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Determination of Cannabinoids in Water and Human Saliva by Solid-Phase Microextraction and Quadrupole Ion Trap Gas Chromatography/Mass Spectrometry

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Solid-phase microextraction (SPME) is applied to the determination of cannabidiol, Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabinol in pure water and human saliva. The inherent extraction behavior of the cannabinoids in pure water is evaluated along with optimization of the method in human saliva. The commercially available poly(dimethylsiloxane) (PDMS) SPME fibers were found to be the best class for the cannabinoid analysis. Partition coefficients were found to be extremely large for all of the cannabinoids (log $K > 4.0$). Equilibrium times for the 7- and 30-μm PDMS fibers were 50 and 240 min, respectively. A shorter extraction time of 10 min with the 30-μm PDMS fiber may be used for multiple extractions from the same vial, thus conserving the sample necessary for analysis and speeding up the total analysis time. Recoveries for the cannabinoids in saliva, relative to pure water, were dramatically improved by a method developed in our laboratory involving addition of glacial acetic acid to the sample vial prior to performing SPME. Using this method, recoveries relative to SPME in pure water ranged from 21 to 47% depending on the cannabinoid. The linear range for spiked saliva samples was established at 5−500 ng/mL ($r^2 > 0.994$) with precisions between 11 and 20% RSD. The ultimate level of detection by SPME for the cannabinoids in saliva was 1.0 ng/mL, with signal-to-noise values of ≥12. A saliva sample collected 30 min after marijuana smoking was subject to SPME and traditional liquid−liquid extraction analysis. Internal standard quantitation results for Δ^9-THC by both methods yielded comparable results, indicating that the SPME method of analysis is highly accurate and precise. The level of Δ^9-THC by SPME was found to be 9.54 ng/mL for the saliva sample.

Recent reports have demonstrated solid-phase microextraction (SPME) as a viable tool for the analysis of drugs in biological fluids. These reports include the analyses of amphetamines by headspace SPME,[1,2] valproic acid in human plasma,[3] phencyclidine,[4] local anesthetics from human blood,[5] ethanol in human breath,[6] benzodiazepines,[7,8] steroids,[9] diphenylmethane antihistaminics,[10] anorectic compounds,[11] tricylic antidepressants,[12] and barbiturates.[13,14] For those cases in which SPME offers sensitive detection of target drugs, there are many benefits to utilizing SPME as an alternate method of analysis. Inherently, SPME is a solvent-free extraction technique, relying on the partition of compounds between the liquid phase and the SPME fiber polymer. Limits of detection are typically equal to or better than traditional solid-phase extraction (SPE) methods which commonly include extra concentration steps prior to instrumental detection. Although not as commercially developed as automated SPE, automation of the SPME technique is being developed by Varian, Inc. The basic theory behind the SPME absorption process has been detailed previously.[15,16]

Initial application reports of SPME were primarily directed at the determination of pesticides and other analytes of environmental interest.[17−22] These constituents are commonly encountered in aqueous matrixes free of high amounts of background con-

(1) Yashiki, M.; Kojima, T.; Miyazaki, T.; Nagasawa, N.; Iwasaki, Y.; Hara, K. *Forensic Sci. Int.* **1995**, *76*, 169−77.
(2) Lord, H. L.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 3899−3906.
(3) Krogh, M.; Johansen, K.; Tonnesen, F.; Rasmussen, K. E. *J. Chromatogr., B: Biomed. Appl.* **1995**, *673*, 299−305.
(4) Ishii, A.; Seno, H.; Kumazawa, T.; Watanabe, K.; Hattori, H.; Suzuki, O. *Chromatographia* **1996**, *43*, 331−333.
(5) Kumazawa, T.; Sato, K.; Seno, H.; Ishii, A.; Suzuki, O. *Chromatographia* **1996**, *43*, 59−62.
(6) Grote, C.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 587−596.
(7) Krogh, M.; Grefslie, H.; Rasmussen, K. E. *J. Chromatogr. B: Biomed. Appl.* **1997**, *689*, 357−364.
(8) Seno, H.; Kumazawa, T.; Ishii, A.; Watanabe, K.; Hattori, H.; Suzuki, O. *Jpn. J. Forensic Toxicol.* **1997**, *15*, 16−20.
(9) Okeyo, P.; Rentz, S. M., Snow, N. H. *J. High Resolut. Chromatogr.* **1997**, *20*, 171−173.
(10) Nishikawa, M.; Seno, H.; Ishii, A.; Suzuki, O.; Kumazawa, T.; Watanabe, K.; Kanako, H. *J. Chromatogr. Sci.* **1997**, *35*, 275−279.
(11) Chiarotti, M.; Strano-Rossi, S.; Marsili, R. *J. Microcolumn Sep.* **1997**, *9*, 249−252.
(12) Lee, X.-P.; Kumazawa, T.; Sato, K.; Suzuki, O. *J. Chromatogr. Sci.* **1997**, *35*, 302−308.
(13) Li, Shu; Weber, S. G. *Anal. Chem.* **1997**, *69*, 1217−1222.
(14) Hall, B.; Brodbelt, J., *J. Chromatogr., A* **1997**, *777*, 275−282.
(15) Louch, D.; Motlagh, S.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 1187−1199.
(16) Zhang, Z.; Yang, M. J.; Pawliszyn, J. *Anal. Chem.* **1994**, *66*, 844A−853A.
(17) Potter, D. W.; Pawliszyn, J. *Environ. Sci. Technol.* **1994**, *28*, 298−305.
(18) Buchholz, K. D.; Pawliszyn, J. *Anal. Chem.* **1994**, *66*, 160−167.
(19) Eisert, R.; Levsen, K. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 1119−1130.
(20) Boyd-Boland, A. A.; Magdic, S.; Pawliszyn J. B. *Analyst* **1996**, *121*, 929−937.
(21) Gorecki, T.; Pawliszyn, J. *Anal. Chem.* **1996**, *68*, 3008, 3014.
(22) Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. *Anal. Chem.* **1996**, *68*, 144−155.

