

CATECOLAMINES/METANEPHRINES IN PLASMA BY LC/MS

Code LC97010

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.

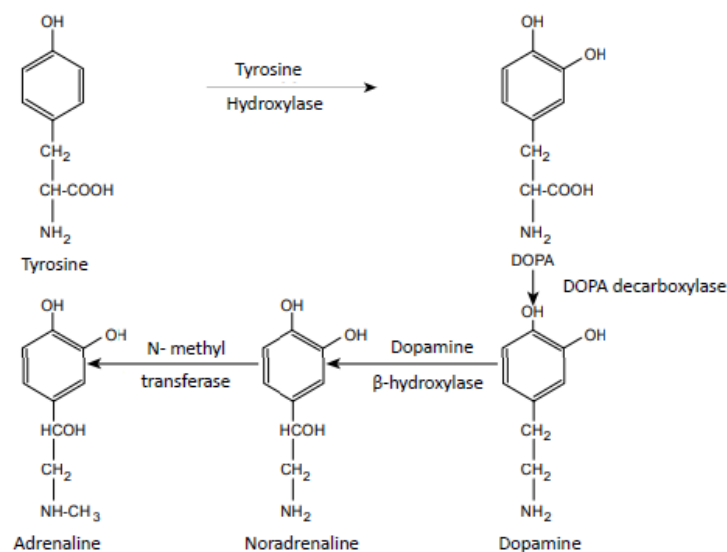


Fig 1. Biosynthesis of catecholamines

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° 006

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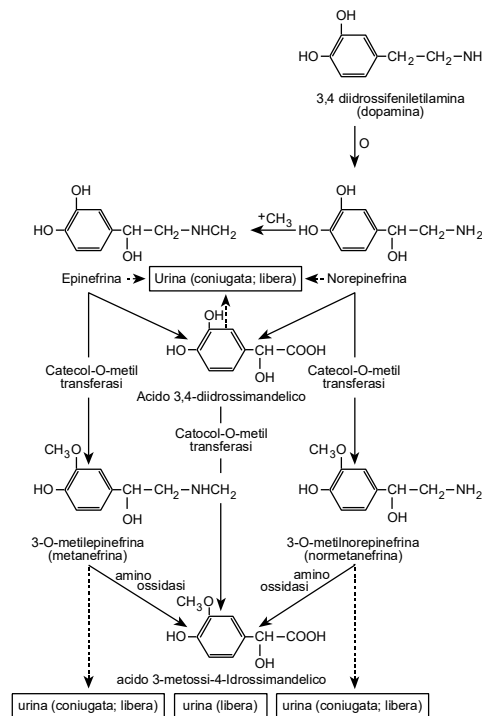


Figura 2: Metabolismo degli ormoni della midollare del surrene

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:

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

Recovery :

>98 %

Normal values :

Analyte	Position	pg/mL	nmol/L (approx.)
Norepinephrine	Supine	70–750	0.41–4.43
	Standing	200–1,700	1.18–10.05
Epinephrine	Supine	≤111	≤0.61
	Standing	≤141	≤0.77
Dopamine	—	<30	<0.20
Metanephrine	—	15.8–100.6	0.08–0.51
Normetanephrine	—	22.0–216.2	0.12–1.18
3-Methoxytyramine	—	<30.1	<0.18

Ref. www.mayocliniclabs.com

Sensitivity (LLOD) :

Metanephrine	1,0 ng/L
Normetanephrine	5,0 ng/L
Adrenaline	10,0 ng/L
Noradrenaline	10,0 ng/L
Dopamine	10,0 ng/L
3-Methoxytyramine	5,0 ng/L

Minimum Concentration analyzable (LLOQ) :

Metanephrine	5,0 ng/L
Normetanephrine	15,0 ng/L
Adrenaline	25,0 ng/L
Noradrenaline	25,0 ng/L
Dopamine	25,0 ng/L
3-Methoxytyramine	15,0 ng/L

Linearity :

