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Gastrointestinal diseases and their impact on drug solubility: Ulcerative Colitis

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Abstract

For poorly soluble compounds, drug product performance in patients with Ulcerative Colitis (UC) compared to healthy subjects can be affected due to differences in drug solubility in GI fluids. A risk assessment tool was developed to identify compounds with a high risk of altered solubility in the GI fluids of UC patients. Pathophysiological changes impacting on the composition of GI fluids in UC patients were considered and UC biorelevant media representative of the stomach, intestine and colon were developed based on alteration of biorelevant media based on healthy subjects and literature data using a Design of Experiment approach. The UC media were characterised and revealed differences in surface tension, osmolality and buffer capacity compared to media based on healthy subjects. The solubility of six drugs was investigated in UC biorelevant media and results were related to media- and drug-dependent factors. A lower drug solubility in UC intestinal media was observed for compounds with a high lipophilicity. In UC simulated colonic fluids, drug solubility was altered for ionisable compounds. Additionally, a higher solubility of neutral lipophilic drugs was observed in UC fasted state colonic media with increased concentrations of soluble proteins. The developed UC biorelevant media offer the possibility to identify the risk of altered drug solubilisation in UC patients without conducting expensive clinical trials. A high risk was related to drug ionization properties and lipophilicity in the current study with all investigated drugs showing differences in solubility in biorelevant media based on UC patients compared to healthy subjects.

Keywords

Gastrointestinal diseases; Ulcerative Colitis; Inflammatory Bowel Disease; Biorelevant media; Physicochemical properties; Solubility

1. Introduction

Ulcerative Colitis (UC), a main type of inflammatory bowel disease (IBD), is an autoinflammatory disorder that affects approximately 2.1 million people in Europe (Burisch et al., 2013). The inflammation manifests itself in ulcerations of the lining of the large intestine, which are confined to the mucosa and submucosa. Typically, the first appearance of the disease is limited to the rectum and further disease progression leads to a proximal extension to the colon. According to the disease location, the Montreal classification system groups UC in Ulcerative proctitis (rectum is affected), left-sided UC (a proportion of the colorectum distal to the splenic flexure is affected) or extensive colitis (entire large intestine is affected) (Silverberg et al., 2005). UC can also be grouped in four different disease states according to symptom severity: mild, moderate, severe or a state of clinical remission (Silverberg et al., 2005).

The different states and locations of UC necessitate different treatment options and drug formulation approaches. The classic step-up approach includes aminosalicylates as first treatment option in mild to moderate UC (Berends et al., 2019). For this treatment, different drug formulations can be used based on disease location with suppositories and enemas for distal UC and/or controlled-release or prodrug formulations of mesalamine, when more proximal parts of the colon are affected. Corticosteroids are used to induce remission in moderate to severe disease states (Berends et al., 2019). Drug formulations include immediate-release formulations of systemic corticosteroids or controlled-release formulations of the topical steroid budesonide (e.g., Uceris® [Santarus, San Diego, CA, USA]). For active UC, the next therapeutic option is a co-treatment with thiopurines such as azathioprine due to their slow onset of therapeutic action. Further treatment options are calcineurin inhibitors for severely active UC or monoclonal antibodies as the last therapeutic option (Berends et al., 2019). Additionally, several new treatment options are emerging: The oral JAK inhibitor tofacitinib

has been approved for the treatment of moderate to severe UC and several other small molecules are enrolling in phase III clinical trials (Ma et al., 2019).

Consequently, drug delivery via the oral route is commonly used in UC for topical as well as systemic drug therapy. Other drugs which are prescribed more often in IBD patients than the general population include e.g., antidepressants, antibiotics and nonsteroidal anti-inflammatory analgesics (Haapamaki et al., 2013). Successful drug delivery via the oral route is dependent on gastrointestinal (GI) physiology and drug/formulation properties. Various processes such as drug release/dissolution, permeation through the GI membrane and gut or hepatic metabolism can be influenced by an altered GI physiology in UC (Bai et al., 2016; Effinger et al., 2019; Hatton et al., 2018, 2019). Since clinical trials to assess drug product performance in UC patients are rarely performed due to high costs, a heterogenous patient population and a high time effort, possible effects on the drug therapy of UC patients are not investigated in most cases. Therefore, alternative tools to predict drug product performance in UC patients are needed.

In vitro release and dissolution testing can be used as surrogate for the *in vivo* performance of poorly soluble compounds with solubility- or dissolution rate-limited absorption (Amidon et al., 1995). For this purpose, biorelevant media have been developed based on healthy subjects to simulate GI fluids of different GI compartments and prandial states and to evaluate drug products *in vitro* (Galia et al., 1998; Jantratid et al., 2008; Markopoulos et al., 2015; Vertzoni et al., 2010a; Vertzoni et al., 2005). For colonic fluids, fasted and fed state conditions were considered as extreme conditions expected in a clinical study setting. Since UC can alter the GI fluid composition of patients, drug product performance could be affected for these drugs. The development of biorelevant media for UC patients would allow the identification of differences in drug solubility or dissolution compared to the biorelevant medium based on

healthy subjects, which would indicate a high risk of altered drug product performance in UC patients.

This study aims to develop a risk assessment tool to identify compounds with a high risk of altered solubility in the GI fluids of UC patients. Pathophysiological changes impacting on the composition of GI fluids in UC patients were considered and UC biorelevant media representative of the stomach, intestine and colon were developed based on alteration of biorelevant media developed based on healthy subjects and literature data using a Design of Experiment approach. Subsequently, the developed UC biorelevant media were characterised according to their surface tension, osmolality, buffer capacity and dynamic viscosity and the solubility of six poorly soluble compounds with different physicochemical properties was determined in UC biorelevant media. To identify if certain drug characteristics contribute to a higher risk of altered drug solubility in GI fluids of UC patients, Partial least Squares (PLS) regression was used to correlate drug properties and media-dependent factors with the relative effect on drug solubility.

2. Materials

Acetic acid High Performance Liquid Chromatography (HPLC) grade, pepsin from porcine gastric mucosa, sodium oleate, α-D-glucose, budesonide, phosphoric acid and sodium hydroxide were purchased from Sigma-Aldrich Company Ltd., Dorset, England. Sulfasalazine, loperamide hydrochloride, dipyridamole, celecoxib, azathioprine, methanol HPLC grade and acetonitrile HPLC grade were purchased from VWR International Ltd, Lutterworth, UK. Tris(hydroxymethyl)aminomethane, hydrochloric acid 36.5–38%, sodium chloride, trifluoroacetic acid (TFA), potassium dihydrogen orthophosphate, bovine serum albumin protease free powder fraction V (BSA), dimethyl sulfoxide and maleic acid were used from Fisher Scientific UK Ltd., Loughborough, England. Other chemicals used included sodium taurocholate (Prodotti Chimici Alimentari S.P.A., Basaluzzo, Italy), egg lecithin–Lipoid EPCS

(Lipoid GmbH, Ludwigshafen, Germany), glyceryl monooleate–Rylo Mg 19 (Danisco, Brabrand, Denmark) and cholic acid sodium salt (VWR International Ltd, Lutterworth, UK). Water was ultra-pure (Milli-Q) laboratory grade.

3. Methods

3.1. Media development

3.1.1. GI pathophysiological changes in UC patients integrated in the experimental design

Information from literature was collected to identify differences in the composition of GI fluids of UC patients compared to healthy subjects. For studies with graphically displayed data, the relevant information was extracted with WebPlotDigitizer (Rohatgi, 2018). Apart from components and properties directly measured in the GI fluids of UC patients, an additional factor, namely the lecithin levels measured in the GI mucosa, was considered as indirect factor due to the limited number of studies performed in UC patients. All factors were integrated with two levels in the experimental design. The low and the high level were selected based on the available information on the respective parameter as described in Section 3.1.1.1-3.1.1.3.

3.1.1.1. Lecithin concentration

The lecithin concentration was included as an indirect factor in the experimental design of UC gastric, intestinal media and fed state colonic UC media.

