

FREE CATECHOLAMINES WITH RESIN IN URINE FAST - Code Z10710
(Derivatization kit for Fluorimetry detection)

BIOCHEMISTRY

Catecholamines are dihydroxyl organic compounds characterized by a phenolic ring (fig.1).

Epinephrine (E), Norepinephrine (NE), and Dopamine are the most important members of this family.

The biosynthetic pathway of catecholamines uses **L-tyrosine** as initial substratum. Chromaffin cells synthesize and store epinephrine in the adrenal medulla, while norepinephrine production occurs in the sympathetic nerve endings. Dopamine is above all a neurotransmitter in the CNS.

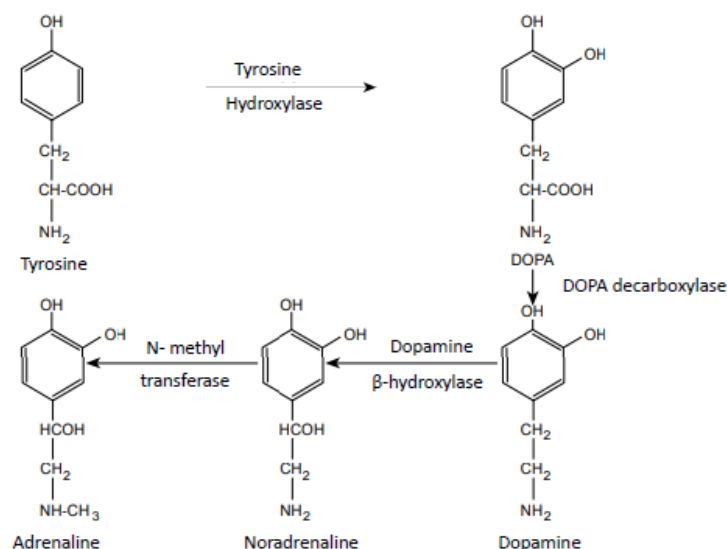


Fig 1. Biosynthesis of catecholamines

CATABOLISM

The biological effects of catecholamines terminate rapidly by uptake into the sympathetic nerve endings. The major changes that occur in these sites include their transformation into meta-O-methylated and deaminated metabolites due to **Catechol-O-methyltransferase (COMT)** and **monoamine Oxidase (MAO)** respectively and, finally, their conjugation with sulphate and glucuronide.

Homovanillic Acid is the major metabolite of **Dopamine** while **Vanillylmandelic Acid** is the main metabolite of norepinephrine and epinephrine. (fig. 2)



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 008	Catecholamines in urine by Fluorimetry - FAST	06/2024
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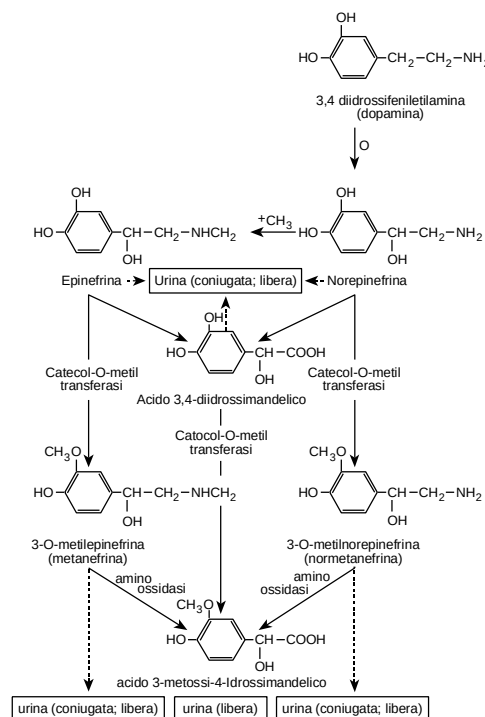


Fig. 2: Metabolism of hormones of suprarenal medulla

CLINICAL BACKGROUND

Catecholamines are often determined in urine for neurological diagnosis and for monitoring the response to therapy in illnesses like **pheochromocytoma** and **neuroblastoma**.

