Research Article

Analytical Performance of an Alternative Wavelength in the O-CPC Calcium Assay

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Keywords

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Abstract

Objective: The o-cresolphthalein complexone (O-CPC) method is widely used for serum calcium measurement but may exhibit analytical limitations related to background absorbance and bias, particularly at low calcium concentrations. This study evaluated the analytical performance of an alternative wavelength (524 nm) compared with the conventional 572 nm measurement.

Methods: Spectral analysis of the calcium–O-CPC complex identified a secondary absorbance feature near 536 nm. Because this wavelength was not available in routine analysers, 524 nm was selected as the nearest operational alternative for analytical evaluation. Performance was assessed using multipoint calibration models, control sera, precision analysis, and comparison with an automated analyzer in 40 samples, including prepared mixtures to extend the analytical range. Agreement between methods was evaluated using regression and Bland–Altman analyses.

Results: Reagent blank absorbance was lower at 524 nm compared with 572 nm (0.048 vs 0.122). Linearity was high at both wavelengths ($R^2 \approx 0.998$). Precision analysis demonstrated comparable CV% values between wavelengths. Regression analysis showed a slope closer to unity and a smaller intercept at 524 nm relative to 572 nm. Bland–Altman analysis demonstrated comparable overall agreement between methods at both wavelengths. In the low concentration subgroup (<9 mg/dL), mean negative bias was reduced from −0.55 mg/dL at 572 nm to −0.30 mg/dL at 524 nm.

Conclusions: Measurement at 524 nm demonstrated comparable overall analytical performance to the conventional 572 nm wavelength and was associated with reduced negative bias in the low concentration range. These findings suggest that alternative wavelength selection may represent a practical approach for optimizing analytical performance in routine O-CPC calcium assays.

Introduction

Accurate measurement of serum calcium is essential in clinical laboratory practice and plays a central role in the evaluation of metabolic, endocrine, and renal disorders [1]. Analytical reliability is particularly important near clinical decision thresholds, where small analytical deviations may influence the classification of hypocalcemia and subsequent clinical interpretation.

In routine laboratories, calcium is commonly measured using colorimetric methods based on metallochromic indicators [2]. Among these, the o-cresolphthalein complexone (O-CPC) method is widely used due to its simplicity, cost-effectiveness, and compatibility with automated systems. In alkaline conditions, O-CPC forms a colored calcium complex typically measured at wavelengths around 570–580 nm, corresponding to the primary absorbance maximum [3].

Despite its widespread use, the O-CPC method may exhibit analytical limitations, particularly in the low concentration range [3]. Background absorbance and signal-to-noise characteristics may contribute to negative bias near clinically relevant decision thresholds [4]. While reagent composition and calibration strategies have been extensively investigated, the analytical impact of wavelength selection has received comparatively less attention in routine clinical applications [4,5].

Wavelength selection is generally treated as a fixed parameter in automated assays; however, it may influence analytical performance by affecting background signal and bias distribution [4]. Identification of alternative measurement regions within the absorbance spectrum may therefore provide a practical approach for evaluating analytical performance without modifications of reagent chemistry or instrumentation [6].

The aim of this study was to evaluate the analytical performance of an alternative wavelength in the O-CPC calcium assay, with particular emphasis on the low concentration range relevant to clinical decision thresholds.

Materials and Methods

Study design and instrumentation

This analytical method comparison study was conducted in the clinical biochemistry laboratory of a tertiary care hospital. Absorbance spectra of the calcium–O-CPC complex were obtained using a spectrophotometer (Philips AU 530). Spectral analysis revealed a secondary absorbance feature near 536 nm in addition to the primary peak at 572 nm. Because 536 nm was not operationally available in routine clinical analysers, 524 nm was selected as the nearest available alternative wavelength for analytical evaluation. The objective was not to maximize absorbance intensity, but to evaluate whether an alternative spectral region with lower background absorbance could influence analytical performance. Measurements performed at 524 nm were compared with the conventional 572 nm wavelength.

Routine measurements were compared with an automated analyzer (Architect c8000, Abbott Laboratories, USA) operating under manufacturer-recommended analytical conditions.

