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Indirect Electrothermal Atomization Atomic Absorption Spectrometric Determination of the Drug Desferrioxamine in Some Pharmaceutical Preparations Using Vanadium (V) as a Mediating Element

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Abstract

A method has been developed for the determination of the drug Desferrioxamine (DFOM) in some pharmaceuticals using indirect electrothermal atomic absorption spectrometry (ETAAS) supplied with zirconium-coated graphite tube and vanadium (V) as a mediating metal. V (V) forms a chelate complex with DFOM at pH range 1-1.5 which could be extracted with benzyl alcohol under certain experimental conditions .Aliquots of the extracts are injected into the coated graphite furnace and the atomic absorption signals are measured. A standard calibration graph was constructed and from which several parameters and figures of merit were found, such as: linear range (0.5-19 µg mL $^{-1)}$ of DFOM, relative standard deviation (1.44-1.68%); limit of detection (0.12 µg mL $^{-1}$) sensitivity. (424.2 pg), recovery %(101.57± 0.147) and relative error (1.57%). The mole ratio method has been used to determine the structure of chelate DFOM: V (V) and found to be 1:1. The developed procedure is applied to analyze desferrioxamine in several commercially available pharmaceuticals using direct and standard additions methods and results are comparable. All statistical calculations are implemented via a Minitab software version 11.

Keywords:

Desferrioxamine; Vanadium (V); Indirect Eelectrothermal Atomic Absorption Spectrometry; Desferal^{TM}; Response Surface Method

1. Introduction

The drug desferrioxamine (DFO) and its derivatives such as desferrioxamine mesylate(DFOM),chemically30-amino-3,14,25-trihydroxy-3,9,14,20,25 pentaazatriacontane-2,10,13,21,24-pentone methanesulphonate (Fig.1), is deemed to be a supreme for the clinical treatment of several diseases specially for those related to the metal intoxication in human subjects, for example, thalassemia [1] (iron over-load) , Alzheimer [2] and renal disorders (aluminum over-load) [3].

In fact, the activity of desferrioxamine lies in its highest efficiency as a special agent for removal of iron and aluminum from patients by formation of the chelates which excreted through feces and urine. However, this drug is not destitute of side-effects encountered upon long-term treatment or high dose.

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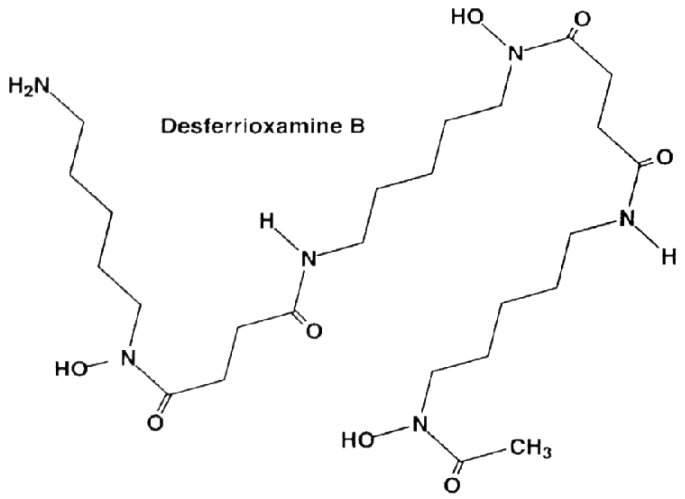

Fig. 1 Structure of Desferrioxamine.

The drug and its formulations are officially listed in British Pharmacopoeia [4] which suggests potentiometric titration method for its assay. Several methods have been reported for the determination of desferrioxamine, such as HPLC [5-6] polarography [7] Voltammetry [8-9] ICP / AES [10] ETAAS [11-12]. The use of AAS is now well-recognized as a technique combines attractive features of both direct detection of metals and indirect determination of organic products such as drugs and medicaments and subjected to many researches [13-16]. To the best of our knowledge, there is no application of ETAAS for the analysis of the drug desferrioxamine as DFOM-V (V) complex in organic solvent.

This work describes an indirect determination of the drug DFOM by using ETAAS combined with zirconium-coated graphite tube under optimized conditions. The suggested method is based on the reaction of the drug with V(V) as a mediating element and measuring the AA signal of the vanadium complex in organic layer. The method is applied successfully for the analyzing of the pharmaceutical preparations containing desferrioxamine mesylate.

