Small scale design of experiment investigation of equilibrium solubility in simulated fasted and fed intestinal fluid.

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Abstract

It is widely recognised that drug solubility within the gastrointestinal tract (GIT) differs from values determined in a simple aqueous buffer and to circumvent this problem measurement in biorelevant fluids is determined. Biorelevant fluids are complex mixtures of components (sodium taurocholate, lecithin, sodium phosphate, sodium chloride, pancreatin and sodium oleate) at various concentrations and pH levels to provide systems simulating fasted (FaSSIF) or fed (FeSSIF) intestinal media. Design of Experiment (DoE) studies have been applied to investigate FaSSIF and FeSSIF and indicate that a drug's equilibrium solubility varies over orders of magnitude, is influenced by the drug type and individual or combinations of media components, with some of these interactions being drug specific. Although providing great detail on the drug media interactions these studies are resource intensive requiring up to ninety individual experiments for FeSSIF. In this paper a low sample number or reduced DoE system has been investigated by restricting components with minimal solubility impact to a single value and only investigating variations in the concentrations of sodium taurocholate, lecithin, sodium oleate, pH and additionally in the case of fed media, monoglyceride. This reduces the experiments required to ten (FaSSIF) and nine (FeSSIF). Twelve poorly soluble drugs (Ibuprofen, Valsartan, Zafirlukast, Indomethacin, Fenofibrate, Felodipine, Probucol, Tadalafil, Carvedilol, Aprepitant, Bromocriptine and Itraconazole) were investigated and the results compared to published DoE studies and literature solubility values in human intestinal fluid (HIF), FaSSIF or FeSSIF. The solubility range determined by the reduced DoE is statistically equivalent to the larger scale published DoE results in over eighty five percent of the cases. The reduced DoE range also covers HIF, FaSSIF or FeSSIF literature solubility values. In addition the reduced DoE provides lowest measured solubility values that agree with the published DoE values in ninety percent of the cases. However, the reduced DoE only identified single and in some cases none of the major components influencing solubility in contrast to the larger published DoE studies which identified multiple individual components and component interactions. The identification of significant components within the reduced DoE was also dependent upon the drug and system under investigation. The study demonstrates that the lower experimental number reduces statistical power of the DoE to resolve the impact of media components on solubility. However, in a situation where only the solubility range is required the reduced DoE can provide the desired information, which will be of benefit during in vitro development studies. Further refinements are possible to extend the reduced DoE protocol to improve biorelevance and application into areas such as PBPK modelling.

Keywords

Fasted State Simulated Intestinal fluid; Fed State Simulated Intestinal Fluid; Orbito; Solubility; Design of Experiment; Biopharmaceutical Classification System

1. Introduction

Poorly soluble drugs (Biopharmaceutical Classification System (BCS) Class II and IV (Amidon et al., 1995)) represent the majority of newly developed molecules over the last twenty years linked to the introduction of high throughput screening systems (Lipinski, 2000). Due to this shift the pharmaceutical industry is constantly striving to facilitate new formulation techniques that can aid the development of compounds with low aqueous solubility (Savjani et al., 2012) while also developing new in-vitro methods that further inform on the gastrointestinal solubility of these compounds (Lennernas et al., 2014).

Gastrointestinal tract (GIT) media contains secretions including bile salt, products of digestion and electrolytes, which influence buffer capacity induce pH variations along the length of the tract and can induce a number of other changes within the tract, which may influence drug solubility (Dressman and Reppas, 2000). These factors within the gastrointestinal environment have been shown to enhance the solubility profile of a drug beyond that predicted during basic solubility studies in simple buffer or acid solutions (Sunesen et al., 2005). In particular, the composition of the primary bile components, bile salt and lecithin, plays an important role in solubility through the formation of mixed micelles (Nielsen et al., 2001). The relationship between bile salts and lecithin is further pronounced in the fed state where concentrations are higher due to, bile release, food ingestion and the presence of lipid digestion products.

Human Intestinal Fluid (HIF) would be the fluid of choice to study drug solubility in the GIT and its subsequent effect upon oral bioavailability; however, sufficient fluid is difficult to obtain, the mixtures are unstable due to loss of CO_2 and as such this approach is not a feasible option in the routine course of drug solubility studies (Kleberg et al., 2010; Reppas and Vertzoni, 2012). A recent publication identified updated concentration ranges in fasted and fed state HIF with Bile salt concentrations ranging from 0.03 to 36.18mM in fasted state and 0.74 to 86.14mM in fed state and phospholipid ranged from 0.01 to 6.33mM in fasted state and 0.6 to 14.39mM in fed state which correspond with previously published literature values (Riethorst 2016, Bergstrom 2014, Fuchs 2014).This same study identified FFA ranges from 0 to 12.67mM in fasted state and 2.1 to 58.7mM in fed state while MAG ranged from 0 to 3.30mM in fasted state and 0 to 34.4mM in fed state. It should be noted that these values include extreme values and outliers. Simulated intestinal fluids (SIF) to mimic gastrointestinal fluid have been used extensively in the last two decades to aid in vitro drug development and formulation studies (Markopoulos et al., 2015; Stappaerts et al., 2014) with varied constituents and compositions based around current literature values. Recent work within our group has focused on the investigation of gastrointestinal solubility of BCS Class II compounds through the use of

simulated fluids in statistical Design of Experiment (DoE) style investigations. This allows media components such as bile salt, pH, oleate, lecithin and mono-glyceride concentrations to be systematically examined to provide an overall view of the individual component's role and interactions within a compound's solubility envelope (Ainousah et al., 2017; Khadra et al., 2015; Perrier et al., 2018; Zhou et al., 2017b). Myers (Myers et al., 2009) described a DoE system as being able to investigate the effects of numerous variable factors to be studied in one single experiment leading to enhanced understanding of the system in a time and consumable efficient manner. Initially, DoE experiments were undertaken with the level of components varied in line with literature values (Bergstrom et al., 2014) with the first DoE study (Khadra et al., 2015) investigating the fasted state environment, whilst the second study (Zhou et al., 2017b) examined the fed state. Although these studies provided novel, interesting and valuable results a subsequent study was carried out to combine both the fasted and fed studies with a reduced experimental load (Perrier et al., 2018) using a 1/8 fraction factorial DoE. This approach reduced the number of samples required for the DoE however, differentiation could no longer be observed between the fasted and fed states leading to the design of a dual level DoE (Ainousah et al., 2017). This was a customised 1/8 fraction factorial design incorporated into the DoE approach described in the Perrier publication, which reduced samples to 32 individual experiments and allowed for results to distinguish between fasted and fed environments. An alternative approach looked to assess the influence of SIF composition on the solubility of BCS class II compounds utilising a DoE with reduced media parameters (Madsen et al., 2017), which also allows for a smaller number of experiments. However, a recent solubility investigation of SIF composition applying an alternate four component mixture design statistical technique (Dunn et al., 2019) indicates that solubility variability is inversely related to the number of amphiphilic components present. Implying that the balance between media component numbers and experimental data point numbers will be important.

Evaluation of all previous DoE protocols alongside pharmaceutical industry requests led to a desire to construct a more concise DoE that further reduced the number of individual experiments required whilst still allowing analysis of component variation within SIF. This study describes a new statistical investigation of equilibrium solubility of oral drugs in fasted (Table 1) and fed (Table 2) SIF using a reduced dual level DoE. The current industrial standard for SIF in solubility studies is either fasted simulated intestinal fluid (FaSSIF) or fed simulated intestinal fluid (FeSSIF) (Fuchs et al., 2015) and as such they have been included as centre points for analysis within this DoE. The resultant DoE comprises of 10 experiments in the fasted state study and 9 in the fed state giving a greatly reduced overall number of experiments, while still allowing the study of five parameters of interest (bile salt, lecithin, fatty

acid, mono-glyceride and pH). Buffer and salt were held at constant values as shown in Table 1 and 2 following previous experiments (Perrier et al., 2018; Zhou et al., 2017b) that showed both these constituents to have no significant solubility impact. The equilibrium solubility of 12 BCS class II drugs was studied: 3 acids (indomethacin, ibuprofen and valsartan), 5 bases (itraconazole, tadalafil, carvedilol, aprepitant and bromocriptine), 3 neutral drugs (fenofibrate, felodipine and probucol), and finally zafirlukast, which behaves in this system as an acid due to its pK_a value of 4 (Madsen et al., 2016; Teague and Valko, 2017).

2. Materials and methods

2.1. Materials

Sodium taurocholate (NaTC), ammonium formate, sodium chloride (NaCl), chloroform, formic acid, monosodium phosphate (NaH2PO4), fenofibrate, indomethacin, itraconazole, bromocriptine, sertraline, valsartan and ibuprofen were purchased from Sigma Aldrich Poole, Dorset UK. Lecithin S PC (phosphatidylcholine (PC) from Soybean "98%") was purchased from Lipoid. Glycerol mono oleate (GMO) was obtained from CRODA Healthcare. The active pharmaceutical ingredients felodipine, probucol, aprepitant, tadalafil, carvedilol and zafirlukast were provided through OrBiTo by Dr. R. Holm Head of Preformulation, Lundbeck, Denmark. Table 3 outlines the physicochemical properties of the selected compounds. Sodium oleate (SO) was obtained from BDH Chemical Ltd. Poole England. FaSSIF V1 and FeSSIF V2 were purchased from Biorelevant.com. The analytical solvents methanol and acetonitrile were of HPLC grade (VWR, UK). All water was ultrapure Milli-Q water.

2.2. Design of experiment and data analysis

A fully customised design of experiment with 4 or 5 factors (fasted or fed with either a component concentration or a system parameter such as pH) and three levels (low, mid and high) was constructed using Minitab®17.2.1. The selection of the different factors used in the design of the experiment were based on a survey of published literature of simulated fasted media variants (Vertzoni 2004, Marques 2011, Jantratid 2008 a, b, Ilardia-Arana 2006, Kleberg 2010, Pederson 2000, Soderlind 2010, Sunesen 2005) and our own previous DOE results. Minitab generated 10 different experiments for fasted and 9 different experiments for fed using various combinations and levels of the 4 or 5 factors as shown in Table 1 and Table 2 (no centre point and no replicate) at either low, high or mid-levels based upon previous literature reported values (Khadra et al., 2015). This design permits the analysis of the impact on solubility of individual factor effects but does not permit the analysis of 2-way or higher factor interactions due to the design of this DOE and its limited number of experiments. The Mann-Whitney test was used in Minitab® to evaluate differences between two data sets.

