Supplementary Material

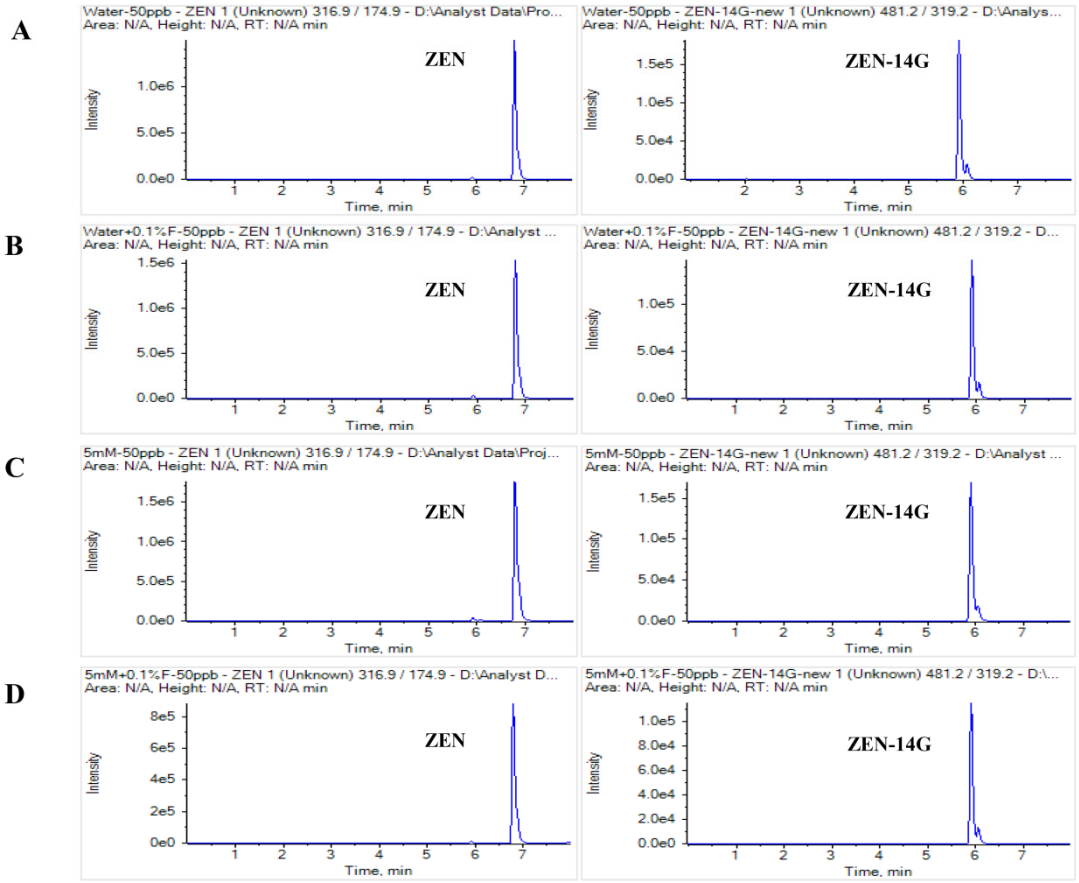

Figure S1. Comparison of ionization efficiencies of zearalenone (ZEN) and zearalenone-14-glucoside (ZEN-14G) among four candidate mobile phases. (A) methanol-water, (B) methanol-water containing 0.1% formic acid, (C) methanol-water containing 5 mmol/L ammonium acetate and (D) methanol-water containing 5 mmol/L ammonium acetate and 0.1% formic acid. The concentration of ZEN and ZEN-14G is 50 μg/L.

