CAPSULES
notes and applications from Bioanalytical Systems, Inc.

SDS: An Alternative Ion Pairing Agent for Catecholamines

Purpose

To develop separations of catecholamines and serotonin (F1) using the ion pairing agent sodium dodecyl (lauryl) sulfate (SDS).

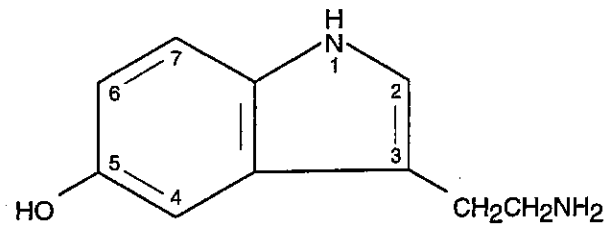

Figure 1. Structure of serotonin

Existing Methods

There are many common reverse-phase separations of the catecholamines. Sodium octyl sulfate (SOS) is a frequently used ion pairing agent, but it retains serotonin on column too long (F2).

Conditions

System: BAS-200B Liquid Chromatograph

Columns: BAS 3 µM C_{18} reverse phase (100 x 3.2 mm) (PN MF- 6213) and BAS 5 µM C_{18} Uni-Jet microbore column (150 x 1 mm)(PN MF-8912)

Temperature: 35 °C

Mobile Phase: 75 mM monochloroacetic acid, 1 mM SDS, 0.7 mM Na_2-EDTA), pH 3.0. Organic modifiers included dimethylacetamide (DMA), tetrahydrofuran (THF) and acetonitrile (ACN) in various mixtures and proportions. Flow rate was 1 (column 1) or 0.08 mL/min (column 2).

Electrode: Cross-flow glassy carbon

Potential: +0.75 V *vs.* Ag/AgCl

Injected Amount: 1 ng (column 1) or 100 pg (column 2) in 5 µL.

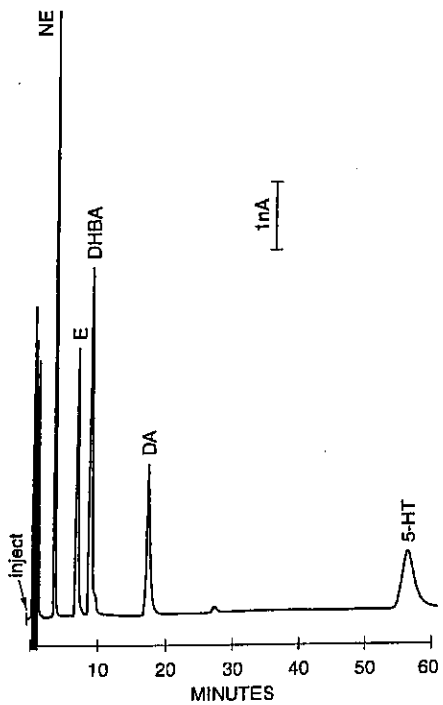

Figure 2. Typical separation of catecholamines using SOS. Mobile phase same as text except for 1 mM SOS in place of SDS, and 10% (v:v) ACN. Key: NE = norepinephrine, E = epinephrine, DHBA = dihyroxybenzylamine, 5-HT = serotonin.

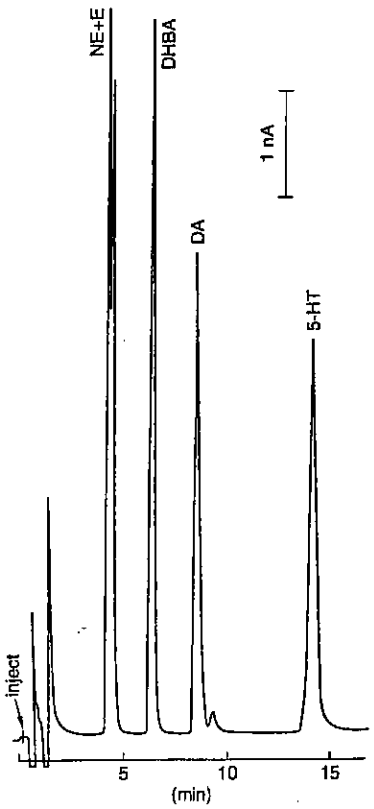

Figure 3. Separation with 20% (v:v) DMA. Peaks as in F2.

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Notes

Use of SDS dramatically reduced the retention time of serotonin, regardless of which organic modifier was added to the mobile phase (F3-F6).

DMA (as the sole organic modifier) brought serotonin off the earliest, but norepinephrine and epinephrine coeluted under these conditions (F3).

A blend of DMA and ACN produced a good separation (F4), as did a blend of ACN and THF (F5).

ACN produced as good a separation as any of the mixes (F6). A similar separation was achieved with a microbore column (F7). Since this mobile phase has only one organic modifier, it is more convenient to make and dispose of than the other mobile phases.

