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Advanced spectrofluorimetric determination of ascorbic acid via serotonin hydrochloride quenching using a twin solar cell device with eight-angled blue radiation sources

Ghufran K. Allawi [a,b,*,1], N.S. Turkey [a,2]

[a] University of Baghdad, College of Science, Department of Chemistry, Baghdad, Iraq
[b] Al-Qasim Green University, College of Food Science, Department of Food Science and Technology, Babylon, Iraq

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ABSTRACT

A novel spectrofluorimetric flow injection analysis (FIA) method was developed for the selective quantification of ascorbic acid via fluorescence quenching of serotonin hydrochloride. The system employs a custom-designed photometric array comprising 16 irradiation sources arranged in a dual-axis matrix—eight aligned horizontally and eight orthogonally, enabling multi-angle excitation and enhanced spectral resolution. Fluorescence signals were captured using a twin-pair solar cell detector, offering high sensitivity and minimal optical interference. The method exhibited a linear calibration range of 0.1–30 μg/mL with a correlation coefficient (r^2) of 0.9966, a limit of detection (LOD) of 0.025 μmol /L, equivalent to 4.403 * 10^{-4} μg/mL per sample (0.1 mL (and limit of quantitative (LOQ) of 99.381 * 10^{-3} μg/ml. Precision and accuracy were confirmed through intra- and inter-day validation, yielding relative standard deviations (RSD) below 2% and recovery rates exceeding 99%. Statistical comparison with conventional fluorometric techniques using a paired *t*-test revealed no significant difference at the 95% confidence level ($p > 0.05$), affirming the method's reliability. The proposed system supports high-throughput analysis of up to 50 samples per hour, with reduced reagent consumption and minimal environmental impact. Its robustness, cost-efficiency, and analytical performance render it a promising tool for routine pharmaceutical quality control and environmental monitoring.

1. Introduction

Ascorbic acid (Vitamin C), a water-soluble organic compound with the molecular formula $C_6H_8O_6$ and IUPAC name (4*R*,5*R*)-5-[(1*S*)-1,2-dihydroxyethyl]-4-hydroxyoxolane-2,3-dione is widely recognised for its essential biological functions and therapeutic applications. It appears as a white to pale yellow crystalline powder, with a melting point of approximately 190–192 °C (decomposes upon heating), and is highly soluble in water. Structurally, it is a lactone containing an enediol group, which imparts strong reducing properties, making it a key antioxidant in biological systems. Medically, ascorbic acid contributes to collagen biosynthesis, enhances iron absorption, supports immune defences, and protects against oxidative stress. Its deficiency leads to scurvy, while excessive intake may result in gastrointestinal disturbances, nephrolithiasis, and iron overload in susceptible individuals [1–3]. Given its clinical and nutritional significance, accurate quantification of ascorbic acid in pharmaceutical formulations and biological matrices is crucial. Traditional methods include titrimetric assays using iodine or 2,6-dichlorophenolindophenol [4,5], spectrophotometry [6,7], high-performance liquid chromatography (HPLC) [6], and electrochemical techniques [5]. These approaches often employ reagents such as ferric ions, Folin–Ciocalteu reagent, and Ortho phenanthroline to enhance sensitivity and selectivity [6,8]. Recently, fluorescence-based analytical techniques have gained prominence due to their high sensitivity, rapid response, and compatibility with automation [9,10]. Fluorescence involves the emission of light by a molecule following excitation, and its intensity is influenced by factors such as pH, temperature, solvent polarity, and the presence of quenching agents like oxygen [11] or the presence of specific quenching agents. Notably, paramagnetic metal ions, such as Cu^{2+} and Fe^{3+}, are well-established efficient quenchers due

* Corresponding author.
E-mail addresses: ghufran.k.allawi@fosci.uoqasim.edu.iq (G.K. Allawi), nagham.turkey@sc.uobaghdad.edu.iq (N.S. Turkey).
[1] https://orcid.org/0000-0001-8747-9701.
[2] https://orcid.org/0009-0002-7925-6633.

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to their ability to enhance intersystem crossing from the singlet to the triplet state. In contrast, diamagnetic ions like Zn^{2+} and Cd^{2+} typically do not cause significant quenching [12]. Fluorescent probes such as fluorescein, rhodamine, coumarin derivatives, and quinine sulphate have been employed to detect ascorbic acid through fluorescence enhancement or quenching mechanisms [13]. The integration of fluorescence detection with flow injection analysis (FIA) has further improved analytical performance by enabling continuous, automated, and high-throughput quantification [14,15]. FIA systems offer advantages such as minimal reagent consumption, reduced analysis time, and enhanced reproducibility. Coupling FIA with fluorescence detection allows for real-time monitoring of reaction kinetics and precise determination of ascorbic acid concentrations, even at trace levels, especially when using fluorophores such as fluorescein, rhodamine B, and quinine sulphate, which have been employed as sensitive indicators in FIA platforms [9,13,15]. Advanced methodologies have emerged, including nanomaterial-based fluorescent sensors such as carbon quantum dots (CQDs) and silicon nanoparticles (SiNPs), which exhibit strong fluorescence and excellent biocompatibility [10,13]. Enzymatic reactions followed by fluorescence measurements often use ascorbate oxidase to catalyse the oxidation of ascorbic acid, producing non-fluorescent products that allow indirect quantification via signal change [11]. Seed-mediated growth of metal nanoparticles—particularly silver nanoparticles (AgNPs)—has also been used to quench the fluorescence of carbon dots, enabling sensitive detection through fluorescence resonance energy transfer (FRET) mechanisms [15]. These techniques have demonstrated exceptional sensitivity and selectivity in complex matrices, making them suitable for pharmaceutical quality control and clinical diagnostics [13,15]. Moreover, fluorescence quenching-based FIA methods provide robust platforms for the determination of ascorbic acid, leveraging the interaction between the analyte and fluorophores such as MnO_2 nanosheets, nitroxide-based probes, and CdTe quantum dots, which respond to redox changes induced by ascorbic acid to generate quantifiable signals [10,15]. Flow injection analysis (FIA) has emerged as a powerful technique in pharmaceutical analytical chemistry, widely applied for the determination of various pharmaceutical compounds due to its automation capabilities, minimal reagent consumption, and rapid throughput. Its adaptability makes it an ideal platform for assessing active pharmaceutical ingredients in complex formulations [16–24].When coupled with fluorescence detection, FIA enables highly sensitive monitoring of reactions involving analytes such as ascorbic acid. These reactions may lead either to fluorescence enhancement, through interaction with fluorogenic probes, or fluorescence quenching, particularly when redox-active substances modulate the emission of pre-existing fluorophores. Such combined methodologies have proven effective for trace-level quantification in pharmaceutical matrices, offering robust, selective, and efficient analytical solutions that support both therapeutic monitoring and formulation quality control. Building on these principles, the present work introduces a novel fluorometric approach for the determination of ascorbic acid in pharmaceutical formulations based on fluorescence quenching under flow-injection conditions.

The novelty of this study lies in the design and integration of a custom irradiation–detection system, which enables highly efficient photon collection and reproducible quenching measurements, rather than in the repetition of conventional fluorescence quenching or flow injection analysis.

The device incorporates eight blue irradiation sources arranged in a matrix within a 100 mm brass housing. These sources are positioned at 0–180° and 0–90° relative to twin solar-cell detectors, which ensures multi-angle excitation and cumulative photon harvesting. The detectors are connected in parallel to maximize photocurrent and signal acquisition. Photons traverse a continuous-flow quartz cell with a 2 mm irradiation path, providing uniform exposure and minimizing sample volume.

The orientation of the irradiation sources relative to the twin solar-cell detectors facilitates multi-angle excitation and cumulative photon harvesting. Parallel connection of the detectors further maximizes photocurrent and signal acquisition.

