PRODUCT MANUAL
DNASWIFT™ MONOLITHIC COLUMN
for
OLIGONUCLEOTIDE PURIFICATION
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PRODUCT MANUAL

FOR
DNASWIFT™ MONOLITHIC COLUMN
FOR
OLIGONUCLEOTIDE PURIFICATION

5 x 150 mm, PEEK
Product No. 066766

DIONEX® Corporation
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GUIDE TO IMPORTANT INFORMATION

SAFETY	***Safety information can help prevent bodily harm.***
WARNING	***Warning information can help prevent equipment harm.***
CAUTION	***Caution information can help prevent problems.***
NOTE	***Note information can help with tips for improved use.***

1. INTRODUCTION

1.1. Design of DNASwift™ Ion Exchange Monoliths:

DNASwift monolithic columns are specifically designed to provide high-resolution, laboratory-scale purification of oligonucleotides by anion-exchange chromatography. DNASwift columns are polymeric monoliths prepared by in column polymerization, followed by functionalization with anion-exchange latexes. The monolith is a cylindrical polymer rod containing uninterrupted, interconnected, through pores, with surface area intermediate between porous bead-based and non-porous anion-exchange columns. However, the latex-covered monolith structure supports capacities comparable to fully porous bead-based columns. This approach results in low mass-transfer distances that produce improved peak shape, even at elevated flow-rates. These coated monoliths represent a new generation of separation media, and this implementation is engineered for oligonucleotide purification.

The DNASwift monolith offers improved resolution for oligonucleotides spanning a wide range of lengths, and can employ a variety of denaturing conditions to limit impurities arising from self-complementarity:

- High temperature, pH 8 or below
- High pH (12.4) at 30° or below
- Organic solvents (such as Acetonitrile)
- Chaotropes (formamide or urea. Note that these may cause excessive pressures)

The quaternary nature of the anion-exchange monolith allows use of elevated pH conditions to optimize selectivity between target and critical oligonucleotide impurities.

1.2. Characteristics of the DNASwift Monolith in 5x150 mm format:

1.2.1. Chromatographic Bed:

Latex Coated Monolith	Anion-exchange (Quaternary ammonium ion, diethyl methyl amine)
Modal Monolith Pore Diameter	3.0 µm
Latex Diameter	~215 nm
Latex Cross-link	3 %

1.2.2. Column Parameters:

Bed Height	130mm
Bed Volume	2.5 mL
Ion exchange capacity (Nitrate)	~150 µeq/column
Total Oligonucleotide binding capacity	~50mg

1.2.3. Typical Operating Parameters:

Operating Flow range	0 to 2.5 mL/min
Maximum flow	3.0 mL/min
Eluent pH range	3-12.4 (at pH >9 eluents must maintain anion (Cl^-) to ^-OH ratios of >1). NOTE: at pH > 8, temperature must remain at ≤ 30 °C.
Cleaning pH	≥ 3 (1mM HCl in 50 mM NaCl) and 14 (1M NaOH in 1.25M NaCl)
Temperature	≤ 85°C
Pressure	≤ 1,500 psi
Organic Solvent Limit	Up to 100% acetonitrile or methanol
Typical eluents	High purity water (18.2 megohm.cm), sodium chloride, sodium perchlorate, buffer mixtures, sodium acetate and sodium hydroxide, acetonitrile, methanol

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2. SYSTEM REQUIREMENTS

DNASwift monoliths are designed to operate with standard HPLC systems that include inert gradient pumps, flow paths, and injection valve materials. Exposure of SST systems to halide salt eluents will result in corrosion of those systems, leading to release of metals that will foul the anion-exchange monolith (in extreme cases this may occur in just a few hours).

WARNING	***A major source of decreasing column performance is due to iron and nickel leaching from stainless steel HPLC systems. The use of halide salts will cause corrosion of stainless steel components leading to column fouling.*** ***DIONEX strongly recommends the use of inert systems and components, including pump heads, and tubing, but especially frits and in-line filters.***

2.1. System Set Up and Operation:

The oligonucleotide chromatography systems should be configured with components providing the following attributes:

a) All components of the fluid path are free from stainless steel, to eliminate column poisoning.
b) Mobile phase components are degassed, or are kept under helium or nitrogen to minimize out-gassing (bubble formation) in the detector cell. On-line degassing of eluents may be provided with the eluent degas option in DIONEX pump modules.
c) Accurate reproducible flow and gradient generation at settings between 0.1 and 3.5 mL/min.
d) Minimal contribution to the background signal by contaminants from the system and reagents.
e) Thermostated column compartment for consistent temperature control of the eluent and column.
f) Minimal system volumes (employ low volume unions and minimal tubing length).

CAUTION	***There may be an increase in back pressure when using tubing with excessively small internal diameter.*** ***The back pressure generated after the column outlet (including the connecting tubing between the column outlet and detector cell inlet, the cell, and cell waste line) must not exceed the maximum allowable pressure limits of the column as excessive back pressure may cause irreversible damage.***

2.1.1. System Plumbing:

The DNASwift monoliths will deliver the best results when all connecting tubing is 0.005-0.01” ID, 1/16 inch OD tubing. Increased tubing ID before the column will increase gradient delay; increased ID between the column and the detector may cause dispersion leading to band broadening and decreased resolution. Record the system pressure at intended flow with the column removed (do not connect the post column components for this measurement). This pressure is separate from that experienced by the column. When evaluating the back pressure on the column, this *pre-column* pressure value should be subtracted from the total pressure when all components are installed.

2.1.2. Injection Loop Size:

The injection loop size may affect the delay time (for example, a 1.0 mL loop will introduce a 0.5 minute delay when operating at 2.0 mL/min).

2.1.3. System Storage in High Salt Eluents:

Eluents high in salts may evaporate and clog the tubing if salt is left in the eluent tubing while the chromatograph is idle. This may produce very high pressure values when the system is restarted. Take the precaution of clearing all eluent lines with water when shutting the system down.

2.1.4. System Void Volume:

For 5 mm ID monoliths, the tubing between the injection valve and detector is recommended to be $\leq$ 0.010” ID PEEK tubing (e.g., P/N 042690). Also, minimize the length of all liquid lines as much as possible, especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing will decrease apparent peak efficiency and resolution.

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2.1.5. Eluent Limitations:

CAUTION	***Use of lithium salts of chloride and perchlorate with solvents such as acetonitrile is NOT recommended. These salts, in the presence of solvent can produce a very fine precipitate that accumulates over several days before it degrades check valve performance. Because it is very fine and not very soluble in water, it may take several days to flush the precipitate out of the system with DI water.***

CAUTION	***For all Ion Exchange columns, NONIONIC detergents are recommended.*** ***Do not use anionic surfactants, which will irreversibly bind to anion exchange monoliths.*** ***Do not use cationic surfactants, which will irreversibly bind to cation exchange monoliths.***

The DNASwift anion exchange columns are compatible with typical eluents such as sodium or potassium chloride or sulfate salts in Tris, phosphate and many other buffers, up to their limits of solubility. Use of organic solvents in the eluent may be helpful for very hydrophobic analytes, but is often unnecessary. If you employ solvents, test their solubility with the salt-containing eluents prior to use. Some combinations of eluent salts and organic solvents are not miscible.

2.2. Chemical Purity Requirements:

Obtaining sensitive, consistent, and accurate results requires eluents that are free of impurities. Chemicals, solvents and deionized water used to prepare eluents must be the highest purity available. Low trace impurities and low particle levels in eluents will extend the life of your ion exchange columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

2.2.1. Deionized Water:

Deionized (DI) water, used to prepare eluents, should be Type I reagent grade water with a specific resistance of 18 megohm/cm. The water should be free from ionized impurities, organics, microorganisms, and particulate matter. Ultra Violet (UV) treatment in the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from UV absorbing components. Contaminated water in eluents may cause high background signals, gradient artifacts, and even sample degradation.

2.2.2. Inorganic Chemicals:

Always use reagent grade or better inorganic chemicals to prepare eluents. Whenever possible, use inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity. These chemicals will detail the purity with an actual lot analysis on each label.

2.2.3. Buffer Components:

Many chemicals may be used as pH buffering components in eluents. For nucleic acid separations, some will serve better than others, when detection is considered. Most organic solvents (e.g., methanol, acetonitrile) will contribute to significant baseline drift at detection wavelengths (260 nm). This may be minimized by treating the column with 40-50% solvent (aqueous) for several hours prior to sample chromatography in their presence.

The buffering components used may affect long-term changes in pressure during chromatography. Buffers that bind to the stationary phase (anions for anion-exchange chromatography) will buffer the stationary phase quickly, producing more stable pressures. Buffers that do not bind to the stationary phase (cations for anion-exchange chromatography) will not efficiently buffer the stationary phase. Thus, stationary phase ionization will occur very slowly, and may result in pressure changes over time. In addition, since these buffers do not effectively buffer the stationary phase, their use may also alter the apparent selectivity of ion-exchange columns. This can be used to advantage as different buffers may produce slightly different selectivity for certain oligonucleotide impurities.

2.2.4. Solvents:

Solvents can be added to the ionic eluents used in DNASwift columns to modify the ion exchange process. The solvents used must be free from ionic impurities; however, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making "ultra high" purity solvents that are compatible with HPLC and spectrophotometric applications. These "ultra high" purity solvents will usually be of sufficient purity to ensure that your chromatography is not affected by ionic impurities in the solvent. At DIONEX, we have obtained consistent results using High Purity Solvents manufactured by *Burdick and Jackson* or *Optima Solvents by Fischer Scientific*.

When using an ionic eluent with solvent, column generated back pressure will depend on the solvent used, the concentration of the solvent, the ionic strength of the eluent, and the flow rate applied. The column backpressure will also vary if the composition of the water-solvent mixture varies. The practical backpressure limit for the DNASwift is 1,500 psi (10.3 MPa). The DNASwift can withstand common HPLC solvents in a concentration range of 0-100%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize out-gassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

2.2.5. Eluent Preparation:

Solvent-Water mixtures are usually specified with a volume to volume basis, but give more reproducible results when prepared on a weight to weight basis. Hence, if a procedure requires an eluent of 90% acetonitrile; prepare the eluent by adding 702g (900 mL, *acetonitrile density is 0.78g/mL*) of acetonitrile to an eluent reservoir. Then add 100.0 g (100 mL) of deionized water. If a buffer concentrate is used to prepare solvent-containing eluents, determine the concentrate's specific gravity to calculate the weight to add. Using this procedure to mix solvents with water will ensure that a *consistent* and true volume/volume eluent is obtained. Premixing water (or eluent concentrate) with solvent will also minimize the possibility of out-gassing which causes bubble formation in the detector cell. If you choose to mix eluents containing solvents with those that do not, use of pumps with eluent degas options is highly recommended. As a second choice, pre-degassing the eluents and covering the eluent reservoir with Helium gas to limit gas dissolution into the eluents is recommended to limit out-gassing.

