New approach development for determination of chlorpromazine HCl in pure and pharmaceutical forms using homemade wave length selector flow injection photometer

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Abstract

A simple , accurate and fast method for determination of chlorpromazine HCl in pure and drug formulations by continuous flow injection spectrophotometric was developed. The method was based on the oxidation of the drug with sodium persulphate to pinkish-red species and the same species was determined using homemade Ayah3Sx3-3D-solar flow injection photometer. Optimum conditions were obtained using the high intensity green light Emitted Diod as source . A 180µl was taken as a reasonable sample volume for the determination of drug in pure and pharmaceutical formulation. The optimum conditions reached were 1ml/min flow rate (10mmole.L^{-1}) for sodium persulphate and 15seconds allowed time for injection. The linear dynamic range for the instrument response versus chlorpromazine HCl concentration was 0.05-0.6 mmole.L^{-1} while the L.O.D was 64.8nM/sample from the stepwise dilution for the minimum concentration of lowest concentration in the linear dynamic range of the calibration graph . The correlation coefficient (r) was 0.9959 while the percentage linearity (%r^2) was 99.19% . R.S.D% for the repeatability (n=5) was < 1.5% for the determination of chlorpromazine HCl with concentrations of 0.15 and 0.18 mmole.L^{-1} . The method was applied successfully for the determination of chlorpromazine HCl in pharmaceutical preparation . Using paired t-test it was shown that there were no significant difference between the proposed method and official method and on that basis the new method can be accepted as an alternative analytical method.

تطوير نمط جديد لتقدير كلوروبرومازين هايدروكلورايد في الصيغة النقية والمستحضرات الصيدلانية باستخدام مطياف التحليل بالحقن الجرياني منتقي الطول ألموجي المصنع محليا

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مفتاح البحث: تقدير كلوربرومازين هايدروكلورايد

الخلاصة

تم تطوير طريقة تحليلية سريعة وحساسة لتقدير كلوروبرومازين هايدوكلورايد في الصيغة النقية والمستحضرات الصيدلانية باستخدام التحليل بالحقن الجرياني الطيفي المستمر. استندت الطريقة على أكسدة الدواء باستخدام بيرسلفات الصوديوم لتكوين ناتج ذي لون احمر- برمنكناتي وقياس امتصاص الناتج الملون باستخدام منظومة مصنعة محليا للتحليل بالحقن الجرياني الطيفي المستمرAyah3Sx3-3D solar FI photometer.تم دراسة الظروف المثلى باستخدام ثنائي الوصلة باعث ذي الضوء الأخضر والشدة العالية كمصدر للتشعيع, كما استخدام حجم أنموذج180 مايكروليتر لتقدير الدواء بصيغته النقية والمستحضرات الصيدلانية. الظروف المثلى التي تم التوصل إليها تتضمن استخدام سرعة جريان 1ml/min لكاشف بيرسلفات الصوديوم 10ملي مول.لتر$^{-1}$ وزمن حقن15 ثانية. تم الحصول على علاقة لتغير الاستجابة الإلية مع تركيز الدواء وكانت حدود منحنى المعايرة 0.05-0.6 مللي مول.لتر$^{-1}$ أما حد الكشف 64.8 نانومولار/ 180 مايكروليتر من التخفيف التدريجي لاقل تركيز في منحنى المعايرة. بلغت قيمة معامل الارتباط(r) 0.9959 ومعامل التقدير(%r^2) 99.19% . الانحراف القياسي النسبي(%R.S.D) للتكرارية < 1.5 % لمحلول الكلوروبرومازين هايدروكلورايد بتراكيز 0.15 و 0.18 مللي مول.لتر$^{-1}$. طبقت الطريقة بنجاح لتقدير الدواء في المستحضرات الصيدلانية.

أجريت مقارنة بين الطريقة المستحدثة والطريقة القياسية باستخدام اختبار t المزدوج وتبين أنة لا يوجد فرق جوهري بين الطريقتين وعلى هذا الأساس بالإمكان استخدام الطريقة المستحدثة كبديل للطريقة القياسية.

1- Introduction

The discovery of antipsychotic agent chlorpromazine HCL in the early 1950s and the advent of even more powerful phenothaizinic psychopharmacological agent represent a landmark in the history of the medical and psychiatric science[1]. Chlorpromazine as shown in fig.1 is used for the control of psychoses including schizophrenia , mania and several disturbed or agitated behavior , it is also used for the relief of nausea , vomiting , preoperative anxiety and intractable hiccups[2-4].

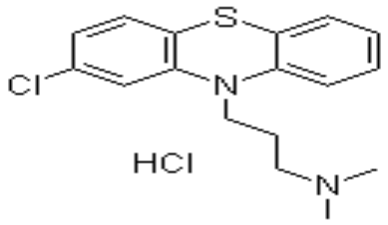

Fig.1- Structure of chlorpromazine HCl.

