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Global Health Commentary

Solubility Determination of Active Pharmaceutical Ingredients Which Have Been Recently Added to the List of Essential Medicines in the Context of the Biopharmaceutics Classification System—Biowaiver

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ABSTRACT

Since the publication of Lindenberg et al., which classified orally administered active pharmaceutical ingredients (APIs) on the 2004 Essential Medicines List (EML) of the World Health Organization according to the Biopharmaceutics Classification System (BCS), various APIs have been added to the EML. In this work, BCS classifications for 16 of the orally administered APIs which were added to the EML after 2004 were determined. To establish a reliable solubility classification for all these compounds, a miniaturized shake-flask method was introduced. This method enables a fast, economical determination of the BCS solubility class while reliably discriminating between "highly soluble" and "not highly soluble" compounds. Nine of the 16 APIs investigated were classified as "highly soluble" compounds, making them potential candidates for an approval of multisource drug products via the BCS-based biowaiver procedure. The choice of dose definition (which currently varies among the guidances pertaining to BCS-based bioequivalence published by various regulatory authorities) had no effect on the solubility classification of any of the 16 substances evaluated. BCS classification of the compounds was then completed using permeability data obtained from the literature. As several APIs decomposed at one or more pH values, a decision tree for determining their solubility was established.

Introduction

The World Health Organization (WHO), which was founded in 1948, is part of the United Nations.[1] Generally regarded as the leading authority on international health, its objective is the achievement of the highest possible level of health for all people.[2] According to its constitution, health "is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."[2] As part of its work for global health, the WHO publishes the WHO Model List of Essential Medicines (EML), which includes medicines that are considered indispensable for a well-functioning health system and which should therefore be made available in dosage forms with assured quality at an affordable price.[3] The first version of the EML was released in 1977 and has since then been updated in regular intervals. The current edition is the 20th Essential Medicines List.[4]

The classification of Active Pharmaceutical Ingredients (APIs) listed on the EML based on the Biopharmaceutics Classification System (BCS)[5] is an essential step in determining whether a multisource product is eligible for approval via a BCS-based biowaiver. This procedure eliminates the need for *in vivo* testing of multisource drug products, and thus reduces development costs and time to approval.

According to the BCS, an API can be assigned to 1 of 4 classes based on its solubility and permeability (Fig. 1). Besides requiring that the API belongs to an eligible BCS class, consideration must be given to therapeutic index, stability of the API under gastrointestinal conditions, eligibility of the dosage form for this procedure, and excipient effects on absorption from the gastrointestinal tract. The risks associated with an incorrect positive decision with respect to bioequivalence (BE) (i.e., the dosage form is deemed to be bioequivalent by the BCS-biowaiver procedure but is actually not bioequivalent) are evaluated. As a last step, the *in vitro* dissolution of the generic product is compared with that of the reference product. Various health authorities such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and

Abbreviations used: WHO, World Health Organization; EML, List of Essential Medicines; APIs, Active Pharmaceutical Ingredients; BCS, Biopharmaceutics Classification System; FDA, Food and Drug Administration; EMA, European Medicines Agency; BE, bioequivalence; IR, immediate-release; D/S, dose/solubility; HPLC, high-pressure liquid chromatography; UV, ultraviolet; BA, bioavailability.

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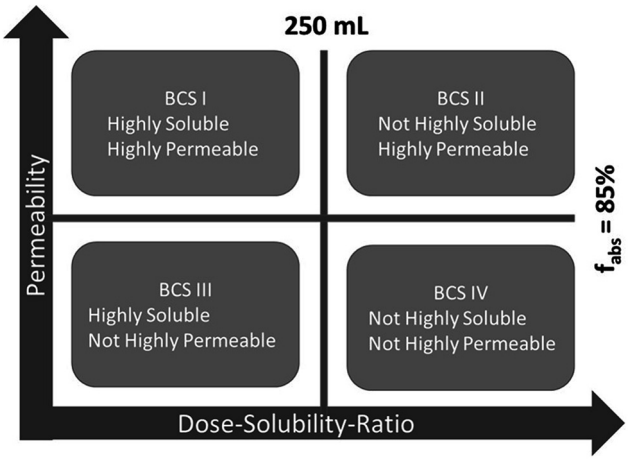

Figure 1. Biopharmaceutics Classification System (modified from Amidon et al.[5]).

the WHO require similar (but not yet fully harmonized) criteria to be fulfilled to grant a biowaiver approval.[6-8] In summary, the BCS-biowaiver procedure is a time- and cost-saving approach for the approval of generic drug products because it is not based on *in vivo* BE studies but on *in vitro* dissolution studies and thus facilitates the realization of WHO's goal to achieve availability of high-quality multisource drug products containing APIs listed on the EML at affordable prices.

In 2004, Lindenberg et al.[9] classified orally administered APIs of the 12th edition of the Essential Medicines List[10] according to the BCS. The classifications were based on solubility and permeability data obtained from the open pharmaceutical literature. Depending on the quality of the data, the APIs were assigned to those with a reliable or a provisional BCS class. Alternatively, it was concluded that the data available were insufficient to reach a conclusion about the BCS class. Experimental solubility data were not obtained in that study because of the large number of APIs under investigation and lack of resources available to experimentally determine the solubility of each API using the standard shake-flask technique.

In 2005, a modification of the shake-flask method was published by Glomme et al.[11] These researchers compared a miniaturized, scaled-down approach with the conventional shake-flask method and showed that the scaled-down method was a cost-effective alternative to the conventional, large-scale approach used in pharmaceutical development. For this reason, scaled-down approaches have become increasingly popular and have been implemented more frequently in the ensuing years.

Combining the scaled-down approach to solubility determination with the need to provide reliable BCS classifications for orally administered APIs that have been added to the EML, the purpose of this study was to experimentally determine the solubility classification of 16 APIs that have been added since the 14th version of the EML.[12] All APIs included in this study are formulated in solid, immediate-release (IR) oral dosage forms and have not previously been reliable classified according to the BCS. Since literature data on the solubility of APIs under BCS-relevant conditions are sparse, and since the definition of the "dose" used for calculating the dose/solubility (D/S) ratio varies between different guidance documents,[6-8] the experimentally determined solubility values of the respective APIs under BCS-relevant conditions are presented in this study. This allows for the calculation of a solubility classification according to the various dose definitions applied across the different jurisdictions. It also enables the BCS classification to be checked in the case where the dosage strength is revised in a future EML version, if the dosage strength is different in a given jurisdiction to the dose recommended by the EML, or if a new dosage strength of the API is added to the products already available.

Materials and Methods

Materials

The 16 APIs included in this study were amiodarone hydrochloride, atazanavir sulfate, cyclizine, dexamethasone, emtricitabine, enalapril maleate, folic acid, hydroxychloroquine sulfate, medroxyprogesterone acetate, mesna, mifepristone, morphine sulfate, oseltamivir phosphate, ribavirin, rifabutin, and succimer.

Folic acid and medroxyprogesterone acetate were already listed on the 12th WHO EML[10] but were also included in this study because of conflicting solubility data in the literature. Dexamethasone was also listed on the 12th EML[10] with a dose strength of 0.5 mg and at that time had been classified as "highly soluble" by Lindenberg et al.[9] It was then withdrawn from the list until the 17th version,[13] when it was listed again, but at a higher dosage strength of 4 mg and for a different indication (see Table 1). It was therefore necessary to confirm the "highly soluble" criterion at the higher dose strength of 4 mg. Because the solubility classification of morphine sulfate as "highly soluble" by Lindenberg et al.[9] was based solely on determinations above pH 5.5 at 35°C,[14] it was necessary to perform further studies for this API to obtain a reliable classification over the whole physiological pH range of 1.2-6.8 at 37°C.

Mifepristone was added in the 14th EML, emtricitabine, and ribavirin in the 15th and amiodarone hydrochloride, atazanavir sulfate, mesna, oseltamivir sulfate, and rifabutin in the 16th edition of the WHO EML (Table 1).[12,15,16] Cyclizine, enalapril maleate, hydroxychloroquine sulfate, and succimer appeared as APIs in solid oral dosage forms for the first time on the 17th WHO Essential Medicines List[13] in March 2011.

All APIs were purchased from suppliers in Germany. Information regarding analytical grade, batch numbers, and details concerning retailer and manufacturer can be found in Table 2. All other chemicals used in the studies were of analytical grade. Dipotassium monohydrogenphosphate, disodium monohydrogenphosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, and sodium hydroxide were obtained from VWR® Prolabo® (Leuven, Belgium). All acids and sodium hydroxide (1 M) were purchased from VWR® Prolabo® (Fontenay-Sous-Bois, France). Ammonium acetate, sodium acetate trihydrate, acetonitrile and methanol were obtained from Merck KGaA (Darmstadt, Germany). Ethanol was purchased from AHK Alkoholhandel GmbH & Co. (Ludwigshafen, Germany). Uniprep™ syringeless filters by Whatman™ (Little Chalfont, UK) were used as small-scale filter systems.

