New Approach for The Turbidimetric Determination of Iron(III) in Drug Samples of Different Origins Using Homemade Linear Array Ayah 5SX1-T-1D-CFI Analyser

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Abstract

A new Turbidimetric method characterized by simplicity, accuracy and speed for determination of iron(III) in drug samples by continuous flow injection analysis. The method was based on the formation of complex for iron(III) with 8-hydroxyquinoline in presence of ammonium acetate as a medium for the formation of deep green precipitate and this precipitate was determined using homemade Linear Array Ayah-5SX1-T-1D continuous flow injection analyser. The optimum parameters were 2.6 mL.min^{-1} flow rate using H_2O as a carrier, 1.9 mL.min^{-1} (14 mmol.L^{-1}) ammonium acetate, 2.4 mL.min^{-1} (14 mmol.L^{-1}) 8-hydroxyquinoline, 60 μL sample volume and open valve for the purge of the sample segment. Data treatment shows that linear range 0.1-8.0 mmol.L^{-1} while L.O.D 4.8×10^{-9} M/sample equivalent to 16.1 pg/sample from the stepwise dilution for minimum concentration of lowest concentration in linear dynamic range of the calibration graph. The correlation coefficient (r) was 0.9911 while percentage linearity (%r^2) C.O.D was 98.24%. R.S.D.% for the repeatability (n=6) was <1% for determination of iron(III) with concentration 4 and 8 mmol.L^{-1}. The method was applied successfully for the determination of iron(III) in pharmaceutical preparations. Using paired t-test between the newly developed method and official method; shows that there were no significant differences between either methods. On this basis the new method can be accepted as an alternative analytical method for determination of iron(III) in pharmaceutical samples.

Key words: Iron(III), 8-hydroxyquinoline, turbidimetric, flow injection analysis, iron-dextran

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نمط جديد لقياس التعكرية لتقدير الحديد(III) في مناشئ مختلفة من العينات الدوائية باستخدام المحلل الحقن الجرياني المستمر Linear Array Ayah 5SX1-T-1D-CFI Analyser المصنوع محلياً

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الخلاصة

استحدثت طريقة بسيطة وسريعة ودقيقة لتقدير الحديد (III) في المستحضرات الصيدلانية من خلال اقتران الحقن الجرياني المستمر مع قياس التعكرية . تستند الطريقة على تكوين راسب بين ايون الحديد (III) والكاشف

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8−هيدروكسي كوينولين بوجود خلات الا مونيوم كوسط، اذ ينتج راسب اخضر غامق، قدر الراسب المتكون باستخدام جهاز Linear Array Ayah 5SX1−T−1D−Continuous Flow Injection Analyser المصنوع محلياً. درست الظروف الفضلى للتفاعل، اذ استخدم 2.6 مل.دقيقة$^{-1}$ كسرعة جريان للتيار ناقل H_2O، 1.9 مل.دقيقة$^{-1}$ (14 مللي مول.لتر$^{-1}$) كتيار لخلات الامونيوم، 2.4 مل.دقيقة$^{-1}$ (14 مللي مول.لتر$^{-1}$) 8−هيدروكسي كوينولين، واستخدم 60 مايكرولتر كحجم لانموذج المحقن مع ترك صمام الحقن مفتوح لخروج مقطع الانموذج . بينت معالجة البيانات المستحصلة المدى الخطي لتقدير الحديد(III) 0.1−8 مللي مول .لتر$^{-1}$ بينما حدود الكشف 4.8×10^{-9} مول.لتر$^{-1}$ والمكافئ الى 16.1 بيكوغرام من التخفيف التدريجي لاقل تركيز في منحني المعايرة . معامل الارتباط (r) 0.9911 والنسبة المئوية للخطية هي 98.24% والانحراف القياسي النسبي المئوي (n=6) اقل من 1% لتركيزين 4 و8 مللي مول.لتر$^{-1}$ لمحلول الحديد(III). طبقت الطريقة لتقدير الحديد (III) في المستحضرات الدوائية . استخدام اختبار t−المزدوج لاجراء مقارنة بين الطريقة المستحدثة والطريقة القياسية ، وجد انه لا يوجد فرق جوهري بين الطريقتين و بذلك يمكن استخدام الطريقة المستحدثة كطريقة بديلة لتقدير الحديد(III) في النماذج الدوائية.

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1- Introduction

Iron is regarded as fourth abundant element in the earth crust. It is found in two oxidation states II and III [1]. Iron is an essential and play an important roles for many metabolic functions that occur in all living cells of plants and animals [2].

The determination of iron in analytical chemistry has become a routine procedure because of its importance in normal living life. There are many techniques reported for the determination of iron in the literature. Some of the most commonly used methods include spectrophotometry [3-10], atomic absorption spectrophotometry [11-14], flow injection method [15-20], inductively coupled plasma [21,22], HPLC [23-25], chemiluminescence [26,27]. This work, describes a simple turbidmetric flow injection method that have been developed for the determination of iron(III) in pharmaceutical preparation. The method uses reaction of Fe(III) with 8-hydroxyquinoline (8-HQ). 8-Hydroxyquinoline exhibit a chelating property to metal ions. Chelation might probably accomplished by amine and hydroxyl groups present in 8-HQ molecules.

