

CANNABINOIDS in whole blood by GC-MS – Code GC90010

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 Delta-9-THC, Delta-9-THC-COOH and 11-OH-THC by GC-MS.



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

Principle of the Method:

The present method consent to determinate Cannabinoids 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.

Recovery : >90 %

Sensitivity (LLOD) : 0,008 ng/ml

Minimum concentration analyzable (LLOQ) : 0,025 ng/ml

Linearity : 0,025 – 2.500 ng/ml

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 1,5 ng/ml	Cm 5 ng/ml	Cs 20 ng/ml	Ci 1,5 ng/ml	Cm 5 ng/ml	Cs 20 ng/ml	Ci 1,5 ng/ml	Cm 5 ng/ml	Cs 20 ng/ml
Delta-9-THC	4,6	10,5	4,5	4,6	1,7	2,7	7,3	12,7	6,3
11-OH-THC	7,5	5,0	4,8	10,4	2,1	2,8	10,4	6,8	8,3
Delta-9-THC-COOH	3,1	5,3	4,9	1,9	3,6	3,8	8,5	7,3	7,3

ANALYTE	Recovery (%)		
	Calibrator		
	Low	Medium	High
Delta-9-THC	90,9	96,8	89,2
11-OH-THC	80,1	94,5	92,8
Delta-9-THC-COOH	80,5	84,1	84,9

ANALYTE	(Coefficient of correlation R2) $\pm$ DS	LOD (ng/ml)	LOQ (ng/ml)
Delta-9-THC	0,9992 $\pm$ 0,0007	0,16	0,5
11-OH-THC	0,9991 $\pm$ 0,0006	0,16	0,5
Delta-9-THC-COOH	0,9996 $\pm$ 0,0002	0,16	0,5

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-Methylenedioxymphetamine 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.

Components of the kit (50 test) :

All the reagents are ready to use and stable 3 years at 2 – 8 °C, except **Reagent A**.

Reagent A – Deuterated Internal Standards, **1 x 750 ul**

Store at -20 °C

Reagent B – Extraction Solution, **1 x 300 ml**

Reagent C – Stabilizing Solution, **1 x 13 ml**

Reagent D – Derivatization Solution, **4 x 1000 ul**

See Warnings

Reagent E – Test Solution THC, **1 x 500 ul**

See Warnings

Calibrator in whole blood – Level 0, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 1, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 2, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 3, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 4, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 5, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

Calibrator in whole blood – Level 6, 3 x 1 ml

Code LC89016 (packed separately – see data sheet)

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 E – Test Solution THC**

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 40 µl of **Reagent D – 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)	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
Reagent B – Extraction Solution	5000 µl	5000 µl	5000 µl	5000 µl	5000 µl	5000 µl	5000 µl	5000 µl	5000 µl
Reagent C – Stabilizing Solution	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl

Seal the tubes and at Vortex for 30 sec.

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

STEP 3 : Transfer the supernatant in a **clean pyrex of 10 ml with screw cap.**

Bringing ashore the eluate with evaporator or nitrogen flow (Max Temperature 50°C)

DERIVATIZATION OF ANALYTES

STEP 4 : Add in each tubes 40 µl of **Reagent D – 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 5 : 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°

CANNABINOIDS in whole blood - Warnings

REAGENT E : TEST SOLUTION

Delta-9-THC Delta-9-THC-COOH 11-OH-THC	About 10.000 ng/ml
Storage: After opening the vial, remove the volume needed, close it with unused reagent and store at -20° C.	

REAGENT D: DERIVATIZATION SOLUTION

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

GAS-CROMATOGRAFIC 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 130 °C x 2 minutes + 20 °C/min till up 300 °C x 5 minutes (run 15.5 minutes)
- Flow in column (Helium): 1 ml/min

SETTAGGIO DETECTOR DI MASSA (4000) :

- Massa Range: 180 – 520
- Massa Temperatura: 190 °C;
- Transfer Line Temperature: 270 °C;
- Manifold Temperature: 80 °C
- Filament on: 5 minutes - Filament off: 15.40 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

CODICE	DESCRIZIONE	CONFEZIONE
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

CANNABINOIDS in whole blood

(Reference Chromatograms/Spectra)

