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# Development And Validation Of A Green Spectrophotometric Method For The Determination Of Antiarrhythmic Procainamide In Injection Solution

**Atef M.A. Homoda***

*Micro-analytical Lab., Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt*

**Abstract**

This study aims to present the spectrophotometric method for the determination of procainamide hydrochloride in its dosage form. It is based on the reaction of procainamide with 1,2-naphthoquinone-4-sulfonic acid (NQS) in alkaline environments, and its maximum light absorption wavelength is 460 nm. Calibration curves and linear regression equations were constructed and computed under optimal conditions. The linear relationships between absorbances and drug concentrations were excellent, with correlation coefficients (0.998) in the range of 1-50µg/ml. The detection level is 0.3 µg/mL. The measured recovery rate ranges from 98.0 to 102.5%. We investigated the optimal conditions and the effects of pH, reaction time, solvent, and foreign ions on procainamide hydrochloride determination. The precision was satisfactory, with relative standard deviation values not exceeding 1.7 %. The assay's accuracy was ≥98.2%. This approach is quick and straightforward, and it can be used to determine the amount of procainamide in a solution for injection. The results acquired by this method matched with those obtained from the official United States Pharmacopeia.

**Keywords:** Spectrophotometric; 1,2-naphthoquinone-4-sulfonic acid; procainamide; determination; pharmaceutical quality control; green method

## 1. Introduction

Procainamide is a well-established antiarrhythmic agent that is crucial in pharmacology and clinical therapy for the successful management of many heart disorders [1]. Precise assessment of procainamide concentrations is essential for enhancing the efficacy and safety of pharmacological treatment. The chemical makeup is p-amino-N-[2(diethylamino)ethyl]benzamide monohydrochloride (scheme1).

Given the necessity for rapid and dependable methods to ascertain trace concentrations of procainamide, various analytical techniques have been documented, including electrochemical sensors [2,3], high-performance liquid chromatography (HPLC) [4], micellar chromatography [5], voltammetry [6], spectrofluorimetry [7], and capillary electrophoresis [8], for the analysis of procainamide in pharmaceutical formulations and biological fluids. The approaches, while well acknowledged for analyzing complicated sample matrices, are arduous and time-intensive, both in terms of sample pre-treatment and the analytical determination of the analyte. Typically, the analytical methods for quantifying procainamide in biological fluids involve several sample preparation procedures, predominantly centrifugation, to mitigate the confounding matrix effects of the materials [9-13].

Spectrophotometric methods are among the most widely used techniques and remain highly popular. Spectrophotometric methods are appealing due to the widespread availability of instruments, the simplicity of procedures, and their speed, precision, and accuracy. Developing a simple, rapid, and sensitive spectrophotometric method is highly desirable for routine quality control, especially in the absence of modern and costly equipment like Gas chromatography (GC), high performance liquid chromatography (HPLC), and high-performance thin layer chromatography (HPTLC). Spectrophotometry, a widely employed analytical technique, serves as an essential tool in this endeavor, offering a reliable means for the quantitative determination of procainamide in diverse biological specimens and pharmaceutical preparations. Various spectrophotometric reagents have been utilized in the analysis of procainamide hydrochloride. Chloanilic acid was employed in the spectrophotometric evaluation of PR through charge-transfer complexation [14]. Additionally, periodate was successfully utilized for the quantification of PR in its pharmaceutical formulation [15]. Flow injection analysis has been employed for the quantification of procainamide hydrochloride with ceric sulfate. The reaction happens in the presence of sulfuric acid, and the product's absorbance was measured at 480 nm [16]. A brown

*Corresponding author e-mail: atefmah06@yahoo.com.; (Atef M.A. Homoda).
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hue was generated by the amalgamation of p-benzoquinone and procainamide in the presence of sodium dihydrogen orthophosphate. The absorbance was subsequently measured at 501 nm [17].

