

BASIC DRUGS OF ABUSE in hair by GC-MS – Code GC49010

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

Method of confirmation by GC-MS

INTRODUCTION

In the early 80 's, a series of studies was begun on the possibility of determining substances of abuse in keratinous array and then use hair analysis as a means of finding the previous use of these substances. Once embedded in the hair, by the systemic circulation and by the contribution of sweat and sebum, substances of abuse remain impounded in the keratinous array in a stable way over time as a function of their lipophilic, molecular weight, the pKa and the steric encumbrance. The analysis of substances of abuse in keratinous array 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 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 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 the use of 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 initial methods to analyse in a short time a large number of samples in a standardized, efficient and economic way and exclude samples that are negative, i.e. samples that did not contain the substance or class of substances, or those in which the concentration is below a threshold value (cut-off).

Confirmation analyses are defined as those that serve to verify false positive due to the non-specificity of screening tests. In the case of keratinous array, because there are no real screening tests we cannot talk about real "confirmatory analysis". The "Society of Hair Testing" (SOHT) recommends the use of 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.

Taking into account 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, MDA, MDE, MDMA, MBDB, Buprenorphine, Norbuprenorphine, Methadone, EDDP) after extraction of molecules from keratinous matrix and solid phase extraction (SPE) column of the analytes of interest (to eliminate the presence of complex matrix interferences) and determination in Gas chromatography/mass spectrometry (GC/MS).



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



CE

N° 005	DRUGS OF ABUSE in hair by GC/MS	09/2024
--------	---------------------------------	---------

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, MBDB, Buprenorphine, Norbuprenorphine, EDDP** after hydrolysis of the keratin matrix, SPE extraction and GC-MS in reading.

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

Cut off values by Society of Hair Testing (SOHT)

Cut-off di screening secondo le raccomandazioni SOHT			Cut-off di conferma secondo le raccomandazioni SOHT	Cut-off di conferma secondo il provvedimento 18/09/2008 sui lavoratori con mansioni a rischio
Classe di sostanza	Cut-off (ng/mg)	Analita	Cut-off (ng/mg)	Cut-off (ng/mg)
Amfetamine	0,2	Amfetamina	0,2	0,2
		Metamfetamina	0,2	0,2
		MDA	0,2	0,2
		MDMA	0,2	0,2
		MDEA	-	0,2
Cannabinoidi	0,1	THC	0,05	0,1
		THC-COOH	0,0002	-
Cocaina	0,5	Cocaina	0,5	0,2
		BEG, EME, CE, NC*	0,05	0,05
Opiacei	0,2	Morfina	0,2	0,2
		Codeina	0,2	0,2
		6-MAM [§]	0,2	0,2
Metadone	0,2	Metadone	0,2	0,2
		EDDP	0,05	-
Buprenorfina	0,01	Buprenorfina	0,01	0,05
		Norbuprenorfina	0,01	-

* BEG: benzoilecgonina, EME: ecgoninametilestere, CE: cocaetilene, NC: norcocaina
[§] 6-MAM – 6-monoacetilmorfina

Cut off values by Italian Forensic Toxicology Group(GTFI)

Valori di cut-off in matrice pilifera
(il GTFI ritiene non validi metodi di screening immunochimici per questa matrice biologica in quanto ancora forieri, per la maggior parte, di risultati "falsi negativi")

Classe di sostanze o sostanza	Cut-off/ conferma (ng/mg)
Opiacei morfina codeina 6-acetilmorfina	0,2 0,2 0,2
Cocaina (cocaina) cocaina benzoilecgonina ecgonina metilestere cocaetilene	0,5 0,05 0,05 0,05
Amfetamina e congeneri amfetamina metamfetamina	0,2 0,2
3,4-Metilendirossimetamfetamina e congeneri MDMA MDA MDEA MBDB	0,2 0,2 0,2 0,2
Metadone metadone EDDP	0,2 0,2
Cannabinoidi THC THC-COOH	0,05 0,001
Buprenorfina buprenorfina norbuprenorfina	0,01 0,01

Cut-off by Gazzetta Ufficiale:

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

0.2 ng/mg
 0.05 ng/mg

Ref. Gazzetta Ufficiale n°236 – 8 ott 2008

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

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
BENZOYLECGONINE	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
ECGONINE METHYL ESTER	0,03	0,2	1
CODEINE	0,15	1	5
NORBUPRENORPHINE	0,0015	0,1	0,5
METHADONE	0,15	1	5
EDDP	0,15	1	5
BUPRENORPHINE	0,007	0,05	0,250
MBDB	0,15	1	5

ANALYTE	Correlation Coefficient R ² ± SD	LOD (ng/mg)	LOQ (ng/mg)
AMPHETAMINE	0,9991 ± 0,0012	0,003	0,1
METHAMPHETAMINE	0,9991 ± 0,0008	0,003	0,1
3,4-MDMA	0,9986 ± 0,0013	0,003	0,1
3,4- MDA	0,9998 ± 0,0001	0,003	0,1
3,4-MDE	0,9979 ± 0,0022	0,003	0,1
BENZOYLECGONINE	0,9996 ± 0,0004	0,016	0,05
COCAETHYLENE	0,9996 ± 0,0006	0,006	0,02
COCAINE	0,9999 ± 0,0001	0,083	0,25
MORPHINE	0,9997 ± 0,0005	0,003	0,1
6-MAM	0,9992 ± 0,0009	0,003	0,1
ECGONINE METHYL ESTER	0,9998 ± 0,0001	0,006	0,02
CODEINE	0,9984 ± 0,0026	0,003	0,1
NORBUPRENORPHINE	0,9973 ± 0,0031	0,003	0,01
METHADONE	0,9996 ± 0,0004	0,003	0,1
EDDP	0,9999 ± 0,0001	0,003	0,1
BUPRENORPHINE	0,9990 ± 0,0013	0,0016	0,005
MBDB	0,9986 ± 0,0012	0,003	0,1

ANALYTE	Recovery (%)		
	Calibrator		
	Low	Medium	High
AMPHETAMINE	129	70	84
METHAMPHETAMINE	90	66	66
3,4-MDMA	76	86	81
3,4- MDA	90	83	109
3,4-MDE	84	80	83
BENZOYLECGONINE	56	91	76
COCAETHYLENE	72	84	71
COCAINE	69	71	61
MORPHINE	71	97	100
6-MAM	55	53	50
ECGONINE METHYL ESTER	70	75	44
CODEINE	75	83	74
NORBUPRENORPHINE	82	97	47
METHADONE	80	74	60
EDDP	50	51	52
BUPRENORPHINE	68	79	65
MBDB	77	76	81

Components of the kit (50 test) :

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

Reagent A – Diluting Solution, 2 x 10 ml

Reagent B – Buffer Solution, 1 x 100 ml

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

Reagent D – Conditioning Solution 2, 1 x 100 ml

Reagent E – Eluting Solution, 1 x 100 ml

Reagent F – Wash Solution 1, 1 x 100 ml

Reagent G – Wash Solution 2, 1 x 100 ml

Reagent H – Derivatization Solution 1 for Amphetamines, 2 x 1 ml

See Warnings. For the sampling of Reagent H, the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Store at –20 °C before opening.

Reagent I – Derivatization Solution 2 for Amphetamines, 1 x 1 ml

Reagent L – Test Solution Amphetamines, 1 x 1 ml

See Warnings

Reagent N – Test Solution Opiates, Methadone, Cocaine, Buprenorphine and metabolites, 1 x 0,5 ml

See Warnings

Reagent M1 – Derivatization Solution 1, 2 x 1 ml (2 ampullas of 1 ml)

See Warnings. For the sampling of Reagent M, the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533

Reagent M2 – Derivatization Solution 2, 2 x 1 ml (2 ampullas of 1 ml)

Store at Room Temperature before and after opening.

See Warnings. For the sampling of Reagent M, the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533

Reagent O – Internal Standard Solution for Basic Drugs, 1 x 1 ml

See Warnings

Reagent P – Internal Standard Solution for Amphetamines, 1 x 1 ml

See Warnings

Reagent Q – Extraction Solution, 1 x 50 ml

Reagent R – Hair Wash Solution, 1 x 250 ml

Reagent S - Blank Hair, 1 x 2000 mg

Reagent T1 – Chemical Standard Solution 1, 1 x 400 µl

Code GC49016 (Packed separately – see data sheets)

Reagent T2 – Chemical Standard Solution 2, 1 x 400 µl

Code GC49016 (Packed separately – see data sheets)

Reagent T3 – Chemical Standard Solution 3, 1 x 400 µl

Code GC49016 (Packed separately – see data sheets)

Reagent V – Stabilizing Solution, 1 x 2,5 ml

Clean-up Columns, 50 pcs

Minimum Instrumental equipment required:

GC-MS Instrument
Operational Computer

Optional Equipment:

Autosampler

Hair collection procedure:

Recommended length: 5 cm, starting from the scalp. Is cut a strand, 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) of each of which is fixed to the proximal end. These aliquots are placed in separate containers non-transparent bearing caps airtight and sealed with tape irremovable and stored at room temperature. The cut of the hair must be performed with clean scissors with a small amount of alcohol and dry well.

Hair collection procedure :

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

PREANALITICAL PROCEDURE for all substances of abuse of basic EXCEPT AMPHETAMINES:

STEP 1 : Pipette in a glass tube with teflon cap :

- 50 µl of Reagent N – Test Solution Opiates, Methadone, Cocaine, Buprenorphine and metabolites

Test Storage: once you open the vial withdrawn the volume needed, reseal and store at -20 ° C.

STEP 2 : Evaporate to dryness with the aid of a stream of helium or nitrogen (in this step the temperature must be < 40 °C)

STEP 3 : Add 25 µl of Reagent M1 - Derivatization Solution 1 + 25 µl of Reagent M2 - Derivatization Solution 2 using the appropriate syringe Hamilton of 50 µl (code ZRE24533)

Seal the tube with the cap

Vortex for 20 sec.

STEP 4 : Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

Open glass tube

STEP 5 : Transferring the sample vials with reduction volume autosampler

NOTE: Before starting the analytical session is advisable to inject 2 µl of this freshly prepared solution in the gas chromatograph to identify the retention time and mass spectrum of all basic substances that must be similar to those shown in Figures below. If the test was successful, proceed to assay. If not, check the functionality of the analytical system.

PREANALITICAL PROCEDURE for AMPHETAMINES:

STEP 1 : Pipette in a glass tube with teflon cap :

- 100 µl of Reagent L – Test Solution Amphetamines

STEP 2 : Evaporate to dryness with the aid of a stream of helium or nitrogen (in this step the temperature must be < 40 °C)

STEP 3 : Add 35 µl of Reagent H – Derivatization Solution 1 for Amphetamines using the appropriate syringe Hamilton of 50 µl (code ZRE24533)

Seal the tube with the cap

Vortex for 20 sec.

STEP 4: Incubate at 80 °C for 5 minutes

Cooling at Room Temperature

Open glass tube

STEP 5 : Add 15 µl of **Reagent I – Derivatization Solution 2 for Amphetamines**

Seal the tube with the cap

Vortex for 20 sec.

STEP 6: Incubate at 80 °C for 5 minutes

Cooling at Room Temperature

Open glass tube

STEP 7 : Transferring the sample vials with reduction volume autosampler

NOTE: Before starting the analytical session is advisable to inject 2 µl of this freshly prepared solution in the gas chromatograph to identify the retention time and mass spectrum of all basic substances that must be similar to those shown in Figures below. If the test was successful, proceed to assay. If not, check the functionality of the analytical system.

N.B.: it is recommended to use different syringes for derivatization reagents H and for Reagent M1 and M2.

ANALYTICAL PROCEDURE :

A) PREPARATION of unknown samples

STEP 1 : Wash hair intact inside a glass tube of 10 ml with 2 ml of **Reagent R – 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 remain in the hair / fur on the bottom of the tube. Alternatively remove with a clean forceps with Acetone (not supplied in the kit), the hair / fur from the tube 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 cm 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 1 A, 1 B, 1 C and dilute each Standard Chemical (T1-T2-T3) with **Reagent A – Diluting Solution** as reported in tab. below:

Tubes	<u>Reagent T1 – Chemical Std. Solution 1</u>	<u>Reagent T2 – Chemical Std. Solution 2</u>	<u>Reagent T3 – Chemical Std. Solution 3</u>	<u>Reagent A – Diluting Sol.</u>
1 A	10 µl	//	//	990 µl
1 B	//	10 µl	//	990 µl
1 C	//	//	10 µl	990 µl

Close the tubes – Vortex for 1 minute

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

Add at each tube (C0-C1-C2-C3-C4-C5) the standard chemical just prepared (1A, 1B, 1C) and the **Reagents T1-T2-T3** referring to the table below:

Calibrator C0	Calibrator C1	Calibrator C2	Calibrator C3	Calibrator C4	Calibrator C5
50 µl of Reagent A – Diluting Sol.	10 µl of 1 A	20 µl of 1 A	100 µl of 1 A	5 µl of <u>Reagent T1</u>	10 µl of <u>Reagent T1</u>
50 µl of Reagent A – Diluting Sol.	10 µl of 1 B	20 µl of 1 B	100 µl of 1 B	5 µl of <u>Reagent T2</u>	10 µl of <u>Reagent T2</u>
50 µl of Reagent A – Diluting Sol.	10 µl of 1 C	25 µl of 1 C	100 µl of 1 C	5 µl of <u>Reagent T3</u>	10 µl of <u>Reagent T3</u>

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 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, Beg, Amphetamine, Methamphetamine, MDA, MDE, MDMA, Methadone, EDDP, 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
Cocaethylene, Ecgonine Methyl Ester	0 ng/mg	0.02 ng/mg	0.04 ng/mg	0.2 ng/mg	1 ng/mg	2 ng/mg

Buprenorphine	0 ng/mg	0.005 ng/mg	0.01 ng/mg	0.05 ng/mg	0.25 ng/mg	0.5 ng/mg
Norbuprenorphine	0 ng/mg	0.01 ng/mg	0.02 ng/mg	0.1 ng/mg	0.5 ng/mg	1 ng/mg
Beg	0 ng/mg	0.05 ng/mg	0.1 ng/mg	0.5 ng/mg	2.5 ng/mg	5 ng/mg

C) PREPARATION OF INTERNAL STANDARDS

Reagent O - Internal standard Sol for basic substances and **Reagent P** - Internal Standard Sol for Amphetamines 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 625 µl of each Standard Internal dilution (**diluted Reagent O** and **diluted Reagent P**). Therefore you fetch 62.5 µl of **Reagent O** - Internal standard Sol for basic substances and 62.5 µl of **Reagent P** - Internal standard Sol for Amphetamine and are diluted respectively with 562.5 µl of H₂O in two glass tubes. Vortex for at least 10 sec. The solutions are now ready for use.

The **Reagent O** - Internal standard Sol for basic substances and the **Reagent P** - Internal standard Sol for Amphetamine 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 O - (Internal Std Sol. for BASIC Substances diluted as shown in STEP C)	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul
Reagent P diluted - (Internal Std Sol. for Amphetamines diluted as shown in STEP C)	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul	25 ul
Reagent Q - Extraction 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 : Add at each tube 2 ml of Reagent B – Buffer Sol. + 45 µl of Reagent V – Stabilizing Solution

Check that the pH is 6.0 ± 0.2 with indicator strips (dip the tip of a pasteur pipette or a pipette in the tube; use a different pipette for each tube). In any case that the pH is not correct, you can make corrections with Sodium hydroxide 0.1 M and Hydrochloric Acid 0.1 M mixing and re-inspecting the pH after each addition.

