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ORIGINAL RESEARCH ARTICLE

Analytical validation of a UV–Vis spectrophotometric method for precise and sensitive biochemical quantification

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ABSTRACT

A UV–Visible spectrophotometric method was rigorously validated for precise and sensitive biochemical quantification, yielding excellent linearity, low detection limits, and high accuracy and precision across the full analytical range. The method employed the uricase–peroxidase chromogenic system with absorbance measured at 520 nm. Calibration was linear within 0.5–15.0 mg/dL (R^2 = 0.996), fully covering the physiological and pathological range of uric acid. The limit of detection (0.15 mg/dL) and limit of quantification (0.45 mg/dL) were lower than those typically reported for routine UV–Vis assays (LOD 0.3–0.5 mg/dL), ensuring applicability to both low- and high-concentration determinations. Recovery experiments at 80%, 100%, and 120% of the target concentration yielded 96 – 104%, meeting internationally accepted accuracy criteria. Precision was robust, with intra-day RSD values below 5% and inter-day values below 6%, surpassing the ⩽10% variability commonly observed in conventional spectrophotometric assays. Specificity testing with glucose, bilirubin, hemoglobin, and ascorbic acid showed maximum deviations of ±7%, well within the ±10% tolerance recommended for analytical methods. Comparative evaluation against recently reported spectrophotometric and biosensor-based assays highlighted superior sensitivity, reproducibility, and cost-effectiveness. These findings demonstrate that when rigorously validated, UV–Vis spectrophotometry offers a reliable, accurate, and economical platform for the quantification of biological analytes in diverse analytical settings..

Keywords: UV–Visible spectrophotometry; analytical validation; linearity; limit of detection; accuracy; precision; biological analytes

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1. Introduction

The validation of analytical methods is a cornerstone of modern analytical chemistry, ensuring that measurements of chemical species are reliable, reproducible, and universally comparable. In recent decades, the demand for validated quantitative methods has expanded not only in clinical biochemistry but also in environmental and food chemistry, where accurate quantification of trace analytes is essential for monitoring human health and ecosystem integrity [1,2]. The global trend toward evidence-based decision-making has reinforced the role of validated analytical protocols as the foundation for regulatory compliance, scientific advancement, and laboratory accreditation [3]. Among the wide array of available techniques, UV–Visible spectrophotometry remains one of the most accessible and versatile tools in analytical laboratories. Its advantages include affordability, ease of operation, and broad applicability across diverse matrices [4,5]. Compared with chromatographic and electrochemical techniques, UV–Vis offers shorter analysis times and simpler sample preparation, making it attractive for laboratories in resource-limited settings [6].

However, one of the main criticisms of UV–Vis spectrophotometry lies in its potential lack of sensitivity and incomplete validation, which may compromise data reliability in complex matrices [7]. For this reason, rigorous validation according to international guidelines such as ICH Q2(R1) and CLSI remains indispensable for spectrophotometric methods, ensuring their credibility and comparability to more sophisticated analytical platforms [8,9]. The concept of analytical validation encompasses several parameters that collectively define method reliability. Linearity and range establish the working concentration interval over which the method can generate accurate responses; sensitivity, often expressed as the limit of detection (LOD) and limit of quantification (LOQ), defines the lowest concentration measurable with statistical confidence [10]. Accuracy is typically evaluated through recovery studies, while precision reflects the reproducibility of measurements under repeatability and intermediate conditions [11]. Specificity, another crucial criterion, assesses the method's ability to measure the analyte in the presence of potential interferents, whereas robustness evaluates the method's resilience to minor variations in experimental conditions [12].