2- Experimental
2-1- Chemicals

All chemicals used were of analytical reagent grade. Distilled water was used throughout this work. Iron(III) chloride anhydrous stock standard solution ($FeCl_3$, 162.21, Fluka, 100 mmol.L^{-1}) was prepared by dissolving 1.6221 g/100 ml distilled water. A stock solution of 8-hydroxyquinolin (BDH, 100 mmol.L^{-1}) 1.4516g in 100 ml of 2 mol.L^{-1} glacial acetic acid. A 1 mol.L^{-1} ammonium acetate as stock solution (CH_3COONH_4, 77.08, BDH) was prepared by dissolving 38.54g in 500 ml distilled water.

2-2- Apparatus and Manifold

The flow system shown in Figure (1) was used for the determination and detection of iron(III). A four channels, variable speed peristaltic pump (Ismatec, Switzerland), the 6-port injection valve (Rheodyne, U.S.A) with a sample loop (0.5 mm i.d., Teflon, variable length) used for sample injection. The instrument response was measured by Ayah 5SX1-T-1D-CFI analyser [28] (homemade) using super white Light Emitting Diode (LED) as source with a solar cell detector. The output signals was recorded by x-t potentiometric recorder (KOMPENSO GRAPH C-1032) Siemens (Germany) and Digital AVO-meter (auto range) (0.00-2000 mV) (China). Peak height was measured for each signal. UV-Vis spectra were measured on UV-Vis. spectrophotometer digital double-beam SHIMADZU type 1800 (Japan).
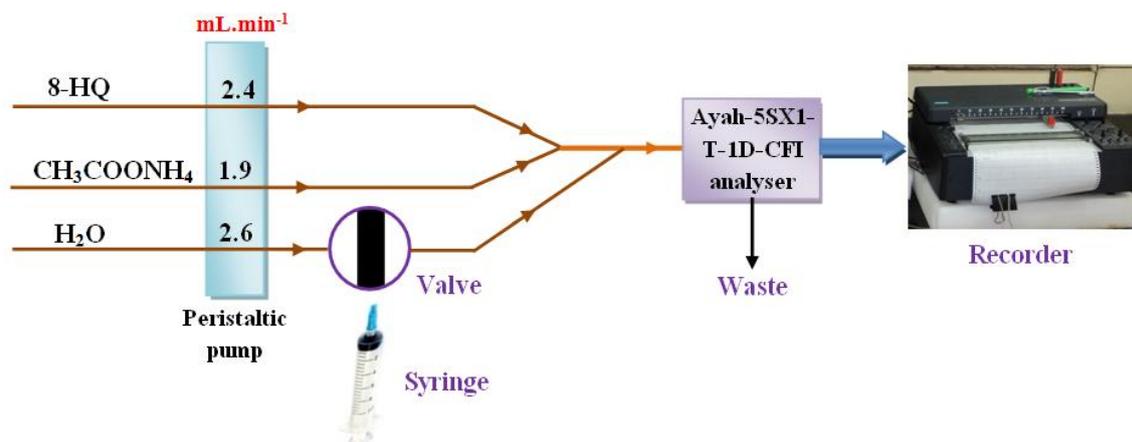
Figure 1- Schematic diagram of flow injection analysis system used for determination of iron(III)

2-3- Methodology

The whole reaction manifold system for iron(III) determination via precipitation with 8-hydroxyquinoline (8-HQ) shown in Figure (1). The manifold system is composed of three lines: first line supplies the (8-HQ) (14 mmol.L-1) solution at 2.4 mL.min^{-1} flow rate, while the second line is for ammonium acetate (14 mmol.L^{-1}) at 1.9 mL.min^{-1} flow rate. Both lines mixes together at a multi Y-junction made from methyl methacrylate polymer as shown in Figure (2). The third line represent the carrier stream (distilled water) leading to the injection valve, which allows the use of 60 µL and a flow rate of 2.6 ml.min^{-1} (loop length 30 cm with 0.5 mm I.D). Both out coming lines meet at a junction as shown in Figure 2. the attenuation of incident light peak of the resulting deep green precipitate is followed using Ayah-5SX1-T-1D-CFI anlayser and the variation of response was monitored using super white Light Emitting Diode (LED). Each solution was assayed in triplicate. The proposed suggested mechanism for complex formation is shown in Scheme no.1.
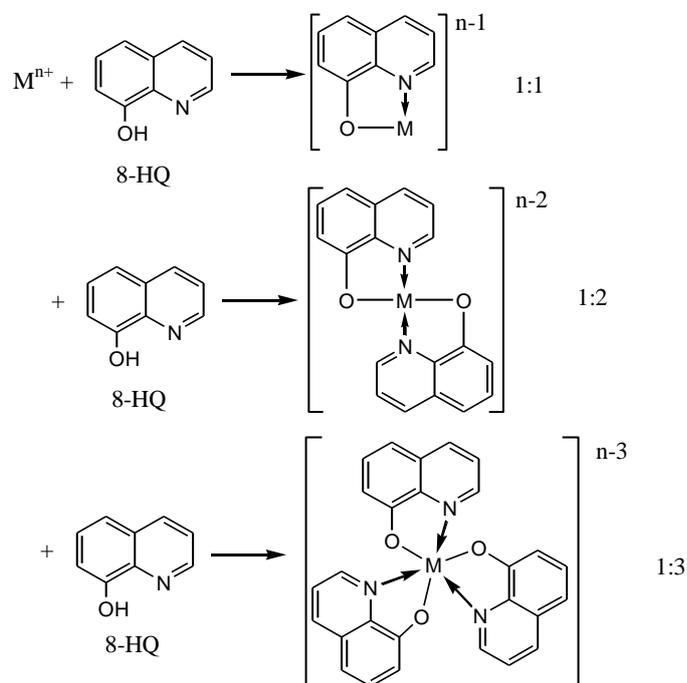
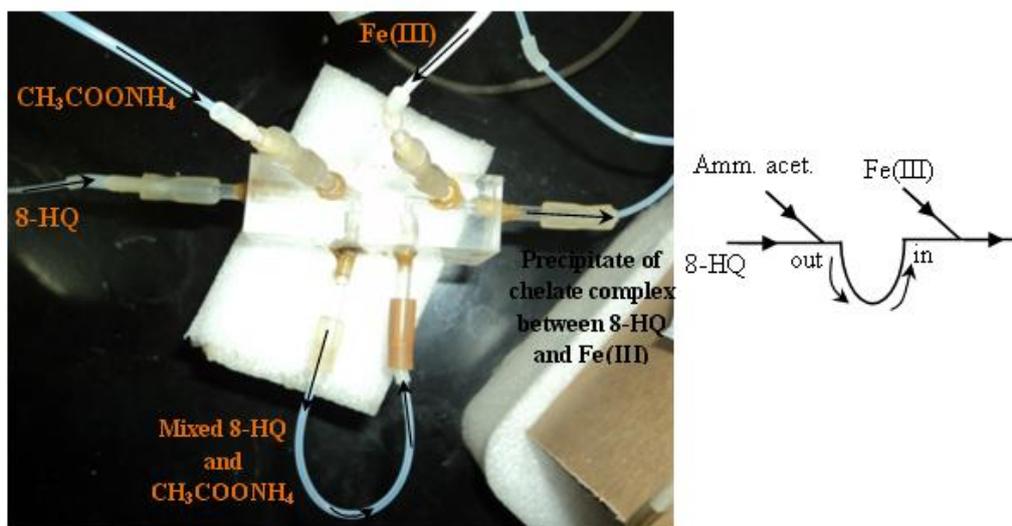**Scheme 1-** Proposed mechanism of complex formation

