

Drugs of Abuse in urine by LC-MS – Code LC74010

Method of Confirmation by LC-MS

INTRODUCTION

The screening of Drugs of Abuse in biological liquids (usually in urine) is generally carried out using immunometric techniques with the utilization of monoclonal antibodies and following marking with various reagents.

As these techniques can't exclude false positives, the positive results of the screening must be confirmed by an alternative technique, generally by chromatographic technique both Gas (GC) and Liquid (LC), both coupled with mass detector (MS).

The present method allows the determination of Morphine, Codeine, 6-Monoacetylmorphine, 3,4-MDA, 3,4-MDE, 3,4-MDMA, Benzoylcegonine, Cocaine, Methadone, EDDP, Amphetamine, Methamphetamine, Norbuprenorphine, Buprenorphine, MBDB and delta-9-THC-COOH by LC-MS.

This approach allows the dosage of other molecules of urine matrix: Ethylmorphine, Dihydrocodeine, Normorphine, Norcodeine, Oxycodone, Diacetylmorphine, Cocaethylene, Ephedrine, Pseudoephedrine, Norpseudoephedrine, Ketamine.



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CE

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° 011	Abuse Drugs by LC/MS	06/2024
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TECHNICAL FEATURES

Principle of the Method :

This method enables a broad package of drugs of abuse in urine after a simple hydrolysis and reading by LC-MS.

Recovery :

100 %

Sensitivity :

Opiates, Cocaine and metabolite, Methadone and metabolite, Amphetamines and metabolites
10,0 ng/ml

Delta-9-THC and Norbuprenorphine metabolite
1,0 ng/ml

Dynamic Range of the Method :

10 – 10.000 ng/ml

1 - 1.000 ng/ml THC- Buprenorphine -
Norbuprenorphine

Cut-off:

Morphine	100 ng/ml
Dihydrocodeine	100 ng/ml
Codeine	100 ng/ml
Ethylmorphine	100 ng/ml
6-Monoacetylmorphine	100 ng/ml
Diacetylmorphine	100 ng/ml
Amphetamine	250 ng/ml
3,4-MDA	250 ng/ml
3,4-MDMA	250 ng/ml
3,4-MDE	250 ng/ml
delta-9-THC-COOH	15 ng/ml
Cocaethylene	100 ng/ml
MBDB	250 ng/ml
Benzoylcegonine	100 ng/ml
Cocaine	100 ng/ml
Methadone	100 ng/ml
Methamphetamine	250 ng/ml
Norbuprenorphine	5 ng/ml
Buprenorphine	5 ng/ml
EDDP	100 ng/ml

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

Screening and confirmation cut-off values in Urine

Substance Class or substance (screening target substance)	Screening cut-off (ng/ml)	Confirmation cut-off (ng/ml)
Opiates (free morphine) Total Morphine ^a Total Codeine ^a 6-Monoacetylmorphine	300	100 100 10
Cocaine metabolite (benzoylecgonine) Cocaine Benzoylecgonine	300	100 100
Amphetamines and congeneris Amphetamine Methamphetamine	500	200 200
3,4-Methylenedioxymethamphetamine and congeneris MDMA MDA MDEa MBDB	500	200 200 200 200
Methadone (Methadone) Methadone EDDP	300	100 100
Cannabinoids (THC-COOH) THC-COOH	50	15
Buprenorphine Total Buprenorphine ^a Total norbuprenorphine ^a	5	5 5

a- the value is reported to the sample subjected to hydrolysis (to check the efficiency of the hydrolysis reaction of morphine-3-glucuronide in the process of method validation is mandatory)

Ref. GUIDELINES FOR LABORATORIES SEARCHING FOR SUBSTANCES OF ABUSE ON BIOLOGICAL SAMPLES TAKEN FROM THE LIVING SUBJECTS WITH "TOXICOLOGICAL AND FORENSIC-MEDICAL-LEGAL PURPOSES" [Rev. n. 4, 6 December 2012 from the Scientific Association Quality Commission "Group of Italian Forensic Toxicologists" (GTFI)].

Components of the kit :

Reagent A – Hydrolysis Solution, **1 x 10 ml**

Reagent B – Hydrolysis Enzyme for Drugs, **2 x 1 ml**

Reagent C – Buffer Solution for Hydrolysis, **1 x 20 ml**

Reagent D – Diluting Solution, **1 x 70 ml**

Reagent E – Internal Standard Solution 1, **1 x 200 ul**

Store at –20 °C

Reagent F – Internal Standard Solution 2, **1 x 500 ul**

Store at –20 °C

Reagent G – Internal Standard Solution 3, **1 x 200 ul**

Store at –20 °C

Reagent M1 – Mobile Phase M1, **1 x 500 ml**

Reagent M2 – Mobile Phase M2, **1 x 500 ml**

All the components are ready-to-use.
Stability: 3 years at 2 – 8 °C except
Reagents E, F and G stored at – 20 °C.

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

Minimum Instrumental equipment required:

LC-MS triple quadrupole instrument with
Divert Valve - Analysis in binary gradient –
Work modality MRM
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.

Centrifuge the urines before processing.

ANALYTICAL PROCEDURE FOR DRUGS (EXCLUDED delta-9-THC-COOH, OPIATES and NORBUPRENORPHINE):

STEP 1 :

In a tube dilute 1:10 in water the **Reagent E – Internal Standard Solution 1** in such a way as to obtain a final volume of diluted Internal Standard that is sufficient to carry out all preparative.

STEP 2 :

In suitable test tubes of 10 ml, dispense in sequence:

	Calibrator	Sample	Controls
Calibrator	100 µl		
Sample		100 µl	
Controls			100 µl
Reagent E – Internal Standard Solution 1 (diluted as in STEP 1)	20 µl	20 µl	20 µl
Reagent D – Diluting Sol	900 µl	900 µl	900 µl

Vortex for 10 sec.

INJECTION :

- Inject 5 - 10 µl of the solution in the LC system

ANALYTICAL PROCEDURE FOR OPIATES, BUPRENORPHINE AND NORBUPRENORPHINE:

STEP 1 : Enzyme Reconstitution

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

Mix by inversion

STEP 2 :

In a tube dilute 1:10 in water the **Reagent F – Internal Standard Solution 2** in such a way as to obtain a final volume of diluted Internal Standard that is sufficient to carry out all preparative.

STEP 3 : Sample hydrolysis

In suitable test tubes of 10 ml, dispense in sequence:

	Calibrator	Sample	Controls
Urine Calibrator	1000 µl		
Sample		1000 µl	
Controls			1000 µl
Reagent B – Reconstituted Enzyme	200 µl	200 µl	200 µl
Reagent F – Internal Standard 2 (diluted as in STEP 2)	50 µl	50 µl	50 µl

Vortex for 10 sec.

