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Application of PFPA/PFPOH derivatization for confirmatory analysis of urinary THC-COOH by GC–MS/MS

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Abstract
This study aimed to develop and evaluate a derivatization method using pentafluoropropionic anhydride (PFPA) and 2,2,3,3-pentafluoro-1-propanol (PFPOH) for the confirmatory analysis of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in human urine by gas chromatography–tandem mass spectrometry (GC–MS/MS). A total of 30 urine specimens were analyzed, comprising 20 cannabinoid-positive samples and 10 cannabinoid-negative samples previously screened using the DRI Cannabinoid immunoassay. Samples were subjected to solid-phase extraction (SPE) followed by PFPA/PFPOH derivatization prior to GC–MS/MS analysis. The developed method successfully confirmed the presence of THC-COOH in all positive samples and the absence of THC-COOH in all negative samples. Excellent calibration linearity was achieved over the concentration range of 10–120 ng/mL, with a coefficient of determination (r^2) of 0.999. Characteristic fragment ions at m/z 445, 459, 607, and 622 provided reliable identification of THC-COOH with library match probabilities exceeding 95%. The method demonstrated satisfactory chromatographic performance and analytical specificity, supporting its suitability for routine forensic and clinical toxicology applications. These findings highlight the value of GC–MS/MS confirmatory testing for cannabinoid detection in urine and support the use of PFPA/PFPOH derivatization as a reliable approach for THC-COOH analysis.

Keywords: THC-COOH, GC–MS/MS, PFPA/PFPOH derivatization, urine, forensic toxicology

Introduction

Cannabis (Cannabis sativa L.) has been used for medicinal, industrial, and recreational purposes for thousands of years and remains one of the most widely consumed psychoactive substances worldwide (Russo, 2007) [1]. In recent years, increasing acceptance of medical cannabis and evolving regulatory frameworks in many countries have stimulated the need for reliable analytical methods to monitor cannabis use and exposure in forensic, clinical, and workplace settings (World Health Organization, 2019) [2]. As cannabis-related policies continue to evolve, accurate and scientifically defensible methods for detecting cannabis biomarkers in biological specimens have become increasingly important.

Δ9-Tetrahydrocannabinol (Δ9-THC), the principal psychoactive constituent of cannabis, undergoes extensive hepatic metabolism following consumption. One of its primary metabolites is 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), an inactive metabolite that is excreted predominantly in urine as glucuronide conjugates (Huestis, 2007) [4]. THC-COOH may remain detectable in urine for several days to weeks depending on frequency of cannabis consumption and individual metabolic characteristics (Lowe *et al*., 2009; Smith *et al*., 2009) [11, 12]. Owing to its specificity and relatively long detection window, urinary THC-COOH is widely recognized as a reliable biomarker for confirming cannabis use in forensic and clinical toxicology (Gjerde *et al*., 2010). Consequently, the determination of THC-COOH plays a critical role in workplace drug testing, impaired-driving investigations, clinical toxicology, and forensic casework. Gas chromatography–mass spectrometry (GC–MS) and gas chromatography–tandem mass spectrometry (GC–MS/MS) remain among the most widely accepted confirmatory techniques for cannabinoid analysis because of their high selectivity, sensitivity, and analytical reliability (Goldberger & Cone, 1994; Moeller *et al*., 2008) [5, 9]. Regulatory guidelines issued by the U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) recommend chromatographic confirmation of presumptive positive immunoassay results to ensure legally defensible and scientifically reliable findings (SAMHSA, 2019). However, direct GC analysis of THC-COOH is challenging because of its low volatility and limited thermal stability. Therefore, derivatization is generally required prior to analysis to improve chromatographic behavior, enhance detector response, and increase analytical sensitivity. The analytical performance of cannabinoid testing is strongly influenced by sample preparation and derivatization procedures. Appropriate derivatization improves analyte volatility, thermal stability, chromatographic behavior, and mass spectral response, thereby facilitating reliable confirmation of urinary THC-COOH by GC–MS-based techniques. Consequently, optimization of derivatization conditions remains an important consideration for forensic and clinical toxicology laboratories engaged in cannabinoid testing. Given the increasing demand for confirmatory cannabinoid testing, the development of robust and reliable analytical methods for urinary THC-COOH determination remains an important objective in forensic and clinical toxicology. Therefore, the present study aimed to develop and evaluate a GC–MS/MS method for the analysis of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine following derivatization with pentafluoropropionic anhydride and 2,2,3,3-pentafluoro-1-propanol (PFPA/PFPOH). The suitability of the developed method for routine forensic and clinical toxicology applications was assessed with respect to chromatographic performance, analytical sensitivity, specificity, and reliability for confirmatory cannabis testing.

