The Application of UHPLC to Reduce Sample Analyses Times

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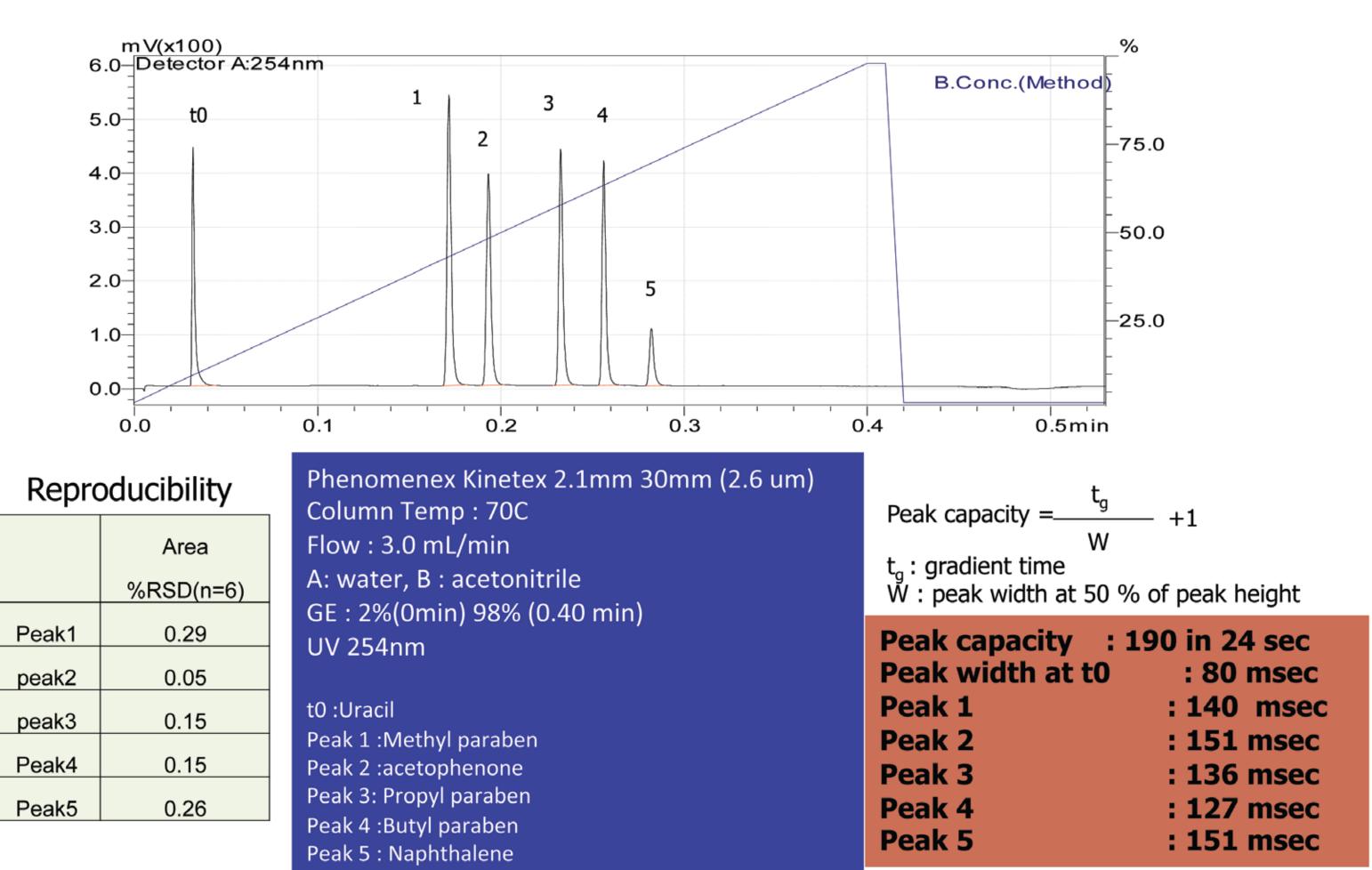
Introduction

The use of UHPLC technology has continued to increase in LC applications as a way to reduce sample analysis turnaround times. Maintaining or increasing resolution of faster separations has been made possible with the use of small particle column technology. UHPLC often provides run times of less than one minute with peak widths of less than 200 milliseconds that have created challenges for system performance and detection methods. A variety of applications in pharmaceutical and environmental areas with small particle columns will be presented that will demonstrate the throughput and performance that can be obtained with current UHPLC technology.

