Profiling the Reactive Metabolites of Xenobiotics Using Metabolomic Technologies

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Table 1. Detected adducts of the tested chemicals using a metabolomic approach.

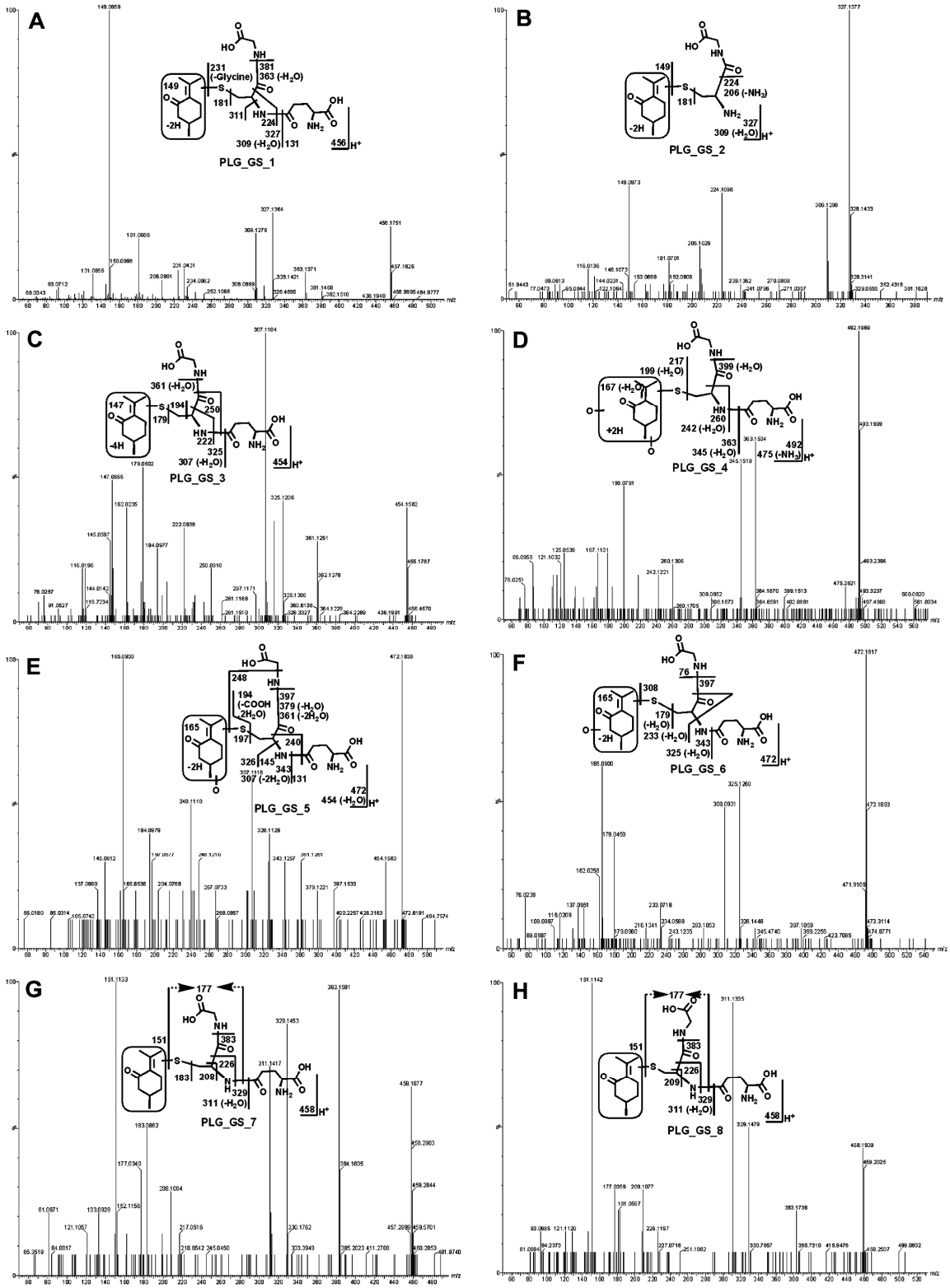
	GSH-conjugates	CN-conjugates	Semicarbazide-conjugates	Description
PLG	PLG_GSH_1	-	-	known
	PLG_GSH_2	-	-	known
	PLG_GSH_3	-	-	new
	PLG_GSH_4	-	-	known
	PLG_GSH_5	-	-	known
	PLG_GSH_6	-	-	new
	PLG_GSH_7	-	-	known
	PLG_GSH_8	-	-	known
	-	-	PLG_pydrazine	known
	-	-	PLG_hydraone_1	new
	-	-	PLG_hydraone_2	new
APAP	APAP_GSH_1	-	-	known
	APAP_GSH_2	-	-	known

