# Application of Two-Dimensional Gas Chromatography with Electron Capture Chemical Ionization Mass Spectrometry to the Detection of 11-nor-$\Delta^9$-Tetrahydrocannabinol-9-carboxylic Acid (THC-COOH) in Hair

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## Abstract

**The proposed federal regulations for the detection in hair of 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), a metabolite of marijuana, require a confirmatory detection level of 0.05 pg/mg. At present, the only way to achieve this on a routine basis has been with the use gas chromatography with tandem mass spectrometry (GC–MS–MS) technology. Tandem MS is an expensive approach and dissuades laboratories from attempting to enter the hair-testing market. A procedure for the determination of THC-COOH in hair using two-dimensional gas chromatography (GC × GC) coupled to mass spectrometry (GC–GC–MS) is described for the first time. The method makes use of several small improvements in the extraction, GC, and MS procedures to allow the required sensitivity to be achieved. The results of this approach demonstrate detection of THC-COOH in hair at a concentration level of 0.05 pg/mg with both a target quantitation ion and a unique confirming qualifier ion, using a single-quadrupole mass selective detector. These two ions and the enhanced separation of the GC–GC provide a high degree of confidence in the determinations. The method has been successfully applied to the detection of THC-COOH in hair specimens from known marijuana users, and it reaches the levels currently proposed in the *Federal Register*.**

## Introduction

In recent years, various changes to the Substance Abuse Mental Health Services Administration (SAMHSA) workplace drug-testing program have been implemented. Some of these changes have included the possibility of using alternative specimens to urine for workplace drug testing (1). Hair has been included in the proposed guidelines because it offers several advantages over urine analysis, including observed specimen collection and longer timeframe of drug detection.

Tetrahydrocannabinol (THC) is the active ingredient in marijuana and is generally administered orally or by smoking, resulting in euphoria and hallucinations. Because its main metabolite, 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), is acidic, its incorporation into the hair shaft is not as extensive as that of more basic drugs such as cocaine or methamphetamine. Hence, the detection of marijuana metabolite, THC-COOH, in hair is extremely difficult because of the very low levels incorporated, the problems with cleanliness of extraction, and the sensitivity of detection. Such problems have restricted the number of publications on this topic. The parent compound, THC, is found in higher concentration in hair samples (2), but for the purposes of the SAMHSA program, and the elimination of potential environmental contamination from marijuana smoke, the detection of the metabolite THC-COOH is mandated.

Various research groups have attempted to analyze THC-COOH in hair using negative ion chemical ionization (NCI) technology, as suggested in this publication. Wilkins et al. (3) digested the hair using sodium hydroxide, then extracted the drug by liquid–liquid methodology. Their analytical method employed gas chromatography–mass spectrometry (GC–MS) in NCI mode, using methane as the reagent gas. The limit of detection was 10 pg of THC-COOH on column, but when applied to hair specimens from self-reported users, they were unable to detect THC-COOH. In a similar procedure, Kintz et al. (4) digested as much as 100 mg of hair using sodium hydroxide, liquid–liquid extraction, and GC–MS-NCI with methane as the reagent gas. They reported a limit of detection of 5 pg/mg, but an average concentration of THC-COOH of 0.12 ng/mg (range 0.02–0.39 ng/mg) in the hair of marijuana users. This is 2400

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times higher than the proposed cut-off under the federal regulations. In an attempt to approach the mandated sensitivity level, Moore et al. (5) reported the use of large volume injection to help improve the detection limit of the marijuana assay for hair analysis. They also digested the hair using sodium hydroxide, followed by solid-phase extraction. Using improved injection technology, they were able to inject up to 25 µL onto the GC column, but the limit of quantitation was still only 0.5 pg/mg, which was 10 times higher than required by the guidelines.

The application of two-dimensional (2d) chromatography to forensic toxicological problems was first described in 2003 (6), and coupling of 2d chromatography to MS for the detection of drugs of abuse was reported for the first time in 2004 (7). Even though the actual technique has been available for many years, with particular usage in the oil and petroleum industry (8), applications in the drugs-of-abuse field have not been widespread.

Our approach to the problem of inadequate detection levels using a single-stage quadrupole MS was to make sufficient small improvements over the entire assay, so that the final required detection limit could be routinely achieved. Modifications were made in three main areas: extraction, GC, and MS.

