Supporting Information

In Vitro and In Vivo Toxicometabolomics of the Synthetic Cathinone PCYP Studied by Means of LC-HRMS/MS

Selina Hemmer[1], Lea Wagmann[1], Benedikt Pulver[2], Folker Westphal[2], Markus R. Meyer[1,*]

[1]Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Center for Molecular Signaling (PZMS), Saarland University, Homburg, Germany
[2]State Bureau of Criminal Investigation Schleswig-Holstein, Kiel, Germany

Corresponding author
*Markus R. Meyer, email: markus.meyer@uks.eu

Table S1. Overview of the peak picking and alignment parameters used for preprocessing for the reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) column and the respective matrices. Pos = positive, neg = negative, ppm = allowed ppm deviation of mass traces for peak picking, snthresh = signal to noise threshold, mzdiff = minimum difference in *m/z* for two peaks to be considered as separate, prefilter 1 = minimum of scan points, prefilter 2 = minimum abundance, bw = bandwidth for grouping of peaks across separate chromatograms.

Column	Matrix	Polarity	Peak width, min	Peak width, max	ppm	sntresh	mzdiff	Prefilter 1	Prefilter 2	bw
RP	pHLM	pos	8.9	100	1.8	10	0.018	7	100	5.0
		neg	8.9	15	1.7	27	0.094	5	100	1.0
	Urine	pos	8.9	19	1.0	12	0.012	7	100	2.5
		neg	7.8	15	2.5	18	-0.098	6	100	4.5
	Plasma	pos	8.9	33	1.3	12	0.1	7	100	1.0
		neg	6.8	100	1.8	16	0.01	5	100	1.0
HILIC	pHLM	pos	7.8	29	1.6	17	0.006	6	100	0.5
		neg	7.8	17	2.5	51	0.01	6	1300	1.0
	Urine	pos	8.9	21	1.9	16	0.02	8	100	1.5
		neg	8.9	35	1.3	15	0.022	8	100	1.5
	Plasma	pos	8.9	46	1.4	6	0.034	6	100	0.2
		neg	8.9	25	2.5	15	0.034	6	100	0.9

Table S2. Overview of the significant features using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) column in pooled human liver microsome incubation. Features are sorted according to *m/z* values, followed by the polarity, the retention time (RT) for the corresponding column in seconds (sec), identity, and the identification level according to MSI. Hyphen (-) means that the feature was not significant using the corresponding column.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
105.0331	Positive	-	192	PCYP artifact	3
146.0812	Positive	-	61	Unknown	4
158.0812	Positive	-	83, 126	Unknown	4
176.0918	Positive	31	126	Unknown	4
218.1539	Positive	-	224	PCYP-M (*N*-dealkyl-)	3
220.1696	Positive	-	247	Unknown	4
271.1884	Positive	314	-	PCYP-M (dehydro-) isotope	3
272.2008	Positive	314	192	PCYP	1
273.2041	Positive	314	192	PCYP isotope	3
274.2164	Positive	314	195	PCYP isotope	3
275.2197	Positive	314	194	PCYP isotope	3
276.223	Positive	314	-	PCYP isotope	3
286.1801	Positive	214, 235	-	PCYP-M (oxo-)	3
288.1957	Positive	280, 302, 255	222, 259, 244	PCYP-M (hydroxy-)	3
289.199	Positive	-	189, 222, 259, 244	PCYP-M (hydroxy-) isotope	3
290.2113	Positive	306	266	PCYP-M (ring opened hydroxy-)	3
291.2146	Positive	-	205	PCYP-M (ring opened hydroxy-) isotope	3
304.1907	Positive	202, 309	234	PCYP-M (dihydroxy-)	3
305.1939	Positive	309	234, 252	PCYP-M (dihydroxy-) isotope	3
306.2063	Positive	216	-	PCYP-M (ring opened dihydroxy)	3
566.5508	Positive	-	42	Unknown	4

Table S3. Overview of the significant features using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) column in rat plasma. Features are sorted according to *m/z* values, followed by the polarity, the retention time (RT) for the corresponding column in seconds (sec), identity, and the identification level according to MSI. Hyphen (-) means that the feature was not significant using the corresponding column.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
146.0599	Positive	-	71	Quinolin-2-ol	2 (NIST msms)
189.0579	Positive	-	66	3-Methyladipic acid $[M+H+H_2O]^+$	2 (NIST msms)
190.0613	Positive	-	67	3-Methyladipic acid $[M+H+H_2O]^+$ isotope	2 (NIST msms)
268.1038	Positive	35,61	248	Adenosine	2 (NIST msms)
269.0878	Positive	-	330	Unknown	4
276.2685	Positive	-	178	Unknown	4
291.2721	Positive	390	-	Unknown	4
304.1904	Positive	306	235	PCYP-M (dihydroxy -)	3
305.1939	Positive	306	235	PCYP-M (dihydroxy-) isotope	3
309.1010	Negative	-	215	Unknown	4
310.1493	Positive	167	-	Unknown	4
312.0945	Negative	-	248	Unknown	4
318.1699	Positive	-	237	PCYP-M (dihydroxy-, oxo)	3
320.1856	Positive	215	290	PCYP-M (trihydroxy-)	3
321.0432	Negative	-	206	Unknown	4
321.1886	Positive	215	-	PCYP-M (trihydroxy-) isotope	3
328.3845	Positive	-	160	Unknown	4
416.3740	Negative	-	159	Unknown	4
562.5880	Negative	24	-	Unknown	4

Table S4. Overview of the significant features using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) column in rat urine. Features are sorted according to *m/z* values, followed by the polarity, the retention time (RT) for the corresponding column in seconds (sec), identity, and the identification level according to MSI. Hyphen (-) means that the feature was not significant using the corresponding column.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
146.0602	Positive	244	70	Quinolin-2-ol	2 (NIST msms)
147.0635	Positive	244	-	Quinolin-2-ol isotope	2 (NIST msms)
148.0965	Positive	-	456	Unknown	4
162.0551	Positive	-	230	Dihydroxyquinoline	3 (NIST msms)
163.0584	Positive	-	230	Dihydroxyquinoline isotope	3 (NIST msms)
185.0437	Positive	-	303	Kynurenic acid $[M\text{-}CH_2O_2+Na]^+$	2 (massbank)
186.0470	Positive	-	303	Kynurenic acid $[M\text{-}CH_2O_2+Na]^+$ isotope	2 (massbank)
189.0582	Positive	156	67	Unknown	4
190.0503	Negative	-	176	Unknown	4
190.0614	Positive	-	67	Unknown isotope	4
208.1183	Negative	-	398	Unknown	4
208.4951	Negative	-	320	Unknown	4
208.9913	Negative	-	320	Unknown	4
211.0401	Positive	156	-	Unknown	4
215.0013	Negative	-	301	Unknown	4
219.9996	Negative	-	248	Unknown	4
220.9920	Negative	-	248	Unknown	4
221.0448	Negative	226	-	Unknown	4
239.9966	Negative	203, 151	232, 271	Unknown	4
240.0539	Positive	-	263	Unknown	4
242.0118	Positive	152	271	Unknown	4
242.0122	Negative	-	245	Unknown	4
242.0123	Negative	157	-	Unknown	4

