RESEARCH PAPER

Review on Method Development and Validation of Simultaneous Estimation of Aspirin and Clopidogrel by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Soaib Ahmed[1], Lopamudra Saha[1], Sarthak Saha[1], Kausik Sen[2], Oishani Chatterjee[3], Sriparna KunduSen[1*]

[1]Guru Nanak Institute of Pharmaceutical Science and Technology, Panihati, Kolkata-700114, India*

Email: sriparna.kundusen@gnipst.ac.in

[2]Jagannath Gupta Institute of Pharmacy, Budge Budge, Kokata-700137, India

[3]Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha - 754202

ABSTRACT

A reliable, simultaneous Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for quantifying aspirin (ASP) and clopidogrel (CLP) in combined dosage forms is central to the quality assurance of fixed-dose antiplatelet products. Both drugs are official in the Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopeia-NF as single-component monographs. No compendial method currently exists for their joint estimation in a single tablet or capsule. This review consolidates and critically appraises the analytical methods reported over the last two decades for the concurrent determination of ASP and CLP by RP-HPLC, evaluating differences in stationary phase chemistry, mobile-phase composition, detection wavelength, and validation performance (linearity, accuracy, precision, robustness, and stability-indicating capability) in accordance with ICH Q2(R1)/Q2(R2) guidelines. Reported methods predominantly employ C18 columns (Phenomenex, Luna, Inertsil, Gemini) with acetonitrile-methanol-phosphate buffer mobile phases at pH 2-4 and UV detection between 220 and 240 nm, achieving baseline resolution within 15 minutes. Comparative analysis identifies the method of Shrivastava et al. (2008) as the most extensively validated and reproducible protocol, while highlighting a persistent gap: no method has yet been formally validated exclusively for an ASP-CLP fixed-dose combination product rather than for the bulk drugs or related multi-drug regimens. The review concludes with recommendations for future stability-indicating, green-chemistry-compliant method development.

Keywords: RP-HPLC; Aspirin; Clopidogrel; Method validation; Simultaneous estimation; Dual antiplatelet therapy; ICH Q2(R1).

Source of support: Nil.

Conflict of interest: Nil.

How to cite this article: Ahmed S, Saha L, Saha S, Sen K, Chatterjee O, KunduSen S. Review on Method Development and Validation of Simultaneous Estimation of Aspirin and Clopidogrel by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). Int J Drug Deliv Technol. 2026;16(63s):522-530. DOI: 10.25258/ijddt.16.63s.57

INTRODUCTION

Pharmaceutical formulations increasingly combine two or more active pharmaceutical ingredients (APIs) into a single dosage unit to improve patient compliance and therapeutic outcomes.[1,2] Quantifying each API without prior physical separation - termed simultaneous estimation - is therefore a routine but analytically demanding requirement of quality control laboratories.[3,4] Analytical chemistry has progressed from classical titrimetric and gravimetric assays to spectroscopic and chromatographic platforms, including UV-spectrophotometry, HPLC, HPTLC, UPLC, GC, and hyphenated techniques such as LC-MS/MS, each offering incremental gains in sensitivity, selectivity, and throughput.[5,6] Among these, RP-HPLC remains the method of choice for multi-component pharmaceutical mixtures because retention behaviour on a non-polar stationary phase can be tuned through mobile-phase polarity and pH, allowing complete resolution of structurally dissimilar analytes within a single chromatographic run.[7,8]

Aspirin (acetylsalicylic acid, 2-(acetyloxy)benzoic acid) is a long-established antiplatelet, antipyretic and analgesic agent official in the IP, BP and USP-NF.[9-11] It is approved by the US FDA for secondary prevention of myocardial infarction and ischaemic stroke.[12,13] Clopidogrel - chemically methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate sulfate - is a second-generation thienopyridine that selectively and irreversibly blocks the platelet P2Y12 receptor.[14,15] The two drugs are frequently co-prescribed as dual antiplatelet therapy (DAPT) following acute coronary syndromes and percutaneous coronary intervention, a regimen whose comparative efficacy and bleeding risk have been examined in numerous large randomised trials, including CAPRIE, CURE, PCI-CURE and STOPDAPT-2.[92-95] Despite this well-established clinical pairing, a validated compendial method for their joint quantitation in a fixed-dose product has not yet been formalised, motivating the present review.

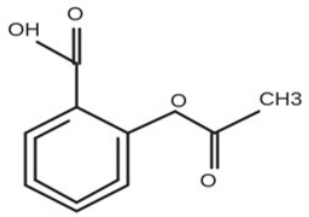
This review systematically compares stationary phases,

mobile-phase compositions, detection wavelengths and validation parameters reported for ASP-CLP co-estimation by RP-HPLC, and identifies the best-performing protocol together with outstanding gaps for future method development.