This system of architecture provides several advantages over existing methodologies:

- Enhanced efficiency: Cumulative photon collection from multiple angles increases sensitivity relative to single-beam excitation.
- Compact integration: The matrix configuration within brass housing ensures mechanical stability and reproducibility.
- Flow compatibility: The quartz flow cell enables continuous monitoring without perturbing the sample, thereby preventing secondary reactions.
- Novel detection principle: The system demonstrates that the observed quenching is purely photophysical, with no chemical reaction involving ascorbic acid. This result distinguishes the present approach from previous reports that frequently assume reactive pathways.

The originality of this work is demonstrated by the unique instrumentation and experimental configuration, which have not been previously reported in the literature for ascorbic acid determination. This innovation establishes a new platform for sensitive, reproducible, and reaction-free fluorescence quenching analysis [25–27]**.** The observed fluorescence quenching is exclusively photophysical, with no chemical interaction between ascorbic acid and the fluorophore. This characteristic distinguishes the proposed system from previously reported reactive quenching methods. The originality of this study is demonstrated by its unique instrumental configuration and detection principle, which have not been previously described for ascorbic acid determination. Under flow-injection conditions, the platform exhibits high sensitivity, reproducibility, and analytical robustness, making it suitable for precise pharmaceutical quality control based on fluorescence attenuation.

2. Experimental

2.1. Reagents & chemicals

Preparation of reagents and solutions: All chemicals utilised were of analytical grade and procured from reputable suppliers, such as British Drug Houses Chemicals limited (UK), Sigma-Aldrich (USA), and Merck (Germany). Serotonin hydrochloride ($C_{10}H_{12}N_2O{\cdot}HCl$, MW = 212.68 g/mol) was prepared as a 50 μM solution by dissolving 10.63 mg in 1 L of deionised water. Ascorbic acid ($C_6H_8O_6$, MW = 176.12 g/mol) was freshly prepared daily at required concentrations. The fluorescent molecules employed in this study were 2,7-dichlorofluorescein (molecular weight = 402.19 g/mol), fluorescein disodium salt (376.28 g/mol), calcein (622.53 g/mol), erythrosin B (879.87 g/mol), and 2-iodo-2-methylfluorescein (528.30 g/mol), all obtained from BDH Chemicals. Each compound was prepared at a final concentration of 1 mM in 250 mL of sterile deionised water. Accordingly, the following masses were weighed: 100.55 mg of 2,7-dichlorofluorescein, 94.07 mg of fluorescein disodium salt, 155.63 mg of calcein, 219.97 mg of erythrosin B, and 132.08 mg of 2-iodo-2-methylfluorescein. Each solution was vortexed thoroughly until fully dissolved and stored under conditions suitable for preserving fluorescence stability. Buffer solutions with varying pH values (2.2–8.0) [28,29] were prepared using two stock solutions at a concentration of 0.1 M: citric acid monohydrate (molecular weight = 210.14 g/mol) and disodium hydrogen phosphate dihydrate (MW = 294.10 g/mol). A precise pH adjustment was achieved by volumetric mixing the stock solutions according to a standardised protocol. All solutions were prepared using sterile deionised water, and pH values were confirmed using a calibrated digital pH meter. All solutions were prepared using sterile deionised water, and pH values were confirmed using a calibrated digital pH meter. Prepare and evaluate ascorbic acid tablet samples from local pharmaceutical products.

To evaluate the pharmaceutical quality of ascorbic acid tablets produced by three Iraqi pharmaceutical companies, Furat Pharma, Kindi Pharma, and Aljazeera, three representative samples were prepared for analysis. The average weight of 20 tablets from each product was found to be 0.634 g, 0.832 g, and 0.639 g, respectively. To obtain a final concentration of 5 mmol/L, precise amounts of 0.11166 g, 0.14653 g, and 0.11254 g were weighed from average weight of each sample accordingly. The weighed portions were finely ground using a mortar and pestle to ensure homogeneity of the active pharmaceutical ingredient. Each powdered sample was then quantitatively transferred into a 100 mL volumetric flask and diluted to volume with distilled water under continuous stirring to facilitate dissolution. The resulting solutions were sonicated for 10 min to enhance solubility and ensure uniform distribution of ascorbic acid. Prior to subsequent analytical procedures, the solutions were filtered through 0.45 μm membrane filters. The final preparations were stored in amber glass containers at a controlled room temperature to minimise oxidative degradation until analysis.

2.2. Apparatus

A custom-built photometric system [25] was developed for fluorescence detection at irradiation angles at 0–90°. The instrument is equipped with a brass incubation chamber (100 mm length × 40 mm width) containing eight perforations on one side, precisely aligned with a tubular flow cell. These perforations, configured at a 0–180° angle, are designed to interface with a twin solar cell detection unit. In addition, a second set of eight identically sized perforations is positioned at a 0–90° angle to accommodate blue light-emitting diode (LED) sources for sample irradiation. The system offers flexible operation modes, enabling the activation of either an LED irradiation port independently or simultaneously. This design facilitates advanced investigation of optical interference phenomena, such as spectral symmetry and self-absorption effects. Fig. 1 illustrates the structural layout of the photometric apparatus. The flow injection system comprises a four-channel peristaltic pump (Ismatec, Switzerland) with adjustable flow rates, connected to a six-port medium-pressure injection valve (IDEX Corporation, USA) equipped with a Teflon sample loop (1 mm i.d., variable length). Data recording was achieved using a potentiometric chart recorder (Siemens, Germany; voltage range 1–5 V) for signal profiling and kinetic pattern analysis. For comparative fluorescence evaluation of serotonin hydrochloride, a classical spectrofluorometric method was applied using a FluoroMax-4 spectrofluorometer (Horiba Scientific, USA). Measurements were conducted in standard 1 cm path length quartz cuvettes, chosen for their high optical transparency and chemical inertness, ensuring reliable fluorescence performance under optimised experimental conditions.

3. Results and discussion

3.1. Methodology for classical method

Spectral fluorescence study of serotonin hydrochloride and its quenching by ascorbic acid. A spectrofluorometric investigation was conducted to evaluate the fluorescence behavior of serotonin hydrochloride under varying pH conditions. Using a classical fluorescence spectrometer (FluoroMax-4, Horiba Scientific, USA) equipped with a 1 cm quartz cuvette, serotonin hydrochloride exhibited a broad excitation spectrum peaking around 400 nm, with fluorescence emission extending from approximately 440 nm up to 500 nm (Fig. 2-a), depending on the excitation conditions and molecular environment [28,29]. The fluorescence intensity was found to be highly pH-dependent, with optimal emission observed at pH 6. This enhancement is attributed to the protonation state of indole nitrogen, which stabilizes the excited state and minimizes non-radiative decay pathways. Protonation suppresses the involvement of the nitrogen lone pair in intramolecular charge transfer and resonance delocalisation, thereby reducing internal conversion and intersystem crossing (ISC) ((i.e., does not play a dominant role in the present system. The continuous fluorescence of serotonin under blue excitation ($\lambda \approx 460$ nm) originates from singlet emission ($S_1 \rightarrow S_0$). The quenching observed upon addition of ascorbic acid follows Stern–Volmer behavior without signatures of triplet involvement. Thus, the mechanism is adequately explained by singlet-state quenching rather than ISC.) [30]. While extended resonance in the ground state may lower the energy gap and facilitate non-radiative relaxation, its attenuation upon protonation favors radiative decay by preserving the singlet excited state. Protonation of the indole moiety stabilizes the singlet excited state by reducing non-radiative pathways such as internal conversion and ISC. This stabilization favors radiative decay, thereby preserving fluorescence intensity until quenched by external agents. Such behavior has been reported for indole derivatives where protonation modulates excited-state lifetimes and enhances singlet emission [31]. Consequently, the reduced resonance and enhanced electronic confinement under mildly acidic conditions contribute to the observed increase in fluorescence quantum yield. Beyond pH 6, a gradual decline in fluorescence was noted, likely due to deprotonation and increased molecular flexibility, which promotes internal conversion and reduces quantum yield. To assess the quenching effect of ascorbic acid, a series of buffered solutions containing a fixed concentration of serotonin hydrochloride (1 μmol/L) were prepared at pH 6 and increasing concentrations of ascorbic acid (0–8 μmol/L) were added (Fig. 2-b). A significant decrease in fluorescence intensity was observed with rising ascorbic acid levels, indicating a dynamic quenching mechanism. This quenching is likely due to electron transfer interactions between the electron-rich ascorbic acid and the excited state of serotonin, as well as possible hydrogen bonding and π–π stacking interactions that facilitate non-radiative relaxation (i.e., Hydrogen bonding and π–π stacking are interaction motifs, not mechanisms by themselves. Their quenching

