A Flow -Injection Method For The Determination Of Total Iron Using 3,4 – Dihydroxybenzaldehyde

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ABSTRACT

A home-made design flow-injection system has been described in a previous work. This system has been applied in the present work for the determination of total iron using the reagent 3,4-dihydroxybenzaldehyde (3,4-DHBA). Optimization of all conditions were as followoms: λ_{max} 440 nm., pH of the carrier stream(C.S) 8.8 and its flow rate with the reagent 1.1 ml.min^{-1}, reagent concentration 0.001M and the Manifold length was 60cm. Optimum, linear range of the calibration curve plotted by the method of Least Square with any one of the two equations $Y = a + bX$ or $Y = bX$; including zero (the blank reading) as one of the standards, was between 0 - 8ppm. total Iron. The accuracy of the method was measured by calculating percentage recovery of 10 μg Iron (II+III) added to samples of Iron-Drugs after dissolution with 0.1MHCl. The error did not exceed 5% with Coefficient of Variation between 2.5 - 3.3%. Paired Comparison by t-test between two calibration curves showed no significant difference at 95% confidence limit.

INTRODUCTION

In 1975, Ruziska and Hansen(1) have discovered that the air - segmented method of automatic analysis could be simplified by omitting air and using much narrower tubings . Thus a new technique called flow-injection analysis (FIA) has appeared .In this method a continuous non-segmented stream is running through narrow tubes (I.D. of the order of about 0.5 - 1 mm.) carrying the analyte , mixing it with proper reagents present in it and converting it into a measureable product before passing through the detector. Since then the method has found wide applications in most areas of Analytical Chemistry. This was mainly because of the simplicity of the method in addition to high sample throughput, effective performance, low cost instrumentations and minimum consumption of chemicals.

Recently, Najib (2) and Matti,et.al. (3) have reported two methods for the determination of total Iron (Iron II + Iron III) in one solution and with one reagent. The reagents were3,4-Dihydroxyphenylacetic acid (3,4,DHPAA) and 3,4-

Dihydroxybenzaldehyde (3,4-DHBA) respectively, which were used without a need to convert one form of Iron to another, as it is normally done in other methods(4-7).

In an attempt to overcome the problem of non availability of proper injection valves, usually used in (FIA), Najib (8, in this Journal) has designed an alternative injection system by a combination of a three-way and a four-way glass distribution valves. This (FIA) system has been used quite successfully for the determination of total iron with (3,4-DHPAA)(8).

The present work describes another application of this system, this time with the reagent (3,4-DHBA). The suitability of the method for (FIA) and the optimization of conditions have been studied for the determination of total Iron in some Iron-Drugs.

EXPERIMENTAL

Chemicals and Preparations: Stock Solutions of Iron II and Iron III (100ppm). These were prepared from appropriate weights of ferrous sulphate hepta hydrate and ferric ammonium sulphate and the two solutions were mixed in a 1:1 ratio. This solution was carefully standardized gravimetrically (9) and renewed after one month. Seriel dilutions were made in the usual way to prepare fresh standard solutions between (0 - 10 ppm) total Iron each time a calibration curve was needed.

Stock Solution of the Reagent (3,4-DHBA) was prepared by weighing (0.045 g) of the substance , dissolved in 50ml. distilled water and filtered. This solution was ignored after one week. The carrier stream was 0.1M NH_4Cl, its pH was adjusted between 8.8 -9 with 2M ammonia solution.

Preparation of samples of Iron- Tablets:

Three procedures (Neither taken from any reference nor was subjected to any study) were applied : (i) Three tablets were crushed to a fine powder with a porcelain mortar and pestol. Equal weights of the powder (0.2 – 0.5 g) were treated separately with 15 mls., 0.1 M HCl or HNO_3 or H_2SO_4, heated gently to drynes then vigorously for another ten minutes. After cooling, 10 mls. of the corresponding acids were added and stirred, filtered and the precipitate was washed several times with distilled water. The pH of these solutions were adjusted to be just over 2 and the volumes were finally completed to 50mls. ready for analysis.

(ii)As in (i)but the coatings were rubbed off on a fine carborandum stone before crushing.