Pheochromocytoma is a catecholamine-producing tumor derived from adrenomedullary chromaffin cells. More than 90% appear to be benign. They are dangerous because of their capacity to store and release catecholamines in large amounts with subsequent production of alarming syndromes including sustained hypertension, resistant to conventional treatment, and hypertensive crisis with malignant hypertension and hypertensive encephalopathy. The diagnosis of pheochromocytoma is established by demonstration of increased urinary excretion of catecholamines or catecholamines metabolites, and their concentration is often determined in urine for monitoring the response to therapy. Correctly diagnosed and properly treated, pheochromocytoma is curable; misdiagnosed or improperly treated, it is fatal.

Neuroblastoma, the second most common solid tumor that occurs during childhood, may appear almost anywhere along the sympathetic nervous system chain. This tumor synthesizes and secretes catecholamines and metabolites like DOPA, dopamine, VMA, and homovanillic acid. Assessment of urinary and plasma catecholamines are useful in establishing a diagnosis and following the results of treatment.

ASSAY PRINCIPLES AND CHARACTERISTICS

The present method allows to analyse Catecholamines in urine without Clean-up procedure. After derivarization 20 μ l of the eluate are injected and read by a Fluorimetric Detector.

TECHNICAL FEATURES

Principle of the Method:

Catecholamines (**Epinephrine, Norepinephrine and Dopamine**) after purification with a specific resin are treated with 2 specific reagents and incubated at 37°C for 30 min. or at 70 °C for 15 min. The eluate is added with 300 µl of H₂O HPLC grade and directly injected into the HPLC System.

Recovery : > 98 %

Sensitivity : < 0,02 µg/l

Linearity : 0,02 - 2.000 µg/l

Normal Urine Values in 24 h :

EPINEPHRINE 2 - 22 µg/24 h

NOREPINEPHRINE 20 - 81 µg/24 h

DOPAMINE 40 - 400 µg/24 h

Components of the kit :

Reagent A – Purification Resin, **1 x 50 ml**

Reagent B – Buffer Solution, **1 x 100 ml**

Reagent B1 – Purification Buffer, **1 x 50 ml**

Reagent C – Eluting Solution, **1 x 200 ml**

Reagent D – Starter Solution, **1 x 2 ml**

Reagent E – Derivatization Solution, **1 x 2 ml**

Reagent G – Test Solution, **1 x 5 ml**

Reagent H – Internal Standard Solution, **1 x 5 ml**

Urine Calibrator Lyophil., **1 x 5 ml**

Reagent M – Mobile Phase, **2 x 500 ml**

All the components are ready-to-use. Stability: 3 years at room temperature ($\leq 20\text{ }^{\circ}\text{C}$), except for the Urine Calibrator lyophil, see Warnings for stability and storage. Store the Internal Standard and the Test Solution at 2-8 °C.

(Shake well by inversion before use; repeat the operation every 10 samples)

Store at 2-8 °C

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Dilute 1:10 before use

Code Z10116 (packed separately – see data sheet)

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 or 50 µl
Fluorimetric Detector $\lambda_{\text{EX}}=360\text{ nm}$ $\lambda_{\text{EM}}=490\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

24 h Urine Collection Procedure:

24-hour urine must be collected into a container with 5 ml (Child) or 10 ml (Adult) of HCl 5 M for each urine litre. After collection, 10 ml of urine should be delivered to the lab with the indication of the total diuresis. **Laboratory should verify that the delivered urine has a pH between 2.5 and 3.5. If the pH is > 7 the sample may not be suitable for testing.** Delayed analyses require sample freezing at -20°C or less. Stable over 2 months.

PREANALYTICAL PROCEDURE

Pipette in a 1,5 ml vial with cap:

- 400 µl of **Reagent C** – Eluting Solution
- 20 µl of **Reagent G** – Test Solution
- 50 µl of **Reagent H** – Internal Standard Solution (diluted 1:10 with H₂O HPLC grade)
- 20 µl of **Reagent D** – Starter Solution
- 20 µl of **Reagent E** – Derivatization Sol.

