Application Note
Clinical & Diagnostics

Serotonin in Plasma

The most selective LC-EC applications for Clinical & Diagnostics analysis

Catecholamines
Serotonin
Metanephrines
VMA
HVA
5-HIAA

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Sulfides
Homocysteine
Glutathione
Disulfides

Vitamins, minerals
A, C, D, E, and K
Iodide
Q10, Ubiquinols

- **Standardized, fast and reliable assay**
- **Kit for standardized sample prep**
- **Robust and reproducible**

Summary

HPLC with electrochemical detection has been established as a fast and reliable method for the determination of serotonin, catecholamines and metabolites in plasma and urine [1 - 5]. The ALEXYS Clinical Analyzer together with a commercially available kit has been evaluated. This dedicated system has proven to be robust and reproducible in routine analysis.

Serotonin in Plasma

Introduction

Serotonin is involved in a variety of physiological processes, including smooth muscle contraction, blood pressure regulation and both central and peripheral nervous system neuro transmission. Abnormalities in serotonin-related processes give rise to various pathological conditions. Aberrations in its central nervous system function are thought to be involved in anorexia, anxiety, depression and schizophrenia. The quantitatively most pronounced aberration in serotonin production is encountered in patients with carcinoid tumors [2]. The diagnostic assessment of the carcinoid syndrome therefore can be performed by the determination of 5-HIAA in urine as well as of serotonin in plasma/serum and urine. The determination of 5-HIAA in urine serves as the basic investigation. The additional determination of serotonin in plasma/serum and urine is considered to provide complementary information.

Figure 1: ALEXYS Clinical Analyzer.

Method

A complete kit contains all the necessary chemicals and materials for sample preparation and analysis. Extracted plasma samples are processed as follows:

- 200 µL of extracted plasma sample is mixed with 10 µL internal standard (IS) and mixed for 5 seconds (vortex mixer).
- 200 µL precipitation reagent is added to the solution and mixed for 5 seconds (vortex mixer).
- The solution is subsequently centrifuged for 1 minute at 10000 x g.
- The supernatant is collected and 20 µL injected in the LC system.

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow rate	1.0 mL/min
Sample	10 µL, supernatant of precipitated plasma solution
Mobile phase	HPLC kit buffer (recycled during experiments)
Temperature	D2 SDC 30°C (separation & detection), AS110: 4°C (sample cooling)
E-cell	550 mV (vs. Ag/AgCl sat'd)
Range	50 nA/V
I-cell	0.2 – 3.0 nA
ADF	0.1 Hz
Analysis time	< 10 minutes

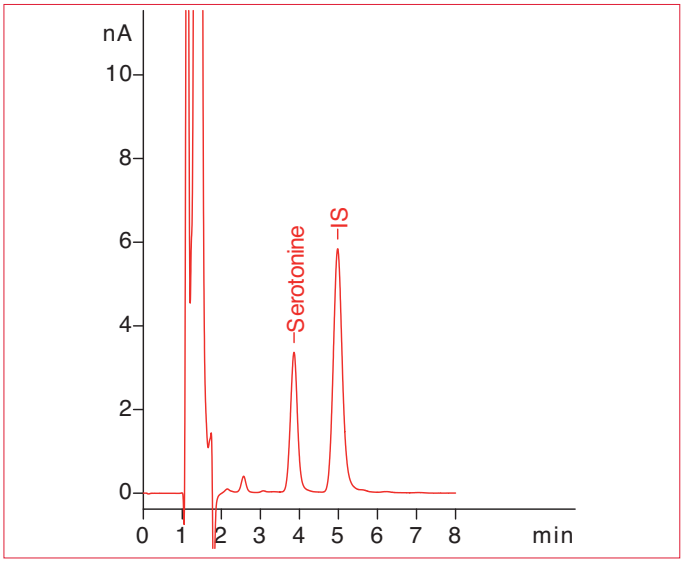

Figure 2: Analysis of 10 µL plasma calibrator with a concentration of 192 µg/L serotonin.

For details about the extraction procedure of plasma from blood samples see reference [11].

The quantification of serotonin in plasma samples is performed by means of a single-point calibration method using a plasma calibrator. The plasma calibrator supplied in the kit is a lyophilized plasma sample with a known amount of serotonin. The plasma calibrator should be reconstituted by adding 5 mL HPLC-grade water and processed the same way as the extracted plasma samples. An example chromatogram of a plasma calibrator analysis is shown in figure 2. An internal standard method is used to compensate for recovery losses during the sample preparation step.

Results

Analysis of controls

For validation of the analytical method 'plasma controls' have been analyzed in both the normal (level I) and the pathological range (level II). The controls are lyophilized plasma samples which should be reconstituted by adding 5 mL HPLC-grade water and have to be processed in the same way as the plasma samples. Both Control I and Control II were analyzed and the analyte concentrations quantified using the plasma calibrator.

Table 2

Measured serotonin concentration in plasma controls level I and II

Component	*Specified (µg/L)*		*Specified (µg/L)*	*RSD (%)*
	Min	*Max*		
Control level I				
	78	116	95	1.8
Control level II				
	231	347	290	2.0

Measured serotonin concentration in plasma controls level I and II, n = 4 (injections) x 3 (days). Concentration range specified is given for reference (source: data sheet supplied with controls).