In the last decade methods based on spectrophotometric determination have been reported for determination folic acid[5], methyldopa[6], promethazine HCL[7], fomatidine[8] and cimetidine[9]. These methods based on oxidative – coupling and redoxy reactions. The widespread uses of chlorpromazine HCL has necessitate the development of rapid , simple and precise methods for it is quality control such sequential and flow injection spectrophotometric were also used for determination of chlorpromazine HCL using a ferric phosphoric solid – phase reactor[10], ammonium metavanadate as colorimetric reagent[11] electro – oxidation of chlorpromazine HCL in sulfuric acid medium[12], redoxy reaction with potassium dichromate and cerium in sulfuric acid media[13,14] and oxidation of chlorpromazine HCL with manganese dioxide entrapped in a polymeric material in a packed – bed reactor[15]. The research work describes the development and the application of flow injection spectrophotometery for determination of chlorpromazine HCL in drugs and pharmaceutical formulations suing sodium persulphate as oxidizing agent.

2- Experimental

2-1- Chemicals

All chemicals used were of analytical reagent grade. Distilled water was used throughout this work. Chlorpromazine HCL stock standard solution ($C_{17}H_{19}ClN_2S$,HCl , 355.3 , SDI , 10mmole.L^{-1}) was prepared by dissolving 0.3553g /100ml distilled water . A stock solution of sodium persulphate ($Na_2S_2O_8$, 238.10 , BDH, 100mmole.L^{-1}: 5.9525g / 250ml distilled water.

2-2 Apparatus and reaction manifold

The flow system used for the determination and detection of chlorpromazine HCl , shown in fig.2. Which comprises the use of a peristaltic pump: four channels, variable speed(Ismatec, USA), the 6-port injection valve (IDEX,V-450, USA) with a sample loop (0.7mm i.d., Teflon , variable lengths)used for sample injection. The instrument response was measured by Ayah 3Sx3-3D Solar FI photometer (home made) using super bright blue , green

and red light Emitted Diod (LED) as source with a detection using solar cell. The output signals was recorded by voltage output potentiometric recorder (KOMPENSOGRAPH) model C-1032 recorder (Siemens, Germany);using the range of 1-500mV. Peak height was measured for each signals .
UV-Vis 3000 spectrophotometer digital double-beam type optima (Japan) were also used to scan the spectrum of product of reactants using 1cm glass cell.

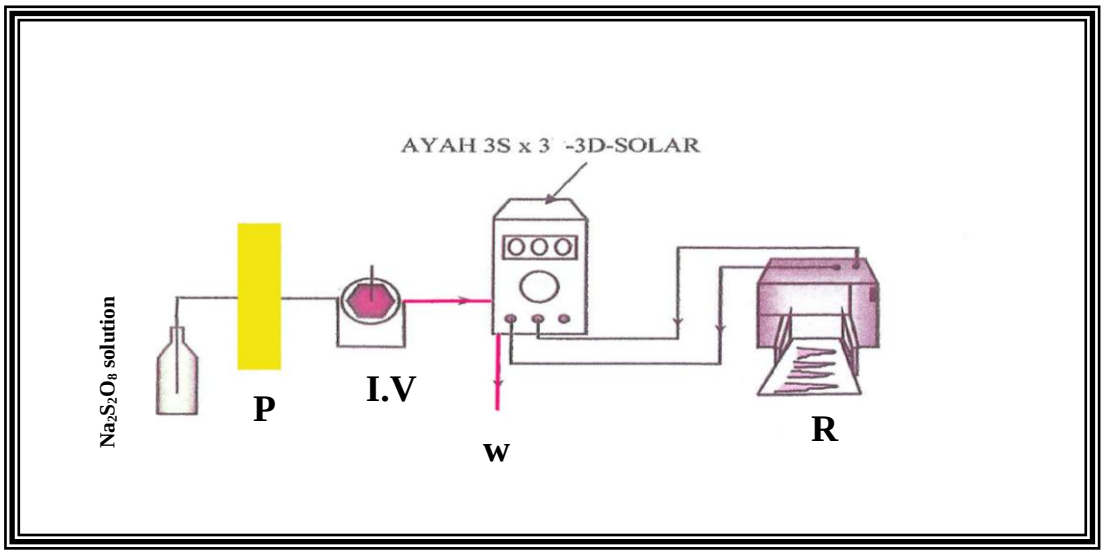

Fig.2- schematic diagram of flow injection analysis system. P,peristaltic pump ; I.V,injection valve ; R, recorder ; W, waste.

2-3- Methodology

The whole reaction manifold system for chlorpromazine HCl determination via oxidation with sodium persulphate is shown in fig.2. The manifold system is simple and composed of one line supplied sodium persulphate solution (10mmole.L^{-1}) at 1ml/min , the same line leading to the injection valve , which allows the use of 180µl of sample (loop length 47cm with 0.7mm I.D). The absorbance peak of the resulting pinkish-red product is followed using Ayah3Sx3-3D solar photometer and the variation of response was monitored using green light Emitted Diod(LED) throughout the reaction. Each solution was assayed in triplicate.

3- Results and discussion

3-1- Spectroscopic study

A dilute aqueous solution of chlorpromazine HCl when mixed with sodium persulphate as oxidizing agent an intense pinkish-red color product was formed immediately, the product shows a maximum absorption at 525nm against reagent blank as shown in fig.3.

