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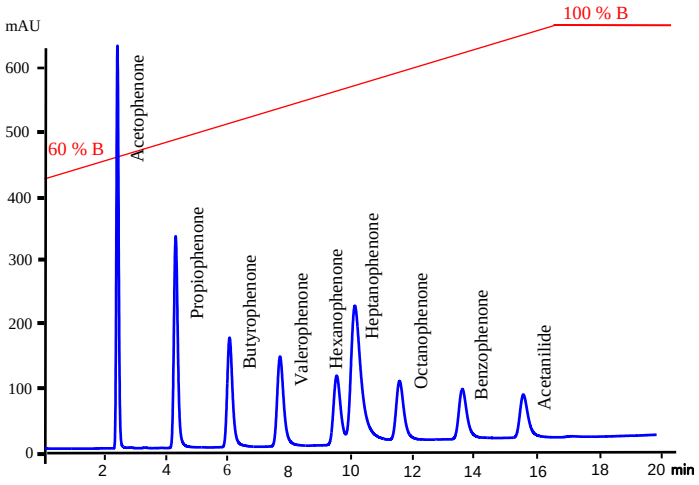
APPLICATION NOTE

STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Separation of 9 Phenones on Narrow Bore column of 2.1 mm ID.

The improvement of mass spectrometers has reached a point where the injection of a mixture allows the detection of its components without the need of any prior separation on an LC column.

The focus is now the contamination of the samples as a result of leaching of the LC columns.

In the present application we are using a Narrow Bore column of 2.1 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace Normal Bore columns of 4 or 4.6 mm ID.



Chromatogram
Separation of 9 Phenones on **STYROS® 2R/NB**
Flow Rate: 0.2 ml/min.

Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R/NB 2. 1 X 150 mm**
Mobile phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.2 ml/min.
Gradient	69 to 100 % B in 18 minutes (~7 cv)
Temperature	60°C
Detection	254 nm
Injection volume	2 µl
Pressure Drop	40 bar (~580 psi) at the start of gradient
Sample:	9 Phenones from Agilent Technologies

The media does not leach and can be used with mass spectrometer.
The size of the column allows minimal splitting to the waste for the hyphenation.

Compared with Normal Bore columns about 50 % less of eluent and sample are needed for the separation.
A total of 4 ml of eluent as well as 2 µl of sample is used for the run.
The separation is satisfactory for the mass spectrometer.
It is also to be noted that the high organic concentration is appropriate in case it is to be used with an electrospray instrument.

As Simulated-Monolith™ the separations can be run at high linear velocities as noted above so can the column regeneration.
This is now an example of small molecules being readily separated with Narrow Bore columns.
It is important to keep in mind the dwell volume of the instrument when using small bore columns as too large of a dwell volume is not helpful in properly achieving the required gradient.

Overall the advantages that Simulated-Monolith™ polymeric columns offer are notable:

- Similar to monolith, Simulated-Monolith™ does not have the restriction of pore size and is considered universal.
- The low pressure drop of the column allows its use in non UHPLC instruments as well.
- It provides the capability of longer columns to provide higher plates. (the column used for this application is 150 mm long, yet the pressure drop is only 40 bar at 0.2 ml/min and ~60 % ACN).
- As a hard gel polymeric it has the mechanical strength of silica without its brittleness and rigidity.
- It is inherently and uniformly hydrophobic and does not need any additional ligand for reversed phases.
- The higher retention of compounds compared to silica is also convenient for its use with mass spectrometers.
- The chemical stability provides a wider range of separation capability that cannot be explored with unstable media.
- The separation is based on fast convective process rather than the slow diffuse one.
- The reconditioning of the column is less time consuming.
- Separations can routinely be run at high linear velocities depending on the complexity of the samples and the requirements of the mass spectrometers.

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