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Regeneration of FLARE C18 Mixed-Mode Column after Exposure to TFA

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Introduction

The FLARE C18 Mixed-Mode (MM), core-shell HPLC column is stable at extremes of pH and at elevated temperature.[1-3] In addition, the column has mixed-mode properties, showing both weak anion exchange and hydrophobic retention mechanisms.[4] Because it contains an amine-based polymer, its stationary phase is protonated (charged) or deprotonated (neutral) over different ranges of pH. This amine-containing polymer is not expected to behave like a molecular amine because the charges on the polymer interact as the column is protonated.[4] Thus, it becomes increasingly difficult to protonate the column as it is more and more charged. Accordingly, one would expect a difference in the column at pH 2 and 7, while no such difference would be expected for a molecular amine.

Changes in the protonation state of the FLARE column change its retention mechanism. Thus, mobile phase pH is a useful lever to control column selectivity. At lower pH (pH < 10), the FLARE column retains acidic analytes through both hydrophobic and ionic interactions. At higher pH (pH > 10), it retains basic analytes through hydrophobic interactions. Neutral analytes are retained over a wide pH range, although this retention is also affected by the protonation state of the column – whether it is more protonated (more hydrophilic) or more deprotonated (more hydrophobic).

A common problem with amine-containing stationary phases is their retention of acidic additives like trifluoroacetic acid (TFA). Given the acid-base properties of TFA and the amines in the FLARE C18 MM stationary phase, one would expect that TFA could be removed from the column at elevated pH. This type of approach would not be an option for silica-based columns because of their lack of stability under these conditions.

A separation was performed on the FLARE C18 MM column at neutral pH (phosphate buffer at pH 7). The same separation was then performed at pH 2 using a TFA-containing mobile phase. With this change in pH, the retention of the neutral analytes dropped. Nevertheless, the column could be regenerated at pH 11, and this regeneration was repeatable. Thus, TFA, a common additive, can be used with the FLARE C18 MM column.

Experimental

Analytes: Alkylbenzene mixture (-ethyl, -butyl, and hexylbenzene)

HPLC system: Waters 1525 Binary HPLC pump with a UV detector

Column: Diamond Analytics FLARE C18 MM column (4.6 x 33 mm, 4 µm)

Injection volume: 5.0 µL

Elution conditions: Isocratic

Detection: UV at 254 nm

Flow rate: 0.7 mL/min

Temperature: 35 °C

Mobile phases: H_2O/ACN (50/50) with 10 mM TFA (pH 2.0), 10 mM phosphate (pH 7.0), or 0.2 % TEA (v/v), pH then adjusted to 11.0 (pH 11).

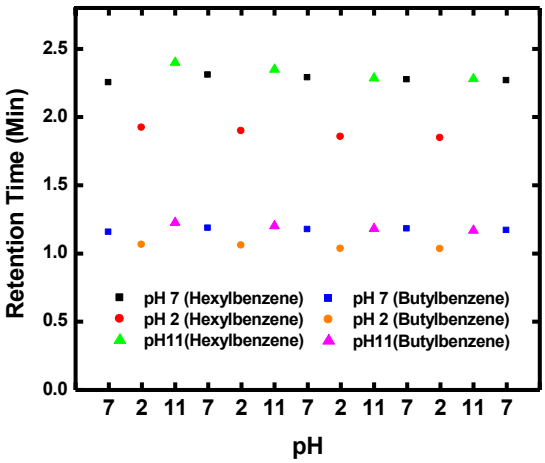

Figure 1. Retention times of hexylbenzene (top points) and butylbenzene (lower points) at different mobile phase pHs.

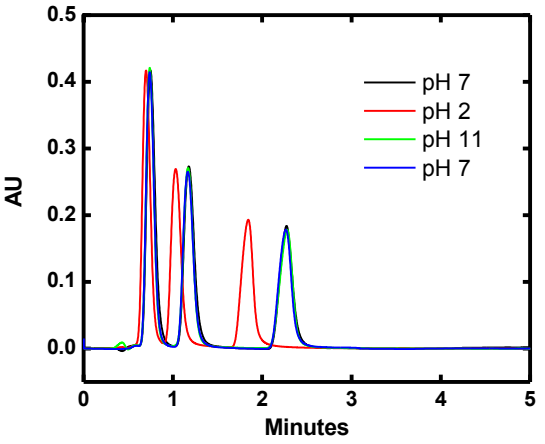

Figure 2. Chromatograms of ethyl-, butyl-, and hexylbenzene at pH 7, 2, 11, and then 7.

Results and Discussion

Figure 1 shows the retention times of hexyl- and butylbenzene at different mobile phase pH values. Initially, the column was tested at pH 7. The same separation was then attempted at pH 2, and, as expected, the retention times of the analytes decreased. A separation was next performed at pH 11. This step regenerated the column. The following run at pH 7 was similar to its previous pH 7 value. This process was repeated four times. Interestingly, after the second cycle, the retention times of the analytes at pH 11 and pH 7 were comparable.

Figure 2 shows the chromatograms of the alkylbenzene test mixture in the fourth cycle. The pH 2 chromatogram shows substantially less retention than the pH 7 chromatogram. However, the subsequent pH 11 and pH 7 chromatograms are almost identical to the original pH 7 chromatogram. Thus, deprotonation at pH 11 can remove the effects of TFA from the FLARE C18 MM column.

References

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