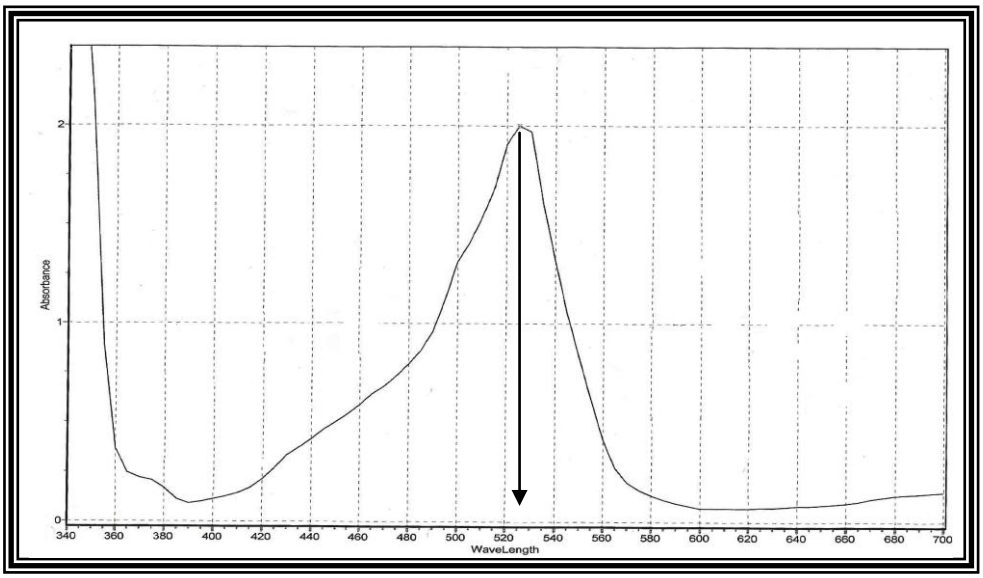

Fig.3- UV-Vis spectropotometric of pikish-red species formed by reaction chlorpromazine HCl (5mM) with sodium persulphate (10mM) against reagent blank.

The same color product of the oxidized species formed of chlorpromazine HCl as mentioned above was measured using Ayah3Sx3-3D solar FI photometer at three different light Emitted Diod (LED) [blue(470nm), green(525nm), and red(635nm)], a maximum response measured in mV obtained when using the high intensity green light Emitted Diod (525nm) as source as shown in fig.4.

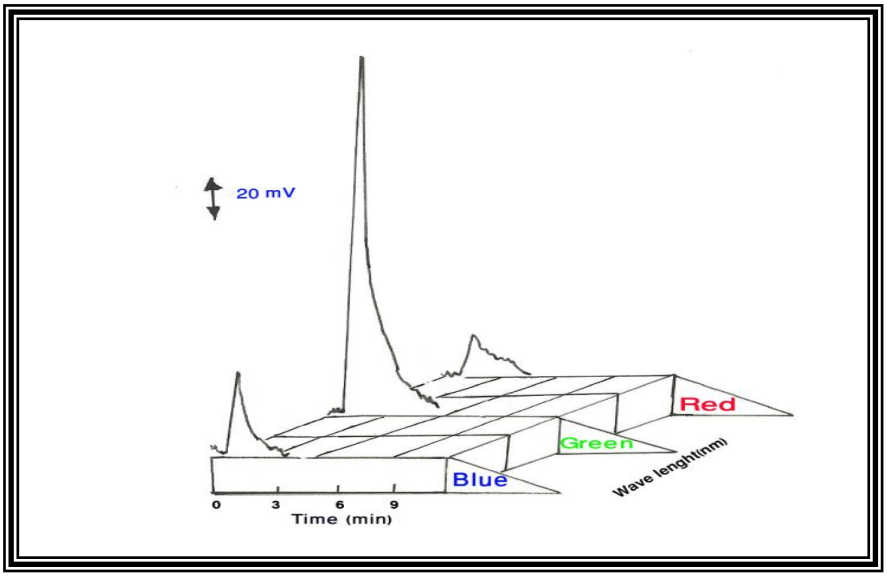

Fig.4- A maximum response measured in mV of pinkish-red species formed by injection 100µl(0.3mM) of chlorpromazine HCl at three different light Emitted Diod (LED) which is blue(470nm), green(525nm) and red(635nm) as source using homemade Ayah3Sx3-3D solar FI photometer.

3-2- Optimization of experimental conditions

A series of experiments were conducted to establish the conditions for the production of maximum well defined repeatable response for the oxidation of chlorpromazine HCl . The physical variables including flow rate , sample volume and allowed permissible time were investigated respectively and chemical variable such as concentration of sodium persulphate were also investigated.

3-2-1- Physical variables

3-2-1-1- Effect of flow rate of sodium persulphate solution.

A set of experiment was carried out for the optimization of the preferred flow rate of sodium persulphate (10mmole.L^{-1}) that extent (0.3-2ml/min) using 100µl of 0.3mmole.$L^{-1}$of sample and 15seconds as purge time for the sample segment and allowed for the carrier to pass through the injection valve in injection mode , after that allowed time the injection valve is returned to the load position as tabulated in table no.1

Table no. 1: Effect of the variation of flow rate of sodium persulphate on the response.

