

# Supporting Information for

## Revealing Brown Carbon Chromophores Produced in Reactions of Methylglyoxal with Ammonium Sulfate

Peng Lin,<sup>a</sup> Julia Laskin,<sup>b</sup> Sergey A. Nizkorodov,<sup>c</sup> Alexander Laskin<sup>a,\*</sup>

<sup>a</sup> Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA.

<sup>b</sup> Physical Sciences Division, Pacific Northwest National Laboratory, Richland, WA.

<sup>c</sup> Department of Chemistry, University of California, Irvine, CA

Appendix I: Separation of BrC by six evaluated HPLC columns

Table S1. Summary of the HPLC columns examined in this study.

Table S2. Organic compounds detected in the MG +AS mixture. Their EICs were evaluated with different HPLC columns in this study.

Figure S1. Fractions of the overall HPLC/PDA signals integrated over the 250-450 nm range and detected at different elution periods. The plot compares the amount of light-absorbing material resolved by different columns during certain periods of the elution time.

Figure S2. Extracted ion chromatograms (EICs) of the 17 products produced from MG reacting with AS obtained from the LC-MS analysis by using different columns.

Figure S3. The effect of sample desalting on the performance of the SM-C18 column for separating BrC compounds produced in MG+AS mixtures. (a) Total ion chromatograms (TIC). Panels (b), (c) and (d) show integrated mass spectra of p1, p2 and p3 regions indicated in panel (a), respectively. The blue asterisks indicate salt adducts.

## 23 **Appendix I: Separation of BrC on different HPLC columns**

24 Reversed-phased chromatography (RPC) is the most common mode of HPLC due to its  
25 broad versatility for the separation of a wide range of analytes.<sup>1</sup> Among the stationary phases  
26 used by RPC, C18 (Octadecylsilane) is the most popular one.<sup>1</sup> The residual free silanol groups  
27 that remain on the surface of the silica gel in the traditional C18 column (e.g., C18 Luna) are  
28 endcapped with nonpolar trimethylsilyl groups (TMS) to eliminate some of their unwanted  
29 effects on the separation, such as peak tailing.<sup>2</sup> This type of stationary phase is usually chosen  
30 as the starting point for RPC method development,<sup>3</sup> and has shown its capability to resolve a  
31 number of nitro-aromatic BrC chromophores.<sup>4-7</sup> As shown in Figure 1a, a majority of the BrC  
32 chromophores from the MG + AS mixture eluted too quickly within the first 5 min, implying  
33 their higher polarity relative to the C18 sorbent. Some of the chromophores eluted between 5  
34 to 15 min, and nearly all of the chromophores eluted within 20 min (Figure S1). Similar elution  
35 behavior was observed for the C18 Fusion column (Figure 1b and Figure S1), in which a  
36 nitrogen-containing polar functional group is embedded within the alkyl chain. This polar  
37 embedding has been suggested to improve dipole-induced interactions of analyte molecules  
38 with the stationary phase.<sup>8</sup> However, our results suggest that it does not improve separation of  
39 BrC compounds in the MG+AS system. This phenomenon indicates that the benefits of polar  
40 embedding C18 stationary phase may be selective with respect to different classes of polar  
41 analytes.

42 For RPC, the elution gradient started with a high fraction of water (90%), and the sample  
43 was also dissolved in water. As a result, poor retention of analytes on the non-polar endcapped  
44 C18 columns may be caused by the poor wetting capability of the sorbent.<sup>8</sup> The C18 Hydro  
45 column was designed to modify the classical C18 chemistry with polar endcapping agent, which  
46 is hydrophilic and allows the silica surface to be wetted with water, facilitating stronger  
47 interactions of analytes with the C18 alkyl chains. This modification improves retention of polar  
48 analytes under highly aqueous conditions.<sup>3</sup> Figure 1c shows the retention behavior of BrC  
49 chromophores on the C18 Hydro column. Compared to the two C18 columns described earlier  
50 (traditional C18 and C18 Fusion), fewer chromophores eluted from the C18 Hydro column  
51 within the first 5 min. A substantial fraction of chromophores eluted after 10-min (Figure S1),  
52 indicating that the retention of some BrC chromophores has improved.