APAP	APAP_GSH_3	-	-	new
	APAP_GSH_4	-	-	known
CLP	-	CLP_CN_1	-	known
	-	CLP_CN_2	-	known
	-	CLP_CN_3	-	known
	-	CLP_CN_4	-	new
	-	CLP_CN_5	-	new
	CLP_GSH_1	-	-	known
	CLP_GSH_2	-	-	known
	CLP_GSH_3	-	-	known
	CLP_GSH_4	-	-	known
	CLP_GSH_5	-	-	known

Supplemental Figure 1. The MS/MS spectra of PLG_GS_1 to PLG_GS_8 and their structural elucidations. The structures of metabolites were elucidated by mass fragmentation with a collision energy ramp ranging from 10 to 40 eV. (**A**) PLG_GS_1. PLG_GS_1, eluted at 4.70 min, had a protonated molecule at *m/z* 456. MS/MS analysis of PLG-GS_1 produced daughter ions at *m/z* 327 (loss of pyroglutamic acid), 309 (loss of pyroglutamic acid and H_2O) and 149. (**B**) PLG_GS_2. PLG_GS_2 was detected at 4.49 min, having a mass of $[M+H]^+$ = 327 *m/z*. MS/MS analysis revealed the fragment ions at *m/z* 309 (loss of H_2O), 224 (loss of glycine unit), and 149. (**C**) PLG_GS_3. PLG_GS_3 (4.04 min) had a mass of $[M+H]^+$ = 454 *m/z*. The corresponding MS/MS analysis showed the major fragment ions at *m/z* 361 (loss of glycine unit), 325 (loss of pyroglutamic acid),

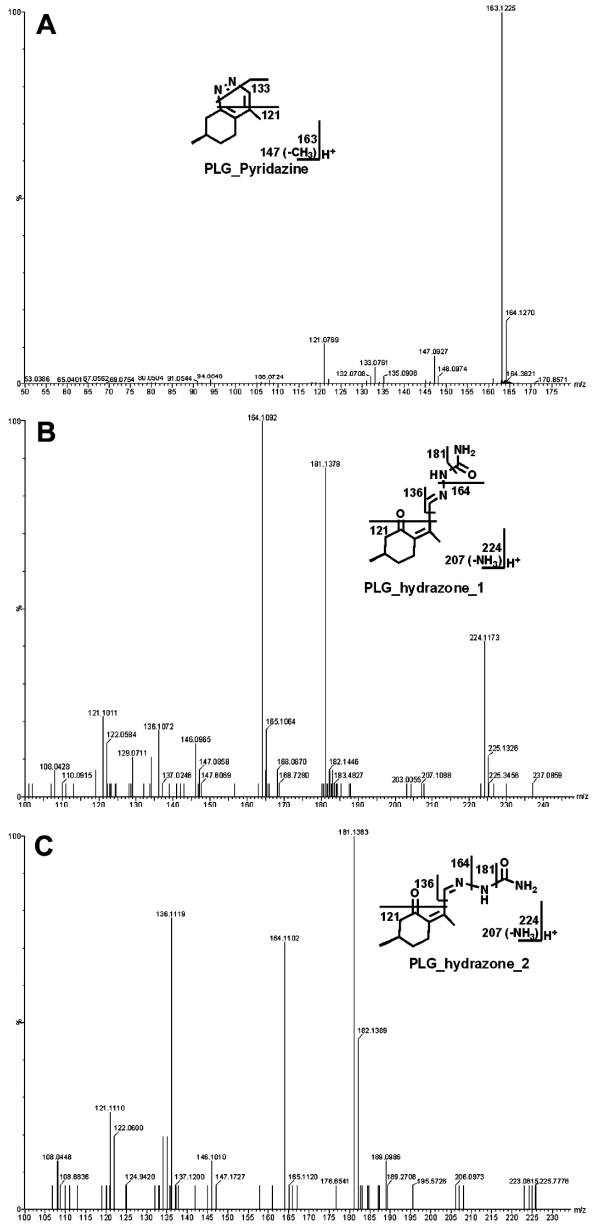
307 (loss of pyroglutamic acid and H_2O), and 147. (**D**) PLG_GS_4. PLG_GS_4 was eluted at 3.58 min and had a $[M+H]^+$ = 492 *m/z*. The corresponding MS/MS displayed the fragment ions at *m/z* 363 (loss of pyroglutamic acid), 345 (loss of pyroglutamic acid and H_2O), 199, and 167. (**E**) PLG_GS_5. PLG_GS_5 was observed at 3.77 min, having a protonated molecule at *m/z* 472. MS/MS analysis of metabolite PLG_GS_5 showed the main fragment ions at *m/z* 454 (loss of H_2O), 397 (loss of glycine unit), 343 (loss of pyroglutamic acid), and 165. (**F**) PLG_GS_6. PLG_GS_6 was eluted at 4.25 min and had a mass of $[M+H]^+$ = 472 *m/z*. The MS/MS of PLG_GS_6 produced the fragment ions at *m/z* 343 (loss of pyroglutamic acid), 325 (loss of pyroglutamic acid and H_2O), and 165. (**G**) PLG_GS_7. PLG_GS_7 was observed at 3.39 min, having a protonated ion at 458 *m/z*. MS/MS analysis of PLG_GS_7 produced the fragment ions at *m/z* 383 (loss of glycine unit), 329 (loss of pyroglutamic acid), 311 (loss of pyroglutamic acid and H_2O), and 151. (**H**) PLG_GS_8. PLG_GS_8 was eluted at 3.64 min and had a mass of $[M+H]^+$ = 458 *m/z*. MS/MS of LPG_GS_8 produced the fragment ions at 383 (loss of loss of glycine unit), 329 (loss of pyroglutamic acid), 311 (loss of pyroglutamic acid and H_2O), and 151.