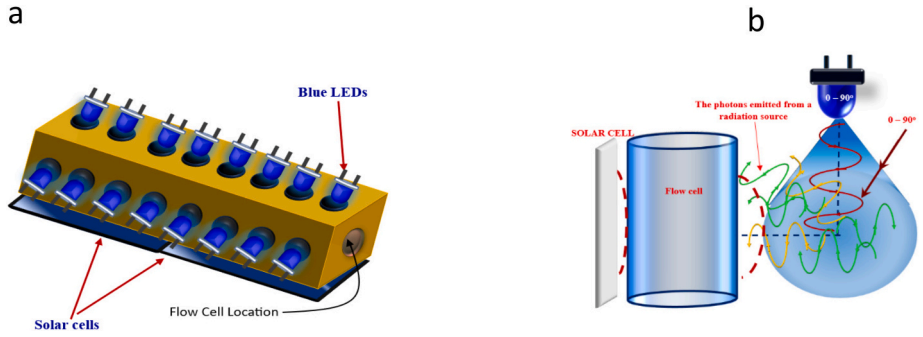


Fig. 1. a-The flow cell comprises eight blue irradiation sources positioned at angles 0–180°, and an additional eight sources oriented at 0–90° relative to twin solar cells. b- Irradiation at angles 0–90° relative to the solar cell, using one of the eight designated radiation sources.

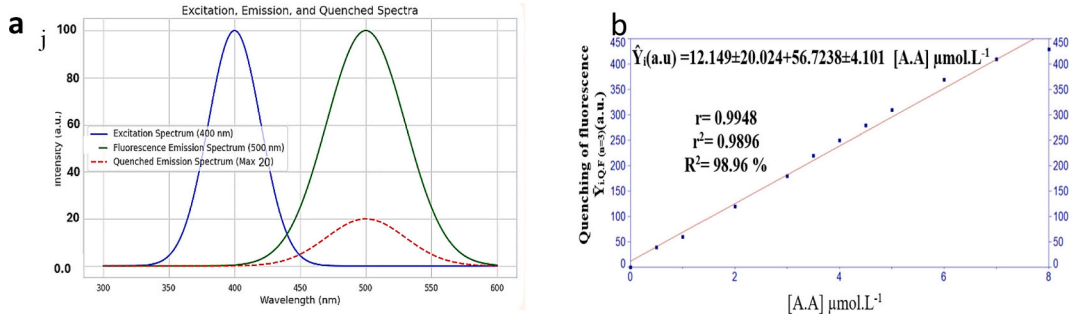


Fig. 2. a-Serotonin hydrochloride excitation (blue), emission (green), and quenched emission (red) spectra. The excitation spectrum shows a distinct peak at 400 nm, while the native fluorescence exhibits maximum intensity at 500 nm at 1 µmol/L. Upon addition of ascorbic acid, a pronounced quenching effect is observed (max ≈20 a.u.) across 440–560 nm, indicating efficient dynamic quenching, presumably via electron transfer. b- A calibration plot demonstrating the concentration-dependent quenching effect of ascorbic acid on the native fluorescence intensity of serotonin hydrochloride, indicating a linear inverse relationship suitable for quantitative analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

roles depend on how they act:

- **Hydrogen bonding:** can facilitate collisional encounters and enhance non-radiative decay during the excited-state lifetime → dynamic if it increases diffusion-controlled contact; static if it stabilizes ground-state complexes.

π–π stacking: typically forms ground-state associated species between aromatics, reducing excitable fluorophore population → static quenching. Mixed behavior is possible if weak stacking coexists with collision-driven encounters*)*.

The proposed interaction mechanism is illustrated in Scheme 1, showing the formation of a transient complex that disrupts fluorescence emission. Supporting evidence for this interaction is reported in the literature [32], where indole-based compounds such as serotonin show sensitivity to fluorescence from redox-active species like ascorbic acid. A calibration curve (Fig. 2-b) was constructed by plotting quenching fluorescence intensity against ascorbic acid concentration, yielding a linear relationship up to 8 µmol/L. Beyond this concentration, no significant quenching was observed, possibly due to saturation of the interaction sites.

While the data as shown in Table 1 and Fig. 2-C using *Stern–Volmer analysis at 298* K

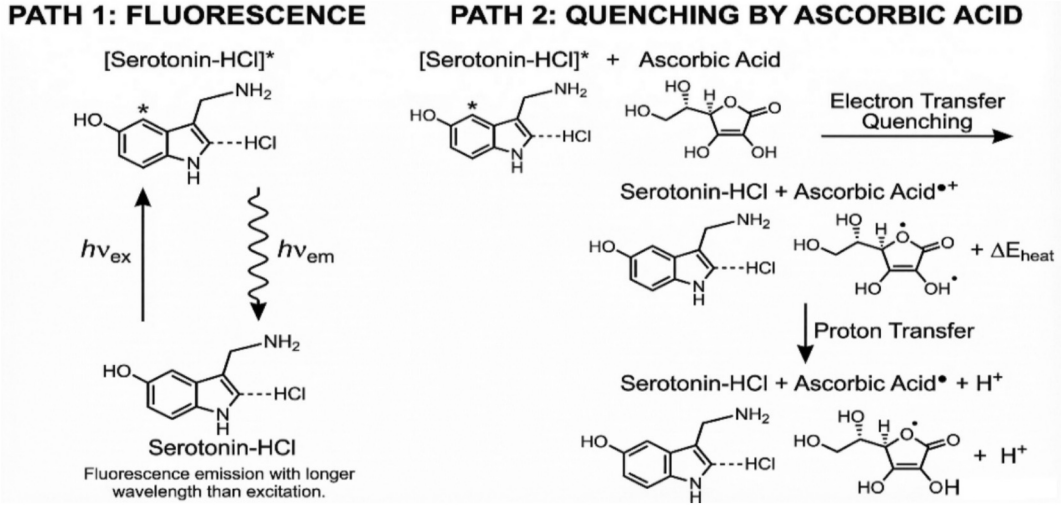
- Y is quenching in intensity units, $Y = \Delta I = I_0 - I$.
- I°: 500 a.u.; Temperature: 298 K; [AA]: micromolar (µmol/L).

Table 1
Stern–Volmer quenching data for ascorbic acid–serotonin hydrochloride system at 298 K, including ΔI, residual fluorescence (I), and Stern–Volmer ratio (I_0/I) across the 0–8 µmol/L concentration range.

[AA] (µM)	Y (ΔI)	$I = 500 - Y$	I°/I
0.0	1	499	1.0020
0.5	40	460	1.0870
1.0	60	440	1.1364
2.0	120	380	1.3158
3.0	180	320	1.5625
3.5	220	280	1.7857
4.0	250	250	2.0000
4.5	280	220	2.2727
5.0	310	190	2.6316
6.0	370	130	3.8462
7.0	410	90	5.5556
8.0	430	70	7.1429

- Computed quantities (remained of fluorescence): $I = 500 - Y$, and Stern–Volmer ratio I_0/I.
- Equation:

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$



Scheme 1. Proposed mechanistic pathway for the fluorescence emission of serotonin hydrochloride and its quenching by ascorbic acid via radical formation.