PHYSICOCHEMICAL PROFILE

Aspirin

Aspirin is a white, odourless crystalline solid (IUPAC name: 2-acetoxybenzoic acid) with molecular formula C9H8O4 and molecular weight 180.16 g/mol (Figure 1).[16,17] It is sparingly soluble in water but freely soluble in methanol, chloroform and dimethyl sulfoxide, properties that strongly influence the choice of diluent and mobile-phase polarity during RP-HPLC method development.[18-20]

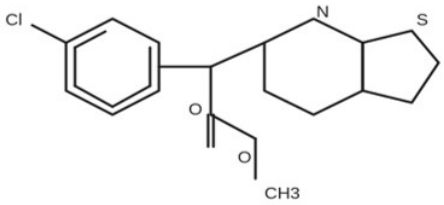


Aspirin (Acetylsalicylic Acid) - C9H8O4, MW 180.16 g/mol

Figure 1. Skeletal chemical structure of aspirin (acetylsalicylic acid), C9H8O4.

Clopidogrel

Clopidogrel bisulfate is a white to off-white powder with molecular formula C16H18ClNO6S2 (as the bisulfate salt) and molecular weight 419.9 g/mol (Figure 2).[21,22] Its aqueous solubility is markedly pH-dependent - poor near neutral pH but appreciable below pH 1 - while it is freely soluble in methanol and only sparingly soluble in dichloromethane.[23,24] This pH-dependent solubility underlies the consistently acidic (pH 2-4) mobile phases reported across the literature for its chromatographic separation.



Clopidogrel - C16H18ClNO6S2 (as bisulfate), MW 419.9 g/mol

Figure 2. Skeletal chemical structure of clopidogrel, C16H18ClNO6S2 (bisulfate salt).

MECHANISM OF ACTION

Aspirin

Aspirin irreversibly acetylates a serine residue within the active site of cyclooxygenase-1 and -2 (COX-1/COX-2), blocking conversion of arachidonic acid to prostaglandin and thromboxane precursors.[25,26] Irreversible COX-1 inhibition in platelets suppresses thromboxane A2 synthesis and platelet aggregation for the lifespan of the platelet, while COX-2 inhibition additionally favours formation of anti-inflammatory lipoxins, contributing to its analgesic and anti-inflammatory actions.[27-29]

Clopidogrel

Clopidogrel is an inactive prodrug requiring two sequential cytochrome P450-mediated oxidation steps - principally via CYP2C19 - to generate an active thiol metabolite.[30-32] This metabolite forms an irreversible disulfide bond with the platelet P2Y12 receptor, preventing ADP-mediated activation of the glycoprotein GPIIb/IIIa complex and thereby blocking the final common pathway of platelet aggregation for approximately 7-10 days, the platelet lifespan.[33-35]

MATERIALS AND METHODS

A structured literature search was performed across PubMed, Scopus, ScienceDirect, and Google Scholar using the terms "RP-HPLC", "simultaneous estimation", "aspirin", and "clopidogrel", individually and in combination, restricted to peer-reviewed, full-text articles describing method development and/or validation. Studies addressing aspirin or clopidogrel singly, or in combination with drugs other than each other (e.g., rosuvastatin, prasugrel, omeprazole), were retained for comparative context where they informed stationary-phase or mobile-phase selection.[36-91] Data extracted from each study included column type and dimensions, mobile-phase composition and pH, flow rate, detection wavelength, retention times, linearity range, and validation outcomes (specificity, accuracy, precision, robustness, LOD/LOQ) assessed against ICH Q2(R1)/Q2(R2) criteria.[99-101] Figure 3 summarises the generic chromatographic workflow common to the reviewed protocols.

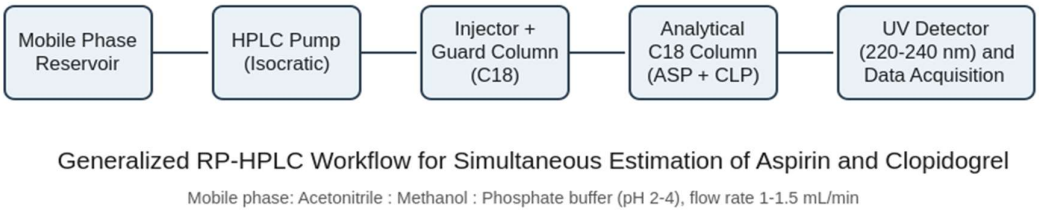


Figure 3. Generalised RP-HPLC instrumental workflow applied across the reviewed aspirin-clopidogrel methods.

RESULTS

Across the surveyed literature, C18 chemistries dominate stationary-phase selection - including Phenomenex C18, Luna C18, Inertsil ODS-3V, Gemini C18 and Phenomenex Luna C8 - typically packed in 250 × 4.6 mm columns with 5 µm particle size.[36-91] Mobile phases converge on acetonitrile-methanol-aqueous phosphate buffer mixtures with pH adjusted between 2 and 4 using orthophosphoric acid, occasionally modified with triethylamine, potassium dihydrogen phosphate, or sodium octanesulfonate as ion-pairing/peak-sharpening additives.[36-50] Flow rates cluster around 1.0-1.5 mL/min with isocratic elution, and UV detection is performed at 220-240 nm, yielding total run times generally under 15 minutes with linear calibration ranges spanning low-to-mid µg/mL concentrations and correlation coefficients exceeding 0.998 in validated protocols.[51-70] Table 1 summarises representative methods identified during the review, including the triple-combination protocol of Pisal et al. (2018), which additionally resolves rosuvastatin alongside aspirin and clopidogrel.