Materials and Methods

1. Instrumentation

The analysis was carried out using a Bruker SCION 436 Gas Chromatograph coupled with a Bruker EVOQ TQ Triple Quadrupole Mass Spectrometer (Bruker Daltonics, Bremen, Germany). Chromatographic separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent J&W Scientific, USA). Sample injection was performed using a MultiPurpose Sampler MPS-DPX robotic autosampler (GERSTEL, Germany). The injector was operated in splitless mode at 250 °C. Helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min, while nitrogen served as the collision gas. The oven temperature program was initiated at 100 °C and held for 2 min, followed by a ramp of 15 °C/min to 280 °C and a final hold of 6 min, resulting in a total run time of approximately 20 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV with the ion source temperature maintained at 250 °C.

Although the EVOQ TQ system is capable of tandem mass spectrometric acquisition, data collection in the present study was performed in selected ion monitoring (SIM) mode. SIM acquisition was selected because the primary objective of this work was the qualitative confirmation and identification of urinary 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) following PFPA/PFPOH derivatization under routine laboratory conditions. Instrument control and data processing were performed using Bruker MS Workstation software.

2. Chemicals and Standards

All solvents and reagents were of analytical grade. Concentrated hydrochloric acid, glacial acetic acid, n-hexane, anhydrous heptane, ethyl acetate, sodium acetate, potassium hydroxide pellets, acetonitrile, and methanol were obtained from Merck (Darmstadt, Germany). The derivatization reagents consisted of pentafluoropropionic anhydride (PFPA, ≥99%, Sigma-Aldrich, USA) and 2,2,3,3-pentafluoro-1-propanol (PFPOH, ≥97%, Sigma-Aldrich, USA). A certified reference standard of (±)-11-nor-9-carboxy-Δ9-THC (THC-COOH, 100 μg/mL in methanol; Cerilliant, Round Rock, TX, USA) and THC-COOH-d_3 internal standard were used for calibration and confirmation.

3. Sample Collection

Thirty urine specimens were included in this study and divided into two groups. Negative samples consisted of ten urine specimens obtained from healthy volunteers with no history of cannabis consumption. These samples were confirmed negative for cannabinoids using an immunoassay screening method (Indiko Analyzer with DRI® Cannabinoid Assay, Thermo Fisher Scientific).

Positive samples consisted of twenty urine specimens previously screened positive for cannabinoids and obtained from the Clinical Pathology and Medical Technology Laboratory, Princess Mother National Institute on Drug Abuse Treatment. All specimens were anonymized prior to analysis and stored at −20 °C until use.(Huestis, 2007; Huestis & Cone, 1998) [4, 10]

4. Sample Preparation and Extraction

Urine samples (0.5 mL) were mixed with 2.5 mL sodium acetate buffer (pH 5.7) and centrifuged at 3,000 rpm for 10 min. Solid-phase extraction (SPE) was performed using THC-specific extraction cartridges that were conditioned sequentially with methanol, ethyl acetate, and sodium acetate buffer. Following sample loading, the cartridges were washed with sodium acetate buffer, 10% acetonitrile in acetate buffer, and hexane. The cartridges were subsequently dried under vacuum. THC-COOH was eluted using 3 mL ethyl acetate and the eluates were evaporated to dryness under a gentle stream of nitrogen at 45 °C. For derivatization, 50 μL PFPA and 50 μL PFPOH were added to the dried residue. The mixture was vortex-mixed and heated at 60 °C for 30 min. After derivatization, the solution was evaporated to dryness, reconstituted with 50 μL anhydrous heptane, transferred into autosampler vials, and subjected to GC–MS/MS analysis.

