

NORBUPRENORPHINE (Buprenorphine's Metabolite) – BUPRENORPHINE in urine
by GC/MS
Code GC44010

Method of Confirmation by GC-MS

INTRODUCTION

Buprenorphine is an analgesic with a long-time action, 25 to 40 times more potent than morphine. It is used in many cases as a drug replacement therapy in the detoxification of heroin addicts with doses ranging from 0.2 to 2 mg. Since 1996, a high number of deaths have been observed as a result of taking buprenorphine, often associated with other psychotropic drugs. Buprenorphine is metabolized through N-dealkylation to norbuprenorphine, which is glucuronidated and excreted in urine. Determinative screening in biological fluids (especially urine) is usually performed using immunometric techniques using monoclonal antibodies and subsequent staining with various reagents. Because these techniques can not rule out false positives, the positive screening must be confirmed by alternative technology. This is usually done with gas chromatography (GC) or liquid chromatography (LC) coupled with both specific and selective detector (UV, Fluorescent, MS). This method can determine buprenorphine and norbuprenorphine in urine (the major metabolite of buprenorphine) after solid phase extraction (SPE) and the taking of fluorimeter readings.

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N° 011	Norbuprenorphine and Buprenorphine in urine by GC/MS	10/2024
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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.

TECHNICAL FEATURES

Principle of the Method :

This method allows to determinate the **Buprenorphine** and the **Norbuprenorphine** in urine (the main metabolite of **Buprenorphine**), after SPE extraction and reading in GC-MS.

Recovery: 100 %

Sensitivity: 2 ng/ml

LOD: Buprenorphine 0,16 ng/ml
Norbuprenorphine 0,3 ng/ml

LOQ: Buprenorphine 0,5 ng/ml
Norbuprenorphine 1,03 ng/ml

Cut-off: 5 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.
To reconstitute with 9,0 ml of Reagent B. After reconstitution, the enzyme is stable 1 year at 2–8 °C.

Reagent A – Hydrolysis Enzyme, 1 x 1 ml

Reagent B – Buffer Solution for hydrolysis, 1 x 10 ml

Reagent C – Conditioning Solution N° 1 and Wash Solution N° 3, 1 x 200 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 L – Test Solution, 1 x 0,5 ml

See Warnings

Reagent P – Internal Standard Solution, 1 x 1 ml

To dilute 1:50 with H₂O before use

Urine Calibrator lyophil.- Level 1, 2 x 6 ml

Code CC44016 (packed separately – see data sheets)

Urine Calibrator lyophil.- Level 2, 2 x 6 ml

Code CC44016 (packed separately – see data sheets)

Urine Calibrator lyophil.- Level 3, 2 x 6 ml

Code CC44016 (packed separately – see data sheets)

Urine Calibrator lyophil.- Level 4, 2 x 6 ml

Code CC44016 (packed separately – see data sheets)

Urine Calibrator lyophil.- Level 5, 2 x 6 ml

Code CC44016 (packed separately – see data sheets)

Reagent M – Derivatization Solution, 4 x 1 ml (4 ampullas of 1 ml)

See Warnings

Clean-up Columns, 50 pcs

Minimum Instrumental equipment required:

GC-MS Instrument
Operational Computer
Autosampler

Optional Equipment:

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 di **Reagent I – Diluting Solution**
- 50 µl di **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 70 µl of **Reagent M - Derivatization Solution**

Vortex for 20 seconds

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 2 µl of this solution in GC to identify the time of retention of Norbuprenorphine and Buprenorphine, that must be similar to that shown in Fig. below. 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 P – Internal Standard Solution** 1:50 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 – Level 1	Calibrator – Level 1	Calibrator – Level 1	Calibrator – Level 1	Calibrator – Level 1	Sample	Controls
Urine Calibrator – Level 1	2,0 ml						
Urine Calibrator – Level 2		2,0 ml					
Urine Calibrator – Level 3			2,0 ml				
Urine Calibrator – Level 4				2,0 ml			
Urine Calibrator – Level 5					2,0 ml		
Sample						2,0 ml	
Controls							2,0 ml
Reagent P – Internal Standard Solution (diluted 1:50 with H₂O HPLC grade)	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
In vortex for 20 seconds

STEP 3 : Incubate at 60 °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	2200 µl						
Hydrolysed Calibrator – Level 2		2200 µl					
Hydrolysed Calibrator – Level 3			2200 µl				
Hydrolysed Calibrator – Level 4				2200 µl			
Hydrolysed Calibrator – Level 5					2200 µl		
Hydrolysed Sample						2200 µl	
Hydrolysed Controls							2200 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.**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 in glass tubes with Teflon caps only