Calibration and linearity assessment

Linearity was evaluated by calibrators at concentrations of 0, 4, 6, 8, 10, and 12 mg/dL. Two multipoint calibration models (0–6–10 mg/dL and 0–4–8 mg/dL) were applied. Linearity was assessed using linear regression analysis and expressed as the coefficient of determination (R^2).

Agreement assessment and precision

Agreement with assigned concentrations was evaluated using two levels of commercial control sera (Bio-Rad Liquicheck, Germany) with assigned values of 8.64 mg/dL and 11.9 mg/dL. Measured values were compared with assigned concentrations and expressed as percentage bias (%). Precision was assessed by repeated measurements and expressed as coefficient of variation (CV%).

Sample selection and preparation

A total of 40 samples were included in the analysis. Twenty routine patient serum samples were analyzed. To extend the analytical measurement range, an additional 20 prepared samples were generated using predefined mixing protocols. Dilution samples were prepared by mixing serum with distilled water at ratios of 1:1 and 2:1 (serum:water), while higher concentration samples were generated by mixing serum with a calcium calibrator (13 mg/dL) at ratios of 1:3 and 1:4. These prepared mixtures were included to ensure distribution across the analytical range; however, potential matrix-related effects were considered during interpretation of the findings.

Method comparison

Spectrophotometric measurements at 524 nm and 572 nm were compared with results obtained from the automated analyzer. Automated measurements were performed in duplicate and averaged.

Agreement between methods was evaluated using linear regression, Passing–Bablok regression, Deming regression, and Bland–Altman analyses.

Statistical analysis

Statistical analyses were performed using SPSS version 24 (IBM Corp., USA). Correlation between methods was assessed using Pearson correlation coefficient (r). Mean bias was calculated as the average difference between methods. Limits of agreement were calculated as mean difference ±1.96 standard deviations. Subgroup analysis was performed for samples with calcium concentrations <9 mg/dL. Method comparison analysis was performed in alignment with CLSI EP09-A3 principles for analytical method comparison. Regression parameters were additionally reported with 95% confidence intervals where applicable.

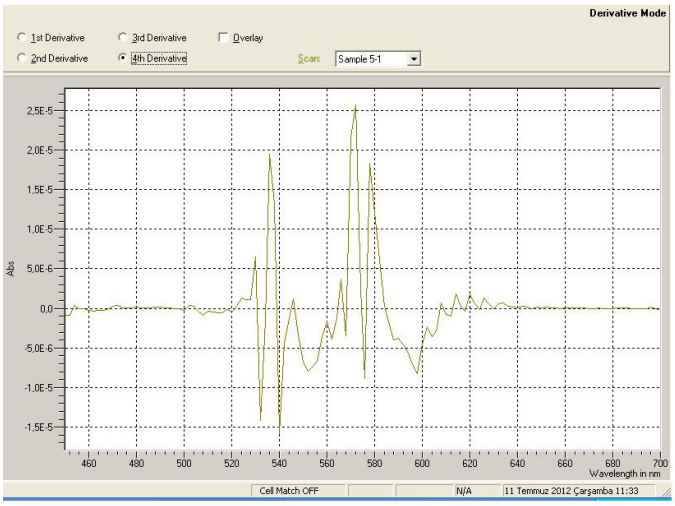
Ethical considerations

The study was performed in the clinical biochemistry laboratory of GATA Haydarpaşa Training and Research Hospital, İstanbul, Turkey. The study used anonymized residual serum samples obtained during routine laboratory practice. According to institutional policy applicable at the time of the study, formal ethics committee approval and informed consent were not required for analytical evaluations performed on anonymized residual samples.

Results

Spectral analysis demonstrated a dominant absorbance peak at 572 nm and a secondary spectral feature near 536 nm (Figure 1). Based on this observation, subsequent analytical evaluations were performed at 524 nm as the nearest operationally available alternative wavelength.

Figure 1: Derivative Spectral Analysis of the Calcium–O-CPC Complex.



Fourth-order derivative spectrum demonstrating the primary absorbance peak at 572 nm and a secondary spectral feature near 536 nm. The secondary feature was used to guide selection of an alternative wavelength for analytical evaluation.

