



DEVELOPMENT AND VALIDATION OF A FOLIN–CIOCALTEU-BASED SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BISACODYL IN BULK PHARMACEUTICAL MATERIAL

Phoomrapee Punnarak, Supakorn Mutthajaratkul, Ammarin Autama, Jankana Burana-osot, Somlak Kongmuang, Sathit Niratisai, Panadda Phattanawasin *

Division of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University, Sanamchandra Palace Campus, Nakhon Pathom

* Corresponding author: phattanawasin_p@su.ac.th

ABSTRACT

A simple colorimetric spectrophotometric method was developed and validated for the indirect quantification of bisacodyl (BIS) in bulk pharmaceutical material using the Folin–Ciocalteu (F–C) reagent. The method was based on hydrolysis of BIS to its phenolic derivative, bis-(p-hydroxyphenyl)-pyridyl-2-methane, followed by a F–C redox reaction to form a molybdenum–tungsten blue chromophore measurable at 740 nm. Experimental variables, including hydrolysis conditions, solvent composition, reagent ratios, and reaction time, were optimized to achieve clear solutions and reproducible color development. The method was validated in accordance with ICH Q2(R2) guidelines and demonstrated acceptable specificity, linearity over 8–24 µg/mL ($R^2 = 0.9979$), accuracy (99.1–101.4%), and precision (intra- and inter-day %RSD < 2%). The limits of detection and quantification were 0.91 and 2.77 µg/mL, respectively. The method was robust with respect to small variations in reaction time and measurement wavelength. Owing to its simplicity and minimal reagent consumption, the method is suitable for routine quality control analysis of BIS in bulk pharmaceutical material and offers a practical alternative to chromatographic methods.

Keywords: bisacodyl, Folin–Ciocalteu reagent, colorimetric analysis

Received: 24 April 2026; Revised: 30 May 2026; Accepted: 31 May 2026

Introduction

Bisacodyl (BIS), chemically known as 4,4'-(2-pyridylmethylene) diphenyl diacetate, is a stimulant laxative commonly used for constipation. After oral administration, intestinal deacetylases convert BIS to bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM) (Fig 1), which stimulates the intestinal mucosa and induces peristalsis.¹ Accurate BIS quantification in pharmaceuticals is essential to ensure product quality, safety, and efficacy. The United States Pharmacopeia (USP) recommends high-performance liquid chromatography (HPLC) for assaying bulk drugs and tablets.² Other studies have used HPLC³⁻⁵, voltammetry⁶, or densitometric thin-layer chromatography (TLC).⁷⁻⁹ Although sensitive and accurate, these methods require costly instruments and technical expertise, which may not be available in resource-limited settings.

Spectrophotometric approaches, including ultraviolet and colorimetric methods, have also been explored for BIS analysis.¹⁰⁻¹⁷ In colorimetric spectrophotometry, the analyte reacts with a chromogenic reagent to produce a colored species, and the absorbance is measured. Reported colorimetric methods for BIS quantification include the use of bromate-bromide mixture with thymol blue and methyl orange¹³, sulfonephthalein acid dyes¹⁴, 2,4-dinitrophenylhydrazine¹⁵, phenanthroline¹⁶, 3-methyl benzothiazolin-2-one hydrazone in the

presence of ceric (IV) ammonium sulfate¹⁷, and 4-aminophenazone in the presence of potassium hexacyanoferrate (III).¹⁷ However, these methods often involve stepwise reactions, multi-component oxidative coupling or complexation, and require strong oxidizing agents or organic solvents.

To address these limitations, a straightforward and economically feasible colorimetric spectrophotometric method using the Folin-Ciocalteu (F-C) reagent was explored. To our knowledge, the F-C reagent has not been previously applied for the colorimetric determination of BIS. The method employs an indirect quantification approach in which BIS is first hydrolyzed to its phenolic derivative, BHPM, enabling selective reaction with the F-C reagent at phenolic sites.¹⁸ In this reaction, BHPM reduces the phosphomolybdate-phosphotungstate complex (yellow) to an intense molybdenum-tungsten blue species, which is measurable spectrophotometrically. The reaction proceeds predominantly in aqueous media, minimizing the use of hazardous oxidants and organic solvents, while providing high sensitivity, good phenolic selectivity, and improved color stability. Therefore, this study aimed to establish a practical spectrophotometric approach for determining BIS in bulk pharmaceutical material using a F-C based reaction system and to evaluate its analytical performance in accordance with ICH guidelines.