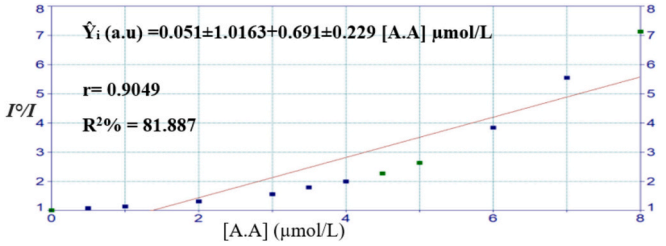


Fig. 2-C. Stern–Volmer Plot (I_0/I vs. [AA]) for the Ascorbic Acid–Serotonin System at 298 K Showing Linear Dynamic-Quenching Behavior at Low Concentrations (0–4 µmol/L) and Upward Curvature at Higher Concentrations (4–8 µmol/L), Indicating Mixed Dynamic–Static Quenching.

- Low-concentration regime (0–4 µmol/L): Observed trend: Approximately linear.
- Estimated slope:

$$K_{SV}^{0-4\,\mu mol/L} \approx \frac{(I_0/I)_{4\,\mu mol/L} - (I_0/I)_{0\,\mu mol/L}}{4-0} = \frac{2.0000 - 1.0020}{4} \approx 0.250\,L \Big/ \mu mol$$

- Interpretation: Consistent with predominantly dynamic quenching at low [AA].
- High-concentration regime (4–8 µmol/L): Observed trend: Upward curvature relative to a single straight line.
- Estimated slope:

$$K_{SV}^{4-8\,\mu mol/L} \approx \frac{7.1429 - 2.0000}{8-4} \approx 1.286\,L \Big/ \mu mol$$

- Interpretation: The upward curvature and higher apparent slope suggest a mixed-mode mechanism (static component increasing due to ground-state association) superimposed on dynamic quenching.
- Single-line estimate (0–8 µmol/L, rough):

$$K_{SV}^{0-8\,\mu mol/L} \approx \frac{7.1429 - 1.0020}{8} \approx 0.768\,L \Big/ \mu mol$$

- This single value shows a specific K_{SV} is more accurate.
- So, Low concentration (0–4 µmol/L): linear regime, $K_{SV} \approx 0.250 L/\mu mol$, consistent with dynamic quenching.
- High concentration (4–8 µmol/L): upward curvature, apparent slope $\approx 1.286\,mM^{-1}$, indicating mixed quenching (static + dynamic).
- Global fit (0–8 µmol/L): $K_{SV} \approx 0.768 L/\mu mol$, but this obscures curvature; regime-specific values are more accurate.
- Temperature dependence: dynamic quenching predicts K_{SV} increases with T; static predicts decrease.
- Viscosity dependence: dynamic quenching follows $K_{SV} \propto 1/\eta$; weak dependence indicates static.

$$K_{SV} \propto D \propto \frac{1}{\eta}$$

where Dis the diffusion coefficient and ηis viscosity.

Lifetime measurements: dynamic quenching requires τ_0/τ to mirror I_0/I; invariant lifetimes with reduced intensity confirm static [33,34].

- Lifetime Stern–Volmer:

$$\frac{\tau_0}{\tau} = 1 + K_{SV}[Q]$$

3.2. The proposed novel method

A single-line continuous flow injection system (Fig. 3) was designed for the determination of ascorbic acid based on fluorescence quenching. The carrier stream consisted of serotonin hydrochloride at a concentration of 5.0 µmol/L, delivered at a flow rate of 1.5 mL/min to produce a stable fluorescence signal.

Excitation was achieved using a custom-built optical unit containing two orthogonal LED arrays, each composed of eight blue LEDs (Excitation wavelength $\lambda \approx 460$ nm, 1.5 W per LED), totalling sixteen LEDs. These arrays were arranged to provide uniform irradiation across the flow cell. Fluorescence detection was performed using a solar sensor (410–1150 nm with Emission maximum, ~500 nm) composed of two photovoltaic cells (2 × 3.78 cm), positioned to capture the emitted light efficiently. The system operated at pH 6.0, which was found optimal for enhancing serotonin fluorescence and maintaining analyte stability. A 100 µL aliquot of ascorbic acid was injected into the carrier stream, resulting in a measurable quenching effect. The change in fluorescence intensity was recorded in real time using a chart recorder, and the degree of quenching was used to quantify the concentration of ascorbic acid.

The proposed fluorescence quenching mechanism is illustrated in the Scheme below (1). Upon photoexcitation, serotonin is promoted to its singlet excited state (S_1). In the absence of quencher, radiative decay ($S_1 \rightarrow S_0$) produces the observed fluorescence. In the presence of ascorbic acid, however, the excited state is efficiently quenched. The Stern–Volmer analysis (see Table 1 and Fig. 2-C) demonstrates a linear regime at low [AA] consistent with dynamic collisional quenching, followed by upward curvature at higher concentrations indicative of additional static contributions due to ground-state complex formation. Thus, the improved scheme now depicts:

1. **Excitation:** $S_0 \rightarrow S_1$.
2. **Radiative decay (fluorescence):** $S_1 \rightarrow S_0$.
3. **Quenching pathway:** S_1 is deactivated by ascorbic acid either through collisional encounters (dynamic) or by formation of non-fluorescent ground-state complexes (static).

This mechanistic assignment is supported by established fluorescence quenching theory, where curvature in Stern–Volmer plots is diagnostic of mixed dynamic and static quenching modes [31,34].

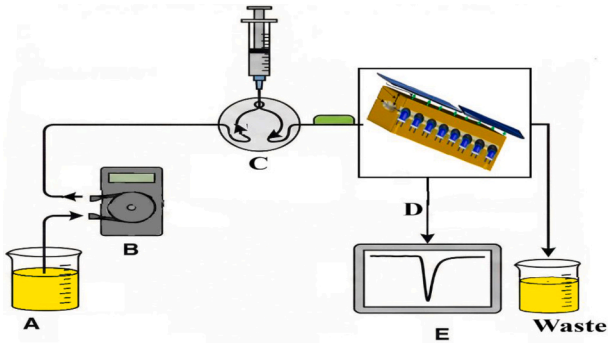


Fig. 3. Single-line flow injection system for the determination of ascorbic acid via quenching of serotonin hydrochloride fluorescence: A–serotonin hydrochloride solution at pH 6, B–peristaltic pump, C–injection valve with sample segment (ascorbic acid as a quencher), D A custom-built fluorescence detector and E-recorder.

3.3. Blue-light-induced fluorescence enhancement of selected organic fluorophores

A comparative fluorescence study was conducted to assess the photophysical response of six organic fluorophores: 2,7-dichlorofluorescein, fluorescein disodium salt, serotonin hydrochloride, calcein, erythrosin B, and 2-iodo-2-methylfluorescein, under excitation by blue LED irradiation (λ ≈ 460 nm). Each compound was prepared at a fixed concentration of 5.0 µmol /L, and fluorescence emission was recorded under identical conditions. Among the tested molecules, serotonin hydrochloride demonstrated the most pronounced fluorescence enhancement, as shown in Fig. 4-A, indicating its superior compatibility with blue-light excitation and its potential utility in fluorescence-based analytical applications. A concentration-dependent fluorescence profile was subsequently established for serotonin hydrochloride across the range of 1.0–10.0 µmol/L. The emission intensity increased steadily with a concentration up to 5.0 µmol/L, followed by a gradual decline at higher concentrations. This attenuation is attributed to fluorescence. Quenching mechanisms, including non-radiative energy dissipation via molecular collisions, thermal relaxation, and internal conversion processes, which collectively reduce the quantum efficiency at elevated concentrations. Accordingly, 5.0 µmol/L was identified as the optimal concentration for maximizing fluorescence yield under blue LED excitation (Fig. 4-B).

