

Rapid, Sensitive, General-Purpose Cleaning-Verification HPLC Methods Using Fused-Core™ Particle (FCP) Columns on Conventional Instrumentation

S. Bannister¹, M. Talbott¹, F. Hanciles¹, R. Henry²
¹Xcelience LLC, Tampa, FL; ²Consultant, State College, PA



Objective

This work was undertaken to investigate the use of rapid gradients using recently introduced FCP columns on conventional instrumentation in the development of general-purpose methods for cleaning validation. The expected benefits include high sensitivity and reductions in the time needed to set up and run the method.

Background

Cleaning Validation
 Verification of the removal of drug residue from multi-product manufacturing equipment is required by GMP regulations and the suitability of applied analytical methods is judged with a combination of sensitivity, selectivity - and because the release of equipment is dependent - speed. The FDA does not set quantitative acceptance specifications, but the commonly used limit is based on not more than 0.1% of a dose carried over into a single dose of the next product. Translation of this into an analytical limit combines the total product contact area, the mass (or volume) of product contacting the surface, the mass (or volume) of each dose unit, the sampled area, and the fraction of the verification sample used for analysis. The requisite limits are commonly measured in ng/mL. The ubiquity of HPLC in drug analysis makes it an attractive choice for cleaning validation. Methods qualified for cleaning validation are often adaptations of drug-substance methods. These methods are capable of determining the drug and its related impurities, but the ability to simultaneously measure multiple closely related analytes comes at the expense of run time and is not needed in cleaning validation.

HPLC Analytical Performance
 Resolution, limits of detection and quantitation, and run-time in HPLC analyses are improved by reducing the width of eluted bands. Contributions to bandwidth include both column (particle size, packing structure and resistance to mass transfer in the stationary and mobile phases) and extracolumn volumes (injection, unswep and tubing). Columns packed with 5-µm fully porous particles have been the standard for conventional HPLC for twenty-five years. Smaller-particle packings (3-µm) have been available almost as long and offer higher efficiency (lower band dispersion) on conventional instrumentation, but require higher pumping pressures due to lower bed permeability. Efficiency can be further increased by the use of particles smaller than 3 µm but only with the use of instrumentation optimized with respect to both pressure and extra-column effects.

Fused-Core™ Particles
 Kirkland, Langlois and DeStefano recently described reverse-phase packings based on 2.7-µm silica particles in which a 0.5-µm layer of 90-Å porous silica has been deposited onto a 1.7-µm solid spherical core¹. Advantages of columns packed with these particles include high efficiency, lower backpressure due to a very narrow particle-size distribution, and smaller efficiency losses with increasing velocity due to improved mass-transfer kinetics in the shallow porous layer. The narrow particle-size distribution allows the use of larger-pore column frits which combined with the greater stability of the packed bed should produce longer column lifetimes in routine use.

Gradient Elution
 The high resolving power of gradient elution in the analysis of closely related substances is the result of the reduction of peak width as a band moves through the column: the back of the band is accelerated by the stronger solvent. A broad gradient will elute a wide range of substances and a steep gradient will elute them quickly. Gradient steepness is analogous to solvent strength in isocratic elution; as it increases, elution times decrease and sensitivity increases because bands are narrower. Since instrument control and data acquisition is entirely in the time domain, gradient steepness is generally expressed in units of %B/min, but G_s is a more general expression.

$$G_s = \frac{V_m (\Delta\%B)}{F \cdot t_G}$$

where V_m is the column dead volume in mL; $\Delta\%B$ is the gradient range; F is the flow rate in mL/min; and t_G is the gradient time in minutes. G_s has units of %B/(column volume) and allows comparisons of separations in which column dimensions and/or flow rate have been changed².

Instruments and Materials

HPLC Columns 150x4.6-mm 5-µm Ascentis® C18; 100x4.6-mm 3.0-µm Ascentis C18; and 100x4.6-mm 2.7-µm Ascentis Express C18 from Supelco (Bellefonte, PA). **Chemicals** Drug Standards were used as received from Sigma (St. Louis, MO); acetonitrile was HPLC grade (Malinkrodt, Phillipsburg, NJ); KH_2PO_4 HPLC grade was from Fisher Scientific, Waltham, MA. Water was drawn as needed from an E-pure® point-of-use purifier (Barnstead/ThermoLyn, Dubuque, IA); H_2PO_4 and triethylamine were HPLC grade (E. Merck, Darmstadt, Germany); and octane sulfonic acid was HPLC grade (Baker, Phillipsburg, NJ). **Instruments** Series 1100 and 1200 HPLC (Agilent Technologies, Santa Clara, CA) and Alliance® 2695 HPLC (Waters Corporation, Milford, MA) all with autosamplers, vacuum degassing modules, quaternary gradient pumps, and photodiode array UV absorbance detectors. **Instrument Control And Data Acquisition** Galaxie™ CDS (Varian, Inc. Walnut Creek, CA) or Empower™ 2 CDS (Waters Corporation)

Effect of Hardware on Band Dispersion

Non-optimized system performance was measured with 60% ACN isocratic elution of 10-µL injections of a four-component test mix in mobile phase using the 5-µm and 2.7-µm columns on conventional instruments with analytical flowcells. Non-optimized system performance (efficiency shown here) is similar to that seen in the column manufacturer's test for the larger-particle conventional column but significant losses are seen for the 2.7-µm FCP column on all three instruments.

