Chemical Science International Journal

23(2): 1-15, 2018; Article no.CSIJ.42128
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

Validation of UV - VIS Spectrophotometry for Phosphorous Molybdenum Determination in Soils of Vietnam

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KTKV and LTTB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GEO and EPO managed the analyses of the study. Author SOA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2018/42128
Editor(s):
(1) Dimitrios P. Nikolelis, Professor, Department of Chemistry, Athens University, Greece.
Reviewers:
(1) Suleiman Bello Physics, Umaru Musa Yaradua University, Nigeria.
(2) I. Ejidike Peter, Vaal University of Technology, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/25024

Original Research Article

Received 20th March 2018
Accepted 1st June 2018
Published 7th June 2018

ABSTRACT

Phosphorus existing under different forms and status in soils is a significantly important nutrient during the growth and development of trees. Spectrophotometry has been developed with many types of reagents to determine phosphorus in environmental samples. Molybdenum blue reaction with ascorbic acid as a reducing agent and antimony tartrate catalyst in strong acid environment gave maximum absorbance wavelength at 890 nm. Under the optimised conditions, this method gave very high recovery efficiency, accuracy and sensitivity (up to 0.06 mgP/L) in determination of

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total phosphorus (around 90%) and bio-available phosphorus (nearly 96%) in soils in Vietnam. The formed blue phosphomolybdenum complex was oxidized by the addition of hexavalent chromium and nitrite ions, leading to the decrease in intensity of blue color, negative error of 10% at 5 mg/L NO_2^- and 3 mg/L Cr^{6+}, which was in contrast to the positive effect of silicate ions at high amount of over 5 mg/L SiO_3^{2-}. χ-squared standard combined with two-way ANOVA analysis highlight the relationship between concentration, time and intensity signals, thus validating the molybdenum blue method before application in phosphorus determination for soil assessment. Olsen and Oniani extraction gave high recovery efficiency despite differences in quantified concentration of bio-available phosphorus. Although the studied soils low amount of total phosphorus (around 0.03 $\%P_2O_5$), they were medium to rich in bio-available phosphorus with the level of 8 to 15 mg P_2O_5 / 100 g soil.

Keywords: Phosphorus; molybdenum blue reaction; olsen extraction; soil assessment.

1. INTRODUCTION

Phosphorus (P) is one of macro elements playing an essential role in all living creatures, more importantly, it is a constituent of bone, nucleic acid and adenosine triphosphate in the process of aicd-base balance, intracellular energy transfer and genetics [1]. Phosphorus and calcium amount have close relationship in the organisms. In general, the average daily demand for human is 0.8 – 1.2 grams of phosphorus and the average ratio of Ca to P in food is about 700 g to maintain the total phosphorus in the body [2]. Terrestrial environment is a main resevoir of many nutrient ions depending on types of soil, especially phosphorus with 8.4 to 40 $\times 10^8$ Tg in sediments and 96000 to 200000 Tg in soils (at the dept of under 0.6 m) [3]. Phosphorus in soil, either total or bio-available phosphorus, is one of the important criteria for soil quality assessment, helping people make plans to use and protect the land. Dissolved inorganic phosphorus in the form of orthophosphate is easily absorbed by primary producing organisms (crops, plants, flowers…). This form is considered to be the main bioavailable phosphorus in soil in addition to other dissolved organic phosphorus species [4,5]. Depending on growing scale and conditions, development stage of specific species, the phosphorus demands are difference. To avoid phosphorus deficiency (Fig. 1), the addition of a calculated phosphorus is necessary via many means, commonly fertilisers and pesticides. These activities indirectly cause soil pollution and reverse effects (Fig. 2) on crops in cases of poor management. However, the excess of phosphorus does not cause serious harmful as nitrogen excess because phosphorus is a mobile element with the ability to transport from old to young organs.

Phosphorus compounds have various applications in fields of industry, environment. For example, phosphoric acid containing up to 70-75% P_2O_5 can be used as raw materials for fertiliser production, calcium phosphate is main ingredients for ceramic industry, monosodium phosphate has been used in baking powders, sodium tripolyphosphate and other polyphosphates are added as food additives. Agricultural and industrial practices have increasingly released phosphorus compounds into the environment. The runoff of large amount of phosphorus from farmland, landfills and phosphorus-contaminated soils has caused serious eutrophication, harmful algale blooms in lakes, rivers, organophosphate pesticide bioaccumulation in aquatic organisms [6,7,8].

Due to its various applications, phosphorus analysis is very essential, thus many methods have been developed and optimised, from very traditional methods [9] using silver nitrate to zinc sulfate and so on to cause orthophotphate, pyrophotphate and metaphotphate precipitations. Ammonium molybdate is currently considered as popular method. The high phosphorus will causes yellow crystals of ammonium phosphomolybdate $(NH_4)_3PO_4.12MoO_3.2H_2O$ easily soluble in alkaline environment, and convertable to $(NH_4)_3[PMo_{12}O_{40}]$ in acid, even to $(NH_4)_3[P(Mo_3O_{10})_4]$ if excess of reagent. The low phosphorus concentrations will produce yellow solution which measured by UV-VIS spectrophotometry. However, the yellow complex is not much stable, and the color can be affected by ferrous ions and color organic substances. Therefore, many reducing agents commonly tin (II) chloride, ascorbic acid with or without tartrate, bismuth as a catalyst to form blue molybdenum complex with stronger light-absorption than yellow form at longer wavelengths. UV-VIS molecular absorption spectroscopy are used in most environmental analysis on account of its simplicity, high economy and accuracy.

Fig. 1. Symptoms of phosphorus deficiency in crops

Fig. 2. Symptoms of phosphorus excess in pink Rosa multiflora

$PO_4^{3-} + 27H^+ + 12MoO_4^{2-} \rightarrow 12H_2O + H_3[P(Mo_{12}O_{40})]$ (phospho molybdenum yellow)
$H_3[P(Mo_{12}O_{40})] + nH^+ + ne^- \rightarrow H_3[P(Mo_{12}O_{40})H_n]$ (phospho molybdenum blue)

The aims of this study are to optimise amount of components in the color developing reagents, investigate effects of intefering ions and validate molybdenum blue method by using χ -squred standard and two-way ANOVA analysis before using UV – VIS method in determination of total phosphorus and bio-available phosphorus determination in soils of Vietnam. Finally, according to Vietnamese standards and Kiecxanop's classification to assess the phosphorus parameters and properties of studied soils.

