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Published by Waters Corporation
34 Maple Street
Milford, MA 01757 USA
Tel: 508-478-2000
Toll-free: 1-800-252-4752
Fax: 508-872-1990

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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=O) or phospho- nyl backbone modified (i.e., phosphorothioate, 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.

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™).

References:


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