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# VALIDATION OF REFLECTOQUANT™ FOR SOLUBLE REACTIVE PHOSPHORUS MONITORING IN SURFACE WATERS

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## ABSTRACT

**High-frequency monitoring of soluble reactive phosphorus (SRP) is essential for managing eutrophication in developing regions, but laboratory-based UV-Vis spectrophotometry (SPEC) is often limited by cost and logistics. This study evaluates the portable Reflectoquant™ (REFL) reflectometric method against SPEC using synthetic standards and 300 filtered river samples from Uruguayan watersheds (0.010–2.400 mg$PO_4$-P $L^{-1}$). Statistical analyses included Spearman correlation, regression models, and Bland-Altman plots. Strong monotonic relationships were found ($\varrho$ = 0.979 for standards; $\varrho$ = 0.890 for rivers). Simple linear regression showed the best fit (adjusted $R^2 \geq 0.972$; root mean square error (RMSE) ≤ 0.018 mg$PO_4$-P $L^{-1}$). Bland-Altman analysis indicated minor overall bias (+0.009 mg $L^{-1}$), with overestimation at trace levels (<0.025 mg $L^{-1}$; bias –0.022 mg $L^{-1}$) and underestimation at higher concentrations (≥0.100 mg $L^{-1}$; bias +0.015 mg $L^{-1}$). While sensitivity is limited at trace levels, REFL's portability and low cost make it an effective field-screening tool in Uruguay, where SRP accounts for 17–100% of total phosphorus and often exceeds the 0.025 mg $L^{-1}$ eutrophication threshold. These results support decentralized monitoring networks and advance United Nations Sustainable Development Goals 6 and 14 in resource-limited settings.**

**Keywords:** soluble reactive phosphorus, reflectometry, method validation, eutrophication monitoring, surface water, Bland-Altman analysis.

## INTRODUCTION

Monitoring soluble reactive phosphorus (SRP) in surface waters is essential for effective assessment and management of eutrophication. Conventional laboratory techniques, such as UV-Vis spectrophotometry (SPEC), are costly, with basic equipment often costing more than US$ 10,000. These methods also require complex sample transport, which may result in analyte degradation within hours or days, and necessitate strict chain-of-custody protocols. Such limitations hinder timely responses to sudden nutrient surges, particularly following rainfall runoff events (Vaughan et al., 2018; Yanu and Jakmunee, 2015). Portable reflectometric systems, including

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Reflectoquant™ (REFL), address these challenges by enabling on-site, frequent screening and minimizing reliance on laboratory confirmation.

Phosphorus in natural waters is present as total phosphorus (TP), which is quantified following acid digestion and hydrolysis; as soluble reactive phosphorus (SRP), primarily orthophosphate defined after 0.45 µm filtration; and as a non-reactive dissolved fraction. The molybdenum blue method is widely employed for SRP determination. In this procedure, orthophosphate reacts in an acidic medium with ammonium molybdate and antimony tartrate to produce phosphomolybdic acid. Subsequent reduction by ascorbic acid yields a blue complex, which is quantified at 885 nm using a spectrophotometer (Murphy and Riley, 1962; Nagul et al., 2015).

The increasing use of portable nutrient analyzers underscores the urgent demand for field-deployable monitoring tools (Vaughan et al., 2018). While Reflectoquant™ provides rapid, multi-analyte detection ($PO_4^{-3}$, $NO_3^-$, $AsO_4^{-3}$) through color-reactive strips and reflectometry, its accuracy and precision at environmentally relevant trace concentrations (< 0.025 mg$PO_4$-P $L^{-1}$) remain underexplored under authentic river-matrix conditions (Hošťálková et al., 2013; Vaughan et al., 2018). Eutrophication risk increases when TP exceeds 0.025 mg $L^{-1}$ in oligotrophic systems, and SRP concentrations above 0.010 mg $L^{-1}$ indicate mesotrophic conditions (Carpenter, 2008; Sharpley and Wang, 2014). In Uruguay and adjacent South American basins, SRP typically accounts for 17–100% of TP, and concentrations exceeding 0.025 mg$PO_4$-P $L^{-1}$ indicate a high likelihood of exceeding eutrophication thresholds (Tables S1 and S2).

