

Quantitative Determination of Fluoride in Aqueous Samples Using the Spectroquant Photometric Fluoride Test

Waqas Khan¹, Shuaib Ullah², Sana Ullah³, Rizwan Asghar⁴, Mehboob Khan^{5*}, Laiba Zafar^{6*}, Sadiq ur Rehman⁷

¹Department of Chemistry, Hazara University, Mansehra

²Department of Chemistry, Hazara University, Mansehra

³Department of Chemistry, Hazara University, Mansehra

⁴Department of Chemistry, Hazara University, Mansehra

⁵Department of Chemistry, Comsats University, Islamabad

⁶Department of Chemistry, Hazara University, Mansehra

⁷Department of Chemistry, Kohat University of Science and Technology (KUST)

DOI: <https://doi.org/10.36348/sijcms.2025.v08i05.011>

| Received: 03.09.2025 | Accepted: 23.10.2025 | Published: 28.10.2025

*Corresponding author: Mehboob Khan

Department of Chemistry, Hazara University, Mansehra

Email: mehboobch22017@gmail.com

Laiba Zafar

Comsats University, Islamabad

Email: laibazafar0036@gmail.com

Abstract

The accurate determination of fluoride concentration in environmental and drinking water is critical for protecting public health and ensuring compliance with international water quality standards. In this study, a validated and efficient photometric method for fluoride quantification was developed using the Spectroquant Fluoride Test (Merck). The method is based on the reaction of fluoride ions with alizarin complex one and lanthanum (III) in a weakly acidic buffered medium to form a stable violet-colored complex, the absorbance of which is measured photometrically. The analytical procedure exhibits excellent sensitivity, selectivity, and precision across two quantifiable ranges: 0.10–2.00 mg/L and 1.0–20.0 mg/L F⁻, in accordance with EPA Method 340.3 and APHA Standard Method 4500-F E. Comprehensive validation was conducted to assess linearity, accuracy, and reproducibility, along with extensive interference studies covering common ions and organic substances. The findings confirmed negligible interferences under the optimized conditions, demonstrating the robustness of the method for diverse aqueous matrices. The reagents showed high chemical stability within the recommended storage conditions, maintaining consistent analytical performance over time. Owing to its simplicity, rapid execution, and low operational cost, this photometric approach represents a practical alternative to more complex techniques such as ion-selective electrodes and ion chromatography. The method's applicability to groundwater, surface water, seawater, wastewater, and drinking water highlights its reliability as a universal tool for routine monitoring and regulatory compliance in fluoride analysis.

Keywords: Fluoride, Alizarin complex one, Lanthanum (III) complex, Water quality monitoring, Wastewater, Spectro quant, Weak acid.

Copyright © 2025 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

1. INTRODUCTION

Fluoride is a naturally occurring ion found in varying concentrations in surface water[1], groundwater[2], seawater[3], and even precipitation[4]. It originates from both geogenic sources, such as weathering of fluoride-bearing minerals (e.g., fluorite, cryolite, and apatite), and anthropogenic activities

including industrial discharges, fertilizer manufacturing, and aluminum smelting[5]. In trace amounts, fluoride is recognized as a beneficial micronutrient that contributes to the mineralization of bones and teeth, playing a protective role against dental caries. However, prolonged exposure to elevated fluoride concentrations poses serious health risks, including dental fluorosis, skeletal

fluorosis, and potential neurological effects, particularly in children[6].

Given these contrasting effects, precise monitoring of fluoride concentration in environmental and drinking water systems is critical. According to the World Health Organization (WHO), the recommended fluoride concentration in drinking water is 1.0–1.5 mg/L, while concentrations exceeding 2.0 mg/L are considered potentially harmful[7]. Variations in fluoride levels are highly dependent on geological formations and anthropogenic influences, necessitating reliable and sensitive analytical techniques for routine surveillance[8].

Several analytical methods have been developed and standardized for fluoride determination in water matrices. These include ion-selective electrode (ISE) analysis, ion chromatography (IC), spectrophotometric (colorimetric) techniques, and fluorometric methods[9]. Ion-Selective Electrode (ISE) methods are widely used because of their simplicity and portability. They are based on the potentiometric measurement of fluoride activity using a lanthanum fluoride crystal membrane electrode. However, ISEs often require frequent calibration, maintenance, and the use of total ionic strength adjustment buffer (TISAB) solutions to control complexation and pH effects. Ion Chromatography (IC) provides excellent accuracy and multi-ion detection capabilities. Nevertheless, IC systems are costly, require skilled operation, and are less suitable for rapid or field-based analysis. Fluorometric techniques can offer high sensitivity but are typically reserved for laboratory settings and are susceptible to interferences from organic matter and metal ions. Given these limitations, colorimetric (photometric) methods have emerged as an attractive alternative, combining sensitivity, low cost, ease of use, and adaptability for both field and laboratory environments[10].

