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Testing Human Hair for Cannabis II. Identification of THC-COOH by GC-MS-NCI As a Unique Proof

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ABSTRACT: To validate information on cannabis use, 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic (THC-COOH) was investigated in human hair. The identification of THC-COOH in hair would document cannabis use more effectively than the detection of the parent drug which might have come from environmental exposure in a smoky atmosphere. Samples (100 mg) were decontaminated with methylene chloride and destroyed by incubation in 1 mL of 1 N sodium hydroxide for 30 min at 95°C in presence of 10 ng of THC-COOH-d_3. After cooling, samples were extracted by n-hexane/ethyl acetate after acidification with acetic acid. After derivatization by PFPA-PFP-OH of the dry extract, drugs were separated on a HP1 capillary column, and detected by mass spectrometry (m/z 602) using negative chemical ionization with methane as reagent gas. Among 30 samples obtained from subjects deceased from fatal heroin overdose, 17 tested positive for THC-COOH, in the range 0.02–0.39 ng/mg, with an average of 0.12 ng/mg.

KEYWORDS: toxicology, cannabis, hair, GC/MS/NCI

Δ9-tetrahydrocannabinol (THC) is a psychoactive drug found as an ingredient of the *Cannabis sativa* plant. It is extensively metabolized in man, one of its major metabolites being 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). Trace levels of this metabolite can be detected and conclusively identified only by using a method of analysis possessing high specificity and low detection limits. Numerous extraction procedures have been published for urine and blood samples [*1,2*].

In contrast, few data are available for cannabis testing in hair, which offers the potential for detection of drug exposure over an extended period of time (see review Ref *3*).

Generally, testing for THC or/and THC-COOH was reported in screening procedure for general unknown analysis in hair [*4–6*].

Only one investigator [*7*] published a full paper dealing with cannabis determination in hair which arosed great controversy [*8*].

The potential contamination of hair by external sources of drugs, generating false positives was presented as the major problem in hair testing, particularly for the interpretation of forensic results [*9*]. Therefore, the identification of THC-COOH in hair would document cannabis use more effectively than detection of THC which might have come from environmental exposure, in a smoky atmosphere.

The detection and quantification of THC-COOH is the most difficult assay to perform due to its low concentration in hair. This is the reason why tandem mass-spectrometry was recently proposed for monitoring THC-COOH [*10*]. However this procedure, due to its cost and high technology is only suitable for research purposes.

It has been shown that some electron capture derivatives give an enhanced sensitivity in negative ion chemical ionization (NCI) mode, compared with registration of positive ions produced by electron impact [*11*].

In the present report, an analytical procedure for THC-COOH in hair of chronic abusers is presented.

Materials and Methods

Chemical Reagents

All chemicals were provided by Merck (Darmstadt, Germany): *n*-hexane, cyclohexane, methylene chloride, methanol and ethyl acetate were HPLC grade; all others were analytical grade. Deuterated internal standard (IS-d_3), THC-COOH-d_3, was purchased from Radian (Austin, USA). Pentafluoropropionic anhydride (PFPA) was purchased from Pierce (Rockford, IL, USA) and 2,2,3,3,3-pentafluoro-1-propanol (PFP-OH) from Aldrich (Gillingham, England).

Materials for Examination

Hair samples were obtained from 30 subjects aged from 19 to 43 years, all of them deceased from fatal heroin overdose. Samples were twice decontaminated in 5 mL of methylene chloride for 2 min at room temperature. About 200 mg of hair were pulverized in a Retsch MM2-type ball mill (Haan, Germany). For screening purposes, only the first 3 cm from the root were pulverized and analyzed.

Sample Extraction

The protein matrix of the hair (about 100 mg) was destroyed by incubation in 1 mL of 1 N sodium hydroxide solution for 30 min at 95°C in presence of 10 ng IS-d_3. The homogenate was extracted with 5 mL of n-hexane/ethyl acetate (9:1, v/v) after acidification by 1 mL of concentrated acetic acid. Organic phase was washed with 1 mL 0.1 N NaOH followed by 1 mL 0.1 N HCl. Organic phase was evaporated to dryness, then derivatizated by propylation-propionylation with 100 μL of PFPA + 75 μL of PFP-OH at 70°C for 30 min. Dry extracts were dissolved in 25 μL of cyclohexane before injection, and transferred to a HP 7673 autosampler.