To evaluate REFL as a practical complement to SPEC for eutrophication surveillance, this study tested two null hypotheses: (1) there is no significant monotonic relationship between REFL and SPEC across the full SRP range, and (2) systematic bias exceeds the 95% limits of agreement (± 0.036 mg $L^{-1}$), indicating that the methods are not equivalent for detecting eutrophic conditions. Rejection of these hypotheses would justify a simple predictive model characterized by statistically significant coefficients ($p < 0.05$), high adjusted $R^2$, and low prediction error. The analysis included synthetic standards (0.01–0.25 mg$PO_4$-P $L^{-1}$) and 300 filtered river samples from Uruguayan watersheds (0.01–2.4 mg$PO_4$-P $L^{-1}$), measured concurrently by both techniques, using Spearman correlation, multiple regression models (linear, logarithmic, square-root, and quadratic), and Bland–Altman analysis. A further objective was to confirm that the Reflectoquant method produces quantitative results of acceptable accuracy and precision within the concentration range most relevant for eutrophication monitoring in Uruguayan surface waters (approximately 0.01–0.1 mg$PO_4$-P $L^{-1}$).

## MATERIALS AND METHODS

### Quantification Methods

#### *Spectrophotometry (SPEC)*

Soluble reactive phosphorus (SRP) was determined by the molybdenum blue method (Murphy and Riley, 1962; Worsfold et al., 2005). A 50 mL sample aliquot, filtered through 0.45 µm glass microfiber filters (Ahlstrom-Munksjö, formerly Munktell), was mixed with 5 mL of reagent solution containing ammonium molybdate (24.28 mM), sulfuric acid (5 N), ascorbic acid (0.31 M), and potassium antimony tartrate (2.2 mM) (Murphy and Riley, 1962; Nagul et al., 2015). After a 30-minute reaction at room temperature, absorbance was measured at 885 nm using a single-beam UV-Vis spectrophotometer (Milton Roy Spectronic 401). Concentrations were calculated by interpolation from a $KH_2PO_4$ calibration curve (0.01–0.25 mg$PO_4$-P $L^{-1}$). Samples exceeding 0.25 mg $L^{-1}$ were diluted with distilled water.

#### *Reflectometry (REFL)*

Reflectometric determination was performed using the Reflectoquant™ orthophosphate test kit (Cat. No. 1.17942.0001, Merck) and an RQflexTM plus 10 reflectometer (Cat. No. 1.16955.0001, Merck KGaA). The kit quantifies concentrations ranging from 0.1 to 5 mg$PO_4$-P $L^{-1}$, equivalent to 0.033 to 1.63 mg $PO_4$-P $L^{-1}$. For samples with concentrations below 0.033 mg$PO_4$-P $L^{-1}$, a 4:1 fortification with a 0.48 mg$PO_4$-P $L^{-1}$ standard solution was applied, and results were corrected by back-calculation. All measurements were conducted according to the manufacturer's instructions using a cuvette adapter.

### Sample collection and preparation

#### *Orthophosphate standard solutions*

A stock solution (250 mg $PO_4$-P $L^{-1}$, equivalent to 81.5 mg$PO_4$-P $L^{-1}$) was prepared and diluted with Milli-Q™ water to produce 13 working standards: 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0.100, 0.150, 0.200, and 0.250 mg$PO_4$-P $L^{-1}$. Each standard was analyzed in triplicate. Data are available at https://gitlab.com/lcarrascol/reflectoquant_prs/snippets/6032465.git.

#### *Surface water samples*

In November 2014, 300 surface water samples were collected from 100 Uruguayan watersheds using Kemmerer bottles as part of the INIA national water-quality of agricultural watersheds

funded by the INIA Grant SA27. One river was excluded as an outlier, as reported by Carrasco-Letelier et al. (2014). Samples were immediately filtered in the field using pre-calcined (500 °C) 0.45 µm glass microfiber discs (Ahlstrom-Munksjö; Worsfold et al., 2005). Filtrates were stored at 4 °C and transported to the laboratory within 8 h. Samples not analyzed on the day of collection were frozen at −20 °C and analyzed within one month. The dataset is available at https://gitlab.com/lcarrascol/reflectoquant_prs/snippets/6032465.git

### Data Analysis

Agreement between methods was evaluated using Spearman's rank correlation, linear regression models (simple, logarithmic, square-root, and second-degree polynomial), and Bland-Altman analysis. Model performance was assessed by adjusted $R^2$, root mean square error (RMSE), mean absolute error (MAE), and residual diagnostics, including the Shapiro-Wilk test for normality, Breusch-Pagan test, and Levene's test for homoscedasticity.

Precision, accuracy, uncertainty, and limit of detection (LOD) were calculated according to ISO 5725 (ISO, 2019, 2023) and EURACHEM guidelines (Cantwell, 2024; Ellison and Williams, 2015) using averages of triplicate samples. The equations used are presented in the Supplementary Material.