2.3. Equilibrium solubility measurements

2.3.1 Preparation of stock solutions for Fasted media experiments and FaSSIF.

Sodium oleate (SO) (73mg) was added to a 5 mL flask under gentle heat, to aid dissolution and made to final volume with water, the solution was then kept at 50°C to aid solubilisation. Bile salt (NaTC, 238mg) was added to a 5 mL flask and made up to final volume with water. Phospholipid (PC)(59mg) was dissolved with a few ml of chloroform and dried under a stream of nitrogen then reconstituted with water to the final volume of 5 mL. Buffer (NaH2PO4,

294mg) was added to a 5 mL flask and made up to final volume with water. Salt (NaCl, 464mg) was added to a 5 mL flask and made up to final volume with water. Stock solutions were designed to be 15 times greater than the highest DoE concentration levels requiring the addition of a fixed volume in each tube as indicated in the supplementary material in supplementary table 1.

To prepare FaSSIF V1, buffer (NaH2PO4, 40mg) was added to NaCl (60mg) in 10 mL flask with 8mL of water, pH was adjusted to 6.5 and FaSSIF V1 powder (20mg) was added, stirred until completely dissolved and made up to final volume with water.

2.3.2 Preparation of stock solutions for Fed media experiments.

NaTC, PC and GMO were weighed into a flask with a few mL of chloroform and stirred to dissolve all the solid material. Chloroform was evaporated off with a stream of nitrogen gas to ensure a dry film is produced. Water (3mL) was added to the dried film and stirred to prepare a homogeneous mixture then made to final volume. The stock solutions have been designed to be 15 times greater than the highest concentration levels, requiring the addition of a fixed volume in each tube as indicated in supplementary material table 2.

2.3.3 Preparation of sodium oleate, buffer, salt and FeSSIF V2 in Fed media.

Buffer (NaH2PO4 4.7g) was added to a 50mL flask and made to final volume with water. Salt (NaCl, 550mg) was added to a 5mL flask and made to final volume with water. Sodium oleate (1.49g) was added to a 10 mL flask under gentle heat to aid dissolution and made to final volume with water, the solution was then kept at 50°C to aid solubilisation. These stock solutions were designed to be 15 times greater than the highest concentration levels, requiring the addition of a fixed volume in each tube as indicated in supplementary material table 3.

To prepare FeSSIF V2, buffer, NaOH (0.03g) and maleic acid (0.06g) was added to NaCl (0.07g) and dissolved in about 8 mL of water. pH was adjusted to 5.8 and FeSSIF V2 powder (0.1g) was added. This was stirred until completely dissolved and made up to volume in 10 mL flask. FeSSIF V2 was chosen instead of V1 as this contained oleate and Glyceryl Mono Oleate and is therefore closest to our original DOE systems allowing more direct comparison.

2.3.4 Preparation of Individual Solutions

An excess of drug (approximately 10mg) above its solubility limit was added to all 15mL centrifuge tubes apart from Ibuprofen which had 20mg added due to higher solubility. The required amount of each stock solution and water was added to each of these tubes as shown in supplementary material table 3, to provide a final volume of 4 mL in the 15 mL centrifuge

tube and pH adjusted to 5, 5.8 or 7 using 0.1 M HCl or 0.1 M KOH (no more than 10% of the final volume was added during pH adjustment). Variation in opacity and emulsion like appearance is observed between tubes. Tubes were shaken for 1 hour at room temperature, pH re-adjusted if required and then placed in an orbital shaker for 24 hr at 37 °C and 240 rpm. Following incubation, the tubes were checked for the presence of solid drug, then centrifuged (13,000 rpm, 5 min) and the supernatant (500 µL) was sampled to determine the solubilised drug concentration by HPLC. The HPLC method has been previously validated to quantify the concentration of the drug of interest (Ref: Perrier 2018). Calibration curves were constructed for each drug and the subsequent equation of the line was used to quantify the drug concentration. Assays conditions are presented in Table 6.

For each drug it is possible to statistically compare the current DoE results in either the fasted or fed state with the corresponding published data providing a possible total of twenty four comparisons. However in four cases, ibuprofen, valsartan, bromocriptine, itraconazole in the fasted state comparable large scale DoE results were not available, reducing the number of possible comparisons to twenty. Due to the small number of data points within the reduced DoE and previous reports that DoE solubility distributions are not universally normally distributed (Ainousah et al., 2017) a non-parametric Mann Whitney comparison was performed. Where a statistically significant difference was observed this has been noted on the relevant figure along with the determined level of significance.

3 Results and Discussion

3.1 Solubility Range

The individual equilibrium solubility measurements from the reduced DoE under fasted and fed conditions for the twelve drugs tested are presented in Figures 1 to 12. Where available previously published data from the larger fasted (Khadra et al., 2015) and fed (Zhou et al., 2017b) DoE studies are plotted alongside as box and whisker plots, along with individual solubility measurements in the relevant simulated or sampled intestinal fluids (Augustijns et al., 2014).

A significant difference was detected in three out of the twenty available comparisons, ibuprofen and probucol in the fed state and tadalafil in the fasted state. In all other comparisons no statistically significant difference was detected indicating agreement between the DoE studies and determination of solubility ranges in eighty five percent of the cases.

For each drug there are also four potential comparisons against reported literature solubility values resulting from either a fasted or fed condition determined in either SIF or HIF media.

This provides a total of forty eight possible comparisons however, only twenty three appropriate literature solubility measurements were available and in twenty of these the reported solubility value lies within the range of the reduced DoE in the respective fasted or fed conditions. Only indomethacin, itraconazole and probucol have reported solubility values outside of the reduced DoE solubility range. This comparison indicates that in approximately eighty five percent of cases the reduced DoE is determining solubility values that are comparable to reported individual literature solubility data in either fasted or fed HIF or SIF.

3.2 Lowest Solubility Values

A key parameter of the original BCS system (Amidon et al., 1995) and modifications (Butler and Dressman, 2010; Rosenberger et al., 2018) is the dose/solubility ratio (Rinaki et al., 2003), with a lower value optimal for drug absorption. This implies that the lowest measured solubility is the critical value since this represents a worst case scenario for oral drug administration. A comparison of the measured lowest solubility values is presented in Table 7 as a ratio of either fasted or fed full DoE lowest value divided by the comparable reduced DoE lowest value. The measured solubility values will be intrinsically influenced by variation in the media component concentrations, ratios and ranges (Dunn et al., 2019) applied within the three DoE systems and they cannot be considered equivalent (see introduction). In addition, differing DoE designs (fasted: fractional factorial and fed: D-optimal) which are also not statistically equivalent have been applied. To allow for these experimental, statistical and concentration differences a ratio within a 10 fold variation (ie a ratio of between 10 to 0.1) has been arbitrarily applied as equivalence. Out of the twenty possible comparisons two or 10% are outside the 10 fold ratio and in all these cases the reduced DoE has a higher solubility value. The two cases are evenly split with one in the fasted state (probucol) and one in the fed state (bromocriptine). For the acidic compounds with the exception of zafirlukast in the fasted systems the ratio is very close to 1, indicating a close agreement between the full and reduced systems, probably related to the comparable pH values employed since pH is the main solubility driver for acidic compounds, especially in fasted media (Dunn et al., 2019), see solubility factor section. For basic and neutral compounds the spread of solubility ratios is greater, probably related to the greater impact of media components, concentrations and ratios (Ainousah et al., 2017; Dunn et al., 2019; Khadra et al., 2015; Zhou et al., 2017a, b) on the solubility of these compound categories in simulated media, see solubility factor section.

3.3 Solubility Factor Analysis

The DoE statistical analysis calculates if there is a relationship between individual media factors, either a component or condition (ie pH) and solubility and can determine if a positive or negative solubility effect is present. Only the significant factors for the reduced DoE are

presented in Table 8 with the significant factors from the published fasted (Khadra et al., 2015) and fed (Zhou et al., 2017b) DoE experiments for comparison. Non-significant factors are not included.

To determine whether the association between factor and solubility is statistically significant, the p-value is compared to the significance level. Standardized effect value is calculated through the Minitab statistical program and further information regarding this can be found within Mintab Support (https://support.minitab.com/en-us/minitab/18/help-and-how-to/modeling-statistics/doe/how-to/factorial/analyze-factorial-design/interpret-the-results/key-results/).

Overall including both fasted or fed, in the comparable cases the reduced DoE detected fourteen significant factors compared to sixty four for the published DoE studies. This can be further broken down to a fasted comparison (eight possible comparisons) where the reduced DoE found no significant factors for four drugs (Itraconazole, ibuprofen, aprepitant and bromocriptine) and eleven factors overall against the larger DoE with all drugs displaying at least one significant factor and a total of twenty eight for the eight drugs where data is available. In the fed state the reduced DoE found no significant factors for nine out of the twelve drugs and only three factors overall against the larger DoE with one drug displaying no significant factors and a total of thirty six for the remaining eleven drugs.

For acidic drugs the most significant individual factor identified in the original fasted (Khadra et al., 2015) and fed (Zhou et al., 2017b) DoE was pH with a pH dependent solubility split easily visible in the data plots especially in the fasted state (Figures 1 to 4). This can be seen as two clear groupings of points in the reduced DoE for indomethacin (pKa = 4.5), ibuprofen (pKa = 4.9) and zafirlukast (pKa = 4) but is not clear for valsartan (pKa = 3.9). pKa values were obtained from PubChem and drugbank unless specified above. This effect can be related to the drug's pKa, the pH of the DoE systems (Table 1 and 2, either pH 5 or 7) and the impact of ionisation on solubility. This is evident in Table 8 where pH is the most common significant factor in the reduced DoE detected in three out of four of the fasted systems but only one out of four for the fed. The reduced detection in the fed system may be due to the fact that increased amphiphile concentrations increase the overall solubility of acidic drugs overwhelming pH induced ionisation (Dunn et al., 2019).

For basic and neutral drugs a range of factors, pH and amphiphiles (bile salt, phospholipid, oleate and in fed state monoglyceride) had equivalent impact on solubility in the published

fasted (Khadra et al., 2015) and fed (Zhou et al., 2017b) DoE systems. For the basic drugs the reduced DoE did not detect a significant factor for three of the drugs in the fasted system and for all drugs in the fed system. Only tadalafil (pH) and carvedilol (bile salt and pH) in the fasted system provided significant factors with in both cases those factors detected in the published studies. For the three neutral drugs the pattern is slightly different with the reduced DoE detecting one significant factor in five out of the possible six cases (fasted and fed) with only probucol in the fed state not detecting a significant factor. In addition for the five cases the factor detected by the reduced DoE was either the primary or secondary factor detected by the published fasted (Khadra et al., 2015) and fed (Zhou et al., 2017b) DoE systems. The results indicate that for the neutral compounds a major significant factor is detected by the reduced DoE but that other factors with a reduced significance are not detected. For the basic compounds the reduced DoE fails to detect significant factors, especially in the fed state, which may indicate a specific issue related to the basic compounds and their solubilisation by the media or that in cases were all factors exert an equivalent solubilisation potential the reduced number of experimental points limits discrimination. In the case of weak bases, factors other than pH have a greater effect on their solubilisation such as pKa and their physicochemical properties.

