

## APPLICATION NOTE

### Trap, Concentrate and Map, Using Acquity UPLC I class Plus.

### STYROS® R Polymeric Compared with C18 Acquity UPLC® BEH. Follow up Study of App. Note 150, 151.

It is important to have a clear assessment of the retentivity and hydrophobic capacity of the column to be used as trap.

We noticed in the previous applications (150 and 151) that the polymeric column with Aryl functionalities displays a clear advantage in retaining the compound to be trapped.

While Uracil and Phenol are retained, it is at a level where Methyl and Butyl Paraben are saturated.

To have a better idea of the full amount of compound trapped in this process, we have omitted Uracil and phenol, including instead Ethyl and Propyl Paraben.

The process is now run in an isocratic mode, therefore skipping the equilibration of the column before each run.

Same solutions are used.:

Solution A: DI H<sub>2</sub>O solution with the compounds to be trapped.

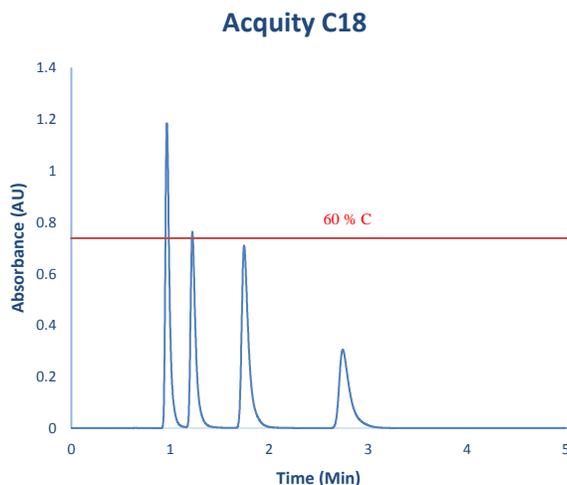
Buffer B: DI H<sub>2</sub>O (for mapping)

Buffer C: MeOH (for mapping)

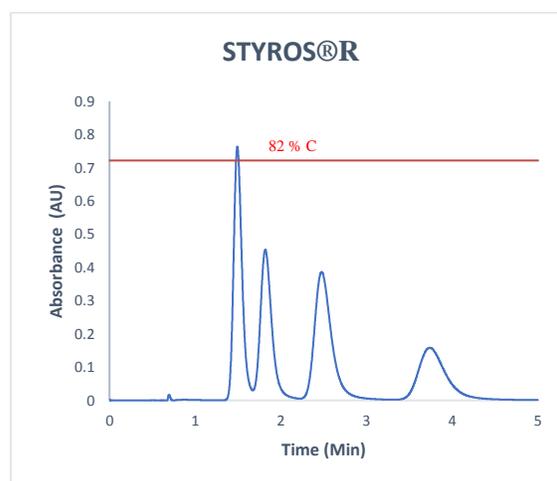
In a first step a C18 column was used. (acquity C18 BEH 1.7µm, 2.1 x 50 mm).

0.1 µl of a solution of 1 mg/ml in a mixture of 60:40 % MeOH: H<sub>2</sub>O is injected.

This is now 0.1 µg of each compound.

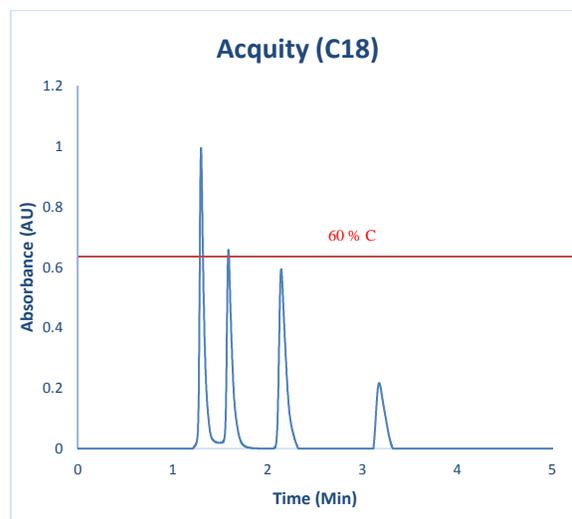


To elute these components from the polymeric column 82 % of MeOH is required. The retentivity is clearly higher and even at these higher organics, the retention times are longer.



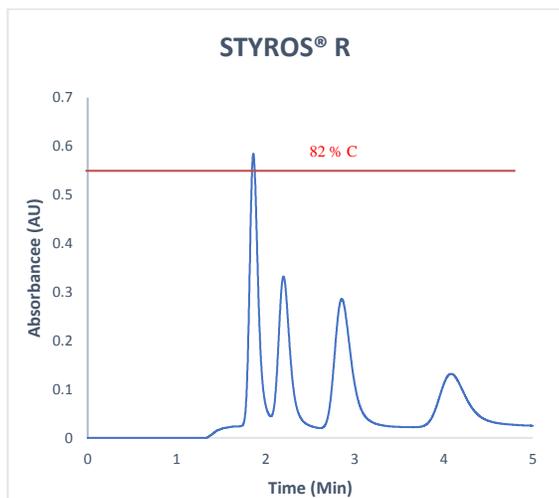
In the subsequent steps 0.5 ml of the previously made sample is diluted in 500 ml of water and run through the columns for 0.3 minutes at 0.3 ml/min with a concentration of 0.1 ng/ml.

That is a total of 0.09 µg to be trapped on each column.



Considering the retention times as well as the peak ratio it is safe to consider the trapping as being complete.

Similar process is done using the STYROS® R polymeric column.



The retention times have shifted similarly without affecting the ratios of the peaks or their relative intensities. What is again clear is the increased hydrophobicity of the polymeric media and its increased retentivity of it.

The following chart depicts the compound involved in this experiment in order of dilution.

1		Methyl Paraben M= 152.15
2		Ethyl Paraben M= 166.176
3		Propyl Paraben M=180.203
4		Butyl Paraben M= 194.23