

Understanding the complexity of porous graphitic carbon (PGC) chromatography: Modulation of mobile-stationary phase interactions overcomes loss of retention and reduces variability

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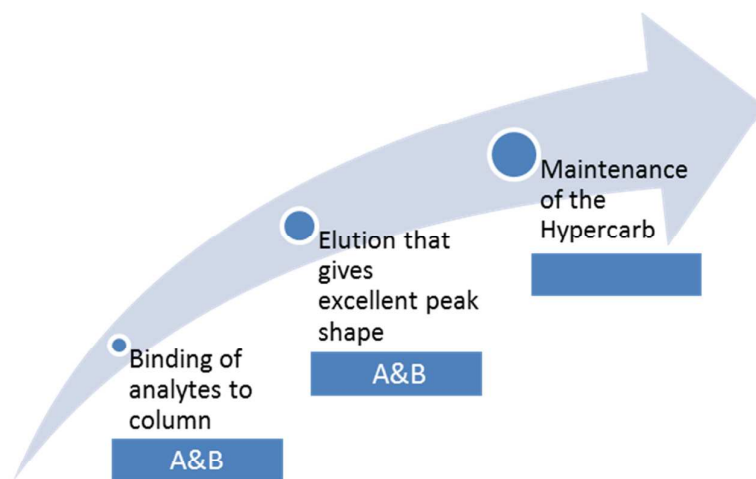
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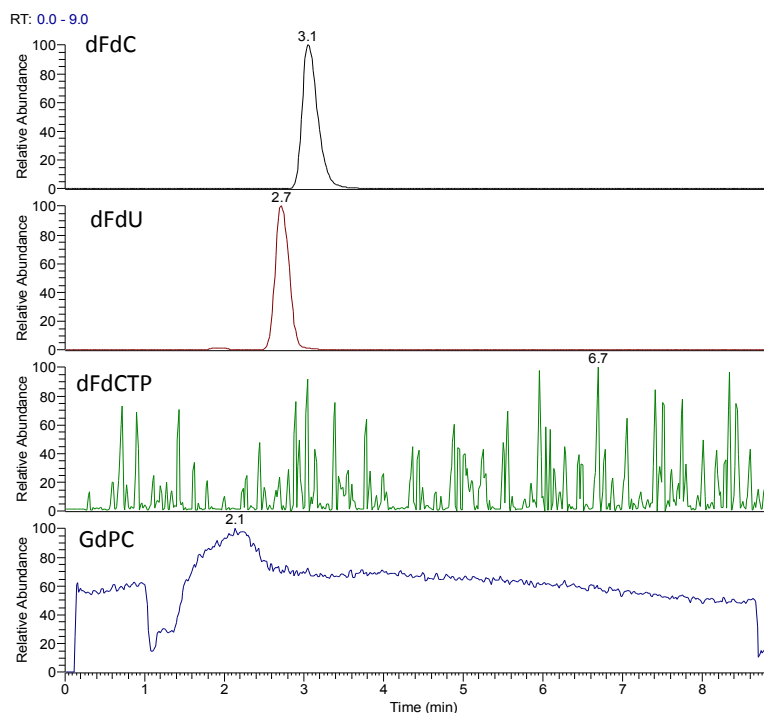
Supporting references.



| Time (min) | A | B | |
|------------|----|----|--|
| 0 | 95 | 5 | |
| 2 | 95 | 5 | |
| 2.2 | 80 | 20 | |
| 7.8 | 80 | 20 | |
| 8.0 | 95 | 5 | |
| 15 | 95 | 5 | |
| | | | |

Figure S-1. Our previously published¹ gradient elution program on Hypercarb™, A: 10 mM ammonium acetate pH10 in water, B: 100% acetonitrile. Showing the absence of a column maintenance step.