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tamination. Thus SPME is not inhibited by its relatively nonselective method of extraction. Analysis of drugs in biological fluids by SPME poses a challenge to detect trace amounts of drugs amid high amounts of other constituents, such as urea and salts found in urine. The presence of proteins and lipids along with the higher viscosity levels of blood and plasma compared to water may also affect the extraction parameters. Often the drawbacks of analyzing drugs in biological fluids may be overcome by extraction in the headspace for those drugs that are sufficiently volatile; however the majority of drugs of abuse are nonvolatile. Therefore, the validation of new SPME methodologies that are compatible with biological fluids and also can confirm the identities of analytes in the presence of numerous other matrix components is an area of ongoing interest.

Analysis for drugs in saliva is attractive to many researchers because sample collection is noninvasive and quantitative measurements may reflect the non-protein-bound fraction of the drug in plasma. Saliva, although a complex mixture, is relatively free of interfering substances and has a much lower content of proteins than other physiological fluids. Furthermore, measurement of cannabinoids in saliva is reported to offer a more accurate value of concentration present during cannabis intoxication. Previous reports indicate that levels of cannabinoids in saliva range from 50 to 1000 ng/mL shortly after marijuana exposure. After 3−4 h, levels fall below 50 ng/mL and remain detectable at a cutoff limit of 1 ng/mL up to 10 h depending on the strength of the marijuana cigarette smoked.[23,24]

Saliva has been successfully used for the detection of cannabinoids, by methods including radioimmunoassay,[25] gas chromatography/mass spectrometry,[26−30] gas chromatography with electron capture detection,[31] tandem immunoaffinity chromatography/high-performance liquid chromatography,[32] and high-performance liquid chromatography with amperometric detection.[23] Most of these techniques require detailed sample preparation or derivatization prior to analysis to achieve low detection limits. Immunoassay techniques are highly sensitive for the cannabinoids; however, cross-reactivity may lead to nonselective results and false positives. In addition, the liquid chromatographic methods mentioned above require specific detectors, as traditional ultraviolet detectors are not sufficiently sensitive.

Gas−liquid chromatography coupled to mass spectrometry stands out as a sensitive and selective method for cannabinoid detection. In an early report, Rosenthal and co-workers reported

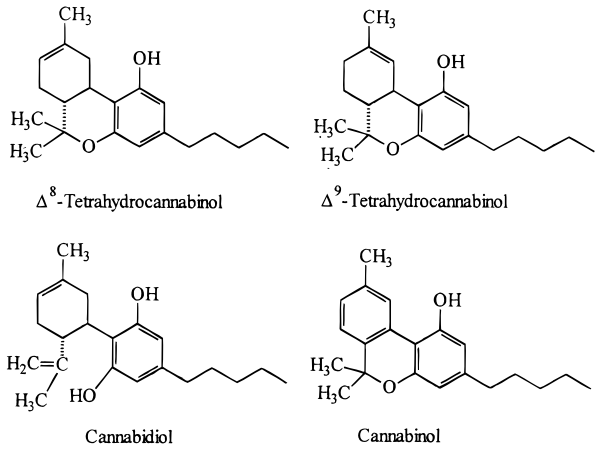

Figure 1. Structures of cannabinoids in the study.

detection limits of 500 pg/mL for underivatized Δ^9-tetrahydrocannabinol (Δ^9-THC) with packed-column GC and selected ion detection.[27] However, the method was based on 3 mL of plasma with a concentration factor of 100×, which translates to a detection level of 100 pg on column. This level was improved to 30 pg with packed-column GC and selected ion monitoring of the trimethylsilyl derivative of Δ^9-THC.[26,28] The method used 3.0 mL of plasma with a lengthy extraction and liquid chromatography sample purification procedure followed by the derivatization reaction. Foltz and co-workers have established a well-accepted method for sensitive measurement of Δ^9-THC in biological matrixes.[29] After a labor-intensive sample preparation involving three separate stages of both centrifugation and evaporation, the increased sensitivity over traditional EI positive ion mass spectrometry was attained after derivatization with trifluroracetic anhydride and detection by negative ion chemical ionization (NICI). A low limit of measurement of 0.2 ng/mL was reported and after correcting for the 40× concentration factor corresponded to 20 pg of Δ^9-THC on column. Rosenfeld and co-workers improved that level to ∼2 pg by a lengthy extraction onto XAD-2 resin particles and a 90-min derivatization with pentafluorobenzyl bromide with detection again by NICI.[30] These methods are especially useful for the detection of Δ^9-THC metabolites where derivatization is crucial to successful chromatography. However, for cannabinoid analysis in saliva where Δ^9-THC is the target analyte and metabolites are generally accepted to be at extremely low levels, Δ^9-THC may be directly analyzed by GC/MS in positive ion mode without derivatization. We have attained detection levels of 20 pg on column with our capillary GC-quadrupole ion trap system as described in this report. Utilization of SPME as the extraction and analyte introduction device significantly reduces the labor and time involved in sample preparation. As illustrated in this study, SPME serves as a sensitive preconcentration device for the cannabinoids. Since the SPME process is based on equilibrium extraction conditions, typically multiple measurements on the same sample may be made to improve precision and accuracy. In addition, since SPME is a solvent-free extraction and injection technique, there is no solvent waste to dispose.

The goal of this study is to outline a successful sensitive SPME method for the determination of a series of cannabinoids, cannabidiol, Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabinol as shown in Figure 1. Δ^9-THC

