

Sensitive Determination of Hydrochlorothiazide Drug in the Various Samples Via Developed Method of CFIA System

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Abstract

Hydrochlorothiazide drug determination in pharmaceutical and biological urine samples using a novel continuous flow-injection analysis technique that is rapid, simple, economical, and sensitive. The complex formed by the reaction of hydrochlorothiazide and O-phenylenediamine with potassium ferricyanide to produce an orange-colored product at 480 nm was the basis for the proposed method. Other chemical and physical parameters that affect the stability and development of the colored product used in this developed method includes sample volume, flow rate, reagent concentrations, and reaction coil. The proposed method's linearity ranges from 5 to 150 $\mu\text{g}\cdot\text{mL}^{-1}$ and its correlation coefficients were 0.9988, and the recovery percentage and error percentage were 100.88 and 0.88, respectively. The detection limit and Limit of quantification values were 2.622 and 7.97. A sample throughput of 90 samples. hour^{-1} . The new approach was effectively employed to determine the presence of hydrochlorothiazide in the pure, biological, and pharmaceutical samples.

Keywords: Biological samples, CFIA System, Hydrochlorothiazide, O-phenylenediamine, Pharmaceutical formulation.

Introduction

Hydrochlorothiazide has a chemical formula of ($\text{C}_7\text{H}_8\text{ClNO}_4\text{S}_2$), with molar mass of 297.7g/ mol. Its formula structure is shown in Fig. 1

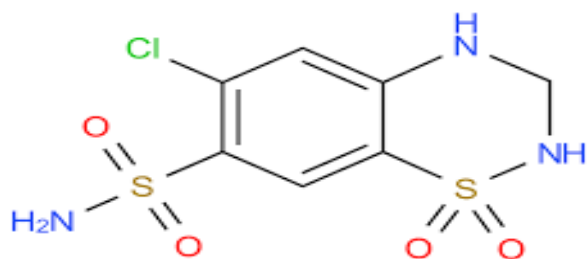


Figure 1. Formula structure of HCTZ¹⁰.

Hydrochlorothiazide is a type of thiazide compound it is used to treat high blood pressure, as well as to treat heart muscle failure. It also reduces blood volume by acting on the kidneys to limit sodium absorption.

Various analytical methods for determining HCTZ using many techniques have been described in the literature, (HPLC) high-performance liquid chromatography¹, (TLC) technique², UV-spectrophotometric³⁻⁵, and electrochemical method^{6,7}. All the methods described above are sensitive but require time-consuming and expensive instrumentation.

In contrast, CFIA/MZ 8,9 techniques were chosen due to their simplicity, cheapness, and sensitivity of semiautomated used for determining the HCTZ pharmaceutical and biological samples. Merging zones technique, A disadvantage of continuous flow injection analysis is the continued consumption of the reagents. If this problem is serious it can be minimized by the introduction of the merging zones technique, which can be achieved by occasional pumping or by multiple injection valves. The suggested FI manifold was made a simple kind using a one-channel manifold in the FIA/merging zones system proposed technique. spectrophotometric drug estimation using the CFIA system was a novel research proposal in this manuscript and was used for the indirect determination of HCTZ by oxidative coupling reaction of [HCTZ] with [OPH] in present $K_3[Fe(CN)_6]$ as oxidizing agent at 480nm

Experimental

Instruments

All absorbance in the batch procedure was measured using a double-beam Shimadzu UV-1800 UV-VIS spectrophotometer with a 1 cm quartz cell. The suggested FI manifold was developed as a simple type with a one-channel manifold in the FIA/merging zones system technique as shown in Fig. 2. The carrier stream distilled water was pumped through

Materials and Methods

Every one of the chemicals and solvents utilized in this work was provided with the analytical grade for this project by the state organization for drug industries and medical equipment in Samara Iraq.

Standard drug solution (M.wt 297.741 g.mol⁻¹): (1000 µg.mL⁻¹)

A stock solution of the drug HCTZ was prepared by taking 0.1g then dissolving it in 10 mL of methanol, then adding 10 mL of concentrated HCL then transferring it to a volumetric flask of 100 mL of distilled water and completing the volume to the mark to prepare a concentration 1000µg.mL⁻¹

Potassium ferricyanide (M.wt 329.24 g.mol⁻¹ SDI): (9 X10⁻³)M

Weighed 0.3g of Potassium ferricyanide then dissolved it in 100 mL of distilled water, prepared fresh daily.

the injection valve seven three-way injection valve, handmade by a peristaltic pump (Master flexC/L, two-channel, USA), which travels at 90° and three Teflon loops (I.d =0.5 mm) into which the sample L1, the oxidizing agent L2, and the reagent L3 were loaded. The reaction coil is made of glass; and used to mix the ingredients (2 mm, I.D.). The modified Optima photometer 301-D+ (VIS-Spectro, single beam) (Japan) was used for all absorbance and spectral measurements during the FIA procedures. The responses (as peak height) were measured using a Kompensograph C1032 (Siemens) or an optical multimeter absorption (DT9205A, OVA, China) for the absorbance measurements. A flow cell quartz silica (1 cm) with an internal volume of 80 µL is used in the detection unit.

