XTerra® COLUMNS

Oligonucleotide Purification Applications Notebook

Waters

www.waters.com

Waters Corporation, 34 Maple Street, Milford, Massachusetts 01757-3696 U.S.A.
Tel: 508 478-2000
Fax: 508 872-1990

HPLC Oligonucleotide Purification with XTerra®, MS C_{18}, 2.5 µm, columns

Click anywhere on Contents; type desired page #; click OK.

Contents

XTerra® Columns Applications Summary

- **Analyze the purity of oligonucleotide synthesis**
  - *Separation performance of XTerra® columns is comparable to capillary gel electrophoresis*
  - *Full length oligonucleotides are resolved from n-1 and other failure sequences*

- **Purify unlabeled oligonucleotides from failure sequences with high product purity**
  - *> 95% product purity for oligonucleotides*
  - *Improved separation performance compared to ion exchange HPLC*
  - *Equivalent or better purity than PAGE*
  - *Superb recovery: typically > 90%* recovery of target product*

- **Purify fluorescently labeled oligonucleotides from failure sequences**
  - *Excellent purification of fluorescent oligonucleotides (> 90% product purity for fluorescently labeled oligonucleotides)*
  - *Outstanding recovery: > 85% of target labeled oligonucleotide recovered*

- **Fast separation, high productivity, easy post-purification handling**
  - *One step purification: easy scale up*
  - *No post-purification desalting or oligonucleotide deprotection is required*
  - *Collected oligonucleotide fraction is simply lyophilized, then ready for use*
  - *Cost effective: column life of approximately 1000 injections*

Table 1: Comparison of purity between available methods

Technique	Published Expected Purity	Actual Purity by HPLC/CGE	Recovery of Target
Desalted (Gel filtration)	60 – 70%	70%	~ 80%
Anion Exchange	85 – 95%	90%	~ 37%
PAGE	85 – 95%	91%	~ 8%
XTerra® Column	> 95%	> 95%	> 90%*

The oligonucleotide purity and recovery obtained with XTerra® columns is compared to other commonly used methods.
* At standard mass loads.

Introduction to XTerra® Columns for Oligonucleotide Purification

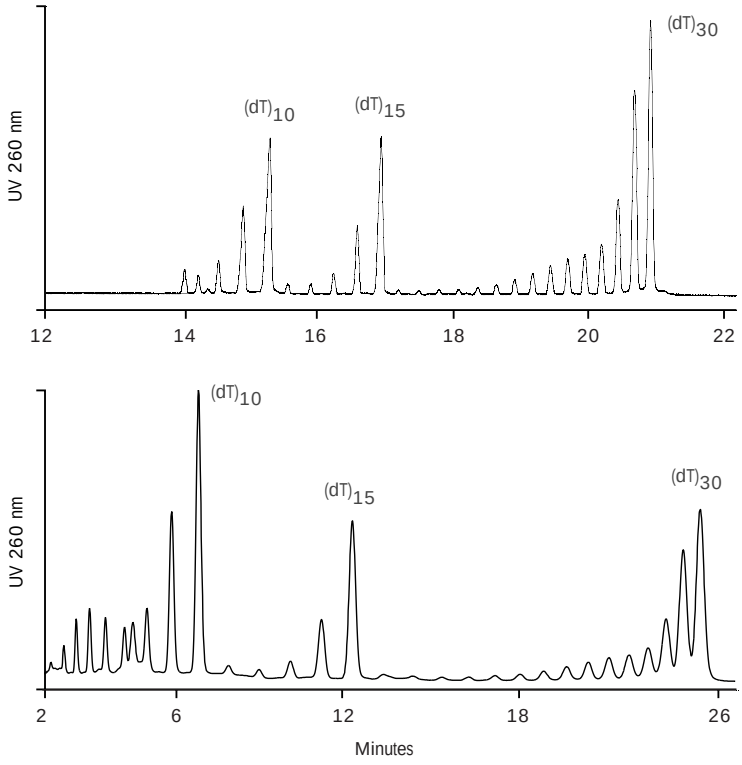
The XTerra® column oligonucleotide purification technology is based on highly efficient reversed-phase, ion-pair HPLC separation of the "trityl off"* target oligonucleotide from failure products. These failure products are produced during synthesis when a nucleotide fails to be coupled to the oligonucleotide chain. Failure sequences can also occur when fluorescent tags are not attached to the oligonucleotide. The XTerra® column oligonucleotide purification technology provides significant advantages over current technologies used to purify oligonucleotides (Table 1). Compared to purification with cartridges, gel electro-phoresis, desalting, or ion exchange chromatography, XTerra® column technology couples the highest recovery with the highest purity. XTerra® columns represent the new standard in oligonucleotide purification.

XTerra® columns are packed with a porous, 2.5 µm, hybrid particle. The small particle size and large surface area of sorbent result in both high separation efficiency and large sample capacity. The 10 mm x 50 mm preparative column can purify up to 0.5 µmole of oligonucleotide without compromising purity or recovery. Higher mass loads, up to 2.5 µmole, can be purified with the same high purity and only moderate reduction in recovery. XTerra® hybrid technology provides a high efficiency of separation and improved pH stability of sorbent. This is particularly beneficial at the elevated temperatures that are used to enhance separation of oligonucleotides. Column lifetime at 60°C with the suggested running buffer is approximately 1000 injections. This lifetime significantly exceeds that for other C_{18} columns available on the market. The XTerra® columns separation performance is comparable to capillary gel electrophoresis (CGE) for resolving full-length oligonucleotides from failure sequences (Figure 1).

The XTerra® column methodology utilizes ion-pairing mobile phases with a C_{18} reversed-phase sorbent. The oligonucleotides are separated according to their length, with longer oligonucleotides eluting later. In addition, RP-HPLC is well suited for separation of modified fluorescent oligonucleotides. The hydrophobicity of fluorescent tags improves the separation of the target product from non-labeled failure products. The properties of the mobile phase allow for easy lyophilization of the oligonucleotide, which is then ready for use*. **No post-collection detritylation or desalting is necessary, that is common for "trityl on" or anion exchange based HPLC purification methods.**

* Oligonucleotide is detritylated at the last step of synthesis. RP-HPLC separates trityl off full length product from failure sequences.

Figure 1: Separation of oligodeoxythymidine ladder CGE vs. "DMT off" RP-HPLC



CGE
PEG sieving matrix
BioCAP™ 75 µm; 27.5/34.5 cm
30° C, 15 kV run, 4s injection at 5kV

RP-HPLC
XTerra® MS C_{18} 2.5 µm
4.6 mm x 50 mm column
A: 5% ACN in 0.1M TEAA,
B: 15% ACN in 0.1M TEAA,
pH 7.0. Gradient from 26.7%B
to 46.7% B in 30 min.
0.5 mL/min, 60° C

Figure 1 shows a comparison of the separation ability of XTerra® columns and capillary gel electrophoresis. An oligo dT ladder was generated by endonuclease digestion of an 30 nt oligo dT molecule that was then spiked with intact 10 nt,15 nt and 30 nt oligo dT molecules as landmarks. The ladder was separated using the conditions described on Page 5. The chromatogram shows that XTerra® columns are capable of separating molecules that differ in size by only one nucleotide.

