

Application

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Rapidly Separate β -Adrenergic- and Calcium Channel-Blocking Drugs by HPLC

Using a 15cm SUPELCOTM LC-8-DB column, a series of isocratic mobile phases, and UV detection, you can quickly monitor small amounts of most β -adrenergic- and calcium channel-blocking drugs. Sharp, symmetrical peaks make quantification easy.

Key Words:

- β -adrenergic drugs • calcium channel-blocking drugs
- SUPELCOTM LC-8-DB column • HPLC

Many drugs and pharmaceuticals are nitrogen-containing compounds. Because they are basic in nature, they exhibit poor peak shapes and long retention times on conventional reversed phase HPLC columns.

SUPELCOTM DB deactivated columns were developed to simplify resolution of basic drugs, improve their peak shapes, and ensure their rapid analyses. The value of these columns is clearly illustrated by analyses of β -adrenergic- and calcium channel-blocking drugs. One SUPELCOTM DB* column and a simple progression of mobile phases can be used to rapidly separate many of the drugs within general classes.

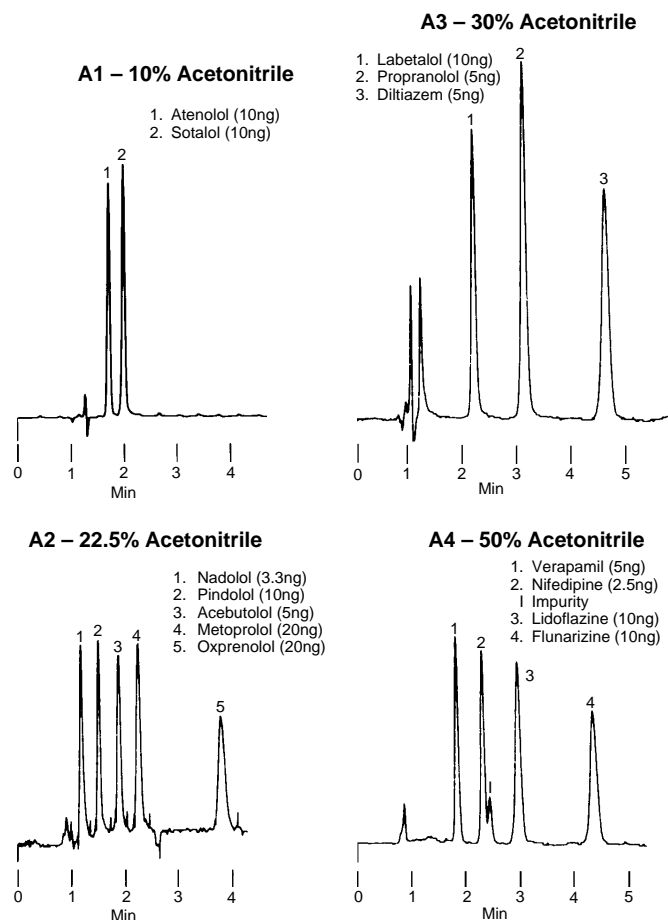
β -Adrenergic-blocking drugs, used in treating diseases characterized by excess nervous activity, decrease the heart rate by slowing sympathetic nerve impulses. Calcium antagonists slow contraction rates for cardiac and vascular smooth muscle cells by retarding the passage of calcium ions across the cell membrane.

To prolong their effectiveness, the drugs usually are dispensed in a slow release oral form. Therefore, the amounts present in a patient's serum normally are very low (1-200ng/mL). Most of the numerous HPLC analyses described for the drugs deal with separating only one or two compounds. Several of these analyses require complex mobile phases. The basic nature of these compounds presents another problem—peak tailing on typical reversed phase columns.

As shown in Figure A, we were able to rapidly resolve the drugs within classes, using a SUPELCOTM LC-8-DB analytical column, an LC-8-DB guard column, and similar analytical conditions (except for mobile phase composition). The analysis is simple and requires no special equipment or reagents, and the fast turn-around time reduces costs. Furthermore, day-to-day values for retention times are reproducible to within $\pm 1.5\%$ (RSD). For any class of these drugs (if only one or two compound are analyzed), analysis time can be considerably shortened.

