

Purification of Oligonucleotides by Ion-pair Chromatography on Hybrid Silica Particles

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Paul Rainville, Martin Gilar, Jeff R. Mazzeo,
and Reb J. Russell II*

Waters Corporation, Chemical Research and Development Department, 34 Maple St., Milford, MA
01757

*send correspondence reb_russell@waters.com

Abstract

Synthetic oligonucleotides are used as primers for DNA sequencing and PCR and are also being investigated as drug candidates. Due to failure sequences, the purity of 25-mer oligonucleotides is typically 80-85%. Higher purity is required, especially for PCR applications. Typically, oligonucleotides are purified by electrophoresis or HPLC. The current techniques have limitations. Slab-gel electrophoresis is a laborious process, although it affords very high purity (>98%). HPLC suffers from the fact that the oligonucleotides are purified in the "trityl on" state. After purification, the DMT protecting group must be removed. To address these limitations, we have developed methods for "trityl off" purification of oligonucleotides using ion-pair reverse phase chromatography on hybrid silica phases (XTerra™ MSC₁₈). We demonstrate purification strategies for synthetic oligonucleotides up to 30-mers on a micromolar scale.

Current Methodology

- Desalting with gel filtration cartridges
 - Lowest purity
 - Simple, high-throughput approach
- PAGE purification
 - Considered to give highest purity
 - Laborious process, requires additional steps post-purification
- HPLC