(iii) As in (i) but the crushed powder was divided into two equal parts , one part was treated with 10 mls.acetone and the other with 10 mls. ethanol to remove the organic matter of the coatings, filtered, washed and left to dry. Three equal weights were then taken from each part and treated with HCl, HNO_3 or H_2SO_4 exactly in the same way as described in (i) above

Apparatus:A single beam Spectrophotometer, Spectronic 20 from (Milton Roy USA). was used. A flow-through cell, 80 µL capacity and 10 mm. path length, fixed in position with a special home- made adaptor. The pH measurements were made with PW9418 pH meter provided with a combined glass-electrode from Philips (England). A three-channel peristaltic pump P-3from (Pharmacia). Two glass distribution valves,

a three and a four-way types, from Vestale (Germany), were used to construct the flow-injection system as described previously somewhere else in this Journal(8) to replace the commercial injection valve.

Calibration Curve and sample Measurements The syringe is filled with, either a standard or a sample containing between (0 - 8 ppm total Iron). The sequence of injection is as follows: Valve (X), Fig 1, is turned to position (1,2 - 3,4) while valve (Y) is in position (5,6,7), this is called L_I position. At this position the syringe can be removed , if wanted, and refilled with the desired solution. After replacing the syringe, the Loop (which has a size of about 100 µL) can be loaded by brief pushings of the plunger several times (4 times) then immediately turning valve (Y) to position (6,7), as shown in Fig. 1 (L_{II} position) and again make few more pushings to the plunger, to be sure of full loading of the Loop. Finally, valve (Y) is turned back to position (5,6). These operations of loading are carried out while the previous injection is still running and about to complete. When everything is ready for next injection , make a quick and smooth turning of the valve (X) in one movement to position (1,4 - 2,3) as shown in Fig. 1 ,(Inj. position). In this way the carrier stream (C.S.) and the reagent (R) will pass through the Loop carrying the analyte with them into the manifold, in which the reaction is completed and finally into the cell. The resulting absorbency (A) is recorded on a chart-recorder. The peak - heights in millimetrers (representing absorbances) are plotted versus concentrations of standard solutions of the analyte (in ppm Total Iron) making use of (C.S. + R) as a blank to zero the instrument. The Calibration Curve is drawn by the method of Least Square Analysis (10) using equations ($Y = a + bX$ or $Y = bX$) including (zero = blank) as one of the standards. Zero - 8 ppm. analyte is then read from the calibration curve and determined as Total Iron within the range.

