ESTIMATION OF MEASUREMENT UNCERTAINTY FOR PHOSPHORUS SPECTROPHOTOMETRIC DETERMINATION IN ORGANO-MINERAL FERTILIZERS

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Abstract

This study evaluates the measurement uncertainty of total phosphorus determinations in organo-mineral ferilizers. Total phosphorus was determined by UV-VIS spectrophotometry using an in-house ammonium molybdate and ammonium metavanadate method. The main parameters controlling the result of the analysis were identified and combined uncertainty (Uc) was calculated. The expanded uncertainty (U) of the method, obtained by multiplying the combined uncertainty by the coverage factor k = 2 (confidence level 95%) was 6.43%. It was noticed, that the main sources of uncertainty were: type A (i.e. repeatability) and type B (i.e. uncertainties related to the analytical balance, volumetric glassware, stock solution and spectrophotometer).

Key words: measurement uncertainty, phosphorus, spectrophotometry, organo-mineral ferilizers.

INTRODUCTION

As the development of agricultural productivity is directly related to the use of fertilizers, it is necessary to analyze them with sensitive techniques in order to monitor their quality. Common fertilizers are either of inorganic or organic composition, of biological or chemical nature (Cox et al., 2003; Viso & Zachariadis, 2018).

Most common mineral fertilizers contain three primary macronutrients that play an important role in plant development: nitrogen (N), phosphorus (P), and potassium (K). Fertilizers may also contain lower levels of the three secondary nutrients, calcium (Ca), sulfur (S), magnesium (Mg), as well as micronutrients, such as: boron (B), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), and molybdenum (Mo) (Pelizzaro et al., 2019). In addition, organo-mineral fertilizers contain materials of biological origin (i.e. animal wastes, agricultural/crop residues, compost, biosolids etc.). Therefore, organo-mineral fertilizers are defined as mixtures of organic and mineral fractions that can be produced in several N, P and K proportions suitable for crop requirements. The use of organic waste in agriculture represents an economical and environmental viable practice mainly because it allows the recovery of several chemical elements, such as N, P, K and microelements (Crusciol et al., 2020).

The quantitative determination of these elements in fertilizers is important for product quality control and regulatory requirements. Fertilizers that are produced or sold within the European Union (EU) must comply with the concentration limits specified in Regulation EC 2003/2003 related to mineral products. In addition, many EU member states have detailed, national rules and standards in place for non-harmonized fertilizers that do not apply to EC-fertilizers. Italy, for example, has regulations that relate to organic fertilizers. In the US, the Environmental Protection Agency sets standards and regulations for some types of fertilizers but States can adopt regulations that are more stringent and/or broader than the Federal regulations. In China, fertilizers are subject to approval by the Ministry of Agriculture in compliance with the Food Safety Law (*Regulation (EC) No. 2003/2003*, 2003; Pelizzaro et al., 2019).

Given the impact of chemical analysis and quality control of fertilizers on agricultural

production, several techniques have been reported in the literature for the determination of these elements in fertilizers (Viso & Zachariadis, 2018; Pelizzaro et al., 2019). Analysis of extracted P in fertilizers is typically performed using (i) gravimetric; (ii) volumetric; and (iii) colorimetric testing methods. More recently, inductively coupled plasma atomic emission spectroscopy (ICP-AES) has been used for the analysis (Viso & Zachariadis, 2018; Pelizzaro et al., 2019). For total phosphorus estimation, the gravimetric quinoline phosphomolybdate method is generally preferred because of the minimal interference of other ions and its accuracy and simplicity (Pelizzaro et al., 2019). Another common method providing acceptable accuracy and simplicity is the spectrophotometric vanadate-molybdate assay (Motsara & Roy 2008).

All measurements are affected by a certain error. The measurement uncertainty gives information on the size of the measurement error. Therefore, the measurement uncertainty is an important part of the reported result (Magnusson et al., 2017).

Many important decisions are based on the results of chemical quantitative analysis; the results are used, for example, to estimate yields, to check materials against specifications or legal (allowable) limits, or to estimate monetary value (Magnusson et al., 2017). Whenever decisions are based on analytical results, it is important to have an indication of the quality of the results. One useful measure used to demonstrate the quality of the results is measurement uncertainty (*Eurachem*, 2012; Magnusson et al., 2017). The data user needs it together with the result to make a correct decision. Also, the laboratory needs it to verify its´ own quality of measurement given that estimation of the measurement uncertainty is required by ISO 17025 (Magnusson et al., 2017; Romanian Standards Association, 2018).

