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Research Article

Rapid, Multi-Sample Assay of Gabapentin in Capsules Using Easy-to-Setup and Low-Cost Smartphone-Based Fluorescence Measurement

Thana Thanayutsiri[1], Thapakorn Charoenying[1], Supusson Pengnam[2], Praneet Opanasopit[1], Tanasait Ngawhirunpat[1], Akhayachatra Chinsriwongkul[3], Theerasak Rojanarata[1*]

[1]Department of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, 73000, Thailand
[2]Department of Biomedicine and Health Informatics, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[3]Newcharoen Pharmaceutical L.P., Pathumthani 12120, Thailand

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Abstract

Background: Pharmacopeial methods for the quantification of gabapentin (GBP), particularly high-performance liquid chromatography (HPLC), require costly instrumentation, unsafe solvents in mobile phase, and time-consuming procedures. While fluorometric methods exist, they also employ expensive fluorometers. This study aimed to develop a cost-effective, eco-friendly GBP assay using smartphone-based digital image fluorometry (SDIF) for rapid multi-sample analysis of GBP in pharmaceutical capsules.

Methods: GBP was reacted with fluorescamine in borate buffer (pH 9.0) for 2 minutes, producing a blue fluorescent product under UV light. Fluorescence intensity was captured using a smartphone inside a dark box with low-cost UV LEDs (395 nm). GBP concentration was determined from green (G) channel intensity using a logarithmic calibration curve. The method was optimized, validated per USP guidelines, and compared with USP HPLC. Greenness and practicality were evaluated.

Results: The SDIF method exhibited excellent linearity ($r^2 = 0.9994$) over GBP concentrations of 0.2–4.0 µg/mL, with high accuracy (98%–102% recovery) and precision (RSD < 2%). No interference was observed from capsule excipients or the impurity GBP-related compound A. Assay results showed no significant difference from the USP HPLC method. Compared to HPLC, the SDIF method demonstrated a greener profile by using acetone instead of acetonitrile. Although requiring derivatization and offering lower automation than HPLC, SDIF achieved a satisfactory Blue Applicability Grade Index (BAGI) score of 65.0, indicating practical applicability. Additionally, the method allowed simultaneous measurement of six samples and six standard solutions, unlike HPLC or fluorometers, which measure only one at a time.

Conclusion: SDIF eliminates the need for fluorometers or HPLC, using inexpensive and simple equipment for rapid, multi-sample GBP analysis. With its green profile and practicality, SDIF offers a viable alternative for routine quality control, particularly in resource-limited settings.

Introduction

Smartphone-based digital image colorimetry (SDIC) has emerged as a powerful analytical tool for quantifying analytes across diverse fields, including environmental monitoring, food safety, clinical diagnostics, and pharmaceutical analysis.[1-3] This technique leverages the advanced imaging capabilities of modern smartphones to capture and analyze the color of test samples, offering a viable alternative to traditional UV-vis spectrophotometry in many applications. At its core, SDIC extracts color information using color space models, such as red-green-blue (RGB), cyan-magenta-yellow-black (CMYK), or grayscale. When a sample undergoes a chromogenic reaction, its color intensity correlates with the analyte concentration. The smartphone camera captures images of the sample, and image-processing algorithms extract RGB values, which are then converted into analyte concentrations using a calibration curve. SDIC offers several advantages that make it an attractive analytical approach. Its accessibility and cost-effectiveness eliminate the need for expensive laboratory equipment, such as UV-Vis spectrophotometers, making it particularly suitable for resource-limited settings. Additionally, its portability enables convenient field measurements. Despite these advantages, certain factors can affect the accuracy and reproducibility of SDIC, including variations in ambient lighting, differences in smartphone camera specifications, and challenges in standardizing image acquisition

***Corresponding Author:** Theerasak Rojanarata, Email: Rojanarata_t@su.ac.th

conditions. However, these limitations can be minimized through proper calibration, controlled lighting setups, and optimized image processing techniques, ensuring reliable and consistent analytical performance.

