

**HyPURITY AQUASTAR™
Columns**

TG03-01



The New Star in the HyPURITY® Family

Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION

Introduction

A new member of the HyPURITY® family, HyPURITY AQUASTAR™ columns are a new silica-based polar end-capped C18 which is designed to offer superior retention of polar compounds and increased sensitivity in 100% aqueous conditions. The robust nature of the bonded phase results in a silica-based column that is unsurpassed for LC and LC/MS applications. This next generation column also offers unmatched batch reproducibility over first generation polar end-capped columns.

HyPURITY AQUASTAR columns combine a C18 ligand with polar end-capping to produce a highly stable material for reversed phase liquid chromatography. HyPURITY AQUASTAR columns have the unique ability to retain and separate polar analytes in 100% aqueous conditions with no phase collapse. Selectivity and sensitivity can be maintained at decreased levels of buffer concentrations, making HyPURITY AQUASTAR ideal for applications involving MS detection. This Technical Guide reviews the new HyPURITY AQUASTAR media and demonstrates its technical advantages over traditional alkyl chain packing materials.

Chromatographic Characterization

The inclusion of polar end-capped functionality within the HyPURITY AQUASTAR media results in additional interactions between the analyte and media

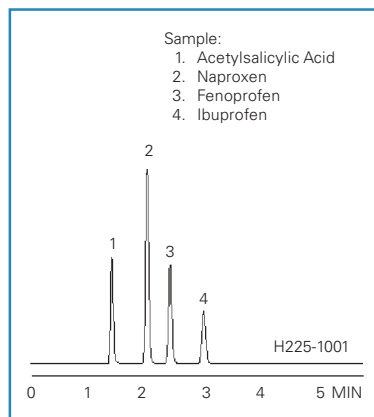
other than purely hydrophobic interactions as seen with traditional alkyl chain media. These additional interactions can give rise to different retention behavior and selectivity compared to an alkyl end-capped media. Generally, analytes with a high degree of polar character will demonstrate the greatest changes in retention and selectivity.

Increased Retention Of Polar Compounds

Polar compounds can be difficult to retain on traditional C18 columns, as they tend to elute at or very close to the void volume. HyPURITY AQUASTAR columns provide additional analyte–ligand interactions compared to traditional C18 columns and show increased retention of analytes containing polar character (figure 1).

Traditional alkyl C18 columns' primary interactions are dispersive. Secondary interactions are usually associated with residual silanols and can be reduced considerably by the use of end-capping, high purity silica and increased density of the derivatized ligand. However, these secondary interactions are in part, responsible for the retention of analytes with polar character, either by hydrogen bonding or ion exchange interactions. The elimination of secondary silanol interactions results in a column that generates good peak shape for basic analytes but shows a decrease in retention for polar compounds in general. HyPURITY AQUASTAR columns provide an unparalleled combination of traditional reversed phase and polar interactions to fully exploit the retention of polar analytes. Figure 2 demonstrates the use of HyPURITY AQUASTAR to retain and separate catecholamines without the need for ion pair reagents in a highly aqueous mobile phase.

figure 1. Analgesics



HyPURITY AQUASTAR, 5µm, 150 x 4.6mm
 Part Number 22505-154630

Eluent: 40% 20mM KH₂PO₄, pH 3 / 60% ACN
 Flow: 1.5 mL/min
 Detection: UV at 233nm
 Temperature: 25°C



Specification			
Phase	Particle Size	Pore Size (Å)	Surface Area(m ² /g)
HyPURITY AQUASTAR	3 and 5µm	190	200

Highly Aqueous Conditions

The wetting characteristic of a media in highly aqueous conditions can be increased by the addition of polar functionality. The additional polar character of the media allows HyPURITY AQUASTAR columns to be used in 100% aqueous conditions without phase collapse and therefore loss of performance.

