

## RESEARCH ARTICLE

# 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

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## ABSTRACT

A new simplicity, accuracy, rapid and sensitive batch and merging zones / flow injection analysis spectrophotometric ways for estimation of Fe (III) as ferric chloride in pure pharmaceutical formulations. The method was based on oxidation-reduction between diphenylamino-4-sulfonic acid sodium salt (DPA-4-SA) with hydrogen peroxide ( $H_2O_2$ ). Ferric chloride was behave as the catalytic effect on this reaction. The measurements were taken at 504 nm, after the reaction was monitored spectrophotometry, a purple-pink complex was formed after 5 min at 25-30° C. The optimized FIA order was able to estimate of Fe (III) with throughput 82 sample/h, flow rate 1.92 mL/min was used distilled water as a carrier, 78.50  $\mu$ L analyte (60  $\mu$ g/mL Fe (III)) and DPA-4-SA ( $1.5 \times 10^{-3}$  M) volume, 7 M  $H_2O_2$  (58.875  $\mu$ L) for  $L_1$  and  $L_2$ , respectively. The colored product showed Beer's Law in the range of concentration 2-30 and 3-75  $\mu$ g/mL and detection limits 1.60,  $9.57 \times 10^{-4}$   $\mu$ g/mL for batch and FIA/ MZ methods, respectively. Repeatability (RSD %) (n =7) were 0.03 and 0.02 for estimation of ferric with concentration 25 &50  $\mu$ g/mL by FIA/MZ method. The open valve was selected as an ideal injection time to finish transporting the sample from the sample loop to the flow cell. The developed CFIA procedure was applied easily and successfully for the quantitative determination of ferric in marketed pharmaceutical preparations without interference from extractable tablet additives and statistical analysis of values comparing with results by united state pharmacopoeia (USP) were also reported.

**Keywords:** Ferric chloride, CFIA/Merging Zones technique, diphenylamino-4-sulfonic acid sodium salt, a green method, Spectrophotometric detection, Iron drugs.

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**Conflict of interest:** None

## INTRODUCTION

Several FI analytical methods were used for determination of ferric and ferrous ions in different samples using various reagents, for Fe (III) like; tiron,<sup>1</sup> chlortetracycline,<sup>2</sup> ammonium or potassium thiocyanate, sodium salicylate,<sup>3,4</sup> O-acetylsalicylhydroxamic acid,<sup>5</sup> nitros-R salt,<sup>6</sup> Chrome Azurol S,<sup>7</sup> norfloxacin.<sup>8</sup> Most analytical techniques using flow injection analysis with spectrophotometric methods.<sup>9,10</sup> The effect of iron is an important element and as a pollutant is well known worldwide. Flow-injection analysis (FIA) is a technique which is well known due to its high sample performance, cost-effective performance, ease of use reliability and versatility, and a wide range of detection systems, low-cost automated and user-friendly analytical methods have become desirable in many sample forms for determining iron trace levels.<sup>11-13</sup>

Catalytic kinetic methods are an appealing alternative for evaluating trace quantities of iron, and these approaches have the overall benefit of combining high accuracy with fairly basic procedures and apparatuses. In several reviews the catalytic action of iron on the oxidation of various organic compounds has been recorded in several kinetic methods.<sup>14,15</sup> The coupling reaction was catalyzed by iron (II, III), and the initial rate increased linearly with iron concentration.<sup>16</sup> Therefore, more sensitive and selective methods are still required which can quickly and conveniently determine the low iron concentrations. This paper describes a green FIA method for iron (III) determination in Iron drugs. The approached was based on using diphenylamine-4-sulfonic acid sodium salt (DPA-4-SA) as redox indicator in a single channel developed FIA system for reaction with Fe (III) (catalytic effect) using hydrogen peroxide as oxidizing agent.

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## EXPERIMENTAL

### Reagents and Solutions

A normal stock solution of Fe (III) was prepared through dissolution 0.025 g of ( $\text{FeCl}_3=162.204 \text{ g/mol}$ ) (Merck) in a few amount of conc.HCL and making up the solution to 100 mL with distilled water. A stock solution of diphenylamine-4-sulfonic acid sodium salt (M.wt=271.26 g/mol, Sigma-Aldrich) 0.01 M prepared by dissolving 0.1356 g in 2 mL of 1M  $\text{H}_2\text{SO}_4$  (CARLO ERBA Reagents) thus ensuring acidic medium then complete the 50 mL with distilled water. Prepared 9M (38.7mL in 50 mL volumetric flask) of hydrogen peroxide solution from 35% (Merck), standardized with 0.0966 M  $\text{KMnO}_4$  in acidic medium of  $\text{H}_2\text{SO}_4$  1:8 (V/V).