### Software

All statistical analyses were performed in R version 4.5.1 (R Core Team, 2025) using the packages readr, dplyr, tidyr, stats, lmtest, BlandAltmanLeh, MethComp, blandr, car, broom, Metrics, ggplot2, and gridExtra.

## RESULTS

### Spearman's correlation

A strong positive monotonic relationship was observed between Reflectoquant™ (REFL) and spectrophotometry (SPEC), both in standard solutions ($\rho$ = 0.98, $p < 2.2 \times 10^{-16}$) and in river samples ($\rho$ = 0.89, $p < 2.2 \times 10^{-16}$).

### Regression analyses

Simple linear regression performed best overall in both datasets (Table 1, Fig. 1).

### Standard solutions

Simple linear regression produced an adjusted $R^2$ of 0.972, an RMSE of 0.01 mg $L^{-1}$, and an MAE of 0.009 mg $L^{-1}$. In comparison, alternative models, including logarithmic, square-root, and quadratic forms, demonstrated inferior fit, with adjusted $R^2$ values not exceeding 0.973 and exhibiting higher RMSE or MAE values as well as less favorable residual diagnostics (Table 1).

### River samples

Simple linear regression yielded the best performance (adjusted $R^2$ = 0.99, RMSE = 0.018 mg $L^{-1}$, MAE = 0.013 mg $L^{-1}$; Fig. 1). Logarithmic, square-root, and quadratic models demonstrated inferior performance, with adjusted $R^2$ values ranging from 0.55 to 0.997, higher errors, and clear violations of residual assumptions. In the low-concentration subset (< 0.025 mg $L^{-1}$, n = 29), all models exhibited poor fit (adjusted $R^2 \leq$ 0.49), primarily due to constant REFL readings of 0.04 mg $L^{-1}$. Table 1 provides a comprehensive comparison of all regression models.

Polynomial regression could not be applied to the rivers_low dataset because SPC_rivers

**Table 1. Performance of different regression models for predicting REFL from SPEC. River low corresponds to a concentration lower than 0.025.**

| Dataset | Model | Adj. $R^2$ | RMSE | MAE | Shapiro - Wilks | Breusch - Pagan |
|---|---|---|---|---|---|---|
| Standards | Simple linear | 0.972 | 0.010 | 0.009 | 0.050 | 0.105 |
| Standards | Logarithmic | 0.856 | 2.634 | 2.583 | 0.002 | 0.097 |
| Standards | Square-root | 0.937 | 0.205 | 0.202 | 0.003 | 0.441 |
| Standards | Polynomial (degree 2) | 0.973 | 0.010 | 0.008 | 0.101 | 0.021 |
| Rivers | Simple linear | 0.995 | 0.018 | 0.013 | <0.001 | <0.001 |
| Rivers | Logarithmic | 0.549 | 2.631 | 2.616 | <0.001 | <0.001 |
| Rivers | Square-root | 0.855 | 0.199 | 0.194 | <0.001 | <0.001 |
| Rivers | Quadratic | 0.997 | 0.0148 | 0.012 | <0.001 | <0.001 |
| Rivers low | Simple linear | 0.455 | 0.000 | 0.000 | <0.001 | 0.547 |
| Rivers low | Logarithmic | 0.455 | 3.259 | 3.259 | <0.001 | 0.547 |
| Rivers low | Square-root | 0.492 | 0.160 | 0.160 | <0.001 | 0.547 |

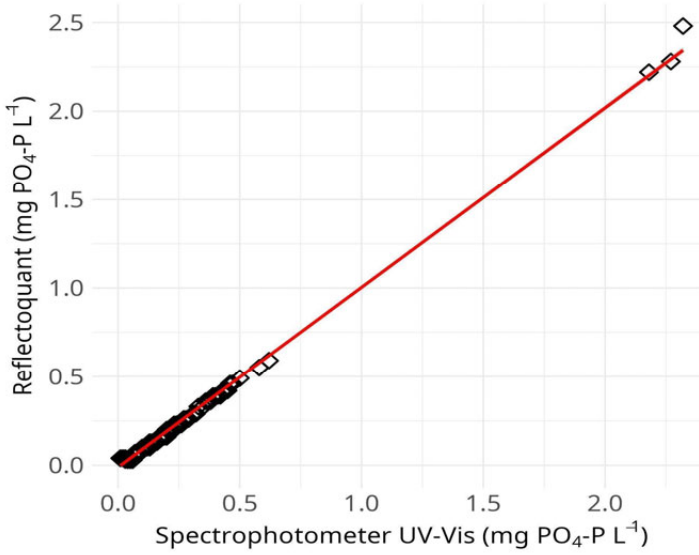


**Fig. 1. Scatter plots of REFL vs. SPEC for river samples, with simple linear regression lines.**

(spectrophotometry measurements for river samples) lacked sufficient unique values to estimate coefficients and perform diagnostic analysis.

