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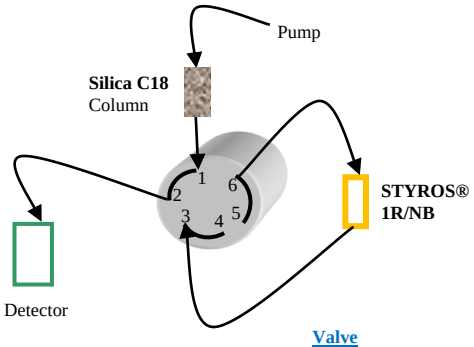
APPLICATION NOTE

Heartcutting Made Simple Using Acquity UPLC *I* class Plus.

Heartcutting by multidimensional liquid chromatography is an important practice now in liquid chromatography and needs an update with the more advanced instruments used in labs.
In this setup the Acquity UPLC *I* class Plus from Waters was used with a single two positions, 6 port valves and a binary pump.
We show here the use of 2 Narrow Bore reversed phase columns of 2.1x50 mm to run the operation and cut any peak from a mixture of 5 components.
The following schematics show the process to be simple.
The initial setup runs through a silica C18 column to find out an acceptable resolution of the compounds to heartcut.

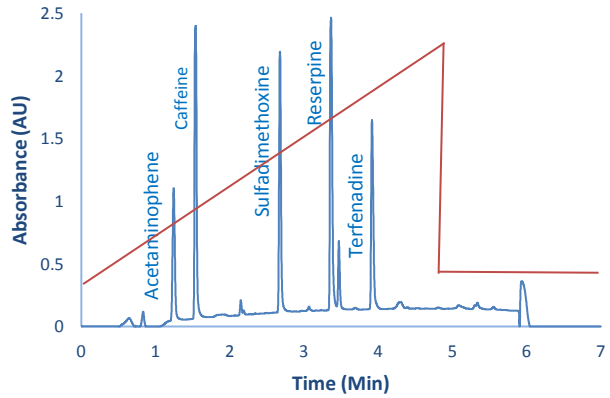


The buffer solutions used are

A: 0.075% TFA in DI H2O,
B: 0.075 % TFA in H2O:ACN 5:95

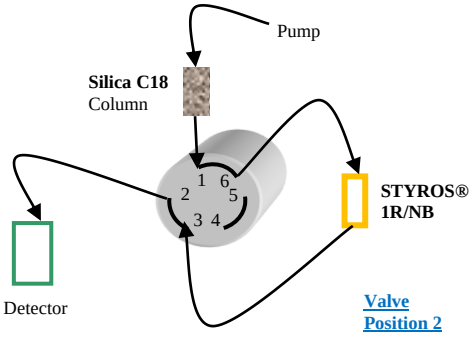
1- Initial run with Acquity UPLC® BEH C18 1.7 µm of 2.1x50 mm column.

Time (minutes)	% of buffer B	% of buffer A	Flow rate (ml/min)
0	10	90	0.2
5	100	0	0.2
5.1	10	90	0.2
7	10	90	0.2



Chromatogram of 5 compounds run with 0.2 µl injection of a 1mg/ml solution of each in buffer B.

The system pressure is now 3,700 psi at the start of the gradient and one column in line.
The separation is done in 7 minutes that includes 2 minutes of equilibration to the start of the gradient.
In the second setup, a SYROS® polymeric reversed phase is switched in line in addition to the initial silica column to capture the peak of interest based in its retention time. The system pressure increases to 4,800 psi.
The schematic of the second setup is shown below



2-. Heartcutting of peaks

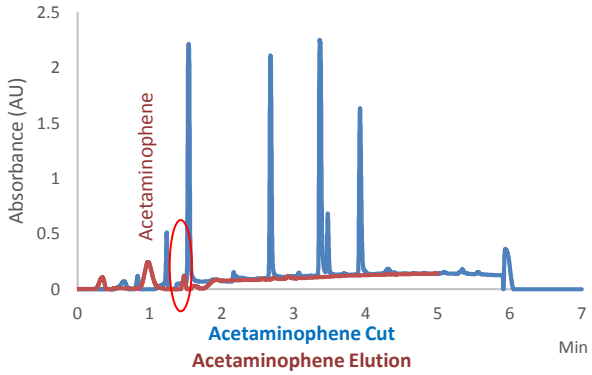
Time (minutes)	% of buffer B	% of buffer A	Flow rate (ml/min)	Valve position
0	10	90	0.2	1
Start of elution of peak of interest.				2
End of elution of peak of interest.				1
5	100	0	0.2	1
5.1	10	90	0.2	2
7	10	90	0.2	2