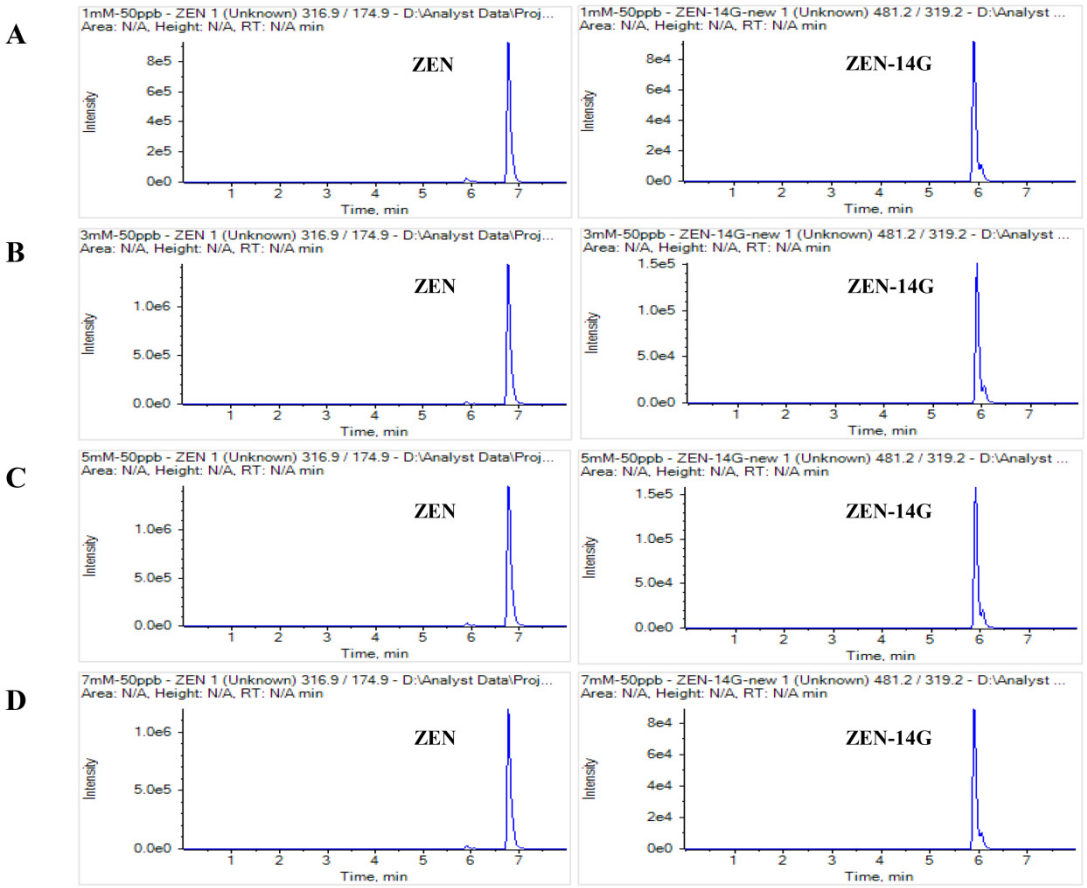

Figure S2. Ionization efficiencies of ZEN and ZEN-14G among different mobile phases were compared. (A) methanol-water containing 1 mmol/L ammonium acetate, (B) methanol-water containing 3 mmol/L ammonium acetate, (C) methanol-water containing 5 mmol/L ammonium acetate, and (D) methanol-water containing 7 mmol/L ammonium acetate. The concentration of ZEN and ZEN-14G is 50 μg/L.

