

OPIATES in urine by GC-MS – Code GC45010
(Morphine, Dihydrocodeine, Codeine, Ethylmorphine, 6-Monoacetylmorphine)
Method of Confirmation by GC-MS

INTRODUCTION

Heroin (diacetylmorphine) is a semi-synthetic drugs of abuse obtained from morphine, a major opium alkaloids, by reaction with acetic anhydride. Inside the body, heroin is rapidly hydrolysed to 6-monoacetylmorphine and then to morphine. It is then conjugated with glucuronic acid in position 3 and 6 of the morphine molecule forming three six glucuronide. The latter two metabolites are excreted in urine together with a small percentage of free morphine. We can also find small amounts of codeine, morphine, opium, and acetylcodeine which is obtained by acetylation of codeine during the preparation of heroin. The methods of screening measure, through an antigen-antibody reaction, the opioid class of substances.

In the event of a positive screening (cut-off > or = 300 ng/ml) analysis is confirmed, usually with gaseous liquid chromatography coupled with a mass spectrometry detector. This method enables the total morphine urine (after hydrolysis of glucuronide), codeine and 6-monoacetylmorphine may be present in small quantities after extraction of these substances from the urine matrix in micro solid phase extraction columns.

The method also allows the determination of dihydrocodeine and of etylmorphine. These two substances are opioids, which provide a positive result with screening methods for this class of opiates, are in fact the active ingredients of certain anti-cough products (eg, dihydrocodeine is the active ingredient in paracodine) or found in association with other active ingredients in pharmaceutical preparations with analgesic-antipyretic (ei. Mindolmerk etylmorphine with propifenazone and contains caffeine). It is important that the method of analysis can identify these two substances and differentiate them from other molecules above.

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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° 014	Opiates in urine by GC/MS	08/2024
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TECHNICAL FEATURES

Principle of the Method :

This method allows to determinate the **Morphine** in urine (the main metabolite of **Heroin**), the **Dihydrocodeine**, the **Codeine**, the **Ethylmorphine** and the **6-Monoacethylmorphine** after hydrolysis of urines and SPE extraction and reading in GC-MS.

Recovery: > 95 %

Sensitivity: < 10,0 ng/ml

Dynamic Range: 10 – 10.000 ng/ml

LOD: 2 ng/ml

LOQ: 6 ng/ml

Cut-off:

Morphine	100 ng/ml
Dihydrocodeine	100 ng/ml
Codeine	100 ng/ml
Ethylmorphine	100 ng/ml
6-Monoacethylmorphine	100 ng/ml

Ref. Procedure applicative del procedimento n.99/cu 30 ottobre 2007

Components of the kit :

All the components are ready-to-use. Stability: 3 years at 2 – 8 °C.

Reagent A – Hydrolysis Enzyme, **1 x 1 ml**

To reconstitute with 9,0 ml of Reagent B. After reconstitution, the enzyme is stable 1 year at 2–8 °C.

Reagent B – Buffer Solution for hydrolysis, **1 x 15 ml**

Reagent C – Conditioning Solution N° 1 and Wash Solution N° 3, **1 x 250 ml**

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

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

Reagent F – Wash Solution N° 1, **1 x 100 ml**

Reagent G – Wash Solution N° 2, **1 x 100 ml**

Reagent H – Eluting Solution, **1 x 50 ml**

Reagent I – Diluting Solution, **1 x 25 ml**

Reagent N – Test Solution, **1 x 0,5 ml**

See Warnings

Reagent S – Internal Standard Solution, **1 x 300 µl**

To dilute 1:10 with H₂O before use

Urine Calibrator lyophil. – Level 1, 2 x 5 ml

Code CC45016 (packed separately – see data sheets)

Urine Calibrator lyophil. – Level 2, 2 x 5 ml

Code CC45016 (packed separately – see data sheets)

Urine Calibrator lyophil. – Level 3, 2 x 5 ml

Code CC45016 (packed separately – see data sheets)

Urine Calibrator lyophil. – Level 4, 2 x 5 ml

Code CC45016 (packed separately – see data sheets)

Urine Calibrator lyophil. – Level 5, 2 x 5 ml

Code CC45016 (packed separately –

Urine Calibrator lyophil. 6-MAM – Level 1, 2 x 5 ml	<u>see data sheets)</u> <u>Code CC45116 (packed separately – see data sheets)</u>
Urine Calibrator lyophil. 6-MAM – Level 2, 2 x 5 ml	<u>Code CC45116 (packed separately – see data sheets)</u>
Urine Calibrator lyophil. 6-MAM – Level 3, 2 x 5 ml	<u>Code CC45116 (packed separately – see data sheets)</u>
Urine Calibrator lyophil. 6-MAM – Level 4, 2 x 5 ml	<u>Code CC45116 (packed separately – see data sheets)</u>
Urine Calibrator lyophil. 6-MAM – Level 5, 2 x 5 ml	<u>Code CC45116 (packed separately – see data sheets)</u>
Reagent M – Derivatization Solution, 3 x 1 ml (3 ampullas of 1 ml)	<u>See Warnings</u>
Clean-up Columns, 50 pcs	

Minimum Instrumental equipment required:

GC-MS Instrument
Operational Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:

Take 10 ml of urine
If stored at -20 °C, the sample is stable about 3 months.