Gas Chromatography/Mass Spectrometry Method

A 1.5 μL portion of the derivatized extract was injected through the column (HP-1 capillary column, 12 m × 0.20 mm i.d.) of a

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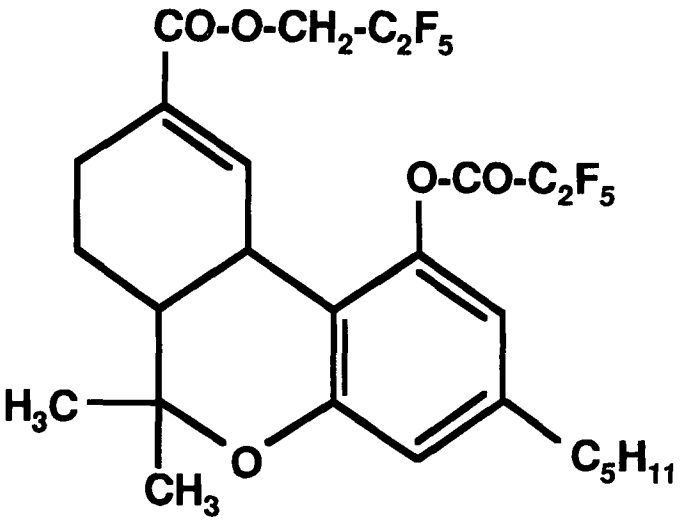

FIG. 1—*Structure of THC-COOH derivative.*

Hewlett Packard 5890 gas chromatograph coupled with a Hewlett Packard 5989 B MS Engine.

Injector temperature was 260°C and splitless injection was employed with a split-valve off-time of 0.75 min. The flow of carrier gas through the column was 1.0 mL/min (Helium, purity grade N55) using an inlet pressure controller. Temperature column was programmed to rise from an initial temperature of 60°C kept 1 min, to 290°C at 30°C/min and kept at 290°C for the final 3 min. The source temperature was 150°C. Mass spectra were recorded in the mass range m/z 400–700.

Methane was used as reactant gas at an apparent pressure of 1.4 torr in the ion source. PFPA derivatizes the phenol moiety of THC-COOH, while PFP-OH converts the carboxylic acid function to the corresponding ester. The structure of the pentafluoropropyl-, pentafluoropropionyl derivatives of THC-COOH is shown in Fig. 1. The mass spectra generated by using NCI is presented in Fig. 2. Under the chromatographic conditions used, there was no interference of the drug tested with any extractable endogenous material present in hair. Figures 3 and 4 are typical total ion and single ion chromatograms, respectively.

Results and Discussion

Analytes were identified and quantified based in comparison with the retention times and the relative abundance of three confirming ions to the deuterated internal standards.