Current HPLC Methodology

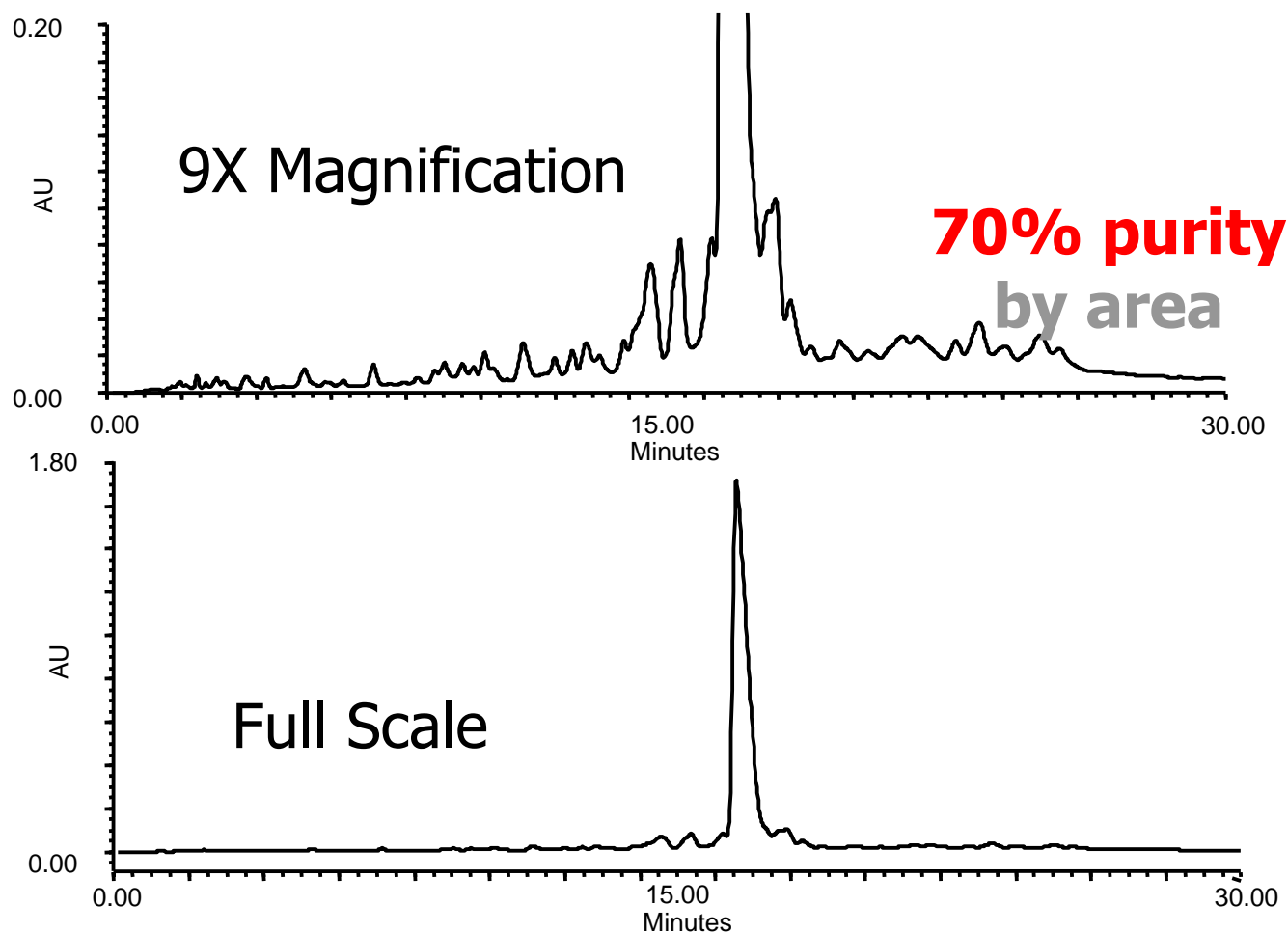
Considered to offer adequate purity

- Two techniques in use
 - Reversed-phase
 - Trityl-on purification
 - Requires deprotection
 - Ion-exchange
 - Trityl-off
 - Requires desalting
 - “Better recovery than gel filtration cartridges but lower purity than PAGE”

Experimental

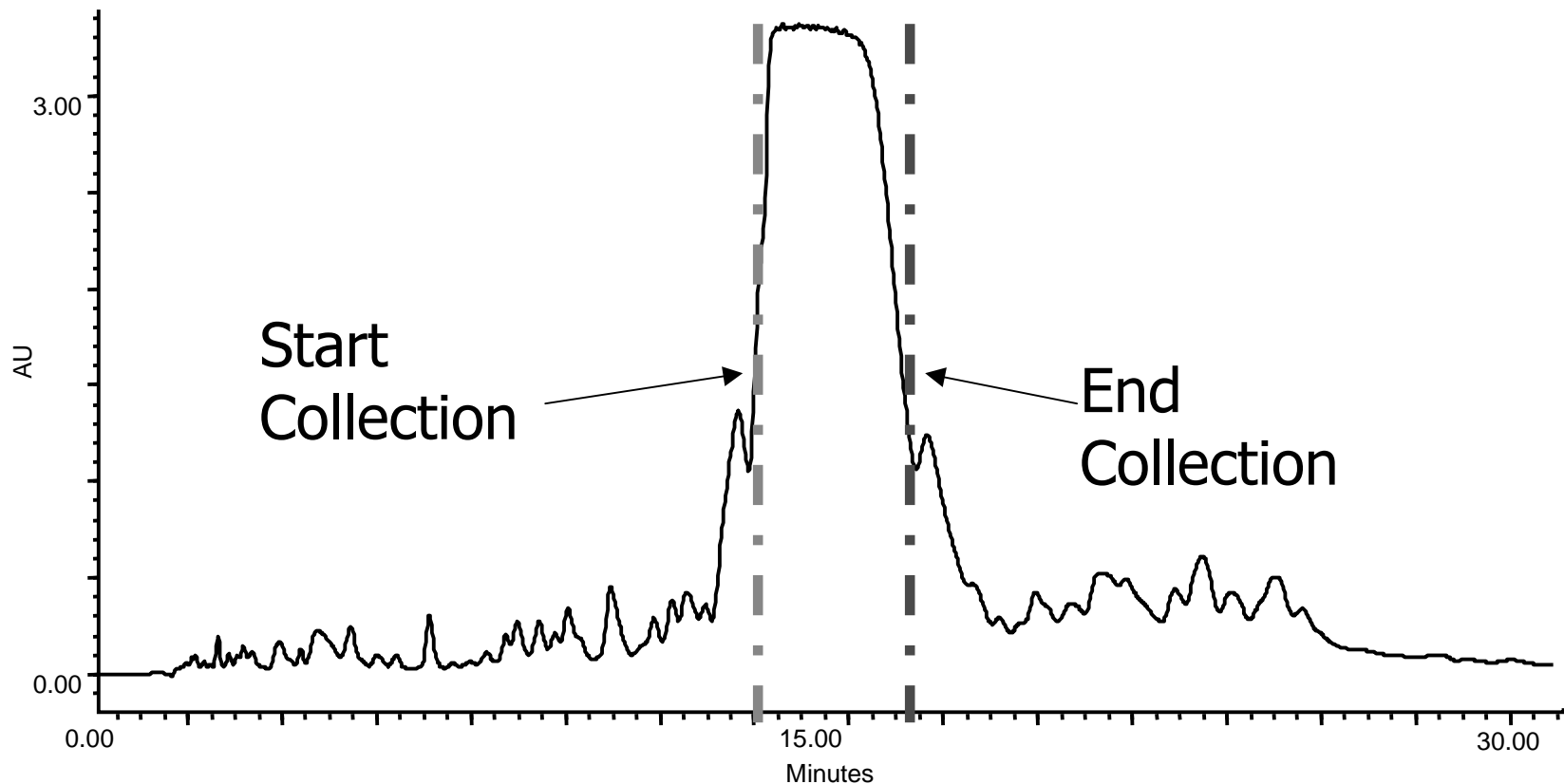
- Test sample: 25mer Oligonucleotide
 - Seq(5'-3')ACCTCTGCACCCATCTCTCTCCTCA
- 0.1 μ mol oligonucleotide single injection
- Purity by UV peak area
- Purity compared to three samples provided by vendor:
 - Desalted
 - PAGE purified
 - Anion-exchange purified
 - Purchased 0.1 μ mol of each
- XTerra™ 4.6 x 50mm MS C₁₈ 2.5 μ m Column
- Waters 2690 HPLC with Waters UV Detector