2. Experimental

2.1. Apparatus

A Shimadzu (AA–670) atomic absorption spectrometer/GFA–4A atomizer system was used for all ETAAS measurements. The atomizer was fitted with high density graphite standard tube (P/N 200-54520). The graphite tube was coated with zirconium according to the procedure described elsewhere [17].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) apparatus . The analytical conditions , data,and the AA signals at 318.4 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).

2.2. Reagents and Materials

Analytical–grade reagents and deionized water were used in the preparation and dilution of solutions, Desferrioxamine Mesylate standard material and desferal drug were of the Novartis pharma AG, Basle, Switzerland. Vanadium Stock Solution (1000 µg mL^{-1}) was prepared by accurately weighing 0.1785 g of V_2O_5 then dissolved in 5 mL of sulphuric acid (2N), and diluted to 100 mL in a volumetric flask with water. A 50 µg mL^{-1} working V solution was prepared by dilution of the stock solution with water. Desferrioxamine Mesylat

Stock Solution (1000 μg mL^{-1}) was prepared by accurately weighing 0.1 g of DFOM standard material, dissolved in water and diluted to 100 mL in a volumetric flask.

2.3. Procedure

2.3.1. Preparation of drug Desferal™ sample:

The content of 10 vials of the drug sample (each contains 500 mg of desferrioxamine) was mixed together, and then 0.1g was diluted to 100 mL. A solution was then prepared by diluting 10 mL to 100 mL with water. The final sample diluted solution was prepared by diluting 25 mL to 50 mL with water.

2.3.2. Determination of Desferrioxamine in Drug Desferal™ sample by Direct Calibration:

Eleven standard solutions were prepared by pipetting (0.05-1.9) mL of 50 μg mL^{-1} of standard DFOM solution into 5-mL volumetric flasks, then 0.7 mL of 50 μg mL^{-1} of vanadium standard solution was added to each flask and after adjusting the pH between 1-1.5, each flasks was diluted to mark with water which correspond to (0.5-19.0) μg mL^{-1} of DFOM. Each flask was extracted with 1 mL of benzyl alcohol using separating funnel after shaking for 5 min. at room temperature. 10-μL of organic aliquots 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 from which the concentration of DFOM in Desferal™ sample was determined by regression

2.3.3. Determination of DFOM in the Drug Desferal™ sample by Standard Additions:

0.2 mL aliquots of the above-prepared final Desferal™ sample solution were pipetted into seven 5-mL calibrated flasks containing(0.0-1.9 mL) of 50 μg mL^{-1} of standard DFOM solution, then 0.7 mL of 50 μg mL^{-1} of vanadium standard solution was added to each flask and after adjusting the pH between 1-1.5, each flasks was diluted to mark with water. The extraction process was carried out for each solution as mentioned in (2.3.2). 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 (Table 1). The standard additions graph was constructed by plotting peak heights versus DFOM concentration. The DFOM content in Desferal™ drug was determined by regression from zero standard additions values.

3. Results and discussion

3.1. Optimization of the graphite furnace program

Table 1 shows the optimum experimental conditions for heating programmer 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 5 μg mL^{-1} as DFOM). It was shown that a period of 20s at 150ºC was suitable of drying the organic extracted complex. The effect of ashing and atomization temperatures on the vanadium AA-signals in the extracted complex [DFOM-V (V)] was studied. It was found that the maximum absorbance signal was achieved at ashing and atomization temperatures of 1300 ºC and 2800ºC respectively (Figure is not shown) and these temperatures were selected as optimal.

Table 1. Optimized experimental parameters and GFA heating cycle for the determination of DFOM (5 μg mL^{-1}) – V (V) by indirect ET-AAS.

Parameter	Instrumental condition
Wavelength (nm)	318.4
Slit band pass (nm)	0.5
H.C.L. current (mA)	5
Signal mode	peak height
B.G. Correction lamp	on
Chart speed (cm/min)	1
GFA-4A heating cycle: Dry (°C/s) ramp	150/20
Ash (°C/s) step	1300/20 *
Atomize (°C/s) step	2800/4 ** gas stop mode
Clean (°C/s) step	2850/4
Cool (°C/s) step	0/20
Purge gas	N_2
Flow rate (L/min)	1.5

* From 700 to 2400/20 for construction of ashing curve
** From 2200 to 3000/4 for construction of atomization curve

3.2. Optimum Extraction Conditions

In all of the following experimental optimizations, the concentration of DFOM standard solution was kept constant (5 μg mL^{-1})

3.2.1. Effect of pH Values.

The effect of pH on the formation of DFOM-V(V) complex is shown in Fig. 2 from which it appears that the best pH ranges occur between (1-1.5) for the formation of chelate complex .

