Sys Rev Pharm 2020;11(10):85-93
A multifaceted review journal in the field of pharmacy

Determination of Cyproheptadine via Diverged(0-90°) Scattered Light Emitted through Irradiation of Low-Pressure Mercury Lamp and Two Solar Cells Detection Using CFIA

Aktham N. Jasim*[1], Nagham Shakir Turkey Al-Awadi[2]

[1]College of Basic Education, University of Mustansiryah /Iraq
[2]Chemistry Department-College of Science. University of Baghdad-Baghdad/Iraq
Email: ms.bio2012@yahoo.com

ABSTRACT

A newly developed analytical method characterized by its speed and sensitivity for the determination of cyproheptadine HCl for cyproheptadine - CdI_2 - H_2O system via continuous flow injection analysis. The method was based on the precipitation of cyproheptadine. HCl with CdI2 to form a white precipitate particle (ion pair), using homemade ISNAG- fluorimeter analyser. Optimum parameter has been studied to increase the sensitivity for newly developed method. Linear dynamic range for the precipitation reaction versus cyproheptadine. HCl concentration was 0.05-20 mmol. L-1 while Linearity was 99.82%. The L.O.Q was 5.679 µg/sample. L.O.D. (S/N=3) = 175.45 ng/sample from the stepwise dilution for the minimum concentration of lowest concentration in the linear dynamic ranged of the calibration graph. R.S.D.% for n=8 (8 & 14 mmol. L^{-1}) was <1%. the method was applied successfully for the determination of cyproheptadine. HCl in three different drugs. A comparison was made between the newly developed method and UV-spectrophotometry as a classical method using paired t-test ,it was shown that there were no significant difference between two methods at 95% confidence level and on that basis the new method can be accepted as an alternative analytical method for the determination of cyproheptadine HCl in different drugs and different samples .

Keywords: Solar cell, CFIA, diverged light, low Hg lamp, cyproheptadine

Correspondence:
Aktham N. Jasim
[1]College of Basic Education, University of Mustansiryah /Iraq
Email: ms.bio2012@yahoo.com

INTRODUCTION

Cyproheptadine hydrochloride 4-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride , is a white to slightly yellowish crystalline solid, with a molecular weight of 350.89, which is soluble in water to the extent of about 4 mg per ml, freely soluble in methanol, sparingly soluble in ethanol, soluble in chloroform, and practically insoluble in ether . It is an antihistamine drug, antagonist of histamine and serotonin with appetite stimulating effect and historically used as prophylactic treatment for migraine. The effects may have some benefit in children with abdominal migraines over a short period. The major reported side effects of cyproheptadine are increased appetite and weight gain, sedation and sleepiness. A β-cell exhibiting ultrastructural changes resulting from two weeks of cyproheptadine treatment with making the effects specific for insulin-producing cell with continued of treatment [1-4]. Several analytical technique have been reported determination of cyproheptadine hydrochloride in pure form and in pharmaceutical formulations such as HPLC[5-7], spectrometry[8-11], flow injection[12,13] , chemiluminescence [14] , LC-MS[15].

Experimental

Chemicals:

All chemicals were use of analytical-reagent and distilled water was used to prepare all the solutions. A standard solution 80mmol/L of cyproheptadine. HCl (molecular formula $C_{21}H_{22}Cl$ N. 1.5 H_2O, molecular weight 350.90 g/mole, SDI-Iraq)was prepared by dissolving 2.8072 g in 100 ml of methanol. A stock solution (150mmol/L)of cadmium iodide) molecular formula of CdI_2, molar mass 366.22 g/mol and Merck-USA) was prepared by dissolving 27.4665 g in 500 ml of distilled water. A 1mol.L^{-1} of sulfuric acid solution (96% w/w, 1.84 g.ml^{-1}, BDH) was prepared by pipetting 14 ml of concentrated sulfuric acid to 250 ml distilled water. A 1 mol. L^{-1} of hydrochloric acid solution (35% w/w, 1.19 g.ml-1, BDH) were prepared by pipetting 22 ml of concentrated hydrochloric acid and completed to 250 mL distilled water. A 1mol.L-1 of nitric acid solution (70%, 1. 42g.ml-1, BDH) was prepared by pipetting 16 ml of concentrated nitric acid and completed to 250 ml. A1mol.L^{-1} of acetic acid solution (99.5%. 1.05g.ml 1, BDH) was prepared by pipetting 15 ml of concentrated acetic acid and completed to 250 ml. Each acid was standardized against standard solution of 1mol.L^{-1} from Na_2CO_3.

