New Turbidimetric-Continuous Flow Injection Analysis Method for The Determination of Chlorpromazine HCl in Pharmaceutical Preparation Using Linear Array Ayah 5SX1-T-1D-CFI Analyser

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Abstract

A new turbidimetric-flow injection method is described for the determination of chlorpromazine HCl in pure and pharmaceutical preparation. The method is characterized by simplicity, sensitivity and fast, it is based on formation of ion pair compound between chlorpromazine HCl and Potassium hexacyanoferrate(III) in an acid medium for the formation of greenish yellow precipitate. This precipitate was determined using homemade Linear Array Ayah 5SX1-T-1D continuous flow injection analyser. Optimum concentrations of chemical reactants, physical instrumental conditions have been investigated. The linear dynamic range of chlorpromazine HCl was 3-30 mmol.L^{-1} while correlation coefficient (r) was 0.9929 and percentage linearity (%r^2) C.O.D was 98.59%. Limit of Detection (S/N=3) 3×10^{-7} M/sample equivalent to 0.12 µg/sample from the stepwise dilution of minimum concentration for the lowest concentration in the linear dynamic range of the calibration graph with R.S.D.% (n=6) < 2% for concentration 8 and 10 mmol.L^{-1}. The method was applied successfully for the determination of chlorpromazine HCl in pharmaceutical drugs. A comparison was made between the developed method with the official method via the use of paired t-test. It shows that there were no significant differences between either methods. Therefore the newly developed method ($K_3[Fe(CN)_6]$-HCl-Chlorpromazine HCl) can be adopted as an alternative method for determination of Chlorpromazine HCl.

Key words: Chlorpromazine HCl (CPZ), Potassium hexacyanoferrate(III), turbidmetric, flow injection analysis.

طريقة جديدة التعكرية–الحقن الجرياني المستمر لتقدير الدواء الكلوربرمازين في المستحضرات الصيدلانية باستخدام Linear Array Ayah 5SX1-T-1D-CFI Analyser

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الخلاصة

وصفت طريقة جديدة لتقدير المادة الدوائية الكلوربرومازين في الحالة النقية وفي المستحضرات الصيدلانية من خلال اقتران الحقن الجرياني بقياس التعكرية، وتتصف هذه الطريقة بالبساطة والحساسية والسرعة في التقدير. تعتمد هذه الطريقة على تكوين مزدوج ايوني بين المادة الدوائية الكلوربومازين والكاشف سداسي سيانات الحديد(III) البوتاسيوم بالوسط الحامضي اذ يتكون راسب اصفر. قدر الراسب المتكون بجهاز Linear Array Ayah 5SX1-T-1D-Continuous Flow Injection Analyser المصنوع محلياً. درست

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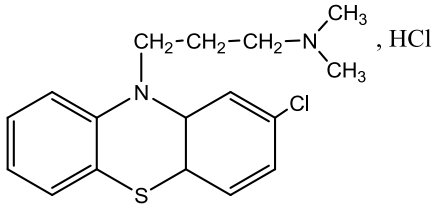
تراكيز المواد الكيميائية الداخلة في التفاعل والمتغيرات الفيزيائية. كان المدى الخطي لتقدير الدواء الكلوربرومازين 3−30 مللي مول.لتر$^{-1}$ بينما معامل الارتباط (r) 0.9929 والنسبة المئوية للخطية هي 98.59%. وبحد كشف (S/N=3) 3×10^{-7} مول.لتر$^{-1}$ والمكافئ الى 0.12 مايكروغرام من التخفيف التدريجي لاقل تركيز في منحني المعايرة والانحراف القياسي النسبي المئوي اقل من 2% لتركيزين 8 و10 مللي مول.لتر$^{-1}$ ولـ n=6 لمحلول الكلوربرومازين. طبقت الطريقة لتقدير الكلوربرومازين في المستحضرات الدوائية. اجريت مقارنة بين الطريقة المستحدثة والطريقة القياسية باستخدام اختبار t−المزدوج. بين انه لا يوجد فرق جوهري بين الطريقتين وبالامكان استخدام نظام: كلوربرومازين−سداسي سيانات الحديد(III) البوتاسيوم− حامض الهيدروكلوريك كطريقة بديلة لتقدير المادة الدوائية الكلوربرومازين.

Introduction

Phenothiazine derivatives are a large group of tricyclic antidepressants which are commonly used for the treatment of psychiatric patients suffering from clinical depression [1]. One of these phenothiazines derivative is chloropromazine HCl. Chlorpromazine HCl (CPZ) chemically known as 3-(2-Chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride. Its characterized as white crystalline powder and very soluble in water, freely soluble in ethanol [2]. The trade names of CPZ is Largactil, Taroctyl, Marazine, Chloractil, Hebanil and Thorazine [3]. Which has a molecular formula of $C_{17}H_{19}ClN_2S$, HCl and the following structure as shown below:



Chlorpromazine HCl

Several analytical methods have been developed for the determination of Chlorpromazine HCl, Spectrophotometric method [4-9], atomic absorption spectrophotometry [10], turbidimetric method [11] flow injection method [12-15], conductimetric [16], chemiluminescence [17-19] and high performance liquid chromatography (HPLC) [20-22].

This paper describes a simple and rapid turbidmetric flow injection method for determination of Chlorpromazine HCl in pharmaceutical preparation. The method uses $K_3[Fe(CN)_6]$ as a precipitating reagent in acidic medium greenish yellow precipitate is formed as ion pair complex. The precipitate is measured by the attenuation of incident light using Ayah 5SX1-T-1D-CFI Analyser.

