Table II. Determination of Total ^{131}I in Formaldehyde-Preserved Milk[a]

^{131}I activity		
added	found[b]	% error
21.3 nCi/L	20.5 ± 1.5 nCi/L	3.8
162.0 pCi/L	164.7 ± 11.8 pCi/L	1.7
81.2 pCi/L	85.9 ± 7.7 pCi/L	5.8
26.2 pCi/L	27.3 ± 2.0 pCi/L	4.2
17.7 pCi/L	19.2 ± 1.4 pCi/L	8.5

[a] ^{131}I activity was radioassayed as the palladous iodide precipitate. [b] Six separate determinations were performed.

the γ-counting system. The iodine-131 in the filtrate was extractable with carbon tetrachloride after oxidation with 30% hydrogen peroxide solution. The percent iodine-131 recovered from the milk protein is the percent iodine-131 in the filtrate.

As shown in Table I, the percent ^{131}I recovered from the milk protein is greater than 90% at both 97 and 120 °C when 8 M HCl was used for the digestion. However, when the same HCl concentration was used and the protein digested at 55 °C, only 27% iodine-131 was recovered. With the digestion temperature remaining at 97 °C, iodine-131 recovery decreased with decreasing HCl concentration. At 1 M HCl, all iodine-131 remained protein-bound after 3 h of digestion at 97 °C. These results indicate that the liberation of iodine-131 from milk protein depends on the digest temperature and the HCl concentration.

The mechanism for the enhancement of protein-bound iodine in milk protein in the presence of formaldehyde is not clear. It may be due to the change in net protein charge and/or protein conformational changes as a result of the reaction of formaldehyde with the primary amino groups of the milk protein which influence the binding of iodine to the protein (*13*). Iodine is bound to the tyrosine residue of the milk protein; when the milk protein is digested with concentrated HCl at high temperature, the more labile amino acid groups such as tyrosine, threonine, and serine are easier attacked by the acid than the more stable amino acid groups. This may explain the relatively short time needed in the digestion step to liberate iodine from milk protein.

The accuracy of the method reported here was tested by analyzing 1-L milk samples spiked with iodine-131 activity which ranged from 21 nCi/L to 17.7 pCi/L. Table II shows the experimental results compared to the known iodine-131 concentration in the milk samples. The experimental results are in good agreement, within experimental error, with the known iodine-131 concentration. Palladous iodide obtained from samples with picocurie iodine-131 concentrations were β-counted for 200 min to minimize the counting errors.

Registry No. ^{131}I, 10043-66-0; formaldehyde, 50-00-0.

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Analysis of Urine for 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic Acid Using Sep-PAK Cartridges for Sample Cleanup

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The widespread use of marihuana requires a rapid and reliable procedure for its detection in urine for routine forensic use. The major active principal of marihuana, Δ^9-tetrahydrocannabinol, rapidly disappears from human blood. It is metabolized mostly to 11-nor- Δ^9-tetrahydrocannabinol-9-carboxylic acid (9-THC-COOH) and excreted in the urine. Most of the metabolite is found in urine as a conjugated form of a glucuronide (*1*).

Urine is generally the preferred specimen for detecting the wrongful use of drugs. The collection is convenient, and the concentrations of drugs and their metabolites are much higher in urine than in the blood. In the case of cannabinoids, the relatively low concentration of 9-THC-COOH requires large amounts of urine as sample. This necessitates an efficient clean-up procedure.

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A number of methods have been reported in the literature for the detection of 9-THC-COOH in urine. These methods include thin-layer chromatography (TLC), enzyme multiplied immunoassay technique (EMIT), radioimmunoassay (RIA), gas chromatography (GC), and gas chromatograhy/mass spectrometry (GC/MS). EMIT and RIA do no require an extraction of samples for analysis, but they do not provide a specific identification of 9-THC-COOH. GC/MS is the preferred technique for forensic identification of drugs such as 9-THC-COOH (*2*). Double liquid-to-liquid extraction procedures are used to prepare samples for GC (*3*) and GC/MS (*4*, *5*). While TLC provides cleaner samples for GC/MS (*6*), this approach is time-consuming. Bond Elute and high-pressure liquid chromatography (HPLC) (*7*) and Bond Elute and TLC (*8*) in combination are used for sample cleanup and detection of 9-THC-COOH. A TLC method (*9*) is used to detect 9-THC-COOH after a liquid-to-liquid extraction of urine specimens.