(23) Thompson, L. K.; Cone, E. J., *J. Chromatogr.* **1987**, *421*, 91−97.
(24) Cone, E. J.; Jenkins, A. J. *Handbook of Analytical Therapeutic Drug Monitoring and Toxicology*; CRC Press: Boca Raton, FL, 1997; Chapter 18.
(25) Gross, S.; Worthy, T. E.; Nerder, L.; Zimmermann, E. G.; Soares, J. R.; Lomax, P. *J. Anal. Toxicol.* **1985**, *9*, 1−5.
(26) Ohlsson, A.; Lindgren, J.-E.; Andersson, S.; Agurell, S.; Gillespie, H.; Hollister, L. E. *Biomed. Mass. Spectrom.* **1986**, *13*, 77−83.
(27) Rosenthal, D.; Harvey, T. M.; Bursey, J. T.; Brine, D. R., Wall, M. E. *Biomed. Mass. Spectrom.* **1978**, *5*, 312−316.
(28) Ohlsson, A.; Lindgren, J.; Wahlen, A.; Agurell, S.; Hollister, L. E.; Gillespie, H. K. *Biomed. Mass. Spectrom.* **1982**, *9*, 6−10.
(29) Foltz, R. L.; McGinnis, K. M.; Chinn, D. M. *Biomed. Mass. Spectrom.* **1983**, *10*, 316−323.
(30) Rosenfeld, J. M.; McLeod, R. A.; Foltz, R. L. *Anal. Chem.* **1986**, *58*, 716−721.
(31) Maseda, C.; Hama, K.; Fukui, Y.; Matsubara, K.; Takahashi, S.; Akane, A. *Forensic Sci. Int.* **1986**, *32*, 259−266.
(32) Kircher, V.; Parlar, H. *J. Chromatogr., B: Biomed. Appl.* **1996**, *677*, 245−255.

is the major psychoactive constituent of cannabis, whereas Δ^8-THC has been identified as a minor active constituent. Cannabidiol and cannabinol are also present in most cannabis preparations and may also be detected in saliva after smoking marijuana. Parameters that effect the extraction of the cannabinoids in water are evaluated, including type of SPME fiber used, extraction time, and viability of multiple extractions from the same vial vs different vials. Partition coefficients for the series of cannabinoids toward the poly(dimethylsiloxane) (PDMS) fibers in pure water are determined to characterize the inherent SPME absorption process and are compared to known octanol−water partition coefficients. Optimization of a SPME method for the analysis of cannabinoids in human saliva is conducted which includes linear range determination along with comparison of detection limits and precisions in both water and saliva. Finally, the method is applied to the quantitation of the cannabinoids in human saliva.

EXPERIMENTAL SECTION

Solid-Phase Microextraction Technique. SPME experiments were performed with a manual fiber holder supplied from Supelco Corp. (Bellofonte, PA). Five commercially available fibers were evaluated for their extraction efficiency toward the cannabinoids: 100-, 30-, and 7-μm film thickness PDMS, 65-μm carbowax−divinylbenzene (DVB), and 85-μm polyacrylate (Supelco). The 65-μm carbowax−DVB and 85-μm polyacrylate were only used for fiber comparison, where as the 100-, 30-, and 7-μm film thickness poly(dimethylsiloxane) were used throughout the study as noted. Manual extractions were undertaken in 4-mL deactivated glass vials on 2.5−4-mL total volume samples. Extractions for partition coefficient determinations for the 30-μm PDMS fiber were conducted in 40-mL deactivated glass vials utilizing 25 mL of solution. A 5% (v/v) dimethyldichlorosilane in toluene (Supelco) solution was used for glassware deactivation. Magnetic stirring at 70% of the maximum setting with a Teflon-coated star head stir bar was used as the method of agitation during the extraction. Air bubbles on the SPME fiber, which will affect precision, were removed by sonication or exposing and retracting the fiber to the solution repetitively. After the desired extraction time, the SPME device was transferred to the injector of the gas chromatograph for a 12-min desorption onto the column. The injector temperature was set to an optimum temperature of 270 °C (except for the carbowax−DVB fiber). Continuous exposure to temperatures over 260 °C was found to cause the carbowax−DVB coating to strip off the silica fiber. Prior to partition coefficient and depletion studies, the mass spectrometer response was calibrated by solvent injection from 500 pg to 50 ng (r^2 > 0.99) for all of the cannabinoids.

SPME performed in human saliva for method development was done on spiked 2-mL samples of cannabis-free saliva. After spiking and prior to SPME, the samples were either diluted with deionized water or treated with glacial acetic acid to clarify the solution in a method which is described as follows. After 2 mL of saliva was spiked with cannabinoids, the solution was allowed to stir for at least 15 min. Next, 1 mL of deionized water and 1 mL of glacial acetic acid were added to the spiked saliva while stirring. The sample was transferred to a test tube and centrifuged for 2 min. Subsequently, the liquid was transferred to the SPME vial for extraction.

Saliva specimens for SPME quantitation after marijuana smoking were treated in the same manner as above except that 1 mL of saliva, 1 mL of deionized water, and 0.5 mL of acetic acid were utilized because of limited quantities of saliva. In addition, for internal standard calibration, 0.030 mL of a 500 ng/mL Δ^9-tetrahydrocannabinol-d_3 was added to the SPME vial prior to acetic acid addition.

For the salt effect experiment, 1 mL of saturated salt solution (15 g of NaCl/50 mL of deionized water) was added with the acetic acid instead of the 1 mL of deionized water as described in the previous paragraph. Four replicates were conducted for this experiment.

Liquid−Liquid Extraction Technique. For quantitative comparison to SPME calibration methods, a previously reported liquid−liquid extraction process was used to prepare extracts for direct injection analysis with a slight modification.[23] To 2.0 mL of saliva, Δ^9-tetrahydrocannabinol-d_3 was added as an internal standard. Deproteinization was carried out by addition of 2.0 mL of methanol and 0.20 mL of 70% perchloric acid. This mixture was vortexed and centrifuged, discarding the precipitate. The supernatant was transferred to a tube containing 1.0 mL of saturated sodium chloride and 0.20 mL of toluene. The mixture was vortexed and centrifuged, the organic phase was removed, and 2 μL was injected into the GC for analysis.

Instrumentation. Gas chromatographic/mass spectrometric analysis was carried out with a Varian Saturn 4D GC/MS system. Separations were conducted on a DB-5ms column (30 m, 0.25 mm i.d., 0.25 μm d_f). The column program consisted of a 0.20 min hold at 50 °C, then ramp to 280 °C at 15 °C/min, and finally hold at 280 °C for 2 min. A septum programmable injector (Varian 1093 SPI) was operated at an isothermal temperature of 270 °C for SPME desorption. A 0.75-mm-i.d. inlet sleeve which accepts any commercially available SPME fiber was used in the injection port (Varian). The transfer line temperature was maintained at 280 °C.