2. MATERIALS AND METHODS

2.1 Materials

Stock solution 1000 mg/L phosphorus was prepared by disolving an exact amount of 2.2097 g KH_2PO_4 dired at 40°C into 500 ml. This stock was diluted to working solutions of 10 mg/L and 5 mg/L phosphorus.

Color developing reagent includes 5N sulfuric acid (S), 3.85% ammonium molybdate tetrahydrate (M), 0.27% potassium antimonyl tartrate hemihydrate (T), 1.76% ascorbic acid (C).

Nitrite solution: $NaNO_2$, 1000 mg/L NO_2^-, 50 mg/L NO_2^-

Silica solution: $Na_2SiO_3.9H2O$, 1000 mg/L SiO_3^{2-}, 50 mg/L SiO_3^{2-}

Dichromate solution: $K_2Cr_2O_7$, 1000 mg/L Cr^{6+}, 10 mg/L Cr^{6+}

Reagents for Olsen phosphorus and Oniani phosphorus extractions in soils included 0.5 M sodium hydrocacbonate pH = 8.5 and 0.05 M sulfuric acid. Other pretreatment reagents were crystal ammonium persulfate, activated coal.

2.2 Optimsation and Validation of Spectrophotometry for Phosphorus Determination

2.2.1 Investigation of roles of components in color development reagent

Determination of optimal wavelength: absorption spectra of color reagent, reaction mixture of 0.6 and 0.8 mg/L phosphorus were produced from the range of 200 to 1000 nm to find out optimal wavelength where absorbance of colored complex was maximum without interferencing peaks. Double distilled water was used as blank samples. Color reagent was prepared by mixing 2.5 mL of (S), 7.5 mL of (M), 2.5 mL of (T) and 1.5 mL of (C).

Effects of components in the color reagent was studied at 2 levels of phosphorus: 0.4 mg/L and/or 1.0 mg/L. Study of effects of sulfuric acid was carried with the same volume of components in color reagent except 5 N sulfuric acid. The amount of sulfuric acid varied from 0.5 mL to 8 mL equivalent from 0.025 M to 0.4 M. Total volume of all solutions was 50 mL in the volumetric flask. After 15 minutes of color development, the absorbance was recorded. The optimal concentration was determined at the time when the absorbance remained unchanged. Study of effects of potassium antimonyl tartrate hemihydrate was tested similarly. The same volume of other components in the color developing reagent was added. Then, antimony solution was added with different volume, from 0.1 mL to 2 mL equivalent from 1.64×10^{-5} M to 3.28×10^{-4} M. Study of effects of ammonium molybdate tetrahydrate was carried with the same procedure above with the amount of molybdate varied from 0.5 mL to 3.5 mL equivalent from 1.042×10^{-3} M đến 7.294×10^{-3} M. Study of effects of ascorbic acid was carried similarly with the amount of ascorbic acid varied from 2 mL to 6 mL equivalent from 2.24×10^{-3} M to 6.72×10^{-3} M.

Study of color stability of phosphorus molybdate complex: The assay was carried with at the concentrationn of 0.1, 0.5 and 0.9 mg/L phosphorus, the optimal amount of color devloping reagent. After mixing, the absorbance was recorded at the optimal wavelength after 5, next 10 and 15 minutes to obtain the optimal time for color development.

2.2.2 Investigation of interferences in molybdenum blue phosphorus method

Ions of elements in the same group of phosphorus (group V) and group VI in the periodic table have been considered to react with the color development reagent. At the fixed phosphorus levels, concentration of SiO_3^{2-} was increased from 0 to 20 mg/L. For nitrite and chromate ions, we increased gradually the concentration of NO_2^- (from 0 to 30 mg/L), Cr^{6+} (from 0 to 5.5 mg/L). In addition to measurement of signals, absorption spectrum was also recorded.

2.2.3 Statistics for validation of molybdenum blue phosphorus method

Under optimisised conditions, the color was developed within 15 minutes after adding 10 mL color reagent into phosphorus standards from 0 mg/L to 3 mg/L, measured absorbance at optimal wavelength. The linear range was drawn to calculate limit of detection (LOD), limit of quantification (LOQ) of this method, recovery yield ($\%Y_R$) representing method accuracy, and used for phosphorus assessment in soil and water.

The absorbance at 4 phosphorus levels within linear range (0.1, 0.5, 0.9 and 1.5 mg/L) was measured 5 times repeatably on one day. Variance values are calculated to draw conclusions whether this method is stable with phosphorus concentrations.

The sensitivity of this method was evaluated by 2-way analysis of variance (ANOVA). Time (D-day) and concentration (C) are two independent factors affecting on absorbance. D factor was investigated at 3 levels - D_1, D_2, D_3; and C factor was studied at 4 levels C_1 = 0.1, C_2 = 0.5, C_3 = 0.9 and C_4 = 1.5 mgP/L.

2.3 Phosphorus Value for Soil Quality Assessment

2.3.1 Sampling

Soil samples were taken at regions in Vienam at a depth of 0–20 cm. Each sample was taken on the same acreage at 10–15 different locations which were then gathered and mixed well in the field to get 1 kg of fresh soil [10,11,12]. Soil samples were transferred to the laboratory, dried in air and ground into pellets sieved through 2 mm. Then, samples were divided into small weights of about 200 g to be crushed and sieved through < 250 µm before analysis [12,13,14,15].

2.3.2 Analysis of total phosphorus in soil

5.0 g of samples with some water was poured to a beaker. If one drop of phenolphthalein made solution pink, drops of 5 N H_2SO_4 was added until colorlessness. 2 mL of H_2SO_4, 0.5 g of $(NH_4)_2S_2O_8$ crystal and water was added until the half of beaker. After heated within 40 minutes, the reaction mixture was cooled to room temperature and neutralised with NaOH 1N to pH 3–4, transferred and filled up with water in 250 mL volumetric flask. Depending on samples, V (mL) varying from 5-25 mL of solution was taken from the 250 mL flask to test phosphorus.

2.3.3 Analysis of bioavailable phosphorus in soil

5.0 g soil samples was taken to 250 mL flask. 100 mL of 0.5 M $NaHCO_3$ was added and mixed

with soil in 30 minutes followed by filter to extract Olsen phosphorus [11]. In the second method, 5.0 g soil samples was taken to 250 mL flask. 100 mL of 0.05 M H_2SO_4 was added and mixed with soil in 5 minutes followed by fine filter to extract Oniani phosphorus. If the filtrate was translucent, the extraction would try again. If the filtrate was colorful, it was filtered with activated coal to remove organic components in soil. After extraction, phosphorus was quantified by standard curve from validated molybdenum blue phosphorus method above.

