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SPECTROPHOTOMETRIC MICRO DETERMINATION OF DRUG DESFERRIOXAMINE IN SOME PHARMACEUTICALS BY CHELTATING WITH VANADIUM (V)

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ABSTRACT

The drug desferrioxamine mesylate (DFOM) forms with vanadium (v) a colored chelate (λ_{max} = 460 nm) complex at pH – range (1- 1.34) which is extractable with Benzyl alcohol as organic solvent .

Under the appropriate experimental conditions a calibration plot was set up from which some analytical parameters were derived and deduced by regression .Standard additions procedure was also adopted .It has been estimated that the concentration of the drug DFOM to be 487.6 mg per unit and 485.1 mg per unit for both calibrations. Under optimal conditions, the developed method has been achieved the following characteristics:

LDR (2.0 – 275 μ g ml $^{-1}$) DFOM, RSD % (0.3- 0.45), sandell sensitivity (0.158 μ g. cm^{-2}), LOD (0.5 μ g ml $^{-1}$), recovery %(101.466 ± 0.763), Erel %(1.466). stability constant (2.1x 10^{7} M $^{-1}$).The mole – ratio method (1:1) approved that DFOM V (v) as a structure of the complex. The developed procedure has been adapted to analyze DFOM in various pharmaceuticals .

INTRODUCTION

Desferrioxamine is a chelator of iron, aluminum and other metals. It is a hexadentate ligand that binds with an extremely favorable stability constant [(1)]. This property of Desferrioxamine makes it ideal for treating diseases such as thalassaemia, in which the body is overloaded with iron [(2).] It is has also been used to treat aluminum toxicity in dialysis patients [(3)].

Various methods have been reported for the determination of desferrioxamine. These include ICP / AES [4], Spectrophotometry [5], ET- AAS [7], Zeeman –ET- AAS [6], HPLC [8].

In this work, a molecular spectrophotometric method for determination of the drug desferrioxamine (DFOM) in some pharmaceutical preparations by chelating with Vanadium (v) has been developed. The complex has a maximum absorption at (460 nm). Benzyl alcohol was used as organic solvent for extraction of chelating complex. This method can be applied successfully to pharmaceutical preparations containing Desferrioxamine.

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EXPRIMENTAL

Apparatus

- All spectral and absorbance measurements were carried out on a Shimadzu UV–Visible 160 a digital double–beam recording spectrophotometer using 1- cm silica cell.
- pH meter, Digital Orion research micro processor analyzer 90

Reagents

All chemicals used were of analytical reagent grade unless otherwise stated, desferrioxamine mesylate standard material and deferral drug were provided from the Novartis pharma AG , Basle , Switzerland .

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

A 0.1gm of DFOM was dissolved in water (DIW) and diluted to 100 ml into volumetric flask.

Vanadium Stock solution (1000 µ g ml $^{-1}$)

A 0.1785 gm of V_2O_5 was dissolved in 5ml of sulfuric acid (2 N), Diluted to100 ml in a volumetric flask with deionized water.

Analytical Procedures

(A) Direct Calibration

1- An appropriate volume of the standard (DFOM) solution so that give(2 – 275 µgml$^{-1)}$ was transferred into a separating funnel and

1.8 ml of 250 μ g ml $^{-1}$ of vanadium stock solution was add and pH – value of solution (pH 1 – 1.34) was adjusted with dil. HCl or NaOH solution.

2- The solutions were set aside for (5 min.) at room temperature and dilute to (5 ml) with deionized water then (4 ml) of benzyl alcohol added and shaked for (7 min), and absorbance of organic layer was measured at (λ_{max} =460 nm) against blank (organic solvent) .The calibration graph was constructed and the unknown DFOM concentration found by regression (Fig 1).

(B) Standard additions

Step (1A) was repeated but in addition appropriate equal volumes of sample solutions were added to each flask. One flask remains free from the standard solution . then step (2B) is applied and a standard calibration plot is constructed from which unknown DFOM was obtained by regression (Fig 2).

