

FREE CATECHOLAMINES/ FREE and TOTAL METANEPHRINES in URINE by LC/MS – Code LC77010

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.

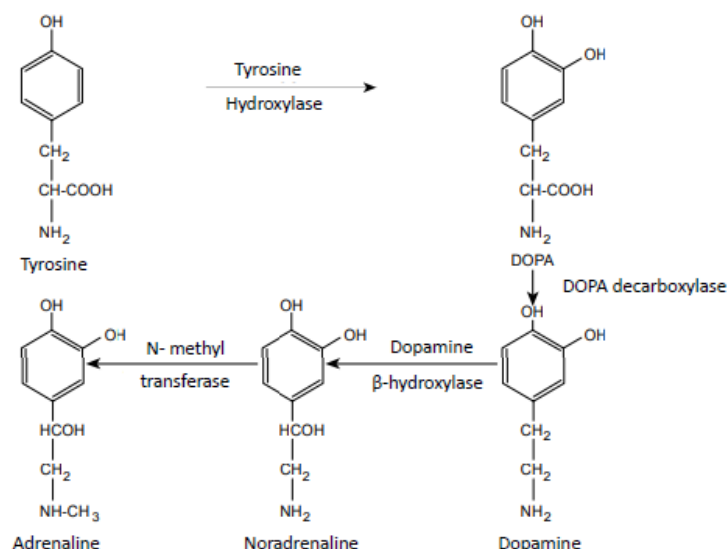


Fig 1. Biosynthesis of catecholamines

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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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° 010	Catecholamines/Metanephrines in urine by LC/MS	06/2024
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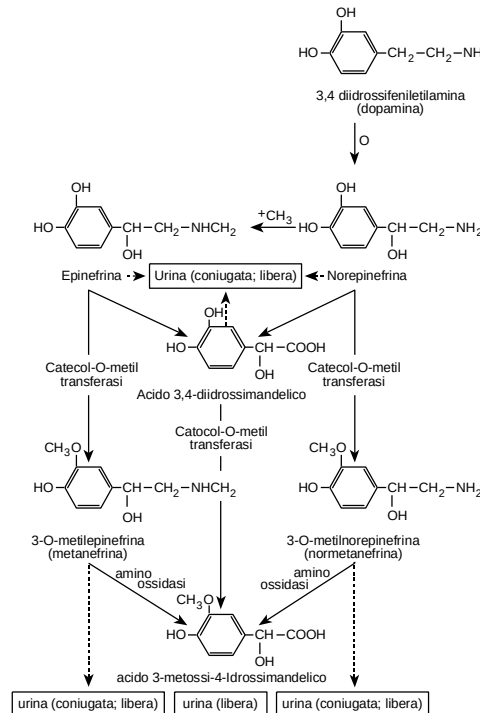


Figura 2: Metabolismo degli ormoni della midollare del surrene

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.

Technical Features

Principle of the Method:

The urinary sample is treated with SPE extraction and injected in LC-MS/MS.

Recovery :

>98 %

Normal values in 24 h Urine:

ADRENALINE	2-22 µg/24 h
NORADRENALINE	20-81 µg/24 h
DOPAMINE	40-400 µg/24 h

TOTAL METANEPHRINES IN URINE

METANEPHRINE	52 - 341 µg/24 h
NORMETANEPHRINE	88 - 444 µg/24 h
3-METHOXYTYRAMINE	55 - 247 µg/24 h

FREE METANEPHRINES IN URINE

METANEPHRINE*	Male < 53.3 µg/24 h Female < 33.3 µg/24 h
NORMETANEPHRINE*	Male < 43.2 µg/24 h Female < 35.2 µg/24 h

* Woo HI Clin Biochem 49 (7-8):573-9

Sensitivity (LLOD) :

Metanephrine	1,0 µg/L
Normetanephrine	2,0 µg/L
Adrenaline	0,5 µg/L
Noradrenaline	2,0 µg/L
Dopamine	5,0 µg/L
3-Methoxytyramine	2,0 µg/L

Minimum concentration analyzable (LLOQ) :