Building on the principles of SDIC, smartphone-based digital image fluorometry (SDIF) offers a promising alternative for fluorescence measurements in the visible light range. Although less documented than SDIC, SDIF has been applied in several fields, including the analysis of Cu^{2+} in mineral and tap water,[4] paper-based determination of β-glucosidase activity in crude almond and human serum,[5] and hydrogen peroxide detection in milk samples.[6] In pharmaceutical quality control, SDIF's applications remains underexplored, with methods only being reported for the analysis of tobramycin[7] and gentamicin[8] formulations. While these SDIF assays offer the advantage of cost-effectiveness by eliminating the need for costly spectrofluorometers, several limitations hinder their widespread adoption. Many require custom-made smartphone attachments (often 3D-printed),[7,8] rely on non-commercial or uncommon fluorogenic reagents, e.g., carbon dots[9,10] or gold nanoparticles,[11] or are limited to single-sample measurement.[7-11] These constraints significantly reduce their practicality for routine pharmaceutical quality control. Given these challenges, there remains a need for rapid and user-friendly SDIF assays that use readily available reagents and simple, easy-to-setup equipment while enabling the simultaneous analysis of multiple samples for pharmaceutical quality control.

Gabapentin (GBP), a structural analog of γ-aminobutyric acid (GABA), is prescribed for the treatment of epilepsy, neuropathic pain, restless legs syndrome, and other conditions. Available in various formulations, including capsules, tablets, and oral solutions, GBP dosage forms requires reliable analytical methods for accurate quantification. The United States Pharmacopeia (USP) and other pharmacopoeias recommend reversed-phase high-performance liquid chromatography (HPLC) as the standard assay method for GBP.[12,13] Although HPLC offers high sensitivity and accuracy, it is time-consuming, requires expensive instrumentation, and relies on unsafe organic solvents, such as acetonitrile and methanol, for the mobile phase.

In addition to HPLC, several colorimetric methods have been developed for the assay of GBP, involving chromogenic reactions with the drug. However, these assays often utilize hazardous chemicals, such as ninhydrin,[14] acetylacetone/formaldehyde,[15] or 2,4-dinitrophenol,[16] which raise concerns about safety and environmental impact. To address these issues, Winotapun et al[17] proposed a greener method using genipin, a naturally derived reagent, that offers enhanced safety and environmental compatibility. Despite these advantages, this method requires a 60-minute heating step, limiting its practicability for rapid analysis.

In terms of fluorometric assays, the use of fluorogenic derivatizing agents, such as fluorescamine, have also been reported.[18] While these methods offer high sensitivity, they require the use of a spectrofluorometer, which is more expensive and less accessible than a UV-vis spectrophotometer, making them unsuitable for routine analysis in many laboratories. Other methods, such as capillary electrophoresis[19] and electrochemical techniques,[20] have also been reported for GBP quantification; however, these methods present challenges related to complexity, cost, and their suitability for pharmaceutical quality control.

Given these limitations, there is a need for more practical, affordable, and environmentally friendly methods to quantify GBP in pharmaceutical formulations. As SDIF offers a potential alternative for drug quantification, the aim of this study was to develop a novel assay for GBP in capsules based on this approach. The method relies on the reaction between GBP's primary amine group and fluorescamine, a common fluorogenic reagent, which produces a blue fluorescent product when excited by UV light at 390-400 nm. The method was optimized and validated following USP guidelines, and its analytical performance was compared to the standard USP HPLC method. Additionally, the greenness and practicality of the method were assessed using established metrics.

Materials and Methods

Chemicals and instrumentation

GBP (purity ≥ 99%), GBP-related compound A (purity ≥ 99%), and fluorescamine (purity ≥ 98%) were obtained from Sigma Aldrich (MO, USA). Acetone, acetonitrile, and boric acid were sourced from Merck (Germany). GBP capsules (400 mg per capsule) were purchased from a drugstore in Thailand, with additional tablet ingredients including corn starch, anhydrous lactose, and talc. An iPhone 11 Pro served as the camera, while 395 nm UV LED lamps provided the UV light source. The standard assay (USP) for GBP capsules was conducted using the Agilent 1220 Infinity LC System (Agilent Technologies, Germany) for comparison with the proposed SDIC method.

