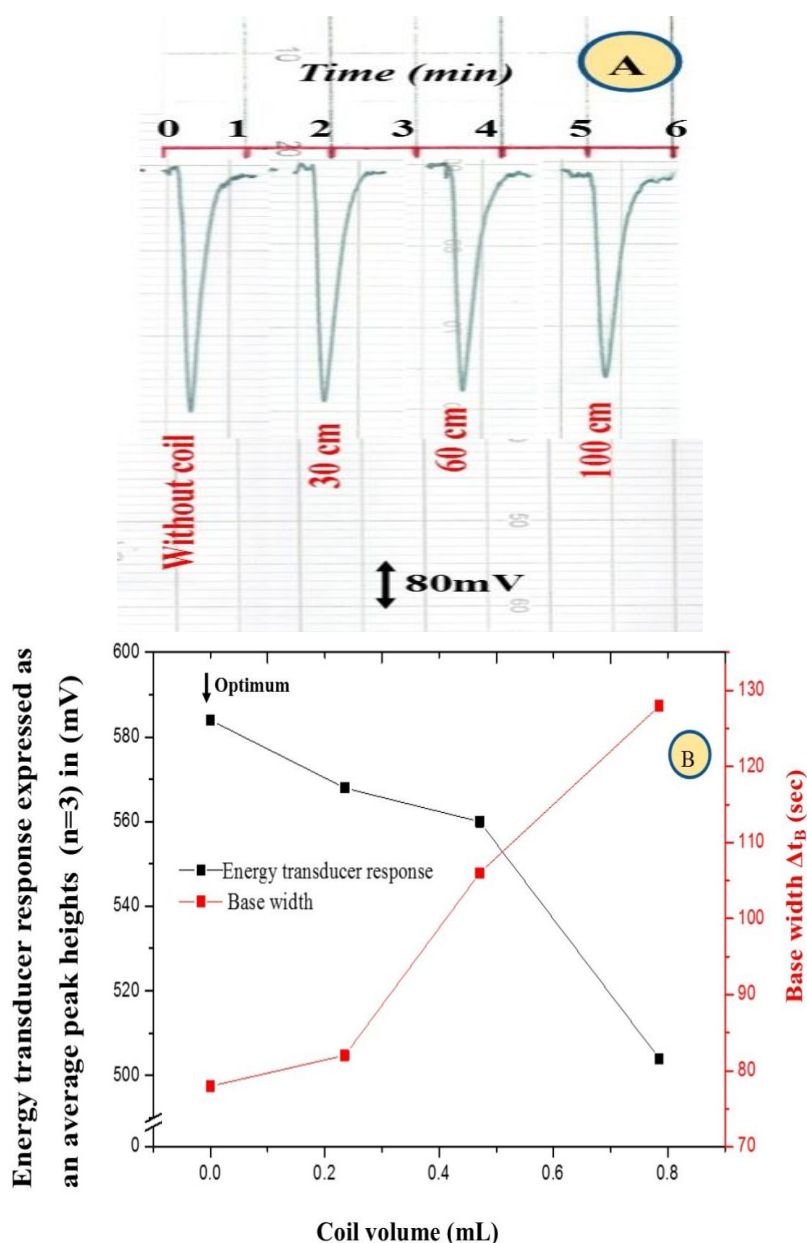


Fig. 10: Effect of reaction coil on :  
(A): Response profile vs. time

(B): Energy transducer response expressed as an average peak heights in mV, using KTF (7 mMol.L<sup>-1</sup>) –Ce(IV)Sulfate (7 mMol.L<sup>-1</sup>) system in 0.5 Mol.L<sup>-1</sup> NaCl as a carrier stream

