

# Diagnostic Fragmentation Pathways for Identification of Phthalate Metabolites in Non-Targeted Analysis Studies

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Table S1: Prioritized candidate molecular ions in the pooled urine spiking Level-0

Precursor ion ( <i>m/z</i> )	Peak	RT (min)	EIC ion ( <i>m/z</i> )	Major fragment ions/confirmed compounds	Classification	Calculated formula	Calculated <i>m/z</i>	Difference (ppm)	DBE	Score
193.0497	#1	4	121			C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0506	4.8	6	95.85
283.0269				C <sub>15</sub> H <sub>18</sub> O <sub>6</sub>		283.0248	-7.35	12	86.33	
285.0407				C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>		285.0405	-0.83	11	99.79	
297.042				C <sub>16</sub> H <sub>10</sub> O <sub>6</sub>		297.0405	-5.16	12	92.32	
223.0601*	#2*	4.12	121, 165	179,135, 119, 107, 71, 59		C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	4.9	6	94.89
223.0603	#3	4.17	121			C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	4	6	96.52
297.0413				C <sub>16</sub> H <sub>10</sub> O <sub>6</sub>		297.0405	-2.81	12	97.58	
255.0654	#4	4.42	121			C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	255.0663	3.45	10	96.96
343.0845				C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>		343.0823	-6.32	11	87.43	
345.081				C <sub>14</sub> H <sub>18</sub> O <sub>10</sub>		345.0827	4.97	6	91.73	
271.0594*				#6*		4.95	165,121	253, 227, 165.0191, 151, 137, 121.0275		C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>
263.0582	#7	5.04	121			C <sub>13</sub> H <sub>12</sub> O <sub>6</sub>	263.0561	-7.91	8	85.59
359.1157				C <sub>18</sub> H <sub>20</sub> O <sub>7</sub>		359.1136	-5.76	10	88.86	
263.1273*	#8*	5.52	121, 165	219, 165, 93		C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	263.1289	5.99	6	91.1
297.1118*				253, 189, 165.0541, 145, 121.0661, 107		C <sub>18</sub> H <sub>18</sub> O <sub>4</sub>	297.1132	4.81	10	93.27
343.1183*				297, 253, 165.0518, 121.0649, 107		C <sub>19</sub> H <sub>20</sub> O <sub>6</sub>	343.1187	1.2	10	99.48
<b>221.0808</b>	#9*	5.84	121,147	Monoisobutyl phthalate (MiBP) (DTXSID5052701)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	5.1	6	94.54
249.1117*				231, 187, 169, 159, 105		C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>	249.1132	6.13	6	91.25
<b>221.0804</b>	#10	6.12	121,147	Monobutyl phthalate (MBP) (DTXSID4040002)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	6.9	6	90.47
341.1037	#14	7.07	121			C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	341.1031	-1.87	11	98.75
349.1995				C <sub>20</sub> H <sub>30</sub> O <sub>5</sub>		349.202	7.27	6	90.81	
279.1217	#17	7.82	121			C <sub>15</sub> H <sub>20</sub> O <sub>5</sub>	279.1238	7.49	6	91.73
307.1178*	#20*	8.97	121,165	292, 263, 248, 195, 180, 165.0207, 137,		C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	2.96	7	97.23
263.1281*				248, 233, 219, 186, 161, 134, 74		C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	263.1289	2.96	6	97.65
265.107	#23	9.8	121			C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	265.1081	4.31	6	97.2
<b>277.143</b>	#25	10.34	121,147*	Mono(2-ethylhexyl) phthalate (MEHP) (DTXSID2025680)	Group I	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	277.1445	5.51	6	95.35

**Red italic bold:** confirmed as phthalate metabolites

\*: Unknown ions in the peak

The screening mass range reported in this table is from *m/z* 175 to *m/z* 360.