Figure 2- New design of multi Y-junction used for determination of iron(III) as part of the manifold

3- Results and Discussion
3-1- Spectroscopic Study

A dilute aqueous solution 8-hydroxyquinole (1 mmol.L^{-1}) precipitating agent when mixed with ammonium acetate (1 mmol.L^{-1}) as reaction medium followed by the addition of iron(III), an intense deep green precipitate product was formed immediately, the product shows a maximum at 460, 601 nm against reagent blank as shown in Figure (3-A) while deep green precipitate appear λ_{max} at 454, 604 nm when changing order of addition by ammonium acetate (1 mmol.L^{-1}) added to iron(III) (1 mmol.L^{-1}) then addition of 8-hydroxyquinoline (1 mmol.L^{-1}) as shown in Figure (3-B).

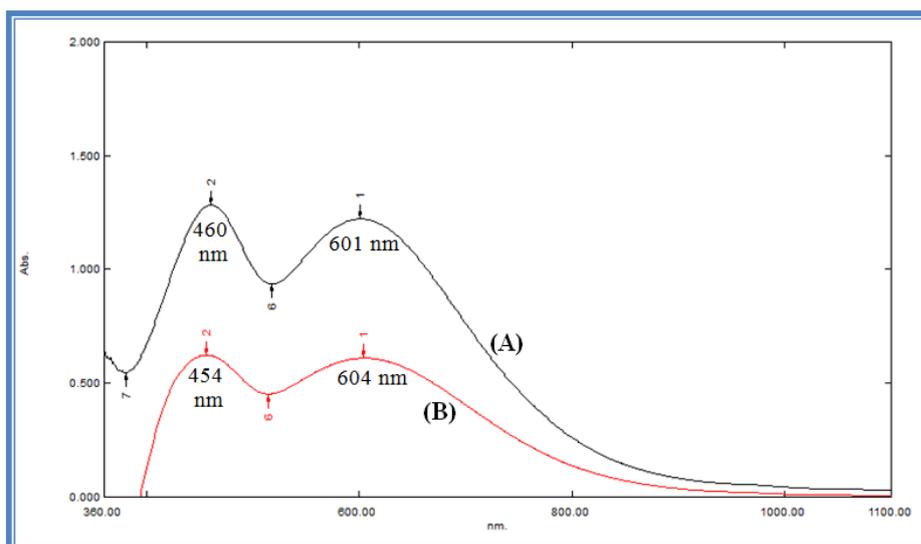**Figure 3-** UV-Vis. Spectrophotometric of bluish-black species formed against reagent blank, order of addition was as; (A) 8-hydroxyquinoline (1 mmol.L^{-1})+CH$_3$COONH$_4$ (1 mmol.L^{-1})+iron(III) (1 mmol.L^{-1}). (B) iron(III) (1 mmol.L^{-1}) +CH$_3$COONH$_4$ (1 mmol.L^{-1})+ 8-hydroxyquinoline (1 mmol.L^{-1}).

3-2-Optimization of Experimental Conditions

A series of experiments were conducted to establish the conditions for the production of maximum repeatable response with good sensitivity for the reaction product. The chemical variables such as concentration of reagents used for the precipitation reaction and physical variables including addition reaction coil1in manifold, intensity of incident light, flow rate, sample volume and allowed permissible sample purge time were investigated respectively.

