VMA, 5-HIAA and HVA IN URINE BY LC/MS

Code LC14610

pro training
ASSISTANCE

CE **This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).**

The declaration of conformity (CE) is available upon request.

INTRODUCTION

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.

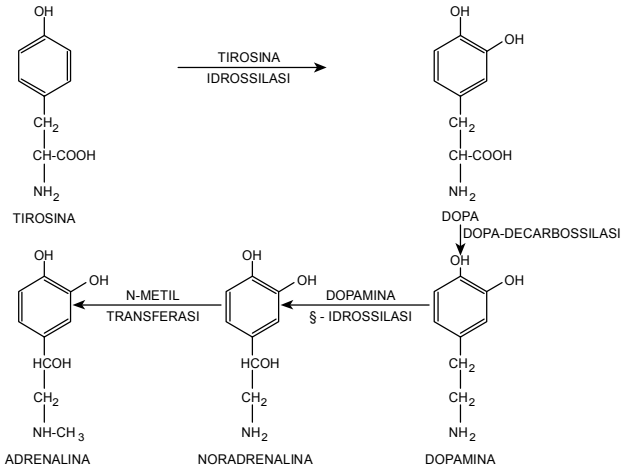


Figura 1: Biosintesi delle catecolamine

Fig 1 : Biosynthesis of catecholamines

CATABOLISM

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

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

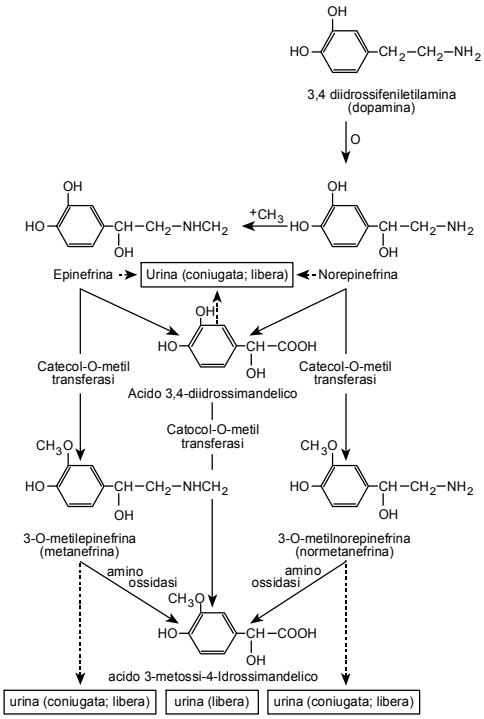


Figura 2: Metabolismo degli ormoni della midollare del surrene

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, pheocromocytoma 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. Assays of urinary and plasma catecholamines are useful in establishing a diagnosis and following the results of treatment.

TECHNICAL FEATURES

PRINCIPLE OF THE METHOD

The sample was diluted and injected into LC-MS/MS.

RECOVERY:	52,8 – 84,4 %
SENSITIVITY (LLOD):	Vanilmandelic Acid 0,008 mg/L 5-HydroxyIndoleAcetic Acid 0,01 mg/L Homovanillic Acid 0,03 mg/L
MINIMUM CONCENTRATION ANALIZABLE (LLOQ):	Vanilmandelic Acid 0,02 mg/L 5-HydroxyIndoleAcetic Acid 0,04 mg/L Homovanillic Acid 0,1 mg/L
LINEARITY:	Vanilmandelic Acid 0,02 - 250 mg/L 5-HydroxyIndoleAcetic Acid 0,04 - 250 mg/L Homovanillic Acid 0,1 - 250 mg/L
NORMAL VALUES IN 24 H URINE:	Vanilmandelic Acid 1,8 – 6,7 mg/24 h 5-HydroxyIndoleAcetic Acid 0,5 – 8,2 mg/24 h Homovanillic Acid 0,5 – 6,2 mg/24 h

Accuracy intra serie (relative error %)
Vanilmandelic Acid:

Ci	Cs
0,4 mg/l	8,2 mg/l
11,79%	5,19%

Accuracy inter serie (relative error %)
Vanilmandelic Acid:

