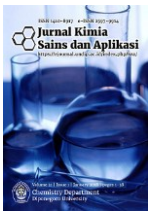
ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 29 (6) (2026): 388-395

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa



Method Evaluation for Chemical Oxygen Demand (COD) in Wastewater Treatment Plant Samples from an Industrial Estate using Closed Reflux Colorimetry

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https://doi.org/10.14710/jksa.29.6.388-395

Article Info

Article history:

Received: 01st March 2026
Revised: 11th June 2026
Accepted: 18th June 2026
Online: 31st July 2026

Keywords:
COD; Environmental monitoring; Uncertainty; Industrial estate

Abstract

This study presents the verification of the APHA Standard Method 5220 D for determining chemical oxygen demand (COD) in wastewater using a spectroscopic closed-reflux colorimetric system. Both the low range (LR: 0–90 mg O_2/L) and high range (HR: 90–700 mg O_2/L) methods were verified to ensure their suitability for wastewater monitoring in the industrial estate, with the LR method applied to effluent samples and the HR method applied to influent samples. Method performance was evaluated using linearity, sensitivity, limit of detection (LOD), limit of quantification (LOQ), limit of linearity (LOL), precision, accuracy, and measurement uncertainty for both LR and HR COD levels. The calibration curves exhibited excellent linearity, with coefficients of determination (R^2) of 0.9991 (LR) and 0.9996 (HR). The LOD ($3S_{yx}/b$) and LOQ ($10S_{yx}/b$) values were 3.34 and 11.14 mg O_2/L for LR, and 15.48 and 51.59 mg O_2/L for HR, respectively. Precision testing produced %RSD values of 2.88% (LR) and 1.37% (HR), both meeting acceptance criteria based on ⅔ CV Horwitz. Accuracy evaluation using potassium hydrogen phthalate (KHP) standards yielded recoveries of 97.50–106.02% (LR) and 98.83–102.92% (HR), consistent with Association of Official Analytical Chemists (AOAC) requirements. Measurement uncertainty was assessed by combining contributions from calibration, precision, accuracy, and instrumental factors, resulting in expanded uncertainties of ±3.83 mg O_2/L for LR and ±13.87 mg O_2/L for HR (k = 2). The findings confirm that the method is reliable and suitable for routine COD analysis in wastewater monitoring.

1. Introduction

Industrial estates play a critical role in driving nationwide economic growth; however, industrial activities inevitably contribute to environmental pollution within these estates [1]. To mitigate such negative impacts, integrated environmental management efforts between tenant companies and estate administrators, particularly through monitoring pollutants at centralized wastewater treatment plants (WWTPs), are essential [2]. The JABABEKA Industrial Estate (JIE), located in Bekasi Regency, Indonesia, represents a modern eco-industrial estate model. JIE operates a centralized WWTP that uses a gravity-based sewer system to convey wastewater from tenant companies, which is then subjected to primary sedimentation, followed by aerobic biological treatment via an activated sludge process in an oxidation ditch to decompose organic pollutants [3].

Chemical oxygen demand (COD) is one of the key pollutant parameters in industrial wastewater [1, 4, 5, 6, 7, 8, 9]. The COD value indicates the oxygen equivalent (mg O_2/L) consumed during the oxidation of non-biodegradable pollutants by strong chemical oxidants such as potassium dichromate ($K_2Cr_2O_7$), potassium permanganate ($KMnO_4$), and hydrogen peroxide (H_2O_2) [10, 11]. A critical challenge in industrial wastewater

analysis is the presence of high chloride ion (Cl^-) concentrations as a major source of interference in COD determination because Cl^- can be oxidized by dichromate ($Cr_2O_7^{2-}$), leading to positive bias [12]. this interference is minimized by the addition of mercuric (II) sulfate ($HgSO_4$) as a complexing agent at $HgSO_4$: Cl^- ratio of 10:1, although the method is generally limited to samples with Cl^- concentrations below 2,000 mg.L^{-1} [13]. The closed-reflux spectroscopic method was specifically selected over the traditional titrimetric method for the JIE wastewater matrix due to its operational efficiency, reduced hazardous reagent volume, enhanced analytical throughput, and suitability for routine monitoring of industrial wastewater [14, 15, 16, 17].

