UV-Vis Spectrophotometric Method Using Natural Reagent from *Vigna unguiculata* subsp. *sesquipedalis* for Tetracycline Determination in Pharmaceutical Samples

Senee Kruanetr[1] and Kanyarak Prasertboonyai[2]*

[1]Center of Excellence for Innovation in Chemistry, Department of Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Mahasarakham, Thailand
[2]Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok (Rayong Campus), Rayong, Thailand

Received: 20 April 2020, Revised: 15 July 2020, Accepted: 4 August 2020

Abstract

The natural reagents from *Vigna unguiculata* subsp. *sesquipedalis* were studied and applied in the determination of tetracycline by UV-Vis spectrophotometry. The method was based on a complexation formation between tetracycline and iron (III) derived from natural plant extract in acetate buffer at pH 5 to give a yellow complex with the optimum absorption at 430 nm. Parameters related to the extraction efficiency of the natural reagent and the factors that affected the determination of tetracycline were examined. Under optimum conditions, linearity was obtained over the range of 1.00 - 20.00 mg l^{-1}. The limit of detection *(*LOD, 3σ*)* and limit of quantification *(*LOQ, 10σ*)*, calculated following IUPAC, were 0.65 and 2.15 mg l^{-1}, respectively. The repeatability and reproducibility for determining 10.00 mg l^{-1} of tetracycline (n=11) were 3.43%and 5.14%, respectively. The proposed method was successfully applied to the determination of tetracycline in pharmaceutical formulations. The results obtained by the proposed method were in good agreement with the label values verified by the student t-test at the 95% confidence level.

Keywords: *Vigna unguiculata* subsp. *sesquipedalis*; tetracycline; UV-Vis spectrophotometer
DOI……..

1. Introduction

Tetracycline (TC) is an antibiotic that is used to treat various bacterial infections such as infections of the skin, intestines, respiratory tract and other body systems [1]. It is often used in treating severe acne, or sexually transmitted diseases such as syphilis, gonorrhea, and chlamydia. In some cases, tetracycline is used when penicillin or other antibiotics cannot be used to treat serious infections [2]. It can be seen that it is effective against a broad spectrum of bacteria, as well as other organisms, including some protozoan parasites. However, overdose with tetracycline can cause liver failure and even death [3].

*Corresponding author: Tel.: (+66) 856939614
E-mail: kanyarak.p@sciee.kmutnb.ac.th

The literature review demonstrated that a wide range of analytical methods, including high-performance liquid chromatography (HPLC) [4-6], capillary electrophoresis [7, 8], electrochemistry [9, 10], spectrofluorometry [11], and chemiluminescence [12], have been used to detect tetracycline in pharmaceutical formulations and environmental samples. Although these methods provide high sensitivity, high selectivity and high precision, they do not provide sufficiently low determination ranges. Moreover, they can be very time consuming and have high operating costs.

UV-Vis spectrophotometry is one option of analytical methods because it is fast, simple, low cost, and offers a wide range of applications that are commonly available in all laboratories set up for quality control for drug analysis. Many researchers have presented the complexation of tetracycline by using various metal ions for tetracycline analysis by UV-Vis spectrophotometric method, and ions used included as lanthanide (III) [13], Manganese (III) and copper (II) [14], aluminium (III) [15], uranium (VI) [16], platinum (II) [17], iron (III) [18]. Moreover, iron (III) is one of the metal ions that strongly chelates tetracycline and it has been shown that the determination of tetracycline was influenced by the presence of iron (III) [19]. In 1988, Sultan *et al.* [18] developed a spectrophotometric method for determining tetracycline that depended on the reaction between iron (III) and tetracycline in H_2SO_4 medium. This method was used for tetracycline detection in drug formulations. In 2008, Palamy and Ruengsitagoon [20] presented an applied flow injection spectrophotometric method for tetracycline detection based on the reaction of tetracycline and iron (III) in acidic medium, and the product was detected at the maximum absorption of 423 nm. However, wastes that result from the use of iron (III) as a chelating agent can be harmful to people and the environment.

Recently, researchers have focused on the design and development of method based on 'green' chemistry as processes that reduce or eliminate the generation of hazardous substances which are economically and environmentally friendly [21]. Natural reagent from plant extract is another alternative for green chemical analysis that is gaining attention. Therefore, plants that have high iron content are another option to develop as a natural extract for tetracycline analysis.

Vigna unguiculata subsp. *sesquipedalis* (Yard-long bean) is an important legume that is grown in tropical and subtropical areas [22]. It offers high nutritional value in both its leaves and seeds, and is resistant to drought and salinity [23]. Yardlong beans are mainly cultivated for their soft and long pods that can be eaten both fresh and cooked [24]. It is widely cultivated for its edible iron-protein-rich seeds, antioxidant capacity and tolerance to mild drought [25].

In this research, we presented a spectrophotometric method for the determination of tetracycline in pharmaceutical formulations by using the natural reagent from *Vigna unguiculata* subsp. *sesquipedalis.* The proposed method depended on the complex reaction between iron (III) content in plant extract and tetracycline in acetate buffer pH 5.0 to form a yellow complex with an optimum absorption at 430 nm. The complexation reaction was shown in Figure 1.