Ci	Cs
0,4 mg/l	8,2 mg/l
10,61%	4,75%

Reproducibility intra serie (coefficient of variation %)
Vanilmandelic Acid:

C LLOQ	Cm	CUP
0,02 mg/l	1,8 mg/l	13,9 mg/l
2,17%	4,32%	2,56%

Reproducibility inter serie (coefficient of variation %)
Vanilmandelic Acid:

C LLOQ	Cm	CUP
0,02 mg/l	1,8 mg/l	13,9 mg/l
8,32%	5,86%	6,05%

Accuracy intra serie (relative error %)
5-HydroxyIndole Acetic Acid:

Ci	Cs
0,4 mg/l	9,8 mg/l
7,48%	4,40%

Accuracy inter serie (relative error %)
5-HydroxyIndole Acetic Acid:

Ci	Cs
0,4 mg/l	9,8 mg/l
5,06%	3,47%

Reproducibility intra serie (coefficient of variation %)
5-HydroxyIndole Acetic Acid:

C LLOQ	Cm	CUP
0,04 mg/l	1,6 mg/l	13,5 mg/l
7,97%	2,24%	0,43%

Reproducibility inter serie (coefficient of variation %) 5-HydroxyIndole Acetic Acid:

C LLOQ	Cm	CUP
0,04 mg/l	1,6 mg/l	13,5 mg/l
5,37%	4,98%	3,11%

Accuracy intra serie (relative error %) Homovanillic Acid:

Ci	Cs
1,0 mg/l	5,3 mg/l
7,12%	9,38%

Accuracy inter serie (relative error %) Homovanillic Acid:

Ci	Cs
1,0 mg/l	5,3 mg/l
8,25%	7,08%

Reproducibility intra serie (coefficient of variation %) Homovanillic Acid:

C LLOQ	Cm	CUP
0,1 mg/l	2,6 mg/l	14,4 mg/l
7,97%	2,64%	1,21%

Reproducibility inter serie (coefficient of variation %) Homovanillic Acid:

C LLOQ	Cm	CUP
0,1 mg/l	2,6 mg/l	14,4 mg/l
6,36%	3,53%	1,71%

Coefficient of Correlation R2 + Dev Std:

0,9974 ± 0,0003 Vanilmandelic Acid
0,9970 ± 0,0007 5-Hydroxyindolacetic Acid
0,9981 ± 0,0013 Homovanillic Acid

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Internal Standard Solution	***1 x 2 ml***	**Store at -20 °C**
Reagent B – Diluting Solution	***1 x 20 ml***	
Calibrator in urine – Level 0	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 1	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 2	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 3	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 4	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 5	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Calibrator in urine – Level 6	***2 x 1 ml***	**Code LC77016** (Packed separately – see data sheet)
Reagent M1 – Mobile Phase M1	***1 x 500 ml***	
Reagent M2 – Mobile Phase M2	***2 x 500 ml***	

All the reagents are ready to use and stable 3 years at 2-8 °C,
except the Reagent A that must be stored at -20 °C.
As regards the preservation method of the Lyophilized Calibration Standard, it is described in the dedicated technical sheet.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC77016	Calibrator in urine for Biogenic Amines	7 x 2 x 1 ml
LC77017	Control in plasma for Biogenic Amines - Level 1	5 x 1 ml
LC77018	Control in plasma for Biogenic Amines - Level 2	5 x 1 ml
LC77013	Control in plasma for Biogenic Amines - Level 3	5 x 1 ml
LC77019	Control in plasma for Biogenic Amines – Levels 1, 2 and 3	3 x 5 x 1 ml
SK14610	Starter kit for VMA/HVA/5-HIAA in urine	1 Pc
S959757902	Zorbax RRHD C18 (50 x 2,1 mm, 1,8 um) Analytical Column	1 Pc

Bibliography

1- Clinica Chimica Acta 398 53-56 ‘ HPLC-mass spectrometry method for quantitative detection of neuroendocrine tumor markers: Vanilmandelic acid, homovanillic acid and 5-hydroxyindoleacetic acid.’
2- JALM 01:04 387-399 ‘Practical LC-MS/MS Method for 5-Hydroxyindoleacetic acid in urine’
3- Current Opinions in Biotechnology 2017, 43:34-40 ‘The future of NMR-based metabolomics’
Chimica Clinica Acta, accepted manuscript ‘Simple dilute-and-shot method for urinary vanilmandelic and homovanillic acid by liquid chromatography tandem mass spectrometry’

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole with binary pump
MRM mode, ESI negative

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.

