

Supplementary material of *Analytical Methods* for

**Simultaneous quantitative analysis of retagliptin and its main active metabolite
in human multi-matrices by liquid chromatography tandem mass spectrometry**

Xiangjun Meng ¹, Lanlan Cai ¹, Tianming Ren ¹, Dong Sun ²*, Jingkai Gu ¹*

¹ Research Center for Drug Metabolism, School of Life Sciences, Jilin University,
Changchun, 130012, P.R. China.

² Beijing institute of modern drug metabolism, Beijing, 102209, P.R. China.

* Corresponding author. E-mail: gujk@jlu.edu.cn, dong_sun@web.de

Tel.: +86 431

Table S1 Concentrations of the calibration standards for retagliptin and retagliptin acid in plasma, urine and feces

Matrices	Plasma		Urine		Feces	
Analytes	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)
Standard 01	0.1	0.3	5	10	1	3
Standard 02	0.3	0.9	10	20	3	10
Standard 03	1	4	50	100	10	30
Standard 04	5	20	250	500	30	100
Standard 05	20	100	1000	2500	100	300
Standard 06	90	450	4500	8500	300	1000
Standard 07	100	500	5000	10000	1000	3000

Table S2 Concentrations of the LLOQ and QC samples for retagliptin and retagliptin acid in plasma, urine and feces

Matrices	Plasma		Urine		Feces	
Analytes	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)	Retagliptin (ng/mL)	Retagliptin acid (ng/mL)
LLOQ	0.1	0.3	5	10	1	3
LQC	0.27	0.81	15	30	2.7	9
MQC	4	16	200	400	24	80
HQC	80	400	4000	8000	800	2400

Table S3 Accuracy and precision for the quantitation of retagliptin in human plasma (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
0.10 (LLOQ)	1	0.10 $\pm$ 0.00	2.00	2.77	0.10 $\pm$ 0.00	0.13	2.89
	2	0.10 $\pm$ 0.00	-1.50	3.59			
	3	0.10 $\pm$ 0.00	-0.10	2.97			
0.27	1	0.27 $\pm$ 0.01	-1.36	3.51	0.26 $\pm$ 0.02	-4.84	6.82
	2	0.25 $\pm$ 0.01	-7.90	4.24			
	3	0.26 $\pm$ 0.03	-5.25	10.00			
4.00	1	3.99 $\pm$ 0.05	-0.33	1.34	4.01 $\pm$ 0.12	0.19	3.08
	2	3.99 $\pm$ 0.05	-0.17	1.17			
	3	4.04 $\pm$ 0.21	1.08	5.21			
80.00	1	82.05 $\pm$ 1.46	2.56	1.78	79.67 $\pm$ 2.53	-0.41	3.17
	2	77.05 $\pm$ 1.10	-3.69	1.42			
	3	79.92 $\pm$ 1.81	-0.10	2.26			

Table S4 Accuracy and precision for the quantitation of retagliptin acid in human plasma (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
0.30 (LLOQ)	1	0.29 $\pm$ 0.03	-4.00	9.33	0.27 $\pm$ 0.02	-9.83	9.23
	2	0.28 $\pm$ 0.01	-6.50	2.77			
	3	0.24 $\pm$ 0.00	-19.00	1.16			
0.81	1	0.87 $\pm$ 0.03	7.30	3.41	0.83 $\pm$ 0.05	2.81	5.95
	2	0.78 $\pm$ 0.03	-3.64	3.64			
	3	0.85 $\pm$ 0.04	4.77	4.54			
16.00	1	15.37 $\pm$ 0.22	-3.96	1.41	15.61 $\pm$ 0.51	-2.47	3.25
	2	15.80 $\pm$ 0.17	-1.25	1.06			
	3	15.65 $\pm$ 0.83	-2.19	5.28			
400.00	1	383.67 $\pm$ 7.76	-4.08	2.02	378.94 $\pm$ 8.65	-5.26	2.28
	2	371.17 $\pm$ 7.11	-7.21	1.92			
	3	382.00 $\pm$ 5.76	-4.5	1.51			