PREANALYTICAL PROCEDURE:

STEP 1 : In a glass-test tube with teflon cap pipette :

- 450 µl of **Reagent I – Diluting Solution**
- 50 µl of **Reagent N – Test Solution**

Vortex for 20 seconds

STEP 2 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 3 : Put in 50 µl of **Reagent M - Derivatization Solution**

Vortex for 20 seconds

STEP 4: Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 5 : Transfer the whole sample in vials goblet form

NOTE : Before starting the analytical session it would be better to inject 1 µl of this solution in GC to identify the time of retention of Opiates, that must be similar to that shown in below Fig. If the Test has given positive result, proceed with the analytical session. If it wouldn't be so, verify the functionality of the analytical system.

ANALYTICAL PROCEDURE:

STEP 1 : Enzyme Reconstitution and Internal Standard dilution

Add at **Reagent A – Hydrolysis Enzyme** (containing 1 ml) 9 ml of **Reagent B – Buffer Solution for hydrolysis.**

Mix by inversion

N.B: Dilute the **Reagent S – Internal Standard Solution** 1:10 with H₂O, preparing the sufficient sample size to cover the assay.

N.B.: this dilution is prepared fresh for each run

STEP 2 : Samples Enzymatic Hydrolysis

Make a Calibration Curve on 5 points:

	Calibrator Opiates + 6MAM Level 1	Calibrator Opiates + 6MAM Level 2	Calibrator Opiates + 6MAM Level 3	Calibrator Opiates + 6MAM Level 4	Calibrator Opiates + 6MAM Level 5	Sample	Controls + Controls 6-MAM
Urine Calibrator Opiates – Level 1	500 µl						
Urine Calibrator 6MAM – Level 1	500 µl						
Urine Calibrator Opiates – Level 2		500 µl					
Urine Calibrator 6MAM – Level 2		500 µl					
Urine Calibrator Opiates – Level 3			500 µl				
Urine Calibrator 6MAM – Level 3			500 µl				
Urine Calibrator Opiates – Level 4				500 µl			
Urine Calibrator 6MAM – Level 4				500 µl			
Urine Calibrator Opiates– Level 5					500 µl		
Urine Calibrator 6MAM – Level 5					500 µl		
Sample						1,0 ml	
Controls							500 µl
Controls 6-MAM							500 µl
Reagent S – Internal Std Sol. (diluted 1:10 with HPLC grade H ₂ O)	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl
Reagent A – Enzyme reconstituted	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl

Control the pH is $\cong$ 5,0 – In case, correct it

N.B: The values of concentration of the Cocaine, Morphine, Dihydrocodeine, Ethylmorphine and 6-MAM calibrators mixed together are the HALF to that shown in the table of warnings!!!!

In vortex for 20 seconds

STEP 3 : Incubate at 50 °C for 3 hours.

STEP 4 : Conditioning of the Clean-up columns

- Pour out in column 1,5 ml of **Reagent C – Conditioning Solution N° 1** and Wash Solution N° 3
Reject the eluate
- Pour out in column 2 ml of **Reagent D – Conditioning Solution 2**
Reject the eluate

STEP 5 : Preparation of the sample

Dispense in test tube :

	Hydrolysed Calibrator – Level 1	Hydrolysed Calibrator – Level 2	Hydrolysed Calibrator – Level 3	Hydrolysed Calibrator – Level 4	Hydrolysed Calibrator – Level 5	Sample	Controls
Hydrolysed Calibrator – Level 1	1200 µl						
Hydrolysed Calibrator – Level 2		1200 µl					
Hydrolysed Calibrator – Level 3			1200 µl				
Hydrolysed Calibrator – Level 4				1200 µl			
Hydrolysed Calibrator – Level 5					1200 µl		
Hydrolysed Sample						1200 µl	
Hydrolysed Controls							1200 µl
Reagent E – Buffer Solution	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl

Vortex for 20 seconds

STEP 6 : Pour the whole sample and the Calibrator, previously prepared, in the Clean-up columns and discharge in a flow of 1 ml/ min (droop by drop).

STEP 7 : Clean-up columns Wash:

- Wash with 2 ml of **Reagent F – Wash Solution 1**
Discharge the percolated liquid
- Wash with 2 ml of **Reagent G – Wash Solution 2**
Discharge the percolated liquid
- Wash with 2 ml of **Reagent C – Conditioning Solution 1 and Wash Solution 3**
Discharge the percolated liquid and dry with a light flux of air

STEP 8 : Elution

- Elute with 1 ml of **Reagent H – Eluting Solution**

Gather the eluate in glass test tubes and vortex

STEP 9 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 10 : Take again 50 µl of **Reagent M - Derivatization Solution**

Vortex for 20 seconds

STEP 11: Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 12 : Transfer the whole sample in vials goblet form

INJECTION :

- Inject 1 µl of the solution in the GC

OPIATES in urine - Warnings

REAGENT N: TEST SOLUTION

Morphine Dihydrocodeine Codeine Ethylmorphine 6-Monoacethylmorphine	about 10.000 ng/ml about 10.000 ng/ml about 10.000 ng/ml about 10.000 ng/ml about 10.000 ng/ml
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REAGENT M: DERIVATIZATION SOLUTION

Once opened an ampulla, transfer the whole content, 1 ml, in a glass vial. Close carefully the cap and store at Room Temperature.

