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Nature's touch in the laboratory: eco-friendly and green dual-mode UV-vis and smartphone RGB analysis of procaine in pharmaceuticals using a non-heated aqueous matcha green tea extract

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Using the principles of green chemistry, a new, simple, rapid, and eco-friendly dual-mode system for procaine detection in pharmaceutical samples was developed in this study. This system relied on the diazotization of procaine, followed by a coupling reaction with catechin-rich molecules present in a non-heated matcha green tea extract (MTE) in an acidic medium containing nitrite. As the concentration of procaine increased, the resulting yellow azo dye became visually more intense, which could be monitored using UV-vis spectrophotometry (λ = 412 nm) and smartphone-assisted RGB analysis (only *B* values varied; *R* and *G* values were constant). The spectrophotometric method allowed the detection of procaine as low as 0.33 mg L^{-1}, with a working concentration range between 1.0 and 8.0 mg L^{-1}. Alternatively, the smartphone-assisted method exhibited linearity over the 2.0–10.0 mg L^{-1} range, with a minimum detectable concentration of 0.67 mg L^{-1}. The performance of the dual-mode system was confirmed by quantifying a real procaine sample, with the testing results showing good agreement with the results obtained using the reference spectrophotometric method. The influences of common ions were investigated, and Na_2EDTA was found to offer an easy method for eliminating Fe^{3+} interference. The total phenolic content of MTE was quantitatively determined using a Folin–Ciocalteu procedure to standardize the MTE.

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1. Introduction

Procaine, also known as 2-dietylaminoethyl *p*-aminobenzoate,[1] commonly marketed as Novocain, belongs to one of the oldest locally acting anesthetics and is used in its hydrochloride form.[2] This ester-type local anesthetic compound was discovered first by Alfred Einhorn in 1950 and consists of 4-aminobenzoic acid.[3,4] It functions as an organic compound and acts widely as an anesthetic for nerves locally and regionally, temporarily inhibiting the flow of neural signals that carry pain perception. It acts as an injection anesthetic and anesthetizes locally on mucous membranes.[4,5] Because of its ability to restrict blood arteries, this drug is used to decrease bleeding.[5] Additionally, due to its chemical structure, it exhibits notable allergic potential.[6] Procaine hydrochloride (procaine) has been detected in pharmaceutical products and biological samples using a variety of techniques, such as high-performance liquid chromatography (HPLC),[7] liquid chromatography–mass spectrometry (LC–MS/MS),[8] gas chromatography (GC),[9] chemiluminescence (CL) detection,[10] fluorimetry,[11] potentiometry,[12] and voltammetry.[13] Although most of these approaches are highly accurate, they are typically expensive, complex, and time-consuming, often require pre-treatments, and need professional operators trained to handle specific techniques.[14] As a versatile and effective alternative method for detecting the target analyte concentration, spectrophotometry offers several benefits, including low cost, simple use, quick analysis, reliability, high sensitivity at low concentrations, and a broad analytical working range.[15] However, it has been reported by Abdulwahab *et al.* that spectrophotometric techniques offer not only practical but also significant environmental advantages. Especially in pharmaceutical quality control applications, a mathematical operation-supported UV-spectrophotometry method is a much more eco-friendly alternative compared to the traditional HPLC-UV method. From a green analytical chemistry perspective, however, UV-spectrophotometry was found to be superior to HPLC-UV, reducing organic solvent spending by more than 20 times, energy consumption by about 10 times, and analysis time by approximately 4 times. This makes it a safer alternative to take into consideration.[16]

The development of smartphone technology in recent years has made them accessible and adaptable platforms for a variety of uses, such as chemical analysis.[17] In the pure sciences, the smartphone has been thoroughly studied. Smartphone-based approaches are beneficial for limited locations and funds because of their affordability, portability, and low energy

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consumption. Studying everything from trace elements to big molecules has been made easier by smartphones with cameras that can detect color changes in reactions within the visible spectrum. The concentration of the target analyte under investigation influences the color analysis.[17,18] Colorimetric methods based on smartphones have potential applications in several fields, including bioanalytics,[19] agricultural analytics,[20] food analysis,[21] environmental monitoring,[22] and health monitoring.[23] The basis for the smartphone analysis of diverse sample types is the detection of color channel (RGB) data using a variety of software programs.[24] Analytical labs are unquestionably crucial to environmental protection since they analyze contaminants in the air, water, and soil. However, studies require a considerable amount of reagents and solvents, leading to the generation of dangerous residue. These factors led to the introduction of green analytical chemistry (GAC) to minimize or eliminate the negative environmental and operator consequences of analytical procedures.[25] Using renewable source reagents instead of toxic synthetic chemicals to determine any analyte concentration is one of the options to create methods that are eco-friendly and in line with green analytical chemistry principles.[26] There have been reports of analytical methods utilizing naturally found plant-sourced reagents to detect analytes.[26–30]

There are numerous varieties of green tea available, including loose leaves, teabags, and powder.[31] Matcha is a powdered form of the tencha variety of Japanese green tea (*Camellia sinensis*).[32] Green teas are often cultivated in direct sunlight, but matcha is grown in 90% shade.[33] The amount of catechins accessible from matcha is therefore expected to differ from catechin levels from other green teas since sunlight alters the composition and concentrations of catechins in tea leaves.[33,34] Green tea contains four primary catechins: (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG). Among these, EGCG is the most abundant and biologically active compound. In particular, matcha provides the highest concentration of these catechins.[32] The findings show that the amount of EGCG found in matcha is 137 times more than that found in China Green Tips green tea and at least three times higher than the highest value reported in the literature for other green teas.[33] In this study, considering all this information, it was aimed to develop a novel and green dual-mode analytical approach for the analysis of procaine in pharmaceutical samples, based on the principles of green chemistry. In this context, a spectrophotometric and smartphone-assisted colorimetric eco-green analytical method was developed based on the diazo-coupling reaction of catechins found in the non-heated aqueous matcha green tea extract (MTE) in the presence of nitrite and procaine in an acidic medium. To the best of current knowledge, the use of a non-heated aqueous matcha green tea extract (MTE), naturally rich in catechins, as an eco-friendly diazo-coupling reagent in combination with nitrite under acidic conditions may represent the first reported application for the determination of procaine in pharmaceuticals. The developed spectrophotometric and smartphone-assisted methods demonstrate a broad working range and reliable selectivity, making them suitable for the accurate quantitative analysis of procaine. Furthermore, the smartphone-assisted method allows for data analysis, making the proposed method portable, user-friendly, highly convenient for field research, and accessible to a wide audience. In contrast to current research, MTE has the benefit of being more affordable and accessible from any local market. In contrast, employing MTE rich in catechins as a coupling reagent for procaine quantification satisfies at least four green analytical chemistry principles,[35] including eliminating or substituting toxic reagents, using reagents sourced from renewable resources, reducing energy consumption, and lowering operator risk because of its nontoxicity.

