Quantitative Determination and Validation of Serotonin in Human Plasma and Human Cerebrospinal fluid

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Introduction:
Serotonin (5-Hydroxytryptamine, 5-HT) is a neurotransmitter involved in many physiological functions (sleep, behavior regulation, serotonin, and gastrointestinal motility). It also has wide pharmacological actions that include enteric neurological disorders, and cardiovascular disease. Depending on the conditions, certain therapies are designed to either promote serotonin availability or to inhibit it.

Serotonin concentrations in cerebrospinal fluids (CSF) are commonly used in epidemiology, but additional indications for use are increasing. This class of drugs increases the amount of interstitial serotonin available after synaptic release. Efforts of this process can be seen in both CSF and plasma. Pharmacological effects include tumor growth and smooth muscle contractions can also lead to observable changes in iontophoretic measurements. Monitoring plasma and cerebral spinal fluid (CSF) serotonin can be a useful marker for a variety of applications.

A wide range of serotonin levels for plasma and CSF have been reported, depending on population, method of collection, and method of analysis. Reported here are two methods for serotonin analysis in human K2-EPO plasma and human CSF. The quantitative ranges for each were based on concentrations found in normal individual.

Methodology:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>LOQ (pg/mL)</th>
<th>LLOQ (pg/mL)</th>
<th>HLOQ (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Plasma</td>
<td>7.3</td>
<td>5.6</td>
<td>200</td>
</tr>
<tr>
<td>Human CSF</td>
<td>200</td>
<td>150</td>
<td>6100</td>
</tr>
</tbody>
</table>


**Representative Chromatograms:**

- Figure 1: Serotonin Plasma Full Chromatogram (50 pg/mL)
- Figure 2: Serotonin CSF Full Chromatogram (50 pg/mL)
- Figure 3: Serotonin Plasma Full Chromatogram (250 pg/mL)
- Figure 4: Serotonin CSF Full Chromatogram (250 pg/mL)


**Validation Data:**

- **Phenomenex Luna C18 3µ, 100**
  - Mobile phase B: 15% solvent B isocratic for 3.5 minutes, 1.0 mL/min
  - Water/formic acid/ammonium hydroxide
  - Organic fraction treatment
  - Reagent added
  - Sample Volume
  - Quantitative Determination and Validation of Dipropionylserotonin (CSF Method)

**Results:**

- Low stability pool had a mean value of 0.748 ng/mL with a CV of 1.2%.
- High stability pool had a mean value of 20.0 ng/mL with a CV of 3.8%.
- The low stability pool had a mean value of 20.0 ng/mL, with a CV of 1.2%.

**Conclusion:**

- Plasma stability testing was done in an authentic, adulterated plasma sample. Plasma lots were analyzed for the presence of indole-based bacteria that had an appropriate concentration to result in a low to high level range. These two samples (low and high) were analyzed to determine the stability of the CSF over a range of time to see if iontophoretic analysis is stable.

**Matrix: Effect in Unaltered Human Plasma**

- Total Amount Found
- %Bias

- | Donor Number | Total Amount Found | %Bias |
- |-------------|--------------------|-------|
- | 1           | 1.12               |       |
- | 2           | 1.13               |       |
- | 3           | 1.53               |       |
- | 4           | 1.34               |       |
- | 5           | 1.00               |       |
- | 6           | 1.49               |       |
- | 7           | 1.95               |       |
- | 8           | 2.05               |       |
- | 9           | 2.55               |       |
- | 10          | 2.75               |       |

- | Donor Number | Total Amount Found | %Bias |
- |-------------|--------------------|-------|
- | 1           | 1.12               |       |
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- | 9           | 2.55               |       |
- | 10          | 2.75               |       |

**Conclusion:**

- Sensitivity is an important biomarker component for neuropharmacological biological study. In method development and CSF are not well described, for various pharmaceuticals. The low molecular weight, endogenous processes, and relatively low concentrations of serotonin make it a challenging component to measure technology of choice. The approach taken with these two methods has demonstrated sensitivity, accuracy, and robustness in human plasma and CSF.

**Figures:**

- Figure 5: Serotonin Plasma Internal Standard (Serotonin-D4)
- Figure 7: Serotonin CSF Control (Scrubbed) Blank Sample
- Figure 9: Serotonin CSF ULOQ (250 pg/mL) Sample

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