

TheReporter

Reprinted from Volume 14, No. 2, 1995

T295022

© 1999 Sigma-Aldrich Co.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

ARGENTINA • Sigma-Aldrich de Argentina, S.A. • Av. Pueyrredon 2446/50 • Piso 5-B • Buenos Aires 1119
AUSTRALIA • Sigma-Aldrich Pty. Ltd. • Unit #2, 14 Anella Avenue • Castle Hill NSW 2154
AUSTRIA • Sigma-Aldrich Handels GmbH • Hebbelplatz 7 • A-1110 Wien
BELGIUM • Sigma-Aldrich N.V./S.A. • K. Cardijnplein 8 • B-2880 Bornem
BRAZIL • Sigma-Aldrich Quimica Brasil Ltda. • Rua Sabara, 566-Conj. 53 • 01239-010 São Paulo, SP
CANADA • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8
CZECH REPUBLIC • Sigma-Aldrich s.r.o. • Pobrezni 46 • 186 21 Praha 8
DENMARK • Sigma-Aldrich Denmark A/S • Vejlegaardsvej 65B • DK-2665 Vallensbaek Strand
FINLAND • Sigma-Aldrich Finland/YA-Kemia Oy • Teerisuonkuja 4 • FIN-00700 Helsinki
FRANCE • Sigma-Aldrich Chimie • Chromatographie Supelco • L'Isle d'Abeau Chesnes - B.P. 701 • 38297 Saint-Quentin Fallavier Cedex
GERMANY • Sigma-Aldrich Chemie GmbH • Geschäftsbereich Supelco • Grünwalder Weg 30 • D-82041 Deisenhofen
GREECE • Sigma-Aldrich (o.m.) Ltd. • 72 Argonafton Str. • 16346 Ilioupoli, Athens
HUNGARY • Sigma-Aldrich Kft. • Nagy Diófa u. 7., IV fl. • H-1067 Budapest
INDIA • Sigma-Aldrich Co. • Survey No. 31/1, Sitharamapalaya • Mahadevapura P.O. • Bangalore 560 048
IRELAND • Sigma-Aldrich Ireland Ltd. • Airton Road • Tallaght • Dublin 24
ISRAEL • Sigma Israel Chemicals Ltd. • Park Rabin • Rohovot 76100
ITALY • Sigma-Aldrich s.r.l. • Via Gallarate, 154 • 20151 Milano
JAPAN • Sigma-Aldrich Japan K.K. • Division Supelco • JL Nihonbashi Building • 1-10-15 Nihonbashi Horidome-cho, Chuo-ku • Tokyo 103
KOREA • Sigma-Aldrich Korea • Samhan Camus Annex, 10th Floor • 17-26 Yoido-dong, Yungdeungpo-ku • Seoul
MALAYSIA • Sigma-Aldrich (M) Sdn. Bhd. • 9-2, Jalan 2/128, Taman Gembira • Off Jalan Kuchai Lama • 58200 Kuala Lumpur • Selangor
MEXICO • Sigma-Aldrich Quimica S.A. de C.V. • Calle 6 North No. 107 • Parque Industrial Toluca 2000 • 50200 Toluca
NETHERLANDS • Sigma-Aldrich Chemie BV • Postbus 27 • 3330 AA Zwijndrecht
NORWAY • Sigma-Aldrich Norway • Sandakerveien 102 • N-0483 Oslo
POLAND • Sigma-Aldrich Sp. z o.o. • Szelagowska 30 • 61-626 Poznań
PORTUGAL • Sigma-Aldrich Quimica, S.A. • P.O. Box 131 • Sintra 2710
RUSSIA • Sigma-Aldrich Russia • TOO Techmedbiochem • Makarenko Str. 2/21 • Building 1, Flat 22 • Moscow 103062
SINGAPORE • Sigma-Aldrich Pte. Ltd. • 102E Pasir Panjang Road • #08-01 Citilink Warehouse • Singapore 118529
SOUTH AFRICA • Sigma-Aldrich (pty) Ltd. • CNR Kelly & Ackerman Streets • Southern Life Industrial Park Unit • Unit 16/17 • Jet Park 1459
SPAIN • Sigma-Aldrich Quimica, S.A. • Apt. Correos 161 • 28100 Alcobendas, Madrid
SWEDEN • Sigma-Aldrich Sweden AB • Solkraftsvägen 14C • 135 70 Stockholm
SWITZERLAND • Supelco Switzerland • Industriestrasse 25 • P.O. Box 260 • CH-9471 Buchs
UNITED KINGDOM • Sigma-Aldrich Company Ltd. • Supelco UK • Fancy Road, Poole • Dorset BH12 4QH
UNITED STATES • Supelco • Supelco Park • Bellefonte, PA 16823-0048 • Phone 800-247-6628 or 814-359-3441 • Fax 800-447-3044 or 814-359-3044 • email:supelco@sial.com

H

This article is archived from a past issue of The Supelco Reporter. Information in the article was appropriate at the time of publication, but product specifications, catalog numbers, and availability may have changed over time.