5. Data Analysis and Method Evaluation

Quantification of THC-COOH was performed using an internal standard calibration approach. THC-COOH-d_3 was added to all calibration standards and urine specimens prior to extraction. Calibration standards were prepared in blank urine at concentrations ranging from 10 to 120 ng/mL. Calibration curves were generated by plotting the peak area ratio of THC-COOH to THC-COOH-d_3 against the corresponding concentration. Linear regression analysis was used to evaluate the relationship between analyte concentration and detector response. Method performance was assessed based on calibration linearity, chromatographic characteristics, mass spectral identification, and successful confirmation of THC-COOH in positive urine specimens.

6. Statistical Analysis

Descriptive statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Calibration data were evaluated using linear regression analysis, and results were reported as mean values and standard deviations where appropriate.

Results and Discussion

1. Confirmation of THC-COOH in Urine Samples

A total of 30 urine specimens were analyzed, including 20 cannabinoid-positive samples and 10 cannabinoid-negative samples as determined by DRI Cannabinoid immunoassay screening (cut-off = 50 ng/mL). Positive specimens contained THC-COOH concentrations ranging from 83 to >100 ng/mL, whereas negative specimens showed concentrations below the screening threshold. Following SPE extraction and PFPA/PFPOH derivatization, all specimens were analyzed by GC–MS/MS operating in SIM mode. THC-COOH was successfully detected in all positive specimens, whereas no detectable THC-COOH was observed in the negative samples. Complete agreement was obtained between the screening and confirmatory results.These findings support previous reports indicating that urinary THC-COOH is a highly specific biomarker of cannabis exposure and remains the preferred target analyte for confirmatory cannabinoid testing (Huestis, 2007[4]; Gjerde *et al*., 2010). The absence of false-positive and false-negative findings further demonstrates the suitability of GC–MS/MS for forensic and clinical toxicology applications.

2. Calibration Performance and Linearity

Calibration standards prepared in blank urine over the concentration range of 10–120 ng/mL demonstrated excellent linearity, yielding a coefficient of determination (r^2) of 0.999. The analytical response remained proportional throughout the calibration range, indicating reliable detector performance and stable derivatization efficiency. The

excellent linearity obtained in this study demonstrates the suitability of the developed analytical procedure for confirmatory analysis of urinary THC-COOH. The successful detection of THC-COOH at the lowest calibration level (10 ng/mL) indicates that the developed analytical procedure is suitable for confirmatory testing at concentrations below the SAMHSA-recommended confirmation threshold of 15 ng/mL.

Table 1: Analytical Characteristics of PFPA/PFPOH-Derivatized THC-COOH

Parameter	Result
Derivatization reagent	PFPA/PFPOH
Calibration range	10–120 ng/mL
Correlation coefficient (r^2)	0.999
Major ions (m/z)	445, 459, 607, 622
Library match	>95%
Positive samples confirmed	20/20
Negative samples confirmed	10/10
Acquisition mode	SIM
Application	Routine cannabinoid confirmation

Table 2: Confirmation Results

Sample Type	Number	Confirmed Positive	Confirmed Negative
Positive urine	20	20	0
Negative urine	10	0	10

3. Chromatographic Performance

The PFPA/PFPOH derivatization procedure generated a stable derivative suitable for GC–MS/MS analysis. Representative chromatograms showed a well-resolved THC-COOH peak with minimal interference from endogenous urinary constituents. The observed chromatographic behavior agrees with previous studies demonstrating that acylation-based derivatization improves analyte volatility and chromatographic performance for cannabinoid metabolites (Musshoff & Madea, 2009) [7] Consistent retention characteristics observed throughout the analytical sequence suggest good derivative stability and reproducibility under routine laboratory conditions.

