always FISCHER ANALYTICS one step ahead

Duhlwiesen 32, 55413 Weiler bei Bingen am Rhein - Germany
Tel. +49-6721-9886710, Fax. +49-6721-9886719

Technical Note
TN0007

Application Note UHPLC Alliance vs. Acquity

Titel
Application Note UHPLC Alliance vs. Acquity

Editor
Oliver Mueller / Parts Engineer

Version
1.04

Updated
13.08.2010

Instruments
Waters Alliance 2690/95

Contents

1 Introduction

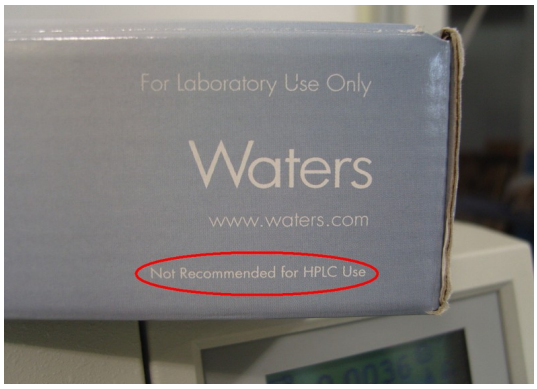
The number for this application note is seven. When I think about seven, I think of 007, the queens' agent. His job is to fight the evil and he always uses his magic items. His car is one of these items which has many special functions.
Our black box (the Alliance UHPLC Kit) is similar to that. It releases all the hidden resources of your Alliance by pressing one button. Most of the customers couldn´t believe it before they saw it running. The best way to convince people is to show them real data.

This application note demonstrates the performance of the UHPLC Alliance 2690/95.
We compare the upgraded Alliance to the binary Acquity. We do the same installation tests that are used for the Acquity with our Alliance 2690.
The question that we want to answer is, “Can the Alliance pass the Acquity installation specs?” and, if it passes, “What are the limits of the Alliance?”

2 Instruments, Methods and Acquity Installation Tests

Our Test-Alliance 2690 was built in 1998 and has run over 45000 samples during it´s lifetime. This is one of our oldest instruments and it has survived all our internal stress tests prior to the release of our upgrade.
It is equipped with our upgrade box, has an optimized dwell volume (Technical Note #0005), the special injector needle (Technical Note #0003) with Titanium Nitride coating and all high pressure seals with ***fischer analytics GmbH*** design. We used the at column dilution technique for sample injection.

Our tests are primarily based on the Acquity UPLC System Installation Checklist (Waters document 715000877 Rev. C). We use the solvents and test samples that are mentioned in this document. We use a short Phenomenex Safety Guard pre-column that is connected to the Acquity BEH C18, 1.7µm, 2.1 x 50mm and the internal solvent heat exchanger in the Alliance column oven.



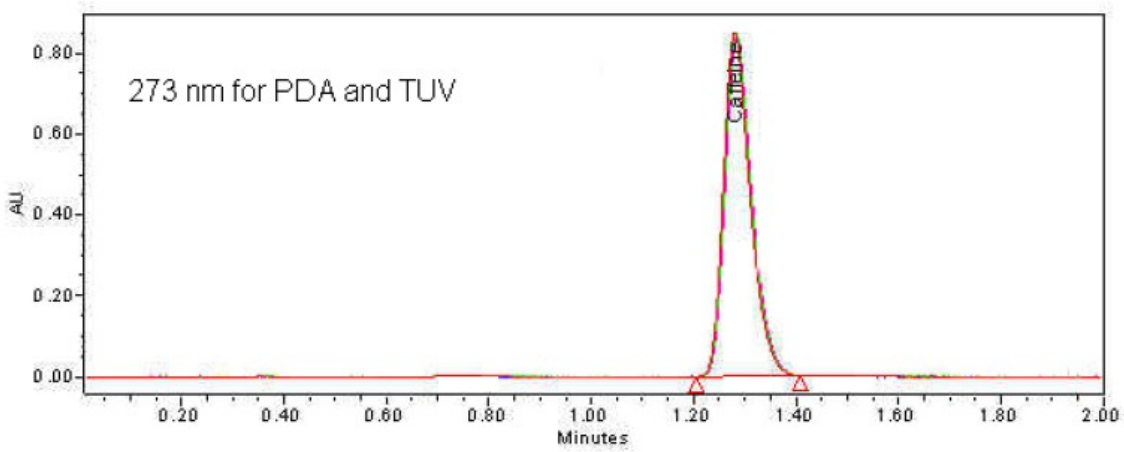
Acquity Column Information on the column box

Please note the information on the column box: “Not recommended for HPLC use”(?)