In the present study, the optimal concentration was not considered as a single discrete value, but rather defined as a functional working segment determined by a slope–intercept segmentation approach. This method evaluates the linearity, stability, and analytical suitability of adjacent concentration intervals using the standard linear regression model: $\hat{Y} = a + b[C]$.

where a (intercept) reflects analytical sensitivity at low concentration levels, and b (slope) represents signal variation throughout the examined interval. Selection of the most reliable working segment was based on two complementary analytical criteria:

1. Higher and consistent intercept values (a) :This indicates enhanced sensitivity and stable detection performance at lower concentrations.
2. Lower slope magnitude (b): Smaller slope values correspond to reduced response variability per unit [21,35].

The optimal working range was selected based on the concentration segment that maintained a stable and elevated intercept with relatively low slope values; specifically, the segment 5–7–9 µmol/L exhibited significant statistical stability, characterized by an increase in the intercept (391.33) and lower slope values (−19). Conversely, the 1–3–5 and 3–5–7 µmol/L segments exhibited markedly higher slope values (Slope = 22and 138, Intercept = 141and 391.33), demonstrating increased signal fluctuation associated with reduced analytical consistency. High slopes indicate large changes in response per unit concentration, whereby minor fluctuations produce substantial analytical deviations, ultimately reducing method reliability within that interval.

Based on these criteria, the concentration region from 5 to 9 µmol/L provided the most consistent regression characteristics, confirming it as the stable working range and supporting the selection of 5.0 µmol/L as the optimal fluorophore concentration for analytical measurements.

3.4. Effect of pH on fluorescence activation and quenching efficiency

In a single-line flow injection system, serotonin hydrochloride was employed as the fluorescent carrier stream and prepared at a concentration of 5.0 µmol/L in buffer media with varying pH values ranging from 3 to 7.6, in addition to distilled water. The buffer solutions were formulated using standardised mixtures of 0.1 M citric acid monohydrate and 0.1 M disodium hydrogen phosphate dihydrate, with all preparations conducted in sterile deionised water and pH values confirmed using a calibrated digital pH meter. Ascorbic acid was injected into the carrier stream at 25.0 µmol /L in 150 µL volumes at 1.3 ml/min. The study revealed that pH 6.0 provided the highest fluorescence intensity for serotonin hydrochloride, along with the most efficient quenching response by ascorbic acid. This dual Optimization is likely attributed to the favourable protonation state of serotonin hydrochloride at a mildly acidic pH, which enhances its quantum yield while simultaneously preserving the redox activity of the ascorbic acid required for dynamic quenching. Based on these findings, serotonin hydrochloride will be prepared in a pH 6.0 medium for all subsequent experiments, as this condition ensures maximum fluorescence activation and optimal quenching performance, as illustrated in Fig. 5.

3.5. Optimization of injection volume and flow rate for quenching efficiency

The effect of sample injection volume and flow rate on the fluorescence quenching efficiency of ascorbic acid was investigated using a single-line flow injection system. Serotonin hydrochloride at 5.0 µmol/L in pH 6.0 buffer served as the fluorescent carrier stream, while ascorbic acid was injected at 25.0 µmol/L in varying volumes ranging from 40 to 200 µL. A gradual increase in quenching efficiency was observed with increasing injection volume, reaching a maximum at 100 µL. Beyond

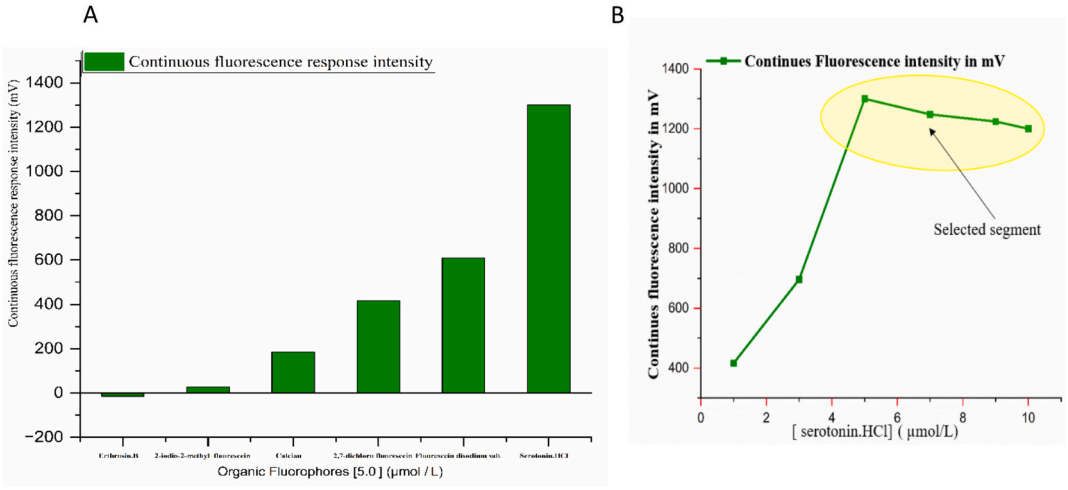


Fig. 4. A) Comparative impact of irradiation sources on the fluorescence activation of distinct fluorophores: percentage contribution analysis, B) the optimal concentration of serotonin hydrochloride was determined to be 5.0 µmol /L.

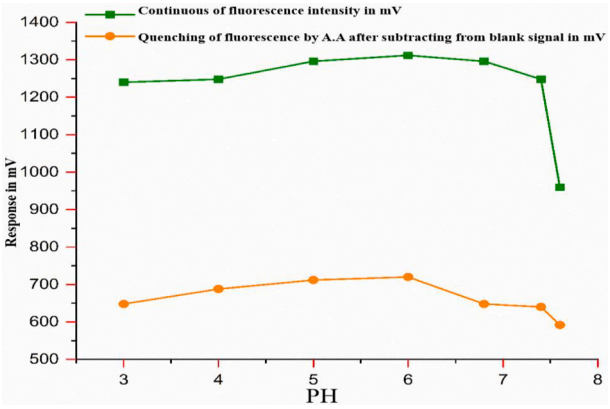


Fig. 5. Effect of solution ph on steady-state fluorescence intensity and quenching efficiency of ascorbic acid.

this volume, a decline in quenching efficiency occurred, likely due to saturation of the interaction sites and increased molecular collisions leading to non-radiative energy loss through internal conversion and thermal dissipation. Additionally, the broadening of the response base was attributed to the extended residence time of the injected segment within the flow cell, resulting in prolonged interaction and signal dispersion. Accordingly, 100 µL was selected as the optimal injection volume for achieving maximum quenching, as illustrated in Fig. 6. Under identical conditions, the influence of flow rate was examined across the range of 0.7 to 2.0 m/ min. Quenching efficiency improved with increasing flow rate up to 1.5 ml/min, likely due to reduced axial dispersion and enhanced mixing dynamics. However, further increases in flow rate led to diminished quenching performance, potentially caused by physical dilution and reduced interaction time between the analyte and the fluorescent carrier stream. Based on these observations, a flow rate of 1.5 mL/min was identified as optimal for maximizing quenching efficiency and will be employed in subsequent analytical procedures, as shown in Fig. 7.

3.6. Calibration curve and analytical performance for ascorbic acid determination

A calibration curve was created to quantify ascorbic acid using fluorescence quenching for serotonin hydrochloride. After optimising all

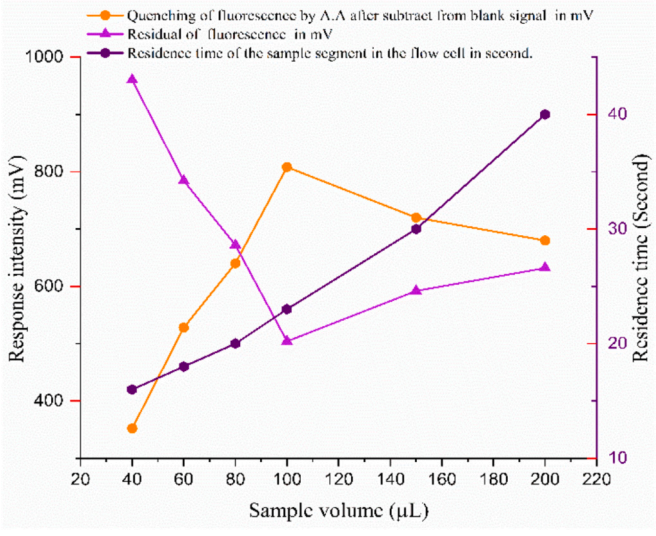


Fig. 6. Effect of sample segment volume on the fluorescence quenching efficiency of ascorbic acid.

