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In Vitro Dissolution Study of Acetylsalicylic Acid and Clopidogrel Bisulfate Solid Dispersions: Validation of the RP-HPLC Method for Simultaneous Analysis

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Abstract: Solid dispersions were prepared via a solvent evaporation method, employing ethanol (96%, *v/v*) as solvent, with three different polymers as carrier: povidone, copovidone, and poloxamer 407. Previously developed reversed-phase HPLC (RP-HPLC) methods were modified and used for the simultaneous determination of acetylsalicylic acid and clopidogrel bisulfate and after release from solid dispersions. Chromatography was carried out on a C-18 column, with a mobile phase of acetonitrile–methanol–phosphate buffer pH 3.0, UV detection at 240 nm, and a run time of 6 min. The method was validated according to International Conference of Harmonisation guidelines and validation included specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method is specific for determination of acetylsalicylic acid and clopidogrel bisulfate. The linearity was provided in the concentration range 0.0275–0.1375 mg/mL for acetylsalicylic acid and 0.0200–0.1000 mg/mL for clopidogrel bisulfate, with a correlation coefficient (R^2 value) of 0.9999 for both active pharmaceutical ingredients (APIs). Accuracy was confirmed by calculated recoveries for acetylsalicylic acid (98.6–101.0%) and clopidogrel bisulfate (100.0–101.6%). The intra-day and the inter-day precision-calculated relative standard deviations are less than 1%, which indicates high precision of the method. The limits of detection and quantification for acetylsalicylic acid were 0.0004 and 0.0012 mg/mL, and for clopidogrel bisulfate 0.0002 mg/mL and 0.0007 mg/mL, respectively. Small variations in chromatographic conditions did not significantly affect qualitative and quantitative system responses, which proved robustness of method. The proposed RP-HPLC method was applied for simultaneous determination of clopidogrel bisulfate and acetylsalicylic acid from solid dispersions.

Keywords: clopidogrel bisulfate; acetylsalicylic acid; solid dispersion; dissolution; RP-HPLC; validation

1. Introduction

According to the World Health Organization (WHO), ischemic heart disease and stroke is the leading cause of adult-acquired disability and death worldwide [1]. Several antiplatelet drugs with different mechanisms of action are currently available for secondary prevention of cardiovascular

events such as ischemic stroke, myocardial infarction, and peripheral vascular disease. The most used oral antiplatelet drugs are acetylsalicylic acid, clopidogrel, dipyridamole, prasugrel, epoprostenol, and streptokinase. A combination of clopidogrel with acetylsalicylic acid may be substantially more effective than either drug alone [2–4]. Dual antiplatelet therapy appears to be safer and more effective in reducing stroke recurrence and combined vascular events in patients with acute ischemic stroke or transient ischemic attack, as compared with monotherapy [3,5]. In a large, randomized, blind, international trial (CURE), the safety and efficacy of the combination of clopidogrel and acetylsalicylic acid in patients with acute coronary syndromes were assessed. This study demonstrated that clopidogrel used with acetylsalicylic acid reduces death or myocardial infarction in patients undergoing intracoronary stent implantation by 77%, compared with acetylsalicylic acid alone [6]. Patient compliance has also shown improvement when a dosage form combining two active pharmaceutical ingredients (APIs) was used [7]. On the market, combination therapy that contains clopidogrel bisulfate and acetylsalicylic acid is available in the form of film-coated tablets.

Acetylsalicylic acid (ASA) (2-acetoxybenzoic acid) is a nonsteroidal anti-inflammatory drug and platelet aggregation inhibitor (Figure 1a). The mechanism of action is that of a cyclooxygenase inhibitor [8]. Acetylsalicylic acid is practically insoluble in water, and freely soluble in ethanol 96% v/v [9].

Clopidogrel bisulfate (CB) (S-methyl 2-(2-chlorophenyl)-6,7-dihydrothieno [3,2-c]pyridine-5(4H)-acetatesulfate) is a thienopyridine antiplatelet drug (Figure 1b). It is an antithrombotic agent that inhibits adenosine diphosphate-mediated platelet aggregation by selectively and irreversibly blocking platelet purinergic P2Y₁₂ receptors [10,11]. Clopidogrel bisulfate is practically insoluble in water at neutral pH, but freely soluble at pH 1. It also dissolves freely in methanol, sparingly in methylene chloride, and is practically insoluble in ethyl ether [11].

