

Universal Reversed Phase HPLC Column Simplifies Method Development

Specially deactivated through a treatment of the silica support, and incorporating a unique silanol shielding mechanism as part of the bonded phase chemistry, SUPELCOSM ABZ⁺Plus HPLC columns enable chromatographers to analyze strong acids or strong bases, using simple buffers and mobile phases. Unique chemical composition ensures symmetric peaks, high efficiency, and special selectivity for a wide spectrum of analytes. At the same time, because it is simple to select and prepare mobile phases to use with this column, method development is less time consuming. The packing material has very low bleed, making SUPELCOSM ABZ⁺Plus columns well suited for gradient analyses and HPLC-MS. Although chemically very different from deactivated C18 columns, SUPELCOSM ABZ⁺Plus columns separate small molecular weight compounds through the same reversed phase mechanism that controls retention on traditional C18 / ODS reversed phase columns.

Key Words:

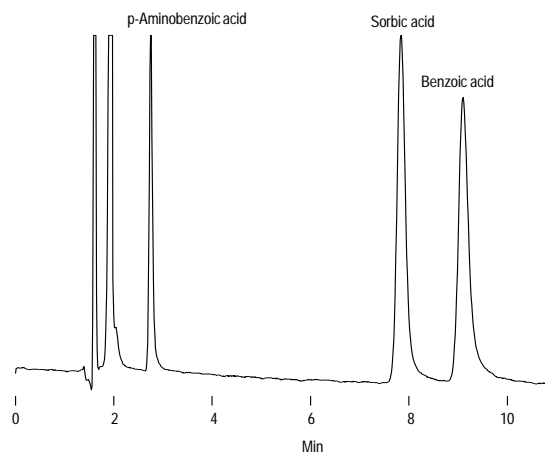
- acidic compounds ● basic compounds
- HPLC method development

Residual silanol groups on the surface of conventional reversed phase silica packings often interact with acidic, basic, or zwitterionic compounds, resulting in low efficiency, tailing peaks, drifting retention time, and irreproducible separations. To obtain acceptable results, particularly for basic compounds, an amine modifier usually must be added to the mobile phase to minimize these interactions. In contrast, SUPELCOSM ABZ⁺Plus columns provide high efficiency and symmetric peaks for a wide range of strong acids and bases, with simple mobile phases. In preparing these columns, we thoroughly purify the silica matrix to reduce the number of highly active isolated free silanol groups. The accessibility of residual silanol groups is further reduced by creating a very high surface coverage (more than 5 $\mu\text{moles}/\text{m}^2$). A unique silanol shielding layer, embedded near the silica surface, significantly reduces interactions between the analytes and remaining silanol groups. As a result, acids, bases, and neutral polar compounds, in various chemical classes, can be analyzed with high efficiency, symmetric peak shape, and constant retention time.

Amine modifiers are unnecessary for chromatography of basic compounds on SUPELCOSM ABZ⁺Plus columns. Method development is further simplified because buffers and organic modifiers have predictable effects on solute retention – a mobile phase

Figure A. Optimal Resolution of Acidic Compounds

Column: SUPELCOSM ABZ⁺Plus, 15cm x 4.6mm, 5 μm particles
 Cat. No.: 59196
 Mobile Phase: acetonitrile:25mM KH_2PO_4 (pH 2.3), 25:75
 Flow Rate: 1mL/min
 Det.: UV, 254nm
 Inj.: 10 μL water (10 $\mu\text{g}/\text{mL}$ p-aminobenzoic acid, 5 $\mu\text{g}/\text{mL}$ sorbic acid, 150 $\mu\text{g}/\text{mL}$ benzoic acid)



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containing a low ionic strength buffer added to acetonitrile or methanol is sufficient for analyzing even the strongest acids or bases (1). Figure A shows an analysis of three acids in a simple phosphate buffer on a SUPELCOSM ABZ⁺Plus column. Efficiency and peak symmetry for sorbic and benzoic acids compare very well with values obtained with other deactivated reversed phase columns (Table 1), and resolution is significantly greater on a SUPELCOSM ABZ⁺Plus column.

