T2453

Determination of Endogenous Serotonin in Human Plasma

Authors:

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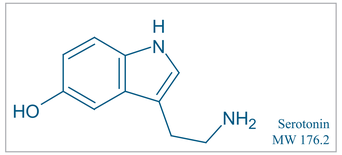
Introduction:

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in many physiological functions (sleep, behavior regulation, hemostasis, and gastrointestinal motility). It also has a role in pathological conditions that include cancer, neurological disorders, and cardiovascular disease. Depending on the condition, certain therapies are designed to either promote serotonin availability or to inhibit it.

Selective serotonin reuptake inhibitors (SSRIs) are commonly used as antidepressants, but additional indications for use are increasing. This class of drug increases the amount of interstitial serotonin available after synaptic release. Effects of this process can be seen in both CSF and plasma. Pathological conditions related to tumor growth and smooth muscle contraction can also lead to observable changes in serotonin in these matrices. Monitoring plasma serotonin can be a useful biomarker for a wide variety of applications.

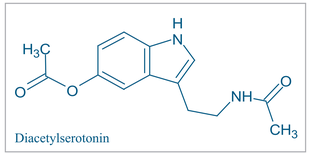


Serotonin
MW 176.2

A wide range of serotonin levels for plasma has been reported, depending on population, method of collection, and method of analysis. Reported here is a method for serotonin analysis in human K_2-EDTA plasma. We have previously reported on success with validating a method for serotonin in human cerebrospinal fluid (CSF). The quantitative range is based on concentrations found in otherwise normal individuals.

Methodology:

Calibration Range	50.0 to 25,000 pg/mL
Sample Extraction	
Sample volume	0.200 mL
Internal standard solution added	20 µL of serotonin-D_4 (200 ng/mL)
Reagent added	0.200 mL 6% ammonium hydroxide solution
Liquid extraction	Isolute SLE+ 400 µL extraction plate with 4 × 0.4 mL ethyl acetate
Organic fraction treatment	0.200 mL acetic anhydride 10 µL pyridine heat 60°C, 30 min. evaporate to dryness
Reconstitute for injection	0.200 mL mobile phase A
LC Analysis	
Column (temp.)	Phenomenex Luna C18 3µ, 100 × 2.0 mm (40°C)
Mobile phase A	Water/formic acid/ ammonium hydroxide (1000:2.0:0.4)
Mobile phase B	Methanol/formic acid/ammonium hydroxide (1000:2.0:0.4)
Gradient conditions	30% to 60% solvent B over 4.5 minutes, linear gradient, 0.3 mL/min
Injection volume	5 µL
MS-MS	
Instrument	Sciex 4000
Ion transitions	261 → 160 Serotonin 265 → 164 Serotonin-D_4



Diacetylserotonin

Elimination Method Used:

In plasma, endogenous serotonin was removed in order to generate blank control matrix. This procedure utilized sodium hypochlorite as a strong oxidizer to attack the sensitive indole substructure of serotonin.

40 mL plasma + 0.4 mL sodium hypochlorite (4%)
Heat at 60°C overnight
Store frozen until use

Results:

Examples of chromatography are presented in figures 1 through 5. Using the mass transitions of the diacetylserotonin on a Sciex 4000 instrument yielded approximately a 20-fold improvement in sensitivity over the underivatized free base, which had a limit of quantitation of approximately 1 ng/mL. Changing the ion transition setting by utilizing a larger derivative reduced the detector noise level experienced with lower mass settings. Also, the derivatized serotonin was better retained on a reverse phase chromatographic system, providing a stronger solvent in the ion source spray and improved desolvation. Improving the sensitivity allowed for a reduction in the sample size needed for analysis, which benefits situations where the same sample will be used for multiple analyses.

Representative Chromatograms:

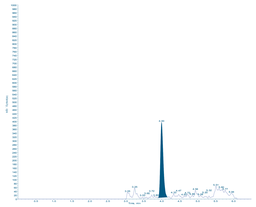


Figure 1. Serotonin Plasma LLOQ (50.0 pg/mL) Sample

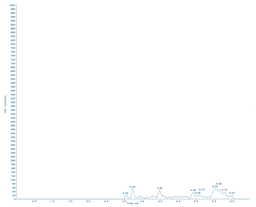


Figure 2. Serotonin Plasma Control (Scrubbed) Blank Sample

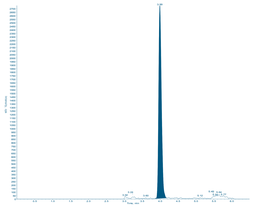


Figure 3. Serotonin Plasma Unaltered Sample at 350 pg/mL

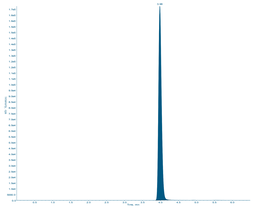


Figure 4. Serotonin Plasma ULOQ (25,000 pg/mL) Sample

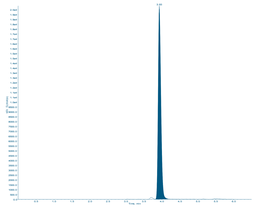


Figure 5. Serotonin Plasma Internal Standard (Serotonin-D_4)

Validation Data:

Extraction recovery for serotonin was found to be consistent at approximately 50%. The matrix factor (relative response in the presence of extracted matrix) was measured to be 0.9 to 1.2.

