RESTEK pharmaceutical

Applications note

The Ultra IBD Column Allows HPLC Separation of Polar and Non-Polar Analytes from the Same Sample

The ability to retain and separate polar and non-polar analytes in the same sample can be difficult in high performance liquid chromatography (HPLC) analyses. Restek's Intrinsically Base Deactivated (IBD) Ultra IBD column can provide the retention needed to simultaneously separate polar and non-polar analytes to more expediently perform analyses within a single method. The Ultra IBD phase is composed of an alkyl chain and a non-ionic functional group. The alkyl chain ensures retention of more hydrophobic analytes, and the intrinsic base deactivation of the functional group reduces tailing often seen with basic analytes. The nature of the functional group also allows retention of charged analytes. The Ultra IBD column is the best choice for separating mixtures of acids, bases, zwitterions, and neutral analytes.

Ultra IBD Column Retains Analytes Across a Wide Range of pH and Mobile Phase Compositions

The base deactivation and polar interaction capability of the Ultra IBD phase remains steady across the pH stability range of the silica. Therefore, the polar retentive capability of the phase is not decreased as the pH is decreased; the retention is stable for a given buffer concentration and aqueous/organic ratio.

Using 100% water can cause chain collapse and loss of retention in standard C18 phases, thereby requiring at least 5% organic in the mobile phase. Because the Ultra IBD phase is immune to stationary phase collapse reverse phase gradients starting with 100% water can be used for maximum retention of analytes. Unlike standard alkyl phases, the Restek Ultra IBD phase results in a rugged HPLC column that can withstand these separation extremes.

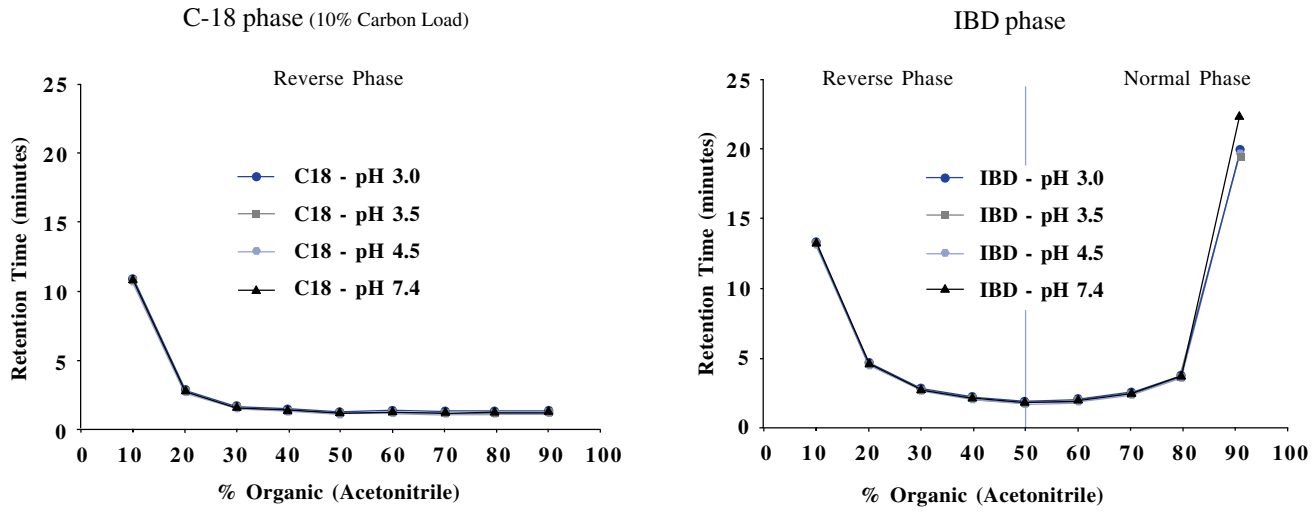
We compared the separation ability of the Ultra IBD column to that of a C18 column using varying pH and mobile phase ratios (Figure 1). Cephaloridine was analyzed because it contains both polar and non-polar (hydrophobic) functional groups. The data demonstrates the ability of the phase to retain the analyte under a wide range of mobile phase conditions.

The comparison revealed that the C18 column cannot successfully retain cephaloridine when acetonitrile exceeds 40% in the mobile phase. Upon reaching 40% acetonitrile, cephaloridine elutes with the solvent front. The C18 column is only capable of hydrophobic interaction by a reverse phase mechanism, and so cannot exhibit the characteristic U-shaped retention profile shown by the Ultra IBD column.