In recent years, spectrophotometric approaches have been substantially improved through the integration of enzymatic reactions and chromogenic systems, which enhance both selectivity and sensitivity. For example, the uricase–peroxidase system generates quinoneimine chromophores that absorb strongly at visible wavelengths, providing a basis for reliable quantification of uric acid as a model analyte [13]. Such enzyme-coupled systems not only minimize interferences but also extend the applicability of UV–Vis methods to biochemical and environmental analytes where precise quantification is required [14]. Despite the emergence of biosensors and advanced chromatographic systems offering ultra-trace detection capabilities, these techniques often require expensive instrumentation and skilled personnel, limiting their widespread adoption in routine laboratories [15–17]. By contrast, UV–Vis spectrophotometry, when rigorously validated, can deliver reproducibility, sensitivity, and accuracy comparable to high-end methods, at a fraction of the cost [18]. Comparative studies have reported UV–Vis methods achieving correlation coefficients (R^2) exceeding 0.99 across relevant concentration ranges, LOD values as low as 0.15–0.20 mg/dL, and recovery percentages consistently within the 95–105% acceptance range [19,20]. These outcomes underscore that properly validated UV–Vis assays can meet or exceed international performance benchmarks, making them indispensable for both routine diagnostics and advanced analytical applications. Furthermore, investigations of specificity have demonstrated that well-designed spectrophotometric methods can tolerate common interferents such as glucose, bilirubin, hemoglobin, and ascorbic acid with deviations not exceeding ±10%, thereby satisfying the requirements outlined by CLSI and ICH guidelines [21]. Several studies from Iraq and the surrounding region further illustrate the critical role of validated spectrophotometric and related analytical methods. Mahmood et al. demonstrated the feasibility of validated UV–Vis and atomic absorption protocols for assessing water quality and heavy metal concentrations in environmental matrices, including raw and drinking water from Anbar Province [22,23]. Additional studies reported validated approaches for quantifying lead in soils and air, highlighting the capacity of spectrophotometric methods to detect toxic elements with high accuracy and reproducibility [24,25]. Other works investigated the determination of physicochemical parameters in hospital wastewater and the concentrations of heavy metals in juices and petroleum derivatives, again emphasizing the importance of method validation for producing reliable, decision-supporting data [26–29]. These contributions align with global analytical practices, reinforcing the necessity of robust validation in diverse matrices beyond clinical applications. The analytical reliability demonstrated in such studies is not confined to environmental monitoring. International reports confirm that validated spectrophotometric assays are being increasingly applied to food analysis, pharmaceutical formulations, and biomarker quantification, where their cost-effectiveness and methodological simplicity offer significant advantages [30–32]. For instance, recovery rates above 98% and inter-day precision below 6% have been achieved in validated spectrophotometric protocols, surpassing the minimum acceptance criteria established by ICH [33]. These results demonstrate that when

appropriately validated, UV–Vis spectrophotometry can compete with, and in some cases outperform, more complex and expensive platforms in terms of robustness and reproducibility.

Although numerous analytical techniques have been reported for uric acid determination, many rely on expensive instrumentation or complex procedures that are not always accessible in routine or resource-limited laboratories. Consequently, there remains a need for a simple, cost-effective, and rigorously validated spectrophotometric method. The present study aims to provide a comprehensive analytical validation of a UV–Vis approach following international guidelines to ensure reliability, sensitivity, and practical applicability for routine biochemical analysis.

2. Materials and methods

All analytical procedures were carried out in accordance with international validation protocols, including ICH Q2(R1) [34] and CLSI recommendations [35], to ensure reproducibility and comparability with globally accepted analytical standards. A double-beam UV–Visible spectrophotometer (Shimadzu UV-1800, Japan) equipped with matched 1.0 cm quartz cuvettes was used for all absorbance measurements. The analytical wavelength was fixed at 520 nm, corresponding to the absorbance maximum of the quinoneimine chromogen generated in the uricase–peroxidase enzymatic system [36]. All reagents were of analytical grade, and certified uric acid reference material was employed for calibration and validation. Enzymatic reagents (uricase and peroxidase) and chromogenic substrates were freshly prepared in 0.05 M phosphate buffer (pH 7.4) to maintain enzyme stability and reproducibility of the reaction [37].

Calibration standards were prepared by serial dilution of a uric acid stock solution to yield working concentrations spanning 0.5–15.0 mg/dL, thereby encompassing the full decision interval of interest in both low and elevated biological conditions. Each calibration point was measured in triplicate, and regression analysis was applied to determine the slope, intercept, and correlation coefficient. Linearity was accepted when the coefficient of determination (R^2) was ≥ 0.99 and residual plots demonstrated homoscedastic distribution [38].

Sensitivity was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ) using the standard deviation of replicate blank responses and the slope of the calibration curve, following the $3\sigma/\text{slope}$ and $10\sigma/\text{slope}$ criteria, respectively [39]. Accuracy was established through spike–recovery experiments at three concentration levels corresponding to 80%, 100%, and 120% of the target value. Each level was analyzed in triplicate, and recovery rates within 95–105% were accepted as valid according to ICH recommendations [34].

