

## **METHADONE and EDDP in urine by GC-MS – Code GC48010**

### **Method of Confirmation by GC-MS**

#### **INTRODUCTION**

Methadone is a synthetic opioid used as a drug of choice in the detoxification of heroin (and / or opiates). Methadone is metabolized in the body as 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), metabolite that is found along with the parent drug in urine.

The screening methods generally measure methadone, through an antigen-antibody reaction, methadone. Because these methods can give false positive results for the presence of foreign substances that may interfere with the antigen-antibody reaction, confirmation analysis is always required.

So if the analysis of a screening is positive (cut-off > or = 300 ng / ml) confirmation is necessary using liquid-gas chromatographic separation, coupled with a mass spectrometry detector.

The following method is used to determine the Methadone and EDDP after solid phase extraction and subsequent determination using gas chromatography - mass spectrometry (Gas Chromatography Mass Spectrometry, GC-MS).

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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° 009 | Methadone and EDDP in urine by GC/MS | 07/2025 |
|--------|--------------------------------------|---------|

## **TECHNICAL FEATURES**

### **Principle of the Method :**

This method allows to determinate the **Methadone** and its main metabolite, EDDP, after solid-liquid extraction and reading by GC/MS.

### **Recovery :**

about 98 %

### **Sensitivity :**

about 20 ng/ml

### **Dynamic Range of the Method :**

20 – 10.000 ng/ml

### **LOD:**

7 ng/ml

### **LOQ:**

20 ng/ml

### **Cut-off :**

Methadone 100 ng/ml  
EDDP 100 ng/ml

*Ref. Provvedimento Gazzetta Ufficiale 236 del 18-09-2008*

### **Components of the kit :**

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

**Reagent A** – Conditioning Solution N° 1, **1 x 50 ml**

**Reagent B** – Conditioning Solution N° 2, **1 x 50 ml**

**Reagent C** – Buffer Solution, **1 x 50 ml**

**Reagent D** – Wash Solution, **1 x 50 ml**

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

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

**Reagent G** – Test Solution Methadone, **1 x 0,5 ml**

**See Warnings**

**Reagent H** – Test Solution EDDP, **1 x 0,5 ml**

**See Warnings**

**Reagent K** – Internal Standard Solution, **1 x 300 ul**

**To dilute 1:10 with H<sub>2</sub>O before use**

**Urine Calibrator lyophil Methadone – Level 1, 2 x 5 ml**

**Code CC48016 (packed separately – see data sheets)**

**Urine Calibrator lyophil Methadone – Level 2, 2 x 5 ml**

**Code CC48016 (packed separately – see data sheets)**

**Urine Calibrator lyophil Methadone – Level 3, 2 x 5 ml**

**Code CC48016 (packed separately – see data sheets)**

**Urine Calibrator lyophil Methadone – Level 4, 2 x 5 ml**

**Code CC48016 (packed separately – see data sheets)**

**Urine Calibrator lyophil Methadone – Level 5, 2 x 5 ml**

**Code CC48016 (packed separately – see data sheets)**

**Urine Calibrator lyophil EDDP – Level 1, 2 x 5 ml**

**Code CC48116 (packed separately – see data sheets)**

**Urine Calibrator lyophil EDDP – Level 2, 2 x 5 ml**

**Code CC48116 (packed separately – see data sheets)**

**Urine Calibrator lyophil EDDP – Level 3, 2 x 5 ml**

**Code CC48116 (packed separately – see data sheets)**

**Urine Calibrator lyophil EDDP – Level 4, 2 x 5 ml**

**Code CC48116 (packed separately – see data sheets)**

**Urine Calibrator lyophil EDDP – Level 5, 2 x 5 ml**

**Code CC48116 (packed  
separately – see data sheets)**

**Reagent M – Derivatization Solution, 5 x 1 ml (5 ampullas of 1 ml)**

**Store at Room Temperature**

**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 :** Pipette in a glass tube with teflon cap:

- 400 µl of **Reagent F** – Diluting Solution
- 50 µl of **Reagent G** – Test Solution Methadone
- 50 µl of **Reagent H** – Test Solution EDDP

**Vortex for 20 sec.**

**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 100 µl of **Reagent M** – Derivatization Solution

**Vortex for 20 sec.**

**STEP 4:** Incubate at 70 °C for 30 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 Methadone and EDDP, that must be similar to that shown in Fig. 1. 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 :** Clean-up Columns Conditioning

