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EVALUATION OF MEASUREMENT UNCERTAINTY TO PHOSPHORUS DETERMINATION BY MOLECULAR SPECTROMETRY IN WATER SAMPLES

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The present work proposes a procedure for the evaluation of measurement uncertainty for the determination of phosphorus from water samples by absorption molecular spectrometry method using ammonium molybdate as analytic reagent. The sources of expanded uncertainty for this determination were the following: uncertainty associated to analyst, uncertainty associated to the preparation of concentration for standard solution, uncertainty associated to the measurement volumes, and the uncertainty associated with the linear fitting least squares method.

Keywords: measurement uncertainty, total phosphorus, surface water, wastewater

1. Introduction

Quality evaluation of analytical results is reflected in the integrated international standards being established as a determinant of competitiveness and comparability of analytical results. The measurement uncertainty is an important parameter in the quality of analytical results and it must be evaluated for any method of analysis following the “in-house-validation” procedure [1].

According to the GUM document [2], where the theoretical principles and methods of expressing the measurement uncertainty are clearly set, the uncertainty can be expressed as a "parameter, a result of a measurement, that characterizes the reasonably spread of values, that could be attributed to the measurand". EURACHEM/CITAC Guide [3] gives practical applications of those principles to quantitative measurements in analytical chemistry.

It is generally recognized that an analytical result is not complete if does not include information about the uncertainty of outcome [4], and therefore more work to clarify the expression of uncertainty in measurement were performed [5,

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6]. Moreover, experimental results depend on all components of uncertainty associated to their used method of analysis [1]. One of the problems faced by analysts is whether the used methodology provides adequate results for the intended purpose [5]. The concept of measurement uncertainty is integrated into the quality management system regarding method validation, internal quality control and participation in external quality assessment programs (inter-laboratories tests) [7].

The process must go through several stages for performing an assessment of uncertainty for a specific analytical method determination. The models for the identification and measurement of any potential sources of uncertainty and the calculation of expanded uncertainty measurement are the first to be considered. In order to decide whether the measurements are adequate for the proposed method, after achieving the estimation of measurement uncertainty, the following step is to appreciate whether the level of uncertainty is acceptable or not [8].

This work presents the calculation of expanded uncertainty measurement related to total phosphorus determination by UV-Vis molecular spectrometry in surface water and wastewater samples.

2. Experimental

Experiments for the calculation of measurement uncertainty in the determination of total phosphorus (TP) in surface water and wastewater have been accomplished by using a visible spectrophotometric method of analysis [9].

2.1. Materials and methods

All reagents used were of analytical purity grade and all solutions were prepared using bidistilled water. Sodium hydroxide and ascorbic acid were both purchased from Sigma-Aldrich (Germany). Sulfuric acid, ammonium heptamolybdate tetrahydrated, and semihydrated antimony potassium tartrate and potassium peroxodisulphate were all purchased from Merck (Germany).

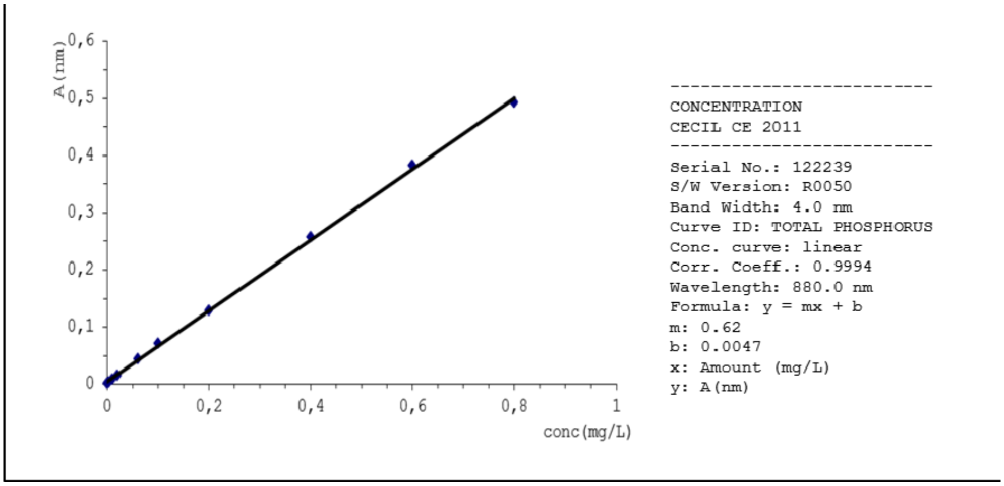
Sample preparation protocol

40 mL of water sample was taken for analysis using the following protocol: water sample was acidified with 0.4 mL of 4.5 mol/L sulfuric acid, and then they were mineralized for 30 minutes on a hot plate. For digestion of the samples was used 5 mL of peroxodisulphate solution (5%) in order to convert most of organophosphorus compounds into orthophosphate. After cooling of the samples, the pH was adjusted to a value of ~3.0 by using sodium hydroxide (2 mol/L). Next step was the addition of acidic antimony molybdate solution (analytic reagent), in the presence of 1 mL of 100 g/L ascorbic acid solution,

when the reaction occurs and leads to formation of blue antimony-phosphomolybdate complex. After 20 minutes, the absorbance of the formed complex is performed at the wavelength of 880 nm, using a CECIL visible absorption spectrophotometer.