UHPLC Requirements for Fast Analysis

- High-speed data acquisition for narrow peak widths of UHPLC for UV and MS detection.
- Low carryover to minimize time needed for rinsing.
- Fast injection speeds to reduce cycle times.
- No loss of system performance at high speeds compared to conventional LC.

UHPLC Peak Widths



UHPLC Impurity Evaluation

Zorbax Eclipse RRI

2.1mm, 100mm, 1.8

B : acetonitrile

: B 25% → 40 % (0.1

→ 70 % (3.0 min)

: 0.8 mL/min

Wide dynamic range and high sensitivity

- Main compound: diazepam
- Two compounds (impurities) were spiked into the diazepam solution.
- The impurity samples were prepared f be 0.5, 0.05, 0.005 % w/w relative to the main compound.

Mobile phase : A : water

Gradient

Flow rate

Column temp. : 30C

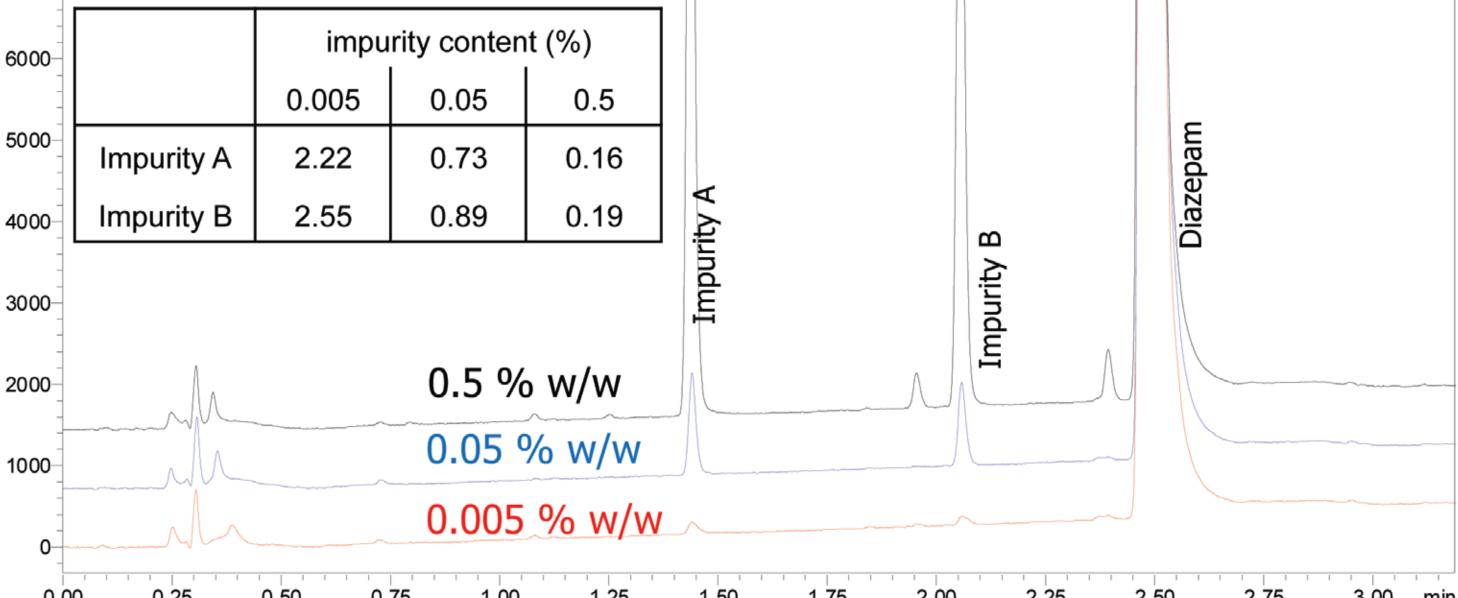
