SUPPORTING INFORMATION

Quantification of P-glycoprotein in the gastrointestinal tract of humans and rodents:
Methodology, Gut Region, Sex and Species Matters

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**Supplementary Figure 1.** Western Blot results of intestinal P-gp expression in male and female human small intestinal tissues

**Supplementary Figure 2.** Western Blot results of intestinal P-gp expression in male and female rat intestinal tract
Supplementary Figure 3. Western Blot calibration of intestinal P-gp expression.

Supplementary Figure 4. Correlation between human small intestinal P-gp following quantification via LC-MS/MS and Western Blot.
Supplementary Figure 5. Correlation between rat small intestinal P-gp following quantification via LC-MS/MS and Western Blot.

Rat small intestine

Rat colon
**Supplementary Figure 6.** Correlation between rat colonic P-gp following quantification via LC-MS/MS and Western Blot.

**Supplementary Figure 7.** Correlation between human small intestinal P-gp following quantification via LC-MS/MS and RT-PCR.
**Supplementary Figure 8.** Correlation between human small intestinal P-gp following quantification via Western Blot and RT-PCR.

Supplementary Figure 9. Correlation between rat small intestinal P-gp following quantification via LC-MS/MS and RT-PCR.
**Supplementary Figure 10.** Correlation between rat small intestinal P-gp following quantification via Western Blot and RT-PCR.

![Graph showing correlation between rat colon P-gp levels and mdr1a expression.](image)

$R^2 = 0.0071$

**Supplementary Figure 11.** Correlation between rat colonic P-gp following quantification via LC-MS/MS and RT-PCR.

![Graph showing correlation between rat colon P-gp levels and mdr1a expression.](image)

$R^2 = 0.9106$
**Supplementary Figure 12.** Correlation between rat colonic P-gp following quantification via Western Blot and RT-PCR.

![1st transition calibration curve](image)

**Supplementary Figure 13.** 1st transition calibration curve from the average ratio of P-glycoprotein at specific concentrations (see Supplementary Table 6).
**Supplementary Figure 14.** 2nd transition calibration curve from the average ratio of P-glycoprotein at specific concentrations (see Supplementary Table 7).

**Supplementary Figure 15.** 3rd transition calibration curve from the average ratio of P-glycoprotein at specific concentrations (see Supplementary Table 8).
Supplementary Figure 16. Comparison of the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} transition quantification values of human intestinal P-glycoprotein (see Supplementary Table 9).
Supplementary Figure 17. Comparison of the 1st, 2nd and 3rd transition quantification values of Wistar rat intestinal P-glycoprotein (see Supplementary Table 10).
**Supplementary Figure 18.** MRM chromatograms for the developed LC-MS/MS method applied to measure all three transitions of proteotypic peptides (left) and their stable isotope labelled internal standard peptides (right) from the spiked HSA matrix.
**Supplementary Figure 19.** MRM chromatograms for the developed LC-MS/MS method applied to measure all three transitions of proteotypic peptides (left) and their stable isotope labelled internal standard peptides (right) from the blank HSA matrix.
Supplementary Figure 20. MRM chromatograms for the developed LC-MS/MS method applied to measure all three transitions of proteotypic peptides (left) and their stable isotope labelled internal standard peptides (right) from 0.1% formic acid water.
Supplementary Figure 21. Comparison of the analytical samples and internal standard transition MS signal ratio, 1/2 transition ratio, 1/3 transition ratio and 2/3 transition ratio respectively; a) human intestinal tissue samples; b) Wistar rat intestinal tissue samples.

