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Optimizing Selectivity Through Intelligent Solvent Selection Using CORTECS Phenyl Columns

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Abstract

When choosing an organic solvent for the mobile phase, an analyst should always consider the properties of the analytes. This is especially true for unique stationary phases such as the CORTECS Phenyl Column. This application note shows two examples where the pi electron differences between methanol and acetonitrile are used to obtain different selectivity. By understanding the interplay between the analytes, the mobile phase, and the stationary phase, analysts can tune chromatographic conditions to have greater success in their separations.

Benefits

By understanding the influence of mobile phase on separations using CORTECS Phenyl Columns, an analyst can optimize the selectivity of the column, increasing method development success.

Introduction

Reversed-phase liquid chromatography (RPLC) is an analytical technique which relies primarily on the interaction of hydrophobic analytes with hydrophobic bonded stationary phases. The analytes are retained through this interaction and are eluted using a water and organic solvent gradient, e.g. water/acetonitrile or water/methanol. The use of various stationary phases can produce very different results when testing the same analytical conditions. Likewise, the use of different mobile phases can produce equally different results when analyzing compounds on the same column.

The difference between solvents such as methanol and acetonitrile must be considered when conducting analytical experiments. Methanol is a protic solvent, due to the hydroxyl (-OH) group, that can both donate and accept hydrogen bonds. Acetonitrile is an aprotic solvent that can accept (but not donate) hydrogen bonds, due to the nitrile (-CN) group. Unlike methanol, acetonitrile has pi electrons which are another source of interaction (pi-pi) between analytes and the stationary phase. This application note shows two examples where the pi electron differences between methanol and acetonitrile are used to obtain different selectivity.

Experimental

System conditions – pharmaceuticals

LC system:	ACQUITY UPLC H-Class System
Columns:	CORTECS UPLC Phenyl, 1.6 µm, 2.1 x 50 mm (p/n 18008379)
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:	Starting at 5% organic, increased linearly to 95% over 4.5 minutes
Flow rate:	0.6 mL/min
Column temp.:	30 °C
Injection volume:	1.0 µL
Detection (UV):	254 nm
MS system:	Xevo TQD

System conditions – sudan dyes

LC system:	ACQUITY UPLC H-Class System
Column:	CORTECS Phenyl 2.7 µm, 2.1 x 100 mm (p/n 186008321)

Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	Methanol
Gradient:	20–100% organic over 5.0 minutes, hold at 100% organic for 4 minutes
Flow rate:	0.4 mL/min
Column temp.:	45 °C
Injection volume:	5 µL
MS system:	Xevo TQD
Data management:	Empower 3 CDS and MassLynx v 4.1

Sample preparation

Pharmaceuticals

Twelve drug and drug-like compounds were combined in a vial to a final concentration of 20 µg/mL per analyte, using water as the solvent. The sample was then placed onto the system for injection.

Sudan dyes

Two dyes, rhodamine B and sudan orange G, were combined in a single vial and diluted to 5 µg/mL each, using methanol as the solvent. MRM transitions were used to detect the individual dyes. The samples were then placed onto the system for injection.

Results and Discussion

CORTECS Phenyl Columns offer a unique 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.

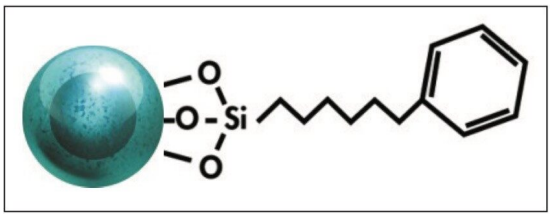

Figure 1. Phenyl ligand attached to the CORTECS solid-core base particle.

A solvent with pi electrons, such as acetonitrile, can interact with analyte pi systems and reduce the pi-pi interactions between the analytes and the phenyl stationary phase. Conversely, a solvent lacking pi electrons, such as methanol, can allow these same analyte and phenyl stationary phase pi-pi interactions to dominate.

An example of how pi electron rich vs. deficient mobile phases can affect separation of a pharmaceutical mixture using CORTECS Phenyl Columns is shown in Figure 2. This chromatography was performed using a 0.1% formic in water and 0.1% formic acid in organic solvent gradient with a flow rate of 0.6 mL/min. The gradient started at 5% organic, increased linearly to 95% over 4.5 minutes. The column was then returned to starting conditions and re-equilibrated for 1 minute. Column temperature was kept at 30 °C and a 1.0 µL injection was performed. UV detection at 254 nm was used to analyze the sample.