- Condition clean up columns by fluxing 1 ml of **Reagent A – Conditioning Solution N° 1**  
*Discharge the percolated liquid*
- Condition clean up columns by fluxing 1 ml of **Reagent B – Conditioning Solution N° 2**  
*Discharge the percolated liquid*

**STEP 2:** Dilute **Reagent K – Internal Standard Solution** 1:10 with Water HPLC grade

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

**STEP 3 : Samples Preparation**

Make a Calibration Curve on 5 points :

|                                                                              | Sample  | Calibrator<br>Methadone<br>+ EDDP<br>Level 1 | Calibrator<br>Methadone<br>+ EDDP<br>Level 2 | Calibrator<br>Methadone<br>+ EDDP<br>Level 3 | Calibrator<br>Methadone<br>+ EDDP<br>Level 4 | Calibrator<br>Methadone<br>+ EDDP<br>Level 5 | Control<br>Methadone<br>+ EDDP |
|------------------------------------------------------------------------------|---------|----------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|--------------------------------|
| Sample                                                                       | 1000 µl |                                              |                                              |                                              |                                              |                                              |                                |
| Calibrator<br>Methadone – Level 1                                            |         | 500 µl                                       |                                              |                                              |                                              |                                              |                                |
| Calibrator EDDP –<br>Level 1                                                 |         | 500 µl                                       |                                              |                                              |                                              |                                              |                                |
| Calibrator<br>Methadone – Level 2                                            |         |                                              | 500 µl                                       |                                              |                                              |                                              |                                |
| Calibrator EDDP –<br>Level 2                                                 |         |                                              | 500 µl                                       |                                              |                                              |                                              |                                |
| Calibrator<br>Methadone – Level 3                                            |         |                                              |                                              | 500 µl                                       |                                              |                                              |                                |
| Calibrator EDDP –<br>Level 3                                                 |         |                                              |                                              | 500 µl                                       |                                              |                                              |                                |
| Calibrator<br>Methadone – Level 4                                            |         |                                              |                                              |                                              | 500 µl                                       |                                              |                                |
| Calibrator EDDP –<br>Level 4                                                 |         |                                              |                                              |                                              | 500 µl                                       |                                              |                                |
| Calibrator<br>Methadone – Level 5                                            |         |                                              |                                              |                                              |                                              | 500 µl                                       |                                |
| Calibrator EDDP –<br>Level 5                                                 |         |                                              |                                              |                                              |                                              | 500 µl                                       |                                |
| Control Methadone                                                            |         |                                              |                                              |                                              |                                              |                                              | 500 µl                         |
| Control EDDP                                                                 |         |                                              |                                              |                                              |                                              |                                              | 500 µl                         |
| Reagent K – Internal<br>Std Solution (diluted<br>1:10 with H <sub>2</sub> O) | 20 µl   | 20 µl                                        | 20 µl                                        | 20 µl                                        | 20 µl                                        | 20 µl                                        | 20 µl                          |
| Reagent C – Buffer<br>Solution                                               | 1000 µl | 1000 µl                                      | 1000 µl                                      | 1000 µl                                      | 1000 µl                                      | 1000 µl                                      | 1000 µl                        |

**Verify that the pH is between 6,5 and 7,5 – Eventually correct it**

**N.B:** The values of concentration of the Methadone and EDDP calibrators mixed together are the **HALF** to that shown in the table of warnings!!!!

**Vortex for 10 sec.**

**STEP 4 :** Dispense all the samples, previously prepared, into clean up columns. Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml / min ( drop by drop ).

**STEP 5 :** Clean-up Column Wash:

- Wash with 1 ml of **Reagent D – Wash Solution**  
*Discharge the percolated liquid and dry with a light flux of air*

**STEP 6** : Eluting **strictly in glass tubes with pyrex caps**

- Elute with 2 ml of **Reagent E – Eluting Solution**, at speed of 1 ml / min.