The reduced DoE was also not powered to detect two way interactions between media factors (see methods section) and these are known to exist from the previous DoE studies (Khadra et al., 2015; Zhou et al., 2017b) along with three way interactions (Dunn et al., 2019).

The striking comparison is that the reduced DoE is not identifying an equivalent number of significant solubilisation factors to the previously published results, with only the most significant factors evident for either acidic or neutral drugs. For basic drugs the reduced DoE fails to detect any significant factors within the media when compared to the previously published studies. This lower detection or increased requirement for significance is most likely related to the lower number of experiments performed within the DoE reducing the statistical power of the method.

In over eighty percent of the cases examined within this reduced DoE study the measured equilibrium solubility range in either fasted or fed media is statistically equivalent to the solubility space previously measured in larger published DoE studies performed within our group (Khadra et al., 2015; Zhou et al., 2017b). In addition the measured lowest solubility value, which represent the highest risk during oral administration also agrees with the previously published DoE values. Although this latter comparison is based on an arbitrary solubility variation of a factor of 10 to allow for experimental, compositional and statistical differences between the studies. Finally, the measured solubility values also correlate well

with published literature solubility values (Augustijns et al., 2014) determined in either fasted or fed, sampled or simulated intestinal fluids. Therefore, the reduced DoE effectively measures the equilibrium gastrointestinal solubility space for the drugs tested and presumably for other similar drugs. In the fasted state, in most cases, the lowest solubility values are driven by the factors affecting solubility for each drug of interest as shown in table 8, for example in Tadalafil; pH, bile salt, lecithin and oleate are detected as factors affecting solubility in the full DOE which corresponds with the lowest solubility values found in both the reduced and full DOE in recipes containing the lowest levels of these factors. The same trend applies in the Fed state, whether reduced or full, for example Tadalafil solubility is driven by the factors influencing solubility, when these factors are present at low levels in the DOE recipes the solubility is comparatively low and when these factors are present in higher levels we see an increase in solubility as expected.

The ability of the reduced DoE to determine which of the media factors influence solubility is lower when compared to the previously published DoE studies performed within our group (Khadra et al., 2015; Perrier et al., 2018; Zhou et al., 2017b). This lower detection or resolution is linked to the smaller number of experiments performed within the reduced DoE which limits the statistical power or resolution of the method. However, differences between the performance of acidic, neutral and basic drugs indicates that category and possibly drug specific variations in solubility are also probably impacting the determination of significant media factors (Dunn et al., 2019; Zhou et al., 2017b). This statistical limitation of the reduced DoE is also evident in the inherent lack of detection of two way interactions between media factors, which the previous published larger studies detected (Khadra et al., 2015; Perrier et al., 2018; Zhou et al., 2017b). During pharmaceutical development this latter limitation may not be critical since assignment of a drug to a BCS classification only requires a solubility determination (with respect to dose) (Amidon et al., 1995; Rosenberger et al., 2018) not identification of the factors controlling solubility. However, it will always remain prudent to check that unique solubility controlling interactions, arising from either a single factor, combination of factors or the invariant media components (eg phosphate) are not present.

4. Conclusions

The reduced DoE effectively determines the gastrointestinal equilibrium solubility envelope for the drugs using a minimal matrix of solubility determinations. It also provides information on the most significant media factors contributing to solubility but this outcome is constrained by the statistical limitations of the small experimental numbers within the reduced DoE and possibly also influenced by the physicochemical properties and behaviours of the drugs.

Further refinement of the reduced DoE concept is possible through a range of modifications based on this study, previous studies and additional literature results. The media mixtures in this research are based around previous simulated recipes (Fuchs et al., 2015) and do not contain cholesterol or lysolecithin (Fuchs and Dressman, 2014; Riethorst et al., 2016, Soderlind 2010), components that are recognised to be present in sampled intestinal media. Additional media components may have added benefit in reducing the solubility variability induced by low numbers of amphiphiles in the media mixture (Dunn et al., 2019). However, the inclusion of additional factors will further erode, if experimental numbers remain constant, the ability to determine factor significance. If only solubility measurement is required this may not be critical. The media factor concentration limits applied within the reduced DoE were based on previously published DoE systems (Khadra et al., 2015; Perrier et al., 2018; Zhou et al., 2017b), which were based on limited data with respect to the analysis of HIF samples (Bergstrom et al., 2014). The factor concentration values could be refined based on recent structured analysis of HIF samples (Riethorst et al., 2016) to provide a more realistic boundary conditions. Finally, if in vitro data is to be combined with PKPB gastrointestinal pharmacokinetic models (Gobeau et al., 2016), which can incorporate up to seven small intestinal compartments (Rowland et al., 2011), the reduced DoE represents a manageable experimental load to determine solubility boundaries in these compartments, assuming media component concentrations and conditions can be determined.

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Supplementary Table 1 Fasted media volumes (µL) – individual component volumes required per sample as shown.

Sample	Bile Salt	Lecithin	Oleate	Buffer	Salt	water	FaSSIF	pH
1	267	267	267	267	267	2665	-	5
2	267	200	136	267	267	2863		
3	68	200	136	267	267	3062		
4	68	53	34	267	267	3311		
5		-					4000	6.5
6	267	267	267	267	267	2665	-	7
7	267	200	136	267	267	2863		
8	68	200	136	267	267	3062		
9	68	53	34	267	267	3311		
10	-	-	137	-		-	3863	6.5

Supplementary Table 2 Stock solution component concentrations (15 X lower and upper limit) in FED media

Stock solution	Bile salt (m M)	Lecithin (mM)	Monoglyceride (mM)	Volume
A - All high	360	72	97.5	5 mL
B - All low	54	7.5	15	
C - mid 1	54	30	75	
D - mid 2	225	30	75	

Supplementary Table 3 Fed media volumes (µl) – individual component volumes required per sample as shown.

Sample	BLM stock	oleate	buffer	salt	FeSSIF V2	Water	pH
1	A 267	267	267	267	-	2932	5
2	D 267	160	267	267	-	3039	5
3	C 267	160	267	267	-	3039	5
4	B 267	54	267	267	-	3145	5
5	-	-	-	-	4000		5.8
6	A 267	267	267	267	-	2932	7
7	D 267	160	267	267	-	3039	7
8	C 267	160	267	267	-	3039	7
9	B 267	54	267	267	-	3145	7

Table 1 Fasted state concentration levels- levels employed in reduced range design of experiment.

Parameter	All Low	All High	Mid 1	Mid 2	FaSSIF V1 with oleate	FaSSIF V1
Bile salt (mM)	1.5	5.9	1.5	5.9	3	3
Lecithin (mM)	0.2	1	0.75	0.75	0.75	0.75
Fatty acid (mM)	0.41	3.2	1.64	1.64	1.64	-
Buffer (mM)	Phosphate 28.4					
Salt (mM)	NaCl 105.9					
pH	5 and 7				6.5	6.5

Table 2 Fed media concentration levels- levels employed in reduced range design of experiment.

Parameter	All Low	All High	Mid 1	Mid 2	FeSSIF V2
Bile salt (mM)	3.6	24	3.6	15	10
Lecithin (mM)	0.5	4.8	2	2	2
Fatty acid (mM)	6.6	32.8	19.7	19.7	0.8
Monoglyceride (mM)	1	6.5	5	5	5
Buffer (mM)	Phosphate 45				Maleic acid 19
Salt (mM)	NaCl 125.5				
pH	5 and 7				5.8

Table 3 Physicochemical properties.

Drug	MW	Log P	pKa	PSA (polar surface area)	Melting point	Intrinsic Solubility (μM)
Indomethacin	357.787	3.8	4.5	68.53	158	16.6
Ibuprofen	206.29	3.51	4.9	37.3	76	245.47
Valsartan	435.519	1.5	3.9	112.07	116	
Itraconazole	705.64	5.66		100.79	166.2	
Tadalafil	389.404	1.64	15.17	74.87	301	19.83
Carvedilol	406.474	3.91	7.8	75.74	114.5	24.6
Aprepitant	534.427	4.8	9.7	75.19	254	1.5
Bromocriptine	750.7	3.2	6.68	118.21	192	3.67
Fenofibrate	360.831	5.24		52.6	80.5	0.81
Felodipine	384.259	3.86		64.63	145	0.276
Probucol	516.844	10		40.46	126	0.0116
Zafirlukast	575.676	6.4	4	115.73	139	

Table 4 Fasted media composition (mM) – Stock mixture concentrations.

Media number	**levels**	**Bile salt**	**Lecithin**	**Oleate**	**pH**
1	All high	5.9	1	3.2	5
2	mid 2	5.9	0.75	1.64	
3	mid 1	1.5	0.75	1.64	
4	All low	1.5	0.2	0.41	
5	fassif v1	3	0.75	0	6.5
6	All high	5.9	1	3.2	7
7	mid 2	5.9	0.75	1.64	
8	mid 1	1.5	0.75	1.64	
9	All low	1.5	0.2	0.41	
10	fassif v1 + oleate	3	0.75	1.64	6.5

Table 5 Fed media composition (mM)- Stock mixture concentrations.

Media	**levels**	**Bile salt**	**Lecithin**	**Oleate**	**Monoglyceride**	**pH**
1	All high	24	4.8	32.8	6.5	5
2	mid 2	15	2	19.7	5	5
3	mid 1	3.6	2	19.7	5	5
4	All low	3.6	0.5	6.6	1	5
5	FeSSIF V2	10	2	0.8	5	5.8
6	All high	24	4.8	32.8	6.5	7
7	mid 2	15	2	19.7	5	7
8	mid 1	3.6	2	19.7	5	7
9	All low	3.6	0.5	6.6	1	7

Table 6 HPLC Assay Conditions.

