Column Bed Stability During Routine Size Exclusion Chromatography

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

Overview

Size Exclusion Chromatography (SEC) is a technique to separate large molecules such as proteins and polymers based on their size in solution. SEC methods are commonly used for routine testing application, most notably for the quantitation of aggregates in a biotherapeutic sample.

The primary failure mode for size exclusion columns is column voiding, wherein the column packed bed compresses and collapses. Column voids can occur because of spikes in backpressure, switching mobile phase (resulting in changes in solvent viscosity), or suboptimal method/sequence start up and shut down.

To demonstrate column method robustness against column voiding during routine testing, a 4.6 mm internal diameter (ID) by 150 mm length column was packed with sub-2 µm, 200 Å media and was run with a viscous mobile phase containing 10 % isopropanol. To further stress the column packed bed, flow rate was modulated between 0.35 mL/min and 0.45 mL/min, the latter resulting in a backpressure of roughly 320 bar. Finally, flow was intermittently stopped for 8 hours to replicate the system start up and shut down which can also result in chromatographic bed collapse. Overall, there was > 100 hours of run time, and 226 injections were performed. A summary of the sequence is indicated below. The robustness sequence was to be run until column failure, which was defined as a drop of 25 % efficiency for uridine, a small molecule which elutes in the total permeation volume.

Upon completion of the sequence, column efficiency showed a nominal drop in uridine efficiency (37231 N to 36257 N, or a 2.6 % drop). It is important to note that no protein samples were injected during the sequence, as the intent of the experiment was to confirm column bed stability. Additionally, peak shape and efficiency of uridine may not be representative of the performance of protein samples. Finally, because column failure was not reached, it is unclear as to when the 25% efficiency benchmark would have been reached.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00F-4787-E0
Dimensions: 150 x 4.6 mm
Mobile Phase: 0.1 M Sodium Phosphate, pH 6.8 + 10 % Isopropanol
Flow Rate: As indicated in Table 1
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Uridine

Table 1. Experimental Conditions and Sequence for SEC Column Robustness

Sequence	Mobile Phase	Flow Rate (mL/min)	Method Run Time (min)	Number of Injections
1	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	6.5	4
2	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.45	6.5	4
3	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	6.5	3
4	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	90	1
5	N/A (no system flow)	0	480	0
Sequence repeated until 100 hours of run time was exceeded				

Have questions or want more details on implementing this method? We would love to help!
Visit www.phenomenex.com/Chat to get in touch with one of our Technical Specialists

Figure 1. Column Efficiency (N) For Uridine (0.35 mL/min Method)

Column efficiency for uridine over hours of run time. Sequence as shown in Table 1. Only a 2.6 % drop in efficiency is observed over the course of the sequence.