Solubility Experiments

The solubility was determined according to the requirements set in the Annex 7 of the WHO technical report series titled "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability," which states that an API is considered "highly soluble" when the D/S ratio is $\leq$250 mL over the pH range of 1.2-6.8 at 37 ± 1°C.[8] The solubility of all substances and the resulting BCS solubility classification was determined according to the study protocol shown in Table 3.

The solubility studies were based on the shake-flask method, which is used to determine the equilibrium solubility of a substance. In this method, an excess of substance is added to a medium with a certain pH-value, creating a suspension (media compositions are listed in Table 4). The suspension is then shaken for a

Table 1
APIs Examined in the Solubility Study, Along With the Year of First Appearance on the EML, Highest Dose Strength and Drug Class Listed on the 20th EML

Drug	First Listed on EML	Dose Strength on 20th EML (mg)	Drug Class Listed in 20th EML
Amiodarone hydrochloride[C]	16 (2010)	400	Antiarrhythmic
Atazanavir sulfate	16 (2010)	300	Protease inhibitor
Cyclizine	17 (2011)	50	Symptom relief in palliative care
Dexamethasone	17 (2011)	4	Antiemetic
Emtricitabine	15 (2007)	200	Protease inhibitor
Enalapril maleate	17 (2011)	5	Antihypertensive
Folic acid	Before 12 (2002)	5	Antianemia
Hydroxychloroquine sulfate[C]	17 (2011)	200	DMARDs
Medroxyprogesterone acetate	12 (2002)	5	Progestogen
Mesna[C]	16 (2010)	600	Cytotoxics and adjuvants
Mifepristone[C]	14 (2005)	200	Oxytocics
Morphine sulfate	Before 12 (2002)	10	Opioid analgesics
Oseltamivir phosphate	16 (2010)	75	Antivirals
Ribavirin	15 (2007)	600	Antivirals
Rifabutin	16 (2010)	150	Antituberculosis
Succimer	17 (2011)	100	Specific antidotes

[C] Included on the complementary list but not included on the main list.

specified time at a defined temperature to produce an equilibrium between the saturated solution and undissolved solid, that is undissolved substance should still be visible at the end of the shaking period. After a final pH measurement to check whether the pH remained unchanged, the sample is filtered and quantified. The shake-flask method can also be conducted in a miniaturized approach with a reduction in both the amount of drug and volume of medium needed, as previously mentioned.[11] Instead of a flask, a Whatman™ Uniprep™ vial with a 3-mL chamber and a plunger with an integrated polytetrafluorethylene filtration membrane (pore size: 0.45 μm) was used for our experiments.

For highly soluble APIs, the approach was further modified. Instead of determining the thermodynamic (equilibrium) solubility as described above, the "minimum solubility" was determined as follows. According to the criteria of the BCS, a drug can be classified as highly soluble if the D/S ratio is equal to or less than 250 mL.[5] In our solubility studies, the highest dose strength listed on the 20th EML[4] was used as the dose for calculating the D/S ratio for each API. To scale down the experiment, the amount of API that would need to go into solution to correspond to a classification as "highly soluble" if completely dissolved in 3 mL of buffer solution was calculated. An amount at least 50% greater than this calculated amount was accurately weighed into the Uniprep™ vials in triplicate. Three milliliters of the appropriate buffer solution was then added to each Uniprep™ vial. A plunger with an integrated polytetrafluorethylene filter system was mounted on each vial, and the unit was closed. All samples were then shaken on an orbital shaker (Heidolph Polymax 1040) for 24 h at a rotational speed of 45 rpm and a temperature of 37 ± 0.5°C. After 24 h, the vials were visually examined for any excess API solid, and the samples were filtered by pushing the plunger into the Uniprep™ vial. Afterward, an aliquot of the sample was withdrawn from the filtrate and diluted with an appropriate

Table 2
Chemical Reference Standards Used for Solubility Determinations

Drug	Analytical Grade/Purity	Batch	Supplier	Source
Amiodarone hydrochloride	99.8%	P500164	Sigma–Aldrich, Germany	RT-Corp, Laramie, WY, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Atazanavir sulfate	100%	Pure API was obtained from Bristol-Myers Squibb	Bristol-Myers Squibb Company, New Brunswick, NJ, USA	Bristol-Myers Squibb Company, New Brunswick, NJ, USA
Cyclizine hydrochloride	USP Reference Standard	H0D321	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Dexamethasone	European Pharmacopeia (Ph. Eur.) 7.0	13352310	Caelo, Germany	Caesar and Lorentz GmbH, Hilden, Germany
Emtricitabine	USP Reference Standard	F0J163	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Enalapril maleate	100.4%	E13Z017	VWR, Germany	Alfa Aesar, Karlsruhe, Germany
Folic acid	100.2%	K45899584537	VWR, Germany	Merck KGaA, Darmstadt, Germany
Hydroxychloroquine sulfate	USP Reference Standard	K0G211	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Medroxyprogesterone 17-acetate	Ph. Eur. Reference Standard	Ph.Eur. CRS # 3.0 Id: 00ESX7	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Mesna	USP Reference Standard	F0H331	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Mifepristone	100%	SLBJ7154V	Sigma–Aldrich, Germany	Sigma–Aldrich, Co, St. Louis, MO, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Morphine sulfate pentahydrate	Analytical grade (>98%)	SLBL1738V	Sigma–Aldrich, Germany	Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Oseltamivir phosphate	USP Reference Standard	R00490	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Ribavirin	Ph. Eur. Reference Standard (99.9%)	Ph.Eur. CRS # 2.0 # 2583198	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Rifabutin	Ph. Eur. Reference Standard (95.5%)	Ph.Eur. CRS # 2.0 Id: 0030C1	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Succimer = meso-2, 3-Dimercaptosuccinic acid	Analytical grade (~98%)	SLBH6371V	Sigma–Aldrich, Germany	Sigma–Aldrich, Co, St. Louis, MO, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany

CRS, chemical reference standards; USP, United States Pharmacopoeia.

Table 3
Study Protocol for Solubility Determination of APIs on the EML

Conditions	Comments
1. Preparation of solubility samples in Uniprep™ syringeless filters	An excess of the API was weighed into Uniprep™ vials in triplicate ($n = 3$ for each buffer). Three milliliters of the buffer solution was added to each Uniprep™ vial.[a] All vials were provisionally sealed with the Uniprep™ plunger.
2. Shaking and incubation	Samples were shaken on an orbital shaker at 45 rpm. Temperature during incubation was maintained at 37 ± 0.5°C. Samples were incubated and shaken for 24 h.
3. Filtration	Status of dissolution, that is, whether any solid could be visually detected, was checked before filtration. The Uniprep™ plunger was pushed into the vial to effect filtration.
4. Sampling and dilution	An aliquot of the filtrate was withdrawn and diluted to an appropriate concentration for analysis (determined in preliminary studies).
5. pH measurement	Any changes to the pH value during the dissolution process were evaluated by a final pH measurement.
6. HPLC analysis	The concentration of dissolved drug was quantified via validated HPLC methods using UV detection (see Table 5). Mean solubility values were calculated.
7. Solubility classification based on the BCS	The highest dose strength listed on the 20th EML was divided by the experimentally obtained solubility values to calculate the dose/solubility ratios for the API. Ratios larger than 250 mL were assigned a classification as "not highly soluble," values ≤250 mL were assigned a classification as "highly soluble."

[a] See Table 4 for buffers.

medium (e.g., organic solvent or mobile phase) to prevent precipitation at room temperature. An appropriate dilution factor was determined in preliminary tests to guarantee that the measured concentration would fall within the validated linear calibration range. For all APIs, a dilution factor between 2 and 100 proved adequate. The pH of the remaining filtrate was checked, and any changes compared with the initial pH of the buffer were recorded.

The amount of dissolved drug in each sample was quantified via high-pressure liquid chromatography (HPLC) analysis with ultraviolet (UV) spectrometric detection. The injection volume was 20 µL, and 2 replicates were performed for each sample. The HPLC systems used consisted of a Hitachi LaChrom pump (LaChrom Elite L-2130 or LaChrom L-7110, respectively), an autosampler (L-2200/L-7200) an UV-detector (L-2400/7400) and a data integrator/organizer unit (D-7000). One system also contained a column oven (VDS optilab). LiChroCART® cartridges filled with LiChrospher® 100 RP-18, LiChrospher® 100 RP-18e, or LiChrospher® 100 RP-8e with 5 µm particle size (Merck Milipore, Darmstadt, Germany) of 2 different lengths (125 mm or 250 mm) were used for analysis. Further details on the HPLC methods such as composition of mobile phase, flow rate, column temperature, run time, retention time, and detection wavelength can be found in Table 5. Each method was validated for the respective API in accordance with the International Conference on Harmonisation guideline Q2(R1),[23] focusing on linearity, repeatability, limit of detection, and limit of quantification.

