

Supporting Information

Lipid compositions in infant formulas affect the solubilisation of antimalarial drugs artefenomel (OZ439) and ferroquine during digestion

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Table S1. Nutritional information of milk and the three infant formulas (IF 1, IF 2 and IF 3) used in this study per 100 mL, with reconstitution of 15.0 g powder (IF 1), 14.0 g powder (IF 2) and 14.6 g powder (IF3).

Nutritional information	Quantity per 100 mL			
	Milk	IF 1	IF 2	IF 3
Total fat	3.8 g	3.8 g	3.8 g	3.8 g
Saturated fat	2.5 g	1.5 g	2.1 g	Not stated
Total protein	3.4 g	1.6 g	1.5 g	1.8 g
Carbohydrate	4.8 g	8.8 g	7.9 g	7.4 g
Sodium	40 mg	25 mg	36 mg	20 mg
Calcium	115 mg	63 mg	60 mg	58 mg
Vitamin A	41 µg	80 µg	60 µg	66 µg
Riboflavin (vitamin B2)	200 µg	90 µg	112 µg	88 µg

Table S2. Average compositions of the free fatty acids (FA) and monoacylglycerols (MAG) as mol% in milk and infant formulas (IF 1, IF 2 and IF 3) after 30 minutes digestion. The species were identified as FA or MAG (X:Y), with X = fatty acid chain length and Y = degree of unsaturation. SEM was standard error of mean based on 3 replicates.

Fatty acids	Mol%±SEM			
	Milk	IF 1	IF 2	IF 3
FA (04:0)	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (06:0)	0.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (08:0)	0.4 ± 0.0	0.5 ± 0.1	5.3 ± 0.5	0.3 ± 0.0
FA (10:0)	1.6 ± 0.1	1.0 ± 0.1	3.5 ± 0.1	1.3 ± 0.0
FA (12:0)	1.4 ± 0.1	1.7 ± 0.1	2.0 ± 0.2	2.2 ± 0.1
FA (14:0)	7.8 ± 0.3	3.6 ± 0.3	4.6 ± 0.2	3.2 ± 0.2
FA (14:1)	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (15:0)	1.9 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
FA (15:1)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (16:0)	38.3 ± 2.0	31.5 ± 0.6	23.9 ± 1.3	26.7 ± 1.0
FA (16:1)	2.8 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.0
FA (17:0)	1.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
FA (17:1)	0.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (18:0)	13.6 ± 1.2	5.2 ± 0.4	6.0 ± 1.3	4.1 ± 0.1

FA (18:1)	25.5 ± 0.1	32.6 ± 1.7	26.0 ± 2.5	26.2 ± 1.2
FA (18:2)	2.1 ± 0.0	9.9 ± 0.6	5.8 ± 0.5	7.7 ± 0.5
FA (18:3)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
FA (19:0)	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (19:1)	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (20:0)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
FA (20:1)	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
FA (20:2)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (20:3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (20:4)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (20:5)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (22:0)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
FA (22:1)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
FA (22:2)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (22:3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (22:4)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (22:5)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
FA (22:6)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monoacylglycerols	Mol%±SEM			
	Milk	IF 1	IF 2	IF 3
MAG (04:0)	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
MAG (06:0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (08:0)	0.1 ± 0.0	0.0 ± 0.0	1.0 ± 0.1	0.0 ± 0.0
MAG (10:0)	0.9 ± 0.1	0.1 ± 0.0	2.1 ± 0.1	0.1 ± 0.0
MAG (12:0)	2.3 ± 0.3	4.2 ± 0.2	6.9 ± 0.2	2.4 ± 0.2
MAG (14:0)	11.7 ± 0.5	0.8 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
MAG (14:1)	2.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (15:0)	0.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (15:1)	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
MAG (16:0)	27.2 ± 3.3	1.3 ± 1.2	5.1 ± 1.6	2.0 ± 1.1
MAG (16:1)	5.0 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
MAG (17:0)	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (17:1)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (18:0)	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
MAG (18:1)	17.2 ± 0.9	30.7 ± 2.1	26.5 ± 1.4	26.1 ± 1.7
MAG (18:2)	6.5 ± 0.6	32.6 ± 2.1	26.4 ± 2.0	28.2 ± 1.4
MAG (18:3)	4.6 ± 0.5	12.1 ± 0.2	17.2 ± 2.0	4.9 ± 0.6
MAG (19:0)	0.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (19:1)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6
MAG (20:0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (20:1)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
MAG (20:2)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (20:3)	0.7 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
MAG (20:4)	1.2 ± 0.3	0.8 ± 0.1	0.0 ± 0.0	0.2 ± 0.0
MAG (20:5)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (22:0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (22:1)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (22:2)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (22:3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MAG (22:4)	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0