Equipment and Reagents

- **Waters XTerra® MS C_{18}, 2.5 µm, 10 mm x 50 mm or 4.6 mm x 50 mm column***
- **Waters Alliance® 2690 or Alliance® 2790 or minimally a ternary HPLC***
- **Waters 996 Photodiode Array UV Detector*** (or a single wave length detector for unmodified oligonucleotides)
- Triethylamine
- Glacial acetic acid
- Water (HPLC grade)
- Acetonitrile (HPLC grade)
- Pipettes
- **Waters Sample Vials***
- Graduated cylinder
- **PEEK tubing, 0.005 I.D.***
- **PEEK fittings***

* See Waters catalog for part number information

Note: Waters offers a kit containing PEEK tubing, fittings and cutter.

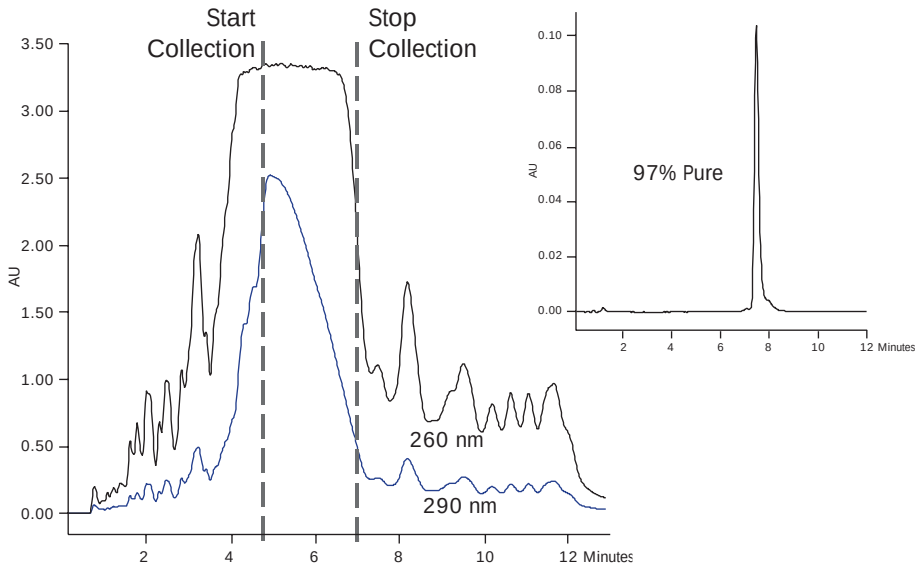
HPLC Instrument

For our experiments we used either a Waters Alliance® 2690 or a Waters Alliance® 2790 HPLC system with a Waters 996 PDA detector. Both systems have the advantage of high throughput and a cooled sample chamber keeping samples at 4° C. The XTerra® column was maintained at a constant temperature either in a water bath or with a built-in air column heater. In order to optimize the separation performance, the column has to be properly installed in the HPLC system. It is important to realize that extra column peak broadening can destroy successful separation. The choice of appropriate column connectors and system tubing is discussed in detail in appendix A. Millennium^{32} software was used for data analysis.

Choice of Column Size

The choice of column size depends on the scale of the synthesis reaction. The 4.6 mm x 50 mm column is excellent for analytical evaluation of oligonucleotide synthesis, and purification of mass loads equal to or below 0.1 µmol. The 10 mm x 50 mm column can be used for any synthesis reaction equal to or below 0.5 µmol. Alternatively, up to 5 fold higher mass loads can be purified on the columns, however, with loads this high (up to 0.5 µmole for the 4.6 mm x 50 mm, and up to 2.5 µmole for the 10 mm x 50 mm column), there is a trade off between purity and recovery. In order to obtain greater than 95% purity, recovery will be reduced. There are no differences in purity or recovery observed between column sizes for synthesis reactions equal to or below 0.1 µmol. However, the elution volume of the purified oligonucleo-tide is substantially larger for the 10 mm x 50 mm column than the 4.6 mm x 50 mm column for the same reaction. The larger elution volume generated by the 10 mm x 50 mm column will increase total lyophilization time needed to remove all solvents. For purifying high mass loads (greater than 0.1 µmole for 4.6 mm x 50 mm and greater than 0.5 µmole for 10 mm x 50 mm) it is recommended that the separation be monitored at 290 nm instead of 260 nm. This will allow for better peak identification (see figure 2 and appendix B)

Figure 2: High mass load purification: XTerra® MS C_{18} 2.5 µm 4.6 mm x 50 mm column, 0.5 µmole of 25 nt oligonucleotide

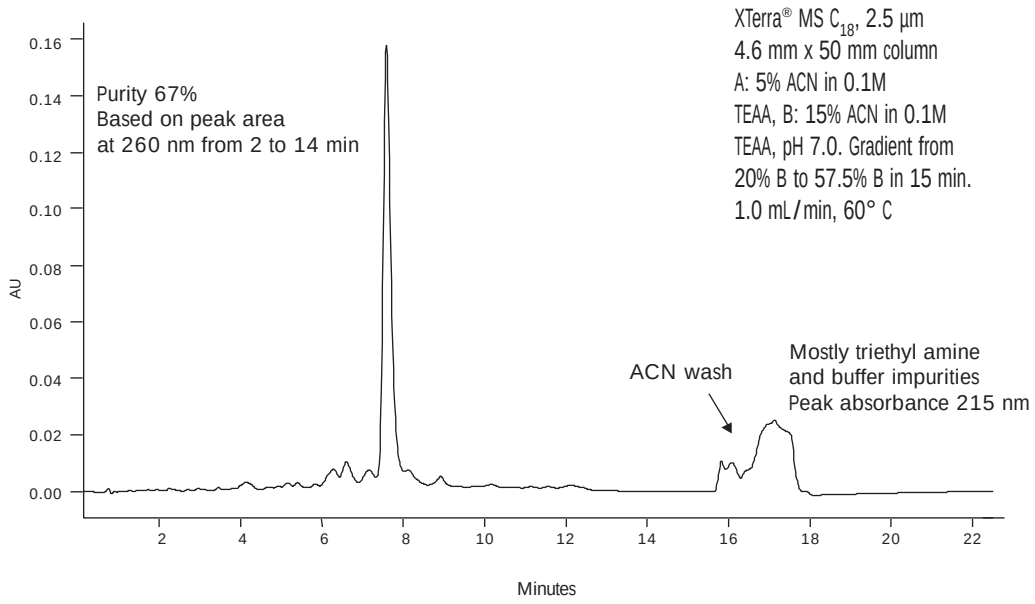


XTerra® MS C_{18}, 2.5 µm, 4.6 mm x 50 mm column A: 5% ACN in 0.1M, TEAA, B: 15% ACN in 0.1M TEAA, pH 7.0. Gradient from 20% B to 57.5% B in 15 min. 1.0 mL/min, 60° C

Method for Analytical Evaluation of Oligonucleotide Purity

There are an increasing number of applications for which oligonucleotide purity is vital. The XTerra® 4.6 mm x 50 mm column is an excellent choice for analytical evaluation of oligonucleotide purity.

