Determination of Desferrioxamine in the Drug Desferal™ as DFOM-Au (III) Complex by Using Indirect Electrothermal Atomic Absorption Spectrometry and Other Techniques.

Ashraf.S. AL-Ayash*, Zuhair A-A Khammas** and Fadhil Jasim*

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Abstract

An indirect method using electrothermal atomic absorption spectrometry (ETAAS) has been established for the determination of DFOM in the drug desferal™as DFOM-Au (III) complex. The formation of this complex at 1:1 mole ratio computed by UV-Vis spectrophotometric technique was confirmed by distinction of the IR absorption spectra between the free DFOM and its complex. Several experimental factors that effect the formation of DFOM-Au (III) complex were optimized using AA responses. Aliquots of the complex extract were injected into a coated GF and the AA-signals measured. A direct calibration graph was constructed and from which the figures of merits were found such as: LDR (0.08-3.5 µg ml^{-1}), m_{0^o} (293.3 pg), LOD (0.0154 µg ml^{-1}), RSD% (2.0-3.5), recovery present (101.54±0.86), and $\%E_{rel}$ (1.54). The established indirect ETAAS was applied to analyze the drug desferal™ for the determination of DFOM using direct and standard additions procedures and DFOM found to be 488 and 484 mg/unit respectively compared to the stated value of 500 mg/unit. All statistical calculations were implemented via the chemsoftware (Minitab version 11).

Introduction

Desferrioxamine (DFO), a trihydroxamate siderophore derived from sterptomyces pilosus, is currently the most clinically useful iron–chelating agent available since its introduction in the 1960, DFO has been extensively used for chelating therapy in iron overloaded states [(1).]

Currently, desferrioxamine is used for treatment of acute iron poisoning and thalassaemia major, as well as aluminum poisoning associated with chronic renal dialysis [(2, 3).]

Accordingly, the determination of the parent DFOM and their metal complexes necessitates the establishment of an accurate, rapid and reliable method.

Various procedures have been described for estimation of desferrioxamine, these include Polarography [(4)] ,ICP/AES [(5)],ESI-MS [(6)] ,pyrolysis–GC–MS[(7)] ,Liquid chromatgraphy[(8)] , Potentiometric [(9)] , ET-AAS [(10)]. The use of AAS is now well-recognized as a technique combines attraction features of both direct detection of metals and indirect determination of organic products such as drugs and medicaments and subjected to many researches [(11-13)].

To the best of our knowledge, there is no application of ETAAS analysis of the drug desferrioxamine as DFOM-Au (III) complex in organic solvent.

In present work, a developed indirect analysis of the drug DFOM as DFOM-Au (III) complex by using ETAAS combined with zirconium-caride coated graphite tube under optimized conditions and be applied simply to pharmaceutical preparation containing desferrioxamine mesylate.

Experimental

Instrumentation

A Shimadzu (AA–670) atomic absorption spectrometer/GFA–4A atomizer system was used for all ET– AAS measurements. The atomizer was fitted with high density graphite standard tube (P/N 200-54520) .

The graphite tube was coated with zirconium-carbide according to the procedure described elsewhere[(14)].

*Chemistry Department/College of Scince/Unversity of Baghedad
**Chemistry Department/ College of Scince/University of Diyala

A high – purity nitrogen (99.9999 %) was employed as the atomizer purge gas .
The graphite tubes were cooled during operation by means of the cool flow CFT-33. The analytical condition, data, and the AA signals at 242.8 nm were displayed on the graphic printer PR-4. The standard and sample solutions (10- µl) were injected with aid of an auto sample changer (ASG-60G).
- Shimadzu UV- Visible Spectrophotometer UV-160A.
-FT-IR Perkin-Elmer, spectrum RXI, PIN099-3421,BSEN 60825 Class 2 Helium (Neon-Ne) Laser.

Reagents

Analytical-grade reagents and deion-ized water were used in the preparation and dilution of solutions; desferrioxamine mesy - late standard material and drug desferal^{TM} were provided from the Novartis pharma AG, Basle, Switzerland.