## Extraction

Marijuana metabolites are incorporated into hair, but not as easily as basic drugs and metabolites. The negatively charged melanin, present in most hair types, does not attract acidic compounds such as THC-COOH as easily as basic compounds. In order to remove the drug from the hair, various approaches were tested.

Sonication of the hair at room temperature for 2 h, in a 50:50 mix of methanol and ethyl acetate, resulted in an extraction recovery of approximately 50% and a clean final extract.

Complete digestion of the hair using sodium hydroxide, followed by liquid–liquid extraction with hexane/ethyl acetate, released a higher amount of bound THC-COOH from the hair, but produced dirty extracts and therefore high background on the detection system

Washing with acetone and base digestion, followed by solid-phase extraction using a strong anion exchange column, improved the extraction recovery, but cleanliness of the extract was again an issue

Finally, the approach that provided the cleanest extract combined with the most efficient extraction, included washing with methylene chloride, base digestion, neutralization, and solid-phase extraction with a cation exchange + hydrophobic phase column. The full procedure is described in this paper.

## GC

*2d GC.* The use of two serial GC columns to separate background from the required peak is established technology and widely used in the oil industry. The application of a prior separating column to our assay allows the background associated with the hair extract to be spread out over a longer time frame. The two columns function optimally when the stationary phases are as different as possible. In previous applications of this technology (9), the determination of the timing for the diversion of the flow from the first column to the main analytical column was made by trial and error. By adding a simple detector system to the restrictor outlet, the time at which the analyte eluted from the first column was determined by injecting a high concentration of the analyte and monitoring the signal of a flame-ionization detector (FID). Once the analyte retention time on the first column had been determined, the pressure switch (Deans switch) was turned on at that time, to divert the flow, and turned off 0.4 min later. This diverts a narrow "window" of the effluent from the first column that contains the analyte and minimal background. The second analytical column is of a different polarity than the first and provides a further separation of the analyte from potential interferences.

*Cryogenic focusing.* Marriott et al. (10) described in detail a method that allowed fast target analysis in multidimensional GC by using a micro-switching valve between two GC columns, with cryogenic trapping and rapid reinjection of trapped solutes in the second dimension. The concept of cryogenic focusing allowed the analyte to be "cold-trapped", then rapidly remobilized at the prevailing column temperature. After the background had been separated, the fraction from the first column was selectively transferred to the analytical column where a cryogenic trap focused the peak of interest. The analytical column passed through the trap, which was cooled to 100°C as the analyte entered, effectively "cold-trapping" the drug. Publications using carbon dioxide (11) or liquid nitrogen cooling of the trap (12) have been reported. For our application, however, the trap was cooled by compressed air, and therefore no additional expensive equipment was required.

The focuser was then heated quickly allowing the peak of interest to advance through the analytical column and enter the MS for analysis. This resulted in a much sharper chromatographic peak, producing an improved signal-to-noise ratio.

## MS

In order to obtain sensitive analysis and low detection limits, a selective detection scheme is desired. Chemical ionization provides more specific and selective ionization of analytes than electron impact ionization by enhancing the signal and lowering the noise generated by potential interferences. The greatest potential gains are found in applying electron capture chemical ionization (ECCI). ECCI is confined to the capture of thermalized electrons by the analyte and resulting fragmentation, rather than negative ion chemical ionization which includes mechanisms of ionization that involve anions in the gas phase (e.g., $Cl^-$, $OH^-$, etc.).

*Reagent gas.* There are several collision gases that can be used for chemical ionization. The most common for ECCI is methane, which is excellent for both positive chemical ionization and ECCI modes. However, it has been reported that applying ammonia as a buffer gas has approximately seven times the thermalizing power of methane when used in the ECCI mode (13) and can significantly improve the sensitivity of an assay. No gas filters are available for ammonia, so use of the highest possible purity of gas is necessary (> 99.998%). Water and oxygen must be eliminated from the system when ammonia is in use because ammonia will react with water to corrode the seals of the pumps. In all CI modes, the presence of

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water decreases the sensitivity of the process. Ammonia gas requires a stainless steel non-corrosive regulator for the tank, but the use of minimal amounts of the gas at a low pressure provided sufficient fragmentation to allow the monitoring of two ions for the drug and internal standard. The reagent gas was also routinely shutoff at the end of a batch to ensure minimum quantities of ammonia were introduced into the system, which reduced maintenance and extended mechanical pump life. Lower temperatures in the ion source produced less fragmentation of the molecule, thereby enhancing the signal of the analyte. The MS ion source was held at 150°C and the quadrupole at 106°C.