Detection : 240 nm

	2000-									Ę		
d to	1500									Diazepam		
)	1000									Dia		
	500											
) C18		0.25	0.50	0.75 1	.00 1.25	1.50	1.75	2.00	2.25 2	.50 2.75	min	
n	6 ^{mAU}				[ZO	ЭМ					
in)	5 4	0.05	%w/v	v		200				E		
,	3- 2- 1-					Impurity A		Impurity B		Diazepam		
	0	m				Π						
	- <u> </u> 0.00	0.25	0.50 0).75 1.0	00 1.25	1.50	1.75	2.00 2	2.25 2.5	50 2.75	min	

UHPLC Sensitivity Evaluation

UHPLC systems can provide higher sensitivities since sharper peaks are obtained. Peak area RSD's were evaluated at low level impurity concentrations down to 0.005%. The wide dynamic range in UHPLC systems allows simultaneous assay and impurity analysis.

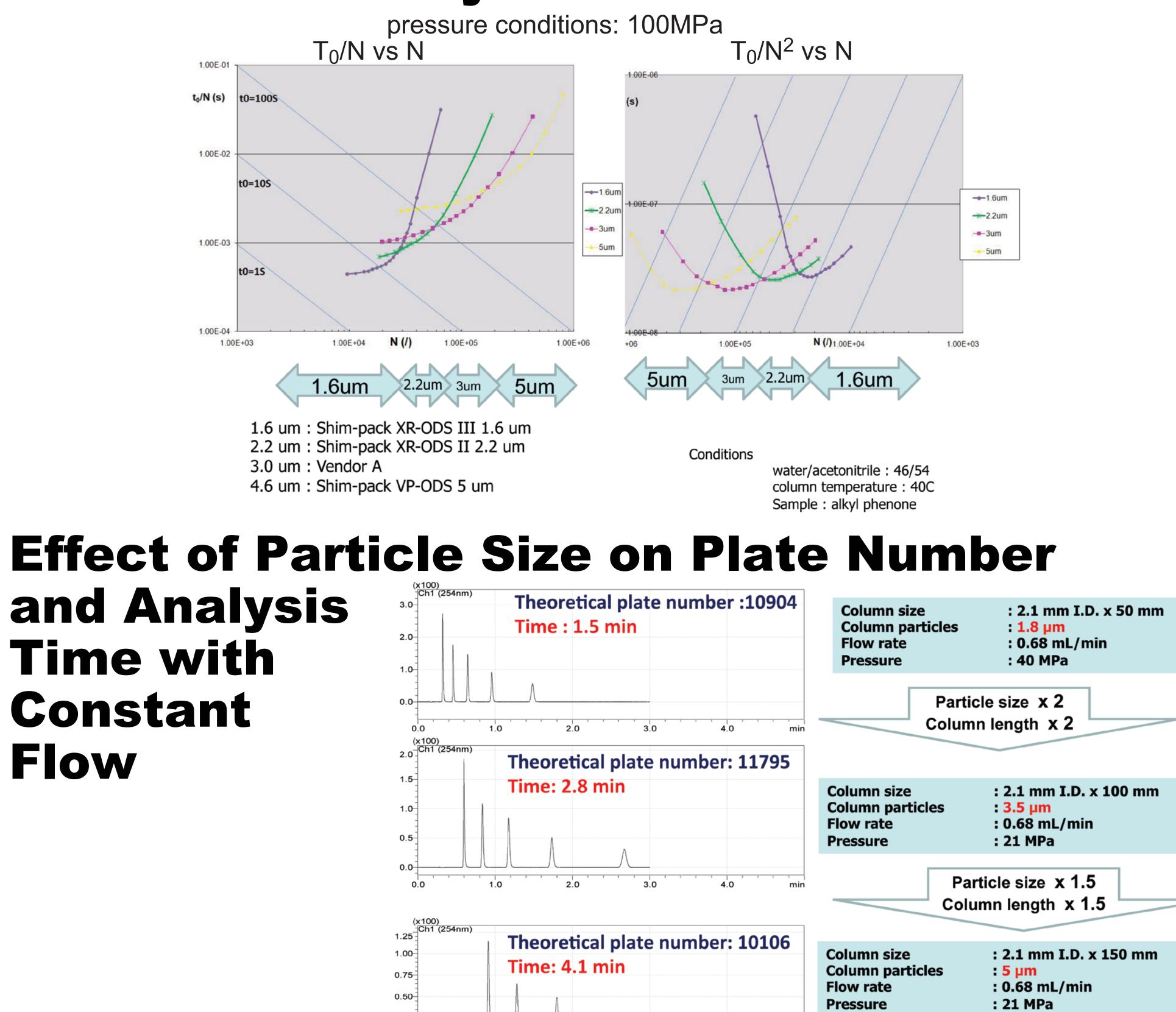
Repeatability of peak area, (% RSD, n=6, 6 uL injection) LOD: 0.001 % (9 pg on column)