Table S5 Accuracy and precision for the quantitation of retagliptin in human urine (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
5.0 (LLOQ)	1	5.0 $\pm$ 0.4	-0.1	8.6	4.8 $\pm$ 0.3	-3.2	5.4
	2	4.8 $\pm$ 0.2	-3.6	3.8			
	3	4.7 $\pm$ 0.2	-6.0	3.6			
15.0	1	15.5 $\pm$ 0.3	3.2	2.2	14.5 $\pm$ 0.9	-3.6	6.2
	2	13.9 $\pm$ 0.7	-7.2	4.8			
	3	14.0 $\pm$ 0.5	-6.8	3.8			
200.0	1	196.3 $\pm$ 3.3	-1.8	1.7	196.3 $\pm$ 3.2	-1.8	1.6
	2	195.0 $\pm$ 1.7	-2.5	1.9			
	3	197.7 $\pm$ 4.0	-1.2	2.0			
4000.0	1	3895.0 $\pm$ 52.4	-2.6	1.3	3767.2 $\pm$ 119.4	-5.8	3.2
	2	3735.0 $\pm$ 31.5	-6.6	0.8			
	3	3671.7 $\pm$ 113.9	-8.2	3.1			

Table S6 Accuracy and precision for the quantitation of retagliptin acid in human urine (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
10.0 (LLOQ)	1	9.7 $\pm$ 0.2	-2.8	2.4	9.6 $\pm$ 0.3	-4.4	2.8
	2	9.6 $\pm$ 0.2	-4.5	2.2			
	3	9.4 $\pm$ 0.3	-5.9	3.0			
30.0	1	31.9 $\pm$ 0.6	6.4	1.8	29.6 $\pm$ 1.9	-1.3	6.4
	2	28.1 $\pm$ 1.0	-6.3	3.4			
	3	28.8 $\pm$ 1.0	-3.9	3.5			
400.0	1	397.0 $\pm$ 3.6	-0.8	0.9	399.7 $\pm$ 8.8	-0.1	2.2
	2	393.8 $\pm$ 2.5	-1.5	0.6			
	3	408.3 $\pm$ 10.2	2.1	2.5			
8000.0	1	7710.0 $\pm$ 108.1	-3.6	1.4	7571.1 $\pm$ 211.9	-5.4	2.8
	2	7610.0 $\pm$ 132.7	-4.9	1.7			
	3	7393.3 $\pm$ 245.8	-7.6	3.3			

Table S7 Accuracy and precision for the quantitation of retaglipin in human feces (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
1.0 (LLOQ)	1	1.1 $\pm$ 0.0	5.5	2.6	1.1 $\pm$ 0.0	6.5	4.1
	2	1.1 $\pm$ 0.1	8.0	6.1			
	3	1.1 $\pm$ 0.0	6.0	3.1			
2.7	1	2.5 $\pm$ 0.1	-6.5	3.5	2.6 $\pm$ 0.1	-5.3	3.8
	2	2.5 $\pm$ 0.1	-6.7	0.3			
	3	2.6 $\pm$ 0.1	-2.7	2.5			
24.0	1	22.9 $\pm$ 0.4	-4.6	1.8	23.5 $\pm$ 0.7	-2.0	3.0
	2	23.8 $\pm$ 0.4	-0.8	1.9			
	3	23.9 $\pm$ 0.8	-0.5	3.3			
800.0	1	799.7 $\pm$ 24.5	0.0	3.1	797.8 $\pm$ 19.9	-0.3	2.5
	2	786.8 $\pm$ 15.2	-1.6	1.9			
	3	807.0 $\pm$ 16.4	0.9	2.0			

Table S8 Accuracy and precision for the quantitation of retagliptin acid in human feces (data are mean $\pm$ SD where appropriate)