Desferrioxamine mesylat Stock solution (1000 µg ml $^{-1)}$

A-0.1000 gm of DFOM was dissolved in water and diluted to 100 ml in a volumetric flask. A 50 µg ml^{-1} was prepared by pipeting 2.5-ml of stock solution into 50-ml volumetric flask and diluted to mark with water.
Gold standard solution (1000 µg ml $^{-1)}$

A Stock gold (III) solution (1000 µgml^{-1}) was purchased from BDH (as Atomic Absorption Standard). A 50-µg ml^{-1} working Au solution was prepared by dilution of the stock solution in water.

Analytical Procedures

Preparation of drug Desferal^{TM} sample

The content of 10 vials of the drug sample (each content 500 mg of DFOM) was mixed together then 0.1g was diluted to 100 ml. This solution was then diluted by transferring 10 ml and diluted to 100 ml with water. The final sample dilute solution was prepared by pipeting 25 ml of the latter and diluted to 50 ml with water.

(A) Determination of DFOM in the Drug Desferal^{TM}Sample by Direct Calibration

Six standard solutions were prepared by pipeting 0.008, 0.05, 0.1, 0.2, 0.3 and 0.35 ml of 50-µg ml^{-1} of standard DFOM solution into 5-ml volumetric flasks, then 0.3 ml of 50 µg ml^{-1} of gold standard solution was added to each flask and after adjusting the pH between 6.5-7, each flask was diluted to mark with water which correspond to 0.08, 0.5, 1.0, 2.0, 3.0 and 3.5 µg ml^{-1} of DFOM. These solutions were immersed in water bath at temperature of 80 ^{0}C for 20 min. then each solution was extracted using separating funnel with 1 ml of carbon tetrachloride after shaking for 4 min.
The organic layer was transferred to test tube for each solution from which10-µl aliquot was injected in graphite furnace and the optimized heating cycle applied (Table 1).The standard calibration graph was constructed by plotting peak heights versus DFOM concentrations (Fig.7) from which the concentration of DFOM in desferal drug sample was determined by regression.

(B) Determination of DFOM in the Drug Desferal^{TM}Sample by Standard Additions

0.2 ml aliquots of the above-prepared final desferal^{TM}sample solution were pipeted into seven 5-ml calibrated flasks containing 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 ml of 50 µg ml^{-1} of standard DFOM solution, then 0.3 ml of 50 µg ml^{-1} of gold standard solution was added to each flask and after adjusting the pH between 6.5-7, each flask was diluted to mark with water**.**The extraction process was carried out for each solution as mentioned as in (A). The organic layer was transferred to test tube for each solution from which 10-µl aliquot was injected in graphite furnace and the optimized heating cycle applied (Table1). The standard additions graph was constructed by plotting peak heights versus DFOM conce-ntrations (Fig.7).The DFOM content in desferal^{TM} drug by regression from zero standard additions values.

Results and Discussion

Optimization of the graphite furnace program

Table (1) shows the optimum experi-mental conditions for heating programme used for the determination of the drug DFOM and the heating cycles used to establish ashing and atomization graphs for the extracted complex(10-µl injection of 2µgml^{-1} as

DFOM) . It was shown that a period of 20s at 100ºC was suitable for drying the organic extracted complex. The effect of ashing and atomization temperatures on the gold AA-signals in the extracted complex [DFOM-Au (III)] was studied. It was found that the maximum absorbance signal was achieved at ashing and atomization temperatures of 500ºC and 2200ºC respectively (Fgure not shown) and these temperatures were selected as optimal throughout this work.

Optimum Conditions

1-Effect of pH Values

The reaction of gold with DFOM doesn't occur directly. It was found that this reaction takes place at certain pH. It appeared that the best pH ranges occur between 6.5 and 7.0 for the formation of chelate complex. At pH more than 7.0, the complex was destroyed and this makes the adjustment of pH very important in extraction process of chelate.

