Application Note
Clinical & Diagnostics

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

Serotonin in Urine

- **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 Urine

Introduction

Serotonin is synthesized by enterochromaffin cells of the intestine and certain neurons of the central nervous system. In blood more than 97% are stored in platelets [1]. Physiological actions of serotonin include the control of circadian rhythms, sleep regulation, sex drive and thermoregulation as well as the influence on melatonin synthesis and on aldosterone regulation [2, 3]. Various diseases are related to a pathologic serotonin metabolism [4, 5]. An elevated plasma concentration of serotonin and an increased renal secretion of the serotonin metabolite 5-hydroxyindoleacetic acid may be found in patients with epilepsy. Migraine is associated with a decreased platelet serotonin concentration. Serotonin metabolism is also disturbed in patients who suffer from schizophrenia, autism or psychotic depression. The determination of plasma serotonin level is of decisive importance for the diagnosis of the carcinoid syndrome which is mainly accompanied by an elevated serotonin production [6].

Figure 1: ALEXYS Clinical Analyzer.

Method

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

- 2 mL acidified urine sample (10 mL concentrated HCl per liter urine) or urine calibrator is mixed with 4 mL stabilizing reagent and 50 µL internal standard (IS) and subsequently adjusted to a pH 4.5 – 6.5 using 0.5M NaOH.
- The mixture is applied to a sample preparation columns to trap the serotonin present in the sample.
- The column is washed with 15 mL HPLC-grade water and subsequently with 2 mL washing solution to remove interfering components.
- 5 mL of eluting reagent is then used to elute serotonin from the extraction column.
- The eluate is collected, mixed (vortex-mixer) and 20 µL injected in the LC system.

The quantification of the serotonin in the urine samples is performed by means of a single-point calibration method using a urine calibrator. The calibrator is a lyophilized urine sample with a known amount of serotonin. The urine calibrator should be processed the same way as the urine samples. An example chromatogram of a urine calibrator analysis is shown in figure 2. An internal standard compensates for recovery losses during the sample preparation step.

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow rate	1.0 mL/min
Sample	20 µL, extracted with sample preparation columns
Mobile phase	HPLC kit buffer (recycled)
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

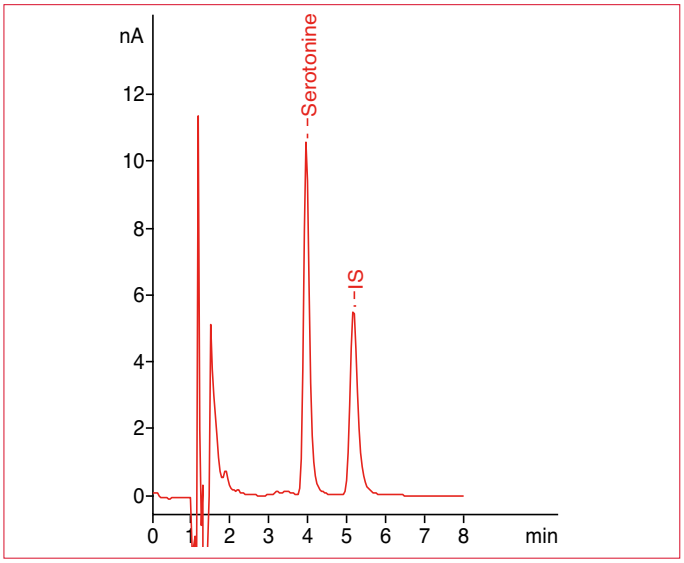

Figure 2: Analysis of 20 µL urine calibrator with a concentration of 346 µg/L serotonin.

Results

Analysis of controls

For validation of the analytical method 'urine controls' have been analyzed in both the normal (level I) and the pathological range (level II).

