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Screening method for 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine using hollow fiber membrane solvent microextraction with in-tube derivatization

Kirsten E. Kramer, Anthony R.J. Andrews*

Clippinger Laboratories, Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701-2979, USA

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Abstract

An efficient and inexpensive screening technique for the simultaneous clean-up, extraction, and derivatization of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) from urine has been developed. Using the principles of solvent microextraction in the form of a 20-μl volume of solvent placed inside a permeable hydrophobic polypropylene hollow fiber membrane, the analyte of interest is preconcentrated inside this tubing as the bulk sample solution is stirred for a given extraction time. The pH of the sample is raised by adding buffer after which the charged moiety is extracted as an ion pair with tetramethylammonium hydrogen sulfate. Using a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide and octane as the extraction solvent allows the GC-unstable carboxylic acid metabolite to be derivatized during the extraction without prior sample clean-up steps such as filtration of the urine. After an 8-min extraction, a 6-μl portion is drawn up with a syringe and directly injected into a gas chromatograph for separation and analysis. Samples as low as 10 ng ml^{-1} were analyzed successfully and the limit of detection was estimated at 1.0 ng ml^{-1} with relative standard deviations lower than 10% in the final protocol.

Keywords: Hollow fiber membrane solvent microextraction; 11-nor-Δ^9-Tetrahydrocannabinol-9-carboxylic acid

1. Introduction

Solvent microextraction (SME) combines the extraction principles of liquid–liquid extraction and the small scale parameters of solid-phase microextraction so that the advantages of both are realized. SME employs a water immiscible drop of organic solvent into which analytes were preconcentrated from a bulk stirred aqueous sample. The theory and principles of this relatively new technique have been published in several papers [1–5]. In our laboratory, SME involves an extraction solvent in the form of a 2-μl drop suspended from a syringe tip which is immersed in a bulk sample solution. The bulk solution is stirred while the analytes partition into the drop for a designated time after which the drop is withdrawn into the syringe and injected directly into a gas chromatograph. SME provides a simple, rapid method of sample clean-up, while greatly reducing the amounts of solvent waste which must be disposed of with traditional liquid–liquid extraction techniques. This technique has been optimized in our

*Corresponding author. Tel.: +1-740-593-1658; fax: +1-740-593-0148.

E-mail address: anthony.andrews@ohio.edu (A.R.J. Andrews).

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laboratory for various environmental pollutants [6,7] and has proven to be an effective screening technique for cocaine and its metabolites [8]. Unfortunately, in complex matrices such as a real urine specimen, the microextraction drop is not stable hanging from the syringe needle. To account for this, as well as to filter out unwanted precipitant from the stirred, urine specimen a polymer membrane tubing serves not only as a filter for the urine, but also as a vessel for the extraction solvent. The tubing allows for more rigorous stirring in the bulk solution, improving extraction efficiencies, and can be filled with more solvent (10–20 μl as opposed to 2 μl). Recently the extraction of benzodiazepines from biological fluid was successfully achieved in this manner [9].

Marijuana is one of five commonly abused drugs according to the Substance Abuse and Mental Health Services Administration (SAMHSA). Δ^9-Tetrahydrocannabinol (THC), responsible for the majority of psychotomimetic actions from marijuana, can be measured from blood samples, but is not normally present in the urine. Rather, the major metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and, to a lesser extent, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) can be analyzed from urine [10]. SAMHSA gives a cut-off level of THC metabolites at 50 ng ml^{-1} and a confirmatory level of 15 ng ml^{-1} [11]. Since the 11-OH-THC form is present in such a small amount, the carboxylic acid is usually the analyte of interest (Fig. 1). Unfortunately, THC-COOH decarboxylates above 80°C unless protected as methyl esters or by silylation. Derivatization options are alkylation, pentafluorobenzyl derivatives for electron capture detection, and silylation. In a study of five different derivatization procedures for three major acidic metabolites of Δ^1-tetrahydrocannabinol, the most successful derivatives involved using trimethylsilyl (TMS) derivatives as opposed to derivatizing with trifluoroethanol (TFE) followed by pentafluoropropionic anhydride (PFPA) or tetramethylammonium hydroxide (TMAH) for mass spectrometry [12]. Preparation and clean-up of THC-COOH from urine samples involves both filtration and extraction, as well as derivatization of GC-unstable species. With hollow fiber membrane solvent microextraction (HFMSME) the possibility of a simple, in-tube derivatization which occurs while the analyte is extracting into the tubing would allow for greatly simplified sample preparation.