The use of GC, HPLC, or TLC as a detection method falls short of identifying 9-THC-COOH for forensic purposes. This paper describes a rapid cleanup of urine hydrolysate using Sep-PAK C_{18} cartridges which yields upon analysis a well-delineated mass spectrum of 9-THC-COOH. Sep-PAK C_{18} cartridges are used in the extraction of drugs such as tricyclic antidepressants (*10*), amphetamines (*11*, *12*), and steroids (*13*) in urine. A small Sep-PAK C_{18} cartridge, 1 cm × 1 cm, containing a octadecylsilane bonded phase, can extract 95% of labeled steroids from a urine hydrolysate equivalent to 100 mL of urine (*12*).

The extraction method presented in this paper facilitates a rapid and reliable identification of 9-THC-COOH for use in law enforcement activities.

EXPERIMENTAL SECTION

Materials and reagents used in this procedure include the following: Sep-PAK C_{18} cartridges, Waters Associates, Milford, MA; Plaspak disposable syringes, 20 mL, Becton-Dickson, Rutherford, NJ; 11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid (8-THC-COOH), gift from U.S. Armed Forces Institute of Pathology, Washington DC; 9-THC-COOH, Research Triangle Institute, Research Triangle Park, NC; tetramethylammonium hydroxide (TMAH) solutions and 5-α-cholestane, Sigma Chemical Co., St. Louis, MO. All other chemicals and solvents were of ACS grade.

Gas Chromatography/Mass Spectrometry. A Finnigan 1020 GC/MS (San Jose, CA) was used. The glass column (6 ft × $^1/_8$ in.) was packed with OV-1 on 100/200 mesh Gas Chrom Q (Applied Science, College Station, PA). The derivative was analyzed at temperatures programmed from 200 to 270 °C at a rate of 5 °C/min. The temperatures of the injector port, jet separator, and ion source were 265 °C, 240 °C, and 80 °C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL/min. Electron impact ionization mode was used with ionization effected at an electron energy of 70 eV. The retention time for 9-THC-COOH was in the range of 5–6 min under these conditions. The spectra were scanned from m/z 45 to 440 with a scan time of 1.95 s.

Alkaline Hydrolysis. The urine was hydrolyzed by adding 1 mL of methanolic potassium hydroxide (10% w/v) to 10 mL of urine in a 50-mL Erlenmeyer flask (*8*). The flask was capped with aluminum foil and placed in an oven at 100 °C for 15–20 min. The flask was cooled in cold water and 1.5 mL of glacial acetic acid was added to adjust the pH to 3.0–4.0.

Extraction. A Sep-PAK cartridge was inserted onto a 20-mL disposable syringe, primed with 2 mL of methanol, and then washed with 5 mL of water. Urine hydrolysate was introduced into the syringe and passed through the cartridge at a rate of approximately 5 mL/min. The cartridge was washed with 5 mL of water and then with 5 mL of acetonitrile:water (4:6 v/v). Two milliliters of methanol was passed through the cartridge to elute 9-THC-COOH into a 13 × 100 mm tube, and the eluant was evaporated to dryness under nitrogen on a hot water bath.

Esterification. The dried extract was treated with 70 μL of aqueous 25% TMAH–dimethyl sulfoxide (1:20), according to a method by Whiting and Manders (*5*). The tube was agitated on a vortex mixer and allowed to stand for 2 min. Five microliters of iodomethane was added to the mixture and the mixture was agitated and allowed to stand for 5 min.

The mixture was then acidified with 0.2 mL of 1 M acetic acid. One milliliter of cyclohexane was added and the tube agitated for 1 min. (If the interface was difficult to see, a drop of 0.2% w/v aqueous methyl orange was added to the mixture.) The mixture was centrifuged when necessary. The upper layer was removed with a Pasteur pipet and collected in a 5-mL conical tube. The lower aqueous layer was extracted again with 1 mL of cyclohexane. The extracts were combined and evaporated to dryness under nitrogen. The residue was dissolved in 20 μL of methanol and a 4–5-μL aliquot was injected into the GC/MS.

