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A Novel Approach of CFIA Technique for Assaying of Furosemide (Sulfa Drug) as Antibacterial Agent in Pharmaceutical and Biological Samples Using Potassium Ferricyanide as Oxidizing Agent

Wasan Ajam*
Department of Chemistry, College of Sciences,
University of Baghdad, Baghdad ,Iraq.

Bushra Basheer
Department of Chemistry, College of Sciences,
University of Baghdad, Baghdad, Iraq.

***CorrespondingAuthor:**wasan.ajam1105d@sc.uobaghdad.edu.iq.

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Abstract

Furosemide drug determination in pharmaceutical and biological urine samples using a novel continuous flow-injection analysis technique that is simple, rapid, sensitive and economical. The complex formed by the reaction of furosemide and O-phenylenediamine with oxidative agent $K_3[Fe(CN)_6]$ to produce an orange-yellow colored product at 460 nm was the basis for the proposed method. The proposed method's linearity ranges (3-100) $\mu g.mL^{-1}$and (1-50) $\mu g.mL^{-1}$ for CFIA/merging zone methods and batch .The detection limit and Limit of quantification values were 2.7502 $\mu g.mL^{-1}$ and 9.1697 $\mu g.mL^{-1}$ the relative standard deviation was 0.7143 %, and the average recovery is 98.80% with a verified sample throughput of 73 h^{-1} . The new approach was effectively employed to determination of furosemide the presence of in the pure, biological, and pharmaceutical samples.

Keywords: Automatization, CFIA/Spectrophotometric system, Pharmaceutical analysis, Furosemide.

1. Introduction

Furosemide has a chemical formula of ($C_{12}H_{11}ClN_2O_5S$) with molar mass of 330.74g/ mol. Its formula structure is shown in Figure 1. furosemide is formally a sulfonamide, an antibacterial agent [1-2]. However, the intense and fast dieresis produced by this drug, has extended its application as a powerful acidic diuretic for diverse treatment in human and veterinary medicine. Furosemide is often classified as a loop diuretic due to its predominate action in the nephron [3-4] .Various analytical methods for determining FUR using many techniques have been described

in the literature, electrochemical sensing method [5–7], HPLC technique [8–10], and spectroscopic methods [11–12]. The method used for the analysis of furosemide in the oxidation-reduction reaction of the FUR and the reagent and [$K_3Fe(CN)_6$] as an oxidizing agent using the flow injection technique to determine the drug in pharmaceutical preparations and biological samples [13–17] The advantages of this method are that it consumes small amounts of the drug and the reagent, has high repeatability with accurate results obtained, is determined in the visible light range, and is a very simple and stable method for drug determination.

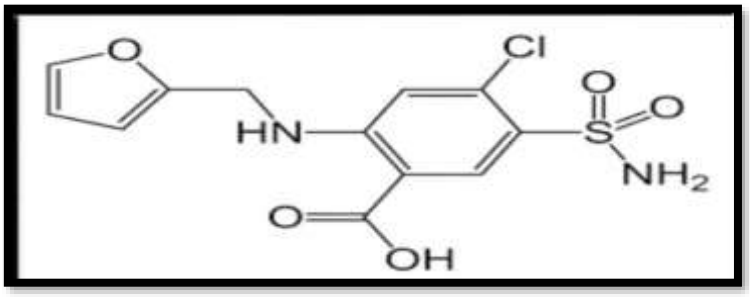

Figure 1. Formula structure of furosemide

2. Apparatus and FIA Manifold

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 Figure 2. The carrier stream distilled water was pumped through 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 [18, 19]. This injection valve was utilized to pass the volumes of reagent solution and sample. The loops were constructed of flexible vinyl and 1 mm for the manifold system. The reaction coil was constructed of glass with an inner diameter of 2 mm. A single-channel manifold system in CIFA is shown in Figure 2. D.W. was used as a carrier stream that was commixed with FUR in loop1, in loop2 was $K_3[Fe(CN)_6]$ and O-Ph in loop 3. In a reaction coil, all the compounds were mixed, and the carrier flow rate was (12.8) $mL.min^{-1}$ with a length of 50 cm and a height absorbance of 460 nm for the yellow complex.

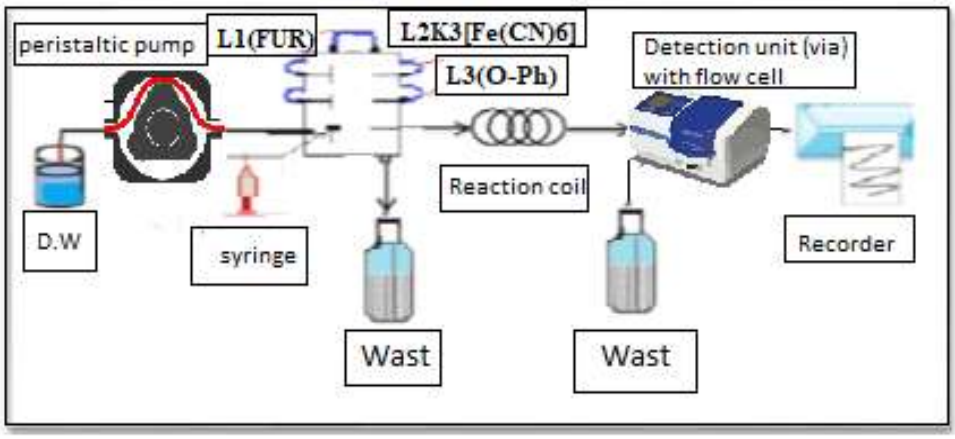

Figure 2. The manifold CFIA system

3. Chemicals and reagent

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 330.745 g.mol^{-1}): (1000 µg.mL^{-1})**

Standard solution furosemide was prepared by dissolving (0.1g) of pure drug in methanol then adding 10 mL of concentration HCl, and 40 mL of distilled water. diluting to the mark in (100ml) volumetric flask with distilled water to prepare 1000 µg.mL^{-1}.The obtained solution was heated at 50C° until it would be clear and yield a light yellowish solution pointed to complete the acidic hydrolysis.