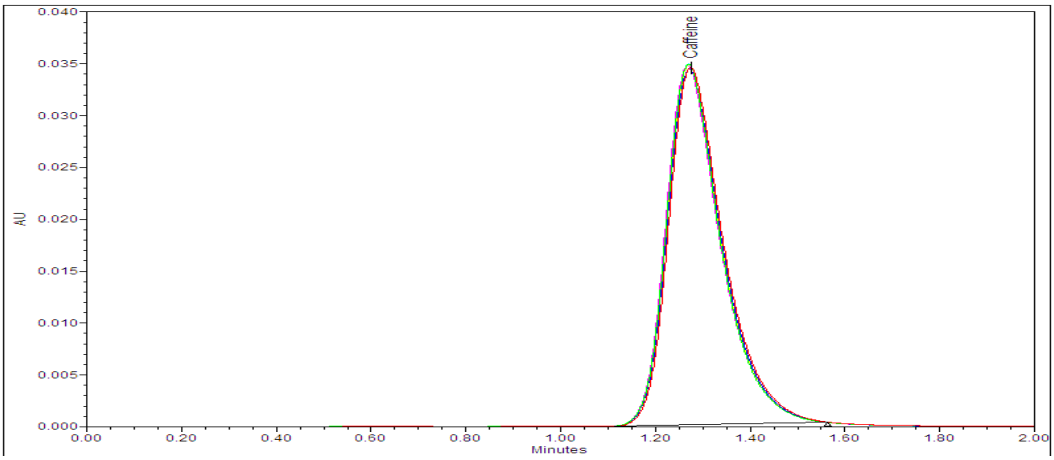
2.1 System Precision Test

For the **system precision test** we injected 5µl Caffeine solution 4 ng/ml. Due to the unexpected late retention time of caffeine (2.3min instead of 1.3min), we adjusted the composition of solvent A from (10% ACN : 90% Water to 15% ACN : 85% Water). Solvent line A and B were put in the same bottle. We used a composition of 50% A and 50% B and a flow of 0.4ml/min. The detector (model 2487) was set to 273nm, sample rate 10Hz, Hamming Filter 0.4s. The injection volume was 5µl, method runtime 2.0min. The temperature of the column oven was set to 30°C.

The chromatogram should look like the one below.



System Precision Chromatogram, 6 Replicates, Waters 715000877 Rev. C, page 19



System Precision Chromatogram, 6 Replicates, UHPLC Alliance

six replicates	binary Acquity Specification	UHPLC Alliance Test Result	pass / fail
Retention Time standard deviation	≤ 1.0 sec	0.12 sec	pass
Peak Area, %RSD	≤ 0.5 %	0.12 %	pass
Peak Height, %RSD	≤ 0.9 %	0.59 %	pass

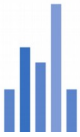
The UHPLC Alliance passed this test without problems

2.2 Gradient Performance Test

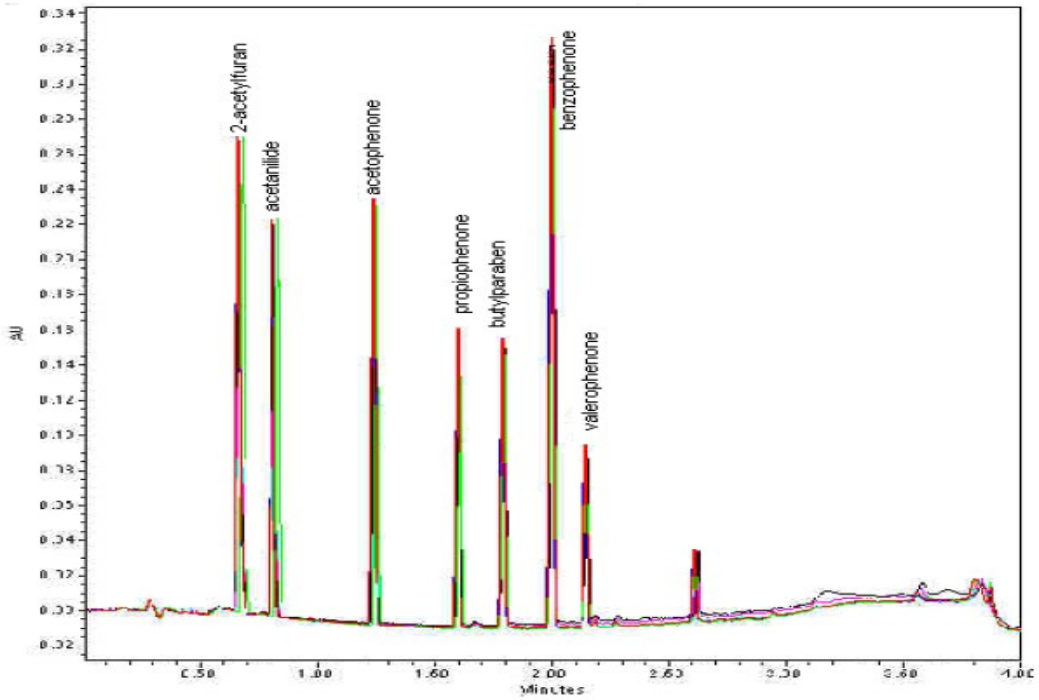
The **gradient performance test** mixture consists of Uracil, 2-Acetylfurane, Acetanilid, Acetophenone, Propiophenone, Butylparabene, Benzophenone, Valerophenone at a concentration of 4 ng/ml, dissolved in 100% Acetonitrile. We used the same composition of solvent A from the system precision test (15% ACN : 85% Water). Solvent B was 100% ACN.