Metanephrine	5,0 – 5.000 ng/L
Normetanephrine	15,0 – 5.000 ng/L
Adrenaline	25,0 – 5.000 ng/L
Noradrenaline	25,0 – 5.000 ng/L
Dopamine	25,0 – 5.000 ng/L
3-Methoxytyramine	15,0 – 5.000 ng/L

Accuracy intra serie (relative error %)
Metanephrine:

Ci	Cs
100 ng/l	500 ng/l
8,24%	7,98%

Accuracy inter serie (relative error %)
Metanephrine:

Ci	Cs
100 ng/l	500 ng/l
10,82%	5,87%

Reproducibility intra serie (coefficient of variation %)
Metanephrine:

C LLOQ	Cm	CUP
5,0 ng/l	250 ng/l	1000 ng/l
3,43%	4,87%	1,09%

Reproducibility inter serie (coefficient of variation %)
Metanephrine:

C LLOQ	Cm	CUP
7,2 ng/l	62,9 ng/l	115,1 ng/l
5,93%	7,36%	2,42%

Accuracy intra serie (relative error %)
Normetanephrine:

Ci	Cs
100 ng/l	500 ng/l
6,31%	1,45%

Accuracy inter serie (relative error %)
Normetanephrine:

Ci	Cs
100 ng/l	500 ng/l
8,84%	1,93%

Reproducibility intra serie (coefficient of variation %)
Normetanephrine:

C LLOQ	Cm	CUP
15 ng/l	250 ng/l	1000 ng/l
7,85%	5,83%	1,80%

Reproducibility inter serie (coefficient of variation %)
Normetanephrine:

C LLOQ	Cm	CUP
7,2 ng/l	62,9 ng/l	115,1 ng/l
7,39%	5,20%	3,95%

Accuracy intra serie (relative error %)
Adrenaline:

Ci	Cs
100 ng/l	500 ng/l
9,43%	6,10%

Accuracy inter serie (relative error %)
Adrenaline:

Ci	Cs
100 ng/l	500 ng/l
6,79%	4,06%

Reproducibility intra serie (coefficient of variation %)
Adrenaline:

C LLOQ	Cm	CUP
25 ng/l	250 ng/l	1000 ng/l
7,06%	0,99%	1,05%

Reproducibility inter serie (coefficient of variation %)
Adrenaline:

C LLOQ	Cm	CUP
7,2 ng/l	62,9 ng/l	115,1 ng/l
7,23%	12,32%	5,12%

Accuracy intra serie (relative error %)
Noradrenaline:

Ci	Cs
100 ng/l	500 ng/l
12,25%	2,24%

Accuracy inter serie (relative error %)
Noradrenaline:

Ci	Cs
100 ng/l	500 ng/l
9,75%	3,30%

Reproducibility intra serie (coefficient of variation %)
Noradrenaline:

C LLOQ	Cm	CUP
25 ng/l	250 ng/l	1000 ng/l
8,34%	3,18%	1,11%

Reproducibility inter serie (coefficient of variation %)
Noradrenaline:

C LLOQ	Cm	CUP
25 ng/l	250 ng/l	1000 ng/l
6,90%	4,69%	3,70%

Accuracy intra serie (relative error %)
Dopamine:

Ci	Cs
100 ng/l	500 ng/l
7,26%	5,39%

Accuracy inter serie (relative error %)
Dopamine:

Ci	Cs
100 ng/l	500 ng/l
9,70%	4,30%

Reproducibility intra serie (coefficient of variation %)
Dopamine:

C LLOQ	Cm	CUP
25 ng/l	250 ng/l	1000 ng/l
5,30%	6,74%	2,48%

Reproducibility inter serie (coefficient of variation %)
Dopamine:

C LLOQ	Cm	CUP
7,2 ng/l	62,9 ng/l	115,1 ng/l
7,18%	5,57%	6,13%

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

Ci	Cs
100 ng/l	500 ng/l
12,05%	0,81%

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

Ci	Cs
100 ng/l	500 ng/l
10,93%	1,76%

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

C LLOQ	Cm	CUP
15 ng/l	250 ng/l	1000 ng/l
7,58%	4,25%	1,47%

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

C LLOQ	Cm	CUP
7,2 ng/l	62,9 ng/l	115,1 ng/l
7,89%	3,53%	1,52%

Coefficient of Correlation R2 + Dev Std

0,9959 ± 0,0035 Metanephine
 0,9988 ± 0,0008 Normetanephine
 0,9983 ± 0,0011 Adrenaline
 0,9983 ± 0,0003 Noradrenaline
 0,9977 ± 0,0017 Dopamine
 0,9970 ± 0,0005 3-Methoxytyramine

Components of the kit :

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 1 ml

Store at -25/-15 °C

Reagent B – Buffer Solution, 1 x 25 ml

Reagent C – Conditioning Solution 1, 1 x 20 ml

Reagent D – Conditioning Solution 2, 1 x 20 ml

Reagent E – Wash Solution 1, 1 x 20 ml

Reagent F – Wash Solution 2, 1 x 20 ml

Reagent G – Soluzione di Eluizione, 1 x 5 ml

Reagent H – Diluting Solution, 1 x 5 ml

Plasma Calibrator – Level 0, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 1, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 2, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 3, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 4, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 5, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Plasma Calibrator – Level 6, 2 x 1 ml

Code LC97016 (packed separately – see data sheets)

Reagent M1 – Mobile Phase M1, 1 x 500 ml

Reagent M2 – Mobile Phase M2, 2 x 500 ml

WASH SOLUTION, 2 x 500 ml

Clean up columns, Well Plates 96

Collect Rack, Well Plates 96

Minimum Instrumental equipment required:

LC-MS/MS Triple Quadrupole with binary pump
 MRM mode, ESI positive
 Operational Software
 Positive pressure manifold for microplates management

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.

ANALYTICAL PROCEDURE

STEP 1 :

INTERNAL STANDARD PREPARATION

Prepare before every analytical session a suitable volume based on the number of samples (considered 50 uL every sample)

- 900 µl of **Reagent H – Diluting Solution**
- 100 µl of **Reagent A – Internal Standard Solution**

STEP 2 :

Preparation of Samples, Calibrator and Controls

	Reconstituted Calibrator	Reconstituted Control	Sample	Reagent B – Buffer Solution	Previously Prepared Internal Standard
Calibrator	250 µL			250 µL	50 µL
Control		250 µL		250 µL	50 µL
Sample			250 µL	250 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 200 ul of **Reagent C – Conditioning Sol. N° 1**
Discharge the eluate
 - Set the pressure to 6 psi and wait for the SPE columns to completely empty;
- Drop in column 200 ul of **Reagent D – Conditioning Sol. N° 2**
Discharge the eluate
 - Set the pressure to 6 psi and wait for the SPE columns to completely empty;

STEP 4 : Samples, Calibrator and Controls Loading

- Pour All Samples, Calibrators and Controls previously prepared in the Clean-up columns
Discharge the eluate
 - Set the pressure to 6 psi and wait for 5 minutes;
 - Set the pressure to 12 psi and wait for the SPE columns to completely empty;

STEP 5: Clean up Columns Washing

- Drop 200 ul of **Reagent E – Wash Solution N° 1** in Clean-up Columns
Discharge the eluate
 - Set the pressure to 12 psi and wait for the SPE columns to completely empty;
- Drop 200 ul of **Reagent F – Wash Solution N° 2** in Clean-up Columns
Discharge the eluate
 - Set the pressure to 12 psi and wait for the SPE columns to completely empty;
 - Dry the column tips on absorbent paper;

STEP 6 : Eluting

Set up the collect rack under the Clean up columns

- Drop 25 µl of **Reagent G – Eluting Solution** *Collect the eluate*
 - Set the pressure to 6 psi and wait for 5 minutes;
 - Set the pressure to 12 psi and wait for the SPE columns to completely empty;
- Drop 25 µl of **Reagent G – Eluting Solution** *Collect the eluate*
 - Set the pressure to 6 psi and wait for 5 minutes;
 - Set the pressure to 12 psi and wait for the SPE columns to completely empty;