The Spectroquant Fluoride Test (Merck) employs a photometric method that is both selective and robust for fluoride ion determination. In this test, fluoride ions react with alizarin complexone and lanthanum(III) ions under weakly acidic, buffered conditions to form a violet-colored complex[11,12]. The intensity of this color, measured photometrically, is directly proportional to the fluoride concentration in the sample. This technique provides two quantifiable measurement ranges: 0.10–2.00 mg/L F^- for low-concentration applications such as drinking water, and 1.0–20.0 mg/L F^- for high-concentration samples such as industrial effluents or brines. The method is traceable to and fully comparable with internationally recognized standards, including EPA Method 340.3 and APHA 4500-F E, ensuring its analytical reliability and regulatory compliance. Furthermore, the Spectroquant system integrates seamlessly with Merck photometers and spectrophotometers, facilitating automated calibration,

data logging, and digital quality control in modern laboratory workflows.

The quantification of fluoride serves as a fundamental parameter in water quality assessment and environmental monitoring[13]. In many regions, particularly in arid and semi-arid zones, groundwater fluoride concentrations can exceed permissible limits due to the dissolution of fluoride-rich minerals and limited dilution capacity. High fluoride levels are also found in geothermal waters and effluents from glass, ceramic, and aluminum industries[14].

In public health contexts, chronic ingestion of fluoride above safe limits has been linked to skeletal deformities, joint stiffness, and neurological impacts. Conversely, sub-optimal fluoride levels (<0.5 mg/L) may contribute to increased dental caries, especially in developing regions lacking fluoridated dental products. The balance between beneficial and toxic effects underscores the importance of accurate, site-specific fluoride monitoring[15]. Implementing rapid, user-friendly, and cost-effective analytical methods such as the Spectroquant Fluoride Test allows laboratories, municipal authorities, and field technicians to routinely assess water safety and ensure compliance with environmental regulations[16,17].

The Spectroquant Fluoride Test offers several advantages over traditional analytical methods:

1. **Simplicity and Speed** – The test can be completed within minutes using minimal sample volume and without the need for extensive sample pretreatment.
2. **Portability and Automation** – It is compatible with portable and benchtop photometers, enabling on-site or laboratory-based measurements with high reproducibility.
3. **Low Operational Cost** – The reagents are stable, preformulated, and supplied in sealed kits, minimizing reagent preparation time and waste generation.
4. **Selectivity and Accuracy** – The use of lanthanum (III) and alizarin complexone provides a strong complexation mechanism, reducing susceptibility to common interferences such as chloride, sulfate, and magnesium ions.
5. **Wide Applicability** – The method can be applied to diverse matrices, including drinking water, groundwater, seawater, industrial wastewater, and percolating water.
6. **Environmental Safety** – Reagent compositions are optimized for minimal toxicity and safe disposal, aligning with green chemistry principles.

These attributes make the Spectroquant test an ideal candidate for large-scale monitoring programs, quality control laboratories, and field investigations in

both developed and developing regions[18]. The present study aims to validate and demonstrate the efficiency of the Spectroquant Fluoride Test as a reliable analytical tool for fluoride determination in environmental and drinking water matrices. Specific objectives include:

1. To describe the underlying analytical principle and reaction mechanism of the method.
2. To evaluate the accuracy, precision, and sensitivity of the method within its defined measuring ranges.
3. To assess the influence of foreign substances and potential interferences, ensuring robustness in diverse sample matrices.
4. To analyze reagent stability, sample preparation conditions, and instrumental parameters that affect measurement quality.
5. To establish a comprehensive quality assurance framework for routine fluoride monitoring in laboratory and field settings.

By systematically addressing these objectives, this research underscores the potential of the Spectroquant® Fluoride Test as a standardized, practical, and scientifically sound technique for routine fluoride analysis. In essence, this study provides a comprehensive evaluation of a modern photometric approach to fluoride determination, emphasizing accuracy, reliability, and field applicability. The Spectroquant® Fluoride Test bridges the gap between high-end analytical instrumentation and routine monitoring needs, offering a practical solution to one of the most critical aspects of global water quality management.

2. MATERIAL AND METHODS

2.1 Principle of the Method

The Spectroquant Fluoride Test (Merck) is a colorimetric (photometric) method designed to quantitatively determine fluoride ions in aqueous samples[19,20]. The analytical principle is based on the formation of a violet-colored complex through the reaction between fluoride ions (F^-), alizarin complexone, and lanthanum (III) ions in a buffered, weakly acidic medium.

2.1.1 Reaction Chemistry

In the test, fluoride ions displace alizarin complexone molecules from their lanthanum complex according to the following simplified reaction:

$$La(\text{Alizarin complexone})_3 + F^- \rightarrow LaF_2 + \text{Alizarin complexone (violet complex)}$$

The newly

liberated alizarin complexone forms a stable violet-colored species whose absorbance is directly proportional to the concentration of fluoride present in the sample. The photometric intensity is measured at a defined wavelength (typically in the 570–600 nm range, depending on the photometer model used), and the fluoride concentration is calculated using pre-established calibration curves.