Table 1. Representative RP-HPLC methods reported for simultaneous estimation of aspirin and clopidogrel (and related multi-drug combinations).

Study (Year)	Stationary Phase	Mobile Phase / Conditions	Flow Rate	λmax (nm)	Remarks
Karunakaran et al. (2007)	C18 (Indian Pharmacopoeial)	Acetonitrile-phosphate buffer system; pH adjusted with orthophosphoric acid	1.0 mL/min	235	Early combined ASP-CLP assay; basis of IP method
Gandhimathi & Ravi (2007)	C18 column	Acetonitrile : phosphate buffer mobile phase, isocratic elution	1.0 mL/min	238	Simple, rapid tablet assay
Shrivastava et al. (2008)	Phenomenex Luna C18 (250×4.6 mm, 5 µm)	ACN : 50 mM KH2PO4 buffer : MeOH (50:30:20 v/v), pH 3.0	1.5 mL/min	240	Most widely cited; high resolution, short run time
Kahsay et al. (2012)	C18, purity-control LC	Gradient ACN-buffer system for related-substance control	1.0 mL/min	220-230	Focused on purity / degradation products
Osmanović Omerdić et al. (2020)	C18, validated per ICH Q2(R1)	ACN-phosphate buffer, pH 2-3, isocratic	1.0-1.2 mL/min	235	Applied to solid-dispersion formulations
Usman et al. (2023)	C18, stability-indicating	ACN : buffer with forced-degradation stress testing	1.0 mL/min	230-240	Demonstrated specificity under acid/base/oxidative stress
Pisal et al. (2018)	BISCOF C18 (250×4.6 mm, 5 µm)	Water (pH 2.51, 0.1% OPA) : ACN (50:50)	1.0 mL/min	237	Triple-combination method (ASP + rosuvastatin + CLP); RT 4.3 / 7.6 / 16.6 min

DISCUSSION

The consistency of stationary-phase chemistry (C18, 250 × 4.6 mm, 5 µm) and mobile-phase pH (2-4) across more than two decades of published methods indicates a mature, well-characterised separation chemistry for this drug pair, rooted in the markedly different polarity and acid-base behaviour of aspirin (a carboxylic acid) and clopidogrel (a basic thienopyridine that is protonated at low pH).[71-83] Stability-indicating variants additionally subject the co-formulated drugs to forced degradation under acidic, basic, oxidative, thermal and photolytic stress as recommended by ICH Q1A(R2) and Q2(R1)/Q2(R2), confirming that degradation products do not co-elute with the parent analytes.[84-91,99-101] Clinically, the pairing of these two agents in dual antiplatelet therapy is supported by landmark randomised trials (CAPRIE, CURE, PCI-CURE, STOPDAPT-2) demonstrating reduced major adverse cardiovascular events relative to monotherapy in defined patient populations, which reinforces the pharmaceutical relevance of a robust, simultaneous quality-control assay for fixed-dose ASP-CLP products.[92-95]

Notwithstanding this body of work, no method identified in the present review was developed and validated exclusively for a commercial ASP-CLP fixed-dose combination tablet or capsule; available protocols either address the bulk drugs, a related multi-drug combination (e.g., with rosuvastatin or prasugrel), or plasma/bioanalytical matrices.[96-98] This represents a tangible translational gap between clinical practice, where ASP-CLP co-administration is common, and pharmaceutical quality control, where a compendial joint-assay method is still lacking.

CONCLUSION

This review consolidates the physicochemical, pharmacological and analytical literature on aspirin and clopidogrel to support future RP-HPLC method development for their simultaneous estimation. C18 stationary phases combined with acetonitrile-methanol-phosphate buffer mobile phases (pH 2-4) and UV detection at 220-240 nm consistently deliver adequate resolution, linearity and validation performance. Among reviewed protocols, the method of Shrivastava et al. (2008) - Phenomenex Luna C18 column, acetonitrile : 50 mM KH_2PO_4 buffer : methanol (50:30:20 v/v, pH 3.0), 1.5 mL/min, UV detection at 240 nm - emerges as the most broadly applicable and reproducible starting point.[70] Future work should prioritise a stability-indicating, ICH Q2(R2)-compliant method validated specifically on a marketed ASP-CLP fixed-dose combination product, ideally incorporating green-chemistry mobile-phase principles to align with current Q1-tier analytical chemistry standards.

