# New approach for the on-line turbidimetric determination of metronidazole in pharmaceutical preparation via the use of a new homemade Ayah 6SX1-T-2D Solar-continuous flow injection analyser

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## Abstract

A newly developed analytical method characterized by its speed and sensitivity for the determination of metronidazole (MZ) in pure and pharmaceutical preparation via turbidimetric measurement (0-180$^o$) by Ayah 6SX1-T-2D Solar-CFI analyser. The method was based upon the formation of greenish yellow precipitate for ion pair compound for the reaction of phosphomolybdic acid with metronidazole in aqueous medium. Turbidity was measured via the reflection of incident light that collides on the surface precipitated particals at 0-180$^o$. Chemical and physical parameters were investigated. Linear dynamic of metronidazole is ranged from 0.05-8 mmol.L$^{-1}$,with correlation coefficient r = 0.9821 . The limit of detection (S/N= 3 )($3S_B$)=171.15 ng/sample from the step wise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.5% for 4, 4.5 mmol.L$^{-1}$ (n=5) concentration of metronidazole. The method was applied successfully for the determination of metronidazole in three pharmaceutical drugs. A comparison was made between the newly developed method analysis with the classical method (HANNA instrument for turbidity measurement) 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.

# نمط جديد لقياس التعكرية الأني لتقدير الميترونيدازول في مستحضراته الدوائية من خلال استخدام محلل جديد مصنع محليا للحقن الجرياني المستمر Ayah 6SX1-T-2D Solar.

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## الخلاصة

طورت طريقة تحليلية جديدة ، تميزت بالسرعه والحساسية لتقدير الميترونيدازول بشكله النقي او على هيئة مستحضرات دوائية عن طريق قياس التعكرية 0-180$^o$ بوساطة محلل الحقن الجرياني المستمر Ayah 6SX1-T-2D Solar. استندت الطريقة على تكوين راسب اصفر مخضر فاتح لمزدوج ايوني بين الميترنيدازول وحامض مولبدات الفسفوريك في الوسط المائي. تم قياس التعكرية عن طريق انعكاس الضوء المسلط والمصطدم بسطوح دقائق الراسب بزواية 0-180$^o$. تم دراسة كافة المتغيرات الفيزيائية والكيميائية. العلاقة الخطية لمنحني المعايرة للميترونيدازول تمتد 0.05-8 مللي مول.لتر$^{-1}$ بمعامل ارتباط r=0.9821. حدود الكشف (3=S/N) ($3S_B$)= 171.15 نانوغم/انموذج من التخفيف التدريجي لاقل تركيز في منحني المعايرة مع انحراف قياسي نسبي مئوي RSD% اقل من 0.5% لتركيز 4 و4.5 مللي مول.لتر$^{-1}$ (n=5) من الميترونيدازول. طبقت الطريقة بنجاح لتعين الميترونيدازول في ثلاثة عقاقير دوائية. اجريت المقارنه بين الطريقة المستحدثة للتحليل والطريقة التقليدية لقياس التعكرية باستخدام الاضافات القياسية بوساطة اختبار t-المزدوخ ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعه 95%.

# Introduction

Metronidazole (MTZ) or 1-(hydroxyethyl)-2-methyl-5- nitroimidazole (Fig 1) is a nitroimidazole derivative which historically introduced in 1960 as the first systemic antitrichomonal agent [1]. Later, it was found that it could be also an effective drug in curing infections caused by anaerobic protozoa; giardiasis, amoebiasis, balantidiasis and also to be applied against Vincent's organisms [2,3]. MTN is an amoebicide at whole sites of infection with Entamoeba histolytica. The effectiveness of MTN against parasites in the bowel lumen is genenerally weak as a result of its rapid absorbtion so it should be used together with luminal amoebicide in the treatment of invasive amoebiasis[4].

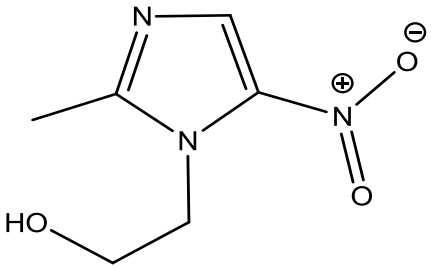



**Figure 1: chemical Structure of metronidazole (MTZ)**

The mechanism of action is explained by a partial reduction of the nitro group. Biological effects are produced via partially reduced metabolites which bind to bacterial and cellular macro-molecules. In bacteria the interaction between reactive metabolites and bacterial DNA inhibits DNA and protein synthesis and leads to the death of the microorganism. In humans and animal inter-action with cellular macromolecules and DNA were demonstrated. Single strand breaks of human DNA were observed following a single therapeutic dose with metronidazole. The same findings were confirmed in human lymphocyte cultures in vitro[5]. Metronidazole is metabolized by oxidation to 2–hydroxymethyl metronidazole and 2–methyl–5–nitroimidazol–1–acetic acid, and by conjugation with glucuronic acid. About 70 to 80% of a dose is excreted in the urine in 48 hr with less than 10% of the dose as unchanged drug, up to 10% as conjugated MTR, about 27% as 2–hydroxymethylmetronidazole, 10% as the conjugated 2–hydroxymethyl metabolite, and 20% as the acid metabolite [6].. Several methods have been reported for metrronidazole assay including chromatographic[7,8], electrochemical[9,10], Spectrophotometric (11-16), high-performance liquid formulations (HPLC) [17,18], voltammetric methods (19, 20).

