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Determination of metoclopramide hydrochloride via quenched continuous fluorescence of fluorescein sodium salt molecule

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Abstract:

A simple and sensitive method using laser diode fluorimeter homemade instrument coupled with flow injection system for the determination of metoclopramide hydrochloride by quenched of continuous fluorescence using fluorescein sodium salt molecule at 405nm laser beam as the irradiation source . Metoclopramide hydrochloride could be determined in the concentration range of 0.05-10 mMol.L^{-1} with detection limits of 76.175ng/sample based on gradual dilution of lowest concentration in calibration graph.

Key words: Laser diode fluorimeter , flow injection analysis , metoclopramide hydrochloride

INTRODUCTION

Metoclopramide hydrochloride (MCP-HCl), chemically known as 4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxybenzamide hydrochloride, is an antiemetic and gastroprokinetic agent. Its action and use is dopamine receptor antagonist and antiemetic, it is primary used to treat nausea and vomiting, to facilitate gastric emptying in patients with gastroparesis. It is also used for the prevention of cancer chemotherapy-induced emesis at much higher doses and used in pregnancy as a second choice for treatment of hypermesis (vomiting of pregnancy) [1-4] . Metoclopramide can be used to treat stomach upset including heartburn, wind, pain, indigestion, sickness and bile regurgitation, to stop nausea and vomiting. Many methods have been reported for the determination of metoclopramide hydrochloride in pharmaceutical formulations and or biological fluids. These methods include, flow injection analysis[5-8] ,spectrophotometry[9-15] , potentiometry[16] , and titrimetry[11] .

Experimental

A stock solution (0.01 Mol.L^{-1}) of fluorescein salt ($C_{20}H_{10}Na_2O_5$, M.Wt 376.27 g.moL^{-1} , Hopkin&William) was prepared by dissolving 1.8814g in 500 mL of distilled water . A stock solution of Metoclopramide hydrochloride ($C_{14}H_{22}Cl.N_3O_2$, M.Wt 354.30 g.moL^{-1} , SDI, 0.1mol.L^{-1}) was prepared by dissolving 3.543g in 100mL of distilled water.

Sample preparation

Thirteen tablets weight ,crushed and grinded . Tablets containing 10 mg of metoclopramide hydrochloride for (Sanofi-aventis France, Actavis UK) and 5mg of SDI Iraq) (were weight (1.336 , 1.384 , 2.432 g) equivalent to 106.29mg of active ingredient 3 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 .

Reagents and chemicals

Apparatus

Laser diode fluorimeter is a homemade instrument that is capable in measuring fluorescence light at two available laser diodes having the wavelength at 405nm (10mW) & 532nm laser diode of not less than 100mW. Each radiation source is fitted with a 2mm flow cell in a block of brass metal equipped with a photo diode detector. The angle between the radiation source at an aperture of 2mm as a maximum radiation area for a flow cell having outside diameter ,4mm inside diameter 2mm (path length for absorption of irradiation). The angle between irradiation source-flow cell- detector is 90^o. The whole instrument composed of five main parts which are as follows :fluorescence cell(composed of cubic (50 mm (L), 50 mm (W), 50 mm (D)) brass metal block) , flow cell(quartz silica having the length of 60mm), detector (photo diode having the diameter of a 4mm which respond to the visible area) , irradiation sources(two laser sources have been used). The first source blue-violet having the wavelength 405nm it's a solid state laser of continuous wave with a light intensity equivalent to 1800-2000 Lux at a distance of 1mm (distance of the source to the detector). Second source green it's a solid state laser with a continuous wave of 532nm with a light intensity more than 2000Lux), and general panel of instrument. All tubes are made of Teflon 1mm inside diameter 2mm outside. Peristaltic pump – 2channel variables speed (Ismatec , Switzerland)and a rotary 6-port injection valve(IDEX corporation ,USA) with a sample loop (0.5mm id, Teflon, variable length) used for sample injection. The output signals was recorded by x-t potentiometric recorder (KOMPENSO GRAPH C-1032) Siemens (Germany).

METHODOLOGY

The flow injection manifold system is composed of the solution as first line by using fluorescein sodium salt (0.5mMol.L^{-1}) as the carrier stream (1.30mL/min) which lead to the injection valve no.$_2$ (where injection valve no.$_1$

was closed) to carry MCP-HCl sample of 43µL (1,5and 7mMol.L^{-1}) and then to the measuring cell. The responses were monitored using 405nm laser beam as the irradiation source thought the reaction as shown in fig1.B$_1$.

B$_1$: Response profile using: a:total continuous response of fluorescence (fluorescein salt molecule) affected by: b: blank (D.W) , variable concentration of MCP-HCl c: 1 mMol.L^{-1} , d: 5mMol.L^{-1} and e: 7mMol.L^{-1}

Variable optimization

A series of experiments were conducted to establish the conditions for the formation quenching fluorescence response with best working optimum reaction parameters .

