Promethazine-HCl Determination Using Entrapped Persulphate in Water Crystals by Flow Injection / Stopped - Flow Technique and Ayah 3SX3-3D Solar Cell Micro Photometer

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Abstract:

A new mode of methodology was adopted in the assay of PM- HCL based on the use of water crystal gel bead made of poly acrylic acid in which the gel beads were left to absorbe persulphate solution that is necessary for the oxidation of PM- HCL in aqua media . The water crystal act as a reserviour for $S_2O_8^{2-}$. Optimum parameters were studied giving to specify the chemical and physical parameters. Two line manifold was used .The flow rates of 1.5 and 1.2 ml.min^{-1} was used , 3 gel beads , 120 µL sample volume , a linear dynamic range extend from 0.01- 18 mmol.L^{-1}. A correlation coefficient (r) of 0.9991while the percentage linearity (r^2%) of 99.82% with R.S.D% at 8 mmol.L^{-1} promethazine-HCl is less than 0.5% (ten replicates) and a detection limit (S/N=3) of 19.25 ng/sample was obtained . Throughput of 20 sample.hr^{-1} can be measured. A successful application on available drug in the market was assayed. A complete satisfactory result was obtained .Using t-test it was shown that there was no significant difference between the Practical value and the quoted value, on that basis the new method can be accepted as an alternative analytical method.

Keywords: Stop-go mode, water gel bead, promethazine hydrochloride, Ayah 3SX3-3D solar micro photometer

تقدير البروميثازين – هيدروكلورايد بأستخدام البيرسلفات المحتواة في بلورات الماء بوساطة تقنية جريان – توقف ومطياف مايكروي Ayah 3SX3-3D solar cell

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الخلاصة :

تم تبني طريقة بنمط جديد لتقدير بروميثازين – هيدروكلورايد بالاستناد على أستخدام بلورات الماء – حبيبات الجل والمتكونة من متعدد حامض الاكريلك والتي فيها حبيبات الجل تترك لامتصاص محلول البيرسلفات الضروري لأكسدة بروميثازين – هيدروكلورايد في الوسط المائي .حيث تتصرف بلورات الماء كخزان لاحتواء ايونات البيرسلفات . تم دراسة الظروف الفضلى لتشخيص المتغيرات الكيميائية والفيزيائية . تم استخدام وحدة متشعبة من خطين وبسرع جريان 1.5 و1.2 مل .دقيقة $^{-1}$, 3 حبات جل , حجم الانموذج 120مايكرولتر , المدى الخطي الفعال يمتد من 0.01 – 18 مللي مول . لتر $^{-1}$ والحصول على معامل ارتباط 0.9991 (r) بينما نسبة الخطية (r^2%) 99.82 مع R.S.D% لتركيز 8 مللي مول . لتر $^{-1}$ من

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البروميثازين – هيدروكلوريد اقل من 0.5 % (تكرار عشرة مرات) وحدود الكشف 19.25 نانوغم / انموذج . معدل النمذجة 20 انموذج / ساعة . طبقت الطريقة بنجاح لفحص عقار متوفر بالسوق ، حيث تم الحصول على نتائج مرضية . تم اجراء اختبار – t للمقارنة بين القيمة المستحصلة عمليا والقيمة الرسمية ولوحظ انه لا يوجد فرق جوهري بين القيمتين، وعلى هذا الاساس بالامكان استخدام الطريقة المقترحة للتحليل بالحقن الجرياني الطيفي كبديل للطريقة القياسية.

Introduction

Promethazine-HCl, 10-[2-(dimethyl- amino) propyl]phenothiazine monochloride ,(PM-HCL) is a prominent compound in the large group of phenothiazine derivatives .Its structure is shown in figure.1. It is widely used as an antihistaminic for the symptomatic relief of hypersensitivity reaction or for enhancing the analgesic, anesthetic and sedative effect of other drugs. It is usually administered in the form of coated tablets or as an injection liquid. Like other phenothiazine , it easily undergoes oxidation in acidic medium in the presence of oxidizing agents [1,2].

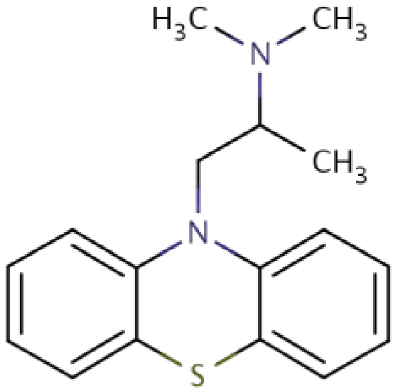


Figure.1-Structure of promethazine-HCl

Many analytical techniques have been employed for the determinations of PM-HCL.The majority of the methods are based on the redox properties of the S-atom.

Hexamine - cobalt (III) tricarbanato cobaltate(III)[3] , molybdophosphoric acid [4] , potassium dichromate (Indirect spectrophotometric) [5] , potassium persulphate [6], etc. ,are used as oxidimetric titrants for the determination of PM-HCL. Numerous flow injection spectrophotometric methods have also been described for the estimation of the drug. Most of these determination method are based on the chemical oxidation of PM-HCL to the colored radical cation using oxidizing agents such as cerium (IV) arsenite as a strongly oxidizing solid - phase reactor [7] , iodic acid in sulphuric or phosphoric acid medium [8] , ceric ions [9] and electroxidation at a gold electrode in sulphuric acid medium [10] . Various other methods, like chemiluminescence of the luminol - H_2O_2- Cr(III) system [11] and using acidic permanganate [12] , Nephelometric titration [13] , Turbidimetric method [14] and Spectrofluorometry [15] , have also been employed.

The widespread uses of these drugs necessitate the development of a rapid, simple and precise method for their quality control assay.

In this study, the use of new mode of stopped- flow technique comprising the entrapment of sodium persulphate inside the water crystal gel bead for the determination of PM-HCL in pure and Pharmaceutical preparation.

A single water gel bead (or many) located in a specially designed cell , which aims to the libration of sodium persulphate from this water gel bead to the carrier stream for the oxidation of PM-HCL in an aqueous medium . The oxidation product yields a pinkish red color measured at 515 nm. This procedure is involved using a homemade [16] FI microphotometer which is equipped with three different light emitting diode [Blue (470 nm), Green (525nm), and Red (635nm)] as a sources and solar cell as a detector. The performance of the proposed procedure was checked by analyzing commercial pharmaceutical formulations. This procedure is simple, rapid, and inexpensive; dose not involves pretreatment procedure or heating steps and has smaller consumption and a higher analysis frequency.

