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ORIGINAL PAPER

Validation of a small-scale method for determination of phosphorus in meat and meat products by molecular absorption spectroscopy

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Abstract

The purpose of this study was to evaluate the applicability of an alternative analytical technique to the reference method for phosphorus detection in meat and meat products established by ISO 2294:1974, revised by ISO 23776:2021. When an analytical method is modified or a new one is developed and implemented, it should be validated before being accepted for routine determinations. Furthermore, it is necessary to demonstrate that the new or alternative method has good performance characteristics in comparison with the reference method. Therefore, a comparison of the performance of the alternative and the reference method should be made. In the present study, a statistical evaluation of the calibration model of the alternative method was made with data from interlaboratory studies made in five consecutive years using fifty analyses of meat and meat products. The statistical parameters evaluated over time for the calibration curve were the y-intercept and slope; correlation and linearity; analytical limits: detection and quantification; working range; and accuracy (precision and trueness). In the interlaboratory tests, the results obtained by the alternative analytical method were compared with those obtained by the reference method, and their performance in these tests was also evaluated. The results obtained by the alternative method indicated better accuracy than the reference method due to lower relative errors, more precision, and a good trueness evaluation through lower absolute values of the z-score. The study demonstrated that the phosphorus alternative method is applicable for the determination of total phosphorus in the matrix of meat and meat products.

Keywords Small-scale method · UV–Vis spectroscopy · Reference method · Phosphorus, meat and meat products

Introduction

The phosphorus content in meat and meat products is an important quantification that is related to product quality; the maximum level in the European Union is 5000 mg (P_2O_5) kg^{-1}, as defined in Regulation (CE) N. 1129/2011 of the European Parliament and of the Council [1] by establishing a Union list of food additives. Thus, reliable analytical data are indispensable, either for food analysts or data users. The standardization of analytical methodologies and the development of a system of quality control in the laboratory can help ensure the validity of analytical measurements and increase the quality and reliability of the results obtained [2]. Despite the wide use of the terms validation and quality assurance, many analysts and laboratories have difficulty knowing exactly what they mean, their differences, and the relationship between them. The purpose of method validation is to determine if the analytical results can be obtained with an acceptable level of uncertainty [3, 4]. Method validation is the first step in quality assurance in a laboratory. Assurance of analytical quality is a complete set of measurements that a laboratory must make to ensure quality results. In these measurements are included: procedures of effective internal quality control, such as the use of reference materials, control charts, etc.; participation in proficiency testing; and

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accreditation to an international standard, often ISO/IEC 17,025 [3, 4]. The method validation is necessary to confirm the suitability for the purpose established for a particular analytical method, i.e., the validation serves to demonstrate the applicability of a certain protocol to a specific type of material and to a concentration range of analyte [4]. The analytical objective reflects the analytical results obtained with an acceptable standard of accuracy [3, 5]. Validation is, in fact, a tool to demonstrate that a particular method actually measures what is intended and is suitable for the intended purpose [6]. Method "revalidation" should be made whenever there is a change in any component of the analytical system or the established method indications are no longer suitable for the intended purpose [3, 7, 8]. It should establish the validation ambit by comparing it with the analytical system proposed and with the analytical requirements requested. The description of the analytical system includes the purpose and type of method, the type and range of analyte concentrations to be measured, the type of matrix material to which the method is applied, and the analytical method protocol. The basis for good analytical results lies in clear specifications of analytical needs [3].

To perform the method validation, it should be done through an indirect assessment carried out by the evidence of its characteristics and a direct assessment performed by comparison with references accepted. In the indirect assessment, it should be conducted a study of the method representativeness, to see if certain characteristics correspond to the purpose of the test/calibration; a study of theoretical fundamentals and principles of the method to evidence its scientific basis; the study of interferences and error sources to outline the application and control its execution; optimization studies of operating conditions and/or method robustness to allow optimization and harmonization in its execution; and the study of the characteristics parameters of the method, such as, application field, trueness, repeatability, intermediate precision, reproducibility; detection and quantification limits, and so on, to know the results quality obtained by it. The indirect assessment is achieved by comparing the method with standardized methods, using standards or certified reference materials, and conducting interlaboratory tests, ISO/IEC 17,025 [9].

Method validation is necessary to demonstrate that the analytical method complies with the established criteria for the different performance characteristics. Reference materials and interlaboratory tests have a very important role in internal quality control. The interlaboratory tests are competence tests, which are an external quality assessment designed to evaluate the performance of the analysis laboratory and reflect the reliability of the analytical results [2].

The purpose of this work is to put into practice an alternative method for the reference method for measuring phosphorus in meat and meat products that can be used to analyze numerous samples at once more quickly, cheaply, and easily. To do this, the current work aims to develop a small-scale alternative analytical approach, validate it, and compare it to the reference method, ISO 2294:1974 [10], revised by ISO 23776:2021 [11], for determining the quantity of phosphorous in meat and meat products,.

Materials and methods

The analytical methods for determination of phosphorous in meat and meat products used in this study were the reference analytical method, ISO 2294:1974 [10], revised by ISO 23776:2021 [11], and an alternative analytical method based on and adapted from the Standard Methods for Water and Wastewater [12].

The quality of the reagents used was analytical grade (ACS, ISO) reagent for analysis. For the preparation and dilution of reagents and samples, deionized water was used. The sample was passed through the meat grinder and mixed. The resultant homogeneous sample was kept in a completely filled and sealed airtight container and stored until analysis. Samples were analyzed within 24 h.