2.2.6. Filtering the Eluent:

To extend the lifetime of your column as well as your HPLC pump, filter all eluent buffers using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents.

2.2.7. Degassing the Eluent:

Before use, degas the eluents. Degassing can be done either by using DIONEX pump degas functions as described in the pump manuals or by placing the eluent reservoir in a sonic bath and drawing vacuum on the filled reservoir with a vacuum pump for 5 minutes while operating the sonic bath.

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3. INFORMATION FOR METHODS DEVELOPMENT

3.1. Elution Order:

The native elution order of oligonucleotide bases from the DNASwift monolith using linear gradients of $NaClO_4$ is as follows Note that these may change in NaCl-based eluents:

DNA > RNA (DNA is more retained than RNA)

3.1.1. Homopolymer Series:

pH 7.0: C > T > G » A
pH 8.5: C > T > G » A
pH 9.5: G > T > C > A
pH 10.0: G > T > C > A
pH 11.0: G > T » C > A
pH 12.2: G > T » C > A

NOTE	***Poly-G will form extensive tetrad ladders at pH values below ~10.5.*** ***These ladders are not readily disrupted, even at 85•C in neutral or mildly basic (pH 7-8.5) salt solutions.***

WARNING	***DIONEX does not recommend using elevated temperatures (>30 •C) at pH values above 8.5.***

3.1.2. Heteropolymer pH Effects:

Elution is influenced by the base composition (especially % G +T), terminal base sequence, pH, solvent concentration, and eluent salt (Cl^- vs. ClO_4^-). At pH 12 each T or G base contributes a negative charge from tautomeric oxygen atoms.

As pH shifts from 9 – 11, hydrogen bond interactions decrease, yielding linear nucleic acids and chromatographic elution in approximate order of *net charge*.

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3.1.3. Effect of pH on Oligonucleotide Retention and Selectivity:

Use of elevated pH offers two advantages over chromatography at neutral pH. First, elevated pH allows control of hydrogen bonding interactions. At pH 11 and above, (pH 12.4 is the recommended upper limit for the DNASwift columns), Watson-Crick and poly-G hydrogen bonds break. Hence, at high pH chromatographic analysis of oligonucleotides with self-complementary sequences results in sharp, well-resolved peaks. Second, for each Thymine (T) and Guanine (G) residue, an increase in oligonucleotide charge is generated with rising pH values due to ionization of the tautomeric oxygen on these bases. Between pH 9 and 11, oxyanion formation on these bases increases retention of oligonucleotides in proportion to the number of T and G residues on the molecule. This offers opportunity to control oligonucleotide selectivity with eluent pH.

Figure 1 illustrates the influence of pH on oligonucleotide selectivity. Here the elution patterns of two 23-base oligonucleotides differing only in their 5’ and 3’ terminal bases are compared between pH 9 and pH 11.

The top trace for each pair of chromatograms has an additional T at the 5’ end of the molecule, and lacks the 3’ A. The oligonucleotide chromatographed in the bottom trace of each pair lacks the 5’ T, but has the 3’A. At pH 9 - 9.5 (bottom 2 pairs of traces) these oligos are unresolved. At pH 10, the 5’ TG-3’G oligonucleotide Dx89 is eluted earlier than the 5’ G-3’ GA oligonucleotide Dx88, and the two are only partially resolved. However, at pH 10.5 and 11 this elution order is reversed, due to the relative contributions of T and A to retention at these pH values

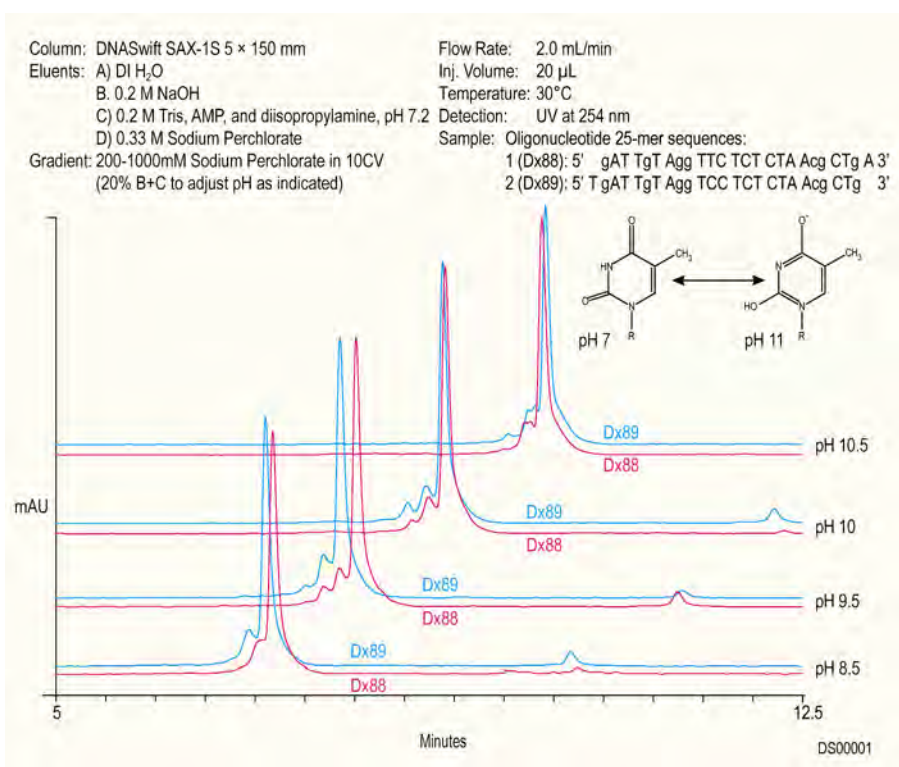

Figure 1
Effect of pH on Selectivity of Dx88 and Dx89 on the DNASwift Column

3.1.4. Effect of Salt Type on Oligonucleotide Elution:

Sodium perchlorate ($NaClO_4$) and sodium chloride (NaCl) are the eluent salts used most commonly with DNASwift columns. Sodium perchlorate is a stronger eluent than sodium chloride, so a higher concentration of sodium chloride than of sodium perchlorate is required for any given separation. For example, ~0.2M $NaClO_4$ may be required to elute a 75-base oligonucleotide at pH 8, while at the same pH and temperature ~0.7M NaCl would be required. In Figure 1 where $NaClO_4$ is the eluent, the best resolution between Dx88 and Dx89 occurs at pH 9.5 where Dx89 elutes first. However, when NaCl is the eluent, the best resolution occurs at pH 10 – 10.5 where Dx88 elutes first (Figure 2). Hence, the combination of salt-form and pH can support retention order reversals coupled to improvements in resolution.

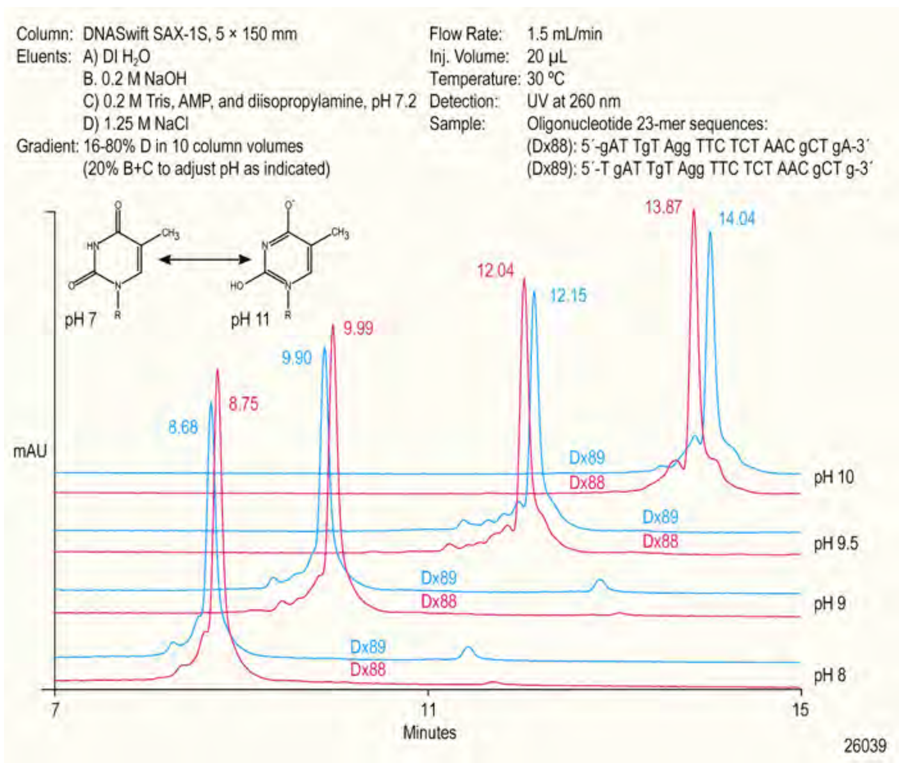

Figure 2

Effect of pH on Selectivity of Dx88 and Dx89

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3.1.5. Sanitation Using NaOH and the Effect of Solvent on Retention:

Figure 3 shows inclusion of solvent reduces retention on the DNASwift anion-exchanger (traces 2-4). This will mask some of the native selectivity of the DNASwift, and reduce retention of oligonucleotides. Removal of the solvent results in complete recovery of the initial chromatographic performance (compare traces 1 and 5). Similarly, treatment of the monolith with 1M NaOH in 1.25M NaCl for sanitation and cleaning does not degrade chromatographic performance (traces 6-8).

