The Benefits of Reversed Phases with Extended Polar Selectivity in Analysing Wide-Polarity -Range Samples

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Reversed phases with extended polar selectivity offer alternative selectivity to current base-deactivated reversed-phase media by allowing residual silanols to play a major role in the retention process. These phases also show reduced hydrophobic retention, which can be helpful with samples that contain both polar and non-polar species. This article describes the use of phases with extended polar selectivity to reduce analysis time for wide-polarity-range samples, including the insecticide pirimicarb, simulated carboxylic acid degradants and phthalate esters.

Chromatographers face a familiar problem when developing chromatographic methods with widely different retention times for the sample components. If they choose a solvent composition that allows reasonable retention of components eluted early in a run, they get unacceptably long retention of the more strongly retained compounds, which leads to increased analysis times and higher detection limits for the longer-retained compounds. In extreme instances, the more strongly retained compounds might never be eluted effectively. The usual solution to this dilemma is to change from a single-eluent system (isocratic elution) to one in which a stronger solvent in the eluent is increased with time (gradient elution). This approach reduces the retention time and the peak width of the late-eluted compounds and provides shorter analysis times and better detection limits. For complex samples, such as tryptic digests of proteins, with a wide polarity range, gradient elution is still the preferred separation mode. However, gradient elution is best avoided, if at all possible, for routine analysis.

Gradient systems require either an additional pump or a proportioning valve system, and both of these devices require some form of electronic control. They result in increased capital costs and system complexity, and they reduce a system's reliability. The change in solvent composition can also cause difficulties because of the changing optical properties of the eluent. These eluent changes can cause baseline shifts in a UV detector, and this effect can limit a detector's use, particularly at low wavelengths. In general, it is better to use isocratic systems for routine analysis.

During the past decade, manufacturers developed many reversed-phase media suitable for analysing difficult compounds, such as amines and chelators.[1] These phases, often called base-deactivated media, have followed similar design criteria based upon maximum phase loading and full endcapping to minimize the effect of silanols. This strategy also maximizes hydrophobic interactions, particularly when base-deactivated silica phases are coupled with high surface-area media (and, by definition, high phase-loading media). These phases can be characterized as providing relatively low retention of polar compounds and high retention of non-polar ones.

The widespread use of these conventional base-deactivated media is testimony to their general applicability, but in specific circumstances their hydrophobic-to-silanophilic balance requires the use of gradient elution.

The metabolism of drugs in the body and the biodegradation of chemicals in the environment share similar chemical strategies in that the parent compound is made more hydrophilic. This change is achieved by the elimination of hydrophobic groups or the introduction of polar groups, such as hydroxyls and carboxylic and sulfonic acids, the precise groups depending upon the structure of the parent molecule and the degradative

environment. The net effect is that the metabolites and degradants are much more polar than the parent compound. This effect leads to the classic elution problem described above. Conditions that resolve the polar degradants and metabolites from the solvent front cause unacceptably high retention for the parent compound. If an eluent is strengthened to obtain earlier elution of the parent compound, the degradants or metabolites are eluted too close to the solvent front. In this instance, the high non-polar–polar selectivity works against the requirements for quick, simple analysis.

My colleagues and I previously described the properties and benefits of reversed-phase media with extended polar selectivity.[2] These phases use low phase loadings to allow the residual silanols to play a more important role in the retention mechanism. The previous publication concentrated on the beneficial changes in selectivity for polar compounds compared with conventional base-deactivated reversed-phase media. An additional facet of the reversed phases with extended polar selectivity is reduced hydrophobic retention. This property leads to a reduced non-polar–polar selectivity, which is beneficial to chromatographic problems associated with degradant and metabolite analysis.

In this article, I illustrate the benefits of reversed phases with extended polar selectivity for analysing polar degradants and homologous series of phthalate esters in which reduced hydrophobic selectivity is beneficial.

Reversed-phase media that use extended polar selectivity can yield more efficient analysis of samples that contain a wide range of polarities by reducing analysis times and minimizing the necessity for gradient elution.

Experimental

Equipment: I used 53 × 7 mm Rocket format columns packed with 3 µm d_p Platinum 100 C18 reversed-phase media with extended polar selectivity, C8 media and C18 media; 3 µm d_p Brava base-deactivated silica C18 media; and 3 µm d_p Adsorbosphere XL octadecylsilyl media (all from Alltech Associates, Deerfield, Illinois, USA).