Reference method

The determination of phosphorus in the reference method is carried out by a gravimetric method that involves three consecutive stages: mineralization of all phosphorus-containing compounds with concentrated sulfuric acid and concentrated nitric acid; precipitation of resultant mineralized phosphorus (inorganic phosphorus) as quinoline phosphomolybdate ($((C_9H_7N)_3H_3PO_4{\cdot}12MoO_3)$); and filtration (filters GF/C 47 mm Whatman), followed by gravimetric measurement of the solid obtained.

Stage 1 *Mineralization*: about 3 g of the sample, to the nearest 0.001 g, was weighed into a 200 mL Erlenmeyer flask. A 20 mL volume of concentrated HNO_3 was added. It was heated on a hot plate for 5 min, cooled, and 5 mL of concentrated H_2SO_4 was added. Gentle heating was maintained until the foaming ceased. Then, the heating was increased. When the mixture began to carbonize, concentrated HNO_3 was added, and heating continued. The procedure was repeated until the evolution of brown fumes ceased. When the liquid became colorless, it was heated until white fumes appeared. It was cooled, 15 mL of water was added, and it was gently boiled for 10 min, avoiding evaporation. A 10 mL volume of concentrated HNO_3 was added.

Stage 2 *Precipitation*: a 50 mL volume of precipitation reagent was added to the liquid in the Erlenmeyer flask, covered with a watch glass, and boiled for 1 min on a heating plate in the fume hood. The Erlenmeyer flask was removed from the heating plate and allowed to cool to room temperature, swirling the contents three or four times while cooling.

Precipitation reagent: solution 1:70 g of sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$) in 150 mL of water; solution 2:60 g of citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$) in 150 mL of water and add 85 mL of concentrated HNO_3; solution 3: add solution 1 to solution 2, while stirring; solution 4: to 100 mL of water, add successively 35 mL of concentrated HNO_3 and 5 mL of quinolone (C_9H_7N). Reagent precipitation: add solution 4 to solution 3 while stirring. Let stand for twenty-four hours, filter, add 280 mL of acetone, and dilute to 1 L with water. Store the reagent in a well-stoppered plastic bottle in the dark.

Stage 3 *Gravimetric measurement*: the contents of the Erlenmeyer flask were filtered under suction through a glass filter, previously conditioned for thirty minutes at a temperature of 260 ± 20 °C, cooled in a desiccator, and weighed to the nearest 1 mg. The precipitate on the filter was washed five times with 25 mL portions of water.

Alternative method

The alternative analytical method consists of a three stage determination: sample preparation for the digestion process, which transforms a solid sample (meat or meat product) into a liquid sample (in aqueous solution) with concentrated nitric acid (HNO_3); sample digestion with sulfuric acid solution and potassium persulfate solution (in the end, all phosphorous is in inorganic form); and a spectrophotometry determination at 880 nm, applying the ascorbic acid method. In this method, molybdate and tartrate react in an acid medium with orthophosphate, resulting in phosphomolybdic acid, which is reduced with ascorbic acid to a blue-colored molybdenum.

Stage 1 *Preparation for digestion*: about 1 g of the sample, to the nearest 0.001 g, was weighed into a 50 mL beaker. Approximately 30 mL of water and 3 mL of concentrated HNO_3 were added. It was heated on a plate until the volume was reduced to approximately 10 mL. Water was added to a volume of approximately 30 mL, heated, and the volume reduced to 10 mL. The addition of water, heating, and volume reduction were repeated. The beaker was removed from the hotplate and allowed to cool slightly. The contents of the beaker were quantitatively transferred to a 250 mL volumetric flask, and the volume was adjusted with water.

Stage 2 *Digestion*: to a 50-mL Erlenmeyer flask, a 25-mL portion, or an appropriate portion of the treated sample was transferred. A volume of 1 mL of sulfuric acid solution (300 mL concentrated H_2SO_4/L water) and 4 mL of potassium persulfate solution (6.25 g $K_2S_2O_8$/100 mL water) was added. The Erlenmeyer flask was covered with aluminum foil and autoclaved for thirty minutes at 121 °C. The digested sample was cooled to room temperature and neutralized with sodium hydroxide solution (NaHO-6 N and 1 N), using one drop of phenolphthalein solution (1 g ($C_{20}H_{14}O_4$)/100 mL ethanol) as an indicator. The solution was quantitatively transferred to a 50-mL volumetric flask and diluted to 50 mL with water. A calibration curve has been constructed by carrying standards through the same persulfate digestion procedure (calibration curve: 0.000; 0.100; 0.200; 0.300; 0.400; and 0.500 mg P-PO_4^{3-}/L).

Standard phosphate solution: 219.5 mg anhydrous KH_2PO_4/1000 mL (1.00 mL = 50, 0 µg P-PO_4^{3-}).

Stage 3 *Spectrophotometry determination*: to the digested samples, 2.0 mL of combined reagent was added and mixed vigorously. After ten minutes but no more than 30 min, the absorbance of each sample at 880 nm was measured, using the reagent blank as the reference solution. The same procedure was carried out for the blank and standards of the calibration curve.

Combined reagent: 50 mL sulfuric acid, 5 N (70 mL concentrated H_2SO_4/500 mL); 5 mL antimony potassium tartrate solution (1.3715 g K(SbO) $C_4H_4O_6{\cdot}1/2H_2O$)/500 mL; store in a glass-stoppered bottle); 15 mL ammonium molybdate solution (20 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$/500 mL; store in a glass-stoppered bottle); and 30 mL ascorbic acid, 0.1 M–1.76 g $C_6H_8O_6$/100 mL; stable for about one week at 4 °C. Mix in the indicated order and after the addition of each reagent. The reagent is stable for four hours.

