SUPPORTING INFORMATION

In vitro human metabolism of the flame retardant resorcinol bis-(diphenylphosphate) (RDP)

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Sample preparation

The following procedures were investigated: a) common solvents for liquid-liquid extraction after HLM incubations reported for other FRs (ethyl acetate, methyl-tert-butylether, MTBE:hexane 1:1 v/v); b) anion exchange solid-phase extraction (SPE) as previously reported for the analysis of PFR metabolites in urine and c) salting-out extraction in mixtures acetonitrile:water with ammonium acetate. Clean-up/concentration experiments were made in duplicate with 0.2 mg/mL HLM, 20 µM RDP and 30 min incubations. Reactions were stopped with 1mL of cold acetonitrile and in ice (1 min) or only in ice (1 min) for subsequent SPE. HLMs were immediately removed by centrifugation (30 s, 8000 rpm) prior to clean-up. For solvent clean-up, two extraction steps with 4 mL of solvent (vortex stirring for 1 min) were carried out in 10 mL glass tubes and the final extracts combined, evaporated to dryness (N_2, 40°C) and reconstituted in 150 µL of methanol. Extracts were finally centrifuged (30 s, 8000 rpm) before analysis. For the salting-out clean-up, an amount of 155 mg of ammonium acetate was added to the mixture of water:acetonitrile 1:1 v/v obtained after stopping the incubation and the extraction done by vortex-mixing for 1 min. This amount of salt was equivalent to around 2 M of salt in water and was selected based on common concentrations used for salting-out procedures with water:acetonitrile in the literature. Two additional extraction steps with 0.8 mL of acetonitrile each were carried out in the 2mL Eppendorf. The final extracts were combined, evaporated to dryness (N_2, 40°C) and reconstituted in 150 µL of methanol. Extracts were finally centrifuged (30 s, 8000 rpm) before analysis.

Figure S-3 shows the apparent recoveries of some RDP metabolites and compounds under the different sample preparation procedures. Both, ethyl acetate and acetonitrile salting-out extractions gave good apparent recoveries for a wider polarity range of compounds, despite the lower solvent volume (3 mL instead of 8 mL) used for the latter. However, cleaner extracts were observed with acetonitrile in comparison with the three other solvent procedures that yielded turbid extracts after evaporation and reconstitution in methanol. This was probably due the co-extraction of non-polar matrix components with low solubility in methanol. These co-extracted matrix components could be anyway mostly separated by precipitation in the final centrifugation step. Furthermore, we selected the salting-out procedure as optimal due to the lower consumption of organic solvent and materials and the simplicity of the procedure. This sample preparation was also suitable for glucuronidated and sulfated metabolites, despite of their higher polarity.

Table S-1. RDP metabolites and hydrolysis products identified in this study: molecular formula, accurate mass, QTOF measured mass errors, retention times (RT) and QQQ-MS main transitions