The international standard method published by the American Public Health Association (APHA), Method 5220 D, describes COD determination by closed-reflux digestion, in which $K_2Cr_2O_7$ reacts with organic matter in the sample, reducing hexavalent chromium ($Cr_2O_7^{2-}$) to trivalent chromium (Cr^{3+}) after digestion. COD concentrations are measured spectroscopically in the visible spectrum. In accordance with SM 5220 D and established laboratory practices, measurements are based on the distinct absorbance behavior of chromium species ($Cr_2O_7^{2-}$ and Cr^{3+} ions) [14]. For LR COD values (≤ 90 mg O_2/L), absorbance is measured at 420 nm, where the intensity of $Cr_2O_7^{2-}$ ion absorption decreases linearly with increasing COD concentrations [18]. Conversely, for HR COD values (approximately 100 to 900 mg O_2/L), measurements are taken at 600–620 nm, corresponding to the increasing absorbance of Cr^{3+} ions [14].

These analytical ranges and wavelength selections are consistent with standardized instrument-specific operating procedures, such as Environmental Protection Agency (EPA) Method 410.4 Revision 2.0, measuring ranging from 3–900 mg O_2/L at 600 nm [16], and Hach Method 8000, which utilize optimized wavelengths ranging from 350 nm for trace levels to 620 nm for high concentration samples to ensure measurement accuracy [15]. This method is widely applicable across various types of wastewater and offers several advantages, including low cost, simplicity, rapid analysis, good sensitivity, and strong linearity [17].

The standard methods for COD measurement must be verified before implementation in the laboratory, in accordance with ISO 17025 requirements [12]. Method verification is essential to demonstrate the validity of analytical testing through the determination of parameters such as linearity, detection limit, precision, accuracy, and estimation of measurement uncertainty [13, 19]. Verification and continuous monitoring of COD measurements are critical for controlling the quality of industrial wastewater in JIE, both before and after treatment by the wastewater treatment plants (WWTPs). According to JIE regulations, influent wastewater from industries must have a COD concentration of ≤800 mg O_2/L before WWTP processing [2], while effluent must comply with a maximum COD limit of ≤100 mg O_2/L, as stipulated in the West Java Governor Regulation No. 6 of 1999 [20, 21]. In addition, this limit is consistent with Appendix I of the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. 68 of 2016 [9], which establishes a maximum COD concentration of 100 mg/L [22]. Monitoring COD values in both effluent and total influent streams also serves as an indicator of WWTP optimization and performance.

2. Experimental

2.1. Materials and Equipment

All chemical materials were purchased from Merck in analytical-grade quality and used without further purification. The chemicals included $K_2Cr_2O_7$, KHP ($C_8H_5KO_4$), silver sulfate (Ag_2SO_4), $HgSO_4$, and sulfuric acid (H_2SO_4). Reagents were prepared using distilled water with a conductivity of ≤1 µS/cm. Due to the hazardous nature of the reagents, all waste containing Hg^{2+}, Ag^+, and $Cr_2O_7^{2-}$ ions must be collected, stored, and managed in accordance with applicable environmental safety procedures and hazardous waste management regulations. The instrument utilized for analysis was a UV-Visible spectrophotometer (HACH, DR6000). Supporting apparatus included an analytical balance with a precision of 0.1 mg, a thermoreactor (WTW, CR 4200), digestion vessels (16 × 100 mm), Class A volumetric pipettes (1 and 20 mL), and Class A volumetric flasks (100 and 1000 mL).