Seal the tubes and mix for 1 minute

Centifuge at 3.500 rpm for 5 minutes

E) EXTRACTION OF ANALYTES

STEP 8 : Column SPE Conditioning

Prepare a number of columns corresponding to the sum of the calibrators, controls and unknown samples

- Add 1,5 ml of **Reagent C – Conditioning Solution 1 and Wash 3**
Pass in the column and discard the eluate without leading to dry the column
- Add 2 ml of **Reagent D – Conditioning Solution 2**
Pass in the column and discard the eluate without leading to dry the column

STEP 9 : Add all the supernatant of the calibrators, controls and unknown samples, prepared in steps 5-7, in columns packed and download at a flow rate of 1 ml / minute (drop by drop) also with the aid of a moderate vacuum.

STEP 10 : Column SPE Washing

- Wash with 2 ml of **Reagent F – Wash Solution 1**
Pass in column and discard the eluate
- Wash with 2 ml of **Reagent G – Wash Solution 2**
Pass in column and discard the eluate
- Wash with 2 ml of **Reagent C – Conditioning Solution 1 and Wash 3**
Pass in column and discard the eluate bringing to dryness under a gentle stream of air

F) ELUTION and DERIVATIZATION OF ANALYTES

Elute **strictly in Pyrex glass tubes with cap**

THERE ARE 2 CASES:

1) **In the case where the sample was positive at screening only for the class of Amphetamine:**

Elute with 1 ml of Reagent E – Eluting Solution

Evaporate to dryness with a gentle stream of air

Collect the eluate of Pyrex tubes with screw cap

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

Derivatization: Add at each tube 35 µl of Reagent H – Derivatization Solution 1 for Amphetamines using the appropriate syringe Hamilton of 50 µl (code ZRE24533)

Seal the tubes with the cap

Incubate at 80°C x 5 min

Cooling at Room Temperature

Open the glass tube

Add 15 µl of Reagent I – Derivatization Solution 2 for Amphetamines

Seal the tubes with the cap

Incubate at 80°C x 5 min

Cooling at Room Temperature

Open the glass tube

Injection: Transfer the derivatized in the vial with reducing volume and inject 2 µl in the gas chromatograph.

2) **In the case where the sample was positive at screening only for the class of Opiates, Methadone, Cocaine, Buprenorphine:**

Elute with 1 ml of Reagent E – Eluting Solution

Evaporate to dryness with a gentle stream of air

Collect the eluate of Pyrex tubes with screw cap

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

Derivatization: Add at each tube 25 µl of Reagent M1 – Derivatization Solution 1 + 25 µl of Reagent M2 – Derivatization Solution 2 using the appropriate syringe Hamilton of 50 µl (code ZRE24533)

Seal the tubes with the cap

Incubate at 70°C x 20 min

Cooling at Room Temperature

Open the glass tube

Injection: Transfer the derivatized in the vial with reducing volume and inject 2 µl in the gas chromatograph.

N.B.: it is recommended to use different syringes for derivatization reagents H and for Reagent M1 and M2.

GENERAL CONSIDERATIONS about QUANTITATIVE 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 it is weighed amount of this hair).

BASIC DRUGS OF ABUSE in hair - Warnings

REAGENT N: TEST SOLUTION OPIATES, METHADONE, COCAINE, BUPRENORPHINE AND METABOLITES

Morphine Codeine 6-Monoacetylmorphine Ecgonine Methyl Ester Norbuprenorphine Methadone BEG Cocaethylene Cocaine Buprenorphine EDDP	About 10.000 ng/ml
Storage: Once you open the vial, withdrawn the necessary volume, close it with the reagent used and stored at -20°C.	

REAGENT L: TEST SOLUTION AMPHETAMINES

Amphetamine Methamphetamine MDA MDMA MDE MBDB	About 10.000 ng/ml
Storage: Once you open the vial, withdrawn the necessary volume, close it with the reagent used and stored at -20°C.	

REAGENT T1: CHEMICAL STANDARD SOLUTION 1

Amphetamine Methamphetamine MDA MDMA MDE EDDP Morphine Codeine MBDB Beg	50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 50.000 ng/ml 25.000 ng/ml
Storage: Once you open the vial, withdrawn the necessary volume, close it with the reagent used and stored at -20°C.	

REAGENT T2: CHEMICAL STANDARD SOLUTION 2

Cocaine Methadone 6-Monoacetylmorphine	125.000 ng/ml 50.000 ng/ml 50.000 ng/ml
Storage: Once you open the vial, withdrawn the necessary volume, close it with the reagent used and stored at -20°C.	

REAGENT T3: CHEMICAL STANDARD SOLUTION 3

Ecgonina Methyl Ester Cocaethylene Buprenorphine Norbuprenorphine	10.000 ng/ml 10.000 ng/ml 2.500 ng/ml 5.000 ng/ml
Storage: Once you open the vial, withdrawn the necessary volume, close it with the reagent used and stored at -20°C.	

REAGENT H: DERIVATIZATION SOLUTION 1 FOR AMPHETAMINES

Once opened ampoule transfer all the content, 1 ml, in a glass vial. Close cap tightly and store at 2-8 °C.

REAGENT I: DERIVATIZATION SOLUTION 2 FOR AMPHETAMINES

Once opened ampoule transfer all the content, 1 ml, in a glass vial. Close cap tightly and store at -20 °C.

REAGENT M1: DERIVATIZATION SOLUTION 1

Once opened ampoule transfer all the content, 1 ml, in a glass vial. Close cap tightly and store at Room Temperature.

REAGENT M2: DERIVATIZATION SOLUTION 2

Once opened ampoule transfer all the content, 1 ml, in a glass vial. Close cap tightly and store at 2-8 °C.

REAGENT O: INTERNAL STANDARD SOLUTION FOR BASIC DRUGS

Nalorphine	100.000 ng/ml
Storage: at – 20 °C	

REAGENT P: INTERNAL STANDARD SOLUTION FOR AMPHETAMINES

MDPA	100.000 ng/ml
Storage: at – 20 °C	

GAS-CHROMATOGRAPH CONDITIONS : (these conditions may be varied depending on the instrument used)

- Column VF-5ms or Rxi-5Sil MS 30 m x 0,25 mm, 0,25 µm (after conditioning according to the indications provided by the manufacturer) - Injector Temperature 280 °C - Split Ratio: - ON split 20 0 min: OFF 1 min: ON split 100 3 min: ON split 20 NB: The split ratio can be varied depending on the instrument used - Heat Schedule for Amphetamines: 110 °C x 3,25 minutes 110-250°C at 20 °C/min 250°C x 1 min 250-300°C at 40 °C/min 301 x 6,5 minutes (run 19 minutes) Helium Gas Flow 1 ml/min - Heat Schedule for other basic molecules (except Amphetamine): 120 °C x 3,25 minutes 120-250°C at 20 °C/min 250°C x 1 min, 250-300°C at 40 °C/min 300 x 6 minutes (run 18 minutes) Helium Gas Flow 1 ml/min

DETECTOR DI MASSA CONDITIONS (4000) : (these conditions may be varied depending on the instrument used)

- Massa Range 80 – 300 m/z (for Amphetamines)
- Massa Range 70 – 540 m/z (for other DRUGS OF ABUSE)
- Massa Temperature: 180 °C;
- Transfer Line Temperature: 270 °C;
- Manifold Temperature: 80 °C
- Filament on: 3 minutes

COLUMN VF-5 MS or Rxi-5Sil MS CONDITIONING

Follow the manufacturer's instructions.

COLUMN VF-5 MS or Rxi-5Sil MS CLEANING

If the analytical performance suffer significant changes (e.g. Low sensitivity or presence of interfering in chromatograms), you must make the cleaning of the column as reported in accordance with the indications of the manufacturer.

AUTOSAMPLER WASH

Wash the syringe before each injection with ACETONE.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
GC49016	Calibrator in hair for DRUGS OF ABUSE	1 x 2000 mg/ 1 x 3 x 0,3 ml/ 1 x 20 ml
GC49019	Control in hair for DRUGS OF ABUSE – Levels 1 and 2	1 x 2000 mg/ 1 x 3 x 0,5 ml/ 1 x 30 ml
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
Z1636/26	Pyrex Tubes of 10 ml with SWL caps	1 x 40 Pcs
SCP8944	VF-5MS Column (30 m x 0,25 mm - 0,25 µm)	1 Pc
S13623	Rxi-5Sil MS Column (30 m x 0,25 mm – 0,25 µm)	1 Pc
ZRE24533	Hamilton Syringe of 50 ul	1 Pc