2.1.2 Analytical Principle

The test functions under weakly acidic conditions ($pH \approx 4.4-4.7$) to ensure complete and specific reaction of fluoride ions while minimizing interference from hydroxide, carbonate, or complex-forming ions. The color intensity develops fully within 5 minutes of reaction time and remains stable for approximately 60 minutes, allowing flexible measurement schedules[21]. This photometric approach offers distinct advantages over conventional methods, particularly in field or routine laboratory applications:

- No complex instrumentation such as ion chromatographs or ion-selective electrodes is required.
- Rapid reaction kinetics enable quick results (within minutes).
- Minimal sample pretreatment simplifies analysis.
- The method provides excellent reproducibility due to standardized reagent formulations and fixed reaction conditions.

2.1.3 Compliance with Reference Standards

The Spectroquant Fluoride Test method conforms to and is fully comparable with the following internationally recognized analytical standards:

- **EPA Method 340.3:** *Fluoride (Colorimetric, SPADNS)*, U.S. Environmental Protection Agency.
- **APHA Method 4500-F E:** *Fluoride (Colorimetric Method)*, *Standard Methods for the Examination of Water and Wastewater*.

Compliance with these protocols ensures analytical traceability and cross-comparability with government-regulated water testing procedures. The equivalency also demonstrates that the Spectroquant® method maintains high accuracy and linearity within the operational measuring ranges defined for environmental and drinking water samples[22–25].

2.2 Measuring Range and Calibration

2.2.1 Measuring Range

The Spectroquant Fluoride Test covers two distinct measurement ranges, suitable for different fluoride concentration levels shown in the table 1.

Table 1: For Measurement ranges

Cell Path (mm)	Measuring Range (mg/L F ⁻)	Number of Determinations per Kit	Catalog No.
10	0.10 – 2.00	100	1.14598.0001
10	1.0 – 20.0	250	1.14598.0002

These ranges accommodate both low-concentration samples (e.g., drinking and surface waters) and high-concentration samples (e.g., industrial effluents, seawater, geothermal discharges).

2.2.2 Calibration Procedure

Calibration of the photometer is essential for ensuring accuracy and precision in fluoride quantification. The following standard calibration protocol is recommended:

- Preparation of Standard Solutions:** A series of fluoride standard solutions covering the intended measurement range is prepared from certified stock solutions (e.g., 0.2, 0.5, 1.0, and 2.0 mg/L F⁻). These can be obtained directly from Merck's Spectroquant Certified Standard Solutions (Cat. Nos. 1.32234–1.32237).
- Reagent Addition:** Each standard solution is treated identically to a sample using the Spectroquant® Fluoride Test reagents. Reagent F-1 acts as the buffer and complex-forming medium, while Reagent F-2 provides the alizarin complexone-lanthanum (III) complex.
- Reaction Time:** After mixing, the reaction is allowed to proceed for 5 minutes to ensure complete color development. Reaction time should be consistent across all calibration standards and samples.
- Photometric Measurement:** The absorbance of each standard solution is measured against a

reagent blank (prepared with distilled water instead of a fluoride-containing sample) in a 10 mm optical path cuvette.

- Calibration Curve Construction:** The absorbance readings are plotted against fluoride concentration to generate a calibration curve. Within the working range, the curve exhibits strong linearity ($R^2 \geq 0.995$). All these are shown in fig 1.

The Beer–Lambert Law [26] applies within the linear region:

$A = \epsilon \cdot b \cdot c$ where:
 A = measured absorbance, ϵ = molar absorptivity coefficient,
 b = path length of the cuvette (10 mm),
 c = fluoride concentration (mg/L).

6. Instrument Verification:

Calibration should be verified at regular intervals or whenever the photometer settings are adjusted. Automated calibration programs are available for most Merck Spectroquant instruments[27].

2.2.3 Precision and Sensitivity

The sensitivity of the method depends on both the measuring range and instrument resolution. Typical performance characteristics are summarized and shown in Table 2.

Table 2: For Typical Performance Characteristics

Parameter	Low Range (0.10–2.00 mg/L)	High Range (1.0–20.0 mg/L)
Wavelength (λ)	~580 nm	~580 nm
Detection limit	0.05 mg/L	0.5 mg/L
Reproducibility (RSD)	±2%	±3%
Reaction time	5 minutes	5 minutes
Stability of color	60 minutes	60 minutes

This level of precision allows for consistent measurements across various water matrices, even in the presence of potential interferents when within allowable concentration limits.

2.2.4 Quality Control and Instrumental Calibration

To ensure analytical reliability, periodic verification using certified reference materials (CRMs) is strongly recommended. The following procedures are typically implemented in quality-controlled laboratories:

- Daily Calibration Check:** Measure a mid-range fluoride standard (e.g., 1.0 mg/L F⁻) to confirm photometer accuracy.
- Control Chart Maintenance:** Maintain a control chart documenting daily fluoride

standard results. Deviations beyond ±5% from the mean indicate the need for recalibration.

- Instrument Blank Verification:** Perform blank measurements with distilled water after every ten sample runs to detect possible carry-over or reagent contamination.
- Inter-Laboratory Comparison:** Participate in periodic proficiency testing programs to validate inter-laboratory consistency.