Statistical evaluation of the alternative method calibration model

A statistical evaluation of the calibration model of the alternative method was made with thirty determinations lasting twenty-four months (Period 1—P1) and its subsequent update with twenty determinations lasting 36 months (Period 2—P2). The statistical parameters evaluated over time for the calibration curve were: y-intercept and slope; correlation and linearity; analytical limits: detection limit and quantification limit; working range; precision (intermediate precision); and trueness. The results obtained in the first period (P1) were utilized as control values for the subsequent period (P2). The trueness values were obtained in interlaboratory tests for orthophosphate determination in natural water, considering the value of the reference laboratory as the true value. The control values for trueness were obtained with 6 interlaboratory determinations. This study consists of the evaluation of the third stage of phosphorous determination in meat and meat products with a spectrophotometry method. The evaluation of sample digestion was made with the reference laboratory samples for the determination of total phosphorus in wastewater. The determination of this parameter involves the second and third stages of the study. The results obtained in interlaboratory tests were evaluated with respect to their performance.

The interlaboratory analyses in meat and meat products were made with reference and alternative methods. The results obtained for phosphorus in interlaboratory tests for that matrix by the 2 methods were compared with each other and with the values of the reference laboratory. The analyses were made in triplicate, and the results were evaluated using statistical parameters. The parameters evaluated were the result's dispersion between replicas, precision (intermediate precision), variation coefficient, and confidence interval.

When these results are compared with the reference value, an evaluation of the trueness and performance of each method is made. Comparing the results from the two procedures with the reference values, their standard deviation intervals, and the z-score values was the statistical parameter used to assess the performance in interlaboratory tests. The last study permitted evaluation of the first stage of the determination: sample preparation for the digestion process (Table 1).

The evaluation and validation of each stage of the analytical procedure required for determining the amount of phosphorus in meat and meat products (Table 1), completed in the reverse sequence, served as the methodology used for the alternative method's validation. The interlaboratory analyses were performed in triplicate.

Results and discussion

Evaluation of the spectrophotometric determination of phosphorous

The data resulting from the statistical evaluation of the calibration model of the alternative method from Period 1 (30 determinations) and from Period 2 (20 determinations) were resumed in Table 2. The statistical parameters presented are those usually utilized to validate an analytical method based on a calibration curve [13, 14]. For easier interpretation, the results were, when possible, presented graphically.

For the calibration curves used in the determination of phosphorus (Absorbance = f (standard concentration)), the linear equation was calculated. The data obtained for the y-intercept and slope are important to analyze the equipment's response to different phosphorous concentrations and its behavior over time. The y-intercept along period 1 (Fig. 1A) showed results in the interval of values between −0.0034 and 0.0038 (Table 2), with the value zero inside that interval. In each determination, the value of zero was included in the y-intercept confidence interval (95%). The values obtained for these parameters were also close to zero.

Table 1 Method validation methodology

Procedure steps	Validation steps	Parameters evaluated
1. Sample preparation	Comparison between the alternative and reference methods	Dispersion between replicas; Intermediate precision; Variation coefficient; Confidence interval (95%)
	Interlaboratory tests for total phosphorus (meat and meat products matrix)	Trueness evaluation; Performance of the alternative and reference methods in the interlaboratory tests
2. Sample digestion	Interlaboratory tests for total phosphorus (wastewater matrix)	Trueness evaluation; Performance in the interlaboratory tests
3. Spectrophotometry	Statistical evaluation of the calibration model (Period 1–30 samples): control values	y-intercept and slope; Correlation and linearity; Analytical limits (detection and quantification limits); Working range; Intermediate precision; Variation coefficient.
	Updated statistical evaluation (Period 2–20 samples)	
	Interlaboratory tests for total phosphorus (natural water matrix)	Trueness evaluation; Performance in the interlaboratory tests

Table 2 Statistical evaluation of calibration model of the alternative method

Parameter	Period 1			Period 2		
	Minimum	Maximum	Medium	Minimum	Maximum	Medium
y-intercept	− 0.0034	0.0038	–	− 0.0006	0.0027	–
Slope	0.343	0.387	–	0.334	0.374	–
R^2	0.99694	0.99998	–	0.99907	0.99997	–
Correlation *t* test[a]	100% of determinations correlated			100% of determinations correlated		
Linearity test[2]	93% linear calibration of determinations			76% linear calibration of determinations		
Detection limit (mg P L^{-1})	0.0030	0.037	0.016	0.0034	0.022	0.0092
Quantification limit (mg P L^{-1})	0.0090	0.112	0.050	0.010	0.068	0.028
Working range (mg P L^{-1})[b]	0.112	0.500	–	0.068	0.500	–
Precision (s_{x0}) (mg P L^{-1})[b]	–	–	0.0057	–	–	0.0033
Variation coefficient (CV) (%)[b]	0.36	5.05	2.22	0.49	3.08	1.23
Trueness (relative error) (%)	1.82	6.98	3.83	0.00	3.75	1.53

[a][14]
[b][13]

When the upgrade was made with data from Period 2, using data from the first period as control values, it was verified that 100% of the values obtained were inside the validation interval (− 0.0006 to 0.0027), the values were nearer to the value zero, and the amplitude between values (0.0033) was lower (Fig. 1B).

The slope results were in the interval between 0.343 and 0.387 (Table 1) in the first period (control values for the second period). The temporal variation results in a maximum relative variation of 11%. This variation becomes 8% if the most distant value is eliminated (0.387), which is also the most distant value from the average value (0.362). When the upgrade of the slope values was made to the second period, it was verified that 95% of the results obtained were inside the interval of the first period (except one value), and the variation was less in the second period (the amplitude between the minimum and maximum values was lower: 0.334–0.374). The amplitude of the values, from the first to the second period, was 0.044 to 0.040 (Fig. 2).