The assay was based on the reaction of the procainamide, via their primary amino groups, with 1,2-naphthoquinone-4-sulphonate (NQS) in an alkaline medium, forming orange colored N-substituted naphthoquinone products. The absorbances of the colored reaction products were measured by spectrophotometric method. The proposed method was proven to meet the principles of the green analytical method, with sensitive, and selective method for determination of procainamide in injection solution.

The suggested approach relied on the interaction between procainamide and 1,2 naphthoquinone-4-sulphonate (NQS) in an alkaline medium. This resulted in the formation of orange-hued N-substituted naphthoquinone derivatives. We quantified the absorbances of the colored reaction products utilizing the spectrophotometric technique. The suggested method adheres to the principles of green analytical chemistry, offering a sensitive and selective approach for quantifying procainamide in injectable solutions.

## 2. Experimental (Materials and Methods)

### 2.1. Apparatus

All the spectrophotometric was conducted spectral and absorbance measurements on a Shimadzu UV/VIS Spectrophotometer, Model 2401 (Kyoto, Japan) using 1 cm quartz cells. A combined Ross glass pH electrode (Orion, La Verne, CA, USA) was used to change the pH on a HANNA pH 211 microprocessor pH meter (made in Europe, Romania).

### 2.2. Reagents and solutions

Procainamide hydrochloride (99.89%) was obtained by Tekeda Chemical Industrial Ltd. (Osaka, Japan). Double-distilled water was used throughout the study. We prepared a stock solution of procainamide (1000 ppm) by dissolving an accurately weighed 0.1 g standard sample of procainamide hydrochloride in water, transferring it into a 100 ml standard flask, dilution to the mark with water, and thorough mixing. We prepared a working solution of 100 ppm by diluting it in double-distilled water. NQS (0.15%) We dissolved an accurately weighed 0.15 g sodium 1,2-naphthoquinone-4-sulfonic in water, transferred it into a 100-ml standard flask, diluted it to the mark with water, and mixed it thoroughly. We stored the solution in the dark and stable conditions at room temperature for at least two weeks. Sodium dihydrogen phosphate 0.05M and 0.1M NaOH solutions were prepared. A suitable amount of NaOH was added to Na2HPO4 solution to give a pH of 11, using a pH meter with a glass electrode to give the actual pH.

### 2.3. Procedure

Suitable aliquots of procainamide solution with concentrations ranging from 10 to 500 μg/mL were transferred into a 10 mL measuring flask, followed by 2 mL of pH 11 buffer solution and 1 mL of 1.5% NQS solution in a 10.0 mL standard flask. The solution was allowed at room temperature for 20 minutes before being completed to the mark and tested for absorbance at 460 nm against a reagent blank. The absorbance obtained was drown against procainamide concentration for construction the calibration graph.

### 2.4. Determination of Procainamide in Injection solution

To validate the suggested approach for detecting procainamide in injectable fluid, the procainamide was first discovered in its pure form. Procainamide was determined using the suggested method at concentrations ranging from 1 to 50 μg/mL. In contrast, we used the preconstructed calibration graph to directly quantify procainamide concentration in the injection fluid.

## 3. Results and discussion

### 3.1. Absorption spectrum

Figure 1 presents the measured absorption spectra of the procainamide funder examination using water as a blank. Procainamide exhibited maximum absorption peaks (λmax) at 279 nm. The molar absorptivity (ε) was determined as 1.17×103. Because procainamide's absorption peaks shift greatly toward the blue, it is not always possible to directly test their absorption in the UV region to determine how much is in a dose form. This is due to the possibility of interference by co-extracted excipients. Additionally, their low molar absorptivity may result in poor sensitivity. As a result, the procainamide needed to be derivatized into additional red-shifted light-absorbing derivatives. NQS interacted with procainamide to derivatize it, producing orange-colored compounds. The reaction brought the procainamide and the NQS reagent together via their main amino groups. Schematic 1 depicts the reaction. We compared the absorption spectra of the reaction mixtures to reagent blanks (Figure 1). The products had an orange hue with a λmax of 460 nm. Procainamide considerably redshifted the max of the procainamide-NQS derivatives by 279 nm, removing possible interferences. Furthermore, we significantly increased the values of molar absorptivity (ε) to 3×104, making the assay extremely sensitive. As a result, we did all following measurements at 460 nm. Reports show that NQS can react with the amino groups of primary and secondary amine derivatives [18-20]. The principal amino group of procainamides, due to the nucleophilicity of the lone pair of nitrogen atoms, tends to attack the electron-deficient center on NQS in an alkaline media, creating an orange-colored N-substituted naphthoquinone product (scheme 1).