**Bland-Altman Analysis**

Bland-Altman analysis for river samples (n = 296 after excluding 4 NA cases, Table 2) revealed an overall mean bias of +0.009 mg $L^{-1}$ (95% limits of agreement: −0.027 to 0.045 mg $L^{-1}$, t-test $p = 5.5 \times 10^{-16}$), indicating that REFL slightly underestimated SPEC overall. The bias was concentration-dependent: at low concentrations (< 0.025 mg $L^{-1}$, n = 29), REFL overestimated SPEC (mean bias = −0.022 mg $L^{-1}$, 95% limits of agreement: −0.031 to −0.014 mg $L^{-1}$, t-test $p < 2.2 \times 10^{-16}$), while at high concentrations (≥ 0.100 mg $L^{-1}$, n = 136), REFL underestimated SPEC (mean bias = +0.015 mg $L^{-1}$, 95% limits of agreement: −0.021 to 0.052 mg $L^{-1}$, t-test $p < 2.2 \times 10^{-16}$). No specific outliers were identified; however, greater scatter in the low-concentration data suggests potential analytical variability (Fig. 2).

Note that the standard deviations exceed the means because SRP concentrations in natural waters are strongly right-skewed (log-normal distribution). Table 3 is descriptive only and does not constitute a formal method comparison.

**Precision, accuracy, uncertainty, and limit of detection**

The SPEC reference method exhibited a pooled repeatability standard deviation ($s_{◎}$) of 8.1 x $10^{-4}$ mg $L^{-1}$ ($CV_{◎}$ = 0.91%) across the 13 concentrations evaluated (0.01–0.25 mg $L^{-1}$). In contrast, REFL exhibited zero variability in triplicates ($CV_{◎}$ = 0.00%) across the ten concentrations evaluated (0.04–0.25 mg $L^{-1}$), reflecting the discrete resolution of the reflectometric system rather than perfect precision.

Bias between methods was small and non-significant (−0.008 mg $L^{-1}$, $t = 2.18$, $p > 0.05$). Combined uncertainty for REFL was 0.0138 mg $L^{-1}$ (relative 13.6%) and expanded uncertainty 0.0276 mg $L^{-1}$ (relative 27.3%). The limit of detection for REFL was 0.033 mg $L^{-1}$ (kit threshold) or 0.060 mg $L^{-1}$ (regression approach).

The linear working range of the Reflectoquant method after sample fortification was 0.033–1.63 mg$PO_4$-P $L^{-1}$. Within this range, the relationship between REFL and the reference spectrophotometric method (SPEC) for standard solutions was described by the linear equation REFL = −0.001 + 0.939 SPEC, with an adjusted coefficient of determination of 0.972 (Table 1). The limit of detection (LOD) estimated from the residual standard deviation of the regression was 0.06 mg $L^{-1}$ (slope = 0.941; residual standard deviation = 0.018 mg $L^{-1}$; Table 4). The corresponding limit of quantification (LOQ) was calculated as 10 x (residual standard deviation/slope), yielding 0.187 mg $L^{-1}$. Reproducibility across different days or operators was not evaluated in this study.

## DISCUSSION

Reflectoquant™ (REFL) is a reliable, field-deployable screening tool for soluble

**Table 2. Bland-Altman metrics (bias, 95% limits of agreement, SD) for river samples and subsets.**

| Subset | n | Mean Bias (mg $L^{-1}$) | 95% Limits of Agreement (mg $L^{-1}$) | SD of Differences (mg $L^{-1}$) |
|---|---|---|---|---|
| All | 296 | +0.009 | -0.027 to 0.045 | 0.018 |
| Low (< 0.025 mg $L^{-1}$) | 29 | -0.022 | -0.031 to -0.014 | 0.004 |
| High (≥ 0.100 mg $L^{-1}$) | 136 | +0.015 | -0.021 to 0.052 | 0.019 |