2.2. Plotting the calibration curve

The stock standard solution of 50 mg/L concentration was prepared in a 1000 mL volumetric flask by dissolving 0.2197 g potassium dihydrogen phosphate (previously brought to constant weight at 105 ºC in the oven) and adding of 10 mL of 4.5 mol/L sulfuric acid. The standard working solution of 2 mg/L was prepared by diluting 20 mL of stock standard solution in 500 mL flask using double distilled water. The calibration curve was plotted (Fig.1) using 7 standard working solutions of 0.004 mg/L, 0.020 mg/L, 0.060 mg/L, 0.100 mg/L, 0.400 mg/L, 0.600 mg/L, 0.800 mg/L as total phosphorus prepared according to the same protocol as the water samples.



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CONCENTRATION
CECIL CE 2011
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Serial No.: 122239
S/W Version: R0050
Band Width: 4.0 nm
Curve ID: TOTAL PHOSPHORUS
Conc. curve: linear
Corr. Coeff.: 0.9994
Wavelength: 880.0 nm
Formula: y = mx + b
m: 0.62
b: 0.0047
x: Amount (mg/L)
y: A(nm)
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Fig.1. Calibration curve for determination of total phosphorus using a spectrometric method

3. Results and Discussion

In order to identify sources of the associated uncertainties involved to the estimation of measurement uncertainty for total phosphorus determination, the flow diagram (Fig.2) and cause-effect approach (Fig.3) were established in accordance with references [10, 11]. Uncertainty associated with mineralization samples was assumed to be negligible.

Total phosphorus concentration in the samples was calculated according to the equation (Eq.1):

$$C_{TP} = \frac{(C - C_0) \times V_{\max}}{V_s} \qquad (1)$$

C_{TP} - total phosphorus concentration, (mg/L);
C - concentration of analyzed sample (mg/L);
C_0 - concentration of blank (mg/L);
V_{max} - reference volume of analyzed sample (50 mL);
V_s - volume of analyzed sample (mL).

