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Solid-Phase Extraction, Identification, and Quantitation of 11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid

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ABSTRACT: A procedure has been developed to extract and recover minute amounts of delta-9-carboxytetrahydrocannabinol (THC-COOH) from urine. A new non-isotopic internal standard is introduced to permit a chromatographic assay of the metabolite. The method affords a 91% recovery of 20 ng/mL of the THC-COOH acid from spiked urine with the assurance of a 3.8% coefficient of variation.

KEYWORDS: toxicology, tetrahydrocannabinol, chromatographic analysis

The C18 bonded-phase adsorption technique has been used by a number of workers [*1–4*] for the cleanup of urine in the isolation of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). The prepacked octadecylsilane cartridge has been shown to be equal or superior in efficacy to liquid-liquid extraction in the isolation of drugs from biological matrices. The sheer simplicity of this procedure has reduced the extraction time at least threefold and has enhanced the reproducibility of the analytical results. The solid-phase extraction procedure used by the authors' laboratory [*4*] was reevaluated and modified to enhance the recovery values of known amounts of THC-COOH added to urine. The chief merit of this procedure is its ability to recover low concentrations of THC-COOH consistently in urine. An inexpensive, nonisotopic internal standard (IS), meclofenamic acid, was introduced into the method. This compound behaves in the same way as THC-COOH throughout the entire process of extraction, derivatization to the dimethyl ester, and quantitative elution from a capillary gas chromatography column. Our use of the ion trap detector for gas chromatography has demonstrated the high sensitivity required for detection of low concentrations of THC-COOH in urine.

Experimental Procedure

Instrumentation

The gas chromatograph (GC) used was a Hewlett-Packard Model 5890 interfaced with a Model 800 Finnigan ion trap detector (ITD). The temperature of the transfer line

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between the two instruments was kept at 270°C. A 30M, SPB-1 capillary column (Supelco), 0.25 mm in inside diameter, was connected directly to the ion trap via the transfer line. The oven temperature was programed to rise from 200 to 270°C at a rate of 5°C/min. The temperatures of the injection port and the ion trap manifold were maintained at 250 and 220°C, respectively. The flow rate of helium was 1 mL/min through the capillary column. Sample injections were split 1:10 in the injection port. A full mass spectrum scan of 50 to 400 *m/z* was employed for identification purposes.

The C18 bonded-phase adsorption columns were Sep-Pak C18 cartridges (Waters Associates, Milford, MA). Trimethylammonium hydroxide (TMAH) was purchased from Sigma Chemical Co., St. Louis, MO, and methyl iodide, reagent grade, from Tokyo Kasei, Tokyo, Japan. The THC-COOH standard was obtained from Alltech Associates, Deerfield, IL. The meclofenamic acid was a gift of Parke-Davis, Ann Arbor, MI. The disposable 20-mL syringe was a product of Becton-Dickinson & Co., Rutherford, NJ.

The derivatizing reagent was prepared by dissolving 20 mg of TMAH in 0.1 mL of methanol, adding 1 mL of dimethylsulfoxide (DMSO), and mixing them. It was prepared fresh for each day's use.

Sample Preparation

Ten millilitres of urine were introduced into a 50-mL Erlenmeyer flask and made alkaline by the addition of 1 mL of 10% potassium hydroxide. Fifty microlitres of 25 ng/µL meclofenamic acid were added to the alkaline urine as the IS. The flask was capped with aluminum foil and placed in an oven at 100°C for 15 min for alkaline hydrolysis. The hydrolysate was cooled to room temperature in a cold water bath and then acidified with 1.5 mL of glacial acetic acid.

A C18 Sep-Pak cartridge was attached to a 20-mL disposable syringe and primed by passing 5 mL of methanol through it, washed with 5 mL of water, and, finally, washed with 5 mL of 1*N* acetic acid. The urine hydrolysate was passed through the column at a rate of 5 mL/min. The column was washed with 2 mL of 40% acetonitrile in 1*N* acetic acid. THC-COOH was eluted with 2 mL of methanol and collected in a 5-mL conical tip glass centrifuge tube. The eluant was evaporated to dryness in a heating block, at approximately 60°C, under a flow of air. The derivatization procedure was similar to that described by Whiting and Manders [5]. The extracted residue was treated with 70 µL of TMAH/DMSO for 2 min, followed by 5 µL of methyl iodide for 5 min, with agitation on the vortex mixer after the addition of each reagent. The mixture was made acidic with 0.2 mL of 1*N* acetic acid, extracted with 1 mL of cyclohexane, and centrifuged at 1000 × *g* for 5 min. The supernatant was transferred to another conical tip glass tube and evaporated to dryness at 60°C under a flow of air. Fifty microlitres of methanol were used to dissolve the residue and a 2 µL aliquot was injected into the GC/ITD.

