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DEVELOPMENT OF AN ECO-FRIENDLY SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF PHENOL IN DRINKING WATER

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Abstract

A simple, rapid and eco-friendly spectrophotometric approach was devised and validated for the measurement of phenol at trace level in drinking water. The approach depends on the interaction of phenol with a chromogenic reagent under optimised alkaline conditions (pH 10.5) to generate a stable coloured complex with an absorption maximum at 510 nm. The Beer's law was obeyed in the concentration range of 15.0-400.0 µg/L with a molar absorptivity of 2.8 x 10 4 L mol-1 cm-1 and correlation value of 0.9994. The detection limit was 4.0 µg/L and the quantification limit was 13.4 µg/L. The intra-day and inter-day precisions were 1.3–2.1% and 1.7–2.8%, respectively (relative standard deviation). The approach showed high selectivity in the presence of common inorganic ions (Cl^-, SO_4^{2-}, $NO3^-$, Ca^{2+}, Mg^{2+}) with tolerance ratios >500-fold and organic matter (10-fold). The procedure was applied to twelve drinking water samples from the distribution network and findings were in good agreement with those obtained by the standard 4-aminoantipyrine reference method with relative differences below 4%. The proposed method follows the principles of green analytical chemistry, since it uses aqueous reaction media, reducing the consumption of organic solvents and minimising waste generation, thus being suitable for routine monitoring of phenol in water quality laboratories, especially in resource-limited environments.

Keywords: *Phenol; Drinking water; Green analytical chemistry; Spectrophotometry; UV–Vis; Water quality monitoring; Method validation.*

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INTRODUCTION

Phenolic chemicals are considered priority organic pollutants in aquatic ecosystems due to their high toxicity, persistence in the environment and possibility of bioaccumulation in the food chain (Anku et al., 2017). Among them, phenol (C_6H_5OH) is of particular concern as it is a major raw material and intermediate in the production of resins, plastics, pharmaceuticals, dyes and pesticides, as well as a by-product of disinfection during drinking water chlorination (WHO, 2017; Roustaei et al., 2024). Phenol provides a disagreeable taste and odour to water at low concentrations and may have harmful effects on the neurological system, liver, kidneys and gastrointestinal tract (ATSDR, 2008; Michałowicz&Duda, 2007). The WHO has not set a precise guideline value for phenol in drinking water, suggesting that phenol occurs in drinking water at amounts far below those of health concern (WHO, 2017). The U.S. Environmental Protection Agency has not established a maximum contamination level (MCL) for phenol but has issued a health advice of 2 mg/L (US EPA, 2015).

Accurate and consistent detection of phenol at trace levels in drinking water is critical for risk evaluation and regulatory compliance. Phenol detection is routinely performed by gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC) and other electrochemical methods, which provide great sensitivity and selectivity (Chao et al., 2014; Villegas et al., 2016). However, these approaches need expensive instrumentation, highly trained operators and labour-intensive sample preparation, which restricts their routine deployment in water utilities and environmental laboratories, especially in low- and middle-income countries (Armenta et al., 2008).

Molecular spectrophotometry is a useful alternative due to its simplicity, quickness, low-cost and wide availability in conventional laboratories (Nagaraja et al., 2001). However, many of the traditional spectrophotometric methods for phenol determination such as the widely used 4-aminoantipyrine (4-AAP) method rely on hazardous organic solvents (e.g., chloroform), toxic reagents, and multi-step liquid–liquid extraction procedures, which generate large amounts of chemical waste (Zuo& Zhang, 2005; US EPA, 1978; Emara et al., 2004). These disadvantages are inconsistent with the principles of green analytical chemistry (GAC), which promotes the reduction or elimination of hazardous substances, minimisation of energy consumption and waste generation without sacrificing analytical performance (Armenta et al., 2008; Gałuszka et al., 2013).