In the first period, the correlation coefficient of the calibration model was high, with 80% of the values obtained having an R^2 higher than 0.999. The *t* test for the correlation coefficient (*R*) [14] was all the time positive (H_1), i.e., there was always a significant correlation; the test for linearity [13] was positive in 93% of the cases, i.e., the calibration function had a linear behavior in 93% of the results (Fig. 3). When the values of the first period were utilized as control values, it was found that all the values of the second period were inside the interval of the control values. It was also found that the correlation was always high ($R^2 > 0.999$), with

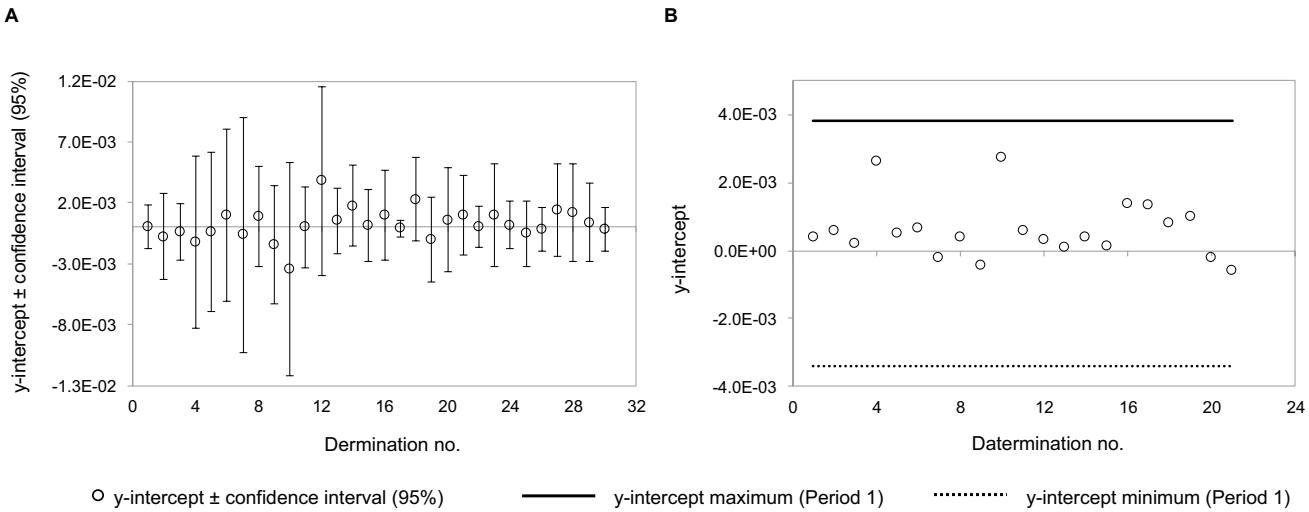

Fig. 1 **A** Evaluation in the y-intercept ± confidence interval (95%) along Period 1; **B** Evaluation in the y-intercept along Period 2

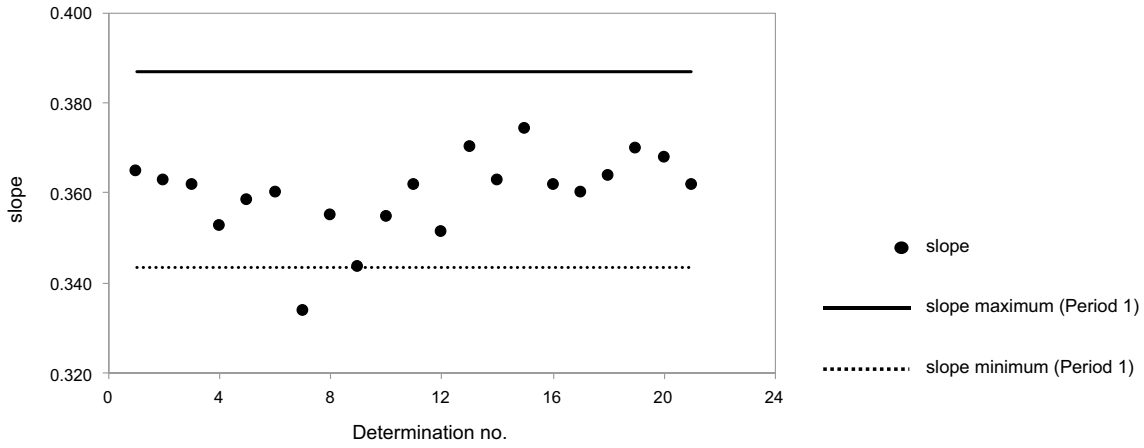

Fig. 2 Evaluation of slope along years Period 2

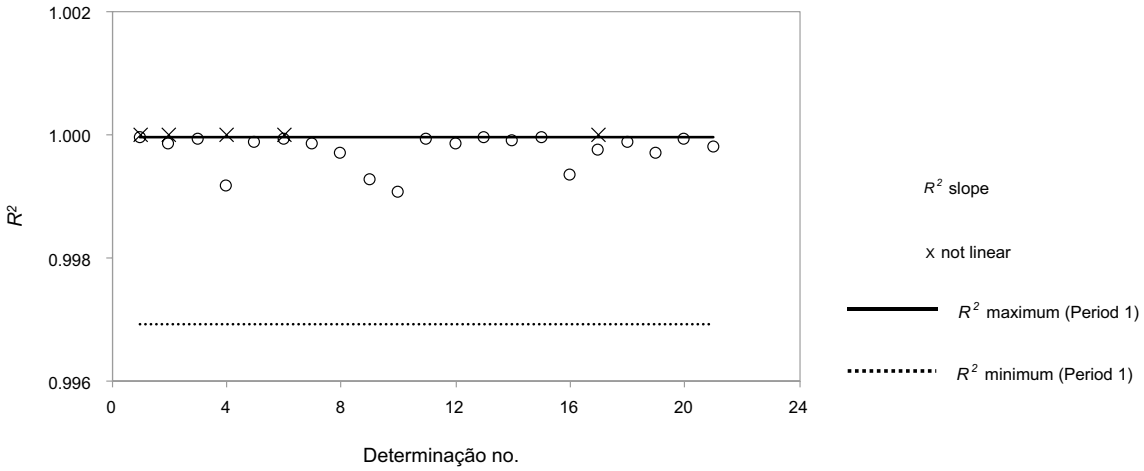

Fig. 3 Evaluation of correlation and linearity along Period 2

the *t* test always positive, albeit the test for linearity was 76% positive. Although this result was obtained for the linearity test, the minimum value presented for the correlation, R^2, in this period was 0.99907.