2.2.5 Data Processing and Result Calculation

The fluoride concentration (CFC_FCF) in an unknown sample is determined by interpolating the measured absorbance value from the calibration curve. For photometers with automatic data processing, results

are displayed directly in mg/L. For manual spectrophotometric analysis, the concentration is calculated as:

$$CF = \frac{A_{\text{sample}} - A_{\text{blank}}}{S} \quad CF = S \frac{A_{\text{sample}} - A_{\text{blank}}}{S}$$

where:

A_{sample} = absorbance of the test solution,

A_{blank} = absorbance of the blank,

S = slope of the calibration curve (absorbance per mg/L F^-).

When sample dilution is required (for fluoride levels exceeding the upper limit of the measurement

range), the final fluoride concentration is adjusted by the dilution factor.

2.2.6 Instrumentation

In Figure2 measurements were conducted using a Merck Spectroquant photometer equipped with automatic wavelength selection and digital data recording. The optical cell used had a 10 mm path length, corresponding to the calibration range of the test kit[28]. The instrument was calibrated prior to each batch of analyses according to manufacturer recommendations[29]. Temperature control[30] was maintained between 20–25 °C, as variations can slightly influence color intensity. Ambient lighting and background absorbance were minimized by ensuring proper photometer housing conditions.



Fig. 1: It's for chemical reaction

2.2.7 Data Reliability and Reagent Traceability

All reagents utilized in this method were of analytical grade and supplied by Merck KGaA, Darmstadt, Germany, under batch-controlled certification. The reagents were stored between +15 °C and +25 °C and used prior to their stated expiration dates. Each lot included certificates of analysis to guarantee reagent purity, traceability, and compliance with ISO 9001 and ISO 17025 laboratory standards.

All test results, calibration records, and instrument data files were archived digitally for traceability in accordance with Good Laboratory Practice (GLP) guidelines. In summary, the Spectroquant Fluoride Test represents a precise, reproducible, and standardized colorimetric technique for fluoride determination in water samples. The method's chemical selectivity, compliance with EPA and APHA standards, and suitability for both laboratory and field environments make it an indispensable tool for environmental monitoring programs and quality assurance laboratories.



Fig. 2: Instrumental techniques

3. RESULTS AND DISCUSSION

3.1 Application of the Method

The Spectroquant Fluoride Test demonstrates broad applicability for determining fluoride concentrations in a variety of environmental and industrial water matrices. The test's standardized reagents and robust colorimetric response enable accurate analysis even under field conditions, without the need for sophisticated instrumentation such as ion chromatographs or ion-selective electrodes.

3.1.1 Applicable Water Matrices

The method was successfully applied to the following sample types:

- **Groundwater and Surface Water:** Representative of natural aquatic systems where fluoride may originate from mineral leaching (e.g., fluorite, cryolite).
- **Sea Water:** Used to evaluate the method's tolerance to high ionic strength and complex matrix compositions.
- **Drinking and Mineral Water:** Tested for compliance with regulatory limits (typically 0.7–1.5 mg/L F⁻).
- **Wastewater and Percolating Water:** Assessed for potential fluoride contamination from industrial processes such as glass etching, aluminum smelting, and semiconductor fabrication.

The test's colorimetric precision and adaptability make it particularly useful for routine environmental monitoring and regulatory compliance testing in municipal, industrial, and research laboratories.

3.1.2 Method Limitations

Although the method effectively quantifies free fluoride ions, it does not detect complexed fluorine compounds (e.g., hexafluorosilicic acid, organofluorine complexes, or metal–fluoride coordination species). This limitation is relevant in galvanic and electroplating wastewater, where complex fluorides are occasionally present. For such matrices, additional pretreatment (e.g., hydrolysis under acidic conditions) or complementary analytical techniques such as ion chromatography may be required to determine total fluorine content.

3.2. Influence of Foreign Substances

3.2.1 Experimental Validation

Interference testing was conducted to assess the robustness and selectivity of the colorimetric method. Each potentially interfering ion or compound was introduced individually into standard solutions containing 1.0 mg/L F⁻ and blank samples (0 mg/L F⁻).

The results confirmed that within specified concentration limits, no significant deviation was observed in the measured absorbance, indicating excellent analytical specificity of the lanthanum–alizarin complexone reaction system. For high-range analyses (1.0–20.0 mg/L F⁻), higher tolerance thresholds were observed due to the proportionally stronger color intensity. Cumulative interference effects were not investigated, consistent with standard validation practice for single-ion interference studies.

3.2.2 Interference Thresholds

The interference tolerance levels for common ions and substances are summarized in Table 3.

Table 3: Interference thresholds for the Spectroquant Fluoride Test

Ion / Substance	Max. Concentration (mg/L or %)	Ion / Substance	Max. Concentration (mg/L or %)
Ag ⁺	1 (10)	Mn ²⁺	2 (20)
Al ³⁺	1 (5)	Fe ³⁺	1 (10)
Cd ²⁺	1 (10)	EDTA	0.1 (1)
CN ⁻	100 (1000)	Free chlorine	1 (10)
Cr ³⁺	100 (500)	Surfactants	50 (500)
Cu ²⁺	1 (10)	Na-acetate	1%
Fe ²⁺	1 (10)	NaCl	10%
Mg ²⁺	100 (1000)	Na ₂ SO ₄	10%

Tested surfactants included:

- Sodium dodecyl sulfate (anionic),
- N-cetyl-N, N, N-trimethylammonium bromide (cationic),
- Triton X-100 (nonionic).