Procedure for fluorescamine-based reaction in the proposed assay

The fluorogenic reaction was performed by adding 100 µL of a 1 mg/mL fluorescamine solution (prepared in acetone) to a mixture containing 500 mL of 200 mM borate buffer, pH 9.0, and 500 µL of either the GBP standard solution (0.2, 0.4, 0.8, 1.6, 3.2, 4.0 µg/mL) or the sample solution (approximately 0.8 µg/mL), both prepared in water, in a 1.5 mL microcentrifuge tube. The mixture was vortexed for 10 s and incubated at room temperature for 2 minutes. Fluorescence intensities were then acquired upon UV excitation by means of smartphone imaging and RGB analysis.

Fluorescence measurement and analysis by SDIF

After the reaction, 1 mL of each resulting solution was transferred to a cuvette. A series of cuvettes containing both standard and sample solutions were then arranged in a row inside a custom-built dark box equipped with LED lamps emitting UV light at 395 nm (Figure 1). Upon UV exposure, the GBP-containing solutions emitted blue fluorescence. For image acquisition, an iPhone 11 Pro camera was set to autofocus mode with a 12 MP resolution, f/1.8 aperture, and flash disabled. The camera was positioned 25 cm from the cuvettes, capturing all standard and sample solutions in a single frame from a front side.

RGB values of the blue fluorescence were extracted from the digital image using the RGB Color Detector, a free mobile application available on both the App Store (iOS) and Play Store (Android). A standard curve was constructed by plotting G value as the analytical signal against the logarithm of GBP concentration. Analyte concentrations were determined using the regression equation derived from the standard curve.

Method validation

To evaluate the analytical performance, the method was validated following the guidelines outlined in <1225> Validation of Compendial Procedures from the USP 43.[12] The relationship between the analytical signal and drug concentration was assessed by generating a standard curve for GBP within a concentration range of 0.2 to 4.0 µM, followed by determining the regression equation and the coefficient of determination (r^2). The standard curve data in the lower concentration range (0.2–0.5 µg/mL), which exhibited linearity, were used to calculate the limit of detection (LOD) and limit of quantitation (LOQ), defined as 3.3 and 10 times the standard deviation of the Y-intercept divided by the slope, respectively. Accuracy was determined by spiking known amounts of standard GBP at three concentration levels (75%, 100%, 125% of the target concentration) into a capsule placebo (n = 3 per level), followed by assay of the spiked samples, with recovery expressed as a percentage. Intra-day and inter-day precision were assessed by analyzing commercial capsules within a single day and across three consecutive days (n = 6), respectively. Results were reported as the percentage relative standard deviation (%RSD) of the labeled amount. Specificity was evaluated by assaying a known quantity of GBP in the presence of excipients commonly used in capsule formulations, and the impurity, 2-azaspiro[4,5]decan-3-one (GBP-related compound A).

HPLC analysis

The USP assay for GBP capsules, based on HPLC,[12] was employed to compare analytical results with those obtained using the SDIC method. Briefly, both the standard and sample solutions were prepared at a concentration of 4.0 mg/mL (23.4 mM) of GBP. The analysis was conducted using a C8 column (4.6 mm × 25 cm, 5 µm particle size; VertiSep™ GES, Vertical Chromatography Co., Ltd., Bangkok, Thailand) with isocratic elution. The mobile phase consisted of 8.8 mM KH_2PO_4 in water and acetonitrile (94:6 v/v), adjusted to pH 6.9 using 5 M KOH. The flow rate was set at 1.2 mL/min, with an injection volume of 50 µL. The column temperature was maintained