Figure 3: Analytical separation of a crude synthesis (unpurified) 25 nt oligo on XTerra® MS C_{18} 2.5 µm 4.6 mm x 50 mm column



Mobile phases for the analytical evaluation of unlabeled oligonucleotides using XTerra® columns

Method for preparing mobile phases can be found in Appendix B.

Mobile Phase A:	5% Acetonitrile, 95% 0.1 M TEAA pH 7.0
Mobile Phase B:	15% Acetonitrile, 85% 0.1 M TEAA pH 7.0
Mobile Phase C:	100% Acetonitrile

XTerra® COLUMNS

HPLC Purification Conditions

1. For the HPLC injection the sample has to be dissolved in a solvent compatible with the mobile phase, ideally 0.1 M TEAA. Any NH_4OH or organic solvents should be eliminated.
2. Before using the column for the first time, equilibrate the column for 15 minutes at initial conditions (80% A: 20% B; appropriate flow rate; column temperature 60° C)
3. Perform analysis following the suggested gradient conditions (see Table 2 or Table 3).

Table 2: Suggested method for analysis and purification of unlabeled oligonucleotides*

Time (minutes)	Flow rate mL/min ID=4.6 mm	Flow rate mL/min ID=10 mm	% A	% B	% C	Column Temperature
0.01	1	4.72	80	20	0	60° C
15	1	4.72	42.5	57.5	0	60° C
15.01	1	4.72	0	0	100	60° C
16	1	4.72	0	0	100	60° C
16.01	1	4.72	100	0	0	60° C
17	1	4.72	100	0	0	60° C
17.01	2	9.44	80	20	0	60° C
22.5	2	9.44	80	20	0	60° C

Table 3: Suggested method for analysis and purification of labeled oligonucleotides*

Time (minutes)	Flow rate mL/min ID=4.6 mm	Flow rate mL/min ID=10 mm	% A	% B	% C	Column Temperature
0.01	1	4.72	100	0	0	60° C
15	1	4.72	0	100	0	60° C
15.01	1	4.72	0	0	100	60° C
16	1	4.72	0	0	100	60° C
16.01	2	9.44	100	0	0	60° C
22.5	2	9.44	100	0	0	60° C

* Appendix B describes methods for the optimization of oligonucleotide purification for non-standard molecules (shorter than 16 nt or those that have a very low or high GC content). This appendix also contains methods for recalculating the gradient for method adaptations.

Method for Purification of Unlabeled Oligonucleotides

The demand for ultra-pure oligonucleotides is ever increasing. However, the current protocols, including, slab gel electrophoresis, anion exchange HPLC and DMT-on HPLC, are either laborious, require extra steps or are not easily scalable to large mass loads. The following method provides a way to obtain exceptional purity coupled with outstanding recovery of target oligonucleotide. This method requires no extra desalting or removal of DMT groups, and is scalable to high mass loads without significant loss of target oligonucleotide.

Mobile phases for the purification of unlabeled oligonucleotides using XTerra® columns

Method for preparing mobile phases can be found in Appendix B. Solutions are adjusted to pH 7.0 using glacial acetic acid.

Mobile Phase A:	5% Acetonitrile, 95% 0.1 M TEAA pH 7.0
Mobile Phase B:	15% Acetonitrile, 85% 0.1 M TEAA pH 7.0
Mobile Phase C:	100% Acetonitrile

HPLC Purification Conditions

1. Choose the column size that is most appropriate for the reaction scale to be purified (see section on choice of column).
2. Before using the column for the first time, equilibrate the column for 15 minutes at initial conditions (80% A: 20% B; appropriate flow rate; column temperature 60° C, see Table 2).
3. Perform an analytical scale separation (see ***Method for Analytical Evaluation of Oligonucleotide Purity***, page 7) and determine the retention time of your oligonucleotide (optional).
4. Prepare a collection tube before injecting your sample. The collection tube should have ample room to handle the estimated volume collected (i.e. 4.6 mm x 50 mm column will give 0.5-2 mLs per peak collection, 10 mm x 50 mm column will give 2-5 mLs per peak collection).

5. Remove all NH_4OH from the oligonucleotide sample and reduce the total volume by evaporation.
6. Prepare sample in 0.1M TEAA, pH 7.0 solution at the correct concentration for the mass load you wish to purify.
7. Perform the purification following the recommended gradient conditions (Table 2, or Appendix B).
8. Collect the purified oligonucleotide. Collect the target peak (it should correspond with the analytically determined retention time) carefully to avoid the unwanted products that come off the column before and after your desired product (see Figure 4).
9. Lyophilize the sample and store under standard conditions.

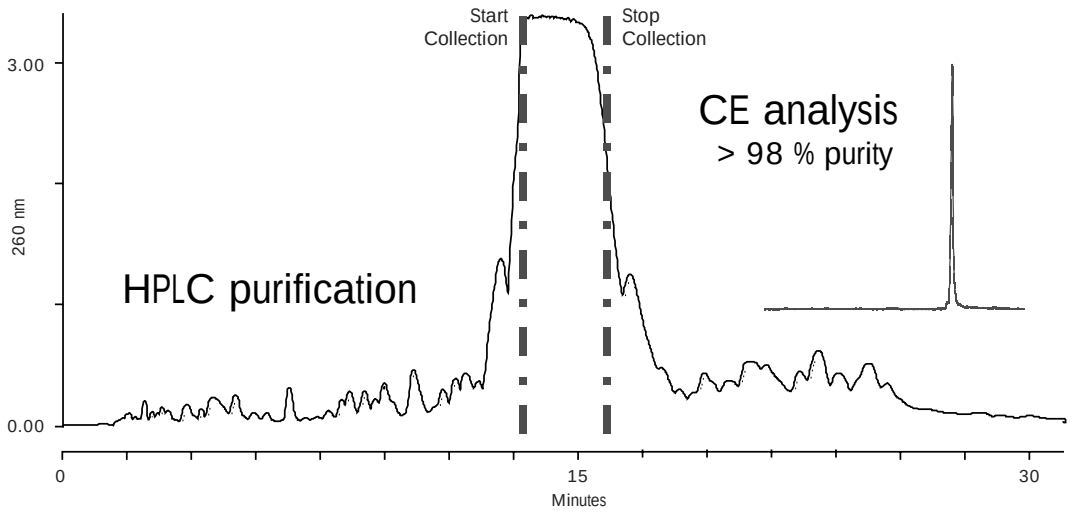
Collection of Purified Oligonucleotide - Manual Collection

NOTE:

Use the shortest possible piece of 0.005 I.D. PEEK tubing connected to the outlet of the UV detector to reduce the delay between the detection and collection. We advise the use of a linear recorder connected to the analog detector output. This will eliminate any possible delays between peak detection and collection due to detector data processing. Unless there is a failure in synthesis, the largest peak is the desired material and clearly stands out from failure products and small molecules. A full length sequence will be retained for a longer time than a failure sequence for a given reaction mixture.
Figure 4 is an example of 0.1 µmol of a crude oligonucleotide synthesis loaded on an XTerra® 4.6 mm x 50 mm column showing suggested collection start and stop points as well as the CGE analysis of the collected peak.