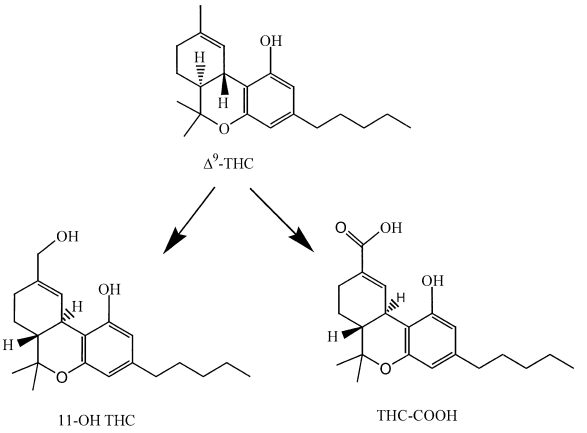

Fig. 1. Structure of Δ^9-THC and its two urinary metabolites, 11-hydroxy-Δ^9-tetrahydro-cannabinol (11-OH THC), and 11-nor-Δ^9-tetrahydro-cannabinol-9-carboxylic acid (THC-COOH), the primary urinary metabolite used to quantitate the ingested amount.

It is possible to extract charged aqueous chemical species into an organic phase by creating a neutral ion pair [13,14]. Therefore, two options are possible for the extraction of THC-COOH: either ensure an acidic medium so that the species is neutral and extract directly into an organic solvent, or alternately, create a basic medium and attempt to extract the target drug as an ion pair. The pK_a of THC-COOH is 3.0, so any pH value one to two units above or below this should create the charged and uncharged forms, respectively.

2. Experimental

2.1. Reagents and materials

THC and THC-COOH were obtained from Radian International (Austin, TX, USA). All solvents used were HPLC grade unless otherwise indicated. Solvents used were chloroform (Sigma, St. Louis, MO, USA), hexane >99.5% (Fluka, Buchs, Switzerland), and octane >99.5% (Fluka). Synthetic urine tablets

were obtained from Alltech (Deerfield, IL, USA) and were dissolved in ultrapure, distilled, deionised (18.2 MΩ) water obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). Trisodium phosphate buffer was made by dissolving 23.8 g trisodium phosphate (Mallinckrodt, Paris, KY, USA) in 250 ml of ultrapure water. All glassware was deactivated using dimethyldichlorosilane (Supelco, Bellefonte, PA, USA) as described by the manufacturer and 7-ml extraction vials were bought pre-silanized (Alltech, State College, PA, USA). The ion pair reagent used was tetramethylammonium hydrogen sulfate (Sigma–Aldrich, St. Louis, MO, USA) consisting of 0.056 g ml^{-1} in aqueous solution. Derivatizing reagents used were N,O bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and BSTFA+1% TMCS (Pierce, Rockford, IL, USA). Accurel® capillary membrane tubing made from polypropylene (type PP Q3/2, 200-μm wall thickness, 0.64-μm pore size, 600-μm inner diameter) was obtained from Akzo Nobel (Wuppertal, Germany). An octagonal 7×2×2-mm micro stir bar (Fisher, Pittsburgh, PA, USA) was used. A 10-μl syringe with Chaney adapter (model 701N, Hamilton, Reno, NE, USA) was used for all extractions and injections.

For adulterant studies, the following reagents were purchased from a local pharmacy unless otherwise indicated: ammonia (Aldrich, St. Louis, MO, USA), L-ascorbic acid (Aldrich), liquid Clorox® bleach, Sun Light® detergent tablets, liquid Clorox® detergent, Drano®, ethanol (Aldrich), ethylene glycol (Fisher), glutaric dialdehyde (Aldrich), hydrogen peroxide (Aldrich), lime solvent, potassium hydroxide (Aldrich), 2-propanol (Fluka), sodium chloride (Aldrich), liquid Clean & Smooth® hand soap, sodium bicarbonate (Aldrich), liquid Vanish®, white vinegar, Visine®.

Urine samples were donated from intra-laboratory volunteers, one of whom was taking the following medications: Claritin® (active ingredients loratadine and pseudoephedrine sulfate), Cephalexin (an antibiotic), oral contraceptives (containing norethindrone and ethinyl estradiol), and Tegretol® (containing carbamazepine). All volunteers gave their samples in full consent of how they would be used. Values for pH were measured with Macherey-Nagel pH strips (Alltech).

