

Research Article

Method Validation and Uncertainty Estimation for Total Phosphorus Determination in Animal Feed Using UV-Vis Spectrophotometer

Debnath Manika¹ and Abrham Ayele^{2*}¹Section of Feed Quality Control, Quality Control Laboratory, Department Of Livestock Services, Bangladesh²College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia***Corresponding author:** Abrham Ayele, College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia**Received:** May 31, 2022; **Accepted:** June 20, 2022;**Published:** June 27, 2022**Abstract**

Objective: The study presents the validation protocol for the determination of total phosphorus content at parts per million (ppm) ($\mu\text{g/L}$) levels in animal feed by a UV-Vis spectrophotometer.

Methods: The measured absorbance of solutions against the blank solution was at 400 nm with the spectrophotometer. A combined ammonium heptamolybdate tetrahydrate and ammonium monovanadate blue solution was used as a coloring reagent for detection. This method was validated by evaluation of statistical parameters such as linearity, sensitivity, limits of detection (LOD) and quantification (LOQ), precision, accuracy, and measurement uncertainty using a matrix blank (MB) against the phosphorus standard.

Results: The Instrumental Detection Limit was 0.066 ppm and the Instrumental Quantification Limit was 0.22 ppm, respectively, while the phosphorus recovery and repeatability percent were 101.15% and 0.11%, respectively. However, the linearity of this method was 0.1 to 30 ppm. The measurement uncertainty of this method was 2.82%, following Commission Regulation (EC).

Conclusion: The estimated parameters in the validation protocol, were found to meet the imposed performance criteria, and the procedure was validated for the intended use.

Keywords: UV-Vis Spectrophotometer; Phosphorus; Method Validation; Animal Feed; Measurement Uncertainty

Introduction

Validation is a matter of huge importance as it attests to the capability of the laboratory to provide reliable results. For any method validation study, the laboratory will require to investigate several performance characteristics- Accuracy, Linearity, Precision, reproducibility, Limit of detection, Limit of Quantification, Measurement uncertainty, etc. Exactly which characteristics are needed will depend on the analytical application. According to Eurachem, [1] method validation is the process whereby the laboratory demonstrates whether or not a method is 'fit for purpose'. Phosphorus is a very essential mineral for animal nutrition. So knowing the Phosphorus content in animal feed is hugely essential for farmers and feed producers. ISO [2] method is applicable to animal feeding stuffs with a phosphorus content of less than 50 g/kg. Following this method, the validation in an analytical laboratory was taken and followed the Commission Regulation (EC) [3] for the statistical parameter analysis. The spectrophotometric molybdenum blue method for phosphorus determination is cheap and eco-friendly due to the application of small volumes of reagents [4]. In the presence of molybdate-vanadate reagent agents, phosphorus in the sample absorb UV light at 400 nm, depending on the phosphorus content light absorption can be stronger.

Materials and Methods

Solutions/Reagents Preparation

Preparing an ammonium molybdate solution was the initial activity. It was done by dissolving 25 g of ammonium molybdate in 300 ml of water. Allow to cool at room temperature after mixing. 1.25 g ammonium monovanadate was dissolved in 330 mL HCL acid once more. The solution was then heated until it boiled, at which point it was dissolved. Allowing the dissolved solution to cool to room temperature was the next step. Finally, the two solutions were properly mixed, and the volume was increased to 1 liter with distilled water. This final solution is kept in the refrigerator for 12 days.

Phosphorus Standard Preparation

Pure phosphorus standard solutions were created for the first time. To accomplish so, standard solutions ranging from 0.5 to 60 ppm were utilized to test the instrument's linearity. For the results of other statistical parameters, however, standards ranging from 0.5 ppm to 15 ppm were utilized to build a calibration curve. Five ml of reagent and the needed amount of pure phosphorus standard were placed in each 25 ml volumetric flask for the calibration curve, and the volume was volumeted up to 25ml with distilled water.

