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OVERVIEW

With over 600 commercially available C₁₈ packing materials on the market, the introduction of a new brand of C₁₈ stationary phase makes choosing a column even more difficult. However, each new column brand often includes additional ligand chemistries that can be explored for optimal methods development. The performance attributes of chemistries such as C₈ and phenyl stationary phases are not as well discussed as C₁₈ stationary phases in the literature. The relative merits of each of these different phases can be exploited in the development of more robust methods.

The recent introduction of the SunFire™ brand of reversed-phase packing materials permits a systematic study of the selectivity, peak shape, and low-pH stability of the various phases in the family: C₁₈, C₈, and a SunFire R&D phenyl. Because these ligands are bonded to the same base-silica particle, the observed differences can be attributed to differences in the surface modification.

Selectivity and peak shape are compared at low and neutral pHs using isocratic conditions. The low-pH stability is compared using 1% TFA (pH 1) as the mobile phase¹.

Using a family of bonded phases based on the same base-silica allows for a systematic study of selectivity, peak shape, and pH stability. Data on the recently introduced brand of SunFire™ silica-based stationary phases is presented along with a SunFire R&D phenyl phase. Relative differences in selectivity, peak shape, and pH stability will be discussed.

Table 1: Physical characteristics of the silica used in this study.

Particle	High purity spherical silica
Particle Diameter	3.5 µm
Pore Size	100 Å
Specific Pore Volume	0.90 cc/g
Specific Surface Area	340 m ² /g

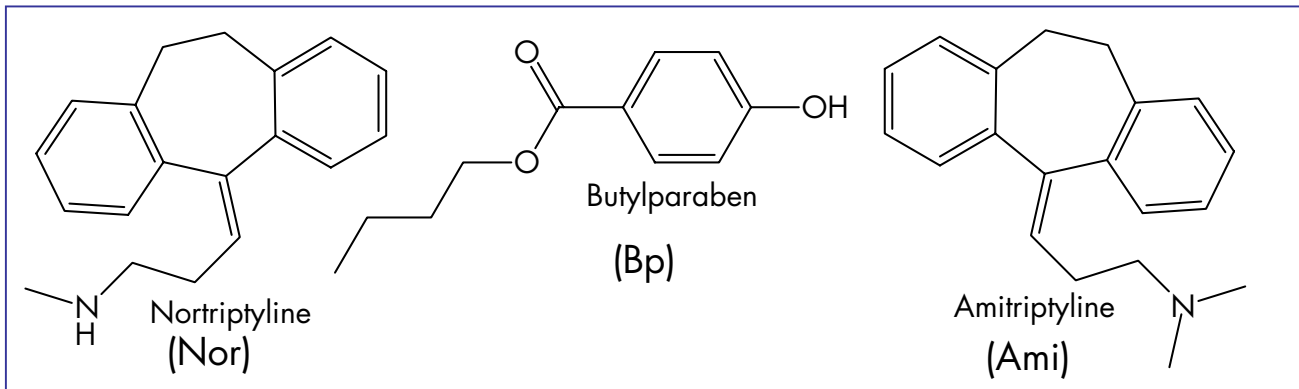


Figure 1: The pH 7 peak shape analytes.

