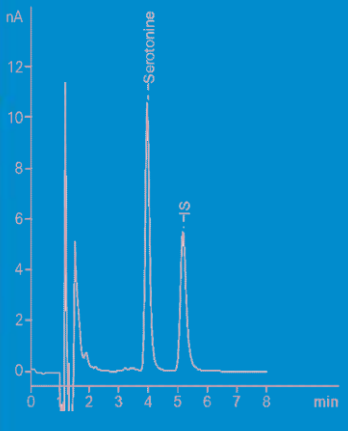
SEROTONIN IN PLASMA

THE SOUNDEST LC-EC APPLICATIONS FOR CLINICAL & DIAGNOSTICS ANALYSIS EVER BUILD

- Catecholamines
- Serotonin
- Metanephrines
- VMA
- HVA
- 5-HIAA Homocysteine
- Glutathione
- (di-)sulfides
- Iodide
- Vitamins A, C, D, E, and K
- Q10
- Ubiquinols



INTRODUCTION

Serotonin 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. 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.

- Standardized, fast and reliable assay
- Kit for standardized sample prep
- Robust & 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. The ALEXYS Clinical Analyzer together with a commerciallya available sample prep kit is dedicated and standardized for routine analysis of serotonin in plasma.

Fig. 1. ALEXYS Clinical Analyzer.

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.

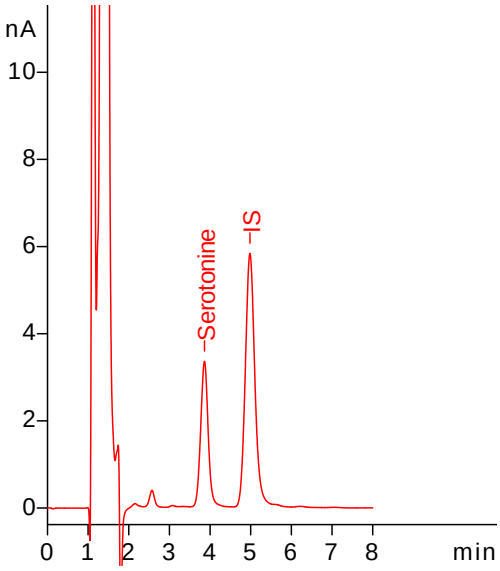


Fig. 1 Analysis of 10 µL ClinCal® plasma calibrator with a concentration of 192 µg/L serotonin.

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

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
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.

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).

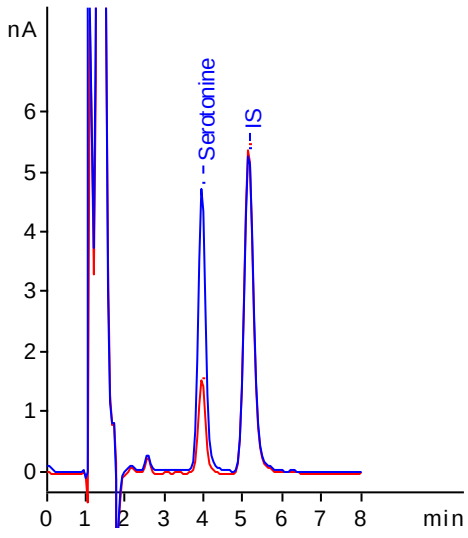


Fig. 2. Overlay of 2 chromatograms of 10µL injections of ClinChek® plasma control level I (red) and II (blue).

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 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].

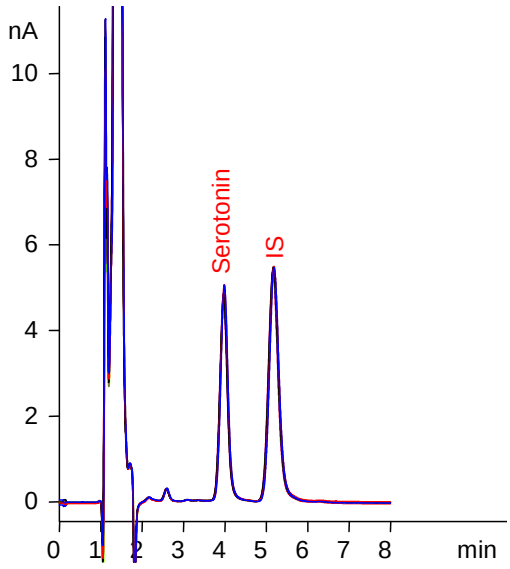


Fig. 3. Overlay of 10 chromatograms of 10 µL injections of plasma sample B.

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

PART NUMBERS AND CONFIGURATIONS	
180.0039C	ALEXYS Clinical Analyzer
110.4105	VT03 3mm GC, salt bridge
RE.6000	ClinRep® complete kit , Serotonin in plasma (for 100
RE.6030	ClinRep® Analytical column
RE.8009	ClinChek® plasma controls, level I, II
RE.6000	ClinRep® complete kit , Serotonin in plasma

References

1. Thomas, Labor und Diagnose: Indikation und Bewertung von Laborbefunden, *TH-Books, Verlagsgesellschaft*, Frankfurt/Main, **5th ed.**, (1998) 1076-1078
2. I.P. Kema, E.G.E. de Vries, F.A.J. Muskiet, *J Chromatogr.* , **B 747**, (2000) 33-48
3. D. Mc Cormic, *Gastroenterol Nurs.*, **25(3)**, (2002) 105-111
4. D. Donaldson, *J R Soc Health.* , **120(2)**, (2000) 78
5. E. Pussard, N. Guigueno, O. Adam, J.-F. Giudicelli, *Clin Chem.*, **42(7)**, (1996) 1086-1091
6. S. Jovanovic, D. Mirkovic, N. Majkic-Singh, *Clin Lab.*, **44** (1998) 263-268
7. M. Picard, D. Olichon, J. Gomber, *J. Chromatogr.*, **341**, (1985) 445-451
8. E. Flachaire, C. Beney, A. Berthier, J. Salandre, C. Quincy, B. Renaud, *Clin. Chem.,* **36 (12)**, (1990) 2117-2120
9. W. G. Meijer, I. P. Kema, M. Volmer, P. H. B. Willemse, E. G. E de Vries, *Clin Chem.*, **46(10)** (2000) 1588-1596.
10. R.T. Peaston and C. Weinkove, *Ann. Clin. Biochem.,* **41**, (2004) 17–38
11. Recipe, Instruction manual for the serotonin in plasma kit, version, **4.0** (2004)

Antec Leyden is an **ISO 9001:2008** certified company. For research purposes only. Specifications mentioned in this application note are subject to change without further notice. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS for the analysis of metanephrines. The actual performance may be affected by factors beyond Antec Leyden's control.

Antec® Proven Performance!

Antec Leyden B.V. - Industrieweg 12 - 2382 NV - Zoeterwoude - The Netherlands - info@myantec.com - www.myantec.com
Antec (USA) - P.O. Box 111418 - Palm Bay FL 32911 - USA - info.usa@myantec.com - www.myantec.com