Journal of Pharmaceutical Sciences 112 (2023) 893–903

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

General Commentary

Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Levocetirizine Dihydrochloride

Naseem A. Charoo[a], Daud B. Abdallah[b], Daoud T. Ahmed[c], Bertil Abrahamsson[d], Rodrigo Cristofoletti[e], Peter Langguth[f], Mehul Mehta[g], Alan Parr[h], James E. Polli[i], Vinod P. Shah[j], Atsushi Kambayashi[k], Jennifer Dressman[l,*]

[a] *Adcan Pharma LLC, Industrial City of Abu Dhabi, United Arab Emirates*
[b] *Department of Pharmaceutics, Faculty of Pharmacy, Al Ribat University, Khartoum, Sudan*
[c] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Omdurman Islamic University, Khartoum, Sudan*
[d] *Oral Product Development, Pharmaceutical Technology & Development, Operations AstraZeneca, Gothenburg, Sweden*
[e] *Center for Pharmacometrics & Systems Pharmacology, Department of Pharmaceutics, University of Florida, Orlando, FL, USA*
[f] *Department of Pharmaceutical Technology and Biopharmaceutics, Johannes Gutenberg University, Mainz, Germany*
[g] *United States Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD, USA*
[h] *Bioceutics LCC, Raleigh-Durham, NC, USA*
[i] *Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD, USA*
[j] *International Pharmaceutical Federation (FIP), The Hague, the Netherlands*
[k] *Pharmaceutical Research and Technology Labs, Astellas Pharma Inc, Analytical Research Laboratories, Yaizu, Japan*
[l] *Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Frankfurt am Main, Germany*

ARTICLE INFO

Article history:
Received 15 December 2022
Revised 21 December 2022
Accepted 21 December 2022
Available online 26 December 2022

Keywords:
Levocetirizine dihydrochloride
Biopharmaceutics classification system
Solubility
Dissolution
Toxicity
Side effects
Pharmacokinetics
Biowaiver
Permeability
Absorption

ABSTRACT

Levocetirizine, a histamine H1-receptor antagonist, is prescribed to treat uncomplicated skin rashes associated with chronic idiopathic urticaria as well as the symptoms of both seasonal and continual allergic rhinitis. In this monograph, the practicality of using Biopharmaceutics Classification System (BCS) based methodologies as a substitute for pharmacokinetic studies in human volunteers to appraise the bioequivalence of immediate-release (IR) oral, solid dosage forms containing levocetirizine dihydrochloride was investigated, using data from the literature and in-house testing.

Levocetirizine's solubility and permeability properties, as well as its dissolution from commercial products, its therapeutic uses, therapeutic index, pharmacokinetics and pharmacodynamic traits, were reviewed in accordance with the BCS, along with any reports in the literature about failure to meet bioequivalence (BE) requirements, bioavailability issues, drug-excipient interactions as well as other relevant information. The data presented in this monograph unequivocally point to classification of levocetirizine in BCS Class 1. For products that are somewhat supra-equivalent or somewhat sub-equivalent, clinical risks are expected to be insignificant in light of levocetirizine's wide therapeutic index and unlikelihood of severe adverse effects. After careful consideration of all the information available, it was concluded that the BCS-based biowaiver can be implemented for products which contain levocetirizine dihydrochloride, provided (a) the test product comprises excipients that are typically found in IR oral, solid drug products that have been approved by a country belonging to or associated with ICH and are used in quantities that are typical for such products, (b) data supporting the BCS-based biowaiver are gathered using ICH-recommended methods, and (c) all *in vitro* dissolution requirements specified in the ICH guidance are met by both the test and comparator products (in this case, the comparator is the innovator product).

Introduction

Allergic rhinitis reportedly afflicts about 10–40% of the population, with a trend to an increase in its prevalence over time. It can severely affect sleep, school and work performance and thus quality of life. Additionally, allergic rhinitis represents a significant risk factor for the development of asthma and it has been estimated that up to 40% of people suffering with allergic rhinitis may later contract asthma.[1-2]

In 1999, the Allergic Rhinitis and its Impact on Asthma (ARIA) initiative originated in a World Health Organisation (WHO) workshop. The first evidence-based guideline for chronic respiratory disease was

* Corresponding author.
E-mail address: jdressman@em.uni-frankfurt.de (J. Dressman).

https://doi.org/10.1016/j.xphs.2022.12.021
0022-3549/

published in 2010 and updated in 2016.[3] The guidelines provide conditional recommendations for the use of either intranasal or oral H1-antihistamines (the newer, less sedating antihistamines i.e. levocetirizine, cetirizine and loratadine) or their combination with an intranasal corticosteroid in patients with seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR).[3] For SAR, Recommendation 1A of the guideline suggests using either an intranasally administered corticosteroid or a combination of an intranasally administered corticosteroid with an orally administered H1-antihistamine. Recommendation 4A recommends either a leukotriene receptor antagonist or an oral H1-antihistamine for SAR. For PAR, the guideline recommends using an intranasal corticosteroid alone (1B), while an oral H1-antihistamine is preferred over a leukotriene receptor antagonist (4B). In terms of whether the H1-antihistamine should be applied nasally or orally for SAR and PAR (Recommendations 6A and 6B), the route of administration is left to the preferences of patients and clinicians.[3]

Cetirizine is a racemic mixture comprising the R and S enantiomers of cetirizine. Levocetirizine is the pharmacologically active enantiomer of cetirizine (R cetirizine).[4-5] This potent histamine H1 receptor antagonist is indicated for the therapy of allergic rhinitis and chronic idiopathic urticaria in both adults and children. The R and S cetirizine isomers show different pharmacological activity, H1 receptor affinity/occupancy and dissociation constants.

Levocetirizine is an excellent candidate for irreversible inhibition of H1 receptors due to its pharmacological attributes, including high affinity/occupancy at the receptor and a long half-life of dissociation. Moreover, its onset of action is rapid, and this is coupled with a long duration of the antihistaminic effect. From a pharmacokinetic standpoint, its bioavailability is high, and it has a low propensity to cause drug interactions due to its minimal hepatic metabolism. Levocetirizine also shows anti-inflammatory effects that enhance both its short and long-term therapeutic benefits.[4-5] By contrast, the S enantiomer (dextracetirizine) does not exhibit any appreciable pharmacodynamic activity, as evidenced by the fact that it does not inhibit wheal and flare responses induced by histamine.

The biopharmaceutical and clinical attributes of levocetirizine dihydrochloride are presented and discussed in this Biowaiver monograph. The risks associated with replacing clinical bioequivalence studies with comparative dissolution testing in accordance with the ICH and associated guidelines for the approval of IR solid oral products which contain levocetirizine dihydrochloride as the sole active pharmaceutical ingredient are determined and discussed.[6-9] The risks of approving such products using the BCS-based biowaiver methodology are first, that an incorrect decision is reached regarding the bioequivalence of the comparator and test products, and second, how an incorrect decision could affect the individual patient and impinge on public health. For these deliberations, several guidances were taken into consideration.[6-9]

Methods

To search readily accessible online literature databases, keywords included: levocetirizine, therapeutic index, toxicity, safety and efficacy, polymorphic forms, solubility, dissolution, permeability, BCS classification, absorption, bioavailability, linear pharmacokinetics, distribution, metabolism, excretion, bioequivalence, and biowaiver. The ICH, WHO, US-FDA, and EMA regulatory guidances were also consulted to obtain the necessary information.[6-9]

All information was thoroughly reviewed and organized using the same approach as in recent biowaiver monographs such as sitagliptin and moxifloxacin.[10-11]

Solubility studies on levocetirizine pure substance and its release from the test formulation (Azal Pharmaceutical Company, Khartoum) were carried out at the Al Ribat University in Khartoum. To determine its solubility in buffer solutions over the pH range 1.2–6.8, an excess of levocetirizine dihydrochloride was added to suitable buffer solutions complying with European Pharmacopoeia specifications. The flasks were then maintained at 37 ± 0.5 °C for 48 h while being mechanically shaken at 100 rpm. (SBS40 Shaker, Bibby Scientific Limited, Staffordshire, ST15 OSA, UK).

