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1 **Gastrointestinal diseases and their impact on drug solubility: Ulcerative**  
2 **Colitis**

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19 **Abstract**

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

39 **Keywords**

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

## 42 **1. Introduction**

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

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

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

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

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

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

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

## 104 **2. Materials**

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

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

### 118 **3. Methods**

#### 119 3.1. Media development

##### 120 3.1.1. GI pathophysiological changes in UC patients integrated in the experimental 121 design

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

##### 130 3.1.1.1. Lecithin concentration

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

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

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

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

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

$$159 \quad x_{UC-BM} = 0.30 * x_{H-BM} \quad (1)$$

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



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

#### 167 3.1.1.2. Fasted state ascending colon fluid

168 The fasted state ascending colon fluid of UC patients in states of relapse and remission (defined  
169 based on the Clinical Rachmilewitz Index (CRI)) has previously been characterised (Vertzoni  
170 et al., 2010b). A higher osmolality was observed in patients with UC in remission compared to  
171 patients in relapse and healthy subjects, as shown in Table 1 (Diakidou et al., 2009; Vertzoni  
172 et al., 2010b). For the experimental design, the osmolality was integrated with a low level of  
173 196 mOsmol/kg, corresponding to the osmolality of FaSSCoF and similar to the osmolality  
174 observed in UC patients in relapse, and a high level of 290 mOsmol/kg representative of UC  
175 patients in remission.

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

182 The concentration of soluble proteins was not significantly different between patients in relapse  
183 and remission but significantly higher compared to healthy subjects [Table 1] (Diakidou et al.,  
184 2009; Vertzoni et al., 2010b). For the experimental design, the concentration of soluble proteins  
185 was integrated using bovine serum albumin with a high level of 19 mg/mL representative of  
186 UC patients in relapse and remission and a low level of 0 mg/mL based on the concentration  
187 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

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

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

209

210 3.1.1.3. Fed state colon fluid

211 Several studies investigated the pH in the colon of UC patients in the fed state (Bosworth et  
 212 al., 2009; Ewe et al., 1999; Nugent et al., 2000; Press et al., 1998; Raimundo et al., 1992). Very  
 213 low pH values (pH 2.3-3.4) observed in a study by Fallingborg et al. (1993) were excluded due  
 214 to analytical uncertainties (e.g., no confirmatory pH measurements, possibly artificial low pH  
 215 values when certain distance to antenna was exceeded) (Press et al., 1998). The highest colonic  
 216 pH value observed in UC patients in the fed state was 7.8 and the lowest was 4.7 (Press et al.,  
 217 1998; Raimundo et al., 1992). Therefore, the pH in the fed state colonic medium was included  
 218 as factor in the experimental design with a low level of 4.7 and a high level of 7.8 representative  
 219 of the pH range observed in UC patients.

220 3.1.2. Development of UC media with Design of Experiments

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

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

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

238 For fasted state colonic UC media, the factors investigated were bile salts, lecithin, pH,  
239 osmolality and soluble proteins. Due to the high number of factors, a fractional factorial design  
240 ( $2^{(5-2)}$ ) was used for the UC fasted state colonic media using Dataplot (NIST, US) (Heckert  
241 and Filliben, 2003). The factor soluble proteins was represented in the UC media by bovine  
242 serum albumin.

243 Each factor changed in UC compared to healthy subjects was integrated in the DoE with two  
244 levels (low and high). Additionally, centre points with medium levels of each parameter were  
245 included for gastric and intestinal media. An overview of the factors and levels of the DoE is  
246 given in Figure 1. For UC-FaSSCoF media with osmolality as factor in the DoE, sodium  
247 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 37°C. 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

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

$$276 \quad \beta = \left( \frac{M_{acid} * V_{acid}}{\Delta pH} \right) * \frac{1000}{V_{sample}} \quad (2)$$

277 where  $\beta$  is the buffer capacity,  $M_{acid}$  is the molarity of the acid,  $\Delta pH$  is the change in pH and  
 278  $V_{acid}$  and  $V_{sample}$  are the volume of the acid added and the volume of the sample, respectively  
 279 (Rabbie et al., 2015).