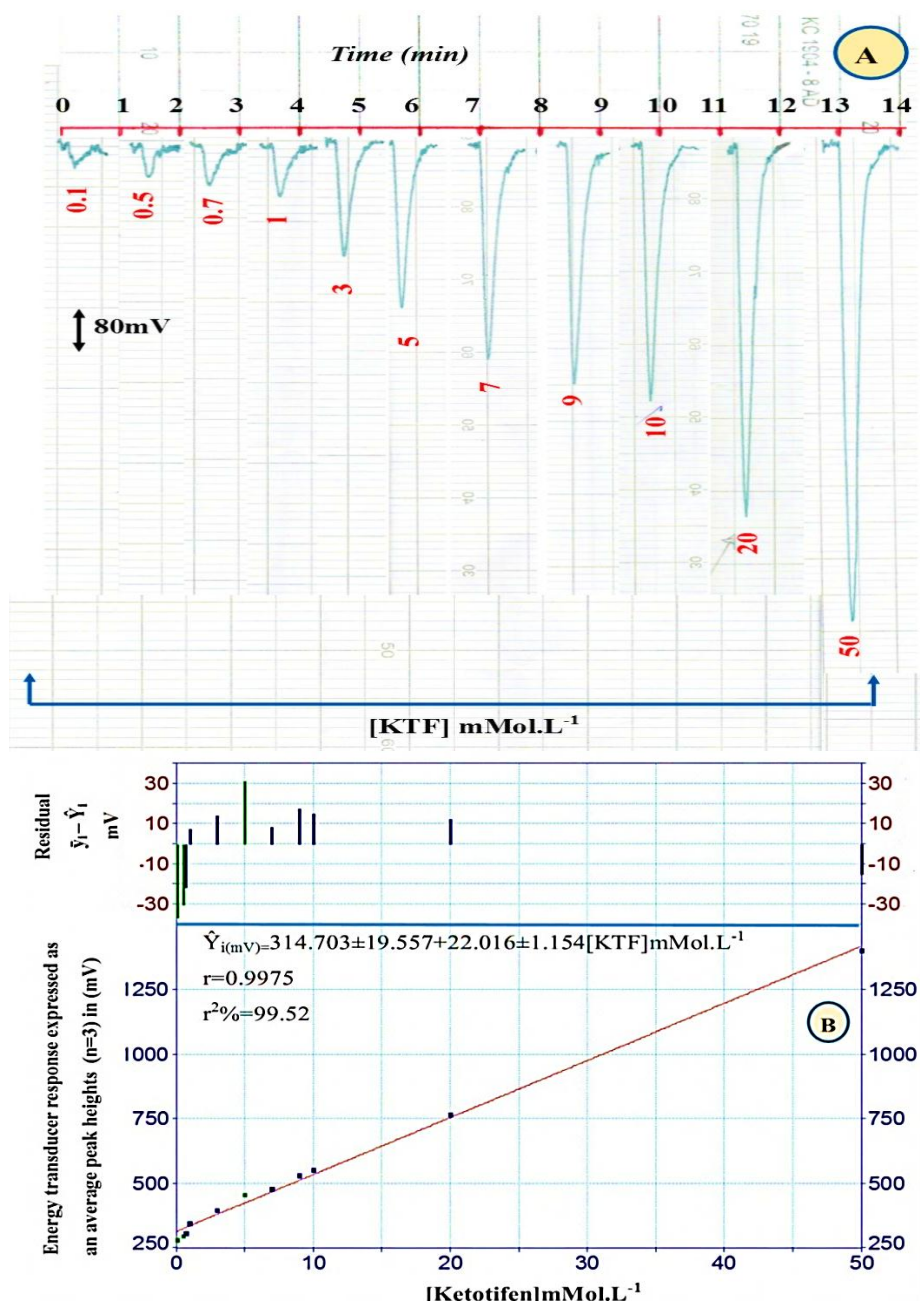
Table 8: Effect of coil length on energy transducer response expressed as an average peak heights (mV) for determination of ketotifen fumarate using optimum parameters (i.e: chemical & physical variable)