The analytical limits, detection and quantification limits [14], obtained in the first period, presented minimum and average values that were quite low. During this period, 80% of the results obtained for the quantification limit were lower than 0.060 mg P L^{-1}. The average values for detection and quantification limits were 0.016 and 0.050 mg P L^{-1}, and the amplitude between the maximum and minimum values was 0.034 and 0.103 mg P L^{-1}, respectively. All values obtained in the second period (100%) were lower than the maximum values obtained in the first period. The maximum and average values for detection and quantification limits decreased in the second period, and the amplitude between their maximum and minimum values also decreased (Fig. 4). The results of the calibration performance seem to indicate improvements over time, since the maximum values for the limits of detection and quantification varied from 0.037 mg P L^{-1} and 0.112 mg P L^{-1} to 0.022 mg P L^{-1} and 0.068 mg P L^{-1}; the average values of 0.016 mg P L^{-1} and 0.103 mg P L^{-1} to 0.0092 mg P L^{-1} and 0.028 mg P L^{-1}; and the amplitudes between maximum and minimum values of 0.034 mg P L^{-1} and 0.103 mg P L^{-1} to 0.019 mg P L^{-1} and 0.058 mg P L^{-1}, respectively.

The operational range was determined by using the maximum value of the quantification limit as the lower limit and the concentration of the standard more concentrated on the calibration curve as the upper limit. For the lower limit, the average value of the quantification limit was not used to ensure that all determined values correspond to a value that is truly quantifiable. By analyzing of the values presented in Table 2, it can be said that the lower limit of the working range decreased from the first to the second period (0.112 mg P L^{-1} to 0.068 mg P L^{-1}),

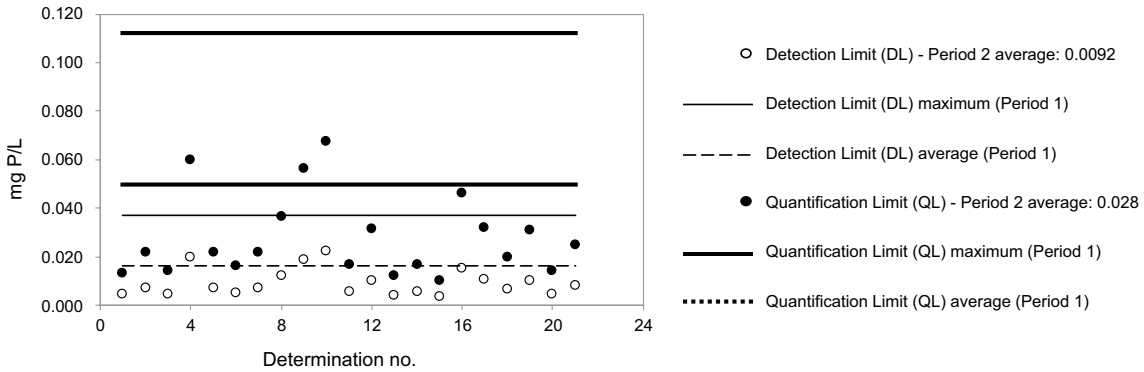

Fig. 4 Evaluation of the detection limit (DL) and quantification limit (QL) during Period 2

resulting in an increase in the working range. Under test conditions, for samples of meat and meat products, the lower limit of the working range decreased from 64.2 mg P_2O_5/kg to 39.0 mg P_2O_5/kg from period 1 to period 2, values well below the limit value of 5000 mg P_2O_5/kg.

Relative to precision parameters calculated according to ISO 8466-1 [13], it was verified that the obtained values for the method's standard deviation (s_{x0}) were small and the values of the variation coefficient were lower than 5.1%. When the average value of standard deviation of the method (0.0057 mg P L^{-1}) and the maximum and average values of variation coefficient (5.05 and 2.22%, respectively) were used as control values for the second period, it was verified that 90% of the obtained values for the method's standard deviation in the second period were lower than the average value of the first period; in the last period, the dispersion of results was lower, with an average value for standard deviation of 0.0033 mg P L^{-1} (compared with 0.0057 mg P L^{-1}); and the maximum and average values of variation coefficient decreased when compared with the first period (Fig. 5).

The method's trueness was evaluated by comparing the laboratory analytical values obtained in interlaboratory tests with the reference values in these tests. The comparison was made with the relative error and the range of uncertainty associated with the reference value ($\bar{X}_{RL} \pm 3S_{RL}$), which is the last criterion used by the interlaboratory organization. The relative error obtained in the orthophosphate analytical determination in interlaboratory tests was always below 7%, with 3.75% being the maximum relative error for the second period. The average value of this parameter decreased from 3.83% in the first period to 1.53% in the second period (Table 2). In the evaluation of data performance by interlaboratory tests, it was observed that all the values obtained for the determination of orthophosphates in natural waters were within the uncertainty intervals associated with the reference values and therefore considered satisfactory (Fig. 6). All determinations of the orthophosphate parameter in natural waters had satisfactory evaluations in the interlaboratory tests (Fig. 6).

