CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

HVA and DOPAC in Brain Tissue

Monitoring the fate of the neurotransmitter dopamine in brain tissue requires determination of its two primary metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Both compounds are formed by the action of sequential enzymatic reactions in the tissue. These reactions are mediated by catechol-O-methyltransferase, monoamine oxidase, and alcohol dehydrogenase.

In 1979 Hefti[1] reported a simple assay for tissue HVA and DOPAC utilizing liquid chromatography/electrochemistry. Tissue is homogenized in dilute perchloric acid. After centrifugation, an aliquot of the supernatant to which an internal standard (vanillic acid, VA) has been added is extracted with diethyl ether. The ether phase containing the dopamine metabolites is dried down, and the residue is reconstituted in dilute acetic acid. This extract is injected into the LC.

Measurements were first carried out on a Bioanalytical Systems LC-304T liquid chromatograph equipped with an LC-22/23 column heating compartment. A cooling coil was inserted between the column and the electrochemical detector to bring the mobile phase back to room temperature. The electrode was packed with CP-O (BAS) carbon paste.

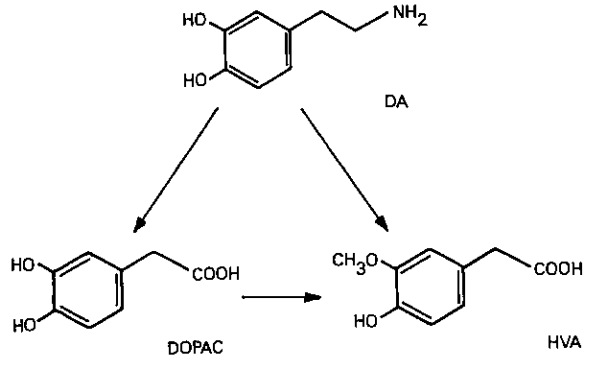

Figure 1. Metabolism of the neurotransmitter dopamine (DA), to 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Vanillic acid (VA) is used as the internal standard in the assay.

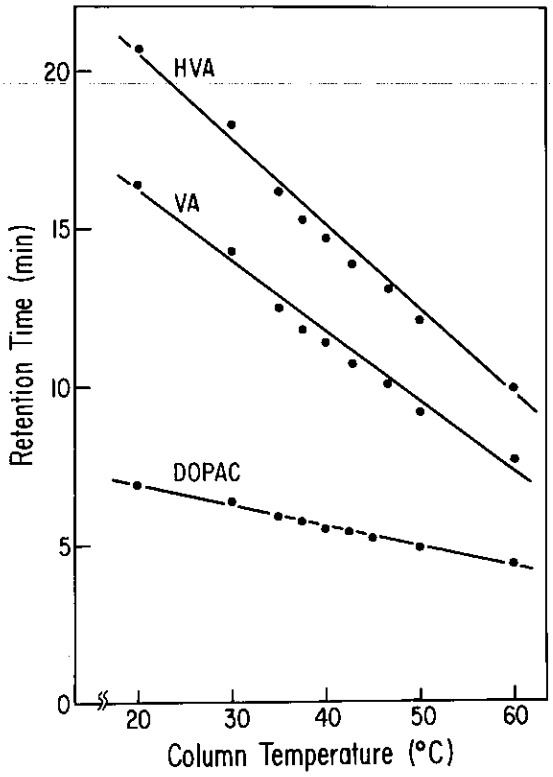

Figure 2. Relationship between column temperature and retention times for DOPAC, HVA, and VA. Stationary phase: µBondapak C_{18} (Waters). Mobile phase: 0.5 M sodium phosphate buffer (pH 5.0).

The column heater allowed easy tunability of the separation; capacity factors or retention times could be varied over a wide range by simply adjusting the LC-22 controller. F2 describes this behavior, and Figure 3 shows the improvement in performance with a 25°C jump in temperature. Analysis times are shortened, and peak symmetry is improved. This decade old work is now quite standard on the more modern BAS 200 and BAS 400 chromatographs.

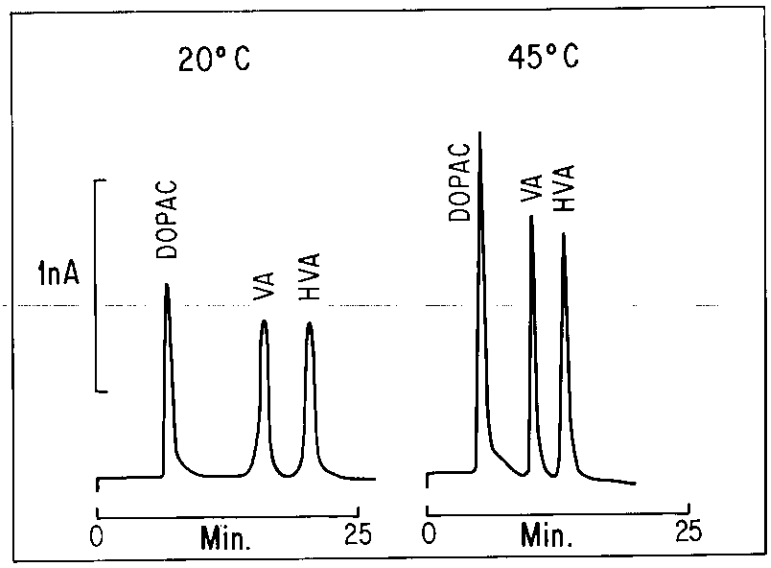

Figure 3. Standard runs (1 nanogram of each) demonstrating improvement in chromatography at increased temperature. Conditions were as described in F2.

References

1. F. Hefti, Life Sciences, 25(1979) 775-782.