

# Mutual Determination of Trifluoperazine Hydrochloride and Vanadium (V) Ions in Real Matrices by Visible Spectrophotometry After Cloud Point Extraction

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**Abstract:** A green method was established for the mutual determination of the drug trifluoperazine hydrochloride (TFPH) and vanadium (V) in real samples by coupling the cloud point extraction with UV-Vis spectrophotometry. The method was based on the formation of the colored V (V)-TFPH complex in acidic medium followed the extraction of the complex by using the surfactant Triton X-114 as an extracting medium. The surfactant-rich phase extracted was dissolved in a small volume of solvent and both the drug TFPH and V (V) ions determined by spectrophotometry at maximum absorption wavelength of 476 nm. Most parameters which impact on the extraction efficiency and detection for both target analytes were optimized. Under the optimum conditions established, the preconcentration factor was found to be of 50 fold making Beer's law obeyed in the concentration range of 2-80  $\mu\text{g mL}^{-1}$  ( $r = 0.9999$ ) for the determination of TFPH and 0.5-10  $\mu\text{g mL}^{-1}$  ( $r = 0.9995$ ) for vanadium (V), giving the detection limits of 1.21 and 0.113  $\mu\text{g mL}^{-1}$  respectively. The mean recovery percentage of 98.94 $\pm$ 2.19 % (in blood serum) and 99.43 $\pm$ 1.40 (in tap water); the precision (RSD %) ranged between 0.48-3.40 % and 0.45-1.49 were obtained for TFPH and V (V) ions respectively. The proposed method was employed for the determination of TFPH in pharmaceuticals and the spiked serum samples, while a V (V) ion was determined in black pepper samples.

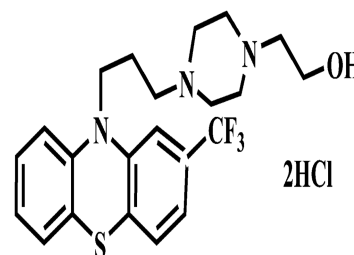
**Keywords:** Trifluoperazine Hydrochloride, Vanadium (V) Ions, Hydrophobic Complex, Cloud Point Extraction, Vis - Spectrophotometry

## 1. Introduction

The exploitation of cloud point extraction methodology for the determination of two target analytes mutually in the same reaction system may really become an attractive avenue after the success that achieved in our recently published work [1]. In fact, that work reflects the importance of this trend, which we believe it will add some benefits and analytical features to the contemporary analytical chemistry by simplifying the analytical procedures in the terms of economy in time and expenses, as well as reducing the analytical chemist efforts. This is what encouraged us to continue to offer other works in this direction for the purpose of devising other new methods before us. In current work, the reaction between the vanadium (V) ions with drug trifluoperazine hydrochloride (TFPH) in acidic medium was adopted as a complexometric reaction system to form a soluble colored V (V)-TFPH complex. The main reason for selection of this reaction because the drug

TFPH and V (V) have a great importance to humans.

Trifluoperazine hydrochloride (TFPH) is one of the very important class of phenothiazine derivatives, where it mainly used as a treatment for Schizophrenia in addition to other psychotic disorders [2]. It chemically known as 10 - [3 - (4 - methylpiperazin - 1 - yl) propyl] - 2 - (trifluoromethyl) - 10H - phenothiazine hydrochloride as showed in Figure 1.



**Figure 1.** The chemical structure of Trifluoperazine hydrochloride ( $\text{C}_{21}\text{H}_{24}\text{F}_3\text{N}_3\text{S} \cdot \text{HCl}$ , M. W. 480.43 g/mol) [3].

TFFH is subjected to many research works, either for the development of the methods of its determination in pure form and pharmaceuticals or as chromogenic reagent for estimation of various target analytes. The literature survey reveals that a number of analytical methods for detection of this drug individually or in combination with other drugs have been used including, indirect atomic absorption spectrometry [4], potentiometry [5], reverse phase high performance liquid chromatography (RP-HPLC) [6-13], solid-phase extraction-high performance liquid chromatography (SPE-HPLC) [14], liquid chromatography – mass spectrometry / mass spectrometry (LC-MS/MS) [15] densitometry [16] and spectrophotometry [17-20]. Also, this drug was proposed as a chelating agent for the determination of some metal ions like  $\text{Cr}^{+6}$  [21],  $\text{Ti}^{3+}$  [22],  $\text{Fe}^{3+}$  [23] ions. In general, one can refer to the analytical applications of the reactions of phenothiazine derivatives with some metal ions through access to the best article published an article in 1999 [24].

Vanadium, on the other hand, is an essential element for cell growth at trace amount ( $\mu\text{g L}^{-1}$ ) level and playing an important role in the regulation of enzymes, but it can be toxic at higher concentration of humans and animals [25]. However, the toxicity of vanadium is dependent on its oxidation state, with vanadium (V) being more toxic than vanadium (IV) and its poisoning symptoms are sometimes fatal [26]. Therefore, the determination of vanadium is so important especially in foods and environmental samples. Among the most instrumental methods reported for the determination of vanadium include electrothermal atomic absorption spectrometry (ETAAS) [27-28], neutron activation analysis (NAA)[29-30] inductively coupled plasma mass spectrometry (ICP-MS) [31-32] inductively coupled plasma optical emission spectrometry (ICP-OES) [33-36] and voltammetry [37].

From the above, it is evident that some of these techniques, whether for TFFH or vanadium determination have some disadvantages in term of non-selectively, expensive instrumentations, time-consuming, unavailable in many laboratories. Because of these two target analytes under study exists at trace concentration level and in complex matrices, the direct determination is very difficult and the separation and pre-concentration of these are a must before their determination. Accordingly, the hyphenation of cloud point extraction(CPE) with UV-Vis spectrophotometry offers fascinating features that pay towards simplification of the analytical procedures in routine analyzes of organic compounds and metal ions in various matrices rather than using sophisticated and expensive instrumentation [1, 38].

