

# Determination of Tetrahydrocannabinol (THC) and Its Main Metabolites Using GC Triple Quadrupole Mass Spectrometry

Tommaso Albertini and Andrea Caruso  
Thermo Fisher Scientific, Milan, Italy

## Overview

**Purpose:** To introduce a novel gas chromatography-triple quadrupole MS system for the determination of the analytes of interest in THC use screening.

**Methods:** Gas chromatography tandem mass spectrometry are applied to the samples prepared by liquid/liquid extraction.

**Results:** This work will show the excellent sensitivity and linearity that such a system can provide for this type of analysis.

## Introduction

Tetrahydrocannabinol (THC), is the main psychoactive constituent of cannabis.

THC intoxication is well established to impair cognitive function on an acute basis, including effects on the ability to plan, organize, solve problems, make decisions, and control impulses. THC is rapidly absorbed through lungs after inhalation and quickly reaches high concentration in blood.

THC is metabolized to produce different byproducts. In particular, hydroxylation of THC generates the psychoactive compound 11-hydroxy tetra hydrocannabinol (11-OH-THC) and further oxidation generates the inactive 11-nor-9-carboxy tetrahydrocannabinol (THCCOOH).

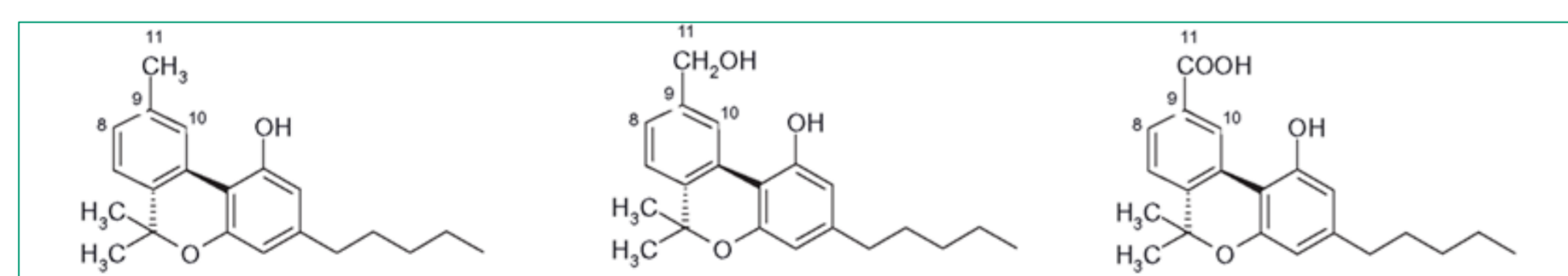
The legality of cannabis varies from country to country, however, in most countries possession and use of cannabis remains illegal. Measurement of cannabinoids is necessary for pharmacokinetic studies, drug treatment, workplace drug testing, and investigating drug impaired driving. THC presence can be assessed in different matrices—urine, blood, hair, nails saliva, or other body fluids. The most commonly tested matrices are urine, blood, and hair.

One of the most effective techniques to monitor the presence of cannabinoids is GC/MS using a triple quadrupole mass spectrometer. The most reliable identification of detected compounds is achievable when operating the mass spectrometer in single reaction monitoring (SRM) mode. In SRM mode, an ion is selected and fragmented to yield fragment ions. One of the generated fragments is selected and analyzed. This technique allows the user to choose which ion to analyze.

In this work, we introduce the Thermo Scientific™ TSQ™ Duo, an innovative triple quadrupole mass spectrometer. Here, we apply the TSQ Duo technology to the analysis of THC and its metabolites in various matrices.

The TSQ Duo GC-MS/MS is tailored to chromatographers and single quadrupole GC-MS users who need easy access to powerful new workflows through MS/MS while continuing to satisfy their current methods requirements. The system accomplishes this by providing excellent performance, in both single quadrupole and triple quadrupole modes, that is easily achievable, even for less experienced users. The TSQ Duo system, coupled with the Thermo Scientific™ TRACE™ 1300 series GC offers analysts a unique choice by allowing the use of single quadrupole methods and enabling the automatic transition, when appropriate, to triple quadrupole on the same.

FIGURE 1. THC and the metabolites.



## Methods

THC, THCOH, and THCCOOH are analyzed in plasma and THC is analyzed in hair. Deuterated THC, THCOH, and THCCOOH are used as internal standards.

### Sample Preparation

The analyte extraction from the matrix was performed via liquid extraction. A 9:1 hexane:ethyl acetate mixture was added to the sample along with a small aliquot of acetic acid. The sample was vortexed and centrifuged, the supernatant was then dried before derivatization with MSTFA. The procedure is the same for both blood and hair. Depending on the analysis, 1 mL of blood or 50 mg of hair were sampled for the extraction.