N.B.: at this step the sample is stable 1 day at 2-8 °C

INJECTION :

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

CATECHOLAMINES/METANEPHRINES - Warnings

COLUMN CONDITIONING

Install the new analytical column ACQUITY UPLC BEH (100 x 2,1 mm, 1,7 um), thermostated at 30 °C. Disconnect the detector and flux the **WASH SOLUTION** set flow at 600 ul / minute for 15 minutes. **Don't recycle the washing solutions**. Condition the column with the Mobile Phase M2 set flow at 600 ul / minute for 15 minutes. **Two injection of 50% Water HPLC grade / 50% Methanol 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 the **WASH SOLUTION** set flow at 600 ul / 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
Adrenaline	184,1>166	8
	184.1>107	20
Noradrenaline	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-Metoxetyramine	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
LC97016	Calibrator for Biogenic Amines in plasma	7 x 2 x 1 ml
LC97019	Control for Biogenic Amines in plasma - Levels 1, 2 and 3	3 x 5 x 1 ml
SK97010	Starter kit for Catecholamines/Metanephrines	11 x 1 ml
LC97011	Mobile Phase M1	4 x 500 ml
LC97021	Mobile Phase M2	4 x 500 ml
S186004801	ACQUITY UPLC BEH (100 x 2,1 mm, 1,7 µm) Analytical Column	1 Pc
S51843550	Clear Glass Vials with reduced volume (goblet form)	1 x 100 Pcs
S51820717	Caps for glass Vials with reduced volume (goblet form)	1 x 100 Pcs

Bibliography

- Eisenhofer G: Free or total metanephrines for diagnosis of pheochromocytoma: what is the difference? Clin Chem 2001 June;47(6):988-989
- Lenders JW, Pacek K, Walther MM, et al: Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 2002 Mar 20;287(11):1427-1434
- Sawka AM, Jaeschke R, Singh RJ, Young WF Jr: A comparison of biochemical tests for pheochromocytoma: measurement of fractionated plasma metanephrines compared to the combination of 24-hour urinary metanephrines and catecholamines. J Clin Endocrinol Metab 2003 Feb;88(2):553-558
- Algeciras-Schimmich A, Preissner CM, Young WF Jr, et al: Plasma chromogranin A or urine fractionated metanephrines follow-up testing improves the diagnostic accuracy of plasma fractionated metanephrines for pheochromocytoma. J Clin Endocrinol Metab Oct 16, 2007;doi:doi:10.1210/jc.2007-1354
- Young WF Jr: Pheochromocytoma and primary aldosteronism. In Endocrine Neoplasms. Edited by A Arnold. Boston, Kluwer Academic Publishers, 1997, pp 239-261
- Hernandez FC, Sanchez M, Alvarez A, et al: A five-year report on experience in the detection of pheochromocytoma. Ann Intern Med 2000;33:649-655
- Pacak K, Linehan WM, Eisenhofer G, et al: Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. Ann Intern Med 2001;134:315-329
- Alexander F: Neuroblastoma. Urol Clin North Am 2000;27:383-392
- McDougall AJ, McLeod JG: Autonomic neuropathy, I. Clinical features, investigation, pathophysiology, and treatment. J Neurol Sci 1996;137:79-88
- Lenders JW, Pacak K, Walther MM, et al: Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 2002;287:1427-1434
- Peaston RT, Graham KS, Chambers E, van der Molen JC, Ball S, Performance of plasma free metanephrines measured by liquid chromatography-tandem mass spectrometry in the diagnosis of pheochromocytoma. Clin Chim Acta 2010; 411:546-5

CATECHOLAMINES/METANEPHRINES IN PLASMA BY LC/MS (Reference chromatograms)