The Saturn system is equipped with a quadrupole ion trap detector which was run in electron ionization (EI) mode and automatic gain control (AGC) applied. For EI experiments, the instrumental parameters were set at the following values: 18-μA filament emission current, electron mulitplier voltage of 2000 V, and an AGC target of 22 000 counts. The manifold temperature was maintained at 200 °C. The mass spectrometer was operated in full-scan mode between 120 and 350 amu. The ions used for quantitation were as follows: cannabidiol (m/z 231^+, 314^+), Δ^8-THC (m/z 231^+, 258^+, 314^+), Δ^9-THC (m/z 231^+, 299^+, 314^+), cannabinol (m/z 238^+, 295^+, 310^+).

Materials. Cannabidiol, Δ^8-THC, Δ^9-THC, and cannabinol used in this study were obtained as 1.0 mg/mL standards in methanol from Sigma (St. Louis, MO). Δ^9-tetrahydrocannabinol-d_3 as a 100 μg/mL solution in methanol was also obtained from Sigma. Human saliva samples for SPME optimization were collected from noncannabis users, and solutions used for SPME were created from pure saliva by dilution with deionized water and/or glacial acetic acid. Saliva was collected at least 1 h after eating and/or drinking, and fresh samples were typically obtained daily as needed or samples were stored at room temperature for a maximum of 1 day. Saliva samples for interference studies were collected 10 min after exposure to the substances and then spiked

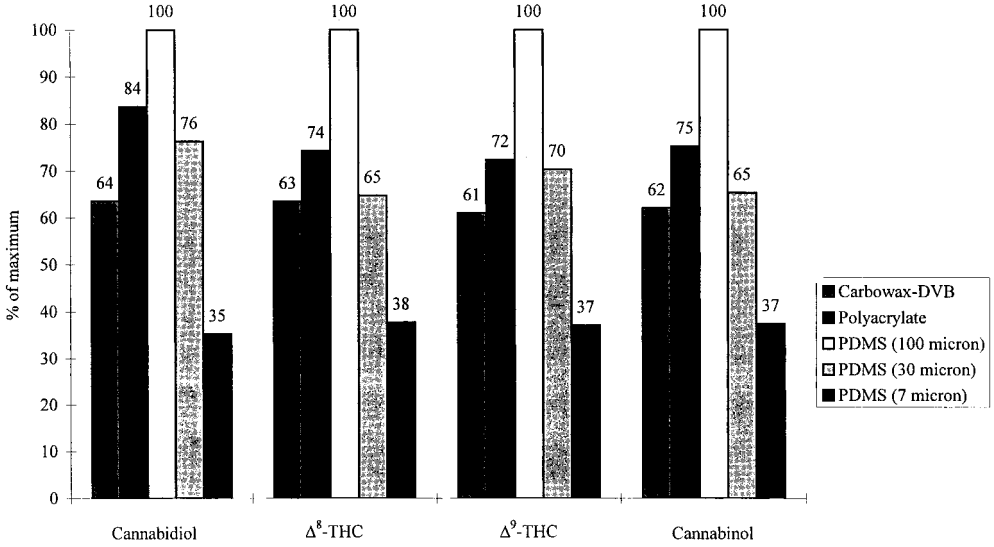

Figure 2. Comparison of five commercial SPME fibers for extraction efficiency. Based on 10-min extractions from 500 ng/mL of each cannabinoid in deionized water.

at 50 ng/mL with the cannabinoids. For the application experiment, saliva samples were collected from a healthy volunteer, who had a history of smoking marijuana occasionally, after smoking from a marijuana cigarette. Saliva samples were collected prior to smoking the marijuana cigarette and 30 min after smoking. Approximately 2 and 4 mL of saliva were collected for the prior and 30-min sample, respectively. The smoking event was held in a social setting, and the subject was asked to refrain from eating 30 min prior to collection of samples. The actual Δ^9-THC content of the marijuana cigarette was unknown.

RESULTS AND DISCUSSION

Fiber Selection and Carry-Over. For the method development procedure, the appropriate fiber was found by comparing the extraction efficiency of five commercial SPME fibers toward the cannabinoids and evaluating the carry-over each fiber exhibited. As illustrated in Figure 2, the 100-μm PDMS fiber showed the highest extraction efficiency for the conditions used in this test. All of the fibers, however, were successful in extracting these cannabinoids. The fact that the cannabinoids were efficiently extracted by all fiber types despite the polarity differences is an indication of the extreme lipophilicity of the cannabinoids. Therefore, all of the fiber coatings are relatively nonpolar to the cannabinoids compared to the aqueous phase. The 65-μm carbowax-DVB fiber was not selected for further study based on higher carry-over of the cannabinoids as a result of requiring a lower desorption temperature (255 °C). For the other fibers, which are more stable at higher temperatures, an optimum desorption temperature of 270 °C was selected to minimize carry-over while maximizing the life of the SPME fiber. Figure 3 illustrates a carryover determination for the 100-μm PDMS fiber expressed as percent carryover vs desorption time. After a 4-min desorption, the amount remaining on the fiber falls to an acceptable level of 1−3% for cannabinoids. Taking the desorption time to 12 min, the carryover levels drop to ≤1%. For the gas chromatograph program utilized in this work to efficiently separate Δ^9-THC and Δ^8-THC, this allowed the analysis of 2−3 samples/h. As discussed later, this analysis time may be shortened if only

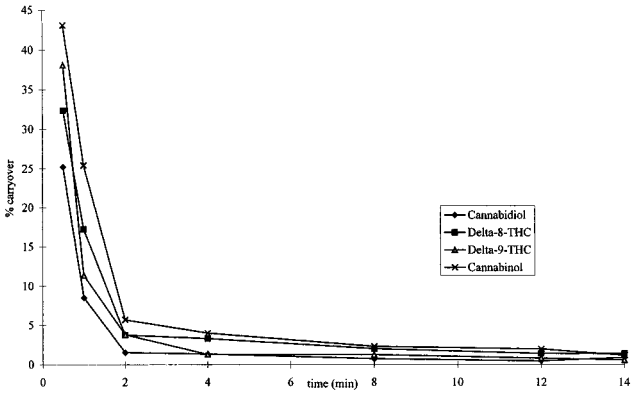

Figure 3. Percent carry-over vs desorption time for the 100-μm PDMS fiber. Based on a 10-min extraction from 500 ng/mL of each cannabinoid in deionized water.

