RESTEK pharmaceutical

Applications note

Allure™ PFP Propyl and Ultra PFP HPLC Columns Provide Improved Analyses of Basic Compounds

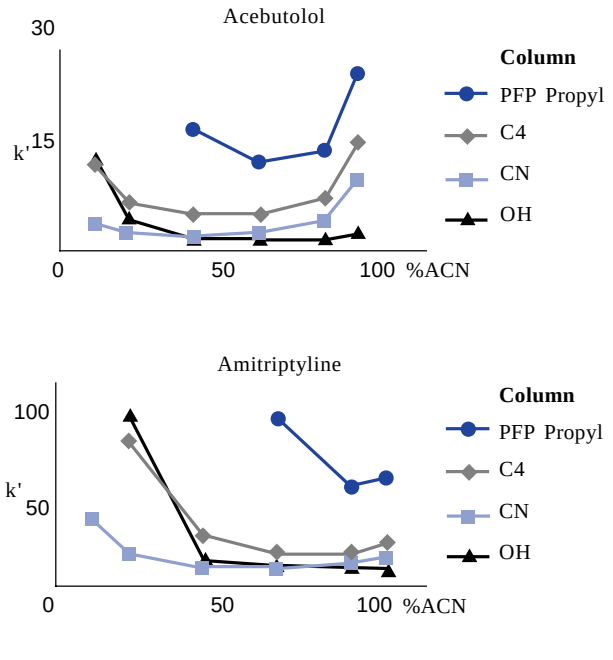
High performance liquid chromatography (HPLC) methods must be optimized to provide the greatest selectivity and sensitivity, and the best peak shape. Unfortunately, many analysts consider switching the stationary phase—the heart of the HPLC system—only as a last resort. Too often analysts coax the stationary phase to perform a non-native separation by using modifiers and ion pairing agents, which leads to reduced sensitivity and equilibration problems (the C18 phase is the most often misused). Selection of the proper stationary phase for your separation can improve LC sensitivity, analyte retention, and peak shape without the use of modifiers or ion pairing agents. For example, Restek's Allure™ PFP Propyl and Ultra PFP HPLC columns easily perform separations of many basic analytes.

Research shows the Allure™ PFP Propyl stationary phase not only provides the greatest retention and capacity factor (k') (Figure 1) for basic analytes such as beta blockers and tricyclic antidepressants, but also the best peak shape. "…The results indicate that both the fluorine groups and the propyl chain are important on the phenyl ring to obtain the best peak shape and retention of the basic solutes when ammonium formate:acetonitrile (10:90) is used as the mobile phase."[1] As peak asymmetry is improved, sensitivity is increased.

Figure 1

Allure™ PFP Propyl demonstrates increased retention of basic analytes

5mM NH_4OAc, pH 4.5:ACN; 30x2.1mm; 0.4mL/min.



Data Courtesy of Shane Needham, Pfizer Inc PLC-0001

Basic analytes are difficult to retain on C18 phases if the analytes have pKa's greater than 8. They can be retained on C8 or C18 columns by using modifiers, but at the expense of sensitivity.[2] Sensitivity can be reduced further on an LC/mass spectrometer (MS) ESI interfaces when the buffer concentrations exceed 50mM. The Allure™ PFP Propyl and Ultra PFP columns eliminate the need for modifiers, and analytes such as cocaine (COC) and its metabolite, ecgonine methyl ester (EME), can be separated and retained using 90% acetonitrile in under 4.5 minutes (Figure 2). As the concentration of the organic solvent in the mobile phase increases, the desolvation process becomes more effective and the LC/MS ESI signal increases.[3] Because of this interaction, using the Allure™ PFP Propyl column with a high organic concentration increases the response of COC and other basic solutes by as much as twelve times over that from a C18 column.[4]

Proper retention also is needed to separate basic analytes from naturally occurring substances in the blood, urine, or body tissues. If the analytes elute too closely to the void volume, ionization suppression can occur.[5] To be cost effective, however, the analytes should be separated in less than 6 minutes. The high selectivity of the Allure™ PFP Propyl column more than adequately separates EME from COC and the column void volume, respectively. (Figure 2).[6]

The Ultra PFP column is similar to the Allure PFP Propyl. The PFP column features a 100 angstrom pore size and is available in 3 and 5 micron particle sizes. Like the PFP Propyl, the PFP has high retention for basic and multi-halogenated analytes. Table I displays the retention of many basic beta-blockers on the PFP phase versus many other phases using 90% acetonitrile as the mobile phase. The HPLC/MS ESI analysis of beta-blockers using the Ultra PFP column shows good intensity (Figure 6). The PFP phase is also able to retain multi-halogenated analytes such as the thyroid hormone, levothyroxine, and purines and pyrimidines (Figures 4 & 5). The PFP phase has the greatest retention, which will improve the separation of one analyte from another.