Traditional alkyl chain C18's, in combination with 100% aqueous conditions, often show some degree of phase collapse which is identified by loss of selectivity, resolution and decreases in retention time. Such media always include at least 5 to 10% organic solvent in the mobile phase to stop these occurrences. Technical Bulletin TB99-01 explains the concepts and theory behind phase collapse and is available from Thermo Electron Corporation and it's authorized dealers. HyPURITY AQUASTAR columns also provide increased sensitivity with LC/MS methodologies. Applications using LC/MS detection often require the use of mobile phase additives to control analyte ionization and retention. However, these additives lead to ion suppression and consequently a reduction in sensitivity. The choice of column in such circumstances is critical. HyPURITY AQUASTAR columns perform exceptionally well and show excellent selectivity even without mobile phase additives.

figure 2. Catecholamines

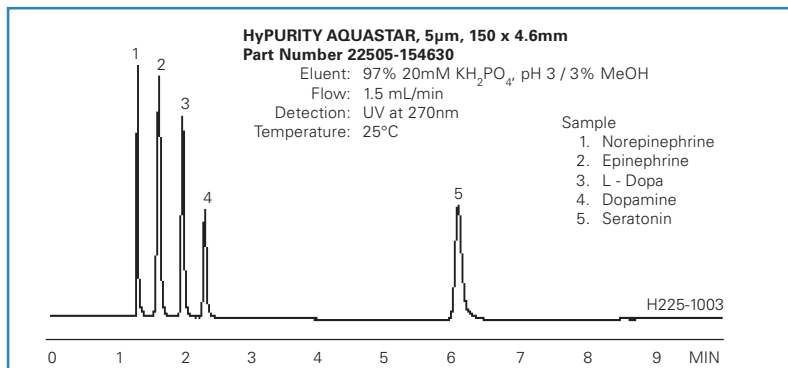
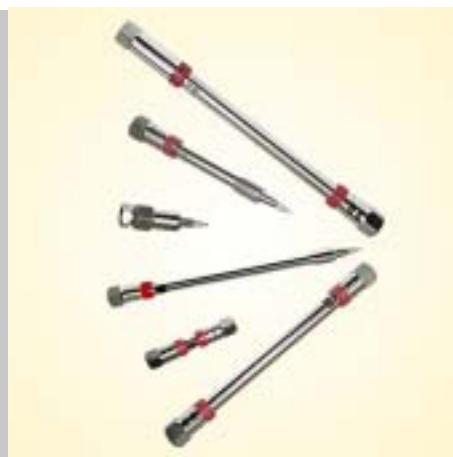
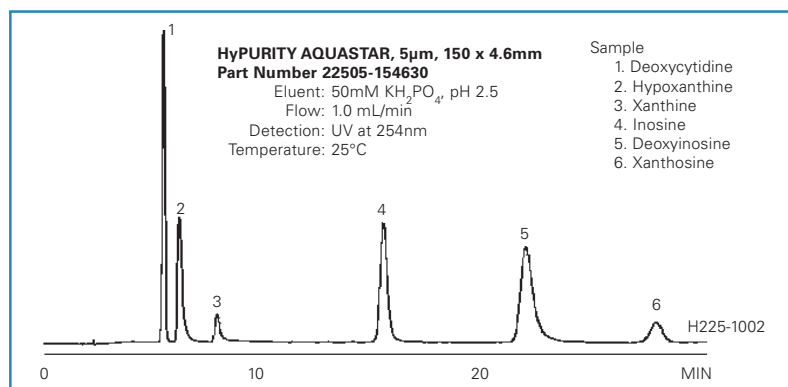


