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# Development and evaluation of a biorelevant medium simulating porcine gastrointestinal fluids

Laura J. Henze[a], Niklas J. Koehl[a], Regina Jansen[a], René Holm[b], Maria Vertzoni[c], Phil D. Whitfield[d], Brendan T. Griffin[a,*]

[a] School of Pharmacy, University College Cork, Cork, Ireland
[b] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium
[c] Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece
[d] Lipidomics Research Facility, Division of Biomedical Sciences, University of the Highlands and Islands, Inverness, United Kingdom

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ABSTRACT

Simulated human intestinal media, have proved to be a useful biopharmaceutics tool as a dissolution media for predicting *in vivo* dissolution and pharmacokinetic profile in humans. During drug product development pre-clinical animal models are also required to assess drug product performance, and there is a need to develop species specific intestinal media to similarly predict *in vivo* pharmacokinetic profiles in each preclinical model. Pigs, are increasingly being used in preclinical drug development, however to date there is a lack of quantitative information about the composition of porcine gastrointestinal (GI) fluids. As a result, a porcine biorelevant medium has not yet been developed, which is essential to improve interpretation and forecast of preclinical results using biorelevant *in vitro* dissolution studies. GI fluid samples, were collected from landrace pigs, and characterized. Fasted State Simulated Intestinal Fluid of pigs (FaSSIFp) was developed based on the physiological composition of the GI fluids in terms of pH, buffer capacity, osmolality, surface tension, as well as the bile salt, phospholipid and free fatty acid content. This study demonstrated that FaSSIFp was superior at predicting the solubility of the six model drugs in porcine intestinal fluids (PIF). A markedly high correlation ($r^2$ 0.98) was observed between the solubility obtained in PIF and FaSSIFp, whereas poor correlation ($r^2$ 0.12) was found for the solubility of the model drugs between human FaSSIF and PIF. This confirms that species specific biorelevant intestinal media are crucial to provide more accurate predictions of pharmacokinetic studies in preclinical models. Additionally, the availability of a species specific intestinal medium offers the potential to improve *in vitro-in silico* approaches to predict *in vivo* absorption and to reduce the overall number of animals needed in oral drug product development testing.

## 1. Introduction

Operating within the conventional drug product development paradigm involves initially *in vitro* screening, preclinical *in vivo* testing followed by clinical evaluation in humans. Reliable and bio-predictive *in vitro* models are essential as a guide during pharmaceutical development of drug products [1]. In order to gain appropriate predictability with *in vitro* models, the models often attempt to mimic biopharmaceutical conditions found in humans. Various advanced dissolution media have been established to mimic the human gastrointestinal (GI) tract, while some of these *in vitro* models are complex such as the TNO Gastro-Intestinal Model (TIM) [2] or the biorelevant gastrointestinal transfer (BioGIT) system [3,4]. Biorelevant media representing the fasted state conditions in human intestine (Fasted State Simulated Intestinal Fluid, FaSSIF), or postprandial conditions (Fed State Simulated Intestinal Fluid, FeSSIF) [5] has been proposed as early as 1998 and have been revised (FaSSIF- V2, and FeSSIF- V2) [6] and improved for the third time (FaSSIF- V3) in 2015 [7]. Markopoulos and co-workers, proposed the concept of categories to classify the level of simulation of the luminal composition, ranging from Level 0 (only aqueous solution with adjusted pH) to Level III (including dietary proteins, enzymes and viscosity effect) [8]. Level II FaSSIF and Level II FeSSIF can provide a first insight *in vitro* to mimic human intraluminal conditions and have been proved useful for the evaluation of solubility of active pharmaceutical ingredients and dissolutions performance of oral drug products [9].

* Corresponding author at: Brendan Griffin, University College Cork, School of Pharmacy, Cavanagh building, College road, Cork, Ireland.
*E-mail address:* Brendan.Griffin@UCC.ie (B.T. Griffin).

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FaSSIF and FeSSIF are designed to match pH, bile and phospholipid concentrations in humans, however given the variations between animal and humans in GI conditions, these media are not readily applicable to preclinical animal models. In order to make more scientifically informed decision in species selection, a thorough understanding of intestinal conditions in each species is required. In addition, species specific *in vitro* models are an essential biopharmaceutics tool to guide preclinical drug development. A species specific *in vitro* biopharmaceutics tool is also in line with the 3 R's principles of replacement, reduction and refinement. Specially, the 'replacement' approach may be considered, and this is where the development of a porcine biorelevant medium is advantageous. Improvements in the correlation from *in vitro* data will help to reduce the number of animals needed and further support the selection of the most suitable animal model.

Key differences in canine and human intestinal fluid compositions, lead to the development of a dissolution media simulating the content of canine GI tract, special designed to improve prediction and interpretation of preclinical results [10]. Additionally, biorelevant media simulating fasted conditions in rats has been proposed [11]. All biorelevant media are designed to simulated species specific properties in the GI tract, including pH, buffer capacity, osmolality, surface tension and hydrodynamic conditions. However, to date there is a clear lack of information about the composition of porcine GI fluids, and in particular limited quantitative physiochemical evaluation of intestinal bile and phospholipids, as well as osmolality estimates. Therefore, currently no porcine biorelevant media exist which limits the potential to mechanistically relate *in vivo* drug absorption profiles, based on preclinical pharmacokinetic studies in pigs, to *in vitro* dissolution behaviour. The use of human biorelevant media for *in vitro* - *in silico* approaches in a preclinical stage, to predict porcine PK profiles, is open to questions. Therefore, species-specific biorelevant *in vitro* testing are needed as an essential tool for integrating *in vitro* data into *in silico* models, in a preclinical setting.

The aim of this study was to characterise the composition of porcine gastric fluid (PGF) and porcine intestinal fluid (PIF) samples under fasted state conditions. A comprehensive physiochemical characterization of PIFs including concentrations of bile salts, phospholipids and fatty acids as well as the pH, buffer capacity and osmolality in the gastric and intestinal contents was conducted with the ultimate aim of establishing for the first time a porcine biorelevant medium, i.e. porcine Fasted State Stimulated Intestinal Fluid (FaSSIFp). For the evaluation of proposed FaSSIFp medium, solubilities of six model drugs with diverse physiochemical properties, were determined. The reliability of FaSSIFp versus FaSSIF to predict solubilites in PIFs was also assessed.

## 2. Materials and methods

### 2.1. Materials

Lipoid E PC S (Phosphatidylcholine) was obtained from Lipoid GmbH (Germany), Sodium taurodeoxycholate; Sodium hydroxide (NaOH) pellets; Chloroform; Sodium chloride (NaCl); Sodium dihydrogen phosphate monohydrate; Sodium oleate were purchased from Sigma Aldrich (Ireland) and sodium taurocholate was ordered from Thermo Scientific Ltd., Alfa Aesar (UK). Celecoxib, danazol, felodipine, ketoconazole, dipyridamole, venetoclax was obtained from Kemprotec Ltd. (UK). Water of HPLC grade was produced using a MilliQ system (Merck KGaA, Germany). All other chemicals and solvents were of analytical or high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

### 2.2. Collection of porcine gastrointestinal samples

Content sample of the stomach and the small intestine for this study have been collected in previous studies [12]. The study was carried out under the licence issued by the Health Products Regulatory Authority (HPRA), Ireland, as directed by the Cruelty to Animals Act, Ireland and EU Statutory Instruments (Licence number AE19130/P058). Local University ethical committee approval was obtained. In the fasted state the final feeding was provided 24 h prior to euthanasia. As part of the study design, any remaining food was removed 16 h before euthanasia. All animals were euthanized by intravenous injection of pentobarbital sodium followed by potassium chloride. The peritoneal cavity was exposed by midline incision and the stomach and small intestine were isolated. Occluding ligatures were applied to the proximal cardiac sphincter and distal to the pyloric sphincter as well as at the proximal and distal ends of the small intestine. Once both ends were secured, both the stomach and small intestine were removed from the peritoneal cavity. The luminal contents were collected from the stomach and intestine and stored in sterile 50 mL sample tubes. Physiochemical characteristics were measured immediately upon collection. The pH was measured using a calibrated Jenway 3510 pH meter. Subsequently, the samples were frozen (−80 °C) until further analysis.