Metanephrine	4,4 µg/L
Normetanephrine	7,3 µg/L
Adrenaline	1,2 µg/L
Noradrenaline	7,2 µg/L
Dopamine	32,5 µg/L
3-Methoxytyramine	6,0 µg/L

Linearity :

Metanephrine	4,4 - 5000 µg/L
Normetanephrine	7,3 - 5000 µg/L
Adrenaline	1,2 - 500 µg/L
Noradrenaline	7,2 - 500 µg/L
Dopamine	32,5 - 500 µg/L
3-Methoxytyramine	6,0 - 5000 µg/L

Accuracy intra serie (relative error %)

Metanephrine:

Ci	Cs
154,4 µg/l	465,5 µg/l
4,94%	1,21%

Accuracy inter serie (relative error %)

Metanephrine:

Ci	Cs
154,4 µg/l	465,5 µg/l
4,10%	1,32%

Reproducibility intra serie (coefficient of variation %) Metanephrine:

C LLOQ	Cm	CUP	
4,4 µg/l	307,2 µg/l	578,6 µg/l	
1,02%	0,28%	0,49%	

Reproducibility inter serie (coefficient of variation %) **Metanephrine:**

C LLOQ	Cm	CUP	
4,4 µg/l	307,2 µg/l	578,6 µg/l	
1,85%	0,28%	2,78%	

Accuracy intra serie (relative error %)
Normetanephrine:

Ci	Cs
187,6 µg/l	567,3 µg/l
6,69%	2,20%

Accuracy inter serie (relative error %)
Normetanephrine:

Ci	Cs
187,6 µg/l	567,3 µg/l
4,81%	1,73%

Reproducibility intra serie (coefficient of variation %) **Normetanephrine:**

C LLOQ	Cm	CUP	
7,3 µg/l	372,4 µg/l	707,5 µg/l	
3,10%	3,55%	1,23%	

Reproducibility inter serie (coefficient of variation %) **Normetanephrine:**

C LLOQ	Cm	CUP	
7,3 µg/l	372,4 µg/l	707,5 µg/l	
3,37%	3,92%	2,07%	

Accuracy intra serie (relative error %)
Adrenaline:

Ci	Cs
14,3 µg/l	40,6 µg/l
3,25%	3,04%

Accuracy inter serie (relative error %)
Adrenaline:

Ci	Cs
14,3 µg/l	40,6 µg/l
4,25%	2,74%

Reproducibility intra serie (coefficient of variation %) **Adrenaline:**

C LLOQ	Cm	CUP	
1,2 µg/l	26,1 µg/l	50,7 µg/l	
2,17%	0,95%	0,46%	

Reproducibility inter serie (coefficient of variation %) **Adrenaline:**

C LLOQ	Cm	CUP	
1,2 µg/l	26,1 µg/l	50,7 µg/l	
3,76%	3,72%	4,28%	

Accuracy intra serie (relative error %)
Noradrenaline:

Ci	Cs
33,1 µg/l	94,7 µg/l
3,78%	1,02%

Accuracy inter serie (relative error %)
Noradrenaline:

Ci	Cs
33,1 µg/l	94,7 µg/l
3,33%	2,28%

Reproducibility intra serie (coefficient of variation %) **Noradrenaline:**

C LLOQ	Cm	CUP	
7,2 µg/l	62,9 µg/l	115,1 µg/l	
3,32%	1,83%	0,68%	

Reproducibility inter serie (coefficient of variation %) Noradrenaline:

C LLOQ	Cm	CUP	
7,2 µg/l	62,9 µg/l	115,1 µg/l	
2,29%	2,00%	3,75%	

Accuracy intra serie (relative error %) Dopamine:

Ci	Cs
495,3 µg/l	1.283,2 µg/l
4,34%	5,49%

Accuracy inter serie (relative error %) Dopamine:

Ci	Cs
495,3 µg/l	1.283,2 µg/l
4,64%	4,38%

Reproducibility intra serie (coefficient of variation %) Dopamine:

C LLOQ	Cm	CUP	
32,5 µg/l	822,6 µg/l	1.598,8 µg/l	
0,55%	1,24%	0,14%	

Reproducibility inter serie (coefficient of variation %) Dopamine:

C LLOQ	Cm	CUP	
32,5 µg/l	822,6 µg/l	1.598,8 µg/l	
2,85%	0,88%	2,41%	

Accuracy intra serie (relative error %) 3- Methoxytyramine:

Ci	Cs
150 µg/l	300 µg/l
7,46%	5,80%

Accuracy inter serie (relative error %) 3- Methoxytyramine:

Ci	Cs
150 µg/l	300 µg/l
8,69%	5,88%

Reproducibility intra serie (coefficient of variation %) 3-Methoxytyramine:

C LLOQ	Cm	CUP	
6 µg/l	240 µg/l	500 µg/l	
1,57%	1,82%	0,31%	

Reproducibility inter serie (coefficient of variation %) 3- Methoxytyramine:

C LLOQ	Cm	CUP	
6 µg/l	240 µg/l	500 µg/l	
9,39%	4,09%	5,82%	

Coefficient of Correlation R2 + Dev Std

0,9979 ± 0,0008 Metanephine
 0,9973 ± 0,0001 Normetanephine
 0,9958 ± 0,0028 Adrenaline
 0,9942 ± 0,0027 Noradrenaline
 0,9937 ± 0,0001 Dopamine
 0,9973 ± 0,0001 3-Methoxytyramine

Components of the kit (100 test) :

All the Reagents are ready to use and stable 3 years at 2–8 °C, except **Reagent A**.

Reagent A – Internal Standard Solution, **1 x 0,8 ml**

Store at – 20 °C

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

Reagent C – Conditioning Solution 1, **1 x 100 ml**

Reagent D – Conditioning Solution 2, **1 x 100 ml**

Reagent E – Wash Solution 1, **1 x 100 ml**

Reagent F – Wash Solution 2, **1 x 100 ml**

Reagent G – Eluting Solution, **1 x 60 ml**

Reagent H – Diluting Solution, **1 x 10 ml**

WASH SOLUTION – **2 x 500 ml**

Calibrator in urine – Level 0, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 1, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 2, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 3, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 4, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 5, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Calibrator in urine – Level 6, 2 x 1 ml

Code LC77016 (packed separately – see data sheets)

Reagent I – Reagent of Hydrolysis for Metanephrines, **1 x 5 ml**

Reagent L – Stabilization Solution for Metanephrines, **1 x 5 ml**

Reagent M1 – Mobile Phase M1, **1 x 500 ml**

Reagent M2 – Mobile Phase M2, **2 x 500 ml**

Clean up Columns, 100 pcs

Minimum Instrumental equipment required:

LC-MS/MS Triple Quadrupole with binary pump
MRM mode, ESI positive
Operational Software

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.

ANALYTICAL PROCEDURE

STEP 1 :

INTERNAL STANDARDS PREPARATION

Prepare before each analytic session a suitable volume based on the number of samples (consider 50 µL per sample)

- 920 µl of **Reagent H – Diluting Solution**
- 80 µl of **Reagent A – Internal Standard Solution**

STEP 2 :

Preparation of Samples, Calibrator and Controls

	Calibrator – Level 0	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Calibrator – Level 6	Sample	Control
Reagent B – Buffer Solution	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl
Calibrator – Level 0	400 µl								
Calibrator – Level 1		400 µl							
Calibrator – Level 2			400 µl						
Calibrator – Level 3				400 µl					
Calibrator – Level 4					400 µl				
Calibrator – Level 5						400 µl			
Calibrator – Level 6							400 µl		
Sample								400 µl	
Control									400 µl
Internal Standard Solution previously prepared	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl

Vortex for 10 sec.

