

AMPHETAMINES in whole blood by GC-MS – Code GC89110

(Amphetamine, Methamphetamine, MDA, MDMA, MDE, MBDB)

Method of Confirmation by GC-MS

INTRODUCTION

The screening of Drugs of Abuse in biological liquids (above all 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 to the screening must be confirmed by an alternative technique, generally by chromatographic technique both Gas (GC) and Liquid (LC), both of them coupled with massa detector (MS).

The present method allows the determination of 3,4-MDA, 3,4-MDE, 3,4-MDMA, Amphetamine, Methamphetamine, MBDB by GC-MS.



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N° 005	Amphetamines in whole blood by GC/MS	08/2024
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TECHNICAL FEATURES

Principle of the Method:

The present method consent to determinate **Amphetamine, Methamphetamine, MDMA, MDA, MDE, MBDB** after blood purification SPE and reading in GC-MS.

The method has been validated in accordance with the criteria laid down at international level by FDA and by ICH.

Concentrations of substances in urine and blood that define the MINIMUM REQUIREMENTS OF PERFORMANCE for confirmatory analysis, for toxicological and forensic purposes, by means of techniques chromatography combined with mass spectrometry.

Class of substances or substances	The method must be able to confirm (ng/ml)
Opiates	
<i>total morphine*</i>	2
<i>total codeine*</i>	2
<i>6-acetylmorphine</i>	2
Cocaine	
<i>cocaine</i>	2
<i>benzoylecgonine</i>	2
<i>cocaethylene</i>	2
<i>nor-cocaine</i>	2
Amphetamines and congeners	
<i>amphetamine</i>	2
<i>methamphetamine</i>	2
3,4-Methylenedioxyamphetamine and congeners	
<i>MDMA</i>	2
<i>MDA</i>	2
<i>MDEA</i>	2
<i>MBDB</i>	2
Methadone	
<i>methadone</i>	2
<i>EDDP</i>	2
Cannabinoids	
<i>THC</i>	1
<i>11-OH-THC</i>	0,1
<i>THC-COOH</i>	2
Buprenorphine	
<i>total buprenorphine *</i>	2
<i>total norbuprenorphine *</i>	2

* the value refers to the sample undergoing hydrolysis.

Guidelines for facilities with laboratories for the detection of Abuse Substances with Toxicological, Forensic and Medical-Legal Purposes
Revision 5 of 29 May 2017.

ANALYTE	Accuracy intra-day			Precision intra-day			CV% inter-day		
	(Error %)			(CV%)			(Error %)		
	Calibrator			Calibrator			Calibrator		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
	Ci 7,5 ng/ml	Cm 20 ng/ml	Cs 100 ng/ml	Ci 7,5 ng/ml	Cm 20 ng/ml	Cs 100 ng/ml	Ci 7,5 ng/ml	Cm 20 ng/ml	Cs 100 ng/ml
AMPHETAMINE	2,5	1,5	4,6	3,9	2,3	1,9	5,9	4,5	5,9
METHAMPHETAMINE	2,8	3,4	2,0	3,0	3,9	2,4	4,1	7,3	6,2
3,4-MDMA	2,3	3,6	2,7	2,8	4,3	2,1	6,7	5,2	3,5
3,4- MDA	6,1	3,5	2,8	3,6	2,5	1,7	6,6	4,8	5,1
3,4-MDE	3,0	4,2	2,8	2,8	5,7	2,1	6,2	4,9	7,2
MBDB	0,5	1,6	3,5	1,9	1,2	2,0	2,6	3,3	4,0

ANALYTE	Recovery (%)		
	Calibrator		
	Low	Medium	High
AMPHETAMINE	88,2	87,9	110,2
METHAMPHETAMINE	72,8	92,4	98,6
3,4-MDMA	78,7	87,5	105,5
3,4- MDA	70,0	89,6	103,3
3,4-MDE	68,4	89,9	102,4
MBDB	70,6	90,5	102,6

ANALYTE	(Coefficient of correlation R2) ± DS	LOD (ng/ml)	LOQ (ng/ml)
AMPHETAMINE	0,9991 ± 0,0007	0,8	2,5
METHAMPHETAMINE	0,9989 ± 0,0008	0,8	2,5
3,4-MDMA	0,9993 ± 0,0005	0,8	2,5
3,4- MDA	0,9998 ± 0,0002	0,8	2,5
3,4-MDE	0,9994 ± 0,0005	0,8	2,5
MBDB	0,9996 ± 0,0003	0,8	2,5

<u>Components of the kit (50 test) :</u>	All the reagents are ready to use and stable 3 years at 2 – 8 °C, except <u>Reagent A.</u> <u>Store at -20 °C</u>
Reagent A – Deuterated Internal Standards, 1 x 750 ul	
Reagent B – Buffer Solution, 1 x 300 ml	
Reagent C – Conditioning Solution 1 and Wash 3, 1 x 250 ml	
Reagent D – Conditioning Solution 2, 1 x 100 ml	
Reagent E – Wash Solution 1, 1 x 50 ml	
Reagent F – Wash Solution 2, 1 x 150 ml	
Reagent G – Wash Solution 4, 1 x 60 ml	
Reagent H – Eluting Solution, 1 x 100 ml	
Reagent I – Derivatization Solution, 3 x 800 ul	<u>Store at 2-8 °C before and after opening.</u> <u>See Warnings</u>
Reagent L – Test Solution Amphetamine, 1 x 500 ul	<u>See Warnings</u>
Calibrator in whole blood – Level 0, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 1, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 2, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 3, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 4, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 5, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Calibrator in whole blood – Level 6, 3 x 1 ml	<u>Code LC89016 (packed separately – see data sheet)</u>
Clean-up Columns, 50 pcs	

Minimum Instrumental equipment required:

Instrument GC-MS
Operational Computer
Sonicator

Optional Equipment:

Autosampler

Whole Blood Collection Procedure:

Tube with anticoagulant EDTA
Stable for about 4 weeks at 2 – 8 °C or 4 months at –20°C.

PREANALYTICAL PROCEDURE :

STEP 1 : In a pyrex tube with screw cap take 50 µl of Reagent L – Test Solution Amphetamines

Test Storage: once you open the vial and tapped the volume needed, close it and store at –20 °C.

STEP 2 : Bring dry with a stream of helium or nitrogen (in this step the working temperature must be < 40 °C)

STEP 3 : Add 40 µl of Reagent I – Derivatization Solution

Seal the tube

Vortex for 30 sec.

STEP 4 : Incubate at 70°C for 30 minutes

Cooling at Room Temperature

Open the glass vial

STEP 5 : Transfer all the sample in glass vials with reduced volume for auto sampler

NOTE : Before starting the run you should inject 2 µl of this solution freshly cooked on the Gas Chromatograph to identify the retention time and the mass spectrum of drugs of abuse that must be similar to those shown in Fig. If the Test is successful you can proceed to run. If it is not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

Dilute the **Reagent A – Deuterated Internal Standards** 1:1 with Water HPLC grade, preparing the volume sufficient to cover the run. It is possible to modulate, if necessary, the dilution factor depending on the sensitivity of the instrument in use.

N.B.: this dilution should be prepared freshly each run

STEP 1 : Sample Preparation

Dispense in tubes of 10 ml:

	Calibrator – Level 0	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Calibrator – Level 6	Controls	Samples
Calibrator – Level 0	1000 µl								
Calibrator – Level 1		1000 µl							
Calibrator – Level 2			1000 µl						
Calibrator – Level 3				1000 µl					
Calibrator – Level 4					1000 µl				
Calibrator – Level 5						1000 µl			
Calibrator – Level 6							1000 µl		
Controls								1000 µl	
Samples									1000 µl
Reagent A – Deuterated internal Std (dilute 1:1 in water HPLC grade)	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl
Reagent B – Buffer Solution	6000 µl	6000 µl	6000 µl	6000 µl	6000 µl	6000 µl	6000 µl	6000 µl	6000 µl

Seal the tubes and at Vortex for 30 sec.