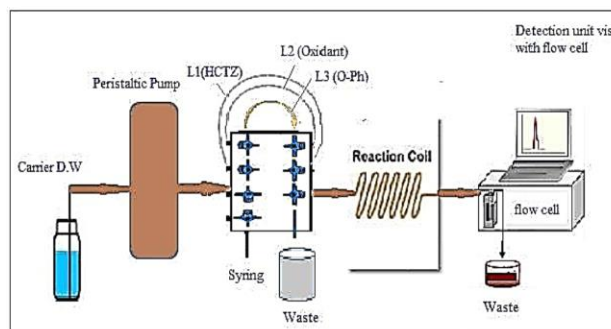


Figure 2. The manifold of CFIA

O-phenylenediamine (M.wt 108.1g.mol⁻¹ SDI): (9.2 X10⁻³)M

Weighed 0.1g of O-phenylenediamine then dissolve in it (100 ml) of ethanol. dilute solutions are prepared by using a standard solution with distilled water.

Preparation of interferences

Dissolving 0.1 g from any one of the interferences including glucose, sodium citrate, cellulose, lactose, and sucrose in 100 mL of distilled water by using a 100 mL standard volumetric flask.

Preparation of pharmaceutical

The standard solutions of pharmaceuticals are prepared by weighing 20 tablets of every three types of companies' drugs.

1. Diuzid (Safa/Iraqi) (50) mg.
2. Actavis (Iran) (50) mg.
3. Esidrex (French) (25) mg.

An average of one tablet weighing (25,50 mg) of HTCZ was accurately weighed and finely crushed. Each weight that was taken in the previous operation was treated as pure material. Additional solutions were diluted to get the concentration inside the linearity of the calibration graph. Serial dilution can be employed to make $100 \mu\text{g. mL}^{-1}$ of the other solution types, and the proposed method is then utilized to quantitatively quantify $10, 15 \mu\text{g. mL}^{-1}$.

Results and Discussion

Batch method Absorption spectra and Chemistry of reaction

Spectrophotometric determination based on oxidative 1mL ($100 \mu\text{g. mL}^{-1}$) of the HCTZ drug with 1mL ($9.2 \times 10^{-3}\text{M}$) of O-Ph reagent in the presence of 1mL ($9 \times 10^{-3}\text{M}$) of the oxidizing agent $\text{K}_3[\text{Fe}(\text{CN})_6]$ and then added to a volumetric flask of 10 mL and the volume completed with distilled water. The appearance of an orange-colored product at $\lambda_{\text{max}} 480\text{ nm}$, against reagent blank is shown in Fig. 3 and scheme 1.

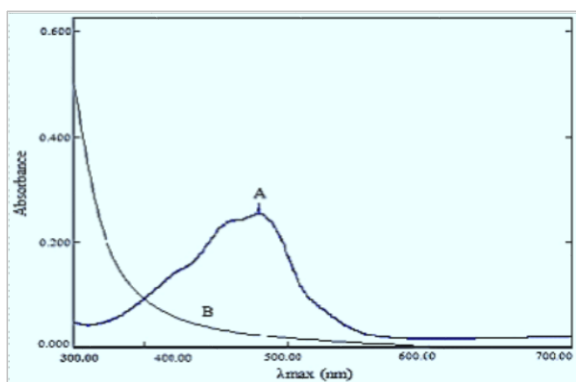
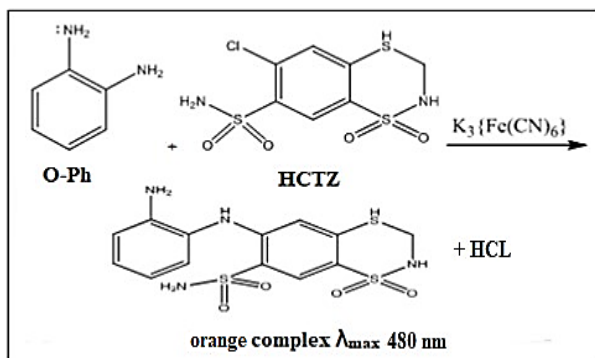


Figure 3. Absorption spectrum of the reaction A\HCTZ ($10\mu\text{g. mL}^{-1}$) B\ blank



Scheme 1. The pathway of reaction for HCTZ and O-Ph.

Biological preparation (Urine)

The samples were taken from different healthy people examined and then centrifuged at 3000 rpm then adding 5 drops of HClO_4 acid were to precipitate the protein and then stored at $20\text{ }^\circ\text{C}$ until use after gentle thawing. For urine sample preparation a volume of 1 mL was converted to a volumetric flask of 10 mL and spiked with 0.1 mL of standard solution $100 \mu\text{g. mL}^{-1}$ and diluted with distilled water.

