APPLICATION NOTE

# The Benefit of Scalability of YMC-Triart Prep in Oligonucleotide Separations

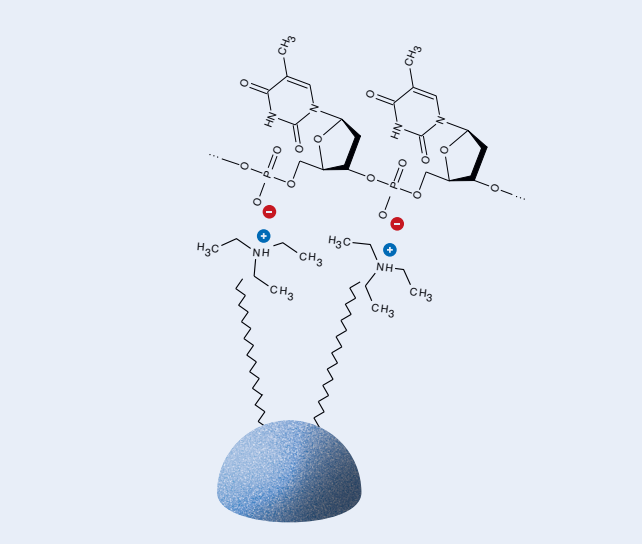
## Introduction

The development of a preparative process typically consists of three steps:

- **Method development at analytical scale**
- **Loadability studies**
- **Final scale-up to the preparative process**

To perform this process development as smoothly as possible several aspects have to be taken into account. It is very important to determine the final process scale before each screening. If the final purification goes beyond an analytical scale, the availability of stationary phases for preparative purposes must be clarified.

After the preparative availability has been clarified, the stationary phase screening begins. This application example uses oligonucleotides to show how a suitable separation is determined step by step. The challenge here is that classic reversed phase liquid chromatography is not directly applicable due to the incompatibility of the highly polar oligonucleotides and the non-polarity of the stationary phase modifications. Therefore, ion-pair reversed-phase chromatography (IP-RP) is a targeted approach. Ion pair reagents are used as intermediaries between the polar oligonucleotides and the non-polar stationary phase. Such ion-pairing reagents are typically alkyl sulfonates, alkyl ammonium salts, or similar compounds such as triethylamine (TEA).



In order to achieve rapid screening with the highest possible resolution, the stationary phases are mostly used in analytical format. In this case it is very important to check the scalability of the particles. This means that comparable separation behaviour should be achieved for all particle sizes used: from the analytical particle size to the preparative quality. Using a fully scalable stationary phase will greatly facilitate method development as reliable results are obtained from analytical to preparative scale.

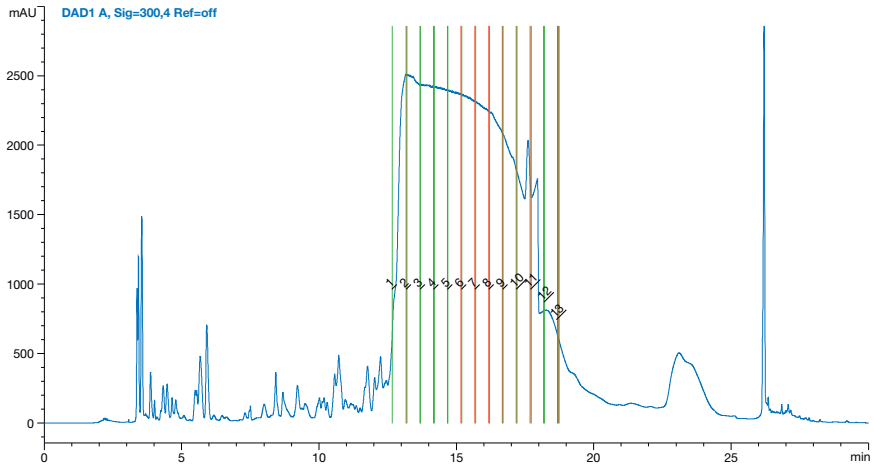
## Comparison study – oligonucleotide separation with analytical and preparative RP phases

Together with one of the leading global pharmaceutical companies worldwide, an IP-RP separation of a 20mer oligonucleotide was tested with different particle sizes in order to examine the scalability of YMC-Triart. The particles sizes used were 3 µm (YMC-Triart C18) as commonly used for analytical applications, and 7 and 10 µm (YMC-Triart Prep C18-S) for preparative purification methods.