2. Materials and methods

2.1. Chemicals and apparatus

In this work, all analytical-grade compounds were utilized. The MTE was bought in a market. Procaine hydrochloride (Procaine) was obtained from Galenik. HCl, $NaNO_2$, $Al_2(SO_4)_3 \cdot 18H_2O$, Na_2EDTA, and ethanol were provided by Sigma-Aldrich. Folin–Ciocalteu's reagent (FC), NaF, $Fe(NO_3)_3 \cdot 9H_2O$, H_2SO_4, Na_2SO_4, $FeCl_2 \cdot 4H_2O$, Na_2CO_3, $NaNO_3$, Na_2SO_4, $Ca(NO_3)_2 \cdot 4H_2O$, KCl, NaCl, $MnSO_4 \cdot H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA·2HCl), H_3PO_4, CH_3COOH, HNO_3, methanol, and gallic acid anhydrous (GA) were obtained from Merck.

Each chemical was weighed using a Precisa XB 220A Analytical Balance. Every absorbance measurement was carried out with the Rayleigh VIS-723G visible spectrophotometer and its 10 mm optically thick glass cuvettes. The Samsung Galaxy S10 Plus (16 MP ultra-wide camera, a 12 MP ultrawide-angle, and a 12 MP telephoto) was utilized with its handy black box, which fitted the phone, for RGB measurements. The Glassco 710 DNAG hot plate was used to stir the matcha green tea solution.

2.2. Preparation of solutions

The preparation of the MTE involved mixing 0.1 g of matcha green tea powder with 50 mL of ultrapure water in a beaker. The mixture was stirred at ambient temperature for roughly 20 min at a speed of 400 rpm. Afterward, it was centrifuged at 5000 rpm for 1 min. The clear supernatant was then separated by decantation, and a freshly prepared solution was used on the same day. For the reagent preparations, procaine and NEDA·2HCl were each dissolved in water to obtain working concentrations of 1000 mg L^{-1}. A concentrated metal salt solution (10 mg mL^{-1}) was also prepared using ultrapure water. GA was dissolved in methanol to reach a concentration of 1000 mg L^{-1}. All prepared stock solutions, including GA, were subsequently diluted with ultrapure water as required and kept refrigerated at 4 °C.

2.3. Folin–Ciocalteu-based total polyphenol determination in MTE

The total phenolic content of the 0.2% MTE (w/v) was estimated using the Folin–Ciocalteu (FC) method[36] because the amount of

phenolic content in the MTE as a coupling reagent is important for detecting procaine.

A milliliter of the sample (standard GA solution or ten-fold diluted 0.2% MTE), 0.1 milliliter of Folin–Ciocalteu's reagent, and 0.9 milliliter of water were added to test tubes. For five minutes, the tubes were left to stand. After this time, 0.4 mL of water and 1 mL of sodium carbonate (7%, w/v) were added, and another 30 min were given to stabilize the blue color that had developed. At 765 nm, the absorbance was measured; every determination was performed in triplicate. The GA equivalent was used to express the data.

2.4. Spectrophotometric and smartphone-assisted determination of procaine

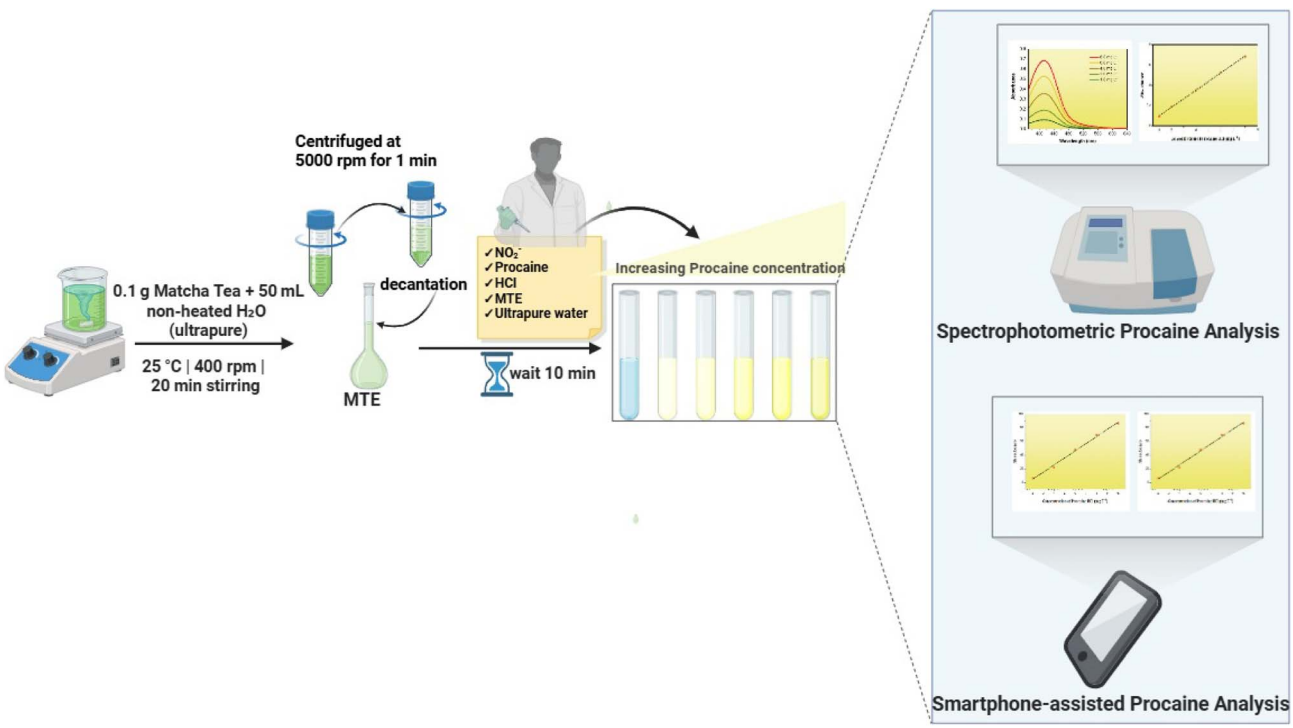
In a typical procedure, 0.1 mL of nitrite solution (30 mg L^{-1}), 0.1 mL of procaine at different concentrations (ranging from 10 to 80 mg L^{-1}), and 0.1 mL of HCl (0.5 mol L^{-1}) were sequentially transferred into a test tube. Following this, 0.4 mL of the MTE was introduced. The final volume was brought up to 1.0 mL using ultrapure water. The reaction mixture was left to stand at room temperature (rt) for approximately 10 min. Absorbance was subsequently recorded at 412 nm, with a blank sample utilized as the reference.

