Tips & Tricks GPC/SEC: How to Test GPC/SEC Columns

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Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) columns are at the heart of a GPC/SEC system. Even when advanced detection, such as on-line light scattering, viscometry, or mass spectrometry (MS), is applied the quality of the results strongly depends on the least expensive part of the system, the separation columns. An easy test to determine the plate count, the peak symmetry, and the resolution of the columns is therefore an essential tool to ensure GPC/SEC system functionality.

Column performance is often described using plate count (or plate count/m), peak symmetry or asymmetry, and specific resolution. It is good practice to regularly monitor these parameters and several national and international standards — such as ISO 13885, DIN 55672, ASTM D 5296-05, and others — provide acceptance criteria for these parameters in dedicated solvents. If column certificates with test data are available to demonstrate column performance, it is good practice to repeat the tests with the specified conditions after column installation. Please note that comparisons of overall column performance can only be done if pertinent variables are kept constant. Mobile phase, test material, flow-rate, temperature, column loading, detector, and system including tubings should be kept the same, otherwise numerical results cannot be compared.

Plate Count and Asymmetry: To measure plate count and asymmetry, a monodisperse test material can be injected onto the gel permeation chromatography/size-exclusion chromatography (GPC/SEC) instrument. Detailed experimental conditions can be found either in a corresponding GPC/SEC standard or on the column quality certificate.

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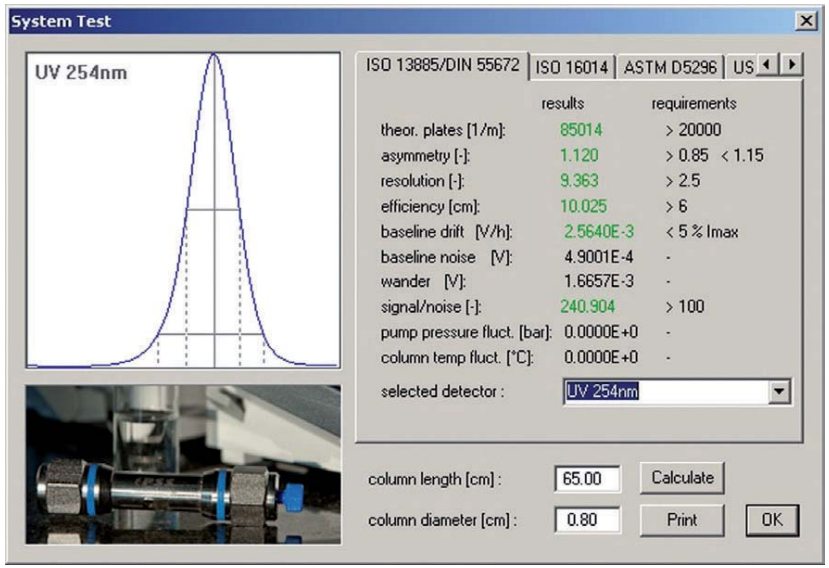
Figure 1: Example of plate count, peak symmetry, and resolution determination based on the acceptance criteria from ISO 13885/DIN 55672.



Alternatively, suitable test materials for a variety of typical GPC/SEC solvents are presented in Table 1. For these materials an injection volume of 20 µL (or if possible less) can be used, whereas concentration should be adapted to the number of columns used: One column = 0.2 g/L; two columns = 0.5 g/L; three columns = 1 g/L. As mentioned before, it is important to use the same test substance with the same instrument and analytical conditions for all tests.

The calculation of the theoretical plate count per metre (N_{th} [1/m]) uses the peak position and the peak width when at half peak height as according to:

$$N_{th} = \left\{\frac{V_p}{\sigma}\right\}^2 = \frac{554}{L} \cdot \left\{\frac{V_p}{W_{1/2}}\right\}^2 \quad [1]$$

where σ is the variance estimated by the half-height method, $w_{1/2}$, and L is the column length in cm.

The asymmetry calculation strongly depends on the GPC/SEC standard applied.

Table 1: Potential test materials to determine plate count, peak symmetry, and resolution.

