

# On-line two-dimensional liquid chromatography hyphenated to mass spectrometry and ion mobility-mass spectrometry for the separation of carbohydrates from lignocellulosic biomass

Carole Reymond<sup>a</sup>, Agnès Le Masle<sup>a,\*</sup>, Cyril Colas<sup>b,c</sup>, Nadège Charon<sup>a</sup>

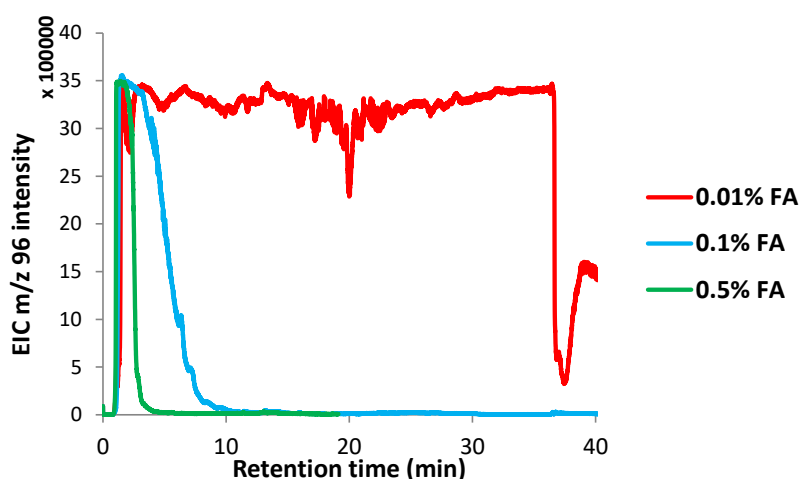
<sup>a</sup> IFP Energies nouvelles, Rond-point de l'échangeur de Solaize, BP 3, 69360 Solaize, France

<sup>b</sup> Institut de Chimie Organique et Analytique, Université d'Orléans, CNRS UMR 7311, Rue de Chartres, 45067 Orléans, France

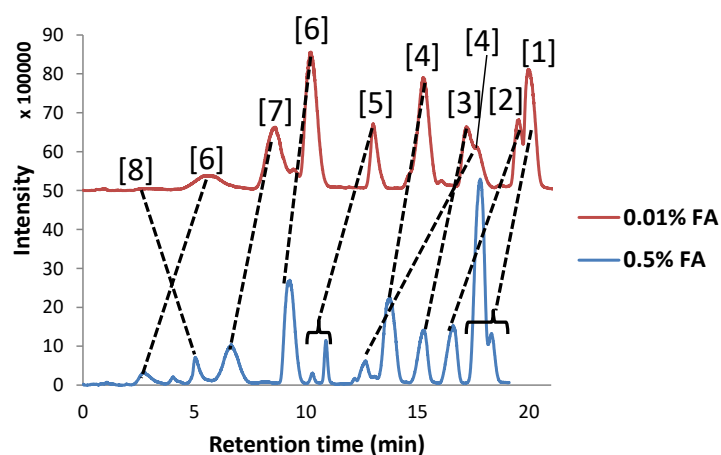
<sup>c</sup> Centre de Biophysique Moléculaire, CNRS UPR 4301, Université d'Orléans, rue Charles Sadron, 45071 Orléans, France

\*Corresponding author. E-mail address: [agnes.le-masle@ifpen.fr](mailto:agnes.le-masle@ifpen.fr) (A. Le Masle)