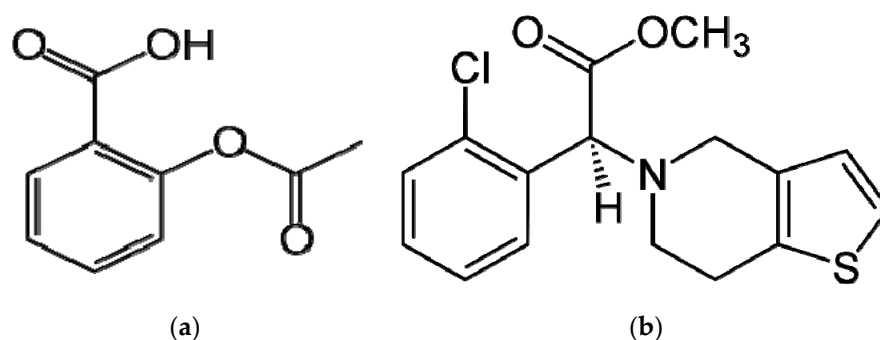


Figure 1. Chemical structure of acetylsalicylic acid (a) and clopidogrel (b).

Development of formulation and validation of the dissolution method for poorly water soluble drugs has been a challenge in the pharmaceutical industry and for scientists, especially for drugs which belong to Biopharmaceutical Classification System (BCS) II or IV groups, like acetylsalicylic acid and clopidogrel bisulfate, with low solubility and/or permeability. Low solubility and dissolution rate are usually a limiting factor for oral drug absorption of these substances and consequently affect the bioavailability and therapeutic efficacy of the drug [12,13].

Formulations of solid dispersions (SD) were often used to increase the solubility and dissolution rate of low solubility drugs [12,14–17]. A solid dispersion is a dispersion of one or more active ingredients in a hydrophilic inert carrier matrix at solid state. Two basic procedures used to prepare solid dispersions are solvent evaporation and melting methods [15,16,18,19].

There are a number of HPLC methods available in the literature for the estimation of ASA and CB alone, and only a few methods are reported for simultaneous determination of both APIs [20–26], but there is no method reported for simultaneous estimation of ASA and CB after release from solid dispersion. This method was required primarily due to the application of excipients in solid dispersions

not used in previous studies to achieve adequate selectivity and also to shorten the retention time compared to the mentioned investigations.

The aim of this study was to modify previously developed [20,21] reversed-phase high-performance liquid chromatography (RP-HPLC) methods and to validate a new method for application in the simultaneous determination of ASA and CB released from solid dispersion. An *in vitro* dissolution method was conducted and validated according to United States Pharmacopeia (USP) and ICH guidelines [27,28]. The proposed RP-HPLC method should be applicable for routine quality control, stability testing, and estimating the concentrations of drugs in other solid dosage forms.

2. Materials and Methods

2.1. Materials

Acetylsalicylic acid, clopidogrel bisulfate, and excipients used for preparation of solid dispersion: povidone (Kollidon[®] 30, BASF, Ludwigshafen, Germany) copovidone (Kollidon[®] VA 64, BASF, Ludwigshafen, Germany) and poloxamer 407 (Kolliphor[®] P 407, BASF, Ludwigshafen, Germany) were pharmaceutical grade. Working standards, as well as reagents, were HPLC grade.

APIs, excipients, and other chemicals were a gift from Bosnalijek d.d. (Sarajevo, Bosnia and Herzegovina).

2.2. Methods

2.2.1. Preparation of Solid Dispersions

Solid dispersions of acetylsalicylic acid and clopidogrel bisulfate with three different hydrophilic polymers (povidone, copovidone, and poloxamer 407) were prepared via the common solvent evaporation method, employing ethanol (96% *v/v*) as solvent. Solid dispersions were prepared in the of CB:ASA:carrier ratio, namely, 1:1.3:2.3, and injected into hard gelatinous capsules that contained a therapeutic dose of acetylsalicylic acid (100 mg) and clopidogrel (75 mg). The amount of polymer in the solid dispersions was chosen through earlier probe screening.

The required quantities of ASA, CB, and polymer were dissolved in the corresponding amount of solvent to get a clear solution. The solvent was then removed by evaporation under reduced pressure (vacuum) on a rotary vacuum evaporator (Rotavapor[®] R-205, Büchi, Switzerland) at a temperature of 55 °C with constant mixing. The mass obtained was crushed, pulverized, and shifted through sieve no. 500 (Ph. Eur.). Solid dispersions were injected into hard gelatinous capsules (size 0) so that they contained a therapeutic dose of ASA and CB.

