pharmaceutical

# Applications note

## 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).

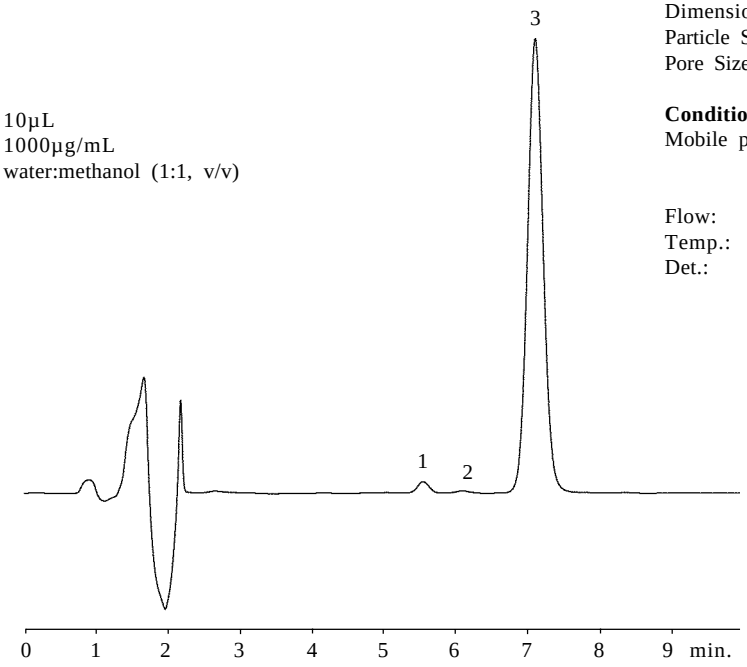
**Figure 1**

*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

pharmaceutical

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

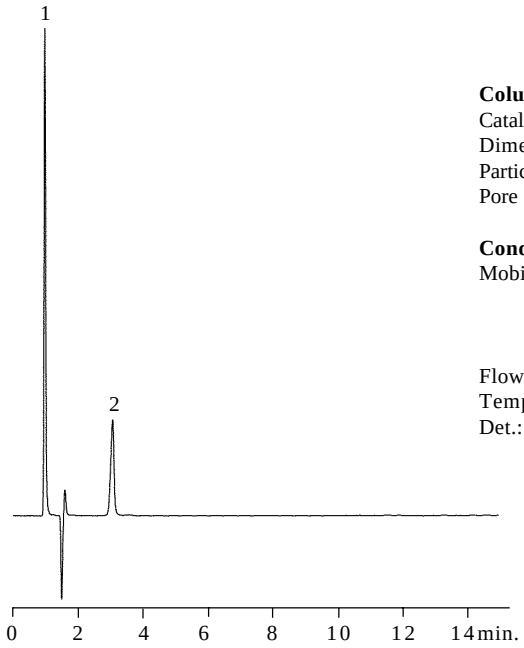
Verapamil, diltiazem, nicardipine, and nifedipine are a group of calcium channel blockers used to treat high blood pressure, angina (chest pain), and/or some arrhythmias. These four compounds all contain a basic amine group. Additionally, nifedipine and nicardipine contain more basic nitrophenol and 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

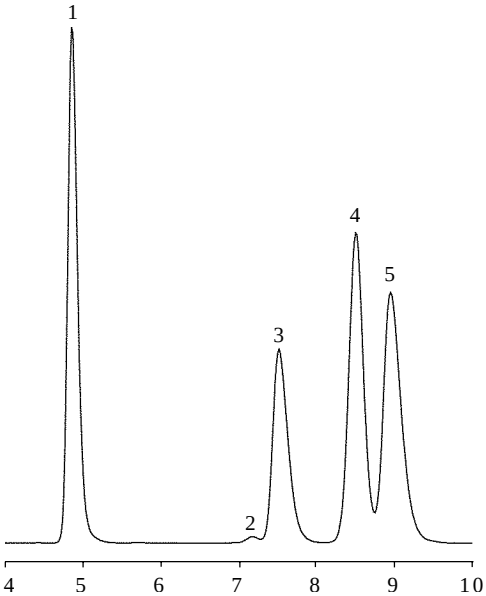
LC_0091

### 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

## 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 mechinism for separation. However, the basic amine groups allow analysis of these compounds using normal phase separation with the Allure™ Basix column.

