

PHARMACEUTICAL TECHNOLOGY

ESTABLISHING SIMILARITY BETWEEN MULTISOURCE BETAHISTINE DIHYDROCHLORIDE ORAL DOSAGE FORMS USING *IN VITRO* METHODSRAMZI SHAWAHNA^{1,2*}, MASHHOUR GHANEM³, AYAT GHANEM¹, ABDUL-FATTAH MANSOUR¹, NEMA AHMAD¹ and ABDEL NASER ZAID¹¹Department of Pharmacy, Faculty of Medicine and Health Sciences, New Campus, Building: 19, Office: 1340, An-Najah National University, P.O. Box 7, Nablus, Palestine²An-Najah BioSciences Unit, Center for Poisons Control, Chemical and Biological Analyses, An-Najah National University, Nablus, Palestine³Pharmacare PLC, Ramallah, Palestine

Abstract: Betahistine dihydrochloride (BHD) is frequently used in treating attacks of vertigo in Ménière's syndrome. This study was conducted to classify BHD into biopharmaceutical classification system (BCS) and assess the suitability of using *in vitro* release profiles in demonstrating similarity between a generic and the innovator product. The solubility of BHD in aqueous media over the pH range of 1-7.5 was determined. Molecular descriptors like PSA, Log P, log D, and pKa were calculated using software packages. Tablets were tested for weight and content uniformity, friability and disintegration. The *in vitro* release profiles and dosage form performance of the generic were compared with the innovator. BHD was very highly soluble in aqueous media over the pH range of 1-7.5 and the dose number ranged from 0.0016 to 0.0048. The high permeability of BHD was predicted by molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors and the low PSA of 24.92 Å. Both, innovator and generic showed good dosage form performance and released more than 85% of their contents in less than 15 min. Our study demonstrated that BHD was a BCS class I drug and thus eligible for biowaiver applications. Similarity between immediate release oral dosage forms can be established using suggested *in vitro* dissolution methods.

Keywords: betahistine, dissolution, biopharmaceutical classification system, biowaiver

Abbreviations: API - active pharmaceutical ingredient, BCS - biopharmaceutical classification system, BE - bioequivalence, BHD - betahistine dihydrochloride, CHMP - Committee for Medicinal Products for Human Use, EMA - European Medicines Agency, FDA - United States Food and Drug Administration, IR - immediate release, Log D - distribution coefficient, Log P - n-octanol/water partition coefficient, MA - marketing authorization, PSA - polar surface area, RLD - reference listed drug, WHO - World Health Organization

Betahistine dihydrochloride (BHD) [N-methyl-2-(pyridin-2-yl)-ethanamine, 2HCl] (1) is a histamine-like (structural analogue) drug that is used clinically to decrease the severity and frequency of attacks of vertigo in the chronic or interim phase of Ménière's syndrome (2). Ménière's syndrome affects many people around the world. Recent studies reported a prevalence of 190 per 100,000 population in the Unites States alone (3, 4). Today, BHD is approved in more than 80 countries and since its first approval in 1968 more than 130 million patients used BHD (5). Pharmacodynamic studies have shown that betahistine acts as a neurotransmitter modulator of the complex histaminergic receptor

system with strong antagonistic effect on the H₂ receptor (6, 7).

Various formulations are currently available on the market place around the world. Recently, there has been a growing concern on the quality and efficacy of generic formulations when clinicians and pharmacists are in difficult situations to choose among alternatives (8). The bioavailability of BHD from different oral formulations is an important parameter to compare the clinical performance of these formulations. Different formulations containing BHD might not have similar bioavailability and this may lead to suboptimal therapy when the formulation has less bioavailabil-

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ity or toxicity when the formulation has more bioavailability.