Physical variables

Flow rate

A set of experimental was carried out for the optimization of the preffered flow rate of fluorescein sodium salt as a carrier stream (0.5mMol.L^{-1}) that extent (0.20-1.75)mL/min using 31µL of 7mMol.L^{-1} of MCP-HCl and open valve mode. The profile obtained were shown in fig.2.A and tabulated in table 1. It was noticed that at slow flow rate, there is an increase in dilution and dispersion which might cause an increase in base of response (Δt_b) . While at higher flow rate (i.e: >1.55 and 1.75mL/min) , although the effect of physical parameter was very crucial on the response and increase in flow rate led to the quenched species to the measuring cell in a minimum time, and causes in obtaining of regular response and sharp maxima, but it is not very high due to departure speed of quenched species from measuring cell at a short time. Therefore a flow rate of 1.30mL/min was adopted for MCP-HCl determination to obtain regular response and minimize the consumption of fluorescein sodium salt solution as shown in fig.2.B.

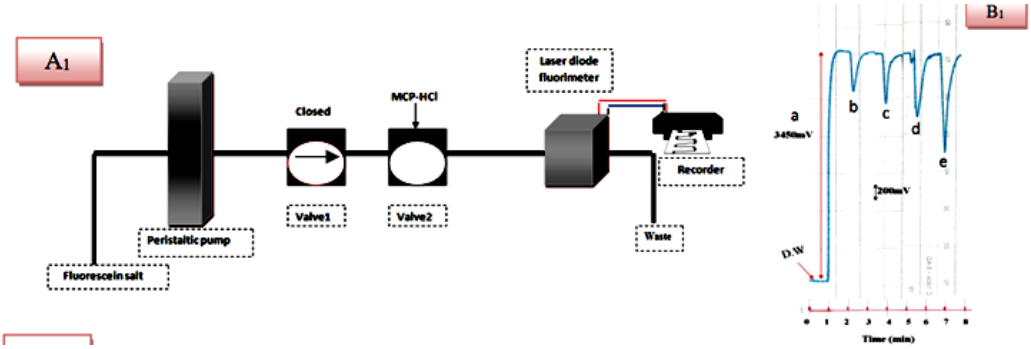


Fig.1: A$_1$ Flowgram for MCP-HCl determination by using one manifold design.

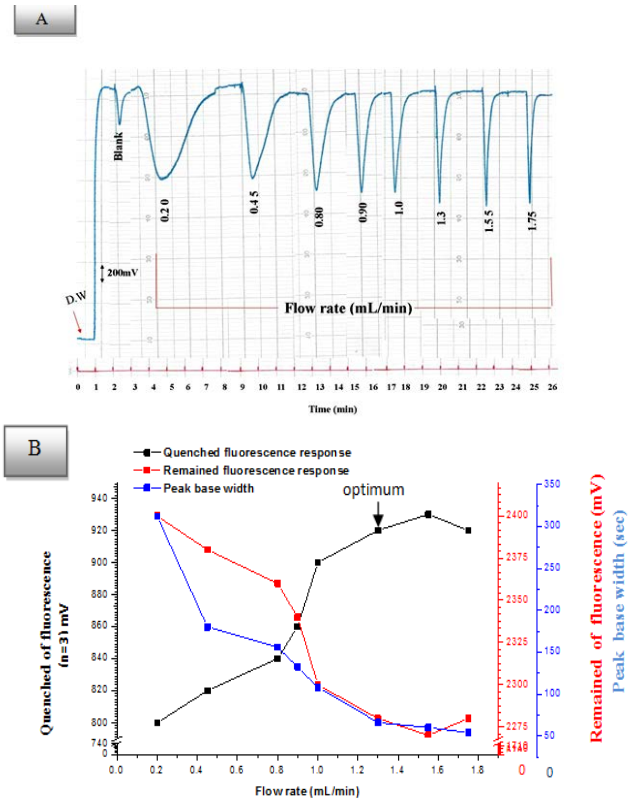


Fig.2: Effect of flow rate on: A-Response profile B- Quenched of fluorescence , remained of fluorescence and peak base width