Compound	Molecular formula (parent compound)	[c]RT (min)	QQQ Transition (*m/z*)
Metabolites			
Hydroxy metabolites			
[a]*para*-HO-TPHP	$C_{18}H_{15}O_5P$	5.1	341.1-249.1
[a]di-HO-TPHP	$C_{18}H_{15}O_6P$	4.6	357.1-249.1
[a]HO-RDP (1)	$C_{30}H_{24}O_9P_2$	7.5	589.1-497.1
[a]di-HO-RDP	$C_{30}H_{24}O_{10}P_2$	6.9	605.1-497.1
[a]HO-RDPn2 (1)	$C_{42}H_{33}O_{13}P_3$	8.8	837.1-93.0
Sulfated and glucuronidated metabolites			
[a]Resorcinyl sulfate	$C_6H_6O_5S$	4.0	188.9-109.0
[a]Phenyl sulfate	$C_6H_6O_4S$	5.3	172.9-93.0
[a]TPHP sulfate	$C_{18}H_{15}O_8PS$	17.3	421.0-249.1
[a]HO-TPHP sulfate (1)	$C_{18}H_{15}O_9PS$	17.1	437.0-249.1
[a]HO-TPHP sulfate (2)	$C_{18}H_{15}O_9PS$	17.3	437.0-249.1
[a]RDP sulfate	$C_{30}H_{24}O_{13}P_2S$	19.4	685.0-497.1
[a]HO-RDP sulfate	$C_{30}H_{24}O_{12}P_2S$	19.3	669.0-497.1
[a]TPHP glucuronide	$C_{24}H_{23}O_{11}P$	16.8	517.1-249.1
[a]RDP glucuronide	$C_{36}H_{32}O_{15}P_2$	15.0	765.1-249.1
[a]HO-TPHP glucuronide (1)	$C_{24}H_{23}O_{12}P$	16.6	533.1-249.1
[a]HO-TPHP-HO glucuronide (2)	$C_{24}H_{23}O_{12}P$	16.8	533.1-249.1
[a]HO-RDP-HO glucuronide (1)	$C_{36}H_{32}O_{16}P_2$	19.1	781.1-497.1
[a]HO-RDP-HO glucuronide (2)	$C_{36}H_{32}O_{16}P_2$	19.2	781.1-497.1
Compounds and hydrolysis products or impurities			
[b]TPHP	$C_{18}H_{15}O_4P$	7.0	327.1-152.1
[b]RDP	$C_{30}H_{24}O_8P_2$	8.8	575.1-481.1
[b]RDPn2	$C_{42}H_{33}O_{12}P_3$	9.9	823.1-481.1
[a,c] RDP-[Phe]	$C_{24}H_{20}O_8P_2$	3.8	497.1-249.0
[a]*meta*-HO-TPHP	$C_{18}H_{15}O_5P$	5.3	341.1-249.0
[a] *meta*-HO-RDP	$C_{30}H_{24}O_9P_2$	7.7	589.1-497.1
[a] *meta*-HO-RDPn2	$C_{42}H_{33}O_{13}P_3$	9.0	837.1-93.0
[a]DPHP	$C_{12}H_{11}O_4P$	2.9	249.1-155.1
[b]TPHP-d_{15}	$C_{18}D_{15}O_4P$	6.9	342.1-82.0

ESI source parameters: gas temperature 320 °C; gas flow 8 L min^{-1}; nebulizer 40 psi, sheat gas heater 250, sheat gas flow 11, capillary 4000 V, Nozzle voltage 500. MS parameters: Frag. 120 V; CE 25 V; Cell Ac. 7 V; dwell time 50 ms. ESI polarity [a]negative mode, [b]positive mode. [c]LC methods conditions for Phase I and hydrolysis products and for Phase II metabolites were different and are explained in the manuscript. [c]Metabolite corresponding to RDP with the loss of a phenyl group. Abbreviations: diphenyl phosphate, DPHP; triphenyl phosphate, TPHP; RDP dimer, RDPn2.

Table S-2. Identification of metabolites and hydrolysis products of RDP by LC-ESI-QTOF