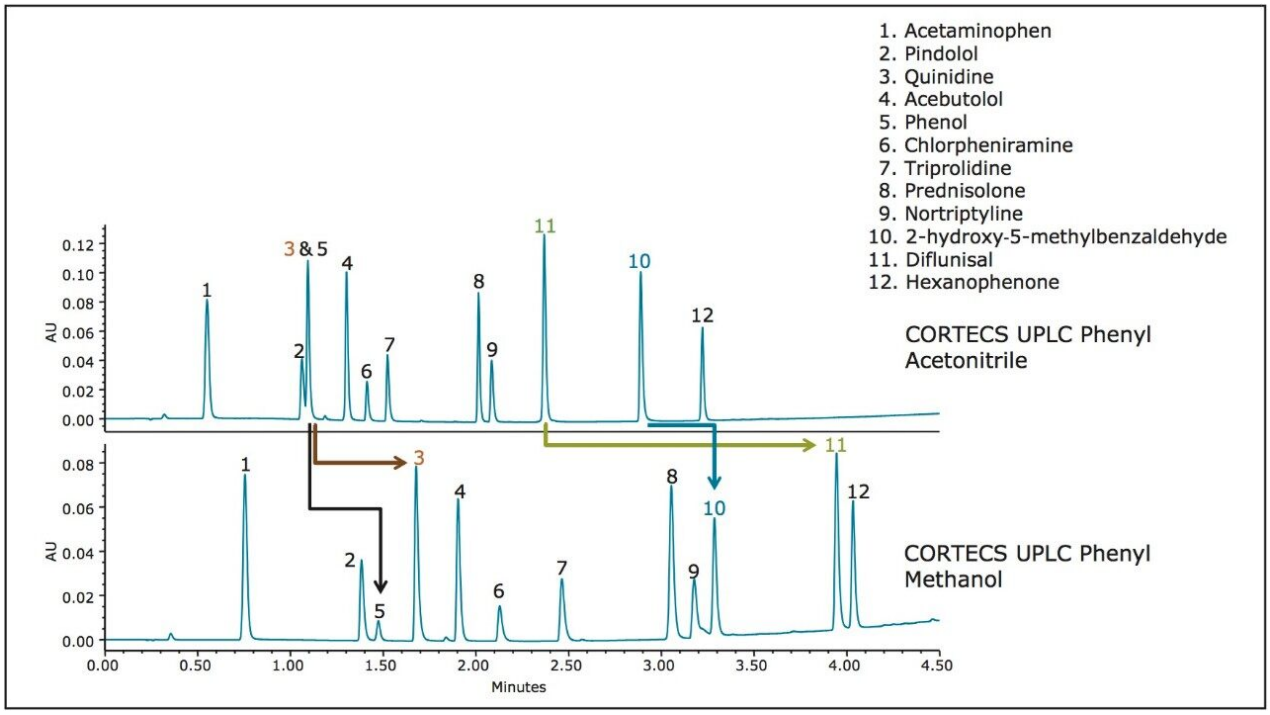

Figure 2. Separation of pharmaceutical compounds using a CORTECS UPLC Phenyl Column, 1.6 µm, 2.1 x 50 mm, (p/n 18008379) with acetonitrile (top) and methanol (bottom) gradients.

Using acetonitrile in the mobile phase does not separate all twelve compounds. Under these conditions, phenol, 5, co-elutes with quinidine, 3, and both partly co-elute with pindolol, 2. When methanol is used, all twelve compounds separate. Quinidine has a significant change in selectivity, with higher retention, due to enhanced pi-pi interaction with the phenyl ligand of the stationary phase.

Diflunisal, 11, and 2-hydroxy-5-methylbenzaldehyde, 10, also change elution order, presenting a unique selectivity difference. Diflunisal, in particular, has a much stronger interaction with the phenyl ligand in the presence of methanol, resulting in a large change in selectivity. The chemical structures of the affected analytes are shown in Figure 3. The use of methanol for this sample provides additional selectivity for compounds which can have strong pi-pi interactions with the phenyl stationary phase.