Table S4. Continued.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
243.0977	Positive	-	472	Unknown	4
243.1817	Positive	-	425	Unknown	4
245.0924	Negative	-	242	Unknown	4
247.9776	Negative	238	-	Unknown	4
250.1439	Positive	-	339	PCYP artifact	3
255.0653	Positive	-	225	Daidzein	2 (massbank)
260.0588	Positive	186	-	Unknown	4
270.0483	Negative	336	-	Unknown	4
271.0390	Negative	-	226	Unknown	4
271.0819	Negative	-	107	Unknown	4
281.1136	Negative	266	329	Unknown	4
283.1290	Positive	266	-	Unknown	4
283.9306	Negative	207, 238	-	Unknown	4
284.1242	Positive	-	468	Unknown	4
285.2286	Positive	-	456	Unknown	4
285.8645	Negative	266	-	Unknown	4
286.0793	Positive	-	455	Unknown	4
287.1139	Positive	208	-	Unknown	4
288.0901	Positive	218	247	Unknown	4
288.1957	Positive	-	222	PCYP-M (hydroxy-)	3
289.0324	Negative	-	354	Unknown	4
290.9998	Negative	-	246	Unknown	4
297.0973	Negative	-	148	Unknown	4
299.8808	Negative	122	-	Unknown	4
302.1422	Positive	-	68	Unknown	4

Table S4. Continued.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
302.2108	Positive	-	366	Unknown	4
303.1704	Positive	-	271	Unknown	4
303.2109	Positive	-	366	Unknown isotope	4
304.0070	Negative	-	320	Unknown	4
304.1910	Positive	305	232	PCYP-M (dihydroxy-)	3
305.1942	Positive	305	-	PCYP-M (dihydroxy-) isotope	3
306.1701	Positive	204	296	PCYP-M (hydroxy + pyrrolidin cleavage with oxidation to COOH)	3
307.0578	Positive	312	-	Unknown	4
307.0749	Positive	249	-	Unknown	4
309.0067	Negative	-	247	Unknown	4
310.0720	Positive	218	247	Unknown	4
311.2119	Positive	320	218	Unknown	4
312.2151	Positive	-	218	Unknown isotope	4
316.1546	Negative	-	236	Unknown	4
317.0329	Negative	-	241	Unknown	4
318.1702	Positive	222	195, 233, 318	PCYP-M (dihydroxy-, oxo)	3
319.1266	Positive	245	-	Unknown	4
319.1734	Positive	222	233, 318	PCYP-M (dihydroxy-, oxo) isotope	3
320.1859	Positive	214, 232	264, 302	PCYP-M (trihydroxy-)	3
321.1892	Positive	214, 232	302	PCYP-M (trihydroxy-) isotope	3
322.2015	Positive	193	-	Unknown	4
323.2048	Positive	192	296	Unknown isotope	4

Table S4. Continued

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
324.9200	Negative	-	234	Unknown	4
325.0855	Positive	206	305	Unknown	4
326.0460	Positive	-	246	Unknown	4
327.1079	Negative	-	176	Unknown	4
327.2069	Positive	243	273	Unknown	4
331.0851	Negative	-	235	Unknown	4
332.1491	Positive	-	86	Unknown	4
334.0101	Negative	-	226	Unknown	4
334.1108	Positive	-	69	Unknown	4
334.1651	Positive	188, 248	115, 324	PCYP-M (trihydroxy-, oxo)	3
335.0223	Positive	-	324	PCYP-M (trihydroxy-, oxo) isotope	3
335.9012	Negative	235	-	Unknown	4
336.1807	Positive	176, 197	323, 341	PCYP-M (tetrahydroxy-)	3
337.1807	Positive	176	323, 341	PCYP-M (tetrahydroxy-) isotope	3
338.0414	Negative	-	241	Unknown	4
341.1861	Positive	210	375	Unknown	4
343.2019	Positive	-	321	Unknown	4
343.8885	Negative	182	-	Unknown	4
346.1433	Positive	-	245	Unknown	4
347.1466	Positive	-	245	Unknown	4
347.2541	Positive	-	374	Unknown	4
349.0703	Negative	-	248	Unknown	4
351.0858	Positive	-	114	Unknown	4
352.0487	Positive	-	225	Unknown	4

Table S4. Continued.

m/z	Polarity	RP RT, sec	HILIC RT, sec	Identity	Identification level according to MSI
353.0329	Negative	-	245	Unknown	4
356.1471	Positive	247	-	Unknown	4
360.1920	Positive	-	202, 344	Unknown	4
361.1952	Positive	-	344	Unknown isotope	4
365.2357	Negative	-	146	Unknown	4
367.0484	Negative	-	240	Unknown	4
371.1338	Negative	225	-	Unknown	4
372.1212	Positive	-	114	Unknown	4
376.1426	Positive	271	-	Unknown	4
380.0548	Negative	-	246	Unknown	4
390.1762	Positive	225	-	Unknown	4
391.0682	Negative	-	223	Unknown	4
426.1333	Positive	-	247	Unknown	4
462.0523	Negative	-	320	Unknown	4
464.2285	Positive	-	393	PCYP-M (hydroxy-glucuronide-)	3
465.2155	Positive	317	360	Unknown	4
466.2189	Positive	-	360	Unknown isotope	4
573.3299	Negative	-	139	Unknown	4

Table S5. Detected PCYP metabolites using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) column in their corresponding matrices namely pooled human liver microsomes (H), rat urine (U), and rat plasma (P) in which the metabolites could be detected. Metabolite identification numbers (ID) match with the labeling of the structure in Figure 1. For each metabolite the calculated exact mass of the protonated molecule and elemental composition are given. Hyphen (-) means that the metabolite was not significant in any matrix of the respective column.

Metabolite-ID	Calculated exact mass, *m/z*	Elemental composition	RP	HILIC
PCYP	272.2009	$C_{18}H_{25}NO$	H	H
M1	288.1958	$C_{18}H_{25}NO_2$	H	H
M2	288.1958	$C_{18}H_{25}NO_2$	H	H
M3	288.1958	$C_{18}H_{25}NO_2$	H	H, U
M4	218.1539	$C_{14}H_{19}NO$	-	H
M5	304.1907	$C_{18}H_{25}NO_3$	H, U, P	H, U, P
M6	320.1856	$C_{18}H_{25}NO_4$	U, P	U, P
M7	336.1805	$C_{18}H_{25}NO_5$	U	U
M8	286.1802	$C_{18}H_{23}NO_2$	H	-
M9	318.1700	$C_{18}H_{23}NO_3$	U, P	U, P
M10	334.1649	$C_{18}H_{25}NO_5$	U	U
M11	290.2115	$C_{18}H_{27}NO_2$	H	H
M12	306.2064	$C_{18}H_{27}NO_3$	H	-
M13	304.1907	$C_{18}H_{25}NO_3$	H	-
M14	320.1856	$C_{18}H_{25}NO_4$	U	U
M15	306.1700	$C_{17}H_{23}NO_4$	U	U
M16	250.1438	$C_{14}H_{19}NO_3$	-	U
M17	464.2279	$C_{24}H_{33}NO_8$	-	U

