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A novel simulated media system for in vitro evaluation of bioequivalent intestinal drug solubility

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ABSTRACT

Orally administered solid drug must dissolve in the gastrointestinal tract before absorption to provide a systemic response. Intestinal solubility is therefore crucial but difficult to measure since human intestinal fluid (HIF) is challenging to obtain, varies between fasted (Fa) and fed (Fe) states and exhibits inter and intra subject variability. A single simulated intestinal fluid (SIF) cannot reflect HIF variability, therefore current approaches are not optimal. In this study we have compared literature Fa/FeHIF drug solubilities to values measured in a novel in vitro simulated nine media system for either the fasted (Fa9SIF) or fed (Fe9SIF) state. The manuscript contains 129 literature sampled human intestinal fluid equilibrium solubility values and 387 simulated intestinal fluid equilibrium solubility values. Statistical comparison does not detect a difference (Fa/Fe9SIF vs Fa/FeHIF), a novel solubility correlation window enclosed 95% of an additional literature Fa/FeHIF data set and solubility behaviour is consistent with previous physicochemical studies. The Fa/Fe9SIF system therefore represents a novel in vitro methodology for bioequivalent intestinal solubility determination. Combined with intestinal permeability this provides an improved, population based, biopharmaceutical assessment that guides formulation development and indicates the presence of food based solubility effects. This transforms predictive ability during drug discovery and development and may represent a methodology applicable to other multicomponent fluids where no single component is responsible for performance.

1. Introduction

Oral drug administration is preferred by patients but solid drug must dissolve in the gastrointestinal tract (GIT) to enable absorption and produce a response. Intestinal solubility controls [1] absorption and the Developability Classification System [2] (DCS) links intestinal solubility, volume and dose administered with permeability to classify absorption behaviour. Most drug development candidates are poorly soluble (DCS Class II and IV) [3] and during drug discovery and development an accurate in vitro intestinal solubility measurement is essential to assess in vivo biopharmaceutical properties [4] and potential formulation strategies.

The gold standard for measuring intestinal solubility is sampled human intestinal fluid (HIF) [2]. However, HIF is a multicomponent system containing in the fasted (Fa) state endogenous solubilising agents e.g. bile salts and phospholipids, with in the fed (Fe) state additional food digestion products such as fatty acids and glycerides [5]. Average bile salt concentration varies from 3 mM in the fasted state to 15 mM in the fed increasing drug solubility and absorption, leading to a potential "food effect" [6]. This prandial variation is superimposed on intra and inter subject variability [5,7], along with population and disease changes [8]. Obtaining HIF requires nasogastric intubation, only provides small volumes (1–2 mL) and exhibits intra and inter subject variability [5]. Drug solubilities measured in sampled Fa/FeHIF are therefore due to HIF compositional variability highly variable [9] and single values are difficult to correlate to in vivo biopharmaceutical performance.

To mitigate HIF availability, fasted and fed simulated intestinal fluid (Fa/FeSIF) based on average HIF component values was introduced as an in vitro surrogate. Several versions were developed [10] by comparing drug Fa/FeHIF solubilities vs Fa/FeSIF and adjusting SIF media composition. However, there is solubility variability between Fa/

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FeSIF recipes [11] and between Fa/FeHIF samples and therefore no consensus on the optimal Fa/FeSIF media.

Statistically guided studies on SIF composition and solubility [12,13] identified the media components driving solubility either individually or in combination [14]. These studies also revealed that drug molecular structure and physicochemical properties influence solubility variability in combination with media variability [14]. Due to these inherent properties of the drug and the media intestinal solubility is therefore a range. A single solubility value determined in a sampled (Fa/FeHIF) or fixed simulated intestinal media (Fa/FeSIF) composition is therefore incapable of representing the potential in vivo solubility range (which can vary by orders of magnitude) due to HIF variability [8].

To capture HIF compositional variability and therefore solubility variability, a study [7] reported a five-dimensional (pH, bile salt, phospholipid, free fatty acid and cholesterol) analysis of Fa/FeHIF samples [5]. The dimensions or media constitutents included were those that had the major individual impact on drug solubility [12,13]. For both prandial states, eight intestinal media that incorporated 95 % of HIF compositional variability were determined along with a centre point (Fa9SIF and Fe9SIF). Each media is a novel FaSIF [15] or FeSIF [16] directly linked to Fa/FeHIF composition with all 9 in combination covering 95 % of either the fasted or fed compositional variability. There is a fed state limitation since the original study [5] administered the liquid feed Ensure Plus™, which is not equivalent to solid meals.

Previous studies have compared Fa/Fe9SIF solubility [15,16] to Design of Experiment (DoE) studies [12,13,17,18], the DCS [2] with calculation of a new solubility population distribution [19,20] and to determine structured solubility behavior [21,22] that identifies the lowest and highest solubility media. Due to Fa/Fe9SIF's derivation [7] from Fa/FeHIF composition [5], measured drug solubility ranges should be bioequivalent and include measured Fa/FeHIF values. In this paper we have compared Fa/Fe9SIF solubility data for twenty three drugs in the fasted and twenty in the fed state to published Fa/FeHIF solubilities (see **Supplementary Tables 3 and 4**). Establishing an in vitro in/ex vivo intestinal solubility correlation along with the ability to determine a drug's intestinal solubility variability will introduce a transformational change throughout drug discovery, development and formulation [4].