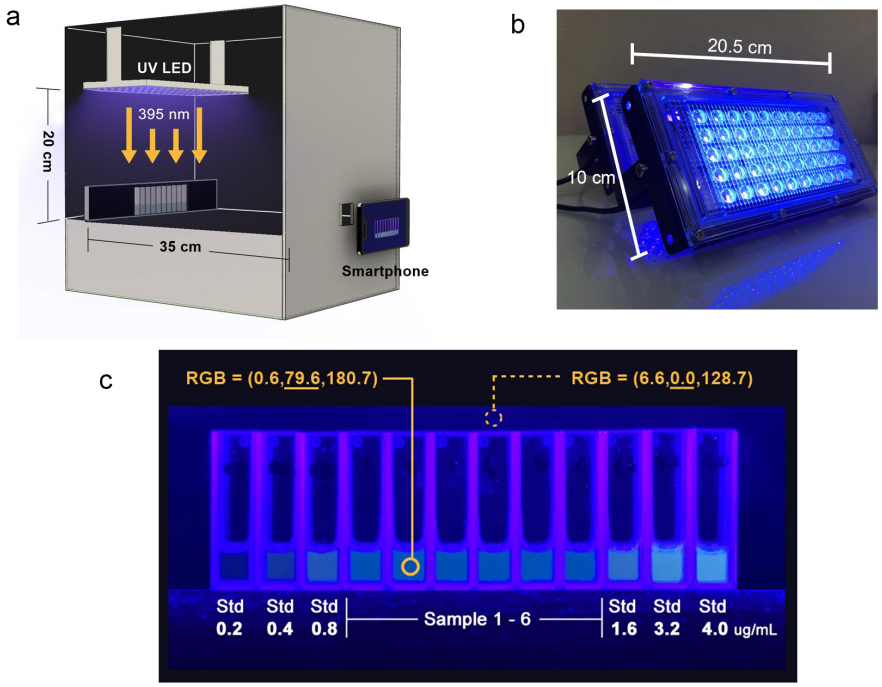


Figure 1. Custom-built photographic chamber for smartphone-based fluorescence measurement (a) using low-cost commercially available UV-LEDs (b), and RGB acquisition from the image (c), showing examples of G values extracted from the fluorogenic reaction of GBP in a cuvette and from the image background with a G value of 0

at 25 °C, and detection was performed at 210 nm using a diode array UV detector. The percentage of the labeled GBP content in the capsule was determined by comparing the peak areas of the sample and standard solutions.

Results and Discussion

Optimization of fluorescamine-based reaction

Since GBP does not inherently fluoresce strongly, a fluorogenic reagent is necessary to enable fluorescence-based analysis. Fluorescamine, a well-known and commercially available reagent, reacts selectively with primary amines, forming a highly blue fluorescent product. The advantage of fluorescamine lies in its lack of intrinsic fluorescence, which ensures a low background signal and enhances sensitivity. Due to its rapid and specific reaction, fluorescamine is widely employed for analyzing amine-containing compounds, including amino acids, peptides, proteins, and pharmaceuticals.[21] Since the presence of a primary amine group in GBP, fluorescamine can react with GBP (Figure 2), offering an ideal choice for the analysis and thus used in this work.

As a standard protocol, the fluorescamine reaction is typically performed in aqueous or buffered solutions, with borate buffer commonly used to maintain an optimal alkaline pH of 8–9. However, other reaction parameters, such as fluorescamine concentration and reaction time, need to be optimized based on the type and concentration of the analyte. Therefore, in this study, these two parameters were initially optimized to achieve efficient quantification of GBP.

As shown in Figure 3, which depicts the results from the experiment using a 4 µg/mL GBP solution—the highest concentration in the standard curve—increasing the fluorescamine concentration from 0.2 to 1 mg/mL led to higher fluorescence, as indicated by the increasing G value, which correlates with the intensity of blue fluorescence. However, increasing the concentration beyond 1 mg/mL did not result in a significant further increase in the analytical signal, as the available GBP had already been consumed. Based on these results, a fluorescamine concentration of 1 mg/mL was selected for the proposed assay, as it provided high sensitivity while minimizing reagent usage. Regarding reaction time, the reaction was found to be complete at room temperature after 2 minutes, as a 1-min incubation resulted in a lower G signal (approximately 90% of the maximum G value). Furthermore, the resulting fluorescence remained stable for at least 15 minutes, indicating that fluorescence measurements can be reliably performed at any time between 2 and 15 minutes after incubation.

Development and optimization of a low-cost smartphone fluorescence imaging system

Traditionally, fluorometric assays using fluorescamine require a fluorometer with excitation and emission wavelengths set at 390 nm (near UV) and 470 nm (blue), respectively. To adapt this for smartphone-based detection, we fabricated a low-cost and easy-to-setup photographic chamber (Figure 1a). A cardboard box, with its interior walls painted black, served as a dark chamber to minimize external light interference, ensuring a stronger and clearer signal from the samples. A panel of UV LEDs (390–400 nm) (Figure 1b) was installed at the top of the box to illuminate the samples, while a small aperture at the front allowed a smartphone camera to capture images of the cuvettes, positioned as shown in Figure 1a. This simple but effective setup facilitated reliable image acquisition. Additionally, the use of a smartphone and UV-LED lamps (costing approximately US$ 6) rendered this method significantly more economical than conventional fluorometric analysis with a fluorometer.