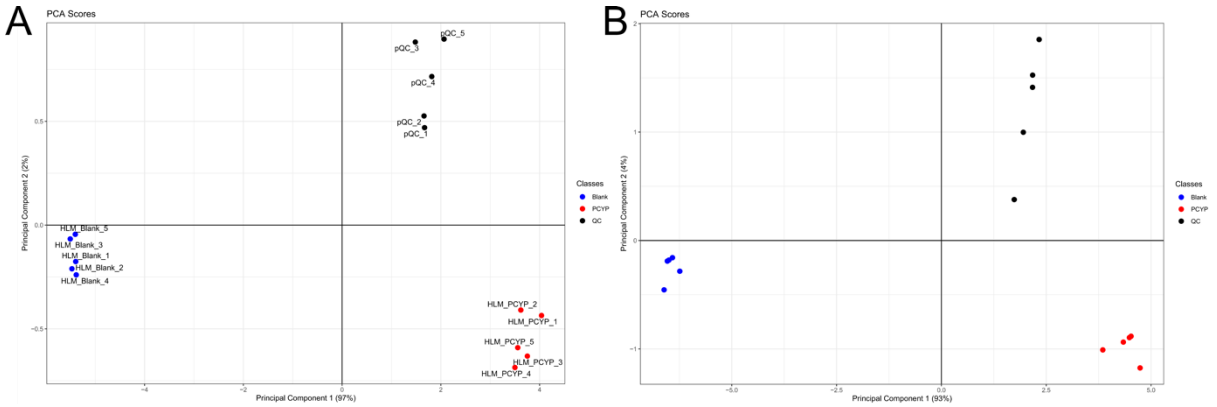

Figure S1. Results of scores of principal component analysis of pooled human liver microsome samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive ionization mode. A = RP pos, B = HILIC pos.

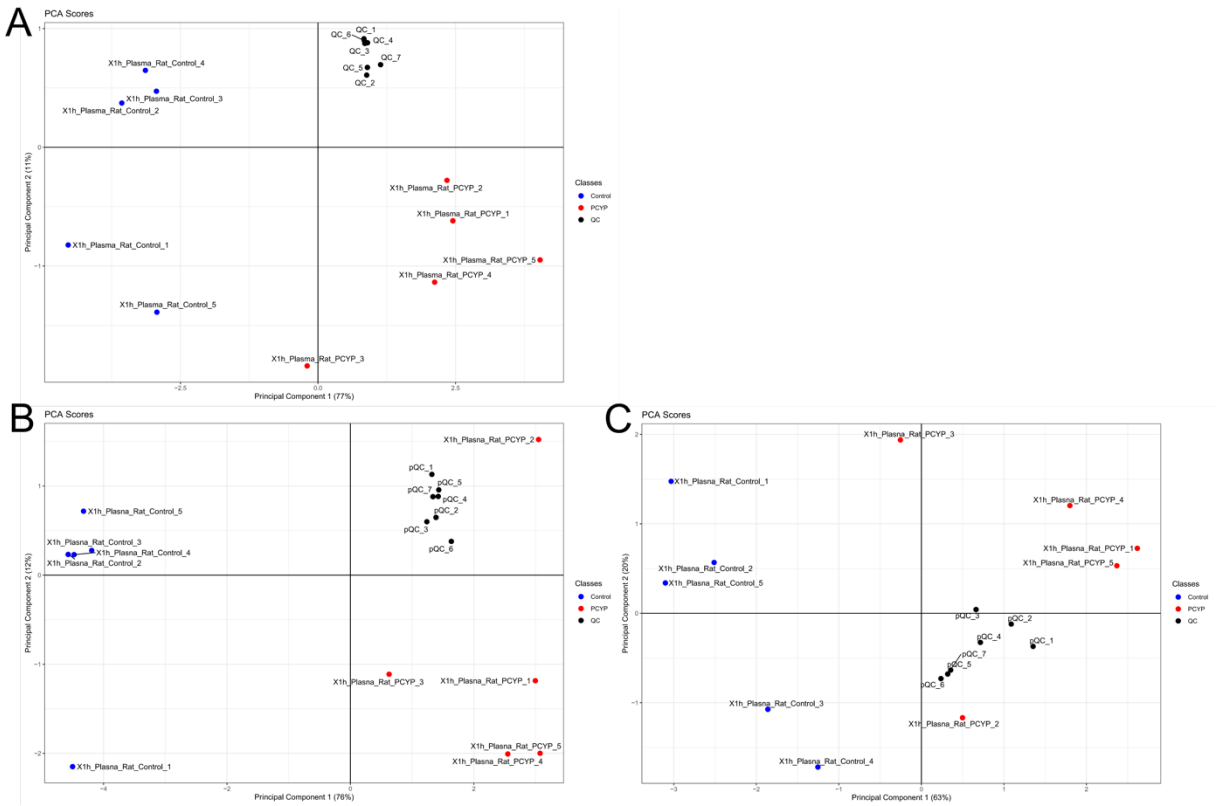

Figure S2. Results of scores of principal component analysis of rat plasma samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive and negative ionization mode. A = PH pos, B = HILIC pos, C = HILIC neg.

