Application Note

ALEXYS - Clinical & Diagnostics

The soundest LC-EC Applications for Clinical & Diagnostics Analysis ever

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

Catecholamines in Plasma

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

Introduction

The catecholamines adrenaline (A), noradrenaline (NA) and dopamine (DA) are metabolic products of the amino acid tyrosine. They are synthesized in the brain, the extra-adrenal chromaffin tissue and the sympathetic nerve endings. Catecholamines play an important role as neurotransmitters and in metabolic regulation by stimulation of several adrenoreceptors [1].

The determination of catecholamines and metabolites is of great importance for the diagnosis and treatment of tumor diseases of the sympathoadrenal system. These tumors, the pheochromocytoma, are causing an elevated catecholamine biosynthesis within the affected tissue. As a result, increased catecholamine concentrations in plasma and urine are observed exceeding by far the normal levels [1-6].

Summary

HPLC with electrochemical detection has been established as a fast and reliable method for the determination of 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.

Figure 1: ALEXYS Clinical Analyzer.

Method

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow rate	1.0 mL/min
Sample	20 µL (unless otherwise stated), extracted with sample preparation columns
Mobile phase	HPLC kit buffer (recycled during experiments)
Temperature	D2 SDC 30°C (separation & detection), AS110: 4°C (sample cooling)
E-cell	500 mV (vs. Ag/AgCl sat'd)
Range	5 nA/V
I-cell	Ca. 0.2 – 3 nA
ADF	0.1 Hz
Analysis time	15 minutes

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

- 1 mL of plasma sample or plasma calibrator and 50 µL internal standard (IS) is pipetted into a sample preparation column.
- After shaking and centrifuging the solid phase suspension, the column is washed with washing solution to remove interfering components.
- After mixing with elution reagent, the catecholamines are eluted from the extraction column and 20 µL is injected in the HPLC system.

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

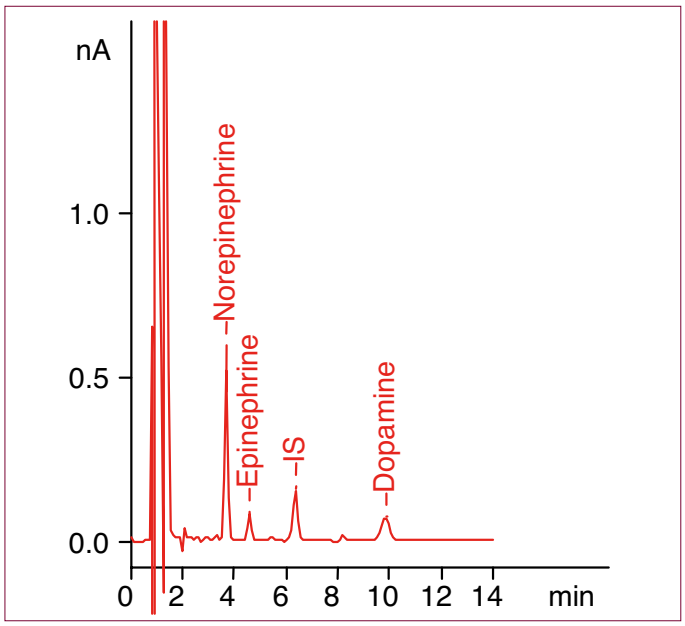

Figure 2: Analysis of 20 µL plasma calibrator. Concentration of catecholamines in the calibrator sample: 1.19 µg/L NA, 275 ng/L A and 212 ng/L DA.

The quantification of the catecholamines in the plasma samples is performed by means of a single-point calibration method using a plasma calibrator. The plasma calibrator is a lyophilized plasma sample with a known amount of catecholamines. The calibrator should be processed the same way as the patient samples. An example chromatogram of a plasma calibrator analysis is shown in figure 2. An internal standard is used to compensate for recovery losses during the sample preparation step. The sample response is interpolated to 100% recovery to establish the real catecholamine concentration in the plasma samples.

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 (see table 2).

Table 2

Measured concentration of plasma controls level I and II			
Component	*Specified (ng/L)*		*Measured (ng/L)*
	Min	*Max*	
Control level I			
NA	255	383	271
A	79	119	83
DA	45	95	55
Control level II			
NA	1522	2284	1858
A	406	608	476
DA	310	466	450

Measured concentration of plasma controls level I and II n= 4 (samples) x 2 (duplicate injections), based on 40 µL injections. Concentration range specified is given for reference (source: data sheet supplied with controls).

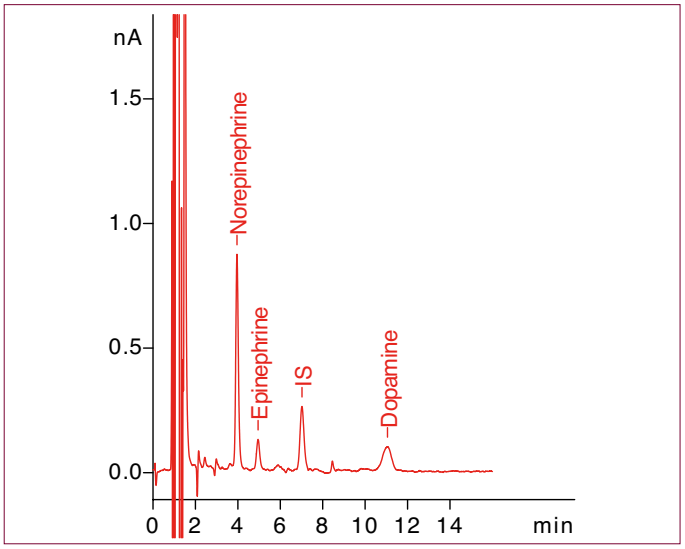

Figure 3: Chromatograms of 20 µL injection of control level II.

Analysis of plasma samples

The plasma control samples were analyzed multiple times to determine the recoveries, LOD, and intra assay precision of the method. The intra-assay precision of the method was determined for sample A (plasma control I) and sample B (plasma control II). The plasma samples were worked-up 4 times and duplicate analysis were performed to determine the relative standard deviation (RSD, %). The RSD's found for all catecholamines (see table II) were typically smaller than 4%. Only for dopamine, which was present in sample A in a low concentration, the RSD was slightly higher, around 8%.

Table 3

Intra-assay precision of plasma sample A and B		
Component	*RSD (%)*	*Conc. (ng/L)*
Sample A		
NA	2.0	271
A	3.3	83
DA	7.7	55
Sample B		
NA	2.0	1858
A	1.8	476
DA	3.3	450

Intra-assay precision of plasma sample A and B, n= 4 (samples) x 2 (duplicate injections, inj. vol. 40 µL)

For all plasma samples, controls and calibrator recoveries typically in the range of 70 – 90 % were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 5 ng/L for all catecholamines. 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 all catecholamines in the concentration range from 10 – 2500 ng/L [11].

Conclusion

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

References

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PART NUMBERS AND CONFIGURATIONS

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