2.2. Preparation of Reagent and Standard Solution

The digestion solution was prepared by dissolving 10.216 g of potassium dichromate ($K_2Cr_2O_7$) for HR analysis and 1.022 g for LR analysis, both previously desiccated at 150°C for 2 hours, along with 33.3 g of mercury(II) sulfate ($HgSO_4$) and 167 mL of concentrated H_2SO_4(p), and then diluting the mixture with distilled water to a final volume of 1000 mL. The sulfuric acid reagent was prepared by dissolving 10.12 g of Ag_2SO_4 in concentrated H_2SO_4(p). The COD standard solution was prepared using KHP, which had been previously dried at 110°C, with a theoretical COD value of 1.176 mg O_2 per mg of KHP [14].

2.3. COD Measurement Procedure

COD measurements were carried out by mixing 2.5 mL of the sample or standard solution, 1.5 mL of digestion solution, and 3.5 mL of sulfuric acid reagent in digestion vessels. Prior to analysis, influent and effluent wastewater samples were thoroughly homogenized and analyzed without filtration to determine total COD. The mixtures were then subjected to reflux digestion for 2 hours at 150°C. Following the digestion process, the solutions were allowed to cool to room temperature before spectrophotometric measurement. In accordance with Standard Methods 5220 D, absorbance for LR COD measurements was determined at 420 nm, whereas absorbance for HR COD measurements was determined at 600 nm [14].

2.4. Verification Methods

2.4.1. Linearity of Methods

Linearity was determined by preparing a COD standard series at concentrations of 90, 200, 300, 400, 500, 600, and 700 mg O_2/L for the HR, and 20, 30, 50, 70,

and 90 mg O_2/L for the LR. Both sets of standard solutions were prepared from a 1000 mg O_2/L COD stock solution. The linear regression equation applied was Y = bX + a, where Y represents the absorbance response, X represents the measured COD concentration, b is the slope, and a is the intercept of the calibration curve [23]. Acceptable linearity was defined by an R-square value (R^2) of ≥ 0.995.

2.4.2. Detection and Linearity Limit

The LOD was estimated from the linear regression analysis using the standard error of estimate (S_{yx}), with the formula LOD = 3.S_{yx}/b and the LOQ were calculated as LOQ = 10.S_{yx}/b [24]. The Limit of Linearity (LOL) was determined by performing 10 replicate measurements (n =10) at both the highest and lowest spiked concentrations within the linear calibration series. The $F_{statistic}$ was used to evaluate LOL acceptance by comparing the standard deviations (SD) of the concentration levels, based on Equation 1.

$$F_{statistic} = \frac{SD_1^2}{SD_2^2}, \text{where } SD_1^2 > SD_2^2 \quad (1)$$

Where, SD_1^2 is the variance of the COD values measured using the first method, SD_2^2 is the variance of the COD values measured using the second method. The calculated $F_{statistic}$ was evaluated against the critical value from the F_{table} at a 99% confidence level (α = 0.01; df = 9.9). If $F_{statistic} \leq F_{table}$, the variance is considered homogeneous and the linearity range is accepted, confirming the LOL.

2.4.3. Precision

The precision determination was performed by conducting 7 replicate COD measurements using an effluent sample for the low range and an influent sample for the high range. The acceptability of precision was assessed by calculating the percentage of relative standard deviation (%RSD) [25]. The %RSD values were considered acceptable if they were less than or equal to two-thirds of the coefficient of variation according to the Horwitz equation (≤ ⅔ %CV Horwitz) [26]. Both calculations can be expressed using Equations 2 and 3.

$$\%RSD = \frac{\sigma}{\bar{C}} \times 100\% = \frac{\frac{\sqrt{\sum_{i=1}^{n}(C_i-\bar{C})^2}}{n-1}}{\bar{C}} \times 100\% \quad (2)$$

$$\%CV\ Horwitz = 2^{1-0.5\log C} \quad (3)$$

Where, C_i is the individual COD measurement value, $\bar{C}$ is the average COD measurement value, C is the concentration (mg O_2/L), and n is the number of replicate measurements.