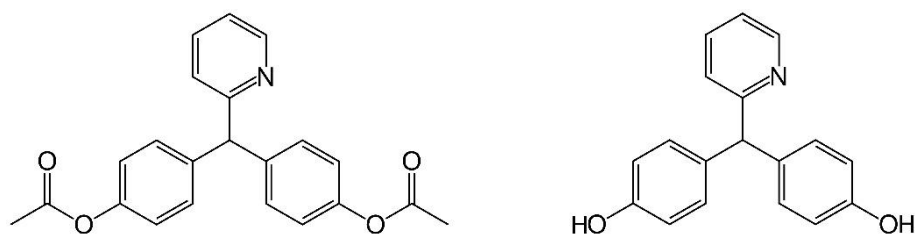


Figure 1 Bisacodyl (BIS) and bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM)

Materials and Methods

1. Chemicals and instrumentation

BIS reference standard (DMScRS) (purity = 100.13%) was purchased from the Department of Medical Sciences. BIS bulk drug was kindly provided by Medicine Supply Company Limited. F–C reagent (commercially stock solution), sodium hydroxide (NaOH), hydrochloric acid (HCl), and ethanol were obtained from Merck (Darmstadt, Germany). Sodium hydroxide solutions (6.0 M), hydrochloric acid solution (2.0 and 6.0 M), and 5% (w/v) NaOH were prepared using distilled water. All spectrophotometric measurements were performed using a UV–Vis spectrophotometer (U-2900, Hitachi, Japan).

2. Optimization of Hydrolysis and F–C Reaction

The experimental conditions were optimized to ensure efficient conversion of BIS to its phenolic derivative, BHPM, and to achieve consistent color development in the subsequent F–C reaction. Hydrolysis of BIS (1 mg/mL) was investigated under acidic and basic conditions using a boiling water bath with constant stirring. Acid hydrolysis was performed using 2.0 M hydrochloric acid for 60 min, whereas basic hydrolysis was carried out using 6.0 M NaOH¹⁹ for 30 min in aqueous and mixed ethanolic–aqueous media containing 20–60% (v/v) ethanol. After cooling to room temperature, all hydrolyzed solutions were neutralized to approximately pH 7.

For the F–C assay, the effect of the alkalizing agent was examined using either 7.5% (w/v) Na₂CO₃ or 5% (w/v) NaOH, with the aim of maximizing color intensity and maintaining solution clarity. Further optimization of the F–C reaction was performed using the hydrolyzed BIS solutions (0.1 and 1 mg/mL) with 5% (w/v) NaOH as the alkalizing agent. The volumes of F–C reagent (25–300 µL) and 5% (w/v) NaOH (100–1000 µL) were systematically varied while keeping the sample volume constant at 200 µL. The reaction mixtures were mixed thoroughly and allowed to react at ambient temperature. Color

development, solution clarity, and the presence of turbidity or precipitation were visually observed.

3. Preparation of Hydrolyzed BIS solutions

A stock standard solution of BIS (1 mg/mL) was prepared by accurately transferring 50 mg of BIS reference standard (DMScRS) into a 50 mL volumetric flask containing 45 mL of 40% (v/v) aqueous ethanol. The solution was subjected to basic hydrolysis by adding 0.5 mL of 6.0 M NaOH and heating in a boiling water bath with constant stirring for 30 min. To minimize solvent loss during heating, the volumetric flask was tightly capped and further sealed with parafilm, thereby providing a closed system that reduced evaporation. After cooling to room temperature, the solution was neutralized with 6.0 M HCl and diluted to volume with 40% (v/v) aqueous ethanol. The stock standard solution was further diluted with 40% (v/v) aqueous ethanol to obtain working standard solutions in the concentration range of 200–600 µg/mL.

A stock sample solution of BIS bulk drug was prepared following the same procedure as described for the standard solution. The resulting solution was further diluted with 40% (v/v) aqueous ethanol to obtain a final concentration of approximately 400 µg/mL.