figure 3. Nucleosides And Bases



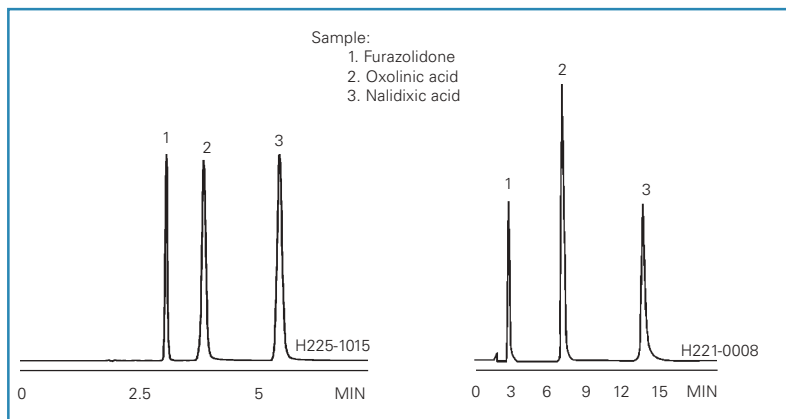
Improvements In Chromatography

Figure 4 shows the analysis of antibacterial compounds using a) HyPURITY AQUASTAR and b) HyPURITY C18 columns. The use of an MS-friendly buffer allows the peak retention times to be reduced by approximately 50% on HyPURITY AQUASTAR columns.

Each antibacterial compound is baseline resolved from its neighbor, even with the faster analysis time, making accurate identification and quantification possible. The faster analysis time means that for high volume analyses, HyPURITY AQUASTAR columns will be able to analyze more samples over a given period of time than a traditional C18 column. Also, the volume of solvent used per analysis is less with HyPURITY AQUASTAR than traditional C18 columns, so overall analysis costs will be lower.

Compounds that are rich in nitrogen and chlorine, for example pesticides, often produce poor peak shape when analyzed on traditional silica based alkyl chain packings. The ideal peak shape is Gaussian with a narrow base, but the peaks produced by these analytes are often non-symmetrical and broad based. Figure 5 demonstrates how, by using a HyPURITY AQUASTAR column in the analysis of pesticides, peak shape can be dramatically improved and fast analyses performed without the traditional use of gradient conditions.

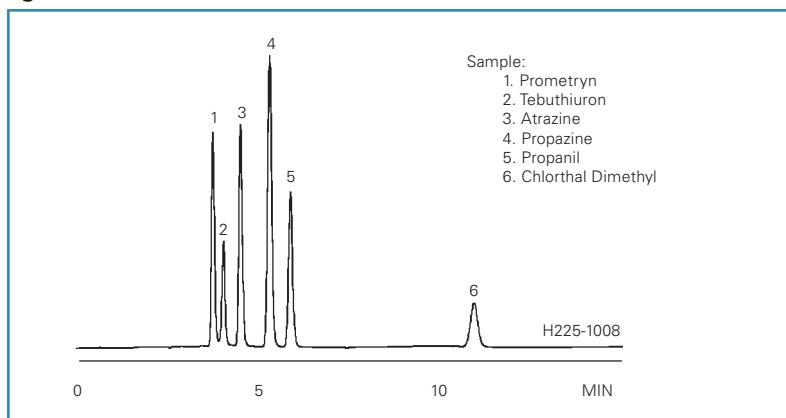
figure 4. Antibacterials



HyPURITY AQUASTAR, 5µm, 150 x 4.6mm
Part Number 22505-154630
 Eluent: 62% H₂O + 0.1% Formic Acid / 38% ACN
 Flow: 1.0 mL/min
 Detection: UV at 254nm
 Temperature: 25°C

HyPURITY C18, 5µm, 150 x 4.6mm
Part Number 22105-154630
 Eluent: 62% 0.1% H₃PO₄ / 38% MeOH
 Flow: 1.0 mL/min
 Detection: UV at 254nm
 Temperature: 25°C

figure 5. Pesticides



HyPURITY AQUASTAR, 5µm, 150 x 4.6mm
Part Number 22505-154630
 Eluent: 97% MeOH / 3% H₂O + 0.1% TFA
 Flow: 0.7 mL/min
 Detection: UV at 254nm
 Temperature: 25°C