Figure 4: Semi-preparative injection of 0.1 µmol of purchased oligonucleotide

(5'-3') ACCTCTGCACCCATCTCTCTCCTCA; 25 nt



HPLC Conditions:
HPLC conditions: XTerra® MS C_{18} 2.5µm 4.6 mm x 50 mm, A: 5% ACN in 0.1 M TEAAc, pH 7; B: 15 % ACN in 0.1 M TEAAc, pH 7; gradient from 20 % B to 60 % B in 32 min, 0.5 mL/min, 50° C, UV 260 nm, 100 µml injection

CE Conditions
PEG sieving matrix BioCAP™ 75 µm; 27.5/34.5 cm 30° C, 15 kV run, 4s injection at 5kV

Method for Purification of Labeled Oligonucleotides

Fluorescent probes, TaqMan™ and molecular beacons can be purified using XTerra® columns in a single step (it is not necessary to combine anion-exchange and RP-HPLC to achieve good purity). One has to be aware, however, that fluorescent groups add to the overall retention of the oligonucleotide. This shift of retention time requires adjustment of the starting mobile phase strength (in comparison to unlabeled oligonucleotides). The individual fluorescent tags affect the retention time of the oligonucleotide differently (doubly labeled oligonucleotides have an even greater effect). This method allows one to purify labeled oligonucleotides from unlabeled oligonucleotides, free dye and failure sequences.

Mobile phases for the purification of labeled oligonucleotides using XTerra® columns

Method for preparing mobile phases can be found in Appendix B.

Mobile Phase A:	5% Acetonitrile, 95% 0.1 M TEAA pH 7.0
Mobile Phase B:	30% Acetonitrile, 70% 0.1 M TEAA pH 7.0
Mobile Phase C:	100% Acetonitrile

HPLC Purification Conditions

1. Choose the column size, which is most appropriate for the reaction scale to be purified (see section on **Choice of Column Size**, Page 6).
2. Before using the column for the first run, equilibrate the column at initial conditions for 15 minutes (appropriate flow rate, 100% A: 0% B and column at 60° C, see Table 3).
3. Perform an analytical scale separation (see **Method for Analytical Evaluation of Oligonucleotide Purity**, Page 7) and determine the retention time of your oligonucleotide (optional).
4. Prepare a collection tube before injecting your sample. The collection tube should have ample room to handle the estimated volume collected (i.e. 4.6 mm x 50 mm column will give 0.5-2 mLs per peak collection, 10 mm x 50 mm column will give 2-5 mLs per peak collection)

5. Remove all NH_4OH from the oligonucleotide sample and reduce the total volume by evaporation.
6. If there is an excess of un-reacted dye in your oligonucleotide sample, removal of unbound fluorescent molecules is recommended. Apply the reaction to a Gel Filtration spin column and collect the flow through portion that contains the oligonucleotide. Unreacted labeling dye is retained on the cartridge.
7. Prepare sample in 0.1M TEAA, pH 7.0 solution.
8. Perform the purification following the recommended gradient conditions (Table 3, and Appendix B).
9. Collect the purified oligonucleotide. The collected fraction should exhibit maximum absorbance at 260 nm (DNA λ max) and the absorbance characteristic of the particular dye label (ie: TAMRA λ max 556 nm, check with dye supplier for absorbance wavelength). Be careful to avoid the unwanted products that come off the column before and after your desired product.
10. Lyophilize the sample and store under standard conditions.

Collection of Purified Oligonucleotide – Manual Collection

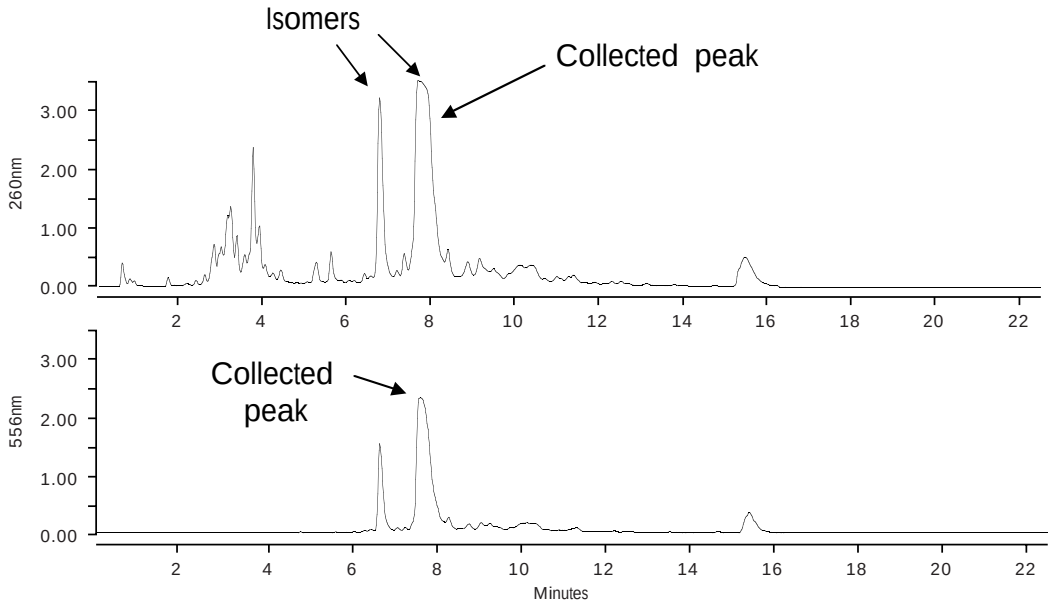
NOTE:

Use the shortest possible piece of 0.005 I.D. PEEK tubing connected to the outlet of the UV detector to reduce the delay between the actual signal and material being collected. Collection is more involved when compared with an unlabelled oligonucleotide. XTerra® columns are so powerful in their resolving power that oligonucleotides labeled with dye isomers will be separated. In **Figure 5A** an oligo-nucleotide is labeled with an isomeric mixture of TAMRA. The difference in retention times is caused by different retention of TAMRA isomers. Both full length labeled peaks can be collected and combined for excellent recovery of target product.