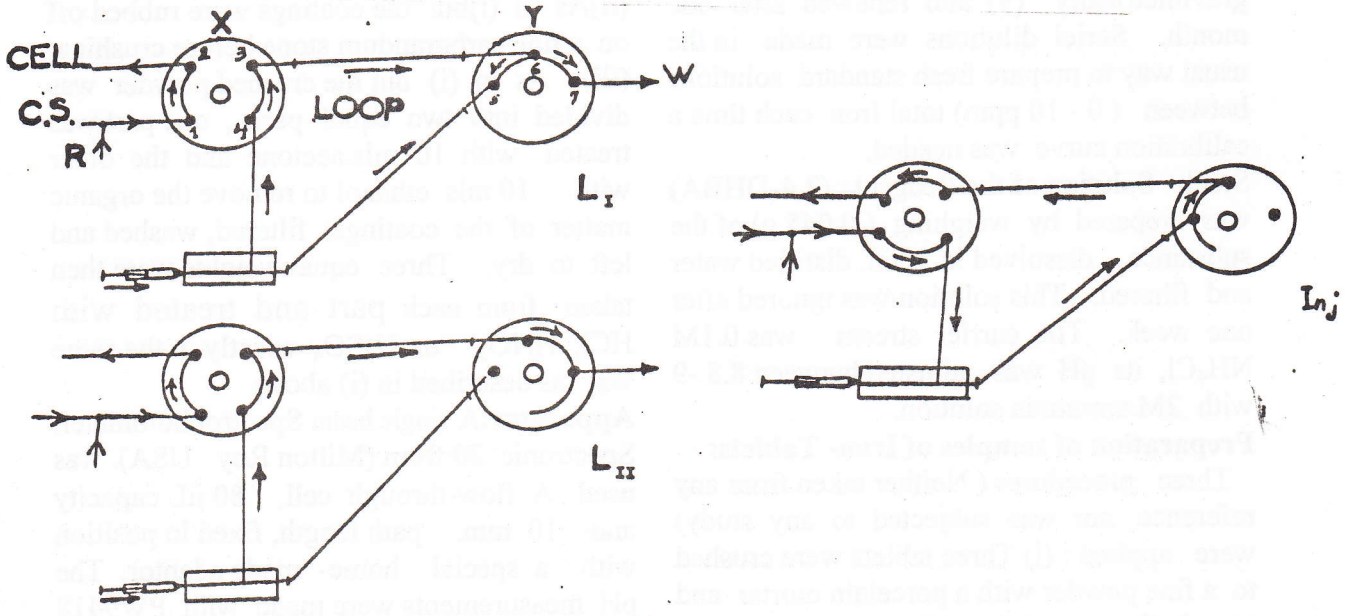

Fig .1.Construction of the Injection System . C.S = Carrier Stream ,R = The Reagent(3, 4 -DHBA),W = Waste . L = Loading . Inj = Injection .

The Recovery Test: The accuracy of the method was tested on real samples of Iron-Drugs after their destruction according to the procedures given in the experimental part. The samples were analysed before and after the addition of 10 μg of total Iron (Fe_{II} + Fe_{III}) and the recovery (%R) were then calculated to represent the accuracy of the method without a need for a comparative method.

RESULTS AND DISCUSSIONS

Optimum Wave Length: Although the original work (3) has given ($\lambda_{max.}$ = 559 nm.), this was found of low sensitivity in the present study. The complete spectrum of the complex was then obtained under the condition of flow-injection in Fig. 2 which shows that the wave-length of (440 nm.) is the optimum. This wave-length has not been studied in the original work and it appeared only as a small shoulder. This large difference can only be due to the condition of flow-injection, which could become the subject of a future work.

Optimum pH: To reach optimum pH at the end of the manifold, the pH of the (C.S.) should be high enough to raise the pH of the analyte solution from a certain starting value. The Iron concentrations having pH values around 2 were tested with varying pH values of the (C.S.) and a fixed length of the manifold and the results are shown in Fig 3. It could be deduced that optimum pH of the (C..S.) between (8.8 - 9) seems to be optimum, providing that pH of the test solutions are not lower than 2. It should be noted here that this high pH is not the pH of the reaction , but rather to adjust pH of the reaction mixture in the manifold to the final optimum value of (4 - 6) needed for the reaction according to the original work (3).

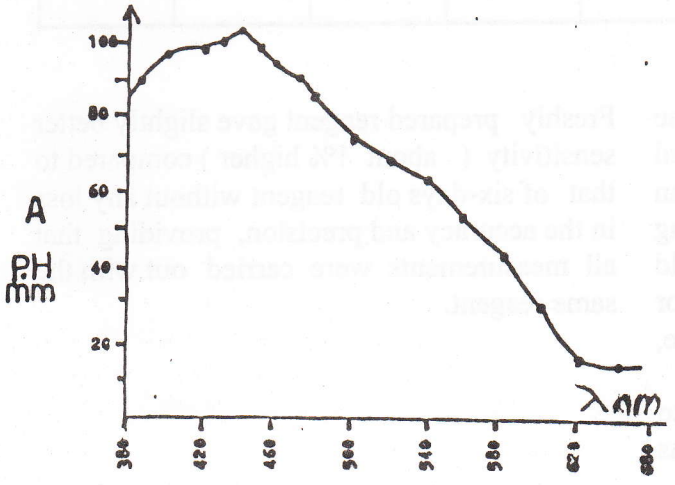

Fig . 2 . Determination of λmax .Iron Concentration is (5 ppm)Total . F.R = 1.1 ml /min , pH of C.S = 9 . P.H = Peak hight (mm)