2.4.4. Accuracy

The accuracy determination was performed by conducting 7 replicate COD measurements using KHP standard solutions, with theoretical COD values of 50 mg O_2/L for the LR and 500 mg O_2/L for the HR. The accuracy was evaluated based on the percent recovery (%R) of the COD standard, with an acceptable range based on the AOAC standard [27]. The %R was calculated using Equation 4.

$$\%R = \frac{C_{measure}}{C_{target}} \times 100\% \quad (4)$$

Where, $C_{measure}$ is the measured COD concentration, and C_{target} is the target concentration value of the in-house COD reference material.

2.4.5. Uncertainty

The uncertainty measurement was conducted using the specification method, which involved identifying sources of component uncertainties through a fishbone diagram. The identified sources of uncertainty were classified as Type A or Type B per the standard evaluations, and each source was used to calculate the corresponding standard uncertainty. All standard uncertainties were then combined, based on their relative contributions, to determine the combined standard uncertainty. Finally, the expanded uncertainty was calculated by applying an appropriate coverage factor to the combined standard uncertainty [12, 17, 28].

3. Results and Discussion

3.1. Verification of Analytical Method for High and Low COD Levels

3.1.1. Linearity Assessment of the COD Method

The laboratory of PT. Jababeka Infrastruktur employed the internationally recognized APHA SM 5220 D for COD determination. Before implementation, this method requires verification. Linearity, one of the key verification parameters, was assessed to determine the analytical capability across various concentration ranges in measuring COD levels in wastewater samples. This linearity was represented through a calibration curve, illustrating the relationship between COD concentration and the absorbance response detected via spectroscopy [29]. Figure 1 presents the calibration curve of COD measurement using the spectroscopic method in high and low concentration ranges. The linear regression equation for HR COD (90 – 700 mg O_2/L) was Y = 0.0003X + 0.0131, while for LR COD (0 – 90 mg O_2/L), it was Y = –0.0019X + 0.2185. The coefficient of determination (R^2) values were obtained as 0.9996 for HR and 0.9991 for LR, both exceeding the threshold of 0.9950, thereby confirming the linearity of the method.

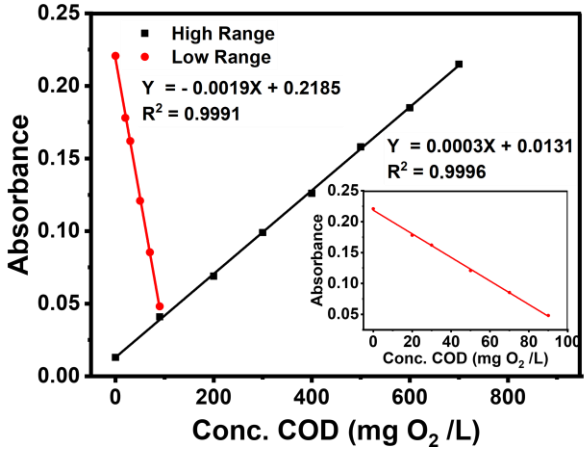

Figure 1. Calibration curves of COD measurement at high and low concentrations

The positive slope observed for the HR calibration curve and the negative slope observed for the LR calibration curve are consistent with the principle of APHA Standard Method 5220 D, in which increasing COD concentration results in increased Cr^{3+} absorbance at 600 nm and decreased residual $Cr_2O_7^{2-}$ absorbance at 420 nm. Statistical evaluation of the calibration models (Table S1) demonstrated acceptable 95% confidence intervals for the slope and intercept, while the residual plots (Figure S1) showed that the regression equations were highly significant, as indicated by large F-values and very low p- values ($p < 0.001$), confirming a strong linear relationship between COD concentration and absorbance response. Furthermore, the 95% confidence intervals for the slope and intercept parameters were relatively narrow, indicating good precision of the regression estimates. Residual plots for the LR and HR calibration models (Figures S1 and S2, respectively) showed a random distribution around zero without systematic trends, suggesting the absence of significant heteroscedasticity and confirming the suitability of the linear regression models.

