

Δ^9 - TETRAHYDROCANNABIN-COOH in urine by GC/MS – Code GC47010

Method of Confirmation by GC-MS

INTRODUCTION

Δ^9 -THC-COOH is the main metabolite of Cannabis. Screening in biological fluids (primarily in urine) is generally performed 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 both Gas (GC) and Liquid (LC), both of them coupled with specific and selective detector (UV, Fluorescence, MS).

The present method allows the determination of Δ^9 -THC-COOH in urine after SPE extraction and reading in GC-MS.



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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° 015	THC in urine by GC/MS	08/2024
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TECHNICAL FEATURES

Principle of the Method :

This method allows to determinate the Δ^9 - **Tetrahydrocannabinin-COOH** (the main metabolite of Cannabis) after SPE extraction and reading in GC-MS.

Recovery: > 90 %

Sensitivity: 2 ng/ml

Cut-off: 15 ng/ml

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

Dynamic Range of the Method: 2 – 2.000 ng/ml

LOD: 1 ng/ml

LOQ: 3 ng/ml

Components of the kit :

All the components are ready-to-use. Stability: 3 years at 2 – 8 °C.

Reagent A – Hydrolysis Reagent, 1 x 20 ml

Reagent B – Conditioning Solution 1, 1 x 100 ml

Reagent C – Conditioning Solution 2, 1 x 100 ml

Reagent D – Washing Solution, 1 x 150 ml

Reagent E – Eluting Solution, 1 x 25 ml

Reagent F – Diluting Solution, 1 x 25 ml

Reagent G – Test Solution, 1 x 0,5 ml

See Warnings

Reagent H – Internal Standard Solution, 1 x 200 µl

To dilute 1:100 in H₂O before use

Urine Calibrator lyophil – Level 1, 2 x 10 ml

Code CC47016 (packed separately – see data sheet)

Urine Calibrator lyophil – Level 2, 2 x 10 ml

Code CC47016 (packed separately – see data sheet)

Urine Calibrator lyophil – Level 3, 2 x 10 ml

Code CC47016 (packed separately – see data sheet)

Urine Calibrator lyophil – Level 4, 2 x 10 ml

Code CC47016 (packed separately – see data sheet)

Urine Calibrator lyophil – Level 5, 2 x 10 ml

Code CC47016 (packed separately – see data sheet)

Reagent M – Derivatization Solution, 3 x 1 ml (3 ampullas of 1 ml)

See Warnings

Clean-up Columns, 50 pcs

Minimum Instrumental equipment required:

GC-MS Instrument
Operational Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:

Take 10 ml of urine

Preanalytical Phase:

If you don't analyze immediately, store at -20 °C.
Stable for about 3 months.

PREANALYTICAL PROCEDURE:

STEP 1 : In a glass-test tube with teflon cap pipette :

- 450 µl of **Reagent F – Diluting Solution**
- 50 µl of **Reagent G – Test Solution**

Vortex for 20 seconds

STEP 2 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 3 : Put in 50 µl of **Reagent M - Derivatization Solution**

Vortex for 20 seconds

STEP 4: Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 5 : Transfer the whole sample in vials goblet form

NOTE : Before starting the analytical session it would be better to inject 2 µl of this solution in GC to identify the time of retention and the massa spectrum of Δ^9 - Tetrahydrocannabin-COOH, that must be similar to that shown in Fig. below. If the Test has given positive result, proceed with the analytical session. If it wouldn't be so, verify the functionality of the analytical system.

ANALYTICAL PROCEDURE:

STEP 1 : Hydrolysis of the sample

N.B: Dilute the **Reagent H – Internal Standard Solution** 1:100 with H₂O, preparing the sufficient sample size to cover the assay.

N.B.: this dilution is prepared fresh for each run

Make a Calibration Curve on 5 points:

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Calibrator – Level 1	3000 µl						
Calibrator – Level 2		3000 µl					
Calibrator – Level 3			3000 µl				
Calibrator – Level 4				3000 µl			
Calibrator – Level 5					3000 µl		
Sample						3000 µl	
Controls							3000 µl
Reagent H – Internal Standard (diluted 1:100 with H ₂ O HPLC grade)	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl
Reagent A – Hydrolysis Reagent	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl

Vortex for 20 seconds

STEP 2 : Incubate at 60 °C for 20 minutes.

STEP 3 : Bring the samples to neutral pH (5<pH<7) with Acetic acid.

STEP 4 : Conditioning of the Clean-up columns

- Condition clean up columns by fluxing 2 ml of **Reagent B – Conditioning Solution 1**
Discharge the percolated liquid
- Condition clean up columns by fluxing 2 ml of **Reagent C – Conditioning Solution 2**
Discharge the percolated liquid

STEP 5 : Dispense all the samples, previously prepared, into clean up columns. Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml / min (drop by drop).