Lecithin is a constituent of the GI mucosa and essential to maintain the normal mucus barrier function. It has been shown that the lecithin concentration in the intestinal mucus barrier of patients with UC was decreased by over 70% compared to healthy subjects (Braun et al., 2009; Ehehalt et al., 2010). Considering the disease state, 6 investigated UC patients were in remission and 15 in relapse (Braun et al., 2009). The lecithin in the colonic mucus barrier is likely to be from secretions by jejunal and ileal enterocytes as investigated in rat intestinal perfusion studies (Ehehalt et al., 2004). Therefore, decreased lecithin concentrations are likely

to be present also in more proximal parts of the GI tract than the colon. The treatment of UC patients with a delayed-release oral formulation of lecithin has shown to increase the amount of lecithin in rectal mucus and reduce inflammatory activity (Stremmel et al., 2010).

Lecithin is also an essential constituent of bile and can emulsify hydrophobic molecules due to its amphiphilic structure. Hepatobiliary manifestations are common in UC patients and include primary sclerosing cholangitis (PSC), small duct PSC, chronic hepatitis, cryptogenic cirrhosis, cholangiocarcinoma and cholelithiasis (Lichtenstein, 2011). The most common of these conditions is PSC with an incidence of 2.5 to 7.5% in patients with UC (Lichtenstein, 2011). PSC leads to the formation of bile duct strictures impeding the flow of bile to the intestine. Consequently, reduced bile salt and lecithin concentrations are likely to be present in the GI fluids of the affected UC patients. Decreased concentrations of bile acids and lecithin were already observed in intrahepatic bile specimens of patients with PSC (Gauss et al., 2013). Decreased lecithin concentrations in UC patients compared to healthy subjects were also observed in gallbladder bile in the fasted state obtained by cholecystokinin-stimulated, duodenal biliary drainage (Marks et al., 1977).

Apart from the ascending colon fluid, no studies investigated the concentration of lecithin in the remaining luminal fluids of UC patients. Therefore, the lecithin levels for the DoE were based on an indirect percental approach in all media except UC-Fasted-State Simulated Colonic Fluid (FaSSCoF) according to

$$x_{UC-BM} = 0.30 * x_{H-BM} \quad (1)$$

where x_{UC-BM} is the low level of the lecithin concentration in UC media, x_{H-BM} is the lecithin concentration in biorelevant media based on healthy subjects and the factor 0.30 represents the ratio of lecithin previously observed in the colonic mucus layer of UC patients compared to healthy subjects (Braun et al., 2009; Ehehalt et al., 2010). Therefore, the low lecithin level in UC biorelevant media is set to 30% of the concentration in corresponding healthy biorelevant

media and the high lecithin level corresponds to the concentration in biorelevant media based on healthy subjects.

3.1.1.2. Fasted state ascending colon fluid

The fasted state ascending colon fluid of UC patients in states of relapse and remission (defined based on the Clinical Rachmilewitz Index (CRI)) has previously been characterised (Vertzoni et al., 2010b). A higher osmolality was observed in patients with UC in remission compared to patients in relapse and healthy subjects, as shown in Table 1 (Diakidou et al., 2009; Vertzoni et al., 2010b). For the experimental design, the osmolality was integrated with a low level of 196 mOsmol/kg, corresponding to the osmolality of FaSSCoF and similar to the osmolality observed in UC patients in relapse, and a high level of 290 mOsmol/kg representative of UC patients in remission.

The mean total bile acid concentration was lower in UC patients in relapse compared to patients in remission and healthy subjects as shown in Table 1 but the difference reached no statistical significance as the power of the test was low (Diakidou et al., 2009; Vertzoni et al., 2010b). For the experimental design, the bile salt concentration was integrated with a low level of 75 μM representative of UC patients in relapse and a high level of 150 μM (bile salt concentration of FaSSCoF, similar bile salt concentration in healthy and UC patients in remission).

The concentration of soluble proteins was not significantly different between patients in relapse and remission but significantly higher compared to healthy subjects [Table 1] (Diakidou et al., 2009; Vertzoni et al., 2010b). For the experimental design, the concentration of soluble proteins was integrated using bovine serum albumin with a high level of 19 mg/mL representative of UC patients in relapse and remission and a low level of 0 mg/mL based on the concentration in FaSSCoF.

188 The lecithin concentrations in the fasted state ascending colon fluid in UC patients in remission
189 and relapse were in the range of 0.13 to 0.62 mM (graphically extracted) (Vertzoni et al.,
190 2010b). While the mean concentration of lecithin in the fasted state ascending colon fluid of
191 UC patients was lower compared to healthy subjects, the difference did not reach statistical
192 significance; a high data variability was observed in this study for the lecithin concentration.
193 For the experimental design, the lecithin concentration was included as factor with the observed
194 range as low and high level.

195 The pH in the fasted state colonic fluid of UC patients was in the range of 5.5 to 7.7 considering
196 both disease states [Table 1] (Vertzoni et al., 2010b). For the experimental design, the pH was
197 included as factor with a low level of 5.5 and a high level of 7.7 representative of the pH range
198 observed in UC patients.

199 The buffer capacity of the fasted state ascending colon fluid was higher in UC patients in
200 relapse and remission compared to healthy subjects as measured with hydrochloric acid [Table
201 1] (Diakidou et al., 2009; Vertzoni et al., 2010b). Due to the high number of factors integrated
202 in the experimental design for the fasted state colonic UC media, the buffer capacity was not
203 included. Consequently, the effect of a higher buffer capacity on, for example, weakly acidic
204 drugs (high dose) that are likely to decrease the luminal pH (depending on the buffer capacity
205 of the GI fluids), which in turn reduces their luminal solubility, is not captured.

206

Table 1: Overview of characteristics of fasted state ascending colon fluid of UC patients and healthy subjects and composition of FaSSCoF.

	UC patients in relapse (Vertzoni et al., 2010b)	**UC patients in remission (Vertzoni et al., 2010b)**	**Healthy subjects (Diakidou et al., 2009)**	**FaSSCoF (Vertzoni et al., 2010a)**
Osmolality [mOsmol/kg]	199.6±127.4	290.1±165.6	80.6±102.5	196
Concentration of bile acid [μM]	75.83±42.96	115.15±100.20	115.2±119.3	150
Concentration of soluble proteins [mg/mL]	18.9±8.1	19.0±10.8	9.8±4.6	0
pH	6.6 (5.5-7.7)	6.5 (6.1-7.3)	7.8 (6.3-8.4)	7.8
Buffer capacity [mmol/L/ΔpH]	32.0±18.1	37.7±15.4	21.4±7.9	16

3.1.1.3. Fed state colon fluid

Several studies investigated the pH in the colon of UC patients in the fed state (Bosworth et al., 2009; Ewe et al., 1999; Nugent et al., 2000; Press et al., 1998; Raimundo et al., 1992). Very low pH values (pH 2.3-3.4) observed in a study by Fallingborg et al. (1993) were excluded due to analytical uncertainties (e.g., no confirmatory pH measurements, possibly artificial low pH values when certain distance to antenna was exceeded) (Press et al., 1998). The highest colonic pH value observed in UC patients in the fed state was 7.8 and the lowest was 4.7 (Press et al., 1998; Raimundo et al., 1992). Therefore, the pH in the fed state colonic medium was included as factor in the experimental design with a low level of 4.7 and a high level of 7.8 representative of the pH range observed in UC patients.

3.1.2. Development of UC media with Design of Experiments

A DoE approach was followed to develop the UC biorelevant media with the aim to assess the impact of each of the factors and to reflect the interindividual variability in UC patients. The development of UC biorelevant media was based on observed differences in UC patients compared to healthy subjects identified in literature (Section 3.1.1) and previously developed

biorelevant media for healthy subjects including Fasted-State Simulated Gastric Fluid (FaSSGF), Fasted-State Simulated Intestinal Fluid-Version 2 (FaSSIF-V2), FaSSCoF, Fed-State Simulated Intestinal Fluid-Version 2 (FeSSIF-V2) and Fed-State Simulated Colonic Fluid (FeSSCoF) (Jantratid et al., 2008; Markopoulos et al., 2015; Vertzoni et al., 2010a; Vertzoni et al., 2005).

