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Technical Report

Ascentis Express F5 HPLC Columns

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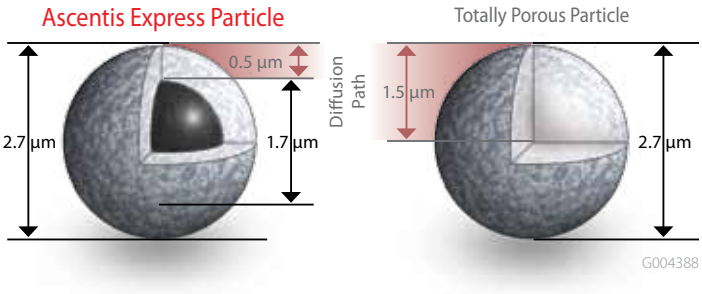
Introduction

Ascentis® Express is a high-speed, high-performance liquid chromatography column based on Fused-Core® (Figure 1) particle technology. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency due to the shallow diffusion paths in the 0.5-micron thick porous shell and the small overall particle size of 2.7 microns. Other features, such as a very narrow particle size distribution and high packing density, result in Ascentis Express columns capable of 240,000 N/m. This is comparable to the efficiency of sub-2 µm particle columns and nearly twice the efficiency possible with 3 µm columns.

The pentafluorophenylpropyl stationary phase of Ascentis Express F5 provides a stable, reversed-phase packing with electron-deficient phenyl rings due to the presence of electronegative fluorines. In addition to forming π-π and mildly steric interactions, F5 phases also retain compounds by polar interactions. As a result of having both polar and non-polar character, F5 phases can show dual-mode retention behavior, sometimes producing a "U-shaped" retention as a function of acetonitrile content of the mobile phase, with retention increasing at both low and high concentrations of ACN (reversed-phase and HILIC retention modes). Ascentis Express F5 can be used for basic, acidic, or neutral compounds with alternate selectivity from C18.

- Alternate selectivity to C18
- Retains bases more and hydrophobes less than C18
- Reversed-phase, HILIC, and 100% aqueous applications
- Stable, low bleed for LC-MS and LC-UV

Figure 1. Comparison of Fused-Core and Standard HPLC Particle



Mechanisms of Retention on Fluorinated Phases

The use of fluorinated stationary phases in liquid chromatography and hyphenated techniques has become significant in recent years. Early applications in the effective separation of paclitaxel provided such phases much notoriety, however more recent studies focusing on orthogonality to traditional alkyl phases has invited even broader attention. Due to the different retention mechanisms fluorinated stationary phases provide, they are often employed for the separations not easily obtained using common C18 phases. Applications in arenas such as biopharmaceutical, pharmaceutical, natural product and environmental analyses are increasingly being reported.

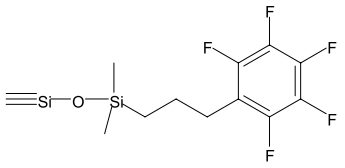
Fluorinated phases have been shown to exhibit greater ion-exchange character than their alkyl counterparts. Fluorinated phases often provide excellent chromatographic results when analytes to be separated differ in their ionization constants or where some ion-exchange is necessary for the retention of polar metabolites or degradation products. A second important attribute of the fluorinated phases lies in their apparent increased shape selectivity relative to common stationary phase chemistries. Fluorinated phases, therefore, are often superior to their alkyl counterparts for the separation of closely related compounds that differ in size and shape.

In order to effectively utilize this interesting and useful tool, it is important to have a basic understanding of the underlying mechanisms that govern retention and selectivity. This report will focus on two main mechanistic features of fluorinated phases that differentiate them from common alkyl phases; increased ionic interactions relative to alkyl phases and shape selectivity.

Practical Implications of Alternative Retention Mechanisms

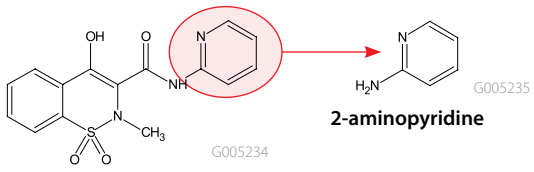
The structure of a popular form of fluorinated phases, pentafluorophenylpropyl (PFP or F5), is shown in Figure 2. The F5 bonded phase exhibits strong dipole potential (polar interaction) from the carbon-fluorine bonds, pi-pi interaction potential and the ability to interact via charge-transfer interactions due to the electronegativity of the fluorine atoms. The relative rigidity of the bonded phase is also believed to provide enhanced shape selectivity of analytes differing in size and spatial attributes.