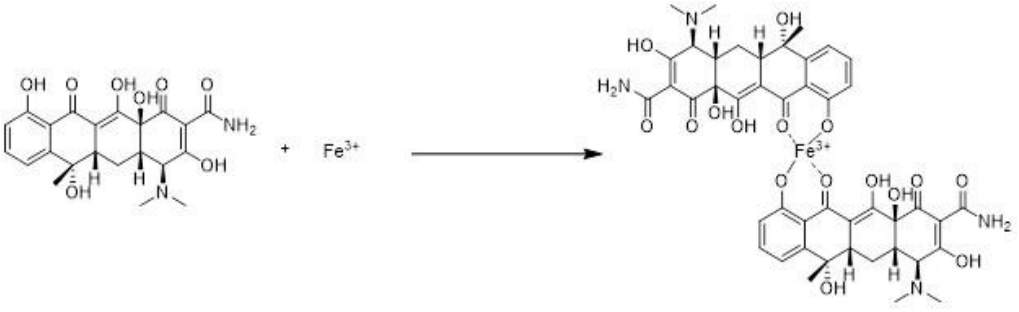

Figure 1. The possible reaction mechanism tetracycline reacts with iron **(**III**)**

2. Materials and Methods

2.1 Reagents and chemicals

All chemicals used in this study were of analytical reagent grade and were not further purified. Double-distilled deionized water (DI water) was used for diluting and adjusting volume of solution throughout the experiments.

Tetracycline standard was purchased from Fluka (Switzerland). Nitric acid (HNO_3) and hydrochloric acid (HCl) were purchased from Merck (Germany). A standard solution of tetracycline (10 mg l^{-1}) was prepared by dissolving 0.01 g of tetracycline in DI water and adjusting volume to 1000 ml.

Acetate buffer solution (0.10 mol l^{-1} at pH 5.0) was prepared by dissolving 5.772 g of sodium acetate in 500 ml of DI water solution. Acetic acid, 1.70 ml, was then added. Finally, a 10 N HCl solution was gently dropped into the solution.

2.2 Apparatus

A spectrophotometer (model Shimadzu 2600, Japan) was used for scanning the spectra of the complex and employed during the investigation for colorimetric studies. A pH meter (Metrohm, Switzerland) was used for measuring the pH of the solutions. A Microwave Plasma Atomic Emission Spectrometer (MP-AES) instrument (Model: 4100, Agilent Inc., Santa Clara, CA, USA) was used for iron (III) determinations (wavelength at 259.94 nm). The sample introduction system consisted of solvent-resistant tubing, a double-pass cyclonic chamber, and an inert flow blurring nebulizer (OneNeb). The wavelength for iron (III) determinations used was at 259.94 nm.

2.3 Plant extraction for iron (III) content

Vigna unguiculata subsp. *sesquipedalis* (collected from Rayong Province, Thailand) was cleaned by washing with tap water and cut into small-sized pieces. The selected plant in the experiment was dried under the sun light for a day. After that, it was stored in a desiccator at room temperature until the weight was constant to be certain that the prepared plant was dried before use in the extraction process.

The parameters that related to the efficiency of preparation of plant extract such as type of acid, volume ratio of acid, acid concentration, *Vigna unguiculata* subsp. *sesquipedalis* concentration and time of digestion were studied (as shown in section 3.1).

The extraction process was as follows; 5.00 g of dry plant was dissolved with acid mixture solution of 1.00 M HNO_3 and 1.00 M HCl at a ratio of 25:75, and heated for 1 h on a hot plate [20]. The solution was cooled down to room temperature and filtered through a Whatman No. 42 filter paper. The extract as natural reagent was then collected and adjusted to the volume of 100 ml with DI water. The amount of iron (III) in *Vigna unguiculata* subsp. *sesquipedalis* extract was analyzed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES 4100, Algilent Technologies).

2.4 Procedure for tetracycline determination

Aliquots (1.00-5.00 ml) of standard tetracycline solution (10 mg l^{-1}) and 2.00 ml aliquots of plant extract were mixed together into 10 ml volumetric flasks. The contents of each flask were then adjusted to the mark with acetate buffer solution pH 5.0 to form a yellow color complex. The product solution was transferred into a cuvette and measured its absorption at 430 nm against a reagent blank using UV-Vis spectrophotometry.

2.5 Sample preparation

Sample was purchased from pharmacies in Rayong, Thailand. Twenty drugs (capsules or tablets containing 250 and 500 mg per capsule or tablet of tetracycline) were mixed together and then ground into a fine powder. A sample was accurately weighed to contain 100 mg of tetracycline and dissolved in DI water and filtered through Whatman No. 42 paper. The filtered solution was transferred to 100 ml volumetric flask and made up to the mark volume by DI water. An aliquot of the sample solution was determined by the proposed method.