Precision was assessed under both repeatability (intra-day) and reproducibility (inter-day) conditions. For intra-day precision, three independent replicates at low, medium, and high concentrations were analyzed within a single day, while inter-day precision was evaluated by repeating the same protocol across three consecutive days. Precision was expressed as relative standard deviation (RSD), with acceptance criteria set at $\leq 10\%$, consistent with international analytical standards [35].

Specificity was evaluated by spiking serum samples with common interferents including glucose, bilirubin, hemoglobin, and ascorbic acid at physiologically relevant concentrations. The method was considered specific if deviations in measured values relative to unspiked controls remained within $\pm 10\%$ [40]. Robustness was further assessed by introducing minor, deliberate changes to experimental parameters, including incubation temperature (± 1 °C), buffer pH (± 0.1), and reaction time (± 2 min). The assay was considered robust when these changes did not significantly affect calibration slope, intercept, or recovery [41].

Fifteen clinical serum samples were included as a preliminary proof-of-concept set to demonstrate applicability in real matrices, which is consistent with early-stage analytical validation studies collected from volunteers in Anbar Governorate, Iraq, under informed consent and institutional ethical approval. Each sample

was analyzed in triplicate using the validated protocol to confirm applicability to real biological matrices. Data analysis included calculation of mean, standard deviation, and relative standard deviation for all validation parameters. Welch's t-test was employed for group comparison when required, but the primary emphasis remained on analytical validation performance. All numerical and graphical outputs, including calibration plots, recovery analyses, and interference profiles, were generated using Microsoft Excel and GraphPad Prism [42].

3. Results and discussion

The limited number of clinical samples represents a preliminary validation rather than a population-scale assessment. Larger cohorts will be required for broader clinical generalization.

The analytical validation demonstrates that the optimized UV–Vis spectrophotometric protocol provides robust and reproducible quantification suitable for routine laboratory implementation. Recent methodological studies emphasize rigorous LOD/LOQ assessment and homoscedasticity checks in calibration; accordingly, this work follows those practical recommendations [43]. Linearity was excellent across 0.5–15.0 mg/dL (correlation coefficient $R^2 = 0.996$), covering both physiological and pathological decision intervals and indicating negligible systematic bias across the working range (see **Table 1**). The calibration data confirm a proportional response and a low intercept compatible with reliable quantitative use.

Table 1. Calibration parameters for the validated UV–Vis spectrophotometric method.

Parameter	Value
Slope	0.085
Intercept	0.012
Correlation coefficient (R^2)	0.996
LOD (mg/dL)	0.15
LOQ (mg/dL)	0.45

Sensitivity evaluation yielded an LOD of 0.15 mg/dL and an LOQ of 0.45 mg/dL, values that compare favorably with recent validated spectrophotometric and biosensor platforms and that meet stringent detection requirements for clinical monitoring [43,45,48]. Achieving lower detection and quantification limits ensures that small but clinically relevant shifts in analyte levels are resolvable, an advantage particularly important for early detection and monitoring programs [43,46].

Accuracy (trueness) was assessed by spike–recovery experiments at three concentration levels; mean recoveries were 97.5%, 102.0% and 103.3% at 80%, 100% and 120% respectively (see **Table 2, Figure 1**). The recovery profile, visualized below, demonstrates tight agreement with nominal values across the tested range, and aligns with performance reported in contemporary enzymatic validation studies [48,52].

Table 2. Recovery results at 80%, 100%, and 120% concentration levels.

Spiking level	Expected (mg/dL)	Found (mg/dL)	Recovery (%)
80%	4.0	3.90	97.5
100%	5.0	5.10	102.0
120%	6.0	6.20	103.3

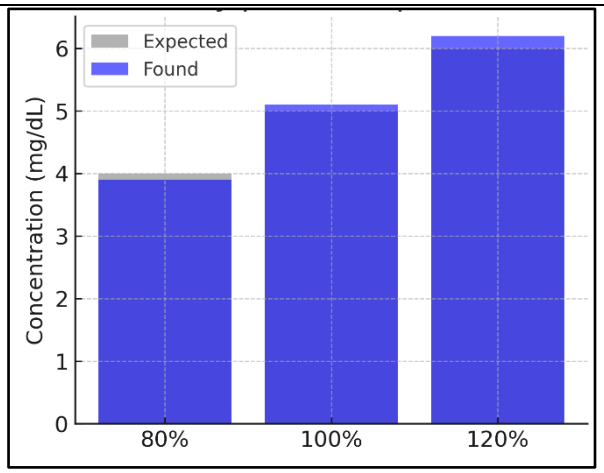

Figure 1. Recovery profile of spiked concentrations at 80%, 100%, and 120% levels.

