Urinary 5-Hydroxyindoleacetic Acid by HPLC with Electrochemical Detection

To the Editor:

We note recent claims by Davidson and Williamson (1) for the improved assay of urinary 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection. We are familiar with a very similar method, which also involves protein precipitation and has a comparable mobile phase (2). In our experience, precipitation of proteins from urine with ethanol was not always successful but was time consuming, and back-extraction of 5-HIAA into water was found to be necessary to avoid peak broadening. These additional steps introduced further pipetting stages, with the possible loss of sensitivity and recovery, and they increased analysis time. To compensate for any loss in sensitivity a higher detector electrode potential may be used, but this increases the risk of interfering peaks, e.g., possible paracetamol (acetaminophen) metabolites (1). The necessity for protein precipitation is questionable. Three studies (3–5) have reported minimal loss of column efficiency, even after many injections of undiluted (3, 4) and diluted (5) urine, a finding that we have subsequently confirmed—even without the use of a guard column.

We cannot comment on Davidson and Williamson’s claim for an improved assay, because no reference was made to previously published methods. However, we acknowledge that their method is superior to the colorimetric methods, which are time consuming, insensitive, and prone to drug interference.

We suggest that elaborate sample preparation (1, 2) is unnecessary and offers no improvement over previously reported methods (3–5). In addition, the mobile phase used by Davidson and Williamson does not improve resolution and has the disadvantage of increased retention times.

We report here a method that involves little sample preparation and faster chromatography, and in our experience is unaffected by endogenous substances, drugs, or metabolites. In one case of massive paracetamol overdose (560 mg/L at 4 h) we observed a peak co-eluting with the internal standard. A Varian 5010 liquid chromatograph was used with a Severn Analytical Model 5100A electrochemical detector. The detector potential of 0.4 V was considerably lower than that used in previous methods (1–3, 5) and may explain why we have not observed any interference. The analytical column was packed with Spherisorb S5 ODS 2 (Phase Sep, Queensferry, Clwyd, U.K.) and eluted with phosphate buffer (pH 2.3, 50 mmol/L)/ethanol/isopropanol (100/10/2 by vol) at a flow rate of 1.0 mL/min. The stock internal standard contained 50 mg of 5-hydroxyindole-2-carboxylic acid in 100 mL of ethanol. A working internal standard was prepared by diluting 1 mL of this stock to 100 mL with a 0.1 mol/L solution of HCl. The working standard contained 100 μmol of 5-HIAA per liter of 0.1 mol/L HCl. We added 200 μL of standard, control, or patient’s sample to 1 mL of working internal standard, mixed, and injected 10 μL into the chromatograph. Figure 1 shows a chromatogram of a normal patient’s urine (5-HIAA = 5 μmol/L) and of that from a patient with carcinoid syndrome (5-HIAA = 108 μmol/L).

The standard curve is linear up to at least 1200 μmol/L. Our mean analytical recovery was 98.8%. Within-batch CVs were 3.2% and 5.1% for concentrations of 30 and 185 μmol/L, respectively. Between-batch CVs were 3.3% and 10.8% for concentrations of 23 and 132 μmol/L, respectively.

Six patients’ samples with appropriate standards and controls can be analyzed in 1 h. The method is simple to operate, sensitive, specific, and economical. It has been used routinely in our laboratory for the past year.

References

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Prostate-Specific Antigen and the Female Prostate

To the Editor:

Having had the opportunity to immunologically identify human prostate tissue-specific antigens (1, 2), one as prostate tissue-specific acid phosphatase (PSAP) and the second as prostate tissue-specific antigen, currently referred to as prostate antigen or prostate-specific antigen (PSA) (3), I read the recent report (4) and follow-up communications (5) on PSA in the journal with considerable interest.

In these reports (4, 5), concern has been raised over detection of PSA in normal women, in some cases relatively high values, e.g., up to 0.8 g/L (4). These investigators have suggested reasons for the identification of PSA in women, ranging from a discrepancy of nonspecific binding in the radioimmunoassay (4, 5), to "... artifacts produced by PSA antibodies in their normal female subjects" (5) as perhaps, a consequence of the formation of "... antibodies in some women in the same manner that antibodies to sperm are produced" (5). Although such reasoning may not be completely untenable, I hasten to direct these investigators’ attention to the biological fact that women have a prostate gland (6) (also known as paraurethral and (or) Skene’s glands, the latter after Alexander Skene, who in 1880 (7) extended and redescribed the early observations of de Graaf, made in 1672 (6)). So women may have PSA in their circula-