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Improving Method Development Using a New Reversed-Phase High-Performance Liquid Chromatography (HPLC) Column with Unique Selectivity

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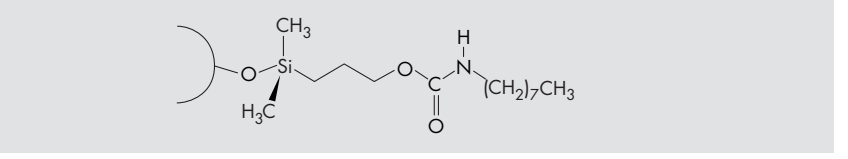
Introduction

The process of developing HPLC methods is a critical step in the work of an analytical chemist. Frequently, the investigator encounters a situation where the selectivity obtainable on a particular column fails to meet the separation requirements, even after systematic optimisation of the mobile phase (1,2). When this happens the chromatographer often resorts to use of unusual mobile phase compositions or additional mobile phase additives. In this paper, we demonstrate an alternative approach: when a separation does not respond to simple variations in mobile phase composition, it is often advantageous to change to a column that has significantly different selectivity characteristics.

The SymmetryShield™ RP_8 Packing Material

Waters has recently developed a unique patented reversed-phase packing material (SymmetryShield ™ RP_8) possessing selectivity characteristics significantly different from traditional C_8 and C_{18} materials (3). The modified selectivity arises from the insertion of a carbamate group into the alkylsilane bonded phase (Figure 1). The incorporation of polar groups into alkylsilane bonded phases has been shown to significantly modify their selectivity characteristics, due to perturbation of both analyte/stationary phase and mobile phase/stationary phase interactions (3-7). These polar functional groups compete with polar analytes for the interaction with the surface silanols. Therefore, one particularly notable feature of all reversed-phases containing embedded polar group is the reduction in both retention and peak tailing of amines.

Figure 1: Structure of the SymmetryShield™ RP_8 bonded phase



This reduced retention can be attributed to a shielding of the silanols by the embedded polar group.

Discussion

In the process of methods development, the investigator often encounters a situation where the selectivity obtainable on a particular packing does not entirely match the requirements of the application. In such a situation, the chromatographer usually struggles for extended periods of time with unusual mobile phase compositions or additional mobile phase additives. An alternative approach that has proven very valuable in our laboratory is the use of a column chemistry that provides similar retention characteristics but different selectivity compared to a classical reversed-phase column. The SymmetryShield™ RP_8 column provides this alternative. The addition of the carbamate group in the bonded phase significantly changes the interaction between polar functional groups in the analyte molecule and the silanol groups present on all bonded-phase packings. As a consequence, switching from a standard C_8 or C_{18} column to a SymmetryShield™ RP_8 column can drastically affect the peak spacing and the elution order of analytes without the need to change mobile phase composition. This allows for a rapid comparison between a standard C_8 column and the SymmetryShield™ RP_8 column.

The orientation of the carbamate group should be emphasised. It is oriented such that after a hydrolytic loss of the ligand at the carbamate function, an interactively neutral alcohol function is formed. Although careful hydrolysis studies have not revealed any stability issues, we purposely designed the phase to avoid any inadvertent secondary interactions. This is not the case for other packing materials available on the market, which also rely on polar functional groups to prevent the interaction between analytes and surface silanols (7).

A selectivity study of SymmetryShield™ RP_8 was made using simple substituted benzenes as samples. It was found that, generally, the methylene group selectivity was lower on the SymmetryShield™ RP_8 column than on a standard Symmetry® C_8 column. At the same time, the selectivity for polar functional groups was significantly different for both phases. There was a reduced retention for very polar functional groups on the SymmetryShield™ RP_8 column, especially for amino-, alcohol- and amide- functions, and an increased retention for phenols and acids at acidic pH. This indicates that the influence of acidic silanols on the retention of analytes is decreased using the SymmetryShield™ RP_8 column. An example of the selectivity differences is shown in Figure 2.