Table S2: Prioritized candidate molecular ions in the pooled urine spiking Level-I

Precursor ion (m/z)	Peak	RT (min)	EIC ion (m/z)	Confirmed compounds	Classification	Calculated formula	Calculated m/z	Difference (ppm)	DBE	Score
193.0497	#1	3.99	121			C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0506	4.8	6	95.85
283.027						C <sub>13</sub> H <sub>8</sub> O <sub>6</sub>	283.0248	-7.7	12	85.22
285.0403						C <sub>13</sub> H <sub>10</sub> O <sub>6</sub>	285.0405	0.56	11	99.9
297.0421						C <sub>16</sub> H <sub>10</sub> O <sub>6</sub>	297.0405	-5.5	12	91.39
223.0603*	#2*	4.11	121, 165			C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	4	6	96.52
223.0623	#3	4.19	121, 165	Mono-3-hydroxybutyl phthalate (3-OH-MBP) (DTXSID601027746) Mono(3-carboxypropyl)phthalate (MCP) (DTXSID8052726)	Group IV	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	-4.92	6	94.83
<b>237.0755</b>						C <sub>12</sub> H <sub>14</sub> O <sub>5</sub>	237.0768	5.66	6	92.82
<b>251.0558</b>						C <sub>12</sub> H <sub>12</sub> O <sub>6</sub>	251.0561	1.24	7	99.6
297.0232						C <sub>12</sub> H <sub>10</sub> O <sub>9</sub>	297.0252	6.73	8	87.7
<b>193.0495</b>	#4	4.43	121, 147	Monoethyl phthalate (MEP) (DTXSID3052696)	Group I	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0506	5.84	6	94
255.0653						C <sub>13</sub> H <sub>12</sub> O <sub>4</sub>	255.0663	3.84	10	96.26
343.0843						C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>	343.0823	-5.74	11	89.37
345.083						C <sub>14</sub> H <sub>18</sub> O <sub>10</sub>	345.0827	-0.81	6	99.76
<b>207.065</b>	#5	4.83	121, 147	Monoisopropyl phthalate (MiPP) (DTXSID90885606)	Group I	C <sub>11</sub> H <sub>12</sub> O <sub>4</sub>	207.0663	6.16	6	92.79
271.0609*	#6*	4.93	121, 165			C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	271.0612	1.09	10	99.66
263.0587	#7	5.04	121			C <sub>13</sub> H <sub>12</sub> O <sub>6</sub>	263.0561	-9.8	8	79.76
359.1151						C <sub>19</sub> H <sub>20</sub> O <sub>7</sub>	359.1136	-4.09	10	94
297.1117*						C <sub>18</sub> H <sub>18</sub> O <sub>4</sub>	297.1132	5.14	10	92.38
343.1162*	#8*	5.52	121, 165			C <sub>19</sub> H <sub>20</sub> O <sub>6</sub>	343.1187	7.3	10	84.01
<b>221.0819</b>	#9*	5.87	121, 147	Monoisobutyl phthalate (MiBP) (DTXSID5052701)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	0.15	6	100
249.1120*						C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>	249.1132	4.93	6	94.14
<b>221.0821</b>	#10	6.12	121, 147	Mono-n-butyl phthalate (MBP) (DTXSID4040002)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	-0.75	6	99.87
<b>293.1494</b>	#11	6.36	121, 147	Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) (DTXSID50873160)	Group I	C <sub>18</sub> H <sub>18</sub> O <sub>4</sub>	297.1132	4.81	10	93.27
<b>307.1165</b>	#12	6.59	121, 165	Mono-2-ethyl-5-carboxypentyl phthalate (MECPP) (DTXSID20873152)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	7.18	7	91.78
349.1989						C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	349.1989	8.99	6	86.71
<b>307.1187</b>	#13	6.94	121, 147, 165	Mono-7-carboxy-n-heptyl phthalate (MCHP)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	0.04	7	100
<b>291.124</b>	#14	7.08	121, 147	Mono-2-ethyl-5-oxohexyl phthalate (MEOHP) (DTXSID00865994)	Group III	C <sub>16</sub> H <sub>20</sub> O <sub>5</sub>	291.1238	-0.69	7	99.85
341.1034						C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	341.1031	-0.99	11	99.82
349.1994						C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>	349.202	7.56	6	90.16
<b>307.1178</b>	#15	7.21	165, 121	Mono-2-carboxy-methylhexyl phthalate (MCMHP) (DTXSID701003038)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	2.96	7	98.49
<b>255.0645</b>	#16	7.5	147	Monobenzyl phthalate (MBzP) (DTXSID9043938)	Group I	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	255.0663	6.96	10	93.25
<b>235.0978</b>	#17	7.85	121, 147	Mono-n-pentyl phthalate (MPP) (DTXSID60179281)	Group I	C <sub>13</sub> H <sub>15</sub> O <sub>4</sub>	235.0976	-0.92	6	99.8
<b>247.0977</b>	#18	8.06	121, 147	Monocyclohexyl phthalate (MCyHP) (DTXSID3052729)	Group I	C <sub>14</sub> H <sub>16</sub> O <sub>4</sub>	247.0976	-0.47	7	99.94
<b>321.132</b>	#19	8.22	121, 165	Mono-1-methyl-7-carboxyheptyl phthalate (MMCHP)	Group II	C <sub>17</sub> H <sub>22</sub> O <sub>6</sub>	321.1344	7.33	7	84.77
307.1167*	#20*	8.96	121, 165			C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	6.53	7	87.95
<b>249.1132</b>	#21	9.46	121, 147	Monoethyl phthalate (MHP) (DTXSID40947420)	Group I	C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>	249.1132	0.13	6	100
<b>335.1512</b>	#22	9.6	121, 147, 165	Monocarboxy-n-nonyl phthalate (MCNP)	Group II	C <sub>18</sub> H <sub>24</sub> O <sub>6</sub>	335.15	-3.53	7	95.74
265.1066	#23	9.8	121			C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	265.1081	5.81	6	95.03
<b>263.1292</b>	#24	10.05	121, 147	Monoheptyl phthalate (MHepP) (DTXSID00947421)	Group I	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	263.1289	-1.2	6	99.61
<b>277.1451</b>	#25	10.33	121, 147	Mono(2-ethylhexyl) phthalate (MEHP) (DTXSID2025680)	Group I	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	277.1445	-2.04	6	98.8
291.121	#26	10.49	121			C <sub>16</sub> H <sub>20</sub> O <sub>5</sub>	291.1238	9.58	7	86.85
<b>291.1598</b>	#27	10.71	121, 147	Monoisononyl phthalate (MiNP) (DTXSID80910057) Mono-n-octyl phthalate (MOP) (DTXSID4052714)	Group I	C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	291.1602	1.31	6	99.48
<b>277.1451</b>						C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	277.1445	-2.04	6	98.8
<b>305.1757</b>	#28	11.81	121, 147	Mono-3,7-dimethyl-1-octyl phthalate (MiDP) (DTXSID70781536)	Group I	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	305.1758	0.43	6	99.94
333.2042						C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	333.2071	8.78	6	87.61