Because most pharmaceutical compounds are basic in nature, manufacturers of deactivated columns have focused on improving the chromatography of basic drugs. The peak shape for the narcotic/analgesic drug codeine, a strongly basic compound, is shown in Figure B. Again, a simple phosphate mobile phase was used. Relevant data for this analysis, from SUPELCOSM ABZ⁺Plus and other deactivated reversed phase columns, are summarized in Table 2. The SUPELCOSM ABZ⁺Plus column and several other columns provide good efficiency and symmetric peaks.

Table 1. Chromatography and Resolution of Acidic Compounds

Column	k' _{sorbic}	k' _{benzoic}	N _{sorbic}	N _{benzoic}	AF10 _{sorbic}	AF10 _{benzoic}	Resolution
SUPEL COSIL ABZ ⁺ Plus [■]	4.3	5.1	68 x 10 ³	68 x 10 ³	1.3	1.4	3.5
YMC-Basic [■]	2.2	2.4	68 x 10 ³	69 x 10 ³	1.2	1.4	0.9
LiChrosorb RP-Select B [▲]	3.2	3.6	49 x 10 ³	38 x 10 ³	2.7	3.7	2.6
Inertsil ODS-2 [■]	2.6	2.7	50 x 10 ³	50 x 10 ³	1.2	1.3	0.8
Hypersil [®] BDS-C18 [■]	2.9	3.0	75 x 10 ³	88 x 10 ³	1.5	1.6	0.3
TSK-GEL [®] ODS-80Ts [■]	2.8	3.0	110 x 10 ³	111 x 10 ³	1.2	1.2	1.5
Nucleosil [®] 100-5 C ₁₈ AB [♦]	2.6	2.7	54 x 10 ³	60 x 10 ³	2.2	2.0	0.4
Zorbax R _x [▲]	3.8	4.2	83 x 10 ³	94 x 10 ³	1.3	1.3	2.6
Waters Symmetry C ₈ [■]	5.3	5.3	48 x 10 ³	34 x 10 ³	5.6	7.4	0.0
Waters Symmetry C ₁₈ [■]	4.9	5.0	63 x 10 ³	61 x 10 ³	2.0	2.2	0.5

[■]15cm column [▲]25cm column [♦]10cm column

k' calculated using the half-height method; N = plates/meter; peak symmetry (AF10) monitored at 10% peak height.

Mobile Phase: acetonitrile:25mM KH₂PO₄ (pH 2.3), 25:75
Flow Rate: 1mL/min

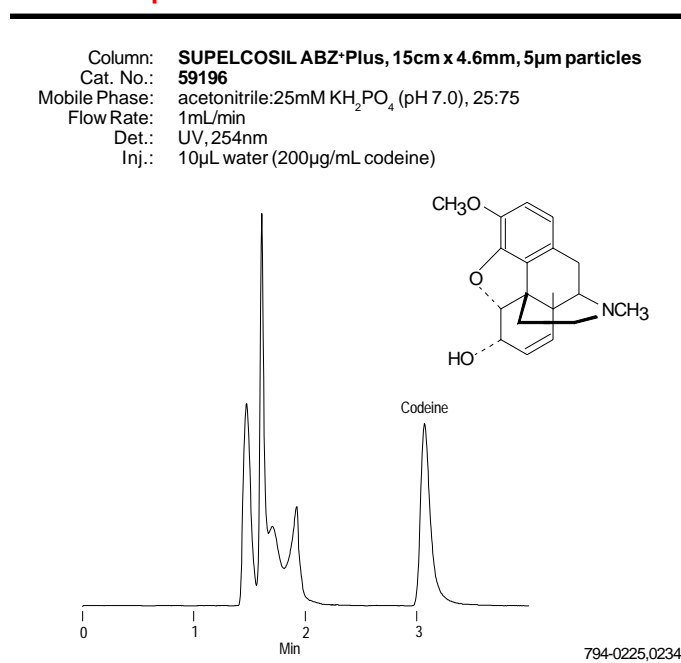
Figure B. Sharp, Symmetric Peak for a Basic Compound

