

BASIC DRUGS OF ABUSE in hair by LC-MS/MS – Code LC49010

(Morphine, Codeine, 6-MAM, Cocaine, BEG, Cocaethylene, Amphetamine, Methamphetamine, 3,4-MDA, 3,4-MDMA, 3,4-MDE, Norbuprenorphine, Methadone, EDDP, Buprenorphine, MBDB, Ecgonine Methyl Ester)

Method of confirmation by LC-MS/MS

INTRODUCTION

In the early 80 's many studies have been carried out on the determination of substances of abuse in keratinous matrix and then use of hair analysis as a means of previous use of these substances. Once incorporate into the hair, by the systemic circulation and by the contribution of sweat and sebum, substances of abuse remain impounded in the keratinous matrix in a stable way over time as a function of their lipophilicity, molecular weight, the pKa and the steric encumbrance. The analysis of substances of abuse in keratinous matrix is an ideal addendum to the analysis of blood or urine as it provides information regarding an earlier time than that covered by other biological matrices and refers to a longer time period (one or more months) as a function of the length of the hair.

Considering an average growth of 1 cm per month, you can learn the history of the consumption by analyzing segmented hair per cm or fractions of cm. It is also possible to highlight external contamination by examination of washing solvents by comparing them with the results obtained by digested hair. The search for substances of abuse in hair can be used to test use, abuse and misuse over time and to determine intensity and its history and then provide analytical data with medico-legal value. Determination in hair may be required in the following cases: deaths related to substances of abuse, assessment of ineligibility for driving, criminal liability, reliance custody of infants, prenatal exposure to substances of abuse and finally as confirmation of sporadic consumption or a dependency in workers.

Immunochemical tests can be used as first screening methods to analyze in a short time a large number of samples in a standardized, efficient and economical way to determine negative samples, i.e. samples that not contain Δ^9 -Tetrahydrocannabinol and its metabolite whose concentration is below a threshold value (cut-off).

Confirmation methods are used to verify false positive due to the non-specificity of screening tests. In the case of keratinous matrix, also no proper first screening tests can be performed. The "Society of Hair Testing" (SOHT) recommends the use of chromatographic separative methodologies coupled to chromatographic separative disclosure in mass spectrometry for the determination of substances of abuse in keratinous matrix for medical-legal purposes and/or clinical trials.

According to the guidelines of SOHT, this method allows to determine all basic substances of abuse (6-MAM, Morphine, Codeine, Ecgonine Methyl Ester, Cocaine, BEG, Cocaethylene, Amphetamine, Methamphetamine, 3,4-MDA, 3,4-MDE, 3,4-MDMA, MBDB, Norbuprenorphine, Methadone, Buprenorphine, EDDP) upon extraction from keratinous matrix and subsequent liquid-liquid extraction to eliminate the presence of complex matrix interferences. Finally, the determination in Liquid chromatography/mass spectrometry (LC/MS/MS) takes places.

 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



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TECHNICAL FEATURES

Principle of the Method :

This method determines **Morphine, Codeine, Ecgonine Methyl Ester, 6-Monoacetylmorphine, Cocaine, Beg, Cocaethylene, Methadone, Amphetamine, Methamphetamine, MDMA, MDA, MDE, Norbuprenorphine, Buprenorphine, EDDP, MBDB** after hydrolysis of the keratin matrix, SPE extraction and LC-MS in reading.

The method was validated according to the criteria established at the international level by the FDA and by the ICH.

ANALYTE	Intra-Day Accuracy			Intra-Day-Precision			Inter-Day-CV%		
	(Error %)			(CV%)			(Error %)		
	Calibrator			Calibrator			Calibrator		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
AMPHETAMINE	11,2%	7,9%	10,5%	2,2%	7,4%	3,7%	4,7%	6,6%	8,8%
METHAMPHETAMINE	14,2%	11,4%	8,9%	8,3%	8,8%	9,2%	13,6%	8,6%	12,8%
3,4-MDMA	10,2%	14,1%	9,9%	4,6%	8,0%	8,0%	11,9%	8,7%	9,5%
3,4-MDA	13,5%	14,9%	15,0%	15,1%	7,3%	4,9%	13,1%	8,1%	6,6%
3,4-MDE	8,2%	10,3%	14,3%	5,5%	4,3%	3,1%	5,8%	4,9%	16,1%
BEG	13,3%	9,2%	11,3%	14,4%	5,2%	9,4%	14,3%	11,3%	13,3%
COCAETHYLENE	11,0%	6,3%	4,6%	10,9%	5,9%	7,3%	13,8%	9,1%	6,2%
COCAINE	7,7%	12,1%	11,3%	4,8%	3,2%	10,1%	10,9%	13,4%	9,4%
MORPHINE	9,2%	10,6%	12,6%	4,5%	12,4%	2,9%	9,2%	13,4%	9,4%
6-MAM	16,6%	12,7%	8,4%	2,5%	7,0%	2,7%	18,4%	13,2%	7,9%
CODEINE	13,7%	12,5%	13,7%	10,5%	4,9%	3,2%	13,2%	6,4%	14,4%
METHADONE	5,5%	14,4%	6,3%	5,9%	5,4%	6,8%	5,6%	7,5%	7,4%
EDDP	12,4%	9,7%	8,1%	3,3%	6,5%	7,8%	13,7%	11,1%	7,8%
BUPRENORPHINE	13,2%	9,4%	8,7%	4,8%	7,4%	6,1%	12,5%	11,5%	7,8%
NORBUPRENORPHINE	8,5%	6,3%	5,8%	8,4%	9,5%	6,0%	11,0%	9,0%	8,8%
MBDB	0,1%	15,1%	9,6%	1,9%	4,0%	3,3%	6,7%	17,1%	4,3%
ECGONINE METHYL ESTER	9,6%	8,9%	4,9%	5,4%	1,7%	3,7%	11,1%	5,4%	5,7%

ANALYTE	(Correlation Coefficient R²) ± DS	LOD (ng/mg)	LOQ (ng/mg)
AMPHETAMINE	0,9994 ± 0,0009	0,03	0,1
METHAMPHETAMINE	0,9998 ± 0,0002	0,03	0,1
3,4-MDMA	0,9993 ± 0,0007	0,03	0,1
3,4-MDA	0,9996 ± 0,0004	0,03	0,1
3,4-MDE	0,9971 ± 0,0015	0,03	0,1
BEG	0,9993 ± 0,0005	0,016	0,05
COCAINE	0,9993 ± 0,0006	0,083	0,25
COCAETHYLENE	0,9962 ± 0,0015	0,006	0,02
MORPHINE	0,9997 ± 0,0003	0,03	0,1
6-MAM	0,9997 ± 0,0004	0,03	0,1
CODEINE	0,9985 ± 0,0009	0,03	0,1
METHADONE	0,9994 ± 0,0007	0,03	0,1
EDDP	0,9992 ± 0,0011	0,03	0,1
BUPRENORPHINE	0,9997 ± 0,0004	0,0016	0,005
NORBUPRENORPHINE	0,9992 ± 0,0010	0,003	0,01
MBDB	0,9993 ± 0,0011	0,03	0,1
ECGONINE METHYL ESTER	0,9990 ± 0,0015	0,006	0,02

ANALYTE	Recovery (%)		
	Calibrator		
	Low	Medium	High
AMPHETAMINE	96,3%	97,1%	97,6%
METHAMPHETAMINE	95,6%	96,7%	96,3%
3,4-MDMA	92,3%	93,5%	95,8%
3,4-MDA	94,6%	95,8%	98,7%
3,4-MDE	96,8%	98,4%	97,4%
BEG	90,3%	91,9%	92,5%
COCAETHYLENE	91,9%	92,4%	92,3%
COCAINE	90,9%	91,3%	91,8%
MORPHINE	89,9%	90,0%	92,8%
6-MAM	88,8%	87,9%	88,0%
CODEINE	95,6%	94,5%	95,2%
METHADONE	92,3%	95,6%	96,6%
EDDP	97,2%	98,8%	99,3%
BUPRENORPHINE	90,5%	92,4%	92,0%
NORBUPRENORPHINE	93,7%	94,3%	92,1%
MBDB	96,9%	98,0%	97,5%
ECGONINE METHYL ESTER	95,3%	96,0%	95,6%

The table below shows for each analyte concentrations corresponding to the calibrators whose values Low, Medium and High cited above refer.