**Red italic bold:** confirmed as phthalate metabolites

\*: Unknown ions in the peak

The screening mass range reported in this table is from m/z 175 to m/z 360.

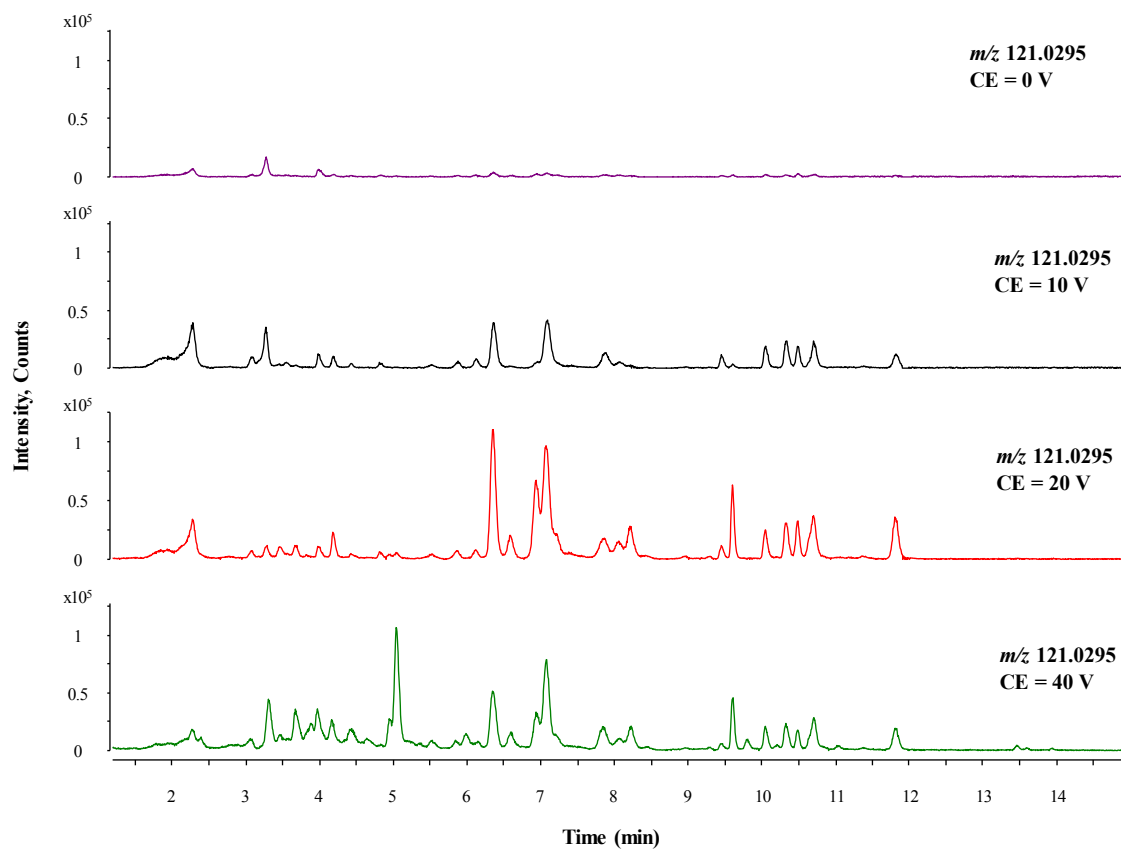
Table S3: Prioritized candidate molecular ions in the pooled urine spiking Level-II