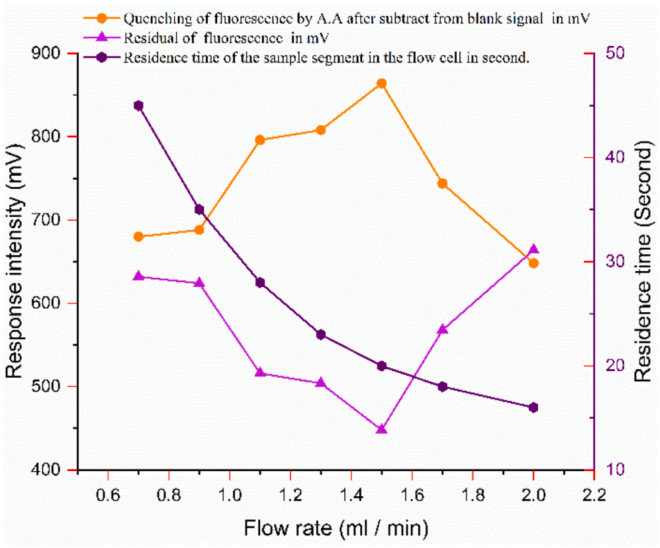


Fig. 7. Influence of flow rate on the fluorescence quenching efficiency of ascorbic acid.

chemical and physical parameters, 100 µL of ascorbic acid solutions ranging from 0.1 to 30.0 µmol/L were injected into a single-line flow system with serotonin hydrochloride at 5.0 µmol/L in a pH 6.0 buffer. Fluorescence intensity decreased gradually as ascorbic acid content increased, indicating greater dynamic quenching via electron transfer and non-radiative relaxation routes. At 30.0 µmol/L, the fluorescence signal decreased from 1312 mV to 372 mV, indicating a quenching efficiency of 71.6%. The linear regression equation was Y(mV) = 32.901 ± 14.556 + 31.393 ± 0.808 [A.A] µmol/L, with a correlation coefficient (r) of 0.9983 and linearity percentage (R^2%) of 99.66% (n = 24). Fig. 8 shows the strong linear relationship between analyte concentration and quenching response. The limit of detection (LOD), determined at a signal-to-noise ratio (S/N) of 3, was 0.025 µmol/L for a 100 µL injection volume. The limit of quantification (LOQ) was calculated according to ICH guidelines using LOQ = 10σ/S, where σ is the standard deviation of the blank, and S is the slope of the calibration curve. The resulting LOQ of 5.643 µmol/L indicates high sensitivity, comparable to or exceeding conventional fluorescence-based techniques (LOD ≈ 0.125 µmol/L, equivalent to 22.015 ng per 1000 µL sample). The method's precision was assessed using intra-day and inter-day repeatability trials at 12.0 and 25.0 µmol/L (n = 8), with relative standard deviations (RSD%) < 1% for intra-assay and 2% for inter-assay values, indicating excellent reproducibility and consistency. These performance criteria, especially sensitivity and linearity, are well positioned among existing analytical approaches. Table 2 provides a comparative review of calibration ranges and detection limits for chosen spectrophotometric, titrimetric, chromatographic, and fluorescence-based methods, highlighting the proposed system's analytical advantages.

3.7. Validation of the developed method using local pharmaceutical samples

The developed single-line flow injection fluorimetric method was applied to determine ascorbic acid in three commercial drug formulations sourced from Iraqi pharmacies: Furat Pharma, Kindi Pharma, and Aljazeera. From each formulation, 0.5 mL of a 50 µmol/L sample was transferred into five 10 mL volumetric flasks, followed by incremental additions of 0.4–1.0 mL of a 50 µmol/L standard solution, yielding final concentrations of 2.0–5.0 µmol/L. The first flask served as the reference. Measurements were performed using an optimised fluorescence quenching system based on serotonin hydrochloride. For comparison, the conventional spectrofluorimetric method was conducted by

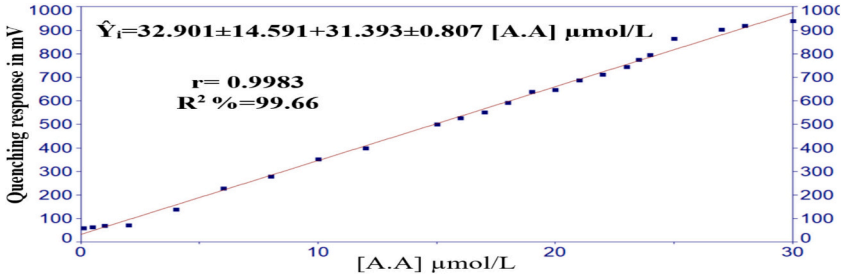


Fig. 8. Calibration curve depicting the relationship between ascorbic acid concentration and fluorescence quenching of serotonin hydrochloride: spectral response profile from the fluorometric detection system.

Table 2
Comparative evaluation of analytical techniques for ascorbic acid quantification.

Method Type	Detection Principle/Reagents	Sample matrix	Calibration Range	LOD	Reference
Spectrophotometry	UV absorbance at 243 nm in acidic medium	herbal antidiabetic mixtures	11.36–68.15 **µmol/L**	**56.78 µmol/L**	[36]
Redox Titration	DCIP[1] as redox indicator; endpoint via color change	foods	0.1–25 µmol/L	**~0.05 µmol/L**	[4]
Fluorescence Quenching	BSA[2] cluster luminescence probe quenched by AA[3]	**Vegetables**	0.1–30 µmol/L	**0.025 µmol/L**	[37]
HPLC[4]	C18[5] column; detection at 254 nm; methanol/ water mobile phase	pharmaceutical Formulations, Biological Samples	2.84–71.0 µmol/L	0.017 µmol/L	[38]
Quantum Dot Fluorescence	MoS_2 QDs[6] quenched by Fe^{3+}; AA restores fluorescence	fruits, beverages, and serum samples	1–150 µmol/L	**0.05 µmol/L**	[38]
Proposed advanced spectrofluorimetric method	Fluorescence quenching of serotonin HCl by ascorbic acid	Pharmaceutical tablet	0.1–30 µmol/L	0.025 µmol/	

1-DCIP: 2,6-Dichlorophenolindophenol,2- **BSA**: Bovine Serum Albumin,3-**AA**: Ascorbic Acid,4-**HPLC**: High Performance Liquid Chromatography,**5-C18**: Octadecylsilane-bonded silica column, **6-MoS_2 QDs**: Molybdenum Disulfide Quantum Dots.

transferring 0.1 mL from each sample into 10 mL volumetric flasks, with standard additions ranging from 0.1 to 0.4 mL, corresponding to final concentrations of 0.5–2.0 µmol /L. Recovery values obtained using the developed method ranged from 99.83% to 100.40%, while the conventional method yielded recoveries between 96.39% and 98.92%, as shown in Table 3. These results confirm the method's high accuracy and suitability for pharmaceutical analysis. Statistical evaluation using the Student's *t*-test showed no significant difference between the two methods (t_calculated (3.7939) < t_tabulated (4.303)). Additionally, one-way ANOVA including the reference value of 500 mg, based on the official monograph for ascorbic acid in the British Pharmacopoeia[35], confirmed that the calculated F-value was less than the tabulated critical value, indicating no statistically significant difference among the three approaches. These findings support the developed method as a reliable, sensitive, and cost-effective alternative for routine medical and environmental applications.