Supplementary Table 1. Statistical difference via one-way ANOVA of P-gp expression in intestinal segments in rats and humans via quantification
Supplementary Table 2. Threshold cycle values for PCR

| Sample | Mean | S.D. | Min | Max | Mean | S.D. | Min | Max | Mean | S.D. | Min | Max | Mean | S.D. | Min | Max | p-value | 2^-ΔCt (10^6) |
|--------|------|------|-----|-----|------|------|-----|-----|------|------|-----|-----|------|------|-----|-----|---------|---------------|
| Rat    |      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Jejunum| M (n = 7) | 21.85 | 0.68 | 21.19 | 23.02 | 31.02 | 0.86 | 30.05 | 32.29 | 9.17 | 0.35 | 1.79 | 0.44 |
|        | F (n = 8) | 22.51 | 0.58 | 21.73 | 23.49 | 33.14 | 0.78 | 32.34 | 34.68 | 10.62 | 0.52 | 0.67 | 0.23 |
|        | M (n = 10)| 22.53 | 0.96 | 20.74 | 23.66 | 31.38 | 1.06 | 29.41 | 32.46 | 8.84 | 0.37 | 2.24 | 0.58 |
|        | F (n = 5) | 22.16 | 0.79 | 21.13 | 23.16 | 31.88 | 0.90 | 30.51 | 32.97 | 9.72 | 0.23 | 1.20 | 0.20 |
| Duodenum| M (n = 6) | 22.77 | 0.68 | 22.24 | 23.66 | 30.96 | 0.96 | 29.97 | 32.46 | 8.19 | 0.39 | 3.54 | 0.87 |
|        | F (n = 6) | 22.01 | 0.48 | 21.23 | 22.46 | 29.86 | 0.70 | 28.96 | 30.72 | 7.85 | 0.37 | 4.45 | 1.11 |
|        |         |       |      |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Jejunum| M (n = 6) | 21.93 | 0.69 | 21.04 | 22.64 | 28.77 | 0.78 | 27.79 | 29.87 | 6.84 | 0.41 | 8.99 | 2.35 |
|        | F (n = 6) | 22.28 | 0.89 | 21.29 | 23.71 | 29.35 | 0.68 | 28.41 | 30.25 | 7.07 | 0.46 | 7.76 | 2.36 |
|        | M (n = 6) | 22.35 | 0.60 | 21.58 | 23.20 | 29.08 | 0.54 | 28.44 | 29.85 | 6.73 | 0.12 | 9.44 | 0.80 |
|        | F (n = 6) | 22.25 | 0.62 | 22.17 | 23.17 | 29.57 | 0.72 | 28.34 | 30.38 | 7.32 | 0.33 | 6.42 | 1.56 |
|        | M (n = 6) | 22.29 | 0.96 | 21.06 | 23.19 | 28.61 | 1.06 | 27.28 | 29.66 | 6.32 | 0.21 | 12.66 | 1.91 |
|        | F (n = 6) | 22.42 | 0.71 | 21.22 | 23.20 | 29.44 | 0.88 | 27.91 | 30.54 | 7.01 | 0.29 | 7.89 | 1.61 |
| Human  |      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Jejunum|      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
|        |        | < 0.001† | 0.002† | < 0.001† | 0.084 | 0.003† | 0.037 | 0.046 | 0.483 | < 0.001† | < 0.001† | 0.016 | 0.516 |
|        |        |        |       |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Human (mdr1) |      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Jejunum|        |      |       |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
|        |        | < 0.001† | 0.002† | < 0.001† | 0.084 | 0.003† | 0.037 | 0.046 | 0.483 | < 0.001† | < 0.001† | 0.016 | 0.516 |
|        |        |        |       |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Rat (mdr1a) |      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
| Jejunum|        |      |       |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |
|        |        | < 0.001† | 0.002† | < 0.001† | 0.084 | 0.003† | 0.037 | 0.046 | 0.483 | < 0.001† | < 0.001† | 0.016 | 0.516 |
|        |        |        |       |     |      |      |     |     |      |      |     |     |      |      |     |     |         |               |