MAG (22:5)	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.1
MAG (22:6)	0.4 ± 0.0	4.7 ± 0.3	0.0 ± 0.0	0.3 ± 0.0

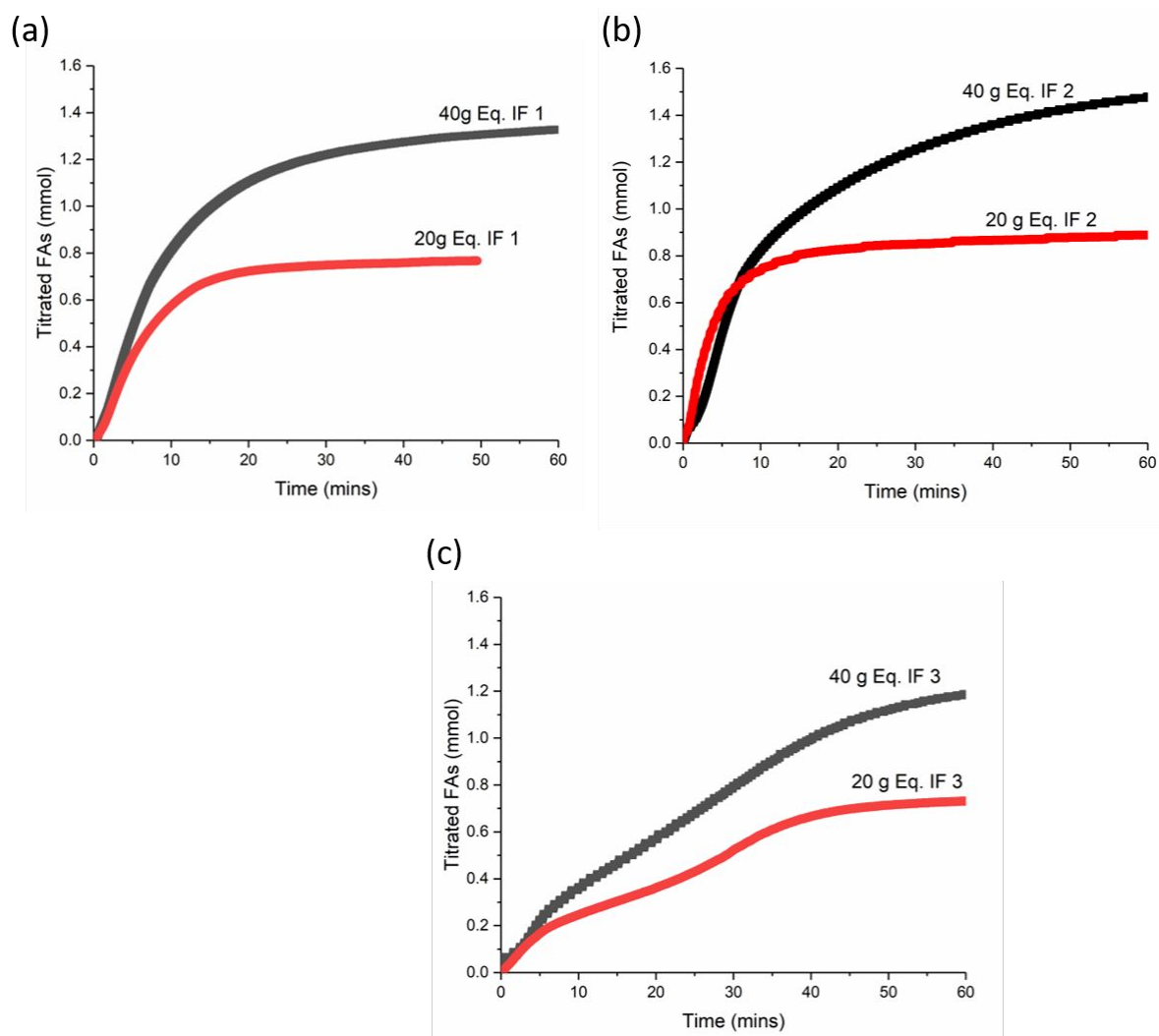


Figure S1. Titrated (ionised) fatty acids during digestion of (a) IF 1, (b) IF 2, and (c) IF 3 at pH 6.5 for 20 g Eq. and 40 g Eq. infant formula powders. The gram equivalents were representative of the amount of IF powder required to administer 800 mg of OZ439-FB and 900 mg FQ-FB in a total volume of 200 mL.

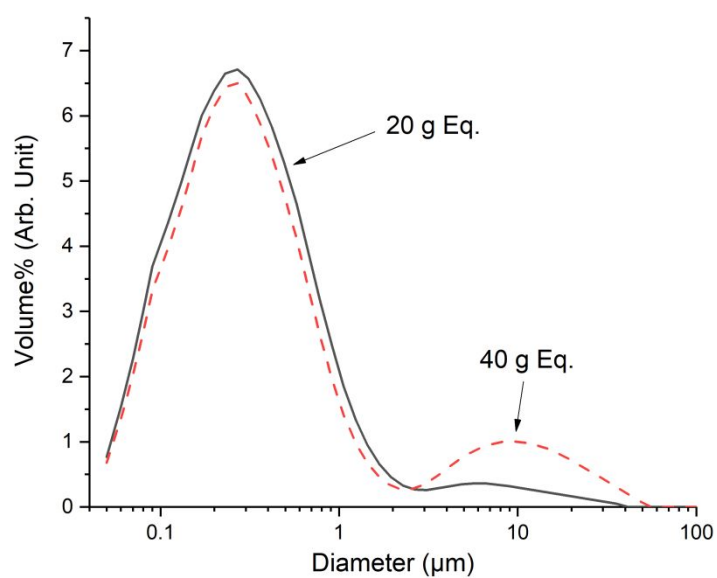


Figure S2. Distribution of the particle sizes in 20 g Eq. (2.7% fat) and 40 g Eq. (5.4% fat) IF 2. The gram equivalents were representative of the amount of IF powder required to administer 800 mg of OZ439-FB and 900 mg FQ-FB in a total volume of 200 mL.