2. Materials and methods

2.1. Materials

Sodium taurocholate, cholesterol, sodium chloride (NaCl), sodium oleate, ammonium formate, formic acid, potassium hydroxide (KOH), hydrochloric acid (HCl), were from Merck Life Science UK Limited, Dorset, UK. Phosphatidylcholine from soybean (PC S) was from Lipoid GmbH, Ludwigshafen, Germany. Chloroform was from Rathburn Chemical Company, Walkerburn, Scotland and sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$) was from Fisher Scientific, Leicestershire, UK. All acetonitrile (ACN) and methanol (MeOH) solvents were HPLC gradient (VWR). All water is ultrapure Milli-Q water.

Aprepitant and felodipine were through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark. Zafirlukast was from Stratech Scientific Ltd, Ely, UK and ibuprofen was obtained from BSAF chemical company. Atazanavir and posaconazole were from Chemshuttle, Burlingame, CA, USA. Carbamazepine, carvedilol, danazol, diazepam, dipyridamole, fenofibrate, furosemide, griseofulvin, indomethacin, itraconazole, naproxen, phenytoin, piroxicam, prednisolone, probucol, tadalafil, valsartan were from Merck Chemicals Ltd, Dorset, UK. See **Supplementary Table 1** for physicochemical data on all drugs.

2.2. Methods

2.2.1. Bioequivalent media stock solutions

For each media recipe (Table 1a Fasted, Table 1b Fed), concentrated stock solutions were prepared [15,16]. The required (x15) weight of bile salt (sodium taurocholate), phospholipid (soyabean lecithin) and free fatty acid (sodium oleate) for each media recipe was dissolved in chloroform (3 mL) – Stock A. The required weight of cholesterol (x1500) for each media recipe was dissolved in chloroform (10 mL) – Stock B. An aliquot of Stock B (0.1 mL) was added to each Stock A, mixed and the chloroform evaporated under a stream of dry nitrogen gas. The dry lipid film was resuspended in water, quantitatively transferred to a volumetric flask (5 mL) and made to volume with water. Stock aqueous solutions of buffer (sodium phosphate monobasic monohydrate; 28.4 mM) and salt (sodium chloride; 105.9 mM) were prepared in water.

2.2.2. Equilibrium solubility measurement

The general equilibrium solubility measurement method has been applied in previous published papers [12,13,15,16,19–22]. In a centrifuge tube (15 mL Corning® tubes), an excess amount of solid drug, exceeding its solubility limit, was weighed, followed by the addition of appropriate concentrated media stock solutions and water. The pH of each tube was adjusted to ± 0.02, using KOH or HCl if necessary, and shaken for an hour at room temperature. The pH was readjusted if needed. The tubes were then placed in an orbital shaker (Labinco L28 Orbital Shaker) and incubated for 24 h at 37 °C and 240 rpm.

Following the 24-hour incubation period, the contents of all tubes were inspected for the presence of solid drug. Then, 1 mL of each solution was transferred to 1.5 mL Eppendorf tubes and centrifuged at 10,000 rpm (RCF approx. 14,000) for 15 min using the Hettich Zentrifugen Mikro 20. Centrifugation is only intended to remove excess solid drug that has not dissolved in the media. Micellar material should remain in the supertnatant as a critical solubilisation component. The supernatant from each tube was analysed for drug content using HPLC. Three separate solubility measurements were taken for each media point to ensure accuracy [15,16].

2.2.3. HPLC analysis

Analysis was performed on a Shimadzu Prominence-i LC-2030C HPLC system using a gradient method for all the drugs. The mobile phase, column (all at 30 °C), retention time, detection wavelengths and injection volume for each drug are provided in **Supplementary Table 2**. For each drug, a concentration curve was prepared using five or six standards that bracketed all the measurement concentrations. For all drugs, the correlation coefficient of the calibration curve was > 0.99 [12,15,16].

2.2.4. Statistical analysis

Statistical analysis was conducted using Graphpad Prism 9 for MacOSX. Correlation analysis were performed using Datagraph 4.7.1 for MacOSX. The variable number of FaHIF measurement values do not permit a simple direct statistical comparison between the data sets. Thirteen drugs have 3 or more available FaHIF values, with seven for FeHIF and these have been compared as a group using a Wilcoxon matched pairs signed rank test, $P < 0.05$ (Two-tailed). Each drug has also been individually compared using a Mann-Whitney test, $P < 0.05$ (Two-tailed). Previous papers have highlighted that the simulated data sets [17] and Fa/FeHIF chemical compositions [7] do not follow a normal distribution and therefore non-parametric statistical comparison is appropriate. The nine media minimum and maximum solubility values (x_{min},y_{max}; x_{max},y_{min}) have been correlated using a power function ($y = ax^b$) to determine a maximum and minimum solubility boundary for each drug category, r^2 reported along with $P < 0.05$ for slope significantly non-zero.

3. Results and discussion

3.1. Equilibrium solubility data sets

One hundred and twenty nine literature Fa/FeHIF equilibrium

solubility values for the measured drugs (**supplementary tables 3 and 4**) are plotted in Figs. 1 and 2. The data are taken from 23 published literature studies and span a single drug value, to a maximum of eight values from four studies for a single drug. The data sets are not balanced (FaHIF 84 values vs Fa9SIF 207 (23x9), FeHIF 45 vs Fe9SIF 180 (20x9)) reflecting issues associated with Fa/FeHIF availability, study drug choices and the multiple research groups performing the research.