The DoE was performed using XLSTAT (Addinsoft, France) with a full factorial design in UC patients for stomach and intestine in the fasted state and intestine and colon in the fed state. For fasted gastric and fasted and fed intestinal media, the lecithin concentration was included as a factor in the experimental design. Additionally, the ratio of bile salts to lecithin was integrated as a factor and set to the ratio in the corresponding biorelevant media based on healthy subjects for the low level. This approach was used to keep a similar composition of the mixed micelles as in healthy biorelevant media in some UC media. For fed state colonic UC media, the pH and the concentration of lecithin and bile salts were the investigated factors.

For fasted state colonic UC media, the factors investigated were bile salts, lecithin, pH, osmolality and soluble proteins. Due to the high number of factors, a fractional factorial design (2^(5-2)) was used for the UC fasted state colonic media using Dataplot (NIST, US) (Heckert and Filliben, 2003). The factor soluble proteins was represented in the UC media by bovine serum albumin.

Each factor changed in UC compared to healthy subjects was integrated in the DoE with two levels (low and high). Additionally, centre points with medium levels of each parameter were included for gastric and intestinal media. An overview of the factors and levels of the DoE is given in Figure 1. For UC-FaSSCoF media with osmolality as factor in the DoE, sodium chloride was added to adjust the osmolality. For all other media, the osmolality was set to the

248 value in corresponding biorelevant media based on healthy subjects by adjusting the
249 concentration of sodium chloride.

250 Biorelevant media were prepared according to the method described in Jantratid et al. (2008)
251 for gastric and intestinal media and Vertzoni et al. (2010a) for colonic media.

252 3.2. Media characterisation

253 Surface tension, osmolality, dynamic viscosity and buffer capacity of biorelevant media
254 previously developed based on healthy subjects and newly developed for UC patients were
255 measured. All measurements were performed in triplicate. The results were reported as mean
256 with standard deviation.

257 3.2.1. Surface tension

258 Surface tension measurements were performed with a ring tensiometer (Sigma 700 Force
259 tensiometer, Attension, UK) and a glass vessel (diameter of 46 mm) filled with 10 mL of each
260 medium at room temperature. The force to pull a Du Noüy ring from the surface of the medium
261 was measured and related to the medium's surface tension (Butt et al., 2004).

262 3.2.2. Osmolality

263 Osmolality was determined with an Advanced Instruments Inc. micro-osmometer Model 3300
264 (Norwood, MA, US) by measuring the freezing-point depression of a 20 µl sample.

265 3.2.3. Dynamic viscosity

266 A Bohlin Rheometer C-VOR (Malvern instruments, UK) with a cone-plate system (4°, 40mm)
267 was used to determine the dynamic viscosity of the media at a temperature of 37ºC. A small
268 amount of sample was placed between the plate and the cone, sheared with different shear
269 stresses (20 points, logarithmically distributed between 0.05 and 0.15 Pa) and the shear rate
270 was measured. Dynamic viscosity corresponds to the ratio of shear stress to shear rate.

271 3.2.4. Buffer capacity

To determine the buffer capacity of the media, small volumes of 0.5 M hydrochloric acid were added to 10 mL of medium until a change of one pH unit was measured with a Mettler Toledo SevenCompact S220 pH meter (Schwerzenbach, Switzerland). Subsequently, equation (2) was used to calculate the buffer capacity according to

$$\beta = \left(\frac{M_{acid} * V_{acid}}{\Delta pH}\right) * \frac{1000}{V_{sample}} \quad (2)$$

where β is the buffer capacity, M_{acid} is the molarity of the acid, ΔpH is the change in pH and V_{acid} and V_{sample} are the volume of the acid added and the volume of the sample, respectively (Rabbie et al., 2015).

3.3. Compound selection

Table 2: Properties of selected compounds for solubility studies.

Drug	**pKa (acid/base)**	**logP**	**BCS class**	**Intrinsic aqueous solubility [mg/mL]**
Azathioprine	7.9 (acid) (Mitra and Narurkar, 1987)	0.1 (Hansch et al., 1995)	IV (Lindenberg et al., 2004)	0.171 (Llinas et al., 2008)
Budesonide	12.0 (acid) (Corey and Fossel, 2016)	2.6 (Bharate et al., 2016)	II (Bhatt et al., 2014)	0.028 (Ali et al., 2010)
Celecoxib	11.1 (acid) (G.D. Searle LLC Division of Pfizer Inc, 2019)	3.5 (G.D. Searle LLC Division of Pfizer Inc, 2019)	II (Paulson et al., 2001)	0.003 - 0.007 (Paulson et al., 2001)
Dipyridamole	6.4 (base) (Pedersen, 1979)	2.2 (Betageri and Dipali, 1993)	II (Zaki et al., 2010)	0.003 (Hopfinger et al., 2009)
Loperamide	8.6 (base) (Manallack, 2007)	5.5 (Dickson et al., 2017)	II (Zaki et al., 2010)	
Sulfasalazine	2.3, 7.9 (acid) (Shalaeva et al., 2008)	2.9 (Graham and Pile, 2015)	II/IV (Lindenberg et al., 2004)	0.29×10^{-3} (Llinas et al., 2008)

For the solubility studies, poorly soluble compounds belonging to class II (low solubility, high permeability) or IV (low solubility, low permeability) of the Biopharmaceutics Classification System (BCS) were selected as presented in Table 2. Azathioprine, budesonide and sulfasalazine are used for the treatment of UC and loperamide can be used as symptomatic treatment of diarrhoea. Additionally, drugs were selected based on their physicochemical characteristics, covering a range of ionization properties (pKa) and lipophilicity (logP). Therefore, several moderately lipophilic drugs were included: dipyridamole and loperamide as weak bases and sulfasalazine as weak acid. Additionally, we included drugs that were mainly neutral over the physiological pH range but varied in their lipophilicity: azathioprine, budesonide and celecoxib. Due to the pKa of 7.9, azathioprine is neutral in gastric and intestinal media, whereas it was considered as weak acid for the colonic media.

3.4. Solubility studies

The shake-flask method was used to determine the solubility of the six investigated drugs (Baka et al., 2008). Therefore, 5 mL of medium were added to an excess amount of drug in a glass tube and placed in a shaking water bath (Grant instruments, UK) at 37 °C with 200 strokes/min. After 24 h, the supernatant was filtered with GF/D membrane filters with a pore size of 2.7 µm (Whatman® Puradisc, diameter 13 mm) and analysed by HPLC/UV. HPLC analysis was performed with an Agilent Technologies 1200 series HPLC system (Santa Clara, CA, US) with a binary pump, autosampler, thermostatted column compartment and diode array detector. The details of the HPLC-UV methods used for the quantitative analysis of the six compounds are described in Effinger et al. (2020).

For biorelevant media including bovine serum albumin, an additional treatment step for protein precipitation was added after sample filtration. 1 mL of protein precipitation reagent was added to 500 µL of sample, the mixture was vortexed for 30 s and centrifuged for 10 min at 12000 rpm and 4°C (Eppendorf Heraeus Fresco 17 centrifuge, ThermoElectron LED GmbH, Germany).

307 The protein precipitation reagent was methanol for all drugs except sulfasalazine, for which
308 dimethyl sulfoxide was used due to the poor solubility of sulfasalazine in methanol. For the
309 sulfasalazine samples with dimethyl sulfoxide, the ratio of the mobile phase used for the HPLC-
310 UV analysis was modified to 60:40 MeOH: Acetic acid 3.3% in H_2O. Solubility studies were
311 performed in triplicate in UC media and healthy media. Average solubility differences between
312 UC media and healthy media were expressed as a % Relative effect on solubility [((S_{UC}-
313 $S_{Healthy}$)/ $S_{Healthy}$) x 100]. Positive values indicate that drug solubility in UC media exceeds the
314 solubility in healthy media, whereas negative values indicate the opposite.