4. Assay Procedure for the proposed F–C Colorimetric Spectrophotometric Method

An aliquot of 200 µL of working standard BIS solutions (200, 300, 400, 500, and 600 µg/mL) or sample solution (approximately 400 µg/mL) was transferred into a test tube, followed by the addition of 4200 µL of distilled water. The colorimetric reaction was initiated by the sequential addition of 150 µL of F–C reagent and subsequently 450 µL of 5% (w/v) NaOH. The final concentrations in the reaction mixtures were 8–24 µg/mL for the standards and 16 µg/mL for the sample solution. The reaction mixtures were mixed thoroughly and allowed to stand at room temperature for 5 min. The absorbance of the resulting blue-colored solution was measured at 740 nm against a reagent blank. The reagent blank was

prepared in the same manner using 40% (v/v) aqueous ethanol in place of the BIS solution.

5. Method Validation

The method was validated in accordance with the International Council for Harmonisation guideline ICH Q2(R2). The validation parameters included specificity, linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. Specificity was evaluated by comparing the absorption spectra of the standard and sample solutions. Linearity and range were evaluated by constructing a calibration curve over the concentration range of 8–24 µg/mL, corresponding to 50–150% of the nominal concentration. Triplicate determinations ($n = 3$) were performed at each concentration level, and absorbance values were plotted against the corresponding concentrations to obtain the linear regression calibration curve and the coefficient of determination (R^2). The LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ represents the standard deviation of the y-intercept, and S is the slope of the calibration curve.

Accuracy was determined by analyzing the hydrolyzed standard solutions at three concentration levels (50%, 100%, and 150% of the nominal concentration). Each level was prepared and analyzed in triplicate ($n = 3$), and accuracy was expressed as percent recovery, calculated by comparing the measured concentrations with the corresponding nominal (theoretical) concentrations of the prepared standard solutions. Precision was evaluated in terms of intra-day (repeatability) and inter-day (intermediate precision). Intra-day precision was assessed by analyzing the sample solution at the nominal concentration six times within the same day ($n = 6$), while inter-day precision was evaluated by repeating the analysis on a different day ($n = 6$). Precision was expressed as the percent relative standard deviation (%RSD) of the assay results.

Robustness was examined by introducing small deliberate variations in reaction time (4, 5, and

6 min) and measurement wavelength (735, 740, and 745 nm). For each condition, six replicate determinations ($n = 6$) were performed, and the results were expressed as %RSD of the assay values.

Results and Discussion

1. Method development and optimization

Method development was based on an indirect quantification strategy in which BIS was converted to its phenolic hydrolysis product, BHPM, serving as the reactive species in the F–C assay. Sample preparation was optimized to ensure efficient hydrolysis and consistent color development. Basic hydrolysis was selected over acidic conditions, as it promoted ester hydrolysis and resulted in more efficient conversion of BIS to BHPM under the studied conditions (Figure 4, step 1).

The influence of solvent composition was also evaluated to obtain a clear and stable solution suitable for colorimetric analysis. Among the solvent systems investigated, basic hydrolysis in a mixed solvent system provided superior performance compared with single-solvent media. Specifically, a 40% (v/v) ethanolic aqueous co-solvent produced a clear stock solution at 1 mg/mL after neutralization, minimizing turbidity due to the limited aqueous solubility of unionized BHPM and avoiding salt precipitation observed in ethanol-rich systems. This solvent composition effectively balanced the solubility of the hydrolysis product, BHPM, and the inorganic salts formed during the reaction and was therefore selected for subsequent F–C reaction optimization.

The reaction conditions of the F–C assay were further optimized to achieve efficient and reproducible color development for BIS determination. A direct comparison between 7.5% (w/v) Na_2CO_3 and 5% (w/v) NaOH was performed, as alkaline media are required in F–C assays for phenolic compounds. Sodium carbonate is conventionally employed in classical methods, whereas sodium hydroxide can be used as an alternative to provide a

stronger alkaline environment, promoting phenolate formation and enhancing color development.¹⁸ The results demonstrated that 5% (w/v) NaOH was superior, particularly for samples containing residual ethanol. The use of 7.5% (w/v) Na₂CO₃ frequently resulted in incomplete color development, characterized by a greenish-blue appearance and occasional turbidity, leading to lower and less reproducible absorbance. This behavior is likely related to its weaker alkalinity and limited ability to maintain sufficiently high pH under ethanolic conditions. In contrast, 5% (w/v) NaOH, as a strong base, effectively deprotonated the phenolic groups of BHPM to form phenolate species, thereby enhancing its reducing capacity toward the F–C reagent and producing a clear and intense blue chromophore with minimal precipitation (Figure 2). Consequently, 5% (w/v) NaOH was selected as the alkalizing agent for subsequent experiments.