### 280 3.3. Compound selection

281 **Table 2:** Properties of selected compounds for solubility studies.

Drug	pKa (acid/base)	logP	BCS class	Intrinsic aqueous solubility [mg/mL]
<b>Azathioprine</b>	7.9 (acid) (Mitra and Narurkar, 1987)	0.1 (Hansch et al., 1995)	IV (Lindenberg et al., 2004)	0.171 (Llinas et al., 2008)
<b>Budesonide</b>	12.0 (acid) (Corey and Fossel, 2016)	2.6 (Bharate et al., 2016)	II (Bhatt et al., 2014)	0.028 (Ali et al., 2010)
<b>Celecoxib</b>	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)
<b>Dipyridamole</b>	6.4 (base) (Pedersen, 1979)	2.2 (Betageri and Dipali, 1993)	II (Zaki et al., 2010)	0.003 (Hopfinger et al., 2009)
<b>Loperamide</b>	8.6 (base) (Manallack, 2007)	5.5 (Dickson et al., 2017)	II (Zaki et al., 2010)	
<b>Sulfasalazine</b>	2.3, 7.9 (acid) (Shalaeva et al., 2008)	2.9 (Graham and Pile, 2015)	II/IV (Lindenberg et al., 2004)	$0.29 \times 10^{-3}$ (Llinas et al., 2008)

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

### 293 3.4. Solubility studies

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

303 For biorelevant media including bovine serum albumin, an additional treatment step for protein  
304 precipitation was added after sample filtration. 1 mL of protein precipitation reagent was added  
305 to 500 µL of sample, the mixture was vortexed for 30 s and centrifuged for 10 min at 12000 rpm  
306 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<sub>2</sub>O. 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 [ $((S_{UC} - S_{Healthy}) / S_{Healthy}) \times 100$ ]. Positive values indicate that drug solubility in UC media exceeds the  
313 solubility in healthy media, whereas negative values indicate the opposite.  
314

### 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,



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

336 The quality assessment of the PLS models was based on the square of coefficient of  
337 determination ( $r^2$ ) and goodness of prediction ( $q^2$ ), both indicating a good fit of the data and a  
338 good predictive ability of the model, respectively, when close to 1. A difference higher than  
339 0.3 between  $r^2$  and  $q^2$  indicates model over-fitting and consequently an inappropriate model  
340 (Eriksson et al., 2008). Models were selected for optimum model predictive ability based on  
341 the lowest predicted residual error sum of squares (PRESS) and the highest  $q^2$ . Usually good  
342 model predictability is given when  $q^2$  is higher than 0.5, in certain cases, however, lower limits  
343 can be accepted since  $q^2$  is dependent on the properties of the data set e.g., number of  
344 observations (Triba et al., 2015). In our models, a high influence on the % Relative effect on  
345 solubility is indicated for the media- and drug-dependent factors with high absolute value of  
346 the standardised coefficients. If the standardised coefficient is positive, this indicates a positive  
347 effect on the % Relative effect on solubility, while a negative standardised coefficient indicates  
348 the opposite. The Variable Importance in Projection (VIP) of a factor summarizes the influence  
349 of each individual independent factor on the PLS model. Factors with  $VIP \geq 0.7$  are considered  
350 as influential to the model, with the factors with a  $VIP > 1$  considered as most influential (Chong  
351 and Jun, 2005; Eriksson et al., 2008).

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

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

## 354 4. Results and discussion

### 355 4.1. Media characterisation

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

358 Surface tension in fasted state gastric media was significantly higher (+24%,  $p < 0.05$ ) in the UC  
359 medium with low lecithin and low bile salt concentrations compared to the healthy medium,  
360 possibly due to the low surfactant concentration being below the critical micellar concentration.

