

Improve Resolving Power and Peak Symmetry for Active Analytes by Using an HPLC Phase with Unique Selectivity

SUPELCOSM ABZ⁺Plus columns provide all of the benefits of silica-based reversed phase HPLC columns: high efficiency, stability, mechanical strength, and a predictable separation mechanism. A polar group incorporated in the SUPELCOSM ABZ⁺Plus phase gives the phase both a high level of silanol deactivation and unique selectivity, significantly different from that of conventional or deactivated C18 reversed phase columns. This unique selectivity enables analysts to resolve many compounds not normally resolved on a C18 or C8 column. SUPELCOSM ABZ⁺Plus columns provide good peak shape and efficiency for compounds with widely different functional groups, even under unbuffered conditions.

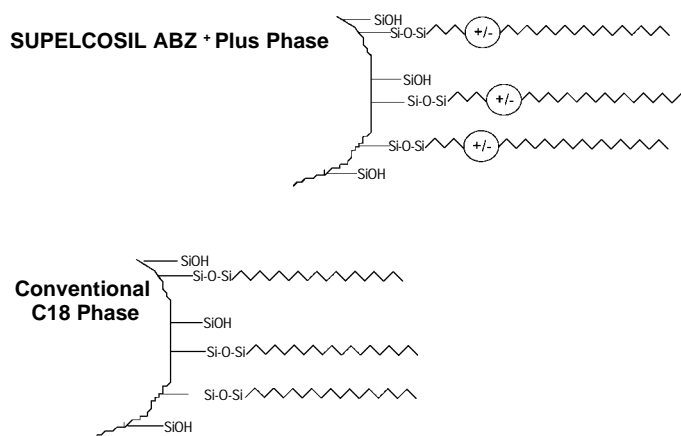
Key Words:

- acidic analytes ● basic analytes ● pharmaceuticals

The goal of most chromatographic separations is to maximize the resolution of analytes and other sample components. Retention time, peak efficiency (and hence symmetry), and peak spacing influence resolution. In most LC separations, mobile phase changes can adjust the relative spacing between peaks, but when the limits to these adjustments are reached, changes in the selective properties of the stationary phase are very effective in increasing — or decreasing — peak spacing.

Most analysts are aware of the difficulties of analyzing basic compounds on silica. Bases can interact with the support by both ion exchange (with -Si-O-) and hydrogen bonding (with -Si-OH) mechanisms, causing prolonged retention times and badly tailing peaks. However, acids also can, and do, interact with silanols (by H-bonding). The SUPELCOSM ABZ⁺Plus phase is part of a family of patented phases that operate by a reversed-phase mechanism, but have different selectivity and better surface shielding than conventional octadecylsilyl (C18) phases. A polar group incorporated in the alkyl chain, near the silica surface (Figure A), appears to act as an electrostatic barrier, repelling similarly charged molecules. Molecular modeling shows that the phase also is highly water-enriched near the silica surface. The water layer makes polar compounds more soluble in the phase and effectively hydrogen bonds with silanol groups on the support surface, making them less reactive. In contrast, a conventional C18 phase has no polar group and a monolayer of water. The unique structure of the SUPELCOSM ABZ⁺Plus phase gives the phase its unique selectivity.