Supplemental Figure 1



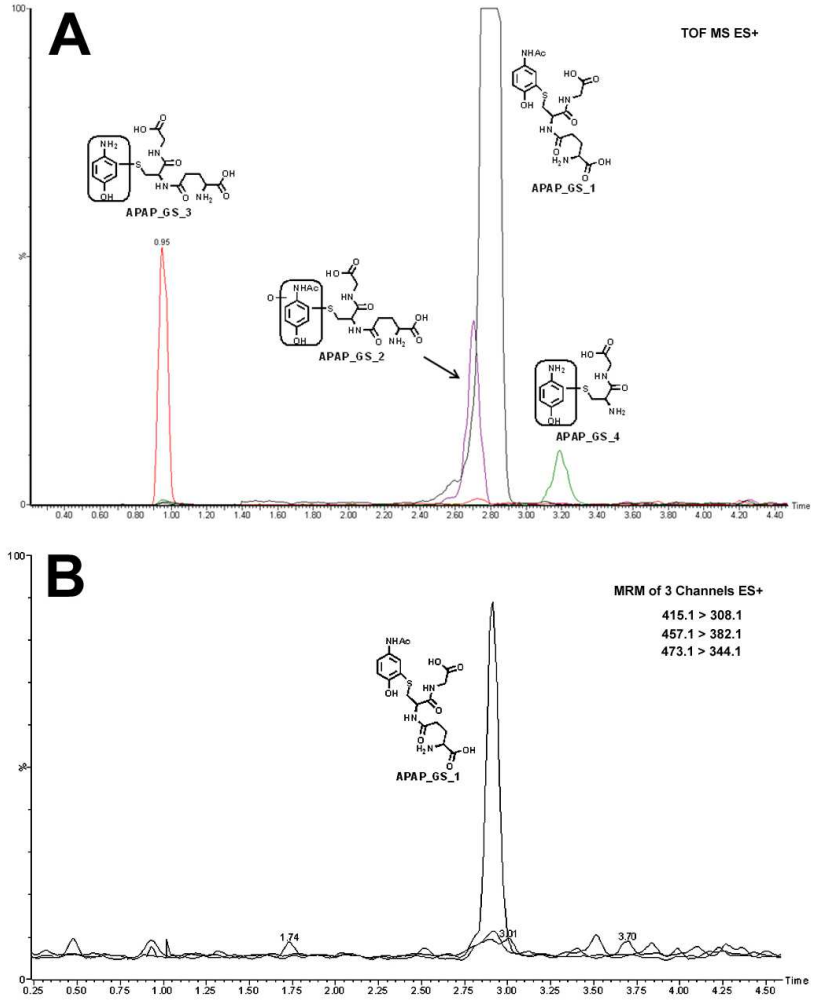
Supplemental Figure 2. The MS/MS spectra and structural elucidation of PLG_Pyridazine and PLG_hydrazones. The structures of metabolites were elucidated by mass fragmentation with a collision energy ramp ranging from 10 to 40 eV. (**A**) PLG_Pyridazine. PLG_Pyridazine, eluted at 3.34 min, had a mass of $[M+H]^+$ = 163 *m/z*,. The MS/MS analysis showed that the ions at *m/z* 147 (loss of CH_3) and 133 (loss of N_2). The ion at *m/z* 121 was interpreted in the inlaid structural diagram. (**B**) PLG_hydrazone_1. PLG_hydrazone_1, eluted at 4.20 min, had a mass of $[M+H]^+$ = 224 *m/z*,. The MS/MS analysis showed that the ions at *m/z* 207 (loss of NH_3), 181 (loss of $CONH_2$), 164 (loss of $NHCONH_2$), and 136. (**C**) PLG_hydrazone_2. PLG_hydrazone_2, eluted at 4.44 min, had a mass of $[M+H]^+$ = 224 *m/z*. The MS/MS of PLG_hydrazone_2 had the similar fragment pattern to that of PLG_hydrazone_1. The ions at *m/z* 181, 164, and 136 were major fragment ions.