The instrumental conditions are reported in Table 1.

The SRM conditions for the two analyses are reported in Tables 2 and 3.

Table 1. Instrument method for the analyzes

Gas Chromatography		Mass Spectrometry	
Plasma	Hair	Plasma	Hair
Injection Volume	1.00 µL	Injection Volume	2.00 µL
Oven Program:		Oven Program:	
150 °C for 3 min	100 °C for 0 min	150 °C for 3 min	100 °C for 0 min
then 40 °C/min to 220 °C for 0 min	then 20 °C/min to 220 °C for 0 min	then 40 °C/min to 220 °C for 0 min	then 20 °C/min to 220 °C for 0 min
then 5 °C/min to 280 °C for 2 min	then 60 °C/min to 240 °C for 0 min	then 5 °C/min to 280 °C for 2 min	then 60 °C/min to 240 °C for 0 min
	then 30 °C/min to 300 °C for 0 min		then 30 °C/min to 300 °C for 0 min
	then 15 °C/min to 310 °C for 4 min		then 15 °C/min to 310 °C for 4 min
Run Time	18.75 min	Run Time	13 min
Front SS Inlet He		Front SS Inlet He	
Mode	Splitless	Mode	Splitless with surge
Temperature	280°C	Temperature	280°C
Split Flow	30mL/min after 1.5 minutes	Split Flow	50mL/min after 2 minutes
		Surge Pressure	165 Kpa Until 0.5 min
Mass Spectrometry		Mass Spectrometry	
Transfer line temperature	300° C	Transfer line temperature	310° C
Source temperature	250° C	Source temperature	280° C

Table 2. SRM transitions for the plasma analysis.

Analyte	R.Time	Window	Mass	Product mass	Collision energy
THC-D3	9.66	2	389	306	15
THC-D3	9.66	2	389	292	15
THC-D3	9.66	2	389	330	15
THC-D3	9.66	2	389	374	15
THC	9.69	2	371	289	15
THC	9.69	2	386	289	15
THC	9.69	2	386	303	15
THC	9.69	2	386	330	15
THCOH-D3	12.28	2	374	268	15
THCOH-D3	12.28	2	374	292	15
THCOH-D3	12.28	2	374	308	15
THCOH	12.32	2	371	289	15
THCOH	12.32	2	371	265	15
THCOH	12.32	2	371	305	15
THCCOOH-D3	13.92	2	374	358	15
THCCOOH-D3	13.92	2	476	358	15
THCCOOH-D3	13.92	2	491	374	15
THCCOOH-D3	13.92	2	491	300	15
THCCOOH	13.95	2	473	355	15
THCCOOH	13.95	2	371	289	15
THCCOOH	13.95	2	488	297	15
THCCOOH	13.95	2	488	371	15

### Software controls

Chromatographic system control and data acquisition, reprocessing, and result storing and reporting were handled by Thermo Scientific™ Dionex™ Chromeleon™ 7.2 chromatography data system (CDS) software.

Chromeleon CDS software offers simple operational functionality thanks to an intuitive graphic interface and powerful tools, such as Eworkflows, AutoSIM, and SIMBridge that enable the user to be productive in a very short time. Data handling is immediate and the results can be collected and reprocessed in real time, increasing laboratory productivity. Chromeleon CDS software also offers a sophisticated and customizable report system and powerful statistical tools for presenting results.

Compliance is made easy with the Chromeleon CDS software thanks to the ability to manage user accounts and authorizations, perform system suitability tests, and trace all actions performed in the software quickly and easily, using the built-in audit trails.

Table 3. SRM transitions for the hair analysis

Analyte	R.Time	Window	Mass	Product mass	Collision energy
THC-D3	10.57	0.3	374	292	10
THC-D3	10.57	0.3	389	374	10
THC	10.57	0.3	371	265	10
THC	10.57	0.3	371	289	10
THC	10.57	0.3	386	315	10
THC	10.57	0.3	386	371	10

## Results

The calibration curves were prepared in matrix. Different curves were prepared for serum and hair at the concentrations reported below. The calibration results are reported in Figure 2 and Table 4.

### Plasma

Level	Conc.	Level	Conc.
1	0.5 ng/mL	1	0,05 ng/mg
2	1.5 ng/mL	2	0,1 ng/mg
3	2.0 ng/mL	3	0,5 ng/mg
4	2.5 ng/mL	4	1 ng/mg
5	3.0 ng/mL	5	2 ng/mg
6	4.0 ng/mL		
7	5.0 ng/mL		
8	10 ng/mL		
9	50 ng/mL		

FIGURE 2.