Materials and Methods

Chemicals

All chemicals used were of analytical reagent grade and distilled water throughout this work. Chlorpromazine HCl (SDI) ($C_{17}H_{19}ClN_2S.HCl$, 355.3 g.mol^{-1}), (0.1 mol.L^{-1}) was prepared by dissolving 3.5530 g/100 mL distilled water. A stock solution of potassium hexacyanoferrate(III) (BDH) ($K_3[Fe(CN)_6]$, 329.24 g.mol^{-1}) (0.2 mol.L^{-1}) 6.5848 g in 100 mL distilled water. A 1 mol.L^{-1} Hydrochloric acid solution was prepared by diluting 88.25 mL of 35% HCl (1.18 g.mL^{-1}, BDH) with distilled water in 1L calibrated flask, Standardized with Na_2CO_3 solution.

Sample Preparation

The adopted procedure was by selecting thirteen tablets randomly from different strips and packets for 25, 100 mg drug dose. The tablets were weighted, crashed, and grinded to fine dust then followed by weighing an amount equivalent to 0.9552g and 1.3597g active ingredient (7, 10 mmol.L^{-1}) for 25, 100mg drug dose. The powder was dissolved in deionized water followed by filtration to remove any undissolved residue affecting the response. The filtrate was completed to 100 mL. 2.85 mL and 4 mL (for 25, 100 mg drug dose) of this solutions was transferred to each of the five 10 mL volumetric flask with addition of gradual increase of the standard chlorpromazine HCl solution to obtained 0, 4, 6, 7, 9, 11 mmol.L^{-1} for 25 mg drug dose and obtained 0, 4, 6, 8, 10, 12 mmol.L^{-1} for 100 mg drug dose. 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.

Apparatus and Manifold

Homemade instrument Linear Array Ayah 5SX1-T-1D-CFI analyser [23] using super white Light Emitting Diode (LED) as a source with a solar cell detector. It's used for measurement of attenuation of incident light. Four channels variable speed Peristaltic pump (Ismatec type ISM796), A rotary 6-port injection valve (Rheodyne, U.S.A) with a sample loop (0.5 mm i.d., Teflon, variable length) used for sample injection.

The output signals were recorded by x-t potentiometric recorder (KOMPENSO GRAPH C-1032) Siemens (Germany) and Digital AVO-meter (auto range) (0.00-2000 mV) (China). Peak height was measured for each signal. UV-Vis spectra were measured on UV-Vis. spectrophotometer digital double-beam SHIMADZU type 1800 (Japan). figure-1 shows the flow system that used for the determination and detection of chlorpromazine HCl.

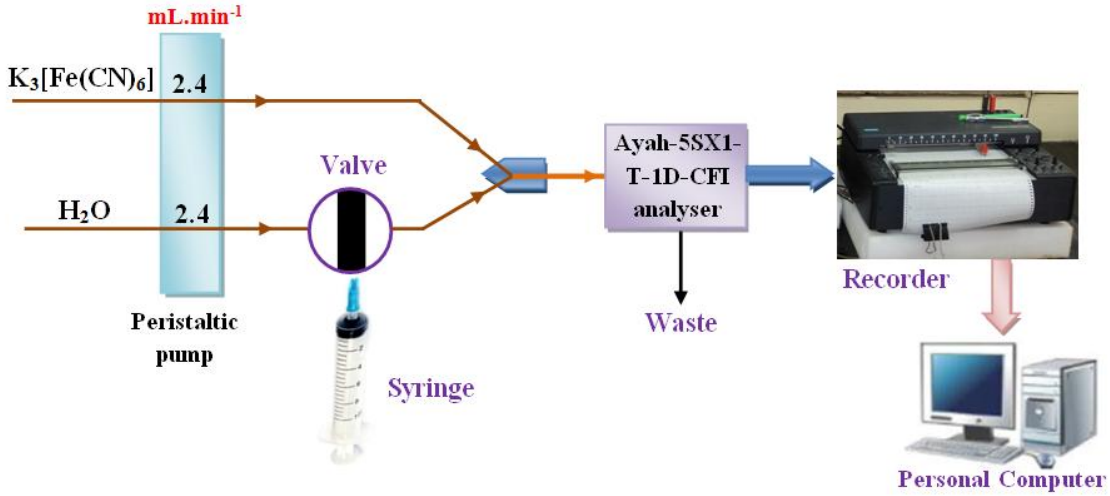
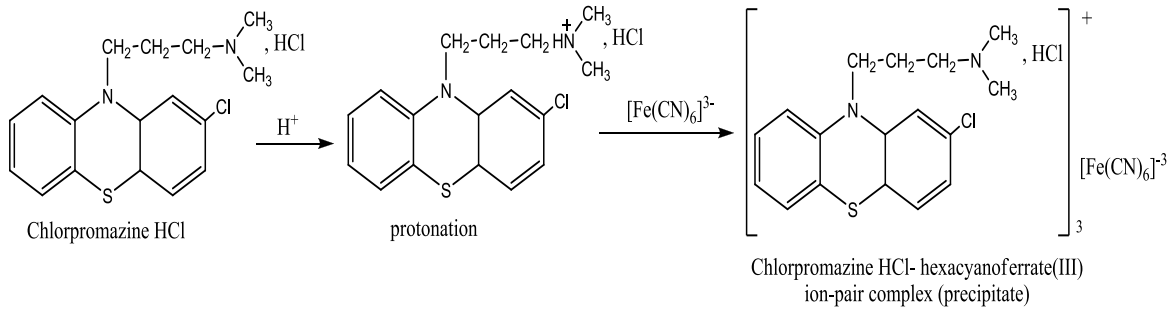


Figure 1-Schematic diagram of flow injection analysis system used for determination Chlorpromazine HCl

Methodology

The manifold system for determination chlorpromazine HCl via precipitation reaction with Potassium hexacyanoferrate(III) to form greenish yellow precipitate as an ion pair is composed of two lines as shown in figure-1. First line supplies the potassium hexacyanoferrate(III) (5 mmol.L^{-1}) solution at 2.4 mL.min^{-1}, while the second line represents the carrier stream (distilled water) leading to the injection valve, which allows the use of 120 µL and a flow rate of 2.4 ml.min^{-1} (loop length 60 cm with 0.5 mm I.D). Both lines mixes together at a Y-junction made from methyl methacrylate polymer. The attenuation of incident light peak of the resulting greenish yellow precipitate is followed using Linear Array Ayah-5SX1-T-1D-CFI anlayser and the variation of response was monitored using super white Light Emitting Diode (LED). Each solution was assayed in triplicate. Survey through the literature [24-26]; three possible mechanism were reported, but the author believe that the most probable mechanism is as follows in scheme no.1.



