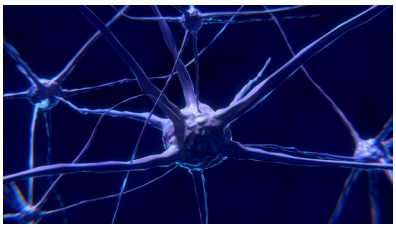
Science Together

KNAUER

DC with GC – Determination of catecholamines in plasma with electrochemical detection



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SUMMARY

Epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine are termed as catecholamines. The determination of catecholamines is primarily used to help detect and rule out pheochromocytomas in symptomatic patients, i.e. in patients with persistent hypertension. It is also used in order to help monitor for recurrence when a pheochromocytoma has been detected and removed[1]. Applying HPLC coupled to electrochemical detection with direct current (DC) using a glassy carbon (GC) working electrode makes it possible to detect even lowest amounts of catecholamines in urine or plasma samples.

INTRODUCTION

Catecholamines can act as neurotransmitters when they are produced in the sympathetic nervous system or the brain. When synthesized in the adrenal medulla, they act as circulating hormones. The endogenous catecholamines include dopamine, epinephrine, and norepinephrine. The specific compound formed depends on the enzymes produced by the synthesizing tissue. All three of these catecholamines are synthesized in a similar fashion, beginning with tyrosine[2]. They break down into vanillylmandelic acid, metanephrine, and normetanephrine. Metanephrine and normetanephrine also may be measured during a catecholamine test. Catecholamines can increase heart rate, blood pressure, breathing rate, muscle strength, and mental alertness. They also reduce the amount of blood reaching the skin and intestines and increase the blood flow to the major organs such as brain, heart and kidneys. Certain rare tumors can increase the amount of catecholamines in the blood. This causes high blood pressure, excessive sweating, headaches, fast heartbeats (palpitations), and tremors[3]. The determination of catecholamines and metabolites is of great importance for the diagnosis and treatment of tumor diseases.

Additional Information

DC with GC – Determination of catecholamines in plasma with electrochemical detection

RESULTS

Following the instructions of the ClinRep® complete kit for determination of catecholamines in plasma, first a standard solution of noradrenaline, adrenaline and dopamine was analyzed. This standard solution already contains the internal standard (IS). **Fig. 1** displays the chromatogram of the mixed catecholamines standard.

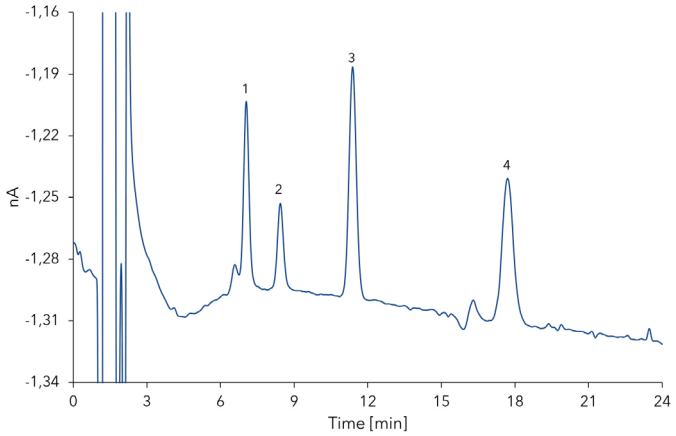


Fig. 1 ClinRep® catecholamines standard: 1 - noradrenaline, 2 - adrenalin, 3 - IS, 4 - dopamine.

With the performance of multiple measurements the relative standard deviation (% RDS) of the method was determined. **Tab. 1** summarizes the calculated values of relative standard deviation for retention time and peak area ascertained with multiple measurements (n=3) of the catecholamine standard. **Fig. 2** shows an overlay of these measurements.

