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METHOD VALIDATION AND UNCERTAINTY ESTIMATION FOR TOTAL PHOSPHORUS DETERMINATION IN WASTEWATER SLUDGE SAMPLES

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ABSTRACT. Analytical method and uncertainty estimation for the measurement of total phosphorus in aquatic media was performed in two different laboratories. Method performance criteria used for validation process were investigated on standard phosphorus samples: concentration linearity domain (by calibration curve), limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy and robustness. In order to announce reliable results, uncertainties sources were identified, and different types of uncertainty were estimated. The validated method was further applied for total phosphorus determination from the sludge resulted at one municipal wastewaters treatment plant. Samples were collected from wastewater treatment sludge, before and after the dehydration process. The results show that the method is suitable and gives trustful results for phosphorus determination from wastewater sludge.

Keywords: *wastewaters, sludge, total phosphorus, method validation, uncertainty estimation.*

INTRODUCTION

Nitrogen and phosphorus containing compounds obtained in the wastewater sludge are nutrients and can be further valorized as fertilizer for agricultural purpose. Consequently, the quality control of the wastewater and wastewater sludge is of great interest for any wastewater treatment plant laboratory. The research group showed interest for analytical procedures for wastewater quality control and previous studies were reported: nutrients and detergents [1], phenolic compounds [2], heavy metals [3], aromatic volatile compounds [4].

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Available phosphorus determination methods for monitoring the effluent quality of wastewater plants are automatic on-line titration unit sensor based [5], colorimetry based on the coloring reagent formation between phosphorus and molybdate [6], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [7] or ion chromatography [8].

This study aims to give the method validation results as well as the uncertainty estimation for total phosphorus (P_t) determination in standard solutions and wastewater sludge samples, which is new and not yet imposed in the Romanian laboratory practices. The validation procedure was carried out in the Wastewater Laboratory (WL) while the reproducibility and uncertainty estimation were studied together with the Chemistry Laboratory (CL).

RESULTS AND DISCUSSION

The study followed specific stages: methods validation for P_t determination, uncertainty estimation for P_t determination in both standard solutions and in wastewaters sludge samples. The validated method was used to determine the total phosphorus content in the sludge produced in two different stages of the wastewater treatment process.

Methods validation for total phosphorous determination

The investigated performance criteria were concentration linearity domain (by calibration curve), limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy and robustness.

Calibration curve

The calibration curve is given in Figure 1.

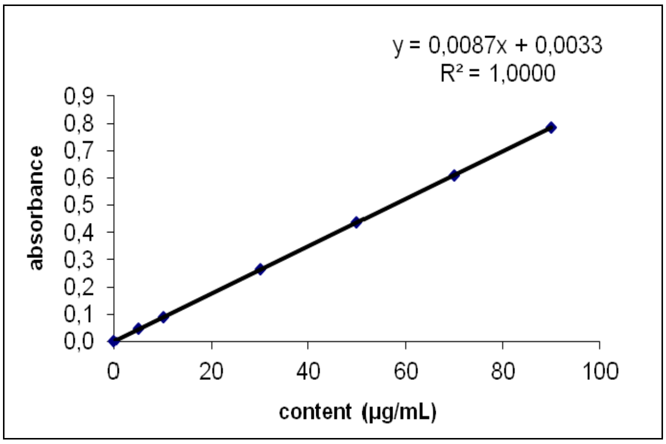

Figure 1. Calibration curves for the P_t determination in RM solutions, in WL.

Calibration curve was plotted with 6 solutions of reference material (RM) of different concentrations, that were spectrometrically determined (at 830 nm, blue phosphomolybdenic complex), for the imposed concentration domain of 10–90 µg P/mL. Very good regression coefficient was obtained.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the equations (1-2) [9,10] using the measured absorbance values for 10 replicates of blank solution, then transformed in concentration values.

$$LOD = \overline{x_{blanck}} + 3 \cdot s_{blank} \tag{1}$$

$$LOQ = \overline{x_{blanck}} + 6 \cdot s_{blank} \tag{2}$$

were: $\overline{x_{blank}}$ – mean absorbance obtained with the blank solutions; s_{blank} – standard deviations of the blank solutions.

The results are: LOD = 0.32 µg/mL and LOQ = 0.49 µg/mL, both lower than the lowest concentration for total phosphorus determination (10–90 µg P/mL), imposed by the Romanian standard method [11].