STEP 6 : Clean-up Columns Wash

- Wash with 3 ml of **Reagent D – Washing Solution**
Discharge the percolated liquid and dry with a light flux of air

STEP 7 : Eluting in glass tubes with teflon caps only

- Elute with 500 µl of **Reagent E – Eluting Solution**
Gather the eluate in glass test tubes and agitate in vortex

STEP 8 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 9 : Take again 50 µl of **Reagent M - Derivatization Solution**

Vortex for 20 seconds

STEP 10 : Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 11 : Transfer the whole sample in vials goblet form

INJECTION :

- Inject 2 µl of the solution in the GC

Δ^9 - TETRAHYDROCANNABIN COOH in urine- WARNINGS

REAGENT G: TEST SOLUTION

Δ^9- Tetrahydrocannabinin-COOH	about 10.000 ng/ml
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REAGENT M: DERIVATIZATION SOLUTION

Once opened an ampulla, transfer the whole content, 1 ml, in a glass vial. Close carefully the cap and store at Room Temperature.

SET OF GAS-CROMATOGRAPH:

- VF-5ms Column 30 m x 0,25 mm, 0,25 μ m (conditioned)
- Injector's Temperature 280 °C
- Split Report Start: ON split 20
 - 0 min: OFF
 - 1 min: ON split 100
 - 3 min: ON split 20
- Temperature 120 °C x 3,25 minutes + 20 °C/min up to a 250 °C x 1 minute + 40 °C/min up to a 300 °C x 3 minutes (run 15 minutes)

SET OF MASSA DETECTOR (4000):

- Range of Massa 50 – 500
- Temperature of Massa: 180 °C;
- Temperature Transfer Line: 270 °C;
- Temperature Manifold: 80 °C
- Filament on: 3 minutes

CONDITIONING OF VF-5 MS COLUMN and SYRINGE

Before use wash the syringe with ACETONE.
Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.

CLEANING OF THE COLUMN

Disconnect the detector. Keep the column at the highest temperature for the recommended time.
(See the manufacturer 's instructions)

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC47016	Calibrator for THC in urine	2 x 5 x 10 ml
CC47019	Control for THC in urine – Levels 1 and 2	2 x 6 x 10 ml
ZRE13623	Rxi-Sil 5MS (30 m x 0,25 mm) ID, DF=0,25 Capillary Column	1 Pc
SCP8944	VF-5ms (30 m x 0,25 mm) ID DF=0,25 Column	1 Pc
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

Δ^9 -TETRAHYDROCANNABIN-COOH
(Reference Chromatograms/Spectrums GC-MS)