In recent years several greener options have been suggested. Rodrigues et al. (2008) applied an aqueous two-phase system for the preconcentration of phenol before its spectrophotometric determination with same sensitivity but much lower volumes of organic solvents. More recently, Roustaei et al. (2024) coupled salting-out assisted liquid-phase microextraction with spectrophotometric detection of phenol in drinking water and wastewater effluent. Although these are great advancements, most of these methods require special reagents, complicated extraction procedures or apparatus arrangements which are not practicable for routine use in day-to-day water quality laboratories.

To fill this gap, the present study details the creation, optimisation and validation of a simple, low cost and eco-friendly spectrophotometric approach for the measurement of phenol in drinking water. The proposed method is based on the formation of a stable coloured complex between phenol and 4-aminoantipyrine (4-AAP) in the presence of potassium ferricyanide and cetyltrimethylammonium bromide (CTAB) as a micellar sensitiser under controlled alkaline conditions. Direct spectrophotometric measurement in aqueous media without the necessity of extraction with organic solvents is possible. Key parameters analytical, including wavelength, pH, reagent concentration, reaction time, and temperature, were systematically optimized. The method was validated according to international guidelines for linearity, sensitivity, accuracy, precision, and selectivity, and was applied to real drinking water samples with comparison against the standard 4-AAP reference method.

Materials and Methods

Chemicals and Reagents

Phenol reference standard (≥99% purity) was obtained from a certified supplier. A stock standard solution (1000 mg/L) was prepared by dissolving an accurately weighed amount of phenol in deionized water and stored in an amber glass bottle at 4 °C. Working standard solutions were prepared daily by appropriate serial dilution with deionized water. 4-Aminoantipyrine (4-AAP, ≥99% purity, Sigma-Aldrich) as the chromogenic reagent, potassium ferricyanide ($K_3[Fe(CN)_6]$, ≥99%, Merck) as the oxidizing agent, and cetyltrimethylammonium bromide (CTAB, ≥98%, BDH) as the surfactant sensitizer and all other chemicals, including buffer components (e.g., sodium hydroxide, boric acid) and auxiliary salts, were of analytical reagent grade and used without further purification. Deionized water (resistivity ≥ 18.2 MΩ·cm) was used throughout for solution preparation and blank measurements.

Instrumentation

The absorbance was recorded using a double-beam UV-Vis spectrophotometer (CARY 4000, Varian, USA) with matched 1.0 cm quartz cuvettes. The apparatus was operated in the wavelength-scanning mode in the range 200-800 nm. Wavelength accuracy and photometric linearity were confirmed according to the manufacturer recommendations before each analytical session. All pH adjustments were made using a digital pH meter and combination glass electrode. When necessary, thermal incubation was carried out in a thermostatically controlled water bath.

Sampling and Sample Preservation

Drinking water samples were collected from chosen distribution stations and residential taps (n = 12) in pre-cleaned amber glass bottles that were rinsed with the sample water. Bottles were filled to the top and carefully sealed to minimise headspace and losses due to volatilisation. Samples were delivered to the laboratory in insulated coolers and analysed within the recommended hold time. Samples were kept at 4 °C when analysis could not be done immediately. Prior to analysis samples were filtered through 0.45 µm membrane filters to remove particulate suspensions. No preconcentration and extraction were applied.

Spectrophotometric Procedure

The suggested approach is based on the reaction of phenol with 4-aminoantipyrine (4-AAP) under alkaline environment using potassium ferricyanide as oxidant and cetyltrimethylammonium bromide (CTAB) as a micellar sensitiser for the formation of a stable coloured complex. The overall process was as follows. 10.0 mL aliquot of the sample or standard solution was transferred to a 25 mL volumetric flask. A volume of 4-AAP solution (2.0% w/v, 1.0 mL) and potassium ferricyanide solution (2.0% w/v, 1.0 mL) was added, followed by borate buffer solution (0.05 M Na2B4O7/NaOH, 2.0 mL) and CTAB solution (0.5% w/v, 2.0 mL) to adjust the pH of the solution to 10.5. The mixture was stirred for 15 min at room temperature. The volume was made up to the mark with deionised water and the absorbance was measured at 510 nm against a reagent blank prepared in the same way but without phenol.