Figure 1

The Ultra IBD column retains analytes by hydrophobic and polar interactions, whereas the C18 column is only capable of retention by hydrophobic interaction

Cephalordine Retention vs % Organic - Aqueous Portion is Ammonium Formate (20 mM)



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By contrast, the Ultra IBD column reaches a minimum retention time at 50% acetonitrile, closely duplicating the hydrophobic interactions of the C18 column. As the organic ratio increases above 50%, polar interactions in the normal phase mode allow retention of the cephaloridine analyte. The retention of cephaloridine reaches a usable maximum at 90% acetonitrile. Furthermore, the U-shaped retention profile is not adversely altered by the pH of the buffer. The separation is a near mirror image across the reverse phase and normal phase modes. Only at the extreme of 90% acetonitrile is a small variation seen in analyte retention due to pH changes. Even then, the variation is less than 2.8 minutes at pH 7.4. The normal phase retention is greater than the reverse phase retention. Both modes, however, provide similar peak shape and retention time (Figure 2).

Note: As with any HPLC column phase capable of performing a reverse or normal phase separation, the user should take care to ensure the retention mechanism is either reverse or normal phase.

Ultra IBD Column Solvent Focusing Ability Improves Detection

Loading multiple samples onto the head of the column concentrates the analyte to improve detection. The injections are then eluted through the system using a decreased solvent level ratio. This ability to load and maintain a sample at the column head is called solvent focusing.*

When cephaloridine was injected with a mobile phase of either 100% acetonitrile or 100% buffer, no peak eluted from the system—even after 90 minutes. To elute the analyte from the system, the mobile phase ratio was altered to 80%. The peak then eluted near its isocratic run time. Cephaloridine remained intact near the start of the column and did not begin appreciably eluting through the column until another mobile phase ratio was selected.

Due to its solvent focusing capabilities, an Ultra IBD column can be loaded at either the 100% aqueous (reverse phase) or 100% organic (normal phase) solvent level to concentrate the sample directly onto the head of the column.

Furthermore, this solvent focusing capability allows use of a gradient system to better remove impurities from a sample mixture that have adsorbed onto the column. More hydrophilic impurities theoretically could be eluted from the analytes of interest with a simple aqueous flush. The process could be reversed using 100% organic for truly neutral analytes.

* The ability to perform solvent focusing will be affected by the chemical nature of the analyte.

Conclusion

The unique functionality of the Ultra IBD column creates an ideal tool for the separation and detection of acids, bases, and zwitterions within complex mixtures. Because the phase interactions are bimodal, a wide range of compounds can be retained and separated by either reverse or normal phase mechanics. At the extreme range of the reverse or normal modes, the solvent focusing ability of the Ultra IBD column can be used to concentrate an analyte or to purify analytes within complex matrices.

See back for product listing.

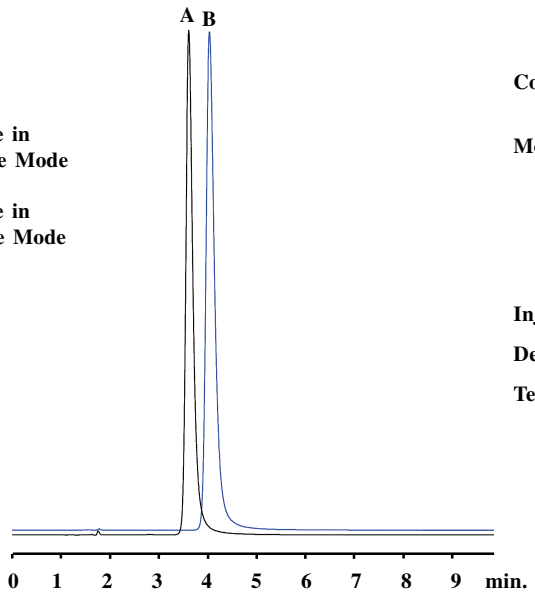
Figure 2

Both normal and reverse phase modes provide good peak shape and retention time on Ultra IBD column.

