

CATECHOLAMINES DUAL KIT FAST –

CODE Z10650

(KIT FOR CATECHOLAMINES IN PLASMA AND IN URINE BY FLUORIMETRY)

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.

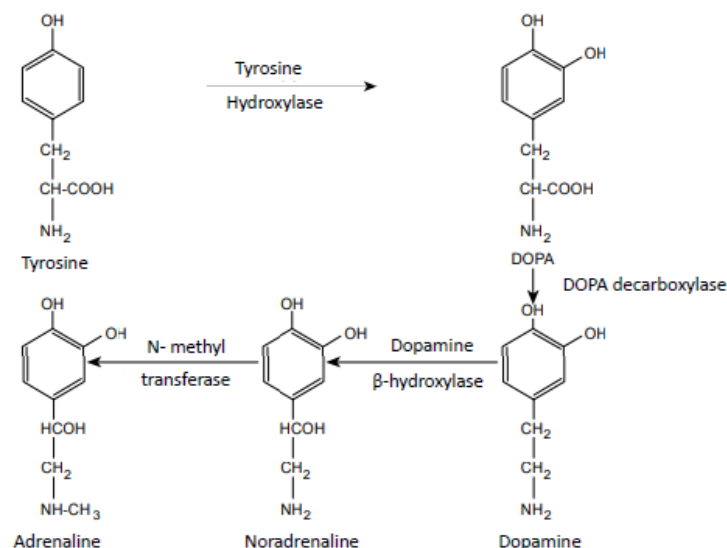


Fig 1. Biosynthesis of catecholamines

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 **Vanillymandelic Acid** is the main metabolite of norepinephrine and epinephrine. (fig. 2)

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CE

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° 012	Catecholamines dual kit in urine by Fluorimetry	06/2024
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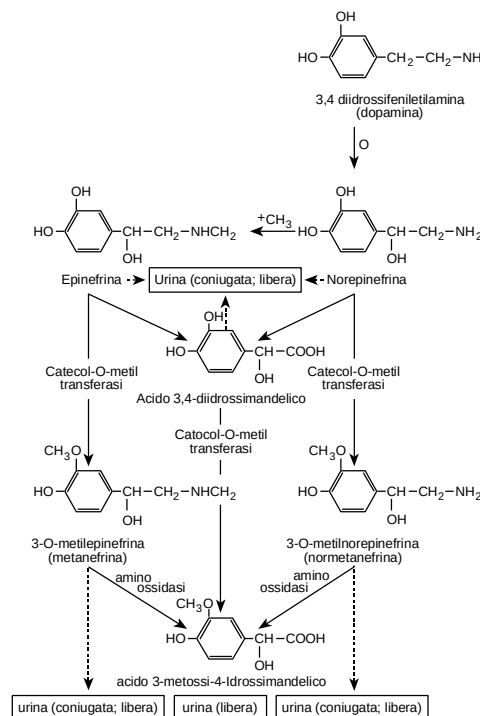


Figura 2: Metabolismo degli omoni della midollare del surrene

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

This method allows to completely purify catecholamines by an organic and specific complexant reagent. This way allows to have a complete extraction using a C-18 solid phase column and after derivatization you can read by a fluorimetric detector.

CATECHOLAMINES DUAL KIT FAST- Technical features

Principle of the Method :

Catecholamines (**Epinephrine, Norepinephrine, and Dopamine**), after purification with Clean up Columns, are derivatized with 2 specific reagents and incubate at 70 °C for 15 minutes. The derivatized solution, is injected in HPLC.