2.2.2. In Vitro Dissolution Method

In vitro dissolution method conditions were: Apparatus 1 (basket) speed of 75 rpm, in 900 mL of phosphate buffer (pH 6.8) at 37 °C ± 0.5 °C. Tests were conducted with a Vankel VK 7010 dissolution bath and a Varian fraction sample collection module. A sample volume of 1.5 mL was filtered (35 µm Micron Full Flow Filter, Agilent) and collected directly into an HPLC vial. The samples were collected at time points of 15, 30, 45, and 60 min. The test was also performed on empty hard gelatinous capsules to prove that they had no effect on the results obtained. Results were expressed as mean value ± standard deviation (S.D.) of three replicates.

Chromatographic Conditions

The final chromatographic conditions were RP-HPLC method, using a C-18 column; 4.6 mm × 150 mm; 5 µm maintained at 30 °C with a mobile phase of acetonitrile–methanol–phosphate buffer pH 3.0 (500:70:430 *v/v/v*). The mobile phase pumped through the column with a flow rate 1.2 mL/min, an injection volume of 20 µL and a run time of 6 min. Detection was carried out at 240 nm by a UV Detector.

Preparation of Standard Stock Solutions

A standard stock solution of ASA was prepared by dissolving 25 mg of ASA in phosphate buffer pH 6.8 (0.25 mg/mL for ASA). A standard stock solution of CB was prepared by dissolving 20 mg of CB in 1 mL of methanol and diluted with phosphate buffer pH 6.8 to 100 mL (0.20 mg/mL for CB). Subsequently, these stock solutions were used to prepare a series of two-component working standard solutions through dilution with phosphate buffer pH 6.8.

Validation of Method

The method was validated according to International Conference of Harmonisation guidelines and validation included specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness.

Specificity was studied by injecting ASA and CB standard solutions, and solutions of polymers, in phosphate buffer pH 6.8 used in formulation studies. Specificity was also determined by injecting solution after disintegration of empty hard gelatinous capsules under the same RP-HPLC conditions to ensure that there was no interference. The chromatograms were examined at retention time of acetylsalicylic acid and clopidogrel.

The linearity of the method was tested in the range 0.0275–0.1375 mg/mL for ASA, and 0.0200–0.1000 mg/mL for CB (Table 1).

The accuracy was tested on three concentration levels: for ASA, 0.0550, 0.1100, and 0.1375 mg/mL; and for CB, 0.0400, 0.0800, and 0.1000 mg/mL (50%, 100% and 125%) of the target concentration (in this study, the target concentration (100%) was 0.1100 mg/mL for ASA and 0.0800 mg/mL for CB). Recovery (%) was calculated for each concentration and mean recovery value should be between 90% and 110% to be accepted.

One level of method precision (as system precision) was tested by analysis of ASA and CB at three concentration levels—50%, 100%, and 125%. The intra-day precision of the method was determined from replicate analysis ($n = 5$) of ASA and CB test standards at concentrations within the linear range of the assay for each drug (0.0550, 0.1100, and 0.1375 mg/mL for ASA and 0.0400, 0.0800, and 0.1000 mg/mL for CB). The inter-day precision of the method was determined by replicate analysis ($n = 5$) of ASA and CB with three different concentrations on two different days. The results were acceptable if the relative standard deviations (RSD) is less than 2% [28].

The values of LOD and LOQ were determined as described before by Alquadeib [29].

Robustness was evaluated by accompanying small variations in chromatographic conditions, such as alteration in the pH of the mobile phase, percentage organic content, changes in temperature, and the impact of these changes on qualitative and quantitative system responses.

3. Results and Discussion

An RP-HPLC method was utilized to provide adequate specificity and a short run time. The optimized chromatographic separation is shown in Figure 2. Chosen RP-HPLC conditions provided satisfactory separation of acetylsalicylic acid and clopidogrel from each other. All the system suitability parameters were optimized by freshly prepared standard solution of acetylsalicylic acid (0.1100 mg/mL) and clopidogrel (0.0800 mg/mL).

The method was validated for simultaneous estimation of acetylsalicylic acid and clopidogrel combinations using specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. Average retention time for acetylsalicylic acid and clopidogrel was 1.9 and 4 min, respectively, and was shorter than other published data [20–26]. Shorter retention time enables shorter analysis time, which is very important in routine analysis, especially in the pharmaceutical industry.