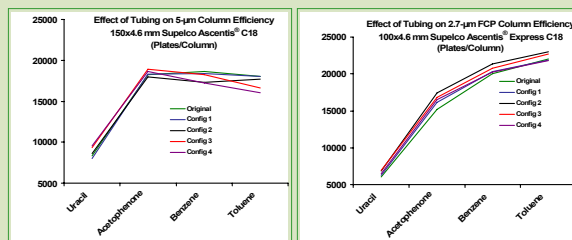
Column	Agilent 1100	Agilent 1200	Waters 2695	Column Manufacturer's Test Data
Ascentis C18 150x4.6-mm 5-µm	16911	18034	16874	18119
Ascentis Express (FCP) C18 100x4.6-mm 2.7-µm	18649	22001	18666	28164

Extracolumn dispersion was investigated in the Agilent 1200 by changes in the flowcell and the length of 0.007" tubing used between the autosampler (AS) and the column compartment (CC), between the column compartment and the column and between the column and the detector.

Changes in Extracolumn Volumes of Agilent 1200 Flowcell (FC) Autosampler (AS) Column Compartment (CC) Column (C) and Detector (D)

Config	FC mm / µL	AS-CC cm / µL	CC-C cm / µL	C-D cm / µL
Original	10 / 13	90 / 22	12 / 3	40 / 10
1	6 / 5	90 / 22	12 / 3	40 / 10
2	6 / 5	90 / 22	12 / 3	20.5 / 5
3	6 / 5	90 / 22	8 / 2	20.5 / 5
4	6 / 5	30 / 7	8 / 2	20.5 / 5

The plate-count data in the figures below show that extracolumn effects due to the components investigated do not contribute significantly to the width of bands eluted from either the 5-µm conventional column or to those eluted from the 2.7-µm FCP column



Effect of Hardware (cont.)

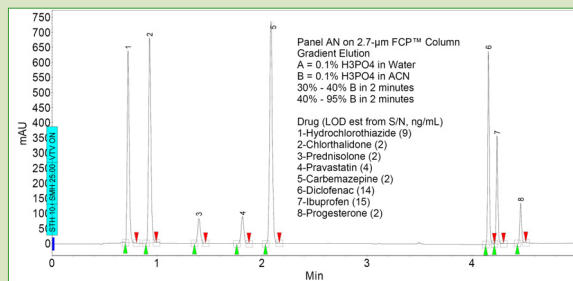
Injection effects were investigated with the 2.7-µm FCP column. Injections (from 1 to 100 µL) of the four-component test mix in MP were made and half-height plates were calculated.

The total effect of precolumn effects was determined by making an injection in very weak solvent so that the band would be concentrated on the head of the column. The test mixture was diluted 1:10 with water and 100 µL were injected. These data suggest that the largest contribution to the dispersion of bands eluted from the 2.7-µm FCP column originates in the injection hardware or the injection process. The table below summarizes the investigation of extracolumn band broadening.

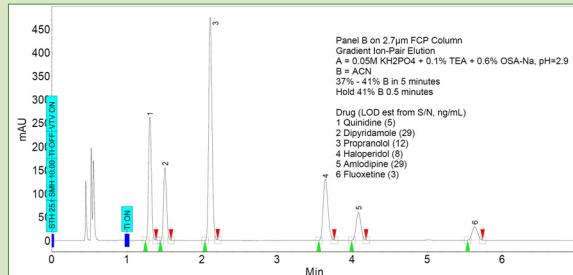
Summary of Toluene Plates from 2.7-µm Supelco Ascentis® Express FCP Column Isocratic Elution with 60% ACN on Agilent 1200 10 µL Injected in Mobile Phase	
Non-Optimized (10 mm / 13 µL flowcell)	22001
Change to 6 mm / 5 µL flowcell	21912
Minimize tubing lengths	22104
*10x sample dilution with 10x volume increase	25738

Separation Development

Using an Agilent 1100 component system with flow rate at 1.76 mL/min and detection at 215nm, a short gradient separation was developed on the 2.7-µm FCP column for each of two panels: eight acidic or neutral drugs (AN) and six basic drugs (B).



