Analytical versus Preparative and Production-Scale HPLC: Some Considerations

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Column dimension is irrelevant; it is the scope of a separation that determines whether it is analytical or preparative HPLC.

While much of the theory and equations that underlie analytical high performance liquid chromatography (HPLC) can also be used in preparative and production-scale HPLC, there is a lot that is different. The first and perhaps most obvious difference is the scope of the separation. In analytical HPLC, the main purpose is the identification and quantification of one or several peaks in the chromatogram, and there is often no need for sample collection after analysis. By contrast, in preparative chromatography, the aim is to isolate a single substance at a certain level of purity. Whether this is performed for further R&D purposes or as a purification step that will eventually be used in production, there is a need for high throughput. In this context, loadability is the key factor. It can be defined as the amount of crude sample per amount of stationary phase that can be loaded onto the column while still reaching purity demands.

When sample loading is increased, the approximation can no longer be made that analytes adsorbed to the stationary phase will not interact with each other. Since the number of available sites on the stationary phase surface can no longer be seen as infinite, the separation has moved from the linear to the non-linear part of the adsorption isotherm. This is one of the most important aspects that differentiates preparative and production HPLC from analytical HPLC. The adsorption isotherm can usually be described as Langmuirian, anti-Langmuirian or S-shaped. Depending on which adsorption isotherm most accurately describes the adsorption behaviour, different peak shapes will be observed when increasing the loading of a component.

Figure 1 shows the peak shape of a preparative purification of a peptide. In this case, a different mobile phase buffer – while keeping

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the same stationary phase – altered the adsorption behaviour resulting in Langmuirian and anti-Langmuirian shaped peaks. The chromatographic properties under nonlinear conditions are difficult to model and therefore are often determined empirically (1).

The Impact of Loading on Purity and Yield

In order to investigate chromatographic behaviour with respect to three important parameters – loading, purity and yield – three injections of different volumes were made on one stationary phase using identical chromatographic conditions. The only restriction was 98 per cent overall purity. The crude sample was a peptide (<5,000 MW) with a starting purity of 82 per cent, which was determined by analytical HPLC. Chromatograms and conditions are displayed in Figure 2.

Fractions were collected every 30 seconds during peak elution, and analysed using the same analytical method used for determining the crude purity. With data from the fraction analysis, an elution profile was reconstructed. The purity and yield of the individual fractions – as well as the overall purity and yield – were calculated.

The results displayed in Table 1 clearly show that sample loading greatly affects purity and yield,

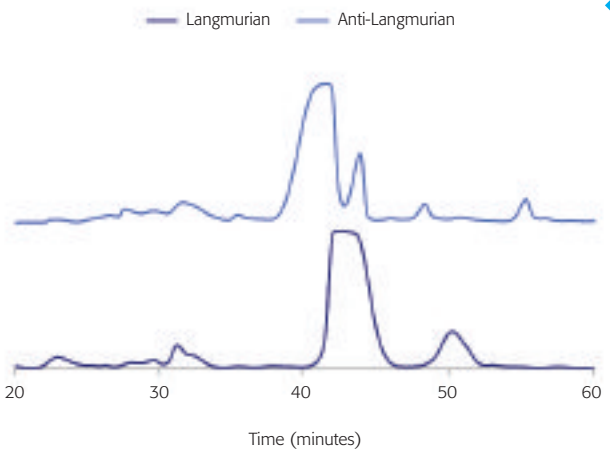

Figure 1: An example showing how the adsorption behaviour of a substance (peptide, MW ~1500) varies depending on mobile phase and stationary phase constituents. In this case, either a Langmuirian or an anti-Langmuirian type behaviour is observed. The separation method was as follows. Stationary phase: Kromasil-100-10-C18 (250 x 4.6 mm), flow rate: 1 ml/min. Mobile phase 1 (Langmuirian): A: 100mM NH_4Ac, pH 4; B: EtOH, 15-24 per cent B during 60 minutes. Mobile phase 2 (anti-Langmuirian): A: 100mM $(NH_4)_2SO4$ pH 3; B: EtOH, 17-26 per cent B in 60 minutes.

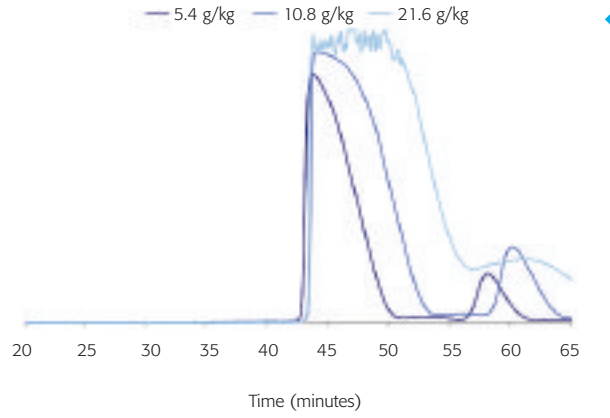

Figure 2: Overlaid chromatograms from the preparative study. The separation method was as follows. Stationary phase: Kromasil-100-10-C18. Mobile phase: A: 200mM NH_4Ac pH 4.0 / EtOH 90/10 (v/v); B: 200mM NH4Ac pH 4.0 / EtOH 10/90 (v/v). Gradient during elution: 22-34 per cent B during 60 minutes. Flow rate: 0.75 ml/min. For the largest injection, the UV detector limit was reached resulting in noise that somewhat distorts peak shape.