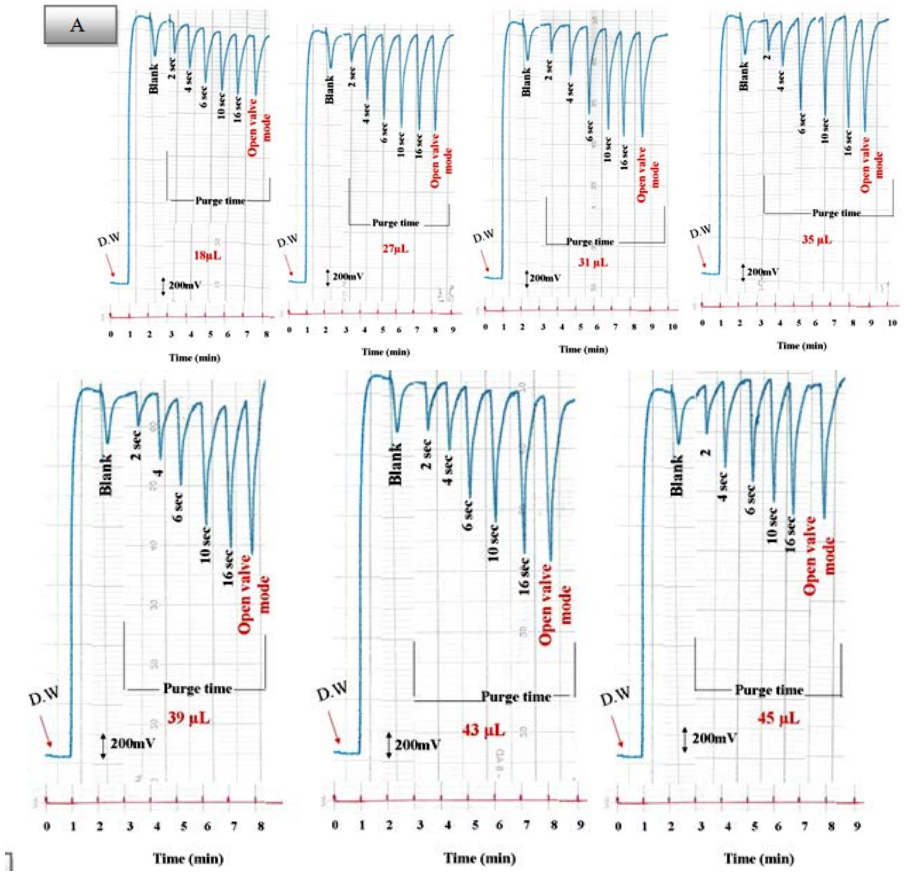
Table 1: Effect for the variation of flow rate for determination of MCP-HCl using 31µL

Speed pump	Flow rate (mL/min)	Total quenched fluorescence expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2, n-1} \sigma_{n-1}/\sqrt{n}$	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	Remained fluorescence $\bar{y}_{Ri}$ (n=3)mV	Δt_b (Sec)	V_{final} (mL)	Concentration in $mMol.L^{-1}$ at flow cell	DF
5	0.20	1100	0.09	1100±2.46	800	2400	312	1.071	0.203	34.55
10	0.45	1120	0.12	1120±3.34	820	2380	180	1.381	0.157	44.55
15	0.80	1140	0.40	1140±11.33	840	2360	156	2.111	0.103	68.09
20	0.90	1160	0.23	1160±6.63	860	2340	132	2.011	0.108	64.87
25	1.00	1200	0.35	1200±10.43	900	2300	108	1.831	0.119	59.06
30	1.30	1220	0.15	1220±4.55	920	2280	66	1.461	0.149	47.13
35	1.55	1230	0.16	1230±4.89	930	2270	60	1.581	0.137	50.99
40	1.75	1220	0.30	1220±9.09	920	2280	54	1.606	0.135	51.81

Response of continuous fluorescence : 3500mV, Response of blank : 300mV. Δt_b (sec) : Time lapse for the fluorescence response within measuring cell or peak base width. V_{final} : Addition volume(mL) at each flow rate to obtain the final volume at flow cell ,DF: Dilution factor at each flow rate at flow cell

Effect of different sample volume with variable purge time

Using (0.5mMol.L^{-1}) fluorescein sodium salt -MCP-HCl (7mMol.L^{-1}) system at flow rate 1.30mL/min for carrier stream. The injection volume was varied from (18-45)µL using different purge time (2-16) sec in addition to open valve mode. All responses profile obtained were shown in fig.3.A and tabulated in table 2 . It was found that any increase in the sample volume up to 43µL led to an increase in the height of responses and showing that the optimum sample volume was 43µL gave a regular response. The increase of sample segment (more than 43µL) probably might increases the time duration of sample segment in front of the detector as illustrated in fig.3.B. At the same time, it was increasing purge time. Therefore; 43µL and open valve mode were chosen as an optimum sample volume and purge time.



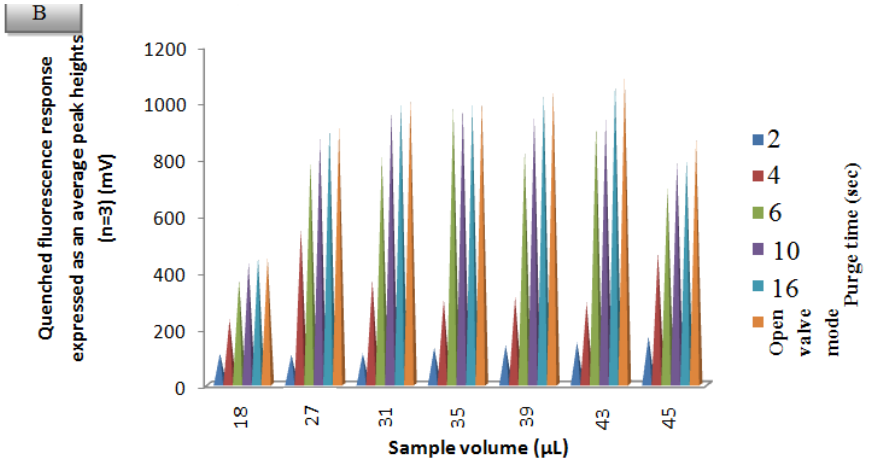


Fig.3: Effect of variable sample volume with different purge time on :