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

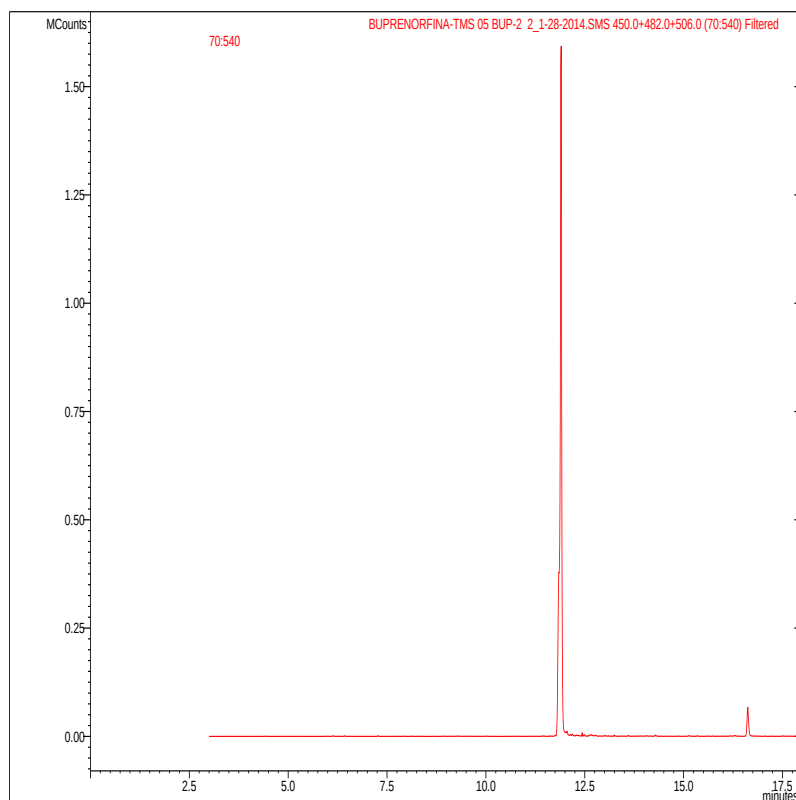


Fig. 1 :	Test Solution Buprenorphine
	R.T. 11.9 Buprenorphine

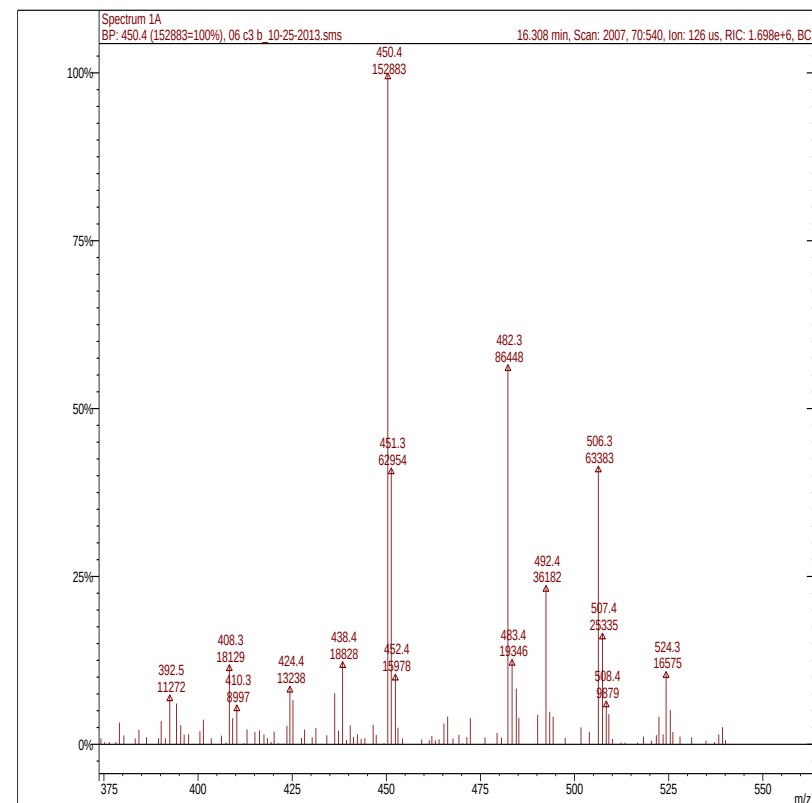


Fig. 2 :	Massa Spectrum of Buprenorphine
	MOLECULAR IONS: 450-482-506

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

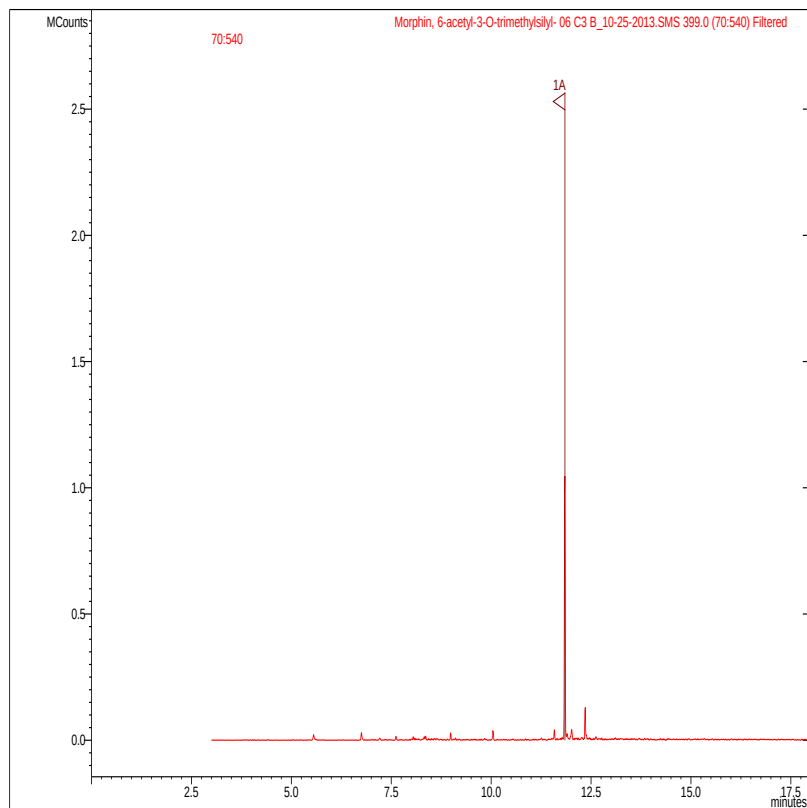


Fig. 3 :	Test Solution 6-MAM
	R.T. 11.84 6-MAM

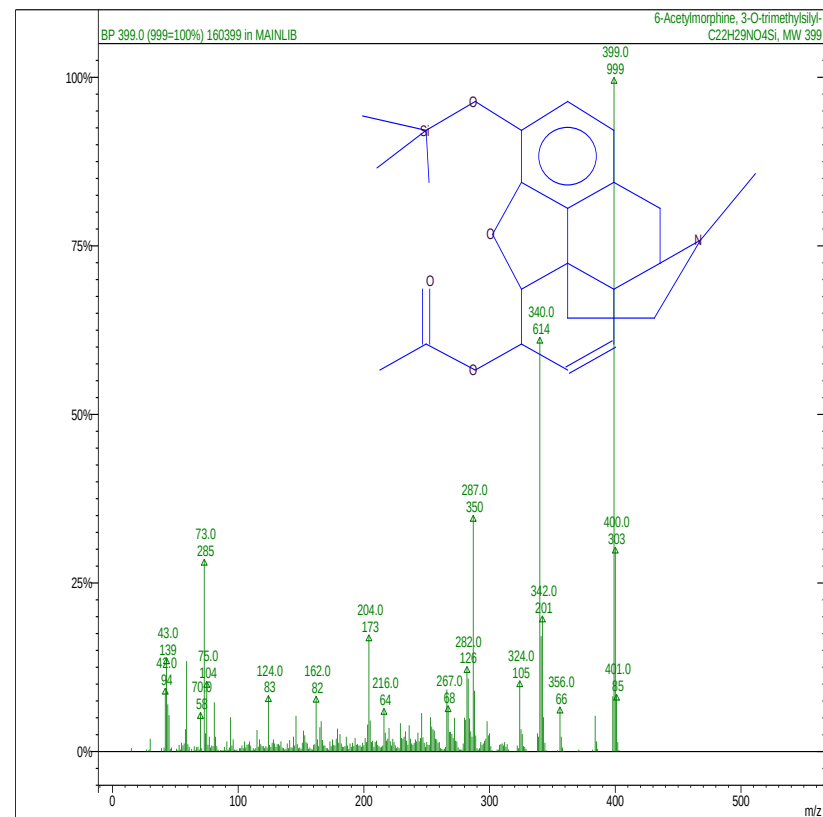


Fig. 4 :	Massa Spectrum of 6-MAM
	MOLECULAR IONS: 399-340-287

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

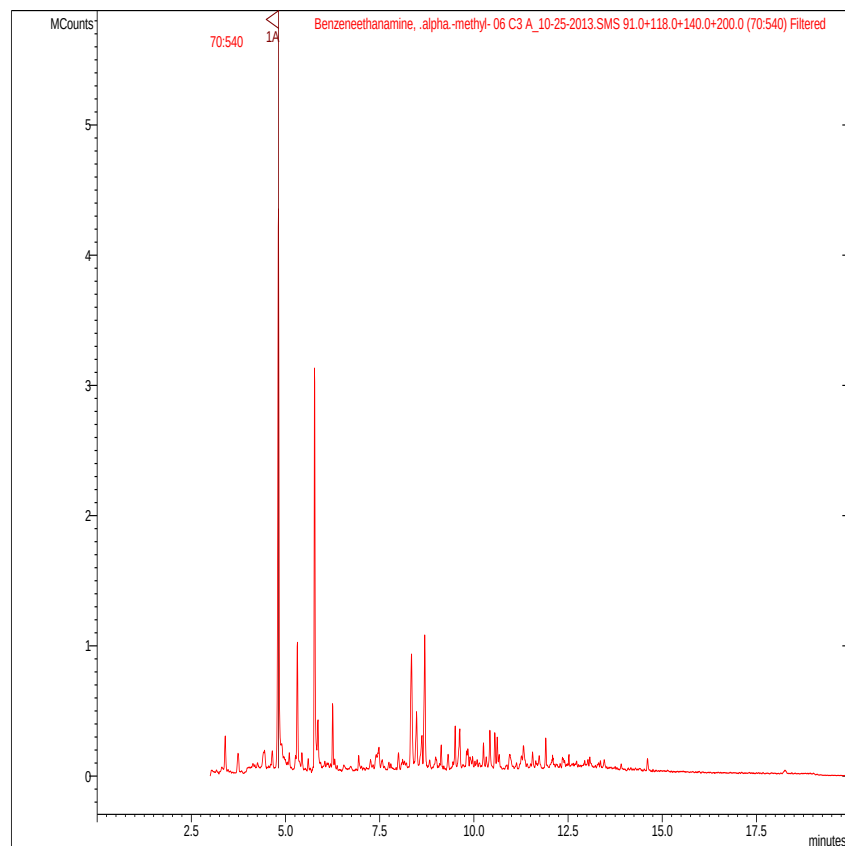


Fig. 5 :	Test Solution Amphetamine
	R.T. 4.81 Amphetamine

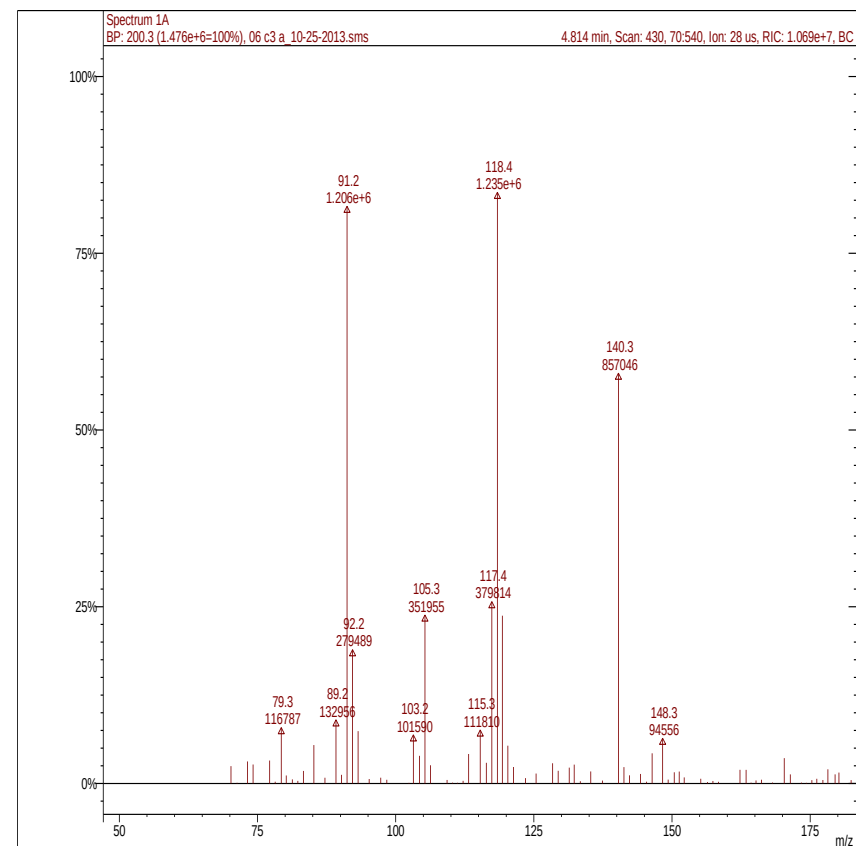


Fig. 6 :	Massa Spectrum of Amphetamine
	MOLECULAR IONS: 91-118-140-200