361 In fasted state intestinal media, a significantly higher surface tension compared to the healthy  
362 medium was observed for both UC media with low lecithin concentrations (+4%, +15%,  
363  $p < 0.05$ ). In fasted state simulated colonic media, the surface tension in three UC media with  
364 low pH (low lecithin/low bile salt/low osmolality/high soluble proteins -11%, low lecithin/high  
365 bile salt/high osmolality/low soluble proteins -26% and high lecithin/low bile salt/high  
366 osmolality/low soluble proteins -31%,  $p < 0.05$ ) was significantly lower compared to the  
367 healthy medium and the surface tension of one UC medium (low lecithin/low bile salt/high  
368 pH/high osmolality/high soluble proteins) was increased by 7% ( $p < 0.05$ ). The surface tension  
369 of UC-FaSSCoF media was in the range of 29.3 mN/m to 46.0 mN/m, which is in accordance  
370 with the surface tension observed in the ascending colon fluid of UC patients in relapse  
371 ( $41.6 \pm 3.1$  mN/m) and in remission ( $40.6 \pm 3.4$  mN/m) (Vertzoni et al., 2010b). In the fed state,  
372 the surface tension of intestinal UC media was significantly decreased compared to the healthy  
373 medium (-7 to -12%,  $p < 0.05$ ). The surface tension of FeSSCoF was significantly higher  
374 compared to six of the UC media including the media with low pH and media with high pH/low  
375 lecithin concentrations ( $p < 0.05$ ). The lower surface tension of the media with low pH could  
376 either be related to the different pH value or the different ion concentration (higher  
377 concentration of sodium chloride) resulting in salt-surfactant synergistic effects that reduce the  
378 surface tension (Alonso et al., 2020).

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

382 The dynamic viscosity of the investigated biorelevant media is presented at three different shear  
383 stresses in Figure 3. All healthy and UC media showed pseudoplastic behaviour. The viscosity  
384 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  
385 range of 3.74 to 4.28 mPas and at 0.06 Pa in the range of 4.59 to 5.99 mPas, respectively.  
386 Significant differences between biorelevant media based on UC patients and healthy subjects  
387 for all three different shear stresses were not observed ( $p < 0.05$ ).

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

#### 395 4.2. Solubility of drugs in UC biorelevant media

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

##### 400 4.2.1. Neutral drugs

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

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

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

#### 428 4.2.2. Weak bases

429 For the investigated weak bases, no significant differences in drug solubilisation were observed  
430 in fasted state gastric UC media. For the dipyridamole studies, the pH of the medium at the end  
431 of the solubility studies was increased in fasted state gastric media (pH:  $3.0 \pm 0.1$ ). The  
432 solubility of loperamide hydrochloride was decreased in all fasted state intestinal UC media  
433 ( $p < 0.05$ ) indicating a lower solubility with a lower concentration of surfactants. For  
434 dipyridamole, a lower solubility was observed in UC-FaSSIF with low lecithin and low bile  
435 salt concentrations, while the solubility was increased in UC-FaSSIF with high bile salt  
436 concentrations and either low or medium lecithin concentration ( $p < 0.05$ ). For dipyridamole,  
437 lower lecithin concentrations seem to promote the solubilisation of dipyridamole, probably due  
438 to electrostatic interactions between dipyridamole and bile salts (Borissevitch et al., 1995). In  
439 fed state intestinal media, the solubility of loperamide hydrochloride was decreased in UC-  
440 FeSSIF with low lecithin concentrations and medium lecithin and medium bile salt  
441 concentrations. For dipyridamole, the solubility was decreased in UC-FeSSIF with low and  
442 medium bile salt concentrations indicating again the importance of bile salts for the  
443 solubilisation of weak bases. In fasted state colonic media, the solubility of loperamide  
444 hydrochloride and dipyridamole was increased in media with low pH due to a higher ionised  
445 fraction of the drug. Additionally, the solubility of dipyridamole was also increased in the UC-  
446 FaSSCoF media with high level of all factors. In fed state colonic media, loperamide  
447 hydrochloride had a lower solubility in all UC media with high pH due to a smaller protonated  
448 fraction of the drug. Similarly, the solubility of dipyridamole was increased in UC media with  
449 low pH due to a higher fraction ionised. Additionally, loperamide hydrochloride had a higher  
450 solubility in UC-FeSSCoF with high bile salt and low lecithin concentrations and low pH. The  
451 solubility of dipyridamole was decreased in the UC-FeSSCoF media with high pH and low  
452 lecithin concentrations.