Sample preparation

Twenty-five tablets were weighted, crushed and grinded. The tablets containing 4 mg of cyproheptadine hydrochloride for (IPI- Periahist, Medico – Citadine, Dankos- Nebor) were weighted i.e. (2.193, 4.158, 4.456 g) equivalent to 70.18 mg of active ingredient 4 mmol. L^{-1} respectively. The powder was dissolved in methanol followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 50 mL with methanol.

Apparatus

Low pressure mercury lamp is used in ISNAG-fluorimeter (16-18), which is characterized by two lambdas (184.9 & 253.7) nm. While the detector that is been used a 2[4 x 2.5cm] (25mm (length), 14mm (width) and 1.2mm(depth)) solar cell as a detector for collecting signal via sample travel through a line of 2mm optical

openture extended for 100mm distance at 410- 1150 nm detection range. The flow system used to determination of cyproheptadine. HCl is shown schematically in figure 2. Peristaltic pump two channels variable speed (Ismatec, Switzerland). Valve 6 – port medium pressure injection valve (I D E X corporation, USA) with sample loop (1mm i.d. Teflon, variable length). The output signals were recorded by potentiometric recorder (Siemens, Germany) (1- 5 Volt,1000-5000 mV). Peak height was measured for each signal. UV spectra were measured with an UV-Vis. (CARY 100 conc) spectrophotometer (Japan).

METHODOLOGY

Fig.1(a) represent a schematic flowgram which shows two lines in manifold system for determination of cyproheptadine hydrochloride by the reaction between cyproheptadine. HCl with cadmium iodide(0.07mmol/L) in aqueous medium to form a white precipitate. The first line is the distilled water (carrier stream) at 1.5mL.min^{-1} flow rate which leads to the injection valve to carry CPH-HCl sample (100µL) while second line supplied with CdI_2 which then passes through reaction coil (250µL) to complete the formation of white precipitate particles and then to ISNAG-fluorimeter to measure diverged scattered light. A proposed mechanism (19,20) for CPH-HCl-CdI_2 system is represented in Fig.1 (b).

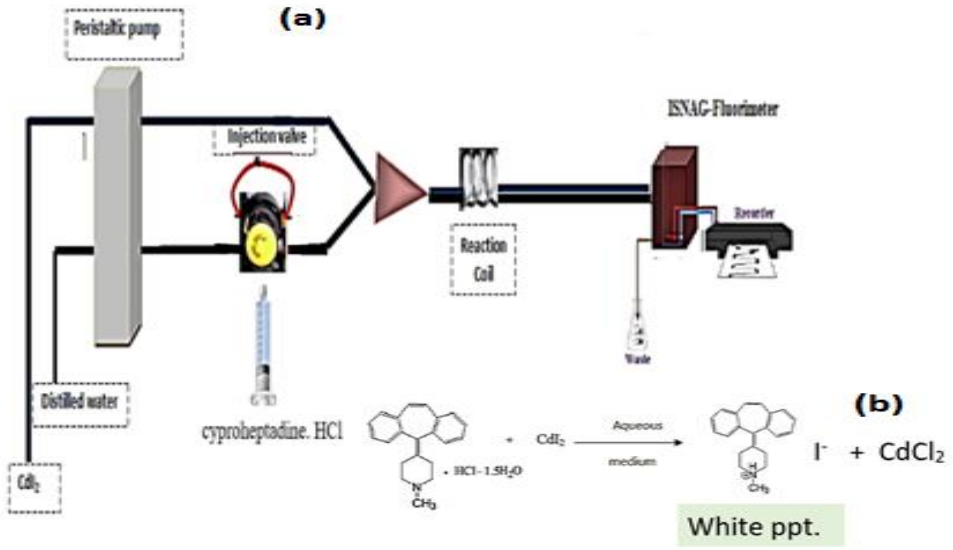


Figure 1: a Flow diagram manifold system used for the determination of cyproheptadine. HCl,) b**)** Proposed mechanism for CPH-HCl-CdI_2 system for determination of CPH-HCl

RESULTS AND DISCUSSION

Concentration of CdI_2

At selected concentration of cyproheptadine. HCl)8 mmol/L, 75µL) was chosen with variable concentrations of precipitating reagent (0.01-0.3mmol/L) at flow rate 1.3mL.min^{-1}. The results obtained are summarized in table1 and shows 0.07mmol/L of CdI_2 was the most suitable for the formation of white crystalline particulates , >0.07mmol/L is a decrease in response (fig.2) which might attributed to irregular formation of precipitate particulates reaction product causing distorted profile .