*p < 0.05*
†p < 0.01
## Supplementary Table 3. Raw RT-PCR data

| Group          | Ct (mdr1) | Ct (β-actin) | ΔCt | ΔCt(-) | 2^ΔCt | Expressive Value | Mean (Ct mdr1) | Mean (Ct β-actin) | Mean (ΔCt) | Mean S.D. (Ct mdr1) | S.D. (Ct β-actin) | Mean S.D. (ΔCt) | S.D. | S.D. |
|----------------|-----------|--------------|-----|--------|-------|-----------------|----------------|-------------------|------------|----------------------|-------------------|------------------|------|------|
| Male Duodenum  | 1         | 33.83        | 23.61 | 8.85   | -8.85 | 0.00217         | 2.17           |                   |            |                      |                   |                  |      |      |
|               | 2         | 32.61        | 22.25 | 8.11   | -8.11 | 0.00363         | 3.63           |                   |            |                      |                   |                  |      |      |
|               | 3         | 32.94        | 22.46 | 8.39   | -8.39 | 0.00299         | 2.99           |                   |            |                      |                   |                  |      |      |
|               | 4         | 32.43        | 22.24 | 7.73   | -7.73 | 0.00471         | 4.71           |                   |            |                      |                   |                  |      |      |
|               | 5         | 33.84        | 23.66 | 8.12   | -8.12 | 0.0036          | 3.6            |                   |            |                      |                   |                  |      |      |
|               | 6         | 32.62        | 22.41 | 7.96   | -7.96 | 0.00401         | 4.01           | 30.96             | 22.77     | 8.19                 | 3.52              | 0.96255         | 0.6757 | 0.38654 | 0.8692 |
| Female Duodenum| 1        | 32.25        | 21.23 | 7.73   | -7.73 | 0.00471         | 4.71           |                   |            |                      |                   |                  |      |      |
|               | 2         | 32.71        | 22.46 | 8.26   | -8.26 | 0.00326         | 3.26           |                   |            |                      |                   |                  |      |      |
|               | 3         | 32.42        | 22.13 | 7.50   | -7.50 | 0.00553         | 5.53           |                   |            |                      |                   |                  |      |      |
|               | 4         | 33.38        | 22.43 | 8.25   | -8.25 | 0.00329         | 3.29           |                   |            |                      |                   |                  |      |      |
|               | 5         | 31.90        | 21.63 | 7.96   | -7.96 | 0.00402         | 4.02           |                   |            |                      |                   |                  |      |      |
|               | 6         | 32.75        | 22.16 | 7.41   | -7.41 | 0.00587         | 5.87           | 29.86             | 22.01     | 7.85                 | 4.45              | 0.69623         | 0.48172 | 0.36556 | 1.1132 |
| Male Jejunum  | 1         | 33.08        | 22.51 | 7.36   | -7.36 | 0.00609         | 6.09           |                   |            |                      |                   |                  |      |      |
|               | 2         | 32.13        | 21.42 | 7.37   | -7.37 | 0.00606         | 6.06           |                   |            |                      |                   |                  |      |      |
|               | 3         | 31.93        | 21.04 | 6.75   | -6.75 | 0.00928         | 9.28           |                   |            |                      |                   |                  |      |      |
|               | 4         | 34.07        | 22.64 | 6.50   | -6.50 | 0.01105         | 11.05          |                   |            |                      |                   |                  |      |      |
|               | 5         | 33.28        | 22.45 | 6.60   | -6.60 | 0.01032         | 10.32          |                   |            |                      |                   |                  |      |      |
|               | 6         | 33.16        | 21.49 | 6.49   | -6.49 | 0.01111         | 11.11          | 28.77             | 21.93     | 6.84                 | 8.99              | 0.77600         | 0.68766 | 0.41225 | 2.3488 |
| Female Jejunum| 1        | 33.68        | 23.71 | 6.54   | -6.54 | 0.01075         | 10.75          |                   |            |                      |                   |                  |      |      |
|               | 2         | 32.54        | 22.67 | 6.65   | -6.65 | 0.00996         | 9.96           |                   |            |                      |                   |                  |      |      |
|               | 3         | 33.14        | 22.27 | 7.68   | -7.68 | 0.00486         | 4.86           |                   |            |                      |                   |                  |      |      |
|               | 4         | 32.28        | 22.35 | 7.00   | -7.00 | 0.00781         | 7.81           |                   |            |                      |                   |                  |      |      |
|               | 5         | 31.90        | 21.29 | 7.54   | -7.54 | 0.00539         | 5.39           |                   |            |                      |                   |                  |      |      |
|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
|   | 6  | 21.40 | 7.01  | -7.01 | 0.00776 | 7.76  | 29.35 | 22.28 | 7.07  | 7.76  |
|   | 1  | 32.76 | 22.58 | 6.75  | -6.75 | 0.00929 | 9.29  |   |   |   |
|   | 2  | 32.44 | 22.63 | 6.57  | -6.57 | 0.01049 | 10.49 |   |   |   |
|   | 3  | 30.68 | 21.76 | 6.68  | -6.68 | 0.00977 | 9.77  |   |   |   |
|   | 4  | 33.52 | 23.20 | 6.65  | -6.65 | 0.00997 | 9.97  |   |   |   |
|   | 5  | 32.29 | 22.35 | 6.83  | -6.83 | 0.00879 | 8.79  |   |   |   |
|   | 6  | 30.90 | 21.58 | 6.91  | -6.91 | 0.00832 | 8.32  |   |   |   |