3. RESULTS AND DISCUSSION

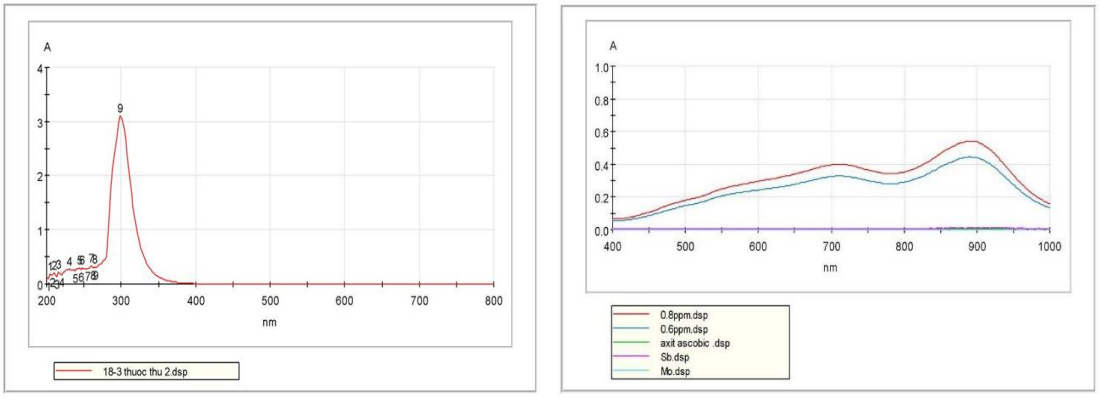
3.1 Optimsation and Validation of Spectrophotometry for Phosphorus Determination

3.1.1 Investigation of roles of components in color development reagent

Each component and mixture of color reagent (S+M, S+M+T, S+M+T+C) had maximum absorbance (peak) at 300 nm (Fig. 3a) while reaction mixture (molybdenum blue phosphorus) had 2 peaks at 720 nm and 890 nm (maximum) in Fig. 3b. Therefore, color reagent did not cause signals at wavelength ranges of the phosphorus complex. To obtain the highest sensitivity, 890 nm was chosen as the optimal wavelength for all next assays. This wavelength is within the range of 650-850 nm or 700-900 nm, in which Mo (VI) complex cause the strong intensity ($\varepsilon \approx$ 26000–34000 L/mol/cm) [16,17].

The molybdenum blue phosphorus formation depends on pH of environment, Fig. 4 shows signals were the lowest in basic environment, higher in neutral and the highest in acid environment. Therefore, acid environment is the best for complex formation and the amount of H_2SO_4 should be optimised. The amount of hydroxyl ions has the close relationship with the amount of molybdenum ions via the ratio $[H^+]/[Mo]$. When [H]/[Mo] ratios were under 50, the absorbance was high but not stable. When [H]/[Mo] ratios were in the range of 50 ad 80, the absorbance was relatively stable. Finally, when [H]/[Mo] ratios were higher than 120, the absorbance had the decreasing trend possibly due to incomplete reaction. Therefore, the optimal [H]/[Mo] ratios should be in the range of 50 and 80, particularly 70, and optimal pH value was 0.76 ([H]=0.3N) for forming stable complex.

In Fig. 5a, potassium antimonyl tartrate had the maximum absorbance at 300 nm, thus, it did not compete absorbing with phosphorus molybdate complex at 890 nm. If potassium antimonyl tartrate was not present in the reaction mixture (line blue in Fig. 5b), very low absorbance signals were produced, maximum of nearly 0.2 abs at the peak of 830 nm. This may be because phosphorus molybdate complex almost did not form or the formed complex changed the structure. When antimonyl tartrate was too low ($1.64–3.28\times10^{-5}$ M), the process of color development was slow, leading to low signals. Particularly, concentrations of antimonyl tartrate increased from 1.64×10^{-5} M to 3.28×10^{-4} M, the corresponding absorbance of reaction mixture increased from 0.210 to 0.2 abs at 0.4 mg/L phosphorus, 0.732 to 0.695 abs at 1.0 mg/L phosphorus. This highlights, amount of antimonyl tartrate was not enough to speed up reaction. When the amount of antimony tartrate was



a) b)

Fig. 3. Absorption spectrum of color reagent (a), molybdenum blue phosphorus (b)

Table 1. Effects of $[H^+]/[Mo]$ ratio at 0.4 mg/L phosphorus

$[H^+]/[Mo]$		20	40	60	80	100	120
$V_{acid\ sulfuric}$	V_{Mo}	1.79	0.89	0.60	0.45	0.36	0.30
0.5 mL	A	2.062	0.439	0.413	0.411	0.386	0.322
$V_{acid\ sulfuric}$	V_{Mo}	3.57	1.79	1.19	0.81	0.71	0.60
1 mL	A	1.994	0.869	0.414	0.412	0.391	0.342
$V_{acid\ sulfuric}$	V_{Mo}	5.36	2.68	1.79	1.34	1.07	0.89
1.5 mL	A	1.991	0.558	0.416	0.414	0.386	0.358
$V_{acid\ sulfuric}$	V_{Mo}	7.14	3.75	2.38	1.79	1.43	1.19
2 mL	A	1.889	0.531	0.413	0.412	0.378	0.341
$V_{acid\ sulfuric}$	V_{Mo}	10.71	5.36	3.57	2.68	2.14	1.79
3 mL	A	1.546	0.678	0.417	0.412	0.368	0.327
$V_{acid\ sulfuric}$	V_{Mo}	14.29	7.14	2.76	3.57	2.86	2.38
4 mL	A	1.202	0.446	0.420	0.416	0.387	0.348