Table 1: Effect of variable concentration [CdI_2] on response obtained in mV

[Cd I_2] mmol/L	Y zi(mV)(n=3) average response	$Y_{Zi(mV)}$ **± tSEM**
0.01	213	213 **±** 1.52
0.03	258	258 ±2.25
0.05	298	298 ± 3.61
0.07	450	450 ±3.73
0.1	455	455 ±3.58
0.3	453	453 ±1.45

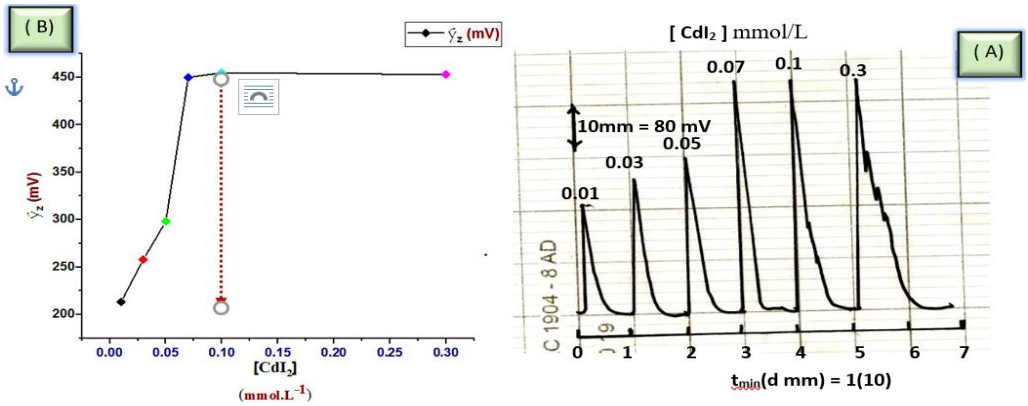


Figure 2: Effect variable concentration of CdI_2 on: **A)** profile vs. time 1(10) **B)** output of response of precipitate particulates for cyproheptadine -CdI_2 - H_2O system

Effect of acid medium

Different acidic medium (HCl, HNO_3, $H2SO_4$, CH_3COOH (0.01mol/L with flow rate1.3mL.min^{-1}) in addition to distilled water) was studied to form of white precipitate particles for cyproheptadine. HCl)8 mmol/L)-CdI_2(0.07mmol/L,75µL) system. The results obtained (Table2) shows the best response recorded was using aqueous medium (H_2O) , while all types of acids used caused a decrease height of response , therefore aqueous medium was regarded as the optimum medium used to obtain an acceptable good response of good S/N for the next studies.

Table 2: Effect of acidity medium as a carrier stream on response obtained(mV) for determination of CPH-HCl

Type of acid media [0.01 mol/l]	Y zi(mV)(n=3) average response	$Y_{Zi(mV)}$± tSEM
H2O	450	450 ± 2.35
HCl	388	388 ±3.45
HNO_3	258	258 ± 3.01
H_2SO_4	411	411 ±3.67
CH_3COOH	432	432 ±3.15

Flow rate

Variation of flow rates (0.5-2mL.min^{-1}) for each line were used for cyproheptadine hydrochloride(8mmol/L)-cadmium iodide (0.07mmol/L,75µL) system. Table 3 sums up the obtained results. Fig.3 shows that the response intensity increases with increasing flow rate and increase peak base width up to 1.5mL.min^{-1}, followed by decrease of peak height which attributed to dilute and dispersion effect. Maximum sensitivity was obtained at 1.5 ml. min^{-1} flow rate of each line that can be supplied to give a better reproducible outcome. So, the best flow rate which gives regular response was 1.5mL.min^{-1}.

Table 3: Effect of flow rate on the variation of diverged light response (mV)

Flow rate (mL.min-1)	Y zi(mV)(n=3) average response	$Y_{Zi(mV)}$± tSEM	Δt_B (sec)
0.5	288	288 ± 3.02	60
0.9	310	310 ±2.32	50
1.2	379	379 ± 2.43	30
1.3	450	450 ±2.73	25
1.5	489	489 ±2.21	20
1.7	476	476±3.33	18
2	398	**398**±4.02	18