This work describes a flow injection turbidimetric method for determination of metronidazole with the aid of Ayah 6SX1-T-2D Solar-CFI analyzer in pharmaceutical formulations. The method is based upon the formation of greenish yellow precipitate for the ion pair compound by phoshomolybidic acid with metronidazole in aqueous medium. The turbidimetry is measured via the reflection of incident light from the surfaces of precipitated particles at 0-180$^{o}$**.** The positive signal from reflection can be recorded by Ayah 6SX1-T-2D Solar supplier with linear array of six super snow white light emitting diode as a source and two solar cell 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 (0.1mol.L$^{-1}$) of metronidazole (171.15g.mol$^{-1}$)was prepared by dissolving 4.27875g in 250 mL of 0.4 mol.L$^{-1}$ sulpharic acid .A stock solution (0.1 mol.L$^{-1}$) of phosphomolybdic acid $H_3PMo_{12}O_{40}$ 1825.25 g.mol$^{-1}$,BDH) was prepared by dissolving 18.2525 g in 100 mL of distilled water. A 1M of sulfuric acid solution(96%, 1.84 g.ml$^{-1}$, BDH) was prepared by pipetting 14 mL of concentrated sulfuric acid and dilute to 250 mLvolumetric flask. A 1 M of hydrochloric acid solution(35%, 1.19 g.ml$^{-1}$, BDH) were prepared by pipetting 21 mL of concentrated hydrochloric acid and completed the volume with distilled water in 250 mL. A 1 M of

nitric acid solution(70%, 1.42 $g.ml^{-1}$, BDH) was prepared by pipetting 16 mL of concentrated nitric acid and completed the volume with distilled water in to 250 mL. A 1 M of Phosphoric acid solution (85%, 1.69 $g.ml^{-1}$,BDH) was prepared by pipetting 17 mL of concentrated phosphoric acid and complete the volume with distilled water to 250 mL. A 1M acetic acid solution (99.5%, 1.05 $g.ml^{-1}$, BDH) was prepared by pipetting 15 ml of concentrated acetic acid and complete the volume with distilled water to 250 mL. Each acid was standardized agenist standard solution of 1 M from $Na_2CO_3$

## Sample preparation

Twenty tablets were weight, crushed and grinded. Tablets containting 500 mg of metronidazole were weight (1.628,1.098, 1.107 ) equivalent to 855.75 mg of active ingredient 50 $mmol.L^{-1}$ for metronidazole (Julphar-Negazole, Ajanta-metrosule, S.D.I-medazole) respectively. The powder was dissolved in 0.4 M sulpharic acid followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 100 ml with sulpharic acid.

## Apparatus

Peristaltic pump – 2 channels (France) an Miniplus 2 type GILSON and rotary 6-port medium pressure injection valve (Teflon),(IDEX corporation, USA) used for injection of sample. The response was measured by a hoemade Ayah 6SX1-T-2D Solar-CFI analyser, which uses six snow white LED for irradiation of the flow cell at 2mm 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 (Kompenso Graph C-1032) Siemens (Germany) or digital AVO-meter (auto range) (0-2 volt) (China). Turbidometric readings under batch conditions were made by HANNA company (U.S.A). The flow diagram for the determination of metronidazole is shown in Figure 2

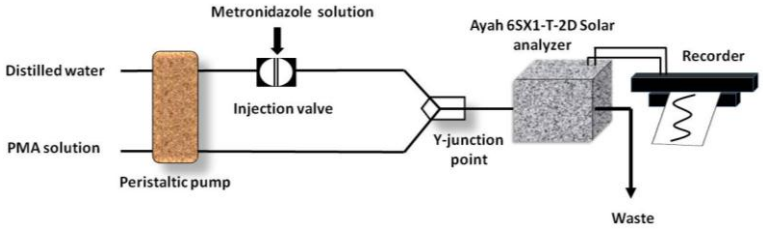
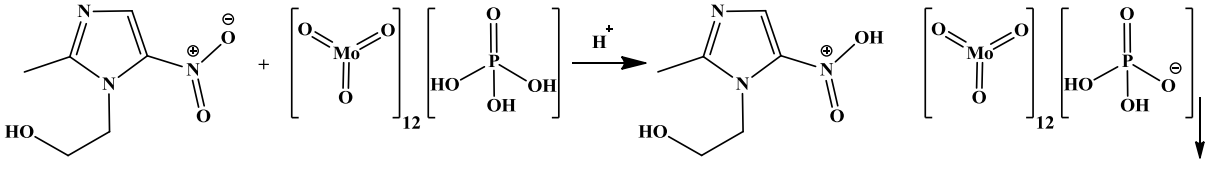



**Fig.2. Flow diagram manifold system used for the determination of Metronidazole**

## Methodology

Flow diagram system for the reaction of MTZ-PMA to form greenish yellow precipitate as an ion pair complex is composed of two lines as shown as in figure 2. The first line is the carrier stream (distilled water) at 1.8 $ml.min^{-1}$ flow rate which leads to the injection valve to carry metronidazole sample, 100µl ; while the second line supplied with phosphomolybdic acid solution (5 $mmol.L^{-1}$) at 1.8 $ml.min^{-1}$.Both of line meet at a junction (Y-junction), with an outlet for reactants product from complex, which passes through a homemade Ayah 6SX1-T-2D Solar-CFI analyser that works with a six snow white light emitting diode will be used as a source. Each solution injected was assayed in triplicate. The response of which was recorded on x-t potentiometric recorder to measurement the turbidity via the reflection of incident light at 0-180$^o$. A proposed mechanism of ion pair for system MTZ-PMA in aqueous medium is presented in schem.3



**MTZ-PMA Ion pair associate**

**Fig. 3:Mechanism of reaction between of MTZ & PMA**

## Results and Discussion

### Study of the optimum parameters:

The flow injection manifold system as shown in Fig.2. was investigated in the relation of chemical and physical variables, in order to obtain optimum conditions for the system. They were optimized by making all variables constant and varying one each at time.

## Chemical variables

### Effect of Phosphomolybdic acid (PMA) Concentration

A series of the precipitating reagent (PMA) solutions (2.5-10) mmol.$L^{-1}$ were prepared, at constant concentration metronidazole (5 mmol.$L^{-1}$) ,45 µl sample volume at 1.6 ml.$min^{-1}$ flow rate for each line and the intensity of incident light of $LED_S$ 1400 mV were used. Table.1 summarizes the total results obtained; it can be shows that an increase in PMA concentration might cause an increase in particls density due to accumulation effect of precipitate particls up to 5 mmol.$L^{-1}$, following this concentration there was a slight increase in the reflected light intensity therefore; 5 mmol.$L^{-1}$ PMA concentration was chosen as the optimum concentration that used for further experiment

