PRACTICAL LIMITS OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-CURRENT STATUS

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INTRODUCTION

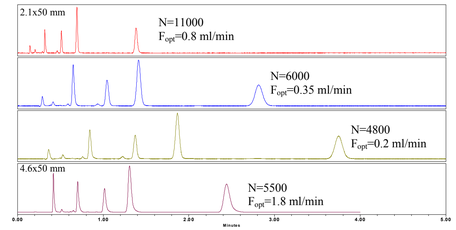
During the last few years instrument and column manufacturers introduced several new approaches to improve performance in HPLC practice. Instrumentation for high temperature isothermal and thermal gradient LC and recently an extended pressure limit (15000 PSI, 1000 atm), low dispersion Ultra Performance Liquid Chromatography (UPLC) instrument became available. A new generation of small particle packed columns (<2.0 µm) are now available from several manufacturers to accommodate the need for shorter analysis time and better resolution.

This presentation gives an overview of different liquid chromatography techniques. It compares performance that can be achieved under traditional HPLC, High Temperature LC and high pressure, Ultra Performance Liquid Chromatography (UPLC) conditions. Advantages and challenges of these techniques are compared. The physico-chemical properties of small particles and the chromatographic performance of small particle packed columns are presented.

COMPARISON OF LC TECHNIQUES

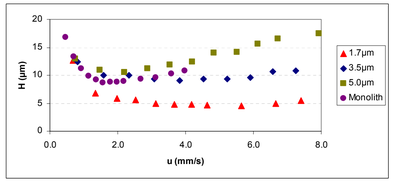
	Current Limits	Particle Size [µm]	Optimum Reduced Linear Velocity (chromatographic)	Interstitial Porosity	Specific Permeability [m^2] B	Optimum Separation Impedance E	Number of Theoretical Plates N	Number of Theoretical Plates Produced in Unit Time at k'=4 N/t	Advantages	Disadvantages
HPLC Porous Particle Packed Columns	6000 PSI 3000 PSI* 10 ml/min 10 Hz	1.8-10	3-5	0.38-0.42	$4x10^{-15}$-$8x10^{-14}$	3000-4500	25000	800	Wide selection of packing materials with different chemistry Multiple vendors of instruments	Slow
HPLC Porous Monolith Columns*		1-2 (skeleton size)		0.6-0.8	$7x10^{-14}$-$1.2x10^{-13}$	500-1000	25000	3000	Fast analysis Minimum sample clean up	Limited availability of stationary phases 4 um particle packed column performance; Solvent consumption
High Temperature (60-200 °C) HPLC Porous Particle Packed Columns		3-10		0.38-0.42	$7x10^{-15}$-$1.2x10^{-13}$	3000-4500	25000		High efficiency separations	Limited availability of stable stationary phases Analyte degradation
High Pressure LC (UPLC) Porous Particle Packed Columns	15000 PSI 2 ml/min 40 Hz	1.5-2.0		0.36-0.40	$2-3.5x10^{-15}$	3500-5500	25000	9000	High efficiency fast separations	Limited availability of stationary phases and packed columns Single vendor of instrument

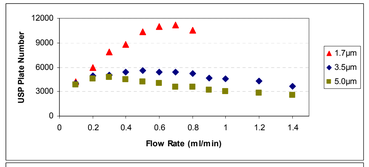
Chromatograms Obtained on 1.7, 3.5 and 5.0 µm Particle Packed and on a Monolith Column at Optimum Flow Rate (F_{opt})

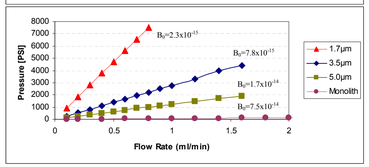


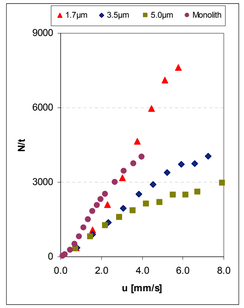
Columns: 2.1x50 mm ACQUITY UPLC™ BEH C18; 2.1x50 mm 3.5 µm XTerra MS C18; 2.1x50 mm 5.0 µm Symmetry C18; 4.6x50 mm Chromolith C18 (Merck)

Chromatography Performance of 1.7, 3.5 and 5 µm Particle Packed Columns and Monolith



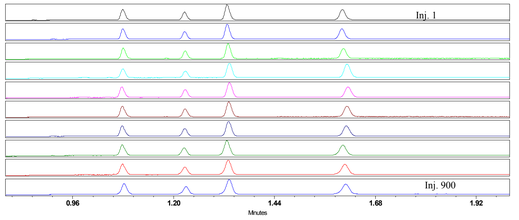






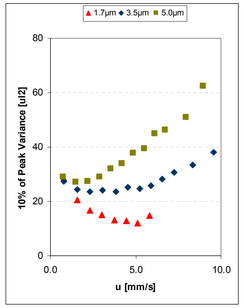
Columns: 2.1x50 mm ACQUITY UPLC™ BEH C18; 2.1x50 mm 3.5 µm XTerra MS C18; 2.1x50 mm 5.0 µm Symmetry C18; 4.6x50 mm Chromolith C18 (Merck) Solvent: Acetonitrile-Water 70:30 (v/v) Temperature: 25.0 °C; Solute: Hexylbenzene

Stability of 1.7 um Particle Packed Columns Under Fast Gradient Conditions



Column: 2.1x100 mm ACQUITY UPLC™ BEH C18; Gradient: from 5% AcN to 95% AcN in 0.5 min, at 95% AcN for 1.2 min, to 5% AcN in 0.1 min; Maximum pressure: 11000 PSI Temperature: 30 °C; Solutes: alkylbenzenes; Instrument: Acquity UPLC (Waters)

Sensitivity to Extra-column Contribution

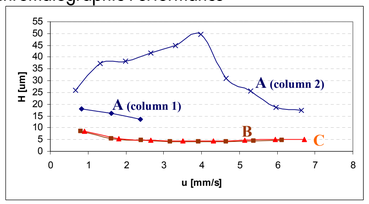


COMPARISON OF POROUS SMALL PARTICLES

Physico-chemical Properties

	Particle Size [µm]	Particle Size Distribution (90/10 ratio)	Specific Surface Area [m^2/g]	Average Pore Diameter [Å]	Specific Pore Volume [cm^3/g]	Particle Shape
Brand 1 **A**	1.44	1.93	174	109	0.53	
Brand 2 **B**	1.75	1.58	185	130	0.68	
Brand 3 **C**	1.79	1.68	196	67	0.36	
Brand 4 **D**	1.44	2.13	188	101	0.52	
Brand 5 **E**	1.66	2.71	201	85	0.51	
Brand 6 **F**	1.70	2.70	182	135	0.66	

Chromatographic Performance



Column size: 2.1x50 mm (except Manufacturer A, Column2: 2.1x100 mm; Solvent: Acetonitrile-Water 70:30 (v/v); Temperature: 30 °C; Solute: Propylbenzene

SUMMARY

Experimental data demonstrate the advantages of using small particles (<2.0 µm) in packed columns. The efficiency and peak capacity in combination with speed of separation can be improved under UPLC conditions compared to traditional HPLC.

Reduced plate heights (2.0-2.5) typical for 3-10 µm particle packed columns could be routinely achieved in high pressure applications. A limited selection of stationary phases with < 2.0 µm particle size is available at this point on the market. The performance of small particle packed columns varies from vendor to vendor.

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