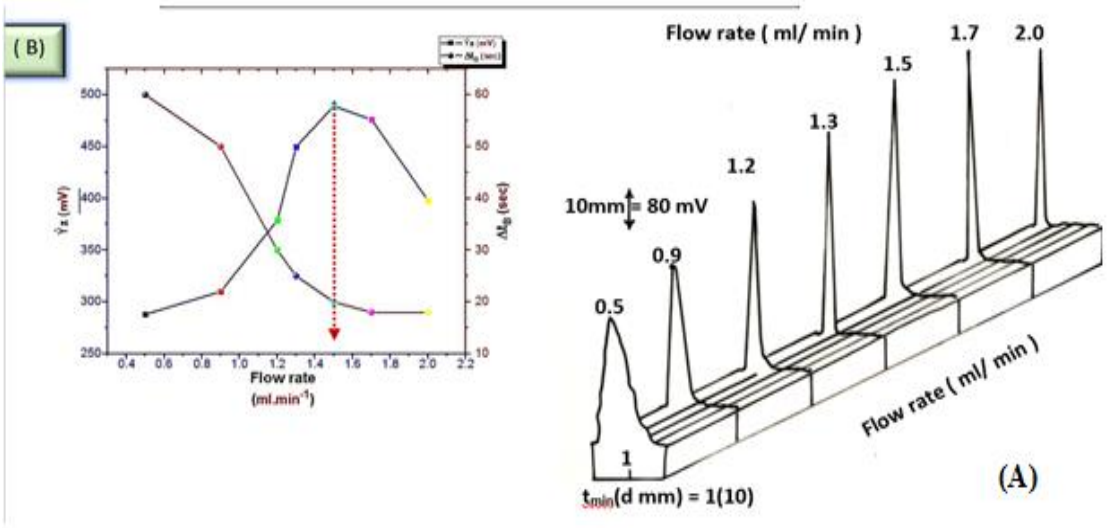


Figure 3: Variation of flow rate on A**)** Profile (80 mV/ 10mm vs. 1(10) plot **B)** response obtained in (mV), peak base width

Sample volume

In order to establish the optimum sample loop, variable sample volume (25-200µL) at cyproheptadine. HCl (8mmol/L)-CdI_2(0.07mmol/L) system, open valve mode, flow rate 1.5mL.min^{-1} were used. All results obtained is shown in fig.4 and Table 4. It was noticed that at an increase in sample volume led to an increase in the response obtained with increase ΔtB (base width) in addition to increase of the particle size causing a slow movement of particles. Therefore, 100µL was chosen as optimum sample volume.

Table 4: Variation of sample volume on the response obtained (mV).

Sample volume (µL)	$Y_{Zi(mV)}$(n=3) average response	$Y_{Zi(mV)}$±tSEM	Δt_B (sec)
25	297	297±2.73	15
50	387	387±2.98	15
75	489	489±3.03	20
100	558	558±2.79	28
200	570	570±3.78	40

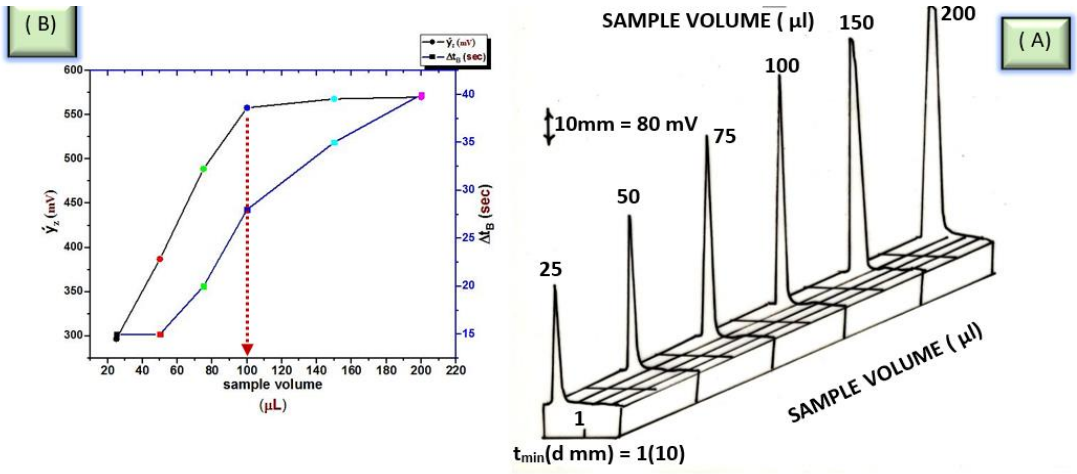


Figure 4: Variation of sample volume on **A)** Profile (80 mV/ 10mm vs. 1(10) plot **B)** response obtained (mV) and peak base width via the use of cyproheptadine. HCl (8mmol/L)-CdI2(0.07mmol/L) system

Reaction coil

Using optimum parameters that were achieved in the previous sections, different coil volumes (0-400µL) were studied. Reaction coil which connected after Y-junction in manifold system. Table 5 and Fig.5 shows that the best reaction coil necessary for completion the formation of white precipitate particles from the reaction between cyproheptadine. HCl and CdI_2 is 250µL. It can be seen that an increase in length of reaction coil causes an accumulation and compactness of the precipitated particles causing obstruction of the scattered light which led to decrease of peak height.