Δ^9-THC is the target analyte. Typically, the 85-μm polyacrylate fiber exhibits a higher background signal throughout the chromatogram than the PDMS fibers, due to the inherent bleeding of polyacrylate polymer material at high injector temperatures. Thus, the series of PDMS fibers were selected as the best available for cannabinoid analysis.

Equilibrium Time and Partition Coefficients. The absorption kinetics of the cannabinoids in pure water were investigated by generating exposure time profiles for the 7- and 30-μm film thickness PDMS fibers, as illustrated for the 7-μm fiber in Figure 4. Equilibrium times were determined under agitated conditions by finding the time necessary for the amount extracted by the fiber to remain constant. In addition, sample volumes for the SPME extractions were adequate so as not to reach exhaustive extraction conditions, which is a concern for compounds with high partition coefficients. For the 7-μm PDMS fiber, equilibrium was reached for all of the compounds at a 50-min extraction time. The equilibrium time for the 30-μm PDMS fiber was significantly higher at 240 min. Experimentally, this result indicates an approximate quadrupling of the equilibrium time along with a quadrupling of the fiber volume coating. This relationship agrees with the theory that the transport limit of the cannabinoids is set by the diffusion through the static aqueous layer just at the

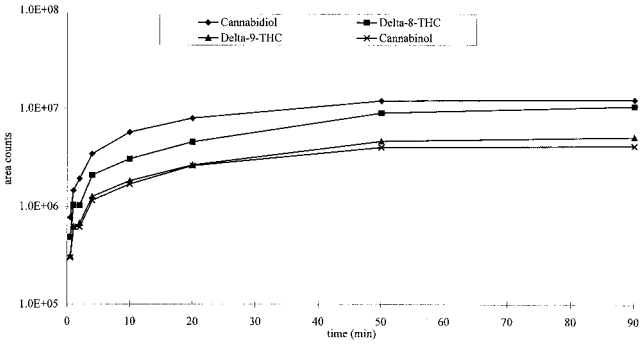

Figure 4. Log of the area counts vs extraction time for the 7-μm PDMS fiber. Leveling out of the signal indicates an equilibrium time of ~50 min.

Table 1. Comparison of SPME Partition Coefficients (K) and Values Reported for the Octanol–Water (K_{ow}) Coefficient

	partition coeff (log K) by SPME		log K_{ow}[a]		
compound	7-μm PDMS[b]	30-μm PDMS ($n = 4$)	HPLC	shake flask	calcd
cannabidiol	4.01	4.32 ± 0.13	5.79		6.92
Δ^8-THC	4.04	4.53 ± 0.13	7.41		7.18
Δ^9-THC	4.03	4.55 ± 0.07	6.97	4.08	7.18
cannabinol	4.00	4.44 ± 0.16	6.20		7.40

[a] Ref 33. [b] Determined from the slope of the linear regression lines ($r > 0.99$).

boundary of the fiber, as established in early SPME development.[15] All of the cannabinoids demonstrate similar absorption kinetics, which is a reflection of their molecular similarity.

The lipophilic nature of the cannabinoids would predict high values for the SPME partition coefficients using the PDMS fibers and thus favorable compounds for SPME studies. To examine the extraction characteristics of cannabinoids in water in more detail, the partition coefficients (K) at equilibrium between water and the 7- and 30-μm PDMS coatings were determined. Partition coefficients for the 7-μm PDMS fiber were found from the slope of the linear range for a nanogram extracted vs concentration curve with standards ranging from 25 to 100 ng/mL ($r^2 > 0.99$), whereas the values of K for the 30-μm PDMS fiber were determined by averaging four independent determinations of different concentration in the range of 2–8-ng/mL. For the 30-μm fiber, the following equation for K calculation was utilized which takes into account analyte depletion:

$$K = NV_{aq}/V_s(V_{aq}C_0 - N)$$

where N is the amount of analyte absorbed by the PDMS coating, V_{aq} is the volume of the aqueous phase, V_s is the volume of the PDMS coating, and C_0 is the initial analyte concentration in the water. Again, solution volumes were chosen such that total analyte depletion would not occur during the extraction. Analyte depletion during the extraction by the 7-μm PDMS fiber was not significant. The values for the SPME partition coefficients are listed in Table 1 along with comparison to previously determined octanol–water coefficients.[33]

SPME has been reported to be a method for the estimation of the octanol–water partition coefficient for compounds with log K_{ow} values of <3.5.[34] This result appears to agree with the results acquired in this study in which the SPME partition coefficients determined are 1–3 orders of magnitude lower than the K_{ow} values determined by HPLC method. The HPLC method is considered the most accurate method in finding the K_{ow} for extremely lipophilic molecules. Nevertheless, the K values determined by SPME for the 30-μm fiber do follow the same trend as the HPLC method, and the magnitudes suggest a potentially sensitive method for the analysis of cannabinoids even in more complex matrixes, such as human saliva.

Depletion Studies and Precisions for SPME in Pure Water. As determined above, equilibrium times for the cannabinoids occur at 50 min and 4 h for the 7 and 30-μm PDMS fibers, respectively. Although extraction at equilibrium times will maximize the detection of the cannabinoids, shorter extraction times may be employed to enhance efficiency without seriously affecting sensitivity because of the large partition coefficients of the cannabinoids. For the majority of the studies to follow, a 10-min extraction time was chosen, which matched well with the 12-min desorption time and chromatographic run, which ended at 17 min, thus allowing time for the GC column to cool between runs.