Recovery : > 98 %

Sensitivity :

PLASMA Catecholamines	< 10 pg/ml	for Norepinephrine
	< 8 pg/ml	for Epinephrine
	< 18 pg/ml	for Dopamine

URINE Catecholamines	< 0,1 µg/litre	for Norepinephrine
	< 0,1 µg/litre	for Epinephrine
	< 0,3 µg/litre	for Dopamine

Linearity :

PLASMA Catecholamines	> 80.000 pg/ml for each Analytes
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URINE Catecholamines	> 8.000 µg/litre for each Analytes
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PLASMA Normal values:

NOREPINEPHRINE

Supine 70-750 pg/ml

Standing: 200-1,700 pg/ml

EPINEPHRINE

Supine: < or =111 pg/ml

Standing: < or =141 pg/ml

DOPAMINE

<30 pg/ml (no postural change)

Ref. www.mayocliniclabs.com

24 h URINE Normal Values :

Norepinephrine	20 - 81	µg/24 h
Epinephrine	2 - 22	µg/24 h
Dopamine	40 - 400	µg/24 h

Plasma Collection Procedure:

Let stay the patient for at least 10 minutes on sample chair. Prepare in un small beaker a water/ice bath. Collect 7 ml of blood with EDTA. Shake smoothly the sample with inversion movement for ca. 1 min. Then place the tube into the water/ice bath previously prepared. Soon after centrifuge at 3.000 rpm for 3 min. Take about 3 ml of Plasma. Plasma samples should be stored at – 70° C for no longer than 1 month.

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 2,0 and 4,0. 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.

CATECHOLAMINES DUAL KIT FAST – Components of the kit

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

Reagent A	Conditioning Solution N° 1	1 x 150 ml	
Reagent B	Conditioning Solution N° 2	1 x 120 ml	
Reagent C	Conditioning Solution N° 3	1 x 120 ml	
Reagent D	Diluting Solution	1 x 200 ml	
Reagent E	Wash Solution	1 x 250 ml	
Reagent F	Eluting Solution	1 x 70 ml	
Reagent G	Test Solution	1 x 5 ml	Store: $2-8\text{ }^{\circ}\text{C}$
Reagent H	Internal Standard Sol.for Catech. in Urine	1 x 10 ml	Store: $2-8\text{ }^{\circ}\text{C}$ Dilute 1:10 before use
Reagent I	Internal Standard Sol.for Catech.in Plasma	1 x 10 ml	Store: $2-8\text{ }^{\circ}\text{C}$ Dilute 1:10 before use
Reagent J	Derivatization Solution	1 x 10 ml	
Reagent L	Starter Solution	1 x 10 ml	
Reagent M	Mobile Phase	3 x 500 ml	
	Plasma Calibrator lyophil.	5 x 1 ml	<u>Code Z25016</u> <u>(packed separately – see data sheet)</u>
	Urine Calibrator lyophil.	1 x 5 ml	<u>Code Z10116</u> <u>(packed separately – see data sheet)</u>
Clean-up Columns		100 pcs	

PREANALYTICAL PROCEDURE

Pipette in a 1,5 ml plastic or glass tube with cap:

- 200 μl of **Reagent F – Eluting Solution**
- 20 μl of **Reagent G – Test Solution**
- 20 μl of **Reagent H – Internal Standard Solution** (diluted 1:10 with Water HPLC grade)
- 20 μl of **Reagent J – Derivatization Solution**
- 20 μl of **Reagent L – Starter Solution**

Close the tube.

- Incubate at $70\text{ }^{\circ}\text{C}$ for 15 minutes

Cooling

- Add 200 μl of H_2O HPLC grade

Vortex for 5 sec.

INJECTION :

- Inject 20 or 50 μl of this solution in the chromatographic system.

Important : Don't use this solution to calibrate!

CATECHOLAMINES DUAL KIT FAST

CONDITIONING AND ACTIVATION OF CLEAN-UP COLUMNS

This phase is the same for Catecholamines in Plasma and for Catecholamines in Urine Purification Procedure.

Place Clean-up Columns on plastic tube of 10 ml to collect eluate and eliminate it.

1 Condition clean up columns by fluxing 1 ml of Reagent A – Conditioning Solution 1

Let percolate solution through clean up column with a flow of 1,0 ml/min (drop by drop) also with the help of a moderate vacuum

Discharge the percolated liquid

2 Condition clean up columns by fluxing 1 ml of Reagent B – Conditioning Solution 2

Let percolate solution through clean up column with a flow of 1,0 ml/min (drop by drop) also with the help of a moderate vacuum

Discharge the percolated liquid

3 Condition clean up columns by fluxing 1 ml of Reagent C – Conditioning Solution 3

Let percolate solution through clean up column with a flow of 1,0 ml/min (drop by drop) also with the help of a moderate vacuum

At this point the Clean-up columns are conditioned and activated and are ready for Catecholamines in Plasma and in Urine Purification procedure.