Permeability Data

To obtain permeability data for BCS classification, a literature search was performed in the bibliographic database PubMed (www.ncbi.nlm.nih.gov, accessed October 20, 2017). The international nonproprietary name of the respective API was searched in combination with one or more of the following key words: absorption, BCS, bioavailability (BA), fraction absorbed, mass balance, perfusion, permeability, pharmacokinetics, and radiolabeled. Permeability data were also obtained from the medical products professional information and the commentary on the European pharmacopoeia for the respective APIs, as well as from the primary sources of permeability data cited in these documents.

Classification of the APIs as "highly permeable" or "not highly permeable" was based on literature permeability or BA data indicative of fraction absorbed *in vivo* ≥85%, in accordance with the guidance documents published by FDA, EMA, and WHO.[6-8]

Results

The results of the solubility studies are shown in Table 6. When the amount of API weighed into the Uniprep™ syringeless filters completely dissolved in 3 mL of buffer solution, the resulting concentration (which represents the minimum solubility of the API) is listed. In all other cases, the mean solubility value and standard deviation calculated from the concentration of API in the saturated solutions at equilibrium sampled at each pH is stated. The D/S ratio was calculated under consideration of the highest dose strength of the pure API (free base or acid, respectively) listed on the 20th version of WHO EML[4] (see first column of Table 6). An API was considered "highly soluble" when the D/S ratio was ≤250 mL at all pH values examined, in accordance with the BCS criteria established by Amidon et al.[5]

Figure 2 shows the D/S ratios of the APIs classified as "highly soluble." With the exception of dexamethasone and cyclizine, the

Table 4
Buffer Compositions Used in Media for Solubility Studies

Buffer	Application
Hydrochloric acid buffer pH 1.2 (5.17.1 Ph.Eur. 8.0)	Amiodarone hydrochloride, atazanavir sulfate, dexamethasone, enalapril maleate, folic acid, hydroxychloroquine, medroxyprogesterone acetate, mifepristone, oseltamivir phosphate, ribavirin, rifabutin
Hydrochloric acid pH 1.2	Cyclizine, emtricitabine, mesna, morphine sulfate pentahydrate, succimer
Phosphate buffer pH 3.0 *R1*[a] (Ph.Eur. 8.0)	Folic acid
Acetate buffer pH 4.5 *R* (Ph.Eur. 8.0)	All substances
Phosphate buffer pH 6.8 *R1* (Ph.Eur. 8.0)	All substances

[a] Buffer with pH close to the solubility minimum of folic acid.

Table 5
HPLC Analysis of the APIs Studied

API	Column and Dimensions	Mobile Phase (V/V)	Flow Rate (mL/min)	Temperature (°C)	Detection Wavelength (nm)	Run Time/Retention Time (min)
Amiodarone hydrochloride	RP-18 (5μm) 125 × 4 mm	Phosphate buffer pH 3.0 R1 (Ph. Eur.)/acetonitrile (1:4)	2.0	40	240	7.0/3.2
Atazanavir sulfate[a]	RP-18e (5μm) 250 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 2.5 (1:1)	1.5	25	288	6.0/3.5
Cyclizine hydrochloride[b]	RP-18 (5μm) 125 × 4 mm	Acetonitrile/potassium dihydrogen phosphate 0.05 M pH 4 (1:1)	1.5	50	225	5.0/1.8
Dexamethasone	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	241	5.0/1.3
Emtricitabine	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	0.75	30	280	5.0/1.5
Enalapril maleate	RP-18 (5μm) 125 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	0.75	25	255	6.0/3.2
Folic acid[c]	RP-8e (5μm) 250 × 4 mm	Methanol/Phosphate buffer pH 6.3 (12:88)	0.6	25	280	30.0/8.2
Hydroxychloroquine sulfate	RP-18 (5μm) 125 × 4	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	1.0	25	255	17.0/2.5
Medroxyprogesterone acetate	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	241	5.0/2.6
Mesna[d]	RP-18 (5μm) 125 × 4 mm	Acetonitrile/phosphate buffer pH 2,3 (2:3)	1.0	25	235	4.0/1.0
Mifepristone	RP-18e (5μm) 250 × 4 mm	Acetonitrile/phosphate buffer pH 2.5 (1:1)	1.0	25	260	8.0/4.2
Morphine sulfate pentahydrate	RP-18 (5μm) 125 × 4 mm	Acetate buffer pH 4/acetonitrile (2:3)	0.75	30	280	10.0/1.85
Oseltamivir phosphate	RP-18 (5μm) 125 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	1.0	25	230	6.0/1.8
Ribavirin[e]	RP-18e (5μm) 250 × 4 mm	Phosphate buffer pH 4.7	1.0	25	207	10.0/4.4
Rifabutin[f]	RP-18e (5μm) 125 × 4 mm	Acetonitrile/ammonium acetate solution pH 4.0 (1:1)	1.0	25	275	15.0/6.2
Succimer = meso-2, 3-dimercaptosuccinic acid	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	255	5.0/1.0

[a] Method adopted from Berlin et al.[17]
[b] The mobile phase was similarly composed as described by El-Gindy et al.[18] Flow rate was obtained from the same publication.
[c] Method adopted from Ph. Eur. 8.0.[19]
[d] Method adopted from Ph. Eur. 8.0.[20] Composition of the mobile phase was modified and a different column length was used.
[e] Method adopted from Belal et al.[21]
[f] Method adopted from Sangshetti et al.[22]

D/S ratios of all APIs classified as "highly soluble" depicted in Figure 2 were based on the observed minimum solubility. Figure 3 shows the D/S ratios of the APIs classified as "not highly soluble."

Table 7 presents the resulting BCS classification of all APIs included in this study, based on permeability data obtained from the literature and on the measured solubility of the highest dose strength listed on the current 20th version of the EML.[4]

Discussion

Solubility Classification and Eligibility for Biowaiver Procedure

Nine of the APIs examined in the present study were conclusively classified as "highly soluble," namely cyclizine, dexamethasone, emtricitabine, enalapril maleate, hydroxychloroquine sulfate, mesna, morphine sulfate pentahydrate, oseltamivir phosphate, and ribavirin. They demonstrated solubility values that would not lead to a change in the solubility classification of the particular drug even if the highest single therapeutic dose would be used for calculation instead of the highest dosage form strength listed on the EML. Considering that the solubility that was determined for most of the highly soluble compounds is a minimum value, the true D/S ratios are expected to be even lower than the ones shown in Figure 3 and Table 6. Furthermore, none of the compounds classified as "highly soluble" showed stability problems in the compendial buffers used. These 9 APIs are therefore possible candidates for a BCS-biowaiver procedure according to the WHO guidance document,[8] as they are either BCS I or BCS III compounds, depending on their permeability classification.

We note that in addition to the BCS I/III classification, further requirements have to be met for IR solid oral dosage forms containing highly soluble APIs to be eligible for a BCS-biowaiver procedure as stated the WHO guidance.[8] Depending on the BCS class, certain considerations regarding excipients and interpretation of the dissolution results have to be followed. Drug products containing BCS I APIs should use well-established excipients in usual amounts with no known influence on the absorption process. In comparative dissolution testing with an appropriate reference product, both the reference product and the multisource product to be approved have to release $\geq$85% of the total drug amount in 15 min (very rapidly dissolving) or in 30 min (rapidly dissolving), in which case there must be an additional comparison of the dissolution profiles via the f_2-test. Dissolution is carried out preferably with the United States Pharmacopoeia II apparatus operating at 50 rpm in $\leq$900 mL dissolution media of pH 1.2, 4.5, and 6.8. For drug products containing BCS III APIs, all excipients used should be qualitatively the same and quantitatively very similar to the reference product, and both drug products have to show very rapid dissolution under the conditions stated previously. In addition, a risk-benefit evaluation is conducted, taking into account the therapeutic index of the drug as well as the possible risk for public health if approval of a product which is actually bioinequivalent is erroneously granted via a BCS-biowaiver procedure. A complete overview of all the points addressed in an assessment of the feasibility of a biowaiver approval

Table 6
Solubility Values and Classification of the APIs According to BCS-Biowaiver Solubility Criteria