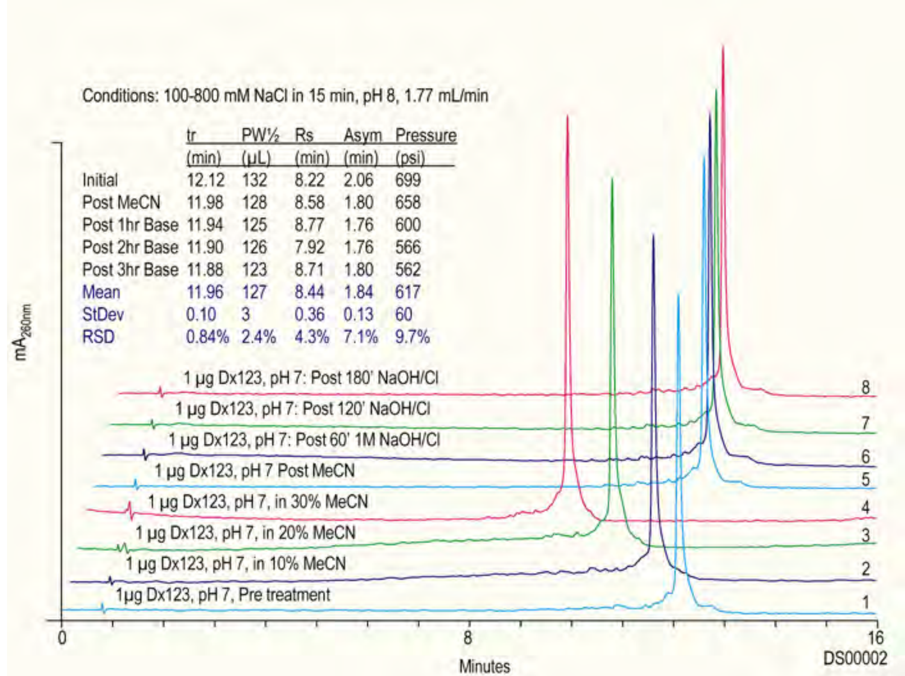

Figure 3
Effect of Solvent and Alkali Exposure on the DNASwift Monolith

3.2. Effects of Temperature on Oligonucleotide Retention and Recovery of Chromatographic Performance After Use at High Temperatures:

WARNING	***DIONEX does NOT recommend combining the use of elevated temperatures with high pH elution systems. Such conditions will accelerate degradation of the DNASwift stationary phase.***

In Figure 4, a 20-base, partially detritylated oligonucleotide is chromatographed on a DNASwift monolith at 30 °C then rechromatographed at increasing temperatures up to 85 °C. After use at each temperature, the column is evaluated again at 30 °C showing return to original chromatographic performance. Note that as the temperature increases, the retention, and resolution also increase. This is typical for latex-based anion-exchange chromatography.

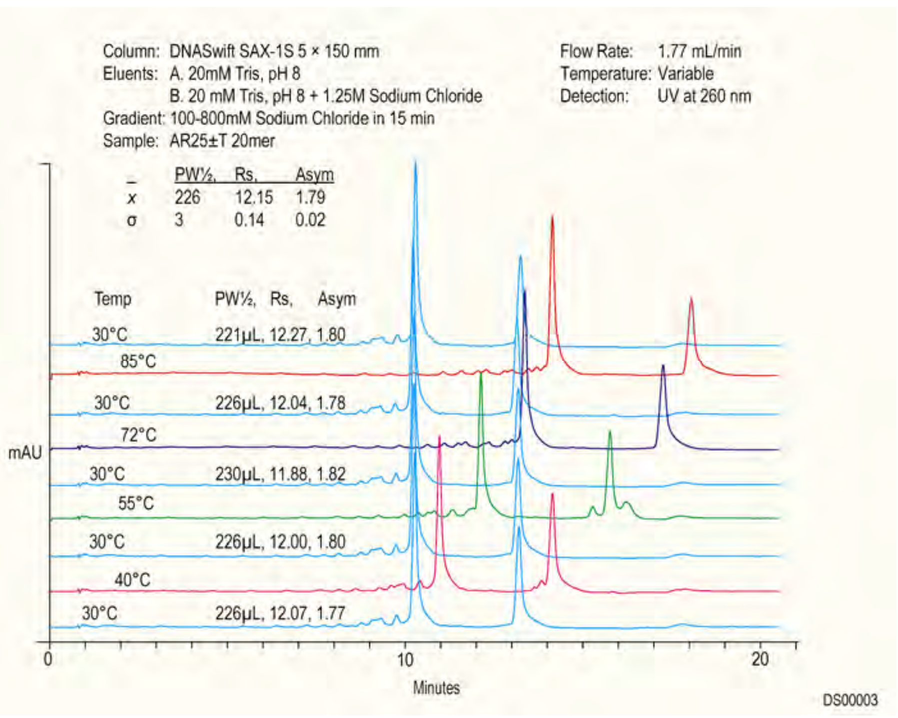

Figure 4
Effect of Exposure of the DNASwift Monolith to High Temperature

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3.3. DNASwift Ruggedness:

Figure 5 shows the stability of DNASwift performance at 3.0mL/min. The DNASwift column was challenged with a 20-base oligonucleotide periodically while running > 300 gradient cycles without any change in chromatographic performance.

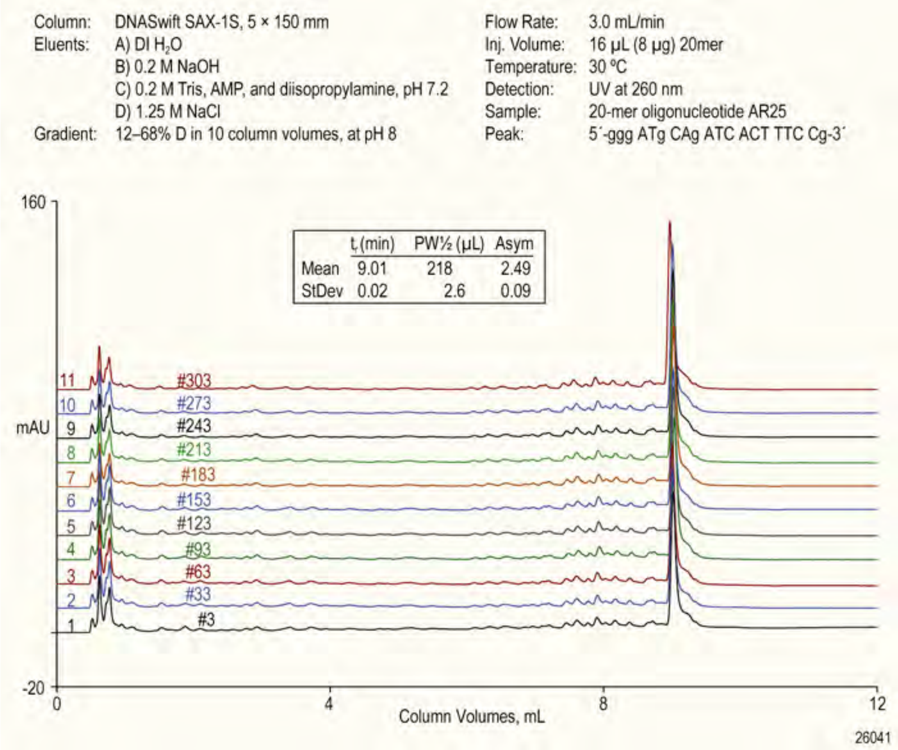


Figure 5
Repeated Injections on the DNASwift Monolith

3.4. Yield / Purity Comparisons:

3.4.1. Identification of Critical Selectivity for Yield / Purity Assessment:

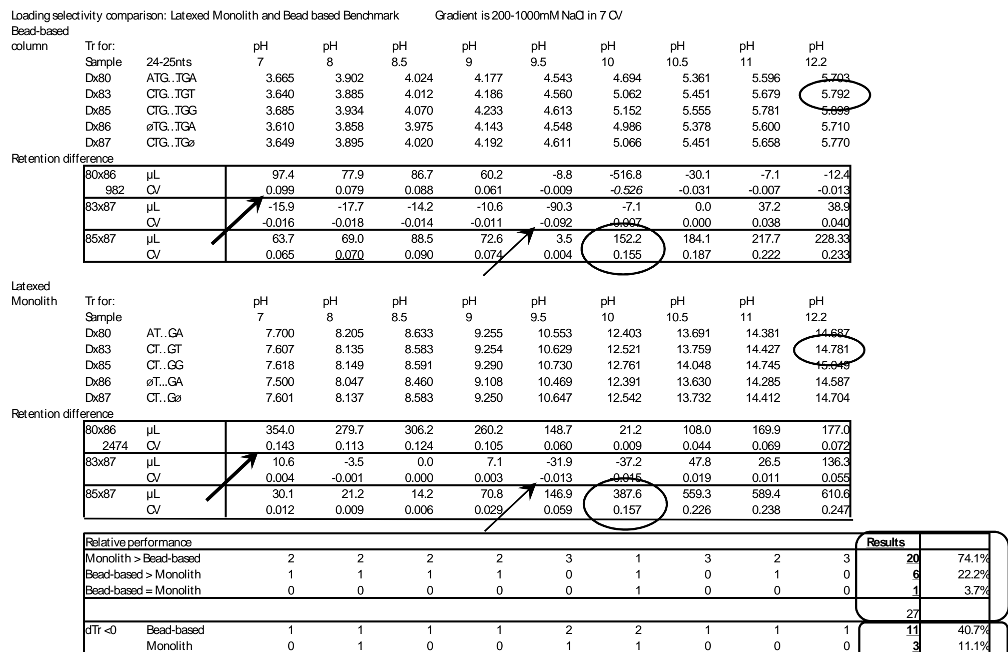
Figure 6 shows the relative selectivity of the DIONEX DNASwift and GE Healthcare Mono-Q products for each of three oligonucleotide pairs (full-length vs. "n-1") at each of nine different pH values using a common NaCl gradient.

For this assessment the retention difference is calculated as the retentions for the full-length less that of the "n-1" oligo (in µL), divided by the column volume (see thick arrows). For a critical assessment, the relative selectivities should be as close as possible. This parameter is closest for both columns when the Dx85 and Dx87 oligonucleotides are chromatographed at pH 10 (large circles, 0.155 vs. 0.157). Comparison of the frequency of superior selectivities of the two columns for all oligonucleotide pairs (upper box) reveals that the DNASwift offers better selectivity in ~ 70% of the time (20 of 27 cases) while the Mono-Q produces better selectivity ~22% of the time (6 of 27 cases), the one "tie" is the Dx85 : Dx87 pair at pH10.