- Incubate at 70 °C for 15 minutes

Cooling at room temperature

INJECTION :

Inject 20 µl of the solution into the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 3. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1: Verify of pH

Pipette in a plastic or glass tube of 10 ml:

- 1 ml of **Reagent B** – Buffer Solution
- 1 ml of **Sample** or **Calibrator** or **Control**

Vortex for 20 seconds

- Verify that the pH of the **Samples**, **Calibrators** and **Controls** is between **7** and **8** ; eventually correct with a little bit of NaOH 2N.
- Shake well by inversion the **Reagent A** (Repeat the operation every 10 samples)

STEP 2 : Samples Purification

Pipette in a plastic or glass tube of 10 ml:

- 500 µl of **Reagent B1** - Purification Buffer
- 500 µl of **Reagent A** – Purification Resin
- 1000 µl of **Samples**, **Calibrators** or **Controls** prepared in the **STEP 1**
- 50 µl of **Reagent H** - Internal Standard (diluted 1:10 with H₂O HPLC grade)

Vortex for 20 seconds

STEP 3 : Centrifuge at 4.000 rpm for 2 minutes

STEP 4 : Take the supernatant and drop it away

STEP 5 : Elution

Pipette in the tube with the precipitate 2 ml of **Reagent C** - Eluting Sol.

Vortex for 20 seconds

STEP 6 : Centrifuge at 4.000 rpm for 2 minutes

STEP 7 : Derivatization

Pipette in plastic tube of 1,5 ml with cap:

- 200 µl of **Sample** from Purification procedure
- 20 µl of **Reagent D** – Starter Solution
- 20 µl of **Reagent E** - Derivatization Sol.

Vortex for 20 seconds

STEP 8 : Incubate at **37 °C** for **30 minutes** or at **70 °C** for **15 minutes**.

STEP 9 : Add 300 µl of H₂O HPLC grade

Vortex for 20 seconds

N.B.: at this step, the sample is stable 7 days at 2-8 °C

INJECTION :

Inject 20 µl of this solution in the chromatographic system.

FREE CATECHOLAMINES in URINE FAST- Warnings

REAGENT G : TEST SOLUTION

NOREPINEPHRINE	about 90,75 µg/l
EPINEPHRINE	about 28,80 µg/l
DOPAMINE	about 545,25 µg/l

FLUORIMETRIC DETECTOR PARAMETERS (Ei. : Jasco Fluorimeter 821 FP)

λ of EXCITATION	360 nm
λ of EMISSION	490 nm
GAIN	1 x 100
INTEGRATION PARAMETERS	5 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µ), the use of Javelin Prefilters (1 x 10 pcs), code S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µ). Disconnect the detector and flush a solution of H₂O : Acetonitrile (20 : 80 v/v) set flow at 0,8 ml / min for 25 min. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1ml / min. for 30 min. Condition further on the column for 30 min. also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run. **It is possible to make analysis at recycling phase, providing that you filter the same mobile phase with a filter of 0,22 µ before any analytical run.** If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flush 30 ml of H₂O: methanol or Acetonitrile (80: 20 v/v) and download. Flush a solution of H₂O: methanol or Acetonitrile (70: 30 v/v) for 30 minutes for download. When the column will be used again, pass 15 ml of H₂O: methanol (20: 80 v/v) before made conditional with the Mobile phase. It is recommended to wash the analytical column at the end of each session.

HPLC PARAMETERS

LOOP	20 or 50 µl
FLOW	1 ml/min
PRESSURE	About 110 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z10116	Urine Calibrator lyophil. for Catecholamines	4 x 5 ml
Z10559	Urine Control lyophil. for Biogenic Amines - Levels 1 and 2	2 x 5 x 5 ml
Z10711	Mobile Phase	4 x 5 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 um) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap for Autosampler	1 x 100 Pcs

FREE CATECHOLAMINES IN URINE BY FLUORIMETRY FAST (Reference Chromatograms)

