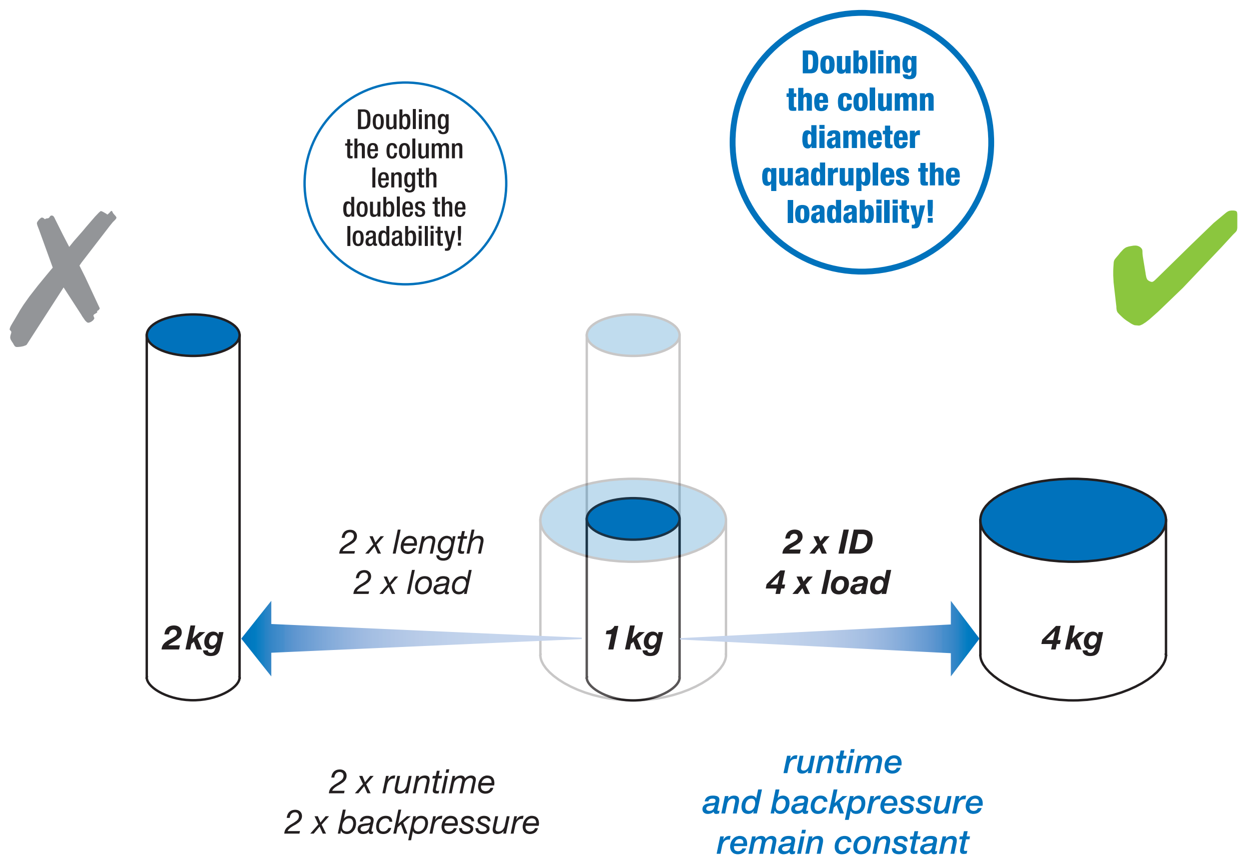
Linear Scale-Up in Preparative LC Method Development

The linear scale-up process is the fundamental basis for cost-efficient preparative LC method development. It saves costs not only by allowing method development on a lab scale in general but also by reducing the required amount of feed in early stages.

Following the rules of the linear scale-up process enables the transfer of a lab scale method to an industrial scale process. To apply the linear scale-up process, the column length has to be maintained as well as the particle size which was used during the method development and loading studies. It's a reliable and fully established approach for the process development of preparative scale HPLC processes.

The general idea is to use the bed length for the chromatographic separation whilst the diameter of the column used defines the capacity. Therefore, by increasing the column diameter the loadability increases as shown in the following diagrams.

