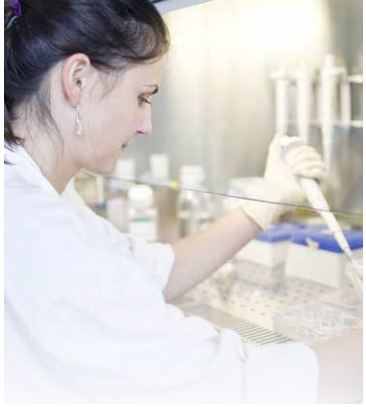
Application Note
Clinical & Diagnostics



HVA, VMA and 5-HIAA 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

The catecholamines noradrenaline, adrenaline and dopamine exercise a number of important functions within the central and peripheral nervous system [1]. Vanillylmandelic acid (VMA) is the major end product of catecholamine metabolism; homovanillic acid (HVA) is the analogue end product of dopamine. Serotonin, another biogenic amine, is mainly located in the enterochromaffin cells of the small intestine. Biochemically, serotonin is degraded by the enzymes monoaminooxidase (MAO) and aldehydedehydrogenase (Ald-DH) to 5-hydroxyindoleacetic acid (5-HIAA).

The analysis of plasma and urinary catecholamines and their metabolites is crucial for the detection and diagnosis of chromaffin cell tumors and a number of other diseases [3-6].

Figure 1: ALEXYS Clinical Analyzer.

Method

A complete kit contains all the necessary chemicals and materials for sample preparation and analysis [14]. Prior to analysis a sample clean-up step is applied using a sample preparation column. The sample preparation procedure consists of the following steps:

- 250 µL urine sample is mixed with 50 µL internal standard (IS) and diluted to a volume of 5 mL.
- 1 mL of the diluted urine is applied to the sample preparation column to trap the acidic metabolites.
- The column is subsequently washed with ammonium solution, followed by a boric acid solution.
- Finally 2 mL of eluting agent is applied to the sample preparation column and 20 µL of the eluate is injected into the LC system for analysis.

Table 1

Set-up	
HPLC	ALEXYS Clinical Analyzer
Flow rate	0.9 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	850 mV (vs. Ag/AgCl sat'd)
Range	50 nA/V
I-cell	2 – 20 nA
ADF	0.1 Hz
Analysis time	18 minutes

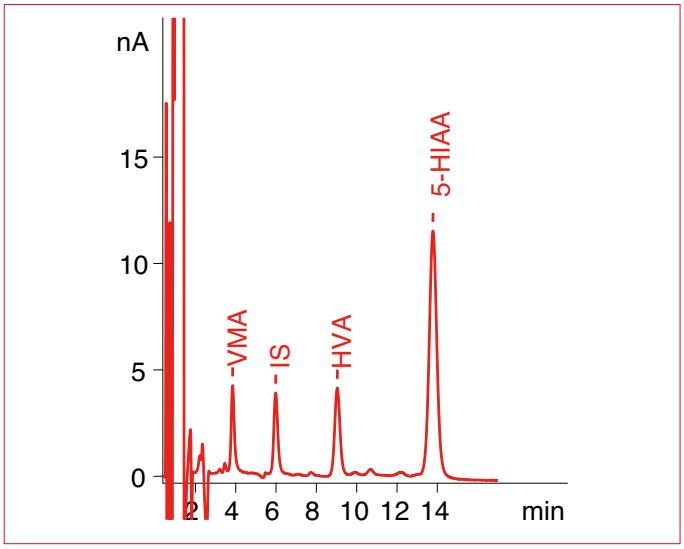


Figure 2: Analysis of 20 µL urine calibrator reconstituted in 0.2M HCl. Concentration of acidic metabolites in the sample: 11 mg/L VMA, 10.2 mg/L HVA and 16.7 mg/L 5-HIAA.

The quantification of the acidic metabolites in the urine samples is performed by means of a single-point calibration method using a urine calibrator. The urine calibrator is a lyophilized urine sample with a known amount of HVA, VMA and 5-HIAA. 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. The lyophilized calibrator was reconstituted in 0.2 M HCl.

An internal standard (IS), iso-VMA, 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 interpolated to 100% recovery to establish the real concentration of HVA, VMA and 5-HIAA in the urine samples. For sample preparation a pH meter, vortex mixer and centrifuge (800 x g) are required.

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).