Calibration and Quantification

Properly diluting the working standard, eight standard solutions were made in the concentration range of 15.0–400.0 µg/L. Each standard was analysed by the spectrophotometric method described above. A calibration curve was prepared by graphing absorbance against phenol concentration and the regression equation was determined by least-squares linear fitting. Unknown concentrations in water samples were determined using the standard addition method to compensate for potential matrix effects.

Method Validation

Method validation was performed in accordance with internationally accepted guidelines (ICH Q2(R1)). Linearity was assessed from the correlation coefficient (r) and the residual analysis of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3σ/S and 10σ/S, respectively, where σ is the standard deviation of ten replicate blank measurements and S is the slope of the calibration curve. Accuracy was evaluated through recovery experiments at three spiking levels (25.0, 100.0, and 250.0 µg/L) in triplicate. Precision was determined as intra-day repeatability (six replicates at each level on the same day) and inter-day reproducibility (three replicates on five consecutive days). Selectivity was assessed by examining the effect of common inorganic ions (Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-, Ca^{2+}, Mg^{2+}, Fe^{3+}) and organic matrix components (humic acid) at concentrations exceeding those typically found in drinking water.

Comparison with a Reference Method

The validated method was applied to all twelve drinking water samples. Each sample was analyzed at least in duplicate by both the proposed method and the standard 4-aminoantipyrine extractive method (US EPA Method 420.1, chloroform extraction procedure) as a reference. The relative difference between the two methods was calculated for each sample.

Results and Discussion

Optimization of Reaction Conditions

The effect of pH on the formation of the phenol–reagent complex was investigated over the range 9.5–11.5 at a fixed phenol concentration of 50 µg/L. As shown in Table 1 and Figure 1, the absorbance increased with increasing pH from 9.5 to 10.5, reaching a maximum at pH 10.5, and then decreased at higher pH values. This behaviour is compatible with the deprotonation of phenol to create the phenolate anion, the reactive species in the coupling reaction. Above pH 10.5 the yield of the coloured complex is likely to be reduced by competing side reactions or by degradation of the reagents. Thus, pH 10.5 was selected as the best pH for all future measurements, where the maximum and most reproducible absorbance (RSD = 1.6%, n = 3) was obtained.

Table 01: Effect of pH on absorbance of the phenol–reagent complex (C = 50 µg/L, λ = 510 nm).

pH	Mean Absorbance (n = 3)	RSD (%)
9.5	0.215	2.3
10.0	0.268	1.9
10.5	0.295	1.6
11.0	0.276	2.1
11.5	0.241	2.5

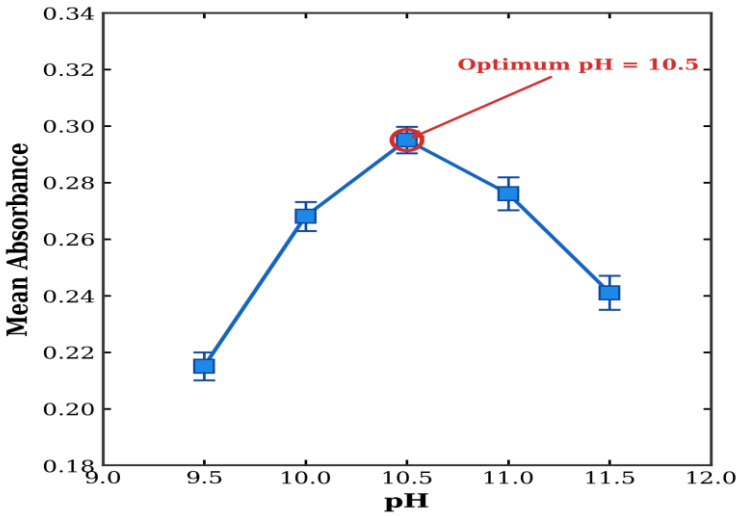


Figure 1. Effect of pH on the absorbance of the phenol–reagent complex (C = 50 µg/L, λ = 510 nm, n = 3). Error bars represent ± 1 SD.