Presitaltic pump speed (indication approximate)	**Flow rate (**ml/min**)**	**Response n=3 Ý(**mV**)**	**Peak base width Δt_B(**min**)**	**t (**sec**)**
3	0.3	186	6	144
5	0.5	198	4.4	84
7	0.7	206	3.4	60
10	1	206	3	36
13	1.3	177	2.6	24
15	1.5	168	2.2	20.4
17	1.7	158	1.6	15.6
20	2	140	1.6	12

t = the arrival time of sample segment to the measuring cell.

It was noticed that at low flow rate there is increase in dilution and dispersion which might cause an increase in base ΔtB as shown in fig5.A. while at higher flow rate , although the effect on physical parameter was not very crucial on the response obtaining regular responses and sharp maxima but it is not very high due to departure of reactants from measuring cell prior to completion of reaction, therefore an indication approximation of 10 which corresponding to a flow rate of 1ml/min was used to obtain maximum response and narrower ΔtB as shown in fig5.B.

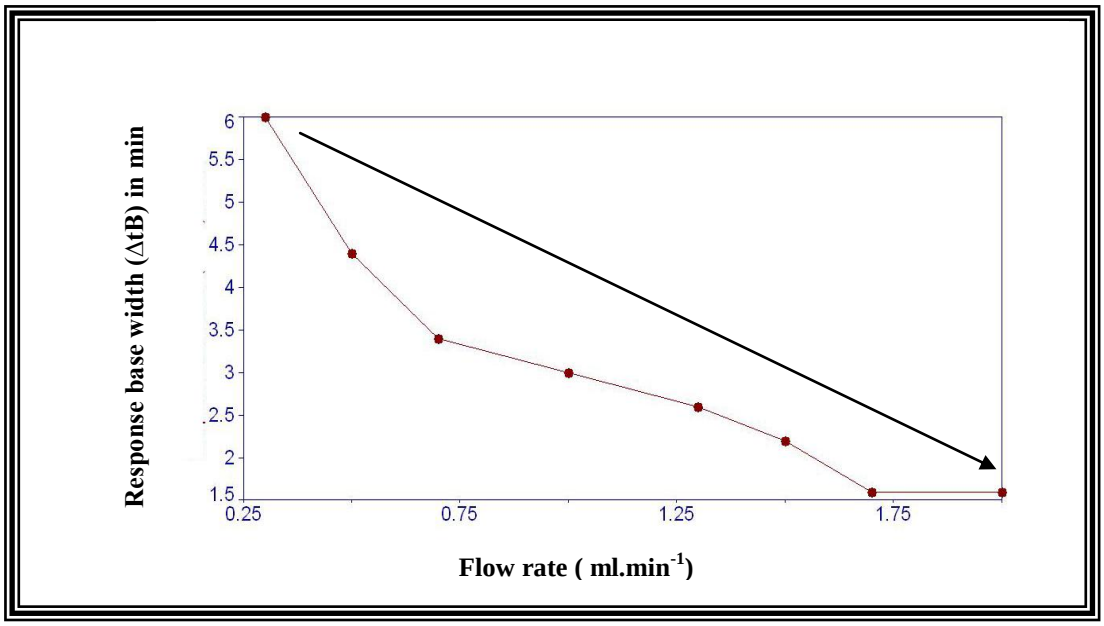

Fig.5A- Variation of ΔtB versus flow rate

The time lapse for the departure of sample segment from injection valve to the measuring cell is 36seconds.