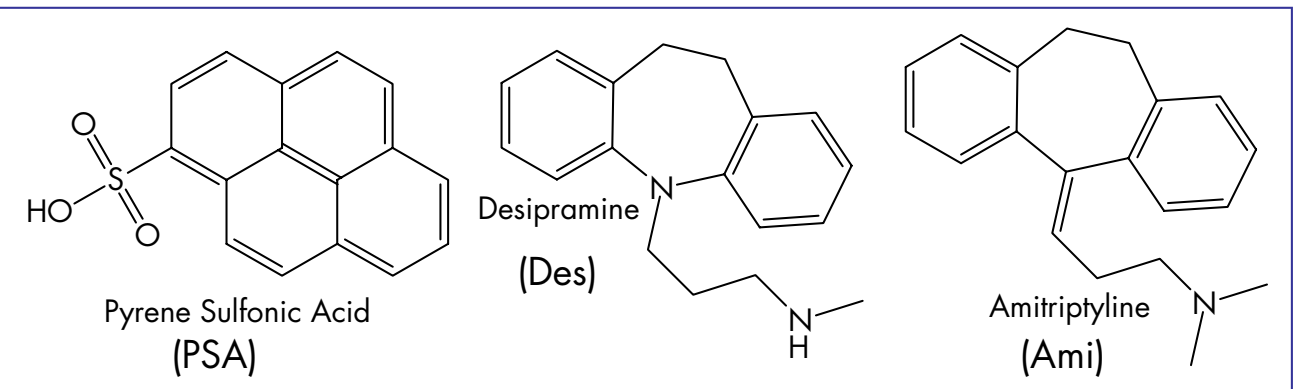


Figure 2: The pH 3 peak shape analytes.

ACCELERATED LOW-pH AGING

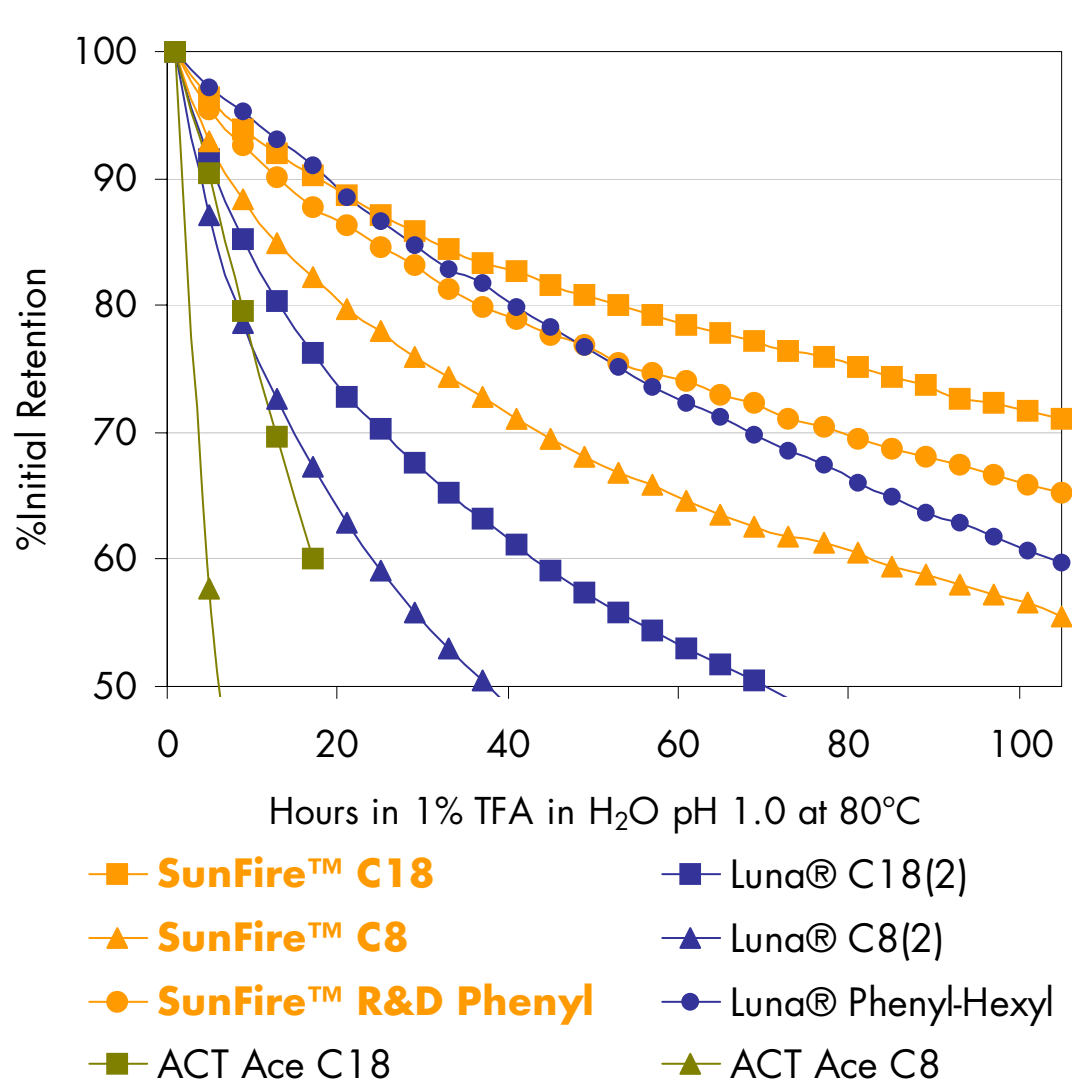


Figure 3: The **1% trifluoroacetic acid (TFA) pH 1.0 stability** of the SunFire stationary phases compared to several benchmark columns. Analyte: benzene.