2.2. Instrumentation

The analysis of extracted THC-TMS derivative was performed with a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, San Diego, CA, USA) equipped with a split/splitless injection port and a pulsed discharge helium ionization detector (PDHID) (Valco Instruments, Houston, TX, USA) connected to a desktop computer with HP ChemStation (Version A.06) software. The column employed was 30 m long with a 250-μm inner diameter 0.25-μm thick (5%-phenyl)-methylpolysiloxane stationary phase (Restek, Bellefonte, PA, USA). The carrier gas consisted of ultra-pure grade helium (Air Products, Parkersburg, WV, USA) at a liner velocity of 2 ml min^{-1}. All samples were injected in the split mode (10:1) with the needle held for 0.2 min in the injector port before removal. The injector temperature was held at 250°C and the detector at 300°C. For THC-TMS detection the oven was ramped from 160 to 280°C at 40°C min^{-1} and from 280 to 300°C at 5°C min^{-1} to give a run time of 7 min. Stir rate was measured with a 631-BL Strobotac® (General Radio, Cambridge, MA, USA). Confirmation of the trimethylsilyl-derivative of THC-COOH was performed on a Finnigan Polaris (GCQ Plus) GC–MS instrument (Finnigan) equipped with Excalibur quantitative analysis software and an RTX-5 30-m×250-μm×0.25-μm 95% dimethyl–5% diphenyl polysiloxane copolymer column (Restek, Bellefonte, PA, USA).

2.3. Derivatization procedures

Derivatizations were first performed in separate vials, outside of the membrane tubing extraction system. Samples of THC-COOH in methanol were evaporated under nitrogen and derivatized using pure BSTFA in a silanized vial. Mass spectra indicated the presence of the trimethylsilyl derivative from the ion scans as given by Radian as well as the in-house library. The derivative structure was confirmed by GC–MS analysis.

In order to calculate preconcentration factors and extraction efficiencies, a calibration was made of THC-TMS derivative by creating a 20 μg ml^{-1} solution of THC-COOH by taking a portion of stock solution (100 μg ml^{-1} in methanol), evaporating the

solvent, and reconstituting in excess BSTFA and hexane. Subsequent dilutions were made down to 0.156 μg ml^{-1}. An eight-point calibration gave an R^2 of 0.9995 using 5 μl as the injection amount and dilution factors were taken into account when analyzing data with different injection volumes.

2.4. Tubing procedure: preconditioning, filling, and recovery

Membrane tubing is cut into 6-cm segments after being singed at one end with a flame to form a seal. The tubing is soaked in the solvent system used for extraction for at least 3 min and a metal tube that fits snugly around the size of the membrane tube is used to lace the tubing through a septum vial lid. The extraction takes place in a 7-ml silanized vial. A similar vial is filled with solvent and used as a model to judge tubing penetration depth (spotted with liquid correction fluid) as well as to serve as a vessel for filling the tube with solvent before it is submerged in the extraction medium. The tube is positioned so that the sealed end rests ~7 mm from the bottom of the vial filled with solvent to ensure that the motion of the stir bar does not disturb the tubing during the extraction. The septum can simply rest upon the vial without need for a screw cap. The tube is filled with ~20 μl of solvent for a 6-cm tubing length. A different needle, with a Chaney adapter set to recover 6 μl, is used for recovery. The needle is penetrated to a depth very close to the bottom of the tube before the plunger is raised, ensuring minimal air gaps and better repeatability. Fig. 2 gives a diagram of the extraction apparatus.

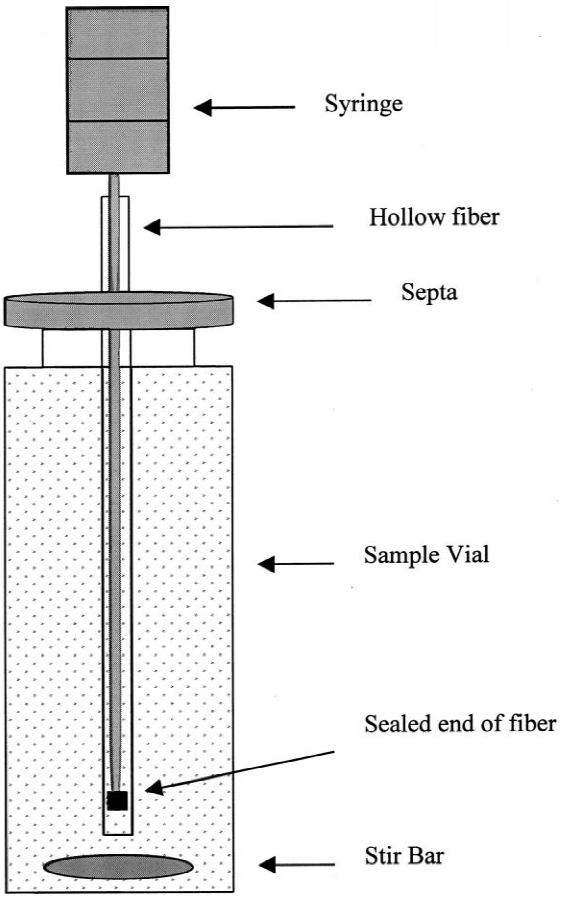

Fig. 2. Hollow fiber membrane solvent microextraction apparatus. Syringe is inserted and withdraws 6 μl of solution after set extraction time.

