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RESEARCH ARTICLE

A Cheap and Simple Method for Determining of Antibiotics in Pharmaceutical Products by Using Prussian Blue Reaction

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method of analysis of Tetracycline and Vancomycin in pharmaceutical dosage forms has been developed and validated. The method is based on the formation of Prussian Blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics (T = 65°C) with the mixture of Fe^{3+} and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the colored complex occurred at λ= 700 nm and the molar absorptivity is 3.2×10^4 $L.mol^{-1}$ cm^{-1}. Reaction conditions have been optimized to obtain PB complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the PB complex were found to increase linearly with increase in concentrations of Tetracycline and Vancomycin, which corroborated with the correlation coefficient values. The linear range of the calibration graph was1.5-25 ppm and 1.8- 24ppm for Tetracycline and Vancomycin respectively. The proposed method was successfully applied to the determination of the selected antibiotics in bulk drugs and pharmaceutical formulations and the results obtained agree well with the labeled contents. Antibiotics are widely used in human and veterinary medicine for the prevention and treatment of bacterial infectious diseases. An important but often disregarded aspect of antibiotic use is the fate of antibiotic residues entering the environment. Pharmaceutical industry wastewater improperly-disposed of unused antibiotics and non-metabolized antibiotics excreted by humans can all enter the sewer system in low concentrations. The validity of the method was tested by the official methods and by the recovery studies of standard addition to pharmaceuticals

Keywords: Spectrophotometric method; Prussian Blue (PB) complex; Vancomycin; Tetracycline; β-lactam antibiotics.

1. INTRODUCTION

Tetracycline and Vancomycin are among the antibiotics widely used in contemporary clinical practice. These drugs have been found very useful in pre and post operative chemotherapy against infections in abdominal, pelvic, orthopaedic, cardiac, pulmonary oesophageal and vascular surgery [1]. Prussian blue [Fe_4 [Fe (CN) $_6$]$_3$] was probably synthesized for the first time by the paint maker Diesbach in Berlin around the year 1706.[2] Most historical sources do not mention a first name of Diesbach. Only Berger refers to him as Johann Jacob Diesbach.[3] It was named "Preußisch blau" and "Berlinisch Blau" in 1709 by its first trader[4] .The pigment replaced the expensive Lapis lazuli and was an important topic in the letters exchanged between Johann Leonhard Frisch[5] and the president of the Royal Academy of Sciences, Gottfried Wilhelm Leibniz, between 1708 and 1716.[4] It is first mentioned in a letter written by Frisch to Leibniz, from March 31, 1708. Not later than 1708, Frisch began to promote and sell the pigment across Europe. By August 1709, the pigment had been termed "Preussisch blau"; by November 1709, the

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German name "Berlinisch Blau" had been used for the first time by Frisch. Frisch himself is the author of the first known publication of Prussian blue in the paper Notitia Coerulei Berolinensis nuper inventi in 1710, as can be deduced from his letters. Diesbach had been working for Frisch since about 1701.In 1731, Georg Ernst Stahl published an account of the first synthesis of Prussian blue[6]. The story involves not only Diesbach but also Johann Konrad Dippel. Diesbach was attempting to create a red lake pigment from cochineal but obtained the blue instead as a result of the contaminated potash he was using. He borrowed the potash from Dippel, who had used it to produce his "animal oil". No other known historical source mentions Dippel in this context. It is therefore difficult to judge the reliability of this story today. In 1724, the recipe was finally published by John Woodward [7][8][9].To date, the "Entombment of Christ", dated 1709 by Pieter van der Werff (Picture Gallery, Sanssouci, Potsdam) is the oldest known painting where Prussian blue was used. Around 1710, painters at the Prussian court were already using the pigment. At around the same time, Prussian blue arrived in Paris, where Antoine Watteau and later his successors Nicolas Lancret and Jean-Baptiste Pater used it in their paintings[10]. "The Great Wave off Kanagawa" by Hokusai, a famous artwork which makes extensive use of Prussian blue. This Prussian blue pigment is significant since it was the first stable and relatively lightfast blue pigment to be widely used following the loss of knowledge regarding the synthesis of Egyptian blue. European painters had previously used a number of pigments such as indigo dye, smalt, and Tyrian purple, which tend to fade, and the extremely expensive ultramarine made from lapis lazuli. Japanese painters and woodblock print artists likewise did not have access to a long-lasting blue pigment until they began to import Prussian blue from Europe.In 1752 the French chemist Pierre J. Macquer made the important step of showing the Prussian blue could be reduced to a salt of iron and a new acid, which could be used to reconstitute the dye[11] . The new acid, hydrogen cyanide, first isolated from Prussian blue in pure form and characterized about 1783 by the Swedish chemist Carl Wilhelm Scheele, was eventually given the name Blausäure (literally "Blue acid") because of its derivation from Prussian blue, and in English became known popularly as Prussic acid. Prussian blue would also give the name to the ferrocyanide and cyanide family of compounds. Ferrocyanide (which is yellow) was coined as Neo Latin for "iron-containing blue material", since it was first isolated from Prussian blue. Cyanide, a colorless anion that forms in the process of making Prussian Blue, was named, in turn, for hydrogen cyanide (also colorless), and ultimately from ferrocyanide. It is for this reason that cyanide, even though the name of a colorless radical, is a Latinized form of the Greek word for "dark blue." Prussian blue is produced by oxidation of ferrous ferrocyanide salts. These white solids have the formula $M_2Fe[Fe(CN)_6]$ where $M^+ = Na^+$ or K^+. The iron in this material is all ferrous, hence the absence of deep color associated with the mixed valency. Oxidation of this white solid with hydrogen peroxide or sodium chlorate produces ferricyanide and affords Prussian blue.[12]. A "soluble" form of PB, $K[Fe^{III}Fe^{II}(CN)_6]$, which is really colloidal, can be made from potassium ferrocyanide and iron (III):