SET OF GAS-CROMATOGRAPH:

- VF-5ms Column 30 m x 0,25 mm, 0,25 µm (conditioned)
- Injector's Temperature 280 °C
- Split Report Start: ON split 20
0 min: OFF
1 min: ON split 100
3 min: ON split 20
- Temperature 120 °C x 3,25 minutes + 20 °C/min up to 250 °C x 1 minute + 40 °C/min up to 300 °C x 3 minutes (run 15 minutes)
- Helium Gas 1 ml/min

SET OF MASSA DETECTOR (4000) :

- Range of Massa 60 – 500
- Temperature of Massa: 180 °C;
- Temperature Transfer Line: 270 °C;
- Temperature Manifold: 80 °C
- Filament on: 5 minutes

CONDITIONING OF VF-5 MS COLUMN and SYRINGE

Before use wash the syringe with ACETONE.
Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.

CLEANING OF THE COLUMN

Disconnect the detector. Keep the column at the highest temperature for the recommended time.
(See the manufacturer 's instructions)

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC45016	Calibrator in urine for Opiates	2 x 5 x 5 ml
CC45019	Control in urine for Opiates – Levels 1 and 2	2 x 6 x 5 ml
CC45116	Calibrator in urine for 6-MAM	2 x 5 x 5 ml
CC48019	Control in urine for EDDP, 6-MAM and Cocaine – Levels 1 and 2	2 x 6 x 5 ml
ZRE13623	Rxi-Sil 5MS (30 m x 0,25 mm) ID, DF=0,25 Capillary Column	1 Pc
SCP8944	VF-5ms (30 m x 0,25 mm) ID DF=0,25 Column	1 Pc
S29057U	Standard glass vial of 2 ml with screw cap	1 x 100 Pcs
S29046U	Caps for Standard glass vials of 2 ml with screw cap	1 x 100 Pcs
S24717	Insert 100 ul for vials of 2 ml	1 x 100 Pcs