3-2-1- Chemical Variables
**3-2-1-1- Effect of 8-hydroxyquinoline Concentration**

A series of solutions were prepared for the range of 3-16 mmol.L^{-1} using preliminary concentration of ammonium acetate 200 mmol.L^{-1}, 10 mmol.L^{-1} of iron(III), sample volume of 60 µl on the carrier stream (distilled water) and 1100 mV intensity of incident light, each measurement was repeated for three successive times. Figure (4-A) was obtained explaining the increase in the attenuation of incident light with increase of 8-HQ concentration, followed by a stability in attenuation of incident light. At high concentration of 8-HQ (i.e. > 14 mmol.L^{-1}) represent to the same growth of particle with increase 8-HQ concentration, therefore 14 mmol.L^{-1} was chosen as optimal concentration of 8-HQ. Figure (4-B) show the profile peak when variation 8-HQ concentration.
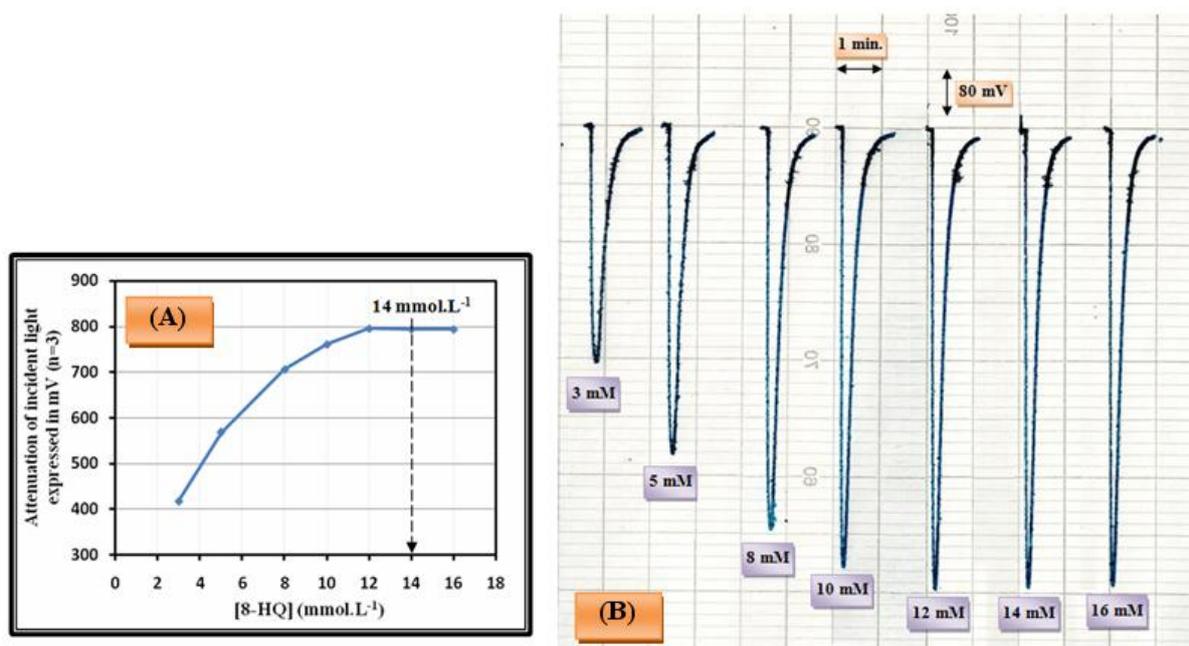
Figure 4- (A) Effect of variation of 8-HQ concentration on precipitation iron(III) for 8-HQ-CH$_3$COONH$_4$-Fe(III). (B): Response profile

3-2-1-2- Effect of CH$_3$COONH$_4$ Concentration

Using optimum concentration of 8-HQ solution 14 mmol.L^{-1}. Series of diluted solutions of ammonium acetate (0-16) mmol.L^{-1} were prepared and concentration of iron(III) 10 mmol.L^{-1} was used. Using sample volume of 60 µL, 1100 mV as the intensity of incident light. Figure 5 shows an increase in attenuation of incident light with the increase of CH$_3$COONH$_4$ for the range 0 to 14 mmol.L^{-1}, and even at a concentration > 14 mmol.L^{-1}. It was noticed that a stable of the obtained response with increase of CH$_3$COONH$_4$ concentration. Therefore, 14 mmol.L^{-1} was chosen as the optimum concentration of ammonium acetate as medium for the formation of precipitate.
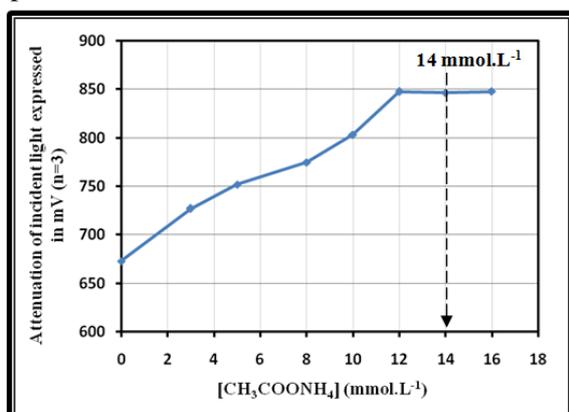
Figure 5- Effect of variation of ammonium acetate concentration on attenuation of incident light for 8-HQ-CH$_3$COONH$_4$-Fe(III) system

3-2-2- Physical Variables
### 3-2-2-1- Effect of Coil Addition
Using optimum concentration for precipitation system with iron(III) solution (10 mmol.L^{-1}) with 60μL as the injected sample volume, 1100 mV as intensity of incident light at a variable coil length (0-40) cm with i.d. 1mm as shown in Table no.1. It was noticed that 10 cm (314 μL) reaction coil was used as a more reproducible and more sensitive measurements.