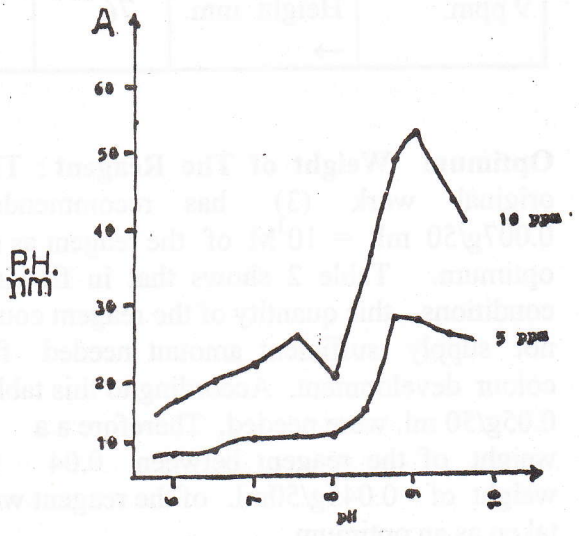

Fig .3 . Optimization of pH at Different Concentration of Total Fe . P.H = Peak Hight (mm)

Optimum Length of The Manifold : Using optimum pH and wave-length, the manifold lengths of 40, 50, 60 and 80 cm. were tested and found that the length of 60 cm. is the optimum. Obviously, when the reaction reaches the optimum, the longer length of the manifold causes excessive dilution and lower sensitivities will be obtained.

Optimum Flow-Rate : At the optimum conditions obtained until now, most flow-rates of the peristaltic pump were tested and the results are given in Table 1. The table shows that the highest speed of the pump, which was (no. 10), giving a flow-rate of 1.1 ml.min^{-1}, seems to be the optimum. The table also shows that this flow-rate is suitable for both concentrations of total Iron tested namely (5 and 9 ppm). Very low flow-rate causes1.1ml.min.$^{-1}$ could not be studied.

Table 1: *A Study for the Optimization of Flow -Rate of the C.S for two Concentrations of Total Iron Namely 5 and 9 ppm*

TotalIron	Pump speed Nos.→	5	6	7	8	9	10
ppm ↓	Flow Rate ml./min→	0.54	0.65	0.76	0.90	1.0	1.1
5 ppm.	Abs. Peak Height mm →	24	24	24.3	24.6	25	25.3
9 ppm.	Abs. Peak Height mm. →	76	79	79	78	80	82

Optimum Weight of The Reagent : The original work (3) has recommended 0.007g/50 ml. = 10^{-3}M of the reagent as an optimum. Table 2 shows that in flowing conditions, this quantity of the reagent could not supply sufficient amount needed for colour development. According to this table, 0.05g/50 ml. were needed. Therefore a a weight of the reagent between 0.04 to weight of 0.045g/50ml. of the reagent was taken as an optimum.

Freshly prepared reagent gave slightly better sensitivity (about 1% higher) compared to that of six-days old reagent without any loss in the accuracy and precision, providing that all measurements were carried out with the same reagent.

Table 2: *A study for the Optimization of the Weight of the Reagent (R) for 10 ppm .Total Iron*

Weight of the Reagent g/50ml	Molarity of the Reagent M mole/L	Abs. Peak Height mm.
0.007	10^{-3} M	22
0.014	$2x10^{-3}$ M	45
0.030	$4.3x10^{-3}$ M	81
0.035	$5.1x10^{-3}$ M	85
0.040	$5.8x10^{-3}$ M	92
0.050	$7.2x10^{-3}$ M	92

Selectivity of The Method: The original work has given a table on the interferences of most cations and anions which was not necessary to be repeated here.

Calibration Curves: The Least Square Regression Analysis for the line of best fit was applied on two equations (9) namely $Y = a + bX$ and $Y = bX$, and in two ways. In the first case, the blank (which had zero value all the time) has been taken as one of the standards, while in the second case the blank has only been used to zero the instrument.The Statistical Functions obtained by these methods are given in table 3 and a typical recorder output for one calibration is shown in Fig. 4. The functions calculated for the evaluation of the calibration curves were: (a , b) from the above equations, the correlation coefficient (r), the relative standard deviation on r (RSD), and the 95% Confidence Interval on the values of r (C.I.) .

Although values of (r) in the table indicate that Beer's Law is obeyed upto 10 ppm total Iron, but in fact they are getting worse after 8 ppm based on the high values of RSD and also the values of (C.I.). The table does not give sharp indications which of the two equations gave better results. It seems that both equations are equally satisfactory between 0 - 8 ppm. total Iron, although, generally, equation $Y=a + bX$ gave slightly narrower (C.I.).