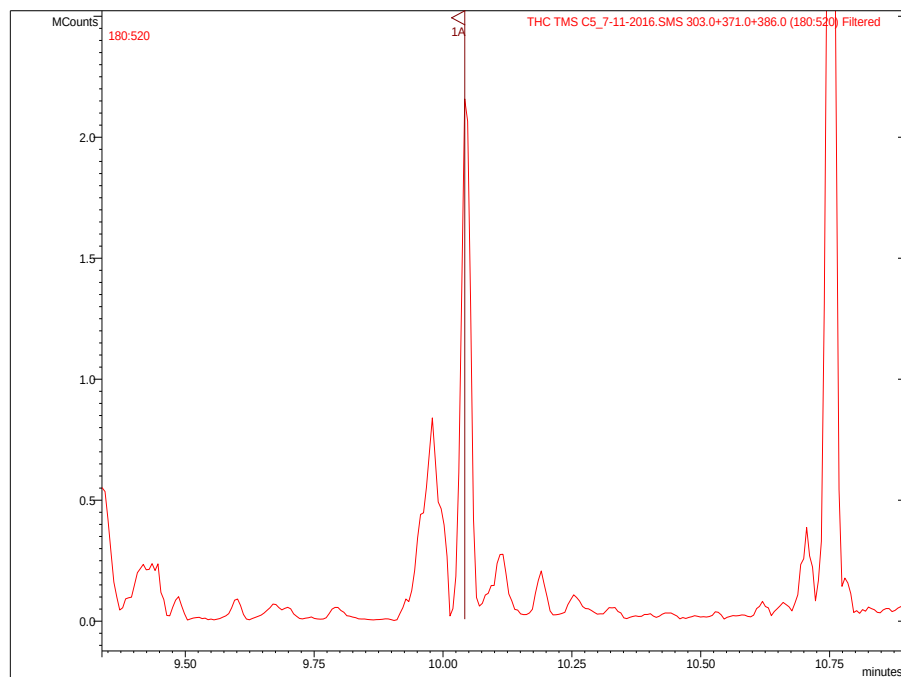


Figure 1: THC; RT 10.042

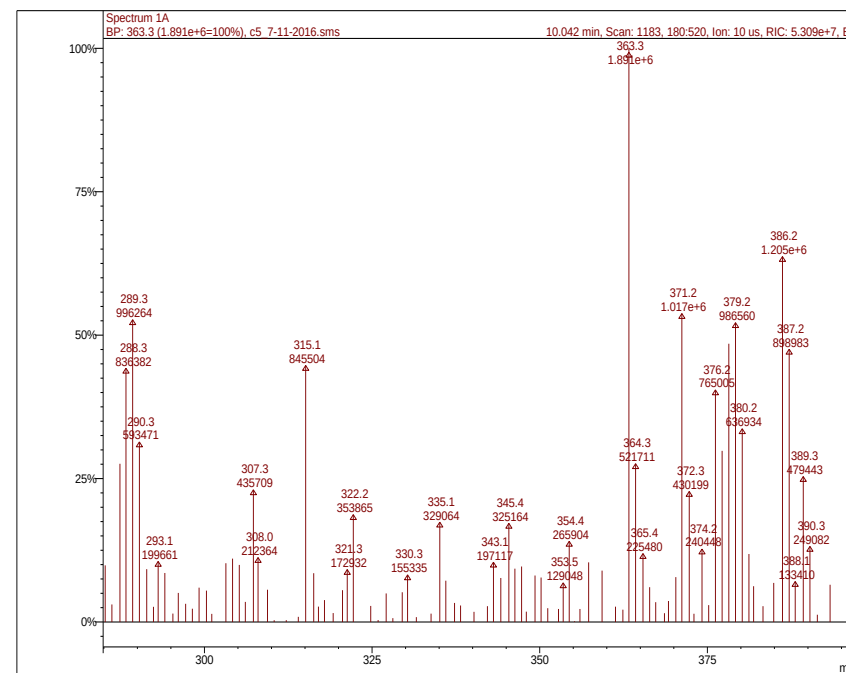


Figure 2: Massa Spectrum THC; Molecular Ions 303 - 371 - 386

CANNABINOIDS in whole blood

(Reference Chromatograms/Spectra)

