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A Flow-Injection Method For The Determination of Total Iron With 3,4-Dihydroxyphenylacetic Acid Using A Specially Home-Designed Injection System.

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ABSTRACT

A special injection system was designed in place of the ordinary commertial rotary injection valve usually used in flow-injection analysis (FIA). The system is a combination of two glass liquid distributing valves, a three-way and a four-way types. The two valves were connected together with PVC tubings of 1 mm. I.D. in such a way to produce an external loop having a volume of about 100 μL. The loop can be loaded with the desired solution without disturbing the flow in the rest of the manifold. The system was used successfully to determine total Iron in iron-containing drugs spectrophotometrically using 3,4-dihydroxyphenyl acetic acid (3,4-DHPAA) as a reagent at 596nm. and a flow-rate of 0.05 mL/min. The carrier stream was a buffer solution of ammonia-ammonium chloride at pH 8.1 and a flow-rate of 1 mL/min. The calibration curve was linear between 0 - 8.3 ppm total Iron with a correlation coefficient $r = 0.99996$ confidence interval $(y \pm ts / \sqrt{n})$ was 72.44±0.6 at 95% confidence limit degree of freedom =5.

Analysis of Iron containing drugs after dissolution with 0.005M HCl or HNO_3 gave errors not exceeding 5% represented by recovery tests of standard Iron solutions added to these samples. At least 20 samples could be determined per hour with coefficient of variation between 1.1 - 1.4%.

INTRODUCTION

Flow Injection Analysis (FIA), which was first described by Ruzisska and Hansen (1) , has become an important analytical technique in laboratories of different fields of science , industry, agriculture .....etc. This is because of its simplicity high sample throughput, effective performance, low cost of instrumentations and chemical consumptions and finally it can easily fit most analytical techniques. Spectrophotometry is one of the most common methods that fits FIA perfectly well, which is also the objective of the present work .The determination of total Iron spectrophotometrically by FIA directly without pretreatment has not yet been reported. Earlier methods used additional steps or units added to the manifold ,mostly to reduce IronIII to IronII such as the use of ascorbic acid (2,3),hydroxylammonium-chloride(4) or reduction column (5) followed by the determination of Iron as Iron (II) .

In a previous work (6), a new reagent 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) has been reported for the determination of total Iron directly without a need for any of such treatments. The reaction of Iron ions with this reagent at the optimum conditions is instantaneous with the formation of a relatively stable colored complex. These properties were exploited in the present work to fit FIA for the determination of total Iron. A simple design was arranged in the present work to replace the commercial type of rotary injection valve, usually used with FIA.

EXPERIMENTAL:

Reagents: Stock solutions of $Iron_{II}$, $Iron_{III}$ (279.25 ppm) and reagent 3,4-DHPAA (0.01M) were prepared as previously given (6).The carrier stream (c.s.) was a buffer solution of 0.1M NH_4Cl (5.53g/L) adjusted to pH 8.1 with 1:1 ammonia solution.

Preparation of samples of Iron-containing drugs: Tablets were crushed to a fine powder and 0.01g for each batch was taken and treated as follows:

Shaken with 5 mL acetone , to remove any organic coatings, then filtered and dried in air. The residue was treated with 0.005M HCl, quantitatively transferred to a 100mL capacity volumetric flask and completed to the mark with the same solution.

b. Shaken with 0.005M HCl until it has completely dissolved, except some small floating material, which were obviously from the organic coatings of the tablets and were removed by filtration.

c. Same as b but using HNO3 instead of HCl.

The injection system: The commercial rotary injection valve usually used in FIA, was neither available nor was possible to obtain it at present, therefore , an alternative injection system was designed. The basic parts of this design is shown in fig. 1 and the complete injection system including the external loop in fig. 2.

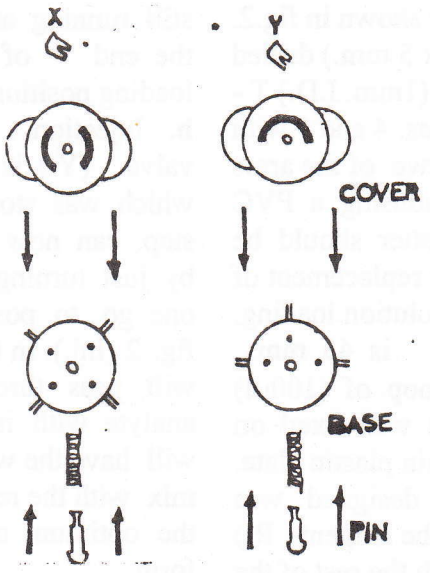

Fig 1: Parts of the liquid distribution valves.
X the four- way. Y The three –way type.