Table 5: Variation of reaction coil volume(µL) on the response obtained(mV)

Coil volume (mL)	Y zi(mV)(n=3) average response	$Y_{Zi(mV)}$± tSEM
0	560	560 ± 2.78
250	643	643 ±3.45
300	587	586 ± 3.09
400	510	510 ±4.98

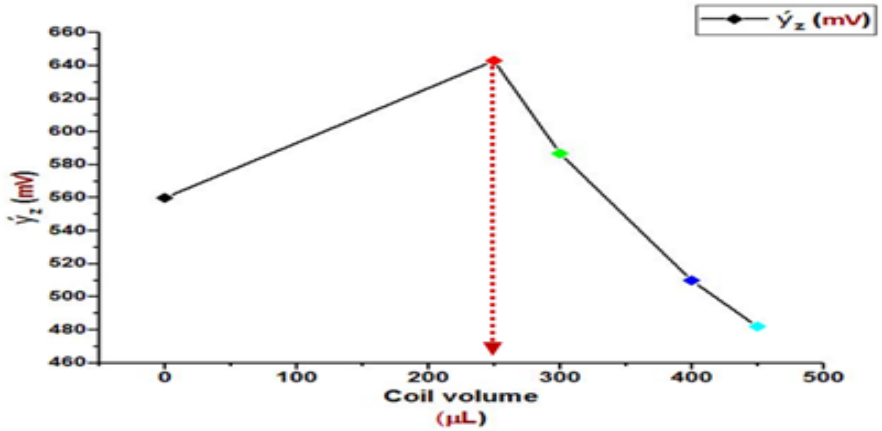


Figure 5: Effect of reaction coil on response obtained from scattered light in mV.

Scatter plot for the variation of S/N – response of the energy transducer response

(S/N) versus concentration of cyproheptadine. HCl using the system of: cyproheptadine. HCl - CdI2(0.07mmol/L) in aqueous medium

Using the established optimum chemical and physical parameters, the calibration curves of continuous flow injection analysis of scatter light measurements were constructed. A series of variable concentration solution (0.05- 25 mmol/L) were prepared. It was noticed that at CFIA method, the energy transducer response ISNAG-fluorimeter via low pressure mercury lamp with cyproheptadine. HCl concentration was ranging from solution (0.05- 20 mmol/L) with correlation coefficient (r): 0.9991, with a capital squared- R of 99.82%. This indicate that the linear equation. chosen:

ŷ Zi (mV)= (a± Sat) + (b± Sbt) [cyproheptadine. HCl] mmol/L

ŷ Zi (mV)= (66.667 ±3.547) + (76.244 ±3.453) [cyproheptadine. HCl]

for n= 13 and confidence level 95%. The first-degree equation of the form ŷ=a+bx (21-23) at optimum conditions was able to explain this much of the obtained results. More than 20 mmol/L a shift from linearity cause a much more intensification caused by the effect of agglomerate formation which form in this short period of time a relatively more intensified massive precipitate which in turn to decrease the diverged light that was received by the ISNAG detector at 0-90° .

The new development methodology were compared with classical methods of determination using Spectrophotometric method based on the measurements of absorbance for the range of concentration (0.5- 50 mmol/L) at max wavelength (λmax =285nm) (9) using quartz cell . , the best linear range extend from 0.05- 10 mmol/L with correlation coefficient of 0.9989 and capital square-R = 99.79%, n= 10 (no. of measurement).for a linear regression equation of the form of :

ŷ Zi = (0.074 ±0.002) + (0.190 ±0.012) [cyproheptadine. HCl] mmol/L

It can be clearly noticed that the new adopted methodology satisfies both the use of low as well as high concentration with high precision and repeatability with minimum of the relative standard deviation.

