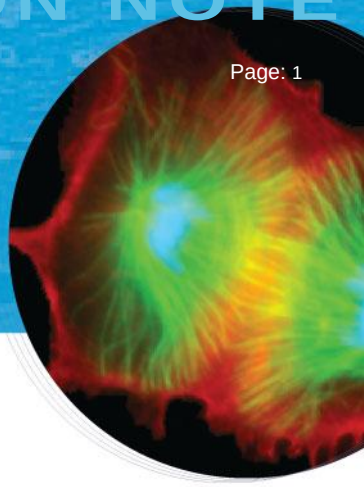
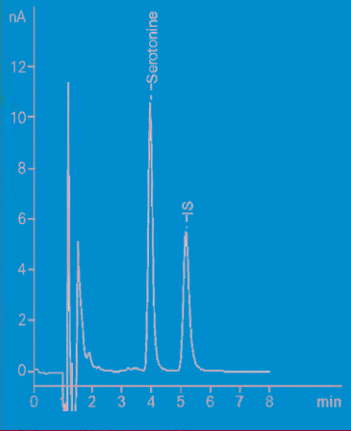
HVA, VMA AND 5-HIAA IN URINE



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

The catecholamines norepinephrine, epinephrine 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 enterochromaffine 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 tumours and a number of other diseases [3-6].

- Urine kit, including sample prep
- Standardized, fast and reliable assay
- 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.

Method

One Recipe ClinRep® complete kit contains all the necessary chemicals and (calibration) materials for sample preparation and analysis of 100 assays [14]. Prior to analysis a sample clean-up step is applied to the urine samples using a ClinRep® 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 a ammonium solution, followed by two washing steps with 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.

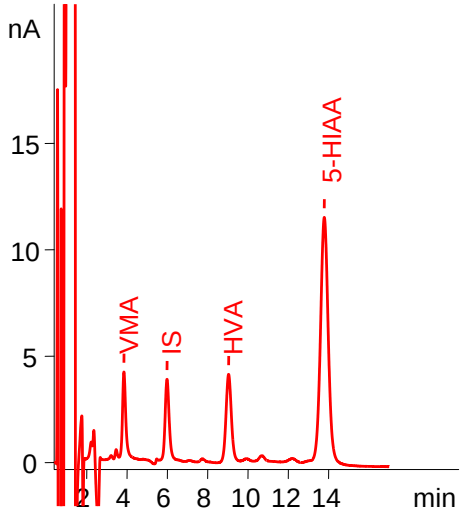


Fig. 1 Analysis of 20 µL ClinCal® urine calibrator reconstituted in 0.2M HCl. Concentration of acidic metabolites in the sample: 11 mg/L VMA, 10.2 mg/L HVA and 16.7 mg/L 5-HIAA.

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 ClinCal® urine calibrator supplied in the ClinRep® kit is a lyophilised urine sample with a known amount of HVA, VMA and 5-HIAA. The urine calibrator should be processed via the same sample preparation method as the urine samples. An example chromatogram of a urine calibrator analysis is shown in figure 1. 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 directly injected standard solution (ClinTest® standard) to determine the recovery. The sample response is then interpolated to 100% recovery to establish the real concentration of HVA, VMA and 5-HIAA in the urine 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 HVA, VMA and 5-HIAA in urine

Furthermore, for sample preparation a pH meter, vortex mixer, and centrifuge (800 x g) are required.

Analysis of ClinChek® controls

For quality control of the analytical determination Recipe ClinChek® urine controls have been used in both the normal (level I) and the pathological range (level II).

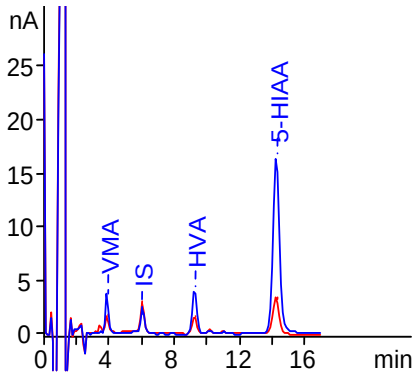


Fig. 2. Overlay of chromatograms of 20µL injections of ClinChek® control level I (red curve) and II (blue curve).

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

Component	Specified conc (mg/l)		Calculated conc (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

The control samples are lyophilised urine samples which have to be processed in the same way as the urine samples. Both Control I and Control II were reconstituted in 0.2 M HCl, analysed and the analyte concentrations quantified using the ClinCal urine calibrator. For both urine controls level I and II the determined concentrations of VMA, HVA and 5-HIAA were within the concentration ranges specified by Recipe on the urine control data sheet (see table I).

Analysis of urine samples

A urine sample (A) was collected from an apparently healthy volunteer and analysed 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 II. Intra-assay precision of urine sample A, n= 5 (samples) x 2 (duplicate injections) for two days.

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

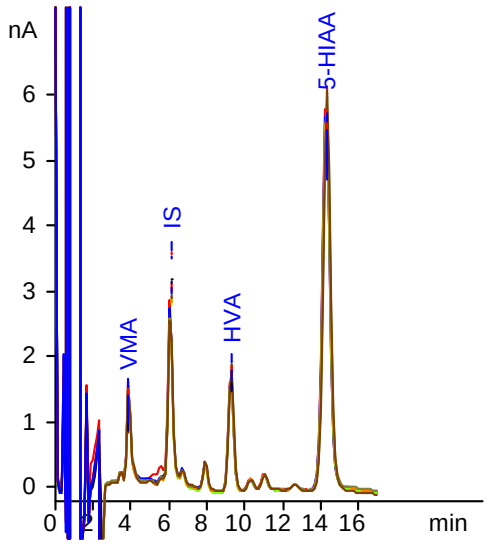


Fig. 3. Overlay of 6 chromatograms of 20uL injections of urine sample A on day 2.

The intra-assay RSD's for HVA and 5-HIAA were typically smaller then 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 directly injected standard. The concentration limit of detection (C_{LOD}) for the method was approximately 20 µg/L for all metabolites. The C_{LOD} 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].

CONCLUSION

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

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PART NUMBERS AND CONFIGURATIONS	
180.0039C	ALEXYS Clinical Analyzer
110.4105	VT03 3mm GC, salt bridge
RE.3000	ClinRep® complete kit , HVA, VMA and 5-HIAA in
RE.3030	ClinRep® Analytical column
RE.8021	ClinChek® urine control, level I
RE.8022	ClinChek® urine control, level II

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