Application Note
Clinical & Diagnostics

Analysis of Catecholamines in Urine and Plasma

The most selective LC-EC applications for Clinical & Diagnostics analysis

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

- **ALEXYS™ Clinical Analyzer for best compatibility with any clinical kit**
- **Standardized, robust and reliable assay**
- **Highest sensitivity due to proprietary SenCell™**

Summary

For the analysis of catecholamines and their metabolites in urine or plasma, the ALEXYS™ Clinical Analyzer based on HPLC with electrochemical detection (LC/ECD) provides a robust and sensitive method. The ALEXYS Analyzer is compatible with all major clinical kits for sample preparation and analysis to assure standardized catecholamines analysis.

In Part I of this application note the analysis of chatecolamines in urine is described, and in Part II the analysis in plasma.

Part I: Catecholamines in Urine

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.

Figure 1: ALEXYS Clinical Analyzer.

Method

The ClinRep®kit of Recipe was used for the analysis of catecholamines. It contains all 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.

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

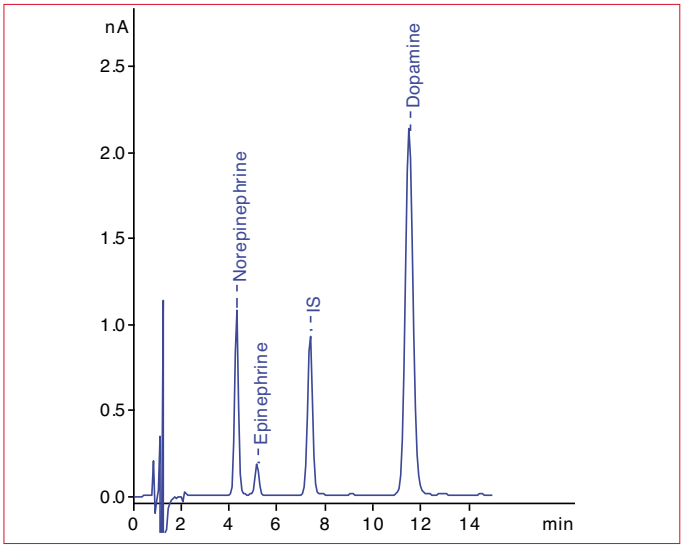

Figure 2: Analysis of 20 µL urine calibrator. Concentration of catecholamines in the calibrator sample: 123 µg/L NA, 29.5 µg/L A and 227 µg/L DA.

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.

Results

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).

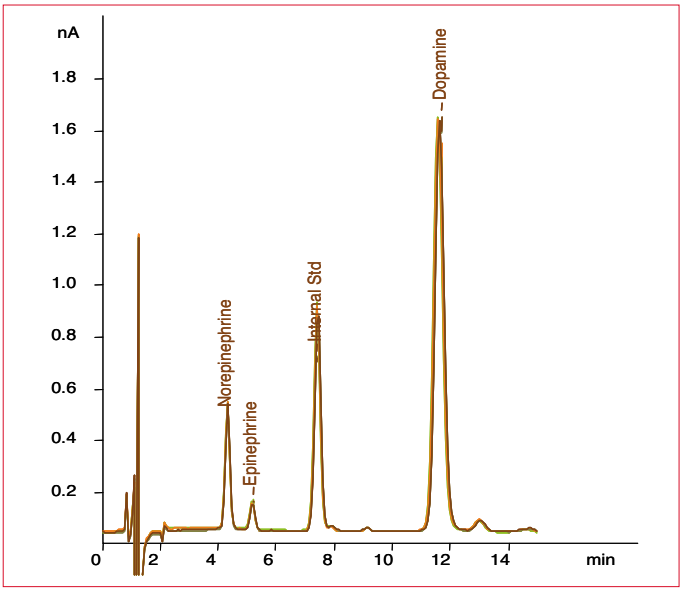

Figure 3: Overlay of 6 chromatograms of 20 µL injections of control level I.

The control samples are lyophilized urine samples which have to be processed in the same way as the urine samples. Both control level I and control level 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).