Apparatus: Agilent Technologies 1260 Series Liquid Chromatography
Software: Clarity Chromatography data system, Column: ACE 3 C18 50x3.0 mm id 3 µm

Drug	Mobile phase	Flow rate (mL/min)	Injection volume (µL)	Detection (nm)	Retention time (min)
Sertraline	MP A – MeOH/H2O) 95:5 v/v MP B – Ammonium Formate pH 3 in (ACN: H2O 9:1) Gradient: **Time (mins) %B** **0 0** **3 100** **4 100** **4.5 30**	1	10	214	0.2
Aprepitant	MP A: 10 mM Ammonium Formate pH3 in H_2O MP B: 10 mM Ammonium Formate pH3 in ACN:H_2O (9:1 v/v) Gradient: **Time (mins) %B** **0 30** **3 100** **4 100** **4.5 30**	1	50	254	3.00
Valsartan			10	254	2.49
Bromocriptine			10	254	2.19
Ibuprofen			10	254	2.84
Carvedilol			10	254	1.86
Felodipine			10	254	3.12
Fenofibrate			10	291	3.7
Indomethacin			10	254	2.75
Probucol			10	254	5.26
Tadalafil			10	290	1.72
Zafirlukast			10	254	3.36
Itraconazole			10	254	3.18

ACN: Acetonitrile, MeOH: Methanol, MP: Mobile Phase

Table 7 Comparison of Low Solubility Point Ratios

	Fasted State				Fed State		
	Solubility (mM)				Solubility (mM)		
Drug	Full DoE	9 DoE	Ratio*		Full DoE	9 DoE	Ratio*
Acidic							
Indomethacin	0.048	0.050	0.96		0.060	0.046	1.3
Ibuprofen	DNA	4.0	-		1.0	1.3	0.81
Valsartan	DNA	1.3	-		2.5	3.1	0.81
Zafirlukast	0.00024	0.00046	0.52		0.0022	0.0031	0.70
Basic							
Itraconazole	DNA		-		8.2×10^{-5}	0.00079	0.10
Tadalafil	0.010	0.0050	2.0		0.051	0.012	4.4
Carvedilol	0.10	0.013	7.7		0.059	0.27	0.22
Aprepitant	0.0023	0.010	0.23		0.04746	0.058	0.8183
Bromocriptine	DNA		-		0.0013	0.030	**0.042**
Neutral							
Fenofibrate	0.0020	0.0029	0.68		0.077	0.014	5.31
Felodipine	0.0023	0.021	0.11		0.0042	0.040	0.10
Probucol	0.00016	0.0086	**0.019**		0.014	0.020	0.73

* Ratio = Full DoE Solubility/9 DoE Solubility
* Ratio range set at 10 fold difference (10 to 0.1) values outside range in bold and underlined
DNA: Data Not Available

Table 8 Factors of significance on compound solubility in the DOE experiment.

	Fasted			Fed	
Drug	Reduced	Full		Reduced	Full
Acidic					
Indomethacin	pH	pH, bile salt, buffer, oleate		pH	pH, oleate, bile salt
Ibuprofen	NF	DNA		NF	pH
Valsartan	pH	DNA		NF	pH, bile salt
Zafirlukast	pH, oleate, bile salt, lecithin	pH, bile salt, oleate		NF	pH, bile salt, oleate
Basic					
Itraconazole	NF	DNA		NF	pH, oleate, bile salt, lecithin
Tadalafil	pH	bile salt, pH, buffer, lecithin, oleate		NF	bile salt, oleate
Carvedilol	bile salt, pH	bile salt, oleate		NF	bile salt, pH, buffer, oleate
Aprepitant	NF	oleate, pH, lecithin		NF	oleate, bile salt, pH
Bromocriptine	NF	DNA		NF	NF
Neutral					
Fenofibrate	oleate	oleate, bile salt, pH, lecithin, buffer		oleate	oleate, bile salt, lecithin, buffer, monoglyceride
Felodipine	oleate	pH, oleate, lecithin, bile salt		bile salt	oleate, bile salt, pH, lecithin
Probucol	pH	pH, oleate		NF	bile salt, monoglyceride, oleate, lecithin, pH

NF: No statistically significant Factors detected.
DNA: Data Not Available.
Fasted significant factors (Khadra et al 2015), fed significant factors (Zhou et al., 2017b).

Figure

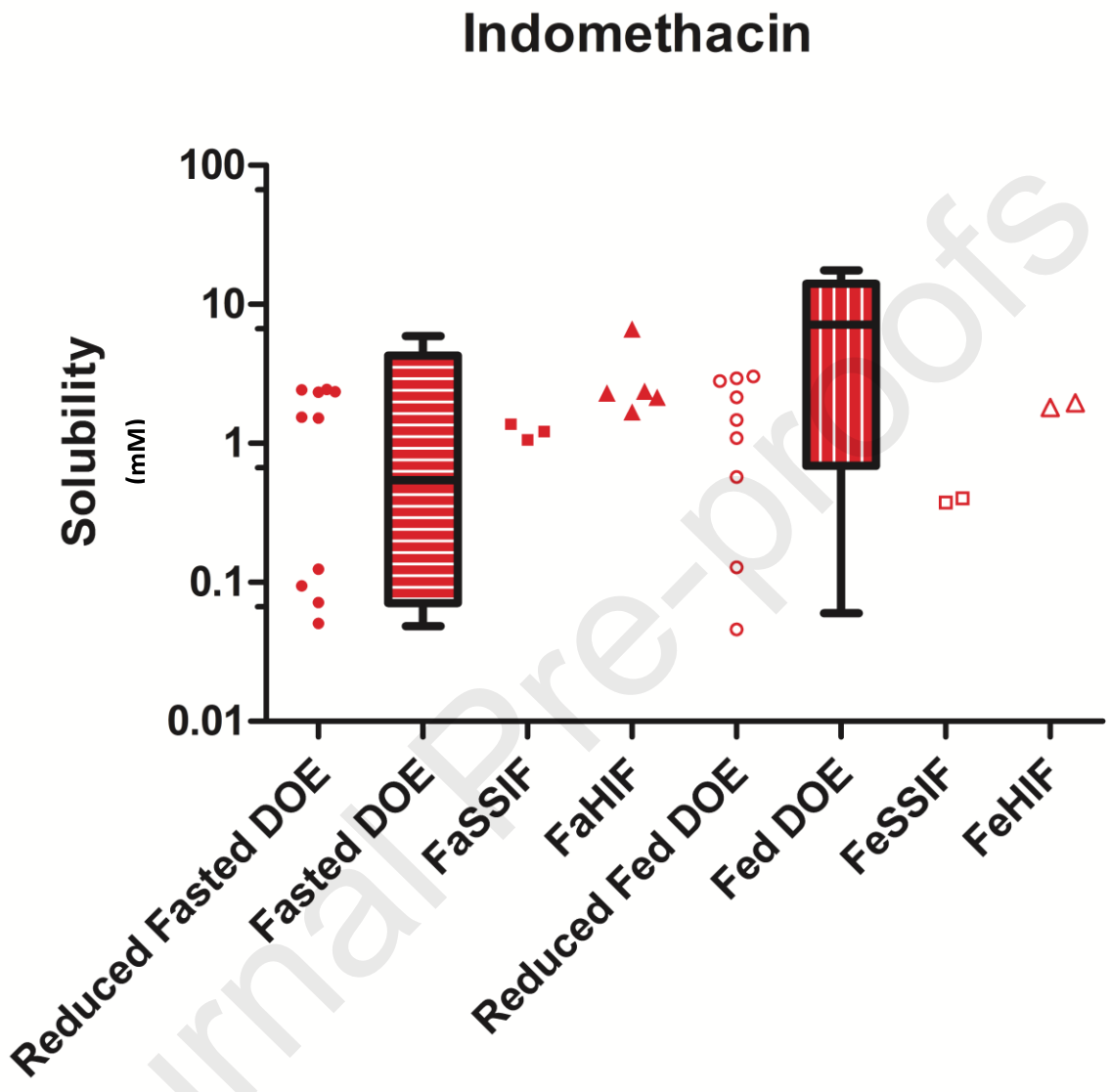

Fig 2. Design of experiment equilibrium solubility measurement of Indomethacin

Legend: Equilibrium solubility measurements for Indomethacin in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in original fasted DOE shown as  , reported solubility values found in fasted simulated intestinal fluid shown as■, reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as  (Khadra et al 2015), reported solubility values found in fed simulated intestinal fluid shown as□, reported solubility values found in fed human intestinal fluid shown as △

Figure

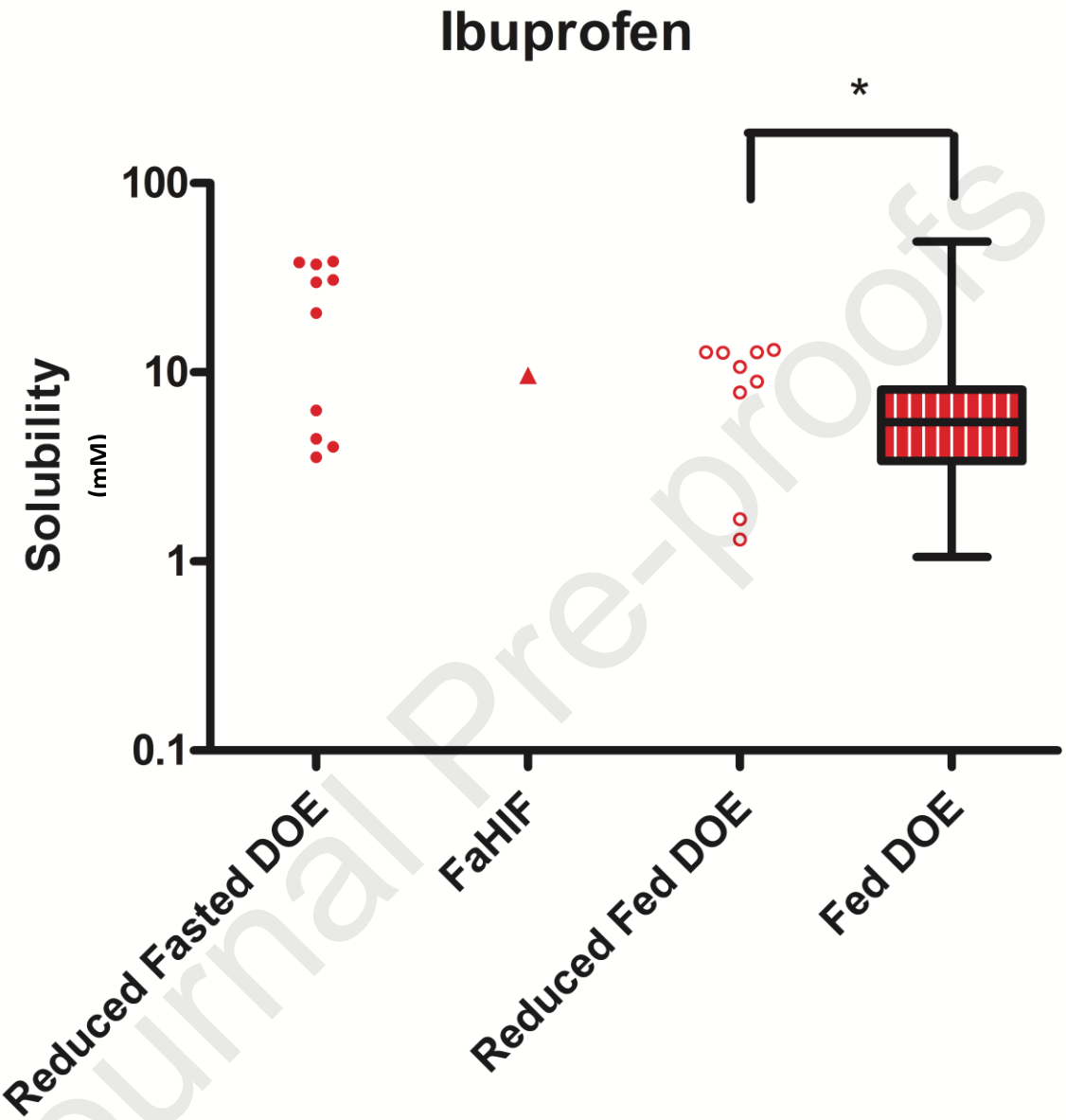

Fig 2. Design of experiment equilibrium solubility measurement of Ibuprofen

Legend: Equilibrium solubility measurements for Ibuprofen in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as ▮ (Khadra et al 2015).