3. Results and Discussion

The experiment was divided into 2 sections, the first section was concerned with studying the optimum conditions for preparing the natural reagent. The second part was an investigation of the suitable conditions for determining tetracycline by the prepared reagent using the univariation method. The parameters that affected the accuracy and sensitivity of analysis of tetracycline were varied while the other were fix constant. The parameter that gave the highest signal with low standard deviation was selected.

3.1 Studying the preparation of the plant extract

The process for the preparation of plant extract containing high amount of iron (III) was a considerable purpose. The highest iron (III) content led to obtain the highest complex resulting in a highest sensitivity. The sensitivity of each studied parameter was examined by selecting the highest slope from the linear relation graph plotted between the absorbance of the iron (III)-complex against three concentrations of the standard tetracycline (1, 2 and 3 mg l^{-1}). The parmeter that provided the highest sensitivity was selected as the optimum condition.

3.1.1 Effect of acid type

Using a suitable acid and/or acid mixture for extraction of plant led to obtain a high efficiency of iron(III) extraction for forming a complex with tetracycline resulting to high absorption signal and high sensitivity. Therefore, the effect of single acid and acid mixture on the plant extraction efficiency was investigated. In this work, 1.00 mol l^{-1} of HNO_3, HCl, H_2SO_4 and acid mixture; HNO_3-HCl were examined. Figure 2 showed that the acid mixture of HNO_3-HCl gave the highest sensitivity. Therefore, the acid mixture between HNO_3-HCl was selected for the plant extract preparation.

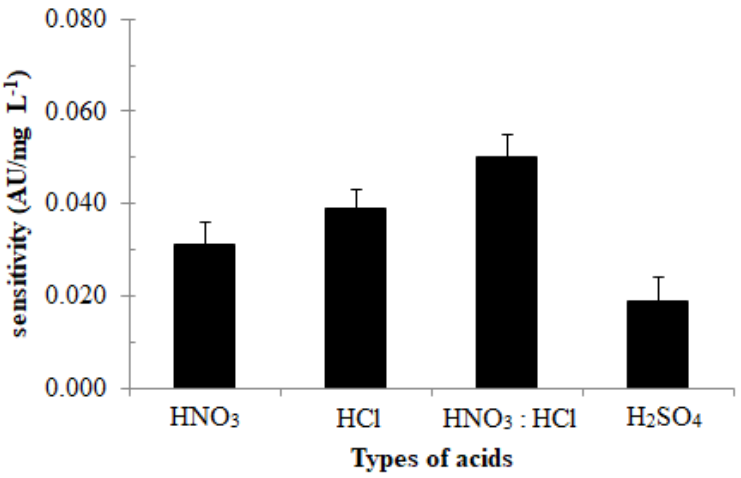

Figure 2. Effect of acid type on sensitivity (*n*=3)

3.1.2 Effect of volume ratio between HNO_3 and HCl

The ratio of mixed acid between HNO_3 and HCl affected the digestion of natural reagents that react with tetracycline to form complexes. Therefore, the ratio of mixed acid between HNO_3 and HCl (at 1.00 mol l^{-1} of each acid) in order to get the suitable acid ratio between HNO_3 and HCl in the determination of tetracycline content was studied. From the experiment, the mixed ratio of HNO_3 and HCl at 25:75, gave the highest sensitivity as showed in Figure 3. However, when the ratio of HNO_3 acid was more than 25, the sensitivity was decreased. Therefore, the mixture ratio of HNO_3 and HCl at 25:75 was chosen as the optimum condition.

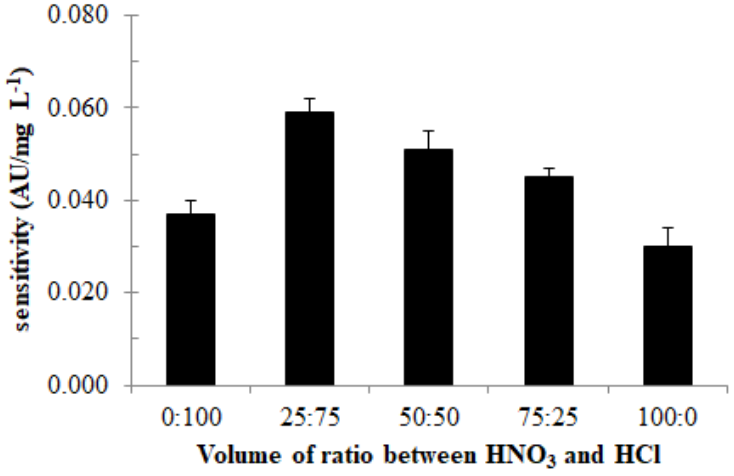

Figure 3. Effect of volume ratio between HNO_3 and HCl on sensitivity (*n*=3)

3.1.3 Effect of acid mixture between HNO_3 and HCl concentration

The concentration of the acid mixture between HNO_3 and HCl affected the digestion of natural reagents that reacted with tetracycline to form complexes. From Figure 4, the acid mixture concentration of HNO_3 and HCl at 1.00 mol l^{-1} provided the highest sensitivity. It can be seen that, the sensitivity was increased at the acid concentration range of 0.50-1.00 mol l^{-1}. However, the sensitivity was decreased at the acid concentration over 1.00 mol l^{-1}. Therefore, the concentration of HNO_3 and HCl at 1.00 mol l^{-1} was chosen as the optimum condition.