STEP 4 : Incubate at 60 °C for 3 hours.

Cooling at Room Temperature

STEP 5 : Take 100 µl of hydrolysed and add 500 µl of **Reagent D – Diluting Solution** (shake before use)

Vortex for 10 sec.

INJECTION :

- Inject 5 -10 µl of the solution in the LC system

ANALYTICAL PROCEDURE SPECIFIC FOR delta-9-THC-COOH:

STEP 1 :

In a tube dilute 1:10 in water the **Reagent G – Internal Standard Solution 3** in such a way as to obtain a final volume of diluted Internal Standard that is sufficient to carry out all preparative.

STEP 2 : Sample Hydrolysis

In suitable test tubes of 10 ml, dispense in sequence:

	Calibrator	Sample	Controls
Urine Calibrator	500 µl		
Sample		500 µl	
Controls			500 µl
Reagent A – Hydrolysis Solution	100 µl	100 µl	100 µl
Reagent G – Internal Std Solution 3 (diluted as in STEP 1)	20 µl	20 µl	20 µl

Vortex for 10 sec.

STEP 3 : Incubate at 60 °C for 20 minutes

Cooling at Room Temperature

Bring at pH between 5 and 7 with about 60-80 µl of Acid Acetic (not included)

STEP 4 : Take 100 µl of hydrolysed and add 500 µl of **Reagent D – Diluting Solution** (shake before use)

Vortex for 10 sec.

INJECTION :

- Inject 5 - 10 µl of the solution in the LC system

Drugs of Abuse in urine - Warnings

REAGENT E – INTERNAL STANDARD SOLUTION 1

The reagent contains the following Deuterated Internal Standard: BEG-D8, COCAINE-D3, METHADONE-D9, EDDP-D3, MDA-D5, MDE-D5, MDMA-D5, AMPHETAMINE-D11, METHAMPHETAMINE-D11. Store at -20°C and stored in freezer immediately after its use.	
Abuse Drugs:	Internal Standard to use for calibrate:
BENZOYLECGONINE	BEG-D8
COCAINE	COCAINE-D3
METHADONE	METHADONE-D9
EDDP	EDDP-D3
3,4-MDA	MDA-D5
3,4-MDE	MDE-D5
3,4-MDMA	MDMA-D5
AMPHETAMINE	AMPHETAMINE-D11
METHAMPHETAMINE	METHAMPHETAMINE-D11
MBDB	MDMA-D5

REAGENT F – INTERNAL STANDARD SOLUTION 2

The reagent contains the following Deuterated Internal Standard: MORPHINE-D6, CODEINE-D6, 6-MAM-D6, NORBUPRENORPHINE-D3 Store at -20°C and stored in freezer immediately after its use.	
Abuse Drugs:	Internal Standard to use for calibrate:
MORPHINE	MORPHINE-D6
CODEINE	CODEINE-D6
6-MONOACETYLMORPHINE	6-MAM-D6
NORBUPRENORPHINE	NORBUPRENORPHINE-D3
BUPRENORPHINE	NORBUPRENORPHINE-D3

REAGENT G – INTERNAL STANDARD SOLUTION 3

The reagent contains the following Deuterated Internal Standard: DELTA-9-THC-COOH-D9. Store at -20°C and stored in freezer immediately after its use.	
Abuse Drugs:	Internal Standard to use for calibrate:
DELTA-9-THC-COOH	DELTA-9-THC-COOH-D9

NEW COLUMN TREATMENT

Install the new analytical column Accucore C18 30 x 2,1 mm – 2,6 um or in alternative RRHD C18 (50 x 2,1 mm. 1.8 um) .
Disconnect the detector and flux 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) at set flow at 300 ul / minute.
Don't recycle the washing solutions..

COLUMN CONDITIONING

Condizionate the new analytical column Accucore C18 30 x 2,1 mm – 2,6 um or in alternative RRHD C18 (50 x 2,1 mm. 1.8 um) with Mobile Phases M1:M2 (95:5) set flow at 400 ul / minute and discharge the first 10 ml. Thermostated at 40°C.
It ISN'T 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.

GRADIENT for UHPLC SYSTEMS (Analytical Column RRHD C18 (50 x 2,1 mm. 1.8 µm))

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (µl/min)
0	95	5	400
3	95	5	400
3.01	80	20	400
5.5	70	30	400
5.51	45	55	400
8	5	95	400
8.01	95	5	400
10	95	5	400

COLUMN CLEANING

Disconnect the detector. Flux a solution made of H₂O : Methanol (20 : 80 v/v) for 30 min and discharge.

AUTOSAMPLER SYRINGE WASHING

Before use wash the syringe with Methanol.

FRAGMENTATIONS

ANALYTE	PARENT ION
Morphine	286.3
Codeine	300.0
Dihydrocodeine	302.0
MDA	180.0
MDMA	194.0
MDE	208.0
BEG	290.0
Cocaine	304.0
6-MAM	328.0
Methadone	310.0
EDDP	278.0
Amphetamine	136.0
Methamphetamine	150.0
Norbuprenorphine	414.0
Ethylmorphine	314.0
Delta-9-THC-COOH	345.0
Cocaethylene	318.1
MBDB	208.0
Buprenorphine	468.1
Normorphine	272.0

Norcodeine	286.0
Oxycodone	316.3
Diacetylmorphine	370.0
Ketamine	238.0
DEUTERATED INTERNAL STANDARD	
Morphine-D6	292
Codeine-D6	306
MDA-D5	185
MDMA-D5	198
MDE-D5	213
BEG-D8	298
Cocaine-D3	307
6-MAM-D6	334
Methadone-D9	319
EDDP-D3	281
Amphetamine-D11	147
Methamphetamine-D11	161
Norbuprenorphine-D3	417
Delta-9-THC-COOH-D9	352
Cocaethylene-D3	321