Precision

Standard solutions of phosphorus RM of 40 µg/mL were prepared ($[P]_{theor}$). Repeatability (RSD_r) was tested with 7 replicates, intermediate precision (RSD_{ip}) with 7 replicates determined by three operators each, and interlaboratory reproducibility (RDS_R) with 7 replicates determined by three operators, in each laboratory (WL and CL).

Precision was evaluated by three criteria: relative standard deviation (RSD%), mean deviation (s_x) and confidence interval (t-distribution test) of the P_t measured concentration ($[P]_{measured}$). The results are given in Table 1.

Table 1. Precision evaluation

$[P]_{theor}$ (µg/mL)	Precision characteristics	n	RSD (%)	s_x	$[P]_{measured}$ ± confidence interval (µg/mL)*
40	repeatability (RSD_r) (WL)	7	0.70	0.1054	39.55 ± 0.2108
40	interim precision (RSD_{ip}) (WL)	21	0.87	0.0745	39.12 ± 0.1489
40	reproducibility (RSD_R) (WL+CL)	42	7.26	0.4498	40.13 ± 0.8996

*Calculated for a tolerance (t-distribution) of t=2, 95% confidence interval.

The obtained results show good results for repeatability and interim precision, as expected, the RSD% for repeatability being lower than the RSD% for interim precision. As expected, reproducibility values are higher than the repeatability and interim precision ones, RSD% = 7.26 is an acceptable result for the reproducibility test. The final result may be expressed as measured concentration together with the confidence interval. To be noticed that, as expected, $RSD_r < RSD_{ip} < RSD_R$.

Accuracy

Accuracy was evaluated by the recovery test (R%), applied on 7 replicates of the standard solutions, according to equation (3).

$$R\% = \frac{C_F - C_U}{C_A}100 \tag{3}$$

where: C_U – concentration determined in the unfortified sample; C_A – concentration of fortification (added solution); C_F – concentration determined in the fortified sample.

Acceptable recovery percentages is a function of the analyte concentration [12]. For the studied analyte concentration (µg/mL) the method is considered accurate if the recovery test gives values in the range of 80% ≤ R ≤ 110%. The results are presented in Table 2.

Table 2. Accuracy evaluation

Volume (mL)	C_U (µg/mL)	C_A (µg/mL)	C_F (µg/mL)	Recovery (%)
2	5	5	10.267	104.30%
4	5	25	29.977	99.91
8	5	85	89.573	99.50

The obtained R% values lower than the accepted upper limit (110%) shows that the method is accurate for all the volumes of tested solutions.

Robustness

Standard solutions of phosphorus RM of 40 µg/mL were prepared ($[P]_{theor}$). Robustness was tested related to: (a) the volume of the sulfuric acid solution (10%) used for the complex formation, (8 mL is the volume required by the standard); (b) the stability of the molybdenic acid; the use of the reagent was tested at 1, 3, 6 days after its preparation (according to the standard it should be freshly prepared and immediately used). The results are given in Table 3 and Table 4, respectively.

Table 3. Robustness evaluation related to the volume of the sulphuric acid solution used for the complex formation.

V_{acid} (mL)*	$[P_t]_{theor}$ (µg/mL)	$[P_t]_{measured}$ (µg/mL)	Colour of the complex solution
0	40	29.73	blue-grey
8	40	39.85	blue
16	40	38.48	colorless
Mean $[P_t]_{measured}$ (µg/mL)		36.02	
RSD%		13.39	

* three replicates for each volume

Total phosphorus determination is influenced by the volume of the sulfuric acid solution used for the complex formation. Not only that the color of the complex solution is not obtained (blue), but also the relative standard deviation of the measurements is high (13.39%), meaning that the method is not robust relative to the volume of the sulfuric acid solution. The molybdenic acid is stable in aqueous solution. Not only that the color of the complex solution is obtained (blue), but also the relative standard deviation of the measurements is low (0.48%), meaning that the method is robust relative to the stability of the molybdenic acid solution.

Table 4. Robustness evaluation related to the stability of the molybdenic acid.

Day*	$[P_t]_{theor}$ (µg/mL)	$[P_t]_{measured}$ (µg/mL)	Colour of the complex solution
1	40	39.58	blue
3	40	39.65	blue
6	40	39.88	blue
Mean $[P_t]_{measured}$ (µg/mL)		39.70	
RSD%		0.48	

* three replicates for each day

Uncertainty estimation for the total phosphorus determination

In order to estimate the uncertainty, the measurand was specified as being the content of total phosphorus in standard solutions and in the mineralized solution obtained from the wastewater sludge, respectively. The uncertainty sources were identified, Ishikawa diagrams were developed and different types of uncertainty were calculated according to equations (4-7): standard uncertainty (u_x); relative standard uncertainty (u_r); combined relative standard uncertainty (u_c); expanded standard uncertainty (U). The final result is announced as the average concentration (C) and the uncertainty contribution (R=C±U).

