

## Analyzing Cardiac Medications by HPLC

In the United States, cardiovascular disease is the leading cause of death. In an effort to reduce mortality from heart disease, several classes of medications are used to decrease high blood pressure, control arrhythmias (abnormal heart rhythms), and treat congestive heart failure. Many of these cardiac medications include beta antagonists, ACE inhibitors, diuretics, or calcium channel blockers.

High performance liquid chromatography (HPLC) is the preferred technique to analyze many of the compounds used in these medications. To maximize the effectiveness of the separation, a chromatographer should choose the column and conditions that best allow amplification of structural differences between matrix components, related compounds, and analytes. Proper HPLC column selection is dictated by the analyte and the sample matrix. In fact, selecting the appropriate analytical column is critical when analyzing cardiac medications because many of them contain basic

compounds, which tend to tail badly on poorly deactivated HPLC phases. Restek's fully end-capped Allure™ Basix, Ultra IBD (intrinsically base deactivated), and Ultra Cyano phases can use the basic nature of these compounds to achieve a separation that will not suffer from the problems normally resulting in peak tailing.

### Angiotensin Converting Enzyme (ACE) Inhibitors

Ancient Egyptians used the ACE inhibitor, digoxin, as a poison. Ancient Romans used it as a wound dressing and heart stimulant. It is extracted primarily from the poisonous foxglove plant in a concentration of up to 0.4% by mass. A commercial digoxin standard claiming 100% purity is shown to be impure when the analysis is performed using the Ultra IBD column. The alternate selectivity of this phase to alkyl stationary phases results in the separation of two unknown impurity peaks in the digoxin standard (Figure 1).

Figure 1

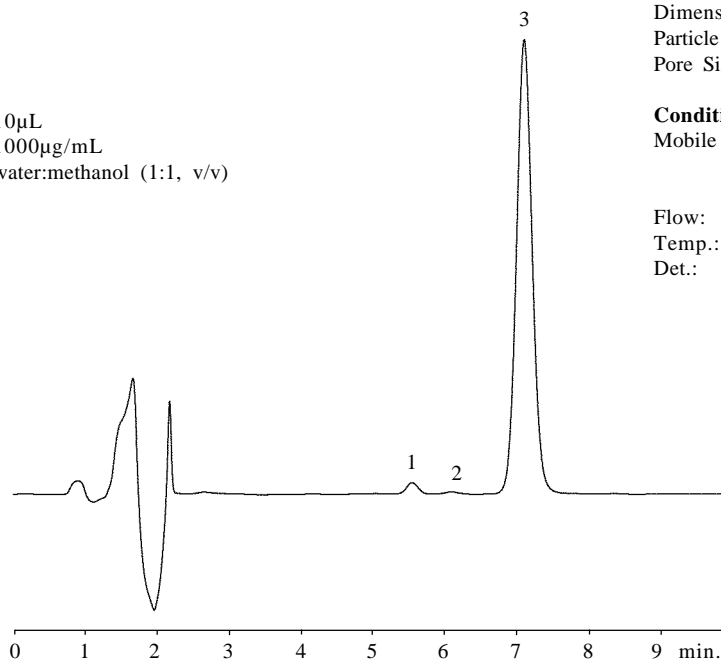
*Ultra IBD column provides alternate selectivity, which separates impurities in a digoxin standard.*

- Peak List:**  
 1. unknown  
 2. unknown  
 3. digoxin

**Sample:**  
 Inj.: 10µL  
 Conc.: 1000µg/mL  
 Solvent: water:methanol (1:1, v/v)

**Column:** Ultra IBD  
 Catalog #: 9175565  
 Dimensions: 150 x 4.6mm  
 Particle Size: 5µm  
 Pore Size: 100Å

**Conditions:**  
 Mobile phase: water with 0.1% (v/v) acetic acid:acetonitrile (65:35, v/v)  
 Flow: 1.0mL/min.  
 Temp.: 27°C  
 Det.: UV @ 220nm



LC\_0068

Enalapril maleate, another common ACE inhibitor, can be separated by polar interaction using the Allure™ Basix phase. The Allure™ Basix column is able to interact with the basic amide and amine of enalapril to provide retention by a normal phase mechanism (Figure 2).

### Calcium Channel Blockers

Verapamil, diltiazem, nifedipine, and nifedipine are a group of calcium channel blockers used to treat high blood

pressure, angina (chest pain), and/or some arrhythmias. These four compounds all contain a basic amine group. Additionally, nifedipine and nicardipine contain more basic nitrophenol and pyridine functional groups. Figure 3 demonstrates how basic functional groups can be used to affect retention and separation of these compounds using the Ultra Cyano column. Also, the Allure™ Basix Column, in the reverse phase mode, easily retains verapamil (Figure 4).