Since 1960s, *in vivo* pharmacokinetic bioequivalence (BE) studies have emerged as “gold standards” in proving similarity and interchangeability between innovator products and their generic versions. BE studies entail comparing the plasma or urine concentration *versus* time profiles of a test *versus* a reference listed drug (RLD) product (8). Sensitive methods to determine the concentration of BHD in biological samples after oral administration are still lacking (9). Previous methods reported in the literature were not sensitive enough to study the pharmacokinetics of BHD in plasma following oral administration (10). Since BHD undergoes almost complete first pass metabolism in human mainly to form 2-pyridylacetic acid (9), this metabolite can be used as a surrogate indication of BHD in human plasma following oral ingestion of BHD oral dosage forms (9). Recently, *in vitro* dissolution testing has emerged as powerful tool in predicting *in vivo* bioavailability of oral drug formulations. The advent of the biopharmaceutical classification system (BCS) and the wide adoption by the regulatory agencies around the globe, especially the United States Food and Drug Administration (FDA), European Medicines Agency (EMA) and the World Health Organization (WHO) have fundamentally changed the drug approval process for immediate release (IR) solid oral formulations (11). Regulatory agencies now can waive the *in vivo* BE studies for IR solid oral formulations containing high solubility (BCS class I and III) drugs and grant the formulation with a marketing authorization (MA) based on a biowaiver application (12, 13). Based on the biowaiver principles, very rapid dissolution or rapid dissolution with similarity factors are enough proof of similarity if the drug has a wide therapeutic index and the formulation contained non-interfering excipients (12-14).

The Focus Group on BCS and Biowaivers of the International Pharmaceutical Federation (FIP) has invited scientists around the world to prepare biowaiver monographs evaluating the suitability of waiving *in vivo* BE studies for drugs listed on the WHO's essential medicines list (15).

In the present study, we attempt to classify BHD as an active pharmaceutical ingredient (API) into its BCS solubility and permeability classes and we compare the *in vitro* dosage form performance of IR oral formulation containing BHD against the RLD. Our results provide rational for the interchangeability between the RLD and generic version based on *in vitro* release profiles. Our results might

be of particular importance for pharmaceutical companies aiming to develop IR solid oral formulations containing BHD as an API, particularly concerned with the difficulty of quantifying pure BHD in biological fluids.

EXPERIMENTAL

Materials

Potassium dihydrogen orthophosphate, hydrochloric acid fuming (37%), and sodium hydroxide pellets GR for synthesis were purchased from Merck (Merck, Germany). BHD working standard was purchased from Refarmed (Refarmed Chemicals Ltd., Switzerland). Purified water was obtained using Millipore Milli-Q Plus (0.45 μm , conductivity: maximum 1.3 $\mu\text{S/cm}$, TOC: maximum 500 ppb) water purification system (Millipore, Bedford, USA). Generic BHD (24 mg) tablets were manufactured by a local pharmaceutical manufacturing company. The RLD for BHD (24 mg) tablets were purchased from a private pharmacy shop (Ramallah, Palestine).

Determination of aqueous solubility

The solubility of BHD was determined using a standard shake flask method in the pH range of 1-7.5 at 37°C (16). Briefly, BHD was added in surplus amounts into aqueous media, the pH of the media was adjusted to 1, 3.5, 4.5, 6.8, and 7.5 using 0.01 M HCl or 0.01 M NaOH (13). The temperature was fixed at 37°C and flasks were shaken for 24 h. Samples were withdrawn and analyzed for BHD solubility using a double beam ultraviolet-visible spectrometer (PG Instruments, U.K.). The dose number was calculated according to the following equation (17, 18):

$$D_0 = \frac{\left(\frac{M_0}{V_0}\right)}{C_s}$$

where, M_0 is the highest dose strength (milligrams), C_s is the solubility (milligrams per milliliter), and $V_0 = 250$ mL.

Determination of permeability

Molecular descriptors like polar surface area (PSA), *n*-octanol/water partition coefficient (log P), distribution-coefficient at pH 6 (log D_6), number of hydrogen bond acceptors, number of hydrogen bond donors and pKa of BHD were calculated using ACD/Labs (ACD/Labs, Advanced Chemistry Development: Toronto, Canada), ChemAxon (ChemAxon, Budapest, Hungary) and ALOGPS (The Virtual Computational Chemistry Laboratory, VCCLAB, Germany) software packages.

Product characteristics and performance

Product characteristics and performance were evaluated by standard weight uniformity, content uniformity, friability, disintegration and dissolution testing.

To evaluate weight uniformity, innovator and generic tablets were weighed individually using analytical balance (Ohaus, USA). The percentage deviation of the individual tablets from the mean was determined according to British Pharmacopeia (19). The friability strength of the uncoated tablets was estimated using a friability tester (Erweka TA3R, Germany). A double beam ultraviolet-visible spectrometer was used to quantify the amounts of BHD. Evaluation of disintegration time of BHD tablets was done according to the procedure described in the United States Pharmacopeia (20). Briefly, one tablet was placed in each of the 6 tubes of the basket of the disintegration apparatus (Erweka, Germany). Each tube was filled with water and the temperature was adjusted at $37 \pm 2^\circ\text{C}$. Disintegration times were noted. According to the United States Pharmacopeia, IR tablets should disintegrate completely in ≤ 30 min.

