■Reproducibility for Alkylphenone and BSA Digest by UHPLC

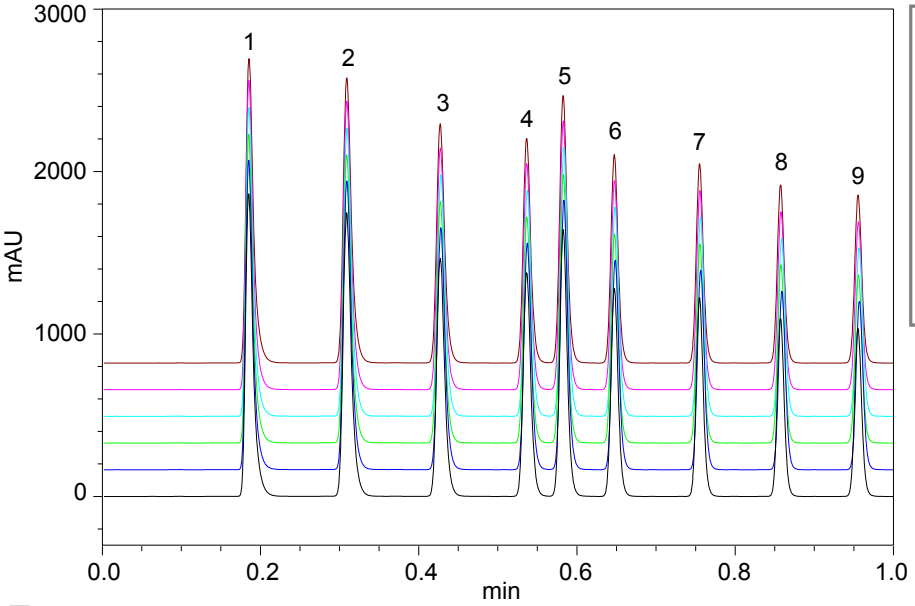
AS/LC-034

Good data reproducibility is extremely important for liquid chromatography analysis.
The good retention time reproducibility of an eluted peak is important for the qualitative analysis of a component whereas the good area reproducibility of an eluted peak is important for quantitative analysis accuracy.
Alkylphenones and BSA digest were used as the model samples for these reproducibility measurements.
Alkylphenones are often used to evaluate the water/acetonitrile gradient with an ODS column and the analysis time is characteristically short.
BSA digest is sometimes used to evaluate the "peptide mapping method" as many peptide peaks are eluted. Peptide mapping is a method to analyze the changes of protein-constituting amino acids by separating peptide fragments formed by digesting proteins with enzymes using LC, etc. and then, comparing the chromatographic patterns. As many peptide peaks will emerge, it is important that high resolution can be achieved and also a good reproducibility is obtained for the peak retention time and the peak area.
With these two contrasting model samples, the evaluation of the reproducibility was performed by using Hitachi ultra high performance chromatograph, ChromasterUltra Rs.

(Optional, Includes the parts prepared by a customer)

ChromasterUltra Rs

■Example of Repeated Measurements of 9 Alkylphenones (100 mg/L each) (n=6)



＜Analytical Conditions＞
Column : LaChromUltra Ⅱ C18 (1.9 μm) 2.0 mm I.D. × 50 mm
Eluents : A) H_2O B) CH_3CN 55 % B (0 min) → 95 % B (0.8 min)
Flow rate : 0.8 mL/min
Column temperature : 40°C
Detection wavelength : UV 247 nm (DAD)
Injection vol. : 2 μL

＜Component Names＞
1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone
7. Hexanophenone
8. Heptanophenon
9. Octanophenone

■Peak Retention Time and Area Reproducibility (n=6)

【Retention Time】

Peak No.	1	2	3	4	5	6	7	8	9
Average	0.185	0.309	0.428	0.537	0.583	0.648	0.756	0.858	0.956
SD	0.00016	0.00017	0.00034	0.00041	0.00044	0.00050	0.00062	0.00071	0.00072
%RSD	**0.084**	**0.054**	**0.079**	**0.076**	**0.076**	**0.077**	**0.082**	**0.083**	**0.076**

【Area Value】

Peak No.	1	2	3	4	5	6	7	8	9
Average	1290537	1333686	1086868	968789	1130751	862629	801602	710796	682375
SD	2307	1668	971	974	1049	1135	754	973	618
%RSD	**0.179**	**0.125**	**0.089**	**0.101**	**0.093**	**0.132**	**0.094**	**0.137**	**0.091**

By using an UHPLC column, it was possible to separate 9 alkylphenones within 1 minute. When the measurement was repeated 6 times, the peak retention time reproducibility (% RSD) was not more than 0.054 - 0.084% and the peak area reproducibility was not more than 0.089 - 0.179%, indicating the extremely good reproducibility. It was also found that the highly reliable analysis is possible even with the short-time gradient analysis.

