Are the results from my screening studies always correct?

The importance of loadability studies for the development of prep LC processes

Abstract

Most phase screenings for preparative processes are carried out with analytical loading. Based on the results the resin offering the best resolution can be found. However, this may not necessarily be the best stationary phase for the preparative process. As preparative processes require high sample feed, loadability should be evaluated during the phase screening as well. Only the combination of these parameters leads to an economic and efficient process.

This expert tip shows the importance of a loadability study. The phase ideally suited for the exemplary process at preparative loading conditions allows more than 40% higher productivity – although at low loading conditions the other phase would have been favoured. This is related to optimised phase characteristics for a high loadability.

Introduction

Typically, screenings are performed with different stationary and mobile phases to identify the best combination. Due to the lack of sufficient feed, injections are typically done on an analytical scale without any overloading of the column. At the end of this screening, the best separation can be identified by comparing the resolution of the target compounds and the impurity eluting next to the target compound.

However, does this result always indicate the most suitable phase for the final process?

In order to achieve the best possible preparative chromatography process, it's important to select the stationary phase with the best combination of selectivity, loadability and lifetime. The selectivity can be evaluated with a screening with analytical loading. However, the loadability is not evaluated without the effective overloading of the stationary phase.

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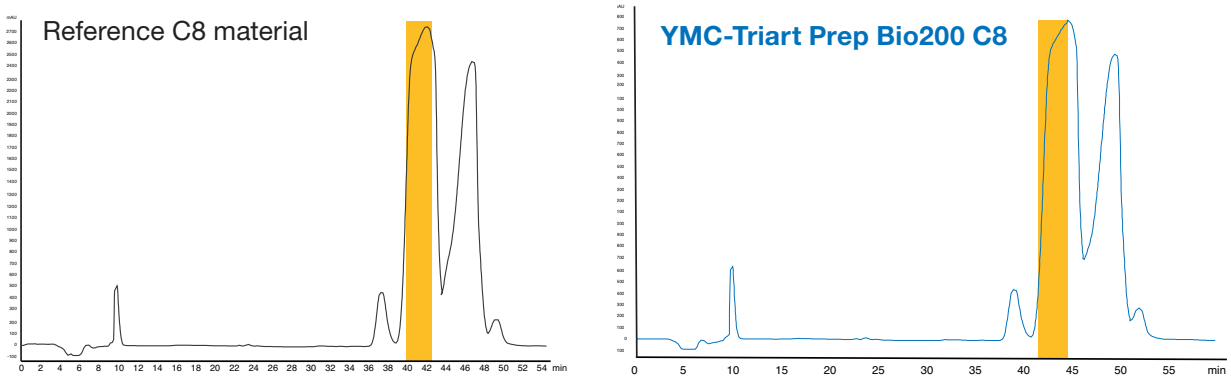
Interesting findings based on loadability studies!

The chromatograms shown below were achieved using the YMC-Triart Prep Bio200 C8 and another C8 phase. As can be seen in the chromatograms for a moderate loading of 8 mg, the resolution is quite similar for both stationary phases. The reference C8 material shows a slightly better resolution. Based on this result, one would typically continue to work with the reference C8 material.

