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Turbidimetric Determination of Metoclopramide Hydrochloride in Pharmaceutical Preparation via the Use of A new Homemade Ayah 6SX1-T-2D Solar Cell-Continuous Flow Injection Analyser

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Abstract:
A newly developed analytical method characterized by its speed and sensitivity for the determination of metoclopramide hydrochloride (MCP-HCl) in pure and pharmaceutical preparation via turbidimetric measurement (0-180^o) by Ayah 6SX1-T-2D Solar cell-CFI Analyser. The method was based on the reaction of phosphomolybdic acid with metoclopramide hydrochloride in acidic medium to form yellowish white precipitate for the ion-pair complex. Turbidity was measured via the reflection of incident light that collides on the surface precipitated particles at 0-180^o. Chemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 0.0005-3 or 0.0005- 4 mMol.L^{-1}, with correlation coefficient r = 0.9947&0.9845 respectively. The limit of detection 3.543 ng/sample from the step wise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 0.3% for 1, 3 mMol.L^{-1} (n=8) concentration of metoclopramide hydrochloride . The method was successfully applied to the determination of metoclopramide hydrochloride 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.

Keywords: Metoclopramide hydrochloride, Flow injection analysis, Turbidity.

التقديرالتعكري لميتاكلوبراميد هايدروكلوريد في المستحضرات الصيدلانية من خلال استخدام محلل جديد مصنع محليا للحقن الجرياني المستمر Ayah 6SX1-T-2D Solar cell

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الخلاصة :
طورت طريقة تحليلية جديدة ,تميزت بالسرعة والحساسية لتقدير الميتاكلوبراميد هيدروكلورايد بشكلة النقي او على هيئة مستحضرات صيدلانية عن طريق قياس التعكرية 0-180º بوساطة محلل الحقن الجرياني المستمر Ayah 6SX1-T-2D Solar cell .استندت الطريقة على تكوين راسب ابيض مصفر لمزدوج ايوني بين الميتاكلوبراميد هيدروكلورايد وحامض مولبدات الفسفوريك في الوسط الحامضي . تم قياس التعكرية عن طريق انعكاس الضوء

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المسلط والمصطدم بسطوح دقائق الراسب بزاوية 0-180º. تم دراسة كافة المتغيرات الكيميائية والفيزيائية . مدى الخطية لمنحني المعايرة للميتاكلوبراميد هيدروكلورايد تمتد 0.0005 - 3 أو 0.0005 - 4 مللي مول. لتر$^{-1}$ بمعامل ارتباط =0.9947 و 0.9845 على التوالي .حدود الكشف 3.543 نانوغرام /نموذج من التخفيف التدريجي لأقل تركيز في منحني المعايرة مع انحراف قيايس نسبي مئوي اقل من 0.3% لتركيز 1,3 مللي مول .لتر$^{-1}$ (n=8) من الميتاكلوبراميد هيدروكلورايد . طبقت الطريقة بنجاح لتعيين الميتاكلوبراميدهيدروكلورايد في ثلاثة مستحضرات صيدلانية . اجريت المقارنة بين الطريقة المستحدثة للتحليل والطريقة التقليدية لقياس التعكرية باستخدام الاضافات القياسية بوساطة اختبار t المزدوج ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعة 95 %.

Introduction:

Metoclopramide(MCP-HCl), 4-amino-5-chloro-2-methoxy-*N*-(2-diethylamino-ethyl) benzamide figure.1, is a dopamine-receptor antagonist,an antiemetic and a stimulant of upper gastrointestinal motility. MCP is available as white or almost white, crystalline powder or crystals, which is very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride,it melts at about 183Cºwith decomposition ,store protected from light [1]. It is used for the management of gastrointestinal motility disorders and gastrointestinal reflux and for the prevention of cancer chemotherapy-induced emesis at much higher doses [2].

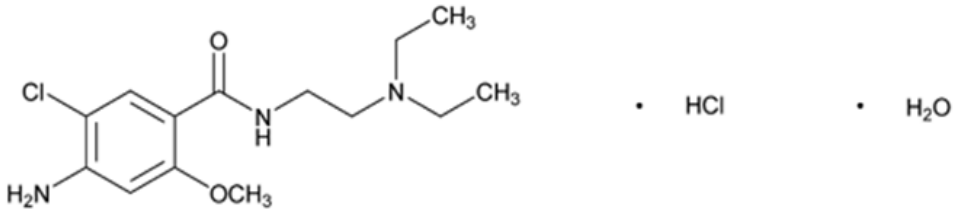


Figure .1-Chemical structure of Metoclopramide hydrochloride

Use of metoclopramide has been associated with extrapyramidal movement disorders .Other effects include drowsiness, depression and headache. Endocrine adverse effects secondary to hyperprolactinaemia may include impotence, amenorrhoea and galactorrhoea [3] .Literature survey revealed that MCP-HCl has been estimated by spectrophotometric [4-11],chemiluminescence [12,13], hydrophilic intereraction chromatography with electrospray ionization tandem mass spectrometric (HILIC/MS/MS)[14], potentiometric [15] , reveresd phase High Performance Liquid Chromatography (R-P-HPLC)[16].

The purpose of this work is to describe a simple, precise and sensitive flow injection turbidimetric method with the use of Ayah 6SX1-T-2D Solar cell –CFI Analyser [17] for determination of metoclopramide hydrochloride in pharmaceutical formulations . The method based on the formation of yellowish white precipitate for the ion-pair compound by phosphomolybdic acid with metoclopramide hydrochloride in acidic medium. The turbidimetry is measured via the reflection of incident light from the surfaces of precipitated particles at 0-180º**.** The positive signal from reflection can be recorded by Ayah 6SX1-T-2D Solar cell supplier with linear array of six super snow-white light emitting diode as a source and two solar cells as a detector.

Experimental

Reagent and chemicals

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solution .A standard solution (0.1Mol.L^{-1}) of metoclopramide hydrochloride (354.3g.mol^{-1}) was prepared by dissolving 8.8575g in 250 ml distilled water . 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 ml volumetric flask. A 1 M of hydrochloric acid solution (35%, 1.19 g.ml^{-1},

BDH) were prepared by pipetting 22 ml of concentrated hydrochloric acid and completed the volume with distilled water in 250 ml. A 1M of nitric acid solution (70% , 1.42g.ml^{-1},BDH)was prepared by pipetting 16 ml of concentrated nitric acid and completed the volume with distilled water to 250 ml . 1M acetic acid solution (99.5%. 1.05g.ml^{-1},BDH) was prepared by pipetting 15 ml of concentrated acetic acid and completed the volume with distilled water to 250 ml. Each acid was standardized against standard solution of 1M from Na_2CO_3 .

Sample preparation

Thirteen tablets weight, crushed and grinded. Tablets containing 10 mg of metoclopramide hydrochloride for (Julphar- premsan , Actavis-metoclopramide) and 5 mg for (NDI-meclodin) were weight (1.089 , 1.158 , 2.009 g) equivalent to 88.575 mg of active ingredient 5 mMol.L^{-1} respectively . The powder was dissolved in distilled water followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 50 ml with distilled water.