Sample Figures of Purified Labeled Oligonucleotides

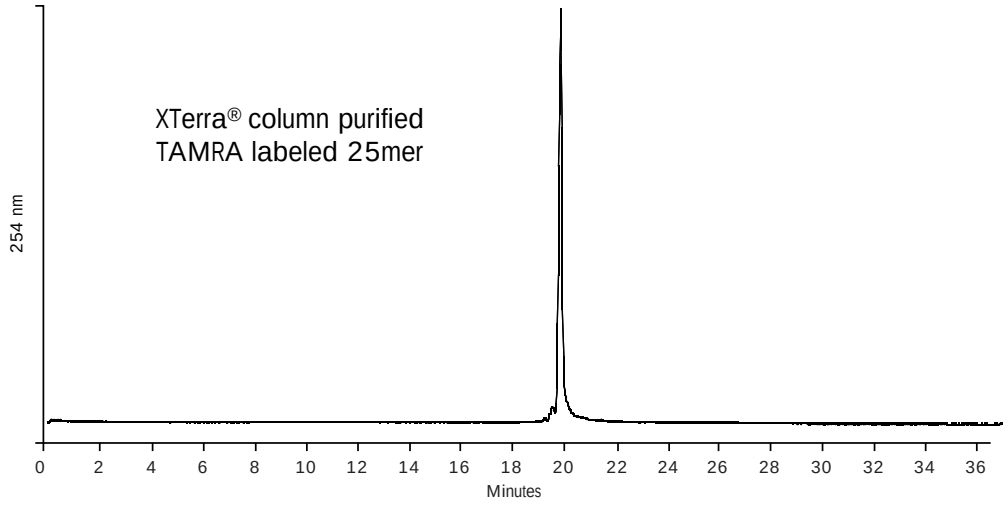
The following figures are examples of purification using several different labeled oligonucleotides. **Figure 5A** is a 25nt oligo labeled with TAMRA dye purified on an XTerra® column. The two labeled oligonucleotides were identified by their absorbance at both 260 nm and 556 nm. The major peak was collected and analyzed by CGE (**Figure 5B**). **Figure 6A** shows the purification of a 25nt oligo labeled with a single isomer of FITC, and **Figure 6B** shows the corresponding CGE analysis of both crude and purified labeled oligonucleotide. **Figure 7A** and **B** show similar results but this time using a 30nt oligo labeled with Cy™ 5. **Figure 8A** shows the purification of a crude 36nt TaqMan™ probe labeled with TAMRA and fluorescein. The chromatogram shows several major peaks, therefore it is important to choose the correct collection window. We utilize online monitoring of full PDA spectra of eluting peaks. Initially we detected unlabeled oligonucleotides followed by failed sequences containing only a single dye. Collection of target TaqMan™ probe starts when the characteristic UV spectrum is detected. **Figure 8B** is the corresponding CGE analysis of the collected fraction.

Figure 5A: Single step purification of TAMRA labeled 25 nt



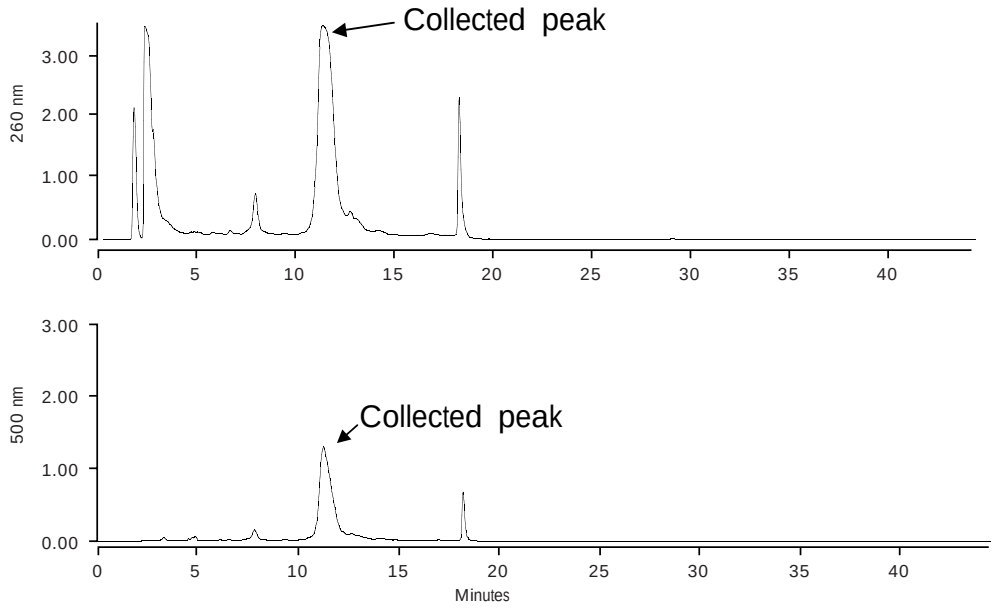
Gradiant conditions:
XTerra® MS C_{18} 2.5 µm 4.6 x 50 mm column, A: 5% acetonitrile in 0.1M TEAA, pH 7.0, B: 30% acetonitrile in 0.1 M TEAA, pH7.0, Flowrate: 1.0 mL/min, Gradient: 0-100% B in 15 min

Figure 5B: CGE purity analysis of TAMRA labeled 25mer



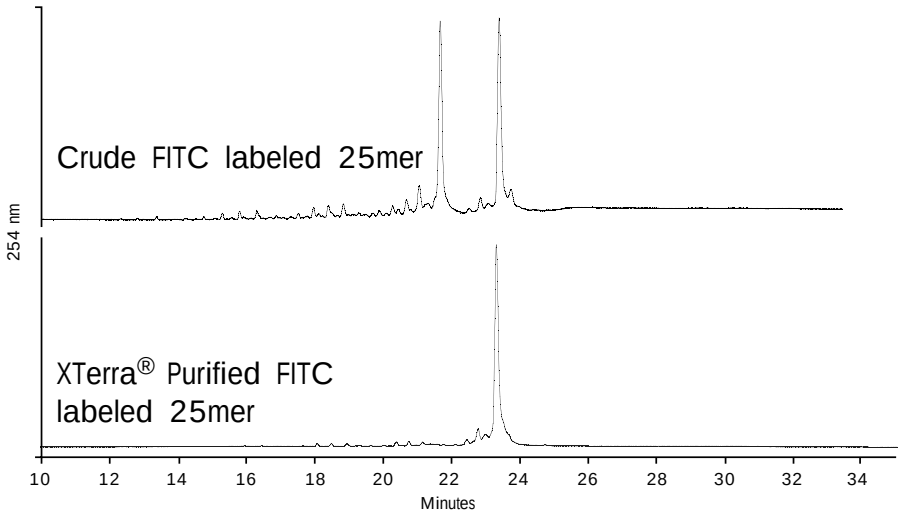
CGE conditions:
BioCAP™ 75 µm ID capillary, 27.5/34.5 cm, PEG sieving matrix, 30° C, 15 kV run, 4s injection at 5 kV

