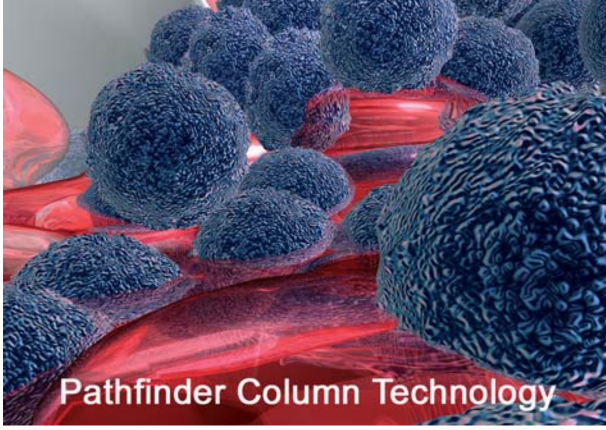
Application Note

Column Classification and Selection of Pathfinder Stationary Phases with Embedded Polar Groups



A procedure is discussed in which several polar embedded Pathfinder columns are classified. The polarity, hydrophobicity and steric selectivity of the columns were considered. In this study procedure two bench mark ODS columns were taken as reference. Pathfinder AS, AP, MR and PS columns showed different selectivity, compared to the other columns ODS.

Introduction

In the development of LC methods various parameters can be used (e.g. type of modifier, pH of the mobile phase, temperature) to optimize a separation. The majority of RPLC separations is nowadays performed on C18 based stationary phases. Many commercial C18 columns have similar retention behaviour. For the chromatographer this is convenient in cases when a manufacturer discontinues the production of a column, or when batch reproducibility problems are observed [1]. The drawback of columns with similar retention behaviour is when a chromatographic method is unsuccessful, all alternatives will also fail.

A good example is the development of pharmaceutical drugs. In this process it is important to know what happens whit the drug candidate and its relating compounds during the synthetic pathway. Therefore a range of chromatographic methods with varying selectivity are used, to get as much as possible information and confirmation.

Pathfinder media is a new generation of HPLC packing material, made from organic and inorganic building blocks: one that forms the internal silica core, and another that forms the external chemically stable and inert polymer capsule (figure 2). Pathfinder technology allows the design of stationary phases with well-defined properties such as hydrophobicity and polarity.

In this application note we present a experimental design in which a small group of analytes was selected to characterize the specific contributions to the overall retention. Based on this design, the properties of the five pathfinder columns and two typical ODS columns are very well highlighted and simplifies column selection.