STEP 3 : Clean up Columns Conditioning

- Put the clean up columns in the manifold with collect rack under them;
- Drop in column 1,0 ml of **Reagent C – Conditioning Sol. N° 1**
Discharge the eluate
- Drop in column 1,0 ml of **Reagent D – Conditioning Sol. N° 2**
Discharge the eluate

STEP 4 : Loading Samples, Calibrator and Controls

- Pour All Samples, Calibrators and Controls previously prepared in the Clean-up columns
Discharge the eluate

STEP 5: Clean up Columns Washing

- Drop 1 ml of **Reagent E – Wash Solution N° 1** in Clean-up Columns
Discharge the eluate
- Drop 1 ml of **Reagent F – Wash Solution N° 2** in Clean-up Columns
Discharge the eluate

STEP 6 : Eluting

Set up the collect tubes under the Clean up columns

- Drop 250 µl of **Reagent G – Eluting Solution** in Clean-up Columns
Collect the eluate
- Drop 250 µl of **Reagent G – Eluting Solution** in Clean-up Columns
Collect the eluate

Put the eluate into the vials

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

INJECTION :

- Inject 2-10 µl of the eluate in the chromatographic system.

PROCEDURE OF HYDROLYSIS FOR TOTAL METANEPHRINES

HYDROLYSIS OF THE SAMPLE

1. In glass tubes of 10 ml with teflon caps transfer:
 - 500 µl of urine Sample or reconstituted urine Calibrator/Control
 - 500 µl of Water HPLC grade
 - 50 µl of **Reagent I – Reagent of Hydrolysis**
2. Close ermetically the tubes with caps and secure the seal.
3. Incubate at 90°C for 30 minutes
4. Cooling, then add 50 µl of **Reagent L – Stabilization Solution**
5. Control that pH is between 4,5 and 6,0, eventually use NaOH 1N or HCl 1N.

At the end of hydrolysis, the sample should be treated as previous ANALYTICAL PROCEDURE (pages 7 – 8)

CATECHOLAMINES/METANEPHRINES - Warnings

COLUMN CONDITIONING

Install the new analytical column ACQUITY UPLC BEH (100 x 2,1 mm, 1,7 μ m), thermostated at 30 °C. Disconnect the detector and flux a solution of Acetonitrile : H₂O (85 : 15 v/v) set flow at 600 μ l / minute for 15 minutes. **Don't recycle the washing solutions**. Condition the column with the Mobile Phase M2 set flow at 600 μ l / minute for 15 minutes. **Two injection of 50% Water HPLC grade / 50% Acetonitrile before proceeding to the analytical series.**

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

Wash with a solution of Acetonitrile : H₂O (85 : 15 v/v) set flow at 600 μ l / minute for 15 minutes. Store the column in this solution. Do not flux a water percentage more than 40% in the analytical column for any reason.

INJECTION NEEDLE WASHING

Use the Wash Solution

PARAMETERS SET ON WATERS XEVO TQS MICRO

Capillary Voltage	0,4
Desolvation Gas (L/hour)	1000
Cone Gas(L/hour)	0
Desolvation Temperature (°C)	500
Source Temperature(°C)	150

Fragmentations (optimized on TRIPLE QUADRUPOLE WATERS XEVO TQS MICRO)

Analyte	Transitions MRM m/z	COLLISION ENERGY
Metanephrine	198.1>180	8
	198.1>165.1	18
Normetanephrine	184.1>166	8
	184.1>134.1	18
3-Methoxytyramine	168.0>91.0	22
	168.0>119.0	20
Dopamine	154.0>91	20
	154.0>119	18
Epinefrine	184,1>166	8
	184.1>107	20
Norepinefrine	170>152	6
	170>107	20

REFERENCE INTERNAL STANDARD

Molecule	Internal Standard
Adrenaline	Adrenaline-d6
Noradrenaline	Noradrenaline-d6
Dopamine	Dopamine-d4
Methanephrine	Methanephrine-d3
Normethanephrine	Normethanephrine-d3
3-Metoxytyramine	Methanephrine-d3

GRADIENT

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	0	100	600
1.0	0	100	600
2.0	10	90	600
2.1	10	90	1000
2.5	30	70	1000
2.6	0	100	1000
3.9	0	100	1000
4.0	0	100	600

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC77016	Calibrator in urine for Catecholamines/Metanephtrines	7 x 2 x 1 ml
LC77019	Control in urine for Biogenic Ammines - Levels 1, 2 and 3	3 x 5 x 1 ml
LC77011	Mobile Phase M1	4 x 500 ml
LC77021	Mobile Phase M2	4 x 500 ml
SK77010	Starter kit for Catecholamines/Metanephtrines	11 x 1 ml
LC77030	Internal Standard Mix Solution	1 x 800 µl
S186004801	ACQUITY UPLC BEH AMIDE Analytical Column (100 x 2,1 mm, 1,7 µm)	1 Pc
S51843550	Glass vials with globet form	100 Pcs
S51820717	Caps for Glass vials with globet form	100 Pcs