Preliminary investigation

Effect of O-phenylenediamine concentration

Through the use of different volumes of the reagent O-phenylenediamine, a volume of 2 mL from ($9.2 \times 10^{-3}\text{ M}$) for O-Ph was the best concentration which has the highest responses the absorbance increases with volume 2 mL then decreased, as shown in Fig. 4-A, while the best volume of $\text{K}_3[\text{Fe}(\text{CN})_6]$ was 4 mL of ($9 \times 10^{-3}\text{ M}$) and this volume was selected for subsequent experiences, as shown in Fig. 4-B.

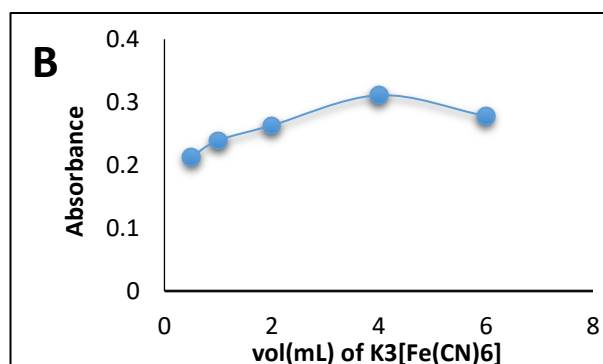
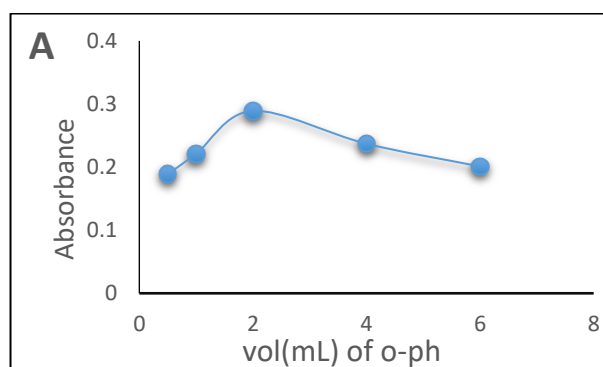


Figure 4. Effect A/ volume of O-Ph, B/ volume of $\text{K}_3[\text{Fe}(\text{CN})_6]$

Calibration curve

After ideal conditions utilizing several concentrations (2-60) $\mu\text{g.mL}^{-1}$ of HCTZ were obtained by diluting the standard solution. The reaction mixture evaluated the maximum absorbance of the orange-colored result at 480 nm in comparison to the reagent blank as shown in Fig. 5. At this range ($>60 \mu\text{g.mL}^{-1}$), Beer's law was not followed, resulting in a negative curvature toward the concentration axis (not shown in Figure).

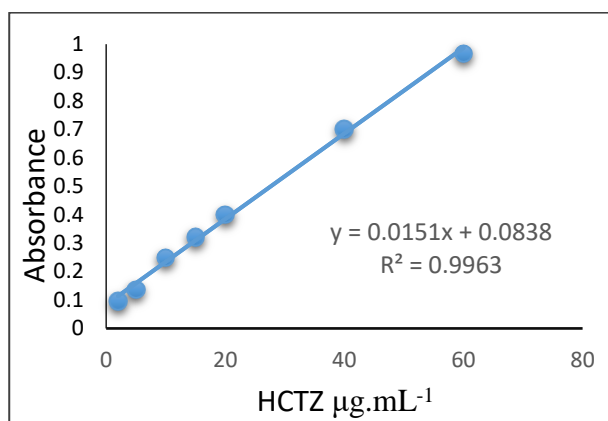


Figure 5. Linear calibration curve of HCTZ - OPh using spectrophotometric method

Study of the Stoichiometry

To investigate the stoichiometric ratio of drug to reagent applied molar ratio method¹⁰ and continuous variation¹¹⁻¹³ by using an equal concentration of HCTZ (3×10^{-2} M) and O-Ph by using increased volumes of O-Ph and added to 1mL of HCTZ drug. The study found that the hydrochlorothiazide to coupling reagent ratio was 1:1. as shown in Fig. 6 A-B.

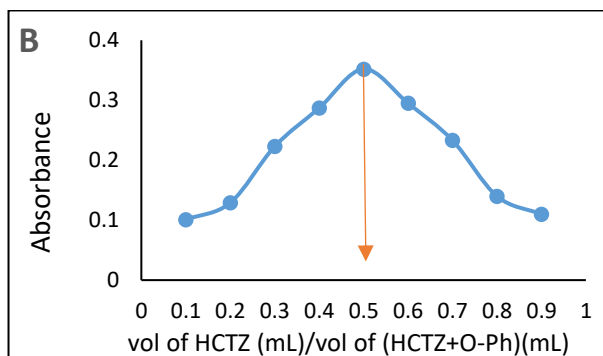
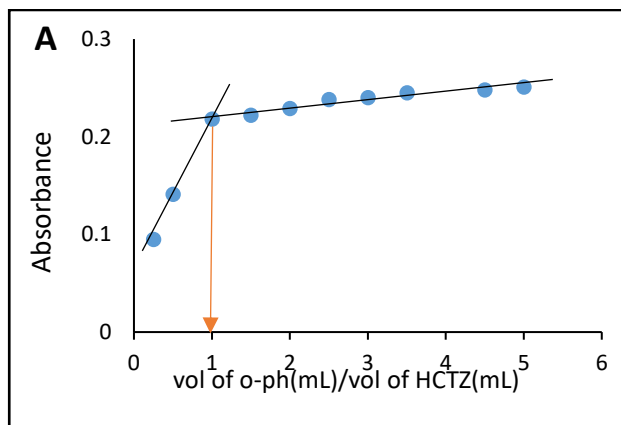