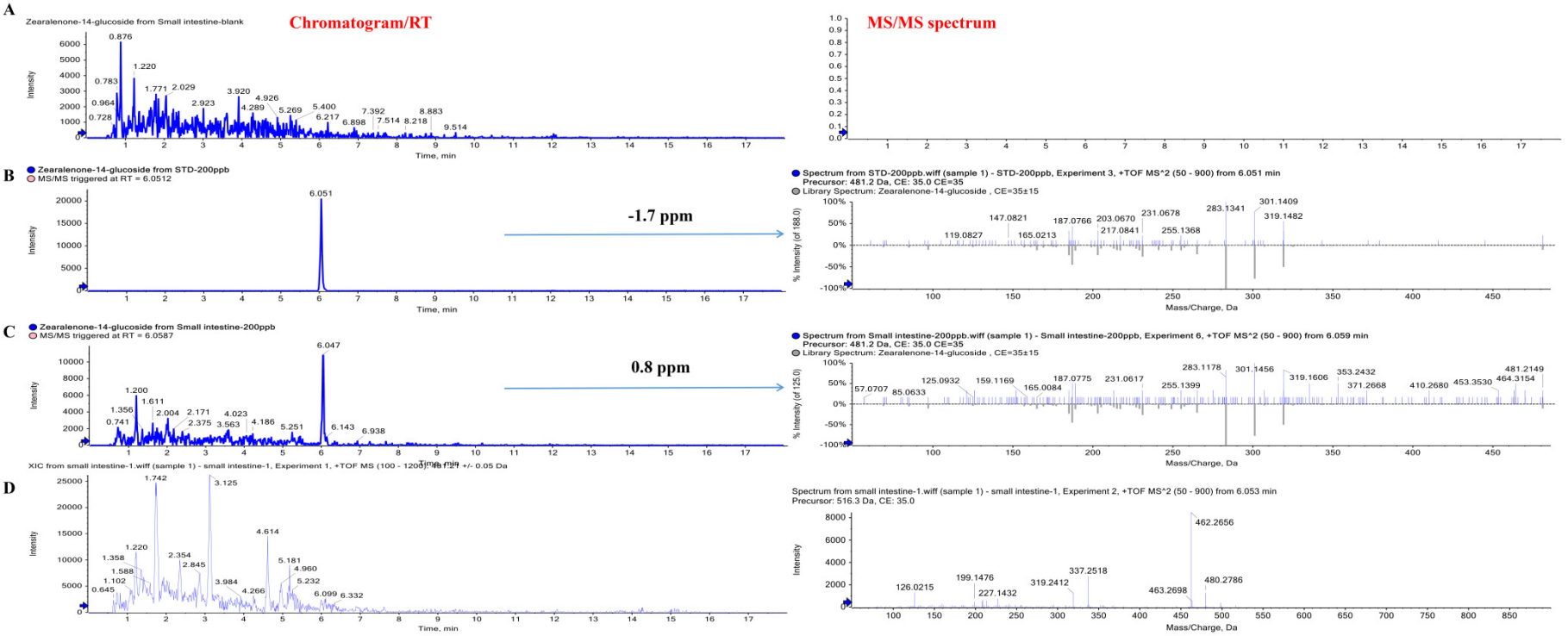

Figure S3. Detection and identification of ZEN-14G by the established UHPLC-Q-TOF/MS method in (A) the blank small intestine, (B) the neat solution (200 μg/L), (C) the spiked small intestine sample (200 μg/L) and (D) the small intestine samples gathered at 24 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

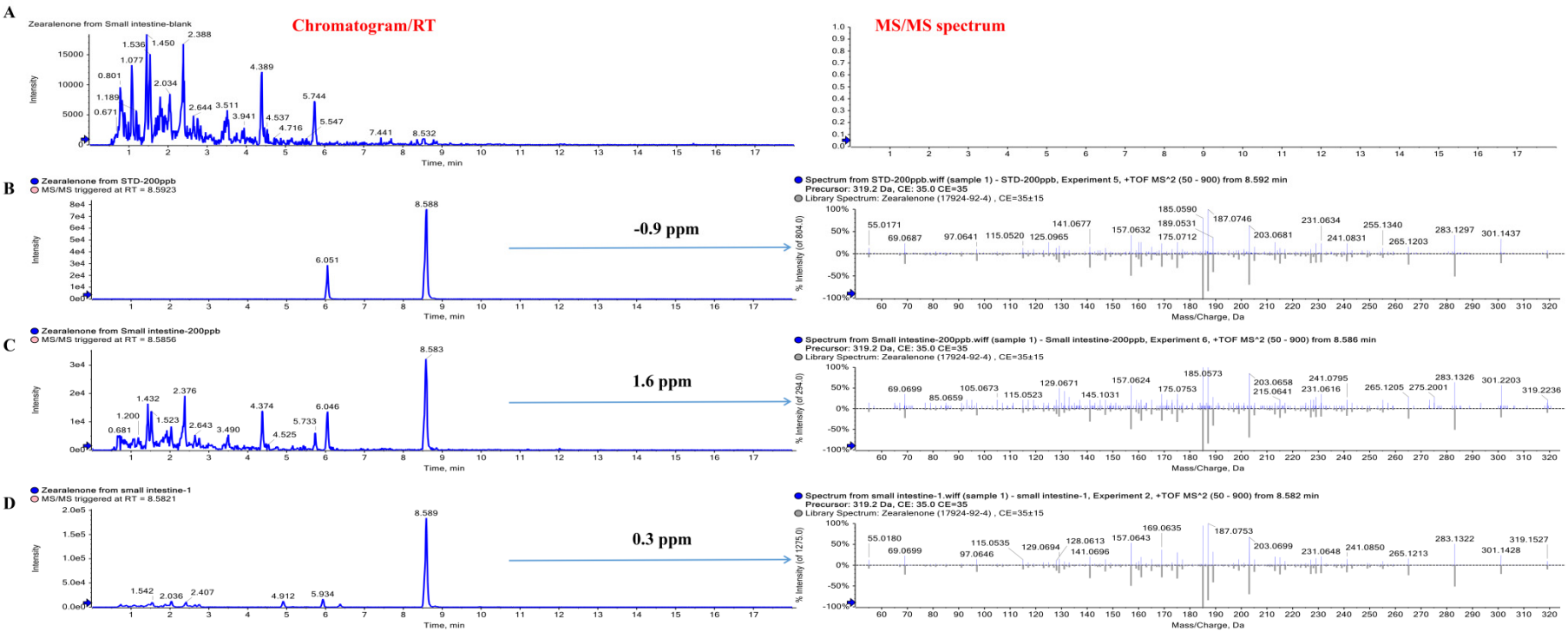

Figure S4. Detection and identification of ZEN by the established UHPLC-Q-TOF/MS method in (A) the blank small intestine, (B) the neat solution (200 μg/L), (C) the spiked small intestine sample (200 μg/L) and (D) the small intestine samples gathered at 24 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