Kinetic Plots

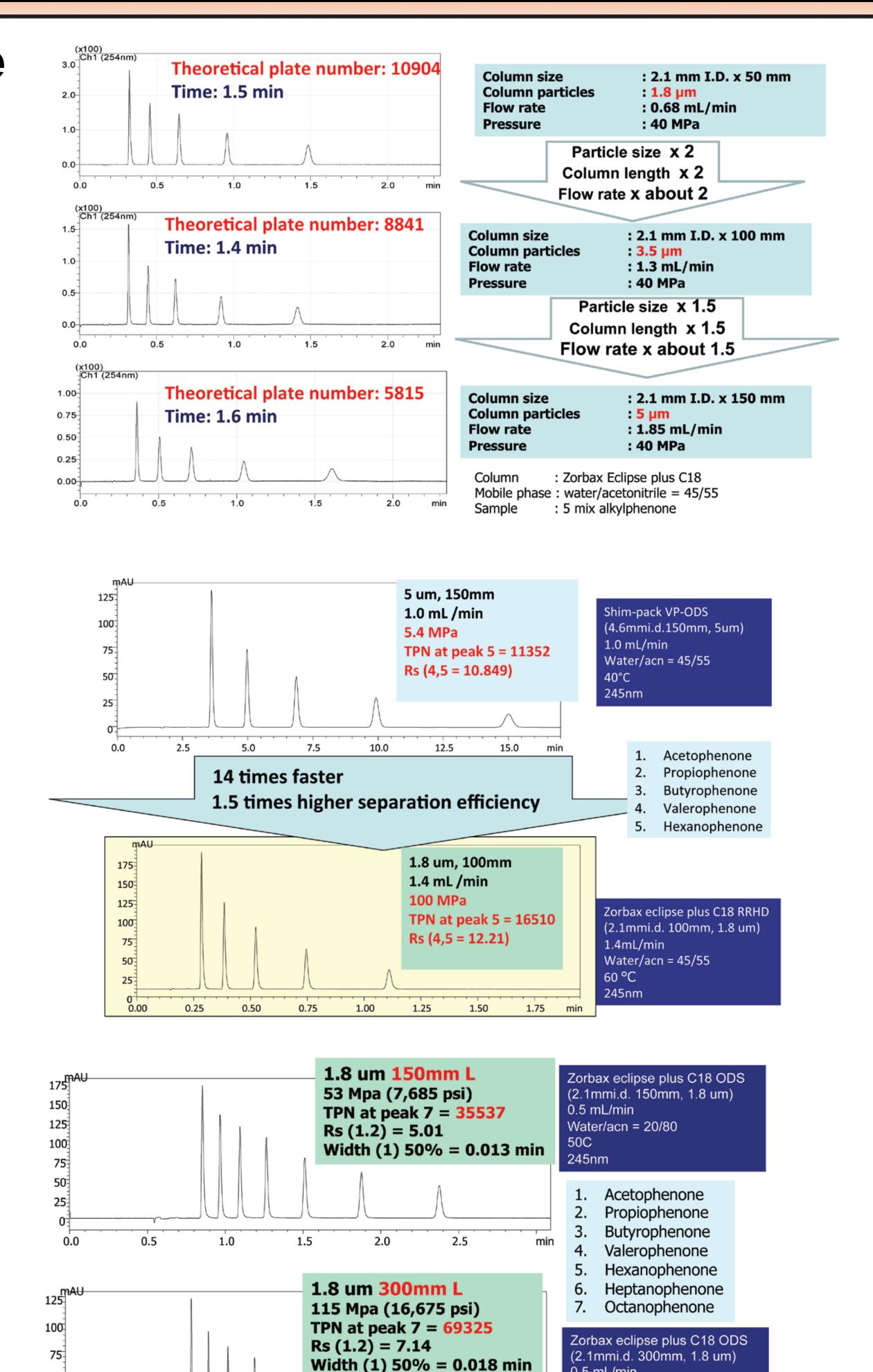
Kinetic plots allow comparisons to determine the optimum particle size to use for your analyses. The following plots compare 1.6, 2.2, 3 and 5 um particle size columns. In general, small particle columns outperform large particles for short run times, while large particles run with low flow rates over an extended period of time can provide the highest efficiency.