Results and Discussion

The retention times for meclofenamic acid and THC-COOH were typically 10 and 15 min, respectively. A mass spectrum of THC-COOH extracted from a urine sample, with a scan range of 50 to 400 *m/z*, is shown in Fig. 1. The 313 base peak ion of the derivatized THC-COOH and 242 base peak ion of the derivatized IS were monitored for quantitation. A plot of the peak area ratios for these ions was linear over the concentration range of 20 to 100 ng/mL of urine, which is the assay range of most case samples. The recovery of THC-COOH added to urine samples was determined to be 97% at a concentration of 100 ng/mL, 93% at 50 ng/mL, and 91% at 20 ng/mL with a coefficient of variation of 2.5, 2.6, and 3.8%, respectively. The correlation of the coefficient for the curve was 0.998. For the low concentrations, 10 mL of urine appears to be optimal. Since the

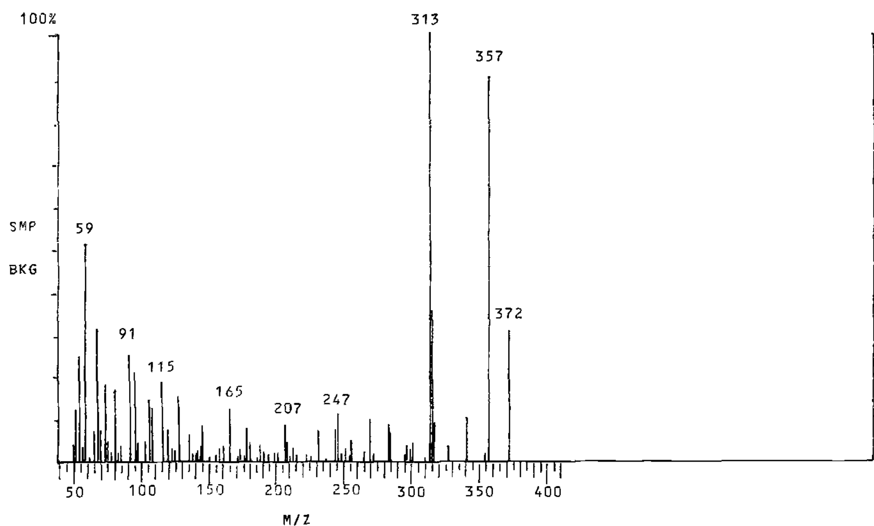

FIG. 1—*A normalized mass spectrum of the dimethyl derivative of THC-COOH found in an actual urine sample.*

authors' laboratory employs an enzyme-multiplied immunoassay technique, EMIT® d.a.u.™, for screening all urine samples for controlled substances, the volume of urine required for extraction can be decreased to correlate with the higher levels obtained for the total cannabinoids by EMIT d.a.u., thus reducing the background noise for spectral analysis. The full mass spectrum of the derivatized THC-COOH in the analyzed sample was examined for forensic science identification. Twenty nanograms per millilitre of THC-COOH in urine appears to be the lowest concentration level at which the metabolite can be absolutely identified by full mass spectral examination.

Generally, we use Sep-Pak cartridges for extracting urine. However, other C18 columns such as the Baker-10 SPE octadecyl disposable column, 3-mL size, can be used interchangably without differences in extraction characteristics. The Baker-10 column is ostensibly designed for processing up to ten samples simultaneously in a manifold system; also, Baker-10 columns can be inserted onto the nub of a disposable syringe for processing individual samples. Since the preparation of this manuscript, J. T. Baker Inc. has replaced Baker-10 SPE with Bakerbond spe columns.

The hydrolysis time of 15 min appears to be optimal for fresh urine samples. In urine samples which have aged in transport, or have been stored under refrigeration for a period of 3 to 5 months, a partial autolysis seems to occur. The hydrolytic step has little effect on the extraction yield of such samples.

The efficiency of extraction over the pH range of 1 to 6 was reevaluated for maximum yield. No significant difference could be observed in the recovery of the tetrahydrocannabinol (THC) metabolite in this pH range.

The amount of acetonitrile used to wash the THC-COOH adsorbed on the column can be critical. An excessive amount can reduce the yield of the metabolite. Two millilitres of wash solution appears to be adequate for fresh urine. Visual observation of the eluting pigments can be an effective method of determining when the pigmentation has been removed. An additional amount of wash, usually 1 mL, may be required to elute heavy pigmentation.

A study was conducted to determine if the silylation of THC-COOH could produce a greater sensitivity than that obtained by methylation. The compounds *N,O*-

bis(trimethylsilyl)acetamide (BSA) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were used to prepare the silyl derivatives of THC-COOH. Comparison of the gas chromatography results for these silyl compounds with those for the methylated compounds using our standard procedure showed no advantage of improved peak responses. The silylated compounds were relatively unstable and had to be chromatographed within minutes after preparation. The methylated THC-COOH was fairly stable in air. The extracted residues, which were derivatized with TMAH/DMSO and kept in a dessicator for a week, exhibited no diminution of THC-COOH concentration.