**CATECHOLAMINES DUAL KIT FAST – ANALYTICAL PROCEDURE FOR CATECHOLAMINES
IN PLASMA**

Calibrator, Samples and Controls Purification.

STEP 1 : Calibrator, Samples and Controls Preparation.

- Pipette in a 10 ml tube:

	Calibrator	Samples	Controls
Reagent D – Diluting Solution	1400 µl	1400 µl	1400 µl
Plasma Calibrator	600 µl	/	/
Samples	/	600 µl	/
Controls	/	/	600 µl
Reagent I – Internal Std for Cat.in Plasma (Diluted 1:10 with H ₂ O HPLC grade)	100 µl	100 µl	100 µl

Vortex for 5 seconds

STEP 2 : Loading of Calibrator, Samples and Controls in Clean-up columns.

- Dispense in the respective Clean-up columns, **2 following loads of 1 ml each one**, the Calibrator, the Samples and the Controls previously prepared.
- Let percolate solution through clean up column with a **flow of 1,0 ml/min** (drop by drop) also with the help of a moderate vacuum.

Discharge the percolated liquid

STEP 3 : Cleaning of Samples through Clean up columns.

Pipette in Clean up columns in sequence:

- 1,0 ml of **Reagent E – Wash Solution** **Discharge the percolated liquid**

When all liquid is percolated, pour again:

- 1,0 ml of **Reagent E – Wash Solution** **Discharge the percolated liquid**

Let dry with a light flux of air!

STEP 4 : Elution by Clean-up columns

Place Clean-up columns in clean tubes. Pipette in Clean-up columns:

- 400 µl of **Reagent F – Eluting Solution** and collect eluate.

Warning! Elute slowly for about 1 minute!

Vortex for 5 seconds

STEP 5 : Procedure of derivatization

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

- 400 µl of **ELUATE** from purification procedure
- 20 µl of **Reagent J – Derivatization Solution**
- 20 µl of **Reagent L – Starter Solution**

STEP 6 : Incubate at 70 °C for 15 minutes.

Cooling

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

INJECTION :

- Inject 20 or 50 µl of this solution in the chromatographic system.

CATECHOLAMINES DUAL KIT FAST – ANALYTICAL PROCEDURE FOR CATECHOLAMINES IN URINE

Calibrator, Samples and Controls Purification.

- Verify that Samples pH is between 6,0 and 7,0 ; eventually correct it with a little bit of NaOH 1N or HCl 1N.

STEP 1: Calibrator, Samples and Controls Preparation.

- Pipette in a 10 ml tube:

	Calibrator	Samples	Controls
Reagent D – Diluting Solution	800 µl	800 µl	800 µl
Urine Calibrator	100 µl	/	/
Samples	/	100 µl	/
Controls	/	/	100 µl
Reagent H – Internal Std for Cat.in Urine (Diluted 1:10 with H ₂ O HPLC grade)	100 µl	100 µl	100 µl

Vortex for 5 seconds

STEP 2 : Loading of Calibrator, Samples and Controls in Clean-up columns.

- Dispense in the respective Clean-up columns 1 ml of the Calibrator, of the Samples and of the Controls previously prepared.
- Let percolate solution through clean up column with a **flow of 1,0 ml/min** (drop by drop) also with the help of a moderate vacuum.

Discharge the percolated liquid

STEP 3 : Cleaning of Samples through Clean up columns.

Pipette in Clean up columns in sequence:

- 1,0 ml of **Reagent E – Wash Solution** **Discharge the percolated liquid**

When all liquid is percolated, pour again:

- 1,0 ml of **Reagent E – Wash Solution** **Discharge the percolated liquid**

Let dry with a light flux of air!

STEP 4 : Elution by Clean-up columns

Place Clean-up columns in clean tubes. Pipette in Clean-up columns:

- 500 µl of **Reagent F – Eluting Solution** and collect eluate.