RGB values for smartphone-assisted colorimetric analysis of procaine were recorded following previously reported studies.[37–39] The original black box (16.5 × 8 × 3 cm^3) in which the mobile phone was sold was used for the RGB analysis. In the process of acquiring RGB data, the smartphone was positioned on the open top of the measurement box, thereby ensuring that the box was sealed and a closed, dark environment was created. The smartphone flash was used as the only light source, ensuring that the data acquired was independent of ambient light. The PhotoMetrix PRO application was downloaded (free) from the Google Play Store for recording RGB values. A region of interest with a size of 96 × 96 pixels was chosen using the flash mode of the software to view and collect the *B* values of the samples within the region of interest. The camera settings of the software were adjusted using the flash mode, exposure value of 0, focus mode set to infinity, white balance adjusted to automatic, and a resolution of 640 × 480 pixels for measurement.

Because high-capacity glass vials (2 mL) were used instead of quartz cuvettes, the same process was followed for the smartphone-assisted colorimetric analysis to detect procaine (2.0–10.0 mg L^{-1} initial concentration range), but two times as much reagent volume was added, as long as the reagent concentrations were the same. When the suggested procedure was applied to the samples, it was found that the adsorption values remained unchanged. Scheme 1 illustrates the preparation step of the proposed method for detecting procaine, utilizing either spectrophotometric or smartphone-assisted analyses.

2.5. Interference analysis

The impact of often-present ions (F^-, Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}, K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, and Al^{3+}) on the analysis of 6.0 mg L^{-1} of procaine was the subject of a thorough investigation for calculating recoveries (%). First, each potentially interfering ion was tested at a concentration 50 times higher than that of the procaine using both the spectrophotometric and smartphone-assisted methods. In the event of interference,



Scheme 1 Schematic representation of dual-mode methods recommended for procaine analysis.

the ratio was gradually decreased until it stopped. It was easy to eliminate the Fe^{3+} interference effect using Na_2EDTA.

2.6. Real sample analysis

In the context of the real sample application, the methods were applied on (i) samples of two different batch numbers of procaine injectable solution obtained from the pharmacy and (ii) a simulated procaine HCl-glycerin topical solution prepared in the laboratory according to the recipe in the magistral guide.[40] The values for procaine recovery (%) and RSD (%) were then estimated.

2.7. Validation of the recommended analytical methods

The recommended methods were validated using the spectrophotometric reference method.[41] The *t*- and *F*-test results obtained from both the recommended and reference spectrophotometric methods were used to determine the confidence level. Microsoft Excel 2024 was used to calculate the means and their standard errors.

3. Results and discussion

3.1. Optimization of the detection system

Three consecutive experiments ($N = 3$) were carried out to obtain the figures that indicated the optimization of all parameters.

In the recommended method for the determination of procaine, HCl, H_2SO_4, HNO_3, H_3PO_4, and CH_3COOH acids, each with the same initial concentration (0.5 mol L^{-1}), were utilized to find the appropriate acid to maximize the reaction efficiency.

In a typical procedure, 0.1 mL of nitrite solution (30 mg L^{-1}), 0.1 mL of procaine (60.0 mg L^{-1}), and 0.1 mL of different types of acids (0.5 mol L^{-1}) were sequentially transferred into a test tube. Following this, 0.4 mL of the MTE was introduced. The final volume was brought up to 1.0 mL using ultrapure water. The reaction mixture was left to stand at rt for approximately 10 min. Absorbance was subsequently recorded at 412 nm, with a blank sample utilized as the reference. As observed in Fig. 1A, the highest absorbance was observed for HCl and was therefore selected as the most suitable acid.

After determining the most suitable acid type, HCl, at the initial concentration range of 0.25–12 mol L^{-1}, was applied in the recommended method mentioned above to find the optimal concentration. When looking at Fig. 1B, it was observed that the absorbance remained constant at concentrations of 0.5 mol L^{-1} HCl and above, so 0.5 mol L^{-1} HCl was selected as the suitable concentration.

The recommended method was used to find the ideal volume of MTE using a volume range of 0.1–0.7 mL of 0.2% MTE solution added. The maximum absorbance was found at 0.4 mL of MTE, as illustrated in Fig. 1C. The ideal volume of MTE was then determined to be 0.4 mL.

In a typical procedure, 0.1 mL of nitrite solution (30 mg L^{-1}), 0.1 mL of procaine (60.0 mg L^{-1}), and 0.1 mL of HCl (0.5 mol L^{-1}) were sequentially transferred into a test tube. Following this, any volume (0.1–0.7 mL) of the MTE was introduced. The final volume was brought up to 1.0 mL using ultrapure water. The reaction mixture was left to stand at rt for approximately 10 min. Absorbance was subsequently recorded at 412 nm, with a blank sample utilized as the reference.