**Collect the eluate and mix at Vortex**

*If there is a turbidity or a precipitate in the eluate, centrifuge at 4000 rpm for 2 min.*

**STEP 7** : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

**STEP 8** : Put in 100 µl of **Reagent M – Derivatization Solution**

**Vortex for 20 sec.**

**STEP 9**: Incubate at 70 °C for 30 minutes

**Cooling at Room Temperature**

**STEP 10** : Transfer the whole sample in vials goblet form

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

**INJECTION :**

- Inject 1 µl of the solution in the GC

### **METHADONE and EDDP in urine - Warnings**

#### **REAGENT G – TEST SOLUTION METHADONE**

|                  |                |
|------------------|----------------|
| <b>Methadone</b> | ~ 50.000 ng/ml |
|------------------|----------------|

#### **REAGENT H – TEST SOLUTION EDDP**

|             |                |
|-------------|----------------|
| <b>EDDP</b> | ~ 10.000 ng/ml |
|-------------|----------------|

#### **SET OF GAS-CROMATOGRAPH :**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>• VF-5ms Column 30 m x 0,25 mm, 0,25 µm (conditioned)</li><li>• Injector Temperature: 280 °C</li><li>• Split Report Start: ON 20<ul style="list-style-type: none"><li>0 min: OFF</li><li>1 min: ON 100</li><li>3 min: ON 20</li></ul></li><li>• Oven Column Temperature 50 °C x 1 minute + 20 °C/min till up 180 °C x 0 minute + 10 °C/min till up 300 °C x 1.5 minute (run 21 minutes)</li><li>• Column Flow: 1 ml/min</li></ul> |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

#### **SET OF MASSA DETECTOR :**

|                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>• Range of Massa 80 – 600</li><li>• Temperature of Massa: 180 °C;</li><li>• Temperature of Transfer Line: 270 °C;</li><li>• Temperature of Manifold: 80 °C</li><li>• Filament on: 6 minutes</li><li>• Operative Method in Electronic Impact (EI)</li></ul> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

#### **CONDITIONING OF VF-5 MS COLUMN and SYRINGE**

|                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Before use wash the syringe with ACETONE.<br>Follow the manufacturer prescription.<br>Do not condition column/s if connected to the Massa Detector. At the beginning of each run to calibrate the masses and RF. |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