Step	Time (min)	Flow (ml/min)	%A	%B	Curve
1	initial	0.5	100	0	initial
2	3.0	0.5	5	95	6
3	3.5	0.5	5	95	6
4	4.0	0.5	100	0	6
5	30.0	0.0	100	0	11

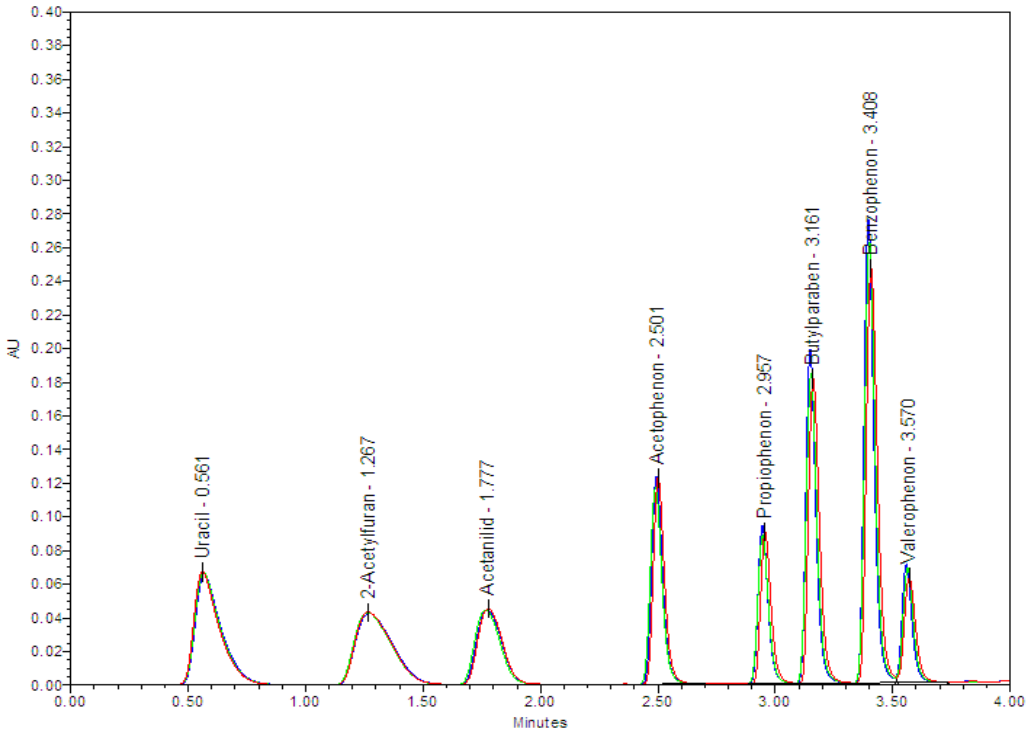
The detector (model 2487) was set to 254nm, sample rate 10Hz, Hamming Filter 0.4s. The injection volume was 10µl, method runtime 6.5min.



The chromatogram below is from the installation checklist.



Gradient Performance Chromatogram, 3 Replicates, Waters 715000877 Rev. C, page 26.



Gradient Performance Chromatogram, 3 Replicates, UHPLC Alliance

Gradient Performance Test (3 Replicates)				pass /fail
Peak	RT mean (min)	Std. Dev. (min)	Limit	pass
Uracil	0.565	0.004	none	pass
2-Acetylfuran	0.1267	0.003	≤ 0.025min 1.5 secs	pass
Acetanilid	1.772	0.006	≤ 0.025min 1.5 secs	pass
Acetophenon	2.495	0.006	≤ 0.025min 1.5 secs	pass
Propiophenon	2.949	0.006	≤ 0.025min 1.5 secs	pass
Butylparaben	3.154	0.006	≤ 0.025min 1.5 secs	pass
Benzophenon	3.402	0.006	≤ 0.025min 1.5 secs	pass
Valerophenon	3.564	0.006	≤ 0.025min 1.5 secs	pass

The UHPLC Alliance passed this test. The chromatogram looks different compared to the one from the installation checklist. The dwell volume difference between the test Alliance and the binary Acquity is only 200µl. At a flow rate of 500µl/min I would expect the retention time shift 24 seconds later, but not 1.4 min.

The reason is that the chromatogram in the document 715000877 Rev. C was acquired at a higher flowrate. I guess about 800µl/min and therefore all the peaks are smaller and the retention times are earlier. Maybe this is the reason why the chromatogram was removed from the document 715000877 in Rev. D.

These Tests show that you obtain a chromatogram with a Acquity column connected to a HPLC System. The binary Acquity system installation specification tests are no problem for the Alliance, it easily passes them. These "mild" conditions can also be accomplished by a standard Alliance.

You can get the same or better chromatographic resolution with a standard HPLC column. The Acquity BEH column will show it´s true performance at higher flow rates and pressures.

From the Van Deemter equation (http://en.wikipedia.org/wiki/Van_Deemter_equation) we know that 1.7µm particles will not alter the number of plate counts much when the flow is increased. What I want to say is, you get UPLC like chromatograms when you increase the flow. For a 2.1mm i.d. UPLC column a minimum flow rate of 0.8ml/min is required.

Compared to a Bond movie, it is quite boring if James would drive his car like an old woman and does not use his special gadgets. Let´s leave this chapter and get ready for action!

3 General Information

The data used for the calculation of the reproducibility is pure raw data. The UV detector used no filter, the data is not smoothed or manipulated in any other way. With the optimized processing method the results are much better.

