

**SMALL MOLECULE** 

# What to Expect from HALO® C30: A Conversation on Retention and Resolution

## NOW THAT'S AN ALKYL CHAIN!

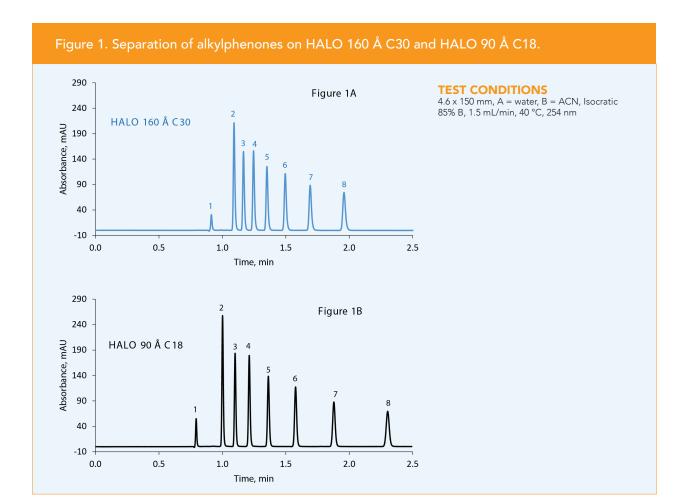
What lies in the power of a 30 carbon alkyl chain? Well, for starters a lot of potential! The HALO® C30 offers high shape selectivity for hydrophobic, long-chain, structurally related isomers. Built on proven Fused-Core® particle technology, you can count on fast, high efficiency, rugged separations. It is also 100% aqueous compatible and finds itself well positioned for isomer separations in applications such as carotenoids, lipids, vitamins, and steroids.

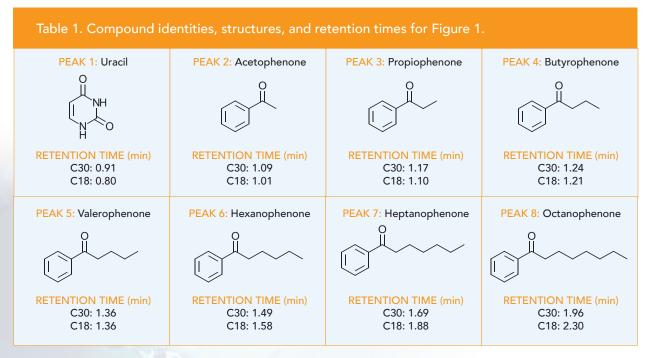
#### RETENTION CHARACTERISTICS

Which bonded phase would you expect to exhibit more retention: A C18 or C30? If all things were equal, then it would be a reasonable response to choose C30. A longer alkyl chain equals more retention, right? What if the base particle was different as it is in the case of HALO 90 Å C18 compared to the HALO 160 Å C30? The HALO® C30 is bonded to 2.7  $\mu$ m Fused-Core® particles with 160 Å pore size and an average surface area of 90 m²/g. The HALO® C18 is bonded to 2.7  $\mu$ m Fused-Core® particles with 90 Å pore size and an average surface area of 135 m²/g. Even though C30 is a longer alkyl chain, because of the pore size, there is 33% less surface area for it to occupy so there is less carbon on the surface. The HALO® C30 has 4.5% carbon load compared to 7.7% with the HALO® C18.

You might say to yourself, ok that makes sense, more surface area means more carbon so surely the retention is higher with the C18. Well, maybe, maybe not - like most things, it's not that simple! Depending on the analyte itself, the retention may or may not be higher on a HALO® C30 column compared to a HALO® C18 column. Consider Figure 1 which shows a separation of a series of alkylphenones on the HALO® C30 compared to the HALO® C18. Before valerophenone (peak 5), the HALO® C30 column shows slightly more retention. But after peak 5, the HALO® C18 column shows more retention. See Table 1 for a list of peak identities and retention times. There are a few things at play here. The analytes are retained by a combination of partitioning into the stationary phase and adsorption onto the stationary phase surface. Since there is more carbon present on the surface of the 90 Å particles, more retention is observed for the longer chain analytes. This retention increases as the alkyl chain length of the analyte increases.







## MORE RETENTION MEANS MORE RESOLUTION... RIGHT?

Not so fast! Figure 2 shows a mix of K vitamins separated on a HALO® C30 column compared to a HALO® C18 column under the same conditions. Retention of vitamin K3 (peak 1) is nearly identical on the two different stationary phases. In contrast, the later eluting vitamins (peaks 2-4) more than double in retention on the HALO® C18 column. This follows the trend of the alkylphenones in Figure 1 which showed the longer the alkyl chain, the more retention is observed using the HALO® C18 column. What is interesting to note is the resolution between the *trans* and *cis* isomers of vitamin K1 (peaks 3 and 4) on the HALO® C30 column. Similar selectivity is *not observed* on the HALO® C18 column even with its increased surface area and retention. This is due to the selectivity advantages of the C30 over the C18 phase for isomers. C30 and C18 bonded phases have different interfacial structures near the solvent-alkyl chain interface which offer an explanation to the different selectivities. (Ref. 1).

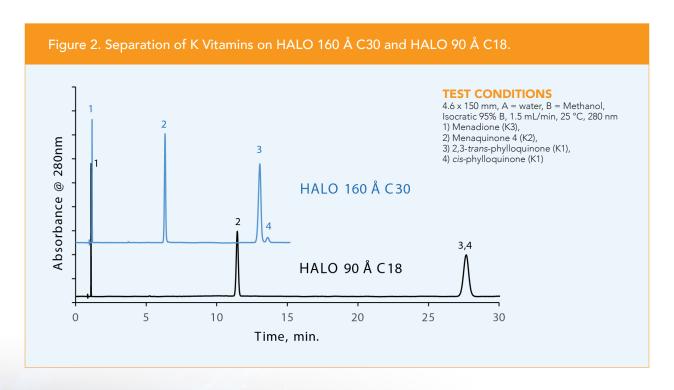


Figure 3. Structures of vitamin K3, K2, and K1.	
VITAMIN K3: Menadione	VITAMIN K2: Menaquinone 4
VITAMIN K1: 2,3-trans-phylloquinone	VITAMIN K1: cis-phylloquinone

# **CONCLUSIONS**

To summarize, the HALO® C30 is a powerful addition to the separation toolbox as the longer alkyl chain can provide more surface for enhanced shape selectivity for isomers. Be aware that retention may or may not be increased on the HALO® C30 column compared to the HALO® C18 column depending on the hydrophobicity of the analyte of interest. However, it should not be a surprise that HALO® C18 exhibits more retention for larger, hydrophobic analytes due to its increased carbon on the packing surface. Finally, keep in mind that increased retention does not always equate to improved resolution as was demonstrated with the vitamin K1 isomer separation.

#### **REFERENCE**

J Chromatogr A, 1223 (2012) 24-34 J.L Rafferty, J.I. Siepmann, and M.R. Schure.