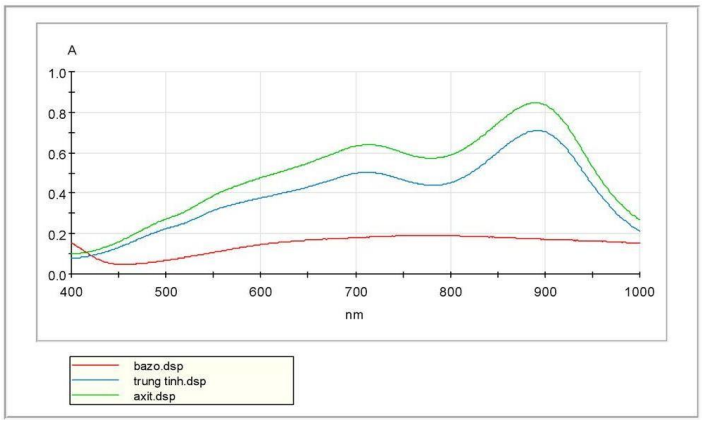
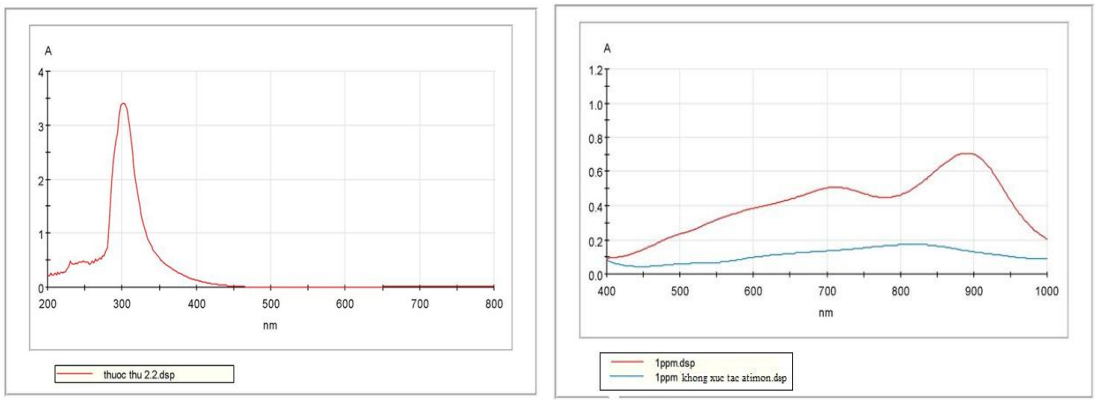


Fig. 4. The role of base (red line), neutral (blue line) and acid (green line) environment in formation of phosphorus molybdate complex



a) **b)**

Fig. 5. a) Absorbance spectrum of only potassium antimonyl tartrate, b) The role of potassium antimonyl tartrate hemihydrate in complex formation

adequate (8.2×10^{-5} M), formation of phosphorus molybdate complex was complete to produce a highly stable phosphorus molybdate complex. Then, if higher amounts of antimony tartrate (more than 2.46×10^{-4} M) were added, color development was quicker; However, signals

stayed unchanged or gradually decreased corresponding to increase in amount of tartrate. Tartrate was also considered to interfere partially the reaction of arsenate or silicate with ammonium molybdate [9], thereby, reducing the effect of these ions. In conclusion, antimonyl tartrate with the optimal amount of 0.082 mM has the role of catalyst, speeds up color development and participate in forming a stable phosphorus molybdate complex.

If increasing amount of ammonium molybdate in the range of low concentrations (Fig. 6), beginning at 1.042×10^{-3} M (0.5 mL) to 3.126×10^{-3} M (1.5 mL), signals were low and increased significantly. When level of amoni molybdate reach to 4.168×10^{-3}M (2 mL), signals were stable despite increase in molybdate amount. This shows that, molybdate concentrations were under 4.168×10^{-3} M (V<2mL) were not enough for a complete reaction. Therefore, 4.168×10^{-3} M ammonium molybdate (equivalent 2.0 mL in 50 mL of mixture reaction) was chosen as optimum.

The similar assay was for ascorbic acid investigation. As a result, ascorbic acid was too low (5.6×10^{-4} M) to cause reduction reaction, leading phosphorus molybdate complex not to be formed. Signals increased followed by the increase in ascorbic amount, until 3.36×10^{-3} M (3 mL) at which signals were relatively stable. This highlights, the residual ascorbic acid did not affect considerably on the complex formation. Therefore, 4.48×10^{-3} M (4 mL) of ascorbic acid was chosen to be adequate to cause yellow molybdate complex to blue molybdate complex.

The colorless solution changed significantly into blue solution within 5 minutes. In Fig. 7, before the thirtieth minute, differences in signals were acceptable, around <5%. After 30 minutes, the absorbance decreased noticeably with high difference percents (>20%). As phosphorus molybdate complex was very stable by the time within 30 minutes, the optimal color development time was within 15 minutes to obtain high sensitivity.

3.1.2 Investigation of interferences in molybdenum blue phosphorus method

Silicate ions have similar reaction with ammonium molybdate to produce $H_4[SiMo_{12}O_{40}]$ or $H_4[Si(Mo_3O_{10})_4]$ in the same environment. A study used molybdenum blue method to determine silica in rice samples at 815 nm [18]. Therefore, silicate ions might affect phosphorus detection at 890 nm. Indeed, only silicate ions gave absorbance spectrum in the UV range (Fig. 8a); however, if silicate ions were present in mixture reaction (Fig. 8b), they competed absorption with phosphorus, and made changed in maximum wavelength (820 nm instead of 890 nm). Studying at two levels 0.4 and 1.0 mgP/L, signals increased gradually following by the increase in silicate concentrations. Low SiO_3^{2-} concentrations (less than 5 mg/L), the difference percentage was small (around 5%), particularly at 1.0 mgP/L, 0.15%; 2.99% and 3.89% for 0.1; 1-3 and 5 mgSi/L, respectively. However, signals increased suddenly and significantly when SiO_3^{2-} concentrations were higher than 10 mg/L (at 0.1 mgP/L, 16.13%; 27.42% and 48.39% for 10; 15