Apparatus

Peristaltic pump – 2 channels variables speed (Ismatec , Switzerland)and rotary 6-port medium pressure injection valve, (IDEX corporation ,USA) with sample loop(0.7mm i.d.Teflon ,different length) The response was measured by a homemade Ayah 6 SX1-T-2D Solar cell-CFI Analyser, which uses a six snow white LED for irradiation of the flow cell at 2 mm path length . Two solar cell used as a detector for collecting signals via sample travel for 60 mm length . The readout of the system composed of x-t potentiometric recorder (Kompenso Graph C-1032) Siemens (Germany) or digital AVO-meter (auto range) (0-2volt) (China). Turbidometric readings under batch conditions were made by HANNA company (U.S.A). The flow diagram for the determination of metoclopramide hydrochloride is shown in figure. 2.

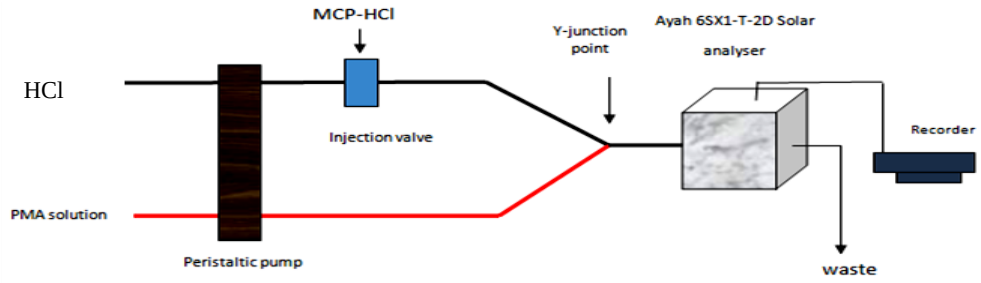
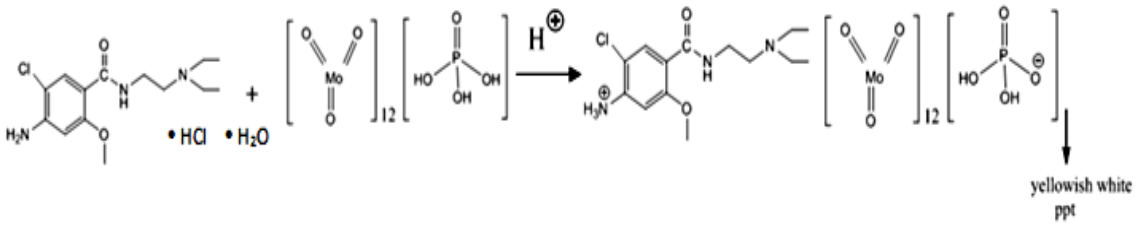


Figure.2-Flow diagram manifold system used for the determination of Metroclopramide hydrochloride

Methodology

The flow system consisting of two lines was used for the determination of MCP-HCl by the reaction between MCP-HCl with phosphomolybdic acid(2 mMol.L^{-1}) in acidic medium(HCl 50 mMol.L^{-1}) to form a yellowish white color precipitate as an ion pair complex form shown in figure. 2 . The first line represent the carrier stream (hydrochloric acid) at 2.2ml.min^{-1} flow rate which lead to the injection valve to carry MCP-HCl, sample volume 100µl;while the second line supplies phosphomolybdic acid solution at 3.1ml.min^{-1} .Both lines meet at a Y-junction ,with an out let for reactants product from complex,which passes through a homemade Ayah 6SX1-T-2D solar cell-CFI Analyser that work with a six snow white light emitting diodes will be used as a source . Each solution injected was assayed in triplicate . The response profile of which was recorded on x-t potentiometric recorder to measure energy transducer response expressed as peak height in mV by reflection of incident light at 0-180º . A probable mechanism of ion pair formation for MCP-HCl-PMA-H_3O^+ system is represented in scheme 1.



MCP-HCl –PMA Ion pair associate

Scheme 1: Mechanism of reaction between of MCP-HCl& PMA

Results and discussion:

Study of the optimum parameters:

The flow injection manifold system as shown in figure.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 variable constant and varying one at a time, i.e fixed variable optimization.

Chemical variables

Phsophomolybdic acid (PMA) concentration

Different concentrations of precipitating reagent (0.5-7) mMol.L-1 were prepared. A 5 mMol.L^{-1} concentration of MCP-HCl and 112µl sample volume was injected through the carrier stream (distilled water) at flow rate 1.8, 2.6 ml.min $^{-1}$ of carrier stream and reagent respectively. The applied voltage to the LEDs was 1.9 volt DC. Each measurement was repeated for three times .the results obtained are summarized in table .1 and figure.3. It was found that 2 mMol.L^{-1} of PMA was the most suitable for a maximum reflection of incident light and was used in all subsequent experiments, more than 2 mMol.L^{-1} mostly causing accumulation of precipitate particles in front of the detector which in turn to a decrease in reflecting surface , this results in a decrease of peak height .

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

[PMA] mMol.L^{-1}	energy transducer response expressed as an average peak heights(n=3) ȳi (mV)	RSD%	Confidence interval at (95%) $\bar{y}i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.5	1360	0	1360±0
0.7	1466.67	0.83	1466.67±30.36
2	1706.67	0.27	1706.67±11.48
5	1525	0.46	1525±17.57
7	1244	0.46	1244±14.05

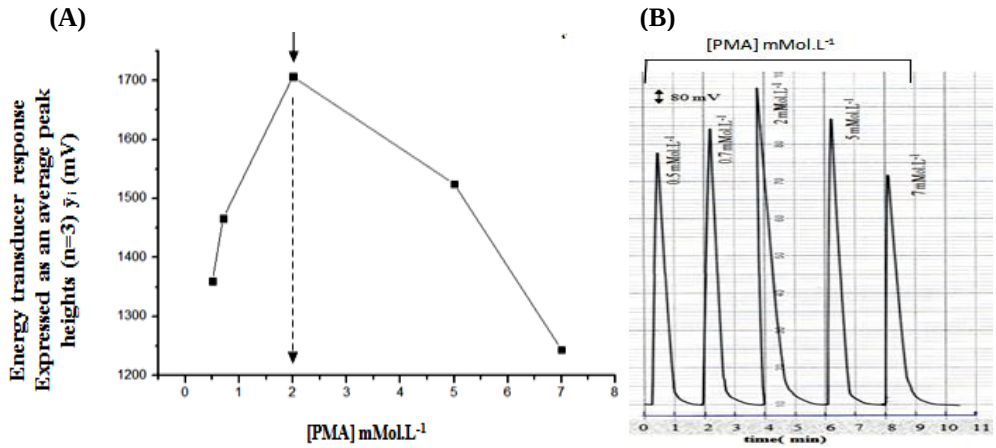


Figure.3-(A) Effect of the [PMA]on energy transducer response by reflection of incident light (B): Response profile versus time

Effect of different acids on the MCP – HCl - PMA System

The ion pair of MCP-HCl (3 mMol.L^{-1})-PMA(2 mMol.L^{-1}) system was studied in different acidic solution media (sulphuric acid, hydrochloric acid , nitric acid and acetic acid) at 10 mMol.L^{-1} concentration in addition to aqueous medium as a carrier stream . The results are summarized in table .2 . The data obtained were plotted as shown in figure. 4. In which, it is expected that acids increase the Solubility of any formed precipitate and thus might causes large pure precipitate. Therefore, It was tried that HCl, H_2SO_4, HNO_3 and CH_3COOH where used as a medium for the entended reaction. All acids used increase response profile to the same level as shown in table .2.