Figure C illustrates how chromatographic results depend on the nature of the analytes and on the column chosen for the analysis. Diphenhydramine (antihistamine) and protriptyline (tricyclic antidepressant) elute in different order on a SUPEL COSIL ABZ⁺Plus column and a conventional deactivated C18 column, apparently without significant effect on peak shape. However, amitriptyline (tricyclic antidepressant), which elutes last from both columns, shows superior peak shape on the SUPEL COSIL ABZ⁺Plus column. The more symmetric peak makes quantification more reliable.

Deactivated columns can exhibit higher bleed than conventional reversed phase columns. SUPEL COSIL ABZ⁺Plus columns show low levels of bleed, comparable to levels from conventional C18 columns. Thus, these columns are well suited to gradient separations. Figure D shows a high performance analysis of 10

Table 2. Chromatography of a Basic Compound

Column	k' _{codeine}	N _{codeine}	AF10 _{codeine}
SUPEL COSIL ABZ ⁺ Plus [■]	1.2	48 x 10 ³	1.5
YMC-Basic [■]	1.1	54 x 10 ³	2.2
LiChrosorb RP-Select B [▲]	2.7	22 x 10 ³	4.4
Inertsil ODS-2 [■]	1.1	39 x 10 ³	1.6
Hypersil BDS-C18 [■]	1.1	74 x 10 ³	1.2
TSK-GEL ODS-80Ts [■]	1.7	64 x 10 ³	4.6
Nucleosil 100-5 C ₁₈ AB [♦]	1.1	13 x 10 ³	5.0
Zorbax R _x [▲]	5.2	5 x 10 ³	5.6
Waters Symmetry C ₈ [■]	1.6	34 x 10 ³	1.7
Waters Symmetry C ₁₈ [■]	1.7	34 x 10 ³	1.7

[■]15cm column [▲]25cm column [♦]10cm column

k' calculated using the half-height method; N = plates/meter; peak symmetry (AF10) monitored at 10% peak height.

Mobile Phase: acetonitrile:25mM KH₂PO₄ (pH 7.0), 25:75 (* = 50:50)
Flow Rate: 1mL/min

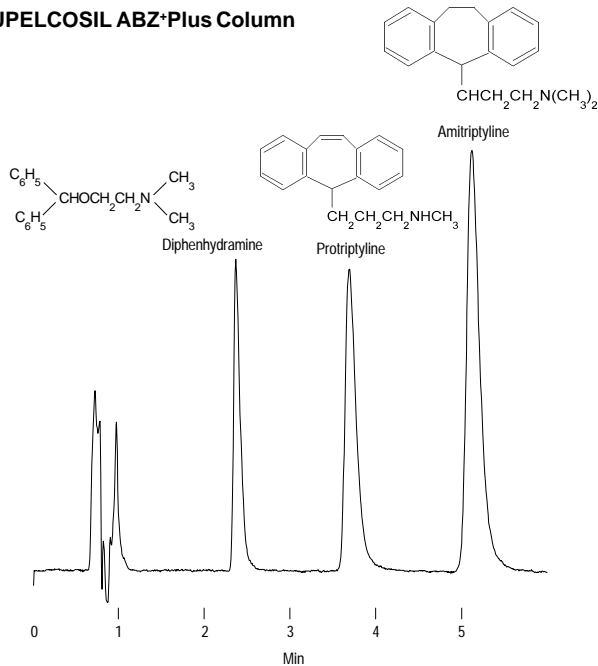
antiarrhythmic drugs, including procainamide, diltiazam, and digoxen, by gradient elution from a SUPEL COSIL ABZ⁺Plus column. Smaller ID columns, suitable for HPLC-MS, can be prepared on request.

