separate apparatuses), but now five samples can be processed automatically overnight, resulting in more than a 50% reduction in the time required of the analyst per sample.

ACKNOWLEDGMENT

We thank Louis Alexander and Ralph O'Connor for their analytical support and especially David Griffin, of Universal Scientific, Inc., for his invaluable assistance in engineering and selecting compatible components.

Registry No. TCDD, 1746-01-6.

LITERATURE CITED

(1) Rappe, C. *Environ. Sci. Technol.* **1984**, *18*, 78-90.
(2) Reggiani, G. *Regul. Toxicol. Pharmacol.* **1981**, *1*, 211-243.
(3) Kimbrough, R. D. *Drug Metab. Rev.* **1982**, *13*, 485-497.
(4) Patterson, D. G.; Holler, J. S.; Lapeza, C. R.; Alexander, L. R.; Groce, D. F.; O'Connor, R. C.; Smith, S. J.; Liddle, J. A.; Needham, L. L. *Anal. Chem.* **1986**, *58*, 705-713.
(5) Little, C. J.; Stahel, O.; Lindner, W.; Frei, R. W. *Am. Lab. (Fairfield, Conn.)* **1984**, *16*, 120-129.
(6) Ramstad, T.; Hahle, N. H.; Matalon, R. *Anal. Chem.* **1977**, *49*, 386-390.
(7) Smith, L. M.; Stalling, D. L.; Johnson, J. L. *Anal. Chem.* **1984**, *56*, 1830-1842.
(8) Tindle, R. C.; Stalling, D. L. *Anal. Chem.* **1972**, *44*, 1768-1773.
(9) Stalling, D. L.; Smith, L. M.; Petty, J. D. *ASTM Spec. Tech. Publ.* **1979**, *686*, 302.

RECEIVED for review September 27, 1985. Accepted November 19, 1985. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Solid-Supported Reagents in the Determination of Cannabinoids in Plasma

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A model for the automation of analytical derivatization reactions in the analysis of Δ^9-tetrahydrocannabinol (Δ^9-THC) and two of its in vivo metabolites was investigated. A solid-supported reagent based on XAD-2 was used to both extract and pentafluorobenzylate Δ^9-THC, 11-hydroxy-Δ^9-THC, and 11-nor-9-carboxy-Δ^9-THC. Optimum conditions for isolation of the analytes from plasma as their pentafluorobenzyl derivatives were developed. Gas chromatographic analysis was carried out using both electron capture detection and mass spectrometry/negative ion chemical ionization detection.

The pharmacology, clinical application, toxicology, and abuse of marijuana and cannabinoid-based drugs continue to be of interest to scientific, medical, and forensic scientists. Such investigations often require quantitative determination of the drugs and/or their metabolites in numerous samples. The demand for analysis of large numbers of cannabinoid-containing samples in these studies suggests the need for automated methods of analysis.

Many methods for determining cannabinoids in biological matrices have been reported and reviewed (*1*); however, few are automated. It is now recognized that automation of sample preparation is an important process, and this area is a focus of current research and development (*2*). For biological samples, a difficulty arises during separation of lipophilic drugs, such as the cannabinoids, from the biological matrix, which is a complex mixture of lipids, proteins, and water. Extraction of biological samples with water-immiscible solvents is commonly used. This usually requires manual separation of the two phases and, if emulsions occur, centrifugation. Such procedures, as any requiring separation of immiscible liquids, are relatively difficult to automate. Consequently, solid adsorbents are used frequently for isolation of analytes in automated and semiautomated techniques for the determination of drugs in biological aqueous samples (*3-5*). A second problem arises from the frequent use of analytical derivatization reactions for the analysis of cannabinoids in biomedical samples (*1*). Such reactions are also difficult to automate.

Recently, derivatization procedures based on solid-supported reagents have been suggested as an approach to automation of methods requiring analytical derivatization reactions for analytes in air (*6, 7*) and aqueous matrices (*8, 9*). We investigated a reagent consisting of pentafluorobenzyl bromide (PFBBr) deposited upon XAD-2 resin (*9*). Pentafluorobenzyl bromide is used in the preparation of electrophoric derivatives for gas chromatographic (GC) analysis with electron capture detection (ECD) or mass spectrometric (MS) detection in the negative ion chemical ionization (NICI) mode. The resin is a polystyrene divinylbenzene cross-linked copolymer compatible with aqueous acid, aqueous base, and all organic solvents. It is the basis for a number of automated and semiautomated sample preparation methods for analysis of organic analytes (*3-5*). We sought to establish the feasibility *of using solid-supported reagents in the determination of* cannabinoids in a biological matrix. In the present work solid-supported reagent was the basis of GC/ECD and GC/MS/NICI techniques for the determination of Δ^9-THC, 11-OH-Δ^9-THC, and 11-nor-9-carboxy-Δ^9-THC from plasma. Both the isolation of the analytes from plasma *and derivatization* were affected by use of solid support demonstrating that solid-supported reactions can be used for the determination of cannabinoids from biological matrices.