Table 3: *Evaluation of the Statistical Parameters (P) for the Equations : (I) Y = a + bX and (II) Y = bX used for plotting the calibration curves. These are (a and b) of the equations, the Correlation Coefficient (r), Relative Standard Deviation (RSD) and Confidence Interval (C.I.).*

P	Ranges ppm→	0 - 7	0.5 - 7	0 - 8	0.5 - 8	0 - 9	0.5 - 9	0 - 10	0.5-10
a	I	- 1.5	- 1.3	- 1.6	- 2.2	0.07	0.08	0.26	0.36
a	II	—	—	—	—	—	—	—	—
b	I	8.9	11.1	8.8	8.9	8.0	8.1	8.0	8.0
b	II	8.9	11.1	8.5	8.9	8.1	8.1	8.0	8.0
r	I II	0.996	0.997	0.999	0.990	0.990	0.984	0.990	0.990
RSD	I	2.0	1.6	2.0	1.9	4.0	4.5	4.1	4.3
RSD	II	2.1	2.3	2.0	2.5	4.0	4.3	4.0	4.0
C.I	I	X ± 4.6	X ± 4.5	X ± 4.7	X ± 4.4	X ± 9.6	X±10.4	X ± 9	X ± 9.6
C.I	II	X ± 4.9	X ± 5.3	X ± 4.7	X ± 5.7	X± 9.0	X± 9.6	X ± 8.5	X ± 9

X = Mean

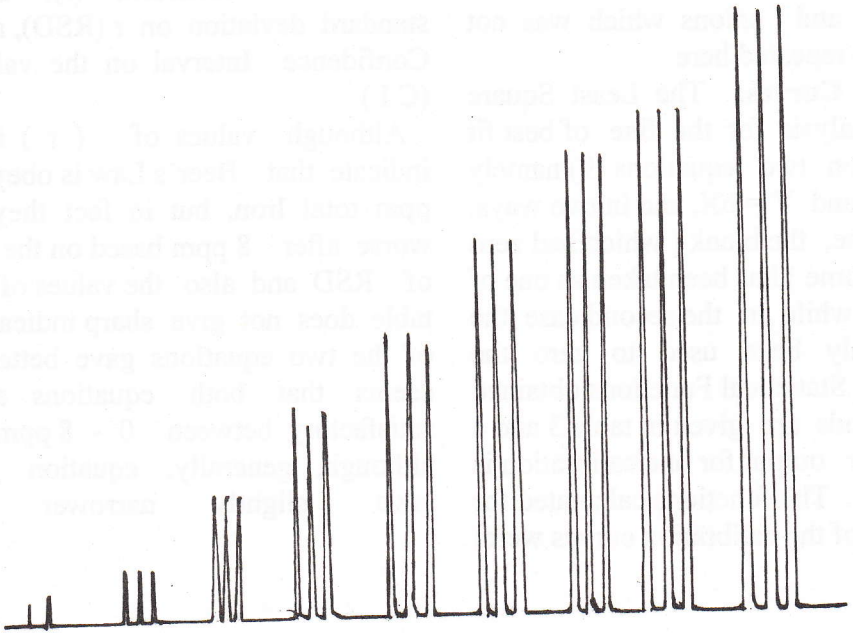

Fig. 4. Recorder Output for the Calibration Curve. Iron - Total Concentration is between 1 - 8 ppm other Conditions as in Fig. 2.

Accuracy and Precision

A known quantity (10 μg) of standard Iron solution was added to different samples of Iron-Drugs. Total Iron in the samples were determined by the present method, before and after the addition of the standard Iron and the recoveries (%R) are given in table 4. The Authors would like to point out that these results are not intended to evaluate methods of Drug dissolution, or, to establish the real quantities of Iron in these samples, but it is meant only as an evaluation of the recovery tests to establish the Accuracy of the method.

Table 4 shows that reasonable accuracy were obtained when the method of dissolution uses HCl with or without ethanol, although the latter was better. In this case also, there was no difference in using any of the two equations given in the previous section. The (%R) given in the table again indicates that best linear range is between (0 - 8 ppm. Total Iron) and, generally , taking the blank as one of the standards for plotting the calibration curve is better than ignoring it. The table also suggests that neither HNO_3 nor the use of acetone followed by acid treatments were satisfactory for sample dissolutions, because of the high error values obtained in these cases.