The unique selectivity of SUPEL COSIL ABZ⁺Plus offers more than peak symmetry. Of the many deactivated reversed phase columns available, only SUPEL COSIL ABZ⁺Plus columns offer both thorough surface deactivation and unique selectivity for polar groups (2,3). We investigated the reversed phase properties of SUPEL COSIL ABZ⁺Plus columns for neutral, acidic, and basic compounds, using homologous series of alkylbenzenes, alkylbenzoic acids, and alkyanilines. There is an approximately linear relationship between the logarithm of the capacity factor (k') and the number of methylene (-CH₂-) groups in the alkyl chain (n). A mix containing benzene (n=0), toluene (n=1), ethylbenzene (n=2), propylbenzene (n=3), butylbenzene (n=4), 1-phenylhexane (n=5) and 1-phenylheptane (n=6) was injected on a SUPEL COSIL ABZ⁺Plus column and on conventional SUPEL COSIL LC-8 and SUPEL COSIL LC-18 reversed phase columns. Figure E shows that the hydrophobic retention and selectivity of the ABZ⁺Plus column (the ratio of the capacity factors for two alkylbenzenes) is similar to that of SUPEL COSIL LC-8 columns while, as expected, the nonpolar analytes are more strongly retained on SUPEL COSIL LC-18 columns.

Figure C. Basic Pharmaceuticals

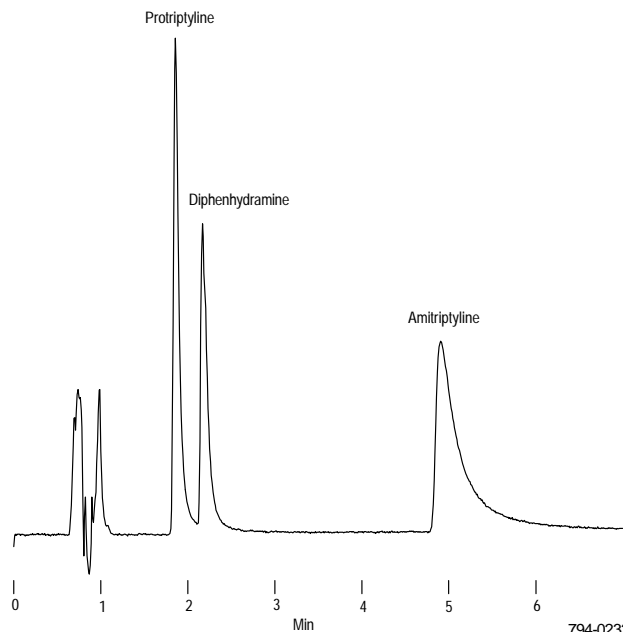
Column: **SUPELCO SIL ABZ+Plus, 15cm x 4.6mm, 5µm particles**
 Cat. No.: **59196**
 Mobile Phase: acetonitrile:50mM K₂HPO₄ (pH 7.0), 40:60
 Flow Rate: 2mL/min
 Det.: UV, 254nm
 Inj.: 10µL water (33µg/mL amitriptyline, protriptyline, 200µg/mL diphenhydramine)

SUPELCO SIL ABZ+Plus Column



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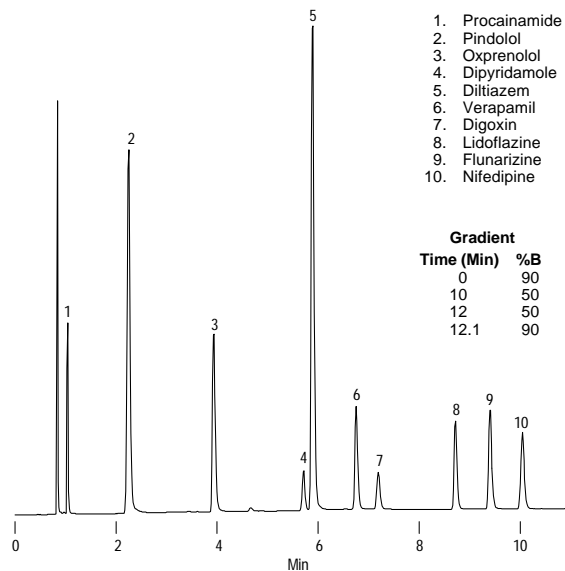
Conventional Deactivated C18 Column