The pH of saturated solutions was measured before and after the solubility experiments using a pH/Ion 510 meter. (Oakton instruments, Vernon Hills, IL 60061, USA). Following filtration through a 0.45 μm PTFE [(Teflon) membrane filter (Sterlitech Corporation, Washington, USA)], the samples were diluted with the corresponding buffer solution immediately to prevent precipitation and to prepare the samples for UV analysis. Absorbance was measured at 230 nm (UV mini-1240 spectrophotometer, Shimadzu, Japan) and solubilities are reported as the average and standard deviation (N=3).[12]

Additional testing had to be performed at all three pH values (1.2, 4.5, and 6.8), as a marked pH shift was observed in the first round of tests. In the second round of tests, smaller amounts of levocetirizine dihydrochloride (approx. 20 mg per flask) were weighed into 100 ml volumetric flasks, and made to volume with buffer. In these studies, the pH of the buffer shifted by less than 0.5 units after 48 h of incubation.[12]

To study dissolution, 5mg film coated tablets (Azal Pharmaceutical Company, Khartoum, Sudan; Batch No.10328; manufacturing date: 8/2021; expiry date: 8/2022) were selected. Testing was carried out in a USP Type 2 dissolution tester (DISSO 2000; Lab India Instruments Pvt. Ltd, Mumbai, India) in either 900 ml of 0.1 N HCl, acetate buffer pH 4.5 or phosphate buffer pH 6.8 at 37 °C. In each case, the dissolution medium was prepared and degassed shortly before the test began.[12] At selected time intervals, a 5 ml sample was withdrawn, and the volume was replaced with pre-warmed buffer. The sample was then filtered through an 0.45μm PTFE (Teflon) membrane filter, suitably diluted and immediately analysed at 230 nm by UV spectrophotometry, as described above.[12]

General Characteristics

Levocetirizine dihydrochloride's structure is shown in Figure 1. Table 1 summarizes its chemical composition and nomenclature. The chemical structure of levocetirizine dihydrochloride has one chiral centre.[4-5] Cetirizine hydrochloride is a racemic mixture of levocetirizine and dextrocetirizine, levocetrizine being the R- and dextrocetirizine being the S- enantiomer.[4-5] Although the racemic mixture (cetirizine) and the R-enantiomer (levocetirizine) both evoke maximum inhibition of wheal and flare induced by histamine, the pure S-enantiomer was not able to inhibit the response to histamine, suggesting stereoselectivity at the H1 receptor.[13] This is supported by the much lower potency of dextrocetirizine than levocetirizine.[14] The inability of human metabolism to bring about chiral inversion of levocetirizine explains the difference in potency of the two enantiomers in clinical trials.[14]

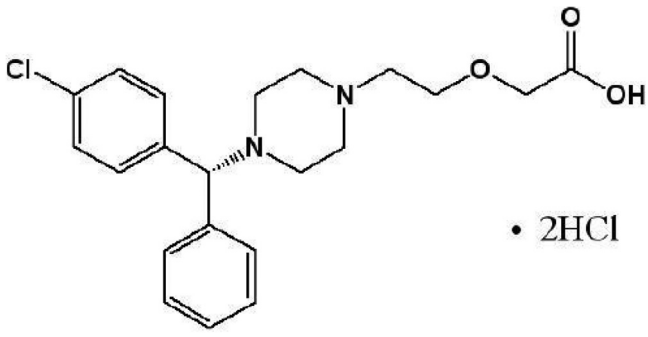

Fig. 1. The Chemical Structure of levocetirizine dihydrochloride.

Table 1
Chemical composition, nomenclature and appearance of levocetirizine dihydrochloride.

INN name	Levocetirizine dihydrochloride
Chemical name	(R)-[2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride
Molecular formula	$C_{21}H_{25}ClN_2O_3$•2HCl
Molecular weight g/mol	461.8
Chemical Abstracts Service (CAS) Registry Number	130018-87-0
Appearance	White to off-white crystalline powder
Melting point	215–220 °C

Therapeutic Uses, Therapeutic Index, Adverse Effects, Toxicity, and Drug Interactions

Levocetirizine is used to alleviate the symptoms of seasonal and perennial allergic rhinitis in young children (6 months to 2 years old),[15] as well as in older children and adults. Treatment for intermittent allergic rhinitis is determined by the patient's history and the severity of the symptoms. Levocetirizine is to be discontinued once the symptoms have subsided, but the treatment may be resumed if symptoms recur. On the other hand, persistent allergic rhinitis can be managed with continuous therapy throughout the period of exposure to allergens.[5,15]

Additionally, levocetirizine is recommended for treating children who are six months of age and older and for adults with milder skin manifestations of chronic idiopathic urticaria.[5,15]

Levocetirizine exhibits antihistamine activity by its selective inhibition of H1 receptors. With a Ki of 3.2 nmol/l, it shows 2-fold higher affinity for H1 receptors in humans than cetirizine, which has a Ki of 6.3 nmol/l. In fact, pharmacodynamic studies performed in healthy human volunteers have revealed that levocetirizine administered at one-half of the usual dose produces comparable activity to cetirizine at the site of action (in the nose or in the skin). The half-life of its dissociation from H1 receptors is 115 ± 38 min. Following a single dose by mouth, it occupies 90% of receptors at 4 h, decreasing to 57% at 24 h.[5,15-16]

In a randomised controlled trial, oral levocetirizine 5mg significantly decreased formation of wheal and flare induced by histamine, compared to placebo and desloratadine 5 mg. The suppression of wheal and flare formation produced by levocetirizine was at its highest over the first 12 h and continued for 24 h.[5,15,17-18]

In placebo-controlled trial using an allergen challenge chamber model, oral levocetirizine 5 mg suppressed pollen-induced symptoms within 1 h after drug administration.[5] *In vivo* skin chamber technique studies revealed that, when the skin was subjected to pollen, levocetirizine 5 mg produced three main inhibitory effects on the skin's reaction. Over the first six hours, VCAM-1 release was reduced, there was less modulation of vascular permeability and eosinophil recruitment was lower than in the placebo group.[5,19]

Levocetirizine is a wide therapeutic index drug with few adverse effects.[20-21] Its safety and efficacy have been confirmed in many placebo-controlled clinical trials conducted in adult patients with SAR, PAR, or persistent allergic rhinitis including nasal obstruction.[5] For instance, the potency of levocetirizine against persistent allergic rhinitis was tested against placebo in a six-month clinical study involving 551 adult patients. Persistent allergic rhinitis was defined in that study as having symptoms that persisted for four days a week during at least four consecutive weeks. The patients were sensitive to dust mites and/or grass pollen. Levocetirizine 5mg was more potent (clinically and statistically) than placebo in terms of a lower overall symptom score throughout the study duration without any signs of tachyphylaxis. Moreover, the quality of life improved significantly during the course of the study in patients taking levocetirizine versus those taking placebo.[5,15] In another clinical study performed in 166 patients with chronic idiopathic urticaria, levocetirizine treatment (5 mg once daily oral administration for six weeks) resulted in a significant decrease in severity of pruritus over the entire duration of treatment compared to placebo. Moreover, the Dermatology Life Quality Index, an indicator of quality of life related to health status, was much higher in the levocetirizine group compared to the placebo group.[5,15]

The safety and efficacy of levocetirizine in the treatment of SAR and PAR have also been confirmed in paediatric patients between 6 and 12 years old. Additionally, several short- and long-term studies have established its clinical safety in paediatric patients below 6 years of age.[5,15]

At concentrations higher than those usually achieved at the therapeutic dose (5 mg orally), levocetirizine is not able to inhibit the CYP 1A2, 2C9, 2C19, 2A1, 2D6, 2E1, and 3A4. Neither does it induce UGT1A or CYP 1A2, 2C9 and 3A4.[5,15] While information on formal *in vivo* studies of metabolically-based drug interactions is scarce, *in vitro* data suggest that levocetirizine is not likely to be either a perpetrator or victim of such interactions. Indeed, studies performed with the racemic mixture, cetirizine, have failed to demonstrate clinically relevant adverse interactions with antipyrine, azithromycin, cimetidine, diazepam, erythromycin, glipizide, ketoconazole or pseudoephedrine. Theophylline (400 mg once a day) was shown to cause a mild decrease (16%) in cetirizine clearance in a multi-dose study, whereas theophylline disposition was not impacted by concomitant administration of cetirizine. In another multi-dose study, ritonavir (600 mg administered twice daily) increased the extent of exposure of cetirizine (10 mg daily dose) by about 40% with a slight impact on ritonavir disposition.[5,15]

Administration with food has no impact on the extent of absorption of levocetirizine. However, the rate of its absorption is reduced by co-administration with food.[5,15] Following administration with a high fat meal, T_{max} was postponed by 1.25 h and the C_{max} was reduced by about 36%. It was concluded that levocetirizine can be administered regardless of food intake.[5,15]

The most common adverse effects observed in patients who are 12 years of age and older are somnolence, nasopharyngitis, fatigue, dry mouth, and pharyngitis. On the other hand, in children between 6 and 12 years old, pyrexia, somnolence, cough, and epistaxis are the commonly seen adverse effects associated with levocetirizine use.[5,15] Two placebo-controlled studies were performed in paediatric patients under six years of age. In one study levocetirizine was administered once daily at a dose of 1.25 mg to patients aged from 6 to 11 months. In a second study, 1.25 mg cetirizine was administered twice daily to patients aged 1–5 years. The adverse effects observed were diarrhoea, constipation, sleep disorders and somnolence.[5,15]

Levocetirizine is contraindicated in patients who are known to have a hypersensitivity to levocetirizine, cetirizine, hydroxyzine or to any other piperazine derivatives. It is also contraindicated in patients with severe renal impairment (creatinine clearance less than 10 ml/min) and patients receiving hemodialysis.[5,15] By the same token, paediatric patients between 6 months and 11 years of age who suffer from impaired renal function should not be administered levocetirizine. Further, it is not recommended for patients with galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption, all of which are rare hereditary problems.[5,15]

Concurrent use with alcohol should be avoided as levocetirizine may further reduce alertness and impair central nervous system performance.[5,15]

Dosage Forms, Dose and Strength

Levocetirizine dihydrochloride is marketed as 5 mg film-coated tablets and a 0.5mg/ml oral solution.[5,15,22] It is available as scored (breakable) tablets which permit administration of 2.5 mg if required by the patient. Orodispersible tablets of levocetirizine are also

available.[23] Levocetirizine dihydrochloride drug substance and tablets are monographed in the USP.[12,24]

For adults and children 12 years and older, a dose of levocetirizine 5 mg once daily in the evening is recommended. The dose may be scaled down by half (2.5 mg once daily in the evening) in some patients, if their symptoms can be adequately controlled by lower doses.[5,15]

A dose of 2.5 mg once daily in the evening is also recommended for the 6–11 year old age group. According to some Prescribers Information documents, the dose should not be increased in this population category as systemic exposure after administration of 5 mg levocetirizine is double that in adults.[5,15] However, levocetirizine products approved in Europe recommend a daily dose of 5 mg for children in the 6 to 12 year age group.[5,15] Perhaps the wide therapeutic index of levocetirizine allows a higher dose administration.