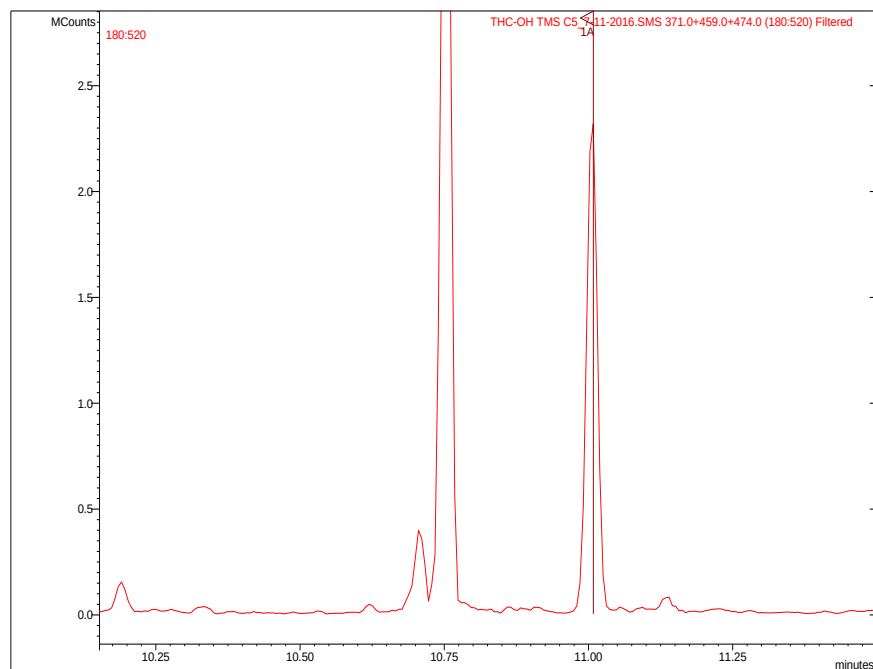


Figure 3: OH-THC; RT 11.008

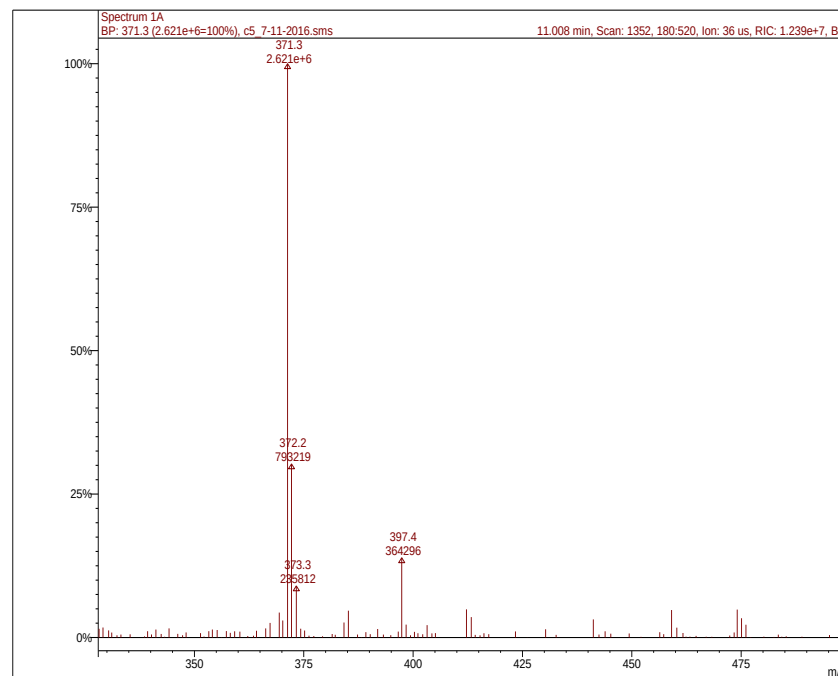


Figure 4: Massa Spectrum OH-THC; Molecular Ions 371 - 459 - 474

CANNABINOIDS in whole blood

(Reference Chromatograms/Spectra)