To ensure method sensitivity and reagent efficiency, the effects of reagent volume on color development were systematically evaluated by varying the volumes of F–C reagent (25–300 µL) and 5% (w/v) NaOH (100–1000 µL) at a fixed sample volume of 200 µL. Multiple reagent volume ratios were investigated to assess their influence on color development. Overall, increasing the relative amounts of F–C reagent and 5% (w/v) NaOH led to a gradual increase in color intensity.

At lower NaOH volumes (e.g., 150 µL in the ratio of 200:150:150 µL), no significant blue coloration was observed. This was attributed to insufficient alkalinity in the reaction system, which limited the deprotonation of phenolic groups of BHPM to their corresponding phenolate ions. As phenolate formation is essential for the reduction of the F–C reagent to the blue molybdenum–tungsten chromophore, incomplete conversion resulted in little or no color development.

However, excessively high reagent levels resulted in turbidity and precipitation, particularly at higher BIS concentrations (Figure 3). Based on these observations, a sample-to-reagent volume ratio (sample solution:F–C reagent:5% (w/v) NaOH) of 200:150:450 µL with water added to a final volume of 5 mL was selected as the optimal condition. This ratio consistently produced a strong and stable blue coloration without precipitation across both low and high BIS concentrations and was therefore employed in all subsequent analyses.

In the F–C method using 5% (w/v) NaOH as the alkalizing agent, BHPM is converted to its phenolate form under alkaline conditions, which enhances its reducing capacity toward the phosphomolybdate–phosphotungstate complex of the F–C reagent, leading to the formation of the characteristic molybdenum–tungsten blue chromophore (Figure 4, step 2). This reaction is based

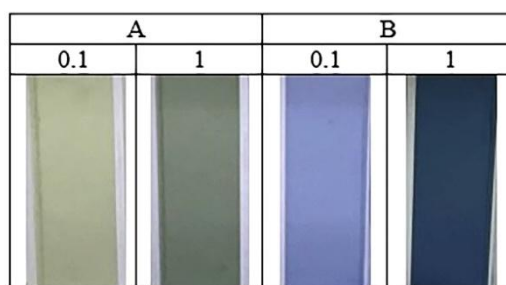


Figure 2 Representative images illustrating observed differences in color development due to alkalizing agent type during method optimization. Comparison of F–C reaction mixtures using 7.5% (w/v) Na₂CO₃ (A) and 5% (w/v) NaOH (B) as alkalizing agents. Reactions were prepared from hydrolyzed BIS standard solutions (0.1 and 1 mg/mL), F–C reagent, and alkalizing agent at a volume ratio of 200:150:450 µL, with water added to a final volume of 5 mL.

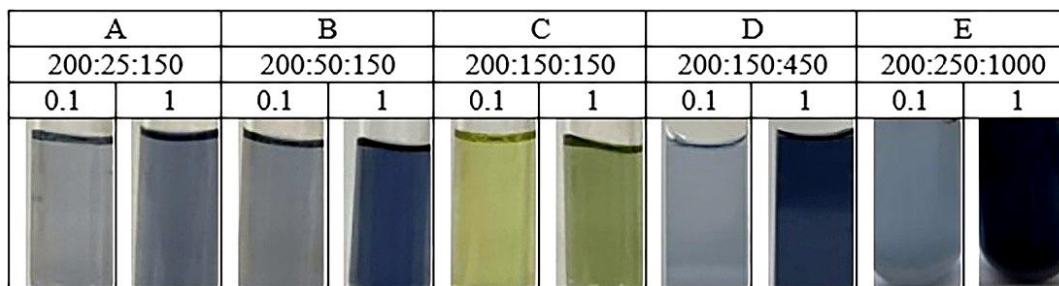
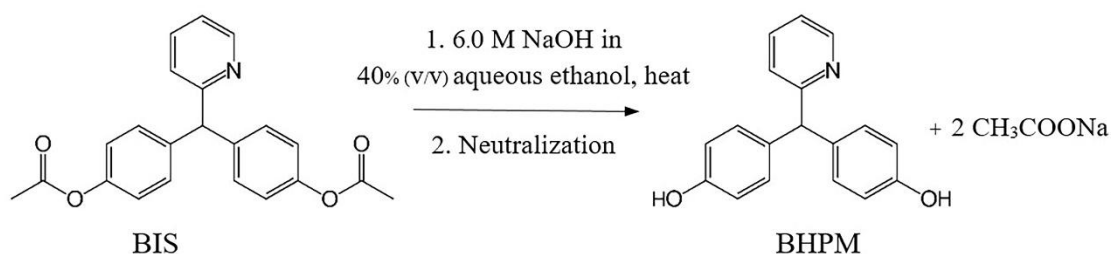