For younger children (6 months to 5 years), a dose of 1.25 mg to be given once daily in the evening is recommended.[25] This dose provides an exposure that is comparable to a 5 mg dose in adults and thus should not be exceeded.[5,15]

Dose adjustments and contraindications for adults and children 12 years and older who have varying degrees of renal impairment can be found in the Prescribers Information documents.[5,15] Although patients suffering solely from hepatic impairment do not need dose adjustment, if they are also suffering from renal impairment, a dose adjustment is recommended.

According to the package insert, levocetirizine can be taken with or without food.

Physicochemical Properties

In both the tablet and liquid oral dosage formulations, levocetirizine is available in the levocetirizine dihydrochloride form.[5,15,22]

Polymorphism

Both morphous and the crystalline form-I are reported in the literature.[26-27] The majority of the commercially available preparations of levocetirizine contain crystalline form I.[28] Since no other crystalline forms have been reported in the open literature, some reports suggest that it does not show polymorphism.[23, 5,15]

Partition Coefficient and pKa

At pH 7.4, levocetirizine shows an octanol-water distribution coefficient of 1.32 ± 0.03.[29] The pKa of levocetirizine is not directly reported in the literature. However, being an R-isomer of cetirizine, its pKa values are likely to be the same as that of cetirizine. Like cetirizine, levocetirizine has three ionisable moieties and will have similar pKa values, i.e., 2.2 for the carboxylic acid group) and 2.9 and 8.0 for the piperazine group.[30]

Solubility

The solubility of levocetirizine is 94.6 g/100 ml in water.[29] Table 2 displays the experimentally measured solubility values at various pH values.

Pharmacokinetic Properties

Absorption and Bioavailability

When administered orally, levocetirizine is absorbed rapidly and almost completely. Recovery in the urine after dosing at 5 mg was just over 85% and in the feces just under 13% by 168 h post dose.[5,14-15] It has also been reported that by 48 h after oral dosing, 77–85.5% is recovered in the urine, again suggesting near complete bioavailability.[14,31-32] Differences arising from genetic polymorphism or concurrent use of drug metabolism inhibitors are anticipated to be negligible in the case of levocetirizine given that the amount of metabolism in humans is less than 14%.

Orally administered tablets achieve peak plasma concentration in adults in 0.9 hours, slightly longer than the T_{peak} after dosing an oral solution, which is 0.5 h.[5,15] Typically, the highest plasma concentration achieved after a single 5 mg dose is 270 ng/ml, which increases to 308 ng/ml after repeated once daily doses of 5 mg.[5,15] Levocetirizine thus shows an accumulation ratio of 1.12 after oral administration at steady state.

The extent of absorption of levocetirizine, as reflected in the AUC, was not impacted when it was administered with a high fat meal. However, the T_{peak} was about 1.25 h longer and the C_{max} approximately 36% lower.[5,15] As these changes are not expected to have any impact on therapeutic success, levocetirizine can be taken with or without food. [5,15]

The 5 mg tablets and 5 mg oral solution have been shown to be bioequivalent.[15] Moreover, the plasma exposure of levocetirizine was equivalent (bioequivalent) when it was administered to healthy Japanese volunteers at 5 mg levocetirizine as an oral solution compared to 10 mg cetirizine as a dry syrup.[15,33]

Table 2
Experimentally determined solubility of levocetirizine dihydrochloride at various pH values and 37 °C and the corresponding dose/solubility ratios for the recommended highest single dose.

Saturation solubility of levocetirizine dihydrochloride					
pH	pH after addition of levocetirizine dihydrochloride	Final pH after 48 h shaking	Average Solubility (±SD) mg/mL	Temperature (°C)	Dose/solubility ratio (ml)
1.2 (0.1N HCl)	0.50	0.50	967.9 ± 0.36	37 ± 0.5	0.005
4.5 (acetate buffer)	0.72	0.72	830.8 ±0.34	37 ± 0.5	0.006
6.8 Phosphate buffer	0.92	0.92	726.2 ± 0.25	37 ± 0.5	0.007

Concentrations of levocetirizine dihydrochloride attained by adding approximately 20 mg levocetirizine dihydrochloride to 100 mL					
pH	**pH after addition of levocetirizine dihydrochloride**	**Final pH after 48 h shaking**	**Average Amount dissolved in 100 ml* (±SD)**	**Temperature (°C)**	**Dose/solubility (ml)**
1.2 (0.1N HCl)	1.25	1.23	20.61 ± 5.14	37 ±0.5	< 24.26
4.5 (acetate buffer)	4.54	4.53	20.60 ± 5.11	37 ±0.5	< 24.27
6.8 Phosphate buffer	6.79	6.79	20.61 ± 5.13	37 ±0.5	< 24.26

* These values do not represent the thermodynamic solubility, instead approximately 20 mg levocetirizine dihydrochloride was added to 100 mL buffer. This ensured that the pH of the buffer did not change by more than 0.5 units during the determination.

In healthy adult subjects, levocetirizine exhibits linearity in its pharmacokinetic parameters over the therapeutic dose range. The inter-subject variability is low.[5,15] Moreover, the pharmacokinetics of levocetirizine are independent of whether it is administered as the R-enantiomer or the cetirizine racemate. Gender-related differences in pharmacokinetics are not deemed to be of clinical importance.[5,15,29-30]

In a single dose, two-way crossover study carried out in 24 healthy human subjects (12 male and 12 female subjects) comparing the pharmacokinetics of levocetirizine with cetirizine, levocetirizine did not undergo chiral inversion, thus demonstrating configurational stability in vivo.[34] The apparent volume of distribution of levocetirizine was 0.41 L/kg, significantly smaller than cetirizine racemate (0.60L/kg) suggesting a positive characteristic with regard to efficacy and safety. The non-renal clearance (9.70 ml/min) of levocetirizine is also significantly lower in comparison to cetirizine racemate (28.70 ml/min) indicating a lower probability of metabolic drug interactions for levocetirizine.[34]

After administration of a single 5 mg dose, the C_{max} and AUC values in pediatric patients between 6 and 11 years old, with body weights of 20 to 40 kg, are twice as high as those in healthy adult subjects.[5,15] The average Tpeak was 1.2 h, at which time a mean concentration of 450 ng/ml was observed. Compared to the adult population, the total clearance was 30% faster and the half-life of elimination was 24% shorter in the paediatric patients.[15,35] The volume of distribution in both central and peripheral compartments were both significantly smaller in the paediatric population compared to adults, explaining the high C_{max} and AUC values observed in the paediatric population.[36]

Like cetirizine, the disposition of levocetirizine depends on renal function. Therefore, in the elderly population (age range 65–74 years), in whom total clearance of levocetirizine is around one-third lower than in younger adults, the dose should be tailored to the renal function. [15]

Permeability

Levocetirizine is reported to be moderately permeable: the permeability of levocetirizine was reported as intermediate with a P_{app} value of 4.38×10^{-6} cm/s.[29] However, in a mass balance study, approximately 86% of the radioactivity was excreted in the urine unchanged. [32] This value would correspond to classification as "highly permeable".[29] The metabolites detected in urine represented a further 3.5% of the dose at 48 hours. Considering almost all radioactivity detected in plasma was assigned to levocetirizine rather than metabolites, it can be inferred that levocetirizine demonstrated adequate intestinal stability.[32]

Levocetirizine permeability (5–100 μM) was investigated across Caco-2 cells using antipyrine (high permeability marker; with apical to basal permeability of 18.80×10^{-6} cm/s at 20 μM), mannitol (low permeability marker; with apical to basal permeability of 2.71×10^{-6} cm/s at 20 μM) and PEG-4000 (zero permeability marker).[37] The transport in the apical to basolateral direction was 4.38×10^{-6} cm/s, while in the basolateral to apical direction it was 7.37×10^{-6} cm/s.[37] The efflux ratio was thus less than 2, suggesting levocetirizine is not a Pgp substrate.[38]