RESULTS AND DICUSSION

Absorption spectra

I - Drug stock solution

A-5 ml of (100 μg ml $^{-1)}$ Desferrioxamine standard solution, was transferred to 10 ml volumetric flask, and diluted to the mark with water , 4 ml of this solution, was transferred to the absorption cell , then the absorption spectrum of this solution was measured in the region between 200 to 1100 nm using water as the reference. Fig (3a) shows the absorption spectra of drug. The maximum absorption was at 213 nm.

II- Vanadium (v) stock solution

Fig (3b) shows the absorption spectra of vanadium (v) and a maximum Absorption was at (304nm) by applied the same procedure described in (I).

III- Red complex of DFOM with vanadium (v)

The absorption spectrum of extracted complex was measured in the region (200 to 1100 nm) using the extracting solvent as the reference.Fig (3c) shows that a wavelength maxima was 460 nm.

Optimum Conditions

1-Effect of pH Values

The Effect of pH on the formation of DFOM –V (v) chelate is shown in Fig (4), from which it appears that the best pH range occur between

(1 – 1.34).

2- Effect of concentration of Vanadium (v) .

The concentration (90 μg ml $^{-1}$) of vanadium (v) was found enough for Complete formation of chelating complex, Fig (5).

3- Effect of Reaction time

Fig. (6) refers that a reaction time of (5 min) is enough for complete Complex formation.

4 – Organic solvent used in the extraction

Since, the method involves the measurement of complex in the organic phase, it is necessary to use a solvent which will extract the chelate complex, but unreacted excess the vanadium (v) used. It was found that DFOM is more soluble in water than in benzyl alcohol, but V (v) – DFOM is more soluble in benzyl alcohol than in water.

5- Effect of extraction time

Fig (7) reveals that the complex of DFOM with vanadium (v), needed (7 min) of shaking to reach a state of equilibrium.

6- Effect of phase ratio

An aqueous–to–organic phase ratio of 5:4 gives the highest extractability and better absorbance, Fig (8).

Extraction Efficiency

Table (1) shows molecular absorbance values for the extracted chelating complex of DFOM with Vanadium (v) after the first and second extraction of the aqueous phase. The extraction efficiency (% E) was found to be 96.33 and the distribution coefficient (D =32.8) was achieved.

The molar ratio of ligand (L) to metal (M)

The molar–ratio method at λ_{max} of 460 nm showed that a 1 : 1 complex was formed **.**Fig (9) shows the molar ratio of the ligand : metal , and the stability constant (K) was calculated and equal to 2.1 ***X***10^{7}.

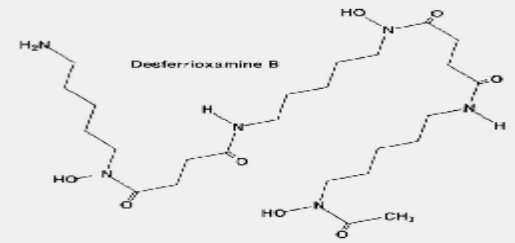
The stability of chelating complex with increasing time

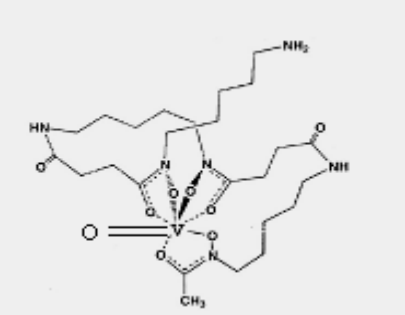
Table (2) shows the stability of complex DFOM with Vanadium (v) at different duration. It was shown that the recovery of the

complex is 91.04% for the duration of 72 hours. 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 vanadium with organic solvent.

The IR spectra for complex and drug

Figs.(10)and(11) illustrate the infrared spectra of free desferrioxamine and complex respectively, Tables (3) and (4) show observed frequencies and band assignment. The spectrum of FT-IR (Fig.11) has disclosed the disappearing of OH wide band that interfered with two NH bands at 3420 and 3300 cm^{-1}.This may be due the formation of covalent bonding with vanadium ion. In addition, shifting toward longer wavelengths was occurred. A new band (Fig.11) was also appeared at 520 cm^{-1} comparing with Fig.10, which belongs to the coordination M-O bond, proving the formation of the complex as shown in the following structure.