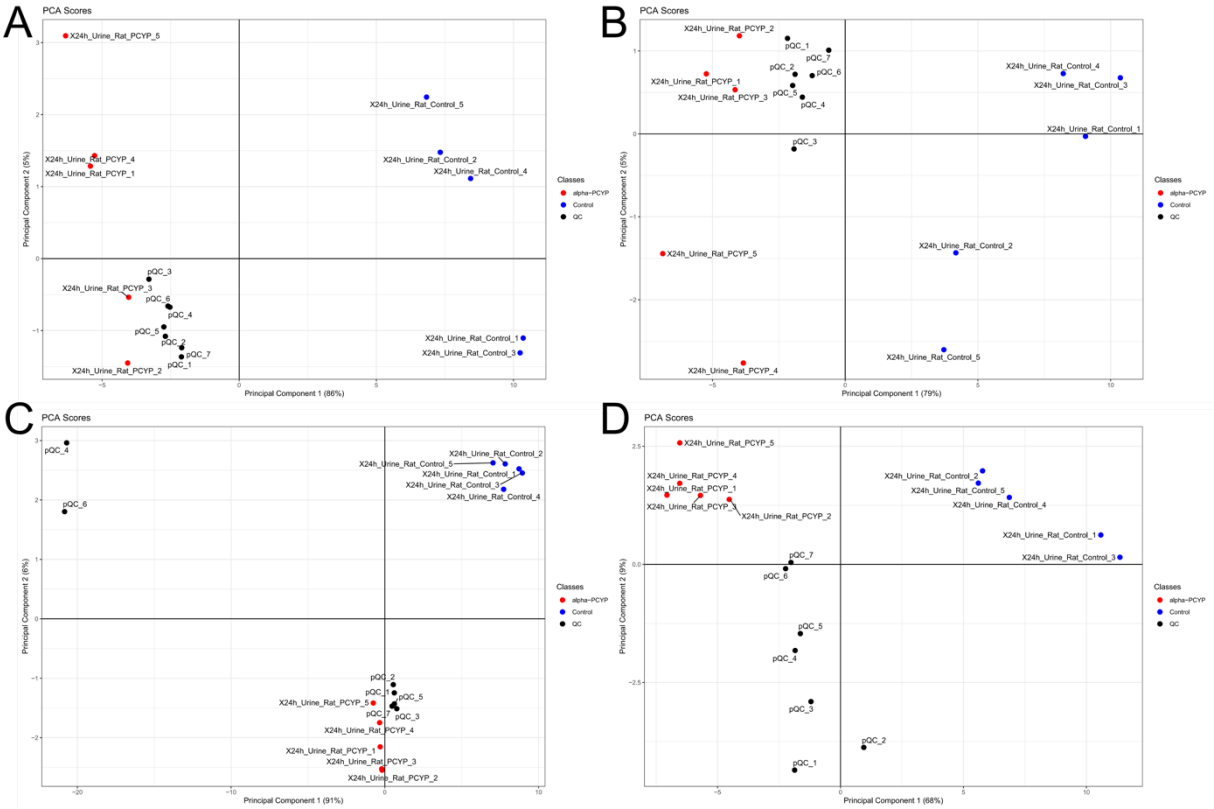

Figure S3. Results of scores of principal component analysis of rat urine samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive and negative ionization mode. A = RP pos, B = RP neg, C = HILIC pos, D = HILIC neg.

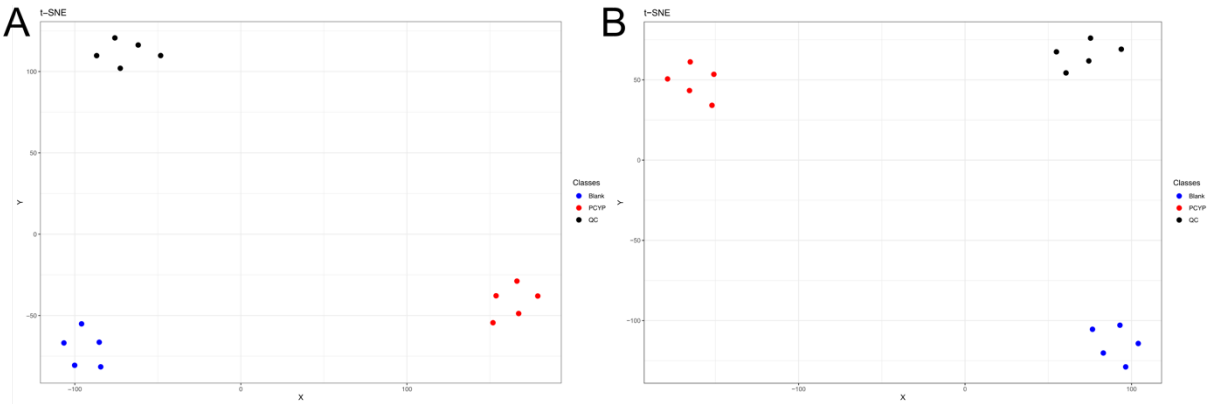

Figure S4. Results of t-distributed stochastic neighborhood embedding (t-SNE) of pooled human liver microsome samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive ionization mode. A = RP pos, B = HILIC pos.

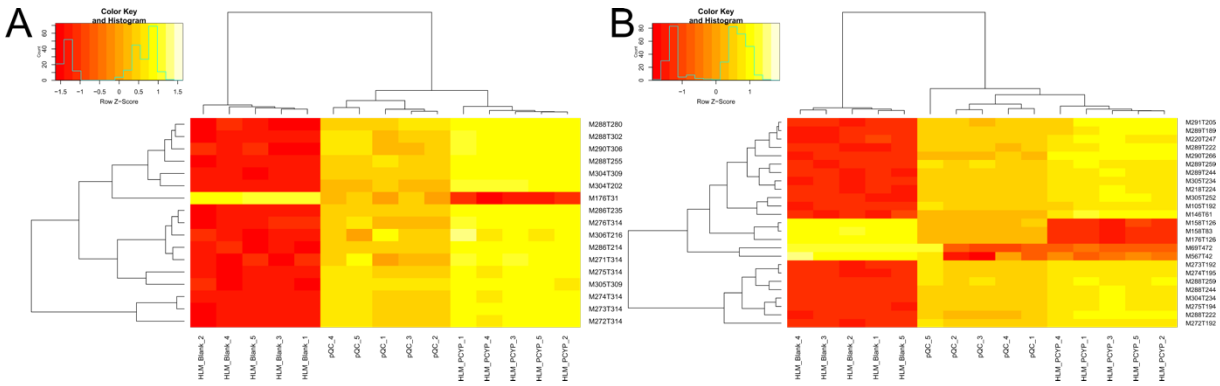

Figure S5. Results of heat map of hierarchical clustering of pooled human liver microsome samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive ionization mode. A = RP pos, B = HILIC pos.