Precursor ion (m/z)	Peak	RT (min)	EIC ion	Confirmed compounds	Classification	Calculated formula	Calculated m/z	Difference (ppm)	DBE	Score
193.0503	#1	3.99	121			C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0506	1.71	6	99.45
283.0276				C <sub>15</sub> H <sub>18</sub> O <sub>6</sub>		283.0248	-9.82	12	78.43	
285.037				C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>		285.0405	12.1	11	71.29	
297.0422				C <sub>16</sub> H <sub>10</sub> O <sub>6</sub>		297.0405	-5.83	12	90.43	
223.0609*	#2*	4.12	121,165		Group I	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	1.33	6	99.61
251.0576				C <sub>12</sub> H <sub>12</sub> O <sub>6</sub>		251.0561	-5.9	7	91.75	
223.0623	<b>237.0763</b>	4.19	121,165		121,165	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0612	-1.8	6	99.28
				Mono-3-hydroxybutyl phthalate (3-OH-MBP) (DTXSID601027746)		C <sub>12</sub> H <sub>14</sub> O <sub>5</sub>	237.0768	2.3	6	98.73
<b>251.056</b>				Mono(3-carboxypropyl)phthalate (MCPP) (DTXSID8052726)		C <sub>12</sub> H <sub>12</sub> O <sub>6</sub>	251.0561	0.44	7	99.95
<b>193.0504</b>	#4	4.43	121,147	Monoethyl phthalate (MEP) (DTXSID3052696)	Group I	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0506	1.2	6	99.73
255.0661				C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>		255.0663	0.71	10	99.87	
343.0851				C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>		343.0823	-8.06	11	81.3	
345.0846				C <sub>14</sub> H <sub>18</sub> O <sub>10</sub>		345.0827	-5.43	6	90.3	
<b>207.066</b>	#5	4.83	121,147	Monoisopropyl phthalate (MiPP) (DTXSID90885606)	Group I	C <sub>11</sub> H <sub>12</sub> O <sub>4</sub>	207.0663	1.36	6	99.62
271.0603*	#6*	4.94	121,165			C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	271.0612	3.3	10	97.01
359.1165	#7	5.05	121			C <sub>19</sub> H <sub>20</sub> O <sub>7</sub>	359.1136	-7.98	10	80.93
297.1125*	#8*	5.52	121,165			C <sub>18</sub> H <sub>18</sub> O <sub>4</sub>	297.1132	2.46	10	98.14
343.1173*				C <sub>19</sub> H <sub>20</sub> O <sub>6</sub>		343.1187	4.1	10	94.23	
<b>221.0816</b>	#9*	5.87	121,147	Monoisobutyl phthalate (MiBP) (DTXSID5052701)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	1.5	6	99.5
249.1124*				C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>		249.1132	3.33	6	97.23	
<b>221.0816</b>	#10	6.12	121, 147	Mono-n-butyl phthalate (MBP) (DTXSID4040002)	Group I	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	221.0819	1.5	6	99.5
<b>293.1415</b>	#11	6.36	121,147	Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) (DTXSID50873160)	Group IV	C <sub>16</sub> H <sub>22</sub> O <sub>5</sub>	293.1394	-6.98	6	87.07
<b>307.1181</b>	#12	6.59	121,165	Mono-2-ethyl-5-carboxypentyl phthalate (MECPP) (DTXSID20873152)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	1.99	7	98.73
349.1993				C <sub>20</sub> H <sub>30</sub> O <sub>5</sub>		349.202	7.85	6	89.49	
<b>307.1187</b>	#13	6.94	121,147,165	Mono-7-carboxy-n-heptyl phthalate (MCHP)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	0.04	7	100
<b>291.1235</b>	#14	7.08	121,147	Mono-2-ethyl-5-oxohexyl phthalate (MEOHP) (DTXSID00865994)	Group III	C <sub>16</sub> H <sub>20</sub> O <sub>5</sub>	291.1238	1.02	7	99.68