### Ileum

|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
|   | 6  | 21.06 | 6.27  | -6.27 | 0.01296 | 12.96 |   |   |   |   |
|   | 1  | 31.69 | 21.06 | 6.27  | -6.27 | 0.01296 | 12.96 |   |   |   |
|   | 2  | 34.43 | 22.17 | 6.55  | -6.55 | 0.0107  | 10.7  |   |   |   |
|   | 3  | 34.12 | 23.07 | 6.07  | -6.07 | 0.01487 | 14.87 |   |   |   |
|   | 4  | 34.42 | 23.02 | 6.48  | -6.48 | 0.01121 | 11.21 |   |   |   |
|   | 5  | 33.50 | 23.19 | 6.47  | -6.47 | 0.01126 | 11.26 |   |   |   |
|   | 6  | 31.46 | 21.22 | 6.06  | -6.06 | 0.01496 | 14.96 | 28.61 | 22.29 |   |   |

### Colon

|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
|   | 6  | 21.22 | 6.69  | -6.69 | 0.00966 | 9.66  |   |   |   |   |
|   | 1  | 31.46 | 21.22 | 6.69  | -6.69 | 0.00966 | 9.66  |   |   |   |
|   | 2  | 32.74 | 22.25 | 7.23  | -7.23 | 0.00665 | 6.65  |   |   |   |
|   | 3  | 33.65 | 22.73 | 6.63  | -6.63 | 0.01009 | 10.09 |   |   |   |
|   | 4  | 33.47 | 22.94 | 7.07  | -7.07 | 0.00744 | 7.44  |   |   |   |
|   | 5  | 34.19 | 23.20 | 7.34  | -7.34 | 0.00619 | 6.19  |   |   |   |
|   | 6  | 32.35 | 22.21 | 7.10  | -7.10 | 0.00728 | 7.28  | 29.44 | 22.42 |   |   |
### Supplementary Table 4. Rat intestinal *mdr1a* and P-gp expression quantified by RT-PCR, Western Blot or LC-MS/MS