Supplemental Figure 2



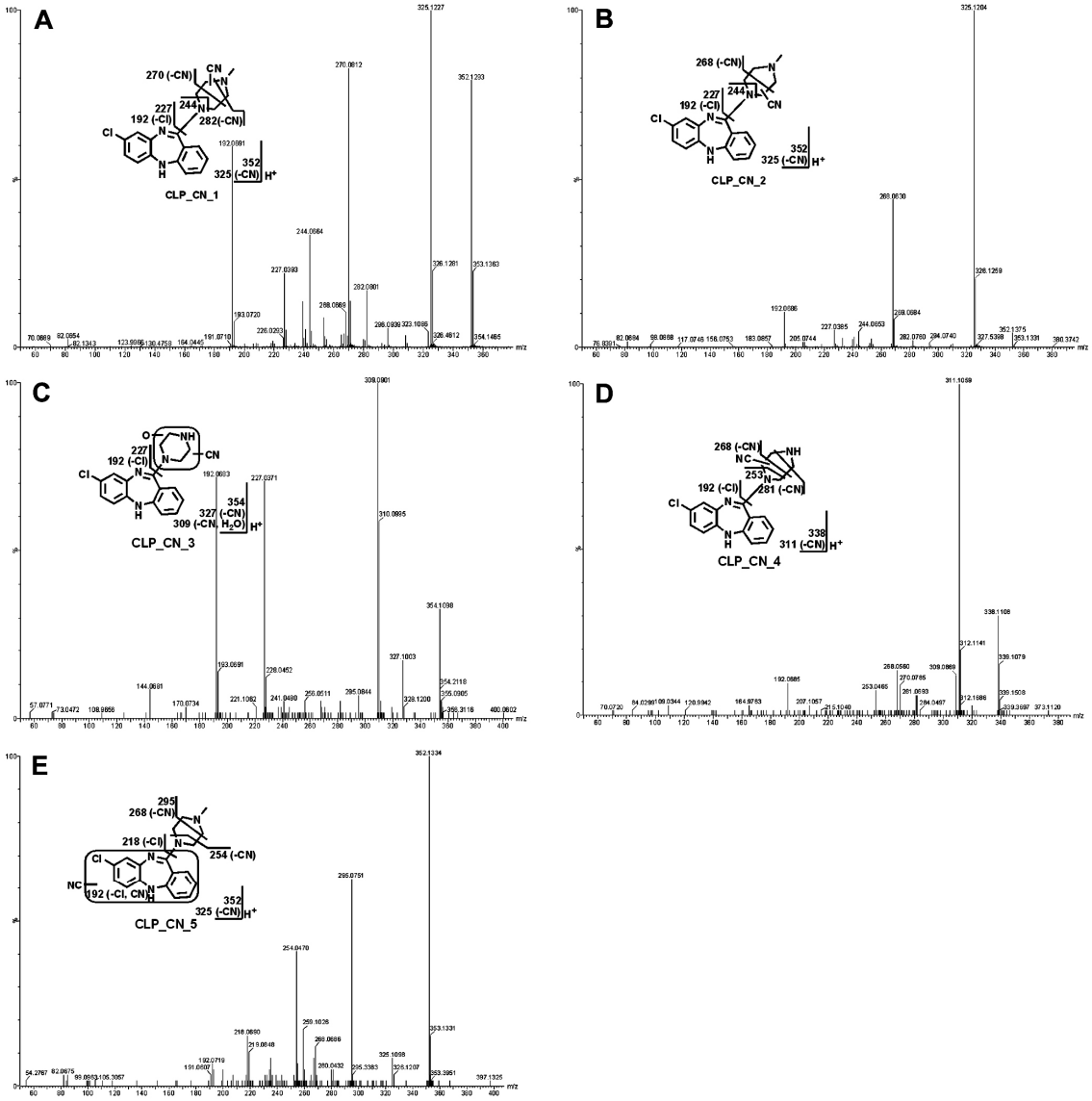
Supplemental Figure 3. The comparison of metabolomic approach (A) and MRM method (B) in identification of APAP_GSH adducts. APAP (30 μM) was incubated in PBS containing HLM (1.0 mg/ml), NADPH (1.0 mM), and GSH (2.5 mM). The total incubation volume was 400 μl. The reactions were initiated by the addition of NADPH and were stopped by the addition of 100 μl of formic acid (10%). After extraction, the residue was reconstituted to 100 μl and 5 μl was analyzed by UPLC-TOFMS (**A**) and UPLC-MS/MS (**B**).

