Technical Guide Version 2

Thermo Scientific HyPURITY HPLC Columns

The Pure Choice for Method Development





Thermo Scientific HyPURITY Columns

The best choice for superior chromatography

For more than 30 years, we have designed and manufactured HPLC media and columns featuring the highest quality program including ISO 9001:2000 certification.

The Thermo Scientific HyPURITY family of columns sets new standards in column performance, characterization and validation. Our quality assurance program has been designed to probe a number of diagnostic chromatographic tests to ensure exceptional column-to-column and batch-to-batch reproducibility. We feature a wide range of stationary phases bonded to the HyPURITY silica including C18, C8, C4, Cyano as well as the unique HyPURITY ADVANCE[™] and HyPURITY AQUASTAR[™] phases.

This versatile range of phases offers exceptional selectivity allowing the operator to resolve compounds at high and low pH and at polarity extremes with excellent peak shape and outstanding column performance time after time.

- HyPURITY C18 columns: one of the most rugged and reliable columns available
- HyPURITY ADVANCE columns for alternative selectivity and exceptional peak shape for bases
- HyPURITY AQUASTAR columns for optimum aqueous stability and polar retention
- Ultra-pure, stable silica enhances column lifetime, performance and reliability
- Superior efficiency and peak shape across a wide range of analyte types
- 190Å pore size provides extensive chromatographic options from small molecule to large peptide analyses

Phase	Particle Size	Pore Size	Carbon Load	End-Capping	Silica Type High Purity
HyPURITY C18	3 and 5 µm	190Å	13%	Yes	High purity base deactivated
HyPURITY C8	5 µm	190Å	8%	Yes	High purity base deactivated
HyPURITY C4	5 µm	190Å	4.5%	Yes	High purity base deactivated
HyPURITY Cyano	5 µm	190Å	4%	Yes	High purity base deactivated
Hypurity Advance	3 and 5 µm	190Å	10%	-	High purity base deactivated
Hypurity Aquastar	3 and 5 µm	190Å	10%	Polar End-Capped	High purity base deactivated

Table 1: HyPURITY phase specifications

Outstanding Silica Quality

HyPURITY silica delivers unparalleled quality and is manufactured in our dedicated, state-of-the-art facility by a team of highly experienced chemists. The silica is essentially metal-free and the C18 is exceptionally stable at high and low pH. The rugged and robust manufacturing process ensures reproducibility and optimum quality, batch after batch. Extra care is taken to ensure an extensively homogenous silica surface – a critical key precursor in the preparation of uniform bonded phases.

Increased Stability and Lifetime

For superior column reliability and longevity, the advanced manufacturing process generates outstanding chromatographic performance across a wide pH range. Silicabased reversed phase columns are generally stable in the pH range 2 to 8. At pH values below 2, the bond holding the alkyl chain to the silica surface starts to dissociate and at pH values higher than 8, silica dissolution occurs. Analyses outside the pH range 2 to 8 have therefore previously resulted in decreased column lifetime and performance. Each HyPURITY C18 batch has undergone rigorous stability trials at pH 0.9 and 10.6 to ensure that demanding analyses at extremes of pH and high temperature can be performed with confidence. This extension to the stable operating range at both high and low pH enhances column lifetime and enables columns to be used with conditions that would previously have resulted in a rapid deterioration of performance and lifetime. Example of the stability of HyPURITY C18 media are shown in Figures 1 and 2.



Figure 1: Analysis of Bases Before and After Exposure to 5.8L of pH 0.9 at 90°C

Thermo Scientific HyPURITY HPLC Columns

Highly Reproducible Columns

Six physical properties are monitored for each batch of silica and bonded phase manufactured to ensure reproducibility of retention and column efficiency, (Figures 3 and 4).

- Surface area
- Pore size
- Carbon load
- Mean particle size
- Particle size distribution
- Full BET adsorption and desorption isotherm

A series of chromatographic tests are also run on the base silica as well as bonded phases to ensure reproducibility. The HyPURITY C18 media has a total of six chromatographic tests probing the nature of the surface, and are further explained on page 7.