Sample Preparation

Matrix Blank (MB), a laboratory-made animal feed sample, was

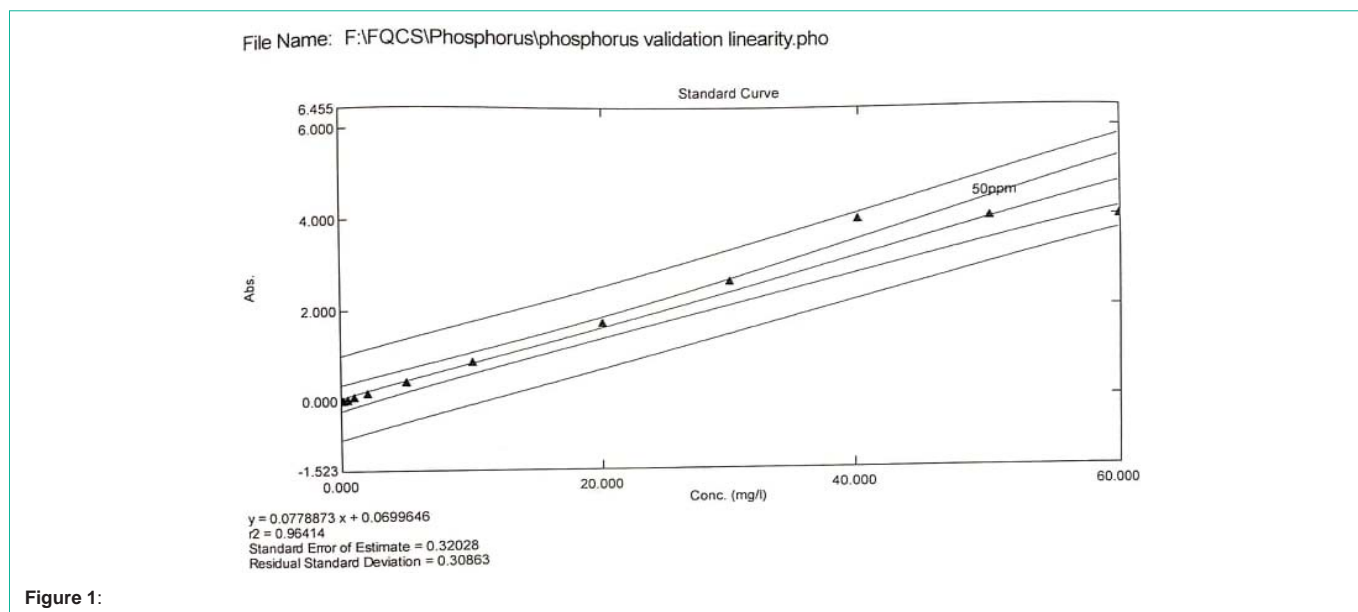


Figure 1:

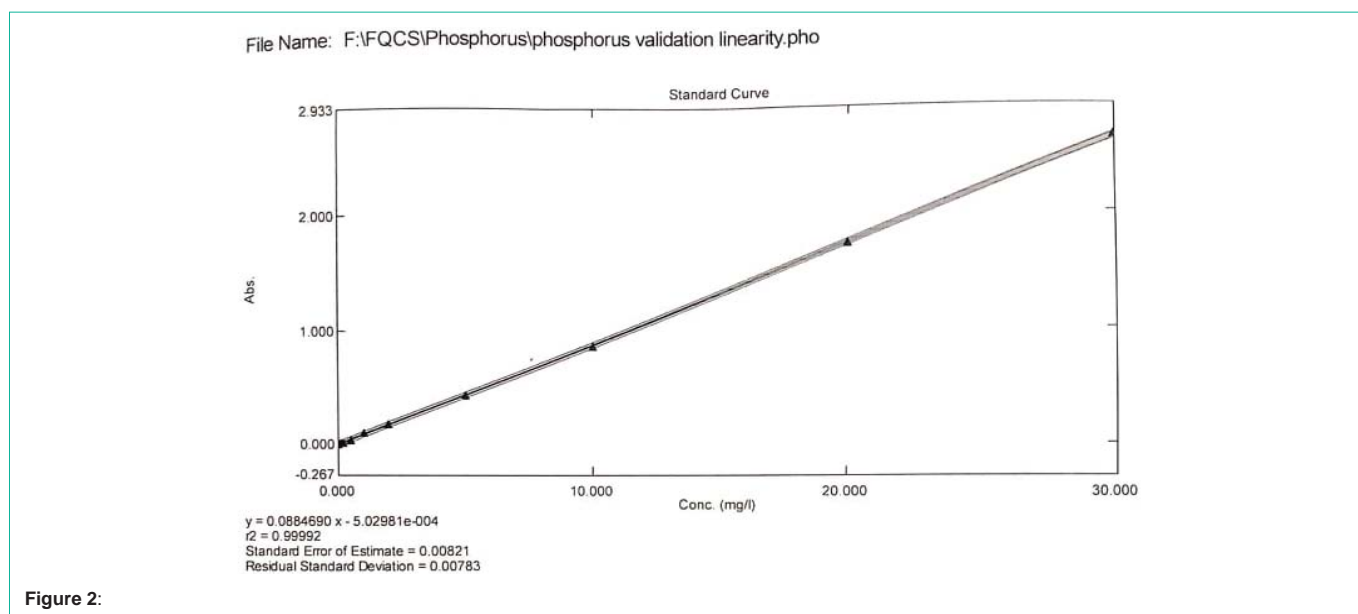


Figure 2:

used for statistical analysis. In a muffle furnace, 0.5g of matrix blank was burned to ash for 2 hours at 550°C. The ash was then digested for 5-6 minutes with a 1:1 concentration of nitric acid solution. The sample was filtered after cooling, and the volume was raised to 50 ml with distilled water. Then 1mL of MB solution was combined with 5mL of ammonium molybdate–vanadate reagent and distilled water was added up to the mark of a 25 mL volumetric flask. To assure color development, all of the prepared solutions were left for 20 minutes and the absorbance was measured at 400 nm against a reagent blank.

Validation Procedure

Statistical Analysis: The generic linear model approach proposed by the Statistics Analysis System Institutes [5] was used to examine all experimental data. The other data was examined automatically using the UV-Vis spectrophotometer’s software. The standard deviation

and regression coefficient were used to express the data’s variability (r2). For the validation of this approach in accordance with the EC standard, the following statistical studies were conducted.

1. Linearity
2. Limit of Detection
3. Limit of Quantification
4. Accuracy
5. Recovery
6. Repeatability/ Precision
7. Reproducibility
8. Measurement Uncertainty

Results and Discussion

Linear Range and Calibration Curve: To determine the linear range and calibration curve fourteen (14) sets of standard (starting from 0.1 ppm to 60ppm) were prepared from the 1000 ppm standard solution. Each data set contains a triplicate measurement reading. According to Figures 1 and 2, it is found that linearity would be up to 30 ppm. Therefore, the calibration curve was prepared up to 30 ppm on three different days and the average absorbance against concentration have been plotted in (Figure 1 & 2), and regression coefficient (r₂), SE, and RSD were calculated. Huang and Zhang [6] discussed the effects of Phosphorus analysis by the molybdate malachite green method resulting in R₂= 0.9512. However, in this validation, the regression coefficient (r₂) of the calibration curve was 0.9992 for up to 30 ppm level, whereas after crossing 30ppm standard in the calibration, the regression coefficient (r₂) was drop down to 0.96414 and the calibration curve was not possessed in a straight line. So, the tested instrument can read and can maintain the linearity of the curve up to 30ppm. In contrast, the linearity was also up to 30 µg of phosphorus/mL during the validation of phosphorus evaluation in dairy products by UV-Vis Spectrophotometer [4]. Therefore, r₂ (0.9992), Standard Error (0.00821), and RSD % (0.00783) are acceptable.

Limit of Detection and Limit of Quantification

The detection limit was first tried to calculate from the Matrix blank analysis data. Phosphorus was detected in the Matrix blank. Therefore, Matrix blank was analyzed 21 times. The CV was 1.41%,

Table 1: LOD and LOQ.

| Sl. No. | Sample ID | (Conc.) ppm | Mean Conc. ppm | SD | CV% | LOD | LOQ |
|---------|-----------|----------------|-------------------|-------|------|-------|------|
| 1 | MB-1 | 1.511 | 1.56 | 0.022 | 1.41 | 0.066 | 0.22 |
| 2 | MB-2 | 1.538 | | | | | |
| 3 | MB-3 | 1.551 | | | | | |
| 4 | MB-4 | 1.548 | | | | | |
| 5 | MB-5 | 1.528 | | | | | |
| 6 | MB-6 | 1.548 | | | | | |
| 7 | MB-7 | 1.579 | | | | | |
| 8 | MB-8 | 1.577 | | | | | |
| 9 | MB-9 | 1.582 | | | | | |
| 10 | MB-10 | 1.606 | | | | | |
| 11 | MB-11 | 1.575 | | | | | |
| 12 | MB-12 | 1.582 | | | | | |
| 13 | MB-13 | 1.572 | | | | | |
| 14 | MB-14 | 1.58 | | | | | |
| 15 | MB-15 | 1.563 | | | | | |
| 16 | MB-16 | 1.547 | | | | | |
| 17 | MB-17 | 1.538 | | | | | |
| 18 | MB-18 | 1.547 | | | | | |
| 19 | MB-19 | 1.54 | | | | | |
| 20 | MB-20 | 1.546 | | | | | |
| 21 | MB-21 | 1.551 | | | | | |

and the Standard deviation was 0.022.