3. Results and discussion

3.1. Method development

Two approaches have been explored for the extraction/derivatization of THC-COOH. The first involves a direct extraction into an organic solvent from an acidic medium (ensuring that the target acid is in its uncharged form) and the second involves creating the charged form (in a basic medium) and extracting the target drug as an ion pair.

There are several factors that affect the outcome of this solvent microextraction system and these were optimized for each type of system. Optimization was investigated from a univariate approach for the following parameters: derivatizing reagent, extraction solvent, pH of sample and ion pair concentration, stir rate, volume of reagent inside tubing and extraction time. It was assumed that after first finding an appropriate extraction solvent all other parameters could be optimized by altering one at a time while holding all others constant. Although parameters were investigated for both acidic and basic extractions, the acidic method was accompanied with extremely high RSD values (possibly due to tube leakage whereby the sample composition was altered from multiple extractions of the same sample). Tube

leakage was lessened with the ion pairing method, most likely due to the ionic environment created when the salt was added to the system, which inhibits solvent solubility in the aqueous medium. Since the basic ion pairing scheme gave far superior results in terms of peak area and repeatability, results will be given for this method only. Also the ability to extract at a pH that is suitable for other drugs of abuse, usually basic, was desired, therefore the ion pairing extraction was considered the most appropriate method for the detection of THC-COOH.

3.2. Derivatizing reagent

Rather than optimize this parameter for response, the interest lay mainly in finding a ''user-friendly'' reagent that could be added to the tubing in one step and did not require specific reaction conditions (heat or catalyst). BSTFA proved to be such a reagent and, although sensitive to moisture, still gave a measurable response for low concentrations of THC-TMS when used in the presence of the aqueous samples. Alkylation (methylation using iodomethane [15]) was also attempted but was not further explored after yielding little or no results. In-tube derivatization is not optimally suited for alkylation because this method requires the addition of a catalyst (usually TMAH) which creates another addition step inside the tubing. Therefore, all research involved forming the trimethylsilyl derivative with BSTFA or BSTFA+1% TMCS (trichloromethylsilane). Fig. 3 illustrates the chromatogram of a TMS-derivative formed from BSTFA after a tubing extraction. Note that, although the subject was taking medications containing loratadine, pseudoephedrine sulfate, cephalexin, norethindrone, ethinyl estradiol, and carbamazepine, these did not produce peaks which interfered with the analysis, as indicated by the blank urine sample.

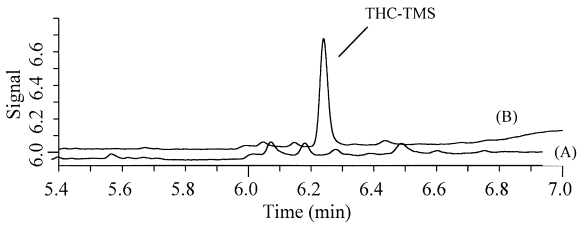

Fig. 3. Chromatogram of blank urine (A) and urine spiked with 50 ng ml^{-1} THC-COOH (B) detected as the THC-TMS derivative after 8-min extraction-derivatization into BSTFA/octane mixture inside hollow fiber tubing.

3.3. Extraction solvent

The most important influence on extraction efficiency has to do with the components inside the tubing, namely the choice of solvent and derivatizing reagent and at optimal proportions. In the interest of maintaining a universal solvent for all drugs, chloroform was chosen as the initial solvent because it performed best for a number of other drugs and metabolites [8], however acidic drugs are traditionally extracted with solvents such as ethyl acetate, acetone–chloroform, or an acetate–chloroform–isopropanol mixture [16]. Switching from the initial chloroform solvent to hexane dramatically decreased noise in the chromatogram and improved peak areas for the desired TMS-derivative. The chloroform could possibly have been more reactive with the BSTFA or the tubing or possibly leaked from the tubing due to its greater solubility, whereby other aqueous sample components could have entered into the tubing. Likewise, switching from hexane to octane dramatically improved peak areas and reduced noise even further. The solubility of hexane is 0.0138 g per 100 ml of water while that of octane is only 0.0015 g per 100 ml water [17] and therefore proved to be the best extraction solvent, remaining in the hydrophobic tubing with minimal possible leakage into the water. Replacing hexane with octane resulted in no ''bubble'' formation on the tube (due to solvent leakage when rigorous stirring is used), improved recovery of sample in terms of amount and uniformity (reduced air gaps in syringe), reduced the background noise in chromatograms (presumably by reducing side reactions), and led to a much greater signal in terms of peak area so that detection limits were much lower. Therefore all optimized parameters are the results of the BSTFA:octane solvent system.