Table 1: Summary of Analytical Performance at 524 nm and 572 nm.

Parameter	524 nm	572 nm
Reagent blank absorbance	0.048	0.122
Linearity (R^2)	0.998	0.998
Regression slope	1.03	1.05
Intercept (mg/dL)	−0.16	−0.53
Correlation (r)	0.965	0.969
Mean bias (mg/dL)	+0.125	−0.082
Bias <9 mg/dL (mg/dL)	−0.30	−0.55

R^2: coefficient of determination; r: Pearson correlation coefficient; bias: mean difference relative to automated analyzer measurements.

Linearity analysis demonstrated excellent linearity at both wavelengths across calibration models (Table 1). Using the 0–6–10 mg/dL calibration model, R^2 values were 0.999 at 524 nm and 0.998 at 572 nm. Similar results were obtained with the 0–4–8 mg/dL calibration model (R^2 = 0.998 at 524 nm and R^2 = 0.996 at 572 nm).

Precision analysis demonstrated comparable repeatability at both wavelengths. At 524 nm, CV values were approximately 2.6% for the low-level control and 2.9% for the high-level control. Corresponding CV values at 572 nm were approximately 2.2% and 3.5%, respectively (Table 2).

Control serum measurements demonstrated numerically lower bias at 524 nm for the low-level control material (Table 2). For the low-level control (8.64 mg/dL), measured values were 8.43 mg/dL at 524 nm and 7.98 mg/dL at 572 nm. For the high-level control (11.9 mg/dL), measured values were 12.08 mg/dL at 524 nm and 11.79 mg/dL at 572 nm. Agreement analysis for the conventional wavelength is presented in Supplementary Figure S1.

Table 2: Control Serum Agreement and Precision Analysis.

Control Level	Target (mg/dL)	524 nm (mg/dL)	572 nm (mg/dL)	Bias 524 (%)	Bias 572 (%)	CV% 524	CV% 572
Low	8.64	8.43	7.98	−2.4%	−7.6%	2.6%	2.2%
High	11.9	12.08	11.79	+1.5%	−0.9%	2.9%	3.5%

Bias (%) was calculated relative to assigned control values. CV: coefficient of variation.

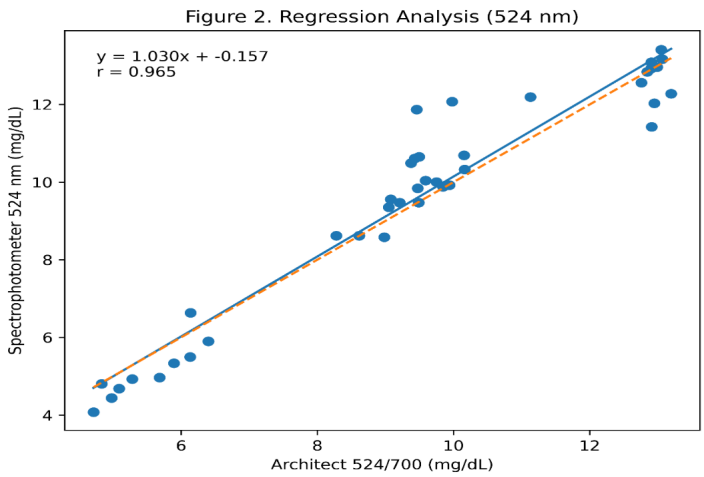
Method comparison analysis in 40 samples demonstrated strong correlation between spectrophotometric and automated measurements at both wavelengths (r = 0.965 at 524 nm; r = 0.969 at 572 nm). Regression analysis demonstrated a slope numerically closer to unity at 524 nm compared with 572 nm (1.03 vs 1.05), together with a smaller intercept magnitude (−0.16 vs −0.53 mg/dL) (Table 3, Figure 2). The corresponding 95% confidence intervals are presented in Table 3. Additional regression analyses using Passing–Bablok and Deming methods yielded comparable results (Supplementary Table S1).

Table 3: Regression and Agreement Analysis Parameters (n = 40).