Structure DFOM and Complex

Calibration Graph **DFOM-V (v)**

Fig (1) shows a calibration graph of desferrioxamine established by plotting the absorbance of complex vs. concentration and shows that Beer's law is obeyed over the DFOM concentration of (275 μ g ml^{-1}) at wave length (460 nm) .

Statistical Calculations

All measurements can be characterized statistically. Table (5) shows the linear range of DFOM – V (v) and detection limit, molar absorptivity (ε), Sandell sensitivity (S) and confidence limits for the concentration and the absorbance. Table (6) reveals that the test statistic t=132.24 is higher than critical value (2.145) in regression

analysis(r=0.9996). This means that the predications based on the estimated regression line Y= -0.0036+0.0063 X should be acceptable. Therefore, all the concentration of DFOM in the analyzed samples was determined from this relationship.

Table (7) shows the accuracy test in term of recovery. Recovery % was shown to be acceptable and found to be 101± 0.763. Good precision as E_{rel} of the method was achieved and found to be 1.466%.

Standard additions procedure was also applied (Fig.2) for the determination of DFOM complex and all the analytical performances were tabulated in Table (8). The two slopes of the direct calibration and standard additions calculated was equal one, indicating the absence of interference effects and use of direct calibration is to be preferred.

Analysis of DOFM in pharmaceutical preparations with vanadium

Two procedures (direct calibration and standard additions) were used to determine DOFM in desferal vials at λ = 460 nm. The results were shown in Table (9) and Table (10). Good agreement in concentration for both calibrations was obtained compared with the stated concentration of 500 mg per unit..

CONCLUSIONS

This study has shown that the method described allows the rapid determination of desferrioxamine. The analytical scheme of the proposed system is simpler than that of other conventional procedures. Moreover, it offers a higher sensitivity compared with other analytical methods and better recovery.

The analytical results obtained for the determination of DFOM in pharmaceuticals have shown good agreement with the given – labeled quantity. The complex formed have stoichiometric ratio of 1: 1.The different FT- I.R absorption spectra (free Desferrioxamine versus chelating confirms the formation of the complexes.

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Table (1) *: Absorbencies of complex after the first and second extraction..*

DFOM	***Vandium (v) g ml^{-1}***	***pH***	***A (Ex . No .1)***	***A (Ex . No . 2)***	***A^{0} (Blank)***
100	90	1 – 1.34	0.64	0.02	0.009

Table (2) : *The Stability of complex DFOM – Vanadium (v)*

Complex	***Con. g ml^{-1}***	***Duration / hr.***					
			0	***1***	***24***	***48***	***72***
DFOM – V (v)	***100***	***Abs.***	0.64	0.642	0.63	0.6	0.57
		Recv. %	102.15	102.4	100.57	95.8	91.04

Table (3) : *Some of Observed Frequencies and Bond Assignments of Free DFOM(Cm^{-1}).*

Drug	$\bar{v}$*(cm^{-1})*	Bond assignment
DFOM	3310 s 3120 w	N – H
	1635 s	N-C= O
	1425	C – N
	1200 – 1300 s	C – O

Table (4) : *Some of Observed Frequencies and Bond Assignments of DFOM – V*

(v) (cm $^{-1}$).

Complex	$\bar{v}$*(cm^{-1})*	Bond assignment
DFOM – V (v)	3420 S 3300 S	N – H
	1700 S	N-C= O
	1520 S	C - N
	1230 – 1300 W	C - O

	520 W	V - O
	980 S	V = O

Table (5): Analytical characteristics of results .

Drug	***Linearity g ml^{-1}***	***D . L g ml^{-1}***	***L . mol^{-1} cm^{-1}***	***S g.cm^{-2}***	***Conf.limit.conc. g ml^{-1} 95% C .I***	***Conf. limit. Abs. 95% C .I***
DFOM	2 – 275	0.5	4152.24	0.158	150.96±1.628814	0.947 ±0.01 026

Table (6) *:Regression equation , correlation coefficient (r) two tailed t – test and confidence limit for the slope and for the intercept at 95 % confidence level and(n – 2)degree of freedom for the calibration graph .*