- **O-phenylendiamine (M.wt 108.1g.mol^{-1} SDI): (1000 µg.mL^{-1})**

It is prepared by dissolving 0.1g of O-phenylendiamine in 100 mL of ethanol. Dilute solutions prepared by using a standard solution with distilled water.

- **Potassium ferricyanide (M.wt 329.24g.mol^{-1} SDI): (0.01 µg.mL^{-1})**

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

- **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.

3.1 Assay procedure for tablets

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

1. Lasix (SDI) Iraqi (40mg)
2. Lasix, Sanofi (French) (40mg)
3. Furosemide/Brawn (Indian)(40mg).

4. Lazine /Syria (40mg).

An average of one tablet weighing (40mg) of FUR was accurately weighed and finely crushed. Each weight that was taken in the previous operation was treated as pure material. [20] Additional solutions were diluted to get the concentration inside the linearity of the calibration graph. Serial dilution can be employed to make 100 μg.mL^{-1} of the other solution types, and the proposed method is then utilized to quantitatively quantify 10, 25 μg.mL^{-1}

3.2 Urine samples preparation

The samples were taken from several healthy individuals, utilized immediately after 5 drops of $HClO_4$ acid were added (to precipitate the protein), and then centrifuged at 3000 rpm. The samples were collected from a healthy volunteer and kept at 20 °C until use after gentle thawing. volume of 1 mL for urine sample preparation, then converted to a volumetric flask of 10 ml and spiked with (2.5, 5) mL of standard solution (100) μg.mL^{-1} and diluted with distilled water to attain (40, 50) μg.mL^{-1} of spiked FUR. A blank solution was prepared in the previous steps, except [21].

3.3 Batch method

Spectrophotometric determination based on oxidative 1mL (100 μg.mL-1) of the FUR drug with 1mL of O-Ph reagent in the presence of 0.5 mL of the oxidizing agent K3[Fe(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 λmax 460 nm, against reagent blank is shown in **Figure 3**.

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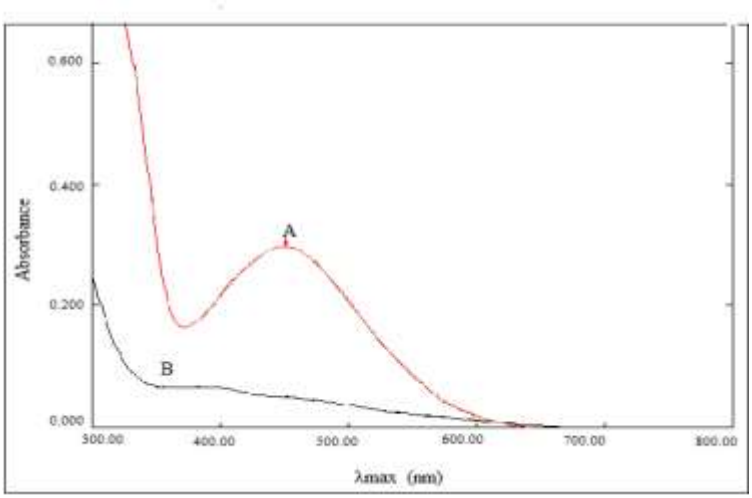
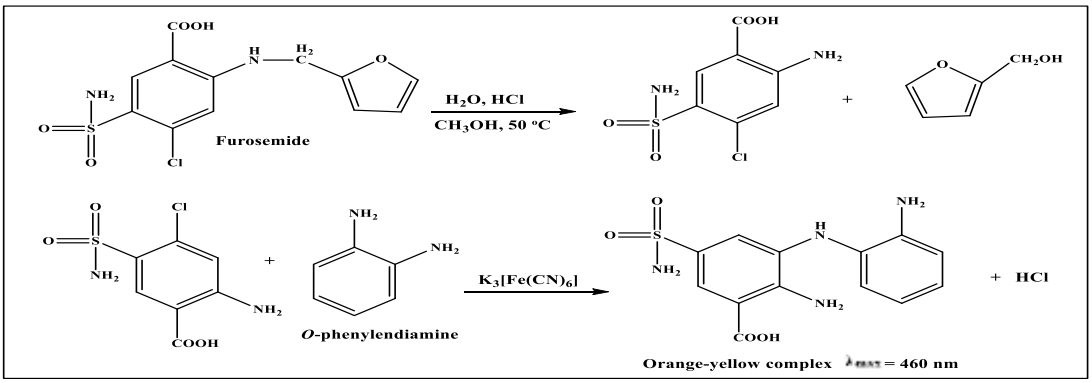


Figure 3. Spectrum of the reaction products of Furosemide (10μg.mL^{-1}) with O-Ph against blank and $K_3[Fe(CN)_6]$

3.4 Mechanism of the proposed method

The suggested mechanism for the hydrolysis with coupling reaction is shown in **scheme (1)** [22]



Scheme 1. The proposed mechanism of the complex between FUR with O-Ph

5. Result and discussion

This study explains the oxidation-reduction reaction between FUR and O-phenylendiamine as a reagent in the presence of the oxidizing agent $K_3[Fe(CN)_6]$. To from colored product orange-yellow at λ_{max} 460 nm opposite reagent blank, which has little absorbance at the same wavelength.

4.1 Stoichiometry study

To investigate the stoichiometric ratio of drug to reagent applied molar ratio method10 and continuous variation by using an equal concentration of FUR ($(3x10^{-4})$ M and O-Ph by using increased volumes of O-Ph and added to 1mL of FUR drug, The study found that the hydrochlorothiazide to coupling reagent ratio was 1:1. as shown in **Figure 6 A-B**.