Figure A. Models of SUPELCOSM ABZ⁺Plus and Conventional C18 Phases



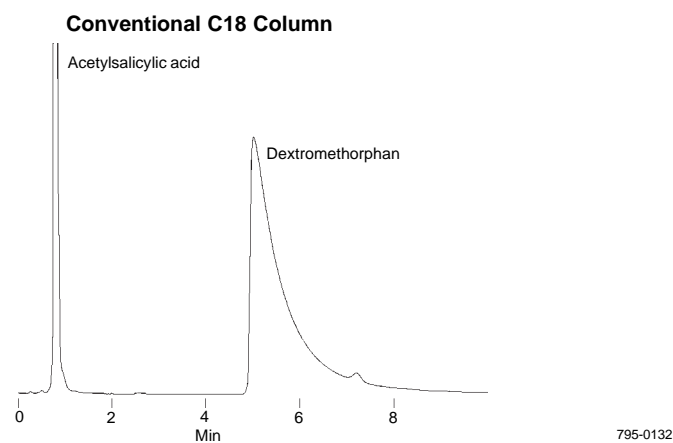
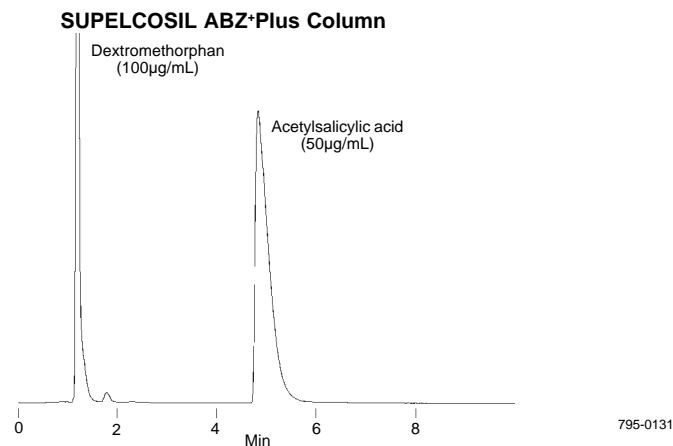
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The SUPELCOSM ABZ⁺Plus phase generally retains acidic compounds longer, relative to conventional C18 phases, and bases for shorter times. (Under most conditions, the polar group attracts acids and repels bases.) This is demonstrated by the reversal of elution order for dextromethorphan and acetylsalicylic acid shown in Figure B. Also, the peaks for both compounds are more nearly symmetric on the SUPELCOSM ABZ⁺Plus column than on the conventional column.

A reversed phase column should elute homologs in order of increasing hydrophobicity (increasing alkyl chain length). Although the selectivity of SUPELCOSM ABZ⁺Plus columns differs from that of C18 columns, SUPELCOSM ABZ⁺Plus columns are reversed phase columns, and behave as such. The retention time on a SUPELCOSM ABZ⁺Plus column increases with increasing carbon number of the alkyl group substituent on alkylbenzoic acid, as expected. Although the polar group in the SUPELCOSM ABZ⁺Plus bonded phase alters selectivity, its interaction with acidic compounds does not dominate; that is, the interaction is neither by ion exchange nor strong adsorption, either of which would cause nonlinear behavior and poor peak shape. The dominant separation mechanism is van der Waals (partitioning) interactions involving the carbon chain. An investigation of the reversed phase properties of SUPELCOSM ABZ⁺Plus columns for neutral, acidic, and basic compounds, using homologous series of alkylbenzenes, alkylbenzoic acids, and alkylanilines, is described in Bulletin 885 (available on request).

Figure B. Elution Order of Acidic and Basic Compounds Reversed

Columns: **15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **59196** (SUPEL COSIL ABZ⁺Plus column)
 Mobile Phase: acetonitrile:25mM potassium phosphate (pH 7.0), 30:70
 Flow Rate: 1.5mL/min
 Det.: UV, 230nm
 Inj.: 10µL

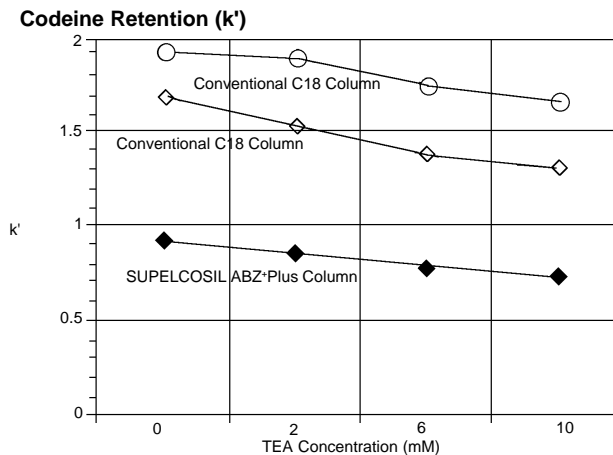
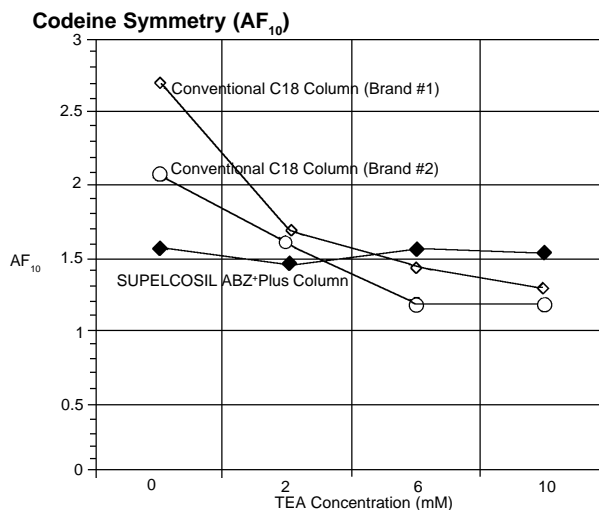