$$K^+ + Fe^{3+} + [Fe^{II}(CN)_6]^{4-} \rightarrow KFe^{III}[Fe^{II}(CN)_6]$$

The similar reaction of potassium ferricyanide and iron(II) results in the same colloidal solution, because $[Fe^{III}(CN)_6]^{3-}$ is converted into ferrocyanide."Insoluble" Prussian blue is produced if in the reactions above an excess of Fe^{3+} or Fe^{2+}, respectively, is added. In the first case:

$$4Fe^{3+} + 3[Fe^{II}(CN)_6]^{4-} \rightarrow Fe^{III}[Fe^{III}Fe^{II}(CN)_6]_3$$

$$Antibiotic + Fe^{3+} \rightarrow Antibiotic^+ + Fe^{2+}$$

The chemical formula of insoluble Prussian blue is $Fe_7(CN)_{18}{\cdot}xH_2O$, where x = 14–16. The structure was determined by using IR spectroscopy, Moessbauer spectroscopy, X-ray crystallography, and neutron crystallography. Since X-ray diffraction cannot distinguish carbon from nitrogen, the location of these lighter elements is deduced by spectroscopic means as well as by observing the distances from the iron atom centers.PB has a cubic lattice structure. Soluble PB crystals contain interstitial K^+ ions; insoluble PB has interstitial water instead. In ideal insoluble PB crystals, the cubic framework is built from Fe(II)-C-N-Fe(III) sequences, with Fe(II)-carbon distances of 1.92 Å and Fe(III)-nitrogen distances of 2.03 Å. One-fourth of the sites of $Fe(CN)_6$ subunits are vacant (empty), leaving three such groups. The empty nitrogen sites are filled with water molecules instead, which are coordinated to Fe(III).The Fe(II) centers, which are low spin, are surrounded by six carbon ligands in an octahedral configuration. The Fe(III) centers, which are high spin, are octahedrally surrounded on average by 4.5 nitrogen atoms and 1.5 oxygen atoms (the oxygen from the six coordinated water molecules). Additional eight (interstitial) water molecules are present in the unit cell, either as isolated molecules or hydrogen bonded to the coordinated water. The composition is notoriously variable due to the presence of lattice defects, allowing it to be hydrated to various degrees as water molecules are incorporated into the structure to occupy cation vacancies. The variability of Prussian blue's composition is attributable to its low solubility, which leads to its rapid precipitation without the time to achieve full equilibrium between solid and liquid[16] [17]. Prussian blue is strongly colored and tends towards black and dark blue when