Data are shown in (Table 1).

Instrument detection limit (IDL) and Instrument quantification limit (IQL) are calculated using following formula: Instrument detection limit (IDL) = 3 s and Instrument quantification limit (IQL) = 10 s

Where, s= standards deviation

In the present case, s is 0.022

- Instrument detection limit (IDL) = 3*0.022= 0.066ppm
- Instrument Quantification limit (IQL) = 10* 0.022= 0.22ppm

Acceptance Criteria [3]: LOD less than one tenth of the maximum level (0.5mg/kg) and LOQ less than one fifth of the maximum level. So, Method LOD and LOQ have met the criteria.

Anna Gliszczynska-Świgło [7] was researched on dairy products to validate the determination of Dairy products. According to them, the method's limit of detection (LOD) was 0.37 ppm and its limit of quantification (LOQ) was 1.13 ppm. An extensive experiment [8] was conducted on the ultra-sonication of minerals in swine feed using UV-Vis spectrophotometer revealed the LOQ of that method of validation was 14ppm. Whereas, in this method, the LOD and LOQ were within the minimum range.

Recovery percentage (%)

Three sets of spike samples (5ppm, 10ppm, and 15ppm) were prepared and each set of spike samples contained seven replicate samples with a triplicate measurement reading. Calculated data are given in (Table 2). The Recovery was found at 96.59% at 5ppm level, 102.41% at 10ppm level, and 104.45% at 15 ppm level respectively, and the overall Recovery was 101.15%. Acceptance Criteria [3]: Recovery % at mass fractions shall be in the range – 20 % to + 10 %. So, Recovery at 5ppm, 10ppm, and 15ppm levels have met the criteria.

Repeatability (Precision) and Stability

Acceptance Criteria [3]: According to the EC guideline, the coefficient of Variation % (CV) of the mean should not exceed 15% for quantitative methods at a range of element mass fractions > 100 µg/kg to 1000 µg/kg (0.1ppm to 1ppm). Here, repeatability (CV% or precision) was found 0.11 % at 5ppm level with mean value 6550 µg/kg (Table 3), 10ppm level with mean value 11200 µg/kg (Table 4), and 15 ppm level with a mean value of 15220 µg/kg (Table 5). The samples were measured in three consecutive days for the calculation of repeatability. So repeatability is also acceptable and concentration remains almost stable with time.

Reproducibility

For a precision check on repeatability, the same analyzer generated three sets of samples once more after 15 days. However, other analysts produced these three sets of samples after 8 days, spiking them at levels of 5 ppm, 10 ppm, and 15 ppm, and testing them as before. The mean concentration, standard deviation, and coefficient of variation (%) for each level of fortified samples were calculated.

The aggregate mean concentrations and CVs of the fortified samples were then calculated. With precision of 2.93 (Table 6), 2.13

Table 2:

| Recovery % | | | | | | | | |
|------------|----------|------------------------|-----------------|--------------|--------------|------------|-----------------|--------------------|
| SI | Code | Measured Concentration | MB Concentation | Standard ppm | MB+spike ppm | Recovery % | Mean Recovery % | Overall Recovery % |
| 1 | 5ppm+MBa | 6.757 | 1.568 | 5.236 | 6.8 | 100.7 | 96.59 | 101.15 |
| 2 | 5ppm+MBb | 6.761 | 1.568 | 5.236 | 6.49 | 95.95 | | |
| 3 | 5ppm+MBc | 6.762 | 1.568 | 5.236 | 6.49 | 95.93 | | |
| 4 | 5ppm+MBd | 6.762 | 1.568 | 5.236 | 6.49 | 95.93 | | |
| 5 | 5ppm+Mbe | 6.762 | 1.568 | 5.236 | 6.49 | 95.93 | | |
| 6 | 5ppm+MBf | 6.765 | 1.568 | 5.236 | 6.49 | 95.89 | | |
| 7 | 5ppm+MBg | 6.774 | 1.568 | 5.236 | 6.49 | 95.76 | | |
| 1 | 5ppm+MBa | 11.548 | 1.568 | 10.27 | 11.84 | 102.51 | 102.41 | |
| 2 | 5ppm+MBb | 11.549 | 1.568 | 10.27 | 11.84 | 102.5 | | |
| 3 | 5ppm+MBc | 11.559 | 1.568 | 10.27 | 11.84 | 102.41 | | |
| 4 | 5ppm+MBd | 11.561 | 1.568 | 10.27 | 11.84 | 102.4 | | |
| 5 | 5ppm+Mbe | 11.564 | 1.568 | 10.27 | 11.84 | 102.37 | | |
| 6 | 5ppm+MBf | 11.563 | 1.568 | 10.27 | 11.84 | 102.38 | | |
| 7 | 5ppm+MBg | 11.571 | 1.568 | 10.27 | 11.84 | 102.31 | | |
| 1 | 5ppm+MBa | 15.695 | 1.568 | 14.846 | 16.41 | 104.58 | 104.45 | |
| 2 | 5ppm+MBb | 15.704 | 1.568 | 14.846 | 16.41 | 104.52 | | |
| 3 | 5ppm+MBc | 15.717 | 1.568 | 14.846 | 16.41 | 104.43 | | |
| 4 | 5ppm+MBd | 15.711 | 1.568 | 14.846 | 16.41 | 104.47 | | |
| 5 | 5ppm+Mbe | 15.715 | 1.568 | 14.846 | 16.41 | 104.45 | | |
| 6 | 5ppm+MBf | 15.724 | 1.568 | 14.846 | 16.41 | 104.39 | | |
| 7 | 5ppm+MBg | 15.732 | 1.568 | 14.846 | 16.41 | 104.34 | | |

Table 3: Repeatability% at 5 ppm level.

| Repeatability % 5ppm Level | | | | | | |
|----------------------------|----|----------|------------------------|------|-------------------|------|
| Day | SI | Code | Measured Concentration | Mean | Standar Deviation | CV% |
| 1 | 1 | 5ppm+MBa | 6.547 | 6.55 | 0.007 | 0.11 |
| | 2 | 5ppm+MBb | 6.551 | | | |
| | 3 | 5ppm+MBc | 6.552 | | | |
| | 4 | 5ppm+MBd | 6.552 | | | |
| | 5 | 5ppm+Mbe | 6.552 | | | |
| | 6 | 5ppm+MBf | 6.555 | | | |
| | 7 | 5ppm+MBg | 6.564 | | | |
| 2 | 1 | 5ppm+MBa | 6.548 | | | |
| | 2 | 5ppm+MBb | 6.552 | | | |
| | 3 | 5ppm+MBc | 6.554 | | | |
| | 4 | 5ppm+MBd | 6.553 | | | |
| | 5 | 5ppm+Mbe | 6.554 | | | |
| | 6 | 5ppm+MBf | 6.557 | | | |
| | 7 | 5ppm+MBg | 6.566 | | | |
| 3 | 1 | 5ppm+MBa | 6.536 | | | |
| | 2 | 5ppm+MBb | 6.54 | | | |
| | 3 | 5ppm+MBc | 6.542 | | | |
| | 4 | 5ppm+MBd | 6.541 | | | |
| | 5 | 5ppm+Mbe | 6.542 | | | |
| | 6 | 5ppm+MBf | 6.545 | | | |
| | 7 | 5ppm+MBg | 6.553 | | | |

Table 4: Repeatability% at 10 ppm level.

| Repeatability % 10ppm Level | | | | | | |
|-----------------------------|----|-----------|------------------------|-------|-------------------|------|
| Day | Sl | Code | Measured Concentration | Mean | Standar Deviation | CV% |
| 1 | 1 | 10ppm+MBa | 11.189 | 11.20 | 0.01 | 0.11 |
| | 2 | 10ppm+MBb | 11.19 | | | |
| | 3 | 10ppm+MBc | 11.2 | | | |
| | 4 | 10ppm+MBd | 11.202 | | | |
| | 5 | 10ppm+Mbe | 11.205 | | | |
| | 6 | 10ppm+MBf | 11.204 | | | |
| | 7 | 10ppm+MBg | 11.212 | | | |
| 2 | 1 | 10ppm+MBa | 11.192 | | | |
| | 2 | 10ppm+MBb | 11.193 | | | |
| | 3 | 10ppm+MBc | 11.202 | | | |
| | 4 | 10ppm+MBd | 11.205 | | | |
| | 5 | 10ppm+Mbe | 11.208 | | | |
| | 6 | 10ppm+MBf | 11.207 | | | |
| | 7 | 10ppm+MBg | 11.215 | | | |
| 3 | 1 | 10ppm+MBa | 11.171 | | | |
| | 2 | 10ppm+MBb | 11.172 | | | |
| | 3 | 10ppm+MBc | 11.182 | | | |
| | 4 | 10ppm+MBd | 11.184 | | | |
| | 5 | 10ppm+Mbe | 11.187 | | | |
| | 6 | 10ppm+MBf | 11.187 | | | |
| | 7 | 10ppm+MBg | 11.194 | | | |