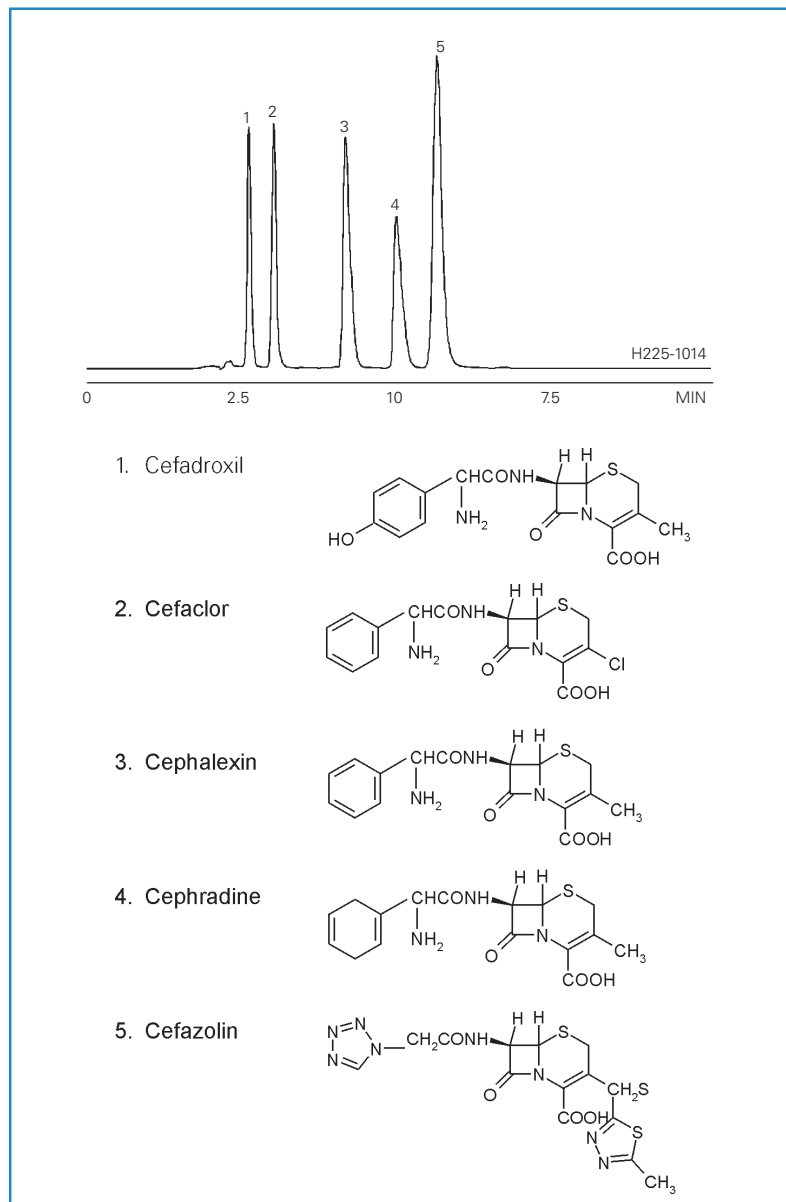
Analysis Of Closely Related Compounds

The family of cephalosporin antibiotics, primarily researched and developed by Professor Sir Edward Abraham at Oxford University, UK, is used in place of penicillin to treat bronchial, skin, and other complaints in patients who are sensitive to penicillin products. Each member of the cephalosporin family is structurally very similar, differing by perhaps as little as one functional group. The structures of the compounds analyzed in figure 6 are shown.

Such small differences make the analysis of a mixture of cephalosporin compounds very difficult using a traditional C18 column. Even under highly aqueous conditions, partial co-elution of compounds may occur.

The analysis of the five named cephalosporin antibiotics using HyPURITY AQUASTAR columns is impressive as all peaks are fully resolved using very simple solvents and show good shape. The additional selectivity shown by HyPURITY AQUASTAR columns over traditional C18's may be due in part to steric interactions of analytes with the stationary phase.

