Determination of Serotonin in Whole Human Blood

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Determination of serotonin in blood involves several challenges. Among the decisions to be made are what fraction of blood to assay, how to stabilize the sample, and what type of detector to employ. BASi has developed and now offers contract services for serotonin employing a Good Scientific Practices (GSP) protocol.

Serotonin (5-hydroxytryptamine, 5-HT) (***F1***) is a heterocyclic amine commonly found in plants and animals. Clinically high serotonin levels can indicate presence of a carcinoid tumor (1). Even within normal limits, variations of serotonin levels have been associated with various psychological conditions including anorexia, anxiety, depression, schizophrenia and others (2). The marketing success of serotonin reuptake inhibitor drugs (SSRIs) attests to the extent and importance of these conditions in our society.

Serotonin can be separated fairly easily on a reverse-phase column, with or without an ion-pairing agent. Electrochemical or fluorometric detection is usually employed for selectivity and sensitivity. We use fluorometric detection as it is not sensitive to the large amount of ascorbic acid added to preserve the samples.

Sample Matrix

The first consideration when designing a protocol to measure blood serotonin is the specific blood fraction to be assayed. More than 95% of serotonin is found in the platelets (2), but its release into the plasma occurs easily (1). Matrix choices include whole blood, platelets, platelet-rich plasma (centrifuged at low g), and platelet-poor plasma (centrifuged at high g), or urine (2,3,4).

Many researchers have focused on measurement of serotonin in whole blood because it is simpler than preparing blood fractions. This is an acceptable shortcut, as serotonin concentration in whole blood is highly correlated with that in platelets. Some clinicians recommend correction for platelet count in the blood, which improves this correlation (1).

Analyte Stability

Serotonin is reported to be unstable in the presence of light, oxygen and low or high pH. Various stabilizing treatments applied at the time of blood collection include the addition of EDTA and any of several reducing agents, such as ascorbic acid, borohydride, metabisulfite and cysteine. Treatments at the time of sample preparation include bubbling with CO and further additions of the reducing agents mentioned above. Of particular concern is stability during and after protein precipitation; the release of oxyhemoglobin and low pH at room temperature are both known to destroy serotonin (4,5).

Sample Collection

Good results depend on good sample-collection practices that prevent blood clotting and oxidation of serotonin. Samples should be collected in EDTA tubes and inverted at least ten times to ensure thorough distribution of the EDTA. Three mL of each sample should then be transferred for shipping into tubes containing 60 mg ascorbic acid and thoroughly mixed by inverting another ten times. Tubes should be frozen in an upright position at -70 °C, and shipped overnight in sufficient dry ice.

Assay Protocol

All reagents and equipment, including centrifuge and autosampler, were maintained at 4 °C throughout, because of the stability issues mentioned above. After thawing, an aliquot of each sample was combined with additional reducing agent and an internal standard (n-methylserotonin), then protein precipitated and clarified by centrifugation. The supernatant was injected into an LC system employing a C_{18} reverse-phase column, an ion-pair mobile phase, and a fluorescence detector. The separation was extremely clean (***F2***).

Precision and Recovery

Within-day precision was 2.9% for a pooled blood sample that was subdivided into six aliquots and analyzed for serotonin. Inter-day precision was 8.0%. Recovery of both serotonin and the internal standard was 70%.

Linearity

Pooled blood was spiked with 5 concentrations of serotonin to determine linearity. Results are shown in ***F3*** (note non-zero intercept due to endogenous serotonin).

References

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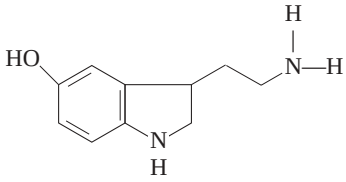
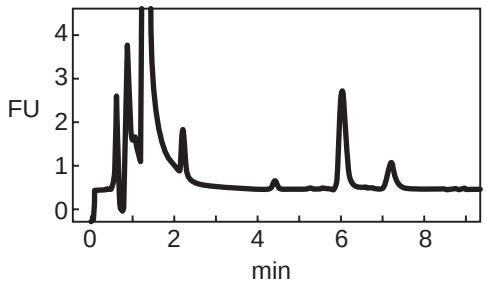
F1. Serotonin (5-hydroxytryptamine, 5-HT)



F2. Unspiked blood sample showing serotonin (6 min) and internal standard (7.2 min) peaks.



F3. Linearity of spiked pooled blood samples.