Figure 2: Enhanced Column Stability at pH 10.6



Figure 3: Excellent Reproducibility of Carbon Load on 5 µm HyPURITY C18 media



Figure 4: Median Pore Diameter for HyPURITY Silica (Å)



Thermo Scientific HyPURITY Column Selection

Table 2: Choosing the Appropriate HyPURITY Column

The choice of the appropriate column for a particular application can be a daunting task. With a range of bonded phases offering different selectivity, the HyPURITY family includes columns to meet most separation needs, including LC/MS compatibility. The chart (Table 2) will help you choose the best HyPURITY column for a particular application.

The HyPURITY column chosen for a particular analysis will depend on the analytes present in the sample, operating conditions, and the selectivity required. Selectivity is controlled by the nature of the stationary phase and the interactions of analytes with the media surface and mobile phase. If you change the surface chemistry of the stationary phase, the selectivity changes. For example, an acidic polar analyte that shows little to no retention using a traditional alkyl chain column may be retained using a column with enhanced polar character where polar groups in the stationary phase offer another type of interaction, such as seen with HyPURITY ADVANCE columns.

Selectivity changes between column packing materials due to changes in the interaction mechanisms predominantly manifest in two ways: either by a change in elution order, by a change in peak separation, or both. In most cases, selectivity changes are a combination of both these factors. This is illustrated in Figure 5 where the peak elution and separation between peaks is changed by moving from a C18 to a polar embedded phase. The different selectivity achievable from six HyPURITY column chemistries is demonstrated in Figure 6. The same dimension of column, test mix and analysis conditions were used with each column. The test mix components are steroids with similar structure. By polar end-capping the alkyl C18 chain, the resolution between peaks 3

and 4 is drastically improved as seen in the comparison of HyPURITY C18 and HyPURITY AQUASTAR columns. By adding a polar embedded group to the alkyl chain, changes in selectivity are possible as illustrated by comparing HyPURITY C8 and HyPURITY ADVANCE columns.



Figure 5: Elution Order Changes Possible using Phases with Alternative Selectivity



Figure 6: Selectivity and Retention Time Differences with Various HyPURITY Phases

Alternative selectivity, including a change in peak elution order, is beneficial when two peaks of a chromatogram coelute or elute very closely. Often the separation of such peaks requires that chromatographic conditions be drastically changed and/or the column chemistry modified. The examples shown in Figures 7 and 8 demonstrate the usefulness of alternative selectivity. Alternative selectivity, which may reduce retention, may offer an opportunity to achieve higher sample throughput by means of a decrease in analysis times.



Figure 7: Enhanced Separation of Structurally Similar Drugs

Figure 8: Separation of Analytes Improved by Adding Polar Character to the Column

Increased Sensitivity with 3 µm Columns

Quantitation of trace components in complex mixtures is generally performed by measurement of peak height, so the chromatographic separation needs to produce sharp, efficient peaks. The accuracy and sensitivity of the analysis is therefore determined by parameters such as particle size. Peak efficiency increases with a decrease in the particle diameter. Figure 9 illustrates the effect of the particle size (3 μ m versus 5 μ m HyPURITY C18) for the analysis of a parent drug and three related substances.

Maintaining all the analysis conditions constant, the increase in peak height with the 3 μ m column is 54, 22 and 42% for peaks 1, 2 and 4 respectively. Therefore, the related substances present at trace levels in this drug were more accurately quantified when the 3 μ m column was used.



Figure 9: Increased Sensitivity using a Smaller Particle Size

HyPURITY C18 Columns

Introduction

HyPURITY C18 columns are based on the latest in silica technology – HyPURITY silica with less than 10 ppm total metals and exceptionally stable at low pH. The HyPURITY silica manufacturing process ensures reproducibility and quality. The homogeneous surface ensures uniform bonding coverage and eliminates silanolanalyte interactions that cause poor peak shapes for bases, acids and chelating compounds. The 190Å pore size and excellent mass transfer properties of HyPURITY C18 makes it ideal for chromatography of large peptides, as well as small molecules, with unbeatable peak shape for all sample types.