Uncertainty sources identification

For the estimation of the uncertainty arising from the determinations in the standard solutions, the calibration curve, the concentration repeatability and the equipment, were found to be the uncertainty sources. Ishikawa diagram is presented in Figure 2.

$$u_x = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2}{n-1}} \tag{4}$$

$$u_r = \frac{u_x}{x} \tag{5}$$

$$u_c = \sqrt{\sum u_r^2} \tag{6}$$

$$U = u_c \cdot k \cdot 100 \tag{7}$$

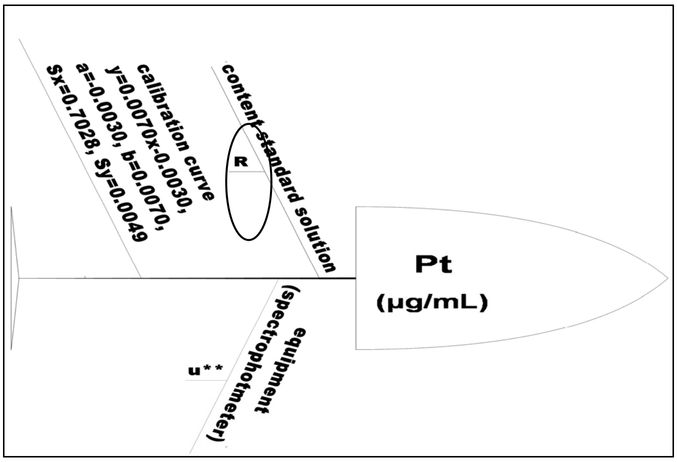

Figure 2. Ishikawa diagram for the P_t determination in RM solutions.

For the estimation of the uncertainty arising from the determinations in the mineralized solutions, the equipments (analytical balance and spectrometer), the calibration curve, the concentration repeatability as well as the different glassware used for volumes measurements were found to be the uncertainty sources. For this estimation Ishikawa diagram is presented in Figure 3.

Using RM solutions of 40 μg/mL, expended standard uncertainty were calculated with k=2 (equation 11), with a confidence level of P=95%. For the standard solutions, 7 replicates were measured in both laboratories and the mean concentrations values were considered. For the mineralized solution 7 replicates were measured in the wastewater laboratory. The final result report will be accordingly with the Table 5 and Table 6, respectively.

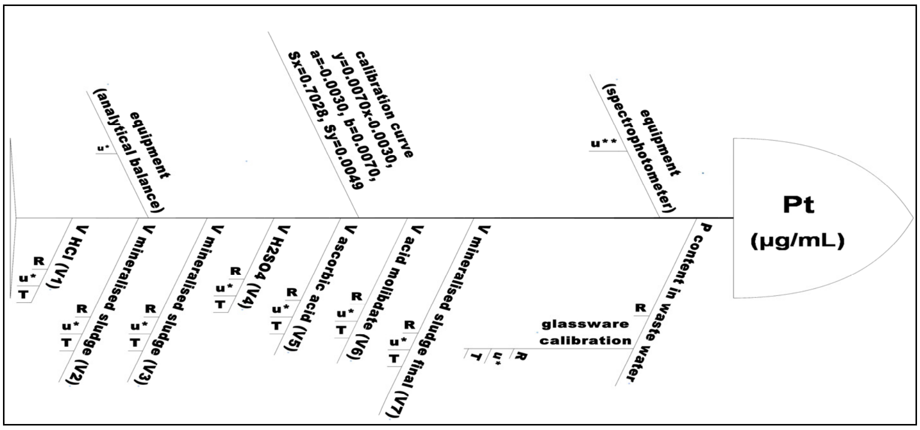

Figure 3. Ishikawa diagram for the P_t determination in mineralized solutions.

Table 5. Uncertainty estimation for the P_t determination in RM solutions.

