



# Tips & Tricks: GPC/SEC

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## The importance of molar mass distributions.

**Q: Why are molar mass distributions (MMDs) important when analysing high-molecular-weight compounds and what is the difference between a chromatogram and a MMD?**

**A:** Synthetic materials, polysaccharides and proteins do not exhibit a definite molar mass, unlike low-molecular-weight substances. They consist of mixtures of chains with different numbers of repeating units, with each chain having its own molar mass. The molar mass of a macromolecule is obtained by averaging the molar mass of the different chains by number ( $M_n$ ) or by weight ( $M_w$ ). However, even if values for  $M_n$ ,  $M_w$  and  $M_w/M_n$  — the polydispersity index (PDI) — are available, macromolecules are still not characterized comprehensively. They can have the same averages but still show significantly different physical properties. This is because they have a different molar mass distribution (MMD) which means the fractions of the defined molar masses are different.

Molar mass distributions (MMDs) can be measured using gel permeation chromatography/size exclusion chromatography (GPC/SEC). GPC/SEC chromatograms show the fractions and the concentration change with molecular size in solution for the sample, but this information is superimposed by the parameters of the analytical equipment (Figure 1).

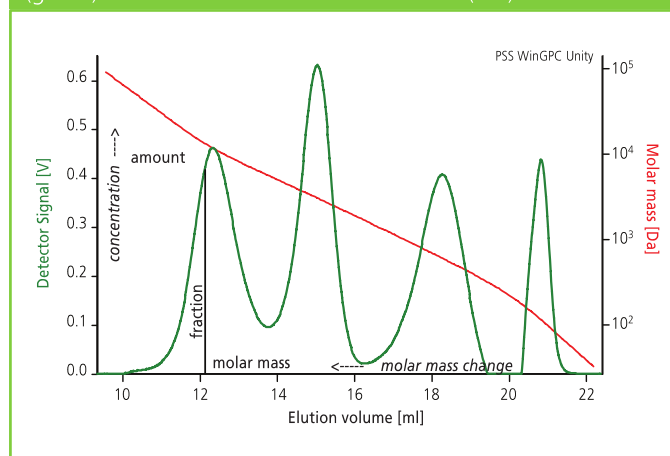
For example, if the same sample is measured in two separate laboratories on two different instruments using different-sized columns, the resulting chromatograms will obviously be different. Without previous knowledge nobody will assume that the chromatograms represent the same sample. If the instrument is properly calibrated, using any kind of calibration (for example, conventional, universal, light scattering), and the samples are correctly evaluated, the influence of the equipment is eliminated. MMDs are obtained that are independent from the instrument. The MMDs measured in the two laboratories are therefore the same.

This is why MMDs allow inter-laboratory comparison and direct comparison of product specifications, while chromatograms don't.

**Q: How are GPC/SEC chromatograms transformed into molar mass distributions?**

**A:** A GPC/SEC chromatogram shows a concentration distribution curve, where the molar mass decreases with higher retention times (or elution volumes). The retention axis (x-axis) is first converted into a molar mass axis via a molar mass calibration. The y-axis is then converted to mass fractions  $w$  in

**Figure 1:** GPC/SEC chromatogram of a sample mixture (green) with an overlaid calibration curve (red).

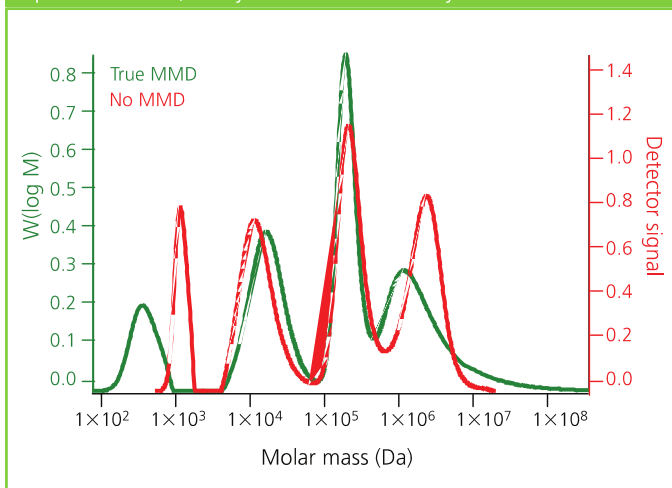


constant molar mass increments [log (M)]. This is necessary because signals in a chromatogram are measured in constant time interval. However, for the MMD a constant molar mass interval is needed.

Unfortunately, many high performance liquid chromatography (HPLC) data systems that also perform GPC/SEC calculate "molar mass diagrams", where only the retention times are converted into molar masses while the y-axis remains the same than in the chromatogram. This can make an inter-laboratory comparison extremely difficult. The determination of fractions above or below certain molar masses, for example below 500 g/mol, can also be faulty. Figure 2 clearly shows that both peak position, which relates to the molar mass, and peak width, which relates to the PDI, can be wrong.

see Figure 1. A typical GPC/SEC calibration curve with a polynomial fit function is used to evaluate the sample mixture and to obtain the MMD (e.g., cubic fit or polynomial 3, which was used for Figure 1). The standards mixture is analysed to obtain the molar mass distribution (MMD). If peak heights and peak widths do not vary, your data system is displaying molar mass-scaled chromatograms, not MMDs (Figure 2).

**Figure 2:** Overlay of the true MMD (green) and the molar mass scaled elugram (red). Green obtained with a GPC/SEC software, red obtained with a HPLC software with GPC option. For red, the y-axis is not correctly transformed.



Does this mean the averages of the molar masses are also wrong? No, most of the time this is not the case. Usually the averages are not calculated from distribution curves and are therefore unaffected by this phenomenon.

It is easy to test if molar mass distributions or molar mass diagrams are shown by the data system. A mixture of polymer standards with the same concentration is injected into a GPC/SEC column or column set (no linear/mixed bed columns)