***Table.1*** Effect of PMA on the measurement of refelection of incident light for the determination MZ

| [PMA] mmol.$L^{-1}$ | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| 2.5 | 1390.00 | 0.719 | 1390.00±24.873 |
| 4 | 2005.00 | 0.432 | 2005.00±21.541 |
| 5 | 2082.00 | 0.166 | 2082.00±8.616 |
| 7.5 | 2092.67 | 0.307 | 2092.67±15.991 |
| 10 | 2113.33 | 0.546 | 2113.33±28.721 |

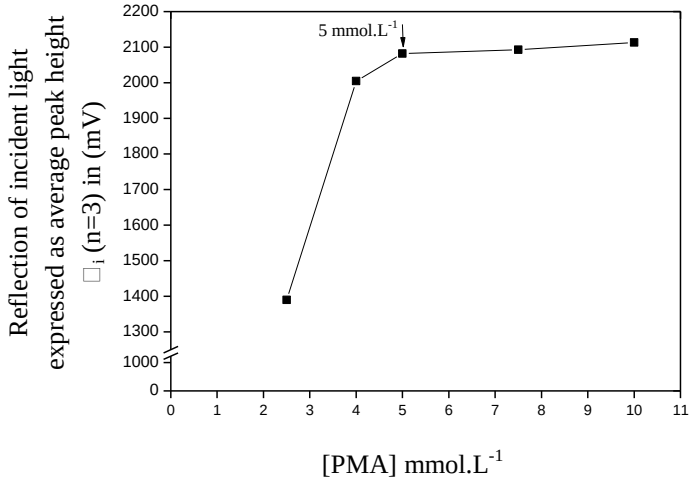



**Fig. 4 Effect of the PMA on Energy transducer response by reflection of incident light**

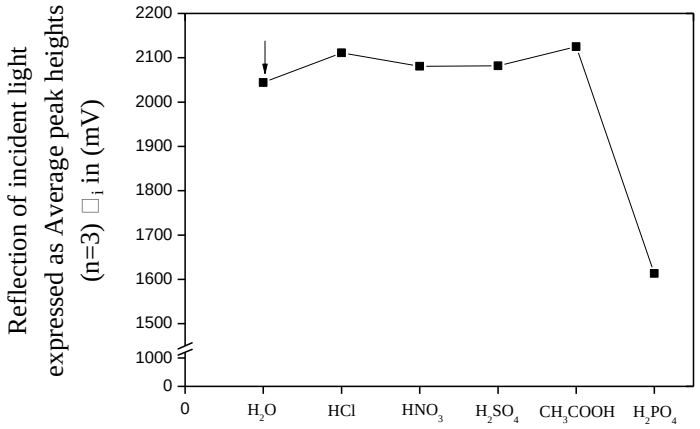
### Effect of acidic media on the increase of incident light intensity

The determination of metronidazole was studied in different acidic media ($HCl$,$H_2SO_4$,$HNO_3$,$CH_3COOH$ & $H_3PO_4$ ) at 50 mmol.$L^{-1}$ in addition to the aqueous medium. The results are summarized in table.2. The data obtained were plotted as shown in Fig.5 (A,B). In which can be seen that there were no significant excess in response obtained from Ayah 6SX1-T-2D solar cell CFI analyser for different acids used; even though there was a ≈ 20% decrease in the response

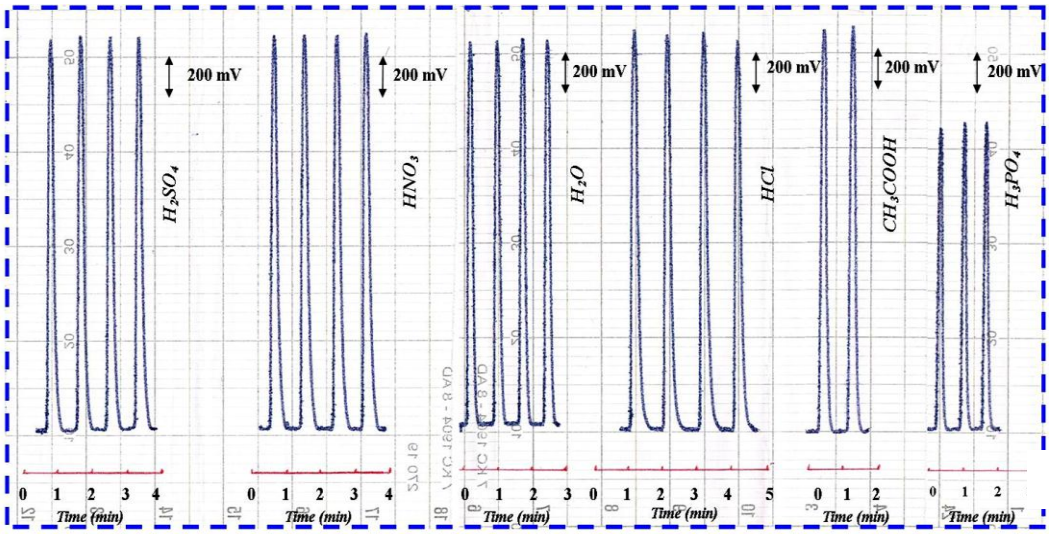
height using phosphoric acid. It was concluded that distilled water can be used equally compared to various acid used.

***Table. 2*** Effect of acidic media on the measurement of refelection of incident light for determination MTZ

| Type of acid | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| $H_2O$ | 2044.00 | 0.339 | 2044.00±17.232 |
| HCl | 2111.33 | 0.383 | 2111.33±20.104 |
| $HNO_3$ | 2081.00 | 0.083 | 2081.00±4.308 |
| $H_2SO_4$ | 2081.67 | 0.139 | 2081.67±7.180 |
| $CH_3COOH$ | 2125.00 | 0.408 | 2125.00±21.541 |
| $H_3PO_4$ | 1613.33 | 0.716 | 1613.33±28.721 |




(A)




(B)

**Fig. 5** A -Effect of the acidic medium on the reflection of light expressed as a positive transducer energy response in mV form MTZ(5 mmol.$L^{-1}$)- PMA(5 mmol.$L^{-1}$), 45µl & flow rate 1.6 ml.$min^{-1}$.
B-response profile