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

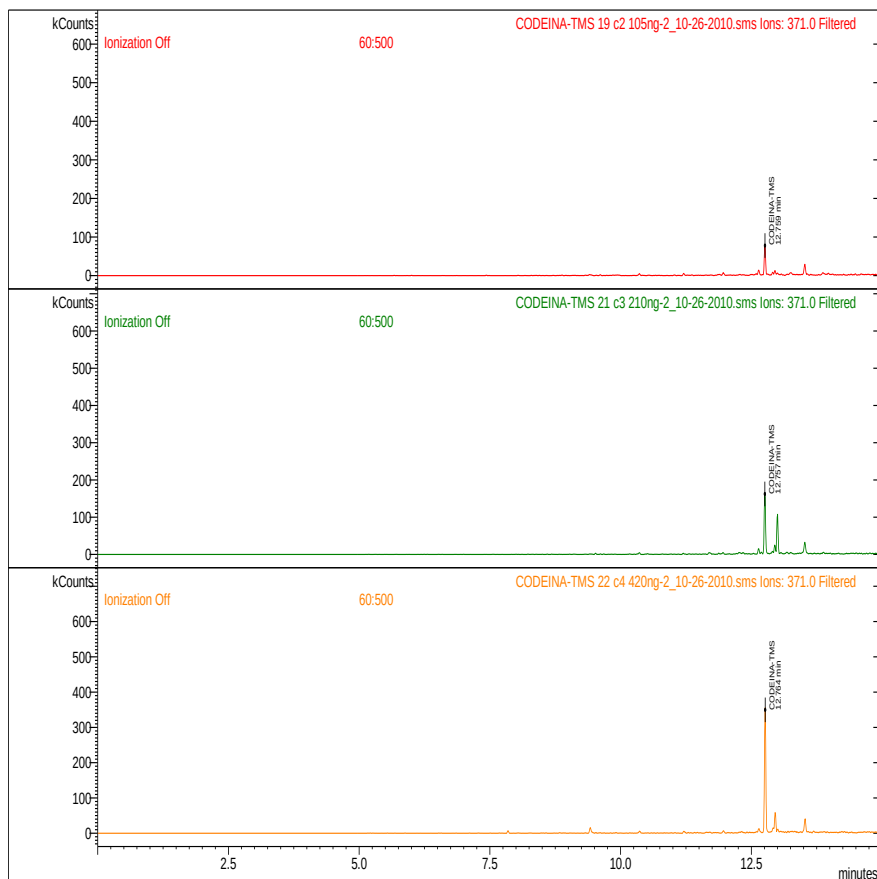


Fig. 1 :	Urine Sample Codeine
	R.T. 12.76 CODEINE

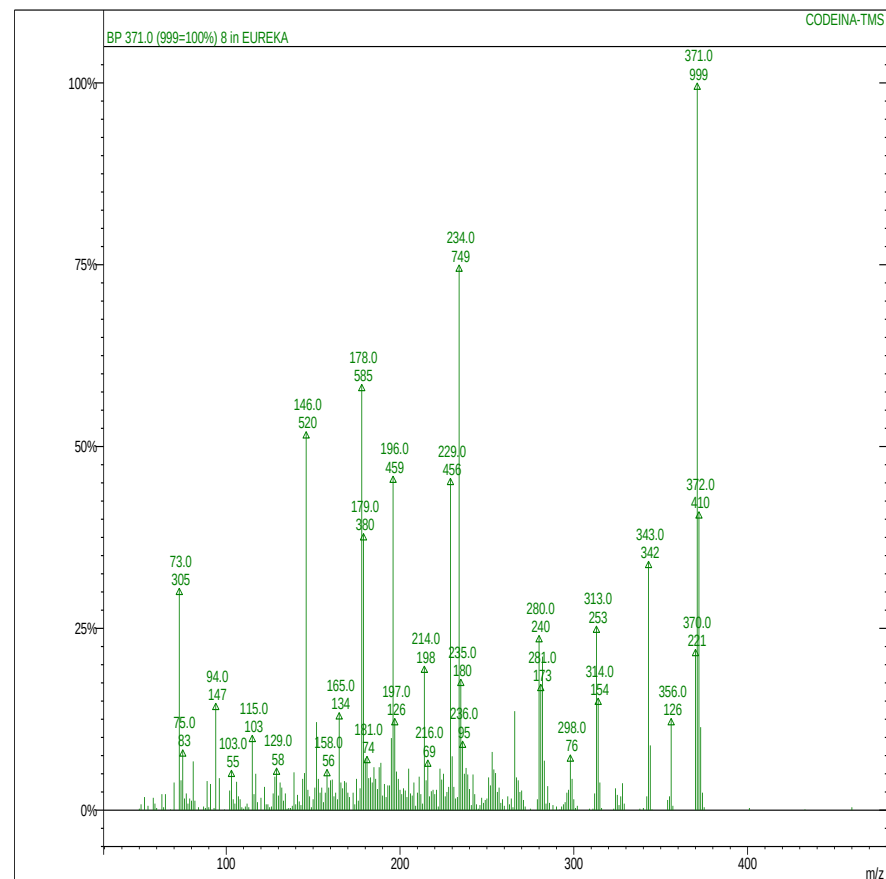


Fig. 2 :	Massa Spectrum Codeine
	MOLECULAR IONS: 371 – 234 - 343

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

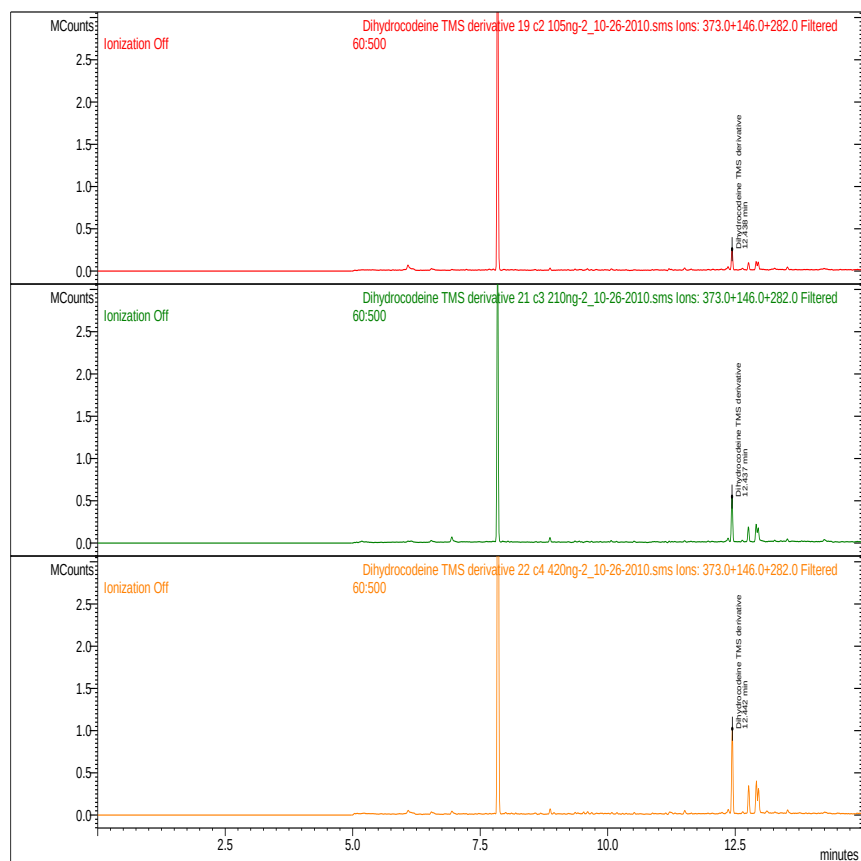