Supplemental Figure 3

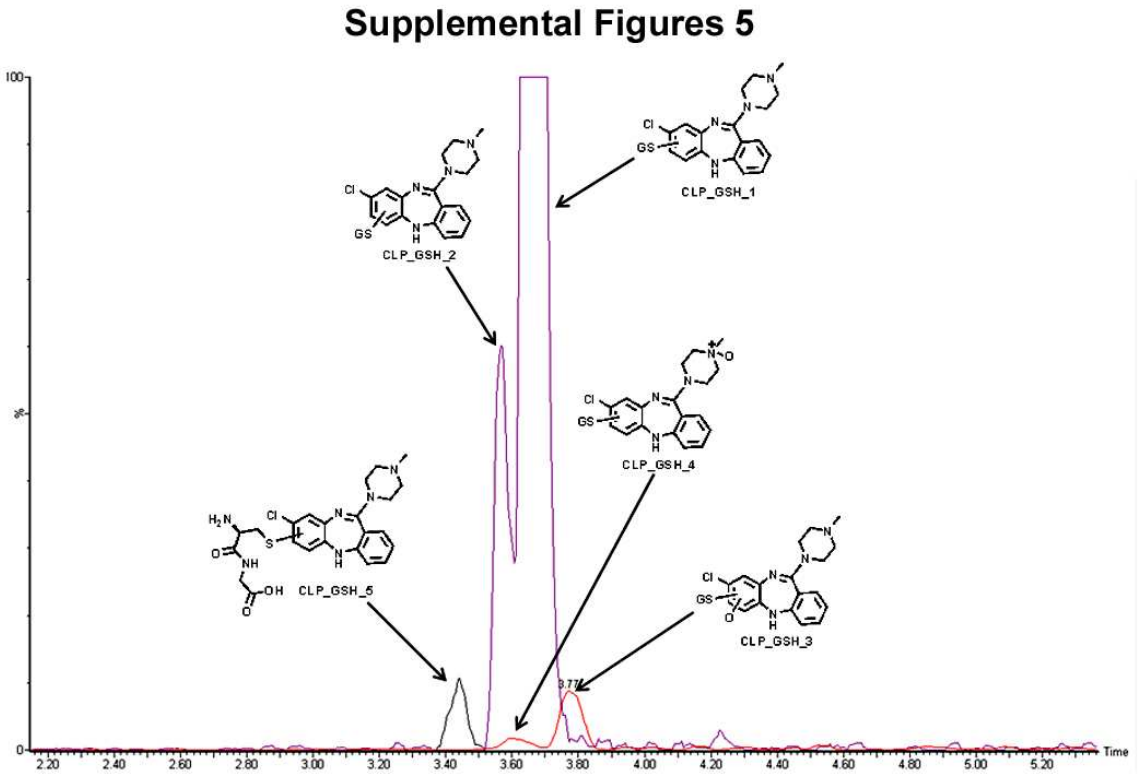


Supplemental Figure 4. The MS/MS spectra of CLP_CN adducts and their structural elucidations. The structures of metabolites were elucidated by mass fragmentation with a collision energy ramp ranging from 10 to 40 eV. (**A**) CLP_CN_1. CLP_CN_1 was eluted at 4.52 min, having a mass of $[M+H]^+$ = 352 *m/z*. MS/MS of CLP_CN_1 produced the ions at *m/z* 325 (loss of CN), 270, 227, and 192. The other ions at *m/z* 282 and 244 were interpreted in the inlaid structural diagram. (**B**) CLP_CN_2. CLP_CN_2, eluted at 5.36 min, had a mass of $[M+H]^+$ = 352 *m/z*,. The MS/MS analysis showed that the ions at *m/z* 325 (loss of CN), 268, 227, and 192. The ion at *m/z* 244 was interpreted in the inlaid structural diagram. (**C**) CLP_CN_3. CLP_CN_3 (5.24 min) corresponded to a protonated molecule at *m/z* 354. MS/MS of CLP_CN_3 produced the fragment ions at *m/z* 327 (loss of CN), 309 (loss of CN and H_2O), 227, and 192. (**D**) CLP_CN_4. CLP_CN_4 was eluted at 4.62 min, having a mass of $[M+H]^+$ = 338 *m/z*. MS/MS analysis of CLP_CN_4 showed the ions at *m/z* 311 (loss of CN), 268, and 192. The other ions at *m/z* 281 and 253 were interpreted in the inlaid structural diagram. (**E**) CLP_CN_5. CLP_CN_5 was eluted at 4.39 min, having a mass of $[M+H]^+$ = 352 *m/z*. MS/MS of CLP_CN_5 produced the ions at *m/z* 325 (loss of CN), 295, 254, and 218. The ion at *m/z* 218 suggested that the CN was attached to the encircled unit. The other ions at *m/z* 268 and 192 were interpreted in the inlaid structural diagram.