EXPERIMENTAL SECTION

Materials. All cannabinoids, including radio- and deuterio-labeled species, were obtained under the auspices of the Canadian Food and Drug Administration and were supplied by the National

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Institute on Drug Abuse (NIDA), USA. XAD-2 resin was obtained from British Drug Houses in the form of 0.3–0.5-mm beads. Gas chromatographic supplies were obtained from Chromatographic Specialties, Brockville, Ontario. Pentafluorobenzyl bromide was purchased from Caledon Laboratories, Georgetown, Ontario. The pentafluorobenzyl (PFB) standards of the cannabinoids were synthesized by reaction with PFBBr in acetone using K_2CO_3 as a catalyst (*9*). All glassware was silanized by treating with 10% dimethyldichlorosilane in toluene for 30 min and washing twice with methanol (MeOH) and twice with water. The glassware was then dried at 100 °C. Thin-layer plates were purchased from Brinkman (Canada) and had a thickness of 0.25 mm of silica gel with F254 fluorescence marker. The reverse-phase TLC plates were prepared by exposing glass-backed silica gel plates to an atmosphere of dichlorodimethylsilane overnight and washing the plate with methanol. The plates were cleaned by developing twice with tetrahydrofuran. Subsequently, the plates were dried at 100 °C overnight.

Instrumentation. Packed-column GC analyses were carried out on a Hewlett-Packard 5710 gas chromatograph equipped with an electron capture detector (ECD) and using a 6 ft × $^1/_8$ in. glass column packed with 1.5% OV-17 on Chromsorb-W 80/100 mesh. The oven was programmed from 270 to 300 °C at 8 °C/min. The carrier gas was 15% argon in methane maintained at a flow rate of 15 mL/min. The detector was maintained at 350 °C.

Capillary GC analysis was carried out on a Hewlett-Packard 5910 gas chromatograph, using direct on-column injection. The column was a J&W DB-17N with a film thickness of 0.15 μm and a length of 15 m. The PFB ester of tetracosanoic acid ($CH_3(CH_2)_{22}CO_2PFB$; PFB–C_{24}) was used as external standard. The injection aliquot was 0.5 μL from a final extract volume of 200 μL of toluene. The oven temperature was programmed from 260 to 300 °C at 6 °C/min and held at 300 °C for 5 min. The average flow rate of the hydrogen carrier gas at 210 °C was 40 cm/s at a head pressure of 6 psi.

For both qualitative and quantitative MS analyses of Δ^9-THC and 11-OH-Δ^9-THC derivatives a DB-15W column with a film thickness of 1 μm and a length of 15 m was used. The carrier gas was H_2 at a linear velocity of 78 cm/s at 200 °C, which was fed directly into the ion source. Samples were injected in the splitless mode in 2-μL aliquots. The oven was maintained at 220 °C for 1 min and then programmed to 310 °C at 20 °C/min. Mass spectrometric analyses of the 9-carboxy metabolite were carried out on a 10-M DB 1701 column with an inner diameter of 0.32 mm and a film thickness of 0.25 μm. The injector port and transfer oven temperature were set at 290 °C, and the column temperature was programmed from 200 to 295 °C at 20 °C/min.

Negative ion chemical ionization MS was performed on a Finnigan MAT Model 4500 GC/MS/data system. The instrument was operated in the normal CI mode with H_2/CH_4 as the CI gas. Hydrogen was introduced as the carrier gas and sufficient CH_4 was bled into the ion source to raise the ion chamber pressure to approximately 0.5 torr. Under these conditions the manifold pressure reading was 4×10^{-5} torr. The temperature of the ion chamber was maintained at 180 °C for the analysis of these analytes as their PFB derivatives.

Procedure. Preparation of Resin. The resin, obtained commercially in the form of 30–50 mesh beads, was prepared by procedures described elsewhere (*6*). Fines and inorganic salts (used to inhibit microbial growth during shipment and storage) were removed by repeatedly suspending the resin in water and aspirating the supernatant until it was clear and at neutral pH. Oligomers left over from the manufacturing process were removed by extracting with MeOH and diethyl ether in a Soxhlet extractor. The residual ether was subsequently removed in vacuo at 21 mm Hg. The dried resin was then stored in a screw-cap vial at room temperature until use. The cleaned and dried resin has a surface area of 300 m^2/g, a pore volume of 500 μL/g, a pore size of 90 nm, and a specific gravity of 1 (*10*).

Gas Chromatographic Analysis. For GC/ECD, quantitation was by the external standard method. In the case of the packed column, the external standard was the PFB derivative of cannabinol (PFB–CNB), and for the capillary column the external standard was PFB tetracosanoate (PFB–C_{24}). Under the described conditions, on the packed column the retention time of the PFB derivative of Δ^9-THC (PFB–THC) was 4.6 min, PFB–CNB was 5.2 min, and 1-OPFB-11-trimethylsiloxy-Δ^9-THC (1-OPFB-11-OTMS-THC) was 6.5 min. On the capillary column, the retention times of the analytes, PFB–THC, 1-OPFB-11-OTMS-THC and 1-OFBF-9-CO_2PFB-THC, and the internal standard, PFB–C_{24}, were 7.45, 9.35, 14.45, and 10.05 min, respectively.

GC/MS/NICI quantitation was by the internal standard method using trideuterated analogues of the three analytes. For the analysis of Δ^9-THC the ions monitored were m/z 313 for the analyte and m/z 316 for the deuterated internal standard; for analysis of the 11-OH-Δ^9-THC the corresponding ions monitored were m/z 401 and m/z 404; and for the analysis of 11-nor-9-carboxy-Δ9-THC the ions monitored were m/z 523 and 526 for the analyte and its deuterted analogue, respectively. The retention times on the GC/MS system were 3.7 min for PFB–THC and 4.3 min for the 1-OPFB-11-OHTMS-THC. The retention time for the PFB derivative of the 9-carboxy metabolite was 5.0 min.