It can be found that the limit of detection of the newly developed methodology of cyproheptadine. HCl, (practically) based on the gradual dilution for the minimum concentration of the calibration graph at injection sample volume of 100 µl was 175.45 ng/sample (using successive dilution) & L.O.Q was 5.679 µg/sample , while the classical method was 35.09 µg/sample . The repeatability and the trustability for 8, 14mmol/L were

RSD% < 1% while classical method < 2% Assessment of the use of CdI_2 as a precipitating agent and low pressure mercury lamp; via the use of ISNAG continue flow fluorimeter for the determination of CPH in the drugs

Three different companies of pharmaceutical preparations (Periahist – Iraq (4mg), Citadine – India (4mg), Nebore – Indonesia, (4mg) were analysis for the determination of cyproheptadine. HCl by continuous flow injection analysis via diverged incident of light in the presence of low pressure Hg lamp that used in ISNAG - fluorimeter achieved in this work and was compared with reference method which in dudes UV-spectrophotometric via the measurement of absorbance at λmax = 285nm by UV-Vis. (CARY 100 conc) spectrophotometer. A series of solutions were prepared of each drugs (4mmol.L^{-1}) (70.18mg , of active ingredient in 50 mL) by transferring 2.5 ml to each five volumetric flask (10 mL) , followed by the addition of gradual volumes of standard cyproheptadine. HCl of 80 m mol.L^{-1} to obtain (0, 1, 2, 3 , 4) mmol.$L^{-1}$for both method .The measurements were conducted and the results were mathematically treated for the standard addition method .The results were tabulated in Tables 6 and 7 ,at confidence level 95% .Paired t-test was used in order to compare between developed method using ISNAG - continue flow fluorimeter with classical method as shown in scheme 1 , the obtained result indicating clearly there was no significant different between two method at 95% confidence level ,since the calculation t-value less than ttab(4.303) for the determination of cyproheptadine. HCl in different drugs. On this basis two assumption statistically is made (21-23):-

Paired t – test:

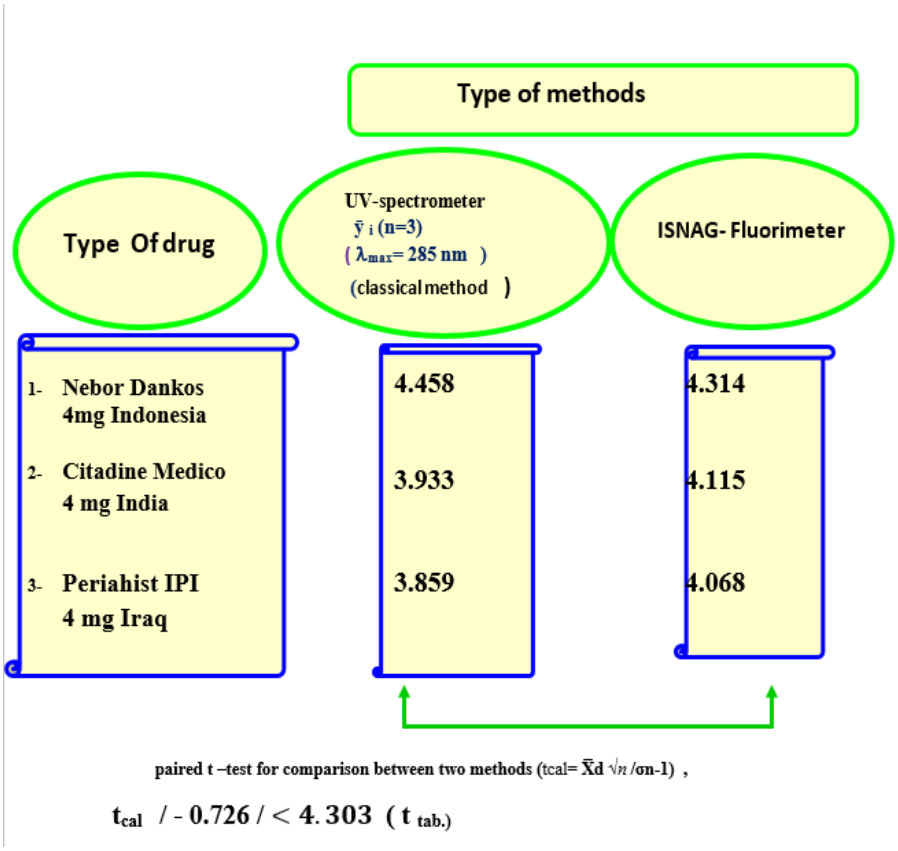
Ho = Null hypothesis = No significant difference between two method:

i.e., μ UV. - SPECTROPHOTOMETRY = μISNAG – Fluorimetry

Against,

The alternative hypothesis H1= a significant difference between two methods:

i.e., μ UV. - SPECTROPHOTOMETRY μISNAG – Fluorimetry



Scheme 1: paired t –test results for energy transducer response (proposed method – ISNAG- Fluorimeter with classical method using UV- spectrometry at 285 nm using standard additions graph for determination of cyproheptadine. HCl in drugs

Table 6: Summary of practical content and efficiency for the determination of cyproheptadine.