A- Response profile

B- Quenched fluorescence response expressed as an average peak heights (mV)

Table 2: Effect of sample volume with different purge time on quenched and remained fluorescence response (mV) for the determination of MCP-HCl

Sample volume (µL)	Purge time (sec)					
	Remained fluorescence $\bar{y}_{Ri}$ (n=3)mV / Quenched fluorescence $\bar{y}_{Oi}$ (n=3)mV					
	2	4	6	10	16	Open valve
18	110 / 3140	230 / 3020	370 / 2880	430 / 2820	450 / 2800	450 / 2800
27	105 / 3145	550 / 2700	790 / 2460	870 / 2380	910 / 2340	910 / 2340
31	110 / 3140	370 / 2880	810 / 2440	970 / 2280	1000 / 2250	1010 / 2240
35	130 / 3120	300 / 2950	980 / 2320	985 / 2280	990 / 2260	1010 / 2240
39	140 / 3170	310 / 2940	830 / 2420	950 / 2300	1030 / 2220	1050 / 2200
43	150 / 3100	290 / 2960	920 / 2330	940 / 2310	1070 / 2180	1090 / 2160
45	170 / 3080	470 / 2780	700 / 2550	790 / 2460	800 / 2450	870 / 2380

Study of the variation of oxonium ion concentration on the total, quenched and remained fluorescence response and statistical parameters

After optimization the chemicals & physical parameters , the calibration curve of quenched and remained) using I^--IO_3^--H_3O^+ system

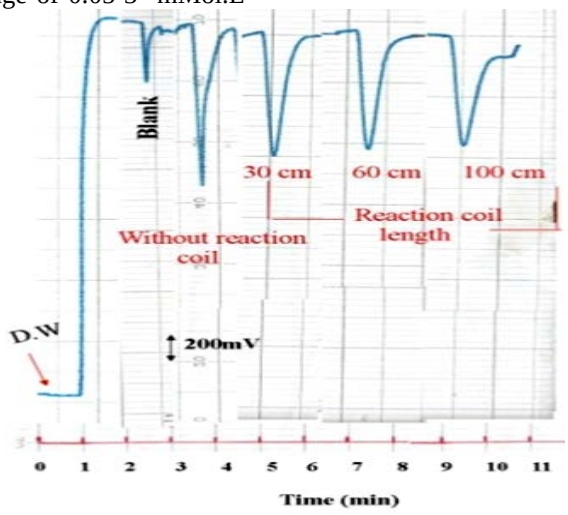
Response of continuous fluorescence : 3500mV,
Response of blank : 300mV
Effect of reaction coil

Variation of reaction coil length (0-100)cm was used, this range of lengths comprises a volume of 0-0.785mL which connected after injection valve no.$_2$ directly in flow system (fig.1.A_1) . Optimum concentration of fluorescein sodium salt (0.5mMol.L^{-1}) and 7mMol.L^{-1} of MCP-HCl with sample volume 43µL were used. Fig.4 and table 3 shows and tabulated all the results obtained. From the obtained results of reaction coil effect of the quenched fluorescence response, peak base width (Δt_b) , dilution effect and MCP-HCl concentration after dilution, it can be seen that an increase in coil length lead to decrease in peak height results in broadening of peak maxima, increase of the Δt_b and increase dilution effect which might be probably attributed to the increase dilution and dispersion effect of the injected sample segment. Therefore; one line manifold system without reaction coil was used for the determination of MCP-HCl using laser diode fluorimeter.

Response of continuous fluorescence : 3500mV,
Response of blank : 300mV V_{final}: Addition volume (mL) at flow cell, DF: Dilution factor at each flow rate
Scatter plot calibration curve

Various concentrations (0.05-100)mMol.L^{-1} of MCP-HCl were prepared by using the parameters achieved above in order to prepare a scatter plot diagram followed by the choice of calibration graph. The quenched fluorescence response as an average peak heights was plotted against the concentration of MCP-HCl , a straight line graph from 0.05 to 10 mMol.L^{-1} of MCP-HCl was obtained at confidence level 95% as shown in fig.5, correlation coefficient r:0.9624 and detection limit 0.005mMol.L^{-1}.

The results tabulated in table 4. 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 using MCP-HCl-PMA($2mMol.L^{-1}$) system as shown in fig.5.C and table 4 [17].



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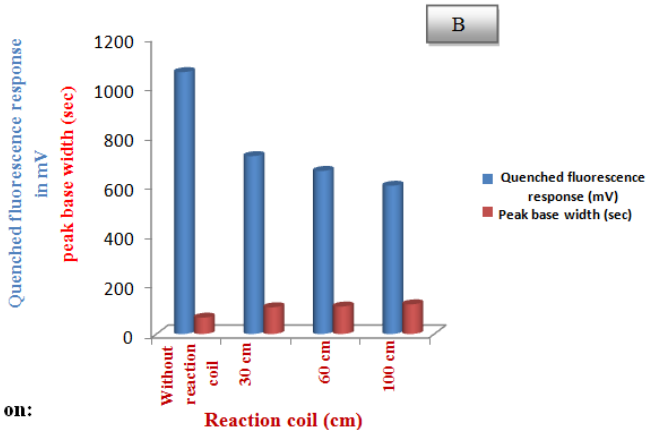