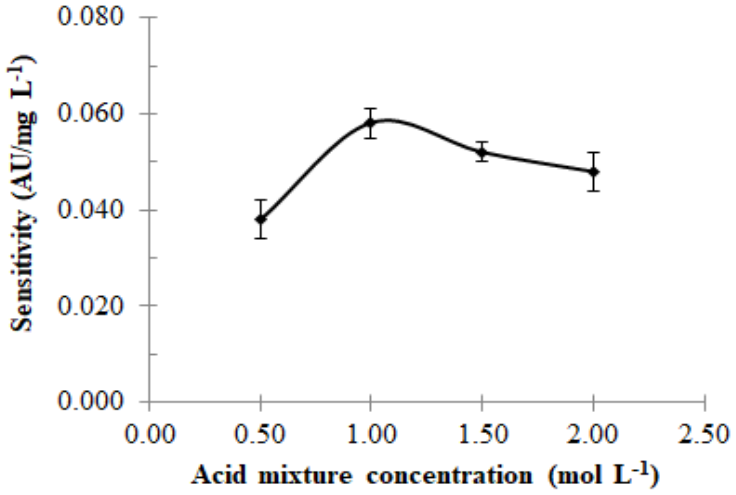

Figure 4. Effect of acid mixture between HNO_3 and HCl on sensitivity (*n*=*3*)

3.1.4 Effect of *Vigna unguiculata* subsp. *sesquipedalis* concentration

The study of the effect of *Vigna unguiculata* subsp. *sesquipedalis* concentration on sensitivity was studied by changing the weight of *Vigna unguiculata* subsp. *sesquipedalis* powder from 0.50-10.00 g (in 100 ml of DI water). Figure 5 showed that sensitivity increased when the weight of *Vigna unguiculata* subsp. *sesquipedalis* powder was increased to 5.00 g which was almost higher than its constant value. Therefore, 5.00 g of *Vigna unguiculata* subsp. *sesquipedalis* were selected.

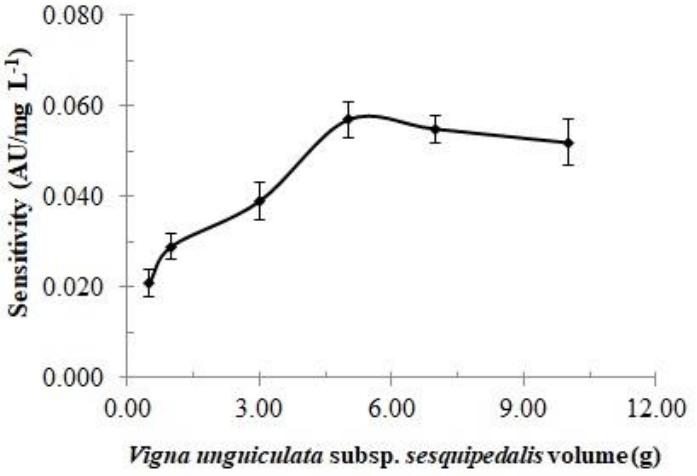

Figure 5. Effect of *Vigna unguiculata* subsp. *sesquipedalis* concentration on sensitivity (*n=3*)

3.1.5 Effect of digestion time for plant extract

The extraction time for the digestion of nature reagents, the type of acid, including the optimum conditions of the above acids affect the amount of iron (III) which is the main component of natural reagent extracts are digested. Therefore, the digestion time between 15-120 min was conducted. From the experimental results (Figure 6), it was found that the natural reagent digestion time at 60 min gave the highest sensitivity. It can be seen that between 15-60 min the sensitivity increased and after 60-120 min the sensitivity decreased. Therefore, the digestion time of 60 min was selected.

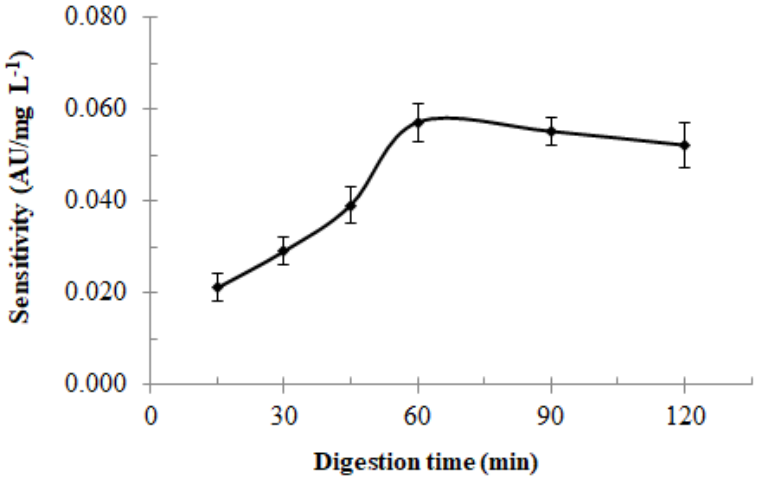

Figure 6. Effect of digestion time for plant extract on sensitivity (*n=3*)

Under the optimum condition, the amount of iron in *Vigna unguiculata* subsp. *sesquipedalis* extract were analyzed by using MP-AES. The results showed that the iron concentration in plant extracts used in the experiment was 49.56 mg l^{-1}.