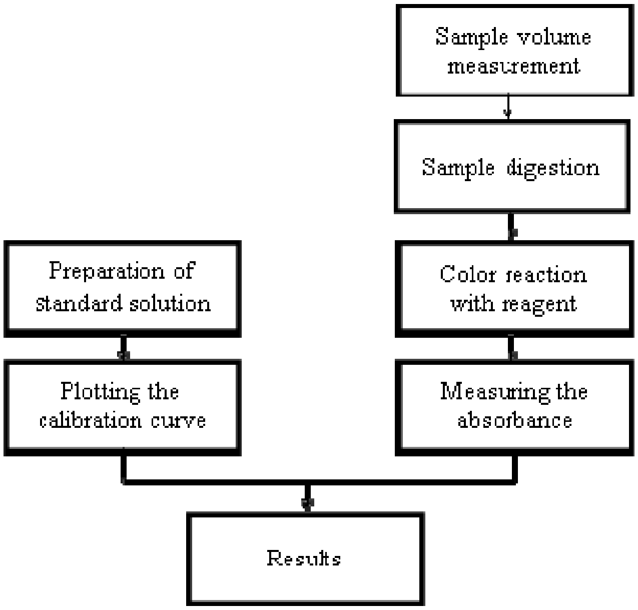

Fig. 2. Flow diagram for determination of phosphorus in water sample

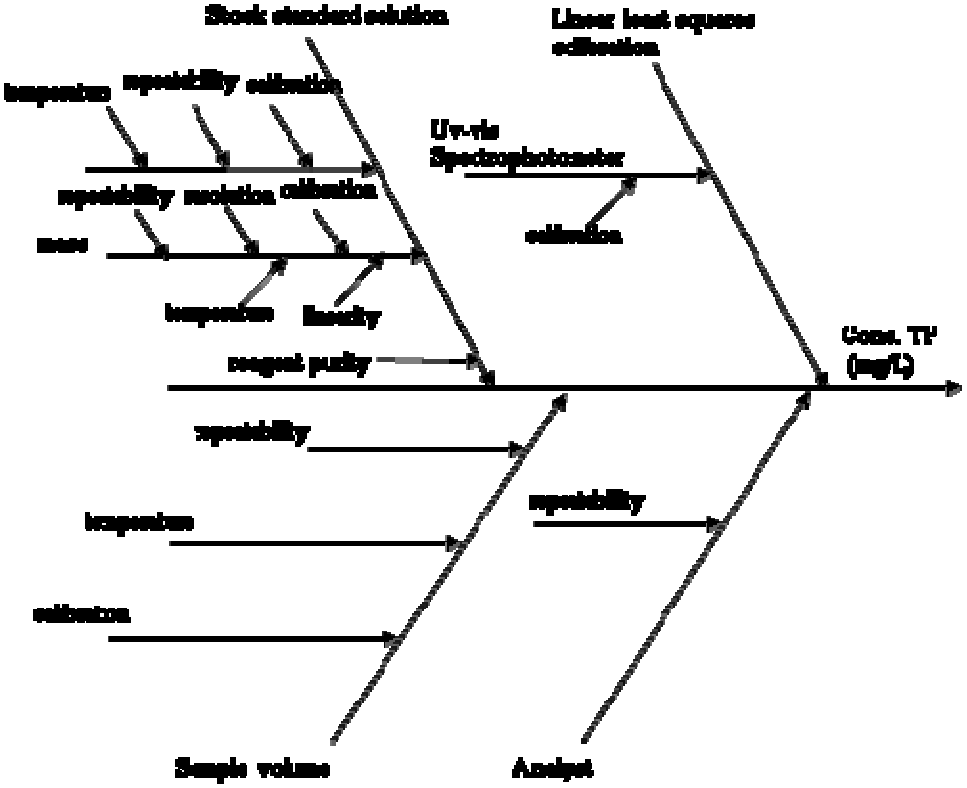

Fig. 3. Cause and effects diagram for measurement uncertainty in total phosphorus determination

3.1. Uncertainty sources

Taking into account the EURACHEM/CITAC Guide CG4 [3], the following associated uncertainties for the total measurement uncertainty in the analysis of total phosphorous in waters by absorption molecular spectrometry should be considered: uncertainty associated to analyst, uncertainty associated to the preparation of concentration for standard solution, uncertainty associated to the measurement volumes, and the uncertainty associated with the linear fitting least squares method.

3.1.1. Uncertainty associated to analyst

To obtain the uncertainty associated the analyst have made a total of 10 determinations using the same equipment working under the same conditions of temperature on a standard sample with a concentration of 0.5 mg/L total phosphorus (C_{TP} = 0.4997 mg/L), was identified uncertainty associated with the deviation standard (U_{as} = 0.00406).

3.1.2. Uncertainty associated with stock solution preparation

The uncertainty associated of the calibration stock solution depends on the mass dihydrogen phosphate, the reagent purity and the volume of the volumetric flask for preparing the solution**.**

Uncertainty associated with reagent mass

Analytical balance (KERN 770-14) has the following technical specifications: linearity = 0.2 mg, resolution = 0.0001 g, uncertainty of the calibration certificate = 0.10 mg whit a coverage factor k = 2, repeatability = 0.000042.
Combined standardized uncertainty associated with reagent mass, U_m= 0.1258.

Uncertainty associated with reagent purity

Quality certificate of dihydrogen phosphate indicates purity (P = 99.95 %). Therefore, uncertainty associated with reagent purity (U_p) is considered to have a rectangular distribution (Table 1) [12].

Table 1

Uncertainty associated with purity reagent

	Mass	**P %**	**Factor = sqrt 3**	**U_p**
KH_2PO_4	$0.2197\cdot10^{3}$	0.9995	1.732	0.0029

3.1.3. Uncertainty associated with the measurement volumes

Three main uncertainty sources are quantified with every volume measurement:

Measurement uncertainty specified in the certificate glassware used (U_1)

Glassware used for measuring volume (pipettes, volumetric flasks and cylinders) has a level of uncertainty indicated in the calibration certificate for a confidence level of 95 % (Table 2, Table 3).

Correction of errors associated with temperature variations (U_2)

In accordance with the calibration manufacturer's certificate glassware used was calibrated to temperature of 20 °C. The variation of temperature inside laboratory is within +/- 4 ºC. Volume variation (dV) of an aqueous solution linked to temperature is calculated using the formula (Eq. 2) [11]:

$$dV = 1 \cdot V \cdot dT \tag{2}$$

1 - variation factor of the liquid (for an aqueous solution $= 2.1 \cdot 10^{-4}$ ºC);
V - volume subject to dilation;
dT - temperature variation.

The dilation of a certain volume of liquid is often higher than of the containing flask, therefore only the dilation of the liquid is counted.

Uncertainty associated with repeatability (U_3) [10]

It has been found experimentally that the error reaches a 0.03 mL for one drop considering the volume up to 100 mL and reaching 0.06 mL for high volumes of 100 mL.