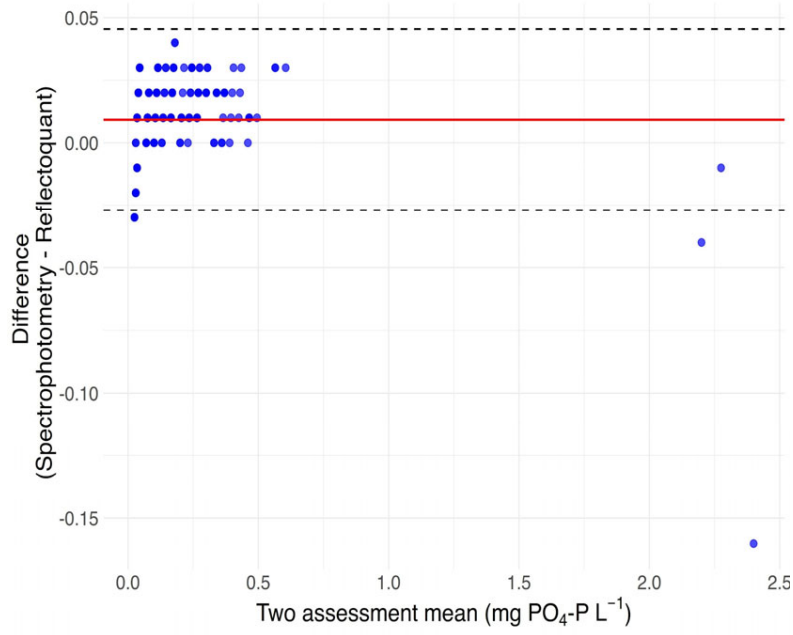


**Fig. 2. Bland-Altman plot comparing spectrometry and Reflectoquant.**

**Table 3. Descriptive statistics of SRP concentrations (mean ± SD, range, mg $L^{-1}$) in standard solutions and river samples.**

| Dataset | Subset | n | SPEC Mean ± SD (Range) | REFL Mean ± SD (Range) |
|---|---|---|---|---|
| Standards | All | 39 | 0.098 ± 0.068 (0.010–0.250) | 0.080 ± 0.058 (0.033–0.228) |
| Rivers | All | 300 | 0.135 ± 0.259 (0.010–2.320) | 0.126 ± 0.248 (0.010–2.480) |

reactive phosphorus (SRP) in surface waters, demonstrating strong agreement with the reference UV-Vis spectrophotometry (SPEC) method, especially at moderate to high concentrations. Robust Spearman correlations ($\rho \geq 0.890$) and the strong performance of simple linear regression (adjusted $R^2 \geq 0.97$, RMSE ≤ 0.018 mg $L^{-1}$) refute the first null hypothesis and confirm a mathematically sound relationship between the two techniques (Cornbleet and Gochman, 1979; Linnet, 1999). The quadratic polynomial model provided only marginal improvement in standards, while logarithmic and square-root transformations performed worse, supporting the use of simple linear models for interpretability in environmental applications (Stöckl et al., 1998; Westgard and Hunt, 1973).

Bland-Altman analysis rejects the second null hypothesis, with an overall bias (+0.009 mg $L^{-1}$) well within the predefined 95% limits of agreement (±0.036 mg $L^{-1}$). However, the bias is concentration-dependent: REFL overestimates at low SRP concentrations (< 0.025 mg $L^{-1}$) and underestimates at high concentrations (≥ 0.1 mg

**Table 4. Coefficient of variation ($CV_r$), accuracy, expanded uncertainty ($U$), and limit of detection (LOD).**

| Metric | SPEC | REFL | Interpretation |
|---|---|---|---|
| $CV_r$ (%) | 0.91 | 0.00 | Acceptable for SPEC (<5%); REFL shows no variability in triplicates. |
| Bias (mg $L^{-1}$) | N/A | -0.008 | Non-significant ($t = 2.18 < t_{crit} = 2.262$, $df = 9$), good agreement in 0.04–0.25 mg $L^{-1}$ range. |
| $U$ (mg $L^{-1}$) | N/A | 0.0276 | At 95% coverage, higher than desirable, limiting precision at low concentrations. |
| LOD (mg $L^{-1}$) | ~0.003 (estimated) | 0.033 (threshold)/ 0.06 (regression) | REFL has limited sensitivity below 0.033 mg $L^{-1}$ ("LOW" indication). |

$L^{-1}$). These patterns, consistent with previous evaluations of portable reflectometric assays (Hošťálková et al., 2013; Major and Barraclough, 2003), are likely attributable to the kit's detection threshold, the fortification procedure for trace levels, and matrix effects in natural waters (Murphy and Riley, 1962; Nagul et al., 2015; Worsfold et al., 2005).