Figure 3 Representative images illustrating observed differences in color development and solution clarity due to different reagent ratios during method optimization. Visual observations were assessed based on color intensity, turbidity, and precipitation. F–C reaction mixtures were prepared using hydrolyzed BIS standard solutions (0.1 and 1 mg/mL) with varying volumes of F–C reagent and 5% (w/v) NaOH.

Step 1: Basic hydrolysis



Step 2: F–C Color Reaction

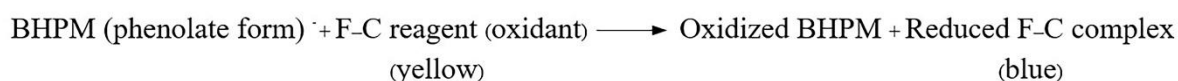
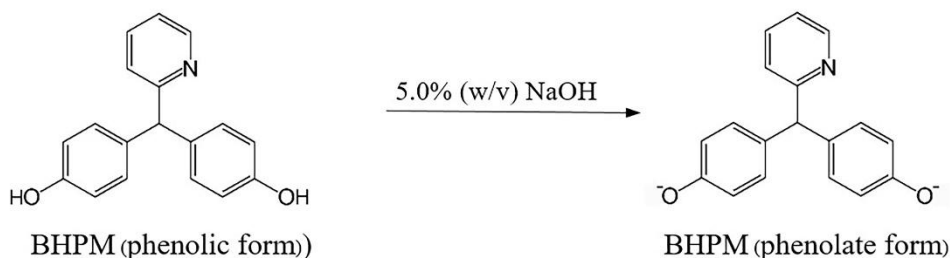


Figure 4 Proposed reaction scheme for the F–C spectrophotometric method for BIS determination, showing (Step 1) basic hydrolysis of BIS to its phenolic derivative (BHPM) and (Step 2) the subsequent color-forming redox reaction with the F–C reagent to produce the molybdenum–tungsten blue chromophore.

on an electron-transfer mechanism in which the phenolic species acts as an electron donor and the F–C reagent acts as an oxidizing agent.¹⁸ The reduction of the yellow phosphomolybdate–phosphotungstate complex results in the formation of blue-colored species, primarily through the reduction of Mo(VI) to

Mo(V), with the intensity of the blue color being proportional to the reducing capacity of the phenolic compound.¹⁸

The resulting blue complex exhibited a broad absorption plateau rather than a distinct absorption maximum (Figure 5). Accordingly, 740 nm was

selected as the analytical wavelength, as it lies within the plateau region and provides relatively high and stable absorbance with minimal variation across neighboring wavelengths.

The time course of blue color formation following complete addition and mixing of all reagents (i.e., from the start of the reaction) was investigated to determine the optimal measurement time. The absorbance profile reflects the progression of blue chromophore formation and subsequent stabilization over time. The absorbance increased rapidly during the initial reaction phase and reached a maximum at approximately 4–6 min, suggesting near completion of the redox reaction between BHPM and the F–C reagent. The absorbance then

remained relatively stable for a short period before gradually decreasing (Figure 6). Therefore, to ensure maximum sensitivity while maintaining signal stability and reproducibility, a measurement time of 5 min after reagent addition was selected for subsequent analyses.

Overall, the method using basic hydrolysis of BIS in a 40% (v/v) ethanolic aqueous co-solvent with the optimized F–C reaction conditions and measurement at 740 nm after 5 min, resulted in clear solutions and stable color development. These conditions were therefore adopted for method validation and the indirect quantitative determination of BIS via BHPM formation.