Table 2

Uncertainty associated with volume measurement

Class A glassware	Volume (mL)	Calibration	Volume variation with temperature	Repeatability
		U_1	U_2	U_3
Pipette	1	0.0040	0.0005	0.0173
Pipette	5	0.1730	0.0024	0.0173
Pipette	10	0.0289	0.0048	0.0173
Pipette	20	0.0173	0.0097	0.0173
Flask	25	0.0231	0.0121	0.0173
Flask	50	0.0346	0.0242	0.0173
Flask	100	0.0577	0.0485	0.0346
Flask	500	0.1443	0.2425	0.0346
Flask	1000	0.2309	0.4850	0.0346

Uncertainty associated with the volume:

$$U_{v1} = \sqrt{U_1^2 \cdot U_2^2 \cdot U_3^2} = 0.0194 \tag{3}$$

Table 3

Uncertainty associated with the sample volume

Class A glassware	**Volume**	**Calibration**	**Volume variation with temp.**	**Repeatability**
	(mL)	**U_1**	**U_2**	**U_3**
cylinder	50	0.2887	0.0242	0.0173

Uncertainty associated with the sample volume:

$$U_{v2} = (\sqrt{U_1^2 \cdot U_2^2 \cdot U_3^2})/50 = 0.0058 \quad (4)$$

3.1.4. Uncertainty associated with the linear fitting least squares method [8]

Total phosphorus was calculated by plotting the calibration straight line using the least squares method.

Slope *b*, as a measurement of sensibility, is calculated using the formula (Eq. 3):

$$b = \frac{SUM(Xi - Xm)(Yi - Ym)}{SUM(Xi - Xm)^2} \quad (5)$$

b - slope;
Xi - standard concentration;
Xm - average of standards used for curve;
Yi - standards absorbance;
Ym - average absorbance used for curve.

The calculation of the uncertainty associated with the fitting linear least squares method is described in the following formula (Eq.6):

$$U_{et} = S_y / b\sqrt{\left\{1/p + 1/n + \left[(C_{TP} - Xm)^2\right] / \sum (Xi - Xm)^2\right\}} \quad (6)$$

U_{et} - uncertainty associated with calibration;
S_y - residual standard deviation;
p - number of measurements performed, C_{TP};
n - number of measurement performed for calibration curve.

$$Uet = 8.2155E - 02\left\{\sqrt{\left\{1/10 + 1/8 + \left[(0.4997 - 0.2480)^2 / 0.092895\right]\right\}}\right\} = 0.00821 \quad (7)$$

Uncertainty associated with the spectrophotometer

Uncertainty associated with the spectrophotometer is specified in the calibration certificate by a trust interval of 95 % = 0.008. Associated uncertainty that was taken into consideration with the spectrophotometer is $U_{uv\text{-}vis} = 0.0133$.

Uncertainty associated with the oven

The uncertainty attributed to the oven (type ECv_{50}), is specified in the certificate of calibration and is represented of extended uncertainty by multiplying the standard uncertainty with coverage factor k = 2. Associated uncertainty that was taken into consideration with the oven is $U_{etv} = 0.0392$.

3.2. Calculation of the combined and expanded uncertainty

Expression of measurement uncertainty is based on combined uncertainty that contribute to the combined standard uncertainty (Table 4) [13].

Table 4

Calculation of the combined standard uncertainty

$(U_{as}/C_{TP})^2$	$(U_{v2}/V)^2$	$(U_m/_m)^2$	$(U_{v1})^2$	$(U_{et})^2$	$(U_{etv})^2$	$(U_{uv\text{-}vis})^2$	$(U_p)^2$
6.592E-05	3.369E-05	0.01583	0.00038	6.74E-05	0.00154	0.00018	8.418E-06
sum	**0.0181**						
sqrt	**0.1345**						

For the calculation of the expanded uncertainty U, combined standard uncertainty (U_c) was multiplied by the coverage factor k, using the relationship (8), considering a normal distribution of the experimental data, k = 2 for a 95 % confidence interval [4, 13, 14].

$$U = U_c \cdot k \tag{8}$$

Combined Uncertainty: $U_c = sqrt \cdot C_{TP} = 0.0672$ mg/L (9)

Extended Uncertainty: $U = U_c \cdot k = 0.1345$ mg/L (10)

4. Conclusions

This study provides a model for the calculation of the expanded measurement uncertainty that has been applied to the data obtained for the method of determination of total phosphorus in surface water samples and wastewater.

The presented computational model allows a rapid calculation of the extended combined uncertainty, being a quantification of a result quality that allows the user to make an accurate assessment of the reliability of the method.

This procedure allows also the identification of the significant sources of uncertainty present in the measurement procedure, showing which parts of the method for determination must be treated with care, or improved to reduce the measurement uncertainty.

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