3.2. Human intestinal fluid experimental protocols

The Fa/FeHIF collection and solubility measurement protocols vary (**supplementary tables 3 and 4**) with potential to influence the SIF vs HIF comparison. The duodenum and jejunum predominate as a sampling location and HIF compositional data indicates minimal differences between these sites [23], although FeHIF comparisons are limited. Subject ages range from 18 to 49 in the fasted and 45 in the fed, with an average study span of 16 and 19 years respectively. Age effects on HIF solubility have been investigated [24] and although samples exhibited a high inter-individual variability, specific age-dependency was not observed. The study utilized to calculate Fa/Fe9SIF [5], sampled from the duodenum with an age range from 18 to 31, parameters consistent with the Fa/FeHIF protocols.

The average subject number per HIF measurement is 10 in the fasted state and 11 in the fed state, with a range of 4 to 20 and sample pooling in 63 % of FaHIF and 74 % of FeHIF measurements. Where Fa/FeHIF samples are not pooled there will be solubility variation due to inter and intra individual compositional variability [7]. Pooling will mitigate variability dependent upon number of samples, but pools will have an unknown composition. Fa/Fe9SIF is based on 20 volunteers and 324 samples comparable to the Fa/FeHIF protocols, but due to the variability unlikely to be identical especially for un-pooled and low number pooled measurements. Seventy nine percent of the fed state protocols use Ensure Plus™ as a standard meal with a mean collection time of 110 min starting on average 10 min after Ensure administration. This is comparable to the study utilized to calculate Fe9SIF [5], although differences in sampling duration (90 min vs 270) for some studies may have an impact [6].

Solubility measurement protocols are consistent with incubation at 37 °C, equilibration time of ≥ 24 h and separation of undissolved drug prior to analysis. In one case, room temperature was applied, which will have a minor impact on solubility [25]. Studies indicate that for slowly dissolving drugs to attain equilibrium solubility requires twelve hours [12] and only 3 h for soluble drugs. One study utilized a 3 h incubation time and it is noticeable that the poorly soluble drugs (phenytoin and itraconazole) exhibit low solubility compared with Fa/Fe9SIF, whilst the soluble (furosemide and dipyridamole) do not. Drug solid form has not been uniformly assessed, which could impact solubility; for atazanavir for example only the crystalline equilibrium solubility values have been utilized.

3.3. Summary

Since the literature Fa/FeHIF solubility data arise from 23 different studies it is inevitable that there will not be absolute consistency between experimental protocols. This will produce variability that can impact the comparison and two main sources can be identified the Fa/FeHIF sampling protocol and the solubility determination in the sampled fluid.

The Fa/Fe9SIF media were calculated to cover 95 % [7] of the compositional variability of a Fa/FeHIF data set taken from 20 volunteers [5]. Literature information on the composition of HIF samples and the impact of sample pooling is limited as well as the potential impacts of changing physiological factors such as sampling site and volunteer status. This issue is further discussed in section 3.2.1 for a Fa/FeHIF study which includes compositional data. The fasted state, as a resting state is likely to exhibit greater compositional consistency than the fed state which will be more dynamic as digestion and intestinal transit occurs [26]. With the additional complication for the fed state of the nature of the meal ingested. The solubility determination protocol is generally consistent as discussed above.

The analysis indicates that although Fa/Fe9SIF were calculated to cover 95 % of Fa/FeHIF compositional space, the solubility comparison limits should be relaxed to allow for the multiple issues discussed above. Irrespective of the comparison and variability problems, realistically the approach applied is all that is possible due to the inherent issues associated with the literature results.

3.4. Comparison of solubility data sets

Previous SIF solubilities are not normally distributed [17] therefore non-parametric statistical comparison is required. There are seventeen fasted drugs and seven fed with three or more Fa/FeHIF values; comparison of prandial groups (Wilcoxon matched by drug pairs test) calculates no significant solubility difference between FaHIF and Fa9SIF or between FeHIF and Fe9SIF (Fig. 1a and Fig. 2a). When drugs are compared individually (Mann-Whitney test) there is no significant

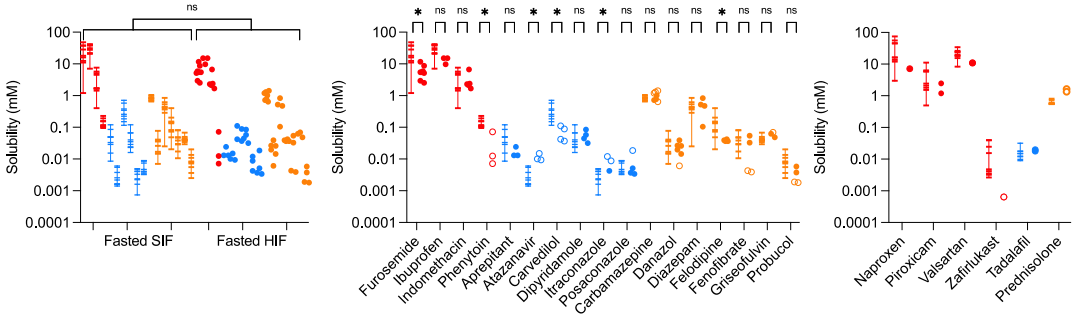

Fig. 1. Comparison plots of Fasted Equilibrium Solubility Values 9 media (Fa9SIF) and literature Fasted Human Intestinal Fluid (FaHIF). Fig. 1a. Drugs with 3 or more FaHIF solubility values. – 9 media, ●FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; ns – no significant difference between media (Wilcoxon matched pairs signed rank test, P = 0.1202 (Two Tailed)(Pairing significantly effective P < 0.0001 (One Tailed) Spearman value = 0.9167)); drug order as per Fig. 1b. Fig. 1b. Drugs with 3 or more FaHIF solubility values. – 9 media, ●FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range; ns – no significant difference between media, * P < 0.05. Mann-Whitney comparison individual P values, furosemide = 0.0079; ibuprofen = 0.0636; indomethacin 0.6993; phenytoin = 0.0091; aprepitant = 0.0955; atazanavir = 0.0091; carvedilol = 0.0028; dipyridamole = 0.414; itraconazole = 0.0364; posaconazole = 0.9399; carbamazepine = 0.351; danazol = 0.607; diazepam = 0.7105; felodipine = 0.0182; fenofibrate = 0.3301; griseofulvin = 0.0636; probucol = 0.0503. Fig. 1c. Drugs with less than 3 FaHIF solubility values. – 9 media, ●FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