Spiked Conc. (ng/mL)	Day No.	Intra-day Conc. (ng/mL)	Intra-day Accuracy (R.E. %)	Intra-day Precision (R.S.D. %)	Inter-day Conc. (ng/mL)	Inter-day Accuracy (R.E. %)	Inter-day Precision (R.S.D. %)
3.0 (LLOQ)	1	3.1 $\pm$ 0.1	4.3	3.5	3.1 $\pm$ 0.1	4.1	2.9
	2	3.2 $\pm$ 0.1	5.0	3.5			
	3	3.1 $\pm$ 0.0	2.8	1.2			
9.0	1	9.1 $\pm$ 0.2	1.4	1.7	8.8 $\pm$ 0.3	-2.7	3.6
	2	8.5 $\pm$ 0.2	-5.5	2.1			
	3	8.6 $\pm$ 0.2	-4.1	2.0			
80.0	1	80.2 $\pm$ 2.2	-0.3	2.8	78.6 $\pm$ 2.3	-1.8	3.0
	2	77.1 $\pm$ 1.3	-3.7	1.7			
	3	78.5 $\pm$ 2.4	-1.9	3.1			
2400.0	1	2455.0 $\pm$ 75.6	2.3	3.1	2420.0 $\pm$ 69.0	0.8	2.9
	2	2383.3 $\pm$ 53.5	-0.7	2.2			
	3	2421.7 $\pm$ 67.4	0.9	2.8			

Table S9 Normalized extraction recovery of retaglipitin in plasma, urine and feces (data were calculated based on an assay of six replicates prepared QC samples)

Matrices	Concentration (ng/mL)	Extraction recovery (%)
plasma	0.27	103.2±3.3
	4.0	102.4±1.4
	80.0	100.0±1.8
urine	15.0	99.0±2.1
	200.0	97.0±1.7
	4000.0	99.9±1.3
feces	2.7	96.6±3.3
	24.0	99.1±1.8
	800.0	98.1±3.0

Table S10 Normalized extraction recovery of retagliptin acid in plasma, urine and feces (data were calculated based on an assay of six replicates prepared QC samples)

Matrices	Concentration (ng/mL)	Extraction recovery (%)
plasma	0.81	106.7±3.6
	16.0	102.7±1.5
	400.0	102.3±2.3
urine	30.0	100.3±1.8
	400.0	96.6±0.8
	8000.0	99.2±1.2
feces	9.0	99.5±1.8
	80.0	99.7±2.7
	2400.0	98.6±2.9

Table S11 Normalized matrix effect of retagliptin in plasma, urine and feces (data were calculated based on an assay of six replicates prepared QC samples)

Matrices	Concentration (ng/mL)	Matrix effect (%)
plasma	0.27	1.03±0.0
	80.0	1.01±0.0
urine	15.0	0.981±0.0
	4000.0	1.01±0.0
feces	2.7	1.02±0.1
	800.0	1.02±0.0

Table S12 Normalized matrix effect of retagliptin acid in plasma, urine and feces (data were calculated based on an assay of six replicates prepared QC samples)

Matrices	Concentration (ng/mL)	Matrix effect (%)
plasma	0.27	1.04±0.0
	80.0	1.03±0.0
urine	15.0	0.998±0.0
	4000.0	1.01±0.0
feces	2.7	1.03±0.0
	800.0	1.00±0.0

Table S13 Stability of retagliptin and retagliptin acid in human plasma and post-extracted plasma samples under various conditions (data were calculated based on an assay of triplicate prepared QC samples)

Analyte	Spiked conc. (ng/mL)	Storage at -80°C for 161 d		Storage at room temperature for 5 h		In human plasma after three freeze/thaw cycles		Post-extracted samples at ambient temperature for 4 h	
		Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)
Retagliptin	0.27	0.26±0.03	-5.56	0.25±0.02	-8.02	0.28±0.02	2.59	0.28±0.01	2.47
	80.00	86.90±7.28	8.63	76.37±2.00	-4.54	80.27±1.14	0.33	76.33±0.42	-4.58
Retagliptin acid	0.81	0.77±0.08	-5.23	0.81±0.06	-0.29	0.83±0.05	2.63	0.89±0.03	10.41
	400.00	372.33±15.95	-6.92	372.33±10.21	-6.92	378.00±3.00	-5.50	375.67±4.62	-6.08

Table S14 Stability of retagliptin and retagliptin acid in human urine and post-extracted urine samples under various conditions (data were calculated based on an assay of triplicate prepared QC samples)