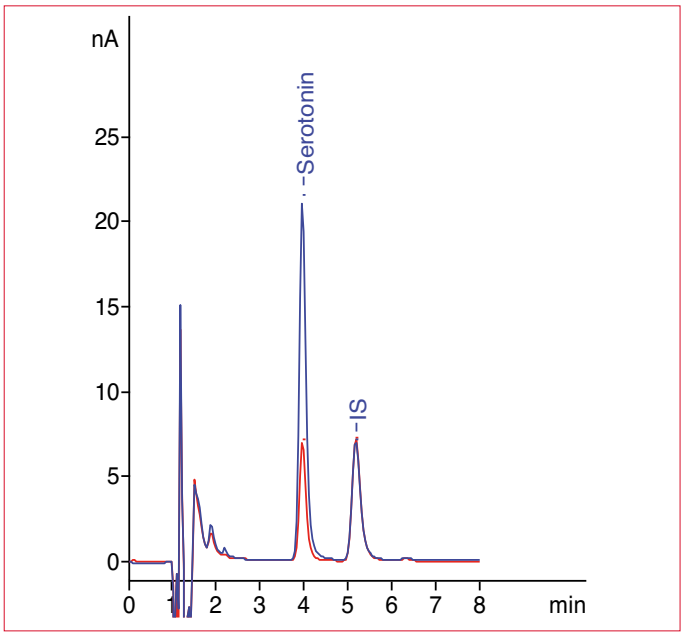

Figure 3: Overlay of 2 chromatograms of 20 µL injections of control level I (red) and II (blue).

Also the control samples are lyophilized urine samples which have to be processed in the same way as the urine samples. Both Control I and Control II were analyzed and the analyte concentrations quantified using the urine calibrator. For both urine controls level I and II the determined serotonin concentrations were within the concentration ranges specified on the urine control data sheet (see table I).

Analysis of urine samples

Urine samples of an apparently healthy volunteer were collected and analyzed multiple times to determine the recoveries, LOD, intra- and inter-assay precision of the method.

The intra-assay precision of the method was determined using two spiked urine samples A and B, matching the serotonin concentration of control level I and level II, respectively. The urine 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. The RSD's found for sample A and B were smaller than 2%.

Table 2

Measured serotonin concentration in urine controls level I and II

Component	*Specified (µg/L)*		*Specified (µg/L)*	*RSD (%)*
	Min	*Max*		
Control level I				
	123	185	180	1.2
Control level II				
	420	630	557	1.0

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

Table 3

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

Component	*RSD (%)*	*Conc. (µg/L)*
Sample A		
Day 1	0.6	190
Day 2	1.4	180
Day 3	0.9	177
Sample B		
Day 1	1.1	591
Day 2	1.6	556
Day 3	1.6	551

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

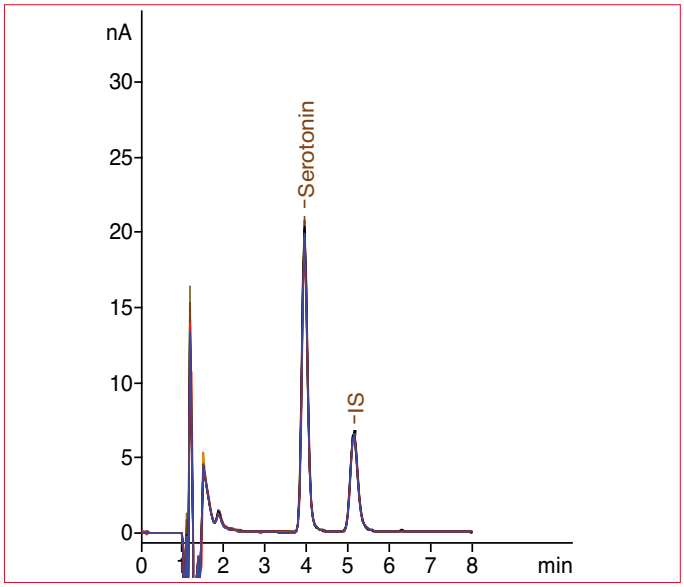

Figure 4: Overlay of 10 chromatograms of 20 µL injections of urine sample B.(red) and II (blue).

For all urine samples, controls and calibrator recoveries typically in the range of 80 – 95% were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 0.3 µg/L for serotonin. The CLOD is calculated based on a 20 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 [14]. 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 and 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		
	3.2	182
Sample B		
	3.5	566

Inter-assay precision for the analysis of serotonin in sample A and B. n= 5 (samples) x 2 (duplicate injections) x 3 (days).

The RSD's for the analysis of sample A & B were smaller than 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 urine.

Ordering information

180.0039W	ALEXYS Clinical Analyzer

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