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC43016	Urine Calibrator for Amphetamines	2 x 5 x 5 ml
CC46016	Urine Calibrator for Benzoylcegonine and Cocaethylene	2 x 5 x 5 ml
CC48016	Urine Calibrator for Methadone	2 x 5 x 5 ml
CC43019	Multiparametric Control for Abuse Drugs – Levels 1 and 2	2 x 6 x 5 ml
CC44016	Urine Calibrator for Buprenorphine and Norbuprenorphine	2 x 5 x 6 ml
CC44019	Urine Control for Buprenorphine and Norbuprenorphine – Levels 1 and 2	2 x 6 x 6 ml
CC45016	Urine Calibrator for Opiates	2 x 5 x 5 ml
CC45019	Urine Control for Opiates – Levels 1 and 2	2 x 6 x 5 ml
CC45116	Urine Calibrator for 6-Monoacetylmorphine	2 x 5 x 5 ml
CC46116	Urine Calibrator for Cocaine	2 x 5 x 5 ml
CC48116	Urine Calibrator for EDDP	2 x 5 x 5 ml
CC48019	Urine Control for EDDP, 6-MAM and Cocaine – Levels 1 and 2	2 x 6 x 5 ml
CC47016	Urine Calibrator for THC	2 x 5 x 10 ml
CC47019	Urine Control for THC – Level 1 and 2	2 x 6 x 10 ml
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs
SK74010	Starter kit for Abuse Drugs in urine	36 x 1 ml
Analytical Column for HPLC Systems		
ZTF17126032130	Analytical Column Accucore C 18 (30 x 2,1 mm. 2.6 um)	1 Pc
Analytical Column for UHPLC Systems		
Z959757902	Analytical Column RRHD C 18 (50 x 2,1 mm. 1.8 um)	1 Pc

Drugs of Abuse in urine

(Reference Chromatograms)

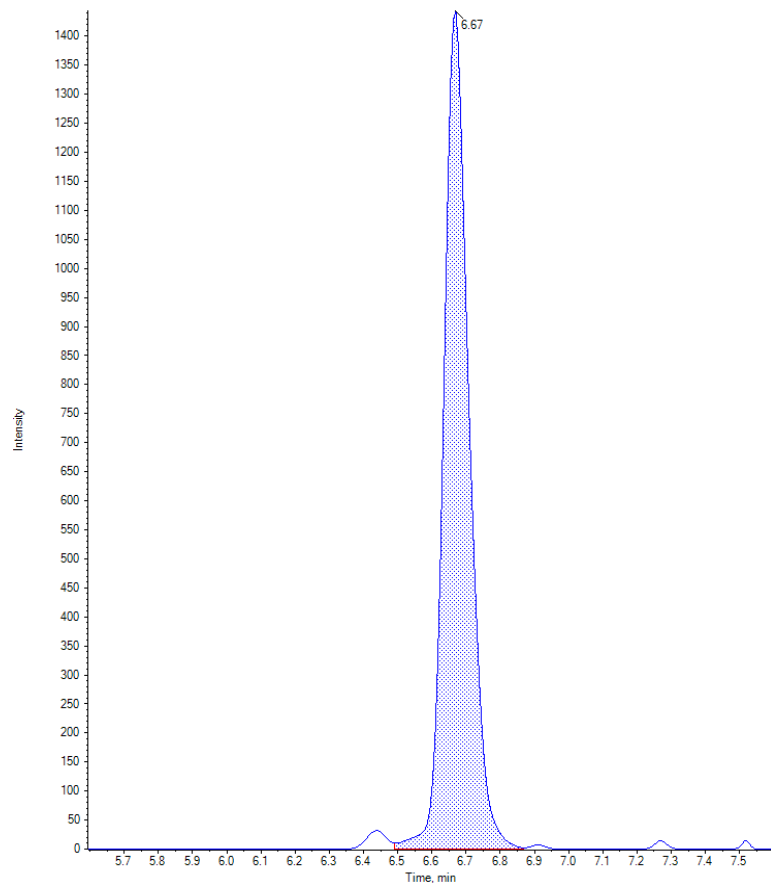


Fig. 1 :	BUPRENORPHINE CALIBRATOR 1
	R.T. 6.67

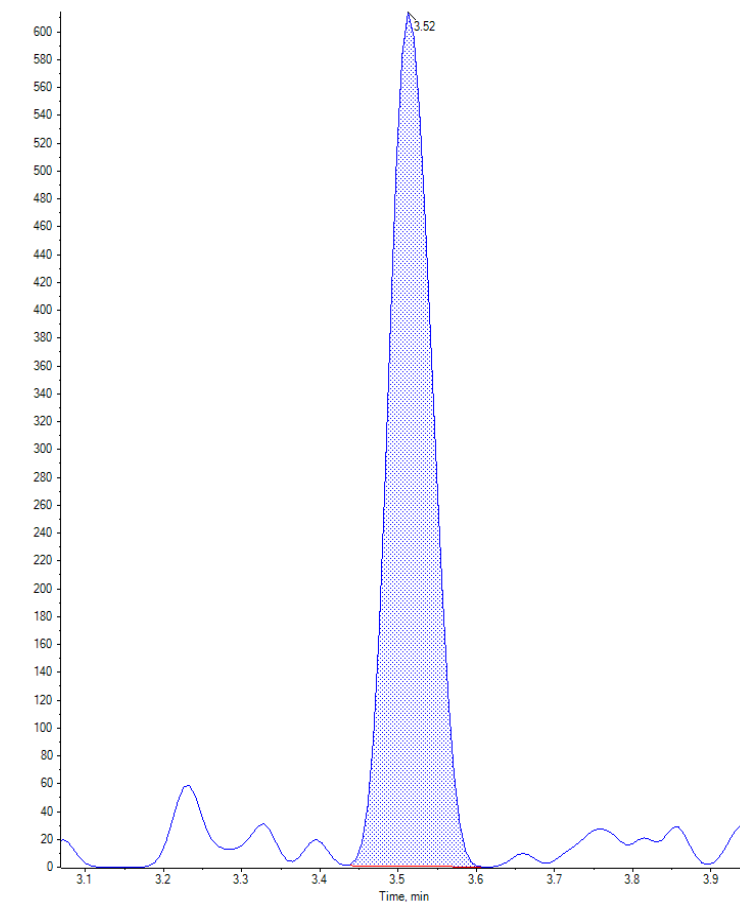


Fig. 2 :	THC- COOH CALIBRATOR 1
	R.T. 3.52

Drugs of Abuse in urine

(Reference Chromatograms)