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%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_n / \sqrt{n}$	t (sec)	Base width $\Delta t_B$ (sec)	V (ml)	Concentration in mMol.L <sup>-1</sup> at flow cell
0	0	584	0.21	584±3.047	36	78	3.907	0.478
30	0.235	568	0.35	568±4.939	41	82	4.094	0.457
60	0.471	560	0.34	560±4.730	52	106	5.214	0.358
100	0.785	504	0.29	504±3.631	63	120	5.867	0.319

V: addition volume. C:Concentration at flow cell.  $\Delta t_B$ : Base width of response  
t: Arrival time from injection valve reaching to the measuring cell

**Scatter Plot Calibration Curve for Variation of KTF Versus Energy Transducer Response**

Using the optimum chemical and physical parameters; a series of KTF solution (0.1-50) mMol.L<sup>-1</sup> were prepared. A scatter plot diagram shows that a linear calibration graph range for the variation of the energy transducer response of Ayah 6SX1-T-1D solar cell CFI Analyser with KTF concentration was ranging from 0.1-50 mMol.L<sup>-1</sup> with correlation coefficient ( r ):0.9975 as shown in **Fig.11A,B**. The results obtained were tabulated in **Table 9**. 50 mMol.L<sup>-1</sup> was chosen as the most suitable upper limit of concentration used; when applying all optimum parameters that were achieved in previous section **(C.F.4.1.B.1)**. Higher concentration gave a higher responses but it was ignored due to the formation of excessive precipitate that might cause blockage of the tubes in the manifold design.



**Fig. 11 : Variation of KTF concentration on :**  
**(A): Response profile versus time.**  
**(B): Calibration graph at range (0.1-50)mMol.L<sup>-1</sup>,**  
**residual ( $\bar{y}_i - \hat{Y}_i$ ),  $\bar{y}_i$  : practical value,  $\hat{Y}_i$ : estimate value at 267  $\mu$ L , 20 sec purge time**

**Table 9: Summary of result for linear regression for the variation of transducer response with KTF concentration using first degree equation**

Measured [KTF] mMol.L <sup>-1</sup>	Range of [KTF] mMol.L <sup>-1</sup> (n)	$\hat{Y}_{i(mv)} = a \pm s_{at} + b \pm s_{bt} [\text{KTF}] \text{mMol.L}^{-1}$ at confidence level 95%, n-2	r r <sup>2</sup> r <sup>2</sup> %	t <sub>tab</sub> at 95%, n-2	Calculated t-value $t_{cal} = \frac{ r \sqrt{n-2}}{\sqrt{1-r^2}}$
0.1-50	0.1-50 (11)	$314.703 \pm 19.557 + 22.016 \pm 1.154 [\text{KTF}] \text{mMol.L}^{-1}$	0.9975 0.9952 99.52%	2.262	<< 43.199

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

### Limit of detection

A study was carried out to determine the limit of detection of Ketotifen fumarate by three different methods at injected sample volume of 267 µl; which tabulated in Table 10.

**Table 10: The limit of detection of KTF at optimum parameters using 267 µL**

Practically based on gradual dilution for the minimum concentration (mMol.L <sup>-1</sup> ) or weight/sample	Theoretical based on the value of slope $X = 3S_B / \text{Slope}$	Theoretical based on the linear equation $\hat{Y} = Y_B + 3S_B$
(0.01 mMol.L <sup>-1</sup> ) 1.136 µg/sample	7.740 µg/sample	0.365 mg/sample

X = value of L.O.D. based on slope, S<sub>B</sub> = standard deviation of blank repeated for 13 times, Y<sub>B</sub> = average response for blank = intercept, L.O.D. = limit of detection,  $\hat{Y}$  = estimate value.