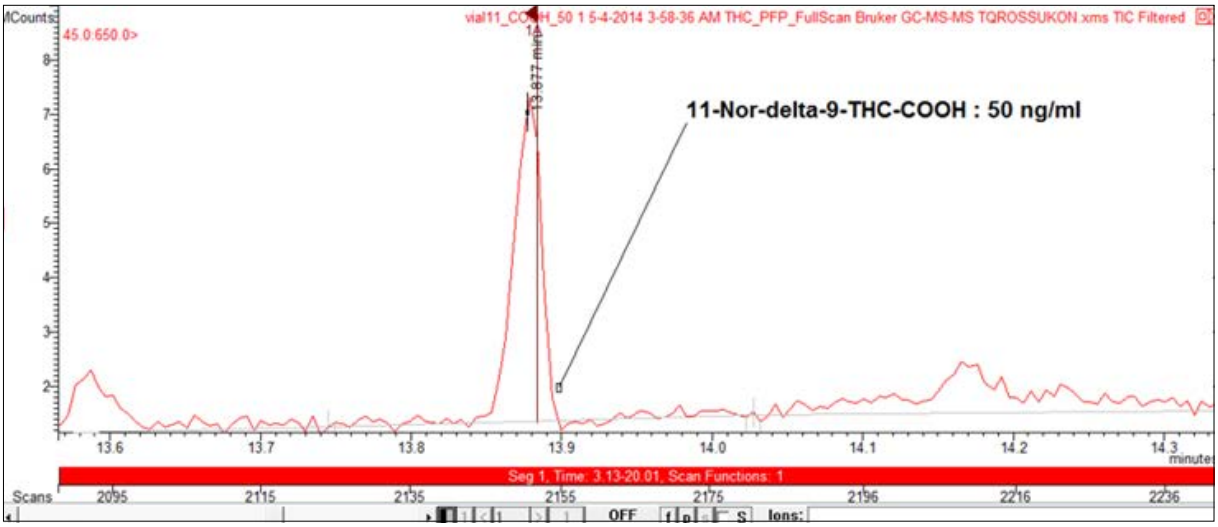

Fig 1: Representative SIM chromatogram of PFPA/PFPOH-derivatized THC-COOH (50 ng/mL) in human urine analyzed by GC–MS/MS. The chromatogram shows a well-resolved analyte peak with minimal matrix interference.

The chromatogram exhibited a well-resolved THC-COOH peak with minimal matrix interference, indicating the suitability of PFPA/PFPOH derivatization for confirmatory cannabinoid analysis. The observed chromatographic behavior is consistent with previous reports demonstrating that acylation-based derivatization enhances analyte volatility and improves chromatographic performance for cannabinoid metabolites during GC–MS analysis (Peters *et al*., 2007; Musshoff & Madea, 2009) [7, 13].

4. Mass Spectral Interpretation

Mass spectral analysis of PFPA/PFPOH-derivatized THC-COOH revealed characteristic fragment ions at m/z 445, 459, 607, and 622. These ions were consistently observed in all positive specimens and matched reference library spectra with similarity scores greater than 95%. The observed fragmentation pattern enabled reliable identification of THC-COOH and was consistent with the expected mass spectral characteristics of PFPA/PFPOH-derivatized cannabinoids. The ion at m/z 445 represented the most abundant fragment and served as the principal confirmation ion, whereas m/z 459, 607, and 622 functioned as qualifier ions. The consistent observation of these characteristic fragment ions in all positive specimens supports the specificity of the PFPA/PFPOH derivatization procedure and its applicability for confirmatory analysis of urinary THC-COOH.