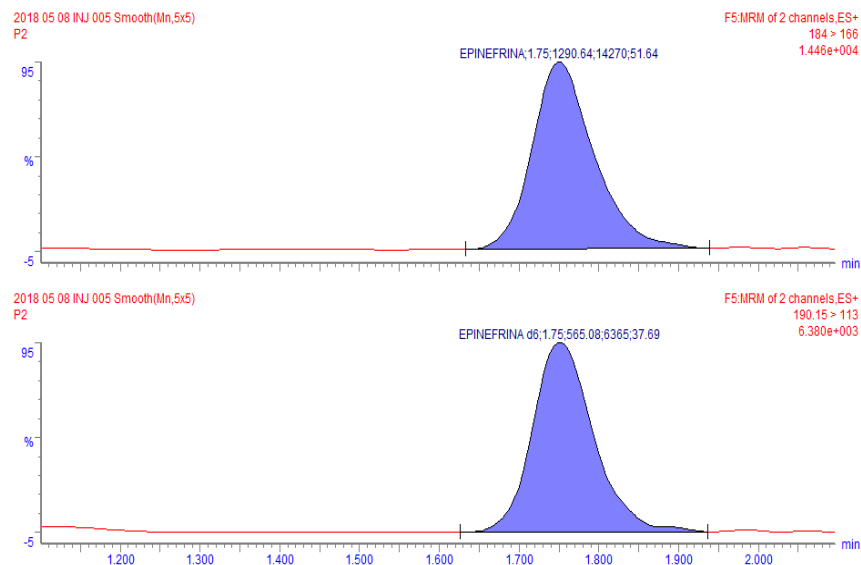


Fig. 1 :	Calibrator in plasma		
	R.T. 1.75	Adrenaline	250 ng/L
	R.T. 1.75	Adrenaline-D6	250 ng/L

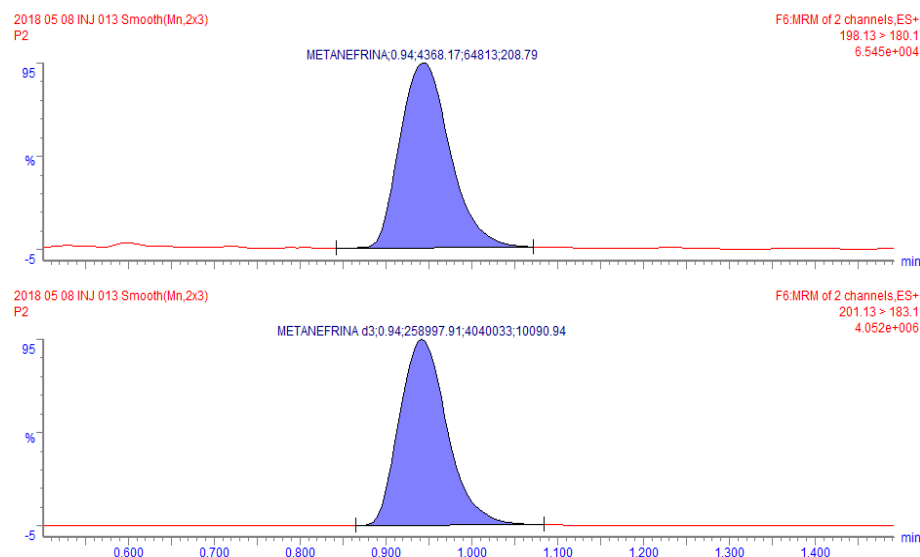


Fig. 2 :	Calibrator in plasma		
	R.T. 0.94	Metanephrine	250 ng/L
	R.T. 0.94	Metanephrine-D3	250 ng/L

CATECHOLAMINES/METANEPHRINES IN PLASMA BY LC/MS (Reference chromatograms)