mixed into oil paints. The exact hue depends on the method of preparation, which dictates the particle size. The intense blue color of Prussian blue is associated with the energy of the transfer of electrons from Fe(II) to Fe(III). Many such mixed-valence compounds absorb certain wave lengths of visible light resulting from intervalence charge transfer. In this case, orange-red light around 680 nanometers in wavelength is absorbed, and the reflected light appears blue as a result.Like most high chroma pigments, Prussian blue cannot be accurately displayed on a computer display.PB is electrochromic—changing from blue to colorless upon reduction. This change is caused by reduction of the Fe(III) to Fe(II) eliminating the intervalence charge transfer that causes Prussian blue's color.Because it is easily made, cheap, non-toxic, and intensely colored, Prussian blue has attracted many applications. The dominant uses are for pigments: approximately 12,000 tonnes of Prussian blue are produced annually for use in black and bluish inks. A variety of other pigments also contain the material[12]. Engineer's blue and the pigment formed on cyanotypes—giving them their common name blueprints. Certain crayons were once colored with Prussian blue (later relabeled Midnight Blue). It is also a popular pigment in paints. Similarly, Prussian blue is the basis for laundry bluing. Prussian blue's ability to incorporate monocations makes it useful as a sequestering agent for certain heavy metal poisons. Pharmaceutical-grade Prussian blue in particular is used for patients who have ingested thallium or radioactive caesium. According to the International Atomic Energy Agency, an adult male can eat at least 10 grams of Prussian blue per day without serious harm. The U.S. Food and Drug Administration (FDA) has determined that the "500 mg Prussian blue capsules, when manufactured under the conditions of an approved New Drug Application (NDA), can be found safe and effective therapy" in certain poisoning cases[18]. Radiogardase (Prussian blue in soluble capsules [19]) is a commercial product for the removal of caesium-137 from the intestine and so indirectly from the bloodstream by intervening in the enterohepatic circulation of caesium-137,[20] reducing the internal residency time (and exposure) by about two-thirds. Prussian blue is a common histopathology stain used by pathologists to detect the presence of iron in biopsy specimens, such as in bone marrow samples. The original stain formula, known historically (1867) as "Perls' Prussian blue" after its inventor, German pathologist Max Perls (1843–1881), used separate solutions of potassium ferrocyanide and acid to stain tissue (these are now used combined, just before staining). Iron deposits in tissue then form the purple Prussian blue dye in place, and are visualized as blue or purple deposits[21] .The formula is also known as Perls Prussian blue and (incorrectly) as Perl's Prussian blue. Prussian blue in oil paint is the traditional material used for spotting metal surfaces such as surface plates and bearings for hand scraping. A thin layer of non-drying paste is applied to a reference surface and transfers to the high spots of the workpiece. The toolmaker then scrapes, stones, or otherwise removes the marked high spots. Prussian blue is preferable because it will not abrade the extremely precise reference surfaces as many ground pigments may. Prussian blue is formed in the Prussian blue assay for total phenols. Samples and phenolic standards are given acidic ferric chloride and ferricyanide which is reduced to ferrocyanide by the phenols. The ferric chloride and ferrocyanide react to form Prussian blue. Comparing the absorbance at 700 nm of the samples to the standards allows for the determination of total phenols[22]. Despite the fact that it is prepared from cyanide salts, Prussian blue is nontoxic because the cyanide groups are tightly bound to Fe. Other polymeric cyanometalates are similarly stable with low toxicity. Owing to the vital importance of antibiotic drugs in biological fluids and pharmaceutical preparations, various spectroscopic,chromategraphic and electrochemical methods for the assay of Vancomycin [1-8] have been reported. Tetracycline was determined by different spectrophotometry, [13-16] HPLC [1719] GC[20]electrophoresis[21]
titrimetry[22] and Circulardichrosim[23].

2. EXPERIMENTAL

2.1. Apparatus and Reagents

Absorbances were measured with a LKB UV_{Vis} 4054 spectrophotometer with 1 cm cells. pH adjustments were made using WTW multilab 540 Ionalyzer (Germany) pH/mV- meter. A water -circulating thermostat (COOLNISC model CTE 21) was used at 65± 3 ℃.All chemicals were of analytical reagent grade and freshly distilled water was used through out. Tetracycline(A) ,Vancomycin(B) (Fig. 2) obtained from Zakaria Pharmaceutical Company (Tabriz,Iran) were of chemically pure laboratory working standards.

2.2. Recommended Procedure

Aliquot portions of drug standard solution 250 mgL^{-1} were transferred into 10 mL volumetric flasks, 2 mL of HCl 0.35 M was added, and the resulting solutions were placed in a thermostat adjusted at 65 ℃ for 45 min. After this period of time, 0.25 mL of 3×10^{-4}M $K_3[Fe(CN)_6]$ and 1 mL of 3×10^{-3} M Fe^{3+} were added and diluted to the volume with distilled water. Absorbance values were measured at 700 nm against a reagent blank after 15 min. The calibration curves were drawn or regression equations calculated[24-29].