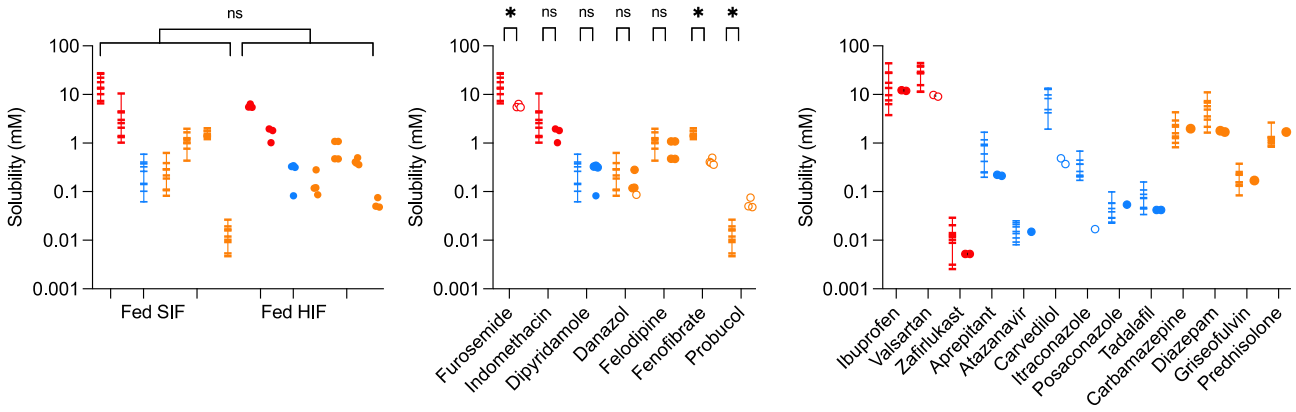

Fig. 2. Comparison plots of Fed Equilibrium Solubility Values 9 media (Fe9SIF) and literature Fed Human Intestinal Fluid (FeHIF). Fig. 2a. Drugs with 3 or more FeHIF solubility values. – 9 media, ●FeHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; ns – no significant difference between media (Wilcoxon matched pairs signed rank test, P = 0.0781 (Two Tailed)(Pairing significantly effective P < 0.014 (One Tailed) Spearman value = 0.9643)); drug order as per Fig. 2b. Fig. 2b. Drugs with 3 or more FeHIF solubility values. – 9 media, ●FeHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range; ns – no significant difference between media, *P < 0.05. Mann-Whitney comparison individual P values, furosemide = 0.0091; indomethacin 0.2091; dipyridamole > 0.9999; danazol = 0.4140; felodipine = 0.2601; fenofibrate = 0.0028; probucol = 0.0091. Fig. 2c. Drugs with less than 3 FeHIF solubility values. – 9 media, ●FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

difference for 11 out of 17 drugs between FaHIF and Fa9SIF (Fig. 1b) and for 4 out of 7 drugs between FeHIF and Fe9SIF (Fig. 2b). The fasted felodipine difference is due to the narrow FaHIF solubility distribution a result possibly due to FaHIF pooling. Furosemide displays a similar behavior but this cannot be attributed to pooling.

For felodipine and furosemide the statistical difference is not significant, FaHIF values within Ha9SIF range, based on this study's aim. Therefore, for fasted 76 % (82 % if the phenytoin result is excluded due to the lower equilibration time) and in the fed 57 % of the drugs with ≥ 3 HIF solubility values there is no individual significant solubility difference between Fa/Fe9SIF and Fa/FeHIF. Comparison of individual FaHIF solubility values with the Fa9SIF range (Fig. 1b/c) indicates that 68 % are within the boundaries and in the fed state the value is 64 % (Fig. 2b/c). One study correlated ten poorly soluble drugs in three different FaSIF media and where a comparison to FaHIF is presented 48 % were within the range [11]. Fa9SIF agreement is higher, expected based on the range coverage compared to individual FaSIF media, although the difference between the studies and drugs examined impacts this comparison.

3.4.1. *Impact of composition on solubility behaviour*

Comparison of solubility behavior determined in fasted [12] and fed [13] state DoE studies reinforces the correlation discussed above. Fa9SIF media composition has minimal impact on carbamazepine solubility [21] a feature that is present for FaHIF solubility values from five studies (Fig. 1b). One study [27] analysed HIF pooled from 16 volunteers for pH, bile salt and phospholipid, three of the five Fa/Fe9SIF components or dimensions, which can be compared with Fa/Fe9SIF values [7]. The pooled fasted bile salt (FaHIF 3.52 mM vs Fa9SIF 1.06–11.45 mM)/phospholipid (0.16 mM vs 0.19–2.48 mM) ratio is low compared to Fa9SIF (Fig. 3a), whilst the bile salt/pH (6.83 vs 5.72–8.04) ratio is in the centre. The pooled fed state pH (FeHIF 5.96 vs Fe9SIF 5.86–6.59)/bile salt (8.91 mM vs 4.94–19.04 mM) ratio (Fig. 3b) is low when compared to Fe9SIF range whilst the bile salt/phospholipid (3.72 mM vs 2.07–7.94 mM) ratio is in the centre. For acidic drugs pH is the major solubility driver [12,13,21,22] hence in the fasted comparisons ibuprofen and valsartan are equivalent, zafirlukast requires bile salt and phospholipid solubilization [12], which in the pooled FaHIF are low and could be linked to the low solubility value. In the FeHIF pool this is reversed where valsartan solubility is low due to the low pH but zafirlukast is equivalent due to the "normal" bile salt phospholipid concentrations. Probucol requires monoglyceride for solubility [13] a component not in Fe9SIF but present in the pooled FeHIF [5], potentially explaining the higher solubility.