Compound	Formula	Target mass	Ions ESI (-)	Ions ESI (+)	Mass error (ppm) ESI(-)	Mass error (ppm) ESI(+)	Identification method
Hydrolysis products or impurities							
DPHP	$C_{12}H_{11}O_4P$	250.0395	[M-H]-	[M+H]+	-3.54	5.58	[a]Meteor
[b]RDP-[Phe]	$C_{24}H_{20}O_8P_2$	498.0633	[M-H]-	[M+H]+ [M+Na]+	-4.35	-2.83	[a]Meteor
***meta*-HO-TPHP**	$C_{18}H_{15}O_5P$	342.0657	[M-H]$^-$ [M+HCOO]-	[M+H]+ [M+Na]+	-4.6	-0.02	[a]Meteor
[c]RDP-n2-[Phe]	$C_{36}H_{29}O_{12}P_3$	746.0872	[M-H]$^-$	[M+H]+ [M+Na]+	-1.72	-2.41	[a]Meteor
HO-RDP (2)	$C_{30}H_{24}O_9P_2$	590.0896	[M-H]$^-$ [M+HCOO]-	[M+H]+ [M+Na]+	-2.89	1.31	[a]Meteor
HO-RDPn2 (2)	$C_{42}H_{33}O_{13}P_3$	838.1134	[M-H]$^-$	[M+H]+ [M+Na]+	2.46	-0.08	[a]Meteor
Hydroxy metabolites							
di-HO-TPHP	$C_{18}H_{15}O_6P$	358.0606	[M-H]- [M+HCOO]- [M+CH_3COO]-	[M+H]+	-5.16	0.51	untargeted
***para*-HO-TPHP**	$C_{18}H_{15}O_5P$	342.0657	[M-H]$^-$ [M+HCOO]-	[M+H]+ [M+Na]+	-2.66	0.31	Meteor
di-HO-RDP	$C_{30}H_{24}O_{10}P_2$	606.0845	[M-H]$^-$	[M-H]$^-$	3.79	-1.34	untargeted
HO-RDP (1)	$C_{30}H_{24}O_9P_2$	590.0896	[M-H]$^-$ [M+HCOO]-	[M+H]+ [M+Na]+	-3.2	-0.29	Meteor
HO-RDPn2 (1)	$C_{42}H_{33}O_{13}P_3$	838.1134	[M-H]$^-$	[M+H]+ [M+Na]+	1.56	2.32	Meteor
Suffated metabolites							
Phenol sulfate	$C_6H_6O_5S$	189.9936	[M-H]$^-$	-	-3.59	n.d.	untargeted
Resorcynil sulfate	$C_6H_6O_4S$	173.9987	[M-H]$^-$	-	-2.88	n.d.	Meteor
TPHP sulfate	$C_{18}H_{15}O_8PS$	422.0225	[M-H]$^-$	[M+H]+ [M+Na]+	-1.61	-0.38	Meteor
HO-TPHP sulfate (1)	$C_{18}H_{15}O_9$ PS	438.0169	[M-H]$^-$	[M+H]+ [M+Na]+	-3.68	-1.54	untargeted
HO-TPHP sulfate (2)	$C_{18}H_{15}O_9$ PS	438.0169	[M-H]$^-$	-	-2.5	n.d.	untargeted
RDP sulfate	$C_{30}H_{24}O_1$ P_2 S	686.0407	[M-H]$^-$	-	-1.13	n.d.	Meteor
HO-RDP sulfate	C_{30} $H_{24}O_{12}P_2$ S	670.0464	[M-H]$^-$	-	-2.67	n.d.	untargeted
Glucuronidated metabolites							
TPHP glucuronide	$C_{24}H_{23}O_{11}P$	518.0978	[M-H]$^-$	[M+H]+ [M+Na]+	-0.87	-1.81	Meteor
RDP glucuronide	$C_{36}H_{32}O_{15}P_2$	766.1216	[M-H]$^-$	[M+H]+ [M+Na]+	-2.23	-1.59	Meteor
HO-TPHP glucuronide (1)	$C_{24}H_{23}O_{12}P$	534.0921	[M-H]$^-$	[M+H]+ [M+Na]+	-0.57	-0.2	untargeted
HO-TPHP-HO glucuronide (2)	$C_{24}H_{23}O_{12}P$	534.0921	[M-H]$^-$	[M+H]+ [M+Na]+	-1.2	-1.8	untargeted
HO-RDP-HO glucuronide (1)	$C_{36}H_{32}O_{16}P_2$	782.116	[M-H]$^-$	[M+H]+ [M+Na]+	-1.43	-1.77	untargeted
HO-RDP-HO glucuronide (2)	$C_{36}H_{32}O_{16}P_2$	782.116	[M-H]$^-$	[M+H]+ [M+Na]+	-1.14	-1.35	untargeted

[a]These compounds were identified by the use of the generated Meteor database in which they are proposed to be formed by phosphatases, however chemical hydrolysis was shown to be the only responsible for their formation and not enzymes under the conditions described in this study; [b]Metabolite corresponding to RDP with the loss of a phenyl group; [c]Metabolite corresponding to RDP-n2 (RDP dimer) with the loss of a phenyl group. Abbreviations: diphenyl phosphate, DPHP; triphenyl phosphate, TPHP; RDP dimer, RDPn2.