3.2.2. Effect of Concentration of V (V).

It was found that the absorbance of DFOM-V (V) complex increases linearly as the concentration of V (V) ion increases and the deviation from this linearity was apparent by curve bending towards the vanadium concentration axis (Fig. 3). Consequently, the optimum concentration of V(V) of 7 μg mL^{-1} was selected for complete formation of chelating complex. It was suggested that the drug Desferrioxime reacts with V (V) and form hexadentate complex according to the following equation [18]:

$$VO_2^+ + H_4DFA^+ \longrightarrow VOHDF+.XH_2O$$

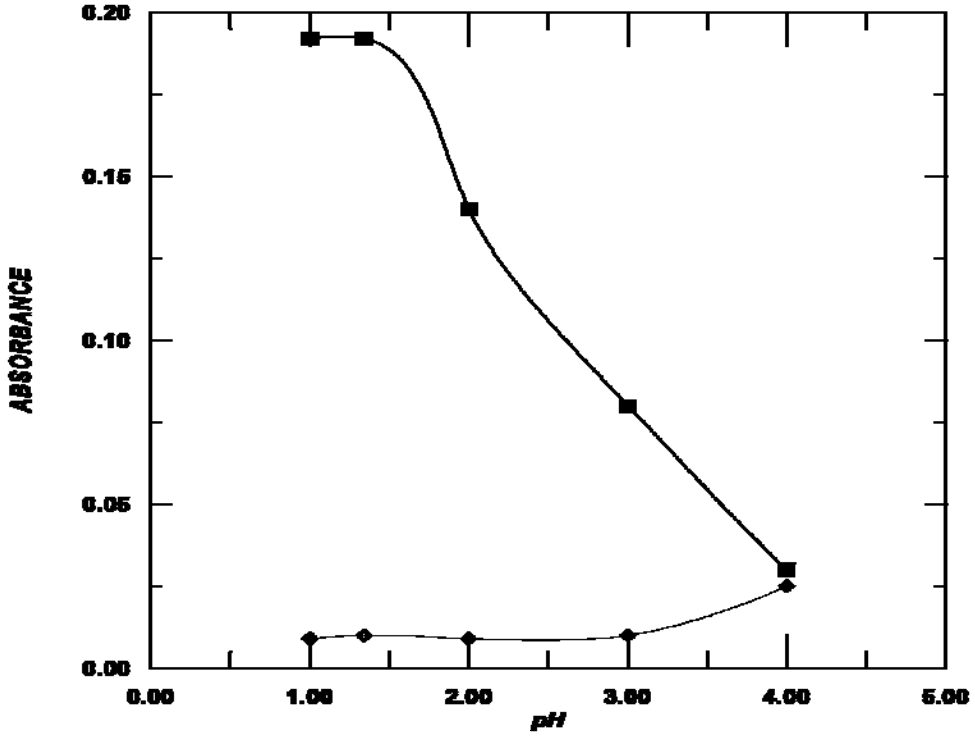

Fig. 2. Effect of pH for the determination of DFOM with V (V) and Blank.