2-Effect of Concentration of Gold (III)

It was found that the absorbance of DFOM-Au (III) complex increases linearly as the concentration of Gold (III) ion increases and the deviation from this linearity was appared by curve bending towards the gold concentration axis (Fig.1).Consequently, the optimum concentration of Au (III) of 3μgml^{-1} was selected for complete formation of chelating complex. It was suggested that the drug Desferrioxamine reacts with Au (III) and form hexadentate complex according to the following equation [15].

$$H_3DFO^- \xrightarrow{pH=6.5-7.0} DFO^{-3}$$

$$DFO^{-3} + Au(III) \xrightarrow{pH=6.5-7.0} DFO-Au(III)$$

3-Effect of Reaction Time

Fig (2) shows the effect of reaction time on the formation of complex before the extraction process. It was shown that the absorbance increases rapidly with the reaction time just up to 20 min. and then reaches a plateau, which indicates that there is no advantages in going beyond 20 min., perhaps lead to partial dissociation of the complex with longer time in aqueous phase.

4- Effect of Extraction Time

It was perceived that the absorbance of the chelating complex increases readily with shaking time and attains a plateau leading to the stability of the absorbance values with increasing time, and hence a 4 min. was chosen as an optimal for complete extraction of the complex (Fig.3).

5-Effect of Phase Ratio

The volume of aqueous phase was varied from 4-8 ml, while keeping the volume of organic phase constant (1ml) and the experiment was conducted to obtain the best organic/aqueous ratio for the extraction of DFOM-Au (III) complex at optimum conditions. The results were revealed that the complex gave maximal atomic absorption signals when the ratio between aqueous and organic phase were 5:1 (Fig.4). The percent extraction (%E) and the distribution ratio (D) of the complex were found to be 95.47% and 84.3 respectively for one stage extraction.

6–Effect of temperature

The reaction of the Au(III) with DFOM was very slow and took about one hour , consequently, the effect of temperatures was studied and found that the best temperature was 80^{o}C (Fig.5) to obtain maximum absorbance and decreased thereafter due to the decomposition of the complex. This factor shorted the reaction time to about 20 min.

7–Selection of Organic Solvents

Since the method encompasses the measurement of complex in organic phase, it is necessary to use a solvent that will extract the chelate complex alone, not excess gold (III) and free ligand (the drug) use. Several organic solvents (such as dichloromethane, chloroform, MIBK,1-octane,o-xylene,toluene, carbon tetrachloride, 1-butanol, cyclohexane, benzene, acetyl acetone, diethyl ether, benzyl alcohol, dichromethane and petroleum ether) have been examined to investigate the suitable one for the extraction of complex and carbon tetrachloride was found to be at best for the extraction the complex at optimum conditions excluding other species in the extraction system, and this solvent doesn't extract the blank but complex only at neutral medium (pH = 6.5 – 7.0) .

Structure of the complex

Several techniques such as FTIR, ETAAS and Molar ratio method have been used to elucidate the structure of DFOM-Au (III) complex formed at optimal conditions. The data revealed that a 1:1 complex was formed with stability constant of $1.2x10^6$ M^{-1} (λ_{max}= 550 nm) and from IR spectra and elemental analysis data [10], the following structure of the complex was suggested (Fig.6):

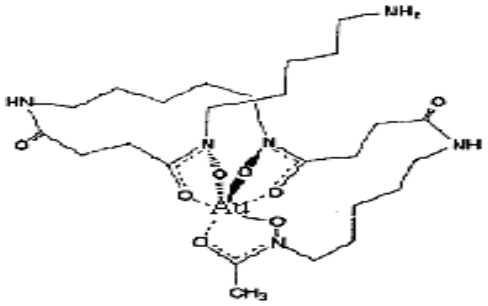

Fig (6) Structure of complex DFOM-Au (III)

To ensure the stability of complex in the organic medium during the measurements by ETAAS, the accuracy in term of recovery% was measured after interval of the complex preparation times.Good recoveries(98.41-100.80%) have been obtained up to 48 hours duration time and remarkable depression occur thereafter (94.44 %).The low recovery may be due to the change in molecular association between the ligand and metal during the time or the interaction between the complex and gold with organic solvent.

Calibration Graph

Using optimum conditions established, direct calibration graph for the indirect determination of DFOM was constructed and the statistical results are illustrated in Table (2). Beer's law was obeyed over the concentration range (0.08-3.50 µg DFOM ml^{-1}), then the calibration line was observed to be bent (Fig.is not shown) toward the concentration axis. This is may be due to the formation of the strong bonding between gold atoms, results in a lower proportion of free atoms being available in the analytical volume within resonance radiation path. The best fit was obtained for a first order equation (Table2)with correlation coefficient of 0.9988 and coefficient of determination (R^2) was 99.76% which suggests statistically valid fit. We use this fitted linear calibration model to estimate the DFOM concentration in the drug samples which appears justified, on statistical basis.