Analyte	Spiked conc. (ng/mL)	Storage at -80°C for 161 d		Storage at room temperature for 5 h		In human urine after three freeze/thaw cycles		Post-extracted samples at ambient temperature for 4 h	
		Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)
Retagliptin	15.0	13.9±0.7	-7.3	13.9±0.3	-7.6	15.0±0.4	-0.2	14.8±1.5	-1.6
	4000.0	4220.0±202.2	5.5	3753.3±55.1	-6.2	3603.3±100.2	-9.9	3750.0±40.0	-6.3
Retagliptin acid	30.0	28.9±1.9	-3.7	29.3±0.8	-2.4	30.1±0.7	0.4	31.8±1.4	5.9
	8000.00	7526.7±165.6	-5.9	7676.7±63.5	-4.0	7250.0±52.0	-9.4	7750.0±134.5	-3.1

Table S15 Stability of retagliptin and retagliptin acid in human feces and post-extracted feces samples under various conditions (data were calculated based on an assay of triplicate prepared QC samples)

Analyte	Spiked conc. (ng/mL)	Storage at -80°C for 30 d		Storage at room temperature for 5 h		In human feces after three freeze/thaw cycles		Post-extracted samples at ambient temperature for 4 h	
		Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)	Mean±SD	RE (%)
Retagliptin	2.7	2.5±0.1	-6.8	2.9±0.2	6.3	2.5±0.1	-5.9	2.6±0.1	-4.6
	800.0	853.7±15.0	6.7	792.7±16.5	-0.9	786.3±4.2	-1.7	807.0±23.4	0.9
Retagliptin acid	9.0	9.3±0.1	3.6	9.1±0.3	0.7	8.6±0.1	-4.5	8.8±0.3	-2.3
	2400.0	2450.0±43.6	2.1	2410.0±43.6	0.4	2423.3±5.8	1.0	2366.7±45.1	-1.4