Drug (Dose)[a]	pH	Solubility (mg/mL)[b] (Mean ± SD)	Dose/Solubility-Ratio (mL)[b] (Mean ± SD)	Solubility Classification[c]
Amiodarone hydrochloride (400 mg)	1.2	$3.4 \pm 0.3 \times 10^{-3}$	$125 \pm 11 \times 10^{3}$	Not highly soluble
	4.5	≥5.0	≤85	
	6.8	$1.02 \pm 0.03 \times 10^{-3}$	$415 \pm 12 \times 10^{3}$	
Atazanavir sulfate (300 mg)	1.2	2.51 ± 0.11	136 ± 11	Not highly soluble
	4.5	$5.21 \pm 0.03 \times 10^{-3}$	$65.5 \pm 0.4 \times 10^{3}$	
	6.8[d]	$<1.0 \times 10^{-3}$	$>0.34 \times 10^{6}$	
Cyclizine (50 mg)	1.2	≥3.75	≤13.2	Highly soluble
	4.5	≥3.94	≤12.8	
	6.8	1.731 ± 0.019	28.9 ± 0.3	
Dexamethasone (4 mg)	1.2	$99.3 \pm 2.7 \times 10^{-3}$	40.3 ± 1.1	Highly soluble
	4.5	$220 \pm 4 \times 10^{-3}$	18.2 ± 0.3	
	6.8	$66.8 \pm 1.8 \times 10^{-3}$	59.9 ± 1.7	
Emtricitabine (200 mg)	1.2	≥3.35	≤60.6	Highly soluble
	4.5	≥3.87	≤51.3	
	6.8	≥3.86	≤51.3	
Enalapril maleate (5 mg)	1.2	≥5.13	≤1.27	Highly soluble
	4.5	≥5.23	≤1.25	
	6.8	≥5.13	≤1.27	
Folic acid (5 mg)	1.2[e]	$15.95 \pm 0.22 \times 10^{-3}$	314 ± 5	Not highly soluble
	3.0	$1.46 \pm 0.04 \times 10^{-3}$	$3.42 \pm 0.09 \times 10^{3}$	
	4.5	$63.6 \pm 0.7 \times 10^{-3}$	78.6 ± 0.8	
	6.8	≥6.47	≤0.773	
Hydroxychloroquine sulfate (200 mg)	1.2	≥4.83	≤53.5	Highly soluble
	4.5	≥4.97	≤52.1	
	6.8	≥4.97	≤52.1	
Medroxyprogesterone acetate (5 mg)	1.2	$0.9 \pm 0.4 \times 10^{-3}$	$7 \pm 4 \times 10^{3}$	Not highly soluble
	4.5	$6.1 \pm 0.4 \times 10^{-3}$	$0.82 \pm 0.05 \times 10^{3}$	
	6.8[d]	$<0.1 \times 10^{-3}$	$>0.05 \times 10^{6}$	
Mesna (600 mg)	1.2	≥3.56	≤166.7	Highly soluble
	4.5	≥4.11	≤146.3	
	6.8	≥3.52	≤171.4	
Mifepristone (200 mg)	1.2	≥4.57	≤43.8	Not highly soluble
	4.5	$69.0 \pm 2.6 \times 10^{-3}$	$2.90 \pm 0.11 \times 10^{3}$	
	6.8[d]	$<1 \times 10^{-3}$	$>0.2 \times 10^{6}$	
Morphine sulfate pentahydrate (10 mg)	1.2	≥4.39	≤3.03	Highly soluble
	4.5	≥3.98	≤3.34	
	6.8	≥3.79	≤3.51	
Oseltamivir phosphate (75 mg)	1.2	≥4.90	≤20.2	Highly soluble
	4.5	≥4.87	≤20.3	
	6.8	≥4.97	≤20.2	
Ribavirin (600 mg)	1.2	≥4.73	≤127	Highly soluble
	4.5	≥4.87	≤123	
	6.8	≥4.93	≤122	
Rifabutin (150 mg)	1.2[e]	0.48 ± 0.07	$0.31 \pm 0.04 \times 10^{3}$	Not highly soluble
	4.5	3.13 ± 0.05	47.9 ± 0.7	
	6.8	$54 \pm 6 \times 10^{-3}$	$2.82 \pm 0.27 \times 10^{3}$	
Succimer (100 mg)	1.2[e]	1.06 ± 0.16	96 ± 14	Not highly soluble
	4.5[e]	0.29 ± 0.03	$0.35 \pm 0.04 \times 10^{3}$	
	6.8[e]	1.3 ± 0.5	85 ± 28	

SD, standard deviation.
[a] The listed dose strengths are the highest strengths found on the 20th WHO EML[4] and refer to the free base or acid, respectively.
[b] If the excess amount weighed into the samples was completely dissolved at the end of the 24-h solubility study, a minimum solubility and maximum dose/solubility-ratio calculated from the sample with the least amount of drug weighed into the Uniprep™ syringeless filters is presented and indicated by "≥" before the solubility value or "≤" before the dose/solubility-ratio, respectively.
[c] Classification is based on the dose strengths in the first column (corrected for the respective salt form) divided by the experimentally obtained solubility values. A dose/solubility-ratio ≤250 mL corresponds to a classification as "highly soluble."
[d] Atazanavir sulfate, medroxyprogesterone acetate, and mifepristone showed solubility values below the limit of quantification at pH 6.8. The solubility value presented equals the limit of quantification for the respective API and minimum dose/solubility-ratios are presented.
[e] Folic acid and rifabutin showed degradation at pH 1.2, succimer showed noticeable degradation at all pH values.

can be found in the various published biowaiver monographs that are available from the web site of the International Pharmaceutical Federation at: *http://www.fip.org/bcs_monographs*.

The remaining 7 APIs investigated, namely amiodarone hydrochloride, atazanavir sulfate, folic acid, medroxyprogesterone acetate, mifepristone, rifabutin, and succimer, were classified as "not highly soluble." All of them, except for succimer (see Degradation Challenges section), failed to comply with the solubility criteria by at least a 10-fold difference. It is worth noting that 5 of these 7 APIs had their lowest solubility values at pH 6.8. Since this pH value represents the physiological environment of the small intestine, poor solubility at pH 6.8 could potentially lead to BA problems *in vivo* due to slow dissolution behavior or precipitation after an initial dissolution in the stomach and therefore reduced availability of dissolved drug substance for absorption. Independent of their permeability classification, an approval of drug products containing these APIs via the BCS-biowaiver is not currently possible according to any of the various regulatory guidances.

Most of the APIs demonstrated pH versus solubility profiles in line with expectations based on the presence or absence of acidic and/or basic functional groups (e.g. mifepristone, a basic molecule with a pKa of 4.89 shows an increase in solubility when pH

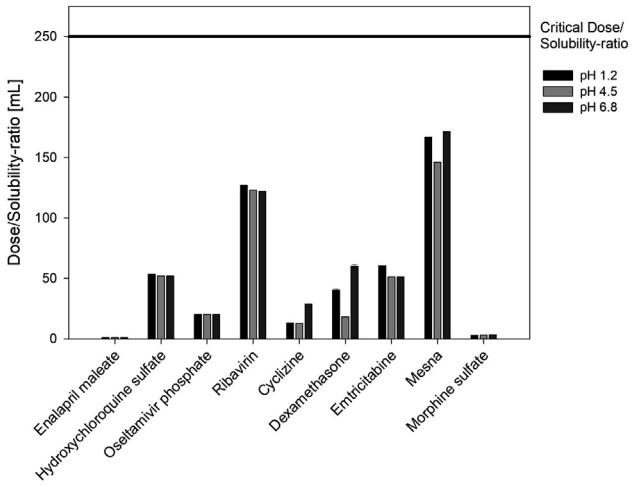

Figure 2. Dose/solubility-ratios of APIs classified as "highly soluble."

decreases). However, amiodarone hydrochloride, dexamethasone, and medroxyprogesterone acetate deviated from the expected behavior. Based on the molecular structure of amiodarone, an increase of solubility with decreasing pH is to be expected due to the basic tertiary amine side chain. As observed in our experiments, amiodarone demonstrated high solubility at pH 4.5 and poorer solubility at pH 1.2 and 6.8. The surprisingly poor solubility at pH 1.2 might be explained by common ion effect, as the salt form of amiodarone used in the experiments is a hydrochloride, and the media also contains chloride ions, thus reducing the degree of dissociation of the salt and the solubility. Dexamethasone and medroxyprogesterone acetate are neutral molecules; and therefore, no influence of media pH on solubility is to be expected. However, both APIs demonstrate the highest solubility at pH 4.5 and lowest solubility at pH 6.8. The observed solubility values, while differing from each other, reside in the same order of magnitude. A visual interaction with the buffer components, for example, the formation of precipitates, was not observed nor was there any change in the appearance or number of peaks in the chromatogram. In any case, measurements at all 3 pH values conclusively indicate high solubility for dexamethasone and poor solubility for medroxyprogesterone acetate, respectively.