Figure S-1. Hydrolysis products with (blue dots) and without (red dots) the presence of HLM (0.2 mg/mL) and with increasing RDP amounts (30 min incubation time, average±standard deviation of three independent experiments)

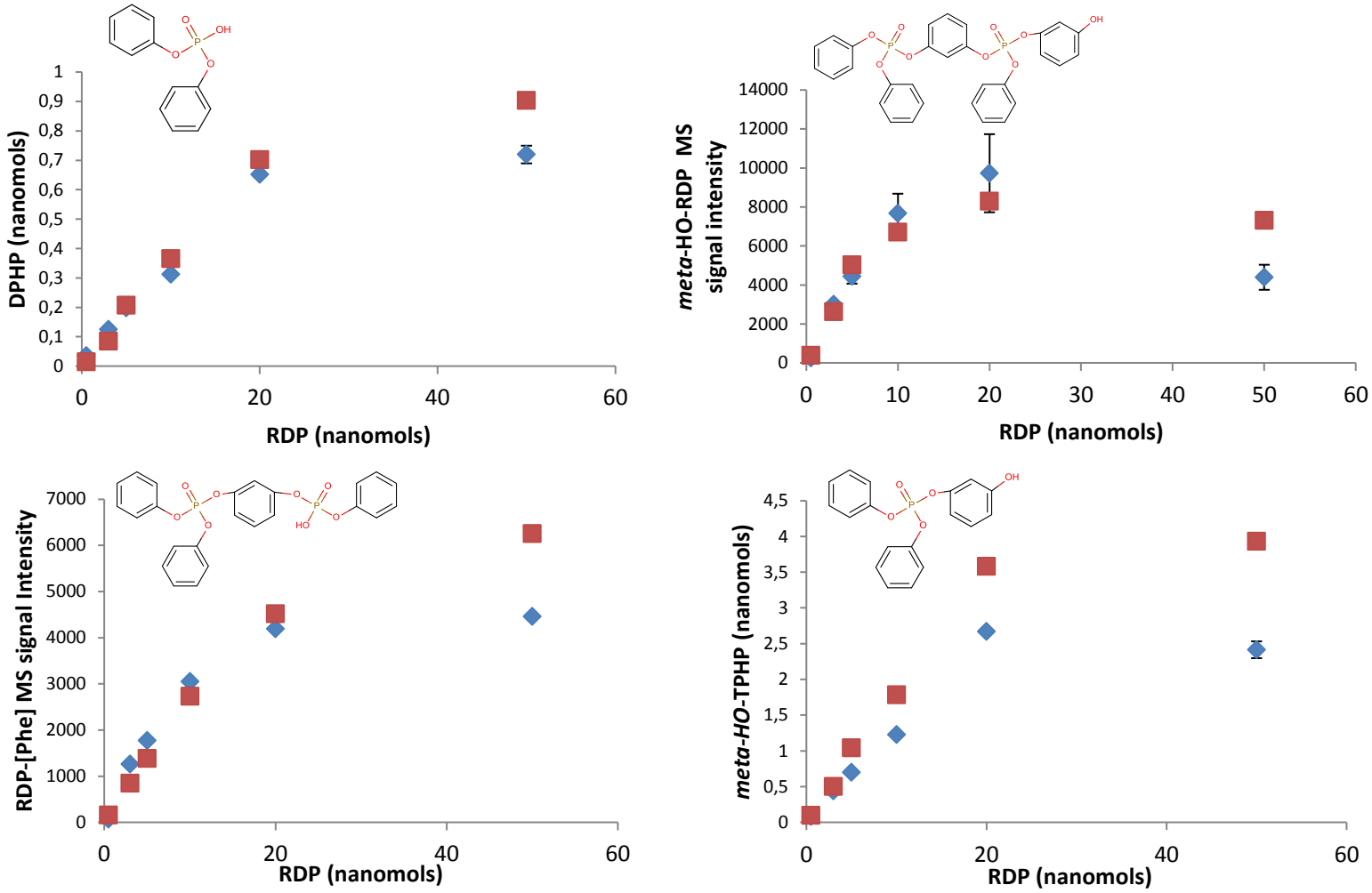

Figure S-2. Formation of hydroxy metabolites by changing influential factors (HLM concentration, time and substrate concentration). The y-axis shows the MS signal intensity (average±standard deviation of three independent experiments). Time incubation experiments were done with 0.3 mg/mL HLM and 20 nanomols of RDP, the HLM experiments were done at 15 min incubation time and with 20 nanomols of HLM and the experiments concerning the amount of RDP (nanomols of RDP) were done with 0.1 mg/mL of HLM and 15 min incubation time