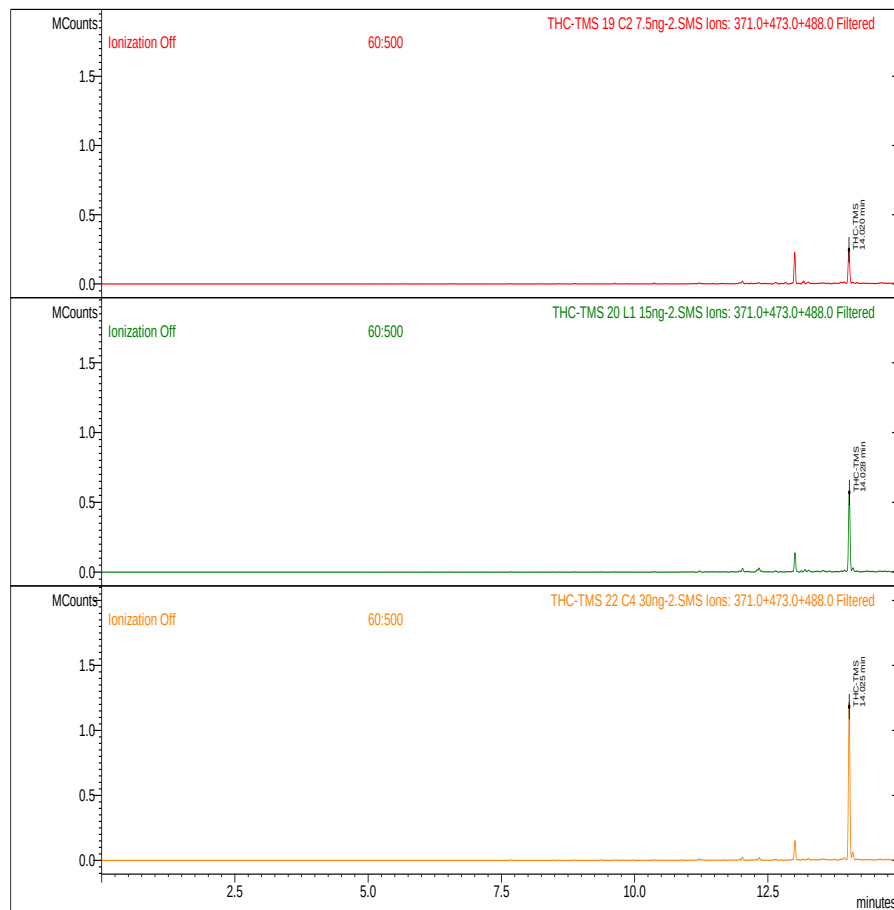


Fig. 1 :	Urine Samples
	R.T. 14.00 THC 7,5 – 15 – 30 ng/ml

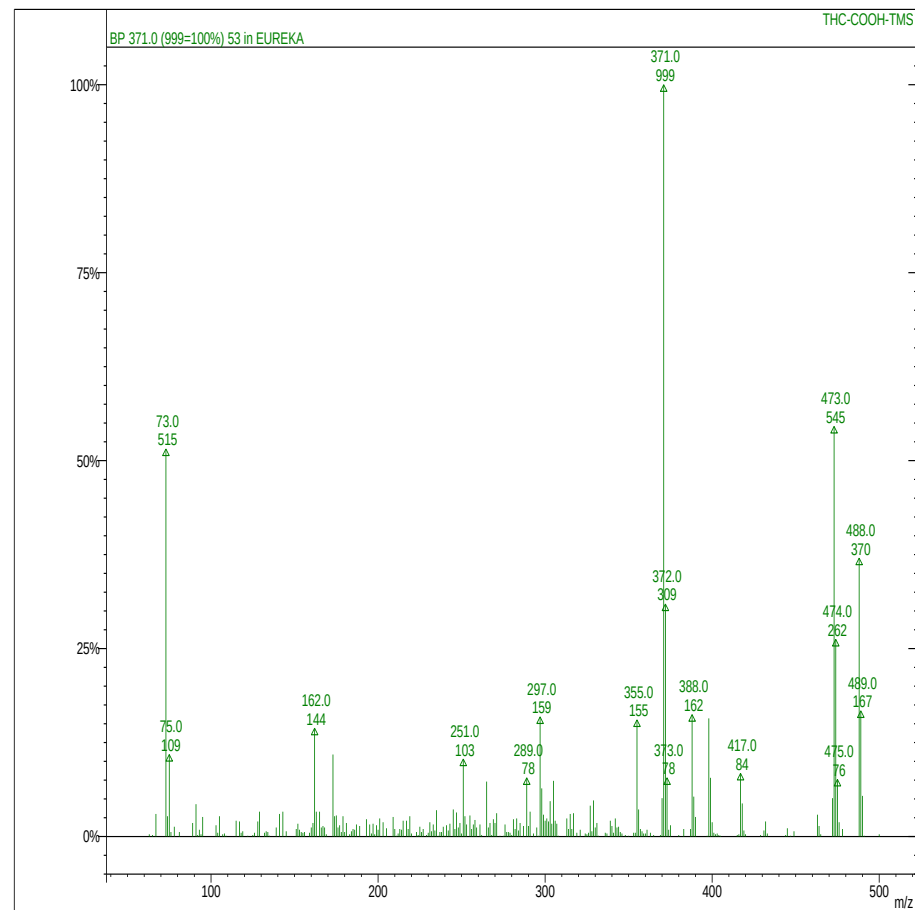


Fig. 2 :	Massa Spectrum of THC
	MOLECULAR IONS : 371 – 473 - 488

Δ^9 -TETRAHYDROCANNABIN-COOH
(Reference Chromatograms/Spectrums GC-MS)