53 Figure 1d shows the retention behavior of BrC chromophores on the Biphenyl column,  
54 which utilizes biphenyl functional groups as the stationary phase favoring separation of  
55 aromatic compounds. As illustrated in Figure S1, similar retention of chromophores was  
56 observed using the Biphenyl and C18 Hydro columns in the first 5 min of the chromatographic  
57 run. Additional chromophores eluted between 5-10 min, but fewer chromophores eluted  
58 between 10-20 min in comparison with the C18 Hydro column. In addition to nonpolar-

59 nonpolar interaction, the biphenyl groups provide some special retention properties and  
60 selectivity to analyte molecules due to  $\pi$ - $\pi$  and steric recognition interactions.<sup>9</sup> The light  
61 absorbance of organic molecules depends on the extent of the double bonds and the  
62 conjugation of the  $\pi$  electrons in the system.<sup>10</sup> Consequently, the improved retention of the  
63 analyte molecules by the Biphenyl column indicates the presence of  $\pi$ - $\pi$  interactions between  
64 the BrC chromophores and biphenyl groups.

65 Over 95% of chromophores (with respect to the overall light absorbance) eluted within  
66 20 min, and a large portion of them eluted before 5 min on the four RPC columns discussed  
67 above. The observed poor retention of chromophores by these columns indicates that many of  
68 the chromophores are relatively polar and/or hydrophilic with respect to the RPC stationary  
69 phases. To overcome the problematic retention and separation of small hydrophilic polar  
70 compounds by conventional RPC, hydrophilic interaction liquid chromatography (HILIC) was  
71 developed and has been successfully used for the analysis of charged and neutral hydrophilic  
72 species.<sup>11, 12</sup> In this study, we examined the retention of BrC mixture on a HILIC column  
73 containing sulfoalkylbetaine zwitterions as the stationary phase. As shown in Figure 1e, BrC  
74 chromophores exhibit the poorest retention and separation on the HILIC column. Over 98% of  
75 the chromophores eluted within the first 10 min and the vast majority of them (~90%) eluted  
76 within 5 min (Figure S1), indicating that it is not easy to retain these analytes with this type of  
77 stationary phase. The retention mechanism of polar analytes on a HILIC phase is complex.<sup>13</sup> It  
78 requires the formation of an aqueous-rich layer adsorbed on the polar surface of the stationary  
79 phase. The retention mainly takes place via the hydrophilic partitioning of the analytes between  
80 the aqueous-rich layer and the bulk mobile phase.<sup>13</sup> It has been demonstrated that the  
81 acetonitrile content in the mobile phase is the largest factor affecting analyte retention.<sup>11</sup>  
82 Therefore, additional efforts may be required to improve the retention of BrC compounds on  
83 the HILIC column by optimizing the acetonitrile content in the mobile phase. There are also  
84 numerous stationary phases for HILIC column, so it may be worthwhile to evaluate the  
85 performance of different HILIC columns and find a more suitable one for separating MG/AS BrC.  
86 For example, good separation of polar compounds has been achieved with a HILIC column  
87 containing an amide stationary phase.<sup>14</sup> Another column that is worth to be considered is the  
88 Atlantis C18 T3 column (Waters, Milford, MA, USA), containing trifunctionally bonded C18 alkyl  
89 chains, which also readily separates low molecular weight polar analytes.<sup>15</sup>

90 The stationary phase of the SM-C18 column is composed of octadecyl functional groups  
91 with cation and anion ligands attached to the silica gel. This mixed mode stationary phase was  
92 designed for combining RPC with anion-exchange (AEX), and cation-exchange (CEX)  
93 chromatography. Such combination enables separation of both neutral and charged analytes by  
94 the same column.<sup>16</sup> As shown in Figure 1f, the SM-C18 column exhibited the best retention and  
95 separation among the six columns examined in this study. The analyzed constituents distributed