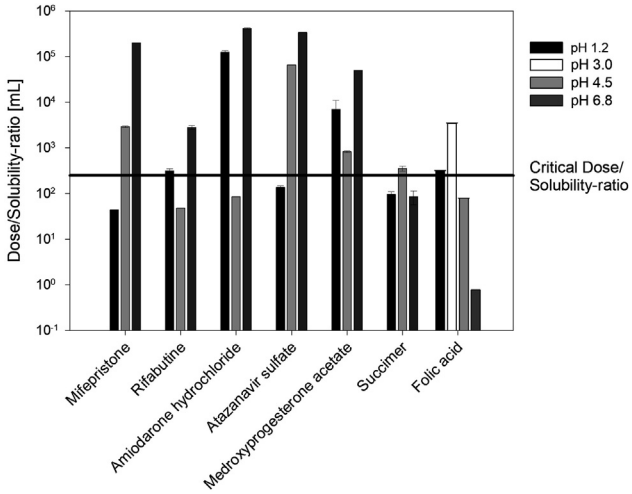

Figure 3. Dose/solubility-ratios of APIs classified as "not highly soluble."

BCS Classification and Possible Influence of Dose Strength Changes

The BCS classifications depicted in Table 7 were obtained using the experimentally determined solubility values and permeability data from the literature. To facilitate comparison with other, previously established classifications of the APIs, we added classifications available from the literature in column 5 of Table 7. The method adopted for classifying varied among the cited publications. For solubility classification, some authors used, whenever possible, solubility data found in the literature obtained from experiments using physiologically relevant conditions (pH 1.2-6.8, 37°C),[9,31,45] whereas others used aqueous solubility data at room temperature without specification of pH,[28-30,39,40] for example, obtained from the United States Pharmacopoeia solubility definitions or the Merck Index. One publication even relied on calculated solubility data derived from physicochemical properties.[29] Regarding the permeability classification, some authors used fraction absorbed and BA data found in the literature,[9,45] others relied solely on *in silico* data correlated to fraction absorbed values,[28,29,39,40] one group used CaCo-2 apparent permeability data for classification,[30] and one group used the Biopharmaceutics Drug Disposition Classification System[76] with ≥70% extent of metabolism as the criterion for high permeability.[31]

Although different approaches for establishing a BCS classification were used, the resulting BCS classifications are mostly in accordance with each other (Table 7), especially with respect to the solubility classifications. The only exceptions were medroxyprogesterone acetate, mifepristone, and folic acid, which were classified as "highly soluble" or "not highly soluble," depending on the reference cited.

Medroxyprogesterone acetate is listed as "highly soluble" in the document "Proposal to waive *in vivo* bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms (Annex 8)"[45] based on its solubility in water at room temperature. Because of the low dose of 5 mg, using the solubility definition "practically insoluble in water (<0.1 mg/mL)" from Clarke's analysis of drugs and poisons still leads to a classification as highly soluble with a D/S ratio of 50 mL. This D/S ratio is an underestimation, as the solubility at pH 6.8 is much lower than 0.1 mg/mL (Table 6), and medroxyprogesterone acetate is therefore correctly classified as "not highly soluble."

Mifepristone was classified provisionally as BCS III or IV by the WHO[45] because no solubility data were available at that time to establish a reliable solubility classification. According to our experiments, mifepristone is clearly to be classified as "not highly soluble" and therefore deemed a BCS IV compound (Table 7).

Folic acid was classified as "not highly soluble" by several authors.[9,31,40] In contrast, others have deemed folic acid to be a highly soluble compound.[28,30,39,45] The pH range considered for classifying the solubility can explain this divergence. If only solubility data in pure water or at pH 1.2, 4.5, or 6.8 are considered, folic acid will be incorrectly classified as borderline highly soluble. But when the solubility at pH 3.0 is taken into consideration, folic acid is clearly classified as not highly soluble because at this pH, the D/S ratio is ≥3 L for a dose of 5 mg (Table 6). This example demonstrates the importance of not only relying on solubility data in pure water or the "standard" pH values proposed in the guidance documents but also solubility values at the pH where the solubility is expected to be lowest.

In the various guidance documents, different definitions of the dose strength to be used for establishing the D/S ratio can be found.[6-8] Although the FDA recommends the highest dosage strength of a marketed IR drug product to be used,[6] the WHO and EMA guidance define that the D/S ratio should be established with

Table 7
BCS Classification of the APIs Based on Measured Solubility Data and Permeability Data From the Literature

Drug Name (WHO EML Dose)	Solubility	Permeability	BCS Class[a]	Previous Classification(s)	Comments
Amiodarone hydrochloride (400 mg)	Low	Low[24-27]	**IV**	II[28-31]	Incomplete absorption (~20%-50%), P-gp inhibitor
Atazanavir sulfate (300 mg)	Low	Low/high[17,32,33]	**IV**/II	IV,[30] II/IV,[17] II[31,33]	Nonlinear pharmacokinetics (range 100-1200 mg), inconclusive f_a data, P-gp Efflux
Cyclizine (50 mg)	High	High/low[34,35]	**I**/III	I[29-31]	No reliable permeability data
Dexamethasone (4 mg)	High	High[36-38]	**I**	III,[30] III/I,[9,39,40] I[28,29,31]	Incomplete BA due to presystemic elimination rather than poor absorption
Emtricitabine (200 mg)	High	High[41]	**I**	III[30,31]	Oral BA $\geq$ 90%, linear kinetics (100-1200 mg)
Enalapril maleate (5 mg)	High	Low[42-44]	**III**	III,[30,40,42,45] III/I,[28] I[29,39]	~60%-70% of a dose is absorbed
Folic acid (5 mg)	Low	Low/high[46-51]	**IV**/II	IV,[40] IV/II,[9] II,[31] III,[28,30,39] III/I[45]	No reliable data for doses $\geq$5 mg, saturable active transport
Hydroxychloroquine sulfate (200 mg)	High	High/low[52,53]	**I**/III	I[30,31]	Rapid and almost complete absorption, BA ~ 67%-74%
Medroxyprogesterone acetate (5 mg)	Low	Low[54]	**IV**	IV,[31] IV/II,[30,39] II,[28,40] III/I[45]	Extent of oral absorption is <10%, positive food effect
Mesna (600 mg)	High	Low/high[55,56]	**III**/I	III/I,[30] I[31]	70% of an oral dose is found in urine (compared with intravenous data)
Mifepristone (200 mg)	Low	Low[57,58]	**IV**	IV/III[45]	Fraction absorbed ~70%, BA ~ 40%, nonlinear pharmacokinetics above 100 mg
Morphine sulfate (10 mg)	High	High/low[59-62]	**I**/III	III,[29,30] III/I,[9,28,40,45] I[31]	BA ~ 30%, high FPE, 90% of a dose is metabolized and found in urine
Oseltamivir phosphate (75 mg)	High	High[63-66]	**I**	III/I,[28,40] I[30,31]	High FPE (~70%-80% of a dose is metabolized in the liver), high BA of metabolized drug (>80%)
Ribavirin (600 mg)	High	Low/high[67-69]	**III**/I	III,[28,30,40] III/I,[69] I[31]	High intestinal FPE, active transport, positive food effect
Rifabutin (150 mg)	Low	Low[70-72]	**IV**	IV,[30] II[31]	Low BA, induces own metabolism, ~ 50%-60% metabolized in urine; highly variable; significant degradation in acidic media
Succimer (100 mg)	Low	Inconclusive[73-75]	**IV**/II	Not classified	Literature data were inconclusive.

FPE, first pass effect.
[a] BCS classifications depicted in **boldface** are the preferred classifications suggested by the authors of this article.

the highest single dose (which could consist of administering multiple dosage forms to achieve a required dose).[7,8] The D/S ratios in Table 6 were calculated based on the highest dose of an IR drug product listed on the WHO EML, which is usually the highest dosage strength of the drug product. A change in the dose definition can only have an impact on the highly soluble compounds, as the D/S ratio can only become larger and not lower. Even when applying the WHO/EMA definition of "dose," all the APIs classified as "highly soluble" by the FDA definition in the present study would remain in that category, further indicating that the solubility classification established for the APIs included in our study is reliable independent of the dose definition used.