Fig. 3 :	Urine Sample Dihydrocodeine
	R.T. 12.44 DIHYDROCODEINE

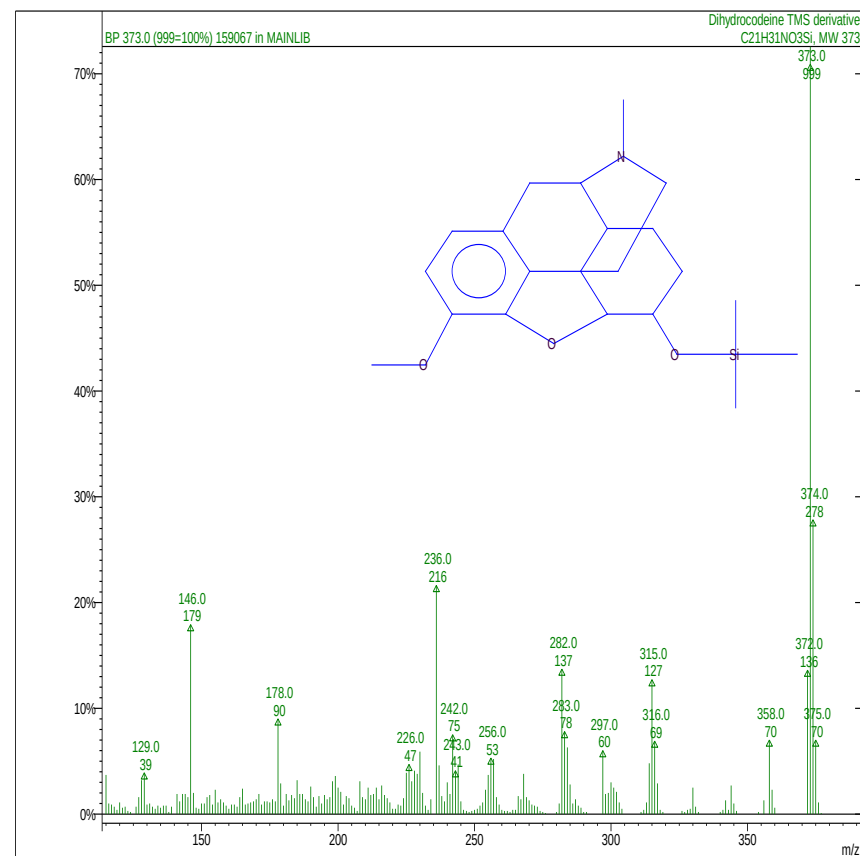


Fig. 4 :	Massa Spectrum Dihydrocodeine
	MOLECULAR IONS: 373 – 146 - 282

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

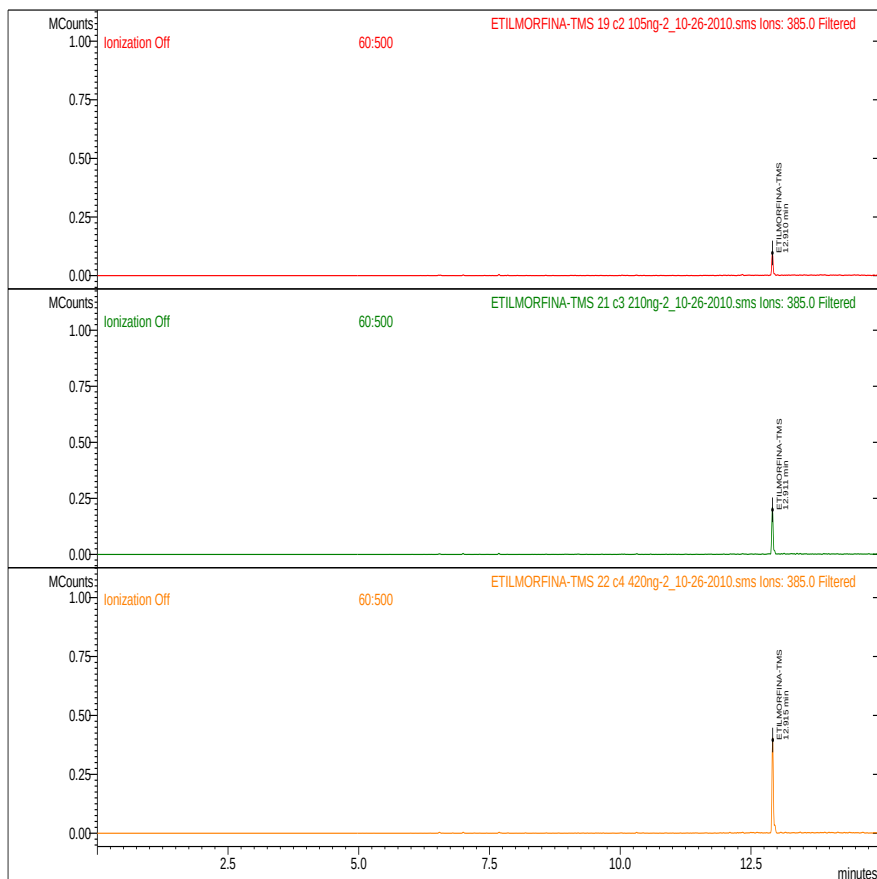


Fig. 5 :	Urine Sample Ethylmorphine
	R.T. 12.91 ETHYLMORPHINE