If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.



Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip.

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

T. Ascah, F. Liu

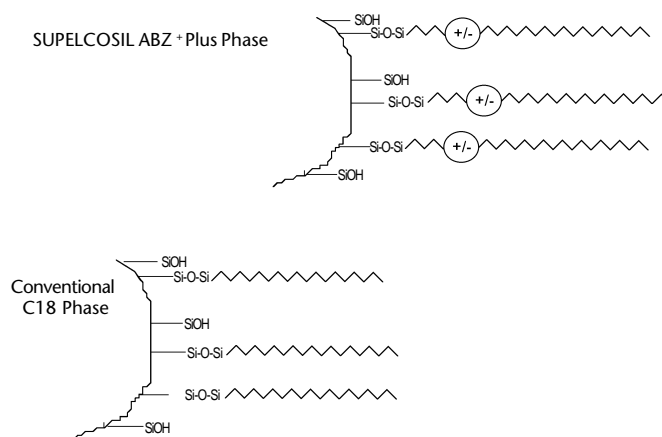
SUPELCOSM ABZ⁺Plus columns provide all of the benefits of silica-based reversed phase HPLC columns: high efficiency, stability, mechanical strength, and a predictable separation mechanism. A polar group incorporated in the SUPELCOSM ABZ⁺Plus phase gives the phase both a high level of silanol deactivation 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.

Most analysts are aware of the difficulties of analyzing basic compounds on silica. Bases can interact with the support by both ion exchange (with $-\text{Si-O}^-$) and hydrogen bonding (with $-\text{Si-OH}$) 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 the newest member 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.

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. Although the selectivity of SUPELCOSM ABZ⁺Plus columns differs from that of C18 columns, SUPELCOSM

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

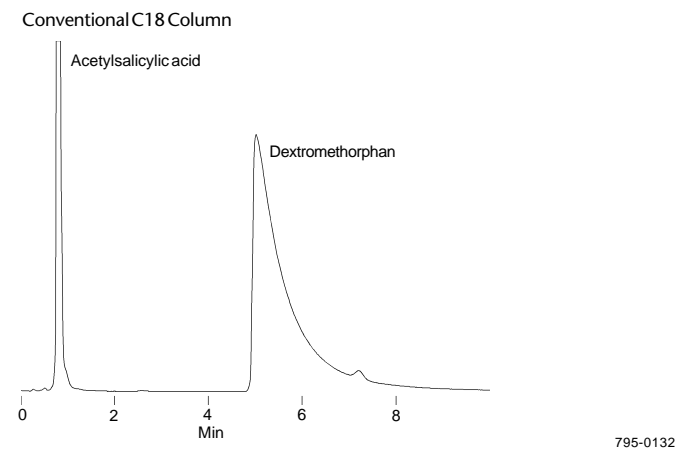
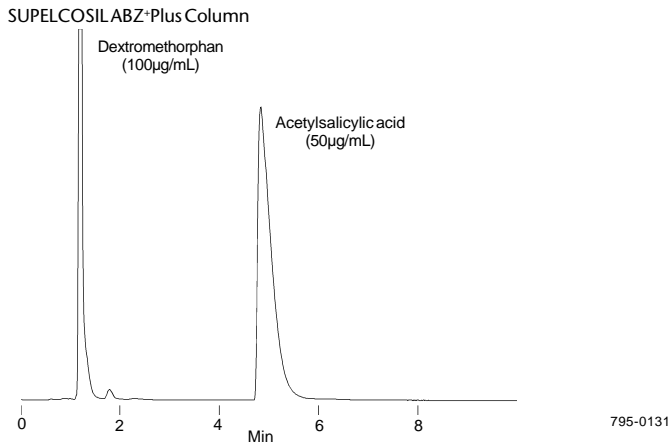


94-0475

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** (SUPELCO SIL ABZ⁺Plus column)
 Mobile Phase: acetonitrile:25mM potassium phosphate (pH 7.0), 30:70
 Flow Rate: 1.5mL/min
 Det.: UV, 230nm
 Inj.: 10µL