Warning! Elute slowly for about 1 minute!

Vortex for 5 seconds

STEP 5 : Procedure of derivatization

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

- 500 µl of **ELUATE** from purification procedure
- 20 µl of **Reagent J – Derivatization Solution**
- 20 µl of **Reagent L – Starter Solution**

STEP 6 : Incubate at 70 °C for 15 minutes.

Cooling

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

INJECTION :

- Inject 20 or 50 µl of this solution in the chromatographic system.

CATECHOLAMINES DUAL KIT FAST - Warnings

REAGENT G : TEST SOLUTION

NOREPINEPHRINE	About 81,35 µg / l
EPINEPHRINE	About 31,92 µg / l
DOPAMINE	About 370,23 µg / l

Conversion coefficient : 1,0 µg/l = 1000 pg/ml

SPECTROFLUORIMETRIC DETECTOR - VARIAN mod. 363 PARAMETERS

λ of EXCITATION	360 nm
λ of EMISSION	490 nm
GAIN FOR CATECHOLAMINES IN URINE	2 - MEDIUM
GAIN FOR CATECHOLAMINES IN PLASMA	1 - HIGH

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µm), 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 µm). 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.

For Catecholamines in Urine it is possible to make analysis at recycling phase, providing that you filter the same mobile phase with a filter of 0,22 µm before any analytical run; for Catecholamines in Plasma it is NOT possible to make analysis at recycling phase.

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 µl or 50 µl
FLOW	1ml/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
Z25016	Plasma Calibrator lyophil. for Catecholamines	4 x 1 ml
Z25019	Plasma Control lyophil. for Catecholamines, Levels 1 and 2	2 x 5 x 1 ml
Z10651	Mobile Phase	4 x 500 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
S51843550	Clear glass vials with reduced volume (globet form) from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul and for Amber vials	1 x 100 Pcs

CATECHOLAMINES DUAL KIT BY FLUORIMETRY FAST

(Reference Chromatograms for *Catecholamines in Urine*)

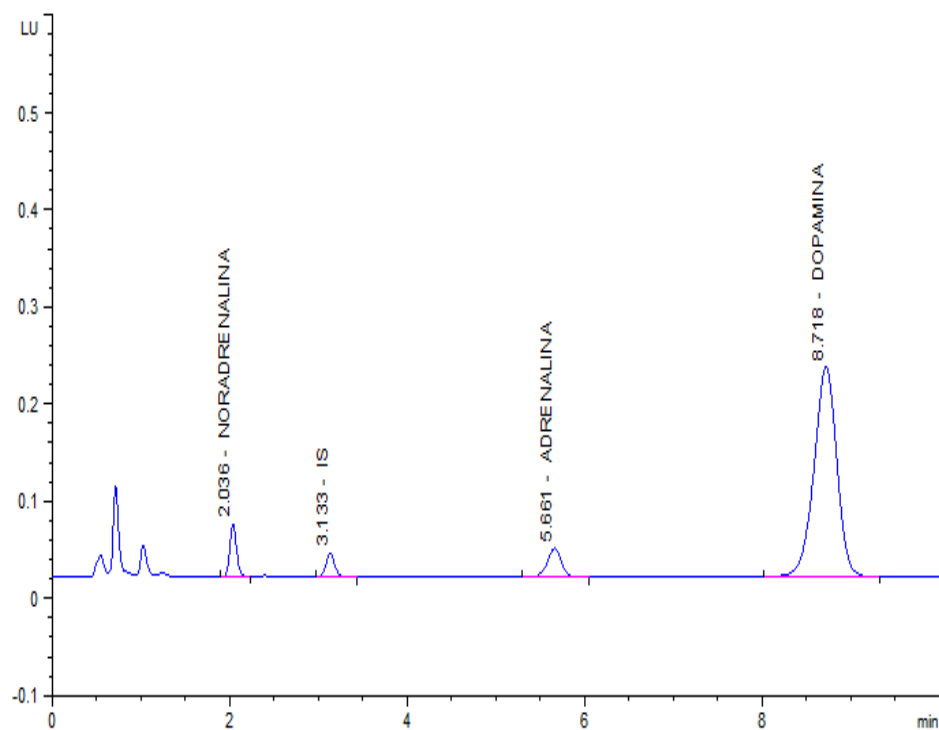


Fig. 1:	Calibrator in urine		
	R.T. 2.03	Noradrenaline	100,5 µg/l
	R.T. 3.13	Internal Standard	
	R.T. 5.66	Adrenaline	24,6 µg/l
	R.T. 8.71	Dopamine	312,8 µg/l