Experimental

Chemicals

All chemicals used were of analytical reagent grade. Distilled water was used to prepare all the solution. Freshly prepared solutions were always used. Standard solution (w/v) of PM- HCL ($C_{17}H_{20}N_2S.HCl$, 320.9 g.mol^{-1}, SDI, 10 mmol.L^{-1}) was prepared by dissolving 0.3209 gm / 100 ml distilled water . A stock solution of sodium persulphate ($Na_2S_2O_8$, 238.10 g.mol^{-1}, BDH, 100 mmol.L^{-1}: 5.9525 g / 250 ml distilled water. Nitric acid(70% , 1.42 g.ml^{-1}, BDH, 1 mol.L^{-1}) was prepared by dilution of 128 ml / 2L, Sulphuric acid(96 % , 1.84 g.ml^{-1}; BDH ,1 mol.L^{-1}) was prepared by dilution of 111ml / 2 L calibrated flask, Hydrochloric acid(35% , 1.18 g.ml^{-1}, BDH ,1 mol.L^{-1}) was prepared by dilution of 176.50 ml in 2L distilled water. Each acid standarized against sodium carbonate, which was prepared by dissolving 28.617 g of $Na_2CO_3.10H_2O$ in L to obtain 0.1 mol.L^{-1}.

Apparatus and reaction manifold

The schematic diagram in figure. 2 shows the system used ,which comprises the use of a peristaltic pump : four channels, variable speed (Ismatic , USA) , a six- way injection valve (IDEX,V-450 , USA) with a sample loop (0.7mm i.d. , Teflon, variable lengths) . The instrument response was measured by Ayah 3SX3-3D Solar cell FI photometer (homemade)[16] using super bright blue (470nm) , green(525nm) and red (635nm) light emitting diode as a source with a detector using three solar cell . The output signals was recorded by recorder (Siemens - Germany) .range (1-500 mV) or (1-500 volt) or digital AVO- meter (auto range) (0-2 volt)
(China) .UV - VIS Spectrophotometer digital double beam (type UV-1800 , Shimadzu, Japan)was also used to scan the spectrum of colored species using Quartz cell .

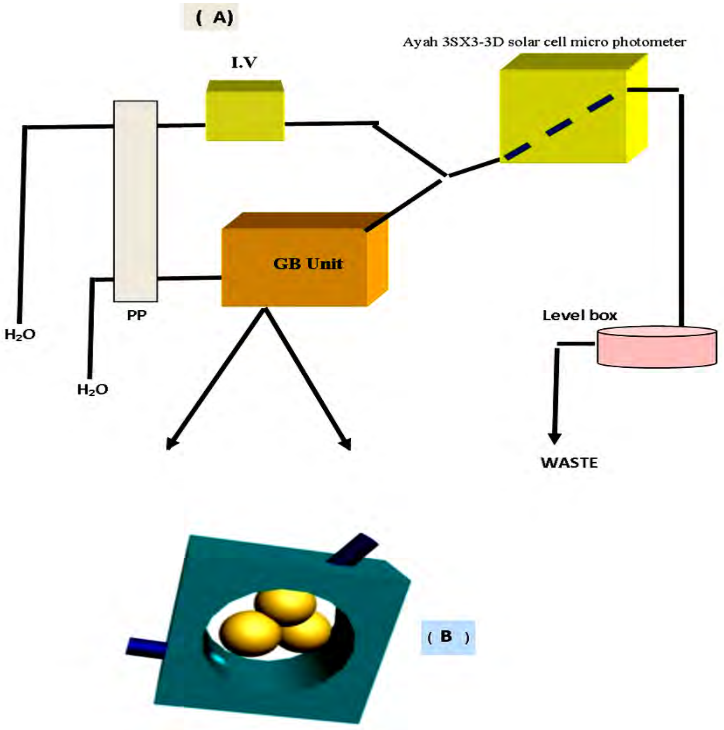


Figure.2 -**A**: Schematic diagram of continuous flow injection analysis system with Ayah 3S$_{BGR}$X3-3D Solar cell CFTA microphotometer, I.V: Injection valve, pp: peristaltic pump
B: Gel bead cell unit (GB Unit)

Gel bead cell unit

Gel bead cell unit made of poly metha methyl acrylate (PMMA) which has a free vacant volume of 33 ml equipped with 3 gel beads , the cell has an 2mm inside diameter for the inlet , with inclined outlet tube having the same inside diameter as the inlet glass tube . The whole system is

Transparent figure.2-B .This cell unit will represent the $S_2O_8^{2-}$ supplier based on stop - go mode of action with open valve mode for introducing sample segment of drug. 2.5 min is required for regeneration of $S_2O_8^{2-}$ ion from the gel bead that is necessary for completion of reaction. Successive recharging of the gel bead is necessary after 45-60 injection. The regeneration process can be accomplished for 2-5 hours if the same gel bead should be used again.

Sample preparation

Thirteen tablets were weighted ($\bar{x}$ = 0.1298 gm, Histazine - Jordan), crushed, grinded and the powder was mixed. Tablets containing 25 mg of PM-HCl . An accurately 0.8331gm equivalent to 160.45 mg of active ingredient to obtain 5 $mmol.L^{-1}$ for pharmaceutical preparation was prepared. Dissolved into a little distilled water, followed by filtration to get rid of undissolved materials, the residue was washed with the same solvent and completed to the volume (100 ml).