### Instrumentation

The measurements of all absorbance in batch procedure were made by using a Shimadzu UV-1800, (Japan) UV-Visible Spectrophotometer double beam and quartz cuvette with an optical length of 1 cm. The proposed FI system was designed is a simple type with one channel manifold as depicted in Figure 1 was used in developed method of FIA/merging zones system for estimation of ferric ion. A one-channel Peristaltic pump (Shenzhen, LabM1) was used to pump the distilled water as a carrier stream through the injection valve (six-three-way injection valve, homemade),<sup>17</sup> that moves at  $90^\circ$  and three loops of Teflon (I.d = 0.5mm). The sample (Fe (III), 60  $\mu\text{g}/\text{mL}$ ) and (DPA-Sulfonate,  $1.5 \times 10^{-3}$  M) reagent solutions  $L_1$ , (7 M  $\text{H}_2\text{O}_2$  solution  $L_2$ , were loaded. Mixing in reaction coil made from glass 2 mm (I.D). In FIA procedure absorbance and spectral control, the modified Optima photometer 301-D<sup>+</sup>, VIS Spectrophotometer single beam (Japan) was used. Kompensograph C1032 (Siemens) was used for measurement of responses as peak height (mV) or optical multimeter absorption (DT9205A, OVA, China) for the absorbance measurements. The detection unit contains a flow cell quartz silica (QS, 1 cm) with an internal volume of 80  $\mu\text{L}$ .

### General procedure

#### A-Batch method

An increasing concentration (2–30)  $\mu\text{g.mL}^{-1}$  of ferric chloride were prepared in a set of 10 mL standard flask. This reaction

occurs in 25–30°C where transfer volumetric flask (10mL) containing different volumes of Fe(III) (250  $\mu\text{g.mL}^{-1}$ ), then added 4mL of DPA-4-SA ( $1 \times 10^{-3}$ M) in water bath the color formed (green) left for 5 minutes and after we added 0.5 mL of (7M)  $\text{H}_2\text{O}_2$  solution, the solution had been diluted to mark with distilled water, the pink-purple complex was formed and measured at  $\lambda_{\text{max}}$  504 nm against reagent blank after 5 min.

#### B- Analysis of flow injection - Process for merging zones

The iron (III) solutions within the concentration range (3-75)  $\mu\text{g.mL}^{-1}$  preparation from stock solution 250 $\mu\text{g.mL}^{-1}$ . The volumes of the sample, reagent and  $\text{H}_2\text{O}_2$  that were injected 78.50  $\mu\text{L}$  in  $L_1$  and 58.875  $\mu\text{L}$  in  $L_2$  consist of Fe (III) with DPA-4-SA solution, which was loaded in  $L_1$  after being kept in a water bath for 5 min at 25-30 °C to form a green median compound, while  $\text{H}_2\text{O}_2$  was loaded in  $L_2$ . The carrier stream was distilled water, the analyte and other loop chemicals injecting as one channel with a flow rate of 1.92  $\text{mL.min}^{-1}$ . The pink-purple complex product absorbance was quantified at  $\lambda_{\text{max}}$  504 nm and ferric ion ( $\mu\text{g.mL}^{-1}$ ) calibration graph was created.

#### C- Preparation of pharmaceutical sample

Prepared pharmaceutical tablet solution by weighing twenty tablets of drug, then powdered using motor. After that weight 0.1 g of 1/Hemafer (tablets) 1000  $\mu\text{g.mL}^{-1}$ , contain 100 mg of Fe (III), UNI-PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece), was dissolved in 100 mL distilled water with stirring and filtered. The stock solution (100  $\mu\text{g.mL}^{-1}$ ) was prepared in 50 mL volumetric flask from the stock solution of dosage form and concluded to the marked with distilled water. The preparation of pharmaceutical syrup and vial solutions 2/Santafer (syrup) 500  $\mu\text{g.mL}^{-1}$ , contain 50 mg of Fe (III), Santa Farm ilac Sanayii A.S. GEBKIM 41455 Dilovasi- Kocaeli – TURKEY, 3/Hemafer (syrup) 500  $\mu\text{g.mL}^{-1}$  contain 50 mg of Fe (III), UNI-PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece, 4/FERPLEX (vials) 400  $\mu\text{g.mL}^{-1}$ , contain 40 mg of Fe (III), ITALFARMACO S.A. –C/San Rafael, 3-28108 Alcobendas Madrid- (Spain), 5/ Hemafer (vials) 1000  $\mu\text{g.mL}^{-1}$ , contain 100 mg of Fe (III), UNI-PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece.), by draw up a certain volume (5 mL) from syrup