The sensitivity and detection limit were also calculated by using the developed analytical procedure. The characteristic mass which is the amount in picogrames needed for 0.0044A was calculated to be 293.3 pg and limit of detection was 0.0154 µg ml^{-1} compared favorably with published value 0.1µgml^{-1} [16] and 0.095µg ml^{-1} [17].

Determination of DFOM in Pharmaceuti- cal preparation

The developed method was applied for the detection of DFOM in one of the selected pharmaceutical preparation containing desferrioxamine (vial) with stated concentration of 500 mg per unit by using direct calibration and standard additions procedures. The DFOM was determined through the atomization of the complex extracted as a result of the reaction of DFOM present in the pharmaceutical preparation with gold (III) and found to be 488.58 and 484.12 mg/unit with relative error of-2.28% and -3.17%. It can be observed from Fig.(7) that the ratio of the slopes of the direct calibration and standard additions is found to be one,which indicates that the interferences resulting from drug constituents are insignificant using the developed procedure.

Since the certificate reference material for the determination of DFOM in drug samples is not available, accuracy has been tested through the recovery percent evaluation.Recoveries were acceptable in the range of 97.2-98.5%with mean value of 98.01% ± 0.70%,indicating that the indirect determination of DFOM using the established method is not highly affected by the presence of other constituents in the drug sample**.**

The Determination of DFOM in the form of chelate with Au(III) was also carried out by using UV-Vis Spectrophotometric techn- ique in our laboratory after taking account all the optimized conditions that performed in

ETAAS. Fig. (8) shows the regression line between the developed ETAAS and UV-Vis Spectrophotometric results after taking the common concentra- tions of the calibr- ation graphs used by these two methods. The r-value (0.9970) was revealed that a systematic error may be occurring in the slope of the individual calibration plots, but still no significant difference occurring in both methods within the concentration range studied(i.e. 0.5–3µg/ml^{-1}).This concludes that no difference in the application of each method for the determination of DFOM in the pharmaceutical preparations.Table(3)also gives the statistical evaluation for comparison of these two methods. The F-value of 4.04 calculated was found to be less than the critical one (9.277) indicating that no significance differe- nce in precision at 95% confident limits between both methods. The detection limit for DFOM by an indirect ETAAS was about 15 times better than those obtained by UV-Vis Spectrophotometric technique (Table 3).Consequently, this low detection limit makes indirect ETAAS technique more applicable for the determination of DFOM in biological fluids than the later one. Finally, the amount of DFOM determined by indirect ETAAS and direct UV-Vis Spectrophotometry using direct calibration procedure were agreed and found to 488.58 mg/unit ($\%E_{rel}$ =-2.28%) and 486.72 ($\%E_{rel}$=-2.65) respectively.

Conclusions

The determination of DFOM using Au(III)as a pairing agent showed low detection limits and highly absolute sensitivities compared with other analytical techniques.The analytical results obtained for the determination of DFO in some pharmaceutical compounds showed good agreement with the given–labeled quantity.From the best of our knowledge from the literatures it was found that no body concerned with the reaction of gold with DFOM,and that confirms the imp ortance of this reaction because the gold is an effective and important element from the biological side.

References:

1- Keberle,H.,1964."The biochemistry of deferroxamine and its relation to iron metabolism."Ann NY.Acad.Sci,119:758-768.

2- Day,J.P.,and Ackrill,P.,1993."The chemistry of deferoxamine chelation for aluminum overloads in renal dialysis patients." Ther Drug Monit, 15: 598-601.

3- Banner,W.Jr.and Tong,T.G.,1986."Iron poisoning."Pediatr Clin North Am,33: 393- 409

4- Romero,R.A.andDay,J.P,1985.Polarographic determination of desferrioxamine B in dialysis samples,Trace Elem.Med,2(1): 1–6.

5- Bourdon,S.,and Houze,P.R.,1987.Quantification of desferrioxamine in blood plasma by inductively coupled plasma atomic emission spectrometry,Clin.Chim,33(1): 132–134 .