Table 1- Effect of variable coils length on attenuation of incident light for 8-HQ-CH$_3$COONH$_4$-Fe(III) reaction system for determination of iron(III)

Coil length (cm)	Coil volume (μL)	Attenuation of incident light $\bar{y}i$ (n=3) (mV)	S.D.	%RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$	arrived time of precipitate to detector (sec.)	Dilution factor	Relative dilution factor
Without	0	847.67	1.53	0.180	847.67±3.79	6	15.83	1.00
10	314	873.50	1.32	0.151	873.50±3.29	9	23.25	1.47
20	628	875.83	1.26	0.144	875.83±3.13	12	30.67	1.94
30	942	873.17	1.04	0.119	873.17±2.59	15	38.08	2.41
40	1256	873.33	1.53	0.175	873.33±3.79	18	45.50	2.87

3-2-2-2- Variation of Incident Light Intensity
Using the system for determination iron(III) via 8-HQ (14 mmol.L^{-1})-CH$_3$COONH$_4$ (14 mmol.L^{-1})-Fe(III) (10 mmol.L^{-1}), 60 μL sample volume with (2.6, 1.9, 2.4) mL.min^{-1} flow rate for carrier, ammonium acetate and 8-HQ respectively. A variable intensity of incident light expressed in mV (750-2200) was used for this study, the plot of attenuation of incident light vs. change in intensity of incident light shown in Figure (6-A). While Figure (6-B) shows profile and response peaks when variation intensity of incident light.
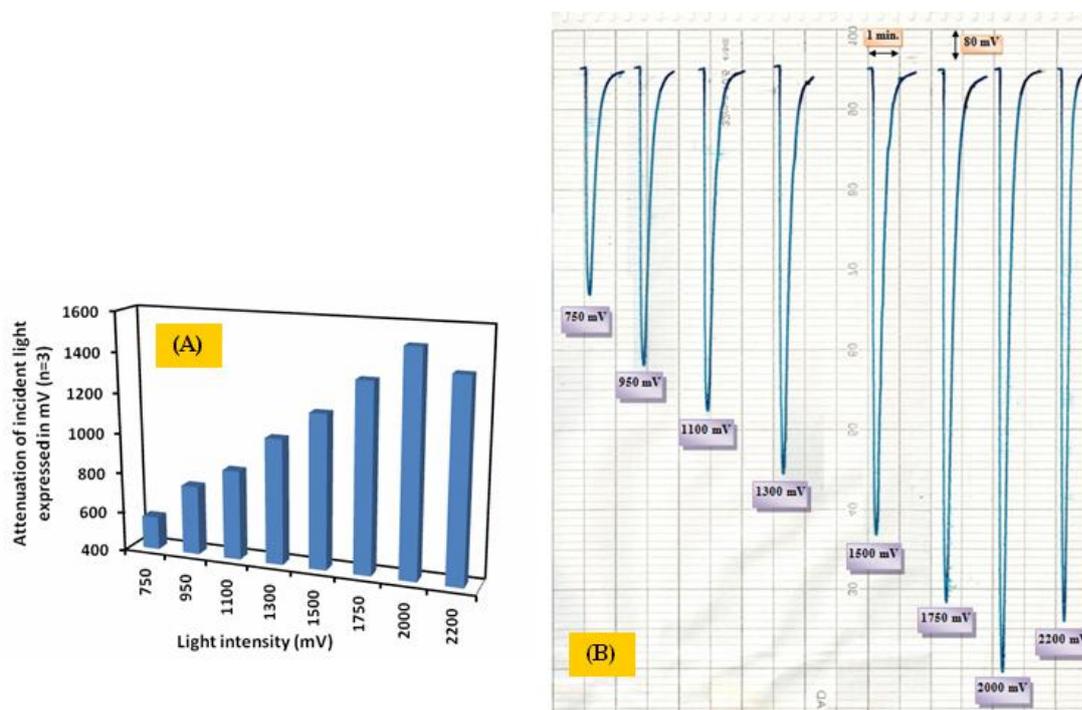
Figure 6- (A) Variation of incident light vs. light intensity expressed in mV. (B) Output response profile of variation of light intensity versus energy transducer of Linear Array Ayah-5SX1-T-1D-CFI analyser

Figure 6 shows the continuation for the increase in attenuation of incident light with increase of intensity of incident light up to 2000 mV then followed by a decrease in attenuation of incident light with increase of intensity of incident light. The decrease in attenuation of incident light above 2000 mV was attributed to the effect of transparency of fine particulate with the increase of incident power of radiation. This effect can be prominent when very fine particle is formed.

3-2-2-3- Effect of Flow Rate

A set of experiments were carried out for the optimization of flow rate of carrier stream (distilled water) using 1 mm i.d. tube, ammonium acetate (14 mmol.L^{-1}) and 8-HQ (14 mmol.L^{-1}) using 0.75 mm i.d. silicon rubber tube; using 60 μL as sample volume and 2000 mV as intensity of incident light at a variable flow rate as tabulated in Table no.2.

It was noticed that at low flow rate there was an increase in dilution and dispersion which might cause an increase in peak base width (Δt_B) as shown in Figure 7. While at higher speed > 30 (indication approximate), although the effect of physical parameter was not very crucial on the responses. Obtaining regular response and sharp maxima, but it was not very high due to the departure of the reactants from measuring cell prior to the completion of precipitation reaction and the precipitate remained for a very short time in the measuring cell, therefore an indication approximate of 30 which corresponding to a flow rate (2.6, 1.9, 2.4) mL.min^{-1} carrier stream (H$_2$O), CH$_3$COONH$_4$, 8-HQ respectively, was used to obtain a maximum response and a narrower base width.