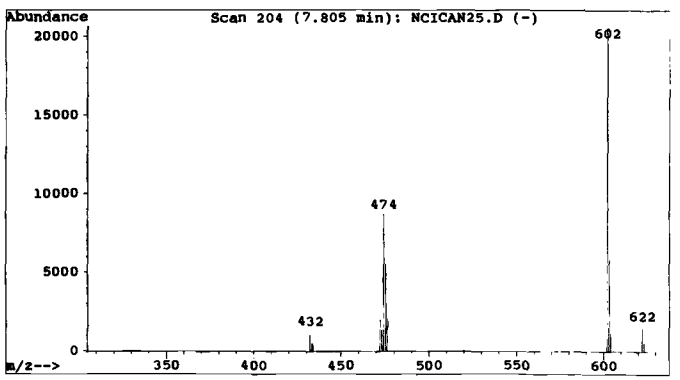

FIG. 2—*Mass spectra of THC-COOH derivative.*

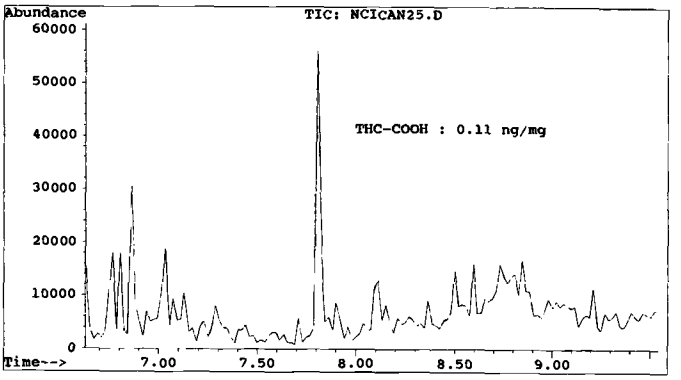

FIG. 3—*Total ion chromatogram of an hair extract (THC-COOH : 0.11 ng/mg).*

The ions chosen for monitoring were the base peaks at m/z 602 (THC-COOH) and 605 (IS-d_3). To be considered positive for THC-COOH, the selected ion monitoring analysis must show coincident peaks in the m/z 602, 622 and 474 ion current profiles. Retention time of THC-COOH was 7.79 min. Standard calibration curves were obtained by adding 1 (0.01 ng/mg), 5 (0.05 ng/mg), 10 (0.10 ng/mg), 50 (0.50 ng/mg), and 100 (1.00 ng/mg), ng of pure standards, prepared in methanol, to 100 mg of pulverized blank control hair (obtained from laboratory personnel). The staff members reported no cannabis exposure during the past year. In all cases, urine, collected at the same time, did not reveal cannabis traces. The use of blank hair was necessary, to be conform in the calibration conditions. The unavailability of such controlled material let us use personnel hair samples.

The correlation coefficient of the calibration curve was r = 0.995, showing linearity for THC-COOH between 0.01 and 1 ng/mg. 10 replicative control hair (100 mg) spiked with 50 ng of THC-COOH, resulting in a final concentration of 0.5 ng/mg were analyzed through the entire procedure in one analysis day. Concentration ranged (% CV) from 10.1%. By this method, concentrations of 5 pg/mg of THC-COOH could be detected. This detection limit (LOD) was evaluated by decreasing concentration of drug, until a response equivalent to three times the background noise was observed. By comparison with our previous paper [*12*], an increase of LOD by a factor of 20 was obtained using NCI. The use of a combination heptafluorobutyric anhydride-hexafluoroisopropanol as derivatizing agents did not improve the LOD, although a base peak ion with a higher mass was obtained (m/z 670). Extraction recovery, determined by comparing the representative peak area of extracted hair with the peak area of a methanolic standard at 0.5 ng/mg was 79.1 ± 3.1%.

Hair samples were obtained from subjects deceased from fatal heroin overdose, as demonstrated by high morphine concentrations in blood, and the presence of 6-monoacetylmorphine in urine. In all cases, it was possible to detect morphine, codeine and 6-monoacetylmorphine in the hair of the 30 addicts. Only 17 hair samples (57%) tested positive for THC-COOH. Concentrations ranged from 0.02 to 0.39 ng/mg. The average was 0.12 ng/mg. By this method, THC was also detected, in larger amount, but not quantified, as there is a potential risk of external contamination by monitoring this analyte. THC-COOH concentrations found are in accordance with the reported data of Hayes et al. [*10*], but largely lower than those of Moeller [*4*] (2 cases, 1.7 and 5.0 ng/mg) and Jurado [*6*] (70 cases, 0.06 − 3.87 ng/mg).