ACKNOWLEDGEMENTS

The authors thank Dr. Abhijit Sengupta (Director, GNIPST) and Dr. Lopamudra Datta (Principal, GNIPST) for institutional guidance and support, and Dr. Sriparna Kundu Sen for assistance with language refinement and proofreading. The authors also acknowledge the Guru Nanak Institute of Pharmaceutical Science and Technology (GNIPST) for providing the facilities necessary to undertake this work.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. J. Vyas A, Anilkumar Jha S, Patel AB, Patel A, Shah SR, Sheth DB. Review on Simultaneous Equation Method (Vierodt's Method). *Asian Journal of Pharmaceutical Analysis*. Published online May 27, 2022:149-156. doi:10.52711/2231-5675.2022.00026
2. R. Aswini, M. M. Eswarudu, P. S. Babu. A NOVEL RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM. *Int J Pharm Sci Res*. 2018;9(12):5161-5167. doi:10.13040/IJPSR.0975-8232.9(12).5161-67
3. Rajput G, Patel P, Gupta G Das, Kurmi B Das. A Mini-Review on Simultaneous Quantification of Active Pharmaceutical Ingredients By UV and Quality by Design Assisted HPLC Method. *Curr Anal Chem*. 2022;18(9):939-955. doi:10.2174/1573411018666220705091354
4. Maddala GS. Simultaneous Estimation Methods for Large- and Medium-size Econometric Models. *Rev Econ Stud*. 1971;38(4):435. doi:10.2307/2296688
5. Wang W, Xu M, Guo Q, Yuan Y, Gu R, Yao J. Rapid separation and on-line detection by coupling high performance liquid chromatography with surface-enhanced Raman spectroscopy. *RSC Adv*. 2015;5(59):47640-47646. doi:10.1039/C5RA05562H
6. Wu TY, Chang FR, Liou JR, et al. Rapid HPLC Quantification Approach for Detection of Active Constituents in Modern Combinatorial Formula, San-Huang-Xie-Xin-Tang (SHXXT). *Front Pharmacol*. 2016;7. doi:10.3389/fphar.2016.00374
7. Shabir G. Development and validation of a reversed-phase HPLC method for the determination of hydroxybenzene in a cream formulation. *Indian J Pharm Sci*. 2010;72(3):307. doi:10.4103/0250-474X.70475
8. Indian Pharmacopoeia Commission. *Indian Pharmacopoeia, Monographs on Drug Substances, Dosage Forms and Pharmaceutical Aids, ASPIRIN*. Vol 2. 9th ed. Indian Pharmacopoeia Commission; 2022.
9. British Pharmacopoeia Commission. *British Pharmacopoeia, Monographs: Medicinal and Pharmaceutical Substances, Aspirin*. Ph. Eur. 11.3 update. British Pharmacopoeia Commission; 2024.
10. United States Pharmacopeia. *USP Monographs, Aspirin Capsules*. USP-NF. United States