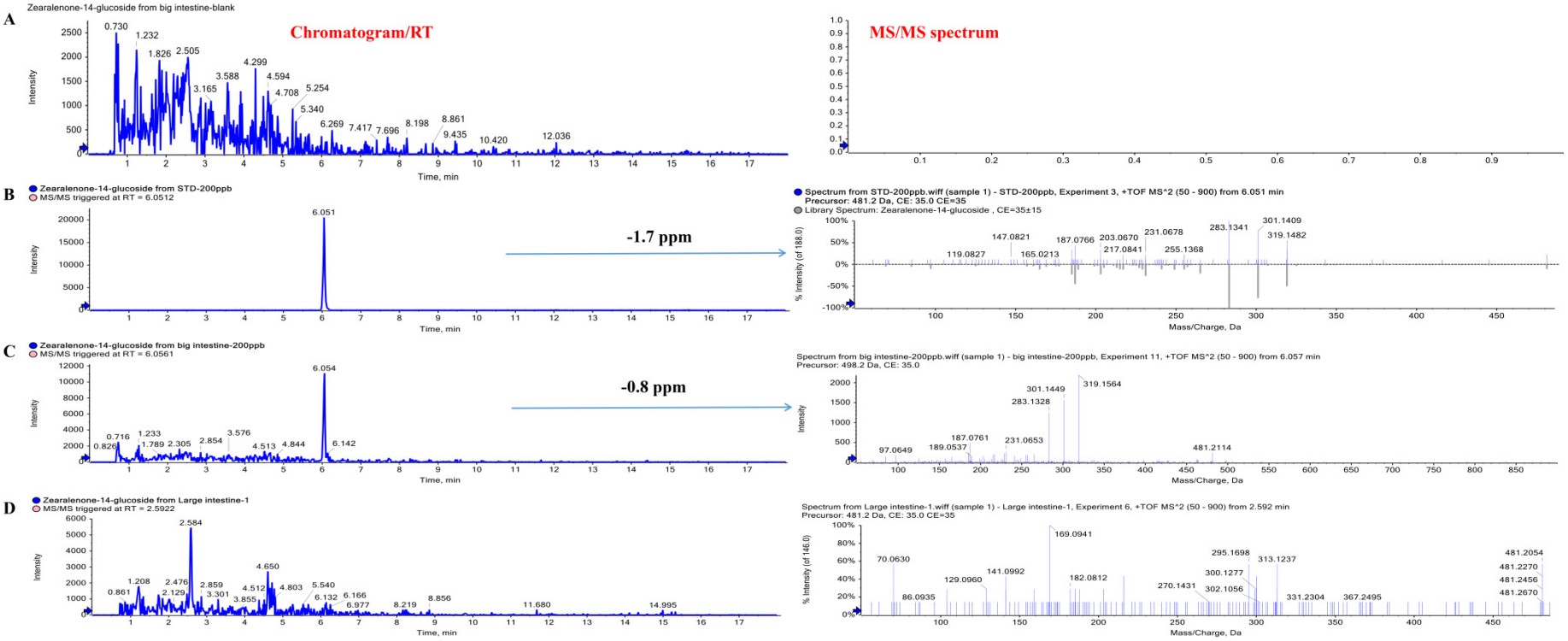

Figure S5. Detection and identification of ZEN-14G by the established UHPLC-Q-TOF/MS method in (A) the blank large intestine, (B) the neat solution (200 μg/L), (C) the spiked large intestine sample (200 μg/L) and (D) the large intestine samples gathered at 24 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

