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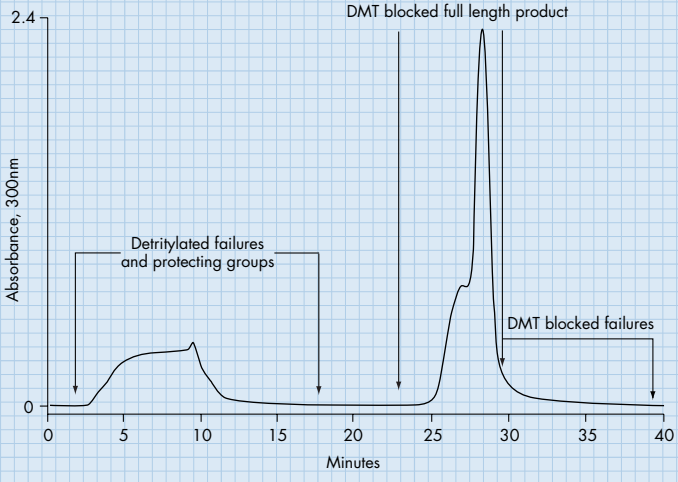
HPLC Purification of Phosphorothioated DNA for Antisense Therapeutic Investigations

Objective:

The objective of this application note is to demonstrate the utility of reversed-phase HPLC for large scale purifications of DMT protected synthetic oligonucleotides.

Details:

Synthetic oligonucleotides are successfully used in a wide variety of applications ranging from use as hybridization probes to utilization as primers for DNA sequencing and the polymerase chain reaction* (PCR). Most recently, studies have shown the effectiveness of using standard (i.e. phosphodiester, P=0) or phosphole backbone modified (i.e. phosphorothioated, P=S) oligonucleotides to selectively inhibit the production of targeted proteins within cells. As such, "antisense and related technology investigations" require milligram to multigram amounts of purified synthetic DNA. Reversed-phase HPLC provides a rapid and cost effective technique for the isolation of either phosphodiester or phosphorothioated full length oligonucleotide products for these applications.



Column:	Bondapak® HC_{18} HA (8 x 100mm Radial-Pak™ cartridge)
Eluent A:	100mM ammonium acetate, pH 7.2/methanol (95:5, v/v)
Eluent B:	Methanal/Milli-Q® water (95:5, v/v)
Sample:	Phosphorothioated 21 mer (DMT protected)
Injection:	12 mLs, containing 624 O.D.260nm units (21 mgs) of oligonucleotide in 70% A and 30% B, injected into column through pump.

Gradient:

Time	%A	%B	Curve
Init	70	30	*
20	65	35	11
24	50	50	11

Flow Rate:	2.0 mL/min.
Detection:	300nm

Milligram quantities of DMT protected synthetic oligonucleotides can be rapidly purified from undesired synthesis reaction by-products using Bondapak HC_{18} HA reversed-phase packing in an 8 x 100mm Radial-Pak cartridge. This same packing is available in a variety of column dimensions and in bulk packaging for gram scale purifications as required for antisense therapeutic investigations.

System:

The HPLC system used consisted of a Waters W600E Multisolvent Delivery System, Waters 486 U.V. Detector. and Waters 860 ExpertEase™ Data System (signal via SIM/LAC/E™.

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* See U.S. Patent No. 4683202 to Cetus Corporation

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