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Scale-Up of Fast Analytical to Fast Preparative Separations

This article describes a simple, reproducible, and predictable process for scaling up from fast analytical to fast preparative HPLC separations. Chromatographers performing lab scale preparative or larger process scale purifications may use the recommendations discussed. Major considerations for preparative work include ease of scale-up, cost, time, and throughput. The former affects the latter points and is the major focus of this report. Scale-up is simplified by using preparative columns that contain the same smaller diameter packing used in the analytical scout columns. In turn, the time and cost required for method development is reduced. Throughput is increased since loading capacity is higher on the more efficient, smaller diameter packing. Backpressure from smaller diameter packings is not excessive for the shorter, wider columns used in fast preparative separations.

Simple Scale-Up

Smaller, 5µm packings offer distinct advantages over 10µm or larger packings for preparative applications. Successful scale-up from analytical to preparative work is quickly and reliably obtained by using two simple formulas. The resulting chromatography for the preparative column is essentially the same as the analytical column (Figure A). These formulas do not necessarily hold true if the preparative column contains larger diameter packing than the analytical packing:

Loading Capacity: $I_p = I_a \times (D_p/D_a)^2 \times L_p/L_a$,

Where,
I_p = Injection load of preparative column
I_a = Injection load of analytical column
L_p = Length of preparative column
L_a = Length of analytical column
D_p = Internal diameter of preparative column
D_a = Internal diameter of analytical column

Flow Rate: $F_p = F_a \times (D_p/D_a)^2$

Where,
F_p = Flow rate of preparative column
F_a = Flow rate of analytical column
D_p = Internal diameter of preparative column
D_a = Internal diameter of analytical column

Cost and Time

Methods developed on 5µm analytical columns can only be easily transferred to preparative scale columns, using the formulas above, when the same 5µm packing is used in the preparative column. Column costs for 10µm preparative columns can be about 40% less expensive than the corresponding 5µm columns. However, scale-up development time and, therefore, expense is often significantly more for 10µm columns. This is due to variances in

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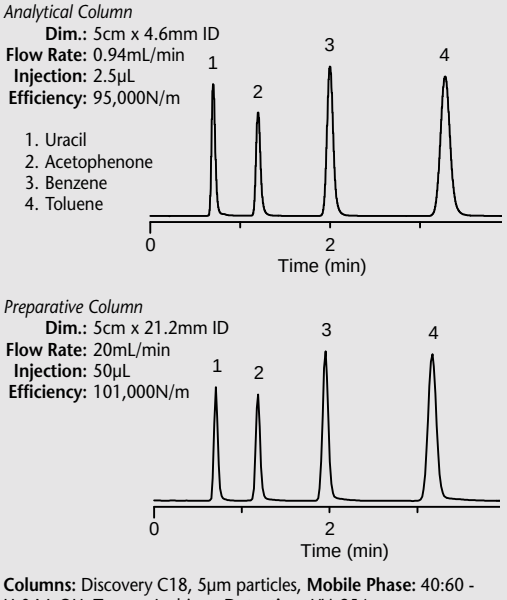
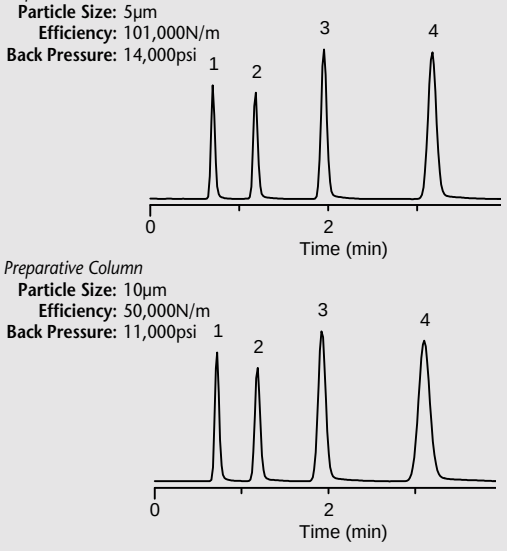
Figure A. Same Chromatography Obtained in Fast Analytical to Fast Preparative Column Scale-Up.



Columns: Discovery C18, 5µm particles, **Mobile Phase:** 40:60 - H_2O:MeOH, **Temp.:** Ambient, **Detection:** UV, 254nm.

Figure B. Efficiency and Backpressure on 5µm vs. 10µm Particles in Preparative Columns.