### 2.3. Physicochemical parameters of porcine gastric fluid (PGF) and porcine intestinal fluid (PIF)

#### 2.3.1. pH and buffer capacity

A calibrated pH meter (model 3510, JENWAY) was used for pH measurements, pH was measured immediately after the collection of the fresh samples without prior processing.

The buffer capacity was calculated using equation (1):

$$\beta = \Delta AB/\Delta pH \quad (1)$$

where △ AB is the amount of acid or base added and △ pH is the change induced by the acid or base added. △ AB and △ pH were determined by titration of PIF and PGF. The volume of 0.1 N hydrochloric acid solution or 0.1 N sodium hydroxide was determined to change the pH by one unit of PIF and PGF, respectively.

#### 2.3.2. Surface tension

The surface tension was conducted using a Lecomte du Nouy Tensiometer (Kruess, Germany, model 6.0). The classical ring system approach (a platinium-iridum ring) was used. Before measurements, the tensiometer was calibrated with Milli-Q water to 71.5 mN $m^{-1}$, at 20 °C.

#### 2.3.3. Osmolality

Osmolality was measured by using the freezing point depression technique (semimicro osmometer Typ Dig L; Knauer, Berlin, Germany).

#### 2.3.4. Phospholipid and bile acid analysis

*2.3.4.1. Sample preparation.* Gastric and intestinal fluid samples were diluted 1:1 (V/V) with isotonic phosphate buffered saline pH 7.4 and then sonicated using a Microson XL 2000 Ultrasonic Cell Disrupter. Extraction of lipids was performed according to the method of Folch *et al.* [13]. In brief, a 10 µL aliquot of each sample was extracted with 3 mL chloroform/methanol (2/1, V/V) and 17:0 lysophosphatidylcholine (Avanti Polar Lipids, Alablaster, AL, USA), 12:0/12:0 phosphatidylcholine (Avanti Polar Lipids), cholesterol-$d_7$ (Avanti Polar Lipids), glycocholic acid-$d_4$ (Cayman Chemicals, Ann Arbour, MI, USA), cholic acid-$d_4$ (Cayman Chemicals) and heptadecanoic acid (Sigma-Aldrich, Poole, UK) were added as internal standards. The mixture was then left to stand on ice for 1 h. The samples were partitioned by the addition of 750 µL of 0.1 M KCl and the mixture was centrifuged to facilitate phase separation. The upper methanolic phase was removed and discarded while the lower chloroform layer was evaporated to dryness under a gentle stream of nitrogen gas.

*2.3.4.2. Quantification of phospholipids and bile acids.* Lipid extracts were reconstituted in 250 µL methanol containing 5 mM ammonium

formate (Sigma, Poole, UK) and analysed by liquid chromatography-mass spectrometry (LC-MS) using a Thermo Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization (HESI) probe and coupled to a Thermo Accela 1250 ultra-high pressure liquid chromatography (UHPLC) system. Samples (2 μL) were injected on to a Thermo Hypersil Gold C18 column (2.1 mm × 100 mm, 1.9 μm) maintained at 50 °C. Mobile phase A consisted of water containing 10 mM ammonium formate and 0.1% (V/V) formic acid. Mobile phase B consisted of 90:10 isopropanol/acetonitrile containing 10 mM ammonium formate and 0.1% (V/V) formic acid. The initial conditions for analysis were 65% A/35% B and the percentage of mobile phase B was increased from 35% to 65% over 4 min, followed by 65%-100% over 15 min held for 2 min before re-equilibration to the starting conditions over 6 min. The flow rate was 400 μL/min. Samples were analysed in positive and negative ion modes over the mass to charge ratio (*m/z*) range 250–2000 at a resolution of 100,000. The signals corresponding to the accurate *m/z* values for $[M + H]^+$ ions of lysophopholipids and diacyl phospholipids and [M−H]- ions of unconjugated and conjugated bile acids were extracted from raw LC-MS data sets with the mass error set to 5 ppm. Quantification was achieved by relating the peak area of each lipid species to the relevant internal standard and the concentrations were normalised to volume.

*2.3.4.3. Quantification of free fatty acids and free cholesterol.* Trimethylsilyl (TMS) ethers of free fatty acids and cholesterol were made by derivatising with 100 μL MSTFA + 1% TCMS (Fisher Scientific) at 37 °C for 30 min. This mixture was evaporated and reconstituted in 1 mL hexane. GC–MS analysis was performed using a Thermo Trace Ultra gas chromatograph fitted with an Agilent J&W DB-5 ms column (30 m × 0.25 mm i.d. × 0.25 μm film) and coupled to a Thermo ISQ mass spectrometer. 1 μL was injected in splitless mode using helium (1 mL/min) as a carrier gas. After a delay of 2 min at 50 °C, the temperature was ramped to 300 °C at 10 °C/min and then held for 8 min. The mass spectrometer was operated in full scan mode over the mass range *m/z* 50–500. The ionisation energy was 70 eV and the source temperature was 220 °C. Quantification was achieved by relating the peak area of individual free fatty acids and cholesterol to the peak area of the relevant internal standard and the concentrations were normalised to volume.

### 2.4. Composition of porcine fasted state simulated intestinal fluid

#### 2.4.1. Buffer

A suitable buffer for the porcine simulated biorelevant media was chosen, based on the pH of the porcine fluids, which were collected post-mortem. The buffer concentration was calculated according to the measured buffer capacity in the pig fluids using the Van Slyke equation:

$$\beta = 2.3C\frac{Ka[H_3O^+]}{(Ka + [H_3O^+])^2} \quad (2)$$

where β is the buffer capacity, C the total buffer concentration (sum of the molar concentration of acid and salt), Ka is the acid dissociation constant, $[H_3O^+]$ is the molar concentration of the hydronium ion.

#### 2.4.2. Osmolality

The amount of sodium chloride, which is needed to adjust the osmolality of the biorelevant medium to physiological conditions, was calculated according to Raoult's law on the basis of the freezing point depression:

$$\Delta T_f = i * K_f * m \quad (3)$$

$\triangle T_f$ is the freezing-point depression, i is the van't Hoff factor, $K_f$ is the cryoscopic constant ($-1.858$ K kg mol$^{-1}$), and m is the concentration in moles of solute per kilogram of solvent (mol kg$^{-1}$) or molality of the solution.

#### 2.4.3. Development of simulated porcine fluids

While chenodeoxycholic and hyodeoxycholate acid are considered the major bile salts, taurocholate (TC) and taurodeoxycholate (TDC) are the most widely utilised bile salts in commercially available simulated intestinal media, and were therefore selected based on commercial considerations. This also facilitates future applications where species specific biorelevant media may be prepared with a common set of bile salts. The ratio of TC: TDC was based on the physiological data of this study, which showed that 35% belong to primary bile acids (TC) and 65% belongs to secondary bile acid (TDC). Additionally, the phospholipid (PL) to bile ratio was also fixed at 0.013 (total PL/ total bile mM), as per the pig intestinal fluids. An approach by Arndt and co-workers was used to determine the representative bile salt concentrations for TC, TDC and PL [10]. In brief, the solubility of celecoxib, danazol, ketoconazole, felodipine, dipyridamole and venetoclax was measured in pig intestinal fluids and in a phosphate buffer (buffer capacity adjusted to the measured data in pig intestinal fluids) at pH 7.0 with varying TC/TDC and PL concentrations (ratios fixed). This resulted in six drug specific 'standard bile concentration versus solubility curves', which were used to calculate the bile and phospholipid concentrations, which would match the *ex vivo* measured solubilites in pig fluids. The final TC/ TDC and PL concentration for the porcine biorelevant media was calculated as the mean of TC/ TDC and phospholipid concentrations obtained with all six drugs.

#### 2.4.4. Example of FaSSIFp preparation

(1) Blank buffer is prepared using the buffer salts calculated for one litre of FaSSIFp medium, containing sodium dihydrogen monophosphate, sodium hydroxide and sodium chloride
(2) The pH is adjusted to pH 7.0 ( ± 0.05) by using sodium hydroxide solution (0.1 M).
(3) Approximately 20% of the buffer volume is transferred into a round-bottom flask.
(4) The required amount of sodium taurocholate and sodium taurodeoxycholate is calculated and dissolved in the buffer in the round-bottom flask under continuous stirring.
(5) The amount of lecithin needed is calculated and added to the bile salt solution using a freshly prepared solution of lecithin in chloroform (usually 100 mg/mL). This produces a white/milky emulsion.
(6) The chloroform is roto evaporated under reduced pressure at 40 °C, for 30 min. This results in a clear solution, which is examined to have no distinctive odour of chloroform.
(7) The sodium oleate is mixed into the concentrated bile salt and phospholipid solution in the round-bottom flask under stirring for approximately 10 min.
(8) Buffer is added (approximately 40% of the final volume) and mixed in.
(9) The mixture is returned to a volumetric flask. After a final pH check, the volume is adjusted.