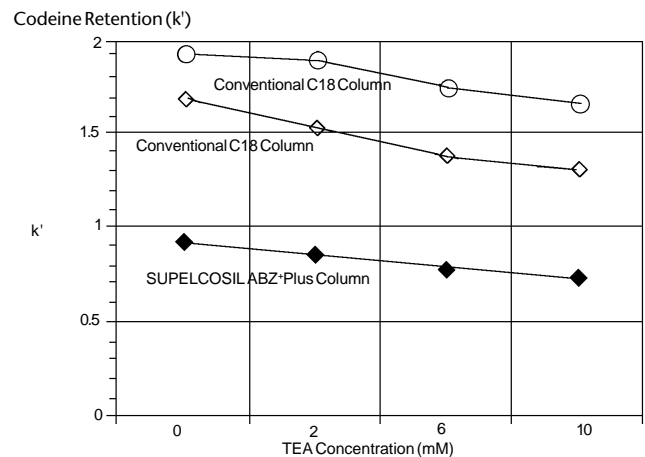
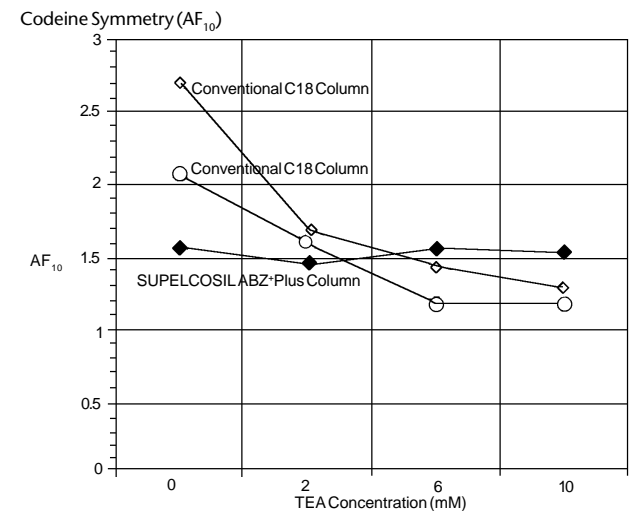
The polar group in the SUPELCO SIL ABZ⁺Plus phase accounts for the phase's high level of silanol deactivation, as well as its selectivity. We added 0-10mM triethylamine (TEA) to an acetonitrile:25mM KH₂PO₄ mobile phase at pH 7 (silanols are more active at pH 7 than at lower pH, where they are protonated), with corresponding adjustment to maintain consistent pH, then measured the effect of TEA on peak shape and *k'* for a base, codeine, for a SUPELCO SIL 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 SUPELCO SIL ABZ⁺Plus column, indicating there is no silanol activity in the SUPELCO SIL 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 SUPELCO SIL 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 SUPELCO SIL 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 SUPELCO SIL 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** (SUPELCO SIL ABZ⁺Plus column)
 Mobile Phase: acetonitrile:25mM KH₂PO₄ plus
 0-10mM triethylamine (pH 7.0), 25:75



94-0506, 0505

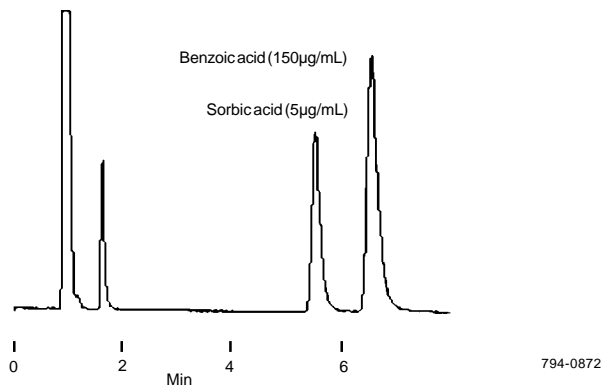
No separation is useful if it cannot be reproduced. Table 1 summarizes results of a ten cardiac drug separation, performed on 4 columns from 2 bonding/silica lots. To further test the reproducibility (stability) of the analysis for these compounds, part of one silica lot was further deactivated. The 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,