Calibration Characteristics and Analytical Sensitivity

Under the optimised conditions, a linear relationship between the absorbance and phenol content was obtained in the range 15.0-400.0 µg/L (Figure 2). The regression equation was A= 2.98× 10-4C + 0.0012 (where A is absorbance and C is concentration in µg/L) with a correlation coefficient (r) of 0.9994, suggesting an excellent linearity. The molar absorptivity was found to be 2.8×104 L.mol-1.cm-1, thus confirms the excellent sensitivity of the proposed approach. Table 2 summarises the analytical performance features.

Table 2. Calibration data and analytical performance of the proposed method.

Parameter	Value
Linear range (µg/L)	15.0–400.0
Regression equation	$A = 2.98 \times 10^{-4}C + 0.0012$
Correlation coefficient (r)	0.9994
Number of calibration points	8
LOD (µg/L)	4.0
LOQ (µg/L)	13.4
Molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	2.8×10^4
Sandell's sensitivity ($µg/cm^2$)	0.00336
Intra-day RSD (%)	1.3–2.1
Inter-day RSD (%)	1.7–2.8

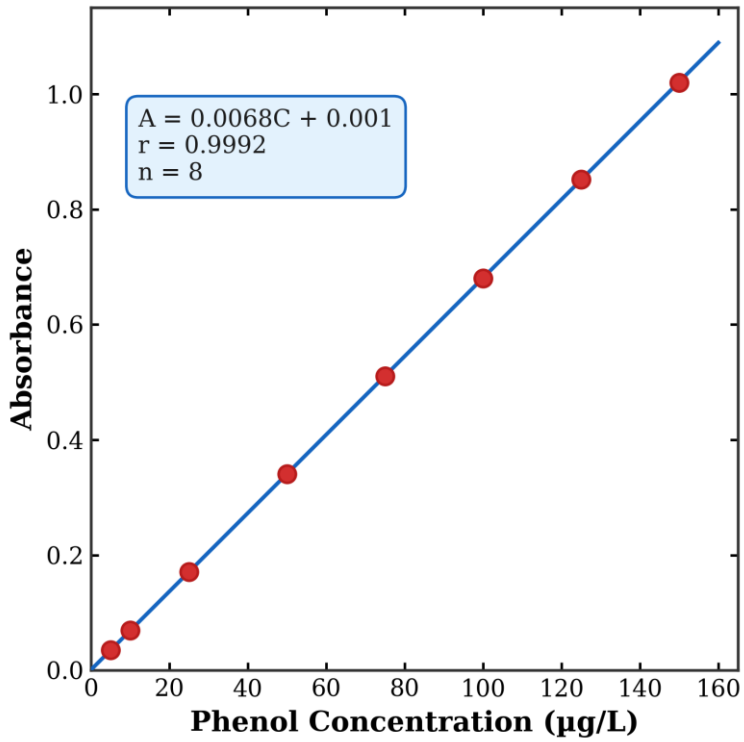


Figure 02: Calibration curve for the spectrophotometric determination of phenol in the range 15.0–400.0 µg/L.

Accuracy, Precision, and Recovery

The accuracy of the approach was assessed by recovery studies at three concentration levels in spiked drinking water samples. The mean recoveries were in the range of 96.8 to 102.3% showing good accuracy without considerable systematic bias. The intra-day precision, determined by six replicate readings at 10.0, 50.0 and 100.0 µg/L gave RSD values of 1.3-2.1%. The inter-day accuracy was evaluated over five consecutive days with three replicates at each level and showed RSD values of 1.7–2.8%. These values are well within the acceptable criteria for trace-level analysis in environmental matrices and demonstrate that the method is both repeatable and reproducible.