- The proprietary bonding process used in the SunFire stationary phases provides substantially improved low-pH stability compared to other similarly derivatized stationary phases.
- Of the SunFire phases, the best low-pH stability is achieved on the C₁₈ phase.

PEAK SHAPE AND SELECTIVITY

The silanol activity of stationary phases has been implicated in the poor peak shape observed at neutral pHs for basic analytes. Poor peak shape for any ionized analyte can also be caused by mass overload². The excellent mass load capacity³ and the use of a proprietary bonding process provide the excellent peak shape observed on SunFire™ phases (Fig. 4).

The selectivity characteristics of reversed-phase packings have been the subject of numerous papers⁴. Most approaches require the analysis of an extensive list of analytes in order to derive descriptive terms for solute-stationary phase interactions. The central principles behind these approaches can be mitigated and applied to a much smaller set of analytes for the purpose of gaining a qualitative understanding of the selectivity differences observed on different stationary phases. Such an approach was taken using our pH 7 (Fig. 5) and pH 3 (Fig. 6) test mixtures. In tables 2 and 3 we report five

[2] D.V. McCalley, *Journal of Chromatography A*, 793 (1998) 31-46

[3] Fang Xia, Jie Y. Cavanaugh, Diane M. Diehl, *LC/GC North American Application Notebook*, 2004, p. 1

[4] Recent review: L.R. Snyder, J.W. Dolan, P.W. Carr, *Journal of Chromatography A*, 1060 (2004) 77-116

[5] a. Neue, U.D.; Serowik, E.; Iraneta, P.C.; Alden, B.A.; Walter, T.H., *Journal of Chromatography A* 849 (1999) 87

b. Neue, U.D.; Alden, B.A.; Walter, T.H., *Journal of Chromatography A* 849 (1999) 101

different terms to describe the selectivity on the C₁₈, C₈, and Phenyl phases. The relative differences between the different phases are shown in Figure 7.