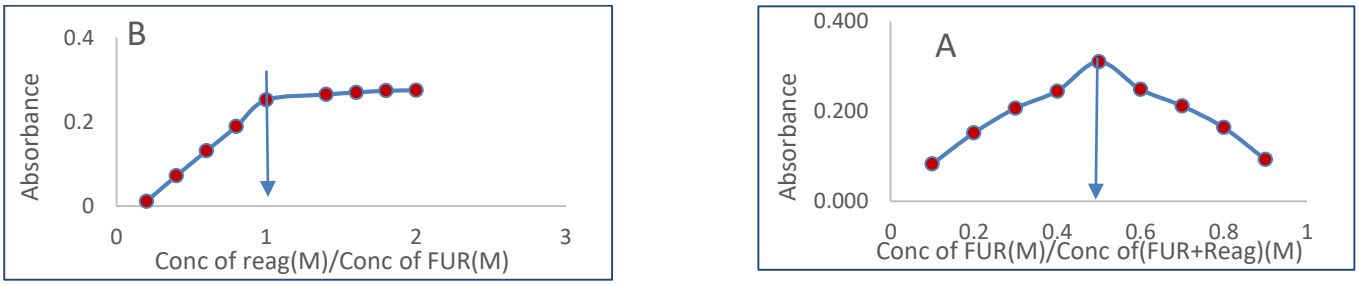


Figure 4. Stoichiometric study between FUR and Reagent Continuous variation (A) mole ratio (B)

4.2 Study of the optimum reaction conditions:

The study of various variables and factors on the color product was done to determine the most suitable conditions for drug determination.

4.3 Effect of reagent concentration

Through the use of different volumes of the reagent O-phenylenediamine, a volume of 1mL from $(8x10^{-3})$ M for O-Ph was the best concentration which has the highest responses the absorbance increases with volume 2mL then decreased, as shown in **Figure 4-A**, while the best volume of K3[Fe(CN)6] was 2mL of (9.2×10^{-3}) M and this volume was selected for subsequent experiences, as shown in **Figure 4-B**.

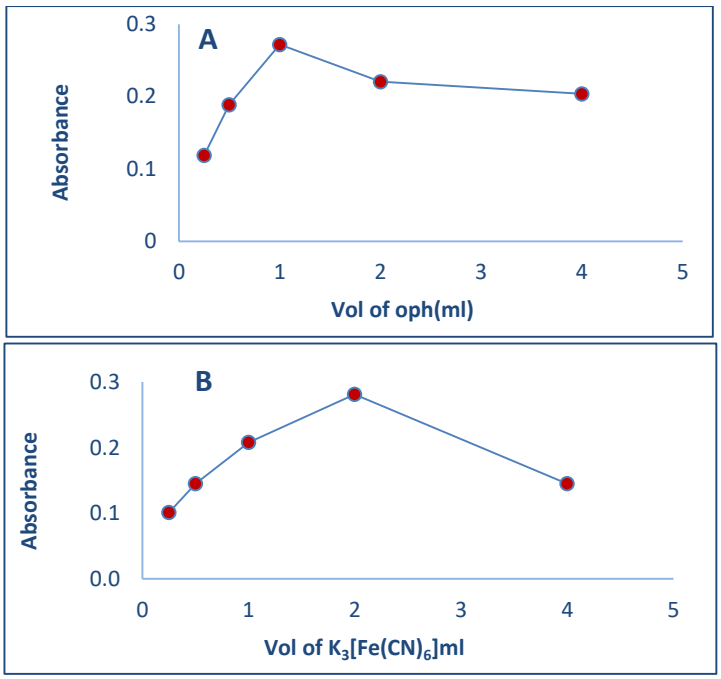


Figure 5. Chemical parameter for batch, A/ volume of Reagent, B/ volume of $K_3[Fe(CN)_6]$

4.5 Calibration curve and linearity

After ideal conditions utilizing several concentrations (1-50) µg.mL^{-1} of FUR were obtained by diluting the standard solution. The reaction mixture evaluated the maximum absorbance of the orange- yellow colored result at 460 nm in comparison to the reagent blank, as shown in **Figure 6.**

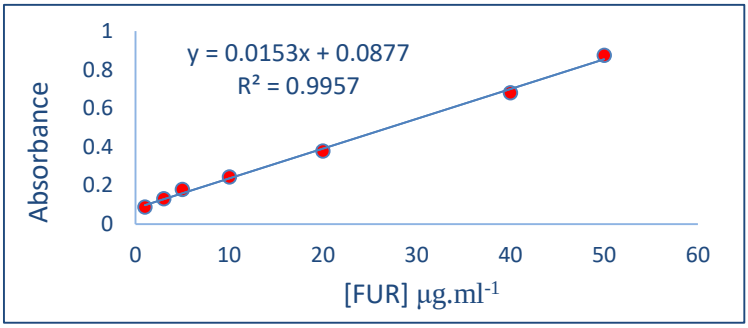


Figure 6. Linear calibration curve of FUR-OPh using spectrophotometric method

4.6 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 FUR

FUR µg.ml^{-1}		Error	Rec%	Erel%	RSD%
Present µ	**Found $\bar{x}$**				
5	4.89	-0.110	97.80	-2.200	1.773
10	10.33	0.3300	103.30	3.300	1.484

4.7 Stable Calculations

The stability constant for the proposed reaction (FUR: OPh) was computed based on the two groups of solutions that had been created; the first group had a stoichiometric amount of FUR and reagent OPh, whereas the second group had a 2-fold excess of OPh. The stoichiometry of the drug to reagent (1:1). According to the relationship, the reaction between FUR and OPh proceeds: While As, Am are the absorbance values of the aqueous solution, which include a sufficient and stoichiometric quantity of reagent [23, 24], C is the molar concentration, and K is the stability constant, where (α) expresses the degree of disintegration (M) of the product, which is equivalent to the concentration of FUR. Where The (ΔG value) spontaneous of complex formation reaction was determined based on K evaluation as in **Table 2** and the equation($\Delta G = -RT \ln K$) where(ΔG), (R), (T) its mean gibbs free energy, general constant of gases (8.314 J. mol^{-1}. K^{-1}), absolute temperature (298 K) shown in the **Table 2.**

Table 2. Stability constants for FUR with O-Ph.