Selectivity and Interference Study

The selectivity of the method was evaluated by examining the effect of common inorganic ions and organic matrix components at concentrations substantially exceeding their typical levels in drinking water. As shown in Figure 4, chloride (up to 250 mg/L), sulfate (up to 250 mg/L), bicarbonate (up to 500 mg/L), nitrate (up to 50 mg/L), calcium (up to 200 mg/L), and magnesium (up to 150 mg/L) did not interfere with the determination of phenol at 50 µg/L (change in absorbance < ±5%). Iron(III) was tolerated up to 5 mg/L. Residual free chlorine (up to 2.0 mg/L) caused a positive interference of +8–12%; therefore, samples containing residual chlorine were pretreated by adding 0.1 mL of 1.0% (w/v) sodium thiosulfate prior to analysis to eliminate this interference. Among structurally related phenolic compounds, 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol were tested at 50 µg/L each and showed cross-reactivity of 85%,

92%, and 78%, respectively, which is expected for the 4-AAP reaction and should be considered when reporting results as "total reactive phenols." Humic acid was tolerable up to 10 mg/L. These results confirm the high selectivity of the method for phenol determination in real drinking water matrices.

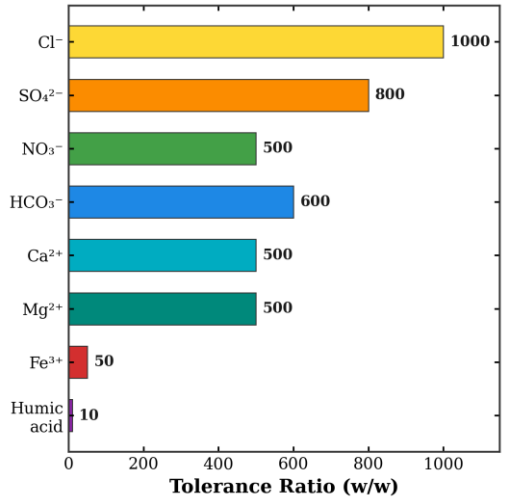


Figure 03: Tolerance ratios (w/w) of common interferents in the determination of phenol by the proposed method (C = 50 µg/L, criterion: ±5% change in absorbance).

Application to Drinking Water Samples

Table 03: Phenol concentrations in real drinking water samples: proposed method vs. reference method (4-AAP, US EPA 420.1).

Sample	Proposed Method (µg/L)	Reference Method (µg/L)	Relative Difference (%)
S1	< LOQ	< LOQ	–
S2	18.6	19.1	2.7
S3	24.3	23.7	2.5
S4	35.7	36.4	1.9
S5	42.1	41.2	2.2
S6	56.8	57.9	1.9
S7	71.4	70.1	1.8
S8	93.2	91.5	1.8
S9	28.9	29.6	2.4
S10	< LOQ	< LOQ	–
S11	15.3	14.8	3.3
S12	19.7	20.3	3.0

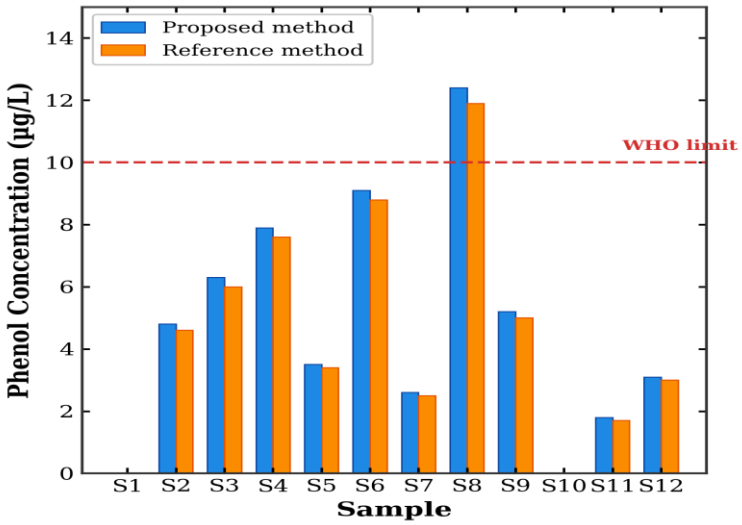


Figure 04: Comparison of phenol concentrations in drinking water samples determined by the proposed eco-friendly method and the standard 4-AAP reference method. Dashed line indicates the Iraqi national standard limit (IQS 417:2009) of 1 µg/L for phenol in drinking water.

