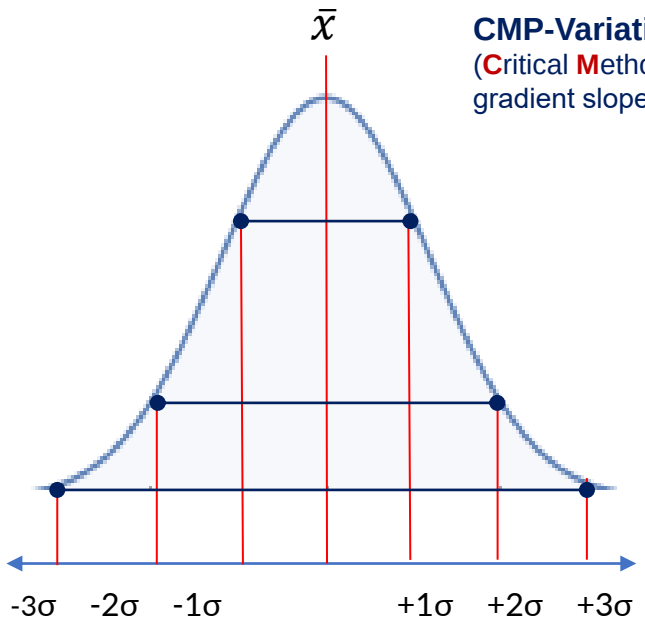
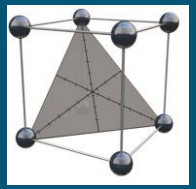


CMP-Variation
(**C**ritical **M**ethod **P**arameter, e.g. flow, temperature, gradient slope, pH, buffer concentration, …)

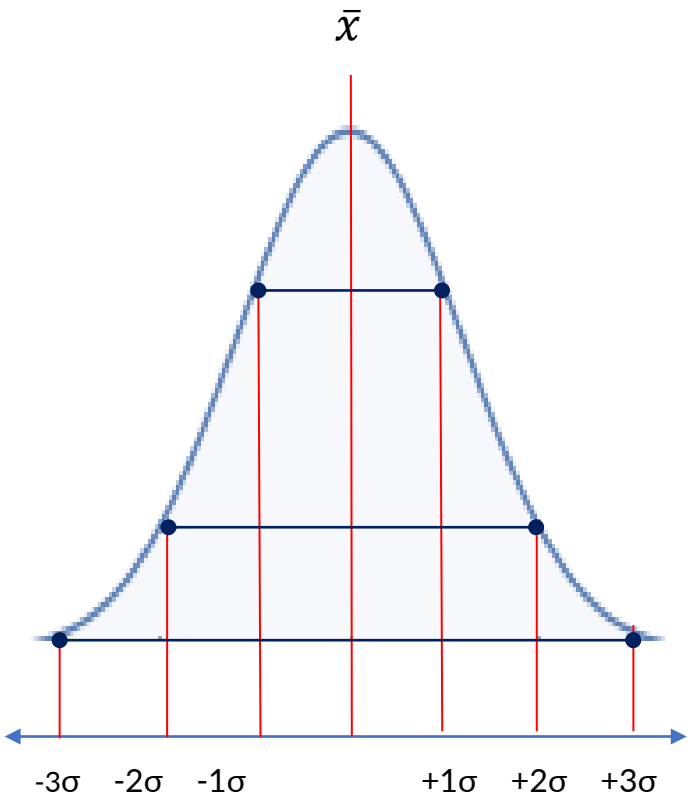
$$\sigma = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

FUSION QBD® - CRITICAL METHOD PARAMETERS

How to Precisely Specify the Maximum Expected Variation of a CMP



What does a +/- σ range stand for?



Standard Deviation (SD)

$$\sigma = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

- **+/- 1σ**: Covers **68.3%** of all observations, 317 of 1000 will be outside.
- **+/- 2σ:** Covers **95.5%** of all observations, 45 of 1000 will be outside.
- **+/- 3σ:** Covers **99.7%** of all observations, 3 of 1000 will be outside.

Why do we need to know the maximum expected variation of a CPM in Fusion QbD®?

