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EVALUATION OF ANALYTICAL METHODOLOGIES FOR NON-INTRUSIVE DRUG TESTING: SUPERCRITICAL FLUID EXTRACTION OF COCAINE FROM HAIR

NIJ Report 601-98

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Evaluation of Analytical Methodologies for Non-Intrusive Drug Testing: Supercritical Fluid Extraction of Cocaine from Hair

NIJ Report 601-98

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FOREWORD

The Office of Law Enforcement Standards (OLES) of the National Institute of Standards and Technology (NIST) furnishes technical support to the National Institute of Justice (NIJ) program to strengthen law enforcement and criminal justice in the United States. OLES's function is to conduct research that will assist law enforcement and criminal justice agencies in the selection and procurement of quality equipment.

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This document covers research conducted by OLES under the sponsorship of the National Institute of Justice. Additional reports as well as other documents are being issued under the OLES program in the areas of protective clothing and equipment, communications systems, emergency equipment, investigative aids, security systems, vehicles, weapons, and analytical techniques and standard reference materials used by the forensic community.

Technical comments and suggestions concerning this report are invited from all interested parties. They may be addressed to the Office of Law Enforcement Standards, National Institute of Standards and Technology, Gaithersburg, MD 20899-8102.

David G. Boyd, Director
Office of Science and Technology
National Institute of Justice

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LIST OF ACRONYMS USED IN THIS REPORT

BSA	*N*,*O*-bis(trimethylsilyl)acetamide
BZE	benzoylecgonine
DMSO	dimethylsulfoxide
FH-P	cocaine- and benzoylecgonine-fortified hair, powdered
FH-S	cocaine-fortified hair, segmented
GC-MS	gas chromatography-mass spectrometry
HP	Hewlett Packard
LC-MS	liquid chromatography-mass spectrometry
LLE	liquid-liquid extraction
MeOH	methanol
m/*z*	mass-to-charge ratio (in mass spectrometry)
NIST	National Institute of Standards and Technology
NMS	National Medical Services, Inc.
NMS-PPC	pooled cocaine-positive control hair, powdered, obtained from National Medical Services, Inc.
RIA	radioimmunoassay
RR-8	genuine drug user hair obtained as part of a round robin study
SF	supercritical fluid
SFE	supercritical fluid extraction
SFE-RIA	supercritical fluid extraction-radioimmunoassay
SPE	solid phase extraction
TEA	triethylamine
TMS	trimethylsilyl

COMMONLY USED SYMBOLS AND ABBREVIATIONS

A	ampere	H	henry	nm	nanometer
ac	alternating current	h	hour	No.	number
AM	amplitude modulation	hf	high frequency	o.d.	outside diameter
cd	candela	Hz	hertz (c/s)	Ω	ohm
cm	centimeter	i.d.	inside diameter	p.	page
CP	chemically pure	in	inch	Pa	pascal
c/s	cycle per second	ir	infrared	pe	probable error
d	day	J	joule	pp.	pages
dB	decibel	L	lambert	ppm	part per million
dc	direct current	L	liter	qt	quart
°C	degree Celsius	lb	pound	rad	radian
°F	degree Fahrenheit	lbf	pound-force	rf	radio frequency
diam	diameter	lbf·in	pound-force inch	rh	relative humidity
emf	electromotive force	lm	lumen	s	second
eq	equation	ln	logarithm (natural)	SD	standard deviation
F	farad	log	logarithm (common)	sec.	section
fc	footcandle	*M*	molar	SWR	standing wave radio
Fig.	figure	m	meter	uhf	ultrahigh frequency
FM	frequency modulation	min	minute	uv	ultraviolet
ft	foot	mm	millimeter	V	volt
ft/s	foot per second	mph	mile per hour	vhf	very high frequency
g	acceleration	m/s	meter per second	W	watt
g	gram	N	newton	λ	wavelength
gr	grain	N·m	newton meter	wt	weight

area=unit^2 (e.g., ft^2, in^2, etc.); volume=unit^3 (e.g., ft^3, m^3, etc.)

