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ClinRep® kit for the analysis of 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 epilepsia. 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].

Serum, plasma or urine serotonin may be determined by fluorescence, gas-chromatographic, mass-spectrometric, and radioimmunologic methods [7, 8]. However, these methods often fail to satisfy the requirements concerning sensitivity and specifity and they are labour-intensive, time consuming and expensive. High performance liquid chromatography (HPLC) with electrochemical detection provides a rapid, accurate and greatly simplified method for the determination of serotonin in biologic material [9-12].

The ClinRep® complete kit for serotonin in urine of Recipe GmbH (Munich, Germany) offers a standardised method for the sample preparation and routine analysis of urinary serotonin. In this application note the results are reported for the analysis of serotonin in urine samples using a ClinRep® kit with an ALEXYS 110 LC-EC system.

Method

A Recipe ClinRep® complete kit contains all the necessary chemicals and (calibration) materials for sample preparation and analysis. Urine samples are processed as follows:

- 2 mL acidified urine sample (10 mL conc. 32% HCl per liter urine) or urine calibrator is mixed with 4 mL stabilising reagent S 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 ClinRep® sample preparation columns to trap the serotonin present in the sample.

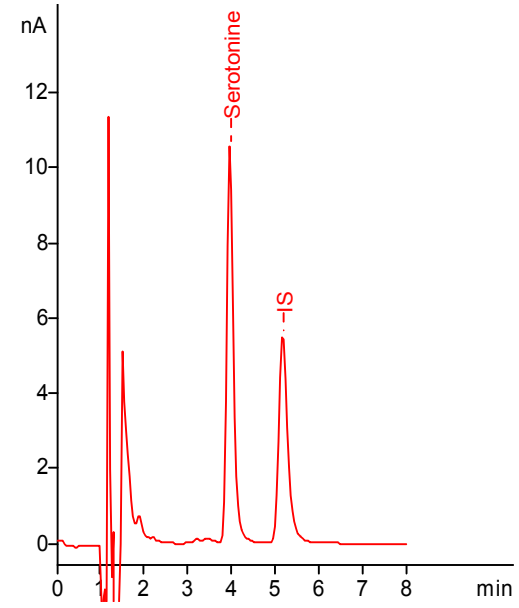

Fig. 1 Analysis of 20 µL ClinCal® urine calibrator with a concentration of 346 µg/L serotonin.

- The column is washed with 15 mL HPLC-grade water and subsequently with 2 mL washing solution W to remove interfering components.
- 5 mL of eluting reagent E 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 lyophilised urine sample with a known amount of serotonin. The urine calibrator should be processed via the same sample preparation method as the urine samples. An example chromatogram of a urine calibrator analysis is shown in figure 1. An internal standard compensates for recovery losses during the sample preparation step.

Set-up

HPLC	ALEXYS 110 system with DECADE II SDC (p/n 190.0035)
Flow cell	GC type flow cell with Ag/AgCl saltbridge REF
Column	ClinRep® Analytical column for serotonin in urine

LC-EC conditions

Flow rate	1.0 mL/min
Sample	20 µl, extracted with ClinRep® sample preparation columns
Mobile phase	ClinRep® serotonin buffer#
Temperature*	$T_{D2\ SDC}$ 30°C (separation & detection), T_{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

#) mobile phase was recycled during experiments. *) minimum actual oven & tray temperature which can be reached is dependent on ambient conditions.

Analysis of ClinChek® controls

For quality control of the analytical determination Recipe ClinChek® urine controls have been used in both the normal (level I) and the pathological range (level II).

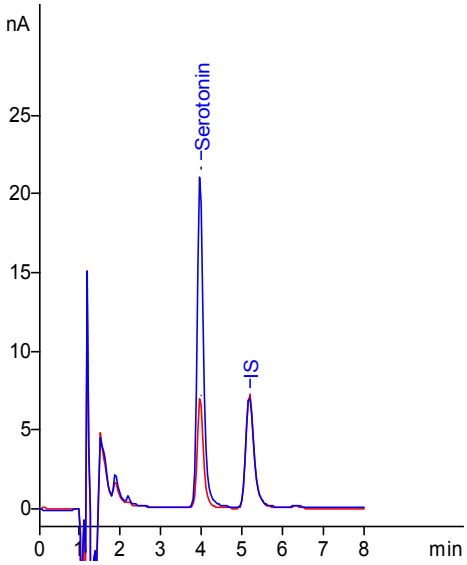

Fig. 2. Overlay of 2 chromatograms of 20uL injections of ClinChek® control level I (red) and II (blue).

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

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

Component	Specified conc (µg/l)		Calculated	RSD
	Min	Max	conc (µg/l)	(%)
Control, level I	123	185	180	1.2
Control, level II	420	630	557	1.0

Analysis of urine samples

Urine samples of an apparently healthy volunteer were collected and analysed 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 then 2%.

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

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

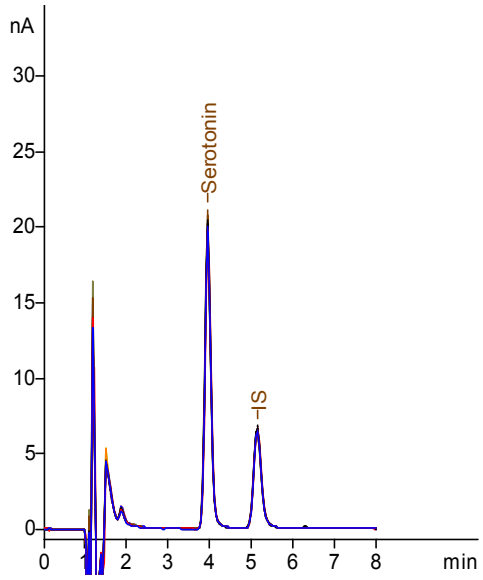

Fig. 3. Overlay of 10 chromatograms of 20uL injections of urine sample B.

For all urine samples, controls and calibrator recoveries typically in the range of 80 – 95% were found, compared to a directly injected standard. The concentration limit of detection (C_{LOD}) for the method was approximately 0.3 µg/L for serotonin. The C_{LOD} 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 analysed (duplicate injection) every single day and the relative standard deviation calculated.

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

Component	RSD (%)	Conc. (µg/l)
Sample A	3.2	182
Sample B	3.5	566

The RSD's for the analysis of sample A and B were smaller then 4%.

Conclusion

The ClinRep® complete kit for serotonin in urine provides a standardised method for the sample preparation of urine samples and fast & reliable analysis of urinary serotonin using LC-EC.

Parts and configuration used

190.0035#	ALEXYS 110 system with DECADE II SDC
7000*	ClinRep® complete kit , Serotonin in urine (for 100 assays)
7030*	ClinRep® Analytical column for Serotonin in urine
8022*	ClinChek® urine controls, level I, II (2x5 x 8 mL)

#) A GC-type flow cell with Ag/AgCl saltbridge REF should be ordered separately.
*) Parts from Recipe GmbH, Sandstrasse 37-39, D80335 Munich, Germany.

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