### 2.5. Solubility studies

The solubility of celecoxib, danazol, ketoconazole, felodipine, dipyridamole and venetoclax was determined. Solubility studies were carried out by the addition of excess of each drug to the media using a shake flask method (200 shakes/min) with a shake time of 24 h at 37 °C. Samples were taken at 1 h, 2 h, 4 h, 5 h and 24 h and added to 1.5 mL centrifuge tubes. Samples were centrifuged at 37 °C, at 11,000 rpm for 10 min (Mikro 200 R, Hettich GmbH, Germany). The supernatant was transferred to a new tube and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC after appropriate dilution with the respective mobile phase. All samples were run in triplicates.

**Table 1**
HPLC methods of six model drugs utilized for solubility measurements.

| | Celecoxib | Ketoconazole | Dipyridamole | Felodipine | Danazol | Venetoclax |
|---|---|---|---|---|---|---|
| Mobile phase (V/V) | Acetonitrile/ $H_2O$ (55:45)[a] | Acetonitrile/ Phosphate buffer 10 mM pH 8.5 (50:50) | Acetonitrile/ $H_2O$ (40:60) | Acetonitrile/ $H_2O$ (55:45) | Acetonitrile/ $H_2O$ (55:45) | Acetonitrile/ $H_2O$ (57:43)[b] |
| Flow rate (mL/min) | 1.0 | 0.8 | 1.0 | 1.0 | 1.0 | 1.0 |
| UV wavelength (nm) | 254 | 232 | 282 | 360 | 286 | 290 |
| Column | I | I | II | II | I | III |
| Column temperature (°C) | 25 | RT | 40 | RT | 20 - 25 | 40 |
| Ret. time (min) | 10.5 | 8.0 | 11.3 | 11.8 | 10.4 | 9.4 |
| Run time (min) | 15.0 | 10.0 | 15.0 | 15.0 | 15.0 | 12.0 |
| Injection volume (µL) | 20 | 50 | 20 | 20 | 50 | 20 |

[a] + 0.15 % trimethylamine and was adjusted to pH3 with orthophosphoric acid.
[b] + 0.5 % trifluoroacetic acid.
I: Waters Symmetry C18 (5µm 4.5 x 150 mm).
II Phenomenex Gemini (5µm 4.6 x 150 mm).
III Zorbax Eclipse Plus-C18 (5 µm, 4.6 x 150 mm) with a Zorbax Eclipse Plus-C18 guard column (5 µm, 4.6 mm x 12.5 mm).

(I) Solubility studies pig intestinal fluids (PIF): On the day of the solubility experiment all samples were brought to room temperature. Fluid samples were pooled by taking 3 mL from each sample to create the PIF samples.
(II) Solubility in FaSSIFp: The porcine biorelevant media was freshly prepared on the day of the solubility experiment. Preparation procedure is outlined below.

### 2.6. Analysis of drug concentrations by HPLC

The drug concentration in the samples was determined by HPLC. The Agilent 1260 series HPLC system comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was controlled, and the data analysed with EZChrom Elite version 3.3.2. The analysis method for each drug is described in Table 1, as previously described [10,14].

### 2.7. Data analysis

Solubility data are presented as mean and standard deviation and corresponded solubility from literature sources are presented as mean values and standard deviations where applicable. *In vitro* solubility data were tested for significance ($p < 0.05$) between PIF and FaSSIFp, using a two-tailed, independent sample *t*-test, assuming Gaussian distribution and equal variance. Correlation were carried out using GraphPad Prism 5. Characterisation results from gastrointestinal porcine fluids are presented as mean and standard deviations, corresponding literature data for human and dogs are presented as mean and standard deviations.

## 3. Results and discussion

### 3.1. Evaluation of porcine gastrointestinal fluid samples in the fasted state

The buffer capacity, osmolality, pH and surface tension of gastrointestinal fluids taken post-mortem from landrace pigs are presented in Table 2. The total phospholipid and bile salt content is presented in Table 3.

#### 3.1.1. pH

In the fasted state the pH values varied from 1.7 to 3.4 in the stomach (Fig. 1), with a mean value of 2.2 ± 0.7 (median: 1.9). These values are similar to reported gastric human data of 1.7–3.3 (median 2.5) under fasted conditions [15], but slightly higher than the fasted pH values in Yucatan minipigs (0.3–1.7) [16]. The pH in the fasted intestinal compartment ranged between 6.3 and 7.9 with a mean pH of 7.0 ± 0.5 (median: 7.0). The observed inter-variability was low indicating a consistent and well buffered pH.

**Table 2**
Overview of buffer capacity, osmolality and surface tension in porcine gastric and small intestinal fluid samples under fasted conditions (mean ± SD) in comparison to literature data for human gastrointestinal fluids (median, range, or mean ± SD).

| | | Landrace pigs | Humans | Canine |
|---|---|---|---|---|
| Buffer capacity [mmol $l^{-1}\triangle pH^{-1}$] | Gastric | 6.1 ± 3.5 | 14.3 ± 9.3[a] | 10.0[b] |
| | Intestinal | 19.4 ± 2.9 | 5.6[c] | 1.4 -4.2[c] |
| Osmolality [mOsm $kg^{-1}$] | Gastric | 99 ± 53 | 220 ± 58[a]<br>155–237[d] | 74.9 ± 6.0[b] |
| | Intestinal | 387 ± 61 | 197[c] | 69–207[c] |
| Surface tension [mN $m^{-1}$] | Gastric | 46.5 ± 1.3 | 31–45, median 36.8[e]<br>34.8 ± 5.2[a] | n.a. |
| | Intestinal | 36. 9 ± 2.6 | 33.6[c] | 31.1[c] |
| pH | Gastric | 2.19 ± 0.67 | 1.2 -6.0, median 1.94, average 2.5 ± 1.4[a]<br>2.9 ± 2.0[d] | 5.5<br>6.8 ± 0.2[f] |
| | Intestinal | 6.97 ± 0.53 | 6.7[c] | 7.1[c]<br>7.34 ± 0.12[b] |

[a] [18] ; [b][10], [c][19], [d][20], [e][21], [f][17].

In fasted dogs the pH is known to be higher compared to humans and pigs, an average pH in the stomach of 6.8 ± 0.2 has been described [17]. Dogs are often pre-treated with pentagastrin (6 µg/kg intramuscular) to decrease gastric pH to 1.7–2.2. Therefore, Arndt and co-workers established two different type of gastric canine media, one composed at pH 6.5, and the other at 1.5, to reflect both conditions [10]. The composition of gastric fluids and especially the pH can have a significant effect on drug solubility. The observed results in pigs support the suitability of pigs for matching pH conditions in the human GI tract, which is particularly relevant in the evaluation of drugs where the solubility is strongly pH dependent.

#### 3.1.2. Buffer capacity, osmolality and surface tension

The buffer capacity of porcine gastric fluids was 6.1 ± 3.5 mmol $l^{-1}\triangle pH^{-1}$. In the literature, values of 14.3 ± 9.3 mmol $l^{-1}\triangle pH^{-1}$ are reported for human gastric fluids [18]. Overall, given the wide intra-variability reported in reported values for humans, the buffer capacity in pigs determined in this study was similar to, albeit at the lower end, of the estimated range in humans [18].