**Figure 2**

*The Allure™ Basix column provides retention by a normal phase mechanism.*

**Peak List:**

1. maleate salt
2. enalapril

**Sample:**

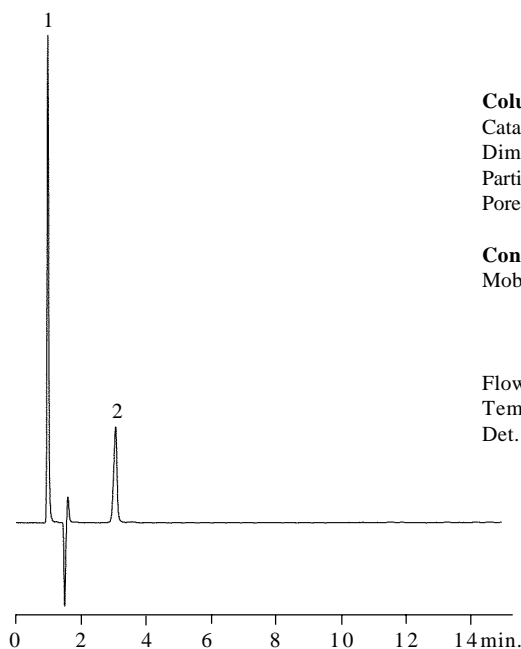
Inj.: 2.5µL  
 Conc.: 1.1mg/mL  
 Solvent: methanol:water  
 (30:70)

**Column: Allure™ Basix**

Catalog #: 9161565  
 Dimensions: 150 x 4.6mm  
 Particle Size: 5µm  
 Pore Size: 60Å

**Conditions:**

Mobile phase: 10mM ammonium formate, pH 2.5: acetonitrile (20:80, v/v)  
 Flow: 1.2mL/min.  
 Temp.: 25°C  
 Det.: UV @ 225nm



LC\_0091

**Figure 3**

*The Ultra Cyano column separates four of the most common calcium channel blockers.*

**Peak List:**

1. diltiazem
2. nifedipine impurity
3. verapamil
4. nifedipine
5. nicardipine

**Sample:**

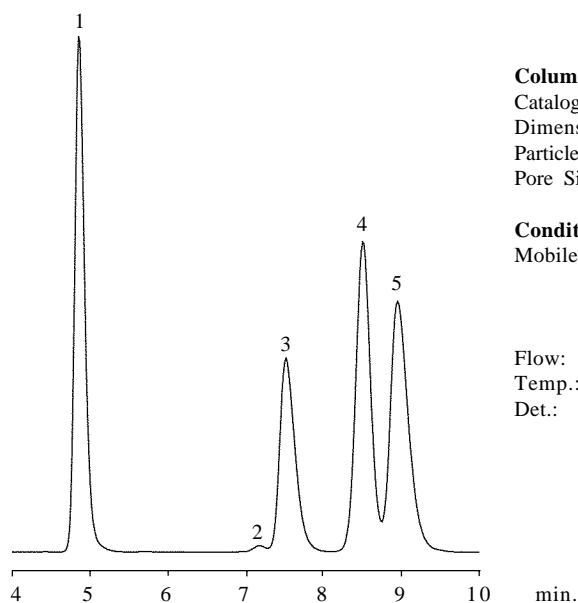
Inj.: 5µL  
 Conc.: 100µg/mL  
 Solvent: acetonitrile:water  
 (1:1)

**Column: Ultra Cyano**

Catalog #: 9106565  
 Dimensions: 150 x 4.6mm  
 Particle Size: 5µm  
 Pore Size: 100Å

**Conditions:**

Mobile phase: 20mM potassium phosphate monobasic, pH 3.0:acetonitrile (70:30, v/v)  
 Flow: 1.2mL/min.  
 Temp.: 30°C (± 1°C)  
 Det.: 235nm



LC\_0062

## Beta Antagonists

Two of the more common beta antagonists are atenolol and metoprolol. The analytical techniques cited in some compendia methods for these compounds use a C18 phase with ion pairing agents. A simpler approach makes use of the nitrogen atom on these compounds as a key mechanism for separation. However, the basic amine groups allow analysis of these compounds using normal phase separation with the Allure™ Basix column.

Because metoprolol is more lipid-soluble than atenolol, it is more hydrophobic. Therefore, an increase in the organic composition of the mobile phase actually will enhance the retention of metoprolol with the Allure™ Basix phase. The Allure™ Basix column performs separation of these components, provides alternate selectivity to alkyl stationary phases, and reveals an impurity in the metoprolol (Figures 5 and 6).

