Application Note: 548

THC-COOH Quantification in Urine Using Dilute and Shoot LC-MS/MS Method for Forensic Toxicology

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Key Words

- **TSQ Quantum Access MAX**
- **Accela Pump**
- **Cannabinoids**
- **Forensic Toxicology**

Introduction

Cannabis sativa is a widely used drug of abuse. Tetrahydrocannabinol (THC) is the major psychoactive chemical compound in the cannabis plant. After smoke inhalation, THC is absorbed and distributed in blood. Subsequently, it is rapidly metabolized to THC-COOH, conjugated with glucuronic acid, and excreted through urine. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered a useful tool to establish the consumption of cannabis by the assessment of THC-COOH in urine for forensic toxicology purposes.

Goal

To develop a reliable and fast analytical method for the quantitative determination of THC-COOH in urine using a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer.

Experimental

Sample Preparation

A urine sample was hydrolyzed with 10M NaOH and heated at 60 °C for 15 minutes. The pH was restored with Fisher Chemical acetic acid. Hydrolyzed samples as well as calibrators were diluted 1:10 in Fisher Chemical water/acetonitrile (1:1). Then, 10 µL were directly injected. Quantitative analysis was performed on the basis of calibration curves prepared in urine, ranging from 7.8 to 1000 ng/mL. Calibrators were injected in duplicate.

UHPLC conditions

Liquid chromatography separation was performed using a Thermo Scientific Accela autosampler and pump. The sample was injected directly on a Thermo Scientific Hypersil GOLD column (50 × 2.1 mm, 1.9 µm). A gradient LC method used mobile phases A (0.1% aqueous formic acid) and B (Fisher Chemical Optima LC/MS acetonitrile) at a flow rate of 300 µL/min. The run time was 6 minutes.

Mass Spectrometry

MS analysis was carried out on a TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer equipped with a Thermo Scientific Ion Max source with a heated electrospray ionization (HESI) probe. The MS conditions were as follows:

Scan type:	SRM
Divert valve:	2 - 4 min to source
Selected ions for quantification:	*m/z* 343 → 299 + 245 for THC-COOH in negative mode

Results and Discussion

Figures 1 and 2 show the ion chromatograms of the lowest and highest calibration points. Excellent linearity ($r^2 = 0.99$) fits for the calibration curve were observed over the range of 7.8-1000 ng/mL urine, with a Coefficient of Variation (%CV) at the lower end of 6.5%. The limit of quantitation (LOQ) was established as 7.8 ng/mL in urine.

Figure 4 reports an ion chromatogram of a real urine sample positive for cannabinoids (225 ng/mL urine), analyzed as described.

To examine the difference between hydrolyzed and non-hydrolyzed urine, we analyzed the same urine sample without the hydrolysis step. When urines were not hydrolyzed, the portion excreted as free THC-COOH was detected at 3.06 minutes, while THC-COOH-glucuronide was detected at 2.58 minutes (Figure 5). The precursor ion *m/z* 343 was generated as result of an in-source fragmentation and a consequent loss of glucuronic acid.

Because THC-COOH is mainly excreted as glucuronic acid conjugate, it is always necessary to perform urine hydrolysis before the LC-MS analysis to obtain an accurate quantification of THC-COOH.