*Derivatization.* The addition of electronegative species to the molecule via derivatization methods allows for enhanced ECCI detection. The THC-COOH was derivatized using fluorinated acylating agents, which reduced the polarity of the functional groups to improve chromatography and added halogenated functionalities. The use of trifluoroacetic anhydride (TFAA) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) allowed the addition of six fluorines [as $CH(CF_3)_2$ 151 μ] to the hydroxyl group of the carboxylic acid; and three fluorines (as $COCF_3$, 97 μ) to the free hydroxyl on the benzene ring. These two derivatized sites add 248 μ to the THC-COOH and when two hydrogen molecules are displaced, the resulting mass is *m/z* 590. The ECCI spectrum shows the molecular ion and a major fragment corresponding to a loss of a derivatization group: $[M - OCH(CF_3)_2]$ = *m/z* 422. The derivatization conditions were varied in order to achieve the most reproducible response. The optimized conditions are described in the procedure; however, longer incubation times (up to 1 h) were tested, as were other fluorinating agents. The addition of more electronegative groups was attempted using pentafluoropropionic anhydride and heptafluorobutyric anhydride in place of the TFAA, but neither anhydride was particularly stable. The ion resulting from loss of one of the added derivatizing agents is reproducible under the same constraints that apply to all fragmentation in MS (i.e., source temperature, collision gas pressure, tuning, etc.). The ion ratios are very reproducible and are, most importantly, invariant with respect to analyte concentration, which is how they were chosen as analytically reliable ions. The spectral confirmation was consistent with the expected form of the derivative based on the chemistry of these acylating agents and their preference for selected functional groups.

## Materials and Methods

### Reagents and consumables

Methanol, acetonitrile, toluene, ethyl acetate, hexane, glacial acetic acid, and methylene chloride were obtained from Spectrum Chemicals (Gardena, CA). All solvents were high-performance liquid chromatography (HPLC) grade or better, and all chemicals were ACS grade. Bond Elut Certify I solid-phase extraction columns (130 mg) from Varian (Walnut Creek, CA) or CSDAU133 extraction columns (130 mg) from United Chemical Technologies (Bristol, PA) were interchangeable for the assay. The positive pressure extraction manifold was obtained from SPEWare (San Pedro, CA). The derivatizing agents were TFAA (Pierce Chemical, Rockford, IL) and HFIP (Campbell Science, Rockton, IL). GC columns were obtained from J&W Scientific (an Agilent Company, Palo Alto, CA).

### Gases

The gases used for the analytical system were obtained from Lehner and Martin (Chino, CA). The quality of the gases is critical to the overall operation of the system. The purity of the carrier gas, helium, was 99.999%. The purity of the ammonia was 99.999% with maximum impurities as follows: carbon monoxide 1 ppm; methane 1 ppm; nitrogen 1 ppm; oxygen 1 ppm; and water 5 ppm. The stainless steel regulator required to avoid corrosion was also purchased from Lehner and Martin. The regulator (Part # SGT 500-15-660-DK-04) was a two-stage with a 2–15 psi delivery range.

### Standards

Trideuterated THC-COOH, which was used as the internal standard (100 μg/mL in methanol), as well as unlabelled drug (1 mg/mL in methanol) were obtained from Cerilliant (Round Rock, TX). The internal standard concentration for THC-COOH-$d_3$ was 1 pg/mg of hair. Initially 5 pg/mg was used, but a 1% contamination of the deuterated surrogate with native THC-COOH may cause a false-positive result. At the concentrations being tested, the purity of the internal standard is an important consideration.

### Sample preparation

Calibrators, controls, or hair specimens (20 mg) were cut finely and weighed into tapered-bottom centrifuge tubes and washed with methylene chloride (1.5 mL). The solvent was decanted, and the hair samples were allowed to dry. The internal standard, THC-COOH-$d_3$ (1 pg/mg), was added to each hair sample. For the calibration curve, unlabelled THC-COOH was added to the hair at concentrations of 0.05, 0.1, 0.5, 1, and 5 pg/mg of hair. Drug-free negative specimens and positive controls at 0.1 pg/mg (2 × cut-off) were included in every batch.