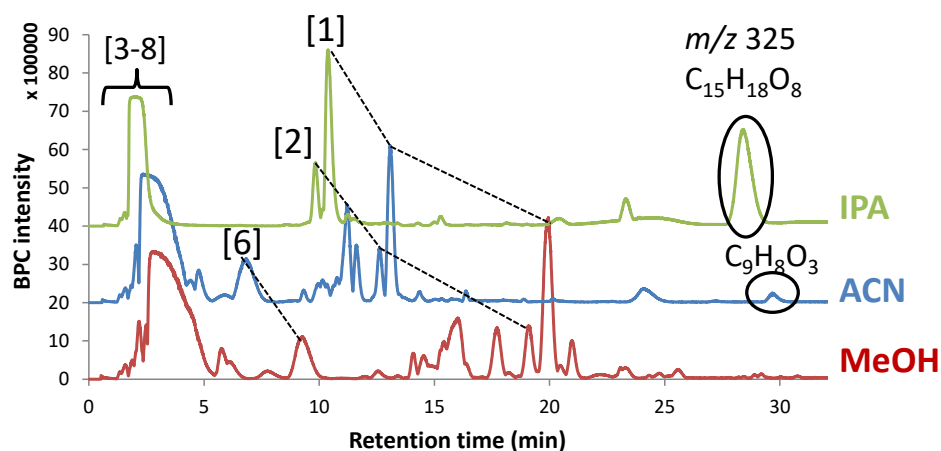
**Figure S1:** Figure 3: EIC of sulfuric acid ( $\pm 0.005$ ) with the Hypercarb column for three percentages of formic acid in the mobile phases



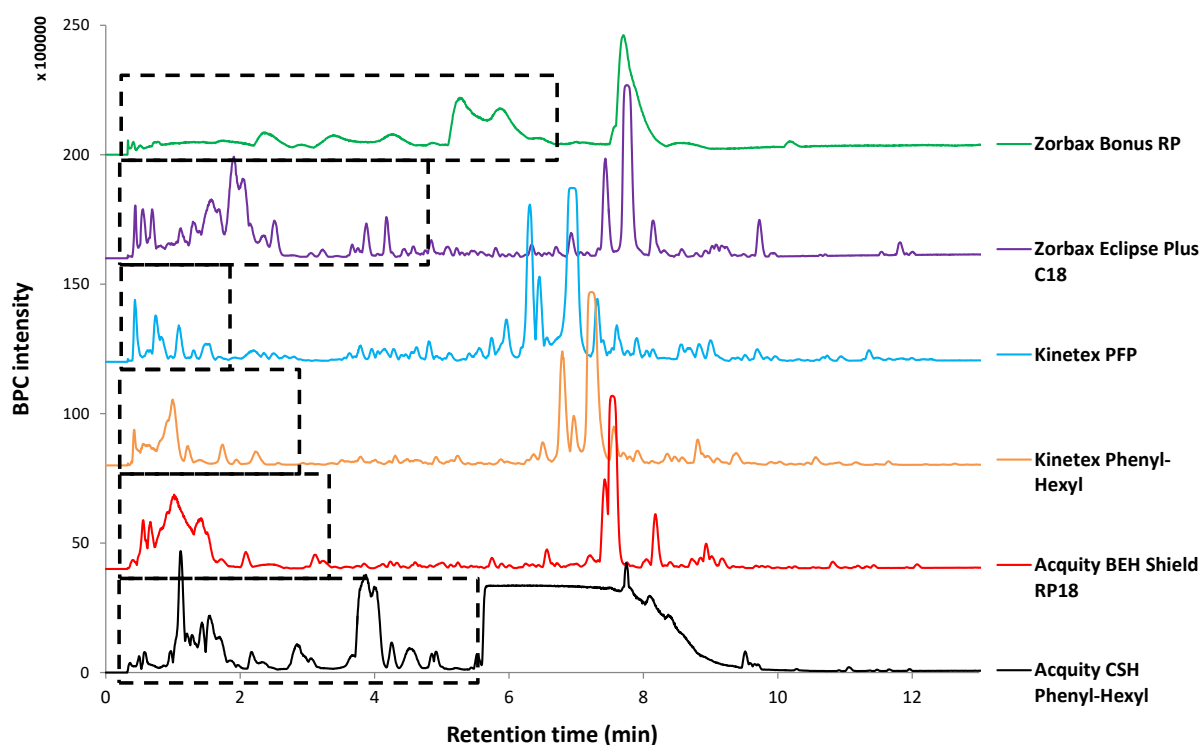
**Figure S2:** EIC of the eight carbohydrates in Table 1 with 0.01% or 0.5% of formic acid in the mobile phases