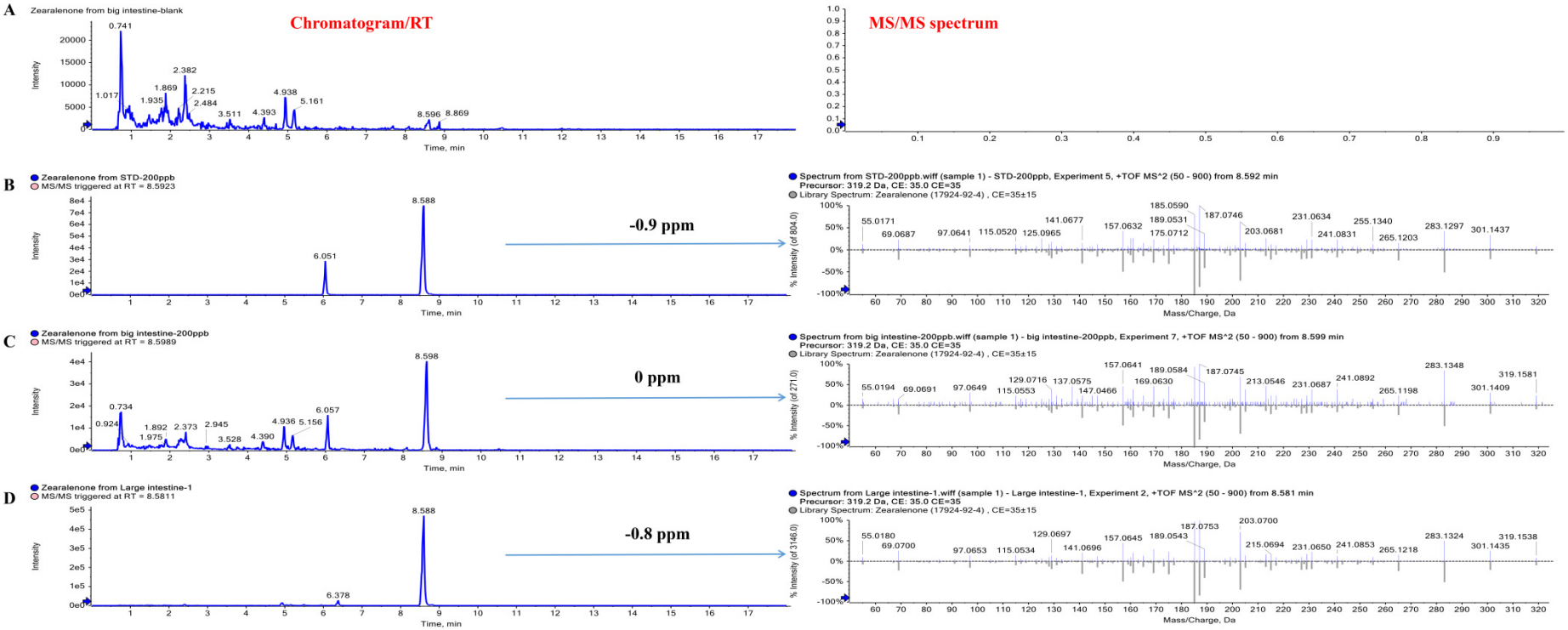

Figure S6. Detection and identification of ZEN by the established UHPLC-Q-TOF/MS method in (A) the blank large intestine, (B) the neat solution (200 μg/L), (C) the spiked large intestine sample (200 μg/L) and (D) the large intestine samples gathered at 24 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

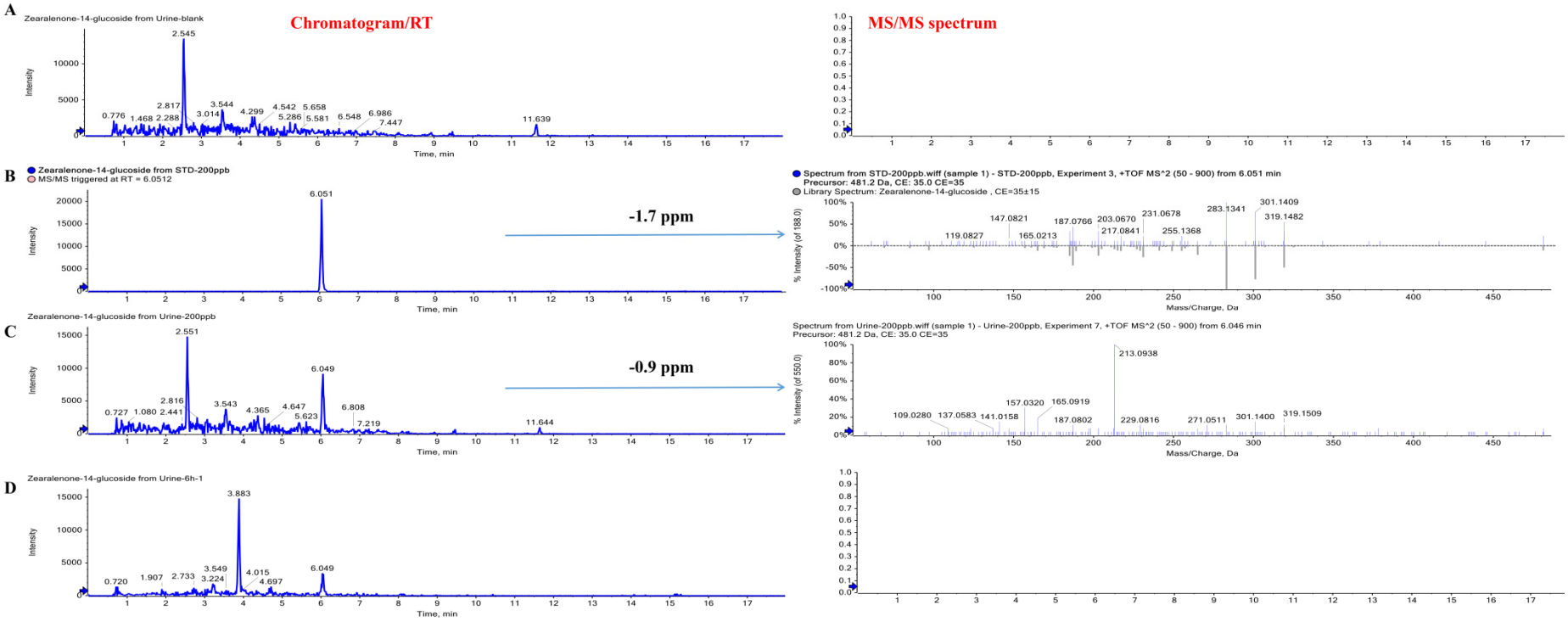

Figure S7. Detection and identification of ZEN-14G by the established UHPLC-Q-TOF/MS method in (A) the blank urine, (B) the neat solution (200 μg/L), (C) the spiked urine sample (200 μg/L) and (D) the urine samples gathered at 6 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