Similarly, *selectivity inversions* (cases where the 24mer elutes *after* the 25mer, see thin arrows) are summed for each column. Since resolution of the 24-base "n-1" oligo after the "target" 25mer will make purification of the 25mer away from the 24mer more difficult, the relative frequency of these selectivity inversions represents a weakness. Hence, a lower frequency of inversions indicates a superior selectivity. Summing and averaging these inversions for both columns (small box) reveals that the DNASwift encounters selectivity inversion for this set of oligonucleotides in 3 of 27 (~11%) cases. The mono-Q encounters them in 11 of these 27 cases (40%).

Competitive "Yield Purity" Assessment:

1) Determine conditions for critical assessment (=Selectivity)
2) Compare relative selectivity for different oligo pairs between columns
3) Evaluate selectivity inversions (these make purification difficult)



Loading selectivity comparison: Latexed Monolith and Bead based Benchmark — Gradient is 200-1000mM NaCl in 7 CV

Bead-based column

Tr for:		pH 7	pH 8	pH 8.5	pH 9	pH 9.5	pH 10	pH 10.5	pH 11	pH 12.2
Sample	24-25nts									
Dx80	ATG..TGA	3.665	3.902	4.024	4.177	4.543	4.694	5.361	5.596	5.703
Dx83	CTG..TGT	3.640	3.885	4.012	4.186	4.560	5.062	5.451	5.679	5.792
Dx85	CTG..TGG	3.685	3.934	4.070	4.233	4.613	5.152	5.555	5.781	5.899
Dx86	øTG..TGA	3.610	3.858	3.975	4.143	4.548	4.986	5.378	5.600	5.710
Dx87	CTG..TGø	3.649	3.895	4.020	4.192	4.611	5.066	5.451	5.658	5.770
Retention difference										
80x86	µL	97.4	77.9	86.7	60.2	-8.8	-516.8	-30.1	-7.1	-12.4
982	CV	0.099	0.079	0.088	0.061	-0.009	*-0.526*	-0.031	-0.007	-0.013
83x87	µL	-15.9	-17.7	-14.2	-10.6	-90.3	-7.1	0.0	37.2	38.9
	CV	-0.016	-0.018	-0.014	-0.011	-0.092	-0.007	0.000	0.038	0.040
85x87	µL	63.7	69.0	88.5	72.6	3.5	152.2	184.1	217.7	228.33
	CV	0.065	0.070	0.090	0.074	0.004	0.155	0.187	0.222	0.233

Latexed Monolith

Tr for:		pH 7	pH 8	pH 8.5	pH 9	pH 9.5	pH 10	pH 10.5	pH 11	pH 12.2
Sample										
Dx80	AT..GA	7.700	8.205	8.633	9.255	10.553	12.403	13.691	14.381	14.687
Dx83	CT..GT	7.607	8.135	8.583	9.254	10.629	12.521	13.759	14.427	14.781
Dx85	CT..GG	7.618	8.149	8.591	9.290	10.730	12.761	14.048	14.745	15.049
Dx86	øT...GA	7.500	8.047	8.460	9.108	10.469	12.391	13.630	14.285	14.587
Dx87	CT..Gø	7.601	8.137	8.583	9.250	10.647	12.542	13.732	14.412	14.704
Retention difference										
80x86	µL	354.0	279.7	306.2	260.2	148.7	21.2	108.0	169.9	177.0
2474	CV	0.143	0.113	0.124	0.105	0.060	0.009	0.044	0.069	0.072
83x87	µL	10.6	-3.5	0.0	7.1	-31.9	-37.2	47.8	26.5	136.3
	CV	0.004	-0.001	0.000	0.003	-0.013	-0.015	0.019	0.011	0.055
85x87	µL	30.1	21.2	14.2	70.8	146.9	387.6	559.3	589.4	610.6
	CV	0.012	0.009	0.006	0.029	0.059	0.157	0.226	0.238	0.247

Relative performance											Results	
Monolith > Bead-based		2	2	2	2	3	1	3	2	3	**20**	74.1%
Bead-based > Monolith		1	1	1	1	0	1	0	1	0	**6**	22.2%
Bead-based = Monolith		0	0	0	0	0	1	0	0	0	**1**	3.7%
											27	
dTr <0	Bead-based	1	1	1	1	2	2	1	1	1	**11**	40.7%
	Monolith	0	1	0	0	1	1	0	0	0	**3**	11.1%

Figure 6
Competitive Yield / Purity Assessment

3.4.2. Yield / Purity Comparison I: 24 and 25 base Oligos in Similar Proportions.

In the chromatograms (to the left in Figure 7) below, 1 µmol samples of Dx 85 (25mer) and Dx87 (24mer) were mixed to contain ~ equal amounts of each oligonucleotide. One half of the mixture (1 µmol total oligo) was chromatographed on each of the indicated columns, and twenty fractions of 0.2 CV were collected starting 4 CV prior to the elution position of an 8 µg sample of the 25mer . The steep gradient at the beginning of the separation was 0.4 CV and the shallow gradient across which the fractions were collected was 10 CV. Each collected fraction was diluted with two volumes of deionized water, and chromatographed on a 2x250mm DNAPac PA200 column at 60 °C using $NaClO_4$ eluent to detect and quantify the amounts of 24mer, 25mer and the sum of "other" components eluting from the columns (example at lower left). Comparing the DNAPac results with a gravimetrically diluted sample of the original material containing the combined sample, the actual recovery (Yield) was calculated for each fraction. The yield calculated from the individual and combined fractions, and the purity similarly calculated was plotted for each column, and the yield – purity curves examined. For this case (combined 24-base and 25-base oligonucleotides) the *yield* at any given purity, and the *purity* at any given yield, were significantly better on the DNASwift than that on the Mono-Q. For this contrived case the purity at 70% yield from the DNASwift was 84%, and from the Mono-Q was 82%. Similarly the Yield at 84% purity was 70% for the DNASwift and ~50% for the Mono-Q.

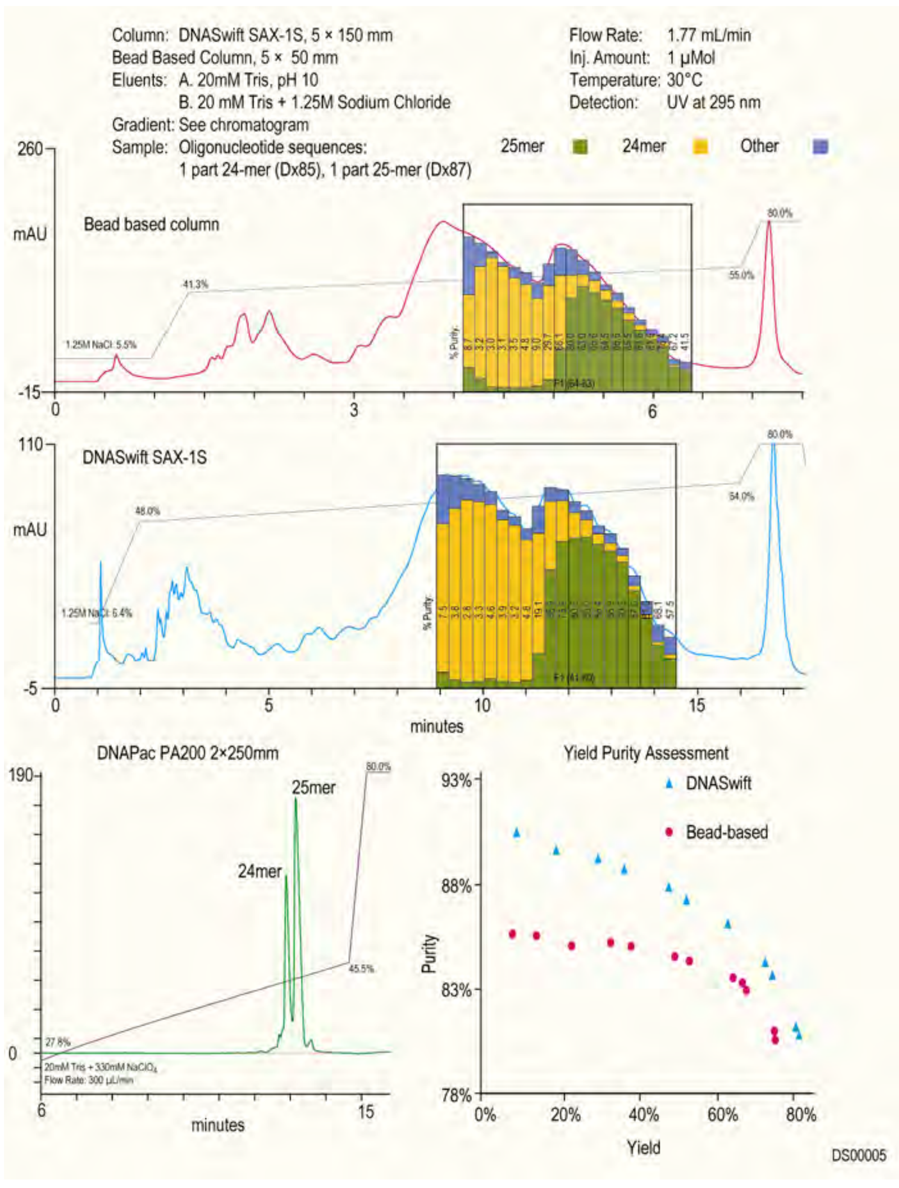


Figure 7
Preparation of Yield-Purity Curves

3.4.3. Yield / Purity Comparison II: 25 Base Oligonucleotide at 2.75 µmol Load (Sample Self-Displacement)

In the chromatograms in Figure 8, a 2.75 µmol sample of the Dx83 25mer was chromatographed on each of the columns, and thirty fractions of 0.2 CV were collected starting 6 CV prior to the elution position of a 1 µg sample of the 25mer. The steep gradient at the beginning of the separation was 0.4 CV and the shallow gradient across which the fractions were collected was 12 CV. Each collected fraction was diluted with two volumes of deionized water, and chromatographed on a 2x250mm DNAPac PA200 column at 60 °C using $NaClO_4$ eluent to detect and quantify the amounts 25mer target and the sum of "other" components eluting from the columns (as in Figure 7). Comparing the DNAPac results with a gravimetrically diluted sample of the original material containing the combined sample, the actual recovery (yield) was calculated for each fraction. The yield calculated from the individual and combined fractions, and the purity similarly calculated was plotted for each column, and the Yield – Purity curves examined. For this case (2.75 µmol injection of the 25-base oligonucleotide) the *yield* at any given purity, and the *purity* at any given yield, were significantly better on the DNASwift than that on the Mono-Q. In this normal case, the yield at 90% purity was ~75% for the DNASwift and ~59% on the Mono-Q. Similarly, the purity at 55% yield was 93.8% from the DNASwift and 90.3% from the Mono-Q.