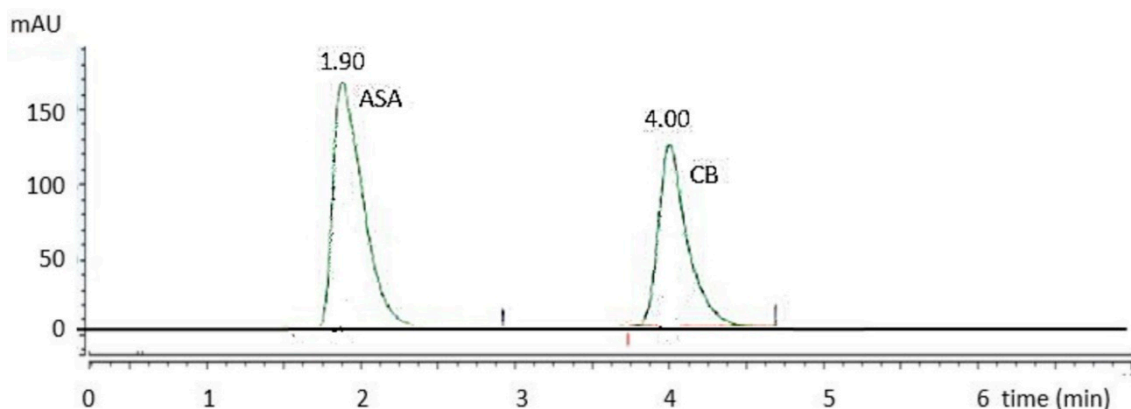


Figure 2. Representative chromatogram of acetylsalicylic acid (ASA) and clopidogrel (CB) mixed standard.

3.1. Specificity

The high degree of specificity of the method was confirmed by analyzing chromatograms obtained by injecting the placebo and standard solution. No peak was identified near ASA and CB retention times. A study to establish the interference of empty hard gelatinous capsules was conducted. Two peaks at retention times of 2.58 and 9.73 min appeared on the capsule chromatogram, so there was no overlap with active substance peaks (1.9 and 4 min) and no interactions, which implies selectivity of the method. The chromatogram of empty hard gelatinous capsules is shown in Figure 3.

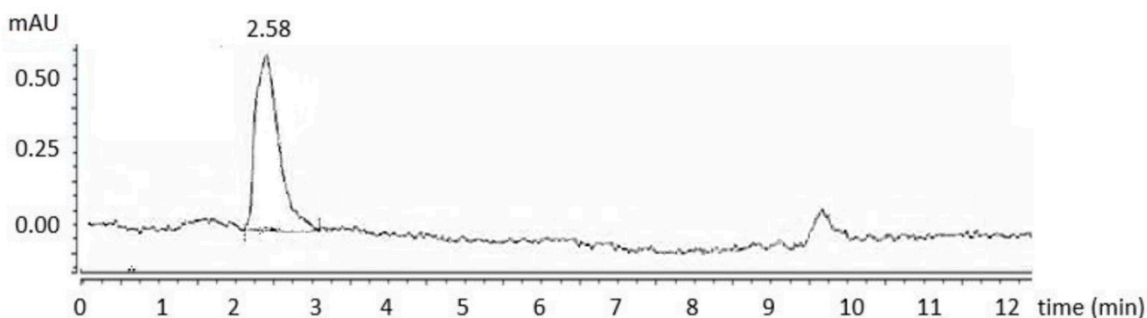


Figure 3. Representative chromatogram of empty hard gelatinous capsules.

3.2. Linearity

Linearity was evident in the concentration range 0.0275–0.1375 mg/mL for acetylsalicylic acid and 0.0200–0.1000 mg/mL for clopidogrel bisulfate with a correlation co-efficient (R^2 value) of 0.9999 (Table 1, Figures 4 and 5).

Table 1. Linearity results for acetylsalicylic acid and clopidogrel bisulfate.

Acetylsalicylic Acid			Clopidogrel Bisulfate		
D (%)	Concentration of Standard Solution (mg/mL)	Peak Area (mAU*s)	D (%)	Concentration of Standard Solution (mg/mL)	Peak Area (mAU*s)
25	0.0275	558,812	25	0.0200	309,513
40	0.0440	888,893	40	0.0320	494,592
45	0.0495	1,001,648	45	0.0360	558,577
50	0.0550	1,118,005	50	0.0400	620,662
100	0.1100	2,191,888	100	0.0800	1,225,652
125	0.1375	2,727,058	125	0.1000	1,526,992
R^2	0.9999		R^2	0.9999	

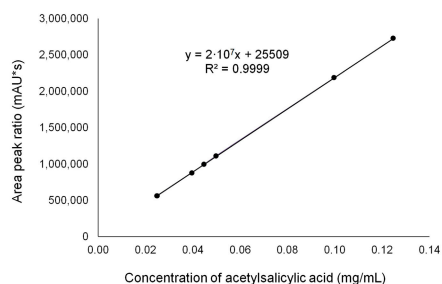


Figure 4. Linearity plot for acetylsalicylic acid.