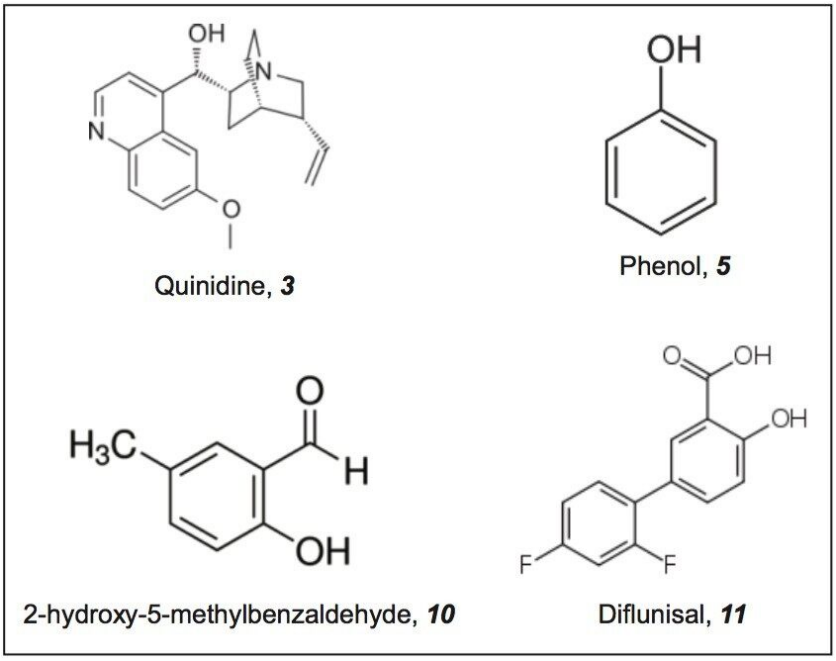

Figure 3. Structures of four analytes susceptible to pi-pi interaction with a phenyl stationary phase.

In some cases, masking pi-pi interactions using acetonitrile may be an effective way to manipulate selectivity when using CORTECS Phenyl Columns. Consider the example of two dyes, Rhodamine B, 13, and Sudan Orange G, 14. The structures of the compounds are shown in Figure 4.

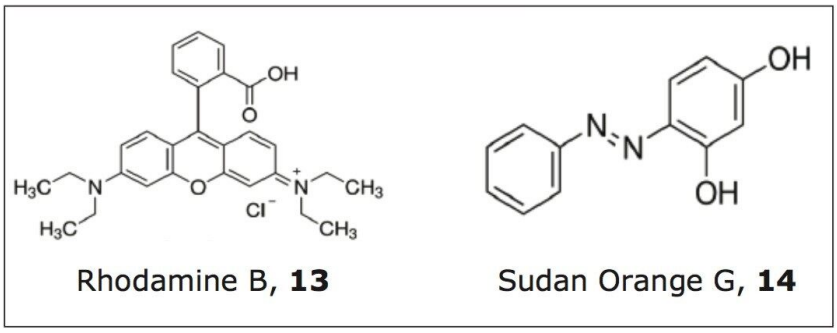

Figure 4. Structure of dyes used in this study.

These compounds were analyzed on an ACQUITY UPLC H-Class System with a CORTECS Phenyl Column, 2.7 µm, 2.1 x 100 mm (p/n 186008321) using methanol and acetonitrile gradients. The column was kept at 45 °C and a 5 µL injection was performed. A flow rate of 0.4 mL/min was used to generate the linear gradient of

20–100% organic over 5 minutes, and a 100% organic hold for 4 minutes. The column was then re-equilibrated to starting conditions for 3 minutes.[1,2] The chromatograms for the separation of the individual compounds using acetonitrile and methanol are shown in Figure 5.