The polar group in the SUPEL COSIL ABZ⁺Plus phase accounts for the phase's high level of silanol deactivation, as well as its selectivity. We added 0-10mM triethylamine (TEA) to an acetonitrile:25mM KH₂PO₄ mobile phase at pH 7 (silanols are more active at pH 7 than at lower pH, where they are protonated), with corresponding adjustment to maintain consistent pH, then measured the effect of TEA on peak shape and *k'* for a base, codeine, for a SUPEL COSIL ABZ⁺Plus column and two conventionally deactivated C18 columns. Figure C shows that TEA improved peak shape on the C18 columns, by reducing analyte-silanol interactions. In contrast, TEA had little effect on peak shape on the SUPEL COSIL ABZ⁺Plus column, indicating there is no silanol activity in the SUPEL COSIL ABZ⁺Plus column under these conditions. Codeine retention decreased modestly on all three columns as the TEA concentration was increased, but interpretation of these data is complicated. In addition to acting as a competing amine, TEA reduces hydrophobic retention by acting as an organic modifier.

We performed several additional analyses to test the surface deactivation of SUPEL COSIL ABZ⁺Plus columns. Selectivity for acids is demonstrated in Figure D. The acids are not resolved on the conventional C18 column, nor on any of almost a dozen other deactivated C18 columns we tested, indicating that most deactivated columns do not break away from traditional selectivity. Pyridine and phenol are often used to indicate analyte-surface interaction in a reversed phase column. The excellent peak shapes in Figure E show that such interactions are minimal in SUPEL COSIL ABZ⁺Plus columns, if present at all. Famotidine, a molecule possessing four primary and three tertiary amine groups, is very difficult to analyze on a column with active surface groups. A SUPEL COSIL ABZ⁺Plus column provides an almost symmetric peak (Figure F).

Figure C. Effect of TEA on Retention and Peak Shape of Codeine

Columns: **15cm x 4.6mm ID, 5µm particles**
 Cat. No.: **59196** (SUPEL COSIL ABZ⁺Plus column)
 Mobile Phase: acetonitrile:25mM KH₂PO₄ plus 0-10mM triethylamine (pH 7.0), 25:75



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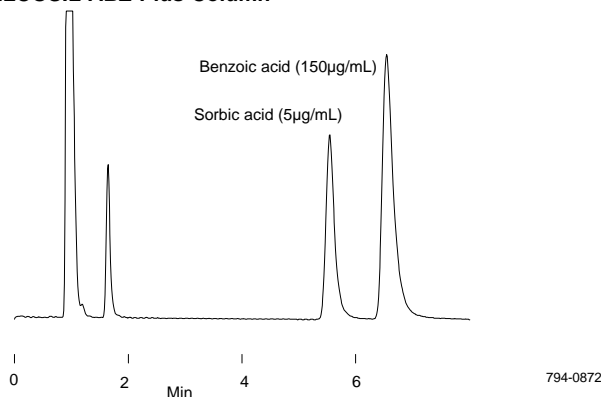
No separation is useful if it cannot be reproduced. Table 1 summarizes results of a ten cardiac drug separation, performed on 4 columns from 2 bonding/silica lots. To further test the reproducibility (stability) of the analysis for these compounds, column B was flushed extensively with an acidic mobile phase. The coefficient of variation (standard deviation/mean), less than 4%, indicates very good reproducibility. This analysis also shows that SUPELCOSIL ABZ⁺Plus columns even are compatible with acidic gradients at low detection wavelengths. Baseline rises from a SUPELCOSIL ABZ⁺Plus column (Figure G) are as small as those from conventional C18 columns operated under the same conditions.