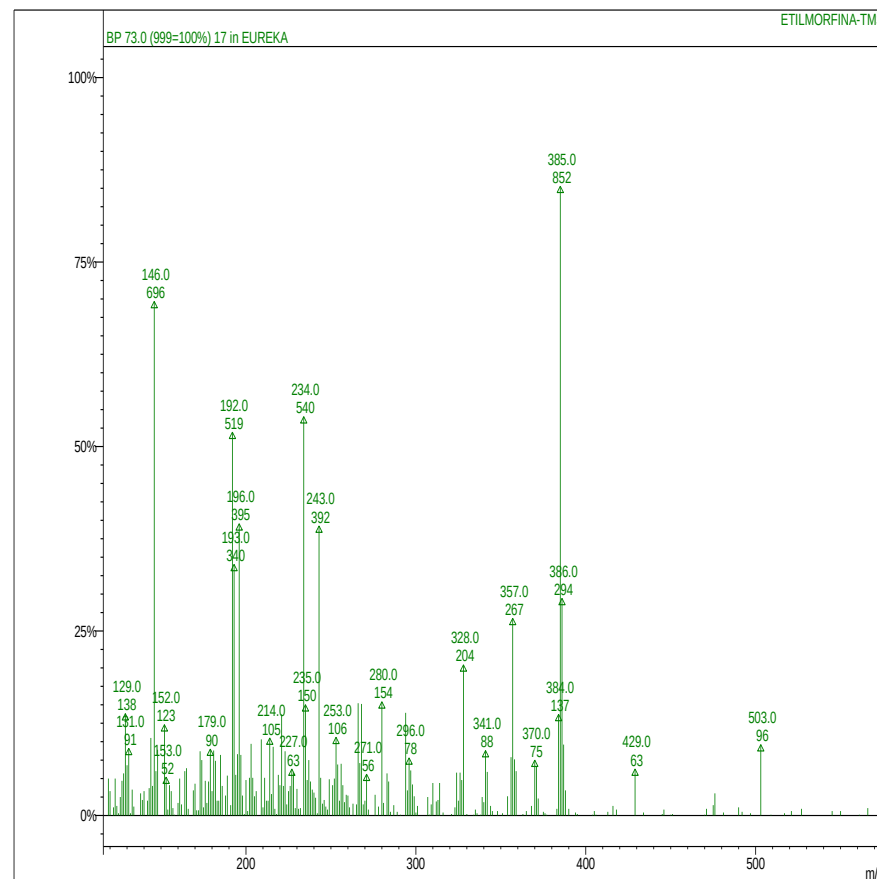


Fig. 6 :	Massa Spectrum Ethylmorphine
	MOLECULAR IONS: 385 – 146 - 73

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

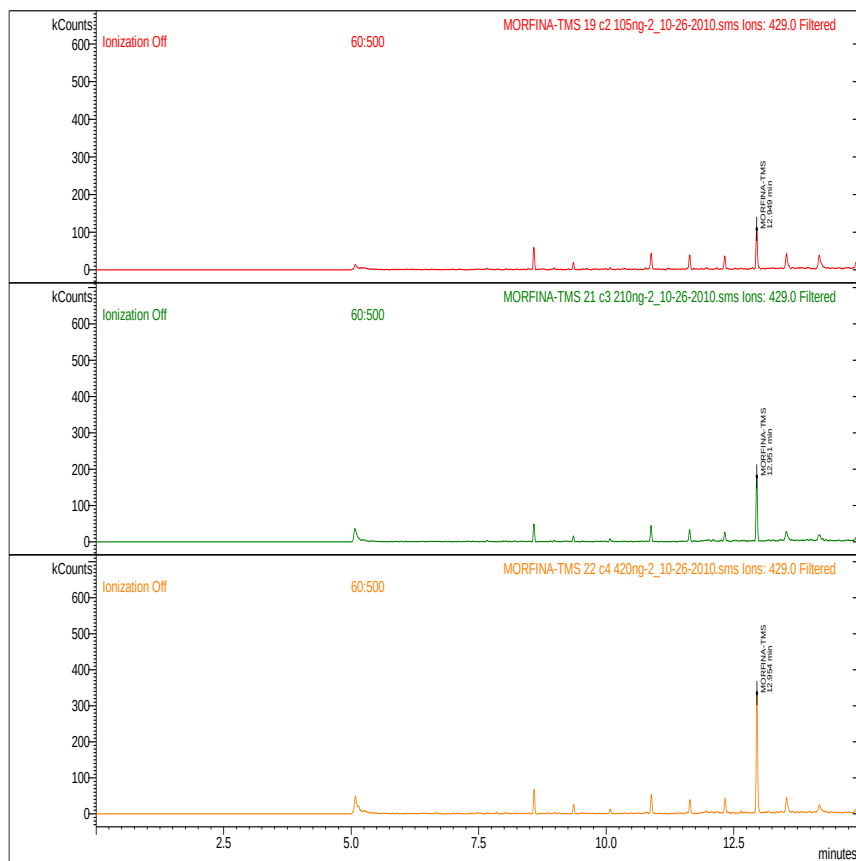


Fig. 7 :	Urine Sample Morphine
	R.T. 12.95 MORPHINE

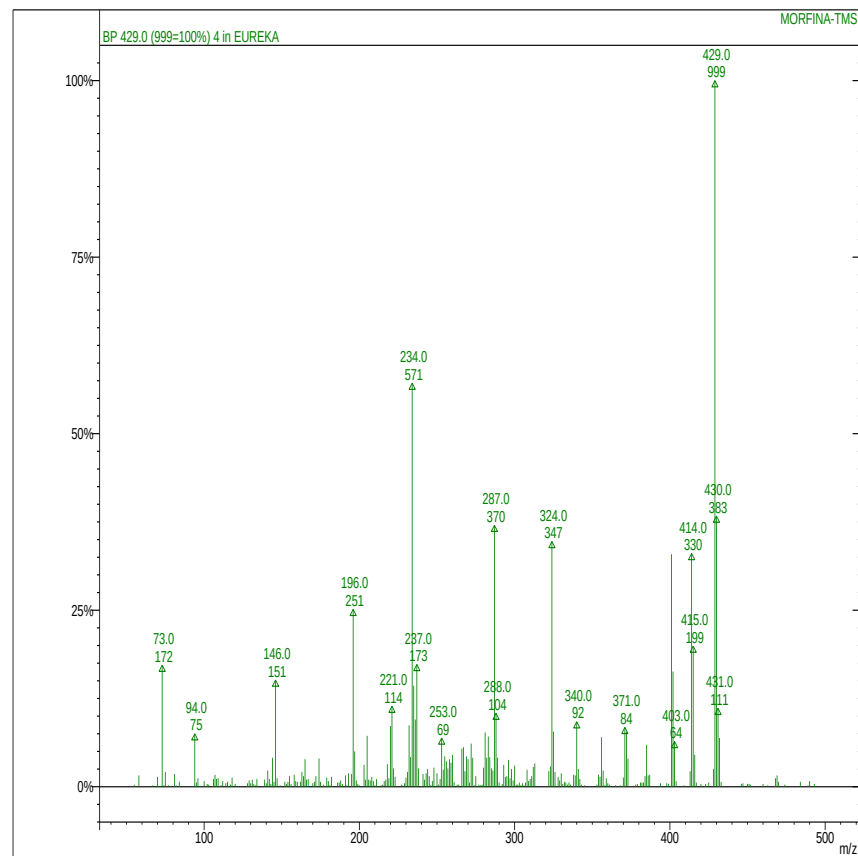


Fig. 8 :	Massa Spectrum Morphine
	MOLECULAR IONS: 429 – 414 - 234

