



Reproducibility of Retention Times and Peak Area in a UHPLC System (X-LC®)

JASCO, Inc.

With recent advances in technology and the use of ultra high pressure chromatography systems becoming more commonplace, it is of use to compare reproducibility of retention time and peak area of UHPLC vs HPLC.

Experimental

The X-LC utilized in this experiment was a JASCO X-LC system consisting of a 3185PU pump, capable of pumping 15,000 PSI, 3080DG degasser, 3067CO column, 3070UV UV/Vis detector, 3159AS autosampler allowing fast cycle time and low carryover with the ability to operate at very high pressures, column X-PressPak C18S (2.1 mm × 50 mm), 2 micron particle size and EZChrom Elite chromatography data system, vers.3.2.1.

A standard mixture contains acetaminophen, caffeine, aspirin, and benzoic acid.

Results and Discussion

The chromatogram in Figure 1 is a series of 15 injections overlaid. Excellent reproducibility is obtained on the X-LC system as well as a shortened analysis time of one min.

Table I lists the retention time and peak area Relative Standard Deviation (RSD) of retention time. The RSD is in a range between 0.09–0.26% and that of peak area in a range between 0.38–0.86%. These results demonstrate that UHPLC and the use of X-LC hardware as being extremely reproducible and reliable, and with confidence one can replace any HPLC analysis with this technique.

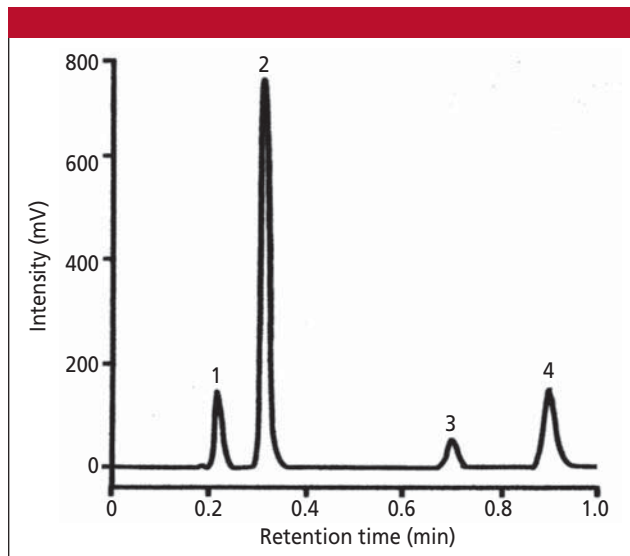


Figure 1: Overlaid chromatograms of the standard mixture.

Peaks: 1=acetaminophen (0.1 mg/mL)
2=caffeine (0.1 mg/mL)
3=aspirin (0.1 mg/mL)
4=benzoic acid (6.0 mg/mL)

Chromatographic conditions:

column=X-Presspak C18S (2,1 mm I.D. × 50 mm L., 2 μm)
mobile phase = H₂O/CH₃CN/CH₃COOH (75/25/3)
column temperature = 45°C
flow rate = 0.6 mL/min
detection wavelength = 275 nm
injection volume = 1 μL

Table I: Reproducibility of retention times and peak area (n=15)

	Reproducibility at hold time			Reproducibility in peak area		
	Average (min)	Standard deviation (min)	Relative standard deviation (%)	Average (μV*sec)	Standard deviation (μV*sec)	Relative standard deviation (%)
Acetaminophen	0.214	0.00055	0.2570	310973	1239	0.3985
Caffeine	0.308	0.00070	0.2273	2E + 06	9656	0.5322
Aspirin	0.698	0.00075	0.1074	188500	1615	0.8568
I.S.	0.895	0.00080	0.0089	536004	2005	0.3740

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