The current work is an extension of our previous attempts to entrench the concept of mutual analysis of the two target analytes at the same time by using CPE coupled with molecular spectrophotometry. The method based on the complexation reaction between vanadium(V) and the drug TFFH in acidic medium to form V(V)-TFFH complex which extracted into a non-ionic surfactant (Triton X-114) followed by their determination spectrophotometrically at specific absorption maximum, in biological, food and environmental samples.

## 2. Materials and Methods

### 2.1. Apparatus

All absorption measurements of the two target analytes and the scanning of the absorption spectra for the complex under study were carried by using a Shimadzu double-beam UV-Vis Spectrophotometer model UV-1800 (Kyoto, Japan) equipped with 5-mm optical path cell. A double-beam atomic absorption spectrometer AA400 (Analytic Jeana, Germany) equipped with a graphite furnace was used for the determination of vanadium according to the instruction manual of the company. Thermostatic water bath model WNB7-45 Experts (England) was employed for CPE experiments. For solution pH measurements, a portable pH/mV/C meter HI 83141 (HANNA, Romania) was used.

### 2.2. Reagents and Materials

The materials and reagents used in this work with high purity, doubly distilled water was used in the preparation of all solutions and for final rinsing of glass wares. Triton X-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL in 100 mL water. A pure grade (99%) of trifluoperazin hydrochloride was obtained from the Directorate General of Drug Supervision / Baghdad-Iraq. A stock solution of 1000  $\mu\text{g mL}^{-1}$  (or 0.002 M) for the drug Trifluoperazin was prepared by dissolving 0.1000 g in a minimum amount of water and dilutes to mark with doubly distilled water in a 100 mL volumetric flask. This solution was stored in the refrigerator and working solutions were daily prepared by appropriate dilutions in water. A stock solution of 1000  $\text{mg L}^{-1}$  (or 0.02 M) for V(V) was prepared by dissolving 0.2296 g of pre-dried ammonium metavanadate ( $\text{NH}_4\text{VO}_3$ , M. wt 116.978  $\text{g mol}^{-1}$ , BDH) in 5 mL doubly distilled water and complete to mark with water in 100 mL volumetric flask. 0.1 M hydrochloric acid solution was prepared by diluted 0.83 mL of 37% HCl (1.19  $\text{g/mL}$ , BDH) with distilled water in 100 mL calibrated flask.

### 2.3. General Procedure for CPE for TFFH

In a series of 10 mL volumetric flasks, an amount of TFFH standard or sample solution matched within the calibration range (2-80  $\mu\text{g mL}^{-1}$ ), an excess of  $4 \times 10^{-4}$  M (or 20  $\mu\text{g mL}^{-1}$ ) vanadium ion solution, pH adjusted to 1.5 with HCl and 0.2 mL of Triton X-114 (10% v/v) were added, mixed and dilute with to mark with water. The content of each flask was transferred into a 10 mL centrifuging tube and all kept in the thermostatic bath at 75 °C for 30 min for clouding. The separation of the surfactant-rich phase was performed by centrifugation at 6000 rpm for 20 min and thereafter cooled down in an ice bath to increase the viscosity of cloud point layer. The aqueous phase was easily poured by inverting the tube. The surfactant-rich phase that contains the complex was dissolved with 1 mL ethanol and the absorbance of the complex measured at 476 nm against a reagent blank prepared under similar conditions. The residual TPPH in aqueous solution was determined by traditional

spectrophotometry at  $\lambda_{\max}$  of 307 nm in order to determine the distribution ratio (D) and extraction efficiency (%E).

#### 2.4. General Procedure for CPE for V(V) Ion

In series of 10 mL volumetric flasks containing known amount vanadium (V) standard or sample solution matched within the calibration range ( $0.5\text{--}10\ \mu\text{g mL}^{-1}$ ), an excess of  $1.2 \times 10^{-4}\ \text{mol L}^{-1}$  TFPH reagent solution, pH adjusted to 1.5 with HCl and 0.2 mL of Triton X-114 (10%) were mixed and diluted to mark with distilled water, then followed the same general CPE for TFPH. The vanadium content was measured at  $\lambda_{\max}$  of 476 nm.

#### 2.5. Preparation of Stelazine Tablet

Ten tablets of Stelazine were crushed to a powder in a clean agate mortar and triturated well. A quantity of fine powder (0.3770 g) was dissolved in a sufficient volume of water with continuous shaking, and then filtered. The filtrate was transferred into a 100mL volumetric flask and diluted to the mark with water. Aliquots of Stelazine sample solution were pipetted into seven 10 mL calibrated flasks containing 0.5, 1.0 and 2.0 mL of  $50\ \mu\text{g mL}^{-1}$  TFPH and each solution followed general CPE procedure for TFPH and the content of drug was measured spectrophotometrically at  $\lambda_{\max}$  of 476 nm.

#### 2.6. Preparation of Blood Serum Sample

Blood sample was selected from one volunteer via withdrawing 5 mL from the vein using medical syringe. The sample was transferred immediately into centrifuging tube and the content centrifuged for 15 min to remove the serum from the whole blood, then kept freezing in a refrigerator until analysis. The serum sample was thawed at ambient temperature, 0.2 mL of serum sample was spiked with 0.2, 0.3 and 0.4 mL of  $1000\ \mu\text{g mL}^{-1}$  TFPH into a 10 mL volumetric flasks and followed general CPE procedure for TFPH. The content in the complex determined spectrophotometrically at  $\lambda_{\max}$  476 nm.