Purity Determination of Purchased, **Desalted** Oligonucleotide Using XTerra™ Methodology



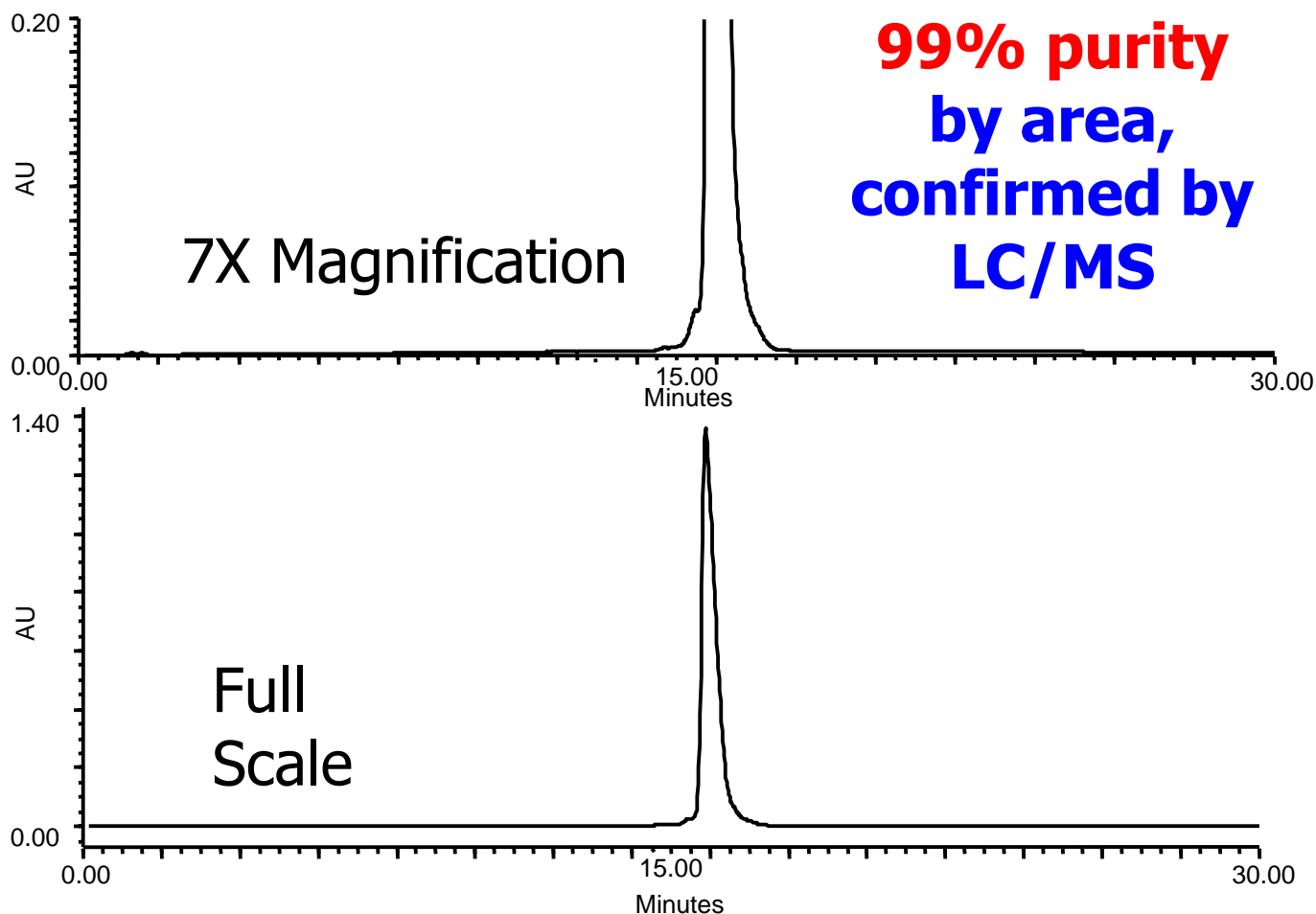
Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Preparative Injection of 0.1 μmol of Purchased, Desalted Oligonucleotide



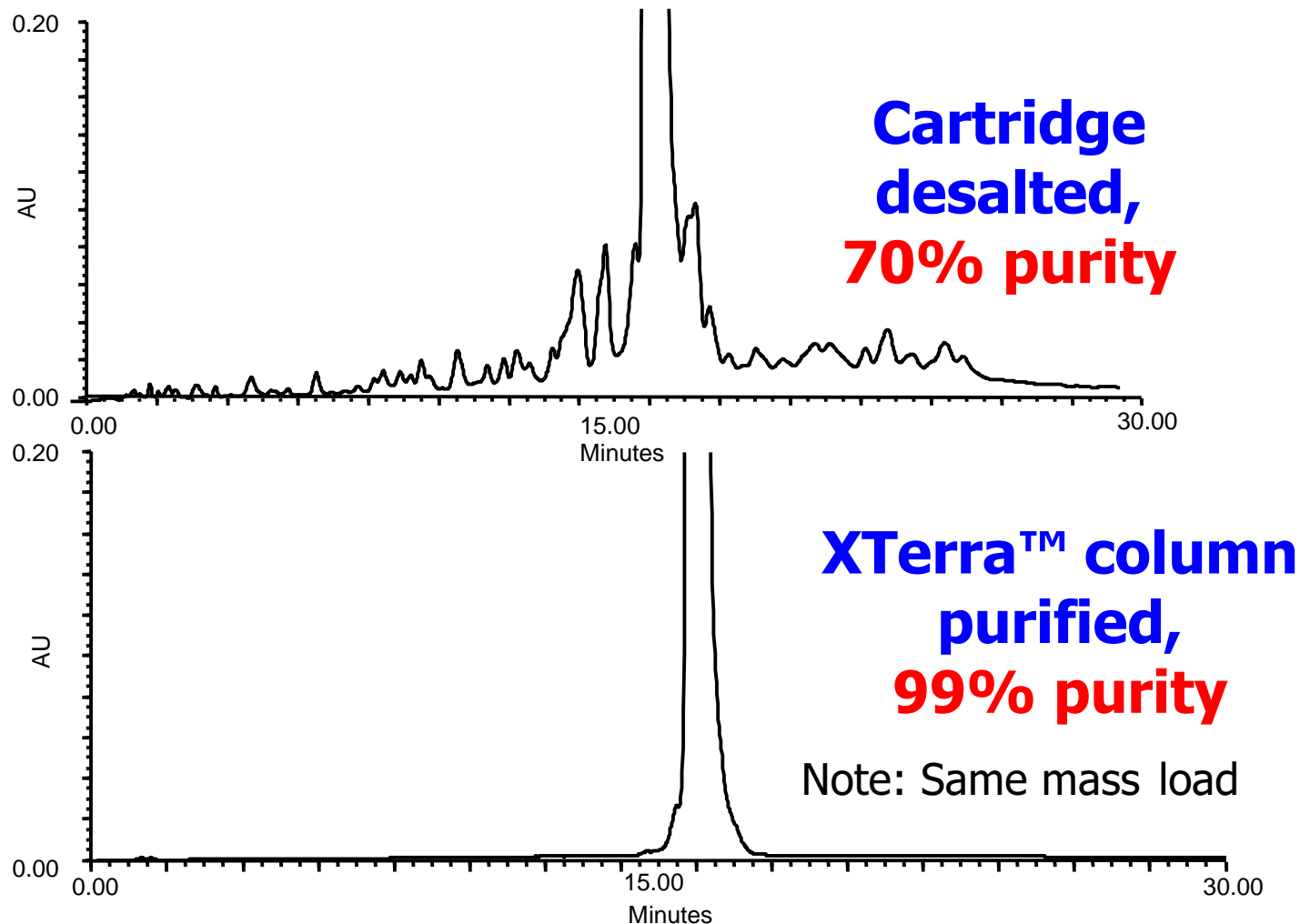
Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Purity Determination of Collected Fraction