ANALYTE	Concentration (ng/mg)		
	Calibrator		
	Low	Medium	High
AMPHETAMINE	0,15	1	5
METHAMPHETAMINE	0,15	1	5
3,4-MDMA	0,15	1	5
3,4-MDA	0,15	1	5
3,4-MDE	0,15	1	5
BEG	0,075	0,5	2,5
COCAETHYLENE	0,03	0,2	1
COCAINE	0,37	2,5	25
MORPHINE	0,15	1	5
6-MAM	0,15	1	5
CODEINE	0,15	1	5
METHADONE	0,15	1	5
EDDP	0,15	1	5
BUPRENORPHINE	0,007	0,05	0,25
NORBUPRENORPHINE	0,015	0,1	0,5
MBDB	0,15	1	5
ECGONINE METHYL ESTER	0,03	0,2	1

Cut-off values according to Society of Hair Testing (SOHT)

Screening cut-offs according to SOHT recommendations			Confirmation cut-off according to SOHT recommendations	Confirmation cut-off in accordance with measure 9/18/2008 on workers with jobs at risk
Class of substance	Cut-off (ng/mg)	Analyte	Cut-off (ng/mg)	Cut-off (ng/mg)
Amphetamines	0,2	Amphetamine Methamphetamine MDA MDMA MDE	0,2 0,2 0,2 0,2 -	0,2 0,2 0,2 0,2 0,2
Cannabinoids	0,1	THC THC-COOH	0,05 0,0002	0,1 -
Cocaine	0,5	Cocaine BEG, EME, CE, NC*	0,5 0,05	0,2 0,05
Opiates	0,2	Morphine Codeine 6-MAM	0,2 0,2 0,2	0,2 0,2 0,2
Methadone	0,2	Methadone EDDP	0,2 0,05	0,2 -
Buprenorphine	0,01	Buprenorphine Norbuprenorphine	0,01 0,01	0,05 -

BEG: Benzoyllecgonine, EME: Ecgonine Methyl Estere, CE: Cocaethylene, NC: Norcocaine

Cut-off values according to Group of Italian Forensic Toxicologists (GTFI)

Substance Class or substance	Confirmation cut-off (ng/mg)
Opiates Morphine Codeine 6-Monoacetylmorphine	0,2 0,2 0,2
Cocaine (cocaine) Cocaine Benzoyllecgonine Ecgonine Methyl Ester Cocaethylene	0,5 0,05 0,05 0,05
Amphetamines and congeneris Amphetamine Methamphetamine	0,2 0,2
3,4-Methylenedioxymethamphetamine and congeneris MDMA MDA MDEa MBDB	0,2 0,2 0,2 0,2
Methadone Methadone EDDP	0,2 0,2
Cannabinoids THC THC-COOH	0,05 0,001
Buprenorphine Buprenorphine Norbuprenorphine	0,01 0,01

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)].

Cut-off :

Opiates, Amphetamine, Cocaine, Methadone:
BEG, Buprenorphine:

0.2 ng/mg

0.05 ng/mg

Ref. Gazzetta Ufficiale n°236 – 8 ott 2008

Components of the kit (50 test) :

All the reagents are ready to use and stable for 3 years at 2 – 8 °C.

Reagent A – Diluting Solution, 1 x 25 ml

Reagent B – Estruent Solution, 1 x 50 ml

Reagent C – Deuterated Internal Standard Solution, 1 x 0,3 ml **See Warnings**

Reagent D – Hair Wash Solution, 1 x 250 ml

Reagent S – Blank Hair, 1 x 2000 mg

Reagent K – Stabilization Solution, 1 x 20 ml

Reagent T1 – Chemical Std Solution 1, 1 x 500 µl

Code CC49016

(Packed separately – see data sheet)

Reagent T2 – Chemical Std Solution 2, 1 x 500 µl

Code CC49016

(Packed separately – see data sheet)

Reagent M1 – Mobile Phase M1, 1 x 500 ml

Reagent M2 – Mobile Phase M2, 1 x 500 ml

Minimum Instrumental equipment required:

Instrument LC-MS/MS triple quadrupole (mode MRM)
Divert Valve at 6 way
Interface ESI (+)
Binary Pump
Loop 100 µl
Operational Computer

Hair collection procedure:

Recommended length: 5 cm, starting from the scalp. A strand is cut, not torn (the bulb has no purpose at the end of these investigations) in the region of the vertex back of the head, of at least 200 mg which is divided into 2 aliquots of similar weight (A and B) each of which is fixed to the proximal end. These aliquots are placed in separate non-transparent containers with airtight caps, sealed with irremovable tape and stored at room temperature. The hair must be cut with scissors that have been cleaned with a small amount of alcohol and well dried.

Hair collection procedure :

And it is necessary to cut 200 mg of hair from the pubic region; the hair thus collected is divided into two aliquots (A and B).

ANALYTICAL PROCEDURE :

A) PREPARATION of unknown samples

STEP 1 : Wash hair intact inside a glass tube of 10 ml with 2 ml of **Reagent D – Hair Wash Solution**

Seal the tube with the cap

Vortex for 1 minute

Centrifuge at 3.500 rpm for 5 minutes

Open glass tube

STEP 2 : Evaporate to dryness the solvent with the aid of a light stream of helium or nitrogen paying attention to keep the hair / fur on the bottom of the tube. Alternatively remove the hair / fur from the tube with forceps cleaned with Acetone (not supplied in the kit), and place on paper towels until completely dry. In this last case the blotter paper should be changed for each sample.

Repeat steps 1 and 2 for two consecutive

STEP 3 : Cut your hair, washed and dried, in segments of 1-2 mm with scissors cleaned with Acetone (not supplied in the kit)

STEP 4 : Weigh an aliquot of sample, between 20 and 50 mg depending on the availability of the sample, in a Pyrex glass tube with screw cap clean and previously named.

B) PREPARATION of CALIBRATORS ON 6 LEVELS

Preparation of the Standard Chemical for enrichment after:

Prepare 3 glass tubes, call them A, B and dilute each Standard Chemical (T1-T2) with **Reagent A – Diluting Solution** as reported in tab. below:

Tubes	Reagent T1 – Chemical Std. Solution 1	Reagent T2 – Chemical Std. Solution 2	Reagent A – Diluting Sol.
A	20 µl	/	1.980 µl
B	/	20 µl	1.980 µl

Close the tubes – Vortex for 1 minute

Prepare 6 glass tubes of 10 ml called C0-C1-C2-C3-C4-C5.

Add at each tube (C0-C1-C2-C3-C4-C5) the standard chemical just prepared (1A, 1B) referring to the table below:

Calibrator C0	Calibrator C1	Calibrator C2	Calibrator C3	Calibrator C4	Calibrator C5
400 µl of Reagent A – Diluting Sol.	40 µl of A	80 µl of A	400 µl of A	20 µl of T1	40 µl of T1
400 µl of Reagent A – Diluting Sol.	40 µl of B	80 µl of B	400 µl of B	20 µl of T2	40 µl of T2

Evaporate to dryness with the aid of a stream of helium or nitrogen (in this step the temperature must be < 40 °C).

Add to each tube of the calibrators C0-C1-C2-C3-C4-C5 just brought to dryness, 50 mg of **Reagent S – Blank Hair** (supplied in kit).

At this point Calibrators of 6 points are ready and the concentrations to be used for the calibration curve will be:

Analyte	Calibrator C0	Calibrator C1	Calibrator C2	Calibrator C3	Calibrator C4	Calibrator C5
Morphine, Codeine, 6-MAM, Amphetamine, Methamphetamine, MDA, MDE, MDMA, Methadone, MBDB	0 ng/mg	0.1 ng/mg	0.2 ng/mg	1 ng/mg	5 ng/mg	10 ng/mg
Cocaine	0 ng/mg	0.25 ng/mg	0.5 ng/mg	2.5 ng/mg	12.5 ng/mg	25 ng/mg
Beg, Cocaethylene, Ecgonine Methyl Ester, EDDP	0 ng/mg	0.025 ng/mg	0.05 ng/mg	0.25 ng/mg	1.25 ng/mg	2.5 ng/mg
Buprenorphine, Norbuprenorphine	0 ng/mg	0.005 ng/mg	0.01 ng/mg	0.05 ng/mg	0.25 ng/mg	0.5 ng/mg

C) PREPARATION OF INTERNAL STANDARDS

Reagent C - Internal standards Sol supplied in the kit should be diluted 1:10 with water before each use, in the necessary amount for the entire assay.