STEP 2 : Put in the sonicator for 15 min

STEP 3 : Centrifuge at 4.000 rpm for 30 minutes for the complete separation of 2 phases

EXTRACTION OF ANALYTES

STEP 4 : SPE Columns Conditioning

Prepare a number of columns that correspond to the sum of calibrators, controls and unknown samples.

- Add 2 ml of **Reagent C – Conditioning Solution 1 and Wash 3**
Pull into the spe column and discharge the eluate without bringing ashore
- Add 2 ml of **Reagent D – Conditioning Solution 2**
Pull into the spe column and discharge the eluate without bringing ashore

STEP 5 : Load 6 ml of the supernatant of calibrators, controls and unknown samples, prepared in the phase 3 in columns packed and download to a flow of 1 ml/minute (drop by drop)

STEP 6 : Wash of SPE Column

- Wash with 1 ml of **Reagent E – Wash Solution 1**
Pull into the spe column and discharge the eluate
- Wash with 3 ml of **Reagent F – Wash Solution 2**
Pull into the spe column and discharge the eluate, bringing dry with an air stream
- Wash with 2 ml of **Reagent C – Conditioning Solution 1 and Wash 3**
Pull into the spe column and discharge the eluate
- Wash with 1 ml of **Reagent G – Wash Solution 4**
Pull into the spe column and discharge the eluate, bringing dry with an air stream

ELUING and DERIVATIZATION OF ANALYTES

Elute rigorously in glass vials

STEP 7 : Elute with 1.5 ml of **Reagent H – Eluting Solution**. *Bringing dry with an air stream.*

Collect the eluate in **pyrex tubes with screw cap**

Bringing ashore the eluate with evaporator or nitrogen flow (Max Temperature 40°C)

STEP 8 : Add in each tubes 40 µl of **Reagent I – Derivatization Solution**

Seal the tube and Vortex for 30 sec.

Incubate at 70°C for 30 min

Cooling at Room Temperature

Open the glass vial

STEP 9 : Transfer the derivatized solution in vials with reduced volume and inject 2 µl in GC-MS

N.B: The sample is stable 24 hours at 2-8 C°

AMPHETAMINES IN WHOLE BLOOD - Warnings

REAGENT L: TEST SOLUTION AMPHETAMINES

Amphetamine Methamphetamine MDA MDMA MDE MBDB	Circa 10.000 ng/ml
Storage: After opening the vial, remove the volume needed, close it with unused reagent and store at -20° C.	

REAGENT I: DERIVATIZATION SOLUTION

Once you open an ampoule transfer all content, 1 ml, in a glass vial. Tighten cap and store at room temperature.

GAS-CROMATOGRAPHIC SYSTEM SET:

- Column Rxi-5Sil MS 30 m x 0,25 mm 0,25 um (conditioned)
- Injector Temperature 250 °C
- Split Ratio: ON split 20
0 min: OFF
1 min: ON split 100
3 min: ON split 20
- Temperature 110 °C x 3.25 minutes + 20 °C/min till up 250 °C x 1 minute + 40 °C/min till up 300 °C x 13.5 minutes (run 26 minutes)
- Flow in column (Helium): 1 ml/min

MASSA DETECTOR SET (4000):

- Massa Range: 70 – 540
- Massa Temperatura: 190 °C;
- Transfer Line Temperature: 270 °C;
- Manifold Temperature: 80 °C
- Filament on: 3 minutes - Filament off: 19 minutes

COLUMN CONDITIONING Rxi-5Sil MS 30 m x 0,25 mm 0,25 um

Follow the manufacturer's instructions. Not affect the column if attached to the Detector.

COLUMN AND SYRINGE CLEANING

Injecting ACETONE before and after the run. Wash the syringe prior to injection with ACETONE (4 times) and after injection with ACETONE (4 times). CHECK THE GOOD FUNCTIONALITY OF THE SYRINGE BEFORE EACH SESSION.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC89016	Calibrator in whole blood for Abuse Drugs	7 x 2 x 1 ml
LC89019	Control in whole blood for Abuse Drugs – Levels 1, 2 and 3	3 x 5 x 1 ml
S29057U	Glass Standard vials of 2 ml	1 x 100 Pcs
S29046U	Caps for Glass Standard vials of 2 ml	1 x 100 Pcs
S24717	Reduced volume for vials of 2 ml	1 x 100 Pcs
Z-1636/26	Pyrex tubes of 10 ml with caps SWL	1 x 40 Pcs
S13623	Restek Rxi-5SILms (30 m x 0,25 mm – 0,25 um) Column	1 Pc
ZRE24533	Hamilton Syringe of 50 ul	1 Pc