The system consists of two glass liquid distributing valves from Vestal , Germany, in which one of them was a three-way while the second was a four-way type. Each valve constructed from two parts, base and cover. A pin together with a spring is passing through a central hole and clipped at the top allowing manual rotation of the cover, as shown in fig.1.The Side tubes branched from the base were cut short ,and PVC tubes of (1mm. I. D.) were inserted through them to reduce the diameter to (1 mm) . The grooves in the flat surface of the cover can connect any two adjacent holes of the base in case of the 4 - way type (fig .1 X) and any two adjacent or three holes of the 3-way type (fig . 1 Y) . A very thin lubrication of the two flat surfaces were occasionally needed to keep the valves air - tight .The complete design of the injection system with the external loop was constructed by connecting the two valves (X and Y) via their side branches using PVC or tygon tubings of(1mm .I .D .)in such a way shown in fig.2. Apiece of Perspex (15 x 10 x 5 mm.) drilled in such a manner to make a (1mm. I.D.) T - shaped connection to join nos. 4 and 5 with a 55 mm. long tubing via two of the arms leaving the third arm for housing a PVC needle of a syringe . The latter should be fixed firmly to allow easy replacement of a (5ml).plastic syringe for solution loading. The distance between 3,6 is 45 mm , making a final external loop of (100µl) capacity. The whole system was fixed on a piece of(10 x 10 cm). thin plastic plate. The injection system so designed was connected to the (C. S.) and the reagent (R) via no. 1 as an inlet and with the rest of the manifold by the outlet no. as shown in fig 2.

Injection Procedure :-

a. Loading : Load 1 , (L_I): It can be noted from fig.2 (L_I) that, to separate the loop from the rest of the manifold completely , the valve X is turned to position (1,2)....(3,4) while valve (Y) should in be position (5,6,7).In this position it is possible to remove the syringe, fill it properly with the desired solution and finally replace it back to its position without disturbing the rest of the manifold. The loop is loaded now by several pushing (4 times is sufficient) of the plunger of the syringe softly.

Load 2, (L_{II}): This was an extra step taken to ensure complete filling of the loop and obtaining highest possible reproducibility. Load 2 is performed as soon as load 1 is complete, by turning valve (Y) to position (6,7) as shown in fig. 2, $L_{II,}$ and again pushing the plunger softly another four times. It should be noted here that loading steps do not take any extra time, because they are performed while the previous injection is still running and just about to complete. At the end of load 2, the valve Y is in its loading position (1,2)....(3,4).

b. Injection : Fig.2, Inj.: Make sure that valve (Y) is in (5,6) position. The solution which was stored in the loop, in the loading step, can now be injected into the manifold by just turning the valve (X) quickly and in one go, to position(1,4).....(2,3) as shown in fig. 2 (Inj.). In this way the (CS) and the (R) will pass through the loop carrying the analyte with it towards the cell. The analyte will have the whole length of the manifold to mix with the reagent and the buffer to reach the optimum condition for the complex to form.