Digital camera images often exhibit non-uniform illumination, where peripheral areas appear dimmer than the central region, leading to differences in color intensity and, ultimately, inaccurate RGB value acquisition.[22] To address this issue, we investigated the maximum number of cuvettes per row captured per frame by analyzing G values extracted from images containing varying numbers of aligned cuvettes, each filled with a 0.8 mg/mL GBP reaction solution—the target drug concentration in the assay. As expected, variation in G values, measured by %RSD, increased with the number of aligned cuvettes (Table 1). To maintain an RSD below 2%, no more than 12 cuvettes should be included in a single shot. Based on this finding, the proposed assay enables simultaneous measurement of six samples and six standard solutions, offering a faster alternative to HPLC or fluorometers, which analyze only one sample at a time.

Beyond its speed, capturing an image of multiple samples alongside standard solutions in a single shot offers the additional advantage of minimizing variations in lighting conditions between shots. By adopting a

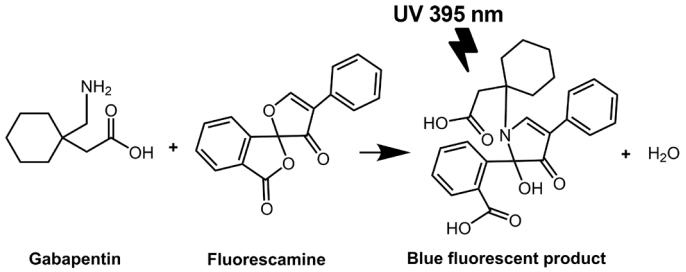


Figure 2. The fluorogenic reaction of GBP with fluorescamine

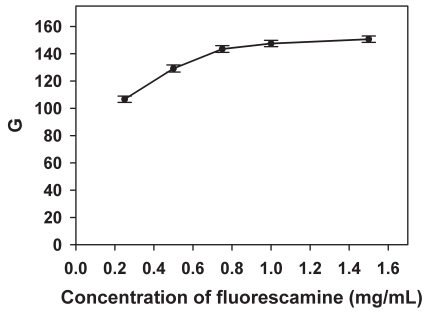


Figure 3. Effect of fluorescamine concentration on G value, studied using 4 µg/mL GBP

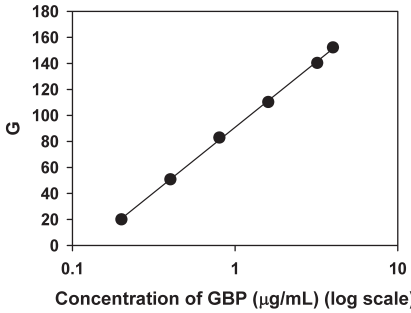


Figure 4. The relationship of concentration of GBP (µg/mL) in a logarithmic scale and G, used as a standard curve for the SDIF assay

Table 1. Effect of cuvette numbers aligned in a row on G values

Number of tube	16	14	12	10	8
Average G	66.54	68.10	68.76	69.06	69.05
SD	4.78	2.21	1.15	1.01	0.59
%RSD	7.19	3.25	1.67	1.46	0.85

calibration technique— where analyte concentrations are determined by comparing the G values of samples to a standard curve derived from the same image—high accuracy was achieved. Unlike some previously reported smartphone-based fluorescence detection devices that capture one sample at a time and require precise design to ensure consistent photographic conditions,[7,8] the proposed method reduces the need for such rigorous device optimization.