This indicate that the negative radicals as chloride , sulphate and nitrate has no significant effect on the precipitate formed ; while the effect is due to a available oxonium ion from ionization , which form a very suitable medium and more convenient atmosphare for precipitate formation and gave a higher response compared with distilled water .Acetic acid confirm ;the effect of proton (oxonium ion) as acetic acid ionizes to 3.3%[18] only ,i.e supplying less hydronium ion to the reaction medium . HCl was chosen due to:

A. The drug was used as hydrochloride salt.
B. Avoidance introduction of other ionic species to the reaction medium.

Table.2-Effect of acidic media on the measurement of energy transducer response via reflection of incident light expressed as an average peak heights for determination of metoclopramide –HCl

Type of medium	energy transducer response expressed as an average peak heights(n=3) $\bar{y}_i$(mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$	Ka
H_2O	1240.00	0	1240±0	10^{-14}
CH_3COOH	1541.33	0.30	1540±11.48	$1.8x10^{-5}$
HCl	1770.33	0.23	1771.5±10.04	10^{6}
H_2SO_4	1770.33	0.23	1771.5±10.04	10^{6}
HNO_3	1800.00	0	1800±0	28

Ka: Ionization constant

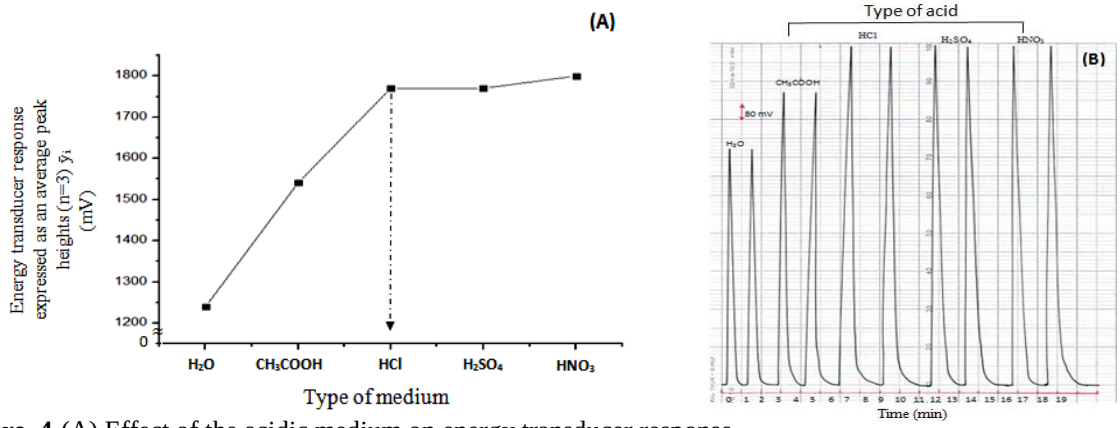


Figure. 4-(A) Effect of the acidic medium on energy transducer response
(B): Response profile versus time using 112µl sample volume, flow rate 1.8,2.6 ml.min^{-1} of carrier stream and reagent ,applied voltage to the LEDs source 1.9 volt DC &open valve (10 sec.).

Effect of HCl concentration

Using MCP-HCl (3 mMol.L^{-1})-PMA(2 mMol.L^{-1})system .A series of solutions were prepared(8-80)mMol.L^{-1} of hydrochloric acid , 112µl at 1.8 and 2.6 ml.min $^{-1}$ flow rate for carrier stream and reagent solution respectively . The results obtained were summarized in table .3. figure.5 was obtained , in which

that, the increase in the reflection of incident light with the increase of HCl concentration for the range (20-50)mMol.L^{-1}, followed by ,increasing acid concentration leads to broadening of the peak response with little decrease in sensitivity ;this might be due to the fines of the precipitate formed at high acid concentration (> 50mMol.l^{-1}) i.e increase solubility or dissociation of some of the precipitate particles . Therefore, 50 mMol.$L^{-1}$of HCl concentrations were chosen as optimum carrier stream.

Table.3-Effect of HCl concentration on the measurement of energy transducer response via reflection incident light for determination of metoclopramide –HCl

[HCl] mMol.L^{-1}	**Energy transducer response expressed as an average peak heights (n=3)** $\bar{y}i$ **(mV)**	**RSD%**	**Confidence interval at (95%)** $\bar{y}i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$	**Δt_B sec**
8	1670.00	0.60	1670±24.84	90
10	1780.00	0.28	1780±12.42	92
20	1806.67	0.64	1806.67± 28.70	96
50	1850.00	0.54	1850±24.84	100
70	1846.67	0.63	1846.67±28.70	106
80	1800.00	0.56	1800±24.84	112

Δt_B: Base width of response

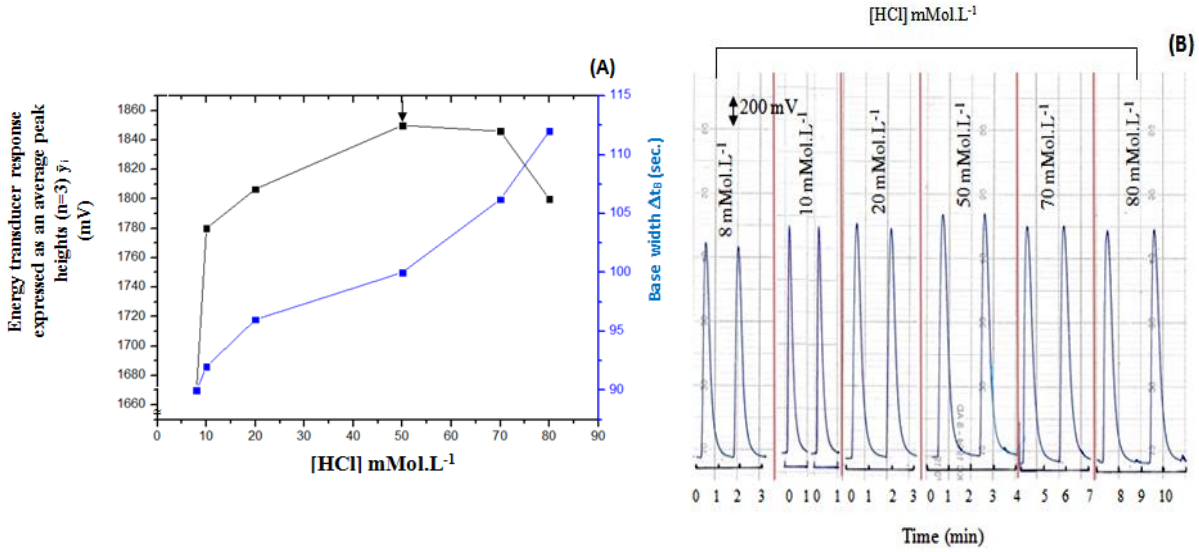


Figure. 5-(A) Effect of the[HCl] on energy transducer response by reflection of incident light (B): Response profile versus time