3.2 Absorption spectra

The optimum absorption wavelength of a yellow color complex forming between tetracycline and iron contents in natural reagent extracts, pure tetracycline solution and standard iron (III) solution were measured over the range of 300-700 nm against reagent blank (acetate buffer pH 5.0). It was found that the maximum absorption signal of the complex was 430 nm while pure tetracycline solution and standard iron (III) solution were absorbed at 275 and 355 nm, respectively. Therefore, the absorption wavelength of 430 nm was selected.

3.3 Optimum conditions of reaction for tetracycline determination

3.3.1 Effect of pH

The stable yellow complex between tetracycline and iron (III) is pH dependent. Therefore, the acetate buffer pH range of 3.0-5.0 was studied. Figure 7 showed that, the sensitivity was increased at the pH range from 3.0-5.0, over of this pH the sensitivity was decreased. Therefore, the optimum pH of 5.0 was selected.

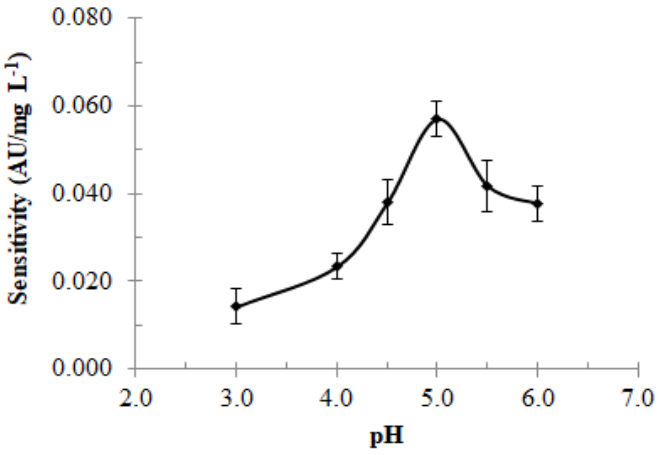

Figure 7. Effect of pH on sensitivity (*n*=3)

3.3.2 Effect of the volume of plant extract

The volume of plant extract affects the reaction because it acts as a reagent, which affects the distribution of the substance and affects the measured signal. In addition, finding the optimum condition in this variable reduces unnecessary use of the natural reagents while maintaining the best sensitivity, accuracy and reproducibility of the procedure for the drug of interest. Therefore, it is important to use the right volume of plant extract for the system environment. Figure 8 showed that the sensitivity increased with increasing volume of plant extract ranging from 1.00-2.00 ml. Above this volume, there was no significant change in sensitivity. So, the plant extract volume of 2.00 ml was selected for determining of tetracycline.

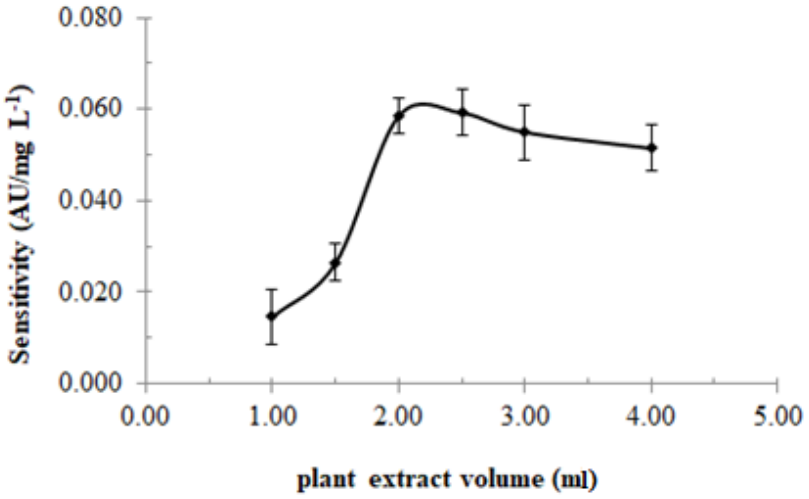

Figure 8. Effect of the volume of plant extract on sensitivity (*n*=*3*)

3.3.3 Effect of reaction time

The effect of reaction time on the sensitivity of the complex was investigated by studying the reaction time up to 60 min. Figure 9 showed that the reaction time did not affect the sensitivity of the complex.