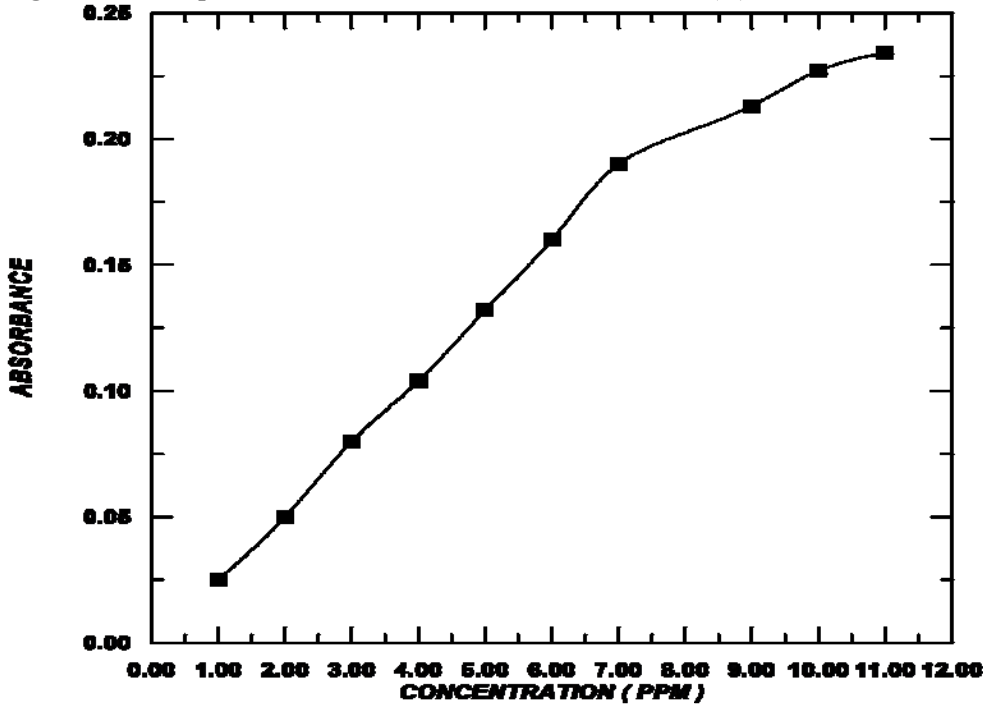

Fig.3. Effect of Concentration of Vanadium on the determination of DFOM.

3.2.3. Effect of Reaction Time.

Fig. 4 displays 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 5 min. and then reaches a plateau, which indicates that there is no advantages in going beyond 5 min., perhaps partial dissociation of the complex with longer time in aqueous phase**,** might occur.

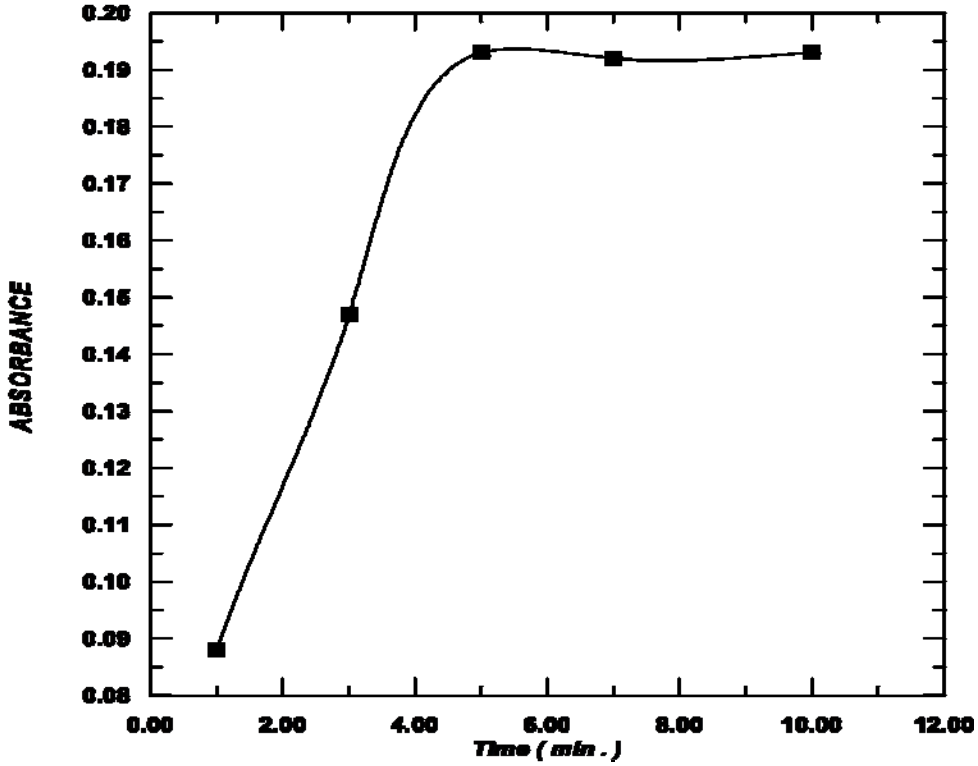

Fig.4. Effect of reaction time on the determination of DFOM-V (V).