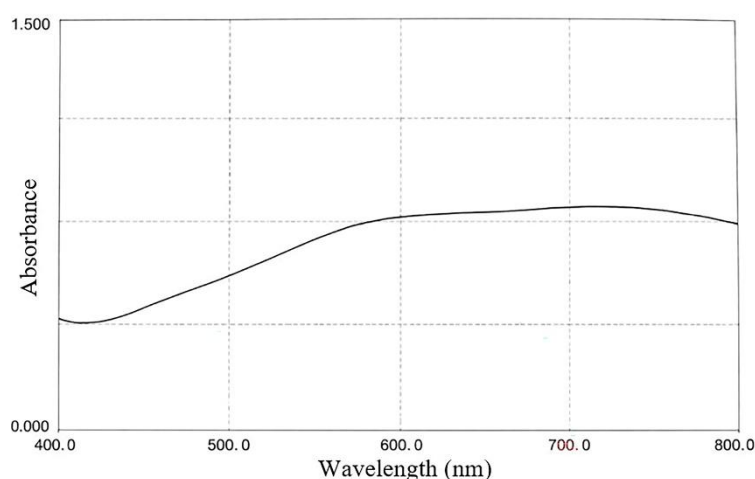


Figure 5 Vis absorption spectrum of the F–C–BHPM complex.

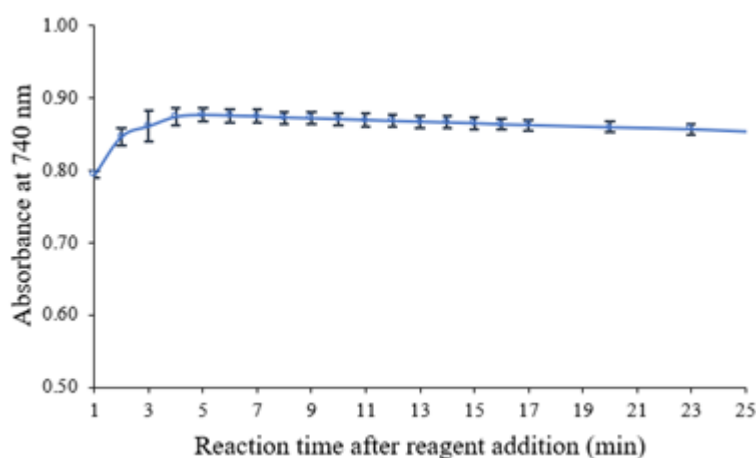


Figure 6 Time course of blue color formation after complete addition of the reagents.

2. Analytical performance

The validation parameters of the proposed F–C method for the BIS assay in bulk drug are summarized in Table 1. A strong linear relationship between color response and BIS concentration was obtained over the range of 8–24 $\mu\text{g/mL}$, with a correlation coefficient (R^2) of 0.9979. Residual analysis was performed in accordance with ICH recommendations to assess the suitability of the linear regression model, and the residuals were randomly distributed, supporting the appropriateness of the linear model over the studied concentration range. The increase in blue color intensity with increasing BIS concentration is illustrated in Figure 7. The LOD and LOQ were determined to be 0.91 and 2.77 $\mu\text{g/mL}$, respectively, indicating adequate sensitivity and reliable quantification at low concentration levels.

The method demonstrated good accuracy, with mean recoveries ranging from 99.1% to 101.4% across the tested concentration levels, and high precision, as indicated by %RSD values below 2% for both intra-day (repeatability) and inter-day (intermediate precision) measurements.

Method specificity was evaluated by comparing the UV–Vis absorption spectra of standard and bulk sample solutions following the F–C reaction. Both spectra exhibited similar broad absorption bands in the visible region, while the blank solution showed negligible absorbance at the measurement wavelength. Robustness was assessed by introducing small deliberate variations in reaction time (4–6 min) and measurement wavelength (735–745 nm). No appreciable changes in analytical response were observed under these conditions, with %RSD values remaining below 2%, indicating that the proposed method is robust.

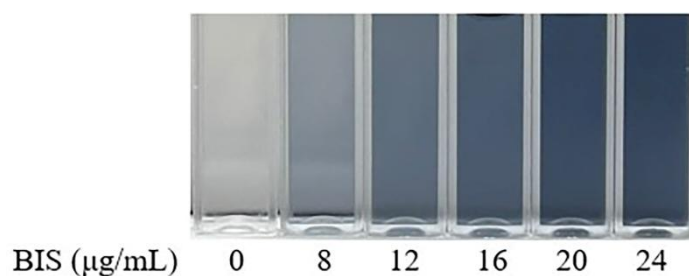


Figure 7 Visual appearance of F–C reaction mixtures at increasing BIS concentrations (0–24 $\mu\text{g/mL}$).