Figure 4

*The Allure™ Basix column offers alternative selectivity for verapamil in the reverse phase mode.*

**Peak List:**

1. toluene (marker)
2. verapamil HCL

**Sample:**

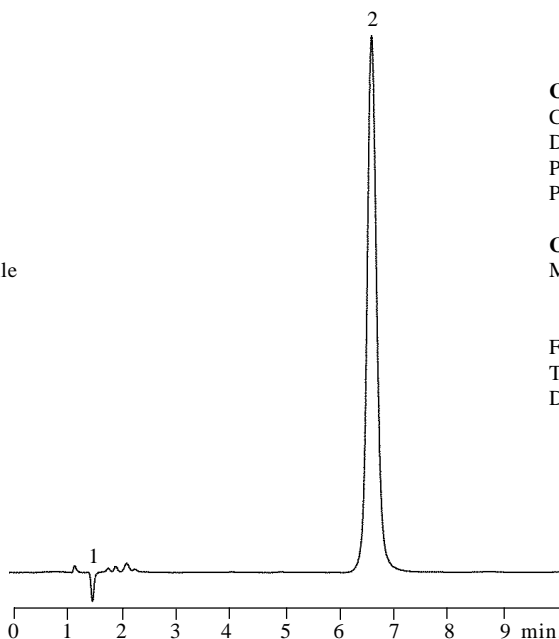
Inj.: 1 µL  
Conc.: 1 mg/mL  
Solvent: water:acetonitrile  
(70:30, v/v)

**Column:** Allure™ Basix

Catalog #: 9161565  
Dimensions: 150 x 4.6mm  
Particle Size: 5 µm  
Pore Size: 60 Å

**Conditions:**

Mobile phase: 20mM ammonium acetate pH 4.5:  
acetonitrile (65:35, v/v)  
Flow: 1.2 mL/min.  
Temp.: 25.0°C  
Det.: UV @ 230nm



LC\_0077

Figure 5

*The Allure™ Basix column separates metoprolol and an impurity in 10 minutes.*

**Peak List:**

1. unknown
2. metoprolol

**Sample:**

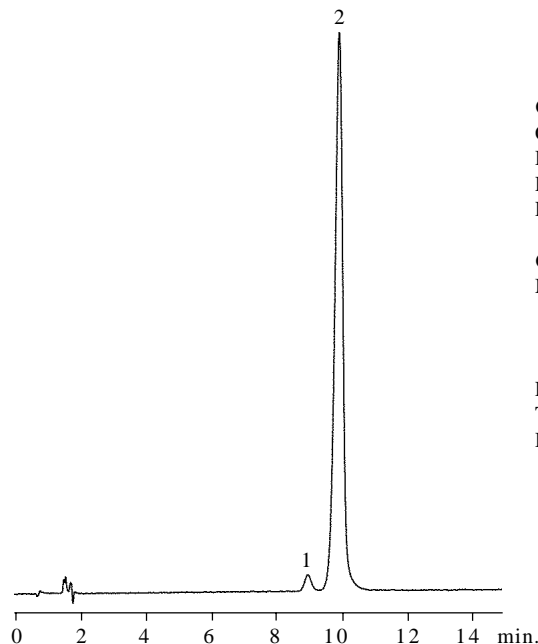
Inj.: 2.5 µL  
Conc.: 1.5 mg/mL  
Solvent: water:methanol  
(70:30)

**Column:** Allure™ Basix

Catalog #: 9161565  
Dimensions: 150 x 4.6mm  
Particle Size: 5 µm  
Pore Size: 60 Å

**Conditions:**

Mobile Phase: 10mM ammonium formate, pH 2.5:  
acetonitrile  
(10:90, v/v)  
Flow: 1.2 mL/min.  
Temp.: 25°C  
Det.: UV @ 225nm



LC\_0090

## Diuretics

Another important class of cardiac and high blood pressure medications are diuretics. These compounds rid the body of excess fluids and salt (sodium). Diuretics such as furosemide are used for the management of edema associated with chronic heart problems. The furosemide molecule contains carboxylic acid and basic sulfa-amine groups. The zwitterionic nature of furosemide makes it an ideal candidate for analysis using an Ultra IBD column (Figure 7).

Enlargement of the baseline reveals that the furosemide standard is not a pure substance (Figure 7, inset). The impurities may possibly be a reason why the therapeutic mechanism of furosemide is not completely understood.