#### 2.7. Preparation of Water Samples

About one liter of drinking and river water samples were collected from the campus of University of Baghdad and river water was first filtered off to remove any suspended materials. A 5 mL of each sample was transferred into 10 mL volumetric flask and spiked with 0.1, 0.3 and 0.5 mL of  $1000\ \mu\text{g mL}^{-1}$  TFPH, then followed the general CPE procedure for TFPH. The content of TFPH in the complex determined spectrophotometrically at  $\lambda_{\max}$  476 nm. Also, 5 mL of each sample was transferred into 10 mL volumetric flask and spiked with 0.2, 0.4 and 0.6 mL of  $100\ \mu\text{g mL}^{-1}$  V(V) ion, then followed the general CPE procedure for V(V). The content V(V) ions in the complex was determined spectrophotometrically at  $\lambda_{\max}$  476 nm.

#### 2.8. Preparation of Black Pepper Samples

The dry ashing method was adopted for preparation of

pepper sample solutions, by taking approximately 5 g of black pepper separately into a porcelain crucible and heated on hotplate under gentle heating till smoke ceased, then the content transferred into a muffle furnace at  $450\text{--}550\ ^\circ\text{C}$  until white ash. The content of each sample was dissolved in 2 mL concentrated HCl and heated on hotplate till dry. Further 2 mL of 4M HCl was added and repeated twice until clear solution. The solution was transferred into 5 mL volumetric flask and completed to mark with water. Each sample solution was subjected to the general CPE procedure for vanadium ion determination.

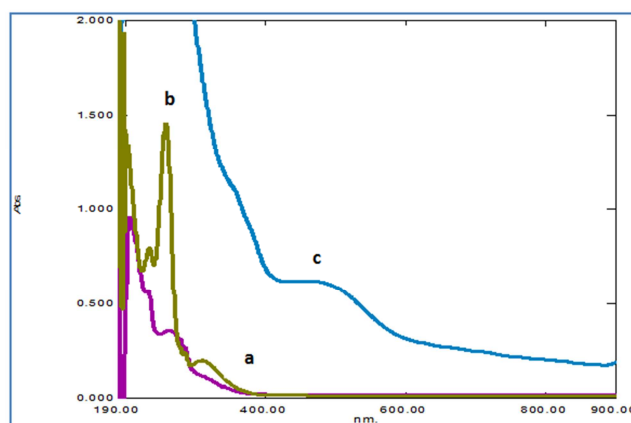
#### 2.9. Statistical Analysis

Excel 2007 (Microsoft Office®) and Minitab version 14 (Minitab Inc., State College, PA, USA) were employed to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

### 3. Results and Discussion

#### 3.1. Absorption Spectra

In a series of preliminary attempts to ensure the formation of the complex between V(V) and TFPH at concentration of  $1 \times 10^{-4}\ \text{M}$  in a strong acidic medium, an orange-purple water soluble complex was formed with a maximum absorption at 476 nm when measured against reagent blank which has negligible absorption at this wavelength. Thereafter, the absorption spectrum of the V(V)-TFPH complex between 190-900 nm was recorded after obtaining the optimal conditions listed below by applying the general CPE procedure. It was observed that there is an absorption band occurred at the same wavelength (i.e. 476 nm) with a remarkable shoulder, indicating the formation of a complex between V(V) ions and TFPH drug as shown in Figure 2c. Whilst pure TFPH solution have two absorption maxima at 257 and 308 nm (Figure 2b) and the V(V) ion displays one distinct band at 233 nm (Figure 2a). Therefore, the wavelength maximum of 476 nm for V(V)-TFPH complex was adopted throughout this study.



**Figure 2.** Absorption spectra (a) V(V) solution (b) TFPH solution and (c) V(V)-TFPH complex in surfactant rich-phase.

### 3.2. Optimization of Experimental Conditions

The effect of various important factors that impact the efficiency of the cloud point extraction procedure was carried via a follow-up series of experiments using classical optimization which suggests observing the effect of one factor at a time (OFAT) on the instrumental response while other factors are maintained at constant level. Thus, a satisfactory sensitivity of the measurement can be achieved. In this regard, the factors such as, HCl concentration, V(V) concentration, Triton X-114 amount, and equilibration temperature and incubation time were studied.

#### 3.2.1. Effect of pH

The complexation reaction between V(V) and TFPH drug was formed in acidic medium. This condition is not just responsible for the stoichiometry of the complex, but has also an important effect in the thermodynamic equilibrium for the formation and stability of the complex extracted by CPE. Consequently, the effect of pH on the formation V(V)-TFPH complex in Triton X-114 medium was studied by measuring the absorbance at  $\lambda_{\max}$  of 476 nm over the range of 1-6. The experiments were conducted with  $50 \mu\text{g mL}^{-1}$  drug TFPH,  $5 \times 10^{-4}$  M V(V) and 0.5 mL of (10% v/v) Triton X-114 followed the CPE procedure, as shown in Figure 3. It was noticed that the absorbance suddenly increased and reached a maximum at pH = 1.5. Later, it decreases at high pH due to the dissociation of the complex and in incomplete extraction in micelle due to the shifting in the formation reaction toward left. Therefore, a pH of 1.5 was chosen for subsequent experiments.