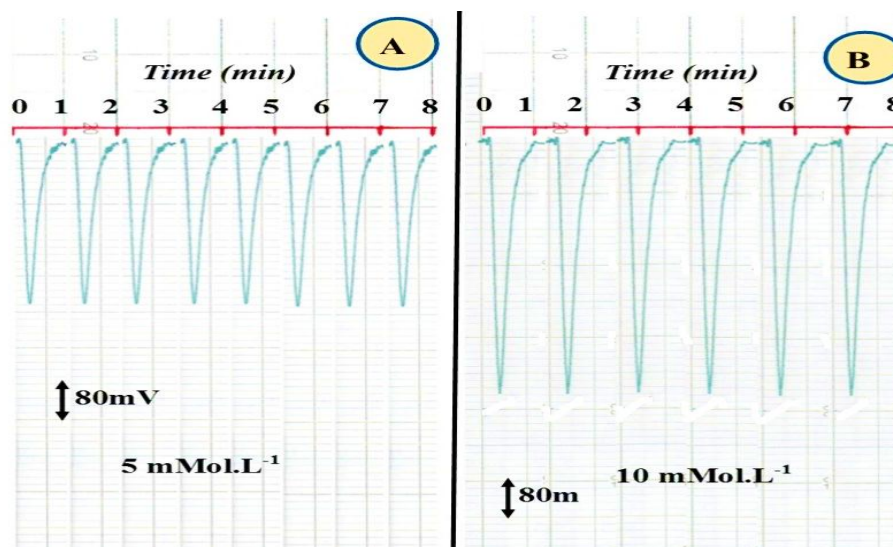
### Repeatability

The repeatability of measurement and the efficiency of homemade Ayah 6SX1-T-1D solar cell CFIA were studied at fixed concentrations of KTF, mainly with two concentrations of 5, 10 mMol.L<sup>-1</sup> at optimum parameters. The value of relative standard deviation (RSD %) for the selected concentrations of KTF were less than 1%. The kind of profile is shown in Fig 12. The repeated measurements for eight and six successive injections were measured and obtained the results were tabulated in Table 11.

**Table 11: Repeatability for the response obtained for the formation of precipitation reaction from KTF-Ce(IV) sulfate (7mMol.L<sup>-1</sup>) system in NaCl (0.5 Mol.L<sup>-1</sup>) as a carrier stream with 267 µl sample volume**

[KTF] mMol.L <sup>-1</sup>	Number of injection	Average response $\bar{y}_i$ (mV)	$\sigma_{n-1}$	RSD %	Confidence interval at (95%) $\bar{y}_{i(mv)} \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
5	8	464	2.41	0.52	$464 \pm 2.015$
10	6	712	5.48	0.77	$712 \pm 5.754$