Fig.4: Effect of reaction coil on:
A-Response profile
B-Quenched fluorescence response(mV) and peak base width (sec)

Table 3: Effect of reaction coil for the determination of MCP-HCl using laser diode fluorimeter

Coil length (cm)	Coil volume (ml) $r^2\pi$ h, r =0.5 mm	Total quenched fluorescence expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$	Quenched fluorescence $\bar{y}_Q$ (n=3)mV	Remained fluorescence $\bar{y}_R$ (n=3)mV	Δt_b (sec)	V_{final} (mL)	Concentr-ation in $mMol.L^{-1}$ at flow cell	DF
Without reaction coil	0	1360	0.24	1360±8.11	1060	2140	66	1.473	0.204	34.26
30	0.235	1020	0.26	1020± 6.59	720	2480	108	2.618	0.115	60.88
60	0.471	960	0.21	960±5.01	660	2540	111	2.919	0.103	67.88
100	0.785	900	0.28	900±6.26	600	2600	120	3.428	0.088	79.72

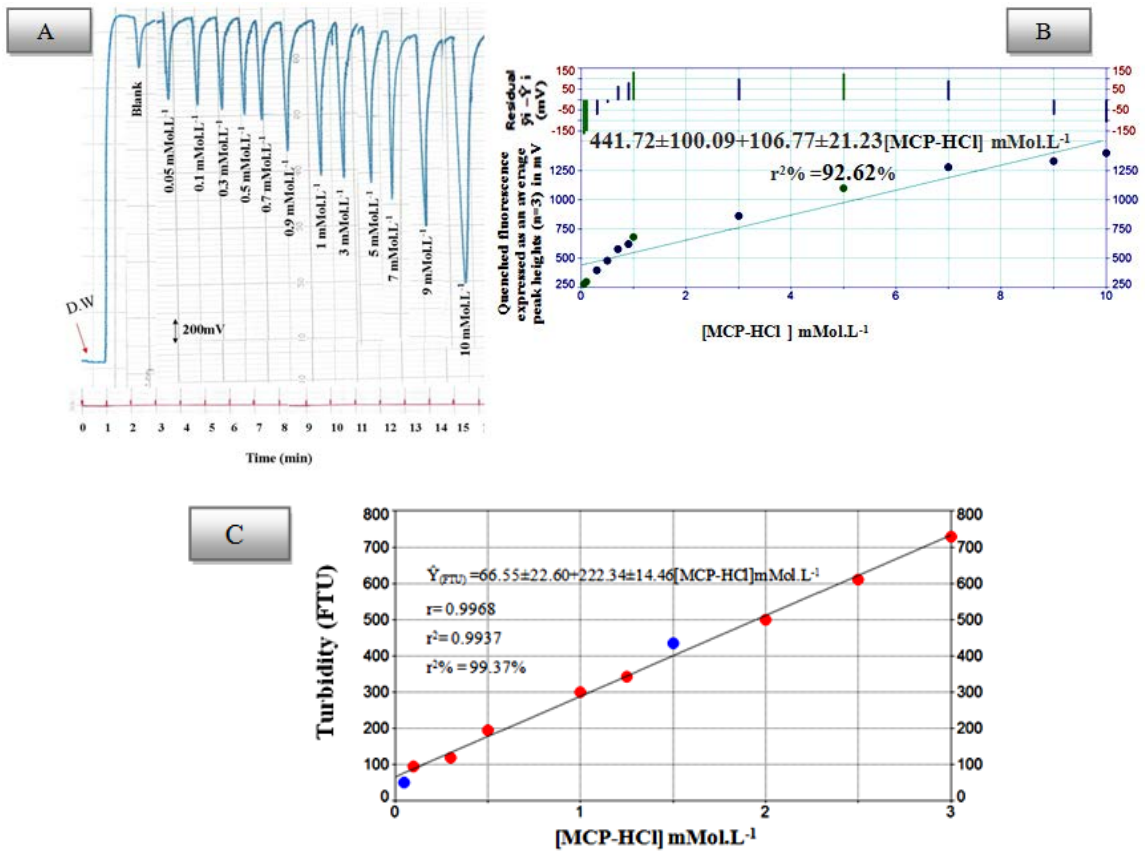

Fig.5 : Calibration graph for the variation of MCP-HCl concentration on :
A-Response profile
B- Quenched fluorescence response expressed by linear equation using laser diode fluorimeter. Residual (ȳi – ŷ i), ȳi : practical value, ŷ i: estimate value.
C- Turbidity in FTU using HANNA instrument

Table 4 : Summary of calibration graph results for the determination of MCP-HClusing laser diode fluorimeter and turbidity measurement by HANNA instrument (classical method)[17]