3.2.4. Effect of Extraction Time

It was perceived that the absorbance of the chelating complex is increased readily with shaking time and attains a plateau with increasing time, and hence a 5 min. was chosen as optimum for complete extraction of the complex (Fig. 5).

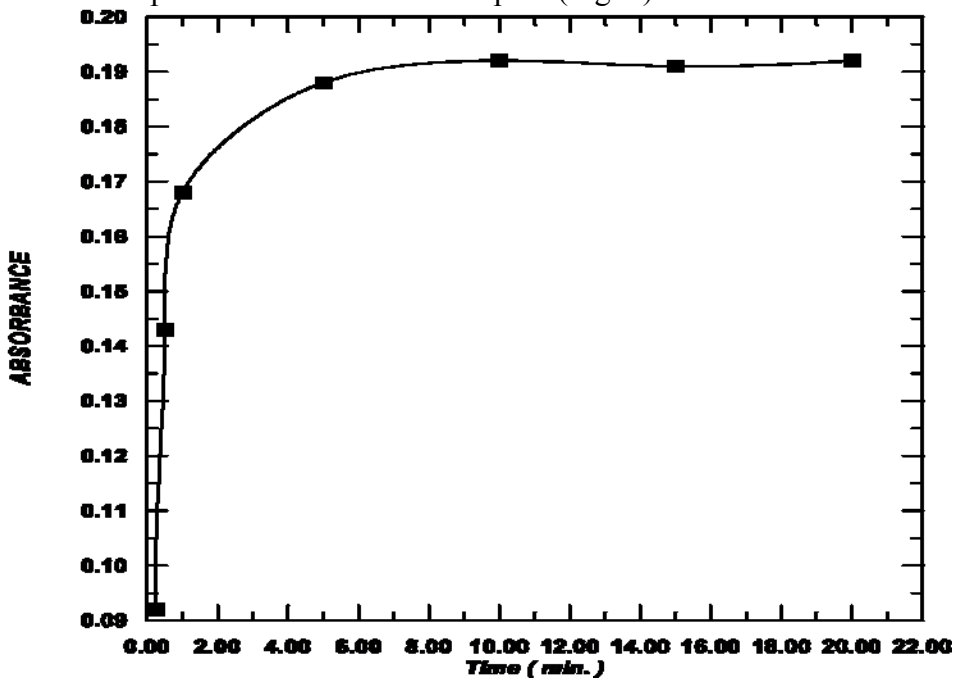

Fig.5. Effect of extraction time on the determination of DFOM-V (V).

3.2.5. Effect of Phase Ratio.

The volume of aqueous phase was varied from 4-8 mL, while keeping the volume of organic phase constant (1 mL) and the experiment was conducted to obtain the best organic/ aqueous ratio for the extraction of DFOM-V (V) complex at optimum conditions. The results revealed that the complex gave maximal atomic absorption signals when the ratio between aqueous and organic phase were 4:1 and 5:1 (Fig.6). The data have also shown that the percent extraction (%E) and the distribution ratio (D) of the complex were 95.5% and 105.4, respectively for one stage extraction.