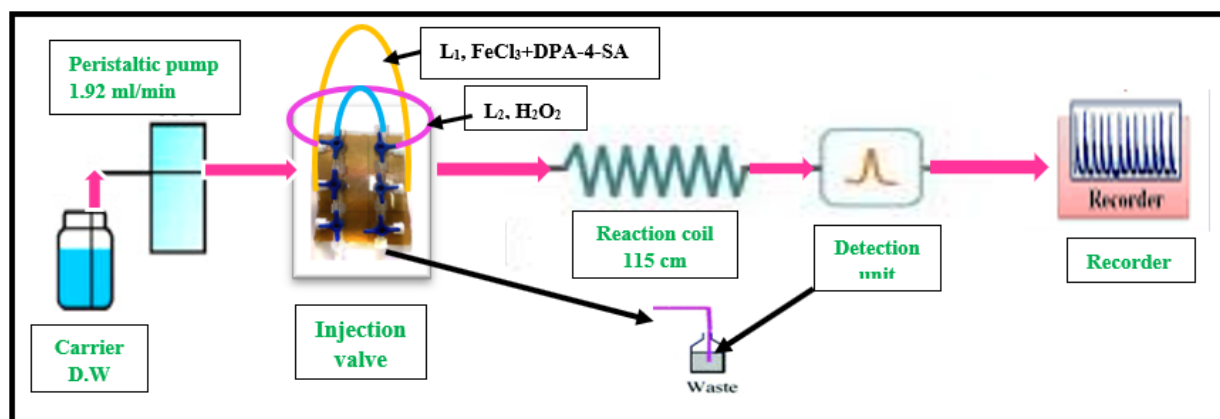


Figure 1: A single channel manifold of FIA-merging zones system for determination of Iron in drugs.

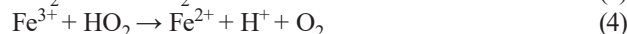
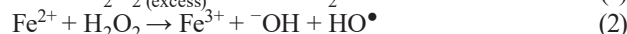
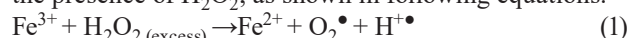
and vial solutions, made up to 50 mL with distilled water to prepared stock solution ( $100 \mu\text{g}\cdot\text{mL}^{-1}$ ).

### Mechanism of the Reaction

The proposed mechanism of the reaction between Fe (III) with DPA-4-SA to formed oxidation product (pink-purple color), has the absorbance maximum at 504 nm. Job's method was used, also mole ratio method have been investigated. The results obtained 1:3 (Iron (III): DPA-4-SA) and according to equation 2. According to research's<sup>18,19</sup> the oxidation of diphenylamine and it is derivatives occurs firstly by an irreversible reaction to colorless diphenylbenzidine and then oxidized reversibly to a blue-violet diquinonedimine and this oxidized product unstable and is destroyed by the irreversible process. In our study the proposed mechanism is based on many facts that follow:

1- The Fe (III) species in the chemical detection solution, in the presence of peroxide which catalyzed the oxidation of Fe (III), then decomposition, and reduction to Fe (II), while the Fe (II) reacted with the peroxide and oxidized to Fe (III) (equation 3). The redox process changed the color indicator from oxidized form to reduced form in the presence of the redox indicator diphenylamine.

2- The Fe(III) catalyzed the decomposition of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  when the solution was exposed to a substance containing  $\text{H}_2\text{O}_2$ . As a result, Fe (III) was reduced to Fe (II), which produced a Fenton reagent in this acidic medium and in the presence of  $\text{H}_2\text{O}_2$ , as shown in following equations:

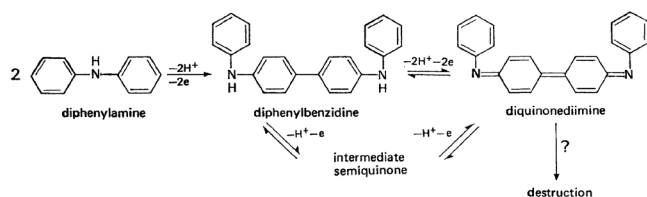


3- In the solution of reaction, diphenylamine acts as the redox color indicating agent responsible for producing the color species that resulted in the color changes observed. The chemical reaction which took place, as shown in Scheme I.

## RESULTS AND DISCUSSION

### Batch Method

Throughout preliminary experiments on the reaction of diphenylamine-4-sulfonic acid sodium salt ( $6 \times 10^{-5} \text{M}$ ) with  $\text{H}_2\text{O}_2$  (9M) and Ferric ion ( $30 \mu\text{g}\cdot\text{mL}^{-1}$ ). This reaction occurs in 25-30°C where transfer volumetric flask (20 mL) containing 2.4 mL of Fe(III)  $250 \mu\text{g}\cdot\text{mL}^{-1}$ , then added 0.24 mL of DPA-4-SA ( $6 \times 10^{-5} \text{M}$ ) in water bath the color formed (green) left for a few minutes and after we added 0.5 mL of 9M  $\text{H}_2\text{O}_2$  solution, the solution had been diluted to mark with distilled water, the



**Scheme I:** The proposed mechanism of the complex formed for determination of iron (III) as ferric chloride with DPA-4-SA and  $\text{H}_2\text{O}_2$ .

pink-purple complex was created and measured at  $\lambda_{\text{max}}$  504 nm against reagent blank after 5 min and reagent blank against distilled water, as shown in Figure 2.

### Optimization of the Experimental Conditions

All conditions were explored by adjusting one factor and keeping the other constant by monitoring the effect generated on the colored product's absorbance strength. The concentration  $25 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe (III) was taken in the final volume of 20 mL, absorbance was measured at 504 nm. The temperature effect was studied with the range 20–60°C. The absorbance of reaction increased with increase of temperature. A temperature of 25–30°C was adopted because gave good absorbance and easy to maintained.