The Precision of the method was tested for 3 and 6 ppm. total Iron on 10 replicates each. The coefficient of variation was 5% for the first one and 2.5% for the second. But when Q-test was applied on both sets 4 outlying results were rejected for the first set making the precision in this case = 3.1%.

Paired comparison by t-test for two calibration curves showed that the difference between them is not significant at 95% confidence limit. This was another confirmation that the precision of the method is quite satisfactory. With all these performances, it is possible to determine at least 20 samples in one hour. Obviously this frequency could have been more if higher flow-rates were available to the authors.

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Table 4: *Evaluatioin of the Accuracy of the Method by the Recovery of 10 µg Standard Iron Solution Added to Different Samples of Iron-Drugs.*
Methods of Sample Dissolution Were by: (a) HCl, (b) HNO_3, (c) Acetone then HCl, (d) Ethanol then HCl.

Range		Y = a + b X						Y = bX			
		0 - 7	. 0.5-7	0 - 8	0.5-8	0 - 9	0.5-9	0 - 6	0 - 7	0 - 8	0 - 9
µg Fe Present Read from Cal. Curve	a	3.33	4.71	3.33	3.43	3.21	3.22	3.03	3.09	3.07	3.23
	b	3.79	5.07	3.79	3.88	3.71	3.71	3.50	3.56	3.54	3.72
	c	5.05	6.06	5.04	5.12	5.11	5.07	4.78	4.87	4.84	5.09
	d	5.74	7.13	5.74	5.84	5.73	5.73	5.40	5.49	5.46	5.74
µg Fe Present Total	a	13.33	14.71	13.33	13.43	13.24	13.22	13.03	13.09	13.07	13.23
	b	13.79	15.07	13.79	13.88	13.71	13.71	13.50	13.56	13.54	13.12
	c	15.05	16.06	15.04	15.12	15.11	15.07	14.78	14.87	14.84	15.09
	d	15.74	17.13	15.74	15.84	15.73	15.73	15.40	15.49	15.46	15.74
µg Fe Found Read from Cal. Curve	a	13.41	14.15	13.40	13.44	13.96	13.96	13.13	13.36	13.28	13.97
	b	14.78	14.78	14.77	14.79	16.45	16.46	14.53	14.78	14.70	14.46
	c	16.67	16.27	16.65	16.65	16.91	17.51	16.45	16.74	16.64	17.51
	d	15.04	15.51	15.03	15.08	15.63	15.65	14.70	14.96	14.87	15.65
% R. = = =	a	100.6	95.8	100.5	100.0	105.7	105.7	100.7	102.1	101.6	105.6
	b	107.2	98.1	107.1	106.5	120	120	107.6	109	108.5	112.6
	c	110.8	101.3	110.7	110.1	112	116	111.3	112.6	112	116
	d	95.6	90.6	95.5	95.2	99.4	99.4	95.5	96.6	96.2	99.4
% Error = = =	a	+ 0.6	- 4.2	+ 0.5	+0.01	+ 5.7	+ 5.7	+ 0.7	+ 2.1	+ 1.6	+ 5.6
	b	+ 7.2	- 1.9	+ 7.1	+ 6.5	+ 20	+ 20	+ 7.6	+ 9	+ 8.5	+12.6
	c	+10.8	+ 1.3	+10.7	+10.1	+ 12	+ 16	+11.3	+12.6	+ 12	+ 16
	d	- 4.4	- 9.5	- 4.5	- 4.7	- 0.6	- 0.6	- 4.5	- 3.4	- 3.8	-0.6

Cal. = Calibration

$$R = \frac{\text{µg Found}}{\text{µg present}} \times 100$$

Captions to the Figures:

Fig. 1. Construction of the Injection System.
C.S. = Carrier Stream, R = the Reagent 3,4-DHBA, W = Waste. L_I and L_{II} = Loadings of the Loop According to the Directions of the Arrows. In case of L_I Valve Y is i position (5,6,7) and (6,7) in L_{II}. In both cases the Manifold is completely separated from the Loop Via Route (1,2) 0f X.
Inj. = Injection Step: X is in Position (2,3) ....(1,4) While Y is in Position (5,6).