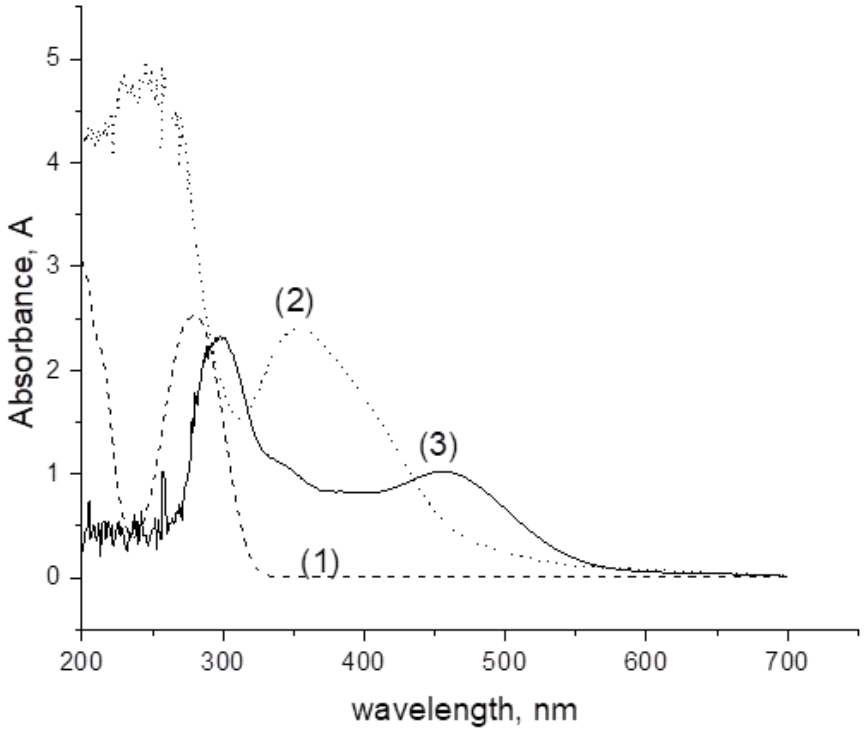
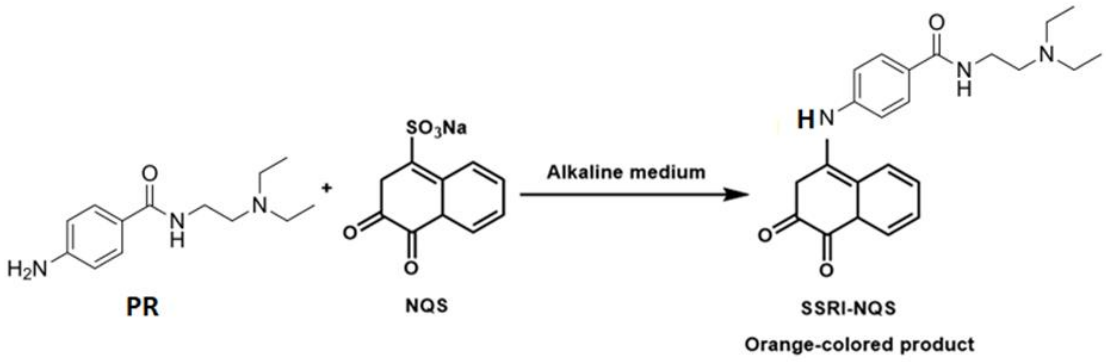