The effect of concentration of DPA-4-SA ( $6 \times 10^{-5}$ – $3 \times 10^{-3}$ ) M on the resulting absorbance a pink-purple complex has been monitored. Throughout the experiment a variable concentrations of DPA-4-SA reagent were used. The absorbance increases with increasing concentration of DPA-4-SA, shows  $1 \times 10^{-3}$  M had was chosen to be the optimum reagent concentration for further experiments; as shown in Figure 3a.

The effect of a concentration of  $\text{H}_2\text{O}_2$  was investigated on the absorbance of the colored product in the range 1 to 7 M. The absorbance was increase with increasing of hydrogen peroxide. A concentration of 7 M gave the highest absorbance and was chosen for further experiments; as shown in Figure 3b.

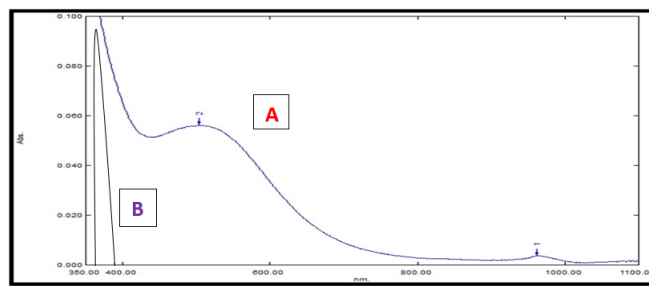
### Calibration curve of classical method

The most suitable reaction conditions proved to create a calibration curve for Fe (III) determination as ferric chloride (2, 3, 5, 8, 12, 15, 20, 25, 30)  $\mu\text{g}\cdot\text{mL}^{-1}$  were analysis with fixed the other parameters. A set of volumetric flask (10 mL) were transfer contain 2 mL of DPA-4-SA reagent ( $1 \times 10^{-3}$ ) M followed by increasing volumes of standard solution  $250 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe (III), keep it for 5 min in water bath at 30°C and followed by adding (0.5 mL) of  $\text{H}_2\text{O}_2$ . The solutions were diluted to mark using distilled water. Standard curve and linear range is built (2-30)  $\mu\text{g}\cdot\text{mL}^{-1}$  for determination of ferric, Figure (3c) shows that.

### Flow injection analysis /Merging Zone technique

#### Optimization of the FIA System Conditions (Chemical and Physical Variables)

For achieve a consistent iron analysis, the batch method was adopted to developed CFIA procedure via the CFIA parameters



**Figure 2:** A- UV-VIS spectrum of colored product formed against reagent blank.

B - Reagent blank against distilled water. ( $30 \mu\text{g}\cdot\text{mL}^{-1}$  of Fe (III))

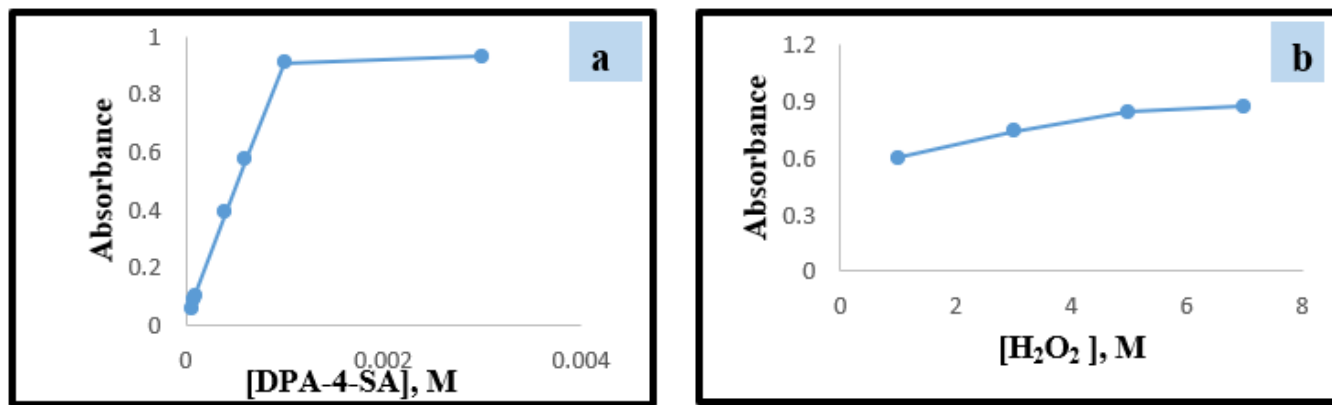


Figure 3: a- Effect of DPA-4-SA concentration, b- Effect of H<sub>2</sub>O<sub>2</sub> concentration, on the reaction between Fe (III) with DPA-4-SA reagent.