As shown in Table 3, the developed FIA–fluorescence method, based on the quenching of serotonin hydrochloride fluorescence by ascorbic acid, exhibited excellent accuracy and precision in the quantification of vitamin C across various commercial tablet formulations. The recovery values ranged from 99.83% to 100.40%, with relative standard deviations (RSD%) consistently below 0.5%, indicating high method reproducibility. Statistical validation using paired *t*-test and one-way ANOVA revealed no significant differences between the developed method and conventional fluorimetry ($p > 0.05$), confirming the analytical reliability of the proposed approach.

3.8. Quantitative assessment of ascorbic acid in real food systems via the developed FIA-twin solar cell system

To verify the analytical resilience and broader utility of the developed FIA-spectrofluorimetric system, the method was employed to quantify ascorbic acid (AA) in various food samples. Analysing food matrices is significantly more challenging than pharmaceutical formulations due to the potential interference from endogenous components such as citric acid, sugars, and photosynthetic pigments.

Table 3
Determination of ascorbic acid in commercial tablets using the developed FIA–fluorescence method: comparison with labeled content and conventional fluorimetry.

Pharmaceutical Product/ Manufacturer/Claimed Value (mg)	FIA–Quenching of Fluorescence by A.A Method Weight of active ingredient at 95% $^{-}Wi_{(mg/tablets)} \pm SD$	(SD) RSD%	Recovery %	$t_{cal.} < t_{tab.}$ Compared to label (500 mg)	Conventional Fluorescence Weight of active ingredient at 95% $^{-}Wi_{(mg/tablets)} \pm SD$ **% Recovery**	RSD %	Statistical Comparison
Vitamin C Aljazeera 500 mg Iraq	502.013 ± 2.135	(2.159) 0.43	100.40%	1.633 < 4.303	485.599 ± 5.365 97.12%	1.10	T-Test 3.7939 < 4.303
Vitamin C Kindi 500 mg Iraq	499.126 ± 1.515	(1.497) 0.30	99.83%	/−0.999/<4.303	481.934 ± 5.892 96.39%	1.22	No significant difference ANOVA
Vitamin C Furat Pharma 500 mg Iraq	500.929 ± 1.098	(1.102) 0.22	100.19%	1.465 < 4.303	494.624 ± 2.325 **98.92%**	0.47	F-value $F_{tab.}(5.14) < F_{cal}(11.275)$

Sample Preparation and Treatment:

Four types of samples were analysed: fresh orange juice, lemon juice, commercial fruit juice, and green bell pepper and obtained from local sources. For the fruit juices (Liquid samples), the samples were filtered and then centrifuged at 4500 rpm for 15 min. For the green bell pepper (solid matrix), 10 g of the sample was finely homogenized in 50 mL of 0.1% (*w/v*) oxalic acid solution to ensure complete extraction and stabilization of AA against photo-oxidation. All extracts were filtered through a 0.45 μm membrane filter and appropriately diluted with deionised water to ensure the final concentration.

Table 4 is that the proposed method demonstrates exceptional robustness. The data summarized in Table 4 demonstrate a high recovery rates (99.56%–100.49%) and low relative standard deviations (RSD $< 1.5\%$). These results confirm that the "serotonin quenching" mechanism is highly selective for ascorbic acid even in the presence of complex botanical matrices such as citric acid and sugars. The multi-angle blue radiation sources combined with the twin solar cell detector effectively eliminated background noise and scattering from pigments. Thus, the proposed system is validated as a robust and reliable tool for the nutritional assessment of vitamin C in various industrial and agricultural applications. (See Table 5.)

Importantly, this study focuses exclusively on the determination of ascorbic acid, and does not involve paracetamol or any other active pharmaceutical ingredient.

Beyond its statistical robustness, the developed method offers substantial economic and operational advantages. It minimizes reagent consumption, shortens analysis time, and reduces chemical waste, making it highly suitable for routine pharmaceutical quality control. The integration of flow injection analysis with fluorescence detection enhances throughput and automation potential, positioning the method as a cost-effective and environmentally sustainable alternative to conventional techniques.

All validation parameters were assessed following the requirements of ICH Q2(R1) and USP The method demonstrated excellent linearity, accuracy, precision, sensitivity, robustness, and system suitability, confirming its appropriateness for quantitative determination of ascorbic acid in pharmaceutical formulations.

Table 4
Determination and recovery study of ascorbic acid in food and vegetable samples via the proposed method ($n = 3$).

Sample Matrix	Found* (μM)	Added (μM)	Total Found (μM)	Recovery (%)	RSD (%)
Fresh Orange Juice	6.20	5.00	11.16	99.64	0.85
		15.00	21.12	99.62	1.10
Fresh Lemon Juice	4.15	5.00	9.11	99.56	1.02
		15.00	19.22	100.36	0.94
Commercial Juice	9.40	5.00	14.36	99.72	1.18
		15.00	24.52	100.49	0.72
Green Bell Pepper	5.30	10.00	15.24	99.60	1.35
		20.00	25.42	100.47	1.15

* Mean of three determinations.

Table 5
Summary of validation parameters for the spectrofluorimetric determination of ascorbic acid according to ICH Q2(R1) guidelines.

Parameter	Value
Accuracy	Recovery 99.83–100.40%; and %RSD $< 0.5\%$ (based on % Recovery).
Precision	Intra-day RSD $< 1\%$; Inter-day RSD $< 2\%$ ($n = 8$).
Specificity	Specific exclusively to ascorbic acid; no interference detected.
Linearity	0.1–30 μmol/L; r = 0.9983; $R^2 = 99.66\%$.
Range	0.1–30 μmol/L.
Detection Limit (LOD)	0.025 μmol/L.
Quantitation Limit (LOQ)	5.643 μmol/L.
Robustness	Variations in pH ±0.2, serotonin ±5%, flow rate ± 0.1 mL/min had no significant effect.
System Suitability	Baseline RSD $< 1\%$ (n = 8); excellent signal stability.

4. Conclusion

The developed FIA–fluorescence method for ascorbic acid quantification demonstrated outstanding analytical performance, with recovery values exceeding 99% and statistical equivalence to conventional fluorimetry. The system integrates a custom-designed spectral array comprising 16 irradiation sources arranged in a dual-axis matrix—eight aligned horizontally and eight orthogonally—providing multi-angle excitation for enhanced photophysical interaction. Detection is achieved via a twin-pair solar cell sensor configuration, enabling precise signal capture with minimal optical loss. This architecture supports high-throughput modelling with up to 50 samples per hour while significantly reducing reagent consumption, analysis time, and environmental impact. The method's sensitivity, scalability, and operational efficiency position it as a robust and economically viable alternative for routine pharmaceutical and environmental applications.

CRediT authorship contribution statement

Ghufran K. Allawi: Writing – review & editing, Visualization, Validation, Software, Resources, Funding acquisition, Formal analysis, Data curation. **N.S. Turkey:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

The authors affirm that the information/data of this research article is available inside the article and the Supplementary Information.