(A)



(B)

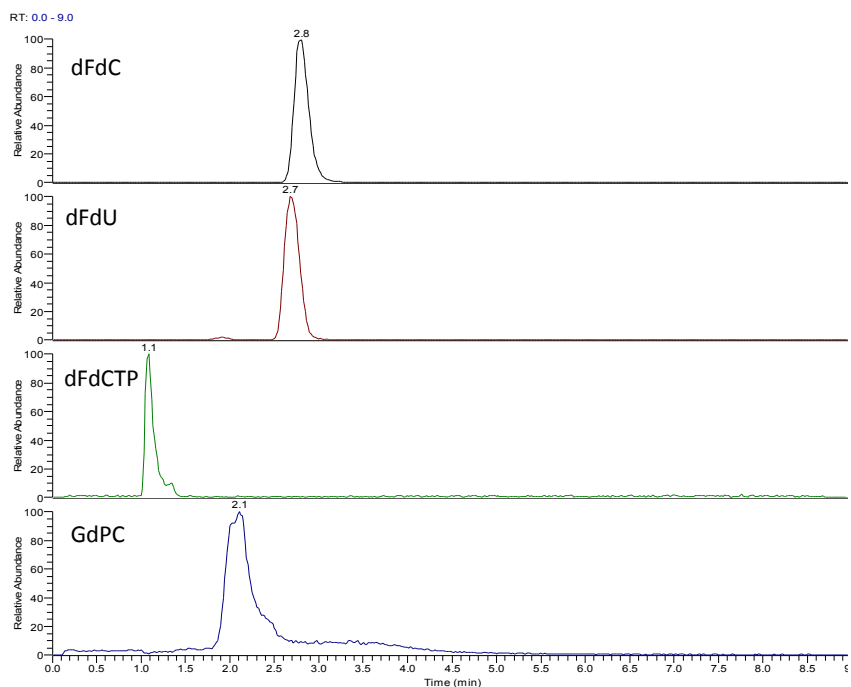
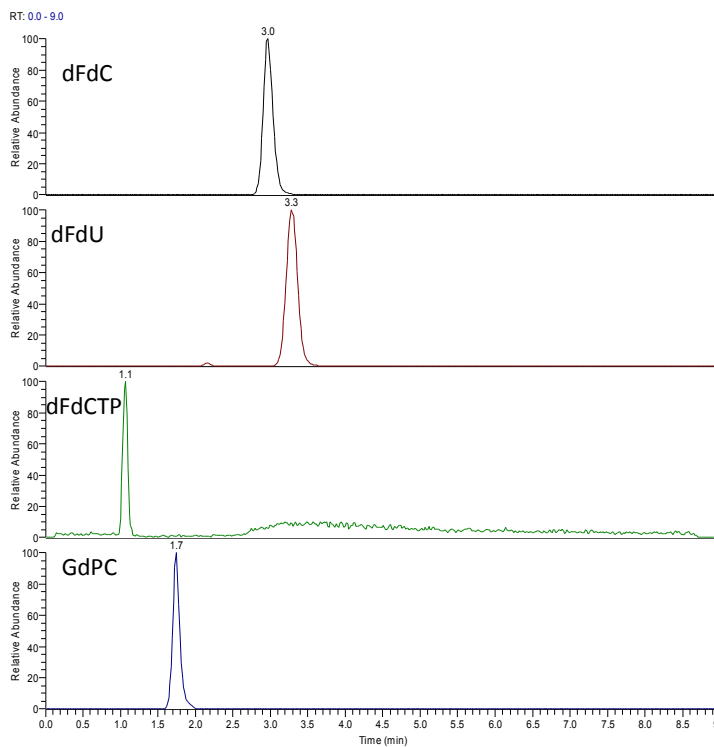


Figure S-2. Effect on retention and peak shape of changing from a mobile phase of (A) methanol:water (95:5) to (B) 10 mM ammonium acetate pH 10 in methanol:water (95:5). Analytes were detected following heated electrospray ionisation using a triple stage quadrupole mass spectrometer.