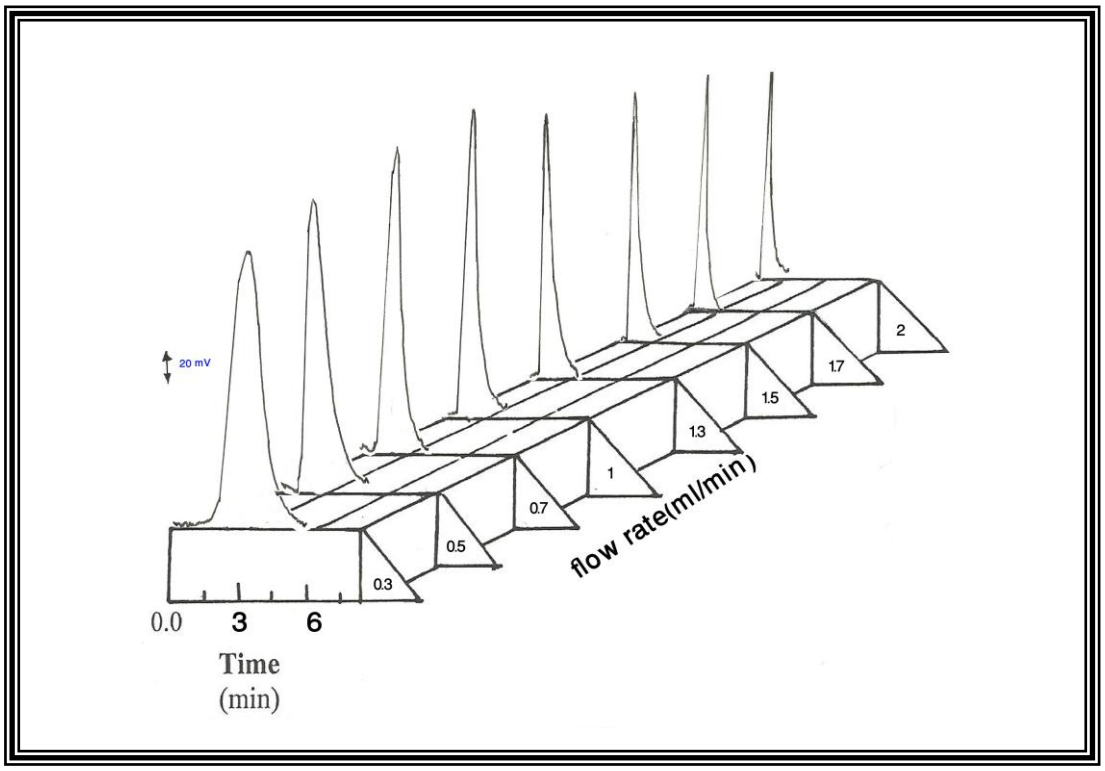

Fig.5B – Variation of flow rate versus energy transducer output response profile Ayah3Sx3-3D FI photometer for the pinkish-red species formed.

3-2-1-2- Effect of sample volume

Using the optimum flow rate of 1ml/min.Variable sample volumes(40,72,100,180,350µl) were injected using open valve mode i.e. allowance for continuation purge of sample from the sample loop in the injection valve. The data obtained were plotted as shown in fig.6A showing that the optimum sample volume is 180µl. Regular clear response were obtained using larger volume i.e. >180µl even though it gave slight higher response but it was characterized by wider ΔtB which was most probably attributed to the continuous relatively longer time duration of sample segment in front of detector as shown in fig.6B.

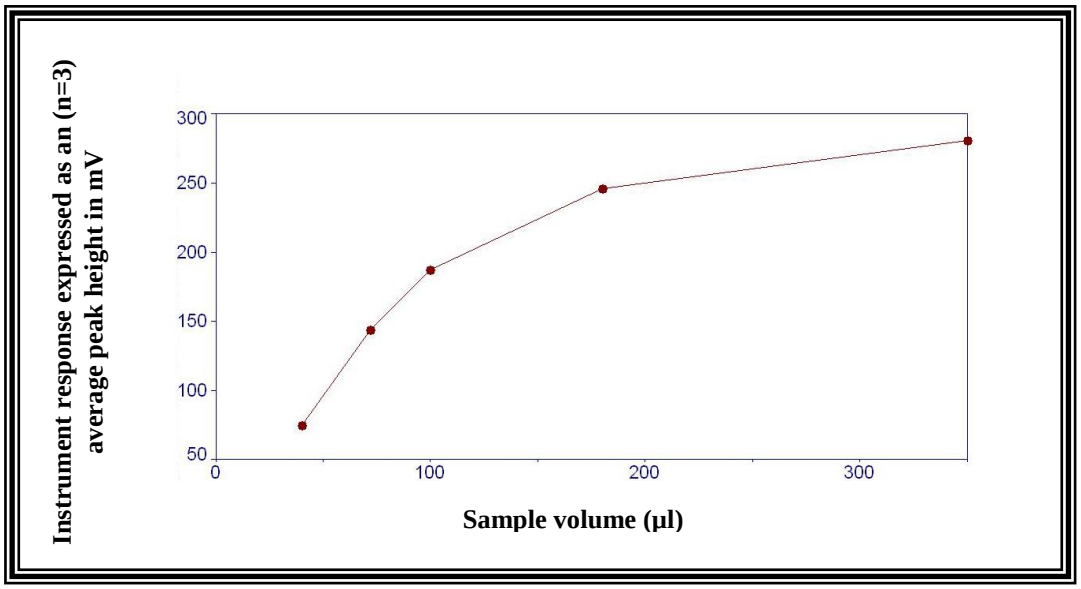

Fig.6A- Variation of injected sample volume on energy transducer response expressed in mV.

