

Improved HPLC Analysis of Analgesics

Analgesics are the largest selling class of pharmaceuticals. Many people use analgesics in prescription form or in simple over-the-counter remedies. Headaches, inflammation, colds, flu, congestion, fever, sneezing, coughing, and muscle aches are among the list of symptoms that can be treated with analgesics.

Symptoms, however, often arrive in multiples. For multiple symptoms, analgesics combined with pain relief, fever reduction, and other beneficial compounds have been created. Many of these compounds can be analyzed simultaneously using high performance liquid chromatography (HPLC), thereby improving laboratory efficiency and productivity. With the selection of the proper LC column phase, separation becomes a simpler and more manageable task that does not rely upon extensive preparation procedures or use of ion pairing agents, which often are described in pharmaceutical compendiums.

Popular compounds for multi-active analgesics include aspirin, salicylic acid, salicylamide, acetaminophen, ibuprofen, naproxen, guaifenesin, codeine, oxycodone, hydrocodone, and caffeine. The Restek Ultra Phenyl,

Allure™ Acidix, and Allure™ Basix HPLC column phases separate mixtures of these pharmaceuticals in a productive and cost effective manner. The selective chemistry of these phases provide powerful separation mechanisms.

The interaction of codeine with active residual silanol sites on improperly deactivated column phases can cause extreme tailing for this compound. A properly end-capped and base-deactivated phenyl phase, such as the Ultra Phenyl phase, can significantly reduce tailing of the codeine peak. In Figure 1, the Ultra Phenyl column provides excellent peak shape and superior resolution of guaifenesin and codeine with a run time of less than six minutes. Furthermore, the method is MS-compatible because no mobile phase additives or ion pairing reagents are needed to reduce tailing.

Acidic silanols also can cause undesirable interaction with analytes containing basic functional groups such as amines and amides. The fully end-capped Allure™ Basix column was developed with specialized functional groups that provide the desired interaction with nitrogen-based functional groups such as amines and amides.

Figure 1

Excellent peak shape and superior resolution of guaifenesin and codeine using Ultra Phenyl column.

Peak List:

1. codeine
2. guaifenesin

Sample:

Inj.: 5µL
 Conc.: codeine 825µg/mL
 guaifenesin 375µg/mL
 Solvent: methanol:water
 (50:50)

Column: Ultra Phenyl

Catalog #: 9105565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:

Mobile Phase: 10mM ammonium
 formate, pH 2.5:
 acetonitrile
 (80:20, v/v)
 Flow: 1.2mL/min.
 Temp.: 25°C
 Det.: UV @ 280nm

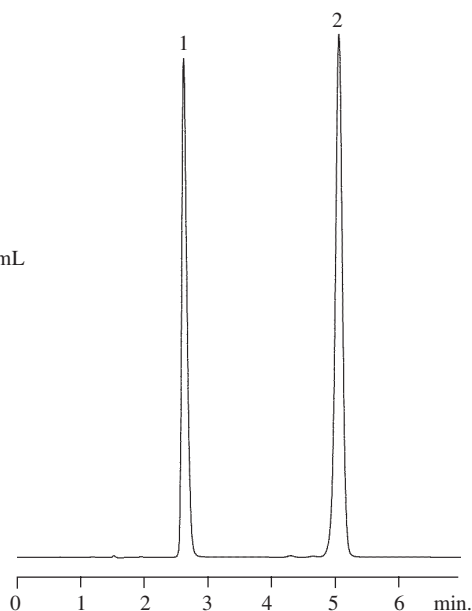


Figure 2 displays a four-component mixture of acetaminophen (a secondary amide); and codeine, oxycodone, and hydrocodone (tertiary amines). Admixtures of acetaminophen and one of the above tertiary amines are used as popular prescription painkillers. The Allure™ Basix column separates all these analytes within 8.5 minutes, showing excellent resolution and peak shape without the need for the docusate sodium modifier recommended in certain method compendiums.

Acetaminophen also is included in many over-the-counter remedies for congestion. In Figure 3, the popular mix of

nitrogen-based compounds, such as acetaminophen, pseudoephedrine, and caffeine are separated. The Allure™ Basix column performs well, separating all compounds in under 9.5 minutes with excellent shape and resolution, and without the need of ethanesulfonic acid as an ion-pairing agent.

Some of the oldest analgesics are not bases, but acids. Acetaminophen falls into the category of a weak acid. Figure 4 displays the analysis of acetaminophen on the Allure™ Acidix column. The selective interaction of this column with acidic solutes, such as acetaminophen, results in excellent peak shape and an analysis time of under four minutes.