Figure A. β -Adrenergic- and Calcium Channel-Blocking Drugs Resolved Within Classes through Simple Mobile Phase Adjustments

Column: SUPELCOTM LC-8-DB, 15cm x 4.6mm ID, 5 μ m particles
Cat. No.: 58347
Mobile Phase: acetonitrile:0.02M KH₂PO₄ buffer (pH to 3.0 with H₃PO₄), 100 μ L/liter triethylamine added (final pH = 3.15)
A1 = 10:90 (200 μ L/liter TEA added, final pH = 3.3)[^]
A2 = 22.5:77.5^{*}
A3 = 30:70
A4 = 50:50
Temp.: 35 $^{\circ}$ C
Flow Rate: 2.0mL/min, pressure range 10-150 bar
Det.: UV, 220nm
A1 = 0.005 AUFS
A2 = 0.005 AUFS
A3 = 0.01 AUFS
A4 = 0.01 AUFS
Inj.: 10 μ L, drug amounts shown on figure

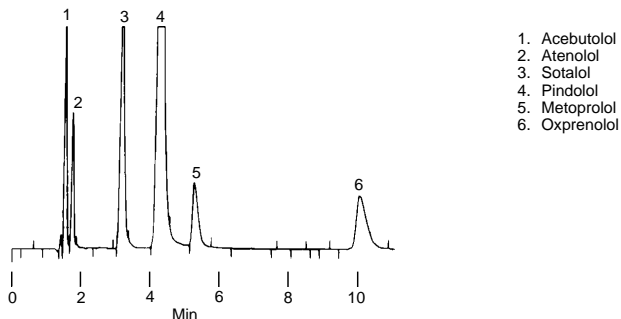


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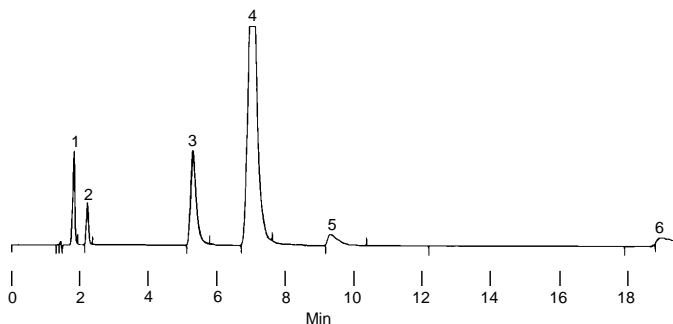
Figure B. β -Adrenergic- and Calcium Channel-Blocking Drugs

Column: B1 = SUPELCOSIL LC-8-DB, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58347
 Column: B2 = SUPELCOSIL LC-8, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58220-U
 Mobile Phase: acetonitrile:0.02M KH₂PO₄ buffer (pH to 3.0 with KOH 200 μ L), 20:80 TEA/liter added (final pH = 3.3)
 Col. Temp.: 35°C
 Flow Rate: 2.0mL/min
 Det.: UV, 254nm, 0.1 AUFS
 Inj.: 10 μ L, mobile phase containing 1 μ g each drug

B1 – Deactivated Column



B2 – Nondeactivated Column



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Figure B illustrates the difference between an analysis of basic drugs on a SUPELCOSIL LC-8-DB column and a conventional LC-8 column. The analysis takes nearly twice as long on the latter, and the poorly shaped metoprolol and oxprenolol peaks are difficult to quantify.

Using a 15cm SUPELCOSIL LC-8-DB column and a series of isocratic mobile phases, you can quickly and efficiently monitor small amounts of most β -adrenergic- or calcium channel-blocking drugs used to treat heart diseases. Although both UV and fluorescence detection have been used in the literature, UV is

generally more convenient. These analyses are rapid and selective, while the sharp, symmetrical peaks make quantification easy.

Because almost any basic compound is better separated on a DB phase than on a nondeactivated phase, SUPELCOSIL DB columns could be invaluable for chromatographers in other industries, including fine chemicals, photography, environmental hygiene, and biochemistry.

Ordering Information:

Description	Cat. No.
SUPELCOSIL LC-8-DB column, 15cm x 4.6mm ID, 5 μ m particles	58347
Supelguard™ LC-8-DB guard column kit**	59553
Supelguard LC-8-DB guard columns, 2cm, pk. of 2	59563

For other SUPELCOSIL DB columns, refer to the Supelco catalog.

▲ The additional TEA was needed for adequate drug retention.

♦ Timolol elutes at about 2 minutes in this system, and is best detected at 295nm UV.

* DB – Deactivated for basic compounds.

**2cm x 4.0mm column, column holder, and hardware to connect the column to 1/6" OD tubing.

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