Selection of RGB signal

To establish the relationship between the blue fluorescence intensity and the drug concentration, a suitable analytical signal was investigated by fitting various functions of red (R), green (G), and blue (B) color intensities with different functions of GBP concentration. A strong linear relationship was observed between the G value and the logarithm of GBP concentration. Additionally, the regression equation for G had a steeper slope compared to similar relationships with R and B (Figure 4 and Supplementary file 1, Table S1). This relationship was described by the regression equation $y = 43.628 \ln(x) + 90.866$, where y represents the G value and x represents the GBP concentration (µg/mL). The high correlation coefficient ($r^2 = 0.9994$) indicated excellent linearity and suitability for quantitative analysis. While calibration curves with a linear relationship between concentration and response are more commonly used in analytical chemistry, calibration curves on a logarithmic scale of concentration are also possible and have been applied in various studies.[23-26] Despite the controversy and concerns regarding the validity of such standard curves,[27-30] the recovery results ("Method validation results" section), studied over the concentration range of 75-125% of the target concentration, confirmed that the log concentration versus analytical signal (G) relationship was reliable and effective for the proposed assay.

The LED lamps used in the assay emitted light in the 390–400 nm range (at the boundary between UV and visible light), producing a violet illumination. However, as shown in Fig. 1c, this violet light did not interfere with the analysis, as a null G value was recorded against this background (Figure 1a). This demonstrates the G channel's selectivity for the analyte's fluorescence and its independence from the excitation light.

Method validation results

The proposed SDIF assay demonstrated good linearity (Table 2). Although the target analyte's naturally high concentration places it in Category I of USP: validation of compendial procedures (analytical procedures for quantitation of major components of bulk drug substances or active ingredients in finished pharmaceutical products), where LOD and LOQ determination is not mandatory, these parameters were nonetheless calculated and found to be 0.059 and 0.179 µg/mL, respectively. The method exhibited excellent accuracy, with recoveries within 98%–102% across all three tested concentrations, and high precision, with %RSD below 2% for both intra-day and inter-day precision. As shown in Table 3, the specificity was confirmed by the near-100% recoveries, meaning that the fluorescamine-based assay was unaffected by common capsule excipients and GBP related compound A (an impurity controlled in the USP monograph for GBP capsules). The substances were tested at the concentrations commonly used for ingredients in the formulation, or at twice the impurity limit for GBP Related Compound A. The absence of primary amine groups in these potentially interfering substances prevented them from reacting with fluorescamine to produce a fluorescent product, ensuring accurate GBP recoveries even in their presence.

Comparison of the assay performance with the standard HPLC methods and other methods

The applicability of the SDIF method was demonstrated by quantifying GBP in the same commercial capsule samples using both the proposed method and the USP method. The results, expressed as a percentage of the labeled amount, were compared between the two methods. The chromatograms obtained from the USP method are shown in (Figure 5), illustrating the

Table 2 Summary of the method validation results

Parameter	Result
Regression equation	y = 43.628 ln(x) + 90.866 when y is G and x is concentration of GBP (µg/mL)
r^2	0.9994
Range	0.2–4.0 µg/mL
LOD	0.059 µg/mL
LOQ	0.179 µg/mL
Accuracy	
% Recovery (n = 3, for each level)	98.65 ± 0.70% (low; spiked with 0.6 µg/mL) 99.90 ± 1.20% (medium; spiked with 0.8 µg/mL) 100.64 ± 0.95% (high; spiked with 1.0 µg/mL)
Precision	
%RSD for intra-day precision (n = 6)	1.75%
%RSD for inter-day precision (n = 18)	1.51%

Table 3. Specificity of the method

Substance	Added amount (mg)*	% Recovery ± SD
GBP Related Compound A	3.2	99.76 ± 2.11
Lactose	200	100.57 ± 2.15
Talcum	4	98.72 ± 1.79
Corn starch	100	98.65 ± 1.91

* mg of substance added to 400 mg of gabapentin to prepare a test mixture.

analysis time of approximately 8 min per sample. No significant difference was observed between the % labeled amounts obtained using the proposed SDIF method (101.35 ± 0.80%) and the USP method (100.90 ± 0.24%), with the calculated t-value (0.0568) being lower than the critical t-value (3.8853). Therefore, the SDIF assay is suitable alternative for analyzing GBP content in capsule formulations, providing results consistent with the standard USP method.