Regre.Eq Y= BX + A	***Corr.Coef. (r)***	***t – test Statistics***	***Tabulated T- test two tailed n- 2 p =0.05***	***Conf.limit. For the Slope b± t S_b***	***Conf.limit. For the Intercept a± t S_a***
Y= 0.0063 X –0.0036	0.9996	*132.24*	*2.145*	0.0063 ± 0.00008	*-0.0036 ±0.0117655*

Table (7)*:shows the relative standard deviation RSD % , $E_{rel\ \%}$, recovery Rec %.*

Amount of DFOM taken g ml^{-1}	***Amount of DFOM found g ml^{-1}***	***Recov. %***	***$E_{rel.\%}$***	***RSD % n= 5***	***Rec. % ± S.D***	***$E_{rel.}$ %***
25	*25.33*	*101.3*	*1.3*	*0.45*	*101.46*	*1.46*
75	*76.33*	*102.3*	*2.3*	*0.4*	±0.763	*6*
225	*227*	*100.8*	*0.8*	*0.3*		

Table (8)*: shows the regression equation , correlation (r) ,two tailed t – test, Confidence limit for X – Value obtained (X_E) at 95% Confidence limit and (n-2) degree freedom for the* ***standard additions calibration*** *graph , Recovery Rec % , E_{rel}*

Sample	**Regre.Eq** ***Y= BX + A***	***Corr.Ccoef.***	***t – test Statistics***	***Tabulated T- test (two tailed) n - 2 p =0.05***	***Conf.limit. For x-value X_E ± t S_{XE}***	***Recov. %***	***$E_{rel.\%}$***

Desferal vial	Y=0.0063X+ 0.1528	0.9992	79.069	2.262	24.253± 0.095501	97	3

Table(9) *:determination DFOM in sample of pharmaceutical preparation by Direct calibration and standard addition .*

Name of pharma ceutical	***Type of preparation***	***Stated Concentration (mg per unit)***	***Found (Direct calb.) (mg per unit)***	***% E_{rel}***	***Found (St.add .calb.) (mg per unit)***	***% E_{rel}***
Desferal	Vial	500	487.6	-2.48	485.079	-2.98

Table (10): *Shows the RSD % , Recovery % , E_{rel} % for the calibration graph .*

Amount of DFOM taken gml^{-1}	***Amount of DFOM found $g\ ml^{-1}$***	***Recov. %***	***E_{rel}. %***	***R.S.D % n=5***	***Rec% ± S.D***	***E_{rel}. % ± S.D***
25	24.6	98.4	-1.6	0.65	98.18 ± 0.608	- 1.81 ± 0.608
75	73.125	97.5	-2.5	0.5		
225	222	98.66	-1.33	0.38		

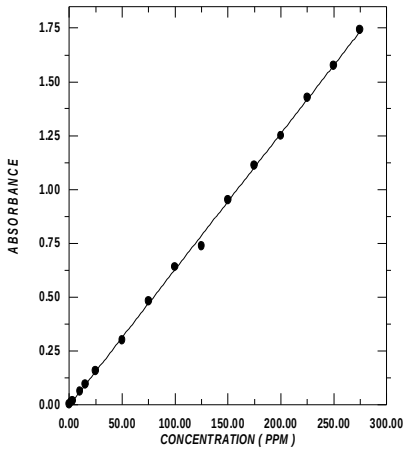


Fig 1: Calibration graph for the determination of DFOM-V(v)

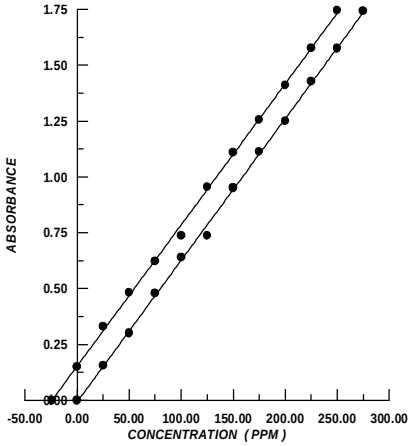
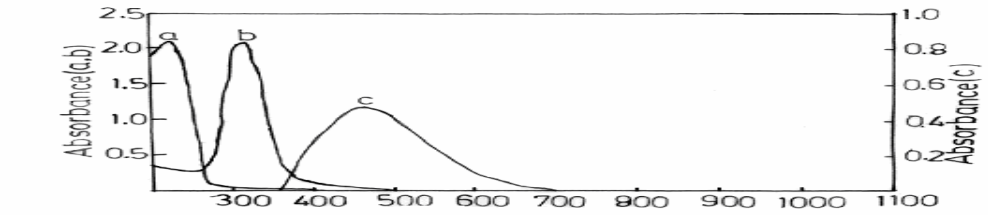