- 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 70 µl of **Reagent M - Derivatization Solution****Vortex for 20 seconds****STEP 11**: Incubate at 70 °C for 30 minutes**Cooling at Room Temperature****STEP 12** : Transfer the whole sample in vials goblet form**INJECTION** :

- Inject 2 µl of the solution in the GC

NORBUPRENORPHINE and BUPRENORPHINE in urine - Warnings

REAGENT L: TEST SOLUTION

Norbuprenorphine	about 10.000 ng/ml
Buprenorphine	about 10.000 ng/ml

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 Factor Four Column 30 m x 0,25 mm, 0,25 µm
- 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 100 °C x 2 minutes + 30 °C/min up to 220 °C x 0 minute + 15 °C/min up to 310 °C x 18 minutes (run 30 minutes)
- Helium Gas 1 ml/min

SET OF MASSA DETECTOR (4000) :

- Range of Massa 350 – 540
- Temperature of Massa: 180 °C;
- Temperature Transfer Line: 270 °C;
- Temperature Manifold: 80 °C
- Filament on: 3 minutes

CONDITIONING OF VF-5 MS Factor Four 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
CC44016	Calibrator in urine for Norbuprenorphine and Buprenorphine	2 x 5 x 6 ml
CC44019	Control for Norbuprenorphine and Buprenorphine – Levels 1 and 2	2 x 6 x 6 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

Norbuprenorphine and Buprenorphine in urine
(Reference Massa Spectra)

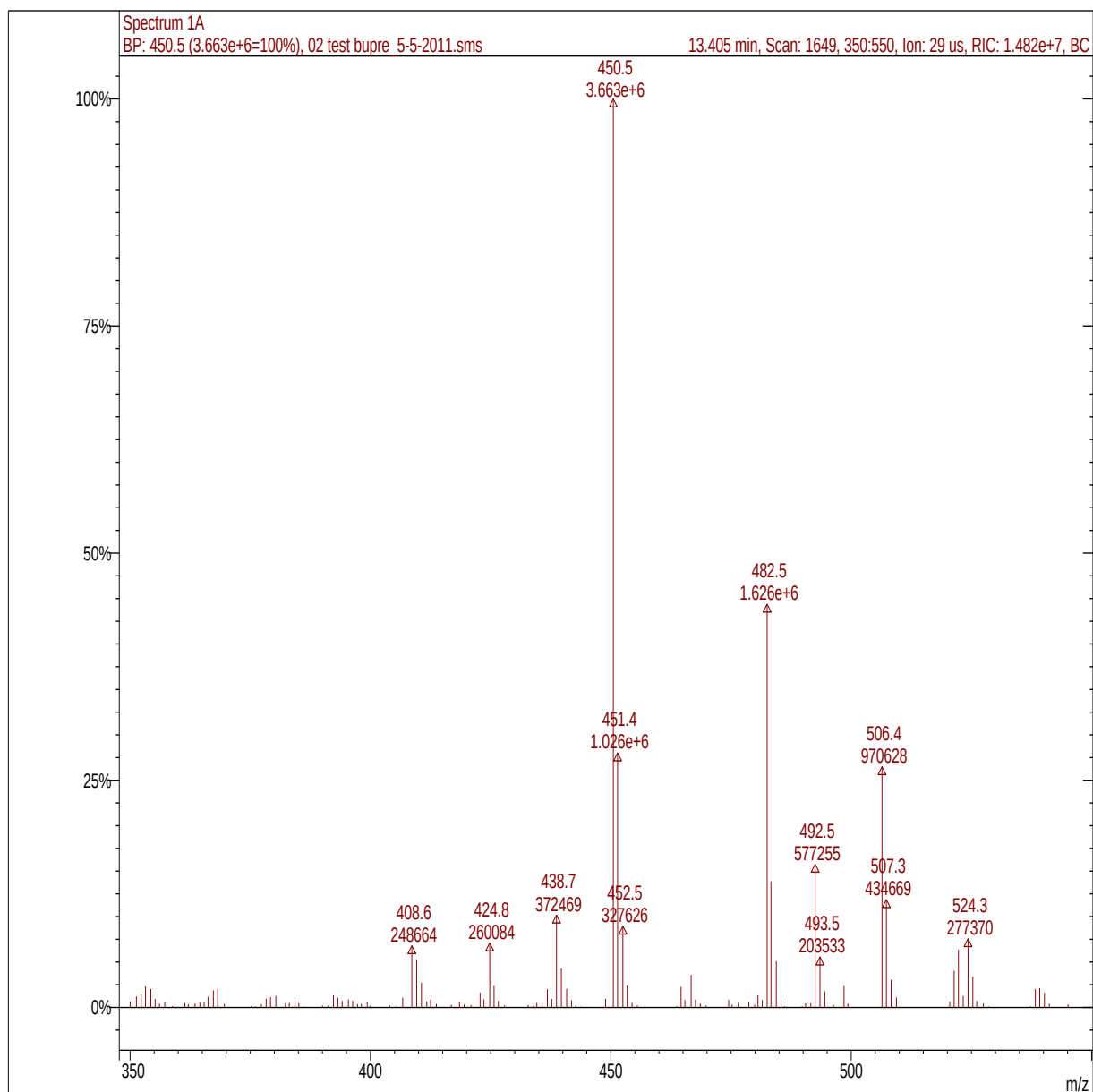


Fig. 1 : *Massa Spectrum of Buprenorphine*

MOLECULAR IONS : 450-482-506

Norbuprenorphine and Buprenorphine in urine

(Reference Massa Spectra)

