SUPPORTING INFORMATION

Demonstrating the involvement of an active efflux mechanism in the intestinal absorption of chlorogenic acid and quinic acid using a Caco-2 bidirectional permeability assay.

Olivier Mortelé[1,3], Jennifer Jörissen[2], Irina Spacova[2], Sarah Lebeer[2], Alexander L.N. van Nuijs[1], Nina Hermans[3]

[1]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[2]Research Group Environmental Ecology and Applied Microbiology (ENdEMIC), Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen

[3]Natural Products and Food – Research & Analysis (NatuRA), University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

*-corresponding authors: olivier.mortele@uantwerpen.be, nina.hermans@uantwerpen.be

1.1. LC-MS/MS analysis

1.1.1. Chlorogenic acid and ferulic acid-D_3

The used mobile phase compositions were ultrapure water with 0.1 % (v/v) formic acid (A) and MeOH (B) with a constant flow of 0.3 mL/min. The gradient was constructed as follows: for 1 min B was used at 2%, followed by an increase to 98% B over 3.5 min. The column was rinsed for 6 min with 98 % B and re-equilibrated at 2% B for 6 min. The LC-stream was directed to the waste after 6 min of analysis-time to limit source contamination.

Chlorogenic acid, and ferulic acid-D_3 as internal standard, were measured in negative MRM mode (Table 1). Gas temperature and flow were set at 200 °C and 14 L/min respectively. A nebulizer pressure of 20 psi was chosen, while sheath gas temperature and flow were set to 400 °C and 11 L/min. A capillary and nozzle voltage of 4000 V and 2000 V respectively were chosen.

1.1.2. Quinic acid

The mobile phase compositions were ultrapure water with 0.04% (v/v) formic acid (A) and MeOH (B) with a constant flow of 0.3 mL/min. Mobile phase B started at 1% for 2 min, followed by an increase to 98% B over 8 min. The column was rinsed for 6 min before 4 min re-equilibration at 1% B. The LC-stream was directed to the waste after 6 min analysis-time.

Quinic acid was analyzed in negative MRM mode. Ferulic acid-D_3 was used as the internal standard (Table 1). Optimized source parameters were set as followed: gas temperature of 200 °C, gas flow of 14 L/min, nebulizer pressure of 50 psi, sheath gas temperature of 400 °C, sheath gas flow of 12 L/min, capillary voltage of 2500 V and a nozzle voltage of 2000 V.

1.1.3. Propranolol and digoxin

Mobile phase A consisted of 20 mM ammoniumacetate in ultrapure water. Acetonitrile was used as mobile phase B. The chromatographic run started at 10% B for 1 min, followed by an increase to 95% B over 9 min. The column was rinsed for 5 min at 95 % B before 5 min re-equilibration at 10% B. The LC-stream was directed to the waste after 8 min of analysis-time to limit source contamination.

Propranolol, propranolol-D_7 (internal standard), digoxin and digoxin-D_3 (internal standard) were analyzed in positive MRM mode (Table 1). Gas temperature, flow and nebulizer pressure were identical to the parameters described for chlorogenic acid. Sheath gas temperature was set to 200 °C with a flow of 11 L/min. Capillary and nozzle voltages were 3500 V and 1500 V.

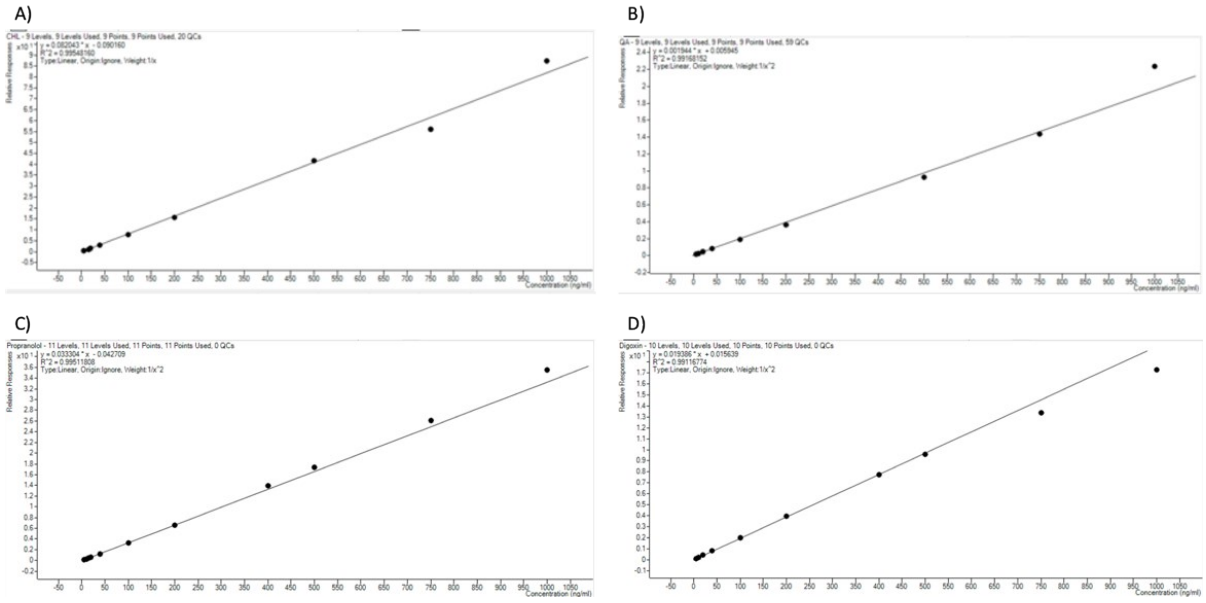

Figure S1: Overview of the calibration curves of chlorogenic acid (A), quinic acid (B), propranolol (C) and digoxin (D).

Table S1: Overview of the calculated P_{app} values and efflux ratios for chlorogenic acid and the positive control compounds

Compound	**P_{app}(A-B) (cm/s) (± SD) x 10^{-6} (n=3)**	**P_{app}(B-A) (cm/s) (± SD) x 10^{-6} (n=3)**	**Efflux ratio**
Chlorogenic acid 10 µM	2.42 (± 0.16)	8.01 (± 0.41)	3.3 (± 0.2)
Chlorogenic acid 50 µM	2.61 (± 0.20)	8.41 (± 0.77)	3.2 (± 0.4)
Propranolol	5.45 (± 0.86)	8.40 (± 0.56)	1.5 (± 0.3)
Digoxin	0.36 (± 0.02)	7.91 (± 0.73)	22.0 (± 2.4)

Table S2: Overview of the calculated Papp values and efflux ratios for quinic acid and the positive control compounds.

Compound	P_{app}(A-B) (cm/s) (± SD) x 10^{-6} (n=3)	P_{app}(B-A) (cm/s) (± SD) x 10^{-6} (n=3)	Efflux ratio
Quinic acid	3.8 (± 0.69)	22.6 (± 1.8)	5.9 (± 1.2)
Propranolol	5.14 (± 0.35)	5.22 (± 0.35)	1.0 (± 0.1)
Digoxin	0.70 (± 0.16)	6.17 (± 0.23)	8.7 (± 2.1)