The proposed SDIF method offers several advantages over other published analytical methods used for gabapentin quantification in pharmaceutical dosage forms, including simplicity, cost-effectiveness, and efficiency. As summarized in Table 4, it requires only a smartphone and LEDs, making it much more affordable compared to methods requiring expensive instruments like UV-vis spectrophotometers,[14,17,31] spectrofluorometers,[18] and HPLC.[32] Additionally, the reaction time is rapid (2 minutes without heating), significantly faster than methods that require lengthy heating or incubation times. The proposed method also supports multi-sample measurement, unlike methods such as HPLC that are limited to single-sample analysis. With a low detection limit (0.059 µg/mL) and a reasonable limit of quantification (0.179 µg/mL), it provides sensitivity comparable to other techniques, making it a practical and efficient choice for gabapentin analysis.

Greenness and practicability of the proposed method

The greenness of the proposed SDIF method was evaluated in comparison with the USP method using two established greenness metrics. While the Green Analytical Procedure Index (GAPI)[33] revealed a similar profile for both methods (5 green, 4 yellow, and 5 red sections), the Analytical Greenness Metric (AGREE) score[34] favored the SDIF method with a score of 0.63 over the USP method which had a score of 0.55 (Table 5), indicating a greener approach. Although both methods utilize organic solvents, the SDIF method's use of acetone as a solvent the fluorescamine solution offers a significant green advantage over the USP method's use of acetonitrile in the HPLC mobile phase. In addition, the 100 µL of fluorescamine solution containing acetone per sample was significantly less than the several milliliters of acetonitrile required for mobile phase and diluent preparation. Acetone is generally considered a greener and safer solvent due to its lower toxicity, ready biodegradability, and reduced contribution to air pollution. In contrast, acetonitrile is classified as toxic and presents greater health risks. Furthermore, bio-based acetone, produced through fermentation of plant-derived materials, offers the potential to further minimize the carbon footprint associated with solvent production. While fluorescamine requires careful handling, its rapid reactivity with water and subsequent degradation into non-fluorescent products minimize concerns about long-term environmental persistence. Additionally, it was used in a low amount, similar to acetone in this assay. These considerations collectively support the SDIF method's alignment with green chemistry principles.

To assess the practicality and applicability of the proposed method, the Blue Applicability Grade Index (BAGI)[35] was employed. This index uses a ten-criteria assessment. The score, ranging from 25 to 100 points, is represented on a blue color scale, with scores nearer to 100 and darker blue shades indicating higher performance in applicability. The proposed method achieved a BAGI score of 65.0 (Table 5), slightly lower than the USP method's score of 72.5. This difference can be attributed to the proposed method's need for derivatization using a reagent not commonly available in QC labs (compared to a common solvent like acetonitrile) and its lower degree of automation compared to semi-automated technique like HPLC. Despite the slightly lower BAGI score, a score above 60, as achieved by the proposed method, is generally considered "practical".

Beyond GBP, several amine-containing drugs have been quantified in pharmaceutical dosage forms using fluorimetric methods based on their reaction with fluorescamine. Examples include tobramycin,[36] lenalidomide,[37] vigabatrin,[18] procaine,[38] and sitagliptin.[39] This highlights the potential for extending the fluorescamine-based SDIF method presented in this study to the quantification of a broader range of drugs, further enhancing its applicability in pharmaceutical analysis.