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

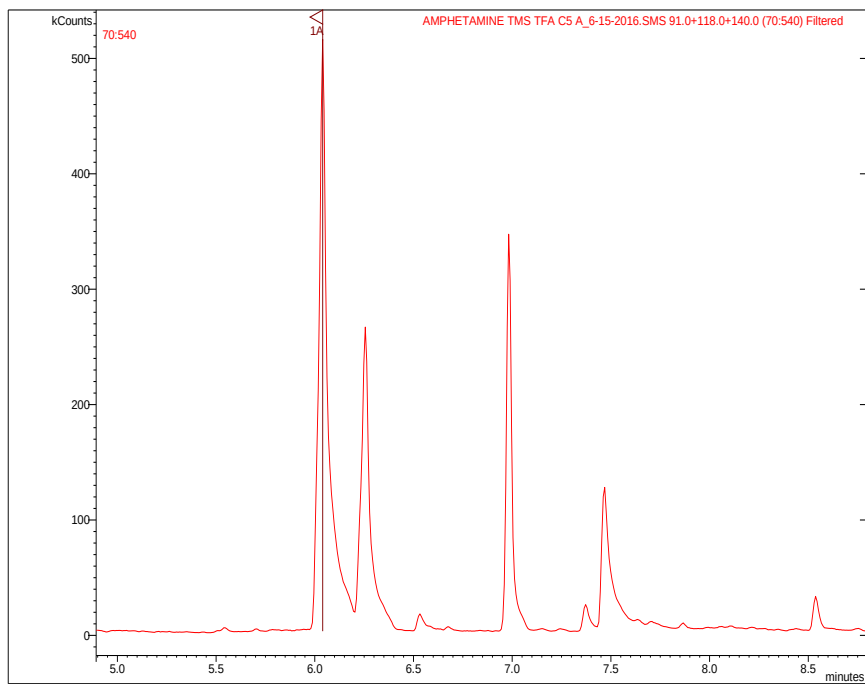


Figure 1: Amphetamine; RT 6.040

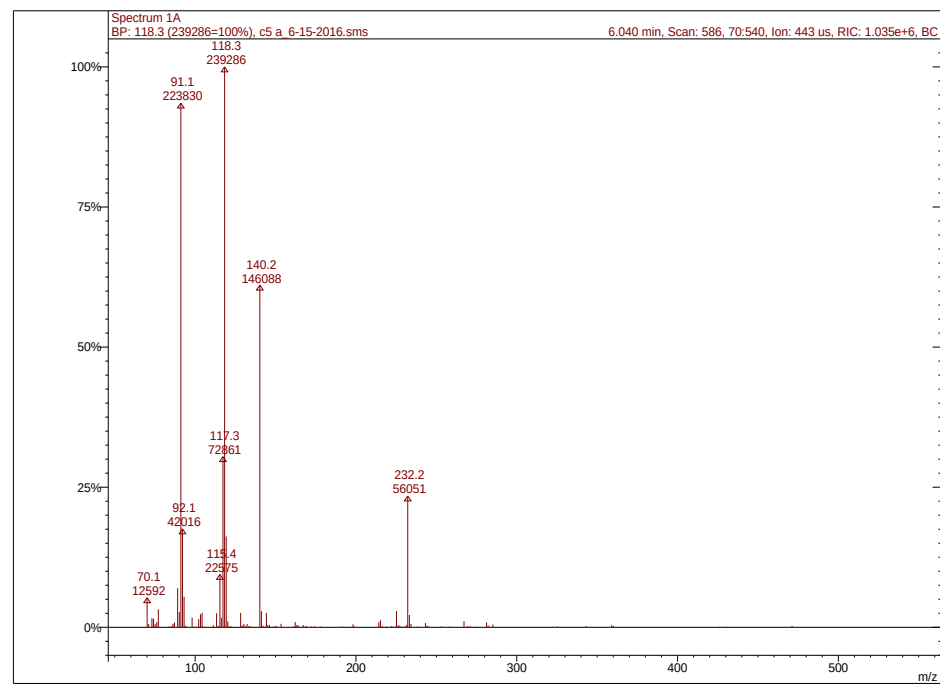


Figure 2 : Massa Spectrum Amphetamine; Molecular Ions 91- 118
- 140

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

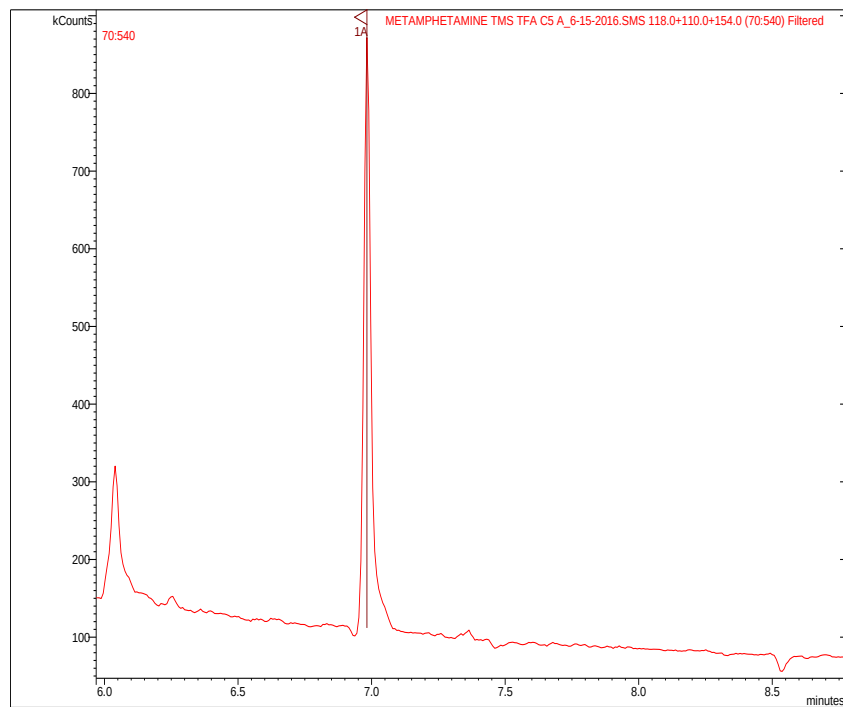


Figure 3 : Methamphetamine; RT 6.982

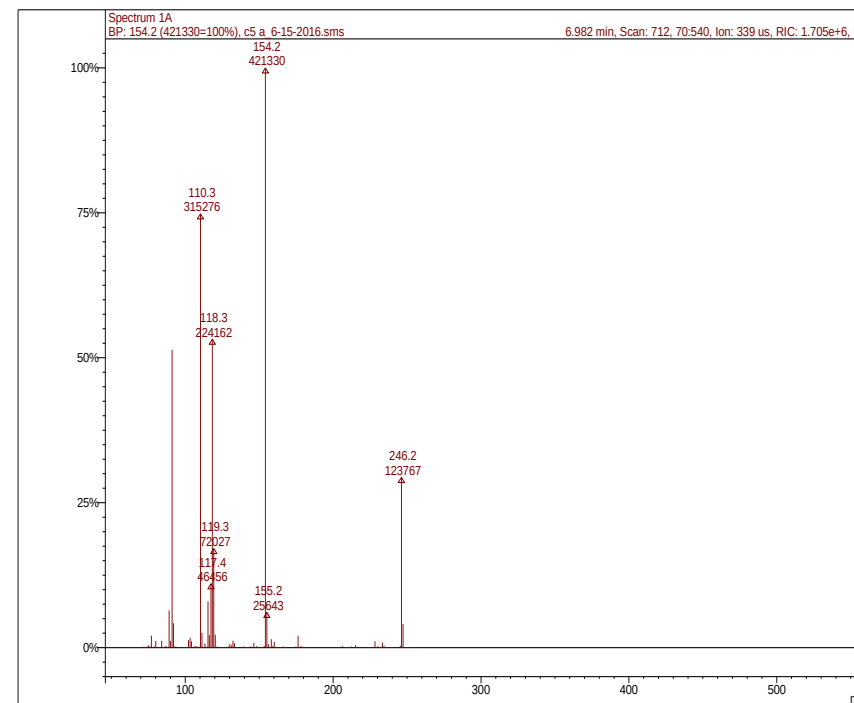