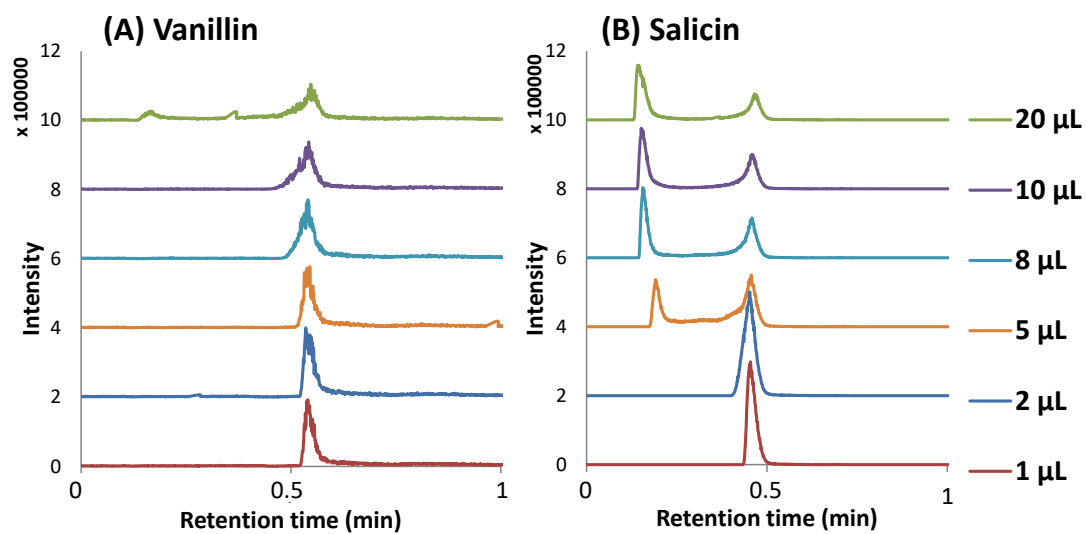
**Figure S3:** BPC between  $m/z$  50-1000 in (-)ESI of the biomass sample using MeOH, ACN or IPA as the organic mobile phase and the Hypercarb column



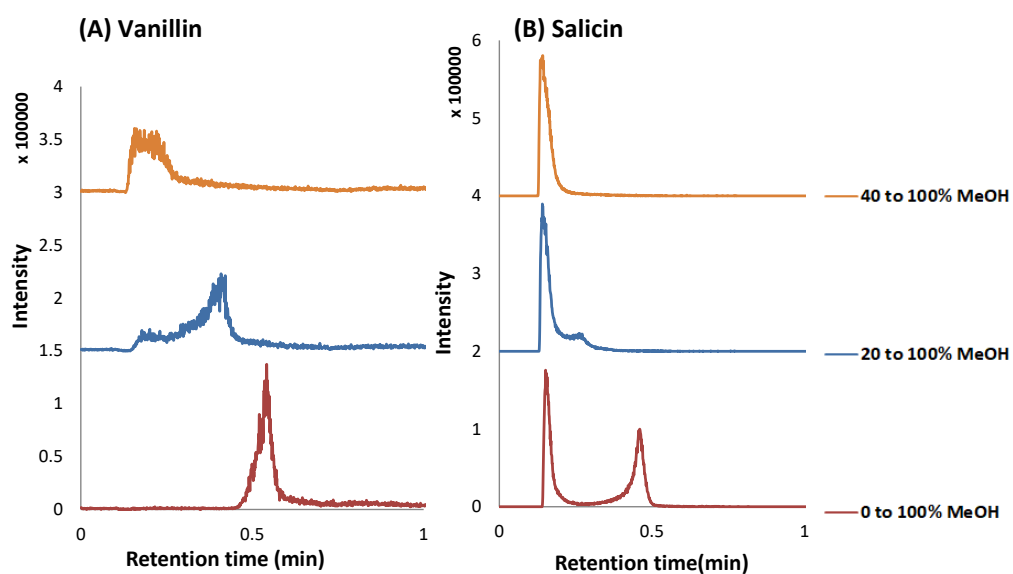
**Figure S4:** BPC between  $m/z$  50-1000 in (-)ESI for the biomass sample with six silica-based columns (water/MeOH gradient + 0.01% of FA). Black boxes correspond to elution windows of analytes of interest