In Uruguay, where SRP typically constitutes 17–100% of total phosphorus and often exceeds eutrophication thresholds (Tables 1 and 2; Aubriot et al., 2017; Beretta-Blanco and Carrasco-Letelier, 2021), REFL's capacity to detect concentrations above 0.025 mg $L^{-1}$ makes it a practical first-line tool for rapid screening during episodic events. However, overestimation at trace levels may result in false-positive eutrophication diagnoses; thus, values near 0.040 mg $L^{-1}$ should be confirmed by SPEC. This method does not replace laboratory analysis for precise low-level quantification or for use in complex matrices.

Limitations of this study include potential SRP degradation during frozen storage, the regional focus on Uruguayan fluvial systems, and reduced resolution of REFL at oligotrophic concentrations. Future research should assess performance in brackish or sediment-rich waters and further refine calibration for trace levels.

Although REFL does not fully replace SPEC, its portability and low cost make it an effective complementary tool for prioritizing samples and supporting evidence-based water quality management.

## CONCLUSIONS

Reflectoquant™ (REFL) serves as a portable screening tool for soluble reactive phosphorus (SRP) in surface waters. Both null hypotheses were rejected, indicating a strong monotonic relationship with spectrophotometry (SPEC) (Spearman's $\rho \geq 0.89$, $p < 2.2 \times 10^{-16}$) and acceptable bias within the 95% limits of agreement (±0.036 mg $L^{-1}$) across 0.01–2.4 mg$PO_4$-P $L^{-1}$. Simple linear regression provided the highest predictive performance (adjusted $R^2 \geq 0.97$, RMSE ≤ 0.018 mg $L^{-1}$).

REFL demonstrates limitations at low concentrations (< 0.025 mg $L^{-1}$), producing constant readings near 0.04 mg $L^{-1}$ and poor model fits (adjusted $R^2 \leq 0.49$). Concentration-dependent biases, such as overestimation at low levels and underestimation at high levels, highlight the need for range-specific calibration and laboratory confirmation.

In summary, REFL complements rather than replaces SPEC by enabling rapid field screening and sample prioritization during eutrophication risk assessment.

**Author contributions**

Participation in Literature Review: Leonidas Carrasco-Letelier and Gabriela Eguren. Study conception and design: Leonidas Carrasco-Letelier and Gabriela Eguren. Methodology development: Leonidas Carrasco-Letelier, Gabriela Eguren, Andrés Hirigoyen, Andrés Beretta-Blanco. Discussion of results: Leonidas Carrasco-Letelier, Andrés Hirigoyen, and Andrés Beretta-Blanco. The first draft of the manuscript was written by Leonidas Carrasco-Letelier and Gabriela Eguren. Revision and approval of final version: Leonidas Carrasco-Letelier, Andrés Hirigoyen, and Andrés Beretta-Blanco. All authors commented on previous versions of the manuscript. All authors read and approved the last version of the manuscript.

**Supplementary Material**

The following materials are available as online supplementary information:

Table S1. Phosphorus concentrations reported in Uruguayan surface waters (post-1980).

Table S2. Phosphorus concentrations reported in Argentina, Brazil, and Paraguay (post-1980).

The entire data set supporting the results of this study, along with the R code, was published at https://gitlab.com/lcarrascol/reflectoquant_prs/snippets/6032465.git

**Generative AI Statement**

The author(s) declared that generative AI was used in the creation of this manuscript. During preparation, the authors used Grok, OpenAI's ChatGPT, and Gemini to enhance the R code for statistical analysis. For readability, the manuscript was checked and refined using Grammarly. After using these tools, the authors reviewed and edited the content as needed and took full responsibility for the published article.

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**Supplementary Material**

## S1. Equations used for method validation

### Repeatability (precision)

The statistical analyses to evaluate the precision, accuracy, uncertainty, and limit of detection (LOD) of the SPEC (reference) and REFL (alternative) methods for phosphate determination were performed using the averages of triplicates obtained on the same day in the same laboratory, considering only the concentrations where both methods provided readings (0.04–0.25 mg $L^{-1}$, n = 10) for comparison, while for SPEC all concentrations (0.01–0.25 mg $L^{-1}$, m = 13) were included. The repeatability was calculated per concentration using the triplicate standard deviation [Eq. 1], and globally by combining the variances of all concentrations [Eq. 2], with the coefficient of variation [Eq. 3] and the associated uncertainty [Eq. 4]. Repeatability with a coefficient of variation of less than 5% in high ranges and 10% in low ranges is considered acceptable, according to ISO 5725 (ISO, 2023, 2019).