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

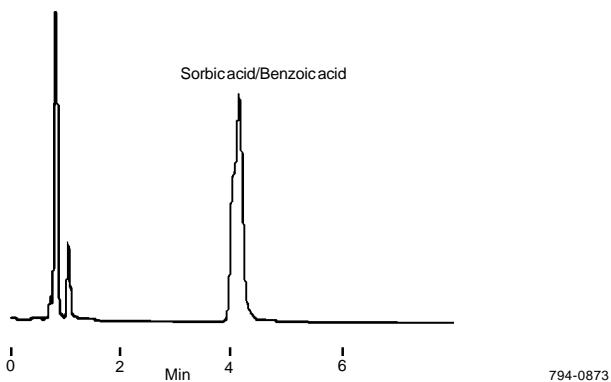


Figure E. Excellent Peak Shape for Pyridine and Phenol

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

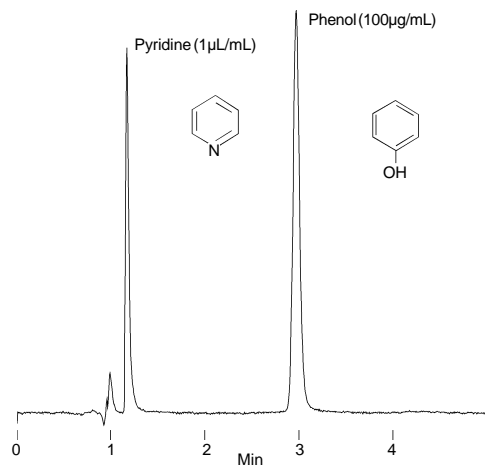


Figure F. Symmetric Peak for an Analyte with Multiple Active Groups

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

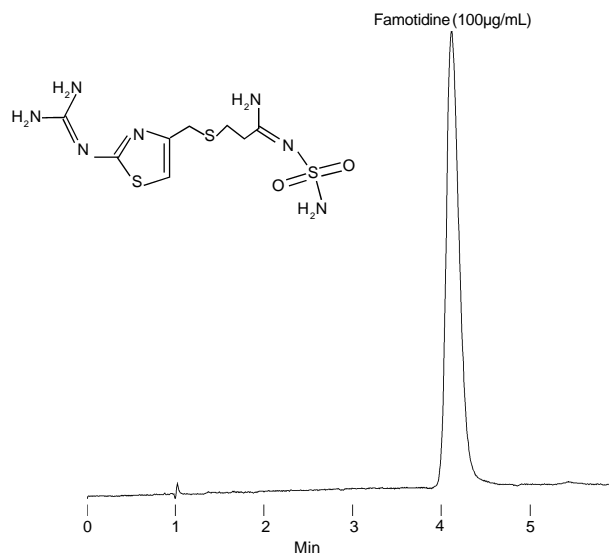


Table 1. Reproducible Analyses of Cardiac Drugs

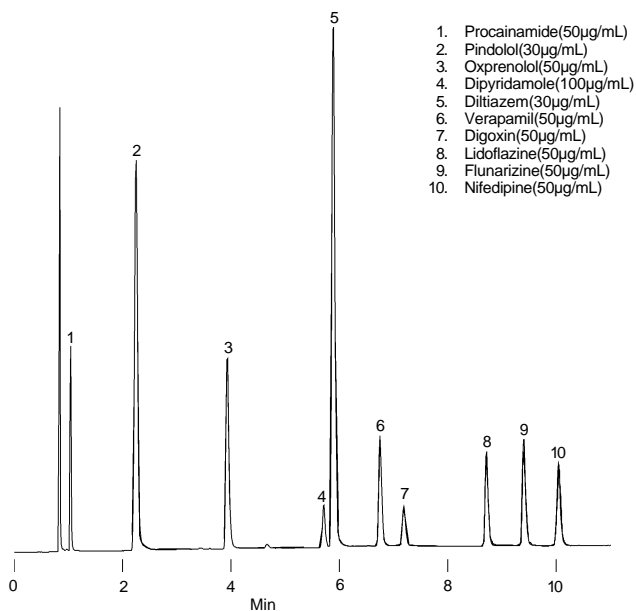
| Drug | Column: A Silica Lot: 1 | B 1 [▼] | C 1 | D 2 | Mean ±%CV |
|--------------|----------------------------|---------------------|--------|--------|------------|
| Procainamide | 1.06 | 1.12 | 1.09 | 1.10 | 1.10 ±2.3 |
| Pindolol | 2.34 | 2.56 | 2.46 | 2.54 | 2.48 ±4.1 |
| Oxprenolol | 4.09 | 4.34 | 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 |

▼Additional deactivation

Conditions listed in Figure G.

Figure G. SUPEL COSIL ABZ⁺Plus Column Provides Flat Baseline in a Low pH Gradient

Column: SUPEL COSIL ABZ⁺Plus, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59196
 Mobile Phase: acetonitrile:25mM potassium phosphate (pH 3.2),
 10:90 to 50:50 in 10 min, hold 2 min, return to 10:90 in 0.1 min
 Flow Rate: 2mL/min
 Det.: UV, 220nm
 Inj.: 20µL



794-0638

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 SUPEL COSIL ABZ⁺Plus phase assures excellent peak shape for all types of compounds, due to effective silanol deactivation and greater solubilization of polar compounds.

Ordering Information:**SUPEL COSIL ABZ⁺Plus Column**

5cm x 4.6mm **59195-U**

15cm x 4.6mm, 5µm particles **59196**

25cm x 4.6mm **59197**

Guard Column Kit
(guard column and holder) **59544-U**

Guard Columns, pk. of 2 **59545-U**

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

SUPEL COSIL is a trademark of Sigma-Aldrich Co.