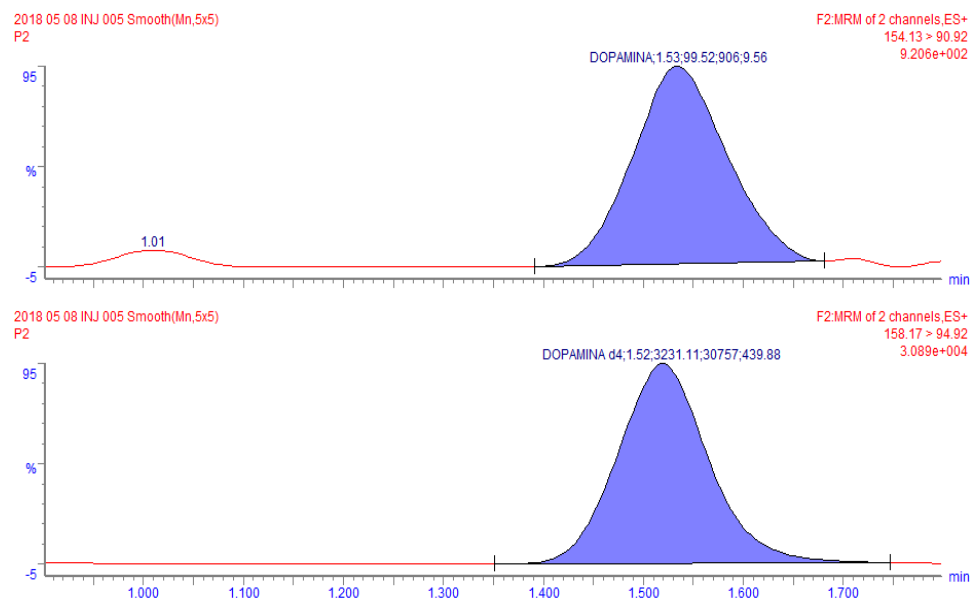


Fig. 3 :	Calibrator in plasma		
	R.T. 1.53	Dopamine	250 ng/L
	R.T. 1.52	Dopamine-D4	250 ng/L

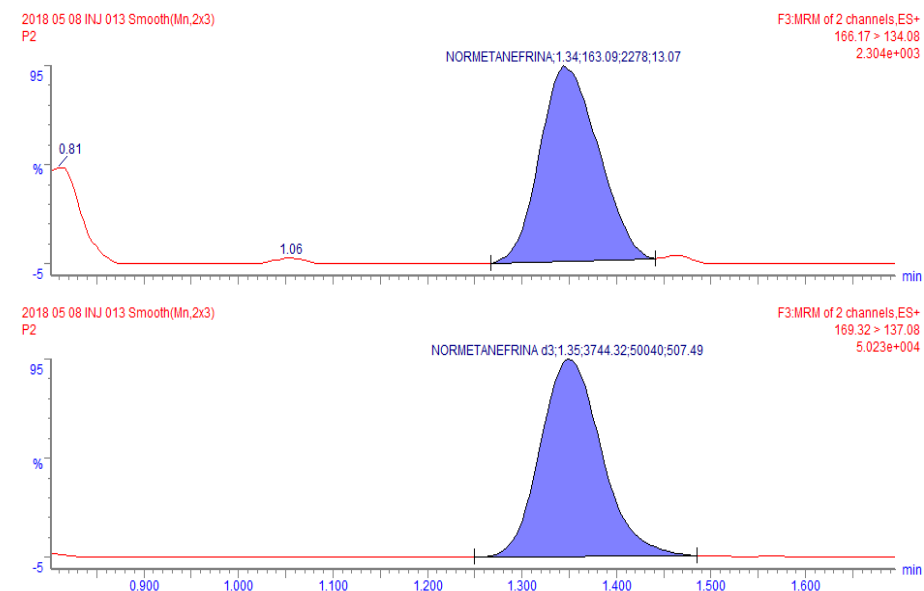


Fig. 4 :	Calibrator in plasma		
	R.T. 1.34	Normetanephrine	250 ng/L
	R.T. 1.35	Normetanephrine-D3	250 ng/L

CATECHOLAMINES/METANEPHRINES IN PLASMA BY LC/MS (Reference chromatograms)