The recommended method was applied by adding NO_2^- in the initial concentration range of 10.0–100.0 mg L^{-1} separately to procaine to find the optimal concentration of NO_2^-. In a typical procedure, 0.1 mL of nitrite solution at different concentrations (ranging from 10.0 to 100.0 mg L^{-1}), 0.1 mL of procaine (60.0 mg L^{-1}), and 0.1 mL of HCl (0.5 mol L^{-1}) were sequentially transferred into a test tube. Following this, 0.4 mL of the MTE was introduced. The final volume was brought up to 1.0 mL using ultrapure water. The reaction mixture was left to stand at rt for approximately 10 min. Absorbance was subsequently recorded at 412 nm, with a blank sample utilized as the reference. As observed in Fig. 1D, 30 mg L^{-1} NO_2^- was selected as the ideal initial concentration since the absorbance remained constant at 30 mg L^{-1} NO_2^- concentration and above.

By analyzing the final procaine concentrations at 6.0 mg L^{-1} for each time interval, the ideal time was established. At 412 nm, the mixed solutions were left independently for various durations, ranging from 1 to 30 min at rt. Then, absorbance readings were taken at 412 nm, using the blank sample as a reference. The ideal time of the diazo-coupling reaction was found to be 10 min, as shown in Fig. 1E.

Here is a brief description of the recommended method: to the test tube, 0.1 mL of 30 mg L^{-1} NO_2^-, 60 mg L^{-1} procaine, (0.5 mol L^{-1}) HCl, and 0.4 mL of MTE were added. The solution was adjusted to 1.0 mL using distilled water and then kept at rt for different time intervals. Then, absorbance readings were taken at 412 nm using the blank sample as a reference.

The recommended method was used on a final concentration of 6.0 mg L^{-1} procaine at temperatures ranging from 25 to 80 °C.

In a typical procedure, 0.1 mL of nitrite solution (30 mg L^{-1}), 0.1 mL of procaine (60.0 mg L^{-1}), and 0.1 mL of HCl (0.5 mol L^{-1}) were sequentially transferred into a test tube. Following this, 0.4 mL of the MTE was introduced. The final volume was brought up to 1.0 mL using ultrapure water. The reaction mixture was left to stand at different temperatures for approximately 10 min. Absorbance was subsequently recorded at 412 nm, with a blank sample utilized as the reference. Since the absorbances were found to be roughly constant as the temperature increased, 25 °C was determined to be the most appropriate temperature, as shown in Fig. 1F.

For RGB-based analytical signal evaluation, the recommended method was applied to procaine solutions of different concentrations. It was found that the *R* and *G* values remained approximately constant under ideal conditions, and the *B* value varied linearly with the final procaine concentration in the range of 2.0 to 10.0 mg L^{-1} (Fig. 1G). Thus, the *B* values were selected and used for the calibration graph.

Besides determining the optimal experimental conditions, the optimization experiments also offer evidence for the robustness of the designed detection system. As illustrated in Fig. 1(B, E, and F), small variations of the optimized reaction time, acid concentration, and temperature did not lead to