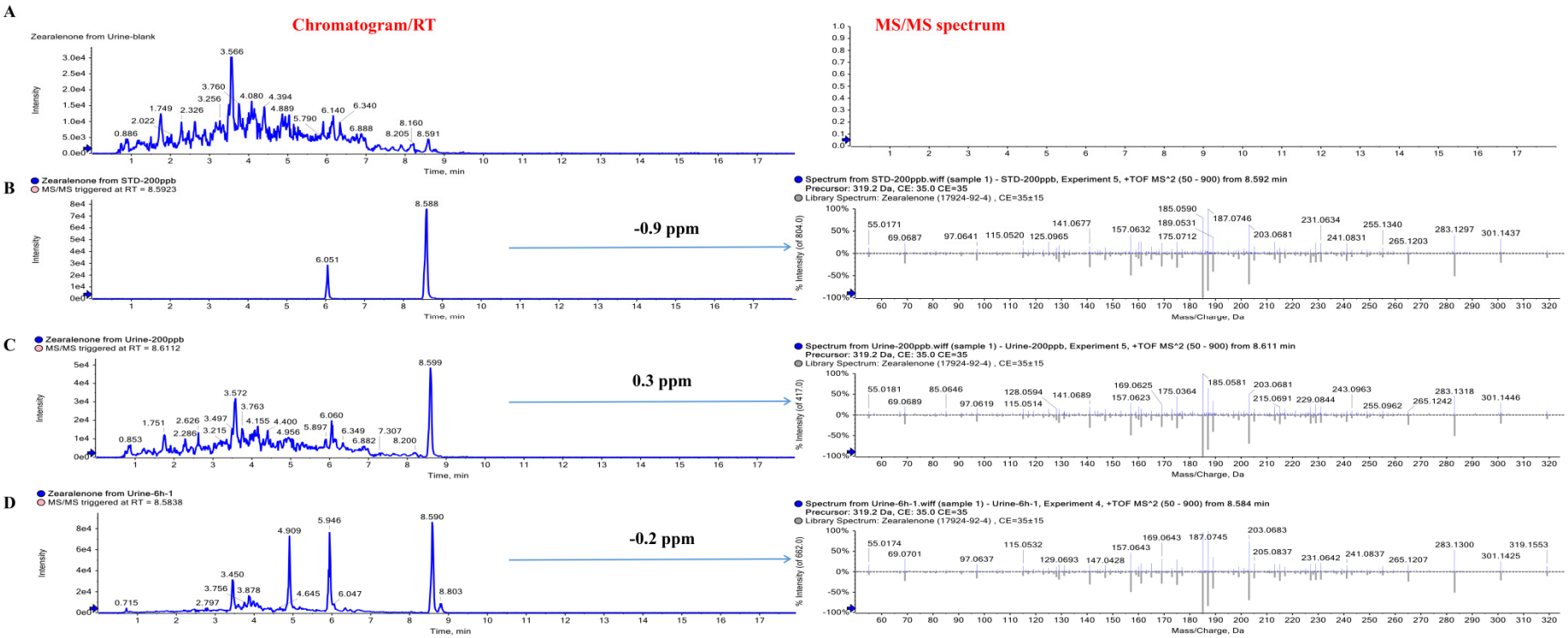

Figure S8. Detection and identification of ZEN by the established UHPLC-Q-TOF/MS method in (A) the blank urine, (B) the neat solution (200 μg/L), (C) the spiked urine sample (200 μg/L) and (D) the urine samples gathered at 6 h after gavage administration of ZEN-14G (4 mg/kg body weight) in rats.

Table S1. Comparison of the re-dissolved effects of zearalenone (ZEN) and zearalenone-14-glucoside (ZEN-14G) among different solvents.

Solvents (Acetonitrile-water containing 5 mmol/L ammonium acetate, v/v)	Recovery (Mean ± SD, %)	
	ZEN	ZEN-14G
10/90	23.4 ± 2.6	86.8 ± 3.6
20/80	59.6 ± 9.4	93.7 ± 3.5
30/70	94.2 ± 3.4	95.9 ± 1.8
40/60	78.0 ± 7.6	87.4 ± 1.3
50/50	79.7 ± 5.8	93.7 ± 2.1

Table S2. Stability of zearalenone (ZEN) and zearalenone-14-glucoside (ZEN-14G) (n = 5).

