

Tips & Tricks: GPC/SEC

Friedhelm Gores and Peter Kilz, PSS Polymer Standards Service GmbH, Mainz, Germany.

Examining accuracy and precision in GPC/SEC.

Any analytical method has its intrinsic inaccuracy. It is important to know these limits and the precision and accuracy of the analytical method to interpret the results in the right way. Furthermore, it is necessary to know how these entities can be influenced and improved with simple tools or proper experimental set-up.

Before discussing the tools, the general definitions of the factors should be clear:

- The **accuracy** of an analytical procedure expresses the closeness of the agreement between the value that is accepted, either as a conventional (true) value or a generally accepted reference value, and the value found.
- The **precision** of an analytical procedure expresses the closeness of the agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the same conditions.

Figure 1 shows the difference between accuracy and precision.

In more detail, precision can be discussed in terms of short- and long-term precision:

- The **repeatability** describes the intra-laboratory empirical variance of the results of multiple measurements of a sample during a short period of time.
- The **intermediate precision** expresses

intra-laboratory variations over a long time period.

- Another important term is the **reproducibility**, that is assessed by means of inter-laboratory deviations.

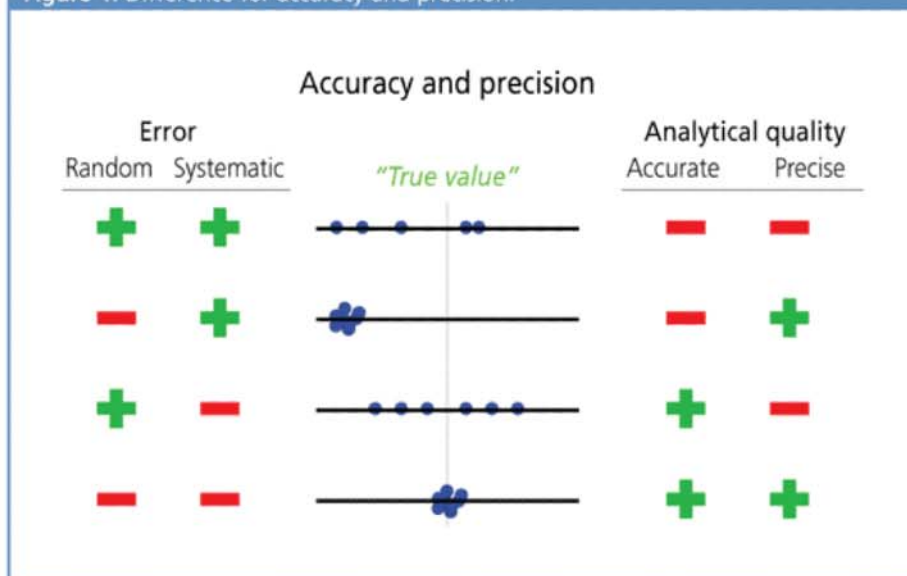
How Accurate is GPC/SEC?

GPC/SEC is a relative method and the molar masses obtained can only be accurate if the calibration standards and the samples are matching with regard to their chemical composition.

Standards such as the ISO 13885 for GPC/SEC do not give any references for accuracy but many analytical laboratories report an accuracy of <5% for M_w and 10–15% for M_n depending on the complexity of the samples. In instances where no matching calibration standards are available deviations of several 100% are possible.

Fortunately many GPC/SEC users only focus on repeatable and precise measurements (e.g., when only quality

Figure 1: Difference for accuracy and precision.



control of products or product comparison is required). However, if accurate results for true molar masses are needed several options are available to overcome the limitation of the missing calibration standards:

- universal calibration with Mark-Houwink coefficients
- broad calibration
- integral calibration
- use of molar mass sensitive detectors as on-line viscometers or light-scattering detectors.

For all of these options reference values are required and the accuracy of the results for the unknown sample depends strongly on the accuracy of the reference values. This is also true for GPC/SEC light-scattering measurements, normally referred to as an absolute method, where the accuracy of the evaluation parameters and constants also influence the accuracy of the results.

To achieve highest accuracy careful calibration of the system and precise evaluation are required. National and international guidelines (e.g., the ISO EN 13885 standard for GPC/SEC)¹ provide valuable information and describe the correct evaluation with separate baseline and integration limits and the proper calibration procedure. From an instrument point of view pumps with a high flow precision are needed, as well as sensitive detectors. The columns used should be in good condition and suited for the molar mass range where the samples are expected. Column sets, a combination of columns with different porosities, can provide more accurate results than single (linear or mixed bed) columns because of the better resolution and efficiency.

How Precise is GPC/SEC?