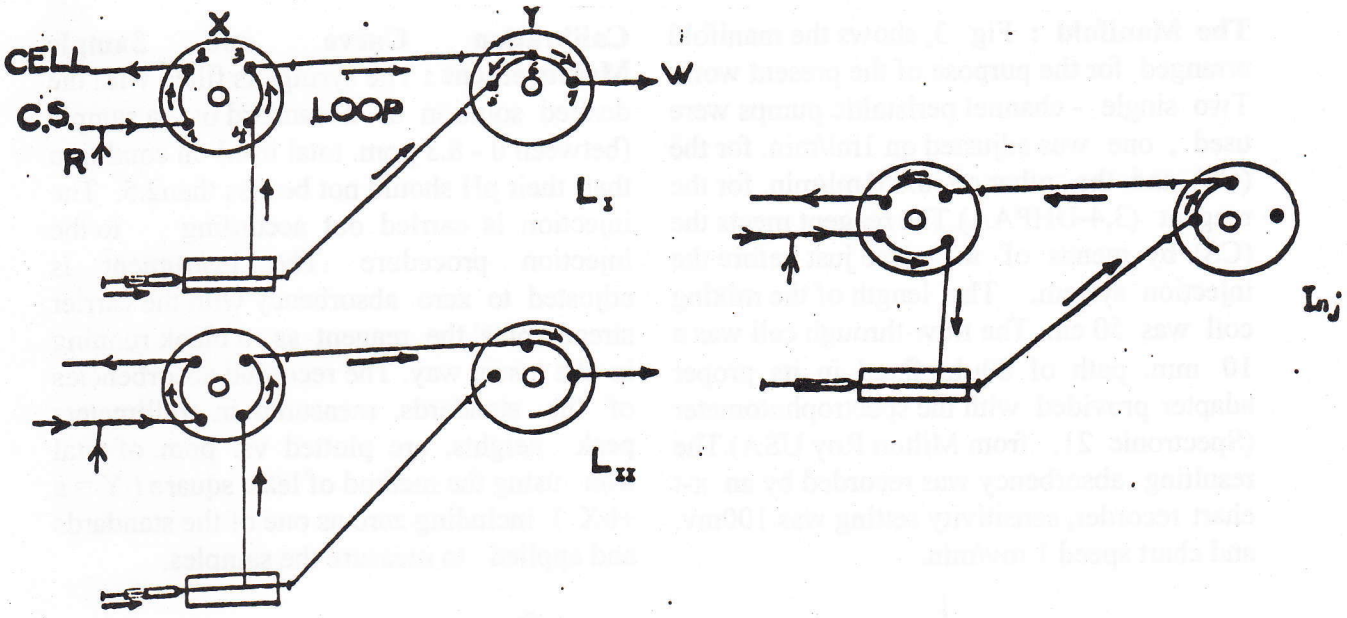

Fig 2: Construction of the injection system.
C .S. Carrier stream. R the reagent 3,4-DHPAA . W waste.
L_I and L_{II} the two loading processes of the Loop, according to the directions of the arrows .In case of L_I valve Y is in position (5,6,7) and (6,7) in L_{II} . The manifold is completely separated from the Loop via the route (1,2) of X in both L_I and L_{II} cases.
Inj Injection step X is in position (2,3) ...(1,4) while Y is in (5, 6)

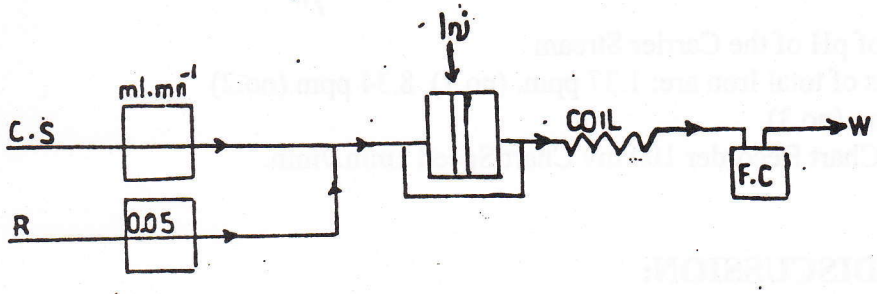

Fig 3: Flow -Injection manifold for the determination of total Iron.
C.S. Carrier stream. R the reagent 3,4- DHPAA. Inj Injection System. F. C. Flow –Through cell. W waste.

The Manifold : Fig 3, shows the manifold arranged for the purpose of the present work. Two single - channel peristaltic pumps were used , one was adjusted on 1ml/min. for the (CS) and the other on 0.051ml/min. for the reagent (3,4-DHPAA).The reagent meets the (CS) by means of a T-piece just before the injection system. The length of the mixing coil was 50 cm. The flow-through cell was a 10 mm. path of 80µl., fixed in its proper adapter provided with the spectrophotometer (Spectronic 21, from Milton Roy USA).The resulting absorbency was recorded by an x-t chart recorder, sensitivity setting was 100mv. and chart speed 1 mv/min.

