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ClinRep® kit for the analysis of 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. Abberations in its central nervous system function are thought to be involved in anorexia, anxiety, depression and schizophrenia. Peripheral aberrations in serotonin-related processes have been implicated in emesis, hypertension, migraine, genesis of cardiac arrhythmias, Raynaud's disease, fibrotic syndromes and some symptoms of the carcinoid syndrome. The quantitatively most pronounced aberration in serotonin production is encountered in patients with carcinoid tumors [2].

Carcinoid tumors develop from enterochromaffine cells and widely spread throughout the gastrointestinal tract, biliary tract and gallbladder, pancreatic ducts, and bronchial tree and are also found in the thymus, thyroid, ovaries, uterus, and salivary glands. Serotonin is produced in excess by carcinoid tumors. Patients suffering from these tumors therefore usually show elevated levels of serotonin in plasma/serum and urine as well as increased urinary 5-HIAA levels [1-4].
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. However, for carcinoid tumors with little serotonin excretion (e.g. AADC-deficient tumors) it is mandatory in order to avoid false negative results [1, 2].

For the determination of these analytes HPLC is considered as method of choice (reference method), being superior to corresponding immunological test procedures due to a higher analytical sensitivity, specificity and precision [2, 5, 6].

The ClinRep® complete kit for serotonin in plasma of Recipe GmbH (Munich, Germany) is standardised for the sample preparation and routine analysis of serotonin in platelet-poor plasma. The determination of serotonin may also be performed from platelet-rich plasma (e.g. [7, 8, 9]). In this case, the obtained results have to be related to the platelet number (indication in nmol/ number of platelets).

In this application note the results are reported for the analysis of serotonin in lyophilised plasma samples using a ClinRep® kit in combination with an ALEXYS 110 system.

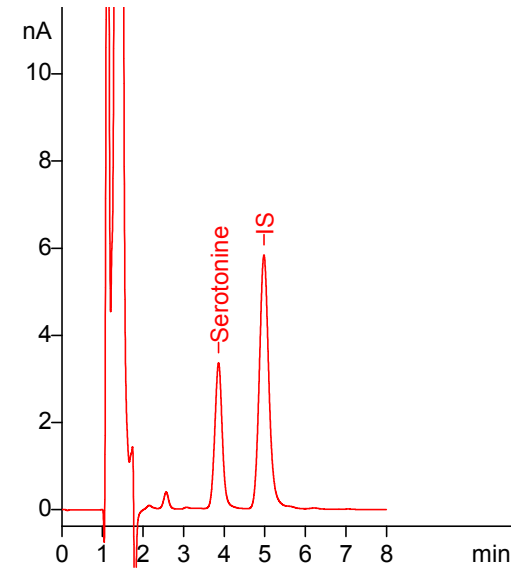

Fig. 1 Analysis of 10 µL ClinCal® plasma calibrator with a concentration of 192 µg/L serotonin.

Method

A Recipe ClinRep® complete kit contains all the necessary chemicals and (calibration) materials for sample preparation and analysis for 100 assays. 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 P is added to the solution and mixed for 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.

*) for details about the extraction procedure of plasma from blood samples see reference [11]. The necessary parts for blood collection and extraction are not provided in the kit.

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 ClinRep® kit is a lyophilised plasma sample with a known amount of serotonin. The plasma calibrator should be reconstituted by adding 5 mL HPLC-grade water and processed via the same

sample preparation method as the extracted plasma samples. An example chromatogram of a plasma calibrator analysis is shown in figure 1. An internal standard method is used to compensate 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 plasma

Furthermore, a centrifuge (10000 x g) and vortex mixer are necessary for sample preparation.

LC-EC conditions

Flow rate	1.0 mL/min
Sample	10 µl, supernatant of precipitated plasma solution
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® plasma controls have been used in both the normal (level I) and the pathological range (level II). The controls are lyophilised 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 analysed and the analyte concentrations quantified using the ClinCal plasma calibrator.

Table I. Calculated serotonin concentration in plasma 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	78	116	95	1.8
Control, level II	231	347	290	2.0

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

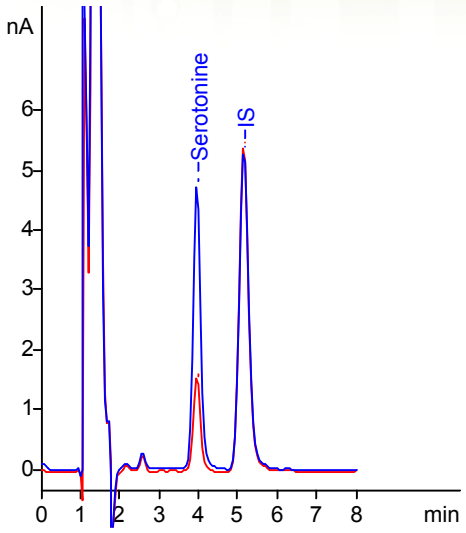

Fig. 2. Overlay of 2 chromatograms of 10uL injections of ClinChek® plasma control level I (red) and II (blue).

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 plasma control samples were 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 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 then 3% (see table II).

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

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

For all plasma samples, controls and calibrator recoveries typically in the range of 80 – 100 % were found, compared to a directly injected standard. The concentration limit of detection (C_{LOD}) for the method was approximately 0.7 µg/L for serotonin. The C_{LOD} 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].

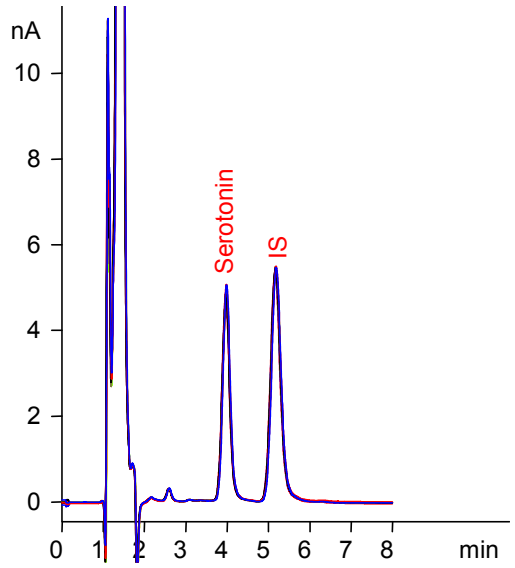

Fig. 3. Overlay of 10 chromatograms of 10uL injections of plasma sample B.

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 analysed (duplicate injection) every single day. From the obtained data 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 2 (days).

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

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

Conclusion

The ClinRep® complete kit for serotonin in plasma provides a standardised method for the analysis of plasma serotonin using LC-EC.

Parts and configuration used

190.0035#	ALEXYS 110 system with DECADE II SDC
6000*	ClinRep® complete kit , Serotonin in plasma (for 100 assays)
6030*	ClinRep® Analytical column for Serotonin in plasma
8009*	ClinChek® plasma controls, level I, II (2x 5 x 5 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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