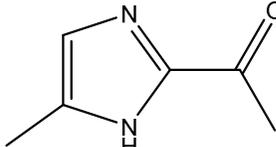
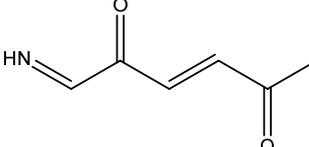
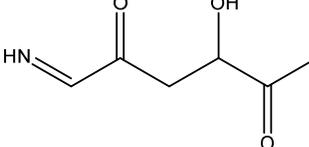
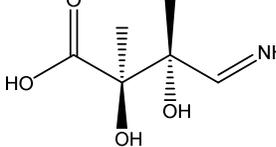
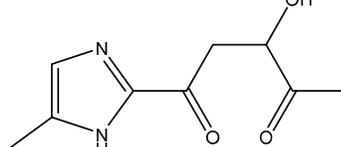
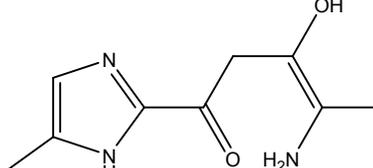
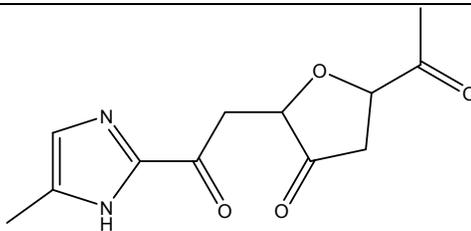
96 more evenly between 3 and 30 min of the RT on this column. Furthermore, ~7 % of  
97 chromophores eluted after 30 min indicating their strong affinity for the stationary phase  
98 (Figure S1).

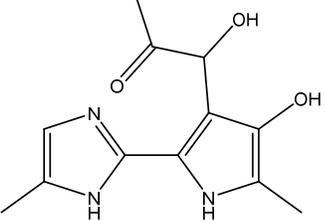
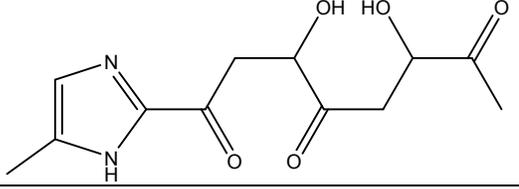
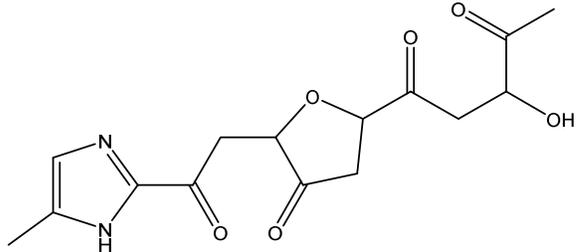
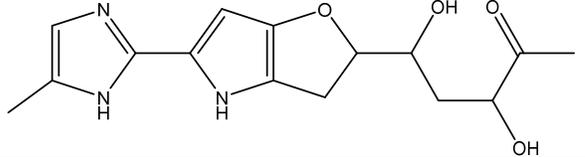
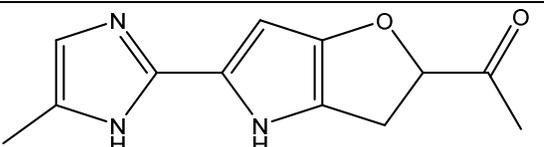
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Table S1. Description of the HPLC columns examined in this study.

column name used in this work	Column manufacture, part number, and parameters	Properties of stationary phase	Expected inteactions with analytes, or benefits for retention and selectivity
<b>C18 Luna</b>	Phenomenex Luna® C18(2), 00F-4252-B0, 100Å, 5µm, 150 × 2.0 mm	Octadecylsilane (C18) with nonpolar trimethylsilyl (TMS) endcapping, no free exposed silanols	Traditional reversed phase column Nonpolar-nonpolar interaction
<b>C18 Fusion</b>	Phenomenex Synergi® Fusion-RP, 00F-4424-B0, 80Å, 4µm, 150 × 2.0 mm	Octadecylsilane (C18) with nonpolar endcapping, polar embedded groups no free exposed silanols	Nonpolar-nonpolar interaction, Less hydrophobic than C18 Luna. Ion exchange or dipole interaction with nitrogen containing embedded groups
<b>C18 Hydro</b>	Phenomenex Synergi® Hydro-RP, 00F-4375-B0, 80Å, 4µm, 150 × 2.0 mm	Octadecylsilane (C18) with polar endcapping	Equivalent hydrophobicity with C18 Luna Enhanced hydrogen bonding for improved retention of polar analytes under highly aqueous conditions
<b>Biphenyl</b>	Phenomenex Kinetex® Biphenyl, 00F-4627-Y0, 100Å, 5µm, 150 × 2.0 mm	Biphenyl with nonpolar TMS endcapping	Nonpolar-nonpolar interaction; π-π and steric recognition interactions. Optimized for retention of aromatics and poly-conjugating systems
<b>HILIC</b>	Thermo Scientific Synchronis® HILIC, 97505-15230, 100Å, 5µm, 150 × 2.1 mm	sulfoalkylbetaine zwitterionic functionality	Hydrophilic partitioning, Hydrogen bonding, Electrostatic interactions <sup>13</sup>
<b>SM-C18</b>	Imtakt Scherzo® SM-C18, SM035, 130Å, 3µm, 150 × 3.0 mm	Octadecylsilane (C18) with anion and cation ligands	Reversed phase + anion exchange + cation exchange, Separation of basic, acidic and neutral analytes at neutral pH condition