Measured [MCP-HCl] mMol.L^{-1}	Linear dynamic range mMol.L^{-1}	Type of measurement	ŷ=(a± S_at)+(b±S_bt) [MCP-HCl]mMol.L^{-1} at confidence level 95%, n-2	r r^2 r^2%	t_{tab} at 95% confidence level, n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
0.05-100	n=12 0.05-10	Total quenched fluorescence	741.72±100.09+106.77±21.23 [MCP-HCl]mMol.L^{-1}	0.9624 0.9262 92.62	2.228<< 11.20	
		Quenched fluorescence	441.72±100.09+106.77±21.23 [MCP-HCl] mMol.L^{-1}			
		Remained fluorescence	2758.28±100.09-106.77±21.23 [MCP-HCl] mMol.L^{-1}			
Turbidity by Hanna instrument (classical method using MCP-HCl-PMA system						
0.0005-10	n=10 0.05-3 D.L=10µMol.L^{-1}	Turbidity	66.55±22.60+222.34±14.46[MCP -HCl]	0.9968 0.9937 99.37%	2.306 << 35.467	

ŷ: estimated response (mV)or FTU for n=3 expressed as an average peak heights of linear equation of the form ŷ= a+bx, [MCP-HCl :
MCP-HCl concentration (mMol.L^{-1}), r :correlation coefficient, r^2: coefficient of determination, r^2%: linearity percentage.
D. L: Detection limit based on the gradual dilution for the minimum concentration for calibration curve
Limit of detection (L.O.D)
Detection limit is calculated from the gradual dilution of the minimum concentration of the used calibration graph which is 0.005mMol.L^{-1}. Table 5 shows the limit of detection conducted by two different methods .

Table 5 : Limit detection of MCP-HCl at optimum parameters using 43µL depending on quenched fluorescence

Practical based on the gradual dilution for the minimum concentration (0.005 mMol.L^{-1})	Theoretical based on the value of slope $x=3S_B/slope$
76.175 ng/ Sample	898.94 ng/Sample

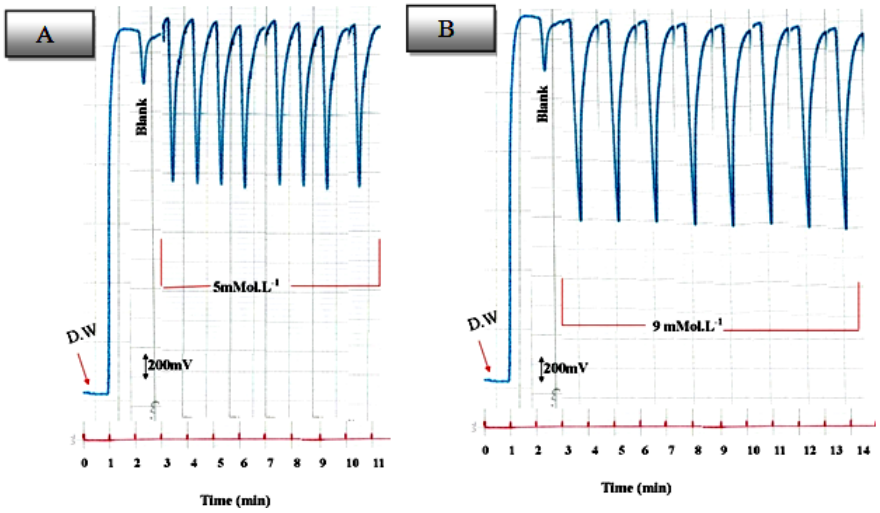

Fig.6 : The quenched fluorescence response profile for eight successive repeatable measurements of MCP-HCl : A- 5mMol.L^{-1} and B- 9mMol.L^{-1}

Table 6: Repeatability results of MCP-HCl at optimum parameters using laser diode fluorimeter

Concentration mMol.L^{-1}	Average of total quenched fluorescence expressed as an average peak heights $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}$ (n=8)mV	RSD %	Confidence interval of the total average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
5	1110	810	0.42	1110±3.89
9	1610	1310	0.17	1610±2.29

Response of continuous fluorescence=3500mV , Response of blank : 300mV, $t_{0.05/2,\ 7=2.365}$. Number of injection = 8

Repeatability

Repeatability of measurements was studied at two variable concentration of MCP-HCl (5 and 9 mMol.L^{-1}) solutions using one manifold system at optimum parameters via quenching of the continuous fluorescence when irradiated by laser beam 405nm . The repeated measurements for eight successive injections were measured as shown in fig.6 and the results tabulated in table 6.

Analysis of pharmaceutical preparation

The established method was used for the determination of MCP-HCl in three different kind of MCP-HCl samples from three different well known manufactures (Sanofi-aventis France-10mg, Actavis UK -10mg and SDI Iraq-5mg) using fluorescein sodium salt-MCP-HCl system. The standard addition method was applied by preparing a series of solutions from each pharmaceutical drug via transferring 10 mL (3mMol.L^{-1}) to five volumetric flask (25mL), followed by the addition of (0 , 0.1 , 0.5, 0.9 and 1.3 mL) from 100mMol.L^{-1} standard solution of MCP-HCl in order to have the concentration range from 0-5.2 mMol.L^{-1} (Fig.7. A_1,$B_1$and $C_1$for the preparation of standard addition calibration plot as shown in fig.7.A_2,$B_2$and C_2. The results were mathematically treated for standard additions method and tabulated in table 7.A,B at confidence interval of 95%.