On the SymmetryShield™ RP_8 column, we find a significant decrease in retention for benzyl alcohol and benzylamine, and a slight increase in retention for phenol and benzoic acid. It is believed that the reason for these selectivity differences is due to the fact that water strongly binds to the polar carbamate group. This tightly bound layer of water prevents interactions between the analytes and the underlying tether function as well as with the surface silanols of the underlying silica. As a consequence, surface silanols do not play as much of a role in the retention of analytes on the SymmetryShield™ RP_8 column as they do on a standard C_8 column. Also, good peak shapes are obtained for polar analytes, in addition to the differences in selectivity discussed above. Thus, the name of the column is quite appropriate: the surface silanols are "shielded" from interacting with the polar functional groups of analytes. Such differences in interaction can result in significant shifts in the selectivity of a separation.

As mentioned above, the SymmetryShield™ RP_8 column often yields a separation when mobile phase modification on a C_8 packing has failed to do so. An example is shown in Figure 3. On the left, the separation of furazolidone impurities is shown on a Symmetry® C_8 column. It appears that there is only a single impurity present. However, as shown in the chromatogram on the right, the SymmetryShield™ RP_8 column cleanly separates the impurity into two well resolved peaks. In this case, the selectivity difference between a SymmetryShield™ RP_8 column and a Symmetry® C_8 column is quite striking.

Another example of selectivity differences is shown in Figure 4 for the Alberta peptide standards. A significant reduction in retention is observed for these peptides on the SymmetryShield™ RP_8 column, as compared to the standard Symmetry® C_8 column.

Figure 2: Selectivity differences between SymmetryShield™ RP_8 and Symmetry® C_8 packings

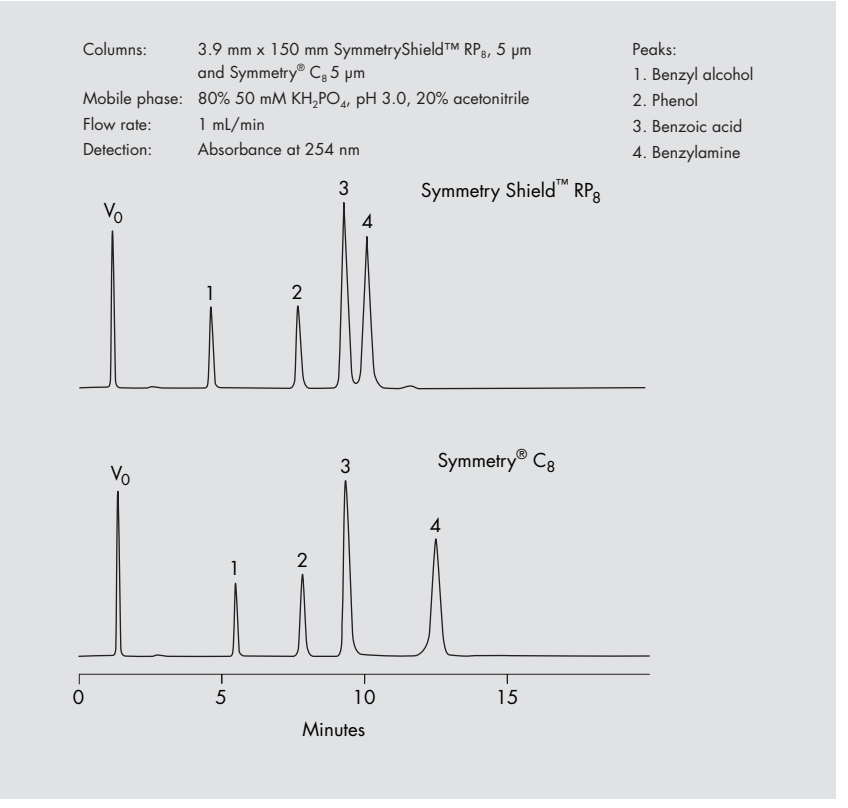
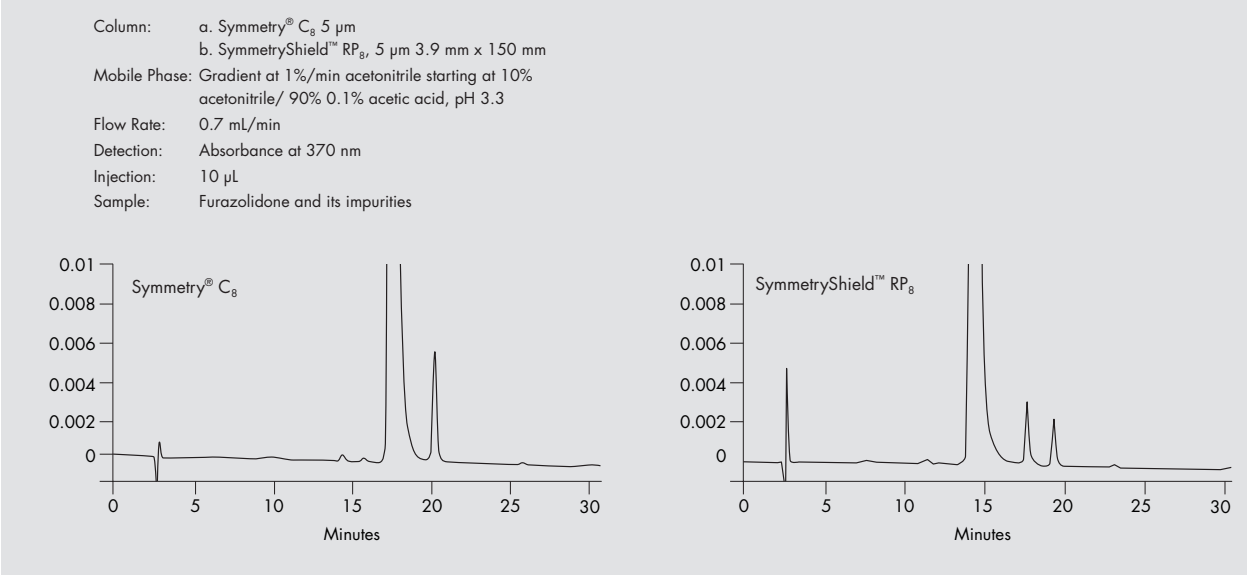