Pharmacopeia; 2024. doi:10.31003/USPNF_M6250_02_01
11. Eikelboom JW, Hirsh J, Spencer FA, Baglin TP, Weitz JI. Antiplatelet Drugs. *Chest*. 2012;141(2):e89S-e119S. doi:10.1378/chest.11-2293
12. Luo GA, Lan QT, Wang ZP, Zhou GH. Determination of three components in aspirin compound tablets by use of UV-PLS method. *Yao Xue Xue Bao*. 1989;24(9):684—689. http://europepmc.org/abstract/MED/2618718
13. Chopra V, Marmur JD, Cavusoglu E. The Role of Clopidogrel in the Management of Patients with Ischemic Heart Disease. *Cardiovasc Drugs Ther*. 2003;17(5/6):467-477. doi:10.1023/B:CARD.0000015862.62649.c8
14. Herbert JM, Savi P. P2Y12, a new platelet ADP receptor, target of clopidogrel. *Semin Vasc Med*. 2003;3(2):113-122. doi:10.1055/s-2003-40669
15. Glaser R. Aspirin. An ab Initio Quantum-Mechanical Study of Conformational Preferences and of Neighboring Group Interactions. *J Org Chem*. 2001;66(3):771-779. doi:10.1021/jo001241s
16. Chandarana C, Kapupara P. Fourier Transform Infrared Spectrophotometry: An Eco-Friendly Green Tool for Simultaneous Quantification of Aspirin and Omeprazole in Pharmaceutical Formulation. *Brazilian Journal of Analytical Chemistry*. 2020;7(28). doi:10.30744/brjac.2179-3425.AR-25-2020
17. Nokhodchi A, Ghafourian T, Nashed N, et al. Solubility Study of Acetylsalicylic Acid in Ethanol + Water Mixtures: Measurement, Mathematical Modeling, and Stability Discussion. *AAPS PharmSciTech*. 2021;23(1):42. doi:10.1208/s12249-021-02192-7
18. Rostamian H, Lotfollahi MN. Statistical modeling of aspirin solubility in organic solvents by Response Surface Methodology and Artificial Neural Networks. *Physica A: Statistical Mechanics and its Applications*. 2020;540:123253. doi:10.1016/j.physa.2019.123253
19. Maia GD, Giulietti M. Solubility of Acetylsalicylic Acid in Ethanol, Acetone, Propylene Glycol, and 2-Propanol. *J Chem Eng Data*. 2008;53(1):256-258. doi:10.1021/je7005693
20. Jassim ZE, Hussein AA. DISSOLUTION METHOD DEVELOPMENT AND ENHANCEMENT OF SOLUBILITY OF CLOPIDOGREL BISULFATE. *International Research Journal of Pharmacy*. 2017;8(6):25-29. doi:10.7897/2230-8407.08691
21. Al-Shami N, Naseef H, Moqadi R, Kanaze F. HPLC Method Development and Validation for the Determination of Apixaban and Clopidogrel in Novel Fixed-Dose Combination Tablets. *J Chem*. 2024;2024:1-11. doi:10.1155/2024/2675736
22. Lestari MLAD, Suciati, Indrayanto G, Brittain HG. Clopidogrel Bisulfate. In: ; 2010:71-115. doi:10.1016/S1871-5125(10)35002-3
23. Florey K. Aspirin. *Analytical Profiles of Drug Substances and Excipients*. 1979;8(C):1-46. doi:10.1016/S0099-5428(08)60112-4
24. Saeed A, Shahzad D, Faisal M, Larik FA, El-Seedi HR, Channar PA. Developments in the synthesis of the antiplatelet and antithrombotic drug (S)-clopidogrel. *Chirality*. 2017;29(11):684-707. doi:10.1002/chir.22742
25. National Center for Biotechnology Information. PubChem Compound Summary for CID 2244, Aspirin. 2024. https://pubchem.ncbi.nlm.nih.gov/compound/Aspirin
26. National Center for Biotechnology Information. PubChem Compound Summary for CID 60606, Clopidogrel. 2024. https://pubchem.ncbi.nlm.nih.gov/compound/Clopidogrel
27. Rao KrK, Lakshmi Kr. Design, development and evaluation of clopidogrel bisulfate floating tablets. *Int J Pharm Investig*. 2014;4(1):19. doi:10.4103/2230-973X.127736
28. Wu D, Wang Z, Zhou L, Hou B, Zhou L. Measurement and Correlation of the Solubility of Aspirin in Four Binary Solvent Mixtures from T = 283.15 to 323.15 K. *J Chem Eng Data*. 2020;65(2):856-868. doi:10.1021/acs.jced.9b01057
29. Catherine S. Physical and Chemical Properties of Aspirin. *Journal of Clinical & Experimental Pharmacology*. 2021;11(7).
30. Beal GD, Szalkowski CR. The melting point of acetylsalicylic acid. *The Journal of the American Pharmaceutical Association (1912)*. 1933;22(1):36-40. doi:10.1002/jps.3080220114
31. ChemicalBook. Clopidogrel Bisulfate | 120202-66-6. 2024. Accessed November 6, 2024. https://www.chemicalbook.com/ChemicalProductProperty_EN_CB1341210.htm
32. Arif H, Aggarwal S. *Salicylic Acid (Aspirin)*. StatPearls Publishing; 2023. Accessed October 28, 2024. https://www.ncbi.nlm.nih.gov/books/NBK519032/
33. Yazan Z, Eskiköy Bayraktepe D, Dinç E. Square wave voltammetric pKa determination of aspirin using multi-way data analysis models. *Chemical Papers*. 2022;76(9):5389-5397. doi:10.1007/s11696-022-02250-9
34. Hansch C, Leo A, Hoekman D. *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*. Vol 2. (Hansch C, Leo A, Hoekman D, eds.). American Chemical Society ; 1995.
35. Dressman JB, Nair A, Abrahamsson B, et al. Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Acetylsalicylic Acid. *J Pharm Sci*. 2012;101(8):2653-2667.