Eg. for an analytic session with 20 unknown samples and 5 calibrators are required 1250 µl of each Standard Internal dilution (**diluted Reagent C**). Therefore you fetch 125 µl of **Reagent C - Internal standard Sol** and is diluted with 1125 µl of H₂O in two glass tubes. Vortex for at least 10 sec. The solutions are now ready for use.

The **Reagent C - Internal standard Sol** undiluted and not used are closed again tightly and kept to the residual quantity in the freezer at -20°C.

D) DIGESTION OF KERATINIC MATRIX

STEP 5 : In each tube previously prepared (phase A and phase B) dispense:

	Calibrator C0 (prepared in Step B)	Calibrator C1 (prepared in Step B)	Calibrator C2 (prepared in Step B)	Calibrator C3 (prepared in Step B)	Calibrator C4 (prepared in Step B)	Calibrator C5 (prepared in Step B)	Controls	Samples (prepared in Step A)
Reagent C diluted - (Internal Std Sol. for BASIC Substances diluted as shown in STEP C)	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul	50 ul
Reagent B – Estraent Sol.	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl

Seal the tubes with the cap - without invert gently mix the vial for 10 seconds

STEP 6 : Incubate at 45 °C for 18 hours

Cooling at Room Temperature

Open the glass tube

STEP 7 : Take 100 µl of hydrolized and add 400 µl of **Reagent K – Stabilization Sol.**

Vortex for 10 sec.

INJECTION :

- Inject 20 µl of solution in LC system

GENERAL CONSIDERATIONS about QUANTITIVE CALCULATION OF UNKNOWN SAMPLES

The calculation of the unknown samples is performed as a function of the equation obtained for the calibration curve where the abscissa axis shows the concentration in ng/mg and along the ordinate the ratio between the areas of the analyte/internal standard. To calculate the concentration in ng/mg hair in the case of unknown samples, please note that once you have the value of concentration on the line, it should be multiplied by the mg of hair that has built the calibration curve (50 mg) and divided by the actual weight of the sample under examination (eg. 20 mg if that is the weight of hair).

DRUGS OF ABUSE IN HAIR - Warnings

REAGENT C: DEUTERATED INTERNAL STANDARD SOLUTION

Solution: Morphine-D6, Codeine-D6, 6-Monoacetylmorphine-D6, Cocaine-D3, Cocaethylene-D3, Benzoyllecgonine-D8, Amphetamine-D11, Methamphetamine-D11, 3,4-MDMA-D5, 3,4-MDA-D5, 3,4-MDE-D5, Methadone-D9, EDDP-D3, Buprenorphine-D4, Norbuprenorphine-D3	
Abuse Drugs:	Internal Standard to use for calibration:
MORPHINE	Morphine-D6
CODEINE	Codeine-D6
6-MONOACETYLMORPHINE	6-Monoacetylmorphine-D6
COCAINE	Cocaine-D3
COCAETHYLENE	Cocaethylene-D3
BENZOYLECGONINE	Benzoyllecgonine-D8
ECGONINE METHYL ESTER	Benzoyllecgonine-D8
AMPHETAMINE	Amphetamine-D11
METHAMPHETAMINE	Methamphetamine-D11
3,4-MDMA	3,4-MDMA-D5
3,4-MDA	3,4-MDA-D5
3,4-MDE	3,4-MDE-D5
MBDB	3,4-MDMA-D5
METHADONE	Methadone-D9
EDDP	EDDP-D3
BUPRENORPHINE	Buprenorphine-D4
NORBUPRENORPHINE	Norbuprenorphine-D3
Storage: at – 20 °C	

COLUMN CONDITIONING

Install a new analytical column Accucore C18 (30 x 2.1 mm, 2.6 µm) or Zorbax RRHD C18 (50 x 2,1 mm, 1.8 µm), both thermostated at 35°C.
 Disconnect the detector and filter a solution of H₂O: ACN or CH₃OH (10 : 90 v/v), set flow at 300 µl / min for 15 min. **Don't recycle the washing solutions.** Condition the column with a solution of Mobile Phase M1: Mobile Phase M2 (95 : 5 v/v), set flow at 300 µl / min for 15 min.
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.

COLUMN CLEANING

Disconnect the detector. Flux a solution made of H₂O : ACN or CH₃OH (5 : 95 v/v) for 15 min at 300 µl/min and discharge. Store in a solution of H₂O : ACN or CH₃OH (5 : 95 v/v).

INJECTION NEEDLE WASH

Wash the syringe before each injection with Methanol.

PARAMETERS SET ON TRIPLE QUADRUPOLE SCIEX 4500

Curtain Gas (CUR): 40
 Collision Gas (CAD): 10
 Ion Spray Voltage: (IS): 4500 V
 Temperature (TEM): 650°C
 Ion Source Gas 1 (GS1): 50
 Ion Source Gas 2 (GS2): 60

GRADIENT FOR HPLC SYSTEMS (ACCUCORE C18)

Time	% M1 (PUMP A)	% M2 (PUMP B)	Flow (ml/min)
0.00 min	95%	5%	0.3
1.00 min	95%	5%	0.3
1.01 min	80%	20%	0.3
3.50 min	70%	30%	0.3
3.51 min	45%	55%	0.3
5.00 min	2%	98%	0.3
7.00 min	2%	98%	0.3
7.01 min	95%	5%	0.3
9.00 min	95%	5%	0.3

GRADIENT FOR UHPLC SYSTEMS (ZORBAX RRHD C18)

Time	% M1 (PUMP A)	% M2 (PUMP B)	Flow (ml/min)
0.00 min	95%	5%	0.4
3.00 min	95%	5%	0.4
3.01 min	80%	20%	0.4
5.50 min	70%	30%	0.4
5.51 min	45%	55%	0.4
8.00 min	2%	98%	0.4
9.00 min	2%	98%	0.4
9.01 min	95%	5%	0.4
12.00 min	95%	5%	0.4

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC49016	Calibrator in hair for Drugs of Abuse	1 x 2000 mg/1 x 2 x 0,5 ml/ 1 x 25 ml
CC49019	Controls in hair for Drugs of Abuse – Levels 1 and 2	1 x 1500 mg/1 x 2 x 0,4 ml/ 1 x 25 ml
LC49011	Mobile Phase M1	4 x 500 ml
LC49021	Mobile Phase M2	4 x 500 ml
SK49010	Starter kit for Drugs of Abuse in hair	32 x 1 ml
LC49030	Deuterated Internal Standard Mix Solution	1 x 300 µl
S29057U	Standard glass Vial of 2 ml with screw cap for Autosampler	1 x 100 Pcs
Z1636/26	10 ml Pyrex Tubes with cap SWL (for hydrolysis, till 300 °C-washable and reusable)	1 x 40 Pcs
Analytical Column for HPLC system		
TF17126032130	Accucore C-18 (30 m x 2.1 mm – 2.6 µm) Analytical Column	1 Pc
Analytical Column for UHPLC system		
Z959757902	RRHD C18 (50 x 2,1 mm. 1.8 µm) Analytical Column	1 Pc

FRAGMENTATIONS

ANALYTE	PARENT ION
Morphine	286.3
Codeine	300.1
6-MAM	328.0
Cocaine	304.2
Cocaethylene	318.1
BEG	290.1
Ecgonine Methyl Ester	200.1
Amphetamine	136.1
Methamphetamine	150.1
3,4-MDMA	194.2
3,4-MDA	180.2
3,4-MDE	208.2
MBDB	208.2
Methadone	310.2
EDDP	278.1
Buprenorphine	468.2
Norbuprenorphine	414.2
Morphine-D6	292,3
Codeine-D6	306.0
6-MAM-D6	334.2
Cocaine-D3	307.1
Cocaethylene-D3	321.2
BEG-D8	298.0
Amphetamine-D11	147.2
Methamphetamine-D11	161,1
MDMA-D5	199,2
MDA-D5	185.0
MDE-D5	213,2
Methadone-D9	319,2
EDDP-D3	281.2
Buprenorphine-D4	472.2
Norbuprenorphine-D3	417.3