The attraction mechanism of pi-acid HPLC phases, such as PFPP and PFP, allow stronger retention of bases than alkyl phases like C18. Alkyl phases like C18 cannot adequately retain strongly basic analytes unless they have significant non-polar functional groups to allow hydrophobic retention.

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Therefore, Restek's Allure™ PFP Propyl and Ultra PFP columns are the best choice for the analysis of basic analytes. COC, EME, quinine, morphine, beta-blockers, and tricyclic antidepressants have been analyzed successfully in urine, blood, and tissue samples. In addition, pyridines, pyrimidines, and multi-halogenated compounds have been successfully separated on the Ultra PFP column.

The Allure™ PFP Propyl and Ultra PFP columns provide superior retention and peak shape for analytes having a pKa >8 without the need for modifers, and they can provide increased sensitivity for LC/MS ESI due to the high level of organic solvent used in the mobile phase.

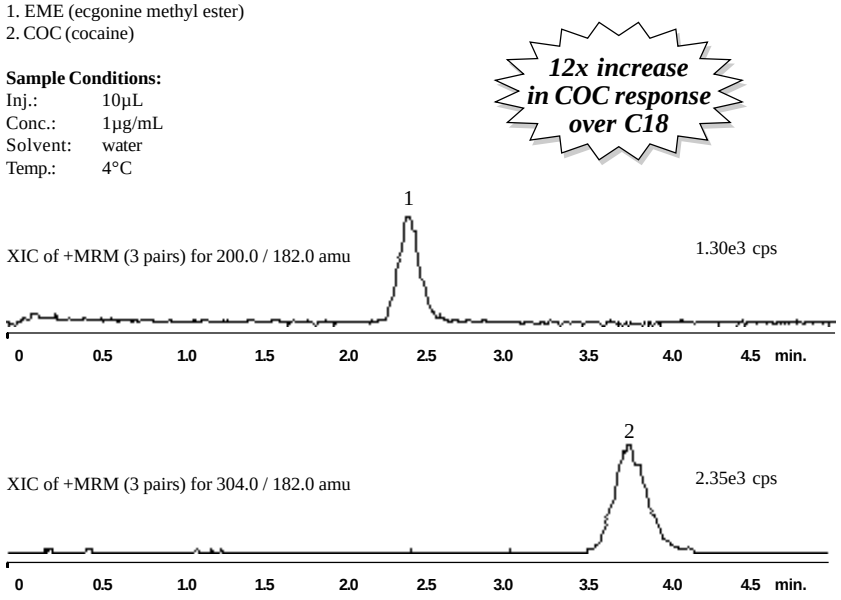
Figure 2

Allure™ PFP Propyl column baseline separates cocaine (COC) and its metabolite, ecgonine methyl ester (EME), with excellent peak shape in less than five minutes.

Peak List:
1. EME (ecgonine methyl ester)
2. COC (cocaine)

Sample Conditions:

Inj.:	10µL
Conc.:	1µg/mL
Solvent:	water
Temp.:	4°C



Column:	**Allure™ PFP Propyl**
Catalog#:	9169532
Dimensions:	30mm x 2.1mm
Particle size:	5µm
Pore size:	60Å
Conditions:	
Mobile phase:	5mM pH 3.0 ammonium formate: acetonitrile (10:90)
Flow:	0.6mL/min.
Column temp.:	ambient
Det.:	PE/Sciex API 3000
Interface:	Turbo Ion Spray, ESI
Interface temp.:	150°C
Ion mode:	positive
ESI probe voltage:	5000V
Orifice:	+/- 71V
Ring:	+/- 265V
Collision gas:	Nitrogen
Collision gas pressure:	2.2 mTorr
Collision gas energy(COC):	28 eV
Collision gas energy(EME):	26 eV
Electron multiplier:	2100 volts
Auxillary gas flow:	7000cc/min.
Nebulizer gas setting:	15 lb/in.^{2}
Curtain gas setting:	12 lb/in.^{2}