The buffer capacity in porcine intestinal samples was 19.4 ± 2.9 mmol $l^{-1}\triangle pH^{-1}$, a 3.2- fold increase relative to gastric fluids. The higher buffering capacity of the intestinal fluids is in line with observations of low inter-pig variability in intestinal pH (Fig. 1). In fact, compared to human intestinal fluids, the buffer capacity of the

**Table 3**
Overview of lipid components in porcine gastric and small intestinal fluid samples under fasted conditions (n = 3, mean ± SD), in comparison to reported data of human intestinal fluids.

| [mM] | Landrace pig | | Human Intestinal Fluids |
|---|---|---|---|
| | *Gastric* | *Intestinal* | |
| Phospholipids total | 0.20 ± 0.18 | 0.37 ± 0.20 | 0.01–6.33, mean 0.95, median 0.58 [a]<br>0.003–2.7 (0.32 ± 0.51)[b] |
| Lysophospholipids | 0.048 ± 0.048 | 0.27 ± 0.18 | – |
| Phosphatidylcholine + Sphingomyelin | 0.15 ± 0.13 | 0.103 ± 0.024 | – |
| Bile Acids | 2.5 ± 1.7 | 28.3 ± 9.6 | 0.03–36.18, mean 4.16, median 3.30[a]<br>2.82[d] |
| Cholesterol | 0.051 ± 0.060 | 1.44 ± 0.77 | 0.00–0.48, mean 0.07, median 0.08 [a]<br>1.8[c] |
| Free Fatty Acids | 0.345 ± 0.081 | 2.8 ± 1.6 | mean 2.07, median 1.75[a] |

[a][22].
[b][23].
[c][25].
[d][19].

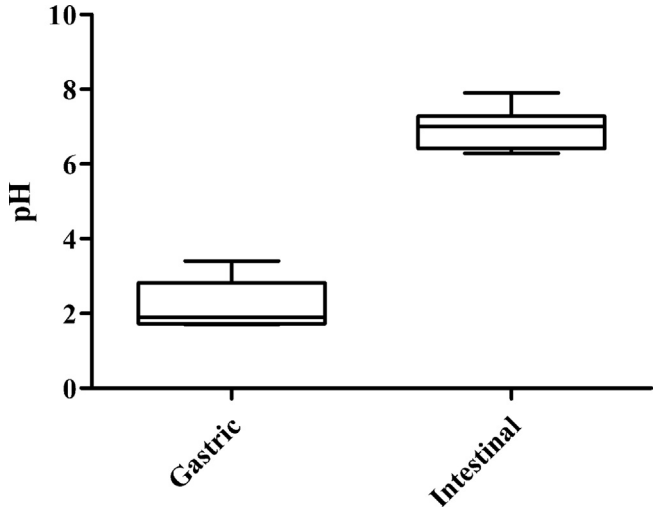


**Fig. 1.** pH values from gastric and intestinal fluid samples of landrace pigs under fasted conditions, (n = 8); Data is presented as box plot (min. to max, line median.).

pig's intestine is 3.4- fold higher and compared to canine intestinal fluids, the pig shows a 6.9- fold higher buffer capacity.

The surface tension was 46.5 ± 1.3 mN $m^{-1}$ in gastric porcine fluids and 36.9 ± 2.6 mN $m^{-1}$ in intestinal porcine fluids. A reduction in surface tension from the stomach to the intestine was expected due to the bile and phospholipid secretion in the upper small intestine. In general, the surface tension was lower than the surface tension of water (72 mN $m^{-1}$), mainly due to the interfacial surface activity of biliary lipids. The surface tension of porcine gastro-intestinal fluids was broadly similar to values previously reported for both humans and dogs.

The osmolality in intestine fluid samples of landrace pigs was 2-fold higher compared to reported literature values for human intestinal fluid samples [19]. However, in gastric fluids of landrace pigs, the determined osmolality of 99.33 ± 53.08 mOsm $kg^{-1}$ was at the lower end of reported human data (Table 2).

### 3.1.3. Phospholipids, cholesterol and fatty acids

The most prevalent phospholipid in the GI tract is phosphatidylcholine, which is hydrolysed to lyso-phosphatidylcholine in the lumen of the small intestine [21]. The total phospholipid amount in the porcine gastric fluids was 0.20 ± 0.18 mM, which increased to 0.37 ± 0.20 mM in intestinal pig fluids. The lyso-phosphatidylcholine concentration increased from 0.05 ± 0.05 mM in gastric fluids to 0.27 ± 0.18 mM in intestinal fluids. By comparison, in humans intestinal phospholipid concentration are reported to be in the range of 0.01–6.33 [22] and 0.003–2.7 [23]. Therefore, the total phospholipid concentration in the porcine intestinal fluids is at the lower end of the range in humans, albeit the variability in reported estimated in humans is relatively high.

The cholesterol concentrations determined in the porcine gastric fluids of 0.051 ± 0.060 mM was 28.3- fold lower compared to the cholesterol concentrations in the intestinal fluids of 1.442 ± 0.772 mM. The higher intestinal cholesterol concentrations are mainly explainable due to the entero-hepatic-circulation, which not only recycles bile salts but also cholesterol via the bile juices [24]. The reported human cholesterol data in the intestine of 1.8 ± 0.21 mM by Heikkila and co-workers compared well to the intestinal porcine concentrations determined in our study [25]. However, Riethorst and co-workers reported a range of 0.00–0.48 mM (median 0.08 mM) for humans, which would suggest that intestinal cholesterol concentrations were possible higher in pigs [22].

The observed total free fatty acid concentration in intestinal pig fluid was 2.82 mM. A mix of palmitic – (24.68%), stearic – (27.88%), oleic – (17.76%) and linoleic (29.68%) acids, was determined. In the intestinal fluids the free fatty acid concentration, which was 2.820 ± 1.633 mM, or approximately 8.2 fold higher compared to the gastric concentration. While up to 40% of lipid digestion occurs in the stomach [26], the majority of digestion is occurring in the small intestine, which would explain the higher free fatty acid levels in the intestine. In humans, the free fatty acids are reported to be approximately 2 mM in intestinal fluids [22], which was similar to the observed data in landrace pigs.

### 3.1.4. Bile acid concentration

In total 97.5% of the determined bile acids in intestinal fluids were conjugated with either glycine 59.3% or taurine 38.2%. Bile acid conjugates in porcine gastric fluids showed the same trend with 99.9% of bile acids conjugated with glycine (96.9%) or taurine (2.9%). The observed data is similar to the estimates described in the literature, that 97.2% of pig bile are conjugated bile acids [27].

The total bile salt concentration in intestinal samples of pigs ranged from 19.43 to 38.44 mM, which resulted in a mean bile salt concentration of 28.33 ± 9.56 mM (median: 27.12 mM) (Table 3). In general, the mean/median bile salt concentrations in porcine intestinal fluids was higher than estimates in humans (median 3.30), and the range of values in pigs was at the higher end of the reported ranges in humans (range 0.03–36.18 mM) [22]. By comparison, bile salt concentrations in canine intestinal fluids (9.39 mM) are generally closer, albeit on average higher, to estimated medium values in humans [14]. The reason for the high porcine bile salt concentrations are unclear, but

may reflect species differences. However, it must also be acknowledged that the sampling technique in the current study was different compared to previous work in humans and dogs. Our studies involved complete intestinal removal, collection of the entire section of intestinal fluids, which are then sampled, reflecting a mean sample of intestinal fluid from the small intestine. In contrast sample are taken by intubation in humans and dogs, which reflect samples from a specific region in the small intestine.

In the porcine gastric fluids, a mean bile salt concentration of 2.50 ± 1.73 mM was observed, which was higher than the gastric bile salt concentration of 0.3 ± 0.3 mM in humans [18]. The high bile concentrations in the stomach of pigs may indicate some gastro-intestinal reflux of bile from the duodenum into the stomach. Alternatively, this could reflect a methodological limitation of the current study during post-mortem sampling. While a ligature was placed between the stomach and intestine below the pyloric sphincter, we cannot exclude the possibly of some reflux occurring immediately after euthanasia, given that the ~5 min delay between laparotomy and placement of this ligature. The observed phospholipids/bile acid ratio in landrace pigs was 0.01 (mM/mM). This was in contrast to reported phospholipids/ bile acid ratio of 0.30 and 0.25 in humans and pigs, respectively [24]. In canine intestinal fluids a higher ratio of 0.86 between phospholipids and bile acids has been reported [14].