Recovery. The efficacy of extraction was tested by determining the recovery of the various amounts of 8-THC-COOH added to the urine. Since the amount of standard 9-THC-COOH available to us was only sufficient for mass spectral examination, 8-THC-COOH was used instead for the recovery study. Three different concentrations were used: 50 ng, 100 ng, and 200 ng in 1 mL of urine. Each of these was extracted under standard conditions. The final extract was dissolved in 50 μL of methanol containing 0.5 mg/mL cholestane and was subjected to gas chromatography under standard conditions. The mean recovery values were 78.8% ± 4.7% standard deviation, 85.3% ± 4.4% standard deviation, and 89.8% ± 3.3% standard deviation for 50 ng, 100 ng, and 200 ng, respectively, based on a replicate of five samples. The calibration was linear over the three concentrations.

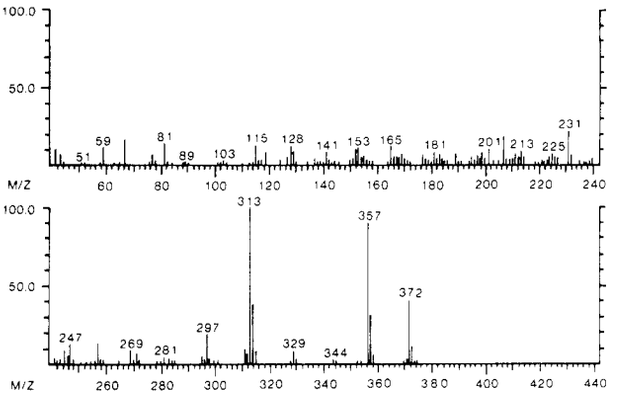

Figure 1. Mass spectrum of 9-THC-COOH extracted from a 20-day-old urine sample which had been sent by mail to this laboratory.

RESULTS AND DISCUSSION

Our method was tested on more than a dozen suspect urine samples received from the field for analysis. The spectra obtained from these analyses showed that they were identical with those of the derivatized standard of 9-THC-COOH. Figure 1 shows a spectrum obtained from one of the urine specimens with peaks at m/z 313, 357, and 372, in the order of relative abundance, which corresponds to the characteristic spectrum of dimethyl-9-THC-COOH. This urine specimen was collected and stored at ambient temperature prior to being sent by mail to this laboratory. Twenty days had passed by the time the analysis was performed. The literature is devoid of data concerning the stability of 9-THC-COOH or its glucuronide in urine specimens over any length of time. It was of interest to note that the compound appears relatively stable over a 20-day period.

The use of Sep-PAK cartridges precludes some of the problems inherent in liquid-to-liquid extractions. It enables a quantitative and reproducible recovery of 9-THC-COOH from a single elution of 2 mL of solvent. It keeps sample manipulation to a minimum. With our method, a sample is hydrolyzed, extracted, and readied for GC/MS in 2 h. A superior cleanup is evidenced by a marked improvement in the reduction of background interferences in the mass spectrum, allowing detection of less than 10 ng/mL of 9-THC-COOH.

For laboratories processing a large number of urine specimens, EMIT and RIA procedures are convenient screening tests for excluding negative samples. The validity of their use was shown in the investigation conducted by O'Conner and Rejent (*14*) by confimring positive results with GC/MS procedures. These screening procedures are not only useful in eliminating negative samples, they also provide a semiquantitative measure of the level of 9-THC-COOH in urine. This information can be useful in reducing the sample volume in a Sep-PAK extraction.

The effect of pH of the hydrolysate sample and its extraction in the cartridge was examined over a range of pH 2.0–8.5. The results indicated that the pH was not critical over this broad range and recovery of 2 μg of 8-THC-COOH in 10 mL of urine was optimal of pH 2.0–5.5.

We found that specimens of freshly voided urine could be analyzed by taking the methanolic eluant from Sep-PAK cartridges, evaporating it to dryness under nitrogen, and an-

alyzing the extract by GC/MS by on-column methylation. A 4:1 ratio of a methanolic solution of the sample extract and 10% TMAH in methanol in tandem in a syringe was injected onto the GC column under standard GC/MS conditions. The results were similar to those obtained from eluants which were further cleaned up in phase transfer alkylation described above.