Data Courtesy of Shane Needham, Pfizer Inc

LC_0126

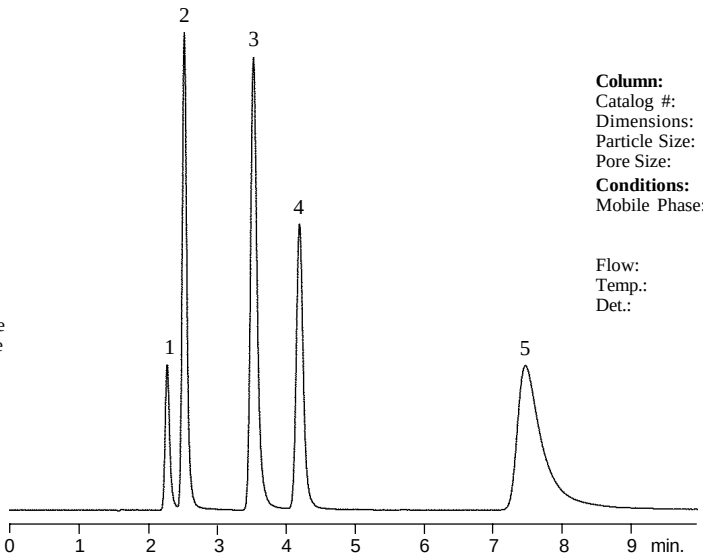
Figure 3

Nucleic bases as guanine, cytosine, uradine, thymine, and adenine are all easily retained upon the Allure™ PFP Propyl.

Peak List:
1. cytosine
2. uracil
3. guanine
4. thymine
5. adenine

Sample:

Inj.:	1.0 mL
Conc.	100µg/mL
Solvent:	20 mM Ammonium Acetate, pH 5.0. Ammonium hydroxide added to keep guanine in solution



Column:	**Allure™ PFP Propyl**
Catalog #:	9169565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	60Å
Conditions:	
Mobile Phase:	20mM Ammonium Acetate, pH 5.0: Methanol (85:15, v/v)
Flow:	1.0mL/min
Temp.:	ambient
Det.:	UV @ 260nm

LC_0137

Figure 4

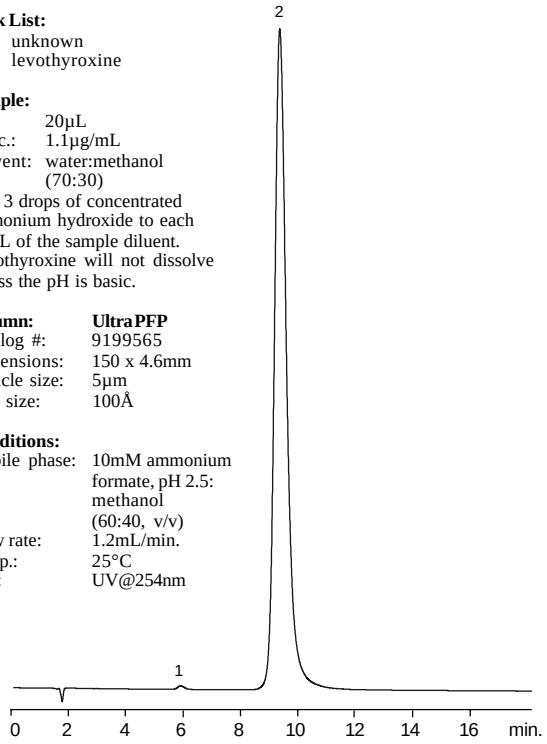
Multi-halogenated analytes as levothyroxine can also take advantage of the Ultra PFP's strong retention

Peak List:
1. unknown
2. levothyroxine

Sample:
Inj.: 20µL
Conc.: 1.1µg/mL
Solvent: water:methanol (70:30)
Add 3 drops of concentrated ammonium hydroxide to each 10mL of the sample diluent. Levothyroxine will not dissolve unless the pH is basic.

Column: **Ultra PFP**
Catalog #: 9199565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 10mM ammonium formate, pH 2.5: methanol (60:40, v/v)
Flow rate: 1.2mL/min.
Temp.: 25°C
Det.: UV@254nm



LC_0093

Figure 5

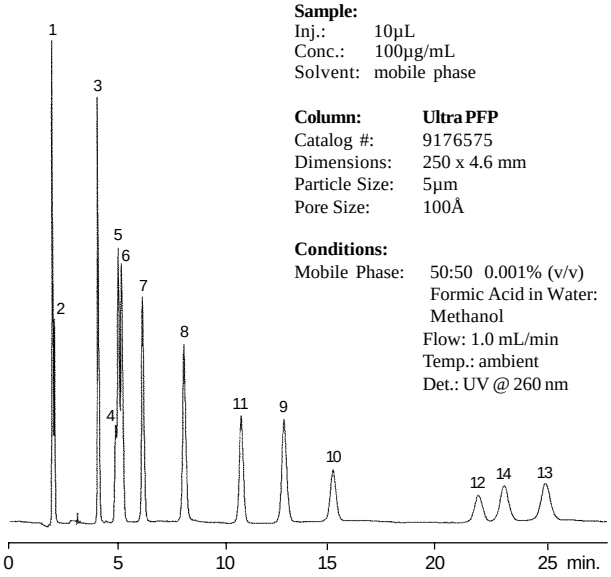
The strong retention of the PFP makes it an excellent phase for the analysis of purines and pyrimidines.

