that of serotonin in CO-treated samples. Three other compounds (N-acetyl serotonin, N-methyl serotonin, and 5-hydroxyindole, all from Sigma Chemical Co.) previously used as internal standards in the determination of serotonin from various tissues were not suitable for the present method. N-Acetyl serotonin was too strongly retained in the present chromatographic system; N-methyl serotonin, in turn, eluted at the same time as 5-hydroxyindoleacetic acid and another unknown substance between serotonin and 6-fluoroserotonin; and 5-hydroxyindole, while having a very similar retention time as 6-fluoroserotonin, showed poor analytical recovery (<10%) from whole blood.

To assess the reproducibility of the method, I analyzed five replicate samples from pooled whole blood on five different days. For the five within-day replicates the mean serotonin concentration was 89.7 ± 1.1 nmol/L and the CV was 2.1% (SD 0.9%, range 0.9–3.2%). The corresponding between-day values were 89.9 ± 0.5 nmol/L and 4.0% (SD 0.9%, range 2.5–4.5%). Thus the assay is precise and reproducible. Concentrations of serotonin in whole-blood samples from normal adult controls (0.92 ± 0.32 µmol/L, mean ± SD; range 0.50–1.70, n = 16) with the present method are in the same range as reported for other methods (e.g., 7). Freezing and storing the samples at −60°C for at least 10 months did not affect the serotonin concentrations measured by the present method.

Because CO is a potentially hazardous substance, I tried other means to prevent the oxidation of serotonin and the internal standard. Flushing with N₂ or the use of a high concentration of Na₂S₂O₃ (1.5 g/L) instead of ascorbic acid, however, was totally ineffective. Further increasing the concentration of ascorbic acid in the sample-diluent solution broadens the solvent front so much that it interferes with the serotonin peak.

The sensitivity of this method allows the use of only 100 to 200 µL of whole blood, an important feature in pediatric practice. No changes are necessary in the dilution of the sample or in the injection volume to monitor serotonin depletion down to about 5 to 10% of the initial value. The method has been used to monitor the depletion of whole-blood serotonin in chronic schizophrenic patients during treatment with fenfluramine, a serotonin-depleting drug, and to compare serotonin concentrations in whole blood of autistic children with those of normal age- and sex-matched controls.

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References