BENEFITS AND APPLICATION OF CORTECS® C_8 AND CORTECS® PHENYL COLUMNS FOR PHARMACEUTICAL ANALYSES

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INTRODUCTION

The use of solid-core particle columns in liquid chromatography has been well documented. The benefits of these columns over columns packed with fully porous particles include higher efficiencies and a slower decrease in efficiency loss above the optimum flow rate. While having highly efficient columns is important, having a range of selectivities is essential.

CORTECS columns, launched in 2013, now include two new stationary phases. The CORTECS C_8 and CORTECS Phenyl columns take advantage of solid-core particles while offering the additional selectivity that is often needed for method development, see Figure 1.

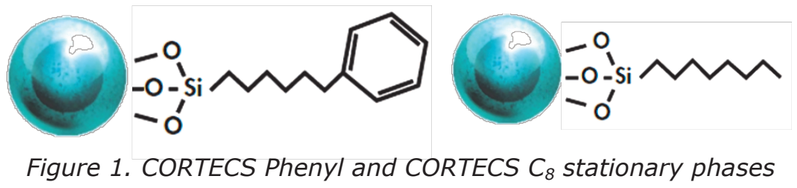

Figure 1. CORTECS Phenyl and CORTECS C_8 stationary phases

This poster will demonstrate the benefits and applications of the newest solid-core columns from Waters Corporation. Examples will be given of decreasing sample analysis time, achieving additional selectivity through intelligent solvent selection, and analysis of eleven structurally similar compounds.

METHODS

All separations were performed on an ACQUITY UPLC H-Class instrument with PDA detection.

Fat Soluble Vitamins Method Conditions

Columns:	CORTECS® UPLC C_8 1.6 µm, 3.0 x 50mm CORTECS® UPLC C_{18} 1.6 µm, 3.0 x 50mm
UV Detection:	285 nm
Column Temp:	30°C
Mobile Phase:	10:90 Water:Methanol
Flow Rate:	1.0 mL/min
Injection:	3.0 µL

Sample Concentration:

- Vitamin D_2 (Ergocalciferol) 1.0 mg/mL
- Vitamin K_2 1.0 mg/mL
- Vitamin E (α-tocopherol) 1.0 mg/mL
- Retinyl Acetate 0.1 mg/mL

Pharmaceutical Mixture Method Conditions

Column:	CORTECS® UPLC Phenyl 1.6 µm, 2.1 x 50mm
UV Detection:	254 nm
Column Temp:	30°C
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in Acetonitrile
Mobile Phase C:	0.1% Formic Acid in Methanol
Gradient Profile:	Linear gradient of 5-95% organic (B/C) over 4.5 minutes. Hold at 95% for 0.25 minutes. Return to starting conditions and re-equilibrate for 1 minute.
Flow Rate:	0.6 mL/min
Injection:	1.0 µL

Sample Concentration:
All compounds at 20 µg/mL

Bisphenol Compound Method Conditions

Column:	CORTECS® UPLC Phenyl 1.6 µm, 2.1 x 100mm
UV Detection:	275 nm
Column Temp:	30°C
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase C:	0.1% Formic Acid in Methanol
Gradient Profile:	Linear gradient from 50-95% C over 8.4 minutes. Hold at 95% C for 1.6 minutes. Return to starting conditions and re-equilibrate for 2 minutes.
Flow Rate:	0.3 mL/min
Injection:	1.0 µL

Sample Concentration:
All compounds at 100 µg/mL except bisphenol S (75 µg/mL)

RESULTS AND DISCUSSION

CORTECS C_8 Benefits and Application

The CORTECS C_8 column offers a slightly different selectivity compared to a C_{18} column. While both ligands are linear alkanes, the C_8 gives less hydrophobic retention. This difference in hydrophobic retention can lead to different separations depending on the analysis.

The decreased hydrophobicity of the CORTECS C_8 column helps to reduce sample run times for fat soluble vitamins, as shown in **Figure 2**.

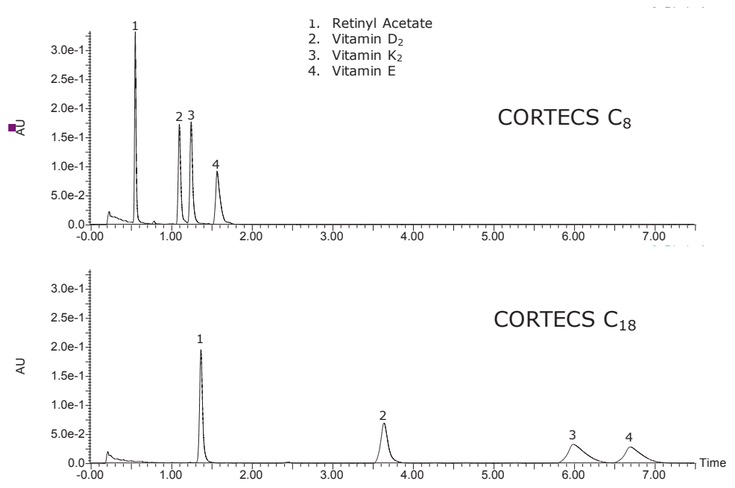

Figure 2. Separation of four fat soluble vitamins on CORTECS UPLC C_8 1.6 µm, 2.1 x 50 mm and CORTECS UPLC C_{18} 1.6µm 2.1 x 50 mm columns.

The CORTECS C_8 column reduces the run time of these analytes approximately 4 times. While all of the compounds still elute in the same order, the overall run time is reduced from 7 minutes to 2 minutes. This translates to both a time and cost savings, with the CORTECS C_8 separation needing 4x less solvent to elute the compounds.