Figure 3: Selectivity comparison for furazolidone impurities



This reduction in retention is due to a decrease in the interactions of these basic peptides with the SymmetryShield™ RP_8 column compared to the Symmetry® C_8 column. This reduced interaction is completely in line with the expectations outlined above. It appears that the SymmetryShield™ RP_8 column provides additional separation capabilities for peptide samples where the options for mobile phase manipulation are more limited.

A further example in Figure 5 shows the separation of phenoxyacid herbicides and phenols on a SymmetryShield™ RP_8 column. The separation of all 16 EPA 555 analytes is accomplished without difficulties using this column. The same separation is not possible using standard reversed-phase columns. Once again, the advantage of the different selectivity achievable with the SymmetryShield™ RP_8 column is well demonstrated for this application.

For separations of compounds with differences in the pK_a, significant variations in elution order have been observed between a standard C_8 column and the SymmetryShield™ RP_8 column. An example is the separation of the weak acid, acetaminophen; the strong base, codeine and caffeine at pH 3 (Figure 6). On the Symmetry® C_8 column, codeine elutes after acetaminophen. Using the identical mobile phase conditions on a SymmetryShield™ RP_8 column, significantly different retention times were observed. Most importantly, the elution order of acetaminophen and codeine changed. The basic compound codeine exhibits less retention than acetaminophen on the SymmetryShield™ RP_8 column, while it elutes after acetaminophen on the Symmetry® C_8 column. This is completely in line with the expectations outlined above.

In addition to the selectivity differences demonstrated, SymmetryShield™ RP_8 columns also perform better than standard reversed-phase columns in highly aqueous mobile phases.

