POPLC®

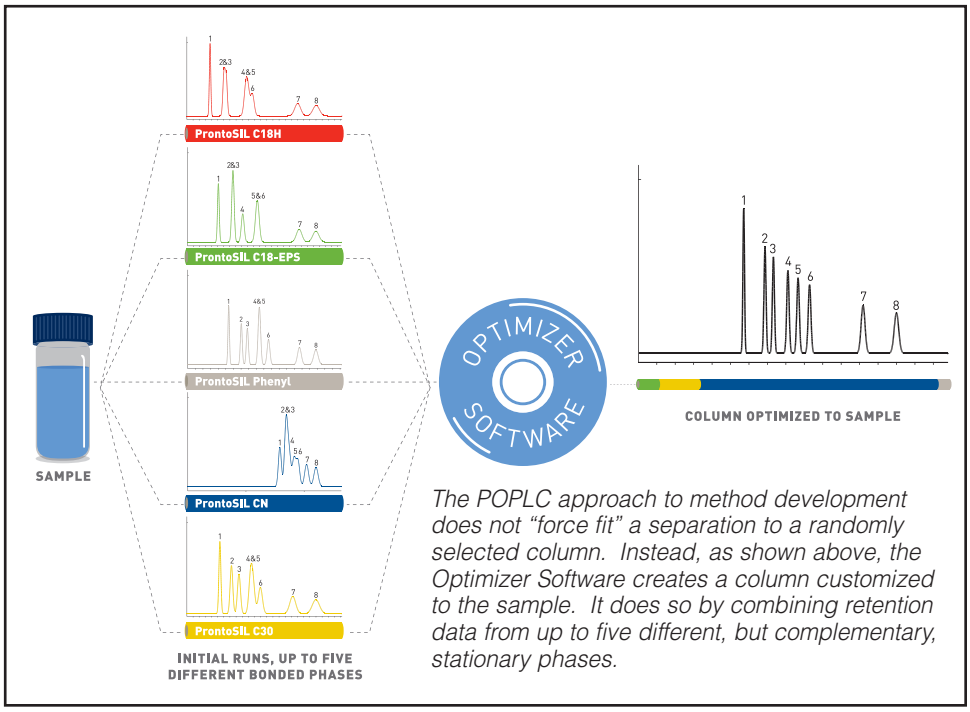
Phase OPtimized Liquid Chromatography

Phase Optimization: A New Approach to HPLC Method Development

Introduction

By some estimates, there are close to 800 different HPLC stationary phases to choose from when beginning an HPLC method development project. Choosing a column usually comes down to an educated guess, and whether it will successfully separate your analytes is usually not obvious until many hours have been spent evaluating different mobile phase conditions. If the column fails, another is chosen and the process is repeated until the right combination of stationary phase and mobile phase is discovered.

FIGURE 1



The POPLC approach to method development does not "force fit" a separation to a randomly selected column. Instead, as shown above, the Optimizer Software creates a column customized to the sample. It does so by combining retention data from up to five different, but complementary, stationary phases.

The reason there are so many stationary phases to choose from is quite simple; selectivity. It is widely accepted that stationary phase selectivity is the most important factor in determining success or failure of a separation. The problem with this classical method development approach is that of the 800 phases available, many offer similar selectivities. A base deactivated C18 from one manufacturer is likely to be quite similar to that from another manufacturer. If your sample separates on the first C18 that you try, your problem is solved. If not, then you begin the arduous task of screening other columns until you find one that works.

In Phase Optimized Liquid Chromatography (POPLC®), a technique developed by Bischoff Chromatography, the stationary phase is first optimized for a given sample. Once you are assured of having the optimum stationary phase, it is a much simpler task to adjust the mobile phase for the desired speed and resolution.