Adsorption of Cannabinoids from Plasma on XAD-2. Two hundred milligrams of XAD-2 was added to 1 mL of plasma containing 25 000 cpm of [^{14}C]-Δ^9-THC and 130 000 cpm of [^{3}H]-11-OH-Δ^9-THC. This was carried out with unmodified plasma and modified plasma containing 15% CH_3CN. Immediately after addition of plasma to the resin a 10-μL aliquot of plasma supernatant was removed for counting. The mixture was shaken for 90 min with 10 μL being removed at 5, 10, 30, 60, and 90 min for counting.

Pentafluorobenzylation of Pure Cannabinoids. *By Solid-Supported Reagent.* Two micrograms each of Δ^9-THC and 11-OH-Δ^9-THC was added to 4 mL of water and 200 mg of resin. The reaction mixture was shaken for 5 min. The buffer was removed by aspiration and 4 mL of 0.1 M NaOH was added followed by 100 μL of a 1:9 (v/v) mixture of PFBBr in 1,1,2-trichloroethylene (TCE). The reaction mixture was shaken for 10 min. The resin was isolated by filtration and washed with water. The PFB derivatives were eluted with successive 20-mL washes of CH_2Cl_2 and ethyl ether. The combined washes were treated with 1 μg of the external standard (PFB–CNB) and evaporated to dryness. The residue was taken up in 200 μL of toluene containing 50 μL of 9:1 BSTFA/TMCS. Aliquots of this solution (1–2 μL) were analyzed by packed-column GC/ECD.

By Extractive Alkylation. The cannabinoids were derivatized in 2-μg amounts using previously described methods (*11*). Prior to analysis the reaction mixture was passed through a column of Florosil to separate excess phase-transfer catalyst.

Pentafluorobenzylation of Cannabinoids from Plasma. One milliliter of plasma containing cannabinoids was transferred via a silanized glass pipet into a silanized 16 × 100 mm glass screw-cap vial containing 200 mg of resin and 170 μL of CH_3CN. Five nanograms each of trideuterated standards was added. The solution was acidified to pH ≤4.0 by the addition of 75 μL of 1 M HCl/mL of plasma solution. The mixture was shaken at room temperature for 1 h at 75 cycles/min. The plasma was then removed by aspiration, and the resin was washed with 4 mL of water. Four milliliters of 20% CH_3CN in 0.1 M NaOH was added to the washed resin followed by 100 μL of a 1:9 mixture (v/v) of PFBBr/TCE. The reaction mixture was then shaken for 90 min at room temperature. The resin was isolated by filtration and washed with water. The PFB derivatives of the cannabinoids were eluted from the resin by successive washes with 10 mL of CH_2Cl_2 and 10 mL of ethyl ether. The combined washes evaporated to dryness at 70 °C under a stream of dry nitrogen. The residue was taken up in 50 μL of BSTFA/TMCS and 200 μL of toluene.

Calculation of Yield. *From Plasma.* For Δ^9-THC and 11-OH-Δ^9-THC the yield was calculated by recovery of radiolabel followed by autoradiography of a thin-layer chromatogram of the reaction mixture (see below for details). For 11-nor-9-carboxy-Δ^9-THC the yield was calculated by comparison to extractive alkylation and determined by GC/ECD using PFB–C_{24} as an external standard.

From Water. For all three analytes the yield was calculated by comparison to extractive alhylation and determined by GC/ECD. The external standard for Δ^9-THC and 11-OH-Δ^9-THC was PFB–CNB, whereas PFB–C_{24} was the external standard for the carboxylic acid metabolite.

Determination of Reaction Mixture Composition. Radiolabeled (^{14}C)-Δ^9-THC and (^{3}H)-11-hydroxy-Δ^9-THC were added to 1 mL of plasma (50 000 dpm ^{14}C and 150 000 dpm ^{3}H). The

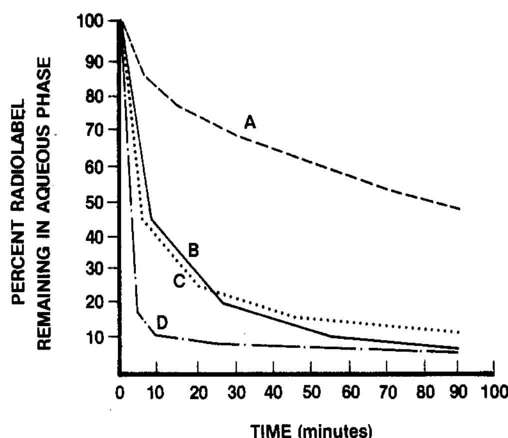

Figure 1. Adsorption of [^{14}C]-Δ^9-THC and [^{3}H]-11-hydroxy-Δ^9-THC from plasma: (A) (– – –) Δ^9-THC from unmodified plasma; (B) (——) Δ^9-THC from 10% CH_3CN in plasma; (C) (···) 11-hydroxy-THC from unmodified plasma; (D) (– · · –) 11-hydroxy-Δ^9-THC from 10% CH_3CN in plasma.

plasma was extracted and derivatized as described above. The extracts were concentrated to dryness and then reconstituted in hexane for spotting on a thin-layer plate. Normal-phase analysis was carried out in thin-layer chromatography using 5% ethyl ether in hexane to develop the plate containing the PFB derivative of Δ^9-THC (PFB–Δ^9-THC). The PFB derivative of 11-OH-Δ^9-THC (PFB–11-OH-Δ^9-THC) was similarly analyzed by using 50% ethyl ether in hexane. Finally, both products were simultaneously analyzed on reverse-phase TLC, the plates being developed in 30% tetrahydrofuran in water. All plates were sprayed with EN^{3}HANCE spray (New England Nuclear Supplies) and analyzed by autoradiography with an exposure time of 1 week.