Scale-up **influence**

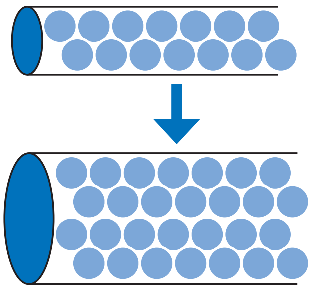


→ **Column length determines retention time**

→ **Diameter determines loadability**

Scale-up Factor (SF)

There are scalable and non-scalable parameters when applying the linear scale-up process. The non-scalable parameters such as the bed length and the particle size (full list see on page 4) have to remain the same. For the calculation of the scalable parameters such as the flow rate and the injection volume, the so-called scale-up factor is applied which is based on the ratio of the cross-section areas (inner diameters squared) of the columns used for both the analytical and preparative scale.



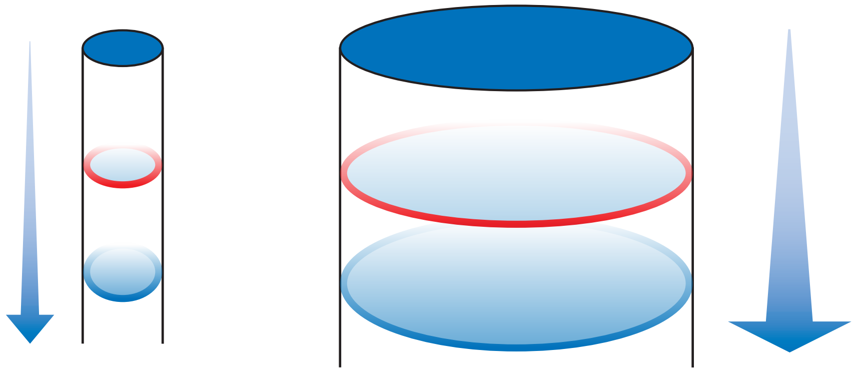
$$\mathbf{SF} = \frac{A_{\text{ID prep.}}}{A_{\text{ID analyt.}}} = \frac{\text{ID}^2_{\text{prep.}}}{\text{ID}^2_{\text{analyt.}}}$$

Adapting the Flow Rate

By applying the scale-up factor (SF), the required flow rate for the intended column dimension can be calculated. In this example, the scale-up shall be converted from a 4.6 mm ID column to a 20 mm ID column. The SF is 18.9. Therefore, if the flow rate for the 4.6 mm ID column is 1 mL/min the flow rate for the 20 mm ID column needs to be 18.9 mL/min to get the same chromatographic results.

$$\mathbf{SF} = \frac{\text{ID}^2_{\text{prep.}}}{\text{ID}^2_{\text{analyt.}}} \rightarrow \text{SF} = \frac{(20\,\text{mm})^2}{(4.6\,\text{mm})^2} = \mathbf{18.9}$$

4.6 mm ID | 20 mm ID



Flow rate: 1 ml/min | **18.9** ml/min

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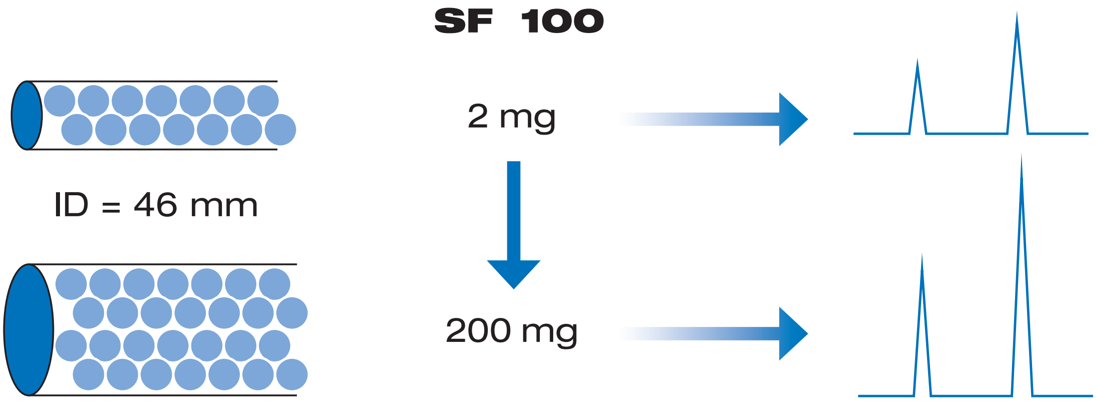
Adapting the Loading

