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 Urine

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

Introduction

The quantitative determination of the urinary catecholamines noradrenaline (NA), adrenaline (A), and dopamine (DA) is a rapid and precise diagnostic method for the identification of pheochromocytoma and other tumor diseases of the nervous system such as neuroblastoma and ganglioneuroma. Approximately, half of all pheochromocytoma patients suffer from permanent hypertension, in others episodic hypertensive crises occur. In about 40 % of the latter group plasma catecholamine concentrations are not raised in the interval between two crises. Nevertheless, determination of catecholamine levels in the 24-hours urine allows the detection of pathologically increased values, even after a hypertensive crisis.

Catecholamines in Urine

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 cell	GC type flow cell with Ag/AgCl salt bridge REF
Column	Analytical column for Catecholamines in urine
Flow rate	1.0 mL/min
Sample	20 µL, extracted with sample preparation columns
Mobile phase	HPLC kit buffer
Temperature	D2 SDC 30°C (separation & detection), AS110 4°C (sample cooling)
E-cell	500 mV (vs. Ag/AgCl sat'd)
Range	10 nA/V
I-cell	Ca. 0.2 – 3.0 nA
ADF	0.1 Hz
Analysis time	15 minutes

A kit for catecholamine analysis contains all the necessary chemicals and materials for sample preparation and analysis. Urine samples are processed prior to analysis:

- 3 mL acidified urine sample (10 mL conc. 32% HCl per liter urine) or urine calibrator is mixed with 10 mL stabilizing reagent and 30 µL internal standard (IS) and subsequently adjusted to a pH 3.0 – 7.0 using 0.5M NaOH.
- The mixture is applied to a sample preparation column to trap the catecholamines.
- The column is subsequently washed with 15 mL HPLC-grade water to remove interfering components.
- 6 mL of eluting reagent is then used to elute the catecholamines from the extraction column.
- The eluate is collected, mixed and 20 µL injected in the LC system.

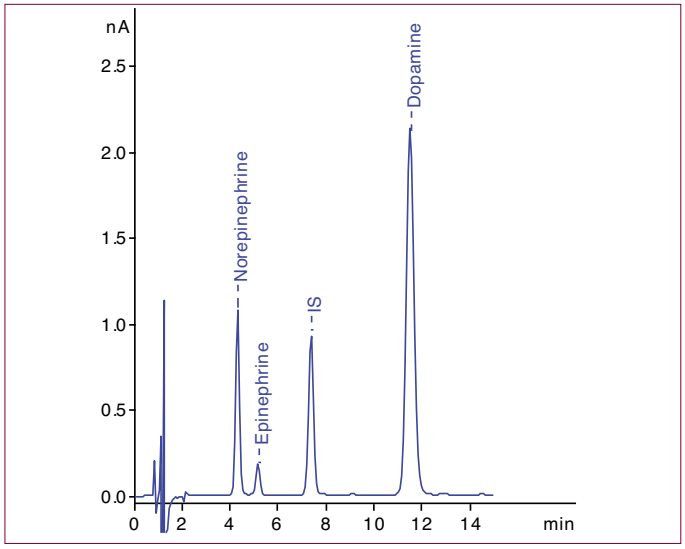

Figure 2: Analysis of 20 µL urine calibrator. Concentration of catecholamines in the calibrator sample: 123 µg/L NA, 29.5 µg/L A and 227 µg/L DA.

The quantification of the catecholamines in the urine samples is performed by means of a single-point calibration method using a urine calibrator. The urine calibrator in the kit consists of lyophilized urine with a known amount of catecholamines. The urine calibrator should be processed exactly the same way as the urine samples. A chromatogram of a urine calibrator analysis is shown in Fig. 2.

An internal standard method is used to compensate for recovery loss during the sample preparation step. To every urine sample, calibrator or control 30 µL of internal standard (IS) solution is added. The IS response of the samples is compared to that of a standard solution (standard) to determine the

recovery. The sample response is then interpolated to 100% recovery to establish the real catecholamine concentration in the urine samples.

Analysis of controls

For validation of the analytical method 'urine controls' have been analyzed in both the normal (level I) and the pathological range (level II).

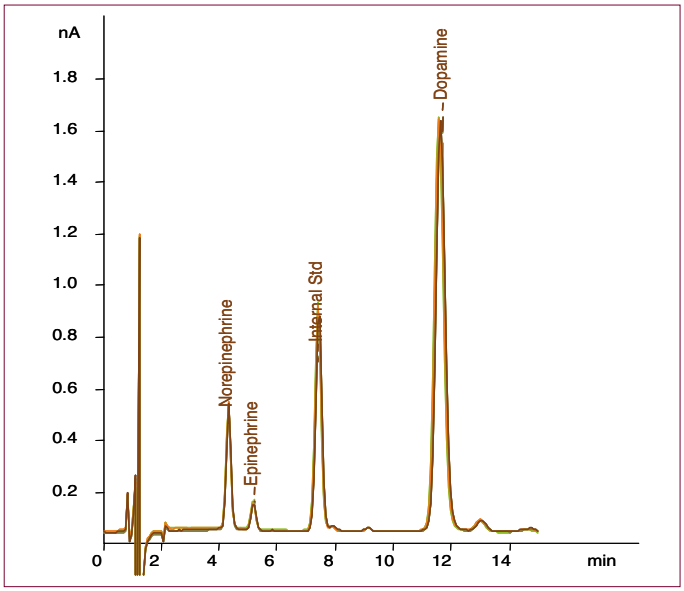

Figure 3: Overlay of 6 chromatograms of 20 µL injections of control level I.