Figure 6A: Single step purification of FITC labeled 25 nt



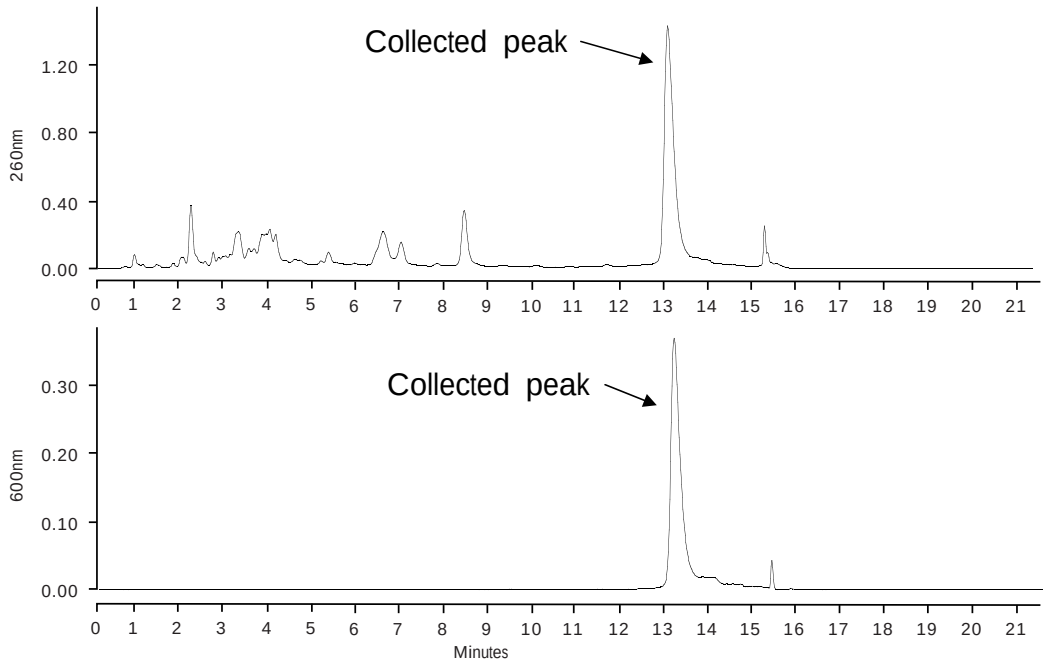
Gradiant conditions:
XTerra® MS C_{18} 2.5 µm 4.6 mm x 75 mm column, A: 0.1M TEAA, pH 7.0, B: acetonitrile, Flowrate: 0.5 mL/min, Gradient: 12.0-37.0% B in 100 min

Figure 6B: CGE purity analysis of FITC labeled 25 mer



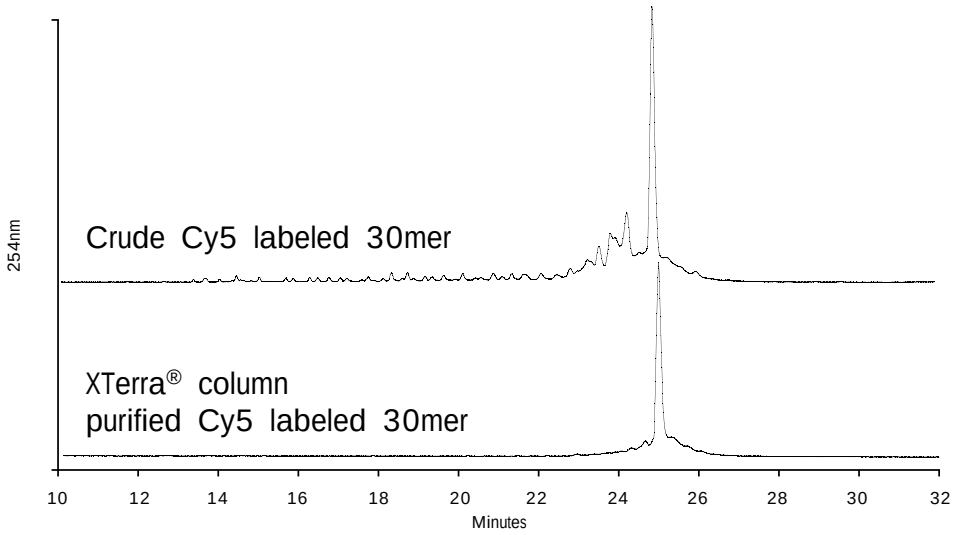
CGE conditions:
BioCAP™ 75 µm ID capillary, 27.5/34.5 cm, PEG sieving matrix, 30° C, 15 kV run, 4s injection at 5 kV

Figure 7A: Single step purification of Cy™ 5 labeled 30 nt



Gradiant conditions:
XTerra® MS C_{18} 2.5 µm 4.6 mm x 50 mm column, A: 5% acetonitrile in 0.1M TEAA, pH 7.0, B: 30% acetonitrile in 0.1 M TEAA, pH 7.0
Flowrate: 1.0 mL/min, Gradient: 0-100%B in 15 min

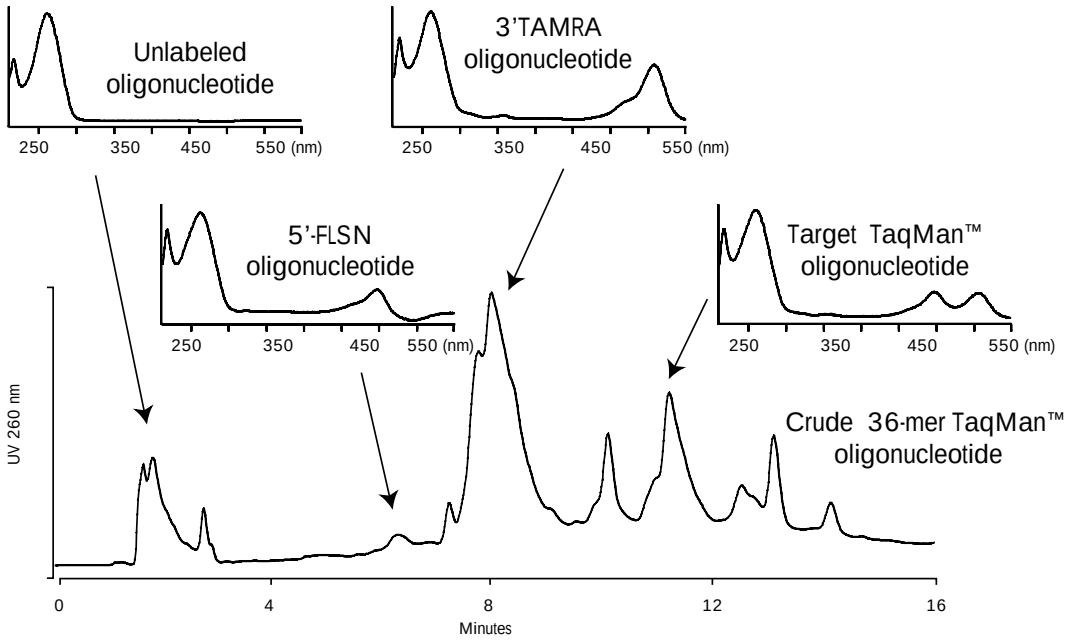
Figure 7B: CGE purity analysis of Cy™ 5 labeled 30 mer



CGE conditions:
BioCAP™ 75 µm ID capillary, 27.5/34.5 cm, PEG sieving matrix, 30 °C, 15 kV run, 4s injection at 5 kV