	Am	**As**	**α**	**K (L.mol^{-1})**	**ΔG (J.mol^{-1})**
1	0.310	0.255	0.177	307475	-76984
2	0.313	0.258	0.176	320212	-77085
Average	313844				-77035

6. FIA/ MZ spectrophotometric determination

An FIA procedure was developed using the batch method for calculatingFUR. 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 FUR with O-Ph in the presence of potassium ferricyanide.

5.1 Optimization of parameters of FIA system

5.2 Chemicals and physical variables

The trace of chemical parameters (volume of reagent, concentration of oxidative agent, and order of addition) and physical variables (the length of reaction coil sampling, flow rate, injected volume of drug, and injection time) were studied.

5.3 Effect of O-phenylendiamine

To determine the best concentration of O-phenylenediamine as a reagent was examined by injecting various concentrations ($4.6x10^{-4}$-$7.3x10^{-3}$) M of OPh by utilizing a seven-three injection valve. The result is shown in **Figure 7.** The (2×10^{-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).

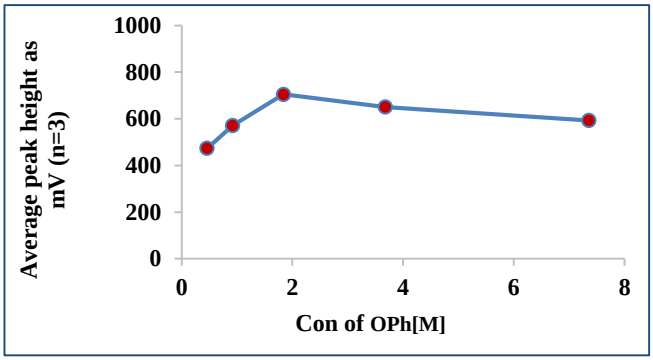


Figure 7. Effect of O-Ph

5.4 Effect of oxidized agent

injecting several concentrations of the oxidizing agent that the best concentration was (4×10^{-4}) M represented as peak height in mV (n=3), in the redox reaction between the (FUR) drug and the reagent, The highest value of absorbance is represented as peak height in mV (n=3) with excellent reproducibility, as shown in **Figure 8**.

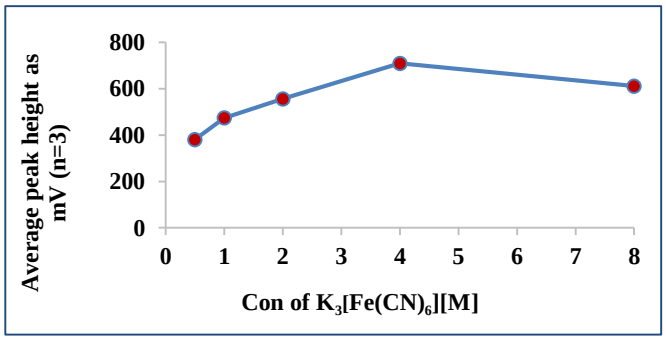


Figure 8. Effect of K_3[$Fe(CN)_6$]

5.5 Effect of mixing coil and injected volume

The effect length of the reaction coil was examined by using different lengths of it (50, 100, and 200 cm). The results showed that the better length of the reaction coil, which gave the highest absorption, was 50 cm, as shown in **Figure 9-A**, which was used in all subsequent experiments. After using different lengths of loops 58.88 (L1), 78.88 (L2), and 78.88 (L3) µL, the results showed that these injection volumes of the drug, oxidizing agent, and reagent are the best volumes, as they showed a high response that expresses a high peak shown in the following figure 9-B.

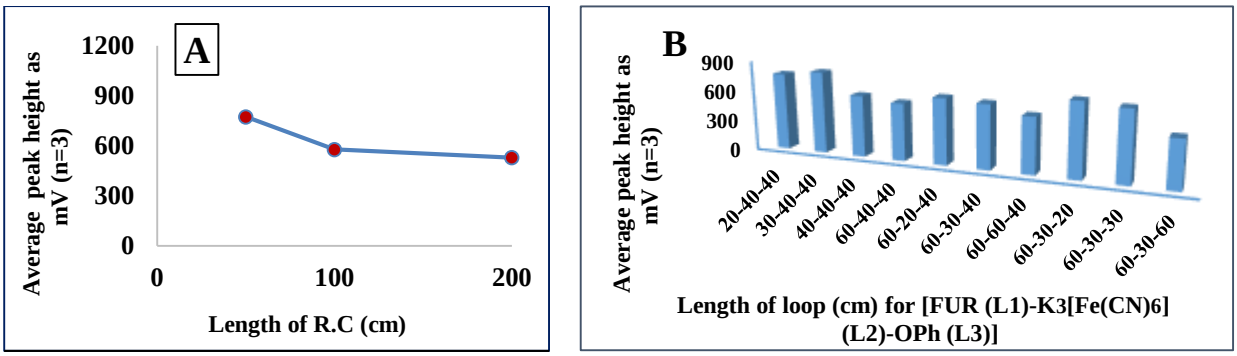


Figure 9. Effect of: A\ Reaction coil, B\ Injected volume

5.6 selecting the best manifold unit

The highest added sequence was D(drug)+O(oxidative agent)+R(reagent), which gave the best absorption value expressed as the highest peak. The results are indicated in **Figure 10**.