Furthermore, quinidine inhibited transport polarity, indicating that levocetirizine is at most a weak Pgp substrate.[32,37] Moreover, no saturation of P-glycoprotein mechanism was observed suggesting levocetirizine was transported mainly by diffusion across Caco-2 cells.[32,37,38]

Levocetirizine had no impact on the Pgp-mediated digoxin transport up to a concentration of 100 μM in Caco-2 cell monolayers, suggesting that drug-drug interactions of this nature are improbable.[37]

However, ranitidine was shown to impact the absorption of levocetirizine, indicating that drug-drug interactions around uptake could occur.[38,39] Along the same lines, the Human Organic Anion Transporter 4 (OAT4) has been reported to mediate levocetirizine uptake but not efflux. Since the uptake rate via OAT4 was about two-fold higher for levocetirizine than racemic cetirizine, uptake of levocetirizine appears to be stereoselective.[40] This OAT4 mediated uptake shows concentration dependence.[29,40] Levocetirizine is not a substrate for OATP1B1 and OATP1B3, transporters involved in hepatobiliary elimination.[29]

Distribution

The volume of distribution of levocetirizine after oral dosing was calculated to be 27 l after administration of an extemporaneous solution, whereas a value of 34 l was reported for the tablet formulation.[5,15,32] These values are commensurate with distribution in the total body water.

The plasma protein binding of levocetirizine is over 90% throughout the concentration range 90–5000 ng/mL, a range which includes plasma levels corresponding to clinical effectiveness.[14-15] The ratio of levocetirizine concentrations between blood and plasma ranges from 0.51 to 0.68, indicative of a limited association with blood cells.[14,32]

Tissue distribution data in rats and dogs revealed highest concentrations of levocetirizine in the kidneys and liver, while the lowest concentrations were detected in the central nervous system.[5,15,32] A comparison of the kinetics of occupancy on H1 receptors in the brain and plasma of guinea pigs also revealed lower levels in the brain than plasma, with a partition coefficient of 0.06–0.08.[41] With a shorter T_{peak} (1–1.5 h *versus* 2–4 h) and terminal half-life (2.1–2.8 h *versus* 4–5.6 h) in plasma in comparison to brain tissue, the H1 occupancy at 1 h following an administration of 1mg/kg dose was 97% in plasma compared to 28–67% in the brain. These results underline the ability of levocetirizine to be an efficient antihistaminic peripherally with little risk of deleterious central effects at therapeutic doses.[41]

Metabolism and Excretion

Levocetirizine does not undergo extensive metabolism. Within 48 h of administration of an oral dose, almost 86% was excreted unchanged, while only 2.4% of the dose was excreted as metabolites, comprising 13 minor metabolites.[14] It undergoes metabolism by aromatic oxidation (mediated by multiple and/or unidentified CYP isoforms), N- and O- dealkylation (mediated by CYP 3A4) and taurine conjugation.[14-15]

Levocetirizine did not impact the CYP 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 activities, even at concentrations much higher than the maximum concentrations associated with a 5 mg oral dose. As it shows low metabolism and little potential for metabolic inhibition, clinically significant metabolic interactions with other drugs is unlikely.[14-15]

Total body clearance, half-life and relative urinary excretion are all independent of dose.[15] In healthy adults, the plasma half-life of levocetirizine is about 8–9 h. The total clearance is about 0.63 ml/min/kg. Both glomerular filtration and active tubular secretion are involved in its excretion.[15] The renal clearance of levocetirizine correlates with creatinine clearance, so as renal impairment becomes more severe, the ability of the kidneys to clear levocetirizine decreases. Therefore, for patients with renal impairment, the dose and/or dosing interval of levocetirizine need to be tailored to the creatinine clearance value.[15]

Patients with anuric end-stage renal disease have a total body clearance that is around 80% lower than that of healthy subjects. During standard four-hour haemodialysis procedures, less than 10% levocetirizine is removed. Its use is thus contraindicated in end stage renal disease.[5,15]

Dosage Form Performance

Bioequivalence

FDA recommends fasting and fed state BE studies for levocetirizine 5 mg tablets.[42] Randomized, open label, two-way crossover

studies were performed to compare orally disintegrating tablets (ODTs) and immediate release tablets (IRTs) with respect to bioavailability.[43] In study 1, the bioavailability of ODTs was compared to IRTs when they were administered with water to 24 fasted subjects. In study 2, involving 48 fasted subjects, the pharmacokinetics of ODTs (taken without water) were compared with IRTs (taken with water). Bioequivalence was demonstrated in both studies.[43] (Table 3). In a further study, the bioequivalence of levocetirizine orally dispersible tablets to levocetirizine IR tablets was also demonstrated[23] (Table 3).

In another two-way crossover design under fasting conditions an oral solution of levocetirizine dihydrochloride (10 ml of 0.5 mg/ml) and levocetirizine tablets 5 mg were shown to be bioequivalent in 24 subjects.[29-30] The geometric mean ratios for C_{max} and $AUC_{0-\alpha}$ and the 90% confidence intervals around them were 1.09 (1.02−1.17) and 1.00 (0.96−1.04), respectively. The solution formulation showed a slightly earlier T_{max} of 0.50 h *versus* 0.67 h for the tablets.[29-30]

Levocetirizine 5 mg tablets (Synthon BV, The Netherlands) were also shown to be bioequivalent to cetirizine 10 mg tablets (Zyrtec 10 mg film coated; UCB GmbH, Germany) in an open label two treatment, two period, single dose, crossover design study involving 24 subjects.[44] The 90% confidence intervals around the geometric mean ratios were 97.35 (93.35−101.52) for C_{max} and 101.36 (97.66−105.21) for $AUC_{0-\alpha}$.[44]

Inhibition of the wheal and flare response induced by histamine was measured pharmacodynamically to compare test (Hetero Drugs Ltd, Hyderabad, India) and reference (Tab Xyzal, 5 mg levocetirizine dihydrochloride tablet formulation; UCB-Pharma AG, Zurich, Switzerland) levocetirizine formulations in 12 fasted healthy male volunteers using a double-blind, balanced, randomized, single dose, crossover design.[45] Histamine was injected intradermally to induce the wheal and flare response. Both formulations significantly reduced the histamine-induced response in all subjects. The maximum reduction in the wheal response ($I_{w\ max\ \%}$) was 82.45% for the reference and 77.9% for the test formulation, while the area under the curve for the plot of antihistaminic activity versus time was 2211 mm^2 /hr for the reference and 2482 mm^2 /hr for the test formulation.[45] Similarly, maximum inhibition of histamine-induced flare response ($I_{f\ max\ \%}$) produced by the reference and test formulations was 88% and 81.58%, respectively. The two formulations were judged to be therapeutically equivalent (using the criterion that the 90% confidence intervals should lie between between 80% and 125%) with respect to the least square mean ratio (%), T *versus* R for peak activity (maximum inhibition of wheal and flare response induced by histamine), and area under the activity versus time plot (AUC_{0-24} mm^2 /h and AUC_{0-24} %/h) for both the non-transformed and log-transformed data. Both formulations were tolerated well.[45]

On the 4th of August 2014, a referral under Article 31 of Directive 2001/83/EC was initiated by the European Commission in response to serious issues discovered during an inspection performed by the French Agency on medicinal products (ANSM) at GVK Biosciences Private Limited, India.[46] The findings raised serious concerns about BE and other clinical studies performed at the centre. As part of its opinion as to whether marketing authorisations for medicinal products, which were partly based on bioequivalence studies performed at GVK's Bio-Hyderabad site from July 2008 on, should be maintained, varied, suspended or withdrawn, the Committee for Medicinal Products for Human Use (CHMP) did accept the BCS-based biowaiver for levocetirizine test products [46] [(Procedures DK/H/1900, DK/H/1901, DK/H/1531): Marketing Authorisation Holders: Alfred E. Tiefenbacher GmbH & Co.KG, Biofarm Sp.z o.o., Delorbis Pharmaceuticals Ltd]. Their decision was based on the fact that levocetirizine doesn't have a narrow therapeutic index drug and may fall either in BCS class 3 (cautious approach) or be a borderline BCS class 1. The test products were also recognized to comply with regulatory requirements pertaining to composition and with respect to their very rapid release of levocetirizine in *in vitro* testing.[46]

Effect of Excipients and Manufacturing Variations

The following excipients were present in the IR film-coated levocetirizine tablets that have been tested in BE trials as of this writing: lactose monohydrate, aspartame (E951), polacrilin potassium, magnesium stearate, silica colloidal anhydrous, mannitol (E421), polyplasdone, sorbitol (E420), syloid, cellulose microcrystalline and blackcurrant flavour, in addition to some coating materials (see Table 3). Only very small amounts of excipients like water, ethanol, coating ingredients, flavoring or coloring agents, taste masking or enhancing agents, and printing ink are present in the final finished product and are therefore not likely to have a significant impact on the critical quality attributes (CQA) of the final product. The lack of impact of excipients is further verified by the successful demonstration of BE of Levocetirizine Athena 5 mg ODT and levocetirizine tablets of Actavis with respect to the reference product (Table 3).