Table 2- Effect of variation of flow rate precipitation system of iron(III)

| Peristaltic pump (indication approximate)	Flow Rate (mL.min^{-1})			Attenuation of incident light ȳi n=3 (mV)	S.D.	%RSD	Confidence interval of the mean $\bar{y}_i \pm t_{(\alpha=0.05/2)} \frac{\sigma_{n-1}}{\sqrt{n}}$	t (sec.)	Δt_B (min.)
	H$_2$O	CH$_3$COONH$_4$	8-HQ						
10	0.8	0.6	0.8	1527.83	2.25	0.148	1527.83±5.60	24	3.4
20	1.6	1.2	1.6	1561.83	1.76	0.112	1561.83±4.36	15	1.6
30	2.6	1.9	2.4	1502.50	1.32	0.088	1502.50±3.29	9	1.2
40	3.4	2.5	3.0	1446.67	1.15	0.080	1446.67±2.87	6	1.0
50	4.3	3.2	3.7	1433.33	1.53	0.107	1433.33±3.79	4	0.9

t= arrival time of sample segment to the measuring cell

Figure 7- Variation of flow rate versus energy transducer output response profile pf linear array ayah-5sx1-T-1D-CFI analyser

3-2-2-4- Effect of Sample Volume

Using the optimum parameters achieved in previous sections. Variable sample volumes (20-100) µL were injected using open valve mode i.e. allowance for continuous purge of sample from the sample loop in the injection valve. The data obtained were plotted as shown in Figure (8-A) showing that the optimum sample volume is 60 µL given regular clear response. Using larger volume i.e. > 60 µL, it gave a slight higher response and it was characterized with the width of their peak maxima as shown in Figure (8-B).
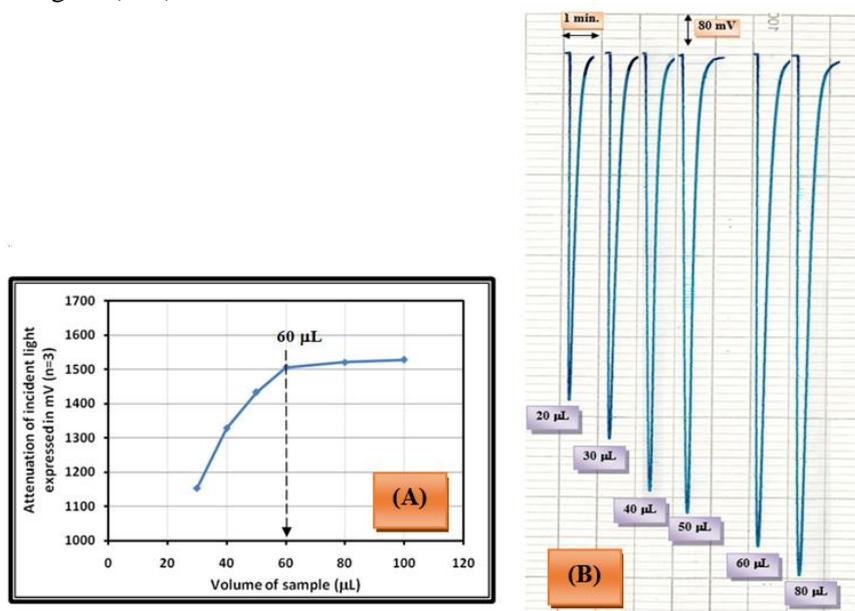
Figure 8- (A) Effect of variation in sample volume versus energy transducer output of Linear Array Ayah-5SX1-T-1D-CFI analyzer. (B) Response profile.

3-2-2-5- Effect of Purge Time

Using different purge time for the sample segment i.e. using 3 to 12 seconds allowed time for the carrier to pass through the injection valve in inject mode, followed by turning the injection valve to the load mode. The volume of sample was 60 µL. Figure (9) shows the continuation of the increase in response with increase of injection time up to 12-15 seconds, after that there was no significant differences in responses. The decrease in responses when using less than that 15 sec was attributed to the incomplete purge of the sample from sample loop in the injection valve, therefore open valve more (12 sec) as purge time was chosen as optimum time to the complete purge of the sample from sample loop in the next studies.
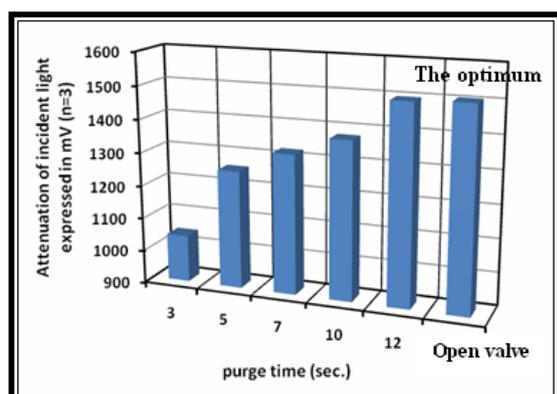
Figure 9- Effect of variation of purge time on attenuation of incident light using optimum conditions