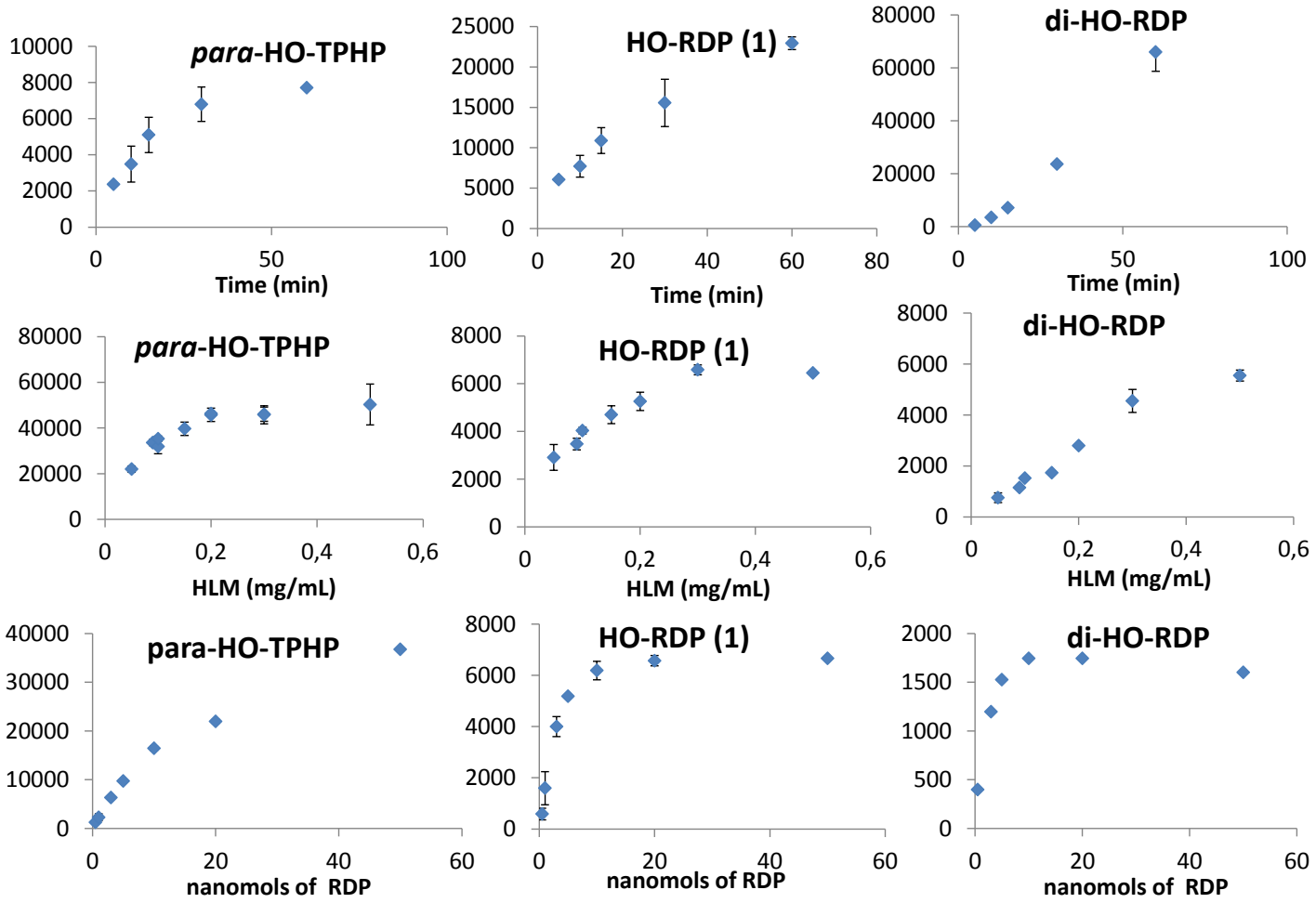

Figure S-3. Apparent recovery of some metabolites and compounds of interest with different sample preparation procedures. The apparent recovery is expressed as MS signal abundance adjusted to a percentage of the acetonitrile method workup (considered as 100%). Values are expressed as an average of three independent experiments