One advantage of SPME is the ability to extract multiple times from the same sample if significant depletion of the analyte does not occur. One method to validate this feature involves determination of the precision of extraction from different vials vs the same vial multiple times. In addition, the actually amount depleted may be determined by extrapolation of a direct injection calibration curve. These results are presented in Table 2. The amounts extracted in nanograms and the percent remaining after both three and six extractions are also presented in Table 2. After three extractions, there is ~10% drop in the initial amount present in the vial. Careful examination indicates that the amounts extracted in extractions 4–6 drop by ~2% relative to extractions 1–3 due to a decrease in concentration of the analytes by previous extractions. Since SPME is an equilibrium process, this result is in accord with theory which states that the amount extracted is dependent on the initial concentration of the analyte (in addition to the fiber volume and partition coefficient). As listed in Table 2, the inherent precision of SPME for the cannabinoids when performing one extraction from six different vials is essentially the same as when extracting up to six times from the same vial. Therefore, although there is a significant depletion up to six extractions, under the SPME conditions utilized, the precisions for extracting from the same vial vs different vials are not significantly different. The improvement of the precisions for the 7-μm PDMS fiber is indicative of sampling at times closer to equilibrium and lessening the amount depleted due to the smaller fiber volume. Thus, with 10-min extraction conditions and stirring, up to six measurements from the same sample are valid, and this result also applies to SPME in saliva as verified by measuring precisions later in the work.

(33) Thomas, B. F.; Compton, D. R.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 624-630.

(34) Dean, J. R.; Tomlinson, W. R.; Makovskaya, V.; Cumming, R.; Hetherridge, M.; Comber, M. *Anal. Chem.* **1996**, *68*, 130–133.

Table 2. SPME Depletion Results and Precisions for the Cannabinoids in Pure Water

	total amt extracted (ng)[a] no. of extractns		% remaining no. of extractns		precision[b]			
					same vial 30-μm fiber		different vials 30-μm fiber	same vial 7-μm fiber
compound	3	6	3	6	($n = 6$)[c]	interday ($n = 3$)	($n = 6$)	($n = 6$)
cannabidiol	88.5	158	88.9	80.3	14	6	16	10
Δ^8-THC	60.2	104	92.5	87.0	18	10	19	7
Δ^9-THC	61.0	104	92.4	87.0	19	10	16	16
cannabinol	69.2	119	91.4	85.1	16	19	19	7

[a] Conditions: 3-μm PDMS fiber used for depletion study; 10-min extraction from the same vial; initial concentration 200 ng/mL (extraction volume 4 mL). [b] Precisions are expressed as relative standard deviations (RSD, %). [c] The RSD represents the average of three separate day determinations of $n = 6$ runs per day.

SPME in Human Saliva: Method Development. To our knowledge, there have been no reports demonstrating the viability of performing SPME in human saliva. Saliva is a viscid material relatively free of interfering substances compared to other biological matrixes, however, still containing levels of proteins and cellular components that should influence SPME performance. A good overview of the use of saliva as an analytical tool in toxicology has recently been reported which includes a description of physiology of the salivary glands as well as a review of existing techniques for the collection and analysis of saliva.[35] Traditional methods of cannabinoid determination in saliva involve preparation, extraction, and analysis steps which are time-consuming and labor intensive. Since SPME is considered a more efficient method of sample preparation for GC analysis, the method development for SPME in saliva was optimized with simplicity of utmost concern.

Initial development of SPME analysis in saliva investigated the recovery of the series of cannabinoids in saliva compared to that obtained in pure water. Performing SPME in pure saliva will inevitably degrade the fiber to a certain degree because of coating of the SPME fiber polymer with protein and cellular material. To minimize this effect, saliva samples were diluted 1:1 with deionized water after spiking with cannabinoids and just prior to extraction. In addition to extending the lifetime of the SPME fiber, dilution prior to analysis is practical since collecting volumes in excess of 2 mL may be tedious and uncomfortable to the donor. As discussed previously, multiple SPME measurements of cannabinoids from the same sample may be used, which allows the collection of small volumes of saliva without suffering loss of sensitivity or precision.

Table 3 lists the first recovery tests of SPME on spiked saliva specimens diluted 1:1 with deionized water for the 30-μm PDMS fiber. Recoveries are in reference to samples prepared in deionized water. A dramatic drop in signal was observed for the extractions in saliva, with recoveries falling to only 5−10%. However, precisions are equal to or better than sampling from pure water. This result indicates that there is not a significant degradation of the performance of the SPME fiber after subsequent extractions in the 1:1 diluted saliva. Presumably, the drop in recovery is an effect of the viscid nature of saliva which hinders the transport of the cannabinoids from the solution to the fiber surface. In addition, binding of the cannabinoids to the saliva proteins may also contribute to the low recoveries. Included in Table 3 is the effect of storage of the saliva for an extended period of time after the spike and before performing SPME. The results show that the recoveries do not significantly change, which is an important situation if a saliva specimen were collected in the field and analyzed in the laboratory later.

Table 3. SPME Recoveries of the Cannabinoids from Water and Saliva: Effect of Storage Time and Concentration

	recovery (%) vs deionized water[a,b]		
		saliva	
compound	deionized water	500 ng/mL spike	20 ng/mL spike
SPME Performed Immediately after Spiking the Water and Saliva			
cannabidiol	100 (10)	9 (8)	6 (18)
Δ^8-THC	100 (11)	7 (9)	7 (23)
Δ^9-THC	100 (14)	8 (8)	6 (23)
cannabinol	100 (12)	7 (6)	8 (19)
SPME Performed after Spiking and Storage of Water and Saliva for 22 h at 24 °C			
cannabidiol	103 (12)	7 (7)	
Δ^8-THC	99 (9)	5 (10)	
Δ^9-THC	83 (9)	6 (6)	
cannabinol	107 (10)	6 (8)	

[a] Relative standard deviations (%) in parentheses represent precision based on four replicate extractions from a 4-mL solution. [b] All samples were diluted 1:1 with deionized water just prior to performing SPME.

Methods of protein precipitation and filtration were attempted to improve sample recoveries. The traditional methods of saliva pretreatment involve dramatic changes in pH from the addition of strong acids, which require the addition of a pH buffer in later steps. A new method devised in our laboratory that involves adding acetic acid to the saliva just prior to SPME was found to clarify the saliva solution and improve recoveries for the cannabinoids. The acetic acid pretreatment step effectively coagulates proteinaceous material and cellular debris for easy removal before SPME is performed. The method is rapid and considerably enhances the extraction efficiency of the cannabinoids. The resulting chromatograms for spiked saliva samples were clean with no interfering peaks during the elution of the cannabinoids.