- The ultimate in chromatographic validation
- Rigorously quality tested throughout manufacturing process
- First-class efficiency and performance
- Highly reproducible, rugged and robust
- Ideal for LC/MS applications

Extensive Chromatographic Testing

HyPURITY silica and bonded phases are subject to extensive quality assurance testing to probe the chemical, physical and chromatographic nature of the media. The physical testing of the media reveals only some of the characteristics of the column. The actual chromatography obtained on a wide range of analytes fully characterizes the chromatographic surface of the HyPURITY C18 media. The chromatographic probes employed cover the broad range of possible analyte/stationary phase surface interactions, and have been chosen only after an in-depth literature survey and extensive consultation. Table 3 lists the tests performed on each batch of HyPURITY C18 after the final media production stage to ensure full characterization and ultimate performance.

This wide range of tests ensures the outstanding reproducibility of both the base silica and bonded phase.

Test	Characteristic Tested	Method
Steric Selectivity Polyaromatics	Bonded Phase Uniformity	Relative Retention of Shape Differentiated
Hydrophobicity	Hydrophobic Character & Surface Coverage of Bonded Phase	Retention & Selectivity of Non-Polar Hydrocarbons
Hydrogen Bonding Capacity	Measure of Activity of Residual Silanols at Silica Surface	Selectivity of Caffeine Relative to Phenol
Ion Exchange Capacity at pH 2.7	Presence of Active Ion Exchange Sites	Benzylamine Retention
lon Exchange Capacity at pH 7.2	Presence of Active Ion Exchange Sites & Dissociated Silanol Sites	Benzylamine Retention
Analysis of Bases at pH 7	Effect of Dissociated Silanols on Basic Analytes	Peak Shape & Selectivity Parameters of Tricyclic Antidepressants
Analysis of Acids & Chelators	Metal/Silanol Sites & Analyte Selectivity Sensitivity to Changes in Surface Silanol Content	Peak Shape & Selectivity Parameters of Acids & Chelators

Table 3: Chromatographic Validation Tests Conducted on HyPURITY C18 columns





Figure 11: Reproducibility of HyPURITY C18 Columns as shown by Steric Selectivity

Steric Selectivity and Hydrophobicity

Steric selectivity is the ability of the stationary phase to recognize and differentiate between molecules with similar molecular formulas but different shapes. It is often indicative of the surface coverage of the bonding chemistry and also provides a characteristic by which different HPLC packings can be compared. The hydrophobicity index provides an indication of the hydrophobic character of the column per unit area.

Hydrogen Bonding Capacity

The function of this test is to provide a measure of the activity of the residual silanol groups on the silica surface. Residual silanol groups are not shielded by the C18 alkyl ligand and are available to form hydrogen bonds with analytes. Caffeine is prone to such bonding and is therefore used as the test probe (Figure 12). HyPURITY C18 columns have an exceptionally low hydrogen bonding capacity, indicating minimal residual silanols present on the silica surface that are available for unwanted secondary interactions.

Ion Exchange Capacity

The retention of protonated amines at pH 2.7 is used to determine the level of ion exchange sites on the silica surface. The majority of the silanol groups (Si-OH) are undissociated at this pH and therefore do not contribute to the retention of protonated amines. Acidic silanols remaining on the silica surface will be in the ionized form (SiO-) and contribute to the retention of protonated amines by an ion exchange mechanism.

At pH 7.6, all of the surface silanol groups are dissociated to form ion exchange sites that increase the retention of protonated amines. To accurately determine the ion exchange capacity of a bonded phase, retention of amines should be measured at both high and low pH. HyPURITY C18 columns possess minimal ion exchange sites at both high and low pH and so are ideal for use at extended pH and with analytes in the protonated form. The careful monitoring of ion exchange capacity ensures reproducible column performance.





Figure 13: Hydrogen Bonding Capacity on HyPURITY C18 Columns Measured as the Separation Factor between Caffeine and Phenol

Figure 12: Hydrogen Bonding Capacity



Analysis of Bases at pH 7

Tricyclic antidepressants (TCAs) are difficult to chromatograph with good peak shape. They are highly basic compounds that can often produce severe peak tailing or irreversibly adsorb to the media surface, particularly at pH 7. The use of this sensitive test on HyPURITY C18 columns ensures the highest standard of reproducibility for selectivity and peak shape for the analysis of basic compounds. The high surface coverage of the bonded phase and minimal ion exchange interactions contribute to the outstanding peak shape and chromatographic performance as illustrated in Figure 15.