Where relevant , significant difference is shown in comparison bars calculated from Mann-Whitney test, * $p \leq 0.05$.

Figure

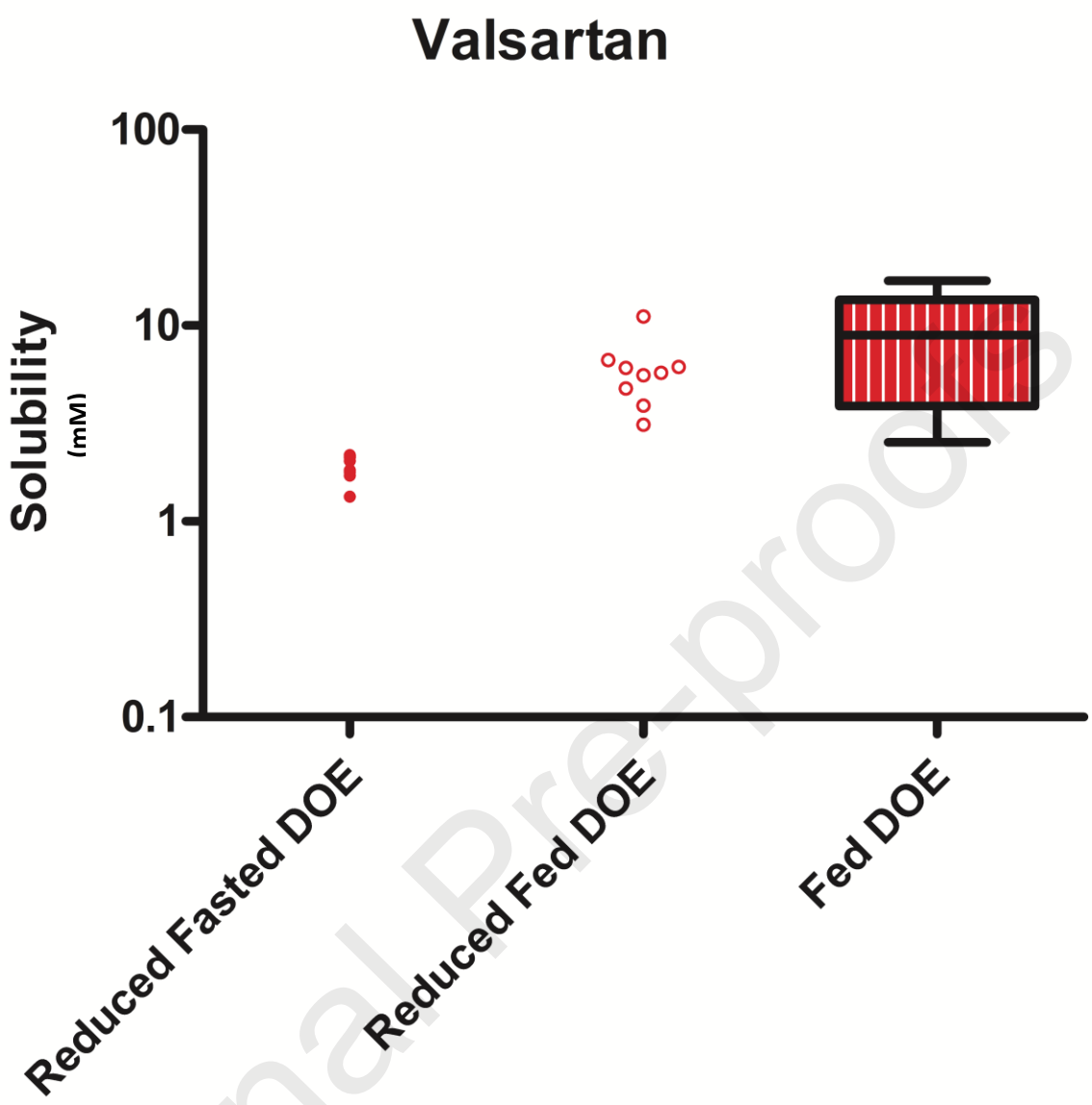

Fig 2. Design of experiment equilibrium solubility measurement of Valsartan

Legend: Equilibrium solubility measurements for Valsartan in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as, solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as  (Khadra et al 2015).

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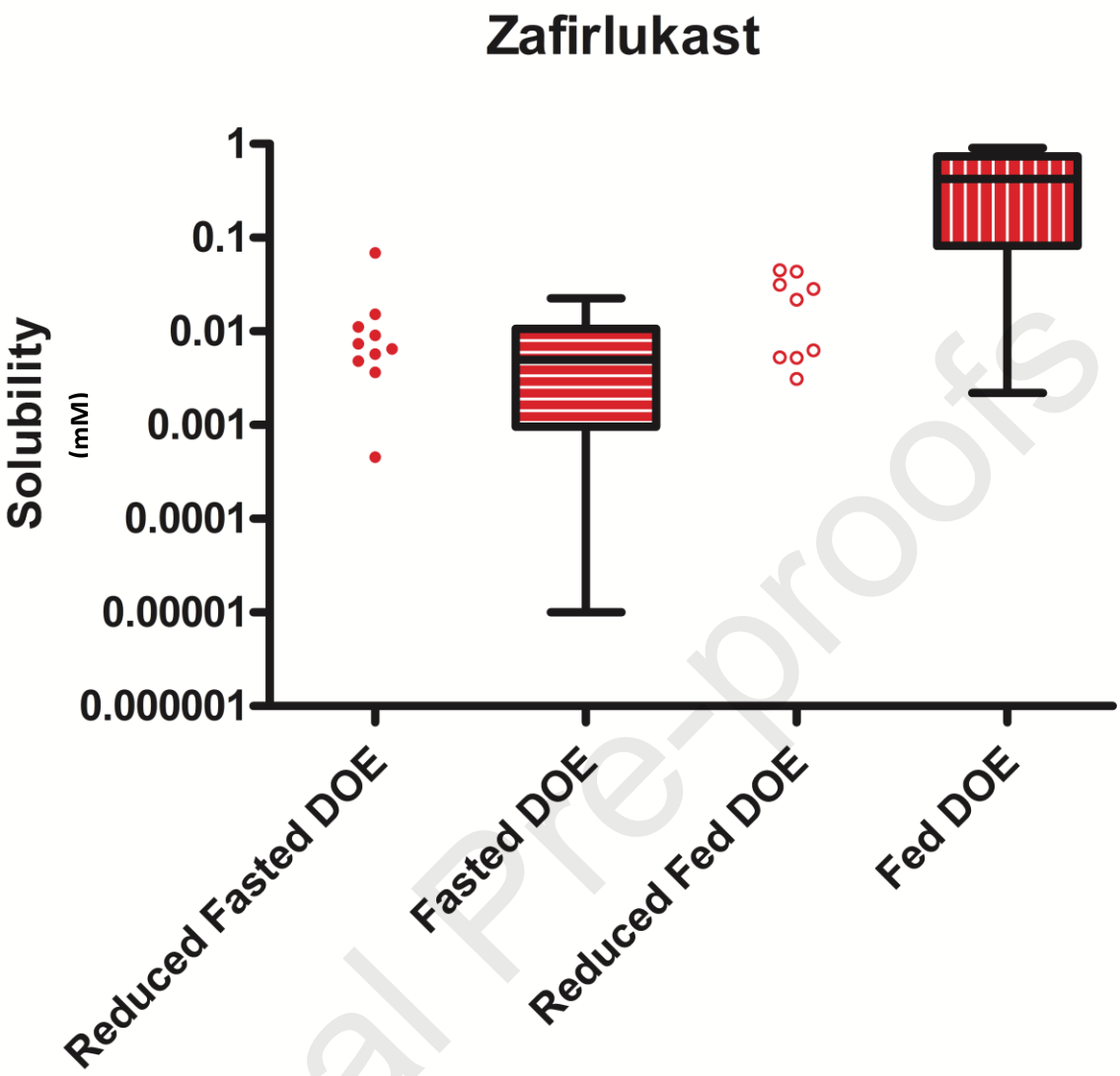

Fig 4. Design of experiment equilibrium solubility measurement of Zafirlukast

Legend: Equilibrium solubility measurements for Zafirlukast in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in original fasted DOE shown as  , solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as  (Khadra et al 2015).