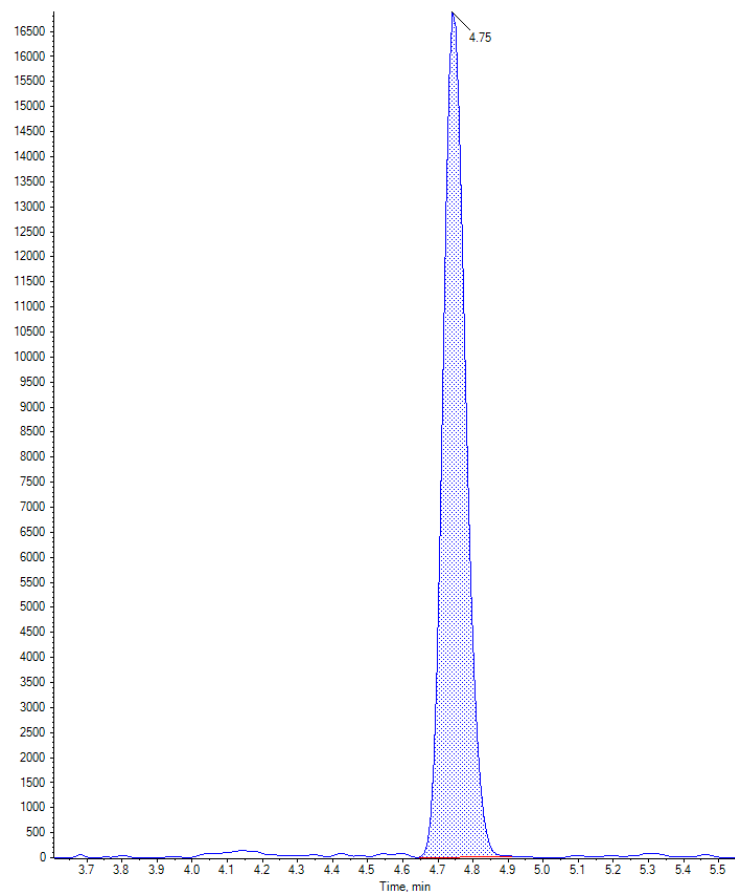


Fig. 3 : **6-MAM CALIBRATOR 1**

R.T. 4.75

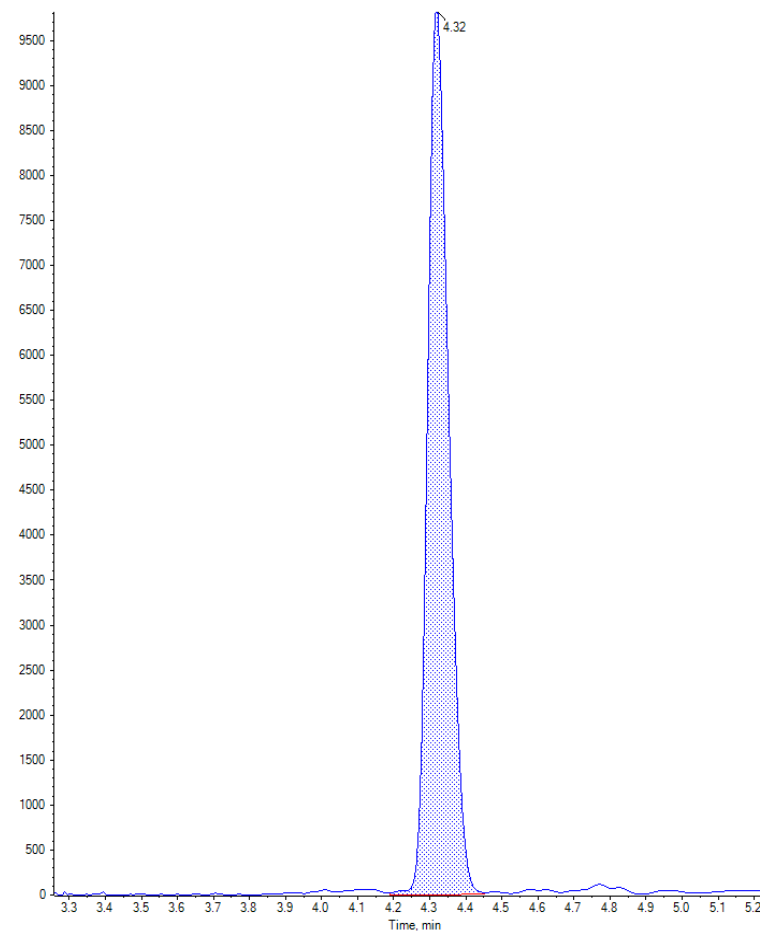


Fig. 4 : **CODEINE CALIBRATOR 1**

R.T. 4.32

Drugs of Abuse in urine

(Reference Chromatograms)