The use of meclofenamic acid offsets the use of deuterated THC-COOH as an internal standard in more than one way. It is discretely separated from THC-COOH chromatographically (see Fig. 2), while the isotopes have the same or nearly the same retention time as that for the natural compound. El Sohly [6] discusses the problem using the base peak in the mass spectrum of the methylated THC-COOH to measure concentration. A difference in the fragmentation mechanism between the natural metabolite and the isotope produces a 4 to 5% error in the measurement of the base peak of the natural THC-COOH. There is a decided difference between meclofenamic acid and THC-COOH in the spectral configuration. The spectrum of the meclofenamic acid is shown in Fig. 3. Moreover, the deuterated THC-COOH is relatively expensive to purchase. Meclofenamic acid is not a controlled drug and is commonly used as an antiinflammatory agent. In the urine, meclofenamic acid behaves in the same way as THC-COOH in each step of our procedure. It is not affected by alkaline hydrolysis, not eluted by acetonitrile solution wash, extracted in acid milieu, and derivatized by methylation for chromatography.

Our quantitative procedure was established primarily to assess the accuracy and precision of the method. We are frequently contacted by investigators and legal officers to interpret the quantitative values from THC-COOH analyses of randomly collected urine samples to determine whether the subject had passively inhaled marijuana smoke. Carefully studied results [7,8] have shown that, under realistic conditions of social smoking, passive absorption of marijuana smoke produces no more than 7 ng of THC-COOH per millilitre of urine. This concentration level is significantly lower than the 20 ng/mL cutoff value, which is the minimum level for our identification procedure based on full-scan mass spectrum examinations. Even at levels lower than 20 ng/mL, the source of THC-COOH in urine cannot be attributed solely to passive inhalation.

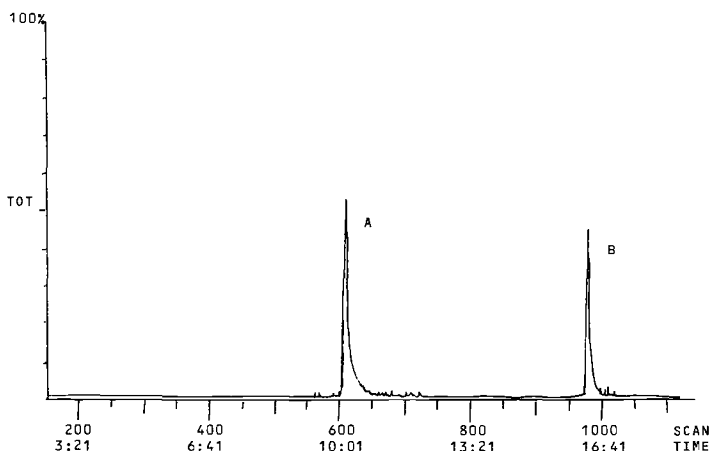

FIG. 2—*Ion chromatogram for* (A) *meclofenamic acid (the internal standard) and* (B) *THC-COOH from a urine extract. GC/ITD was used in the selected ion monitoring mode to delineate the peaks.*

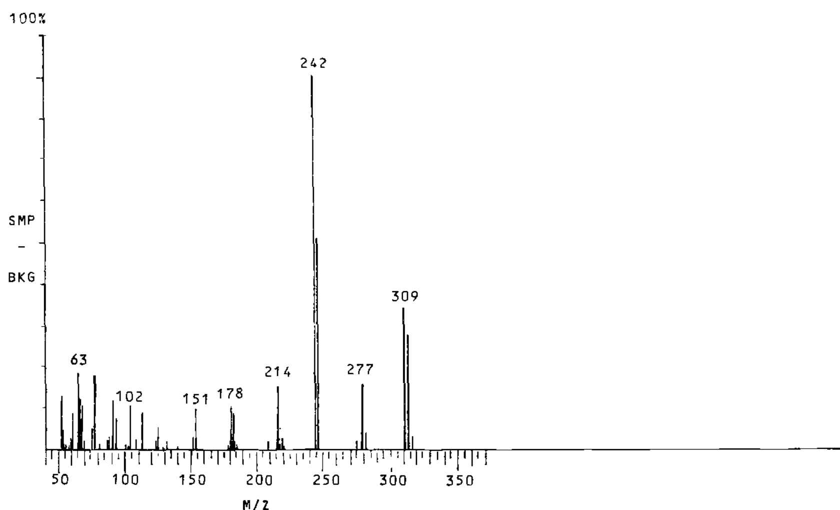

FIG. 3—*A normalized mass spectrum for the internal standard, methylated meclofenamic acid, extracted from urine.*

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