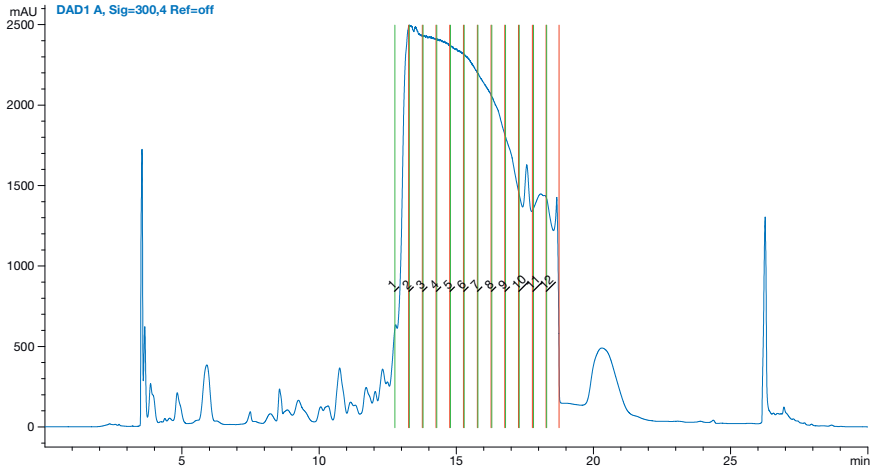
## Conditions

| | |
|---|---|
| Column: | YMC-Triart C18 (3 µm), YMC-Triart Prep C18-S (7, 10 µm) 250 x 4.6 mm ID |
| Eluent: | A) 100 mM triethylammonium acetate |
| | B) Acetonitrile |
| Gradient: | 8%B (0–2 min), 8–23%B (2–22 min), 23–80%B (22–23 min), 80%B (23–28 min), 80–8%B (28–28.1 min), 8%B (28.1–35 min) |
| Flow rate: | 1.3 mL/min |
| Temperature: | 45 °C |
| Injection: | 50 mg (75 mg/mL crude) |
| Sample: | 20 mer oligonucleotide |

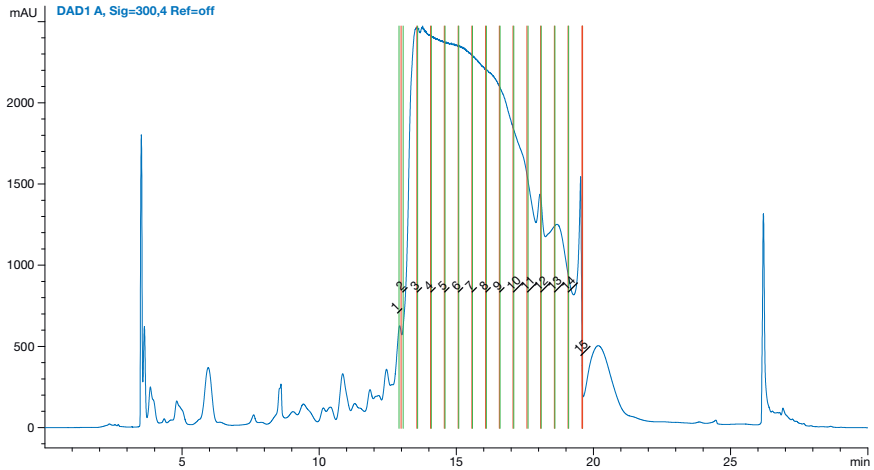
**3 µm**




**7 µm**




**10 µm**



### Results of the comparison study

Due to the fully scalable properties of YMC-Triart particles, comparable chromatograms were obtained for the three particle sizes tested. All particle sizes showed good selectivity for this oligonucleotide separation.

Furthermore, purity and yield were maintained when scaling up from 3 µm to 10 µm. Depending on the selection of the collected fractions, the calculated yield varies from 57% to 100% whereas the minimum purity reached was about 89%.

| | Pool | 100% yield[1] | Best result[2] |
|---|---|---|---|
| 3 µm | Purity: | 88.8 | 93.0 |
| | Yield%: | 100.0 | 89.9 |
| 7 µm | Purity: | 88.6 | 93.1 |
| | Yield%: | 100.0 | 90.1 |
| 10 µm | Purity: | 88.7 | 93.1 |
| | Yield%: | 100.0 | 91.3 |

1 100% yield, fractions collected: 1 to 11 (3 µm and 7 µm), 2 to 12 (10 µm)
2 Best result, fractions collected: 3 to 9 (3 µm and 7 µm), 4 to 11 (10 µm)

- ✔ **Easy method development due to full scalability of YMC-Triart**
- ✔ **Comparable yield and purity for 3 µm, 7 µm and 10 µm**
- ✔ **Excellent selectivity for oligonucleotide separations with YMC-Triart C18**
- ✔ **YMC-Triart C18 is available from analytical to preparative scale sizes**