453 4.2.3. Weak acids

454 For the investigated weak acids, most differences were observed due to pH changes. For  
455 azathioprine, a hydrophilic compound with a log P of 0.1, the solubility was significantly  
456 decreased in UC-FaSSCoF with low pH, while the solubility was increased in UC-FeSSCoF  
457 with high pH. For sulfasalazine, the solubility in the fasted state gastric media was below the  
458 limit of quantification. In intestinal fasted and fed state media, the solubility of sulfasalazine  
459 was significantly decreased in UC media with low lecithin and low bile salt concentration and  
460 medium lecithin and medium bile salt concentration. In fasted state colonic media,  
461 sulfasalazine solubility was decreased in UC media with low pH and other media with high  
462 pH, low osmolality and low concentration of soluble proteins. In fed state colonic media, the  
463 solubility of sulfasalazine was increased in UC media with high pH and decreased in UC media  
464 with low pH. Differences in the pH of the investigated media compared to their initial pH were  
465 observed in some UC media for the sulfasalazine studies (final pH in UC-FaSSIF  $6.2 \pm 0.1$ ,  
466 UC-FeSSIF  $5.7 \pm 0.1$ , UC-FaSSCoF with high pH  $6.7 \pm 0.1$ , UC-FeSSCoF with high pH  $6.6 \pm$   
467  $0.1$ ).

#### 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) (probuocol 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

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

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## 557 **7. Declaration of interest**

558 None.

559

560 **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
<b>Bile salts [mM]</b>	0.024	0.08	0.90	3.0	0.075	0.15			3.0	10	0.18	0.6
<b>Lecithin [mM]</b>	0.006	0.02	0.06	0.2	0.130	0.62			0.6	2	0.15	0.5
<b>Bile salts/Lecithin</b>	4:1	40:3	15:1	50:1	15:62	15:13			5:1	50:3	6:5	4:1
<b>pH</b>					5.5	7.7					4.7	7.8
<b>Osmolality [mOsm/kg]</b>					196	290						
<b>Soluble proteins [mg/ml]</b>					0	19						