The instrumentation used included a model 301 pump, a model 330 column heater and a model 200 UV/vis detector (all from Alltech Associates) and a model 7125 injection valve (Rheodyne, Rohnert Park, California, USA).

Chemicals: The eluent volumes were measured before mixing. The eluent was UV 190–grade acetonitrile (Romil, Cambridge, UK) with either water or potassium phosphate buffer (pH 3).

The samples were purchased from Sigma (Poole, UK), except for the pirimicarb and related products and the anticonvulsants, and they were used as received. Pirimicarb and its related products were provided by F. Moffat of Zeneca Agrochemicals (Bracknell, UK). The anticonvulsants were supplied by D. Wiles of the department of biochemistry at Dumfries and Galloway Royal Infirmary (Dumfries, UK).

Results and Discussion

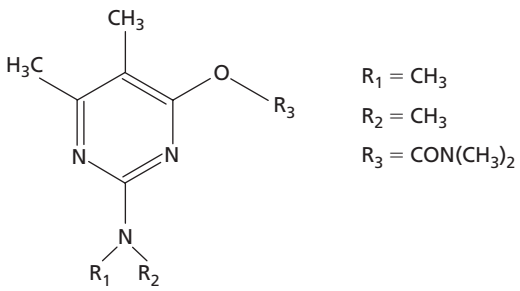
The previous article discussed how the common design criteria of commercial base-deactivated reversed-phase media — neutral, purified silica; maximum phase loading; and fully endcapped — led to a large number of products that had different absolute retentions but similar selectivities.[2] The extended polar selectivity approach provides different selectivity based upon how the residual silanols affect the retention mechanism and cause polar compounds to be retained longer relative to non-polar compounds. In that publication, my co-authors and I also showed how the relative extended polar selectivity effect of substituents on a benzene ring followed the intuitive series, based upon the functional group polarity[2]; that is,

$$CH_3 < I < Br < Cl < F < CHO < COOH < CH_2OH < NH_2$$

Earlier, my colleagues and I focused on how a separation of polar compounds benefited by having a different selectivity from that provided by conventional base-deactivated silica phases. However, an important corollary of the reversed phase with extended polar selectivity is the reduced retention of hydrophobic species, which can be advantageous when their retention is excessive on a conventional reversed-phase column. The typical base-deactivated silica phases with high phase loading, full endcapping, and, often, high surface area provide high hydrophobicity. This hydrophobicity can be excellent if small differences in the hydrophobic part of a molecule form the basis for a separation (e.g., in the separation of linear and branched alkyl chains). However, if the sample contains both highly polar and non-polar species, then the highly hydrophobic phase increases the likelihood that chromatographers will need to use gradient elution. Many analyses have this characteristic, particularly those used in monitoring metabolism, degradation and by-product formation in which the compounds of interest are usually much less polar than the parent compound.

Pirimicarb and related products: Figure 1 shows the structure of the insecticide pirimicarb. Products related to pirimicarb feature a loss or oxidation of R_1 to CHO, a loss of R_2 and a loss of R_3, all of which result in a range of compounds that are significantly more polar than pirimicarb. I used 20% acetonitrile eluent to separate the parent compound and related products on a base-deactivated, fully endcapped C18 column and on a C18 reversed-phase column with extended polar selectivity. Under these conditions, the greater polar interaction of the reversed phase with extended polar selectivity provided longer retention of all compounds and allowed baseline resolution of the two most polar products with a pirimicarb retention time of 4.5 min (Figure 2).

Figure 1: The structure of pirimicarb.



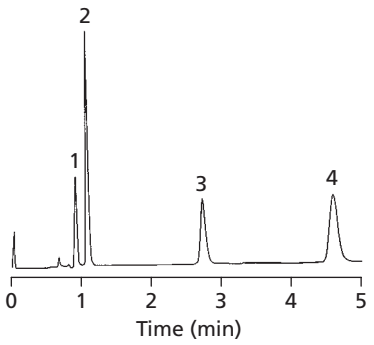
The more conventional C18 phase provided a shorter total analysis time, but it failed to resolve the two most polar products, which were eluted close to the solvent front (Figure 3). Weakening the solvent to 10% acetonitrile improved the resolution of these two peaks, but the retention time of pirimicarb increased to 15 min (Figure 4). The standard C18 phase required a weaker eluent to achieve retention and resolution of the early peaks, which caused excessive retention of the parent compound.