Figure 4 : Massa Spectrum Methamphetamine; Molecular Ions 110 - 118 - 154

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

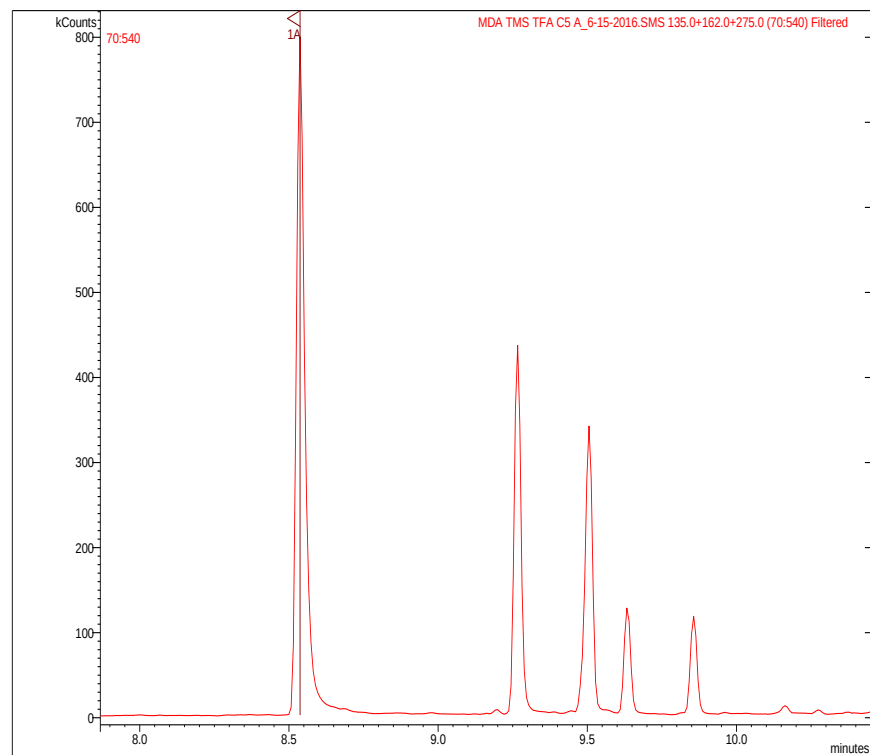


Figure 5 : MDA; RT 8.537

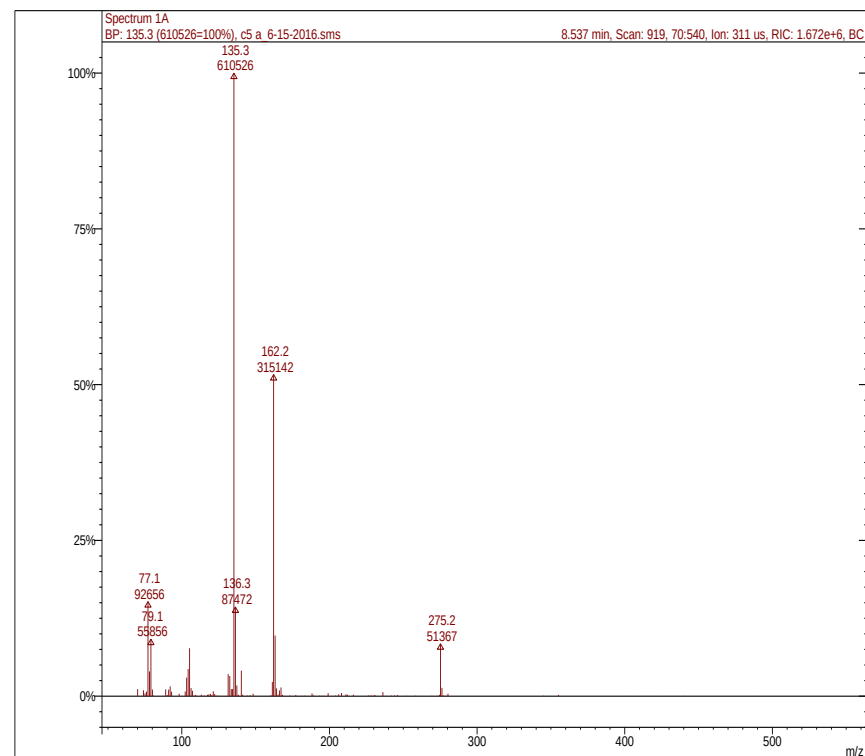


Figure 6 : Massa Spectrum MDA; Molecular Ions 135 - 162 - 275

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

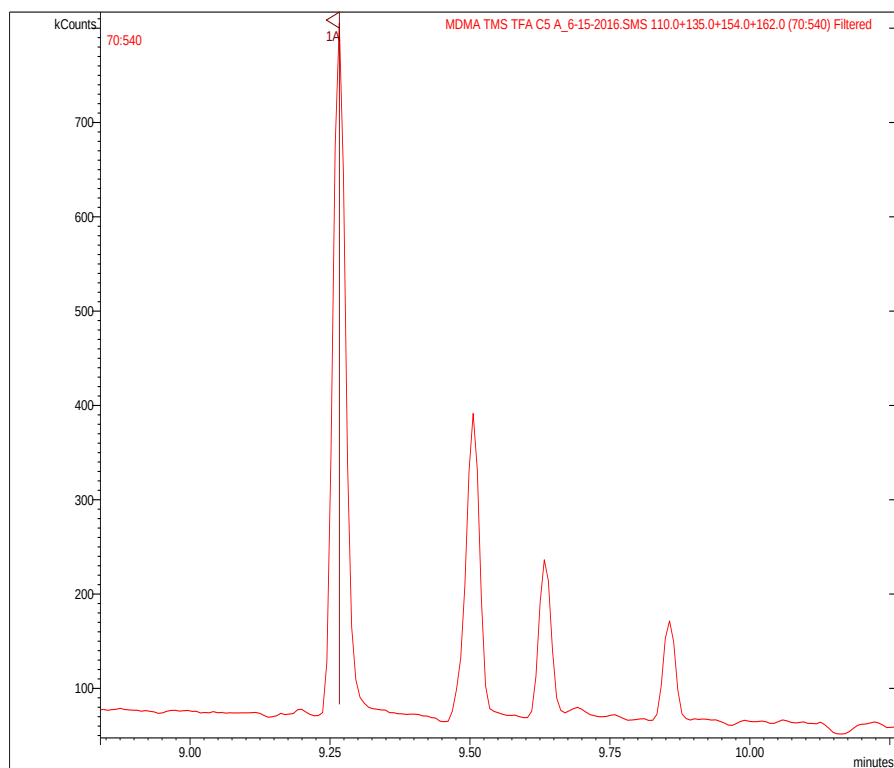


Figure 7 : MDMA; RT 9.267