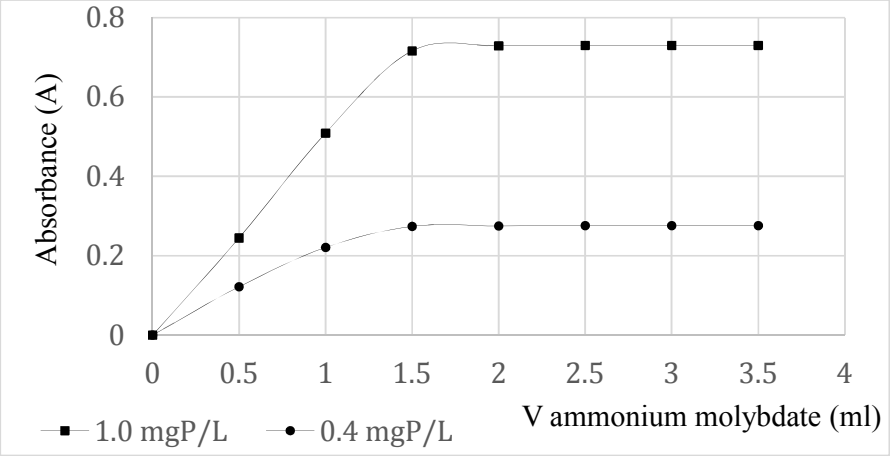


Fig. 6. The effect of ammonium molybdate tetrahydrate in complex formation

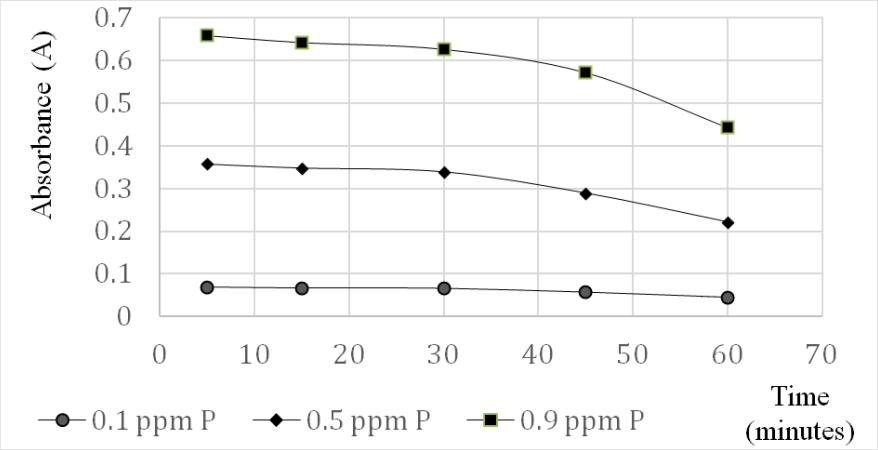
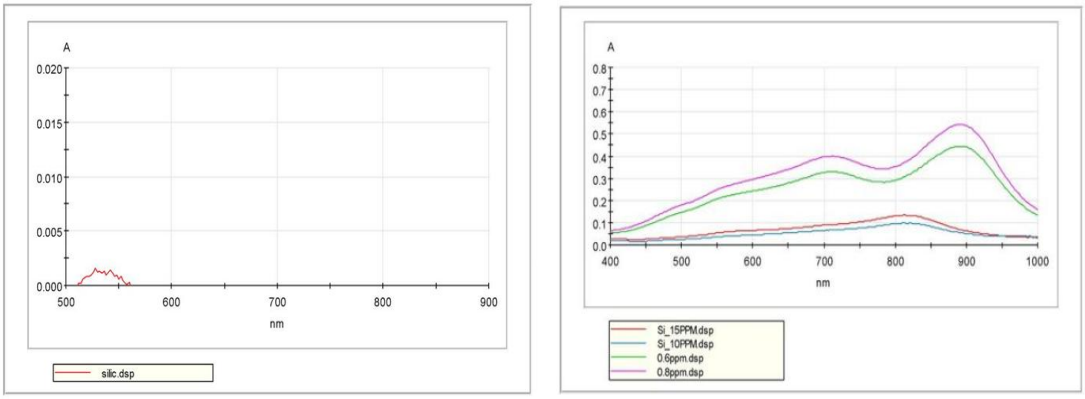


Fig. 7. The stability of phosphorus molybdate complex



a) **b)**

Fig. 8. a) Absorbance spectrum of silicate ions, b) The effect of silicate ions in complex formation

and 20 mgSi/L). Therefore, the influence threshold of silicate ions was 5-10 mg/L to allow the difference of under 20%.

Although effects of nitrite (Fig. 9) and hexavalent chromium (Fig. 10) ions could not be seen clearly due to no overlapped spectrum, their effects were obserbved by measuring the absorbance. Only nitrite or chromium ions had the maximum absorbance at 700 nm and 780 nm, respectively; However, the molybdate complex caused the maximum intensity at 814 nm in the presence of nitrite ions [19]. Nitrite and hexavalent chromium ions are oxidising agents, thus, they can react with ascorbic acid and/or oxidise blue phosphorus molybdenum complex, leading to negative error. Both of these ions made signals of phosphorus molybdate complex decrease; however, at the same concentrations, the action of nitrite was less than hexavalent chromium, this could be explained by the stronger oxidising property of hexavalent chromium for phosphorus molybdate complex. For example (Fig. 11), hexavalent chromium interfered about 2-4% low at 1 mgCr^{6+}/L and 44% low at 5 mgCr^{6+}/L, while nitrite interfered about 4% low at 1 mgNO_2^-/L and 10% low at 5 mgNO_2^-/L ppm. In conclusion, the influence threshold of nitrite ions was 5 mg/L to allow the acceptable difference of under 10%, while the threshold of hexavalent chromium ions was 3 mg/L to allow the acceptable difference of within 10-15%.

3.1.3 Statistics for validation of molybdenum blue phosphorus method

The standard curve was investigated over the phosphorus concentration range of 0 and 3

mgP/L to find out the linear range followed by Beer–Lambert law. When the phosphorus amount was too low (0.01 mg/L), the color development was too slow under the help of strong vibration (until 20 minutes to apprear color solution). From the Table 2 showing regression between phosphorus concentration and the number of sample (N), regression equation, A=0.7455C+0.0197 (N=9, R^2=0.999) was calculated in the range of 0.02 to 1.0 mgP/L.

The error of regression coefficients S(a) and S(b), the confidence intervals of the regression coefficients U(a) and U(b) were calculated as below:

$S^2_{model} = 0.069805$; $S^2_{residue} = 8.3669 \times 10^{-5}$; $S^2_{total} = 0.069878$

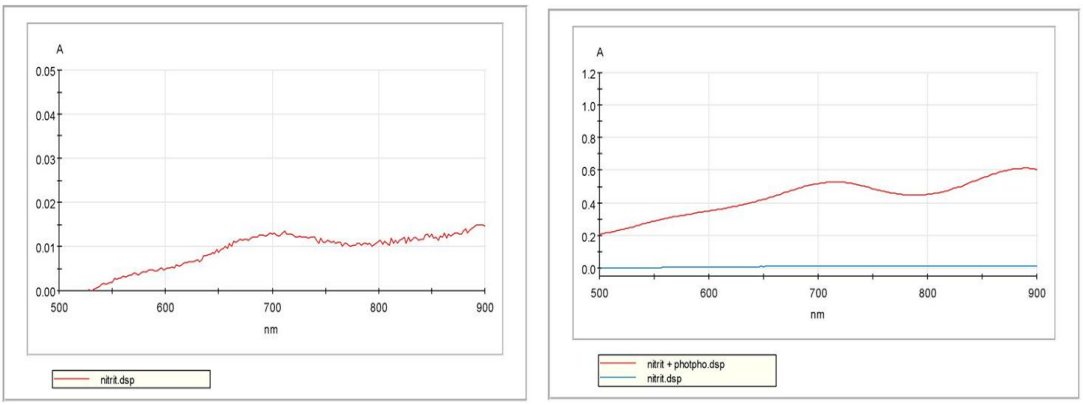
$S(a) = 8.3469 \times 10^{-5}$; $S(b) = 3.8234 \times 10^{-5}$

