Indirect Spectrofluorimetric Determination of Captopril and Clopidogrel Bisulfate in Pure and Pharmaceutical Formulations using 4,5-Dibromofluorescein

Reem A. Al-Luhaiby†,‡*, Subhi M. J. Al-Mtwaiti‡**

† Department of Pharmaceutical Chemistry, College of Pharmacy, Ninevah University, Al-Jawsaq, Mosul 41002, Iraq; *e-mail: reem.23esp52@student.uomosul.edu.iq;
‡ Department of Chemistry, College of Education for Pure Sciences, University of Mosul, Al-Majmoaa Street, Mosul 41002, Iraq; **e-mail: drsubhi74@uomosul.edu.iq

Received: April 10, 2025; Accepted: July 10, 2025
DOI: 10.17721/moca.2025.221-227

A novel, simple, and selective indirect spectrofluorimetric method was developed for the quantitative determination of captopril (CPL) and clopidogrel bisulfate (CLO) in both bulk materials and pharmaceutical formulations. The method is based on the fluorescence quenching of 4,5-dibromo fluorescein (DBF) at 540 nm (λex = 330 nm) caused by the formation of non-fluorescent ion-pair complexes between DBF and the studied drugs. The decrease in fluorescence intensity (ΔF) showed a linear correlation with drug concentration in the ranges of 0.4–14 μg/mL for CPL and 0.4–8 μg/mL for CLO. The method demonstrated high accuracy, with mean recoveries of 99.7% and relative standard deviations ranging from 1.11 to 4.12%. Application to commercial tablets confirmed the suitability of the method, with results in excellent agreement with certified values and those obtained by the standard addition technique. Common pharmaceutical excipients did not interfere with the assay. Owing to its simplicity, sensitivity, and lack of prior extraction steps, the proposed method represents a practical alternative to more complex and solvent-intensive techniques.

Keywords: spectrofluorimetry, captopril, clopidogrel bisulfate, 4,5-dibromofluorescein

Introduction

Captopril (CPL), 1-(3-mercapto-2-D-methyl-1-oxopropyl)-L-proline ($C_9H_{15}NO_3S$, molecular weight 217.29 g/mol), is one of the first angiotensin-converting enzyme (ACE) inhibitors introduced for clinical use. It is widely prescribed for the treatment of hypertension, chronic heart failure, and diabetic nephropathy [1]. Because of its importance in cardiovascular therapy, many analytical methods have been developed for CPL determination. Reported techniques include spectrophotometry [2–5], spectrofluorimetry [6,7], atomic absorption spectroscopy [8], FT-Raman spectroscopy [9], and chromatography [10–12]. Each of these methods has its own merits, but some require expensive instruments, long analysis times, or lack sensitivity.

Clopidogrel bisulfate (CLO), methyl-2-chloro-phenyl-(4,5,6,7-tetrahydrothieno[3,2-c]pyridin-5-yl) acetate bisulfate ($C_{16}H_{16}ClNO_2S$, molecular weight 321.82 g/mol), is a widely used antiplatelet drug. It prevents platelet aggregation by blocking ADP binding to its receptor and is prescribed to reduce the risk of myocardial infarction and ischemic stroke [13]. Compared with aspirin, CLO shows higher efficacy and a lower risk of gastrointestinal bleeding. Several analytical techniques have been reported for CLO determination, including spectrophotometry [14–22], spectrofluorimetry [23], chromatography [24–29], and voltammetry [30]. As with CPL, many of these methods face challenges such as cost, complex operation, or limited sensitivity.

High-performance liquid chromatography (HPLC) is considered a reference method for pharmaceutical analysis, but it has disadvantages. It consumes large amounts of organic solvents, increases analysis cost, and requires trained personnel. Electrochemical techniques also offer high sensitivity, but they often require specialized electrode preparation and expertise. Conventional spectrophotometric methods are easier but usually indirect and less sensitive.