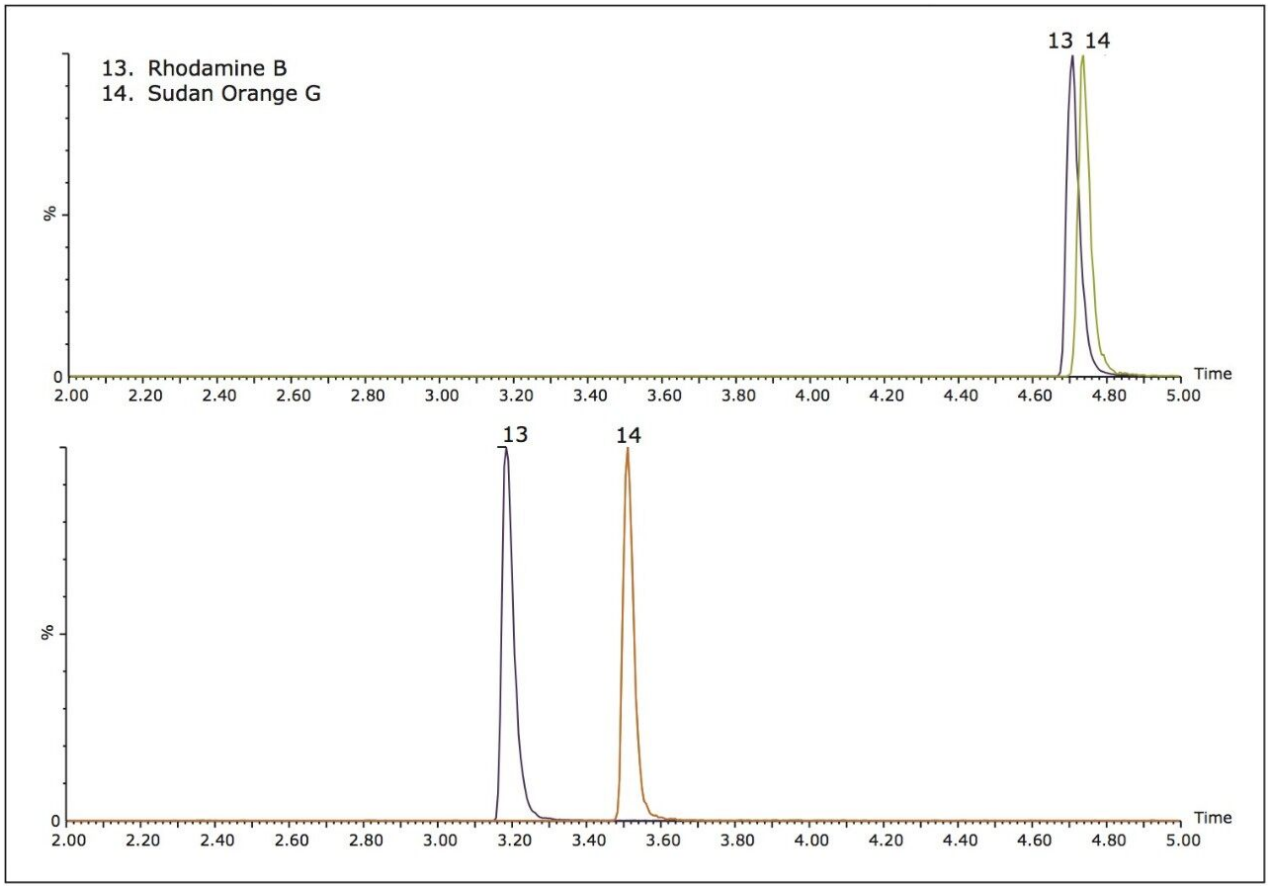

Figure 5. Chromatograms for the separation of the standard injections of rhodamine B and sudan orange G using a CORTECS Phenyl Column and a methanol gradient (top), and an acetonitrile gradient (bottom).

With methanol in the mobile phase, these compounds co-elute. Under these chromatographic conditions, the extensive pi electron system of each analyte is free to interact strongly with the pi electrons of the phenyl stationary phase and this effect dominates over simple hydrophobic interactions. In contrast, the pi electron rich acetonitrile solvent provided the selectivity needed to separate these two compounds. The acetonitrile masks pi-pi interactions between analytes and the phenyl stationary phase causing the two dyes to be separated based on hydrophobicity. Since rhodamine B is significantly less hydrophobic, it becomes significantly less retained and separates from the sudan orange G when acetonitrile is used in the mobile phase.

Conclusion

When choosing an organic solvent for the mobile phase, an analyst should always consider the properties of the analytes. This is especially true for unique stationary phases such as the CORTECS Phenyl Column. Pi electron deficient solvents such as methanol, can help to strengthen the pi-pi interaction of an analyte and the phenyl stationary phase, whereas pi electron rich solvents, such as acetonitrile, can minimize that interaction and allow other influences to dominate. By understanding the interplay between the analytes, the mobile phase, and the stationary phase, analysts can tune chromatographic conditions to have greater success in their separations.

References

1. Rapid Analysis of Sudan Dyes and Other Prohibited Dyes in Chili Powder Using the ACQUTIY UPLC H-Class System with Xevo TQD. Waters Application Note. Part Number: 720004975EN.
2. LC-MS Determination of Sudan Dyes in Chili Oleoresin Using the CORTECS C_{18}, 2.7µm Column. Waters Application Note. Part Number: 720005070EN.

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