RESULTS AND DISCUSSION

The model for automation was a procedure for the isolation of the cannabinoids as their PFB derivatives directly from plasma. In this example the resin served both as an adsorbent and the solid base of a solid-supported reagent. Preliminary experiments suggested that direct extraction/derivatization from plasma was not feasible and that some preliminary isolation was required. Thus we developed a method based on adsorption of analytes from plasma unto the resin followed by subsequent derivatization.

Adsorbtion of Δ^9-THC and 11-hydroxy-Δ^9-THC by XAD-2 directly from unmodified plasma was inefficient, and methods of improving adsorption were investigated. These included saturating the plasma with sodium chloride, acidifying the plasma, or adding 15% CH_3CN as a cosolvent to the plasma. A significant effect on adsorption efficiency was observed only with the use of CH_3CN as a cosolvent (Figure 1).

In contrast, adsorption of both Δ^9-THC and 11-OH-Δ^9-THC from H_2O and from 15% CH_3CN/H_2O was complete and quantitative within 5 min. This effect of cosolvent suggests that plasma components, most likely lipoproteins, inhibit the recovery of the highly lipophilic analytes from plasma. Similar observations were made regarding extraction of Δ^9-THC from plasma using water-immiscible organic solvents (*12*). The extraction of Δ^9-THC and 11-hydroxy-Δ^9-THC was independent of pH, but efficient extraction of the carboxylic acid metabolite required acidification of the plasma. Consequently, all extractions were carried out from acidified plasma diluted with 15% CH_3CN.

In previous studies, PFBBr was impregnated on the resin prior to the reaction and ionized analytes were initially present in aqueous solution. By use of the present procedure, however, analytes were adsorbed as lipophilic, neutral species and, subsequently, would have to be ionized in situ on a hydrophobic surface. In this instance, the resin could in fact be impregnated with reagent and the reaction affected *subsequent* to the adsorption of the analytes. This was achieved simply by adding PFBBr to the aqueous phase and shaking the mixture. Moreover, with this procedure significantly less PFBBr was required than when the analyte was present in solution as an ionized species (*6*). When as little as 10 μL of PFBBr was used, 58% yield was obtained in 10 min (Table I).

Table I. Effect on Yield of Coimpregnation of TCE with 10 μL of PFBBR on 200 mG XAD-2 in a Reaction Time of 10 min

TCE vol	0	90
% yield of PFB-Δ^9-THC	58 ± 6[a]	80 ± 3

[a] Average (±) relative standard deviation, $n = 5$.

Initially, two alternatives were considered to increase the reaction yield: increasing the amount of PFBBr and increasing reaction time. The former option, while based upon and consistent with previous observation, was considered a priori a means of last resort. It had already been demonstrated that derivatization of cannabinoids with large excess of PFBBr resulted in such high backgrounds as to severely limit the GC/MS/NICI sensitivity (*1*). The second alternative, prolonged reaction time, surprisingly, did not increase the yield. This led to consideration of a third alternative.

Reduced yield may have been due to the fact that while 10 μL constitutes greater than 10^5 M excess, it compromises only 5% of the pore volume. Thus some beads and/or pores may have been impregnated with less than optimal amounts of PFBBr resulting in slow or no reaction at these sites. Accordingly, the 10 μL of PFBBr was diluted with 90 μL of TCE. This solvent was selected to dilute the reagent because, like PFBBr, it is immiscible with water and is a halogenated unsaturated hydrocarbon; thus, to some extent at least, the solvent effect would be reduced. Moreover, this solvent is volatile and thus easily removed, reducing problems with subsequent GC/ECD analysis. As a result of adding 90 μL of TCE with 10 μL of PFBBr the total volume of water immiscible organic solvent was equal to the total pore volume for 200 mg of resin. Assuming that a bead cannot adsorb more liquid than its pore volume, the addition of 100 μL of hydrophobic organic solvent would result in a homogeneous impregnation of the beads. Although the PFBBr would be diluted on each bead and in each pore, there was still a very large excess of reagent. As a result all of the adsorbed analyte molecules would be exposed to sufficient reagent to increase the reaction rate. While the mechanism for the effect of TCE is still speculative, the result of adding PFBBr in TCE was a marked increase in yield over a reaction time of 10 min (Table I).

The NICI mass spectra of the PFB derivatives of Δ^9-THC and 11-OH-Δ^9-THC derived from the resin showed only a single major ion resulting from the loss of the PFB group (*1*), identifying the products as the 1-OPFB derivatives of the monofunctional phenols (Figure 2). However, the carboxylic acid metabolite has two functionalities that can be derivatized: the phenolic and the carboxy group. This can give rise to at least three reaction products. The reaction product of the solid-supported reagent had the identical retention time on two capillary columns as the synthetically prepared bis-PFB derivative. Mass spectrometric analysis of the product from solid-supported reagent confirmed that the bis-PFB derivative formed (Figure 2). These data demonstrate that the solid-supported reagent gives the same reaction product of the polyfunctional molecule as does the more standard derivatization procedures.