When working with Fusion QbD knowledge about the maximum expected variation of a study factor is required for:

- **Defining suitable Study Level Settings*.**
- **Setting realistic conditions for Robustness Simulation*.**

***Described in previous technology notes.**

How to determine the Maximum Expected Variation?

For most study factors vendor specs are available. Please check for any specs referring to precision, not accuracy!

Note: Precision is usually not reported as **±3σ variation** in the vendors specs, but as **±1σ**.

Example Pump Flow Rate:

A flow precision is often indicated as e.g.

$$\leq 0.075\ \%RSD\ or\ 0.01\ mL\ SD\ (whichever\ is\ greater)$$

This means precision depends on the actual setpoint value, but has at least 0.01mL SD (standard deviation/ 1σ). Remeber the following equation:

$$\%\mathrm{RSD} = \frac{SD}{\bar{X}} \times 100\%$$

$$\bar{X} = \frac{SD}{\%RSD} \times 100\% \quad \rightarrow \quad \bar{X} = \frac{0.01mL}{0.075\%} \times 100\% = 13.\bar{3}\ \mathrm{mL}$$

This means, you will need pretty high flow rates in order to obtain an SD greater than 0.01mL and for common LC chromatography we could use 0.01 mL SD.

Still this is not the maximum expected variation, but only 68.3% of all observations, as SD represents ±1σ variation.

Therefore, multiply this value by 3: ±0.03 mL is our value!

What about Buffer Concentration?

When preparing a buffer solution **several preparation steps** are involved. Each step provides an **additional source of analytical error**. We will perform weighings and need to dilute the solution to target concentration.

Therefore, we first need to identify the possible error of each step. We need to know the uncertainety of the **analytical balance** and the expected error of all **measuring devices** used for dissolution and diluting the buffer.

But how to determine such a propagated error?

Example: Determination of the Expected Maximum Variation for a Buffer Concentration

Let us demonstrate the calculation for the preparation of a 20mM Ammonium Acetate Buffer.

Preparation should be as follows:

- 1M Stock Solution:
  - Weigh about 1mol (77.083g) and dilute to 1000mL.
- 20mM Target Solution:
  - Take an aliquot of 10mL and dilute to 500mL.

The following uncertainties are indcated by the vendors:

- Analytical Balance: 0.00012g
- 1000mL Measuring Flask: 0.5 mL
- 10mL Pipette: 0.1mL
- 500mL measuring Flask: 0.25mL

Although not explicitly specified, we expect that the given uncertainty expresses the SD.*

* Check with your vendor, if you are in doubt, and good luck! But for our needs this also stands for a worst case scenario.

Example: Determination of the Expected Maximum Variation for a Buffer Concentration

The propagated error in a case were different units are involved is calculated by summing up the individual relative errors.

The final analytical error is then calculated by multiplying the target concentration level with the resulting relative error, applying the following equation:

$$\sigma_x = \bar{x}\sqrt{\left(\frac{\sigma_a}{\bar{a}}\right)^2 + \left(\frac{\sigma_b}{\bar{b}}\right)^2 + \left(\frac{\sigma_c}{\bar{c}}\right)^2}$$

For our example the calculation will be as follows*:

$$\sigma_x = 20\text{mM} \times \sqrt{\left(\frac{0.00012\text{g}}{77.083}\right)^2 + \left(\frac{0.5\text{mL}}{1000.0\text{mL}}\right)^2 + \left(\frac{0.1\text{mL}}{10.0\text{mL}}\right)^2 + \left(\frac{0.25\text{mL}}{500.00\text{mL}}\right)^2} = 20\text{mM} \times 0.011 = 0.22\text{mM}$$

Again this is the 1σ variation and therefore we still need to multiply this value by 3 to obtain the expected maximum variation:

3 × 0.22mM = 0.66mM is our value!

*target molar mass of 77.083 used for simplicity reason, but remeber to enter the actual value reported by your balance.

Contact us for more Information

Fusion QbD® is a mature LC method development software especially designed for AQbD approaches in the pharmaceutical industry.

If you want to get the full understanding of how the design region is modelled in Fusion QbD, please contact us for our **training or consultancy services**.

Cromingo e.K. – All Fusion QbD products and services from a single source in close collaboration with S-Matrix Corporation.

Reach out to us directly for further information **info@cromingo.com**