5. Forensic and Clinical Toxicological Significance

The increasing use of cannabis for medical purposes and the expansion of cannabinoid testing programs have increased the demand for reliable confirmatory analytical methods. Although immunoassay screening provides rapid preliminary results, chromatographic confirmation remains essential for the definitive identification of urinary THC-COOH. Urinary THC-COOH is widely recognized as the preferred biomarker for confirming cannabis exposure because of its specificity and extended detection window compared with the parent compound, thereby providing reliable evidence of prior cannabis use (Verstraete, 2004; Huestis & Cone, 1998; Lowe *et al*., 2009) [8, 10, 11]. The present study demonstrated that PFPA/PFPOH derivatization combined with GC–MS/MS operated in selected ion monitoring (SIM) mode provides reliable qualitative confirmation of urinary THC-COOH. Following solid-phase extraction and derivatization, characteristic fragment ions at m/z 445, 459, 607, and 622 enabled

consistent identification of THC-COOH in all positive specimens, while no THC-COOH was detected in negative samples. Complete agreement between immunoassay screening and GC–MS/MS confirmation was observed. These findings support the applicability of PFPA/PFPOH derivatization coupled GC–MS/MS as a practical analytical approach for routine confirmatory cannabinoid testing in forensic and clinical toxicology laboratories. The method provided reliable identification of THC-COOH and demonstrated suitability for the confirmation of cannabis exposure in urine specimens.

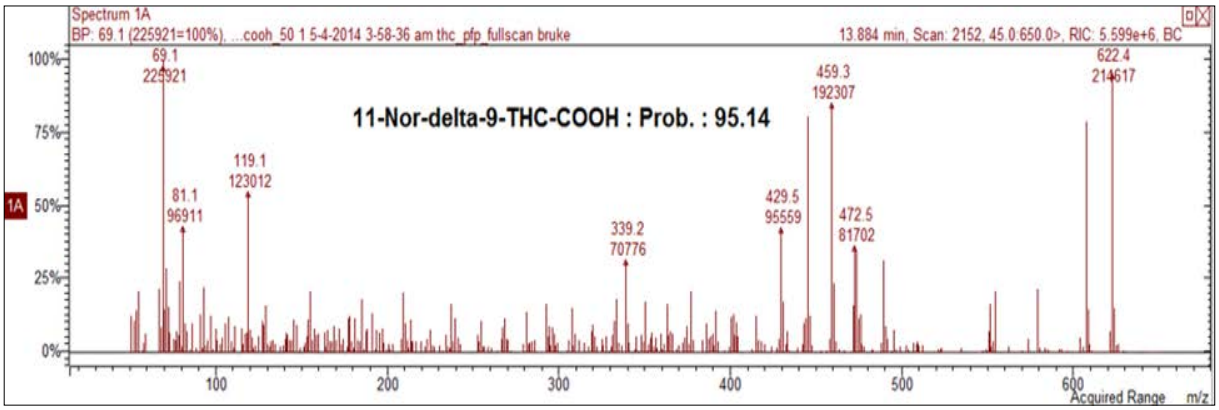

Fig 2: Mass spectrum of PFPA/PFPOH-derivatized THC-COOH obtained by GC–MS/MS analysis. Characteristic fragment ions were observed at m/z 445, 459, 607, and 622, confirming the identity of THC-COOH

Conclusion

The present study demonstrated the successful application of PFPA/PFPOH derivatization for the confirmatory analysis of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in urine using GC–MS/MS operated in selected ion monitoring (SIM) mode. The method showed excellent calibration linearity over the concentration range of 10–120 ng/mL (r^2 = 0.999) and produced characteristic fragment ions at m/z 445, 459, 607, and 622, enabling reliable identification of THC-COOH. All cannabinoid-positive urine specimens identified by immunoassay screening were successfully confirmed by GC–MS/MS, whereas all cannabinoid-negative specimens remained negative. In addition, the PFPA/PFPOH derivatization procedure generated well-resolved chromatographic peaks with minimal matrix interference, supporting its suitability for qualitative confirmation of urinary THC-COOH. Overall, the findings indicate that PFPA/PFPOH derivatization combined with GC–MS/MS provides a reliable analytical approach for the confirmatory identification of THC-COOH in urine and may be applied as a routine method for cannabinoid confirmation in forensic and clinical toxicology laboratories.

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