These examples illustrate the issue of reconciling different drugs' solubility behavior in media of defined against unknown composition. The results indicate that increasing the number of HIF values increases compositional coverage and provides a greater chance of agreement with Fa/Fe9SIF, multiple drugs have solubility values outside the Fa/Fe9SIF range but this is not statistically significant (Figs. 1 and 2). Highlighting that the single value comparison is a stringent test and multiple value comparisons provide greater coverage. This implies that a larger HIF composition data set is required to improve the analysis using more or different dimensions [7], and that HIF solubility measurement should be linked to chemical composition [27]. This latter modification would permit a systematic comparison of HIF and SIF solubility.

3.5. *Solubility correlation boundary*

To extend the literature Fa/FeHIF comparison for the drugs measured using Fa/Fe9SIF, upper and lower correlation boundaries have been calculated based on the minimum and maximum solubility values (x_{min},y_{max}; x_{max},y_{min} where min or max represents the Fa/Fe9SIF minimum and maximum solubilities) and plotted graphically (Fig. 4a). The acidic and basic drug correlations are statistically significant and for neutral drugs in the fed state but not the fasted, this is not critical since the relationship defines a boundary with a span equal to the average solubility range for each drug category. The boundaries shape reflects drug category solubility behavior previously determined by DoE studies [12,13]. Acidic drug solubility is pH driven and the similarity of pH ranges between Fa9SIF (5.72 – 8.04) and Fe9SIF (5.97 – 6.59) leads to contiguous boundaries with fed (lower pH range) inside the fasted. Basic and neutral drug solubility is driven by pH and total amphiphile content (pH x TAC) and the difference between Fa9SIF (15.1 – 122.4) and Fe9SIF (109.1 – 493.1) is reflected in the boundaries. The boundary changes between fasted and fed states for these drug classes is indicative of solubility changes between fasted and fed states and the presence of a food effect, see next section.

An additional literature [9] Fa/FeSIF vs Fa/FeHIF solubility data set of 66 values for 25 drugs has been plotted with the boundaries (Fig. 4b) and 95 % are inside. This is a first exploration of this relationship and reinforces the statistical conclusion that Fa/Fe9SIF provide an in vitro in