Scheme no.1- Probable proposed mechanism for the reaction between chlorpromazine HCl with potassium hexacyanoferrate(III).

Results and Discussion

Spectroscopic Study

A dilute aqueous solution of chlorpromazine HCl (1 mmol.L^{-1}) when mixed with hydrochloric acid HCl (0.1 mol.L^{-1}) as reaction medium then followed by the addition of potassium hexacyanoferrate(III) as precipitating agent, an intense greenish yellow precipitate product was formed immediately, the product shows two maximum peak at 451, 521 nm against reagent blank as shown in figure-2.

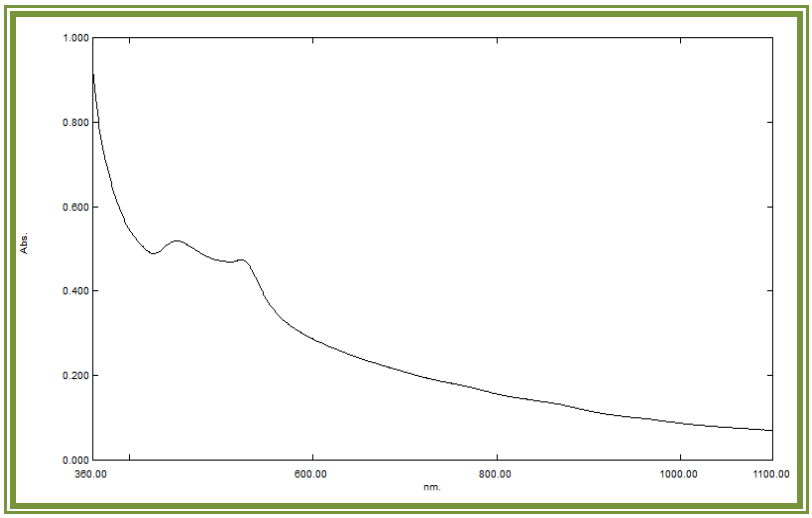


Figure 2- UV-Vis. Spectrophotometric of greenish yellow species formed reaction of Chlorpromazine HCl and Potassium hexcyanoferrate(III) against reagent blank

Variable Optimization

The chemical parameters (mainly such as concentration of reagents used for the precipitation reaction and pH of the reaction medium) as well as physical parameters (flow rate, intensity of incident light, sample volume, purge time) were investigated.

Optimization of Chemical Parameter

Effect of Potassium hexacyanoferrate(III) Concentration

A series of solutions (0.5-10 mmol.L^{-1}) were prepared. Using 80 µl of 10 mmol.L^{-1} chlorpromazine HCl as injected sample volume. Flow rate of 1.8 ml.min^{-1} was used as a carrier stream flow. Incident light intensity was set at 1100 mV. Each measurement was repeated for three successive times. table-1 summarizes the results obtained. It can be seen that an increase in $K_3[Fe(CN)_6]$ causes an increase in the attenuation of the incident light on particle surface. figure-3 shows this increase in the attenuation of incident light with increasing $K_3[Fe(CN)_6]$ concentration, therefore 5 mmol.L^{-1} was regarded as the optimum concentration for further work.

Table 1- Effect of $K_3[Fe(CN)_6]$ on the measurement of attenuation of incident light

Concentration of $K_3[Fe(CN)_6)$ mmol.L^{-1}	**Attenuation of incident light $\bar{y}i$ (n=3) (mV)**	**Standard Deviation σ_{n-1}**	**Repeatability %RSD**	**Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$**
0.5	33.00	1.73	5.249	33.00±4.30
0.7	56.67	1.15	2.038	56.67±2.87
1	81.00	0.87	1.025	81.00±2.15
5	87.83	0.76	0.870	87.83±1.90
10	86.50	1.32	1.529	86.50±3.29

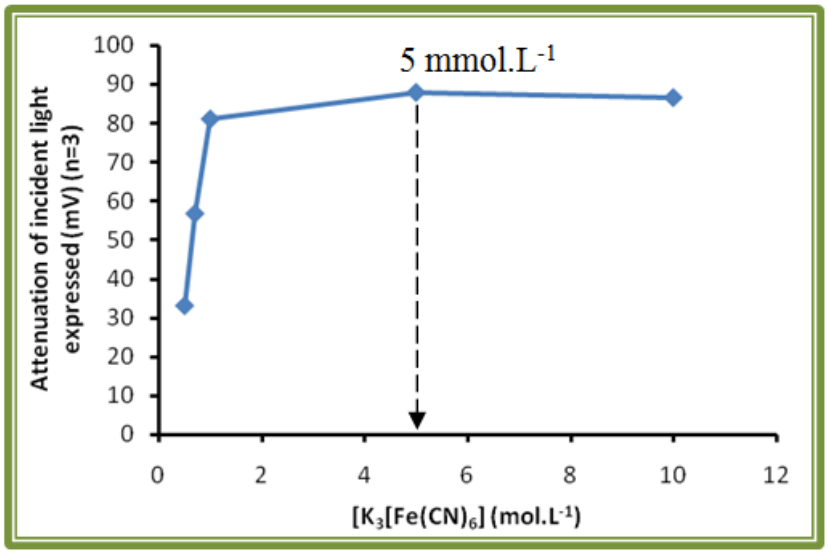


Figure 3- Effect of variation of $K_3[Fe(CN)_6]$ concentration on precipitation chlorpromazine HCl

Effect of Acidity on Precipitation Reaction

The effect of acidity of the reaction medium on the sensitivity in general was studied by using optimum concentration of $K_3[Fe(CN)_6]$ solution 5 mmol.L^{-1}. Series of diluted solutions of hydrochloric acid HCl (1-150) mmol.L^{-1} were prepared and 120 µL sample volume, 1100 mV as the intensity of incident light were used. It can be seen that there is an increase in sensitivity of response within increase of acidity medium as shown in figure-4. Therefore, 0.1 mol.L^{-1} of HCl was regarded as the optimum acid concentration medium for the work conducted in this research.