The diuretic admixture of triamterene and hydrochlorothiazide (HCTZ) is used to remove excess fluid while attempting to limit the amount of potassium displaced from the body. The highly-charged HCTZ contains numerous amine and sulfonic groups. The high charge is the reason it elutes in

Figure 6

The Allure™ Basix column retains atenolol without ion pairing agents.

**Peak List:**

1. uracil (marker)
2. atenolol

**Sample:**

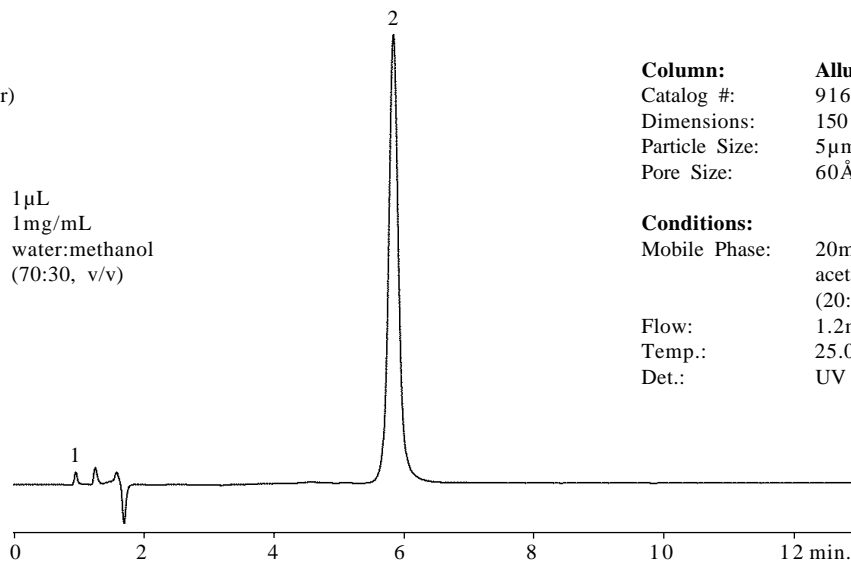
Inj.: 1 µL  
Conc.: 1 mg/mL  
Solvent: water:methanol  
(70:30, v/v)

**Column:** Allure™ Basix

Catalog #: 9161565  
Dimensions: 150 x 4.6mm  
Particle Size: 5 µm  
Pore Size: 60 Å

**Conditions:**

Mobile Phase: 20mM ammonium acetate pH 4.5:acetonitrile (20:80, v/v)  
Flow: 1.2mL/min.  
Temp.: 25.0°C  
Det.: UV @ 225nm



LC\_0072

Figure 7

The Ultra IBD column separates impurities in furosemide.

**Peak List:**

1. uracil (marker)
2. furosemide

**Sample:**

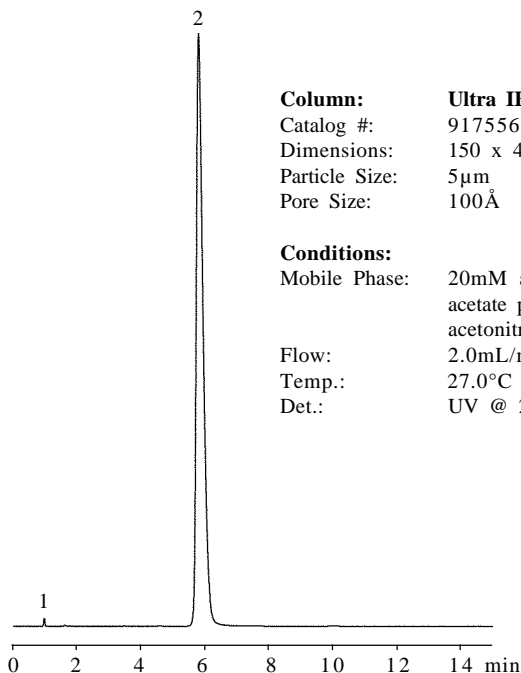
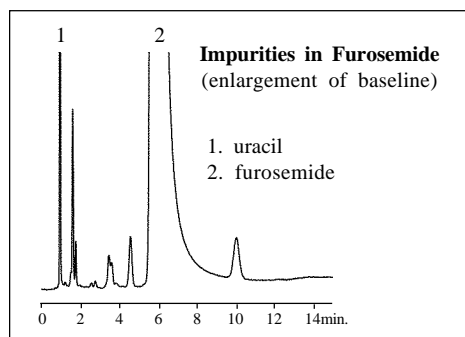
Inj.: 5 µL  
Conc.: 1000 µg/mL  
Solvent: acetonitrile:water  
(60:40, v/v)

**Column:** Ultra IBD

Catalog #: 9175565  
Dimensions: 150 x 4.6mm  
Particle Size: 5 µm  
Pore Size: 100 Å

**Conditions:**

Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (70:30, v/v)  
Flow: 2.0mL/min.  
Temp.: 27.0°C  
Det.: UV @ 280nm



LC\_0071

less than 3 minutes on the Allure™ Basix in reverse phase mode (Figure 8). The retention of HCTZ would increase if the organic modifier exceeded 50%, but solubility may become a concern. Triamterene is less charged than HCTZ. The amides present in the triamterene pteridine structures and the amino groups make this molecule fairly polar. Under the mobile phase conditions shown in Figure 8, triamterene is easily resolved from the HCTZ.