Similarly, levocetirizine 0.5 mg/ml solutions have been formulated with excipients including: sweeteners like maltitol solution, glycerin and saccharin, pH adjusting substances such as sodium acetate trihydrate and glacial acetic acid and preservatives like methylparaben, and propylparaben, purified water and flavouring agents.[15]

A drug-excipient interaction between the carboxylic acid function on cetirizine and hydroxyl groups on excipients such as sorbitol, mannitol and glycerol to form monoesters has been reported.[47-48] The authors reported the presence of 0.1−0.3% cetirizine esters in two marketed formulations.[47] The USP monograph for cetirizine tablets limits levocetirizine lactose esters to not more than 0.5% and any unspecified degradation products at not more than 0.2%.[24] Similar limits would also apply to levocetirizine finished formulations.

Summarizing, the excipients employed to manufacture levocetirizine oral IR solid dosage forms up to this point (Table 3) are often found in solid formulations for oral ingestion. It is therefore notable that none of the levocetirizine tablets had issues with BE in the studies conducted to date.

Dissolution Studies

Levocetirizine tablets are designed to be swallowed, releasing the active ingredient rapidly in the gastrointestinal tract.[5,15] Levocetirizine has a broad therapeutic window and its T_{max} is not critical to the intended use. It is a highly soluble drug and the excipients typically chosen for its formulation, as well as the quantities used, are appropriate for the design of IR drug products, their labelled function and the target population.[20-21] Therefore, according to the FDA guidance "Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances", either paddle or basket dissolution methods can be used for routine testing.[49]

The release specification recommended for highly soluble drugs is that at least 80% (Q) of the label amount is released within 30 min.[49]

The dissolution method recommended by USP for Levocetirizine Tablets is to use apparatus 2 (paddle), 900 ml water and a stirring rate of 50 rpm.[12] The same specification is recommended by FDA. Since levocetirizine meets the "highly soluble" criteria, the above dissolution methods can be used for routine dissolution testing of products containing this drug.

Levocetirizine tablets were reported to be rapidly dissolving, with over 85% being released within 15 min at pH 1, 4.5, 6.5 and in water. [29-30,37]

However, water is not considered to be a reliable dissolution medium for BCS-based biowaiver purposes due to the probability of a shift in pH during dissolution studies. Therefore, further dissolution studies were performed on levocetirizine 5 mg tablets (manufactured by Azal Pharmaceutical Company, Khartoum) in three different pH media, using the paddle method with stirring at 50 rpm. The volume

Table 3
Summary of bioequivalence studies conducted on levocetirizine 5 mg tablets.

Subjects	Test formulation manufacturer or license holder	Composition of test formulation	Reference product	Fasting/ fed study	Study design	Bioequivalence criteria/ Statistics	90% CI of C_{max} (Log Transformed Values)	90% CI of $AUC_{0-\infty}$ (Log Transformed Values)	Results	*In Vitro* Results	References
Part 1: 24 Part 2: 48 Healthy Japanese male subjects	Levocetirizine ODTs	n.a.	Levocetirizine IRTs	Fasting	Single−center, single−dose, open−label, randomized, 2−way crossover study	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	Part 1 0.934 (0.875−0.998) Part 2 0.85 (0.815−0.902)	Part 1 0.975 (0.948−1.003) Part 2 0.978 (0.958−0.998)	BE	-	[43]
22 Healthy Chinese subjects	Levocetirizine tablets, 5 mg, Suzhou Downray Pharmaceutical Co., Ltd	n.a.	Levocetirizine tablets, 5 mg, UCB Farchim S. A.,	Fasting	single-center, single-dose, open-label randomized two-arm self- cross	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	100.8 (94.75-107.24)	99.8 (97.13−102.50)	BE		[58]
23 Healthy Chinese subjects	Levocetirizine tablets, 5 mg, Suzhou Downray Pharmaceutical Co., Ltd	n.a.	Levocetirizine tablets, 5 mg, UCB Farchim S. A.,	Fed	single-center, single-dose, open-label randomized two-arm self- cross	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	107.1 (99.98−114.69)	101.1 (98.36−103.98)	BE		[58]
26 healthy Thai male subjects	Levocetirizine dihydrochloride 5 mg tablets of GPO, Thailand	n.a.	(Xyzal®) 5 mg tablets, UCB Farchim SA, Bulle-Switzerland	Fasting	A randomized, open-label, two-treatment, two-period, two-sequence, single dose, crossover	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	100.6 (95.56−105.89)	99.0 (95.81−102.31)	BE		[59]
24 subjects	Levocetirizine Athena 5 mg ODT manufactured by Athena Drug Delivery Solutions Pvt. Ltd., India.	Polacrilin potassium, Lactose monohydrate, Aspartame (E951), Silica, colloidal anhydrous, Magnesium stearate, Blackcurrant flavour, Pharmaburst B2 (contains Mannitol (E421), Polyplasdone, Sorbitol (E420) and Syloid)	Xyzal® 5 mg tablet manufactured by Aesica Pharmaceuticals s.r.l. Via Praglia 15 10044 Pianezza (TO), Italie for UCB Pharma S.A.,	Fasting	open label, balanced, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	90.9 (86.84−95.14)	98.5 (94.76−102.42)	BE	-	[23]
26 healthy subjects (male & female)	Avocet® Silom Medical Co., Ltd., Thailand	n.a.	Xyzal® UCB Pharma	Fasting	single-dose, randomized-sequence, open-label, two-way crossover	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	96.00 (87.39−103.05%)	102.00 (95.21 −111.42%)	BE	-	[60]
26 subjects	Levocetirizine dihydrochloride 5 mg tablets, Actavis Group PTC ehf	Core: Lactose monohydrate, cellulose microcrystalline, magnesium stearate Coat: Hypromellose, Titanium dioxide, macrogol 400	Zyrtec 10 mg film coated; UCB GmbH, Germany	Fasting	open label, balanced, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover	90% Confidence interval (C_{max}, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$) /ANOVA	97.35 (93.35−101.52)	101.36 (97.66−105.21)	BE	Dissolution profiles similar to reference	[61]

n.a. composition not available

Table 4
Mean (%) drug release of levocetirizine dihydrochloride in different pH media.

Time (min)	% Dissolved in 0.1 N HCl (±SD)	% Dissolved in pH 4.5 acetate buffer (±SD)	% Dissolved in pH 6.8 phosphate buffer (±SD)
10	45.6 ± 0.37	26.4 ± 0.07	20.0 ± 0.42
15	102.4 ± 0.22	83.0 ± 0.33	77.60 ±0.02
30	96.3 ± 0.11	93.8 ± 0.06	99.3 ± 0.41

of the dissolution medium was 900 ml, which is adequate to obtain sink conditions for highly soluble drugs like levocetirizine dihydrochloride. Over 85% levocetirizine was released within 30 min in all three media, verifying the "rapid" release behaviour of levocetirizine tablets, as reported in the FDA review (Table 4). [29-30,37]

Discussion

Solubility of Levocetirizine Dihydrochloride According to the BCS Classification

The highest dose strength of levocetirizine in a tablet dosage form commercially available is 5 mg, while the liquid formulation is available in a concentration of 5 mg per 10 ml. The maximum single dose of levocetirizine is 5 mg, to be taken once a day.[5,15,22]

At 37°C, drugs must have a dose to solubility ratio at or below 250 ml over the pH range 1.0 to 6.8 (relevant gastrointestinal pH range) to be considered "highly soluble". [6-9]

The initial values for the saturation solubility of levocetirizine at 37 °C were 967.9 at a starting pH of 1.2, 830.8 at pH 4.5 and 726.2 mg/ml at pH 6.8 (Table 2, upper part). However, these solubility values were considered unsuitable for calculating the dose/solubility ratio due to the large shift in pH after addition of a substantial excess levocetirizine hydrochloride.

To obtain more reliable solubility values, additional solubility studies were carried out, with the aim of demonstrating that a multiple of the highest recommended single dose of levocetirizine dihydrochloride could be dissolved in 250 ml or less of buffers across the pH 1.2–6.8 range. In each case, it was attempted to dissolve 20 mg of levocetirizine in 100 ml of medium, corresponding to one dose of 5 mg per 25 ml. In all three media, the entire amount was dissolved, indicating that the dose/solubility ratio in this pH range does not exceed 25 ml (Table 2, lower part), which is just one-tenth of the 250 ml limit. Therefore, levocetirizine dihydrochloride meets the "highly soluble" definition according to all regulatory authorities.[6-9]

Permeability of Levocetirizine Dihydrochloride According to the BCS Classification

Drugs can be classified as "highly permeable" if 85% or more of the dose is absorbed in humans. The most reliable approaches to estimate permeability and acceptable to regulatory authorities are if the absolute bioavailability of the compound in question is 85% or higher, or if mass balance studies indicate at least 85% absorption. The latter approach is deemed particularly appropriate for drugs that are subject to first pass metabolism.[6-9]

Levocetirizine exhibits rapid absorption and shows near complete bioavailability in humans with a bioavailability of more than 85%. Therefore, levocetirizine should be regarded as a "highly permeable" drug substance. Despite these clinical data, levocetirizine has been categorised as either borderline BCS Class 1 or as a BCS Class 3 drug substance.[45-46] No specific justification was provided for provisionally assigning it to BCS Class 3.