References

[1] D. Vitamin, "Health Professional Fact Sheet," *Natl. Institutes Heal.*, 2020.
[2] S.J. Devaki, R.L. Raveendran, Vitamin C: Sources, functions, sensing and analysis, in: Vitamin C, IntechOpen, 2017.
[3] A. Meščić Macan, T. Gazivoda Kraljević, S. Raić-Malić, Therapeutic perspective of vitamin C and its derivatives, Antioxidants 8 (8) (2019) 247, https://doi.org/10.3390/antiox8080247.
[4] S. Popescu, T.-Ștefania Chiș, M.G. Chiricuțiță, A.-C. Vârlan, L. Pîrvulescu, A. Velciov, Vitamin C determination in foods. Titrimetric methods-A review, J. Agroaliment. Process. Technol. 30 (4) (2024) 471–476, https://doi.org/10.59463/JAPT.2024.2.49.
[5] S. Raman, et al., Different methods used for determination of vitamin C: a review, Int. J. Curr. Microbiol. Appl. Sci. 12 (9) (2023) 56–66.
[6] A.M. Abass, F.H. Abdulla, Role of analytical chemistry for the determination of ascorbic acid: a review, Plant Arch. 20 (2) (2020) 9067–9072.
[7] A.P. Desai, S. Desai, UV spectroscopic method for determination of vitamin C (ascorbic acid) content in different fruits in South Gujarat region, Int. J. Environ. Sci. Nat. Resour. 22 (2) (2019) 41–44.
[8] A.N. Alene, T.M. Tekaligne, A.T. Habte, Determination of Vitamin C (ascorbic acid) in some fresh fruits: a comparative evaluation of methods, J. Food Process. Technol. 15 (4) (2024) 1115.
[9] T. Wang, H. Luo, X. Jing, J. Yang, M. Huo, Y. Wang, Synthesis of fluorescent carbon dots and their application in ascorbic acid detection, Molecules 26 (5) (2021) 1246.

[10] Y. Matsuoka, M. Yamato, K. Yamada, Fluorescence probe for the convenient and sensitive detection of ascorbic acid, J. Clin. Biochem. Nutr. 58 (1) (2016) 16–22.
[11] S. Sugiarti, N. Darmawan, Synthesis of fluorescence carbon nanoparticles from ascorbic acid, Indones. J. Chem. 15 (2) (2015) 141–145.
[12] J.R. Lakowicz, Quenching of fluorescence, in: Principles of Fluorescence Spectroscopy, Springer, 1983, pp. 257–301.
[13] K. Xing, et al., A turn-on fluorescent probe for sensitive detection of ascorbic acid based on SiNP–MnO 2 nanocomposites, New J. Chem. 43 (24) (2019) 9466–9471.
[14] S.H. Nannuri, A.N. Nikam, A. Pandey, S. Mutalik, S.D. George, Subcellular imaging and diagnosis of cancer using engineered nanoparticles, Curr. Pharm. Des. 28 (9) (2022) 690–710.
[15] J. Liu, L. Wang, H. Bao, A novel fluorescent probe for ascorbic acid based on seed-mediated growth of silver nanoparticles quenching of carbon dots fluorescence, Anal. Bioanal. Chem. 411 (4) (2019) 877–883.
[16] S.F. Hameed, N.S. Turkie, A novel approach for study of surface morphology & roughness analysis for characterization of precipitation product at a nanoscale level via the reaction of fluconazole with phosphomolybidic acid, Chem. Methodol. 6 (2022) 385–397.
[17] N.S. Turkey, G. Fadhel, Chlorpromazine-HCl determination via its oxidation with sodium nitrite in sulfanilic acid medium via CFIA technique through long distance chasing photometer NAG-ADF-300-2, J. Med. Chem. Sci. 5 (3) (2022).
[18] O.A. Yassin, N.S. Turkey, New method for estimation Mebeverine hydrochloride drugs preparation by a new analyser: Ayah 6S. X1 (WSLEDs)-T.-two solar cells complied with CFIA, Baghdad Sci. J. 18 (3) (2021) 10, https://doi.org/10.21123/bsj.2021.18.3.0565.
[19] I. M. S. Ali, N. Shakir, and A. A. H. Fares, "Combined CFA-AFM Analytical approach for precipitation reaction regarding crystal growth building single and multiple monolayers based on surface area calculation with image surface roughness analysis (unseen surface)," in *Journal of Physics: Conference Series*, IOP Publishing, 2021, p. 12031. doi:https://doi.org/10.1088/1742-6596/2063/1/012031.
[20] E.N. Mezaal, N.S. Turkie, A Assessment of long distance chasing photometer (NAG-ADF-300-2) by estimating the drug atenolol with ammonium molybdate via continuous flow injection analysis, Baghdad Sci. J. 17 (1) (2020) 78.
[21] N.S. Turkie, G. Khalid, Ciprofloxacin assessment via the formation of precipitate reaction product with ammonium metavanadate as a reagent using ISNAG-fluorimeter, Eurasian Chem. Commun. 4 (1) (2021) 52–66.
[22] M.H. Athab, N.S. AL-Awadi, Continuous flow injection-turbidimetric analysis for determination of cobalt (II) ion in alloys using a microcell with irradiation sources at 0–180° using a single solar cell as a detector, Baghdad Sci. J. 22 (10) (2025) 3239–3251.
[23] A.N. Jasim, A. Kamel, N.S. Al-Awadi, H.F. Abd-Alrazack, Online column preconcentration for speciation and selective determination of Cr (III) in natural water samples using flow injection with chemiluminescence detection, Luminescence 38 (3) (2023) 360–368, https://doi.org/10.1002/bio.4464.
[24] B.S. Mohammed, N.S. Turkey, Utilizing an atomic force microscopy with continuous flow injection analysis using NAG-4 (sources) x3 with three solar cells (NAG-4SX3-3D) analyzer for studying the surface morphology of the precipitate of the cyproheptadine-HCl and loratadine, Baghdad Sci. J. 22 (6) (2025) 1801–1814.
[25] I. M. A. Shakir and N. S. T. Al-Awadie, "Photometric instrument for excited fluorescence, attenuation and to measure the interference effects at the same wavelength using adjacent solar cells," Patent No. 6615., 2021.
[26] G.K. Allawi, N.S. Turkey, Development of a multi-source blue light irradiation platform for photometric quantification of ascorbic acid via continuous absorbance quenching of the ferric–thiocyanate complex, RSC Adv. 15 (2025) 40655–40665, https://doi.org/10.1039/d5ra07160g.
[27] G.K. Allawi, N.S. Turkey, A dual-angle optoelectronic flow injection platform for ferrous ion (Fe 2+) determination using blue LED excitation and solar cell detection, RSC Adv. 16 (4) (2026) 3314–3324.
[28] A. Jain, R. Jain, S. Jain, Basic Techniques in Biochemistry, Microbiology and Molecular Biology, Springer, New York, US, 2020.
[29] T.C. McIlvaine, A buffer solution for colorimetric comparison, J. Biol. Chem. 49 (1) (1921) 183–186.
[30] H.-L. Liu, K. Zhan, K.-L. Zhong, X.-L. Chen, X.-H. Xia, A novel indole derivative with superior photophysical performance for fluorescent probe, pH-sensing, and logic gates, Int. J. Mol. Sci. 24 (2) (2023) 1711.
[31] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3 ed, Springer, Boston, MA, 2006.
[32] G.A. Hernández-Mendoza, et al., Fluorescence of serotonin in the visible spectrum upon multiphotonic photoconversion, Biomed. Opt. Express 11 (3) (2020) 1432–1448.
[33] S. Lehrer, Solute perturbation of protein fluorescence. Quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion, Biochemistry 10 (17) (1971) 3254–3263.
[34] B. Valeur, M.N. Berberan-Santos, Molecular Fluorescence: Principles and Applications, John Wiley & Sons, 2013.
[35] A. de Juan, S. Mas, M. Maeder, R. Tauler, A perspective on modeling evolution, J. Chemometr. 34 (7) (2020) e3205.
[36] A. Savych, S. Marchyshyn, L. Mosula, L. Kryskiw, Spectrophotometric determination of L-ascorbic acid in the herbal antidiabetic mixtures, Development 26 (27) (2021) 28–29.
[37] J. Song, X. Guo, H. Chen, Y. Tang, L. Han, Ascorbic acid-caused quenching effect of protein clusteroluminescence probe: the fast fluorescent detection of ascorbic acid in vegetables, Molecules 28 (5) (2023) 2162.
[38] N.R. Ahmed, High performance liquid chromatographic method for determination of ascorbic acid in pure form, pharmaceutical formulations, biological samples and in environmental water samples, Al Mustansiriyah J. Pharm. Sci. 14 (1) (2014) 92–98.