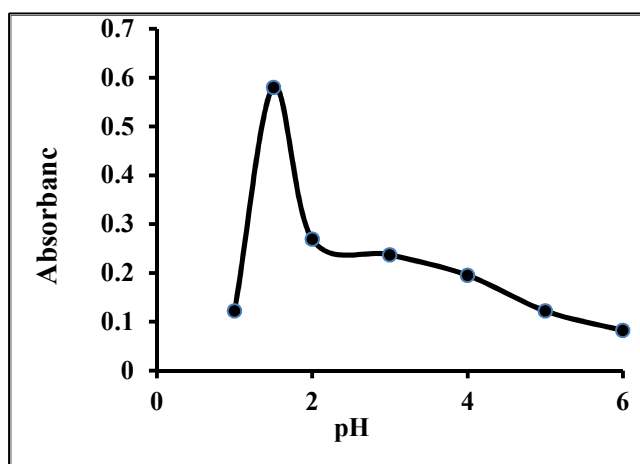


Figure 3. Effect of pH on the formation of V(V) - TFPH complex.

#### 3.2.2. Effect of V(V) Ions Concentration

The influence of V(V) concentration plays a major role in the formation of the V(V)-TFPH complex in acidic medium and its stability after CPE process. These experiment were carried out by measuring the absorbance of the solutions containing  $50 \mu\text{g mL}^{-1}$  TFPH, varying concentration of the V(V) solution ranged from  $1 \times 10^{-4}$  -  $8 \times 10^{-4}$  M at pH 1.5 and 0.5 mL of (10% v/v) of Triton X-114 at temperature  $75^\circ\text{C}$  for 30 min. The results are displayed in Figure 4. It was shown

that the concentration of V(V) ions at  $4 \times 10^{-4}$  M was enough to obtain the maximum absorbance and it was used in the subsequent experiments. At higher V(V) ions concentration, a decrease in the absorbance of the complex was obvious due the deviation of the equilibrium toward the backward reaction because of law of mass action. Whilst at lower concentration of V(V) ions, no there any way for complex formation completion which leads to less amount of the drug complex extracted into the cloud point layer. Thus,  $4 \times 10^{-4}$  M of V(V) ion concentration was as optimum throughout this study.

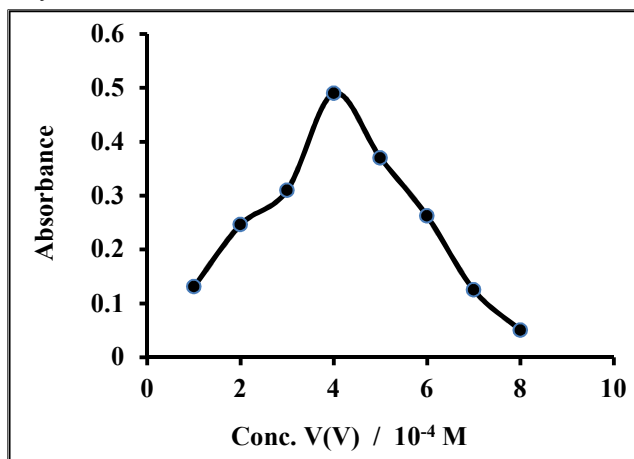


Figure 4. Effect of V(V) concentration in the formation V(V) - TFPH complex by CPE.

#### 3.2.3. Effect of Surfactant Amount

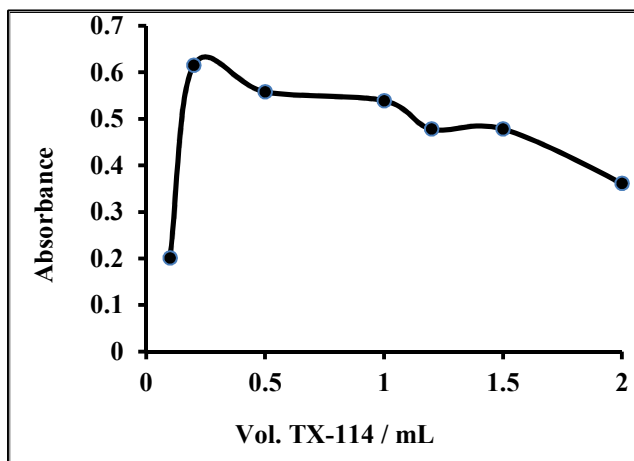


Figure 5. Effect of Triton X-114 amount on the absorbance of V(V) - TFPH complex.

The amount of a non-ionic surfactant has a great significant for maximizing extraction efficiency by minimizing the phase volume ratio ( $V_s/V_a$ ), thus improving its perconcentration ability [39]. Figure 5 displays the effect of the Triton X-114 amount on the absorbance of the extracted V(V)-TFP complex within the surfactant volume range of 0.1-2 mL of 10% (v/v) Triton X-114 at constant other parameters. It was appeared that the absorbance the V(V)-TFPH complex increases rapidly with increasing the Triton X-114 amount up to 0.2 mL of 10% (v/v) and then

markedly decreased at higher amounts. For low amount of Triton X-114, low sensitivity or extraction efficiency occurs owing to the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively. While at higher amount led to deteriorating the detection signal and thus inefficient extraction. Therefore, a volume of 0.2 mL of 10% Triton X-114 was found to be enough to obtain the point cloud layer with the smaller volume and higher viscosity and was used as the optimum amount.

### 3.2.4. Effect of Temperature and Time

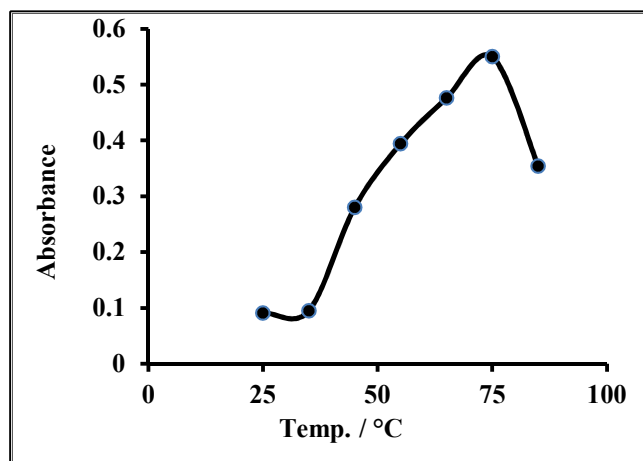


Figure 6. Effect of equilibration temperature on the CPE of V(V)-TFPH complex.