According to the Eurachem/CITAC Guide (2012) the measurement uncertainty is a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand. There are general rules for evaluating and expressing uncertainty for a wide range of measurements (Barwick &

Ellison, 2000; *Eurachem*, 2012). The international guidelines require the identification of all possible sources of uncertainty associated with the procedure; the estimation of their magnitude from either experimental or published data (quality control charts, validation, proficiency testing, certified reference materials etc.); and the combination of these individual uncertainties to give standard and expanded uncertainties of the procedure (Cox et al., 2003; Barwick & Ellison, 2000; Vetter, 2001; *Eurachem,* 2012; Ionescu et al., 2014; Magnusson et al., 2017; Romanian Standards Association, 2018).

This paper is focused on estimation the measurement uncertainty related to total phosphorus determination by UV-VIS spectrophotometry in organo-mineral fertilizer samples.

MATERIALS AND METHODS

Reagents and solutions

For the preparation of the working standard solutions and acid digestion of fertilizer samples Certipur Phosphorus 1000 mg/L standard, NH_4VO_3, $(NH_4)_6Mo_7O_{24}*4H_2O$, HNO_3 (65%), HCl (37%), and H_2SO_4 (95-97%) of analytical grade were obtained from Merck (Germany). All solutions were prepared using distilled water.

Ammonium molybdate solution (5%) was prepared by dissolving 50 g of $(NH_4)_6Mo_7O_{24}*4H_2O$ in 500 mL distilled water. The final volume of the solution was adjusted to 1000 mL. Ammonium vanadate solution (0.25%) was obtained by dissolving 2.5 g of NH_4VO_3 in 500 mL distilled water and adding 20 ml concentrated nitric acid. Finally, the solution was diluted to 1000 mL (Romanian Standards Association, 1998).

An interlaboratory comparisons organo-mineral sample (ILC) obtained from Bipea (France) was used as secondary/external reference material in the experimental trials.

All weighing operations were carried out using METTLER TOLEDO AG204 analytical balance. Acid digestion was performed by using a simple heated sand bath. The absorbance of the sample solutions was measured with a UV-VIS spectrophotometer (CECIL Instruments).

Preparation of Working Standard Solutions
Working standard solutions containing 0.00, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05 mg/mL were prepared by proper dilutions of the 1000 mg/L phosphorus standard (5 mL hydrocloric acid was added to each standard).

The calibration curve was prepared by pipetting 10 mL of the above mentioned standard solutions in 25 mL Erlenmayer flasks and addition of 2 mL of each colorimetric reagent (5% molibdate solution and 0.25% vanadate solution). These solutions contain 0.00, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg of phosphorus.

After 30 min, the color development is complete and the absorbance can be measured at 470 nm.