Calibration Curve and Sample Measurement : The syringe is filled with the desired solution of a standard or a sample (between 0 - 8.3 ppm. total iron) on condition that their pH should not be less than2.5. The injection is carried out according to the injection procedure .The instrument is adjusted to zero absorbency with the carrier stream plus the reagent as a blank running in the usual way. The recorded absorbencies of the standards, measured in millimeters peak heights, are plotted vs. ppm. of total iron using the method of least square (Y = a +bX) including zero as one of the standards and applied to measure the samples.

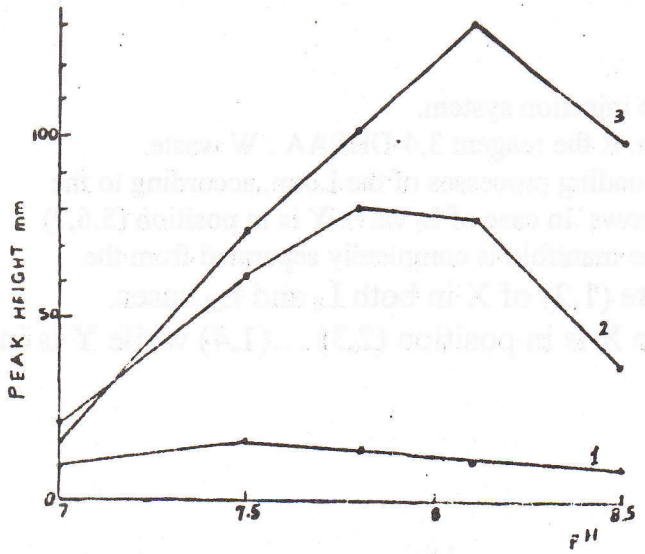

Fig 4: Optimization of pH of the Carrier Stream.
Concentrations of total Iron are: 1.37 ppm. (no.1), 8.34 ppm.(no.2)
And 20.86 ppm. (no.3).
Sensitivity of Chart Recorder 100 mv Chart Speed 1mm. /min.

RESULTS AND DISCUSSION:

Optimum ph of The Carrier Stream : The original work (6) recommended pH between 6.5 - 7.8 for complete color development .Thus for a certain length of the manifold, pH of the (C.S.) should be high enough to reach this optimum just before entering the cell. A study of changing pH of the (C.S.) between 7-8.5 for three different concentrations of

total iron is shown in fig .4. It shows that for 8.5 ppm. iron and lower, pH of the (C.S.) between (7.8-8.1) is optimum while for 20 ppm iron the pH should not be lower than 8 . Therefor this pH was used for all subsequent measurements.

It was also found that the buffer solution prepared in this work from 0.1M NH_4Cl and 1:1 NH_3 had the same effect as that recommend in the original work.

Optimum Flow - Rate (F.R.) : Keeping the (C.S.) at a (F.R.) of 1ml./min., it was found that (F.R.)s of 0.04 ml./min. and higher of the reagent all gave same results shown in fig.5A. In another attempt fig.5B, the (F.R.) of the (C.S.) was changed between 0.5 - 2 ml./min. at a fixed (F.R.) of 0.05 ml./min. reagent using different concentrations of iron. This figure suggests that a (F.R.) of 1ml./min. (C.S.) is optimum. A simple calculation showed that the (F.R.) of 0.05 ml./min. reagent gave an excess of 50 times that of iron in the manifold, which is the same as that recommended in the original work.