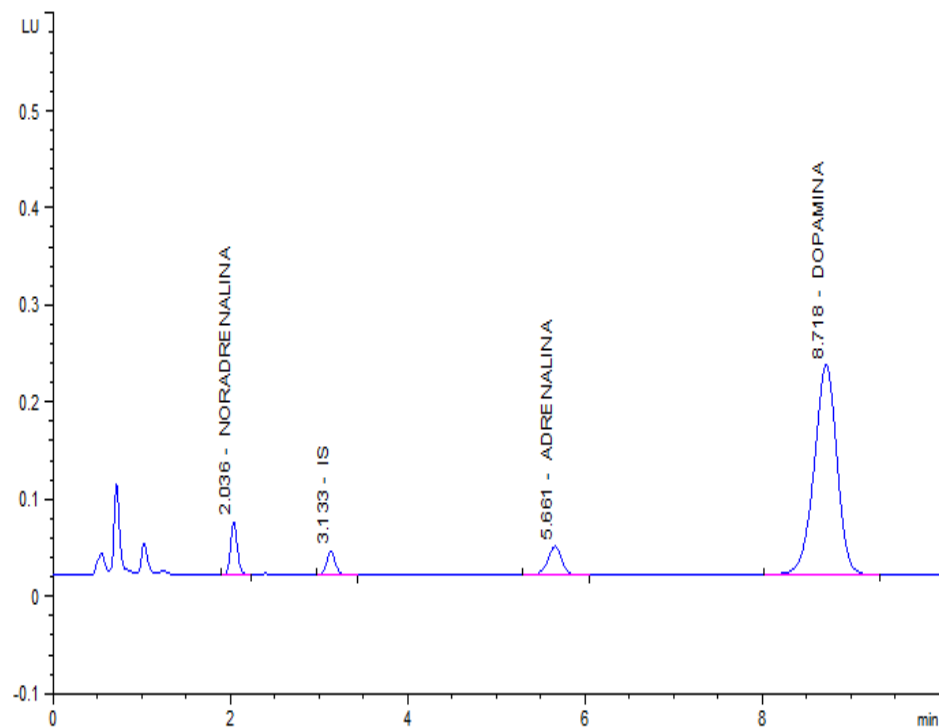


Fig. 3:	Calibrator in urine		
	R.T. 2.03	Noradrenaline	100,5 µg/l
	R.T. 3.13	Internal Standard	
	R.T. 5.66	Adrenaline	24,6 µg/l
	R.T. 8.71	Dopamine	312,8 µg/l

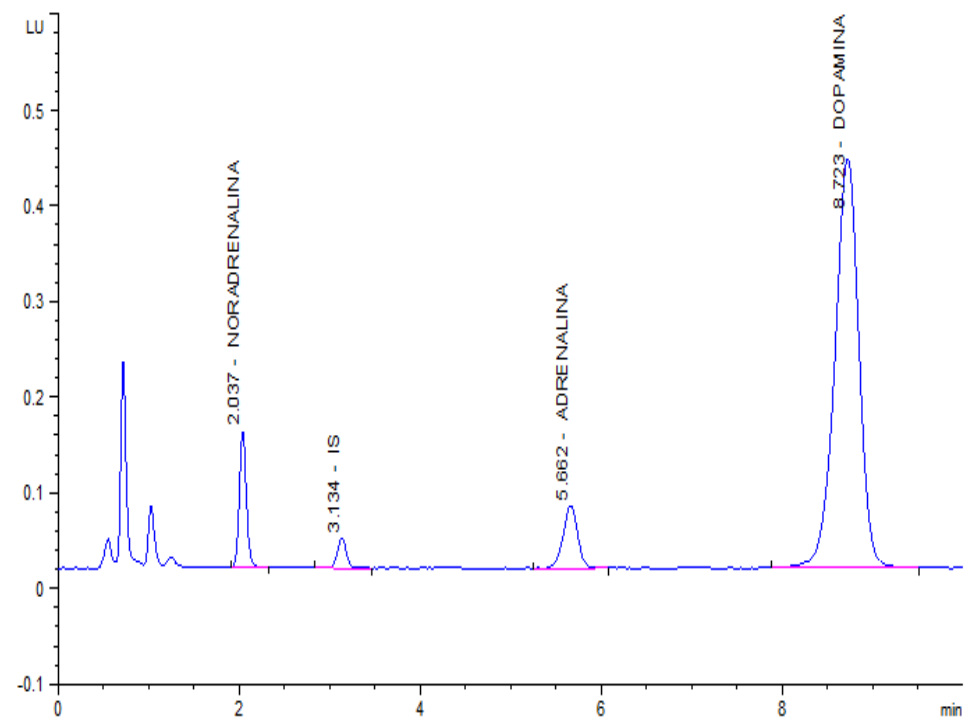


Fig. 4:	High Control		
	R.T. 2.03	Noradrenaline	187,8 µg/l
	R.T. 3.13	Internal Standard	
	R.T. 5.66	Adrenaline	52,7 µg/l
	R.T. 8.72	Dopamine	639,6 µg/l