Figure

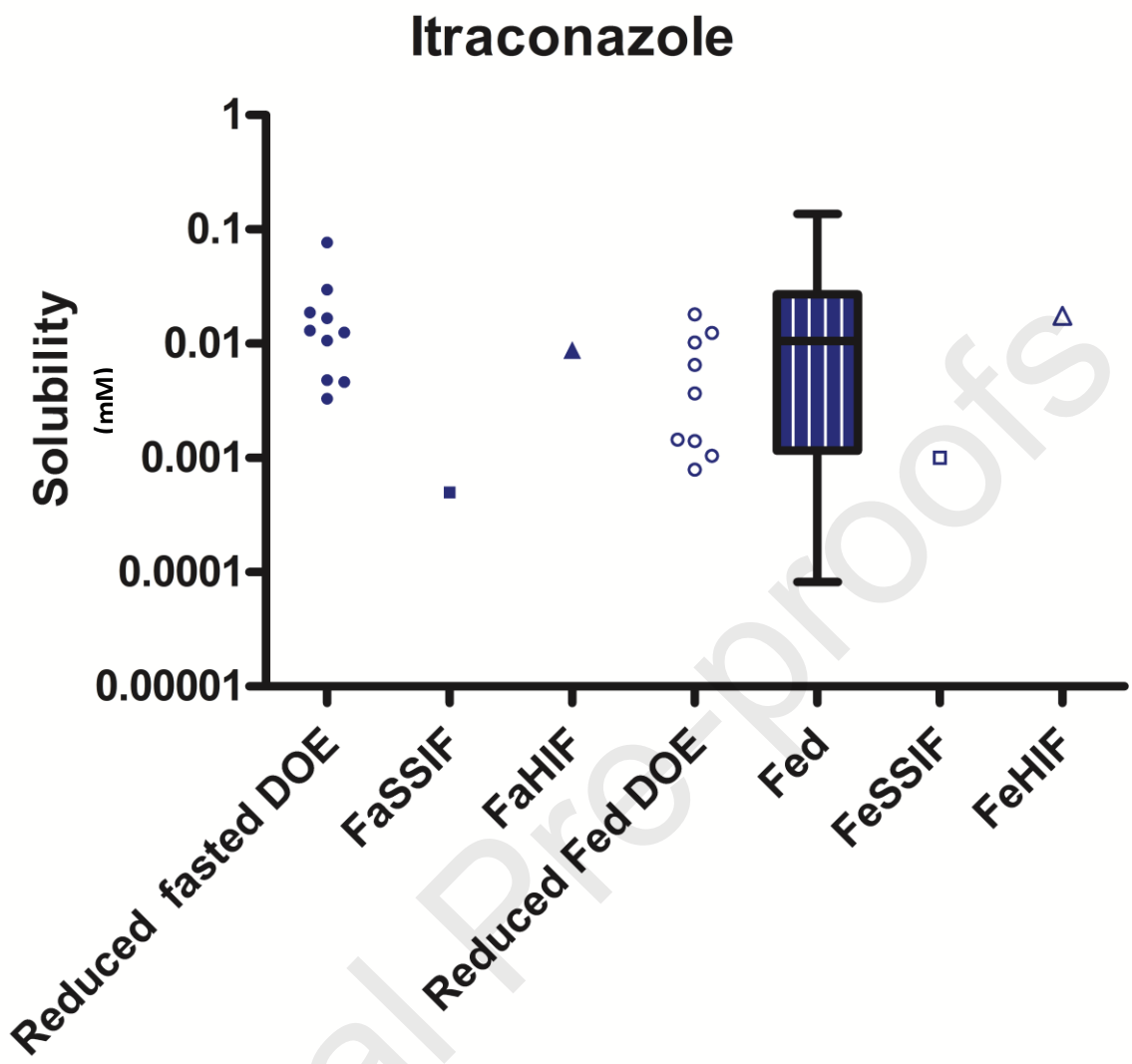

Fig 5. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Itraconazole in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ● reported solubility values found in fasted simulated intestinal fluid shown as ■ reported solubility values for fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○ solubility values found in original Fed DOE shown as  (Khadra et al 2014), reported solubility values found in fed intestinal simulated fluid shown as □ and reported solubility values found in fed human intestinal fluid shown as △.

Figure

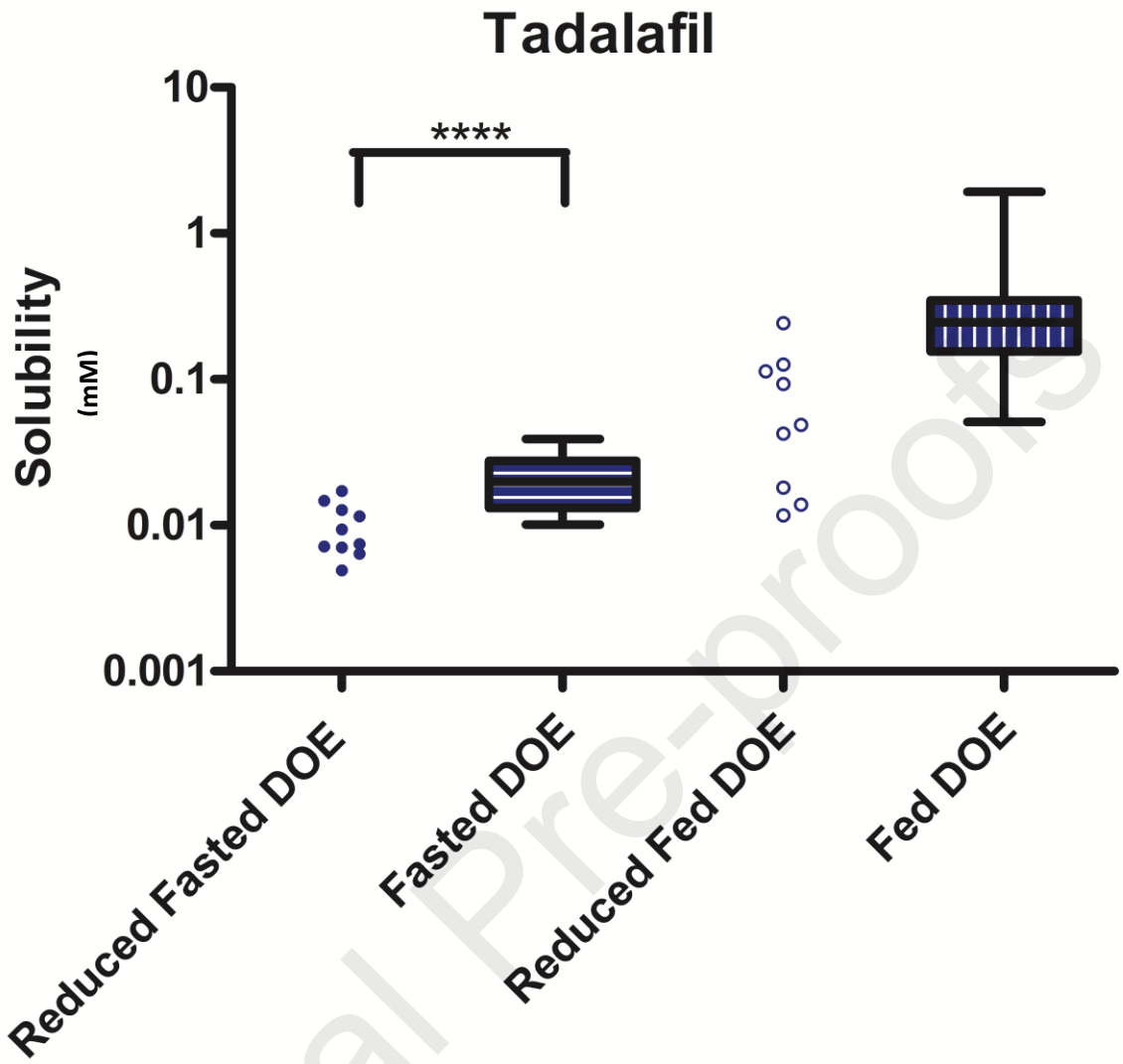

Fig 6. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Tadalafil in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ● reported solubility values found in original fasted DOE shown as  , solubility values found in reduced fed DOE shown as ○ and solubility values found in original Fed DOE shown as 

Where relevant, significant difference is shown in comparison bars calculated from Mann-Whitney test, **** $p \leq 0.0001$.

Figure

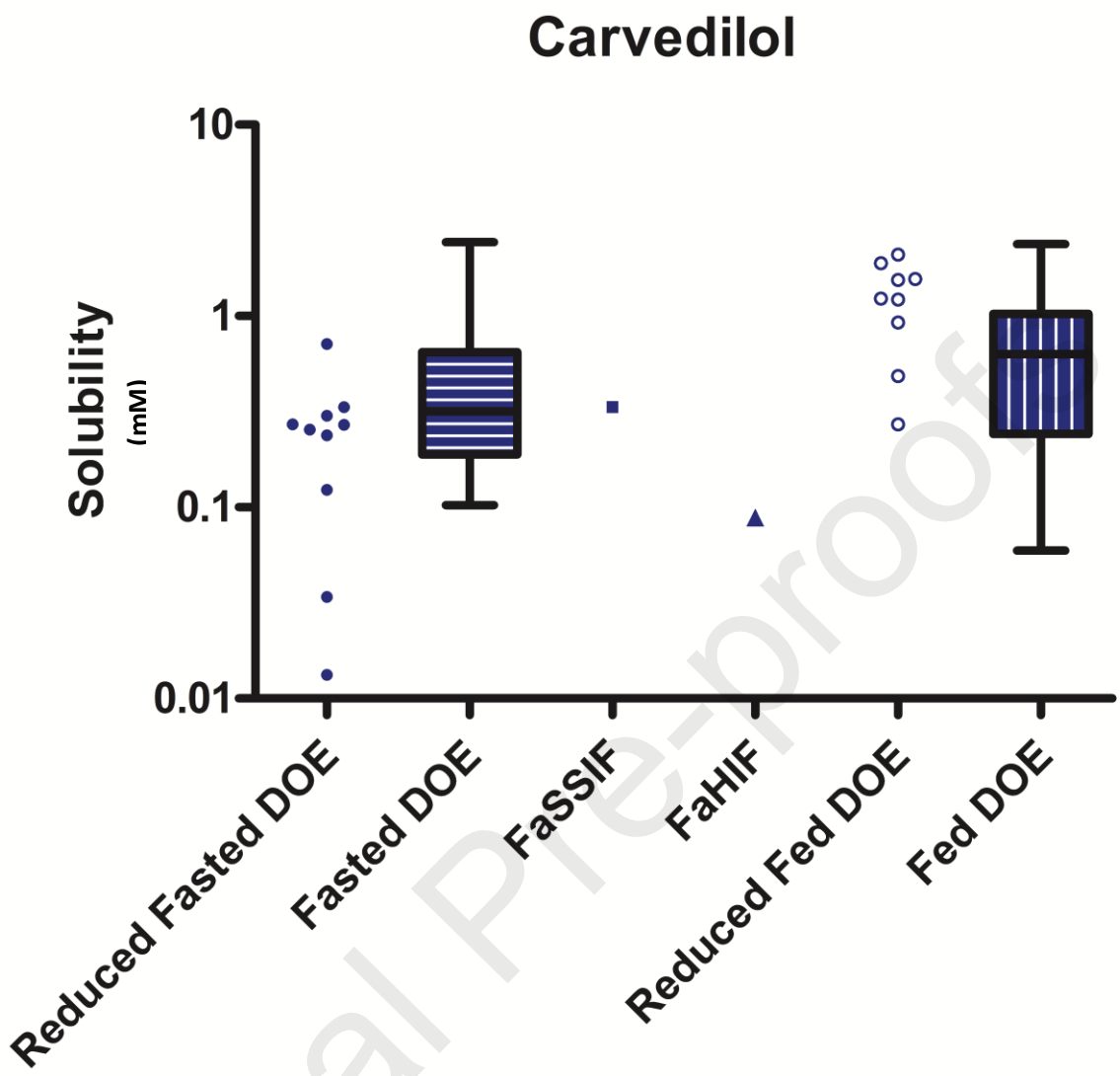

Fig 7. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Carvedilol in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ● reported solubility values found in original fasted DOE shown as  reported solubility values found in fasted simulated intestinal fluid shown as ■ reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○ and solubility values found in original Fed DOE shown as  (Khadra et al 2014).