Deionized water (0.5 mL) and 2N sodium hydroxide (0.5 mL) were added, and the hair was heated at 75°C for 15 min. The sample was allowed to cool and then centrifuged (2500 rpm, 15 min). The supernatant was poured into glass tubes already containing acetic acid (1 mL), 1M acetic acid (3 mL), and 0.1M sodium acetate buffer (pH 4, 2 mL). The tubes were capped and mixed.

Bond Elut Certify I, or CleanScreen CSDAU133 columns (130 mg capacity) were conditioned with hexane/ethyl acetate (75:25, v/v; 2 mL), methanol (3 mL), deionized water (3 mL), and 0.1M hydrochloric acid (1 mL). The acidified samples were loaded onto the respective columns and allowed to dry. The columns were washed with deionized water (2 × 3 mL) and allowed to dry for 5 min. The columns were washed with 0.1M hydrochloric acid/acetonitrile (70:30 v/v; 3 mL) and allowed to dry at 30 psi for 10 min. The columns were finally rinsed with hexane/ethyl acetate (75:25 v/v; 3 mL) in order to elute the THC-COOH into silanized glass tubes. The entire extraction procedure was carried out using a positive pressure manifold, which allows the flowrate through the columns to be highly uniform.

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The eluent was evaporated to dryness under nitrogen at 40°C and reconstituted in TFAA (50 µL) and HFIP (30 µL). The mixture was transferred to autosampler vials with glass inserts and capped. The vials were heated at 70°C for 15 min, then left at room temperature for 10 min. Finally, the extracts were evaporated to dryness in a vacuum oven. The samples were finally reconstituted in toluene (25 µL) for injection into the GC–MS system.

The elimination of moisture during the derivatization was essential. All derivatizing agents were stored under desiccants as recommended by the manufacturer, and the final solvent was moisture-free. All glassware was previously silanized to minimize losses due to adhesion of the THC-COOH to active sites on the glassware.

### GC analysis

The development of the analytical procedure is the most important part of this assay. An Agilent Technologies 6890 GC, containing two GC columns in series, a cryofocusing trap, and a Deans switch, coupled to an Agilent Technologies 5973 inert mass selective detector operating in ECCI mode was used for analysis. (The concept of 2d chromatography was discussed previously in the Introduction.)

The extract was injected onto the first column, which was used for the separation of the background associated with the hair extract from the drug. The pressure set-points for the primary injection port and for the auxiliary pressure control module were calculated using Deans switch software provided with the system. The Deans switch allowed all the material from the injection to be vented through the FID, until directly before the analyte eluted from the first column. Deans switch methods are run using constant pressure mode, not constant flow, because the outlet pressure to the primary column for the 6890 GC system cannot be operated at a pressure greater than 4 psi if the constant flow mode is selected. The switch was turned on, to allow the effluent to enter onto the analytical column (column 2) for 15–30 s, after which the switch is turned off and the flow returns to the FID vent.

The second (analytical) column is passed through a cryogenic focusing device that allowed "cold-trapping" of the drug, then rapid release, after the background has been separated. It is critical to cool the oven immediately after the analyte has been passed into the analytical column. Rapid cooling of the oven allows separation of the drug from the background on the second column. Finally, the analyte enters the MS, operating in ECCI mode, for detection.

*Columns.* The entire operation functioned optimally when the phases of the two columns were as different as possible. The primary GC column was a DB-35 MS (40 m × 0.18-mm i.d., 0.18-µm film thickness). The inlet pressure was 55.4 psi, the helium flow was 1.6 mL/min, and the average linear velocity was 40 cm/s. The length of the restrictor column was calculated by software and was dependant on the dimensions of the column and the pressure. In this case, the restrictor length was 0.303 m. The restrictor tubing was connected to the Deans switch, and the other end was attached to the FID.

In a Deans switch mode, the flow from the primary column plus a switching flow are passed onto the secondary column. The secondary column was a DB-1 stationary phase (15 m × 0.25 mm i.d., 0.25-mm film thickness) operating at a flowrate of 2 mL/min.

*Settings.* The Deans switch (auxiliary port #3) was programmed to operate at a pressure of 18.1 psi. It allowed all the flow from the primary column to vent through the FID for 11 min. For 0.4 min, the flow was then switched to allow the carrier gas to enter the secondary analytical column. At 11.4 min, the flow was returned to the FID vent.