To optimize the ratio of derivatizing reagent:solvent (BSTFA:octane), the ratios were combined in the following proportions: 1:1, 3:1, 5:1, and 7:1 (v/v) BSTFA:octane with the concentration of THC-COOH held at 30 ng ml^{-1} in an 8-ml urine sample.

The 5:1 mixture gave the greatest peak areas. The tubing is preconditioned in pure octane in the hope that this would function as a protective layer for the BSTFA mixture placed in the tubing, although the extent to which moisture comes in contact or effects the BSTFA is unknown. It was also found that pure BSTFA performed much better and cleaner than BSTFA+1% TMCS which is often added as a catalyst to improve reaction rates of sterically-hindered functional groups. In this case, however, the peak areas were markedly decreased with the catalyst present. This was possibly due to competitive reactions among other organic constituents from the biological matrix or the increased susceptibility to the aqueous environment.

3.4. Optimization of pH and ion pair concentration

Sample pH was optimized before ion pair optimization because it was assumed that pH was a factor that would have a greater effect on the extraction, altering the actual charge on the drug moiety. Optimum pH was 8.0 (usually corresponding to ~50 μl of pH 13 phosphate buffer added to an 8-ml urine sample) when the ion pair reagent was held at a 10-μl addition and the concentration of THC-COOH at 30 ng ml^{-1}. However, the extraction was still effective when the pH was not altered (Fig. 4), most likely because natural urine is basic enough for the drug to be charged. At a pH of 8.0, the amount of ion pair reagent (0.056 g ml^{-1} tetramethylammonium hydrogen sulfate) added to an 8-ml urine sample was 100 μl, but peak areas were not much larger than with only 25-μl additions (Fig. 5). It is interesting to note that the extraction took place to a significant extent without any ion pair added to the system, presumably from any natural salts present in urine. However, it cannot be assumed that all urine samples will have a substantial amount of the species necessary for the extraction to take place. Therefore, ion pair reagent should be added until a large number of different urine samples can be studied to verify that no ion pair is necessary in a urine matrix. Adding ion pair did significantly improve the extractions from a pure water matrix.

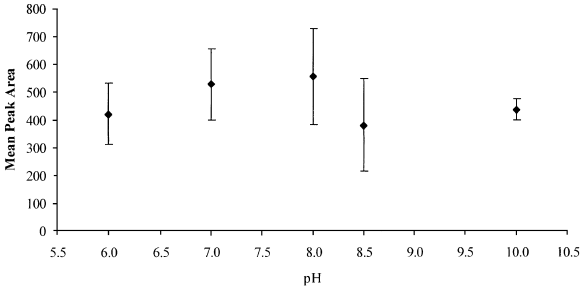

Fig. 4. Optimization of pH for the extraction of THC-COOH from 8-ml urine samples into BSTFA:octane solvent system inside hollow fiber. Phosphate buffer (pH 13) was added to urine to vary the pH while the ion pairing reagent (0.056 g ml^{-1} TMAHS) remained constant. Results were based on the mean of triplicate 8-min extractions with error bars based on a 95% confidence interval.

3.5. Stir rate and hydrodynamics

The stir rate was varied from 1 to 4.5 in 0.5-level increments for the ion pair protocol above using 10-μl ion pair at pH 6. For a 7×2-mm stir bar, the optimum stir rate was 3.5 (1540 rpm), which was the highest rate possible without causing the tubing to move or sway. With the 7-mm stir bar, complete stirring from base to surface was achieved, confirmed visually by the addition of 10-μl quantities of $KMnO_4$. Even with a stir rate of only 2, the colored drop spread uniformly in 1 or 2 s. Therefore, the 7-mm stir bar was used for all subsequent extractions at a stir rate of 3.5, much greater than is possible with a microdrop, which is in the order of hundreds of rpm [6].