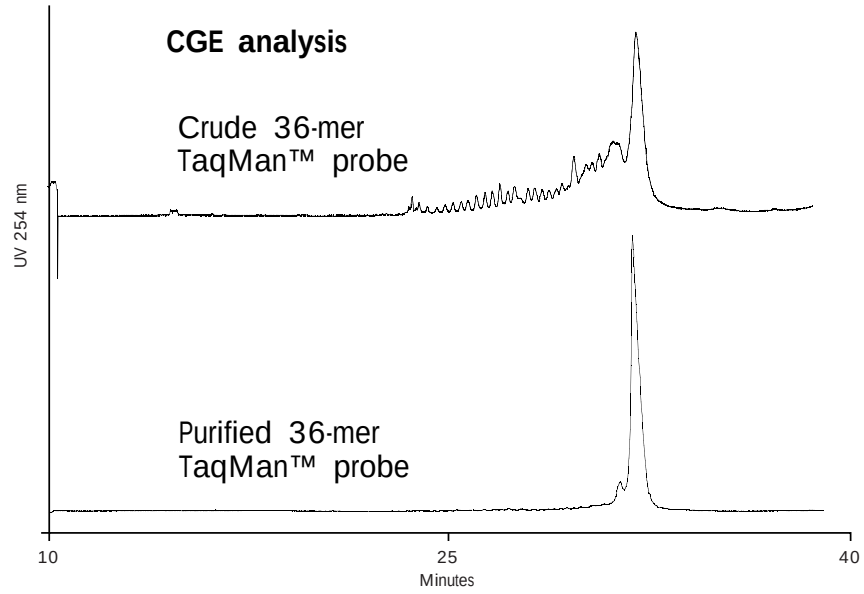
Figure 8A: HPLC purification of TaqMan™ 36 nt oligonucleotide

On-line UV-VIS spectrum monitoring



XTerra® MS C_{18} 2.5 µm 4.6 mm x 50 mm column, A : 5% ACN in 0.1 M TEAA, pH 7 B : 40% ACN in 0.1 M TEAA, pH 7 Flow rate 0.5 mL/min gradient starts at 17.1 % B, at 30 min 60% B (0.5 % ACN/min) Column temperature 60 °C

Figure 8B: Purity control of TaqMan™ 36 nt oligonucleotide



BioCAP™ 75 µm ID capillary, 27,5/34,5 cm, PEG sieving matrix with 15 % IPA in 0,2 M Tris Boric acid buffer, pH 8.3, UV 254 nm, 15 kV run, 5s injection at 5 kV, 30° C

Click anywhere on Appendix list; type desired page #; click OK.

Appendix A

Appendix B

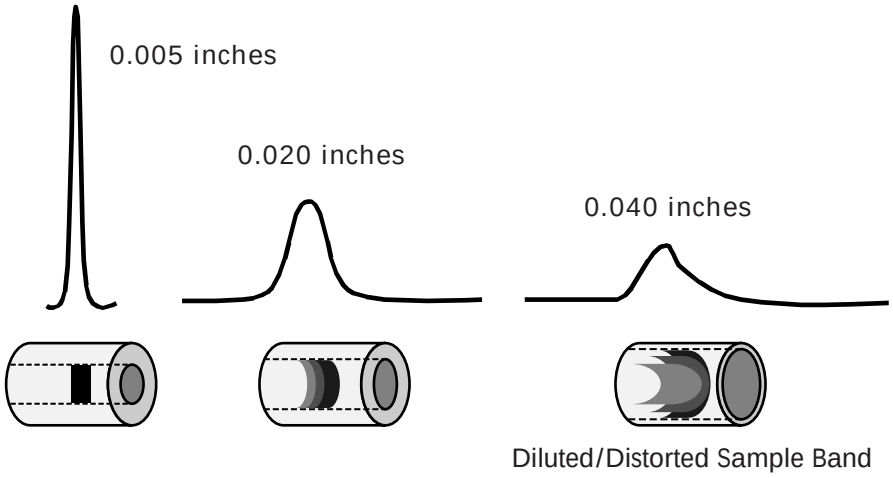
Appendix A

Minimization of Band Spreading

In order to achieve optimal separation performance, it is necessary to follow several simple steps for proper column installation and HPLC instrumentation setup.

The following figure shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen in Figure A1, the larger tubing diameter causes excessive peak broadening and lower sensitivity. We recommend that tubing with 0.005 inch diameter be used from the column to the detector for this application.

Figure A1: Effect of Connecting Tubing on System Band spreading



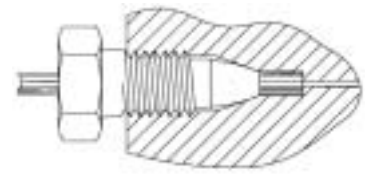
Connecting the Column to the HPLC Instrument

The following figures show the influence of ferrule depth on chromatographic peak shape. XTerra® columns are equipped with a Waters style end-fitting which requires the 0.130'' ferrule depth as seen in Figure A2. If you are presently using a non-Waters style column, it is critical that you reset the ferrule depth for optimal performance. Figure A3 shows how the dead volume created by incorrect ferrule depth can affect the peak shape.

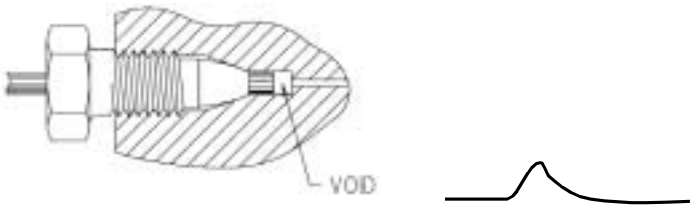
Figure A2: Ferrule depth settings

Waters® ferrule setting **Many other brands**

Figure A3:



Proper connection



Improper connection

XTerra® COLUMNS

Appendix B

Condition Modification for Non-Standard Lengths and Sequence Composition

There is a trade off between speed and selectivity of separation. When the gradient is very shallow, it may take a long time before the oligonucleotide elutes. This is especially true for modified oligonucleotides, where retention is not known. Therefore, the choice of starting mobile phase strength is important. Our recommendation is to use 1 % ACN/min as a scouting gradient for nonstandard oligonucleotides.

Most unmodified oligonucleotides will elute within the standard gradient, however, because retention is affected by the sequence composition as well as length some of the oligonucleotides will require different starting conditions. Short oligonucleotides or oligonucleotides with high G + C ratios (i.e.: above 70%) should have a starting mobile phase of 5.25 % ACN and use the same slope as the standard protocol. Long oligonucleotides or oligonucleotides with high A + T ratio's (i.e.: above 60%) should have a starting mobile phase composition of 7.5 % ACN, again with the same slope as the standard protocol. Table B1 gives a range of gradients and conditions that may be useful for purifying non-standard oligonucleotides.