3.1 Alliance UHPLC Tests at 8000 PSI, 1ml/min

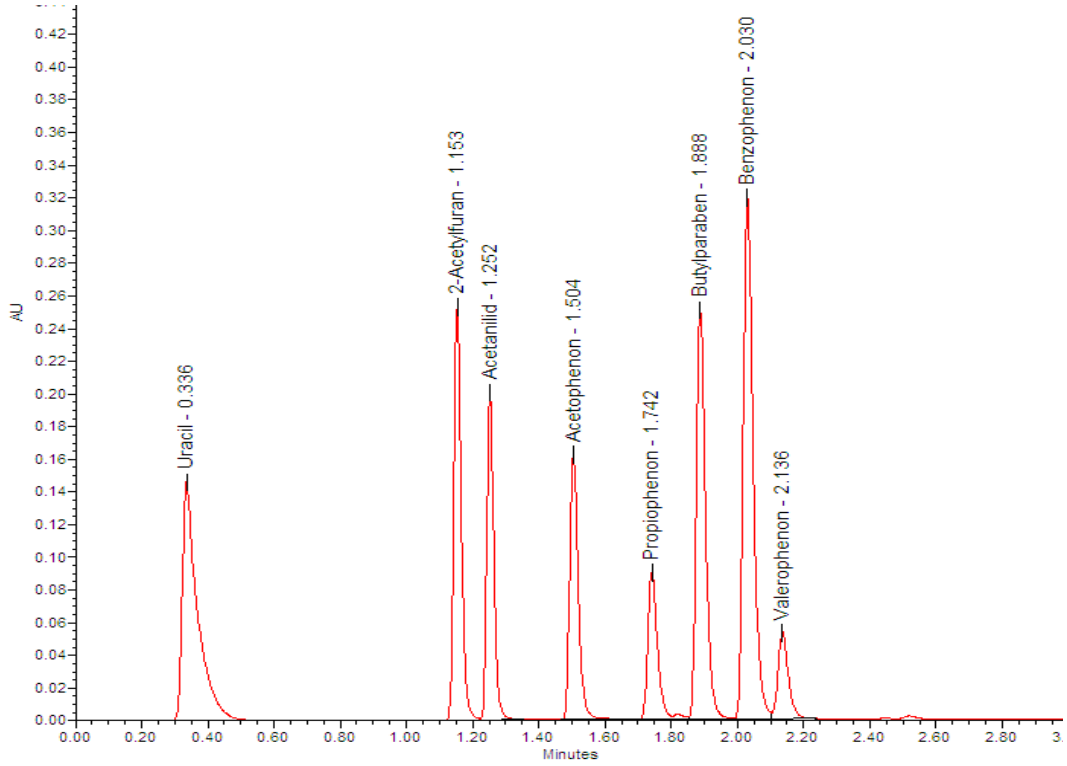
Now we are using a flow of 1.0ml/min. The pressure is about 8000 PSI at initial conditions. The column temperature is set to 40°C. Higher flow rates are possible, but require higher temperatures.

For the next experiment we use pure Water in channel A and pure ACN in channel B. The sample is dissolved in pure ACN and the injection volume is 10µl. I use pure Water in the beginning of the gradient to have a better focussing of 2-Acetylfuran and Acetanilid. Pure water can be used for a short period of time without damaging the stationary phase.

Time	Flow [ml/min]	A [%]	B [%]	Curve
0.0	1.0	100	0	-
0.1	1.0	100	0	6
0.2	1.0	80	20	6
2.5	1.0	0	100	6
3.0	1.0	100	0	6
5	1.0	100	0	6
15	0	95	5	6