Figure 6. Stoichiometric ratio of drug to the reagent (a) Mole ratio (b) Continuous variation

Precision and Accuracy

The accuracy and precision of the suggested method have been verified by measuring the (RSD) relative standard deviation proposed and (RE) relative error values as shown in Table 1 which shows good results for accuracy and precision.

Table 1. Precision and accuracy for HCTZ

HCTZ $\mu\text{g.mL}^{-1}$					
Present μ	Found \bar{x}	Error	Rec%	Erel %	RSD %
10	10.12	0.120	101.2	1.200	0.608
20	19.84	0	0	-0.800	1.833
		-	99.20	0.200	1.220
		0.160	100.2		
		0 \bar{x}	00		

Calculations of stability constant

It was found that the suggested interaction (HCTZ: O-PH) has an observed stability constant values. The proposed mechanism and drug-reagent stoichiometry ratio was 1:1 used to evaluate the spontaneous complex formation process through the negative values of Gibbs free energy. as shown in Table 2.

Table 2. Stability constant of the reaction

	Am	As	α	K (Lmol^{-1})	ΔG (J.mol^{-1})
1	0.423	0.413	0.024	11577	-97394
2	0.426	0.415	0.026	81160	-96513
				98466	-96954
Average					

FIA-Spectrophotometric determination

An FIA procedure was developed using the batch method for calculating HCTZ. The estimation manifold used for hydrochlorothiazide was made to

provide a variety of reaction conditions for magnifying the absorbance signal produced by the oxidative reaction of HCTZ with O-Ph in the presence of potassium ferricyanide.

Optimization of chemical and physical parameters

Several experiments were carried out to determine the requirements for producing a reaction product with high sensitivity and maximum repetition response.

Effect of chemical variable

To determine the best concentration of O-phenylenediamine as a reagent was examined by injecting various concentrations (9×10^{-4} – 7×10^{-3}) M of OPh by utilizing a seven-three injection valve. The result is shown in Fig. 7-A. The (3.7×10^{-3}) M of O-Ph was the best concentration because gives the greater value of responses and high repeatability measured as peak height in mV ($n=3$). by injecting several concentrations of the oxidizing agent that the best concentration was (6.5×10^{-3}) M represented as peak height in mV ($n=3$), in the redox reaction between the (HCTZ) drug and the reagent, as shown in Fig. 7-B. The results in Fig. 7-C appear that the best sequence of chemicals is (D in L1 + O in L2 + R in L3) where D is HCTZ, O is $K_3[Fe(CN)_6]$ R is O-Ph.

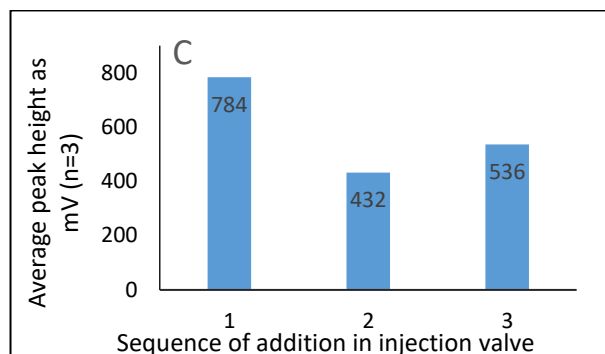
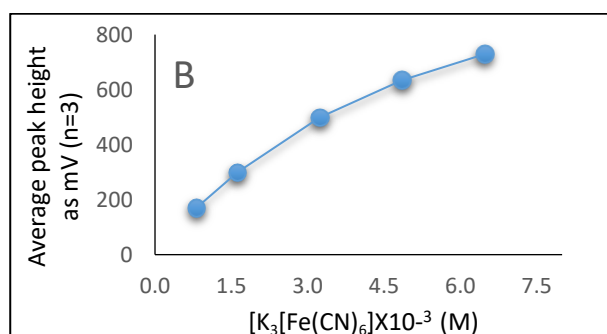
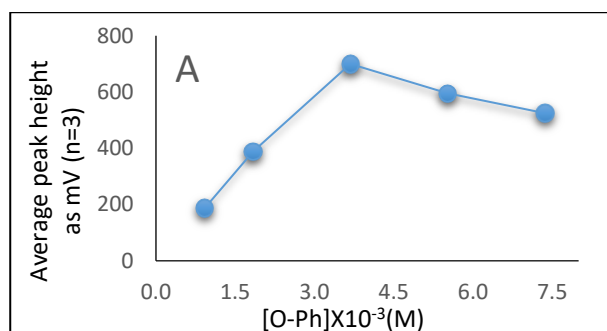


Figure 7. Effect of concentration A\ O-Ph, B\ $K_3[Fe(CN)_6]$, C\ sequence of chemicals.