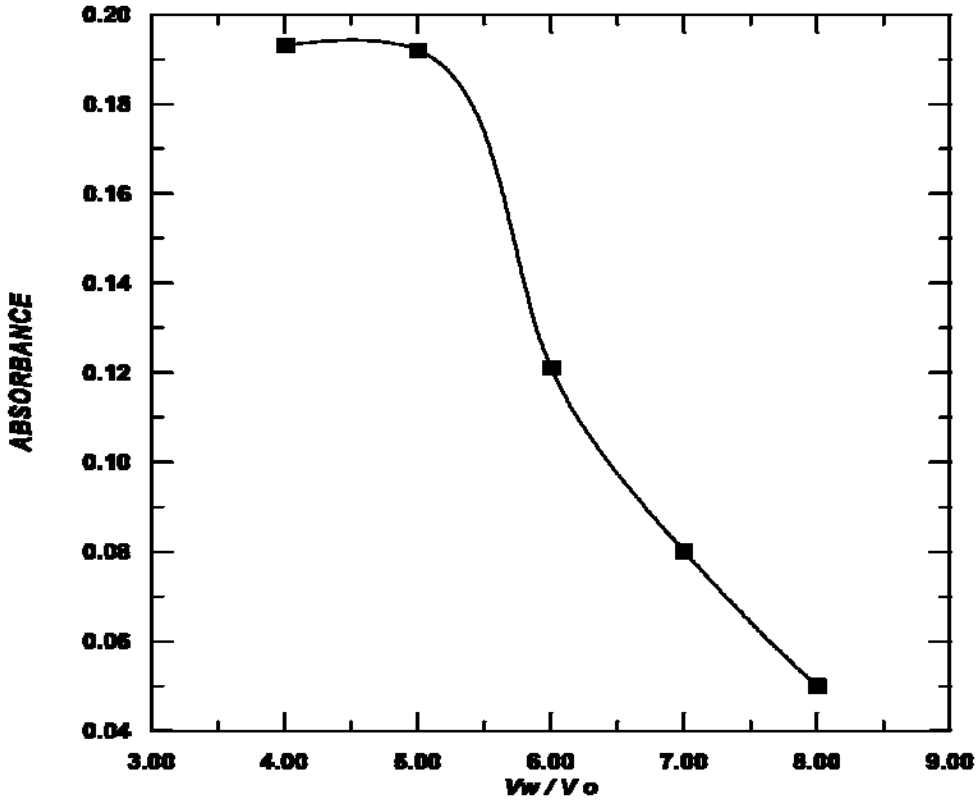

Fig.6. Effect of phase ratio on the determination of DFOM-V (V).

3.2.6. Selection of Organic Solvents.

Since the method encompasses the measurement of complex in organic phase, it is necessary to use a solvent that will only extract the chelate complex, but not excess V(V) or free ligand (the drug) used. Several organic solvents (such as dichloromethane, chloroform, MIBK, 1-octane, o-xylene, toluene, carbon tetrachloride, 1-butanol, cyclohexane, benzene, acetylacetone, diethyl ether, benzyl alcohol, dichloromethane, and petroleum ether) have been examined to investigate the suitable one for the extraction of complex. Benzyl alcohol was found to be the best for the extraction the complex at optimum conditions excluding other species in the extraction system. The molar – ratio method (λ_{max} at 460 nm) showed that a 1:1 complex was formed, with stability constant of $k=2.1X10^7\ M^{-1}$ [12].

3.3. Structure of the complex.

Several techniques as FTIR, ETAAS and Molar ratio method have been used to elucidate the structure of DFOM-V(V) complex formed under optimal conditions. The data revealed that a 1:1 complex was formed with stability constant of $2.1x10^7\ M^{-1}$ (λ_{max}= 480 nm) and from IR spectra and elemental analysis data [13], shown that the structure of the drug

DFOM with V (V) as complex is displayed in Fig. 7. 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 (98.1-100.8%) have been obtained up to 48 hours duration time and remarkable depression occurs thereafter (92.8%).The low recovery may be due to the change in molecular association between the ligand and metal ion during the time or the interaction between the complex and vanadium ion with organic solvent.

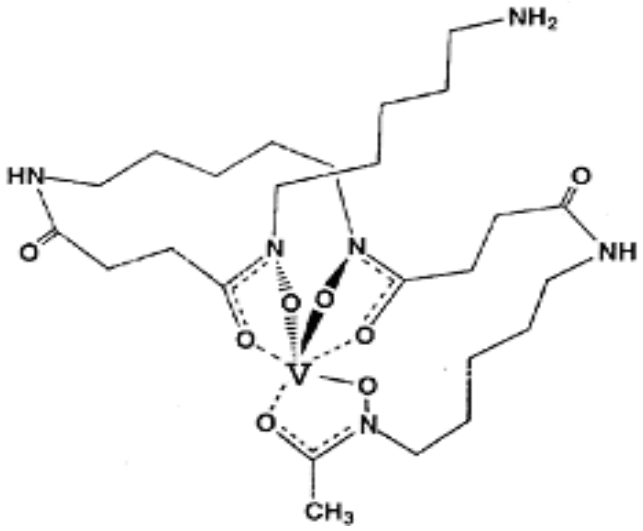

Fig. 7 The structure of DFOM-V (V).