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

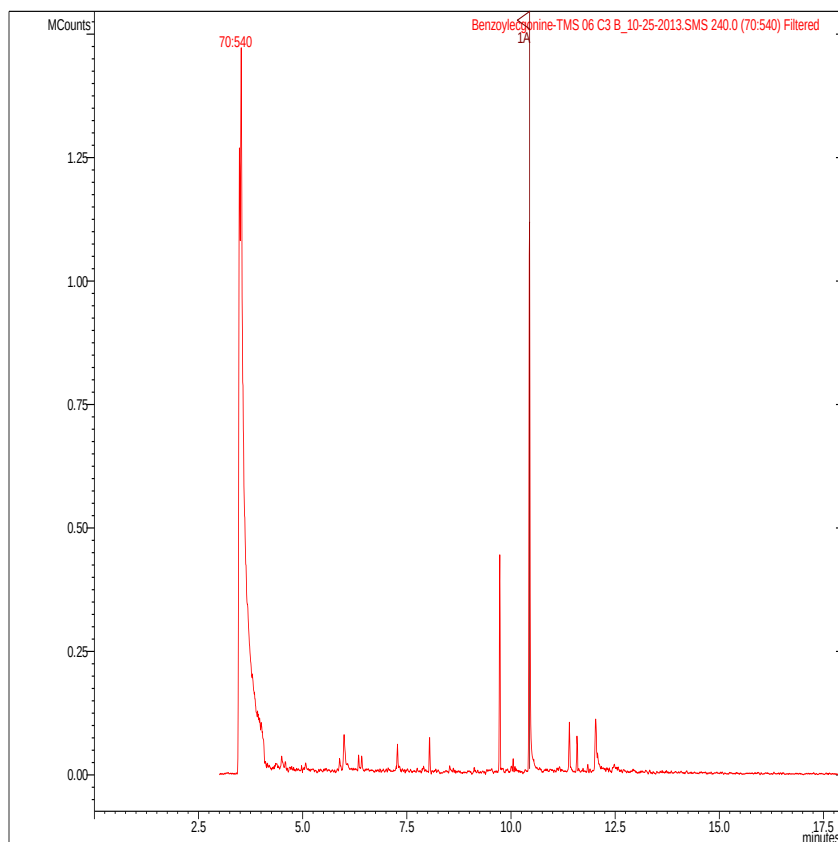


Fig. 7 :	Test Solution Beg
	R.T. 10.44 Beg

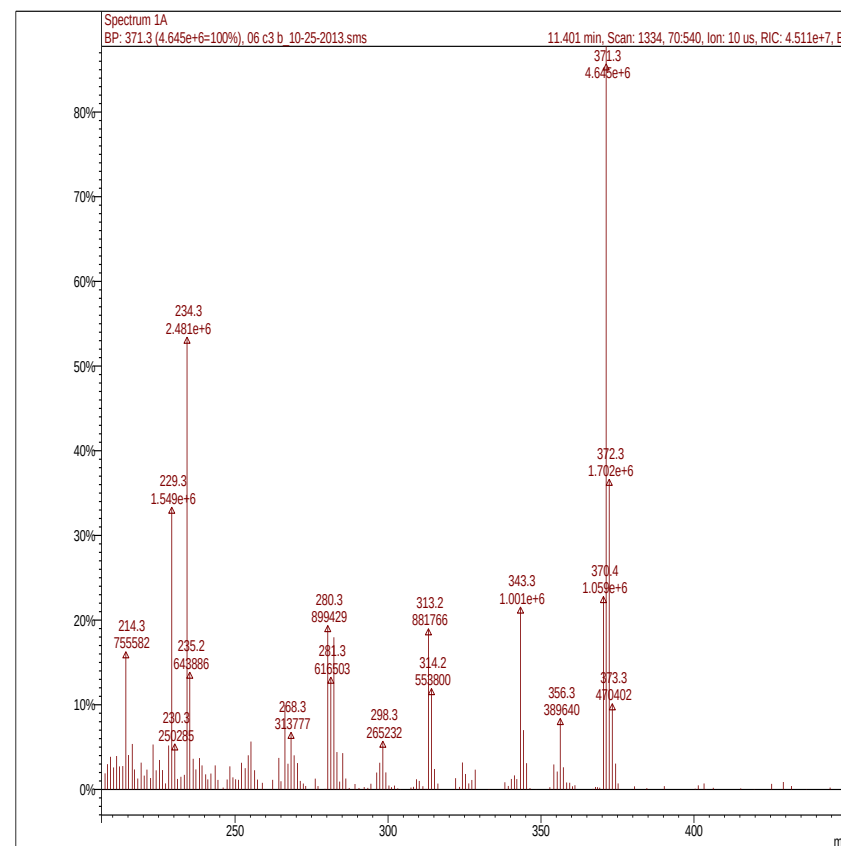


Fig. 8 :	Massa Spectrum of Beg
	MOLECULAR IONS: 240-361-82

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

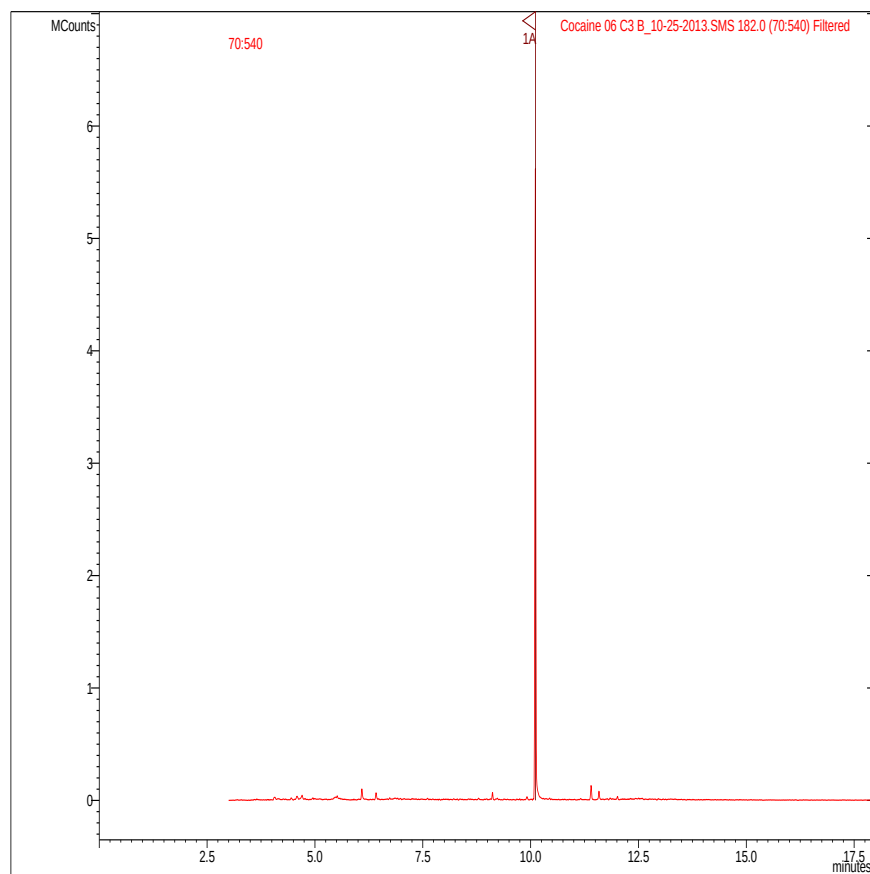


Fig. 9 :	Test Solution Cocaine
	R.T. 10.11 Cocaine

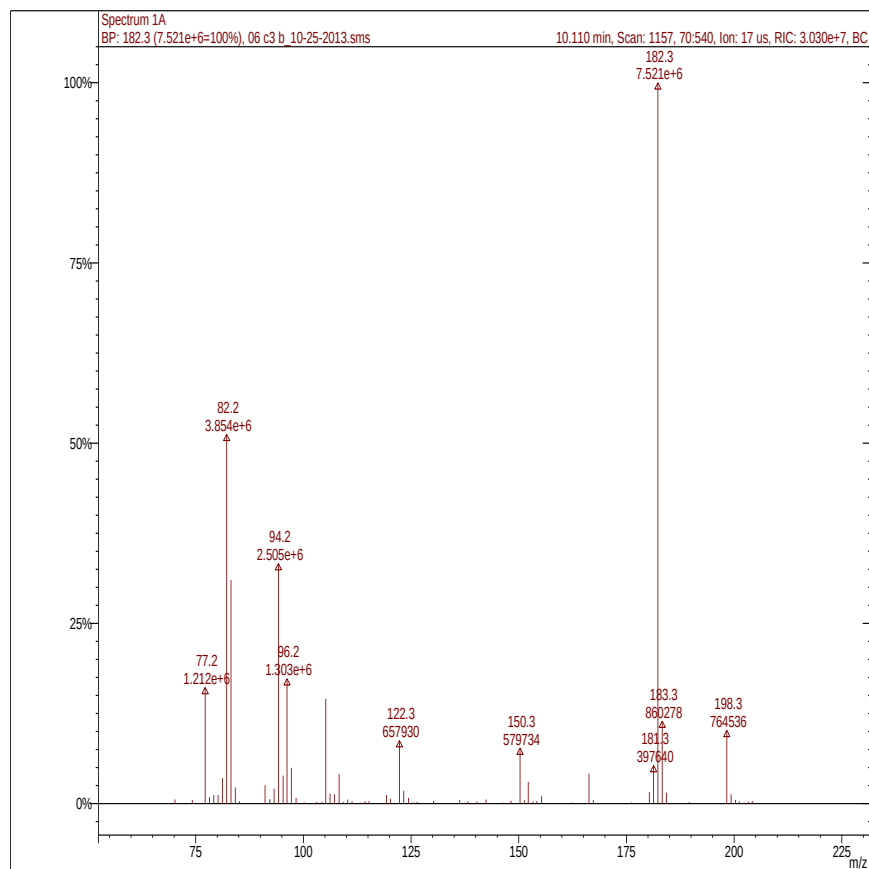


Fig. 10 :	Massa Spectrum of Cocaine
	MOLECULAR IONS: 182-94-82

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

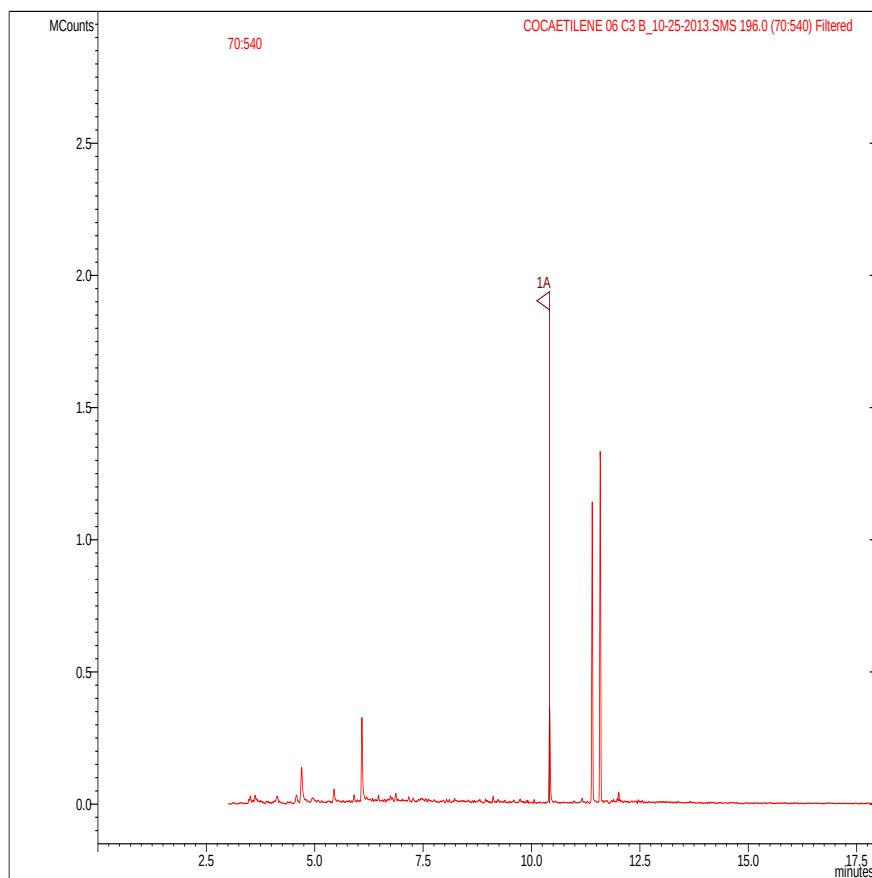


Fig. 11 : *Test Solution Cocaethylene*
R.T. 10.42 Cocaethylene

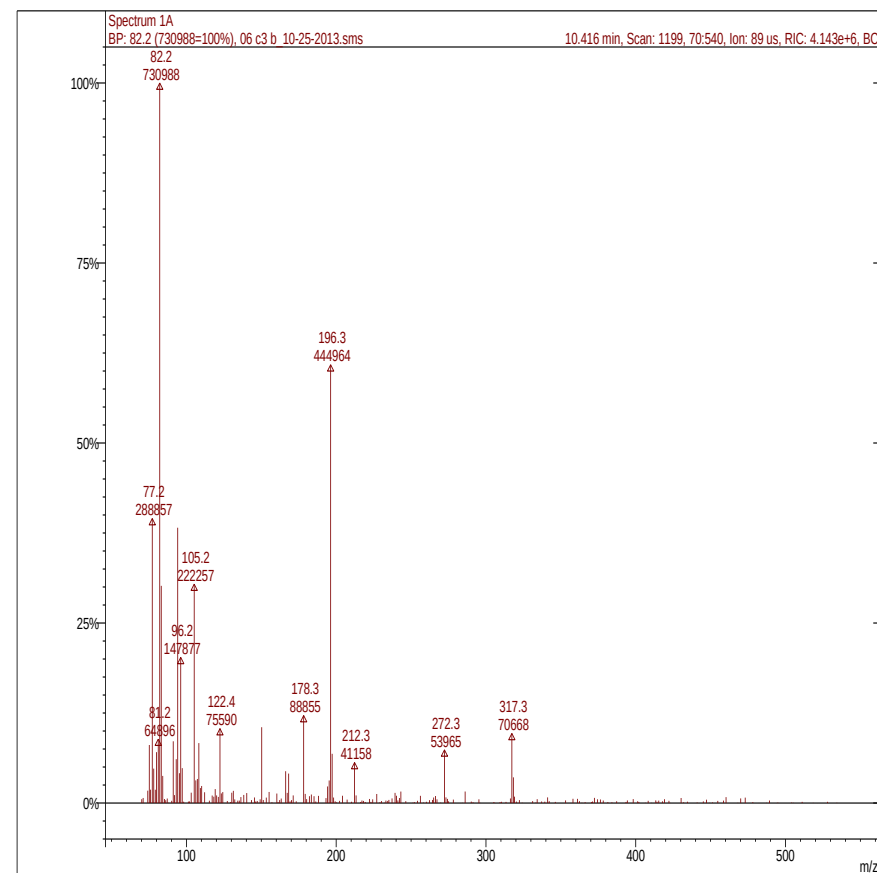


Fig. 12 : *Massa Spectrum of Cocaethylene*
MOLECULAR IONS: 196-94-82

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