Gradient Table for the 8000 PSI Reproducibility Test

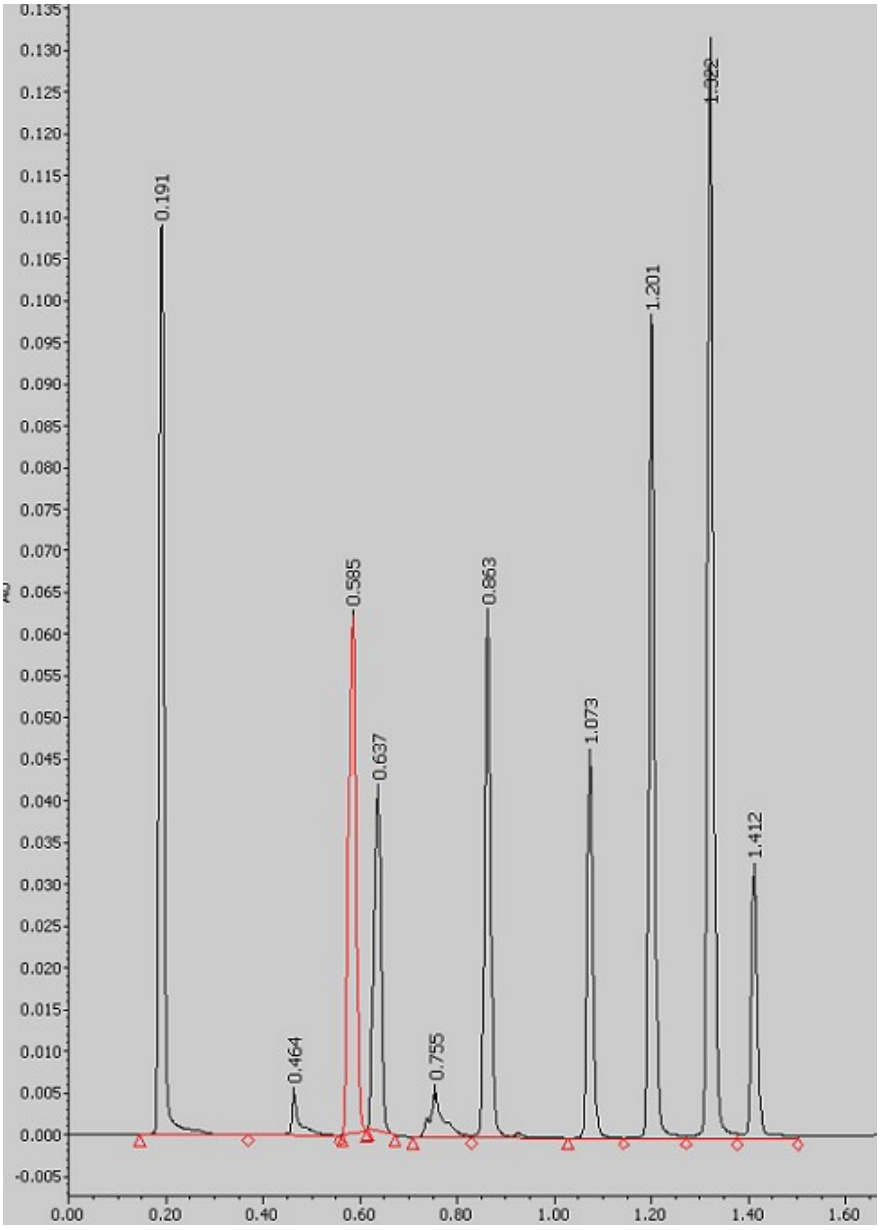
We obtain the following chromatogram:



Chromatogram at 8000 PSI and 1ml/min

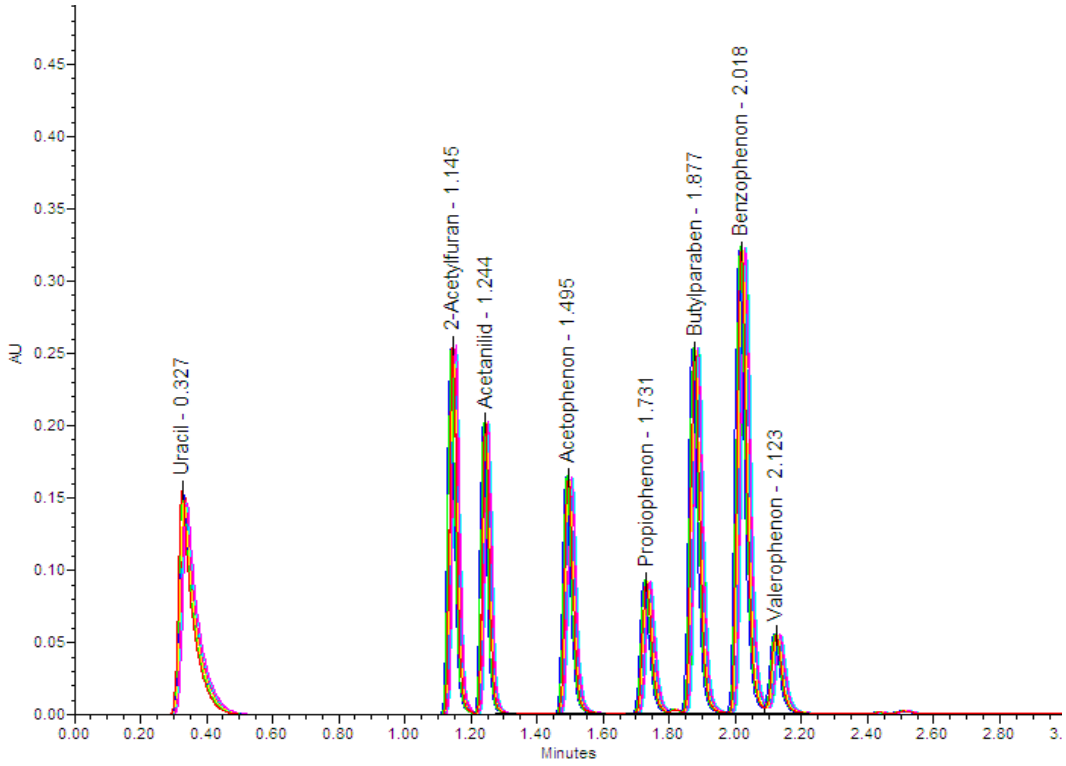
The peaks have a nice shape and we are close to the chromatogram that was printed in the Acquity installation checklist. Remember, we use a standard HPLC system for this test.

The capillary from the column to the detector has a standard i.d. of 0.009 inch, the UPLC detectors use i.d. 0.004 or 0.0025 inch. Our flow cell has 10µl volume, the UPLC has ~0.4µl, with the same pathlength of 10mm. This dwell volume difference is responsible for the peak broadening. Most of the peak broadening is caused by the tubing from the column outlet to the detector. For that reason the UPLC has a column compartment that directly under the UV detector. If a mass spectrometer is used it can swing towards the ion source that the distance is decreased. If we would connect a UPLC detector with a very short capillary, you would get the same results as on a true Acquity system.