Parameter	524 nm	572 nm
Slope (95% CI)	1.03 (0.94-1.12)	1.05 (0.96-1.13)
Intercept (mg/dL, 95% CI)	−0.16 (-1.05 to 0.74)	−0.53 (-1.38 to 0.32)
Correlation (r)	0.965	0.969
Standard Error of Estimate	0.045	0.043
Mean Bias (mg/dL)	+0.125	−0.082
Lower Limits of Agreement (mg/dL)	−1.38	−1.55
Upper Limits of Agreement (mg/dL)	+1.63	+1.38

CI: confidence interval; limits of agreement calculated using Bland–Altman analysis.

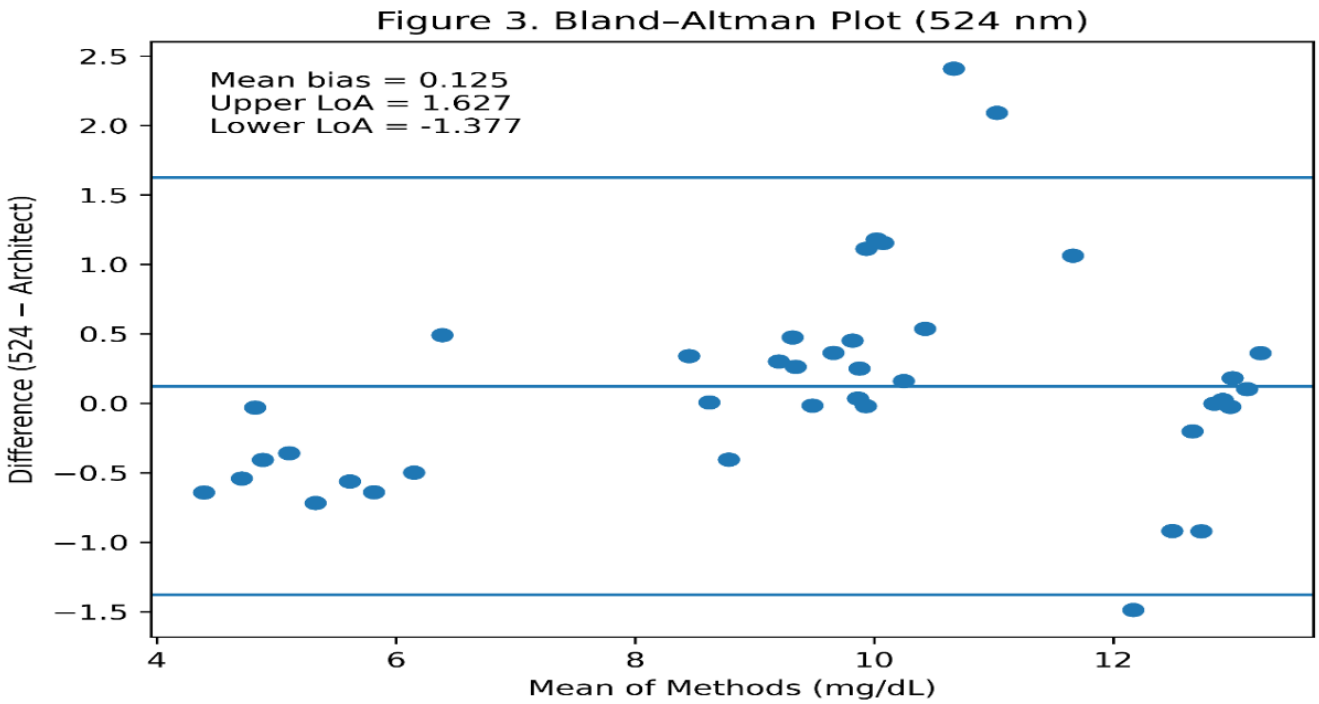
Figure 2: Regression Analysis between Spectrophotometric Measurement at 524 nm and Architect Autoanalyzer Results.



Linear regression analysis comparing spectrophotometric measurements performed at 524 nm with Architect autoanalyzer results in 40 samples. The solid line represents the regression line, and the dashed line represents the identity line (y = x).