$U(a) = \pm t_{0.95;7}.S(a) = 0.000196$

$U(b) = \pm t_{0.95;7}.S(b) = 9.023 \times 10^{-5}$

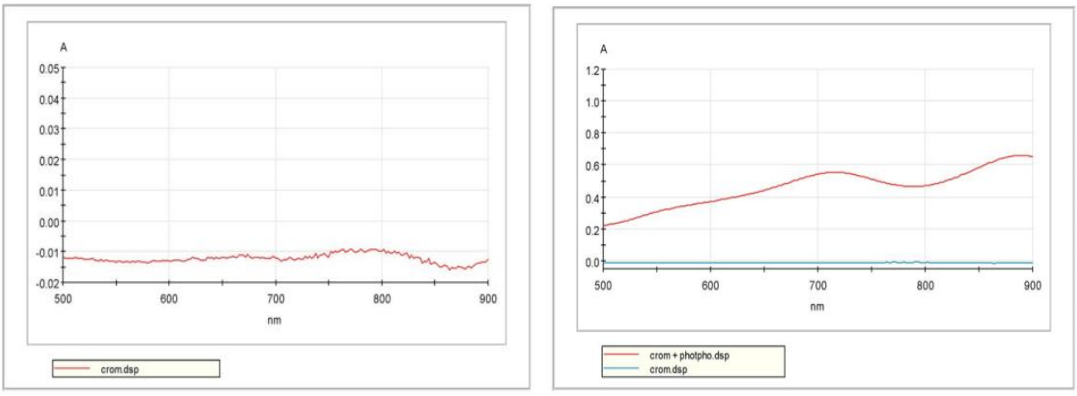
Table 2. Regression between P concentration and number of samples

Concentration range (mgP/L)	N	R^2
0.01 – 3.00	33	0.9877
0.01 – 2.40	30	0.9963
0.01 – 2.00	28	0.9992
0.05 – 3.00	31	0.9866
0.05 – 2.40	28	0.997
0.05 – 2.00	26	0.9991



a) b)

Fig. 9. a) Absorbance spectrum of nitrite ions, b) The effect of nitrite ions in complex formation



a) b)

Fig. 10. a) Absorbance spectrum of hexavalent chromium ions, b) The effect of hexavalent chromium ions in complex formation

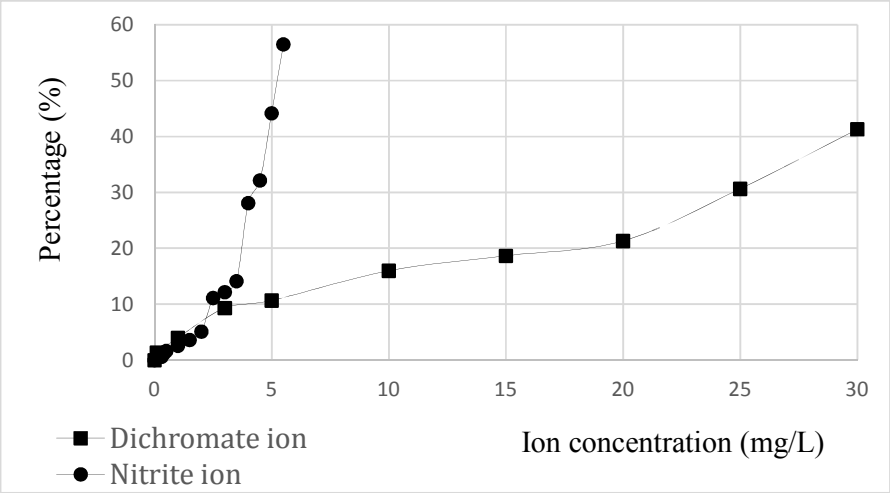


Fig. 11. Effect level of nitrite and hexavalent chromium on phosphorus molybdate complex

The suitability of regression equation was assessed via regression efficiency R.

$$F_{cal} = \frac{S^2_{model}}{S^2_{residue}} = \frac{0.0698047}{8.3669\times10^{-5}} = 834.29 \quad > \quad F_{theory} = F_{0.95,8,7} = 3.73 \text{ (Fisher standard)}$$

As F_{cal} was higher than F_{theory} and $R^2 = \frac{S^2_{model}}{S^2_{total}} \times 100\% = \frac{0.069805}{0.069878} \times 100\% = 99.89\% \rightarrow 100\%$, there was a strong compatibility between regression equation with the practice.

Limit of detection (LOD) and limit of quantification (LOQ) of this method were determined from absorbance average ($\bar{A}$) and standard deviation (SD) from triplicate measurements of 30 blank samples containing only 10 mL color developing reagent, without phosphorus.

$$LOD = \frac{3.SD}{a} = \frac{3\times0.0011}{0.7455} = 0.0045 \text{ mgP/L}$$

$$LOQ = \frac{10.SD}{a} = \frac{10\times0.0011}{0.7455} = 0.061 \text{ mgP/L}$$

Finally, the method accuracy was verified by recovery yield (%Y_R). 5 mg/L of phosphorus solution was diluted to 0.4 mg/L (C_o). Absrobance of triplicate was recorded to calculate quantified concentration (C_Q). As a result, the average of C_Q was 0.389 and Y_R value was 97.46 ± 1.27 (%).

Molybdenum blue complex was prepared with 4 phosphorus levels (0.1, 0.5, 0.9 and 1.5 mgP/L) and the abosrbance was measured. Each phosphous level was prepared repeatably 5 times. Solution containing 0.1 mgP/L gave 0.072, 0.073, 0.071, 0.072, 0.075 absorbance. Solution with 0.5 mgP/L produced 0.371, 0.369, 0.370, 0.369, 0.372 absorbance. Solution containing 0.9 mgP/L produced 0.674, 0.675, 0.673, 0.675, 0.678 absorbance. Finally, complex of 1.5 mgP/L gave 1.110, 1.108, 1.109, 1.110, 1.110 absorbance. This data is evaluated with χ-squared standard. The hypothesis H_o is "variances are unchanged if changing concentrations".