Table 5: Repeatability% at 15 ppm level.

| Repeatability % 15ppm Level | | | | | | |
|-----------------------------|----|-----------|------------------------|-------|-------------------|------|
| Day | Sl | Code | Measured Concentration | Mean | Standar Deviation | CV% |
| 1 | 1 | 15ppm+MBa | 15.208 | 15.22 | 0.02 | 0.11 |
| | 2 | 15ppm+MBb | 15.216 | | | |
| | 3 | 15ppm+MBc | 15.229 | | | |
| | 4 | 15ppm+MBd | 15.224 | | | |
| | 5 | 15ppm+Mbe | 15.227 | | | |
| | 6 | 15ppm+MBf | 15.235 | | | |
| | 7 | 15ppm+MBg | 15.244 | | | |
| 2 | 1 | 15ppm+MBa | 15.212 | | | |
| | 2 | 15ppm+MBb | 15.22 | | | |
| | 3 | 15ppm+MBc | 15.233 | | | |
| | 4 | 15ppm+MBd | 15.227 | | | |
| | 5 | 15ppm+Mbe | 15.231 | | | |
| | 6 | 15ppm+MBf | 15.239 | | | |
| | 7 | 15ppm+MBg | 15.247 | | | |
| 3 | 1 | 15ppm+MBa | 15.184 | | | |
| | 2 | 15ppm+MBb | 15.192 | | | |
| | 3 | 15ppm+MBc | 15.204 | | | |
| | 4 | 15ppm+MBd | 15.199 | | | |
| | 5 | 15ppm+Mbe | 15.203 | | | |
| | 6 | 15ppm+MBf | 15.211 | | | |
| | 7 | 15ppm+MBg | 15.219 | | | |

(Table 7), and 2.13 (Table 8) percent at 5 ppm, 10 ppm, and 15 ppm concentration levels, respectively, the overall mean concentrations were 6.8 ppm, 11.66 ppm, and 15.85 ppm, demonstrating acceptable reproducibility. According to EC [3] regulations, the coefficient of variation percent (CV) of the mean for quantitative methods at a range of element mass fractions of >100 g/kg to 1000 g/kg should not be higher than 15%. Calculated data are given in (Table 6-8).

Measurement Uncertainty

In general, the uncertainty is calculated by estimating the errors associated with the various stages of the analysis, e.g. pre-analytical effects, homogenization, weighing, pipetting, injection, extraction, derivatization, recovery, calibration curves. However, validation data e.g. accuracy and precision under, repeatability/reproducibility conditions already accounted for many of these factors [9].

Calculated the measurement uncertainty at 95% confidence interval is as follows:

$$1. \quad U = k * RSD$$

where: U = uncertainty, k = coverage factor (for 95% and 50 points, use 2; for less than 50 points, use the appropriate t statistic for 95%) [9].

$$\text{Here, } RSD = CV\% = 1.41$$

$$\text{So, } U = 2 \times 1.41 = 2.82\%$$

2. Calculated the measurement uncertainty interval for a measured value is as follows:

$$\text{Interval} = U * c$$

where: c = concentration

In contrast, the expanded uncertainty was 13.1% on the determination of phosphorus in different types of waste using the ICP-MS technique [10].

Conclusions

The study provided a procedure for the validation of the determination of phosphorus by UV-VIS spectrophotometer in the 400 nm range. The estimated parameters in the validation protocol, such as, limit of detection, internal repeatability, reproducibility, recovery, linearity of the working concentration range, and uncertainty) were found to meet the imposed performance criteria, and the procedure was validated for the intended use.

Recommendation

It is recommended that further research with a reference certified sample may use for revision of this validation procedure.