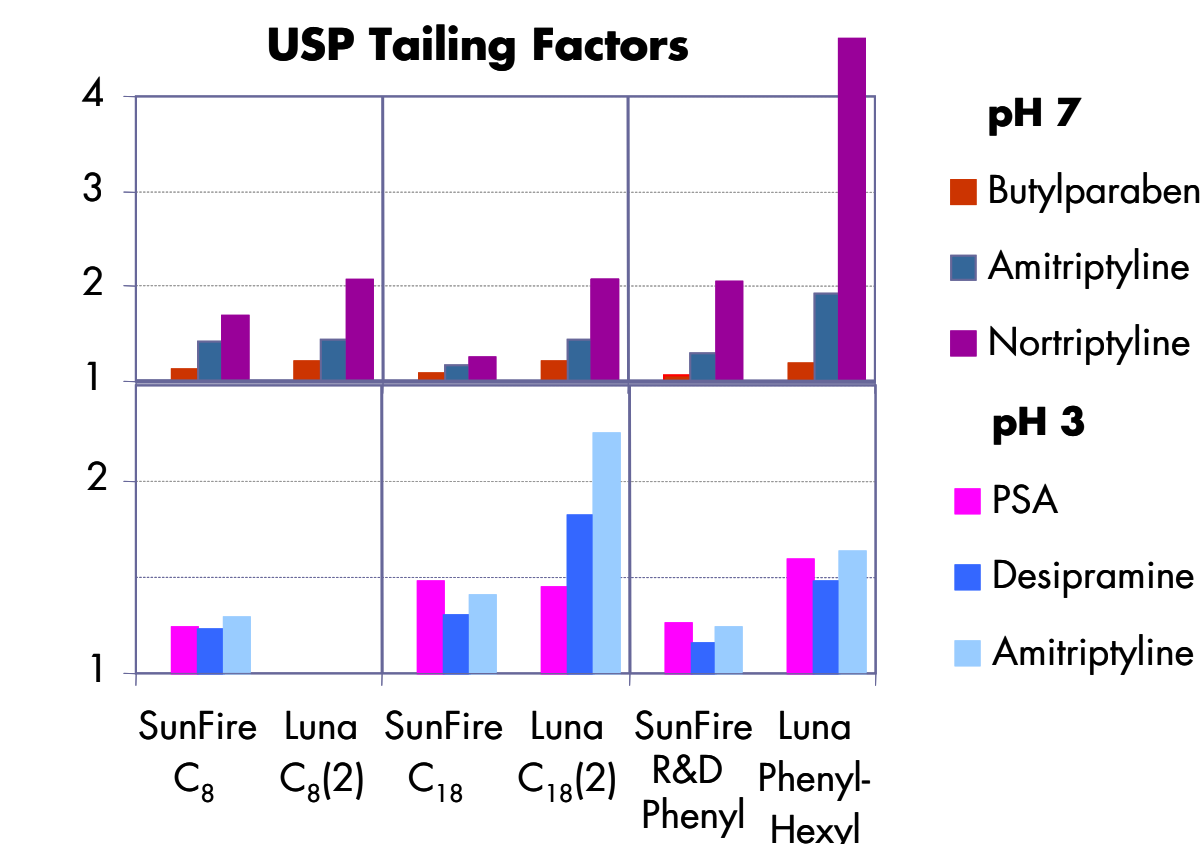


Figure 4: Peak shape comparison for acids (pyrene sulfonic acid (PSA) and butylparaben), 2° amines (desipramine and nortriptyline, injected separately), and a 3° amine (amitriptyline) at pH 3 and pH 7.

- The excellent mass load capacity and the use of a proprietary bonding process provide excellent peak shape on the SunFire phases at both pHs.
- At pH 7, tailing factors were best on SunFire stationary phases compared to those on the benchmark brand.
- At pH 3, although the data set is not complete, SunFire stationary phases provided lower tailing factors for all analytes except for pyrene sulfonic acid on the C₁₈.

Table 2: Selectivity descriptors and data of SunFire™ phases using the pH 7 test mixture.

Property	Description	C ₁₈	C ₈	R&D Phenyl
Retentivity	k' Ace	20.1	9.12	12.5
Activity	Relative Retention			
2° amine - Pro	Pro/Ace	0.141	0.208	0.298
3° amine - Ami	Ami/Ace	1.40	1.58	2.02
H-bond acceptor	Bp/Ace	0.220	0.316	0.253
H-bond donor	Dpp/Ace	0.416	0.596	0.626

Table 3: Selectivity descriptors and data of SunFire™ phases using the pH 3 test mixture.

Property	Description	C ₁₈	C ₈	R&D Phenyl
Retentivity	k' Tol	19.8	14.1	10.7
Activity	Relative Retention			
2° amine - Des	Des/Tol	0.110	0.141	0.240
3° amine - Ami	Ami/Tol	0.151	0.184	0.324
H-bond acceptor	Bp/Tol	0.817	0.905	0.908
Acid - PSA	PSA/Tol	0.171	0.251	0.367

$$\text{Normalized Relative Property} = \frac{\text{Property} - \text{Property Average}}{\text{Property Average}} \quad \text{Eq. 1}$$