The size (n_j) is 5; thus the degree of freedom (f_j) is 4. The dgree (k) is 4 because the experiment was designed at 4 phosphorus levels: 0.1, 0.5, 0.9 and 1.5 mgP/L. The total size (N) is 20 because 4 phosphorus levels were measured repeatably 5 times). The value f_{th} is 16 by subtracting k from N.

$S^2_{th} = \frac{1}{f_{th}} \times \sum_{j=1}^{k} f_j S^2_j = \frac{1}{16} \times 22.38\times10^{-3} = 1.40\times10^{-3}$

$B = 2.303(f_{th} lg S^2_{th} - \sum_{j=1}^{k} f_j lg S^2_j) = 2.303\times(16\times\lg(1.40\times10^{-3}) - (-45.912)) = 0.576$

$C = 1 + \frac{1}{(3k-3)} \times \left[\left(\sum_{j=1}^{k} \frac{1}{f_j}\right) - \frac{1}{f_{th}}\right] = 1 + \frac{1}{(3\times4-3)} \times \left[\left(\sum_{j=1}^{k} \frac{1}{4}\right) - \frac{1}{16}\right] = 1.104$

$\chi^2_{TN} = \frac{B}{C} = \frac{0.576}{1.104} = 0.522 < \chi^2_{LT} = \chi^2_{P=0.95,f=k-1=3} = 0.7815$

As χ^2_{TN} was lower than χ^2_{LT}, the hypothesis is accepted. It could be concluded that molybdenum blue phosphorus method has high stability, in the meanwhile, this method does not depend on concentrations. Finally, the sensitivy of method is investigated via ANOVA analysis. Table 3 describes the absorbance of each phosphorus concentration measured 5 times everyday.

ANOVA analysis (Table 4) shows effects of concentrations and days carrying out the assays on absorbance produced by molybdenum blue phosphorus. For factor "concentration", F_C = 26779.16 is higher than $F_{LT} = F_{(0.95,3,54)}$ = 2.78, and P – value reach 0.0000, thus, this factor does not cause effects on signals. In contratst, for factor "day", F_D = 2.43 is lower than $F_{LT} = F_{(0.95,2,54)}$ = 3.16, and P – value was 0.0972, thus, this factor does cause effects on signals at the confidence level of 90.28%.

To sum up, combination χ-squared standard with ANOVA can conclude that molybdenum blue phosphorus method is stable and very sensitive. The method can detect and distinguish low changes in concentration, detect very low amount of phosphorus in samples, up to 0.0045 mgP/L. In contrast, although one concentration value is measured at different days, the absorbance is not much different if measuring at the same experiment conditions.

3.2 Phosphorus Value for Soil Quality Assessment

There are several methods to transfer phosphorus from the soil into the water phase in the form of soluble orthophotphat. In soil samples taken from Phan Thiet, the total phosphorus concentration was 1.112 ± 0.022, converted into 0.0319 ± 0.0015 (% P_2O_5) with the recovery of yield of 90.05%. This result shows that combination of strong acids particularly sulfuric acid with strong oxidants such as ammonium persulfate results in high recovery efficiency in total phosphorus extraction. In addition, Olsen and Oniani phosphorus concentrations were 0.257 ± 0.003 and 0.287 ± 0.006 mgP/L, converted into 6.3542 ± 0.1173 (94.20%) and 7.0433 ± 0.1762 mg P_2O_5 / 100g soil (94.05%), respectively. The extracts before phosphorus analysis were tested. Olsen extract had only 0.0073 mg/L nitrite, almost no presence of aluminum and ferrous ions, while Oniani extract had 0.0174 mg/L nitrite, and aluminum and ferrous ions. Compared with Vietnamese standard, this soil sample with pH H_2O of 6.14 and pH KCl of 5.24 was classified as coastal sandy soil [20]. With the total phosphorus in the

Table 3. Relationship between absorbance, concentrations and days of experiment

	0.1 mgP/L	0.5 mgP/L	0.9 mgP/L	1.5 mgP/L
Day 1	0.069	0.361	0.639	1.089
	0.068	0.361	0.641	1.088
	0.069	0.358	0.641	1.089
	0.067	0.359	0.636	1.087
	0.068	0.358	0.639	1.089
Day 2	0.069	0.355	0.685	1.083
	0.068	0.358	0.687	1.082
	0.059	0.357	0.645	1.083
	0.066	0.356	0.685	1.084
	0.057	0.358	0.686	1.083
Day 3	0.061	0.361	0.651	1.085
	0.062	0.369	0.644	1.083
	0.064	0.353	0.645	1.084
	0.066	0.370	0.642	1.085
	0.066	0.361	0.643	1.083

Table 4. Two-way ANOVA analysis

Factors	Sum of squares	Df	Mean square	F - ratio	P - value
Concentration	8.51696	3	2.83899	26779.16	0.0000
Day	0.0000516133	2	0.000258067	2.43	0.0972
Residue	0.0057248	54	0.000106015		
Total (corrected)	8.5232	59			

Table 5. Description of soil samples

Marker		Sampling location		Description of samples (Fig. 12)
TG	TG1	Tien Giang province, in the garden, surrounding	On the surface	The soil had ligh brown color. The frequency of watering was low, the tree
	TG2	young papaya tree. The underground was connected with a nearby	At the depth of 0.5m	used from regular rain and underground water. fertiliser had been added 3 years before sampling. There were leaves, no
	TG3	small branch of Tien River	At the depth of 1.0m	roots in the soil. The soil clotted into particles in which some large particles were hard and difficult to disolve in water
PT		Phan Thiet city, along the coast		The soil had ligh brown color, very fine and small particles with sand particles
TN		Tay Ninh province, in the garden, on the surface		No trees were grown on the soil. The soil clotted into small, soft, porous particles

a) b) c)

Fig. 12. Images of soil samples a) Tien Giang (TG), Phan Thiet (PT), Tay Ninh (TN)