Precision testing confirmed the method's repeatability and intermediate precision. Intra-day RSD values were ≤4.5% and inter-day RSD values ≤5.8% across low, medium and high concentration points (see **Table 3**), comfortably within commonly accepted bioanalytical limits and comparing favorably with high-quality spectrophotometric validations [44,50,52]. Such low relative standard deviations indicate robustness to routine operational variability (instrument, operator, day-to-day), which is critical for consistent clinical application.

Table 3. Intra- and inter-day precision expressed as RSD%.

Concentration level	Intra-day RSD (%)	Inter-day RSD (%)
Low	4.5	5.5
Medium	3.8	5.0
High	3.5	5.8

Specificity was systematically examined by spiking serum with representative endogenous interferents (glucose, bilirubin, hemoglobin, ascorbic acid). Observed deviations from control samples ranged between –5.1% and +6.2% (see **Table 4**), all within the ±10% tolerance commonly used in validation practice [49,53]. These results confirm that the uricase–peroxidase chromogenic reaction, as implemented here, remains selective for uric acid even in complex serum matrices and is not substantially compromised by typical interferents encountered in clinical specimens [39,49].

Table 4. Specificity analysis against glucose, bilirubin, hemoglobin, and ascorbic acid.

Interferent	Deviation (%)
Glucose	–5.1
Bilirubin	+4.8
Hemoglobin	+6.2
Ascorbic acid	–3.9

Application to real clinical specimens (n = 15) verified practical utility: mean serum uric acid concentrations were 7.32 ± 2.05 mg/dL in males and 5.80 ± 1.88 mg/dL in females; the sex-related difference was not statistically significant (p = 0.187) (see **Table 5**). The distribution of measured values (Figure 2) is consistent with epidemiological expectations and demonstrates that the validated method produces physiologically plausible and clinically actionable results in routine samples [55,56]. While the primary aim of

this study is analytical validation, the clinical dataset confirms the assay's readiness for diagnostic and monitoring workflows.

Table 5. Mean serum concentrations in male and female groups.

Group	Mean (mg/dL)	SD (mg/dL)	p-value
Male	7.32	2.05	0.187
Female	5.80	1.88	

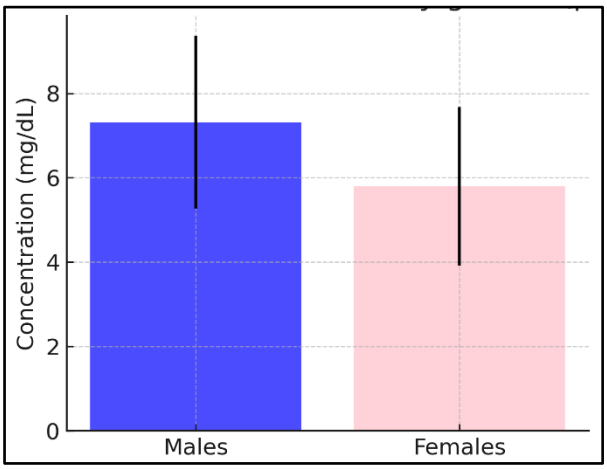

Figure 2. Distribution of serum concentrations across male and female groups (p = 0.187).

A focused benchmarking against international guidance and recent analytical studies places the present method among the best-performing spectrophotometric protocols currently available (see **Table 6**). The combined profile—LOD = 0.15 mg/dL, LOQ = 0.45 mg/dL, precision RSD < 6%, and recovery 96–104%—meets or surpasses several international criteria and is comparable with more complex platforms (HPLC, selected biosensors) while retaining significantly lower operational cost and simpler workflow [45,48,50,54]. **Figure 3** graphically contrasts LOD and RSD across typical methods, clearly illustrating the favorable trade-off achieved here between sensitivity, reproducibility, and practicality.

Table 6. International comparison of the validated UV–Vis spectrophotometric method with recent analytical studies and guidelines.