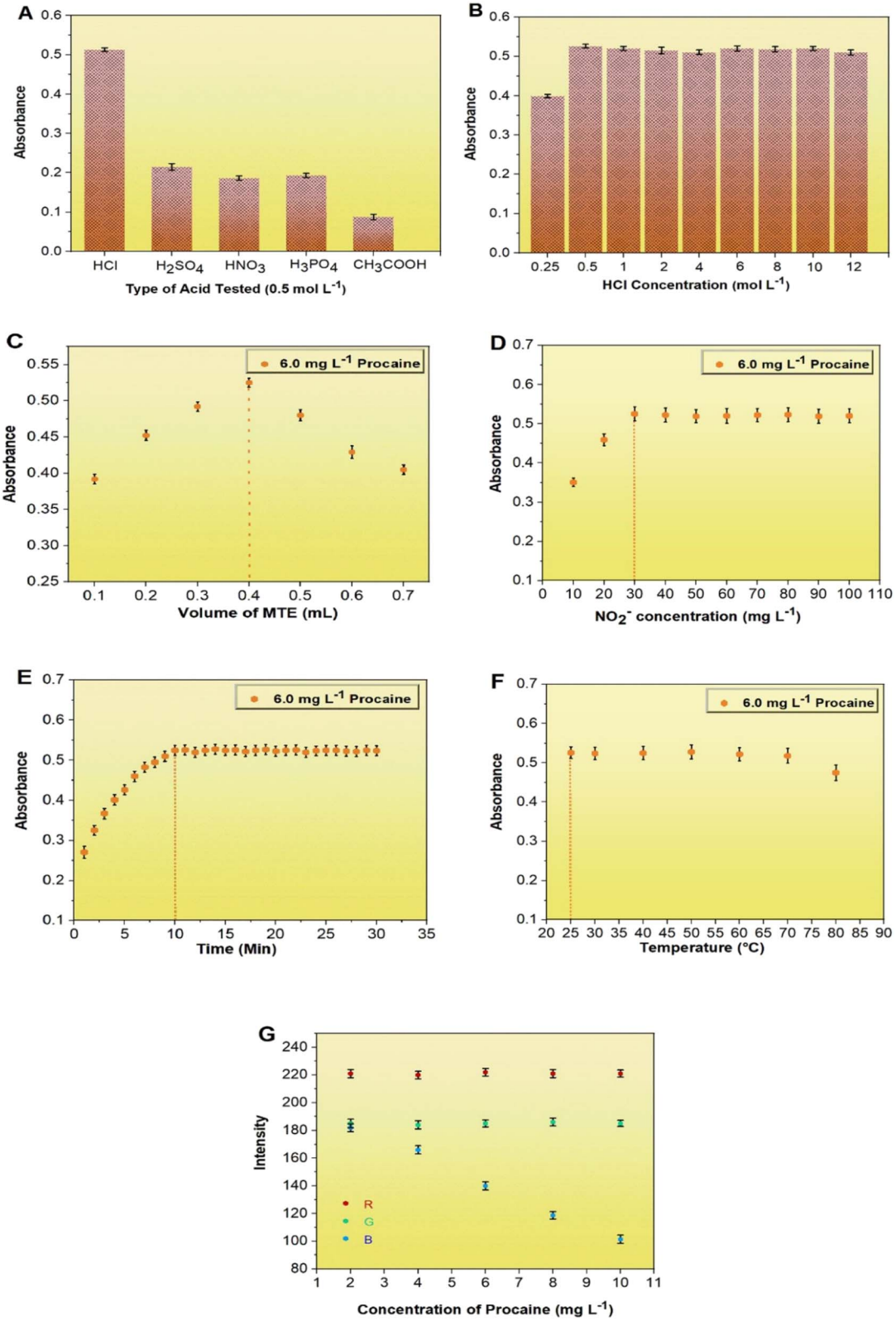


Fig. 1 Optimization of the experimental conditions for the absorbance-based determination of procaine. (A) Effect of different acids (HCl, H_2SO_4, HNO_3, H_3PO_4, and CH_3COOH) at a concentration of 0.05 mol L^{-1} on absorbance. (B) Effect of HCl concentration on absorbance. (C) Effect of the MTE volume on absorbance at a procaine concentration of 6.0 mg L^{-1}. (D) Effect of the NO_2^- concentration on absorbance at a procaine concentration of 6.0 mg L^{-1}. (E) Effect of the reaction time on absorbance at a procaine concentration of 6.0 mg L^{-1}. (F) Effect of temperature on absorbance at a procaine concentration of 6.0 mg L^{-1}. (G) Variation of RGB color intensity values as a function of procaine concentration.

a significant difference in absorbance, and thus, the method is robust against small variations of experimental conditions.

3.2. Folin–Ciocalteu-based total polyphenol determination in MTE

Using the FC method, a calibration curve was developed for assessing the total polyphenol content in the 0.2% MTE (w/v).[36] A variety of GA standard solutions with final concentrations varying from 1.5 to 7.5 mg L^{-1} were used for evaluation.

$$\text{Absorbance (765 nm)} = (3.30 \times 10^{-2} \pm 1.41 \times 10^{-3})\, C + (1.13 \times 10^{-1} \pm 1.021 \times 10^{-2}) \quad (N = 5,\ r = 0.9964)$$

The GA standard solution (in mg L^{-1}) at the final concentration is denoted by C.

A 10-fold diluted 0.2% MTE was tested according to the FC method, and the total polyphenol concentration was measured to be 93.50 ± 0.47 mg GAE per g sample.

3.3. Investigation of the reaction mechanism and working principle of the recommended method

The azo dye solution synthesized from procaine solution at an initial concentration of 60.0 mg L^{-1} was dropped onto the watch glass and allowed to dry at rt; similarly, a 0.2% MTE solution was dropped onto the another watch glass and allowed to dry at rt. The FTIR-ATR spectra of the dried samples and the standard procaine were then measured.

According to the FT-IR spectra of procaine, characteristic peaks were shown at 3351 and 3308 cm^{-1} (–N–H stretching of NH_2), 3050 and 2982 cm^{-1} (–C–H stretching of $–CH_2$), 2586 and 2488 cm^{-1} (–N–H stretching), and 1694 cm^{-1} (–C═O, carbonyl group).[42,43] In the spectrum of the MTE, the intense bands at 3134 and 3038 cm^{-1} indicate the presence of the –OH group.[44] The observed peaks at 2920 and 2807 cm^{-1} are related to the symmetric and antisymmetric C–H stretching of the CH_2 groups, respectively,[45] while the peak at 1696 cm^{-1} is associated with carbonyl (C═O) ester.[46,47] The vibrational movements of C═C groups in the aromatic rings mainly occupy the range between 1644 and 1400 cm^{-1}.[45] The observed band at 1070 cm^{-1} is related to the C–O–C group.[44] An analysis of the FT-IR spectra of the azo dye (Fig. 2A) reveals that the azo dye is synthesized when the NH_2 (–N–H stretching) vibration peaks at 3351 and 3308 cm^{-1} disappear and the –N═N– vibration peak forms at 1501 cm^{-1}.[48,49]

As illustrated in Fig. 2B, the highest absorbance at 412 nm was recorded in mixture (a), which consisted of (as final concentration) 3.0 mg L^{-1} NO_2^-, 6.0 mg L^{-1} procaine, 0.05 mol L^{-1} HCl, and 0.08% MTE. In comparison, no absorbance was observed at the same wavelength in mixture (b), composed of 3.0 mg L^{-1} NO_2^-, 6.0 mg L^{-1} procaine, and 0.05 mol L^{-1} HCl, indicating that procaine alone does not undergo diazotization under acidic conditions. In addition, no noticeable absorption at 412 nm was observed in mixture (c), which included (as final concentration) 3.0 mg L^{-1} NO_2^-, 0.05 mol L^{-1} HCl, and 0.08% MTE. This absence suggested that NO_2^- did not undergo a nitrosation reaction with the catechin-based compounds present in the MTE. It was clear from combining all of the results that a diazo-coupling reaction, rather than diazotization or nitrosation reactions, was responsible for the color change from nearly colorless to dark yellow. Scheme 2 presents a schematic of the proposed mechanism[37,50,51] (due to the complexity of the polyphenolic content in MTE, catechin is illustrated as a representative structure).

3.4. Analytical parameters

Since the recommended method was carried out under ideal conditions for procaine at various concentrations, it was observed that the color changed from light yellow to dark yellow, depending on the procaine concentration, as a result of