(A)



(B)

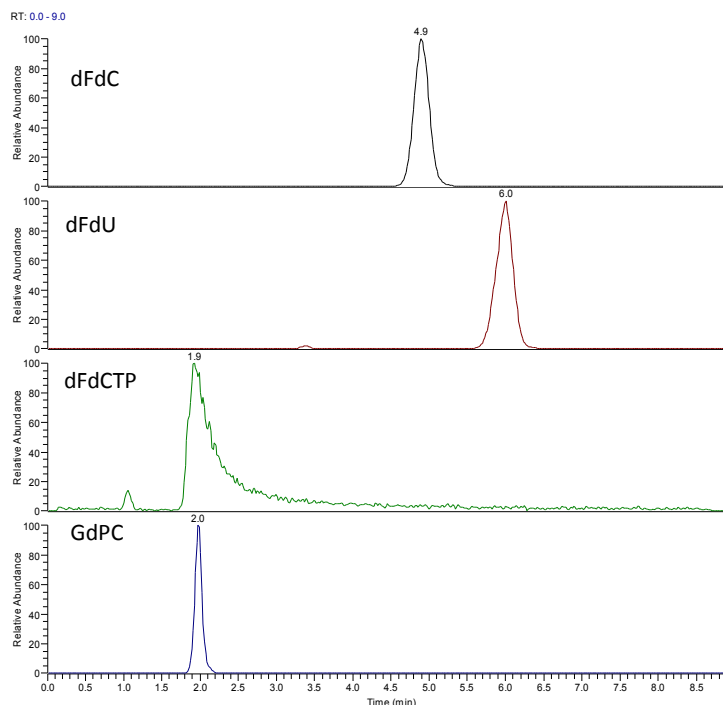


Figure S-3. Effect on retention and peak shape of changing from (A) 10 mM ammonium acetate pH 10 in methanol:water (70:30) to (B) 10 mM ammonium acetate pH 10 in methanol:water (1:1). Analytes were detected following heated electrospray ionisation using a triple stage quadrupole mass spectrometer.