Columns: Discovery C18, 5cm x 21.2mm ID, **Mobile Phase:** 40:60 - H_2O:MeOH, **Flow Rate:** 20mL/min, **Temp.:** Ambient, **Detection:** UV, 254nm, **Inj.:** 50µL

CONTENTS

SUPELCO
a member of the Sigma-Aldrich family

T200010

Preparative HPLC Column

Preparative HPLC Columns

Discovery and SUPELCOSIL phases in columns up to 50.8mm ID.

Choose **5µm particles** when you need high efficiency, because of a complex sample matrix or closely-eluting peaks. Economical columns containing **12µm particles** are a good choice when compounds of interest are widely spaced or when high throughput or lower back pressure is required. Flow rate and sample volume are related to the column volume, and are proportional to the ratio of the square of the column radius. For fixed column length and linear velocity, the numbers in Table 1 show a progressive increase in sample capacity and optimum flow rate as column ID increases. Sample capacity, however, also is dependent on the elution volume and resolution of the separation. The higher the resolution and longer the retention, the more sample can be purified per injection.

50.8mm ID columns available on request. Other dimensions and packing materials available.

Packing Characteristics

Composition: spherical silica
Pore Size: 120Å
Pore Volume: 0.6mL/g (5µm pkgs), 0.7mL/g (12µm pkgs)
Surface Area: 170m²/g (5µm pkgs), 190m²/g (12µm pkgs)
pH Range: 2-7.5

Table 1. Relative Flow Rates and Sample Capacities of SUPELCOSIL Preparative Columns

COLUMN ID:	4.6mm	10mm	21.2mm	50.8mm
Sample Capacity[1]:	1	4.8	21.2	122
Flow Rate[1]:	1	4.8	21.2	122
Optimum Flow Rate (mL/min):	0.7	3.4	14.8	21.4

[1]Relative to 4.6mm column

For more information, request T495004.

Phase LENGTH (cm)	/ d_p / PORES (µm)	(Å)	4.6mm ID COLUMN CAT. NO.	10mm ID COLUMN CAT. NO.	21.2mm ID COLUMN CAT. NO.
Discovery RP-AmideC16					
5	5	180	505005	569325-U	569328-U
10	5	180	569323-U	569333-U	569327-U
25	5	180	505064	569324-U	569326-U
SUPELCOSIL ABZ⁺Plus					
10	5	120	custom	custom	59148
25	5	120	59197	59179	54855
25	12	120	59156	custom	59174
SUPELCOSIL ABZ					
25	5	120	59142	59170	54853
Suplex pKb					
25	5	120	58934	59172	custom
Discovery C18					
5	5	180	504947	569225-U	569228-U
10	5	180	569223-U	569233-U	569227-U
25	5	180	504971	569224-U	569226-U
SUPELCOSIL LC-18-DB					
25	5	120	58355-U	58358	custom
SUPELCOSIL LC-18					
25	5	120	58298	58368	54849
25	12	120	59182	custom	59185
Discovery C8					
5	5	180	59352-U	569430-U	569433-U
10	5	180	569423-U	569428-U	569432-U
25	5	180	59354-U	569429-U	569431-U
SUPELCOSIL LC-8-DB					
25	5	120	58354	58357	custom
SUPELCOSIL LC-8					
25	5	120	58297	58367	54845
25	12	120	59181	custom	59184
Discovery Cyano					
5	5	180	59355-U	569530-U	569533-U
10	5	180	569520-U	569528-U	569532-U
25	5	180	59357-U	569529-U	569531-U
SUPELCOSIL LC-CN					
25	5	120	58231	58369	custom
SUPELCOSIL LC-1					
25	5	120	58296	custom	custom
SUPELCOSIL LC-Si					
25	5	120	58295	58365	54843
25	12	120	59180-U	custom	59183

Injectors

Rheodyne Model 7725 and 7725i Injectors

The Rheodyne Model 7725 injector allows you to inject 1µL-5mL samples with accuracy and precision. The rugged, easily maintained design offers many advanced features:

- Patented continuous flow design – flow is uninterrupted when you switch from LOAD to INJECT
- Easy seal adjustment using pressure screw on front of injector
- Easy access to fittings

Injector includes a 20µL sample loop and is supplied with a VESPEL rotor seal that can be replaced with a Tefzel rotor seal for operation at pH 0-14. Factory set at 5000psi (345 bar), adjustable to 7000psi (483 bar). Model 7725i has an internal position sensing switch.