figure 6. Cephalosporin Antibiotics



HyPURITY AQUASTAR, 5 μ m, 150 x 4.6mm

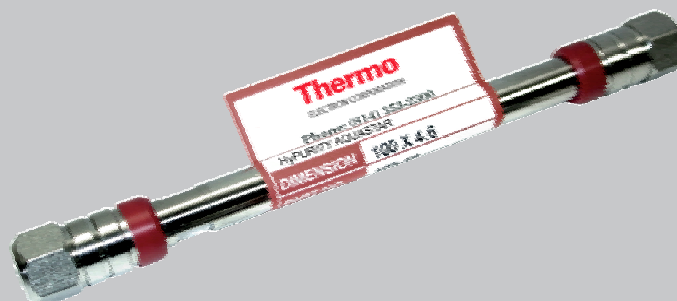
Part Number 22505-154630

Eluent: 85% 0.5% glacial acetic acid / 15% ACN

Flow: 1.0 mL/min

Detection: UV at 254nm

Temperature: 25°C

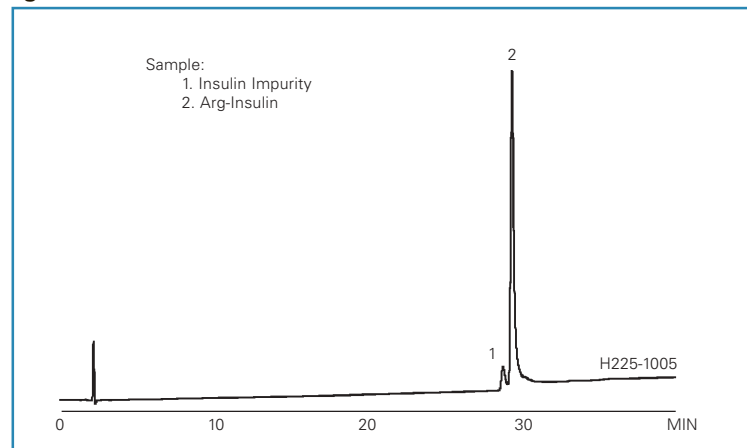


Alternative Selectivity

Although alternative selectivity, or a change in peak elution order, is not always a desired feature of a column packing material, it is something that can prove of benefit when two peaks in a chromatogram co-elute or elute very closely. Often the separation of such peaks requires that chromatographic conditions be drastically changed and/or the column chemistry modified to effect better separation.

Alternative selectivity can also prove useful in the decrease of analysis time, for instance in the analysis of insulin from pancreatic extract using pharmacopoeia methods. They require that the level of a named impurity is typically less than 5%. The analysis on a traditional C18 column elutes the impurity after the main insulin peak. Using a HyPURITY AQUASTAR column, the impurity elutes prior to the main insulin peak as illustrated in figure 7, so analysis times can be decreased and higher sample throughput achieved.

figure 7. Insulin in Pancreatic Extract

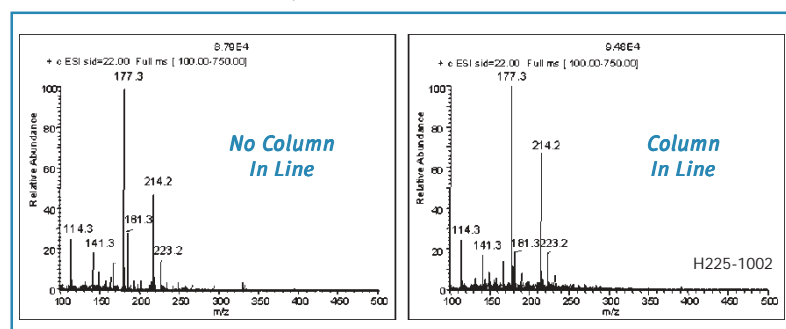


HyPURITY AQUASTAR, 5µm, 150 x 4.6mm
 Part Number 22505-154630
 Eluent A: 20mM KH₂HO₄ pH3
 Eluent B: ACN
 Gradient: 15 to 40% B over 45 minutes
 Flow: 1.0 mL/min
 Detection: UV at 210nm
 Temperature: 25°C

Use With LC/MS Detectors

Modified phases, such as those with polar embedded functionality, are unsuitable for use with LC/MS applications, as the phase tends to "bleed". Spurious trace peaks are produced by ligands or partial ligands which wash off the column during use (type and density are determined by the analysis conditions used). However, HyPURITY AQUASTAR is a polar end-capped media. Tests have shown that HyPURITY AQUASTAR shows no evidence of phase bleed under normal conditions.