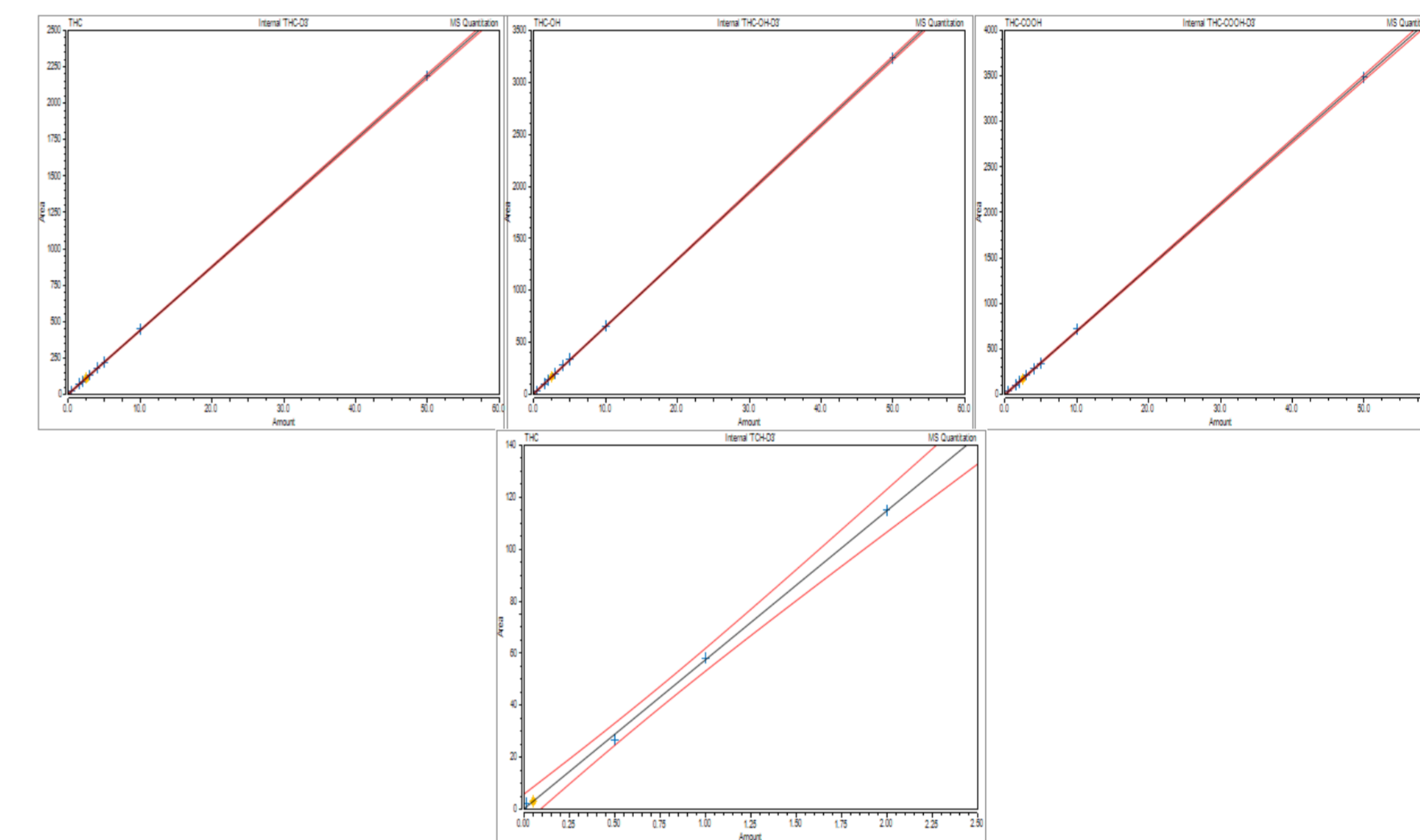


Table 4. Calibration results

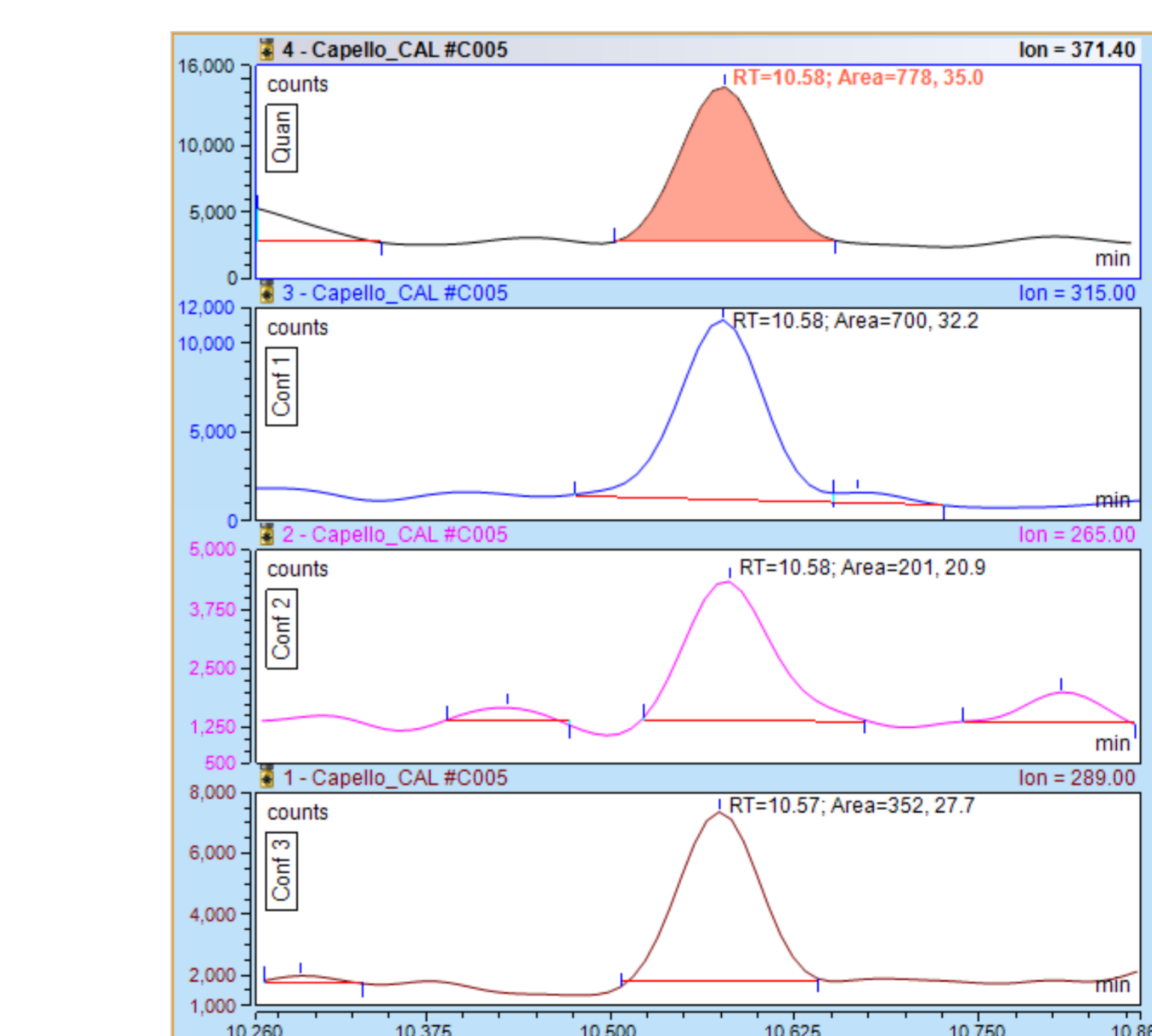
Peak Name	Ret.Time	Cal.Type	Number of Points	Coeff.of Determination
THC	9.64 Lin, WithOffset		9	0.999
THC-OH	12.29 Lin, WithOffset		9	0.999
THC-COOH	13.96 Lin, WithOffset		9	0.999

Peak Name	Ret.Time	Cal.Type	Number of Points	Coeff.of Determination
THC	10.58 Lin, WithOffset		5	0.999

Figure 3 shows the quantitation and confirming ions for THC in hair at the lower concentration, provided by the calibration point at 0.05 ng/mL — well below the cutoff level for THC in hair defined for gas chromatographic confirmation tests. Both peak shape and signal-to-noise ratio are appreciable for such a low concentration.

FIGURE 3. THC components for 0.05 ng/mL concentration



The TSQ Duo GC-MS/MS system is a robust and reliable system for the analysis of THC and its metabolites for forensic investigation. The system shows excellent performance in terms of sensitivity and linearity and quick and easy setup and operation. The MS and GC controls are fully integrated in the Chromeleon 7.2 CDS software, offering a powerful and easy-to-use tool for data collection and reprocessing. Chromeleon CDS software also offers sophisticated auditing and user authorization system that is well-suited for regulated environments.

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