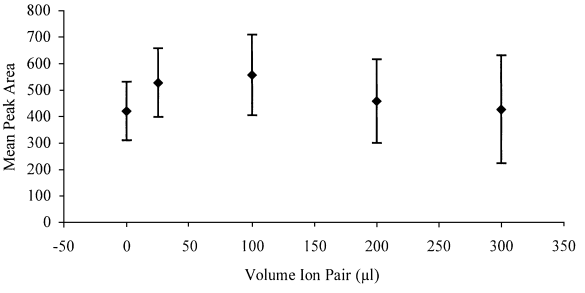

Fig. 5. Optimization of ion pairing reagent (0.056 g ml^{-1}) added to 8-ml sample of urine keeping pH at 8.0 and extracting for 8 min. The results are the means of triplicate extractions with error bars based on a 95% confidence interval.

3.6. Volume of solvent inside tubing

Aqueous samples were spiked with 30 ng ml^{-1} THC-COOH and the extractions proceeded according to the optimized conditions, varying the total volume of the BSTFA:solvent inside the tubing. For volumes of 10, 15, 20, and 25 μl, the response tapered off above 20 μl so this was chosen as a convenient filling volume since10 μl is a common syringe volume. The larger volume of reagent allows a greater amount to be recovered and injected. Octane has a low enough volatility that enough is left in the tubing after an extraction, so that a Chaney adapter could be set to collect 6 μl with little or no air gaps in the syringe.

3.7. Extraction time

For 8-ml urine samples spiked with 30 ng ml^{-1} and all other parameters held constant, the BSTFA: octane solvent system could be left as long as 11 min and still have a 6-μl recoverable portion. The average peak area was greatest after a 10-min extraction, but RSDs increased above 8 min, so an 8-min extraction was chosen as optimum. An 8-min extraction gave an RSD of 6.6% while 9-, 10-, and 11-min extractions gave RSDs of 21, 17, and 22%, respectively, most likely due to variations in the extent of the reaction occurring after longer periods of time.

3.8. Concentration dependence

Peak area calibration graphs over the concentration range 10–250 ng ml^{-1} ($n=3$ at each concentration) were obtained for four different extraction methods. Comparisons of extraction protocol and RSD values are summarized in Table 1. The ion pairing calibrations were quite linear with hexane but still accompanied by high RSDs. The RSDs greatly improved (averaging 6.1%) when the Chaney adapter was set at 6 μl rather than simply trying to extract all possible in the 10-μl syringe (which caused air gaps). To test concentration dependence, a co-worker spiked urine samples, which were analyzed in triplicate and the concentration calculated from a calibration graph obtained on the same day. Values for blind trials averaged a 3.7% relative error for five separate trials, the results of which are summarized in Table 2.

Table 1
Concentration dependence and linearity

Extraction method	R^2	% RSDs for THC-COOH concentrations (ng ml^{-1})				
		10	20	50	100	250
A	0.9319	22.9	44.0	29.1	18.2	10.8
B	0.9994	11.9	22.7	22.1	8.3	12.0
C	0.9986	15.4	18.2	16.1	12.7	8.1
D	0.9968	5.2	6.6	3.0	6.8	8.9

Calibrations corresponding to particular extraction method used. Method A corresponds to the direct acidic extraction (pH 2.0), and no set injection volume. Method B involved the basic, ion pairing with BSTFA+1% TMCS:hexane as the extraction solvent and no set injection volume. Method C was the BSTFA:hexane solvent when the Chaney adapter was set at 6-μl recovery volumes. Method D indicates the BSTFA:octane solvent system when the Chaney adapter was set to 6 μl, resulting in greatly improved relative standard deviations (RSDs) for this extraction system.

3.9. Adulteration studies

The main advantages of solvent micro-extractions for detection of THC-COOH in urine are the low solvent consumption in terms of reagent and disposal costs, ease of operation, and decreased analysis time. However SME, when coupled to GC, also provides the additional benefits of a chromatographic data analysis which is useful when contamination or adulteration of the specimen is possible. Although many chemical species may successfully taint an immunoassay screening, SME relies on entirely different principles and therefore many adulterants are unsuccessful at interfering or may even slightly improve the extraction. The ability to view an entire

Table 2
Blind trials

Unknown solution (ng ml^{-1})	Predicted concentration (ng ml^{-1})	Relative error (%)
31.3	32.6	−4.2
93.8	86.2	8.1
46.9	44.1	6.0
68.8	68.6	0.2
0.0	0.0	0.0

All results are taken as the mean of three consecutive extractions from the same sample.

chromatographic ''finger-print'' of a typical blank urine specimen is helpful. In this case, if a certain adulterant was able to somehow deteriorate the indicative peak, other retention times could be monitored where specific adulterant peaks have been known to elute.