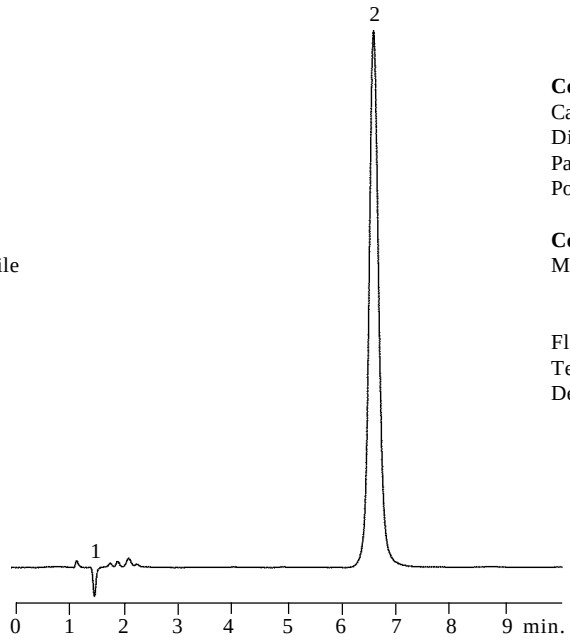
Because metoprolol is more lipid-soluble than atenolol, it is more hydrophobic. Therefore, an increase in the organic composition of the mobile phase actually will enhance the retention of metoprolol with the Allure™ Basix phase. The Allure™ Basix column performs separation of these components, provides 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.: 1µL
Conc.: 1mg/mL
Solvent: water:acetonitrile (70:30, v/v)




**Column:** **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

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

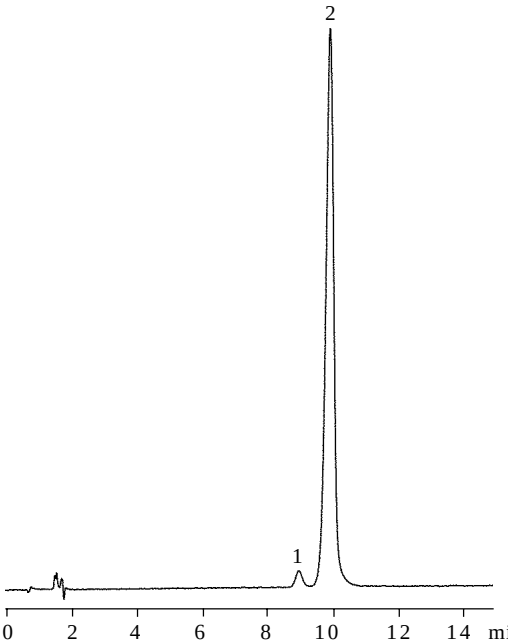
LC_0077

### Figure 5

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

**Peak List:**
1. unknown
2. metoprolol

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




**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 (10:90, v/v)
Flow: 1.2mL/min.
Temp.: 25°C
Det.: UV @ 225nm

LC_0090

## Diuretics

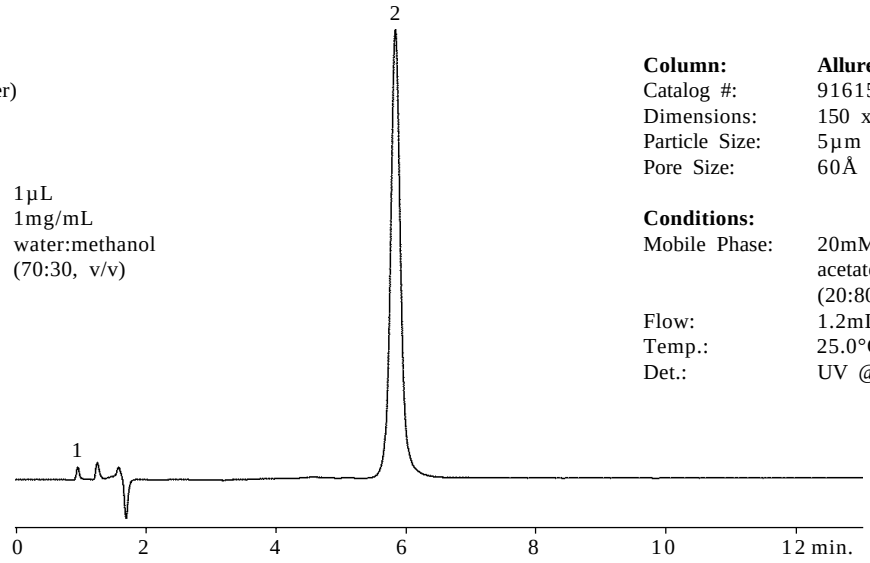
Another important class of cardiac and high blood pressure medications are diuretics. These compounds rid the body of excess fluids and salt (sodium). Diuretics such as furosemide are used for the management of edema associated with chronic heart problems. The furosemide molecule contains carboxylic 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.: 1µL
Conc.: 1mg/mL
Solvent: water:methanol (70:30, v/v)