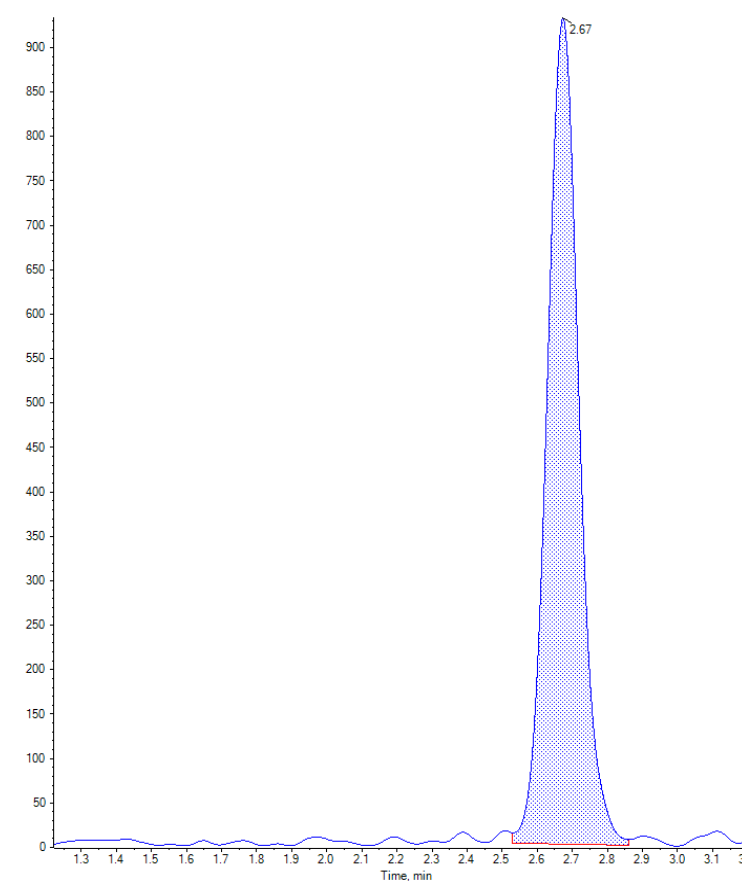


Fig. 5 :	MORPHINE CALIBRATOR 1
	R.T. 2.67

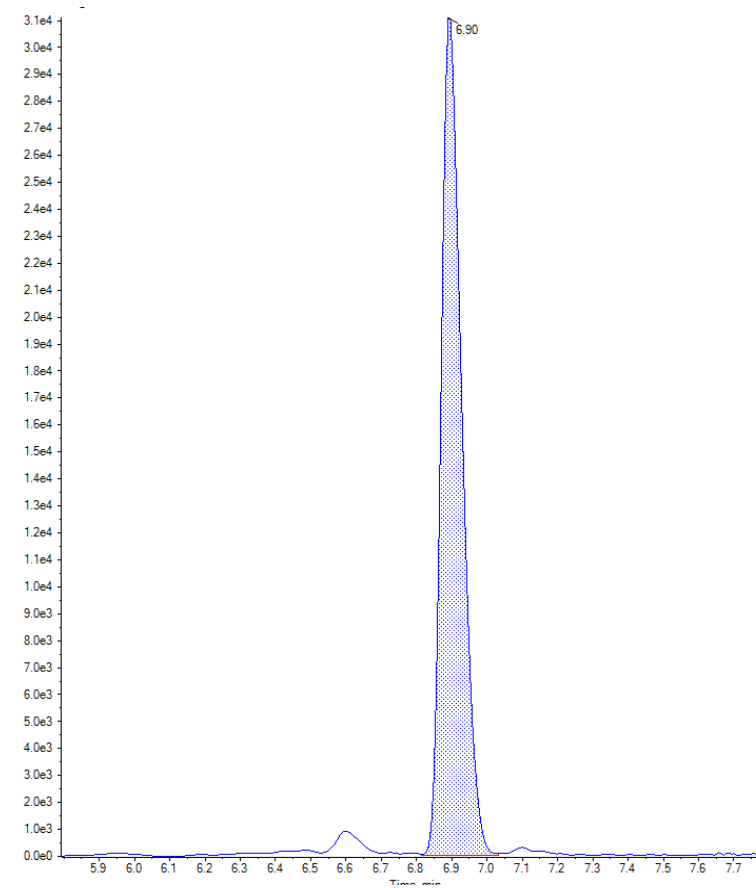


Fig. 6 :	METHADONE CALIBRATOR 1
	R.T. 6.9

Drugs of Abuse in urine

(Reference Chromatograms)

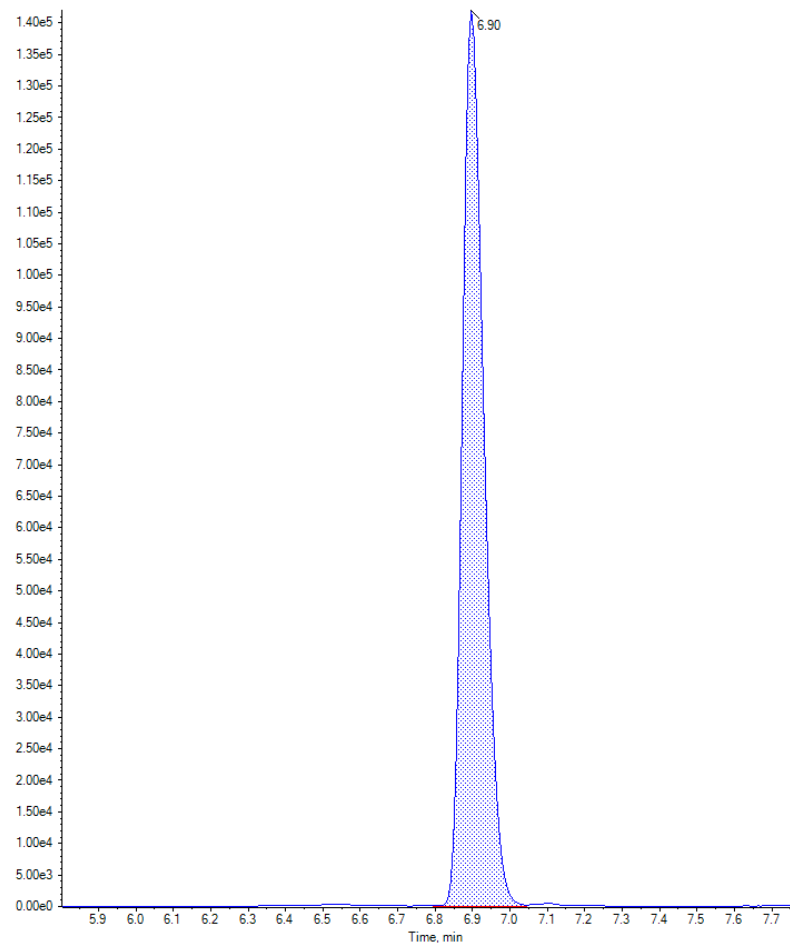


Fig. 7 :	METHADONE REAL SAMPLE
	R.T. 6.9

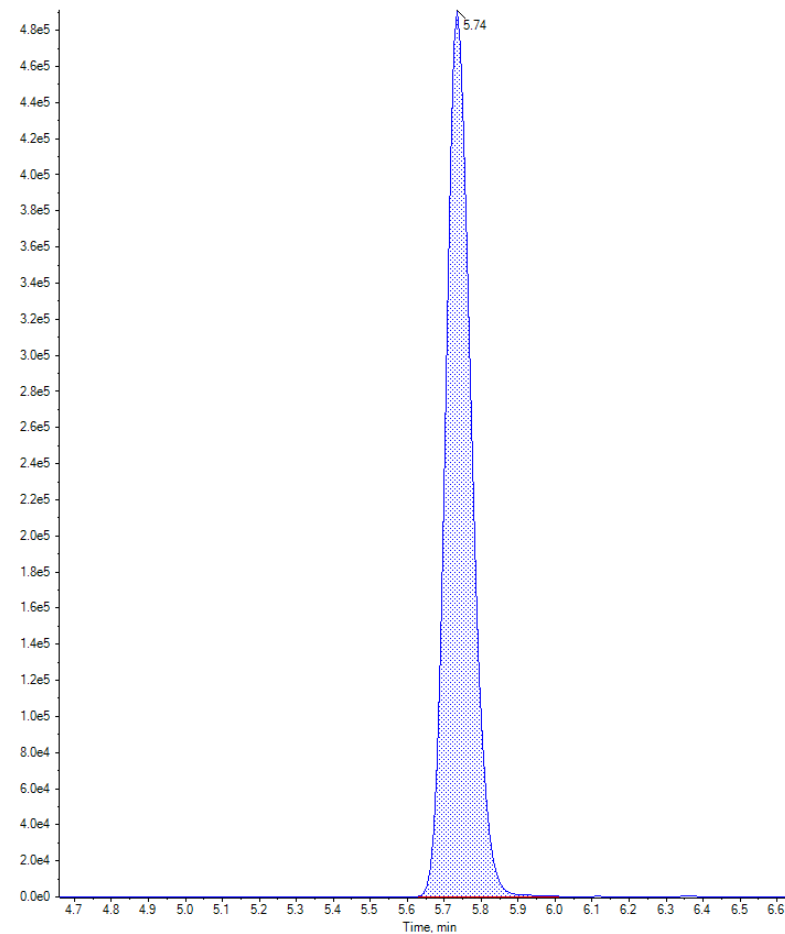


Fig. 8 :	BEG CALIBRATOR 1
	R.T. 5.74

Drugs of Abuse in urine

(Reference Chromatograms)

