Introduction

Catecholamines 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 adreno receptors [1]. In clinical chemistry, the term "catecholamines" is usually constricted to the compounds epinephrine (also called adrenaline, abbreviated as A), norepinephrine (noradrenaline, NA) and dopamine (DA), see figure 1.

Fig. 1 Structural formulas of epinephrine, norepinephrine and dopamine.

The determination of catecholamines and catecholamine metabolites is of great importance for the diagnosis and management 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, due to their enhanced excretion, in urine are observed. These concentrations are exceeding by far the values being obtained for the normal range [1-6].

Today, LC-EC has been established as a fast and reliable method for the determination of catecholamines and metabolites in plasma and urine [1, 5, 7-9].

In this application note the results are reported for the analysis of catecholamines in lyophilised plasma samples using a ClinRep® kit (Recipe GmbH, Munich, Germany) in combination with an ALEXYS 110 system.

Method

The Recipe ClinRep® complete kit contains all the necessary chemicals and (calibration) materials for sample preparation and analysis of 250 plasma assays, excluding the analytical column. Extracted plasma* samples are processed as follows:

- 1 mL of plasma sample or plasma calibrator and 50 µL internal standard (IS) is pipetted into a ClinRep® sample preparation column.

Fig. 2 Analysis of 20 µL ClinCal® plasma calibrator. Concentration of catecholamines in the calibrator sample: 1,19 µg/L NA, 275 ng/L A and 212 ng/L DA.

- 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 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 the catecholamines in the plasma samples is performed by means of a single-point calibration method using a plasma calibrator. The ClinCal® plasma calibrator supplied in the ClinRep® kit is a lyophilised plasma sample with a known amount of catecholamines. The plasma calibrator should be processed via the same sample preparation method as the patient plasma 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.
Set-up

HPLC
ALEXYS 110 LC-EC 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 catecholamines in urine

Furthermore, a centrifuge (1000 x g) and vortex mixer are necessary for sample preparation.

LC-EC conditions

Flow rate
1.0 mL/min

Sample
20 µl (unless otherwise stated), extracted with ClinRep® sample preparation columns

Mobile phase
ClinRep® catecholamine buffer

Temperature
T$_{D2}$ SDC 30°C (separation & detection), T$_{AS110}$ 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

* 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 validation 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 (see table I).

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Specified conc (ng/L)</th>
<th>Calculated conc (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control level I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>255 – 383</td>
<td>271</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>79 – 119</td>
<td>83</td>
</tr>
<tr>
<td>Dopamine</td>
<td>45 – 95</td>
<td>55</td>
</tr>
<tr>
<td>Control level II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1522 – 2284</td>
<td>1858</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>406 – 608</td>
<td>476</td>
</tr>
<tr>
<td>Dopamine</td>
<td>310 – 466</td>
<td>450</td>
</tr>
</tbody>
</table>

Analysis of plasma samples

The plasma control samples were analysed 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%.

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

Table II. Intra-assay precision of plasma sample A and B, n= 4 (samples) x 2 (duplicate injections, Inj. Vol. 40 uL)

<table>
<thead>
<tr>
<th>Component</th>
<th>RSD (%)</th>
<th>Conc. (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2.0</td>
<td>271</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>3.3</td>
<td>83</td>
</tr>
<tr>
<td>Dopamine</td>
<td>7.7</td>
<td>55</td>
</tr>
<tr>
<td>Sample B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2.0</td>
<td>1858</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.8</td>
<td>476</td>
</tr>
<tr>
<td>Dopamine</td>
<td>3.3</td>
<td>450</td>
</tr>
</tbody>
</table>
Conclusion

The ClinRep® complete kit for catecholamines in plasma provides a standardised, fast and reliable method for sample preparation and analysis of plasma catecholamines using LC-EC.

Parts and configuration used

<table>
<thead>
<tr>
<th>Part Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>190.0035</td>
<td>ALEXYS 110 LC-EC system with DECADE II SDC</td>
</tr>
<tr>
<td>1000*</td>
<td>ClinRep® complete kit, Catecholamines in plasma</td>
</tr>
<tr>
<td>1030*</td>
<td>ClinRep® Analytical column for catecholamines in plasma</td>
</tr>
<tr>
<td>8010*</td>
<td>ClinChek® plasma control, level I</td>
</tr>
<tr>
<td>8011*</td>
<td>ClinChek® plasma control, level II</td>
</tr>
</tbody>
</table>

*) A GC-type flow cell with Ag/AgCl saltbridge REF should be ordered separately.

*) Parts from Recipe GmbH, Sandstrasse 37-39, D80335 Munich, Germany.

References


Antec Leyden is an ISO 9001:2000 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 110 for the analysis of catecholamines. The actual performance may be affected by factors beyond Antec Leyden’s control. Antec Leyden, Industrieweg 12, 2382 NV Zoeterwoude, The Netherlands ● sales@antecleyden.com ● www.antecleyden.com