Bland–Altman analysis demonstrated comparable overall agreement between methods at both wavelengths, with a mean bias of +0.125 mg/dL at 524 nm and −0.082 mg/dL at 572 nm (Figure 3).

Figure 3: Bland–Altman Analysis of Measurements at 524 nm.



Bland–Altman plot showing mean bias and limits of agreement between spectrophotometric measurements performed at 524 nm and Architect autoanalyzer results in 40 samples.

In the low concentration subgroup (<9 mg/dL), mean negative bias was lower at 524 nm compared with 572 nm (−0.30 vs −0.55 mg/dL) (Table 1).

No statistically significant concentration-dependent bias was observed for either wavelength.

Discussion

In this study, we evaluated the analytical performance of an alternative wavelength in the O-CPC calcium assay under routine laboratory conditions. Measurements performed at 524 nm demonstrated comparable overall agreement with the conventional 572 nm wavelength, together with lower negative bias in the low concentration range.

Spectral analysis identified a secondary absorbance feature near 536 nm in addition to the primary peak at 572 nm. Because this wavelength is not operationally available in routine analysers, 524 nm was selected as the nearest practical alternative for analytical evaluation. The objective was not to maximize absorbance intensity, but to assess whether an alternative spectral region with lower background absorbance could influence analytical performance characteristics. Previous studies evaluating derivative spectrophotometric approaches and wavelength optimization strategies have similarly demonstrated that alternative spectral regions may influence analytical signal characteristics and background interference [4,6].

One of the most consistent findings was the substantial reduction in reagent blank absorbance at 524 nm. Lower background absorbance may improve signal-to-background characteristics, particularly in measurements near the lower analytical range [7]. In the subgroup analysis, negative bias in samples with calcium concentrations below 9 mg/dL was lower at 524 nm compared with 572 nm. Precision profiles were comparable between wavelengths, suggesting that this observation was not solely attributable to analytical imprecision.

Although the absolute difference in bias was modest, small analytical deviations near clinical decision thresholds may influence classification of borderline hypocalcemia. In this context, reduction of negative bias in the low concentration range may still be analytically relevant, particularly when considered within acceptable total allowable error limits for calcium measurement [5].

The calcium–O-CPC reaction may demonstrate concentration-dependent nonlinearity related to complex stoichiometry at low calcium concentrations and reagent limitation at higher concentrations, supporting the routine use of two-point or multipoint calibration strategies [8]. In this context, the consistent linearity observed at 524 nm across both 0–6–10 and 0–4–8 calibration schemes suggests stable analytical response characteristics across the evaluated measurement range.

Regression analyses demonstrated a slope numerically closer to unity and a smaller intercept magnitude at 524 nm relative to 572 nm. However, confidence intervals overlapped between methods, indicating broadly comparable overall analytical agreement.

Importantly, the present findings do not suggest replacing the conventional wavelength solely on the basis of correlation equivalence. Instead, they demonstrate that wavelength selection represents a controllable analytical parameter that can be adjusted to improve performance in specific concentration ranges. Because this approach does not require modification of reagent composition or instrumentation, it may represent a practical strategy for analytical optimization in routine laboratory settings.

From a clinical laboratory perspective, even modest differences in analytical performance may have meaningful implications when applied across large testing volumes. In particular, reduced negative bias in the low concentration range may support more consistent classification near hypocalcemia decision thresholds. The primary contribution of this study is not the introduction of a new analytical method, but the demonstration that wavelength selection may influence analytical characteristics within clinically relevant concentration ranges.

This study has several limitations. The automated analyzer was used as a comparative method rather than a definitive reference method such as isotope dilution mass spectrometry or atomic absorption spectroscopy [9]. Second, the sample size was modest and the study should therefore be considered an exploratory analytical evaluation rather than a full-scale clinical validation study. Third, prepared mixture samples were included to extend the analytical measurement range; however, dilution-related matrix effects may have influenced analytical behavior and limit direct comparability with native patient samples [10,11]. In addition, potential analytical interference from hemolysis, lipemia, bilirubin, or magnesium was not specifically evaluated. Because wavelength availability differs among analytical platforms, the findings may be partially instrument-dependent and require validation in other automated systems before broader generalization. Finally, because the study was performed using a single analytical platform, external validation on other systems is required.