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

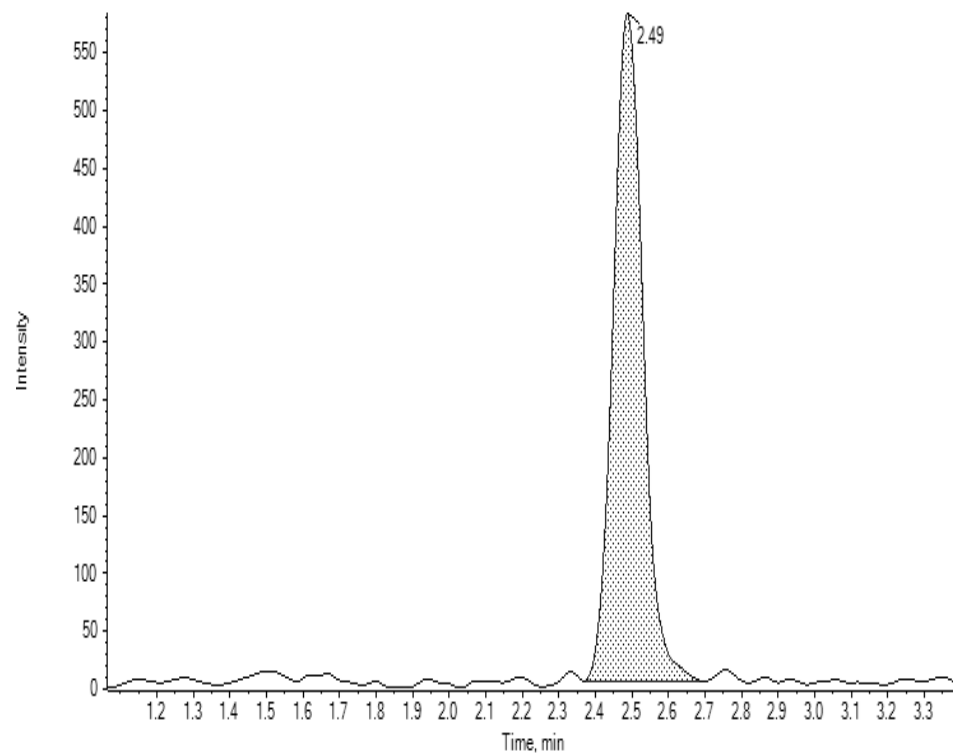


Fig. 1 :	<i>Hair Calibrator</i>
	R.T. 2.49 Morphine

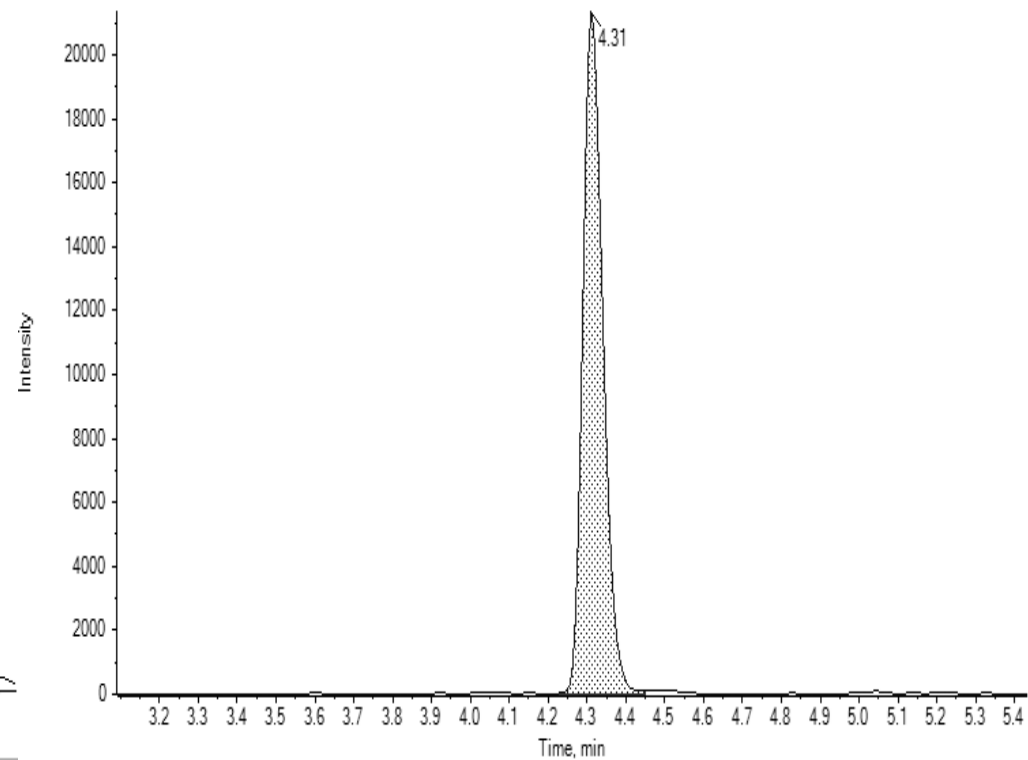


Fig. 2 :	<i>Hair Calibrator</i>
	R.T. 4.31 Codeine

DRUGS OF ABUSE IN HAIR (Reference Chromatograms)