Figure 2

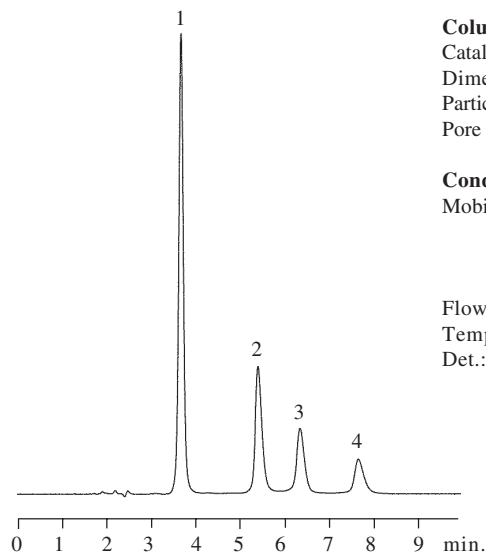
The Allure™ Basix column separates acetaminophen and tertiary amines within 8.5 minutes, resulting in excellent resolution and peak shape.

Peak List:

1. acetaminophen
2. codeine HCl
3. oxycodone HCl
4. hydrocodone bitartrate

Sample:

Inj.: 5 µL
 Conc.: 100 µg/mL
 Solvent: mobile phase



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 (85:15, v/v)
 Flow: 1.0mL/min.
 Temp.: 30°C
 Det.: UV @ 280nm

Figure 3

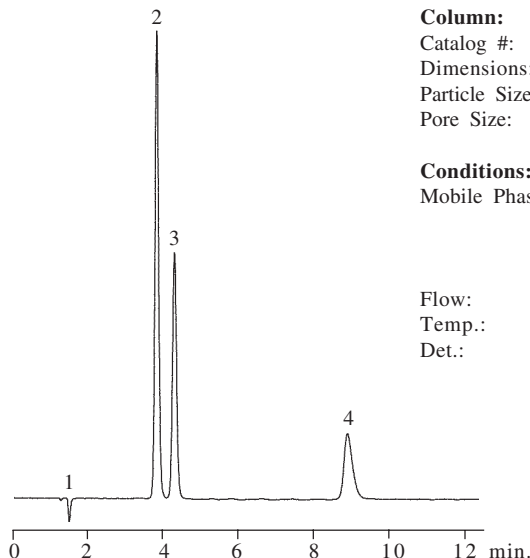
The Allure™ Basix column separates acetaminophen mixed with nitrogen-based compounds in under 9.5 minutes, with excellent resolution and peak shape.

Peak List:

1. sample solvent (marker)
2. caffeine
3. acetaminophen
4. pseudoephedrine

Sample:

Inj.: 5 µL
 Conc.: 100 µg/mL
 Solvent: mobile phase



Column: Allure™ Basix

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

Conditions:

Mobile Phase: 10mM ammonium acetate, pH 6.9: methanol (75:25, v/v)
 Flow: 1.0mL/min.
 Temp.: 25°C
 Det.: UV @ 210nm

Figure 5 displays the analysis of aspirin and salicylic acid, which also are easily separated on the Allure™ Acidix phase without the need of 1-heptane sulfonic acid or special solid phase supports referenced by the USP. This separation uses LC/MS compatible buffers and will out-perform the reproducibility of any method employing ion-pair reagents.

Ibuprofen and naproxen are medications frequently recommended for pain caused by inflammation. Figure 6 displays the separation of these closely related carboxylic acids in under seven minutes using an Allure™ Acidix column.

The Restek Ultra Phenyl, Allure™ Acidix, and Allure™ Basix columns provide excellent separation of analgesics without the need of additional mobile phase modifiers. The Food and Drug Administration (FDA) is now requiring “grandfathered” methods that suffer from low accuracy and precision to be upgraded to state-of-the-art methods. These phases provide the means to update many of the older methods used in pharmaceutical compendia, which will reduce laboratory costs, improve laboratory productivity, and minimize additive obstacles to LC/MS compatibility.

Figure 4

Acetaminophen elutes with excellent peak shape in under 4 minutes using the Allure™ Acidix column.