**Technique employed for gene and protein expression of intestinal P-gp**

| Technique         | Sex     | Rat Sample | Duodenum | Jejunum | Ileum | Colon |
|-------------------|---------|------------|----------|---------|-------|-------|
| RT-PCR            | Male    | 1          | 2.17     | 6.09    | 8.79  | 14.87 |
|                   |         | 2          | 3.60     | 9.28    | 9.97  | 11.21 |
|                   |         | 3          | 3.63     | 11.05   | 9.29  | 12.96 |
|                   |         | 4          | 2.99     | 6.06    | 8.32  | 11.26 |
|                   |         | 5          | 4.71     | 11.11   | 9.77  | 14.96 |
|                   |         | 6          | 4.01     | 10.32   | 10.49 | 10.70 |
|                   | Female  | 1          | 3.26     | 7.76    | 5.04  | 7.44  |
|                   |         | 2          | 3.29     | 4.86    | 5.49  | 7.28  |
|                   |         | 3          | 4.02     | 5.39    | 5.54  | 6.19  |
|                   |         | 4          | 4.71     | 7.81    | 8.79  | 9.66  |
|                   |         | 5          | 5.53     | 10.75   | 5.66  | 6.65  |
|                   |         | 6          | 5.87     | 9.96    | 8.00  | 10.09 |
| Western Blot      | Male    | 1          | 0.40     | 0.51    | 0.63  | 0.82  |
|                   |         | 2          | 0.48     | 0.64    | 0.84  | 0.96  |
|                   |         | 3          | 0.58     | 1.00    | 1.22  | 1.58  |
|                   |         | 4          | 0.47     | 0.48    | 0.54  | 0.99  |
|                   |         | 5          | 0.80     | 1.42    | 1.52  | 1.65  |
|                   |         | 6          | 0.70     | 0.83    | 0.96  | 1.66  |
|                   | Female  | 1          | 0.10     | 0.41    | 0.49  | 0.72  |
|                   |         | 2          | 0.21     | 0.37    | 0.38  | 0.58  |
|                   |         | 3          | 0.22     | 0.40    | 0.51  | 0.90  |
|                   |         | 4          | 0.23     | 0.55    | 0.73  | 0.81  |
|                   |         | 5          | 0.34     | 0.74    | 0.62  | 1.25  |
|                   |         | 6          | 0.47     | 0.60    | 0.67  | 0.99  |
| LC-MS/MS          | Male    | 1          | 2.04     | 3.16    | 3.37  | 3.09  |
|                   |         | 2          | 2.05     | 3.18    | 3.38  | 1.82  |
|                   |         | 3          | 2.06     | 3.45    | 5.17  | 2.11  |
|                   |         | 4          | 2.04     | 2.24    | 2.51  | 2.00  |
|                   |         | 5          | 2.27     | 3.92    | 4.18  | 3.20  |
|                   |         | 6          | 2.11     | 3.26    | 3.38  | 1.68  |
|                   | Female  | 1          | 1.37     | 1.81    | 2.41  | 2.00  |
|                   |         | 2          | 1.57     | 1.61    | 1.91  | 1.78  |
|   | 1.81 | 1.92 | 2.23 | 1.75 |
|---|------|------|------|------|
| 4 | 2.15 | 2.20 | 3.38 | 2.85 |
| 5 | 2.31 | 2.24 | 2.46 | 2.44 |
| 6 | 2.54 | 1.95 | 2.24 | 1.77 |
Supplementary Table 5. Human intestinal *mdrl* and P-gp expression quantified by RT-PCR, Western Blot or LC-MS/MS

| Number | Intestinal region | Sample | RT-PCR | Western Blot | LC-MS/MS |
|--------|-------------------|--------|--------|--------------|----------|
| 1      | Jejunum           | M1     | 1.79   | 1.63         | 2.89     |
| 2      |                   | M2     | 1.83   | 1.77         | 2.97     |
| 3      |                   | M3     | 1.28   | 1.29         | 2.22     |
| 4      |                   | M4     | 1.62   | 1.53         | 2.81     |
| 5      |                   | M5     | 2.47   | 2.04         | 3.32     |
| 6      |                   | M6     | 1.31   | 1.36         | 2.41     |
| 7      |                   | M7     | 2.20   | 1.84         | 3.05     |
| 8      |                   | F1     | 0.39   | 0.37         | 0.59     |
| 9      |                   | F2     | 0.53   | 0.71         | 1.60     |
| 10     |                   | F3     | 0.95   | 1.06         | 1.95     |
| 11     |                   | F4     | 0.52   | 0.61         | 1.14     |
| 12     |                   | F5     | 0.89   | 0.91         | 1.86     |
| 13     |                   | F6     | 0.91   | 0.95         | 1.94     |
| 14     |                   | F7     | 0.43   | 0.42         | 0.68     |
| 15     |                   | F8     | 0.75   | 0.81         | 1.85     |
| 1      | Ileum             | M1     | 3.34   | 3.09         | 6.43     |
| 2      |                   | M2     | 2.45   | 2.11         | 4.99     |
| 3      |                   | M3     | 2.00   | 1.94         | 4.11     |
| 4      |                   | M4     | 1.37   | 1.03         | 3.87     |
|     |     |     |     |
|-----|-----|-----|-----|
| 5   | M5  | 1.71| 1.59| 3.94|
| 6   | M6  | 2.96| 2.26| 5.01|
| 7   | M7  | 2.25| 2.10| 4.55|
| 8   | M8  | 2.24| 2.06| 4.40|
| 9   | M9  | 2.21| 1.99| 4.28|
| 10  | M10 | 1.90| 1.86| 4.02|
| 11  | F1  | 1.28| 1.23| 2.88|
| 12  | F2  | 1.50| 1.44| 3.53|
| 13  | F3  | 1.15| 1.08| 2.71|
| 14  | F4  | 0.97| 0.95| 2.31|
| 15  | F5  | 1.11| 1.06| 2.52|
**Supplementary Table 6.** Average ratio of the first transition for a calibration curve