At a retention time of 11.0 min, the analyte of interest was switched onto the analytical column. However, because the analyte was to be "cold-trapped" using a cryogenic focuser, it was necessary for the trap to be cool before the THC-COOH arrived. Therefore, the trap (operated through the Back Inlet software of the 6890 GC system) was cooled from the oven temperature of 280°C to 100°C beginning at a run time of 10 min. The ramp rate for cooling was as high as it was possible to set the software and was set at 777°C/min. It was held at 100°C for 1.5 min, thereby allowing the THC-COOH to trap in the cryofocuser. At a retention time of 11.5 min, the focuser was heated at a rate of 777°C/min to a final temperature of 280°C.

*Injection and oven parameters.* The mode of injection is a critical parameter. The front inlet was operated in pulsed splitless mode at an initial temperature of 200°C. The pressure was 55.4 psi and the pulse time was 1 min. The purge flow was 50 mL/min and the purge time was 1 min. The injection volume was 2 µL.

The oven was programmed from 100°C for 0.5 min; ramped at 50°C/min to 200°C; ramped at 10°C to 260°C, held for 2.9 min, then cooled at 120°C/min to 220°C. The post-run temperature was raised to 320°C and held for 3 min for a total run time of 15.23 min. The post-run column pressure was raised to 99 psi to vent background material.

*FID parameters.* The FID was set at 250°C with the hydrogen and air flows at 40 mL/min and 450 mL/min, respectively.

*MS parameters.* The instrument was tuned in NCI mode using ammonia. The flow of the ammonia collision gas into the source was maintained between $8.0 \times 10^{-5}$ and $1.0 \times 10^{-4}$ Torr. The MS source was held at 150°C, the quadrupole at 106°C, the transfer line at 280°C, and it was operated at 800 eV over tune. The mass selective detector was operated in selected ion monitoring mode with four ions in a single group. Ions 593.1 and 425.1 were monitored for THC-COOH-$d_3$ and 590.1 and 422.1 for THC-COOH with a dwell time of 100 ms for each ion. The retention time of THC-COOH was 13.92 min.

## Results and Discussion

The analytical procedure described details the determination of THC-COOH in hair specimens at the proposed regulatory levels using a single-quadrupole MS operated in the ECCI mode.

The precision of the assay was determined by analyzing six hair specimens containing THC-COOH at concentrations of 0.05, 0.1, and 0.5 pg/mg. The interday precision of the assay at the three concentrations was 0.4%, 1.04%, and 1.21%,

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respectively. At 1 pg/mg, the coefficient of variation was 1.3% and 1.61% for intraday ($n$ = 5) and interday ($n$ = 6), respectively. The accuracy of the assay at 0.05 pg/mg was –0.0075 and at 0.1 pg/mg + 0.014. Interference studies revealed that cocaine, norcocaine, cocaethylene, benzoylecgonine, methamphetamine, amphetamine, MDMA, MDA, morphine, codeine, 6-acetylmorphine, hydrocodone, hydromorphone, phencyclidine, cannabinol, THC, 11-hydroxy-THC, and cannabidiol did not interfere with the assay when injected at concentrations higher than 10 ng/mg. The limit of quantitation of the system was 0.05 pg/mg. The correlation coefficient was 0.996 for the calibration curve, and the upper limit of linearity was 5 pg/mg.

The method was further validated using fortified hair specimens, and by analyzing samples which had previously been sent to a certified laboratory for testing. The specimens were found positive for THC-COOH using a GC–MS–MS triple stage quadrupole analytical method (LabOne, Salt Lake City, UT).

Thirteen hair specimens from self-reported marijuana users, which had previously been found to contain THC-COOH using GC–MS–MS, were extracted and analyzed according to the reported procedure (Table I). Ten specimens from drug-free individuals were also analyzed and were found to be negative by both procedures. The self-reported usage of marijuana from the subjects is also provided in Table I. Although we appreciate that self-reported drug use is notoriously unreliable, the subjects had no reason to fabricate marijuana usage. The mean concentration of THC-COOH detected in hair overall was 0.898 pg/mg using GC–MS–MS and 0.922 pg/mg using GC–GC–MS. When subjects admitted smoking once per day, the mean concentration detected in hair was 0.626 pg/mg ($n$ = 5). When subjects reported smoking less frequently than once per day, the mean was 0.595 pg/mg ($n$ = 2), and when use of marijuana more than once a day was reported, the mean value detected was 1.146 pg/mg ($n$ = 5). One of the subjects did not report a frequency of use, although admitted marijuana usage. The unreliability of self-report was further indicated when subject # 10 admitted daily use of marijuana, but the hair concentration was 1.69 pg/mg, which was significantly higher than the mean of other self-reported daily users.