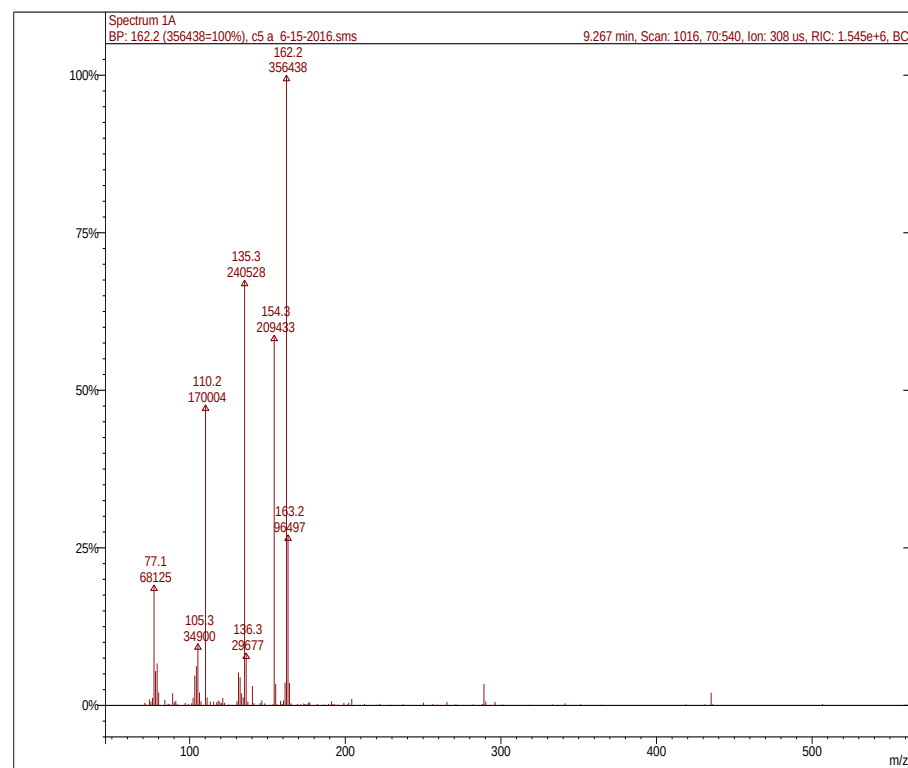


Figure8 : Massa Spectrum MDMA; Molecular Ions 110 - 135 - 154 - 162

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

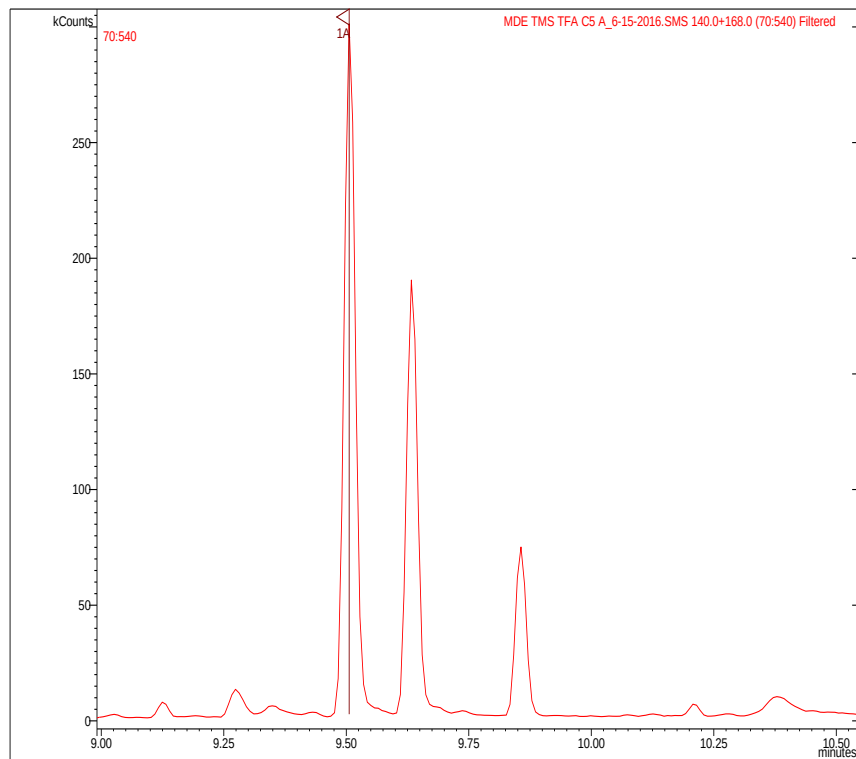


Figure 9 : MDE; RT 9.506

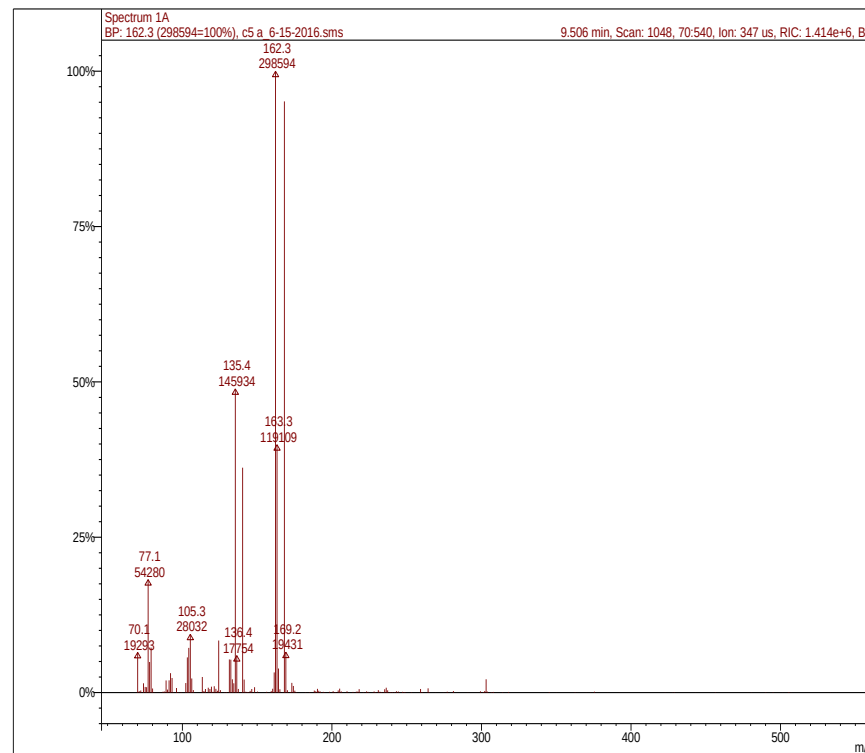


Figure 10 : Mass Spectrum MDE; Molecular Ions 135 - 140 - 162 - 168

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

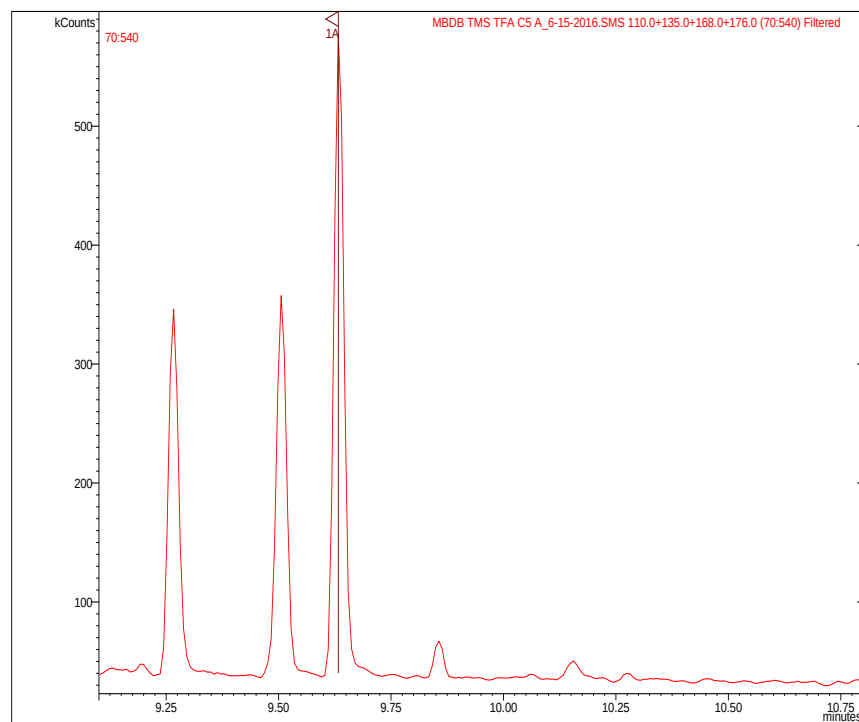