The influence of the definition of "dose" on the BCS classification was also investigated in a review of published biowaiver monographs.[77] The impact of the difference between the 2 definitions varied among the 24 individual APIs; as for some, the dose considered did not change (highest single dose = highest dosage strength, 6 APIs), whereas for other APIs, the highest single dose was as much as 5 times the highest dosage strength (e.g., ethambutol hydrochloride, isoniazid).[77] Of the BCS classifications of 24 APIs examined, 2 changed when using the EMA/WHO rather than the US-FDA dose definition and 2 had to be reevaluated.[77]

Choice of Experimental Conditions and Challenges

The experimental conditions of a solubility study must be chosen carefully. One crucial aspect is the influence of the solid state form of the evaluated substance on the solubility. Different polymorphic forms might show different values for solubility. With respect to APIs with several polymorphic forms, it is recommended that the solid state form of the chosen material is identified in the solubility report. Since a full solid state characterization of the examined APIs was outside the scope of this study, pharmacopoeial reference standards were chosen as study material wherever possible (see Table 2).

In contrast to the proposed method for solubility determination in the various guidance documents,[6-8] we determined a minimum solubility after 24 h rather than a thermodynamic equilibrium solubility for the highly soluble APIs. Since some APIs on the EML are rather expensive, a more cost-effective method was chosen to establish solubility classifications. The scaled-down approach, adopted from Glomme et al.,[11] yielded various advantages when compared with the conventional shake-flask method. In most cases, about 15 mg of API per sample was sufficient to establish a reliable solubility classification. Determining the equilibrium solubility of highly soluble compounds would have sometimes required using more than 600 mg per sample to exceed the maximum dose strength—even using the scaled-down experiment—for example, for ribavirin. Using such excessive amounts of API to determine the equilibrium solubility is not only wasteful but can also lead to a change in the pH of the buffer solutions if the drug has acidic or basic properties, as is the case for several of the APIs investigated in our study. The resultant high concentration of dissolved drug would likely exceed the buffer capacity of media and would therefore require adjustment of the pH during the incubation period, changing the total volume and introducing an additional source of variability.

In addition to using a scaled down version of the shake-flask method, we selected a 24-h time frame to determine the solubility. A 24-h time point was selected because experience in our laboratories has shown that most APIs achieve their equilibrium solubility within this time frame. Furthermore, since the physiological transit time of drugs through the absorptive compartments of the gastrointestinal tract is rarely more than 24 h, extending the solubility determinations to more than 24 h seems unnecessary. Figures 2 and 3 show that relevant and reliable values were obtained for all APIs using the (minimum) solubility at 24 h approach, with the exception of succimer and ribafutin. In the case of succimer and ribafutin, the solubility measurements were complicated by their degradation under the experimental conditions. In these cases, a considerably shorter time frame for the solubility measurement, for example corresponding to upper gastrointestinal transit time, may be more appropriate.

Degradation Challenges

During the 24-h incubation at 37°C, degradation was observed for 3 drugs, which were later categorized as "not highly soluble": folic acid, rifabutin, and succimer. In additional studies to quantify the extent of degradation, it was found that after 4 h at pH 1.2°C and 37°C, about 5% of the total amount of folic acid in a solution of known concentration and about 30% of the total amount of rifabutin in a solution of known concentration had degraded. The relative extent of degradation was estimated for each API from its peak area in the chromatogram at each individual time point divided by the peak area at the beginning of the degradation study ($t = 0$ h). Degradation rates of folic acid and rifabutin at acidic pH values observed in this study are in accordance with results of degradation studies found in the literature.[22,78-81] Succimer dissolved directly after immersion in the different buffers, but a large amount had sedimented after the 24-h incubation. Immediately after adding the media, hydrogen sulfide was detected organoleptically, especially with the more acidic media. In contrast to succimer solutions prepared in organic solvents, these samples showed degradation peaks in the HPLC analysis. It was inferred that succimer undergoes hydrolysis and that the obtained values, although indicating that succimer is not highly soluble, do not reflect the true thermodynamic solubility of succimer. The instability of succimer in aqueous media at physiological pH values, the fact that no reliable permeability data are available in the open literature and that it shows borderline solubility behavior make it impossible to reliably classify succimer according to the BCS. Following a worst-case approach, succimer is conservatively classified as BCS IV, and thus unsuitable for a BCS-biowaiver approval.

The possibility of degradation during the solubility determination requires a stability indicating analytical procedure such as analysis via HPLC, which is also recommended in the FDA draft guidance document.[6] Analytical methods solely based on UV-Vis spectroscopy may lead to biased results if the investigated drug demonstrates instability in the test media.

The various guidance documents state no specific consequence for the BCS classification of an API if degradation during solubility measurement occurs. In the solubility section of the FDA draft guidance document, it is stated that the occurrence of degradation should simply be reported,[6] as degradation may also have an influence on the amount of drug available for absorption. In the section discussing permeability of the same document, it is stated that instability in the gastrointestinal tract should be taken into consideration. Here, degradation to an extent $\geq$5% is considered significant,[6] and the FDA recommends degradation studies to be carried out in simulated gastric or intestinal fluids at 37°C for a period of 1 h or 3 h, respectively.[6] When a compound shows a large degree of degradation in acidic media for example rifabutin ($\geq$30% in 4 h at pH 1.2), it is to be assumed that this could also influence the permeability criterion as defined by the BCS. In fact, if the compound shows degradation to an extent greater than 15% under conditions corresponding to those before or at the site of absorption, it is reasonable to infer that the fraction of dose absorbed *in vivo* cannot be equal or higher than 85%. For this reason, we propose that an otherwise highly permeable drug showing degradation to an extent $\geq$15% over 1 h in simulated gastric fluid (reflecting a rather slow gastric emptying time in the fasted state) at a temperature of 37°C (the conditions stated in the FDA draft guidance document[6]), the drug should be classified as "not highly permeable." For the investigation of degradation under intestinal conditions, the FDA recommendation of experiments in simulated intestinal fluid at 37°C for 3 h seems appropriate. Since degradation to an extent of $\geq$15% in 3 h measured *in vitro* could be compensated or even negated by rapid absorption *in vivo*, no reliable assumption can be made here about the fraction of the dose available for absorption. The potential influence of intestinal degradation of an API has therefore to be discussed individually for each API. Both degradation experiments should be carried out using stability indicating dissolution testing, a method which is described and implemented in the biowaiver monograph for acetylsalicylic acid.[82]

Impact of Degradation on the BCS Solubility Classification

Significant degradation during the 24-h solubility measurement in any of the media can have an influence on the resulting solubility classification. If decomposition occurs, the 24-h solubility approach could yield either higher or lower solubility values compared with solubility measurements of shorter duration and might therefore lead to the wrong BCS classification. For APIs that show degradation at pH values that are relevant for the BCS classification (i.e., pH 1 to pH 6.8), additional solubility measurements should be carried out. The appropriate time period for the additional solubility experiments can be inferred from the degradation studies discussed in the previous section: for substances showing degradation a pH 1.2, the maximum time period for the supplementary solubility determination should be 1 h, as a longer exposition to this media pH is unlikely *in vivo*. For degradation at other pH values, a maximum of 3 h as a time period for additional solubility experiments is reasonable because this time frame corresponds to approximately the period in which the majority of the uptake of an API in an IR formulation from the small intestine is expected.

If a drug shows a rate of degradation higher than 15% in 1 h under gastric conditions or 3 h under intestinal conditions, the duration of the solubility experiments should be no longer than the time required for 15% decomposition (in other words, until the time when 85% of the drug is still intact). For the APIs investigated in our study, such additional solubility experiments were not necessary even for the APIs showing degradation such as folic acid and rifabutin. Although both of these APIs degraded in acidic media (pH 1.2), they had already shown poor solubility at other pH values and thus it could be concluded that they were not "highly soluble" within the BCS definition.

Nevertheless, consideration of degradation might be important for other APIs, which are highly soluble over the entire pH range required for BCS. For example, the impact of hydrolysis on solubility measurement is discussed in the biowaiver monograph of acetylsalicylic acid.[82] In that publication, the authors chose a duration of 15-45 min (depending on the media pH) for the solubility experiments to ensure that the extent of degradation during the study would be less than 2%. If a 24-h solubility determination would have been used, the degradation of acetylsalicylic acid would

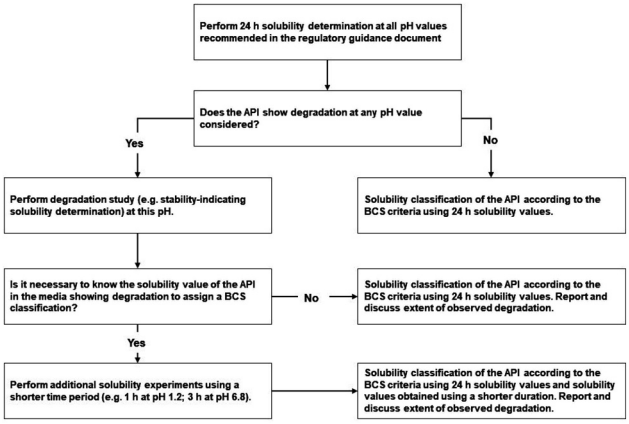

Figure 4. Decision tree for performing solubility determinations in the context of the BCS-based biowaiver.

have been almost complete, resulting in an underestimation of the solubility and potentially in an erroneous solubility classification as "not highly soluble." For acetylsalicylic acid, it would have also been possible to determine the solubility over 1 h at pH 1.2 and over 3 h at pH 6.8, as recommended earlier in this paragraph because, using a worst-case assumption, the time to 10% degradation is 3.17 h at pH 6.8 for acetylsalicylic acid, indicating that more than 90% of the API would remain intact for at least 3 h.