Figure 2. Chemical Structure of Pentafluorophenylpropyl Bonded Phase



A common short-coming of traditional alkyl phases such as C18 (ODS) and C8 (octyl) is their inability to retain polar compounds. Because the F5 phase exhibits ion-exchange and polar interactions, retention of polar compounds are often achieved. Figure 3 shows the structures of the anti-inflammatory drug piroxicam and a potential synthetic impurity, 2-aminopyridine (2-AMP). 2-AMP is relatively polar (log P = 0.53±0.27) and thus difficult to retain on a conventional alkyl column. Figure 4 shows the separation of the two analytes using a C18 phase. Retention of piroxicam is easily achieved; however, 2-AMP is unretained and thus not quantifiable. Efforts to lower mobile phase organic content and raise pH to improve retention were ineffective using the alkyl phase. It is possible that retention of 2-AMP may be accomplished through the addition of ion-pair reagents; however, such methods are often difficult to validate and suffer from robustness and ruggedness issues. Figure 5 shows the separation of 2-AMP and piroxicam using a fluorinated phase. In this case, 2-AMP is well retained and separated from the parent molecule using a simple mobile phase. The retention of 2-AMP demonstrates the availability and utility of the polar and ionic interactions the F5 phase exhibits.

Figure 3. Structures of Piroxicam and its Potential Impurity, 2-aminopyridine



Piroxicam

2-aminopyridine

Figure 4. Separation of Piroxicam and 2-Aminopyridine on a C18 Column

column: Ascentis Express C18, 10 cm x 4.6 mm, I.D. 2.7 µm particle size (53827-U)
mobile phase A: 10 mM ammonium formate (aq), pH 3 with concentrated formic acid
mobile phase B: acetonitrile
mobile phase ratio: A:B 75:25, v/v
flow rate: 0.8 mL/min.
temp.: 35 °C
pressure: 1635 psi
det.: UV PDA at 230 nm and MS in ESI (+), SIR (single ion recording mode)
injection: 5 µL
sample: 10 µg/mL 2-AMP and 100 µg/mL piroxicam in 90:10 10 mM ammonium formate (aq), pH 3 with concentrated formic acid:acetonitrile

1. 2-Aminopyridine
2. Piroxicam

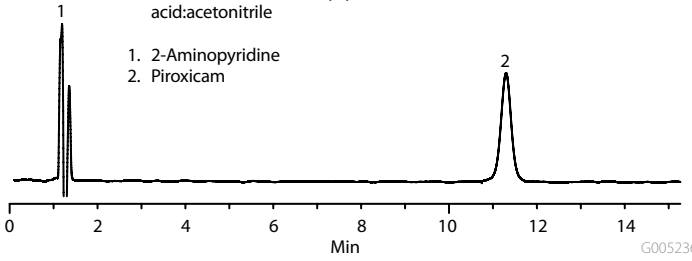
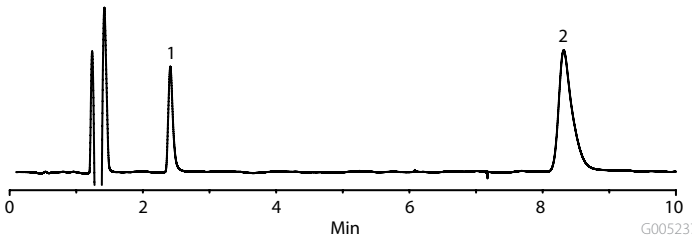


Figure 5. Separation of Piroxicam and 2-Aminopyridine on an F5 Column

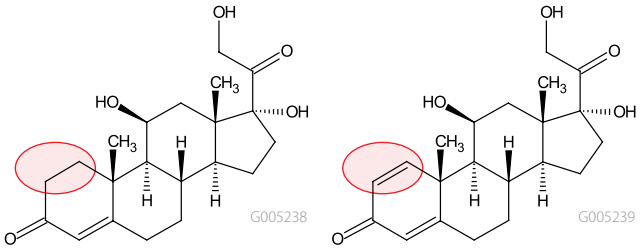
column: Ascentis Express F5, 10 cm x 4.6 mm, I.D. 2.7 µm particle size (53590-U)
mobile phase A: 10 mM ammonium formate (aq), pH 3 with concentrated formic acid
mobile phase B: acetonitrile
mobile phase ratio: A:B 75:25, v/v
flow rate: 8 mL/min.
temp.: 35 °C
pressure: 1635 psi
det.: UV PDA at 230 nm and MS in ESI (+), SIR (single ion recording mode)
injection: 5 µL
sample: 10 µg/mL 2-AMP and 100 µg/mL piroxicam in 90:10 10 mM ammonium formate (aq), pH 3 with concentrated formic acid:acetonitrile

1. 2-Aminopyridine
2. Piroxicam



Chromatographers are often faced with the challenge of separating compounds that are very similar in their solubilities. Separation on non-polar phases such as C18 is driven by differential partitioning of analytes, therefore, the alkyl phases are often ineffective in meeting this challenge. Hydrocortisone and prednisolone (see Figure 6) differ by a single double bond. Their solubilites are very similar; however, their shapes differ significantly. Figure 7 shows a comparison of their separation (along with prednisone internal standard (IS) using both a C18 and an F5 stationary phase. The fluorinated phase, apparently due to the enhanced shape selectivity, is shown to provide the separation of these closely related compounds.