$$S_{i,j} = \sqrt{\frac{\sum_{i=1}^{3}\left(x_{ij}-\overline{x_j}\right)^2}{3-1}} \qquad \text{eq. 1}$$

$$S_r = \sqrt{\frac{\sum_{j=1}^{m}\sum_{i=1}^{3}\left(x_{ij}-\overline{x_j}\right)^2}{m(3-1)}} \qquad \text{eq. 2}$$

$$CV_r = \frac{S_r}{\overline{X_{global}}} \times 100\% \qquad \text{eq. 3}$$

$$u(s_r) = \frac{S_r}{\sqrt{2(m(3-1))}} \qquad \text{eq. 4}$$

### Accuracy (bias)

The accuracy was evaluated through the bias, calculated as the average difference between the averages of REFL and SPEC [Eq. 5], with its uncertainty determined by the standard deviation of the differences [Eq. 6]; the significance of the bias was verified with a t-test [Eq. 7], compared to the critical value at a 95% confidence level and degrees of freedom df = n - 1, being non-significant if the calculated value is less than the critical value.

$$Bias = \frac{1}{n}\sum\left(x_{REFL,j} - x_{SPEC,j}\right) \qquad \text{eq. 5}$$

$$u(Bias) = \frac{s_d}{\sqrt{n}}, s_d = \sqrt{\frac{\sum\left(d_j-\bar{d}\right)^2}{n-1}} \qquad \text{eq. 6}$$

$$t = \frac{|Bias|}{u(Bias)} \qquad \text{eq. 7}$$

### Combined uncertainty

The combined robust uncertainty was estimated by integrating the bias uncertainty and a robust estimator of variability based on the median absolute deviation [Eq. 8], and the expanded uncertainty was obtained by applying a coverage factor [Eq. 9]; a relative uncertainty less than 10% in high ranges and 20% near the LOD is considered acceptable, according to EURACHEM (Cantwell, 2024).

$$u_c = \sqrt{u(Bias)^2 + s_{robust}^2}\ ,\ s_{robust} = 1.4826 \times median\left(\left|d_j - median(d_j)\right|\right) \quad \text{eq. 8}$$

$$U = 2u_c \quad \text{eq. 9}$$

**Limit of Detection (LOD)**

The LOD of REFL was determined through two approaches: the minimum detectable concentration (threshold of "LOW" at 0.033 mg $L^{-1}$) and the linear regression of the averages against the reference concentrations, calculating the slope, the intercept, and the residual standard deviation [Eq. 10], and the LOD [Eq. 11], considering an acceptable LOD below the regulatory limit for the analyte (e.g., <0.05 mg $L^{-1}$ for phosphate in drinking water). All calculations were performed in R, organizing the triplicates into lists, computing averages, applying the statistical equations, and verifying significance using Student's t-tables.

$$s_y = \sqrt{\frac{\sum(y_i - (bx_i + a))^2}{n-2}} \quad \text{eq. 10}$$

$$LOD = \frac{3.3\ s_y}{b} \quad \text{eq. 11}$$

## S2. Tables

**Table S1. Phosphorus concentration in water expressed in ($10^{-3}$ mg $PO_4$–P $L^{-1}$) for some cases of Uruguay (Post-1980).**

| Water Body | TP | SRP | SRP/TP (%) | Department | Year(s) | References |
|---|---|---|---|---|---|---|
| Río Santa Lucía rivers | 0.050 - 0.180 | 0.020 - 0.080 | ~76% | Canelones, Montevideo | 2004-2016 | Aubriot et al., 2017 |
| Santa Lucía river | 0.300 - 0.900 | 0.680 (median) | ~88% | Canelones | 2008-2009 | Barreto et al., 2017 |
| Río Negro and Río Uruguay rivers | 0.050 - 0.150 means: 0.086 (Río Negro) and 0.099 (Río Uruguay) | 0.020 - 0.080 | ~40 - 60% (estimated) | Río Negro, Soriano | 2009-2018 | Beretta-Blanco and Carrasco-Letelier, 2021 |
| Queguay River | Rivers: 0.044 – 0.051; Streams: 0.051 – 0.097; Floodplain lakes: 0.074 - 0.109 | n.d. | - | Paysandú | 2019-2021 | Lucas et al., 2022 |
| Salto Grande Reservoir (Argentine - Uruguay) | 0.030 - 0.090 | 0.010 - 0.065 | ~23-39% | Salto (Uruguay), Entre Ríos (Argentine) | 2000-2001 | De León and Chalar, 2003 |
| Paso Severino Reservoir (Santa Lucía river) | 0.380 (annual mean) | n.d. | - | Florida | 2013-2015 | Arocena et al., 2016 |
| Canelón Grande and Canelón Chico streams | > 0.100 | n.d. | - | Canelones | 2004-2016 | Aubriot et al., 2017 |
| Coastal Lagoons | 0.060 - 0.300 | 0.010 - 0.100 | ~17-33% | Maldonado, Rocha | 2007-2011 | Rodríguez-Gallego, 2010 |
| Río Negro river | 0.158 | 0.010 - 0.050 | ~20–40% | Río Negro | 2009–2010 | MVOTMA-DINAMA, 2020 |
| Laguna de Rocha lagoon | 0.046 - 0.167 | 0.011 - 0.065 | ~23–39% | Rocha | 2017 | MVOTMA, 2018 |
| Streams – Laguna Merín Basin | 0.046 - 0.150 | 1.100 - 1.300 | >100% | Cerro Largo, Treinta y Tres | 2015–2019 | Ministerio de Ambiente, 2021 |
| 99 Nationwide (various rivers) | 0.005 - 0.110 (Autumn) | 0.002 - 0.030 (Autumn) | ~20–30% | 15 departments | 2014 | Carrasco-Letelier et al., 2014 |