The oral solution of levocetirizine dihydrochloride and levocetirizine tablets have been shown to be bioequivalent.[5,15, 28-30] The EMA Guidance from 2010 states that „*reported bioequivalence between aqueous and solid formulations of a particular compound administered via the oral route may be supportive as it indicates that absorption limitations due to (immediate release) formulation characteristics may be considered negligible*".[7]

BCS Classification

Levocetirizine demonstrates high solubility and high permeability. According to the ICH M9 guidance on BCS-based biowaivers, drugs can be considered highly permeable provided 85% or more of the administered dose is recovered in the urine as the parent drug (unchanged form).[8] In the case of levocetirizine, various clinical studies have demonstrated recovery of more than 85% parent drug in urine. Moreover, considering levocetirizine exhibits linear pharmacokinetics, it is not likely that permeability would be limited by saturation of transporters in the intestines. Therefore, levocetirizine can be duly assigned to BCS Class 1.

Dissolution

In media at pH 1.2, pH 4.5, and pH 6.8, more than 85% of the levocetirizine dose dissolved within 30 min, in line with levocetirizine's high solubility over this pH range. (Table 4). Hence, levocetirizine hydrochloride meets the dissolution criteria stipulated by regulatory guidelines for approval of products containing BCS Class 1 drugs.[6-9]

Risks with Respect to Excipient and/or Manufacturing Variations

Table 3 shows the excipients used to manufacture IR levocetirizine tablets and oral solution formulations that have received market authorizations in various countries. Each of the BE studies reported up to present in the open literature succeeded in meeting the BE criteria for C_{max} and AUC. Moreover, tablet and solution dosage forms have also been shown to be bioequivalent. Although we recognize that a bias towards publishing positive results exists, in this case the lack of publications reporting non-bioequivalent outcomes is more probably due to levocetirizine being a BCS class 1 drug. In addition to the assignment of levocetirizine to BCS Class 1, fast disintegration of tablets and rapid dissolution from both the comparator and generic products likely contribute to the absence of non-bioequivalent results published in the open literature.

Formulation of generic versions of BCS class 1 drugs offers substantial flexibility with regard to the wide variety of excipients that can be used, as bioavailability and other critical quality attributes are not usually influenced by the choice of excipients or variations in processing. The risk of a false positive BCS-biowaiver decision can be mitigated by formulating the tablets with excipients that are present in the levocetirizine tablets that have been assessed to date und applying these excipients in amounts applicable to the manufacture of IR tablets (Table 3).

It should be noted that sodium lauryl sulphate, an excipient considered to be critical, is not present in any of the formulations that have obtained a marketing authorization thus far. One oro-dispersible tablet dosage form contains mannitol which, by virtue of its osmotic effects, can reduce GI transit time and hence time available for absorption if co-administered in large amounts.[23] However, the impact of this reduced GI transit time is more of concern in cases where the drug relies on transporters located in the proximal small intestine for uptake (e.g. peptide transporters) and drugs which are poorly permeable. Furthermore, solid oral dosage forms of highly permeable drugs like levocetirizine are unlikely to contain enough mannitol in a formulation to impact upper GI transit and hence time available for absorption. The above reasoning is fully consistent with the demonstration of bioequivalence between an oro-dispersible formulation containing mannitol and the IR levocetirizine dosage form

Xyzal®.[23] The results also corroborate the argument that, although critical excipients, e.g. sodium lauryl sulphate and mannitol, may impact drug permeability, BCS class 1 drugs like levocetirizine are unlikely to be impacted due to their inherently high permeability characteristics. Moreover, since levocetirizine is already rapidly dissolving, without any wetting issues, it is not likely that sodium lauryl sulphate would enhance its dissolution rate in a way that would have an impact on the absorption rate.

Surrogate Techniques for in Vivo *Bioequivalence (BE) Testing*

According to the FDA guidance document, either the basket method (100rpm, 500 ml of 0.1N HCl) or the paddle method (50 rpm, 500 ml of 0.1 N HCl) can be used for highly soluble drugs like levocetirizine.[49] On the other hand, USP recommends dissolution in 900 ml water using apparatus 2 (paddle apparatus at 50 rpm).[12] The solubility of levocetirizine is not pH dependent. It is reported in the literature that levocetirizine tablets are rapidly dissolving with over 85% drug released in 15 min at pH values between 1.2 and 6.8.[29-30,37] The solubility and dissolution data presented in Tables 2 and 4 generally support the reported results although our dissolution experiments revealed that 85% drug release is first assured at all three pH values within 30 min (Table 4). Therefore, meeting the dissolution specifications (including similarity of the test and reference profiles, as tested by the f2 statistic) using the paddle method in 0.1 N HCl, pH 4.5 and pH 6.8 media (500 ml) would serve as a reliable assurance of the BE of the product with each and every batch release, consequently minimizing the risk of a batch which is not BE being released to the market.

Risks Associated with Applying the BCS-Based Biowaiver (Patient's Risks Associated with Lack of Bioequivalence)

Approval via the biowaiver of a multisource drug product entails some risks from the viewpoint of the patient. Therefore, it cannot be overemphasized that the risks associated with a positive but false waiver decision must be thoroughly assessed before admitting levocetirizine products to this procedure. In this regard two factors must be considered: first, the probability that a false positive decision could occur, and second, what this would mean in terms of therapeutic outcome.

So far, in studies run in conjunction with application for marketing authorization, several have confirmed levocetirizine products to be pharmacokinetically bioequivalent to the reference product, while on the other hand, there are no published studies reporting failure to achieve bioequivalence. This clinical experience suggests the risk of manufacturing tablets of levocetirizine that are not bioequivalent is low. Moreover, tablet dosage forms of levocetirizine have been shown bioequivalent to solution dosage forms.[15,29-30,33]

Theoretically, the incidence of adverse effects could increase if a false positive biowaiver decision is reached for a generic version of levocetirizine that has higher AUC /C_{max} values than those of the comparator product. However, the wide therapeutic window of levocetirizine and general lack of side effects speaks against this scenario.[20-21] For example, in two placebo-controlled clinical trials performed on 428 patients (age group 12 years and older), the treatment group received levocetirizine 5 mg once daily for 4 or 6 months.[15] The patients in the treatment group demonstrated a similar safety profile to that reported in short term studies. Only ten patients receiving levocetirizine quit the study due to somnolence, fatigue or asthenia, in comparison to two patients in the placebo group.[15]

The current treatment guideline, published jointly by the European Academy of Allergology and Clinical Immunology, the Global Allergy and Asthma European Network and the European Dermatology Forum), for chronic urticaria in patients who respond inadequately to approved doses of antihistamine is to invoke up-dosing of second generation H1 antihistamines, including levocetirizine, up to four times.[50] It must be emphasized here that this is an off-label dose. However, clinical evidence supports the safety and efficacy of these increased dose levels of levocetirizine. For instance, an uncontrolled, nonrandomized, single-center clinical trial was performed with levocetirizine 5 mg, 10 mg, or 20 mg daily in 20 patients for 4 weeks. Based on how much control was achieved in urticaria, doses were increased in the first 2 weeks. Levocetirizine was found to be more effective at higher doses against urticaria in patients for whom the standard 5 mg dose was inadequate. The adverse events recorded consisted of mild sedation only at the 10 mg and 20 mg doses.[51] Similarly, Staevska et al. performed a double-blind, randomized, crossover study in 80 patients and analysed the therapeutic success of increasing doses of levocetirizine and desloratadine in patients in whom control was not achieved at the usual dose.[52] 75% of patients experienced an improvement in chronic urticaria at higher doses without compromising safety (no serious adverse effects).[52]

H1 antihistamines can potentially block hERG (human Ether-a-go-go-Related Gene) voltage-gated K^+ channels, leading to a prolongation of the QT interval and thus putting the patient at more risk for ventricular fibrillation and sudden death.[53] However, the safety profile of second generation H1 antihistamines is excellent, with no cardiotoxicity even at doses as high as four-fold their usual dose, subject to screening patients for known potential risk factors for cardiotoxicity (inherited long QT interval syndrome, advanced age, cardiovascular irregularities, subnormal plasma levels of potassium or magnesium, or concomitant use of drugs that directly prolong the QT interval or inhibit metabolism of second generation H_1 antihistamines) before initiation of therapy.[53]

The safety of levocetirizine over 18 months of therapy was also verified in young atopic children (12–24 months) in a randomized, double-blinded study. The children were given either levocetirizine 0.125 mg/kg or a placebo formulation twice a day over the course of the study. Adverse events documented were similar between treatment and placebo groups.[54] Oral drops of levocetirizine have also been shown to be safe in infants and children with allergic rhinitis and chronic idiopathic urticaria.[55]

The acute maximal non-lethal oral dose (AMNOD) of levocetirizine reported in mice and rats is 240 mg/kg. Extrapolating the mouse AMNOD to humans corresponds to approximately 200 times and 230 times higher than the maximum daily oral doses recommended for adults and children, respectively, on a mg/m^2 basis.[15] Similarly, rat AMNOD would correspond to 390 times and 460 times the maximum daily oral doses recommended in adults and children, respectively.[15]

At a blood concentration higher than C_{max} levels achieved at the usual dose, levocetirizine neither inhibits nor induces CYP isoenzymes.[15] Therefore, it is also improbable that a generic or reformulated levocetirizine tablet with moderate supra-bioavailability would produce or be subject to metabolic interactions.