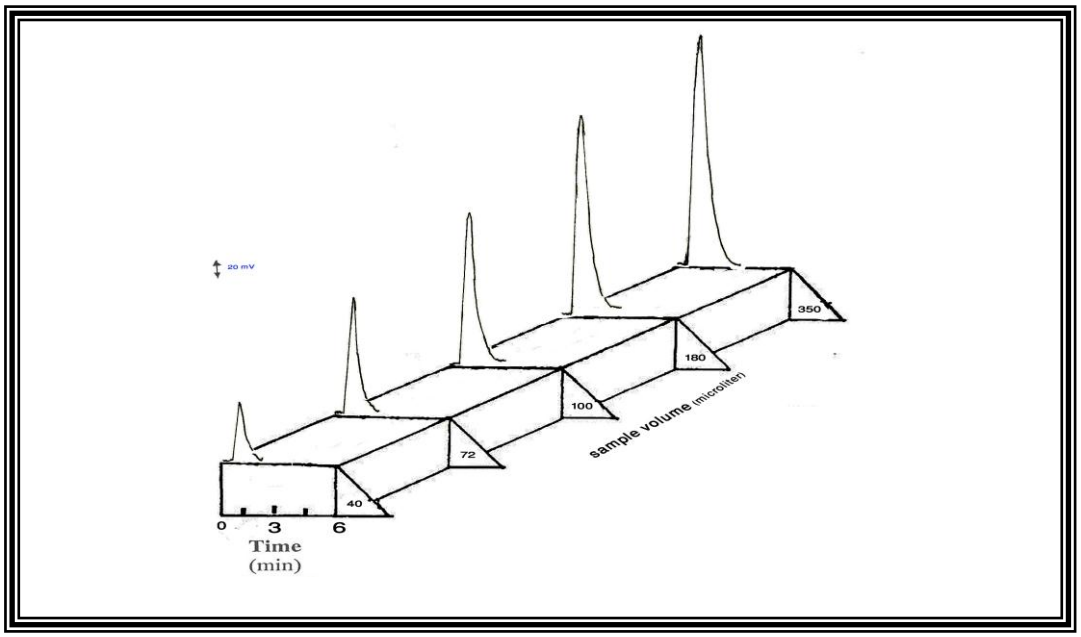

Fig.6B – Variation of sample volume versus energy transducer output response profile of Ayah3Sx3-3D-solar FI photometer for pinkish-red species.

3-2-1-3- Effect of purge time

Using different purge time for the sample segment i.e. using 3 to 24 seconds allowed permissible time for the carrier solution to passing through the injection valve in injection mode followed by turning the injection valve to the load position. Sample volume of 180µl were used. Fig no 7 shows the continuation of the increase in response with increase of injection time up to 15-18 seconds , after that there was no significant differences in responses. The decrease in responses when using less that 15-18sec was attributed to the incomplete purge of the sample from sample loop in the injection valve , therefore 15 seconds as purge time was chosen as optimum time to the complete purge of the sample from sample loop in the next studies.

Fig.7- Variation of energy transducer response expressed in mV versus time of injection , A sample volume of 180µl was used.

3-2-2/ Effect of sodium persulphate concentration

Using the optimum variable achieved in previous sections. A series of sodium persulphate solution were prepared ranging 1-13mmole.L^{-1} to establish the optimum concentration that can be used. The study was conducted using 0.3mmole.L^{-1} of chlorpromazine HCl ,each measurement was repeated for three of successive times. A repeatability of <0.5% was obtained. Fig no 8 was obtained and it was noticed that 10mmole.L^{-1} was the optimum concentration for sodium persulphate solution.

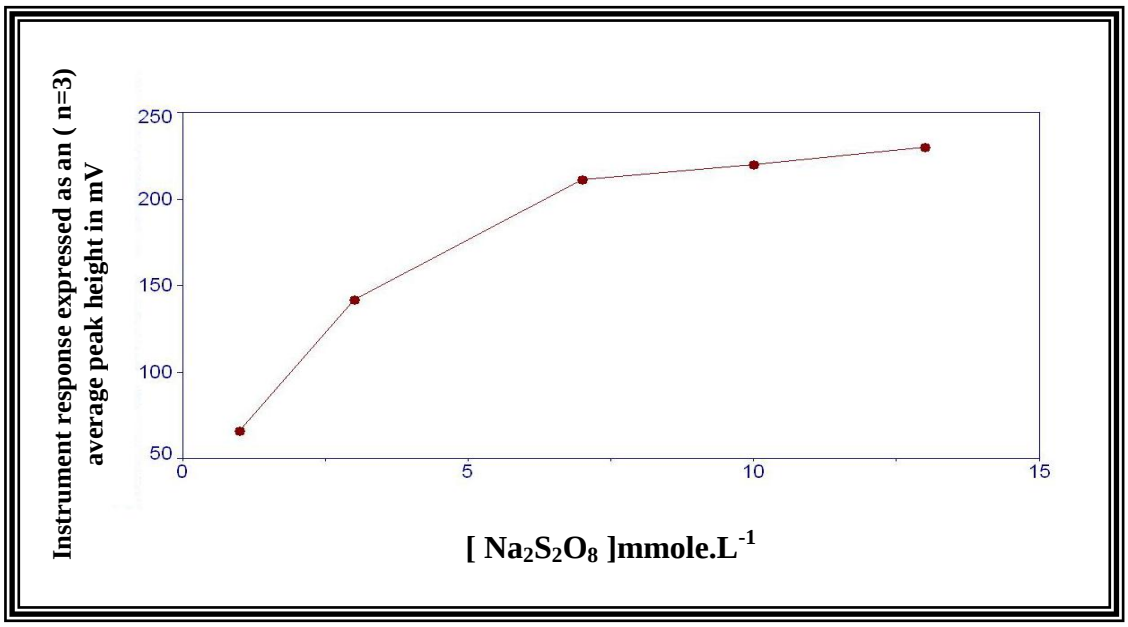

Fig.8 – Variation in energy transducer response in mV versus sodium persulphate solution concentration.