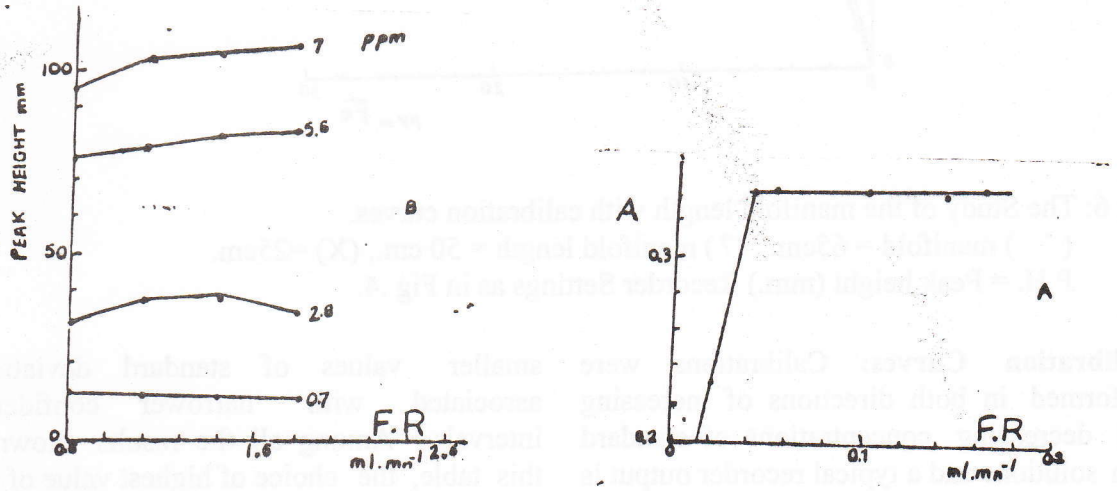

Fig 5:-Optimum Flow - Rate (F.R.). A for the reagent (R).
B For the carrier stream buffer (C.S.) recorder settings, as in Fig.4

Optimum Length of The Manifold : Correct length of the manifold would allow perfect mixing of the reagent and the (C. S) so that the optimum is reached .For this purpose a calibration curve was repeated with manifolds of 25,50 and 65cm.long and the results are shown in fig. 6. It was found that the best linear range between (0 - 8.3 ppm. iron) was obtained with the manifold length of 50 cm. The range of linearity became quite narrow (0 - 3 ppm iron) with slightly higher sensitivity when the manifold length of 25 cm. was used .

An intermediate range between the two (0 - 6 ppm. iron) was obtained with the manifold length of 65 cm . Accordingly a manifold of 50 cm. was regarded optimum for all subsequent measurements.

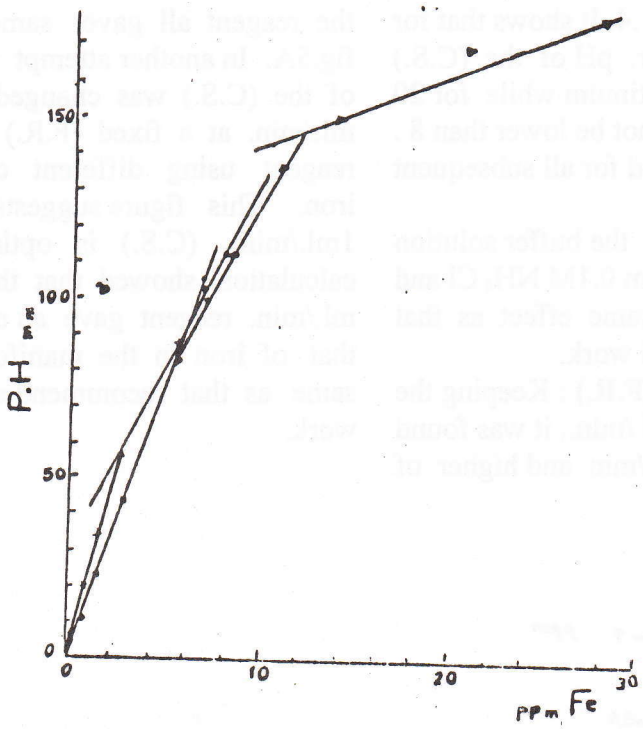

Fig 6: The Study of the manifold length with calibration curves.
(ˇ) manifold = 65cm. , (?) manifold length = 50 cm., (X) =25cm.
P.H. = Peak height (mm.) Recorder Settings as in Fig .4.

Calibration Curves: Calibrations were performed in both directions of increasing and decreasing concentrations of standard iron solutions and a typical recorder output is shown in fig.7. Statistical evaluations for the functions obtained from the applications of the method of least square (7) using both equations (Y = a + bX) and (Y=bX) with and without including the blank's value, which was zero all the time, are shown in table 1.

In general, good straight lines were obtained in all cases indicated from the high values of the correlation coefficients (r) given in the table, but better when equation (Y = a + bX) was used. This was also confirmed from smaller values of standard deviations associated with narrower confidence intervals. Among all the results shown in this table, the choice of highest value of (r), smallest (S), and narrowest (C.I.) goes to (AII). It seems that the recommended procedure of this work would be for the range (0 - 8.3 ppm total iron) carried out in the increasing direction of concentrations taking blank's reading as one of the standards and using the latter equation to draw the calibration curve by the method of least square. This procedure was applied for the determination of total iron in samples of some iron-containing drugs and the results obtained are shown in table 2.