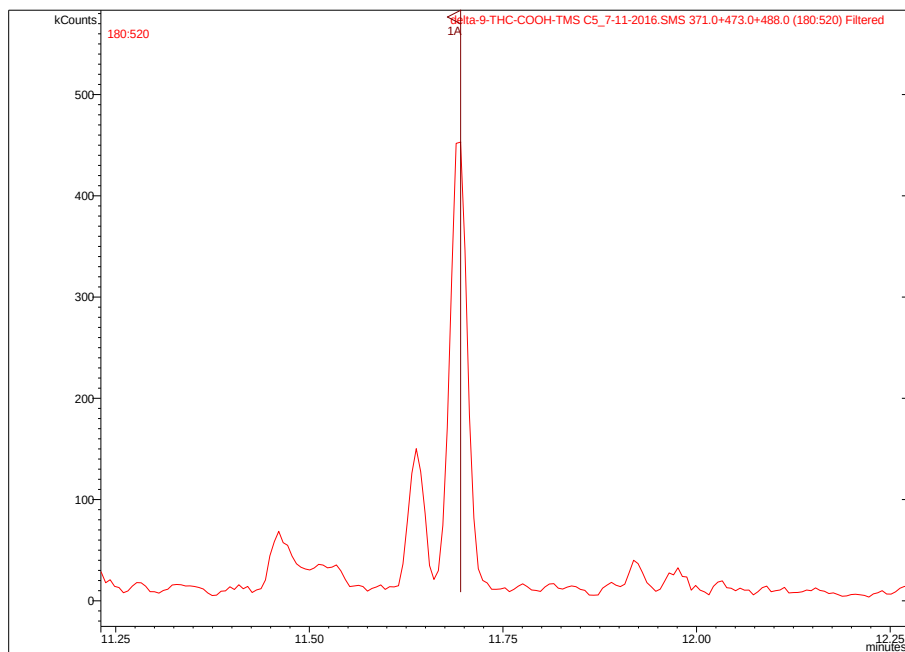


Figure 5: THC-COOH; RT 11.695

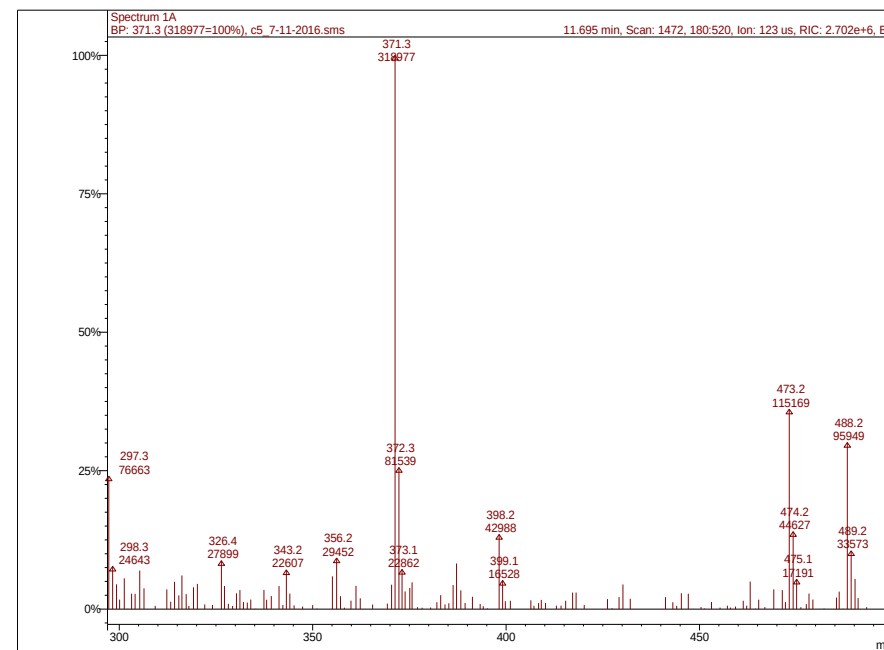


Figure 6: Mass Spectrum THC-COOH; Molecular Ions
371 - 473 - 488

CANNABINOIDS in whole blood

(Reference Chromatograms/Spectra)