The chromatograms obtained for the reaction product mixture from extractive alkylation and solid-supported reagent (Figure 3) are both relatively clean. However the extractive

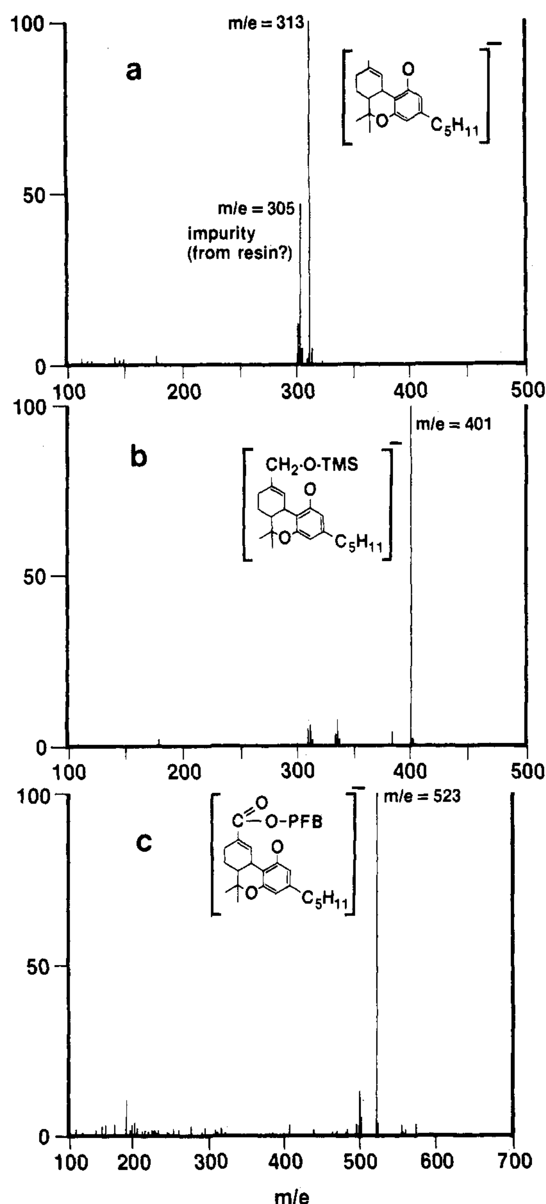

Figure 2. GC/MS/NICI spectra of the PFB derivatives synthesized by solid-supported reagent: (a) PFB–THC, (b) 11-OTMS–PFB–THC, (c) bis-PFB–11-nor-9-carboxy-THC.

alkylation product mixture contains significantly more material eluting close to the solvent front, even though chromatographic "cleanup" on Florosil was a prerequisite for GC/ECD analysis of the extractive alkylation reaction product. (In the absence of the cleanup the solvent front interfered with the chromatographic peaks from the analytes.) These data suggest that solid-supported reagent gives a reaction product mixture with fewer electrophoric compounds. Cleaner reaction mixtures are generally important (*1*) for any procedure.

Having optimized conditions for adsorption from plasma and derivatization, it was possible to combine the two steps in a "one pot" sample preparation. Some modification of reaction conditions was necessary, however, because plasma constituents reduced the reaction rate (Table II). Coadsorption of plasma lipids with analyte may have inhibited access of hydroxide ion to nonionized analyte thus reducing reaction rate. If so, it was argued that addition of a water-miscible organic solvent (CH_3CN) would enhance access of the base. The exact mechanism for reduction of reaction rate by plasma constituents remains hypothetical; nevertheless, addition of 20% CH_3CN to 0.1 M NaOH significantly improved the yield in a 10-min reaction time (Table II).

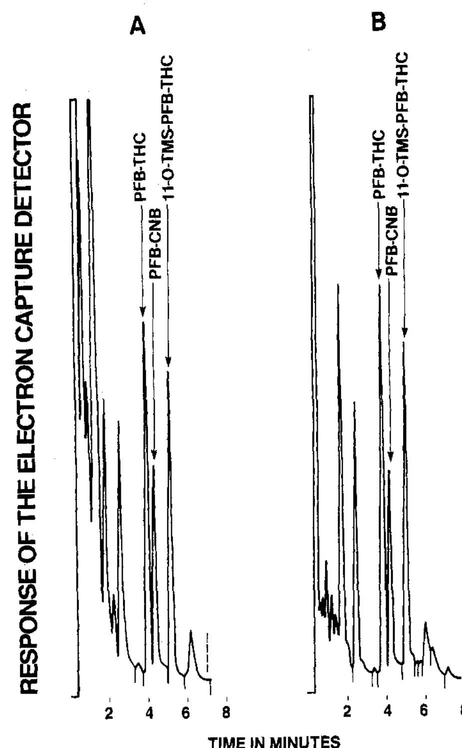

Figure 3. Packed-column GC/ECD traces for reaction mixture from pentafluorobenzylation of Δ^9-THC and 11-hydroxy-Δ^9-THC using (A) extractive alkylation and (B) solid-supported reaction.