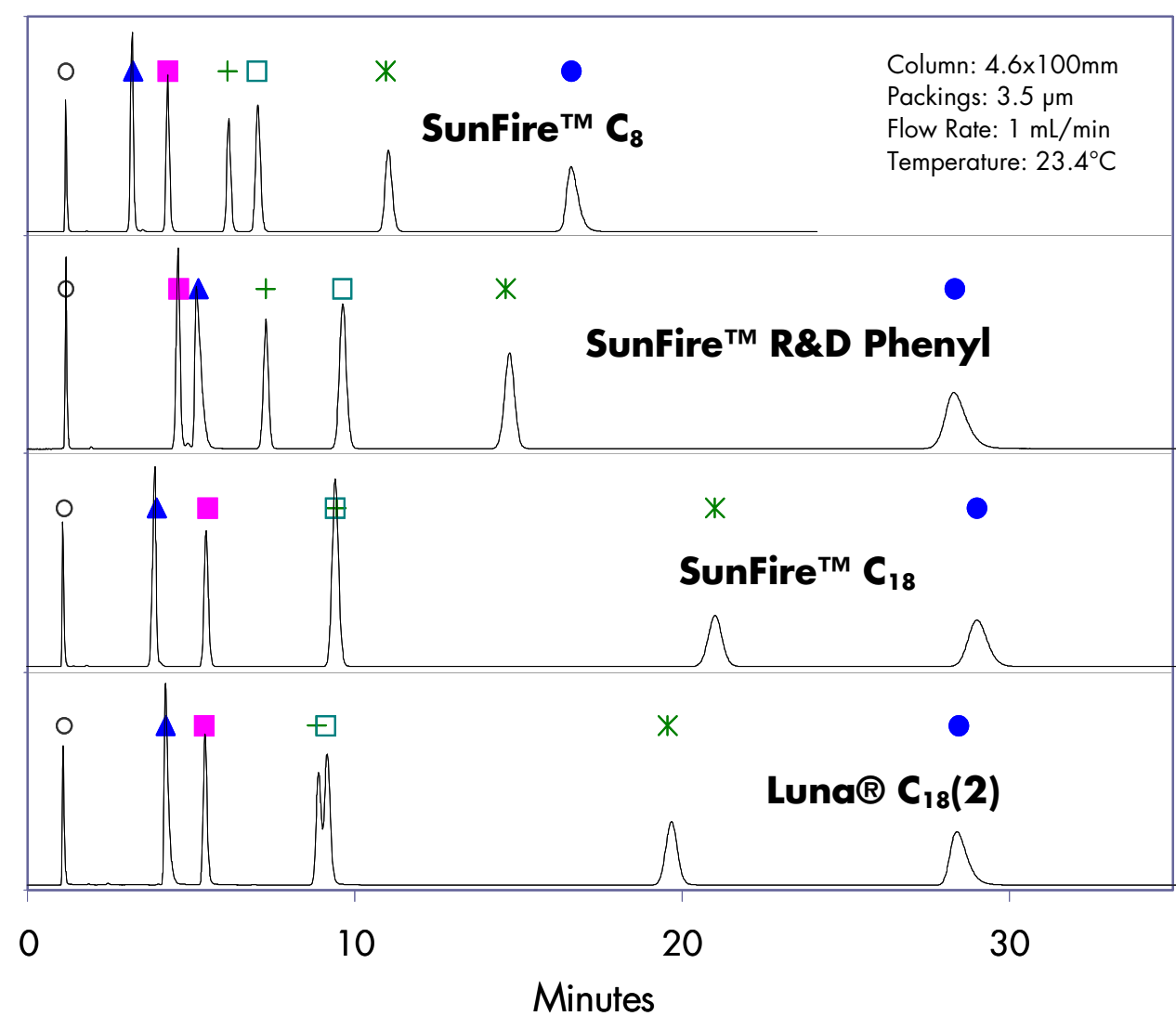


Figure 5: Selectivity and peak shape of SunFire™ stationary phases compared to Luna® C₁₈(2) in 65/35 methanol/20mM K₂HPO₄, pH 7.0 (v/v). Test is described in detail elsewhere⁵. Analytes: ○ Uracil, ▲ Propranolol (Pro), ▲ Butylparaben (Bp), □ Dipropylphthalate (Dpp), + Naphthalene, * Acenaphthene (Ace), and ● Amitriptyline (Ami).