doi:10.1002/jps.23212
36. Dolores M, Hiplito EAM, Rosas JAG, Villaseor A, Arellano RL. Development and Validation of an Alternate Stability-indicating UV Spectrophotometric Analytical Method for Aspirin in Tablets. *Indian J Pharm Sci*. 2016;78(6). doi:10.4172/pharmaceutical-sciences.1000187
37. Zimmermann P, Curtis N. The effect of aspirin on antibiotic susceptibility. *Expert Opin Ther Targets*. 2018;22(11):967-972. doi:10.1080/14728222.2018.1527314
38. Lei J, Zhou Y, Xie D, Zhang Y. Mechanistic Insights into a Classic Wonder Drug—Aspirin. *J Am Chem Soc*. 2015;137(1):70-73. doi:10.1021/ja5112964
39. Tóth L, Muszbek L, Komáromi I. Mechanism of the irreversible inhibition of human cyclooxygenase-1 by aspirin as predicted by QM/MM calculations. *J Mol Graph Model*. 2013;40:99-109. doi:10.1016/j.jmgm.2012.12.013
40. Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase Inhibitors and the Antiplatelet Effects of Aspirin. *New England Journal of Medicine*. 2001;345(25):1809-1817. doi:10.1056/NEJMoa003199
41. Ratchford SM, Lavin KM, Perkins RK, Jemiolo B, Trappe SW, Trappe TA. Aspirin as a COX inhibitor and anti-inflammatory drug in human skeletal muscle. *J Appl Physiol*. 2017;123(6):1610-1616. doi:10.1152/japplphysiol.01119.2016
42. Hawkey CJ. COX-1 and COX-2 inhibitors. *Best Pract Res Clin Gastroenterol*. 2001;15(5):801-820. doi:10.1053/bega.2001.0236
43. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res*. 2003;110(5-6):255-258. doi:10.1016/S0049-3848(03)00379-7
44. Warner TD, Mitchell JA. Cyclooxygenase-3 (COX-3): Filling in the gaps toward a COX continuum? *Proceedings of the National Academy of Sciences*. 2002;99(21):13371-13373. doi:10.1073/pnas.222543099
45. Sangkuhl K, Klein TE, Altman RB. Clopidogrel pathway. *Pharmacogenet Genomics*. 2010;20(7):463-465. doi:10.1097/FPC.0b013e3283385420
46. Polasek TM, Doogue MP, Miners JO. Metabolic activation of clopidogrel: *in vitro* data provide conflicting evidence for the contributions of CYP2C19 and PON1. *Ther Adv Drug Saf*. 2011;2(6):253-261. doi:10.1177/2042098611422559
47. Savi P, Pereillo JM, Uzabiaga MF, et al. Identification and biological activity of the active metabolite of clopidogrel. *Thromb Haemost*. 2000;84(5):891-896.
48. Savi P, Herbert JM, Pflieger AM, et al. Importance of hepatic metabolism in the antiaggregating activity of the thienopyridine clopidogrel. *Biochem Pharmacol*. 1992;44(3):527-532. doi:10.1016/0006-2952(92)90445-O
49. Cadavid AP. Aspirin: The Mechanism of Action Revisited in the Context of Pregnancy Complications. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.00261
50. Vickers JD. Binding of polymerizing fibrin to integrin alpha IIb beta3 on chymotrypsin-treated rabbit platelets decreases phosphatidylinositol 4,5-bisphosphate and increases cytoskeletal actin. *Platelets*. 1999;10(4):228-237. doi:10.1080/09537109976077
51. Ford I. Coming safely to a stop: a review of platelet activity after cessation of antiplatelet drugs. *Ther Adv Drug Saf*. 2015;6(4):141-150. doi:10.1177/2042098615588085
52. Nakhonsri V, John S, Panumasmontol H, et al. The Diversity of CYP2C19 Polymorphisms in the Thai Population: Implications for Precision Medicine. *Appl Clin Genet*. 2024;Volume 17:95-105. doi:10.2147/TACG.S463965
53. Vani R, Sunita M. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF OMEPRAZOLE AND ASPIRIN USING REVERSE PHASE HPLC METHOD IN BULK AND DOSAGE FORM. *Universal Journal of Pharmaceutical Research*. 2017;2(4):25-29. doi:10.22270/ujpr.v2i4.R6
54. Yaasir Ahmed A, Tarique M, Abdul Azeem S, et al. HPLC Method Validation for The Estimation of Aspirin in Bulk and Tablet Dosage Form as Per IP. *Bulletin of Environment, Pharmacology and Life Sciences*. 2021;10(11):108-113.
55. Belov VYu, Kursakov S V., Sevast'yanov VI, Antonov EN, Bogorodskii SÉ, Popov VK. Development of an HPLC-UV Method for Quantitative Determination of Acetylsalicylic Acid and Its Main Metabolite. *Pharm Chem J*. 2018;52(2):151-155. doi:10.1007/s11094-018-1781-x
56. Krishnaiah V, Reddy R. Development and validation of HPLC method for the simultaneous determination of aspirin. *J Chem Pharm Res*. 2012;4(3):2349-2353.
57. Kowalska M, Woźniak M, Kijek M, Mitrosz P, Szakiel J, Turek P. Management of validation of HPLC method for determination of acetylsalicylic acid impurities in a new pharmaceutical product. *Sci Rep*. 2022;12(1):1. doi:10.1038/s41598-021-99269-x
58. Yaasir Ahmed A, Patel Z, Khan R, Patel H, Abdul Azeem S. HPLC Method Validation For The Estimation Of Aspirin In Bulk And Tablet Dosage Form As Per USP. *International Journal of Research in Advent Technology*. 2019;7(4S). doi:10.13140/RG.2.2.18620.87680
59. Anupama B, Jagathi V, Vishwanadh A. Determination of Clopidogrel Bisulphate in Pharmaceutical Dosage Forms by RP-HPLC. *Asian J Research Chem*. 2011;4(2).