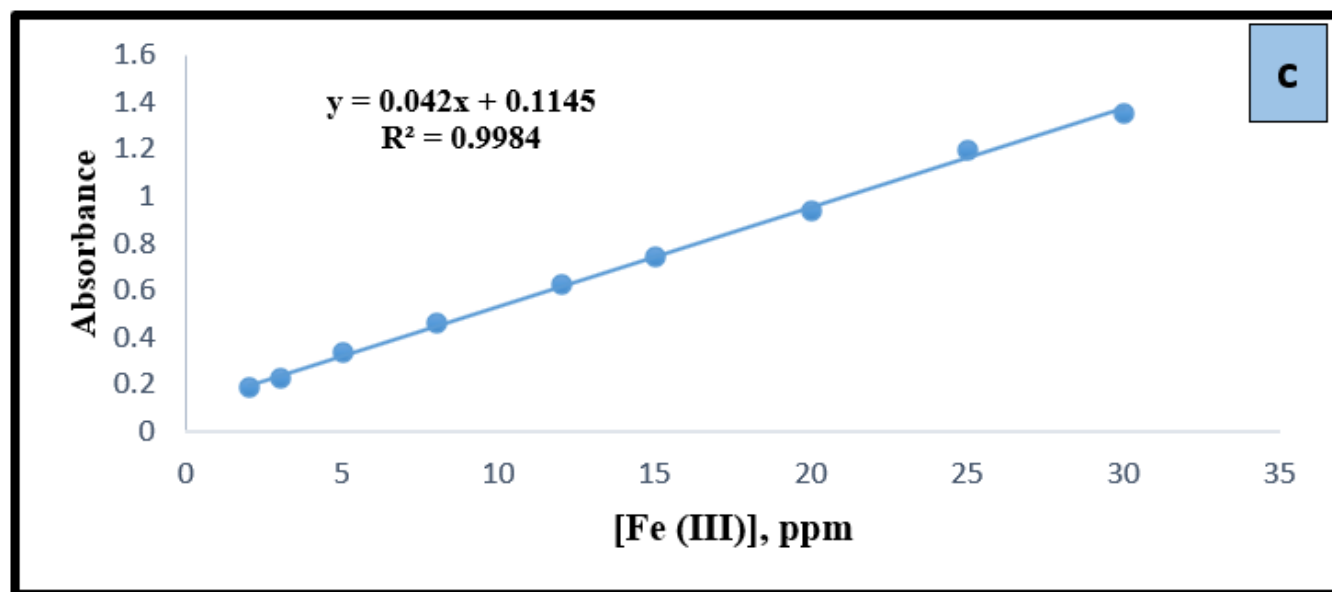


Figure 3: c- linear calibration curve of Fe (III) with DPA-4-SA using H<sub>2</sub>O<sub>2</sub> as oxidizing agent across batch method.

were studied to gain maximum sensitivity and reproducibility centered on optimum batch measurement experimental conditions.

Effect of the varying DPA-4-SA concentration was investigated in the range ( $2 \times 10^{-4}$ – $1.8 \times 10^{-3}$ ) M for determination of Fe (III) ( $70 \mu\text{g.mL}^{-1}$ ) by using six-three way homemade injection valve, using distilled water as carrier of chemicals. The results obtained that concentration  $1.5 \times 10^{-3}$  M had the highest absorbance value which calculated by average peak height in mV ( $n = 3$ ), as shown in Figure 4a.

The influence of H<sub>2</sub>O<sub>2</sub> concentration was investigated in a range (1–7) M for estimation of  $70 \mu\text{g.mL}^{-1}$  Fe (III), the absorbance increasing with concentration of H<sub>2</sub>O<sub>2</sub> increase. A concentration 7M H<sub>2</sub>O<sub>2</sub> that gave the best and highest peak of absorbance was selected to be optimum concentration of hydrogen peroxide to form the color product which calculated by average peak height in mV ( $n = 3$ ), as shown in Figure (4b). The optimal flow rate was examined by measuring peak height at flow rate 1.6-1.92-2.28-2.64 and 2.86 mL.min<sup>-1</sup>. Figure (4c) shows that the maximum absorbance value was at a flow rate of 1.92 mL.min<sup>-1</sup> with lower dispersion. In a lower flow rate

the dispersion will be the highest level while in a greater flow rate, the reaction may be not complete.

The volumes of the sample with reagent and hydrogen peroxide injected were tested using a variety of sample with reagent (L<sub>1</sub>) and H<sub>2</sub>O<sub>2</sub> (L<sub>2</sub>) volumes {58.875, 78.50, 98.125, 127.563 and 147.188} μL, ideal volumes that provided maximum response in mV; 78.50 μL for L<sub>1</sub> and 58.875 for L<sub>2</sub> were used in subsequent experiments as shown in Figure (4d, 4e).

The effect of length of the reaction coil was evaluated by using a coil length (85, 100, 115, 180 and 250) cm having (I.d. 2 mm) which was put directly in the flow technique after the injection valve. Maximum length absorbance was obtained at 115 cm. With the other length of the reaction coil, the absorbance decreased up to 115 cm, as shown in Figure (4f).