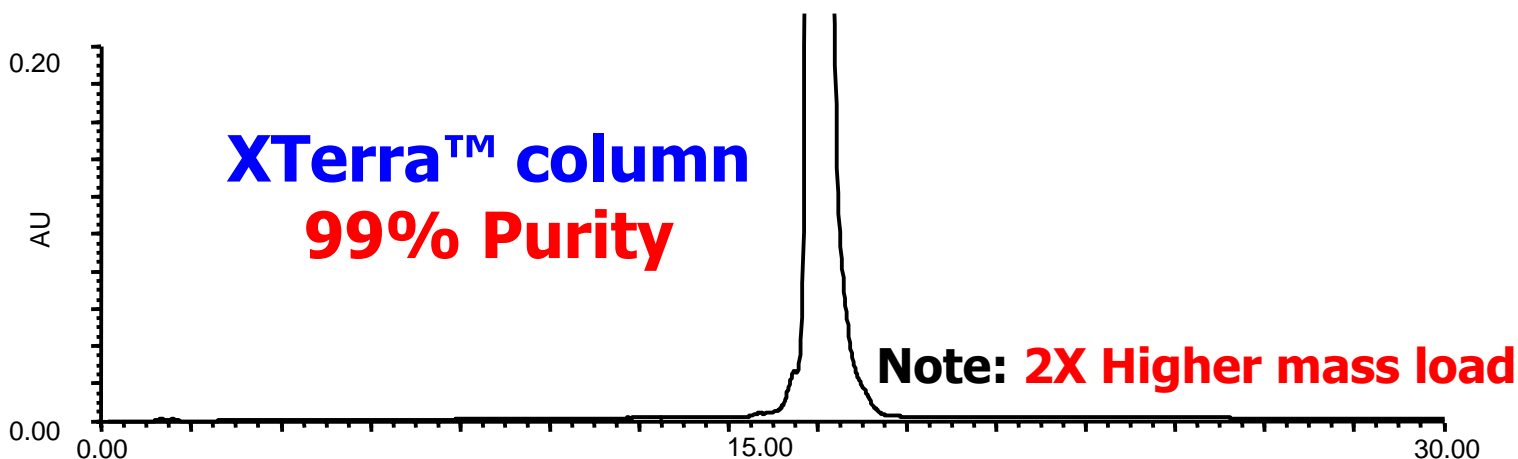
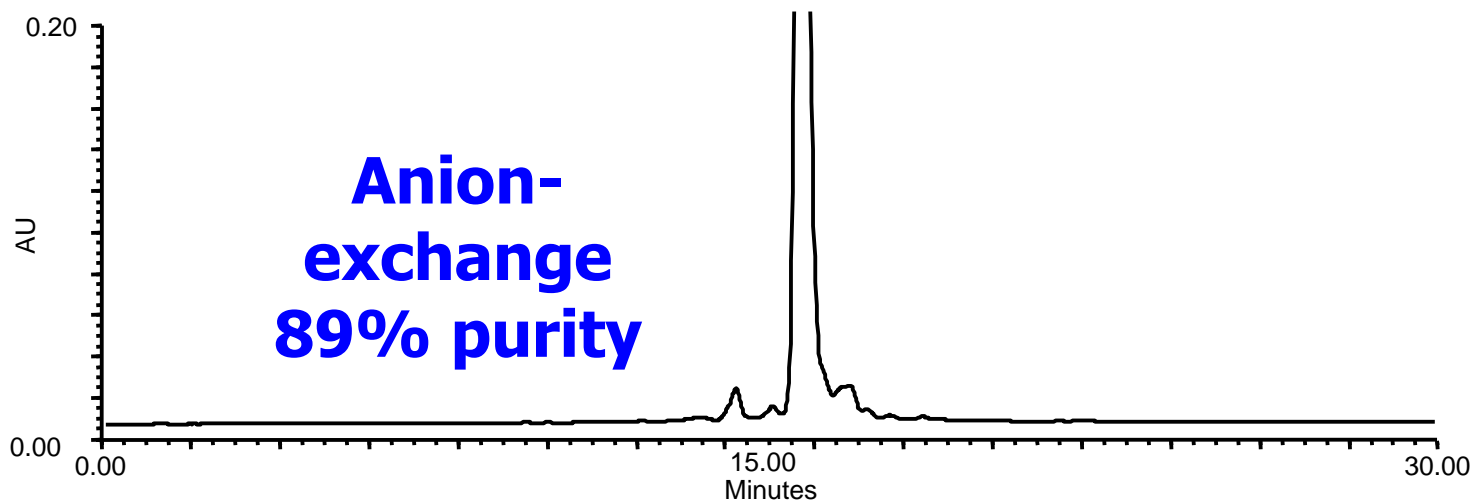
Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Cartridge-desalted Vs. XTerra™ Column Purified Oligonucleotide