**Figure 1. The absorption spectra of (1) procainamide (50 µg/ml), (2) NQS (0.15%), and (3) procainamide-NQS.**




**Scheme 1. The chemical reaction of the procainamide with NQS reagent**.

### 3.2. Optimization of the proposed method

#### 3.2.1. Effect of NQS Concentration

We investigated the effects of NQS concentration (0.05 -1%, w/v) on its reaction with procainamide and discovered that the responses were concentration-dependent. The absorbances of the procainamide reaction solutions increased with increasing NQS concentration. We found that the maximum absorption intensities occurred at NQS concentrations 0.15 % (w/v, %). Procainamide with NQS concentration was the most effective at 0.15% (w/v) and had no effect on absorption values with increasing NQS concentration.

#### 3.2.2. Influence of pH

We investigated the effect of pH on the absorbance of procainamide-NQS products by running the reaction in several buffer solutions with varying pH levels (range from 4 to 13). The results revealed that the absorbances were rather low at acidic pH values, indicating the difficulty of the reaction under such conditions (Figure 2). We attributed the poor

reactivity at acidic pH to NQS amino groups that exist as acid salts, decreasing their nucleophilic substitution capabilities. When the pH increased, the absorbances increased rapidly because the amino groups of NQA in the acid salts became free, allowing for nucleophilic substitution processes. We obtained the highest absorption values in the pH range of 8 to 12. The testing revealed that the product's absorbance was maximum at pH 11, and there was a strong linear relationship between that absorbance and procainamide hydrochloride at pH 11. We chose a pH 11 potassium dihydrogen phosphate/NaOH solution to adjust the system's pH. The buffer solution clearly had no effect on the determination of procainamide hydrochloride.

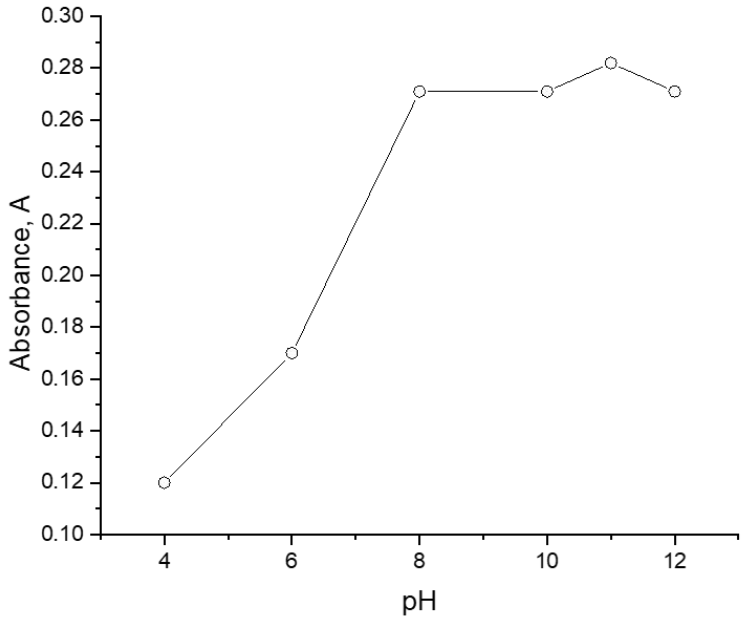



**Figure 2. Effect of different pHs on the absorbance of procainamide with NQS.**

**3.2.3. Effect of Reaction Time**

We examined the impact of time on the production of reaction products by allowing the reactions to proceed for different durations. The findings indicated that the NQS reactions were completed in under 20 minutes, and extending the reaction time to 60 minutes did not influence the outcomes (Figure 3). Consequently, we performed all additional trials with a response duration of 20 minutes.