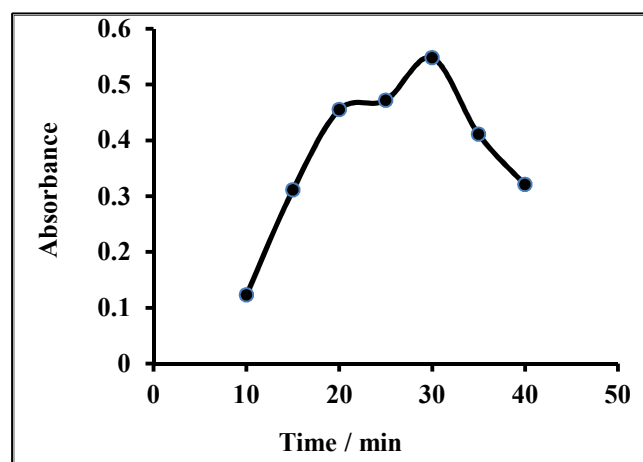


Figure 7. Effect of incubation time on the CPE of V(V)-TFPH complex.

Figure 6 shows the impact of equilibration temperature on the extraction of V(V)-TFPH complex by varying the temperature from 25 to 85 °C at incubation time of 30 min and keeping other variables constant. It was observed that the formation of cloud point layer (i.e. agglomeration of surfactant micelles) was achieved at 75 °C, giving the maximum absorbance for V(V)-TFPH complex and decreased thereafter. Thus, an equilibration temperature of 75 was selected as optimal. Figure 7 represents the influence of the incubation time from 10 to 40 min at previously optimized parameters in order to attain the equilibrium between two phases (i.e. surfactant-rich and bulk aqueous

phases) via the gathering the surfactant micelles[1]. It was noticed that the incubation time of 30 min was sufficient for the maximum absorbance of V(V)-TFPH complex. The effect of centrifugation rate and time was also considered and found that a centrifugal time of 20 min at 3500 rpm was sufficient to easily separate the two phases.

### 3.3. Optimization Parameters for V(V) Using TFPH Drug

The constant parameters as with TFPH drug determination were optimized, but a discrete variable here is the concentration of TFPH as chelating agent. At constant and trace concentration of V(V) of  $5 \mu\text{g mL}^{-1}$ , the optimum conditions of most parameters were similar to that obtained in the case TFPH optimization, but here it needs  $1.2 \times 10^{-4}$  M concentration of TFPH as optimal for the formation of V(V)-TFPH complex as shown in Figure 8.

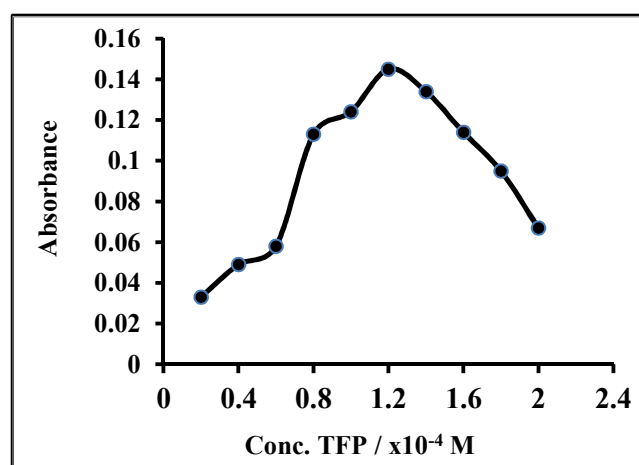


Figure 8. Effect of TFPH concentration for the formation of V(V)-TFPH complex by CPE.

### 3.4. Stoichiometry of the V(V)-TFPH Complex

In a preliminary study, it was observed that an orange-purple color is instantly formed when V(V) ion solution is added to a certain amount of TFPH drug in the presence of a wide concentration of HCl, indicative the complex formation. But, in this study the optimum pH of 1.5 was found to be enough for the stable colored complex formation which appeared in visible region with maximum absorbance at 476 nm. Consequently, mole ratio method was used to measure the stoichiometry of V:TFPH ratio at optimum acid medium. This method was conducted at constant amount of V(V) with varying amount of TFPH at optimum conditions as showed in Figure 9. Obviously, the plotted curve exhibits a maximum for a mole ratio of V(V)-TFPH complex via the point of intersection of the two lines which equal to 0.95, indicating that the expected ratio of V:TFPH in the complex was of about 1:1. The Job plot (continuous variation method) was also confirmed similar result to the former method which exhibited that the mole fraction close to 0.45, indicating again that the ratio of V:drug in the complex is 1:1 as displayed in Figure 10.

The formation constant ( $K_f$ ) of the V(V)-TFPH complex can



be determined assuming only a single complex is present by using the data of continuous variation calculated according to the procedure adopted elsewhere [40] found to be of  $3.19 \times 10^5 \text{ L mol}^{-1}$  at 476 nm. Depending on the above result it can be concluded that the most probable structure of the complex formed between V(V) and TFPH in dilute acidic medium as displayed in Figure 11.