Figure 6. Structures of Hydrocortisone and Prednisolone

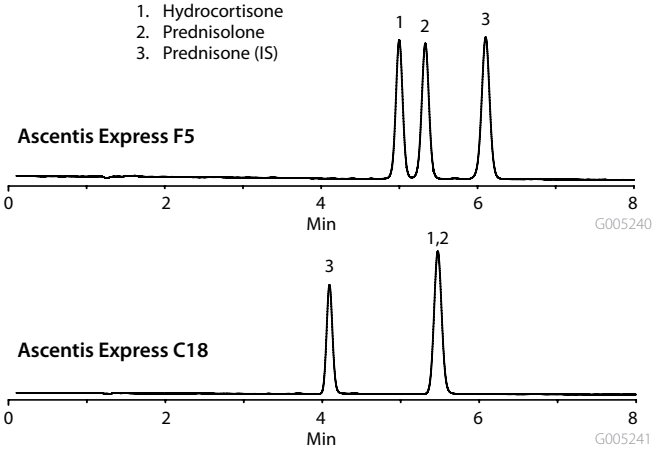


Hydrocortisone **Prednisolone**

Figure 7. Comparison of C18 and F5 for the Separation of Closely Related Steroids

column(s): Ascentis Express F5 (53590-U) and Ascentis Express C18, 10 cm x 4.6 mm, I.D., 2.7 µm particle size (53827-U)
mobile phase A: water
mobile phase B: methanol
mobile phase ratio: A:B 50:50, v/v
flow rate: 0.8 mL/min.
temp.: 35 °C
pressure: ~2400 psi
det.: UV at 240 nm
injection: 5 µL
sample: 10 µg/mL each in 90:10 water:methanol

1. Hydrocortisone
2. Prednisolone
3. Prednisone (IS)



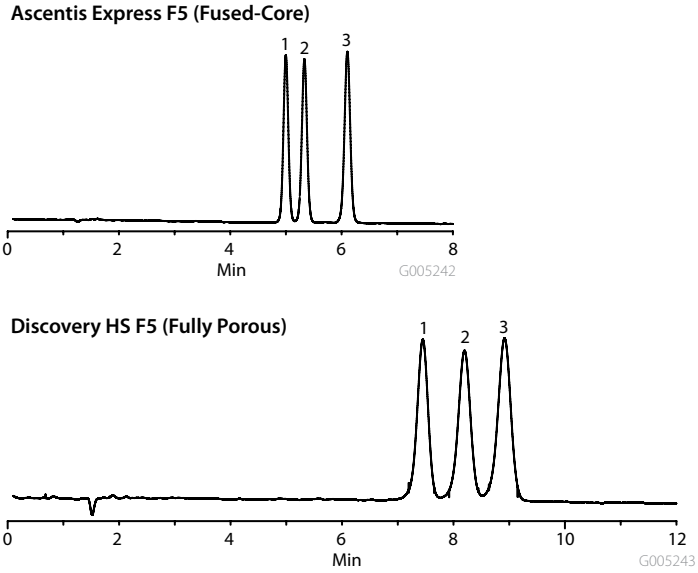
Alternate Selectivity with High Efficiency

The F5 bonded phase has recently been introduced on the highly efficient Fused-Core technology platform with the brand name Ascentis Express F5. Figure 8 shows a comparison of the fully-porous Discovery® HS F5 and the new Ascentis Express F5. Similar overall selectivity is observed due to the similar interactions provided by the F5 moiety. A dramatic increase in efficiency due to the Fused-Core particle support is demonstrated when using the Ascentis Express F5.

Figure 8. Comparison of Fluorinated Phases Based on Totally Porous and Fused-Core Particle Technologies

column(s): Ascentis Express F5, 10 cm x 4.6 mm, I.D., 2.7 µm particle size (53590-U), Discovery HS F5, 10 cm x 4.6 mm, I.D., 5 µm particle size (567515-U)
mobile phase A: water
mobile phase B: methanol
mobile phase ratio: A:B 50:50, v/v
flow rate: 0.8 mL/min
temp.: 35 °C
pressure: ~2400 psi Express, ~1000 psi Discovery
det.: UV at 240 nm
injection: 5 µL
sample: 10 µg/mL each in 90:10 water:methanol

1. Hydrocortisone
2. Prednisolone
3. Prednisone (IS)



Conclusions

Fluorinated stationary phases exhibit increased ionic and polar interactions relative to common alkyl phases. The rigidity of the F5 bonded phase is also believed to provide increased shape selectivity over commonly used alkyl phases. These alternative mechanisms of retention often provide selectivity not readily achieved on the more traditional phases. Retention and selectivity of highly polar and ionic species as well as separation of closely related neutral compounds have been used to demonstrate the power of the bonded phase chemistry. The recent combination of the selectivity provided by the fluorinated bonded phase and the efficiency of Fused-Core particle technology provides even greater resolution power.

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