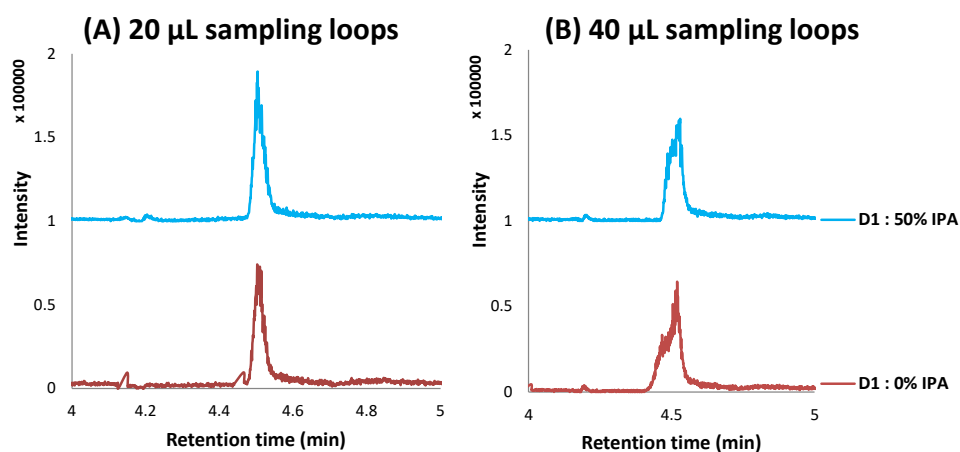
**Figure S5:** EIC of (A) vanillin and (B) salicin prepared in 50/50 water/IPA (v/v) for several injection volumes (D2 gradient: from 0 to 100% of MeOH in 1 min at 1.25 mL/min)



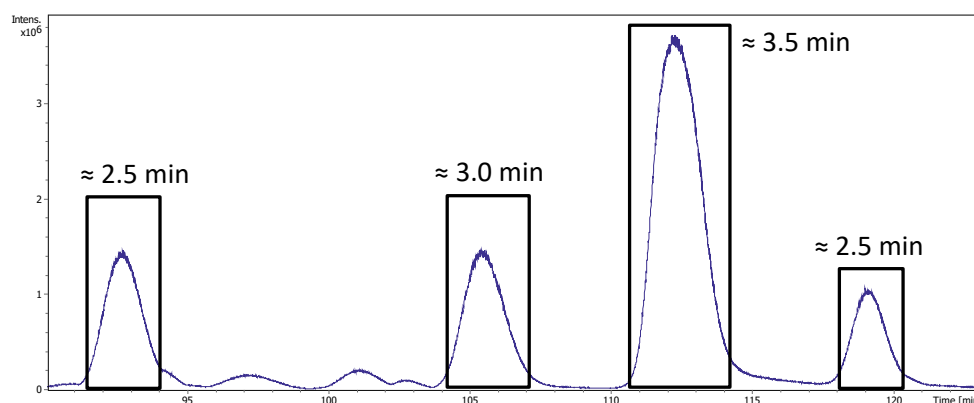
**Figure S6:** EIC of (A) vanillin and (B) salicin prepared in 50/50 water/IPA (v/v) for several initial %MeOH in the second dimension ( $V_{inj}$  10  $\mu\text{L}$ )



**Figure S7:** EIC of vanillin in (-)ESI for several D1 mobile phase compositions using (A) 20  $\mu\text{L}$  and (B) 40  $\mu\text{L}$  sampling loops ( $V_{\text{inj}}$  D1 2  $\mu\text{L}$ , D1 flow rate 10  $\mu\text{L}/\text{min}$ , modulation time 1 min, D2 gradient from 0 to 100% MeOH)