Bile acids differ in the number and position as well as the stereochemistry of hydroxyl groups to the steroidal structure. While the impact of bile acid structure is generally not considered in biopharmaceutical sense, a recent study showed that drug solubilisation capacity in bile salt micelles is influenced by the type of bile acid [28]. In particular, the ratio of primary bile acids (synthesized in the liver) to secondary bile acids (i.e. primary bile acids that have been enzymatically modified by intestinal gut microbiota) was shown to affect the solubilisation capacity of bile micelles for poorly water soluble drugs. In general, human and pig individual bile acids differ in their composition and synthesis [24]. One important difference of bile composition and synthesis between pigs and humans is the 6α-hydroxylation of chenodeoxy cholate to hyocholic acid. In humans hyocholic acid are only present in small amounts [29,30], whereas in pigs it was one of the key bile acids. While in humans the most common bile acids are cholic acid, chenodeoxycholic and deoxycholic acid, in pigs chenodeoxycholic acid (CDC) and hyodeoxycholate acid (HDC) are described to be one of the major bile acids. The concentration of 11 individual bile salts in the porcine fluids are represented in Table S1 (supplementary data).

The analysis demonstrated that the four dominant bile acids in fasted intestinal fluids were glycohyodeoxycholic acid (GHDC) 37%, taurohyodeoxycholic acid (THDC) 24%, glycochenodeoxycholic acid (GCDC) 19% and taurochenodeoxycholic acid (TCDC) 11% (Fig. 2), which is in line with porcine bile acid data previously published [27,31,32]. The dominant bile acids in gastric fluids were broadly in line with the major intestinal bile acids, albeit minimal taurine conjugated were found in the stomach (Fig. 2A). Interestingly, in both gastric and intestinal fluids the observed ratio of primary: secondary bile acids were ~1:2.

### 3.2. Solubility studies in gastric fluid samples

Gastric fluid samples were collected from three male landrace pigs post-mortem, after an overnight fasting period. For the four model drugs illustrated in Fig. 3, the solubility was compared to human gastric fluid (HGF), a biorelevant media simulating the fasted human gastric conditions (FaSSGF, Level II) and canine gastric fluid (CGF) [10,33,34]. For weakly basic drugs such as ketoconazole the solubility is highly pH dependent. The ketoconazole solubility observed in PGF was 5756.56 ± 647.69 μg/mL. By comparison to solubilities reported in HGF was 9025 μg/mL (pH 1.8) and 9054 μg/mL in FaSSGF (pH 1.6) [34], PGF solubility was 1.5- fold lower to HGF, which can be a result of the pH difference in pig gastric fluids, as the measured pH in porcine gastric fluid (PGF) was 2.19 ± 0.67 (Table 1.). The solubility of celecoxib, danazol and felodipine is pH independent over the physiological pH range. Celecoxib solubility in PGF was 4.57 ± 1.58 μg/mL, slightly higher compared to values observed in FaSSGF and CGF [10,33]. The same trend could also be detected for danazol. In the case of felodipine, a similar solubility in PGF (1.43 ± 0.08 μg/mL) and FaSSGF (1.40 μg/mL) was observed, while the solubility in canine gastric fluid was higher (2.51 ± 0.48 μg/mL) and lower in human gastric fluids (0.40 μg/mL) [34].

### 3.3. Development of a biorelevant porcine fasted state simulated intestinal media (FaSSIFp)

Based on the characterisation of the fasted intestinal porcine fluids a novel biorelevant media representing the fasted intestinal state in landrace pigs was developed (FaSSIFp). The pH of the media was set to 7.0 using a phosphate buffer and the buffer salt concentrations were adjusted to match the experimentally determined buffer capacity of 19 mmol $l^{-1}\triangle pH^{-1}$. While a range of buffers salts have been used to simulate intestinal fluids, the most common is phosphate buffers, including simulated human and canine intestinal [7]. The phospholipids were represented by lecithin and used at a ratio of 0.013 (phospholipid mM/ bile mM) as determined in this study (see section 3.1.4). Based on previous reports that the ratio of primary: secondary bile salts may impact solubilisation capacity in simulated intestinal media, the bile salts were added at a physiological ratio of 35% primary bile acids and 65% secondary bile acids (as discussed in Section 3.1.4). However, due to limited availability of sufficient quantities of common porcine bile salts such as taurohyodeoxycholate, glycohyodeoxycholate and glycochenodeoxycholate, sodium taurocholate and sodium taurodeoxycholate were used as the primary and secondary bile salts respectively in FaSSIFp. A high concentration of bile acids seems to influence drug solubilisation and therefore it appears to be important to take physiological ratios of primary: secondary bile acids into account [28], but at physiological relevant concentrations it has been hypothesized that the steroid structure of the bile salts have a negligible impact on the drug solubilisation, suggesting a flexibility of bile acid selection in preparation of simulated media [35]. In order to determine the effective concentrations of the TC: TDC (35:65) bile salt mixture that achieve similar overall drug solubilities as porcine intestinal fluids (PIF), the previously reported method for determined effective bile salt concentrations was used [10].

For an overall assessment of the impact of selecting TC and TDC overall, on drug solubility, an indirect approach of solubility measurement was employed using a diverse set of different drugs, i.e. celecoxib (BCS II), danazol (BCS II), ketoconazole (BCS II), felodipine (BCS II), dipyridamole (BSC II) and venetoclax (BSC IV). Solubility was measured *ex vivo* in porcine intestinal fluids (PIF) and compared to the solubility in simulated buffers with increasing PL and BS concentrations, while the ratio of PL:TC:TDC (1.3: 34.6: 64.1) remained fixed. This generated six drug specific 'standard bile concentration versus solubility curves' (Fig. 4). Using this standard calibration curve, the experimentally determined solubility in PIF was used to work back the effective concentration of PL:TC:TDC that achieves the same drug concentration in the simulated media.

The effective concentrations of PL:TC:TDC were similar for five drugs (celecoxib, danazol, felodipine, dipyridamole and venetoclax). Ketoconazole showed a higher concentration of PL:TC:TDC, reflecting the higher inherent solubility in pig intestinal fluids. The final TC, TDC and PL concentration for the porcine biorelevant media was calculated as the mean of concentrations obtained with all six drugs. A representative total bile salt concentration of 14.97 mM (5.25 mM sodium taurocholate and 9.72 mM sodium taurodeoxycholate) and a total phospholipid concentration of 0.20 mM was determined using this approach (Supplementary data, Table S.2). Subsequently, 2.82 mM

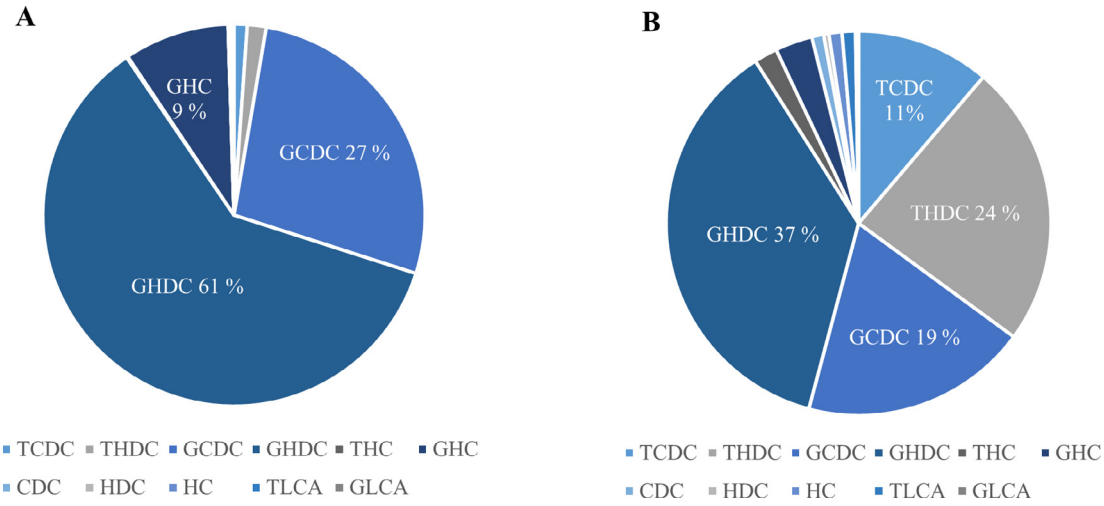


**Fig. 2.** Percentage of bile acids from porcine gastric (A) and intestinal fluid (B) samples under fasted conditions, (n = 3). Abbreviations: TCDC = Taurochenodeoxycholic acid; THDC = Taurohyodeoxycholic acid; GCDC = Glycochenodeoxycholic acid; GHDC = Glycohyodeoxycholic acid; THC = Taurohyocholic acid; GHC = Glycohyocholic acid; CDC = Chenodeoxycholic acid; HDC = Hyodeoxycholic acid; HC = Hyocholic acid; TLCA = Taurolithocholic acid; GLCA = Glycolithocholic.

sodium oleate was added to prepare the final media to match concentration of free fatty acids determined in this study (see section 3.1.3). Finally, the osmolality was adjusted to 386.67 mOsm $kg^{-1}$ as determined in PIF using 134.32 mM sodium chloride. The final media composition is summarised in Table 4.

