CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of 5-HIAA in Urine

Purpose
Determination of 5-HIAA (5-hydroxyindole-3-acetic acid) in urine by direct injection of a diluted sample.

An elevated concentration in urine of the serotonin metabolite 5-HIAA was correlated with the presence of carcinoid tumors over thirty years ago. The determination of 5-HIAA in urine is still important in the diagnosis of carcinoid syndrome.

Existing Methods
Colorimetric, LCUV, LCF, and LCEC. Colorimetric procedures are time consuming and can be biased either by interferences or incomplete extraction. LC methods can potentially improve the speed and specificity of the assay, but require sample cleanup, either by solvent extraction or solid phase pretreatment. Each of these procedures has its particular disadvantages in terms of convenience, time, and recovery of analyte.

Reference
The procedure outlined below, from the BAS research laboratories, describes the determination of 5-HIAA using a short high-efficiency reverse-phase analytical column and an electrochemical detector. The chromatographic system provides good resolution of 5-HIAA and other components found in urine in relatively short run times. This in combination with the inherent specificity and sensitivity of the electrochemical detector allows quantitation of 5-HIAA in urine by direct injection of a diluted sample.

Conditions
System: LC-154 (BAS)
Electrode: Glassy Carbon
Potential: +0.60 V vs. Ag/AgCl
Column: 3 µm, C-18, 40 x 3.2 mm (BAS, P/N MF6215)

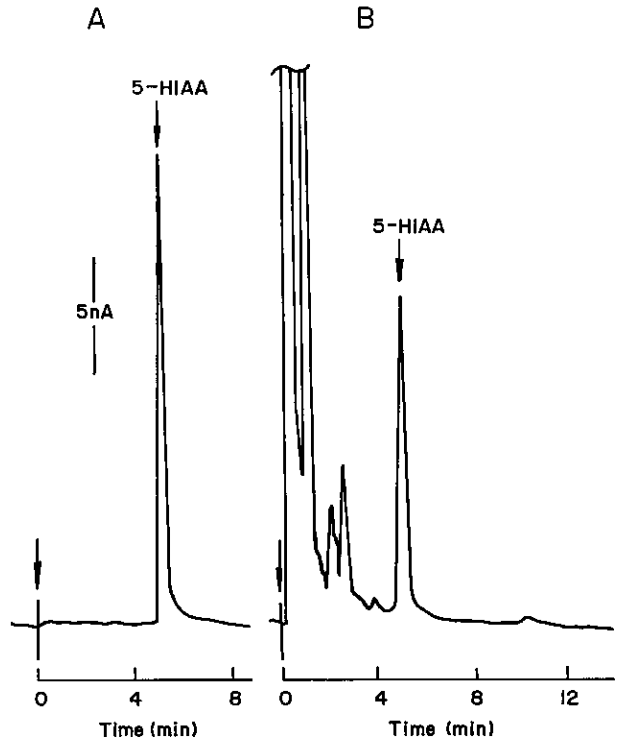

Figure 1. Chromatograms of A) standard (1 µg/mL) and B) diluted urine sample.

Mobile Phase: 0.05 M monochloroacetic acid, 0.5 g/L Na_2EDTA, and 1.0 g/L diethylamine•HCl (pH 3.0); mixed with acetonitrile 100:2. Flow rate was 1.0 mL/min.
Linear Range: 1 to 50 ng injected in 5 µL. This corresponds to 2 to 100 µg/mL of 5-HIAA in the sample.

Sample Preparation
Urine samples were acidified (pH 3.0 with 6 M HCl) and 1 mL aliquots were frozen prior to use (or for storage). After thawing at room temperature the samples were briefly vortexed and filtered through a

0.45 µm membrane. A 100 µL aliquot of the filtrate was added to 900 µL of mobile phase, mixed, and 5 µL directly injected onto the column. A number of standards and samples, typically required for a complete day of injections, were prepared in a single batch and refrigerated until used.

Comments

Typical chromatograms of a 5-HIAA standard and diluted urine sample are presented in Figure 1. Peak heights of 5-HIAA in the urine samples were compared to that obtained for a 1 µg/mL working standard (prepared in mobile phase). Using the conditions outlined above, total run times of only 10 minutes are necessary for urine samples.

Detector linearity is demonstrated in Figure 2. This type of linear response over a wide concentration range (2 to 100 µg/mL) permits the use of a one-point calibration in the procedure.

The absence of sample cleanup, short run times, and economical column used make this procedure very attractive to the clinical lab. Furthermore, the instrumentation used in this assay can be found in most labs.

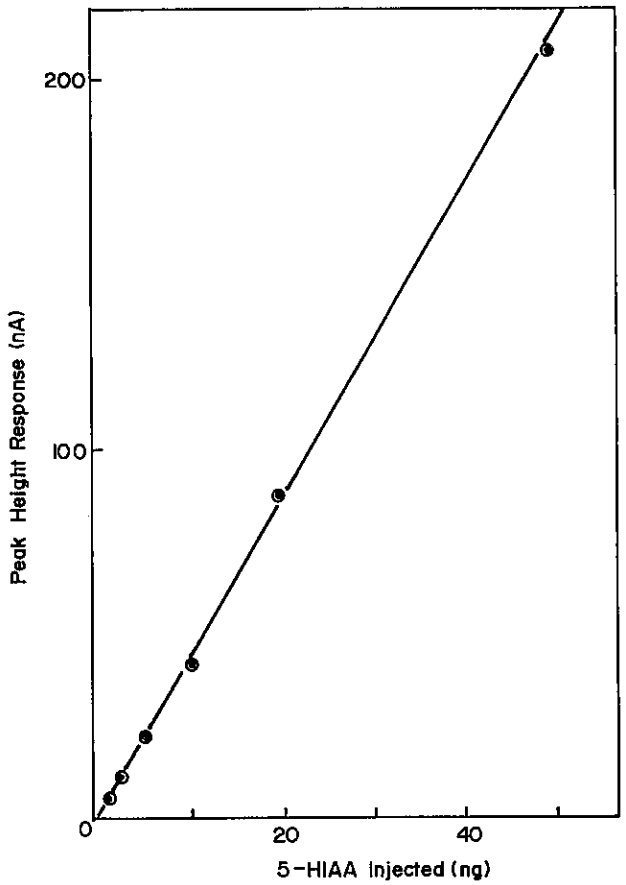

Figure 2. Linearity curve for 5-HIAA. Slope, +4.16; Y-intercept, +1.03; correlation coefficient, 0.9998.