**Figure S8:** BPC from  $m/z$  50 to 1000 of the biomass sample analyzed under optimized first-dimensional conditions



**Figure S9:** EIC of vanillin in (-)ESI using a sampling rate of 48 s (D1: 50% IPA at 10  $\mu\text{L}/\text{min}$ , 20  $\mu\text{L}$  sampling loops, D2: gradient from 0 to 100% ACN at 2  $\text{mL}/\text{min}$ )

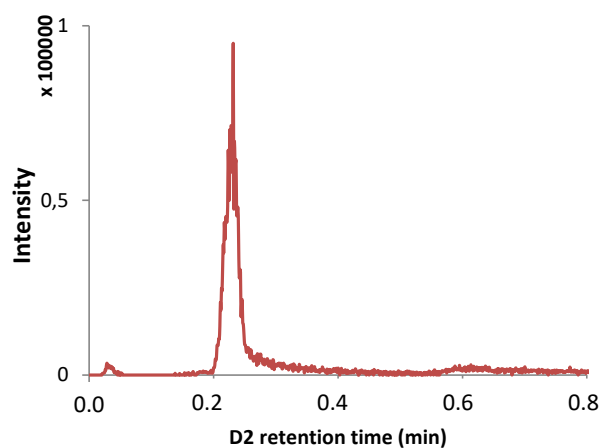


Figure S10: Labeled peaks in the window of interest

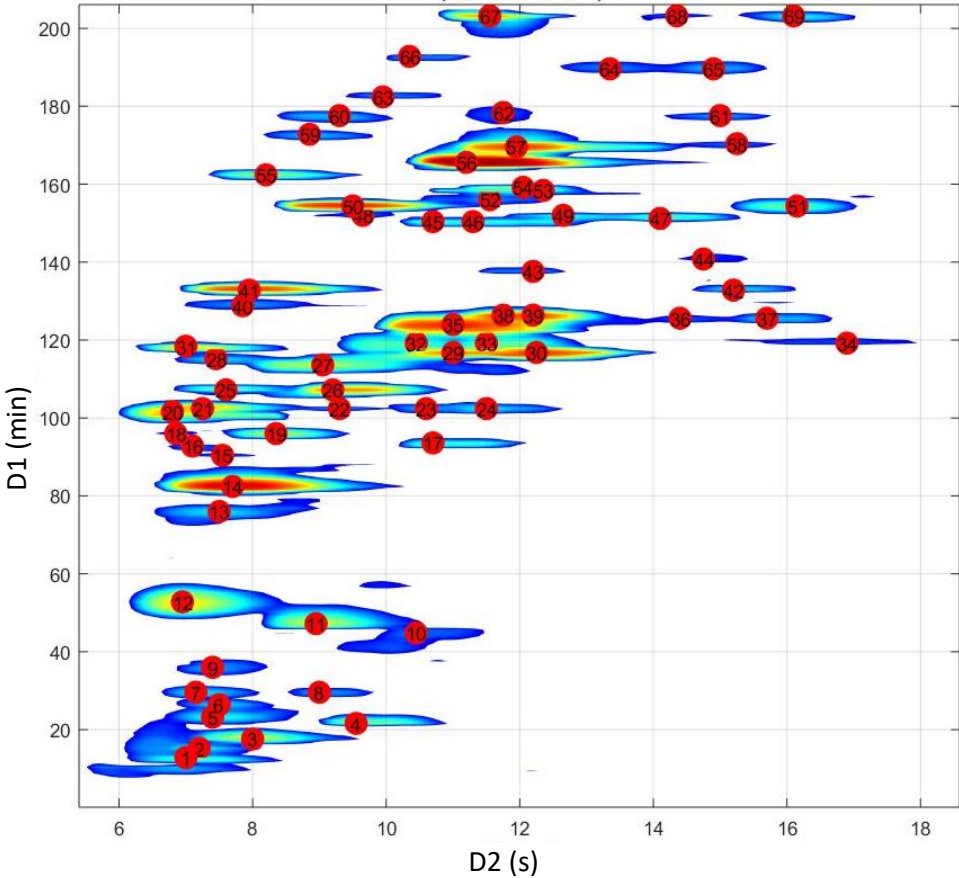
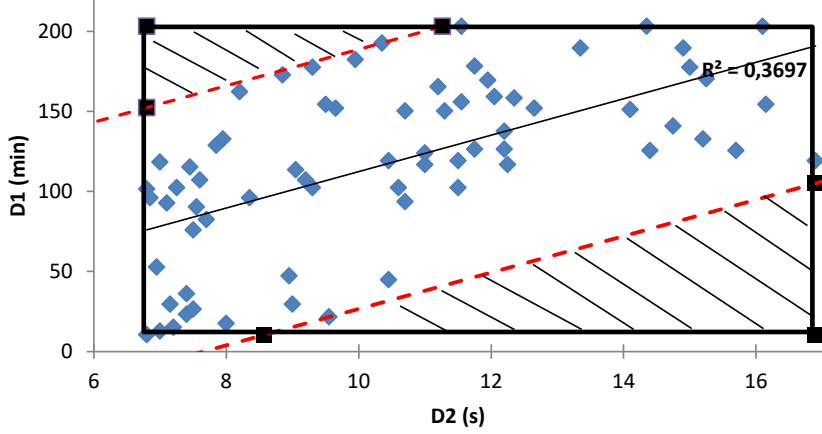
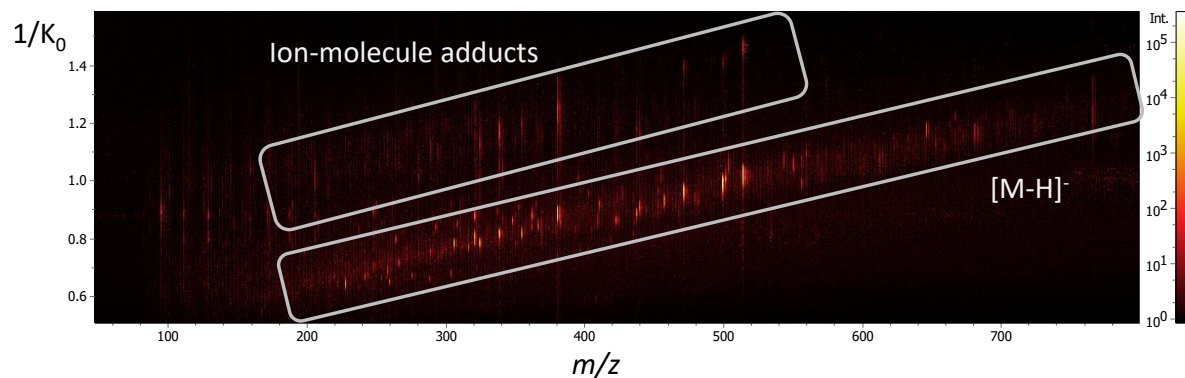