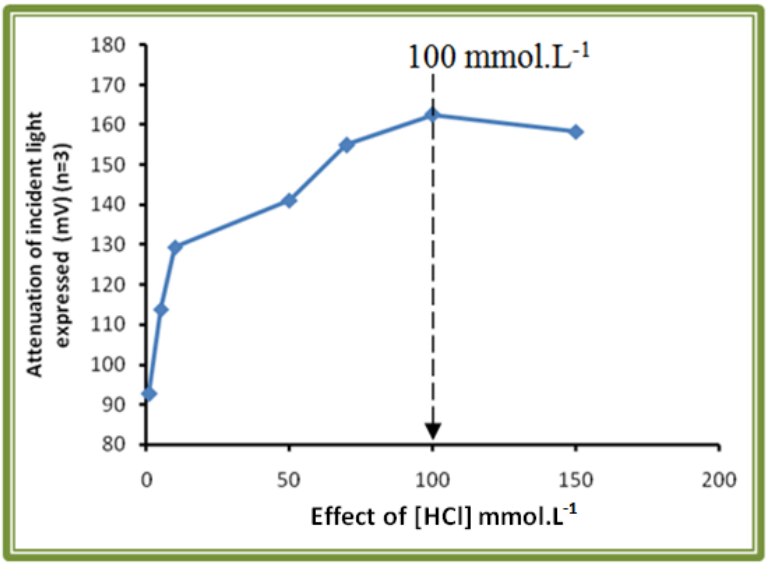


Figure 4- Effect of variation of Hydrochloric acid concentration on attenuation of incident light for determination of chlorpromazine HCl

Optimization of Physical Parameter

Effect of Flow Rate

A set of experiments was conducted for optimizing the flow rate of carrier stream 0.1 mol.L^{-1} and Potassium hexacyanoferrate(III) (5 mmol.L^{-1}); using 80 µL as sample volume and 1100 mV as intensity of incident light at a variable flow rate as tabulated in table-2. and figure-5. It was noticed that at low flow rate there is an increase in dispersion and dilution. A 2.4 ml. min^{-1} carrier stream flow rate was chosen on the basis of the best sensitivity and repeatability.

Table 2- Effect of variation of flow rate precipitation system of chlorpromazine HCl

Peristaltic pump (indication approximate)	Flow Rate (mL.min^{-1})		Attenuation of incident light $\bar{y}_i$ (n=3) (mV)	Standard Deviation σ_{n-1}	%RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$	t (sec.)
	0.1MHCl carrier stream	$K_3[Fe(CN)_6]$ precipitation agent					
10	0.6	0.6	113.33	2.08	1.837	113.33±5.17	42
20	1.2	1.2	107.33	1.53	1.423	107.33±3.79	24
30	1.85	1.8	102.67	1.15	1.125	102.67±2.87	15
40	2.4	2.4	117.33	1.53	1.302	117.33±3.79	12
50	3.1	3.0	91.83	1.26	1.370	91.83±3.13	9

t= arrival time of sample segment to the measuring cell

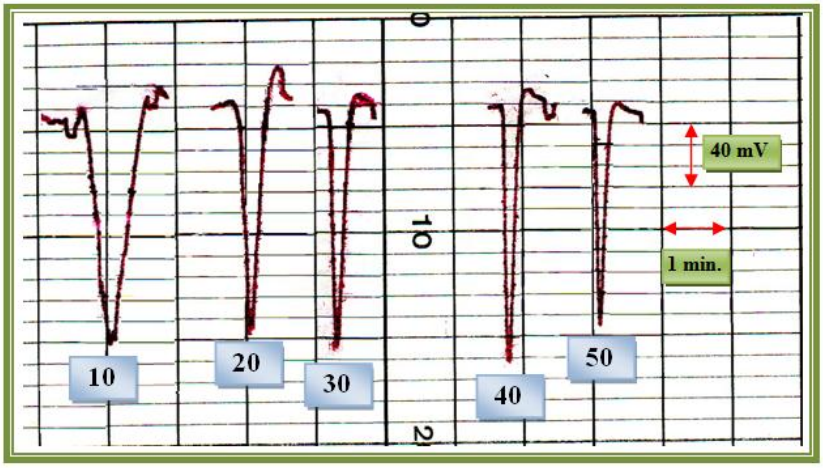


Figure 5- Effect of variation of flow rate versus energy transducer output response profile of Linear Array Ayah-5SX1-T-1D-CFI analyser

Variation of Incident Light Intensity

A variable intensity of light source of white LEDs expressed in mV (756-2030) was used for this study, and the system used for determination chlorpromazine HCl via $K_3[Fe(CN)_6]$ (5 mmol.L^{-1})-HCl (100 mmol.L^{-1})-CPZ (10 mmol.L^{-1}), 80 µL sample volume. The obtained results tabulated in table-3 shows that an increase in the attenuation of incident light. The intensity of 1754 mV was selected as the optimum voltage output that can be supplied to give a better response. figure-6-A shows the plot of attenuation of incident light vs. change in intensity of incident light while figure-6-B shows profile and response peaks with variation intensity of the incident light.