3.4. Optimization by using a Chemometrics Tools

The Response Surface Method (RSM) using Screening Design (SD) was also applied to estimate the effects of factors for the extraction of chelating complex on statistical basis. Three main factors were selected, the concentration of V (V) ions (Cppm), the pH and volume of an aqueous phase (V_w). Table 2 shows the coding of these factors at two levels and Table 3 represents the 2^3-screeing design and factor levels for the estimation of the above mentioned factors.

Table 2. Coding factors at two levels

Factor	+1	-1
pH	4	1.5
Vw	8	4
C_{ppm}	120	90

The factors effect was calculated as the difference between the responses of a factor at high and low level. These differences were then tested against the experimental error expressed by the standard deviation multiplied by the student's t-value. The factor effects were evaluated according to the relationships described elsewhere [19] . Data have shown that the comparison of the experimental error with absolute differences reveal that the main factors pH and volume of aqueous phase show a significant effect (DpH and DV_w are higher than 0.0154), while the effect of V (V) concentration can be neglected in the studied ranged between 90 and 120 µg mL^{-1} (i.e. there is a minimal influence by the concentration of V (V).

From the above study, the factors pH and V_w were found to significantly influence on the extraction of the chelating complex DFOM-V (V). A design at three levels, a Box-Benhnken design was run at optimal V (V) concentration in order to study the relationship

between the response (AA-signal) and the significant two factors. Table 4 shows the coding of the two factors at three levels, and Table 5 describes the factors at three levels according to Box-Behnken.

Table 3. 2^3- Screening design and factor levels for estimation of the factors pH values, the volume of aqueous phase and the concentration of V (V).

Coded factor level							
Run	pH	V_w	C_{ppm}	pH C_{ppm}	pH.V_w	C_{ppm}.V_w	Response (Absorbance)
1	-1	+1	+1	-1	-1	+1	0.410
2	-1	-1	+1	-1	+1	-1	0.640
3	-1	-1	-1	+1	+1	+1	0.641
4	-1	+1	-1	+1	-1	-1	0.407
5	+1	+1	+1	+1	+1	+1	0.110
6	+1	-1	+1	+1	-1	-1	0.190
7	+1	-1	-1	-1	-1	+1	0.181
8	+1	+1	-1	-1	+1	-1	0.120

Table 4. Coding the two factors at three levels

Factor	level		
	+1	0	-1
pH	4	1.5	1
V_w	8	4	4

Table 5. Factor levels and Box-Behnken design for studding the DFOM determination by indirect ETAAS.

Box-Benhnken level			Response (Absorbance)
Run	pH	V_w	
1	+1	+1	0.106
2	+1	- 1	0.112
3	- 1	+1	0.335
4	+1	0	0.190
5	- 1	0	0.600
6	0	+1	0.400
7	0	- 1	0.650
8	- 1	- 1	0.600
9	0	0	0.650

The response surface was drawn graphically as a counter plot (Fig. 8). It can concluded that the curved dependences in the direction of both factors lead to a maximum absorbance at coded level of pH and V_w to the range close to the optimal values. Then, the surface starts to fall-off slightly in the case of increasing factor value from the optimal limit. However , the response surface was observed to be depressed extremely toward the least factor value , hence , inferring that it is necessary to maintain the pH at level higher than 1 and lower than 4 , and the same situation for volume of aqueous phase.