Figure 14: Ion Exchange Tests at pH 2.7 and 7.6



Figure 15: Excellent Peak Shape of Basic Compounds

Analysis of Acids, Alcohols and Chelators

Surface metal interactions can cause changes in selectivity or peak shape for chelating solutes. The presence of metal ions can be attributed not only to the base silica but also can be traced to the column hardware, such as frits. This only happens if the column is stored in a solvent that will promote leaching such as a strong acid or base. This test probes the variability of the HyPURITY surface by determining the presence of metal ions. Comparison of peak symmetry of two regioisomers, 2,3- and 2,7-dihydroxynapthalene is monitored. The former chelates while the latter does not. Acids and alcohols are also included in this test mixture to illustrate the applicability of the HyPURITY C18 media to a range of analytes where both chelating and hydrogen bonding secondary interactions are possible. Without careful control of the media surface, these interactions can change the overall selectivity and performance in terms of peak shape. This test gives the chromatographer confidence in the lot-to-lot reproducibility of the HyPURITY C18 media.



Figure 16: Outstanding Peak Shape for Acids, Alcohols and Chelating Compounds

Its excellent peak shapes make HyPURITY C18 columns the ideal choice for use with demanding LC/MS applications (Figure 18). Mobile phase modifier compatibility and enhanced stability and lifetime make HyPURITY C18 columns the best allround choice for LC/MS method development.



(ii) 20(S)-protopanaxatriols

Figure 19: Structure of Ginsenosides



Figure 17: High Throughput Fast Analysis using Short Columns



Figure 18: Analysis of Ginseng

Ginsenoside	R ₁	R ₂	Mass	Ginsenoside	R ₁	R ₂	Mass
Rb1	Glc(b1-2)Glc	Glc(b1-6)Glc	1108.6	Re	Rha(a1-2)Glc	Glc	946.5
Rb2	Glc(b1-2)Glc	Ara(pyr)(a1-6)Glc	1078.6	Rf	Glc(b1-2)Glc	HY	800.5
Rc	Glc(b1-2)Glc	Ara(fur)(a1-6)Glc	1078.6	Rg1	Glc	Glc	800.5
Rd	Glc(b1-2)Glc	Glc	946.5				



HyPURITY ADVANCE Columns

Introduction

HyPURITY ADVANCE media is a reversed phase material with polar embedded groups close to the silica surface. These groups block surface silanols from interacting with polar analytes, and also provide alternative selectivity compared to C18 and C8 phases. HyPURITY ADVANCE columns offer improved chromatographic performance with broad applicability and improved peak shape.

- A polar embedded stationary phase for alternate selectivity
- Ideal for high throughput fast analyses
- Enhanced aqueous stability
- Exceptional peak shape for acids, bases and chelators

Alternative Selectivity through Alternative Retention Mechanisms

Multiple interactions occur between analytes and the HyPURITY ADVANCE bonded phase to determine selectivity. The C8 chain portion of the HyPURITY ADVANCE media retains analytes according to the hydrophobic interactions that occur in standard reversed phase chromatography. In addition, dipoledipole interactions occur between the polar embedded group and the polar compounds. Retention mechanisms for HyPURITY ADVANCE columns are illustrated in Figure 20.

The unique polar embedded chemistry of HyPURITY ADVANCE columns can also act to positively repel ionized basic species, resulting in excellent peak shape for these typically problematic compounds. The additional interactions shown may result in a change of elution order for some compounds when compared to a C18 or C8 phase.



Figure 20: HyPURITY ADVANCE Retention Mechanisms



Figure 21: Improved Selectivity using HyPURITY ADVANCE Columns

Changes in elution order can also result from changing the organic solvent in the mobile phase. As in Figure 22, using methanol causes the coelution of peaks 3 and 4, but by changing to acetonitrile, peaks 3 and 4 separate.