Table II. Percent Yield of PFB Derivative from Analyte in Plasma as a Function of CH_3CN Concentration of Derivatization Mixture

	% yield at the following times and CH_3CN concn[a]		
	10 min		90 min
analyte	0 (v/v)	1/5 (v/v)	1/5 (v/v)
Δ^9-THC	31 (14)[b,d]	58 (14)[b,d]	96 ± 5[c,d]
11-OH-Δ^9-THC	ND[e]	ND	85 ± 6[c]
11-nor-9-carboxy-Δ^9-THC	ND	ND	65 ± 8[c,d]

[a] In 0.1 N NaOH. [b] Average of three results (range). [c] Average of five results ± relative standard deviation. [d] Determined by capillary GC/ECD and calculated relative to extractive alkylation. [e] Not done.

The yield in a 10-min reaction time for analytes initially adsorbed from plasma was still lower than for pure compounds even in the presence of CH_3CN. Thus a 90-min reaction time was used for the final method. Under the described conditions the entire procedure was carried out in one tube. Thin-layer chromatography of the reaction mixture followed by autoradiography of the chromatogram showed that virtually all of the radiolabel was in the spots corresponding to the PFB derivatives of Δ^9-THC and 11-OH-Δ^9-THC. These spots were scraped and counted. When no carrier was added the mass of analyte was less than 25 pg. Under these conditions the corresponding recoveries were 85% for the [^{14}C]-Δ^9-THC and 88% for the [^{3}H]-11-OH-Δ9-THC, both recovered as the PFB derivative. When 2 μg of "carrier" consisting of unlabeled analyte was initially added to the plasma 91% of the ^{14}C radiolabel and 80% of the tritium radiolabel were recovered in regions corresponding to PFB–Δ^9-THC and PFB–11-OH-Δ^9-THC, respectively. Thus the radiolabel recovered gives a direct determination of the yield of the PFB derivatives. The solid-supported reagent accordingly appears to give high and reproducible recoveries of the PFB derivatives of both Δ^9 and 11-OH-Δ^9-THC in plasma (Table II) as measured by the radiolabel method. Furthermore, the recovery is invariate

Table III. Calibration Curves for Three Analytes Using GC/MS/NICI

analyte	range, ng/mL	slope	intercept	r^2
Δ^9-THC	0.3–100	0.19	0.095	1.00[a]
11-OH-Δ^9-THC	1–100	0.087	0.079	1.00[b]
11-nor-9-carboxy-Δ^9-THC	0.3–100	0.069	0.029	1.00[a]

[a] Six concentrations analyzed in triplicate. [b] Five concentrations analyzed in triplicate.

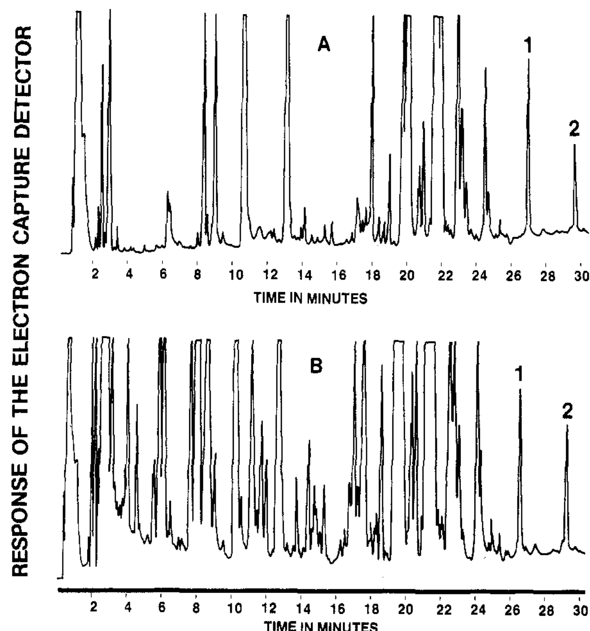

Figure 4. Capillary column gas chromatograph/ECD trace for 11-nor-9-carboxy-Δ^9-THC prepared as the bis(pentafluorobenzyl) derivative from plasma using resin mediated reactions: (A) 10 μL of PFBBr; (B) 40 μL of PFBBr; (1) external standard, PFB tetracosanoate; (2) derivatized analyte, bis-PFB derivative of 11-nor-9-carboxy-Δ^9-THC.

over a concentration range from picograms (no carrier added) to micrograms per milliliter (2 μg of carrier added). This ranges includes the concentrations normally found after smoking a cigarette of Δ^9-THC (*1*). Although the yield for 11-nor-9-carboxy-Δ^9-THC was determined relative to extractive alkylation, it appears that the recovery of the PFB derivatives is also likely to be adequate for an analytical method. Finally, for combined GC/MS/NICI the calibration curves were linear for all three analytes (Table III). The intercepts were positive indicating interferences to the analysis, but these were below 100 pg/mL and interferences below this concentration were in fact found.

As expected, numerous plasma constituents can be derivatized to electrophoric products producing extraneous peaks in the GC/ECD determination of cannabinoid from plasma. The quantity of interfering electrophoric derivatives is significantly reduced by fourfold reduction in the amount of PFBBr with only a 30% reduction in the yield of 11-nor-9-carboxy-Δ^9-THC. A similar decrease was not observed for the other two analytes. The reduction of interferences is particularly evident in the early part of the chromatogram. This result is demonstrated with the carboxy metabolite, the derivative of which appears in an interference-free region of the chromatogram on a DB-1 column (Figure 4).