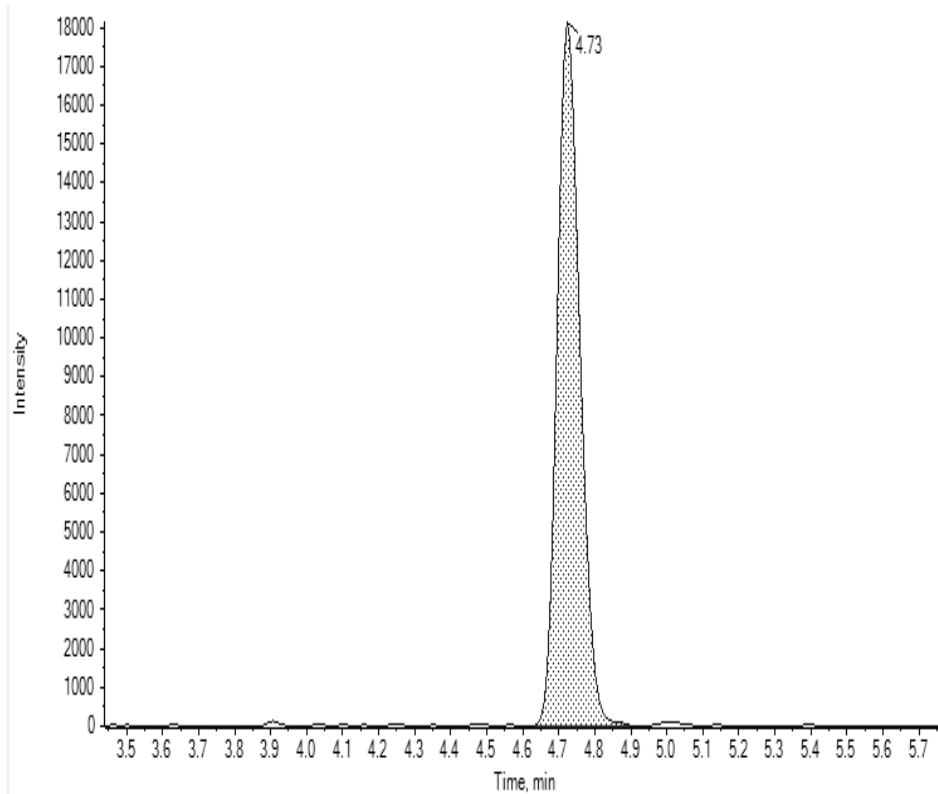


Fig. 3 :	Hair Calibrator
	R.T. 4.73 6-MAM

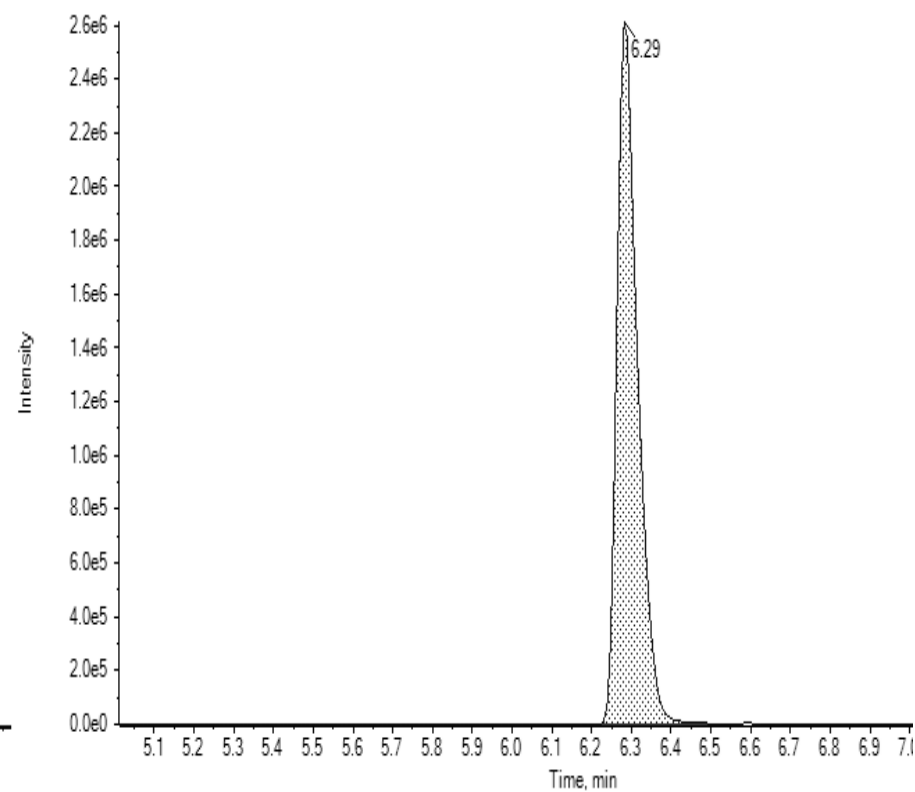


Fig. 4 :	Hair Calibrator
	R.T. 6.29 Cocaine

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

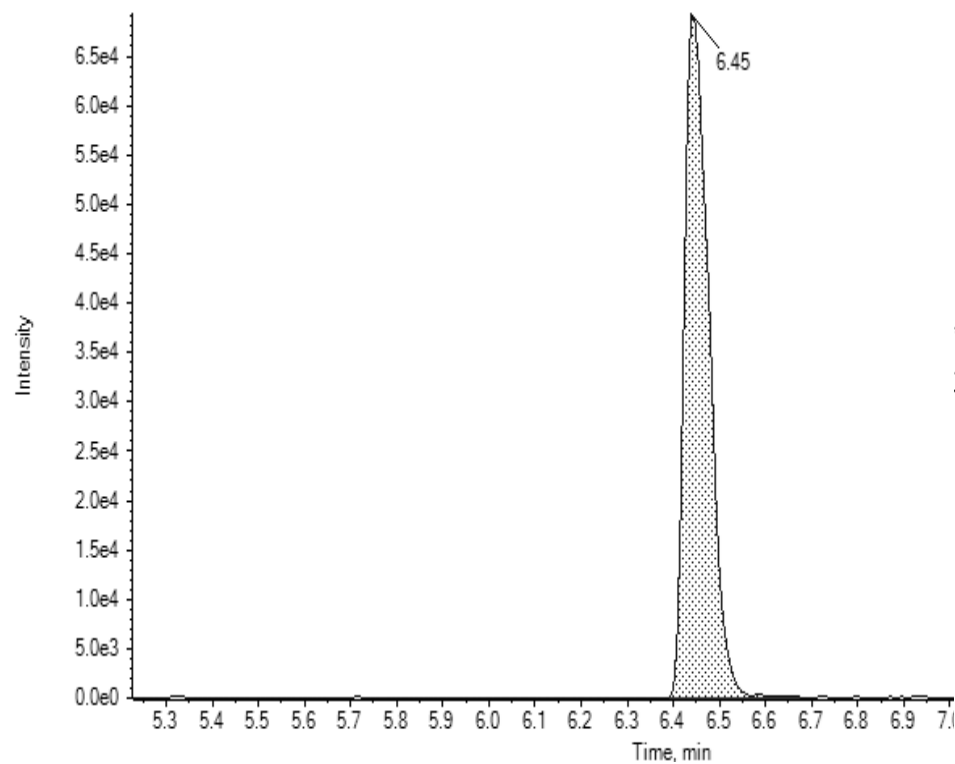


Fig. 5 :	<i>Hair Calibrator</i>
	R.T. 6.45 Cocaethylene

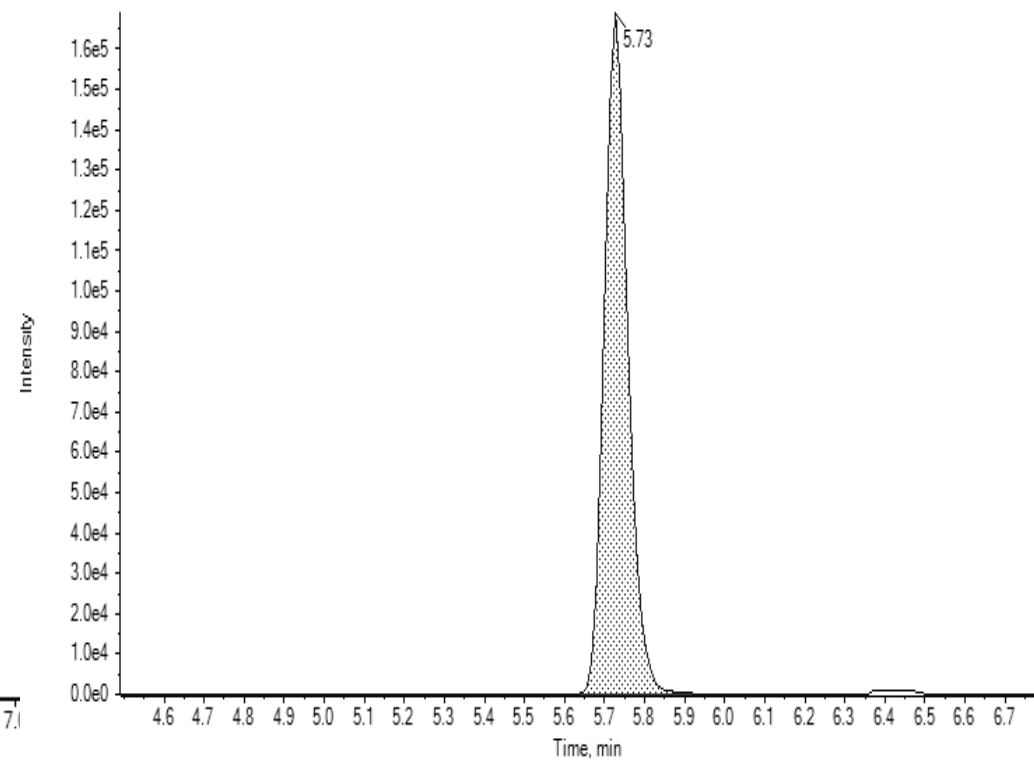


Fig. 6 :	<i>Hair Calibrator</i>