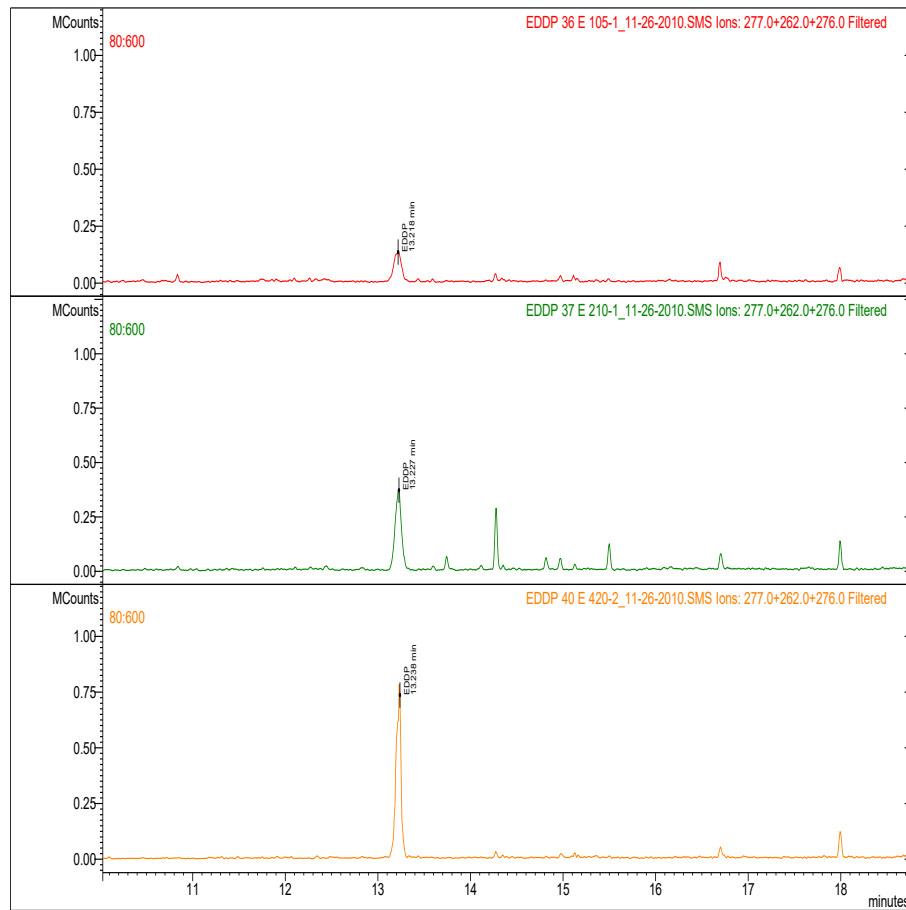
#### **CLEANING OF THE COLUMN**

|                                                                                                                                                                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Disconnect the detector. Keep the column at the highest temperature for the recommended time (see the manufacturer 's instructions) with a speed of 30 °C/min. |
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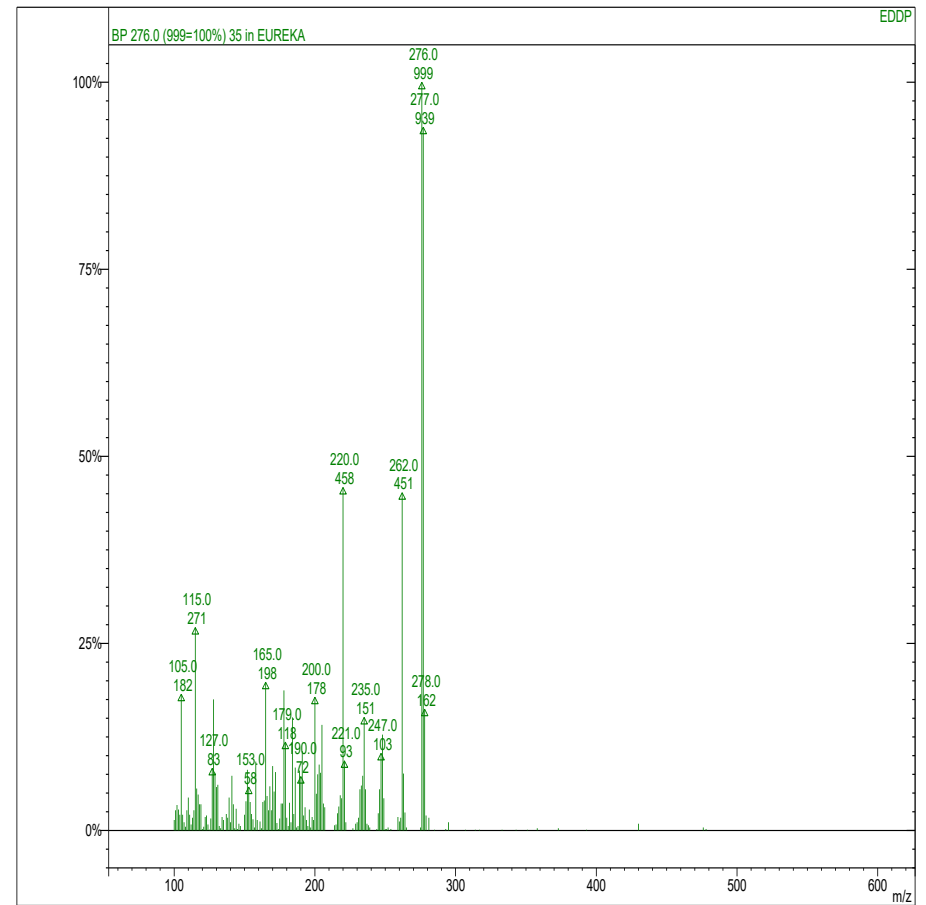
#### **ACCESSORIES AND CONSUMABLES**

| <b>CODICE</b>   | <b>DESCRIZIONE</b>                                            | <b>CONFEZIONE</b> |
|-----------------|---------------------------------------------------------------|-------------------|
| <b>CC48016</b>  | Calibrator in urine for Methadone                             | 2 x 5 x 5 ml      |
| <b>CC48116</b>  | Calibrator in urine for EDDP                                  | 2 x 5 x 5 ml      |
| <b>CC43019</b>  | Multiparametric Control for Drugs of Abuse – Levels 1 and 2   | 2 x 6 x 5 ml      |
| <b>CC48019</b>  | Control in urine for EDDP, 6-MAM and Cocaine – Levels 1 and 2 | 2 x 6 x 5 ml      |
| <b>ZRE13623</b> | Rxi-Sil 5MS (30 m x 0,25 mm) ID, DF=0,25 Capillary Column     | 1 Pc              |
| <b>SCP8944</b>  | VF-5ms (30 m x 0,25 mm) ID DF=0,25 Column                     | 1 Pc              |
| <b>S29057U</b>  | Standard glass vial of 2 ml with screw cap                    | 1 x 100 Pcs       |
| <b>S29046U</b>  | Caps for Standard glass vials of 2 ml with screw cap          | 1 x 100 Pcs       |
| <b>S24717</b>   | Insert 100 ul for vials of 2 ml                               | 1 x 100 Pcs       |