The weak incorporation rate of THC-COOH in hair is not sur-

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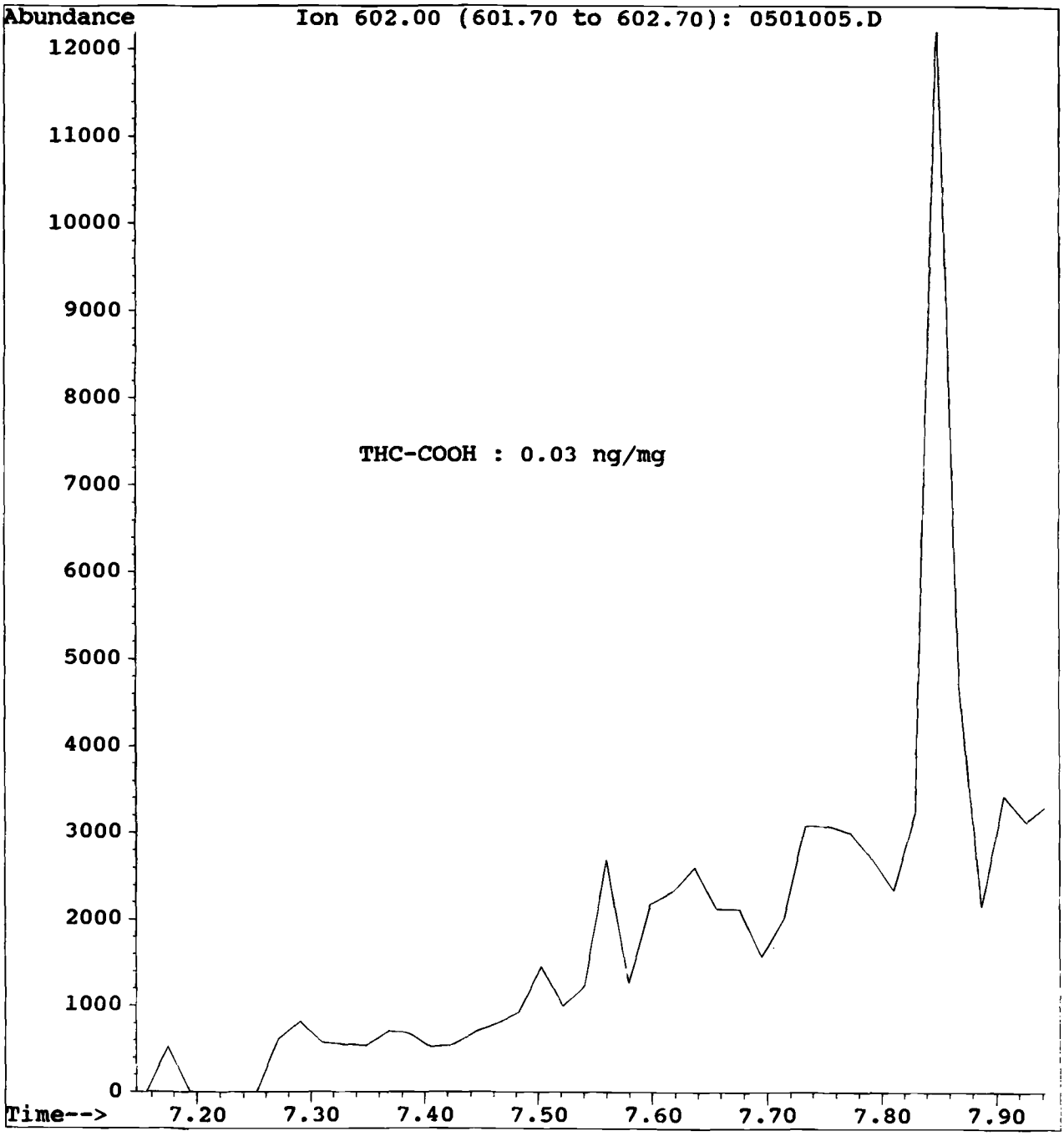

FIG. 4—*Single ion monitoring chromatogram with a THC-COOH concentration of 0.03 ng/mg.*

prising taking into consideration the three main factors which influence drug incorporation in hair, that are lipophilicity, melanin affinity and membrane permeability [*13*]. In spite of its high melanin affinity, THC-COOH is less incorporated into hair than basic drugs, as membrane permeability is based on the pH gradient between blood (pH 7.4) and hair matrix (pH <5).

Therefore, basic drugs such as cocaine, opiates or amphetamines are incorporated into hair in preference to acidic drugs, like THC-COOH. For forensic purposes, it was necessary to determine a cut-off to be considered positive. Hayes et al. [*10*] reported a positive specimen at 0.5 pg/10 mg hair using tandem mass-spectrometry. Based on our previous consideration [*14*], the positive cut-off was fixed at 0.05 ng/mg, which represents 10 times the limit of detection. In conclusion, a method for testing cannabis in hair is presented, which is based on the detection of THC-COOH by negative chemical ionization, mass spectrometry.

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