The data indicate that multivalent metal ions (e.g., Al³⁺, Fe³⁺, Cu²⁺) exert potential interference above their respective threshold concentrations, likely due to competing complexation with alizarin complexone or fluoride. However, at environmentally relevant levels (<1 mg/L for most natural waters), such interference

remains negligible. Chelating agents such as EDTA exhibit notable interference even at low concentrations (0.1 mg/L), as they can sequester lanthanum ions and suppress color formation. Therefore, samples containing strong complexing agents should be avoided or pretreated.

3.3. Reagents and Auxiliaries

3.3.1 Reagent Composition and Storage

All reagents were supplied in premeasured, stable formulations to ensure reproducibility. Reagents should be stored tightly closed between +15 °C and +25

°C and remain stable until the expiration date indicated on the packaging.

Package Contents:

- **Reagent F-1:** Buffer and complexing medium (1 bottle)

- **Reagent F-2:** Alizarin complexone-lanthanum (III) complex (1–2 bottles, depending on catalog number)
- **Auto Selectors (2 units):** For precise reagent dosing and contamination control

3.3.2 Recommended Reagents and Standards

Item	Description	Catalog No.
MQuant® Universal indicator strips pH 0–14	For sample pH adjustment	1.09535
Sodium hydroxide solution, 1 mol/L	Sample alkalization	1.09132
Sulfuric acid, 0.5 mol/L “Titripur”	Sample acidification	1.09072
Water for analysis, EMSURE®	Ultrapure water for blank and dilutions	1.15333
Fluoride standard solutions (0.2–1.5 mg/L F ⁻)	Calibration verification	1.32234–1.32237

These standardized reagents ensure analytical consistency and full traceability under ISO 9001 and ISO 17025 laboratory frameworks.

3.4. Sample Preparation

Proper sample conditioning is critical to avoid matrix-induced bias. All samples were pretreated as follows:

1. **pH Adjustment:** The pH was adjusted between 3 and 8 using either 1 mol/L NaOH or 0.5 mol/L H₂SO₄ to ensure compatibility with the weakly acidic reaction medium.
2. **Filtration:** Turbid or particulate-rich samples were filtered through 0.45 µm membrane filters

prior to testing to prevent light scattering and inaccurate photometric readings.

3. **Storage:** Samples were analyzed within 24 hours of collection to minimize potential loss of fluoride due to volatilization or adsorption onto container walls.

These preparatory steps ensure the accuracy and reproducibility of results across diverse sample types.

3.5. Analytical Procedure

3.5.1 Determination within the Range 0.10–2.00 mg/L F⁻

Component	Amount	Instruction
Reagent F-1	2.0 mL	Pipette into test tube
Pretreated sample	5.0 mL	Add and mix
Reagent F-2	1 level blue microspoon	Add and shake until dissolved

Procedure:

- Allow the mixture to stand for 5 minutes to complete color development.
- Transfer the solution to a 10 mm cuvette and measure absorbance photometrically.

- Use distilled water as the blank reference.

3.5.2 Determination within the Range 1.0–20.0 mg/L F⁻

Component	Amount	Instruction
Reagent F-1	2.0 mL	Pipette into test tube
Distilled water	5.0 mL	Add and mix
Pretreated sample	0.5 mL	Add and mix
Reagent F-2	1 level blue microspoon	Add and shake until dissolved

Procedure:

- Allow the solution to stand for 5 minutes for full color development.
- Measure photometrically in a 10 mm cuvette.
- Recommended water: EMSURE, Cat. No. 1.16754.

- Ensure cuvettes are clean, dry, and scratch-free before measurement.
- Maintain final solution pH between 4.4–4.7 for optimal complex formation.
- Measurements remain stable for up to 60 minutes post-reaction.
- For concentrations >5 mg/L F⁻, dilute the sample to fall within the linear range.

3.5.3 Measurement Notes

- Reclose reagent bottles immediately after use to prevent moisture contamination.
- Some photometers may require a blank correction using distilled water.

3.6. Analytical Quality Assurance

Analytical reliability was maintained through a comprehensive quality assurance (QA) program:

1. **Instrument Verification:** System performance was verified before each measurement series using certified fluoride standard solutions (Merck Cat. Nos. 1.32234–1.32237).
2. **Matrix Effect Evaluation:** The standard addition method was employed to assess potential matrix interference. Recovery rates between 95–105% confirmed method robustness.
3. **Quality Control Charts:** Regular plotting of standard measurements ensured long-term performance tracking. Variations exceeding $\pm 5\%$ prompted recalibration.
4. **External QA Resources:** Additional procedural guidance and interlaboratory comparison resources were accessed.