ANALYTICAL PROCEDURE

STEP 1

Dispense in a vial:

- 180 µl of **Reagent B –** Diluting Solution
- 20 µl of **Reagent A –** Internal Standard Solution
- 20 µl of **Calibrator (C0-C6)/Controls/Samples**

Vortex for 10 sec.

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

INJECTION

- Inject 2 µl of solution in HPLC system.

HVA, 5-HIAA and VMA in LC/MS - Warnings

COLUMN CONDITIONING

Install the new analytical column Zorbax RRHD C18 (50 x 2,1 mm, 1,8 um), termostatated at 60 °C. Disconnect the detector and flux a solution of Mobile Phase M2 : Mobile Phase M1 (90 : 10 v/v) set flow at 400 ul / minute for 20 minutes. Condizionate the column with a solution of Mobile Phase M2 : Mobile Phase M1 (90 : 10 v/v) set flow at 400 ul / minute for 15 minutes. **Don't recycle the washing solutions**. Condizionate the column with a solution of Mobile Phase M2 set flow at 400 ul / 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 and STORAGE

Wash with a solution of Mobile Phase M2 : Mobile phase M1 (90 : 10 v/v) set flow at 400 ul / minute for 20 minutes. Store the column in this solution.

INJECTION NEEDLE WASHING

Wash with a solution of Methanol : H_2O (85 : 15 v/v).

PARAMETERS SET ON WATERS XEVO TQS MICRO

Capillary Voltage (kV)	3,0
Desolvation Gas (L/hour)	1000
Desolvation Temperature (°C)	600
Source Temperature(°C)	150

Fragmentations (optimized on TRIPLE QUADRUPOLE WATERS XEVO TQS MICRO)

Analyte	Transitions MRM m/z	COLLISION ENERGY
HVA	181.0>121.9	15
5-HIAA	190>145.9	5
VMA	197.0>137.9	10
HVA-D5	186.0>142.0	6
5-HIAA-13C6	196.0>152.0	10
VMA-D3	200.0>137.0	18

GRADIENT

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	100	0	400
0.5	100	0	400
3.5	70	30	400
3.51	0	100	400
4.50	0	100	400
4.51	100	0	400
6.0	100	0	400

VMA, 5-HIAA and HVA BY LC/MS

(Reference Chromatograms)