Table 3- Effect of Intensity of light source (white LED) on attenuation of incident light

Intensity of incident light (mV)	Attenuation of incident light $\bar{y}_i$ (n=3) (mV)	Standard Deviation σ_{n-1}	%RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
756	83.50	1.80	2.159	83.50±4.48
1024	106.33	1.53	1.437	106.33±3.79
1224	130.67	1.15	0.884	130.67±2.87
1500	133.50	1.32	0.991	133.50±3.29
1754	158.17	1.26	0.796	158.17±3.13
2030	151.33	1.53	1.009	151.33±3.79

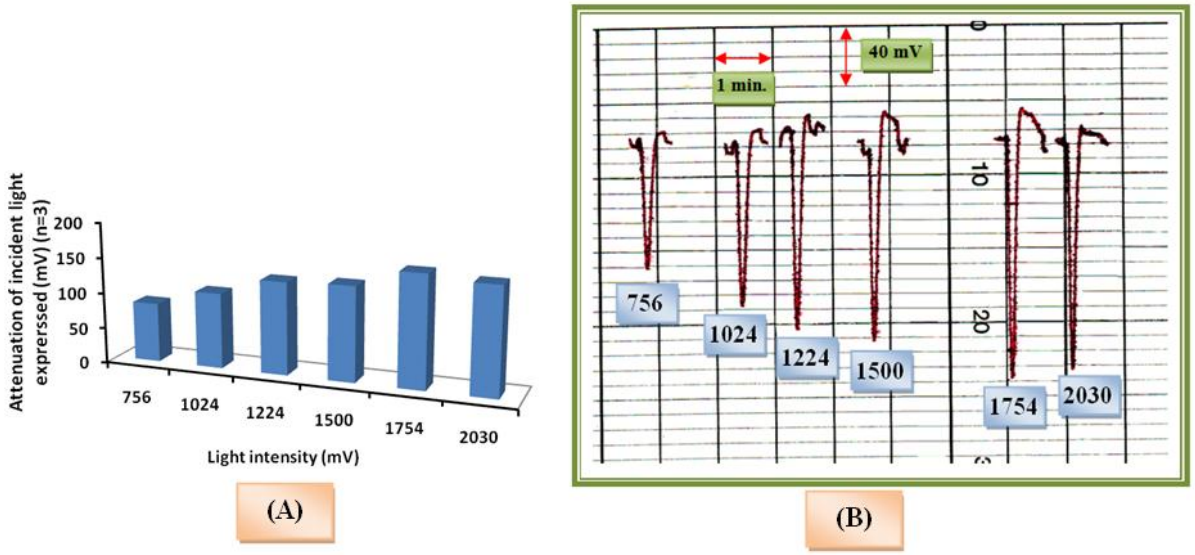


Figure 6- Variation of incident light intensity (white light source LEDs)
(A) Attenuation of incident Light.
(B) Response profile due to the variation of light source intensity.

Effect of Sample Volume

Under the optimum parameters achieved in previous sections. Different sample volumes (30-160) µL were injected using open valve mode i.e. allowance for continuous purge of sample from the sample loop in the injection valve. figure-7-A show that an increasing in the sample volume led to a significant increase in sensitivity, that the optimum sample volume 120 µl gave regular responses of the attenuation of incident light. Larger volume i.e > 120 µL, it gave a slight higher response and it was characterized with the width of their peak maxima which was can be probably attributed to long time duration of sample segment in front of detector as shown in figure 7-B.

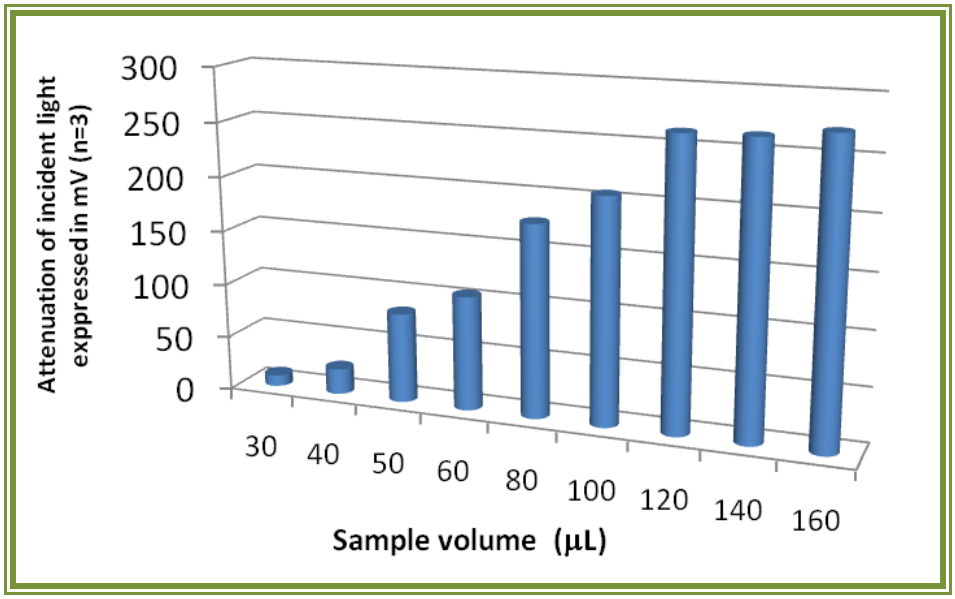


Figure 7-A- Effect of variation of injection sample volume against attenuation of incident light