The orthophosphate determination in natural waters only involves the third stage of the analytical procedure studied, i.e., molecular absorption spectrophotometry (MAS). The use of the ascorbic acid spectrophotometric method for the determination of orthophosphates appears to be valid for several concentration ranges (Fig. 6) and seems to be

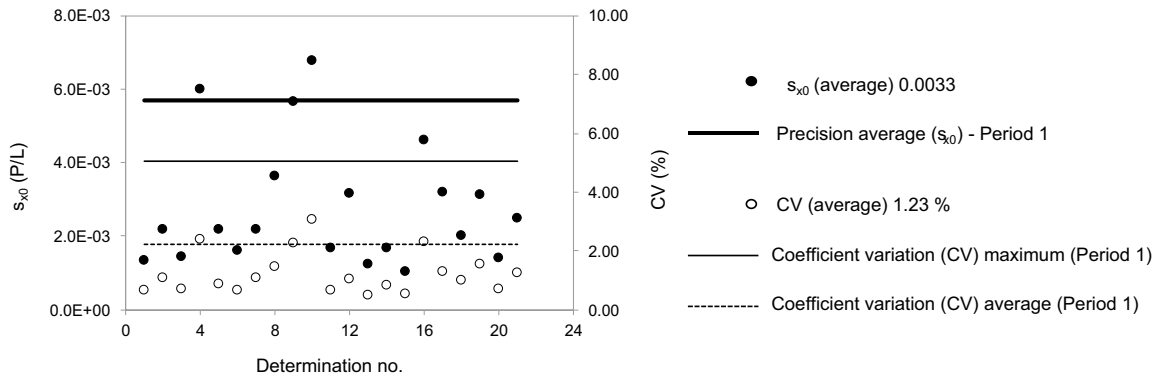

Fig. 5 Evaluation of the precision (s_{x0}) and variation coefficient (CV) along Period 2

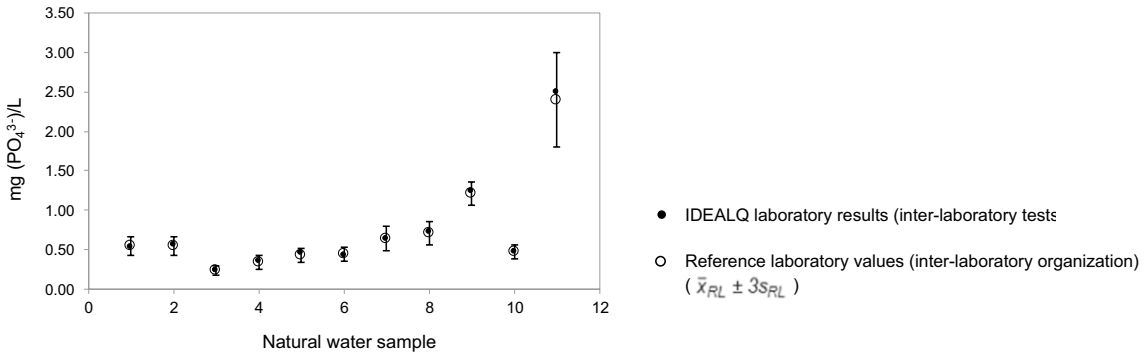

Fig. 6 Performance evaluation for the results of orthophosphate determinations in natural waters in interlaboratory tests

suitable for quantitatively determining inorganic phosphorus at various concentration ranges.

Evaluation of the sample digestion for phosphorus determination

The results obtained for the total phosphorus determination in the wastewater matrix in interlaboratory tests were taken into account for the evaluation of the digestion stage by the persulfate method. In the comparative study of the laboratory results and the reference values of these tests for the total phosphorus in wastewater, the relative error for the experimental determinations was always less than 5%. When the performance of the experimental results in the interlaboratory tests was evaluated, it was verified that the values of all the analytical determinations were within the uncertainty intervals associated with the reference values and, therefore, considered to have satisfactory performance. The total phosphorous values from the IDEALQ laboratory were always satisfactory in the wastewater matrix analysis (Fig. 7).

The determination of total phosphorus in wastewater involves sample digestion by persulfate and molecular absorption spectrophotometry (MAS) by the ascorbic acid method. The method used for the digestion of the samples was appropriate since the digestion seems to be complete for the several ranges of phosphorus concentrations, i.e., the persulfate digestion method appears to be suitable to mineralize all the phosphorus present in the samples for different ranges of phosphorus concentrations (Table 3).

Evaluation of the sample preparation for the digestion process for phosphorous determination in meat and meat products

The laboratory results obtained for total phosphorus in interlaboratory tests for meat and meat products by two analytical methods, an alternative method (AM) and the reference method [10], were compared with each other and with the values of the reference laboratory. A performance evaluation of the results obtained by the two methods in interlaboratory tests was also carried out. The reference values of these tests were obtained by ISO 2294:1974 [10]. The analytical results obtained by the IDEALQ laboratory were carried out in triplicate.

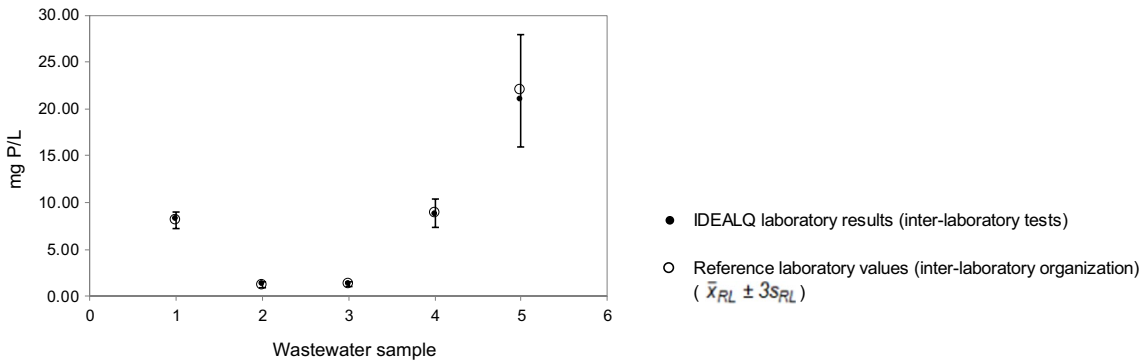

Fig. 7 Performance evaluation for the results of total phosphorus determinations in wastewaters in the interlaboratory tests

a) Analysis of the results obtained by the 2 methods.