Experimental conditions

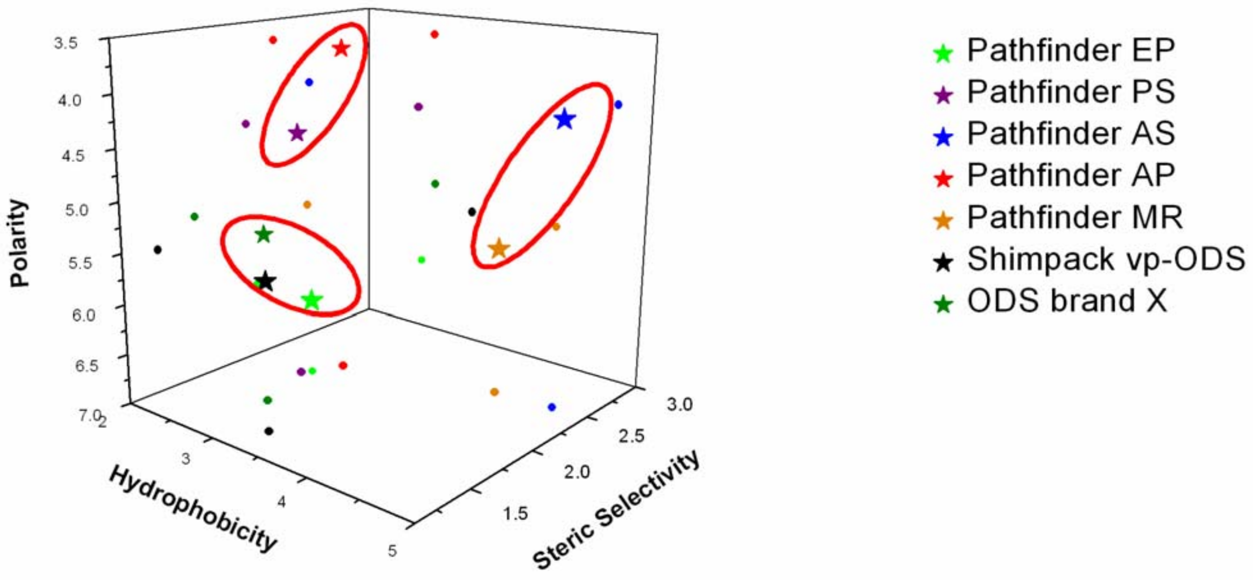


Figure 1. An 3-dimensional column classification design in which five Pathfinder columns and two bench mark ODS columns are displayed.

Instrumentation

A Shimadzu Prominence® liquid chromatograph equipped with a model LC-20AD pump, gradient mixer, SIL-20AC autosampler with cooling function, SPD-M20A diode array detector and a CTO-20AC oven. A CBM-20A system controller was used to control the modules

Hydrophobicity

Mobile Phase : H_2O:ACN (45:65)

The retention factor of ethylbenzene is an indicator of ligand density.

Shape selectivity:

Mobile Phase : H_2O:MeOH (20:80)

Retention factor ratio between triphenylene and o-terphenyl. This descriptor is a measure of the ability to separate compounds based on their molecular shape.

Polarity

Mobile Phase : H_2O:ACN (30:60)

Ratio between the retention factors of phenol and benzene $\alpha_{p/b}$.

Further experimental conditions

Temperature: 30°C
Flow: 1.0 ml/min
Detection : UV@254
Injection volume: 5 µl

Column dimensions

Dimensions: 4.6 mm x 150 mm
Particle size: 5.0 µm

Results and Discussion

When developing a design to classify stationary phases it should reflect the most important properties of the column. In this study the hydropobicity, the polarity and the shape selectivity were considered[3].

Ethylbenzene is an absolute non polar compound and is therefore a good indicator for the hydrophobicity of a packing. Hydrophobicty is a consequence of the type of ligand and the ligand density of a packing. As can be seen in the 3D plot (figure 1) Pathfinder MR, PS, EP and both ODS columns show similar hydrophobicity. Pathfinder AP and AS show extended retention behaviour compared to the others. The high surface coverage is a the advantages of the polymer encapsulated technology. Much higher bonding densities can be realised, since the coverage is not more dependent on the available amount of silanollic sites.

The polarity of columns was measured by calculating the relative retention between benzene and phenol. The difference between these two compounds is an OH-group what makes phenol much more polar than benzene. The smaller the alpha value, the higher the polarity of the packing. Retention of phenol is less influenced by the hydrophobic model, which retains benzene. Also reversed, the higher the alpha value is, the more phenol is retained by the hydrophobic model. In figure 1 it can be seen that the two ODS columns, Pathfinder EP and AS have a similar surface polarity. The Pathfinder PS, AP and especially Pathfinder MR have a significant higher surface polarity. With these columns chromatographers will always observe a more retention for polar analytes compared with traditional ODS columns.

The steric selectivity is defined as the ability of a column to separate compounds with the same polarity and hydrophobicity but different spatial conformation. As measurement for shape selectivity often o-terphenyl and triphenylene are used. Both molecules have the same molecular weight and size, only triphenylene is planar and o-terphenyl is twisted out of plane. Figure 1 clearly demonstrates that all Pathfinder columns have a higher shape selectivity compared with traditional ODS columns. This exceptional high shape selectivity can attributed to the high ligand density and the bonding chemistry. This is also discussed in literature by Sander & Wise [4] and Engelhardt[5].