Dissolution of RLD and generic BHD tablets was evaluated using United States Pharmacopeia paddle 2 semi-automated dissolution testing system (PT-DT70, PharmaTest, Germany). In accordance with the test standards recommended by the FDA, a total of 12 tablets were tested. In the dissolution apparatus, 1 tablet was placed in each vessel with a paddle stirrer at 50 rpm filled with 900 mL of dissolution media in three different pH points (0.1 M HCl, phosphate buffer pH = 4.5, and phosphate buffer pH = 6.8, respectively) and the temperature was adjusted at $37 \pm 0.5^\circ\text{C}$ (21). Aliquots of 10 mL from each dissolution vessel were removed after 10, 15, 20, 30 min) and substituted by the same volume. The amounts of BHD released were determined spectrophotometrically.

RESULTS AND DISCUSSION

Interchangeability between a RLD and its generic versions is based on a proof of similarity (8). In this study, we attempted to classify BHD into BCS and assessed the similarity of a generic product containing BHD as an API with the RLD using *in vitro* release method. Pharmaceutical companies intending to formulate and market multisource IR solid oral dosage forms containing BHD might benefit from these results and apply for a waiver of *in vivo* BE studies (biowaiver) applications for the regulatory authorities, particularly when considering the difficulty of quantifying pure BHD in biological fluids.

BCS class

Our results showed that BHD was very highly soluble in water and the commercial dose of 24 mg can dissolve in less than 2 mL of water over the physiologically relevant pH range specified by the regulatory agencies (both FDA and EMA). According to the FDA guidelines, a drug can be considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1–7.5. This can be demonstrated through the calculation of the dose number of the drug of interest. Table 1 shows the solubility data and dose numbers at different pH points. Our results showed that BHD can unequivocally be assigned a “high solubility” BCS class. These solubility results were consistent with those reported in the literature (22–24). The estimated aqueous solubility of BHD was reported to be around 49 mg/mL (25). Similarly, in a previous application to waive *in vivo* BE requirements for BHD, the European Committee for Medicinal Products for Human Use (CHMP) evaluated the solubility data and concluded that BHD exhibit high solubility over the pH range specified in the EMA

Table 1. Solubility and dose number of BHD in aqueous media at 37°C over the pH range of 1-7.5.

pH of the aqueous media	Solubility (mg/mL)	Dose number for 24 mg strength
1	20	0.0048
3.5	30	0.0032
4.5	40	0.0024
6.8	60	0.0016
7.5	50	0.00192

guidelines (12). Our results showed that the dose number was less than 1 all over the pH range specified by the FDA too (1-7.5).

Table 2 shows calculated molecular descriptors of BHD using different software packages. Analyzing the chemical structure of BHD, ChemAxon predicted 2 hydrogen bond acceptors, 1 hydrogen bond donor, and a PSA of 24.92 Å. ChemAxon showed that BHD is a basic compound with a calculated pKa of 9.77.

The rest of molecular descriptors are shown in Table 2. Various attempts failed to quantify effectively pure BHD in the plasma (26). However, absorption was said to be rapid and complete following oral administration of radio-labeled betahistine with a peak plasma concentration reached 1 h after oral administration (9). The predicted PSA of BHD was less than 60 Å. Having a molecular weight of less than 500 (approximately 209), hydrogen bond acceptors of less than 10, and hydrogen bond donors of less than 5, BHD violates Lipinski's rule of five by only having a log *P* of less than 1 (27). Taken together, molecular weight, PSA with the number of hydrogen bond acceptors and hydrogen bond donors might explain the observed rapid and complete absorption of BHD *in vivo* (9, 27). Previous studies showed that PSA and log *D* best described intestinal oral absorption (28, 29). Although the reported log *P* values did

not differ widely, it seems that partition coefficient data do not explain passive permeability, especially, when radio-labeled BHD showed rapid and complete absorption (9, 30-32). The extremely low concentrations of BHD in plasma following oral administration could be explained by the complete metabolism into 2-pyridylacetic acid (9, 30-32). BHD was shown to be excreted almost completely (80-90%) as 2-pyridylacetic acid in the urine within 24 to 48 h (26, 33). Taken together, our results suggest that BHD can be classified under BCS class I (high solubility and high permeability).