## Physical variables

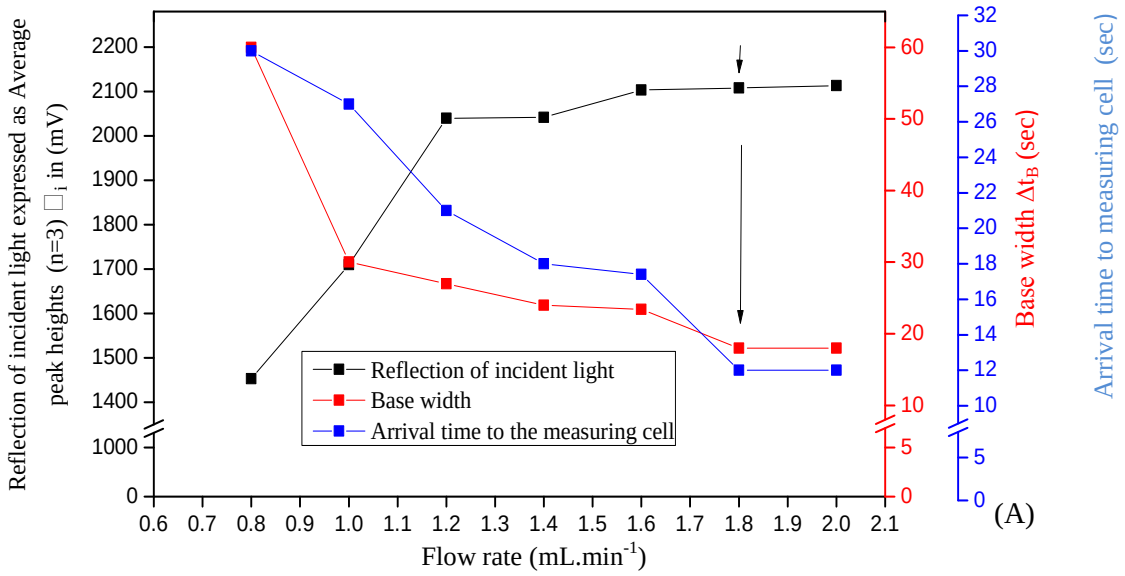
### Effect of flow rate

Using MTZ(5 mmol.$L^{-1}$)-PMA(5 mmol.$L^{-1}$) system with variable flow rates (0.8-2 mL.$min^{-1}$) controlled by the peristaltic pump as shown in Fig.2 The results obtained were summarized in Table.3. It was noticed that at slow flow rates, there were an increase in peak base width ($\Delta t_B$) as

shown in Fig.6 (A,B) this might be due to the dispersion and dilution which causes an irregular response.While at higher flow rate > 1.2 for the carrier stream and PMA, although the effect of physical parameter was very crucial on the response obtaining regular response and very sharp maxima but it is not very high due to departure speed of reflecting surfaces from measuring cell at a short time. Therefore; the best flow rate for the completion of the reaction between the MTZ and PMA was 1.8 mL.min$^{-1}$ to obtain a regular response, narrower $\Delta t_B$ and minimize the consumption of reactants solution as shown in Fig. (6 A,B).

***Table.3*** Effect of the variation of flow rate on the measurement of refelection of incident light for determination of MTZ (5 mmol.L$^{-1}$) ,PMA (5 mmol.L$^{-1}$), 45µl

| Flow rate mL.min$^{-1}$ | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ | Base width $\Delta t_B$ (sec) | t Arrival time to the measuring cell (sec) |
|---|---|---|---|---|---|
| 0.8 | 1453.33 | 0.397 | 1453.33±14.360 | 60 | 30 |
| 1 | 1710.00 | 0.585 | 1710.00±24.873 | 30 | 27 |
| 1.2 | 2040.00 | 0 | 2040.00±0 | 27 | 21 |
| 1.4 | 2041.67 | 0.141 | 2041.67±7.180 | 24 | 18 |
| 1.6 | 2103.33 | 0.274 | 2103.33±14.360 | 23.4 | 17.4 |
| 1.8 | 2108.00 | 0.164 | 2108.00±8.616 | 18 | 12 |
| 2 | 2113.33 | 0.273 | 2013.33±14.360 | 18 | 12 |



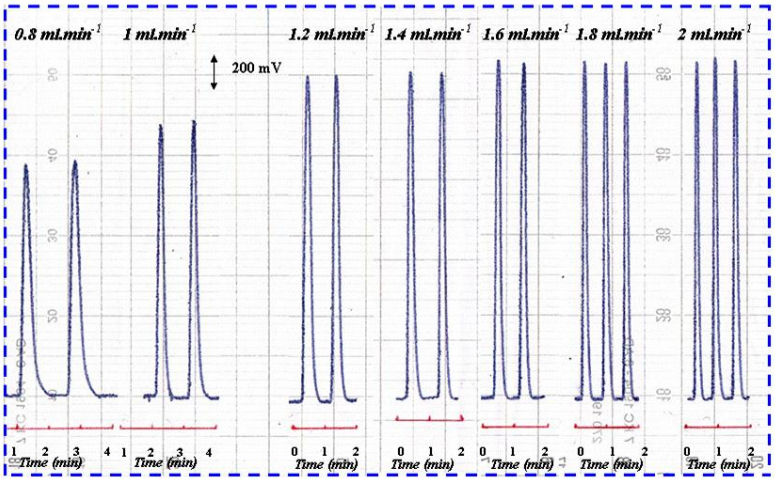




**Figure 6-** Effect of the variation of the flow rate on the

A- Transducer energy response expressed as peak height in mV for Relection of incident light for MTZ (5 mmol.L$^{-1}$) - PMA (5 mmol.L$^{-1}$) 45µl

B- Response profile

## Effect of sample volume

Using MTZ(5 mmol.L$^{-1}$)-PMA(5 mmol.L$^{-1}$) system with the optimum flow rate and variable volume (26-200) µL were used for this study, the plot of change in sample volume vs. Reflection of incident light and ΔtB is shown in Fig. 7 A. It was noticed that an increasing of sample volume up to 100 µl lead to a significant increase in response height & more preceptibe than low volume as shown in Fig. 7 B. While a larger sample volume i.e: more than 100 µl even though it gave a slightly higher response but it was characterized with wider in $\Delta t_B$ & response maxima which was might be attributed to the continuous relatively longer time duration of precipitate particles segment in front of the detector and increase of particle size causing a slow movement of precipitate particles therefore; 100 µl was chosen as an optimum sample volume. All results was tabulated in table. 4

