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Separation of Free Fatty Acids by Mixed-Mode Anion Exchange Chromatography

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GOAL

To demonstrate the separation of free fatty acids from C3:0–C18:0.

BACKGROUND

Fatty acids are present in foods, as part of biological systems and microbiota fermentation of complex fiber, and represent an important reservoir for energy and cell wall components for mammals.[1] Their analysis spans multiple disciplines and can be performed in several ways, including gas chromatography (following derivatization of the carboxylic acid moiety),[2] reversed phase liquid chromatography,[3] and supercritical fluid chromatography,[4] among others. Here, we show the separation of free fatty acids using a mixed-mode reversed phase/anion exchange column, the Waters™ Atlantis™ PREMIER BEH C_{18} AX.

THE SOLUTION

The Atlantis PREMIER BEH C_{18} AX is a mixed-mode reversed phase/anion exchange column having C18 ligand as well as tertiary alkylamine moieties bonded to a bridged-ethyl hybrid (BEH) silica particle.[5] At pH below 8.0, the surface of the stationary phase is positively charged and can undergo ion exchange with negatively charged molecules, such as acids. A free fatty acid standard mix from Nu-Chek Prep, Inc. (GLC-412) was prepared together with 10 µL of concentrated propionic acid (Sigma Aldrich) and brought to 1 mL with 3:1 methanol/chloroform. The sample was further diluted 100x with methanol and placed in an autosampler vial for analysis.

The Atlantis PREMIER BEH C_{18} AX Column provides excellent separation of free fatty acids.

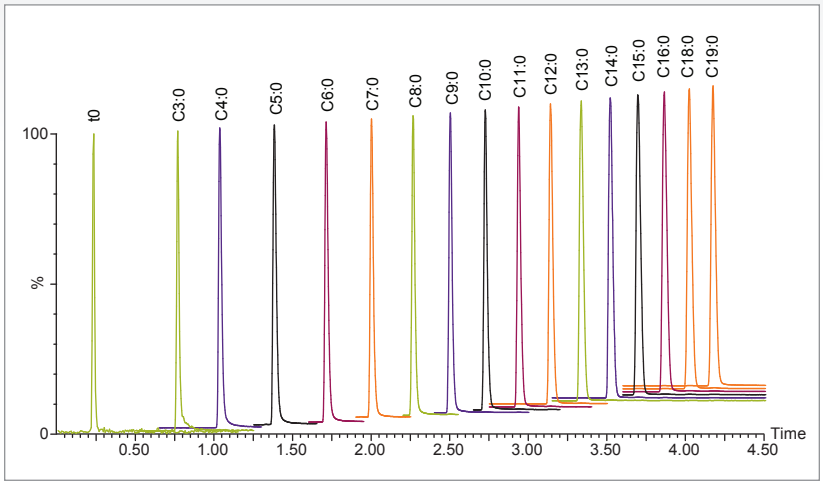

Figure 1. Separation of free fatty acids in a standard mix.

The mobile phase consisted of aqueous 1 mM ammonium formate, pH 3.0 in A, and 50:50 acetonitrile/isopropanol containing 1 mM ammonium formate, pH 3.0, in B. The gradient was programmed as 0–100% B over six minutes using a gradient curve of five. The flow rate was 0.6 mL/min, column temperature was set to 60 °C, and injection volume was 3 µL. Figure 1 shows the separation of free fatty acids from C3:0 propionic acid to C18:0 steric acid. The k' of propionic acid is 2.36 and increases in order of chain length. With the pH at 3.0, lower than the pKa of the carboxylic moiety, the aliphatic acids are uncharged and the retention is achieved by interaction with the C18 ligand. However, the pore size of the Atlantis BEH C_{18} AX material is 95 Å, smaller than the 130 Å of other BEH particles.[5] The smaller pore size particles have greater surface area, which leads to greater retention. This is illustrated

in Figure 2, where retention of fatty acids is increased in the Atlantis PREMIER BEH C_{18} AX Column with surface area of 270 m^2/g compared to the BEH C_{18} Column with surface area of 185 m^2/g. Additionally, the peak shape for propionic acid improved. To test injection reproducibility, retention time RSD were calculated for five replicate injections. In Figure 3, the injections are stacked and retention time RSD ranged from 0.02–0.14% for all the analytes.