Reference Chromatogram, a binary UPLC with PDA was used

Now lets have a look at the reproducibility for our UHPLC system.



Reproducibility Test at 8000 PSI and 1ml/min, 10 Replicates, raw data

Name / RSD [%]	Ret.Time	Area	Height
Uracil	1.52	1.03	2.69
2- Acetylfuran	0.53	0.71	0.53
Acetanilid	0.47	0.76	0.39
Acetophenon	0.43	0.68	0.55
Propiophenon	0.40	1.95	0.80
Butylparaben	0.36	0.46	0.48
Benzophenon	0.36	0.56	0.43
Valerophenon	0.35	1.04	1.21

Reproducibility Results Table at 8000 PSI and 1ml/min, 10 Replicates

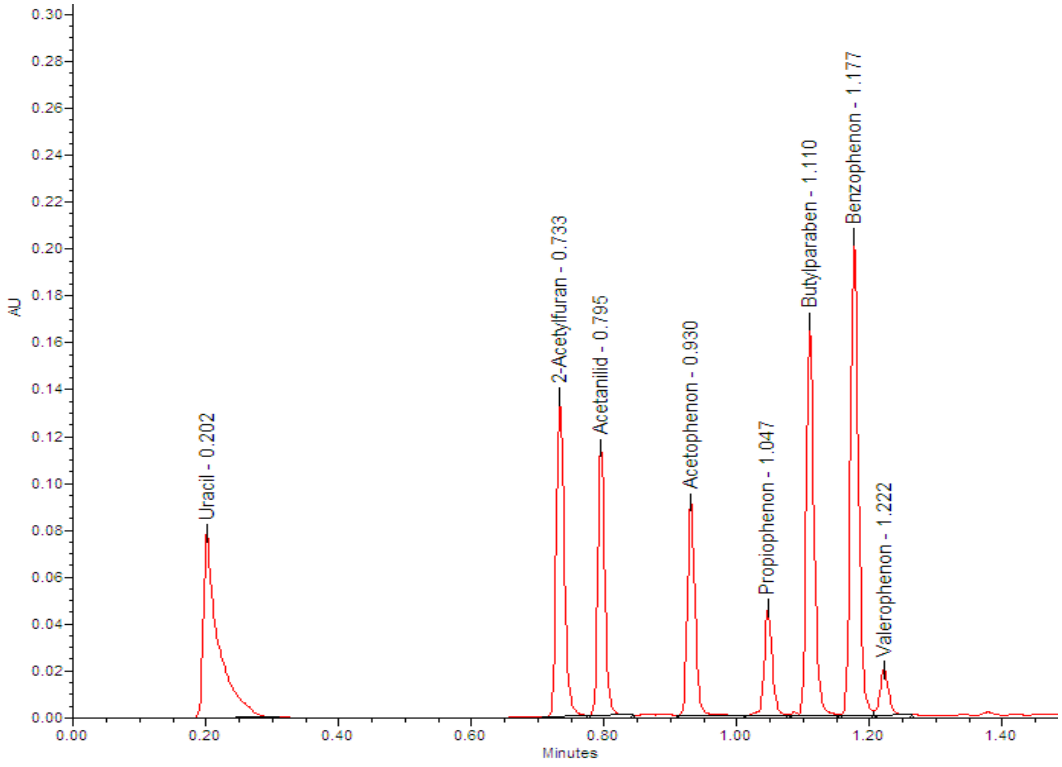
3.2 Alliance UHPLC Tests at 15000 PSI, 2ml/min

Now we are running the Alliance under real "ultra high pressure". The flow rate is 2ml/min and we start with 100% Water. The column oven is set to 60°C to lower the viscosity of the mobile phase. We inject 10µl sample. These conditions are over the specification of the Acquity, it can do only 9000 PSI at 2ml/min!

Time	Flow [ml/min]	A [%]	B [%]	Curve
0.0	2	100	0	-
0.1	2	100	0	6
0.2	2	80	20	6
1.25	2	0	100	6
1.5	2	100	0	6
2.5	2	100	0	6
15	0	95	5	6