#### Calibration Curve

A linear calibration graph for evaluating individually within the range of (3-75) μg mL<sup>-1</sup> of Fe (III) solutions had been prepared with sufficient dilution of stock solution ( $250 \mu\text{g.mL}^{-1}$ ) injected into L<sub>1</sub> with DPA-4-SA reagent ( $1 \times 10^{-3}$ M), and a solution of H<sub>2</sub>O<sub>2</sub> (7 M) was injected into L<sub>2</sub> using the suggested

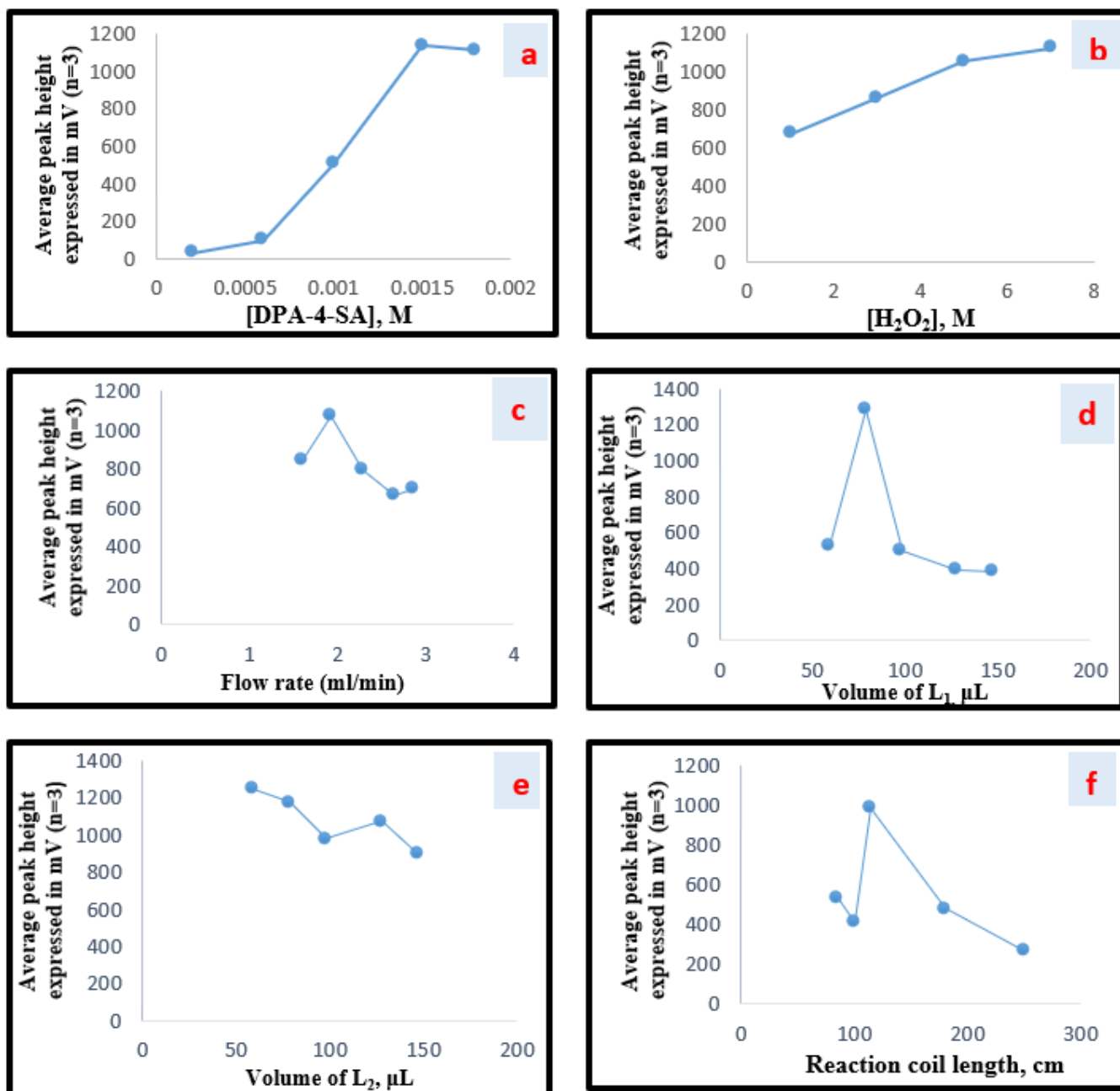


Figure 4: a- Effect of DPA-4-SA concentration, b- Effect of H<sub>2</sub>O<sub>2</sub> concentration, c- Effect of flow rate, d-Effect of (sample and reagent) volume, e-Effect of H<sub>2</sub>O<sub>2</sub> volume, f- Effect of reaction coil length, on the absorbance of reaction product using the developed FIA method.

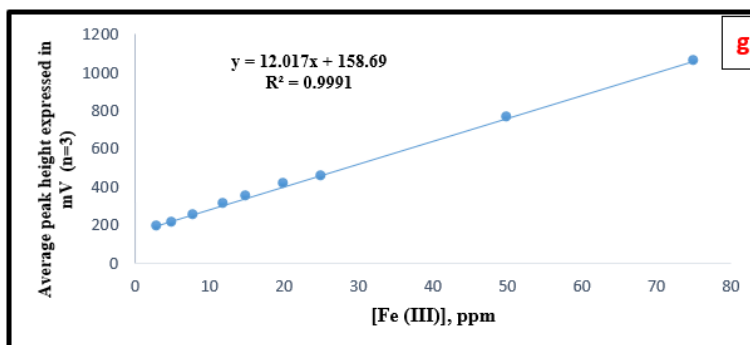


Figure 4: g- Linear calibration graph for determination of ferric ion using of DPA-4-SA as a redox indicator in present of hydrogen peroxide was measured at  $\lambda_{max}$  504 nm, using the developed FIA method.