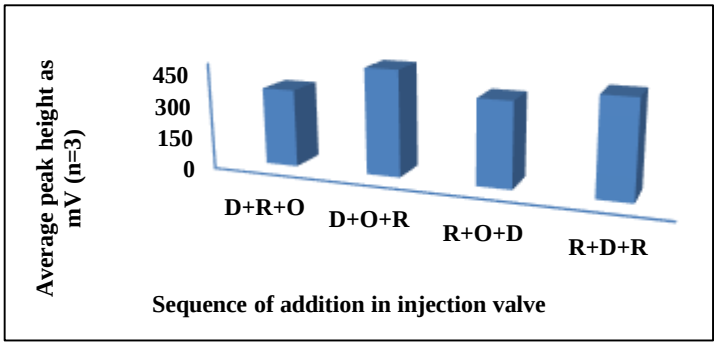


Figure 10. Effect of the sequence of chemicals.

5.7 Effect of optimum total flow rate and sample through-put

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. several flow speeds and it was found that the better speed is 9.2 mL.min^{-1} with a sample through-put 73 sample/h^{-1}. The results are indicated in **Figure 11**.

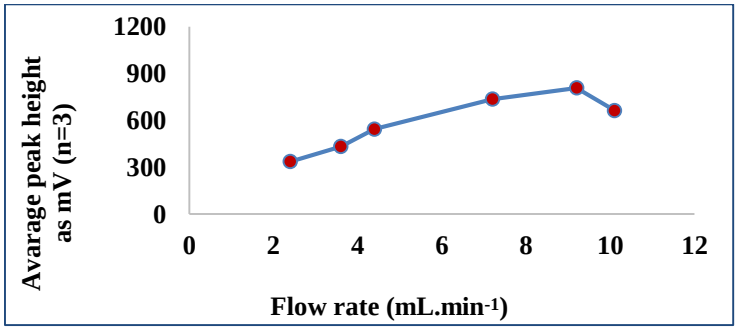


Figure 11. Effect of flow rate of distilled water in developed system

5.8 Purge Time

The effects of the purge time for the sample to be injected by distilled water as a carrier stream were investigated using the best chemical and physical features previously investigated. For this experiment, an open valve (injection mode) and times of 5, 10, 15, and 20 seconds were used. To get the highest response intensity with the least amount of dispersion, the purge time—the amount

of time between the injection of the sample and the start of the termination of the signal—should be larger than 20 seconds. **Figure 12** illustrates the ideal injection timing for moving the drug from the drug loop to the flow cell: when the valve is open.

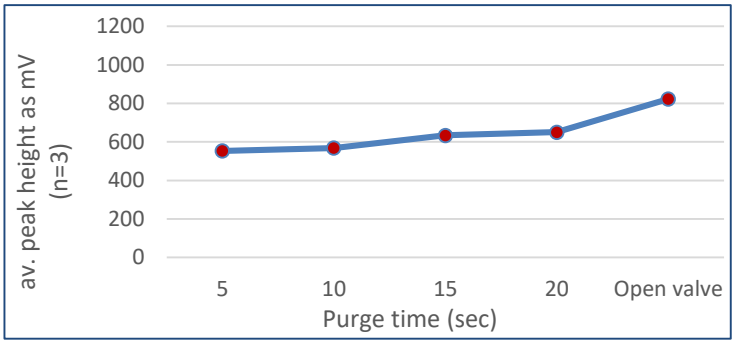


Figure 12. Effect of purge time

5.9 Study of dispersion

The most important aspect of the flow injection technique is to control the physical dispersion phenomenon, which can be calculated through the law (D = Co/Cmax). D refers to the dispersion coefficient. While C_0 is the peak height without using dilution reactions outside the FIA system, C is the peak height with the use of dilution interactions inside the FIA system. The dispersion of the FUR-OPh reaction was 1.167 and 1.170 for concentrations 25, 50. It is illustrated in Figure 13 as well as in **Table 3**.

Table 3. Dispersion value of FUR

[FUR] µg.ml^{-1}	C_o (cm)	C max (cm)	D
25	9.1	7.8	1.167
50	11.7	10.0	1.170

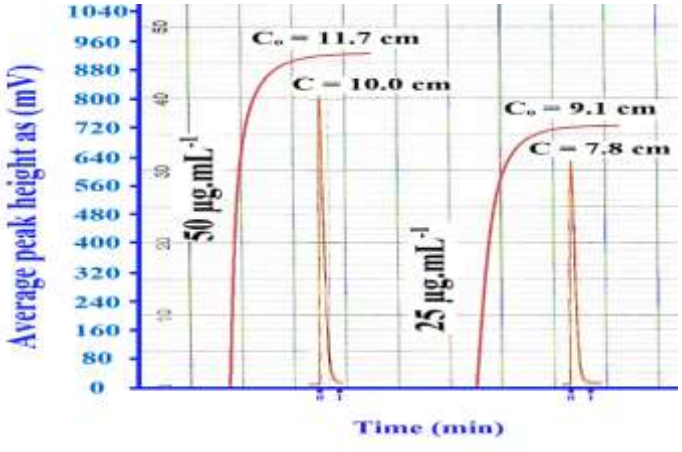


Figure 13. Dispersion of FUR in CFIA system

5.10 Calibration curve

Utilizing the ideal experimental for FUR drug evaluation, a linear calibration curve was created in the concentration range of 3-100 µg.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 460 nm in contrast to the reagent blank as shown in **Table 4 and Figure 14**.

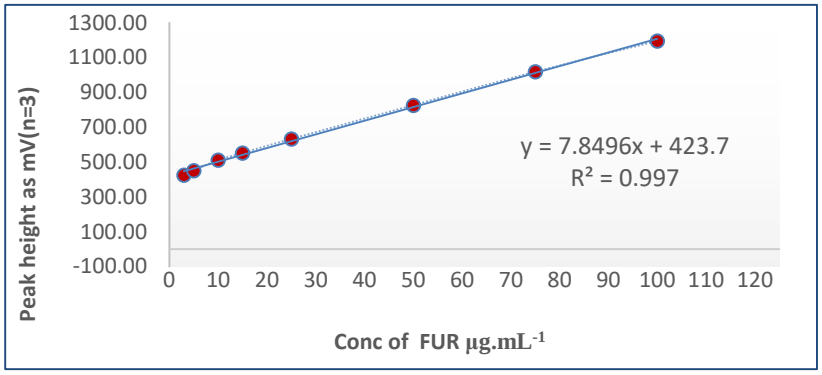


Figure 14. Calibration curve of FUR using the developed CFIA system.