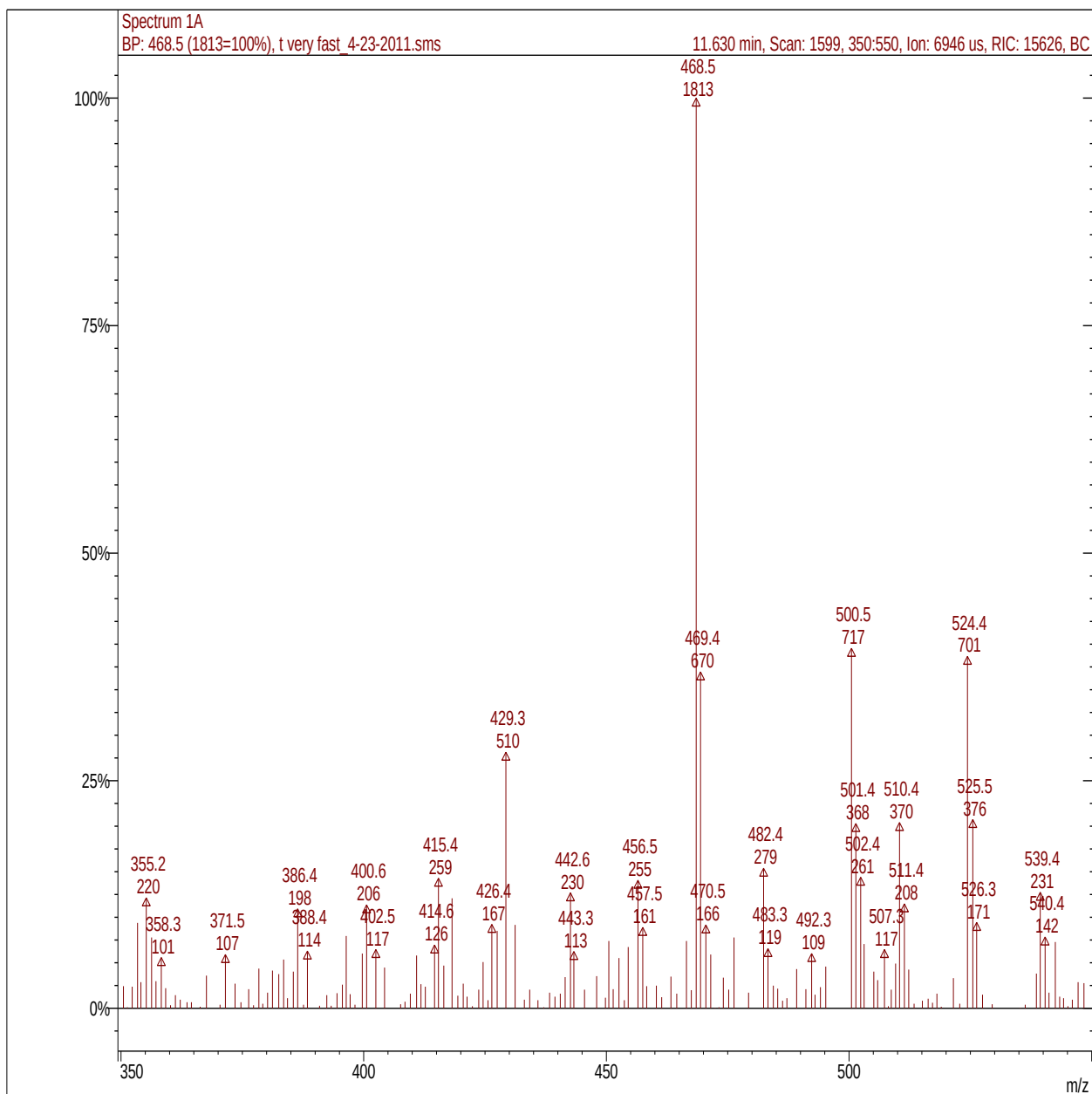


Fig. 2 :	Massa Spectrum of Norbuprenorphine
	MOLECULAR IONS : 468-500-524

(Reference Massa Spectra)



MOLECULAR IONS : 455-440-414