Figure 4: Difference in selectivity for polar basic peptides

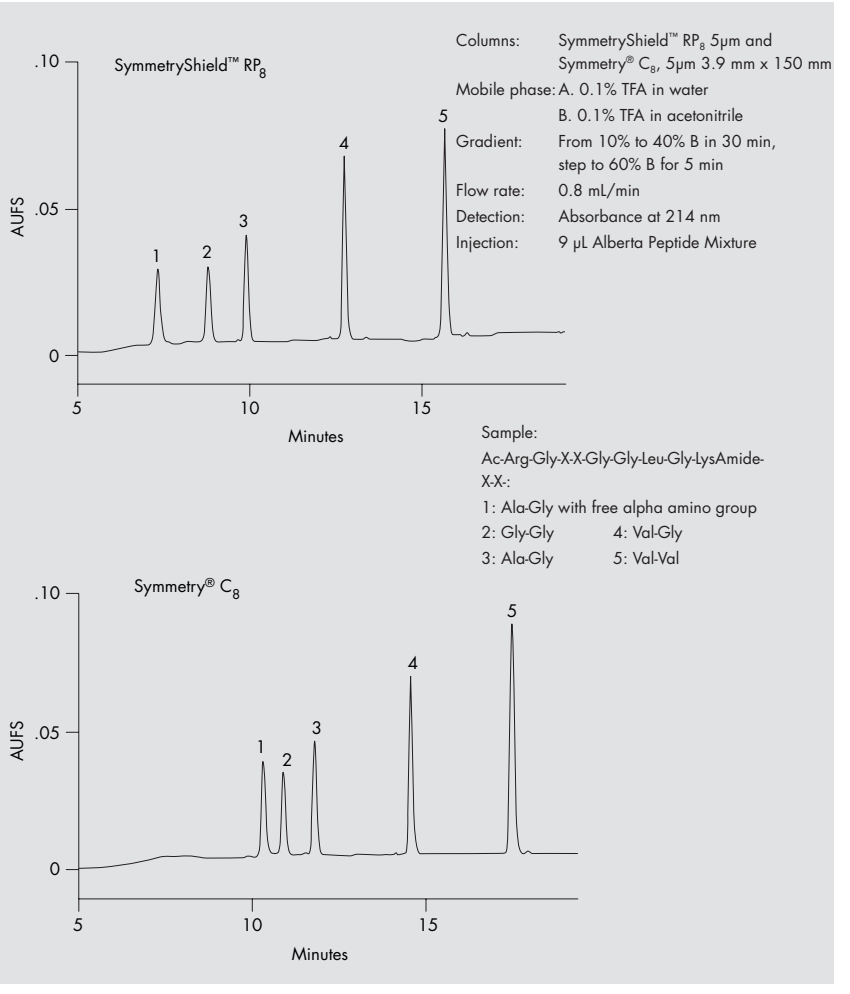
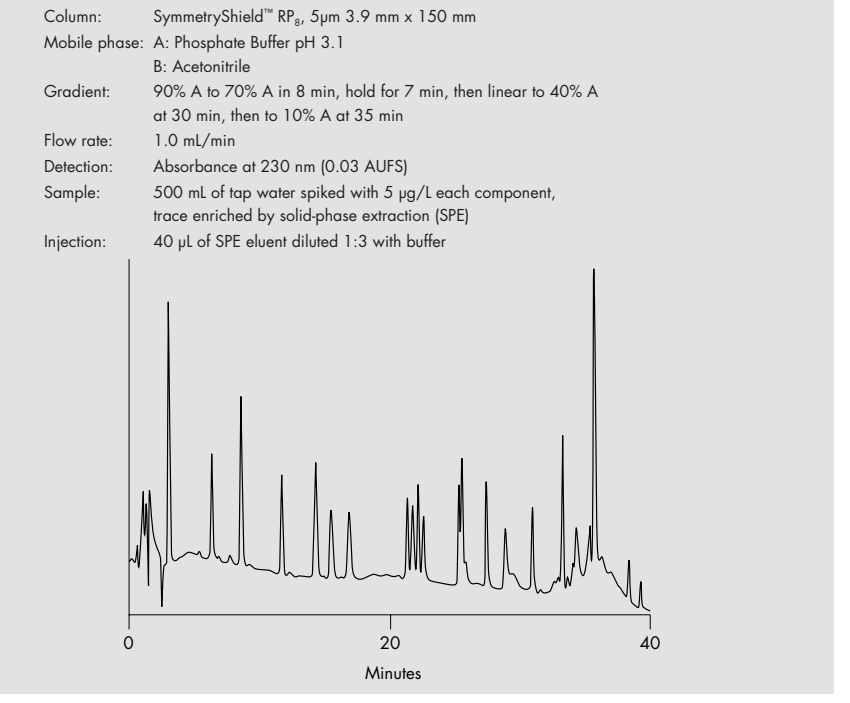
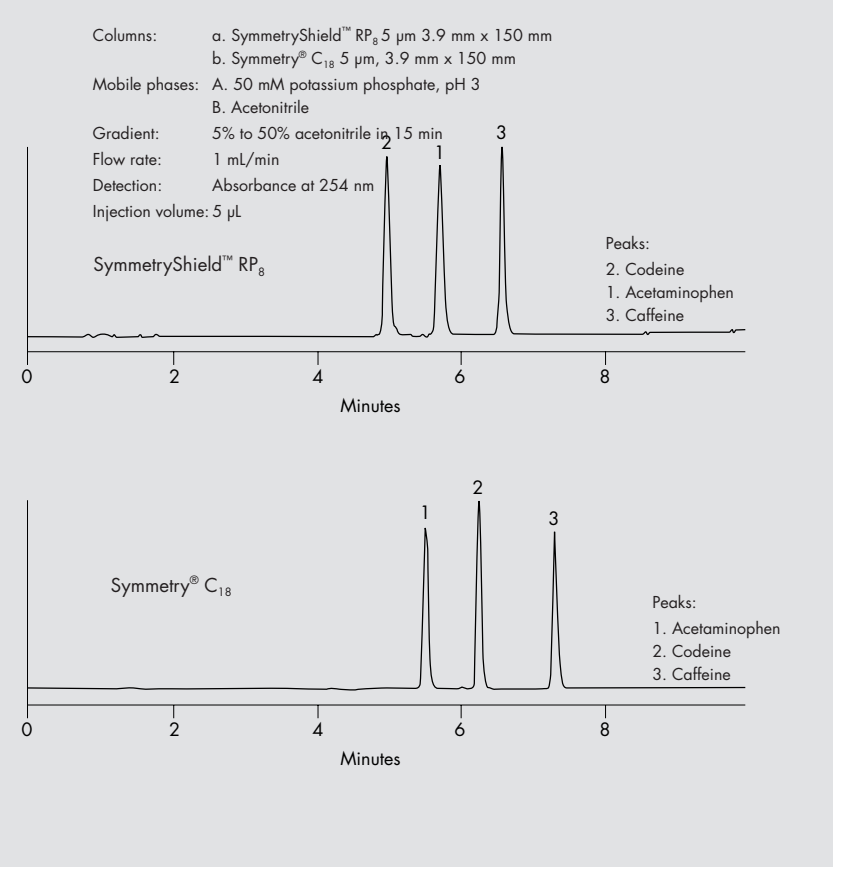