Table 4. The S.E.M for FUR-OPh

conc. [FUR] µg.mL^{-1}	peak height (mV)			Mean response ($\bar{y}$) (mV)	SD	*RSD%	S.E.M			*E/y%
3	424	424	425	424	0.84	0.198	424	±	2.086	0.49
5	449.6	450.5	450.4	450	0.487	0.108	450	±	1.208	0.27
10	511.2	511.4	512	512	0.423	0.083	512	±	1.051	0.21
15	552	551.2	552.8	552	0.8	0.145	552	±	1.986	0.36
25	632.9	632	632.8	633	0.487	0.077	633	±	1.208	0.19
50	824	828	826.4	826	2.013	0.244	826	±	4.998	0.61
75	1018	1018	1019	1018	0.721	0.071	1018	±	1.791	0.18
100	1194	1194	1194	1194	0.462	0.039	1194	±	1.147	0.10

* $Ey\% = ttabSD/\sqrt{n} \times 100\% y$, S.E.M=$\bar{y}$ ± t0.05 ($\sigma n - 1\sqrt{n}$)

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

To compute (yi – ŷi)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 (yi) and the appraiser's ŷi values (S2) 2. Calculate the sum of squares of the variance of values ŷi 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 **Tables (5, 6).**

Table 5. Analysis of a nova for proposed method

Source of Variation	Sum. of Squares SS	df	Mean of Squares MS	F (S_1^2/S_2^2)	F crit
Between Groups	1967734.215	1	1967734.215	48.91339414	4.600109937
Within Groups	563205.222	14	40228.94443		
Total	2530939.437	15			

Table 6. The repeatability of the proposed method

[FUR]µg.mL^{-1}	Found	Error	Rec%	Erel%	RSD%
25	24.630	-0.370	98.520	-1.480	0.528
50	49.540	-0.460	99.080	-0.920	0.901

6.1Methods validation

The analytical characteristics of the new technique (CFIA/MZ) include the detection limit LOD, LOQ[27-28],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 7**. 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 73samples/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 (7). Analytical properties of calibration curve for FUR

Parameters	FIA approach	Batch approach
λmax (nm)	460	460
Regression equation	y=7.8496x+423.7	y=0.0153x+0.0877
Linear range (µg mL^{-1})	3-110 ppm	1 - 50 ppm
Average of recovery (%)	98.80	99.55
Error % Erel %	-1.2000	-0.4500
(RSD %)	0.7143	1.3800
Slope (b) (mL/µg)	7.8496	0.0561
Intercept (a)	423.7000	0.0875
Linearity R^2	0.997	0.9957
Correlation coefficient (r)	0.9985	0.9977
Standard deviation of slope (Sb)	0.1491	0.0040
Standard deviation of intercept (Sa)	7.2861	0.0116
(LOD)* (µg/mL) Limit of detection	2.7509	0.5344
(LOQ)** (µg/mL) Limit of quantification	9.1697	1.7814
Ɛ = b*M.Wt*1000		1855.27
Sandell s sensitivity (S)		0.0178
Sample through put (h^{-1})	73	0.0214
Standard deviation of the residuals	14.2197	
Confidence limit of slope (b)	7.85±0.3652	
Confidence limit of intercept (a)	423.7±17.8509	

Table 7. Interferences effect on [FUR–$K_3[Fe(CN)_6]$-O-Ph] FIA system.

Type of Interference	conc.of Interferences μg.mL^{-1}	mean (mV)	Found conc. of FUR μg.mL^{-1}	Erel%	Rec%
Standard	5	504	10.23	2.2982	102.30
sucrose	10	502	10.12	0.2599	100.26
	15	502	9.99	-0.0798	99.92
cellulose	5	502	9.96	-0.4196	99.58
	10	502	10.03	0.2599	100.26
	15	502	9.99	-0.0798	99.92
lactose	5	501	9.79	-2.1182	97.88
	10	503	10.13	1.2790	101.28
	15	502	10.03	0.2599	100.26
glucose	5	503	10.09	0.9393	100.94
	10	502	9.96	-0.4196	99.58
	15	503	10.09	0.9393	100.94
Sodium	5	503	10.16	1.6188	101.62
citrate	10	503	10.16	1.6188	101.62
	15	502	10.03	0.2599	100.26
		503	10.09	0.9393	100.94

6.2 Effect of interferences

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 100μg.mL^{-1} of FUR 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 FUR by using the CFIA system.

6.3 Urine samples

The FIA method used successfully to determine FUR with accuracy in samples of spiked human urine. (100) μg.mL^{-1} of FUR's precision and accuracy were assessed. Every concentration was subjected to three analyses. The urine sample results in **Table 8** were observed with satisfactory precision and accuracy.

Table 8. Determination of FUR in urine samples using proposed method

[FUR]μg.mL^{-1}						
Present μ	**y**	**Found $\bar{x}$**	**Erel%**	**Rec%**	**RSD%**	**SD**
10	510	9.91	-0.9000	99.10	0.33	1.67
10	508	10.12	1.2000	101.20	0.42	2.12
10	509	10.07	0.7000	100.70	0.19	0.97
10	511	10.30	3.0000	103.00	0.39	2.01
10	510	9.91	-0.9000	99.10	0.24	1.21
10	509	10.07	0.7000	100.70	0.64	3.23
10	510	9.91	-0.9000	99.10	0.31	1.60

6.4 Applications and assessment of suggested method

Under the proposed method, the first types of medications containing FUR that are equipped with different sources when added conventionally have been investigated. Student F-test and t-test results from the numeration comparison between the suggested approach and the spectrophotometric approach [31] revealed that the calculated t-test values were 1.3026 and 0.5337 and the F-test values were 0.5399 and 0.4678, which were less than the theoretical t-test (2.45) and F-test (9.28) via FIA, as shown in **Table 10**.

