Recently, we described a specific and sensitive method for the assay of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) in cerebrospinal fluid, plasma, tissue, and urine (1). Depending on the matrix and whether 5-HIAA and HVA were to be analyzed together or separately and whether the detection was by fluorometry or by HPLC with electrochemistry (LCEC), we purified specimens by using solvent extraction, anion-exchange resin, or both. Although we could account for all of the HVA after either extraction procedure, such was not the case with 5-HIAA. That is, 5-HIAA was recovered after solvent extraction but was "lost" in the resin. It was not eluted from the resin under conditions in which we recovered all of the HVA. This problem was finally resolved by eluting with a solution of 0.1 mol/L NaOH containing 1.5 mol of NaCl per liter, which quantitatively eluted both metabolites as well as vanillylmandelic acid (VMA).

The results of a typical analysis of the three metabolites from urine, shown in Table 1, were obtained with the following procedure: To 1.0 mL of urine add 2.5 mL of distilled water and standards for 5-HIAA, HVA, and VMA; adjust the pH to 2.0–2.5 and dilute to 5.0 mL with distilled water. (Centrifuge, if necessary, because sediment interferes with the resin step.) Treat a separate 1.0 mL of urine similarly but without the added standards, to determine the endogenous concentrations of the three metabolites. Transfer the clear solutions to the prepared anion-exchange resin column (1). After the samples have passed through the resin, add the indicated washes and eluents (Table 1 stepwise. Collect and acidify each fraction separately, and inject a 100-μL aliquot of each into the LCEC system (1).

In our current procedure the initial effluent, the 10-mL water wash, and the first 5 mL of NaCl (Table 1, steps 1, 2, 3) are discarded. Then the three metabolites are eluted in 10 mL of alkaline NaCl (steps 4 & 5 combined); the 10-mL eluate is acidified and a 100-μL aliquot analyzed by LCEC. As also shown, HVA and VMA can be separated from 5-HIAA and recovered quantitatively in the first 5 mL of alkaline NaCl (step 4), whereas most of the 5-HIAA can be recovered in the second 5 mL of alkaline NaCl (step 5). We emphasize that the indicated dimensions of the column are critical to the success of the method; the amount of resin can vary widely, depending on its moisture content.

If LCEC is not available, the 5-HIAA and HVA in the resin extract can be separated and concentrated by selective solvent extraction and detected by fluorometry (1). To quantify VMA by other means, however, its dilution in the 10 mL of resin eluate, together with the relative insensitivity of the spectrophotometric method for vanillin (2) and the lack of a suitable fluorometric method, require that an aliquot (e.g., 3.0 mL) of the urine itself be processed (2).

As we pointed out previously (1), organic solvent extraction of 5-HIAA and HVA in the supernatant fluid remaining after precipitation of protein from cerebrospinal fluid, plasma, or tissue yields a product of sufficient purity for detection by fluorometry or LCEC without the resin step that is necessary for urine. Again, because of the relative insensitivity of the vanillin spectrophotometric method, LCEC must be used for detecting VMA extracted from these sources.

References

Determination of Magnesium in Serum by the Technicon SMAC with a Calmagite Method with Blank Correction, Sue Brown and W. Greg Miller (Dept. of Pathol., Section of Clin. Chem., Medical College of Virginia Hospitals, Virginia Commonwealth University, Richmond, VA 23298-0697)

We have adapted the calmagite (1) with EDTA blank (2) method to the SMAC continuous-flow analyzer (Technicon Instruments, Tarrytown, NY). The SMAC manifold diagram
is shown in Figure 1. Absorbance of the Mg-calmagite complex is measured in flow cell 1. EDTA is added to specifically remove magnesium from the calmagite complex. A second flow cell measures the absorbance of the sample blank after incubation for 185 s in 343 cm of coiled polyethylene tubing.

Abernethy and Fowler (1) used Empigen BB surfactant to decrease the absorbance signal of the reagent blank. However, we omitted Empigen BB because it produced a positive bias at low magnesium concentrations and a negative bias at high concentrations when patient's sera were compared between the SMAC and atomic absorption spectrometry (AAS) methods.