Typical chromatograms are shown in Figure 1. Figure 1A shows a negative hair specimen, and Figure 1B is from hair sample # 13, containing 1.21 pg/mg.

This is the first procedure reported which uses a single-quadrupole MS to approach the required limit of quantitation for SAMHSA hair-testing purposes. Various attempts to reach low detection levels have been published. Sachs and Dressler (14) described the use of GC–MS in NCI mode to achieve a limit of detection of 0.3 pg/mg of THC-COOH following clean-up using HPLC. The concept of our method is similar, in that the specimen is cleaned up prior to introduction into the analytical column. The difference is that in the reported procedure, the clean-up column is in the same oven as the analytical column, and separate collection and injection procedures are not required. Backofen et al. (15) used electrochemical detection and headspace solid-phase extraction coupled to single-stage MS or tandem MS. THC-COOH could not be detected using the single quadrupole method (16) and the GC–MS–MS method reported detection of THC, cannabinol, and cannabidiol in the hair of a marijuana user (17).

Procedures using single stage GC–MS systems were described in the introduction. However, even tandem MSs have often not been adequate to reach these mandatory levels on a routine basis. In 2000, Uhl (18) reported a detection limit of 0.16 pg/mg

**Table I. Results of Hair Analysis for THC-COOH using GC–MS–MS and GC–GC–MS**

| Sample ID | GC–MS–MS (pg/mg) | GC–GC–MS (pg/mg) | Self-Reported Frequency of Marijuana Intake |
|---|---|---|---|
| 1 | 1.98 | 1.94 | No frequency reported |
| 8 | 0.33 | 0.95 | 2x week |
| 5 | 0.2 | 0.24 | 3x week |
| 2 | 1.66 | 1.21 | 7x week |
| 3 | 0.11 | 0.11 | 7x week |
| 6 | 0.09 | 0.09 | 7x week |
| 10 | 1.69 | 1.4 | 7x week |
| 11 | 0.37 | 0.32 | 7x week |
| 13 | 1.23 | 1.21 | 21x week |
| 4 | 0.71 | 0.9 | 28x week |
| 7 | 0.31 | 1.15 | 28x week |
| 9 | 1.45 | 1.12 | 56x week |
| 12 | 1.50 | 1.35 | 56x week |
| **Mean** | **0.898** | **0.922** | |