**Table.4** Effect of the variation of sample volume on the measurement of refelection of incident light for determination of : metronidazole (5 mmol.L$^{-1}$) ,PMA (5 mmol.L$^{-1}$) & flow rate 1.8 ml.min$^{-1}$

| Sample volume µl | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ | Base width $\Delta t_B$ (sec) | t Arrival time to the measuring cell (sec) |
|---|---|---|---|---|---|
| 26 | 1915.00 | 0.261 | 1917.33±12.436 | 12 | 18 |
| 45 | 2103.33 | 0.274 | 2101.67±14.360 | 15 | 21 |
| 100 | 2159.00 | 0.080 | 2158.33±4.308 | 18 | 24 |
| 140 | 2195.00 | 0.228 | 2195.00±12.436 | 19.2 | 30 |
| 200 | 2219.00 | 0.078 | 2119.00±4.308 | 21 | 54 |

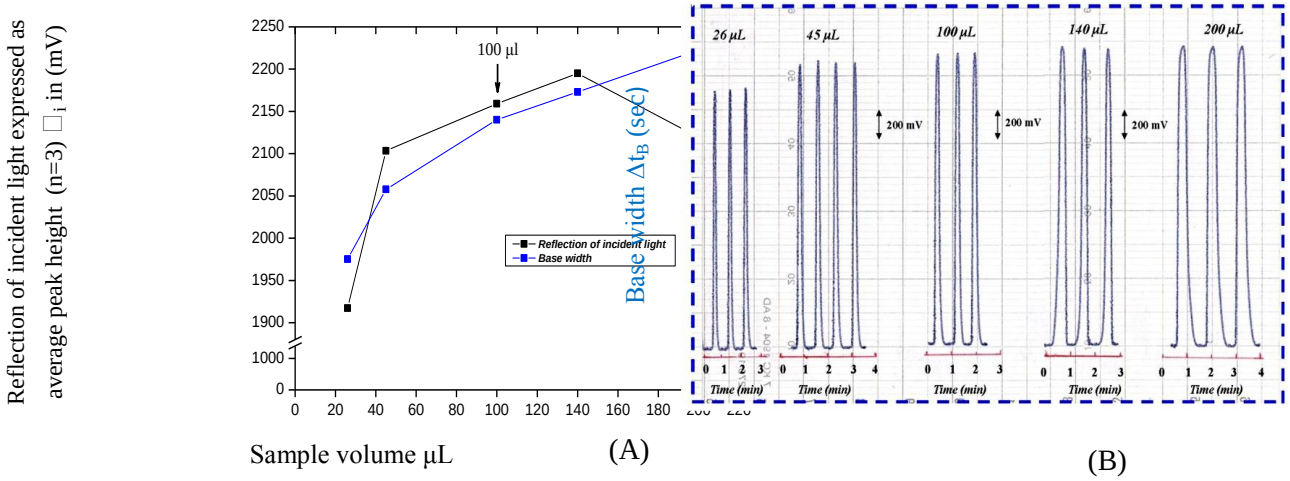



**Fig.7** Effect of the variation of sample volume on the

A- Transducer energy response expressed as peak height in mV for relection of incident light for metrondiazole5 mmol.L$^{-1}$), PMA (5 mmol.L$^{-1}$) & flow rate 1.8 ml.min$^{-1}$

B- Response profile

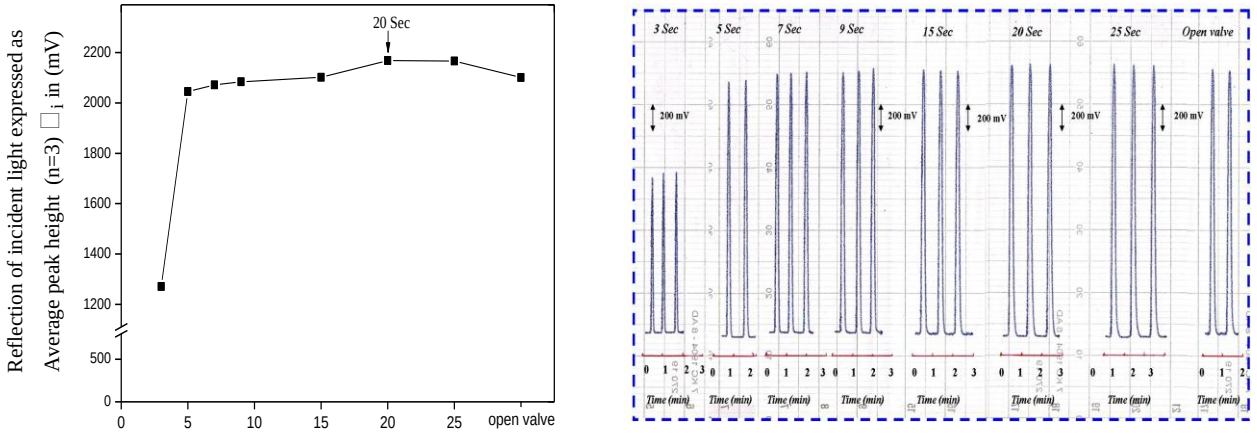
## Effect of Purg time

A study was carried out to determine the optimum duration of the injection time i.e. allowed permissible time for purging of the sample from the injection valve unit. 3-25 seconds were used in this study in addition to allowed the injection valve in the open mode.Sample volume 100 µl and MTZ(5 mmol.L$^{-1}$)- of PMA (5 mmol.L$^{-1}$) were used. It can be seen from Fig. (8 A,B) that there is an increase on the reflection of incident light with increasing the allowed permissible time up to 20

sec, while beyond 20 sec ( i.e: continuous passed the carrier stream through out the injection valve) there were no significant increase in the response height which indicated that 20 sec of purge time as an optimum for completely removed of the sample segment from the injection valve by the carrier stream. The obtained results were tabulated in table.5.