Fig. 2. Determination of λ_{max} Iron Concentration is 5 ppm. Total. F.R.= 1.1ml/min., pH of C.S. = 9.

Fig. 3. Optimization of pH at different concetration of Total Fe.

Fig. 4. Recorder Output for the Calibration Curve. Iron - Total Concentration is between 1 - 8 ppm. Other Conditions as in Fig. 2.

طريقة الحقن الجريان لتقدير الحديد الكلي
باستعمال الكاشف ٣،٤- ثنائي هايدروكسي بنزالديهايد

فاضل محمد نجيب و آريان رفيق محمد
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الخلاصة

استخدم تصميم خاص وصفناه سابقاً لتقدير الحديد الكلي باستعمال الكاشف (٣،٤- ثنائي هايدروكسي بنزالديهايد) (3,4-DHBA) بطريقة التحلل الطيفي المرئي. أفضل الظروف كانت: الطول الموجي 440 nm و pH المحلول الحامل (C.S.) = 8.8، تركيز المحلول الكاشف 0.001M وسرعة الجريان لمزيج المحلول الحامل مع الكاشف كانت1mL/min. وطول المانيفولد من نقطة الحقن للخلية 60cm .

قانون بير-لامبرت مرسومة بطريقة ال(Least square) مستعملاً إحدى المعادلتين (Y = bX أو Y = a + bX) تتراوح ما بين0-8 ppm حديد كلي (Fe_{III} + Fe_{II}) مستضمناً البلانك كأحد المحاليل القياسية.دقة الطريقة مقاسة بنسبة استعادة 10µg من محلول الحديد القياسي مضافة لنماذج الأدوية الحاوية على الحديد تراوحت بين 95-105% و معامل التكرارية ما بين 2.5-3.5% وتبينت من مقارنة منحني المعايرة لمرتين بطريقة t-test للمقارنة الزوجية، بان الفرق بين المعايرتين غير معنوي عند درجة الثقة 95%.

(ریکەیەک بو ئینجەکت کردنی شلەی نەوەستاو بو دوزینەوەی)
کوی ئاسن بە بەکارهینانی ٣،٤- دووهایدروکسی بە نزئە لدیهاید

فاضل محمد نجیب ناریان رفیق محمد
بەشی کیمیا/ کولیجی زانست/ زانکوی سلیمانی/ کوردستان / عراق

کـورتە

ئەو ئامیرە تایبەتمەندەی کە لەوپیش باسمان کردبوو بو (ئینجەکت) لە جیاتی ڤالڤی بازار. بەکارمان هینا بو دوزینەوەی کوی ئاسن لە دەرماندا بەلام ئەم جارەیان بە بەکارهینانی ٣،٤-دووانە هایدروکسی بەنزئەلدیهاید (3,4- DHBA) بە ریکەی شەپەنکی هیلی بینراو لە شەپولی (440nm) ،و بەفەر(pH)ی کیراوەی هەلکر (C.S 8.8) = وە پەیتی ناسەرەوەکە (0.001M) ە.وخیرایی تیپە ربوونی کیراوەی هەلکر و ناسەرەوەکە (1.1mL/min)وە دریژی ری پەوی کارلیکە کە لە شوینی تیکەل کردنی تا شانەی ئامیرەکە(60cm)بو. ولە (0-8 ppm) ی کوی ئاسنی (Fe_{III},Fe_{II}).هیلی راستی بیر - لامبرت کە بە (Least square) رەسم کراوە وە بلانکیش یەکیکە لە جیکیرکراوە کان وپاسادانی یە کیک لەم دوو هاوکیشەیە (Y=bX یان Y=a+bX).دەکات راستی ئەم ریکەیەمان دەستکەوتنەوەی (10µg) ئاسن بووکە تیکەل کرابوو لەکەل دەرمانی دیاری کراووە کەوتبوە نیوان (95-105%) ، وهاوکولکەیی دووبارەبوونەوەی لە نیوان (2.5-3.5%) دابو.

جووت بەراورد کردنی دوو هیلی راستی بیر-لامبرت بە ریکەی(t-test) کەجیاوازی بەهاداری نیە لە دەرەجەی باوەری 95% دا .