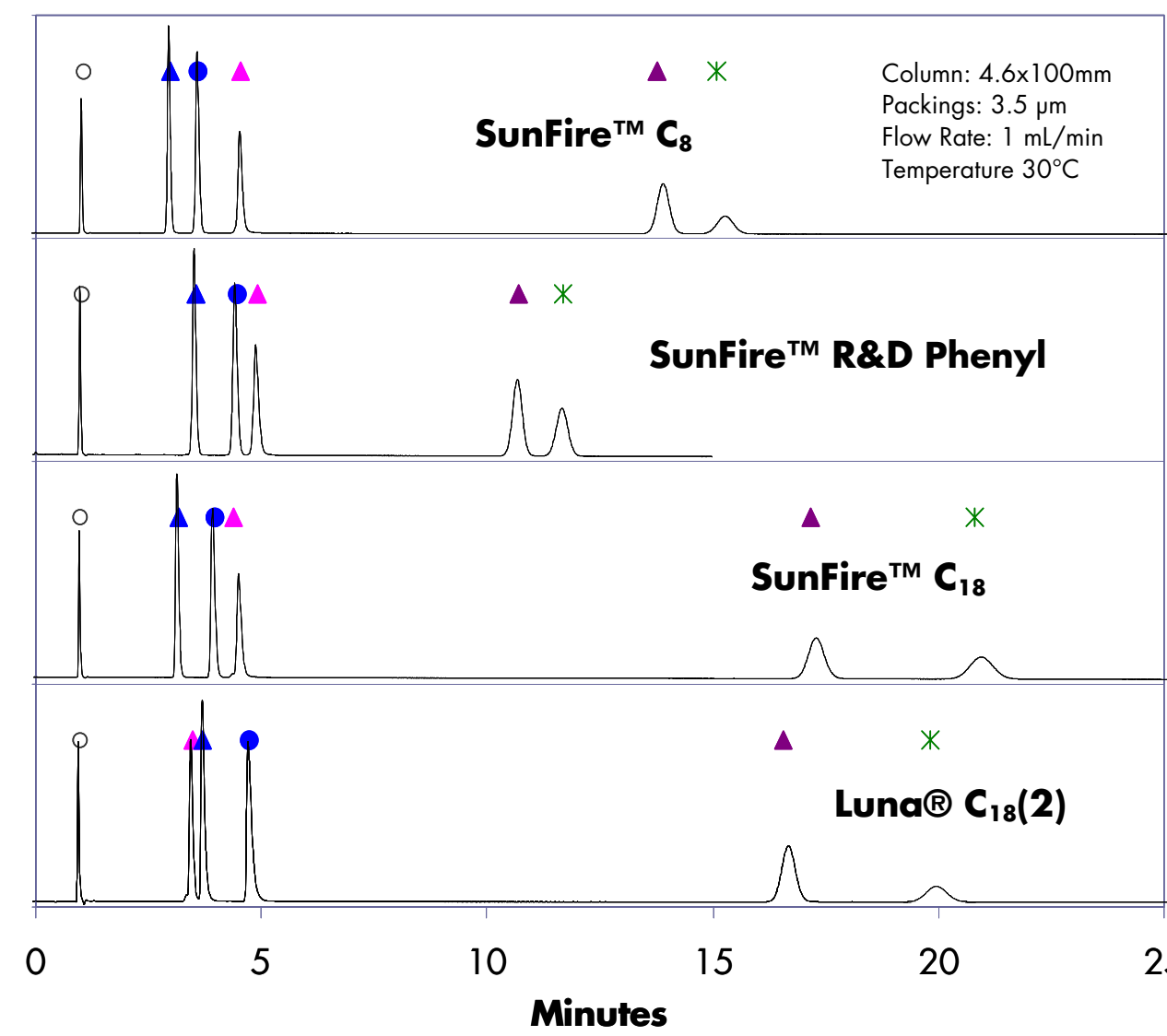


Figure 6: Selectivity and peak shape of SunFire™ stationary phases compared to Luna® C₁₈(2) in 35/65 acetonitrile/15.4mM HCOONH₄, pH 3.0 (v/v). Analytes: ○ Uracil, ▲ Desipramine (Des), ● Amitriptyline (Ami), ▲ Pyrene Sulfonic Acid (PSA), ▲ Butylparaben (Bp), and * Toluene (Tol).

CONCLUSIONS

Stability

- The proprietary bonding process used in the SunFire stationary phases provides substantially improved low-pH stability compared to other similarly derivatized stationary phases (Fig. 3).
- Of the SunFire phases, the best low-pH stability is achieved on the C₁₈ phase (Fig. 3).