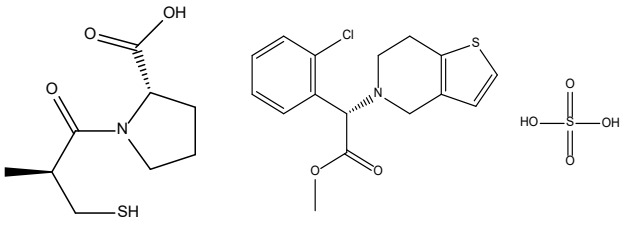
Spectrofluorimetric methods offer an attractive alternative. They are fast, simple, and sensitive. The instruments are relatively inexpensive and easy to operate, making them suitable for routine quality control in resource-limited laboratories. In many cases, fluorimetry provides detection limits comparable to chromatographic techniques. For example, sensitive fluorimetric assays have been reported for the determination of sulfonamides in pharmaceutical formulations [6], antihistamines in plasma [7], and non-steroidal anti-inflammatory drugs in biological fluids [23]. In all of these studies, fluorescence quenching or enhancement by specific dyes provided reliable analytical responses without the need for complex

sample preparation.

Compared with HPLC, fluorimetric assays generally require smaller volumes of solvents and simpler sample handling, which reduces both cost and environmental impact. Recent studies have shown that fluorimetric methods can provide recoveries above 98% and relative standard deviations below 3% in drug formulations, with performance comparable to HPLC [14,19]. Another strength is their versatility: they have been applied in clinical labs for therapeutic drug monitoring [18], in forensic labs for detecting drugs of abuse [12], and in environmental studies for tracing pharmaceuticals in water [22].

4,5-Dibromofluorescein (DBF) is a fluorescent dye that can interact with analytes containing functional groups, such as carboxyl, thiol, or sulfonate groups. These interactions form ion-pair complexes that reduce (quench) the fluorescence of DBF. This quenching effect can be used to indirectly quantify the analyte. For CPL, the carboxyl group interacts with DBF, while for CLO, the bisulfate group plays a key role in complex formation. Both interactions produce measurable decreases in fluorescence, which makes DBF a suitable probe for their determination.

The purpose of this study is to propose a simple, sensitive, and rapid spectrofluorimetric method for the determination of CPL and CLO in pure form and pharmaceutical tablets, based on fluorescence quenching of DBF. The method avoids time-consuming extraction, does not require large volumes of organic solvents, and therefore meets the principles of green analytical chemistry. It provides a reliable alternative to existing methods and can be applied for routine pharmaceutical quality control.



Captopril Clopidogrel bisulfate

Figure 1. Chemical structures of CPL and CLO.

Experimental part

Materials and reagents

All chemicals and reagents were of analytical grade and used without further purification. Pharmaceutically pure captopril (CPL, ≥99%) and clopidogrel bisulfate (CLO, ≥99%) were kindly supplied by the State Company for Drugs Industry and Medical Applications (SDI, Samarra, Iraq). 4,5-Dibromofluorescein (DBF, ≥98%) was obtained from BDH (UK). Acetonitrile (HPLC grade, ≥99.9%) was purchased from Sigma-Aldrich (Germany). Distilled–deionized water (resistivity ≥18.2 MΩ·cm) was used throughout all experiments.

Instrumentation

Fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer (Japan) equipped with a xenon lamp and 1 cm path-length quartz cells. An analytical balance (Kern ABS 120-4, Germany) was employed for weighing. Dissolutions were assisted by an ultrasonic bath (Power Sonic 405, Korea). Reaction mixtures were thermostated using a BS-11 Lab Companion water bath (Korea). pH values were measured with a Thermo-RL 060 portable pH meter (Singapore) equipped with a combined glass electrode.

Preparation of solutions

Stock standard solutions of CPL and CLO (100 μg/mL) were prepared by accurately weighing 0.0100 g of each drug into 100 mL calibrated flasks and dissolving in distilled water. A stock solution of DBF dye (100 μg/mL) was prepared by dissolving 0.0100 g in 50 mL acetonitrile, transferring into a 100 mL volumetric flask, and diluting to volume with distilled water.