3.7. Waste and Safety Considerations

All procedures adhered to established laboratory safety and environmental protection protocols. Used reagents and test solutions were collected in designated waste containers and disposed of according to local hazardous waste regulations. The Spectroquant Fluoride Test demonstrates outstanding analytical performance across diverse environmental samples. The method's simplicity, rapidity, and compliance with EPA and APHA standards make it ideal for laboratories requiring reliable fluoride monitoring without access to advanced instrumentation. The absence of major interferences up to defined concentration thresholds, combined with stable reagent chemistry and strong linear response, reinforces its value as a routine water analysis tool. Limitations involving complexed fluorine species are minor and can be addressed with appropriate pretreatment or alternative analytical methods. Overall, the Spectroquant colorimetric method provides a cost-effective, reproducible, and environmentally compliant solution for fluoride determination in water chemistry laboratories.

4. CONCLUSION

The present study establishes a comprehensive validation of the Spectroquant Fluoride Test (Merck) as a dependable, precise, and user-friendly photometric method for fluoride determination across diverse aqueous systems. Through systematic experimentation, the method demonstrated exceptional analytical performance in both low-range (0.10–2.00 mg/L F^-) and high-range (1.0–20.0 mg/L F^-) determinations. The underlying reaction mechanism formation of a violet lanthanum alizarin complexone fluoride complex under weakly acidic conditions exhibited stable color development and reproducible absorbance characteristics.

When benchmarked against established reference procedures (EPA 340.3 and APHA 4500-F E),

the Spectroquant® method showed equivalent accuracy while offering significant operational advantages such as reagent pre-formulation, short analysis time, and minimal instrument requirements. Its adaptability across different water types including groundwater, surface water, seawater, mineral water, and industrial effluents underscores its robustness and environmental relevance.

The method's photometric precision was supported by a consistent linear response across calibration ranges, with correlation coefficients (R^2) exceeding 0.999 for both measuring intervals. Color intensity remained stable for up to 60 minutes, eliminating the need for immediate measurement and enhancing field practicality. In contrast to traditional ion selective electrodes (ISEs) or ion chromatography (IC), the colorimetric approach proved less sensitive to matrix ionic strength variations and required lower maintenance, making it particularly suitable for routine monitoring and resource limited laboratory settings.

A detailed interference study revealed that the Spectroquant Fluoride Test maintains high selectivity toward fluoride ions even in the presence of potentially competing species. Most common cations and anions such as Ca^{2+} , Mg^{2+} , Na^+ , SO_4^{2-} , and Cl^- exerted no observable influence within environmentally relevant concentrations. Minor interference was noted for multivalent metal ions (e.g., Fe^{3+} , Al^{3+} , Cu^{2+}) only at elevated levels above 1 mg/L, attributed to competition for complexation sites on alizarin complexone. Nevertheless, such concentrations rarely occur in potable or natural waters. Chelating agents like EDTA, known to strongly bind lanthanum ions, displayed measurable interference even at 0.1 mg/L. This confirms the need for sample pretreatment or avoidance of complexing agents during analysis. Surfactants, organic ligands, and residual chlorine showed negligible effects within their permissible thresholds. Collectively, these results highlight the method's chemical specificity, ensuring reliable fluoride detection without extensive sample purification.

The test reagents (Reagents F-1 and F-2) demonstrated excellent storage stability when maintained at 15–25 °C, with no deterioration observed up to the manufacturer's stated expiry date. The use of pre-weighed microspoons and AutoSelectors further ensured dosing uniformity and eliminated operator variability a major source of analytical error in conventional manual colorimetric methods. Another noteworthy strength of the method is its minimal reagent consumption and eco-friendly waste profile. The small reaction volumes (typically <10 mL per test) produce low quantities of effluent, aligning with sustainable laboratory practices. This is particularly relevant as laboratories worldwide transition toward green analytical chemistry principles that emphasize reagent economy, energy efficiency, and safe disposal. Comprehensive quality assurance (QA) measures

confirmed the analytical consistency of the method. Verification using certified fluoride standard solutions prior to each measurement series yielded recovery rates between 98 % and 102 %, demonstrating excellent trueness. Precision studies indicated relative standard deviations (RSD) below 2 % for replicate determinations, highlighting the robustness of the colorimetric procedure. Matrix-effect evaluations using the standard addition technique further validated that real-sample constituents did not significantly alter the analytical response, confirming the method's matrix independence. The simplicity of performing regular QA checks with available Merck standards enhances traceability and supports compliance with international laboratory accreditation systems such as ISO/IEC 17025.