3-3- Calibration graph

A series of iron(III) solution ranging from 0.1-15 mmol.L^{-1} were prepared for the purpose of using them for the preparation of scatter plot diagram followed by the choice of calibration graph. All
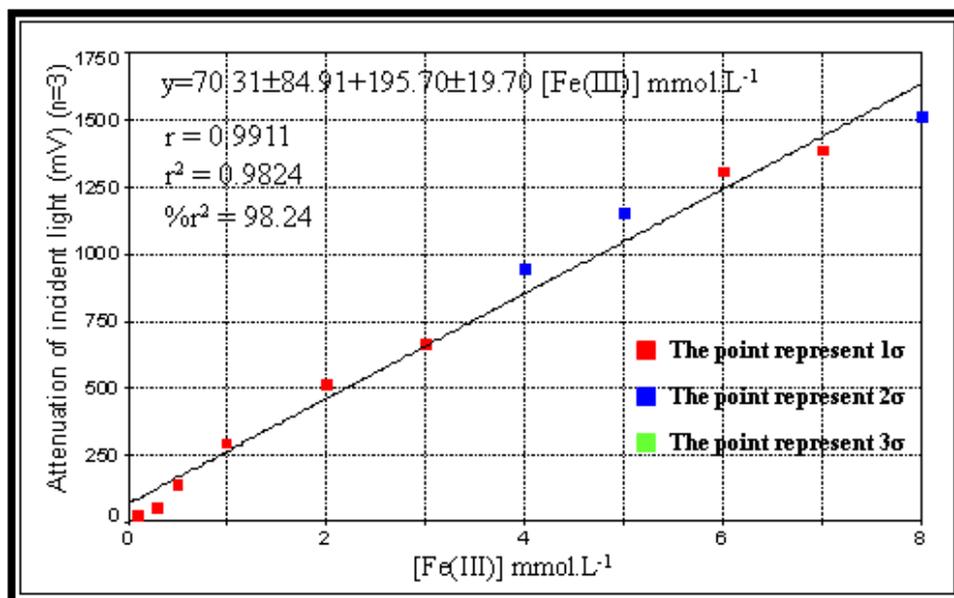
physical and chemical variables were fixed at their optimum values. The results obtained were tabulated in Table (3-A) while their representation was in Figure 10 which shows the variation of response with concentration of iron(III). Analysis of variance was carried out as shown in Table (3-B) which indicate that $F_{tab.} = F_{v_2}^{v_1} = F_9^1 = 5.12 << F_{Stat.} = 504.87$ therefore, it can be concluded that there is a significant relation between the concentrations of iron(III) and the response obtained.

Table 3-A- A statistical summary of calibration graph for the determination of iron(III) using 8-HQ-CH_3COONH_4-Fe(III)

Measured Fe(III) mmol.L^{-1}	Fe(III) range for n=11 mmol.L^{-1}	$y^{\wedge}$(mV)=a±S_at+b±S_bt [Fe(III)]mmol.L^{-1} at confidence interval 95%, n-2	r, r^2, r^2%	$t_{tab.}$	$t_{cal} = \frac{\|r\|\sqrt{n-2}}{\sqrt{1-r^2}}$ at 95%, n-2
0.1-15	0.1-8	70.31±84.91+195.70±19.70 [Fe(III)] mmol.L^{-1}	0.9911 0.9824 98.24		2.262 << 22.46

Table 3-B- ANOVA for linear equation results [29-31]

Source	Sum of squares	D_f	Mean square	$F_{stat.} = S_1^2/S_2^2$
Regr. $(\hat{y}_i - \bar{y})$	3085753	v_1=1	3085753	
Error $(y_i - \hat{y}_i)$	55007.3	v_2=9	6111.922	504.8745
Total $(y_i - \bar{y})$	3140760	10		

Figure 10- Linear calibration graph for the energy transducer response expressed in mV with iron(III) concentration in mmol.L^{-1}

Using three different ways for the study of the detection limit for iron(III). The results obtained is tabulated in Table no.4 using a sample volume of 60 μL.

Table 4- Limit of detection of iron(III) at optimum parameter for 8-HQ-CH_3COONH_4-Fe(III)

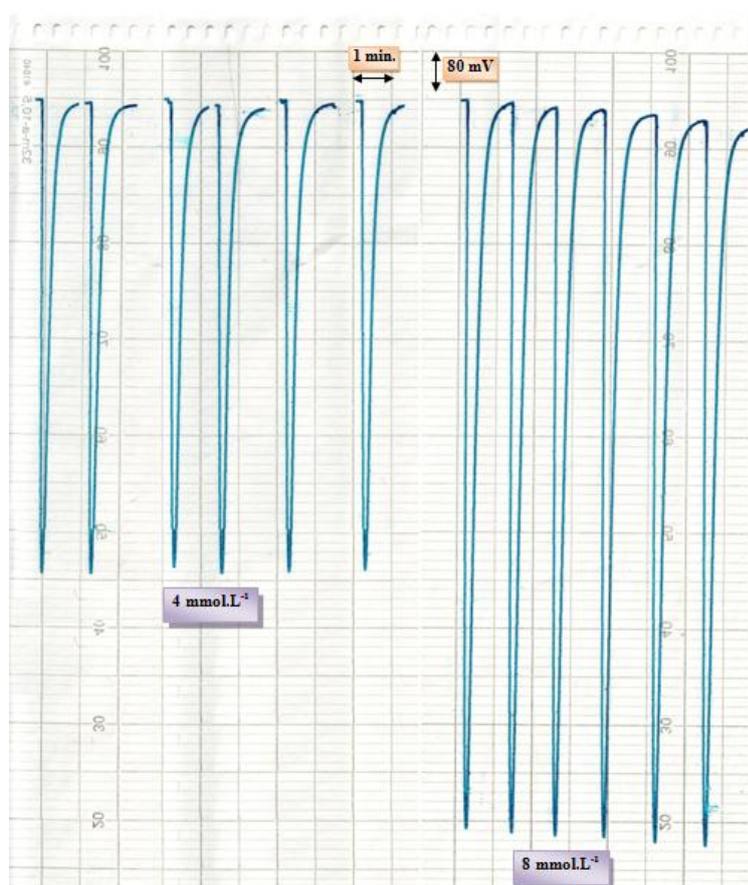| Gradual dilution for the minimum concentration in calibration graph	Based on the value of slope $x = \dfrac{3S_B}{\text{slope}}$	Linear equation $\hat{y}\ (mV) = y_B + 3S_B$	Limit of Quantity L.O.Q $\hat{y}\ (mV) = y_B + 10S_B$
4.8×10^{-9} M/sample 16.1 pg/sample	4.59×10^{-4} M/sample 1.54 µg/sample	1.19×10^{-3} M/sample 4.02 µg/sample	3.99×10^{-3} M/sample 13.40 µg/sample