Kinetic Plot Analysis



Mobile phase : water/acetonitrile = 45/55

Effect of Particle Size on Plate Number and **Analysis Time** with Constant Pressure



Ultra-high Pressure Evaluation

Increasing

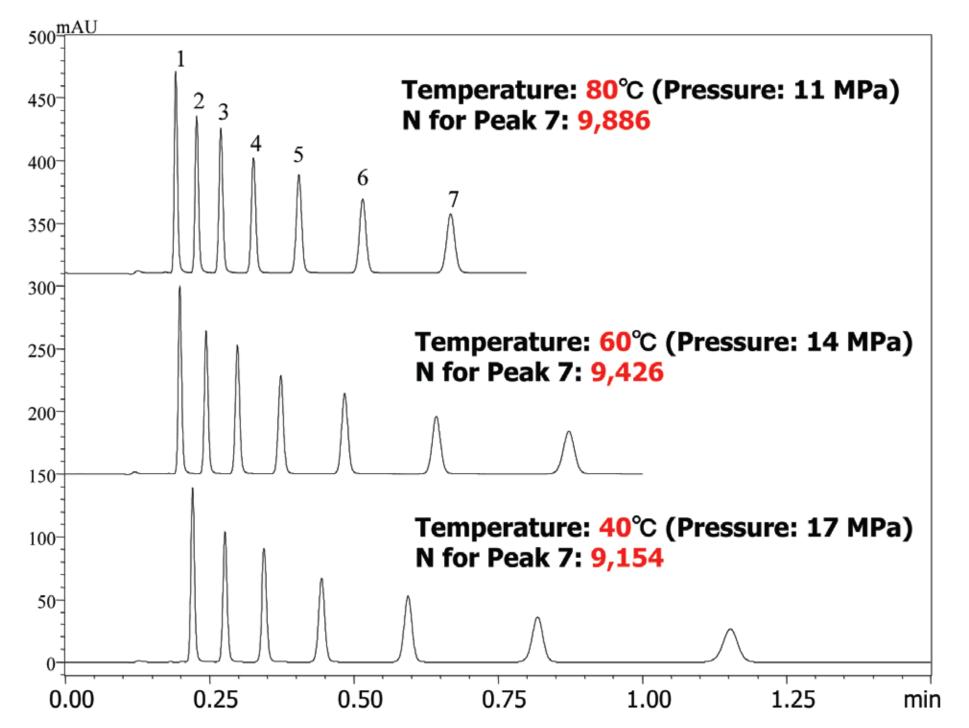
Speed and

Resolution

Theoretical plate number is not compromised at ultra-high pressures.

