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ClinRep® kit for the analysis of Catecholamines in Urine

Introduction

The catecholamines norepinephrine (NA), epinephrine (A) and dopamine (DA) are synthesised in the chromaffin cells of the adrenal medulla and the sympathetic nervous system and play an important part as neurotransmitters and in metabolic regulation. Tumors of the sympathoadrenal system cause an elevation in production and in urinary excretion of catecholamines and their metabolites.

The quantitative determination of urinary catecholamines 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 rest episodic hypertensive crises occurs. 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.

Today, LC-EC has been established as a fast and reliable method for the determination of catecholamines and metabolites in plasma and urine [1-5].

The ClinRep® complete kit for catecholamines in urine of Recipe GmbH (Munich, Germany) is a standardised kit for the sample preparation and routine analysis of urinary catecholamines. In this application note the results are reported from analysing urine samples using a ClinRep® kit with an ALEXYS 110 LC-EC system.

Method

One Recipe ClinRep® complete kit contains all the necessary chemicals and (calibration) materials for sample preparation and analysis of 100 assays, excluding the analytical column. Urine samples are processed using the following sample preparation procedure prior to analysis:

- 3 mL acidified urine sample (10 mL conc. 32% HCl per liter urine) or urine calibrator is mixed with 10 mL stabilising reagent S 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 ClinRep® sample preparation columns to trap the catecholamines present in the sample.
- The column is subsequently washed with 15 mL HPLC-grade water to remove interfering components.
- 6 mL of eluting reagent E is then used to elute the catecholamines from the extraction column.

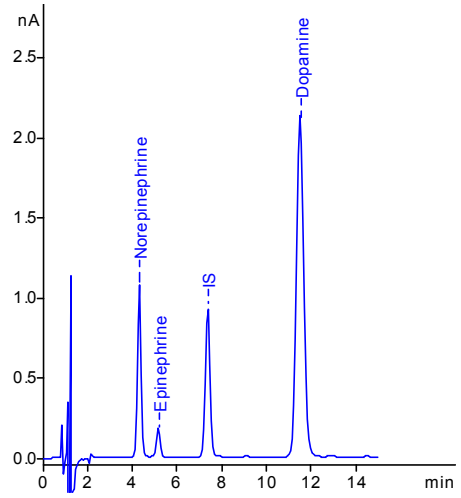

Fig. 1 Analysis of 20 µL ClinCal® urine calibrator. Concetration of catecholamines in the calibrator sample: 123 µg/L NA, 29.5 µg/L A and 227 µg/L Dopamine.

- The eluate is collected, mixed and 20 µL injected in the LC system.

The quantification of the catecholamines 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 catecholamines. 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.

An internal standard method is used to compensate for recovery losses 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 directly injected standard solution (ClinTest® standard) to determine the recovery. The sample response is then interpolated to 100% recovery to establish the real catecholamine concentration in the urine samples.

Set-up

HPLC	ALEXYS 110 LC-EC system with DECADE II SDC (p/n 190.0035)
Flow cell	GC type flow cell with Ag/AgCl saltbridge REF
Column	ClinRep® Analytical column for catecholamines in urine

LC-EC conditions

Flow rate	1.0 mL/min
Sample	20 µl, extracted with ClinRep® sample preparation columns
Mobile phase	ClinRep® catecholamine buffer#
Temperature*	$T_{D2\ SDC}$ 30°C (separation & detection), T_{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

#) mobile phase was recycled during experiments. *) minimum actual oven & tray temperature which can be reached is dependent on ambient conditions.

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).

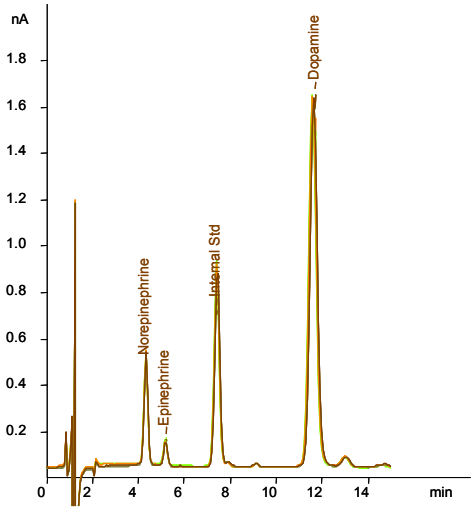

Fig. 2. Overlay of 6 chromatograms of 20uL injections of ClinChek® control level I.