www.ajrconline.org
60. Sahu S, Sarangi SP, Sahoo HB. Development and validation methods for the estimation of Clopidogrel in bulk and pharmaceutical dosage forms. *International Journal of Research in Pharmaceutical Sciences*. 2012;3(2):224-227. https://www.researchgate.net/publication/268187036
61. Nagavi JB, Gurupadayya B. Stability Indicating RP-HPLC Method Development and Validation for the estimation of Clopidogrel bisulphate. *American Journal of PharmTech Research*. 2014;4(6). http://www.ajptr.com/
62. Sivarama Krishna V, Ravi Kumar D, Balamuralikrishna K, Rambabu C. Development and validation of stability indicating RP-HPLC method for the determination of clopidogrel bisulphate in bulk and its dosage forms. *Der Pharma Chemica*. 2014;6(2):366-374. http://derpharmachemica.com/archive.html
63. Sahoo NK, Sahu M, Rao PS, Indira JN, Rani SN, Ghosh GK. Validation of assay for bulk clopidogrel and for some tablet forms by reverse-phase high-performance liquid chromatography. *Journal of Taibah University for Science*. 2014;8(4):331-336. doi:10.1016/j.jtusci.2014.02.001
64. Soliman B, Mohamed Mabrouk M, Hammad S, Kamal A. Development and Validation of RP-HPLC Method for Analysis of Counterfeit Clopidogrel. *Journal of Advanced Medical and Pharmaceutical Research*. 2024;0(0):34-41. doi:10.21608/jampr.2024.255346.1061
65. Puri M, Kumar Walia Y. RP-HPLC Analysis of Clopidogrel Bisulphate in Pharmaceutical Dosage form: Method Development and Validation. *Res J Pharm Technol*. Published online July 24, 2024:3109-3114. doi:10.52711/0974-360X.2024.00486
66. Anosova LS, Remezova IP, Agafonov AM. Development and validation of a methodology for HPLC analysis of clopidogrel. *Aspirantskiy Vestnik Povolzhiya*. 2022;22(4):33-39. doi:10.55531/2072-2354.2022.22.4.33-39
67. Ghotekar DS, Kushare VN. Development of a Validated Stability Indicating RP-HPLC Method for Assay of Clopidogrel. Published online 2019. Accessed October 13, 2024. www.ijsrst.com
68. VENKATESWARARAO Y, SUJANA K. A NOVEL STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF CLOPIDOGREL IN BULK AND ITS DOSAGE FORMS . *International Journal of Pharmacy Research & Technology*. 2019;9(2). doi:10.31838/ijprt/09.02.01
69. Dimple B, Vinodh M, Vinayak M. DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CLOPIDOGREL BISULPHATE. *Malaysian Journal of Analytical Sciences*. 2013;17(3):387-393.
70. Ammar Al-Khayat M, Haidar S, Mando H. DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF CLOPIDOGREL IN TABLETS. *Int J Pharm Sci Rev Res*. 2012;14(2):1-5.
71. Kumar VP, Sunandamma Y. Simultaneous Determination of Clopidogrel and Pioglitazone by High Performance Liquid Chromatography in Bulk Drug and Dosage Forms. *International Journal of Pharmaceutical and Life Sciences*. 2013;2(1):1-9. doi:10.3329/ijpls.v2i1.14580
72. Mohammad MAA, Elkady EF, Fouad MA, Salem WA. Analysis of Aspirin, Prasugrel and Clopidogrel in Counterfeit Pharmaceutical and Herbal Products: Plackett–Burman Screening and Box–Behnken Optimization. *J Chromatogr Sci*. 2021;59(8):730-747. doi:10.1093/chromsci/bmaa113
73. Tiwari PK, Jain A, Dubey B, Pandey G, Dhakad S. Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel and Rosuvastatin in Pharmaceutical Dosage Form. *Journal of Drug Delivery and Therapeutics*. 2019;9(4-s):432-438. doi:10.22270/jddt.v9i4-s.3351
74. Vivek Sagar P, Samidha T, Vamshi Krishna M, Shobha Rani S. A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND PRASUGREL IN TABLET DOSAGE FORM. *Int J Pharm Sci Res*. 2014;5(11):4858. doi:10.13040/IJPSR.0975-8232.5(11).4858-64
75. Sarkis N, Bitar Y, Sarraj MM. Development and validation of RP-HPLC method for simultaneous estimation of aspirin and rivaroxaban in synthetic mixture. *Res J Pharm Technol*. 2020;13(11):5459-5465. doi:10.5958/0974-360X.2020.00953.1
76. Ministry of Health & Family Welfare G of I. *Indian Pharmacopeia*. Vol 2. 9th ed. Indian Pharmacopoeia Commission; 2022.
77. Karunakaran A, Ayyappan T, Raghu Raman V, Vetrichelvan T, Sankar ASK, Nagavalli D. RP-HPLC analysis of aspirin and clopidogrel bisulphate in combination. *Indian J Pharm Sci*. 2007;69(4):597. doi:10.4103/0250-474X.36958
78. Gandhimathi M, Ravi T. High performance liquid chromatographic determination of aspirin and clopidogrel in tablets. *Indian J Pharm Sci*. 2007;69(1):123. doi:10.4103/0250-474X.32125
79. Shrivastava P, Basniwal P, Jain D, Shrivastava S. Concurrent estimation of clopidogrel bisulfate and aspirin in tablets by validated RP-HPLC method. *Indian J Pharm Sci*. 2008;70(5):667. doi:10.4103/0250-474X.45414
80. Panda SS. Ion-Pairing RP-HPLC Method for Simultaneous determination of Aspirin and

Clopidogrel bisulphate in Tablet and Capsule Dosage Form. *International Journal of PharmTech Research CODEN*. 2010;2(1):269-273.