Matrix	Mycotoxin	Spiked Level (μg/L)	Recovery (Mean ± SD, %)		
			Short-Term [a]	Long-Term [b]	Freeze-Thaw [c]
Plasma	ZEN	LLOQ	89.9 ± 4.6	90.9 ± 7.2	91.8 ± 4.6
		5	94.0 ± 1.8	88.3 ± 7.1	87.1 ± 9.3
		20	92.7 ± 4.4	89.4 ± 8.1	87.3 ± 6.8
		50	94.0 ± 3.8	94.0 ± 3.8	96.5 ± 6.5
	ZEN-14G	LLOQ	104.1 ± 3.9	102.4 ± 5.4	101.8 ± 5.5
		5	99.7 ± 5.9	93.7 ± 3.7	93.6 ± 4.0
		20	94.6 ± 1.6	95.7 ± 6.8	95.7 ± 4.0
		50	92.7 ± 2.3	92.5 ± 6.5	91.6 ± 6.1
Liver	ZEN	LLOQ	90.5 ± 8.6	88.4 ± 8.4	89.0 ± 6.2
		5	95.5 ± 7.4	98.5 ± 6.5	98.2 ± 3.4
		20	98.9 ± 7.8	100.0 ± 8.9	93.2 ± 2.5
		50	99.7 ± 2.7	101.0 ± 2.3	98.2 ± 7.1
	ZEN-14G	LLOQ	94.9 ± 5.4	93.6 ± 7.1	97.0 ± 5.6
		5	96.7 ± 0.3	95.6 ± 8.9	95.7 ± 6.8
		20	94.9 ± 7.0	94.2 ± 7.8	98.9 ± 5.2
		50	100.5 ± 4.4	100.4 ± 3.7	97.4 ± 3.4
Kidney	ZEN	LLOQ	86.6 ± 10.8	84.8 ± 10.6	86.6 ± 10.7
		5	91.3 ± 4.0	94.6 ± 4.2	98.4 ± 1.2
		20	94.1 ± 5.5	96.9 ± 5.8	91.1 ± 5.2
		50	102.8 ± 5.7	102.2 ± 2.3	100.9 ± 5.0
	ZEN-14G	LLOQ	88.1 ± 3.1	86.4 ± 3.0	89.4 ± 3.1
		5	96.3 ± 7.1	99.0 ± 7.6	99.4 ± 3.7
		20	96.6 ± 7.6	96.6 ± 9.3	95.5 ± 6.3
		50	99.2 ± 1.5	101.5 ± 3.6	98.2 ± 1.2
Stomach	ZEN	LLOQ	89.0 ± 8.1	85.9 ± 5.0	90.1 ± 8.2

		5	93.7 ± 6.0	90.6 ± 6.9	93.3 ± 8.2
		20	100.2 ± 4.6	102.0 ± 6.9	98.5 ± 6.1
		50	94.1 ± 5.8	95.4 ± 5.2	96.6 ± 3.3
	ZEN-14G	LLOQ	86.2 ± 1.8	81.7 ± 1.7	87.9 ± 8.1
		5	93.5 ± 1.8	98.4 ± 5.9	99.0 ± 4.3
		20	95.3 ± 4.8	104.1 ± 4.9	98.8 ± 6.2
		50	94.9 ± 4.0	97.5 ± 7.4	97.5 ± 5.2
Small intestine	ZEN	LLOQ	88.7 ± 6.6	85.0 ± 6.4	85.9 ± 7.5
		5	96.3 ± 4.4	93.6 ± 2.2	99.7 ± 3.3
		20	99.6 ± 2.1	101.0 ± 7.4	97.9 ± 5.3
		50	98.0 ± 3.4	98.9 ± 6.8	98.8 ± 6.1
	ZEN-14G	LLOQ	87.2 ± 6.9	86.3 ± 6.3	87.9 ± 6.0
		5	95.1 ± 6.8	98.2 ± 5.8	96.8 ± 6.9
		20	97.6 ± 5.5	100.0 ± 1.5	96.9 ± 2.0
		50	94.8 ± 5.5	95.1 ± 5.5	94.1 ± 4.8
Large intestine	ZEN	LLOQ	91.8 ± 4.2	85.5 ± 3.0	86.5 ± 4.5
		5	93.0 ± 5.2	90.4 ± 5.0	90.5 ± 3.8
		20	95.0 ± 8.6	101.3 ± 4.4	100.4 ± 2.7
		50	95.6 ± 3.8	97.6 ± 8.1	95.8 ± 3.1
	ZEN-14G	LLOQ	92.3 ± 1.1	86.0 ± 1.0	82.5 ± 1.0
		5	97.3 ± 2.9	99.6 ± 4.0	95.0 ± 3.9
		20	96.8 ± 1.9	100.0 ± 7.5	98.9 ± 5.2
		50	95.0 ± 8.4	96.1 ± 4.1	96.9 ± 4.0
Ovary	ZEN	LLOQ	89.8 ± 5.6	91.3 ± 5.2	90.9 ± 5.2
		5	96.9 ± 5.6	95.2 ± 4.4	90.0 ± 9.3
		20	96.3 ± 2.1	97.7 ± 1.7	95.6 ± 9.5
		50	100.5 ± 3.2	102.3 ± 3.9	97.6 ± 6.2
	ZEN-14G	LLOQ	90.3 ± 6.8	86.1 ± 5.0	96.7 ± 7.3
		5	96.7 ± 3.8	98.1 ± 5.8	94.3 ± 2.5