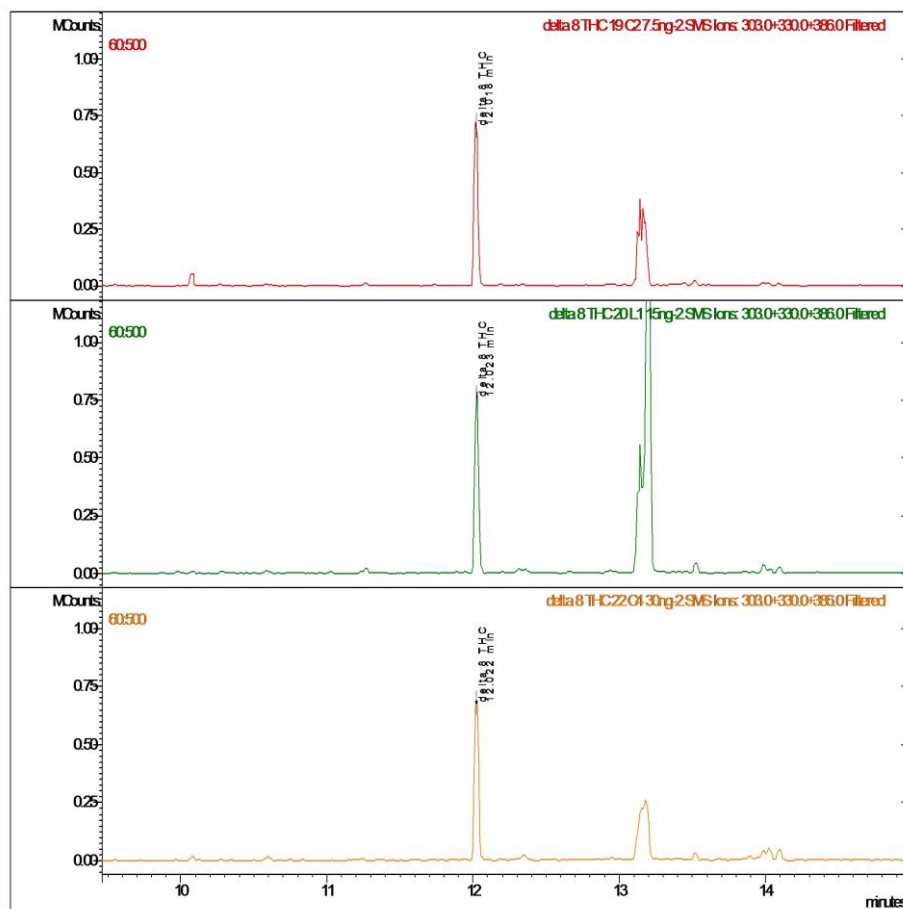


Fig. 3 :	Urine Samples with Internal Standard
	R.T. 12.00 Internal Standard

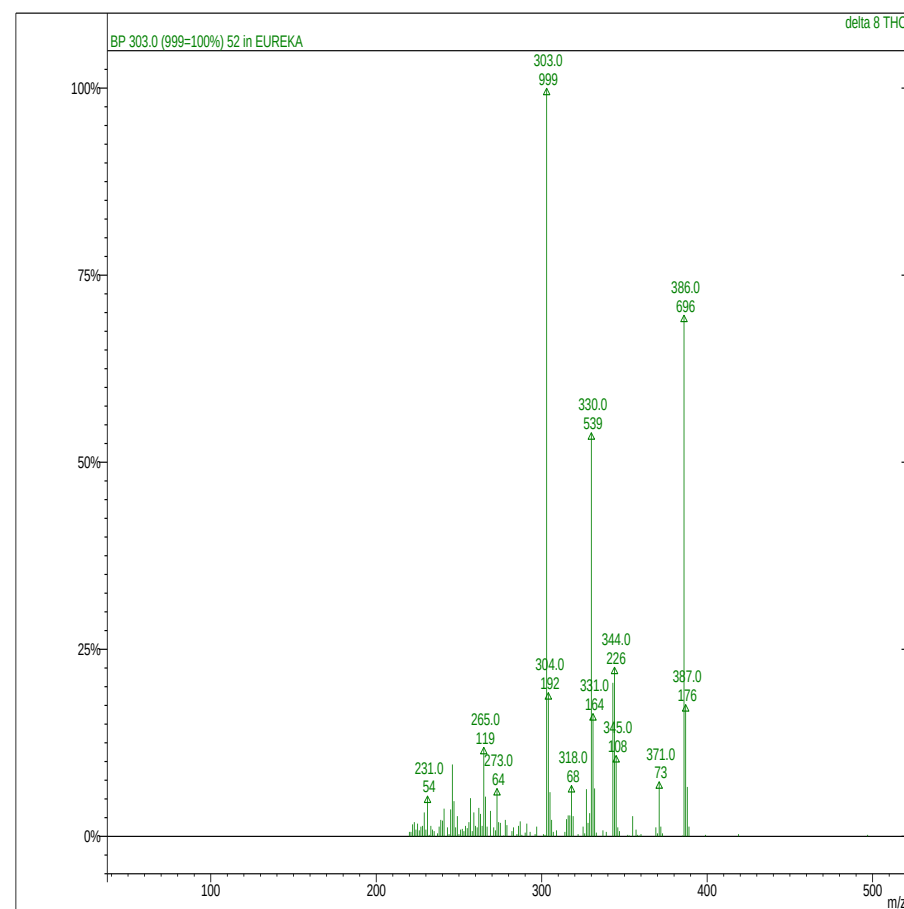


Fig. 4 :	Massa Spectrum of Internal Standard
	MOLECULAR IONS : 303 – 330 – 386