As described above precision can be discussed as short-term precision,

Table 1: Repeatability and reproducibility results from round robin tests of complex samples in different GPC/SEC eluents.

| | THF | | | DMA | | | H ₂ O | | |
|--------------------------------|-------------------------|----|----|-----------------|-----|-----|------------------|-----|-----|
| | Precision/Repeatability | | | Reproducibility | | | | | |
| M _n | 3% | 2% | 2% | 15% | 15% | 15% | 15% | 15% | 15% |
| M _w | 2% | 2% | 2% | 10% | 15% | 15% | 15% | 15% | 15% |
| M _z | 3% | 3% | 3% | 15% | 24% | 24% | 24% | 24% | 24% |
| M _w /M _n | 3% | 3% | 3% | 15% | 24% | 24% | 24% | 24% | 24% |

“repeatability” and as long-term precision, “intermediate precision”. Several round robin tests provide results for repeatability and interlaboratory reproducibility as shown in Table 1. These results were obtained with complex samples with broad molecular weight distributions.²

Repeatability is an important element of method validation. The repeatability can be improved when working with standardized calculation algorithms. Separate baseline and integration limits increase the repeatability, especially when samples with a broad molecular weight distribution and a high amount of low molecular weight content and oligomers are investigated. The use of a low molecular weight internal standard as a flow marker is also recommended.²

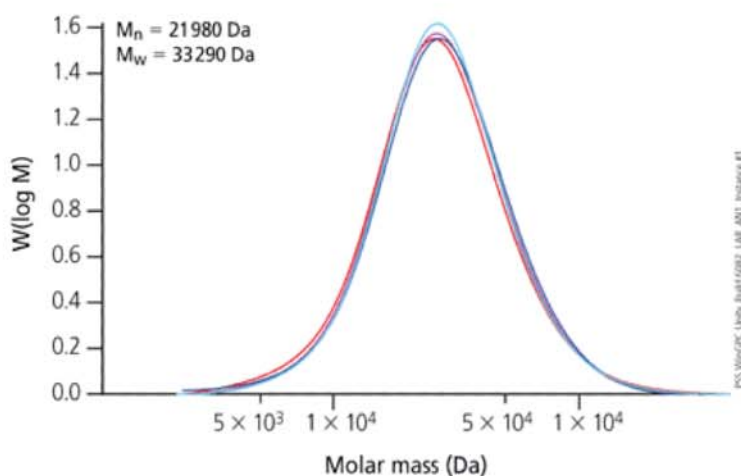
Typical variations that influence the intermediate precision are different days, different equipment and operators. The separation columns play an important role here. It is essential that the appropriate column material is used that allows interaction-free size-exclusion chromatography.³ If that is not the situation, slight recipe changes by the column manufacturer or even new columns made from a different batch might lead to different interactions. This

would then lead to systematic deviations and, therefore, a low intermediate precision.

The intermediate precision can be improved by establishing strict workflows for system set-up, sample preparation, calibration and evaluation. It can be beneficial to use a dedicated column set for each product and avoid running different applications on the same columns. This can be especially important if the samples contain reactive groups (e.g., isocyanate, amine, polyol) or in aqueous applications with polyelectrolytes (e.g., polyanions, polycations) to prevent formation of polymer complexes on the column surface. For rigorous quality control applications (e.g., in pharmaceutical applications) reservation of column batches from the manufacturer helps to avoid potential problems.

Typical deviations for the reproducibility are also given in Table 1. In case of critical GPC/SEC applications (e.g., aqueous SEC for polyelectrolytes or GPC/SEC–light-scattering coupling) the deviation might be even higher. However, these values can be dramatically reduced by using the same equipment, the same data analysis software, column sets produced from the same column batch,

Figure 2: Repeatability of an aqueous GPC/SEC measurement with two concentrations and duplicate injections.



Concentration 1, measurement 1 and 2:

$M_n = 21300$ (-3.1%), $M_w = 33050$ (-0.7%)

$M_n = 22400$ (1.4%), $M_w = 33130$ (-0.5%)

Concentration 2, measurement 1 and 2:

$M_n = 21400$ (-2.6%), $M_w = 33460$ (0.5%)

$M_n = 22800$ (3.7%), $M_w = 33530$ (0.7%)

the same calibration standards and the same calibration fit. This can lead to reproducibility deviations in the range of repeatability.

References

1. ISO EN 13885, GPC in Tetrahydrofurane.
2. P. Kilz and D. Held, in Quantification in LC and GC — A practical guide to Good Chromatographic Data, Eds., S. Kromidas and H.-J. Kuss, Wiley-VCH, Weinheim, 2008 (in press).
3. Thorsten Hofe and Günter Reinhold, *The Column*, **3**(12), 30–33 (2007).

Friedhelm Gores is the head of the contract analysis laboratory at the PSS Polymer Standards Service headquarters in Mainz, Germany. He is responsible for method development and hyphenated techniques including GPC/SEC coupled with light scattering and viscometry detection.

Peter Kilz is the head of the PSS software development department. He is responsible for software development, system integration and customer support.