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Figure D. Cardiac Drugs

Column: **SUPELCO SIL ABZ+Plus, 15cm x 4.6mm, 5µm particles**
 Cat. No.: **59196**
 Mobile Phase: acetonitrile:25mM KH₂PO₄ (pH 3.0), gradient profile on figure
 Flow Rate: 2mL/min
 Det.: UV, 220nm
 Inj.: 20µL methanol:water, 50:50 (30µg/mL pindolol, diltiazem, 100µg/mL dipyridamole, 50µg/mL other analytes)



Gradient	
Time (Min)	%B
0	90
10	50
12	50
12.1	90

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Acids, such as alkylbenzoic acids (n=0-5), are retained much more strongly on SUPELCO SIL ABZ+Plus columns than on C8 or C18 columns (Figure F). Retention of the smallest acids is shorter on SUPELCO SIL LC-18 columns than on SUPELCO SIL LC-8 columns, but more hydrophobic acids are, as expected, retained longer on C18 columns. The slopes of the log k' vs. n plots, which are proportional to the selectivity, are roughly the same for SUPELCO SIL ABZ+Plus and SUPELCO SIL LC-18 columns. Both columns are more selective for acids than SUPELCO SIL LC-8 columns.

The plots in Figure G indicate that capacity factors and selectivities for alkyaniline compounds (aniline, 4-ethylaniline, 4-propylaniline, 4-butyraniline, 4-pentyraniline) are almost identical for SUPELCO SIL ABZ+Plus columns and deactivated SUPELCO SIL LC-18-DB columns, and are greater than values for SUPELCO SIL LC-8-DB columns. The special selectivities of SUPELCO SIL ABZ+Plus columns for compounds with polar charged functional groups are attributed to the unique chemical structure of the bonded phase. The chromatograms in Figure C show that selectivity for basic compounds on SUPELCO SIL ABZ+Plus columns can be so different from selectivity of conventional deactivated columns that the order of elution changes. Analysts can take advantage of this unique chemical selectivity.

SUPELCO SIL ABZ+Plus columns provide high efficiency for polar, nonpolar, and charged analytes, symmetric peaks for the most difficult compounds, hydrophobic selectivity similar to that of SUPELCO SIL LC-8 columns, and special selectivity for polar and charged compounds. They ensure a flat baseline for gradient analyses, preparative applications, and HPLC-MS. If you are struggling with complicated mobile phases or asymmetric, difficult-to-quantify analyte peaks, we highly recommend these columns to you.

Figure E. Log k' vs. Number of Methylene Groups (Alkylbenzenes)

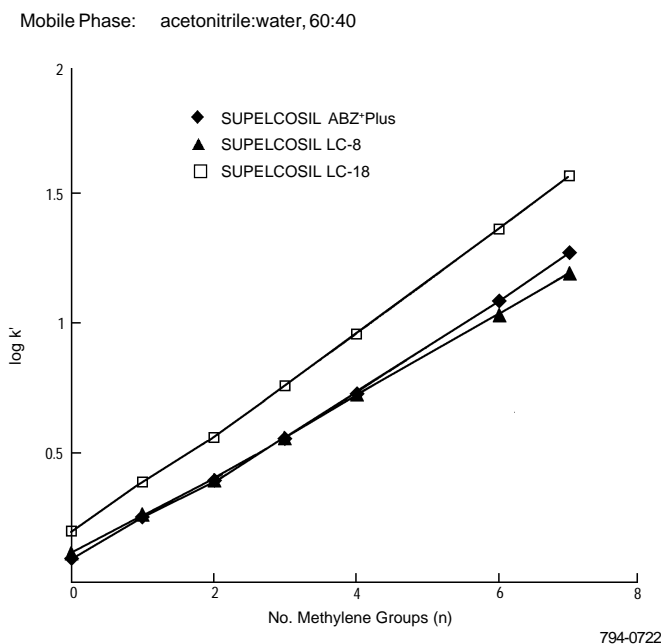


Figure G. Log k' vs. Number of Methylene Groups (Alkylanilines)