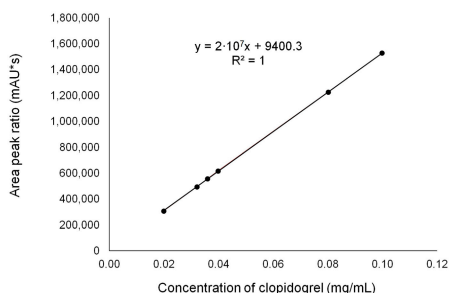


Figure 5. Linearity plot for clopidogrel.

3.3. Accuracy

The accuracy of the method was determined by recovery experiments. The accuracy studies were carried out at three concentrations: 0.0550, 0.1100, and 0.1375 mg/mL for ASA (50%, 100%, and 125%) and 0.0400, 0.0800, and 0.1000 mg/mL for CB (50%, 100%, and 125%). The percentage recovery was calculated from the data obtained. The results are shown in Tables 2 and 3. Mean recoveries of acetylsalicylic acid and clopidogrel were 98.6–101.0% and 100.0–101.6%, respectively, confirming the accuracy of the method.

Table 2. Accuracy results for acetylsalicylic acid.

Level (%)	Concentration of Standard Solution (mg/mL)	Obtained Concentration (mg/mL)	Recovery(%)
50	0.0550	0.0556	101.8
100	0.1100	0.1089	99.0
125	0.1375	0.1356	98.6
Mean recovery (%)			99.5

Table 3. Accuracy results for clopidogrel.

Level (%)	Concentration of Standard Solution (mg/mL)	Obtained Concentration (mg/mL)	Recovery(%)
50	0.0400	0.0406	101.6
100	0.0800	0.0802	100.3
125	0.1000	0.1000	100.0
Mean recovery (%)			100.6

3.4. Precision

System precision studies were evaluated by five replicate measurements at three concentrations (intra-day) and on two different days over a week (inter-day). The % RSD was found to be less than 1% for both substances, which indicates high precision of method (Tables 4 and 5). In practical analysis, that means that average results are statistically equivalent [30]. RSD values were lower than results shown in two other mentioned methods [20,21].

Table 4. Precision results for acetylsalicylic acid.

Injections	Peak Area (mAU*s) Acetylsalicylic Acid					
	50% 1. day	50% 2. day	100% 1. day	100% 2. day	125% 1. day	125% 2. day
1	1,118,005	1,119,005	2,196,838	2,148,464	2,727,058	2,730,364
2	1,116,341	1,121,366	2,208,228	2,146,395	2,730,215	2,725,634
3	1,121,242	1,123,216	2,212,075	2,149,707	2,729,241	2,719,863
4	1,115,786	1,118,345	2,216,265	2,148,082	2,715,653	2,740,793
5	1,110,211	1,115,256	2,214,434	2,139,806	2,715,934	2,720,256
Mean area peak (mAU*s)	1,116,317	1,119,438	2,209,568	2,146,491	2,723,620	2,727,382
RSD (%)	0.36	0.29	0.35	0.18	0.27	0.32

Table 5. Precision results for clopidogrel bisulfate.

Injections	Peak Area (mAU*s) Clopidogrel Bisulfate					
	50% 1. day	50% 2. day	100% 1. day	100% 2. day	125% 1. day	125% 2. day
1	620,662	621,251	1,220,200	1,227,386	1,526,992	1,525,632
2	619,432	619,534	1,225,194	1,215,347	1,519,965	1,518,649
3	619,578	620,097	1,219,874	1,238,342	1,528,761	1,526,489
4	621,392	619,987	1,218,465	1,237,379	1,523,235	1,517,398
5	620,978	621,872	1,224,411	1,210,359	1,527,691	1,527,821
Mean area peak (mAU*s)	620,408	620,548	1,221,629	1,227,563	1,525,329	1,523,198
RSD (%)	0.14	0.16	0.24	0.84	0.24	0.32

3.5. LOD and LOQ

The limits of detection and quantification were 0.0004 and 0.0012 mg/mL for ASA, and 0.0002 and 0.0007 mg/mL for CB, respectively.