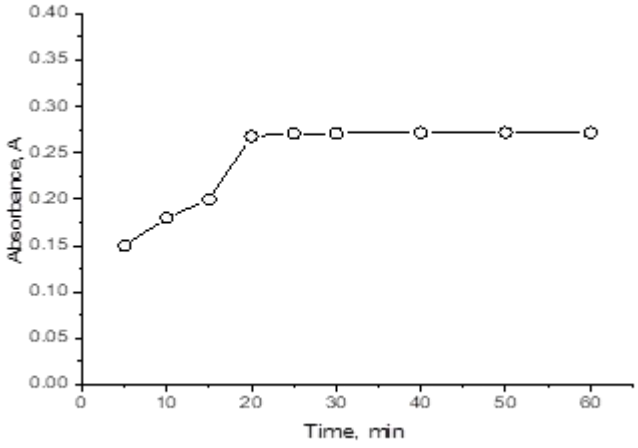



**Figure 3. Effect of reaction time on the absorbance of the reaction product of procainamide-NQS.**

**3.2.4. Effect of Solvent**

The addition of water to the reaction solution produced a translucent solution, indicating the product's solubility and highlighting water's efficacy as a diluent. We evaluated various solvents including water, methanol, ethanol, acetone, and acetonitrile. The maximum absorbance readings were recorded with water, methanol, and ethanol, while lower absorbance values were seen with the remaining solvents (Figure 4). We employed water as a solvent to create an eco-friendly and cost-effective test.

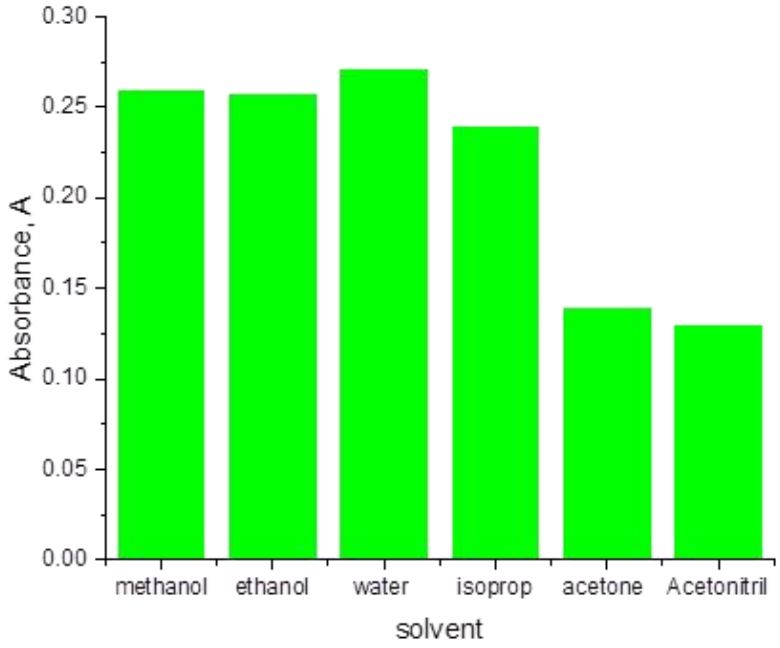



**Figure 4. Effect of different solvents on the absorbance of the reaction product of procainamide-NQS**

**3.4. Validation of the proposed method.**

**3.4.1. Calibration graph**

We developed calibration curves for quantifying procainamide via its interaction with NQS by graphing absorbances against concentrations, employing the ideal conditions specified in Table 1. The regression equation is A = ax - b, where A denotes the maximum absorbance and C signifies the quantity of procainamide in µg/ml. Linear relationships with minimal intercepts (1-50 µg/ml) and elevated correlation coefficients (0.9996) were established for procainamide within the concentration range of 1-50 µg/ml. The International Conference of Harmonization (ICH) recommendations [21] for verifying analytical processes informed the establishment of the limits of detection (LODs) and limits of quantification (LOQs). The subsequent formula was employed: LOD or LOQ is defined as 3.3 times the standard deviation of noise, whereas LOQ is defined as 10 times the standard deviation of noise.