Supplemental Figure 4



Supplemental Figure 5. The chromatograms and structures of CLP_GSH adducts. All samples were analyzed by UPLC-TOFMS.



Supplemental Figure 6. The MS/MS spectra of CLP_GSH adducts and their structural elucidations. The structures of trapped metabolites were elucidated by mass fragmentation with a collision energy ramp ranging from 10 to 40 eV. (**A**) CLP_GSH_1. CLP_GSH_1 was eluted at 3.65 min, having a mass of $[M+H]^+$ = 632 *m/z*. MS/MS of CLP_GSH_1 produced the ions at *m/z* 614 (loss of H_2O), 503 (loss of pyroglutamic acid), 328 (loss of GSH). The other ions at *m/z* 446, 359, and 302 were interpreted in the inlaid structural diagram. (**B**) CLP_GSH_2. CLP_GSH_2, eluted at 3.57 min, had a mass of $[M+H]^+$ = 632 *m/z*. The MS/MS analysis showed ions at *m/z* 503 (loss of pyroglutamic acid), 328 (loss of GSH), 359, and 302. (**C**) CLP_GSH_3. CLP_GSH_3 (3.77 min) had a mass of $[M+H]^+$ = *m/z* 648. MS/MS of CLP_GSH_3 produced the fragment ions at *m/z* 630 (loss of H_2O), 519 (loss of pyroglutamic acid), 375, 300, and 275. (**D**) CLP_GSH_4. CLP_GSH_4 was eluted at 3.62 min, having a mass of $[M+H]^+$ = 648 *m/z*. MS/MS analysis of CLP_GSH_4 showed ions at *m/z* 519 (loss of pyroglutamic acid), and 302. (**E**) CLP_GSH_5. CLP_GSH_5 was eluted at 3.45 min, having a mass of $[M+H]^+$ = 503 *m/z*. MS/MS analysis of CLP_GSH_5 showed ions at *m/z* 446, 328 (loss of GSH), and 302.

Supplement Figure 6