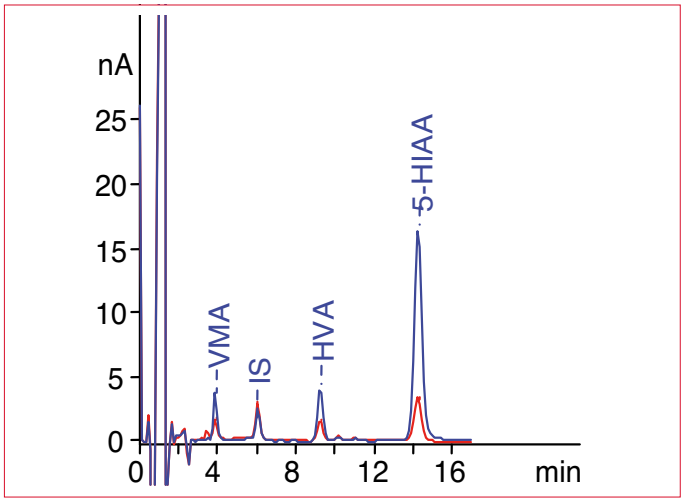


Figure 3: Overlay of chromatograms of 20 µL injections of control level I (red curve) and II (blue curve).

Table 2

Measured concentration of urine controls level I and II

Component	*Specified (mg/L)*		*Specified (mg/L)*	*RSD (%)*
	Min	*Max*		
Control level I				
VMA	4.4	6.6	6.5	0.1
HVA	4.0	6.1	4.6	0.1
5-HIAA	4.1	6.9	5.0	0.1
Control level II				
VMA	13.2	19.8	16.4	0.2
HVA	12.2	18.2	14.1	0.2
5-HIAA	21.0	31.4	28.9	0.6

Measured concentration of urine controls level I and II (n=2). Concentration range specified is given for reference (source: data sheet supplied with controls).

The control samples (lyophilized urine) have been processed in the same way as the urine samples. Both Control I and Control II were reconstituted in 0.2 M HCl, analyzed and the analyte concentrations quantified using the urine calibrator. For both urine controls level I and II the measured concentrations were within specification (see table 2).

Analysis of urine samples

A urine sample (A) was collected from an apparently healthy volunteer and analyzed multiple times to determine the recoveries, LOD and intra-assay precision of the method. 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. (mg/L)*
Day 1		
VMA	8.3	5.5
HVA	3.5	4.9
5-HIAA	2.1	8.5
Day 2		
VMA	4.6	6.2
HVA	3.3	5.4
5-HIAA	2.7	7.7

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

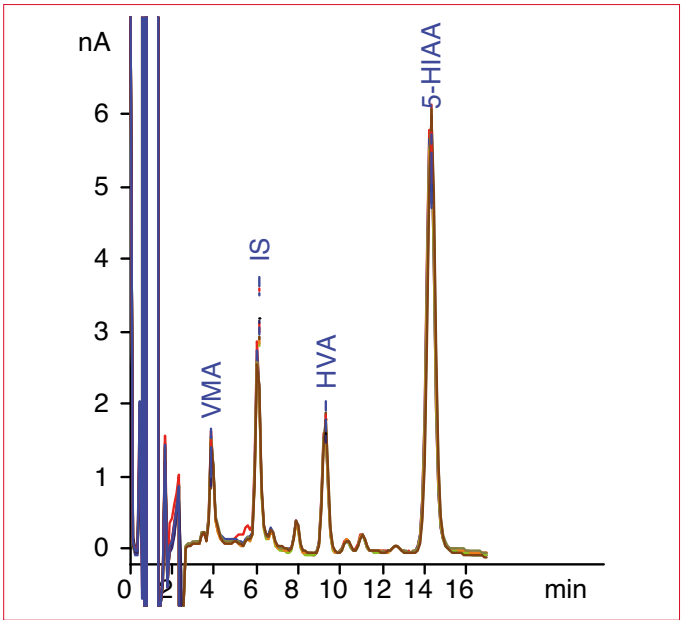


Figure 4: Overlay of 6 chromatograms of 20 µL injections of urine sample A on day 2.

The intra-assay RSD's for HVA and 5-HIAA were typically smaller than 4%. The RSD for VMA was larger on day 1. This due to an interfering peak in this urine sample, which complicated peak integration.

For all urine samples, controls and calibrator recoveries typically in the range of 55 – 75% were found, compared to a standard. The concentration limit of detection (CLOD) for the method was approximately 20 µg/L for all metabolites. 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 HVA, VMA and 5-HIAA in the concentration range from 0.1 – 300 mg/L [from ref. 18].

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 urinary catecholamine metabolites.

Ordering information

180.0039W	ALEXYS Clinical Analyzer

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