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

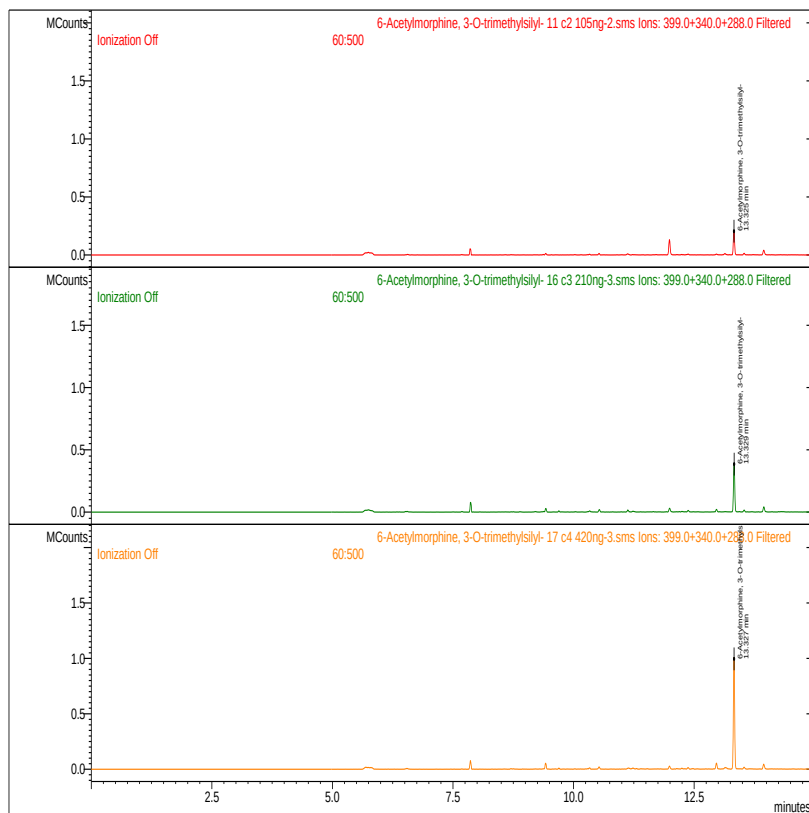


Fig. 9 :	Urine Sample 6-Monoacetylmorphine
	R.T. 13.32 6-MONOACETYLMORPHINE

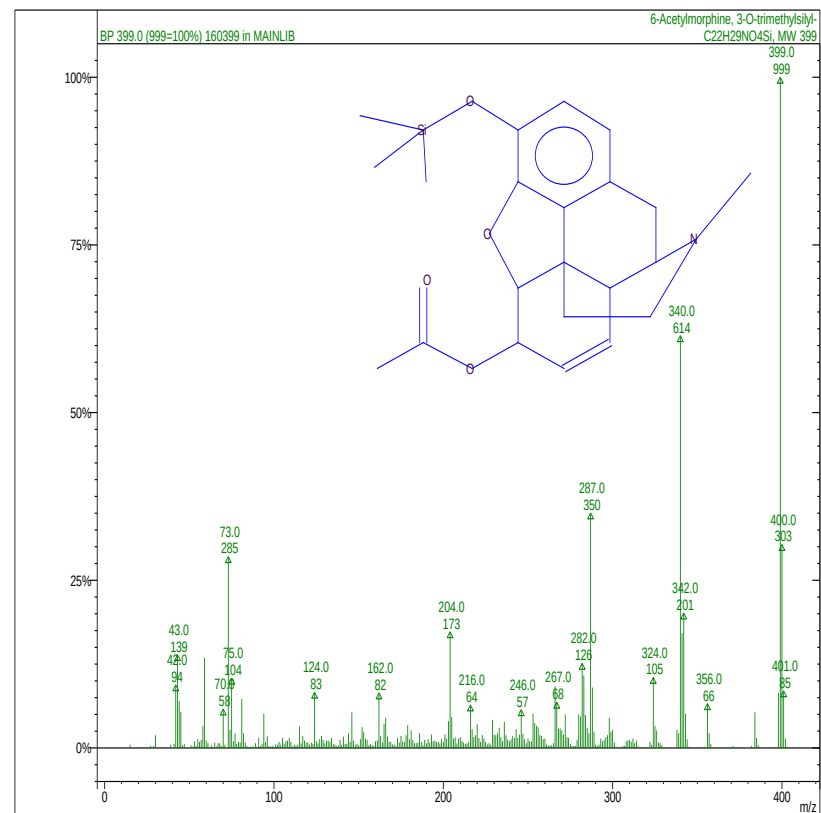


Fig. 10 :	Massa Spectrum 6-Monoacetylmorphine
	MOLECULAR IONS: 399 – 340 - 288

Opiates in urine

(Reference Chromatograms/Spectrum GC-MS)