Physical parameters of the FIA system

Effect of optimum total flow rate

The sampling rate was calculated based on the time needed to load the chemicals into the seven-three-way valve's loops in addition to the time needed for the highest response appearing. used for this system [HCTZ- $K_3[Fe(CN)_6]$ - O-Ph] several flow speeds and it was found that the better speed is $11 \text{ mL} \cdot \text{min}^{-1}$ with a sample through-put 90 sample/h^{-1} . as shown in Fig. 8.

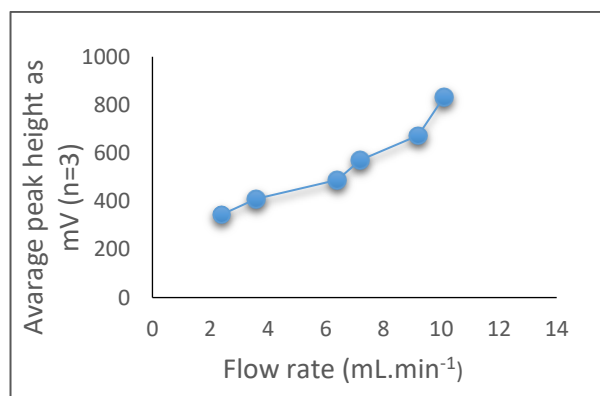


Figure 8. Effect of flow rate in a developed system

The effect of mixing coil

The influence of the length of the reaction coil on the development of the colored product was investigated by utilizing different lengths from the reaction coil (50,100,200) cm. The length of 50 cm provided the best absorption with acceptable repeatability. Absorbance decreased when a coil length greater than 50 cm was used, as shown in Fig. 9.

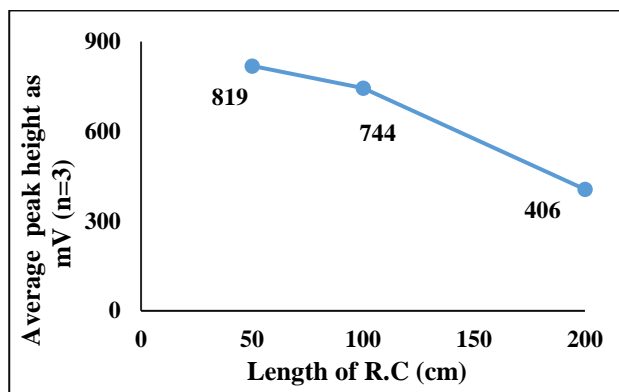


Figure 9: Effect of reaction coil

Effect of injected sample volume

Different volumes of injector loops were examined in this study. The volume of injection drug(L1) was 78.50 μL and the volume of injection reagent (L2) also studied was 78.50 μL and the volume of injection oxidizing agent(L3) was 78.50 μL was used in the next experiments. all the volume above was the best as shown in Fig. 10.

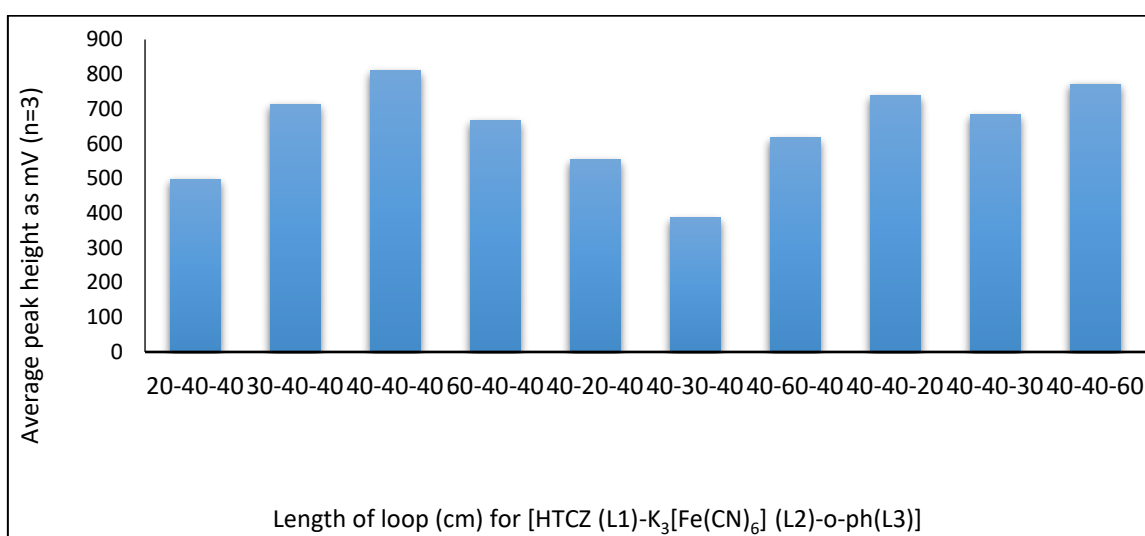


Figure 10. Effect of Injected volume.