Results for recoveries after the acetic acid addition along with intra- and interday precisions are presented in Table 4 for the 30-μm PDMS fiber. Recoveries of the cannabinoids improve by a

(35) Hold, K. M.; de Boer, D.; Zuidema, J.; Maes, R. A. A. Saliva as an Analytical Tool in Toxicology. *Int. J. Drug Testing* **1996**, *1* (1) http://big.stpt.usf.edu/~journal/hold.html.

Table 4. SPME Recoveries of the Cannabinoids from Saliva: Effect of Acetic Acid Clarification Step

	saliva spiked at 500 ng/mL			saliva spiked at 50 ng/mL			
		precision[b]			precision[b]		
compound	rec (%) vs deionized water[a]	intraday	interday	rec (%) vs deionized water[a,b]	intraday	interday	rec (%) in water with acetic acid[c]
cannabidiol	21	10	11	22	15	18	34 (6)
Δ^8-THC	47	5	7	28	18	14	88 (6)
Δ^9-THC	35	5	7	23	12	7	93 (4)
cannabinol	42	4	4	41	24	25	76 (8)

[a] Deionized water controls were run for each trial and both concentrations. Each trial was conducted on a different day. Just prior to SPME, 1 mL of deionized water and 1 mL of acetic acid was added as described in the Experimental Section. [b] Precisions are expressed as RSD (%). [c] Recovery is in reference to a deionized water spike at 50 ng/mL. RSDs (%) listed in parentheses ($n = 4$).

Table 5. Calibration Results for SPME of Cannabinoids Spiked in Saliva with Acetic Acid Addition[a]

				precision[b]			
						lowest std tested	
compound	linear range (ng/mL)	slope of regression line (log−log plot)	r^2	(500 ng/mL) ($n = 6$)	(10 ng/mL) ($n = 6$)	(ng/mL)	S/N[c]
cannabidiol	5−500	0.970	0.995	11	14	1.0	12
Δ^8-THC	5−500	0.975	0.996	14	14	1.0	30
Δ^9-THC	5−500	0.998	0.997	15	17	1.0	15
cannabinol	5−500	0.945	0.995	14	20	1.0	80

[a] Saliva samples were spiked at different concentrations of the cannabinoids prior to acetic acid addition as explained in the Experimental Section. Based on 10-min extractions with the 100-μm PDMS fiber. [b] Precisions expressed as RSDs (%). [c] Signal-to-noise ratios.

factor of 4−7 times relative to the case of performing SPME in diluted saliva. Precisions, especially at the higher concentration spike, are much better than those obtained by performing SPME on the cannabinoids in pure water, presumably due to a buffering effect of the acetic acid. The precisions listed in Table 4 are meant to demonstrate that the addition of acetic acid will not dramatically affect the reproducibility. It should be noted that these precisions are not based on the use of an internal standard but on the inherent change in area counts extraction to extraction. Addition of acetic acid resulted in a pH in the extraction solution of ~2.2. This pH is high enough not to damage the PDMS coating on the SPME fiber. However, there is an effect on extraction efficiency for the cannabinoids just upon the addition of acetic acid. This factor was tested by adding acetic acid to a deionized water solution spiked with cannabinoids. The results present in Table 4 list the recoveries of a side-by-side comparison of the recoveries of the four cannabinoids spiked in pure water. Δ^8-THC and Δ^9-THC are not dramatically affected by the addition of acetic acid; however, the extraction efficiency of cannabidiol and cannabinol drops in accord with the presence of exposed polar hydroxyl functionalities which tend to increase the partition of these compounds into the aqueous phase rather than the PDMS polymer. The drop in extraction efficiency due to the presence of acetic acid is not significant enough to negate the benefits obtained by treating the saliva with acetic acid prior to SPME.

In previous SPME studies, the addition of salt to the analyte solutions has proven to improve extraction efficiencies by altering the solvation environment of the target analytes. To test the effect of salt on the extraction efficiency of the cannabinoids in saliva, 1 mL of a saturated salt solution was added to the extraction vial in two ways. In the first case, the salt solution was added prior to the acetic acid. In the second case, salt was added after the acetic acid and debris removal step. In both cases, the addition of salt resulted in signals dropping ≥70% relative to a saliva sample treated with deionized water and acetic acid. Therefore, the addition of salt to the reaction vial as a means for enhancing extraction efficiencies was not considered further.

Calibration Results in Saliva. The linear range of the method was established by spiking six saliva samples covering the range of 5−500 ng/mL. Acetic acid was added to each calibration standard just prior to performing SPME. The results including linear range, slope of the log−log regression line, precisions, and detection limits are presented in Table 5. A 100-μm PDMS fiber was utilized for the data listed in Table 5. All four of the cannabinoids exhibited good linearity with regression coefficients greater than 0.99. The precisions are similar to results of SPME in pure water with the 30-μm PDMS fiber, as listed in Table 2. The precision values verify that up to six extractions may be performed on the same vial, as determined earlier for the studies in pure water. Again, the precision values are based on the inherent change in area counts of the signal, not referenced to an internal standard. The area count values do not consistently drop, indicating that a significant concentration reduction does not occur extraction to extraction. The detection limit of 1 ng/mL is presented as the lowest standard analyzed in Table 5, with its corresponding signal-to-noise ratio. The results indicate that there was no observable degradation to the SPME fiber after at least 30 runs.