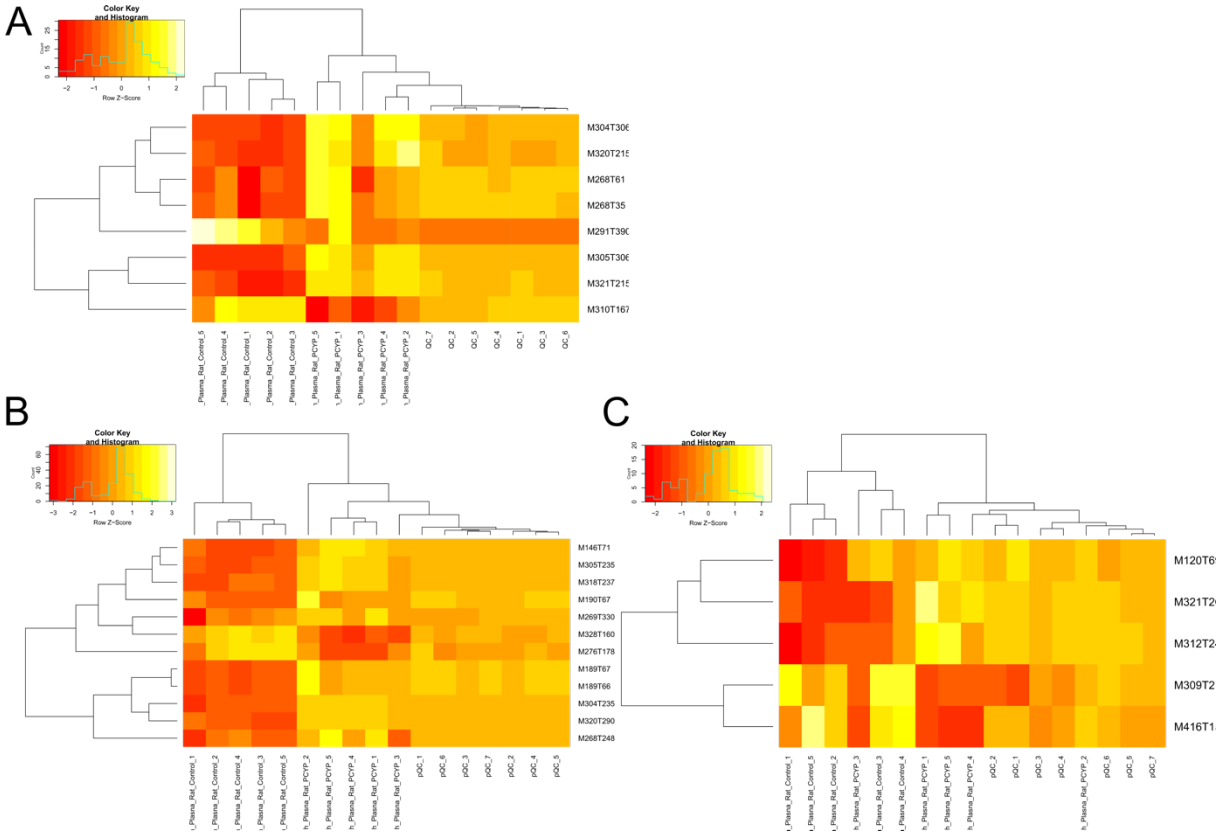

Figure S6. Results of heat map of hierarchical clustering of rat plasma samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive and negative ionization mode. A = RP pos, B = HILIC pos, C = HILIC neg.

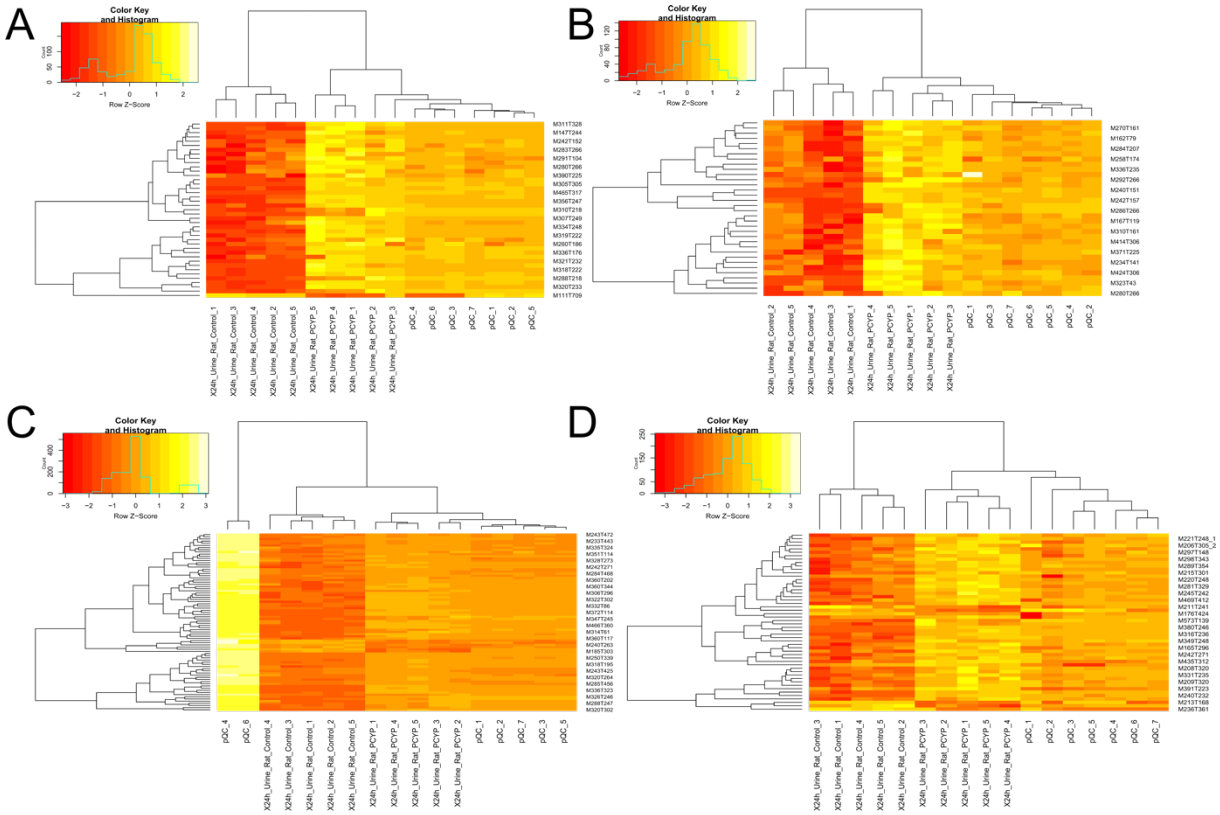

Figure S7. Results of heat map of hierarchical clustering of rat urine samples after analysis using reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) in positive and negative ionization mode. A = RP pos, B = RP neg, C = HILIC pos, D = HILIC neg.