Figure

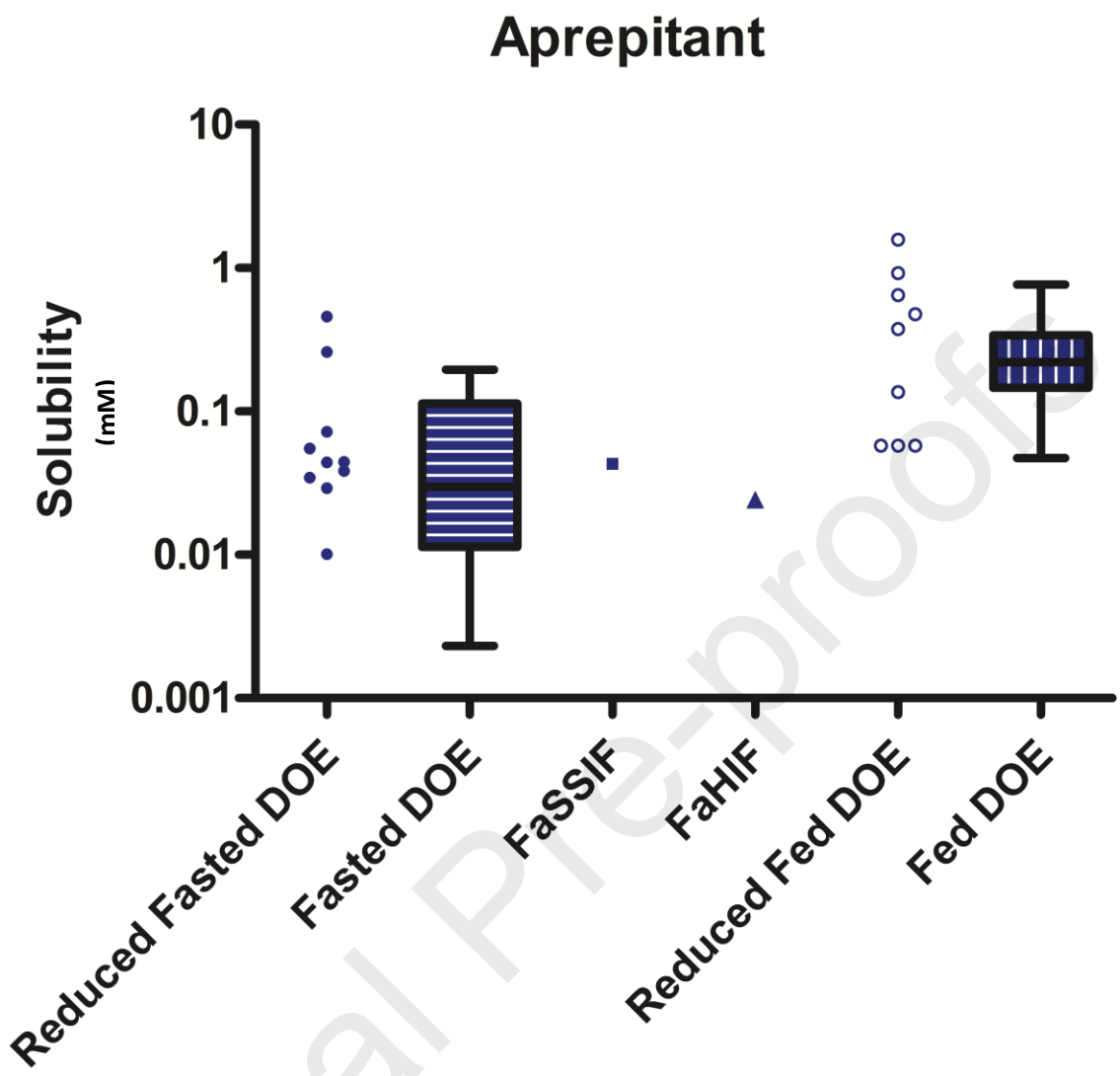

Fig 8. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Aprepitant in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ● reported solubility values found in original fasted DOE shown as  reported solubility values found in fasted simulated intestinal fluid shown as ■ reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○ and solubility values found in original Fed DOE shown as  (Khadra et al 2014).

Figure

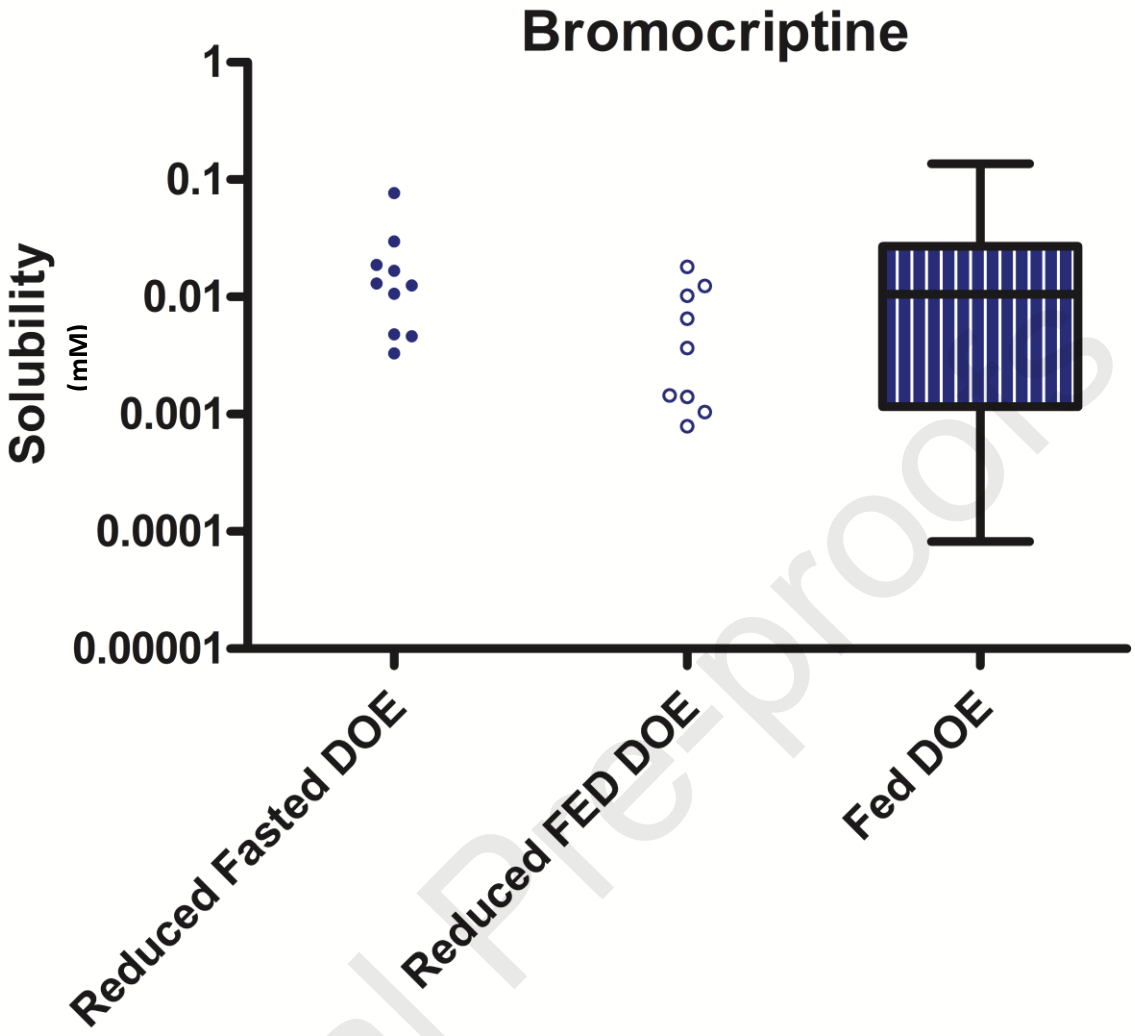

Fig 9. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Bromocriptine in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ● solubility values found in reduced fed DOE shown as ○ and solubility values found in original Fed DOE shown as  (Khadra et al 2014).

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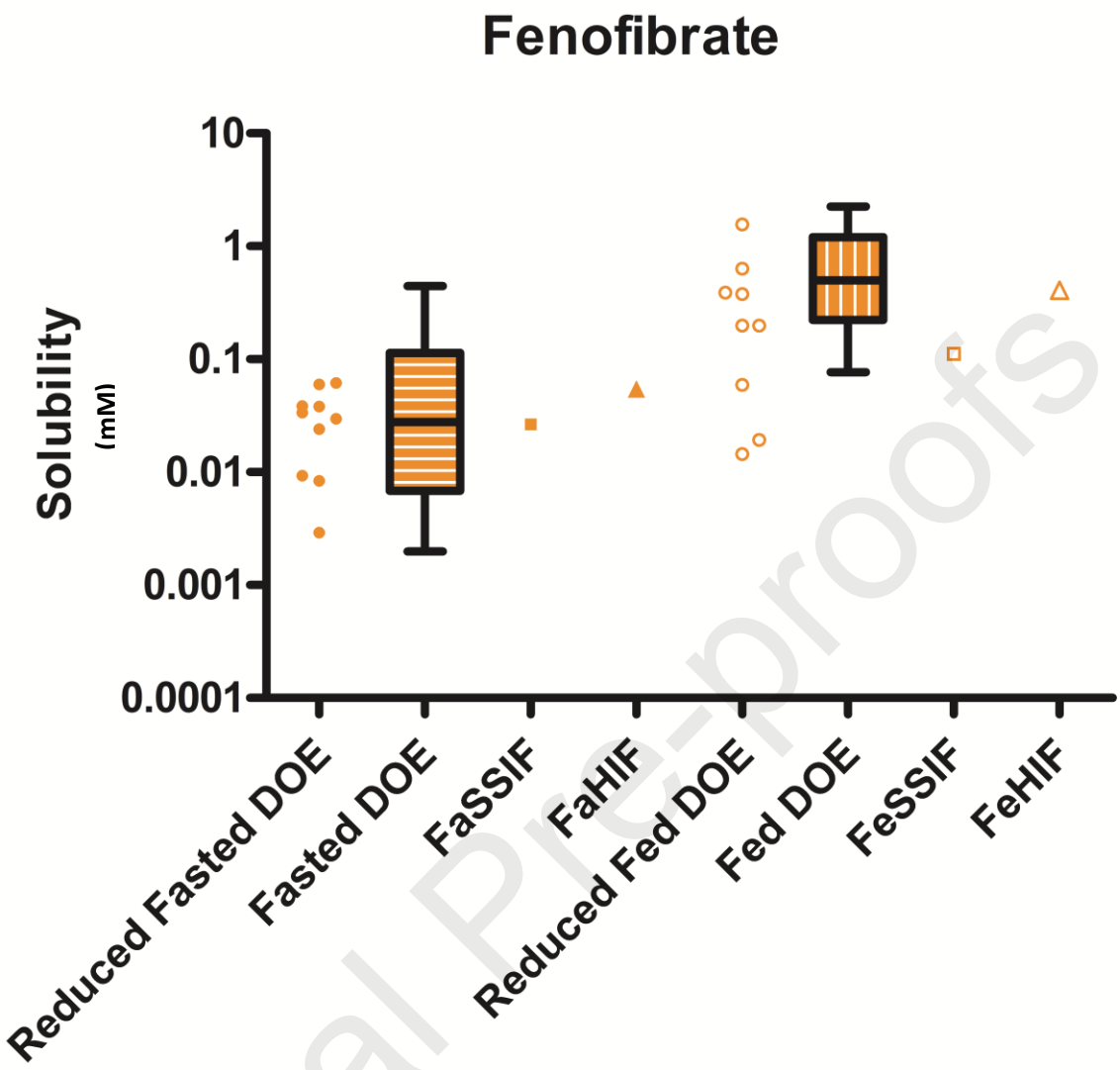