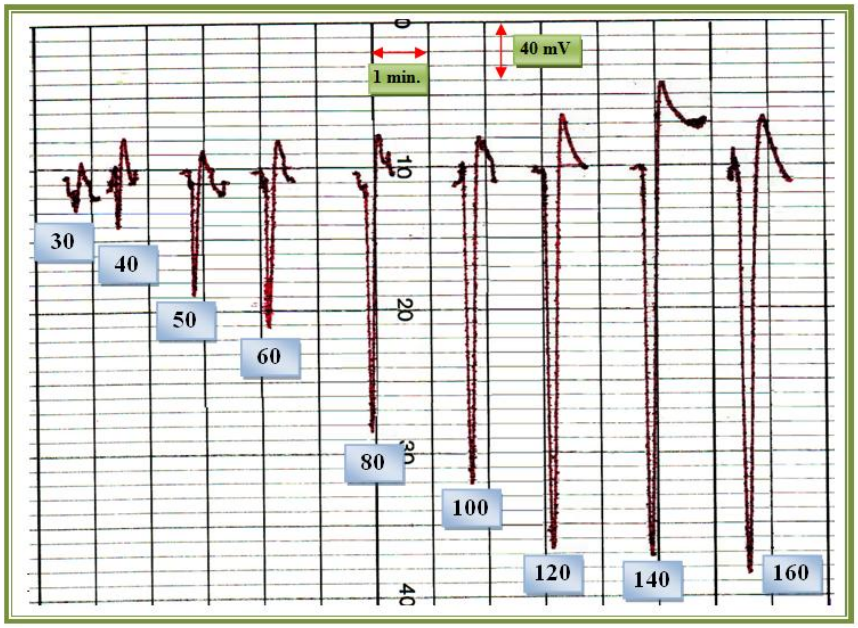


Figure 7-B- Response profile of variation in sample volume.

Effect of Purge Time

A study was made to determine the optimum duration of the injection time by using different purge time for the sample segment i.e. using 5 to 20 seconds allowed time for the carrier to pass through the injection valve. 120 µL sample volume was used. table-4 shows the continuation of the increase in response with increasing purge time. The decrease in response when using less than open valve was attributed to the incomplete purge of the sample from sample loop in the injection valve.

Table 4- Purge time effect on the attenuation of incident light

Purge time (sec.)	Attenuation of incident light $\bar{y}i$ (n=3)	Standard Deviation σ_{n-1}	%RSD	Confidence interval of the mean
5	118.50	1.32	1.12	118.50±3.29
7	178.33	2.08	1.17	178.33±5.17
10	205.33	1.53	0.74	205.33±3.79
12	208.83	1.61	0.77	208.83±3.99
20	206.33	2.08	1.01	206.33±5.17
Open valve > 20	223.50	1.50	0.67	223.50±3.73

Variation of Chlorpromazine HCl Concentration

The results for variation of Chlorpromazine HCl concentration was obtained under the optimized conditions. A series of variable concentration ranging from 1 to 35 mmol.L^{-1} were prepared for the purpose of using them for the preparation of scatter plot diagram followed by the choice of calibration graph. The obtained results were tabulated in table-5-A while their representation was in figure-8 which shows the variation of response with concentration of Chlorpromazine HCl. Analysis of variance was carried out as shown in table-5-B which indicated that $F_{tab.} = F^{v_1}_{v_2} = F^1_8 = 5.32 << F_{Stat.} = 562.42$ therefore, it can be concluded that there is a significant relation between the concentrations of Chlorpromazine HCl and the response obtained.

Table 5-A- Summary of calibration graph results for the estimation of Chlorpromazine HCl by FIA method

Measured CPZ mmol.L^{-1}	**CPZ range for n=10 mmol.L^{-1}**	**y^(mV)=a±S_at+b±S_bt [CPZ)]mmol.L^{-1} at confidence interval 95%, n-2**	**r, r^2, r^2%**	**$t_{tab.}$**	**$t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$**
				at 95%, n-2	
1-35	3-30	-56.17±38.92+24.42±2.37 [CPZ] mmol.L^{-1}	0.9928 0.9857 98.57	2.306 << 23.71	

Table 5-B- ANOVA for linear equation results [27-29]

Source	**Sum of squares**	**D_f**	**Mean square**	**$F_{stat.}=S_1^2/S_2^2$**
Regr. $(\hat{y}_i-\bar{y})$	433221	ν_1=1	433221	562.4216
Error $(y_i-\hat{y}_i)$	6162.224	ν_2=8	770.278	
Total $(y_i-\bar{y})$	1335473	9		

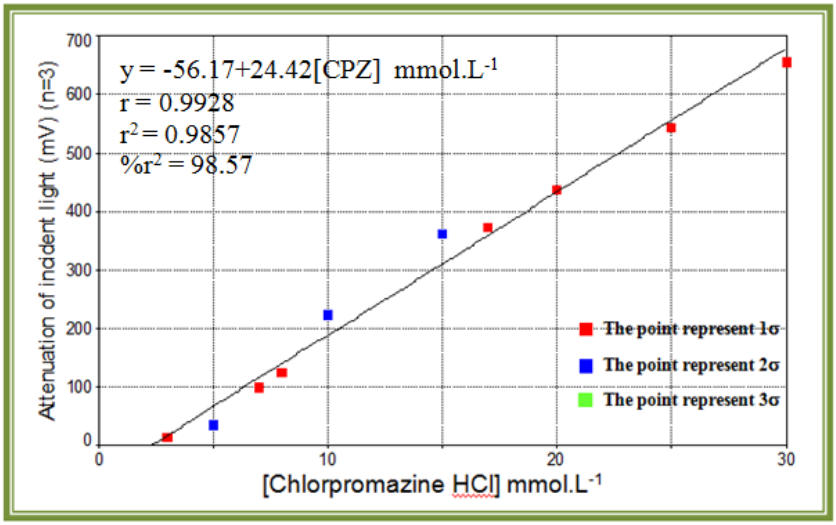


Figure 8- Calibration graph for Chlorpromazine HCl concentration in mmol.L^{-1}

Limit of detection for chlorpromazine HCl was conducted though using three different approach as tabulated in table-6 at injected sample volume of (120 µl).