Also noteworthy is that under these *sample self-displacement* conditions (15-25% of the total oligonucleotide binding capacity), the Mono-Q exhibits substantially more overload than the DNASwift in that the signal maximum occurs ~33% into the shallow gradient, while that in the DNASwift occurs ~ 50% into the gradient. Between the injection and the signal maxima, the number of observable peaks (early eluting components) from the DNASwift is ~2x that from the Mono-Q, reflecting the improved peak shape from the latexed monolith.

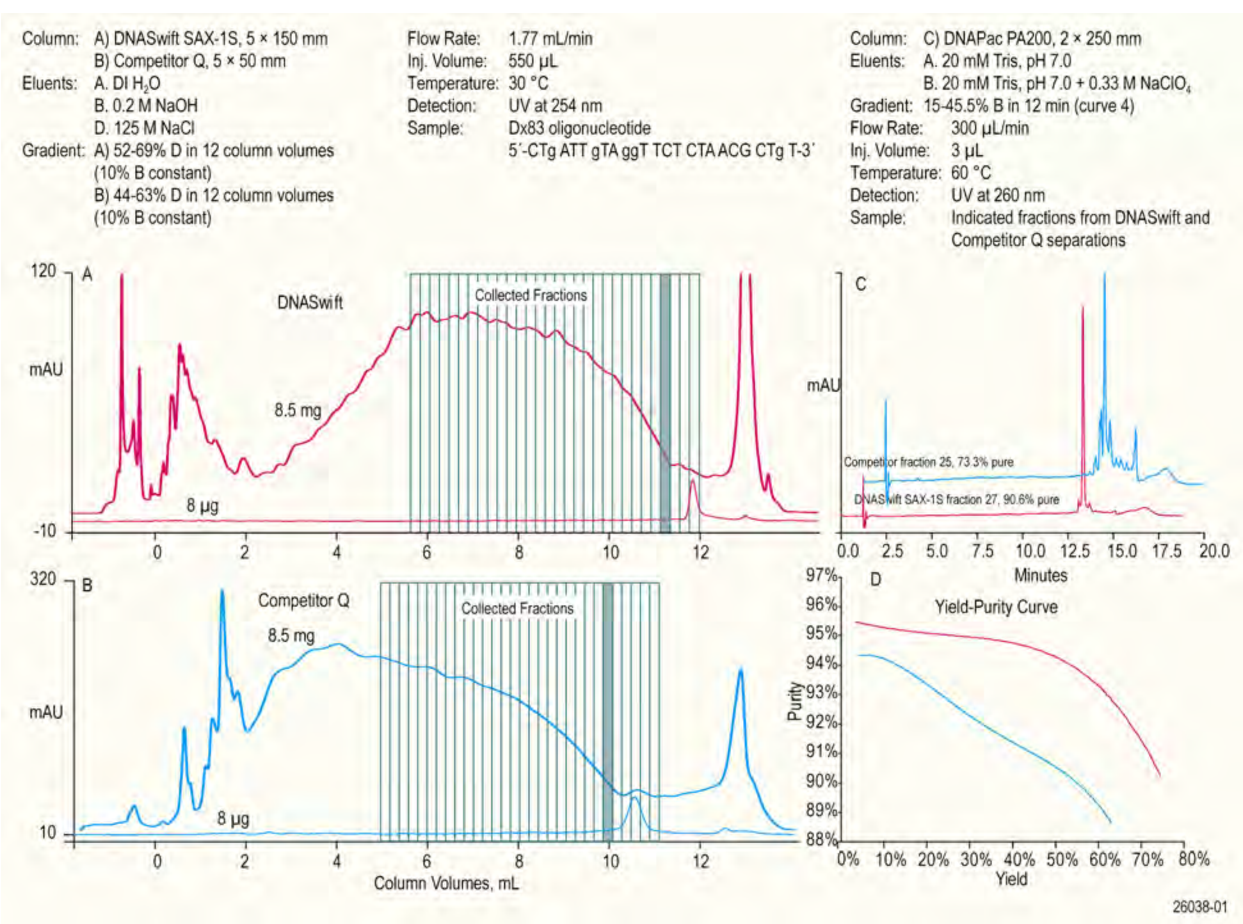


Figure 8
Yield Purity Curves for 25mers at pH 12

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3.5. Loading Capacity Comparisons

3.5.1. DNASwift vs. DNAPac and Mono-Q

Figure 9 shows the relative capacities of the nonporous DNAPac PA200 4x250 mm column and the DNASwift 5x150 mm monolith. The figure shows the DNAPac column to give much better peak shape at low injection amounts, but much poorer peak shapes when 150 µg or more sample is injected. Conversely, the DNASwift produces better PW½ at all sample loads when compared to the GE Healthcare Mono-Q.

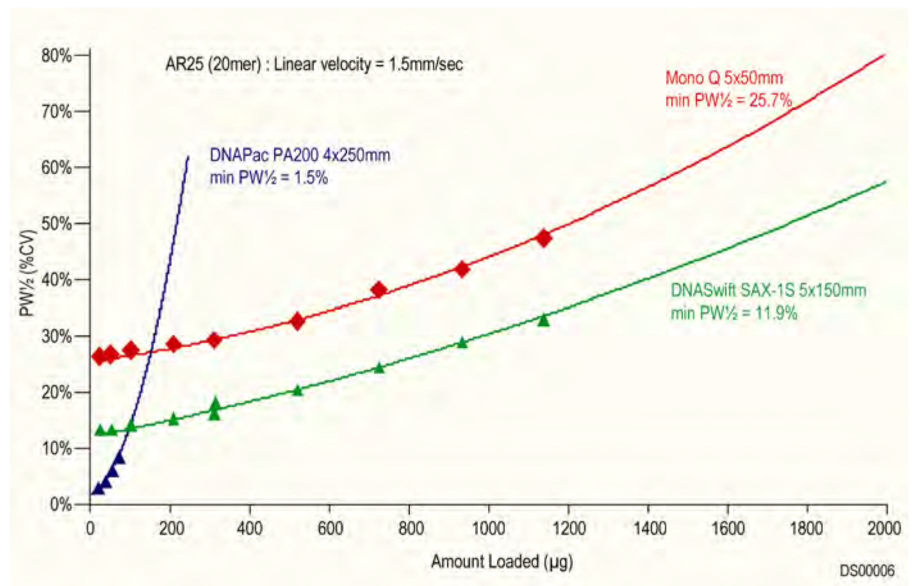

Figure 9
Comparison of Peak Width at Half Height vs. Sample Load of DNASwift (5x150 mm), Mono-Q (5x50 mm) and DNAPac PA200 (4x250 mm)

3.6. Resolution of Oligonucleotide Isomers (those having identical length, sequence and mass)

3.6.1. RNA linkage Isomers

During synthesis and release from the synthesizer, some RNA linkages may undergo a phosphoryl migration from 3´-5´ to 2´-5´ linkages. This change does not alter the RNA's charge, hydrophobicity, or mass, so identification of this impurity can be very difficult. Since the presence of 2´-5´ linkages is known to alter a variety of cellular biochemical activities, regulatory agencies may require reporting of their presence in RNA species intended for therapeutic use, and lacking 2´-OH protecting groups.
The DNASwift can resolve RNAs harboring one or more 2´-5´ linkages from identical sequences with all normal linkages as shown in Figure 10. Here, a 21-base RNA sequence without any 2´-5´ linkages (Dio-1) elutes at 13.19 min. Eleven other RNAs with the same sequence, but with intentionally introduced 2´-5´ linkages, elute at 9.97 – 14.99min. Confirmation of the presence of the aberrant linkages can be obtained by treatment of RNAs purified on the DNASwift with Phosphodiesterase-II, an exonuclease incapable of cleaving the 2´-5´ linkages[1].

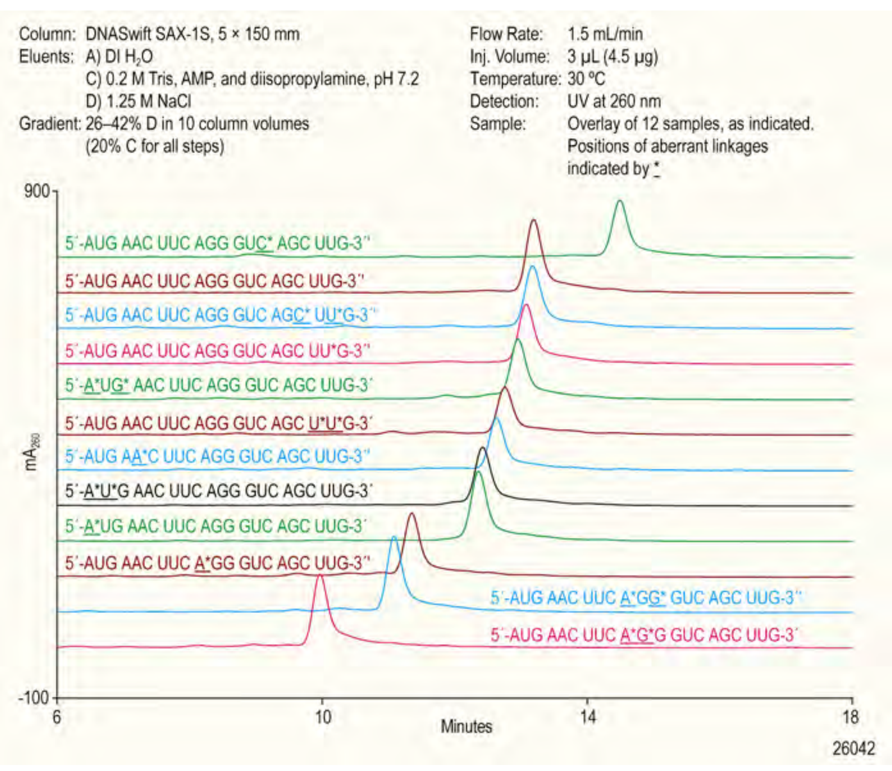

Figure 10
DNASwift resolution of Eleven different Aberrant Linkage Isomers in a Single Sequence of RNA

[1] J.R.Thayer, S.Rao, N.Puri, C.A.Burnett, M.Young, ***Anal. Biochem.*** 361 (2007) 132-139.