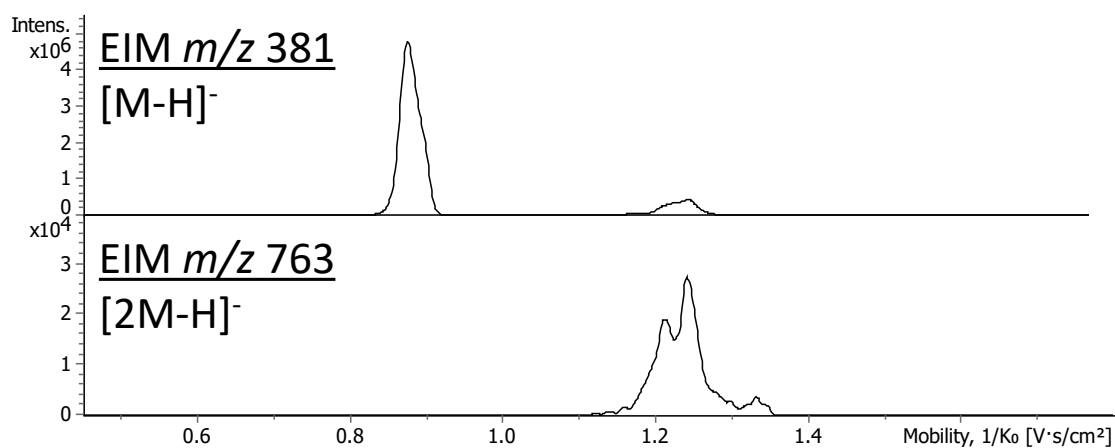
Figure S11: Methodology to measure the occupation of the separation space



**Figure S12:**  $1/K_0$  as a function of  $m/z$  for the elution window of interest (D1: 0-205 min)



**Figure S13:** EIM of  $m/z$  381 and 763



**Table S1:** Retention times in D1 and D2 for labeled peaks in Figure S7. The main ion under each peak was identified and the DBE was calculated.