Lab	U source	u_x	u_r	u_c	U	U (%)	Result (μg/mL)
WL	calibration curve	0.2210	0.0056	0.35	0.71	1.81	39.49 ± 0.71
	repeatability	0.2789	0.0071				
	equipment	0.0225	0.0006				
CL	calibration curve	0.1480	0.0037	1.27	2.54	6.39	39.78 ± 2.54
	repeatability	1.2629	0.0317				
	equipment	0.0152	0.0004				

To be noticed that $U_{standard,(WL)}<U_{standard,(CL)}$; $U_{standard,(WL)}<U_{mineralized,(WL)}$. For the expanded uncertainty estimations of the P_t determined in standard solution (WL, CL), as well as in the mineralized solution obtained from the wastewater sludge (WL), the higher contribution to the uncertainty has the repeatability. The fact that the extended standard uncertainty obtained for the standard solutions are lower than those for mineralized samples can be explained by the contribution brought to the uncertainty estimation by the sample preparation from the sludge.

Total phosphorus content determination in the wastewater sludge

According to the European Union regulation related to the environment protection, especially of the soil quality, when sewage sludge is used in agriculture, soil and sludge analysis should include among other parameters phosphorus determinations too [13].

Therefore the phosphorus content in the sludge was of great interest for our study. Using the validated method, the total phosphorus content in the wastewater sludge was determined at WL, in two different stages of the treatment: before and after the dehydration process.

Table 6. Uncertainty estimation for the P_t determination in mineralized solutions.

U source	x (unit)	u_x	u_r	u_c	U	U (%)	Result (µg/mL)
analytical balance	200 mg	0.2	0.0010	0.68	1.37	2.60	52.70 ± 1.37
spectrometer	52.70 µg/mL	0.0225	0.0004				
repeatability	52.70 µg/mL	0.5314	0.0101				
calibration curve	52.70 µg/mL	0.2210	0.0042				
V_1	25 mL	0.0437	0.0017				
V_2	100 mL	0.1488	0.0015				
V_3	5 mL	0.0086	0.0017				
V_4	8 mL	0.0377	0.0047				
V_5	1 mL	0.0033	0.0033				
V_6	2 mL	0.0046	0.0023				
V_7	100 mL	0.1488	0.0015				

The measurements were done in three replicates and the results are given in Table 7., as R=C±U.

Table 7. Total phosphorus content in the wastewater sludge

Wastewater treatment stage	P_t content (µg/mL)
before dehydration the sludge	58.04 ± 2.58
after dehydration the sludge	52.70 ± 1.37

The expanded standard uncertainties are increasing with the increase of P_t content in the mineralized sample. The results demonstrate that a part of the phosphorus is lost during the dehydration process.

CONCLUSIONS

The total phosphorus determination method [11] was validated to the following performances criteria: calibration curve, LOD, LOQ, precision (repeatability, intermediate precision, and reproducibility), accuracy (recovery test) and robustness. The method is sensitive, precise, accurate, not robust against the volume of the sulfuric acid solution, but is robust against the molybdenic acid, both used for the colored complex formation. Based on the identified sources of uncertainty, for both standard and mineralized solutions, the Ishikawa diagrams were designed, and different types of uncertainties were estimated showing that reliable results are to be announced. The higher contribution to the expanded uncertainty had the repeatability. Using the validated method, the total phosphorus content in the sludge produced during the wastewater treatment, before and after dehydration, was determined. Further work will be done for more comparative studies, both for proficiency testing (PT) and inter-laboratory comparisons (ILC).

EXPERIMENTAL SECTION

The Romanian standard method [11] was used, based on the spectrometric determination of the blue phosphomolybdenic complex. Sludge samples are mineralized with concentrated sulfuric acid and perchloric acid in order to obtain orthophosphate from all different forms of phosphorus content in the sludge. Orthophosphate ions are further transformed by ammonium molybdate in phosphomolibdenic complex, than reduced by ascorbic acid and suphuric acid to a blue complex, that absorbs at 830 nm.

All the reagents were of analytical grade, and were purchased as follows: ascorbic acid, ammonium molybdate tetrahydrate and the phosphorus reference material (RM), monopotassium phosphate, all from Merck, Germany.

The two laboratories used the following equipments: a molecular absorption UV-VIS spectrometer Jasco 550 with double beam optical system (CL) and a molecular absorption UV-VIS spectrometer Secomam 750I (WL). Nabertherm L9/C6 oven was used for sludge mineralization. The determination domain imposed by the standard is 10–90 μg P/mL in the solution obtained after mineralization. Data processing was carried out using specific procedures [9-10, 12], while the uncertainty estimation was based on the EURACHEM guides [14-16].

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