3.6.2. Resolution of Phosphorothioate Isomers.

Many therapeutic oligonucleotides are designed with phosphorothioate linkages to protect them from nuclease digestion. Unless these are introduced with stereochemical specification, this produces diastereomeric oligonucleotides. Each phosphorothioate linkage exists in one of two conformations (R and S), so two such linkages will result in four possible isomers (R-R, R-S, S-R, and S-S). Using Tris-buffered NaCl eluent, the DNASwift monolith elutes DNA without phosphorothioate linkages as a single peak (Figure 11 bottom trace). In the same sequence with two thioate linkages The DNASwift resolves three different isomers. (likely R-R and S-S isomers from the R-S and S-R isomers but not the R-S and S-R isomers from one another). With RNA oligos, the DNASwift elutes same sequence without phosphorothioate linkages as a single peak, and resolves all four phosphorothioate isomers (Figure 11, top trace)

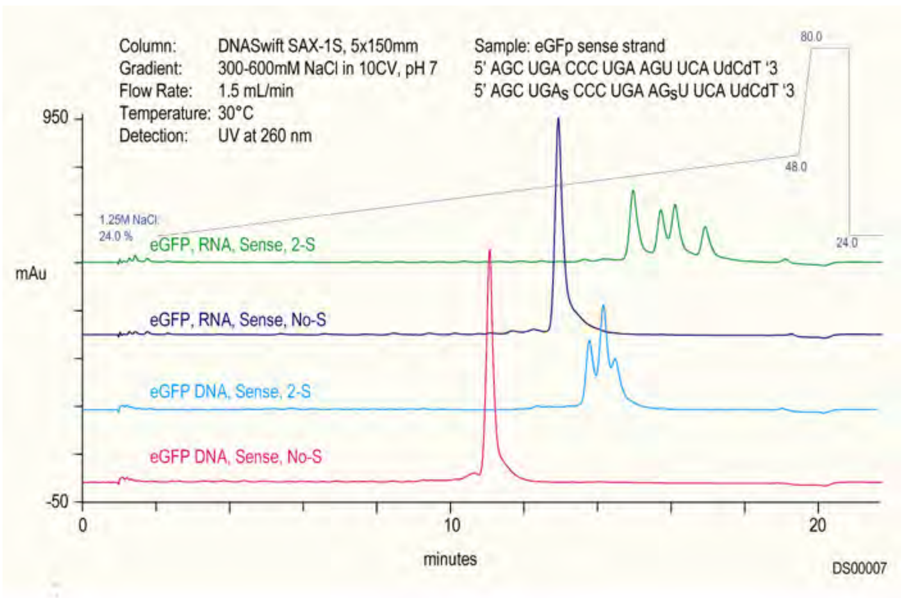

Figure 11
DNASwift Resolution of DNA (bottom two traces) and RNA (top two traces) Harboring no Phosphorothioate Linkages (bottom and third trace) or Two Phosphorothioate Linkages (second and top traces)
Sequence and Phosphorothioate Sites as Indicated in the Figure

4. SPECIFICATIONS

Table 1

Parameter	**DNASwift SAX-1S**
Column dimension	5x150 mm
Base Matrix Material	Poly(meth)acrylate
Surface chemistry	Quaternary amine
Nucleic Acid binding capacity per mL of polymer	~20mg/mL 20-mer
Protein binding capacity per column	~50 mg 25-mer
Bed Height	130mm
Bed volume	2.5 mL
pH Range	6 – 12.4 for operation 3 – 14 for cleaning NOTE: Salt equimolar to NaOH required.
Recommended Flow rate	0.5 – 2.5 mL/min
Maximum Flow rate	3 mL/min
Operating column pressure	< 1500 psi 10.3 MPa
Temperature	85 °C Note: maintain eluent pH $\leq$ 8 above 30 °C
Solvent Compatibility	Most common organic solvents (e.g. MeCN, MeOH)

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5. INSTALLATION

5.1. Operation:

The following sections outline guidelines for proper operation.

5.1.1. QuickStart for DNASwift:

DNASwift Monolith columns offer improved separation of DNA and RNA Oligonucleotides. Conditioning of the column bed is **required** prior to initial use and after long-term storage if the column is allowed to dry. The QuickStart process will ensure extended column lifetime and reproducibility.

I. Preparation:
   a. Eluent Preparation
      The following eluents are recommended, but the column may be used with any eluents suitable for analysis. Typically Eluent A is a low salt buffer and Eluent B is a high salt buffer.

Eluent Composition	Example 1:	Example 2:
A	20 mM Tris, pH 8.0	20 mM Tris, pH 8.0
B	20 mM Tris, pH 8.0, 1.25M NaCl	20 mM Tris, pH 8.0, 0.33M $NaClO_4$

   b. Column Installation
      Install the column on the instrument in the correct flow direction.

WARNING	**Sudden increases in flow rates may damage dry monolithic columns. When new or potentially dry, increase the flow rate slowly using a linear flow gradient or stepwise increments in flow rate.** **If the eluent composition generates back pressure in excess of the maximum pressure, reduce the flow rate to ensure the upstream back pressure is less than the maximum pressure.** **The maximum pressure limit for the DNASwift monolith is 1500 psi (10.4 MPa)**

II. Flow Rate Start-Up (Ramping)
    Using a linear or stepwise flow gradient, increase the flow rate of Eluent A starting from 0.00 mL/min to the desired flow rate using the flow rates given below.
      Use a change of ≤0.50 mL/min, every 30 seconds

III. Column Conditioning - Use the guidelines below to determine the proper startup conditions:

Removal of Storage Solution:
- Using the desired flow rate, run a 25 mL (~ 10 column volumes) binary gradient from 100% A to 100% B.
- Pump another 25 mL (~10 column volumes) of 100% B through the column.

Column Equilibration:
- Equilibration from 100% B to the starting eluent composition should include at least a 1 minute reverse gradient to the initial conditions.
- Pump at least 25mL (~10 column volumes) of this eluent composition through the column.

IV. Storage:
   a. For short-term storage, <3 days, store the column in buffer B.
   b. For long-term storage, >3 days, use Buffer B containing 20% Acetonitrile to avoid microbial growth on the column.
   c. In all cases the column should be tightly sealed with end plugs to prevent the column from drying out.

For additional information, please refer to the manual, DNASwift Product Manual Doc. No. 065227.

5.2. Sample Preparation:

For best results, dissolve the samples in the initial run buffer or in pure deionized water. The salt concentration should be determined so the column is not overloaded by the injected sample.

Generally, from 0.05 to 10 mg of oligonucleotide may be loaded on the DNASwift monolith for purification purposes.

For oligonucleotide *analyses*, DIONEX recommends loading no more than 120 µg per mL of column volume (300 µg on the DNASwift) although more can be loaded for oligonucleotide purification.

If the sample may contain particulates, filter it through a 0.2 µm syringe filter before loading it into the injection valve.

5.3. Column Equilibration:

Before performing a run after storage, wash and equilibrate the column using protocols described in the QuickStart (Section 5.1.1). When switching to a different buffer type, use an eluent volume of 20 times the column volume (approximately 50 mL) to ensure the monolith is well equilibrated.

6. TROUBLESHOOTING

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using DNASwift columns.

If you cannot solve the problem on your own, contact the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390).

Table 2
DNASwift Ion Exchange Column Troubleshooting Summary

Observation	Cause	Action	Reference Section
High back pressure	Blocked component	Isolate and clear blocked component.	6.1.1
		In-Line Filter	6.1.1.1
		Crimped Tubing	6.1.1.2
	Plugged column bed supports (frit)	Clean, regenerate or replace column, reverse column flow to flush out particles trapped at inlet frit.	6.1.1.3
	Other system modules	Disconnect, and replace	System module manual
	Incorrect Flow-rate	Correct or calibrate pump flow	6.1.1.4
High background noise	Contaminated eluents	Remake eluents	6.2.1
	Contaminated column	Clean column	6.2.2
	Contaminated hardware	Clean component	6.2.3
	Pump lost prime, air bubbles in line	Re-prime pump; ensure there are no air bubbles. Degas buffers	6.2.4
	Not enough backpressure at cell outlet when going from larger ID to narrow ID column	Add backpressure tubing to cell outlet	6.2.4
Poor resolution	Poor efficiency due to large system void volumes	Re-plumb system	6.3
	Contamination of column or frit	Clean column, reverse column flow to flush out particles trapped at inlet frit	6.3
	Column Overloading	Inject smaller volume and/or reduce sample concentration	6.3
	Inadequate temperature control	Install or Verify operation of column oven	6.3
	Inadequate Gradient due to system dead volumes when extrapolating gradients from different column ID	Minimize dead volumes, modify gradient times.	6.3
	Un-equilibrated system	Lengthen first eluent time before inject	6.3
Decreased retention times	Flow rate too fast	Recalibrate pump or reduce flow	6.1.1.4

Observation	**Cause**	**Action**	**Reference Section**
	Contaminated eluents	Remake eluents	6.4.1
	Column contamination	Clean column	6.4.2
Poor front end resolution	Incorrect or contaminated eluents	Prepare clean eluents	6.5.1
	Column overloading	Reduce sample size or concentration	6.5.2
	Insufficient column equilibration	Increase pre-equilibration time	6.5.3
	Sluggish injection valve	Service valve	6.5.4
	Large system void volumes	Re-plumb system	6.5.5
Spurious peaks	Sample contamination	Pre-treat Desalt the samples	6.5.2
	Sluggish injection valve	Service valve	6.2.2
	Column Contamination	Clean Column	6.6.1
	Contaminated eluents	Test contamination, and remake eluents	6.4.1
Small Peak Areas/ Decreased detection sensitivity	Incorrectly installed (or controlled) injection valve.	Reinstall injection valve	6.8
	Sample degradation	Prepare fresh sample	6.7
	Column deterioration or contamination	Clean or replace column	6.7
	Low detector lamp intensity	Replace UV lamp in detector	6.7
Other instrument problems	High Background	Check system	6.9.1
	No (small) peaks, poor area reproducibility	Maintain Sampler, or valve	6.9.2
	Incorrect or variable retention times	Check System	6.9.3

6.1. High Back Pressure

NOTE	***Inclusion of solvent in eluent may increase column backpressure by 10-30%.***

6.1.1. Finding the Source of High System Pressure:

If the system pressure is very high, it is advisable to find out what is causing the high system pressure.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure. It should not exceed 50 psi (0.34 MPa). Continue adding the system components (injection valve, column, and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of ~600 psi (4.1 MPa) at a flow rate of 1.0 mL/min when the 5-mm DNASwift column is connected. No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

6.1.1.1. In-line Filters:

The system should be used with a high pressure in-line filter eluent filter. The filter should be positioned between the pump and the injection valve. Make sure you have an inert high pressure in-line filter in place and that it is not contaminated.