ID	D1 (min)	D2 (s)	$m/z$	Ion formula	DBE
1	12.8	7.00	179.0562	$C_6H_{11}O_6$	1
2	15.2	7.20	195.0511	$C_6H_{11}O_7$	1
3	17.6	8.00	133.0142	$C_4H_5O_5$	2
4	21.6	9.55	128.0375	$C_5H_6O_3N$	3
5	23.2	7.40	339.0936	$C_{12}H_{19}O_{11}$	3
6	26.4	7.50	207.5111	$C_7H_{11}O_7$	2
7	29.6	7.15	267.0720	$C_9H_{15}O_9$	2
8	29.6	9.00	207.5112	$C_7H_{11}O_7$	2
9	36.0	7.40	383.0834	$C_{13}H_{16}O_{13}$	4
10	44.8	10.45	381.1042	$C_{14}H_{21}O_{12}$	4
11	47.2	8.95	191.0199	$C_6H_7O_7$	3
12	52.8	6.95	325.0783	$C_{11}H_{17}O_{11}$	3
13	76.0	7.50	281.0882	$C_{10}H_{17}O_9$	2
14	82.4	7.70	339.0934	$C_{12}H_{19}O_{11}$	3
15	90.4	7.55	307.0675	$C_{11}H_{15}O_{10}$	4
16	92.8	7.10	341.1091	$C_{12}H_{21}O_{11}$	2