**Methadone and EDDP in urine**  
(Reference Chromatograms and Spectrums)

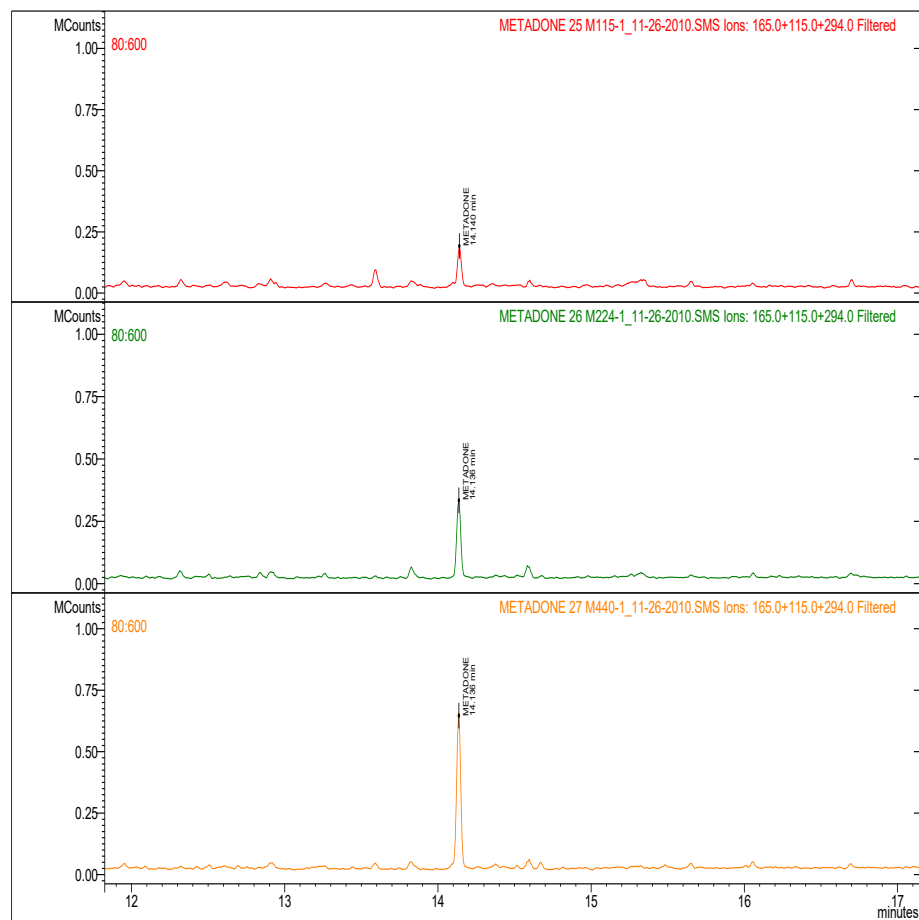


|               |                                  |
|---------------|----------------------------------|
| <b>Fig. 1</b> | <b>Urine Samples EDDP</b>        |
|               | R.T. 13.7 EDDP 105-210-420 ng/ml |