Fig 10. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Fenofibrate in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in original fasted DOE shown as  , reported solubility values found in fasted simulated intestinal fluid shown as ■, reported solubility values in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○ solubility values found in original Fed DOE shown as  (Khadra et al 2014) reported solubility values found in fed intestinal simulated fluid shown as ▫ and reported solubility values found in fed human intestinal fluid shown as △.

Figure

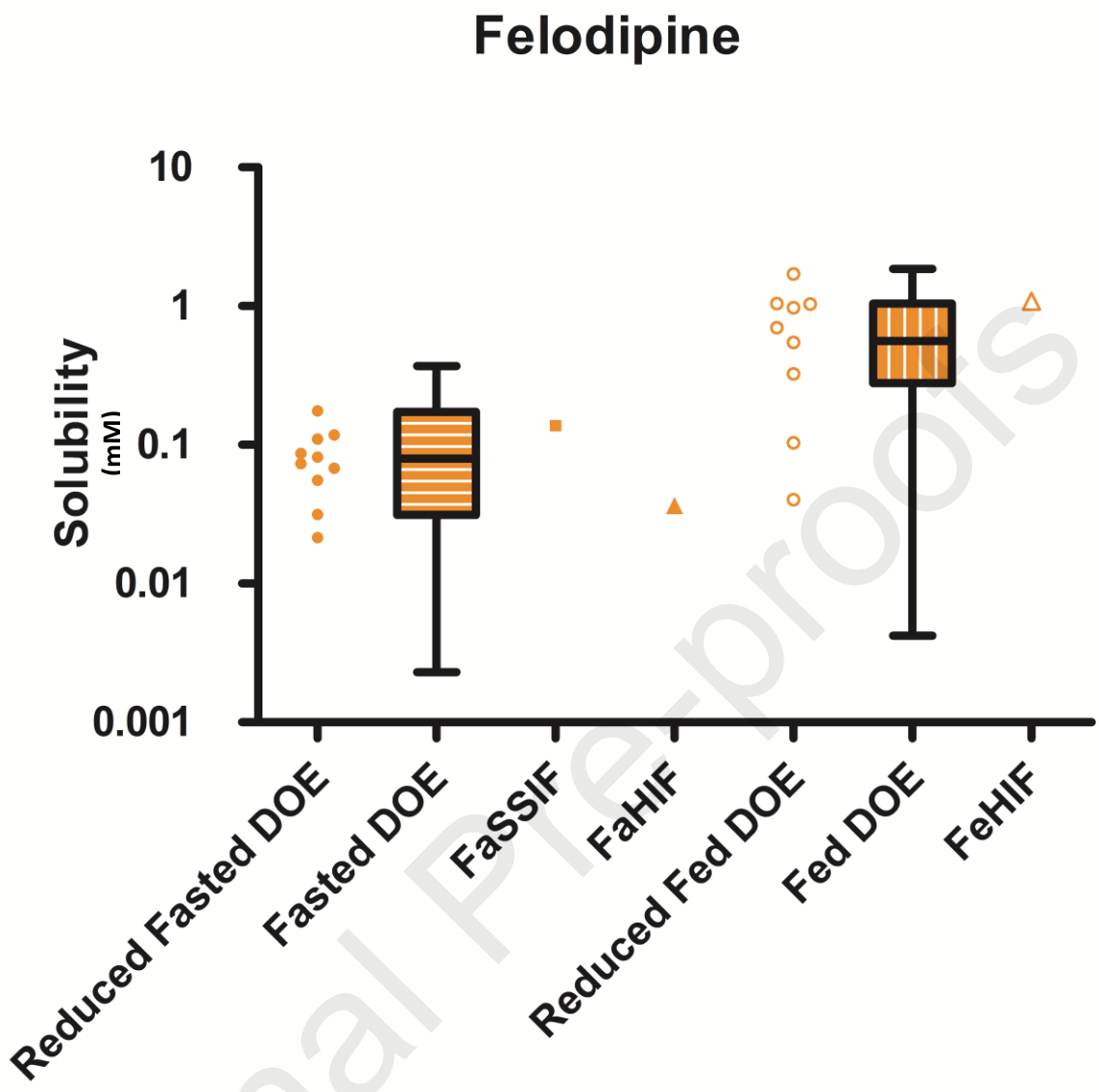

Fig 11. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurements for Felodipine in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in original fasted DOE shown as  , reported solubility values found in fasted simulated intestinal fluid shown as ■ , reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as  (Khadra et al 2014) and reported solubility values found in fed human intestinal fluid shown as △.

Figure

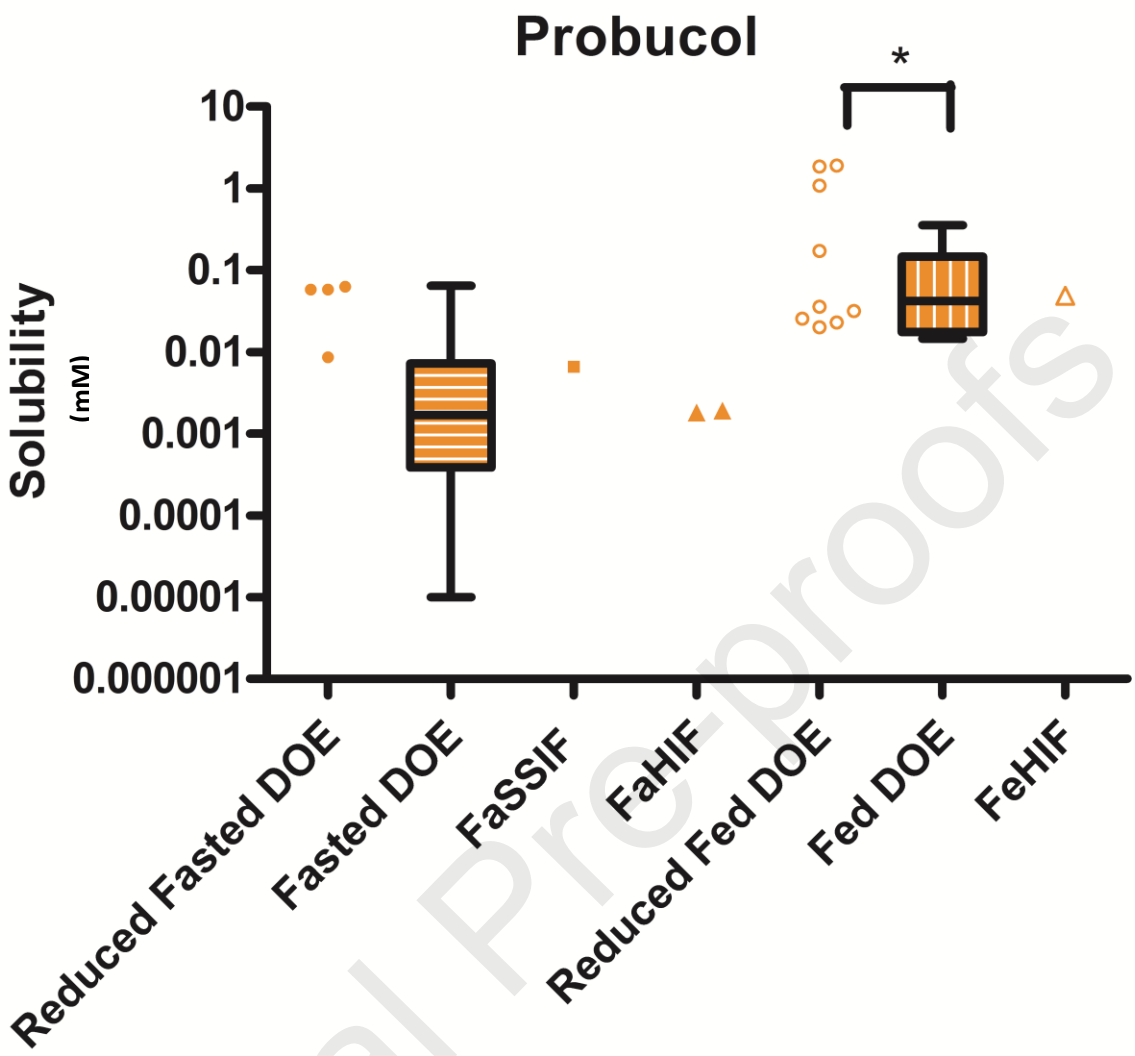

Fig 12. Design of experiment equilibrium solubility measurements. Legend: Equilibrium solubility measurement of Probucol in DOE media compositions detailed in table 1. Solubility values found in reduced fasted DOE shown as ●, reported solubility values found in original fasted DOE shown as  , reported solubility values found in fasted simulated intestinal fluid shown as ■ , reported solubility values found in fasted human intestinal fluid shown as ▲ (Augustijns et al 2014), solubility values found in reduced fed DOE shown as ○, solubility values found in original Fed DOE shown as  (Khadra et al 2014) and reported solubility values found in fed human intestinal fluid shown as △.

Where relevant , significant difference is shown in comparison bars calculated from Mann-Whitney test, * $p \leq 0.05$.

Graphical Abstract