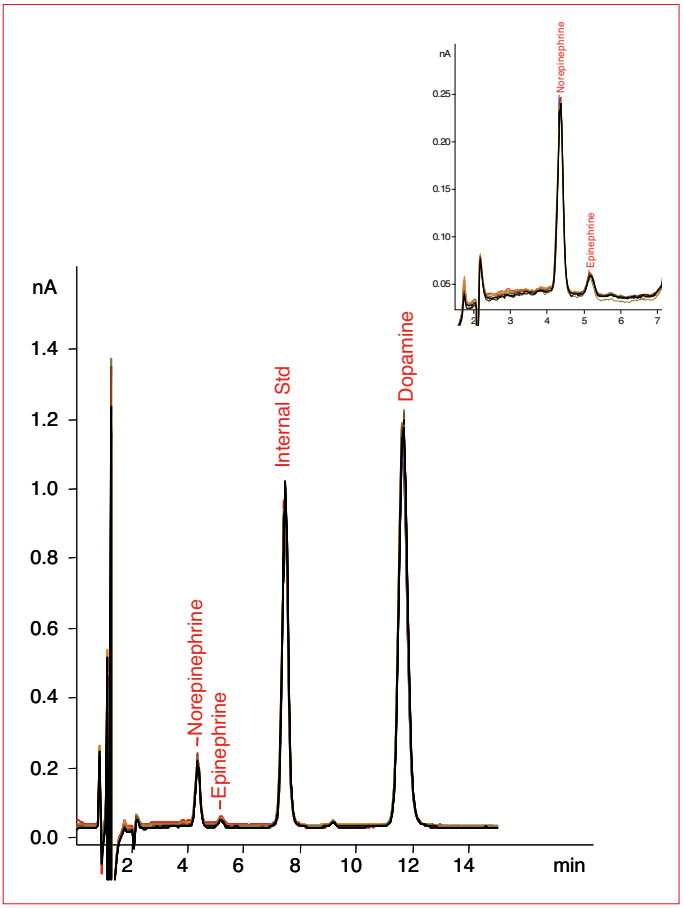

Figure 4: Overlay of 10 chromatograms of 20 µL injections of urine sample B. Zoom in on NA and A peaks.

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 [1].

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

1. Recipe, Instruction manual for catecholamine in urine, version 3.4 (2005)

Ordering information

180.0039W	ALEXYS Clinical Analyzer

Recipe ClinRep kits are availabe from Antec Scientific (USA)

2000-AS	ClinRep kit for analysis of catecholamines in urine
2030-AS	Analytical column

Conclusion

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

Part II: Catecholamines in Plasma

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].

Figure 1: ALEXYS Clinical Analyzer.

Method

The ClinRep®kit of Recipe was used for the analysis of catecholamines in plasma. It contains all 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 [7].

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 and column	ClinRep kit buffer (recycled during experiments) and column (Recipe)*
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

* Available from Antec Scientific (USA)

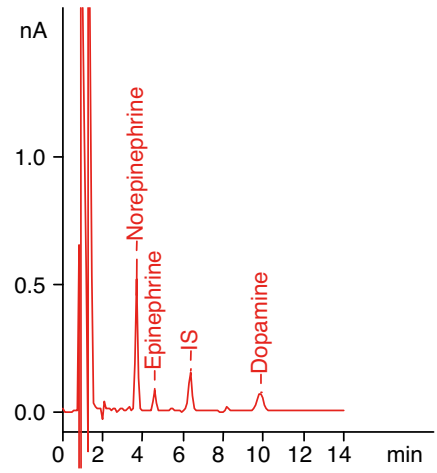

Figure 2: Analysis of 20 µL plasma calibrator. Concentration of catecholamines in the calibrator sample: 1.19 µg/L NA, 275 ng/L A and 212 ng/L DA.

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 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).

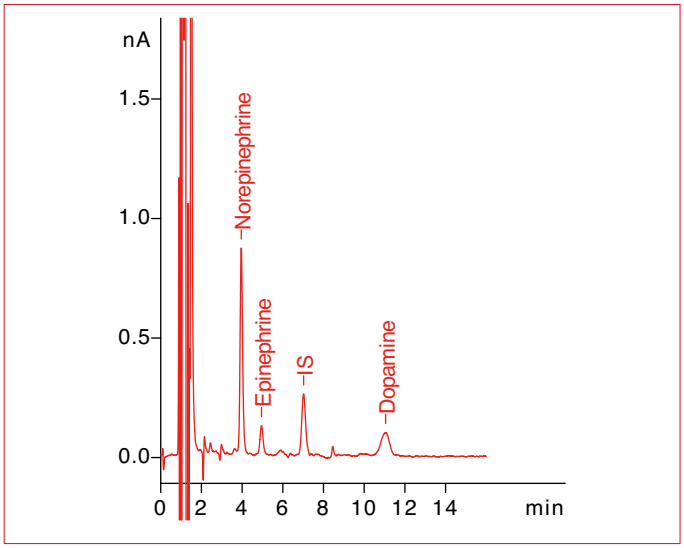

Figure 3: Chromatograms of 20 µL injection of control level II.

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)

Conclusion

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

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 [7].

References

1. L. Thomas, Labor und Diagnose, 5. Auflage, TH-Books, Verlagsgesellschaft, Frankfurt/Main 1998, S. 1062 - 1075.
2. R.W. Gifford, W.M. Manger, E.L. Bravo, *Endocrinol. Metab. Clin. North. Am.*, 23(2), (1994) 387-404.
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4. M. Walther, H.R. Keiser, W.M. Linehan, *World J. Urol.* „ 17(1), (1999) 35-39
5. T.G. Rosano, T.A. Swift, L.W. Hayes, *Clin. Chem.* , 37(10/2) , (1991) 1854-1867
6. EL. Bravo, R.W. Gifford, *N. Engl. J. Med.*, 31(20), (1984) 1298-1303
7. Recipe, Instruction manual for catecholamine in plasma, version 3.2 (2006)

Ordering information

180.0039W	ALEXYS Clinical Analyzer

Recipe ClinRep kits are availabe from Antec Scientific (USA)

1000-AS	ClinRep kit for analysis of catecholamines in plasma
1030-AS	Analytical column

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