Table 1:- Statistical treatment of the results of the calibration curves using the method of least square according to reference (7).

Ppm.Iron	AI	AII	AIII	AIV	BI	BII	BIII	BIV
Range	0 - 7	0 - 8.3	0.7 - 7	0.7 - 8.3	0 - 7	0 - 8.3	0.7 - 7	0.7 - 8.3
n	6	7	5	6	6	7	5	6
r UP	0.9993	0.99996	0.9968	0.9980	All the Same			
r down	0.9997	0.9992	0.9992	0.9999				
S UP	2.2	0.6	3.6	3.9	1.4	2.9	6.0	5.5
S down	1.4	2.3	2.2	4.6	4.2	5.1	4.7	5.6
C.I UP	58.45±2.5	72.44±0.6	68.18±4.9	82.02±4.5	58.45±1.5	72.44±2.6	68.18±7.6	82.82±5.7
C.I down	58.47±1.6	72.44±2.3	70.14±3.1	83.95±5.3	58.47±4.5	71.96±4.5	70.14±5.8	83.95±14

A_I - A_{IV} (Method A) Using equation $Y = a + b X$
B_I . B_{IV} (Method B) Using equation $Y = b X$ ..... Ref (7).
n = Number of points involved in drawing the calibration curve .
r = Correlation coefficient .
S = Standard deviation of the straight line.
C.I= Confidence interval ($y \pm ts/\sqrt{n}$) where y is the mean absorbency as (mm. Peak height),t values obtained from statistical t tables at(95%) confidence limit , degree of freedom (n - 2) for method (A) and (n - 1)for method (B) ..... Ref. (7).
UP & down = refer to increasing and decreasing directions of concentration measurements respectively

It has to be pointed out that the procedures used in this work for sample dissolution have neither been taken from any reference nor was the Author's intention to study them in the present work. They were only used to test the accuracy of the present method whatever the method of dissolution was. Variations between the methods of sample dissolution, a,b and c can be seen from the different values of iron obtained for the same sample. The differences between the samples for each method are, obviously, due to the origin of each of the three drug types and the composition of the coatings. But, since all these were not the objectives of the present work, no further studies were under taken at this stage.

To evaluate the suitability of the method to real samples of drug, a recovery test was performed. A quantity of 27.82 µg iron was added to the samples of drugs after dissolution and the percentage recovery (%R) of total iron were determined from the calibration curve as shown in table 2. It is shown that recoveries ranged between 95% - 105% could be obtained which were quite reasonable for such analysis. The last two columns of the table were devoted to the precision of the method. Eleven repeated determinations of two samples in random gave the results shown in the table with calculated coefficient of variation between 1.1 - 1.4%.

Table 2: Determination of total iron in samples of iron containing drugs . using the recommended procedure of the present work . The last two columns are devoted for the examination of the precision of the method .

Methods Of Sample prpn		* Iron found In Sample. ppm	Iron Found In10 ml µg	Amount of Iron added µg	Total Iron Present µg	Total Iron * Recovered µg	Recovery %	E %	Precision Examination Peak height mm	mm
Sample I	a	2.124	21.24	27.82	49.06	47.00	95.8	-4.2	21.5	66.0
	b	9.35	93.5	27.82	121.32	122.28	100.8	0.8	21.5	68.0
	c	8.24	82.4	27.82	110.22	112.74	102.2	2.2	21.0	65.5
Sample II	a	8.011	80.11	27.82	107.95	113.04	104.7	4.7	22.0	65.0
	b	7.65	76.5	27.82	104.32	109.6	105.0	5.0	21.0	65.5
	c	7.042	70.42	27.82	98.24	102.1	103.9	3.9	23.0	64.7
Sample III	a	3.82	38.2	27.02	66.02	66.6	100. 85	0.85	22.0	66.5
	b	5.901	-59.01	27.82	86.83	82.6	95.13	-4.9	22.0	66.0
									21.5	65.0
									21.0	66.5
									21.5	67.5
* Each result is a mean of three replicates.									Mean $\bar{X}$	21.64 66.82
									Standa rd Deviation S. D.	0.3 0.7
									Coefficient of Variation C. V %	1.4 1.1

It was possible to run at least 20 samples per hour. Each injection required 30 seconds to reach the cell and starts the peak. A maximum of three minutes, including the above 30 seconds, were required to wash back the manifold to the base line. Loading of the Loop can be performed 15 seconds before the end of the previous injection and starting a new one.