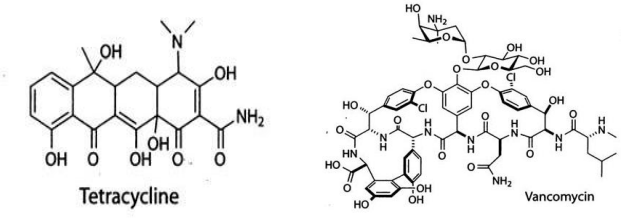
Fig.1. Different concentrations of antibiotics for drawing calibration curve.

2.3. Determination in Pharmaceutical Preparations

The pharmaceutical preparations were obtained from local sources in various forms (tablet, capsule, oral suspension and vial). Accurately weighed quantities of powdered tablet, vial or oral suspension equiv a lent to 250 mg L^{-1} of drugs A and B were transferred into 100 mL volumetric flasks, and then the proposed spectrophotometric method was per -formed on aliquot portions of the resulting sample solutions.

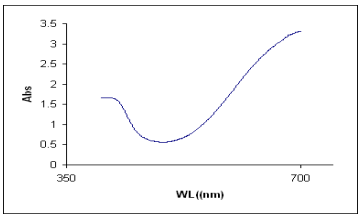
3. RESULTS AND DISCUS SION

Preliminary studies were designed to examine the re action between the selected antibiotics and chromogenic reagents at room temperature. The obtained results confirmed that no consider able interactions occurred under these condition,but by increasing the temperature, the color of PB complex gradually appears in solution. Considering that the PB reaction had been employed only in qualitative tests, a first experiment was made to obtain the visible absorption spectrum of this com pound. Fig.3A shows the absorption spectrum for chromogenic reagents,for example Fe(III) mixed with hexacyanoferrate(III) in acidic media(blank solution), and a weak band with wave length peak at 420 nm, which is related to the formation of the complex ferric hexacyanoferrate[32].After adding acidic hydrolysis products of studied antibiotics to this mixture, the absorption spectrum changed as depicted in Fig. 3B for Tetracycline, due to the formation of Prussian Blue ($Fe_4[Fe(CN)_6]_3$), with the maximum absorbance peak at 700 nm. The molar absorptivity of the complex compound formed is 3.2×10^4 L mol^{-1} cm^{-1}.

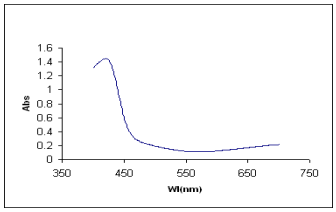


A **B**

Fig.2. Structure of antibiotics (Tetracycline(A), Vancomycin(B))



A



B

Fig.3. Visible absorption spectra of (A) chromogenic reagents (Fe(III) + $[Fe(CN)_6]^{3-}$) and (B) (A) + acidic hydrolysis products of Tetracycline .

The acidic hydrolysis of antibiotic drugs containing β-lactam ring have been extensively studied The results obtained from these studies have shown that the major products resulting from the acidic hydrolysis at 65℃ in water.Acidic hydrolysis conditions such as nature and concentration of acid, temperature and heating time on the formation of PB complex was studied by measuring the absorbance values at λ= 700 nm. Among HNO3, H_2SO_4 and HCl, hydrochloric acid was selected for further experiments due to providing high intensity color and also nearly fast reaction. The effect of various concentrations of HCl used in the acidic hydrolysis step of Tetracycline is shown in Fig. 4. As can be seen, by increasing the concentration of HCl the absorbance values in crease and level off at about 0.3 M. Therefore, the 0.35 M of HCl was selected as optimum acid concentration. Fig. 5 shows that the hydrolysis of Tetracycline is completed at 65 ℃ after 45 min and increase of heating time and temperature did not considerably alter the color. Similar be hav iors were ob served for Vancomycin. The effect of the concentration of the hexacynoferrate(III) ion was studied for solutions containing a fixed concentration of antibiotic and varying amounts of $[Fe(CN)_6]^{3-}$. As shown in Fig. 6, in the case of both selected antibiotics, the analytical signals increase with increasing there agent concentration up to 3×10^{-4} M, above which they remain virtually constant.The effect of Fe(III) concentration was studied in the range 4.0×10^{-4} - 4.0×10^{-3} M (Fig. 7). The maximum color intensity remained constant at concentrations higher than 3×10^{-3} M for both antibiotics. There fore, the concentration selected was 3×10^{-3} M.