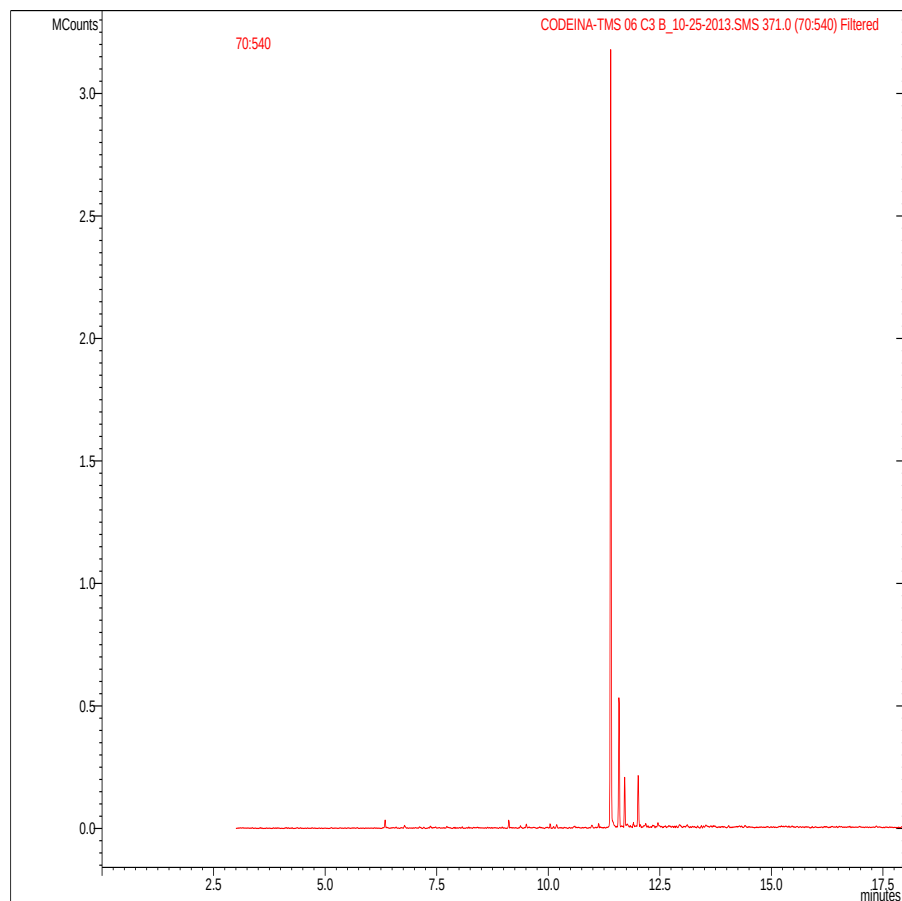


Fig. 13 :	Test Solution Codeine
	R.T. 11.40 Codeine

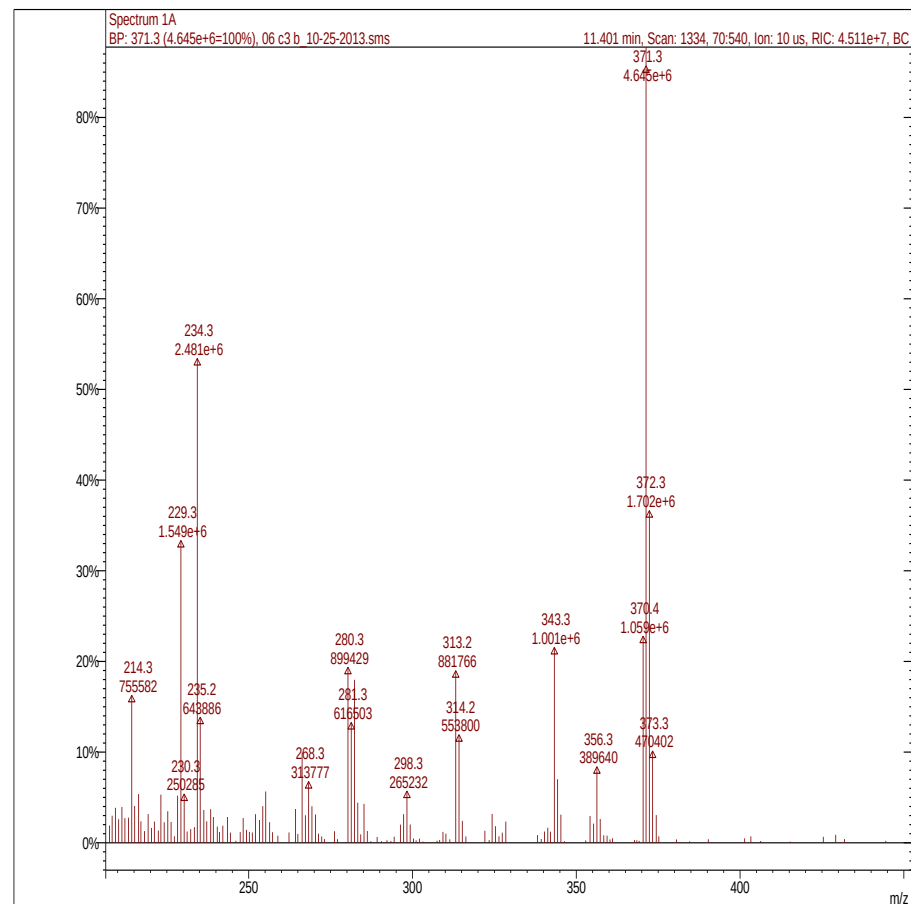


Fig. 14 :	Massa Spectrum of Codeine
	MOLECULAR IONS: 371-234-343

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

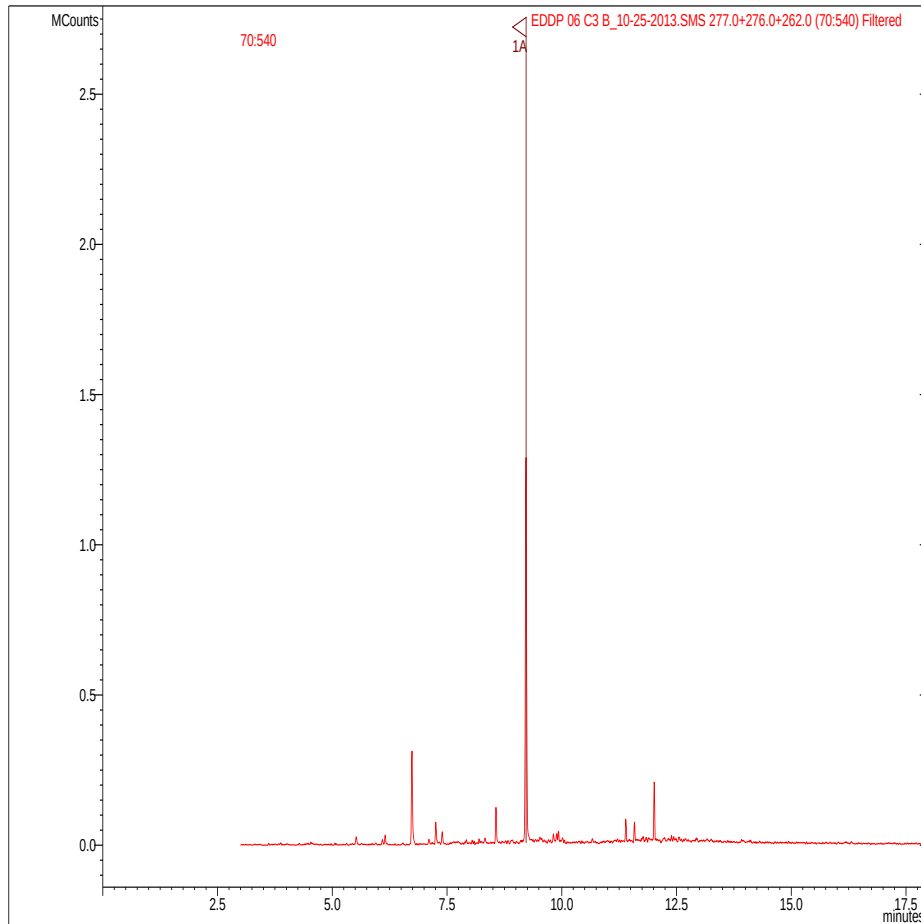


Fig. 15 : Test Solution EDDP
R.T. 9.22 EDDP

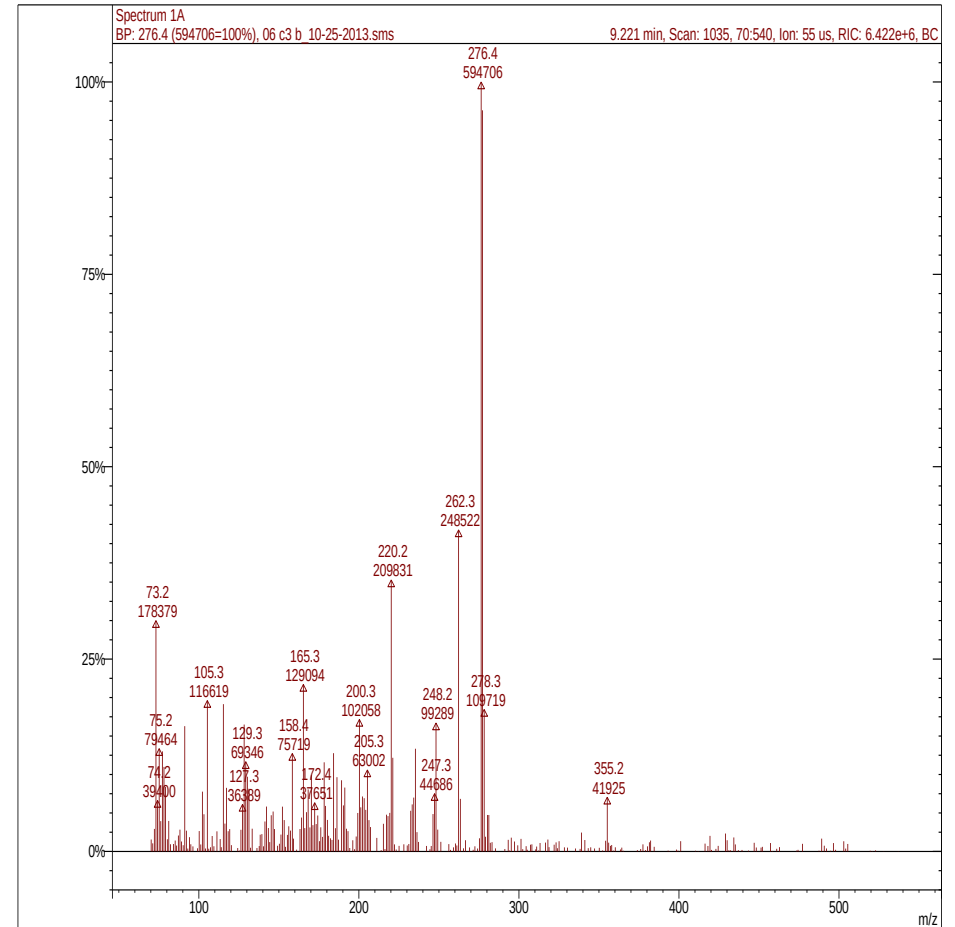


Fig. 16 : Massa Spectrum of EDDP
MOLECULAR IONS: 276-277-262

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

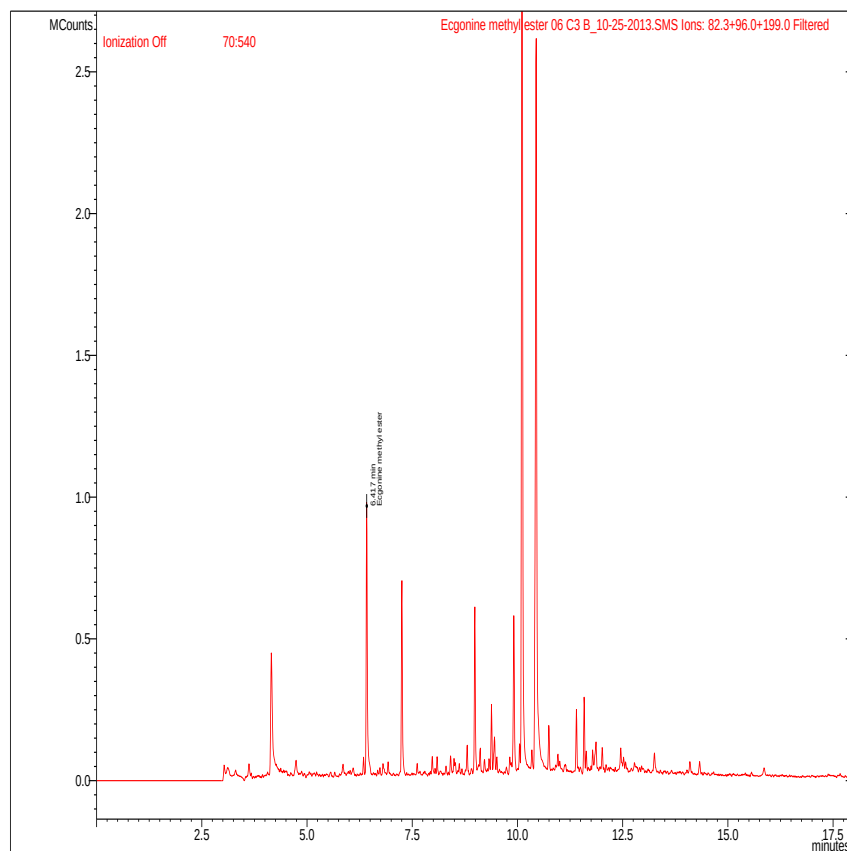


Fig. 17 :	Test Solution Ecgonine Methyl Ester
	R.T. 6.42 Ecgonine Methyl Ester

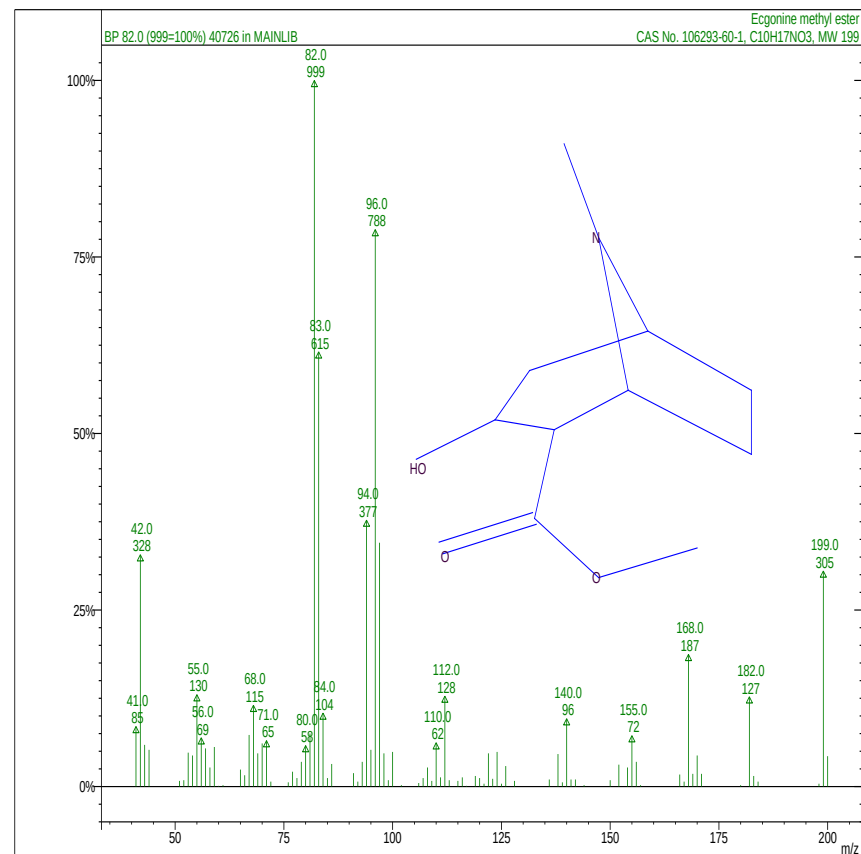


Fig. 18 :	Massa Spectrum of Ecgonine Methyl Ester
	MOLECULAR IONS: 82-96-199

DRUGS OF ABUSE IN HAIR (Reference Chromatograms/Spectra)