Tab. 1 Relative standard deviation of method (n=3)

Substance	Replicate	Retention time [min]	Area [nA.s]
Noradrenalin	1	7.025	1.262
	2	7.050	1.259
	3	7.050	1.252
%RSD		0.20	0.42
Adrenalin	1	8.417	0.719
	2	8.442	0.716
	3	8.442	0.714
%RSD		0.17	0.35
Dopamin	1	17.650	1.918
	2	17.700	1.973
	3	17.700	1.919
%RSD		0.16	1.62

The next step was to perform a single-point calibration with the corresponding plasma calibrator. Here the internal standard needs to be added and the calibrator was prepared according to the described sample preparation procedure. Finally, a ClinCheck® plasma control sample (Level II) was measured to verify the method and calibration **(Fig. 3)**. Therefore, the sample with known amounts of catecholamines was set up, again following the described preparation procedure.

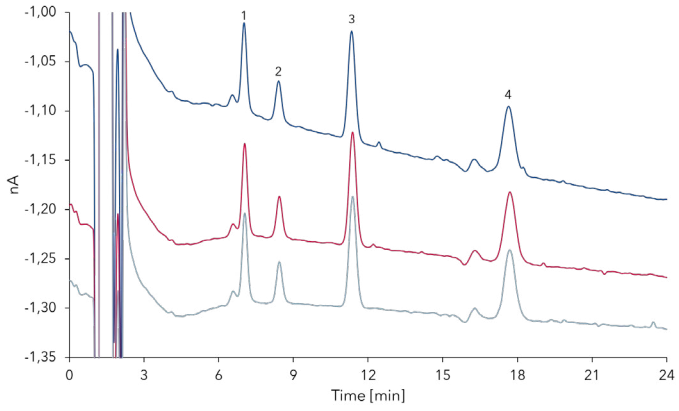


Fig. 2 Overlay of multiple measurements of standard solution: 1 - noradrenaline, 2 - adrenalin, 3 - IS, 4 - dopamine.

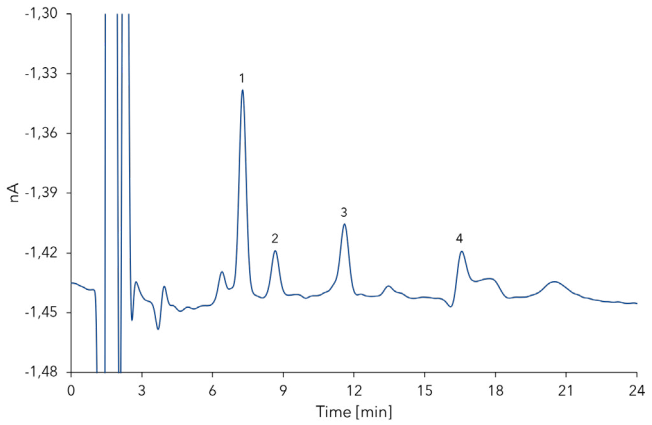


Fig. 3 Chromatogram of plasma control sample: 1 - noradrenaline, 2 - adrenalin, 3 - IS, 4 - dopamine.

RESULTS

The exact calculation of values considering the recovery is specified in the ClinRep® instructions. **Tab. 2** shows the comparison of the specified and measured values of the plasma control sample. The measured results for the plasma control sample were within the required specifications.

Tab. 2 Comparison of specified and measured values of the plasma control sample

Substance	Specification [ng/L]		Value [ng/L]	% RSD
	min	max		
Plasma control sample				
Noradrenalin	1778	2667	2004 ± 29	1.45
Adrenalin	475	713	591 ± 3.5	0.59
Dopamin	423	704	537 ± 27	5.10

SAMPLE PREPARATIONS

Sample preparation was performed according to the ClinRep® instructions included in the ClinRep® complete kit for the determination of catecholamines in plasma. **Fig. 4** shows the schematic procedure of sample preparation[1].