Table S2. Organic compounds detected in the MG +AS mixture. Their EICs were evaluated with different HPLC columns in this study.

neutral formula	theoretical mass of [M+H] <sup>+</sup>	measured m/z	tentative structure	remarks & references
C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	109.0760	109.0758		<sup>c</sup> This study
C <sub>6</sub> H <sub>8</sub> ON <sub>2</sub>	125.0709	125.0707		<sup>b</sup> Galloway et al., 2009; Yu et al., 2011 <sup>17, 18</sup>
C <sub>6</sub> H <sub>7</sub> O <sub>2</sub> N	126.0549	126.0548		<sup>a</sup> Sareen et al., 2010 <sup>19</sup>
C <sub>6</sub> H <sub>9</sub> O <sub>3</sub> N	144.0655	144.0654		<sup>a</sup> Sareen et al., 2010 <sup>19</sup>
C <sub>6</sub> H <sub>11</sub> O <sub>4</sub> N	162.0761	162.0757		<sup>a</sup> Sareen et al., 2010 <sup>19</sup>
C <sub>9</sub> H <sub>12</sub> ON <sub>2</sub>	165.1022	165.1019		<sup>c</sup> This study
C <sub>9</sub> H <sub>12</sub> O <sub>3</sub> N <sub>2</sub>	197.0921	197.0916		<sup>b</sup> Sareen et al., 2010 <sup>19</sup>
C <sub>9</sub> H <sub>13</sub> O <sub>2</sub> N <sub>3</sub>	196.1081	196.1086		<sup>b</sup> Sareen et al., 2010 <sup>19</sup>
C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> N <sub>2</sub>	183.1128	183.1124		<sup>c</sup> This study
C <sub>12</sub> H <sub>14</sub> O <sub>4</sub> N <sub>2</sub>	251.1026	251.1019		<sup>b</sup> Sareen et al., 2010; Kampf et al., 2012 <sup>19, 20</sup>
C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	167.0703	167.0699		<sup>c</sup> This study
C <sub>9</sub> H <sub>11</sub> O <sub>2</sub> N	166.0863	166.0868		<sup>c</sup> This study

$C_{12}H_{16}O_3N_3$	250.1186	250.1192		<sup>b</sup> Bones et al., 2010; Amarnath et al., 1994 <sup>21, 22</sup>
$C_{12}H_{16}O_5N_2$	269.1132	269.1125		<sup>a</sup> Lee et al., 2015 <sup>23</sup>
$C_{15}H_{18}N_2O_6$	323.1238	323.1243		<sup>a</sup> Lee et al., 2015 <sup>23</sup>
$C_{15}H_{19}O_4N_3$	306.1448	306.1444		<sup>a</sup> Lee et al., 2015 <sup>23</sup>
$C_{12}H_{13}O_2N_3$	232.1080	232.1074		<sup>a</sup> Lee et al., 2015 <sup>23</sup>