Peak List:
1. cytosine
2. 5-methylcytosine
3. uracil
4. uric acid
5. hypoxanthine
6. xanthine
7. thymine
8. purine
9. 6-methylpurine
10. theobromine
11. ethyluracil
12. 1,7-dimethylxanthine
13. theophylline
14. propyluracil

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Solvent: mobile phase

Column: **Ultra PFP**
Catalog #: 9176575
Dimensions: 250 x 4.6 mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 50:50 0.001% (v/v) Formic Acid in Water: Methanol
Flow: 1.0 mL/min
Temp.: ambient
Det.: UV @ 260 nm

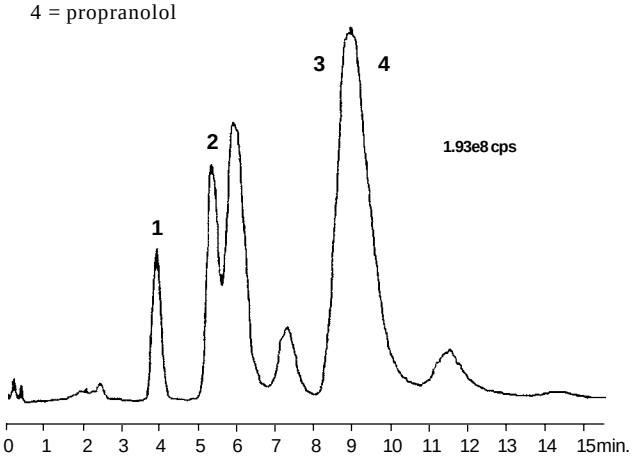


LC_0136

Figure 6

HPLC/MS ESI analysis of beta-blockers on the Ultra PFP column shows good intensity

Peak list:
1 = atenolol
2 = acetbutolol
3 = alprenolol
4 = propranolol



Data courtesy of Shane Needham, Pfizer Inc

Table I

Retention Data of Basic Solutes with 90% Acetonitrile

	Retention Time (min.)			
β-blockers	**CN**	**OH**	**PFP**	**C4***
Acebutolol	3.22	2.02	6.32	<0.5
Alprenolol	3.79	1.74	9.99	<0.5
Atenolol	3.04	2.15	4.63	<0.5
Labetolol	3.56	1.32	4.93	<0.5
Metoprolol	3.42	1.83	7.70	<0.5
Nadolol	3.37	2.15	5.58	<0.5
Oxprenolol	3.81	1.78	9.20	<0.5
Pindolol	3.64	1.81	6.60	<0.5
Propranolol	3.27	1.78	10.3	<0.5
Sotalol	3.12	1.69	5.09	<0.5
Timolol	3.37	1.74	7.53	<0.5

*<20% acetonitrile needed for adequate retention

Data courtesy of Shane Needham, Pfizer Inc

References

1. S. R. Needham, P.R. Brown, and K. Duff, *Rapid Commun. Mass Spectrom.* 13, 2231-2236 (1999).
2. J. Svensson., *J. Anal. Toxicol.*, 10 (1986) 122-124.
3. P. Sjoberg and K. Markides., *J. Chromatogr. A.*, 855 (1999) 317-327.
4. S. R. Needham, *op. cit.*
5. B. Matuszewski, M. Constanzer, and C. Chavez-Eng. *Anal. Chem* 70 (1998) 882-889.
6. S. R. Needham, *op. cit.*

References not available from Restek.

Product Listing

Allure™ PFP Propyl, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9169531	9169532	9169533	9169535
50mm	9169551	9169552	9169553	9169555
100mm	9169511	9169512	9169513	9169515
150mm	9169561	9169562	9169563	9169565
200mm	9169521	9169522	9169523	9169525
250mm	9169571	9169572	9169573	9169575

Allure™ PFP Propyl, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9169532-700	9169533-700	9169535-700
50mm	—	9169552-700	9169553-700	9169555-700
100mm	—	9169512-700	9169513-700	9169515-700
150mm	—	9169562-700	9169563-700	9169565-700
200mm	—	9169522-700	9169523-700	9169525-700
250mm	—	9169572-700	9169573-700	9169575-700

Ultra PFP, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9176531	9176532	9176533	9176535
50mm	9176551	9176552	9176553	9176555
100mm	9176511	9176512	9176513	9176515
150mm	9176561	9176562	9176563	9176565
200mm	9176521	9176522	9176523	9176525
250mm	9176571	9176572	9176573	9176575

Ultra PFP, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9176532-700	9176533-700	9176535-700
50mm	—	9176552-700	9176553-700	9176555-700
100mm	—	9176512-700	9176513-700	9176515-700
150mm	—	9176562-700	9176563-700	9176565-700
200mm	—	9176522-700	9176523-700	9176525-700
250mm	—	9176572-700	9176573-700	9176575-700

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