$t_{0.025, n-1}$ ,  $t_{0.025, 5} = 2.571$ ,  $t_{0.025, 7} = 2.365$

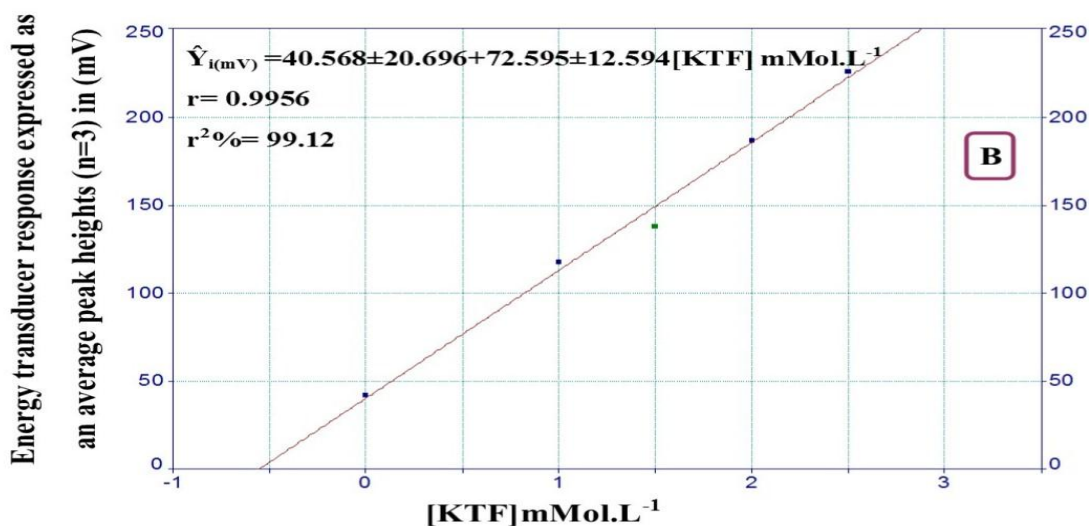
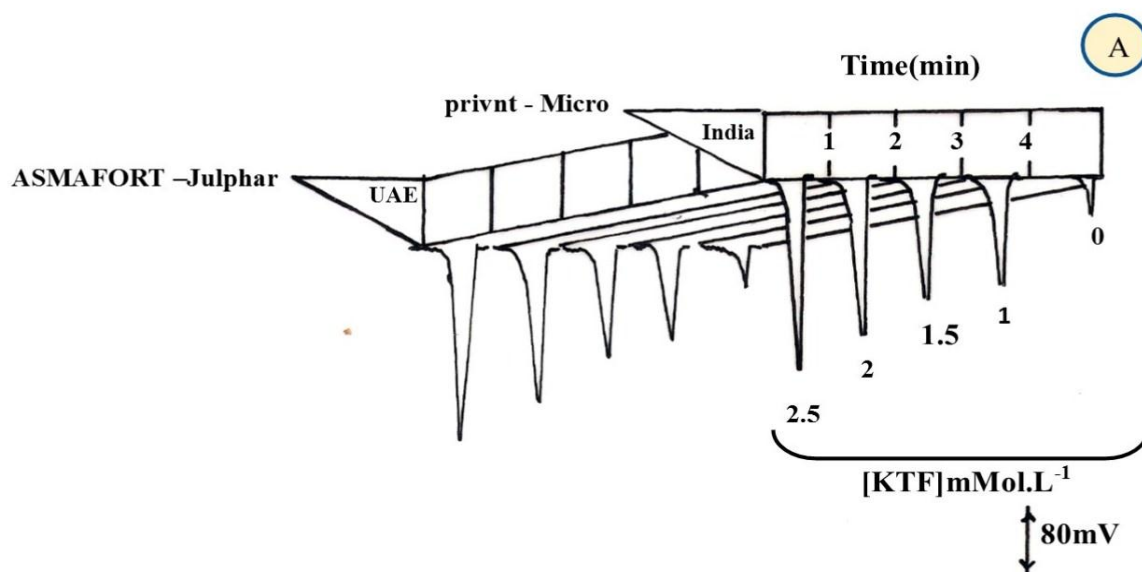


**Fig.12: Response – time of profile for eight and six successive repeatable measurement of 5 & 10 mMol.L<sup>-1</sup> of KTF**



**Analysis of pharmaceutical preparation**

The established method was used for the determination of KTF in two different kind of 1 mg KTF from two different drug manufactures (privnt - Micro-India) and (Asmafort –Julphar – UAE) using Ayah 6SX1-T-1D- Solar cell CFI Analysis which was compared with Quoted value [1 mg]. A series of solutions were prepared of each pharmaceutical drug (5 mMol.L<sup>-1</sup>) by transferring 1.2 mL to each of the five volumetric flask (10 mL), 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<sup>-1</sup> to obtain (0, 1, 1.5, 2, 2.5) mMol.L<sup>-1</sup> .Flask no.1 is the sample. The measurements were conducted using Ayah 6SX1-T-2D solar cell CFI Analyser method. **Fig.13.A.**shows response profile and **13.B,C** standard addition calibration graph using two different samples . Results were mathematically treated for the standard addition method. The results were tabulated in **Table 12. A** at confidence interval 95%. The practically content of active ingredient & efficiency of determination of KTF in each drug tabulated in **Table 12.B** .