Dosage form performance and *in vitro* release characteristics

Visually inspected BHD tablets showed no defects in physical appearance. Overall, all tablets were of good quality and did not show any signs of defects with respect to shape, color, presence of black spots or preached edges. Previous studies showed that the organoleptic properties of a pharmaceutical product are very important for ensuring patient compliance and confidence in the therapy (34). As shown in Table 3, the tested tablets were within the accepted range for weight uniformity and disintegration time specified by the British Pharmacopeia (19). In this study, the content uniformity was also assessed to ascertain the quality of dosage form and ensure that

Table 2. Predicted molecular descriptors of BHD using different software.

Property	Software		
	ACD/Labs	ChemAxon	ALOGPS
Log <i>P</i>	0.10 ± 0.21	0.63	0.59
Log <i>D</i> at pH 6 (log <i>D</i> _{6.0})	-2.99	-2	-
Number of hydrogen bond acceptors	2	2	-
Number of hydrogen bond donors	1	1	-
PSA (Å)	24.92	24.92	-
pKa	-	7.4	-

Table 3. Characteristics of RLD and generic BHD tablets.

Parameter	RLD	Generic
Weight average (mg)	440	443
Content assay (%)	98.9	98.5
Friability (% of loss)	< 1%	< 1%
Disintegration time (min)	4.4	4.65

RLD = reference listed drug

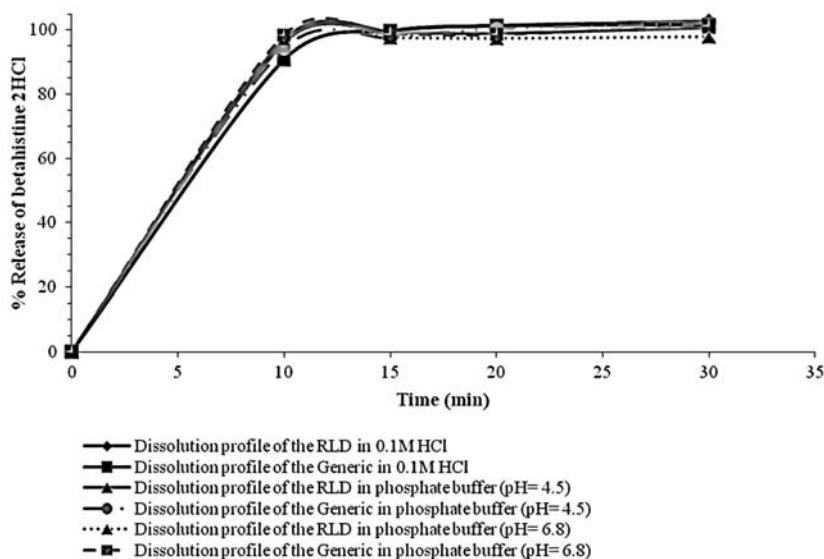


Figure 1. Dissolution profile of RLD and generic BHD in: 0.1 M HCl, phosphate buffer pH = 4.5 and phosphate buffer pH = 6.8

Table 4. Excipients included in multisource IR solid oral formulations with a MA in Canada (CA)^a, Czech Republic (CZ)^b, France (FR)^c, and United Kingdom (UK)^d, and the minimal and maximal amount of that excipient present per dosage unit in solid oral drug products with a MA in the US^e

Excipients	Drug formulations containing that excipient with a MA granted by the named country	Range present in solid oral dosage forms with a MA in the US (mg)
Povidone	CA (1), CZ (4-7, 9-12), FR (13-15, 17,19-23, 25), UK (26)	0.17-80
Microcrystalline cellulose	CA (1-3), CZ (4-12), FR (13-25), UK (26)	0.75-1385
Lactose monohydrate	CA (1), CZ (4-7, 9-12), FR (13-15, 17-25)	4.9-614.2
Colloidal anhydrous silica	CA (2), CZ (4-10), FR (13-25), UK (26)	0.50-100
Crospovidone	CA (1), CZ (4-7, 9-12), FR (13-15, 17-23, 25), UK (26)	2-792
Stearic acid	CA (1, 2), CZ (4-12), FR (13-25), UK (26)	0.9-72
Hydrogenated vegetable oil	CA (1, 11, 12)	0.93-37.6
Maize starch	CA (1, 11, 12)	9.9-1135
Mannitol	CA (2, 3, 8, 16, 18, 24), UK (26)	10-454
Talc	CA (2, 8, 16, 18, 24), UK (26)	0.1-220
Sodium stearyl fumarate	CA (2)	0.85-29.3