Purge time

The Purge time is the amount of time required for all sample segments to be injected via the carrier stream that was examined. The time utilized was from (5-15 sec and open valve mode). The open valve gives the height response in a period needed by the sample segment to transfer the drug from the injection valve to the flow cell, as shown in Fig. 11.

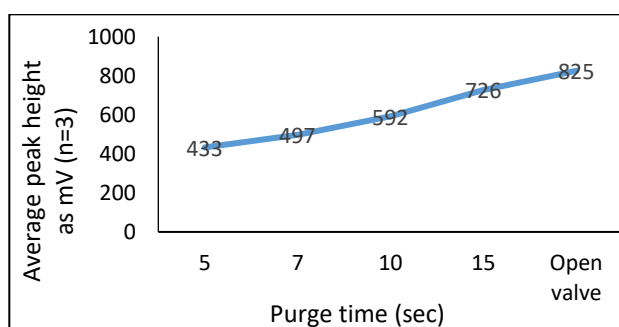


Figure 11. Effect of purge time

Dispersion of zone

The dispersion was calculated using a relationship $=C_0/C_{max}$ (D; dispersion, C_0 the peak without dilution, C the peak after dilution. The dispersion of the HCTZ–OPh reaction was 1.245,1.448 for concentration 10,25. It is illustrated in Fig.12. The results were obtained by conducting two experiments, In the first experiment, all of the components are combined in a suitable beaker, and the resulting solution was then run through the flow injection system (as carrier stream) (C_0). The second experiment comprised injecting HCTZ, $K_3[Fe(CN)_6]$, and O-Ph into L1, L2, and L3, respectively. The system uses distilled water as a carrier to move the components to the reaction coil, where they are subsequently pushed by the component that was injected.

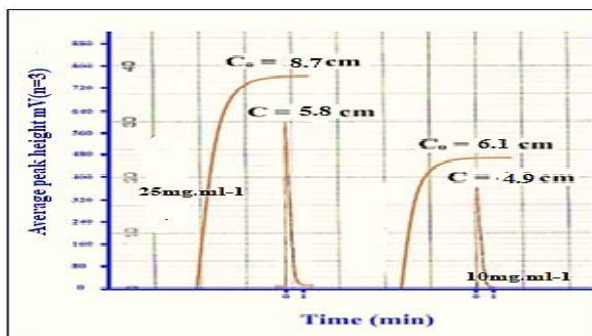


Figure 12. Dispersion of HCTZ in CFIA system.

Linearity and Calibration curve

Utilizing the ideal experimental for HCTZ drug evaluation, a linear calibration curve was created in the concentration range of 5-150 $\mu\text{g.mL}^{-1}$ over this range Beer's law was not followed, and the reaction

mixture measured the maximum absorbance of the orange-colored result at 480 nm in contrast to the reagent blank as indicated in Table 3 and Fig. 13.

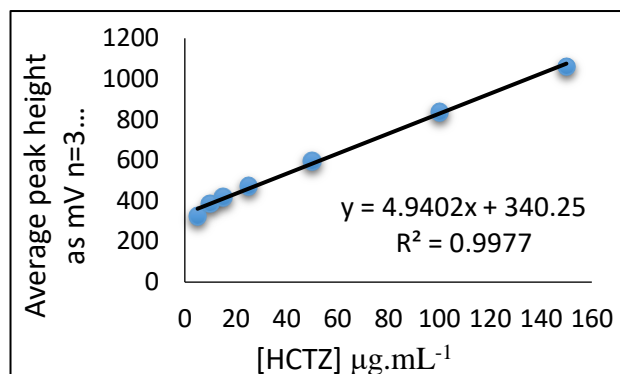


Figure 13. Calibration curve of HCTZ utilizes the suggested method

Table 3. Calibration table as S.E.M for HCTZ

Conc. HCTZ $\mu\text{g.mL}^{-1}$	peak height (mV)			Average response (\bar{y}) (mV)	\hat{y}	SD	*RSD%	S.E.M	*E/y%
5	352	347	352	350	364	2.88	0.82	350.8 \pm 7.143	2.04
10	391	391	377	387	389	8.13	2.10	387.15 \pm 20.18	5.21
15	416	426	417	420	414	5.57	1.33	420.05 \pm 13.82	3.29
25	476	472	475	474	463	1.67	0.35	474.67 \pm 4.134	0.87
50	594	604	594	597	587	5.91	0.99	597.97 \pm 14.68	2.45
100	840	844	839	841	834	2.70	0.32	841.44 \pm 6.715	0.8
150	1067	1071	1063	1067	1081	3.96	0.37	1067.1 \pm 9.833	0.92

Analysis of variation (ANOVA) of the linear equation and Repeatability

To compute $(y_i - \hat{y}_i)^2$ for $(n-2)$ degrees of freedom, calculate the assumed error, called-for regression, and the sum of squares of the difference between the response's (y_i) and the appraiser's \hat{y}_i values (S_2)².