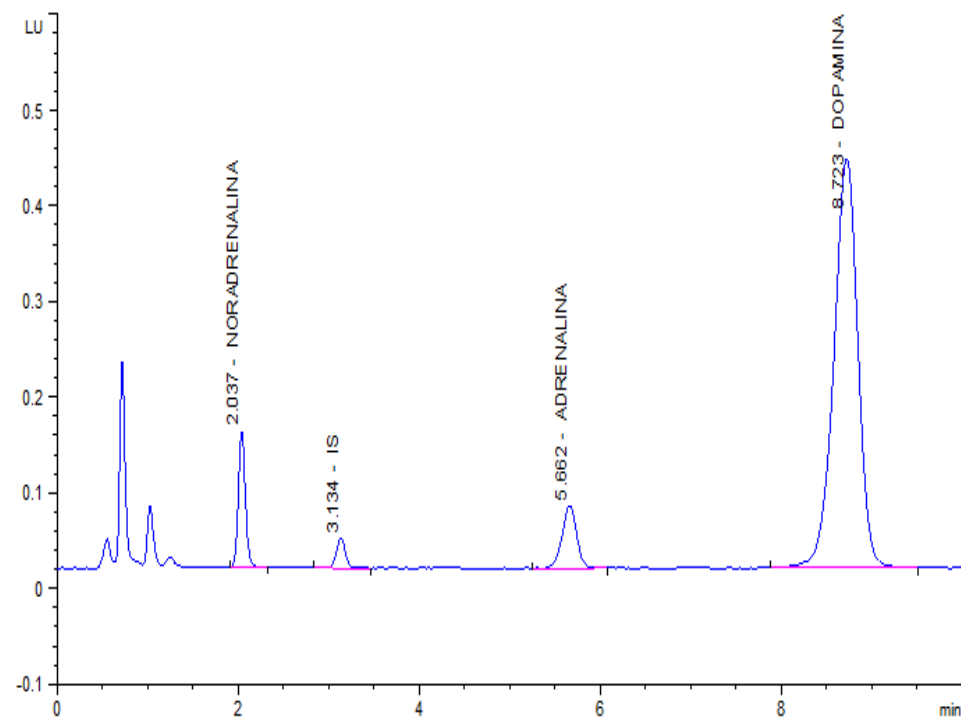


Fig. 2:	High Control		
	R.T. 2.03	Noradrenaline	187,8 µg/l
	R.T. 3.13	Internal Standard	
	R.T. 5.66	Adrenaline	52,7 µg/l
	R.T. 8.72	Dopamine	639,6 µg/l

CATECHOLAMINES DUAL KIT BY FLUORIMETRY FAST

(Reference Chromatograms for *Catecholamines in Plasma*)