Figure 11 : MBDB; RT 9.633

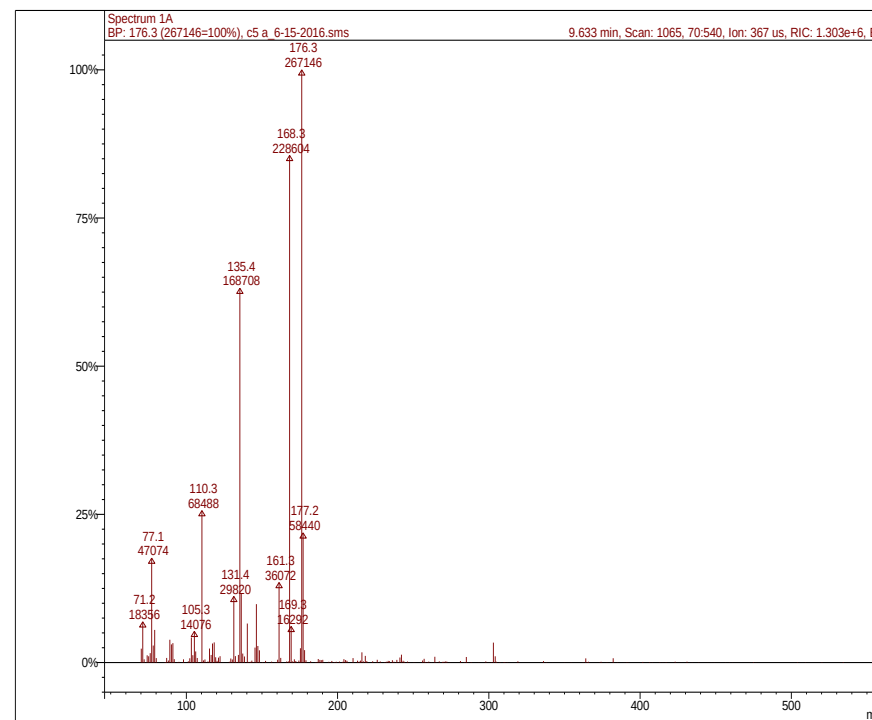


Figure 12 : Massa Spectrum MBDB; Molecular Ions 110 - 135 - 168 - 176

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

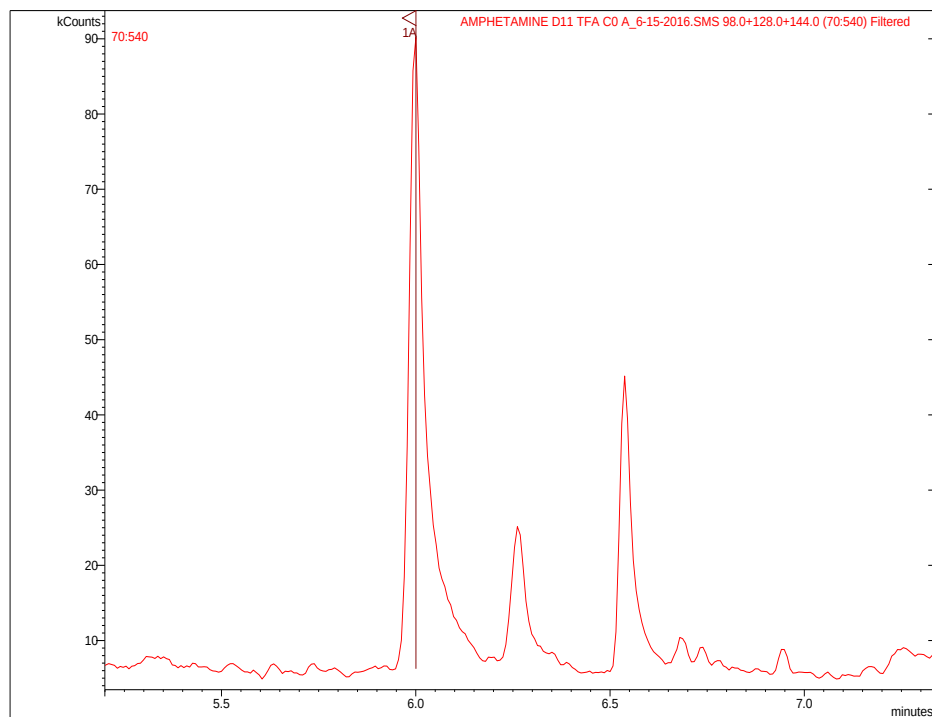


Figure 13 : Amphetamine D11 (deuterated IS); RT 6.000

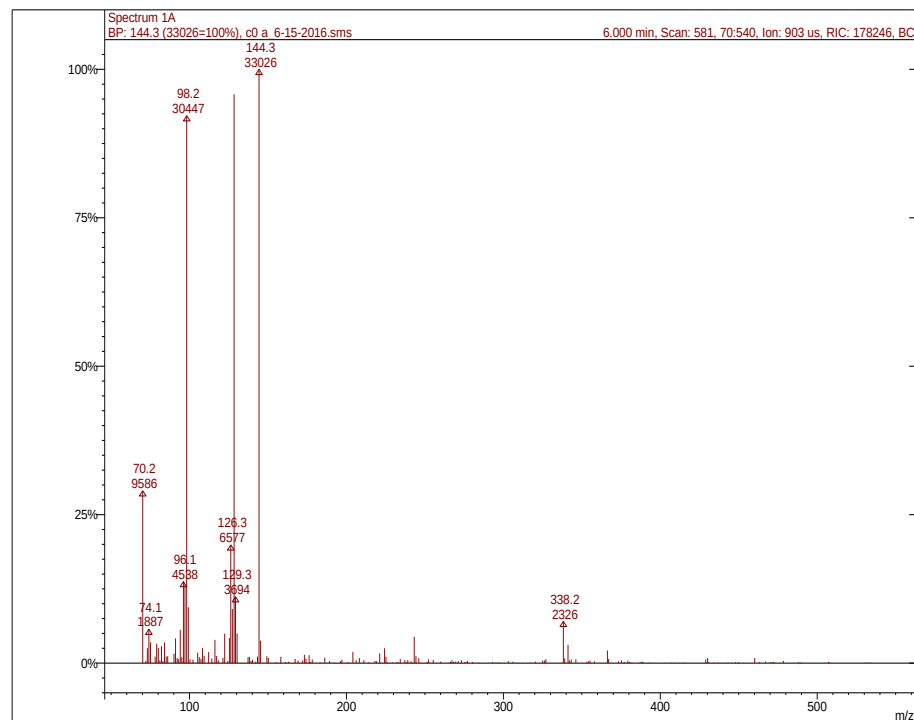


Figure 14 : Massa Spectrum Amphetamine D11 (deuterated IS);
Molecular Ions 98 - 128 - 144