Figure 5: Separation of phenoxyacid herbicides and phenols on a SymmetryShield™ RP_8 column



This improvement arises because the embedded polar functional group in the bonded phase ensures that the packing remains well wetted even in mobile phases containing 100% water. In contrast, in highly aqueous (> 90% water) mobile phases, high coverage C_8 and C_{18} columns often show erratic retention (8). This has been attributed to poor wettability of the bonded alkylsilane groups, preventing the partitioning of analyte into the stationary phase (9). Since the SymmetryShield™ RP_8 packing does not exhibit this problem, it can be used with advantage in the separation of very polar analytes, which require mobile phases with a high water content. These advantageous wetting properties can be readily demonstrated using very polar analytes, such as the antibiotic amoxicillin. The mobile phase consisted of 99.9% water and 0.1% glacial acetic acid. Good retention was achieved when the separation of this analyte was run on a Symmetry® C_8 column. However, when the flow was stopped for a short time (5 min), then resumed, the retention of this compound was lost completely. It was only possible to regain the original retention of the compound by reconditioning the stationary phase first with methanol, then with the aqueous mobile phase. When the same experiment is performed using a SymmetryShield™ RP_8 column, no loss of retention is observed. Fundamentally, this shows that the surface remains wetted even when a mobile phase containing 100% water is used. This wetting phenomenon is due to the polar functionality incorporated into the stationary phase. As mentioned already in the discussion of the retention mechanism, the polar carbamate group of the SymmetryShield™ RP_8 column binds water very strongly, thereby preventing the "hydrophobic collapse" observed with standard reversed-phase columns in mobile phases with a very high water content. Consequently, the SymmetryShield™ RP_8 column can be used advantageously for the analysis of very polar compounds that require mobile phase compositions approaching 100% water.