Preparation of Gel bead for use

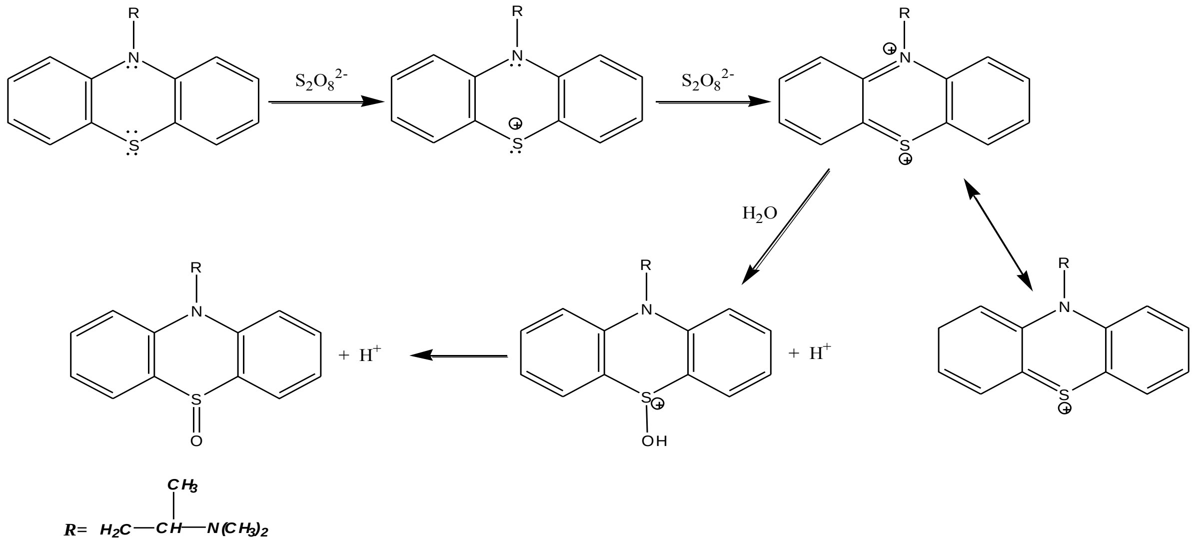
Commercial gel beads were selected on the basis of their individual weight (wt. range 34.9 - 38) mg. 100 gel bead were chosen as most possible representation of the bulk of gel beads with a confidence interval of 37.24± 0.25. The gel beads washed with distilled water from excessive dust that were or might be present on the gel bead surface, followed by soaking of gel bead in distilled water for overnight usually 16-19 hrs. is required to reach the most possible expansion of gel beads.

The gel beads is left to loss the water after being washed by a homemade unit based on 2- Lamps, 60 watt as a heating source in a container equipped with a section fan that will draw hot air passing over gel beads as shown in figure.3.The unit is equipped with two probes for temperature control (inlet and outlet) with relative humidity measurement and a timer.

Figure.3-Gel bead evaporation unit

Methodology:

The manifold unit that was used for the determination of PM - HCL composed of two lines figure.2 ,both carrying distilled water . The first line representing the carrier stream that passes through the injection valve carrying the sample loop segment content (i.e. PM -HCL , 100 µL) at flow rate of 1.5 $ml.min^{-1}$ .While the second line at 1.2 $ml.min^{-1}$ which is supplied by distilled water to pass through the gel bead cell unit (that is equipped with 3 beads , that was already soaked in potassium persulphate solution (2 $mmol.L^{-1}$)) . This gel bead cell unit will serve as a reservoir for persulphate ion solution . 2.5 min is required as optimum parameter for stop - go mode of operation. Both lines mixes at the Y-junction leading to the formation of colored cationic radical. The output signal of the resulting pinkish - red product is measured using Ayah 3SX3-3D solar cell FI photometer and the variation of response was monitored using green light emitted diode throughout the reaction. Each solution was assayed in triplicate. A proposed mechanism of oxidation of PM-HCL in aqueous medium is presented in scheme 1 [17].



Scheme 1: Proposed mechanism of reaction between PM-HCl and sodium persulphate.

Results and discussion

Spectroscopic study

A literature survey showed that the scanning for the colored product resulted from the oxidation of PM-HCL with $S_2O_8^{2-}$ gave λ_{max} at 515 nm [18] . While a measurement for the colored product using Ayah 3SX3-3D solar cell FI photometer which is shown in figure.4 which shows clearly that the green LED is the most suitable source that can be used , using the optimum experimental parameters: 3 gel beads were used which were previously soaked and embedded in 1.5 $mmol.L^{-1}$ $S_2O_8^{2-}$, using stop flow (3 minutes) - go as a working methodology , 3 $mmol.L^{-1}$ of PM-HCL , 80 µL and three different light emitting diode (LED) of red , green and blue as a different irradiation sources , showed that highest output signal was through the use of green LED . This was fixed as the LED to be used through this work .

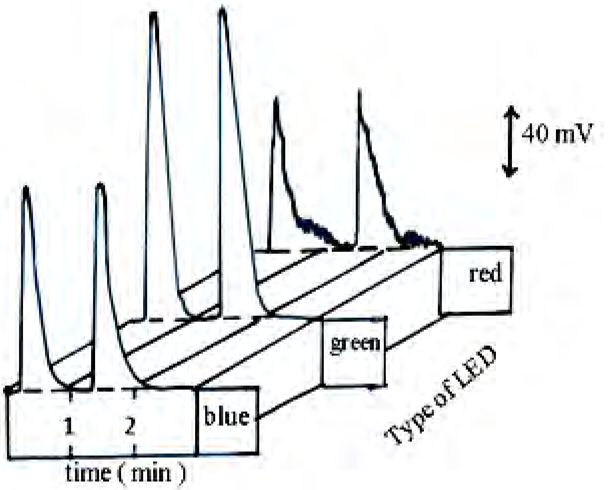


Figure 4-Variation of type of the light emitting diode [blue 470 nm, green 525 nm, and red 635 nm] on transducer energy response profile versus time for two successive injected samples of PM- HCL

Optimum conditions

Chemical variable

Effect of number of gel beads and the concentration of $S_2O_8^{-2}$ on the output signal

A set of one to four gel beads were taken , kept and soaked in variable concentration of $S_2O_8^{2-}$ (range 0.5- 4 $mmol.L^{-1}$) . Twenty four beaker of 100 ml arranged in a set of four beakers were used , containing six variable concentration of $S_2O_8^{2-}$ for each of the four sets of gel bead (i.e. one single gel bead in six different beaker , each of the six beakers containing 0.5 mmol.L^{-1} for the first beaker , 1 mmol.L^{-1} for the second beaker , 2 mmol.L^{-1} for the third beaker , 3 mmol.L^{-1} for the fourth beaker , 3.5 mmol.L^{-1} for the fifth beaker ,and 4 mmol.L^{-1} for the sixth beaker .Table 1 sum up the data that were obtained , containing no. of gel bead , variable concentration of $S_2O_8^{2-}$, and the responses obtained when subjected to the use in the assay method that were established throughout this research work .From figure.5-A,B ,it can be seen that the most suitable , acceptable gel bead number that will be used throughout this research work will be three gel beads , using 2 mmol.L^{-1} of $S_2O_8^{2-}$. The mechanism of supplying $S_2O_8^{2-}$ from gel bead is governed by the diffusion process through the gel bead body structure . A 3 minutes was regarded as time lag necessary for refreshed $S_2O_8^{2-}$ supply . figure.6 shows the most probable mechanism of $S_2O_8^{2-}$ departure via diffusion from gel bead . While using more than three gel beads will result in having broad peaks , therefore it was avoided .