For the preparation of pharmaceutical formulations, five tablets of each drug were accurately weighed, finely powdered, and homogenized. An amount equivalent to a single tablet was transferred into a 100 mL volumetric flask, dissolved in 5 mL ethanol, and diluted to volume with distilled water. The suspensions were sonicated for 5 min and filtered. The obtained stock solutions corresponded to 250 μg/mL CPL and 750 μg/mL CLO, respectively. Aliquots were further diluted with distilled water to obtain working solutions of 100 μg/mL. Final concentrations of 2–8 μg/mL CPL and 2–4 μg/mL CLO were prepared for analysis.

General procedure

Aliquots of CPL or CLO stock solutions (100 μg/mL) were transferred into a series of 25 mL volumetric flasks containing 8 μg/mL DBF solution. The mixtures were diluted to the mark with distilled water, equilibrated in a thermostated water bath at 30 °C for 10 min, and the fluorescence intensity was measured at λex = 330 nm and λem = 540 nm against a reagent blank. Under these conditions, DBF alone produced a characteristic emission spectrum (Figure S1). A standard calibration curve was also constructed for DBF under identical conditions (Figure S2).

In addition, fluorescence measurements were performed by incremental addition of CPL or CLO to DBF solution under neutral, acidic (0.1 M HCl), and basic (0.1 M NaOH) media. These experiments were designed to assess the influence of medium on possible ion-pair complex formation between DBF and the studied drugs.

Results and Discussion

Optimization of experimental conditions

Effect of buffer solution

The influence of buffer solutions on the fluorescence response of DBF in the presence of CPL or CLO was evaluated. Buffers with pH 4.8, close to the natural

aqueous conditions of the system, were tested. The addition of 1 mL of buffer solutions caused a noticeable decrease in the extent of complex formation, leading to reduced quenching efficiency. Consequently, all subsequent experiments were performed in aqueous medium without the use of external buffers.

Effect of solvent

To investigate the role of solvent environment, the interaction of DBF with CPL and CLO was examined in water, ethanol, methanol, and acetonitrile. As summarized in Table 1, the solvents produced negligible changes in the excitation and emission wavelengths but significantly reduced the quenching efficiency (ΔF) compared to distilled water.

Table 1. Effect of solvents on the fluorescence quenching of DBF in the presence of CPL and CLO.

Solvent	ΔF	λ_{em} (nm)	λ_{ex} (nm)
	CLO		
Water	140.22	540	330
Ethanol	34.64	550	330
Methanol	26.30	550	332
Acetonitrile	98.46	550	332
	CPL		
Water	47.95	540	330
Ethanol	41.60	550	332
Methanol	32.41	547	332
Acetonitrile	46.78	553	332

For instance, ΔF values for CLO decreased from 140.22 in water to 26.30 in methanol. These findings confirm that water provides the most favorable medium, and it was therefore selected for the analytical procedure.

Effect of temperature

The effect of temperature on the stability of the drug–dye complexes was investigated at 20, 30, and 40 °C. As shown in Figure 2, maximum quenching efficiency was achieved at 30 °C, where the fluorescence intensity decreased markedly within 5 min and remained stable for more than 120 min. Higher or lower temperatures did not improve the quenching effect. Therefore, 30 °C was chosen as the optimum temperature for all further experiments.

Under the optimized conditions (aqueous medium, 30 °C, λ_{ex} = 330 nm), the fluorescence emission spectrum of DBF was recorded in the absence and presence of increasing concentrations of CPL and CLO. As illustrated in Figures S3, progressive addition of the drugs resulted in a concentration-dependent decrease in fluorescence intensity of DBF, confirming the formation of non-fluorescent ion-pair complexes.

Validation of the proposed method

Linearity and sensitivity

Under the optimized experimental conditions, calibration curves were constructed for CPL and CLO. To a series of 25 mL volumetric flasks containing 8 μg/mL DBF, increasing aliquots of the drug stock solutions (100 μg/mL) were added separately, diluted to volume with distilled water, and incubated at 30 °C for 10 min. The decrease in fluorescence intensity ($\Delta F = F_0 - F$) was measured at λ_{ex} = 330 nm and λ_{em} = 540 nm.