6.1.1.2. System Flow Path:

Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls have collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support (frit), a plugged high pressure in-line filter, or the detector cell.

6.1.1.3. Clogged Column Bed Support (Frit) Assemblies:

If the column inlet frit or the media is determined to be the cause of the high back pressure, clean the column in the reversed direction, or regenerate the columns using the methods described in Section 8.2

6.1.1.4. Flow Rate:

Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the actual pump flow at various flow rates, if necessary by collecting the flow of deionized water into a pre-weighed graduated cylinder. Calculate the flow rate based on the collected volume of deionized water.

6.2. High Background or Noise:

6.2.1. Contamination of Eluents:

Make sure that all eluents are made correctly, and from chemicals with the recommended purity.

Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm.cm.

6.2.2. Contaminated Column:

Remove the DNASwift column from the system. If the background noise decreases, then the column itself is the cause of the high background. Clean the column as instructed in Appendix B - Column Care.

6.2.3. Contaminated Hardware:

To eliminate the hardware as the source of the high background signal, bypass the column and pump deionized water with a specific resistance of 18.2 megohm.cm through the system. The background signal should be less than 0.1 mA_{260}. If it is not, check the detector cell by injecting deionized water directly into it. See the appropriate detector manual for further details.

6.2.4. Air Bubbles in Detector:

If the pump loses prime, or a large injector loop filled with air is activated, air can enter the system. The air can dissolve at high pressure, but comes out of solution when it elutes from the column (low back pressure), and become trapped in the detector cell. Ensure that the pump is not pumping air, and that the injection system is working properly, and then clear the bubble by applying increased back pressure to the detector cell.

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6.3. Poor Peak Resolution/Efficiency:

Poor peak resolution or efficiency can be due any or all of the following factors:

Extra-column volumes can cause sample band dispersion and will decrease peak efficiencies. Ensure that system void volumes have been minimized. Make sure you are using the appropriate tubing to make all eluent liquid line connections between the injection valve and the detector cell inlet on the system. Make all tubing lengths as short as possible. Check for leaks. Also, changes in system dead volume due to changes in column scale-up or down can result in unexpected changes due to flow and dead volume changes. In such cases you may need to adjust the gradient delay and equilibration times.

Contamination of the column media or frit due to binding of the sample or eluent components can be responsible for the loss of column efficiency. Please refer to Section 8 - Column Care.

A contaminated flow cell may contribute to dispersion. Ensure this is clean.

a) Verify that 0.01" ID or smaller tubing is installed for all connections between injector and detector.
b) Verify that the shortest possible length of 0.010" I.D. tubing (black) is installed between the column and detector.
c) Check for proper installation of ferrules on all PEEK tubing, starting with the injector outlet and all other connectors to the detector cell inlet.
d) Check temperature settings in your method and/or the actual temperature in your column oven.
e) The column may be overloaded. Try a smaller injection volume, or reduced sample concentration.

If all of the above does not lead to an improved separation, the bed of the main separator column may have been damaged and column should be replaced.

If you use a column thermostat, the temperature may have changed. Check the column temperature and/or thermostat.

6.4. Decreased Peak Retention Times:

6.4.1. Eluents:

Eluent contaminants may be decreasing the capacity of the column. Use de-ionized water with a specific resistance of 18.2 megohm.cm. Sterilize by filtering through a 0.2-µm, or 0.45-µm filter. Also, check for changes in eluent concentration and pH. If column cleanup and use of clean fresh eluents fail to restore retention, the column cleanup process, (section 8.2) should be performed.

Even with adequate system and column efficiency, resolution of peaks will be compromised if the analytes elute too early. The gradient and eluent composition should be confirmed. See also section 6.5.3.

Check to ensure that the eluent compositions and concentrations are correct. For isocratic analysis, an eluent that is too strong will cause the peaks to elute too early. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

For gradient analysis, remake the eluents or adjust the proportions and times in the gradient program to obtain the required peak resolutions.

Check the eluent flow rate. If it is different from the flow rate specified by the analytical protocol, recalibrate the pump.

6.4.2. Column Contamination:

Column contamination can lead to a loss of column capacity because fewer of the binding sites will be available for the sample compounds. Polymers or metal ions might be concentrating on the column. Refer to section 8.2 - Column Cleanup Procedure, for column cleanup recommendations.

Possible sources of column contamination are impurities in chemicals or components in the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm.cm, and the solvents should be of HPLC grade.

After cleaning equilibrate the column with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column.

6.5. Loss of Front End Resolution:

If poor resolution and efficiency is observed for very early eluting peaks (near the system void volume) compared to the later eluting peaks, check the following:

6.5.1. Eluents:

Improper eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity. Eluents prepared by mass rather than volume will provide more reproducible eluents.

6.5.2. Sample Concentration or Contamination:

Column overloading may be the problem. Reduce the amount of sample injected onto the column by either diluting the sample or injecting a smaller volume onto the column. If the sample contains very high salt levels or high ionic strength the sample may elute earlier than expected. Desalt the sample.

6.5.3. Column Equilibration:

The column may not be equilibrated with the first eluent. Increase the amount of time the first eluent runs through the columns before injection.

6.5.4. Injection Valve:

Sluggish operation of the injection valve may be the problem. Check the valve operation to make sure there are no leaks or partially plugged port faces. Refer to the valve manual for instructions.

6.5.5. Dead Volumes:

Improperly swept out volumes anywhere in the system prior to the columns may be the problem. Swap components (one at a time) in the system prior to the analytical column and test for front-end resolution after every system change.

6.6. Spurious Peaks:

6.6.1. Column Contamination:

The column may be contaminated. If the samples contain an appreciable level of ionic components and the column is used with a weak eluent system, these components may remain on the analytical column. The retention times for the analytes in subsequent injections will then decrease, and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in section 8.

6.6.2. Injection Valve:

The injection valve may be creating a baseline disturbance. This baseline upset can show up as a peak of varying size and shape. This may happen when the injection valve needs to be cleaned or torque reapplied (see valve/autosampler manual). Check to see there are no restrictions in the tubing connected to the valve. Also, check the valve port faces for blockage and replace them if necessary. Refer to the valve/autosampler manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest. If cleaning and reapplication of the torque to the valve does not help, and the valve is known to be the problem, replace the valve.

6.6.3. Eluents:

The eluent may be contaminated. When performing gradient chromatography, contaminants in the eluent may accumulate on the column until the eluent strength is sufficient to elute them. Increasing the equilibration time at low eluent strength will result in more pronounced contaminant peaks. Spurious peaks due to the eluent may also be observed during a blank (no injection) run. If either of these is observed, remake the eluents. If the problem persists, prepare the eluents from higher purity chemicals.

6.7. Decreased Detection Sensitivity:

Detection sensitivity may be caused by sample degradation, column degradation leading to increased peak width (lower peak height), or limitations to light throughput in the absorbance detector (e.g., dirty or improperly installed flow cell, near failure of D_2 lamp).

6.8. Small Peak Areas:

Small peak areas can occur when the injection valve is incorrectly installed or controlled. Plumb the injection valve's sample loop so that it is inserted into the flow path when the inject command is issued.

6.9. System Problems:

6.9.1. High Detection Background Caused by the System:

a) Verify the problem is not related to the detector or column.
b) Prepare new eluents with freshly filtered deionized water.
c) Rinse all eluent lines with the new eluents (at least 40mL using the priming syringe)
d) If new eluent introduces high background without the column set installed, your deionized water source, or eluent components have become contaminated.

6.9.2. No Peaks, Poor Peak Area Reproducibility or Unexpectedly Small Peak Area:

a) Check the position and filling levels of sample vials in the autosampler.
b) Check injector needle height setting.
c) Check each line of the schedule for proper injector parameters. Employ full loop methods if other injection modes (partial loop fill) do not provide acceptable reproducibility.
d) Service the injection valve (check for leaks, rotor fragments, or sediments inside the valve)
e) Check sampling needle for bits of vial septa clogging the flow path.

6.9.3. Incorrect or Variable Retention Times:

a) Check your eluent preparation procedure for possible errors.
b) Prime the pump if necessary.
c) Measure the flow rate by weighing out the eluent collected during exactly five minutes of flow. Recalibrate the pump if necessary.
d) Set the eluent composition for 100% for each eluent and draw out at least 40mL of eluent from each of the lines to verify pump is primed.
e) Check and/or service the pump's proportioning valve. With the pumping turned off, the flow through the pump outlet tubing (disconnected from the injector) should be zero in all eluent positions. Check this separately for each eluent line.

For further information on system troubleshooting please refer to the appropriate system component manual.