Method description
The measuring principle is based on the photometric yellow method (molybdate-vanadate), which has been used for years to measure orthophosphate in mineral fertilizers (Romanian Standards Association, 1998).
The flow sheet for total phosphorus extraction and determination in organo-mineral fertilizers is illustrated in Figure 1.
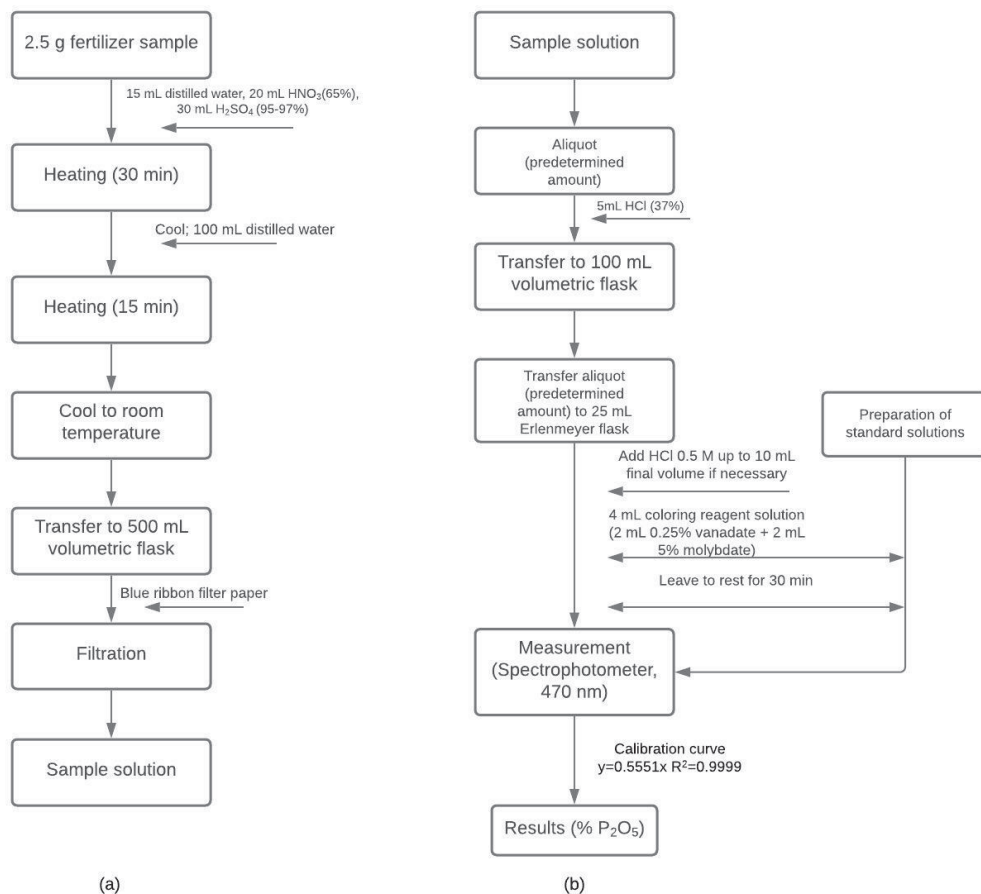

(a)   (b)

Figure 1. Flow sheet for total phosphorus extraction (a) and determination (b) in organo-mineral fertilizers

RESULTS AND DISCUSSIONS

The measurement uncertainty was calculated by following an internal procedure and international agreed approaches (*Eurachem*, 2012). The evaluation of measurement uncertainty of total phosphorus determination consisted the following steps: specification of the measurand, identification of uncertainty sources, quantification of uncertainty components and calculation of combined and expanded uncertainty.

Specification of the measurand

$$\text{Phosphorus }(\%\ P_2O_5) = \frac{c \times r_1 \times r_2 \times 100}{m \times 1000} \qquad (1)$$

where:

c - concentration of phosphorus corresponding to the absorbance read on the calibration curve (mg);

r_1 - the ratio between the initial volume (v1) of the sample solution and aliquot (a1);

r_2 - the ratio between the volume (v2) at which the aliquot 1 (a1) was transferred and the volume used for colorimetry (a2);

m - mass of the sample (g).

Identification of uncertainty sources

Figure 2 illustrates the cause and effect diagram for the standard uncertainty of the method.
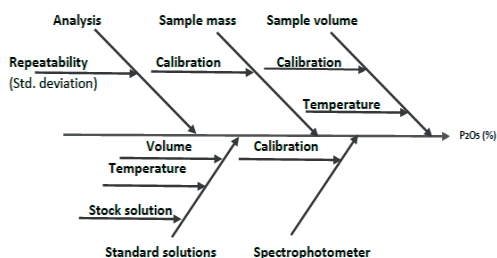

Figure 2. Cause and effect diagram for total phosphorus determination

As schematized in Figure 2, the following sources of were identified: (a) uncertainty from the standard and standard dilution to obtain the working calibration solutions; (b) uncertainty from the spectrophotometer; (c) the dilution factor includes the contributions of uncertainties from the volumetric flasks and pipettes and the volume expansion at the temperature of the laboratory; (d) uncertainty of the sample mass / analytical balance (obtained from the calibration certificate); (e) repeatability, evaluated by measuring 10 sample replicates of the analyte. The measurements were conducted by the same analyst using the same UV-VIS instrument under the same working conditions.

Quantification of individual components
Repeatability

The characteristics of the ILC sample used as secondary reference material in the experiments are given in Table 1.

Table 1. Characteristics of the ILC sample used as secondary reference material

Statistical parameter	% total phosphorus
Assigned value, x_{pt}	3.39
Standard uncertainty of the assigned value, $u(x_{pt})$	0.06
Robust standard deviation of the results, $s(x_{pt})$	0.30
Tolerance value, VT	0.80
Maximum value	4.19
Minimum value	2.59

All steps of the measurement procedure (Figure 1) were included in the uncertainty associated with the repeatability of the overall experiment. Repeatability data is given in Table 2.