The calibration plots exhibited linear relationships in the ranges of 0.4–14 μg/mL for CPL and 0.4–8 μg/mL for CLO, Figure S4. Negative deviations were observed beyond these ranges, indicating the limits of linear applicability. The determination coefficients (R^2) were greater than 0.996, confirming excellent linearity. Analytical performance parameters are summarized in Table 2.

Accuracy and precision

The method demonstrated excellent accuracy, with mean recovery values of 99.7%. Precision, expressed as relative standard deviation (RSD), ranged from 1.11% to 4.12% for five different concentrations of CPL and CLO, confirming the reproducibility of the procedure.

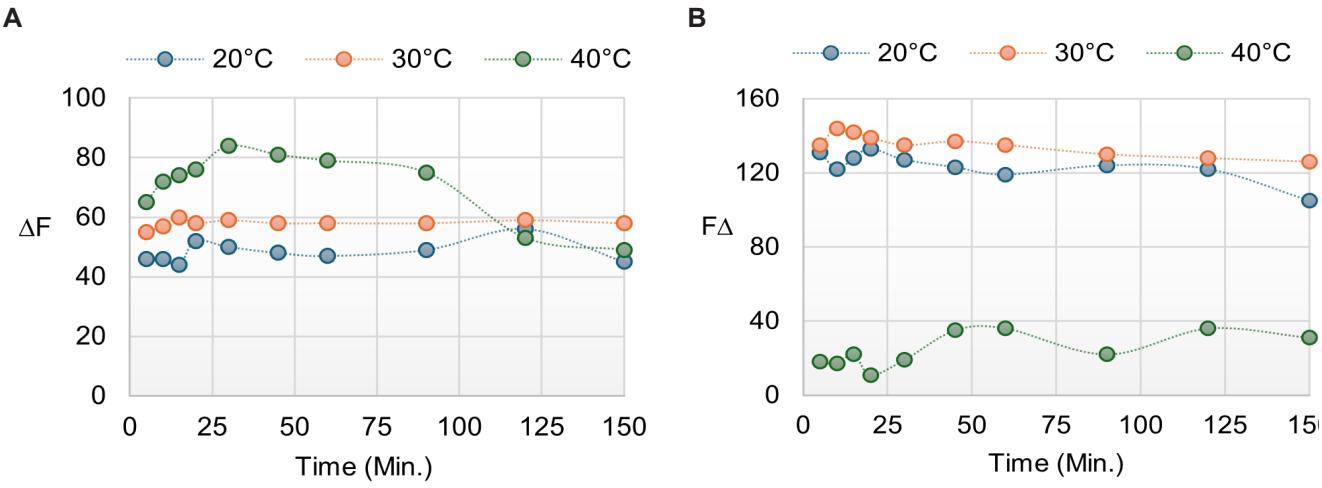


Figure 2. Effect of temperature on the fluorescence quenching of DBF in the presence of captopril (A) and clopidogrel bisulfate (B).

Table 2. Analytical parameters for the calibration of CPL and CLO.

Parameter	CPL	CLO
Linearity range (μg/mL)	0.4–14	0.4–8
Slope	11.237	25.809
Intercept	6.3506	20.222
R^2	0.9988	0.9962
LOD (μg/mL)	0.263	0.061
LOQ (μg/mL)	0.796	0.185

** LOD and LOQ values were calculated as 3.3σ/s and 10σ/s, respectively, where σ is the standard deviation of replicate blank measurements and s is the slope of the calibration curve.*

Stoichiometry of the complex

The molar ratio method measures changes in fluorescence intensity at a fixed dye concentration while gradually increasing the drug concentration. By plotting the response against the drug-to-dye ratio, the inflection point reveals the stoichiometry of the complex. Both experiments were performed with equimolar dilute solutions (2×10^{-4} mol/L) under neutral aqueous conditions. The results (Figures S4 and S5) consistently showed a 1:1 drug-to-dye ratio for both captopril and clopidogrel bisulfate, indicating that each drug molecule binds to one DBF molecule to form a stable ion-pair complex. The consistency between the two independent methods confirms that DBF fluorescence quenching by CPL and CLO results from specific complex formation rather than nonspecific effects. This reproducible stoichiometry supports the analytical reliability of the proposed spectrofluorimetric method.