In Table 4, the results obtained for the total phosphorous in meat and meat products using the two methods studied are presented.

The phosphorus results obtained by the two analytical methods were slightly different; however, the alternative method determinations appeared to be more precise (replicates with a lower standard deviation and related parameters such as the coefficient of variation and 95% confidence interval).

The average values obtained for the determinations by the two methods were similar, with maximum coefficients of variation of 2.5% and 4.1% for the alternative and reference methods, respectively. The precision of the results was high in both methods since the dispersion of the replicated values within the analytical results was low (average of the standard deviation: 0.0086% and 0.017% for the alternative and reference methods, respectively), and the data seems to indicate a lower dispersion of values in the alternative method (lower standard deviation values associated with average values). Similar considerations can be assessed from the results obtained for coefficients of variation (coefficients of variation: 1.6% and 3.2% for alternative and reference methods, respectively). Although the average values obtained by the two methods were similar, the confidence interval of the

Table 3 Trueness evaluated by the interlaboratory tests in wastewater

Parameter	Period 1 and Period 2			
	Minimum	Maximum	Medium	
Natural water: orthophosphate				Determination stage
Trueness (relative error) (%)	0.00	6.98	2.78	– Spectrophotometric determination
Performance evaluation	All satisfactory determinations			
Wastewater: total phosphorus				Determination stage
Trueness (relative error) (%)	1.23	4.55	2.45	– Sample digestion
Performance evaluation	All satisfactory determinations	– Spectrophotometric determination		

Table 4 Precision evaluated by the interlaboratory tests in meat and meat products

Sample	Alternative method IM phosphorous-880 nm				Reference method ISO 2294:1974; NP 1842:1982			
	Average % P_2O_5	s % P_2O_5	CV % P_2O_5	CI % P_2O_5	Average % P_2O_5	s % P_2O_5	CV % P_2O_5	CI % P_2O_5
1	0.434	0.0079	1.8	± 0.020	0.420	0.0076	1.8	0.019
2	0.431	0.0047	1.1	± 0.012	0.42	0.017	**4.1**	0.043
3	0.696	0.0079	1.1	± 0.020	0.67	0.023	3.4	0.056
4	0.50	0.012	**2.5**	± 0.030	0.50	0.018	3.5	0.044

s standard deviation, *CV* coefficient of variation, *CI* confidence interval (95%)

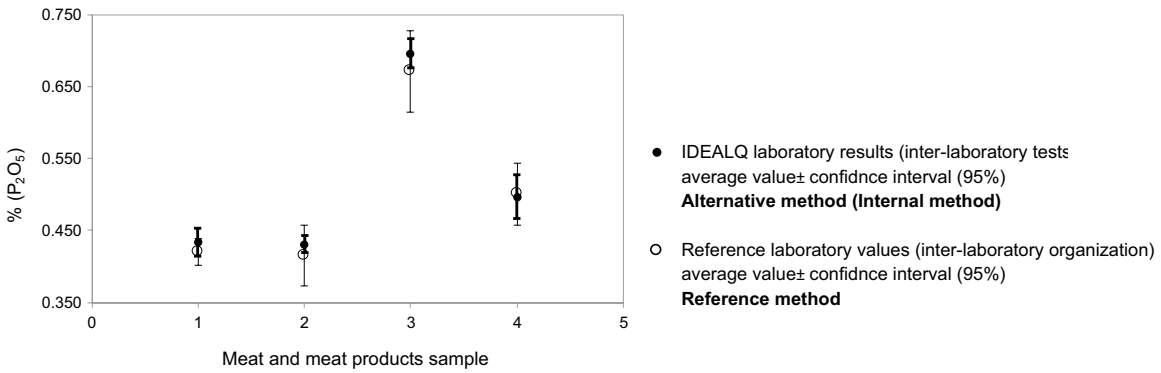

Fig. 8 Average values and confidence intervals (95%) for total phosphorous determinations in meat and meat products

alternative method was lower, appearing frequently within the confidence interval of the reference method (Fig. 8).

b) Trueness evaluation with interlaboratory tests.

The trueness evaluation [15] was initially used to compare the experimental results from the two analytical procedures to the reference value. The truthfulness can be assessed by the relative error (RE) and z-score. In this study, the reference value of the interlaboratory session was considered the true value of the phosphorus. The relative error of all determinations was always less than 7%, with the values shown by the alternative method having determinations generally lower, i.e., determinations with more trueness. The maximum error obtained by the alternative method was 4.44%, and by the reference method it was 6.67% (Table 5). Values below the 10% barrier were, generally defined as the maximum acceptable value.

The performance evaluation of the results in interlaboratory tests showed that all the results were within the standard deviation range associated with the reference value ($\bar{X}_{RL} \pm 3S_{RL}$), with the values obtained by the alternative method closer to the reference laboratory values (Fig. 9).

