CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Urinary Metanephrines by LCEC

The principal basic catecholamine metabolites are the "metanephrines", formed by the action of the enzyme catechol-O-methyltransferase on the catecholamines to give the corresponding 3-methoxy analogs. The compounds formed, normetanephrine (from norepinephrine), metanephrine (from epinephrine), and 3- methoxytyramine (from dopamine), are clinically interesting not only from their stature as catecholamine metabolites but also more specifically for their diagnostic value in diseases such as pheochromocytoma and neuroblastoma. Urinary concentrations of these compounds in healthy individuals range from 50 to 500 ng/mL, whereas in pheochromocytoma, for example, levels are generally greated than 2 µg/mL and usually even much more than that.

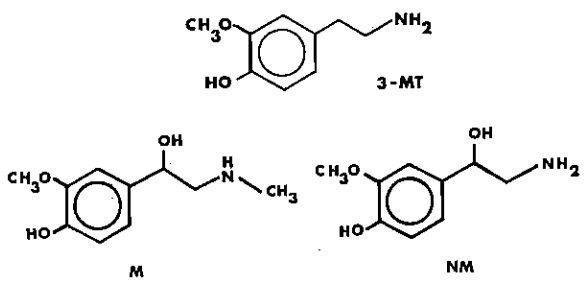

Figure 1. Structures of normetanephrine (NM), metanephrine (M), and 3- methoxytyramine (3-MT).

The suitability of these compounds for LCEC was assessed by using cyclic voltammetry with a Bioanalytical Systems CV-1A instrument. Solutions of normetanephrine, metanephrine, and 3-methoxytyramine were made in possible LC mobile phases at approximately millimolar concentrations. A sample response curve (a voltammogram) is illustrated in Figure 2. A positive scan of the working electrode potential was initiated at 0.0 volts. No faradaic current was measurable until approximately +0.6 V, whereupon a large wave due to oxidation of the ortho methoxy-hydroxy groups on the aromatic ring became evident. Thus, by setting the working electrode potential on the LCEC controller at some potential past the peak potential shown by cyclic voltammetry, electrochemical detection was possible. Since the peak potentials of the cyclic voltammograms were all about + 750 mV, the working electrode on the LC-4 detector was set at + 850 mV.

The low levels and frequent interferences seen in urine prevent direct injection of these compounds onto the analytical column. However, with a relatively brief extraction procedure, quantitation free from interferences is easy to accomplish. Hydrolyzed urine (to destroy sulfate and glucuronide conjugates) is first passed through miniature cation exchange columns, then eluted with pH 10 ammonia buffer. After extracting the buffered eluate twice with an ethyl acetate/acetone mixture, the extracts are then combined, evaporated under nitrogen, and brought up in dilute acid prior to injection. Only 4 mL of urine is required for analysis, and no tedious timing steps are necessary.

For the chromatography, a Bioanalytical Systems LC-304 reverse phase system was employed. A sample chromatogram is shown in Figure 3. In addition to the clean separation of normetanephrine, metanephrine, 3-methoxytyramine, and the internal standard (3-methoxy-4-hydroxybenzylamine), peaks for tyramine and serotonin (5-HT) are also evident. The new LCEC method is the first ever to separate and quantitate at normal levels all of the basic O-methylated metabolites simultaneously, without the necessity of performing derivatization prior to separation. The additional benefits of an internal standard (for better precision) and a detector capable of quantitation at ±10% r.s.d. at the 200 pg (injected) level for each of the three compounds make the assay very attractive to biomedical researchers interested in tyrosine metabolism.

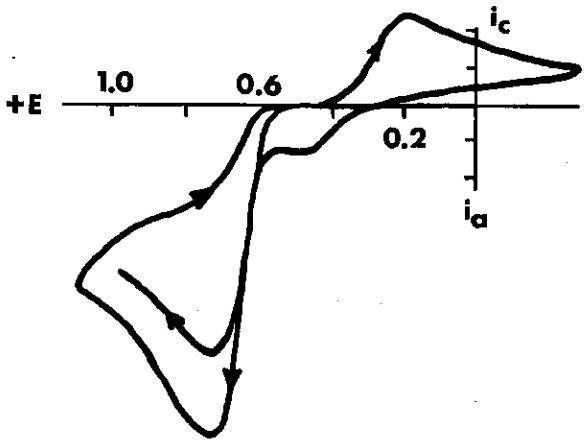

Figure 2. Cyclic voltammogram of 0.5 x 10^{-3}M solution of 3-methoxytyramine in the mobile phase. Scan rate 250 mV sec^{-1}.

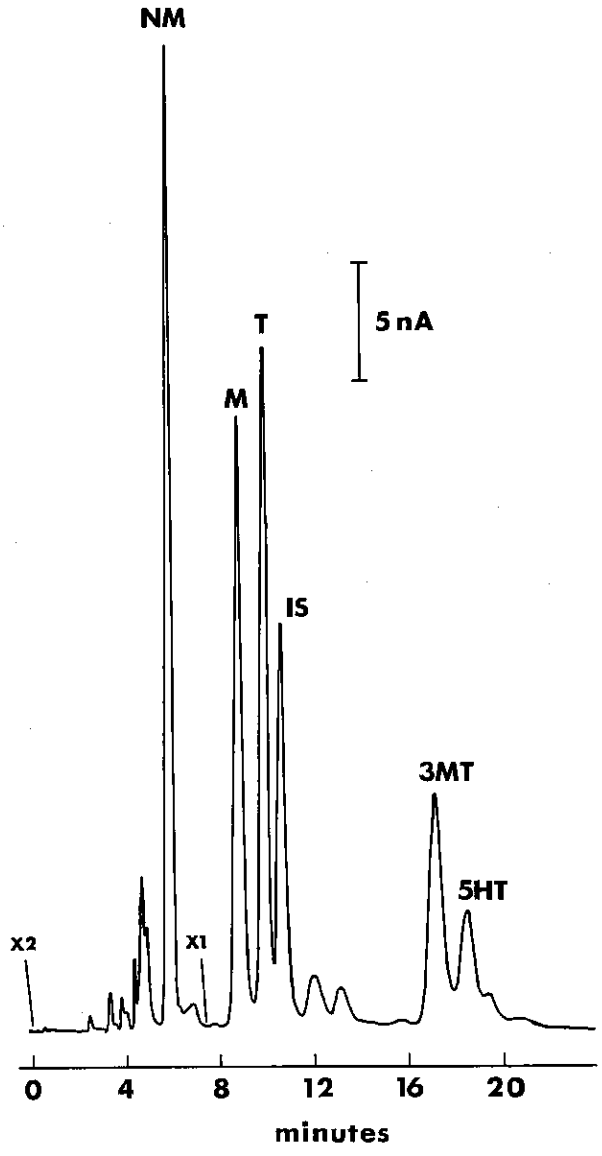

Figure 3. Typical chromatogram of an actual urine sample from a healthy individual. Concentrations: 320 ng/mL NM, 170 ng/mL M, and 160 ng/mL 3-MT. Reprinted from reference 3, courtesy of the American Association of Clinical Chemistry.

References

1. S. E. Gitlow, M. Mendlowitz, L. M. Bertani, Am. J. Cardiol. 26(1970) 270.

2. I. Molnar, C. Horvath, Clin. Chem. 22 (1976) 1497.

3. R. E. Shoup, P. T. Kissinger, Clin. Chem. 23(1977) 1268