In conclusion, measurement at 524 nm demonstrated comparable overall analytical performance to the conventional 572 nm wavelength, together with lower negative bias in the low concentration range. These findings suggest that alternative wavelength selection may represent a practical and cost-neutral approach for analytical optimization of routine O-CPC calcium assays.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval and informed consent were waived according to institutional policy applicable at the time of the study because anonymized residual samples obtained during routine laboratory practice were used.

Author contributions

S.H. designed the study and wrote the manuscript. Ö.Ö. contributed to data analysis. O.M.İ. and M.G. supervised the study and reviewed the manuscript. All authors approved the final version.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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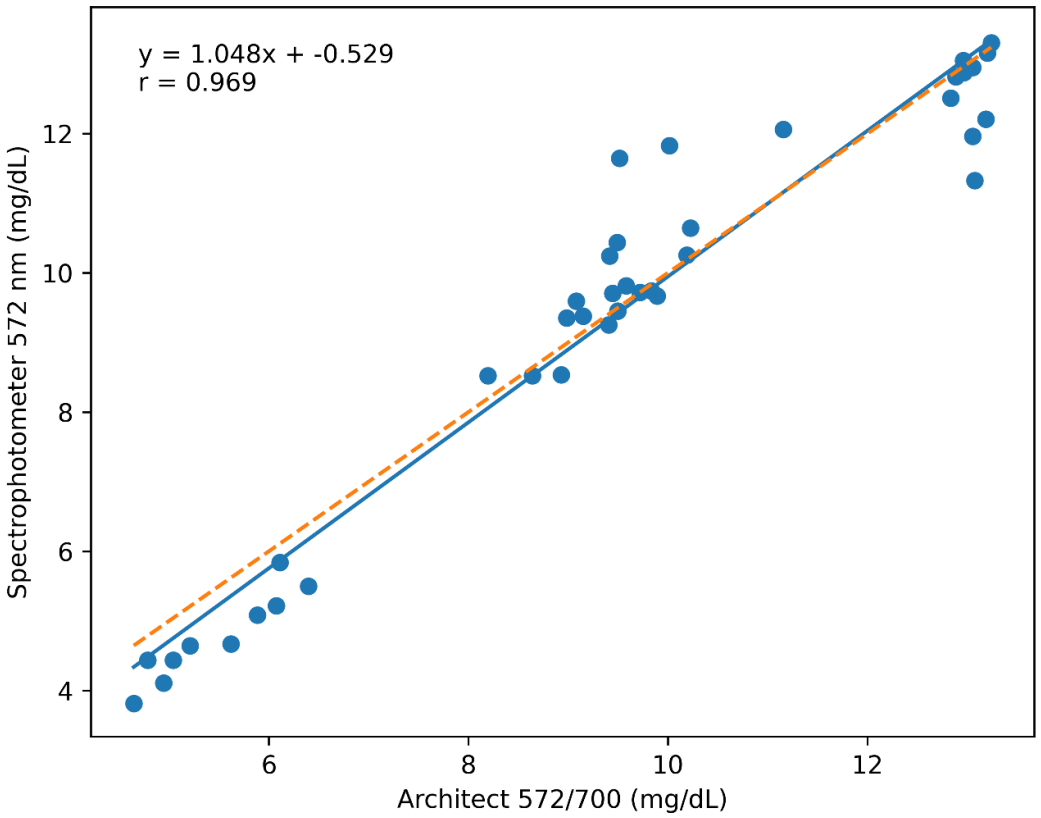
Supplementary Table S1: Agreement analysis using alternative regression models.