For both plasma controls level I and II the determined serotonin concentrations were within the specified concentration ranges (see table 2).

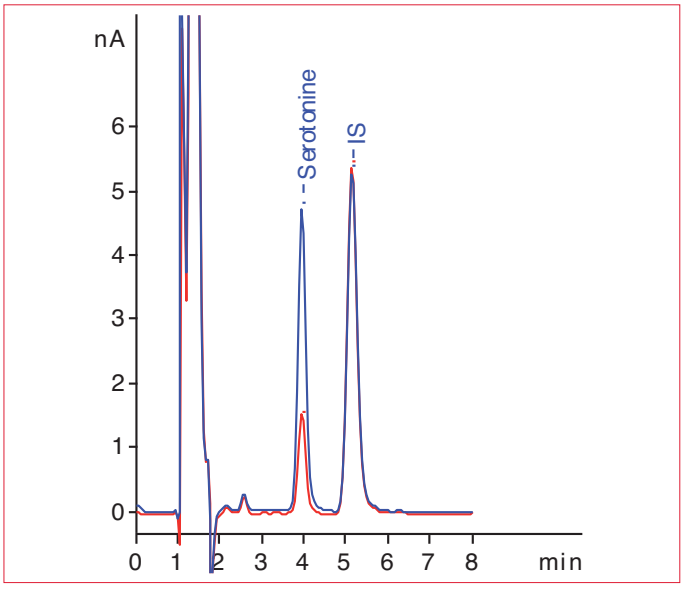

Figure 3: Overlay of 2 chromatograms of 10 µL injections of plasma control level I (red) and II (blue).

Analysis of plasma samples

Plasma controls, level I (sample A) and level II (sample B), were used for the statistical evaluation of the method. The intra-assay precision of the method was determined for sample A and B. The plasma samples were worked-up 5 times and duplicate analysis were performed to determine the relative standard deviation (RSD, %). This procedure was repeated for 3 days. For plasma sample B an overlay is shown in figure 3 of 10 chromatograms (5 samples x 2 duplicate injections) recorded at day 1. The RSD's found for sample A and B were smaller than 3% (see table 3).

Table 3

Intra-assay precision for the analysis of serotonin in plasma sample A and B

Component	*RSD (%)*	*Conc. (µg/L)*
Sample A		
Day 1	1.7	93
Day 2	2.7	91
Day 3	1.0	95
Sample B		
Day 1	1.1	294
Day 2	1.8	295
Day 3	1.6	296

Intra-assay precision for the analysis of serotonin in plasma sample A and B, n= 5 (samples) x 2 (injections).

For all plasma samples, controls and calibrator recoveries typically in the range of 80 – 100 % were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 0.7 µg/L for serotonin. The CLOD is calculated based on a 10 µL injection and defined as the concentration that gives a signal that is three times the peak-to-peak noise. The method is linear for the determination of serotonin in the concentration range from 1 – 1000 µg/L [11].

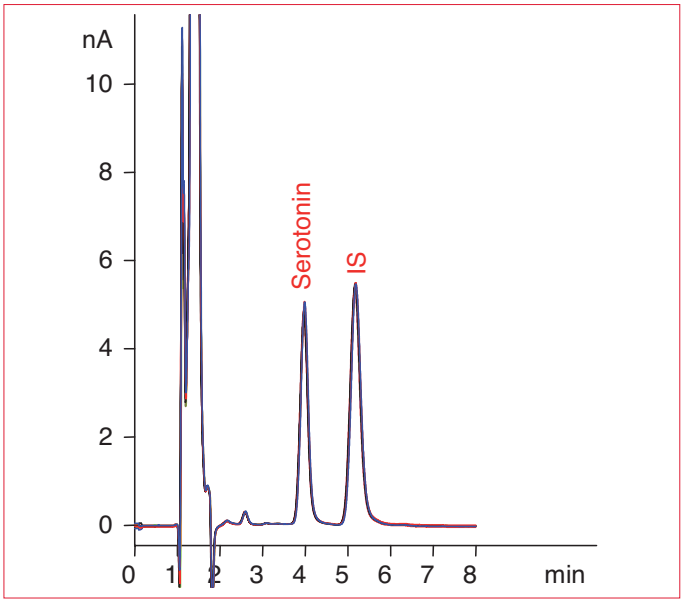

Figure 4: Overlay of 10 chromatograms of 10 µL injections of plasma sample B.

The inter-assay precision of the method was determined over a time period of three days for sample A and B. Both samples were worked-up 5 times and analyzed (duplicate injection) every single day. From the obtained data the relative standard deviation calculated.

Table 4

Inter-assay precision for the analysis of serotonin in sample A and B

Component	*RSD (%)*	*Conc. (µg/L)*
Sample A		
	2.4	93
Sample B		
	1.5	295

Inter-assay precision for the analysis of serotonin in sample A and B. n= 5 (samples) x 2 (duplicate injections) x 2 (days).

The RSD's found for the analysis of sample A and B were smaller than 3% (see table 4).

References

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Conclusion

The ALEXYS Clinical Analyzer in combination with a commercially available kit provides a standardized method for fast and reliable analysis of serotonin in plasma.

Ordering information

180.0039E	ALEXYS Clinical Analyzer

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.