Physical variables

Intensity of light

Variation of light source intensity on the efficiency for determination of MCP-HCl at 3mMol.l^{-1} was studied .While keeping all other variables fixed (i.e:112µl sample volume , PMA 2mMol.L^{-1} , 50 mMol.L^{-1} of HCl , open valve (10sec)&1.8, 2.6 ml.min^{-1} flow rate for carrier stream and reagent line respectively . The applied voltage to the LEDs was used(0.204-2.280)volt DC by variation of light intensity knob (in the front panel of Ayah 6SX1-T-2D solar cell CFI Analyser figure.2. The whole process was monitored by AVO-meter .The results were tabulated in table .4 which shows that an increase on the energy transducer response with increase intensity of light source .Therefore the intensity of 2 volt DC was selected as the optimum voltage that can be supplied to give a better peak height and for the sake of the

compromise between sensitivity and instrument life time . Figure.6 shows the effect of variation of light intensity on energy transducer response.

Table 4-Effect of intensity of light on the measurement of energy transducer response via reflection of incident light for determination of metoclopramide –HCl

Applied voltage expressed as intensity of light Volt	**Energy transducer response expressed an average peak heights (n=3) ȳi (mV)**	**RSD%**	**Confidence interval at(95%) $\bar{y}i \pm t_{0.05/2\ ,n-1}\sigma_{n-1}/\sqrt{n}$**
0.204	0	0	0
0.439	140	0.7	140±2.43
0.918	620	0.17	620±2.56
1.162	970	0.15	970±3.56
1.361	1190	0.10	1190±2.10
1.573	1460	0.07	1460±2.61
1.905	1850	0.06	1850±3.08
2.000	1890	0.08	1890±3.93
2.240	1970	0.05	1970±2.41
2.280	1960	0.06	1960±2.81

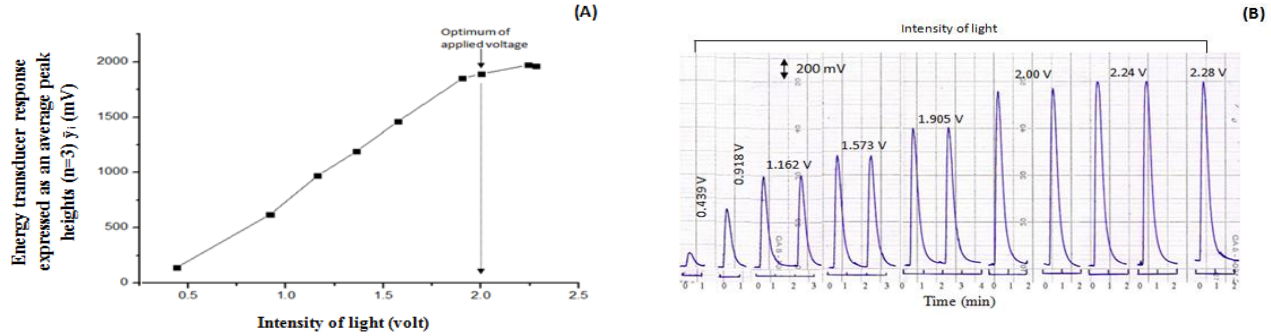


Figure.6-(A) Effect of light intensity on energy transducer response expressed as an average peak heights in mV (B): Response profile versus time

Flow rate

Variation of flow rates (0.25- 2.8),(0.5- 4)ml.min^{-1} for carrier stream and reagent respectively controlled by the peristaltic pump for determination of MCP-HCl at 3mMol.L^{-1} was studied . While keeping all other variables constant (i.e . 112µl sample volume , PMA(2mMol.L^{-1}), 50mMol.L^{-1}HCl , open valve (10sec.)&intensity of light 2 volt DC. The results obtained were summarized in table .5 .It can be recognized that at low flow rate a response peak broadening occur , increase in peak base width (Δt_B) with little increase in peak height as shown in figure. 7 while at higher flow rate little broadening occur and lesser measurement time no much gain in sensitivity . This prove that the allowed time of reaction is the same and it is fast ,and the total precipitate formed is the same , but the compact movement in high pump speed cause the accumulation of precipitation at very short location while at low flow rate, the location of the precipitation particles is distributed among longer distance in front of detector which might be attributed to increase of dilution and dispersion . While at higher flow rate >2.2 ml.min^{-1} for carrier stream, although the effect of physical parameter was not very crucial on concerning response peak heights to obtain sharp maxima and regular response but it was not very high due to departure speed of reflecting surfaces from measuring cell at a short time , therefore the best flow rate was (2.2,3.1)ml.min^{-1}.

Table .5-Effect of the variation of flow rate on the energy transducer response

Pump speed(approximate)	Flow rate ml .min^{-1}		Energy transducer response expressed as an average peak height (n=3) $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$	Base width ΔtB(sec)	t sec.	V_{add} ml	Concentration in mMol.L^{-1} at flow cell	*Df
	Carrier stream	Rea gent								
5	0.25	0.5	2193.33	0.53	2193.33±28.69	540	99	6.862	0.049	61.23
10	0.6	0.85	2030.00	0.50	2030±24.84	291	51	7.145	0.047	63.83
20	1.25	1.8	2020.00	0	2020±0	171	24	8.805	0.038	78.95
25	1.6	2.3	1996.67	0.29	1996.67±14.33	135	23	8.887	0.038	78.95
30	1.8	2.6	1890.00	0.26	1890±12.42	123	21	9.132	0.037	81.08
35	2.2	3.1	1926.67	0.30	1926.67±14.33	70	17	6.295	0.053	56.60
40	2.4	3.5	1900.00	0	1900±0	69	12	6.897	0.049	61.22
45	2.8	4.0	1880.00	0	1800±0	48	11	5.552	0.061	49.18

*Df: Dilution factor at flow cell

V_{add}: addition volume

t (sec): Arrival time from injection valve reading to the measuring cell

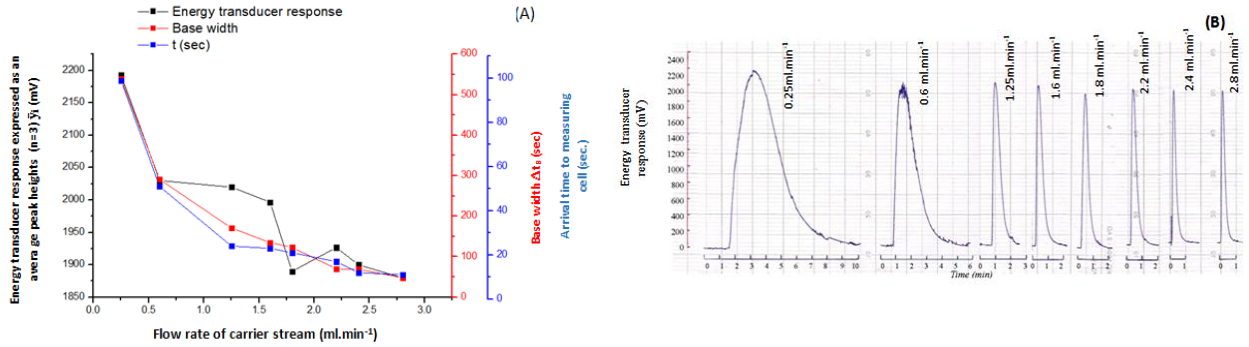


Figure. 7-(A) Effect of the variation of flow rate onenergy transducer response expressed as an average peak heights in mV, base width (Δt_B) and departure time of sample segment from injection valve to the measuring cell (t) in sec.