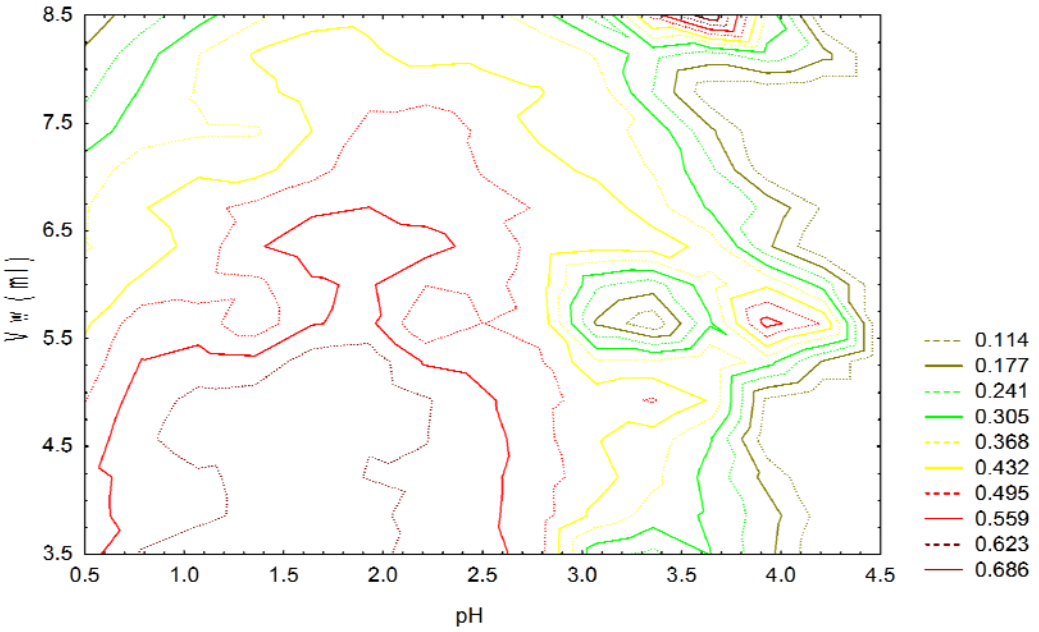

Fig.8. Contour plot of absorbance versus the factors pH and volume of aqueous phase.

3.5 Calibration Graphs

Using optimum conditions established, direct calibration graph for the indirect determination of DFOM was constructed and the statistical results are illustrated in Table 7.

Table 7. Representative Statistical results For the Analysis of DFOM by Indirect ETAAS

Range of concentration (µg mL^{-1})	0.15- 19
Detection limit (µg mL^{-1}) for n=13	0.12
Characteristic mass (pg)	424.2
Regression line	Abs= 0.0375 (conc.)-0.001
Correlation coefficient (r)	0.9992
Coefficient of determination (R^2)	99.84%
C.L. for the slope($b \pm ts_b$) at 95%	0.0376 ± 0.009
C.L. for the intercept ($a \pm ts_a$)	0.001 ± 0.008

Beer's law was obeyed over the concentration range (0.5-19 µg DFOM m L^{-1}), then the calibration line was observed to be bent toward the concentration axis. This may be due to the formation of the strong bonding between vanadium 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 (Table 7) with correlation coefficient of 0.9992 and coefficient of determination (R^2) was 99.84% which suggests a 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 424.2 pg and limit of detection was 0.12 µg mL^{-1} compared favorably with published value 0.1 µg mL^{-1} [5,20] and 0.095 µg mL^{-1} [21]. Comparison of the developed method (indirect ETAAS) and UV-VIS spectrophotometric method [12] using *f*-test at 95% confidence limit

showed no significant difference in precision within the same range of linearity for both methods, but the former gave lower detection limit (Table 8) making this method more convenient and applicable for the determination of DFOM in biological samples as well.

Table 8. Comparison between UV-Vis Spectrophotometric and Indirect ETAAS methods for the determination of DFOM.

Method	Linearity ($\mu g\ mL^{-1}$)	D.L ($\mu g\ mL^{-1}$)	RSD (%)	r	Calculated F	Tabulated F
UV-Vis[13]	2-275	0.50	0.38	0.9996	2.43	6.388
Indirect ETAAS	0.5- 19	0.12	1.42	0.9992		

3.6. Determination of DFOM in Pharmaceutical preparation

The developed method was applied to 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 V (V) and found to be 486.8 and 490 mg / unit with relative error of (-2.64%) and(-2%) respectively . The results found by both procedures were agreed with stated concentration value and in good agreement with results obtained by direct UV-Vis spectrophotometric method [12]. It was observed that the ratio of the slopes of the direct calibration (Abs= 0.0375 (conc.)-0.001, r =0.9992) and standard additions (Abs= 0.037 (concn) -0.001, r =0.9993) 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 in the range of 96.47-97.77% with a mean value of 97.17±0.65, 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**.**

4. Conclusion

The determination of DFOM using V (V) as a pairing agent showed low detection limits and highly absolute sensitivities compared with other analytical methods. The analytical results obtained for the determination of DFO in some pharmaceutical compounds showed good agreement with the given–labeled quantity. The procedure is a simple and rapid since it is a single process. The selectivity of the method, in general, has not been tested. This important characteristic parameter has to be preformed. Further work is needed to apply this method for the analysis of desferal drug in biological samples rather than the pure or pharmaceutical preparations.

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