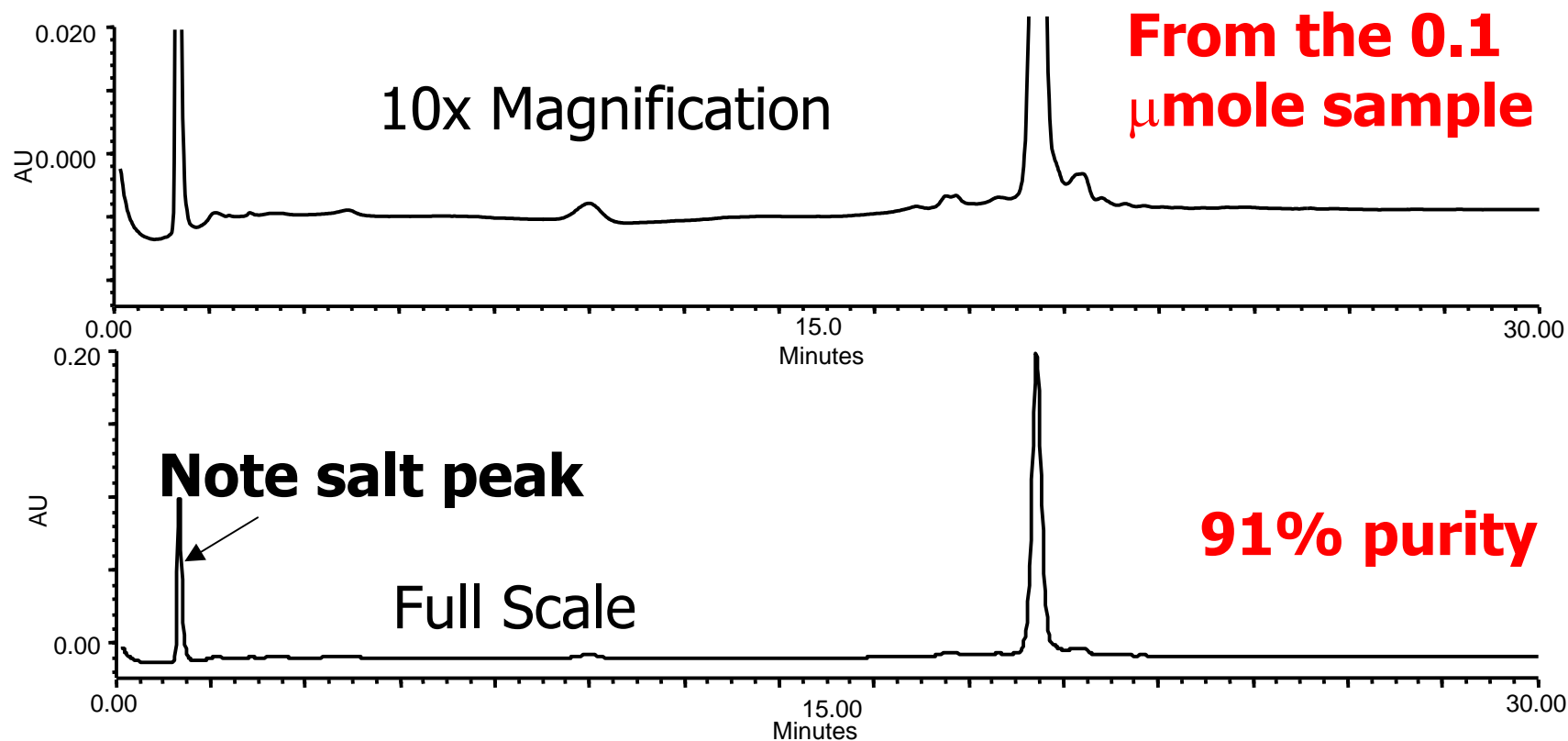
Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Purchased **Anion-Exchange** Purified Oligonucleotide vs. XTerra™ Column Purified



Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Purity Determination of **PAGE** Purified Oligonucleotide



Mobile Phase: 0.1 M Triethylammonium Acetate pH7.0 with a 0.125%/min gradient 5% to 15% ACN.
Flow Rate: 0.5 ml / min

Purity Determination Results*

<u>Technique</u>	<u>Expected Purity</u>	<u>Percent Purity</u>
Desalted	60-70%	70%
Anion-Exchange	85-95%	89%
PAGE	85-95%	91%
XTerra™	>95%	99%

* Using XTerra columns, confirmed by LC/MS

Typical Results Range for 0.1 μmol

<u>Technique</u>	<u>Percent Purity</u>
Desalted	60-70%
Anion-Exchange	85-95%
PAGE	85-95%
XTerra™	>95%

Purity and Recovery for Triplicate Injections

Injection	Purity (% area)	Recovery (% OD)
1	95.75	63.33
2	96.75	70.00
3	98.78	66.66

Conclusions

Capabilities of the XTerra™ Column Methodology

- Purification of oligonucleotides up to 25mers with high resolution from n-1 products
- Loading capacity up to 0.1 μmol per injection for the 4.6 x 50 mm column dimension
- Highest purity (>95%) of methods tested
- Highest recovery (60-70%) of methods tested

Conclusions Continued

- Trityl-off oligonucleotide is purified
 - No deprotection required after purification
- Volatile mobile phase is used
 - No desalting necessary
 - Collected fraction is lyophilized, and your DNA is ready
- Use existing HPLC hardware