| STDS | Concentration (fmol) | Target peak area (635.3/771.3) | Target peak area (640.3/781.4) | Ratio | Average ratio |
|------|----------------------|---------------------------------|---------------------------------|-------|---------------|
| 1    | 500                  | 1067                            | 1069                            | 1269  | 0.8423956     | 0.92146047 |
|      |                      | 1372                            |                                 | 1.0811663 |                |
|      |                      | 509                             |                                 | 0.4104839 |                |
| 2    | 250                  | 457                             | 1240                            | 623   | 0.3685484     | 0.42715054 |
|      |                      |                                 |                                 | 0.5024194 |                |
|      |                      | 251                             |                                 | 0.2079536 |                |
| 3    | 125                  | 241                             | 1207                            | 266   | 0.1996686     | 0.20933444 |
|      |                      |                                 |                                 | 0.2203811 |                |
|      |                      | 123                             |                                 | 0.1000000 |                |
| 4    | 62.50                | 114                             | 1230                            | 106   | 0.0926829     | 0.09295393 |
|      |                      |                                 |                                 | 0.0861789 |                |
|      |                      | 50                              |                                 | 0.0394633 |                |
| 5    | 31.25                | 48                              | 1267                            | 39    | 0.0378848     | 0.03604315 |
|      |                      |                                 |                                 | 0.0307814 |                |
|      |                      | 27                              |                                 | 0.0225188 |                |
| 6    | 15.625               | 20                              | 1199                            | 20    | 0.0166806     | 0.01973867 |
|      |                      |                                 |                                 | 0.0200167 |                |
### Supplementary Table 7. Average ratio of the second transition for a calibration curve

| STDs | Concentration (fmol) | Target peak area (635.3/900.5) | Target peak area (640.3/910.5) | Ratio | Average ratio |
|------|----------------------|---------------------------------|---------------------------------|-------|---------------|
| 1    | 500                  | 1659                            | 1602                            | 1.04012539 | 1.00438871    | 1.05350052 |
|      |                      | 1780                            | 1595                            | 1.11598746 |               |               |
|      |                      | 899                             |                                 | 0.62430556 |               |               |
| 2    | 250                  | 699                             | 1440                            | 0.48541667 | 0.58101852    |               |
|      |                      | 912                             |                                 | 0.63333333 |               |               |
|      |                      | 367                             |                                 | 0.24049803 |               |               |
| 3    | 125                  | 358                             | 1526                            | 0.23460026 | 0.24093491    |               |
|      |                      | 378                             |                                 | 0.24770642 |               |               |
|      |                      | 200                             |                                 | 0.1332445 |               |               |
| 4    | 62.50                | 209                             | 1501                            | 0.13924051 | 0.1221408     |               |
|      |                      | 141                             |                                 | 0.09393738 |               |               |
|      |                      | 75                              |                                 | 0.05175983 |               |               |
| 5    | 31.25                | 73                              | 1449                            | 0.05037957 | 0.05221992    |               |
|      |                      | 79                              |                                 | 0.05452036 |               |               |
|      |                      | 34                              |                                 | 0.02278820 |               |               |
| 6    | 15.625               | 37                              | 1492                            | 0.02479893 | 0.02457551    |               |
|      |                      | 39                              |                                 | 0.02613941 |               |               |
Supplementary Table 8. Average ratio of the third transition for a calibration curve