There are a variety of common products used when attempting to mask a target drug in a urine sample and 19 such adulterants were tested, each of which reflect various degrees of interference (both positive and negative) depending on immunoassay used [11]. Each adulterant was added to the THC-COOH spiked 8-ml urine sample (30 ng ml^{-1}) in varying concentrations, after which the pH was immediately measured. The adulterant was judged as a potential threat only if there existed a specific concentration that showed no suspicious pH values which could diminish the THC-TMS peak to an undetectable level. In almost every case either the adulterant did not mask the THC-TMS peak or it needed to be added in such high amounts that some other indicator marked a suspicious sample that could be further analyzed by a second more accurate method. Suspect samples would have indicators such as abnormal pH levels or display incredibly ''dirty'' early portions of the chromatogram such as ten or more well-defined peaks that were absent in the blank or gave responses much greater ($S/N>>3$) than peaks from a blank run. Results for various adulterants are shown in Table 3 and an example of a chromatogram characterized as ''dirty'' is displayed in Fig. 6. Note that samples were often adulterated at

Table 3
Effects of common adulterants on extraction/derivatization of THC-COOH

Adulterant, concentration	Effect on peak area (%)	Adverse characteristics
Ammonia, 25%	−46	pH 12[a], early peaks higher than blank
L-Ascorbic acid (sat'd)	−42	pH 2[a], after 600-μl buffer, pH only 4.5
Bleach, liquid Clorox®		
0.6%	−10	Chromatogram must be monitored
25%	Undetectable	(t=3.05 min) for bleach
Detergent, solid (sat'd)	−52	pH 9.0, very dirty chromatogram
Detergent, liquid, five drops	−85	Very dirty chromatogram
Drano®, 5.9%	−90	pH 14[a], dirty chromatogram
Ethanol, 25%	−44	
Ethylene glycol, 25%	−28	
Glutaric dialdehyde, 6.3%	−14	Dirty chromatogram
Hydrogen peroxide, 1.5%	+41	Bubbles vigorously, turns clear when stirred
Lime solvent, 1.2%	−29	pH 3.0[a], dirty chromatogram
Potassium hydroxide (0.036 g)	−89	pH 12.5[a], dirty chromatogram
2-Propanol		
25%	−60	Monitor just after solvent peak (t=1.7
50%	Undetectable	min) for 2-propanol
Salt (NaCl), sat'd	−25	
Soap, liquid hand (0.03 g)	−27	Very dirty chromatogram
Sodium bicarbonate (sat'd)	+19	pH 8.5
Vanish®, 5.9%	−80	Blue solution, very dirty chromatogram
Vinegar, 50%	+47	pH 3.0[a]
Visine®, 25%	−8	

Results are based on the mean of three consecutive extractions and compared to the average of three urine samples containing drug but no adulterant, analyzed on the same day. All amounts of adulterant are for an 8-ml urine sample spiked at 30 ng ml^{-1}. Influences on peak area are calculated as a comparison to the blank.

[a] Extractions that were performed at an abnormal pH because the optimal pH could not be achieved after the addition of adulterant.

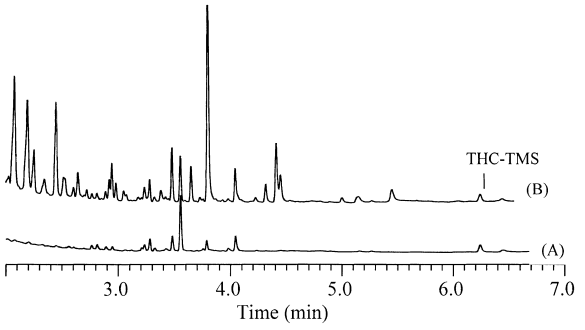

Fig. 6. Example of a chromatogram characterized as ''dirty'' caused by adulteration of the urine by liquid hand soap (B) over a non-adulterated blank (A).

a ridiculously high level to show that, even at amounts that grossly altered pH, appearance, or gave suspicious chromatograms (with peak height and frequency high above a typical blank urine), the analyte was still detectable at a concentration of 30 ng ml^{-1}, below the cut-off level.

Monitoring certain retention times for any potential adulterant can be a useful strategy for targeting suspicious samples which could later be confirmed as adulterated by mass spectral data. Therefore, SME offers a very attractive alternative as a screening method, especially for its ability to detect or be unaffected by adulterated samples.

4. Calculations

4.1. Detection limits, preconcentration factors, and extraction efficiencies

Based on a signal-to-noise ratio of 3, the limit of detection (LOD) was calculated to be 1.0 ng ml^{-1} THC-COOH in urine prior to extraction based on a five-point calibration of signal peak heights. Preconcentration factors, also called enrichment factors, are defined as the concentration in the extracted portion divided by the bulk sample concentration. Extraction efficiencies (the total amount of drug in the extracted portion divided by the total amount in the bulk sample multiplied by 100) are summarized in Table 4. Although extraction efficiencies were rather low (2 –3%), the term ''extraction efficiency'' in this case must take into account not only the extraction but also the derivatization with BSTFA.