Table 2. Repeatability of the spectrophotometric method for determination of phosphorus in ILC sample

Sample	% total phosphorus
Replicate 1	3.54
Replicate 2	3.60
Replicate 3	3.64
Replicate 4	3.54
Replicate 5	3.64
Replicate 6	3.40
Replicate 7	3.54
Replicate 8	3.60
Replicate 9	3.64
Replicate 10	3.35
Statistical parameter	% phosphorus
Average value	3.55
Standard deviation, n = 10	0.11

Sample mass, dilution factor and spectrophotometer contribution

The uncertainty related to the calibration of all equipment was evaluated using the data from the calibration certificates. The standard uncertainty associated with the mass of the sample was calculated (Eq. 2), using the data from the calibration certificate (i.e. linearity and eccentricity) (De Oliveira, 2016). This contribution was counted twice, once for the tare and once for the gross weight, because each is an independent observation and the linearity effects are not correlated (*Eurachem*, 2012). Standard uncertainties were calculated according to the following equations:

$$u(m) = \sqrt{\left(\frac{U_{eccentricity}}{2}\right)^2 + \left(\frac{U_{linearity}}{2}\right)^2} \qquad (2)$$

$$u(v) = \sqrt{\left(\frac{U_{calibration}}{2}\right)^2 + \left(\frac{V_{temp}}{\sqrt{3}}\right)^2} \qquad (3)$$

$$u(x) = \frac{U_{calibration}}{2} \qquad (4)$$

For all volumetric equipment the uncertainty consists of 2 components: calibration uncertainty and uncertainty due to the volume expansion at laboratory temperature (20 ± 4ºC), calculated by means of Eq. 5 (Drolc & Ros, 2002; *Eurachem*, 2012):

$$V_{temp} = V_{calibration} \times Dt \times 2.1 \times 10^{-4} \qquad (5)$$

where:

V_{temp} - uncertainty due to the volume expansion at laboratory temperature;

$V_{calibration}$ - volumetric glassware uncertainty according to calibration certificate;

$2.1 \times 10^{-4}\,°C^{-1}$ - coefficient of volume expansion for water;

Dt - temperature variation in the laboratory (20 ± 4ºC).

Standard uncertainty due to sample dilution was estimated by summing the uncertainties of the volumetric glassware to the volume expansion (Table 3). The relative uncertainties due the dilution factor of the sample (volumetric flasks+aliquots) were combined into one contribution for the overall experiment. Standard uncertainty related to the spectrophotometer was calculated by means of Eq. (4).

Working standard solutions and stock solution contribution

An example for the preparation of one working standard solution is summarized in Table 4. The same procedure was repeated for all standards. Standard uncertainty associated to the stock solution was estimated by means of Eq. 4 (Table 4). The relative uncertainty related to the dilution of the standards was obtained by the sum of the relative uncertainties due to the dilution factor and stock solution.

The relative contribution of each component, as well as the combined and expanded uncertainties are summarized in Table 5. The combined standard uncertainty of the method was estimated according to Eq. (6). The expanded uncertainty (U) was obtained by multiplying the combined uncertainty with a coverage factor of 2 (Table 5).

$$u(P_2O_5) = \bar{x}\sqrt{\left(\frac{u(r)}{\bar{x}}\right)^2 + \left(\frac{u(c)}{c}\right)^2 + \left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(v)}{v}\right)^2 + \left(\frac{u(au)}{au}\right)^2} \qquad (6)$$

Table 3. Standard uncertainties related to sample mass, dilution factor and spectrophotometer

Source/ Quantity	Uncertainty components		Distribution		Eq./us
Analytical balance					
Mass of the sample	Eccentricity	Linearity	normal	normal	Eq. (2)
2.5 g	0.0005 g	0.0002 g	2	2	0.0004 g
Dilution factor (volume)					
Volumetric flask (v1)	Calibration	Volume expansion	normal	rectangular	Eq. (3)
500 mL	0.05 mL	0.2425 mL	2	$\sqrt{3}$	0.1422 mL
Volumetric flask (v2)	Calibration	Volume expansion	normal	rectangular	Eq. (3)
100 mL	0.03 mL	0.0485 mL	2	$\sqrt{3}$	0.0318 mL
Aliquot 1	Calibration	Volume expansion	normal	rectangular	Eq. (3)
10 mL	0.023 mL	0.0048 mL	2	$\sqrt{3}$	0.0118 mL
Aliquot 2	Calibration	Volume expansion	normal	rectangular	Eq. (3)
10 mL	0.023 mL	0.0048 mL	2	$\sqrt{3}$	0.0118 mL
Spectrophotometer	Calibration		normal		Eq. (4)
0.225 AU	0.005 AU		2		0.0025 AU