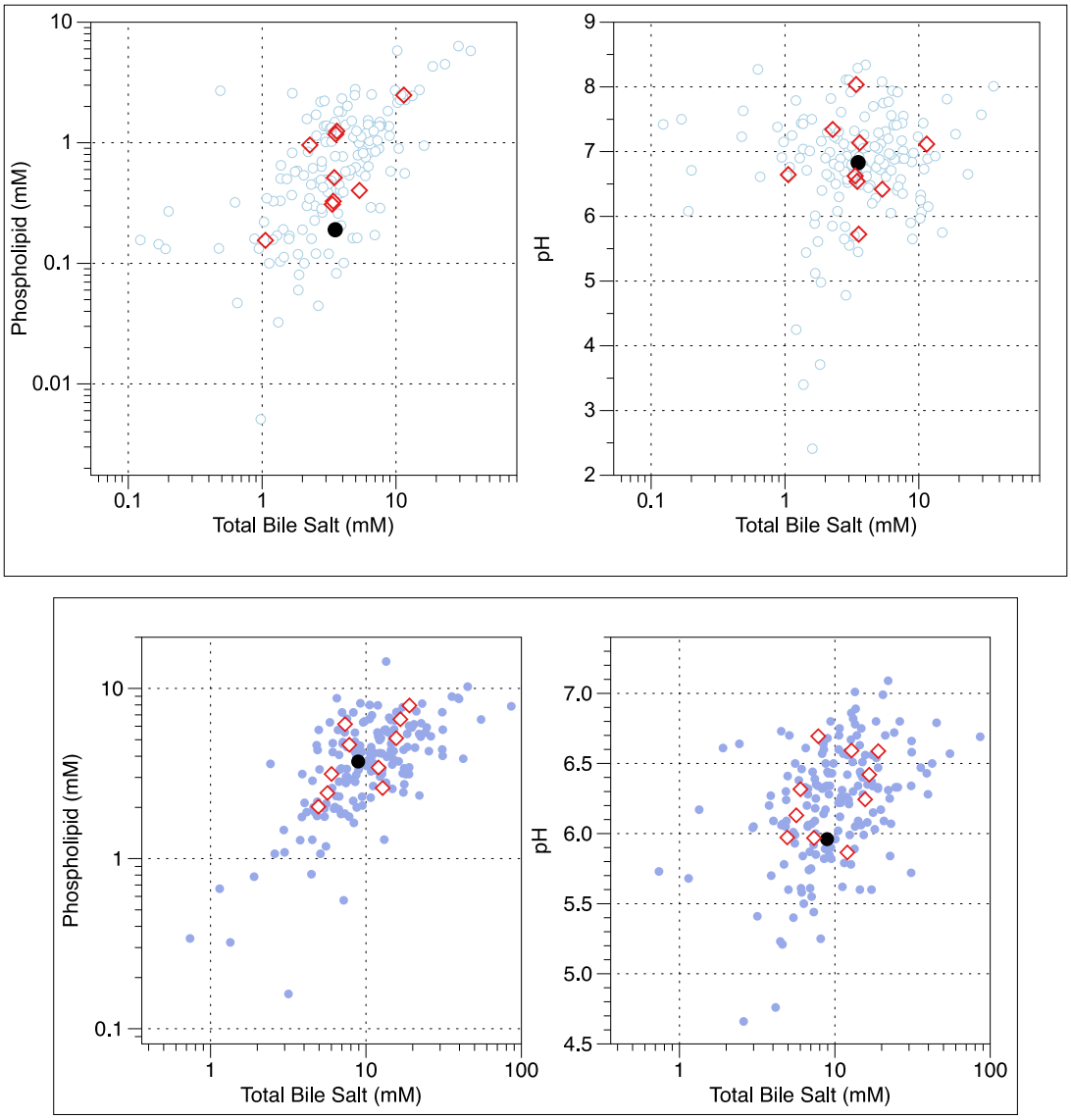

Fig. 3. Compositional comparison Fa/Fe9SIF and sampled pooled FaHIF. Fig. 3a. Dahlgren Pooled FaHIF Composition vs FaHIF Data Set and Fa9SIF Composition. ○Bile salt, phospholipid and pH individual sample values from Pyper[7]. Fa9SIF ◇nine media points; ●Dahlgren[27] pooled FaHIF values. Fig. 3b. Dahlgren Pooled FeHIF Composition vs FeHIF Data Set and Fe9SIF Composition. ●Bile salt, phospholipid and pH individual sample values from Pyper[7]. Fe9SIF ◇nine media points; ●Dahlgren[27] pooled FeHIF values.

vivo solubility correlation, but should be treated with caution. A wide enough boundary will accommodate any data, especially if centered on the equivalence line around which correlation is unavoidable. In addition, the boundary is based on the study drugs which may not be a representative sample.

3.6. Potential biopharmaceutical application

The DCS [2] applies a single poorly characterised Fa/FeHIF or Fa/FeSIF solubility measurement to evaluate a drug's potential biopharmaceutical performance. Fa/Fe9SIF is an advance by providing a bioequivalent solubility range (see above) linked to an intestinal solubility population distribution, which can be applied to provide DCS [19,20] boundary limits. Absorption depends on the solubility, intestinal permeability interplay (other issues e.g. first pass metabolism are not considered in this paper), which along with intestinal transit time and surface area can be utilized to calculate a Solubility Limited Absorbable Dose (SLAD) [2]. The utility of a bioequivalent Fa/Fe9SIF solubility range can be visualized by calculating the Dose/SLAD ratio and plotting against the intestinal solubility population distribution (Fig. 5).

Dose/SLAD < 1, indicates that intestinal equilibrium solubility, permeability and transit time is sufficient to permit complete absorption and the highest value for ibuprofen provides a > 10 fold solubility excess or safety factor. Dose/SLAD > 1, indicates that intestinal solubility, permeability and transit time is not sufficient to permit complete absorption and for griseofulvin that the maximum solubility deficit is > 10 fold. This provides a performance level, supersaturated concentration and time relationship, for formulation strategies, for example amorphous systems [28], to ensure complete absorption. The Fa9SIF, Fe9SIF griseofulvin Dose/SLAD curves, indicate that there is a fed state induced solubility difference and since the curves do not overlap is detecting in vitro the known griseofulvin food effect [29]. Other drugs also display this phenomenon (Fig. 4a, e.g dipyridamole) indicating that this result is worthy of further examination for the in vitro detection of solubility based food effects.

Fa/Fe9SIF display structured solubility distributions that permit identification of the minimum and maximum solubility media for the drug categories [21,22]. This permits a pick-n-mix, drug development