In this situation, the analysis time using the C18 reversed phase with extended polar selectivity was reduced by a factor of 3.5, with a similar savings factor in solvent. The separation on the standard phase would be a strong candidate for gradient elution to improve the resolution at the early part of the chromatogram and to shorten the total analysis time.

Simulated degradation of carboxylic acids: I observed a similar effect with a range of carboxylic acids, which were chosen to simulate the chain-shortening and polar-group addition that occurs in degradation studies. A conventional base-deactivated silica C18 phase of moderate retention was used for comparison. In this instance, I observed similar retention and selectivity for acetylsalicylic and cinnamic acids, presumably because the increased polar retention of the C18 reversed phase with extended polar selectivity was counterbalanced by the greater hydrophobicity of the base-deactivated silica C18 (see Figure 5). However, the latter medium exerted its influence with the longer chain acids and caused an analysis time twice that of the C18 reversed phase with extended polar selectivity.

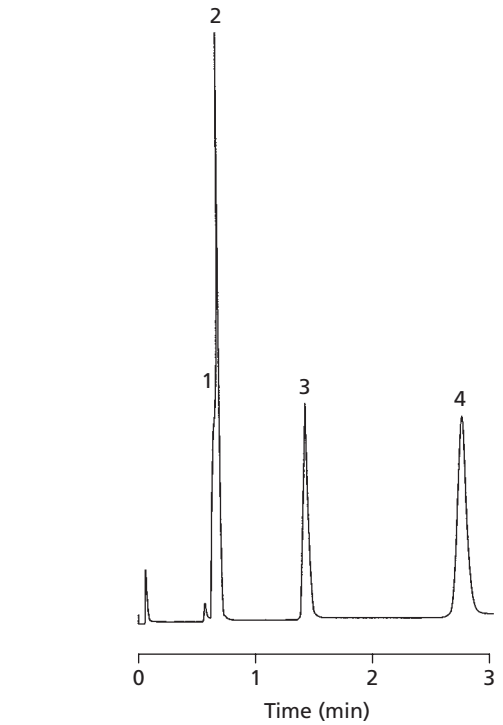
Changing to a C8 reversed phase with extended polar selectivity required a reduction in eluent from 50% to 40% organic solvent to obtain similar retention for the acetylsalicylic

Figure 2: Separation of pirimicarb and its polar degradation products on a reversed-phase column with extended polar selectivity.



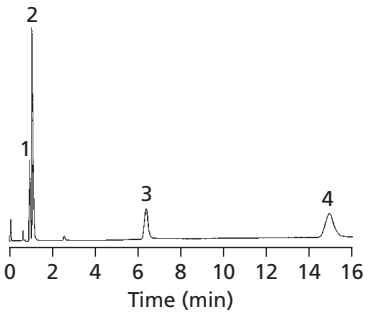
Column: 53 × 7 mm, 3 µm d_p Platinum EPS C18; mobile phase: 20:80 (v/v) acetonitrile–20 mM monobasic potassium phosphate (pH 3); flow-rate: 2.3 mL/min; temperature: 35 °C. Peaks: 1–3 = polar degradation products, 4 = pirimicarb.

Figure 3: Separation of pirimicarb and its polar degradation products on a fully endcapped, base-deactivated phase.



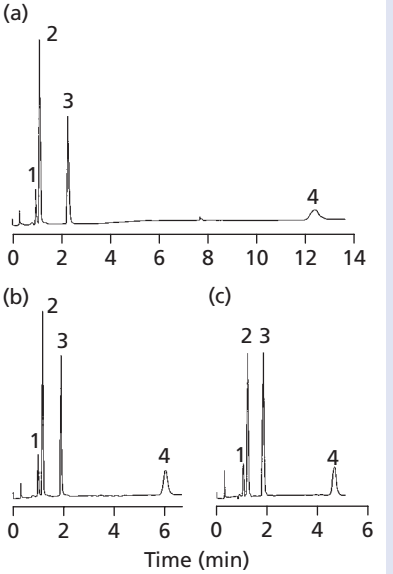
Column: 3 µm d_p Platinum C18. Peak identifications and other conditions were the same as those described in Figure 2.