**Table 1:** Summary of optical characteristics of the proposed method compared with the classical method for estimation of ferric ion as ferric chloride.

Parameters	Batch method	CFIA method
Linear range ( $\mu\text{g.mL}^{-1}$ )	2-30	3-75
Correlation coefficient, $r^2$	0.9984	0.9991
Regression equation $y = b x + a$ ; $y = \text{absorbance}$ , $x = \text{concentration } (\mu\text{g.mL}^{-1})$	$y = 0.042x + 0.1145$	$y = 12.017x + 158.69$
Linearity ( $r^2\%$ )	99.84	99.91
Slope (b), $\text{L.mg}^{-1}$	0.042	12.017
Intercept (a = $y - b x$ )	0.1145	158.69
*Relative Standard deviation (RSD %)	1.72 (at 15 ppm)	0.03 (at 25 ppm)
Standard deviation of the residuals, $S_{y/x}$	$3.7343 \times 10^{-3}$	4.0137
Standard deviation of the slope, $S_b$	$2.5169 \times 10^{-4}$	0.0395
Standard deviation of the intercept, $S_a$	$7.1081 \times 10^{-3}$	3.2384
Confidence limit of intercept (a) = $a \pm S_a$	$0.1145 \pm 0.0131$	$158.69 \pm 5.9458$
Confidence limit of slope (b) = $b \pm S_b$	$0.042 \pm 4.62 \times 10^{-4}$	$12.017 \pm 0.0726$
Molar absorptivity ( $\epsilon$ ) ( $\text{L.mole}^{-1}.\text{cm}^{-1}$ ) $\epsilon = b \times M \times 1000$	6812.568	1949205.468
Sandell's sensitivity (S) ( $\mu\text{g cm}^{-2}$ ) $S = M / \epsilon$ , M=M.wt of drug	0.02381	$8.3215 \times 10^{-5}$
Sample throughput ( $\text{h}^{-1}$ )	10	82
Limit of quantification (LOQ), $\mu\text{g.mL}^{-1}$	5.34	$3.19 \times 10^{-3}$
Limit of detection (LOD), $\mu\text{g.mL}^{-1}$	1.60	$9.57 \times 10^{-4}$
K = Stability constant ( $\text{L}^2.\text{mol}^{-2}$ or $\text{M}^{-2}$ ) [20]	$0.263 \times 10^{30}$	-----

\*Average of three determinations

**Table 2:** Interferences effect of some ions on Ferric determination  $8 \mu\text{g.mL}^{-1}$ 

Interference	Conc. $\mu\text{g.mL}^{-1}$	Peak height mV	Erel. %	Rec %	Interference	Conc. $\mu\text{g.mL}^{-1}$	Peak height mV	Erel. %	Rec %
Standard	8	228	0.00	100%		4	228	0.00	100.00
	4	224	-1.75	98.25	$\text{Cr}_2\text{O}_7^{-2}$	8	225	-1.31	98.69
Na (I)	8	225	-1.31	98.69		16	222	-2.63	97.37
	16	222	-2.63	97.37		4	226	-0.88	99.12
	4	222	-2.63	97.37	$\text{CrO}_4^{-2}$	8	226	-0.88	99.12
Cu (II)	8	220	-3.50	96.49		16	224	-1.75	98.25
	16	220	-3.50	96.49		4	226	-0.88	99.12
	4	225	-1.31	98.68	$\text{NO}_3^{-1}$	8	223	-2.19	97.81
Zn (II)	8	225	-1.31	98.68		16	219	-3.94	96.06
	16	220	-3.50	96.49		4	230	0.88	100.88
	4	223	-2.19	97.81	$\text{IO}_4^{-1}$	8	227	-0.43	99.56
Pb (II)	8	220	-3.50	96.49		16	228	0.00	100.00
	16	220	-3.50	96.49		4	225	-1.31	98.68
	4	230	0.88	100.80	$\text{Br}^{-1}$	8	224	-1.75	98.25
Bi (III)	8	228	0.00	100.00		16	220	-3.50	96.49
	16	226	-0.88	99.12		4	234	2.63	102.63
	4	229	0.43	100.43	$\text{Cl}^{-1}$	8	230	0.88	100.80
Al (III)	8	227	-0.43	99.57		16	231	1.31	101.31
	16	222	-2.63	97.37		4	227	-0.43	99.56
	4	225	-1.31	98.68	$\text{I}^{-1}$	8	220	-3.50	96.49
$\text{MnO}_4^{-1}$	8	227	-0.43	99.56		16	220	-3.50	96.49
	16	232	1.75	101.75		4	226	-0.88	99.12
	4	224	-1.75	98.25	$\text{C}_2\text{O}_4^{-2}$	8	221	-3.07	96.93
$\text{CO}_3^{-2}$	8	221	-3.07	96.93		16	218	-3.47	96.53
	16	220	-3.50	96.49					

**Table 3:** Applications of the proposed methods compared with official method (USP) for estimation of Iron (III) in medicinal formulations.