Table10. Applications of the suggested methods via with approved method for determination of FUR in preparation pharmaceuticals

Dosage form	Proposed FIA method					Official method (theoretical)				
	conc. of FUR µg.mL^{-1}		Erel %	Rec %	RSD %	conc. of FUR µg.mL^{-1}		Erel %	Rec %	RSD %
	Present	Found				Present	Found			
Laxis (SDI) Iraqi(40mg) Laxis Sanofi(French)(40mg) Furosemide/Brawn(Indian)(40mg) Lazine /Syria	10	10.07	0.70 - 0.44	100.70 99.56	0.84	10	10.01	0.12 1.32 - 1.60	100.12 101.32	1.16
	25	24.89	1.00	101.00	0.50	25	25.33	0.16	98.40	0.72
	10	10.10	0.44	100.44	0.84	10	9.84	0.70	100.16	1.18
	25	25.11	-	99.50	0.50	25	25.04	0.44	100.70	0.73
	10	9.95	0.50	100.40	0.85	10	10.07	-	98.50	1.15
	25	25.10	0.40	101.50	0.50	25	25.11	1.50	99.56	0.73
	10	10.15	1.50	100.72	0.85	10	9.85	-		1.17
	25	25.18	0.72		0.50	25	24.89	0.44		0.74

$ttab$=2.45 for $n1$=$n2$=4,$n1$+$n2$−2=6,at 95% $confidence\ level$ $Ftab$=9.28 for $n1$−1=$n2$−1=3,at 95% $confidence\ level$

7. Conclusions

FIA designs based on the combining zone approach and spectrophotometric detection wrer used successfully to find furosemide in both its pure and pharmaceutical forms. The construction of a wonderful lab-made valve, which is a crucial part of the system that was built, was easy, affordable, and efficient, with components that were readily available and cheap to clean, replace, and repair. The procedure is based on the development of an orange-yellow condensation adduct when FUR and O-Ph combine with the oxidizing agent $K_3[Fe(CN)_6]$ that has been provided. The approach offers a high sample throughput and a low detection limit. The suggested techniques had the best application for the pharmaceutical preparation and adhered to Beer's law. By examining the assay of FUR, the FIA method's broad application for everyday quality monitoring is successfully demonstrated.

References

1. Naveed, S.; Qamar, F.; Zainab, S. Simple UV Spectrophotometric Assay of Furosemide. *JIPBS* **2014**,*3*, 97-101.
2. Khan, T.M.; Patel, R.; Siddiqui, A.H. Furosemide. *In Stat Pearls*. **2020**, *12*.
3. Miles, J.A.; Hanumanthu, B.K.; Patel, K.; Chen, M.; Siegel, RM.; Kokkinos's, D.G. Torsemide Versus Furosemide and Intermediate-Term Outcomes in Patients with Heart Failure: An Updated Meta-Analysis. *Journal of Cardiovascular Medicine*. **2019**,*20*, 379-388.
4. Rahhal, A.; Saad, MO.; Tawengi, K.; Assi, AA.; Habra, M.; Ahmed, D. Torsemide Versus Furosemide After Acute Decompensated Heart Failure: *BMC cardiovascular disorders*. **2019**,*1*.
5. Heidarimoghadam, R.; Farmany, A. Rapid determination of furosemide in drug and blood plasma of wrestlers by a carboxyl-MWCNT sensor. *Materials Science and Engineering* .**2016** ,*58*,1242-1245.
6. Kimura, S.; Kiriyam, A.; Nishimura, E.; Sakata, S.; Inoue, D.; Furubayashi, T.; Yutani, R.; Tanaka ,A.; Kusamori, K.; Katsumi, H.;Iga, K. Dec 1; Novel Strategy for the Systemic Delivery of Furosemide Based on a New Drug Transport Mechanism. *Biological and Pharmaceutical Bulletin.* **2018**,*41*,1769-1777.
7. Emam, A.A.; A.; E.A.; Naguib, I.A.; Abdallah, F.F.; Ali, N.W. Successive Ratio Subtraction as a Novel Manipulation of Ratio Spectra for Quantitative Determination of a Mixture of Furosemide, Spironolactone and Canrenone. Spectrochimica Acta Part A. *Molecular and Biomolecular Spectroscopy.* **2018**,*192*,427-436.
8. Naguib, IA.; A.; EA.; Emam, AA.; Ali, NW.; Abdallah, FF. Development and Validation of HPTLC and Green HPLC Methods for Determination of Furosemide, Spironolactone and Canrenone, in Pure Forms, Tablets and Spiked human Plasma. *Biomedical Chromatography*. **2018**,*32*,4304.
9. Sora, D.I.; Udrescu, S.; Albu, F.; David, V.; Medvedovici, A. Analytical Issues in HPLC/MS/MS Simultaneous Assay of Furosemide, Spironolactone and Canrenone in Human Plasma Samples. *Journal of pharmaceutical and biomedical analysis.* **2010**, *52*,734-740.
10. Ram, V.R.; Dave, P.N.; Joshi, H.S. Development and Validation of a Stability-Indicating HPLC Assay Method for Simultaneous Determination of Spironolactone and Furosemide in Tablet Formulation. *Journal of chromatographic science*. **2012**,*50*,721-726.
11. Ferraro, M.C.; Castellano, P.M.; Kaufman, T.S. A Spectrophotometric-Partial Least Squares (PLS-1) Method for the Simultaneous Determination of Furosemide and Amiloride Hydrochloride in Pharmaceutical Formulations. *Journal of pharmaceutical and biomedical analysis.* **2001**,26,443-451.
12. Ali, H.; Abass, S. Use of Acidic Hydrolysis and Diazo Coupling Reaction for Spectrophotometric Determination of Furosemide in Urine and Pharmaceutical Formulation. *Ibn AL-Haitham Journal for Pure and Applied Science.* **2018**, *24*,221-234.
13. Qassim, B.B.; Asmaa ,A.; Zyda. Sensitive Simultaneous Estimation of Atorvastatin. Ca in Pure and Dosage Forms Via Developed CFIA Using 1, 2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent. *Indian Journal of Forensic Medicine & Toxicology* .**2020**, *14*,2161.
14. Kadhim, M.M. Effect of Sr/Mg Co-substitution on Corrosion Resistance Properties of Hydroxyapatite Coated on Ti–6Al–4V Dental Alloys. *Journal of Physics and Chemistry of Solids* .**2022**,*161*,110450.
15. Abdullah, H.J.; Qassim, B.B. Development and Validation CFIA/MZ System as A Green Method for Determination of Thiol Drug (D-PEN). *Egyptian Journal of Chemistry*. **2022**,*65*,1-2.