Table 1 Validation parameters of the proposed F–C method

Parameters	Results
Linearity	0.9979 (8–24 $\mu\text{g/mL}$)
LOD	0.91 $\mu\text{g/mL}$
LOQ	2.77 $\mu\text{g/mL}$
Accuracy (%Recovery, $n = 3$ for each level)	101.4 $\pm$ 1.3 % (at 50% level), 99.8 $\pm$ 1.9 % (at 100% level), 99.1 $\pm$ 1.0 % (at 150% level)
Inter-day precision ($n=12$)	0.9 % RSD
Intra-day precision ($n=6$)	0.7 % RSD
Robustness	
▪ reaction time (4, 5, and 6 min) ($n=18$)	1.7 % RSD
▪ measurement wavelength (735, 740, and 745 nm) ($n=18$)	1.6 % RSD

The analytical performance of the proposed F–C method was compared with previously reported colorimetric spectrophotometric methods for the determination of BIS. The method exhibited moderate sensitivity, with LOD and LOQ values of 0.91 and 2.77 µg/mL, respectively. While some reported methods demonstrated lower detection limits^{15,16}, other earlier studies did not explicitly report LOD and LOQ values and instead described linear concentration ranges within the low µg/mL range.^{13,14,17} Nevertheless, the LOQ achieved by the proposed method was sufficient for reliable quantification within the working concentration range of the assay. The method also demonstrated good accuracy (99.1–101.4%) and precision (RSD < 2%), which are consistent with previous reports, where recoveries were generally close to 100% and precision values typically remained below 2% RSD.

In terms of detection principle, most previously reported methods rely on oxidative coupling reactions, dye systems, ion-pair formation, or charge-transfer complexation.^{13–17} For example, methods involving bromate–bromide oxidation¹³, sulfonephthalein dye complexes¹⁴, or cerium(IV)-mediated reactions¹⁷, often require multiple reaction steps, strict control of reaction conditions, and/or the use of strong oxidizing agents or organic solvents. These requirements may increase procedural complexity, particularly for routine analytical applications.

Regarding specificity, several previously reported methods demonstrated minimal interference from common pharmaceutical excipients or relied on relatively selective reaction conditions.^{13,16,17} For example, oxidative coupling methods reported negligible interference from tablet excipients.^{13,17} In contrast, the proposed method is based on the reaction between the F–C reagent and hydrolyzed phenolic derivatives of BIS, which may exhibit broader reactivity toward other phenolic compounds. Consequently, interference from phenolic substances may occur if the method is

applied to more complex matrices without prior separation. Furthermore, because the F–C reagent responds to phenolic species in a non-specific manner, interference from structurally related compounds, degradation products, or specified impurities that generate phenolic derivatives under the hydrolysis conditions cannot be completely excluded. Therefore, the proposed method should not be regarded as a stability-indicating method and may not be suitable for applications requiring discrimination between BIS and related substances. However, because the intended application of the method is the analysis of BIS in bulk pharmaceutical material, where interfering matrix components are expected to be limited, the broader reactivity of the F–C reagent may have limited impact on its applicability within this context.

Additionally, the proposed method employs a relatively simple indirect approach involving basic hydrolysis of BIS to its phenolic derivative, followed by a F–C redox reaction performed in a predominantly aqueous medium. Color development is rapid within 5 min, and the method reduces reliance on hazardous reagents while minimizing organic solvent consumption, potentially reducing environmental burden and maintaining cost-effectiveness.

Conclusion

A simple and cost-effective F–C based colorimetric spectrophotometric method was developed and validated for the indirect determination of BIS in bulk pharmaceutical material through basic hydrolysis to its phenolic derivative, BHPM. The method demonstrated satisfactory analytical performance in accordance with ICH Q2(R2) guidelines, with acceptable specificity, accuracy, precision, sensitivity, and robustness within the intended application scope. Owing to its straightforward procedure, rapid color development, and minimal reagent consumption, the proposed method may provide a practical and cost-effective

alternative for routine quality control analysis of BIS in bulk pharmaceutical material, particularly in resource-limited settings. Further comparative studies with compendial chromatographic methods would be valuable to evaluate analytical equivalence. The application of the proposed method to pharmaceutical dosage forms or more complex matrices may be explored in future studies following appropriate sample preparation and assessment of potential matrix interference.