Outstanding Sample Throughput

HyPURITY ADVANCE columns are designed to provide high sample throughput. Fast analyses use less solvent and their turnaround time is shorter, therefore providing an overall decrease in the real cost per sample analysis.



Not all polar embedded phases are the same. The polar group and the attached alkyl ligand often differ and result in different elution profiles for the same analytes under the same. Using a HyPURITY ADVANCE column, the analysis time for a basic analyte mixture can be reduced by almost 50% without compromising baseline resolution and performance.







Figure 23: Similar Polar Embedded Chemistries Generate Markedly Different Results



Enhanced Peak Shape

The embedded polar group within the alkyl chain in HyPURITY ADVANCE columns inhibits secondary interactions between the analyte and any silanol groups present at the silica surface. This results in exceptional peaks shapes for a wide variety of compounds as shown in Figures 24 and 25. This polar group also inhibits water molecules from collapsing the alkyl chains and causing a loss of column performance in highly aqueous mobile phases.



Figure 24: Excellent Peak Shape for Basic Compounds



Figure 25: Excellent Peak Shape for Acidic Compounds

HyPURITY AQUASTAR Columns

Introduction

Thermo Scientific HyPURITY AQUASTAR columns are a silica-based polar end-capped C18 that is designed to offer superior retention of polar compounds and increased sensitivity in 100% aqueous conditions. The robust nature of the bonded phase makes the column is ideal for HPLC and LC/MS applications. This next generation column also offers unmatched batch reproducibility over previous polar end-capped columns.

- Increased retention of polar compounds
- Chromatographic improvements over end-capped C18
- Optimized for LC/MS applications
- Unaffected by 100% aqueous conditions
- Ideal for critical pairs analyses

Increased Polar Analyte Retention

Dispersive interactions are the primary mechanism of retention in traditional alkyl chain columns. Secondary interactions are usually associated with residual silanols and can be reduced by the use of end-capping, high purity silica and increased density of the derivatized ligand.



Figure 26: Impressive Polar Analyte Retention on a Silica-Based Column

With HyPURITY AQUASTAR columns, dispersive interactions are reduced but still play a major role in retention of both polar and non polar compounds. The additional of polar end-capping provides a controlled level of secondary interaction to give an additional mechanism by which polar compounds can be retained. This additional mechanism often will result in quite different selectivity or retention behavior over the more traditional C18 packing materials.

Chromatographic Improvements

Changing to an MS-friendly buffer allows analyses to easily transfer from UV detection to MS detection. The analysis time on HyPURITY AQUASTAR is roughly 50% of that on HyPURITY C18, allowing higher sample throughput and reduced solvent costs (Figure 27).

Compounds that are rich in nitrogen and chlorine such as pesticides, often exhibit poor peak shape on traditional C18 columns. The peak shape is dramatically improved using HyPURITY AQUASTAR and the requirement for complex gradients diminished (Figure 28).



Figure 27: Higher Productivity Achievable using a Polar End-Capped Column



Enhanced Separation Performance

Structurally similar analytes such as cephalosporin antibiotics can be separated using a simple solvent mixture on HyPURITY AQUASTAR columns as shown in Figure 29.



Figure 29: Enhanced Performance in Separation of Closely Related Analytes

Analyses in 100% Aqueous Conditions

The wetting characteristic of a media in highly aqueous conditions can be increased by the addition of polar functionality. The polar end-capping of the HyPURITY AQUASTAR phase allows it to be used for routine analysis in 100% aqueous conditions without the risk of phase collapse, and the resulting decrease in performance.



Figure 30: No Phase Collapse in 100% Aqueous Conditions

HyPURITY columns are available in other column formats. Please contact your local customer support for more details.