Proposed reaction mechanism

The quenching of 4,5-dibromo fluorescein (DBF) fluorescence by captopril (CPL) and clopidogrel bisulfate (CLO) is attributed to the formation of stable ion-pair complexes. This mechanism is supported by the structural features of both drugs, stoichiometric evidence from Job's and molar ratio methods (Figures S4 and S5), and earlier reports on dye–drug interactions [32].

For CPL, the free carboxyl group and thiol functionality are central to complex formation. The carboxyl group interacts electrostatically with the anionic DBF moiety, while hydrogen bonding likely provides extra stabilization. This results in a 1:1 complex that quenches DBF fluorescence through non-radiative energy transfer (Figure S7a).

For CLO, the sulfonate counter-ion interacts strongly with DBF via electrostatic attraction. In addition, π–π stacking between the aromatic ring of CLO and the xanthene ring of DBF may further stabilize the complex. The stoichiometry was again 1:1, with clear quenching of DBF emission at 540 nm (Figure S7b).

In both systems, the observed quenching reflects the formation of non-fluorescent complexes that act as efficient energy sinks. Notably, this effect occurred only in neutral aqueous medium. Under strongly acidic or basic conditions, protonation or deprotonation prevented stable complex formation, and DBF fluorescence was preserved.

Overall, these findings confirm that CPL and CLO interact with DBF through complementary electrostatic and hydrogen-bonding interactions. The resulting quenching, directly proportional to drug concentration, provides the analytical basis for the proposed spectrofluorimetric method.

Application to pharmaceutical preparations

The applicability of the proposed spectrofluorimetric method was assessed by analyzing commercial tablet formulations of CPL and CLO from different manufacturers. As shown in Table 3, the method provided highly accurate results, with mean recovery values close to 100% (average 101.4%) and relative standard deviations (RSD) below 2.1%. Statistical comparison using the *t*-test confirmed that there was no significant difference between the certified values and the results obtained by the present method, indicating excellent agreement.

To confirm the reliability of the proposed spectrofluorimetric procedure in the presence of formulation excipients, the standard addition method was applied to commercial tablet preparations of CPL and CLO. Calibration curves constructed by spiking known amounts of pure drug into tablet extracts showed excellent linearity, with correlation coefficients ($R^2 \geq 0.995$) for all tested formulations.

The recovery values obtained (Table 4) were in close agreement with those determined by the direct calibration procedure, confirming that common pharmaceutical excipients did not interfere with the assay. These results demonstrate the robustness of the method for routine application to quality control of commercial products.

Representative standard addition calibration plots are presented in Figure S8, which illustrate the linear response of fluorescence quenching (ΔF) with increasing drug concentration for different commercial sources of captopril and clopidogrel bisulfate tablets.

Comparison with reported methods

A comparison of the proposed method with earlier procedures for CPL and CLO determination is shown in Table S1. For CPL, the DBF-based fluorimetric method achieved a lower LOD (0.263 μg/mL) and a wider linear range (0.4–14 μg/mL) than the ortho-phthaldehyde fluorimetric method [7]. For CLO, it showed higher sensitivity (LOD = 0.061 μg/mL) than the reported charge-transfer method with Chloranel [33], while maintaining similar accuracy and correlation. Overall, these findings demonstrate the superior sensitivity and practical value of the proposed method for routine pharmaceutical analysis.

Conclusion

A simple, rapid, and sensitive indirect spectrofluorimetric method was developed for determining captopril (CPL) and clopidogrel bisulfate (CLO) in bulk and tablet formulations. The assay is based on fluorescence quenching of 4,5-dibromo fluorescein (DBF) at λex = 330 nm and λem = 540 nm through the formation of 1:1 ion-pair complexes with the drugs at 30 °C.

The method showed excellent linearity over 0.4–14 μg/mL for CPL and 0.4–8 μg/mL for CLO, with low detection limits (0.263 and 0.061 μg/mL, respectively). High accuracy (mean recovery ≈ 99.7%) and precision (RSD = 1.11–4.12%) were achieved. Application to commercial tablets confirmed agreement with certified values, with no interference from common excipients.