Calculate the sum of squares of the variance of values \hat{y}_i from the average value (due to regression) and then divide that result by the square root of the degree of freedom (1) to obtain the value (F), as shown in Table 4. The good repeatability of the approach is shown in Table 5.

Table 4. Analysis of ANOVA for approach.

Source of Variation	Sum. Of Squares SS	df	Mean of Squares MS	F (S_1^2/S_2^2)	F crit
Between Groups	496776050	1	496776049.6	12.24905572	4.747225347
Within Groups					
Total	486675278	12			
	983451328	13	40556273.17		

Table 5. The repeatability of the suggested FIA method was good

[HCTZ] $\mu\text{g.mL}^{-1}$	Found	Error	Rec%	Erel%	RSD%
15	14.929	-0.071	99.524	-0.476	0.895
25	25.22	0.220	100.88	0.88	0.788
		\bar{x}	102.202	0.202	0.841

Analytical characteristics

The analytical characteristics of the new technique (CFIA/MZ) include the detection limit LOD, LOQ¹⁵⁻¹⁷, correlation coefficient (r), relative standard deviation, linear range, Standard deviation of the residuals (Sy/x), intercept (Sa) slope (Sb) with 95% confidence limits for (n-2) degrees were acquired under ideal circumstances as indicated in Table 6.

Comparing the proposed FIA to the batch approach, it was found to have excellent repeatability and reproducibility on tiny subjects. Due to its speed sample throughput of 90samples/h. The developed FIA/approach which includes my thesis was a simpler and semiautomated technique than the classic method since it produced calibration curves with large linear ranges.

Table 6. Analytical characteristic of the suggested method

Parameters	FIA method	Batch method
λ_{max} (nm)	480	480
Regression equation; $y = bx + a$; $y =$ absorbance; $x =$ concentration ($\mu\text{g. mL}^{-1}$)	$y = 4.9402x + 340.25$	$y = 0.0151x + 0.0838$
Linear range ($\mu\text{g mL}^{-1}$)	5 - 150 $\mu\text{g mL}^{-1}$	
Average of recovery (%)	100.88	2 - 60 $\mu\text{g mL}^{-1}$
Average of Relative Error (Erel %)	0.88	103.03
Average of Relative standard deviation (RSD %)	0.841	0.72
Slope (b); ($\text{mL. } \mu\text{g-1}$) $b = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sum (x_i - \bar{x})^2}$	4.94	1.41
Intercept (a); ($a = y - b x$)	340.25	0.015
Linearity ($r^2\%$)	0.9977	0.08
Correlation coefficient (r): $r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{[\sum (x_i - \bar{x})^2 (\sum (y_i - \bar{y})^2)]^{0.5}}$	99.7700	0.9963
Standard deviation of slope (Sb)	0.9988	
Standard deviation of intercept (Sa)	0.087	0.9981
Limit of detection (LOD)*	2.622	0.001
Limit of quantification (LOQ)**	7.977	0.345
Sample throughput (h-1)	90	1.151
The standard deviation of the residuals; $Sy/x = [\sum (y_i - \hat{y}_i)^2 / (n - 2)]^{0.5}$; $\hat{y}_i = bx_i + a$	11.6934	7
Confidence limit of slope (b) = $b \pm tSb$	4.9402 \pm 0.213	0.0206
Confidence limit of intercept (a) = $a \pm tSa$	340.25 \pm 15.32	-----

Interferences effect

The impact of several types of interferences; cellulose, sodium citrate, glucose, lactose, and sucrose was examined for selectivity of the suggested method, by estimating the concentration of

100 $\mu\text{g.mL}^{-1}$ of HCTZ in a presence of the interferences. The results appear in Table 7. where it was found that there was no impact from any of the excipients on the determination of HCTZ by using the CFIA system.

Table 7. Interferences effect on complex product for HCTZ

Interference	Conc. of Interferences $\mu\text{g. mL}^{-1}$	Average response (\bar{y}) (mV)	Found Conc. Of HCTZ $\mu\text{g.mL}^{-1}$	Erel%	Rec%
Standard sucrose		389	9.87	-1.2928	98.71
	10	386	9.27	-7.2845	92.72
	15	388	9.63	-3.6841	96.32
	25	390	10.17	1.6760	101.68
cellulose	10	386	9.28	-7.2305	92.77
	15	391	10.17	1.7322	101.73
	25	389	9.80	-2.0485	97.95
lactose	10	385	9.13	-8.6879	91.31
	15	388	9.57	-4.2616	95.74
	25	390	10.03	0.2796	100.28
glucose	10	388	9.57	-4.3426	95.66
	15	391	10.29	2.8636	102.86
	25	389	9.93	-0.7249	99.28
Sodium citrate	10	386	9.35	-6.4748	93.53
	15	390	10.01	0.1215	100.12
	25	388	9.62	-3.7812	96.22

* Average of five determinations.