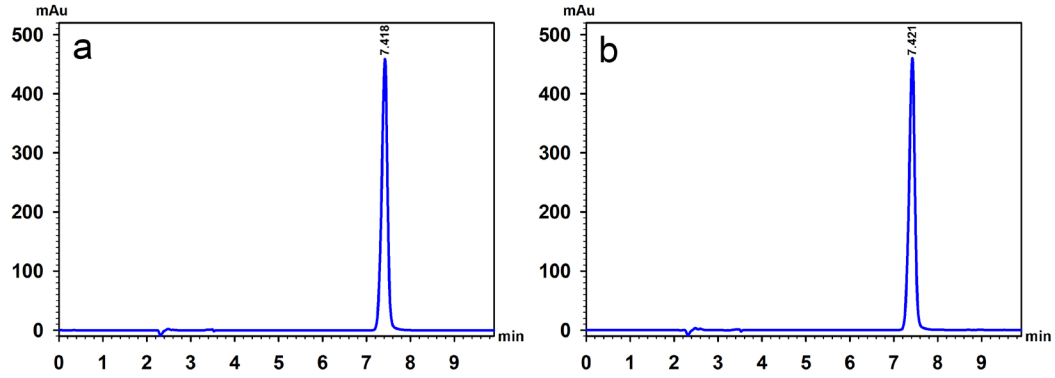


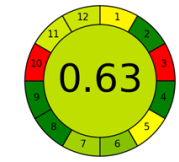
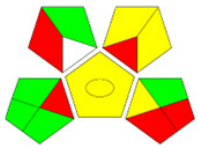
Figure 5. HPLC chromatograms of a 4 mg/mL standard GBP solution (a) and a sample solution containing a nominal 4 mg/mL of gabapentin prepared from commercial capsules (b), analyzed using the USP method

Table 4. Comparison of some analytical methods for gabapentin quantification in pharmaceutical dosage forms

Method	Instrument required	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Remarks
Proposed SDIF in this study	Smartphone/LEDs	0.2–4.0	0.059	0.179	Use simple and inexpensive equipment, reaction time of 2 min without heating, multi-sample measurement
Colorimetry using genipin[17]	UV-vis spectrophotometer	25.7–85.7	0.685	2.400	Heating for 60 min required for color formation
Colorimetry using sodium 1, 2-naphthoquinone-4-sulfonate[31]	UV-vis spectrophotometer	7.5-75	2.46	7.46	Reaction time of 20 min
Colorimetry using ninhydrin[14]	UV-vis spectrophotometer	2-30	0.16	0.454	Heating for 20 min required for color formation
Fluorometry using fluorescamine[18]	Spectrofluorometer	0.1-1.0	0.06	0.20	Expensive instrument required
HPLC[32]	HPLC	2.5-7.5	NP	NP	Expensive and sophisticated instrument required, single sample analysis

NP = Not reported.

Table 5. Assessment of greenness using GAPI and AGREE, and practicality using BAGI

Assessment of greenness	Smartphone method	USP method
AGREE*		
GAPI		
BAGI		

* 1 = Sample treatment, 2 = Sample amount, 3 = Device positioning, 4 = Sample preparation Stages, 5 = Automation, miniaturization, 6 = Derivatization, 7 = Waste, 8 = Analysis throughput, 9 = Energy consumption, 10 = Source of reagents, 11 = Toxicity, 12 = Operator's safety.

Conclusion

This study developed and validated a novel, easy-to-setup, fluorometer-free, SDIF method for rapid, multi-sample GBP analysis in capsules. The method utilizes the reaction between GBP and fluorescamine, a readily available reagent, generating a blue fluorescent product detectable with a simple, low-cost setup. The method exhibited excellent analytical performance, comparable to the USP HPLC method, demonstrating good linearity, accuracy, precision, and specificity. Importantly, the SDIF method offers significant advantages in cost-effectiveness and reduced environmental negative impact due to the elimination of expensive instrumentation and the use of the greener solvent, acetone, compared to the acetonitrile used in HPLC. While requiring a derivatization step, the method achieved a BAGI score indicating practical applicability. This SDIF assay provides a viable alternative for routine GBP quality control, especially in resource-limited settings, and highlights the potential of SDIF for expanding smartphone-based analytical techniques to fluorescence-based assays. Future work may focus on automating image analysis and exploring the application of this SDIF approach to other pharmaceuticals.

Authors' Contribution

Conceptualization: Theerasak Rojanarata.
Formal analysis: Thana Thanayutsiri, Thapakorn Charoenying, Supusson Pengnam, Praneet Opanasopit, Tanasait Ngawhirunpat, Theerasak Rojanarata.
Funding acquisition: Theerasak Rojanarata.
Investigation: Thana Thanayutsiri, Thapakorn Charoenying, Supusson Pengnam
Methodology: Thana Thanayutsiri, Theerasak Rojanarata.
Project administration: Theerasak Rojanarata.
Resources: Akhayachatra Chinsriwongkul.

Visualization: Thana Thanayutsiri.
Writing–original draft: Thana Thanayutsiri, Praneet Opanasopit.
Writing–review & editing: Theerasak Rojanarata.

Competing Interests

The authors declare that there is no conflict of interest.

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Supplementary Files

Supplementary file contains Table S1.

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