3.1.2. LOD and LOL in COD Measurement

The LOD and LOQ represent the lowest concentration of COD in a sample at which the measurement signal can be consistently detected (LOD) and reliably quantified (LOQ), respectively [30]. The LOD is commonly estimated as $3.S_{yx}/b$, whereas the LOQ is estimated as $10.S_{yx}/b$ [25]. Based on the S_{yx}/b ratio derived from the linearity test, the LOD values for COD measurement were 15.48 mg O_2/L for the HR and 3.34 mg O_2/L for the LR. Correspondingly, the LOQ values were 51.59 mg O_2/L (HR) and 11.14 mg O_2/L (LR), which are approximately 3.33 times the corresponding LOD values, consistent with the applied calculation approach.

LOL refers to the highest COD concentration at which the analytical response remains linear as the COD level increases. Figure 2 illustrates the LOL determination, based on the lowest and the highest signals within the linear calibration series. The calculated $F_{statistics}$ were 3.51 (HR) and 1.99 (LR), while the critical F_{table} value at $\alpha = 0.01$ (degree of freedom (df) = 9; 9) was 5.35. Since the $F_{statistics}$ were less than the F_{table} value, the LOL tests results were accepted.

3.1.3. Assessment of Precision in COD Measurement

Precision is defined as the closeness of repeated measurement values obtained under specified conditions, using the same analyst and performed over short time intervals [31]. Table 1 presents the precision results of COD standard measurements at concentrations of 50 mg O_2/L (LR) and 500 mg O_2/L (HR), with average measured COD values (n = 7) of 50.74 ± 1.46 mg O_2/L (LR) and 502.03 ± 6.88 mg O_2/L (HR), respectively. The precision for both concentration ranges was considered acceptable, as indicated by %RSD values of 2.88% (LR) and 1.37% (HR) [32], which were ≤ ⅔ CV Horwitz values of 5.91% (LR) and 4.18% (HR) [27], as shown in Table 1.

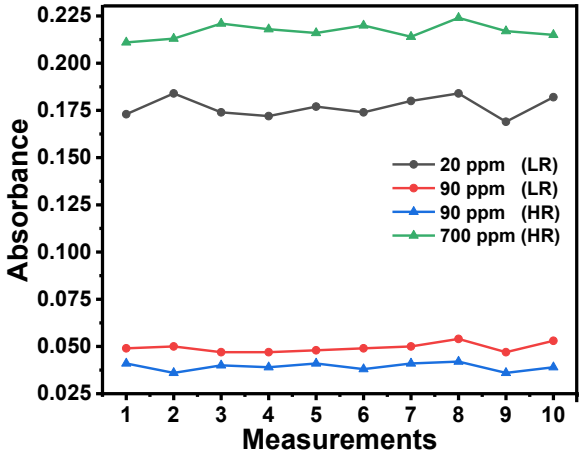

Figure 2. COD linearity limits at high and low concentrations via spectroscopy

Table 1. Precision and accuracy assessment with COD standard solutions

No.	Absorbance (a.u)		COD Level (mg O_2/L)		%Recovery		%Bias	
	LR	HR	LR	HR	LR	HR	LR	HR
1	0.120	0.155	51.22	494.07	101.29	98.83	1.29	-1.17
2	0.115	0.156	53.62	497.56	106.02	99.53	6.02	-0.47
3	0.123	0.156	49.79	497.56	98.45	99.53	-1.55	-0.47
4	0.123	0.158	49.79	504.52	98.45	100.92	-1.55	0.92
5	0.122	0.157	50.26	501.04	99.40	100.22	-0.60	0.22
6	0.120	0.158	51.22	504.52	101.29	100.92	1.29	0.92
7	0.124	0.161	49.31	514.96	97.50	103.01	-2.50	3.01
		Average	50.74	502.03	100.34	100.42	0.34	0.42
		SD	1.46	6.88				
		%RSD	2.88	1.37				
		$CV_{Horwitz}$	8.86	6.28				
		⅔ $CV_{Horwitz}$	5.91	4.18				

3.1.4. Assessment of Accuracy in COD Measurement

Accuracy, or trueness, refers to the closeness of the average measured value to the true value of an analyte [4]. Accuracy was assessed using the %R of COD standard solutions at concentrations of 50 mg O_2/L (LR) and 500 mg O_2/L (HR), yielding recoveries (n = 7) of 97.50–106.02% (LR) and 98.83–103.01% (HR), respectively. According to AOAC standards, these values fall within the acceptable recovery ranges of 80–110% for LR and 90–107% for HR [27], as shown in Table 1.