The POPLC Approach to Method Development

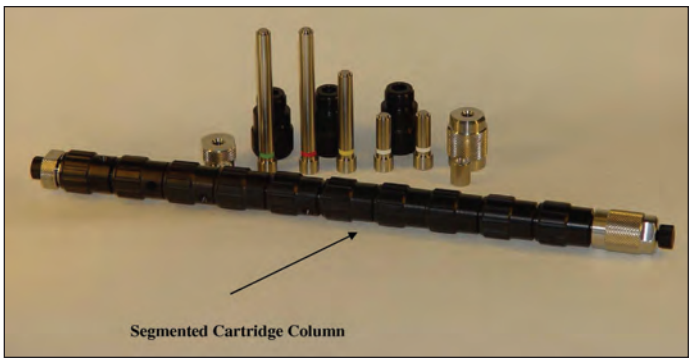
In POPLC, rather than making a rough estimate of the appropriate column and then optimizing the mobile phase, one does the reverse. A rough choice of mobile phase is made, and then the sample is run isocratically on short columns of three or more different bonded phases. (Figure 1) The mobile phase chosen is based on previous experience with the sample, or determined by running a scouting gradient and choosing an organic composition near the mid-point of the chromatogram. Retention times for all components on each stationary phase are input to the POPLC Optimizer software. The software is based on the simple principle that retention times are additive. In other words, the retention time of an analyte on a column made up of segments of different stationary phases will be the sum of its retention times on the individual stationary phases. Armed with these data, the software can determine the optimum column, consisting of various length segments of the different stationary phases used. Based on the requirements specified by the investigator, the method can be optimized for speed, resolution or both. The user can specify maximum column length, desired analysis time and required resolution. Once the optimum column has been indentified, the various segments are assembled using the POPLink coupling system (Figure 2). Mobile phase optimization is then carried out either manually or computer aided by using other programs.

The POPLink system is ideal for quickly combining various stationary phase segments. Up to ten segments can be linked with no detectable loss in efficiency. Once a column combination has been finalized, the segments can be linked inside a solid stainless steel tube similar in appearance to a conventional HPLC column. A real benefit of this design is that as the column wears, there is no need to replace the entire column, only the first segment, as the inlet end is where column failure normally occurs.

Complementary Selectivities

Rather than work with hundreds of random stationary phases, the POPLC approach uses up to five different stationary phase types which offer complementary, or even orthogonal selectivity. The POPLC system includes column segments of varying lengths and stationary phases which can be coupled together to form a custom column optimized for your specific sample (Figure 2).

FIGURE 2



Column segments of varying lengths can be coupled with the POPLink system. Individual cartridges are color coded by stationary phase so that the column sequence can be viewed from the outside.

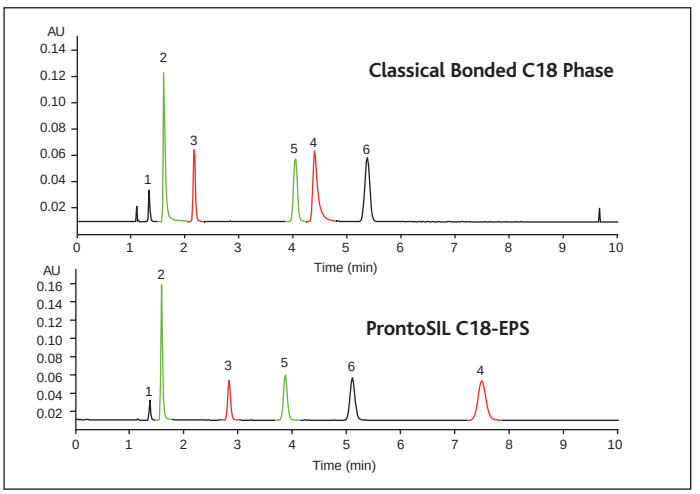
The stationary phases included in the POPLC system are as follows:

ProntoSIL C18 EPS-2: This is a C18 phase with an embedded amide group. As such, it offers additional polar selectivity over a conventional C18 column. In general, it will show greater retention for acids, and slightly less retention for bases. See Figure 3.

ProntoSIL C18 SH-2: This is a classic C18 stationary phase with maximum carbon loading and effective endcapping. It separates purely by hydrophobic interaction. See Figure 3.

FIGURE 3

Comparison of Selectivity
Acid, Basic and Neutral Solutes



This figure illustrates differences in selectivity for a classically bonded C18 phase (top) and a polar embedded phase (bottom). Acidic compounds are retained longer while bases are retained slightly less on the polar embedded phase.