16. Mohamed, M.R.; Qassim, B.B. A New Indirect Sensitive and Green CFIA Method for Assay of α-Cypermethrin Insecticide in Pure Form, Biological and Industrial Samples Using N-Bromo Succinamide as Oxidizing Agent in Acidic Medium. *REVISTA GEINTEC-GESTAO INOVACAO E TECNOLOGIAS.* **2021**,*11*, 4033-4057.

17. Abdullah, H.J.; Qassim, B.B. High Throughput Flow Injection /MZ Technique for Indirect Assay of Hydrosulfurnyl Group in Tiopronin drugs and Biological Samples Using 2,2`-Dipyridyl as a Selection OAR. *International Journal of Mechanical Engineering*. **2022**,*7*, 0974-5823.

18. 18. Hamed, L.L.; Qassim, B.B. Direct and New Flow Injection method for Assay of Iron as Ferrous Sulfate in Pure and Dosage Forms Through the Complexation with 2, 2`Dipyridyl Reagent. *International Journal of Pharmaceutical Research.***2020**,*12*,1329-1338.

19. Qassim, B.B.; Hamed, L.L. Simple Green Method High Throughput Flow Injection Technique for Spectrophotometry Determination of Fe (III) in Iron Drugs Through the Reaction between DPA-4-Sulfonat with Hydrogen Peroxide using a Modified Detection Unit. *International Journal of Drug Delivery Technology*. **2020**, *10*, 563-570.

20. Sheej, A.; O.A.; Al-Enizzi, M.S.; Saeed, A.M.. Using 9-Chloroacridine as A Chromogenic Reagent for The Detection of Tetracycline. *Egyptian Journal of Chemistry* .**2021**.

21. Qassim, B.B.; Zydan, A.A. Novel Approach of Oxidation-Reduction Reaction with Kmno4 for Simultaneous Determination of Simvastatin Drug in Either Pharmaceutics Preparation or Human Urine Using Homemade fia-Stopped-Flow/Merging Zone Technique. *BioChem.* **2020**, *20*.

22. 22 Abdullah, A.I., Abass, SM. Azo Coupling Reaction for Indirect Spectrophotometric Determination of Furosemide Using Resorcinol as A Reagent. In IOP Conference Series: *Materials Science and Engineering* **2021**, *012077*,1058.

23. Tawfeeq, A.H.; Qassim, B.B. A Novel Method of CFIA/Merging Zones Technique for Assay of Doxycycline in Bulk and Pharmaceutical preparation Depending on Azo Dye Formation. *Research Journal of Pharmacy and Technology*.**2021**, *14*, 67-74.

24. Qassim, B.; Alwan A.A. Indirect Way for the Assay of Captopril Drug in Dosage FormsUsing1, 10-Phenanthroline as a Selective Spectrophotometric Agent for Fe (II) Via Homemade CFIA/Merging Zones Technique. *Ibn AL-Haitham Journal for Pure and Applied Science*. **2018**, 294-320.

25. Ruzicka, J.; Hansen, E.H. Flow Injection Analysis. *John Wiley & Sons*. **1988**.

26. Ellison, S.L.; Barwick,,V.J.; Farrant, T.J.D. Practical Statistics for the Analytical Scientist: A Bench Guide. *Royal Society of Chemistry.* **2009**.

27. Suarez, W.T.; Madi, A.A.; Figueiredo-Filho, L.C.S.; Fatibello-Filho, O. Flow-injection Spectrophotometric System for Captopril Determination in Pharmaceuticals. *Journal of the Brazilian Chemical Society*. **2007**, *18*, 1215-1219.

28. Tzanavaras, P.D.; Suarez, W.; Cavalheiro, E.T.G.; Fatibello-Filho, O. Flow and Sequential Injection Manifolds for the Spectrophotometric Determination of Captopril Based on Its Oxidation by Fe (III). *Microchimica Acta* .**2003**, *142*, 55-62.

29. Yaseen, S.M.; Qassim, B.B.; Al-Lami, N.O. Spectrophotometric Determination of Co (II) in Vitamin B12 Using 2-(biphenyl-4-yl)-3-((2-(2, 4-dinitrophenyl) hydrazono) methyl) imidazo [1, 2-a] pyridine as Ligand by Flow Injection–Merging Zone Analysis. *Al-Nahrain Journal of Science.* **2020**, *23*, 24-38.

30. Yaseen, S.M., Qasim, B.B.; Al-lame, N.O. Spectrophotometric Determination of Cu (+ II) by Complexation with 2-(4-biphenyl) Imidazo [1, 2-] Pyrimidine-3-Hydrazone and Studying Characteristics of prepared complex. *Egyptian Journal of Chemistry*. **2021**, *64*, 4-5 .

31. Alfred-Ugbenbo, D.; Zdoryk, O.A.; Georgiyants, V.A. Validation of Analytical Method for Determination of Furosemide in Extemporaneous Syrup. *Medical and Clinical Chemistry*. **2017**,*12*, 7859.