Table 1: Results from the preparative study. Load is defined as gram crude sample per kg packing material. Cycle time is defined as the elution time for the last collected peak with an additional 15 minutes for column washing and re-equilibration. Productivity is defined as amount of purified product per kg packing material and hour. Note that for the largest injection (3), the target purity of 98 per cent was not reached.

Preparative study	1	2	3
Load (g_{crude}/kg_{resin})	5.4	10.8	21.6
Purity (per cent)	98	98	95
Yield (per cent)	99	81	77
Cycle time (hours)	1.3	1.3	1.3
Productivity ($g_{product}/kg_{resin}/h$)	3.4	5.5	10.5

and must be carefully chosen depending on which parameter is the most important for the separation. In this case, sample loading could be doubled (from 5.4 g/kg to 10.8 g/kg) and still reach a 98 per cent purity. However, increasing the loading even further resulted in a much poorer separation. In fact, no single fraction reached the purity target.

Introducing the Loading/Yield/Purity Triangle

The results clearly show that optimising one of the parameters – loading, yield or purity – will greatly affect the others. This is why it is of the utmost importance to clearly define the scope of a specific purification before commencing method development. While purity (including overall purity and single impurity levels) is often defined by regulatory agencies, the other parameters can be altered more freely. The relationship between these

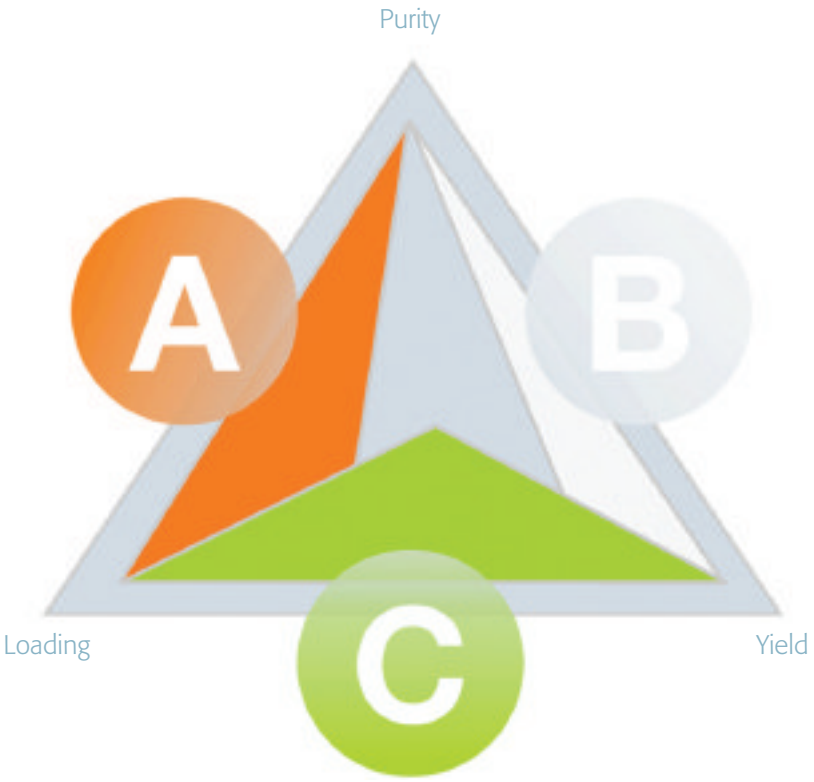

Figure 3: The loading/yield/purity triangle

Images: Akzo Nobel Separation Products

three parameters can be represented by the triangle, as shown in Figure 3. It becomes evident that one cannot perform the separation in all three corners at the same time. Instead, most preparative HPLC separations are performed along one of the sides of the triangle, which is why the definition of purification goals is so important.

On side A, yield has been sacrificed for maximised loading and high purity. This is the area to be close to if the value of the crude from upstream processes is low.

On side B, one sacrifices loading and, as a direct consequence, productivity in order to keep purity and yield high. This is a good choice if the value of the crude coming from upstream processes is high, and if productivity can be kept somewhat low. Due to the low loading, more injections are needed in order to purify a given amount of crude sample.

On side C, at first it may seem intuitively wrong, but sacrificing purity for high loading and yield is not that uncommon in preparative HPLC. Purification along this line is performed for quick purification in the R&D phase, which is when a small amount of medium purity sample is needed to perform further studies. Since these separations are usually performed on a small scale, semi-preparative columns – such as dynamic axial compression (DAC), pre-packed or spring type are typically used. However, performing a separation using this method is not a good idea when purifying a substance for the market due to purity restrictions.

Sometimes a two-step HPLC purification method is needed. In the first step, one typically targets the HPLC purification close to side C of the triangle, in order to remove separated impurities more easily. Collected medium purity fractions from the first step can then be re-introduced into the column using another method that operates more closely to side B. Note that a two-step process always increases work-load and solvent consumption, which is often the single largest contributor to the cost of a purification. In these cases, one should always try to improve the HPLC purification method or evaluate the possibility of altering upstream processes.

Reference

1. Guiochon G, Golshan-Shirazi S and Katti AM, *Fundamentals of Preparative and Nonlinear Chromatography*, 1994

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