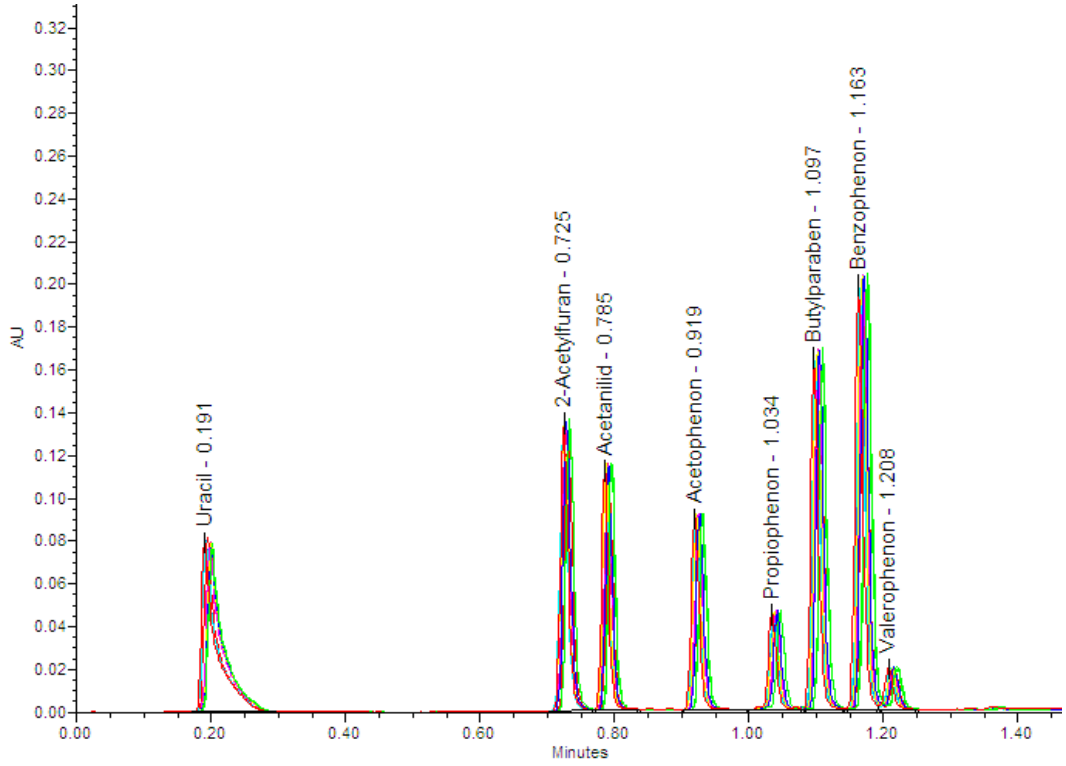
Gradient Table for the 15000 PSI Reproducibility Test

We obtain the following chromatogram



Chromatogram at 15000 PSI and 2ml/min

Nice, we saved another minute. The peak widths at baseline are 0.05min = 3sec, the widths at half height are between 1-2sec. At this level the data acquisition rate of 10Hz is still sufficient. Due to this extreme conditions the reproducibility deteriorate a little.



Reproducibility Test at 15000 PSI and 2ml/min, 10 Replicates

Name / RSD [%]	Ret.Time	Area	Height
Uracil	2.08	1.26	2.06
2- Acetylfuran	0.48	1.23	1.95
Acetanilid	0.48	2.69	2.15
Acetophenon	0.43	2.31	2.18
Propiophenon	0.41	2.44	2.18
Butylparaben	0.38	0.59	1.79
Benzophenon	0.37	2.53	1.92
Valerophenon	0.35	2.60	2.19

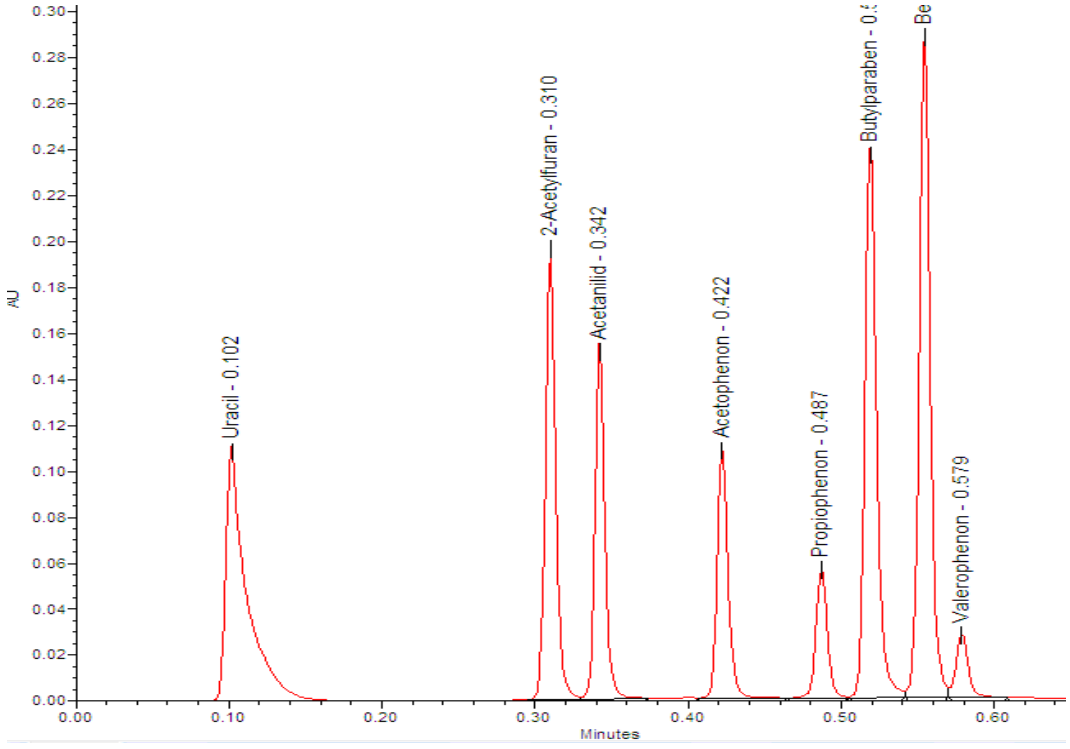
Reproducibility Results Table at 15000 PSI and 2ml/min, 10 Replicates, raw data