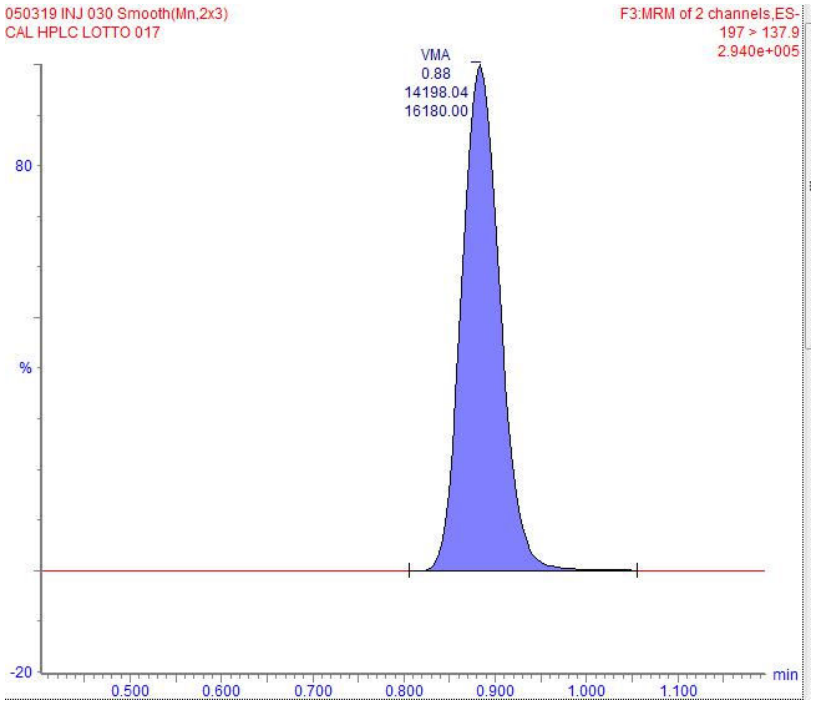


Fig. 3 :	*Urine Calibrator*
	R.T. 0.88 VMA

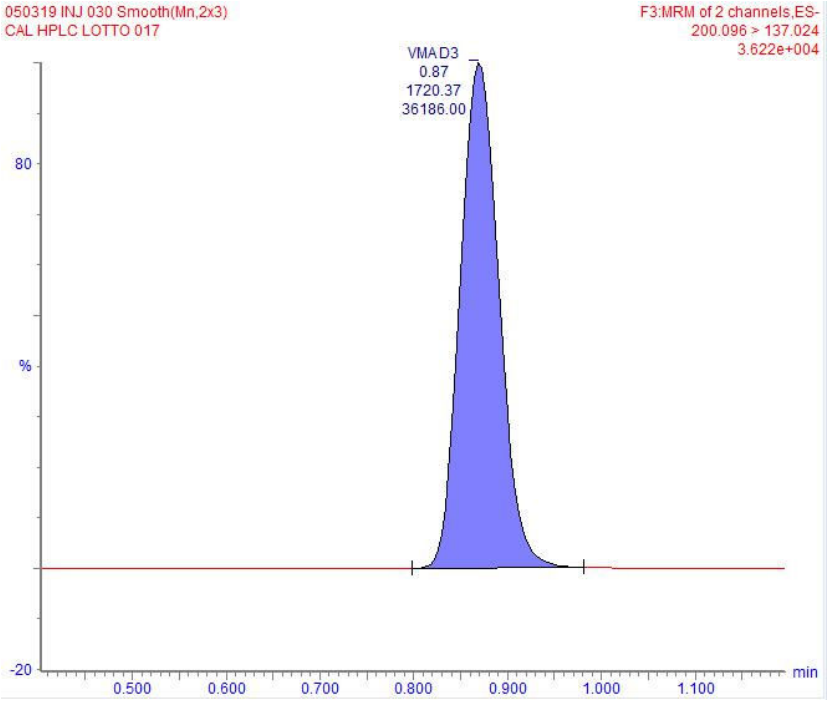


Fig. 4 :	*Urine Calibrator*
	R.T. 0.87 VMA-D3

VMA, 5-HIAA and HVA BY LC/MS

(Reference Chromatograms)