81. Sultana N, Ali KA, Arayne MS, Nawaz M. Simultaneous Determination of Clopidogrel and Aspirin by RP-HPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique. *J Chromatogr Sci*. 2011;49(2):165-169. doi:10.1093/chrsci/49.2.165
82. Kahsay G, Van Schepdael A, Adams E. Development and validation of a liquid chromatographic method for purity control of clopidogrel–acetylsalicylic acid in combined oral dosage forms. *J Pharm Biomed Anal*. 2012;61:271-276. doi:10.1016/j.jpba.2011.12.019
83. Kahsay G, Lakshmi Dhulipalla R, Van Nyen G, Punie X, Van Schepdael A, Adams E. Analysis of Purity of Clopidogrel and Acetylsalicylic Acid in Combined Oral Dosage Forms. *Curr Pharm Anal*. 2013;9(1):82-91. doi:10.2174/157341213804806070
84. Chatrabhuji PM, Pandya C V., Patel MC. Development and validation of RP-HPLC-UV method for simultaneous quantitation of clopidogrel bisulphate and aspirin in bulk drug. *Analytical Chemistry: An Indian Journal*. 2014;15(2). https://www.researchgate.net/publication/267021279
85. Kumar PP, Srikanth I, Anusha K, Srinivasa Rao D, Reddy KC. LC method development and validation of aspirin and clopidogrel in pure API'S and its pharmaceutical dosage forms. *International Journal of Pharmacy and Analytical Research*. 2014;3(4):391-396. doi:https://doi.org/10.61096/ijpar.v3.iss4.2014.391-396
86. Mahesh H, Babu KS. A Novel Stability Indicating HPLC Assay Method for Simultaneous Estimation of Clopidogrel and Aspirin in Tablet dosage form by Using Core shell Technology column. *Res J Pharm Technol*. 2015;8(2):172. doi:10.5958/0974-360X.2015.00031.1
87. Gousuddin M, Sengupta P, Datt Tripathi V, Das A. STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ASPIRIN AND CLOPIDOGREL IN DOSAGE FORM. *Malaysian Journal of Analytical Science*. 2016;20(2):247-257. doi:10.17576/mjas-2016-2002-05
88. Chhonker YS, Pandey CP, Chandasana H, et al. Simultaneous quantitation of acetylsalicylic acid and clopidogrel along with their metabolites in human plasma using liquid chromatography tandem mass spectrometry. *Biomedical Chromatography*. 2016;30(3):466-473. doi:10.1002/bmc.3573
89. Osmanović Omerdić E, Alagić-Džambić L, Krstić M, Pašić-Kulenović M, Odović J, Vasiljević D. In Vitro Dissolution Study of Acetylsalicylic Acid and Clopidogrel Bisulfate Solid Dispersions: Validation of the RP-HPLC Method for Simultaneous Analysis. *Applied Sciences*. 2020;10(14):4792. doi:10.3390/app10144792
90. Sai Sreekanth ND, Adilakshmi N. Method development and validation of aspirin and clopidogrel pharmaceutical dosage forms by developing new RP HPLC method. *Journal of Applied Pharmaceutical Research*. 2020;8(3):38-47. doi:10.18231/j.joapr.2020.v.8.i.3.38.47
91. Usman S, Akram M, Shah F, KVRNS Ramesh R, Islam Q. Stability Indicating HPLC Method for Simultaneous Assessment of Clopidogrel Bisulfate and Aspirin: Development and Validation. *Int J Pharm Investig*. 2023;13(2):270-278. doi:10.5530/ijpi.13.2.037.
92. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet. 1996;348(9038):1329-1339. doi:10.1016/S0140-6736(96)09457-3
93. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345(7):494-502. doi:10.1056/NEJMoa010746
94. Mehta SR, Yusuf S, Peters RJ, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358(9281):527-533. doi:10.1016/S0140-6736(01)05701-4
95. Watanabe H, Domei T, Morimoto T, et al. Effect of 1-month dual antiplatelet therapy followed by clopidogrel vs 12-month dual antiplatelet therapy on cardiovascular and bleeding events in patients receiving percutaneous coronary intervention: the STOPDAPT-2 randomized clinical trial. JAMA. 2019;321(24):2414-2427. doi:10.1001/jama.2019.8145
96. Pisal P, Nigade G, Kale A, Pawar S. Development and validation of stability indicating RP-HPLC method for simultaneous determination of aspirin, rosuvastatin, clopidogrel in bulk and pharmaceutical dosage form. Int J Pharm Pharm Sci. 2018;10(10):50-56. doi:10.22159/ijpps.2018v10i10.28329
97. Singh RD, Yadav H, Hinge M, Patel A. Development and validation of analytical methods for simultaneous estimation of rosuvastatin, clopidogrel and aspirin in the pharmaceutical dosage form. J Pharm Sci Biosci Res. 2016;6(2):197-205.
98. Panchal A, Sanghvi G, Vachhani A, Seth N, Vaishnavi D. Simultaneous determination of

aspirin and rosuvastatin calcium in capsules by using an RP-HPLC coupled with photodiode array detection. Int Lett Chem Phys Astron. 2014;33:218-230. doi:10.18052/www.scipress.com/ILCPA.33.218

99. International Council for Harmonisation. ICH Harmonised Tripartite Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology. ICH; 2005.
100. International Council for Harmonisation. ICH Harmonised Guideline Q2(R2): Validation of Analytical Procedures. ICH; 2023.
101. US Food and Drug Administration, Center for Drug Evaluation and Research. Q2(R2) Validation of Analytical Procedures: Guidance for Industry. Silver Spring, MD: FDA; 2024.