Table 4
Extraction efficiencies and preconcentration factors

THC-COOH (ng ml^{-1} in urine)	Preconcentration factor	Extraction efficiency (%)
10	60.4	4.5
20	43.7	3.3
50	44.6	3.3
100	34.4	2.6
250	36.0	2.7

Results calculated from most successful calibration using BSTFA:octane as the mean of triplicate extractions.

Therefore higher amounts may be extracted, but only the successfully reacted drugs are detected. Ordinarily derivatizations of THC-COOH with BSTFA are performed at 100°C for 20 min [18], therefore the 8-min room temperature extraction is not expected to yield the highest possible signal, but to give a detectable signal in a reasonable amount of time.

In research with extracting cocaine with SME in the form of a 2-μl solvent drop suspended from a syringe, extraction efficiencies were between 0.75 and 1.75% for four different metabolites [8] using a 6-min extraction time. Much higher extraction efficiencies (28–69% depending on solvent used) were achieved in a study of benzodiazepines from urine using 6 cm of tubing, however the extraction time was 50 min [9]. A greater amount of surface area that is exposed to the sample results in better extraction efficiencies, therefore the 6 cm of tubing offers significant improvement over a 2-μl extraction drop. A 2-μl extraction drop, calculated as a sphere, has a surface area of 7.7 mm^2. Estimating that ~41 mm of tubing length is exposed to the urine solution, the outer surface area of the fiber (excluding the bottom heat-sealed end) is 129 mm^2. However, as the porosity of the polypropylene fiber is 70%, the actual amount of exposed octane would be ~90 mm^2. The improved extraction efficiencies could also be due to factors other than surface area, namely the higher stir rate and larger sample volumes that are possible using the membrane tubing.

5. Conclusions

A rapid, inexpensive screening method has been

developed for the simultaneous filtration, extraction, and derivatization of Δ^9-THC-COOH from real urine samples using hollow fiber membrane solvent microextraction (HFMSME). The membrane tubing acts as a vessel for the mixture of extraction solvent (octane) and derivatization reagent (BSTFA). Clean and efficient sample clean-up occurs as the drug is extracted into the permeable tubing for a set extraction time, withdrawn into a syringe, then directly injected into a GC for analysis of the TMS-derivative.

This technique may be characterized currently as only semi-quantitative but the speed and ease of the extraction/derivatization more than makes up for any variability from run to run. The cost of the tubing is less than 1 cent per 6-cm tube, which allows it to be discarded and replaced between extractions, eliminating the possibility of sample carry-over, which is possible in solid-phase microextraction. Only 20 μl of reagent is used for each extraction, minimizing the cost of organic reagents and waste disposal. There is no reason why this method could not be coupled directly to a GC–MS analysis system for use as a confirmatory analysis method.

References

[1] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[2] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 2935.
[3] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[4] M.H. Ma, F.F. Cantwell, Anal. Chem. 70 (1998) 3912.
[5] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[6] L.S. De Jager, A.R.J. Andrews, Chromatographia 50 (1999) 733.
[7] H. Zhang, A.R.J. Andrews, J. Environ. Monit. 2 (2001) 656.
[8] L.S. De Jager, A.R.J. Andrews, J. Chromatogr. A 911 (2001) 97.
[9] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 749 (2000) 85.
[10] I. Tebbett (Ed.), Gas Chromatography in Forensic Science, E. Horwood, New York, 1992.
[11] R.H. Liu, B. Goldgerger, A Handbook of Workplace Drug Testing, AACC Press, Washington, DC, 1995.
[12] M. Szirmai, O. Beck, N. Stephansson, M.M. Halldin, J. Anal. Toxicol. 20 (1996) 573.
[13] D.R. Knapp, Handbook of Analytical Derivatization Reactions, Wiley, New York, 1979.
[14] H.H. Maurer, J. Chromatogr. B 733 (1999) 3.
[15] J.D. Whiting, W.W. Manders, J. Anal. Toxicol. 6 (1982) 49.
[16] O.H. Drummer, J. Chromatogr. B 733 (1999) 27.
[17] L. Scheflan, M.B. Jacobs, in: The Handbook of Solvents, Van Nostrand, New York, 1953.
[18] C.J.M. Langen, G.A. de Bijil, A.C.G. Egberts, J. Anal. Toxicol. 24 (2000) 433.