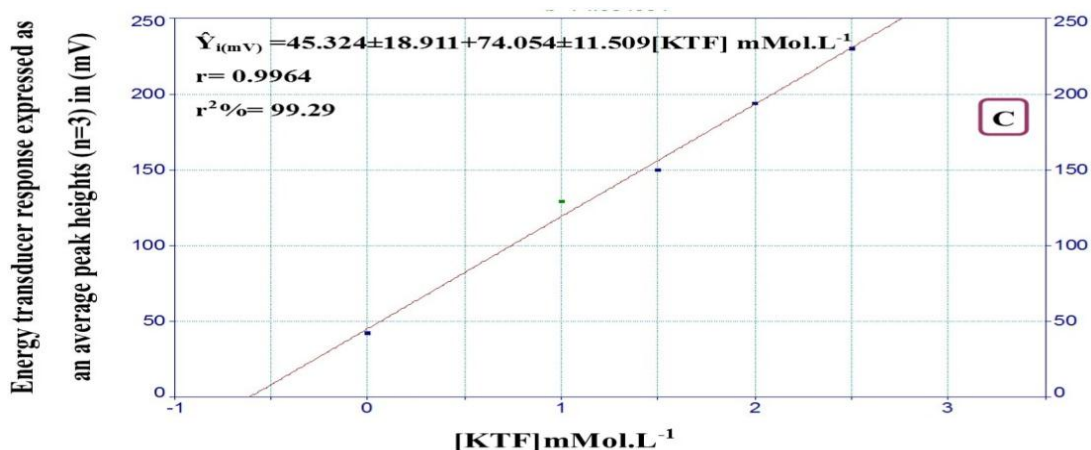


Fig. 13: Standard addition calibration graph and sample of response profile versus time for two pharmaceutical preparations (A): profile versus time. (B): standard addition calibration graphs for Asmafort, UAE. (C): standard addition calibration graphs for Privent, INDIA.

Table 12 A: Results for the determination of KTF in pharmaceutical preparation by standard addition method using Ayah 6SX1-T-1D Solar cell Analyser

sample no	commerical name ,content ,company,country	Ayah 6SX1-T-1D solar CFIA (mV)					Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_{at}+b\pm s_{bt}$ [KTF] mMol.L <sup>-1</sup>	r r <sup>2</sup> r <sup>2</sup> %	Practical conc. mMol.L <sup>-1</sup>	
		[KTF] mMol.L <sup>-1</sup>							in 10 ml	In 25ml
		0	1	1.5	2	2.5				
1	ASMAFORT ,1mg, Julphar, UAE	42	118	138	187	226	40.568±20.696+72.595±12.594[KTF] mMol.L <sup>-1</sup>	0.9956 0.9912 99.12%	0.559	4.657
2	PRIVNT ,1mg, MICRO ,India	42	129	150	194	230	45.324±18.911+74.054±11.509[KTF] mMol.L <sup>-1</sup>	0.9964 0.9929 99.29%	0.612	5.100