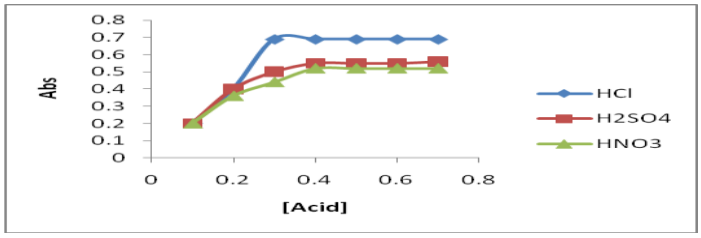


Fig.4. Effect of concentration of HCl used for the hydrolysis of 10 ppm Tetracycline within a period of 60 min

at T = 65 ℃ on the absorbance of PB complex.

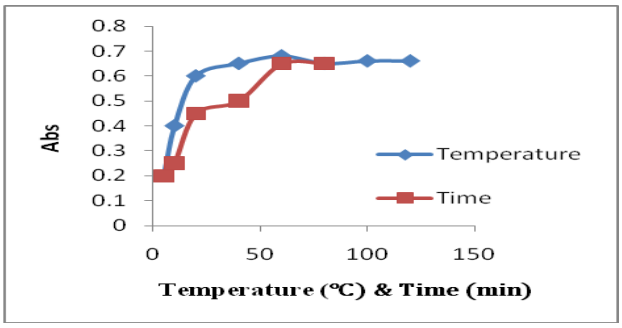


Fig.5. Effect of acidic hydrolysis temperature within a period of 60 min and time of acidic hydrolysis at 65 ℃ on the absorbance of PB complex related to the reaction of hydrolyzed Tetracycline (10 ppm) with chromogenic mixture.

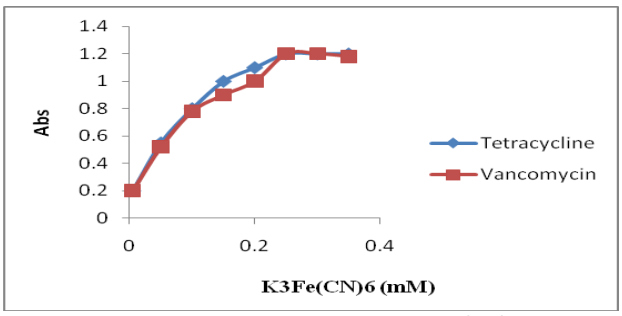


Fig. 6. Ef fect of the concentration of $K_3[Fe(CN)_6]$, Vancomycin; Tetracycline.

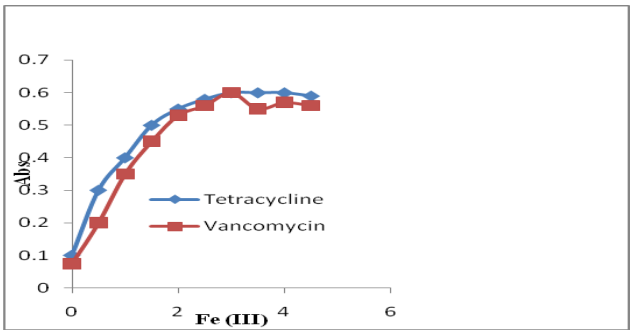


Fig. 7. Ef fect of different concentrations of [Fe(III)]on the absorbance of PB complex produced correspond ing to: Tetracycline and Vacomycin.