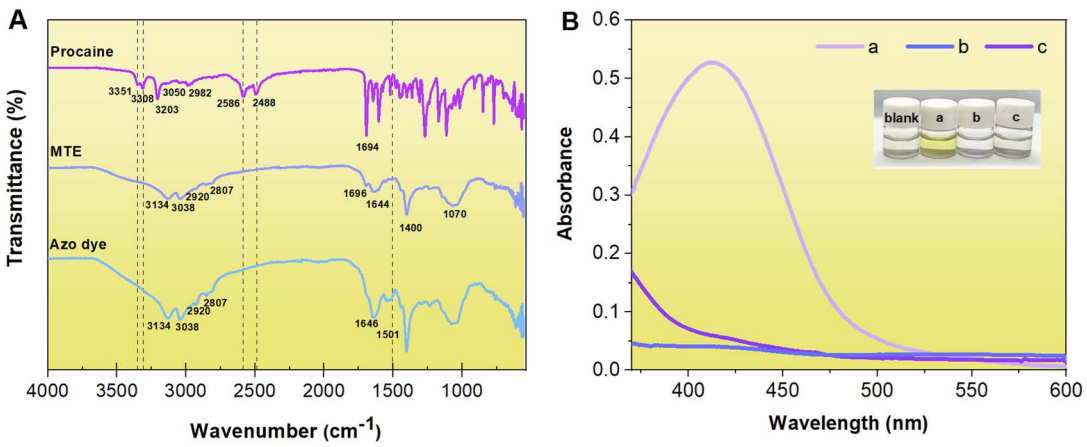
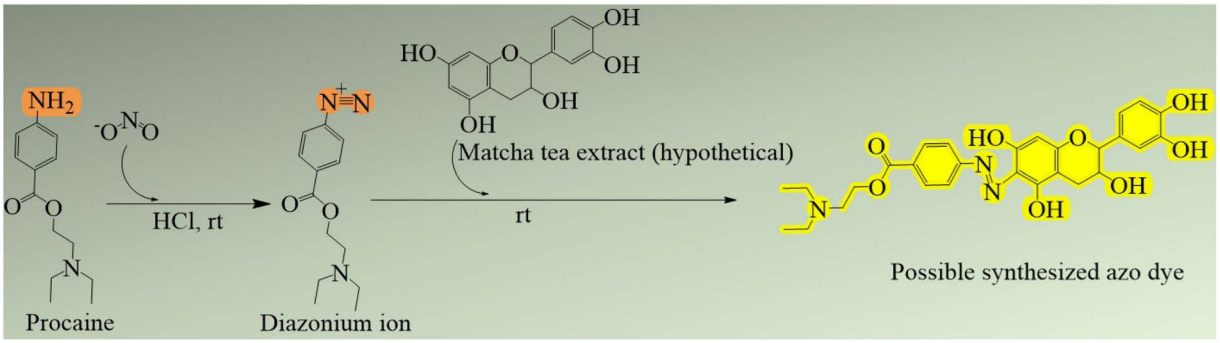


Fig. 2 (A) FTIR-ATR spectra of procaine, MTE, and azo-dye (dried at room temperature). (B) Spectra of the color differences of the mixtures, confirming the diazo-coupling reaction. The inset shows the visual color change for: (a) the mixture of 3.0 mg L^{-1} NO_2^-, 6.0 mg L^{-1} procaine, 0.05 mol L^{-1} HCl, and 0.08% MTE; (b) 3.0 mg L^{-1} NO_2^-, 6.0 mg L^{-1} procaine, and 0.05 mol L^{-1} HCl; and (c) 3.0 mg L^{-1} NO_2^-, 0.05 mol L^{-1} HCl, and 0.08% MTE. Absorbance measurements were performed using aqueous solutions prepared in ultrapure water under the optimized experimental conditions.



Scheme 2 Probable diazo-coupling reaction mechanism for the analysis of procaine. The reaction between the procaine-derived diazonium ion and the polyphenolic compounds in MTE (represented by catechin) results in the formation of a colored azo-dye.

the diazotization and coupling reaction, with maximum absorbance occurring at 412 nm.

Equation for the linear calibration of procaine:

$$A_{412\ \mathrm{nm}} = (8.42 \times 10^{-2} \pm 1.25 \times 10^{-3})\ C + (1.52 \times 10^{-2} \pm 6.17 \times 10^{-3})\ (N = 5,\ r = 0.9995)$$

In the context of the current analysis, the final level of procaine (mg L^{-1}) is represented as C.

A linear relationship was observed between absorbance and concentration in the range of 1.0–8.0 mg L^{-1} (Fig. 3A and B). Additionally, 0.33 mg L^{-1} and 1.0 mg L^{-1} were the values for LOD and LOQ, respectively. For the spectrophotometric method, the expressions LOD = $3\sigma_{bl}/m$ and LOQ = $10\sigma_{bl}/m$ were used to determine the limits of detection and quantification (mg L^{-1}), respectively, where m is the slope of the calibration curve, and σ_{bl} is the standard deviation of the blank solution (N = 5). The coefficients of variation (CVs) for within-day (three