**Table 1. Analytical characteristics of the developed spectrophotometric method.**

| Parameters | Spectrophotometric method |
|---|---|
| Linear detection range (µg/ml) | 1-50 |
| Correlation coefficient (r) | 0.9993 |
| Wavelength, nm | 460 |
| Molar absorptivity, ε | $0.2\times10^4$ |
| Regression equation | y = 0.0168× - 0.0118 |
| Lower detection limit (LOD, µg/mL) | 0.3 |
| Lower quantification limit (LOQ, µg/mL) | 1 |
| Working pH range | alkaline medium 8-12(optimum 11) |

**3.4.2. Accuracy and precision**

We evaluated five distinct procainamide working standard solutions to determine the reproducibility of the proposed spectrophotometric approach [22]. The assay demonstrated acceptable repeatability, with the relative standard deviation (RSD) not surpassing 3.0%. This degree of precision is enough for the normal examination of the examined pharmaceuticals in

quality control laboratories. We performed recovery studies to evaluate the accuracy of the proposed assay by including known quantities of procainamide into the sample and assessing them with the proposed assay. The recovery values ranged from 97.5% to 99%. Table 2 shows inter- and intra-day precision and accuracy of the proposed spectrophotometric method, demonstrating the assay's accuracy. Table 3 compares the proposed method with previously published spectrophotometric methods. Based on the results, it is clear that the current study is more sensitive and has a lower detection limit than previous investigations.

**Table 2. Inter- and intra-day precision and accuracy of the proposed spectrophotometric method.**

| Concentration **(µg/ml)** | **Recovery, %** | **RSD, %** | **Recovery, %** | **RSD, %** |
|---|---|---|---|---|
| | **Intra-day** | | **Inter-day** | |
| 1.0 | 97.5 | 2.2 | 97.3 | 2.5 |
| 10 | 97.8 | 2.4 | 97.4 | 2.8 |
| 20 | 98.3 | 2.1 | 97,8 | 2.5 |
| 30 | 98.4 | 2.4 | 98.4 | 2.4 |
| 40 | 99.4 | 2.3 | 98.3 | 2.7 |
| 50 | 99,3 | 2.4 | 98.6 | 2,7 |
| Average | **98.5** | **2.3** | **98.0** | **2.6** |

* Average of 5 measurements ±RSD.
*RSD%, Relative standard deviation %

**Table 3.** A comparison between the proposed method and other published spectrophotometric methods

| Spectrophotometric method | Reagent | $\lambda_{max.}$ nm | Calibration range, ppm | LOD, ppm | Reference |
|---|---|---|---|---|---|
| Procainamide- chloranilic acid | chloranilic acid | 520 | 1000-10000 | 1000 | 14 |
| Procainamide-periodate | Sod.periodate | 531 | 50 -700 | 25 | 15 |
| Procainamide- cerium (IV) in sulphuric acid | cerium (IV) in Sulphur acid | 480 | 100-600 | 75 | 16 |
| Procainamide- p-benzoquinone | p-benzoquinone | 501 | | | 17 |
| Procainamide-1,2-naphthoquinone-4-sulfonic acid | 1,2-naphthoquinone-4-sulfonic acid | 460 | 1-50 | 0.3 | The present study |

**3.5. Application of the Proposed**

Table 4, illustrates the implementation of the proposed spectrophotometric technique for assessing procainamide in a pure solution. The mean recovery was 98%, with a relative standard deviation of 2.3%. Table 5, illustrates the quantification of procainamide in injectable solution, with the results consistent with those documented in the United States Pharmacopeia [23].

