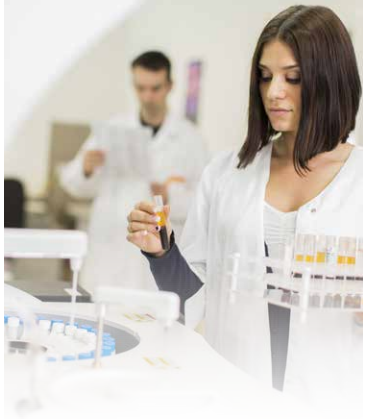
Application Note
Clinical & Diagnostics



Metanephrines in Urine

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

- **Standardized, fast and reliable assay**
- **Kit for standardized sample prep**
- **Robust and 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 [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.

Introduction

Catecholamines exert numerous physiological actions in the cardio-vascular system and in the intermediary metabolism. Various tumors of neurogenic origin are responsible for a substantial rise in catecholamine production [1]. Catecholamines are primarily inactivated (up to 90 %) by the re-uptake into the adrenergic nerve endings. The remaining catecholamines are metabolized in the cells of the target organs or in the liver. Catecholamine metabolism by catechol-o-methyltransferase results in the formation of the methoxy analogues nor-metanephrine (N-Meta), metanephrine (Meta) and 3-methoxytyramine (3-Meth). The metabolites are released into the blood stream and excreted mainly by the kidney. The quantitative determination of the catecholamines and their metabolites is of great clinical significance for the diagnosis and treatment of neurogenic tumors [2-8].

Figure 1: ALEXYS Clinical Analyzer.

Method

A complete kit contains all the necessary chemicals and materials for sample preparation. Prior to analysis the urine samples are first acid hydrolyzed to free the conjugated metanephrines followed by a sample clean-up step on an extraction column. The sample preparation procedure can be summarized as follows:

- Acidified urine is mixed with 20 µL internal standard (IS) and hydrolyzed for 30 minutes.
- After hydrolyses the sample is diluted and applied to a sample preparation column to trap the unconjugated metanephrines.
- The column is then washed followed by elution of the metanephrines and 20 µL injection in the LC system.

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow rate	1.0 mL/min
Sample	20 µL, extracted with sample preparation columns
Mobile phase	HPLC kit buffer (recycled during experiments)
Temperature	D2 SDC 30°C (separation & detection), AS110: 4°C (sample cooling)
E-cell	720 mV (vs. Ag/AgCl sat'd)
Range	50 nA/V
I-cell	0.1 – 3.0 nA
ADF	0.1 Hz
Analysis time	20 minutes

The quantification of the metanephrines in the urine samples is performed by means of a single-point calibration method using a urine calibrator. The urine calibrator supplied in the kit is a lyophilized urine sample with a known amount of metanephrines. The urine calibrator should be processed the same way as the urine samples. An example chromatogram of a urine calibrator analysis is shown in figure 2.

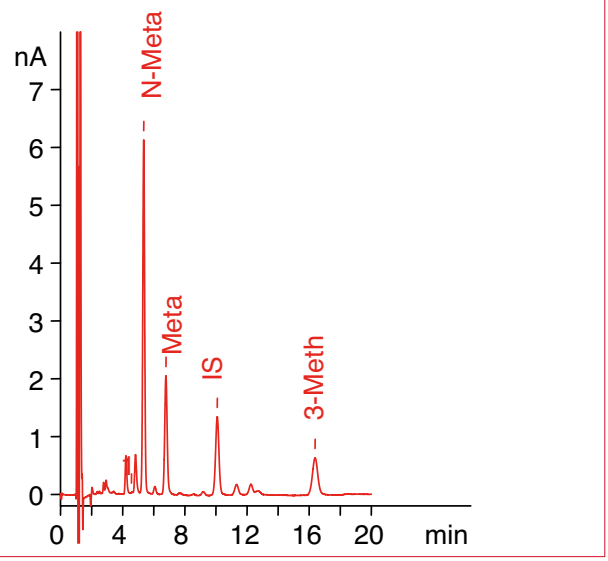


Figure 2: Analysis of 20 µL urine calibrator. Concentration of metanephrines in the calibrator sample: 930 µg/L N-Meta, 530 µg/L Meta and 181 µg/L 3-Meth.

The IS is used to compensate for recovery losses during the sample preparation step. The IS response of the samples is compared to that of a standard solution to determine the recovery. The sample response is then interpolated to 100% recovery to establish the real metanephrine concentration in the urine samples. For sample preparation (hydrolysis and extraction) a water bath, pH meter, vortex mixer, hydrolysis tubes and column rack were used.