315 3.5. Statistical analysis

316 All statistical analysis was performed using XLSTAT (Addinsoft, France). To identify
317 statistically significant differences of media properties and drug solubility between UC
318 biorelevant media and the corresponding healthy media, one-way analysis of variance
319 (ANOVA) with a post-hoc Tukey's test was applied with a significance level of $p \leq 0.05$.
320 Multivariate statistical analysis was used to identify drug properties that result in a high risk of
321 altered drug solubility in UC. Therefore, the % Relative effect on drug solubility was correlated
322 with media-dependent factors of the DoE and drug physicochemical properties by Partial Least
323 Squares (PLS) regression. Media-dependent factors were for gastric and intestinal UC media
324 the bile salt and lecithin concentration. For fasted state colonic UC media, the media-dependent
325 factors were osmolality, pH and the concentrations of bile salts, of lecithin and of soluble
326 proteins. For fed state colonic UC media, media-dependent factors were pH and bile salt and
327 lecithin concentration. In terms of drug-dependent parameters, the partition coefficient, log P,
328 was included for all UC media. For media with pH as media-dependent factor (colonic UC
329 media), a categorical variable discriminating between weak acids, weak bases and neutral
330 compounds was introduced. For the gastric and intestinal UC media, the % Fraction ionised
331 (calculated using Advanced Chemistry Development, Inc. (ACD/Labs) Software V11.02,

Toronto, On, Canada and defined for anionic species as negative and cationic species as positive) was used as additional drug-dependent factor (Advanced Chemistry Development Inc., 2019). Interactions between media-dependent and drug-dependent factors were included in the model as shown in Table 3.

The quality assessment of the PLS models was based on the square of coefficient of determination (r^2) and goodness of prediction (q^2), both indicating a good fit of the data and a good predictive ability of the model, respectively, when close to 1. A difference higher than 0.3 between r^2 and q^2 indicates model over-fitting and consequently an inappropriate model (Eriksson et al., 2008). Models were selected for optimum model predictive ability based on the lowest predicted residual error sum of squares (PRESS) and the highest q^2. Usually good model predictability is given when q^2 is higher than 0.5, in certain cases, however, lower limits can be accepted since q^2 is dependent on the properties of the data set e.g., number of observations (Triba et al., 2015). In our models, a high influence on the % Relative effect on solubility is indicated for the media- and drug-dependent factors with high absolute value of the standardised coefficients. If the standardised coefficient is positive, this indicates a positive effect on the % Relative effect on solubility, while a negative standardised coefficient indicates the opposite. The Variable Importance in Projection (VIP) of a factor summarizes the influence of each individual independent factor on the PLS model. Factors with VIP $\geq$0.7 are considered as influential to the model, with the factors with a VIP>1 considered as most influential (Chong and Jun, 2005; Eriksson et al., 2008).

352 **Table 3:** Predictive factors of the different UC biorelevant media in the PLS model.

Medium	**Media-dependent factors**	**Drug-dependent factors**	**Interactions**
UC-FaSSGF **UC-FaSSIF** **UC-FeSSIF**	Bile salts Lecithin	LogP % Fraction ionised	Bile salts*logP Bile salts*% Fraction ionised Lecithin*logP Lecithin*%Fraction ionised
UC-FaSSCoF	Bile salts Lecithin Osmolality pH Soluble proteins	Categorical variable (weak acid, weak base, neutral) LogP	Bile salts*weak acid/weak base/neutral Bile salts*logP Lecithin*weak acid/weak base/neutral Lecithin*logP Osmolality*weak acid/weak base/neutral Osmolality*logP pH*weak acid/weak base/neutral pH*LogP Soluble proteins*weak acid/weak base/neutral Soluble proteins*logP
UC-FeSSCoF	Bile salts Lecithin pH	Categorical variable (weak acid, weak base, neutral) Log P	Bile salts*weak acid/weak base/neutral Bile salts*logP Lecithin*weak acid/weak base/neutral Lecithin*logP pH*weak acid/weak base/neutral pH*logP

353

4. Results and discussion

4.1. Media characterisation

The surface tension and osmolality of healthy and UC biorelevant media are presented in Figure 2.

Surface tension in fasted state gastric media was significantly higher (+24%, $p<0.05$) in the UC medium with low lecithin and low bile salt concentrations compared to the healthy medium, possibly due to the low surfactant concentration being below the critical micellar concentration. In fasted state intestinal media, a significantly higher surface tension compared to the healthy medium was observed for both UC media with low lecithin concentrations (+4%, +15%, $p<0.05$). In fasted state simulated colonic media, the surface tension in three UC media with low pH (low lecithin/low bile salt/low osmolality/high soluble proteins -11%, low lecithin/high bile salt/high osmolality/low soluble proteins -26% and high lecithin/low bile salt/high osmolality/low soluble proteins -31%, $p <0.05$) was significantly lower compared to the healthy medium and the surface tension of one UC medium (low lecithin/low bile salt/high pH/high osmolality/high soluble proteins) was increased by 7% ($p <0.05$). The surface tension of UC-FaSSCoF media was in the range of 29.3 mN/m to 46.0 mN/m, which is in accordance with the surface tension observed in the ascending colon fluid of UC patients in relapse (41.6±3.1 mN/m) and in remission (40.6±3.4 mN/m) (Vertzoni et al., 2010b). In the fed state, the surface tension of intestinal UC media was significantly decreased compared to the healthy medium (-7 to -12%, $p<0.05$). The surface tension of FeSSCoF was significantly higher compared to six of the UC media including the media with low pH and media with high pH/low lecithin concentrations ($p<0.05$). The lower surface tension of the media with low pH could either be related to the different pH value or the different ion concentration (higher concentration of sodium chloride) resulting in salt-surfactant synergistic effects that reduce the surface tension (Alonso et al., 2020).

Osmolality in UC biorelevant media was only different according to the specified levels for fasted state colonic media when osmolality was included as factor in the experimental design (Figure 2).

The dynamic viscosity of the investigated biorelevant media is presented at three different shear stresses in Figure 3. All healthy and UC media showed pseudoplastic behaviour. The viscosity at an applied shear stress of 0.15 Pa was in the range of 3.23 to 3.50 mPas, at 0.08 Pa in the range of 3.74 to 4.28 mPas and at 0.06 Pa in the range of 4.59 to 5.99 mPas, respectively. Significant differences between biorelevant media based on UC patients and healthy subjects for all three different shear stresses were not observed ($p<0.05$).

The buffer capacity was not significantly different in healthy fasted and fed state intestinal media compared to UC media (Figure 4). In fasted state colonic media, the healthy medium had a significantly lower buffer capacity compared to all UC media ($p<0.05$) and the increase was more pronounced for UC-FaSSCoF media with low pH compared to UC-FaSSCoF media with high pH. In contrast, in the fed state colonic media the buffer capacity was significantly lower in the UC media ($p<0.05$), whereby the decrease was more pronounced for UC-FeSSCoF media with low pH compared to UC-FeSSCoF media with high pH.

4.2. Solubility of drugs in UC biorelevant media

The % Relative effect of UC on the solubility of six different drugs, as investigated with biorelevant media based on UC patients and healthy subjects simulating stomach, small intestine and colon in the fasted state and small intestine and colon in the fed state, is shown in Figure 5.

4.2.1. Neutral drugs

For all investigated neutral drugs, the final pH of the UC media after 24 h was similar to the initial pH of the media (within a standard deviation of 0.1). Differences in drug solubility in

gastric and intestinal media were observed for the investigated neutral drugs due to decreased lecithin or bile salt concentrations. For budesonide, the decrease was significant in all gastric fasted state UC media, in the fasted state intestinal UC medium with low lecithin and high bile salt concentrations and in the fed state intestinal medium with low lecithin and low bile salt concentrations ($p<0.05$). For celecoxib, a significantly reduced solubility was observed in the fasted state gastric UC medium with low lecithin and low bile salt concentrations and in all fasted and fed state intestinal UC media ($p<0.05$). These findings are consistent with lower concentrations of bile salts and lecithin resulting in a decreased concentration of mixed micelles available for drug solubilisation of lipophilic compounds (Wiedmann and Kamel, 2002).