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

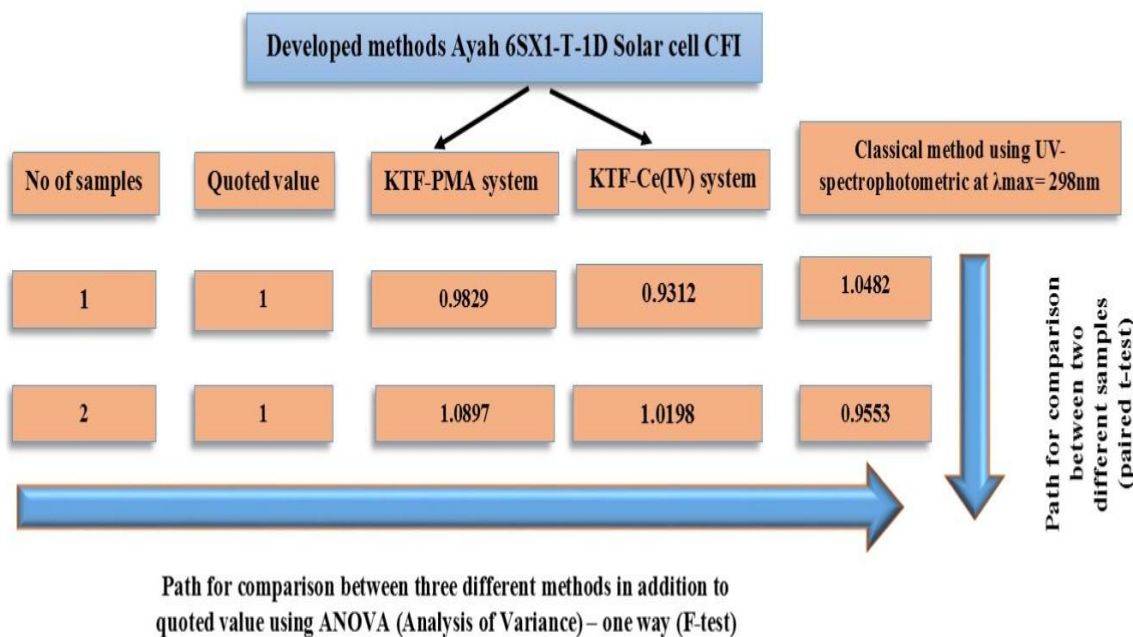
Table 12 B: Summary of data for paired t-test, practical content and efficiency for determination of KTF in two samples of pharmaceutical preparation

Sample no	Confidence interval for the average weight of tablets $\bar{w}_i \pm 1.96 \sigma_{n-1} / \sqrt{n}$ (g)	content for the active ingredient $\bar{w}_i \pm 1.96 \sigma_{n-1} / \sqrt{n}$ at 95% (mg)	Sample weight equivalent to 0.0532 g (5 mMol.L <sup>-1</sup> ) of the active ingredient w <sub>i</sub> (g)	Practical content of active ingredient		Efficiency of determination (Rec%)
				In 25 ml of sample $\bar{w}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$ at 95%, (mg)	in tablets $\bar{w}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$ at 95% ,(mg)	
				Ayah 6SX1-T-1D solar CFIA (mV)		
1	0.1165±0.0000741	1 ±0.000636	6.1978	49.5373± 3.279	0.9312 ± 0.062	93. 12%
2	0.1315 ±0.0000566	1 ±0.000431	6.9958	54.2544±4.546	1.0198 ± 0.085	101.98%

Xd: Difference between two method ,  $\bar{X}_d$ : difference mean ,  $\sigma_{n-1}$ :Difference standard deviation , n=3 for individual & n=2 for comparison between two method,  $\mu$  (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.

**Analysis of Date Using Proposed Hypothesis**

-Statistical of data was used for the comparison between two different samples and four different methods as shown is **scheme 2**



**Scheme 2: Summed up the results for three different methods in addition to quoted value and two different samples of KTF**

From the results were tabulated in the **scheme.2**, that the two samples from different manufacturer were subjected to a paired t-test to evaluate if there were a difference in the mean of the both samples. Therefore the assumption were made at α=0.05 level of significant, taking into account that neglecting differences between four methods.

Null hypothesis  $H_0: \mu_{Asmafort} = \mu_{Privent}$

i.e: there is no significant difference between the mean of the two samples while the alternative hypothesis.