Mobile phase (sorted by polarity)	Plate count test material	Resolution test material	Recommended operating temperature [°C]
Toluene	Butylated Hydroxytoluene, (BHT)	Polystyrene	25–40
Tetrahydrofuran (THF)	BHT	Polystyrene	25–40
Chloroform	BHT	Polystyrene	25–40
1,2,4-Trichloro-benzene (TCB)	BHT	Polystyrene	140–160
Dimethylacetamide (DMAc)	Methyl isobutyrate, (e.g. mmp1)	Polymethyl methacrylate	60–80
N-Methylpyrrolidone (NMP)	Methyl isobutyrate	Polymethyl methacrylate	60–80
Dimethyl sulphoxide (DMSO)	Methyl isobutyrate	Polymethyl methacrylate	60–80
Dimethylformamide, (DMF)	Methyl isobutyrate	Polymethyl methacrylate	60–80
Hexafluoro isopropanol (HFIP)	Methyl isobutyrate	Polymethyl methacrylate	25–40
Water	Ethylene glycole	Pullulan	25–60

* Small PMMA signals as a result of small dn/dc of PMMA in DMSO; if S/N ratio is not sufficient use pullulan.

The peak asymmetry in DIN 56672 and ISO/EN 13885 are defined as $A = w_l/w_r$ where w_l and w_r are the peak widths on the left and right side of the peak maximum (measured in 10% of the peak height). In ASTM asymmetry is defined as $A' = w_r/w_l$, therefore $A' = 1/A$.

In general, resolution is the most important factor because it gives direct information about the performance of a separation in a dedicated molar mass region. To measure resolution, a mixture of polymer standards should be injected onto

the system and analyzed. Alternatively, if a calibration curve is available the required information, such as the slope (D), can also be taken from this calibration.

Specific resolution (R_{sp}) specifies the quality of resolution of two peaks (denoted as R_s), whose molecular weight differs by one order in magnitude:

$$R_{sp} = \frac{R_s}{\lg M_1 / M_2} = \frac{0.579}{\sigma \cdot D} \quad [2]$$

Figure 1 shows the results of a test to determine plate count, peak symmetry, and resolution using butylated hydroxytoluene (BHT) in tetrahydrofuran (THF). Numbers in green indicate that the acceptance criteria of the applied standard ISO 13885/DIN 55672 are met.

Regular plate count determination is important if a lot of different samples and samples with unknown composition or origin are analyzed. Testing before and after analysis can help to identify column damage because of inappropriate sample preparation or can detect samples that cannot be analyzed using the applied conditions. In quality control, where the same type of sample is analyzed, a check-out sample of the same chemistry can be analyzed in addition to ensure that the actual application is running properly.

Mismatch Tests

In contrast to resolution tests, pore size mismatch tests require the injection of test materials with broad yet homogeneous molar mass distribution. These materials should be injected if unexpected shoulders occur in chromatograms. If the shoulder is visible at the same elution volume for the test material and the sample, a pore size mismatch is the most probable reason. Pore size mismatch can occur either in a column bank or in a single column with blended pore sizes.[1]

Actions If the Plate Count Test Fails

If the plate count or asymmetry tests fail for freshly installed columns it may be necessary to check column connections. If the column supplier has changed it may be that the different stop depth of the column heads is responsible for the bad performance. Unfortunately the stop depth (length of the end of the ferrule compared to the tubing end) varies between suppliers, so that it will often be a problem if existing tubing is used with new columns.

If this is not the reason and if several columns are installed it is often worth testing each column individually, and even a test without the precolumn or guard column should be performed. Within a column bank or a column combination a single column, or even the precolumn, can be defective and the reason for an out-of-specification plate count and asymmetry test. If defective column(s) are identified they need to be replaced. If tests are inconclusive or ambiguous, contacting the column supplier with the results from the individual tests can help find the cause.

Reference

1. T. Hofe, *The Column* **4**(4), 20–23 (2008) [http://www.nxtbook.com/nxtbooks/advanstaruk/thecolumn0408/index.php?startid=20#/20].

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