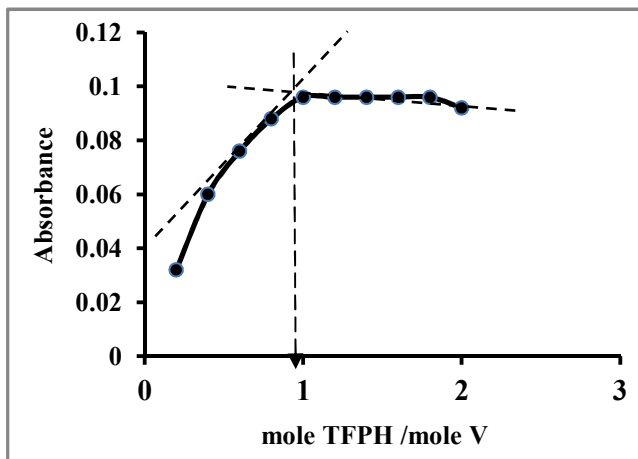


Figure 9. Mole ratio method for V(V)-TFPH complex.

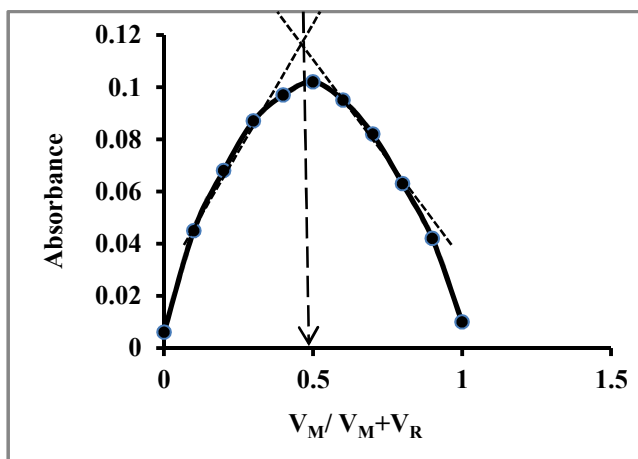


Figure 10. Continuous variation method for V(V)-TFPH complex.

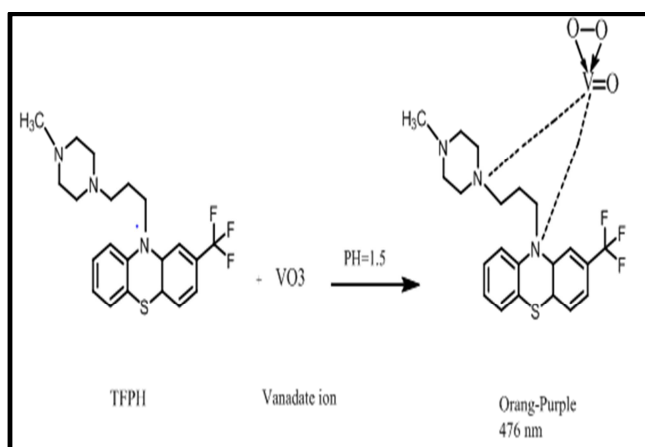


Figure 11. The probable structure of the complex formed between V(V) and TFPH in acidic medium.

For the purpose of verification of complexation reaction

between V(V) ions and TFPH drug from thermodynamic point of view, the standard free energy ( $\Delta G^\circ$ ) can be calculated from the following relationship [41];

$$\Delta G^\circ = -2.303 RT \log K$$

where R is the gas constant ( $1.987 \text{ cal mol}^{-1} \text{ degree}^{-1}$ ), T is the optimum temperature in Kelvin ( $348^\circ \text{K}$ ), and K is the formation constant of V-TFPH complex ( $\text{L mol}^{-1}$ ). It found that the standard free energy ( $\Delta G^\circ$ ) for the complexation reaction of between V(V) ions TFP drug was of a negative value and equal to  $(-8.764) \text{ kcal mol}^{-1}$ , indicating that the complexation reaction is a spontaneous.

### 3.5. Calibration Curves for TFPH Drug and V(V) Ions

Under the optimized conditions established by CPE procedure for the determination of TFPH using V(V) ions, and for determination of V(V) ion using TFPH were constructed. All figures of merit for target analytes such as linear range, correlation coefficient, molar absorptivity, Sandell's sensitivity, detection limit and quantification limit were calculated according to the statistical equations which were summarized in Table 1 and 2. The statistical evaluation for the two calibration curves reveals that that the linear regression equations  $[y=(0.0111 \pm 0.00012)x - (0.00068 \pm 0.0056)]$  and  $[y=(0.0308 \pm 0.0032)x + (0.0058 \pm 0.0018)]$  for TFPH and V(V) ions respectively, were statistically valid. This because of the ratio (MSreg/MSerror) for 1 and 7 dof, and 1 and 8 dof, larger than critical value ( $F_{1,7} = 5.59$  and  $F_{1,8} = 5.32$  at  $\alpha=0.05$ ), indicating that the predication based on the regression line is satisfactory as listed in Table 3.

Table 1. The statistical data and analytical figures of merits for TFPH using V(V) ions by CPE- Spectrophotometry.

Parameter	TFPH
Colour of complex	Orange-purple
$\lambda_{\text{max}}$ (nm)	476
Regression equation	$y=0.0111x-0.00068$
Correlation coefficient(r)	0.9999
Coefficient of determination ( $R^2$ )	99.98%
C.L. for the slope ( $b \pm ts_b$ ) at 95%	$0.0111 \pm 0.00012$
C.L. for the intercept ( $a \pm ts_a$ ) at 95%	$-0.00068 \pm 0.0056$
Concentration range ( $\mu\text{g mL}^{-1}$ )	2-80
Limit of Detection ( $\mu\text{g mL}^{-1}$ )	1.21
Limit of Quantitation ( $\mu\text{g mL}^{-1}$ )	4.04
Sandell's sensitivity ( $\text{mg cm}^{-2}/0.001\text{A.U}$ )	0.090
Molar absorptivity ( $\text{L.mol}^{-1}.\text{cm}^{-1}$ )	$6.4 \times 10^3$
Compisition of complex (V-TFPH)*	1:1
RSD% (n=3)	1.18 at $15 \mu\text{g mL}^{-1}$
RSD% (n=3)	0.37 at $60 \mu\text{g mL}^{-1}$
Preconcentration factor	50
Enrichment factor	19
Distribution ratio(D)	18
Extraction efficiency(%E)	94.73