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Table 6: Reproducibility Precision Check data for P in feed at 5ppm level.

| Day | SI | Code | Measured Concentration | Concentration | MB | Recovery% | Mean Recovery% | Overall Recovery% | Mean | SD | CV% |
|-------|----|----------|------------------------|---------------|-------|-----------|----------------|-------------------|------|------|---------|
| Day 1 | 1 | 5ppm+MBa | 6.179 | 5.348 | 1.560 | 111.80 | 103.99 | 100.71 | 6.80 | 0.20 | 2.92936 |
| | 2 | 5ppm+MBb | 6.724 | 5.348 | 1.560 | 102.74 | | | | | |
| | 3 | 5ppm+MBc | 6.725 | 5.348 | 1.560 | 102.72 | | | | | |
| | 4 | 5ppm+MBd | 6.724 | 5.348 | 1.560 | 102.74 | | | | | |
| | 5 | 5ppm+Mbe | 6.725 | 5.348 | 1.560 | 102.72 | | | | | |
| | 6 | 5ppm+MBf | 6.728 | 5.348 | 1.560 | 102.68 | | | | | |
| | 7 | 5ppm+MBg | 6.737 | 5.348 | 1.560 | 102.54 | | | | | |
| Day 2 | 1 | 5ppm+MBa | 6.705 | 5.218 | 1.557 | 101.04 | 100.94 | 100.71 | 6.80 | 0.20 | 2.92936 |
| | 2 | 5ppm+MBb | 6.709 | 5.218 | 1.557 | 100.98 | | | | | |
| | 3 | 5ppm+MBc | 6.711 | 5.218 | 1.557 | 100.95 | | | | | |
| | 4 | 5ppm+MBd | 6.71 | 5.218 | 1.557 | 100.97 | | | | | |
| | 5 | 5ppm+Mbe | 6.711 | 5.218 | 1.557 | 100.95 | | | | | |
| | 6 | 5ppm+MBf | 6.714 | 5.218 | 1.557 | 100.91 | | | | | |
| | 7 | 5ppm+MBg | 6.723 | 5.218 | 1.557 | 100.77 | | | | | |
| Day 3 | 1 | 5ppm+MBa | 7.02 | 5.20 | 1.630 | 97.31 | 97.21 | 100.71 | 6.80 | 0.20 | 2.92936 |
| | 2 | 5ppm+MBb | 7.024 | 5.20 | 1.630 | 97.25 | | | | | |
| | 3 | 5ppm+MBc | 7.026 | 5.20 | 1.630 | 97.22 | | | | | |
| | 4 | 5ppm+MBd | 7.025 | 5.20 | 1.630 | 97.24 | | | | | |
| | 5 | 5ppm+Mbe | 7.026 | 5.20 | 1.630 | 97.22 | | | | | |
| | 6 | 5ppm+MBf | 7.029 | 5.20 | 1.630 | 97.18 | | | | | |
| | 7 | 5ppm+MBg | 7.038 | 5.20 | 1.630 | 97.06 | | | | | |

Table 7: Reproducibility Precision Check data for P in feed at 10 ppm level.

| Day | SI | Code | Measured Concentration | Concentration | MB | Recovery% | Mean Recovery% | Overall Recovery% | Mean | SD | CV% |
|-------|----|-----------|------------------------|---------------|-------|-----------|----------------|-------------------|-------|------|---------|
| Day 1 | 1 | 10ppm+MBa | 11.484 | 10.196 | 1.560 | 102.37 | 102.27 | 100.77 | 11.66 | 0.25 | 2.13156 |
| | 2 | 10ppm+MBb | 11.485 | 10.196 | 1.560 | 102.36 | | | | | |
| | 3 | 10ppm+MBc | 11.495 | 10.196 | 1.560 | 102.27 | | | | | |
| | 4 | 10ppm+MBd | 11.497 | 10.196 | 1.560 | 102.25 | | | | | |
| | 5 | 10ppm+Mbe | 11.5 | 10.196 | 1.560 | 102.23 | | | | | |
| | 6 | 10ppm+MBf | 11.5 | 10.196 | 1.560 | 102.23 | | | | | |
| | 7 | 10ppm+MBg | 11.508 | 10.196 | 1.560 | 102.16 | | | | | |
| Day 2 | 1 | 10ppm+MBa | 11.46 | 10.192 | 1.557 | 102.52 | 102.42 | 100.77 | 11.66 | 0.25 | 2.13156 |
| | 2 | 10ppm+MBb | 11.461 | 10.192 | 1.557 | 102.51 | | | | | |
| | 3 | 10ppm+MBc | 11.471 | 10.192 | 1.557 | 102.42 | | | | | |
| | 4 | 10ppm+MBd | 11.473 | 10.192 | 1.557 | 102.41 | | | | | |
| | 5 | 10ppm+Mbe | 11.476 | 10.192 | 1.557 | 102.38 | | | | | |
| | 6 | 10ppm+MBf | 11.476 | 10.192 | 1.557 | 102.38 | | | | | |
| | 7 | 10ppm+MBg | 11.483 | 10.192 | 1.557 | 102.32 | | | | | |
| Day 3 | 1 | 10ppm+MBa | 11.998 | 10.093 | 1.630 | 97.71 | 97.61 | 100.77 | 11.66 | 0.25 | 2.13156 |
| | 2 | 10ppm+MBb | 11.999 | 10.093 | 1.630 | 97.70 | | | | | |
| | 3 | 10ppm+MBc | 12.009 | 10.093 | 1.630 | 97.62 | | | | | |
| | 4 | 10ppm+MBd | 12.012 | 10.093 | 1.630 | 97.59 | | | | | |
| | 5 | 10ppm+Mbe | 12.015 | 10.093 | 1.630 | 97.57 | | | | | |
| | 6 | 10ppm+MBf | 12.014 | 10.093 | 1.630 | 97.58 | | | | | |
| | 7 | 10ppm+MBg | 12.023 | 10.093 | 1.630 | 97.50 | | | | | |

