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APPLICATION NOTE

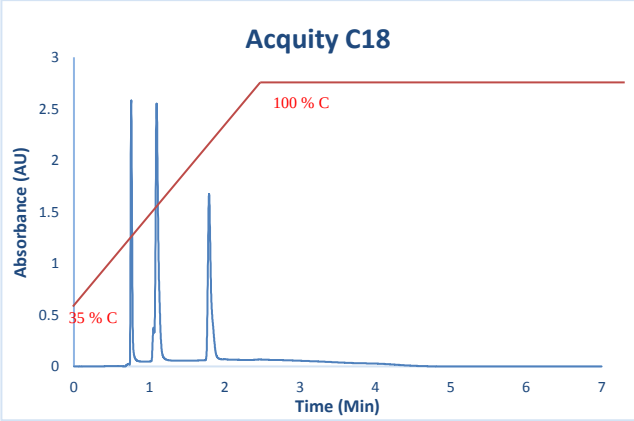
Trap, Concentrate and Map, Using Acquity UPLC *I* class Plus.
STYROS® R Polymeric Compared with C18 Acquity UPLC® BEH. Folow up Study of App. Note 150.

To properly identify the eluted compounds, a limited number of products from the previous experiment were chosen in known amounts and rerun under similar conditions.

Same setup as Application Note 150, and same solution and buffers:

Solution A: DI H2O solution with the compounds to be trapped.
Buffer B: DI H2O (for mapping)
Buffer C: MeOH (for mapping)

In a first step a C18 column was used. (acquityC18 BEH 1.7µm). Since a gradient is run an equilibration step is required.
Using the Inlet Prerun of the Acquity *I* class, a 5 minutes run of the initial solvent gradient (H2O) is included at 0.3 ml/min followed by 2µl injection of a sample with 3 mg/ml each of Uracil and Phenol, and 2 mg/ml each of Methyl Paraben and Butyl Paraben. That is the equivalent 0.4 and 0.6 µg of each.



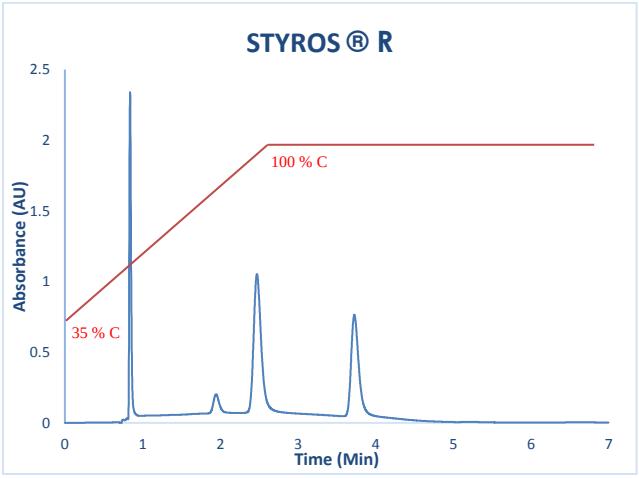
Same gradient step to an initial 35% MeOH, followed by the increase of MeOH to 100 % in 3 minutes and run for 7 minutes as shown in the chromatograms.

It is now clear that the Phenol and Methyl Paraben co-elute under the present conditions at 1.1 minute.

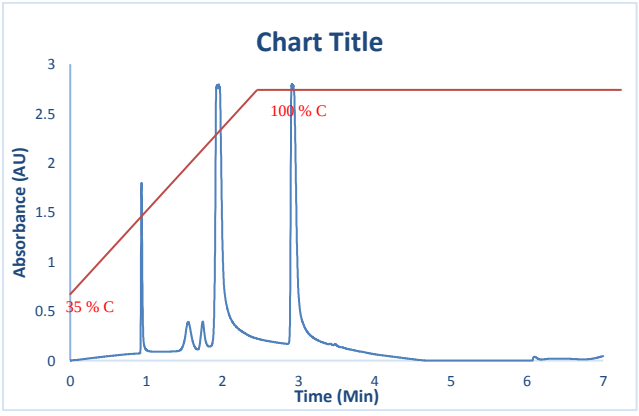
Under similar conditions, the STYROS® R polymeric column shows the compounds well separated.

Phenol elutes at 1.95 minutes and Methyl Paraben elutes at 2.47 minutes as they are more retained.

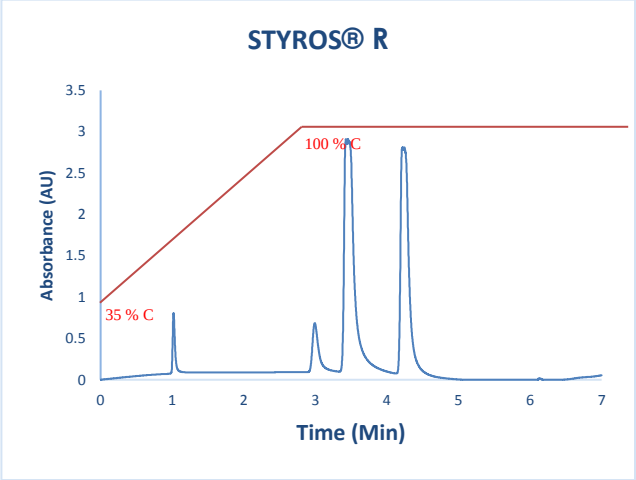
Uracil and Butyl Paraben have a longer retention time: they elute at 0.84 and 3.73 minutes, respectively.



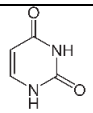
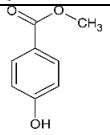
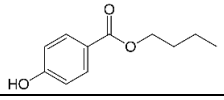
In the subsequent steps 0.5 ml of the previously made sample is diluted in 500 ml of water and run through the columns for 2 minutes at 0.3 ml/min.
The concentration of the compounds is now 4-6 ng/ml.
The 0.6 ml injected during the 2 minutes contains 2.4 and 3.6 ng.
While the amount injected is such that it saturates the peaks that identify Methyl and Butyl Paraben, it is just enough to show the presence of Uracil and Phenol.



Similar process is done using the STYROS® R polymeric column.
Similar phenomenon occurs, however at a lesser extent considering the higher retention of STYROS ® R.
The Methyl and Butyl Paraben get trapped on the column while excess amount of Uracil and Phenol are required to be trapped in order the be detected during the process of mapping.
However, here again the elution of the compounds is unambiguous.



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

1		Uracil M=112.09
2		Phenol M= 94.11
3		Methyl Paraben M= 152.15
4		Butyl Paraben M= 194.23