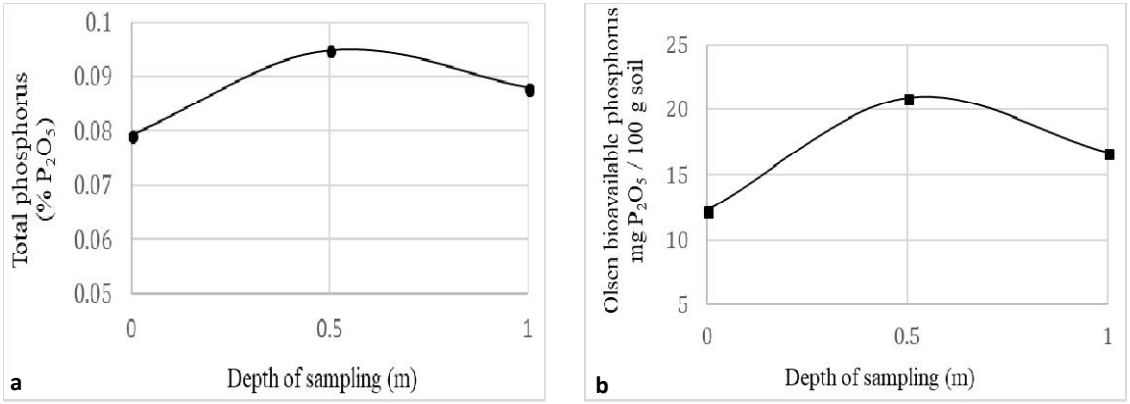


Fig. 13. The effect of sampling location on a) total phosphorus, b) bioavailable phosphorus concentrations

range of 0.01% and 0.05% and its color at the sampling location, this soil was classified into total phosphorus poor soils [21,22]. According to Kiecxanop's classificatin [23], soil samples taken from Phan Thiet had Olsen and Oniani phosphorus in the range of 3 to 8 mg P_2O_5 / 100g soil, thus, they were considered to have an average amount of bioavailable phosphorus.

In soil samples taken from Tay Ninh, the total phosphorus concentration was 1.254 ± 0.005, converted into 0.0357 ± 0.0007 (% P_2O_5) with the

recovery of yield of 89.58%. Furthermore, Olsen and Oniani phosphorus concentrations were 0.411 ± 0.024 and 0.584 ± 0.0017 mgP/L, converted into 9.8891 ± 1.3014 (93.88%) and 13.5971 ± 0.9434 mg P_2O_5 / 100 g soil (93.82%), respectively. Olsen extract contained only 0.066 mg/L nitrite, almost no aluminum and ferrous ions, whereas Oniani extract had 0.208 mg/L nitrite, and ferrous ions. This shows that, using $NaHCO_3$ as the extracting agent in Olsen method, metal cations such as Fe^{3+} and silicate ions could be removed under precipitation and filtration in alkaline environment of carbonate buffer [13]. In addition, Olsen method could be used in basic, neutral or acid soils [13,14]. This soil sample with pH H_2O of 5.11 and pH KCl of 4.31 was classified as ferralsols. With total phosphorus in the range of 0.01% and 0.05% and its color at the sampling location, this soil was classified into total phosphorus poor ferralsols. According to Kiecxanop, samples taken from Tay Ninh contained Olsen and Oniani phosphorus in the range of 8 to 15 mg P_2O_5 / 100 g soil, thus, they were considered to be relatively rich in bioavailable phosphorus.

For soil samples taken from Tien Giang, pH H_2O was 6.95 and pH KCl of 5.88, thus, these soil samples were classified as alluvium soils. With total phosphorus in the range of 0.05% and 0.10% and its color at the sampling location, this soil was classified into soils with average amount of total phosphorus. According to Kiecxanop, soil samples taken from Tien Giang contained very high Olsen phosphorus, more than 15 mg P_2O_5 / 100 g soil, thus, they were considered to be very rich in bioavailable phosphorus. This could be because of their locations where the soils were fertilise with lots of alluvium from the river nearby.

Phosphorus is a nutrient element very important for crops, especially legumes. The soil sample taken at the depth of 0.5 m (TG2) with the beginning Olsen phosphorus of 20.0592 mg P_2O_5 / 100g was also used to grow black bean plants. Fertiliser was not used during the plant proliferation. Olsen phosphorus analysis shows the decrease in bioavailable phosphorus in soil during the growth of plants. Particularly, after one week, many green leaves appeared and phosphorus was 18.4381; after two weeks, the plants changed leaves and phosphorus was 17.2794; since the third week, beans were produced and P amount was only 15.2396 to 13.9175 mg P_2O_5 / 100g. As we can see, each stage of development had different demand of phosphorus dose. The amount of bioavailable phosphorus reduced remarkably in the period of bean production. Phosphorus could make trunk rigider, foliage and green leaves healthier, grains and beans larger and increase pest resistance. Thus, black beans are sorted into phosphorus rich food which needs to be studied further.

4. CONCLUSION

UV-VIS spectrophotometry with phosphomolybdenum - ascorbic acid was optimised and validated for determination of total and bioavailable phosphorus in this study. A stable molybdenum blue phosphorus complex was formed with the maximum absorption at 890 nm under these following conditions: acid environment with pH from 0.76 to 1.2 equivalent to final hydroxyl concentration of 0.3 N, the optimal [H]/[Mo] ratios in the range of 50 and 80, the optimal final ammonium molybdate concentration of 4.168×10^{-3} M. Antimonyl tartrate was an indispensable component in the color development reagent with the optimal amount of 0.082 mM to speed up complex formation, color development and hide interferences such as arsenate, silicate and heavy metal ions. The residues of ascorbic acid did not cause significant effect, with optimal amount of 4.48×10^{-3} M to reduce quickly yellow molybdate complex to blue molybdate complex stable within 20 minutes. The absorbance should be recorded within 15 minutes to obtain the high accuracy within the range of 0.02 to 1.0 mgP/L. With its high sensitivity, the method could determine the lowest amount of phosphorus, up to 0.061 mgP/L (LOQ) and 0.0045 mgP/L (LOD), and detect very low changes in concentration of phosphorus.

Silicate ions increased signals 20% at 15 mgSiO_3^{2-}/mL, in contrast, nitrite and hexavalent chromium ions caused negative error of 10%. The samples were ideal if silicate, nitrite and dichromate ions were lower than 5, 5 and 3 mg/L, respectively. Although Oniani and Olsen methods gave the similar bioavailable phosphorus amount and reliable results with high recovery efficiency (>90%), Olsen method was better because the extract contained low nitrite and heavy metal ions. Although arsenate gave the same reaction with phosphorus, it was not abundant in normal soils. Thus, this procedure should be further optimised to find out the influence threshold if quantifying phosphorus in arsenate polluted areas.

ACKNOWLEDGEMENT

The authors would like to express their profound gratitude to Dr. Vu Dinh Le for his guidance about statistic analysis, and MSc. Tai Van Ho for giving valuable information at Faculty of Chemical Engineering, Industrial University of Ho Chi Minh City, Vietnam.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25024