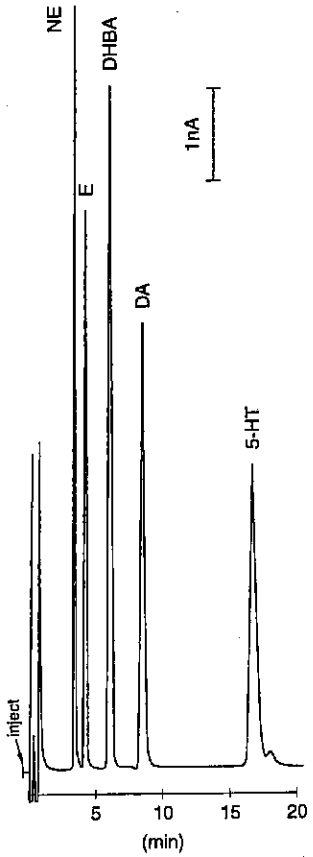

Figure 4. Separation with 4.5% DMA (v:v) and 13% ACN. Peaks as in F2.

An additional feature of an SDS mobile phase is that it does not retain any of the acidic catecholamine metabolites (F6, F7). Thus, it would be ideal for samples in which trace amounts of catecholamines are obscured by high levels of metabolites.

One drawback of an SDS mobile phase is that it is very foamy, and therefore difficult to filter. For this reason we recommend SOS when determining catecholamines in the absence of serotonin, and when high levels of metabolites are not a problem.

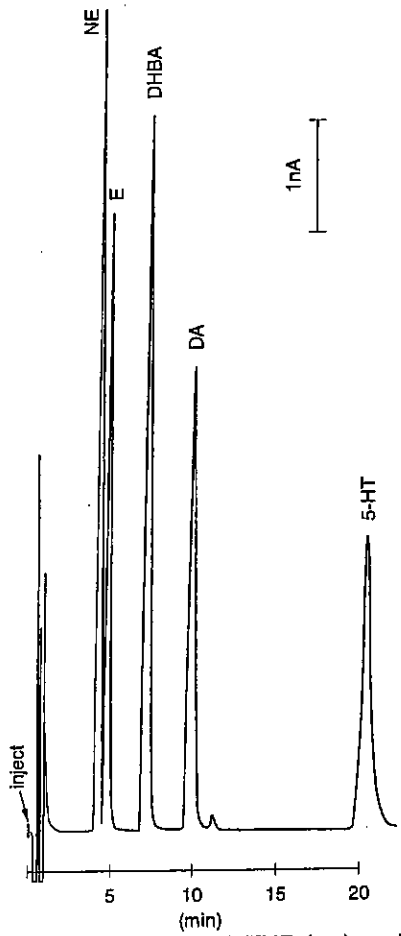

Figure 5. Separation with 3% THF (v:v) and 11% ACN. Peaks as in F2.

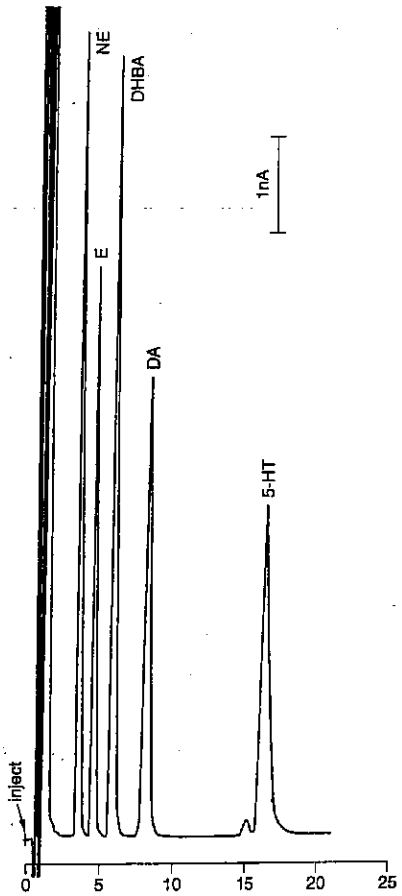

Figure 6. Separation with 16% ACN (v:v). Peaks as in F2. The following compounds were injected, but eluted in the void: DOPAC, 5-HIAA, HVA, L-DOPA, VMA.

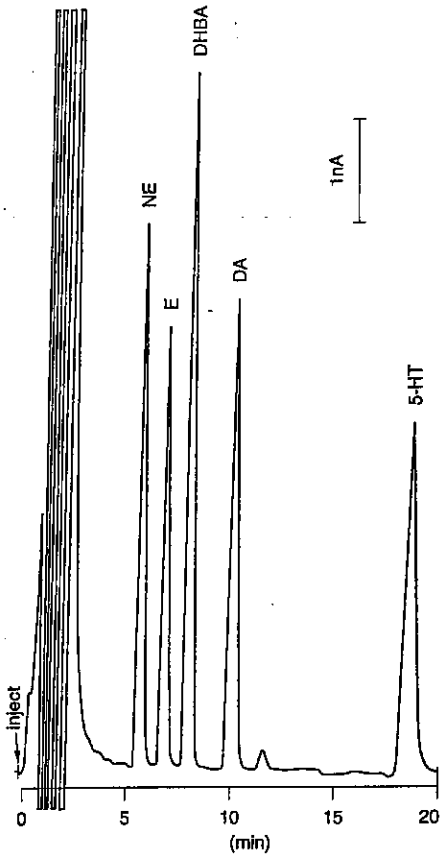

Figure 7. Microbore separation with 20% ACN (v:v). Peaks as in F2. The following compounds were injected, but eluted in the void: DOPAC, 5-HIAA, HVA, L-DOPA, VMA.