(B): Response profile versus time

Sample volume

Using MCP-HCl (3mMol.L^{-1})-PMA(2mMol.L^{-1}) system and variable sample volumes (70-112)µl were used ,while keeping all other changeable constant (i.e . 50mMol.L^{-1}of HCl , flow rates (2.2,3.1)ml .min^{-1} for carrier stream and reagent respectively , open valve (10 sec.)& applied voltage to the LEDs was 2volt DC . The plot of change in sample volume vs. reflection of incident light and ΔtB is shown in figure.8- A . It was noticed that an increase of sample volume up to 100µl lead to a significant increase in response height(gave an increase of ≈ 20%) & more perceptible than low volume as shown in figure.8- B .While a larger sample volume i.e :more than 100µl even though it gave a slightly higher response(add only 2%) but it was characterized with wider Δt_B which might be attributed to the continuous relatively longer time

duration of precipitate particles segment in front of the detector and increase of the particles size causing a slow movement of precipitate particles so;100µl was the best sample volume . All results were tabulate in table 6.

Table 6-Effect of the variation of sample volume on the transducer energy response determination of MCP-HCl

Loop length Cm r=0.35 mm	**Sample volume µl $V=r^2h\pi$**	**Energy transducer response expressed as an average peak heights(n=3) $\bar{y}i$ (mV)**	**RSD%**	**Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$**	**Base width Δt_B sec.**	***t sec.**
18	70	1576.67	0.37	1576.67±14.35	51	13
19	73	1616.67	0.95	1616.67±37.95	54	13
21	81	1690.00	0	1690±0	57	14
22	85	1800.00	0.56	1800±24.84	60	15
26	100	1890.00	0.26	1890±12.42	66	16
27	104	1895.00	0.37	1895±17.56	68	16
29	112	1926.00	0.3	1926±14.33	70	17

*t (sec.): Departure time for sample segment from injection valve to the measuring cell.

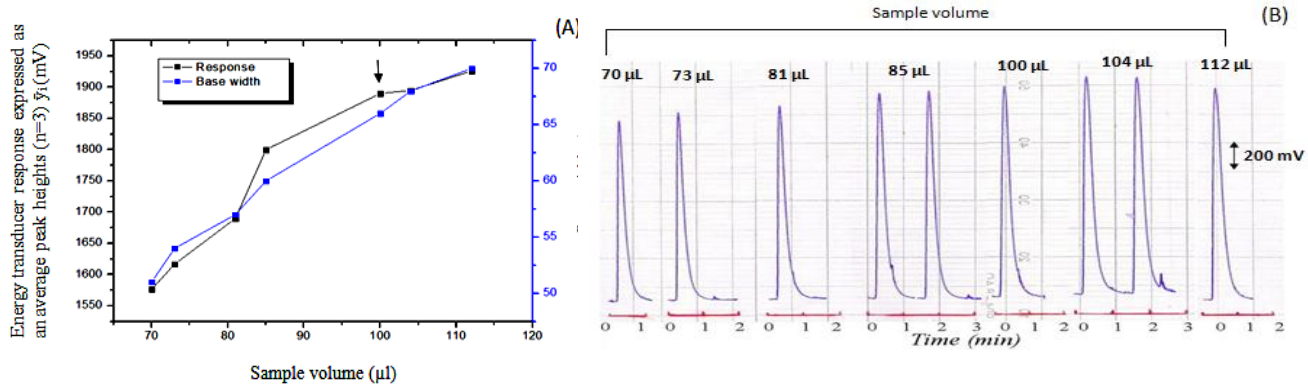


Figure. 8-(A) Effect of the variation of sample volume on energy transducer response expressed as an average peakheights in mV

(B): Sample of response profile versus time

Purge 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 segment from the injection valve. 2-13 seconds were used in this study . The optimum physical and chemical parameters achieved in previous section were kept constant . Figure . 9 shows the continuation of the increase the height of response with increase of purge time up to 6 sec, after that there was no longer significant difference in peak height but increase of Δt_B,which might be attributed to the resistance of flow due to the continuous passage of carrier stream through the injection valve which leads to the slow movement of reflecting particles , therefore 6sec. as a purge time was chosen as optimum to completely purge of sample segment from sample loop . The obtained results were tabulated in table .7.

Table.7-Effect of variation purge time on the transducer energy response for determination of MCP-HCl

Purge time count	Purge time sec	Energy transducer response expressed an average peak heights (n=3) $\bar{y}_i$ (mV)	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$	t sec.
5	2	1030	1030±2.43	13
10	3	1380	1380±2.68	13
15	5	1770	1770±2.78	14
20	6	1920	1920±2.14	15
25	8	1900	1900±4.17	15
30(Open valve)	10	1890	1890±4.55	16
40	13	1850	1850±4.52	16

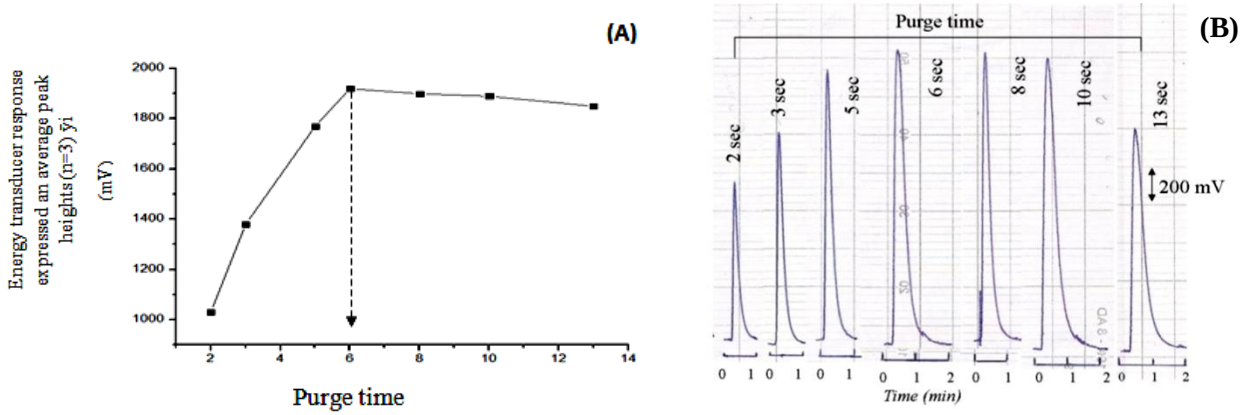


Figure. 9-Effect of the variation of purge time on

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

(B): Response profile versus time

Scatter plot calibration curve for variation of MCP-HCl versus energy transducer response