An overview of the experimental procedure proposed in this section is depicted in Figure 4 as a decision tree.

Conclusions

The experimental study protocol elaborated in these studies, which is based on a miniaturized shake-flask method, is a fast and cost-effective approach for establishing a reliable solubility classification of APIs listed on the WHO EML in the context of the BCS-based biowaiver and enabled all APIs studied to be clearly classified into 1 of the 2 solubility categories. Of the 16 APIs, 3 were assigned to BCS class I, 1 to class III and 4 to class IV. For 8 APIs, permeability could not be well defined from the literature, resulting in 5 class I/III classifications and 3 class II/IV classifications. The resulting solubility and BCS classification were in accordance with other, previously proposed, classifications, suggesting that although the current results were obtained using a scaled-down method and that experiments were conducted over 24 h rather than requiring thermodynamic equilibrium to be reached, the scaled-down methodology provides an accurate BCS classification. In particular, using the "minimum solubility" approach can dramatically cut down the amount of API required to obtain a solubility classification for "highly soluble" drugs while avoiding issues with maintenance of the target pH value when studying weak acids and bases. Thus, it is proposed that some flexibility in the determination of solubility for BCS purposes be allowed in future guidances. We would further like to emphasize the importance of stating the experimental conditions in conjunction with the solubility classification as either "highly soluble" or "not highly soluble," to enable calculations based on other "dose" definitions and to allow better assessment of the quality of the data.

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References

1. United Nations. Specialized agencies. Available at: http://www.un.org/en/sections/about-un/funds-programmes-specialized-agencies-and-others/index.html. Accessed November 6, 2017.
2. World Health Organization (WHO). Constitution of the World Health Organization. Available at: http://apps.who.int/gb/bd/PDF/bd47/EN/constitution-en.pdf?ua=1. Accessed November 6, 2017.
3. World Health Organization (WHO). Essential medicines and health products. Available at: http://www.who.int/medicines/about/en/. Accessed November 6, 2017.
4. World Health Organization (WHO). WHO model List of Essential Medicines. 20th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
5. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res*. 1995;12(3):413-420.
6. U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Evaluation and Research (CDER). Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Guidance for industry; Draft guidance. Available at: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070246.Pdf. Accessed November 6, 2017.
7. European Medicines Agency (EMA), Committee for Proprietary Medicinal Products (CPMP). Guideline on the investigation of bioequivalence. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf. Accessed November 6, 2017.
8. World Health Organization (WHO). Annex 7. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Available at: http://apps.who.int/medicinedocs/documents/s21898en/s21898en.pdf. Accessed November 6, 2017.
9. Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the World Health Organization model List of Essential Medicines according to the Biopharmaceutics Classification System. *Eur J Pharm Biopharm*. 2004;58(2):265-278.
10. World Health Organization (WHO). WHO model List of Essential Medicines. 12th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
11. Glomme A, März J, Dressman JB. Comparison of a miniaturized shake-flask solubility method with automated potentiometric acid/base titrations and calculated solubilities. *J Pharm Sci*. 2005;94(1):1-16.
12. World Health Organization (WHO). WHO model List of Essential Medicines. 14th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
13. World Health Organization (WHO). WHO model List of Essential Medicines. 17th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
14. Roy SD, Flynn GL. Solubility behavior of narcotic analgesics in aqueous media: solubilities and dissociation constants of morphine, fentanyl, and sufentanil. *Pharm Res*. 1989;6(2):147-151.
15. World Health Organization (WHO). WHO model List of Essential Medicines. 16th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
16. World Health Organization (WHO). WHO Model List of Essential Medicines. 15th list. Available at: http://www.who.int/medicines/publications/essentialmedicines/en/. Accessed November 6, 2017.
17. Berlin M, Ruff A, Kesisoglou F, et al. Advances and challenges in PBPK modeling—analysis of factors contributing to the oral absorption of atazanavir, a poorly soluble weak base. *Eur J Pharm Biopharm*. 2015;93:267-280.
18. El-Gindy A, Emara S, Mostafa A. Spectrophotometric and LC determination of two binary mixtures containing antihistamins. *Farmacognosia*. 2004;59(9):713-722.
19. European Pharmacopoeia. *General Monographs—Folic Acid*. 8th ed. Strasbourg, France: European Directorate for the Quality of Medicines & Healthcare; 2014.
20. European Pharmacopoeia. *General Monographs—Mesna*. 8th ed. Strasbourg, France: European Directorate for the Quality of Medicines & Healthcare; 2014.
21. Belal F, Sharaf El-Din MK, Eid MI, El-Gamal RM. Validated stability-indicating liquid chromatographic method for the determination of ribavirin in the presence of its degradation products: application to degradation kinetics. *J Chromatogr Sci*. 2015;53(4):603-611.
22. Sangshetti N. Stability-indicating (liquid chromatographic) LC method for the determination of rifabutin in bulk drug and in pharmaceutical dosage form. *Afr J Pharm Pharmacol*. 2011;5(3):298-305.
23. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH harmonised tripartite guideline. Validation of analytical procedures: text and methodology Q2(R1). Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed November 6, 2017.