Table 1-Variation of $S_2O_8^{2-}$ concentration on the transducer energy response

[$S_2O_8^{2-}$] mmol.L^{-1}	**0.5**	**1**	**2**	**3**	**3.5**	**4**
No.of bead	**Confidence interval of the average transducer energy response expressed as peak heights, n=3, $_i$ (mV) $_i \pm t_{0.05/2,\,n-1}\,\sigma_{n-1}/\sqrt{n}$**					
1	**20 ± 0.49**	**40 ± 2. 03**	**80± 2.07**	**88 ± 0.77**	**90 ±0.46**	**95 ± 0.33**
2	**55 ± 1.22**	**100 ± 1.43**	**140± 1. 34**	**148 ± 0.47**	**150 ± 0.45**	**165 ± 1.67**
3	**109 ± 1.34**	**130 ± 0.36**	**180± 1.48**	**175 ± 0.89**	**170 ± 1.23**	**155 ± 1.98**
4	**150 ± 1.03**	**180 ± 1.33**	**120± 1.00**	**116 ± 0.59**	**100 ± 1.66**	**90 ± 2.34**

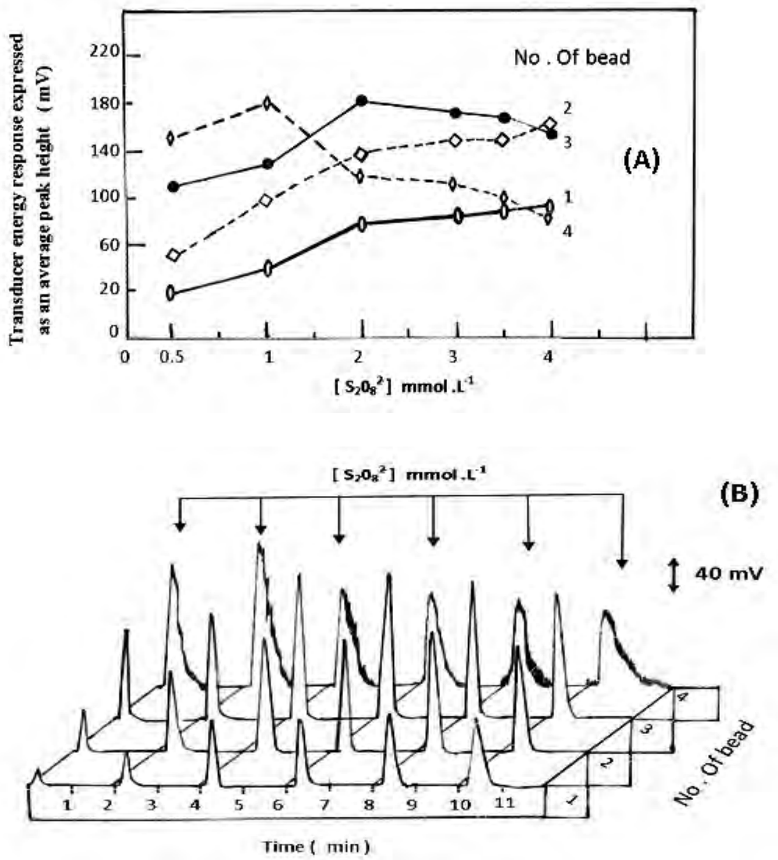


Figure 5-Variation of number of gel beads and the concentration of S_2O8^{-2} on the: A**:** Transducer energy response. **B**: Response profile versus time . Using 80 µL, 3 mmol.L^{-1} of PM-HCL

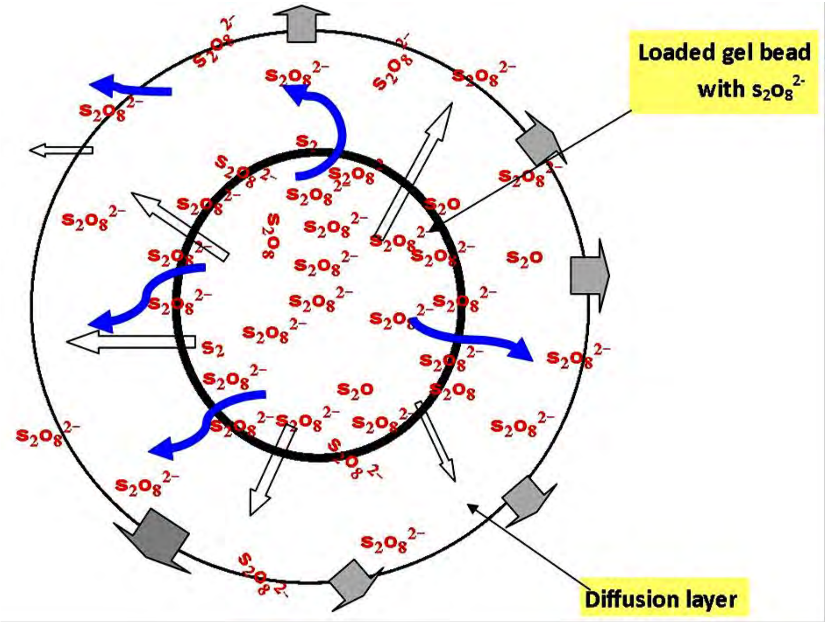


Figure.6-The probable mechanism of $S_2O_8^{2-}$ departure from gel bead via diffusion

Effect of acid medium on the formation of colored speciec for the PM-HCL reaction system

Using optimum parameters that were achieved in the previously conducted experiments at 3 mmol.L^{-1} PM-HCL as the injected used concentration . Series of different acids (HNO_3 ,H_2SO_4 , and HCL) solution were prepared in addition to distilled water . The measurements were carried out via the normal methodology that was adopted through this research work. Figure.7 shows the kind of maximum detector response versus time profile was through the use of distilled water as a carrier stream compared with the different acids . This might be attributed to the dissociation of formed colored species , especially when using HCL or H_2SO_4 , while other acids e.g. HNO_3 did not show any improvement in the detector response . On the basis of the above study, distilled water was fixed as the carrier stream as the most favorable medium for conducting the oxidation of PM-HCL with $S_2O_8^{2-}$