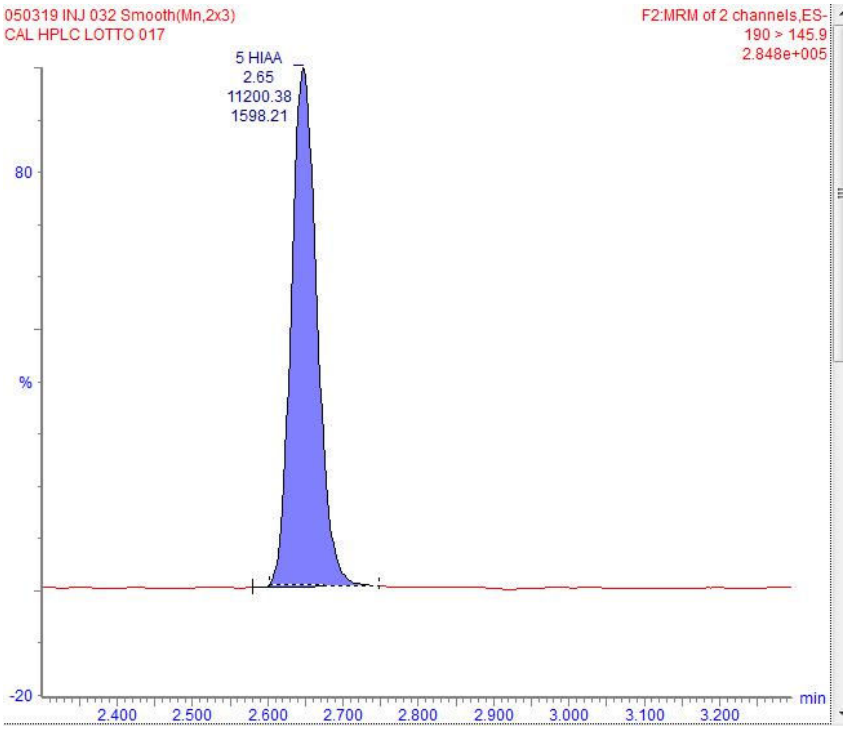


Fig. 5 :	*Urine Calibrator*
	R.T. 2.65 5-HIAA

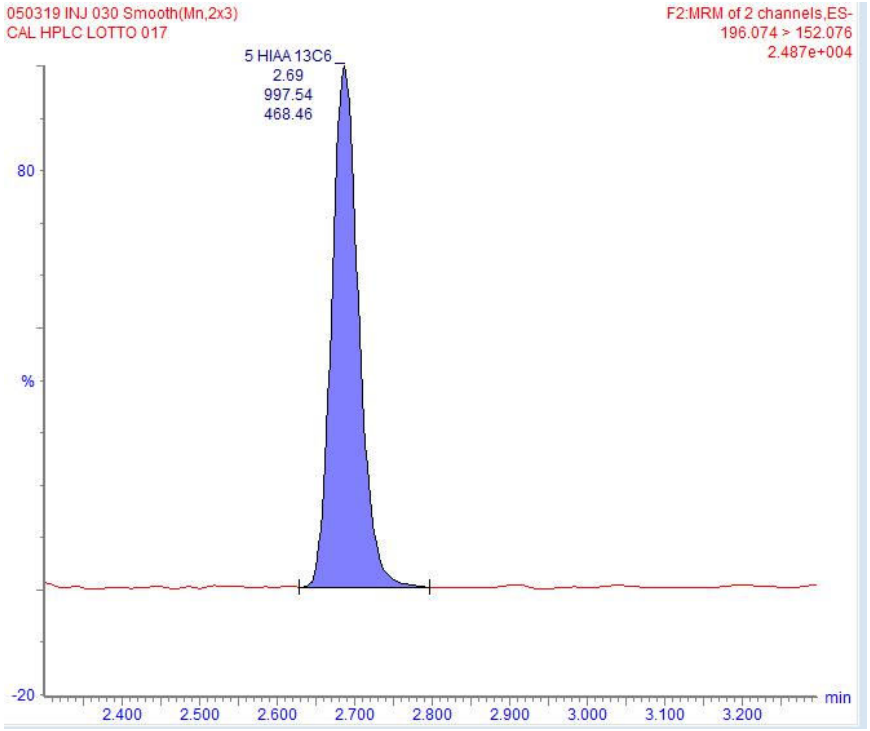
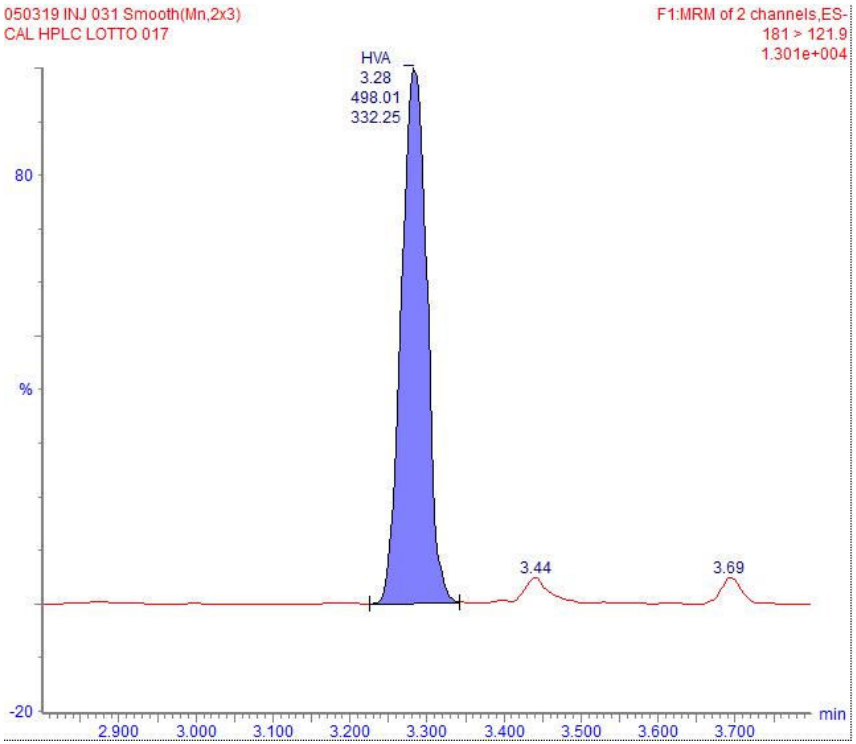