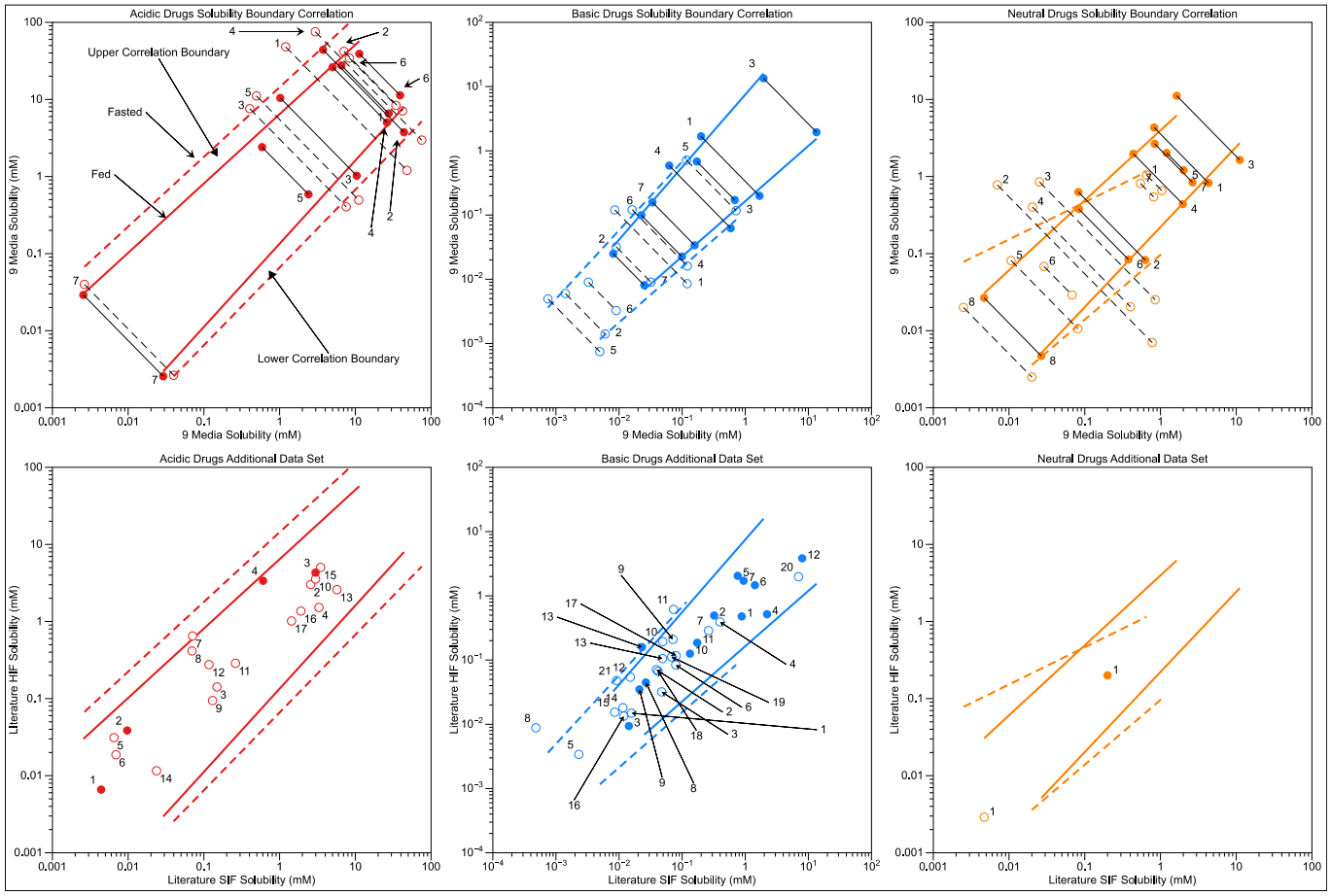

Fig. 4. Solubility Boundary Correlation. Fig. 4a. Solubility boundary correlation – Upper Panel. Acidic, basic and neutral, fasted and fed, upper and lower solubility correlation boundaries based on the minimum and maximum solubility for individual drugs (see numbers) in each Fa/Fe9SIF state plotted as x_{min},y_{max} and x_{max},y_{min} (fasted open symbol, fed closed symbol, fasted drug points connected by dashed black line, fed solid black line); best fitting power correlation line ($y = A\ x^B$) (fasted – dashed coloured line; fed – solid coloured line). Acidic Drugs 1- Furosemide, 2-Ibuprofen, 3-Indomethacin, 4-Naproxen, 5-Piroxicam, 6-Valsartan, 7-Zafirlukast. Basic Drugs 1-Aprepitant, 2-Atazanavir, 3-Carvedilol, 4-Dipyridamole, 5-Itraconazole, 6-Posaconazole, 7-Tadalafil. Neutral Drugs 1-Carbamazepine, 2-Danazol, 3-Diazepam, 4-Felodipine, 5-Fenofibrate, 6-Griseofulvin, 7-Prednisolone, 8-Probucol. Acidic Drugs Lower Correlation Boundary: Fasted y = 0.066013*xˆ1.009, R^2 = 0.9122, P = 0.0008; Fed y = 0.13456*xˆ1.0792, R^2 = 0.9707, P < 0.0001. Upper Correlation Boundary: Fasted y = 14.389*xˆ0.90412, R^2 = 0.9122, P = 0.0008; Fed y = 6.4331*xˆ0.89946, R^2 = 0.8995, P < 0.0001. Basic Drugs Lower Correlation Boundary: Fasted y = 0.11225*xˆ0.86083, R^2 = 0.9200, P = 0.0006; Fed y = 0.16457*xˆ0.8606, R^2 = 0.9716, P = < 0.0001. Upper Correlation Boundary: Fasted y = 7.9449*xˆ1.0687, R^2 = 0.9200, P = 0.0006; Fed y = 7.4946*xˆ1.1289, R^2 = 0.9716, P < 0.0001. Neutral Drugs Lower Correlation Boundary: Fasted y = 0.096135*xˆ0.84039, R^2 = 0.4058, P = 0.0894; Fed y = 0.22133*xˆ1.0385, R^2 = 0.9420, P < 0.0001. Upper Correlation Boundary: Fasted y = 1.41*xˆ0.48289, R^2 = 0.4058, P = 0.0894; Fed y = 3.9606*xˆ0.90705, R^2 = 0.9420, P < 0.0001. Fig. 4b. Additional literature data comparison. Acidic, basic and neutral, fasted and fed, upper and lower solubility correlation boundaries based on the minimum and maximum solubility for individual drugs in each Fa/Fe9SIF state Fasted open symbol, fed closed symbol, Fasted – dashed coloured line; Fed – solid coloured line. Additional solubility data from [9]. Fasted – open symbols, Fed – closed symbols. Acidic Drugs Fasted 1-Atovaquone, 2-Diclofenac, 3-Diethylstilbestrol, 4-Flufenamic acid, 5&6-Glibenclamide, 7, 8&9-Glipizide, 10-Hydrochlorothiazide, 11-Irbesartan, 12-Nimesulide, 13-Probenecid, 14-Rimonabant, 15&16-Sulfasalazine, 17-Warfarin. Acidic Drug Fed 1-Glibenclamide, 2-Glipizide, 3-Hydrochlorothiazide, 4-Sulfasalazine. Basic Drugs Fasted 1&2-AZD0865, 3-Cinnarizine, 4-Darunavir, 5-Etravirine, 6-Indinavir, 7-Irbesartan, 8-Itraconazole, 9,10,11,12&13-Ketoconazole, 14,15&16-Loviride, 17,18&19-Nifedipine, 20-Quinidine, 21-Ritonavir. Basic Drug Fed 1-Cinnarizine, 2-Darunavir, 3-Etravirine, 4-Indinavir, 5,6&7-Ketoconazole, 8&9-Loviride, 10&11-Nifedipine, 12-Quinidine, 13-Ritonavir. Neutral Drugs Fasted 1-Cyclosporine. Neutral Drugs Fed 1-Cyclosporine.