### *3.4. Solubility of six model drugs in porcine gastrointestinal fluids (PIF) and corresponding porcine biorelevant media (FaSSIFp)*

The solubility of six model drugs was determined in FaSSIFp and compared to the solubilities that were determined in PIF and no statistically significant difference was observed (Fig. 5). The correlation of solubility in PIF and FaSSIFp is illustrated in Fig. 6B. The porcine biorelevant media was able to forecast the solubility in PIF for the

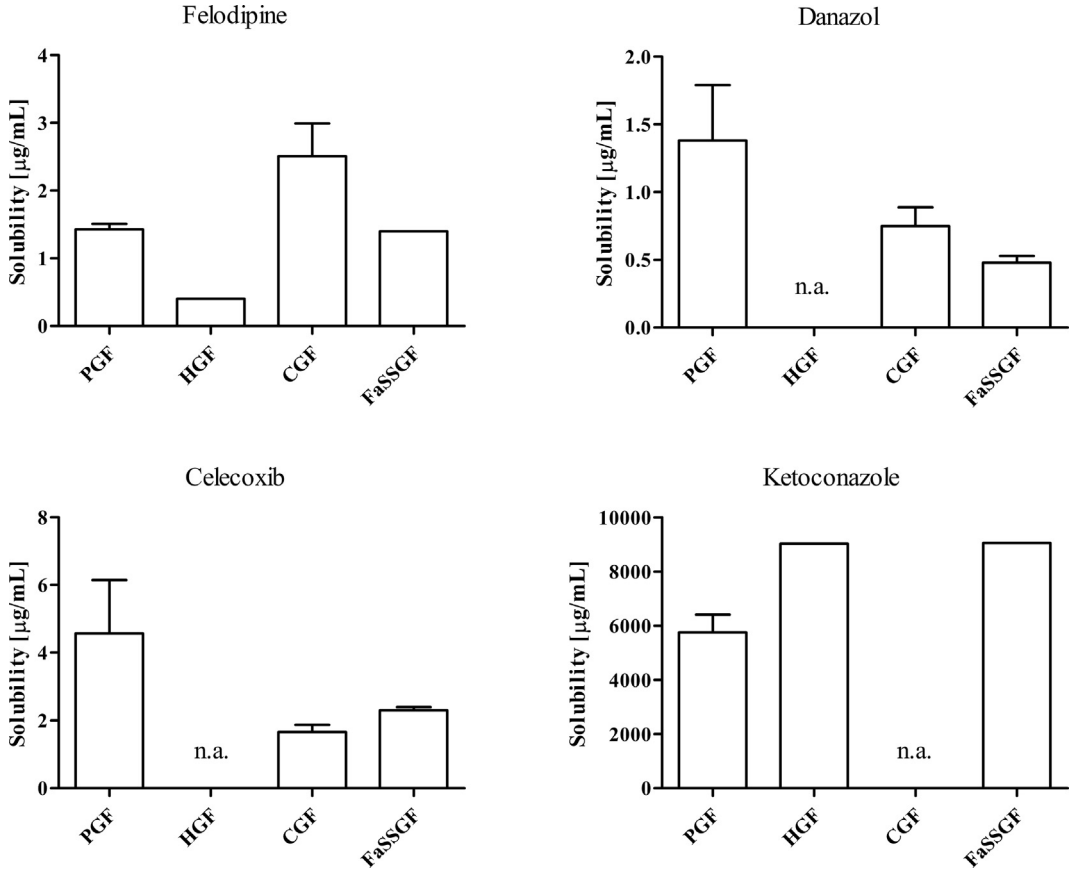


**Fig. 3.** Solubility of felodipine, danazol, celecoxib and ketoconazole in pig gastric fluids (PGF), compared to reported values, in human gastric fluid (HGF), canine gastric fluid (CGF) and human Fasted State Simulated Gastric Fluid (FaSSGF), [10,33,34]; data is presented as mean ± SD, n = 3.

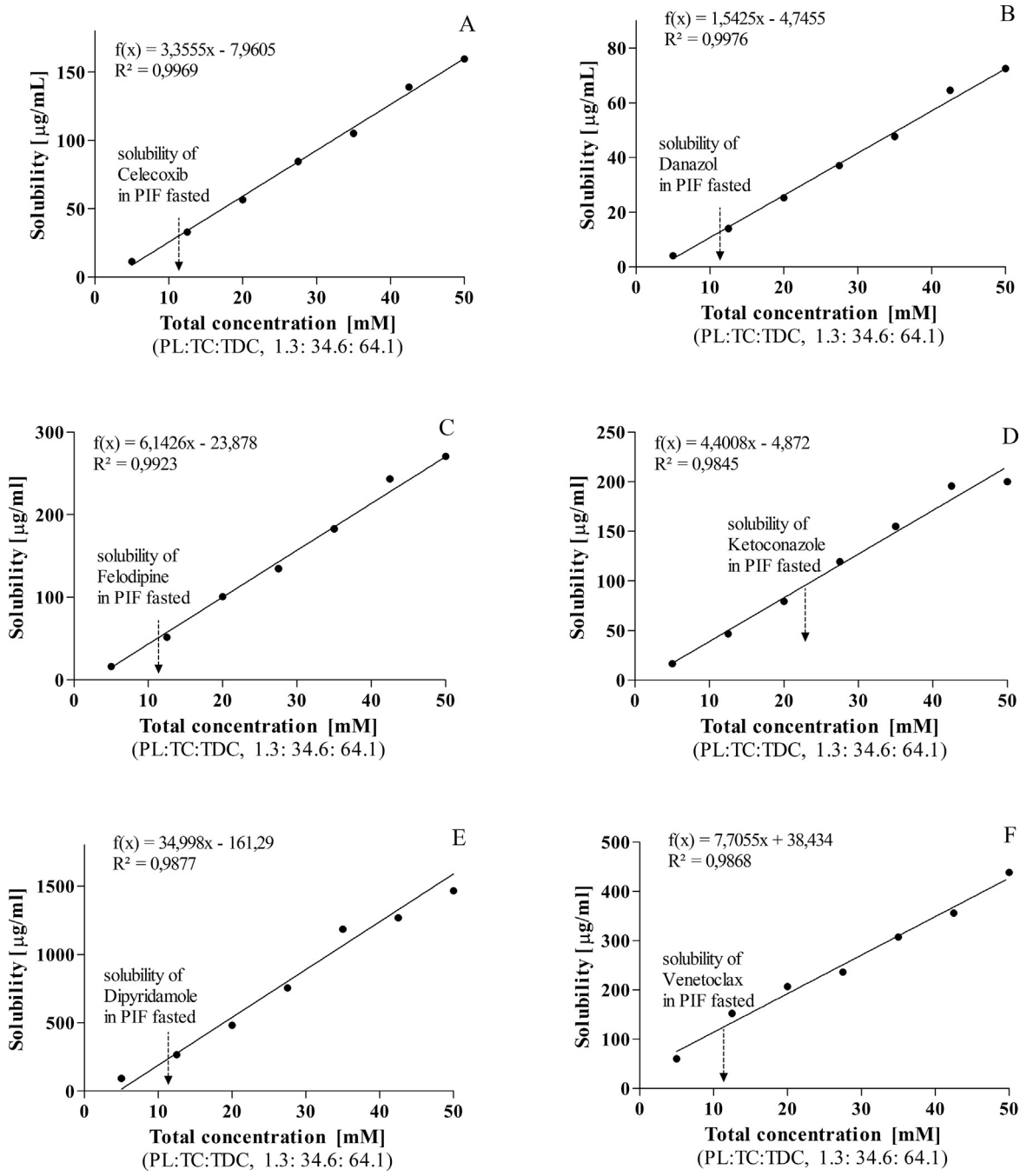


**Fig. 4.** Total phospholipid and bile salt concentration versus solubility standard curves for six drugs. Estimation of effective bile salt (BS) and phospholipid (PL) concentration in porcine intestinal fluid (PIF). BS concentration is represented by sodium taurocholate (TC) and sodium taurodeoxycholate (TDC) and PL concentration is represented by lecithin, at a fixed ratio of 1.3:34.6:64.1 (PL:TC:TDC); A: Celecoxib; B: Danazol; C: Felodipine; D: Ketoconazole, E: Dipyridamole; F: Venetoclax.