Compared with previous methods, this approach offers higher sensitivity, lower solvent use, and simpler operation, in line with green analytical chemistry principles. Its robustness makes it suitable for routine quality control and, with further validation, potentially applicable to biological samples such as blood and urine.

Table 3. Determination of CPL and CLO in pharmaceutical preparations using the proposed method.

Pharmaceutical preparation	Certified value (mg)	Amount taken (μg/mL)	Drug content found (mg)	Recovery %	Average recovery %	*t*-test
		Captopril				
Captoneer Tab 25 mg (Pioneer, Iraq)	25	2	26.00	104.00	101.28	1.09
		4	24.37	97.48		
		8	25.59	102.36		
Aceprotin Tab 50 mg (Medochemie, Cyprus)	50	2	50.25	100.50	98.66	1.42
		4	49.12	98.24		
		8	48.62	97.24		
		Clopidogrel bisulfate				
Plavineer Tab 75 mg (Pioneer, Iraq)	75	2	76.12	101.49	102.87	2.08
		3	77.74	103.65		
		4	77.62	103.49		
Plagrine Tab 75 mg (Micro, India)	75	2	76.12	101.49	102.79	1.06
		3	77.74	103.65		
		4	77.43	103.24		

Average of five determinations

Table 4. Results of the standard addition procedure for the determination of CPL and CLO.

Pharmaceutical preparation	Certified value (mg)	Amount taken (μg/mL)	Drug content found (mg)	Recovery % (standard addition)	Recovery % (present method)
		Captopril			
Captoneer Tab 25 mg (Pioneer, Iraq)	25	2	24.98	96.07	104.0
		4	25.01	102.62	97.5
Aceprotin Tab 50 mg (Medochemie, Cyprus)	50	2	49.98	99.46	100.5
		4	50.02	101.83	98.2
		Clopidogrel bisulfate			
Plavineer Tab 75 mg (Pioneer, Iraq)	75	2	75.01	98.54	101.5
		3	75.02	96.50	103.6
Plagrine Tab 75 mg (Micro, India)	75	2	74.97	98.48	101.5
		3	75.02	96.50	103.6

Average of five determinations

References

1. Davis, R.; Ribner, H. S.; Keung, E.; Sonnenblick, E. H.; LeJemtel, T. H. Treatment of Chronic Congestive Heart Failure with Captopril, an Oral Inhibitor of Angiotensin-Converting Enzyme. *N. Engl. J. Med.* **1979**, *301* (3), 117–121. https://doi.org/10.1056/NEJM197907193010301.

2. El-Enany, N.; Belal, F.; Rizk, M. Novel Spectrophotometric Method for the Assay of Captopril in Dosage Forms Using 2,6-Dichloroquinone-4-Chlorimide. *Int. J. Biomed. Sci.* **2008**, *4* (2), 147.

3. Tu, C.; Wen, X. Spectrophotometric Determination of Captopril in Pharmaceutical Sample by Phosphomolybdic Blue. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *218* (1), 012141. https://doi.org/10.1088/1755-1315/218/1/012141.

4. Kokhasmail, D. M.; Tahir, T. F.; Azeez, K. F. Spectrophotometric Indirect Determination of Captopril through Redox Reaction with N-Bromosuccinimide and RB Dye in Pharmaceutical Products. *ARO Sci. J. Koya Univ.* **2020**, *8* (2), 8–14. https://doi.org/10.14500/aro.10662.

5. Dawood, N. H.; Ali, N. H. Spectrophotometric Determination of Captopril in Pharmaceutical Formulations Based on Ion-Pair Reaction with Congo Red. *J. Pharm. Negat. Results* **2022**, *13*, 1–7.

6. El-Didamony, A. M. Spectrofluorimetric Determination of the Hypertensive Drug Captopril Based on Its Oxidation with Cerium(IV). *J. Chin. Chem. Soc.* **2009**, *56* (4), 755–762. https://doi.org/10.1002/jccs.200900112.