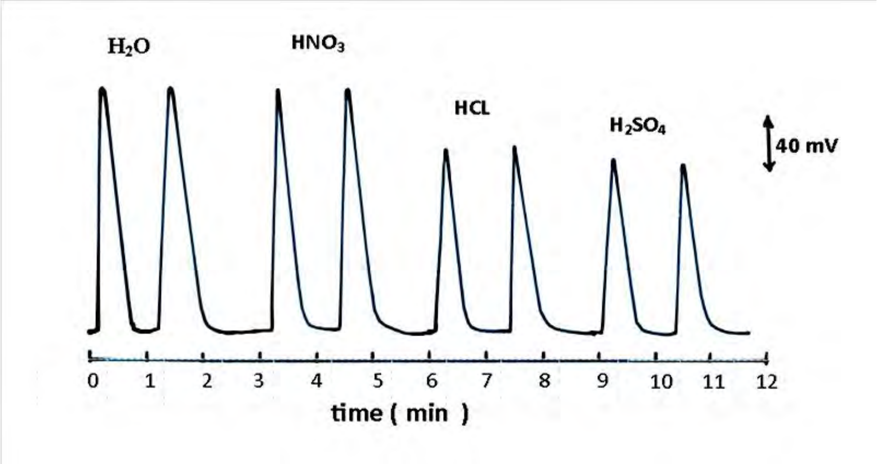


Figure 7-Response versus medium variation using Ayah 3S$_{BGR}$X 3-3D solar cell CFIA microphotometer, 80 µL , 3 mmol.L^{-1} of PM-HCL

Physical variable Effect of stop -go mode of operation on the amount of oxidation ($S_2O_8^{2-}$) supplied through diffusion via the gel beads

Fixing all studied variable at their optimum values. 3 mmol.L^{-1} of PM-HCL of 80 µL was used for this study , Variable hault periods of time were used from 0.5 up to 3 minutes in 0.5 min (30 seconds) increment . Table 2 and figure.8 tabulate and shows that the most suitable lag of time to give the time required for the many equilibrium processes **that** occur from the core of the gel bead up to the surface of the gel bead to the surrounding environment was 2.5-minute i.e 150 seconds. Above 2.5 minutes, it was noticed that a decrease in signal response. Also a broadening of the peak maxima and its base width Δt_B as shown in figure.8. This behaviour could be attributed to increased amount of colored species that will attenuate the incident light for a longer period of time in front of the detector . A very important step in the whole determination procedure is that the sample should be injected at the same moments when solution is allowed to flow in the cycle of stop - go mode.

Table 2-Effect of stop -go on mode of operation

Hault period of time (min)	Transducer energy response expressed as peak heights n=3, $_{i}$ (mV)	RSD%	Confidence interval of the average response $_{i} \pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$
0.5	**70.68**	**1.20**	**70.68 ± 2.04**
1	**90.79**	**1.08**	**90.79 ± 2.43**
1.5	**120.49**	**0.17**	**120.49 ± 0.52**
2	**166.98**	**0.09**	**166.98 ± 0.37**
2.5	**188.45**	**0.51**	**188.45 ± 2.41**
3	**192.57**	**0.46**	**192.57 ± 2.21**

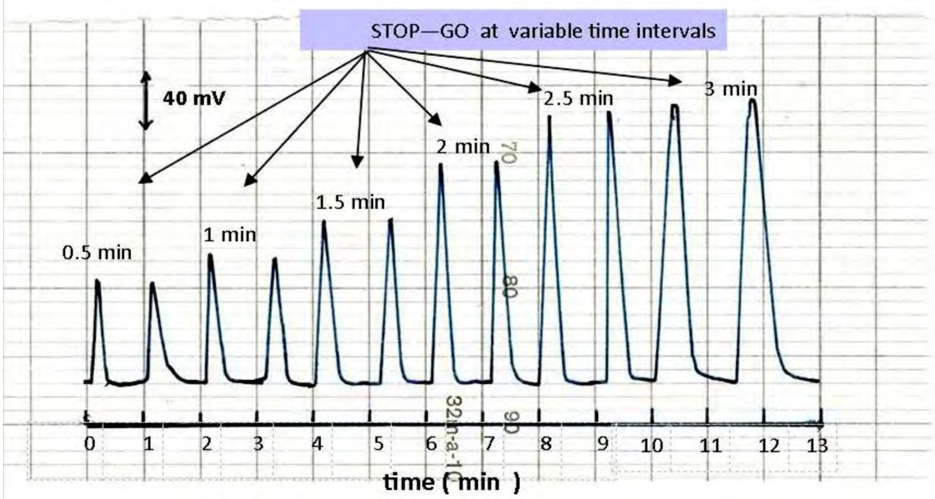


Figure 8-Effect of stop - go mode for various time interval (0.5 - 3) min on response profile using 80 µL , 3 mmol.L^{-1} of PM-HCL

Flow rate and Sample segment volume effect on the produced colored species of PM-HCL

Adjusting all achieved optimum parameters that were studied in previous sections with variable flow rate extended from 0.9 - 2.5 ml.min^{-1} and 0.5 - 2.4 ml.min^{-1} for each of carrier stream and the second flow lines that enters to the gel beads unit , using variable volume of sample segment which extends from 50 - 200 µL with open valve mode . Figure.9-A, B shows kind of response profile that were obtained. While all results were tabulated in table 3 which indicate clearly that the optimum flow rate is 1.5 and 1.2 ml.min^{-1} for each of the individual lines successively. An increase of flow rate more than these values although sharp edges responses were obtained but it was characterized by low height responses, this might be attributed to the incomplete formation of colored species with its quick departure of the flow cell affecting the sensitivity of the measurements.

Even though that 100 µL is the optimum sample volume to obtain sharp profile , but an increase of sample segment more than 100 µL leads to slight increase in peak heights . For the sake of a compromise between peak height and sample volume consumption, 120 µL was the choice.