***Table.5*** Effect of the variation of purg time on the measurement of reflection of incident light of MZ (5mmol.$L^{-1}$)-PMA(5 mmol.$L^{-1}$),100 µl & 1.8 ml.$min^{-1}$

| Purge time Sec | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| 3 | 1271.00 | 0.821 | 1271.00±25.968 |
| 5 | 2045.00 | 0.244 | 2045.00±12.436 |
| 7 | 2071.67 | 0.139 | 2071.67±7.180 |
| 9 | 2084.33 | 0.360 | 2084.33±18.668 |
| 15 | 2102.00 | 0.095 | 2102.00±4.975 |
| 20 | 2168.67 | 0.107 | 2168.00±5.744 |
| 25 | 2167.00 | 0.092 | 2167.00±4.975 |
| Open valve | 2101.33 | 0.145 | 2101.33±7.599 |




(A) (B)

**Fig. 8** Effect of the variation of purge time on the

A- Transducer energy response expressed as peak height in mV for Relection of incident light for MTZ(5 mmol.$L^{-1}$), PMA(5 mmol.$L^{-1}$) 100µl & flow rate 1.8 ml.$min^{-1}$ B- Response profile

## Intensity of light

Variable intensity of light source was used 0.57-2.21 volt by changing of light intensity channel in Ayah 6SX1-T-2D-solar cell CFI analyser operation where read by AVO-meter. The results tabulated in table.6 which shows that an increase on the reflection of incident light with increased intensity of source light. Therefore; the intensity of 2 volt was selected as the best voltage that can be supplied to give a better reproducible outcome and increase in the response height expressed by reflection of incident light as shown in Fig.9.

***Table 6:*** Effect of intensity of light on the measurement of refelection of incident light expressed as transducer energy response

| Intensity of light Volt | Reflection of incident light expressed as average peak heights (n=3) $\square_i$ in (mV) | RSD% | Confidence interval at (95%) $\square_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$ |
|---|---|---|---|
| 0.57 | 397.00 | 0.756 | 397.00±7.462 |
| 0.98 | 996.67 | 0.289 | 996.67±7.180 |
| 1.46 | 1646.00 | 0.219 | 1646.00±8.968 |
| 1.80 | 1946.00 | 0.272 | 1947.00±13.161 |
| 2.00 | 2177.67 | 0.116 | 2177.67±6.259 |

| | | | |
|---|---|---|---|
| 2.21 | 2177.33 | 0.096 | 2177.33±5.178 |

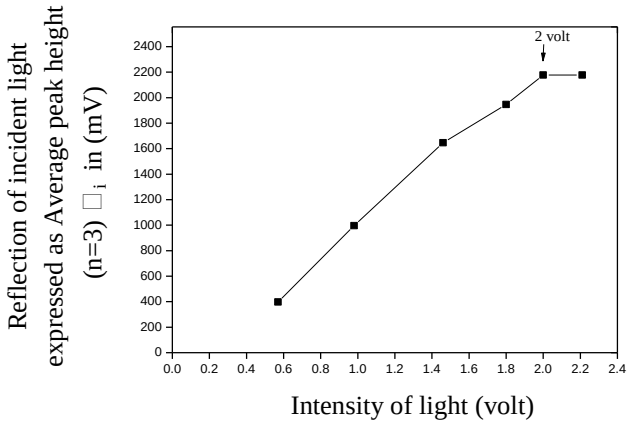



**Figure .9** Effect of the varation of light intensity on the reflection of light expressed as a positive transducer energy response.

## Calibration curve for variation of MTZ concentration versus energy tranducer response

A series of MTZ solution ranging from 0.01-15 mmol.L$^{-1}$ were prepared, using the parameters established in previous experiment. Each measurement was repeated three times. The reflection of incident light expressed as average peak height for n=3 in mV was plotted against the concentration of MTZ as shown in Fig. 10, which noticed that a straight- line graph ranged from 0.05-8 mmol.L$^{-1}$. Above 8 mmol.L$^{-1}$ of MTZ the value for correlation coefficient will deviate from linearity, it might be attributed to the accumulation of precipitate particles that cause a long duration of the precipitate in the flow cell and an increased exposure time in front of the detector which leads to increase in the $\Delta t_B$ and width the response maxima. All results of the linear regression analysis was summarized in table.7

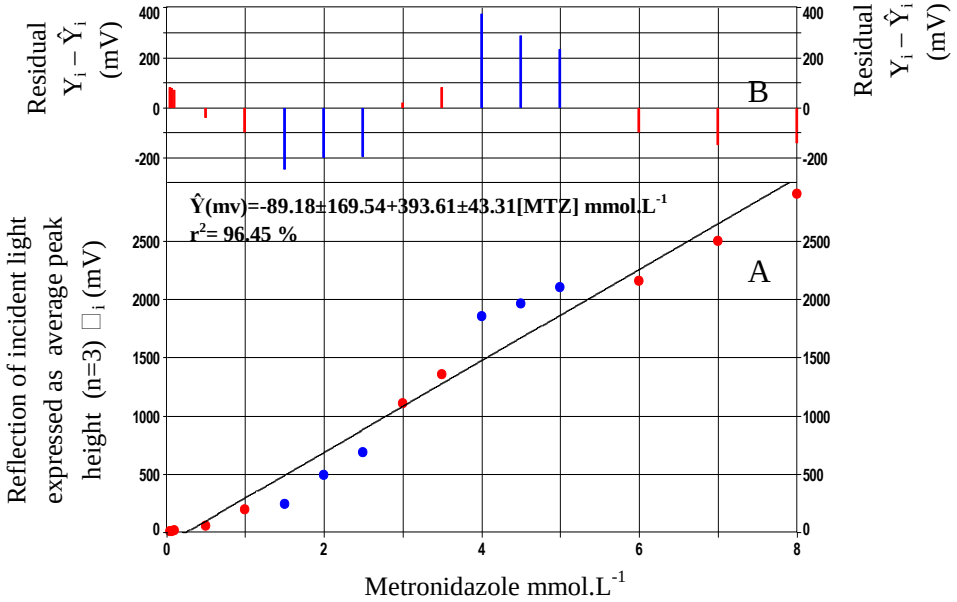



Fig.10. Calibration graph for the variation of Metronidazole concentration on the A: Reflection of incident light expressed by linear equation using Ayah 6SX1-T-2D Solar- CFI analyser B: residual (□i – Ŷi), □i: practical value, Ŷi: estimate value.