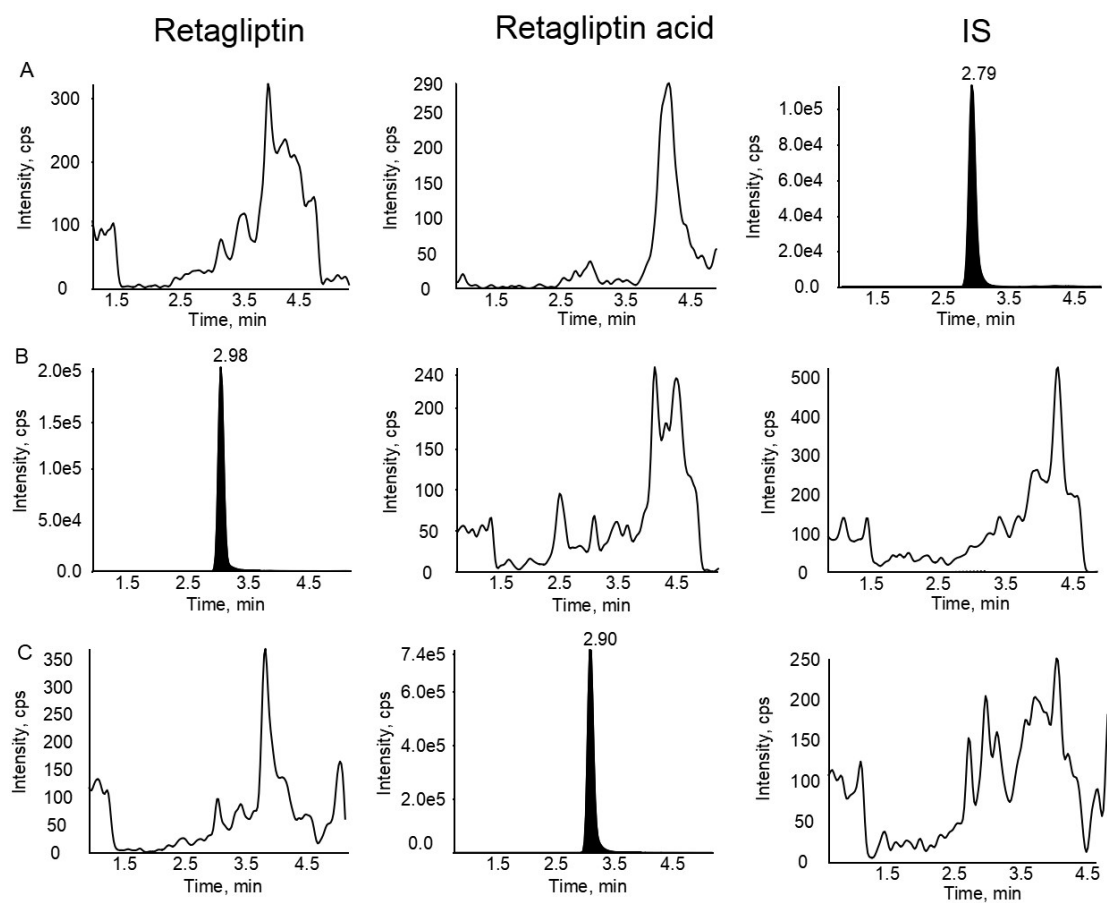


Fig. S1 Plasma samples contains only IS (A), retagliptin (B) and retagliptin acid (C)

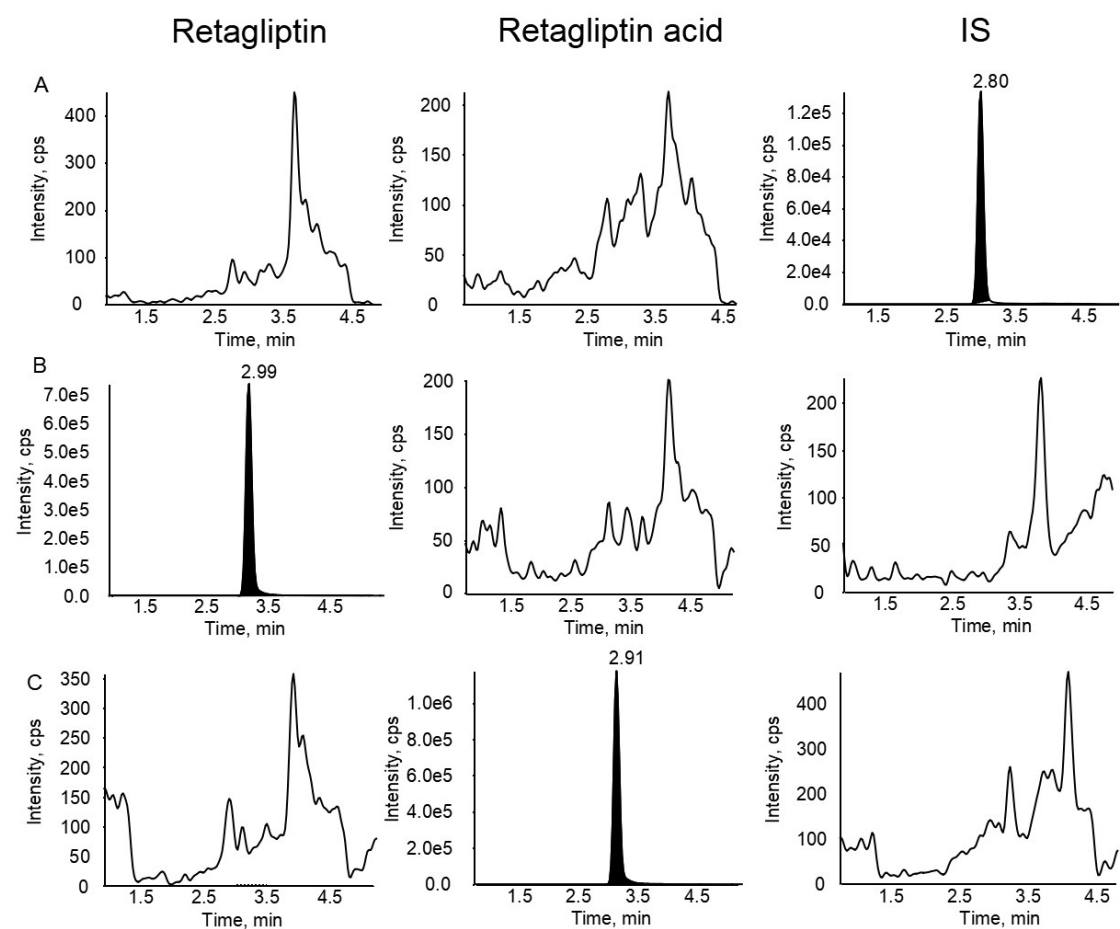


Fig. S2 Urine samples contains only IS (A), retagliptin (B) and retagliptin acid (C)