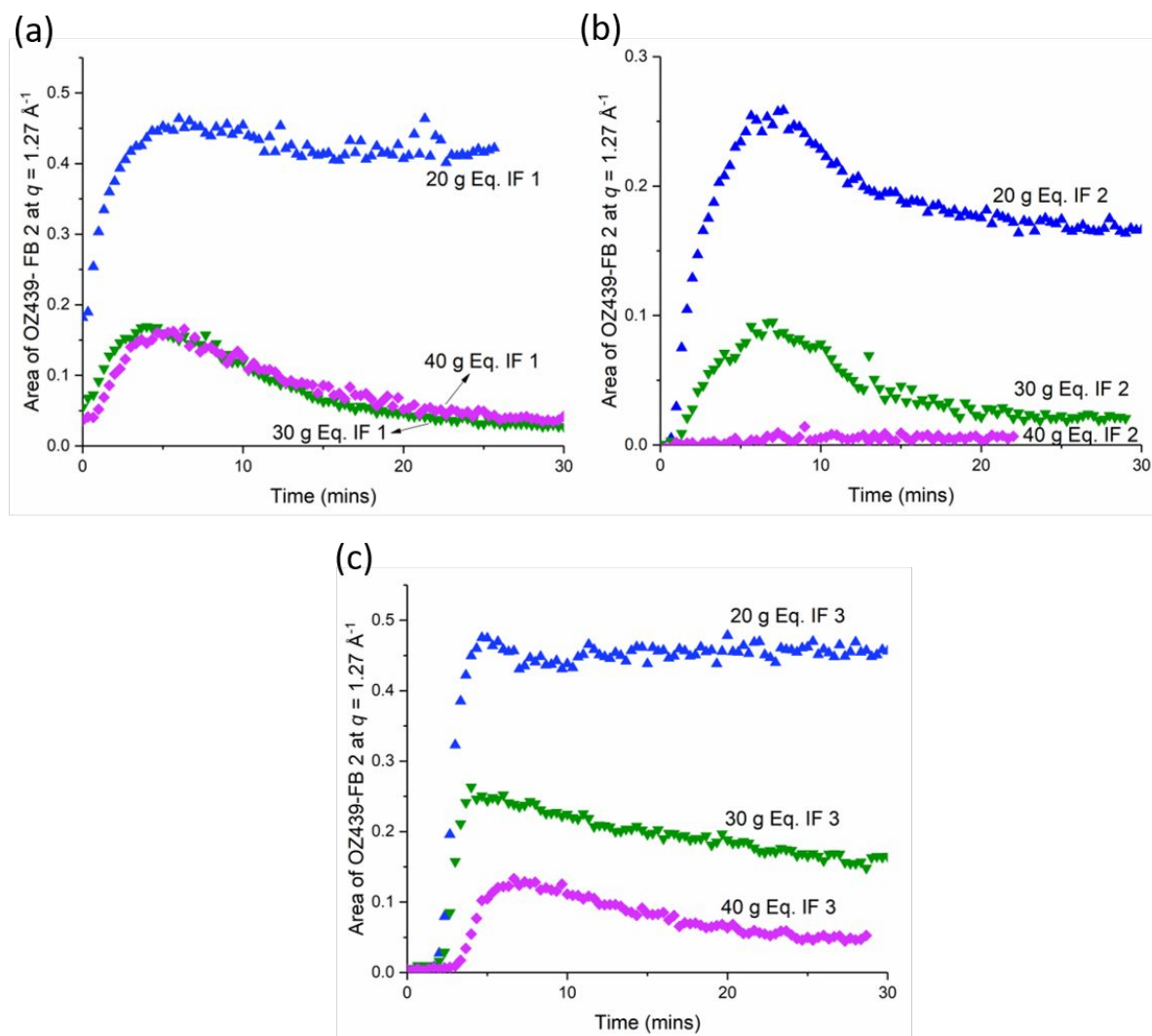


Figure S3. Amount of crystalline OZ439-FB 2 present during digestion in (a) IF 1, (b) IF 2 and (c) IF 3 at 20 g, 30 g, and 40 g Eq. powders. The gram equivalents were representative of the amount of IF powder required to administer 800 mg of OZ439-FB and 900 mg FQ-FB in a total volume of 200 mL.

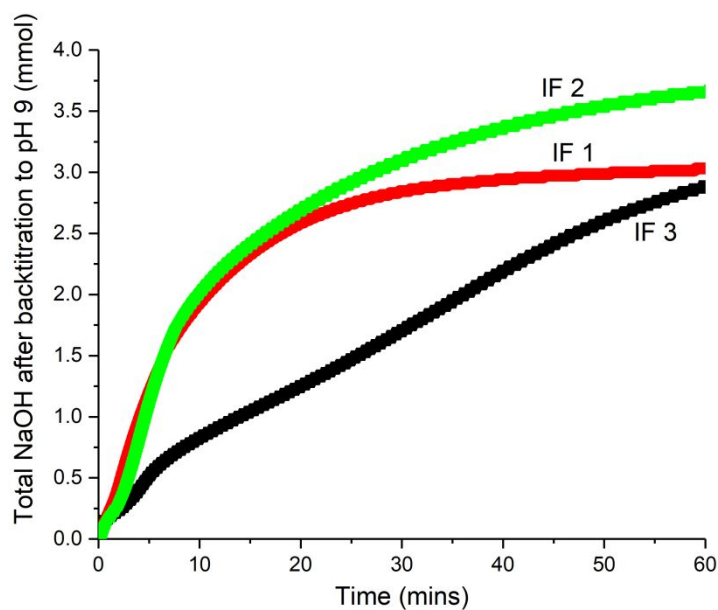


Figure S4. Total amount of sodium hydroxide added during forward titration (digestion at pH 6.5) and backtitration to pH 9 after 1 hour lipolysis of infant formulas at 40 g Eq. IF 1, IF 2, and IF 3. The fat contents were 5.1, 5.4, and 5.2% respectively. The gram equivalents were representative of the amount of IF powder required to administer 800 mg of OZ439-FB and 900 mg FQ-FB in a total volume of 200 mL.