The control samples are lyophilized urine samples which have to be processed in the same way as the urine samples. Both Control I and Control II were analyzed and the analyte concentrations quantified using the urine calibrator. For both urine controls level I and II the determined concentrations were within the concentration ranges specified by on the urine control data sheet (see table 2).

Table 2

Measured concentration of urine controls level I and II				
Component	*Specified (µg/L)*		*Specified (µg/L)*	*RSD (%)*
	Min	*Max*		
Control level I				
NA	44	66	58.7	0.8
A	14	21	18.7	0.8
DA	120	180	176.6	0.8
Control level II				
NA	125	187	156.9	0.2
A	29	43	35.1	1.5
DA	186	278	236.9	0.5

Measured concentration of urine controls level I and II (n=6). Concentration range specified is given for reference (source: data sheet supplied with controls).

Analysis of urine samples

Urine samples were collected from an apparently healthy volunteer and analyzed multiple times to determine the recoveries, LOD, intra- and inter-assay precision of the method. The intra-assay precision of the method was determined using two urine samples (A and B). The urine samples were worked-up 5 times and duplicate analysis were performed to determine the relative standard deviation (RSD, %).

Table 3

Intra-assay precision of urine sample A and B		
Component	*RSD (%)*	*Conc. (µg/L)*
Sample A		
NA	2.8	30.6
A	1.7	16.0
DA	3.7	102.5
Sample B		
NA	2.5	20.7
A	14	3.6
DA	2.3	115.2

Intra-assay precision of urine sample A and B, n= 5 (samples) x 2 (duplicate injections).

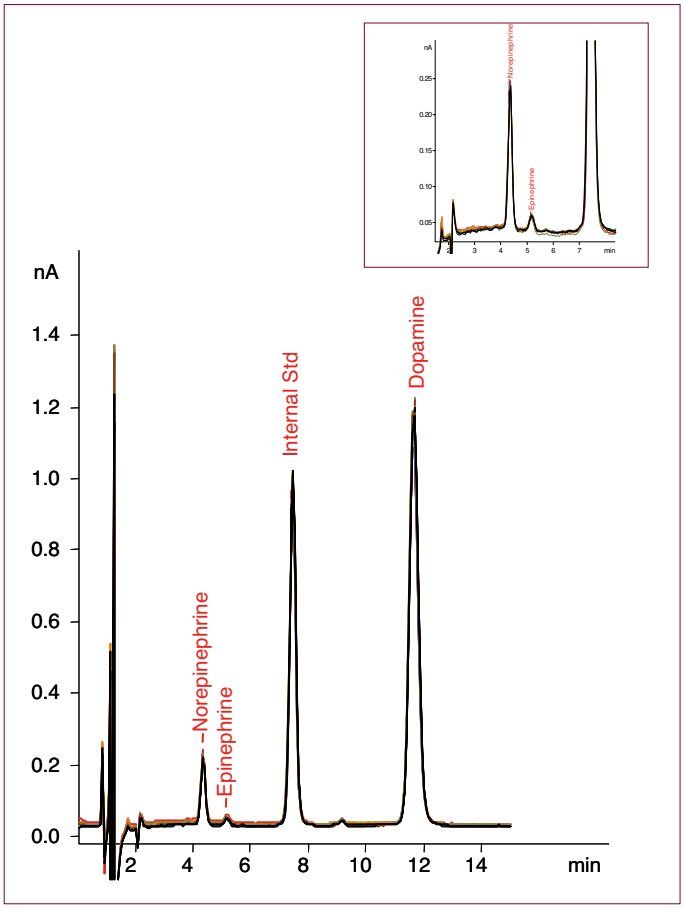

Figure 4: Overlay of 10 chromatograms of 20 µL injections of urine sample B. Zoom in on NA and A peaks.

The RSD's for all components were typically smaller than 4%. Only for low concentrations of adrenaline, near the limit of quantitation, a RSD of 14 % was found.

For all urine samples, controls and calibrator recoveries typically in the range of 85 – 95% were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 1 µg/L for all catecholamines. The CLOD here is 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 the catecholamines in the concentration range from 1 – 1000 µg/L [6].

To determine the inter-assay precision a urine sample (C) was worked-up 4 times and analyzed (duplicate injection), this procedure was repeated the next day and the relative standard deviation calculated.

Table 4

Inter-assay precision		
Component	*RSD (%)*	*Conc. (µg/L)*
Sample C		
NA	3.8	48.7
A	6.2	5.1
DA	3.2	225.1

Inter-assay precision (urine sample C, n= 4 (samples) x 2 (duplicate injections) x 2 (days).

The RSD's for noradrenaline and dopamine were smaller than 4%. For adrenaline, which was present in the sample in a significantly lower concentration, the RSD was slightly higher, 6.2%.

References

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Conclusion

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

PART NUMBERS AND CONFIGURATIONS

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