CORTECS Phenyl Benefits and Application

CORTECS Phenyl columns offer a different selectivity compared to traditional C_{18} stationary phases. The phenyl-hexyl group, shown in Figure 1, interacts with analytes through pi-pi interactions as well as the hydrophobicity of the stationary phase. While this additional separation mechanism can be present, using different mobile phases can hinder or augment its effectiveness.

Mobile phase solvents containing pi electrons, such as acetonitrile, can hinder the pi-pi interaction of the analyte with the stationary phase. By removing this interaction, analytes will be separated strictly by their hydrophobicity. Likewise, a solvent that does not contain pi electrons, such as methanol or isopropanol, allows the pi-pi interactions to take place, thereby creating a different selectivity for the column. The effect of solvents on a separation using CORTECS Phenyl columns is shown in **Figure 3**.

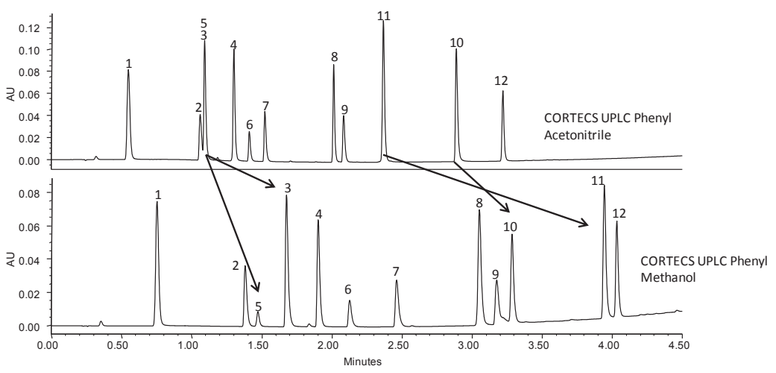

Figure 3. Separation of 12 pharmaceutical related compounds. *1) Acetaminophen 2) Pindolol 3)Quinidine 4)Acebutolol 5) Phenol 6) Chlorpheniramine 7)Triprolidine 8)Prednisolone 9) Nortriptyline 10)2-hydroxy-5-methylbenzaldehyde 11)Diflunisal and 12)Hexanophenone.*

For this mixture of analytes, the use of acetonitrile does not produce an acceptable separation. Quinidine, **3**, and Phenol, **5**, co-elute completely. Additionally these two compounds are partially co-eluting with Pindolol, **2**. Using methanol as the strong solvent causes a drastic change in the separation. Quinidine and Phenol are fully separated not only from each other but also from Pindolol. Additionally, Diflunisal, **11**, and 2-hydroxy-5-methylbenzaldehyde, **10**, have changed elution order. Diflunisal, in particular, has a much stronger interaction with the phenyl ligand in the presence of methanol, resulting in a large change in selectivity.

Taking this phenomenon into consideration is important for an analyst, as it can have a drastic effect on the success or failure of a method. Another example of using CORTECS Phenyl columns is the separation of bisphenol related compounds.

Bisphenol A (BPA) is a known component of many plastics and resins, ranging from drink bottles to receipt paper. Over the years the use of BPA has been reduced due to the hazardous nature of the compound. BPA has begun being replaced with other bisphenol compounds such as bisphenol S, bisphenol F, and bisphenol AF[1]. A separation of 11 different bisphenol compounds, including BPA, was developed on a CORTECS UPLC Phenyl column and is shown in **Figure 4**.

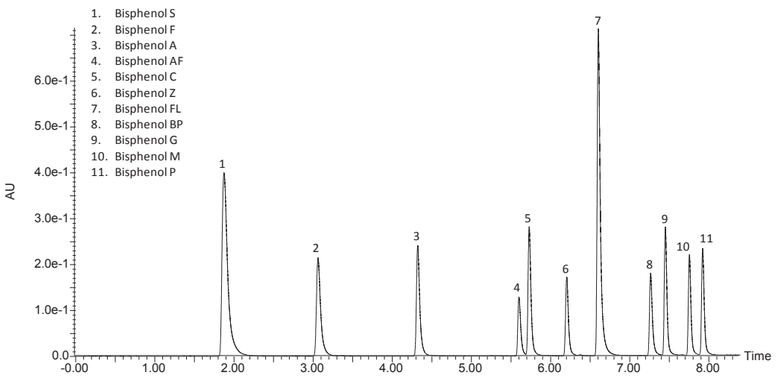

Figure 4. Separation of 11 bisphenol compounds.

The separation shows that all 11 compounds are baseline resolved, and separate under 8 minutes on a CORTECS UPLC Phenyl, 1.6 µm, 2.1 x 100 mm column. BPA is well resolved from the other compounds, as are bisphenols S, F, and AF. This separation could be used for the analysis of "real world" samples to identify and quantify any bisphenols in a sample.

CORTECS C_8 and Phenyl columns offer additional choices for method development for LC separations. With two particle sizes, the CORTECS columns are suitable for use on UPLC, UHPLC, and HPLC instrumentation, making them ideal for method modernization and transfer between instruments or locations.

CONCLUSION

- **The new CORTECS C_8 and CORTECS Phenyl columns provide alternative selectivities in addition to the advantages of solid-core particles (higher efficiency).**
- **CORTECS C_8 columns can provide faster analysis of extremely hydrophobic compounds compared to C_{18} columns.**
- **CORTECS Phenyl columns offer an additional separation mechanism, pi-pi interactions between the stationary phase and the analytes, compared to C_{18} and C_8 columns.**
- **Understanding the effects of solvent on the pi-pi interactions between an analyte and the CORTECS Phenyl stationary phase can lead to better chromatography.**

References

1.Yang, Y et al. Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A*. 1328 (2014). 26-34.

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