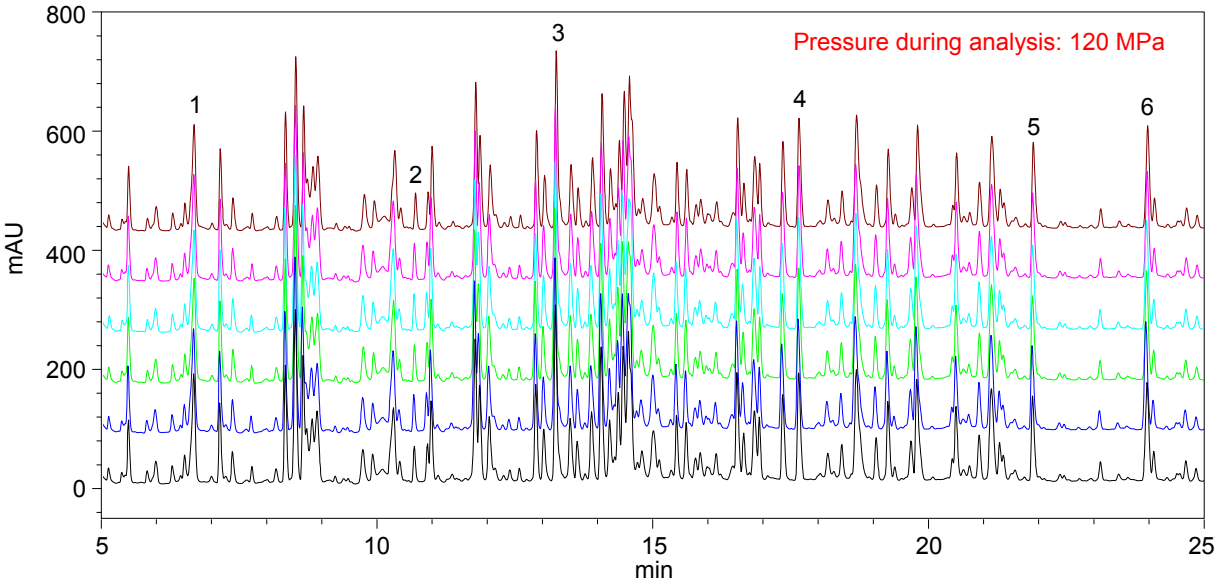
◆New Double Cork Mixer and Low System Volume Structure◆

Structure of Double Cork Mixer (Volume: 55 μL)

The binary pump is installed with a new double cork mixer (volume of 55 μL). With the application of the micro-fluid technology, the efficient solvent mixing and low volume were achieved. As a result, a stable baseline can be obtained even with a gradient analysis and analysis can be performed with good reproducibility. The system design with low mixer and tubing volumes also allows the excellent tracking capability for a gradient program with a short analysis time.

■Reproducibility for Alkylphenone and BSA Digest by UHPLC

■Analysis Example of BSA (Bovine Serum Albumin) Digest



<Analytical Conditions>

Column	:	LaChromUltra IIC18(1.9 μm) 3.0 mm I.D. × 250 mm
Eluents	:	A) 0.05 % TFA / H_2O(v/v)
		B) 0.05 % TFA / CH_3CN(v/v)
		5 % B (0 min) → 45 % B(30 min)
Flow rate	:	0.85 mL/min
Column temperature	:	40°C
Detection wavelength	:	UV 214 nm(DAD)
Injection vol.	:	5 μL

■Peak Retention Time and Area Reproducibility (n=6)

【Retention Time】

Peak No.	1	2	3	4	5	6
Average	6.680	10.679	13.234	17.648	21.888	23.960
SD	0.004	0.011	0.010	0.006	0.007	0.012
%RSD	**0.060**	**0.103**	**0.076**	**0.034**	**0.032**	**0.050**

【Area Value】

Peak No.	1	2	3	4	5	6
Average	762565	173294	1086195	711222	515865	688446
SD	5203	1727	8110	2389	2425	14254
%RSD	**0.682**	**0.997**	**0.747**	**0.336**	**0.470**	**2.070**

By using an UHPLC column, peptides were separated. When the measurements of the above 6 eluted peaks were repeated 6 times, the peak retention time reproducibility (% RSD) was 0.032 - 0.103% and the peak area reproducibility was 0.336 – 2.070%, indicating the extremely good reproducibility.
The pressure at the time of the analysis was 120 MPa. It was indicated that with the new LBT control technology, the highly reliable chromatographic pattern analysis can be performed even under this high pressure level.

◆New LBT Control Technology of 6170 Binary Pump◆

LBT Control (Liquid Beat Technology Control)
LBT control is a control technology to precisely control the pressure to lessen the pressure variation during the solvent delivery while automatically correcting for the bulk modulus of the solvent. As a result, solvents can be delivered stably regardless of solvent types under the wide pressure range from a low pressure to a high pressure of 140 MPa.

Main system configuration : ChromasterUltra Rs DAD system
(6170 Binary Pump, 6270 Autosampler, 6310 Column Oven, 6430 Diode Array Detector, Organizer)

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.