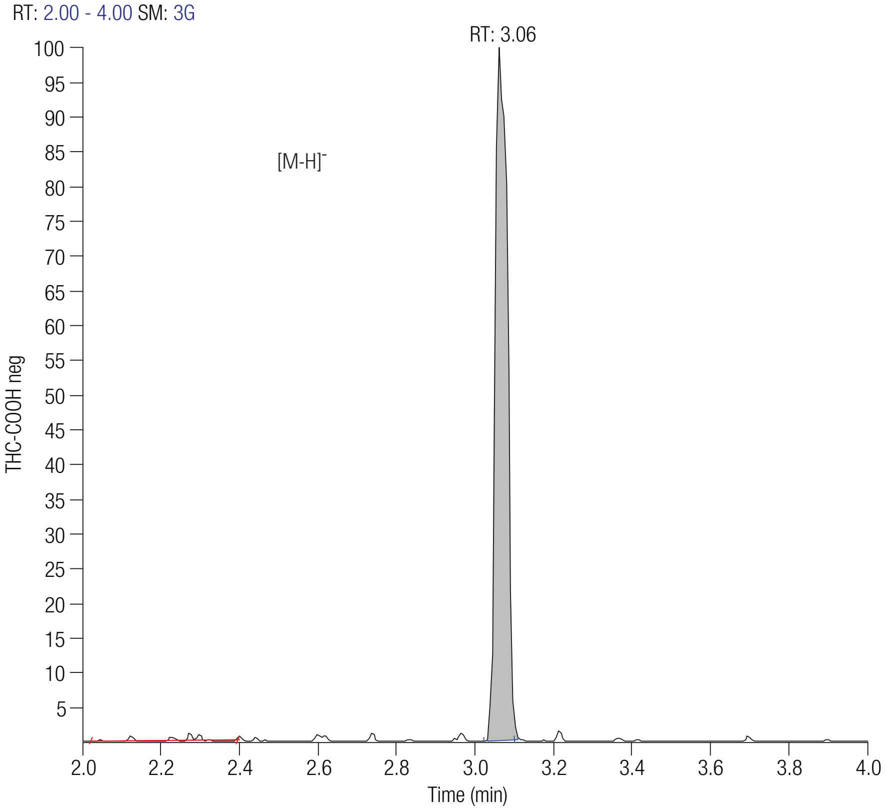

Figure 1. Ion chromatogram of 7.8 ng/mL urine calibration standard

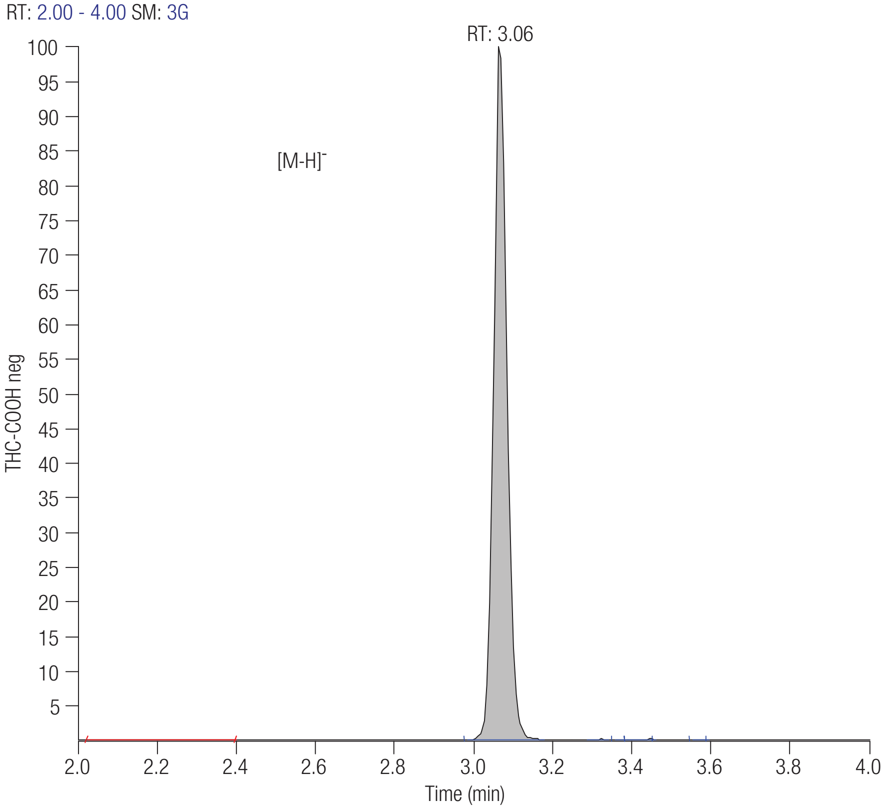

Figure 2. Ion chromatogram of 1000 ng/mL urine calibration standard

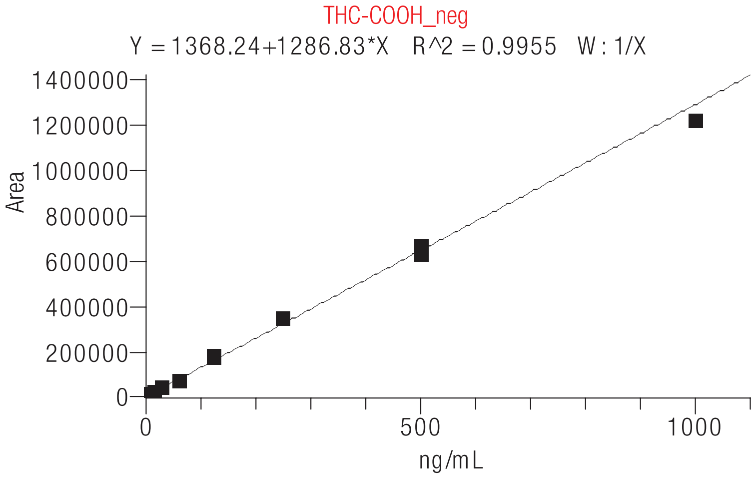

Figure 3. Calibration curve of THC-COOH in negative ionization mode

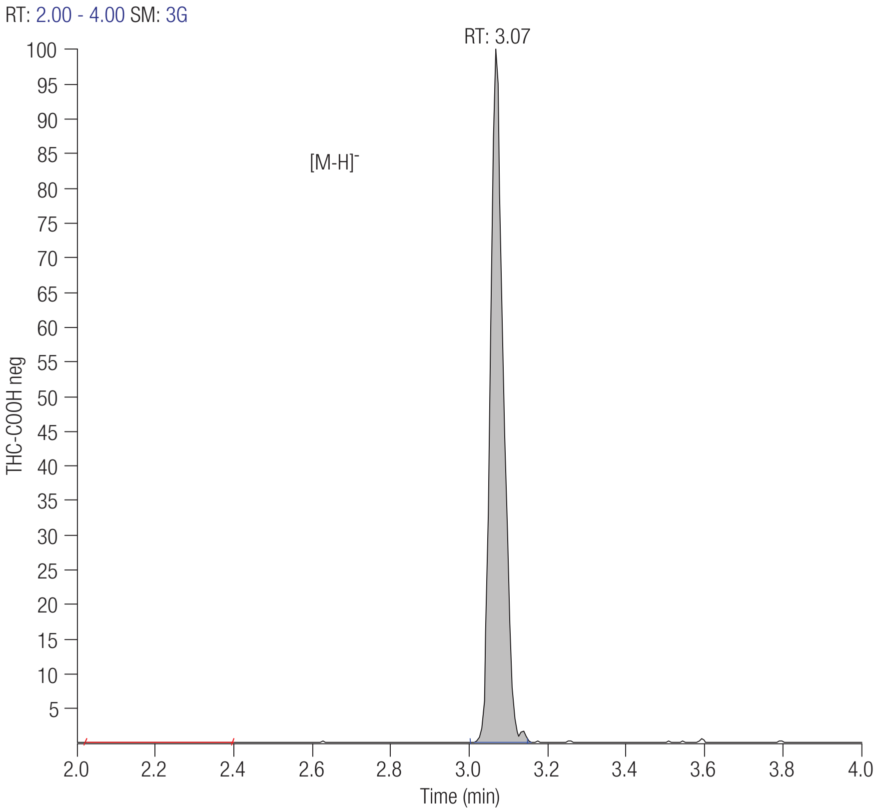

Figure 4. Ion chromatogram of urine sample containing 225 ng/mL. Sample was hydrolyzed and diluted 1:10 before the analysis.

