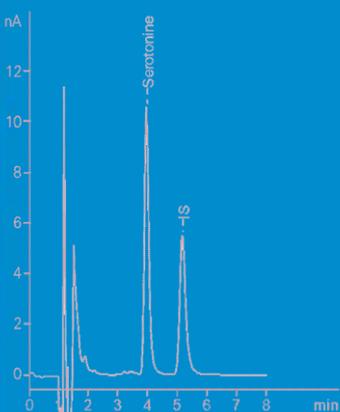# CATECHOLAMINES IN PLASMA

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

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 neuro-transmitters 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 (noradrena-line, NA) and dopamine (DA) The determination of cate-cholamines and catechola-mine metabolites is of great importance for the diagnosis and management of tumor diseases of the sym-pathoadrenal system. These tumors, the pheochromocy-toma, are causing an elevated catecholamine biosynthesis within the affected tissue. As a result, increased cate-cholamine concentrations in plasma and, due to their enhanced excretion, in urine are observed. These concen-trations are exceeding by far the values being obtained for the normal range [1-6].

- 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 catecholamines and metabolites in plasma and urine [12, 13]. The ALEXYS Clinical Analyzer together with a commerciallya available sample prep kit is dedicated and standardized for routine analysis of urinary catecholamines.

Fig. 1. ALEXYS Clinical Analyzer.

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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.
- 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.
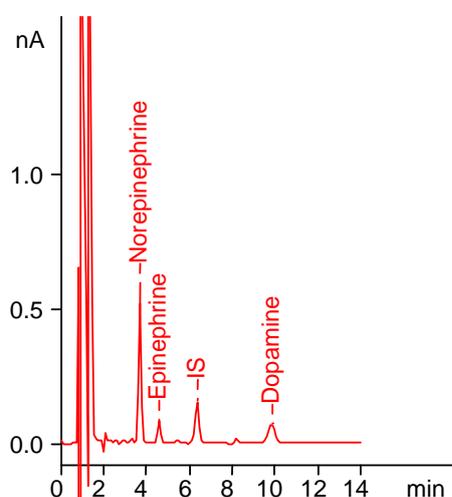

Fig. 2 Analysis of 20 μL ClinCal® plasma calibrator. Concentration of catecholamines in the calibrator sample: 1,19 μg/L NA, 275 ng/L A and 212 ng/L DA.

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.

Table 1	
Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow cell	GC type flow cell with Ag/AgCl saltbridge REF
Column	ClinRep® Analytical column for catecholamines in plasma

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

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).

Component	Specified conc (ng/L)		Calculated
	Min	Max	conc (ng/L)
Control level I			
Norepinephrine	255	383	271
Epinephrine	79	119	83
Dopamine	45	95	55
Control level II			
Norepinephrine	1522	2284	1858
Epinephrine	406	608	476
Dopamine	310	466	450

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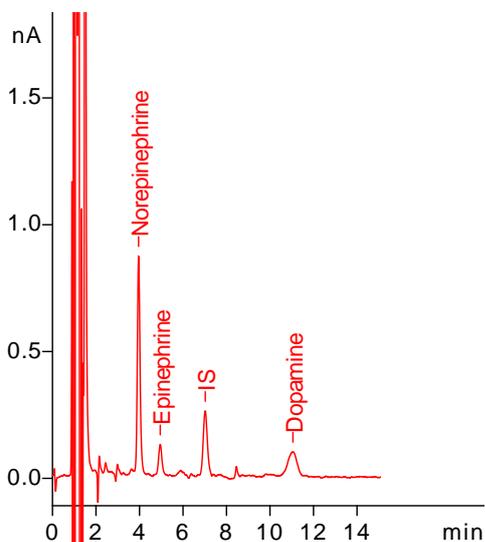*Fig. 2. Chromatograms of 20uL injection of ClinChek® control level II.*

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 then 4%. Only for dopamine, which was present in sample A in a low concentration, the RSD was slightly higher, around 8%.

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

Component	RSD (%)	Conc. (ng/L)
Sample A		
Norepinephrine	2.0	271
Epinephrine	3.3	83
Dopamine	7.7	55
Sample B		
Norepinephrine	2.0	1858
Epinephrine	1.8	476
Dopamine	3.3	450

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].

CONCLUSION

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

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PART NUMBERS AND CONFIGURATIONS	
180.0039C	ALEXYS Clinical Analyzer
110.4105	VT03 3mm GC, salt bridge
RE.1000	ClinRep® complete kit , Catecholamines in plasma
RE.1030	ClinRep® Analytical column
RE.8010	ClinChek® plasma control, level I
RE.8011	ClinChek® plasma control, level II

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