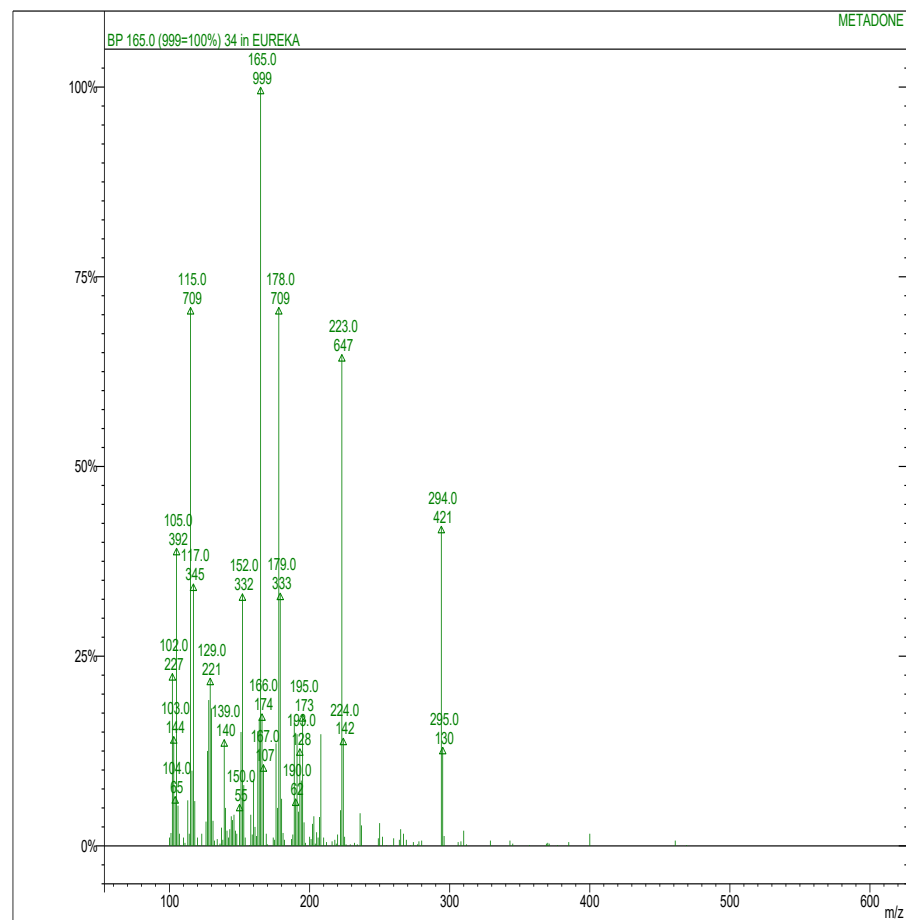


|               |                             |
|---------------|-----------------------------|
| <b>Fig. 2</b> | <b>Massa Spectrum EDDP</b>  |
|               | MOLECULAR IONS: 277-262-276 |