The time course of the plasma concentration for the three analytes in a rabbit following intravenous and subcutaneous injection of Δ^9-THC was determined by use of both GC/MS/NICI with deuterated internal standards and capillary GC/ECD with an external standard (Figure 5). The concentration ranges found for these analytes in this experiment

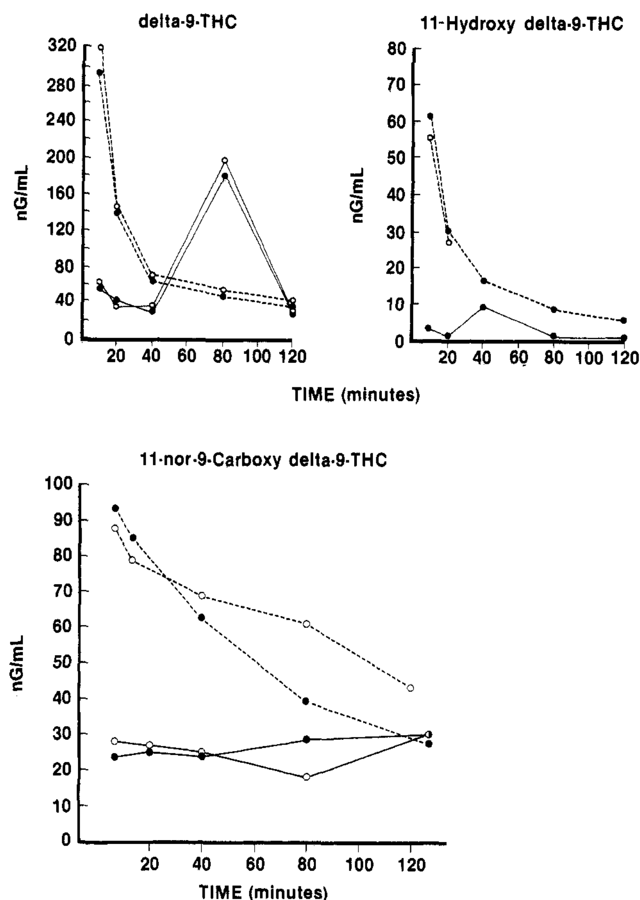

Figure 5. Plasma concentrations of Δ^9-THC, 11-OH-Δ^9-THC, and 11-nor-9-carboxy-THC using capillary column GC/ECD (●) and GC/MS/NICI (O) following intravenous (- - -) and subcutaneous (——) injections.

are similar to those observed in man after smoking a marijuana (*1*).

The plasma concentrations in a rabbit determined by use of both GC/ECD and GC/MS/NICI generally correlated quite well (Figure 5). For Δ^9-THC the coefficient of correlation between the two instrumental methods was 0.987 (n = 10 concentrations determined in duplicate). Concentration of 11-OH-Δ^9-THC could be detected only at two time points by GC/ECD (Figure 5); these values were 56 [9] ng/mL and 27.5 [1] ng/mL, and the corresponding values determined by GC/MS/NICI were 63 [4] and 29.5 [1] (values reported as average of two points [range]). In the case of 11-nor-9-carboxy-Δ^9-THC there was some discrepancy between the GC/ECD and GC/MS/NICI data, particularly at the 80- and 120-min time points. This was attributed to an interference of 15 ng/mL that was detected in the blank by GC/ECD analysis but was not present in the mass spectrometric determinations. The coefficient of correlation between the two techniques for detection of 11-nor-9-carboxy-Δ^9-THC was 0.95 (n = 10 concentrations analyzed in duplicate). The mass spectrometric method was more sensitive and specific as no interference to the three analytes could be detected above 100 pg/mL. the practical limit of quantitation was thus taken at 300 pg/mL.

The generally close correlation between the two methods of instrumental analysis suggests that a combination of solid-supported techniques for the isolation and derivatization of cannabinoids from plasma followed by GC/ECD analysis should be investigated as a possible analytical method for preliminary screening of forensic samples as well as in those instances where the short-lived metabolites are of no pharmacologic or toxicological interest.

Registry No. XAD-2 resin, 9060-05-3; Δ^9-THC, 1972-08-3; 11-OH-Δ^9-THC, 36557-05-8; 11-nor-9-carboxy-Δ^9-THC, 56354-06-4.

LITERATURE CITED

(1) Foltz., R. L. *Adv. Anal. Toxicol.* **1984**, *1*, 125–157.
(2) Borman, S. A. *Anal. Chem.* **1985**, *57*, 651A.
(3) St. Onge, L. M.; Dolar, E.; Anglim, M. A.; Leist, C. J., Jr. *Clin. Chem.* (*Winston-Salem, N.C.*) **1979**, *25*, 1373–1376.
(4) Hux, R. A.; Mohammed, H. Y.; Cantwell, F. F. *Anal. Chem.* **1982**, *54*, 113–117.
(5) Balkon, J. B.; Donnelly, B.; Prendes, D. *J. Forensic Sci.* **1982**, *27*, 23.
(6) Kennedy, E. R.; Hill, R. *Anal. Chem.* **1982**, *54*, 7739–1742.
(7) Lipori, E. *Anal. Chem.* **1984**, *56*, 1820–1826.
(8) Zhu, A. N.; Xu, Gui-Yun *J. Chromatogr.* **1984**, *314*, 421–428.
(9) Rosenfeld, J. M.; Mureika-Russell, M.; Phatak, A. *J. Chromatogr.* **1984**, *283*, 127–135.
(10) Poole, C. F.; Schuette, S. A. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1983**, *6*, 526–547.
(11) Rosenfeld, J. M.; Taguchi, V. Y. *Anal. Chem.* **1976**, *47*, 726–729.
(12) Rosenfeld, J. M. *Anal. Lett.* **1978**, *10*, 917–925.