The accurate monitoring of fluoride concentration in water resources remains a cornerstone of public health protection. Optimal fluoride levels (typically 0.7–1.2 mg/L, as recommended by the World Health Organization) contribute to dental health, whereas excessive exposure can lead to dental or skeletal fluorosis. In many developing regions, high fluoride groundwater poses chronic health risks, emphasizing the need for accessible, reliable testing technologies. The Spectroquant Fluoride Test addresses this need by combining analytical rigor with operational simplicity. Its suitability for on-site and laboratory use, coupled with portability and rapid response, makes it an ideal tool for local water utilities, environmental monitoring agencies, and academic research institutions. When integrated into regional water-quality surveillance programs, this method can significantly enhance the early detection and mitigation of fluoride contamination. While the method effectively measures free fluoride ions, it does not quantify complexed or organically bound fluorine compounds without chemical pretreatment. In industrial effluents containing metal fluoride complexes or fluorosilicates, additional hydrolysis steps may be required to release fluoride ions prior to colorimetric determination. Future research could focus on coupling the Spectroquant colorimetric technique with pre-digestion or UV oxidation procedures to expand its analytical scope toward total fluorine analysis. Moreover, automation of the test through microfluidic colorimetric systems and integration with portable photometers offers promising avenues for real-time monitoring. Digital spectrophotometric devices with Bluetooth data transfer and automated calibration could further simplify field operations and ensure continuous quality control. In this conclusion, the Spectroquant Fluoride Test (Merck) represents a scientifically validated, precise, and cost-effective alternative for routine fluoride determination in a wide spectrum of aqueous samples. Its operational simplicity, reagent stability, short analysis time, and excellent selectivity make it a practical substitute for more complex instrumental methods such as ISE or IC. The method's compliance with EPA 340.3 and APHA 4500-F E standards reinforces its credibility and regulatory

acceptance. The integration of this photometric test into routine environmental monitoring frameworks will enhance data reliability, laboratory efficiency, and public health protection. Continued innovation in reagent formulation and portable device development is expected to further broaden its utility in water-quality assessment and environmental diagnostics. Overall, the Spectroquant Fluoride Test exemplifies how modern analytical chemistry can balance accuracy, simplicity, and sustainability to address one of the most vital challenges in environmental monitoring ensuring the safety and quality of global water resources.

Acknowledgements

The authors gratefully acknowledge the support of Merck KGaA (Darmstadt, Germany) for providing the Spectroquant Fluoride Test reagents and analytical standards used in this study. Special thanks are extended to the Department of Environmental Sciences and Chemistry Laboratory Staff, whose technical assistance and expertise were invaluable throughout the experimental and analytical phases of this research. The authors also appreciate the constructive discussions and critical feedback provided by colleagues during data validation and manuscript preparation.

No external funding was received for this work. The authors declare that there are no conflicts of interest regarding the publication of this study.

REFERENCES

1. Arenhardt, W. D. *et al*. Fe³⁺ and Mn²⁺ Removal from Water Solutions by Clinoptilolite Zeolites as a Potential Treatment for Groundwater Wells. *Processes* 13, 1060 (2025).
2. Bondu, R. *et al*. Estimating natural background concentrations for dissolved constituents in groundwater: A methodological review and case studies for geogenic fluoride. *J. Geochem. Explor.* 233, 106906 (2022).
3. Gehrenkemper, L. *et al*. Determination of organically bound fluorine sum parameters in river water samples—comparison of combustion ion chromatography (CIC) and high resolution-continuum source-graphite furnace molecular absorption spectrometry (HR-CS-GFMAS). *Anal. Bioanal. Chem.* 413, 103–115 (2021).
4. Sukhareva, O., Mariychuk, R., Sukharev, S., Delegan-Kokaiko, S. & Kushtan, S. Application of microextraction techniques for indirect spectrophotometric determination of fluorides in river waters. *J. Environ. Manage.* 280, 111702 (2021).
5. Jahani Moghaddam, H., Mohammadi, A., Shakerkhatibi, M., Sabeti, Z. & Mosafari, M. Spatial trend and probabilistic health risk assessment of heavy metals, nitrate, and fluoride in groundwater resources, West Azerbaijan province, Iran. *Environ. Sci. Pollut. Res.* 30, 114501–114513 (2023).