3.1.5. Evaluation of Uncertainty in High and Low Range COD Measurement

The determination of COD values using the spectroscopic method with closed reflux in both high- and low-concentration ranges was expressed as a mathematical model. Therefore, the sources of measurement uncertainty must be properly identified using a cause-and-effect (fishbone) diagram to prevent overestimation or underestimation of the results, as illustrated in Figure 3. The sources of measurement uncertainty in COD analysis using the spectroscopic method include several key components. The uncertainty arising from the calibration curve (μ_{Cx}) is associated with the relationship between COD level and absorbance. For low-range measurements, absorbance is measured at 420 nm, where a decrease in $Cr_2O_7^{2-}$ ions is observed. In high-range measurements, absorbance is measured at 600 nm, corresponding to an increase in Cr^{3+} ions. The accuracy of the calibration curve directly influences the reliability of the concentration estimates.

The uncertainty of the calibration curve ($\mu c_{(spectro)}$) (Eq. (5)) was calculated using the standard error of estimate ($S_{y/x}$) (Eq. (6)), the slope of the regression line (b), and the bias between measured absorbance and COD concentrations. This evaluation was based on the average absorbance and the average COD concentration values within the linearity range (Eq. (7)). The formulas used to calculate components of calibration curve uncertainty are presented in Table 2.

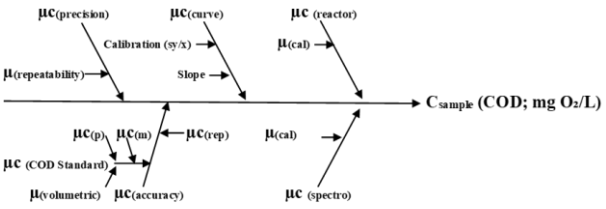

Figure 3. Fishbone diagram of COD measurement via spectroscopy for low and high ranges

Repeatability (precision) of the COD level refers to the consistency of measurement results under the same conditions. This component of uncertainty ($\mu_{(precision)}$) was evaluated through multiple replicate measurements. A higher standard deviation indicates greater variability, contributing to overall measurement uncertainty. The standard deviation (SD) of concentration obtained from repeatability tests was used to calculate the uncertainty contribution by dividing it by the square root of the number of replicates. Table 3 presents the formulas for calculating the precision uncertainty components (Eqs. (8)–Eq. (9)).

The uncertainty of the accuracy component ($\mu_{(accuracy)}$) was evaluated using an in-house KHP standard solution across both low and high COD ranges. The recovery percentage, calculated by comparing the theoretical COD with the measured COD, indicates how closely the measurement aligns with the true value. The uncertainty of accuracy arises from two main sources: the uncertainty in the theoretical COD value ($\mu c_{(COD\ Standard)}$) (Eq. (10) – Eq. (11)) and the uncertainty in the measured COD value ($\mu_{(repeatability)}$). Theoretical COD uncertainty is influenced by the purity of the KHP ($\mu c_{(purity)}$) (Eq. (12)), the weighing process ($\mu c_{(m)}$) (Eq. (13)), and the use of volumetric flasks during dilution ($\mu c_{(volumetric)}$) (Eq. (14)). Meanwhile, the uncertainty in the measured COD value primarily results from precision or repeatability errors.

The manufacturer's specification for KHP purity is (100 ± 0.05)% with the assumption of a normal distribution at a 95% confidence level, using a coverage factor (K) of 2. The SD of the analytical balance, obtained from calibration data, is 0.26 mg, assuming a normal distribution at a 95% confidence level with a K of 2. The formulas used to calculate the components of accuracy-related uncertainty (Eq. (15) – Eq. (16)) are presented in Table 3.