In fasted state colonic UC media, budesonide solubility was significantly higher in media with high pH, high osmolality and high soluble proteins ($p<0.05$). For celecoxib, the solubility was increased in all UC media with high concentrations of soluble proteins and one other UC medium (high bile salt and lecithin concentrations, high pH, low concentration of soluble proteins and a low osmolality), while the solubility was decreased in media with low concentrations of lecithin and soluble proteins ($p<0.05$). The positive effect of soluble proteins, represented by bovine serum albumin, on the solubility of non-ionised compounds has previously been reported for danazol, felodipine and prednisolone (Fadda et al., 2010; Vertzoni et al., 2010a). Additionally, it was shown that the octanol:water partition coefficient is positively correlated to the BSA:water partition coefficient for neutral compounds (Endo and Goss, 2011). In fed state colonic media, the solubility of budesonide and celecoxib was significantly decreased in UC media with low lecithin concentrations ($p<0.05$) indicating lower solubilisation due to decreased surfactant concentration. For celecoxib, this was also the case for UC media with low pH. This could be due to the low pH (4.7) resulting in more sodium cholate (pKa 5.13) being present in its unionised form and hindering the formation of micelles (Posa et al., 2017).

4.2.2. Weak bases

For the investigated weak bases, no significant differences in drug solubilisation were observed in fasted state gastric UC media. For the dipyridamole studies, the pH of the medium at the end of the solubility studies was increased in fasted state gastric media (pH: 3.0 ± 0.1). The solubility of loperamide hydrochloride was decreased in all fasted state intestinal UC media ($p<0.05$) indicating a lower solubility with a lower concentration of surfactants. For dipyridamole, a lower solubility was observed in UC-FaSSIF with low lecithin and low bile salt concentrations, while the solubility was increased in UC-FaSSIF with high bile salt concentrations and either low or medium lecithin concentration ($p<0.05$). For dipyridamole, lower lecithin concentrations seem to promote the solubilisation of dipyridamole, probably due to electrostatic interactions between dipyridamole and bile salts (Borissevitch et al., 1995). In fed state intestinal media, the solubility of loperamide hydrochloride was decreased in UC-FeSSIF with low lecithin concentrations and medium lecithin and medium bile salt concentrations. For dipyridamole, the solubility was decreased in UC-FeSSIF with low and medium bile salt concentrations indicating again the importance of bile salts for the solubilisation of weak bases. In fasted state colonic media, the solubility of loperamide hydrochloride and dipyridamole was increased in media with low pH due to a higher ionised fraction of the drug. Additionally, the solubility of dipyridamole was also increased in the UC-FaSSCoF media with high level of all factors. In fed state colonic media, loperamide hydrochloride had a lower solubility in all UC media with high pH due to a smaller protonated fraction of the drug. Similarly, the solubility of dipyridamole was increased in UC media with low pH due to a higher fraction ionised. Additionally, loperamide hydrochloride had a higher solubility in UC-FeSSCoF with high bile salt and low lecithin concentrations and low pH. The solubility of dipyridamole was decreased in the UC-FeSSCoF media with high pH and low lecithin concentrations.

4.2.3. Weak acids

For the investigated weak acids, most differences were observed due to pH changes. For azathioprine, a hydrophilic compound with a log P of 0.1, the solubility was significantly decreased in UC-FaSSCoF with low pH, while the solubility was increased in UC-FeSSCoF with high pH. For sulfasalazine, the solubility in the fasted state gastric media was below the limit of quantification. In intestinal fasted and fed state media, the solubility of sulfasalazine was significantly decreased in UC media with low lecithin and low bile salt concentration and medium lecithin and medium bile salt concentration. In fasted state colonic media, sulfasalazine solubility was decreased in UC media with low pH and other media with high pH, low osmolality and low concentration of soluble proteins. In fed state colonic media, the solubility of sulfasalazine was increased in UC media with high pH and decreased in UC media with low pH. Differences in the pH of the investigated media compared to their initial pH were observed in some UC media for the sulfasalazine studies (final pH in UC-FaSSIF 6.2± 0.1, UC-FeSSIF 5.7± 0.1, UC-FaSSCoF with high pH 6.7± 0.1, UC-FeSSCoF with high pH 6.6± 0.1).

468 4.3. Multivariate statistical analysis

469 Successful PLS models were developed for small intestinal and colonic UC media in the fasted
470 and fed state. The plots of the standardised coefficients of the respective drug- and media-
471 dependent factors are shown in Figure 6. For the fasted state gastric media, it was not possible
472 to develop a predictive PLS model (q^2 -0.04, r^2 0.09).

473 For fasted state intestinal media, the developed PLS model for the % Relative effect of UC on
474 drug solubility showed a good fit of the experimental data (r^2 0.76) and a high predictive power
475 (q^2 0.70). The model depicted a positive effect of bile salts, lecithin and the interaction between
476 lecithin and log P, while log P had a negative impact. Consequently, in the luminal fluids of
477 UC patients with low bile salt and lecithin concentrations a high risk of reduced drug solubility
478 is expected for compounds with a high lipophilicity. This is in accordance with another study,
479 where a positive effect of bile salt and lecithin concentration on drug solubility in fasted state
480 simulated fluids has previously been shown for seven out of twelve compounds with a clogP
481 in the range of 1.43 to 6.15 (ACD/Labs) including three neutral compounds (felodipine clogP
482 4.83, griseofulvin clogP 3.53, fenofibrate clogP 4.80), three weak bases (tadalafil clogP 1.43,
483 zafirlukast clogP 6.15, aprepitant clogP 4.80) and one weak acid (phenytoin clogP 2.52)
484 (Advanced Chemistry Development Inc., 2019; Khadra et al., 2015). It should be noted that
485 five drugs with a clogP of 1.71-10.27 (ACD/Labs) (probucol clogP 10.27, carvedilol clogP
486 4.11, piroxicam clogP 1.71, indomethacin clogP 3.1, naproxen clogP 3.0) didn't follow this
487 pattern in the respective study indicating drug-specific effects in certain cases (Khadra et al.,
488 2015). Therefore, a difference in luminal drug solubility in UC patients may not be fully
489 predicted for certain drugs by the sole use of drug properties employed in the current study.

490 For fed state intestinal media, the model quality of the developed PLS model was accurate with
491 a high predictability (r^2 0.73, q^2 0.66). As for the PLS model of the fasted state, bile salts and
492 lecithin had a positive effect on the % Relative effect on drug solubility with a higher impact

493 of the bile salt concentration. The interaction between lecithin and log P had also a positive
494 influence (VIP>0.7). In contrast, log P had a negative impact. In another study, a positive
495 impact of higher bile salt concentration on drug solubility in fed state simulated intestinal media
496 was observed for nine of thirteen compounds (itraconazole, probucol, felodipine, tadalafil,
497 aprepitant, carvedilol, zafirlukast, indomethacin, phenytoin) with a clogP in the range of 1.43
498 to 10.27 (ACD/Labs) (Zhou et al., 2017). In the same study, a positive effect of lecithin on the
499 solubility of eight out of thirteen compounds was also observed (itraconazole, probucol,
500 felodipine, fenofibrate, carvedilol, zafirlukast, indomethacin, phenytoin) (Zhou et al., 2017).
501 However, bile salts or lecithin had a negative impact on drug solubility for certain lipophilic
502 drugs in the respective study indicating again drug-specific effects in some cases (Zhou et al.,
503 2017).
504 For fasted state colonic media, the PLS model with good model quality (r^2 0.90, q^2 0.82)
505 revealed a positive effect of log P, weak base and the interplay between soluble proteins and
506 neutral drugs and a lower positive influence (VIP>0.7) of soluble proteins and the interplay
507 between log P and neutral drugs. In contrast, the model showed a negative influence of pH,
508 weak acids, the interplay between pH and log P and the interplay between pH and weak base.
509 This indicates that differences in drug ionisation determine the drug solubility in the fasted
510 state colonic fluid of UC patients. Additionally, a higher drug solubility of neutral lipophilic
511 compounds is expected in the fasted state colonic fluids of UC patients.
512 For fed state colonic media, the predictive power of the developed PLS model was acceptable
513 (q^2 0.49, r^2 0.71). Most influential variables of the model with positive impact were the
514 categorical variable weak acid and the interplay between pH/logP and pH/weak acid.
515 Additionally, a positive effect of log P was influential to the model (VIP>0.7). A negative
516 impact on the % Relative effect on drug solubility was observed for the categorical variable
517 neutral and the interplay between pH and weak base. Differences in ionisation are therefore,

518 expected to be the major influence on drug solubility in the fed state colonic fluid of UC
519 patients.
520 Given the high number of UC media and the solubility studies of six compounds, the statistical
521 models were acceptable. Further studies with more compounds would additionally increase the
522 confidence in the models.