stage or requirement based approach for intestinal solubility measurement [4]. A total intestinal solubility range screen can be assessed with two measurements, both prandial states with four, providing assessment of potential food effect and eighteen to provide the full assessment.

4. Conclusions

The in vitro in vivo comparison of intestinal solubility is in principle simple but confounded by multiple factors associated with HIF's natural variability and limited availability. Twenty three drugs are not a comprehensive or structured sample and arises due to published study choices, which limits comparison. This could be ameliorated by targeting additional drugs with multiple Fa/FeHIF (≥3) measurements or optimally a compositional assessment of Fa/FeHIF prior to solubility measurement.

Statistical comparison does not detect a significant solubility difference between Fa9SIF and FaHIF or Fe9SIF and FeHIF data sets. The result indicates that the Fa/Fe9SIF solubility range can be considered bioequivalent to Fa/FeHIF. A novel comparison based on solubility boundaries encompasses 95 % of an additional solubility data set, further reinforcing the statistical conclusion of in vitro in vivo correlation. Solubility differences and behavior can be linked to SIF DoE study results and the influence of media components, indicating that further intestinal fluid composition assessment can refine the approach delivering the potential to measure in vitro intestinal solubility in multiple population and patient groups or species.

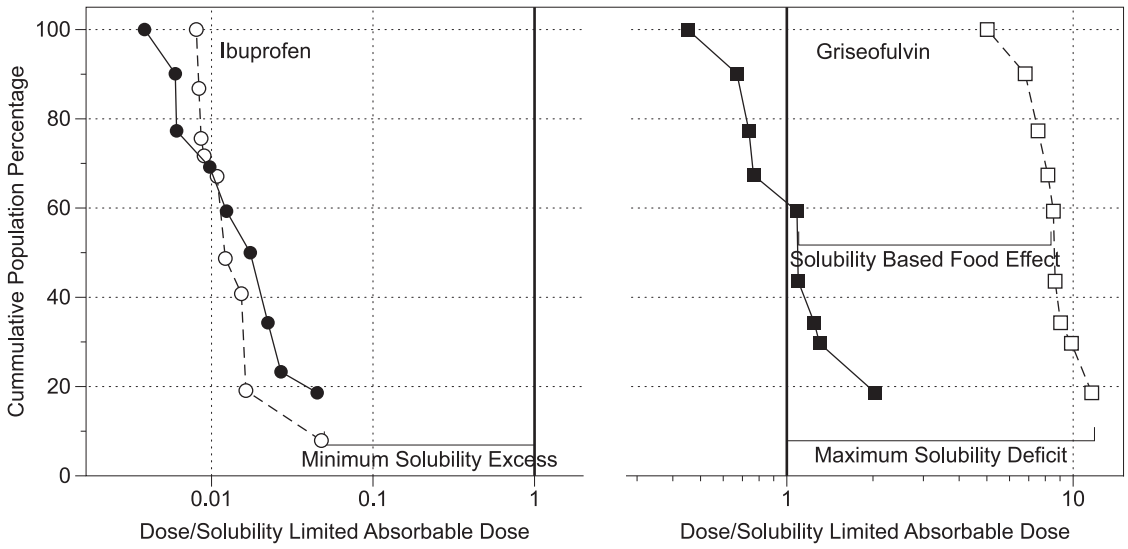

Fig. 5. Biopharmaceutical analysis. Ibuprofen Fasted ○; Fed ●Dose = 400 mg: Griseofulvin Fasted□; Fed ■Dose = 500 mg.

An in vitro bioequivalent solubility range measurement incorporating population distribution information [19,20] expands DCS [2] approaches to biopharmaceutical performance assessment. A novel graphical analysis utilising the administered dose divided by the solubility limited absorbable dose permits the calculation of drug and dose related solubility safety margins, formulation performance requirements and potential solubility based food effects. Since equilibrium solubility [1] is a key parameter controlling oral absorption an in vitro bioequivalent measurement can be applied to refine PBPK [30] and in silico modelling with potential to generate individual or disease related intestinal solubility profiles and reduce in vivo testing. The Fa/Fe9SIF system is therefore worthy of further investigation with linkage of system results to in vivo performance a key next stage and may also represent a methodology applicable to other multicomponent biological fluids where no single component is responsible for performance.

CRediT authorship contribution statement

Qamar Abuhassan: Writing – original draft, Data curation. **Maria Inès Silva:** Writing – original draft, Data curation. **Rana Abu-Rajab Tamimi:** Data curation. **Ibrahim Khadra:** Supervision. **Hannah K. Batchelor:** Supervision. **Kate Pyper:** Formal analysis, Conceptualization. **Gavin W. Halbert:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2024.114302.

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