chosen model drugs with good accuracy. Celecoxib, a weak acid, had a solubility in PIF (34.5 ± 4.85 µg/mL) that matched the solubility in FaSSIFp (36.3 ± 4.3 µg/mL). Also, for the neutral compound felodipine had a solubility in PIF (48.2 ± 9.2 µg/mL), which was similar to FaSSIFp (64 ± 18 µg/mL). In the case of the neutral compound danazol, the solubility in PIF of 13.2 ± 2.4 µg/mL was slightly overestimated in FaSSIFp with a solubility of 19.34 ± 0.96 µg/mL. The solubility of weak bases, such as ketoconazole, dipyridamole and venetoclax is highly dependent on the pH with the general trend of higher solubility at lower pHs. Venetoclax had a solubility of 163 ± 43 µg/mL in PIF, which was similar to FaSSIFp (154 ± 15 µg/mL) and also the solubility of dipyridamole in PIF (266 ± 41 µg/mL) correlated well with the solubility in FaSSIFp (272 ± 19 µg/mL), albeit showing a higher variability. Ketoconazole had a solubility of 91.7 ± 4.2 µg/mL in PIF, while the solubility in FaSSIFp was slightly lower (71 ± 13 µg/mL). Overall, despite slight variations, FaSSIFp was well able to capture drug specific high or low solubility resulting in a correlation of $r^2$ 0.9825.

While the overall bile salt and lecithin concentrations in the final assembled FaSSIFp were lower compared to concentration determined quantitatively in section 3.1.4 (~28 mM), for practical and availability considerations it was not feasible to use the full range of porcine bile acids. However, our approach is physiologically relevant in the context of applying a fixed ratio of primary: secondary bile acids as well as

**Table 4**
Composition and physiochemical properties of Fasted State Simulated Intestinal Fluid porcine (FaSSIFp).

| Composition | FaSSIFp | mM |
|---|---|---|
| Buffer | Sodium dihydrogen phosphate | 35.71 |
| | Sodium hydroxide | 13.62 |
| | Sodium chloride | 135.32 |
| Bile salts | Sodium taurocholate | 5.25 |
| | Sodium taurodeoxycholate | 9.72 |
| Phospholipid | Lecithin | 0.20 |
| Fatty acid | Sodium oleate | 2.82 |
| pH | 7.0 | |
| Buffer capacity [mmol $l^{-1}\triangle pH^{-1}$] | 19.4 | |
| Osmolality [mOsm $kg^{-1}$] | 387 | |

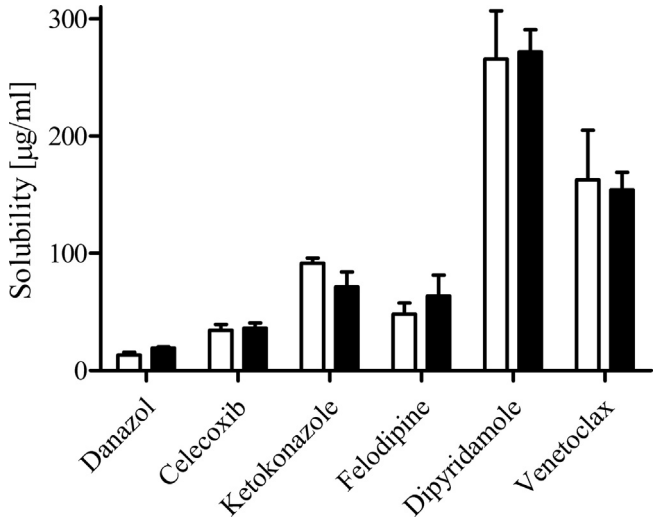


**Fig. 5.** Comparison of the solubility of six model drugs in pig intestinal fluids (PIF) (white bars), and in porcine Fasted State Simulated Intestinal Fluid (FaSSIFp) (black bars), data is presented as mean ± SD, n = 3.

matching the bile acid: phospholipid ratio determined for pig intestinal fluids. In addition, it should be noted that the use of the measured PIF total bile and phospholipid concentrations of approximately 28 mM and 0.374 mM, respectively, would overestimated the *in vivo* solubility of the drugs (based on the standard bile concentration versus solubility curves in section 3.3). The effective bile salt and phospholipid concentrations of 14.97 mM and 0.20 mM for the composition of FaSSIFp resulted in a good overall correlation, which supports the feasibility of the applied development approach.

### *3.5. Comparison of the measured solubility in porcine intestinal fluids to human biorelevant media*

FaSSIFp contains phospholipids and bile salts at the physiological ratio of 0.013 (mM/mM) with an effective bile acid concentration of 14.97 mM (5.25 mM sodium taurocholate and 9.72 mM sodium taurodeoxycholate) and a phospholipid concentration of 0.20 mM (lecithin). In comparison to FaSSIF, which has a taurocholate concentration of 3 mM and a phospholipid concentration of 0.75 mM, FaSSIFp shows a 5.0-fold higher bile salt and a 3.8-fold lower phospholipid content. In comparison to FaSSIF-V2, where the phospholipid content had been changed to 0.2 mM, FaSSIFp still demonstrates a 5.0-fold higher bile salt concentration but an identical phospholipid concentration. FaSSIF-V3, which has a bile salt concentration (taurocholate and glycocholate) of 2.8 mM and a phospholipid concentration of 0.07 mM, FaSSIFp shows a 5.3-fold higher bile salt and a 2.9-fold higher phospholipid content.

It has been reported that the amount of lecithin displays a significant role in estimations of *in vivo* solubility, especially for neutral drugs [35]. Therefore, in many cases FaSSIF overestimated drug solubilites of neutral compounds when compared to human intestinal fluids (HIF). In FaSSIF-V2, this was corrected by reducing the phospholipid content, which seemed more appropriate for reliable solubility estimations [35]. For example, in the case of the highly lipophilic (log*P* 3.56) BCS class II drug felodipine [34], the solubility in FaSSIF (47.6 ± 1.1 µg/mL) [10] overestimated the solubility in HIF (14.00 µg/mL) [35,36]. When compared to PIF (48.25 ± 9.25 µg/mL) the solubility of felodipine in FaSSIF was similar and in fact due to the high variability also similar to the felodipine solubility in FaSSIFp (63.64 ± 17.80 µg/mL). For another model drug celecoxib, FaSSIF solubility (46.2 ± 0.03 µg/mL) [10] was even higher when compared to PIF (34.46 ± 4.81 mg/mL). In this case, using FaSSIF would have overestimated *in vivo* solubility for celecoxib, while FaSSIFp showed a similar solubility (36.29 ± 4.28 mg/mL) when compared to PIF. Interestingly, in the case of the highly lipophilic (log*P* 4.53) drug danazol, FaSSIFp slightly overestimated the solubility in PIF (13.19 ± 2.36 µg/mL), nevertheless FaSSIFp would capture the trend towards a higher solubility more accurately than human FaSSIF. Clarysse and co-workers reported that FaSSIF underestimated the solubility (5.4 ± 0.3 µg/mL) compared to HIF (reported range: 2.04 ± 1.5 up to 13.2 ± 14.1 µg/mL) [35–41]. In general, for neutral compounds the developed porcine media (FaSSIFp) showed the capability to predict the solubility in PIF, and displayed an advantage over the commonly used human biorelevant media in predicting *in vivo* conditions in pigs (Fig. 6). Furthermore, this study confirms that using human biorelevant media in estimating *in vivo* solubility in pigs is not reliable given the overall poor level of correlation $r^2 = 0.12$.