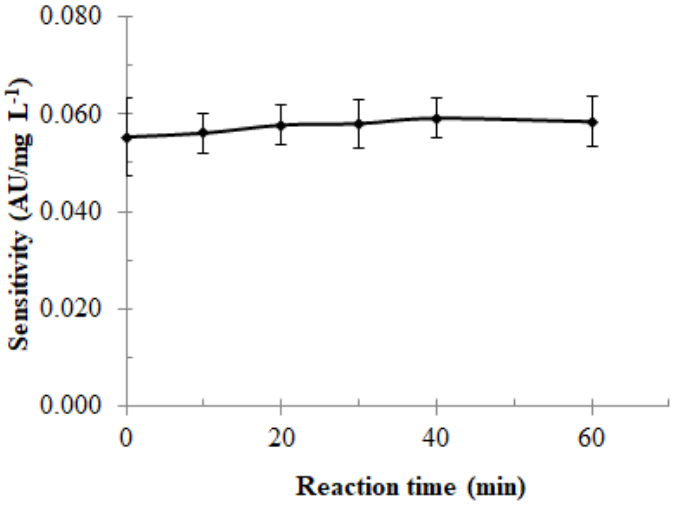

Figure 9. Effect of reaction time on sensitivity (*n*=*3*)

3.4 Validation method

The validation of the proposed method was evaluated under the optimum conditions (Table 1) obtained from the previous section. The results showed that the linearity range was in the range of 1.00-20.00 mg l^{-1} (Figure 10) with the regression equation: $y = 0.061x + 0.004$ (R^2= 0.9890), where Y and X present absorbance and TC concentrations in mg l^{-1}, respectively. The limit of detection (LOD, 3σ) and limit of quantification (LOQ, 10σ), calculated following IUPAC were 0.65 and 2.15 mg l^{-1}, respectively. The repeatability and reproducibility for 10.00 mg l^{-1} of tetracycline (n=11) was examined as the percentage relative standard deviation (%RSD), which was calculated using the equation ($SD/\overline{X}$) x 100, where SD is standard deviation and $\overline{X}$ is an average of the measurement data. It was found that the percentage relative standard deviations were 3.43% and 5.14%, respectively.

Table 1. Variable range studies and optimum conditions for tetracycline determination

Parameter studied	Range studied	Optimum level
- Effect of acid type	HNO_3, HCl, HNO_3 + HCl, H_2SO_4	HNO_3 + HCl
- Effect of volume ratio between HNO_3 and HCl (%v/v)	100:0, 75:25, 50:50, 25:75, 0:100	25:75
- Effect of acid mixture between HNO_3 and HCl concentration (mol l^{-1})	0.50 – 2.00	1.00
- Effect of *Vigna unguiculata subsp. sesquipedalis* concentration (g)	0.50-10.00	5.00
- Effect of digestion time (min)	15-120	60
Wavelength (nm)	350-700	430
- Effect of pH	3.00-7.00	5.00
- Effect of natural reagent volume (ml)	1.00-4.00	2.00
- Effect of reaction time (min)	0-60	0

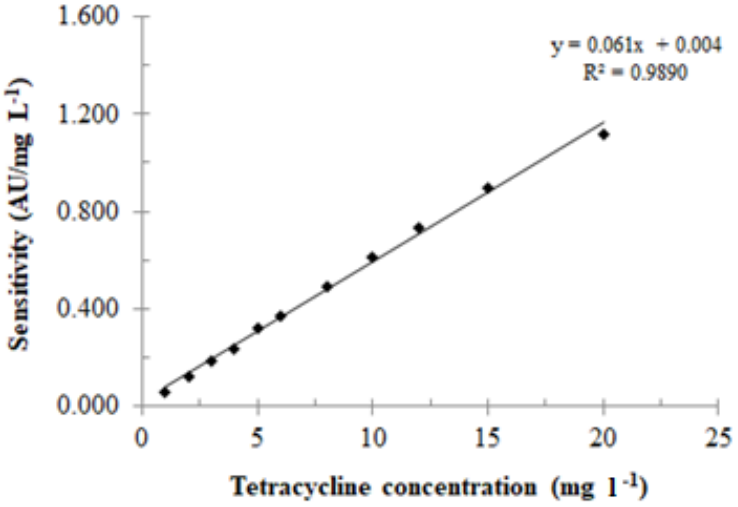

Figure 10. The calibration curve of spectrophotometric method for determination of tetracycline

3.5 Effects of interferences

Under the conditions selected above, the effects of many substances are used as generic excipients in drug preparation for this test method of tetracycline. The tolerance ratio was taken as the maximum amount causing an error not greater than 5% for determining the analyte of interest. The mixture of 5.00 mg l^{-1} tetracycline solutions with excipients at different concentrations were filtered if necessary before analysis. The tolerance values of the investigated species were >500 mg l^{-1} for starch; 400 mg l^{-1} for glucose, sucrose and lactose; 250 mg l^{-1} for fructose, citric acid and sodium benzoate. So, it could be considered that there was no interference and there was a specificity in drug analysis by the proposed method.