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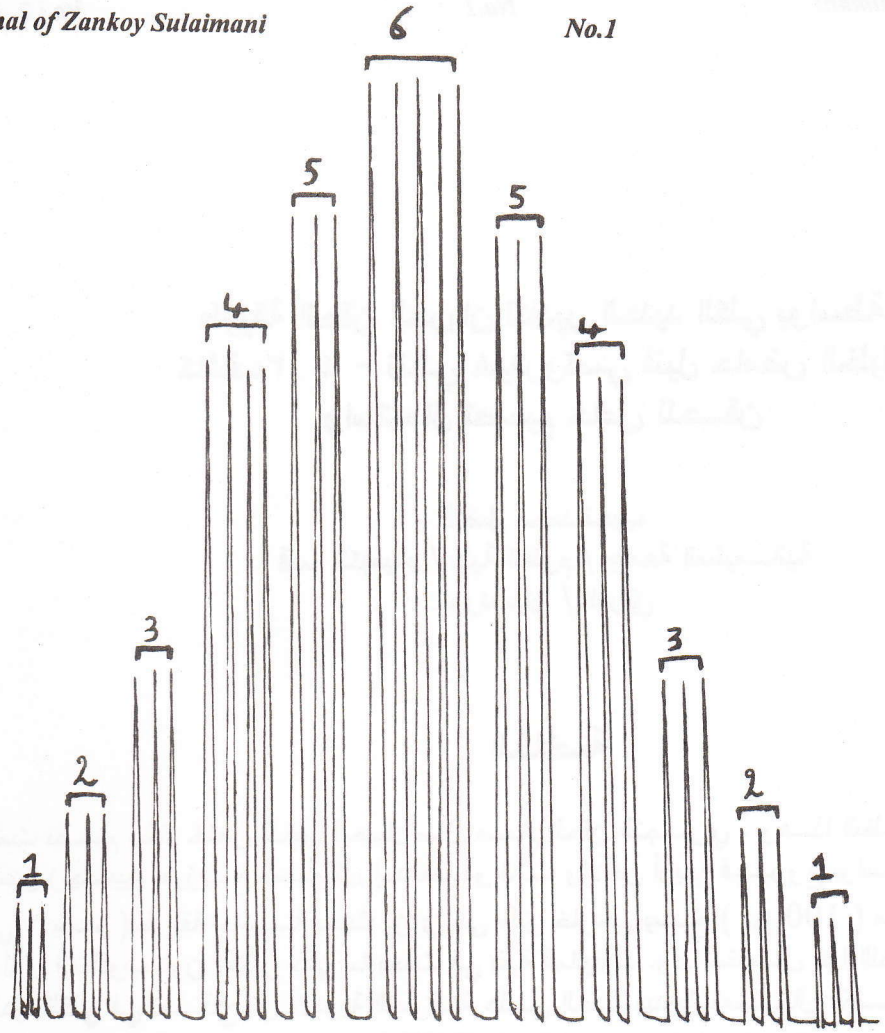

Fig .7: Recorder's output for calibrations in both ascending and descending directions . Total concentrations of Iron are:
(1= 0.7), (2 = 1.4), (3 = 2.8), (4 = 5.6), (5 = 7) and (6 = 8.3) ppm

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طريقة الحقن الجريان لتقدير الحديد الكلي بواسطة كاشف٣, ٤ - ثنائي هايدروكسي فنيل حامض الخليك واستعمال تصميم خاص للحقن

