

Monitor Homologous Catecholamines by HPLC Using a SUPELCOSM LC-ABZ Column

The HPLC analysis of catecholamines and their metabolites, neurochemicals that include both bases and acids, is difficult because of poor peak shape due to silanol interaction. The SUPELCOSM LC-ABZ column features a unique deactivation process that allows the analysis of both types of compounds without the need for silanol-suppressing additives. The excellent peak shape improves detection at the low levels at which these compounds are monitored.

Key Words:

- catecholamines
- deactivated HPLC phases

The biogenic amines norepinephrine (NEPI), epinephrine (EPI), and dopamine (DA) are the primary human neurotransmitters and the most commonly analyzed catecholamines. Two acidic metabolites of catecholamines, homovanillic acid (HMA) and vanilmandelic acid (VMA), are frequently monitored in urine for the diagnosis and treatment of patients with pheochromocytoma and related neurogenic tumors. VMA is the end product of both epinephrine (adrenaline) and norepinephrine (noradrenaline) catabolism, while HVA is a dopamine catabolite. Because these compounds are typically analyzed at low levels, good peak shape is essential.

These neurotransmitters and their metabolites comprise a mixture of functional groups — amines, phenolic OH, and carboxylic acids — which can interact with the silanols in a silica-based HPLC packing, causing poor peak shape, retention drift, and column-to-column irreproducibility. The SUPELCOSM LC-ABZ column eliminates these problems with a specially deactivated reversed phase silica packing. This packing features an embedded polar group that, via electrostatic shielding, prevents silanol interaction of either acids or bases. The column provides excellent peak shape for acids, bases, neutrals, and zwitterions without silanol-deactivation techniques or amine modifiers.

We analyzed a mixture of EPI, NEPI, and DA in water using a SUPELCOSM LC-ABZ column (Figure A). An ion pairing reagent (heptane sulfonic acid) which associates with the primary amine group was used to obtain sufficient retention on this unique reversed phase column. A mixture of DA, dihydroxyphenylacetic acid (DOPAC), and catecholamine metabolites in water (Figure B) was also analyzed. DA was not retained due to the low pH and the absence of ion pairing reagents. DOPAC, a catecholamine structurally similar to DA, was included in this analysis because it has a convenient retention time, falling between VMA and HMA. Both analyses produced well-resolved, symmetrical peaks, suitable for quantitative monitoring of these compounds.

Figure A. Epinephrine, Norepinephrine, and Dopamine

Column: SUPELCOSM LC-ABZ, 5cm x 4.6mm ID, 5µm particles
 Cat. No.: 59141
 Mobile Phase: acetonitrile:25mM potassium phosphate containing 5.4mM heptane sulfonic acid, 5:95, pH 2.0
 Flow Rate: 2mL/min
 Det.: UV, 270nm, 0.01 AUFS
 Inj.: 10µL of 12.5µg/mL each compound in water

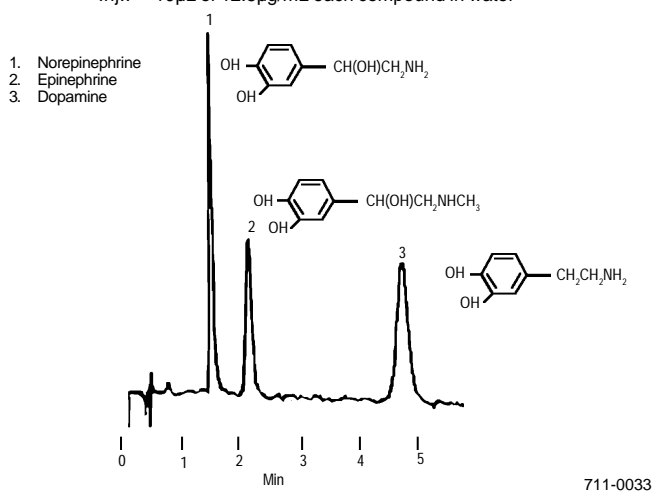


Figure B. Vanilmandelic and Homovanillic Acids

Column: SUPELCOSM LC-ABZ, 5cm x 4.6mm ID, 5µm particles
 Cat. No.: 59141
 Mobile Phase: acetonitrile:25mM potassium phosphate, 5:95, pH 2.2
 Flow Rate: 2mL/min
 Det.: UV, 270nm, 0.01 AUFS
 Inj.: 10µL of 12.5µg/mL each compound in water