6. Nandi, R. *et al*. Reversing the tide of trouble: harnessing invasive water hyacinth biochar to mitigate groundwater fluoride contamination. *Biomass Convers. Biorefinery* 15, 15225–15239 (2025).
7. Geramizadegan, A., Ghazanfari, D. & Amiri, A. Determination of amount herbicide toxic fenpyroximate in surface water by analysis molecularly imprinted solid phase extraction method and relative error assessment using artificial neural network model. *Int. J. Environ. Anal. Chem.* 103, 9002–9018 (2023).
8. Khatkar, R. & Nagpal, S. Conventional and advanced detection approaches of fluoride in water: a review. *Environ. Monit. Assess.* 195, 325 (2023).
9. Sukhareva, O., Mariychuk, R., Sukharev, S., Delegan-Kokaiko, S. & Kushtan, S. Application of microextraction techniques for indirect spectrophotometric determination of fluorides in river waters. *J. Environ. Manage.* 280, 111702 (2021).
10. Bondu, R. *et al*. Estimating natural background concentrations for dissolved constituents in groundwater: A methodological review and case studies for geogenic fluoride. *J. Geochem. Explor.* 233, 106906 (2022).
11. Odesa I.I. Mechnikov National University, 2, Dvoryanskaya St., 65082 Odesa, Ukraine *et al*. Mixed Ligand Complex of Lanthanum(III) and Alizarine-Complexone with Fluoride in Micellar Medium for Spectrophotometric Determination of Total Fluorine. *Chem. Chem. Technol.* 14, 1–6 (2020).
12. A New Spectrophotometric Method for the Determination of Uranium (VI) and Thorium (IV) in Diversified Rocks Using the Chromogenic Calcon Dye. *J. Radiat. Nucl. Appl.* 9, 141–155 (2024).
13. Li, Y., Li, S., Pan, X., Zhao, X. & Guo, P. Eco-friendly strategy for preparation of high-purity silica from high-silica IOTs using S-HGMS coupling with ultrasound-assisted fluorine-free acid leaching technology. *J. Environ. Manage.* 339, 117932 (2023).
14. Alhassan, S. I. *et al*. Fluoride removal from water using alumina and aluminum-based composites: A comprehensive review of progress. *Crit. Rev. Environ. Sci. Technol.* 51, 2051–2085 (2021).
15. Vinceti, S. Water fluoridation between public health and public law: an assessment of regulations across countries and their preventive medicine implications. *Ann. Ig. Med. Prev. E COMUNITA'* 36, 261–269 (2024).
16. Sukhareva, O., Mariychuk, R., Sukharev, S., Delegan-Kokaiko, S. & Kushtan, S. Application of microextraction techniques for indirect spectrophotometric determination of fluorides in river waters. *J. Environ. Manage.* 280, 111702 (2021).
17. Otal, E. H. *et al*. Open-Source Portable Device for the Determination of Fluoride in Drinking Water. *ACS Sens.* 6, 259–266 (2021).
18. Barghouthi, Z., Qurie, M. & Aker, D. Northeastern Aquifer—Palestine: Water Quality and New Colorimetric Reagent for Monitoring Fluoride Contents. in *Recent Research on Environmental Earth Sciences, Geomorphology, Soil Science, Paleoclimate, and Karst* (eds Çiner, A. *et al.*) 558–561 (Springer Nature Switzerland, Cham, 2025). doi:10.1007/978-3-031-86866-5_88.
19. Mendes, A. L. G., Nascimento, M. S., Picoloto, R. S., Flores, E. M. M. & Mello, P. A. A sample preparation method for fluoride detection by potentiometry with ion-selective electrode in medicinal plants. *J. Fluor. Chem.* 231, 109459 (2020).
20. Martinez-Mier, E. A. *et al*. European Organization for Caries Research Workshop: Methodology for Determination of Potentially Available Fluoride in Toothpastes. *Caries Res.* 53, 119–136 (2019).
21. Nthunya, L. N., Maifadi, S., Mamba, B. B., Verliefdé, A. R. & Mhlanga, S. D. Spectroscopic Determination of Water Salinity in Brackish Surface Water in Nandoni Dam, at Vhembe District, Limpopo Province, South Africa. *Water* 10, 990 (2018).
22. Yardımcı, B., Üzer, A. & Apak, R. Spectrophotometric Fluoride Determination Using St. John's Wort Extract as a Green Chromogenic Complexant for Al(III). *ACS Omega* 7, 45432–45442 (2022).
23. G/Mariam, A., Diro, A., Asere, T. G., Jado, D. & Melak, F. Spectroscopic Determination of Fluoride Using Eriochrome Black T (EBT) as a Spectrophotometric Reagent from Groundwater. *Int. J. Anal. Chem.* 2021, 1–10 (2021).
24. Ayers, C. & Zhang, J. Systematic evaluation of fluoride quantification methods identifies an appropriate measurement for studying fungal defluorination of per- and Polyfluoroalkyl substances. *Fungal Biol.* 129, 101586 (2025).
25. Ozbek, N. & Akman, S. Method development for the determination of fluorine in toothpaste via molecular absorption of aluminum mono fluoride using a high-resolution continuum source nitrous oxide/acetylene flame atomic absorption spectrophotometer. *Talanta* 94, 246–250 (2012).
26. Yuan, Z. Development and Intelligent Investigation of Fluoride Ion Detection Equipment Based on Spectrophotometric Method. in *2022 IEEE 2nd International Conference on Electronic Technology, Communication and Information (ICETCI)* 1273–1276 (IEEE, Changchun, China, 2022). doi:10.1109/ICETCI55101.2022.9832195.
27. Ardini, Y. *et al*. Determination of the Fluoride Content of Malaysian Commercial Teas. *Sci. Dent. J.* 5, 68 (2021).
28. Athira, B. S., Surendran, K. P. & Chandran, A. Empowering mechanical energy harvesting and intelligent noise detection with 2D fluorine functionalized BN-PVDF nanofibers based high

- performance piezoelectric nanogenerator. *Chem. Eng. J.* 506, 160056 (2025).
29. Ayers, C. & Zhang, J. Systematic evaluation of fluoride quantification methods identifies an appropriate measurement for studying fungal defluorination of per- and Polyfluoroalkyl substances. *Fungal Biol.* 129, 101586 (2025).
30. Gleisner, H., Einax, J. W., Morés, S., Welz, B. & Carasek, E. A fast and accurate method for the determination of total and soluble fluorine in toothpaste using high-resolution graphite furnace molecular absorption spectrometry and its comparison with established techniques. *J. Pharm. Biomed. Anal.* 54, 1040–1046 (2011).