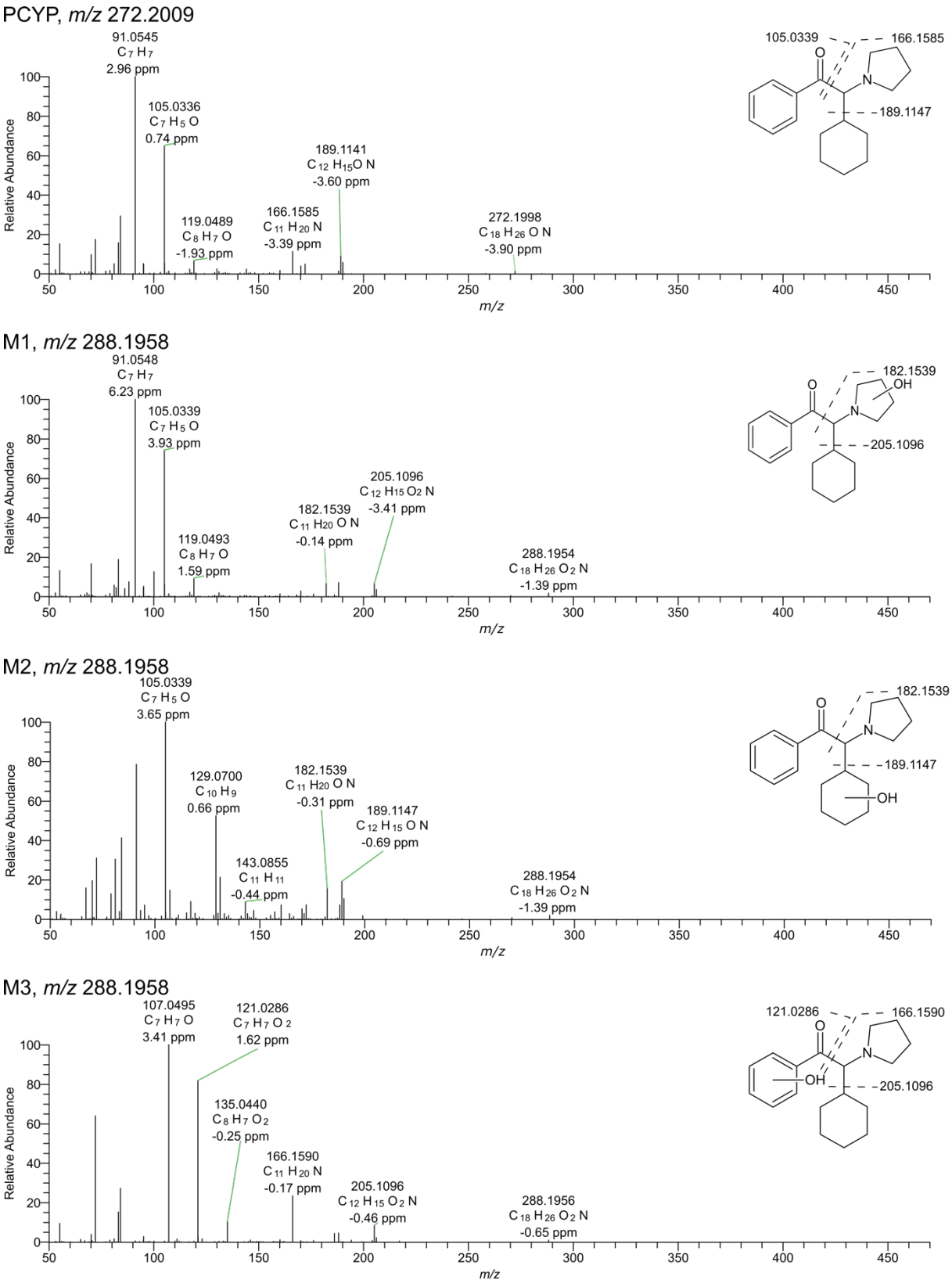

Figure S8. LC-HRMS/MS spectra of the PCYP metabolites detected in positive ionization mode. Fragments with accurate mass, calculated elemental formula, and mass error value in parts per million (ppm).

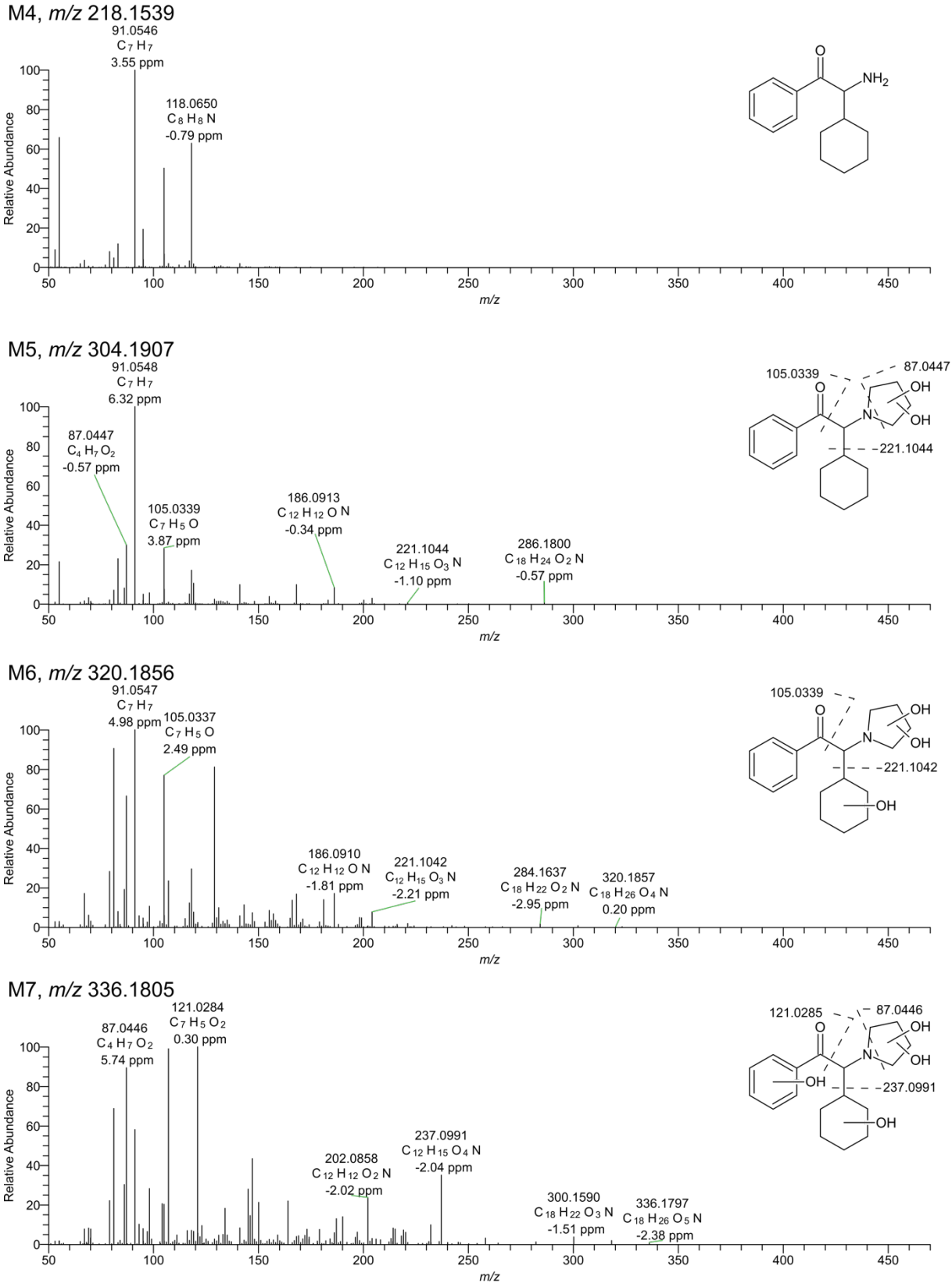

Figure S8. Continued.