[cyproheptadine. HCl] mmol /L					**Equation of standard addition at 95% for n-2** $\hat{Y}_i = a \pm s_a t + b \pm s_b t[x]$ **r, R²%**	**Practical content mmol. L⁻¹ in 10 ml & (50 ml)** **$W_{i\,(}$mg), Rec%**
0.0	**0.125 ml**	**0.25 ml**	**0.375 ml**	**0.5 ml**		
0	**1**	**2**	**3**	**4**		
ȳi for n= 3						
80	**158**	**298**	**360**	**400**	90.80 ±10.11+ 84.20 ± 2.12 [cyproheptadine. HCl] mmol /L 0.9798, 96.00 %	1.078, 4.314 4.314, 107.85%
0.21	**0.39**	**0.63**	**0.79**	**0.97**	0.214 ±0.081+ 0.192 ± 0.012 [cyproheptadine. HCl] mmol /L 0.9981, 99.61 %	1.114, 4.458 4.458, 111.46%
87	**190**	**280**	**360**	**455**	93.20 ±9.32+ 90.60 ± 7.65 [cyproheptadine. HCl] mmol /L 0.9992, 99.84%	1.029, 4.115 4.115, 102.87%
0.18	**0.36**	**0.52**	**0.71**	**0.90**	0.176 ±0.022+ 0.179 ± 0.023 [cyproheptadine. HCl] mmol /L 0.9995, 99.90 %	0.980, 3.933 4.068, 101.71%
93	**210**	**305**	**399**	**497**	101.40 ±3.35+ 99.70 ± 4.65 [cyproheptadine. HCl] mmol /L 0.9991, 99.82%	1.0171, 4.068 3.933, 98.33%
0.18	**0.29**	**0.47**	**0.64**	**0.80**	0.1534 ±0.032+ 0.1590 ± 0.046 [cyproheptadine. HCl] mmol /L 0.9960, 99.21 %	0.9648, 3.859 3.859, 96.48%

$t_{0.025}$, = 1.96 at 95 %, n= no. of sample =3: r: correlation coefficient, r^2: coefficient of determination& R^2%: percentage capital R- square, $t_{0.025,\,3}$= 3.182. For n-2 using standard addition.

Table 7: Summary of practical content for determination of cyproheptadine depending on commercial names.

Commercial name, content and company country	**Confidence interval for the average weight Wi±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95% (g)**	**Sample weight equivalent to 70.180 mg (4mmol.L⁻¹) of the active ingredient(g)**	**Theoretical content of the active ingredient at 95% (mg) μ±1.96 σ_{n-1} /**
Nebor Dankos 4mg Indonesia	0.255 ± 0.0019	4.456	4 0.0299
Citadine Medico 4 mg India	0.237± 0.0018	4.158	4 0.0303
Periahist IPI 4 mg Iraq	0.125± 0.0021	2.193	4 0.0672
Nebor Dankos 4mg Indonesia	0.255 ± 0.0019	4.456	4 0.0299

Supplementary date:
Energy transducer response $\bar{y}_i$ (n=3) in mV
(Newly developed methodology)

$\hat{Y}_i$ (mV)= a±s_at+b±s_bt [cyproheptadine. HCl**] mmol /L**

UV-spectrometer $\bar{y}_i$ (n=3) at
λ_{max}= 285 nm (classical method)
$\hat{Y}_i$ = a±s_at+b±s_bt[cyproheptadine. HCl] mmol /L

CONCLUSION

The present paper introduces an alternative high, simple, rapid, inexpensive & sensitivity flow method for ON – Line determination of cyproheptadine. HCl via precipitation reaction of CdI2 - cyproheptadine. HCl – aqueous medium system. The manipulation is very simple, and sequential measurement was permitted with high sample frequency, up to 60 samples per hour.An improved linearity and detection limit compared with the available literatures cited in the introduction .The %R.S.D was <1% and good agreements 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. The proposed method and the uv-method were applied to the determination of cyproheptadine. HCl in three drugs.