3-3- Performance of chlorpromazine HCl measurement system.

Fixing all the achieved parameters whether it is physical or chemicals . A series of solutions for chlorpromazine HCl 0.01-0.9mmole.L^{-1} were prepared, a calibration graph for the variation of instrument response with chlorpromazine HCl for 0.05-0.6mmole.L^{-1} as shown if fig.9. Above 0.6mmole.L^{-1} the value for correlation will decrease most probably due to the un-oxidized chlorpromazine HCl .The obtained results were tabulated in table no .2.

Table no.2 : Summery of calibration graph results for the determination of chlorpromazine HCl using sodium persulphate as oxidizing agent.

Measured **[Chlopromazine HCl]**mmole.L^{-1}	Linear dynamic range n=16	**Ý(**mV**)**=a±S_at + b±S_bt **[Chlorpromazine HCl]** mmole.L^{-1} at confidence interval 95%, n-2	**r** **r^2** **%r^2**	t_{tab}	$t_{cal=/r/}\sqrt{n-2}\,/\sqrt{1-r^2}$
				at 95% , n-2	
0.01-0.9	0.05-0.6	10.34±10.33 + 666.24±666.14 **[chlorpromazine HCl]** mmole.L^{-1}	0.9959 0.9919 99.19%	2.131«41.40	

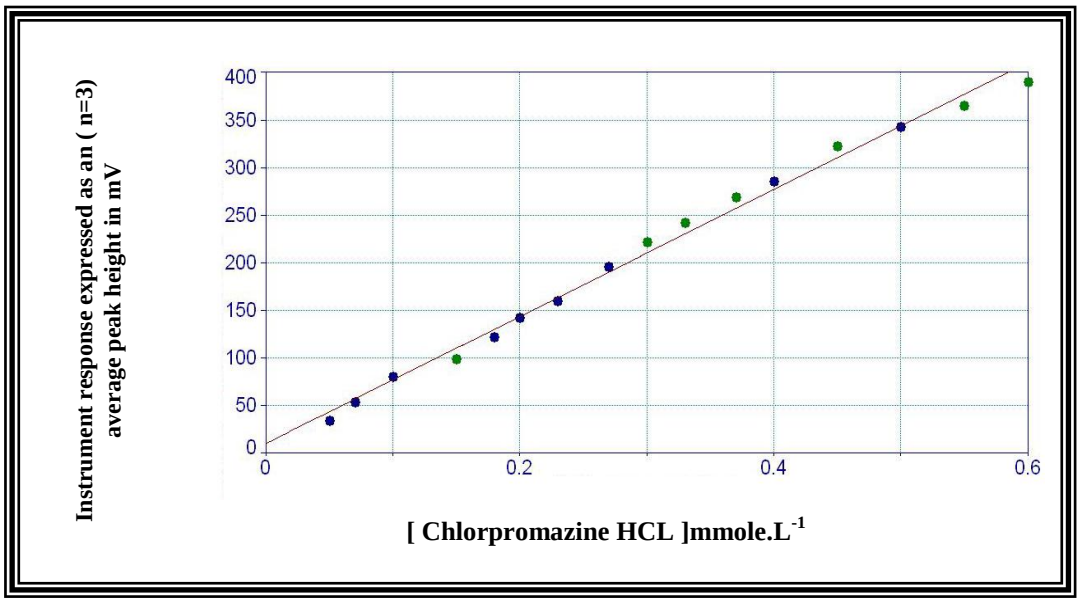

Fig.9 – linear calibration graph for the energy transducer response expressed in mV with chlorpromazine HCl concentration expressed in mmole.L^{-1}.

Limit of detection for chlorpromazine HCl was conducted through three methods as tabulated in table no.3 at injected sample volume of 180µl.

Table no.3: limit of detection of chlorpromazine HCl at optimum parameter.

Gradual dilution for the minimum concentration	Based on the value of slope $x = 3S_B$ / slope	Linear equation $\hat{Y}(mV) = Y_B + 3S_B$
64.8nM	8.58µM	8.10µM

X = value of L.O.D based on slope.

S_B = standard deviation of blank solution.

Y_B = average response for the blank solution (equivalent to intercept in straight line equation).

L.O.D = limit of detection.

3-4- The repeatability of chlorpromazine HCl results.

The repeatability was studied for the determination of chlorpromazine HCl via measurements of oxidized chlorpromazine HCl at concentration (0.15 ,0.18mmole.L^{-1}) of five successive injected sample measurements as shown in fig 10(A,B).