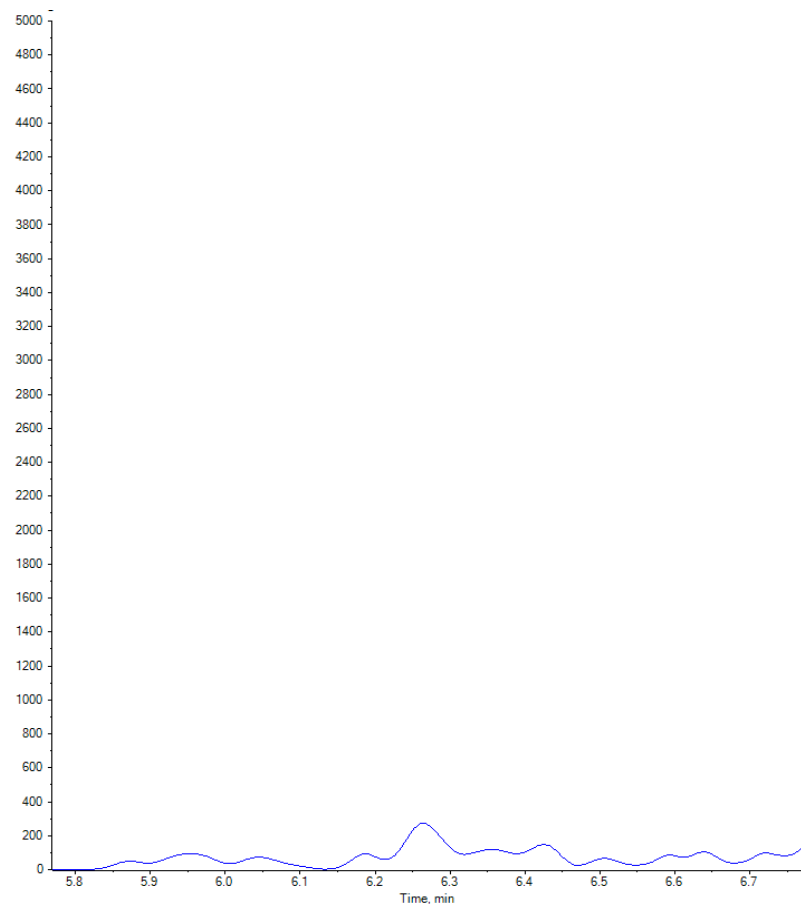


Fig. 9 :	COCAINE PLASMA BLANK
	R.T. 6.27

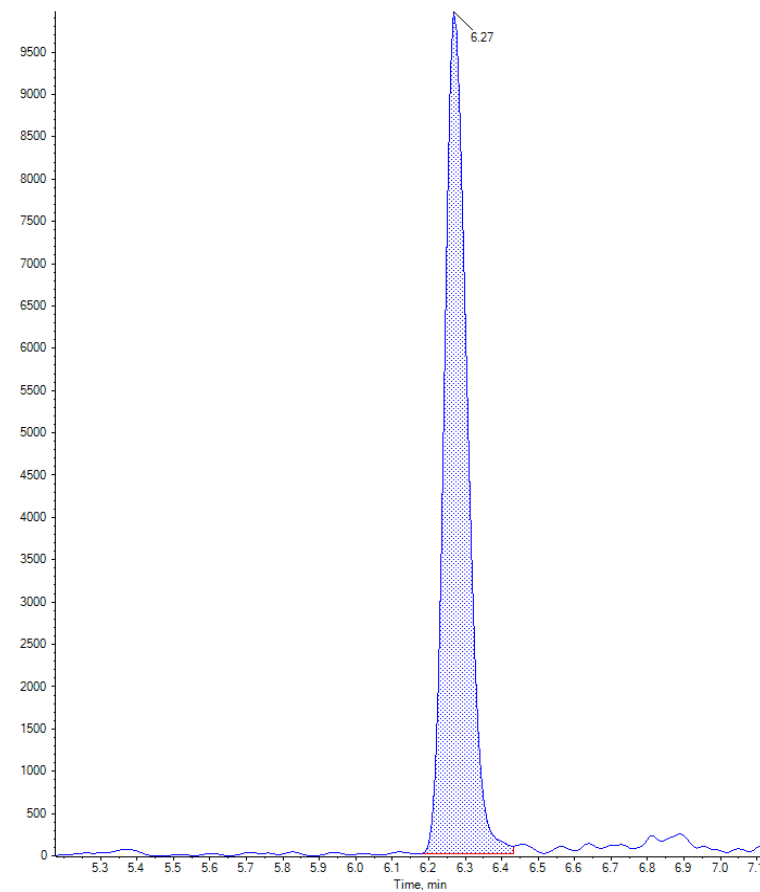


Fig. 10 :	COCAINE CALIBRATOR 1
	R.T. 6.27

Drugs of Abuse in urine

(Reference Chromatograms)