***Table 7-*** Summary of linear regression for the variation of energy tranducer response with Metronidazole concentration using first degree equation of the from $\hat{Y} = a+bx$ at optimum conditions.

| Measured [MTZ] $mmol.L^{-1}$ | Range of [MTZ] $mmol.L^{-1}$ n=16 | $\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t[MTZ]$ $mmol.L^{-1}$ At confidence interval 95%, n-2 | r $r^2$% | $t_{tab}$ at 95 %, n-2 | Calculated t-value $\frac{/r/\sqrt{n-2}}{\sqrt{1-r_2}}$ |
|---|---|---|---|---|---|
| 0.01-15 | 0.05-8 | $-89.18\pm169.54+393.61\pm43.31[MTZ]mmol.L^{-1}$ | 0.9821 96.45 | 2.145<<19.498 | |

## Limit of detection

A study was carried out to determine the limit of detection of metronidazole by three different methods at injected sample volume 100 µl; which tabulated in table.8

***Table 8-*** limit of detection for Metronidazole at optimum parameters depend on different approach

| Practically Based on the gradual dilution for the minimum concentration | TheoreticalBased on the value of slope $x=3s_B/slope$ | Theoretical based on the linear equation $\acute{Y}=Y_B+3s_B$ |
|---|---|---|
| 171.15 ng/sample | 1.305 ng/sample | 29.696 µg/sample |

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

## Repeatability

The reality and repeatability of the proposed method was studied at a selected concentration of metronidazole (4 and 4.5 $mmol.L^{-1}$). Five successive measurements were carried out for this experiment. Table.9 tabulated the average obtained response of five measurements, relative standard deviation, and the confidence interval of average response at 95% confidence. A value of relative standard deviation range from 0.095-0.213 indicate clearly that the proposed method and the instrument was used the most suitable for the determination of MTZ. While figure(4.41) shows the kind of profile obtained.

***Table 9-*** repeatability of energy transducer response for MTZ at optimum parameters.

| [MTZ] $mmol.L^{-1}$ | Average response $\square_i$(mV) | RSD % | $\square \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ At confidence interval 95% | Number Of injection |
|---|---|---|---|---|
| 4 | 1879.2 | 0.095 | 1879.2±4.449 | 5 |
| 4.5 | 2098 | 0.213 | 1425±11.123 | 5 |

$t_{0.05/2,n-1}$=2.776

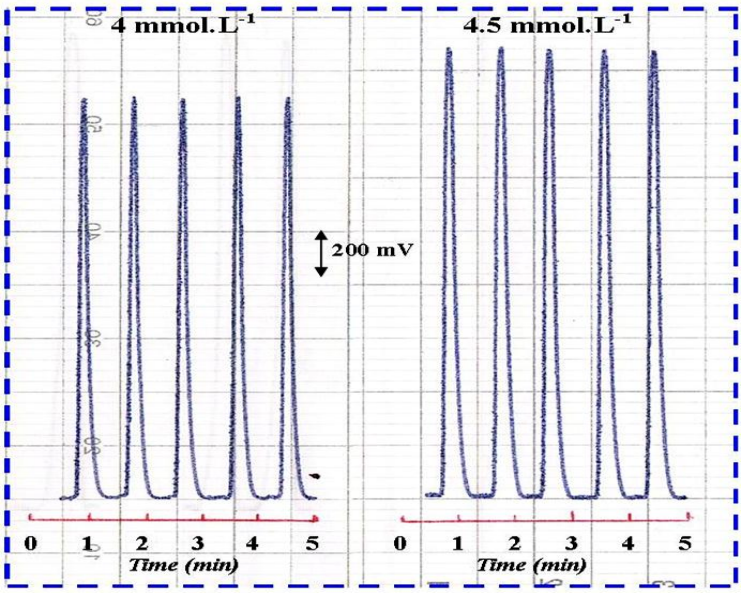



**Fig.11** Response-time of five successive repeatable measurements of MTZ concentration (4,4.5 $mmol.L^{-1}$)

## Analysis of pharmaceutical preparation

The CFIA via reflection of incident light expressed as ($T_{0\text{-}180}{}^{o}$) method using Ayah 6SX1-T-2D solar cell-CFIA analyser achieved in this work was used for the analysis of MTZ in three different of pharmaceutical preparation (Negazole-Julphar, Metrosul-Ajanta and Medazol-SDI) and was compared with classical method via the measurement of turbidity by HANNA instrument, linearity calibration curve was obtained for the concentration range of (0.5-4.5) mmol.$L^{-1}$, correlation coefficient was 0.9797 and limit of detection was 0.4279 mg/sample as tabulated in table.10

***Table 10-*** :Summary of linear regression for determination of MTZ-PMA system using classical method and detection limit

| Measured MTZ mmol.$L^{-1}$ | range mmol.$L^{-1}$ n=9 | $\hat{Y}=a\pm s_a t+b\pm s_b t[x]$ At confidence interval 95%, n-2 | r $r^2$% | $t_{tab}$ at 95 % n-2 | Calculated t-value $\frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$ | Practically Based on the gradual dilution for the minimum concentration |
|---|---|---|---|---|---|---|
| 0.5-8 | 0.5-4.5 | -179.60±101.456+197.46±36.06[x] | 0.9797 95.99 | 2.365<<12.951 | | 0.4279 mg/sample |

X= [MTZ] mmol.$L^{-1}$

A series of solution were prepared of each pharmaceutical drug (50 mmol.$L^{-1}$) by transferring 0.7 mL to each of the five volumetric flask (25 mL), followed by the addition of gradual volumes of standard MTZ (0, 2.5, 3.75, 5, 6.25) mL of 10 mmol.$L^{-1}$ to obtained (0, 1, 1.5, 2, 2.5) mmol.$L^{-1}$ flask no.1 is the sample. The measurement were conducted by both methods. Results were mathematically treated for the standard addition method. The results were tabulated in table.11, at confidence interval 95 %. Paired t- test [21,22] was used as shown in table 12- A,B which shows a comparison. Treatment of data were subjected at two difference paths.