SUMMARY

The Atlantis PREMIER BEH C_{18} AX Column provides good retention and separation of free fatty acids with excellent injection reproducibility.

References

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4. Yang Y. et al. Advances of Supercritical Fluid Chromatography in Lipid Profiling *J. Pharm. Anal.* **2019**, 9, 1–8.
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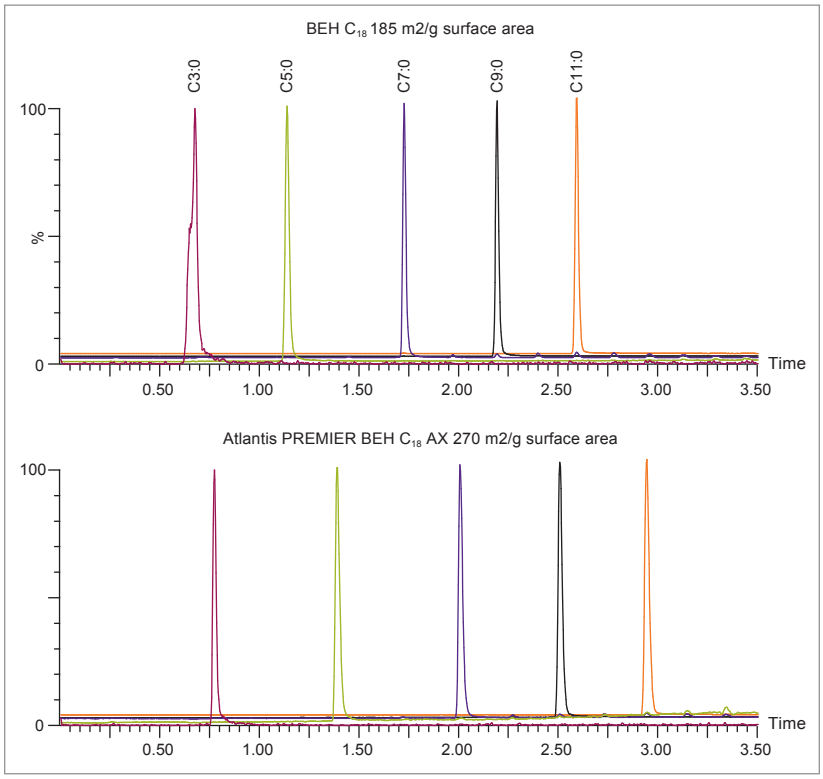

Figure 2. Effect of surface area on retention of free fatty acids. Comparison of the BEH C_{18}, 2.1 mm x 50 mm, 1.7 µm column (top) with the Atlantis PREMIER BEH C_{18} AX 2.1 mm x 50 mm, 1.7 µm column (bottom). Retention is increased with higher surface area.

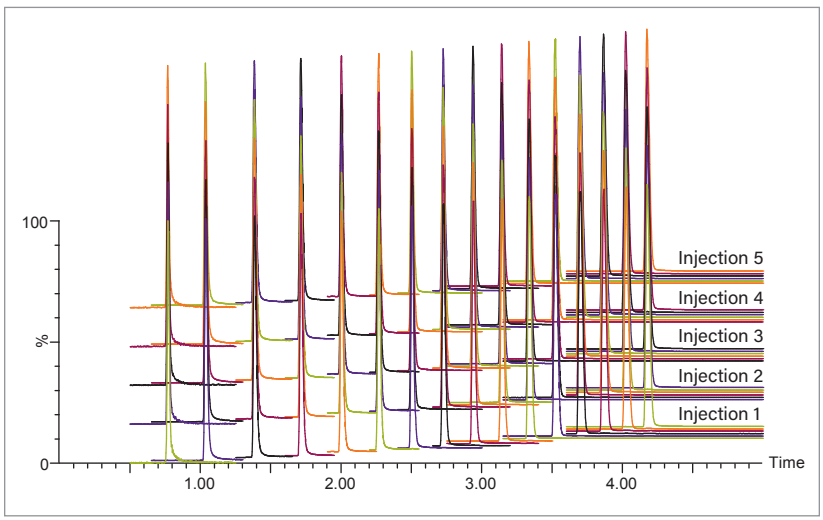

Figure 3. Injection reproducibility of free fatty acids.

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