Peak List:

- 1. acetaminophen

Sample:

Inj.: 10µL
 Conc.: 1mg/mL
 Solvent: water:acetonitrile
 (1:1, v/v)

Column:

Allure™ Acidix
 Catalog #: 9162565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 60Å

Conditions:

Mobile Phase: 20mM ammonium acetate pH 4.5:
 acetonitrile (95:5, v/v)
 Flow: 1.0mL/min.
 Temp.: ambient
 Det.: UV @ 220nm

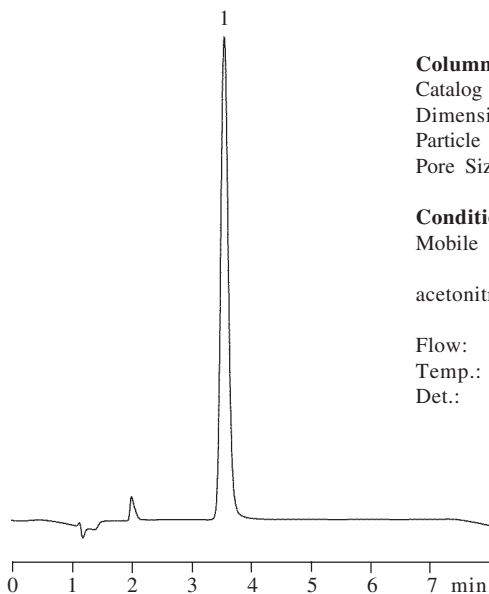


Figure 5

Aspirin and salicylic acid are easily separated on the Allure™ Acidix column.

Peak List:

- 1. toluene
- 2. unknown
- 3. salicylic acid
- 4. aspirin

Sample:

Inj.: 10µL
 Conc.: 200µg/mL
 Solvent: buffer : acetonitrile
 (1:1, v/v)

Column:

Allure™ Acidix
 Catalog #: 9162565
 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: 1.0mL/min.
 Temp.: ambient
 Det.: UV @ 220nm

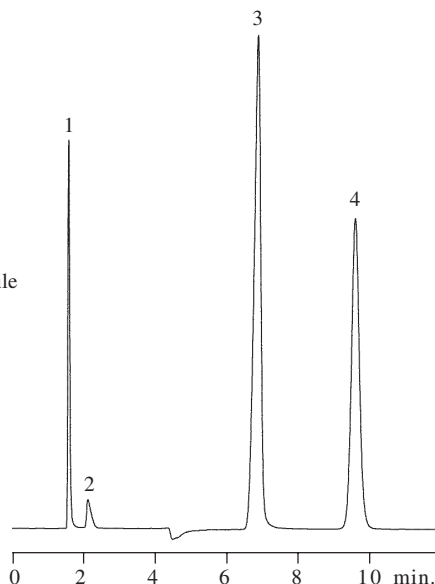


Figure 6

Closely related carboxylic acids are quickly separated in under 7 minutes using the Allure™ Acidix column.

Peak List:

1. unknown
2. unknown
3. uracil
4. ibuprofen
5. naproxen

Sample:

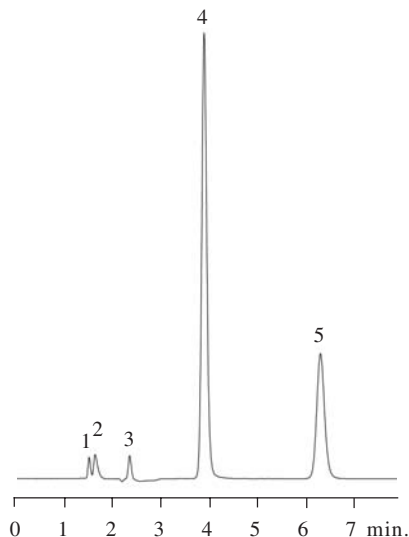
Inj.: 10µL
 Conc.: 100µg/mL
 (ibuprofen and naproxen)
 Solvent: ammonium acetate
 buffer : acetonitrile
 (1:1, v/v)

Column: Allure™ Acidix

Catalog #: 9162565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 60Å

Conditions:

Mobile Phase: 20mM ammonium acetate pH 4.5:
 acetonitrile (30:70, v/v)
 Flow: 1.0mL/min.
 Temp.: ambient
 Det.: UV @ 220nm



Allure™ Acidix Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9162531	9162532	9162533	9162535
50mm length	9162551	9162552	9162553	9162555
100mm length	9162511	9162512	9162513	9162515
150mm length	9162561	9162562	9162563	9162565
200mm length	9162521	9162522	9162523	9162525
250mm length	9162571	9162572	9162573	9162575

Allure™ Basix 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 Phenyl Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9105531	9105533	9105533	9105535
50mm length	9105551	9105552	9105553	9105555
100mm length	9105511	9105512	9105513	9105515
150mm length	9105561	9105562	9105563	9105565
200mm length	9105521	9105522	9105523	9105525
250mm length	9105571	9105572	9105573	9105575

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