First: comparing individual mean with Quated value as discribed by the manufacturer. Having reference value of 500 mg to be compared with practically found value using Ayah 6SX1-T-2D solar cell-CFIA analyser. Table no 12. A shows the t-test value obtained

Assuming $H_o$: $\mu_{Jul}= \mu_{500}$ for Julphar company

$H_1$: $\mu_{Jul}\neq \mu_{500}$

$H_o$: $\mu_{SDI}= \mu_{500}$ for SDI company

$H_1$: $\mu_{SDI}\neq \mu_{500}$

$H_o$: $\mu_{Ai}= \mu_{500}$ for Ajanta company

$H_1$: $\mu_{Aj}\neq \mu_{500}$

i.e: $H_o$= Null hypothesis , $H_1$= Alternative hypothesis. That indicate

Null hypothesis for Julphar,SDI that there were no significant difference between Quated value and partically found value; while for Ajanta company the alternative hypothesis can be accepted in favour of rejecting Null hypothesis.

Secondary: A paired t-test was conducted between the sample from three different manufacturer by either method of analysis i.e. using Ayah 6SX1-T-2D solar cell CFIA analyser with classical method. Our hypothesis is as follows:

Null hypothesis: $H_o$:$\mu_{new\ method}= \mu_{clasical\ method}$

Alternativelly hypothesis $H_1$: $\mu_{new\ method}\neq \mu_{clasical\ method}$

A t-value for n-1 degree of freedom= 4.303. Any value of (t) calculated should be less than 4.303 in order to accept $H_o$ i.e there is no significant difference between the two method of analysis. Calculated t-value= 0.908 for n-1 at α 0.05 (95%), two tailed test indicate that since 0.908<4.303 therefore; $H_o$ is accepted in favour of $H_1$ as shown that in table 12.B

***Table (11 )*:** Result for the determination of Metronidazole in pharmaceutical preparation by standard addition method using Ayah 6SX1-T-2D Solar analyser-CFI & Classical method turbidity measurement .

| Sample no | Commerical name ,content ,country,company | Confidence interval for the average weight $\bar{W}\pm1.96\,\sigma_{n-1}/\sqrt{n}$ at 95% (g) | Sample weight equivalent to 855.75 mg (50 mmol.$L^{-1}$)of the active ingredient (g) | Theoretical content for the active ingredient at 95%( mg) | Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t[x]$ $\hat{Y}_{(FTU)}=a\pm s_a t+b\pm s_b t[x]$ | r $r^2$% | Practical conc. (mmol.$L^{-1}$) and what is equivalent of active ingredient (mg) | Pratical content of active ingredient at 95% (mg) | Efficiency of determination (%Rec) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *AYAH 6SX1-T-2D Solar CFIA* | | | | |
| | | | | | *HANNA instrument (classical method turbidity measurement)* | | | | |
| 1 | Julphar 500 mg Negazole U.A.E | 0.95131±0.0064 | 1.628 | 500±3.3638 | 748.32±80.31+520.05±48.88[x] | 0.9987<br>99.74 | 51.43<br>880.21 | 514.34±14.92 | 102.87% |
| | | | | | 109.89±65.29+76.65±39.74[x] | 92.62<br>0.9624 | 51.07<br>874.06 | 510.75±16.17 | 102.15% |
| 2 | SDI 500 mg Medazol Iraq | 0.64708±0.0078 | 1.107 | 500±6.0271 | 774.31±274.02+553.16±166.77[x] | 0.9868<br>97.38 | 49.96<br>855.13 | 499.83±7.46 | 99.97% |
| | | | | | 178.03±68.44+125.84±41.65[x] | 0.9841<br>96.85 | 50.54<br>864.99 | 505.59±16.91 | 101.12% |
| 3 | Ajanta 500 mg Metrosul india | 0.64178±0.0062 | 1.098 | 500±4.908 | 840.72±296.05+569.62±31.98[x] | 97.12<br>0.9855 | 52.79<br>903.43 | 528.06±6.96 | 105.61% |
| | | | | | 160.76±104.88+118.46±63.831[x] | 92.08<br>0.9596 | 48.57<br>831.29 | 485.89±17.41 | 97.19% |

$\hat{Y}_i$=Estimated response in (mV) or (FTU). [ X]= MTZ conc.(mmol.$L^{-1}$), r= Correlation coefficient, $r^2$%= Linearity percentage, $t_{0.025,\,\infty}$ = 1.96 at 95 %,

**Table .12-A**: Results of paired t-test for the comparision between partical content from Ayah 6SX1-T-2D solar cell CFIA analyser with Quated value

| Sample no | Practically content of MTZ (mg) | Quated value (mg) | $(\bar{X}-\mu)\sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-T-2D solar analyser with Quoted | |
|---|---|---|---|---|
| | | | $t_{cal}$ | $t_{tab}$ |
| 1 | 514.34 | 500 | 3.821<4.303 | |
| 2 | 499.83 | 500 | \|-0.098\|<<4.303 | |
| 3 | 528.06 | 500 | 17.35>>4.303 | |

**Table.12-**B Paired t-test for Ayah 6SX1-T-2D solar analyser with classical method using standard addition method for determination of MTZ in pharmaceutical preparation

| Sample no | A moment found $\bar{X}$(mg) | | Xd | $\bar{X}d$ | $\sigma_{n-1}$ | $t_{cal}=\bar{X}d\sqrt{n}/\sigma_{n-1}$ at 95 % | $t_{tab}$ at 95% |
|---|---|---|---|---|---|---|---|
| | Proposed method Ayah 6SX1-T-2D-CFIA solar analyser | Classical method | | | | | |
| 1 | 514.34 | 510.75 | 3.59 | 13.33 | 25.40 | 0.908<<4.303 | |
| 2 | 499.83 | 505.59 | -5.76 | | | | |
| 3 | 528.06 | 485.89 | 42.17 | | | | |

**Conclusion**

The suggested methods is simple sensitivite. Application of the proposed methods to the analysis of metronidazole in pure and pharmaceutical preparation. It was shown that with no doubt that newly developed method is a good as the classical method. An alternative analytical methods is found through this research work which based on simple parameter conditions.

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