523 4.4. Drugs at risk of altered solubility in luminal fluids of UC patients

524 Considering intestinal fluids in the fasted and fed state, compounds with a higher lipophilicity
525 are expected to show a lower drug solubility in UC patients compared to healthy subjects. This
526 is especially expected for UC patients with low concentrations of bile salts and lecithin in their
527 intestinal fluids.

528 In terms of fasted state colonic fluids of UC patients, a high risk of altered drug solubility is
529 indicated for weak bases and weak acids. For weak acids, a lower drug solubility is expected
530 in fasted state colonic fluids of UC patients compared to healthy subjects. For weak bases, a
531 higher drug solubility is expected in UC patients with a low pH in their fasted state colonic
532 fluids. Additionally, neutral moderately lipophilic drugs are expected to have a higher
533 solubility in UC patients with increased concentrations of soluble proteins (relapse and
534 remission) in their fasted state colonic fluids.

535 Regarding the fed state colonic fluid of UC patients, the altered colonic pH in UC patients
536 poses a risk for ionisable drugs. For weak acids, a higher drug solubility is expected in UC
537 patients with increased pH in their fed state colonic fluids, whereas for weak bases a lower
538 drug solubility is expected. In addition, a lower solubility of neutral moderately lipophilic drugs
539 is expected in the fed state colonic fluids of UC patients with low lecithin concentration.

540 **5. Conclusion**

Biorelevant media were developed as an *in vitro* tool to assess drug solubility and dissolution in UC patients for different GI compartments and prandial states based on literature data investigating pathophysiological changes in UC. The characterisation of UC biorelevant media revealed differences in terms of surface tension, buffer capacity and osmolality compared to biorelevant media based on healthy subjects. Differences in drug solubility were observed for the six investigated compounds in UC media compared to biorelevant media based on healthy subjects and suggest differences in drug ionisation, micellar solubilisation and interaction with soluble proteins as main influencing factors. Additional studies are needed to characterise the gastrointestinal fluids of UC patients and solubility studies of additional compounds in UC biorelevant media would create a bigger database for the biopharmaceutics risk assessment in UC patients.

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7. Declaration of interest

None.

8. References

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738 **Figure Legends**

739 Figure 1: Design of experiments for the development of Ulcerative Colitis media.

740 Figure 2: Surface tension (blue, left y-axis) and osmolality (black, right y-axis) of UC
741 biorelevant media according to the Design of Experiments (green: high level, yellow:
742 medium level, red: low level, white: healthy level) and healthy media.

743 Figure 3: Dynamic viscosity of biorelevant media based on UC patients and healthy subjects
744 at different shear stress (0.06 Pa: blue, 0.08 Pa: red, 0.15 Pa: black) according to the Design
745 of Experiments (green: high level, yellow: medium level, red: low level, white: healthy
746 level).

747 Figure 4: % Relative effect on buffer capacity in biorelevant media based on UC patients
748 compared to healthy subjects according to the Design of Experiments (green: high level,
749 yellow: medium level, red: low level).

750 Figure 5: % Relative effect on solubility of investigated drugs in biorelevant media based on
751 UC patients compared to healthy subjects according to Design of Experiments for (a) neutral
752 drugs, (b) weak bases and (c) weak acids (green: high level, yellow: medium level, red: low
753 level).

754 Figure 6: Standardised coefficients of the PLS regression of drug solubility in UC simulated
755 gastrointestinal fluids in the fasted state (left) and fed state (right) and different compartments
756 of the GI tract (top: small intestine, bottom: colon). Red colour denotes coefficients of VIP
757 values > 1 and blue > 0.7.

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	Ulcerative colitis											
Prandial state	**Fasted state**						**Fed state**					
Compartment	**stomach**		**intestine**		**colon**		**stomach**		**intestine**		**colon**	
Level	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**	**low**	**high**
Bile salts [mM]	0.024	0.08	0.90	3.0	0.075	0.15			3.0	10	0.18	0.6
Lecithin [mM]	0.006	0.02	0.06	0.2	0.130	0.62			0.6	2	0.15	0.5
Bile salts/Lecithin	4:1	40:3	15:1	50:1	15:62	15:13			5:1	50:3	6:5	4:1
pH					5.5	7.7					4.7	7.8
Osmolality [mOsm/kg]					196	290						
Soluble proteins [mg/ml]					0	19						

	no changes
	decrease
	increase
	Value in healthy biorelevant media

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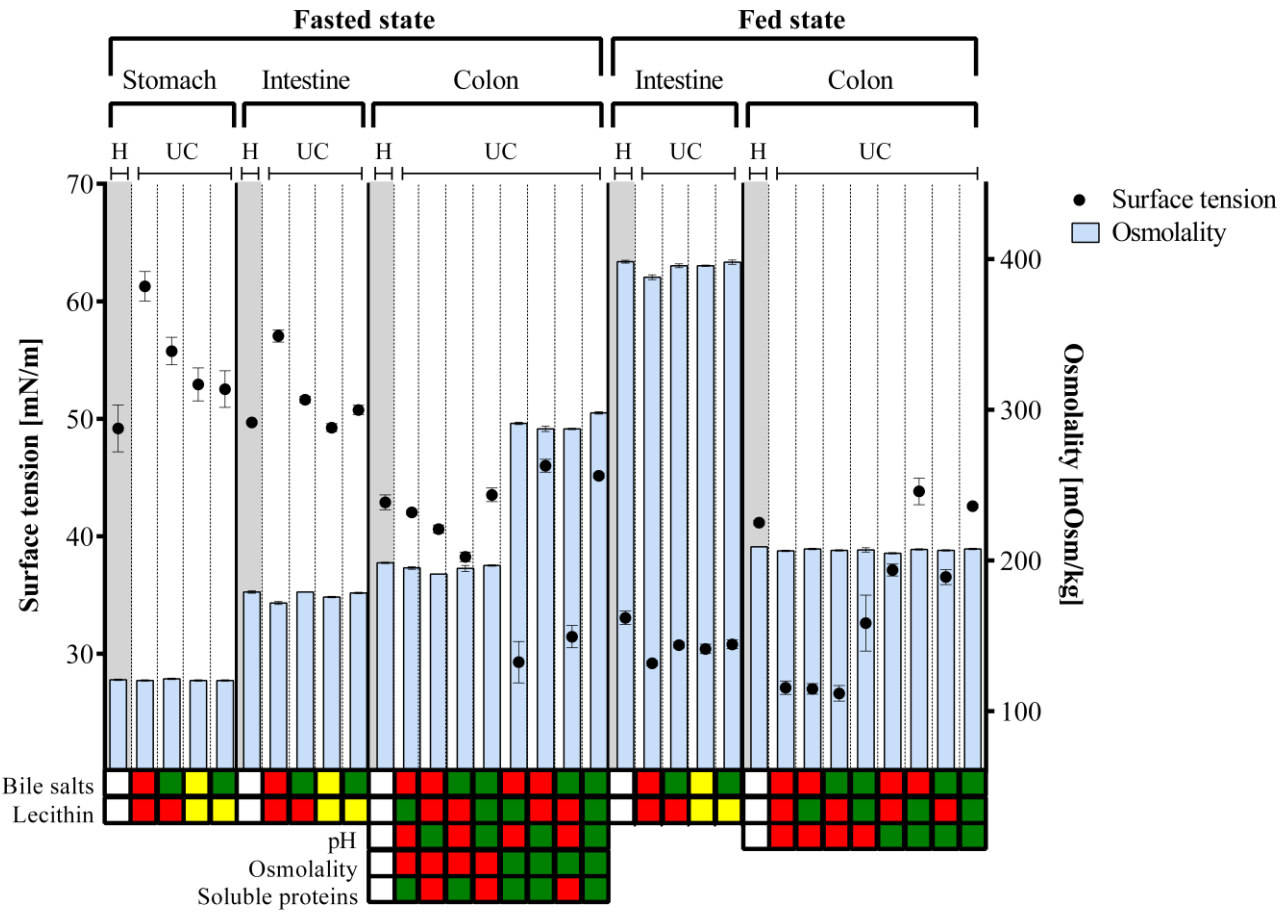