Using the optimum chemical and Physical parameters a series of MCP-HCl solution (0.0005-7) mMol.L^{-1}were prepared .A scatter plot diagram shows that a linear calibration graph range for the variation of the energy transducer response of Ayah 6SXI-T-2D solar cell CFI Analyser with MCP-HCl concentration was ranging from 0.0005-3 mMol.L^{-1} or 0.0005-4 mMol.L^{-1} with correlation coefficient (r) :0.9947& 0.9845 respectively as shown in figure .10-A,B,C . The results obtained were tabulated in table. 8. It was noticed, above 4 mMol.L^{-1} a broad in the peak maxima was observed and increase of the base width (Δt_B) , this cause a deviation of correlation coefficient . It might be attributed to the an increase in precipitate particulats and its compactness, thus leading to decrease interstitial spaces and reflecting surface , in addition to an increase of particle size causing a slow movement of precipitate particles leading to a longer time duration of precipitate segment in front of the detector .

Table.8 summary of liner regression for the variation of energy transducer response with MCP-HCl concentration using first degree equation $\hat{y}=a+bx$ [19,20] at optimum conditions .

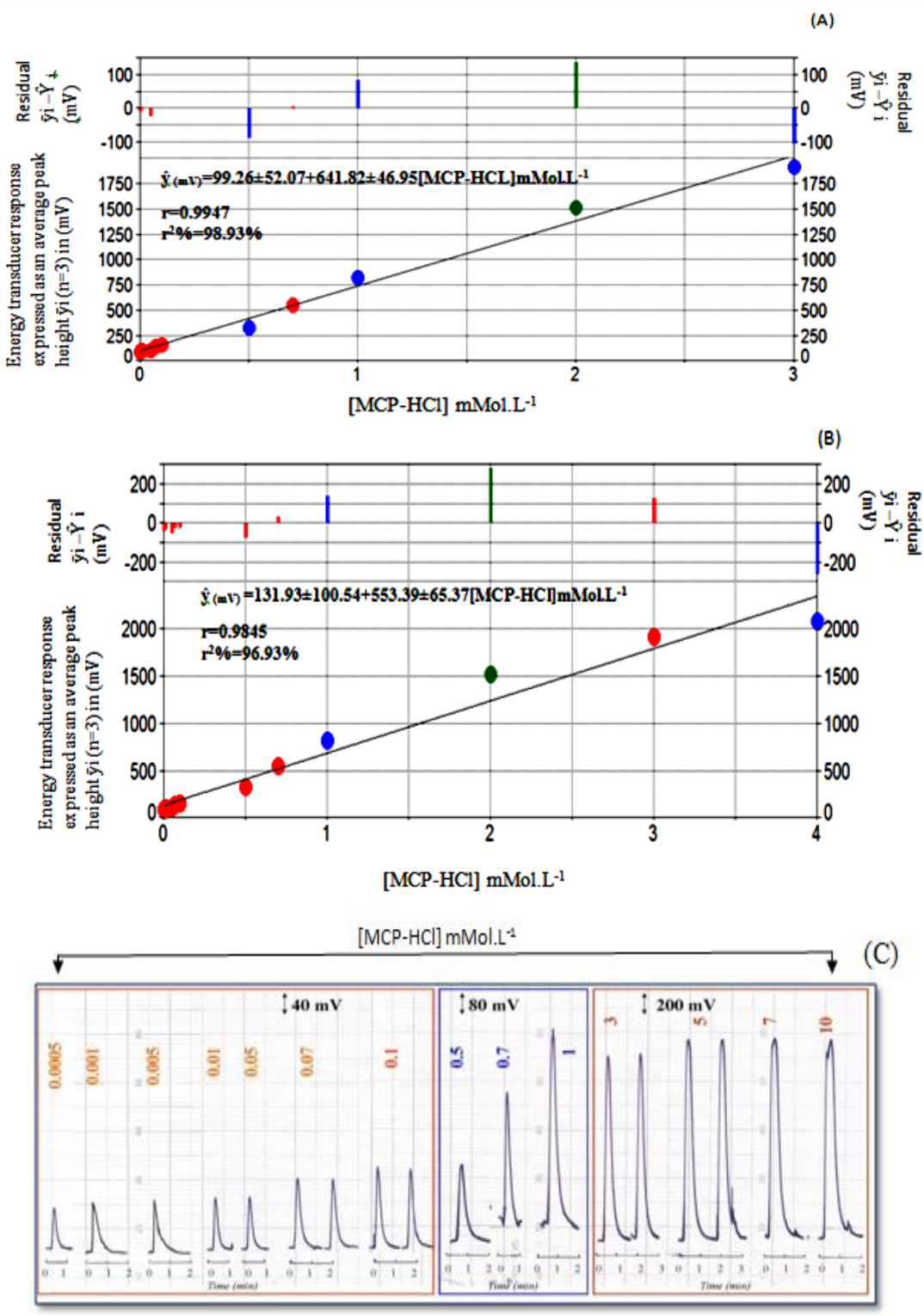


Figure. 10 A,B -Calibration graph for the variation of Metoclopramide hydrochloride concentration on the energy transducer response by reflection of incident light expressed by linear equation (a+bx)using Ayah 6SX1-T- 2D solar cell –CFI Analyser.

(C): Response profile versus time

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

Measured [MCP-HCl] mMol.L^{-1}	**Range of [MCP-HCl] mMol.L^{-1} (n)**	**$\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t$[MCP-HCl]mMol.L^{-1} at confidence level 95%,n-2**	**r r^2%**	**t_{tab} at 95%,n-2**	**Calculated t-value = $/r/\frac{\sqrt{n-2}}{\sqrt{1-r^2}}$**
0.0005-7	0.0005-3 (12)	$\hat{Y}$=99.26±52.07+641.82±46.95[X]	0.9947 98.93	2.228<< 30.46	
	0.0005-4 (13)	$\hat{Y}$=131.93±100.54+553.39±65.37[X]	0.9845 96.93	2.201<< 18.63	

[X]=[MCP-HCl] mMol.L^{-1} , $\hat{Y}$=estimate value , r = correlation coefficient
r^2% = Linearity percentage, r^2= coefficient of determination (C.O.D)

Limit of detection

In general, terms, the L.O.D of an analyte may be described as that: concentration, which gives an instrument signal (y) significantly different from the blank or background signal. This description gives the analyst a good deal of freedom to decide the exact definition of L.O.D.

There is an increasing trend to define the L.O.D. as: the analyte concentration giving a signal equal to the blank signal, y_B plus three standard deviation of the blank S_B.