^aSource of data: www.hc-sc.gc.ca (accessed on 01-10-2014); ^bSource of data: www.sukl.cz (accessed on 01-10-2014); ^cSource of data: www.theriaque.org (accessed on 01-10-2014); ^dSource of data: www.mhra.gov.uk (accessed on 01-10-2014); ^eSource of data: FDA's Inactive Ingredient Database, <http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm> (version date: 29-03-2013). 1. Betahistine dihydrochloride tablets, 24 mg (Cobalt Pharmaceuticals); 2. Betahistine dihydrochloride tablets, 24 mg (Abbott Laboratories); 3. Betahistine dihydrochloride tablets, 24 mg (Teva Canada Limited); 4. Betahistine dihydrochloride tablets, 24 mg (Avefarm); 5. Betahistine dihydrochloride tablets, 24 mg (Actavis Group); 6. Betahistine dihydrochloride tablets, mg (Generics [UK] Limited, Potters Bar); 7. Betahistine dihydrochloride tablets, 24 mg (Ratiopharm GmbH); 8. Betahistine dihydrochloride tablets, 24 mg (Abbott Healthcare Products BV); 9. Betahistine dihydrochloride tablets, 24 mg (EGIS Pharmaceuticals PLC); 10. Betahistine dihydrochloride tablets, 24 mg (PharmaSwiss); 11. Betahistine dihydrochloride tablets, 24 mg (MEDOCHEMIE Ltd.); 12. Betahistine dihydrochloride tablets, 24 mg (HENNIG Arzneimittel GmbH & Co.); 13. Betahistine dihydrochloride tablets, 24 mg (ACTAVIS); 14. Betahistine dihydrochloride tablets, 24 mg (ARROW); 15. Betahistine dihydrochloride tablets, 24 mg (BIOGARAN); 16. Betahistine dihydrochloride tablets, 24 mg (BIPHAR); 17. Betahistine dihydrochloride tablets, 24 mg (EG); 18. Betahistine dihydrochloride tablets, 24 mg (MYLAN); 19. Betahistine dihydrochloride tablets, 24 mg (RANBAXY); 20. Betahistine dihydrochloride tablets, 24 mg (RATIOPHARM); 21. Betahistine dihydrochloride tablets, 24 mg (SANDOZ); 22. Betahistine dihydrochloride tablets, 24 mg (TEVA); 23. Betahistine dihydrochloride tablets, 24 mg (ZENTIVA); 24. Betahistine dihydrochloride tablets, 24 mg (BETASERC); 25. Betahistine dihydrochloride tablets, 24 mg (LECTIL); 26. Betahistine dihydrochloride tablets, 24 mg (Milpharm Limited).

Table 5. Summary of different bioequivalence studies of solid oral products containing BHD.

Strength on which BE performed (mg)	Subjects	Test Formulation Manufacturer or License Holder	Composition	Reference product	Study design	Prandial	Bioequivalence Criteria/Statistics	Results	Ref.
24 1	7	pms-Betahistine, Pharmascience Inc.	BHD 24 mg, crosopvidone, colloida lactose anhydrous silica, monohydrate, microcrystalline cellulose, povidone and stearic acid	Serc [®] , Solvay Pharma Inc.	A blind, randomized, two-way crossover study in healthy volunteers	Fasting	90% CI (C_{min} , AUC_{0-12} , $AUC_{0-\infty}$) [*]	Bioequivalent	(27)
24	24	Teva-Betahistine, Teva Canada Limited.	BHD 24 mg, crosopvidone, colloidal lactose anhydrous silica, monohydrate, microcrystalline cellulose, povidone and stearic acid	Serc [®] , Solvay Pharma Inc.	A blinded, single-dose, randomized, two-period, two-sequence, two-treatment, crossover study in healthy volunteers	Fasting	90% CI (C_{min} , AUC_{0-12} , $AUC_{0-\infty}$) [*]	Bioequivalent	(38)
24	36	Vertisan, HENNIG Arzneimittel GmbH & Co.	BHD 24 mg, lactose monohydrate, corn starch, microcrystalline cellulose, citric acid, povidone, crosopvidone type A, hydrogenated vegetable oil	Serc [®] , Solvay Pharma Inc.	A randomized, two-way crossover study in healthy volunteers	Fed	90% CI (C_{min} , AUC_{0-12} , $AUC_{0-\infty}$) [*]	Bioequivalent	(38)

*Since BHD undergoes almost compete first-pass metabolism in human, bioequivalence was established using parameters of the main metabolite, 2-pyridylacetic acid.

all tablets contain the same amounts of the API (BHD). Table 3 shows that the generic and RLD BHD tablets contain similar amounts in the range permitted by the British Pharmacopeia. Weight uniformity and content assay are important parameters ensuring the uniformity of dose for all patients and hence similar *in vivo* performance.