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الخلاصة

تمت تصميم نظام خاص للحقن ليحل محل صمام الحقن التجاري . وهذا النظام عبارة عن دمج صمامين زجاجيين لتوزيع السوائل إحداهما ذو ثلاث والآخر أربعة فروع بواسطة أنابيب ذو قطر داخلي(١ ملم)بطريقة خاصة بحيث يؤدي إلى ملتو خارجي يسع (100 µl) محلول ويسمح بملئه بالمحلول المطلوب دون التأثير على ما يحدث في بقية المانيفولد .وقد استعمل هذا النظام بنجاح تام لتقدير الحديد الكلي في نماذج من الأدوية الحاوية على الحديد باستعمال الكاشف٣ ،٤ ـ ثنائي هيدروكسي فنيل حامض الخليك (DHPAA- 4, 3) بطريقة التحليل الطيفي المرئي وفي طول موجي (596 nm) وسرعة جريان الكاشف (0.05 L/min) . المحلول الحامل(C.S) عبارة عن بفر (NH_3 + NH_4Cl) في (pH=8.1) وسرعة جريان (1mL/min) منحني المعايرة خط مستقيم ما بين (0 - 8.3 ppm) حديد كلي (Fe_{II} + Fe_{III}) متضمنا البلانك كأحد المحاليل القياسية ومرسوم بطريقة المربع الأدنى (Least square) ومعامل ارتباط (r) مساويا إلى (0.99996) ومدى الثقة ($y \pm ts/\sqrt{n}$) كان (72.44 ± 0.6) . وان تحليل نماذج الأدوية حاوية على حديد بعد إذابتها في (0.005M) من (HCl أو HNO_3) أعطى نسبة خطاء لم تتجاوز ألى (5 %) مستعملا طريقة الاستعادة لمقادير معينة من الحديد القياسي المضاف لتلك النماذج ومعامل التكرارية كان بين (1.1 - 1.4)% .

ریکەی تیرژاندنی بەردەوام (Flow-Injection) بو دیاری کردنی کوی ئاسن بەهوی ناسەرەوەی٣ ،٤ دوانە هایدروکسی فەنیل ئەستیک ئەسید (3,4 - DHPAA) بە بەکارهینانی سیستەمیکی تایبەت بو تیرژاندن .

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کوردستانی / عیراق

کورتە

لەم توژینەوەیەدا سیستەمیکی تایبەت دانرا بو دروستکردنی زمانە (Valve)ی تیرژاندن لە جیاتی زمانەی تیرژاندنی بازرگانی.ئەمەیش بەبەستنی دوو زمانەی شوشەیی بو دابەش کردنی شلەیەکیان -٣- ریکەیەو ئەوی دیکەیان چوار ریکەیەبەهوی بوریپلاستیکی باریک کەتیرەی ناوەوەی(یەکملم) ەو بەیەکەوە دەبەستریت بەجوریک ئەبیتەهوی پیک هینانی لوپیک لەدەرەوە دروست بیت قەبارەکەی نزیکەی (100 µl) دەبی ،ریکەدەدات بەکیراوی مەبەست پر ببیتەوەبەبی ئەوەی کارلە پاشماوەی مانیفولید بکات.ئەم سستمە بەسەرکەوتویی لەدیارکردنیکوی ئاسن لە نمونەی ئەو دەرمانانەی کەئاسنیان تیدایەبەناسەرەوەی (٣ ،٤ – دووانە هایدروکسی فەنیل ئەستیک ئەسید) بەکار هینرا بەریکەی شەبەنکی بینراو کەدریژی شەپولەکەی (596 nm) ، خیرایی ناسەرەوەکە (0.05ml / min) بوو کیراوەی هەلگر (C.S) بریتیبە لەبەفەری ($NH_3 + NH_4Cl$) لە (pH=8.1) وبەخیرای (1ml/ min) . هیڵی راست کەکیشرابیت بەریکەی (Least Square) ئەکەویتە نیوان (0 ¦ 8.3ppm) ، بلانکیش یەکیکەلەستاندەرەکان،کوی ئاسن (Fe^{III} + Fe^{II}) کە (r = 0.99996) وئاستی بروا پی هینان (Confidence Interval) واتە ($y \pm ts/\sqrt{n}$) یەکسان بووبە (72.44 ± 0.6) لە (95 %) ئاستی بروا پی هینان .بەشی کردنەوەی نموونەیی ئەو دەرمانانەی کەئاسنیان تیدایەودوای تواندنەوەیان لە (0.005M) لە (HCl یان HNO_3) ریژەی هەئەلە (5 %) . تیپەڕینەکردبوو بەبەکارهینانی ریکەی دەست کەوتنەوەی بریکی زانراولەئاسنی پیوانەی کەخراوەتەسەر ئەونمونانە وهاوکوڵکەی دووبارە بوونەوە لە نیوان (1.4 % ¦ 1.1) بوو .