From the results obtained can be a comparison at two different paths :

First test : Comparison of newly developed method with official quoted value (10 mg or 5 mg) as shown in table 7- B (column 8) by calculated t- values of each individual company and these comparison with tabulated t-value (4.303) .

A hypothesis can be estimated as follow:

Null hypotheses: There is no significant difference between the means obtained from three source of three different companies $\bar{x}$ and quoted value (μ_0)

i.e. H_o : $\bar{x} = \mu_0$

against :

Alternative hypothesis : there is a significant difference between the means and quoted value

i.e. H_1 : $\bar{x} \neq \mu_0$

For three different companies.

It was noticed that there were no significant difference between the quoted value and the measured value (($t_{calculated}$) are < t_{tab} (4.303) at confidence level 95 %); Null hypothesis will be accepted and will reject the alternative hypothesis.

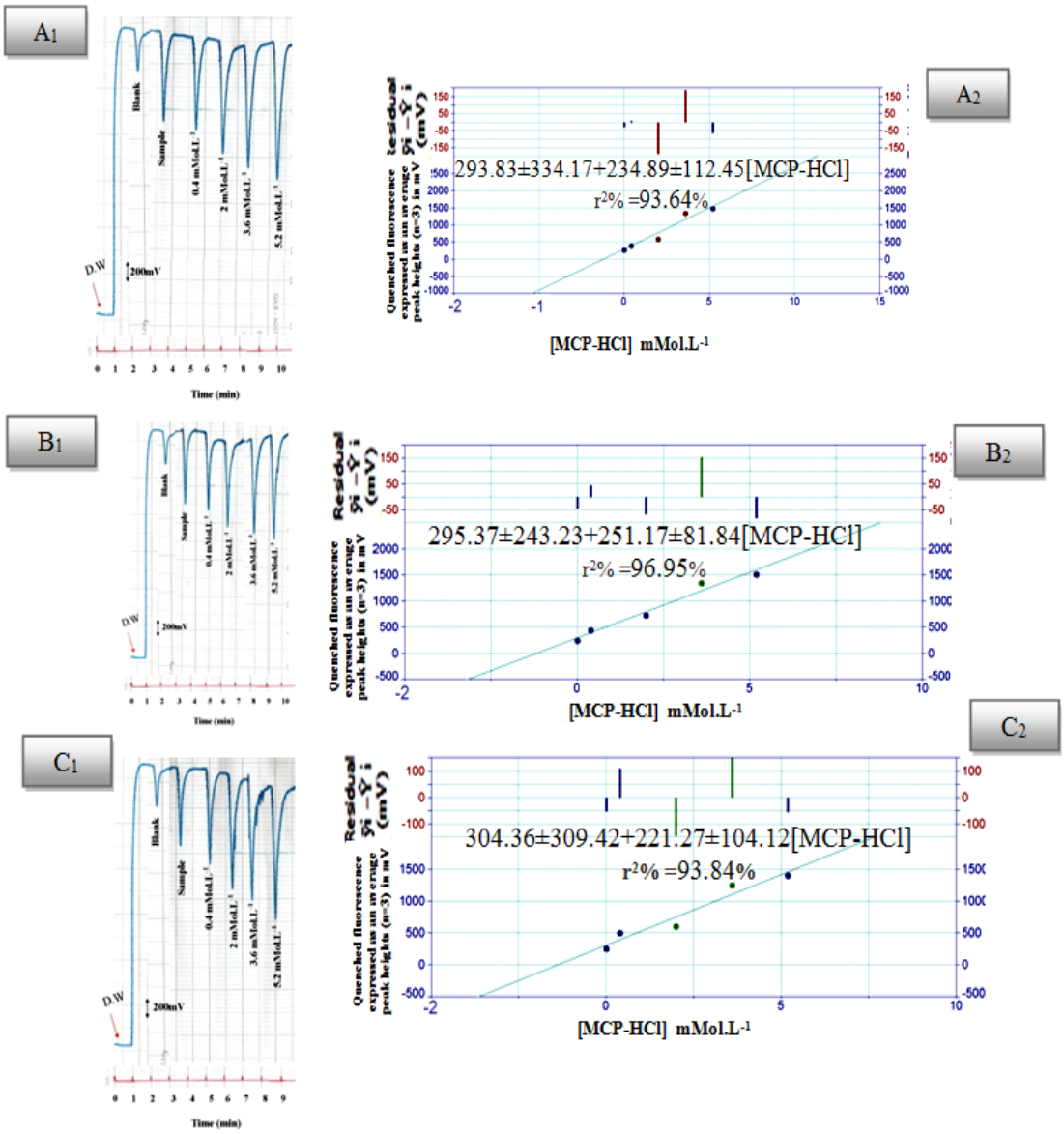

Fig.7: S**ample of responses profile and standard addition calibration graph for three pharmaceutical preparations A_1,A_2: Sanofi-aventis France, B_1 , B_2: Actavis UK and C_1 , C_2 : SDI Iraq. Using laser diode fluorimeter and fluorescein salt-MCP-HCl system**

Second test : Table 8 analysis of variance (ANOVA one way) for the comparison between different of the means of the three pharmaceutical samples using developed & classical method means (expressed as an average of weight)

Null hypothesis : H_o : $\mu_{sample1} = \mu_{sample2} = \mu_{sample3}$

Against

Alternative hypothesis: H_1 : $\mu_{sample1} \neq \mu_{sample2} \neq \mu_{sample3}$

The results was tabulated in table 8, from this study shows; since calculation F_{value} of 168.549 >> $F_{\alpha/2\ ,\ 2,6}$ (5.14) at 95% confidence level and sig<0.05 . Therefore ; H_0 is rejected and accepted H_1 , indicating that there is a significant difference between the performance of the three different pharmaceutical samples.