Savory et al. (3) reported that calmagite reagent is not stable at room temperature because the pH decreases as carbon dioxide is absorbed. They increased the buffering capacity of the reagent (2-amino-2-methyl-1-propanol, AMP) to 0.1 mol/L and attached a carbon dioxide absorbant to the Technicon RA-1000 reagent boat. The SMAC system calibrates itself every 20 min, and can compensate for minor variations in reagent pH throughout the day. We found that increasing the AMP concentration in the buffer from 0.01 to 0.1 mol/L made special handling of the reagent unnecessary.

A manifold with 76 cm of tubing between the fluid-injection block and flow cell 1 (4) produced a sample-to-sample carryover of 14%. Typical SMAC channels have carryover factors of 2% to 5%. Shortening the tubing to 29 cm reduced carryover to 7%. Further shortening the tubing to 15 cm yielded poor-quality curves, which could be attributed to inadequate mixing. Use of glass and various types of tubing in the manifold did not decrease carryover. Patients' samples analyzed after a high-concentration quality-control sample (magnesium concentration, 49 mg/L) had a positive bias of 3 mg/L (1 mol of mg = 24.3 g).

A comparison with AAS results for freshly collected patient's sera with magnesium concentrations from 8 to 79 mg/L demonstrated a positive bias at values >45 mg/L. Deming regression analysis for values ≤45 mg/L gave the following: y(SMAC) = 1.07x(AAS) – 1 mg/L; S_{xy} = 0.6 mg/L, mean x = 21 mg/L, mean y = 21 mg/L, n = 235 over 16 days. Samples with concentrations >45 mg/L should be diluted and re-assayed with the SMAC. Equivolume dilutions give satisfactory results, but greater dilutions of serum give erroneous results, probably owing to a protein matrix effect. Day-to-day imprecision (CV) over six months was 3.3% at a mean of 26 mg/L (n = 271). This sample-blank-corrected magnesium method is not affected by metals (copper, iron, copper) or common spectrophotometric interferents (hemolysis, lipemia, bilirubin).

![Polyethylene tubing 1.14 mm ID](Image 1)

- Sample from riser
- 343 cm
- 29 cm
- 568 μL/min
- 482 μL/min
- 74 μL/min
- EDTA blank reagent
- Air
- Polyethylene tubing 1.14 mm ID
- FC 2: 520 nm
- FC 1: 520 nm
- 37 μL/min

References

Long-Term In Vitro Stability of Cyclosporine in
Whole-Blood Samples, Mary Carole Smith and Gregory
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Although the reagent manufacturer's recommendation for sample storage is 2–8 °C for samples not tested immediately, unrefrigerated EDTA-anticoagulated whole-blood samples for cyclosporine (CsA, cyclosporin A) determination are received by mail from remote locations. Two to seven days are required for delivery, and afternoon peak temperatures range from 32 to 38 °C. We therefore undertook a study of long-term in vitro CsA stability at non-standard storage temperatures. Previous studies of in vitro stability have concentrated on the partitioning of CsA between plasma and blood cells and the effects of temperature and hematocrit on the equilibration of this partitioning rather than on degradation (1).

In this study, we tested 10 different samples from eight patients, seven of whom had received kidney transplants and the other a heart transplant. Specimens were analyzed on the day of collection and repeatedly for nine to 13 days after storage at room temperature (20–24 °C) and at 37 °C (in a water bath). The samples stored at 37 °C were equilibrated to room temperature before assay. CsA concentrations were determined with the CYCLO-Trac™ SP 125I radioimmunoassay (INCSTAR Corp., Stillwater, MN 55082). This kit involves a monoclonal antibody to CsA, an 125I-labeled CsA derivative as tracer, and separation of the bound and free fractions by a second antibody. The test is performed on a methanol extraction of EDTA-treated whole blood, with samples being equilibrated at room temperature for 2 h just before analysis. Figure 1 illustrates the results.

The daily values obtained through the testing interval did not differ significantly, and there was no downward trend to suggest an active catalytic process. The interassay CV for multiple determinations of each sample over the testing period (2.3–10.6%; mean, 5.4%) was well within the range expected for interassay variation (1–3). In our laboratory, the average interassay CV for three concentrations of whole-blood control is 6.3%.

Because mixed-function oxidases are located primarily in the liver, CsA is unlikely to be further metabolized in vitro. CsA in an EDTA-containing tube is potentially susceptible to intra- or extra-cellular degradation; how-