	R.T. 5.73 Beg

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

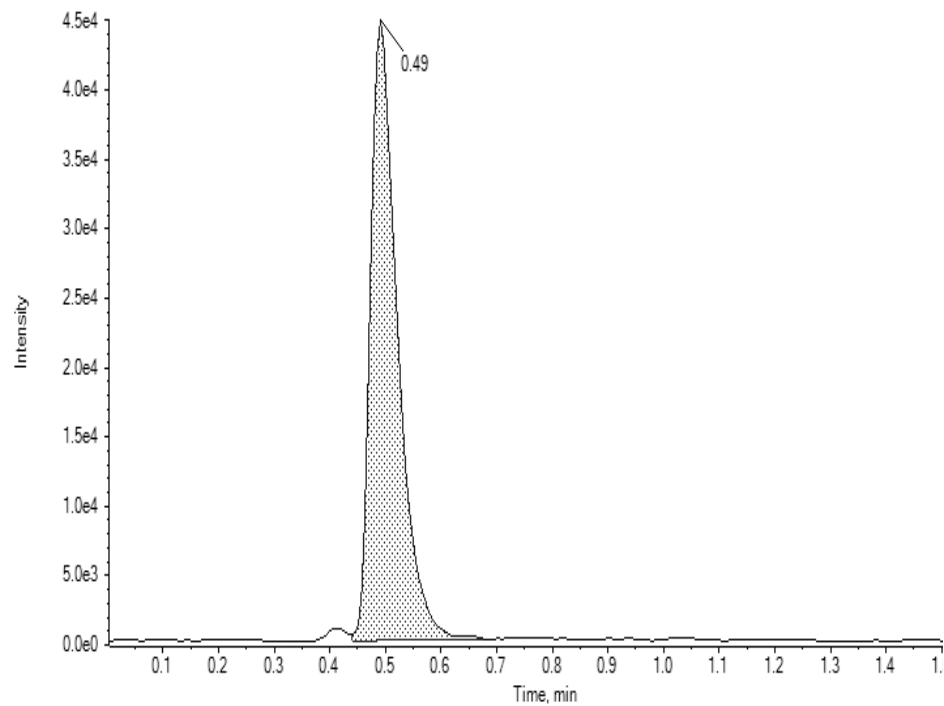


Fig. 7 :	Hair Calibrator
	R.T. 0.49 Ecgonine Methyl Ester

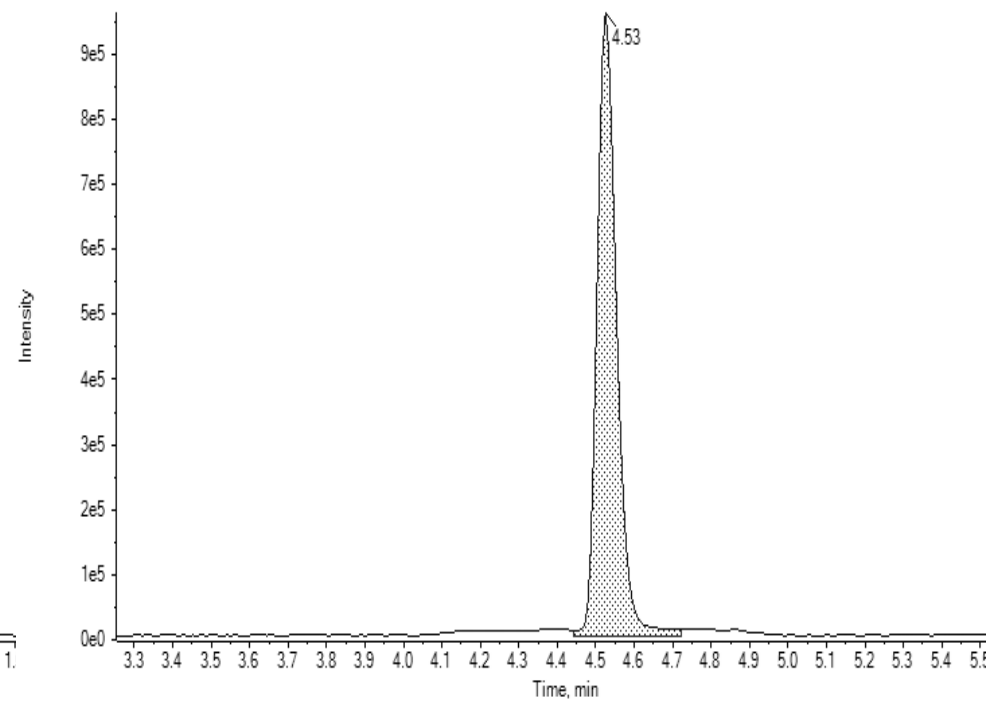


Fig. 8 :	Hair Calibrator
	R.T. 4.53 Amphetamine

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

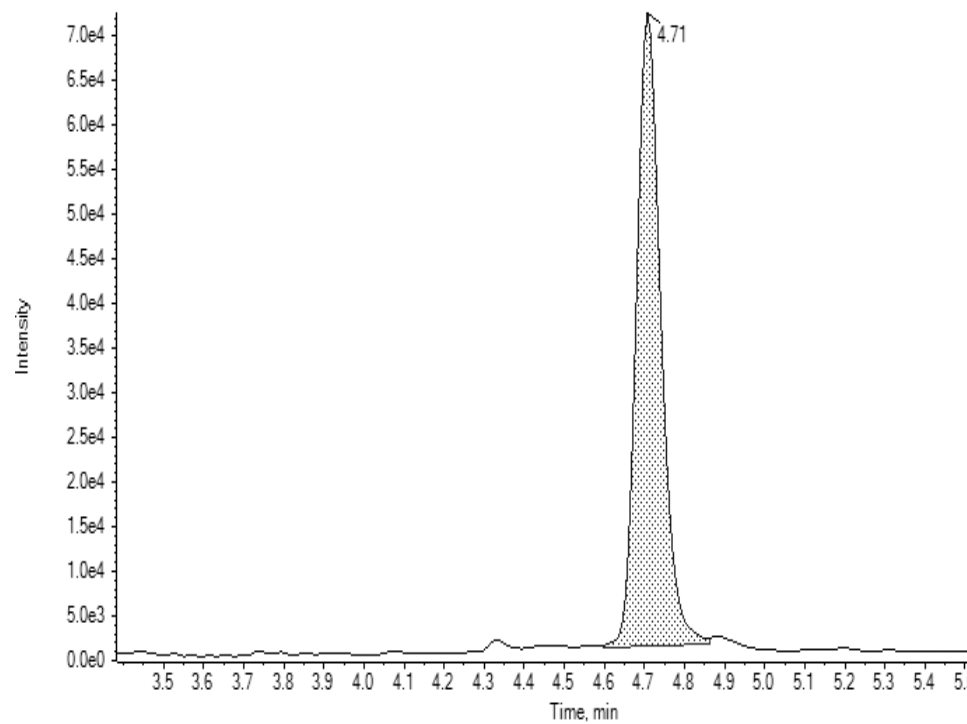


Fig. 9 :	Hair Calibrator