L.O.D = y_B+ $3S_B$

We have been using three approaches for the expression of L.O.D

1. (Gradual dilution).

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

2. Theoretically (slope method)

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

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

3. Theoretically (Linear equation)method

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

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

The last two methods are an output of a linear regression graph treatments where they obtained (real) results are subjected to statistical treatments; these method can be used as an approximate indication but should not unless otherwise defined.

A study was carried out to calculate the limit of detection of MCP-HCl through three methods as tabulated in table.9.

Table.9-Limit of detection for MCP-HCl at optimum parameters using 100µl as an injection sample

Practically based on the gradual dilution for the minimum concentration (0.0001 mMol^{-1})	Theoretical based on the value of slope x=$3S_B$/slope for n=13	Theoretical based on the linear equation $\hat{Y}=Y_B+3S_B$
3.543 ng	57.96 ng	11.13 µg

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 relative standard deviation expressed as percentage, which is equally to the repeatability of the measurement. A repeated measurements for eight successive injections were measured at fixed

concentrations of MCP-HCl , while mainly two concentrations were used and the obtained results is tabulated in table 10 . The percentage of relative standard deviation less than 0.3 %, indicate a reliable measurement can be achieved using this method. Figure.11 is shown response profile of repeatability at 1 and 3 mMol.L^{-1} respectively.

Table.10-Repeatability of MCP-HCl at optimum parameters with 100µl sample volume.

[MCP-HCl] mMol.L^{-1}	Average response $\bar{y}_i$ (mV) n*=8	RSD %	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
1	824	0.24	824±1.664
3	1920	0.12	1920±1.990

n*=number of injection

$t_{0.05/2,7}$=2.365

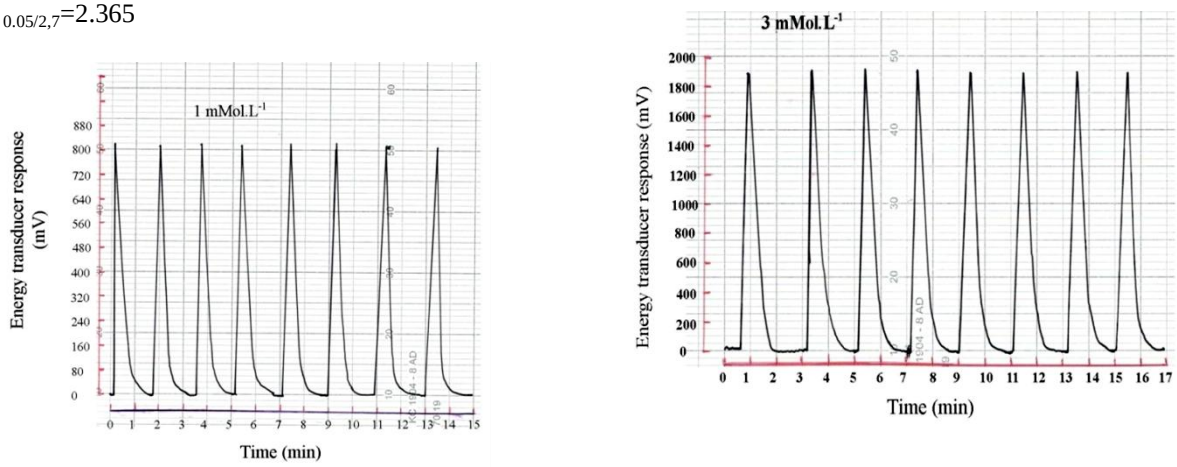


Figure 11-Response – time of profile for eight successive repeatable measurement of MCP-HCl concentration (1 and 3 mMol.L^{-1})

Assessment of the use of Ayah 6SX1-T-2D solar cell CFI Analyser for the determination of MCP-HCl in the pharmaceutical preparation.

The CFIA via reflection of incident light expressed as ($T_{0\text{-}180^o}$) method using Ayah 6SX1-T-2D solar cell –CFI Analyser achieved in this work was used for the analysis of MCP-HCl in the three different drug manufactures (Julphar –UAE -10 mg , actavis-UK -10 mg , NDI –Iraq-5mg)and was compared with classical method via the measurement of turbidity by HANNA instrument . A linearity calibration curve was obtained for the concentration range of 0.05-3 mMol.L^{-1} of MCP-HCl as shown in figure. 12, correlation coefficient was 0.9968 and limit of detection was 10µMol.L^{-1} as tabulated in table 11.

Table.11-Summary of linear regression for MCP-HCl using turbidity measurement by HANNA instrument (classical method).

[MCP-HCl] mMol.L^{-1} measured	Rang at calibration curve n=10	Linear regression at confidence interval 95%,n-2 $\hat{Y}_{(FTU)}=a\pm s_a t+b\pm s_b t[x]$	r r^2 r^2%	t_{tab} at 95%,n-2	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$	D.L
0.0005-10	0.05-3	66.55±22.60+222.34±14.46[x]	0.9968 0.9937 99.37%	2.306 << 35.467		10µMol.L^{-1}

D. L: Detection limit based on the gradual dilution for the minimum concentration for calibration curve

[X]: [MCP-HCL] mMol.L^{-1},$\hat{Y}$: Estimate value

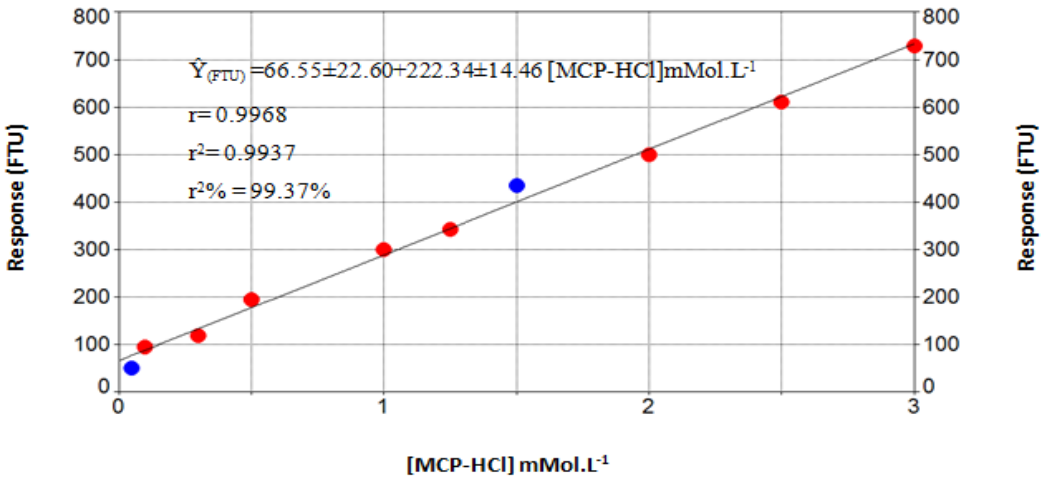


Figure. 12 -Calibration graph for the variation of MCP-HCl concentration versus turbidity in FTU using HANNA instrument