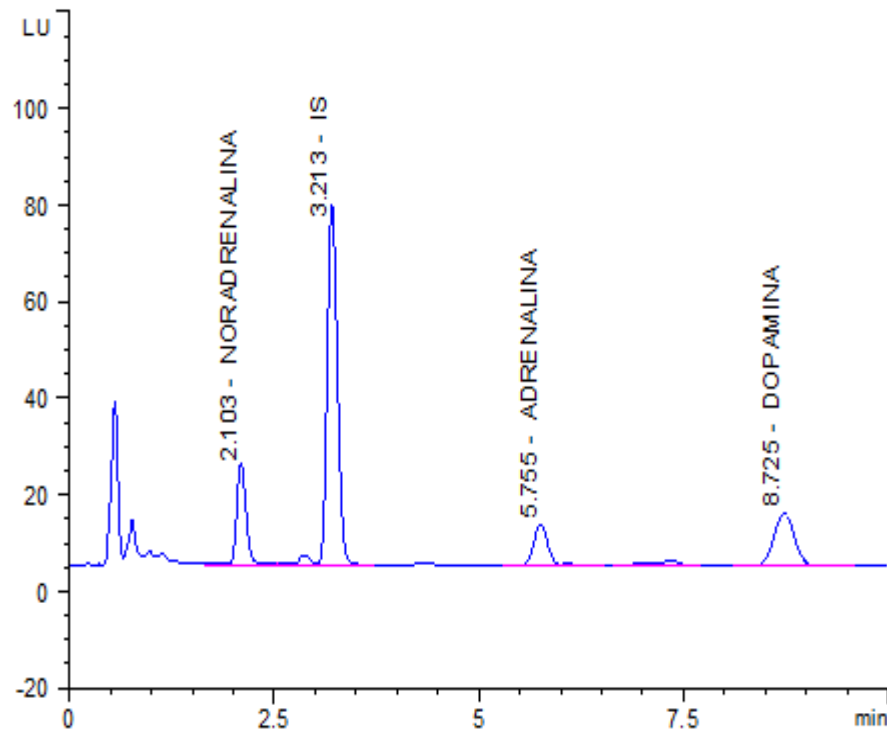


Fig. 1:	Calibrator in plasma			
	R.T.	2.10	Noradrenaline	832 ng/l
	R.T.	3.21	Internal Standard	
	R.T.	5.75	Adrenaline	185 ng/l
	R.T.	8.72	Dopamine	726 ng/l

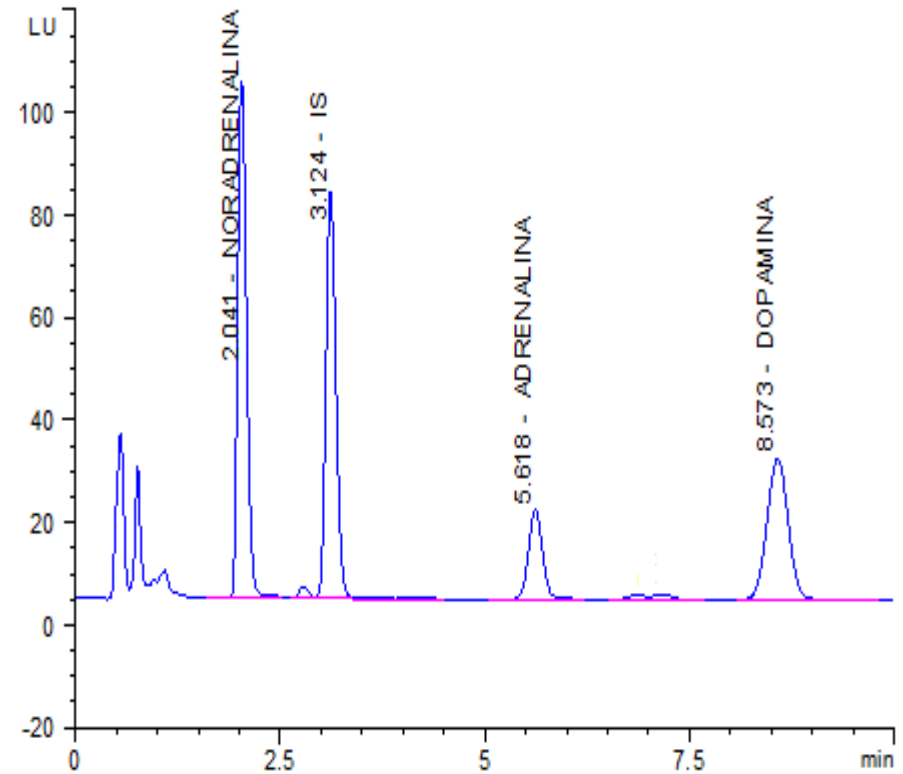


Fig. 2:	Control in plasma level 2			
	R.T.	2.04	Noradrenaline	3.243 ng/l
	R.T.	3.12	Internal Standard	
	R.T.	5.61	Adrenaline	440 ng/l
	R.T.	8.57	Dopamine	1.936 ng/l