Table 4. Standard uncertainties related to the preparation of working standard solutions and to the stock standard solution

Source/Quantity	Uncertainty components		Distribution		Eq./us
Dilution factor (volume)[i]					
Burette	Calibration	Volume expansion	normal	rectangular	Eq. (3)
1 mL	0.015 mL	0.00049 mL	2	$\sqrt{3}$	0.0075 mL
Volumetric flask	Calibration	Volume expansion	normal	rectangular	Eq. (3)
100 mL	0.03 mL	0.0485 mL	2	$\sqrt{3}$	0.0318 mL
Stock solution[ii]					
Concentration	Calibration		normal		Eq. (4)
1004 mg/kg	6 mg/kg		2		3 mg/kg

[i] Standard uncertainty related to the preparation of one working standard solution
[ii] Standard uncertainty related to the stock standard solution

Table 5. Expanded and combined uncertainties of the method for phosphorus determination in organo-mineral fertilizer by UV VIS spectrophotometry

Uncertainty component	Value	Unit	Type
Phosphorus content mean ($\bar{x}$)	3.55	% P_2O_5	A
Relative standard uncertainty of the sample repeatability u(r)/ $\bar{x}$	0.0285	-	A
Relative standard uncertainty of the calibration solutions u(c)/c	0.0096	-	A&B
Relative standard uncertainty of the sample mass u(m)/m	0.0002	-	B
Relative standard uncertainty of the dilution factor of the sample u(v)/v	0.0017	-	B
Relative standard uncertainty of the spectrophotometer u(au)/au	0.0111	-	B
Combined standard uncertainty (u_c)	0.1140	% P_2O_5	A+B
Combined uncertainty ($U_c = (u_c \times 100)/ \bar{x}$	3.21	%	
Expanded uncertainty, $k=2$ ($U = U_c \times 2$)	6.43	%	

Uncertainty budget

The uncertainty budget as relative contributions of the different components are shown in Figure 3. As shown in Figure 3, the main sources of uncertainty of the result of measurement were identified as contributions from repeatability, standard solutions preparation and spectrophotometer, while the contribution of the sample mass/ analytical balance has no influence on the overall uncertainty.
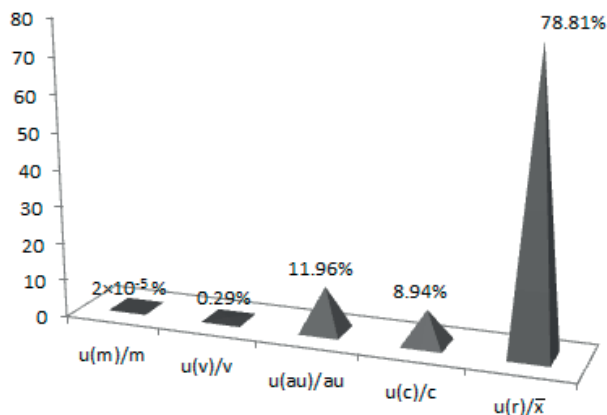

Figure 3. The uncertainty contributions as relative uncertainties (u(m)/m: uncertainty related to sample mass uncertainty; u(v)/v: uncertainty related to sample dilution; u(au)/au: spectrophotometer uncertainty; u(c)/c: uncertainty associated to the standard solutions; u(r))/x average: uncertainty associated to the repeatability)

CONCLUSIONS

This study evaluated the measurement uncertainty of the result of total phosphorus determinations in organo-mineral fertilizers.

The identified uncertainty components were the repeatability, standard solution dilutions, spectrophotometer, sample mass (anaytical balance) and sample dilution factor. It was observed that the largest contribution comes from the analysis process (i.e. repeatability, 78.8%).

The result of measurement and the expanded uncertainty estimated by using Eurachem guidelines was 3.55 ± 0.23 % P_2O_5.

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