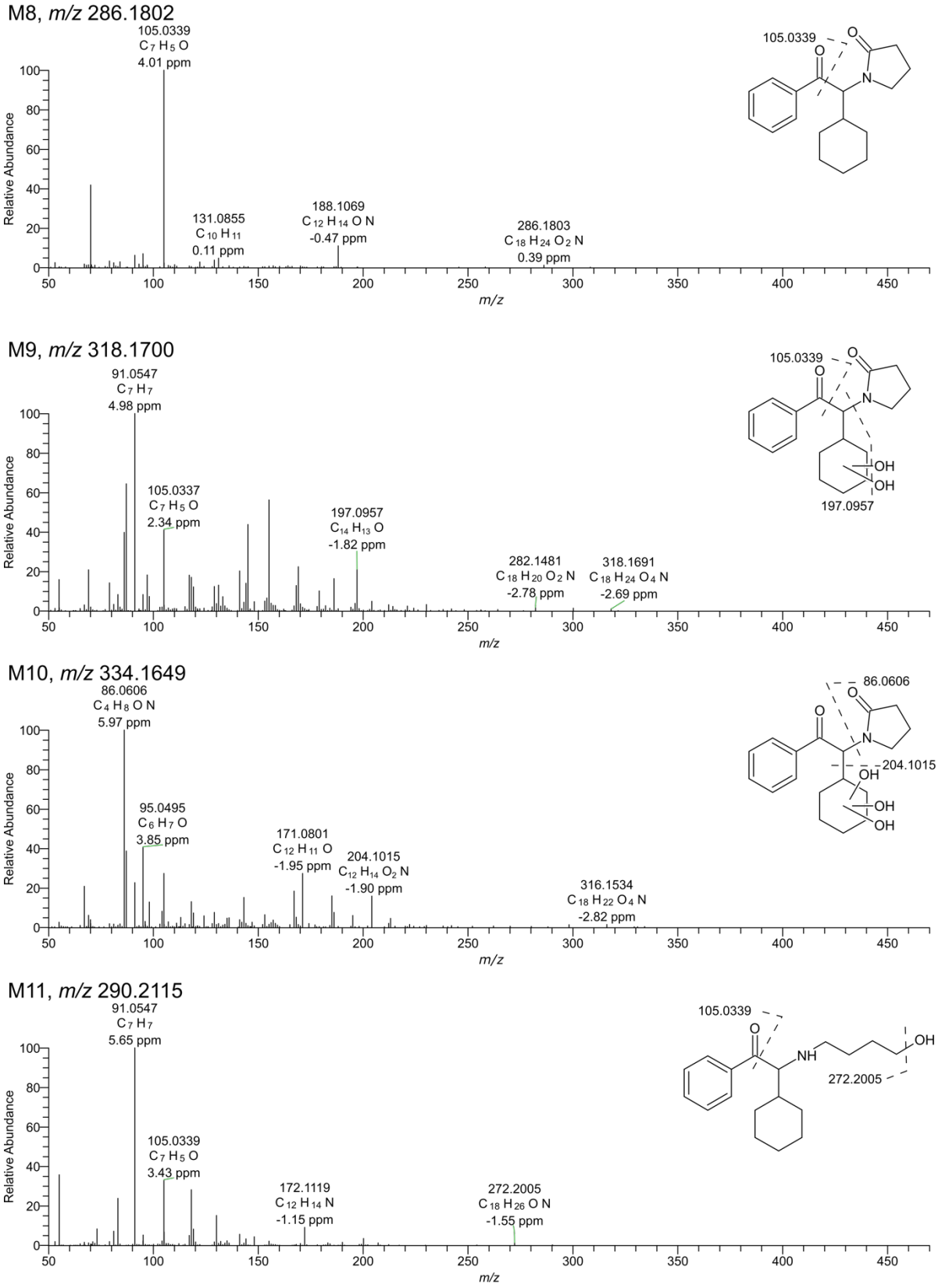

Figure S8. Continued.

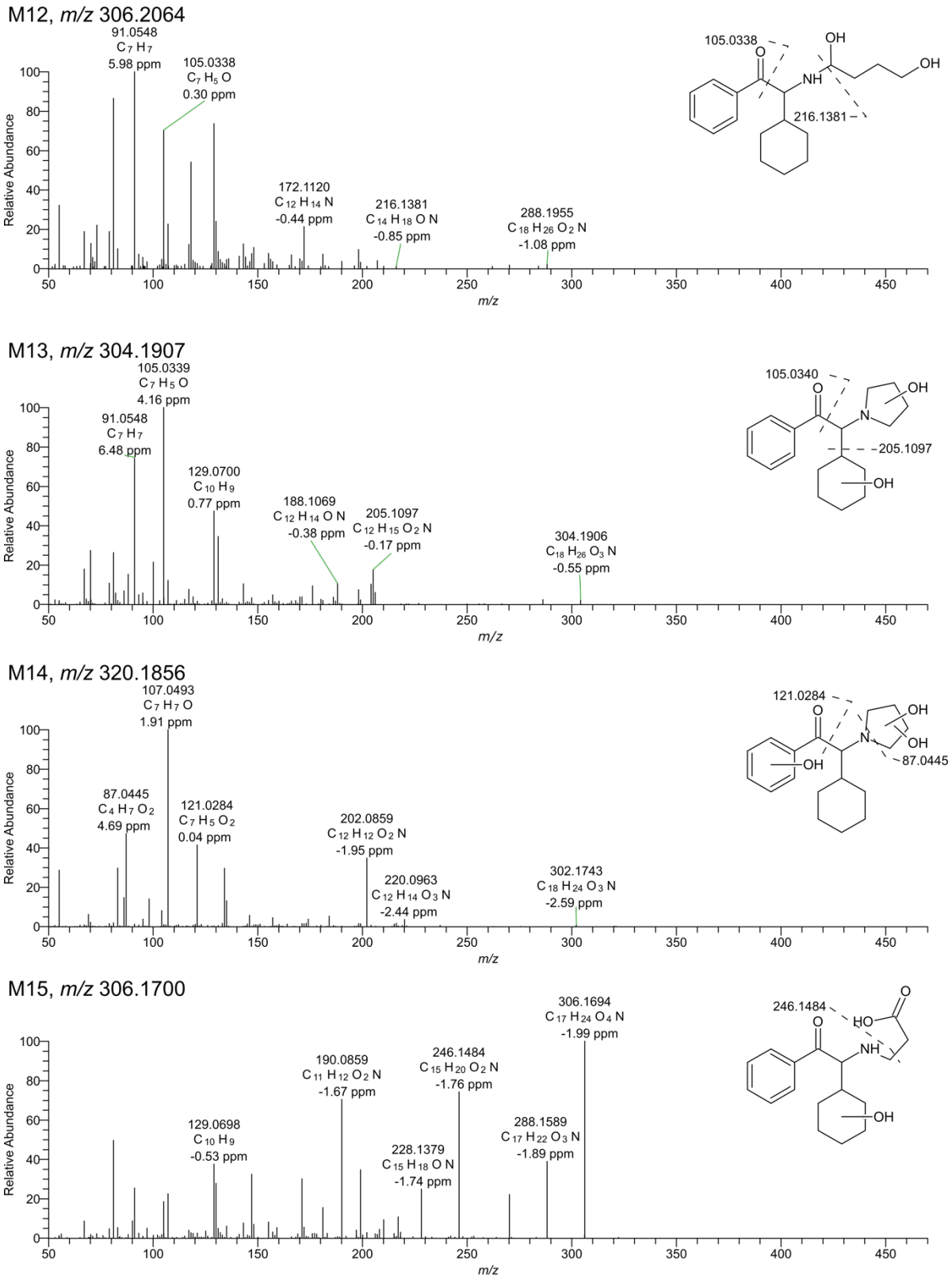

Figure S8. Continued.