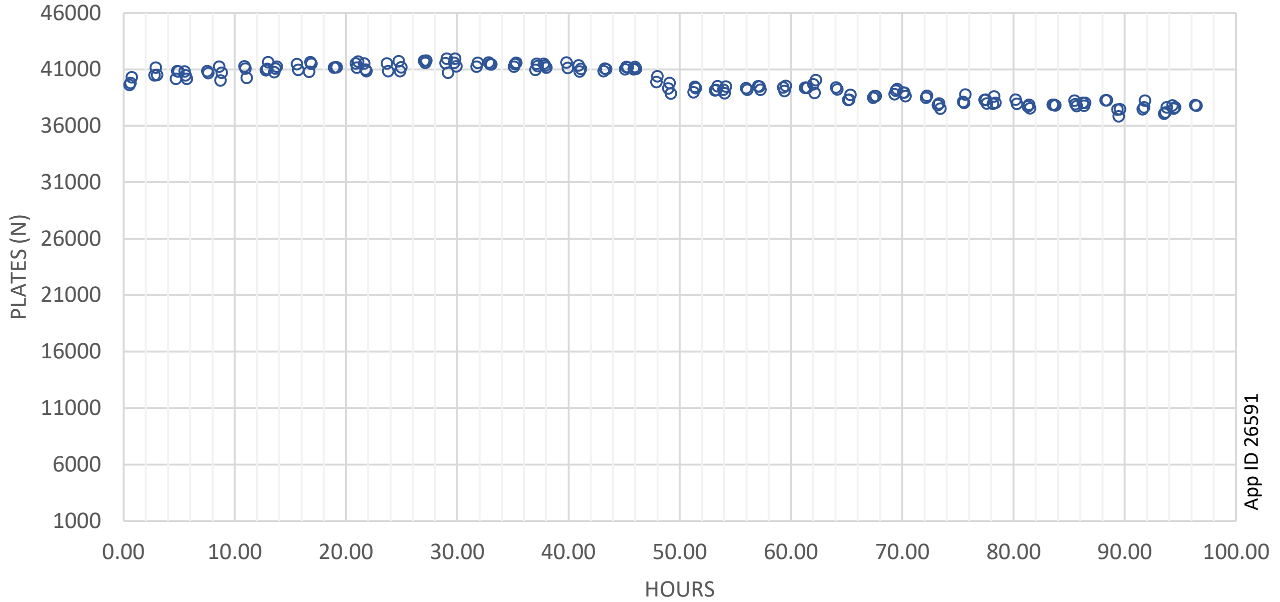
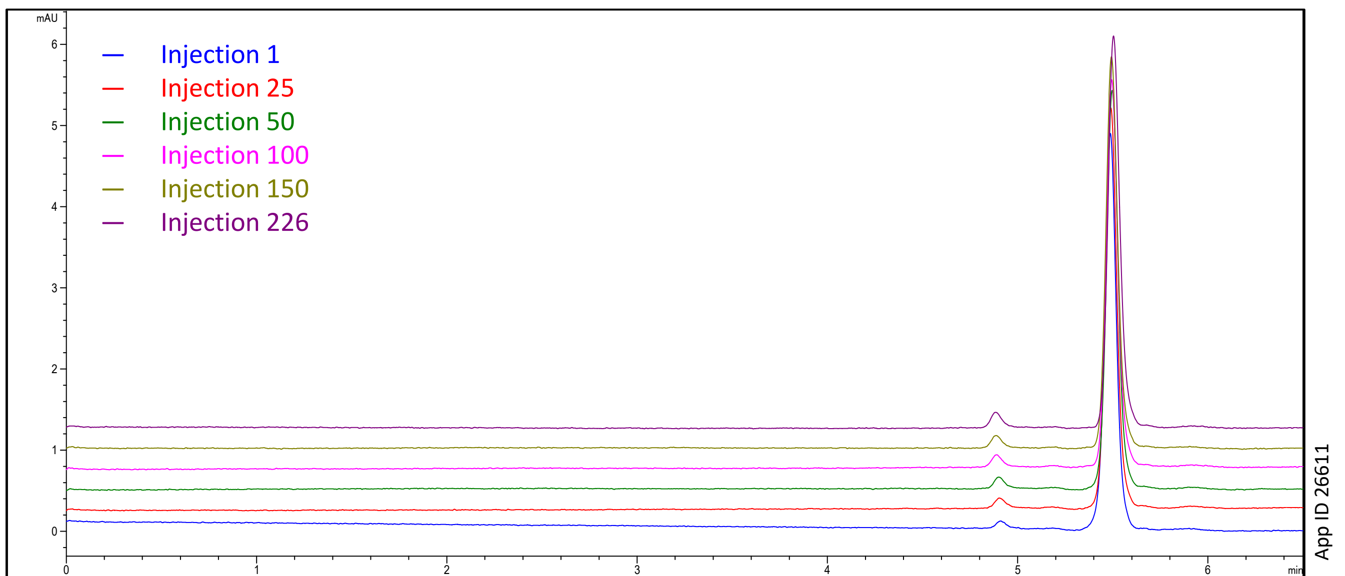


Figure 2. Chromatographic Overlay of Uridine Peak

Overlay of uridine chromatograms. Nominal changes in plates (37 k to 36 k) and retention time (5.4 min to 5.5 min) were observed through the assessment of bed stability during the sequence.



Have questions or want more details on implementing this method? We would love to help!
Visit ***www.phenomenex.com/Chat*** *to get in touch with one of our Technical Specialists*

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

Australia
t: +61 (0)2-9428-6444
auinfo@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
info@phenomenex.com

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™ GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.
www.phenomenex.com/behappy

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.
Trademarks
Biozen and BE-HAPPY are trademarks of Phenomenex.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.
© 2021 Phenomenex, Inc. All rights reserved.

AN39690621_W

Have questions or want more details on implementing this method? We would love to help!
Visit www.phenomenex.com/Chat to get in touch with one of our Technical Specialists

phenomenex®