6- Gledhill,M.,2001."Electrospray ionisation mass spectrometry of hydroxamate siderophores",Analyst,126(8):1359– 1362.

7- Richard,M.,Higashi,Teresa,W–M.Fan and Andrew,N.Lane.,1998."Association of desferrioxa mine with humic substances and their interaction with cadmium(II)as studied by pyrolysis-gas chromatography–mass spectrometry and nuclear magnetic resonance spectro-scopy",Analyst, 123,(5) : 911–918.

8- Menendezfraga,P.,Blancogonzaleze,E.,Sanzmedel,A.,and Connataandia, J.B.,1997 ."Mic- ellar versus reversed phase liquid chromatography for the determination of desferrioxamine and its chelates with aluminium and iron in uremic serum", Talanta, 45 (1): 25 – 33.

9- Hou, Z.G. ,Whisenhunt, D.W.,and Raymond,K.N.,1994."Potentiometric,spectroph otometric, and H-1-NMR study of 4 desferrioxamine-B derivatives and their ferric complexes", American chemical society, 116 (3): 840-846

10- Jasim,F.,Khammas,Z.A-A.,And Al-Ayash ,A.S.,2005."Spectrophotometric micro determination of drug desferrioxamine in

some phamaceuticals by chelating with vanadi- um(v)",Al-fath, J., 23: 25 -37.

11- Garcia–Vargas,M.,Milla,M.,and Pere Bustamante ,1983.Atomic-absorption spectroscopy as a tool for the determ ination of inorganic anions and organic compounds. A review Analyst, 108(1293): 1417-1449.

12- Clark,E.R.,andYacoub,A.K.,1984.Indirect determination of organic compounds by atomicabsorp-tion spectrophotometry,31 (1) :15-17 .

13- Hassan,S.S.M.,1984."Organic Analysis Using Atomic Absorption Spectrometry" Ellis Hordwood Ltd, Chichester, West Sussex, pp.242.

14- Khammas,Z.A-A.,And Jasim,F.,2001." Determination of gallium in biotite and bauxite with ET-AAS using Zr-carbide coated graphite tube and Pd matrix modifier" , Iraqi J. Sci., 42 (1) :87 - 105.

15- Buglyo,P.,Culdu, N., Kiss, T.S.,Micera, F. and Sanna,D.,1995.Vanadium(IV) and vanadium (V) complexes of deferoxamine B in aqueous solution J.Inorg.Biochem., 60(1) :45-59 .

16- Allain,P.,Mauras,Y.,Beaudeau,G.and Hingouet,P,1986.Indirect micro-scale method for the determination of desferrio xamine and its aluminium and iron chelated forms in biological samples by atomic absorption spectrometry with electrothermal atomization,Analyst,111(5) :531-533.

Table (1):Optimized Experimental Parameters and GFA heating cycleFor determination of DFOM(2 µg ml $^{-1)}$–Au (III) by indirect ET-AAS

Parameters	Instrumental Conditions
Wavelength (nm)	248.2
Slit band pass (nm)	0.5
H.C.L. current (mA)	6
Singal mode	Peak height
B.G.Correction lamp	On
Chart speed (cm / min)	1
Injected volume (µl)	10
GFA- 4 A Heating cycle :Dry- (^{0}C / S) ramp	100 / 20
Ash – (^{0}C / S) step	500 / 20 *
Atomize– (^{0}C / S) step	2200 / 4 ** Gas stop mode
Clean – (^{0}C / S) step	2300 / 4
Cool– (^{0}C / S) step	0 / 20
Purge Gas	N_2
Flow rate(liter / min)	1.5

* From 300 to 1500/20 for construction of ashing curve

**From 1000 to 2600 / 4 for construction of atomization

Table (2): Representative Statistical results For the Analysis of DFOMBy Indirect ETAAS.