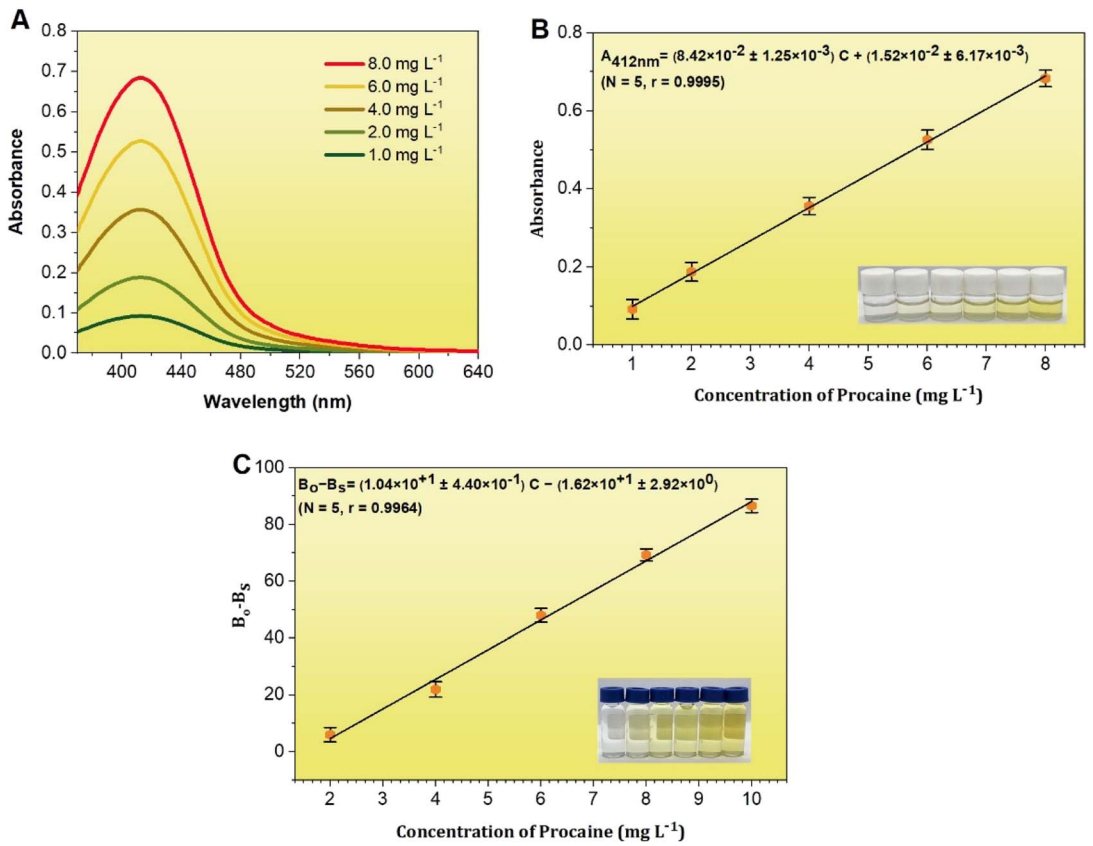


Fig. 3 (A) Matching solution colors of the UV-vis absorption spectra of the recommended-method sensing system with varying procaine concentrations, (B) calibration response for the detection of procaine concentrations ranging from 1.0 to 8.0 mg L^{-1} (inset: the photograph of procaine at different concentrations for calibration), (C) with an inset photo showing a broad range of procaine concentrations, the calibration equation and calibration plot were created using the B values for the smartphone-assisted method.

time points) and between-day (three consecutive days) computations of procaine were found to be 1.09% and 1.31%, respectively, based on five replicate measurements ($N = 5$) for the evaluation of precision. It was possible to generate a calibration plot that linearly correlated B values with procaine concentrations ranging from 2.0 to 10.0 mg L^{-1} in smartphone-assisted measurements (Fig. 3C) because of the gradual increase in the solution color, which changed from pale yellow to deep yellow. It was clear from the study that while the B value varied as the quantity of procaine increased, the R and G values approximately remained constant. The calibration curve was constructed by subtracting the B values of the samples (B_s) containing procaine from the B value of the blank solution (B_o) to take the analytical signal, as per the literature, using the channel (RGB) subtracting method.[52,53]

The equation for the linear calibration of procaine provided using a smartphone is as follows:

$$B_o - B_s = (1.04 \times 10^{+1} \pm 4.40 \times 10^{-1})\ C - (1.62 \times 10^{+1} \pm 2.92 \times 10^{0})\ (N = 5, r = 0.9964)$$

In the context of the current analysis, the final level of procaine (mg L^{-1}) is represented as C.

LOD and LOQ values were calculated as 0.67 and 2.0 mg L^{-1}, respectively, as reported in the recommended spectrophotometric method. For the smartphone-assisted method, the expressions LOD = $3\sigma_{bl}/m$ and LOQ = $10\sigma_{bl}/m$ were used to determine the limits of detection and quantification (mg L^{-1}), respectively, where m is the slope of the calibration curve, and σ_{bl} is the standard deviation of the blank signal (B_o, $N = 5$).

The coefficients of variation (CVs) for within-day (three time points) and between-day (three consecutive days) computations of procaine were found to be 1.45% and 1.78%, respectively, based on five replicate measurements ($N = 5$) for the evaluation of precision. The recommended spectrophotometric and smartphone-assisted methods showed good precision according to the results of the coefficients of variation (CVs) of within-day and between-day.

3.5. Method comparison for procaine detection

The spectrophotometric and smartphone-assisted colorimetric methods developed in this study were compared with different analytical methods reported in the literature (Table 1). The spectrophotometric method based on MTE obtained without heating, as developed in this study, offers a sensitivity comparable to those of some literature methods, with a LOD value of 0.33 mg L^{-1}. In addition, these methods can be considered an eco-friendly and sustainable analysis option without the use of toxic reagents. The smartphone-assisted colorimetric method developed is applicable in the field with an LOD value of 0.67 mg L^{-1}, especially due to its portability, low cost, and rapid application advantages; additionally, it exhibits better or similar performance than some classical spectrophotometric and potentiometric methods. In conclusion, the methods proposed in this study offer both eco-friendly and user-oriented approaches in the determination of procaine; they attract attention with their competitive LOD values compared to those of some methods reported in the literature, as shown in Table 1.

3.6. Analytical eco-scale for evaluating the recommended analytical method's greenness

The analytical eco-scale has been used to evaluate the greenness of the recommended methods in accordance with the green chemistry principles.[60,61] Penalty points (PPs) are deducted from 100 points to determine the eco-scale. The closer the analytical eco-scale is to 100 points, the more eco-friendly the procedure becomes. Since the effect of hazardous chemicals relies on their amount, authors recommend that the total penalty points (PPs) need to be estimated by multiplying the sub-total PPs for a given amount and hazard.[60] According to the safety data sheet (SDS) report,[62] there are no warnings or danger statements for the commercial 0.1 M (6900 mg L^{-1}) $NaNO_2$ solution. In the recommended methods, only a $NaNO_2$ solution containing 30 mg L^{-1} NO_2^- (0.1 mL) was used, and since this concentration was considerably lower than 0.1 M (6900 mg L^{-1}) for the commercial $NaNO_2$ solution, the hazard level could be accepted as "none". 0.5 M HCl (0.1 mL) was used in this study. Since there is only a "warning" in the SDS information[63] for 0.5 M commercial HCl solution, it falls into the "less severe" category for the GHS and was assigned a hazard penalty score of 1,[60] which was considered a less severe hazard in the analytical eco-scale calculation. Additionally, using just a small amount of this diluted HCl (0.1 mL) reduces any possible risks. In accordance with the analytical eco-scale assessment, the degree of greenness of an analytical method based on

Table 1 Method comparison for procaine detection

Method	Sensing reagent	LOD	Ref.
Spectrophotometry	5,7-Dichloro-4,6-dinitrobenzofuroxan	1.5 μg mL^{-1}	54
Colorimetry	Chromotropic acid	0.9 μmol L^{-1}	55
Flow injection-based spectrophotometry	8-Hydroxyquinoline	0.75 μg mL^{-1}	56
Spectrophotometry	1,2-Naphthoquinone-4-sulfonic acid	0.28 μg mL^{-1}	57
Spectrophotometry	2,5-Dimethylphenol	0.26 μg mL^{-1} (method A) 0.16 μg mL^{-1} (method B)	14
Flow injection-based spectrophotometry	Cerium(IV) sulfate tetrahydrate	0.75 mg L^{-1}	58
Single-sweep polarography	Potassium peroxymonosulfate	6×10^{-6} mol L^{-1}	59
Potentiometry	Procaine (Pr)–tetraphenylborate (TPB) ion pair	3.18×10^{-5} mol L^{-1}	12
Spectrophotometry	Aqueous matcha tea extract	0.33 mg L^{-1}	This assay
Smartphone-based colorimetric		0.67 mg L^{-1}	