The same applies for the injection volume. In this example, the loading for a 4.6 mm ID column is 2 mg. When performing a scale-up to a column that has a 10 times larger ID, the SF of 100 has to be used. Thereby, the loading for a 46 mm ID column would need to be 200 mg to get to the same chromatographic result.

$$\mathbf{SF} = \frac{ID^2_{prep.}}{ID^2_{analyt.}} = \frac{m_{prep.}}{m_{analyt.}} \rightarrow \mathbf{SF} = \frac{(46\,mm)^2}{(4.6\,mm)^2} = \frac{200\,mg}{2\,mg} = \mathbf{100}$$

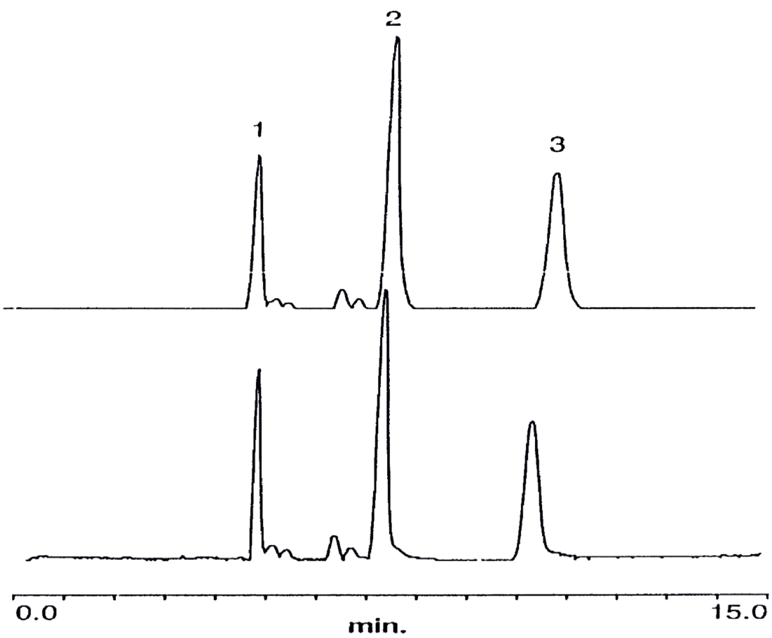


Practical example

The chromatograms below show the scale-up from a 4.6 mm ID to a 150 mm ID column. The scale-up factor is 1,063. The flow rate was increased from 1 mL/min to 1 L/min and the injection volume was increased from 20 µL to 20 mL. As can be seen from the chromatograms, the separation is the same for both column dimensions. The linear scale-up was done successfully.

250 x 4.6 mm ID
1 mL /min
20 µL Injection

250 x 150 mm ID
1 L /min
20 mL Injection



Non-scalable parameters

Column	Chromatography	Sample
Length	Stationary Phase	Yield
Run Time	Gradient	Concentration
Performance	Temperature	Purity

Reference values

			Lab scale → Production scale								
Column inner diameter [mm ID]			**4.6**	10	20	30	50	100	200	500	1,000
Cross sectional area ratio			1.0	4.7	19	42	118	473	1,890	11,800	47,300
Example of calculation	Flow rate [ml/min]		0.5	2.4	9.5	21	60	235	950	6,000 (6 L)	24,000 (24 L)
			1.0	4.7	19	42	120	470	1,900	12,000 (12 L)	47,000 (47 L)
	Loading [mg]		5	25	100	220	600	2,500	10,000	60,000 (60 g)	240,000 (240 g)
HIGH ↑ Column efficency, Pressure, Costs ↓ LOW	Particle size [µm]	5	+++	+++	+++	+++	++	+	+		
		10	++	+++	+++	+++	+++	++	++	++	++
		10–20	+	++	++	++	+++	+++	+++	++	++
		15–30		+	+	+	++	+++	+++	+++	++
		50~					+	++	++	+++	+++

+++ Most appropriate, ++ Appropriate, + Depending on purpose

More information concerning the strategies which should be used during the development of preparative scale HPLC processes can be found on the YMC website **www.ymc.eu** or please contact your YMC representative.