	R.T. 4.71 Methamphetamine

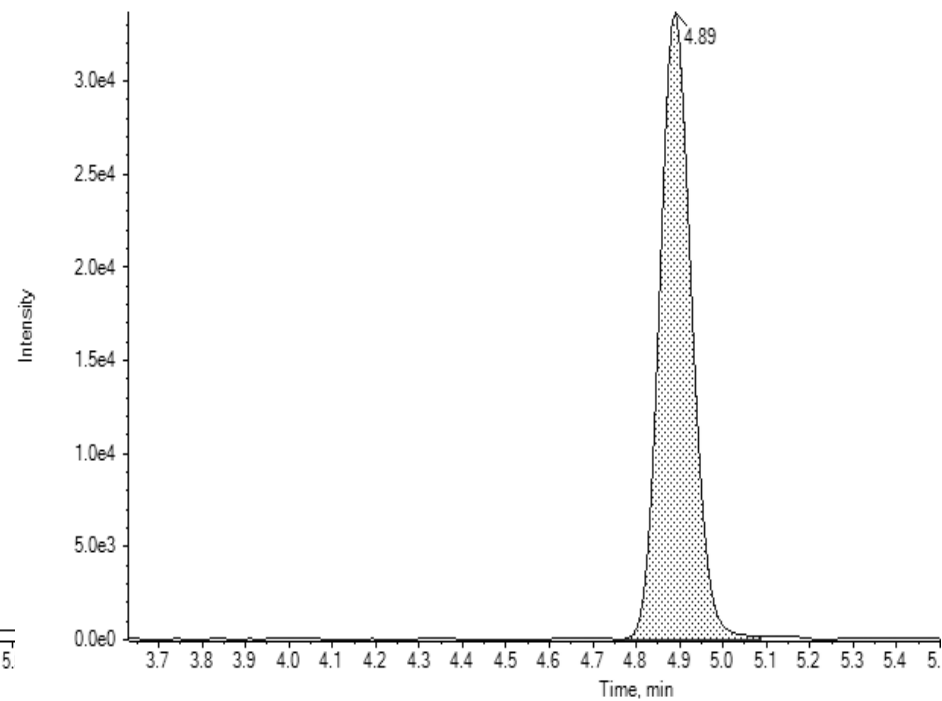


Fig. 10 :	Hair Calibrator
	R.T. 4.89 3,4-MDMA

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

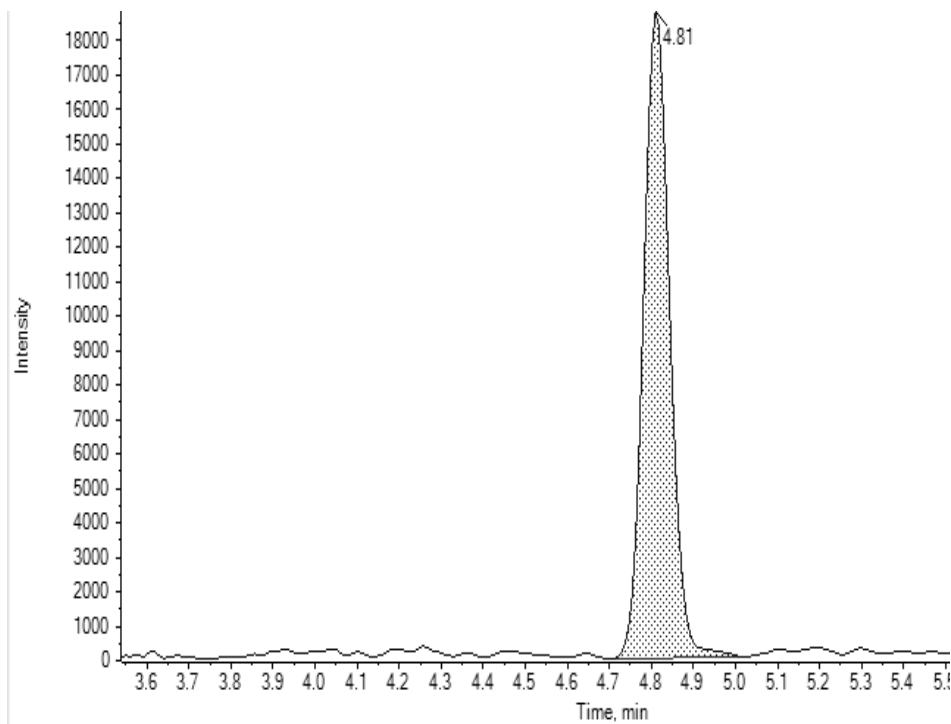


Fig. 11 :	<i>Hair Calibrator</i>
	R.T. 4.81 3,4-MDA

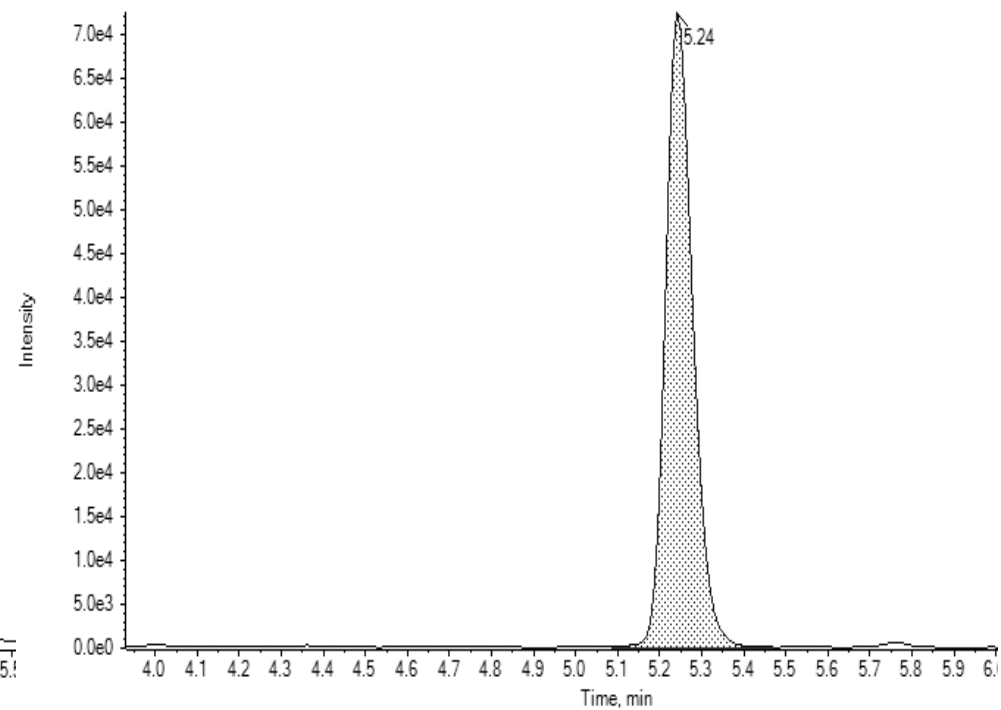


Fig. 12 :	<i>Hair Calibrator</i>
	R.T. 5.24 3,4-MDE

DRUGS OF ABUSE IN HAIR (Reference Chromatograms)