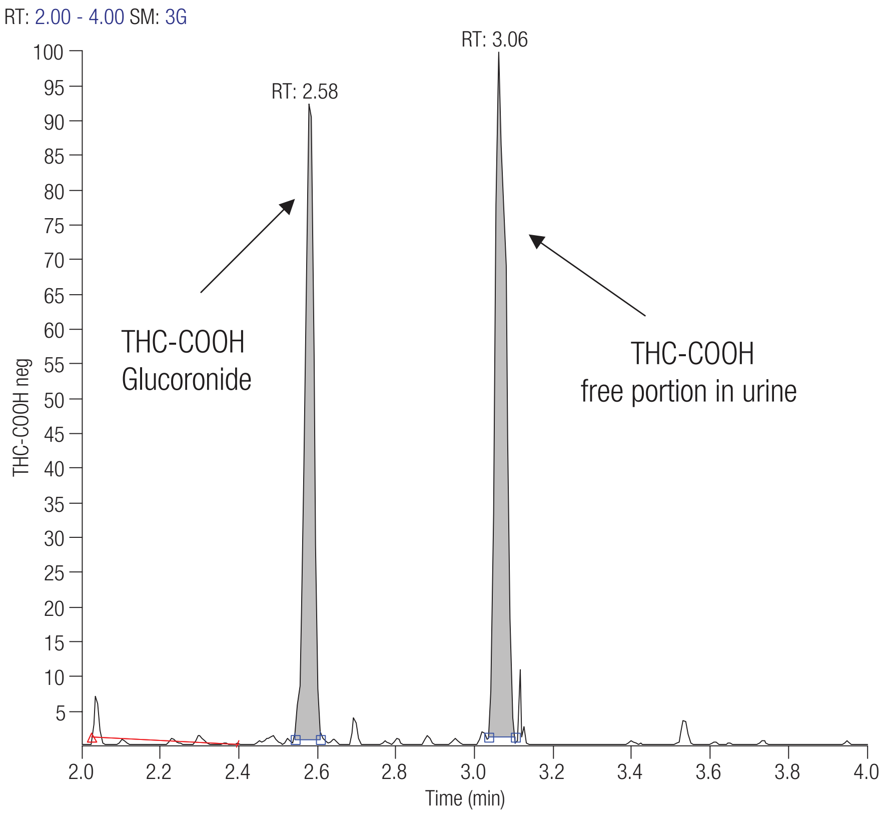

Figure 5. Ion chromatograms of urine sample containing 225 ng/mL. Sample was diluted 1:10 before the analysis; no hydrolysis was performed.

Conclusion

A robust 6-minute method for the quantification of THC-COOH with a dynamic range of 7.8-1000 ng/mL urine has been developed using the TSQ Quantum Access MAX mass spectrometer for forensic toxicology purposes.

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