

High-Throughput Methods for Size Exclusion Chromatography

Dr. Ivan Lebedev, Brian Rivera, and Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Flow rate is a common method parameter for most adsorptive LC methods. However, SEC is a unique separation modality in that lower flow rates often yield improvements in separation.

The use of sub-2 μm particles allows the method developer some flexibility in increasing flow rate while still maintaining acceptable chromatographic performance. Typically, a resolution from monomer and aggregates greater than 1.8 is desirable. If fragment is present in the sample, then maintaining resolution to allow for auto-integration is often preferred. **Figure 1** compares SEC chromatograms when running a flow rate of 0.35 mL/min, a standard linear velocity, and a flow rate of 0.6 mL/min. Acceptable resolution is obtained and a nominal change in percent purity is observed.

LC Conditions

Column: Biozen™ 1.8 μm dSEC-2, 200 Å
Dimensions: 150 x 4.6 mm
Part No.: [00F-4787-E0](#)
Mobile Phase: 50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate: As Indicated
Injection Volume: 10 μL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Cetuximab, 2 mg/mL

In summary, the use of sub-2 μm particles for SEC methods may be a practical way to slightly increase throughput for applications that demand it. However, optimal chromatography for SEC methods is typically using at or below standard linear velocities.

Figure 1. SEC chromatogram overlays for Cetuximab run at varying flow rates.

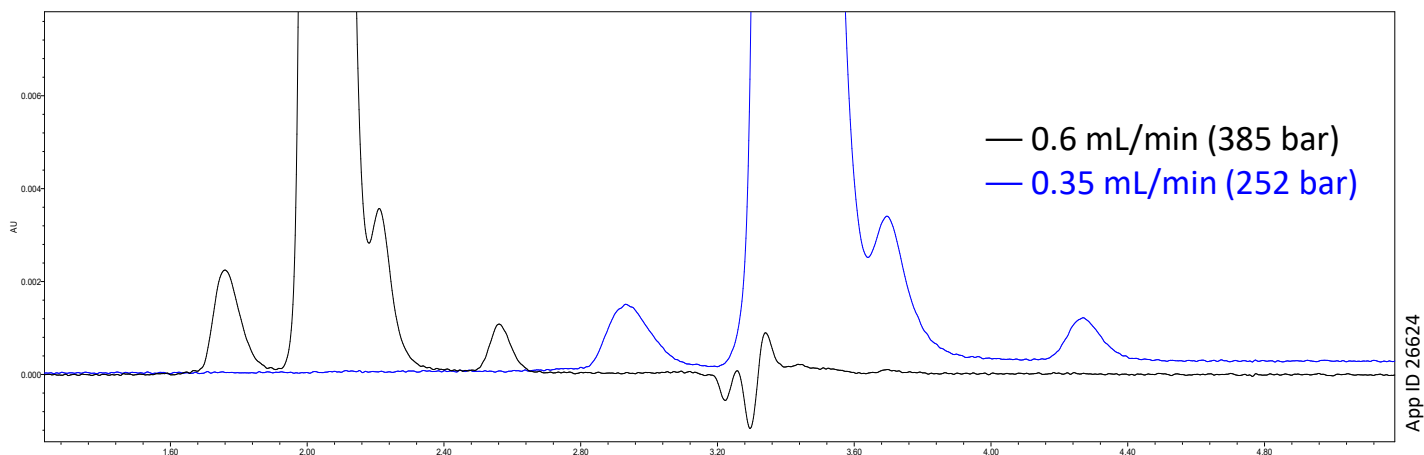


Table 1. Summary of chromatographic results with modulating flow rate

Flow Rate (mL/min)	Rs 1,2 (HMW, Monomer)	% Purity Monomer
0.35	2.20	98.4
0.6	2.15	98.8



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t: +61 (0)2-9428-6444
auiinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
cz-info@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong

t: +852 6012 8162
hkinfo@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia

t: +62 21 5010 9707
indoinfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Japan

t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Poland

t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
sk-info@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
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Corporate Office USA
t: +1 (310) 212-0555
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