Weak bases display higher solubility at acidic pH, due to their ionization characteristics. Upon oral administration in the fasted state, concentration gained in the gastric compartment, can exceed solubility in the contents of the intestinal lumen, and precipitation in the intestinal lumen may occur [10]. In the case of the weak base dipyridamole, solubility values in PIF showed high variability (range: 240–313 µg/mL) and an approximately 9-fold higher solubility compared to HIF. While such high variability has not been reported in HIF solubility studies of dipyridamole (29 ± 0.4 µg/mL) [35], large variability has also been reported in canine intestinal fluids (range: 25–95 µg/mL,) where it was suggested to reflect fluctuations in bile concentrations in intestinal fluids [10,14]. In comparison to HIF and PIF, FaSSIF (19 ± 0.4 µg/mL) underestimated dipyridamole solubility, while FaSSIFp (272 ± 19 µg/mL) matched the solubility of PIF. Similar results were obtained for the weak base venetoclax. While the PIF (163 ± 43 µg/mL) and FaSSIFp (153.97 ± 15.08 µg/mL) solubilites were similar, the solubilites measured in FaSSIF (5.2 ± 0.1 µg/mL) under predicted the scenario in pig intestinal fluids showing a 30-fold lower solubility. In the case of the weak base ketoconazole, solubility in PIF 91.7 ± 4.2 µg/mL was in the range of reported solubility values in HIF. Multiple independent studies reported the solubility of ketoconazole in HIF, obtained results ranged from 28.8 ± 3.0 µg/mL up to 326 ± 366 µg/mL [19,36,39–41]. However, FaSSIF (26 ± 5.2 µg/mL) underestimated the solubility in PIF, as the solubility in PIF was 3.5- fold higher [40]. In general, also in the case of weak bases FaSSIFp demonstrated a superior predictability of *in vivo* solubilities in PIF compared to the human media FaSSIF.

In summary, for all six model drugs, the use of FaSSIFp resulted in a better correlation to the PIF solubility than the values reported with FaSSIF (Fig. 6). While drug solubilites in HIF have been extensively investigated and reviewed [42], this study provided a first insight into PIF drug solubilities using 6 model drugs. A limitation of the current proposed porcine media is that this was designed based on the landrace pig model, and while there are general similarities across the various species of pigs [27], inter-species differences may lead to different intestinal conditions and potentially limit reliability of the FaSSIFp for

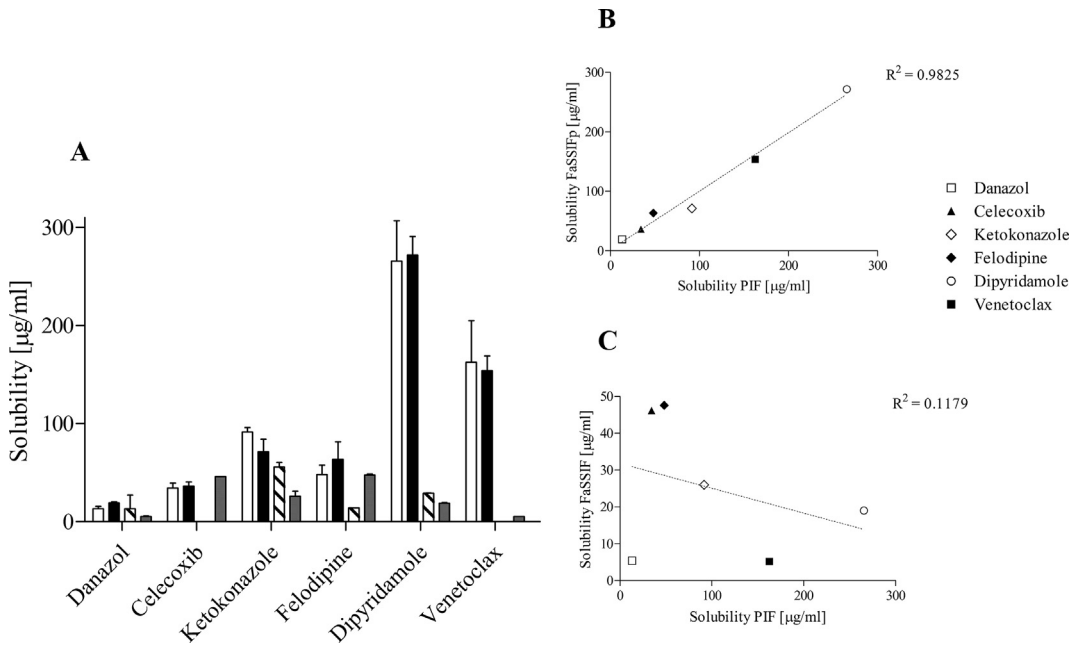


**Fig. 6.** A: Comparison of the solubility of six model drugs in pig intestinal fluids (PIF) (white bars), and in porcine Fasted State Simulated Intestinal Fluid (FaSSIFp) (black bars), to published literature of human intestinal fluid samples (HIF) (dashed bars) and human Fasted State Simulated Intestinal Fluid (FaSSIF) (grey bars); data is presented as mean ± SD, n = 3; B: Correlation of the solubility of six model drugs in pig intestinal fluids (PIF) and in porcine Fasted State Simulated Intestinal Fluid (FaSSIFp); C: Correlation of solubility of six model drugs in pig intestinal fluids (PIF) and human Fasted State Simulated Intestinal Fluid (FaSSIF).

other species. Nevertheless, landrace pigs have been widely used for preclinical evaluations [12,43–45], and therefore FaSSIFp specially designed on landrace pig model, provide a useful *in vitro* tool. Last it should be considered that FaSSIFp contains taurocholate (TC) and taurodeoxycholate (TDC) as bile acids, which is distinctly different to the variety of determined pig bile components in this study. The use of a highly complex media may offer a more precise simulated media. While quantitatively observed total bile acid concentration ranged between 19.43 and 38.44 mM, an effective bile acid concentration of 14.97 mM (5.25 mM sodium taurocholate and 9.72 mM sodium taurodeoxycholate) in FaSSIFp was used to estimate the *in vivo* solubility. While this latter approach suggested that the working concentration of TC and TDC is was lower than the total sum of the range of individual bile salts determined by mass spectrometry in PIF, we believe that this approach was more accurate in terms of predicting drug solubility in the pig intestine and as a tool for modelling drug absorption *in vivo*. However, there are also applications where the absolute concentration of bile salts reported in Table 3 may be more applicable such as specific input parameters for PBPK modelling, to utilize and integrate intestinal bile porcine concentrations into the applied absorption model. Nevertheless, FaSSIFp was markedly superior in predicting the solubility of the model drugs in pig intestinal fluids compared to human biorelevant media.

The key impact of this study is that by integrating species specific *in vitro* testing with *in silico* PBPK modelling, more reliable insights can be achieved in preclinical pharmacokinetic studies to assess the impact of drug formulation *in vivo* [46]. As an example, Walsh and co-workers emphasised that using the canine fasted simulated intestinal media, resulted in a better predictability of the plasma concentration profiles in dogs [46]. In comparison human intestinal media was assessed, with the result that FaSSIF significantly under predicted the observed absorption in dogs due to a lower solubility. Improvements of biorelevant media based on species-specific findings will further enhance the correlation, interpretation and extrapolation from *in vitro* data and can be employed to allow drug formulation characterisation in advance of *in vivo* preclinical testing. Based on the correlations of *in vivo* solubility in PIF and the capability of FaSSIFp to estimate the *in vivo* solubility, the established porcine biorelevant media seems to be a promising tool for predicting *in vivo* performance of oral dosage forms in pigs.

## 4. Conclusion

In summary, by comprehensively characterising the GI fluids in landrace pigs, including lipid composition, pH, buffer capacity, osmolality and surface tension, this study established the basis for a designing a media that simulated intestinal conditions in fasted pigs. The final FaSSIFp was designed to mimic PIF by matching both the BS:PL and primary: secondary ratio found in porcine GI fluids. The solubility of drugs in porcine simulated media were highly correlated ($r^2$ 0.98) to solubility obtained in PIF, whereas poor correlation ($r^2$ 0.12) was obtained using human simulated fluids. Therefore, the resulting porcine biorelevant media, specially designed for pigs used in pharmacokinetic studies, can be a useful tool in predicting *in vivo* performance early in preclinical studies of drugs. Porcine biorelevant media can be recommended to achieve more accurate predictions and interpretation of pharmacokinetic studies after oral administration of new drug candidates.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2020.06.009.

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