**Table 4. Direct determinations of procainamide using spectrophotometric method**.

| **Concentration Added (µg/ml)** | **Recovery, %** | **RSD, %** |
|---|---|---|
| 1 | 97.8 | 2.7 |
| 10 | 97.6 | 2.4 |
| 20 | 98.3 | 2.3 |
| 30 | 98.5 | 2.2 |
| 40 | 98.9 | 1.9 |
| 50 | 99.1 | 1.9 |
| | **98.4%** | **2.3%** |

* Average of 5 measurements ± RSD

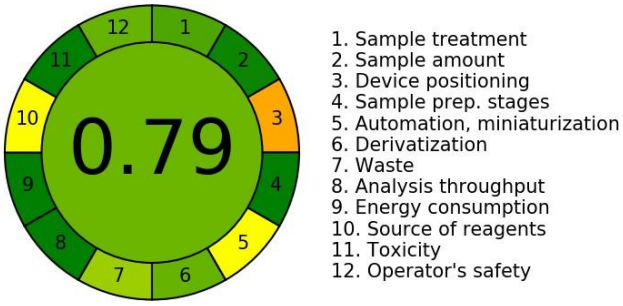
**Table 5. Determination of procainamide in injection solution using the spectrophotometric method.**

| **Injection solution** | **Spectrophotometric method** | | **Reported method [23]** | |
|---|---|---|---|---|
| | **Recovery, %** | **RSD, %** | **Recovery, %** | **RSD, %** |
| Procainamide Injection | 97.5 | 2.5 | 98.0 | 2.4 |

* Average of 5 measurements ± RSD.

### 3.6. The Greenness of the proposed method

The greenness of the analytical process relates to its environmental impact and sustainability. An eco-friendly analytical approach aims to reduce or eradicate the use of hazardous substances, minimize waste production, save energy and natural resources, and promote comprehensive environmental stewardship. The environmental sustainability of an analytical method is assessed by its commitment to minimizing ecological consequences, fostering sustainability, and incorporating environmentally friendly activities throughout the analytical process. The environmental sustainability of the suggested technique was assessed utilizing AGREE software tools [24]. The AGREE green program scores range from 0 to 1.0. The proposed approach earned a score of 0.83, signifying that it is assessed as favorable for its environmental impact (Figure 5).




| Criteria | Score | Weight |
|---|---|---|
| 1. Direct analytical techniques should be applied to avoid sample treatment. | 0.85 | 2 |
| 2. Minimal sample size and minimal number of samples are goals. | 0.98 | 2 |
| 3. If possible, measurements should be performed in situ. | 0.33 | 2 |
| 4. Integration of analytical processes and operations saves energy and reduces the use of reagents. | 1.0 | 2 |
| 5. Automated and miniaturized methods should be selected. | 0.5 | 2 |
| 6. Derivatization should be avoided. | 0.8 | 2 |
| 7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided. | 0.69 | 2 |
| 8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time. | 1.0 | 2 |
| 9. The use of energy should be minimized. | 1.0 | 2 |
| 10. Reagents obtained from renewable sources should be preferred. | 0.5 | 2 |
| 11. Toxic reagents should be eliminated or replaced. | 1.0 | 2 |
| 12. Operator's safety should be increased. | 0.8 | 2 |

**Figure 5. AGREE pictogram for the greenness evaluation of the proposed method for the quantitation of procainamide.**

## 4. Conclusion

The findings of this paper unequivocally indicated that the green spectrophotometric approach is capable of quantifying procainamide hydrochloride. The spectrophotometric technique relies on the reaction between NQS and procainamide in an alkaline environment at pH 11. The ICH technique functioned as the validation for the procedure. The results obtained were consistent with those of the dead-stop titration. The primary advantage of this approach is its capacity to detect

procainamide hydrochloride within the visible light spectrum, thus circumventing possible interference. Furthermore, the approach is expeditious and straightforward, exhibiting an extensive linear range. The suggested assay provides an environmentally friendly spectrophotometric method for procainamide. These benefits encompass the utilization of minimal quantities of samples and solvents, rendering the process both economical and environmentally sustainable. The green spectrophotometric method rendered the proposed technique user-friendly.

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