In figure 1 the three parameters are diplayed in a three dimensional design. Chromatographers can select a column based on the properties of the analytes. On the plane of each dimension, the individual results are plotted. These are visualized by dots. The stars in the 3D-plot, are the combination of the individual parameters. The two ODS columns show similar selectivity for all tests. The Pathfinder EP column shows the highest similarity with the two other bench mark column. A different group of columns are the Pathfinder PS and MR. These columns show both a high degree of polarity.

With novel columns designed for the separation of polar analytes the increased surface polarity is often compromised by a reduced hydrophobicity (data not shown [6]) As can be seen in figure 1 the hydrophobicity of Pathfinder columns is comparable or even higher than traditional ODS columns. This results in extended retentively for polar analytes.

The above developed design, is a useful tool for the selection of a suitable column for a particular application. For example, if one wants to separate ascorbic acid, which is added to foodstuffs as anti oxidant, a Pathfinder MR would be the best candidate. Ascorbic acid is a highly polar compound and often poor retained on traditional ODS columns. Another example is the separation of PAH compounds, these compounds have close related structures with similar molecular weight and polarity. In these cases it is important that the selected column is able to separate compounds based on their 3 dimensional arrangement, an AS or AP column would probably give the best results.

Conclusion

In this application note we proposed a design for the classification and selection of Pathfinder columns. We have visualized the most important properties of molecules: there hydrophobicity, polarity and shape.

We also showed that there is a significant difference between the traditional ODS columns and the Pathfinder columns. The Pathfinder EP column shows the highest similarity with the two bench mark ODS columns.

References

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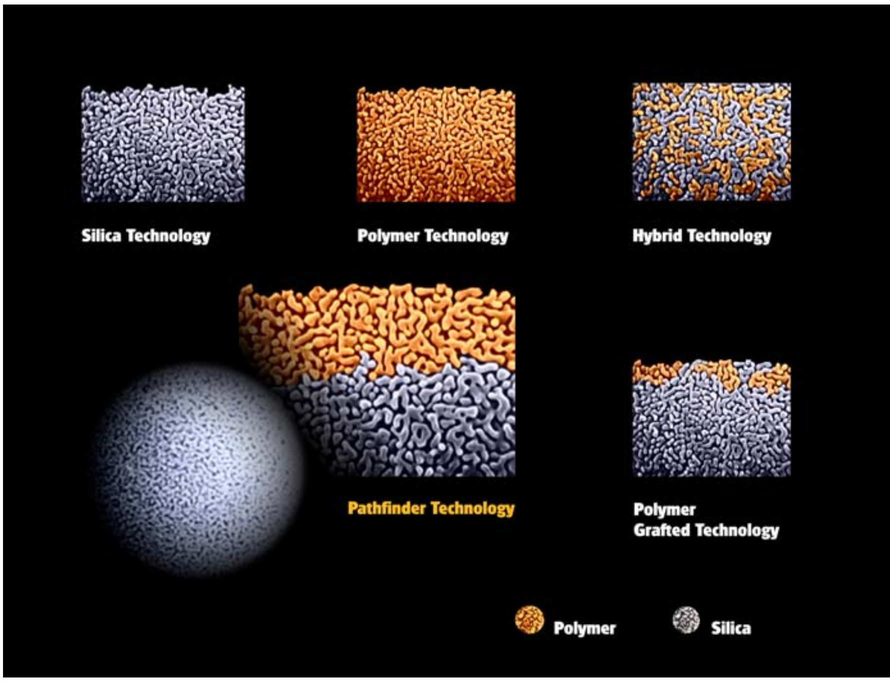

Figure 2. Overview of the most common supports used for reversed phase chromatography. Unlike other hybrid materials, where polymeric groups exist through the whole particle, Pathfinder technology only covers the surface layer with polymer. This preserves the high mass transfer characteristics of silica based materials and shares the chemical stability of polymeric stationary phases.