Table B1: Gradient suggestions for non-standard oligonucleotides

	Gradient 1 Standard oligonucleotides	Gradient 2 High GC Content or Short Oligos	Gradient 3 High AT Content or Long Oligos
Initial % ACN	7 (80% A)	5.25 (97.5% A)	7.5 (75% A)
Final % ACN	10.75 (42.5% A)	9 (60% A)	12.5 (25% A)
Time	15 min	15 min	20 min

The slope in all cases is 0.25 % ACN per minute (mL)

Pre-mixing of Mobile Phases

We suggest that the mobile phases are premixed instead of allowing the equipment to mix them for the following reason. Premixing allows for a more accurate shallow gradient to be generated. This is important because most of the oligonucleotides will elute in a very narrow range of acetonitrile (8% to 10%).

Premixed mobile phases:

Mobile Phase A:

5 % ACN, 95% 0.1 M TEAA, pH 7 (v:v)

Mobile Phase B (for standard oligonucleotides):

15 % ACN, 85% 0.1 M TEAA, pH 7 (v:v)

Mobile Phase B (for labelled oligonucleotides)

30 % ACN, 70% 0.1 M TEAA, pH 7 (v:v)

0.1 M TEAA buffer preparation:

950 ml of water + 5.6 mL of acetic acid + 13.86 mL of TEA, adjust pH to 7.0 with acetic acid, add water to the final volume of 1 L

Note:

Precision: mobile phase weighting is more accurate than volumetric mixing

d_{ACN} = 0.786, d_{TEAA} = 0.9959 (for 0.1 M TEAA)

500 mL of 5% ACN m.p. = 19.65 g of ACN + 473.05 g of 0.1 M TEAA

500 mL of 15% ACN m.p. = 58.95 g of ACN + 425.31 g of 0.1 M TEAA

500 mL of 30% ACN m.p. = 117.90 g of ACN + 348.6 g of 0.1 M TEAA

Note: protect m.p. reservoirs from evaporation.

Formulas for calculating the mobile phase percentage of A and B % for desired ACN % starting conditions

Mobile phase A: 5% ACN, 95% 0.1 M TEAA, pH7 (v:v)
Mobile phase B: 15% ACN, 85% 0.1 M TEAA, pH7 (v:v)
ACN_A- volumetric fraction of ACN in mobile phase A
ACN_B- volumetric fraction of ACN in mobile phase B
100 = % A + % B ACN_A=0.05 ACN_B=0.15

$$\% A = \frac{(ACN_B * 100) - \text{desirable \% ACN}}{ACN_B - ACN_A}$$

Example:

If desired ACN % = 8%

$$\% A = \frac{(ACN_B * 100) - \text{desirable \% ACN}}{ACN_B - ACN_A}$$

$$\% A = \frac{(0.15 * 100) - 8}{0.15 - 0.05}$$

% A = 70%

Flow rate and mass load scale up calculations based on internal diameter on the column

$$FR_2 = FR_1 \frac{(ID_2)^2}{(ID_1)^2}$$

Example:

To up scale from 4.6 x 50 mm to 10 x 50 mm column the calculation would be:

$FR_1 = 1$ $ID_1 = 4.6$ mm $ID_2 = 10$ mm

$$FR_2 = 1 \frac{(10)^2}{(4.6)^2}$$

$FR_2 = 4.725$ mL/min

The same calculation can be applied to the mass load of the column.

Click anywhere on list for up-to-date contact information for your nearest Waters office.

Waters

Australia
Waters Australia Pty. Limited
Tel: 2 9933 1777
Fax: 2 9898 1455

Austria and European Export
(Central Europe, CIS, Middle East,
India and India Subcontinent
Waters Ges.m.b.H.
Tel: 43 1 877 1807
Fax: 43 1 877 1808

Belgium and Luxemburg
Waters S.A.-N.V.
Tel: 32 2 726 1000
Fax: 32 2 726 1100

Brazil
Waters Comercial LTDA
Tel: 011 55 11 543 7788
Fax: 011 55 11 530 6413

Canada
Waters Limited
Tel: 800 252 4752
Fax: 905 678 9237

China
Waters China Limited
Representative Office
Tel: 86 10 6526 0828
Fax: 86 10 6526 0830

Czech Republic
Waters Ges.m.b.H
Tel: 42 02 617 11384
Fax: 42 02 617 11386

Denmark
Waters A/S
Tel: 45 46 59 8080
Fax: 45 46 59 8585

Finland
Waters Oy
Tel: 90 506 4140
Fax: 90 5064 1411

France
Waters S.A
Tel: 1 3048 7200
Fax: 1 3048 7211

Germany
Waters GmbH
Tel: 49 6196 400600
Fax: 49 6196 482388

Hungary
Waters kft
Tel: 36 1 350 5086
Fax : 36 1 350 5087

India
Waters Pvt. Ltd.
Tel: 91 80 837 1900
Fax: 91 80 839 2157

Italy
Waters S.P.A.
Tel: 39 02 274 211
Fax: 39 02 250 1827

Japan
Nihon Waters K.K.
Tel: 3 3471 7191
Fax: 3 3471 7116

Malaysia
Waters Asia Ltd.
Tel: 3 704 8600
Fax: 3 704 8599

Mexico
Waters S.A. DE C.V.
Tel: 525 524 8383
Fax : 525 524 9376

The Netherlands
Waters Chromatography B.V.
Tel: 31 076 508 7200
Fax: 31 076 508 7280

Norway
Waters AS
Tel: 47 6 384 6050
Fax: 47 6 384 6051

Poland
Waters Sp z.o.o
Tel: 48 22 833 4400
Fax: 48 22 833 0987

Puerto Rico
Waters Technologies Corporation
Tel: 787 747 8445
Fax: 787 747 8448

Russia/CIS
Waters Ges.m.b.H.
Representation Office
Tel: 7 095 931 9193
Fax: 7 095 336 7000

Singapore
Waters Asia Ltd. (Asia Headquarters)
Tel: 65 278 7997
Fax: 65 278 7557

Spain
Waters Chromatografia S.A.
Tel: 34 93 600 9300
Fax: 34 93 325 9896

Sweden
Waters Sverige AB
Tel: 46 8 623 0090
Fax: 46 8 623 0095

Switzerland
Waters AG
Tel: 41 62 889 2030
Fax: 41 62 889 2059

Taiwan
Waters Asia Ltd.
Tel: 886 2 2543 1918
Fax: 886 2 2543 1898

UK and Ireland
Waters Ltd.
Tel: 44 208 238 6100
Fax: 44 208 207 7070

All Other Countries

Waters Corporation
34 Maple Street
Milford, MA 01757 USA
Tel: 508 478 2000
800 252 4752
Fax: 508 872 1990

www.waters.com

Ordering Information

Description	Particle Size	Dimensions	Part Number
XTerra® MS C_{18} Column	2.5 µm	4.6 mm x 50 mm	186000602
XTerra® MS C_{18} Column	2.5 µm	10 mm x 50 mm	186000982

c.shop
c.shop.waters.com

Order online at c.shop.waters.com

Additional column configurations and chemistries are available. Contact your local Waters office for details.