\*Job's and mole ratio methods

**Table 2.** The statistical data and analytical figures of merits for V(V) using TFPH by CPE- Spectrophotometry.

Parameter	V(V) ion
Colour of complex	Orange-purple
$\lambda_{\text{max}}$ (nm)	476
Regression equation	$y = 0.0308x + 0.0058$
Correlation coefficient(r)	0.9995
Coefficient of determination ( $R^2$ )	99.90%
C.L. for the slope ( $b \pm ts_b$ ) at 95%	$0.0308 \pm 0.0032$
C.L. for the intercept ( $a \pm ts_a$ ) at 95%	$0.0058 \pm 0.0018$
Concentration range ( $\mu\text{g mL}^{-1}$ )	0.5-10
Limit of Detection ( $\mu\text{g mL}^{-1}$ )	0.113
Limit of Quantitation ( $\mu\text{g mL}^{-1}$ )	0.380
Sandell's sensitivity ( $\text{mg cm}^{-2}/0.001\text{A.U.}$ )	0.032
Molar absorptivity ( $\text{L.mol}^{-1}.\text{cm}^{-1}$ )	$1.78 \times 10^4$
Composition of complex (V-TFPH)*	1:1
RSD% (n=3) at $2 \mu\text{g mL}^{-1}$	2.14
RSD% (n=3) at $10 \mu\text{g mL}^{-1}$	0.44
Preconcentration factor	50

Concerning the limit of detection for the drug TFPH, our finding was in harmony with that obtained by Karimi et al [20] using indirect spectrophotometric method and principal

component regression (PCR), partial least squares (PLS) and orthogonal signal correction (OSC)-PLS multivariate calibration methods and was 8-fold better than that obtained by Navyasri et al [12] and 2-fold better than that obtained by Pattanayak and Ash Rani [11] whom using RP-HPLC method. However, it was worse than that obtained by Sharma et al [6] Patel and Patel [7], Parvataneni and Nagarjuna [13] using the developed RP-HPLC, and Waleed [4] using indirect flame AAS. Regarding the detection limit of vanadium, it observed that it was not good as with those obtained by Liu and Jiang [32], Okamoto et al [35] and Khammas et al [37] whom used more sophisticated instrumentation such as ICP-MS, ETA-ICP-OES and CPE-spectrophotometry using newly prepared organic reagent, respectively. However, by considering the detection limit of V(V) ions is  $113 \mu\text{g/L}$  in aqueous solution and 5 g of sample in 5 mL, the method detection limit is  $0.113 \mu\text{g/g}$ . This finding has encouraged the application of the proposed method successfully in the estimation of V(V) ion species in water,, black pepper and rice samples to test its applicability and reliability.

**Table 3.** Analysis of Variance of regression line for TFPH and V(V) ion.

Analyte	Source	dof	SS	MS	F	significance F(p-value)
TFPH	Regression	1	0.043858	0.043858	28929.4	6.41E-14
	Residual Error	7	1.06E-05	1.52E-06		
	Total	8	0.043868			
V(V)	Regression	1	0.075654	0.075654	6654.072	2.29E-10
	Residual Error	6	6.82E-05	1.14E-05		
	Total	7	0.075722			

Dof=degrees of freedom, SS: sum of squares, MS: mean of squares, F (Fisher F-test)

### 3.6. Accuracy and Precision

Since the certificate reference materials (CRM's) that define exactly the true value are not available for the determination of TFPH and/or V (V) ions, the trueness of the developed method was evaluated with accuracy test in the term of recovery percentage by spiking three blank water (tap and river) samples collected from the University Campus with standard solutions of TFPH and/or V (V) ions and subjected to the general CPE procedure according to each target analyte. The results are tabulated in Tables 4 and 5. The data revealed that the accuracies of the proposed method were within acceptable level for the determination of both TFPH drug

using V (V) ions, and V (V) ions using TFPH as chelating agent, indicative the established method is unbiased. This can confirm that the proposed method is relatively free from matrix interferences. In the meantime, an intra-precision (i.e. repeatability) of the proposed method was also carried out with the same spiked samples for three replicate measurements and the RSD% found to be in the range of 0.84-3.40% (Table 3) for TFPH using V (V) ions and 0.45-1.49% (Table 4) for V (V) ion determination using TFPH, indicative the proposed method is highly free from random errors.

**Table 4.** The accuracy and precision of the proposed method for the determination of TFPH using V(V) in tap and river water.

sample	Amount TFPH taken ( $\mu\text{g mL}^{-1}$ )	Amount TFPH found ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	$E_{\text{rel}}$ (%)	Mean Rec. $\pm ts/\sqrt{n}$ (%)	RSD (%) (n=3)
Tap water	10	10.2	102.0	2.00	99.93 $\pm$ 2.88	2.70
	30	29.6	98.0	-1.30		1.50
	50	49.9	99.8	-0.20		0.90
River water	10	09.94	99.4	-0.60	99.33 $\pm$ 3.45	3.40
	20	19.38	96.9	-3.10		3.19
	40	40.70	101.7	1.75		0.84

**Table 5.** The accuracy and precision of the proposed method for the determination of V(V) using TFPH in tap and river water.

sample	Amount of V(V) taken ( $\mu\text{g mL}^{-1}$ )	Amount of V(V) found ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	$E_{\text{rel}}$ (%)	Mean Rec. $\pm$ ts/ $\sqrt{n}$ (%)	RSD (%) (n=3)
Tap water	2	2.02	101.0	1.00	99.43 $\pm$ 1.40	1.49
	4	3.96	99.0	-1.00		0.83
	6	5.90	98.3	-1.60		0.45

### 3.7. Applications

Of the results obtained in the above-mentioned, it was shown that the established method gave satisfactory figures of merit for the standard aqueous solutions. Also, it has achieved promising results in terms of accuracy and precision in real samples for both target analytes which means that this method is free from crucial interferences, and encourage to test its applicability to a variety of samples below.

