Catecholamines in Plasma

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.

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

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

Electrochemistry Discover the difference
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 adrenoceptors [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].

Method

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].

Table 1

<table>
<thead>
<tr>
<th>Set-up</th>
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<tbody>
<tr>
<td>HPLC</td>
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<td>Flow rate</td>
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<tr>
<td>Sample</td>
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<tr>
<td>Mobile phase</td>
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<tr>
<td>Temperature</td>
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<td>E-cell</td>
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<td>Range</td>
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<td>I-cell</td>
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<td>ADF</td>
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<td>Analysis time</td>
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Figure 1: ALEXYS Clinical Analyzer.

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.

Results

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 samp-
Conclusion

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

References


Ordering information

180.0039E ALEXYS Clinical Analyzer

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For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec’s control. Specifications mentioned in this application note are subject to change without further notice.

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