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

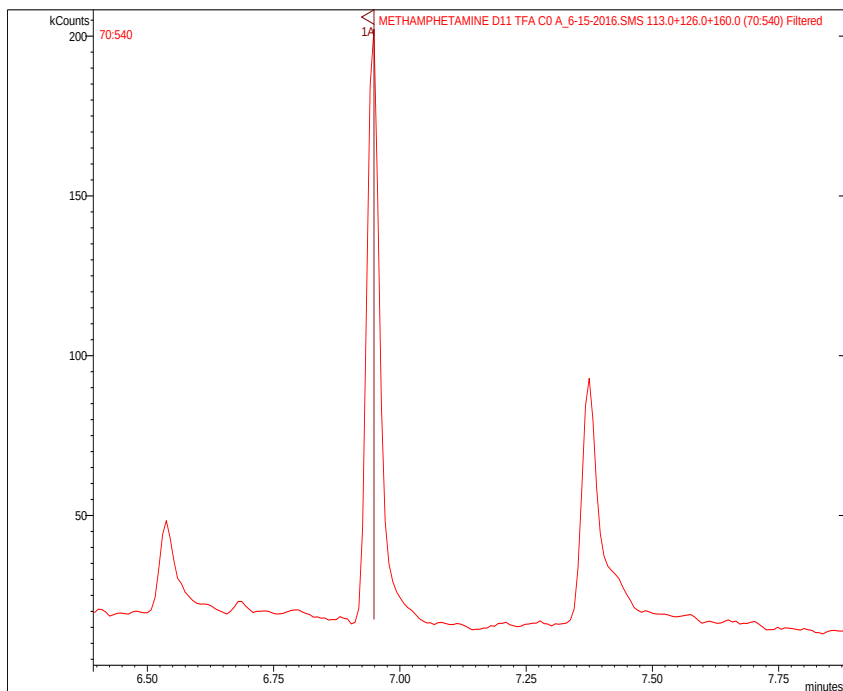


Figure 15 : Methamphetamine D3 (deuterated IS); RT 6.949

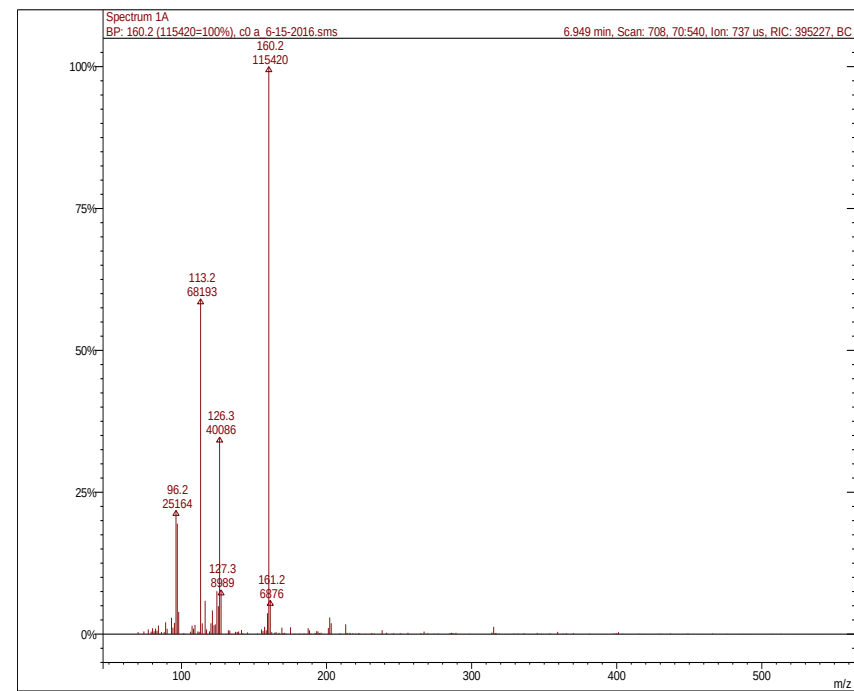


Figure 16 : Massa Spectrum Methamphetamine D3 (deuterated IS); Ioni molecolari 113 - 126 - 160

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

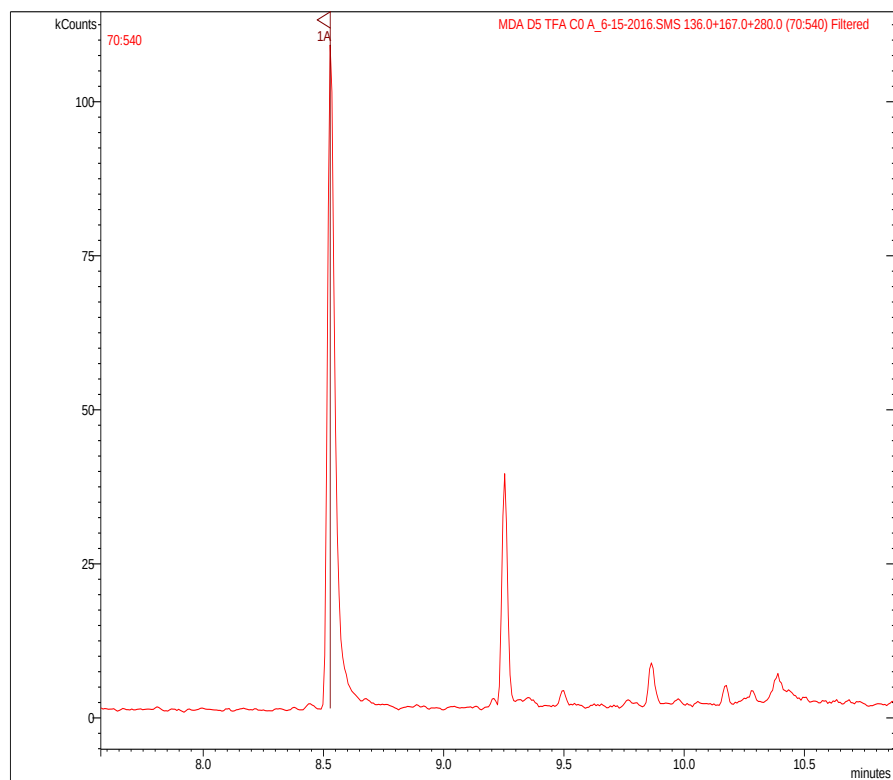


Figure 17 : MDA D5 (deuterated IS); RT 8.528

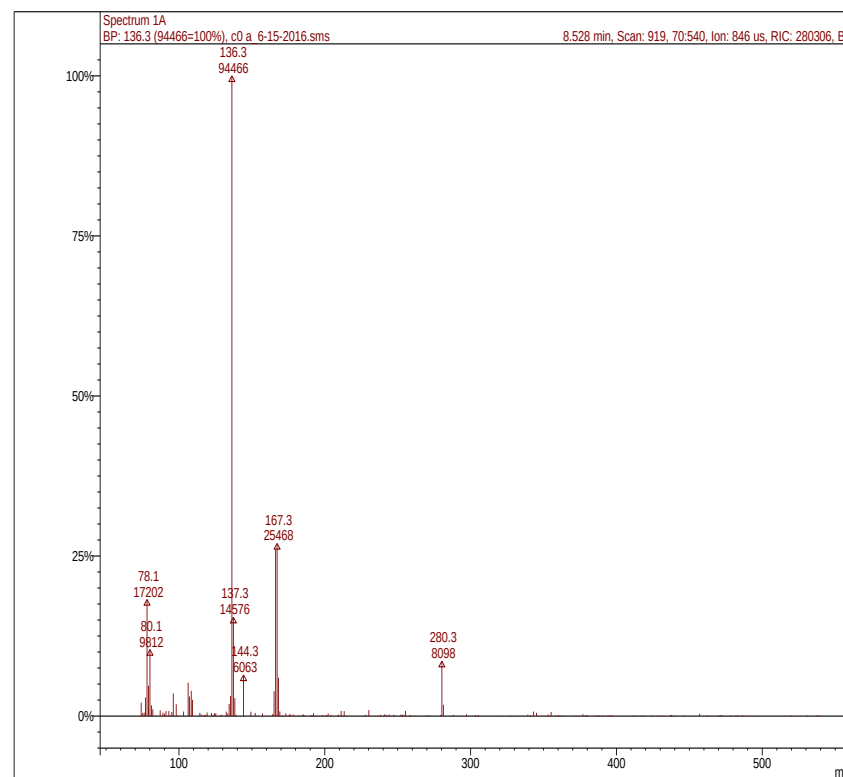


Figure 18 : Massa Spectrum MDA D5 (deuterated IS);
Molecular Ions 136 - 167 - 280