Figure 6: Gradient separation of acetaminophen, codeine and caffeine



Conclusion

The SymmetryShield™ RP_8 column represents a powerful new tool for reversed-phase separations. The selectivity of this column for compounds with polar functional groups can be significantly different from the selectivity of standard reversed-phase columns. This property can be used advantageously in methods development. This is especially true in circumstances where a good separation with standard reversed-phase columns appears to be difficult to achieve. The difference in the selectivity characteristics observed between the SymmetryShield™ RP_8 column and standard reversed-phase column is primarily due to the shielding of the surface silanols by the carbamate group incorporated in column. Another advantage of this column is its good wettability in highly aqueous mobile phases. The SymmetryShield™ RP_8 column significantly enhances the tools available to the methods development chemist and opens a new dimension in reversed-phase selectivity.

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Ordering Information

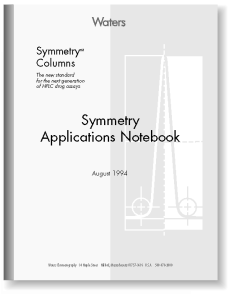
SymmetryShield™ RP_8 5 µm Columns						Sentry™ Guard Column**
Dimension	2.1 x 150	3.0 x 150	3.9 x 150	4.6 x 150	4.6 x 250	
Part No.	WAT094245	WAT094243	WAT200655	WAT200662	WAT200670	WAT200675
SymmetryShield™ RP_8 5 µm Cartridge Columns*						
Dimension	3.9 x 50		3.9 x 150	4.6 x 150	4.6 x 250	
Part No.	WAT094248		WAT200658	WAT200665	WAT200661	
SymmetryShield™ RP_8 3.5 µm Columns						
Dimension	2.1 x 50	4.6 x 50	4.6 x 75	4.6 x 100	4.6 x 150	
Part No.	WAT094257	WAT094260	WAT094263	WAT094266	WAT094269	
SymmetryShield™ RP_8 3.5 µm Cartridge Columns*						
Dimension			4.6 x 75	4.6 x 100		
Part No.			WAT094272	WAT094275		
SymmetryShield™ RP_8 5 µm Method Validation Kits (three columns from 3 different batch materials)						
Dimension	2.1 x 150	3.0 x 150	3.9 x 150	4.6 x 150	4.6 x 250	
Part No.	WAT094254	WAT094251	WAT210594	WAT210588	WAT210591	
SymmetryShield™ RP_8 5 µm Method Validation Kits (three cartridge columns* from 3 different batch materials)						
Dimension			3.9 x 150	4.6 x 150	4.6 x 250	
Part No.			WAT210582	WAT210585	WAT210579	
SymmetryShield™ RP_8 3.5 µm Method Validation Kits (three columns from 3 different batch materials)						
Dimension			4.6 x 150			
Part No.			WAT094278			

Description	Part No.
Endfittings	WAT037525
Integrated Guard Holder (for Waters steel cartridge columns only)	WAT046905
Universal Guard Holder (for any HPLC column)	WAT046910

* Requires reusable endfittings

** Guard columns require the appropriate Sentry Guard Holder. (3.9 x 50 mm cartridge columns must use the Universal Guard Holder)

For complete Symmetry® ordering information, please check box 5 on the business reply card.



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