and bars shows standard deviations. Abbreviations: methyl-tert-butylether, MTBE; solid-phase extraction; diphenyl phosphate, DPHP; triphenyl phosphate, TPHP; phenyl, [Phe]; RDP dimer, RDPn2

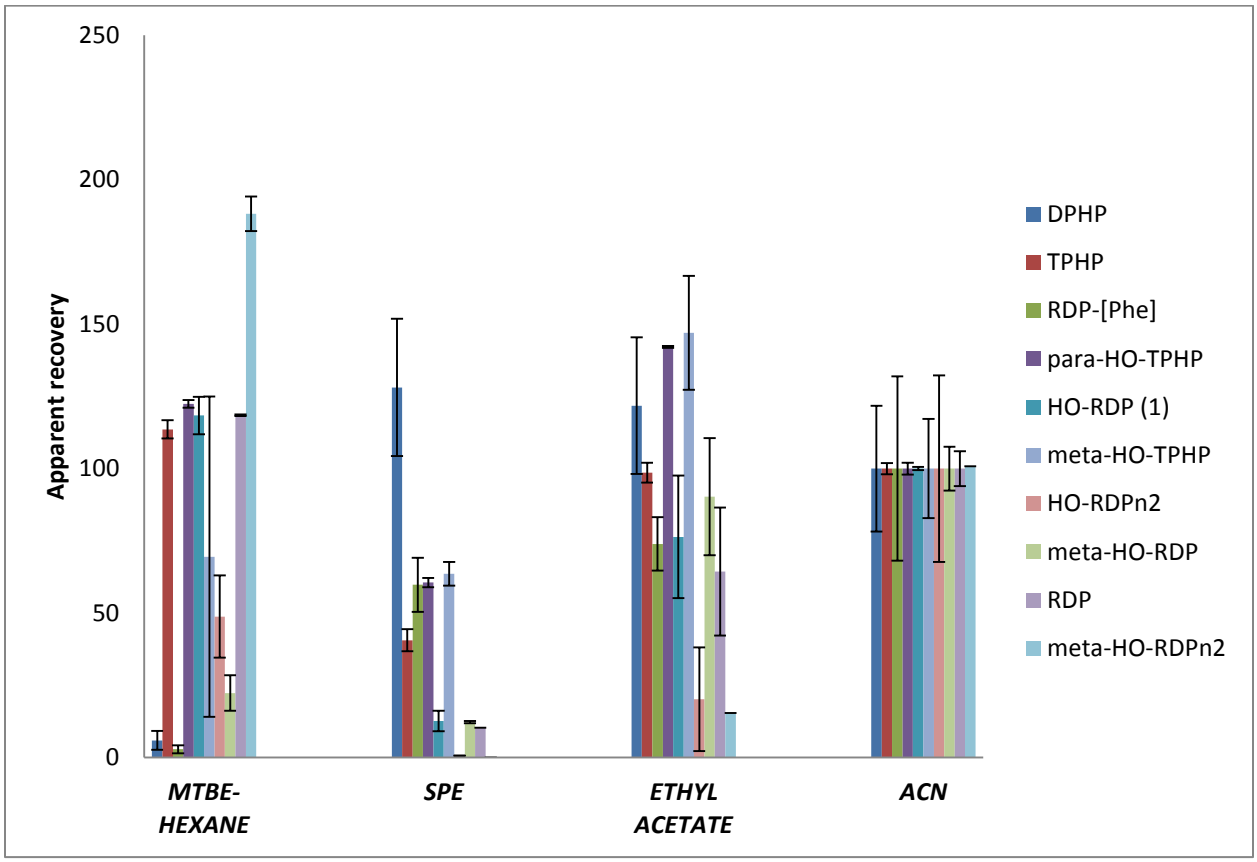

Figure S-4. Extracted ion chromatograms (LC-QQQ-ESI-) of the hydroxy metabolites and the hydrolysis products or impurities of RDP