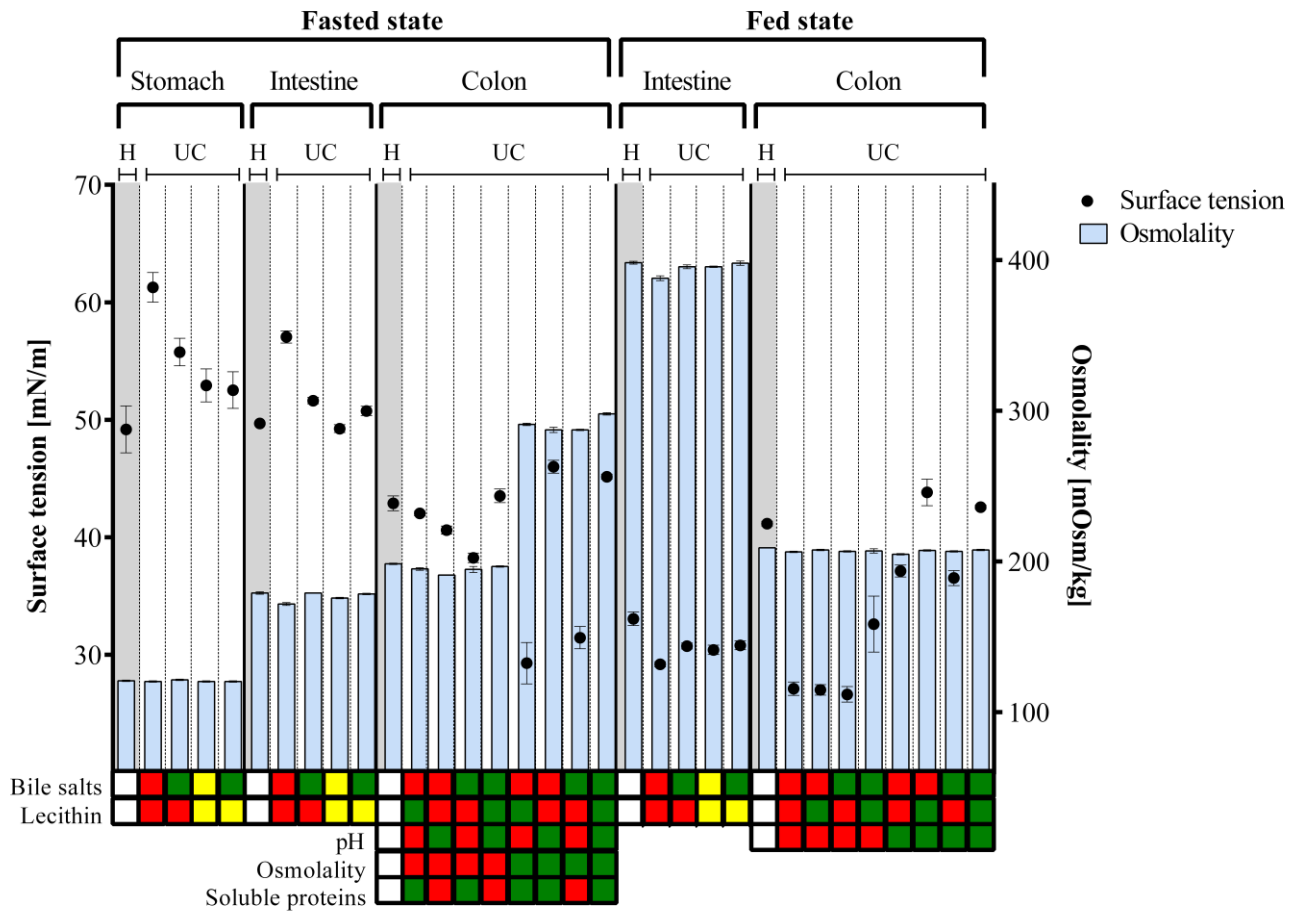
	no changes
	decrease
	increase
	Value in healthy biorelevant media