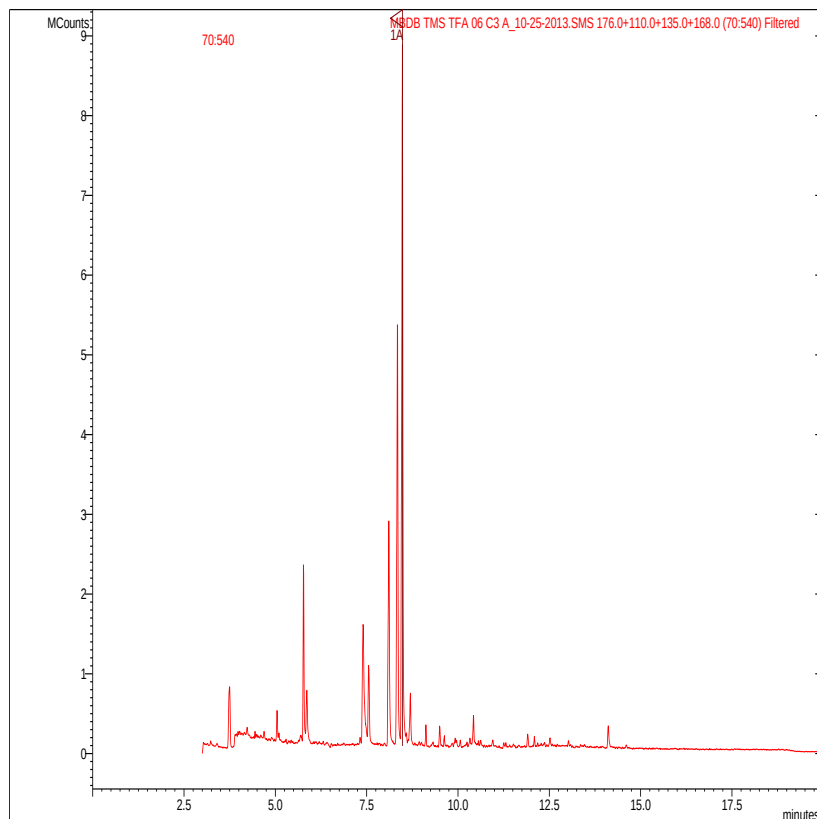


Fig. 19 :	Test Solution MBDB
	R.T. 8.48 MBDB

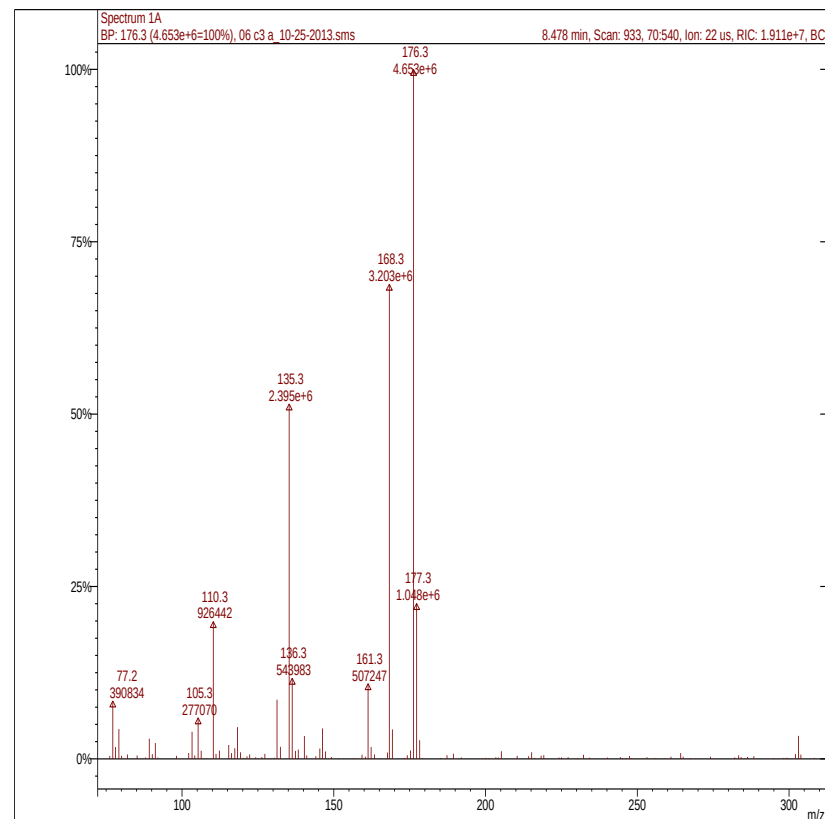


Fig. 20 :	Massa Spectrum of MBDB
	MOLECULAR IONS: 176-168-135-110

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

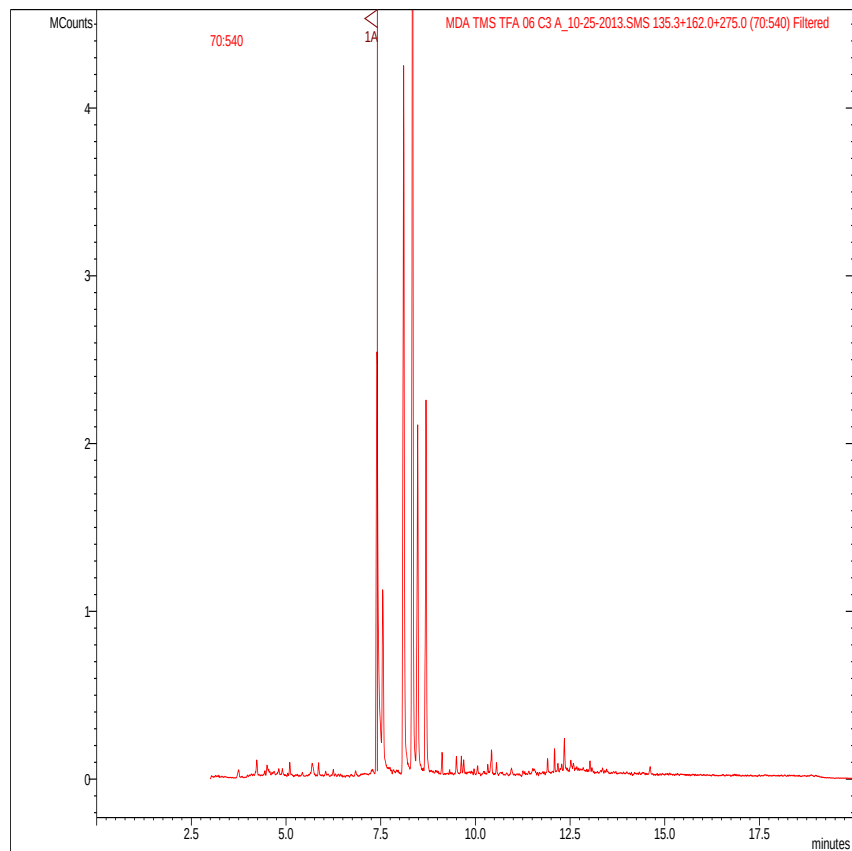


Fig. 21 :	Test Solution of MDA
	R.T. 7.40 MDA

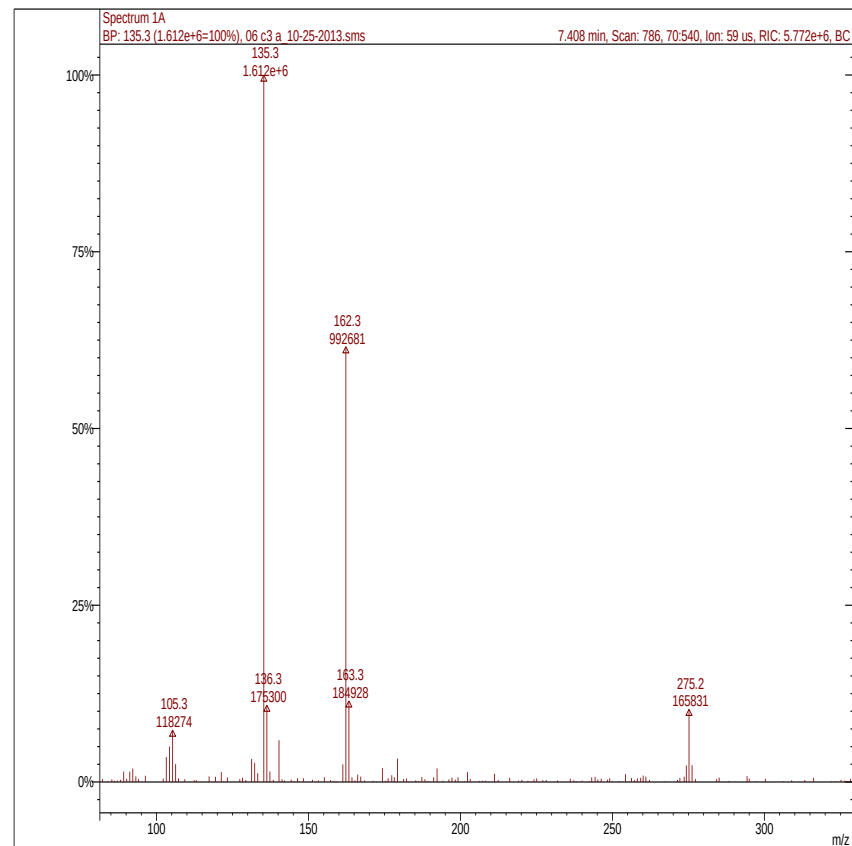


Fig. 22 :	Massa Spectrum of MDA
	MOLECULAR IONS: 135-162-275

DRUGS OF ABUSE IN HAIR (Reference Chromatograms/Spectra)

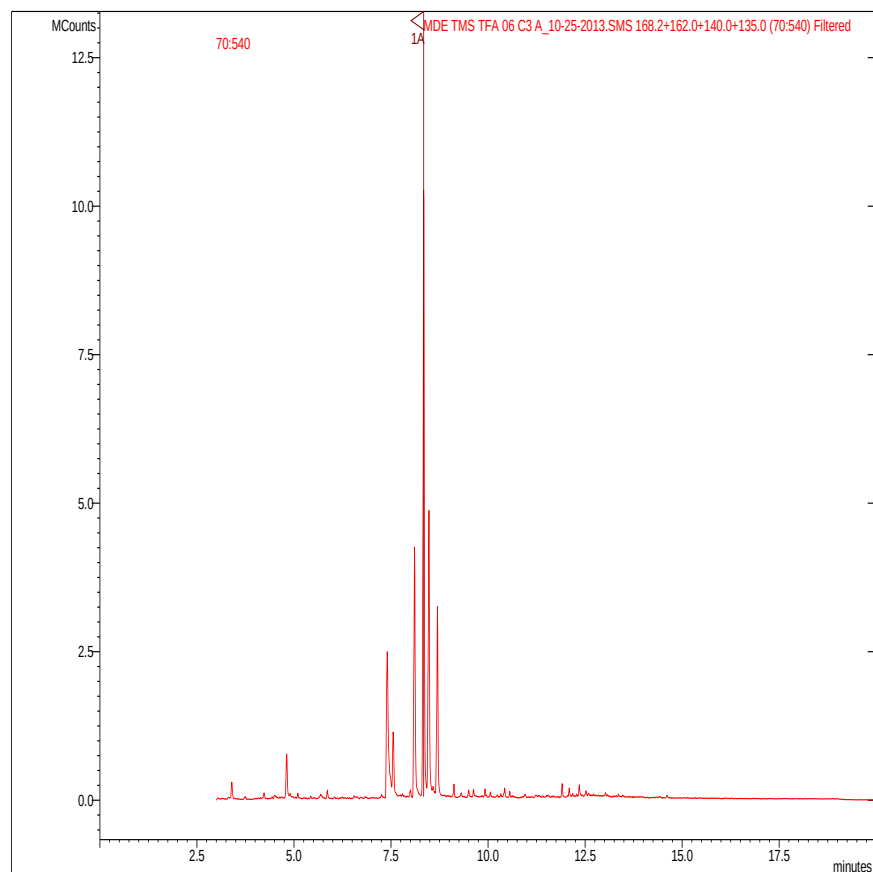


Fig. 23 :	Test Solution MDE
	R.T. 8.34 MDE

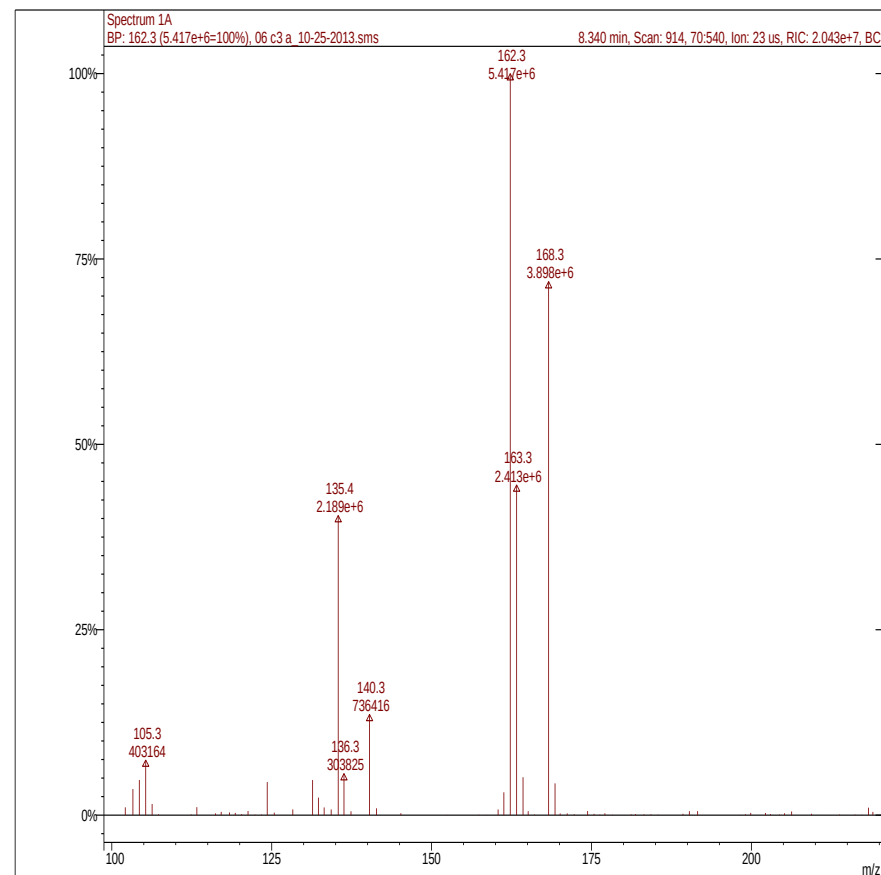


Fig. 24 :	Massa Spectrum of MDE
	MOLECULAR IONS: 162-135-140-168

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

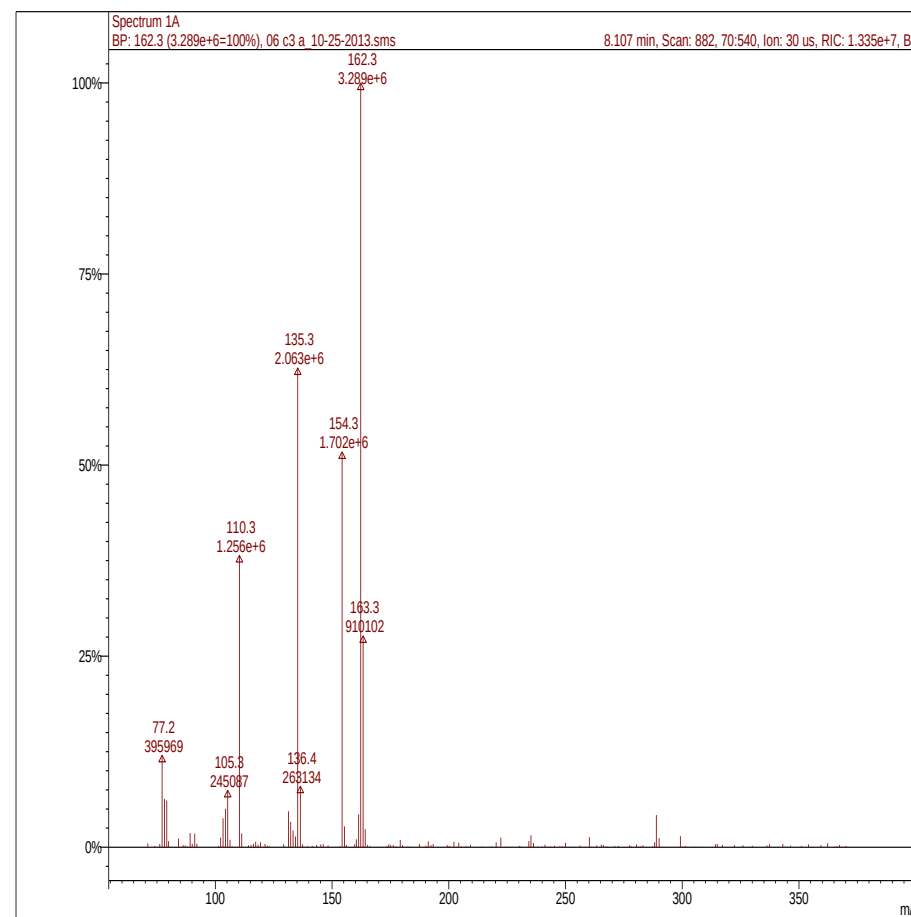
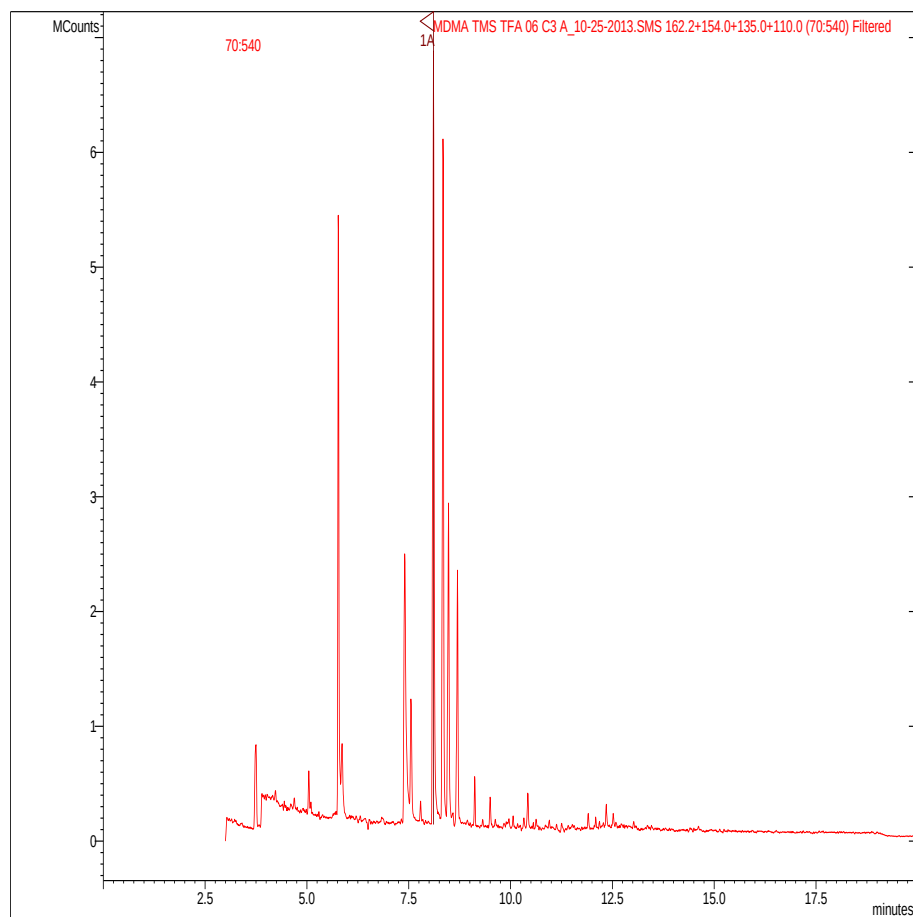