Table 6- Limit of detection of chlorpromazine HCl at optimum parameters for $K_3[Fe(CN)_6]$-HCl-CPZ

Gradual dilution for the minimum concentration in calibration graph	**Based on the value of slope** $x=\frac{3S_B}{slope}$	**Linear equation y^(mV) = y_B+3S_B**
3×10^{-7} M/sample 0.12 µg/sample	3.68×10^{-6} M/sample 0.15 µg/sample	1.10×10^{-3} M/sample 47.2 µg/sample

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)

Repeatability Study

Six successive injected samples measurements were carried out for repeatability study for the determination of chlorpromazine HCl via measurements of the precipitate formed by the reaction of

potassium hexcyanoferrate(III) with Chlorpromazine HCl at concentration of 8 and 10 mmol.L^{-1} in acidic medium. table-7 show the results obtained while figure-9 show the response profile.

Table 7- Repeatability of Chlorpromazine HCl results

[CPZ] mmol.L^{-1}	Number of measuring (n)	Attenuation of incident light $\bar{y}i$ (n=6) (mV)	Standard Deviation σ_{n-1}	Repeatability R.S.D.%	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$
8	6	121.16	2.40	1.98	121.16±2.72
10	7	223.33	3.26	1.46	223.33±3.70

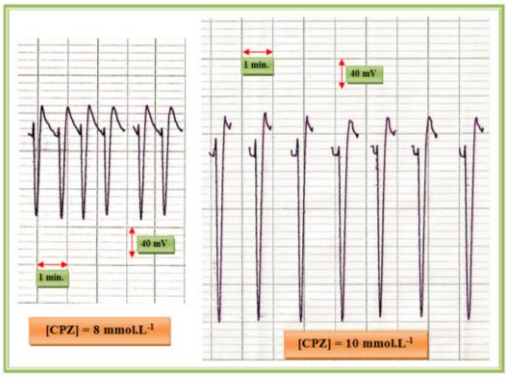


Figure 9- Response profile of repeatable measurements of Chlorpromazine HCl concentrations (8, 10 mmol.L^{-1}) in Ayah-5SX1-T-1D-CFI analyser

Analytical Application

The developed method was applied to drugs available in the market as tablets form; named Largactil. It was compared with official method. table-8 shows the names of the company supplier, drug dose and results at confidence interval 95%, paired t-test was used as shown in table-9. The obtained results indication clearly that there was no significant difference between developed method FIA with official method [2] at 95% confidence interval as the calculated t-test value is less than tabulated t-test value.

Table 8- Results for the determination of chlorpromazine HCl in pharmaceutical preparation

Commercial name Content Country	Confidence interval for average volume at 95% $\bar{w} \pm 1.96 \frac{\sigma_{n-1}}{\sqrt{n}}$ (mg)	Weight of sample (g) that equivalence to (355 mg) of active ingredient to obtain 0.01mol.L^{-1} (g)	Theoretical content of active ingredient at 95% n=∞ (mg)	found content of active ingredient at 95 n=∞ (mg)	% Recovery
Largactil 25 mg Oubari pharma-aleppo-syria company under license from Aventis Laboratory-France	0.1070± 0.000538	1.0644	25±0.231	24.80±0.135	96.76
Largactil 100 mg Oubari pharma-aleppo-syria company under license from Aventis Laboratory-France	0.4264 ± .006436	1.5149	100±2.776	98.57±1.115	95.75

Table 9- Paired t-test results for turbidmetric-CFI method with quoted value using standard additions method for the determination of Chlorpromazine HCl in pharmaceutical preparation

Sample no.	Practical content (mg)		D	$\bar{x}_d$	σ_{n-1}	Paired t-test $t = \frac{\bar{x}_d \sqrt{n}}{\sigma_{n-1}}$	$t_{tab.}$ at 95% confidence interval n-1
	Quoted value	New method					
1	25	24.94	0.06	0.2	0.135	2.56 < 4.303	
	25	24.67	0.33				
	25	24.79	0.21				
2	100	98.12	1.88	1.43	1.115	2.22 < 4.303	
	100	99.84	0.16				
	100	97.75	2.25				

Conclusion

The proposed turbidmetric flow-injection method is simple, rapid, inexpensive and sensitive for the determination of chlorpromazine HCl. The method based on reaction between chlorpromazine with potassium hexacyanoferrate(III) in acidic medium to yield greenish yellow precipitate as ion pair complex. The new method can be used to determine of chlorpromazine HCl in pure and pharmaceutical preparation without the need for heating or extraction. The precipitate is measured via the attenuation of incident light. The proposed method uses cheaper instruments and reagents with those spectrophotometry, fluorimetry, HPLC and other turbidimetric-FIA method with different precipitating agents. The %R.S.D was < 2% and good agreements were observed for all samples, which is an indication of satisfactory accuracy of the proposed method. The standard additions method was used to avoid matrix effects.

References

1. Karpinska J., Starczweska B. and Puzanowska-Tarasiewicz H. **1996.** Analytical properties of 2- and 10 disubstituted phenothiazine derivatives. *Analytical Science*, 12, pp.161-170.
2. British Pharmacopoeia. **2013.** version 17.0, 7th Ed., The stationary office, London, U.K.
3. Ajil W. A. **2006.** Determination of Micro Amount of Chloropromazine Hydrochloride in The Pharmaceutical Preparation "Epichlor" by Molecular and Flame Atomic Absorption Spectrophotometry Using Palladium and Rhodium as Mediating Metals. M.Sc. Thesis. Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.
4. Hassouna M.E.M., Adawi A.M. and Ali E.A. **2012.** Extractive spectrophotometric determination of chlorpromazine and trifluoperazine hydrochloride in pharmaceutical preparations. *Egyptian Journal of Forensic Sciences*, 2(2), pp.62-68.
5. Al-Kaffiji M.J. and Al-Anbakey, **2013.** New chromogenic reagent for the spectrophotometric determination of chlorpromazine HCl in aqueous and pharmaceutical formations. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 606-611.
6. El-Ansar A.L., El-Hawary W.F., Issa Y.M. and Ahmed A.F. **2001.** Preparation, characterization, and thermodynamic studies of promazine, chlorpromazine, promethazine, imipramine, and ciprofloxacin ion-associates with some metal complex ions. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, 31(3), pp.441-456.
7. Al-Hayani N.K.A. and Mohammad F.K. **2013.** A simple spectrophotometric assay for stability determination of chlorpromazine in veterinary injectable solutions. *International journal of the Bioflux Society*, 5(2), pp.66-68.
8. Basavaiah K. and Swamy J.M. **2002.** Spectrophotometric determination of some phenothiazines using hexacyanoferrate(III) and Ferriin. *Chemia Analityczna (Warsaw) (Chemical Analysis)*, 47, pp.139.
9. Aman T., Anwar J., Ahmad A. and Latif L. **2003.** Determination of five phenothiazines in pure and pharmaceutical preparations using vanadium pentoxide as a chromogenic reagent. *Analytical Letters*, 36(14), pp.2961-2974.