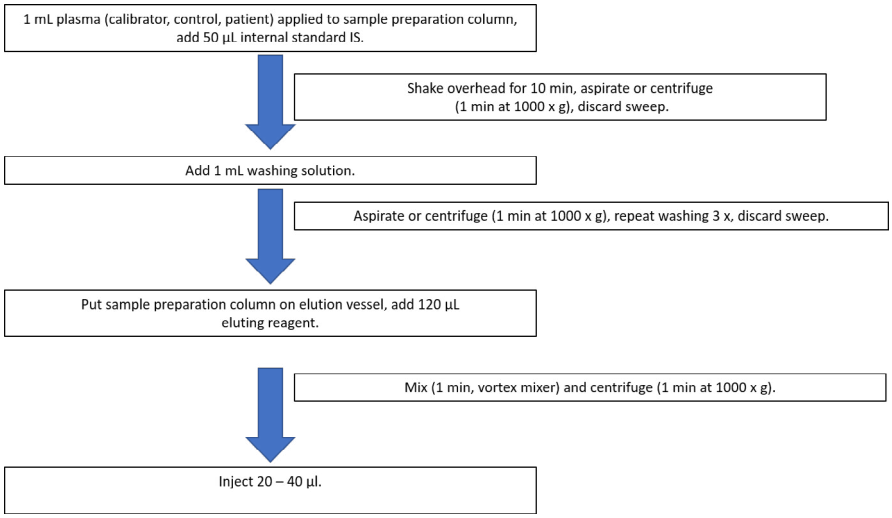


Fig. 4 Schematic sample preparation procedure

CONCLUSION

The ClinRep® HPLC complete kit for determination of catecholamines in plasma is easy to handle, due to the detailed instructions included. In combination with DC electrochemical detection, using the glassy carbon working electrode and the salt-bridge reference electrode, it is possible to quantify low amounts of catecholamines in urine or plasma samples.

MATERIALS AND METHODS

Tab. 3 Instrument setup

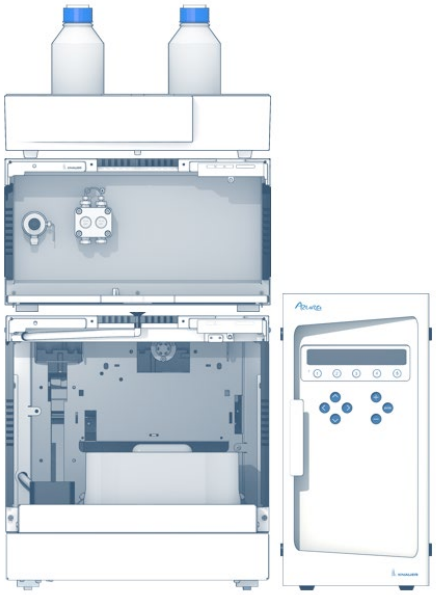
Column temperature	30 °C
Injection volume	20 µL
Injection mode	Partial loop
Detection	ECD (DC mode)

Tab. 4 Pump parameters

Eluent (A)	ClinRep® mobile phase for catecholamines in plasma
Flow rate	1 mL/min
Gradient	isocratic

Tab. 5 ECD settings (DC mode)

E cell	500 mV
Cell temperature	30 °C
Range	5 nA
Polarity	+
Compensation	On
AST position	2
Filter	0.02 Hz



Tab. 6 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, isocratic	APH30EA
Autosampler	AZURA AS 6.1L	AAA01AA
Detector	AZURA ECD 2.1	A1651
Flow cell	SenCell GC Salt-Bridge high sensitivity electrochemical flow cell	A1652
Column	Eurospher II 100-5 C8, 150 x 4 mm ID with precolumn	15WE081E2J
Software	ClarityChrom 8.1 – Workstation, autosampler control included	A1670
Software	ClarityChrom 8.1 – System suitability extension (SST)	A1677

REFERENCES

[1] Recipe, Catecholamines. https://recipe.de/products/catecholamines-plasma (February 2, 2020).

[2] Simmons, J.P. Wohl, J.S. Vasoactive catecholamines. In: Silverstein, D., Hopper, K. Small animal critical care medicine. Elsevier (2009).

[3] Universtiy of Michigan, Catecholamines in blood. https://www.uofmhealth.org/health-library/tw12861 (February 2, 2020).

RELATED KNAUER APPLICATIONS

VPH0017J – Determination of Catecholamines II

VPH0009J – HPLC method for the determination of Amineptine and its main Metabolite in human plasma