SUPELCOSIL ABZ⁺Plus columns provide all of the benefits of silica-based reversed phase HPLC columns: high efficiency, stability, mechanical strength, and a predictable separation mechanism. However, their selectivity is significantly different from that of conventional C18 reversed phase columns – including columns promoted as highly deactivated. This unique selectivity often enables an analyst to resolve compounds not normally resolved on a C18 or C8 reversed phase column. The SUPELCOSIL ABZ⁺Plus phase assures excellent peak shape for all types of compounds, due to effective silanol deactivation and greater solubilization of polar compounds.

Figure D. A SUPELCOSIL ABZ⁺Plus Column Is Selective for Sorbic and Benzoic Acids

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59196 (SUPELCOSIL ABZ⁺Plus column)
 Mobile Phase: acetonitrile:25mM potassium phosphate (pH 2.3), 20:80
 Flow Rate: 2mL/min
 Det.: UV, 254nm
 Inj.: 10µL

SUPELCOSIL ABZ⁺Plus Column



Conventional C18 Column

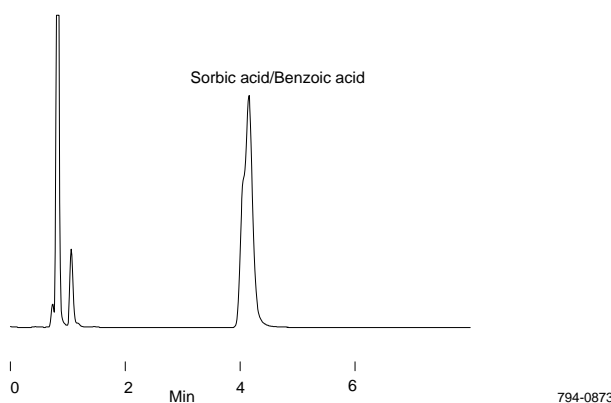


Figure E. Excellent Peak Shape for Pyridine and Phenol

Column: SUPELCOSIL ABZ⁺Plus, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59196
 Mobile Phase: acetonitrile:10mM potassium phosphate, 30:70
 Flow Rate: 2mL/min
 Det.: UV, 254nm
 Inj.: 10µL

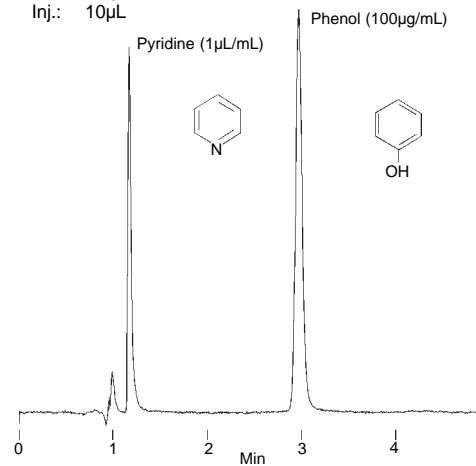


Figure F. Symmetric Peak for an Analyte with Multiple Active Groups

Column: SUPELCOSIL ABZ⁺Plus, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59196
 Mobile Phase: acetonitrile:10mM potassium phosphate, 5:95
 Flow Rate: 2mL/min
 Det.: UV, 254nm
 Inj.: 10µL