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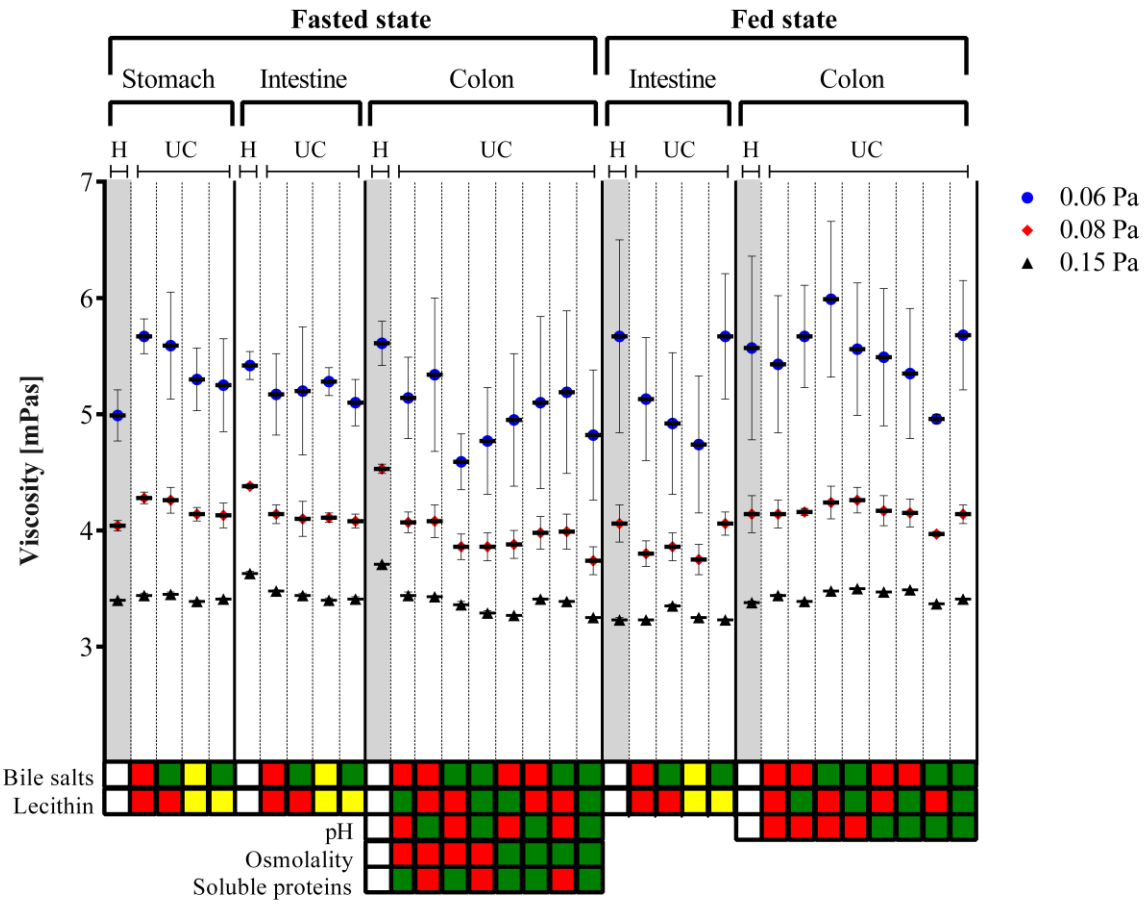

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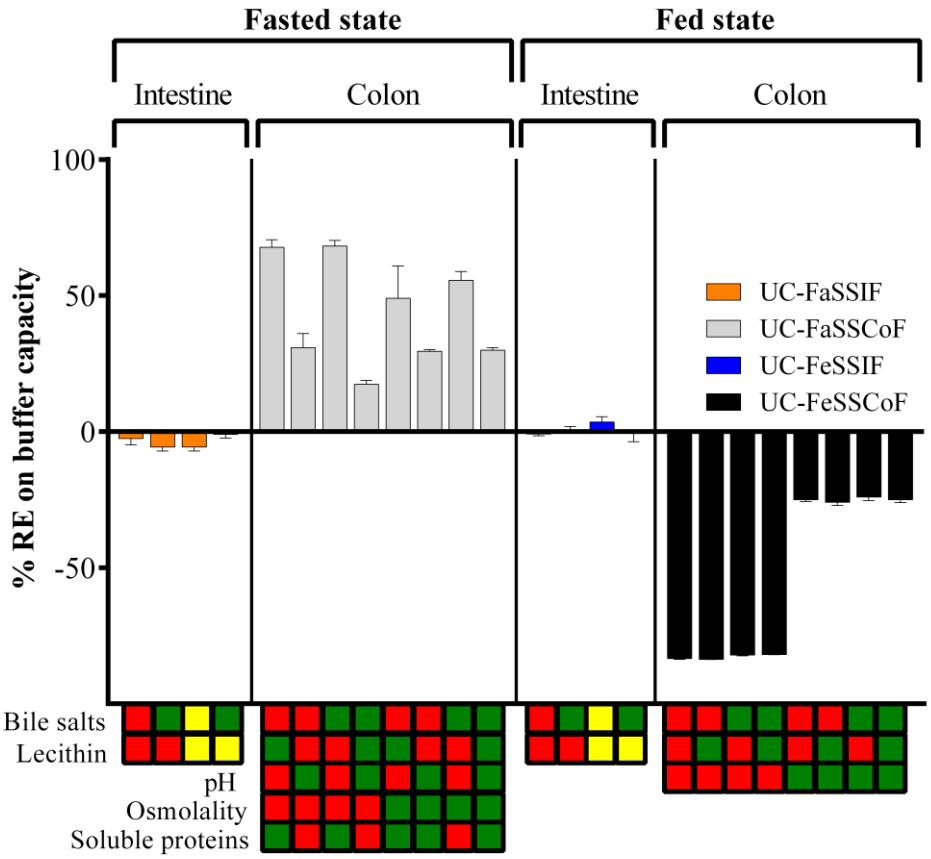

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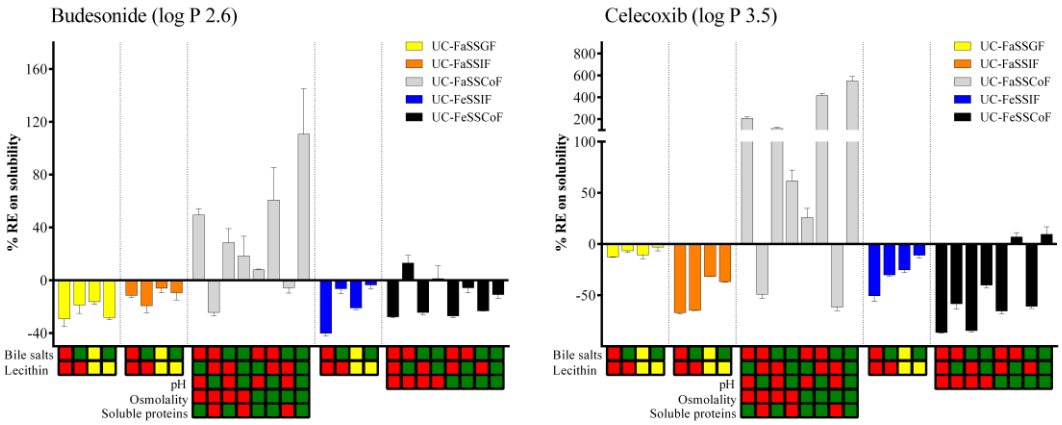
(a) Neutral drugs