The advent of these disposable cartridges presages a wider use in analytical toxicology because of a number of inherent advantages. They provide a rapid extraction of a relatively large volume of urine with a small volume of solvent. The recovery of a polar cannabinoid compound is virtually quantitative and the results show excellent cleanup and reproducibility. We feel that further adaptions of these cartridges to the extraction of other drugs-of-abuse and organic poisons in biological materials are in the offing.

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Registry No. 9-THC-COOH, 56354-06-4.

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Recovery of Dichlorobenzenes, Nitrobenzene, and Naphthalene during Evaporative Concentration

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Extraction of several semivolatile compounds from a water sample, notably dichlorobenzenes, nitrobenzene, and naphthalene, can be achieved with methylene chloride, as indicated by the analytical methods proposed in the *Federal Register* (*1*) and implemented by the Environmental Protection Agency (*2*). Concentration of the extract is carried out with a Kuderna–Danish concentrator (K-D) equipped with a Snyder column and a graduated receiver. In this laboratory, the extract is concentrated further in a warm water bath by passing nitrogen over the extract surface. The total process combines the best macro and micro concentration techniques that were evaluated by Erickson et al. (*3*) for a variety of organic compounds. The procedure is neither labor-intensive nor time-consuming. However, it can lead to both low and nonreproducible recoveries of these compounds, typically from about 40 to 90%, as reported by the EPA (*2*) and the U.S. Geological Survey (*4*). Clearly, meaningful determinations cannot be performed with these compounds until losses are significantly reduced.

The work of Higgins and Guerin (*5*) showed that losses of naphthalene from spiked solvent systems could be minimized by the use of a Tenax-GC trapping system during evaporative concentration and suggested the possibility that the same trap might be employed for the benzene derivatives as well.

Although the Tenax trapping system proved to be technically feasible, the entire procedure was complicated and required far too much time for a production laboratory. Therefore, a simpler and faster technique was sought.

To establish whether conditions in the last stage of evaporative analyte concentration could be standardized to improve the recovery of the benzene derivatives and naphthalene, a precision pressure regulator and a toggle cutoff valve were added to the nitrogen line of the evaporative concentrator. Subsequent testing established that these minor alterations significantly improved recoveries of all compounds.

EXPERIMENTAL SECTION

Materials. Naphthalene, nitrobenzene, and *o*- and *p*-dichlorobenzene standards were obtained from EPA, Research Triangle Park, NC. Solvents (hexane, isooctane, methanol, methylene chloride, and toluene) were from Burdick and Jackson, Muskegon, MI.

Apparatus. The following were employed: a 250-mL K-D with a 10-mL graduated concentrator tube and a one-ball Snyder column; an evaporative concentrator (N-Evap, Organomation Associates, Inc.) with a 1 to 10 psi pressure regulator (Model 8601, Brooks Instruments) and an in-line togggle cutoff valve. Analyses were performed with a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector (FID). Separations were accomplished with a 0.2 mm i.d. by 12 m fused silica capillary column with a thin film coating of OV-101. Conditions of analyses were as follows: column flow, 1.7 mL/min nitrogen, splitless injection with injector at 200 °C; initial oven temperature 45 °C for 4 min and then programmed at 5 °C/min for 7 min; FID at 275 °C, hydrogen 30 mL/min, air 410 mL/min, nitrogen makeup 28 mL/min.

Procedure. Analyte standards were prepared in toluene. About 75 mL of solution (50 mL of hexane and 25 mL of methylene chloride) was placed in the K–D. (In this laboratory, water samples are extracted with methylene chloride. Sufficient hexane is then added to bring the methylene chloride to the surface of the water. This technique has been found to be helpful to eliminate emulsion difficulties which are frequently encountered with environmental samples.) Standard solutions were transferred to this mixture with volumetric micropipets. A boiling chip and about 2 mL of isooctane "keeper" (to prevent total solvent and analyte loss) were added to the solution. A Snyder column was