24. Pfeiffer A, Vidon N, Bovet M, et al. Intestinal absorption of amiodarone in man. *J Clin Pharmacol*. 1990;30(7):615-620.
25. Andreasen F, Agerbaek H, Bjerregaard P, Gotzsche H. Pharmacokinetics of amiodarone after intravenous and oral administration. *Eur J Clin Pharmacol*. 1981;19(4):293-299.
26. STADApharm GmbH. Amiodaron STADA 200 mg tabletten—summary of product characteristics. Available at: http://fachinformation.srz.de/pdf/stadapharm/amiodaronstada200mgtabletten.pdf. Accessed November 6, 2017.
27. Anastasiou-Nana M, Levis GM, Moulopoulos S. Pharmacokinetics of amiodarone after intravenous and oral administration. *Int J Clin Pharmacol Ther Toxicol*. 1982;20(11):524-529.
28. Takagi T, Ramachandran C, Bermejo M, et al. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol Pharm*. 2006;3(6):631-643.
29. Shawahna R, Rahman N. Evaluation of the use of partition coefficients and molecular surface properties as predictors of drug absorption: a provisional biopharmaceutical classification of the list of national essential medicines of Pakistan. *Daru*. 2011;19(2):83-99.
30. Pham-The H, Garrigues T, Bermejo M, et al. Provisional classification and in silico study of biopharmaceutical system based on caco-2 cell permeability and dose number. *Mol Pharm*. 2013;10(6):2445-2461.
31. Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. *AAPS J*. 2011;13(4):519-547.
32. Bristol-Myers Squibb Pharmaceuticals Ltd. REYATAZ Capsules—summary of product characteristics. Available at: http://www.ema.europa.eu/docs/de_DE/document_library/EPAR_-_Product_Information/human/000494/WC500056380.pdf. Accessed November 6, 2017.
33. Singh G, Pai RS. Atazanavir-loaded Eudragit RL 100 nanoparticles to improve oral bioavailability: optimization and in vitro/in vivo appraisal. *Drug Deliv*. 2016;23(2):532-539.
34. WABOSAN. Echnatol B6 dragées—summary of product characteristics. Available at: http://www.wabosan.at/daten/echnatolb6.pdf. Accessed November 6, 2017.
35. Surmann P, Moser U. *Commentary on Ph. Eur. Monographs—Cyclizine Hydrochloride*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2009.
36. Duggan DE, Yeh KC, Matalia N, et al. Bioavailability of oral dexamethasone. *Clin Pharmacol Ther*. 1975;18(2):205-209.
37. Neubeck M, Högger P. *Commentary on Ph. Eur. Monographs—Dexamethason*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2014.
38. Brophy TR, McCafferty J, Tyrer JH, Eadie MJ. Bioavailability of oral dexamethasone during high dose steroid therapy in neurological patients. *Eur J Clin Pharmacol*. 1983;24(1):103-108.
39. Kasim NA, Whitehouse M, Ramachandran C, et al. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol Pharm*. 2004;1(1):85-96.
40. Dahan A, Wolk O, Kim YH, et al. Purely in silico BCS classification: science based quality standards for the world's drugs. *Mol Pharm*. 2013;10(11):4378-4390.
41. Wang LH, Begley J, St Claire III RL, et al. Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once daily dosing for the treatment of HIV infection. *AIDS Res Hum Retroviruses*. 2004;20(11):1173-1182.
42. Verbeeck RK, Kanfer I, Löbenberg R, et al. Biowaiver monographs for immediate-release solid oral dosage forms: enalapril. *J Pharm Sci*. 2017;106:1933-1943.
43. Ebel S, Neubeck M. *Commentary on Ph. Eur. Monographs—Enalapril Maleate*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2011.
44. Sandoz Pharmaceuticals GmbH. Enalapril sandoz 2,5 mg tabletten—summary of product characteristics. Available at: http://www.sz-produkte.de/pdf/fi/2010_10_2_5_mg_012987_d858_internet.pdf. Accessed November 6, 2017.
45. World Health Organization (WHO). Annex 8. Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms. Available at: http://www.who.int/medicines/areas/quality_safety/quality_assurance/ProposalWaiveVivoBioequivalenceRequirementsModelListEssentialMedicinesImmediateReleaseSolidOralDosageFormsTRS937Annex8.pdf. Accessed November 6, 2017.
46. Menke A, Weimann HJ, Achtert G, et al. Absolute bioavailability of folic acid after oral administration of a folic acid tablet formulation in healthy volunteers. *Arzneimittelforschung*. 1994;44(9):1063-1067.
47. Wright AJ, Finglas PM, Dainty JR, et al. Single oral doses of ^{13}C forms of pteroylmonoglutamic acid and 5-formyltetrahydrofolic acid elicit differences in short-term kinetics of labelled and unlabelled folates in plasma: potential problems in interpretation of folate bioavailability studies. *Br J Nutr*. 2003;90(2):363-371.
48. Lin Y, Dueker SR, Follett JR, et al. Quantitation of in vivo human folate metabolism. *Am J Clin Nutr*. 2004;80(3):680-691.
49. Iyengar L, Babu S. Folic acid absorption in pregnancy. *Br J Obstet Gynaecol*. 1975;82(1):20-23.
50. Nguyen P, Boskovic R, Yazdani P, et al. Comparing folic acid pharmacokinetics among women of childbearing age: single dose ingestion of 1.1 versus 5 mg folic acid. *Can J Clin Pharmacol*. 2008;15(2):e314-e322.
51. Patanwala I, King MJ, Barrett DA, et al. Folic acid handling by the human gut: implications for food fortification and supplementation. *Am J Clin Nutr*. 2014;100(2):593-599.
52. Furst DE. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. *Lupus*. 1996;5(S1):S11-S15.
53. Sanofi-Aventis Deutschland GmbH. Quensyl - summary of product characteristics. Available at: https://mein.sanofi.de/produkte/Quensyl/Downloads?id=1e8a9f80-25e3-417e-a339-7fa9ea9984d9. Accessed November 6, 2017.
54. Etienne M-C, Milano G, René N, et al. Improved bioavailability of a new oral preparation of medroxyprogesterone acetate. *J Pharm Sci*. 1991;80(12):1130-1132.
55. Rücker G, Brodschelm W. *Commentary on Ph. Eur. Monographs - Mesna*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2006.
56. Baxter Oncology GmbH. Uromitexan—summary of product characteristics. Available at: http://www.baxter.de/de_DE/assets/downloads/fachinformation/uromitexan_tabletten.pdf. Accessed November 6, 2017.
57. Sarkar NN. Mifepristone: bioavailability, pharmacokinetics and use-effectiveness. *Eur J Obstet Gynecol Reprod Biol*. 2002;101(2):113-120.
58. Liao A, Pang X, Li H, et al. Bioavailability of mifepristone in capsule versus tablet form in healthy nonpregnant women. *Contraception*. 2008;77(6):431-434.
59. Center for Drug Evaluation and Research (CDER). Summary review. Application number 201517Orig1s000. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/201517Orig1s000SumR.pdf. Accessed November 6, 2017.
60. Scriba B, Müller A, Neubeck M. *Commentary on Ph. Eur. Monographs—Morphine Sulfate*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2012.
61. Hasselström J, Säwe J. Morphine pharmacokinetics and metabolism in humans. Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. *Clin Pharm*. 1993;24(4):344-354.
62. Tzvetkov MV, dos Santos Pereira JN, Meineke I, et al. Morphine is a substrate of the organic cation transporter OCT1 and polymorphisms in OCT1 gene affect morphine pharmacokinetics after codeine administration. *Biochem Pharmacol*. 2013;86(5):666-678.
63. Gehring A, Neubeck M. *Commentary on Ph. Eur. Monographs—Oseltamivir Phosphate*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2012.
64. Davies BE. Pharmacokinetics of oseltamivir: an oral antiviral for the treatment and prophylaxis of influenza in diverse populations. *J Antimicrob Chemother*. 2010;65(S2):ii5-ii10.
65. Incecayir T, Sun J, Tsume Y, et al. Carrier-mediated prodrug uptake to improve the oral bioavailability of polar drugs: an application to an oseltamivir analogue. *J Pharm Sci*. 2016;105(2):925-934.
66. Patel K, Rayner CR, Giraudon M, et al. Pharmacokinetics and safety of oseltamivir in patients with end-stage renal disease treated with automated peritoneal dialysis. *Br J Clin Pharmacol*. 2015;79(4):624-635.
67. Merck Sharp & Dohme Ltd. Rebetol—summary of product characteristics. Available at: https://www.msd.de/fileadmin/files/fachinformationen/rebetol_kapseln.pdf. Accessed November 6, 2017.
68. Dixit NM, Perelson AS. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol Life Sci*. 2006;63(7-8):832-842.
69. Goodarzi N, Barazesh Morgani A, Abrahamsson B, et al. Biowaiver monographs for immediate release solid oral dosage forms: ribavirin. *J Pharm Sci*. 2016;105(4):1362-1369.
70. Battaglia R, Pianezzola E, Salgarollo G, et al. Absorption, disposition and preliminary metabolic pathway of ^{14}C-rifabutin in animals and man. *J Antimicrob Chemother*. 1990;26(6):813-822.
71. Handbook of anti-tuberculosis agents. Rifabutin. *Tuberculosis*. 2008;88(2):145-147.
72. Skinner MH, Blaschke TF. Clinical pharmacokinetics of rifabutin. *Clin Pharm*. 1995;28(2):115-125.
73. Aposhian HV, Aposhian MM. meso-2,3-Dimercaptosuccinic acid: chemical, pharmacological and toxicological properties of an orally effective metal chelating agent. *Annu Rev Pharmacol Toxicol*. 1990;30:279-306.
74. Dart RC, Hurlbut KM, Maiorino RM, et al. Pharmacokinetics of meso-2,3-dimercaptosuccinic acid in patients with lead poisoning and in healthy adults. *J Pediatr*. 1994;125(2):309-316.
75. Pigman EA, Lott JR, Fernando Q, Blanchard J. Use of the Caco-2 cell model to assess the relative lead-chelating ability of diasterioisomers of 2,3-dimercaptosuccinic acid. *Environ Health Perspect*. 1999;107(2):111-115.
76. Wu C-Y, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res*. 2005;22(1):11-23.
77. Sediq A, Kubbinga M, Langguth P, Dressman J. The impact of the EMA change in definition of “dose” on the BCS dose-solubility ratio: a review of the biowaiver monographs. *J Pharm Sci*. 2014;103(1):65-70.
78. Pfeiffer C, Diehl JF, Schwack W. Nahrungsfolate—eine aktuelle Übersicht Stabilität, Physiologische Bedeutung, Bioverfügbarkeit, analytische Bestimmungsmethoden, Einfluß der Lebensmittelbehandlung. *Z Ernährungswiss*. 1994;33(2):85-119.
79. de Brouwer V, Zhang G-F, Storozhenko S, Straeten DV, Lambert WE. pH stability of individual folates during critical sample preparation steps in prevision of the analysis of plant folates. *Phytochem Anal*. 2007;18(6):496-508.
80. Preu L, Liebmann-Gülicher B. *Commentary on Ph. Eur. Monographs—Folic Acid*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2011.
81. Schirmeister T, Hofner A. *Commentary on Ph. Eur. Monographs—Rifabutin*. Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2005.
82. Dressman JB, Nair A, Abrahamsson B, et al. Biowaiver monograph for immediate-release solid oral dosage forms: acetylsalicylic acid. *J Pharm Sci*. 2012;101(8):2653-2667.