UHPLC with Elevated Temperatures

Column efficiency improves as column temperature increases Increased theoretical plate number Decreased column pressure



Shim-pack XR-ODS (3.0mm i.d., 50mmL, 2.2 um) Flow rate : 1.5 mL/min

5 mL/min

ter/acn = 20/80

Mobile phase: water/acetonitrile (3/7, v/v), Detection: absorbance at 245 nm. Peaks; 1: acetophenone 2: propiophenone 3: butyrophenone 4: balenophenone 5: hexanophenone 6: heptanophenone 7: octanophenone

UHPLC/MS Requirements

Mass spectrometer requirements for UHPLC:



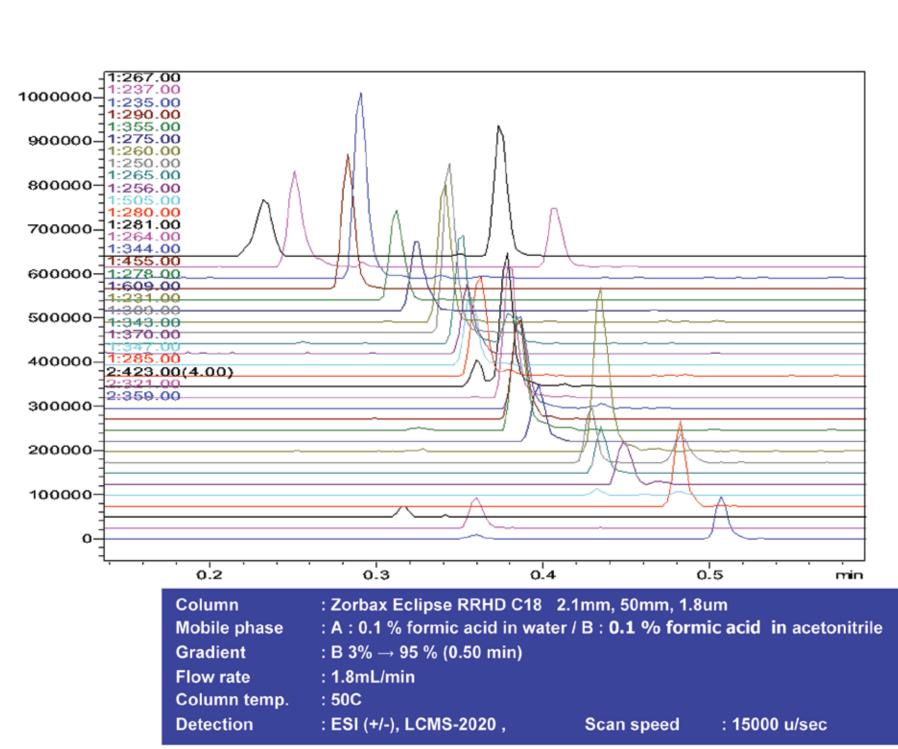
What makes an MS instrument suitable for UHPLC? The ability to acquire data at high speed without sacrificing data quality.

The three things that enable high-speed analysis:

- The ability to perform scan measurement at high speeds scanning up to 15,000 u/sec
- The ability to switch between positive and negative ion measurement at high speed polarity switching in 15 msec
- High sensitivity in high-speed measurement design to prevent loss of sensitivity in high-speed analysis

UHPLC with High Speed MS

Nexera-LCMS-2020 Analysis of 30 pharmaceuticals by high-speed polarity switching (15 msec) and high-speed scanning technology (15,000 u/sec)