Instrumental uncertainty was assessed based on the resolution of the instruments used. The thermo-reactor ($\mu c_{(reactor)}$), which was used for sample digestion, has a temperature accuracy of ±1°C. The $\mu c_{(reactor)}$ was 0.5°C with the digestion temperature of 150°C. Meanwhile, the UV- Vis spectrophotometer ($\mu c_{(spectroscopy)}$) used for absorbance measurement has a wavelength accuracy of ±1 nm. The $\mu c_{(spectro)}$ was 0.5 nm with the wavelength 420 nm for LR COD and HR COD of 600 nm. Both resolution limits contribute to the overall measurement uncertainty and can affect the accuracy of COD results.

Table 2. Components of calibration curve uncertainty in LR and HR COD measurement

Components	Unit	Formula	Eq.	LR COD	HR COD
$\mu c_{(spectro)}$	mg O_2/L	$\mu c_{(curve)} = \frac{sy/x}{b} \times \sqrt{1 + \frac{1}{n} + \frac{(y_i - y_r)^2}{b^2 \sum(x_i - x_r)^2}}$	(5)	1.726	5.603
sy/x	a.u	$sy/x = \sqrt{\frac{\sum(y_i - y_c)^2}{(n-2)}}$	(6)	0.0034	0.0015
$C_{(sample)}$	mg O_2/L	$C_{(sample)} = \frac{(y-a)}{b}$	(7)	50.74	502.03

Table 3. The uncertainty of precision, accuracy, and instrumentation in LR and HR COD measurement

Component	Unit	Formula	Eq.	LR COD	HR COD
$\mu c_{(precision)}$	mg O_2/L	$\mu c_{(precision)} = \frac{SD}{\sqrt{n}}$	(8)	0.553	2.600
$C_{measured}$	mg O_2/L	$\bar{C}_{measured} = \frac{\sum_{i=1}^{n} C_i}{n}$	(9)	50.74	502.03
$\mu c_{(COD\ Standard)}$	mg O_2/L	$\mu c_{(COD\ Standard)} = m \times 1.176 \sqrt{\left(\frac{\mu c_{(purity)}}{p}\right)^2 + \left(\frac{\mu c_{(m)}}{m}\right)^2 + \left(\frac{\mu c_{(volumetric)}}{V}\right)^2}$	(10)	0.220	0.711
COD Standard	mg O_2/L	$COD\ theoretical = m_{KHP} \times 1.176 \frac{mgO_2}{mg\ KHP}$	(11)	1000	1000
$\mu c_{(purity)}$	%	$\mu c_{(purity)} = \frac{SD}{k}$	(12)	0.025	0.025
Purity of KHP	%	-		100	100
$\mu c_{(m)}$	mg	$\mu c_{(m)} = \sqrt{\mu_{m\ gross}{}^2 + \mu_{m\ tare}{}^2} = \sqrt{2 \times (\frac{SD}{K})^2}$	(13)	0.184	0.184
Mass of KHP	mg	-		43.0	425.1
$\mu c_{(volumetric)}$	mL	$\mu c_{(volumetric)} = \sqrt{\mu_{cal}{}^2 + \mu_{temp}{}^2}$	(14)	0.711	0.711
Volume	mL	-		1000	1000
$\mu_{(accuracy)}$	%	$\mu c_{(accuracy)} = \%R \times \sqrt{\left(\frac{\mu c_{(precision)}}{C_{Measure}}\right)^2 + \left(\frac{\mu c_{(COD\ Standard)}}{C_{theoretical}}\right)^2}$	(15)	1.176	0.527
Accuracy	%	$\%R = \frac{C_{measure}}{C_{target}} \times 100\%$	(16)	100.34	100.42

The formulas used to calculate each component of uncertainty are presented in Table 4. These include uncertainties arising from the calibration curve, repeatability (precision), accuracy, and instrumental resolution. Each component was evaluated individually and combined to estimate the overall measurement uncertainty, typically using the root-sum-square (RSS) method. The distributions of source uncertainty of LR and HR COD were presented in Figure 4.