3.6. Robustness

There were no changes in the chromatographic response for varied chromatographic parameters, thus confirming the robustness of the method.

3.7. In Vitro Dissolution Data

The development of the present RP-HPLC method has made it a possibility to prove that the selected polymers for solid dispersion preparations have an influence on the dissolution rate of poorly soluble drugs, like ASA and CB. The percentage of dissolved drugs from solid dispersions and pure APIs is presented in Table 6. All three prepared solid dispersions showed an improvement in the percentage of the released drug after 60 min for both active substances, compared to the pure APIs. The reason for such improvements could be because APIs in SD are surrounded by the molecules of the hydrophilic polymers, which improves wetting and dissolution [19,31,32].

After 60 min, in case of ASA, the SD containing povidone and poloxamer 407 had the highest improvement of dissolution rate (98.63% and 97.46%, respectively), in comparison with pure ASA (82.02%). For CB, the highest improvement of dissolution rate was for SD with poloxamer 407, 59.56% (32.20% for pure CB). SD with poloxamer 407 had the most significant results for both APIs (59.56% for CB and 97.46% for ASA). An increase in the ratio of polymers could have the effect of increasing the dissolution rate of CB, and promising results could be obtained with a SD using poloxamer 407.

Table 6. Percent (%) of dissolved drugs from solid dispersions and pure APIs.

API	Percent (%) of Dissolved Drugs *							
	Pure Drug		Polymer in SD					
	ASA	CB	Povidone		Copovidone		Poloxamer 407	
Time (min)	ASA	CB	ASA	CB	ASA	CB	ASA	CB
0	0	0	0	0	0	0	0	0
15	61.40 (0.21)	26.81 (0.07)	58.98 (0.93)	27.87 (0.04)	17.58 (0.56)	10.38 (0.86)	28.44 (0.46)	19.71 (0.36)
30	76.02 (0.50)	28.05 (0.03)	84.98 (0.52)	38.90 (0.32)	52.43 (0.48)	25.20 (0.15)	64.33 (0.33)	39.71 (0.33)
45	80.58 (0.28)	32.07 (0.02)	97.11 (0.54)	39.03 (0.68)	71.95 (0.55)	34.65 (0.04)	86.39 (0.30)	54.12 (0.16)
60	82.02 (0.08)	32.20 (0.12)	98.63 (0.77)	39.65 (0.54)	85.51 (0.64)	39.68 (0.71)	97.46 (0.37)	59.56 (0.03)

* Mean value (\pm standard deviation).

4. Conclusions

The proposed RP-HPLC method was validated for simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate. All the validation parameters for both active substances meet the criteria, according to ICH guidelines, which indicates the validity of the method. This analytical method is simple, selective, accurate, precise, robust, and linear. The method was successfully modified with a significantly shorter retention time, higher precision, high accuracy, and low detection and quantification limits. Because of the shorter retention time, this method shortens analysis time, which is very important in research, and also in the pharmaceutical industry.

This RP-HPLC method has been successfully used to determine acetylsalicylic acid and clopidogrel bisulfate after release from solid dispersions. All prepared solid dispersions showed improvement in the percent of dissolved drug, compared to pure APIs, but the best results for both APIs occurred in solid dispersions with poloxamer 407. In further research, the developed method would be very helpful in understanding how the chosen hydrophilic polymer ratio in solid dispersions affects the dissolution rate of ASA and CB.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

- World Health Organization. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2016. Geneva, 2018. Available online: <https://apps.who.int/iris/bitstream/handle/10665/272596/9789241565585-eng.pdf?ua=1> (accessed on 26 June 2020).
- Prasad, K.; Siemieniuk, R.; Hao, Q.; Guyatt, G.; O'Donnell, M.; Lytvyn, L.; Heen, A.F. Dual antiplatelet therapy with aspirin and clopidogrel for acute high risk transient ischemic attack and minor ischemic stroke: A clinical practice guideline. *BMJ* **2019**, *364*, I103. [[CrossRef](#)]