Parameters	Λ (nm)	Type of acid	[HCl] (M)	$K_3[Fe(CN)_6]$	Fe (III) (M)	Time (min)	Temperature (℃)
Optimized Amount	700	HCl	0.35	3×10^{-4}	3×10^{-3}	60	65

Table 1. Parameters optimized

Drug	Regression Equation	L.R (ppm)	n	R^2	D.L(ppm)	Quantitation Limit(ppm)
Tetracycline	Y=0.0244X+ 0.055	1.5-25	6	0.9972	0.543	1.2
Vancomycin	Y=0.0235X +0.0665	2-24	6	0.9943	0.647	1.4

Table2. Assay Parameters and Regression Analysis

Y= Absorbance, X= Concentration (ppm), L.R Linear Range. D.L= Detection Limit

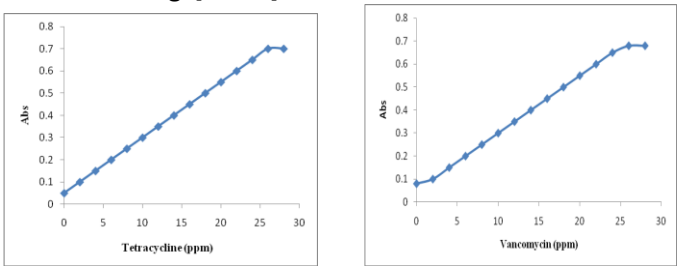
Pharmaceutical producted	Labeled Amount	Amount Found	Value Antibiotics calculated (Recovery)± SD	HPLC Method	RSD%
Capsul(Tetracycline)	250mg	248.8mg	249± 2.3mg	249.1±1.2	2.2
Ointment(Tetracycline)	0.3%(3.5mg)	0.31%(3.501mg)	3.51± 0.4	3.49 ± 0.3	2.1
Solution(Tetracycline)	60mg mL^{-1}	61.2 mg mL^{-1}	61.2± 2.3	61.2± 2.3	2.4
Injection(Tetracycline)	0.3%(3.5mg)	0.294%(3.479mg)	3.479±1.2	3.49 ±1.2	1.1
Tablet(Tetracycline)	100mg	99.8mg	99.8 ± 2.3mg	99.9± 1.3mg	1.2
Capsul(Vancomycin)	150mg	149.3mg	149.8 ± 2.3mg	149.9± 2.1mg	1.3
Ointment(Vancomycin)	3%	2.96%	2.96%±1.3%	2.98%±1.2%	1.2
Solution(Vancomycin)	50mg	51.2mg	50.8 ± 1.2mg	49.8±1.1mg	1.1
Tablet(Vancomycin)	100mg	98.2mg	99.8 ± 2.2mg	99.9± 1.3mg	1.3

Table 3. Analysis of some pharmaceutical preparations.

4. ANALYTICAL APPLI CATIONS

After the optimization of all parameters effective on acidic hydrolysis and PB reaction for the determination of selected antibiotics, the relation between absorbance and concentration of drugs was studied. The Beer's law limit, regression equation, Y = a + bX, and correlation coefficient for all the sys tems are given in Ta ble 2. A linear relationship was found between the absorbance at λ= 700 nm and the concentration of the colored PB complex in the concentration range 1.5-25ppm for Tetracycline, 2-24 ppm for Vacomycin. Also, the calculated detection and quantitation limits of the three analyzed samples are presented in Table 2 and indicate the high sensitivity of the pro posed method. A regression analysis of Beer's law plots reveals a good correla tion. A sample of the resulting calibration graphs for Tetracycline is shown in Figure. 8. The effects of common excipients and other sub stances were tested for possible interferences in the as say. It was observed that talc, glucose, starch, lactose and magnesium stearate did not interfere with the determination at the levels found in dos age forms[33-40].In order to establish the validity of the analytical method,the proprietary drugs containing antibiot ics were analyzed.The same samples were also assessed by the official BP[30] or USP[31] methods (Table 3). The recoveries and relative standard deviation ranging from 0. 5-1.2% reveal that similar degrees of accuracy and precision are afforded by both methods. In addition, to test the accuracy of the proposed method,recovery experiments were per formed on the samples prepared from dosage forms and pure drugs (Table 3). The results were found to be sat is factory and confirm that the proposed method is free from interferences by oral and

tablet fillers or vial additions usually formulated with the examined drugs[41-51].



A B

Fig. 8. Calibration curve for Tetracycline (A) and Vancomycin (B)

5. CONCLUSION

A simple, inexpensive, precise and sensitive spectrophotometric method is proposed for the Determination of Tetracycline and Vancomycin and obtained results compare with HPLC method. The intensity of color of the Prussian Blue complex formed is proportional to the amount of antibiotic drug in sample[51-53]. The other advantages of the present method over the previously described methods include low detection limit with high accuracy, precision, and non-interference from the associated substances in the dosage forms. Therefore, the proposed method is suit able for the analysis of the mentioned antibiotics in pharmaceutical preparations.

6. ACKNOWLEGMENTS

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