Method	Slope	Intercept
Linear regression	0.98	0.14
Passing–Bablok	0.98	0.20
Deming	0.98	0.10

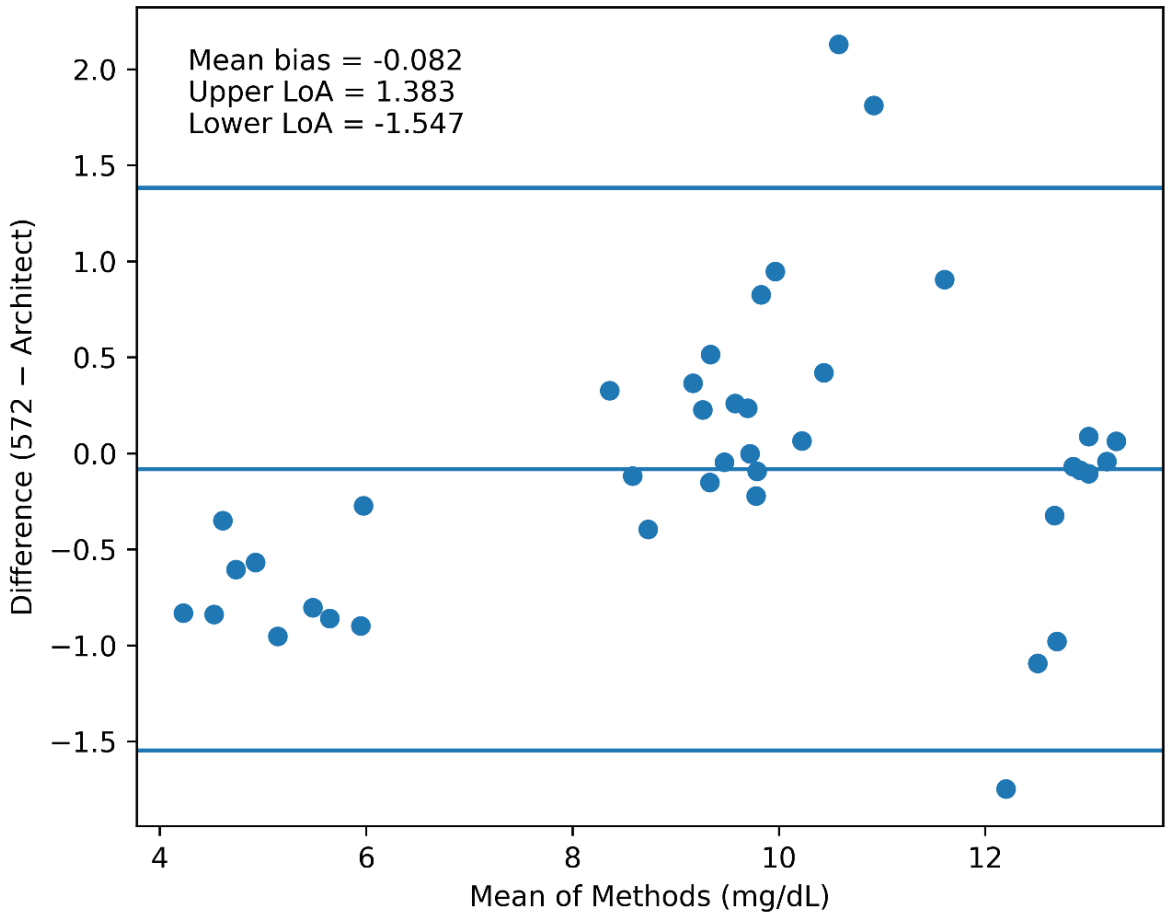
Comparison of regression parameters obtained using different analytical models (linear regression, Passing–Bablok, and Deming regression) for spectrophotometric measurements at 524 nm. Slope and intercept values are presented. Results are presented for comparison purposes and reflect model-specific estimates obtained using different regression approaches. Minor differences in slope and intercept values are expected due to methodological differences between regression models.

Supplementary Figure 1: Regression Analysis between Spectrophotometric Measurement at 572 nm and Architect 572/700 nm Method.



Linear regression analysis showing agreement between spectrophotometric measurements performed at the conventional 572 nm wavelength and Architect autoanalyzer results (572/700 nm). The solid line represents the regression line, and the dashed line represents the identity line.

Supplementary Figure 2: Bland–Altman Agreement Analysis for 572 nm Measurement.



Bland–Altman plot demonstrating mean bias and limits of agreement between spectrophotometric measurements at 572 nm and Architect 572/700 nm results.