		20	98.6 ± 6.9	101.7 ± 3.3	100.3 ± 5.1
		50	98.5 ± 4.0	99.4 ± 2.4	101.3 ± 3.5
Urine	ZEN	LLOQ	85.1 ± 6.0	89.6 ± 8.9	85.2 ± 6.9
		5	91.9 ± 10.2	89.6 ± 7.4	90.6 ± 5.3
		20	92.7 ± 4.8	98.9 ± 4.8	96.5 ± 9.7
		50	98.5 ± 3.4	101.2 ± 4.4	97.1 ± 2.4
	ZEN-14G	LLOQ	90.5 ± 7.9	89.4 ± 7.8	88.3 ± 7.7
		5	94.9 ± 4.9	97.7 ± 3.9	96.9 ± 4.8
		20	100.2 ± 5.4	101.2 ± 5.8	98.5 ± 1.9
		50	96.3 ± 5.1	96.0 ± 6.2	96.6 ± 4.5

"a": stored at room temperature for 8 h; "b": stored at −20 °C for 20 days; "c": undergo three freeze-thaw cycles.

Table S3. The screening detection limits (SDLs) obtained by UHPLC-Q-TOF/MS for the investigated zearalenone (ZEN) and zearalenone-14-glucoside (ZEN-14G) in different matrix.

Matrix	Mycotoxin	SDL (μg/L)
Solvent	ZEN	/
	ZEN-14G	/
Plasma	ZEN	50
	ZEN-14G	200
Liver	ZEN	50
	ZEN-14G	200
Kidney	ZEN	100
	ZEN-14G	200
Stomach	ZEN	200
	ZEN-14G	200
Small intestine	ZEN	200
	ZEN-14G	200
Large intestine	ZEN	50
	ZEN-14G	100
Ovary	ZEN	100
	ZEN-14G	100
Urine	ZEN	200
	ZEN-14G	200

Table S4. In-parallel analysis of the concentration of zearalenone (ZEN) and zearalenone-14-glucoside (ZEN-14G) in plasma (10 min), liver (24 h), and urine (24 h) samples after gavage administration of ZEN-14G (dose of 4 mg/kg body weight, n = 3) to rats by the current established method and previously reported LC-MS/MS method (Mean ± SD, μg/L).

Mycotoxin	Plasma	Liver	Urine	Methods	References
ZEN	3.1 ± 0.3	-	-	LC-MS/MS	[1]
ZEN-14G	4.8 ± 0.8	-	-		
ZEN	-	94.7 ± 0.8	-	LC-MS/MS	[2]
ZEN-14G	-	/	-		
ZEN	-	-	918.2 ± 29.3	LC-MS/MS	[3]
ZEN-14G	-	-	8.2 ± 2.4		
ZEN	2.8 ± 0.2	97.1 ± 7.6	977.5 ± 98.0	LC-MS/MS	The current established method
ZEN-14G	4.4 ± 1.2	/	7.6 ± 3.0		

"/": low than the lower limit of quantification;"-": no detection.

Table S5. Comparison of solubility of zearalenone-14-glucoside (ZEN-14G) with the concentration of 100 μg/L in different solvents for gavage administration.

Solvents	Solubility (Mean ± SD, μg/L)
(Ethanol/Water, V/V)	ZEN-14G
10/90	82.5 ± 2.1
20/80	81.9 ± 5.4
25/75	99.8 ± 1.8
30/70	99.9 ± 2.6
40/60	99.7 ± 0.9

References

1. Dellafiora, L.; Galaverna, G.; Righi, F.; Cozzini, P.; Dall'Asta, C. Assessing the hydrolytic fate of the masked mycotoxin zearalenone-14-glucoside - a warning light for the need to look at the "maskedome". Food Chem. Toxicol. 2017, 9, 9-16. https://doi.org/10.1016/j.fct.2016.11.013.
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3. Warth, B.; Petchkongkaew, A.; Sulyok, M.; Krska, R. Utilising an LC-MS/MS-based multi-biomarker approach to assess mycotoxin exposure in the Bangkok metropolitan area and surrounding provinces. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk. Assess. 2014, 31, 040-6. https://doi.org/10.1080/19440049. 2014.969329.