10. El-Ansar A.L., El-Hawary W.F., Issa Y.M. and Ahmed A.F. **1999.** Application of ion-pairs in pharmaceutical analysis. Atomic absorption spectrometric determination of promazine, chlorpromazine, promethazine, imipramine and ciprofloxacin hydrochlorides with sodium cobalt nitrite. *Analytical Letters*, 32(11), pp.2255-2269.
11. Farhadi K., Savojbolagh A. K., Farajzadeh M. and Maleki R. **2003.** Development of turbidimetric methods for the determination of some N-substituted phenothiazine derivatives using sodium dodecyl sulfate and mercury(II) chloride. *Analytical Letters*, 36(10), pp.2183-2198.
12. Al-Hashimi I.M.S. and Al-Kaffije M.J.H. **2012.** New approach development for determination of chlorpromazine HCl in pure and pharmaceutical forms using homemade wavelength selector flow injection photometer. *Karbala Journal of Pharmaceutical Science*, 3, pp.86-97.
13. Nesmerak K., Cerveny V., Hranicek J. and Rychlovsky P. **2013.** A spectrofluorimetric determination of phenothiazine derivatives after their photooxidation or chemical or electrochemical oxidation in a flow injection arrangement, *Microchemical Journal*, 106, pp.226-232.
14. Feng S. Li C., Fan J. and Chen X. **2007.** Sequential injection technique for the determination of chlorpromazine hydrochloride in pure form and pharmaceutical formulations, *Journal of Analytical Chemistry*, 62(3), pp.233-237.
15. Kojlo A., Calaltayud J.M. **1995.** FIA-Spectrophotometric determination of N-subsituted phenothiazine derivatives by oxidation with a soild-phase reactor of manganese dioxide incorporated in polyester resin beads. *Talanta*, 42(7), pp.909-913.
16. Issa Y.M., El-Hawary W.F. and Ahmed A.F. **2000.** Ion-pair formation in pharmaceutical analysis. Conductimetric determination of promazine, promethazine, imipramine and ciprofloxacin hydrochlorides in pure form, drug formulations and urine, *Mikrochim Acta*, 134, pp.9-14.
17. Halaburda P. and Mateo J.V.G. **2012.** Chemiluminometric determination of phenothiazines by means of a combined multi-commutated/multi-pumped flow assembly. *Talanta*, 96, pp.202-209.
18. Wenbing S., Jidong Y. and Yuming H. **2004.** Ion-pair complex based solvent extraction combined with chemiluminescence determination of chlorpromazine hydrochloride with luminal in reverse micelles. *Journal of Pharmaceutical and Biomedical Analysis*, 36(1), pp.197-203.
19. Tomas P., Carmen M., Antonio S. and Teresa S. M. **1999.** Flow injection chemilumescent determination of phenothiazines in pharmaceutical preparations. *Laboratory Automation & Information Management*, 34(2), pp.149-158.
20. Mckay G., Geddes J., Cowper M.J. and Gurnsey T.S. **1983.** Recent advances in the analysis of phenothiazine drugs and their metabolites using high performance liquid chromatography. *Progress in Neuropsycho pharamacology & Biological Psychiatry*, 7(4-6), pp.703-707.
21. Mehta A.C. **1981.** High-performance liquid chromatographic determination of chlorpromazine and thioridazine hydrochloride in pharmaceutical formulations. *The Analyst*, 106(1267), pp.1119-1122.
22. Chagonda, L.F.S. and Millership J.S. **1988.** High-performace liquid chromatographic determination of chlorpromazine and its degradation products in pharmaceutical dosage forms: a stability-indicating assay. *The Analyst*, 113(2), pp.233-237.
23. Issam M.A. Shakir Al-Hashimi and Nagam Skakir Turkie Al-Awadi. **2013.** Linear Array Ayah 5SX1-T-1D-CFI Analyser, Iraq Patent, Patent No. 3615.
24. Puzanowska-Tarasiewicz H., Karpinska J. and Kuzmicka L. **2009.** Analytical applications of reactions of iron(III) and hexacyanoferrate(III) with 2,10-disubsituted phenothiazine. *International Journal of Analytical Chemistry*, 2009, p.8.
25. Puzanowska-Tarasiewicz H., Kuzmicka, Karpinska J. and Mielech-Lukasiewicz K. **2005.** Efficient oxidizing agents for determination of 2,10-disubstituted phenothiazine. *Analytical Science*, 21, pp.1149-1153.
26. El-Brashy A. M., El-Sayed Metwally M. and El-Sepai F. A. **2005.** Spectrophotometric determination of some fluoroquinolone antibacterials by ion-pair complex formation with cobalt(II) tetrathiocyante. *Journal of the Chinese Chemical Society*, 52, pp.77-84.
27. Miler J.C. and Miller J.N. **2010.** *Statistics for analytical chemistry.* 6nd Ed., John Wiley and N.y. Sons.
28. Bluman A.G. **1997.** *Elementary Statistics.* 3rd Ed., WCB/MC Graw-Hill, New York.
29. Murdoeh J. and Barnes J.A. **1974.** *Statistical tables*. 2nd Ed., Macmillan, pp.8.