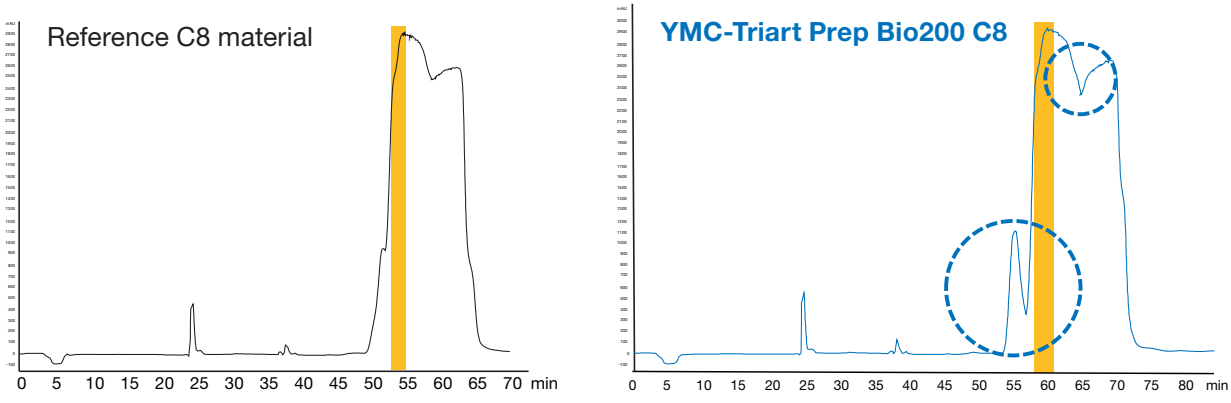
Loading: 8 mg



Fraction with ≥ 99% purity of target

However, on increasing the loading the picture changes. For a loading of 30 mg, the YMC-Triart Prep Bio200 C8 phase still shows a good separation whereas the reference C8 material shows strong overloading effects.

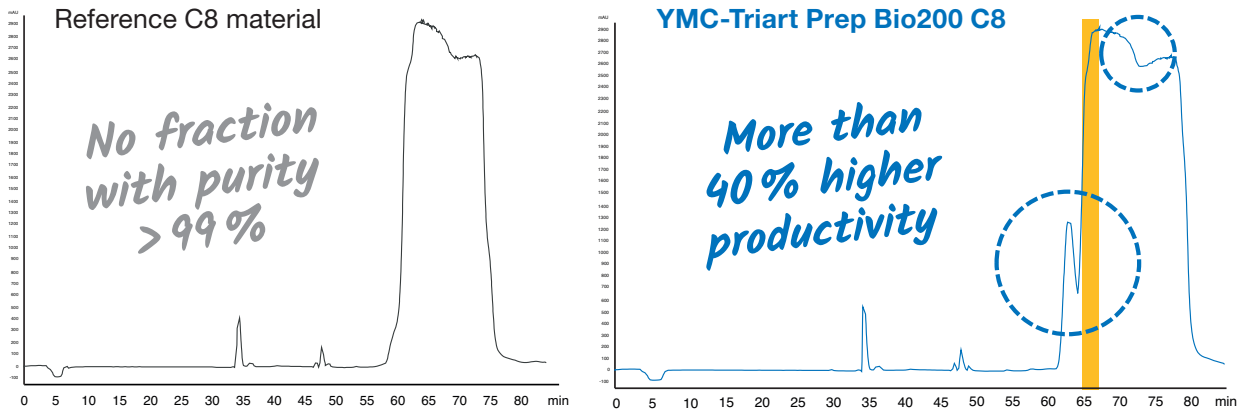
Loading: 30 mg



Fraction with ≥ 99% purity of target

Finally, with a loading of 45 mg, there is no fraction with the target purity of 99 % with the reference C8 material whereas the separation with the YMC-Triar Pre Bio200 C8 remains stable to reach the required purity.
This demonstrates that the achievable productivity is 42.5 % higher with the YMC-Triart Prep Bio200 C8.

Loading: 45 mg



Fraction with ≥ 99% purity of target

Chromatographic conditions

Column Size:	250 x 4.6 mm ID
Eluent:	A) buffer*/acetonitrile (90/10)
	B) buffer*/acetonitrile (60/40)
Gradient:	0%B (0-5 min) 0-45% (5-10 min)
	45–55% (10–58 min) 100%B (58–100 min)
Flow rate:	1.0 mL/min
Temperature:	ambient
Detection:	UV at 214 nm
Sample:	insulin in 0.1 N HCl left at 40 °C for 16 hr
Sample conc.:	2 mg/mL in 0.1% CH_3COOH (Insulin purity about 75 %)
buffer:	20 mM CH_3COONH_4-CH_3COOH, 200 mM $(NH_4)_2SO_4$ (pH 4.5)

Conclusions

Phase screenings without the evaluation of the loadability of the stationary phases does not answer the question which phase is the most suitable one for a preparative process. Instead, the evaluation of the loadability must be part of the process development programme in the phase selection stage.