Iternative hypothesis  $H_1: \mu_{Asmafort} \neq \mu_{Privent}$

At a significant level of α=0.05 (confidence of 95%). Any value < 0.05 will reject Null hypothesis and accepted the alternative hypothesis. Reversing: any value of significant > 0.05 will accept Null hypothesis. Therefore, since the value of  $t_{cal} (|-0.5585|) < t_{tab} (3.182)$ , So; Null hypothesis will be accepted and will rejected the alternative hypothesis; as shown in **Table 13.A**.

**Table 13.A: paired t-test for the comparison between two samples for the analysis of KTF in pharmaceutical drugs**

Type of samples		Xd	$\bar{X}_d$ ( $\sigma_{n-1}$ )	Paired t- test $=\bar{X}_d\sqrt{n} / \sigma_{n-1}$	$t_{tab}$ (95% confidence level)
ASMAFORT, Julphar	PRIVENT, MICRO				
1.0000	1.0000	0	-0.02562 (0.09176)	-0.5585  < 3.182	
0.9829	1.0897	-0.1068			
0.9312	1.0198	-0.0886			
1.0482	0.9553	0.0929			

Xd: Difference between two method ,  $\bar{X}_d$ : difference mean ,  $\sigma_{n-1}$ :Difference standard deviation , n=3 for individual & n=2 for comparison between two method.  $\mu$  (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.

- One way ANOVA (F–test) treatment (that is used to compare three or more means but it contains only on variable) was used for the results obtained from determination of KTF in different pharmaceutical drugs by different four methods.

**Assumption for the F- test depended on**

- First estimate is called :between-group variance
- Second estimate is called :within-group variance

The ANOVA test was carried out at of  $\alpha = 0.05$  (95% confidence level). Test of hypothesis i.e. ANOVA and comparison of different methods in addition to quoted value 1mg of KTF are given in **scheme 4.2**.

The following hypothesis should be used :

**H<sub>0</sub>( Null hypothesis )**

For sample (Asmafort, julphar)

$\mu$  quoted value =  $\mu$  developed method (KTF-PMA) =  $\mu$  developed method (KTF-Ce(IV) ) =  $\mu$  classical method

**For sample (Privnt, INDIA)**

$\mu$  quoted value =  $\mu$  developed method (KTF-PMA) =  $\mu$  developed method (KTF-Ce(IV) ) =  $\mu$  classical method

**Against****H1( Alternative hypothesis )**

For sample (Asmafort, julphar)

$\mu$  quoted value  $\neq \mu$  developed method (KTF-PMA)  $\neq \mu$  developed method (KTF-Ce(IV) )  $\neq \mu$  classical method

**For sample (Privnt, INDIA)**

$\mu$  quoted value  $\neq \mu$  developed method (KTF-PMA)  $\neq \mu$  developed method (KTF-Ce(IV) )  $\neq \mu$  classical method

i.e : . At least one mean is different from the others . Taking into account that there was no significant difference between two samples. From the results obtained that were tabulated in **Table 4.24.B** shows that there is no significant difference between the means obtained in all data, since the value of Sig > 0.05 (0.787>0.05) and  $F_{cal} (0.359 < F_{tab} ( 6.59 )$  : therefore Null hypothesis will be accepted and will rejected the Alternative hypothesis .These mean that there is no significant difference between four different methods for two samples.

**Table 13.B: ANOVA results of four methods for comparison between two different samples of KTF**

Source	Sum of squares Ssq	df	Mean square Msq	F <sub>cal</sub>	F <sub>tab</sub>	Sig
Between groups	0.004	3	0.001	0.359 << 6.59	6.59	0.787
Within groups	0.014	4	0.003			
Total	0.018	7				

$$F_{tab} = F_{0.95, U_1, U_2} = F_{0.95, 3, 4} = 6.59$$

df = degree of freedom

$$F_{cal} = Msq_B / Msq_W, w : \text{within groups}, B: \text{Between groups}$$

**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 greenish blue color precipitate as an ion- pair compound for the reaction of ketotifen fumarate with Ce(IV) Sulfate 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.

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