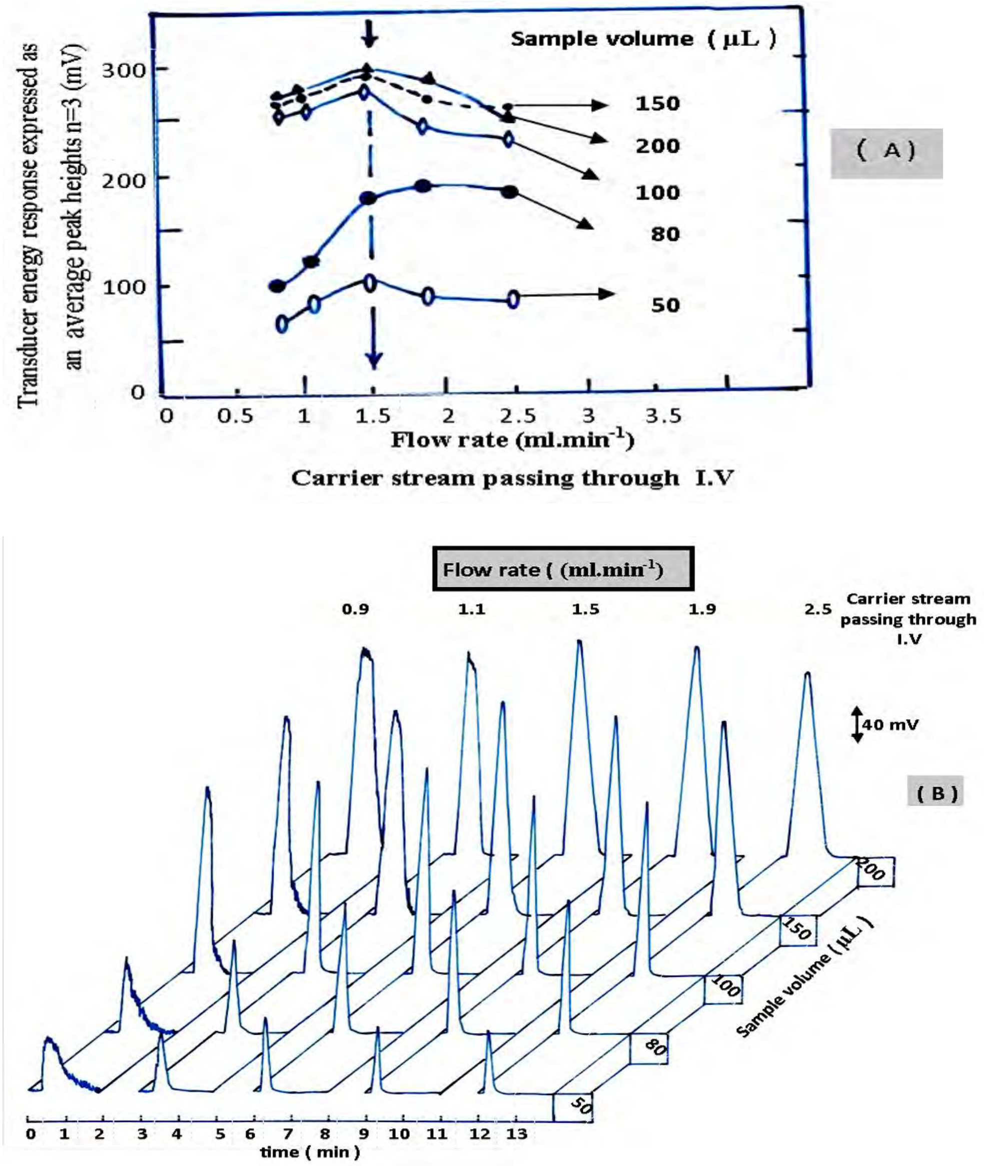


Figure 9-Effect of Flow rate and Sample segment volume on the
A: transducer energy response for the PM- HCL- $S_2O_8^{2-}$ (G B)system.
B: - response Profile using Ayah $3S_{BGR}$ x 3-3D solar cell CFIA microphotometer for the determination of PM-HCL (3 $mmol.L^{-1}$)

Table 3-Variation of injected sample volume and flow rate on transducer energy response

Sample volume (µL)		**50**	**80**	**100**	**150**	**200**
Flow rate ($ml.min^{-1}$)		**Confidence interval of the average transducer energy response expressed as peak heights,** **n=3, $_i$(mV)** **$_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$**				
Carrier stream (first line)*	**Carrier stream (second line)****					
0.9	0.5	68 ± 0.45	100 ± 0.98	250 ± 0.49	258 ± 0.45	268 ± 1.07
1.1	0.9	77 ± 0.95	125 ± 0.78	255 ± 0.39	268 ± 0.85	270 ± 0.98
1.5	1.2	100 ± 1.05	175 ± 0.59	270 ± 0.99	280 ± 0.93	288 ± 1.45
1.9	1.7	90 ± 0.88	188 ± 0.89	240 ± 1.45	268 ± 1.45	278 ± 1.22
2.5	2.4	`88 ± 1.89	178 ± 1.45	230 ± 1.05	260 ± 1.06	250 ± 1.35

*: **Carrier stream passing through I.V , **: Carrier stream passing through G.B Unit**

Scatter plot for the variation of response versus concentration to evaluate of the used methodology

A series of solutions having the concentration range of 0.005 mmol.L^{-1} to 20 mmol.L^{-1} using all achieved reaction parameters , whether it is physical or chemical , it was found that the linear range corresponding to a linear equation of the form : response = intercept + slope [conc.] for the concentration range of 0.01 - 18 mmol.L^{-1} with a correlation coefficient of r = 0.9991 which indicate an excellent correlation between obtained response versus concentration range depicted . Table 4 tabulate all results , while Fig. 10 explain schematically the plot of response versus concentration of PM-HCL . At a concentration above 18 mmol.L^{-1} a decline in r value caused by a deviation from linearity with the used linear equation .

Table 4-Summed up calibration graph data for the determination of PM-HCL

Measured [PM-HCL] mmol.L^{-1}	Linear dynamic range mmol.L^{-1} n = 13	$\hat{Y}i$ (mV) = a ± ts_a +b ± ts_b [PM-HCL] mmol.L^{-1} at confidence level 95% , n – 2	r r^2 %	t_{tab}	$t_{cal} = /r/ \sqrt{n-2} / \sqrt{1-r^2}$
0.005-20	0.01-18	12.92 ± 18.77 + 91.54 ± 2.59 [PM-HCL] mmol.L^{-1}	0.9991 99.82	2.201 << 77.34	

