5%). Recoveries were similar when samples were sonicated before or after addition of perchloric acid. The compounds were determined in seven 250-μL portions of a pooled whole-blood sample; the within-day CVs were 4.3% for 5-HT and 2.1% for TRP. Determinations made on 20-μL samples of pooled whole blood (prepared by adding 10 μL of ascorbic and perchloric acid) gave CVs of 9.7% for 5-HT and 6.8% for TRP. Portions of a specimen of whole blood stored at −80 °C were analyzed during six months; the CV was 10% for 5-HT, with no significant loss of 5-HT or TRP. Recently, we have found 5-hydroxytryptophan (100–500 μg/L added) to be an excellent internal standard. It is eluted at 5 min, is completely resolved from 5-HT, and is analytically recovered from plasma and whole blood in yields intermediate to those of 5-HT and TRP. Whole blood and plasma samples analyzed to date have had values of <1 to 500 μg/L, with a mean of ~200 μg/L. The absolute detection limit of 5 pg for 5-HT allows a concentration of 1 ng of 5-HT per milliliter to be measured with a 20-μL injection.

The method is more rapid than present LC-F methods (2, 3) for TRP in plasma and is simpler than the LC-amperometric (electrochemical) methods (4, 5) for 5-HT in plasma, and it allows both compounds to be easily determined in either plasma or whole blood. Because nearly all of the serotonin in blood is bound to platelets, measurements in plasma are complicated by difficulties in obtaining plasma with high and consistent yields of platelets. By measuring 5-HT in whole blood, one can assume that the entire platelet population has been assayed, and inter-laboratory results will be more comparable.

We are grateful for the cooperation of Peter McPhedran and the support of Bennett Shaywitz. This research was supported by NIMH MH030929, NICHD grant HD-03008, the William T. Grant Foundation, Mr. Leonard Berger, the Gatespost Foundation, and The Solomon R. & Rebecca D. Baker Foundation, Inc.

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Sublimation Losses of Salicylic Acid from Plasma during Analysis

To the Editor:

We would like to describe a simple way to avoid sublimation losses during the assay of salicylic acid in biological fluids. Most of the numerous chromatographic methods for salicylic acid analysis (1–6) require extraction with an organic solvent, which then is evaporated. Although several authors have been concerned about sublimation losses during the evaporation process, the problem has not been satisfactorily solved. Peng et al. (4) suggested using an ice bath during the evaporation of the extracting solvent. Amick and Mason (5) evaporated the solvent under reduced pressure, removing the samples immediately after the sample went to dryness. Other recommendations (6, 8) are much the same.

In our laboratory, we use a liquid-chromatographic method for the simultaneous analysis for aspirin and salicylic acid in plasma, with 4-methoxyphenylacetic acid as internal standard. The method is based primarily on the procedure of Lo and Bye (6). On assay validation, the precision for the aspirin assay was very good, but acceptable precision for salicylic acid was difficult to achieve. This problem was attributed to sublimation losses. Only by carefully watching the samples during the evaporation step and removing each sample as soon as it went to dryness were we able to obtain good precision. Because this was so critical, an alternative approach was highly desirable. We overcame this problem by using extra-long test tubes (20 × 150 mm) for the evaporation step, carrying out the evaporation in an ice water bath under a gentle stream of nitrogen, and dissolving the sample residue in 0.5 mL of the mobile phase (methanol and 850 mg/L phosphoric acid, 45/55 by vol), being certain that the entire test tube walls are rinsed thoroughly. The last step was extremely important to recover sublimed salicylic acid from the upper test walls.

Sublimation losses were then negligible (<2%), even when samples were left for as long as 30 min after they were dry.

References

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On the Interpretation of Diagnostic Tests: A Common Logical Fallacy

To the Editor:

Important and very useful relationships between the prevalence of a disease and the sensitivity and specificity of a diagnostic test for that disease have been described (1), but one variable that has been ignored is the prevalence of testing. The proportion of the popula-