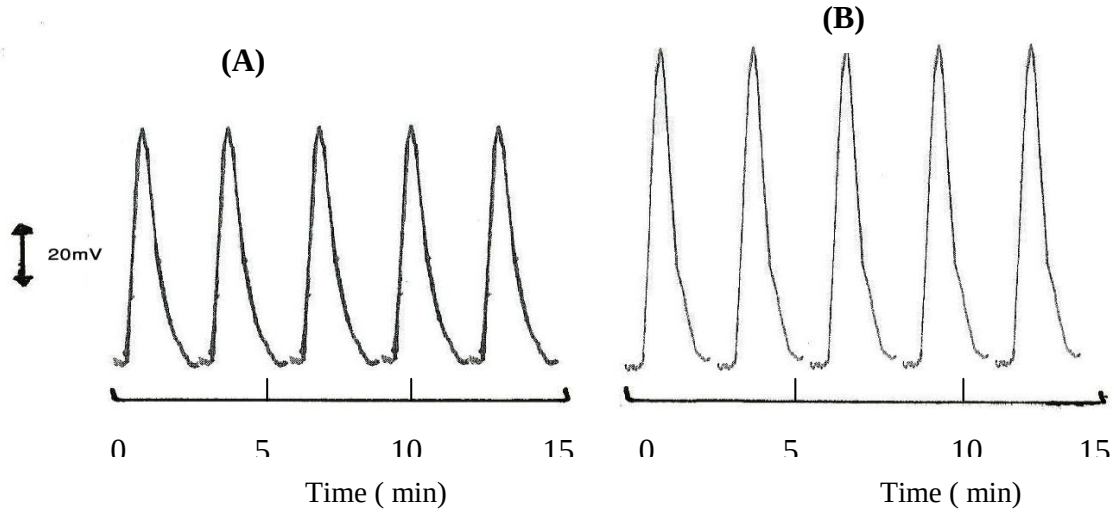

Fig.10- successive repeatable measurement for chlorpromazine HCl [(A)0.15,(B)0.18mmole.L^{-1})]

The results obtained are tabulated in table no.4.

Table no.4: Repeatability of chlorpromazine HCl.

[chlorpromazine HCl] mmole.L^{-1}	$\acute{Y}_i$(mV) n=5	σ_{n-1}	RSD%	Confidence interval of the mean $\acute{Y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
0.15	97	1.41	1.45	97 ± 1.75
0.18	123.2	1.09	0.88	123.2 ± 1.35

3-5- Analysis of pharmaceutical preparation.

The proposed method achieved in this work was used for the analysis of chlorpromazine HCl in pharmaceutical preparation and was compared with the official method. Thirteen tablets were weight crushed and grinded. 638.8mg(0.0888g active ingredient)(0.0025mole.L^{-1}) from pharmaceutical preparation, dissolved in 100ml of distilled water, followed by filtration to get rid of undissolved material followed by complete the volume to 100ml with distilled water ; 1.5ml was drown to each of five 25ml volumetric flasks followed by the addition of gradual volumes of standard chlorpromazine HCl (0,1,2,3,4)ml of 2.5mmole.L^{-1} to obtain (0.150,0.25,0.35,0.45 and 0.55)mmole.L^{-1}. Flask no.1 is the sample flask volume. The measurement were conducted by proposed method and the results were mathematically treated for the standard addition method. The results were tabulated in table no.5 at confidence interval 95%, paired t-test was used as shown in table no.6. The obtained results indication clearly that there was no significant difference between newly developed method FIA with official method[16] at 95% confidence interval as the calculated t value is less than tabulated t value.

Table no.5: Results for the determination of chlorpromazine HCl in pharmaceutical preparation using proposed method.

Commercial name, Content & country	**Confidence interval for the average weight at 95%** $\acute{w} \pm 1.96\ \sigma_{n-1}/\sqrt{n}$	**Sample weight(1.3323mg) equivalent to 0.15mmole.L^{-1} of active ingredient(g)**	**Theoretical content of active ingredient at 95% n=∞ (mg)**	**Practical content of active ingredient at 95% n=∞ (mg)**	**%Recovery**
Largcitel 50mg SDI Iraq	0.3596±0.0102	0.0095	50±0.0014	50.09±0.78	100.18

Table no.6: paired t-test results for FIA proposed method with official method for the determination of chlorpromazine HCl in pharmaceutical oreparation.

Measurement no	Proposed method (FIA)	Official method	d (mg)	X_d	σ_{n-1}	t_{tab} at 95%, n-1	$t_{cal} = X_d \sqrt{n} / \sigma_{n-1}$
1 2 3	50.09 49.85 51.30	50 50 50	0.09 -0.15 1.3	0.41	0.77	4.303 »0.929	

n = number of measurements.

3-6- Conclusion

The proposed FIA method is simple, rapid and inexpensive with high sensitivity for the determination of chlorpromazine HCl based on it is oxidation to pinkish-red product with sodium persulphate solution. From the experimental point of view, the manipulation is very simple and sequential measurement was permitted with sample frequency up to 20 samples per hour. The proposed method uses cheaper instruments and reagents with those of spectrophotometery , HPLC , flourometry and other FIA method with different oxidizing reagents. The %RSD was <1.5% and good were observed for all samples , which is an indication of satisfactory accuracy of the proposed method . The standard addition method was used to avoid matrix effects. Also this method can be applied to the micro determination of chlorpromazine HCl in pure as well as in pharmaceutical preparations without the need for heating or extraction.

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