REFERENCES

1. British pharmacopeia. 2012. 7th edition. The stationery effects. London.
2. Feås X, Ye L, Regal P, Fente C and Cepeda A. 2009. Application of dummy molecularly imprinted solid-phase 2009, 32, p1740 – 1747extraction in the analysis of cyproheptadine in bovine urine. Journal of Separation of Science, 32(10), p1740-1747.
3. Gupta R.2012. 2nd edition. Veterinary toxicology: Basic and clinical principles. Academic press, United States. America.
4. Hassan Y and Al-Badr A. 1981. Cyproheptadine. Analytical Profiles of Drug Substances, 9, p.155-179.
5. Gogory W and Cheryl L. 1983. High-performance liquid chromatography determination of cyproheptadine hydrochloride tablet formulations. Journal of Pharmaceutical Sciences, 72(10), p.1212-1213.
6. Alaa E, Fawzy E, Ahmed M and Moustafa M.2004. HPLC and chemometric methods for simultaneous determination of cyproheptadine hydrochloride, multivitamins, and sorbic acid. Journal of Pharmaceutical and Biomedical Analysis, 35(4), p.703-713.
7. Jianwen Y, Zongnan W, Tong Z, Qingyong L, Yuman Z and Limin H.2015. Determination of cyproheptadine in feeds using molecularly imprinted solid-phase extraction coupled with HPLC. Journal of Chromatography B, 990(15), p.39-44.
8. Sayanna T and Venkata R.2015. Determination of cyproheptadine hydrochloride in pure and pharmaceutical forms: A spectrophotometric study. Oriental Journal of Chemistry, 31(3), p.1779-1786.
9. Vinod T, Seethamma M and Venkateshwarla G.2014. Quantitative determination of drugs pharmaceuticals using iodine as analytical reagent: A spectrophotometric study. Journal of Applied Science, 7(5), p.7-15.
10. Basavaiah K and Charan V.2002. The use of chloranilic for spectrophotometric determination of three antihistamines. Turkish Journal of Chemistry,26, p.653-661.
11. Raghu M and Basavaiah K.2012. Senstive and selective methods for the determination of cyproheptadine in tablets using N-bromo succinimide and two dyes. Chemical Industry& Chemical Engineering Quarterly ,18(3), p. 449-458.
12. Nagam S and Kefah H.2015. A new approach for the turbidimetric determination of cyproheptadine hydrochloride in pharmaceutical preparations by using homemade linear array Ayah 6SX1-T-2D solar cell CFI analyser. Journal of Kerbala University, 13(3), p.61-80.
13. Nagam S and Issam M.2015. Novel study by potassium hexacyanoferrate and sodium nitroprusside using atomic force microscopy. Iraqi Journal of Science, 56, p.2745-2761.
14. Liu R and Lu J.2009. Determination of cyproheptadine hydrochloride with riboflavin chemiluminescence system. Chinese Journal of Analytical Chemistry, 37(2), p.267-270.
15. Min L, Eric S and Diana M.2003. LC-MS and NMR dtermination of a dichloromethane artifact adduct, cyproheptadine chloromethochloride. Journal of Pharmaceutical and Biomedical Analysis. 31(1), p.29-38.
16. IRQ. PATENT. No. 3884. Shakir ,I.M.A. and Al-Awadie ,N.S.T., ISNAG Fluorimeter for fluorescence measurement at the visible region of electromagnetic radiation using mercury tube lamp as a source for irradiation for continuous flow analysis using solar cells .2014, patent, International classification HO1J61/00 .
17. Nagham S.T.Al-Awadi & Hayder Q. Munshid , 2019 , New Approach for the determination of ciprofloxacin hydrochloride using fluorescence resonance energy transfer (FRET) and continuous flow injection analysis via ISNAG-fluorimeter ,Journal of pharm. Science and research ,.11(4),pp:1563-1570.
18. Nagham S.T.Al-Awadi & Hussein F. A.2018.Newly developed method for determination indomethacin using phosphotungstic acid by continue flow injection analysis via homemade ISNAG-fluorimeter, Journal of applied chemistry, 11(2),pp:25-39.
19. Amina, M.E.; Mohamed, E.M. and Fawzi, A.E. 2005. Spectrophotometric determination of some fluoroquinolone antibacterials by ion-pair complex

formation with cobalt (II) tetrathiocyanate.Journal of the Chinese chemical society.52, pp:77-84.
20. Vogel, A. I., 1979 "Textbook of Macro and Semimicro Qualitative Inorganic Analysis", 5th Ed., Longman, London.
21. Jaisingh, L. R., 2000, "Statistics for the Utterly Confused", 2nd Ed., McGraw-Hill, London.
22. Bluman, A.G. 1997.Elementary statistics .3rd edition. WCB/MC Graw –Hill, New York
23. J.M. Miller and J.C.Miller, 2005 "Statistical and chemometric for analytical chemistr", 5thed, person education limited