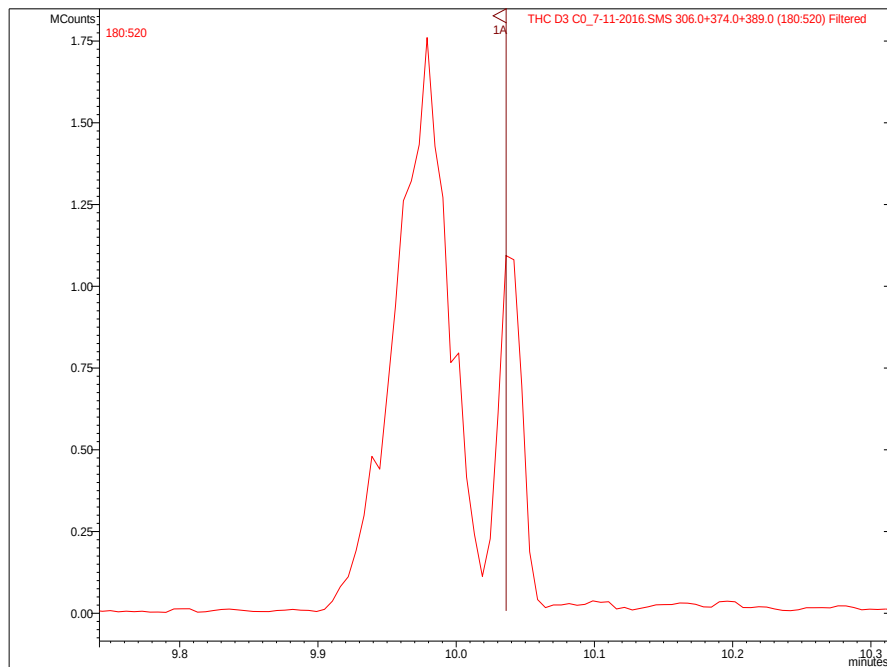


Figure 7: THC D3 (deuterated IS); RT 10.036

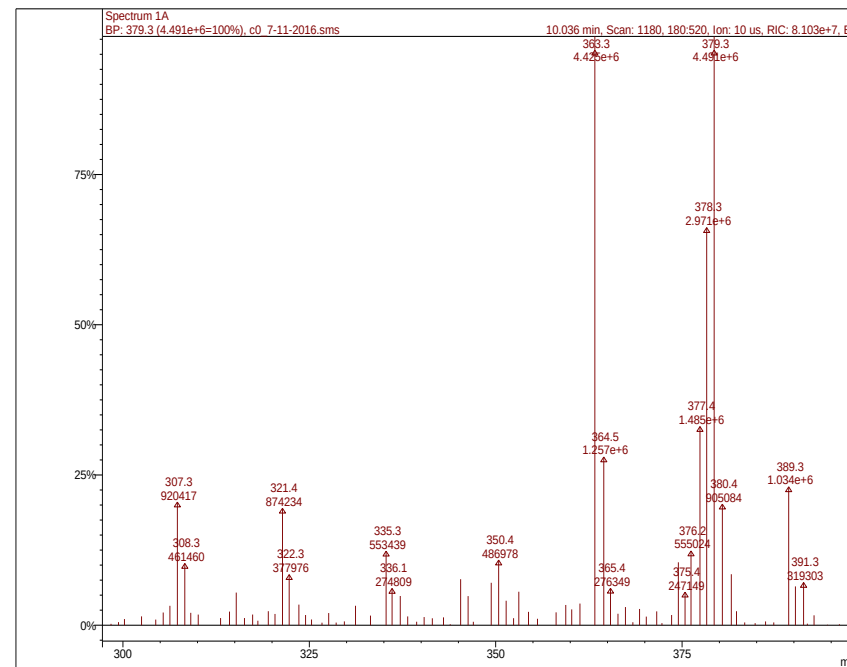


Figure 8: Mass Spectrum THC D3 (deuterated IS);
Molecular Ions 306 - 374 - 389

CANNABINOIDS in whole blood

(Reference Chromatograms/Spectra)