CATECHOLAMINES/METANEPHRINES IN URINE (Reference Chromatograms)

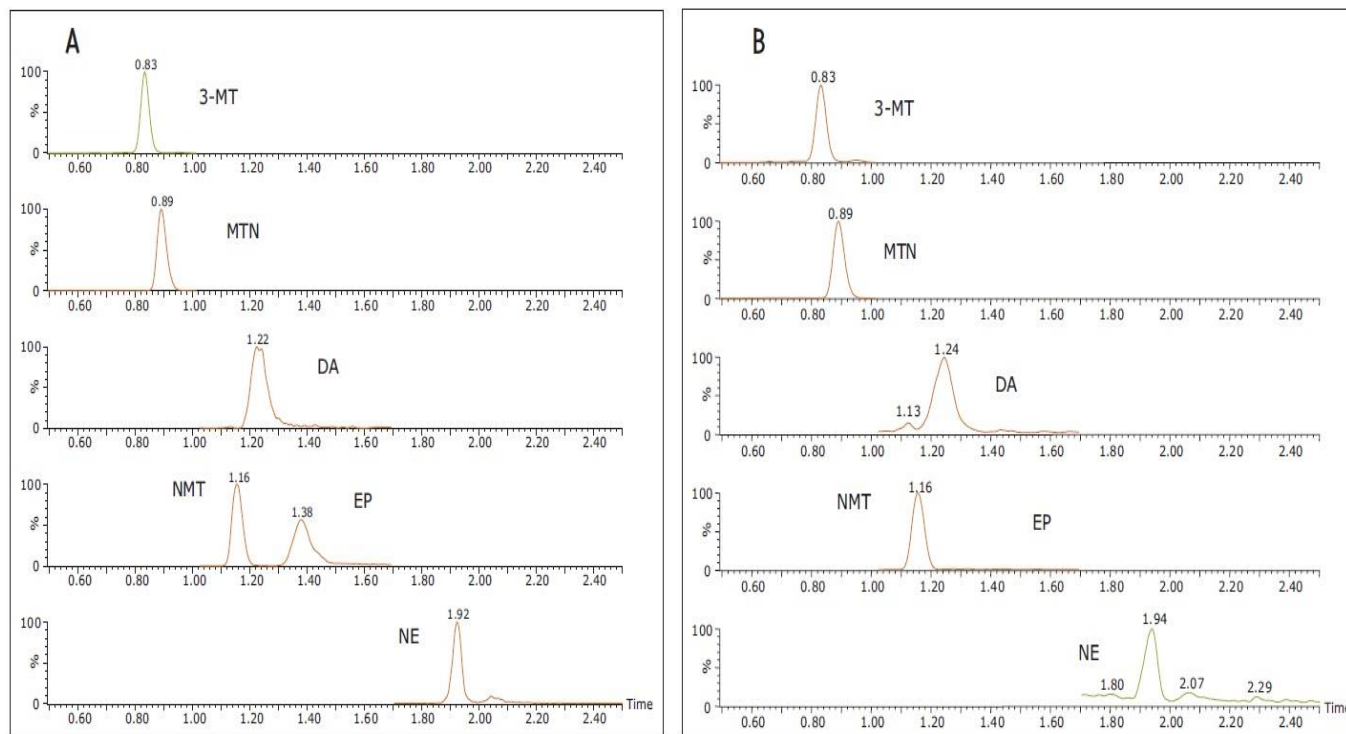


Figure 2. Chromatography of catecholamines and metanephrines on the ACQUITY UPLC BEH Amide Column, 1.7 μ m, 2.1 x 100 mm.

A. Representative 50 ng/mL calibration standard.

B. Unspiked urine sample showing chromatography of endogenous catecholamines and metanephrines. Chromatographic conditions are detailed in the methods section.