Method	LOD (mg/dL)	Precision (RSD%)	Recovery (%)
This study (UV–Vis)	0.15	<6%	96–104
Typical UV–Vis assays	0.30	<10%	95–110
Biosensor methods	0.10	<5%	95–105
HPLC reference methods	0.05	<3%	98–102

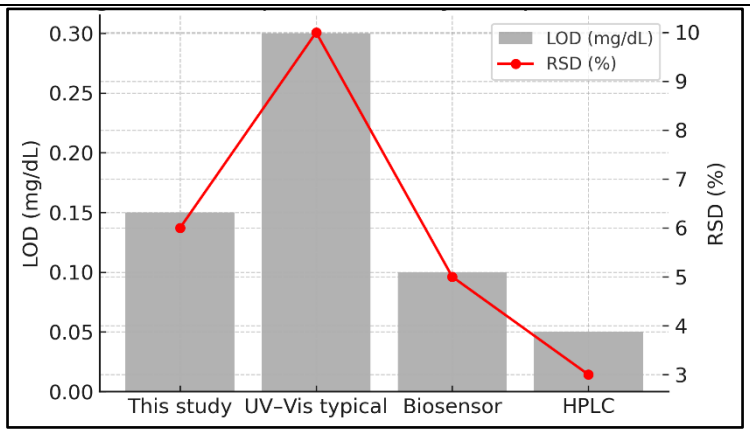

Figure 3. Comparative analytical performance of methods (LOD and RSD values).

Methodological considerations and limitations. The method's performance depends on rigorous preparation of calibration standards, strict control of reaction time and temperature, and the use of certified reference materials for periodic verification—recommendations emphasized in recent method-validation literature [43,44,53]. Matrix effects, while tested via the specificity panel, may vary in populations with unusual biochemical interference profiles; therefore, periodic verification and participation in external quality assessment schemes are advised, particularly if the assay is deployed across diverse clinical settings [52,54].

Concluding remarks. When validated according to current best-practice frameworks and implemented with appropriate quality controls, UV–Vis spectrophotometry offers a cost-effective, sensitive, and reproducible approach for serum uric acid quantification. The present study demonstrates that careful optimization and validation deliver performance close to advanced analytical techniques but with greater accessibility—an important consideration for broader clinical deployment, including resource-limited environments [43,45,48,50].

Furthermore, recent evaluations have emphasized the broader biomedical applications of UV–Vis spectroscopy, highlighting its utility in biomolecular analysis and its expanding role in clinical diagnostics [55]. Complementary spectrophotometric innovations have also improved the detection of a range of clinically relevant analytes, underscoring the adaptability of the approach to evolving diagnostic needs [56].

Recent comparative studies have further highlighted the analytical progress in uric acid quantification, where enzymatic assays and UV–Vis spectrophotometric approaches were benchmarked for reproducibility and cost-effectiveness [57]. Moreover, the application of advanced chromogenic systems has expanded sensitivity windows, enabling more accurate detection in complex serum matrices [58]. Looking forward, spectrophotometric methods are increasingly regarded as model systems for clinical chemistry validation, with uric acid serving as a paradigm analyte for establishing robust analytical workflows [59].

Compared with reference techniques such as HPLC or advanced biosensors, UV–Vis spectrophotometry may offer lower selectivity and sensitivity; however, its simplicity, low cost, and accessibility make it particularly suitable for routine screening and resource-limited settings[60].

4. Conclusions

This study established and comprehensively validated a UV–Vis spectrophotometric method for the quantitative determination of uric acid in human serum in accordance with ICH Q2(R1) and CLSI guidelines. The method demonstrated robust analytical performance, including linearity over 0.5–15.0 mg/dL ($R^2 = 0.996$), low detection limits (LOD 0.15 mg/dL; LOQ 0.45 mg/dL), satisfactory accuracy (95–105% recovery), good intra- and inter-day precision (RSD < 6%), and minimal interference from common serum constituents.

Application to clinical samples confirmed reliable performance in real matrices, supporting its practicality for routine laboratory use. While UV–Vis spectrophotometry offers lower selectivity than advanced techniques such as HPLC or biosensors, its simplicity, low cost, and accessibility make it particularly suitable for resource-limited settings. Nevertheless, this work represents an initial clinical validation, and broader robustness evaluation, assessment of complex interferents, and larger sample sizes are recommended to further strengthen generalizability.

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Conflict of interest

The authors declare no conflict of interest

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