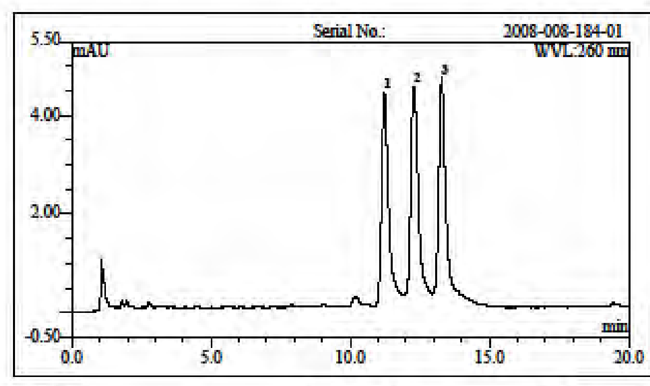
7. QUALITY ASSURANCE REPORTS

7.1. DNASwift (5x150 mm):

DNASwift™ SAX-1S
5 x 150 mm
Product No. 066766

Date: 13-May-09 08:14
Serial No. :
Lot No. :

Buffer A:	20 mM Tris, pH 7.0, Adjusted with MSA
Buffer B:	0.33M Sodium Perchlorate in Buffer A
Gradient:	27% to 37%B in 18 minutes, 1 minute Hold, 100%B in 1 minute, 1 minute Hold
Flow Rate:	1.50 mL/min
Temperature:	30 °C
Detection:	UV 260 nm
Injection Volume:	10 µL
Storage Solution:	250mM NaCl in 40 mM Tris, pH 7.5 (MSA Adjusted) in 20% Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)
1	dT22	11.22	1.8	2.34	0.252
2	dT23	12.27	1.6	2.28	0.279
3	dT24	13.28	1.5	n.a.	0.243

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=1100	212
dT22-24	Average PW@10%	<=0.66	Passed
T1/T2	Ret.Time Ratio	0.89-1.11	Passed

T1 = Ret. Time of dT24-dT23
T2 = Ret. Time of dT23-dT22

Production Reference:
Datasource: MonoBio
Directory: Production\DNASwift_Validation
Sequence: DNASWIFT_GRADIENT_VALIDATION_3-OLIGO
Sample No.: 7

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8. COLUMN CARE

8.1. New Column Equilibration:

The columns are shipped in the storage solution containing 20% acetonitrile to suppress microbial growth. Before use, please refer to QuickStart (Section 7) for cleaning of the storage solution.

8.2. Column Cleanup:

If the column inlet frit or the media is fouled by sample or eluent contaminants, (precipitates and hydrophobically bound components,) these may be removed by a strong solvent, solubilizing agents, acid, base, or if the contaminant is a protein, by pepsin treatment.

CAUTION	***Always ensure that the cleanup protocol used does not switch directly between eluents that can react or precipitate when mixed together. Choose a flow rate that will not create higher column back pressure than maximum pressure in column specifications.***

8.2.1. Column Cleanup Procedures:

WARNING	***If your eluent composition generates back pressure in excess of the maximum operation pressure, adjust the flow rate to ensure the backpressure of the column is less than the recommended maximum operation pressure.***

8.2.1.1. High Salt Wash to Remove Ionic Components:

This cleanup procedure should be performed when component retention decreases. This solution will protonate most organic acids (1 mM HCl = pH 3). The $NaClO_4$ will still be largely ionized so it will elute the organic acids. The solvent will aid in removing components bound primarily by hydrophobic interactions.

1. Rinse column with 10 volumes (~ 25 mL) of deionized water.
2. Inject 5 x 1 mL of the wash solution containing:
   0.4 M $NaClO_4$
   1 mM HCl
   30% CH_3CN
3. Rinse column with 15 volumes (~ 38 mL) of deionized water.
4. Regenerate column with 1 volume (~ 3 mL) of high concentration eluent used for column wash at the end of each chromatographic run.
5. Equilibrate column with 2 volumes (~ 6 mL) of eluent used at the start of each chromatographic run.

8.2.1.2. Organic Solvent Wash to Remove Non-Ionic Components

Use this approach when band broadening without loss of retention occurs, or when the background signal is elevated. This solution will elute neutral compounds bound primarily by hydrophobic interactions (e.g., non-ionic detergents).

1. Rinse column with 10 volumes (~ 25 mL) of deionized water.
2. Inject 5 x 1 mL of 100% CH_3CN.
3. Rinse column with 10 volumes (~ 25 mL) of deionized water.
4. Regenerate column with 1 volume (~ 2.5 mL) of high concentration eluent used for column wash at the end of each chromatographic run.
5. Equilibrate column with 2 volumes (~ 5 mL) of eluent used at the start of each chromatographic run.Equilibrate the column with eluent before resuming normal operation.

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8.2.1.3. Column Sanitation:

This cleanup procedure should be performed between purifications of different oligonucleotides to prevent cross contamination.

WARNING	***The DNASwift is a hydroxide-selective chromatographic phase eluents containing NaOH in the absence of competing salts will create a stationary-phase pH capable of degrading the column. DIONEX recommends use of 1.25M NaCl with 1M NaOH for sanitation.*** ***This will help maintain the stationary phase pH close to that of the mobile phase.***

1. Rinse column with 10 volumes (~ 25 mL) of deionized water.
2. Inject 5 x 1 mL of the wash solution containing:
   1 M NaOH
   1.25M NaCl
3. Rinse column with 15 volumes (~ 38 mL) of deionized water.
4. Regenerate column with 1 volume (~ 3 mL) of high concentration eluent used for column wash at the end of each chromatographic run.
5. Equilibrate column with 2 volumes (~ 6 mL) of eluent used at the start of each chromatographic run.

8.3. Column Storage:

8.3.1. Short Term Storage:

For short term storage (less than 3 days), use the low salt concentration eluent (pH = 6 - 8) as the column storage solution.

8.3.2. Long Term Storage:

For long term storage, use 0.05 M NaCl in 0.01 M Tris.HCl, pH 7.6 + 20% MeCN to avoid microbial growth on the column.

Flush the column with at least 5 CV (~13 mL) of the storage eluent. Cap both ends securely using the plugs supplied with the column.

9. DENATURING AGENTS AND DETERGENTS

9.1. DNASwift Column Operational Parameters:

CAUTION	***Use of these chaotropes will increase back pressure, and may reduce column lifetime***

CAUTION	***Do not use anionic or cationic detergents. These will bind irreversibly to, and foul the column.***

Chaotrope Limit:	30% formamide, 6 M Urea
Typical Eluents:	High purity water (18 megohm.cm), sodium chloride, sodium perchlorate, buffers, sodium acetate and sodium hydroxide.
Detergent Compatibility:	Nonionic or zwitterionic detergents.

10. APPLICATION SPECIFIC RECOMMENDATIONS

From the observations detailed in the preceding sections, the following suggestions can be made:

10.1. Minimizing Base-Specific Retention:

For synthetic ODNs where the goal is to evaluate purity, determine the coupling efficiency, or purify the full-length component from “n-1” and “n+1” impurities in the sample, eluent systems minimizing base-specific retention would produce the best results. Hence, solvent-containing $NaClO_4$ eluent at pH 7 or below, where pH-induced ionization is further minimized would be the logical choice. We have also had success with the inclusion of 20% acetonitrile in the eluent.

10.2. Maximizing Base-Specific Retention:

When multiple possible ODNs of similar length in the same solution must be resolved, eluents maximizing base-specific retention would provide the best probability of success. Examples of such samples include: identification of all primers in a multiplex PCR amplification cocktail, QA/QC of multiple primers in amplification-based diagnostic kits, identification of the different components in “n-1” or “n+1” impurities when troubleshooting nucleic acid synthesis protocols. For these applications, NaCl without solvent, at pH values between 9 and 12 would be most likely to produce the desired separations.

10.3. Exploitation of Interactions Between the Stationary Phase and ODN Derivatives:

When hydrophobic interactions between some bases and the phase are suspected, interactions between the phase and additional ODN derivatives may also be exploited. Examples of such derivatives include numerous fluorescent dyes, and the “Trityl” group used to protect the oligo from unwanted base additions at each elongation step during ODN synthesis. For these applications, addition of solvent at concentrations between 5 and 30% may be helpful.

10.4. Denaturing Conditions for Control of Secondary Structure:

Single-stranded nucleic acids may contain inter-, and/or intra-, strand hydrogen bonding. Such interactions, if sufficiently strong, result in spurious peaks and a general inability to distinguish between the oligonucleotide components in the sample. There are three common methods to restrict these interactions; high temperature, addition of chaotropic agents such as urea or formamide, and use of high pH. Both the temperature used and the concentration of chaotropic agent used depend upon the extent of hydrogen bonding. For pH-based control of secondary structure, values between pH 11 – 12.4 are typically effective at controlling both Watson-Crick, and non Watson-Crick oligonucleotide hydrogen-bonding interactions.

While the DNASwift can be used with any of the above methods for controlling secondary structure, there are certain considerations that should be taken into account when deciding which approach to use:

a) The use of a chaotropic agent, such as formamide or urea, tends to dramatically increase pressure, and reduce the lifetime of the column.
b) The use of elevated temperature at pH values above 8 tends to reduce the lifetime of the column.
c) Elevating the temperature of the DNASwift will increase the retention time of the oligonucleotide. This means that more eluent will be required to elute the oligonucleotide, and thus the amount of salt eluting with it will be increased.
d) Increasing the pH of the eluent will also generally increase retention of oligonucleotides, but in a manner that allows control of oligonucleotide selectivity.

10.5. Effect of High Temperature and High pH on Column Lifetime:

Because the DNASwift is a *hydroxide-selective* chromatographic phase, eluents containing NaOH in the absence of competing salts will result in stationary-phase pH values far in excess of the mobile-phase pH. These can reach levels capable of degrading the column. Therefore, DIONEX recommends inclusion of a competing salt (e.g., NaCl) at concentrations at least equimolar to the eluent [NaOH]. This will help maintain the stationary phase pH close to that of the mobile phase. Fortunately, the required sodium hydroxide concentrations tend to be < 25mM therefore the required salt does not interfere with oligonucleotide retention and resolution. Use of elevated temperatures can amplify this effect. DIONEX strongly recommends avoiding the combined use of elevated temperatures with elevated pH.

10.6. Effect of Fluorophores and Capture Probes on Oligonucleotide Retention:

Oligonucleotides derivatized with different fluorescent or affinity probes can be resolved from one another on the DNASwift column. Thus, post-labeling purification of labeled oligonucleotides from their unlabeled parents is readily accomplished on the DNASwift monolith.

NOTES

For further information call 1-800-DIONEX-0 (1-800-346-6390)