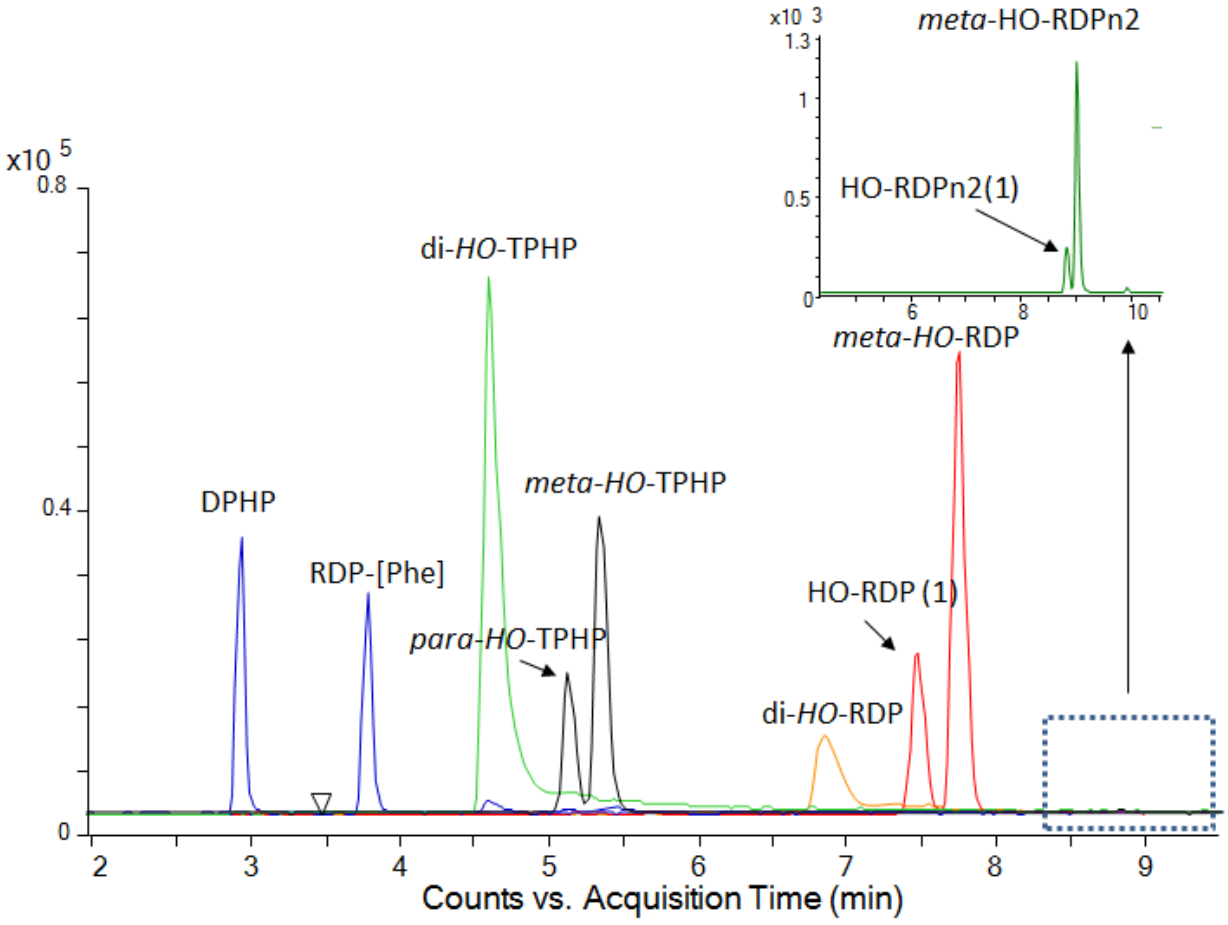

Figure S-5. Extracted ion chromatograms (LC-QQQ-ESI-) of the sulfated and glucuronidated metabolites of RDP