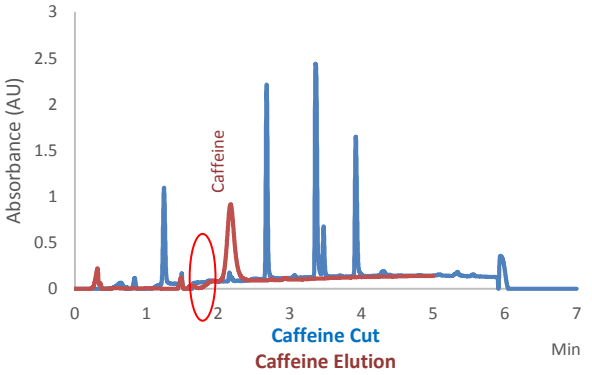
The next setup involves the elution of the retained peak from the STYROS® polymeric column using the same original gradient and the second setup

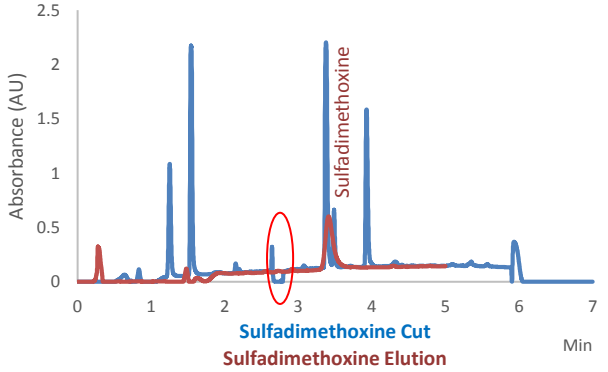
3-. . Elution of retained peak in position 2.

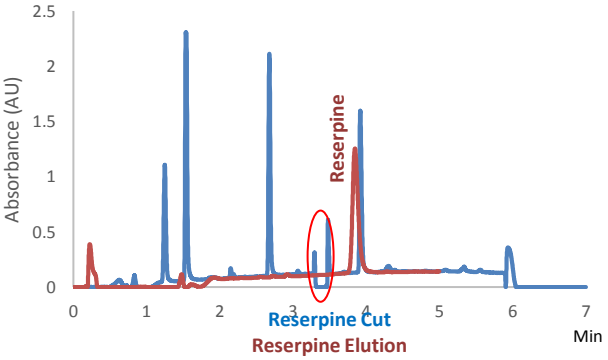
Time (minutes)	% of buffer B	% of buffer A	Flow rate (ml/min)
0	10	90	0.2
5	100	0	0.2
5.1	10	90	0.2
7	10	90	0.2

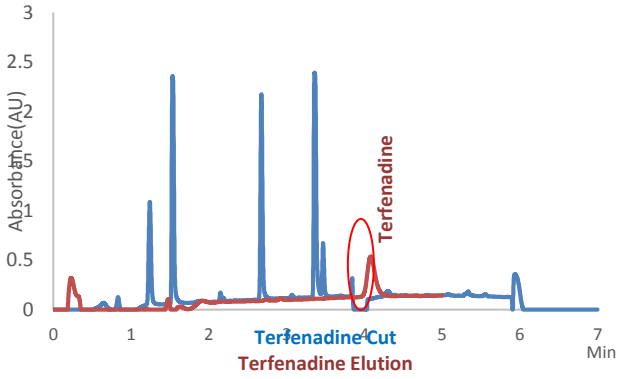
Shown in the following chromatograms, are the cutting and elution of each of the 5 peaks, superimposed.



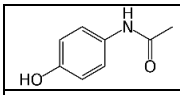
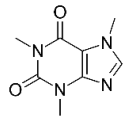
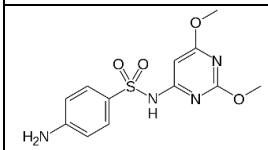
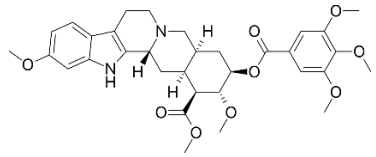
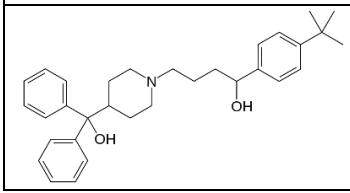








The process is used for all the peaks from the chromatogram in an automated setup.
It is important to preequilibrate both columns, as shown in the initial chromatogram, to the start of the gradian for at least 2 minutes.
This needs to be done at the start of each gradient run to elute the retained peak.
The use of Narrow Bore columns requires minimal use of solvents therefore the automated heartcutting can be run around the clock without any concern of running out of buffers or overfilling the waste.
The compound used consist of the following:

	Acetaminophene M=151.16
	Caffeine M=194.19
	Sulfadimethoxine M=310.33
	Reserpine M=608.68
	Terfenadine M=471.67