### Summary

The Allure™ Basix, Ultra CN, and Ultra IBD columns can be used to achieve an alternate and effective selectivity to alkyl stationary phases for many types of cardiac medications.

Because all three of these phases display a characteristic U-shaped profile for retention versus percentage of organic solvent in mobile phase, the mobile phase content can be modified to use either the normal or reverse phase mode if solubility problems arise. The selectivity of the Allure™ Basix and Ultra CN phases are based upon non-ionic polar interactions from basic functional groups as well as hydrophobic interaction. Basic molecules—especially those containing electron-deficient nitrogen complexes—can be retained readily by a normal phase mechanism. The selectivity for the IBD phase can be adjusted for acids, bases, zwitterionic, or neutral molecules.

**Figure 8**

*The Allure™ Basix column easily resolves the diuretic admixture of triamterene and hydrochlorothiazide (HCTZ)*

**Peak List:**

1. hydrochlorothiazide
2. triamterene

**Sample:**

Inj.: 1 µL  
 Conc.: 500 µg/mL  
 Solvent: methanol:water:  
 tetrahydrofuran  
 (30:35:35)

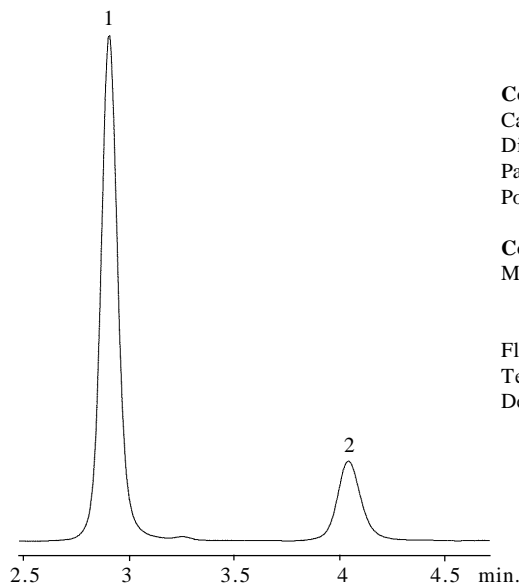
**Column:**

**Allure™ Basix**

Catalog #: 9161565  
 Dimensions: 150 x 4.6mm  
 Particle Size: 5 µm  
 Pore Size: 60 Å

**Conditions:**

Mobile Phase: 20mM ammonium  
 acetate, pH 4.5:  
 acetonitrile (65:35, v/v)  
 Flow: 1.2mL/min  
 Temp.: 25°C  
 Det.: UV @ 225nm



LC\_0086

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**Application Notes:**

- (#59511) Improved HPLC Analysis of Analgesics
- (#59512) The Ultra IBD Column Allows HPLC Separation of Polar and Non-Polar Analytes from the Same Sample
- (#59510) HPLC Stationary Phase Selection for the Analysis of Steroids
- (#59118) Allure™ PFP Propyl HPLC Column Provides Improved LC/MS Analyses of Basic Compounds

**Fast Facts:**

- (#59728) HPLC Mobile Phase Accessories
- (#59896) Trident™ Integral HPLC Guard Column System
- (#59302) HPLC and LC/MS Column Kits
- (#59303) Allure™ Acidix HPLC Columns
- (#59314) Trident™ Direct Guard Column System
- (#59614A) Ultra IBD HPLC Columns

## ■ Allure™ Basix, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

## ■ Ultra Cyano, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106331	9106332	9106333	9106335
50mm length	9106351	9106352	9106353	9106355
100mm length	9106311	9106312	9106313	9106315

## ■ Ultra Cyano, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106531	9106532	9106533	9106535
50mm length	9106551	9106552	9106553	9106555
100mm length	9106511	9106512	9106513	9106515
150mm length	9106561	9106562	9106563	9106565
200mm length	9106521	9106522	9106523	9106525
250mm length	9106571	9106572	9106573	9106575

## ■ 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

## ■ 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