Urine samples

suggested method has been utilized to evaluate biological samples (urine) taken from seven different healthy individuals. A series of solutions from each biological sample spiked with $10 \mu\text{g. mL}^{-1}$ of HCTZ were verified for accuracy and precision. as shown in Table 8.

Table 8. Determination of HCTZ in biological samples using propose

HCTZ $\mu\text{g.mL}^{-1}$		Erel%	Rec%	RSD %
Present μ	Found \bar{x}			
10	9.67	-3.3440	96.66	4.05
10	9.87	-1.3198	98.68	0.24
10	10.27	2.7286	102.73	1.06
10	9.87	-1.3198	98.68	0.95
10	9.46	-5.3682	94.63	1.22
10	9.67	-3.3440	96.66	1.47
10	9.87	-1.3198	98.68	1.98

Pharmaceutical applications

The proposed method investigated three pharmaceutical tablets containing HCTZ, as shown in Table 9. The statistical results were compared between the suggested method and the official method according to the pharmacopeias using F-test and student t-test showed that the calculated F-test¹⁸⁻¹⁹ values were 6.9705 and 2.0757, t-test values were 0.7560 and 1.0615 less than the theoretical(critical) F-test (19.00) and t-test (2.78) via CFIA/MZ, so there is no fundamental difference between the proposed method for estimating drugs and the official method.

Table 9. Application of the proposed methods for determination of HCTZ in pharmaceutical formulations.

Type commercial pharmaceutical	Classical method					Official method				
	Conc. of HTCZ $\mu\text{g.mL}^{-1}$		Erel %	Rec %	RSD %	Conc. of HTCZ $\mu\text{g.mL}^{-1}$		Erel %	Rec %	RSD %
	Present	Found				Present	Found			
Diuzid (Safa / Iraqi) (50) mg	10	10.21	2.10	102.10	1.99	10	9.89	-1.10	98.90	0.74
Actavis (Iran) (50) mg	15	15.14	0.93	100.93	0.79	15	15.02	0.13	100.13	0.55
Esidrex (French) (25)mg	10	10.12	1.20	101.20	2.01	10	9.92	-0.80	99.20	0.78
	15	14.91	0.60	99.40	0.80	15	15.11	0.73	100.73	0.51
	10	9.75	-2.50	97.50	2.09	10	10.02	0.20	100.20	0.77
	15	15.18	1.20	101.20	0.78	15	14.92	-0.53	99.47	0.55

*t*_{tab}=2.78 for $n_1=n_2=3, n_1+n_2-2=4$, at 95% confidence

*F*_{tab}=19.00 for $n_1-1=n_2-1=2$, at 95% confidence

Conclusion

The flow injection-merging zone technique has the advantage of accurately and rapidly determining hydrochlorothiazide drugs in the pharmaceutical and biological samples. The aim of this approach is it proposes an improved easy and high sampling rate (throughput of 90 samples. hour¹) and detection limit

value was 2.622 with a recovery percentage of 100.88 and repeatability good for a drug in this method, it was found that there is no effect of the common interferences. Therefore, this approach is effective for evaluating the drug in all its pure and pharmaceutical forms.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

W.A.H contributed to the sample preparation included data collection, interpretation, analysis,

correction and to the writing of the manuscript and B.B.Q. analyzed the data.

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التقدير الحساس لعقار هايدروكلوروثيازيد في العينات المختلفة عبر طريقة مطورة لنظام تحليل الحقن الجرياني المستمر

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الخلاصة

تحديد عقار هايدروكلوروثيازيد في العينات الصيدلانية والبايولوجية الادرار باستخدام تقنية جديدة لتحليل الحقن الجرياني المستمر وهي سريعة , بسيطة , اقتصادية وحساسة. المعقد المتكون بواسطة تفاعل هايدروكلوروثيازيد مع اورثوفانيلين داي امين مع فيروسيانيد البوتاسيوم لانتاج ناتج ملون برتقالي عند طول موجي 480 نانومتر كان الاساس للطريقة المطورة . العوامل الكيميائية و الفيزيائية الأخرى التي لها تؤثر على استقرارية وتطور الناتج الملون المستخدم في هذه الطريقة المطورة تضمنت حجم العينة، معدل التدفق، تركيز الكاشف وملف التفاعل، خطية الطريقة المقترحة كانت 5-150 مايكروغرام. مل⁻¹ ومعامل الارتباط كان 0.9988 و كانت قيم النسبة المئوية للأسترداد والخطأ النسبي %100.88, 0.88 على التوالي. قيم حد الكشف والحد الكمي 2.622, 7.97. نمذجة العينة 90 نموذج لكل ساعة تم استخدام النهج الجديد بشكل فعال لتحديد الهايدروكلوروثيازيد في العينات النقية، البايولوجية و الصيدلانية

الكلمات المفتاحية: عينات بايولوجية، نظام تحليل الحقن الجرياني المستمر، هايدروكلوروثيازيد، اورثوفانيلين داي امين ، مستحضرات الصيدلانية.