RECEIVED for review August 1, 1985. Accepted October 25, 1985. This work was supported by the National Institute on Drug Abuse of the USA and was presented in part at the Satellite Symposium on Cannabinoids held at Oxford, England, August 1984.

Extraction of Anthracene and Benzo[*a*]pyrene from Soil

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Extraction of ^{14}C labeled benzo[*a*]pyrene and anthracene from contaminated soil samples by Soxhlet and Polytron techniques was studied in a replicated 2^4 factorial experiment. Soxhlet extraction gave higher recoveries than Polytron extraction. Percent recoveries from both techniques were greater at a 50 µg/g contamination level than at 5 µg/g. Pyrolysis of the extracted residue followed by trapping of $^{14}CO_2$ corroborated the extraction results. In addition, analysis of the residue showed a significant increase in ^{14}C due to soil sterilization with $HgCl_2$ and an interaction effect between PAH and concentration. The effects of PAH, concentration, and $HgCl_2$ treatment on extraction and sorption were the same with both extraction methods.

The extraction of ^{14}C labeled polynuclear aromatic hydrocarbons (PAH's) from contaminated soil samples has been studied as part of the Wastewater Technology Centre's (WTC) program to determine the fate of PAH's applied to land. This program, including the Donnybrook sandy loam, has been described by Bulman et al. (*1*). The soil samples were spiked with labeled and unlabeled benzo[*a*]pyrene, or anthracene at 5 and 50 µg/g soil. The samples were incubated in biometer flasks at 20 °C for 3 and 5 months for anthracene and benzo[*a*]pyrene, respectively, allowing degradation to be monitored and the PAH to interact with the soil matrix. These samples provided a unique opportunity to assess the effects of extraction method on PAH extractability and sorption onto soil under conditions that approach "natural incorporation" of the spikes as suggested by MacDougall et al. (*2*). Haddock et al. (*3*) compared a 1- and 8-day "spike time" for anthracene and found significantly reduced recovery at the longer time.

A common problem encountered in the interpretation of PAH data is the variability resulting from different extraction procedures. In this study, subsamples were taken from each flask and extracted by one of two methods: by overnight Soxhlet extraction with 1:1 hexane:acetone (Maybury et al. (*4*)) or by extraction in a Polytron homogenizer (Brinkman Instruments, Ltd.) with three successive 25-mL portions of acetone (Afghan and Wilkinson (*5*)). Both of these extractions are routinely used in the WTC laboratory.

EXPERIMENTAL SECTION

Radiochemicals. ^{14}C-labeled chemicals used as tracers were obtained from California Bionuclear Corp., Sun Valley, CA. Benzo[*a*]pyrene (7,10-^{14}C, CBN 114) and anthracene (9-^{14}C, CBN 086) were purchased at 98% radiochemical purity and were used as received. The labeled compounds were dissolved in toluene, 1 mL of which was pipetted evenly over each 50 g biometer flask soil sample. The moist soil was mixed for several minutes with a glass rod and then left open to air overnight to evaporate the toluene. Benzo[*a*]pyrene was added to the soil samples at 2.76×10^5 dpm/g while anthracene was added at 2.40×10^5 dpm/g.

Instruments. All samples were counted for ^{14}C on a LKB Model 1217 liquid scintillation counter. The external standard ratio (ESR) method of automatic window setting and the ESR method of quench correction for disintegrations per minute calculation was used. Chemiluminescence was routinely monitored by the instrument.

Combustion analysis of the samples was carried out in a Biological Material Oxidizer (BMO/R. J. Harvey Instrument Corp.).

Standards and Reagents. All samples were counted in 22-mL plastic vials with 10 mL of Amersham PCS liquid scintillator and varying volumes of sample. Standards were prepared by dissolving one LKB-Wallac ^{14}C–O Internal Standard pellet (^{14}C = 105 400 dpm) in 10 mL of PCS and adding various volumes of blank sample to the PCS. Several standards formed the quench calibration curve which was stored in the instrument memory for automatic disintegrations per minute calculation. The CO_2 trapping solution was Oxisorb CO_2 (New England Nuclear) mixed 1:2 with PCS.

Methods. *Soxhlet.* A 10-g soil sample of 29% moisture content was weighed into a Soxhlet extraction tube and extracted 16–18 h with 300 mL of 1:1 hexane:acetone. The recovered solvent was evaporated to 15 mL on a rotovap. One milliliter was taken for ^{14}C counting, 50 µL for thin-layer chromatography, and the soil residue reserved for combustion analysis.

Polytron. A 10-g soil sample of 29% moisture content was weighed into a 40-mL centrifuge tube and 25 mL of acetone added. The sample was extracted 2 min with a Polytron homogenizer (a high-velocity mixer/shearer). The tube was then centrifuged for 5 min in a clinical centrifuge at 2000 rpm and the supernatant decanted. This procedure was repeated two more times with acetone and the three extracts combined. The volume of recovered acetone was recorded and a 1-mL sample taken for ^{14}C counting. The soil residue was reserved for combustion analysis.

Thin-Layer Chromatography (TLC). With a microliter syringe, 50 µL of the Soxhlet extract was applied to the preadsorbent area

0003-2700/86/0358-0721$01.50/0 Published 1986 by the American Chemical Society