3.6 Application

The proposed method was applied for determination of tetracycline in pharmaceutical formulations available doses of 250 and 500 mg per capsule/tablet. The results obtained were satisfactory while compared to the label values (Table 2). Statistical analysis of the results from both values using the t-test [26] at the 95% confidence level were not significant. Indicating that this method was accurate.

Table 2. Accuracy of the proposed method compared with label value for determination of tetracycline in pharmaceutical sample

Pharmaceutical sample	**Tetracycline found (mg)**	
	Label value	**Proposed method[a]**
TC-1	250.00	242.11 ± 0.38
TC-2	250.00	247.21 ± 0.46
TC-3	250.00	241.09 ± 0.49
TC-4	250.00	245.25 ± 0.31
TC-5	500.00	496.02 ± 0.43

[a] = Average from three determinations

Table 3 showed the comparison of the analytical characteristics of the proposed method for determining tetracycline with the previous reports. The proposed method was provided a lower sensitivity than the previous report due to the limitation of the phototube detector in the UV-Vis spectrometer. Although this method was lower sensitivity than the previous reports, the advantages of this method was provided a wide linearity range, rapid and simple, using non-toxic natural reagent and environmentally friendly. To improve the sensitivity for further studies, it should be replaced with a high sensitivity detector such as photomultiplier tube or photodiode array detector.

Table 3. Comparison of the analytical characteristics of the proposed method for determining tetracycline with the previous reports

Analytical method	Linear range	LOD	%RSD	Reference
HPLC	50-5000 μg kg^{-1}	15.30 μg kg^{-1}	4.90%	[5]
CE	-	0.50 μg ml^{-1}	4.37%	[7]
Electrochemistry	2.0×10^{-5}-3.1×10^{-4} mol l^{-1}	3.6×10^{-7} mol l^{-1}	6.50%	[10]
Spectrofluorometry	0.05-100 μg ml^{-1}	0.029 μg ml^{-1}	3.53%	[11]
Chemiluminescence	5.0×10^{-5}-5.0×10^{-4} mol l^{-1}	2.0×10^{-6} mol l^{-1}	3.70%	[12]
This work	1.00 – 20.00 mg l^{-1}	0.65 mg l^{-1}	3.43%	

4. Conclusions

A simple, precise and green UV-Vis spectrophotometric method for tetracycline determination was demonstrated by the reaction between tetracycline and iron (III) contents in *Vigna unguiculata* subsp. *sesquipedalis* extraction as a natural reagent in an acetate buffer solution pH 5.0. The proposed method was successfully applied to the determination of tetracycline in pharmaceutical formulations. The results obtained by the proposed method were in good agreement with the assigned value from the label verified by student t-test at 95% confidence level. The benefit of the proposed method over the previous studies were used non-toxic/hazardous reagents, generates less waste, simple and cost-effective.

5. Acknowledgements

The authors would like to express their sincere gratitude and appreciation to the Faculty of Science, Energy and Environment, King Mongkut's University of Technology, North Bangkok (Rayong Campus) for providing all the necessary equipment and laboratory facilities. This research was funded by contract No. SciEE 01/61. Senee Kruanetr wishes to thank the Center of Excellence for Innovation in Chemistry, Department of Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University for facilities and financial support.

References

[1] Chopra, I. and Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, 65(2), 232-260.

[2] Chopra, I., Hawkey, P.M. and Hinton, M., 1992. Tetracyclines, molecular and clinical aspects. *Journal of Antimicrobial Chemotherapy*, 29(3), 245-277.

[3] Katiyar, S.K. and Elend T.D., 1991. Enhanced antiparasitic activity of lipophilic tetracyclines: role of uptake. *Antimicrobial Agents and Chemotherapy*, 35, 2075-2080.

[4] Cinquina, A.L., Longo, F., Anastasi, G., Giannetti, L. and Cozzoani, R., 2003. Validation of a high-performance liquid chromatography method for the determination of oxytetracycline,

tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *Journal of Chromatography A*, 987(1-2), 227-233.

[5] Arabsorkhi, B. and Sereshti H., 2018. Determination of tetracycline and cefotaxime residues in honey by micro-solid phase extraction based on electrospun nanofibers coupled with HPLC. *Microchemical Journal*, 140, 241-247.

[6] Charoenraks, T., Chuanuwatanakul, S., Honda, K., Yamaguchi, Y. and Chailapakul, O., 2005. Analysis of tetracycline antibiotics using HPLC with pulsed amperometric detection. *Analytical Sciences*, 21(3), 241-247.

[7] Hsiao, Y.M., Ko, J.L. and Lo, C.C., 2001. Determination of tetracycline and streptomycin in mixed fungicide products by capillary zone electrophoresis. *Journal of Agricultural and Food Chemistry*, 49(4), 1669-1674.

[8] Islas, G., Rodriguez, J.A., Perez-Silva, I., Miranda, J.M. and Ibarra, I.S., 2018. Solid-phase extraction and large-volume sample stacking-capillary electrophoresis for determination of tetracycline residues in milk. *Journal of Analytical Methods in Chemistry*, 2018, 1-7.