Iron (III) medicinal formulations	Proposed methods								USP Official Method recovery%
	Batch				CFIA/ merging zone				
	Present conc. $\mu\text{g.mL}^{-1}$	*Rec %	Mean	*RSD %	Present conc. $\mu\text{g.mL}^{-1}$	*Rec %	Mean	*RSD%	
Santafer (syrup) 50mg Santa Farm ilac Sanayii A.S. GEBKIM 41455 Dilovasi-Kocaeli – TURKEY	10	96.40	97.87	3.47	20	100.88	100.17	0.06	100.04
	15	99.33		0.17	50	99.45		1.39	
Hemafer (syrup) 50mg UNI PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece	10	96.63	98.52	3.13	20	98.53	98.95	0.18	98.90
	15	100.40		0.24	50	99.37		1.71	
FERPLEX (vials) 40mg ITALFARMACO S.A. –C/San Rafael, 3- 28108 Alcobendas Madrid- (Spain)	10	91.00	95.30	4.54	20	100.97	99.23	0.05	99.43
	15	99.60		2.47	50	97.48		1.98	
Hemafer (vials) 100mg UNI-PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece.	10	97.30	98.22	1.18	20	98.73	97.27	0.12	97.75
	15	99.14		0.05	50	95.80		3.20	
Hemafer (tablets) 100mg UNI-PHARMA KLEON TSETIS Pharmaceutical Laboratories S.A. Greece	10	99.30	100.32	1.19	20	100.50	99.83	0.70	99.30
	15	101.33		0.03	50	99.15		1.65	

$t_{\text{crit}}=2.31$  for  $(n_1=n_2=5)$ ,  $(n_1+n_2-2=8)$

$F_{\text{crit}}=6.388$  for  $(n_1-1=n_2-1=4)$  at 95% confidence

\*Average of four determinations

method under the optimum physical and chemical conditions for determination of ferric chloride, as shown in Figure 4g.

#### Analytical Parameters

The analytical features like linear range, detection limit, correlation coefficient for each approach and relative standard deviation<sup>20,21</sup> were calculated at the improved conditions of the developed FIA contrasted with batch method, as shown in Table 1. These small points suggested that the developed FIA was highly reproducible and repeatable in comparison to the batch method for determination of Fe (III) in drugs. FIA/MZ was simple and easier than the classical method because that was rapid (sample throughput of 82 s/h), linear scale of calibration curve were gotten. Selectivity, sensitivity, economy of samples and reagents, throughput, cost-effectiveness, simplicity and eco-friendliness as possible.

#### Study of Interferences

The selectivity of proposed method (Flow injection analysis/ MZ) was examined. A sample of pure iron (III) spiked with half, equal and double fold excess of selected interference ions excipients were analyzed. The results mentioned in Table 2 showing that all ions tested have a major impact on iron (III) determination based on recovery values and the small values of error that result from the concentration of interference ion that effect on the peak height of Fe (III) with increase the concentration of some interference ions, the peak height of ferric will be decrease.

#### Analysis of Ferric in Pharmaceutical Samples

The suggested procedure was carried out to the analysis of some pharmaceutical formulations that contain Fe (III). Standard method was carried out by preparing a series of solution from each pharmaceutical drug by transferring 10 mL of 100 ppm of pharmaceutical drug to each of standard flask (20 mL); followed by the addition of increasing concentrations from 250 ppm of  $\text{FeCl}_3$  solution. The statistical comparison between the proposed method with official method (USP)<sup>22</sup> using the student t-test and F-test.<sup>23</sup> Five pharmaceutical drugs were analyzed and results were mathematically treated, shown in Table 3. By applying the F-test and t-test at 95% confidence limits, the result acquired by the proposed methods. Estimation of F-test valuations were (1.7543) and (1.9972), t-tests were 0.0182 and 0.5624 respectively for FIA and batch methods, the results refers to, there is no significant in accuracy between the proposed method with the official method, therefore was adopted for determination of Fe (III) in Iron drugs.

#### CONCLUSION

Analysis via a green flow injection / MZ method was able to evaluate the ferric in fine forms of pharmaceutical drug with the level  $\mu\text{g.mL}^{-1}$ , because the proposed homemade CFIA/MZ method is automation process provides feasibility, cheap and sensitive. Main advantages of this method are its large dynamic range, adequate sensitivity and its suitable for routine apply in pharmaceutical specify control laboratories due to

their facility and result in diminishing reagents consumption when compared with batch method. The procedure has good linearity, high analytical frequency with throughput 82 sample/h with non-interference of syrup additives makes the method suitable this allows estimating the drug in syrup complex quality control for routine Iron analysis. Provided that the measurement requires time as an experimental variable, care is required to ensure that reagent mixing occurs at times in order to achieve highly accurate results using a manual method. These disadvantages in the manual method can be overcome by the use of flow-injection analysis (FIA), in which the reaction time can be easily regulated by changing the solution flow rate and reaction coil length.

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