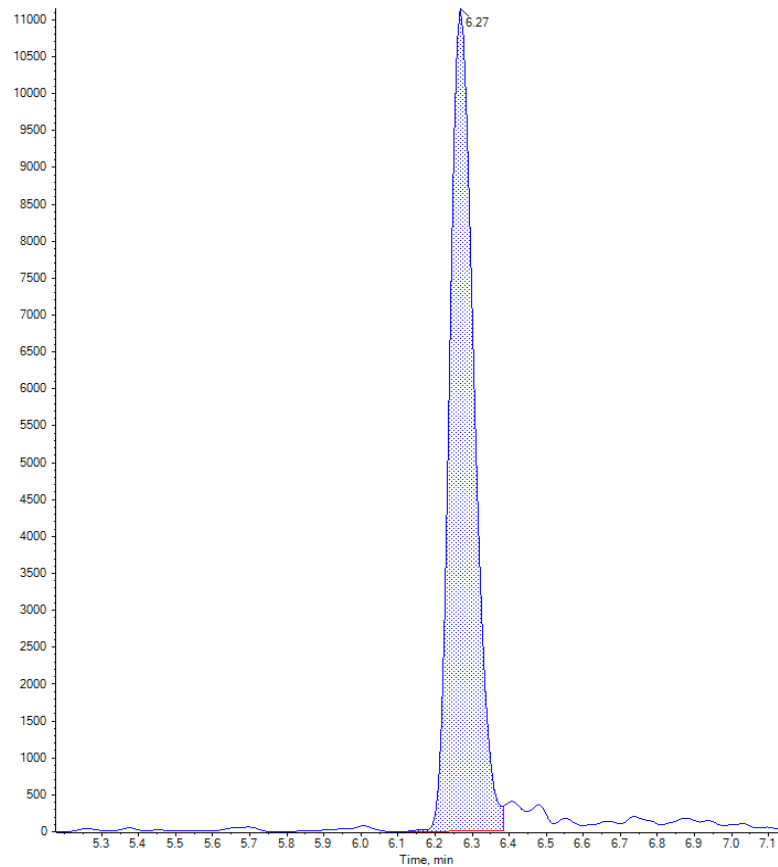


Fig. 11 :	COCAINE REAL SAMPLE
	R.T. 6.27

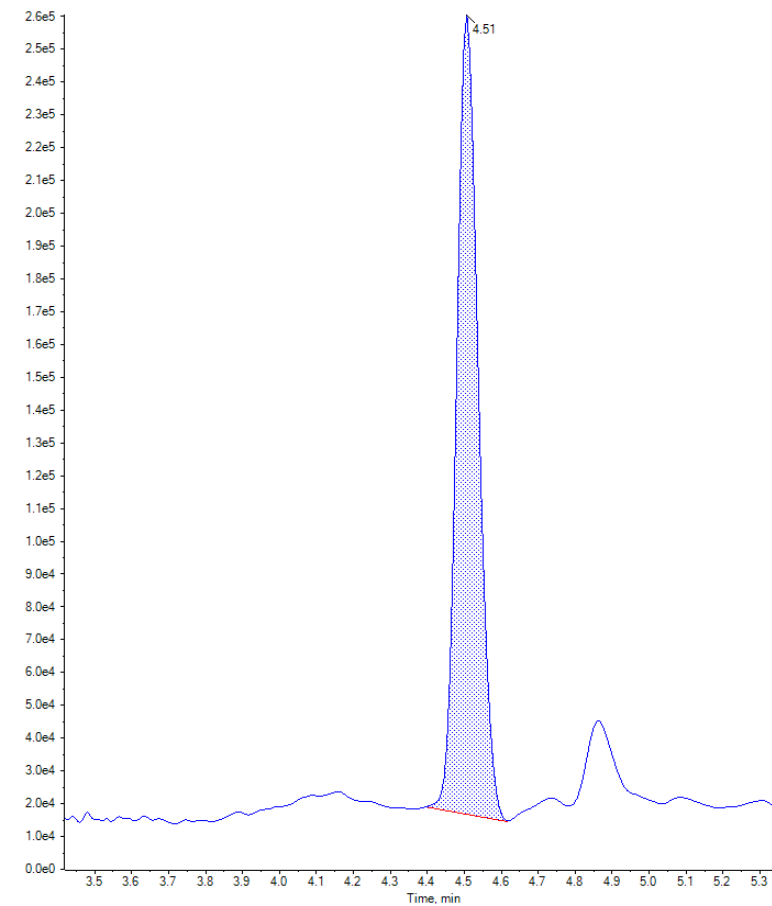


Fig. 12 :	AMPHETAMINE CALIBRATOR 1
	R.T. 4.51

Drugs of Abuse in urine

(Reference Chromatograms)

