Ion-Pair Reversed-Phase Purification of De-Protected Oligonucleotides - Choice of Pore Size

Application Note

Authors

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Introduction

Ion exchange, or ion-pair reversed-phase chromatography, can be used for the analysis and purification of de-protected synthetic oligonucleotides. The choice of which technique to use will often depend on the availability of equipment, and the pre- and post-steps in the purification scheme. For ion-exchange chromatography, essentially aqueous eluents are used, but the volumes required are large and non-volatile salts are required for elution. For ion-pair reversed-phase chromatography organic solvents are required, but the eluents are essentially volatile and smaller volumes are obtained. This application note focuses on the media and ion-pair requirements for IP-RP chromatography.

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Materials and Methods

Separation of Phosphodiester in a Thiolated Oligonucleotide

Column:	PLRP-S 100Å, 8 µm, 150 x 4.6 mm (Part number PL1512-3800)
Mobile Phase:	0.1 mM Triethylammonium acetate (TEAA) in Water : ACN 0.025 mM TBuABr in Water : ACN
Temperature:	Ambient or 60 °C

Determination of Binding Capacity

The dynamic binding capacity was determined for each pore size of PLRP-S by frontal loading analysis. The oligonucleotide solution, prepared in binding eluent, was pumped though the column and the breakthrough curve was obtained by monitoring the column eluent at 260 nm. The volume of oligonucleotide solution required to saturate the column was determined at 20% of the adsorption of the oligonucleotide solution, when no column was in place.

Columns:	PLRP-S 100Å, 3 µm, 50 x 4.6 mm (Part number PL1512-1300) PLRP-S 300Å, 3 µm, 50 x 4.6 mm (Part number PL1512-1301) PLRP-S 1000Å, 5 µm, 50 x 4.6 mm (Part number PL1512-1502) PLRP-S 4000Å, 5 µm, 50 x 4.6 mm (Part number PL1512-1503)
Binding Eluent:	Acetonitrile 0.1 M TEAA pH 7.0 (1:99 v/v) or 0.025% TBuABr (1:99 v/v)
Wash Eluent:	Acetonitrile 0.1 M TEAA pH 7.0 (50:50 v/v) or 0.025% TBuABr (99:1 v/v)
Linear Velocity:	360 cm/h
Detector:	UV 260 nm

Results and Discussion

A high percentage of the synthetic oligonucleotides that require purification after synthesis are in the range of 25 to 35 nucleotides long. For efficient purification, that is high yield with good purity, good resolution a high capacity material must be achieved. The PLRP-S range of reversed-phase materials has a high surface area and is ideal for IP-RP chromatography. For good economic purification, the correct pore size must be chosen to achieve efficient mass transfer and maximum capacity.

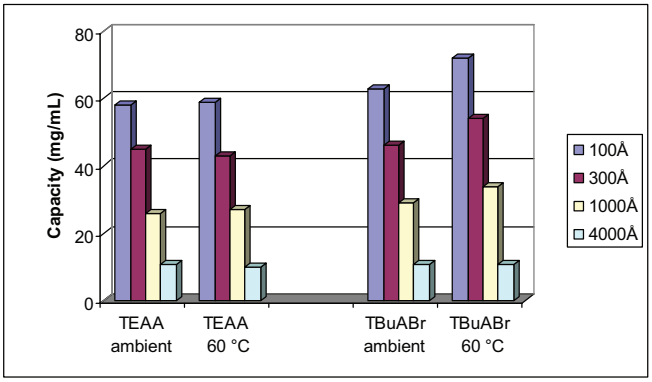

Figure 1. Dynamic capacity of four pore sizes of PLRP-S, at ambient and 60 °C with TEAM and TBuABr ion-pairing agents.

The capacity of a reversed-phase material will depend on the available surface area, which for any given pore size will be related to the solute size. The dynamic capacity for a 20mer oligonucleotide was determined for the four PLRP-S materials, 100Å, 300Å, 1000Å and 4000Å, using two ion-pairing agents, TEAA and TBuABr, as shown in Figure 1. Oligonucleotide chromatography is often performed at elevated temperature and so the dynamic capacity was done at both ambient and 60 °C. In all cases, the dynamic capacity decreases as the pore size increases, due to a reduction in the available surface area. The TEAA dynamic capacity is independent of temperature and less than the TBuABr, which shows an increase in capacity at 60 °C.

In Figure 2 it is clear that the PLRP-S 100Å material has good mass transfer characteristics for a 20mer oligonucleotide. The frontal loading curves are sharp.

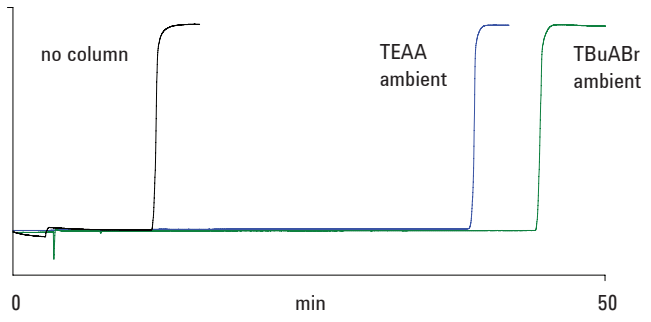

Figure 2. Frontal loading curves for a de-protected 20mer oligonucleotide produced at ambient temperature using TEAA and TBuABr ion-pairing agents. Oligonucleotide loaded at a linear velocity of 360 cm/h and a concentration of 4 mg/mL binding buffer.

Resolution must also be considered when choosing ion-pairing agents. With both TEAA (A) and TBuABr (B) it is possible to resolve thiolated 18 and 20mer oligonucleotides, but only the TEAA resolves the phosphodiester component, as illustrated in Figure 3.

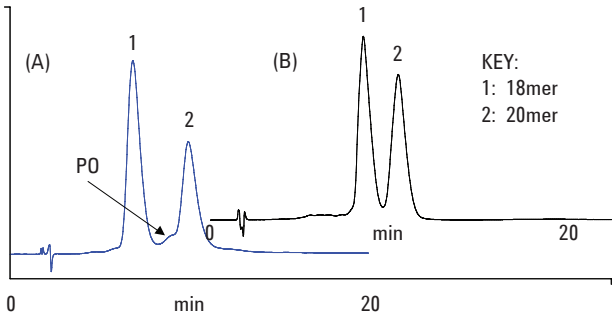

Figure 3. Resolution of thiolated 18mer and 20mer oligonucleotides.

Conclusions

For the purification of typical 15 to 20mer phosphodiester and phosphorothioate oligonucleotides, the small pore high surface area PLRP-S 100Å is recommended. PLRP-S has the highest dynamic capacity and good mass transfer characteristics.

To resolve residual phosphodiesters from phosphorothioate oligonucleotides using ion-pair reversed phase HPLC, the TEAA ion-pairing agent is preferred.

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