Description	Cat. No.
Model 7725 Injector	**57620-U**
Model 7725i Injector	**57621**
Replacement Components	
VESPEL Rotor Seal	**58830-U**
Tefzel Rotor Seal	**57633**
Stator Face Assembly	**57634**
Needle Port Cleaner	**57635**
Valve Angle Bracket (for all metal Rheodyne valves)	**57636**
RheBuild Kit for 7725/7725i/7726	**55049**
Sample Loops (wide-angle ports; fittings included)	
2µL	**57622**
5µL	**57623**
10µL	**57624**
20µL	**57625**
50µL	**57626**
100µL	**57627**
200µL	**57628-U**
500µL	**57629-U**
1mL	**57630**
2mL	**57631**
5mL	**57632**

Note: Use VESPEL seals to pH 10, Tefzel seals to pH 14.

*All literature mentioned in this issue can be obtained from the website, **www.sigma-aldrich.com/TheReporter**, by completing the Literature Request section on the reply card, or by calling our Technical Service Department.*

LITERATURE

Good News From Supelco!
Custom Resin and Media Processing Services

Supelco supplies most types of chromatographic resin from the major manufacturers of adsorbents and ion exchangers. We carry an extensive inventory of these media for your convenience. Now you can purchase small quantities of the leading resins without having to stock large amounts of resin due to minimum order levels required by resin producers.

But there's more good news…Supelco will also process your resin into the exact form for your application. You define the exact process to follow in producing your low pressure chromatography media and Supelco takes it from there. Our capabilities include: GMP manufacture, sieving and grinding, blending of different resins, cleaning (aqueous and organic), acid/base wash, ionic conversion, drying, sterilization, cartridge packaging (from 1mL to 4L range) and unit-use packaging.

And there's more good news…Supelco will test your processed resin to assure that it meets your requirements and provide a Certificate of Analysis in most cases. We can perform the following tests on the final resin: USP or EPA extractables, sterility/endotoxicity, moisture content, exchange capacity, conductivity and pH.

When you factor in the costs for raw resin and expense of handling and disposing of large volumes of solvents, the value of operator time for processing the media and the convenience of purchasing your resin in the exact form you need it makes sense to consider Supelco's Custom Resin and Media Processing Service.

Here's how it works:

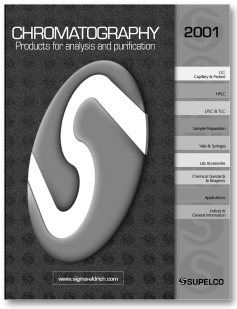
1. We work with you to define the product specification, including testing required, the expected demand, and other relevant information.
2. The Supelco Resin Team reviews the project, conducts a feasibility study and determines if we can produce the resin to the specification.
3. If feasible, we give you an estimate of price and delivery.
4. We refine the process until the product is exactly what you want.
5. We assign a catalog number (at your request) to make it easier to purchase the resin in the future.

To see how this service can work for you please contact Supelco at 800-652-2572, ext. 9.

T400049

The 2001 SUPELCO Chromatography Catalog

Be sure to check the box on the literature request form to reserve your copy of this valuable resource. Catalogs are scheduled to be mailed in January, 2001.

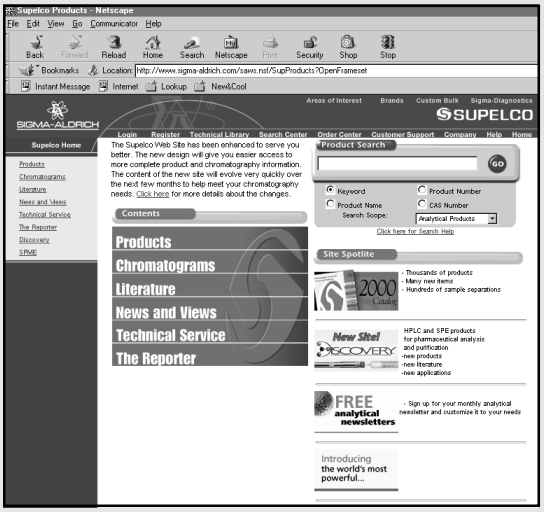


LC PERFORMANCE TIP

Turn to the Supelco Web Site First for All Your Application Needs

If you are trying to develop a method, need to know how best to analyze for a particular compound or have applications questions, visit the Sigma-Aldrich web site at **www.sigma-aldrich.com**. At the Supelco section of the site we have hundreds of chromatograms on the many applications we have done, including applications on our Discovery family of columns and SPE materials. You can search the applications section by alphabetical name, technology, compound class or industry type. You will also have access to all of Supelco's other technical information such as Bulletins, Trouble Shooting Guides and Reporter articles. Having difficulty finding the information you need? You can contact our Technical Service Department right from the web site through email. Finally, you can sign up for free newsletters, view the other Sigma-Aldrich brands' web sites and even order directly on line.