Figure 4: Separation of pirimicarb and its polar degradation products on a fully endcapped, base-deactivated phase.



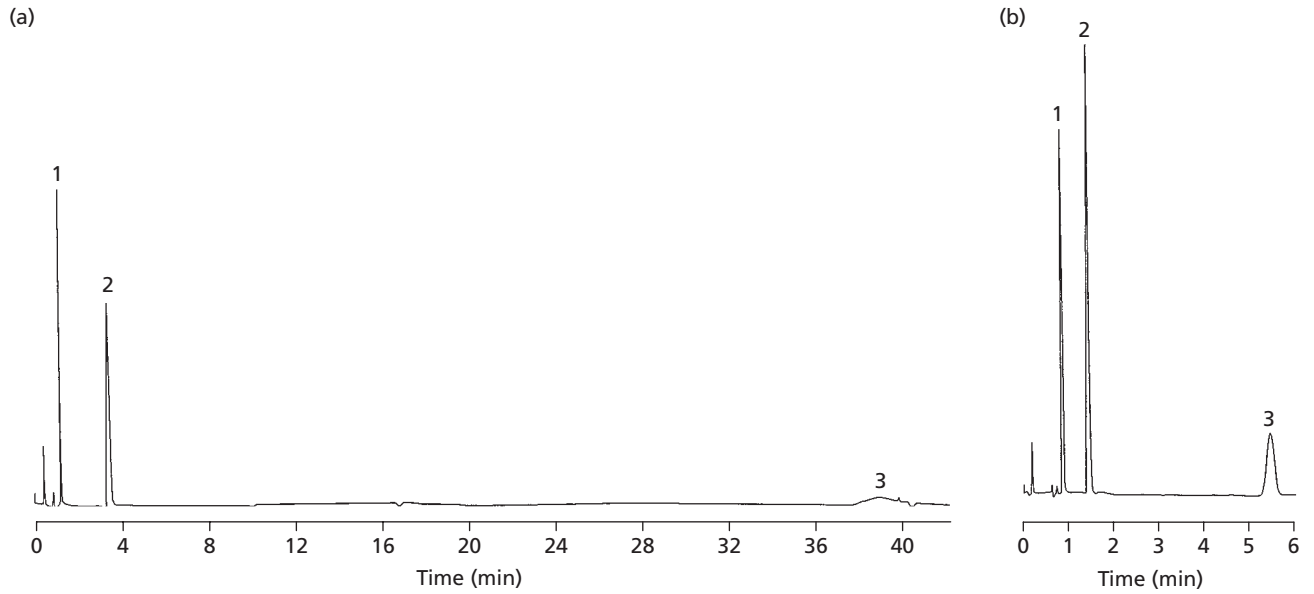
Column: 3 µm d_p Platinum C18; mobile phase: 10:90 (v/v) acetonitrile–20 mM monobasic potassium phosphate (pH 3). Peak identifications and other conditions were the same as those described in Figure 2.

Figure 5: Separation of a four-compound mixture using (a) a base-deactivated silica C18 column, (b) a C18 column with extended polar selectivity and (c) a C8 column with extended polar selectivity.



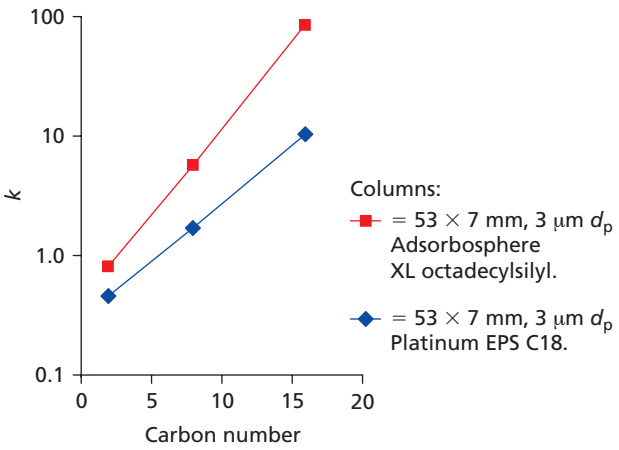
Column: (a) 100 × 4.6 mm, 5 µm d_p Brava base-deactivated silica C18; (b) 100 × 4.6 mm, 5 µm d_p Platinum EPS C18; (c) 100 × 4.6 mm, 5 µm d_p Platinum EPS C8. Mobile phase: (a, b) 50:50 (v/v) acetonitrile–50 mM monobasic potassium phosphate (pH 3); (c) 40:60 (v/v) acetonitrile–50 mM monobasic potassium phosphate (pH 3). Flow-rate: 2.0 mL/min; temperature: 35 °C. Peaks: 1 = acetylsalicylic acid, 2 = cinnamic acid, 3 = 4-butylbenzoic acid, 4 = 4-octylbenzoic acid.