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

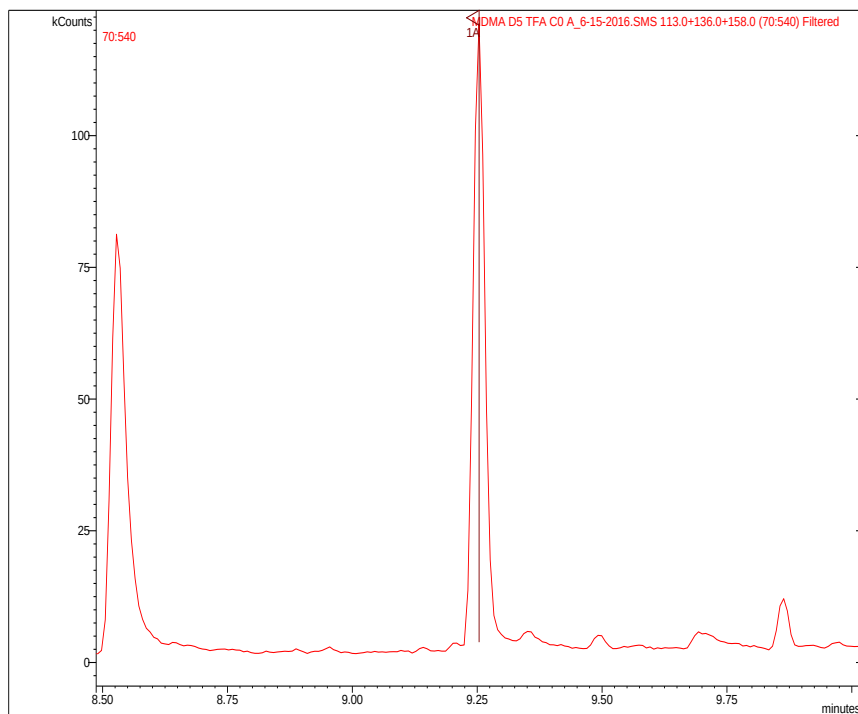


Figure 19 : MDMA D5 (deuterated IS); RT 9.254

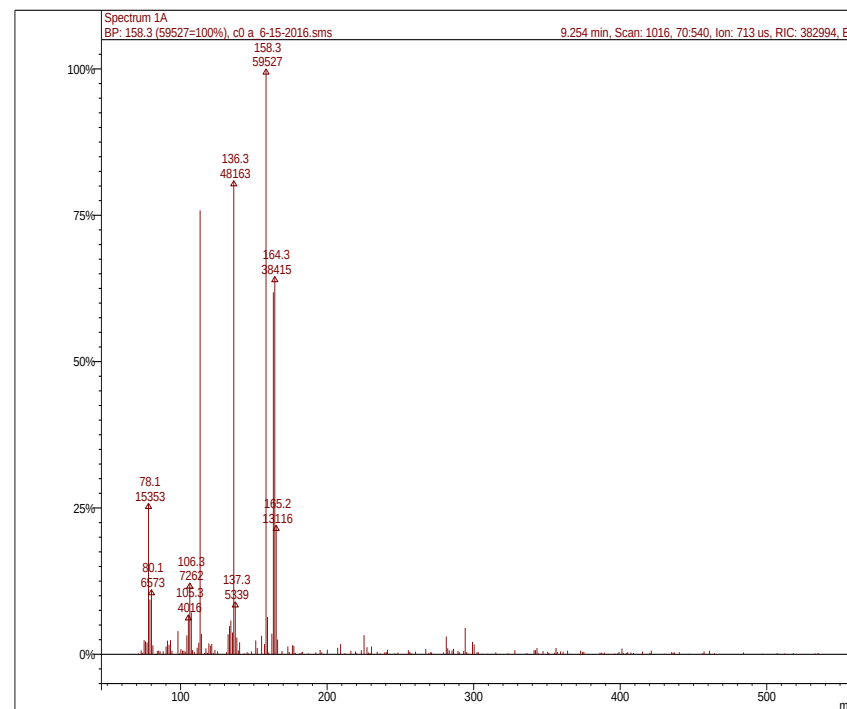


Figure 20 : Massa Spectrum MDMA D5 (deuterated IS); Molecular Ions 113 - 136 - 158

ABUSE DRUGS IN WHOLE BLOOD

(Reference Chromatograms/Spectra)

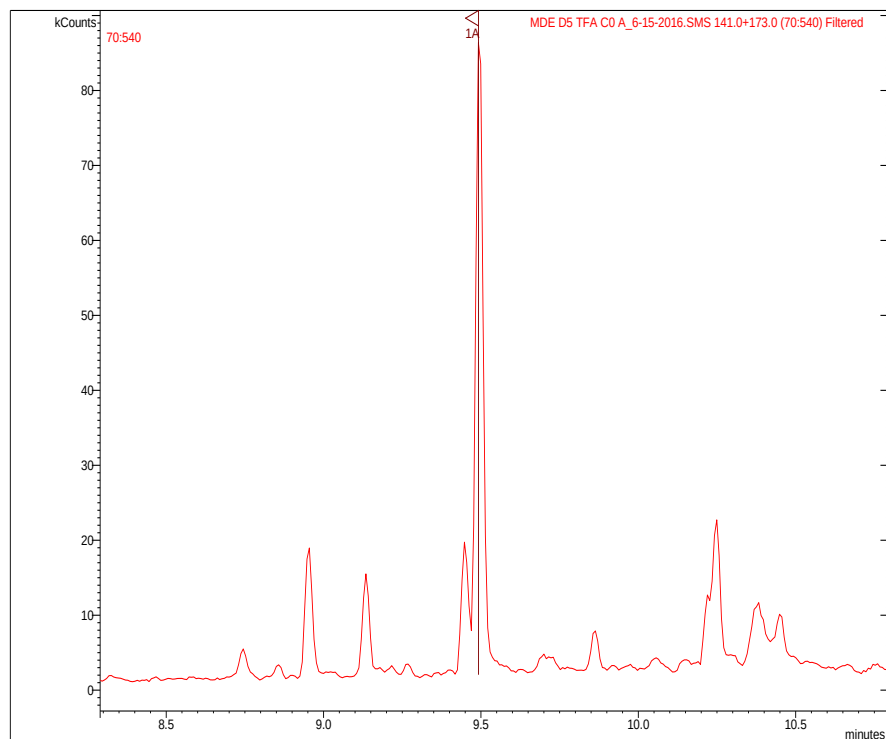


Figure 21 : MDE D5 (deuterated IS); RT 9.492

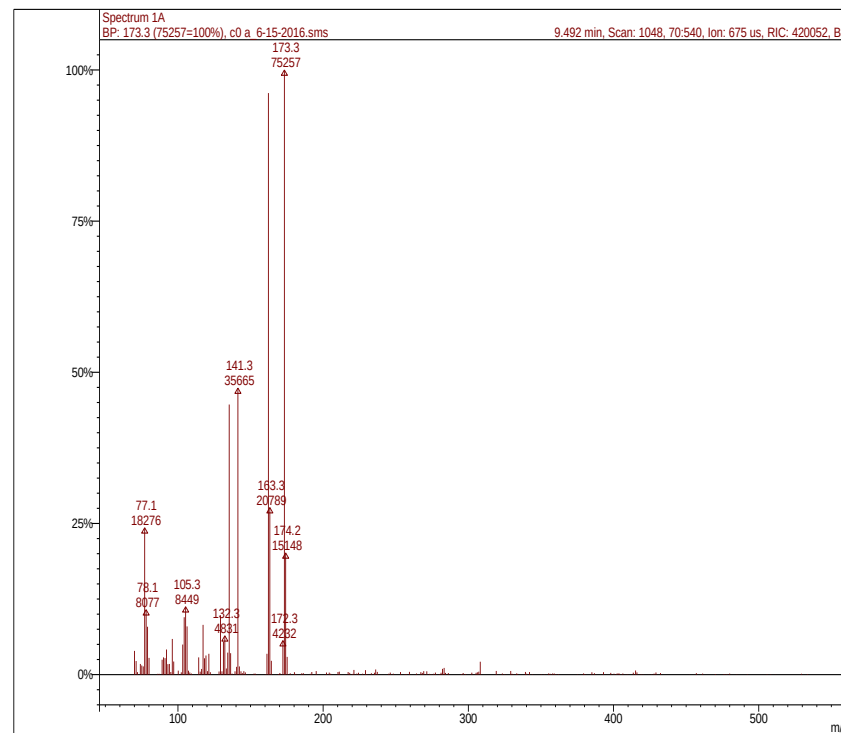


Figure 22 : Massa Spectrum MDE D5 (deuterated IS); Molecular Ions 141 - 173