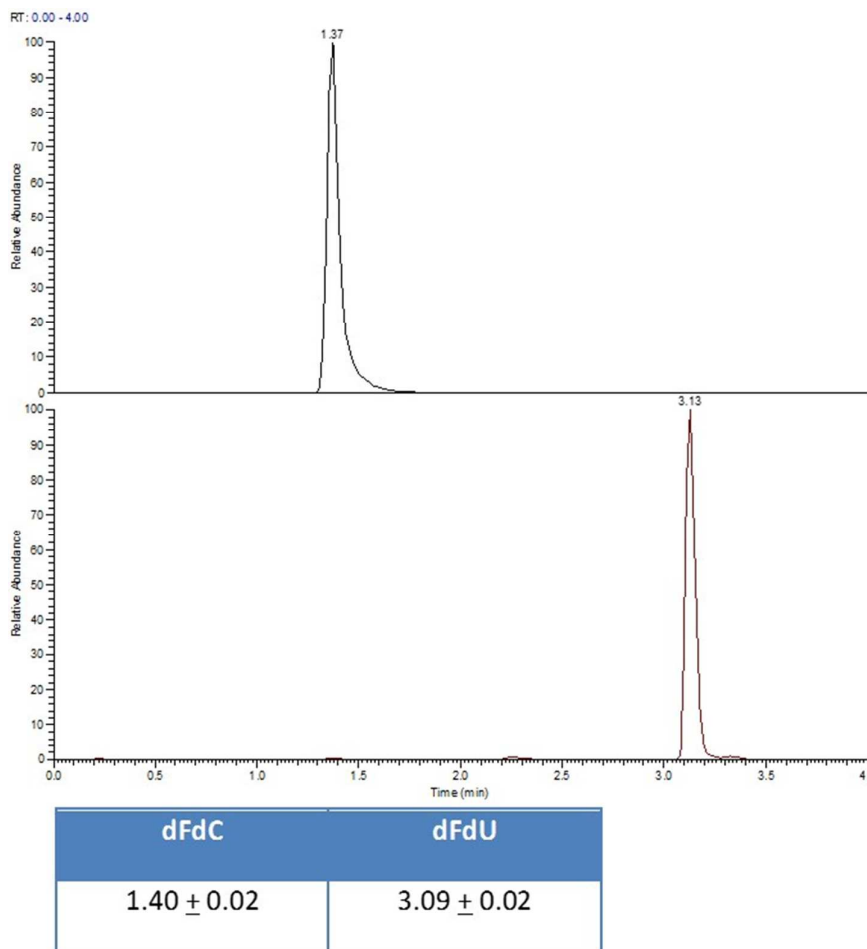


Figure S-4. Typical chromatogram on the Acquity T3 (C18) column, of dFdC and dFdU, 200 ng/ml spiked in water, detected following heated electrospray ionisation using a triple stage quadrupole mass spectrometer. The average retention time (t_R (min)) and standard deviation are shown for 95 injections of dFdC and dFdU.

| Compound | t _R (min) | Reference |
|---|----------------------|--------------|
| Cytidine-5'-monophospho-N-acetylneuraminic acid | 1.9 ± 0.06 (N = 10) | ² |
| Uridine 5'-diphosphoglucose | 2.3 ± 0.08 (N = 10) | ² |
| Uridine 5'-diphosphogalactose | 2.6 ± 0.09 (N = 10) | ² |
| Guanosine 5'-diphospho-β-L-fucose | 5.9 ± 0.15 (N = 10) | ² |
| Guanosine 5'-diphospho-D-mannose | 4.8 ± 0.15 (N = 10) | ² |
| Uridine 5'-diphospho-N-acetylneuraminic acid | 2.3 ± 0.09 (N = 10) | ² |
| | | |
| | | |
| | | |

Table S-1 Average retention times (t_R) and standard deviation (N= number of runs) on PGC reported by others.

| Compound | t _R (min) | | | Reference |
|-----------------------------|----------------------|----------|----------|----------------|
| | Column 1 | Column 2 | Column 3 | |
| Uridine 5'-diphosphoglucose | 11.76 | 10.40 | 8.20 | ³ |
| gemcitabine | 7.75 | 5.02* | | ^{4,5} |
| Gemcitabine triphosphate | 6.89 | 8.32* | | ^{4,5} |

Table S-2 Average retention times (t_R) on different (age/usage) or the same (after re-generation) PGC columns, as reported by others.

*- not clear whether this was the same or different column but these are the retention times after treating the column with hydrogen peroxide^{4,6}

Column number: 10170107

| dFdC | dFdU | GdPC | dFdCTP |
|-------------|-------------|-------------|-------------|
| 5.23 ± 0.02 | 5.04 ± 0.02 | 4.11 ± 0.02 | 3.93 ± 0.03 |

Column number: 0610524V6

| dFdC | dFdU | GdPC | dFdCTP |
|-------------|-------------|-------------|-------------|
| 5.16 ± 0.02 | 5.02 ± 0.02 | 4.07 ± 0.02 | 3.89 ± 0.02 |

Column number: 10065922

| dFdC | dFdU | GdPC | dFdCTP |
|-------------|-------------|-------------|-------------|
| 5.30 ± 0.01 | 5.21 ± 0.01 | 4.13 ± 0.02 | 3.93 ± 0.01 |

Table S-3. Average retention times (t_R (min)) and standard deviation of 18 injections of dFdC and metabolites extracted from tumour homogenate on 3 different PGC columns using the gradient shown in Figure 1 but with 100% water instead of methanol:water (95:5) used as solution "C" for flushing in the column maintenance step.

References

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