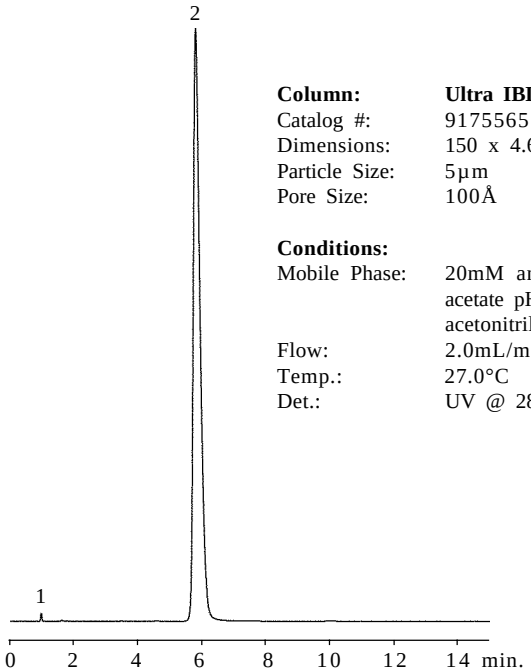
**Column:** **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

**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

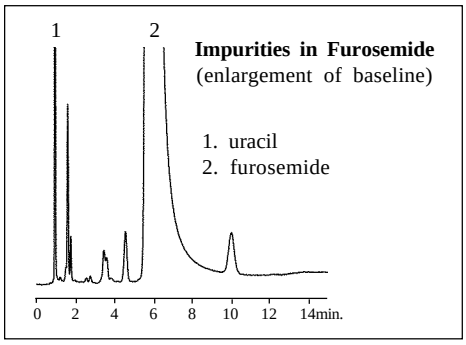
**Figure 7**

*The Ultra IBD column separates impurities in furosemide.*




**Peak List:**
1. uracil (marker)
2. furosemide

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




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

**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 triamerene 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.

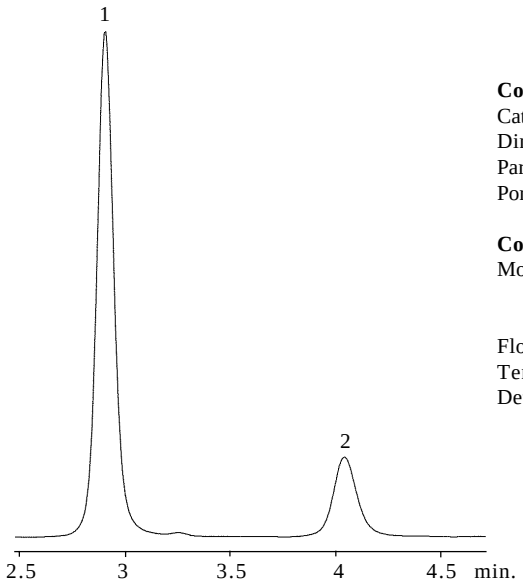
Because all three of these phases display a characteristic U-shaped profile for retention versus percentage of organic solvent in mobile phase, the mobile phase content can be modified to use either the normal or reverse phase mode if solubility problems arise. The selectivity of the Allure™ Basix and 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.: 1µL
Conc.: 500µg/mL
Solvent: methanol:water: tetrahydrofuran (30:35:35)




**Column:** **Allure™ Basix**
Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 60Å

**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

## *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 |

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