All tablet products were subjected to dissolution testing using dissolution media at pH of 1.2, 4.5 and 6.8. The dissolution profiles of the RLD and generic products are shown in Figure 1. All tested products showed very rapid dissolution and released $\geq 85\%$ of their BHD contents in ≤ 15 min. Very rapid dissolving tablets are considered essentially similar without a need for similarity (f_2) and difference (f_1) factors (35).

Excipients

The excipients contained in several multisource IR solid oral formulations containing BHD 24 mg are listed in Table 4. The table does not include colorants, water, and ingredients present in the coating and polish. During our literature review, we did not find any evidence on possible effects of various excipients on the release and absorption of BHD. Since many of the multisource IR solid oral formulations containing BHD 24 mg are marketed in ICH associated countries, we assume that these formulations already passed rigorous BE studies and hence the combination of these various excipients do not affect the absorption of BHD. We therefore believe that there is no risk of bioequivalence as a result of incorporating excipients or manufacturing process parameters.

In vivo BE

Multisource BHD formulations were generally reported to perform well *in vivo*. In previous BE studies, plasma levels of the metabolite, 2-pyridylacetic acid, were compared in healthy volunteers and all formulations met the BE criteria compared to the RLD (33, 36, 37). Table 5 summarizes different BE studies conducted for some multisource IR solid oral formulations containing BHD as an active ingredient. Results showed that generics were bioequivalents compared to the RLD.

Therapeutic index and patient's risk due to bioequivalence

Since its approval, clinical and postmarketing studies showed that BHD has a good safety profile (39). Safety surveillance data of more than 35 years revealed that the adverse drug reactions associated with the use of BHD are generally mild and serious reactions are very rare (39). The most commonly

reported signs and symptoms were mild cutaneous hypersensitivity reactions (39). A few reported overdose cases (with up to 640 mg), showed that patients suffered mild to moderate symptoms of nausea, dry mouth, dyspepsia, abdominal pain and somnolence (27). Previous studies showed that even very high doses of 480 mg/day were beneficial for patients with severe Ménière's disease (40). The oral LD₀ for BHD was 3040 mg/kg in the albino rat (27). It is therefore unlikely that BHD would cause serious adverse reactions at concentrations moderately above or below the therapeutic concentrations as a result of bioinequivalence.

Furthermore, we searched two official lists of narrow therapeutic index drugs, one published by the National Institute of Health Sciences in Japan (<http://www.nihs.go.jp>) and the other by the United States FDA (<http://ecapps.health.state.pa.us>) and BHD was not found in these two lists. We therefore conclude that fluctuations in plasma levels and bioinequivalence might not present serious risk of toxicity since there is a wide difference between the usual therapeutic dose and toxic doses. So, it can be assumed that BHD is not a narrow therapeutic index drug and eligible for biowaiver applications.

CONCLUSION

In conclusion, our investigation suggests that BHD is a high solubility, high permeability drug substance and hence assigned a BCS class I drug. BHD is not a narrow therapeutic index drug and the risks associated with bioinequivalence are manageable. Therefore, we can consider BHD as a suitable candidate for biowaiver applications and it is therefore suitable to use *in vitro* dissolution testing to surrogate *in vivo* BE testing. Establishing similarity between multisource IR oral formulations containing BHD as an API by comparing *in vitro* release profiles in dissolution media at the pH points of 1.2, 4.5 and 6.8 (at $37 \pm 0.5^\circ\text{C}$) is scientifically justified when: 1) formulations contain only excipients that are well known and used in normal amounts known not to interfere with the release and absorption of the API, and (2) both the test and RLD formulation enable very rapid dissolution of BHD, or, rapid dissolution with similarity of the dissolution profiles demonstrated at least at pH 1.2, 4.5, and 6.8 for BHD.

Competing interests

All authors declare that this study was conducted for the purpose of scientific research only. All authors declare no competing interests.

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