A series of solution were prepared of each pharmaceutical drug (5mMol.L^{-1}) by transferring 2 ml to each six volumetric flask (25 ml), followed by the addition of gradual volumes of standard MCP-HCl (0, 2, 2.5 , 3 ,3.5 , 4) ml of 10 mMol.L^{-1} to obtain (0, 0.8, 1, 1.2, 1.4, 1.6) mMol.L^{-1} . Flask no.1 is the sample .The measurements were conducted by both methods. Results were mathematically treated for the standard addition method. The results were tabulated in table 12-A ,at confidence level 95% ; showing practically concentration of MCP-HCl in each pharmaceutical drug using two method of analysis. Table 12-B was shown a practical content of active ingredient at 95% confidence level &efficiency of determination in addition to paired t-test, which shows a comparison at two difference paths:

First: comparing individual between mean ($\bar{x}$) with quoted value (μ or U_0)([British pharmacopeia] as described by the manufacturer), which depend on the results that was obtained an individual dependant t-test table 12-B colume 8 was conducted for the analysis of measuring the active metoclopramide-HCl material that is present in pharmaceutical preparation . Three sources of three different companies and manufacturer were used; Julphar (UAE), Actavis (UK) &NDI (Iraq).Assuming the following assumptions:

Null hypothesis: H_0 UAE (Julphar) $\bar{x} - U_0 = 0$

Alternative hypothesis: H_1 $\bar{x} - U_0 <> 0$

A calculated t-value shows that at 0.05 probability a value of calculated t- value of – 0.213 was obtained while at DF (n- 1) of the critical t-value was 4.303 this clearly indicate that the H_0 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 metoclopramide and mecloden drug .Table, no.12-B column 8 tabulates the summary of these calculations based on standard addition method.

Secondary: A paired t- test was conducted between the samples from three different manufacturers by either method of analysis i.e. using Ayah 6SX1-T-2D Solar cell CFI Analyser with classical method as shown in table 12-B column 11.

Our hypothesis is as follows:

Null hypothesis: H_O: $\mu_{developed\ method} = \mu_{classical\ method}$

against

Alternatively hypothesis H_1: $\mu_{developed\ method} \neq \mu_{classical\ method}$

Since $t_{calculated}$= 0.415<<t_{tab} (4.303), therefore H_0 is accepted against H_1.These indicated ,there is no significant difference between two method at 95% confidence level for the determination of MCP-HCl in pharmaceutical preparation.

Table.12-A: Standard addition results for the determination of MCP-HCl in three pharmaceutical preparations

No. of sample	Commercial name, Company Content Country	Sample weight equivalent to 88.575 mg (5 mMol.L^{-1}) of the active ingredient (g)	[MCP-HCl] mMol.L^{-1}						Equation of standard addition at 95% for n-2 $\hat{Y}_{(mV)}$= a±s_at+b±s_bt[x] $\hat{Y}_{(FTU)}$= a±s_at+b±s_bt[x]	r r^2 r^2%	Practical concentration mMol.l^{-1} in 25ml 50ml
			0	0.8	1	1.2	1.4	1.6			
			Energy transducer response expressed as an average peak heights $\bar{y}_i$ (n=3) in mV								
			Ayah 6SX1-T- 2D Solar cell CFIA								
			HANNA instrument (classical method) Turbidity measurement								
1	Premsan Julphar 10 mg Ras Al Khaimah U.A.E	1.0886	290	720	920	1060	1180	1340	259.58±96.94+658.75±86.14[x]	0.9956 0.9912 99.12	0.394 4.925
			143	600	780	825	830	874	201.33±200.34+474.00±178.02 [x]	0.9653 0.9318 93.18	0.425 5.309
2	Metoclopramide Actavis 10 mg UK	1.1577	300	800	1000	1140	1280	1400	286.67±66.87+700±59.43[x]	0.9981 0.9963 99.63	0.409 5.113
			200	577	581	861	875	919	194.96±168.78+473.88±149.96 [x]	0.9749 0.9506 95.06	0.411 5.143
3	Meclodin NDI 5mg Iraq	2.0088	285	783	976	1116	1255	1413	264.21±67.29+707.13±95.80[x]	0.9982 0.9963 99.63	0.374 4.675
			100	613	652	724	751	810	165.46±178.58+442.88±158.68 [x]	0.9683 0.9375 93.75	0.376 4.670

$\hat{Y}$= Estimated response in mV or FTU , [x] = [MCP-HCl] mMol.L^{-1} , r= Correlation coefficient , r^2%= Linearity percentage

Table. 12-B-Summary of results for paired t-test, practical content and efficiency of determination of MCP-HCl in three samples of pharmaceutical preparation .

Sample no.	Confidence interval for the average weight $\overline{Wi}\pm1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight equivalent to 88.575 mg ($5mMol.L^{-1}$) of the active ingredient(g)	Theoretical content of the active ingredient at 95% (mg)	Practical concentration ($mMol.L^{-1}$)and what is equivalent of active ingredient (mg)	Practical content $\overline{Wi}\pm4.303\sigma_{n-1}/\sqrt{n}$ (mg) for (n=3) ,at 95%	Efficiency of determination (Rec. %)	Paired t-test			
							Individual comparison $(\overline{X}-\mu)\sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-T-2D Solar cell-CFIAnalyser with Quoted value $t_{0.05/2,2}=4.303$	Comparison between two method		
				Ayah 6SX1-T- 2D Solar cell CFIA				Xd	$\overline{X}$ d (σ_{n-1})	$t_{cal}=\overline{x}_d\sqrt{n}/\sigma_{n-1}$ at 95 %
				HANNA instrument (classical method) Turbidity measurement						
1	$0.1229\pm1.56\times10^{-3}$	1.0886	10±0.1302	4.925 87.246	9.849 ± 3.06	98.49	|0.213|<<4.303	0.77	0.415 0.503	1.43<< 4.303
				5.309 94.056	10.619±0.986 10.619- 9.849	106.19				
2	$0.1307\pm3.15\times10^{-4}$	1.1577	10±0.0230	5.113 90.572	10.226±2.43	102.26	0.394<<4.303	0.059		
				5.143 91.108	10.285±1.100 10.285-10.226	102.85				
3	$0.1134\pm1.64\times10^{-3}$	2.0088	5±0.0705	4.670 82.730	4.670 ± 4.92	93.40	|0.284|<<4.303	0		
				4.670 82.73	4.670±1.086 4.670-4.670	93.40				

Xd: Difference between two Method, $\overline{x}$ d: difference mean , σ_{n-1}**:**Difference standard deviation , n= no. of sample =3

Conclusion

The suggested methods are simple, sensitivities and rapid. Application of the proposed methods to the analysis of metoclopramide hydrochloride in pure and pharmaceutical preparation based on formation yellowish white precipitate for ion- pair compound for the reaction of metoclopramide hydrochloride with phosphomolbdic acid in acidic medium . It was shown that with no doubt that newly developed method is a good as the classical method. An alternative analytical method is found through this research work, which based on simple parameter conditions.

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