[9] Palaharn, S., Charoenraks, T., Wangfuengkanagul, N., Grudpan, K. and Chailapakul, O., 2003. Flow injection analysis of tetracycline in pharmaceutical formulation with pulsed amperometric detection. *Analytica Chimica Acta*, 49, 191-197.

[10] Wong, A., Scontri, M., Materon, E.M., Lanza, M.R.V. and Sotomayor, M.D.P.T., 2015. Development and application of an electrochemical sensor modified with multi-walled carbon nanotubes and graphene oxide for the sensitive and selective detection of tetracycline. *Journal of Electroanalytical Chemistry*, 757, 250-257.

[11] Sun, C.Y., Su, R.F., Bie, J.X., Sun, H.J., Qiao, S.G., Ma, X.Y., Sun R. and Zhang, T.H., 2018. Label- free fluorescent sensor based on aptamer and thiazole orange for the detection of tetracycline. *Dyes and Pigments*, 149, 867-875.

[12] Townshend, A., Ruengsitagoon, W., Thongpoon, C. and Liawruangrath, S., 2005. Flow injection chemiluminescence determination of tetracycline. *Analytica Chimica*, 541, 103-109.

[13] Karthikeyan, G., Mohanraj, K., Elango, P.K. and Girishkumar, K., 2004. Synthesis, spectroscopic characterization and antibacterial activity of lanthanide-tetracycline complexes. *Transition Metal Chemistry*, 29(1), 86-90.

[14] Chen, R.W. and Huang, H.C., 2009. Transformation of tetracyclines mediated by Mn (II) and Cu (II) ions in the presence of oxygen. *Environmental Science and Technology*, 43, 401-407.

[15] Liawruangrath, S., Liawruangrath, B., Watanesk, S. and Ruengsitagoon, W., 2006. Flow injection spectrophotometric determination of tetracycline in a pharmaceutical preparation by complexation with aluminium (III). *Analytical Science*, 22(1), 15-19.

[16] Mahgoub, S.A., Khairy, M.E. and Kasem, A., 1974. Complex formation of uranyl acetate with tetracycline and its utilization for their microdetermination. *Journal of Pharmaceutical Sciences*, 63(9), 1451-1455.

[17] Silva, P. P., De Paula, F.C.S., Guerra, W., Silveira, J. N., Botelho, F.V., Vieira, L.Q., Bortolotto, T., Fischer, F.L., Bussi, G., Terenzi H. and Pereira-Maia, E.C. 2010. Platinum (II) compounds of tetracyclines as potential anticancer agents: cytotoxicity, uptake and interactions with DNA. *Journal of the Brazilian Chemical Society*, 21(7), 1237-1246.

[18] Sultan, M.S., Alzamil, Z.I. and Alarfaj, A.N., 1988. Complexometric-spectrophotometric assay of tetracyclines in drug formulations. *Talanta*, 35(5), 375.

[19] Grenier, D., Huot, M. and Mayrand, D., 2000. Iron-chelating activity of tetracyclines and its impact on the susceptibility of actinobacillus actinomycetemcomitans to these antibiotics. *Antimicrobial Agents and Chemotherapy*, 44(3), 763-766.

[20] Palamy, S. and Ruengsitagoon, W., 2017. A novel flow injection spectrophotometric method using plant extracts as green reagent for the determination of doxycycline. *Spectrochimica Acta Part A*, 171, 200-206.

[21] Settheeworrarit, T., Hartwell, S.K., Lapanatnoppakhun, S., Jakmunee, J., Christian, G.D. and Grudpan, K., 2005. Exploiting guava leaf extract as an alternative natural reagent for flow injection determination of iron. *Talanta*, 68, 262-267.

[22] Iriti, M. and Varoni, E., 2017. Pulses, healthy, and sustainable food sources for feeding the planet. *International Journal of Molecular Science*, 18(2), 1-8.

[23] Kongjaimun, A., Kaga, A., Tomooka, N., Somta, P., Vaughan, D.A. and Srinives, P., 2012. The genetics of domestication of yardlong bean, *Vigna unguiculata* (L.) Walp. ssp. *unguiculata cv.-gr. sesquipedalis. Annals of Botany*, 109 (6), 1185-1200.

[24] Kongjaimun, A., Somta, P., Tomooka, N., Kaga, A., Vaughan, A.D. and Srinives, P., 2013. QTL mapping of pod tenderness and total soluble solid in yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata cv.-gr. sesquipedalis*]. *Euphytica*, 189(2), 217-223.

[25] Kumar, S., Yadav, S.S, Tripura, P. and Jatav, S.H., 2017. Use of phosphorus for maximization of mungbean (*Vigna radiata* L.) (Wilszeck) productivity under semi-arid condition of Rajasthan, India. *International Journal of Current Microbiology and Applied Sciences,* 6(2), 612-617.

[26] Miller, J.C. and Miller, J.N. 1993. *Statistics for Analytical Chemistry*, 3rd ed. New York: Ellis Horwood PTR Prentice Hall.