Fig. 6 :	*Urine Calibrator*
	R.T. 2.69 5-HIAA-13C6

VMA, 5-HIAA and HVA BY LC/MS

(Reference Chromatograms)



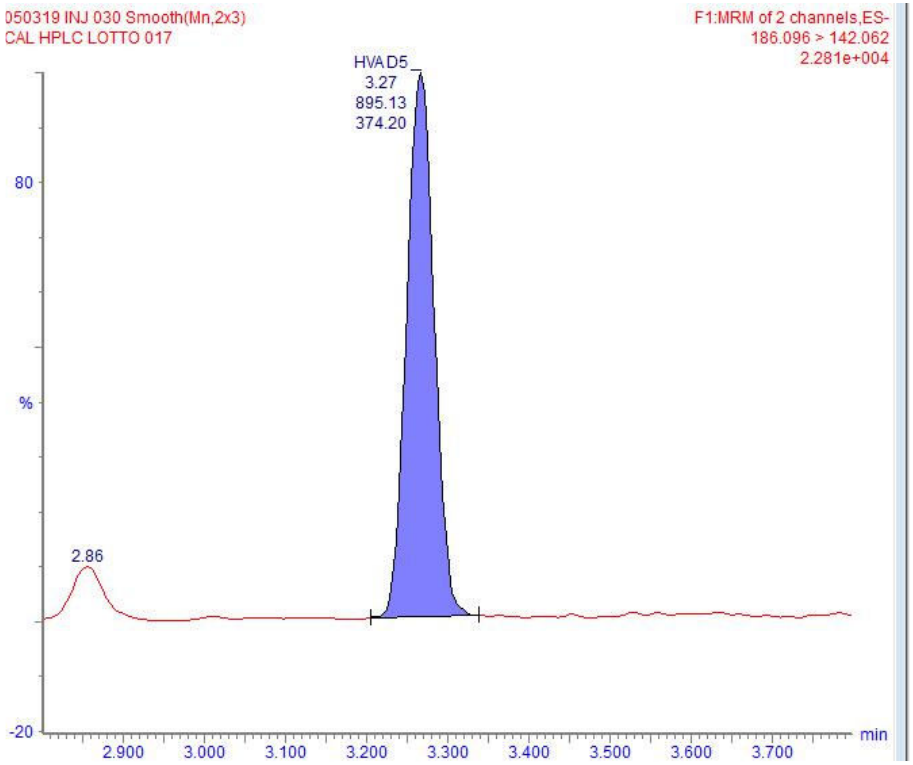


Fig. 7 :	***Urine Calibrator***
	R.T. 3.28 HVA

Fig. 8 :	***Urine Calibrator***
	R.T. 3.27 HVA-D5

eureka kit

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