Table 2 Calculating the penalty points (PPs) of the analytical eco-scale for procaine analysis

Reagents					
Amount		Hazard level	Sub-total PPs for the amount	Sub-total PPs for the hazard level	Total PPs (amount PP × hazard PP)
NO_2^-	0.1 mL (30 mg L^{-1})	None	1 (<10 mL (g))	0	0
HCl	0.1 mL (0.5 mol L^{-1})	Less severe	1 (<10 mL (g))	1	1
MTE	0.4 mL (0.2% (w/v))	None	1 (<10 mL (g))	0	0
					$\sum$ 1
Instruments					
UV-vis spectrophotometer or smartphone (≤0.1 kWh per sample)					0
Occupational hazard					0
Waste (1–10 mL (g))					3
No waste treatment					3
					$\sum$ 6
					Total penalty points: 7
					Analytical eco-scale total score: 93

the total penalty points is classified as excellent green analysis if its total penalty points are above 75 points, acceptable green analysis if it is above 50 points, and inadequate green analysis if it is below 50 points.[60] The recommended methods are seen as an excellent green analysis because the greenness of the scientific method was 93, as shown in Table 2. These findings made it clear that the recommended method fitted with sustainability, green analytical chemistry, and environmental friendliness requirements.

3.7. Interference analysis

As presented in Table S1, the recommended methods were tested on procaine (6.0 mg L^{-1}) under conditions where potential interferents were introduced at 50-fold mass ratio relative to procaine. Furthermore, as shown in Fig. S1, the recovery (%) values of the procaine acquired by the spectrophotometric and smartphone-assisted methods ranged from 87.55% to 106.95% and 88.43% to 110.65%, respectively. Solely Fe^{3+} exhibited an interfering effect, which was effectively eliminated by utilizing Na_2EDTA as a masking agent (Fe^{3+} : EDTA ratio 1 : 5 (w/w)).

3.8. Analyzing real pharmaceutical samples with the recommended methods

To determine the recovery (%) and RSD (%) values for procaine, pharmaceutical samples including two different batch numbers of injection solutions (referred to as injection A and injection B) and a simulated glycerin-based topical solution (details on the ingredients are provided in Table S2), which is utilized externally as a local anesthetic for mouth sores, especially in children,[40] were subjected to the recommended spectrophotometric and smartphone-assisted methods. Procaine recoveries (%) and RSD% values for the pharmaceutical samples were from 99.10% to 101.0% and 2.70% to 2.90% for the spectrophotometric analysis, respectively, whereas these values for the smartphone-assisted analysis ranged from 97.60% to 98.76% and 3.86% to 4.01% (Table 3). These results proved that the recommended methods could be used to measure the amount of procaine with acceptable precision (RSD% <5%) for pharmaceutical samples.

3.9. Validation of the recommended analytical methods

To determine the calibration equation, working solutions of procaine at concentrations ranging from 2.0 to 12.0 mg L^{-1} were investigated utilizing the spectrophotometric reference method, as detailed in a previous article,[41] as well as three repeated analyses for every standard concentration. According to the following equation, the absorbance readings demonstrated a significant linear dependency on concentration:

$$\text{Absorbance} = (3.30 \times 10^{2-} \pm 1.41 \times 10^{-3})\, C + (1.14 \times 10^{-1} \pm 1.02 \times 10^{-2})\ (N = 5,\ r = 0.9964)$$

Table 3 Results of procaine analysis in pharmaceutical samples

	Spectrophotometric method				Smartphone-assisted method			
Pharmaceutical samples	Labeled (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD% ($N = 3$)	Labeled (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD% ($N = 3$)
Injection A	10 000	9910	99.10	2.70	10 000	9810	98.10	3.92
Injection B	10 000	10 100	101.0	2.90	10 000	9760	97.60	3.86
Simulated glycerin-based topical solution	21 010	20 900	99.48	2.84	21 010	20 750	98.76	4.01

In the context of the current analysis, the final level of procaine (mg L^{-1}) is represented as C.

The precision and accuracy of the results with repeated analyses ($N = 5$) did not significantly differ between the reference method and the recommended methods when applied to commercial procaine injectable solution. The F-test was used to compare the variances, and the t-test was used to compare the population means. In terms of the t- and F-test, 95% confidence levels (nominal 0.05 significance level) were used to validate the results. Statistical parameters of both the recommended and reference methods are shown in Table S3.

4. Conclusions

In this study, rapid, simple, and low-cost methods that can be considered environmentally benign under the studied conditions were developed for the colorimetric determination of procaine, a local anesthetic drug. The main improvement of the proposed methods for the determination of procaine in pharmaceutical samples lies in the replacement of traditional toxic and synthetic diazo-coupling agents with a non-heated aqueous MTE, rich in catechins and easily accessible, thereby reducing chemical hazards and improving the sustainability of the analytical procedures, in line with green chemistry principles. Procaine is typically provided as an injection, and both recommended methods show satisfactory detection limits and range of concentrations for determining procaine in injections and topical solutions with good precision, good recoveries, and quite low (<5.0%) RSD% findings.

In comparison with many existing spectrophotometric methods, the proposed methods offer advantages, such as simple sample handling, low reagent consumption, and the absence of organic solvents and external energy sources, making them suitable for application in both laboratory and field settings. Furthermore, the integration of a smartphone-assisted colorimetric system is an additional improvement that allows for portable, convenient, and on-site analysis without the need for complex equipment. The originality of this work is based on the use of a natural and renewable coupling reagent, coupled with a dual-mode detection strategy, which makes this work a more environmentally friendly alternative to existing approaches for procaine analysis.

Conflicts of interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the findings of this study are included within the article, and additional data are available from the corresponding author upon reasonable request.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6ay00184j.

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