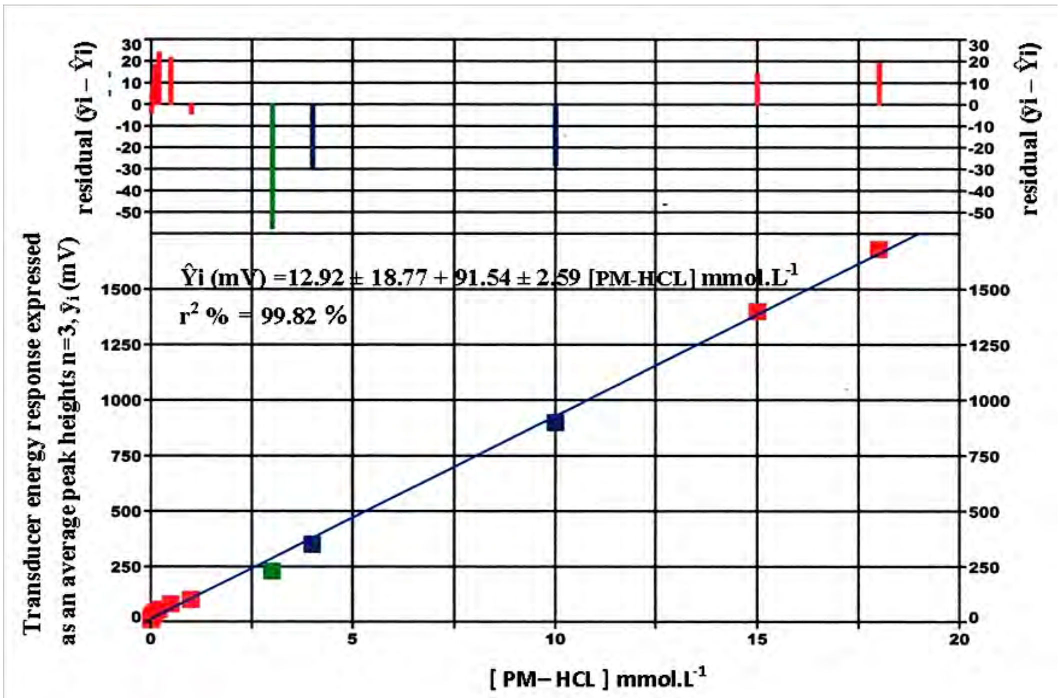


Figure.10-Calibration graph for the variation of PM-HCL concentration on: A-transducer energy response expressed by linear equation. B- residual (i – Ŷi), i: practical value, Ŷi: estimate value.

Limit of detection (L. O. D)

The minimum detectable concentration of analyte that the adopted methodology can detect above distilled water signal at 95% confidence level was 19.25 ng / sample at 3σ .

Repeatability:

A repeated ten successive measurements of a single concentration (8 mmol.L^{-1}) of analyte gave a variation of RSD % less than 0.5% . Results were tabulated in table. 5. This indicate the trustability of the used method even though it indicate the success of the used gel bead mode of chemical reaction

Figure. 11 shows a kind of responses-time, it indicate stability and regularity between the supply of gel bead to $S_2O_8^{2-}$ with stable equilibrium mode of diffusion process inside gel bead.

Table 5-Repeatability of PM-HCL results obtained for the formation of colored species from PM-HCL - $S_2O_8^{2-}$ system.

[PM-HCL] mmol.L^{-1}	no. of injection	Average transducer energy response expressed as peak heights i (mV)	RSD %	Confidence interval of the average response 95 % confidence i (mV)±$t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$
8	10	708..93	0.478	708.93 ± 2.42

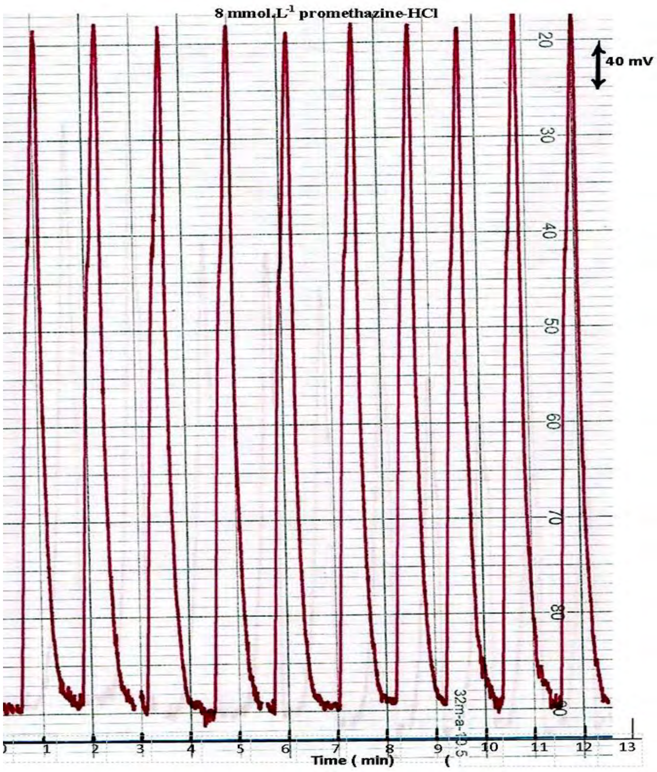


Figure 11-A profile of ten successive measurements of PM-HCL

Application of the adopted methodology for the assay PM - HCL in pharmaceutical drug

For the insurance of the efficient working of gel beads for its action in supplying **$S_2O_8^{2-}$** . A 25 mg of active PM-HCL in histazine (Jordan) was tested via the use of standard addition method . Which was applied by preparing a series of solutions from pharmaceutical drug (Quoted value-25 mg),via transferring 5 ml, (5 mmol.L^{-1}) to five volumetric flask (25 ml) , followed by the addition of (0 , 0.5 , 1, 1. 5 , 2 , and 2.5) from standard solution of PM-HCL (10 mmol.L^{-1}) in order to have the concentration range from 0 – 1 mmol.L^{-1} , for the preparation of standard additions calibration plot. All the six solutions were measured using linear array Ayah $3S_{BGR}X3$-3D Solar cell CFTA microphotometer .The obtained results were summed up in table 6 . A comparison was made using t-test [19] as shown in table 7, on the assumption of :

(Null Hypothesis) H_o: $\mu_{\text{assayed Quoted value}} = \mu_{\text{Ayah3Sx3-3D}}$

There is no significant difference between the means of the determination using Ayah $3S_{BGR}X3$-3D Solar cell CFTA microphotometer with assayed Quoted value

Against

(Alternative Hypothesis) H_1: $\mu_{\text{assayed Quoted value}} \neq \mu_{\text{Ayah3Sx3-3D}}$

there is a significant difference between the mean of the determination using Ayah $3S_{BGR}X3$-3D Solar cell CFTA microphotometer compared to assayed Quoted value .

$t_{\text{calculate}} = 0.22$ while the critical $t_{\text{value}} = 4.303$ at $\alpha = 0.05$ (95% confidence level)

Since $t_{\text{Cal.}} = 0.22 << t_{\text{tab.}}(4.303)$.