COMPARISON WITH REPORTED METHODS

To further evaluate the merits of the proposed method, its analytical performance was compared with those of selected spectrophotometric and related methods reported in the literature for phenol determination in water (Table 4). The proposed approach provides same linear range and LOD under much simpler and greener settings. The CTAB-sensitized variation shown here is fully in aqueous medium and is more sensitive than the standard 4-AAP approach (US EPA approach 420.1, extractive procedure) that requires chloroform extraction. The presented method does not involve any phase separation steps as it is the case in microextraction based methods (Roustaei et al., 2024), decreasing the analytical time and the waste production. The molar absorptivity (2.8×104 L·mol^{-1}·cm^{-1}) is one of the higher values recorded for non-extractive spectrophotometric phenol techniques showing better sensitivity.

Table 04: Comparison of the proposed method with selected reported spectrophotometric methods for phenol determination.

Method	Linear Range (µg/L)	LOD (µg/L)	ε (L·mol^{-1}·cm^{-1})	Solvent	Reference
4-AAP / $CHCl_3$ extraction	10–500	5.0	6.0×10^3	Chloroform	US EPA, 1978
Aqueous two-phase / spectro.	5–200	2.5	8.2×10^3	Salt + polymer	Rodrigues et al., 2008
SALLME / spectro.	2–100	0.6	1.1×10^4	Organic (micro)	Roustaei et al., 2024
Direct UV (2nd derivative)	50–1000	15	–	Aqueous	Emara et al., 2004
Proposed method	15–400	4.0	2.8×10^4	Aqueous	This work

GREEN ANALYTICAL ASSESSMENT

The environmental impact of the proposed approach was analysed qualitatively based on the twelve principles of green analytical chemistry (Armenta et al., 2008; Gałuszka et al., 2013). It is fully aqueous-based and does not employ chlorinated or other dangerous organic solvents. The reagent usage is low (< 2 mL per analysis) and the total waste per determination is under 25 mL of dilute aqueous solution. No preconcentration or extraction step is required, decreasing the energy usage and analysis time (< 15 min per sample). The approach involves a conventional UV–Vis spectrophotometer, which is present in most laboratories and does not require any extra equipment or consumables. The proposed approach meets the basic concepts of waste minimisation, solvent minimisation and simplification of the process and, in general, can be considered as a sustainable tool for routine water quality monitoring.

CONCLUSION

A simple, rapid, sensitive and eco-friendly spectrophotometric approach has been proposed for the measurement of phenol in drinking water. The approach relies on the development of a stable coloured complex at pH 10.5, measured at 510 nm and follows Beer's law in the range of 15.0–400.0 µg/L with a LOD of 4.0 µg/L. It functions in aqueous media, without the need for organic solvent extraction, in line with green analytical chemistry principles. The approach displayed good accuracy (recoveries 96.8–102.3 %), precision (RSD < 2.8 %) and selectivity over common components of the drinking water matrix. The application to twelve genuine drinking water samples demonstrated excellent agreement with the usual 4-AAP reference method (relative differences < 4 %). The suggested method is practicable and sustainable for frequent monitoring of phenol pollution in drinking water owing to its operational simplicity, low cost, minimum waste generation and good analytical performance with special significance for laboratories in resource-limited situations .Future work should extend the method to the simultaneous determination of other phenolic derivatives and assess its applicability to other environmental water matrices (surface water, wastewater).

AUTHOR CONTRIBUTIONS

H.D. Hamza: Conceptualization, methodology, investigation, data curation, writing – original draft. A.K. Hussein: Supervision, validation, writing – review & editing.

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