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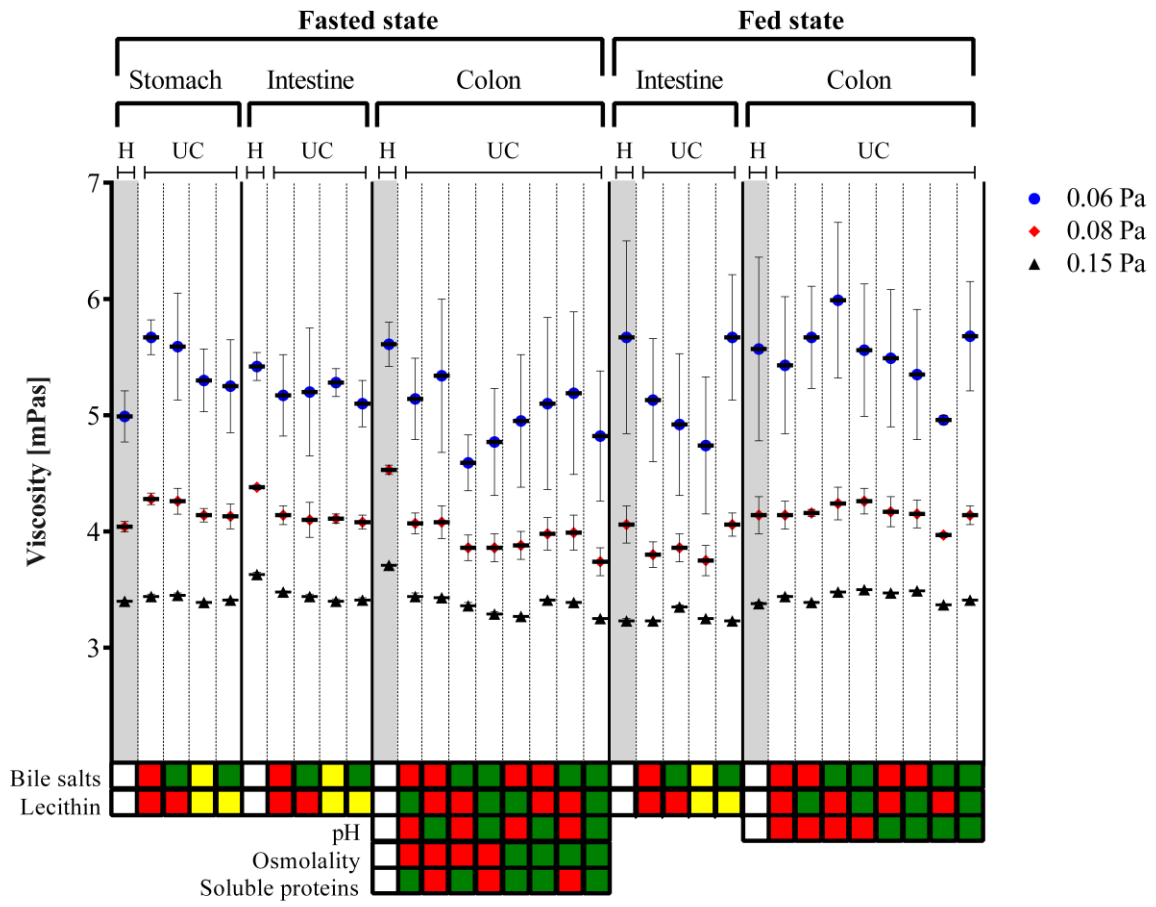