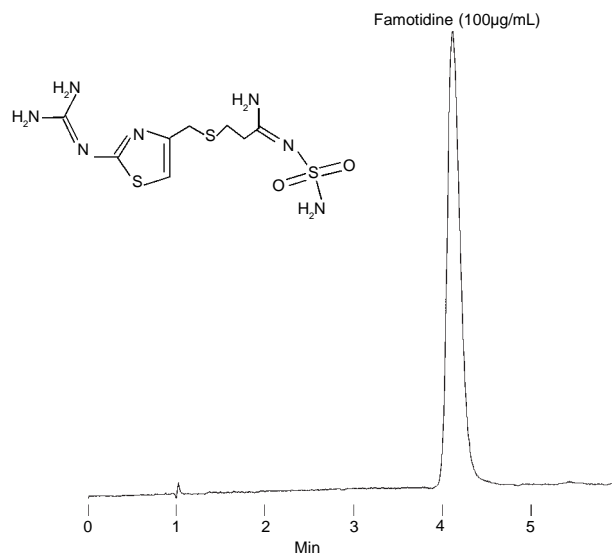


Table 1. Reproducible Analyses of Cardiac Drugs

Column:	A	B	C	D	
Silica Lot:	1	1 [▼]	1	2	
Drug	Retention Time (Min)				Mean ±%CV
Procainamide	1.06	1.12	1.09	1.10	1.10 ±2.3
Pindolol	2.34	2.56	2.46	2.54	2.48 ±4.1
Oxprenolol	4.09	4.34	4.25	4.28	4.24 ±2.4
Dipyridamole	5.81	6.09	5.98	6.14	6.01 ±2.5
Diltiazem	6.01	6.27	6.17	6.25	6.17 ±1.9
Verapamil	6.87	7.19	7.08	7.21	7.09 ±2.2
Digoxin	7.19	7.19	7.17	7.19	7.18 ±0.1
Flunarizine	9.48	9.94	9.79	10.10	9.83 ±2.7
Lidoflazine	8.82	9.18	9.06	9.26	9.08 ±2.2
Nifedipine	10.02	10.02	10.00	10.00	10.01 ±0.1

[▼]Acid-washed

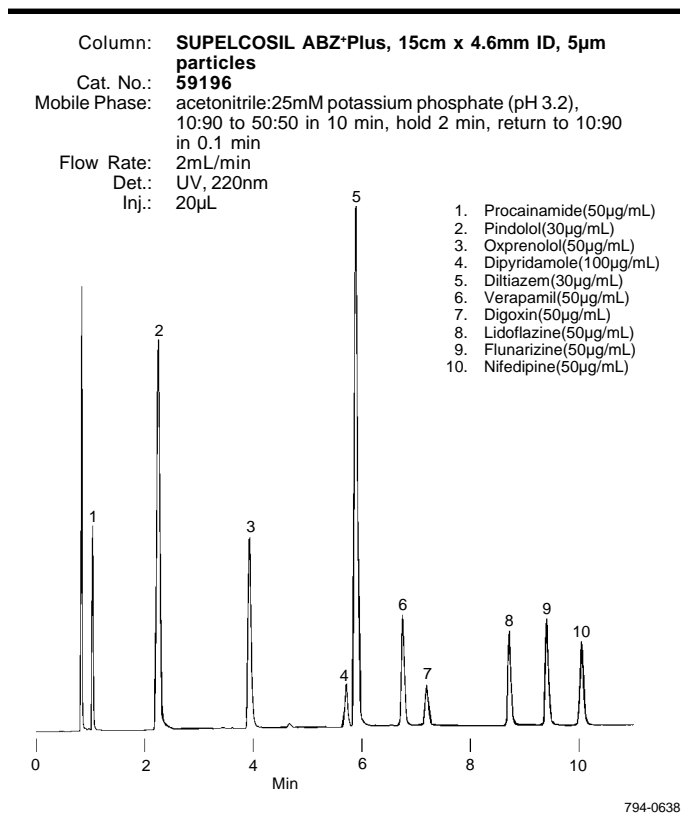
Conditions listed in Figure G.

Ordering Information:

Description	Cat. No.
SUPELCO SIL ABZ ⁺ Plus Columns	
5cm x 4.6mm, 5µm particles	59195-U
15cm x 4.6mm, 5µm particles	59196
25cm x 4.6mm, 5µm particles	59197
Guard Column Kit (guard column and holder)	59544-U
Guard Columns, pk. of 2	59545-U

For descriptions and a complete list of SUPELCO SIL ABZ⁺Plus analytical columns, preparative columns, and guard columns, please request Product Specification 494128.

Figure G. SUPELCO SIL ABZ⁺Plus Column Provides Flat Baseline in a Low pH Gradient



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