| STDs | Concentration (fmol) | Target peak area (635.3/971.6) | Target peak area (640.3/981.5) | Ratio | Average ratio |
|------|----------------------|-------------------------------|-------------------------------|-------|---------------|
| 1    | 500                  | 2480                          | 2461                          | 0.8775646 |
|      |                      | 3135                          | 3076                          | 0.87084218 | 0.95258316    |
|      |                      | 1129                          | 1112                          | 0.39697609 |
| 2    | 250                  | 1110                          | 1107                          | 0.38924051 | 0.42217534    |
|      |                      | 1366                          | 1359                          | 0.48030942 |
|      |                      | 581                           | 574                           | 0.22865014 |
| 3    | 125                  | 550                           | 550                           | 0.21645022 | 0.22261577    |
|      |                      | 566                           | 563                           | 0.22274695 |
|      |                      | 312                           | 307                           | 0.12154266 |
| 4    | 62.50                | 284                           | 284                           | 0.11063498 | 0.10167511    |
|      |                      | 187                           | 187                           | 0.07284768 |
|      |                      | 116                           | 116                           | 0.04178674 |
| 5    | 31.25                | 103                           | 103                           | 0.03710375 | 0.03758405    |
|      |                      | 94                            | 94                            | 0.03386167 |
|      |                      | 46                            | 46                            | 0.01762452 |
| 6    | 15.625               | 48                            | 48                            | 0.01839080 | 0.01634738    |
|      |                      | 34                            | 34                            | 0.01302682 |
## Supplementary Table 9. Comparison of transitions 1 – 3 in human intestinal samples

| Sex     | Site     | Number | Transition 1 | Transition 2 | Transition 3 |
|---------|----------|--------|--------------|--------------|--------------|
| Male    | Jejunum  | 1      | 1.39         | 1.19         | 0.98         |
|         |          | 2      | 3.01         | 3.21         | 3.44         |
|         |          | 3      | 2.66         | 2.56         | 2.25         |
|         |          | 4      | 2.76         | 2.96         | 2.82         |
|         |          | 5      | 2.85         | 3.15         | 2.95         |
|         |          | 6      | 1.94         | 2.04         | 2.40         |
|         |          | 7      | 2.52         | 2.87         | 3.07         |
|         |          | 8      | 6.64         | 6.74         | 6.11         |
| Male    | Ileum    | 9      | 4.01         | 4.51         | 4.30         |
|         |          | 10     | 3.44         | 3.82         | 4.02         |
|         |          | 11     | 3.99         | 4.09         | 3.65         |
|         |          | 12     | 4.18         | 4.31         | 4.25         |
|         |          | 13     | 4.98         | 5.08         | 4.94         |
|         |          | 14     | 3.32         | 3.33         | 3.67         |
|         |          | 15     | 4.56         | 4.88         | 5.10         |
|         |          | 16     | 4.24         | 4.18         | 4.04         |
|         |          | 17     | 4.01         | 3.91         | 4.13         |
| Female  | Jejunum  | 18     | 0.41         | 0.67         | 0.52         |
|         |          | 19     | 1.77         | 1.99         | 1.71         |
|         |          | 20     | 1.87         | 2.11         | 1.77         |
|         |          | 21     | 0.98         | 1.17         | 1.11         |
|         |          | 22     | 2.21         | 2.01         | 1.89         |
| Female  | Ileum    | 23     | 1.56         | 1.96         | 1.76         |
|         |          | 24     | 0.78         | 0.71         | 0.65         |
|         |          | 25     | 1.66         | 1.57         | 1.63         |
| Female  | Ileum    | 26     | 2.81         | 2.79         | 2.63         |
|    |      |      |      |
|----|------|------|------|
| 27 | 3.31 | 3.67 | 3.39 |
| 28 | 2.98 | 2.89 | 2.87 |
| 29 | 2.44 | 2.40 | 2.22 |
| 30 | 2.31 | 2.63 | 2.41 |
**Supplementary Table 10.** Comparison of transitions 1 – 3 in Wistar rat intestinal samples