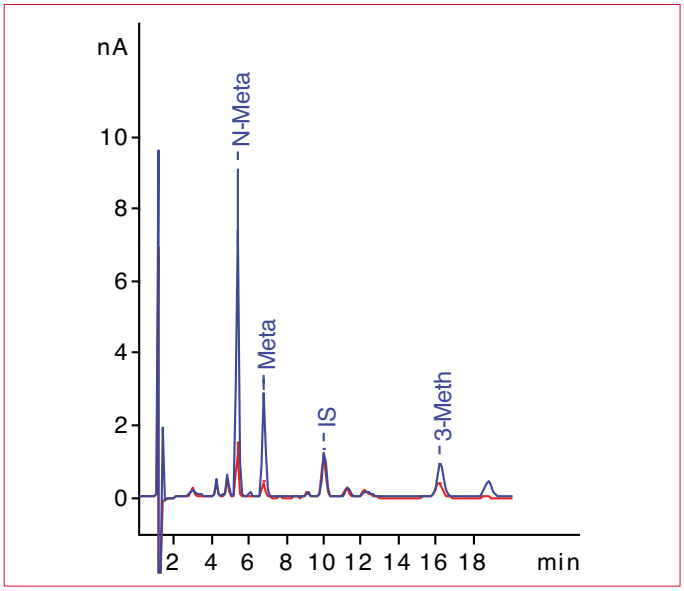


Figure 3: Overlay of chromatograms of 20 µL injections of control level I and II.

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). 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 metanephrine concentrations were within the concentration ranges specified 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				
Nor-Meta	238	358	269	0.8
Meta	112	168	137	0.5
3-Meth	110	164	132	2.0
Control level II				
Nor-Meta	1222	1832	1414	0.1
Meta	722	1084	839	0.3
3-Meth	222	332	275	1.4

Measured concentration of urine controls level I and II (n=4). Concentration range specified is given for reference (source: data sheet supplied with controls).

Analysis of urine samples

A urine sample (A) was 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 urine sample A. The urine sample was worked-up 5 times on two different days and duplicate analysis were performed to determine the relative standard deviation (RSD, %).

Table 3

Intra-assay precision of urine sample A

Component	*RSD (%)*	*Conc. (µg/L)*
Day 1		
Nor-Meta	3.0	451
Meta	3.9	201
3-Meth	4.5	220
Day 2		
Nor-Meta	2.4	502
Meta	4.1	231
3-Meth	4.1	216

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

The intra-assay RSD's for all components were typically smaller than 5 %. For all urine samples, controls and calibrator recoveries typically in the range of 50 – 80% were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 0.5 µg/L for all metanephrines.

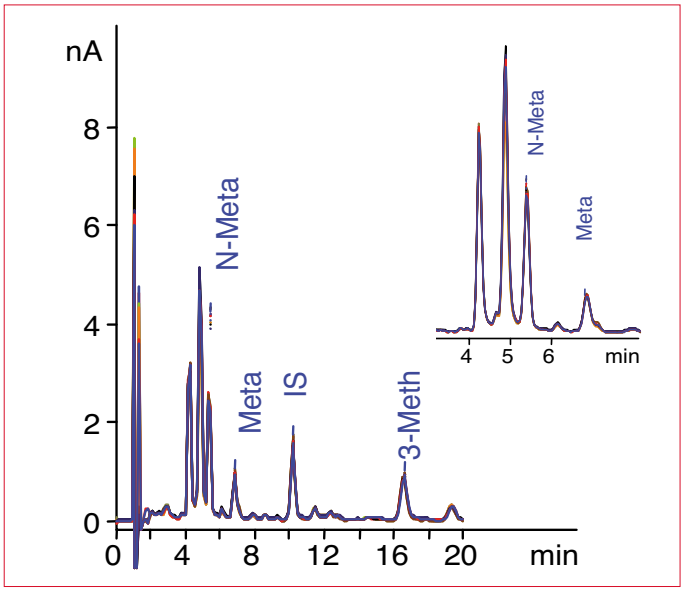


Figure 4: Overlay of 10 chromatograms of 20 µL injections of urine sample A. Top-right: zoom in on N-Meta and Meta peaks.

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 metanephrines in the concentration range from 5 – 7500 µg/L [18]. To determine the inter-assay RSD's the results of two days were averaged for sample A, see table 4.

Table 4

Inter-assay precision of urine sample A

Component	*RSD (%)*	*Conc. (µg/L)*
Sample A		
Nor-Meta	6.1	476
Meta	7.9	216
3-Meth	3.4	217

Inter-assay precision of urine sample A, n= 5 (samples) x 2 (duplicate injections) x 2 (days).

The inter-assay RSD's for the metanephrines were typically smaller than 8 %.

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 and metabolites.

Ordering information

180.0039W	ALEXYS Clinical Analyzer

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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