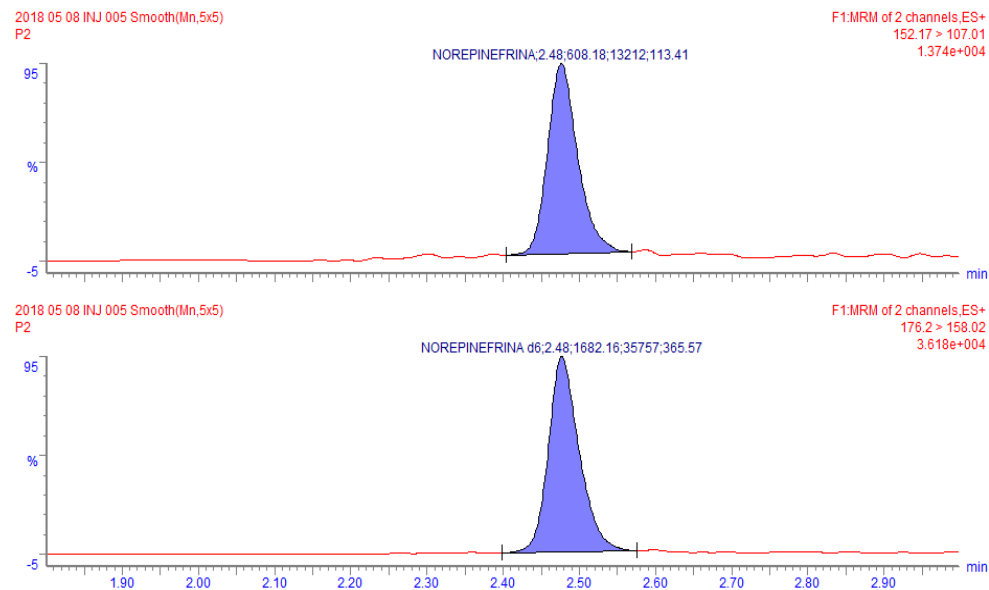


Fig. 5 :	Calibrator in plasma		
	R.T. 2.48	Noradrenaline	250 ng/L
	R.T. 2.48	Noradrenaline-D6	250 ng/L

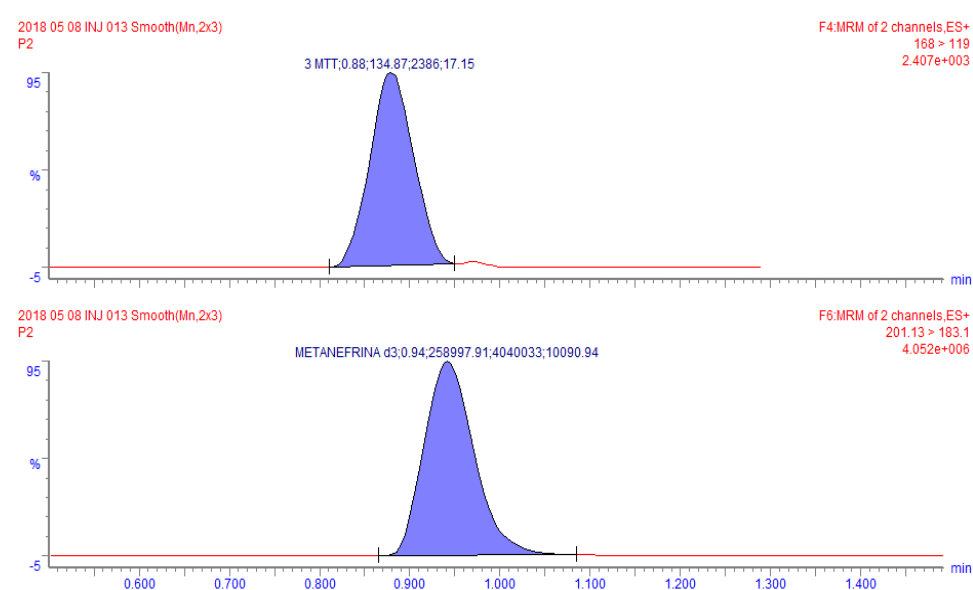


Fig. 6 :	Calibrator in plasma		
	R.T. 0.88	3-Methoxytyramine	250 ng/L
	R.T. 0.94	Metanephrine-D3	250 ng/L