Fig 2: Analysis of DFOM pharmaceutical By standard



Wavelength (nm)

Fig 3: Absorption spectrum (a) Drug (b) ion (c) complex

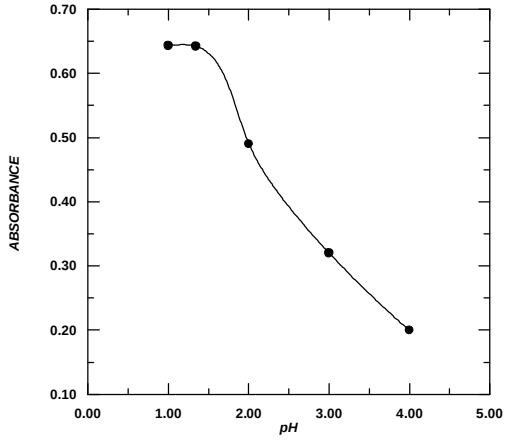


Fig 4: Effect of pH

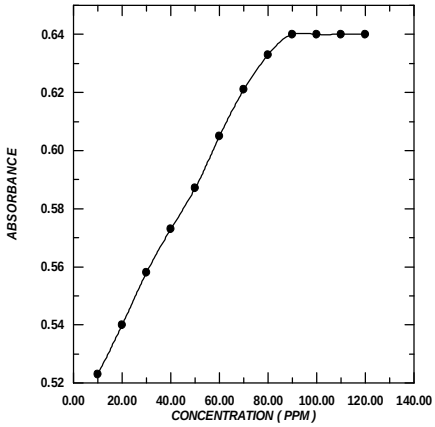
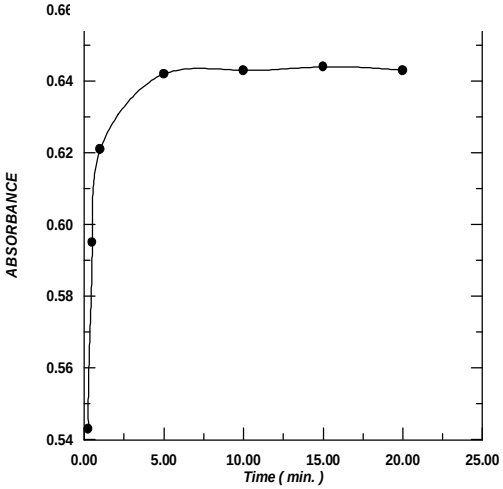