3.3 Alliance UHPLC Tests at 8000 PSI, 4ml/min

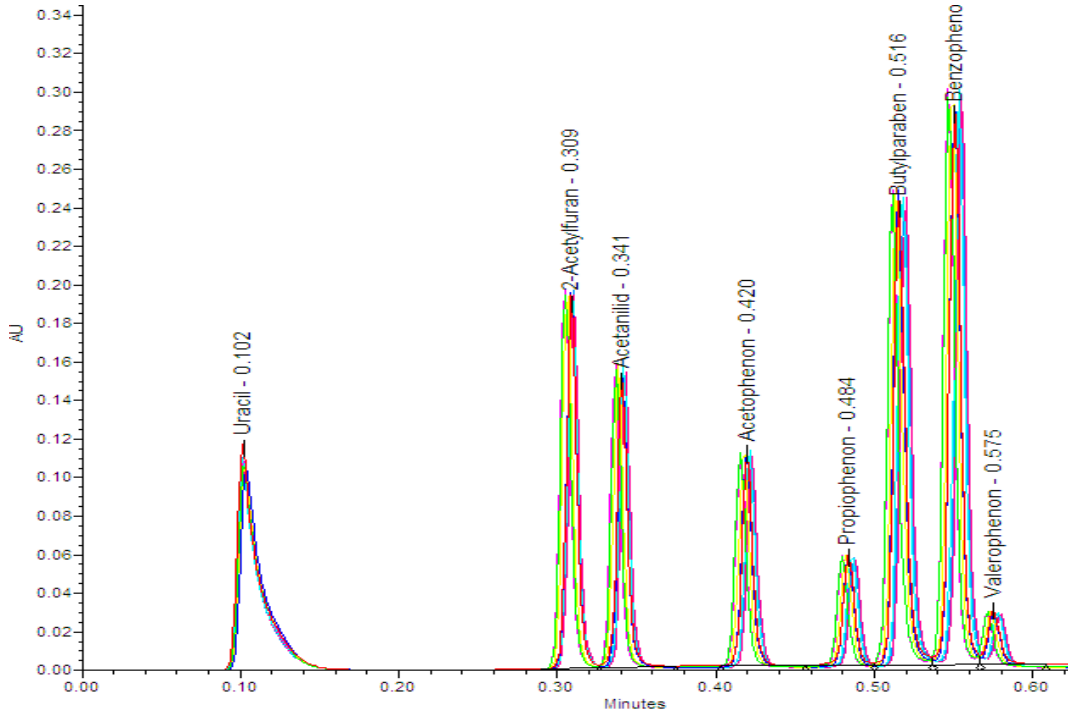
Maybe you will ask why the pressure is only 8000 PSI at a flow rate of 4ml/min? The answer is, we changed to a Symmetry C18, 3.5µm, 2.1x50mm column. This allows us to run the double flow rate of the Acquity on our Alliance. If you really have the need for speed you can run your analysis under turboflow like conditions. Maybe you are impressed by the previous results, but we have still not reached the bottom.

Time	Flow [ml/min]	A [%]	B [%]	Curve
0.0	4.0	100	0	-
0.1	4.0	80	20	6
0.6	4.0	0	100	6
1.0	4.0	0	100	6
1.2	4.0	100	0	6
15	0	95	5	11

Gradient Table for the 8000 PSI Ultra Flow Test



Chromatogram at 8000 PSI and 4ml/min



Reproducibility Test at 8000 PSI and 4ml/min, 10 Replicates

Name / RSD [%]	Ret.Time	Area	Height
Uracil	0.73	3.36	3.25
2- Acetylfuran	0.71	0.4	0.54
Acetanilid	0.71	1.07	0.98
Acetophenon	0.68	0.65	0.69
Propiophenon	0.63	1.32	1.02
Butylparaben	0.62	0.78	0.81
Benzophenon	0.58	0.82	0.85
Valerophenon	0.55	2.25	1.19

Reproducibility Results Table at 8000 PSI and 4ml/min, 10 Replicates, raw data

4 Discussion

The Performance Tests in chapter 3 show that the concept of the Alliance can compete with the Acquity. It further shows that UPLC and HPLC can be combined in a perfect way by using the upgraded Alliance.

For the analyst in the lab an extended pressure range makes separation problems easier by using the latest column technology. Higher flow rates are also possible to reduce the analysis time.

Please have a look at the chromatogram in chapter 3.3. It shows a separation of the 8 component mix in 0.6min, that are only 36 seconds! This was done on a Alliance 2690 with a 2487 UV detector. For our example, an optimized method would not take longer than 1.5min total cycle time and 40 samples per hour could be run.

Any questions left? Feel free to contact us.

5 Appendix

Raw Data Reports

TN0007_1_UHPLC_System_Precision.pdf

TN0007_2_UHPLC_Gradient_Performance.pdf

TN0007_3_Overlay_Report_UV_1ml_min.pdf

TN0007_4_Overlay_Report_UV_2ml_min.pdf

TN0007_5_Overlay_Report_UV_Ultra_Fast.pdf