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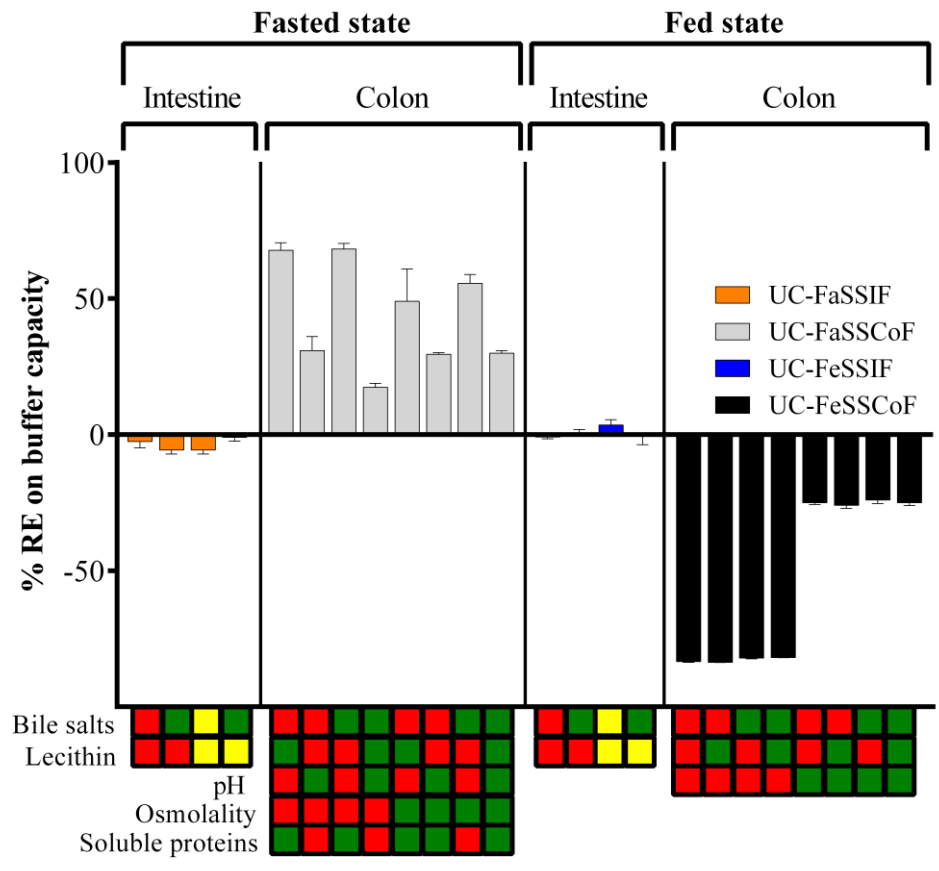
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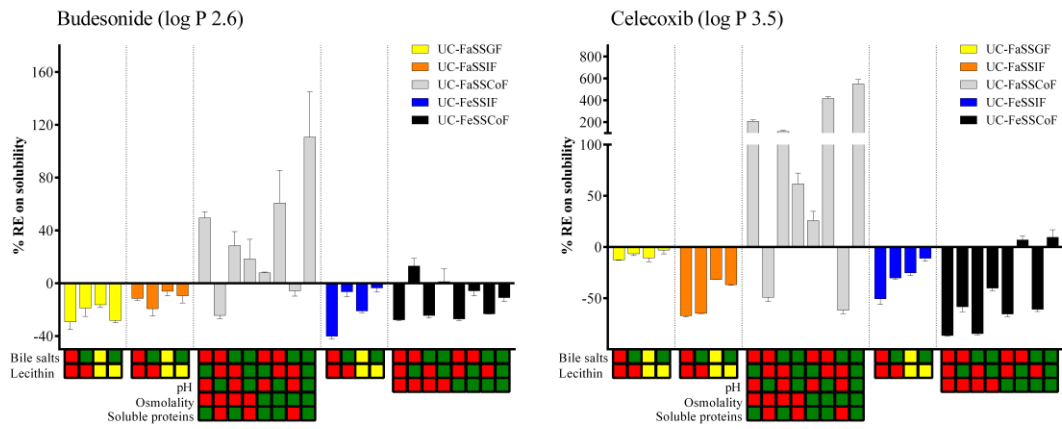
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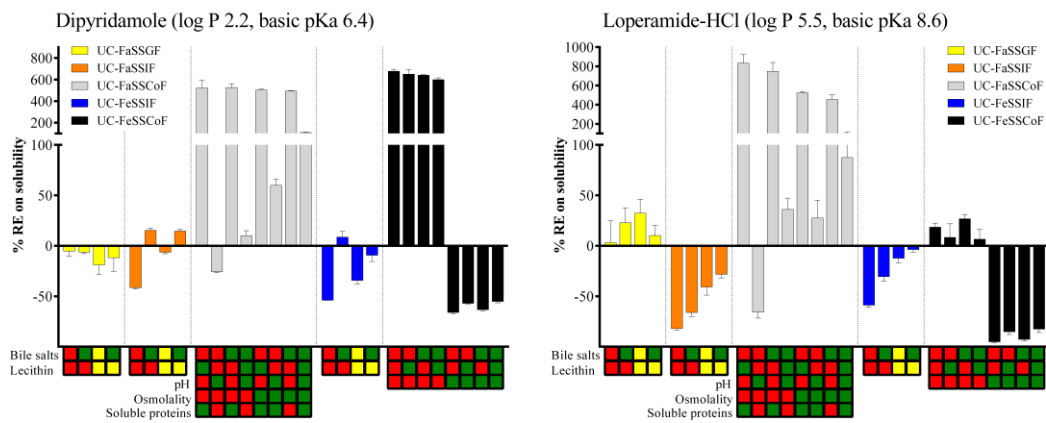
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(a) Neutral drugs



(b) Weak bases



(c) Weak acids