Table 8: Reproducibility Precision Check data for P in feed at 15ppm level.

| Day | SI | Code | Measured Concentration | Concentration | MB | Recovery% | Mean Recovery% | Overall Recovery% | Mean | SD | CV% |
|-------|----|-----------|------------------------|---------------|-------|-----------|----------------|-------------------|-------|------|---------|
| Day 1 | 1 | 15ppm+MBa | 15.609 | 14.764 | 1.560 | 104.58 | 104.46 | 103.38 | 15.85 | 0.34 | 2.13225 |
| | 2 | 15ppm+MBb | 15.617 | 14.764 | 1.560 | 104.53 | | | | | |
| | 3 | 15ppm+MBc | 15.63 | 14.764 | 1.560 | 104.44 | | | | | |
| | 4 | 15ppm+MBd | 15.625 | 14.764 | 1.560 | 104.47 | | | | | |
| | 5 | 15ppm+Mbe | 15.629 | 14.764 | 1.560 | 104.45 | | | | | |
| | 6 | 15ppm+MBf | 15.637 | 14.764 | 1.560 | 104.39 | | | | | |
| | 7 | 15ppm+MBg | 15.646 | 14.764 | 1.560 | 104.33 | | | | | |
| Day 2 | 1 | 15ppm+MBa | 15.576 | 14.778 | 1.557 | 104.87 | 104.75 | | | | |
| | 2 | 15ppm+MBb | 15.584 | 14.778 | 1.557 | 104.82 | | | | | |
| | 3 | 15ppm+MBc | 15.597 | 14.778 | 1.557 | 104.73 | | | | | |
| | 4 | 15ppm+MBd | 15.592 | 14.778 | 1.557 | 104.77 | | | | | |
| | 5 | 15ppm+Mbe | 15.596 | 14.778 | 1.557 | 104.74 | | | | | |
| | 6 | 15ppm+MBf | 15.604 | 14.778 | 1.557 | 104.68 | | | | | |
| | 7 | 15ppm+MBg | 15.613 | 14.778 | 1.557 | 104.62 | | | | | |
| Day 3 | 1 | 15ppm+MBa | 16.308 | 14.851 | 1.630 | 101.06 | 100.94 | | | | |
| | 2 | 15ppm+MBb | 16.316 | 14.851 | 1.630 | 101.01 | | | | | |
| | 3 | 15ppm+MBc | 16.33 | 14.851 | 1.630 | 100.92 | | | | | |
| | 4 | 15ppm+MBd | 16.324 | 14.851 | 1.630 | 100.96 | | | | | |
| | 5 | 15ppm+Mbe | 16.328 | 14.851 | 1.630 | 100.94 | | | | | |
| | 6 | 15ppm+MBf | 16.337 | 14.851 | 1.630 | 100.88 | | | | | |
| | 7 | 15ppm+MBg | 16.346 | 14.851 | 1.630 | 100.83 | | | | | |

Author Responsibilities

Debnath Manika is the researcher's data collector, laboratory analyst, and information creator. Manika was also in charge of the manuscript's writing and quality control. She makes a significant contribution to the data analysis of the manuscript. Abrham Ayele was responsible for the computation of statistical factors such as measurement of uncertainty. Both authors prepare the paper according to the publisher's guidelines.

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