5 µm HyPURITY Columns

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
Hypurity C18	30	22105-034630	22105-034030	22105-033030	22105-032130	22105-031030
	50	22105-054630	22105-054030	22105-053030	22105-052130	22105-051030
	100	22105-104630	22105-104030	22105-103030	22105-102130	22105-101030
	150	22105-154630	22105-154030	22105-153030	22105-152130	22105-151030
	250	22105-254630	22105-254030	22105-253030	22105-252130	22105-251030
HyPURITY C8	50	22205-054630	22205-054030	22205-053030	22205-052130	22205-051030
	100	22205-104630	22205-104030	22205-103030	22205-102130	22205-101030
	150	22205-154630	22205-154030	22205-153030	22205-152130	22205-151030
	250	22205-254630	22205-254030	22205-253030	22205-252130	22205-251030
HyPURITY C4	50	22405-054630	22405-054030	22405-053030	22405-052130	22405-051030
	100	22405-104630	22405-104030	22405-103030	22405-102130	22405-101030
	150	22405-154630	22405-154030	22405-153030	22405-152130	22405-151030
	250	22405-254630	22405-254030	22405-253030	22405-252130	22405-251030
HyPURITY Cyano	50	22805-054630	22805-054030	22805-053030	22805-052130	22805-051030
	100	22805-104630	22805-104030	22805-103030	22805-102130	22805-101030
	150	22805-154630	22805-154030	22805-153030	22805-152130	22805-151030
	250	22805-254630	22805-254030	22805-253030	22805-252130	22805-251030
Hypurity Advance	50	21005-054630	21005-054030	21005-053030	21005-052130	21005-051030
	100	21005-104630	21005-104030	21005-103030	21005-102130	21005-101030
	150	21005-154630	21005-154030	21005-153030	21005-152130	21005-151030
	250	21005-254630	21005-254030	21005-253030	21005-252130	21005-251030
Hypurity Aquastar	50	22505-054630	22505-054030	22505-053030	22505-052130	22505-051030
	100	22505-104630	22505-104030	22505-103030	22505-102130	22505-101030
	150	22505-154630	22505-154030	22505-153030	22505-152130	22505-151030
	250	22505-254630	22505-254030	22505-253030	22505-252130	22505-251030

Other column dimensions and hardware designs are available. Please call Customer Service for more information.

5 µm HyPURITY Drop-In Guard Cartridges

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
HyPURITY C18	10	22105-014001	22105-014001	22105-013001	22105-012101	22105-011001
Hypurity C8	10	22205-014001	22205-014001	22205-013001	22205-012101	22205-011001
Hypurity C4	10	22405-014001	22405-014001	22405-013001	22405-012101	22405-011001
HyPURITY Cyano	10	22805-014001	22805-014001	22805-013001	22805-012101	22805-011001
Hypurity Advance	10	21005-014001	21005-014001	21005-013001	21005-012101	21005-011001
Hypurity Aquastar	10	22505-014001	22505-014001	22505-013001	22505-012101	22505-011001
UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder		850-00	850-00	852-00	852-00	851-00

3 µm HyPURITY Columns

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
HyPURITY C18	30	22103-034630	22103-034030	22103-033030	22103-032130	22103-031030
	50	22103-054630	22103-054030	22103-053030	22103-052130	22103-051030
	100	22103-104630	22103-104030	22103-103030	22103-102130	22103-101030
	150	22103-154630	22103-154030	22103-153030	22103-152130	22103-151030
Hypurity Advance	50	21003-054630	21003-054030	21003-053030	21003-052130	21003-051030
	100	21003-104630	21003-104030	21003-103030	21003-102130	21003-101030
	150	21003-154630	21003-154030	21003-153030	21003-152130	21003-151030
Hypurity Aquastar	30	22503-034630	22503-034030	22503-033030	22503-032130	22503-031030
	50	22503-054630	22503-054030	22503-053030	22503-052130	22503-051030
	100	22503-104630	22503-104030	22503-103030	22503-102130	22503-101030
	150	22503-154630	22503-154030	22503-153030	22503-152130	22503-151030

Other column dimensions are available. Please call customer service for more information.

3µm HyPURITY Drop-In Guard Cartridges

Description	Length (mm)	4.6 mm ID	4.0 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
HyPURITY C18	10	22103-014001	22103-014001	22103-013001	22103-012101	22103-011001
Hypurity Advance	10	21003-014001	21003-014001	21003-013001	21003-012101	21003-011001
Hypurity Aquastar	10	22503-014001	22503-014001	22503-013001	22503-012101	22503-011001
UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder		850-00	850-00	852-00	852-00	851-00

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