Of course, the risks associated with failure to achieve therapeutic blood levels of levocetirizine due to a false positive biowaiver decision for a sub-bioavailable product are also part of the risk analysis. Levocetirizine has been shown to suppress production of Osteopontin (OPN) and OPN-induced chemotactic factor, GM-CSF, RANTES, and Eotaxin from nasal epithelial cells at blood levels (0.05 μM) far lower than those achieved after a 5 mg dose (about 0.348 μM). It is believed that this attenuating effect of levocetirizine and other antihistamines on the OPN production from nasal epithelial cells may be the mechanism of their effect on allergic diseases.[56] Based on these data, it appears that the risk associated with administration of moderately sub-bioavailable levocetirizine products is low.

The plot of plasma levocetirizine and fexofenadine concentration *versus* the % suppression of wheal, flare or pruritus responses yielded a counter-clockwise hysteresis loop.[57] Although the C_{max} was attained within 1–2 h, maximum suppression of the flare response

was seen at 4 h. Moreover, inhibition by both levocetirizine and fexofenadine was maintained despite plasma drug levels falling to low levels, indicating that the duration of action of both drugs has little dependency on plasma drug concentration. The main reason for prolonged duration is the slow clearance of these drugs from the extravascular space. Levocetirizine also exhibited low inter-subject variability in comparison to fexofenadine.[57]

In addition to the above-mentioned drug characteristics, compliance of the drug product to critical quality attributes, such as specifications of content uniformity and dissolution performance, would help to prevent blood levels from a generic or re-formulated version of levocetirizine from falling outside the limits for bioequivalence to the comparator product.

Conclusion

Levocetirizine is assigned to BCS Class 1. This classification is clearly supported by evidence accessible in the open literature as well as out experimental results. Taking into account its wide therapeutic window and the absence of severe adverse effects, the clinical risks ensuing from either a moderately supra- or sub-equivalent dose were deemed acceptable. In light of the classification and the clinical evidence reviewed, implementation of the BCS-based biowaiver for oral IR solid formulations of levocetirizine dihydrochloride as the single active pharmaceutical ingredient is recommended, contingent on the product meeting all of the following criteria: (a) the test product is formulated exclusively with excipients that are often used in oral IR solid drug products approved in ICH or associated countries and used in amounts appropriate for this kind of product, (b) data submitted to support approval via the BCS-based biowaiver are generated using the methods stipulated by the WHO, FDA, EMA or ICH and (c) both products – test and comparator (which is the innovator product in this case) - meet the *in vitro* dissolution specifications set by the WHO, FDA, EMA and/or ICH guidance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

No funding was received for this work.

References

1. Guerra S, Sherrill DL, Martinez FD, Barbee RA. Rhinitis as an independent risk factor for adult-onset asthma. *J Allergy Clin Immunol*. 2002;109:419–425.
2. Shaaban R, Zureik M, Soussan D, et al. Rhinitis and onset of asthma: a longitudinal population-based study. *Lancet*. 2008;372:1049–1057.
3. Brożek JL, Bousquet J, Agache I, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017;140(4):950–958. Oct.
4. Walsh GM. The anti-inflammatory effects of levocetirizine—are they clinically relevant or just an interesting additional effect? *Allergy Asthma Clin Immunol*. 2009;5(1):14.. Dec 17.
5. Summary of product characeristics. Levocetirizine 5 mg film-coated tablets. Available at: https://www.medicines.org.uk/emc/product/9917#gref. (Accessed 13 October 2022).
6. U.S. Food and Drug Administration, Department of health and human services center for devices and radiological health. In: guidance for industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Silver Spring, MD: FDA; 2017. Available at: https://www.fda.gov/downloads/Drugs/Guidances/ucm070246.pdf. (Accessed 13 October 2022).
7. European Medicines Agency (EMA), 2010. Guideline on the investigation of bioequivalence. Doc.Ref.:CPMP/EWP/QWP/1401/98 Rev. 1/Corr. Available at: https://www.ema.europa.eu/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf. (Accessed 13 October 2022).
8. International Conference on Harmonization (ICH). Biopharmaceutics classification system-based biowaivers. M9, 2020. Available at: https://database.ich.org/sites/default/files/M9_Guideline_Step4_2019_1116.pdf (Accessed 23 October 2022).
9. World Health Organization (WHO). *Expert Committee on Specifications for Pharmaceutical Preparations. Fifty -first report. World Health Organization technical report series*. Geneva, Switzerland: World Health Organization; 2017:.1003.. Available at; http://www.who.int/medicines/areas/quality_safety/quality_assurance/expert_-committee/WHO_TRS_1003_full-version.pdf. Accessed 13 October 2022.
10. Charoo NA, Abdallah DB, Bakheit AA, et al. Biowaiver monograph for immediate-release solid oral dosage forms: sitagliptin phosphate monohydrate. *J Pharm Sci*. 2022;111(1):2–13. Jan.
11. Charoo NA, Abdallah DB, Parveen T, et al. Biowaiver monograph for immediate-release solid oral dosage forms: moxifloxacin hydrochloride. *J Pharm Sci*. 2020;109(9):2654–2675. Sep.
12. USP39NF. Levocetirizine dihydrochloride tablets. P4541.
13. Devalia JL, De Vos C, Hanotte F, Baltes E. A randomized, double-blind, crossover comparison among cetirizine, levocetirizine, and ucb 28557 on histamine-induced cutaneous responses in healthy adult volunteers. *Allergy*. 2001;56:50–57.
14. Benedetti MS, Plisnier M, Kaise J, et al. Absorption, distribution, metabolism and excretion of [14C]levocetirizine, the R enantiomer of cetirizine, in healthy volunteers. *Eur J Clin Pharmacol*. 2001;57:571–582.
15. Xyzal®. FDA, 1995. Highlights of prescribing information. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/022064s009lbl.pdf. (Accessed 13 October 2022).
16. Simons KJ, Benedetti MS, Simons FE, Gillard M, Baltes E. Relevance of H1-receptor occupancy to H1-antihistamine dosing in children. *J Allergy Clin Immunol*. 2007;119(6):1551–1554. Jun.
17. Denham KJ, Boutsiouki P, Clough GF, Church MK. Comparison of the effects of desloratadine and levocetirizine on histamine-induced wheal, flare and itch in human skin. *Inflamm Res*. 2003:(10):424–427. Oct; 52.
18. Popov TA, Dumitrascu D, Bachvarova A, Bocsan C, Dimitrov V, Church MK. A comparison of levocetirizine and desloratadine in the histamine-induced wheal and flare response in human skin in vivo. *Inflamm Res*. 2006;55(6):241–244. Jun.
19. Ioana Corina Bocsan 1, Ioana Adriana Muntean 2,3,*. Nicolae Miron 4, Irena Pintea 2,3, Carmen Teodora Dobrican 2,3, Corina Ureche 5,*, Anca Dana Buzoianu 1 and Diana Deleanu. Are Markers of Allergic Inflammation in Grass Pollen Allergy Influenced by H1 Antihistamines. *J. Clin. Med*. 2022;11:113.
20. Pampura AN, Papadopoulos NG, Spičák V, Kurzawa R. Evidence for clinical safety, efficacy, and parent and physician perceptions of levocetirizine for the treatment of children with allergic disease. *Int Arch Allergy Immunol*. 2011;155(4):367–378.
21. Simons FER. The value of broad therapeutic index for antihistamines. *Adv Stud Med*. 2002;2(24):872–876.
22. FDA. 2022. Orange book: approved drug products with therapeutic equivalence evaluations. Available at: https://www.accessdata.fda.gov/scripts/cder/ob/index.cfm. (Accessed 30 March 2022).
23. Public Assessment Report. Levocetirizine Substipharm 5 mg orodispersible tablet Evianzin 5 mg orodispersible tablet Loritozin 5 mg orodispersible tablet (Levocetirizine dihydrochloride) ES/H/0398/001/DC ES/H/0399/001/DC ES/H/0400/001/DC. Available at: https://cima.aemps.es/cima/pdfs/ipe/83352/IPE_83352.pdf. (Accessed 13 October 2022).
24. USP39NF. Levocetirizine dihydrochloride. P4539.
25. Xyzal. Levocetirizine dihydrochloride. Available at: https://www.rxlist.com/xyzal-drug.htm#description. (Accessed 17 October 2022).
26. Reddy MS, Srinivasan TR, Uppala MB, Vaddadi PR, Joga R. 2003. Polymorphic forms of dihydrochloride salts of cetirizine and processes for preparation thereof. WO2004050647A3, WIPO (PCT).
27. Célal A, Emile C, Didier B. Piperazine derivatives and their use as synthesis intermediates. 2004. WO2004/065360.
28. Public Assessment Report. Levocetirizina IPCA. 5 mg film-coated tablets (Levocetirizine dihydrochloride). PT/H/0940/001/DC. Available at: https://mri.cts-mrp.eu/Human/Downloads/PT_H_0940_001_FinalPI_1of5.pdf. (Accessed 13 October 2022).
29. FDA. 2007. Clinical pharmacology review. Levocetirizine dihydrochloride. NDA: 22-157. Available at: https://www.fda.gov/media/71515/download. (Accessed 23 October 2022).
30. FDA, 2009. Clinical pharmacology and biopharmaceutics review(s). Zyrtec® (cetirizine HCl) Orally Disintegrating Tablets (10 mg). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022578Orig1s000ClinPharmR.pdf. (Accessed 13 October 2022).
31. Molimard M, Diquet B, Strolin Benedetti M. Comparison of pharmacokinetics and metabolism of desloratadine, fexofenadine, levocetirizine and mizolastine in humans. *Fndametal Clin Pharmacol*. 2004;8(4):399–411.
32. FDA, 2006. Clinical pharmacology and biopharmaceutics review. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2007/022064s000_ClinPharmR.pdf. (Accessed 13 October 2022).
33. Ino H, Hara K, Honma G, Doi Y, Fukase H. Comparison of levocetirizine pharmacokinetics after single doses of levocetirizine oral solution and cetirizine dry syrup in healthy Japanese male subjects. *J Drug Assess*. 2014;3(1):38–42.
34. Baltes E, Coupez R, Giezek H, Voss G, Meyerhoff C, Strolin Benedetti M. Absorption and disposition of levocetirizine, the eutomer of cetirizine, administered alone or as cetirizine to healthy volunteers. *Fundam Clin Pharmacol*. 2001;15(4):269–277. Aug.
35. Simons ER, Simons KJ. Levocetirizine: Pharmacokinetics and pharmacodynamics in children age 6 to 11 years. *J Aller Clin Immunol*. 2005;116(2):355–361.
36. FDA 2009. Clinical Pharmacology Review. NDA: 22-064, S017 (pediatric supplement). Available at: https://www.fda.gov/media/77886/download.