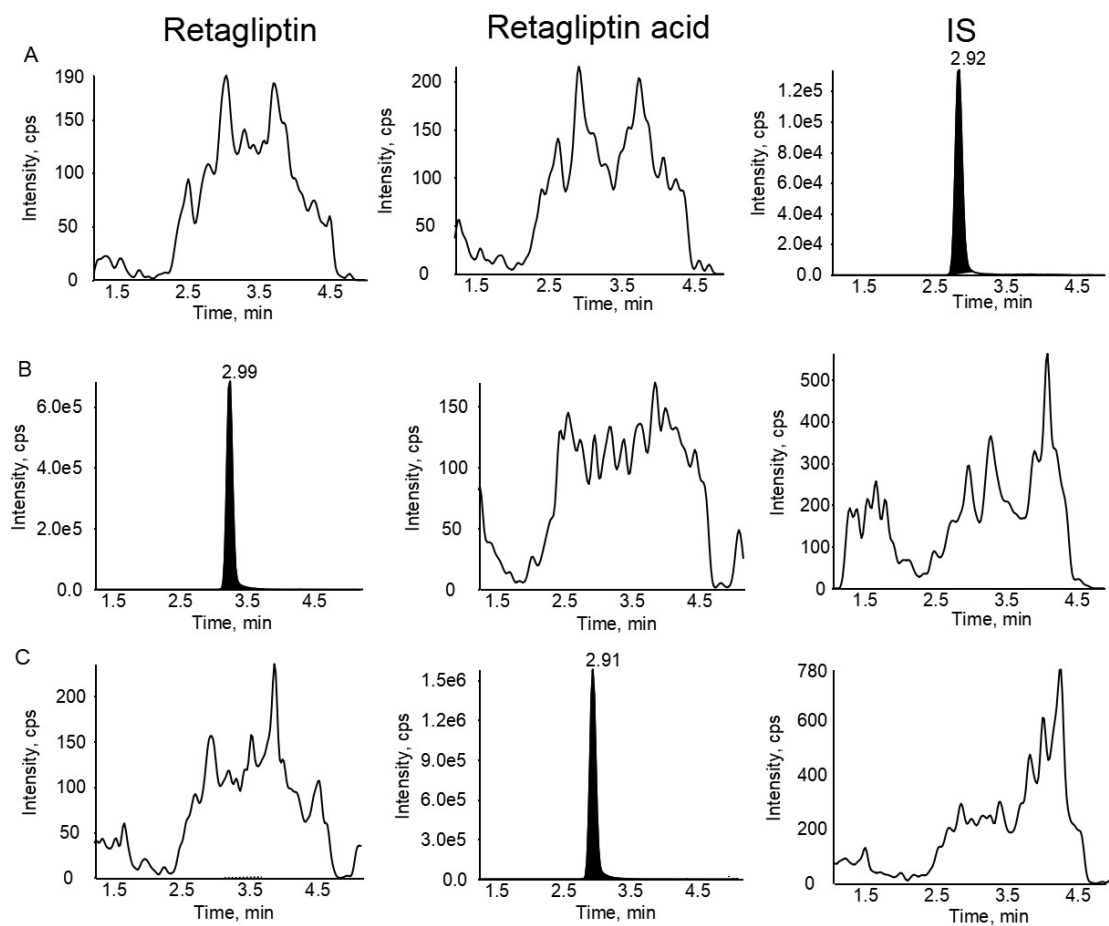


Fig. S3 Feces samples contains only IS (A), retagliptin (B) and retagliptin acid (C)