Range of concentration (µg.ml^{-1})	0.08-3.5
Detection limit (µg.ml^{-1}) for n=13	0.0154
Characteristic mass (pg)	293.3
Regression line	Abs.=0.126(conc) + 0.001
Correlation coefficient (r)	0.9988
Coefficient of determination (R^2)	99.76%
C.L. for the slope (b±ts$_b$) at 95%	0.126±0.0084
C.L. for the intercept(a±ts$_a$) at 95%	0.001±0.0165

Table (3): comparison between UV-Vis and ETAAS techniques for the determination of DFOM in the form of DFOM-Au (III)

Techique	Linearity (µgml^{-1})	D.L. (µgml^{-1})	RSD %	Corr. Coef (r)	Calculated F-test	Tabulated F-test
UV-Vis. method	1-40	0.23	1.89	0.9984	4.09	9.277
ET-AAS method	0.08-3.5	0.0154	2.66	0.9988		

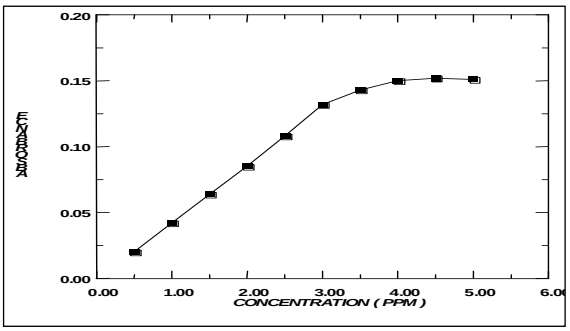

Fig.(1): Effect of Concn.of Gold on the determination of DFOM-Au(III)

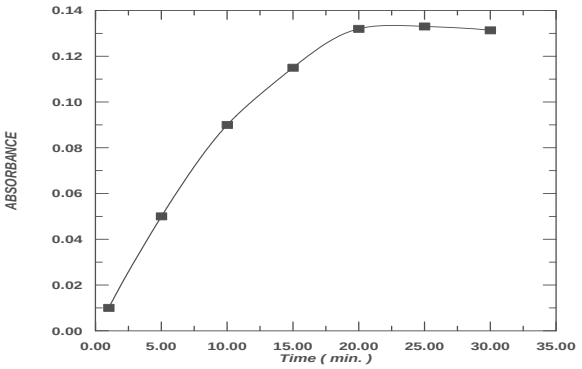

Fig.(2): Effect of reaction time on the determination of DFOM-Au(III)

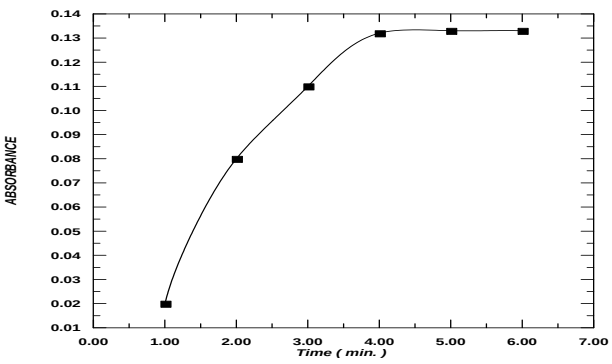

Fig.(3): Effect of extraction time on the determination of DFOM-Au(III)

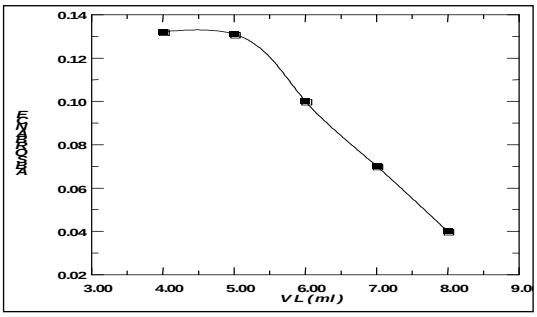

Fig.(4): Effect of phase ratio on the determination of DFOM-Au(III)

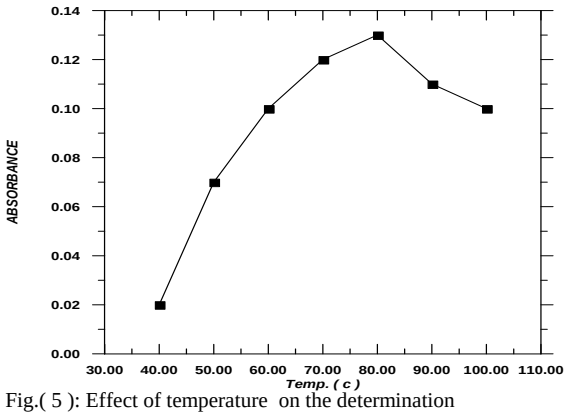

Fig.(5): Effect of temperature on the determination of DFOM-Au(III)