When the z-score criterion was used as a tool for performance evaluation, the results of all determinations were accepted as satisfactory by the organizing entity (Table 4; Fig. 10). The alternative method showed lower values of the z-score for all the determinations, appearing to indicate values with more trueness. The acceptance criterion was |z-score| ≤2 for an acceptable result, 2<| z-score| <3 for a questionable result, and |z-score| ≥3 for an unacceptable result.

The total phosphorus determination in meat or meat products involves all stages of the procedure in the study: spectrophotometric quantification by the ascorbic acid method, sample digestion by the persulfate method, and sample treatment for digestion.

As previously mentioned, spectrophotometric measurement by the ascorbic acid method appears to be adequate for the quantification of inorganic phosphorus, and the digestion of the sample by the persulfate method seems appropriate for the total mineralization of the samples. Consequently, the results obtained at this stage of the study seem to indicate that the procedure used in preparing the sample for digestion can be applied to this type of sample.

Conclusions

The statistical parameters evaluated in the spectrophotometric method of ascorbic acid showed an optimization trend for the method over time. In the interlaboratory tests, the results obtained by the alternative method appeared to be normally more precise, with lower relative errors seeming

Table 5 Comparison of the experimental results with the reference values

Sample	Reference laboratory (RL)	*s* (RL)	IDEALQ laboratory (AM)	RE (AM) (%)	*z-score* (AM)	IDEALQ Laboratory (RM)	RE (RM) (%)	*z-score* (RM)
1	0.45	0.018	0.434	4.44	− 1.11	0.420	6.67	− 1.67
2	0.43	0.022	0.431	0.00	0.00	0.42	2.33	− 0.45
3	0.69	0.013	0.696	1.45	0.77	0.67	2.90	− 1.54
4	0.51	0.015	0.50	1.96	-0.67	0.50	1.96	− 0.67

|z| ≤2—acceptable result, 2< |z| <3—questionable result, |z| ≥3—unacceptable result

AM alternative method, *RM* reference method, *RE* relative error

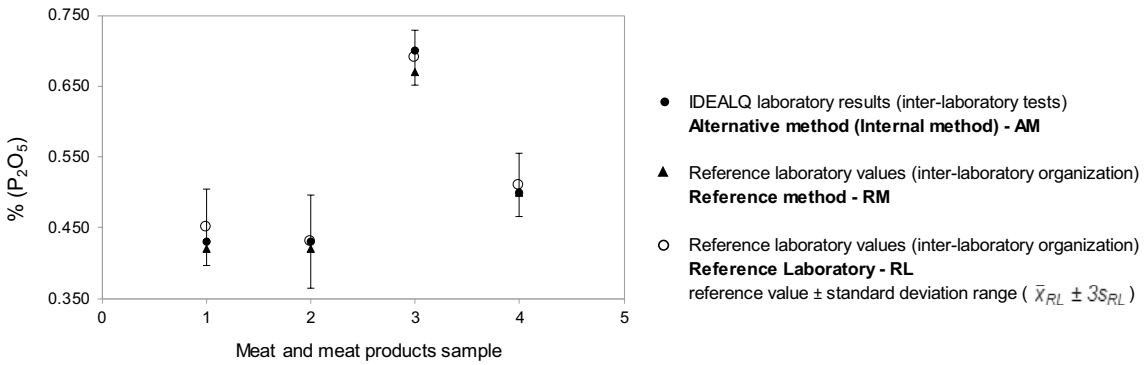

Fig. 9 Analysis of the results of the interlaboratory tests

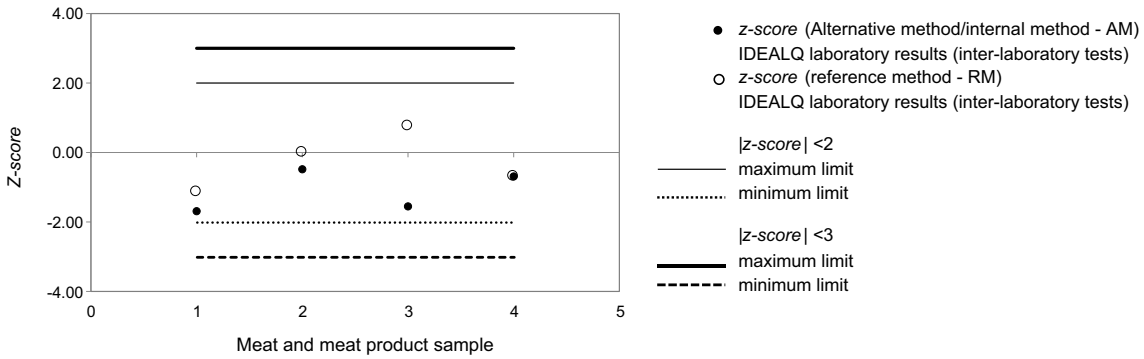

Fig. 10 Evaluation of performance in the interlaboratory tests (Z-score)

to indicate better accuracy and consequently lower absolute values of the z-score.

It should be noted that all outputs in interlaboratory tests, whether in determining orthophosphates or total phosphorous, had satisfactory performances. Satisfactory results for the determination of orthophosphate in natural waters allowed us to demonstrate the acceptability of the calibration model for the quantification of inorganic phosphorus in diverse concentration ranges, the third stage of the analytical procedure. Satisfactory results for the determination of total phosphorus in wastewater allowed us to demonstrate the efficiency of the digestion method to mineralize all the phosphorus present in the sample, the second stage of the analytical procedure. Satisfactory results for the determination of total phosphorus in meat and meat products allowed us to show that the conditions selected in the treatment of the sample for digestion were adequate to obtain representative samples, the first step of the analytical procedure. The new approach uses less reagent and enhances workflow efficiency with reduced staff time commitment, which lowers the cost of the analysis. In conclusion, the alternative analytical method is applicable for the determination of total phosphorus in the meat and meat products matrix.

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