Peak Shape

- The excellent mass load capacity³ and the use of a proprietary bonding process provide excellent peak shape on the SunFire phases at both pHs.
- At pH 7, tailing factors were lower on SunFire stationary phases compared to those on the benchmark brand (Fig. 4).
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Selectivity

There are distinct differences in the nature of the interactions on the different stationary phases (Fig. 5, 6, and 7). This information can be useful in methods development aimed at resolving particular classes of analytes. Because samples are often not simple mixtures, choosing phases that are opposite in activity for your class of compound may be of strategic value.

- The hydrophobic retentivity of the SunFire phases changes as a function of organic modifier:
 - pH 7 Test with MeOH: C₈ < Phenyl < C₁₈
 - pH 3 Test with MeCN: Phenyl < C₈ < C₁₈
- The activity for 2° and 3° amines is highest on the SunFire R&D Phenyl in both pH 3 and pH 7 mobile phases (Tables 2 and 3, Fig. 7).
- 2° versus 3° amine activity differences for each of the phases are more pronounced at pH 7. This may be due to the fact that amitriptyline is only about 50% ionized in the pH 7 mobile phase.
- At pH 7, the H-bond acceptor activity was greatest on the SunFire C₈, while the H-bond donor activity was greatest on the SunFire R&D Phenyl (Fig. 7).
- The relative H-bond acceptor activity of the phases at pH 3 differs from that at pH 7. This may be due to the partially ionized state of butylparaben at pH 7.

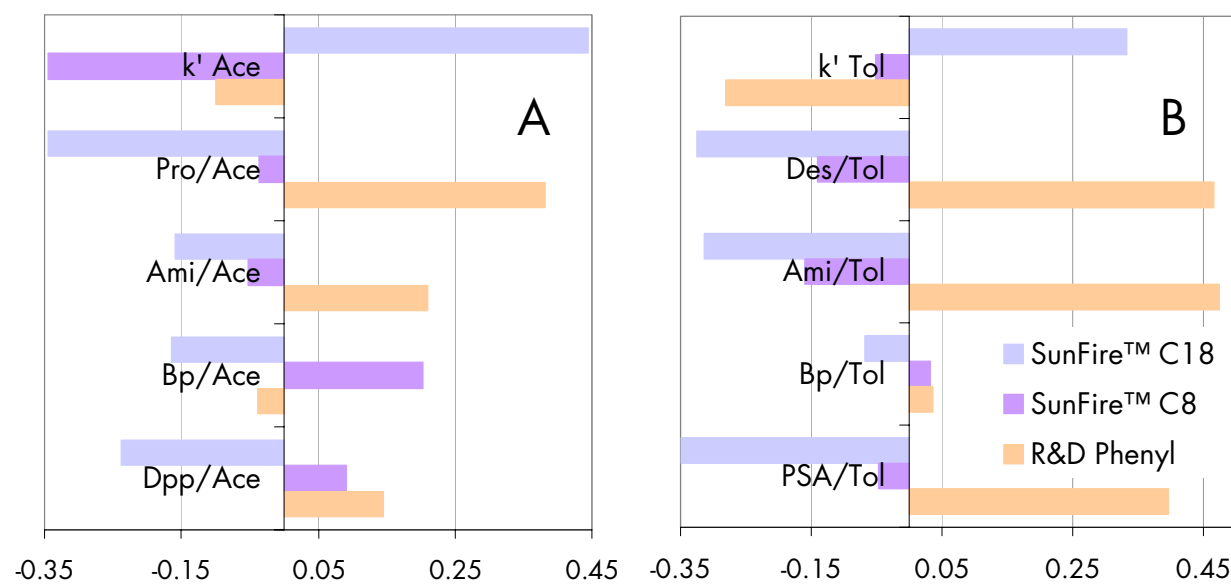


Figure 7: Normalized relative retentivity and activity properties (eq. 1) of SunFire stationary phases at (A) pH 7 and (B) pH 3.

[1] Trammell, B.C.; Boissel, C.A.; Carignan, C.; O'Shea, D.J.; Hudalla, C.J.; Neue, U.D.; Iraneta, P.C., *Journal of Chromatography A*, 1060 (2004) 153-163