*Visit **www.sigma-aldrich.com** and meet your needs!*

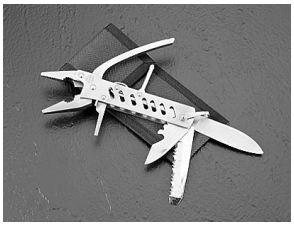


NEW SERVICE!

LC Answers by email

If you are trying to develop a method, looking for state-of-the-art column recommendations and separation strategies, or seeking trouble-shooting advice, contact Keith Duff at **kduff@sial.com** for a fast, accurate response.

Win a free Barlow Multitool, or Executive Discovery Pen if your question is chosen to appear in a future "LC Performance Tip."



Scale-Up of Fast Analytical…

(continued from page 1)

chromatographic performance between 5µm and 10µm columns.

Throughput

Peak efficiency is superior on 5µm columns compared to 10µm columns (Figure B). Preparative columns packed with 5µm packings can withstand higher sample loads than 10µm columns since the peaks are innately narrower on the 5µm columns. This allows more crude sample to be purified in fewer injections. Also, faster flow rates do not broaden peak widths as much on 5µm columns as they do on 10µm columns. Each individual preparative run can be run faster on 5µm than on 10µm preparative columns.

Normal Operating Backpressure

Short, wide columns are commonly used in fast preparative separations. A typical fast preparative column size is 5cm x 21.2mm. Backpressure is directly proportional to the length of the column and to the square of the particle radius. The short length of fast preparative columns helps to compensate for the higher backpressure associated with the smaller 5µm particle size. This allows fast preparative separations to be run under normal operating backpressures.

For more information, request T495004, and see Keith J. Duff and Richard Ludwig, "Packing and Evaluation of Small Particle Preparative Columns", American Laboratory, 32KK-33MM, March (1994).

CASE STUDY 5

Detection Wavelength Adjustment for Preparative HPLC

Detector response is much higher in preparative HPLC than standard analytical scale runs due to the large amount of sample that is injected. To compensate, small volume preparative HPLC detector cells are available from instrument manufacturers, which reduces the response. Still, peaks can (and often do) go off scale, even with a setting at the highest attenuation. The peaks appear as shown in Figure C, where they are "gated" or run off of the top of the chromatogram. Adjustments should be made to keep the response within the detector linear range of about 2AU or less. The best solution is to use a wavelength setting that is somewhat away from the lambda maxima of the peaks of interest, particularly of the largest peak. Most modern multi-wavelength and diode array detectors are capable of scanning the UV absorbance of analytes. Alternatively, a scanning UV instrument is helpful in choosing a good preparative HPLC detection wavelength. Keep in mind that peak heights/areas may change disproportionately to each other when switching the detection wavelength. Some peaks of interest might disappear entirely if a proper wavelength is not chosen. In the example shown, the detector wavelength was switched from 205nm to 230nm (Figure C and Figure D, respectively) to keep the largest peak within a reasonable detection limit.

For more information, request T400183.

Figure C. "Gated" Peek due to Too Strong of a Detector Response @ **205nm**

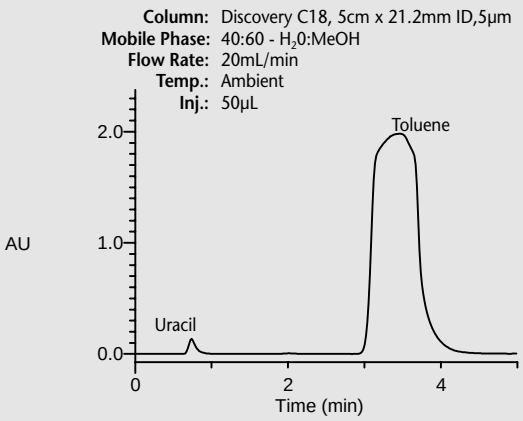
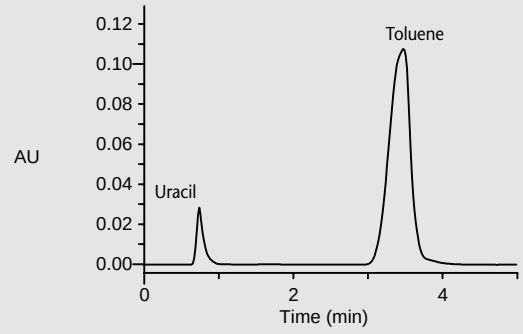


Figure D. Good Detector Response Obtained @ **230nm**



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