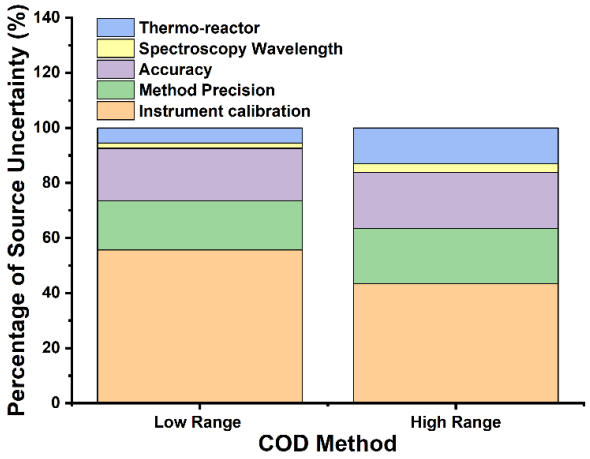

Figure 4. The distributions of source uncertainty of LR and HR COD

Table 4. The combined uncertainty of the LR and HR COD measurement

The source's uncertainty	COD	X	μ_x	μ_x/X	$(\mu_x/X)^2$
Instrument calibration	LR	50.74	1.726	0.03401	0.00116
	HR	502.03	5.603	0.01116	0.00012
Method precision	LR	50.74	0.553	0.01089	0.00012
	HR	502.03	2.600	0.00518	0.00003
Accuracy	LR	100.34	1.176	0.01172	0.00014
	HR	100.42	0.527	0.00525	0.00003
Spectroscopy wavelength	LR	420	0.500	0.00119	0.00000
	HR	600	0.500	0.00083	0.00000
Thermo-reactor	LR	150	0.500	0.00333	0.00000
	HR	150	0.500	0.00333	0.00000
The combined uncertainty	LR	50.74	1.916	0.03776	–
	HR	502.03	6.934	0.01381	–

The expanded uncertainty (U) for both the HR and LR COD measurements was calculated using a coverage factor of k = 2 and the combined uncertainty. For the LR COD result of 50.74 mg O_2/L, the expanded uncertainty was 3.83 mg O_2/L. For the HR COD result of 502.03 mg O_2/L, the expanded uncertainty was 13.87 mg O_2/L. These values correspond to valid COD concentration ranges of 46.91–54.57 mg O_2/L for the LR method and 488.16–515.90 mg O_2/L for the HR method. This demonstrates that the measurements are reliable and provide high confidence in the reported COD values.

4. Conclusion

The verification of the APHA Standard Method 5220 D for COD determination demonstrated that the spectroscopic measurement system provides reliable performance across both low (0–90 mg O_2/L) and high (90–700 mg O_2/L) concentration ranges. The method showed excellent linearity with R^2 values of 0.9991 (LR) and 0.9996 (HR), and adequate sensitivity, as indicated by LOD and LOQ values of 3.34 and 11.14 mg O_2/L (LR) and 15.48 and 51.59 mg O_2/L (HR). The linearity limit was accepted for both ranges based on F-test results, indicating a stable analytical response throughout the calibration interval. Precision and accuracy met the required criteria, as reflected by %RSD values below ⅔ of Horwitz %CV and recoveries within AOAC acceptance limits. The estimation of measurement uncertainty, incorporating calibration, repeatability, and accuracy components, produced expanded uncertainties of ±3.83 mg O_2/L for LR and ±13.87 mg O_2/L for HR, confirming that the reported COD values fall within robust confidence intervals. These results demonstrate that the method is suitable for routine COD monitoring of both influent wastewater (≤800 mg O_2/L) and treated effluent (≤100 mg O_2/L) in the JIE, provided that appropriate dilution, control of chloride interference using $HgSO_4$, and routine quality control measures are maintained.

Acknowledgement

The authors gratefully acknowledge PT. Jababeka Infrastruktur for providing laboratory facilities, instrumental support, and technical assistance throughout this study.

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