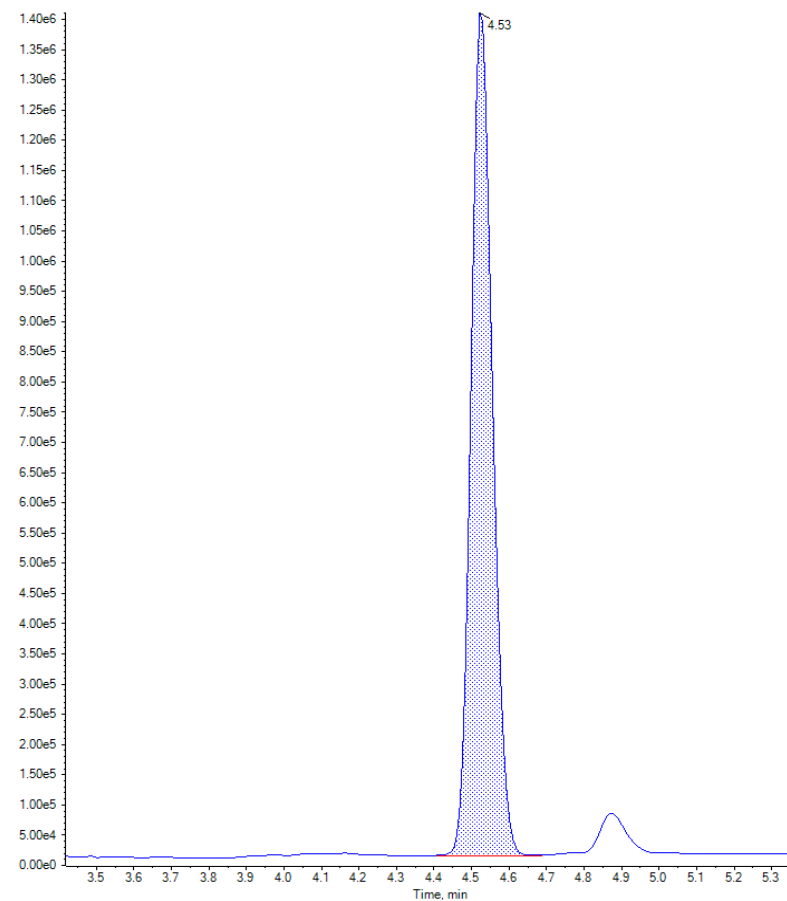


Fig. 13 :	AMPHETAMINE REAL SAMPLE
	R.T. 4.53

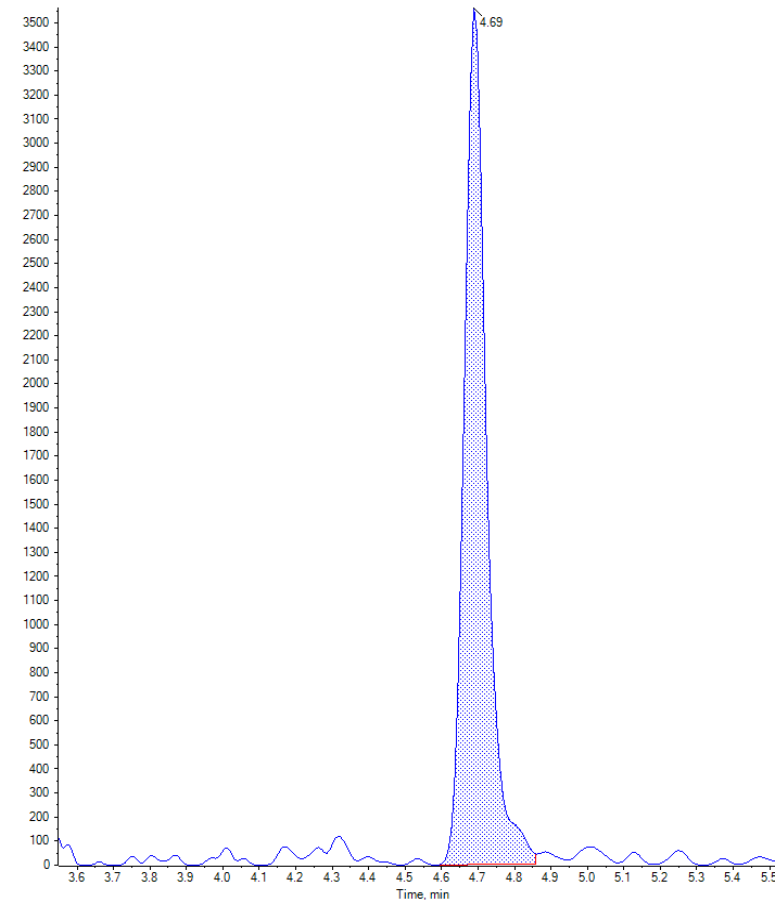


Fig. 14 :	METHAMPHETAMINE CALIBRATOR 1
	R.T. 4.69

Drugs of Abuse in urine

(Reference Chromatograms)

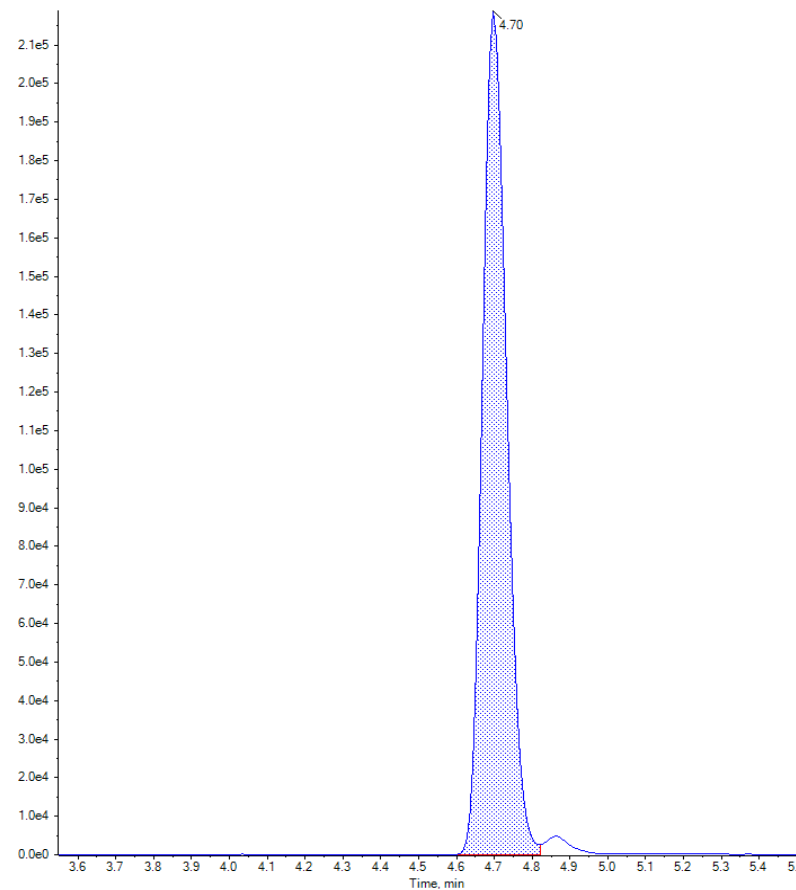


Fig. 15 :	METHAMPHETAMINE REAL SAMPLE
	R.T. 4.69

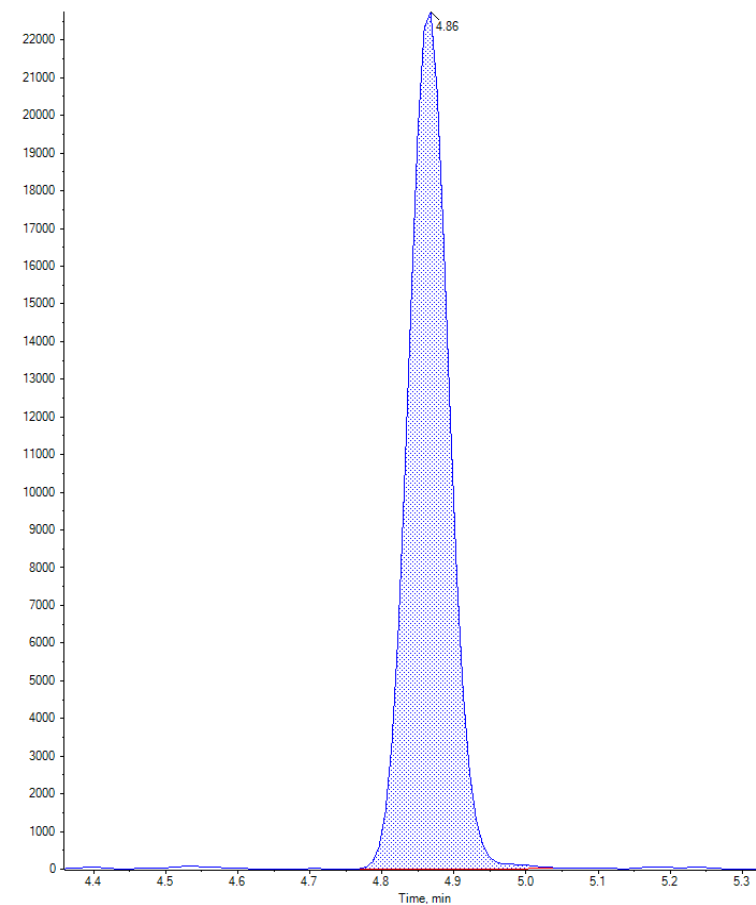


Fig. 16 :	MDMA CALIBRATOR 1
	R.T. 4.86