Fig 5 : effect of concentration of Vanadium



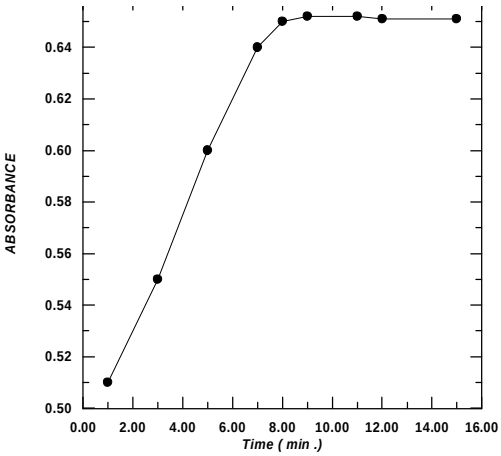


Fig 6 : Effect of reaction time

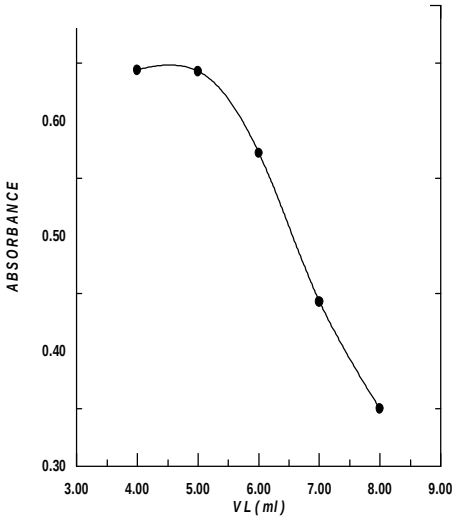


Fig 7: Effect of extraction time

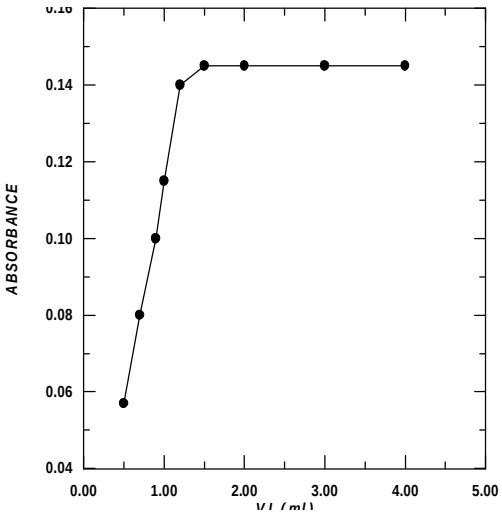


Fig 8: Effect of phase ratio

Fig 9: Molar ratio plot, DFOM-V(v)

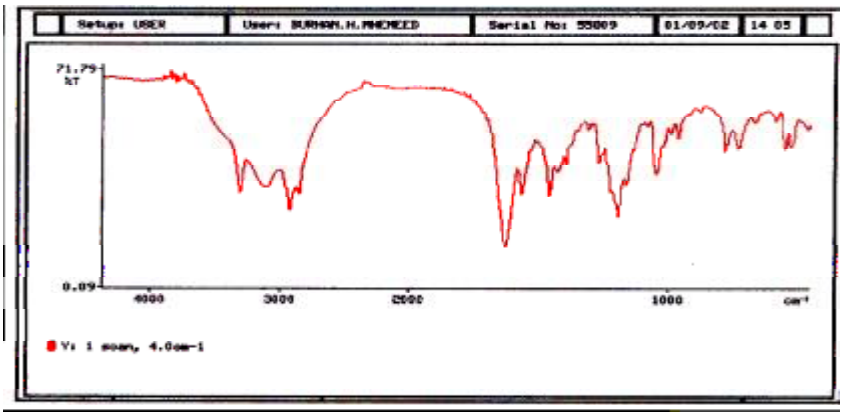


Fig 10 : FT-IR spectrum of DFOM

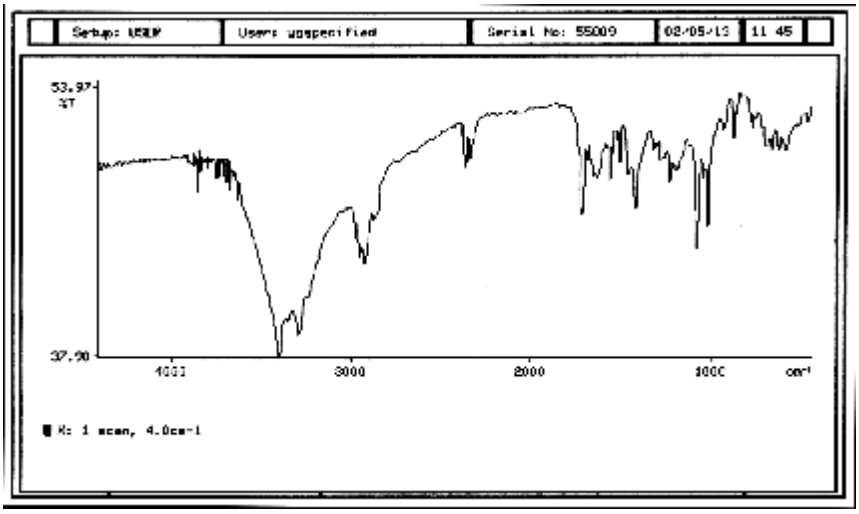


Fig 11: FT-IR spectrum of DFOM-V(v)