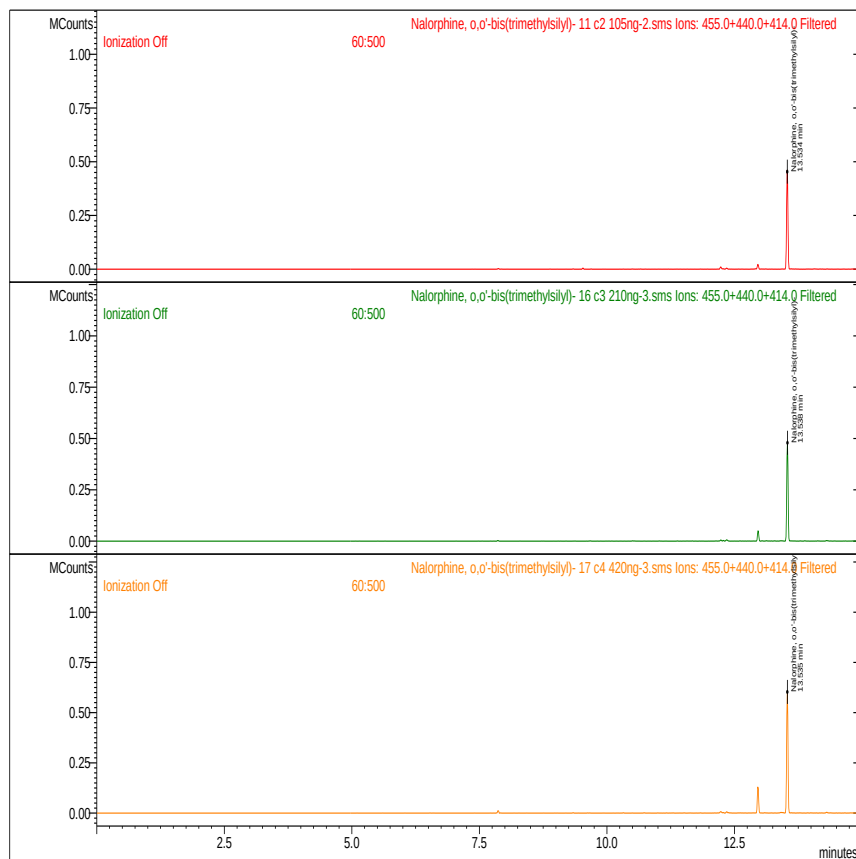


Fig. 11 :	Urine Samples with Internal Standard
	R.T. 13.52 INTERNAL STANDARD

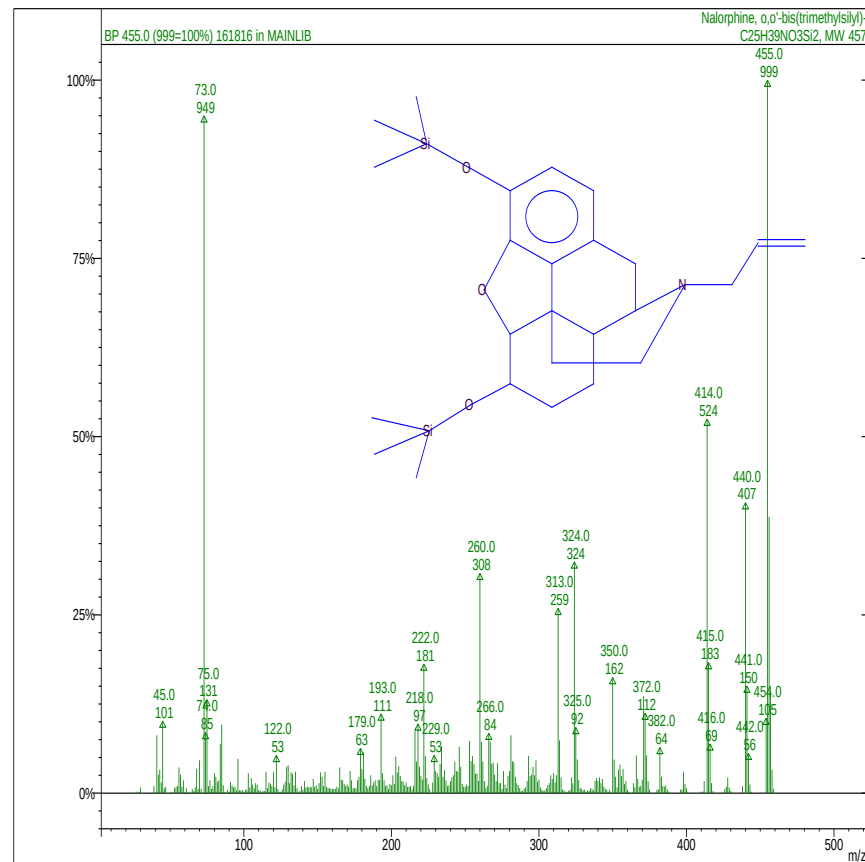


Fig. 12 :	Massa Spectrum Internal Standard
	MOLECULAR IONS: 455 – 440 - 414