37. PMDA. Zyzar Tablets 5mg Galaxosmithkline Co. Ltd. Available at: https://www.pmda.go.jp/drugs/2010/P201000063/34027800_22200AMX00949_I100_1.pdf. (Accessed 9 October 2022).
38. FDA, 2020. In Vitro drug interaction studies — cytochrome P450 enzyme- and transporter-mediated drug interactions guidance for industry. Available at: https://www.fda.gov/media/134582/download. (Accessed 9 October 2022).
39. He Y, Liu Y, Zeng S. Stereoselective and multiple carrier-mediated transport of cetirizine across Caco-2 cell monolayers with potential drug interaction. *Chirality*. 2010;22(7):684–692. Jul.
40. Noguchi S, Nishimura T, Mukaida S, Benet LZ, Nakashima E, Tomi M. Cellular uptake of levocetirizine by organic anion transporter 4. *J Pharm Sci*. 2017;106(9):2895–2898. Sep.
41. Gupta A, Gillard M, Christophe B, Chatelain P, Massingham R, Hammarlund-Udenaes M. Peripheral and central H1 histamine receptor occupancy by levocetirizine, a non-sedating antihistamine; a time course study in the guinea pig. *Br J Pharmacol*. 2007;151:1129–1136. 2007.
42. FDA. 2008. Draft Guidance on Levocetirizine Dihydrochloride. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Levocetirizine%20Dihydrochloride_draft_Oral%20tab_RLD%2022064_RC10-08.pdf. (Accessed 13 October 2022).
43. Ino H, Shiramoto M, Eto T, et al. Levocetirizine oral disintegrating tablet: a randomized open-label crossover bioequivalence study in healthy Japanese volunteers. *Clin Pharmacol Drug Dev*. 2020;9(7):805–812. Oct.
44. Public assessment report (PAR). Hexal AG. UK/H/2333/01/DC. Available at: https://www.geneesmiddeleninformatiebank.nl/pars/h102859.pdf. (Accessed 23 October 2022).
45. Usharani PM, Mur N, Reddy KLN, Kumar T. Randomized, double-blind, crossover comparison between two levocetirizine formulations on histamine-induced cutaneous response in healthy male human adult volunteers. *J Appl Res*. 2005;5(1):149–159.
46. EMA, 2015. Final assessment report following the re-examination procedure. Referral under Article 31 of Directive 2001/83/EC Medicinal products for which the clinical part of bioequivalence studies has been carried out by GVK Biosciences Hyderabad during the time period 2004-2014 Procedure number: Available at: EMEA/H/A-31/1408. https://www.ema.europa.eu/en/documents/referral/gvk-biosciences-article-31-referral-gvk-biosciences-final-assessment-report-following-re-examination_en.pdf. (Accessed 13 October 2022).
47. Yu H, Cornett C, Larsen J, Hansen SH. Reaction between drug substances and pharmaceutical excipients: formation of esters between cetirizine and polyols. *J Pharm Biomed Anal*. 2010;53(3):745–750. Nov 2.
48. Sharma N, Rao SS, Ullas G, Durga N, Kumar A, Malleswara Reddy A. Optimization and validation of reverse phase liquid chromatographic method for estimation of cetirizine mannitol ester impurity in cetirizine hydrochloride chewable tablet. *J Liq Chromatog Rel Technol*. 2011;34(18):2157–2168.
49. FDA. Dissolution testing and acceptance criteria for immediate-release solid oral dosage form drug products containing high solubility drug substances. 2018. Available at: https://www.fda.gov/media/92988/download. (Accessed 7 September 2022).
50. Zuberbier T, Aberer W, Asero R, et al. European academy of allergy and clinical immunology; global allergy and asthma European Network; European dermatology forum; World Allergy Organization. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*. 2014;69(7):868–887. Jul.
51. Godse KV. Updosing of antihistamines to improve control of chronic urticaria. *Indian J Dermatol Venereol Leprol*. 2010;76(1):61–62.
52. Staevska M, Popov TA, Kralimarkova T, et al. The effectiveness of levocetirizine and desloratadine in up to 4 times conventional doses in difficult-to-treat urticaria. *J Allergy Clin Immunol*. 2010;125(3):676–682. Mar.
53. Cataldi M, Maurer M, Taglialatela M, Church MK. Cardiac safety of second-generation H_1-antihistamines when updosed in chronic spontaneous urticarial. *Clin Exper Allergy*. 2019;49:1615–1623.
54. Simons FE. Early Prevention of Asthma in Atopic Children (EPAAC) Study Group. Safety of levocetirizine treatment in young atopic children: an 18-month study. *Pediatr Allergy Immunol*. 2007;18(6):535–542. Sep.
55. Hampel F, Ratner P, Haeusler JM. Safety and tolerability of levocetirizine dihydrochloride in infants and children with allergic rhinitis or chronic urticaria. *Allergy Asthma Proc*. 2010;31(4):290–295. Jul-Aug.
56. Komatsuzaki T, Suzaki I, Hirano K, Kanai K, Asano K, Suzaki H. Suppression of osteopontin functions by levocetirizine, a histamine receptor antagonist, in vitro. *BioMed* Res Internat. 2013:12.. Article ID 735835pages.
57. Schoepke N, Church MK, Maurer M. The inhibition by levocetirizine and fexofenadine of the histamine-induced wheal and flare response in healthy Caucasian and Japanese volunteers. *Acta Derm Venereol*. 2013;93(3):286–293. May.
58. Cheng Y, Lin BJ, Guo JH, et al. The effect of food on the pharmacokinetic properties and bioequivalence of two formulations of levocetirizine dihydrochloride in healthy Chinese volunteers. *Drug Des Devel Ther*. 2019;18(13):3625–3634. Oct.
59. Teerawonganan P, Yoosakul E, Chuasuwan B, et al. Bioequivalence study of levocetirizine dihydrochloride 5 mg tablets in healthy thai male volunteers. *Isan J Pharmaceut Sci*. 2015;11(1):79–88.
60. Jantarat C, Keskanokwong T. Pharmacokinetic and bioequivalence evaluation of two formulations of levocetirizine 5 mg in healthy Thai volunteers. *Thai J. Pharm. Sci.*. 2013;37:1–11.
61. Public assessment report. Levocetirizine dihydrochloride 5 mg film coated tablets. UK/H/2337/01/DC. Actavis. Available at: https://www.geneesmiddeleninformatiebank.nl/pars/h102860.pdf. (Accessed 13 October 2022).