The sensitive linearity of the method after the acetic acid pretreatment demonstrates that a calibration curve may be used for accurate quantitation if a pool of saliva is obtained that is representative of the saliva sample under question. This action is especially valid if deuterated internal standard is included to correct for any variations in saliva composition and/or sample preparation. In addition, standard addition is also a common method for quantitation by SPME in complex matrixes. Variation

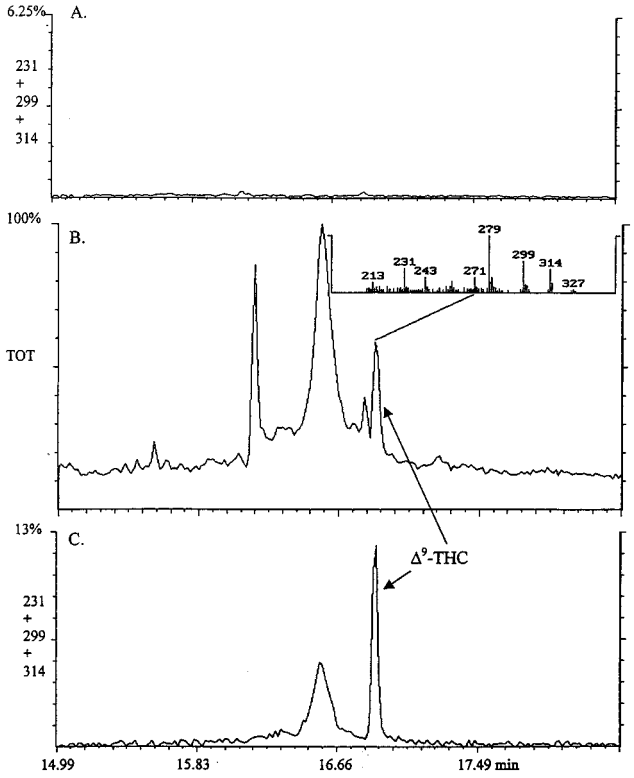

Figure 5. Chromatograms after performing SPME on human saliva samples prior to and after marijuana smoking.

in saliva composition is certainly to be expected, and interferences should be considered. Three possible interferences on the SPME performance were examined by collecting and spiking saliva samples after drinking coffee or beer or smoking a cigarette. None of these substances affected the recovery of the cannabinoids relative to SPME in "clean" saliva. Prior to SPME, all of these saliva samples were treated with acetic acid. Although these results do not cover all possible interference scenarios, research is being conducted in the other stages of saliva analysis which may be beneficial to the SPME method. For example, passive saliva collection as an ultrafiltrate via a device placed in the mouth is being studied to produce a clear sample.[36] Also, it has been reported that freezing the saliva sample at −40 °C for at least 24 h before analysis effectively breaks down cellular components and suspended particles, providing a clear liquid which was easier to process.[37]

Analysis of a Marijuana Smoker's Saliva. For final verification of the SPME method, saliva specimens were collected after smoking of marijuana and quantified by both SPME and a liquid−liquid method of extraction. Figure 5 illustrates the chromatograms and mass spectrum obtained for saliva samples collected both prior to marijuana smoking and 30 min after the smoking event utilizing a 100-μm PDMS SPME fiber with a 10-min extraction time. The saliva sample collected prior to the smoking event contained no detectable amount of any of the cannabinoids included in this study. However, 30 min after smoking, Δ^9-THC was detected at a significant level above the baseline. Of the other cannabinoids in this study, only a trace level of cannabinol was detected, beyond the limit of quantitation. Thus only Δ^9-THC was quantified in this sample. Table 6 summarizes the quantitation results for Δ^9-THC by external standard, internal standard, and standard addition SPME methods along with a liquid−liquid extraction technique with internal standard calibration. Results by the SPME methods and the liquid−liquid technique are all in reasonable agreement, indicating relatively accurate quantitation results. The internal standard SPME method is believed to be the most accurate value based upon compensation for any matrix effects occurring during the sample preparation and extraction. Standard addition methods are also accurate; however, in this case, limited saliva quantity allowed only two single-point standard addition determinations. The liquid−liquid extract yielded extremely low levels of signal despite a concentration factor of 10. Although the direct injection internal standard calibration curve was linear at these low levels, the ultimate accuracy of the liquid−liquid extraction method is difficult to ascertain since limits of detection were being approached. In contrast, the SPME quantitation method yielded signals that were simple to work with even at the low concentrations.

Table 6. Quantitation Results for Δ^9-THC in a Marijuana Smoker's Saliva by SPME and Liquid−Liquid Extraction

	SPME (ng/mL)[a,b]				liquid−liquid extracn (ng/mL)	
			std additn			
compound	ext std	int std	trial 1	trial 2	trial 1	trial 2
Δ^9-THC	7.70 (16)	9.54 (8.1)	12.2	7.12	7.50	9.52

[a] Values in parentheses represent RSD (%; $n = 3$). [b] SPME conditions: 100-μm PDMS fiber for 10-min extraction times.

In comparison to a previously reported liquid−liquid method of Δ^9-THC analysis,[29] the SPME method retains excellent specificity and accuracy. The precisions of 8.6−11.1% RSD reported in the previous liquid−liquid extraction method utilizing an internal standard are directly comparable to the internal standard SPME precision of 8.1% RSD in the present method. Typically, Δ^8-THC is not of interest when saliva is tested for the presence of cannabinoids since it is present at extremely low levels. Thus, the analysis time may be shortened by utilizing a shorter GC column or faster ramp allowing up to four analyses per hour. Using a relatively rapid sample preparation procedure of ∼3 min (thus freeing up analysts' time) and traditional positive ion quadrupole ion trap mass spectrometric detection, the SPME method achieves sensitivity equal to or better than many previous techniques of cannabinoid analysis in saliva.[26−29]

CONCLUSIONS

Outlined in this work was a successful and sensitive SPME method for the determination of cannabinol, Δ^8-THC, Δ^9-THC, and cannabidiol in both pure water and human saliva. Extraction efficiencies of SPME performed in saliva relative to pure water were considerably enhanced by a rapid presample preparation involving the addition of acetic acid and removal of the resulting protein and cellular debris. This simple pretreatment exploits the advantages of the SPME-GC/MS method to attain sensitive and

(36) Schramm, W.; Smith, R. H. *Clin. Chem.* **1991**, *37*, 114−115.
(37) Wolff, K.; Hay, A.; Raistrick, D. *Clin. Chem.* **1991**, *37*, 1297−1298.

quick confirmation of Δ^9-THC presence in a marijuana smoker's saliva. This method may be directed toward screening analysis with a low cutoff, as well as highly accurate quantitation with the use of deuterated internal standards or standard addition. In a clinical setting, this SPME technique may be utilized as a measurement tool for research in the pharmokinetics of cannabinoid in saliva after various means of marijuana exposure. Automating the SPME injection procedure could allow this procedure to be more readily accepted in a clinical setting.

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