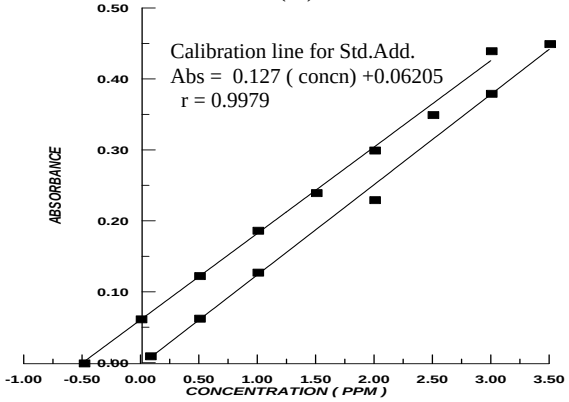

Fig. (7): Determination of DFOM in pharmaceuticals by using direct and standard additions procedures.

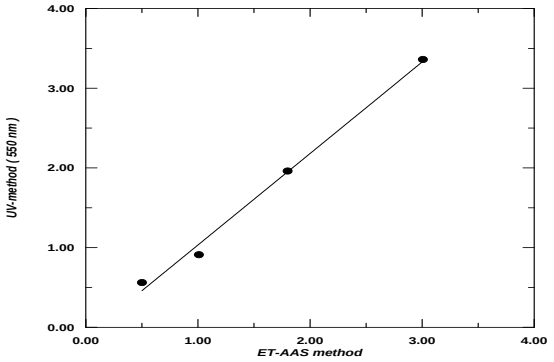

Fig.(8) : Comparison between ETAAS and UV- Vis. Spectrophotometric techniques using regression line

التعيين غير المباشر للدسفيروكسامين في المستحضر الصيدلاني الدسفرال بصيغة المعقد DFOM-Au(III) باستخدام مطيافية الامتصاص الذري الكهروحراري وتقنيات اخرى

اشرف سعد العياش *، د. زهير عبد الاميرخماس**، د. فاضل جاسم محمد*

* قسم الكيمياء - كلية العلوم - جامعة بغداد
** قسم الكيمياء - كلية العلوم - جامعة ديالى

الخلاصة :

استحدث طريقة تحليلية غير مباشرة لتعيين المادة الفعالة الدسفيروكسامين في دواء الدسفرال من خلال تفاعل هذا المركب مع عنصر الذهب الثلاثي لتكون معقد مخلبي يجري استخلاصه بمذيب رابع كلوريد الكربون وحقن حجم معيين من هذا المستخلص في الفرن الغرافيتي المطلي بكربيد الزركونيوم . جرى دراسة معرفة تكوين وتركيب المعقد (DFOM-Au(III) بالتقنيات المعروفة مثل مطيافية الاشعة تحت الحمراء ومطيافية الاشعة الفوبنفسجية-المرئية وتبين ان النسبة المولية للعضيدة-الفلز هي 1:1.

لقد تم دراسة العوامل التجريبية التي تؤثر على تكوين هذا المعقد من خلال حساب الممتصية الذرية بالفرن الكرافيتي للحصول على الظروف الامثلية . كما تم تثبيت ارقام الاستحقاق التحليلية (figures of merits) من منحنيات المعايرة كالمدى الخطي والحساسية وحد الكشف ونسبة الاسترداد بالمائة والدقة المعبر عنها بالاستنساخيه . جرى تطبيق هذه الطريقة لتعيين الدسفيروكسامين في دواء الدسفرال ووجد ان تركيزه هو 488 و 484 ملغم / وحدة في هذا الدواء باستعمال المعايرة المباشرة واضافات القياس على التوالي مقارنة بالكمية المصرحة على العبوة وهي 500 ملغم / وحدة .

كانت نتائج مطيافية الامتصاص الذري غير المباشرة ومطيافية الاشعة الفوق البنفسجية-المرئية المباشرة متفقة إذ نفذت معظم الحسابات الاحصائية باستخدام البرنامج الحاسوبي (Minitab version 11) .