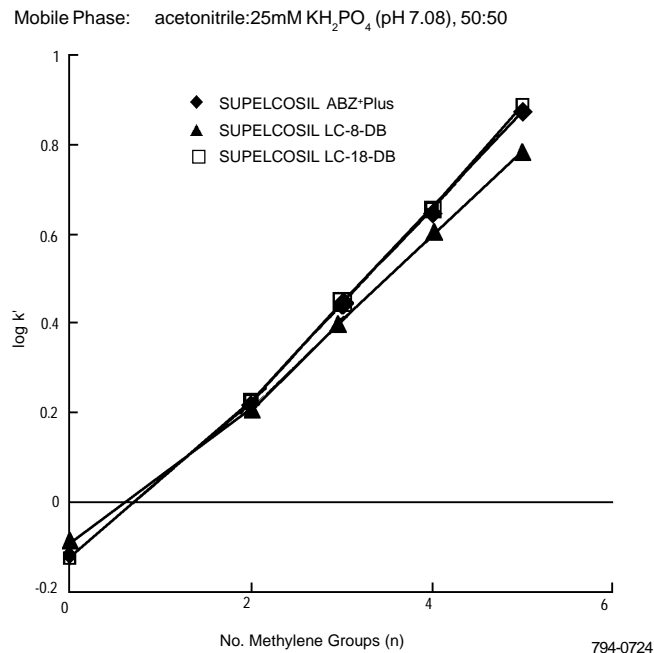
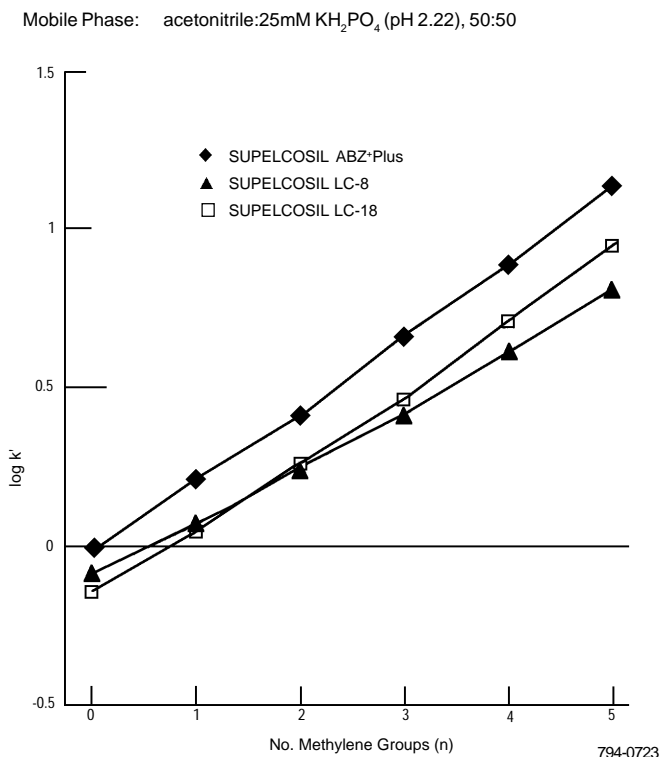


Figure F. Log k' vs. Number of Methylene Groups (Alkylbenzoic Acids)



Ordering Information:

Description	Cat. No.
SUPELCOSIL ABZ*Plus Columns	
5cm x 4.6mm	59195-U
15cm x 4.6mm	59196
25cm x 4.6mm	59197
25cm x 2.1mm	57927
Supelguard™ ABZ*Plus Guard Column Kit	
2cm x 4.6mm guard column, holder, connecting hardware	59534-U
Replacement 4.6mm columns, pk. of 2	59535-U
2cm x 2.1mm guard column, holder, connecting hardware	59604
Replacement 2.1mm columns, pk. of 2	59605

References

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References not available from Supelco.

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