Fig. 25 :	Test Solution MDMA
	R.T. 8.10 MDMA

Fig. 26 :	Massa Spectrum of MDMA
	MOLECULAR IONS: 162-110-135-154

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

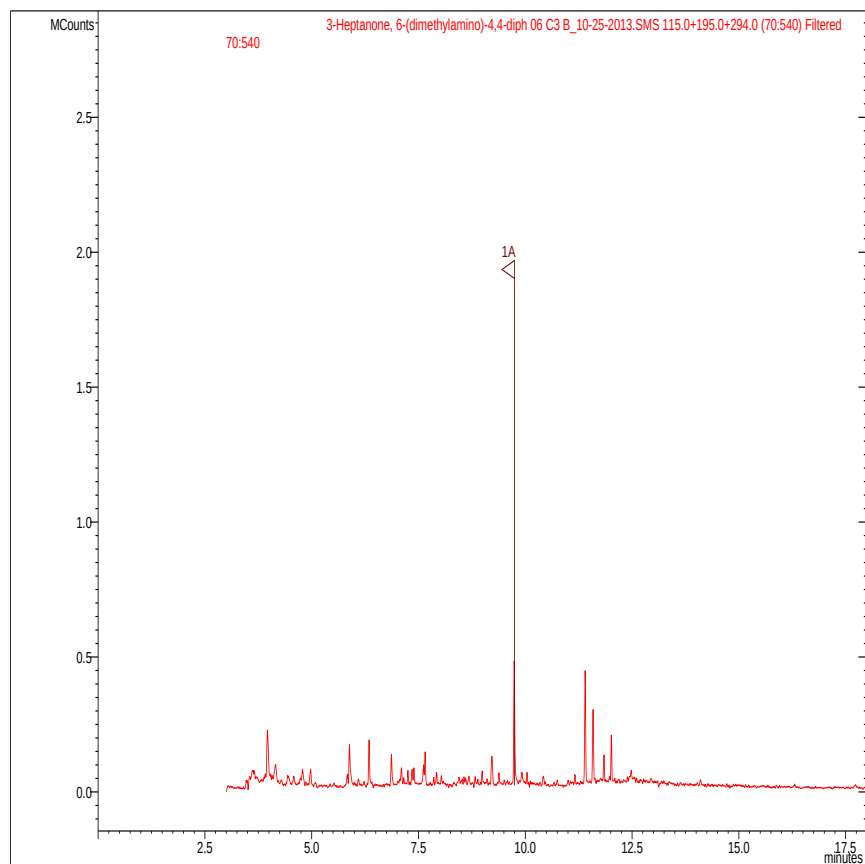


Fig. 27 :	Test Solution Methadone
	R.T. 9.75 Methadone

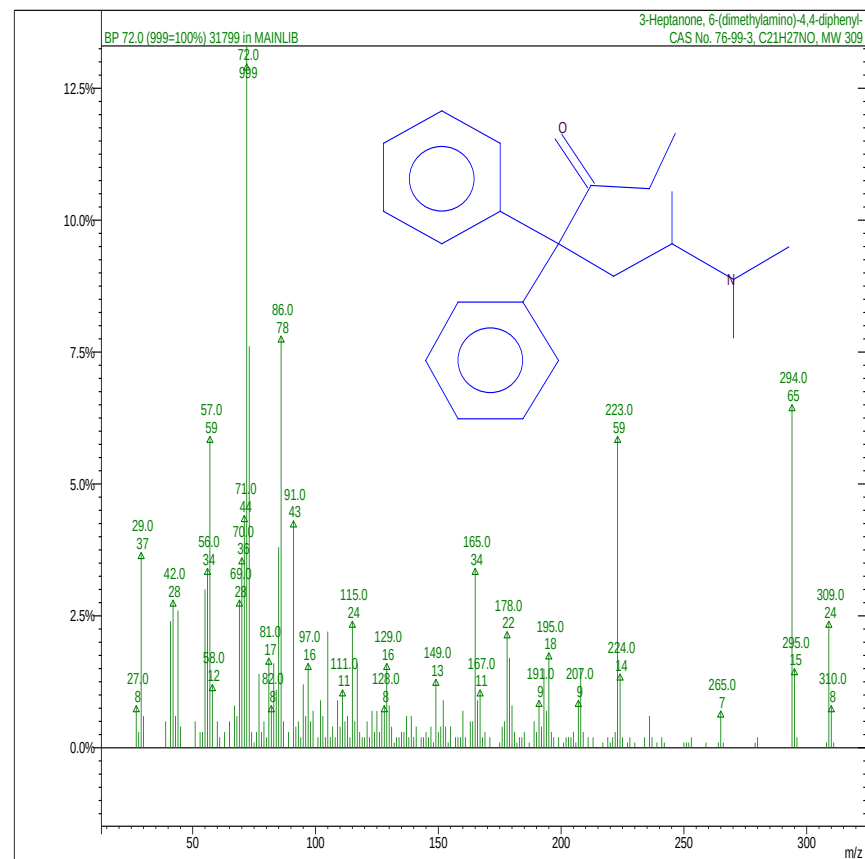


Fig. 28 :	Massa Spectrum of Methadone
	MOLECULAR IONS: 165-115-294-72

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

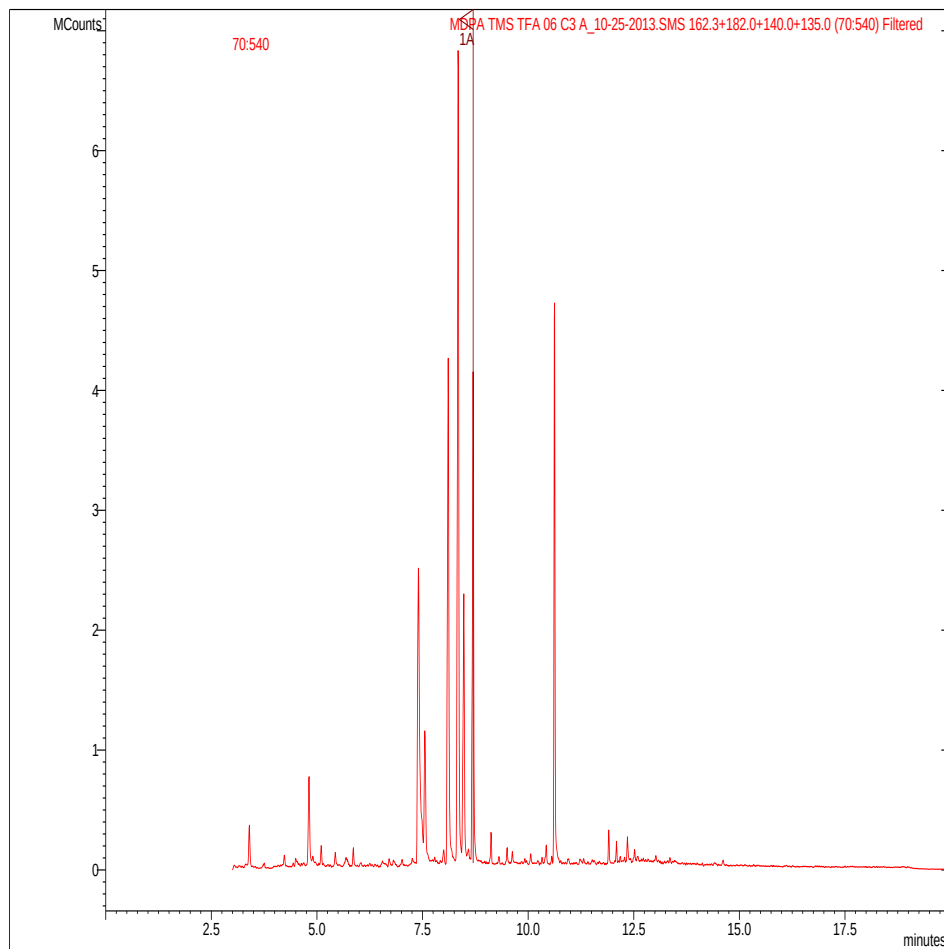


Fig. 29 :	Internal Standard MDPA
	R.T. 8.70 MDPA

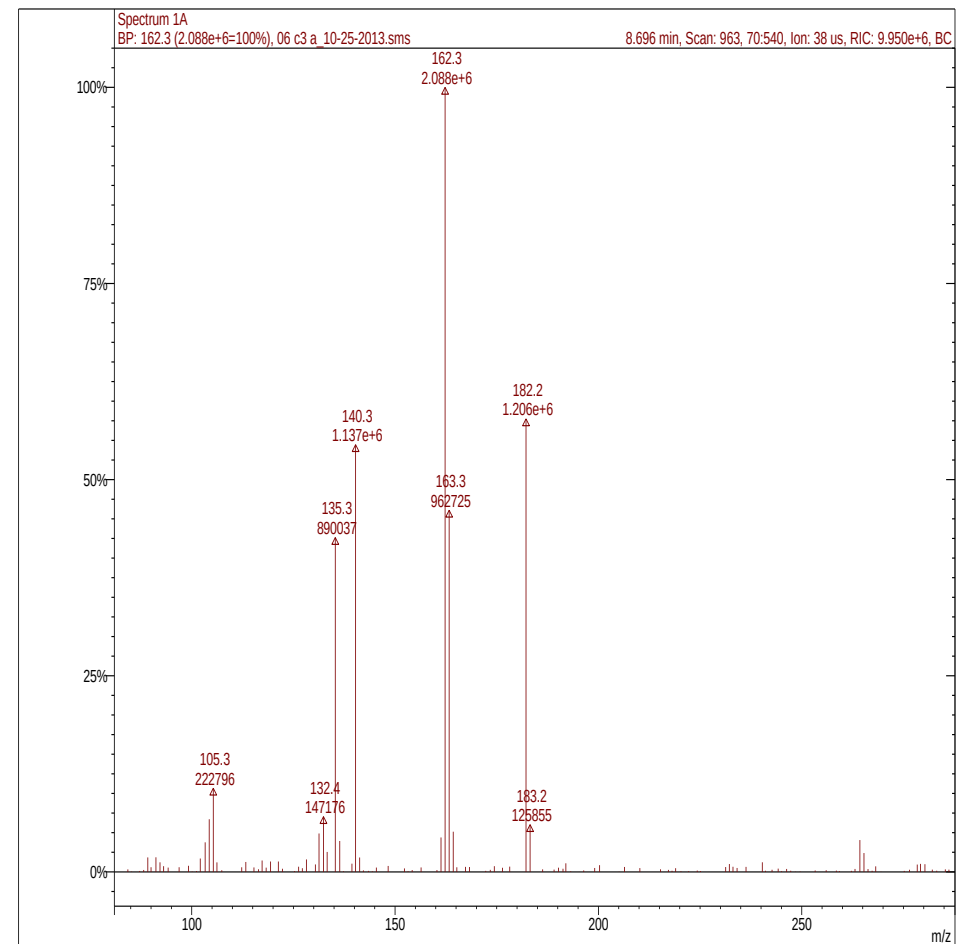


Fig. 30 :	Massa Spectrum of MDPA
	MOLECULAR IONS: 162-182-140-135

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

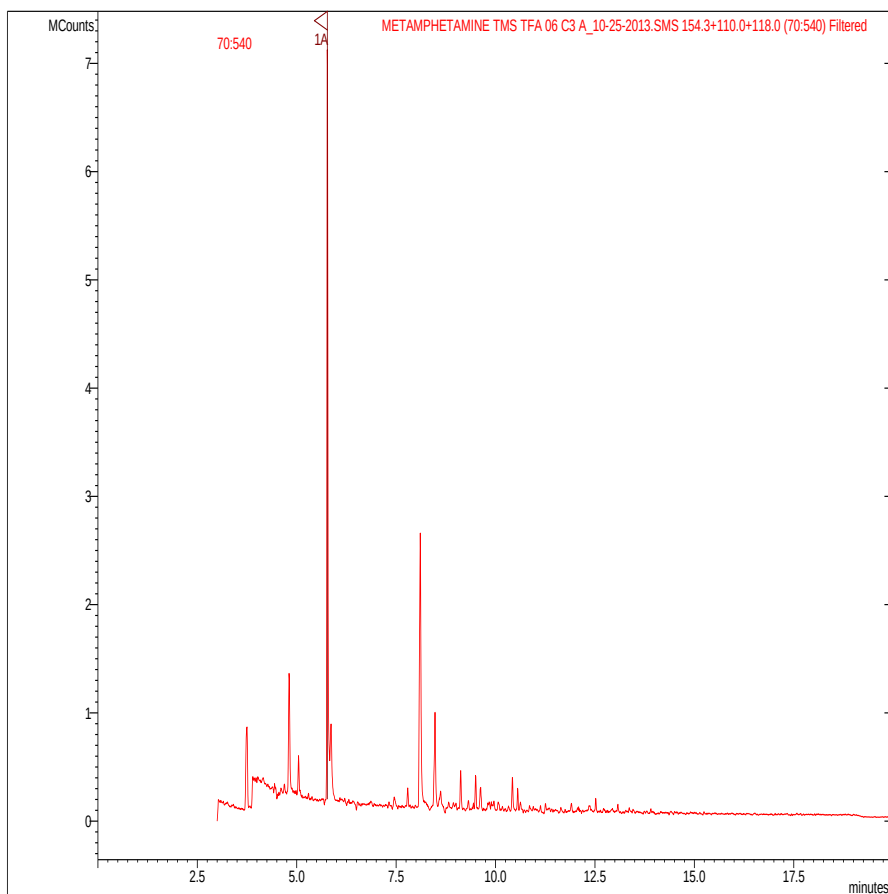


Fig. 31 :	Test Solution Methamphetamine
	R.T. 5.77 Methamphetamine

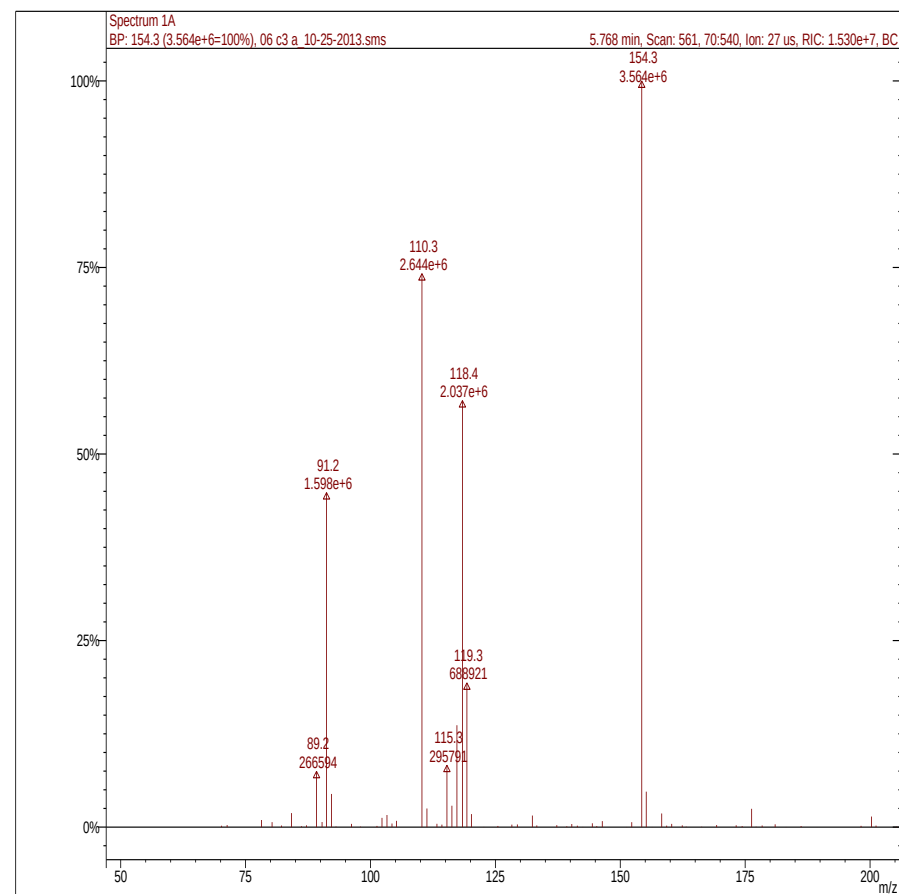


Fig. 32 :	Massa Spectrum of Methamphetamine