Acknowledgements

The authors extend their sincere gratitude to Medicine Supply Company Limited, Thailand, for providing the drug, and to the Faculty of Pharmacy, Silpakorn University, for financial assistance and for providing facilities to support the senior project of academic year 2025.

References

- Corsetti M, Landes S, Lange R. Bisacodyl: A review of pharmacology and clinical evidence to guide use in clinical practice in patients with constipation. *Neurogastroenterol Motil.* 2021;33(10):e14123.
- United States Pharmacopeial Convention. Bisacodyl monograph. In: United States Pharmacopeia 43–National Formulary 38 (USP43–NF38). Rockville (MD): United States Pharmacopeial Convention; 2020.
- Bradshaw KM, Burnett J, Sidhu AS. High-performance liquid chromatographic determination of bisacodyl in pharmaceutical dosage forms marketed in Australia. *J Pharm Biomed Anal.* 1995;13(11):1355–62.
- Jabar EG, Taghi HS, Hasson KJ, Alkhalifa II. Evaluation of innovated formula of bisacodyl suppository following the dissolution profile and stability data by using a developed HPLC method. *J Adv Pharm Educ Res.* 2020;10(2):5–10.
- Valenti LP, Lau-Cam CA. Reverse-phase liquid chromatographic determination of bisacodyl in dosage forms. *J Assoc Off Anal Chem.* 1985;68(3):529–32.
- Falone MF, Salamanca-Neto CAR, Moraes JT, Sartori ER. Electrochemical evaluation and voltammetric determination of

- laxative drug bisacodyl on boron-doped diamond electrode. *Measurement.* 2019;137:464–9.
- Ibrahim MM, Kelani KM, Ramadan NK, Elzanfaly ES. Smartphone as a portable detector for thin-layer chromatographic determination of some gastrointestinal tract drugs. *ACS Omega.* 2022;7(27):23815–20.
- Perkins SL, Livesey JF. A rapid high-performance thin-layer chromatographic urine screen for laxative abuse. *Clin Biochem.* 1993;26(3):179–81.
- Duncan A, Phillips JJ. Evaluation of thin-layer chromatography methods for laxative detection. *Ann Clin Biochem.* 2001;38(1):64–6.
- Ahmed NR, Al-Etewi MJE. Eco-friendly method for the estimation of bisacodyl in pharmaceutical preparations and environmental wastewater samples: Application to content uniformity testing. *J Phys Chem Res.* 2023;5(2):1–3.
- Rivai H, Pratama N, Asra R. Development and validation of bisacodyl analysis method in tablet with absorbance method and area under curves method in ultraviolet spectrophotometry. *Int J Pharm Sci Med.* 2017;2(12):1–8.
- Elvis AM, Deepali MG. Development and validation of UV spectrophotometric method for determination of bisacodyl in suppositories. *Int J Pharm Tech Res.* 2011;3(1):193–6.
- Baraka MM, Elsadek ME, Ibrahim AM. Spectrophotometric determination of bisacodyl in pure form and tablet form. *Asian J Pharm Anal Med Chem.* 2015;3(1):1–13.
- Abdel-Hay MH, Sabry SM, Barary MH, Belal TS. Spectrophotometric determination of bisacodyl and piribedil. *Anal Lett.* 2004;37(2):247–62.
- Mohammed IS, Nasser KA, Mhemeed AH. Spectrophotometric determination of bisacodyl in pure and pharmaceutical preparation via oxidative coupling organic reaction. *Baghdad Sci J.* 2017;14(1):181–7.
- Ahmad NR, Majid NA. Indirect spectrophotometric method for the determination of bisacodyl in commercial dosage forms and in environmental water samples. *Iraqi J Pharm.* 2011;11(2):77–84.
- Sastry CSP, Prasad AVSS, Naidu PY. Spectrophotometric methods for the determination of bisacodyl in pharmaceutical preparations. *Indian Drugs.* 1997;34(11):637–40.
- Pérez M, Domínguez-López I, Lamuela-Raventós RM. The chemistry behind the Folin–Ciocalteu method for the

estimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern. *J Agric Food Chem.* 2023;71(46):17543–553.

19. de Wolff FA, de Haas EJ, Verweij M. A screening method for establishing laxative abuse. *Clin Chem.* 1981;27(6):914–7.