341.1045				C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>		341.1031	-4.2	11	93.99	
<b>307.1185</b>	#15	7.21	165,121	Mono-2-carboxy-methylhexyl phthalate (MCMHP) (DTXSID701003038)	Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	0.69	7	99.85
<b>255.0658</b>	#16	7.5	147	Monobenzyl phthalate (MBzP) (DTXSID9043938)	Group I	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	255.0663	1.88	10	99.07
<b>235.0972</b>	#17	7.86	121,147	Mono-n-pentyl phthalate (MPP) (DTXSID60179281)	Group I	C <sub>13</sub> H <sub>16</sub> O <sub>4</sub>	235.0976	1.62	6	99.37
<b>247.0976</b>	#18	8.06	121,147	Monocyclohexyl phthalate (MCyHP) (DTXSID3052729)	Group I	C <sub>14</sub> H <sub>16</sub> O <sub>4</sub>	247.0976	-0.07	7	100
291.1593	#19	8.22	121,165		Group II	C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	291.1602	3.02	6	98.49
<b>321.1342</b>				Mono-1-methyl-7-carboxyheptyl phthalate (MMCHP)		C <sub>17</sub> H <sub>21</sub> O <sub>6</sub>	321.1344	0.5	7	99.91
307.1183*	#20*	8.96	121,165		Group II	C <sub>16</sub> H <sub>20</sub> O <sub>6</sub>	307.1187	1.34	7	99.42
263.1287*				C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>		263.1289	0.69	6	99.87	
<b>249.1129</b>	#21	9.46	121,147	Monoethyl phthalate (MHP) (DTXSID40947420)	Group I	C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>	249.1132	1.33	6	99.55
<b>335.1495</b>	#22	9.6	121,147,165	Monocarboxy-n-nonyl phthalate (MCNP)	Group II	C <sub>18</sub> H <sub>24</sub> O <sub>6</sub>	335.15	1.52	7	99.18
265.1074	#23	9.8	121			C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	265.1081	2.81	6	98.79
<b>263.129</b>	#24	10.05	121,147	Monoheptyl phthalate (MHepP) (DTXSID00947421)	Group I	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	263.1289	-0.44	6	99.95
<b>277.1441</b>	#25	10.33	121,147	Mono(2-ethylhexyl) phthalate (MEHP) (DTXSID2025680)	Group I	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	277.1445	1.56	6	99.3
291.1227	#26	10.49	121			C <sub>16</sub> H <sub>20</sub> O <sub>5</sub>	291.1238	3.76	7	95.85
<b>277.145</b>	#27	10.71	121,147	Mono-n-octyl phthalate (MOP) (DTXSID4052714)	Group I	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	277.1445	-1.68	6	99.19
<b>291.1594</b>				Monoisononyl phthalate (MiNP) (DTXSID80910057)		C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	291.1602	2.68	6	97.84
<b>305.1756</b>	#28	11.82	121,147	Mono-3,7-dimethyl-1-octyl phthalate (MiDP) (DTXSID70781536)	Group I	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	305.1758	0.76	6	99.81
333.2048				C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>		333.2071	6.98	6	91.73	

**Red italic bold:** confirmed as phthalate metabolites

\*: Unknown ions in the peak

The screening mass range reported in this table is from m/z 175 to m/z 360.



**Figure S1.** The TIC and EICs of  $m/z$  121.0295 from the DIA data of the pooled urine sample spiked with 100 ppb of 24 metabolite standards at different CEs. A: TIC Scan (CE=0V); B: EIC at  $m/z$  121.0295, CE=10V; C: EIC at  $m/z$  121.0295, CE=20V; D: EIC at  $m/z$  121.0295, CE=40V.