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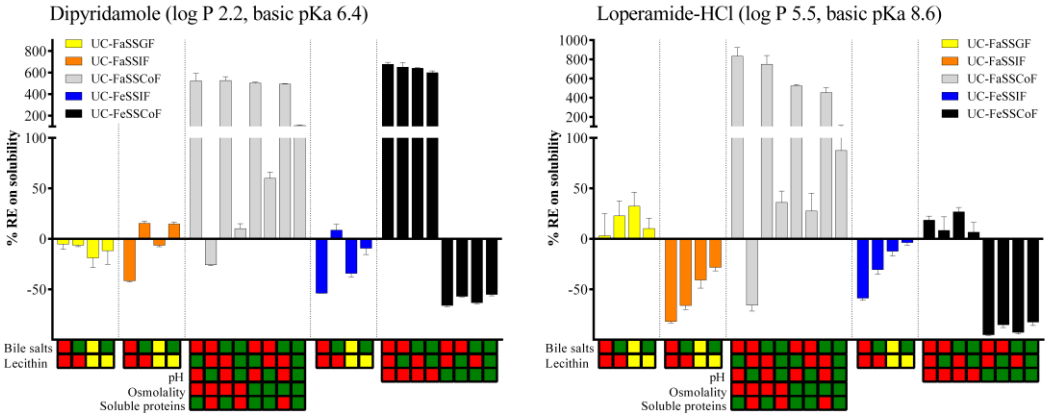
(b) Weak bases



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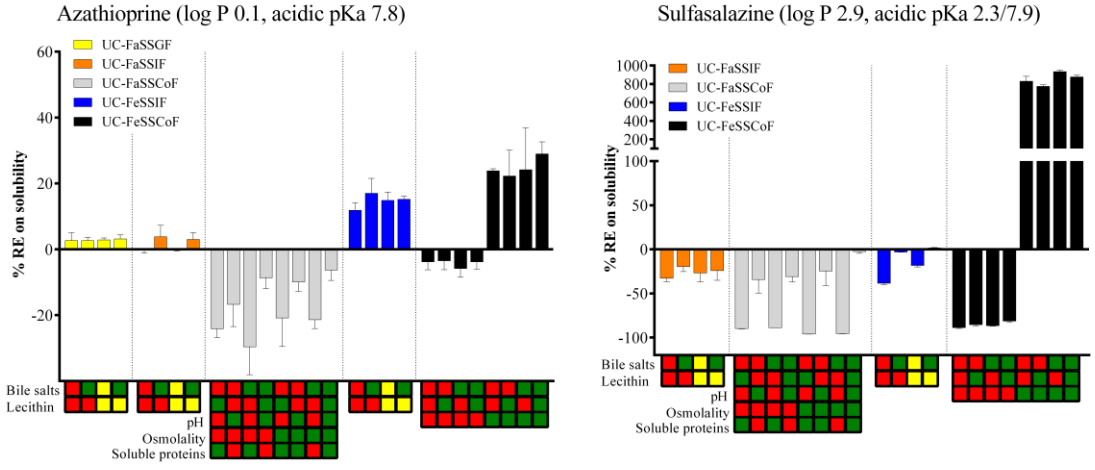
(c) Weak acids



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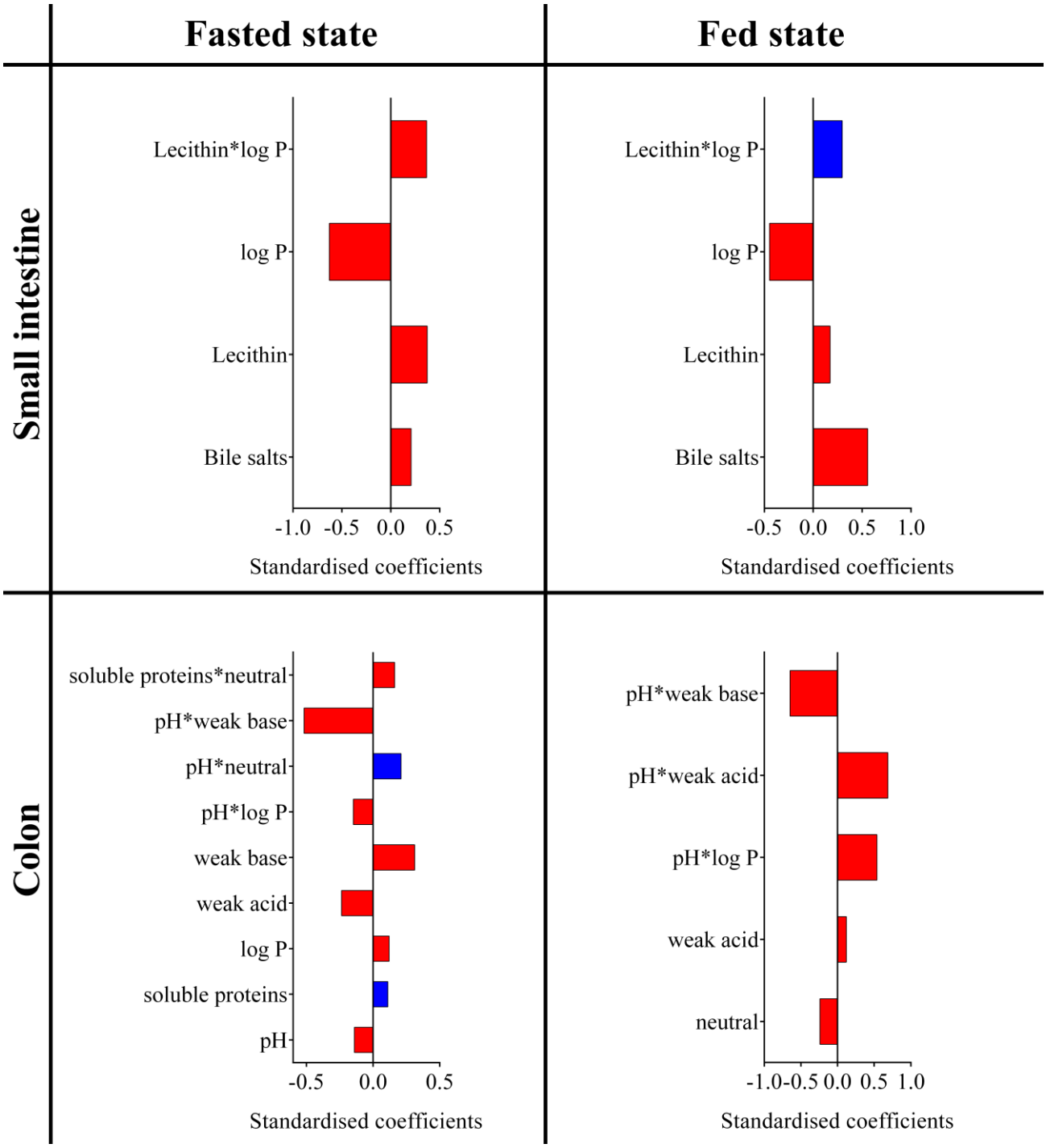
Fasted state
Fed state
Small intestine
Colon
Lecithin*log P
log P
Lecithin
Bile salts
-1.0 -0.5 0.0 0.5
Standardised coefficients
-0.5 0.0 0.5 1.0
Standardised coefficients
soluble proteins*neutral
pH*weak base
pH*neutral
pH*log P
weak base
weak acid
log P
soluble proteins
pH
-0.5 0.0 0.5
Standardised coefficients
pH*weak base
pH*weak acid
pH*log P
weak acid
neutral
-1.0-0.5 0.0 0.5 1.0
Standardised coefficients

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