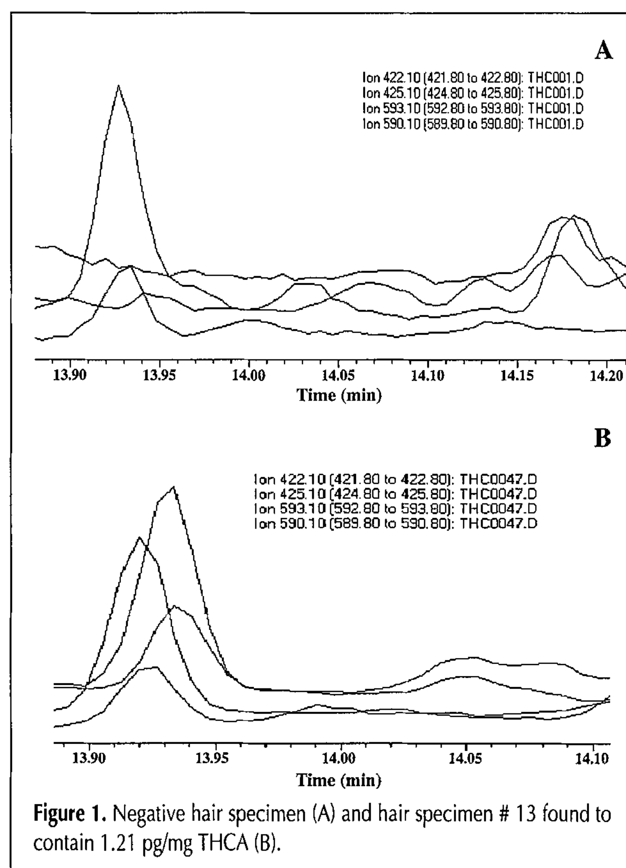


**Figure 1.** Negative hair specimen (A) and hair specimen # 13 found to contain 1.21 pg/mg THCA (B).

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using GC–MS–MS, which does not reach the 0.05 pg/mg requirement. It is difficult to assess the "state of the art" regarding mass spectral criteria in hair analysis for THCA because SAMHSA drug-testing programs to date have been based on three ion, two ratio electron impact MS. The use of electron impact generates many fragments, and the selected ions for monitoring must be chosen intelligently. ECCI is a much more selective ionization procedure, affecting only compounds with sufficient electronegativity to capture ionized electrons. Because less fragmentation occurs at low source and quadropole temperatures, fewer ions are produced (in this case, two), but their selectivity is critical. The procedure is highly selective and specific because only compounds that are stable through base hydrolysis, extract using the specific solid phase procedure, derivatize to the same molecular weight, are retained to the same extent on both chromatographic columns (with widely different phases), elute in the same "heart-cut" time window that transfers the flow to the second column, and then fragment in exactly the same way as THCA, producing two distinct ions with a stable ratio, could interfere with the assay. The presence of the molecular ion and an associated fragment are adequate to assign positivity or negativity to extracts analyzed using this procedure. The final assay used optimized extraction, derivatization and analytical methods, in order to achieve the sensitivity required (5,9,19).

Because the primary GC column used in this procedure (DB-35 MS, 40 m × 0.18-mm i.d., 0.18-µm film thickness) was not an easily obtainable column and required custom design, the method was modified to use a standard column. Using a DB-35 MS column (30 m × 0.25-mm i.d., 0.25-µm film thickness) as the primary, the inlet pressure was 32.6 psi for 6.6 min, and the pressure was lowered to 0.5 psi, allowing an average velocity of 64 cm/s. The secondary column remained the same. The Deans switch operated at a pressure of 15.2 psi, lowered to 10 psi for 5 min. The pressure was then raised to 19.17 psi at a rate of 20 psi/min, where it remained for 1.1 min. The pressure was lowered to 2 psi after the flow to the analytical column had occurred. The "heart-cut" from column one to column two occurred at 6.3 min for 0.2 min. At 6.5 min, the flow was returned to the FID vent. The cryofocuser was cooled from the oven temperature of 280°C to 100°C at 777°C/min, beginning at a run time of 5.5 min where it was held at 100°C for 1.5 min. At 7.0 min, the focuser was heated at a rate of 777°C/min to a final temperature of 280°C. The front inlet was operated in pulsed splitless mode at an initial temperature of 220°C. The pressure was 32.63 psi, and the pulse time was 1 min. The purge flow was 20 mL/min, and the purge time was 1 min. The oven was programmed from 150°C for 0.5 min; ramped at 50°C/min to 230°C; ramped at 10°C to 275°C, then cooled at 120°C/min to 200°C, where it was held for 1 min. The oven was then heated at 10°C/min to 240°C. Using this slightly modified procedure to incorporate a widely available standard primary column, the retention time of the THC-COOH was 11.3 min. The within-day precision of the assay was 0.51%, 0.51%, and 2.42% at THC-COOH concentrations of 0.05, 0.1, and 0.5 pg/mg, respectively ($n$ = 6). Interday precision was 0.11% at 0.05 pg/mg, 1.53% at 0.1 pg/mg, and 5.53% at 0.5 pg/mg ($n$ = 3). The other parameters and assay performance were as described with the longer primary column.

## Conclusions

This procedure makes use of small improvements in methodology: cleaner and more efficient extraction, addition of multiple electronegative species to the molecule during derivatization, 2d chromatography to minimize matrix background, cryogenic focusing, and soft fragmentation ECCI MS detection, to allow the identification of very low amounts of THC-COOH in hair. The results correlated extremely well with analytical results from a triple-quadrupole MS procedure. This is the first reported procedure using a single-quadrupole MS to routinely detect the SAMHSA proposed concentrations for THCA in hair samples.

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Manuscript received April 28, 2005;
revision received October 31, 2005.

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