3. Geeganage, C.M.; Diener, H.C.; Algra, A.; Chen, C.; Topol, E.J.; Dengler, R.; Markus, H.S.; Bath, M.W.; Bath, P.M.W. Dual or mono antiplatelet therapy for patients with acute ischemic stroke or transient ischemic attack: Systematic review and meta-analysis of randomized controlled trials. *Stroke* **2015**, *43*, 1058–1066. [[CrossRef](#)] [[PubMed](#)]
4. The ESPRIT Study Group. Aspirin plus dipyridamole vs aspirin alone after cerebral ischemia of arterial origin (ESPRIT): Randomized controlled trial. *Lancet* **2006**, *367*, 1665–1673. [[CrossRef](#)]
5. Squizzato, A.; Bellesini, M.; Takeda, A.; Middeldorp, S.; Donadini, M.P. Clopidogrel plus aspirin versus aspirin alone for preventing cardiovascular events. *Cochrane Database Syst. Rev.* **2017**, *12*, CD005158. [[CrossRef](#)]
6. CURE Study Investigators, The Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial programme; rationale, design and baseline characteristics including a meta-analysis of the effects of thienopyridines in vascular disease. *Eur. Heart J.* **2000**, *21*, 2033–2041. [[CrossRef](#)]
7. Trofimov, E.S.; Poskrebsheva, A.S. The role of treatment compliance in combination therapy for heart failure and possibilities of its correction: Generalization of Russian and world experience. *Ter. Arkh.* **2014**, *86*, 119–123. Available online: <https://www.ncbi.nlm.nih.gov/pubmed/25518517> (accessed on 25 May 2020).
8. Patrono, C.; Rocca, B. Aspirin and other COX-1 inhibitors. *Hand. Exp. Pharmacol.* **2012**, *210*, 137–164. [[CrossRef](#)]
9. *European Pharmacopoeia*, 10th ed.; Council of Europe: Strasbourg, France, 2019.
10. Bali, D.E.; Osman, M.A.; El Maghraby, G.M. Enhancement of dissolution rate and intestinal stability of clopidogrel hydrogen sulfate. *Eur. J. Drug Metab. Pharmacokinet.* **2016**, *41*, 807–818. [[CrossRef](#)] [[PubMed](#)]
11. Evaluation of medicines for Human Use. European Medicines Agency, 2009. Doc. Ref EMEA/661461/2009. Available online: https://www.ema.europa.eu/en/documents/assessment-report/clopidogrel-apatex-epar-public-assessment-report_en.pdf (accessed on 1 June 2020).
12. Singh, A.; Worku, Z.A.; Van der Mooter, G. Polymeric Delivery Systems for Poorly Soluble Drugs. In *Encyclopedia of Pharmaceutical Science and Technology*, 4th ed.; Swarbrick, J., Ed.; CRC Press: London, UK; New York, NY, USA, 2013; Volume IV.
13. Krstić, M.; Ražić, S.; Vasiljević, D.; Spasojević, D.; Ibrić, S. Application of experimental design in the examination of the dissolution rate of carbamazepine from formulations. Characterization of the optimal formulation by DSC, TGA, FT-IR and PXRD analysis. *J. Serb. Chem. Soc.* **2015**, *80*, 209–222. [[CrossRef](#)]
14. Mogal, S.A.; Gurjar, P.N.; Yamgar, D.S. Solid dispersion or improving solubility of some poorly soluble drugs. *Pharm. Lett.* **2012**, *4*, 1574–1586. Available online: <https://www.scholarsresearchlibrary.com/articles/solid-dispersion-technique-for-improving-solubility-of-some-poorly-soluble-drugs.pdf> (accessed on 22 May 2020).
15. Sotthivirata, S.; McKelveya, C.; Moser, J.; Rege, B.; Xu, W.; Zhang, D. Development of amorphous solid dispersion formulations of a poorly water-soluble drug, MK-0364. *Int. J. Pharm.* **2013**, *452*, 73–81. [[CrossRef](#)] [[PubMed](#)]
16. Vasconcelos, V.; Marques, S.; Das Neves, J.; Sarmiento, B. Amorphous solid dispersions: Rational selection of manufacturing process. *Adv. Drug Deliv. Rev.* **2016**, *100*, 85–101. [[CrossRef](#)]
17. Krstić, M.; Đuriš, J.; Petrović, O.; Lazarević, N.; Cvijić, S.; Ibrić, S. Application of the melt granulation technique in development of lipid matrix tablets with immediate release of carbamazepine. *J. Drug Deliv. Sci. Technol.* **2017**, *39*, 467–474. [[CrossRef](#)]
18. Krstić, M.; Lukić, I.; Bušatlić, A.; Lazarević, N.; Vasiljević, D. Solid dispersion with carbamazepine: Optimization of formulation, characterisation and examination of long-term stability. *Chem. Ind.* **2018**, *72*, 191–204. [[CrossRef](#)]
19. Krstić, M.; Manić, L.; Martić, N.; Vasiljević, D.; Đogo Mračević, S.; Vukmirović, S.; Rasković, A. Binary polymeric amorphous carvedilol solid dispersions: In vitro and in vivo characterisation. *Eur. J. Pharm. Sci.* **2020**, *150*, 105343. [[CrossRef](#)] [[PubMed](#)]
20. Gousuddin, M.; Sengupta, P.; Tripathi, V.D. Das A. Stability indicating RP-HPLC method for simultaneous determination of Aspirin and Clopidogrel in dosage form. *Malays. J. Anal. Sci.* **2016**, *20*, 247–257. [[CrossRef](#)]
21. Sultana, N.; Arayne, M.S.; Ali, K.A.; Nawaz, M. Simultaneous Determination of Clopidogrel and Aspirin by RP-HPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique. *J. Chrom. Sci.* **2011**, *49*, 165–169. [[CrossRef](#)]