Table 7-A: Standard addition results for the determination of MCP-HCl in three pharmaceutical preparation using laser diode fluorimeter

No. of sample	Commercial name, Company Content Country	Sample weight equivalent to 106.29 mg (3 mMol.L^{-1}) of the active ingredient (g)	Laser diode fluorimeter (Quenched fluorescence response mV) [MCP-HCl] mMol.L^{-1} 0 mL	0.1mL	0.5mL	0.9mL	1.3mL	Equation of standard addition at 95% for n-2 $\hat{Y}_{i\ (mV)}= a\pm s_a t+b\pm s_b t[x]$	r r^2 r^2%	Practical concentration mMol.L^{-1} in 25mL / In prepared sample 100mL (3mMol.L^{-1}) $\bar{x}$(mMol.L^{-1})
			0	0.4	2	3.6	5.2			
1	Sanofi-aventis Metoclopramide 10mg France	1.3361	300	400	630	1350	1420	293.83±334.17+234.89±112.45[MCP-HCl]	0.9677 0.9364 93.64	1.251 / 3.128
2	Metoclopramide Actavis 10 mg UK	1.3835	250	440	730	1350	1520	295.37±243.23+251.17±81.84[MCP-HCl]	0.9846 0.9695 96.95	1.176 / 2.940
3	Meclodin SDI 5mg Iraq	2.4319	250	500	600	1250	1400	304.36±309.42+221.27±104.12[MCP-HCl]	0.9687 0.9384 93.84	1.376 / 3.440

$\hat{Y}$= Estimated response in mV for laser diode fluorimeter , [x] = [MCP-HCl] mMol.L^{-1} .r :correlation coefficient, r^2: coefficient of determination& r^2%: linearity percentage

Table 7-B:Summary of results for individual t-test, practical content and efficiency for determination of MCP-HCl in three samples of pharmaceutical preparation using fluorescein sodium salt-MCP-HCl system

Sample no.	Confidence interval for the average weight $\overline{Wi}\pm1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight equivalent to 106.29 mg (3mMol.L^{-1}) of the active ingredient (g)	Theoretical content of the active ingredient at 95% (mg) $\mu\pm1.96\ \sigma_{n-1}/\sqrt{n}$	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. %)	Individual t-test: Individual comparison $(\bar{x}-\mu)\sqrt{n}/\sigma_{n-1}$ Laser diode fluorimeter with Quoted value $t_{0.05/2,2}$=4.303
				Laser diode fluorimeter			
1	0.1257±0.0008	1.3361	10±0.064	3.128 110.825	10.426± 2.48	104.26	0.738<<4.303
2	0.13016±0.0004	1.3835	10±0.031	2.940 104.164	9.799±2.11	97.99	|-0.409|<<4.303
3	0.1144±0.0025	2.4319	5±0.109	3.440 121.879	5.733± 1.74	114.66	1.814<<4.303

n= no. of sample =3

$t_{0.025,\infty}$ = 1.96 at 95 % , μ : quoted value (10 or 5mg)

$\overline{X}$ = $\overline{W}_i$ = practical content(mg)

Table 8: ANOVA results of two methods and quoted value for comparison between three different samples

No. of sample	Quoted value (mg) / Practically content of MCP-HCl $\overline{Wi}\pm4.303\sigma_{n-1}/\sqrt{n}$ / Proposed method (laser diode fluorimeter) / Classical method(turbidity measurements)[317]	Source	Sum of Squares (SSq)	df	Mean Square (MSq)	F_{cal}	F_{tab}	Sig.
	10mg							
1	10.426± 2.48 / 10.619±0.986	Between group	SS_B =51.236	2	MS_B=25.618	168.549>> 5.14		0.00
	10mg							
2	9.799±2.11 / 10.285±1.100	Within groups	SS_W =0.912	6	MS_W = 0.152			
	5mg							
3	5.733± 1.74 / 4.670±1.086	Total	52.148	8				

df=degree of freedom

F_{tab} = 9.55

F_{cal} = MS_B / MS_W

CONCLUSION

Determination of metoclopramide hydrochloride using one line manifold design and one valve for the injection of sample to quench fluorescein salt molecule (continuous fluorescence light) when irradiated by laser source at 405nm. The method was successfully applied for the estimation of metoclopramide hydrochloride in drugs and characterized by sensitivity , rapid measurement and use with a small volume of samples.

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