	MOLECULAR IONS: 154-110-118-91

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

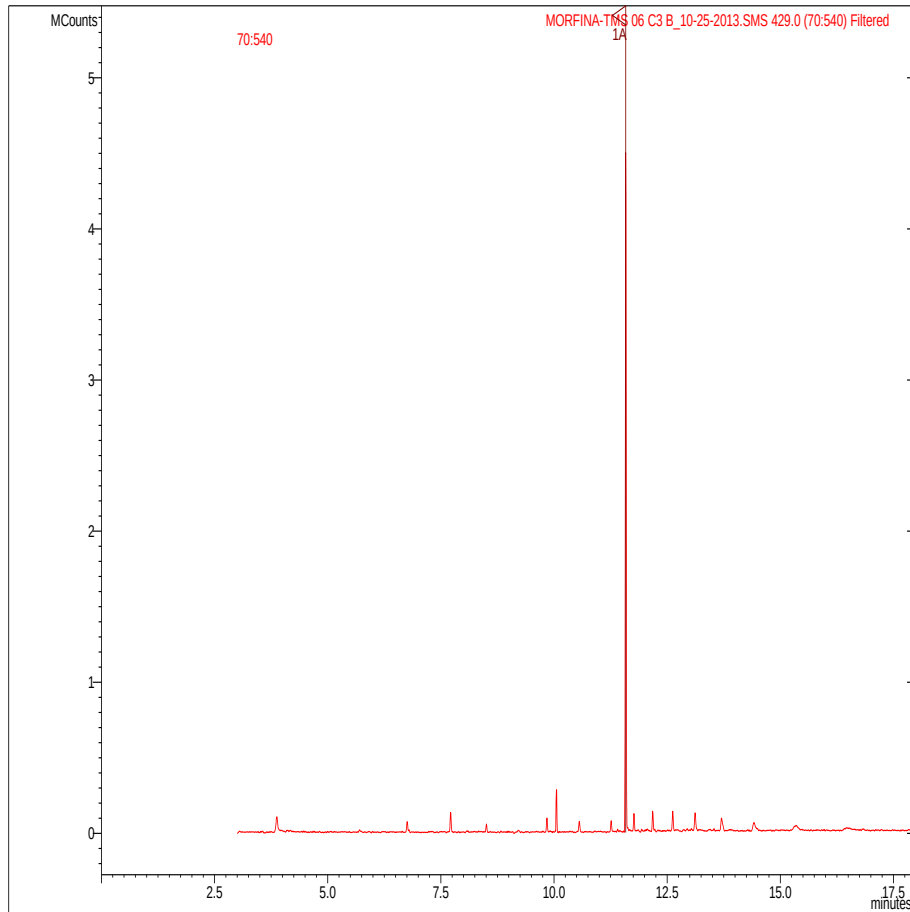


Fig. 33 :	Test Solution Morphine
	R.T. 11.58 Morphine

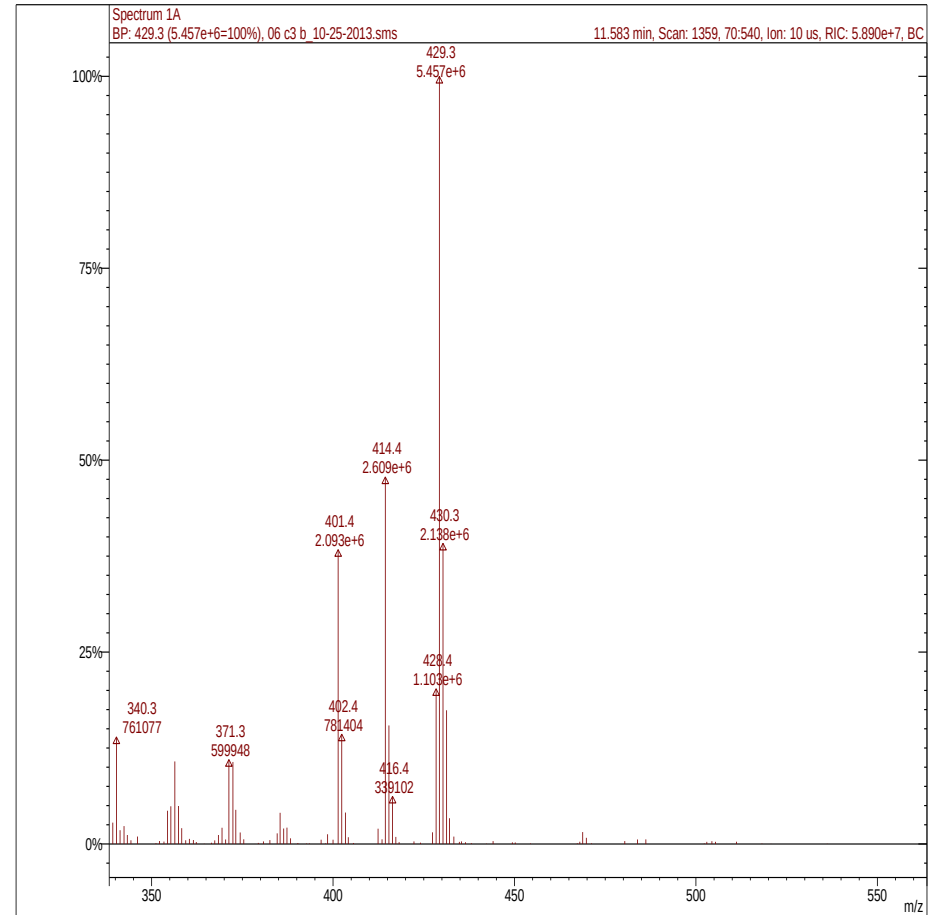


Fig. 34 :	Massa Spectrum of Morphine
	MOLECULAR IONS: 429-414-401

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

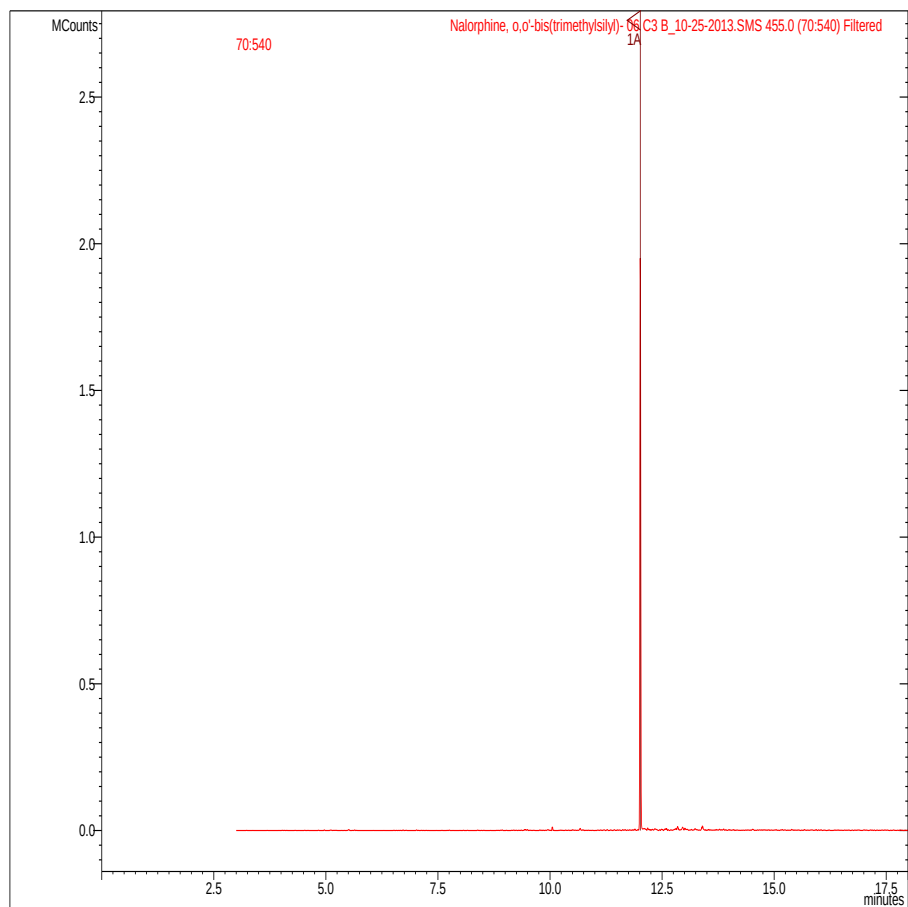


Fig. 35 : *Internal Standard Nalorphine*
R.T. 12.01 Nalorphine

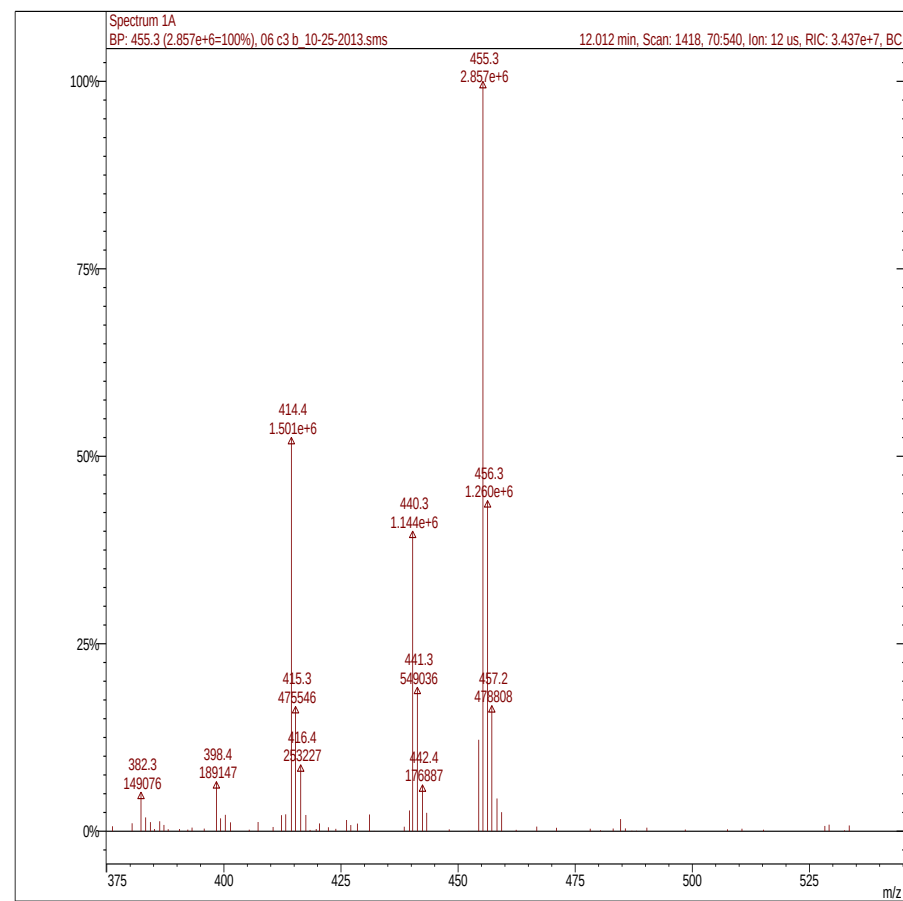


Fig. 36 : *Massa Spectrum of Nalorphine*
MOLECULAR IONS: 455-440-414

DRUGS OF ABUSE IN HAIR

(Reference Chromatograms/Spectra)

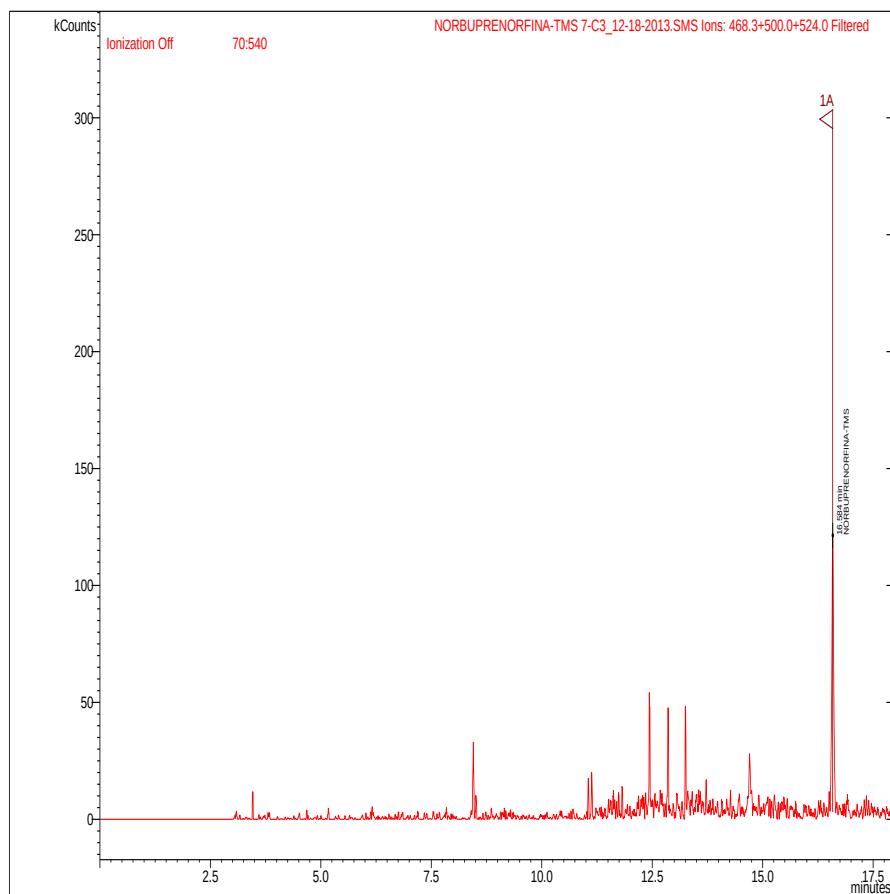


Fig. 37 :	Test Solution Norbuprenorphine
	R.T. 16.6 Norbuprenorphine

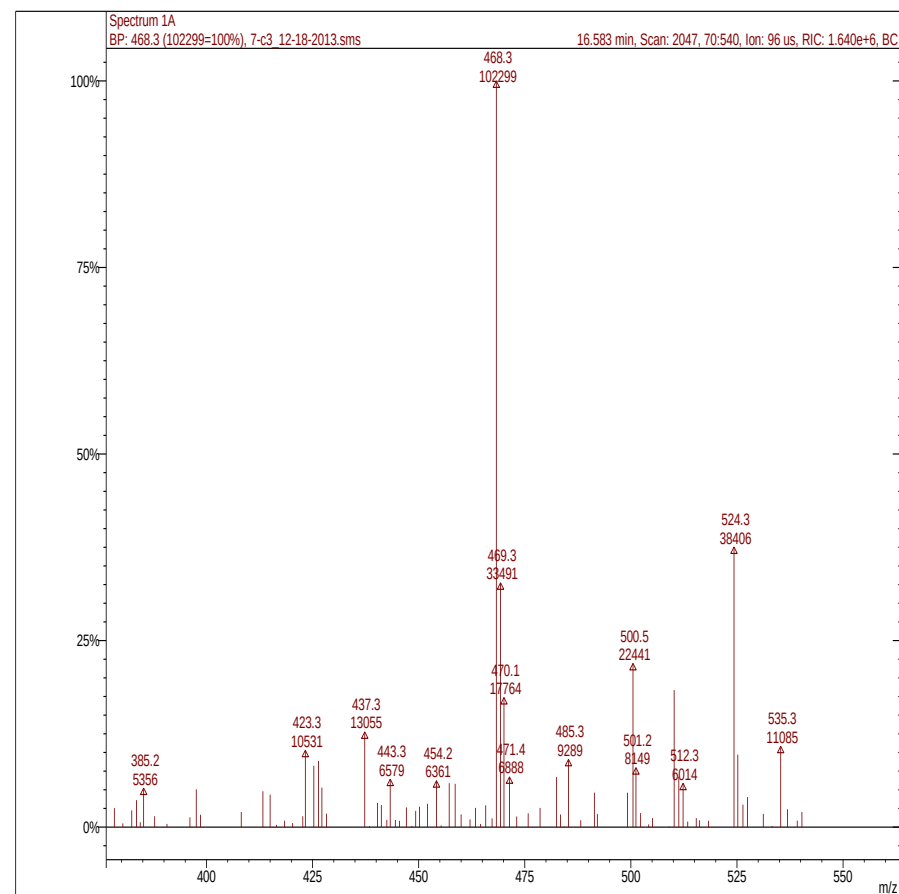


Fig. 38 :	Massa Spectrum of Norbuprenorphine
	MOLECULAR IONS: 468-500-524