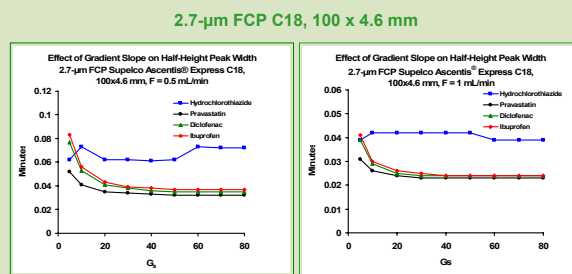
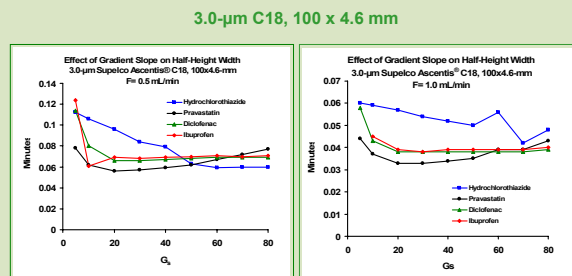
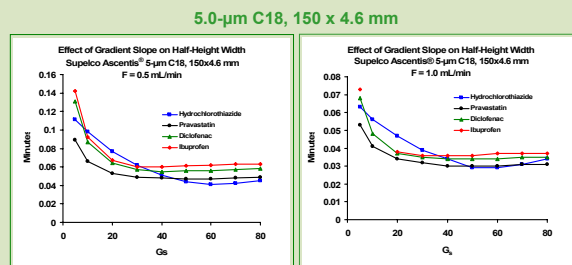
and six basic drugs (B) eluted with a shallow gradient in which the weak solvent contained an ion-pairing reagent.



Effects of F_s , G_s and d_p on Band Dispersion

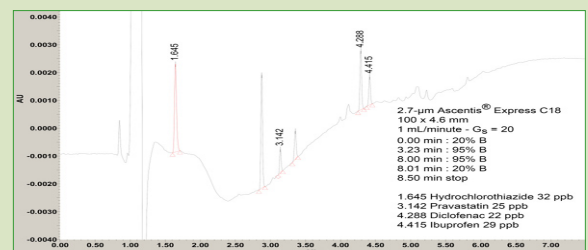
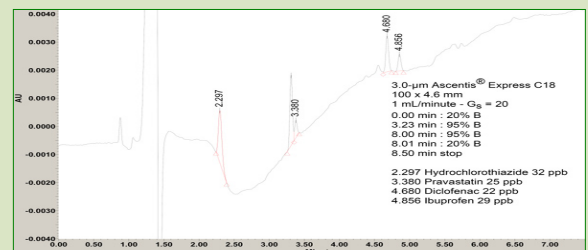
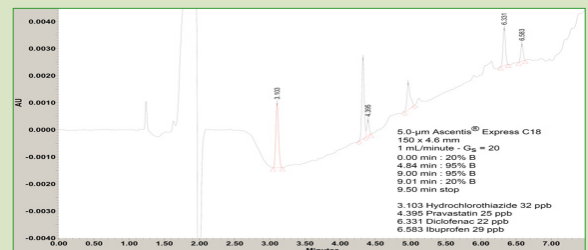
After demonstrating the utility of short gradients for the analysis of a wide variety of compounds, the effects of flow rate and gradient steepness on band width (and therefore on sensitivity and run time) were investigated using four acidic compounds (hydrochlorothiazide, pravastatin, diclofenac and ibuprofen).

These experiments were conducted on an Agilent 1100 HPLC with a standard analytical flowcell (10-mm path length and 13-µL volume) with absorbance detection at 235 nm. Solvent A was water, B was ACN, both had 0.1%(v/v) H_3PO_4 . Flow was set at either 0.5 or 1.0 mL/min for a 20% B to 95% B single-segment gradient. Gradient times were adjusted to achieve G_s values from 5 to 80.



On each column the band width (in minutes) at 1.0 mL/min is approximately half of that at 0.5 mL/min; the volumetric band width is only slightly affected by flow rate. The effect of gradient steepness is much more significant. Band width decreases rapidly as G_s is increased to approximately 20% per column volume on each column at both flow rates, but no further band width (or sensitivity) advantage is seen with further increases in slope.

Demonstration of Selectivity and Sensitivity



Conclusions

2.7-µm FCP reverse-phase particles provide high separation performance on conventional HPLC hardware.

This performance is readily adaptable to analyses such as cleaning validation in which sensitivity, speed of analysis and versatility are performance criteria.

References

- J. J. Kirkland, T. J. Langlois, and J. J. DeStefano, "Fused Core Particles for HPLC Columns," American Laboratory 39 (8), 18-21 (2007).
- L.R. Snyder, J.J. Kirkland, and J.L. Glajch, "Gradient Elution," in *Practical HPLC Method Development*, 2nd edition, Wiley-Interscience, New York, 1997, pp. 350-401

Trademarks and Copyright

Xcelience and the stylized printing of Xcelience are registered trademarks of Xcelience, LLC; Fused-Core is a trademark of Advanced Materials Technology, Inc.; Ascentis is a registered trademark of Sigma-Aldrich Co; Alliance and Empower are trademarks of Waters Corporation; Galaxie is a trademark of Varian, Inc. Copyright 2007 Xcelience, LLC