Peak List:

A. Cephaloridine in Reverse Phase Mode

B. Cephaloridine in Normal Phase Mode



Column:	Ultra IBD, 5µm, 4.6mm x 150mm, 100 Å (cat.# 9175565)
Mobile Phase:	
Reverse Phase	20mM Ammonium Acetate, pH 4.0:Acetonitrile (80:20)
Normal Phase:	20mM Ammonium Acetate, pH 4.0:Acetonitrile (20:80)
Inj.:	5uL, 1.0mg/mL Cephaloridine
Det.:	254nm
Temp.:	27°C

Application notes: LC-0101 & LC-0102

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Trident™ Integral HPLC Guard Column System Offers Maximum Protection Against Contaminants and Particulate Matter

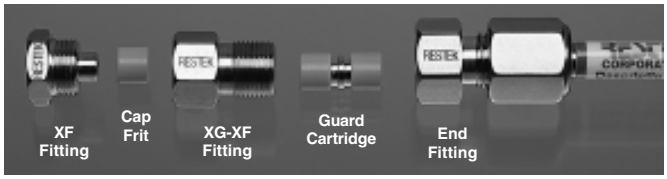
High backpressure is one of the most common problems encountered when performing HPLC analysis. Normal column backpressure is observed after a new column has been installed and equilibrated with the mobile phase. Unfortunately, this pressure will often increase with use because of particulates collecting on the column inlet frit.

The source of these particles can be from sample impurities, mobile phase contaminants, and the injector or autosampler rotor seal. The presence of particles can result in increased backpressure, split peaks, tailing, and eventually over-pressure shut-down. In some circumstances, these problems can be corrected by back-flushing the column. However, in many cases it results in an unusable column.

To reduce backpressure problems, all samples and mobile phase solvents must be filtered before use; and rotor seals should be changed on a routine basis. Along with these preventative measures, it is advisable to use column prefilters such as the Trident™ column protection system. When using a prefilter, particles build up on its inexpensive, replaceable frit, instead of the permanent frit at the head of the column.

The system's foundation consists of the analytical column configured with our exclusive Trident™ end fitting and XF fitting. This configuration contains the standard internal frit as well as a replaceable cap frit, which can be easily changed without disturbing the packed bed. Changing the external frit can reverse the effects of accumulated particles, such as high backpressure or peak distortion. To obtain this basic configuration, simply order any Restek HPLC column that has a (-700) suffix catalog number.

For maximum protection against contaminants and particulate matter, the system can be configured with both an integral guard cartridge and a replaceable external frit. To obtain this configuration, simply order any Restek HPLC column that has a (-700) suffix catalog number, the XG-XF male fitting (cat.# 25026), and the appropriate pack of guard cartridges (see right).



Trident™ HPLC Guard Column Fittings and Frits

Description	cat.#
XG-XF Fitting for 1cm Guard Cartridge	25026
Replacement XF Filter Fitting	25024
Replacement Cap Frits, 2µm	25022
Replacement Cap Frits, 0.5µm	25023

Trident™ HPLC Guard Column Cartridges

	(10 x 2.1mm)	(10 x 4.0mm)
Guard Cartridge	**cat.#**	**cat.#**
Allure™ Acidix	916250212	916250210
Allure™ Basix	916150212	916150210
Allure™ C18	916450212	916450210
Allure™ Silica	916050212	916050210
Ultra Amino	910750212	910750210
Ultra C1	910150212	910150210
Ultra C4	910250212	910250210
Ultra C8	910350212	910350210
Ultra C18	917450212	917450210
Ultra Cyano	910650212	910650210
Ultra IBD	917550212	917550210
Ultra Phenyl	910550212	910550210
Ultra Silica	910050212	910050210

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Product Listing

Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365
200mm length	9175321	9175322	9175323	9175325

Ultra IBD, 3µm Columns with Trident™ Inlet

Particle Size: 3µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9175332-700	9175333-700	9175335-700
50mm length	—	9175352-700	9175353-700	9175355-700
100mm length	—	9175312-700	9175313-700	9175315-700
150mm length	—	9175362-700	9175363-700	9175365-700
200mm length	—	9175322-700	9175323-700	9175325-700

Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

Ultra IBD, 5µm Columns with Trident™ Inlet

Particle Size: 5µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9175532-700	9175533-700	9175535-700
50mm length	—	9175552-700	9175553-700	9175555-700
100mm length	—	9175512-700	9175513-700	9175515-700
150mm length	—	9175562-700	9175563-700	9175565-700
200mm length	—	9175522-700	9175523-700	9175525-700
250mm length	—	9175572-700	9175573-700	9175575-700

Ultra IBD Guard Cartridges

Dimensions	cat.#	Qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

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