Figure 6: Separation of phthalate esters using (a) a standard octadecylsilyl phase and (b) a reversed phase with extended polar selectivity.



Column: (a) 53 × 7 mm, 3 µm d_p Platinum EPS C18; (b) 53 × 7 mm, 3 µm d_p Adsorbosphere XL octadecylsilyl. Mobile phase: 65:35 (v/v) acetonitrile–water; flow-rate: 2.3 mL/min; temperature: 35 °C. Peaks: 1 = dimethylphthalate, 2 = dibutylphthalate, 3 = dioctylphthalate.

Figure 7: Plots of log *k* versus alkyl-chain carbon number for a C18 reversed phase with extended polar selectivity and a standard octadecylsilyl phase.



and cinnamic acids because of the lower phase loading of the C8, but the change resulted in an additional reduction in total analysis time to 4.5 min. This change demonstrates that, as might be expected, the extended polar selectivity effect is more pronounced with the shorter alkyl chain C8 media and that both the reversed phases with extended polar selectivity led to reduced analysis times.

Phthalate esters: The two previous examples illustrate the ability of the C18 reversed phase with extended polar selectivity to retain polar compounds and keep the retention of the more hydrophobic species in check. Similar benefits are available if the differences are in the hydrophobic region of the target compounds and are demonstrated with phthalate esters. Analysing these plasticizers has become more important as scientists' awareness of their potential safety hazard has grown.

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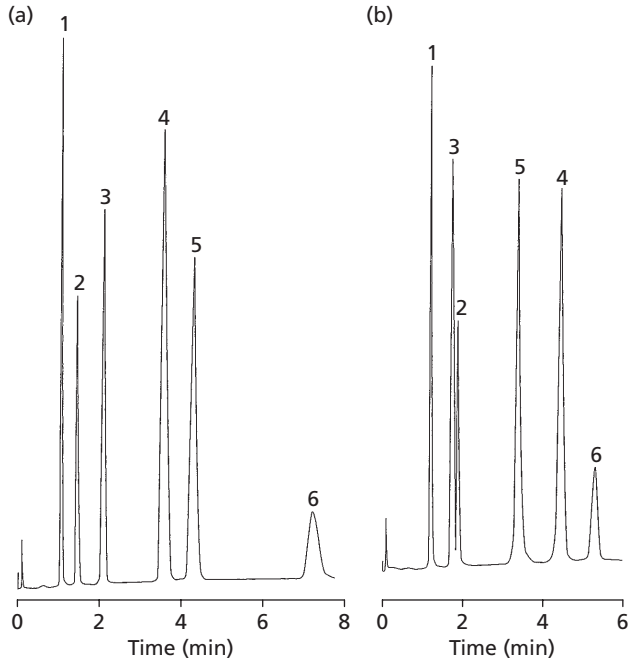
I separated the phthalate esters on the C18 reversed phase with extended polar selectivity and a standard octadecylsilyl phase. I chose a solvent composition to yield similar retention for the first peak, dimethylphthalate. The chromatograms of Figure 6 clearly show the excessive retention of the dioctylphthalate on the standard octadecylsilyl column, and this retention causes an analysis time more than sixfold longer than that of the separation using the C18 reversed phase with extended polar selectivity.

The large difference in the retention of the dimethyl and dioctylphthalates on conventional phases has required the use of gradient elution. However, the reduced hydrophobic selectivity of the C18 reversed phase with extended polar selectivity allows the ready isocratic separation of these compounds. Figure 7 shows this effect in which linear plots are achieved for log retention factor (k) versus total alkyl-chain carbon number for the C18 reversed phase with extended polar selectivity and the standard octadecylsilyl phase.