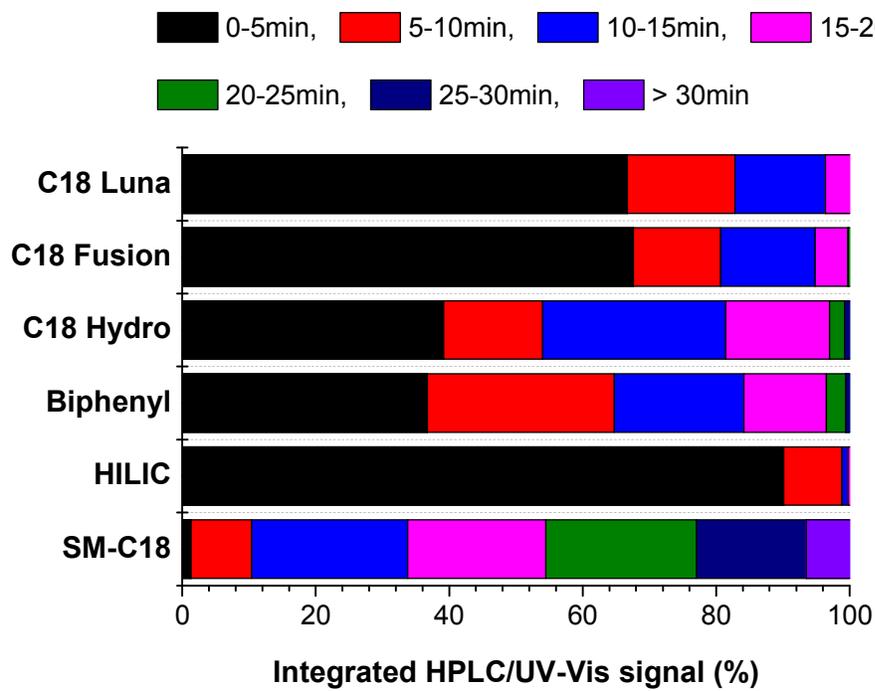
remarks

<sup>a</sup> BrC compounds identified in previous studies, for which molecular structures were proposed based on the reported reaction mechanisms;

<sup>b</sup> BrC compounds that have not been reported before, but for which their elemental formulas and structures can be inferred based on previously reported reaction mechanisms and structurally-related products.

<sup>c</sup> New light-absorbing nitrogen-containing compounds observed in this study.

Figure S1. Fractions of the overall HPLC/PDA signals integrated over the 250-450 nm range and detected at different elution periods. The plot compares the amount of light-absorbing material resolved by different columns during certain periods of the elution time.