**Table S2. Phosphorus concentration in water expressed in ($10^{-3}$ mg $PO_4$–P $L^{-1}$) for some cases of Argentina, Brazil, and Paraguay (Post-1980).**

| Water Body | TP | SRP | Year(s) | References |
|---|---|---|---|---|
| Ypacaraí Lake (Paraguay) | 0.124 - 0.256 | n.d. | 1988; 2012 - 17 | López Moreira M. et al., 2018 |
| Paraná lakes (Argentina) | 0.005 to 0.004 | 0.015 - 0.124 | 1992 - 1997 | Carignan and Vaithiyanathan, 1999 |
| Salado River (Argentina) | 0.200 - 1.320 | n.d. | 2006 - 2007 | Moscuzza et al., 2007 |
| Pampulha Reservoir (Brazil) | 0.100 - 0.300 | n.d. | 1990s - 2000s | Pinto-Coelho and Barcelos Greco, 1999 |
| Orós Reservoir (Brazil) | 0.030 - 0.140 | n.d. | 2008 - 2012 | Andrade et al., 2020 |

## S3. Data availability

Standard solutions dataset ("ss"), river samples dataset ("Rivers"), and full R code used for statistical analysis and figure generation is available in the GitLab repository (https://gitlab.com/lcarrascol/reflectoquant_prs/snippets/6032465.git).

## S4. Model performance summary

| Model / Dataset | n | Adj. $R^2$ | RMSE (mg $L^{-1}$) | MAE (mg $L^{-1}$) | Shapiro-Wilk p | Breusch-Pagan p | Levene's p | Notes |
|---|---|---|---|---|---|---|---|---|
| Standards – Simple linear | 30 | 0.9720 | 0.0103 | 0.0091 | 0.0500 | 0.1047 | 0.4410 | Best fit for standards |
| Standards – Logarithmic | 30 | 0.8563 | 2.6341 | 2.5827 | 0.0017 | 0.0974 | 0.1489 | Poor performance |
| Standards – Square root | 30 | 0.9374 | 0.2049 | 0.2024 | 0.0033 | 0.4406 | 0.1533 | Acceptable but inferior |
| Standards – Polynomial (deg 2) | 30 | 0.9726 | 0.0100 | 0.0084 | 0.1014 | 0.0212 | 0.9049 | Marginal gain vs. simple |
| Rivers – Simple linear | 296 | 0.9948 | 0.0181 | 0.0130 | <0.0001 | <0.0001 | 0.0640 | Best overall model |
| Rivers – Logarithmic | 296 | 0.5488 | 2.6308 | 2.6161 | <0.0001 | <0.0001 | 0.0054 | Strongly rejected |
| Rivers – Square root | 296 | 0.8545 | 0.1990 | 0.1945 | <0.0001 | <0.0001 | 0.0788 | Not recommended |
| Rivers – Polynomial (deg 2) | 296 | 0.9965 | 0.0148 | 0.0116 | <0.0001 | <0.0001 | 0.1295 | Slight improvement but residuals violate assumptions |
| Rivers low (<0.025) – Simple | 29 | 0.4551 | 0.0000* | 0.0000* | <0.0001 | 0.5469 | NA | Constant REFL values (~0.040) |
| Rivers low – Logarithmic | 29 | 0.4551 | 3.2589 | 3.2589 | <0.0001 | 0.5469 | NA | Useless |
| Rivers low – Square root | 29 | 0.4924 | 0.1600 | 0.1600 | <0.0001 | 0.5469 | NA | Still poor |