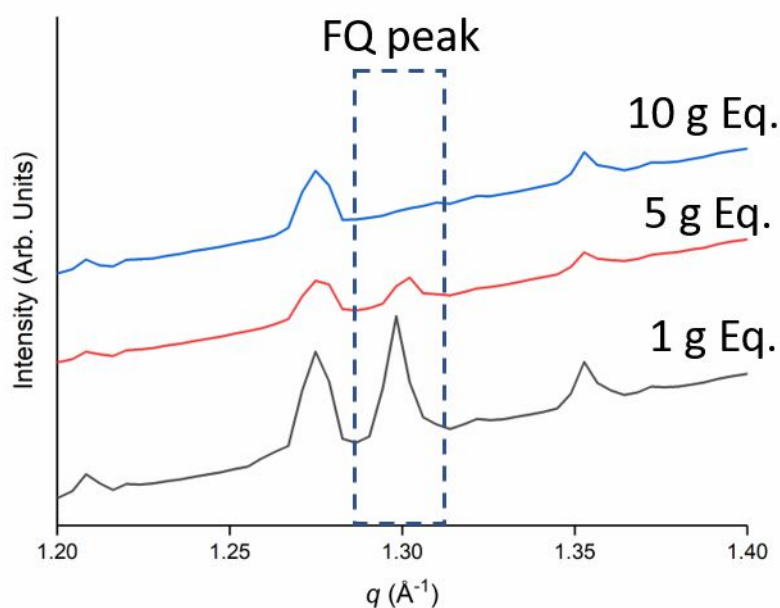


Figure S5. Dependence of solubilisation of ferroquine on fat content of IF2 present. X-ray scattering profiles of OZ439+FQ in IF 2 (characteristic peak from FQ in dashed box) at 1 g (0.1% fat), 5 g (0.7% fat), and 10 g (1.4% fat) Eq. IF 2 powder at the end of digestion. The gram equivalents were representative of the amount of IF powder required to administer 800 mg of OZ439-FB and 900 mg FQ-FB in a total volume of 200 mL.

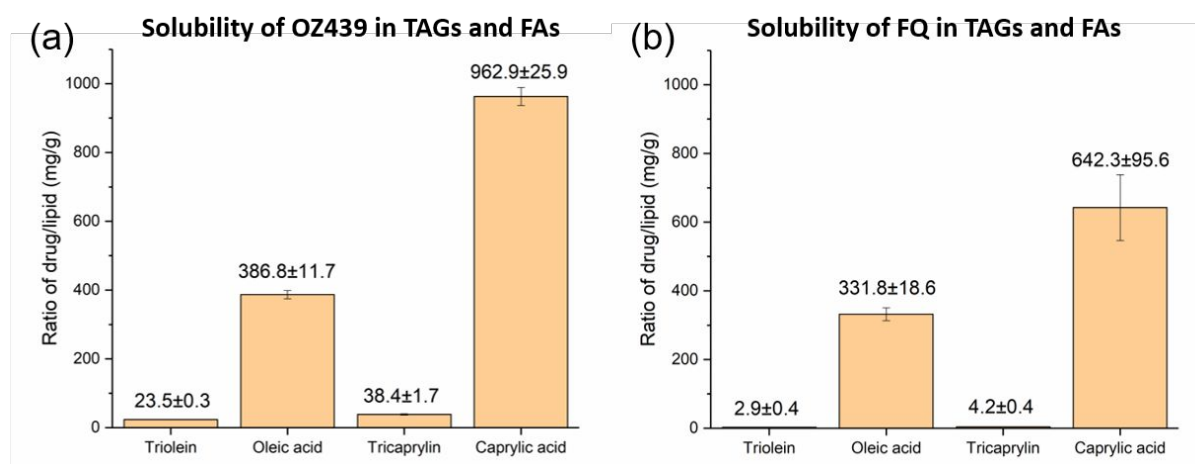


Figure S6. Equilibrium solubility of (a) OZ439-FB 2 and (b) FQ-FB in triacylglycerols (triolein and tricaprylin) and fatty acids (oleic acid and caprylic acid) in mg drug/g lipid.