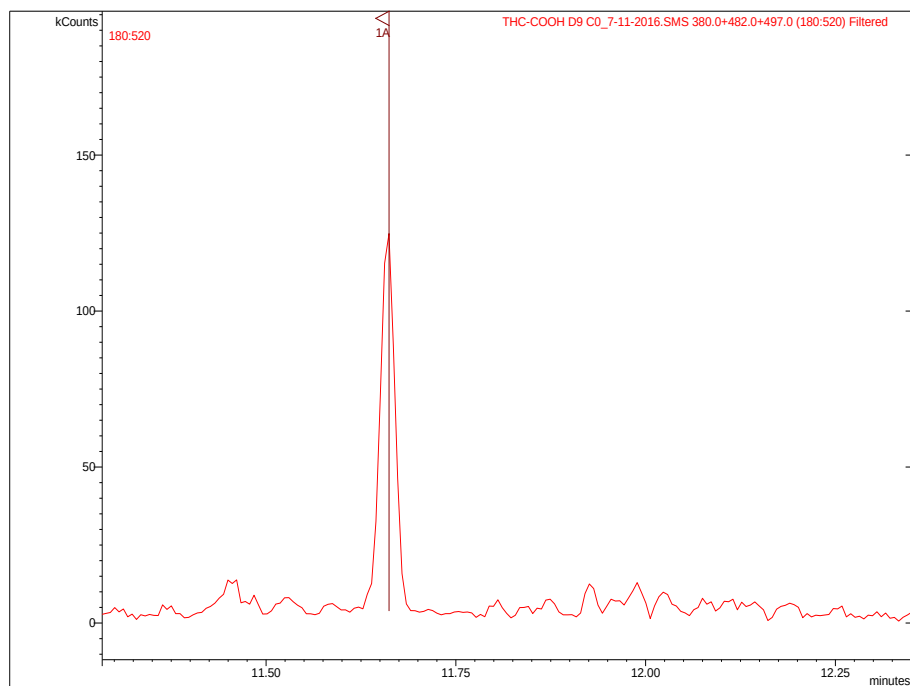


Figure 9: THC-COOH D9 (deuterated IS); RT 11.662

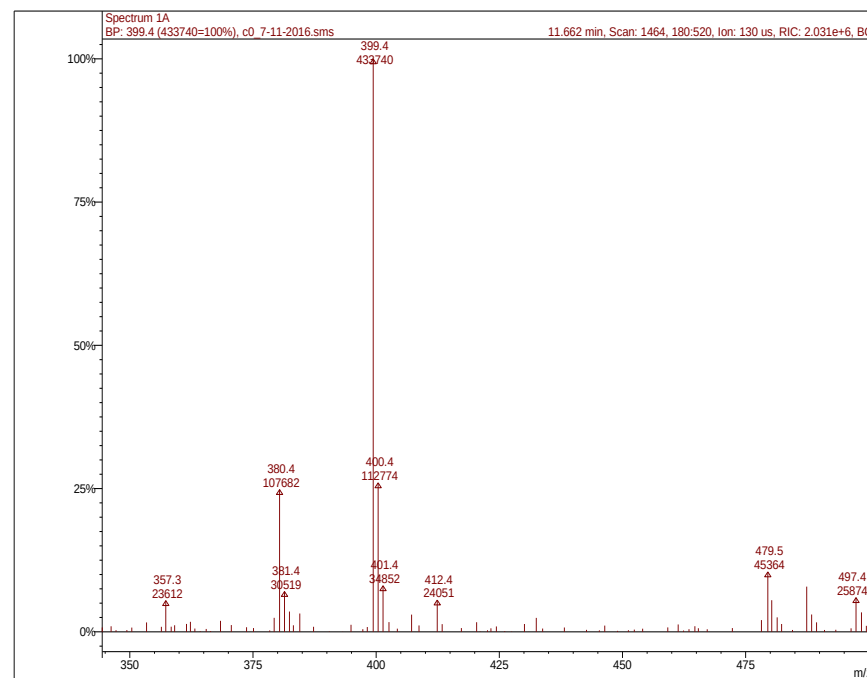


Figure 10: Massa Spectrum THC-COOH D9 (deuterated IS); Molecular Ions 380 - 482 - 497