Table I. Calculated concentration of urine controls level I and II (n=6). Concentration range specified by Recipe is given for reference (source: data sheet supplied with controls).

Component	Specified conc (µg/l)		Calculated	RSD
	Min	Max	conc (µg/l)	(%)
Control level I				
Noradrenaline	44	66	58.7	0.8
Adrenaline	14	21	18.7	0.8
Dopamine	120	180	176.6	0.8
Control level II				
Noradrenaline	125	187	156.9	0.2
Adrenaline	29	43	35.1	1.5
Dopamine	186	278	236.9	0.5

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 concentrations were within the concentration ranges specified by Recipe on the urine control data sheet (see table I).

Analysis of urine samples

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

Component	RSD (%)	Conc. (µg/l)
Sample A		
Norepinephrine	2.8	30.6
Epinephrine	1.7	16.0
Dopamine	3.7	102.5
Sample B		
Norepinephrine	2.5	20.7
Epinephrine	14	3.6
Dopamine	2.3	115.2

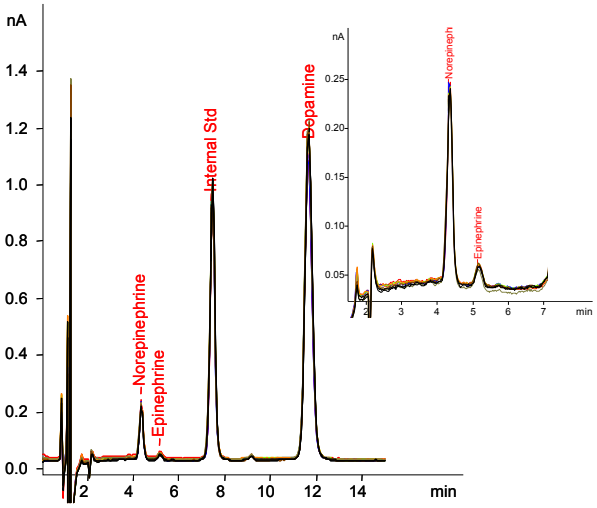

Fig. 3. Overlay of 10 chromatograms of 20uL injections of urine sample B. Top-right: zoom in on NA and A peaks.

The RSD's for all components were typically smaller then 4%. Only for low concentrations of epinephrine, 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 directly injected standard. The concentration limit of detection (C_{LOD}) for the method was approximately 1 µg/L for all cate-

cholamines. 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 catecholamines in the concentration range from 1 – 1000 µg/L [6].

To determine the inter-assay precision an urine sample (C) was worked-up 4 times and analysed (duplicate injection), this procedure was repeated the next day and the relative standard deviation calculated.

Table III. Inter-assay precision (urine sample C, n= 4 (samples) x 2 (duplicate injections) x 2 (days).

Component	RSD (%)	Conc. (µg/l)
Sample C		
Norepinephrine	3.8	48.7
Epinephrine	6.2	5.1
Dopamine	3.2	225.1

The RSD's for norepinephrine and dopamine were smaller then 4%. For epinephrine, which was present in the sample in a significantly lower concentration, the RSD was slightly higher, 6.2%.

Conclusion

The ClinRep® complete kit for catecholamines in urine provides a standardised method for the sample preparation of urine samples and fast & reliable analysis of urinary catecholamines using LC-EC.

Parts and configuration used

190.0035#	ALEXYS 110 LC-EC system with DECADE II SDC
2000*	ClinRep® complete kit , Catecholamine in urine
2030*	ClinRep® Analytical column for catecholamines in urine
8021*	ClinChek® urine control, level I
8022*	ClinChek® urine control, level II

#) A GC-type flow cell with Ag/AgCl saltbridge REF should be ordered separately.
*) Parts from Recipe GmbH, Sandstrasse 37-39, D80335 Munich, Germany.

References

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