Therefore, H_1 is rejected in favour for the) H_o , which indicate that there is no significant difference between the means of the two method . Therefore , the adopted methodology in using the new method can be regarded as an alternative analysis method .

Table 6-Results for the determination of PM- HCL in pharmaceutical drug using proposed method.

Commercial name, content and country	Confidence interval for the average weight at 95% $\bar{w} \pm 1.96\frac{\sigma_{n-1}}{\sqrt{n}}$ (g)	Sample weight equivalent to 160.45 mg , (5 $mmol.L^{-1}$) of active ingredient (g)	confidence interval for the theoretical content of active ingredient at 95% n=∞ (mg)	Equation of standard addition curve at 95% for n-2 $\hat{Y}_{(mV)}=a \pm s_a t + b \pm s_b t[x]$ r , r^2%	Practical content of active ingredient at 95% n=∞ (mg)	Recovery %
Histazine 25 mg United pharmaceutical Jordanie (Jordan)	0.1298 ± 0.0012	0.8331	25 ± 0.1375	73.09±11.42+72.14 ±7.05[x] 0.9895 , 0.9791 %	25.24 ± 0.593	100.99%

$\hat{Y}i$ = estimated value for energy transducer response (mV), $t_{0.025,\infty}$ = 1.96 at 95 %,
X: [PM-HCL] $mmol.L^{-1}$.

Table 7-t-test results for proposed method with official method [20] for determination of PM-HCl in pharmaceutical preparation.

Practical content ($\bar{x}$) mg	Official method 'Quoted value (μ)	σ_{n-1}	n	$\lvert t_{cal.} \rvert = \frac{(\bar{x}-\mu)\sqrt{n}}{S}$	**t_{tab} at 95%, n-1**
25.24 mg	25 mg	1.88	3	0.22<< 4.303	

$t_{critical} = t_{tab} = t_{\propto/2,\, n-1} = t_{0.05/2} = 4.303$

Conclusion:

Through the work that has been conducted , it can be clearly noticed that a clear trust of liable determination can be processed through the methodology adopted in this research work . Repeatable measurement can be made easily giving a complete confidence in the assay method . Therefore the adopted method can be regarded as an alternative simple method for the determination of PM-HCL.

References:

1. Martindale, **1993**. The *extra pharmacopoeia*, 30th Ed., pharmaceutical press, London, pp. 1194-1197.
2. Sethi,P.D., **1997** *Quantitative analysis of drugs in pharmaceutical formulations* ,3rd.Ed.,CBS publishers and distributors.New Delhi .
3. Walash, M. I., Belal, F.and Aly, F. A., **1988**, Evaluation of certain pharmaceuticals with hexa-ammine cobalt (III) tricarbonato cobaltate(III), *Talant*, 35(4), 320-322.
4. Gowda, H. S., Ramappa, P. G. and Nayak, A. N., **1979**, Rapid spectrophotometric determination of phenothiazines with molybdophosphoric acid, *Analytical Chimica Acta*, 108, 277-283.
5. Basavaiah, K., **2004**, Indirect spectrophotometric determination of some biological important phenothiazines using potassium dichromate, iron(II) and 1,10-phenanthroline, *Indian Journal of Chemical Technology* , 11, 632-638.
6. Saif, M. J. and Anwar, J., **2005**, A new spectrophotometric method for the determination of promethazine-HCl from pure and pharmaceutical preparations, *Talanta,* 67, 869-872.
7. Calatayud , J. M and Mateo, V. G., **1992**, Cerium(IV) arsenite as a solid phase reactor for use in flow-injection analysis. Spectrophotometric determination of promethazine, *Analytical. Chimica. Acta,* 264, 283-289.
8. Revanasiddaappa, H. D. and Ramappa, P. G., **1996**, Spectrophotometric determination of some phenothiazine drugs, *Talanta*, 43, 1291-1296.

9. Calatayud, J. M. and Sancho, T. G., **1992**, Spectrophotometric determination of promethazine by flow injection analysis and oxidation by Ce(IV), *Journal of pharmaceutical and Biomedical Analysis* 10(1), 37-42.

10. Daniel, D. and Rolfgutz,I.G., **2003**, Flow injection spectroelectroanalytical method for the determination of promethazine-HCl in pharmaceutical preparations, *Analytical. Chimica. Acta*, 494, 215-224.

11. Alwarthan, A.A., Al-Tamrah, S.A. and Akel, A.A. ,**1993**,Determination of promethazine by it inhibition of chemiluminescence of the luminol-hydrogen peroxide-chromium(III) system, *Analytica Chimica. Acta*, 282,169-174.

12. Sultan, S. M., Hassan Y. A. M. and Abulkibash A. M., **2003**, Chemiluminescence assay of promethazine-HCl using acidic permanganate employing flow injection mode operated with syringe and peristaltic pumps, *Talanta*, 59, 1073-1080.

13. Zhang, Q., Zhan, X., Li, C., Lin T., Li L., Yin X., He N. and Shi, Y., **2005**, determination of promethazine-HCl and its preparations by highly accurate nephelometric titration, *International Journal of Pharmaceutics,* 302, 10-17.

14. Calatayud, J. M., Sarrion, S. N., Sampedro, A. S.and Benito, C. G., **1992**, Determination of promethazine-HCl with bromophenol blue by a turbidimetric method and flow injection analysis, *Microchemical Journal*, 45,129-136.

15. Czerniak, A. S., Dembinski, B. and Kurzawa, M., **2001**, spectrofluorometric determination of 2- and, 10-disubstituted phenothiazines, *Acta Poloniae Pharmaceutica-Drug research*, 58(4), 235-240.

16. Issam, M.A.S. and Nagam, S.T., **2012**, Microphotometer Ayah 3SX3-3D- Solar cell, *patent*, present to central organization for standardization and quality control ,Baghdad - Iraq , NO.3424, G01N29/00.

17. Smith, J. G.,**2006**, *Organic chemistry* , ,1 st Ed., McGraw Hill Companies ,New York ,898.

18. Issam, M.A.S.,and,Nagam, S.T., **2013**, Flow injection analysis for the photometric determination of PM-HCL in pure and pharmaceutical preparation via oxidation by persulphate using Ayah 3SX3-3D Solar micro photometer ,*Journal of Baghdad for science* ,10(4),pp: 1-7.

19. Miller, J. C.and Miller, J. N., **2010**, "*Statistics and chemometrics for analytical chemistry*", 6th Ed., pearson education limited, England.

20. *The Brithish pharmacopoeia*, **1993**, Her majesty's stationary office, London, 1902.