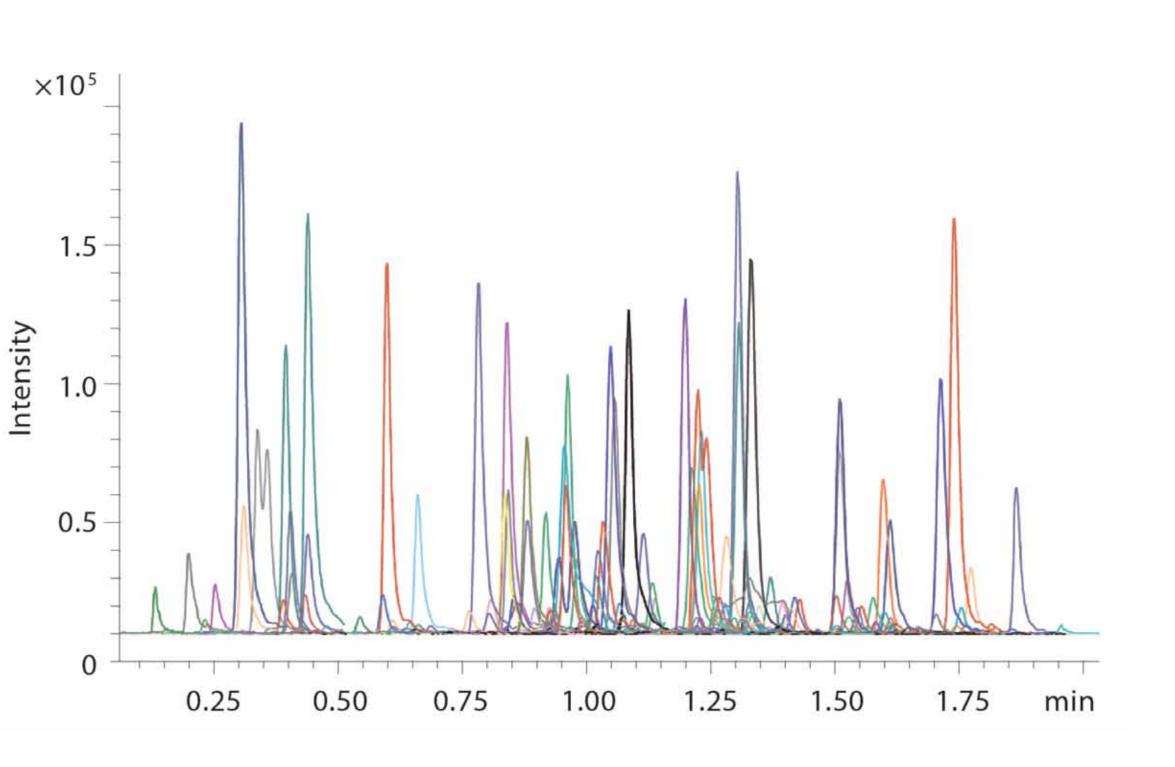


Atenolol (267) Procaine (237) Lidocaine (237) Atropine (290) Yohimbine (355 Chlorpheniramine (Propranolol (260) Alprenolol (250) Tetracaine (265) Diphenhydramine (Doxepin (280) Dipyridamol (505 Desipramine (26 Imipramine (281 5. Nortriptyline (26 Amitriptyline (27 Dibucaine (344 Verapamil (455 Reservine (609) Carbamazepine (2 Isopropylantipyrine Alprazolam (309) Cilostazol (370 Nifedipine (347 Diazepam (285 Warfarin (309) **Negative** 1. Cefuroxime (42) Chloramphenicol (321
Nitrendipine (359)

UHPLC/MS/MS Data

Standard Chromatogram of 226 Pesticides in Two Minutes

Ultra fast polarity switching (15 msec), a high-speed scanning rate of 15,000 u/sec, and Ultra fast MRM transitions allows full spectrum scans within a series of MRM measurements, providing confirmation of target compounds with information-rich product ion spectra.



Conclusions

- Reproducibility data with UHPLC conditions can match or exceed those of conventional HPLC conditions.
- Columns with 1.6 um particle size provided the highest performance for fast analytical runs in the comparison.
- Increased temperature can provide benefits in speeding up analysis times without loss of performance.
- MS scanning speeds of 15,000 u/sec and polarity switching speeds of 15 msec can meet the needs of narrow UHPLC peak widths.