17	93.6	10.70	321.0831	C <sub>12</sub> H <sub>17</sub> O <sub>10</sub>	4
18	96.0	6.85	317.0881	C <sub>13</sub> H <sub>17</sub> O <sub>9</sub>	5
19	96.0	8.35	307.0674	C <sub>11</sub> H <sub>15</sub> O <sub>10</sub>	4
20	101.6	6.80	355.0880	C <sub>12</sub> H <sub>19</sub> O <sub>12</sub>	3
21	102.4	7.25	341.1091	C <sub>12</sub> H <sub>21</sub> O <sub>11</sub>	2
22	102.4	9.30	341.1091	C <sub>12</sub> H <sub>21</sub> O <sub>11</sub>	2
23	102.4	10.60	367.0882	C <sub>13</sub> H <sub>19</sub> O <sub>12</sub>	4
24	102.4	11.50	367.0885	C <sub>13</sub> H <sub>19</sub> O <sub>12</sub>	4
25	107.2	7.60	341.1091	C <sub>12</sub> H <sub>21</sub> O <sub>11</sub>	2
26	107.2	9.20	321.0830	C <sub>12</sub> H <sub>17</sub> O <sub>10</sub>	4
27	113.6	9.05	367.0884	C <sub>13</sub> H <sub>19</sub> O <sub>12</sub>	4
28	115.2	7.45	355.0875	C <sub>12</sub> H <sub>19</sub> O <sub>12</sub>	3
29	116.8	11.00	323.0987	C <sub>12</sub> H <sub>19</sub> O <sub>10</sub>	3
30	116.8	12.25	381.1040	C <sub>14</sub> H <sub>21</sub> O <sub>12</sub>	4
31	118.4	7.00	457.1205	C <sub>16</sub> H <sub>25</sub> O <sub>15</sub>	4
32	119.2	10.45	369.1042	C <sub>13</sub> H <sub>21</sub> O <sub>12</sub>	3
33	119.2	11.50	369.1040	C <sub>13</sub> H <sub>21</sub> O <sub>12</sub>	3
34	119.2	16.90	253.0719	C <sub>12</sub> H <sub>13</sub> O <sub>6</sub>	6
35	124.0	11.00	381.1041	C <sub>14</sub> H <sub>21</sub> O <sub>12</sub>	4
36	125.6	14.40	205.1130		
37	125.6	15.70	205.1129		
38	126.4	11.75	381.1041	C <sub>14</sub> H <sub>21</sub> O <sub>12</sub>	4
39	126.4	12.20	381.1042	C <sub>14</sub> H <sub>21</sub> O <sub>12</sub>	4
40	128.8	7.85	459.1350	C <sub>16</sub> H <sub>27</sub> O <sub>15</sub>	3
41	132.8	7.95	471.1489	C <sub>17</sub> H <sub>27</sub> O <sub>15</sub>	4
42	132.8	15.20	363.0935	C <sub>14</sub> H <sub>19</sub> O <sub>11</sub>	5
43	137.6	12.20	349.0780	C <sub>13</sub> H <sub>17</sub> O <sub>11</sub>	5
44	140.8	14.75	411.1145	C <sub>24</sub> H <sub>37</sub> O <sub>20</sub>	4
45	150.4	10.70	499.1304	C <sub>18</sub> H <sub>27</sub> O <sub>16</sub>	5
46	150.4	11.30	499.1302	C <sub>18</sub> H <sub>27</sub> O <sub>16</sub>	5
47	151.2	14.10	363.0936	C <sub>14</sub> H <sub>19</sub> O <sub>11</sub>	5
48	152.0	9.65	457.1201	C <sub>16</sub> H <sub>25</sub> O <sub>15</sub>	4
49	152.0	12.65	363.0938	C <sub>14</sub> H <sub>19</sub> O <sub>11</sub>	5
50	154.4	9.50	499.1304	C <sub>18</sub> H <sub>27</sub> O <sub>16</sub>	5
51	154.4	16.15	411.1146	C <sub>24</sub> H <sub>37</sub> O <sub>20</sub>	4
52	156.0	11.55	501.1463	C <sub>18</sub> H <sub>29</sub> O <sub>16</sub>	4
53	158.4	12.35	501.1463	C <sub>18</sub> H <sub>29</sub> O <sub>16</sub>	4
54	159.2	12.05	501.1463	C <sub>18</sub> H <sub>29</sub> O <sub>16</sub>	4
55	162.4	8.20	549.1675	C <sub>19</sub> H <sub>33</sub> O <sub>18</sub>	3
56	165.6	11.20	513.1455	C <sub>19</sub> H <sub>29</sub> O <sub>16</sub>	5
57	169.6	11.95	513.1458	C <sub>19</sub> H <sub>29</sub> O <sub>16</sub>	5
58	170.4	15.25	411.1147	C <sub>24</sub> H <sub>37</sub> O <sub>20</sub>	4
59	172.8	8.85	503.1617	C <sub>18</sub> H <sub>31</sub> O <sub>16</sub>	3
60	177.6	9.30	503.1620	C <sub>18</sub> H <sub>31</sub> O <sub>16</sub>	3
61	177.6	15.00	555.1570	C <sub>21</sub> H <sub>31</sub> O <sub>17</sub>	6
62	178.4	11.75	513.1459	C <sub>19</sub> H <sub>29</sub> O <sub>16</sub>	5
63	182.4	9.95	503.1619	C <sub>18</sub> H <sub>31</sub> O <sub>16</sub>	3
64	189.6	13.35	543.1567	C <sub>20</sub> H <sub>31</sub> O <sub>17</sub>	5
65	189.6	14.90	543.1569	C <sub>20</sub> H <sub>31</sub> O <sub>17</sub>	5
66	192.8	10.35	631.1730	C <sub>23</sub> H <sub>35</sub> O <sub>20</sub>	6
67	203.2	11.55	645.1883	C <sub>24</sub> H <sub>37</sub> O <sub>20</sub>	6
68	203.2	14.35	543.1568	C <sub>20</sub> H <sub>31</sub> O <sub>17</sub>	5
69	203.2	16.10	543.1568	C <sub>20</sub> H <sub>31</sub> O <sub>17</sub>	5