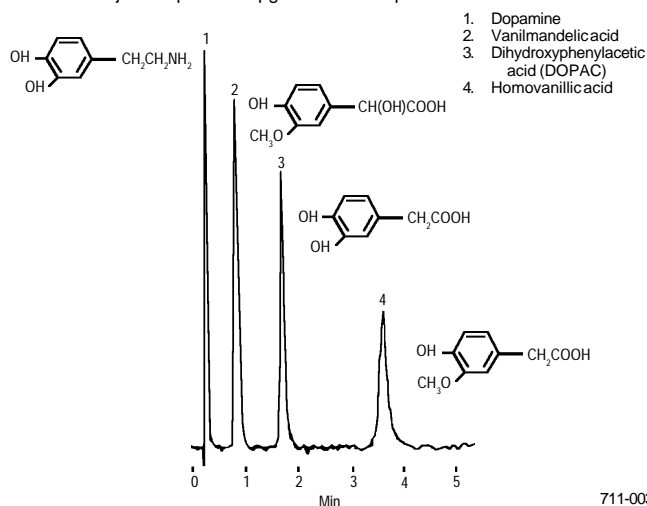
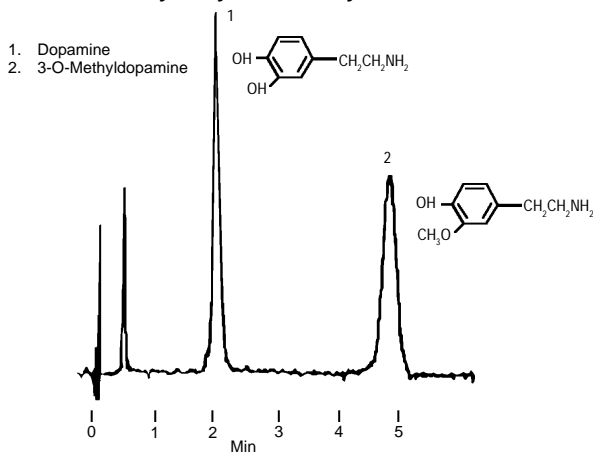


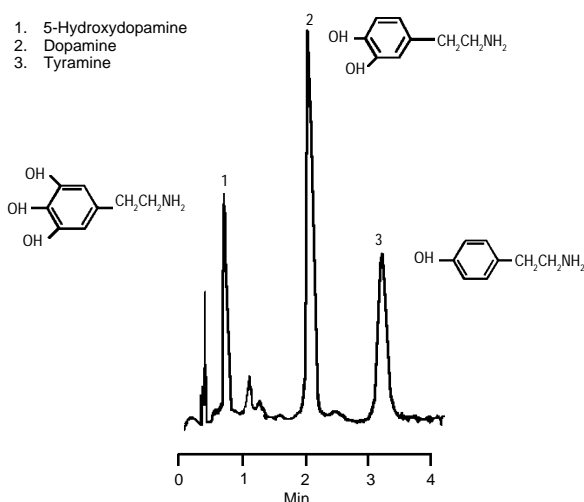
Figure C. Catecholamine Homologs

Column: **SUPELCOSIL LC-ABZ, 5cm x 4.6mm ID, 5µm particles**
 Cat. No.: **59141**
 Mobile Phase: acetonitrile:25mM potassium phosphate containing 5.4mM heptane sulfonic acid, 5:95, pH 2.0
 Flow Rate: 2mL/min
 Det.: UV, 270nm, 0.01 AUFS
 Inj.: 10µL of 12.5µg/mL each compound in water

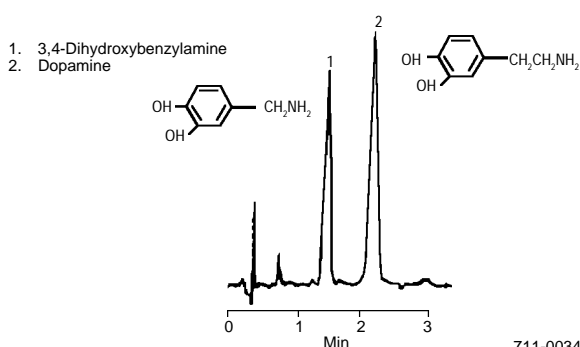
Hydroxy vs. Methoxy Substitution



Mono-, Di-, and Trihydroxy Substitution



Methylamine vs. Ethylamine Substitution



711-0034.0035.0040

To determine the effects of small changes in molecular structure on hydrophobic retention, we analyzed a series of homologous catecholamines (Figure C). The conversion of a hydroxyl to a methoxy group decreased hydrophobic retention, as did the addition of hydroxyl groups, which also made the compounds more polar. The addition of one methylene group in the alkyl chain produced a large effect on hydrophobic retention. The homologs, representing a range of polarities, were separated as effectively as the parent compounds.

SUPELCOSIL LC-ABZ columns offer the benefits of reversed phase HPLC on silica without using the extreme mobile phase conditions that are often necessary to improve the chromatography of acids and bases. Compounds with acidic and basic functionalities can be analyzed using the same column, often with little or no modification to the conditions. In addition, unique polar-nonpolar selectivity, relative to conventional reversed phase columns, offers opportunities for new analyses of previously well-characterized samples.

Ordering Information:

Description	Cat. No.
SUPELCOSIL LC-ABZ Columns	
5µm packing, 100Å pores	
5cm x 4.6mm	59141
15cm x 4.6mm	59140-U
25cm x 4.6mm	59142
Additional sizes are listed in the Supelco catalog.	
Supelguard™ LC-ABZ Guard Column Kit	
2cm x 4.6mm cartridge-type column, column holder, hardware for connecting to 1/16" tubing	59544-U
Supelguard LC-ABZ Guard Columns	
pk. of 2	59545-U
Ion Pair Reagent	
1-Heptane sulfonate (sodium salt), 99.9+%, 10g	39,7075
Other ion pair reagents are listed in the Supelco catalog.	

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Note 26