7. Derayea, S. M.; Nagy, D. M.; El-Din, K. M. B.; Attia, T. Z.; Samir, E.; Mohamed, A. A.; Hamad, A. A. Green Innovative Fluorescence Approach for the Feasible and Reliable Assay of Thiol-Containing Drugs; Captopril as a Model. *RSC Adv.* **2022**, *12* (27), 17607–17616. https://doi.org/10.1039/D2RA02408J.

8. El Reis, M. A.; Abou Attia, F. M.; Kenawy, I. M. M. Indirect Determination of Captopril by AAS. *J. Pharm. Biomed. Anal.* **2000**, *23* (2–3), 249–254. https://doi.org/10.1016/S0731-7085(00)00286-7.

9. Mazurek, S.; Szostak, R. Quantitative Determination of Captopril and Prednisolone in Tablets by FT-Raman Spectroscopy. *J. Pharm. Biomed. Anal.* **2006**, *40* (5), 1225–1230. https://doi.org/10.1016/j.jpba.2005.03.047.

10. Kuśmierek, K.; Bald, E. A Simple Liquid Chromatography Method for the Determination of Captopril in Urine. *Chromatographia* **2007**, *66*, 71–74. https://doi.org/10.1365/s10337-007-0254-6.

11. Sultana, N.; Arayne, M. S.; Naveed, S. RP-HPLC Method for Simultaneous Determination of Captopril and Diuretics: Application in Pharmaceutical Dosage Forms and Human Serum. *J. Chromatogr. Sep. Tech.* **2011**, *2* (109), 1–5. http://dx.doi.org/10.4172/2157-7064.1000109.

12. Rastkari, N.; Khoobi, M.; Shafiee, A.; Khoshayand, M. R.; Ahmadkhaniha, R. Development and Validation of a Simple and Sensitive HPLC–UV Method for the Determination of Captopril in Human Plasma Using a New Derivatizing Reagent 2-Naphthyl Propiolate. *J. Chromatogr. B* **2013**, *932*, 144–151. https://doi.org/10.1016/j.jchromb.2013.06.019.

13. Chan, F. K.; Ching, J. Y.; Hung, L. C.; Wong, V. W.; Leung, V. K.; Kung, N. N.; et al. Clopidogrel versus Aspirin and Esomeprazole to Prevent Recurrent Ulcer Bleeding. *N. Engl. J. Med.* **2005**, *352* (3), 238–244. https://doi.org/10.1056/NEJMoa042087.

14. Prasanan, L.; Naidu, G.; Fathima, N.; Chakravathy, I.; Hug, G. Spectrophotometric Determination of Clopidogrel in the Presence of Aspirin by Charge Transfer Complex Method Using 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone (ODQ). *Int. J. Pharm. Res.* **2018**, *12* (2), 138–147.

15. Antypenko, L.; Gladysheva, S.; Vasyuk, S. Development and Validation of Clopidogrel Bisulphate Determination in Bulk by UV Spectrophotometric Method. *Scripta Sci. Pharm.* **2016**, *3* (2), 17–22. http://dx.doi.org/10.14748/ssp.v3i2.1704.

16. Babu, K.; Seklar, D.; Kumari, N.; Rao, V. Novel Spectrophotometric Methods for the Determination of Clopidogrel Bisulphate in Bulk and Pharmaceutical Formulations. *Der Pharm. Lett.* **2015**, *7* (3), 241–246.

17. Harbi, S.; Salim, A.; Missa, Y. Quantitative Determination of Clopidogrel Bisulphate Using Validated Spectrophotometric Methods. *Asian J. Adv. Res.* **2020**, *4* (1), 14–24.

18. Saberin, H.; Yousry, I.; Alyaa, S. Spectrophotometric Determination of Clopidogrel Bisulfate. *Amazon Sci. Publ.* **2015**, ISBN 3659744921.

19. Vishwas, T. S.; Gurupadayya, B. M.; Jain, R. Simultaneous Estimation of Atorvastatin and Clopidogrel by Simultaneous Equation Method in Capsule Dosage Form. *Int. J. Pharm. Sci. Res.* **2019**, *10* (7), 3305–3310. https://doi.org/10.21276/ijcpa.