#### 3.7.1. Determination of TFPH Using V(V) as Mediating Metal Ion

The proposed CPE- Spectrophotometry was applied for the determination of TFPH as an active ingredient of Stelazine tablet containing 5 mg TFPH per unit (Cairo Pharmaceuticals and Chemical Industries/Egypt). The results summarized in Table 6 have revealed that the calculated t-value of 2.23 for TFPH determination using V(V) as mediating metal ion was

less than the t-critical (4.303) at  $\alpha=0.05$  level and (n-1) degrees of freedom, indicating the acceptance of null hypothesis ( $H_0$ ) and concluding there is no evidence for systematic and random errors at the 95% confidence level, thus manufacturer's claim can be acceptable. This finding is a good agreement with results of our previously reported method based on indirect FAAS [4].

The applicability of the developed method was examined by spiking 20, 30 and 40  $\mu\text{g mL}^{-1}$  of TFPH standard solutions to serum sample and the preconcentrated according to general CPE procedure, then determined by Vis-spectrophotometry. The average recovery obtained within 98.94 $\pm$ 5.42% as illustrated in Table 7, seems to suggest again that the developed method is feasible and reliable, at least, for the sample analysed for trace level of TFPH drug.

**Table 6.** Determination of TFPH in pharmaceutical formulation by the proposed method and statistical comparison with quoted values.

Commercial name*, and content	Practical content (proposed** method) (mg/tablet)	Average TFPH (mg /tablet) ( $\bar{x} \pm \text{ts}/\sqrt{n}$ ) at $\alpha=0.05$	$t = (\bar{x} - \mu)\sqrt{n/s}$ Proposed method* versus quoted value at $\alpha=0.05$	$E_{\text{rel}}$ (%)	RSD (%) n=3
Stelazine	4.80	4.89 $\pm$ 0.21	$t_{\text{cal}}=2.23$	-2.2	4.79
Tablet,	4.90		2.23<4.303		
Quoted value (5mg /tablet)	4.97				

\*Cairo Pharmaceuticals and Chemical Industries/Egypt

\*\*Mean of three determinations

**Table 7.** Determination of TFPH using V (V) in blood serum by the prposed method.

Amount of TFPH taken ( $\mu\text{g mL}^{-1}$ )	Amount of TFPH found ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	$E_{\text{rel}}$ (%)	Mean Rec. $\pm$ ts/ $\sqrt{n}$ (%)	RSD % (n=3)
20	19.38	96.90	-3.10	98.94 $\pm$ 5.42	1.58
30	29.60	98.66	-1.33		0.93
40	40.50	101.25	1.25		0.48

**Table 8.** Determination of vanadium ions in black pepper samples with statistical paired t-test at 95% confidence level.

Sample	Proposed method $\bar{x} \pm t s/\sqrt{n}$ ( $\mu\text{g g}^{-1}$ )	ETAAS method $\bar{x} \pm t s/\sqrt{n}$ ( $\mu\text{g g}^{-1}$ )	Paired t-test at 95% C.I   $t = \bar{x}_d/\sqrt{n/s_d}$
Malwiya (Iraqi brand)	0.528 $\pm$ 0.039	0.490 $\pm$ 0.133	$\bar{x}_d = -0.059333$
Amercana (USA)	0.696 $\pm$ 0.071	0.789 $\pm$ 0.172	$S_d = 0.085617$
Meshwar (Syrian brand)	0.958 $\pm$ 0.105	1.081 $\pm$ 0.212	$t_{\text{cal.}}(n=3) = 1.20$ $t_{\text{crit at 95\% CI, dof, 2}} = 4.303$

#### 3.7.2. Determination of V(V) Ion Using TFPH Chelating Agent

The established method revealed that the detection limit of the method was of 0.113  $\mu\text{g/g}$ , by considering 113  $\mu\text{g/L}$  and 5 g of food sample in 5 mL solution. This finding encouraged to analyze vanadium ions in black pepper collected from Iraqi local markets. Table 8 summarized the content of vanadium ions measured in triplicate by the developed method and ETASS method.

The results in Table 8 showed that the calculated t-value (1.20) for vanadium determination using TFPH drug as the chelating agent are less than t-tabulated (4.303) at 95% confidence interval and (n-1) degrees of freedom, indicative the acceptance of null hypothesis ( $H_0$ ), and concluding no significant difference between the proposed method and ETAAS. So, our findings were in harmony with that reported by López-García et al [42] who claimed that the black pepper contains vanadium between 431 and 987  $\mu\text{g kg}^{-1}$ .



## 4. Conclusions

This study shows the competence of the mutual detection of both target analytes in the same reaction system by coupling CPE and Vis-spectrophotometry. The inherent benefits of the proposed method are time-saving, lessening in reagents used and analyst effort. This work also reveals the possibility of the determination of the drug TPH in variety of biological and environmental matrices other than in drug formulations due to its sensitivity and reliability. In addition, TFPH can be considered a good analytical reagent that produces a sensitive colour with vanadium (V), giving a complex absorbed at  $\lambda_{\text{max}}$  of 476 nm with 1:1 stoichiometry at pH = 1.5, and reasonable molar absorptivity that permits the detection of vanadium ions from 0.5-10  $\mu\text{g mL}^{-1}$ .

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