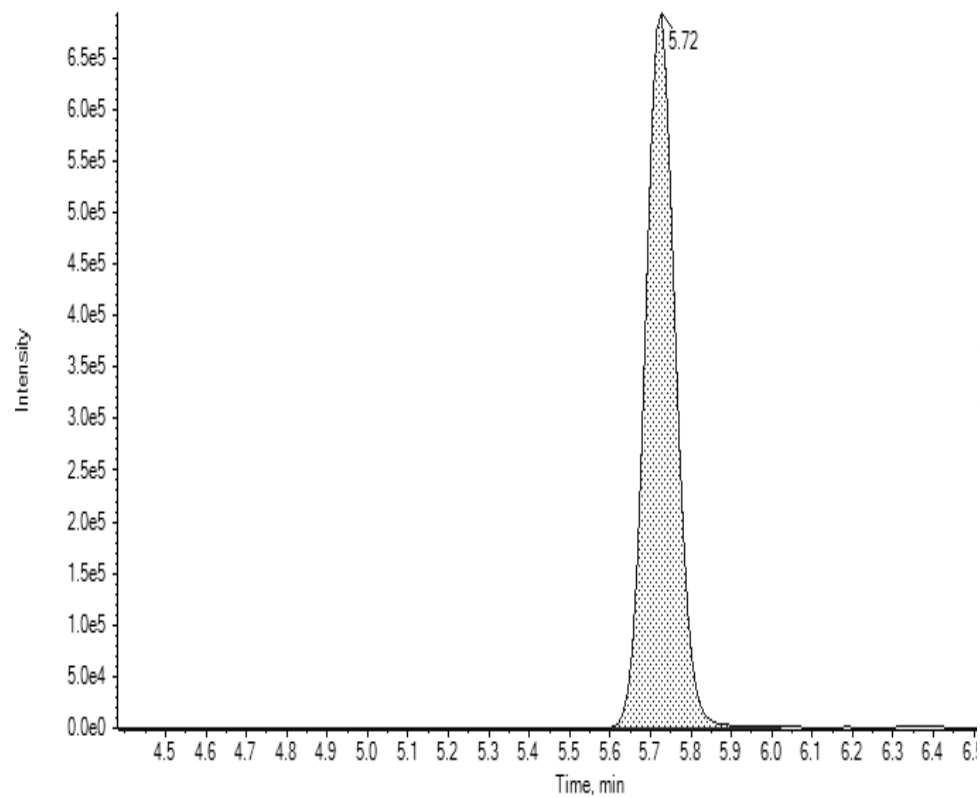


Fig. 13 :	<i>Hair Calibrator</i>
	R.T. 5.72 3,4-MBDB

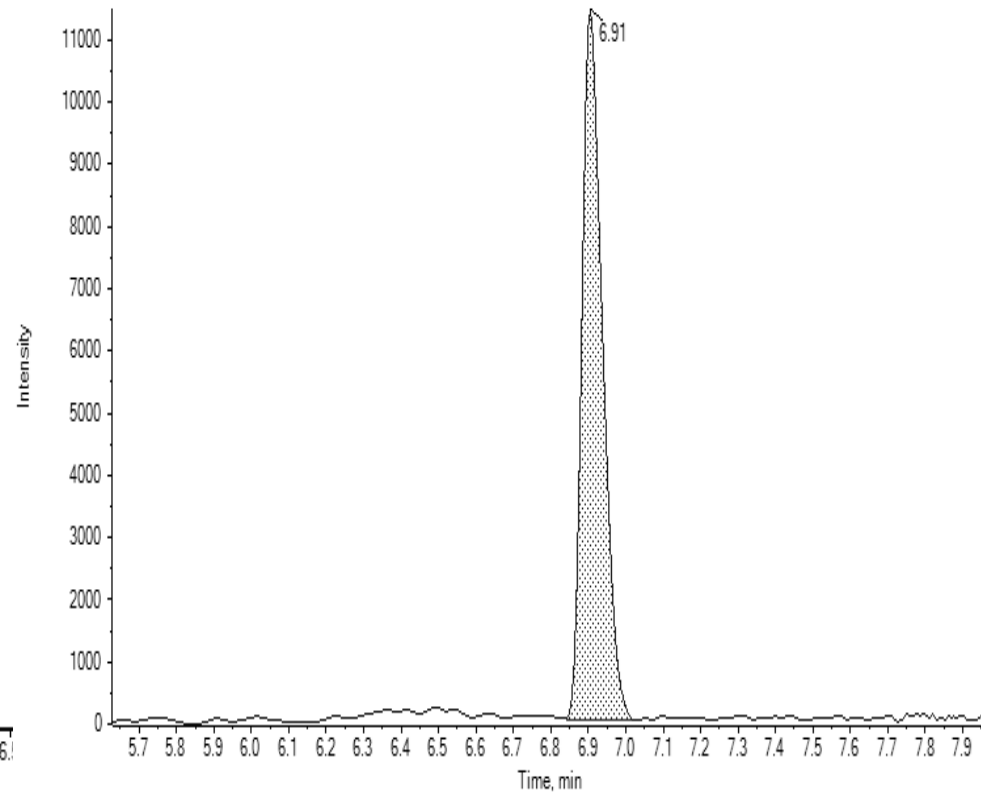


Fig. 14 :	<i>Hair Calibrator</i>
	R.T. 6.91 Methadone

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

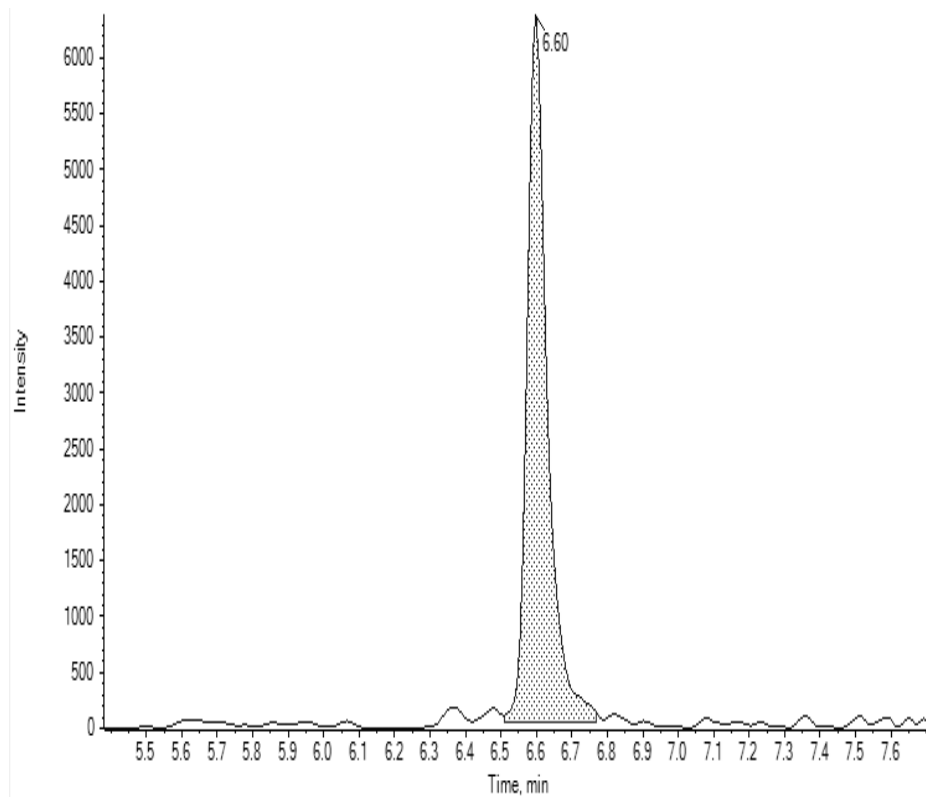


Fig. 15 :	<i>Hair Calibrator</i>
	R.T. 6.60 EDDP

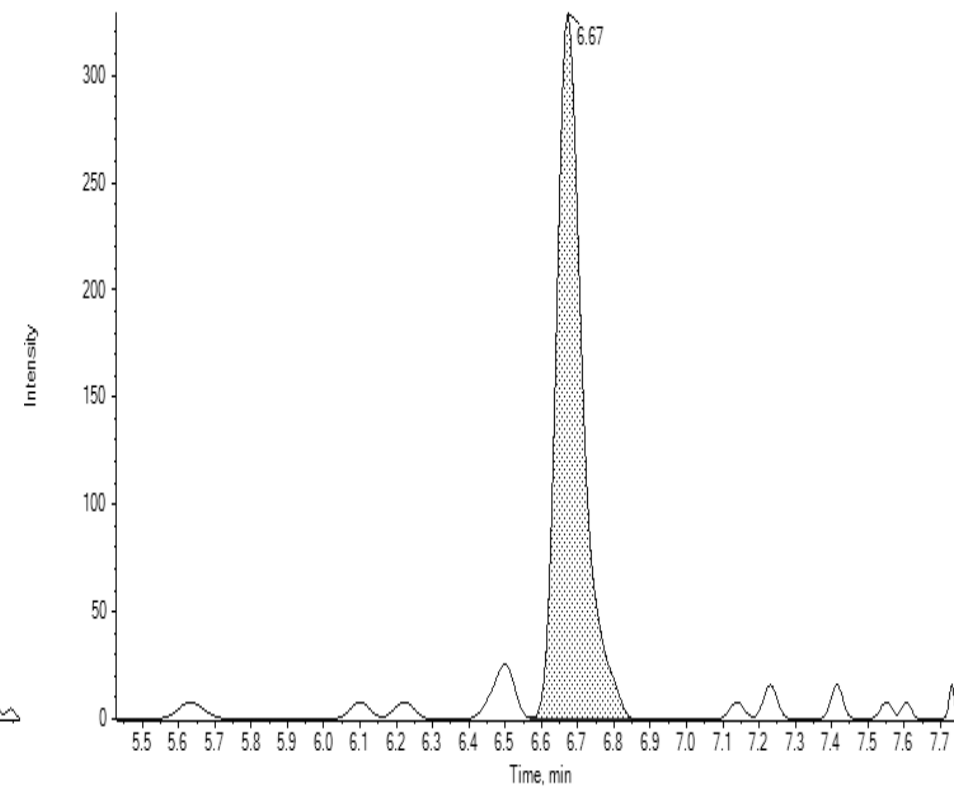


Fig. 16 :	<i>Hair Calibrator</i>
	R.T. 6.67 Buprenorphine

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms)

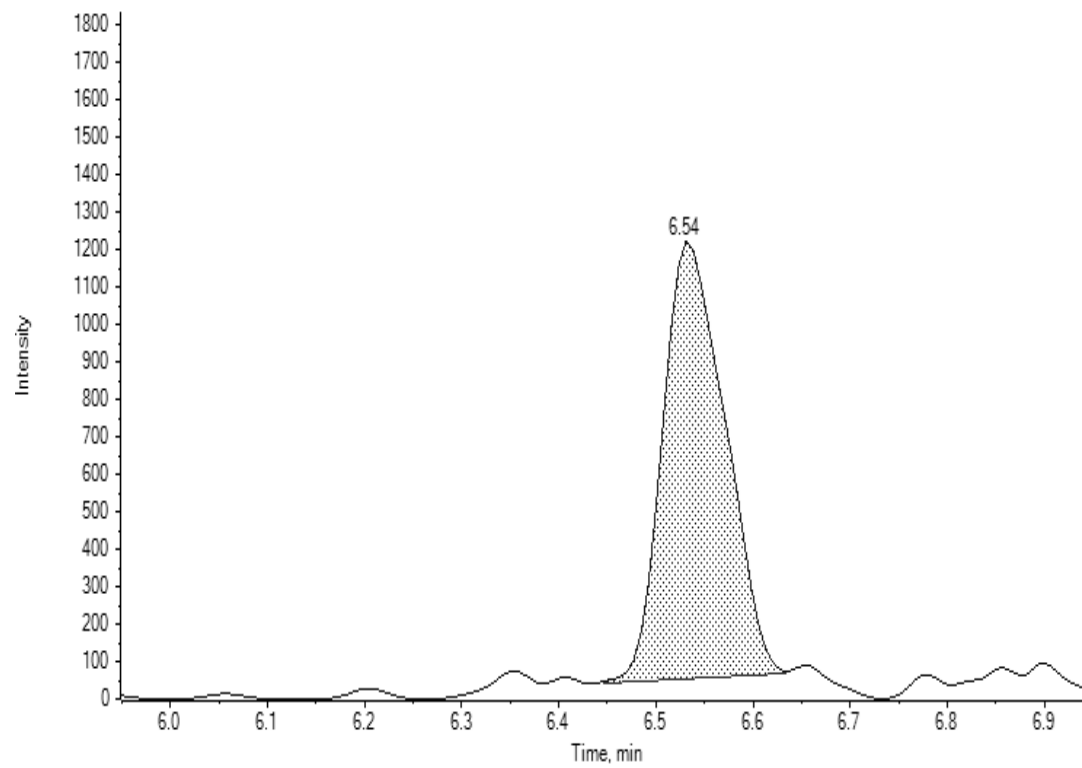


Fig. 17 :	<i>Hair Calibrator</i>
	R.T. 6.54 Norbuprenorphine