Anticonvulsants: The above examples may leave the impression that reversed phases with extended polar selectivity are superior to conventional endcapped base-deactivated silica phases. This statement is not always true. The phases have different selectivity that can improve separation in some applications; however, many times the standard selectivity yields a better separation.

Figure 8: Separation of anticonvulsants using (a) a conventional base-deactivated silica C18 column and (b) a reversed-phase column with extended polar selectivity.



Column: (a) 53 × 7 mm, 3 µm d_p Platinum C18; (b) 53 × 7 mm, 3 µm d_p Platinum EPS C18. Mobile phase: 30:70 (v/v) acetonitrile–30 mM monobasic potassium phosphate (pH 3); flow-rate: 2.3 mL/min; temperature: 23 °C. Peaks: 1 = primidone, 2 = lamotrigine, 3 = phenobarbitone, 4 = carbamazepine, 5 = phenytoin, 6 = 5-(*p*-methylphenyl)-5-phenylhydantoin.

Figure 9: Plots of *k* versus base-silica batch number for five test compounds separated using a C18 reversed phase with extended polar selectivity.

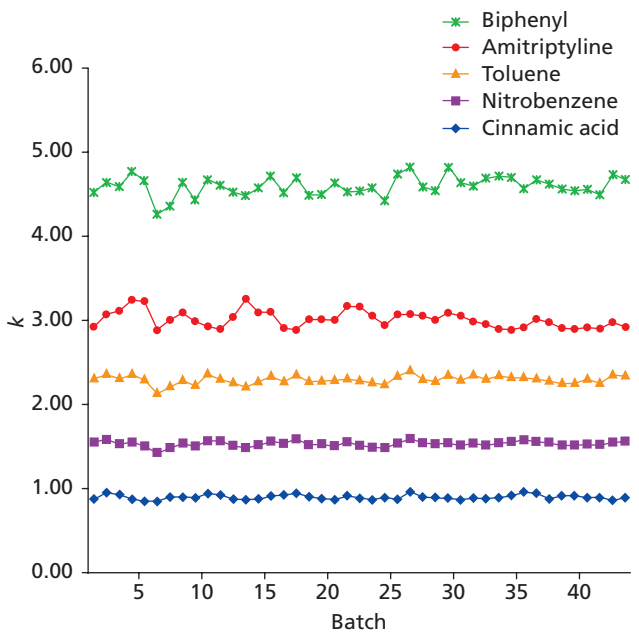


Figure 8 illustrates this scenario with the separation of a range of anticonvulsants. Despite the C18 reversed phase with extended polar selectivity providing a lower total analysis time because of its less hydrophobic retention of the final compound (5-[p-methylphenyl]-5-phenylhydantoin), the standard C18 phase yields improved resolution between lamotrigine and phenobarbitone and a better basis for a more rugged method. This chromatogram also illustrates the marked change in selectivity caused by the extended polar selectivity effect with peak reversals on two pairs of peaks. Except for the phthalate esters, the other examples discussed previously were either bases or acids, and in all instances I obtained good peak shapes with the reversed phases with extended polar selectivity, which have high residual silanol levels.

Because reversed phases with extended polar selectivity are based upon the interplay of both alkyl chain and residual silanol with samples, chromatographers can legitimately raise concerns regarding their reproducibility. Improved selectivity is useless if it cannot be reproduced batch to batch and year to year. The previous article by my colleagues and I provided data for the retention of acidic, basic and neutral species and resulting relative standard deviations (RSDs) of 2.5–3.5% for 10 batches of the C18 reversed phase with extended polar selectivity.[2] Updated data for more than 40 batches show RSDs remained within a similar 2.3–3.4% range (Figure 9). The data represent 3-, 5- and 10 mm d_p media from different batches of base silica and silane and includes process, sampling and test method variability. The results span a four-year period and indicate that the process and product are under control.

Conclusion

Reversed-phase media that use extended polar selectivity can yield more efficient analysis of samples that contain a wide range of polarities by reducing analysis times and minimizing the necessity for gradient elution. This improvement is achieved by a combination of increased retention of polar species, which are retained away from the solvent front, and decreased retention of non-polar compounds, which reduces the total analysis time.

References

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(2) I. Chappell, S.C. McGee and R.J. Weigand, *LC•GC Int.*, **11**(7), 440–444 (1998).