figure 8. Phase Bleed Study



HyPURITY AQUASTAR, 5µm, 150 x 4.6mm
 Part Number 22505-154630
 Eluent: MeOH / H₂O
 Gradient: 5 to 100% H₂O over 5 minutes
 Flow: 0.7 mL/min
 Detection: +ve ESI full MS



HyPURITY AQUASTAR™ Standard Columns

Description	Particle Size (µm)	Length (mm)	Standard Bore (4.6mm)	Standard Bore (4.0mm)	Small Bore (3.0mm)	Small Bore (2.1mm)	Microbore (1.0mm)
HyPURITY AQUASTAR	3	30	22503-034630	22503-034030	22503-033030	22503-032130	22503-031030
	3	50	22503-054630	22503-054030	22503-053030	22503-052130	22503-051030
	3	100	22503-104630	22503-104030	22503-103030	22503-102130	22503-101030
	3	125	22503-124630	22503-124030	22503-123030	22503-122130	22503-121030
	3	150	22503-154630	22503-154030	22503-153030	22503-152130	22503-151030
	3	200	22503-204630	22503-204030	22503-203030	22503-202130	22503-201030
	3	250	22503-254630	22503-254030	22503-253030	22503-252130	22503-251030
HyPURITY AQUASTAR	5	30	22505-034630	22505-034030	22505-033030	22505-032130	22505-031030
	5	50	22505-054630	22505-054030	22505-053030	22505-052130	22505-051030
	5	100	22505-104630	22505-104030	22505-103030	22505-102130	22505-101030
	5	125	22505-124630	22505-124030	22505-123030	22505-122130	22505-121030
	5	150	22505-154630	22505-154030	22505-153030	22505-152130	22505-151030
	5	200	22505-204630	22505-204030	22505-203030	22505-202130	22505-201030
	5	250	22505-254630	22505-254030	22505-253030	22505-252130	22505-251030

Drop-In Guard Cartridges for UNIGUARD® Holder (pk/4)

Note : 4.0mm Drop-Ins are used for both 4.0 and 4.6mm analytical columns

Description	Particle Size (µm)	Length (mm)	Standard Bore (4.6mm)	Standard Bore (4.0mm)	Small Bore (3.0mm)	Small Bore (2.1mm)	Microbore (1.0mm)
HyPURITY AQUASTAR	3	10	22503-014001	22503-014001	22503-013001	22503-012101	22503-011001
	5	10	22505-014001	22505-014001	22505-013001	22505-012101	22505-011001
UNIGUARD Direct-Connect Drop-In Guard Cartridge Holder		10	850-00	850-00	852-00	852-00	851-00

KAPPA® Capillary Columns

Description	Particle Size (µm)	Length (mm)	500µm ID	320µm ID	180µm ID	100µm ID
HyPURITY AQUASTAR	3	50	22503-050565	22503-050365	22503-050265	-
	3	100	22503-100565	22503-100365	22503-100265	-
	3	150	22503-150565	22503-150365	22503-150265	-
	3	250	22503-250565	22503-250365	22503-250265	-
KAPPA Guard	3	30	22503-030515	22503-030315	22503-030215	-
HyPURITY AQUASTAR	5	50	22505-050565	22505-050365	22505-050265	22505-050165
	5	100	22505-100565	22505-100365	22505-100265	22505-100165
	5	150	22505-150565	22505-150365	22505-150265	22505-150165
	5	250	22505-250565	22505-250365	22505-250265	22505-250165
KAPPA Guard	5	30	22505-030515	22505-030315	22505-030215	22505-030115

DASH™ Columns

Description	Particle Size (µm)	Dimension (mm)	Pack of 1	Pack of 3	Pack of 10
HyPURITY AQUASTAR	3	20 x 2.1	22503-022150	22503-022151	22503-022152
	5	20 x 2.1	22505-022150	22505-022151	22505-022152

The Next Step
in HPLC Columns



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