x= value of L.O.D. based on slope, S_B = standard deviation of blank solution
y_B= average response for the blank solution (equivalent to intercept in straight line equation)

3-4- The Repeatability of Iron(III) Results

The repeatability was studied for determination of iron(III) via measurements of the precipitate formatted by the reaction of iron(III) with 8-HQ at concentration of 4 and 8 mmol.L^{-1} of six successive injected sample measurements as shown in Figure (11) and the results obtained are tabulated in Table no.5.

Table 5- Repeatability result

[Fe(III)] mmol.L^{-1}	Number of measuring (n)	$\overline{y_i}$ (n=6) mV	σ_{n-1}	Repeatability R.S.D.%	Confidence interval of the mean $\overline{y_i} \pm t_{(\alpha=0.05/2)}\dfrac{\sigma_{n-1}}{\sqrt{n}}$
4	6	938.66	6.53	0.695	938.66±6.85
8	6	1508.00	4.38	0.290	1508.00±4.59

Figure 11- Response time profile of six successive repeatable measurements of iron (III) concentrations (4, 8 mmol.L^{-1}) in Linear Array Ayah-5SX1-T-1D-CFI analyser

3-4- Application of The Method

The turbidimetric-flow injection method achieved in this work was used for the analysis of iron(III) in three different pharmaceutical preparations and it was compared with official method. Volumes of thirteen ampoules were measured. Collected in container. 2 mL (0.035 mol.L^{-1}) from each preparation, dissolved in distilled water and diluted to 50 mL. 0.28 mL was drawn to each of six 10 mL volumetric flask followed by the addition of gradual volumes of standard iron(III) (0, 0.5, 1, 1.5, 2, 2.5) mL at 20 mmol.L^{-1} to obtain 1, 3, 5, 7 and 9 mmol.L^{-1}. Flask no.1 is the sample flask volume. The measurements were conducted by both methods and the results were mathematically treated (for standard addition method). The results were tabulated in Table no.6 at confidence interval 95%. Paired t-test was used as shown in Table no.7. The obtained results indicate clearly that there was no significant difference between newly developed method FIA with the official method [32] at 95% confidence interval as calculated t value is less than tabulated t value.

Table 6- Results for the determination of Iron(III) in pharmaceutical preparations

Commercial name Content Country	Confidence interval for average volume at 95% $\bar{V} \pm 1.96 \frac{\sigma_{n-1}}{\sqrt{n}}$ (mL)	Sample volume equivalent to 1 mmol.L^{-1} of the active ingredient (mL)	Quoted content of active ingredient at 95% n=∞ (mg)	found content of active ingredient at 95 n=∞ (mg)	% Recovery
Santafer3 Santafarma 100mg/2mL Turky	1.996±0.0340	0.28	100±3.59	106.86±0.50	106.86
Iron-dextran Kontan pharmaceutical 100mg/2mL China	2.003±0.043	0.28	100±2.79	97.87±1.12	97.87
Iron dextran-Cox Cox-pharmacetical LTD 100mg/2mL G.B	1.984±0.0338	0.28	100±2.78	104.86±0.69	104.86

Table 7- Paired t-test results for turbidmetric-CFI method with quoted value using standard addition method for the determination of iron(III) in pharmaceutical preparations

Sample no.	Practical content (mg)		d	$\bar{x}_d$	σ_{n-1}	Paired t-test $t = \frac{\bar{x}_d \sqrt{n}}{\sigma_{n-1}}$	$t_{tab.}$ at 95% confidence interval n-1
	New method	Quoted value					
1	100.86	100	0.86	0.536	0.50	1.85 < 4.303	
	99.96	100	-0.04				
	100.79	100	0.79				
2	98.86	100	-1.14	-1.473	1.12	2.26 < 4.303	
	99.45	100	-0.55				
	97.27	100	-2.73				
3	101.61	100	1.61	0.95	0.69	2.35 < 4.303	
	100.22	100	0.22				
	101.02	100	1.02				

Conclusion

The proposed turbidmetric flow-injection method is simple, rapid, inexpensive and sensitive for the determination of iron(III) based on the 8-HQ-CH_3COONH_4-Fe(III) system. From the experimental point of view, the manipulation is very simple, and sequential measurement was permitted with high sample frequency, up to 60 samples per hour. The proposed method uses cheaper instruments and reagents with those spectrophotometry, fluorimetry, HPLC and other turbidimetric-FIA method with different precipitating agents. 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 additions method was used to avoid matrix effects. Also this method can be applied to the micro determination of iron(III) in pharmaceutical preparations without the need for heating or extraction.

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