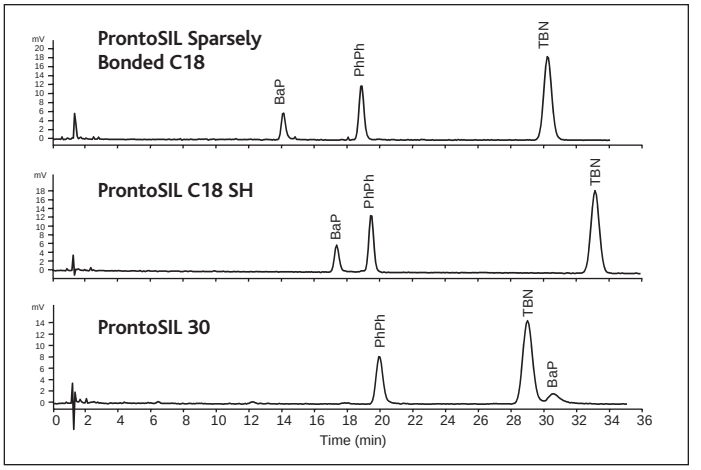
ProntoSIL Phenyl-2: This modern phenyl phase offers orthogonal selectivity to a C18 phase through $\pi - \pi$ interactions.

ProntoSIL CN-2: This is an endcapped cyanopropyl phase with very low hydrophobic interaction. Polar selectivity is excellent.

ProntoSIL C30: This non-endcapped C30 has very high shape selectivity in that it can separate planar and non-planar analytes such as cis – trans isomers and fused ring aromatic compounds. See Figure 4.

FIGURE 4

Influence of Shape Selectivity



Benzo(α)pyrene (BαP) is lower molecular weight than tetrabenzonaphthalene (TBN) and, as predicted, elutes earlier on columns with low shape selectivity. The bottom chromatogram, however, illustrates the high degree of shape selectivity of ProntoSIL C30. Since BαP is a planar molecule, it has greater access to the stationary phase, and is retained longer than non-planar TBN.

Example – Separation of Triazine Pesticides

The structures of eight triazine pesticides are shown in Figure 5. POPLC was used to develop an isocratic method with baseline resolution of all pesticides. Figure 6 shows the separation of a triazine pesticide mixture on four different stationary phases. Notice that no individual stationary phase adequately separated all analytes. Retention data were then entered into the Optimizer Software along with criteria regarding the required separation. The user could specify a minimum resolution, a maximum run time, or the "best" separation possible given these stationary phases. In this case, the computer simulated best separation is shown in Figure 7 along with the actual chromatogram obtained.

FIGURE 5

Structures of Triazine Pesticides

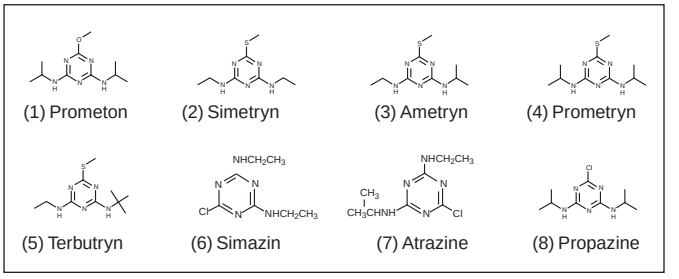
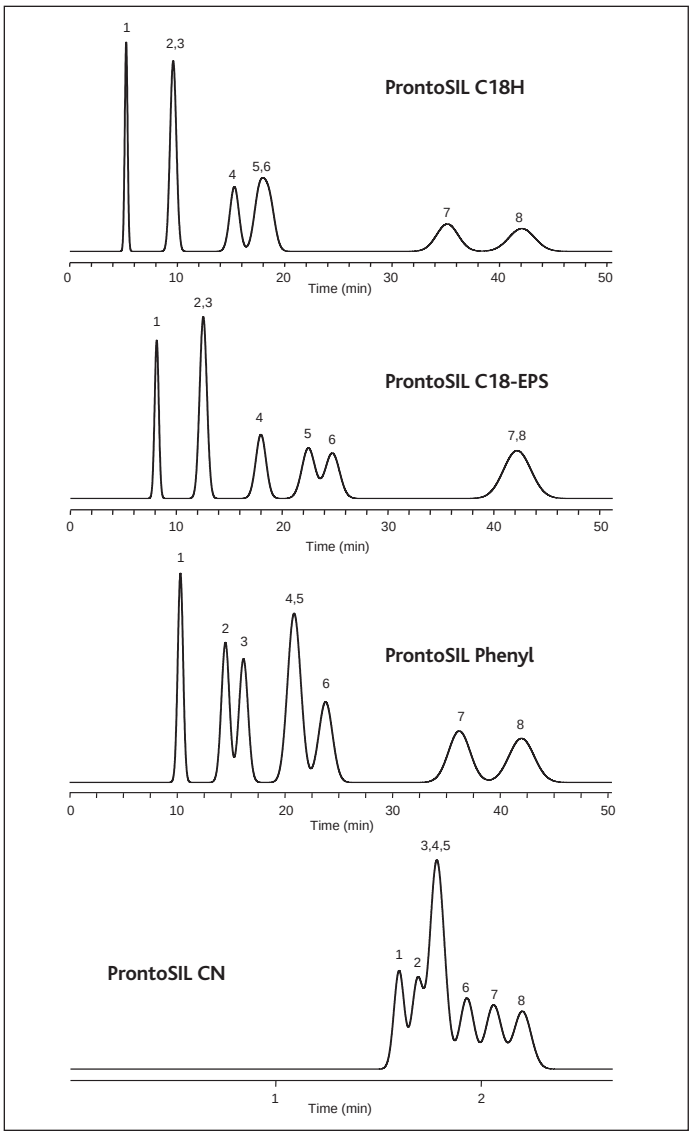