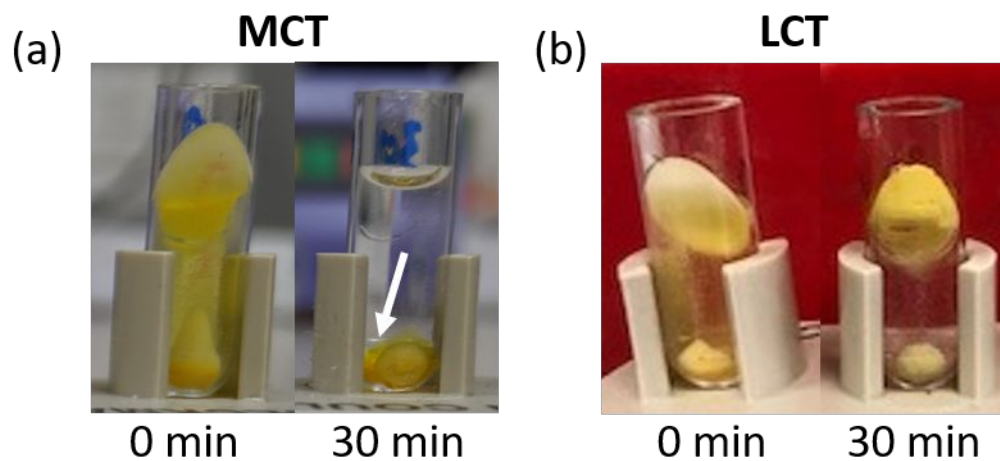


Figure S7. Visual observations of the separated digested phases of OZ439+FQ in medium chain triacylglycerols (MCT) and long chain triacylglycerols (LCT) after ultracentrifugation at 0 minute (before digestion) and 30 minutes digestion. Arrow in panel (a) points to a sedimented oily layer.

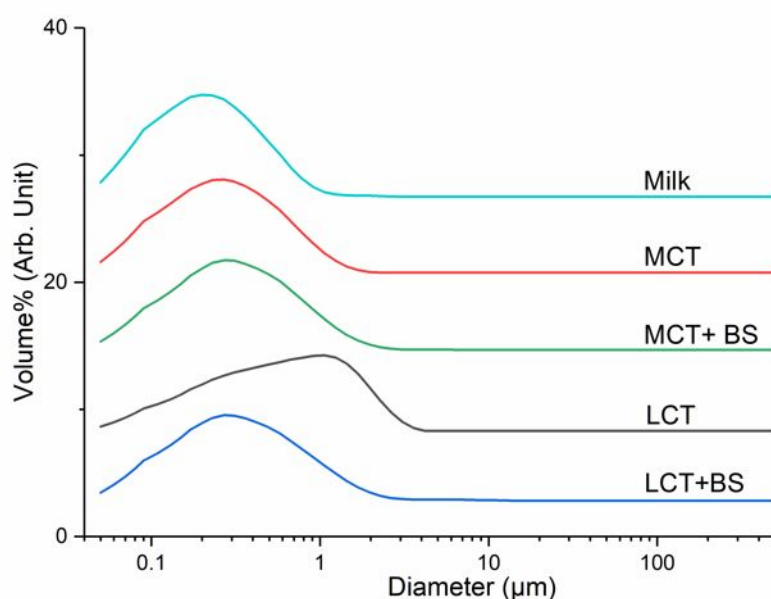


Figure S8. Distribution of the particle sizes in milk, medium chain triacylglycerols (MCT), and long chain triacylglycerols (LCT) and the lipid emulsions in presence of bile salts (BS).

Table S3. Effects of bile salts (NaTDC/DOPC) on the amount of sodium hydroxide added during forward titration (digestion at pH 6.5) and forward + back titration (digestion at pH 6.5 and pH increment to 9) after 1 hr digestion of OZ439+FQ in medium chain – and long chain triacylglycerols. The amount NaOH added have been subtracted from the blank digestions to account for titrated volumes from the MFGM emulsifier, pancreatin, and the drugs.

Formulation	Digestion media	NaOH added in forward titration at pH 6.5 (mmol)	Total NaOH added: forward + back titration to pH 9 (mmol)
MCT	Tris	1.03 ± 0.05	3.00 ± 0.39
	NaTDC/DOPC in tris	1.30 ± 0.04	3.94 ± 0.35
LCT	Tris	0.22 ± 0.01	1.16 ± 0.05
	NaTDC/DOPC in tris	0.44 ± 0.00	1.42 ± 0.11