CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination Of Serotonin In Blood

Purpose

Determination of serotonin (5-hydroxytryptamine) in whole blood.

Serotonin plays a role in a variety of physiological functions, such as sleep regulation and behaviour, and pathological conditions, such as depression, mental retardation, infantile autism, and psychiatric disorders. This indoleamine is also implicated in haemostasis, thrombosis, and cardiovascular diseases. Clinically serotonin has been used to diagnose abdominal carcinoid which is a metastasizing tumor of the argentaffin (enterochromaffin) cells. An excess amount of serotonin has been linked with this disease. Decreased blood serotonin has been reported in some cases of Down's Syndrome and in untreated phenylketonuria.

Existing Methods

Various analytical methods including thin-layer chromatography, LCF, LCUV, GC-MS, and radioenzymatic and radioimmunological assay. The following LCEC procedure is simple, rapid, and sensitive (as well as specific). The equipment is less expensive to purchase and relatively easy to maintain.

Reference

The following procedure results from the extensive studies carried out by Dr. Peter Chou of American Medical Laboratories, Inc., Fairfax VA 22030. It is part of their repertoire of in-house analytical procedures. It has been thoroughly tested with real world samples. We thank Dr. Chou for making this information available to BAS. Dr. Chou is Technical Director of Research and Development at AML.

Reagents

0.2 M Phosphate Buffer, pH 6.5: 17.84 g KH_2PO_4, 9.76 g Na_2HPO_4, make to a final volume of 1 L with deionized water. Filter and refrigerate.

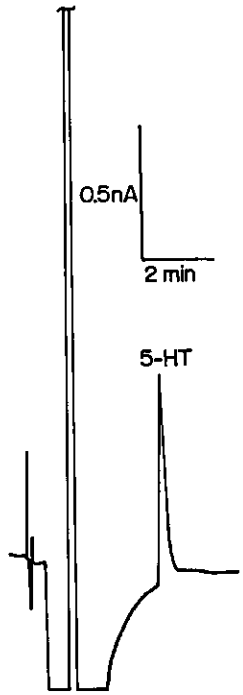

Figure 1. Chromatogram of serotonin in whole blood. Human blood was processed as described under sample preparation. The large negative response is due to the high concentration of ammonium acetate in the injected sample.

0.1 M Acetate buffer, pH 5.0: 1.5 mL of 6 M acetic acid, 1.3 g sodium acetate (anhydrous), make to 250 mL with deionized water. Filter and refrigerate.

3 M Ammonium acetate: 50.7 mL conc. NH_4OH, 43.1 mL glacial acetic acid, make to 250 mL with deionized water.

Stock standard solution of serotonin: Dissolve 10 mg serotonin creatinine sulfate complex (Sigma) in the 0.1 M acetate buffer to a final volume of 10 mL. NOTE: The final concentration of serotonin must be calculated as the free base.

Working standard solutions: dilute the stock solution 1:1000 with acetate buffer.

Cation-exchange resin: 25 g of Amberlite CG-50 (H^+), 100-200 mesh (Sigma), was washed 3 times with successive 100 mL volumes of 2 N HCl-deionized water - 2 M NaCl - water. Lastly, it was equilibrated with 0.2 M phosphate buffer.

Procedure

Sample requirements. Serotonin in blood is unstable and care must be taken to protect specimens from oxidation in order to obtain meaningful results.

Draw 10 mL of blood and transfer to a container having 10 mg Na_2EDTA and 75 mg ascorbic acid. Mix well and freeze, if the blood is not immediately processed. Monoamine oxidase inhibitor drugs should be discontinued for at least one week prior to sampling.

Sample preparation.

1. For standards and aqueous control samples add 400, 800, and 1200 µL of working standard solution to 2 mL of deionized water plus 2 mL of sodium acetate buffer.

2. For patient and blood control samples add 1 mL of whole blood, 1 mL of deionized water, and 200 µL of 4 M $HClO_4$. Vortex for 2 minutes and centrifuge at 1,600 x g for 10 minutes.

3. Transfer the supernatant to a clean container and add 2 mL of the sodium acetate buffer. Adjust to pH 5.0 ± 0.1 with 6 N NaOH, if necessary.

4. Prepare a column for each sample by adding regenerated CG-50 resin to a height of 5 mm into a polypropylene column (Bio-Rad Labs). Wash the resin bed with 3 mL of phosphate buffer.

5. Apply all samples to their respective columns. Wash all columns with two-1 mL aliquots of deionized water. Discard washes.

6. Add and collect three-1 mL washes of 3 M ammonium acetate buffer.

7. Vortex and inject 20 µL of each sample into the LCEC system.

Conditions

System: BAS 400
Detector: BAS LC 4B/17A
Electrode: Glassy carbon
Potential: + 0.6 V vs Ag/AgCl
Column: Phase II, C_{18}, 100 x 3.2 mm (BAS P/N MF-6213)
Mobile Phase: 0.05 M monochloroacetic acid, pH 3.0 (with NaOH), 1 mM Na_2EDTA, 1.25% acetonitrile, filter through a 0.2 µm membrane and degas for 10 minutes under aspirator vacuum. Flow rate was 1 mL/min.

Comments

Although the precision of extraction of replicate samples is very good the addition of an internal standard may be of benefit. Suggested internal standards are 5-hydroxy-N-dimethyltryptamine, 6-hydroxytryptamine, or N-methyl-5-hydroxytryptamine, these compounds should have a capacity factor greater than 5-HT.