| Sex    | Site     | Number | Transition 1 | Transition 2 | Transition 3 |
|--------|----------|--------|--------------|--------------|--------------|
| Male   | Duodenum | 1      | 2.22         | 2.13         | 1.96         |
|        |          | 2      | 2.33         | 2.23         | 1.98         |
|        |          | 3      | 2.14         | 2.09         | 2.01         |
|        |          | 4      | 2.10         | 2.17         | 1.91         |
|        |          | 5      | 2.13         | 2.33         | 2.21         |
|        |          | 6      | 2.22         | 2.19         | 1.93         |
|        |          | 7      | 3.01         | 3.24         | 3.08         |
|        |          | 8      | 2.90         | 2.99         | 3.36         |
|        | Jejunum  | 9      | 2.31         | 2.37         | 2.11         |
|        |          | 10     | 3.71         | 3.89         | 3.95         |
|        |          | 11     | 3.21         | 3.32         | 3.20         |
| Male   | Ileum    | 12     | 3.27         | 3.49         | 3.41         |
|        |          | 13     | 3.53         | 3.50         | 3.26         |
|        |          | 14     | 2.50         | 2.68         | 2.34         |
|        |          | 15     | 4.12         | 4.21         | 4.15         |
|        |          | 16     | 5.15         | 5.05         | 5.29         |
|        |          | 17     | 3.16         | 3.45         | 3.29         |
|        |          | 18     | 3.73         | 4.01         | 3.95         |
|        |          | 19     | 1.87         | 1.91         | 1.74         |
|        |          | 20     | 1.92         | 1.72         | 1.65         |
|        | Colon    | 21     | 1.93         | 2.21         | 2.02         |
|        |          | 22     | 2.88         | 3.29         | 3.12         |
|        |          | 23     | 3.10         | 3.20         | 2.97         |
| Female | Duodenum | 25     | 2.29         | 2.39         | 2.22         |
|        |          | 26     | 2.54         | 2.68         | 2.41         |
**Supplementary Table 11.** The accuracy and precision of QC samples with the current method, calculated as % recovery and the relative standard deviation (CV%) respectively.

|        | QC low     | QC medium   | QC high    |
|--------|------------|-------------|------------|
| Accuracy | 91.8 – 104.1% | 92.9 – 107.6% | 90.5 – 111.4% |
| Intra-day | 11.1%       | 6.1%        | 9.8%       |
| Precision | 11.3%       | 14.6%       | 13.8%      |
**Supplementary Table 12.** The results of peptide stability assessment in different processing conditions.

| Stability       | QC low  | QC medium | QC high |
|-----------------|---------|-----------|---------|
| 2 hours at RT   | 96.1%   | 93.4%     | 99.6%   |
| 24 hours at 4°C | 91.1%   | 92.8%     | 98.7%   |
| 4 hours at 37°C | 94.1%   | 91.2%     | 100.9%  |

**Supplementary Table 13.** Verification of interference-free transitions for the analyte and internal standard

|                      | Transition ratio 1/2 | Transition ratio 1/3 | Transition ratio 2/3 |
|----------------------|----------------------|----------------------|----------------------|
|                      | Analytical samples   | Internal standards   | Analytical samples   | Internal standards   | Analytical samples   | Internal standards   |
| Human tissue         |                      |                      |                      |
| Range                | 0.54 – 0.85          | 0.66 – 0.90          | 0.30 – 0.50          | 0.35 – 0.50          | 0.49 – 0.70          | 0.52 – 0.65          |
| Average              | 0.68                 | 0.77                 | 0.40                 | 0.43                 | 0.59                 | 0.56                 |
| Wistar rat tissue    |                      |                      |                      |
| Range                | 0.59 – 0.96          | 0.66 – 0.93          | 0.32 – 0.58          | 0.39 – 0.53          | 0.51 – 0.76          | 0.51 – 0.64          |
| Average              | 0.72                 | 0.80                 | 0.45                 | 0.46                 | 0.63                 | 0.58                 |