FIGURE 6
Separation of Triazine Pesticides

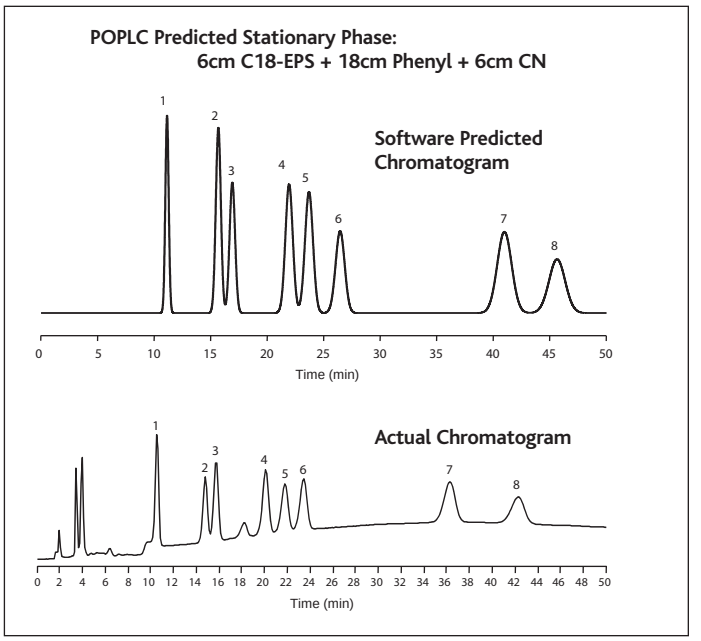


The above chromatograms show the separation of a mixture of eight triazine pesticides chromatographed under the same mobile phase conditions on four different stationary phases.

Summary

Sationary phase selectivity is highly important in HPLC method development yet conventional method development procedures randomly select a column with the hope that methodical mobile phase changes will provide adequate resolution of the sample. In phase optimized liquid chromatography (POPLC), column selectivity is optimized for a given sample by using a combination of stationary phases with complementary or orthogonal selectivity. This process requires a minimum number of experiments and is aided by a very powerful software package. Once the appropriate type and number of stationary phases is determined, these are combined through a unique connection system called POPLink. Methods thus created are isocratic, with the user selecting conditions optimal for speed, resolution or both. By having a column with optimum selectivity for a given sample, the operator can now, if desired, further refine the separation by optimizing the mobile phase either manually or through a computer-aided method development program.

FIGURE 7
Separation of Triazine Pesticides



The lower chromatogram represents an actual pesticide sample analyzed on the column combination suggested by the optimizer software. The top trace is the predicted chromatogram.

POPLC® Kits

Description	Part Number
POPLC Basic Kit 150-3	MSCQ-1503-3
POPLC Basic Kit 250-3	MSCQ-2503-3
POPLC Basic Kit 150-5	MSCQ-1503-5
POPLC Basic Kit 250-5	MSCQ-2503-5

Note: All Kits have the POPLC Optimizer Software

Differences in the Kits

The 250-3 and 150-3 Kits contain the following stationary phases:

- C18 SH-2
- C18 EPS-2
- Phenyl-2

The 250-5 and 150-5 Kits contain the following stationary phases:

- C18 SH-2
- C18 EPS-2
- Phenyl-2
- CN-2
- C30

The 150 Kits (150-3 and 150-5) contain the following POPLink cartridges of each of the stationary phases in the Kit:

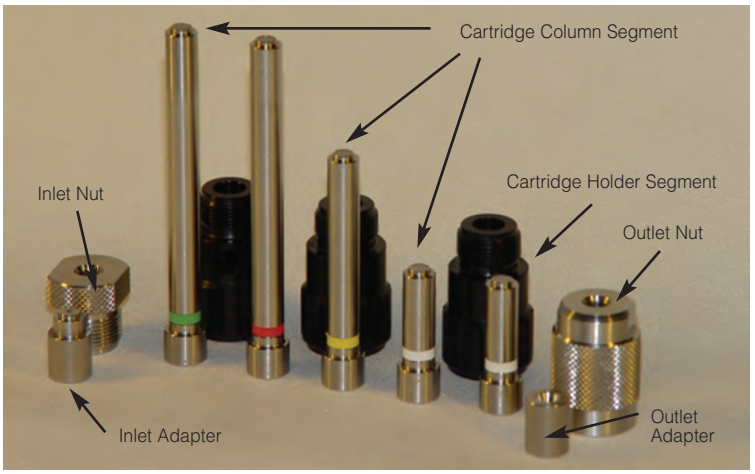
- 1 each 1 cm
- 1 each 2 cm
- 1 each 4 cm
- 1 each 8 cm

The 250 Kits (250-3 and 250-5) contain the following POPLink cartridges of each of the stationary phases in the Kit:

- 1 each 1 cm
- 1 each 2 cm
- 2 each 4 cm
- 1 each 6 cm
- 1 each 8 cm

Note: All Kits have the necessary POPLink holder segments and inlet and outlet fittings for configuring columns. POPLink cartridges are packed with 5 micron particles and have an internal diameter of 3.0 mm.

FIGURE 8
Column Segments and POPLink Connecting Hardware



POPLink Column Segment Cartridges

Description	Part Number
ProntoSIL C18 SH-2, 1cm, 3 each	UB03E182PS050-3
ProntoSIL C18 SH-2, 2cm, 3 each	UC03E182PS050-3
ProntoSIL C18 SH-2, 4cm, 2 each	UD03E182PS050-2
ProntoSIL C18 SH-2, 6cm, 1 each	UE03E182PS050-1
ProntoSIL C18 SH-2, 8cm, 1 each	UF03E182PS050-1
ProntoSIL C18 EPS-2, 1cm, 3 each	UB03E18BPS050-3
ProntoSIL C18 EPS-2, 2cm, 3 each	UC03E18BPS050-3
ProntoSIL C18 EPS-2, 4cm, 2 each	UD03E18BPS050-2
ProntoSIL C18 EPS-2, 6cm, 1 each	UE03E18BPS050-1
ProntoSIL C18 EPS-2, 8cm, 1 each	UF03E18BPS050-1
ProntoSIL Phenyl-2, 1cm, 3 each	UB03E052PS050-3
ProntoSIL Phenyl-2, 2cm, 3 each	UC03E052PS050-3
ProntoSIL Phenyl-2, 4cm, 2 each	UD03E052PS050-2
ProntoSIL Phenyl-2, 6cm, 1 each	UE03E052PS050-1
ProntoSIL Phenyl-2, 8cm, 1 each	UF03E052PS050-1
ProntoSIL CN-2, 1cm, 3 each	UB03E202PS050-3
ProntoSIL CN-2, 2cm, 3 each	UC03E202PS050-3
ProntoSIL, CN-2, 4cm, 2 each	UD03E202PS050-2
ProntoSIL CN-2, 6cm, 1 each	UE03E202PS050-1
ProntoSIL CN-2, 8cm, 1 each	UF03E202PS050-1
ProntoSIL C30, 1cm, 3 each	UB03H300PS050-3
ProntoSIL C30, 2cm, 3 each	UC03H300PS050-3
ProntoSIL C30, 4cm, 2 each	UD03H300PS050-2
ProntoSIL C30, 6cm, 1 each	UE03H300PS050-1
ProntoSIL C30, 8cm, 1 each	UF03H300PS050-1

POPLink Holder Segments and Inlet and Outlet Port Assemblies

Description	Part Number
POPLink Holder Segments 1cm, 3 each	MSCH10-08-03
POPLink Holder Segments 2cm, 3 each	MSCH20-08-03
POPLink Holder Fitting Assy, Inlet Port	MSCI-08
POPLink Holder Fitting Assy, Outlet Port	MSCO-08

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BISCHOFF CHROMATOGRAPHY

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