22. Panda, S.S. Ion-Pairing RP-HPLC Method for Simultaneous determination of Aspirin and Clopidogrel bisulphate in Tablet and Capsule Dosage Form. *Int. J. PharmTech Res.* **2015**, *2*, 269–273. Available online: <https://pdfs.semanticscholar.org/b944/405fc0be30ab7c415115193e258152cbb7c7.pdf> (accessed on 3 June 2020).
23. Chatrabhuji, P.M.; Pandya, C.V.; Patel, M.C. Development and validation of RP-HPLC-UV method for simultaneous quantitation of clopidogrel bisulfate and aspirin in bulk drug. *Anal. Chem. Ind. J.* **2014**, *15*, 43–48. Available online: https://www.academia.edu/8821280/Development_and_validation_of_RP-HPLC-UV_method_for_simultaneous_quantitation_of_clopidogrel_bisulphate_and_aspirin_in_bulk_drug (accessed on 5 June 2020).
24. Al Masud, A.; Begum, I. Development and validation of a RP-HPLC method for simultaneous estimation of aspirin and clopidogrel in combined tablet dosage form. *Int. J. Pharm. Sci. Res.* **2016**, *7*, 4443–4448. [[CrossRef](#)]
25. Shrivastava, P.K.; Basniwal, P.K.; Jain, D.; Shrivastava, S.K. Concurrent Estimation of Clopidogrel Bisulfate and Aspirin in Tablets by Validated RP-HPLC Method. *Ind. J. Pharm. Sci.* **2008**, *70*, 667–669. [[CrossRef](#)]
26. Anandakumar, K.; Ayyappan, T.; Raghu Raman, V.; Vetrichelvan, T.; Sankar, A.S.K.; Nagavalli, D. RP-HPLC analysis of aspirin and clopidogrel bisulphate in combination. *Ind. J. Pharm. Sci.* **2007**, *69*, 597–599. [[CrossRef](#)]
27. *The United States Pharmacopeia 42th Rev, and the National Formulary 37th*; United States Pharmacopeial Convention: Rockville, MD, USA, 2019.
28. Validation of analytical procedures: Text and Methodology Q2 (R1), Step 4, International Conference on harmonization. 2005. Available online: https://database.ich.org/sites/default/files/Q2_R1_Guideline.pdf (accessed on 3 June 2020).
29. Alquadeib, T.B. Development and validation of a new HPLC analytical method for the determination diclofenac in tablets. *Saudi Pharm. J.* **2018**, *27*, 66–70. [[CrossRef](#)]
30. Epstein, M. Validation of HPLC Techniques for Pharmaceutical Analysis. *Pharm. Chem. J.* **2004**, *38*, 212–228. [[CrossRef](#)]
31. Chutimaworapan, S.; Rutthidej, G.C.; Zonemochi, E.; Oguchi, T.; Yamamoto, K. Effect of water soluble carriers on dissolution characteristics of nifedipine solid dispersions. *Drug Dev. Ind. Pharm.* **2000**, *26*, 1141–1150. [[CrossRef](#)] [[PubMed](#)]
32. Vojinović, T.; Medarević, Đ.; Vranić, E.; Potpara, Z.; Krstić, M.; Đuriš, J.; Ibrić, S. Development of ternary solid dispersions with hydrophilic polymer and surface adsorbent for improving dissolution rate of carbamazepine. *Saudi Pharm. J.* **2018**, *26*, 725–732. [[CrossRef](#)]



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