20. Elsonbaty, A.; Elssa, M.; Hassan, W.; Abdulwahab, S. Separation-Free Spectrophotometric Platforms for Rapid Assessment of Combined Antiplatelet Therapy in Complex Matrices. *Bioanalysis* **2020**, *12* (5), 335–348. https://doi.org/10.4155/bio-2019-0293.

21. Mohamed, S.; Magdy, A.; Ahmed, A. Exploring the Nature of the Clopidogrel–Bromocresol Green Interaction via Spectrophotometric Measurements and Quantum Chemical Calculations. *RSC Adv.* **2018**, *8*, 29104–29114. https://doi.org/10.1039/C8RA05187A.

22. Al-Rashidee, N.; Mohammad, M.; Alobeady, M. A. Spectrophotometric Determination of Atorvastatin, Clopidogrel, and Rosuvastatin Using N-Bromosuccinimide and Congo Red Dye. *J. Glob. Sci. Res.* **2022**, *8* (2), 2926–2935. https://doi.org/10.5281/jgsr.2023.7698619.

23. Al-Rashidee, N. H. Spectrophotometric Determination of Some Pharmaceutical Compounds Used in the Treatment of Heart Diseases and the Follow-Up of Some Biochemical Changes. Ph.D. Thesis, University of Mosul, 2023.

24. Nagavi, J.; Gurupadayya, B. Stability-

Indicating UFLC Method for Uncoupling and Estimation of Impurities in Clopidogrel, Aspirin, and Omeprazole in Their Tablet Dosage Form Using PDA Detection. *Iran. J. Pharm. Sci.* **2016**, *12* (2), 43–60. https://doi.org/10.22037/ijps.v12.40777.

25. Sivagami, B.; Reddy, P.; Babumin, N. Method Validation and Stability Evaluation for the Simultaneous Estimation of Clopidogrel and Atorvastatin in Tablet Dosage Forms by RP-HPLC. *Austin J. Anal. Chem. Pharm. Chem.* **2018**, *5* (2), 1–8.

26. Nagarajan, S. Method Development and Validation of Clopidogrel Bisulphate by RP-HPLC in Bulk and Pharmaceutical Dosage Forms. *Int. J. Pharm. Anal. Res.* **2019**, *1* (1), 1–7.

27. Chandar, P.; Babualla, S.; Devi, V.; Srevannela, Ch. Development and Validation of HPLC Method for Determination of Clopidogrel in Human Plasma and Its Application to Pharmacokinetic Study. *Int. J. Sci. Eng. Res.* **2015**, *6* (2), 1–8.

28. Awdhut, D.; Kakde, B. Stability-Indicating Method Development and Validation for Estimation of Clopidogrel Bisulphate in Pharmaceutical Dosage Form by RP-HPLC. *Asian J. Pharm. Res.* **2020**, *10* (4), 244–248. https://doi.org/10.5958/2231-5691.2020.00044.1.

29. Naim, M.; Patel, N.; Ahmed, A. Stability-Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Clopidogrel and Rivaroxaban in Pharmaceutical Dosage Form. *Glob. J. Med. Res.* **2020**, *20* (1), 1–7.

30. Dermiș, S. A.; Aydoğan, E. Electrochemical Study of the Antiplatelet Agent Clopidogrel and Its Determination Using Differential Pulse Voltammetry in Bulk Form and Pharmaceutical Preparations with a Glassy Carbon Electrode. *Pharmazie* **2010**, *65* (3), 175–181. https://doi.org/10.1691/ph.2010.9123.

31. De Levie, R. *Principles of Quantitative Chemical Analysis*; McGraw-Hill International Edition: Singapore, 1997; p. 498.

32. Dena, A. S. A.; Hassan, W. M. I. Experimental and Quantum Mechanical Studies on Ion-Pair of Levocetirizine and Bromocresol Green in Aqueous Solution. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2016**, *163*, 108–114. https://doi.org/10.1016/j.saa.2016.03.030.

33. Kelechi, O.; Justina, N.; Alkachukwu, I. Spectrophotometric Determination of Clopidogrel in Pure Powder and Tablet. *Res. J. Chem. Environ. Sci.* **2015**, *3* (1), 65–69.