Analyzing quality control samples over three days generated precision and accuracy data. Plasma was fortified to target concentrations across the calibration range. The precision and accuracy for the assay was acceptable with an overall percent bias of less than 12% across all levels and precision less than 5%. The quality control data are presented at right.

Plasma Precision and Accuracy

	LLOQ (50.0 pg/mL)	Low (150 pg/mL)	Mid (5000 pg/mL)	High (20,000 pg/mL)	5X Dilution (125,000 pg/mL)
Run 1	49.9	155	4490	17,400	126,000
	48.1	156	4650	16,600	123,000
	48.2	155	4560	18,100	127,000
	51.3	143	4610	17,300	125,000
	47.6	148	4450	18,300	125,000
	47.4	139	4520	17,500	126,000
Run 2	51.1	159	4600	17,700	
	50.7	147	4660	18,200	
	49.1	151	4480	18,100	
	49.6	150	4670	17,500	
	52.3	154	4750	15,800	
	50.4	151	4800	18,300	
Run 3	52.4	151	4810	18,100	
	51.6	153	4740	17,200	
	52.2	144	4710	18,000	
	53.1	165	4670	17,400	
	55.6	160	4570	18,000	
	48.5	158	4640	18,000	
Overall %CV	**4.3**	**4.3**	**2.2**	**3.3**	**1.1**
Overall %Bias	**1.0**	**2.0**	**-7.0**	**-11.5**	**0.0**

Plasma Serotonin Stability

	High Stability (20,000 pg/mL)	% Bias	Low Stability (748 pg/mL)	% Bias
24 hrs @ RT	18,300	-8.5	760	1.6
	19,400	-3.0	721	-3.6
	17,900	-10.5	755	0.9
5 cycle freeze/thaw	18,100	-9.5	762	1.9
	18,300	-8.5	757	1.2
	17,700	-11.5	782	4.5
124 days @ -20°C	18,400	-8.0	759	1.5
	17,900	-10.5	773	3.3
	18,400	-8.0	771	3.1
89 hrs extract stab.	18,500	-7.5	783	4.7
	18,400	-8.0	798	6.7
	18,600	-7.0	758	1.3

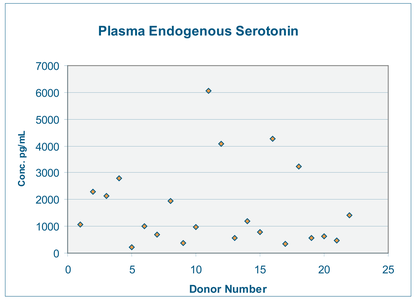
Plasma stability testing was done in unaltered, authentic plasma samples. Plasma lots were screened to find individual batches that had an appropriate concentration to represent a low and high serotonin level. Of the two selected lots (low and high), the lot with the highest level was fortified with a standard solution to adjust the concentration to the upper end of the calibration curve. These two pools were originally analyzed over the course of two runs to calculate mean anchor values for each prior to stressing. The high stability pool had a mean value of 20,000 pg/mL with a CV of 3.8%. The low stability pool had a mean value of 748 pg/mL with a CV of 1.2%.

Matrix Effect on Quantitation

Plasma Lot #	Endogenous Amount Found (pg/mL)	Amount Added (pg/mL)	Total Amount Expected (pg/mL)	Total Amount Found (pg/mL)	% Bias
1	345	1000	1340	1320	-1.9
2	3220	1000	4220	4430	5.0
3	564	1000	1560	1540	-1.5
4	628	1000	1630	1640	0.74
5	461	1000	1460	1420	-2.8
6	1410	1000	2410	2460	2.1

Matrix effect in plasma was tested by measuring endogenous levels in six different lots of unaltered matrix. These same lots were fortified with additional serotonin by spiking an aliquot of each with a standard solution. A target value was calculated for each matrix lot and compared to the found value. These results are presented to the left.

Twenty-two different unaltered plasma samples were tested prior to validation in order to confirm an appropriate calibration range was chosen. All samples produced measurable values within the range of quantitation.



Conclusion:

Serotonin in human plasma is an important biochemical index for diseases of the central and peripheral nervous systems and is extremely helpful in monitoring certain drug therapies. In patients where conversion to serotonin is inhibited, the concentrations can be extremely low and a significant challenge to measure. As with our method for serotonin in human CSF, this method has demonstrated excellent sensitivity, accuracy, and robustness. The data presented here also demonstrates its ability to measure a wide range of baseline levels.

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