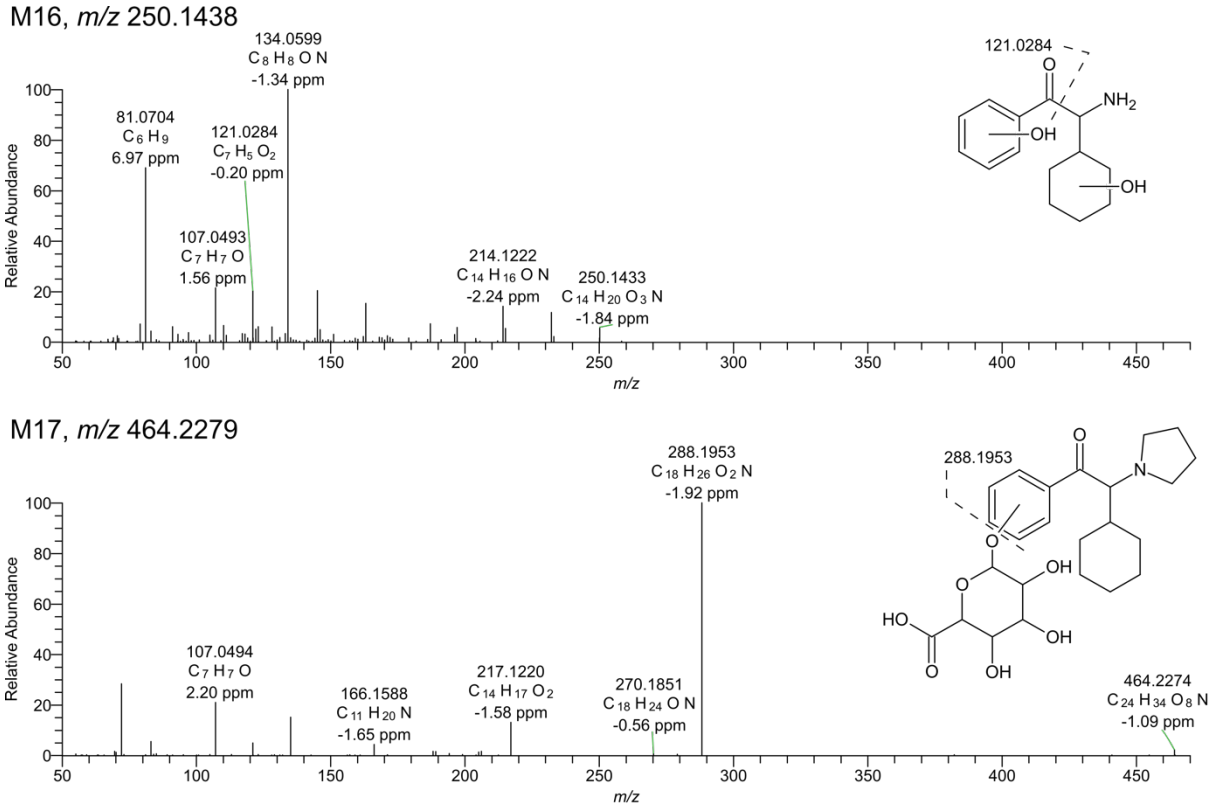

Figure S8. Continued.

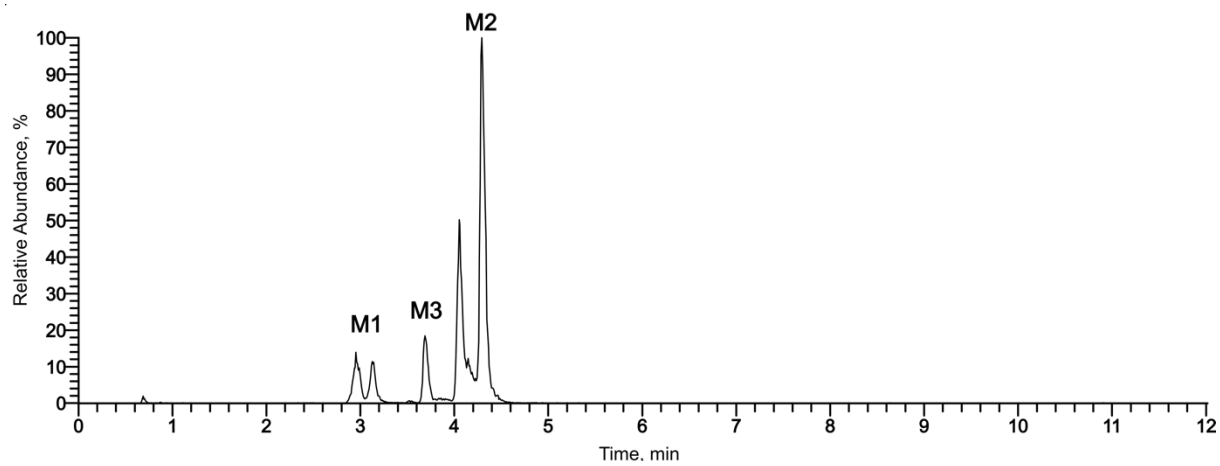

Figure S9. Reconstructed ion chromatogram of *m/z* 288.1958 after analysis of one QC sample of pooled human liver microsome in full scan in positive ionization mode using hydrophilic interaction chromatography (HILIC). Metabolite identification number (M) match with the metabolites listed in Table S5.

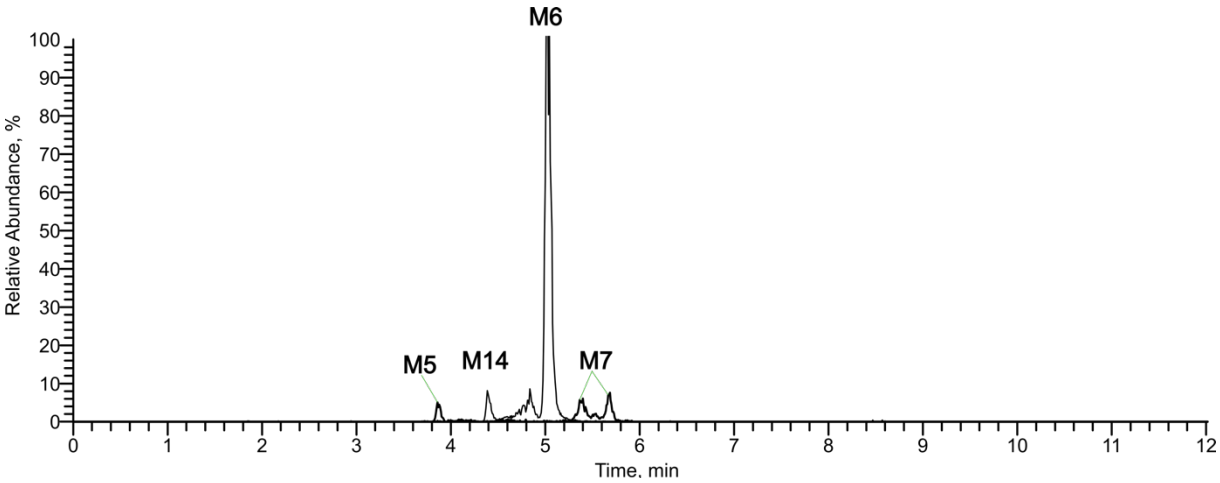

Figure S10. Reconstructed ion chromatograms of *m/z* 304.1856, *m/z* 320.1856, and *m/z* 336.1805 after analysis of one QC sample of rat urine in full scan in positive ionization mode using hydrophilic interaction chromatography (HILIC). Metabolite identification numbers (M) match with the metabolites listed in Table S5.