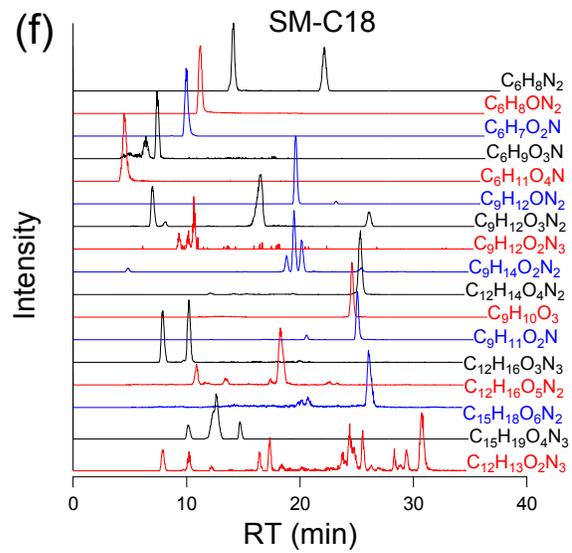
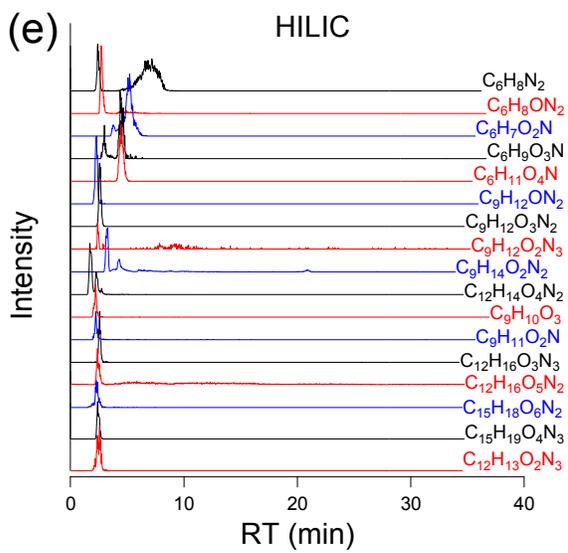
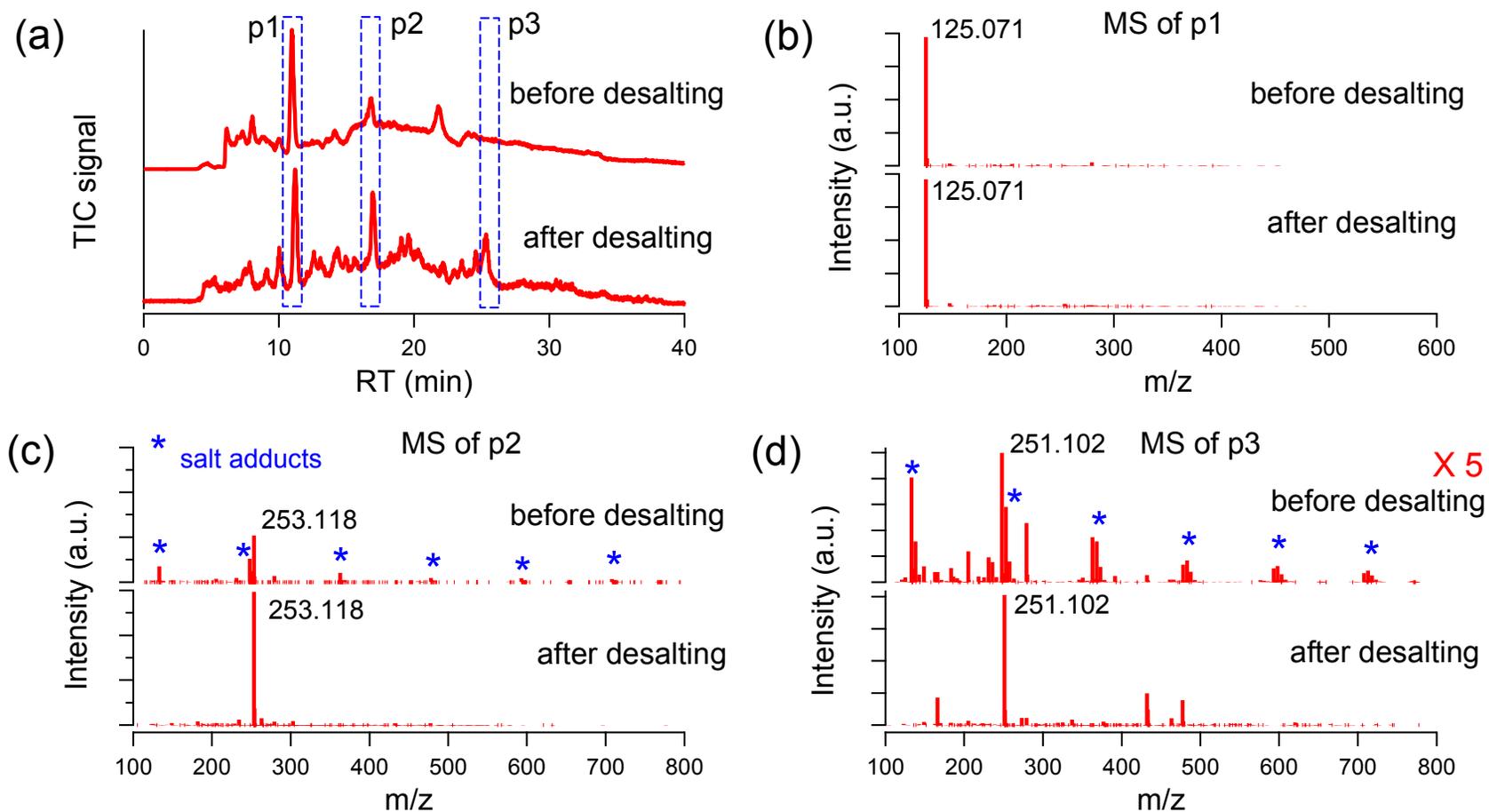


Figure S3. The effect of sample desalting on the performance of the SM-C18 column for separating BrC compounds produced in MG+AS mixtures. (a) Total ion chromatograms (TIC). Panels (b), (c) and (d) show integrated mass spectra of p1, p2 and p3 regions indicated in panel (a), respectively. The blue asterisks indicate salt adducts.



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