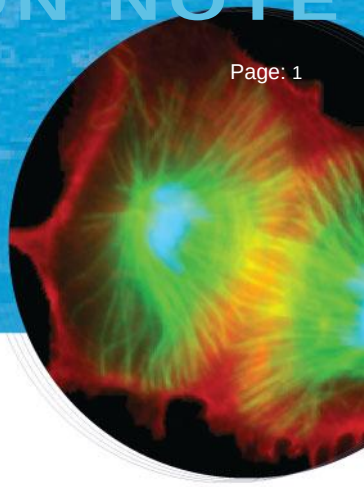
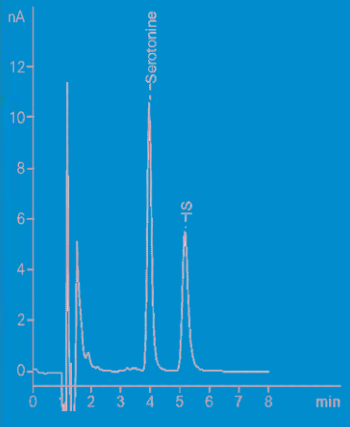
METANEPHRINES IN URINE



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

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 metabolised 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 normetanephrine (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].

- Standardized, fast and reliable assay
- Kit for standardized sample prep
- 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. Prior to analysis the urine samples are first acid hydrolysed 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 ClinRep® sample preparation column to trap the unconjugated methanephrines.
- The column is then washed followed by elution of the metanephrines and 20 µL injection in the LC system.

The quantification of the metanephrines 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 metanephrines. 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.

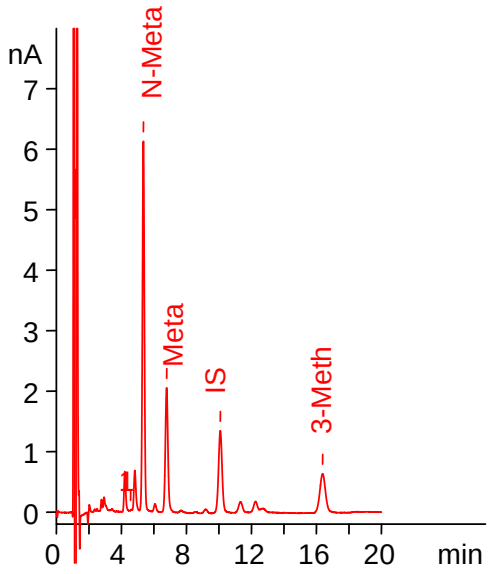


Fig. 1 Analysis of 20 µL ClinCal® urine calibrator. Concentration of metanephrines in the calibrator sample: 930 µg/L N-Meta, 530 µg/L Meta and 181 µg/L 3-Meth.

An 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 directly injected standard solution (ClinTest® standard) to determine the recovery. The sample response is then interpolated to 100% recovery to establish the real metanephrine concentration 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 metanephrines in urine

Furthermore, for sample preparation (hydrolysis and extraction) a water batch, pH meter, vortex mixer, hydrolysis tubes and column rack 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).

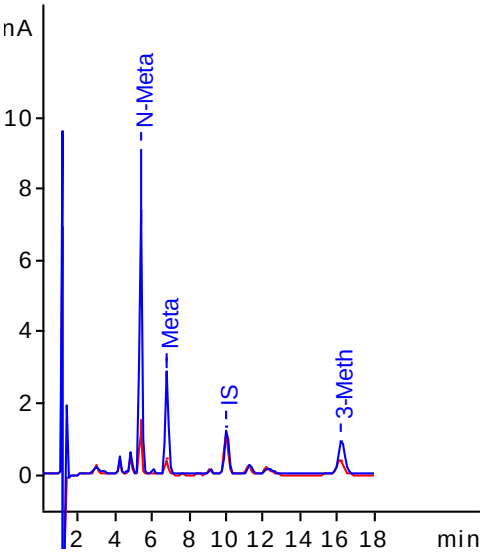


Fig. 2. Overlay of chromatograms of 20 µL injections of ClinChek® control level I and II.

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 analysed and the analyte concentrations quantified using the ClinCal urine calibrator. For both urine controls level I and II the determined metanephrine concentrations were within the concentration ranges specified by Recipe on the urine control data sheet (see table I).

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

Component	Specified conc (µg/l)		Calculated conc (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

Analysis of urine samples

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

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

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

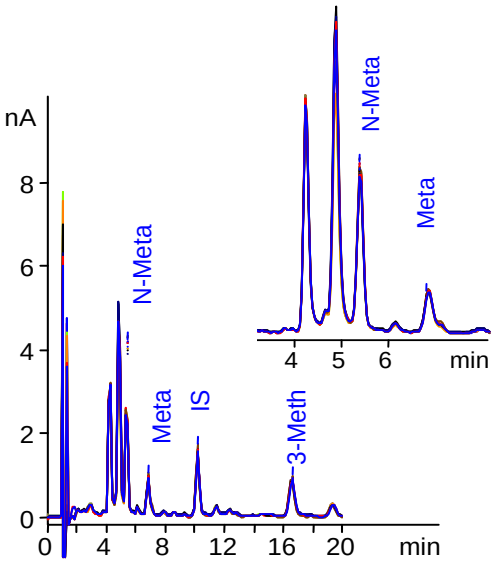


Fig. 3. Overlay of 10 chromatograms of 20uL injections of urine sample A. Top-right: zoom in on N-Meta and Meta A peaks.

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 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 III.

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

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

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

CONCLUSION

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

PART NUMBERS AND CONFIGURATIONS	
180.0039C	ALEXYS Clinical Analyzer
110.4105	VT03 3mm GC, salt bridge
RE.4000	ClinRep® complete kit , Metanephrines in urine
RE.4030	ClinRep® Analytical column
RE.8021	ClinChek® urine control, level I
RE.8022	ClinChek® urine control, level II

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