**Methadone and EDDP in urine**  
(Reference Chromatograms and Spectrums)

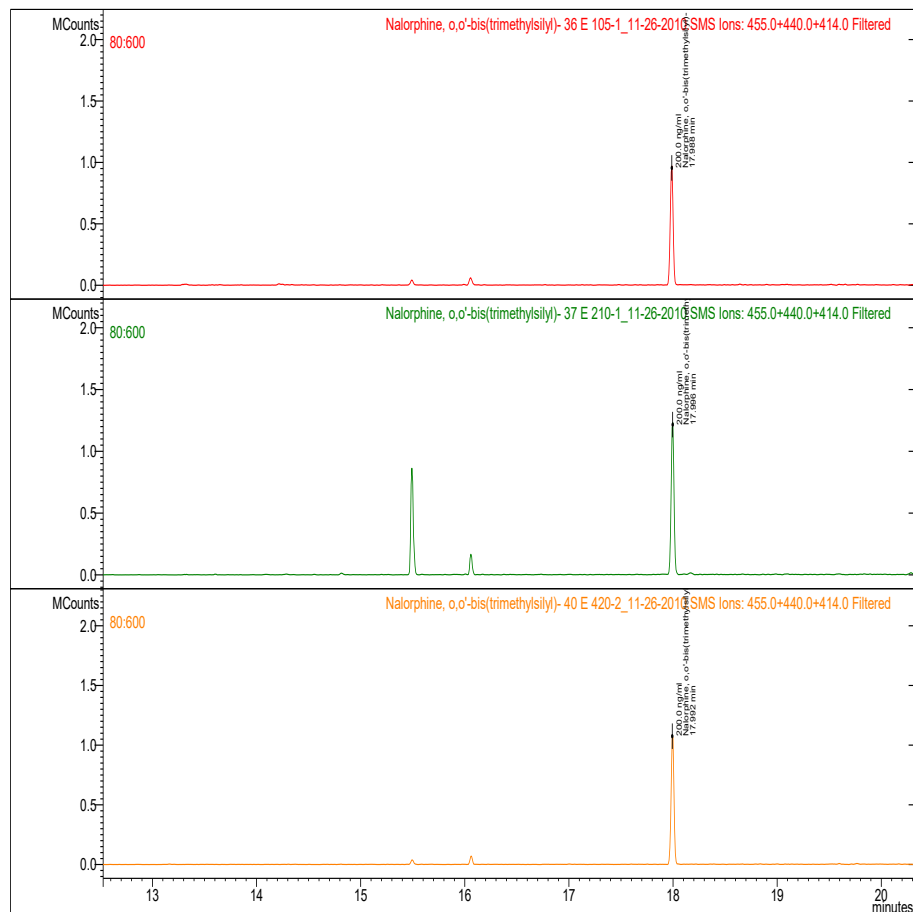


|               |                                       |
|---------------|---------------------------------------|
| <b>Fig. 3</b> | <b>Urine Samples Methadone</b>        |
|               | R.T. 14.6 Methadone 115-224-440 ng/ml |

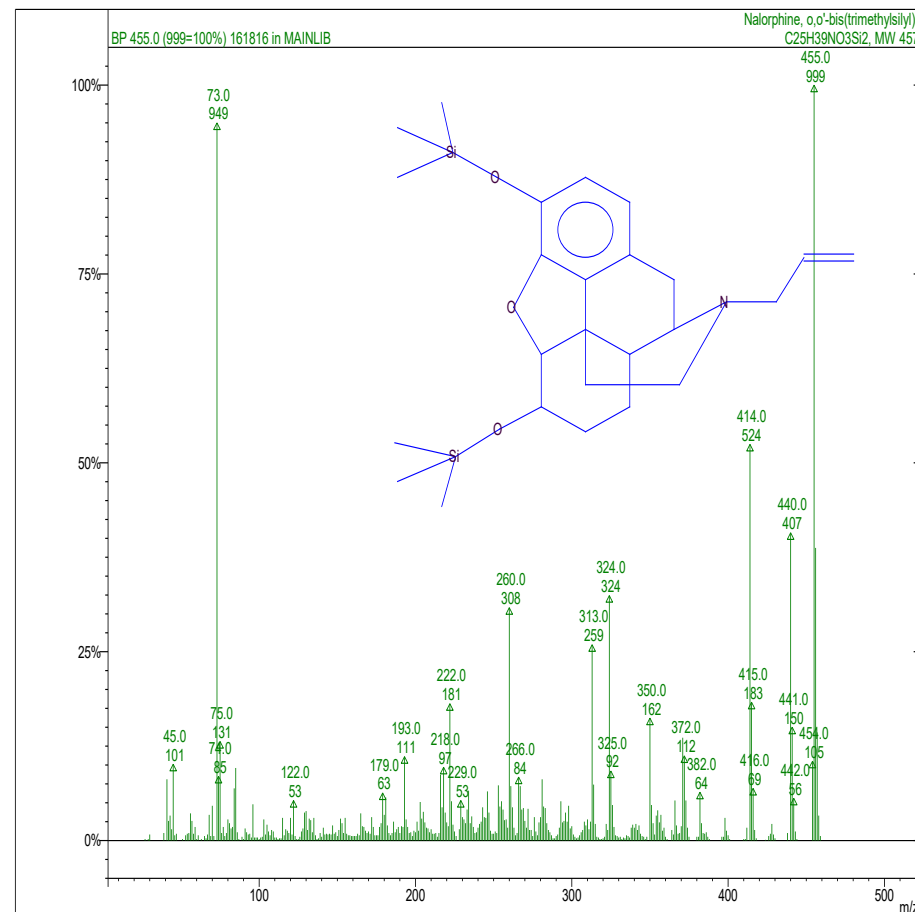


|               |                                 |
|---------------|---------------------------------|
| <b>Fig. 4</b> | <b>Massa Spectrum Methadone</b> |
|               | MOLECULAR IONS: 115-165-294     |

**Methadone and EDDP in urine**  
(Reference Chromatograms and Spectrums)



|               |                                             |
|---------------|---------------------------------------------|
| <b>Fig. 5</b> | <b>Urine Samples with Internal Standard</b> |
|               | R.T. 18.8 Internal Standard                 |



|               |                                           |
|---------------|-------------------------------------------|
| <b>Fig. 6</b> | <b>Mass Spectrum of Internal Standard</b> |
|               | MOLECULAR IONS: 455-440-414               |