PREFIXES

d	deci (10^{-1})	da	deka (10)
c	centi (10^{-2})	h	hecto (10^2)
m	milli (10^{-3})	k	kilo (10^3)
μ	micro (10^{-6})	M	mega (10^6)
n	nano (10^{-9})	G	giga (10^9)
p	pico (10^{-12})	T	tera (10^{12})

COMMON CONVERSIONS
(See ASTM E380)

ft/s×0.3048000=m/s
ft×0.3048=m
ft·lbf×1.355818=J
gr×0.06479891=g
in×2.54=cm
kWh×3 600 000=J
lb×0.4535924=kg
lbf×4.448222=N
lbf/ft×14.59390=N/m
lbf·in×0.1129848=N·m
lbf/in^2×6894.757=Pa
mph×1.609344=km/h
qt×0.9463529=L

Temperature: $(T_{°F}-32)\times 5/9=T_{°C}$

Temperature: $(T_{°C}\times 9/5)+32=T_{°F}$

EVALUATION OF ANALYTICAL METHODOLOGIES FOR NON-INTRUSIVE DRUG TESTING: SUPERCRITICAL FLUID EXTRACTION OF COCAINE FROM HAIR[1]

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Supercritical fluid extraction (SFE) was investigated as an environmentally friendly alternative to currently used wet chemical procedures in hair drug testing protocols. The SFE behavior of cocaine and its primary metabolite benzoylecgonine was studied and found to be highly dependent upon the nature of the matrix and the manner in which the target drug analytes are incorporated into or on the matrix. The dependence of extractability on hair/drug binding interactions allows the differentiation of cocaine present at different discrete sites in hair based on differences in SFE behavior, suggesting the potential for distinguishing exogenous (i.e., environmental) from endogenous (i.e., physiological) sources of drugs in hair by employing sequential extraction under differing SFE conditions. SFE using pure carbon dioxide was found to be superior to existing liquid decontamination methods for the removal of vapor-deposited cocaine from the surface of hair. Addition of an SFE modifier mixture containing triethylamine and water allowed the recovery of "matrix-bound" cocaine from hair binding sites, and extraction efficiencies compared favorably with existing liquid-based extraction procedures. Extraction times in SFE were approximately 30 min to 50 min, compared with several hours to one day for currently used liquid methods.

Based on the extraction results observed on drug-fortified and drug user hair, a displacement SFE mechanism is proposed in which triethylamine competes with cocaine hair binding sites. The SFE results additionally provide insight into the nature of hair-drug binding interactions and lend support to a current model for drug incorporation in hair.

Key words: supercritical fluid extraction; drugs; hair; cocaine; benzoylecgonine.

1. INTRODUCTION AND SCOPE

In recent years, human hair has received considerable attention as a toxicological specimen for evidence of chronic drug use in clinical and forensic investigations, as well as workplace drug testing programs. Compared with urinalysis, hair analysis has the potential for greatly expanding the time window for drug detection. Additionally, proponents of hair drug testing argue that sample acquisition is less intrusive. Despite these advantages, widespread acceptance of hair as a reliable drug testing medium has been hindered by a lack of uniformity in analytical hair drug testing protocols, a limited understanding of the mechanisms of drug incorporation in hair, and continued controversy over the ability of laboratories to reliably distinguish passive environmental contamination from active drug use. Laboratories engaged in hair drug testing currently employ operationally-defined rinse protocols to remove environmental contamination from the hair surface; however, the significance and effectiveness of these decontamination procedures have been the subject of much debate [1–5][4]. Following the rinsing steps, a variety of wet chemical extraction procedures (e.g., acid incubation, alkaline hydrolysis, enzyme dissolution, organic solvent extraction) are

[1] Portions of this manuscript have been published in Morrision, J. F.; Chesler, S. N.; Yoo, W. J.; Selavka, C. M. *Anal. Chem. 1998, 70, 163–172.*
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[4] Numbers in brackets refer to references in Section 5.

employed for release of "matrix-bound" drugs from the hair [6, 7]. Typically, the target drug analytes are subsequently isolated from the crude extracts by liquid-liquid extraction (LLE) or solid phase extraction (SPE) methods. These wet-chemical procedures are often labor- and time-intensive, involving multiple manipulations that are not easily automated.

Continued growth in the field of hair drug testing will be fostered by the development of independent confirmatory extraction technologies which provide alternatives to liquid-based methods. An ideal extraction method should simplify sample preparation steps, provide rapid, efficient recoveries of target analytes with the potential for automation, and also accurately address the environmental contamination issue. The following report describes the results of our investigation of supercritical fluid extraction (SFE) as an environmentally "friendly" sample preparation method which overcomes some of the limitations of currently used liquid-based hair drug testing methodologies while simultaneously contributing to a greater fundamental understanding of drug-hair binding mechanisms.

A supercritical fluid (SF) is a substance which exists above a critical temperature and pressure (see Fig. 1) which possesses properties intermediate between those of gases and liquids. The most commonly used SF in analytical applications is carbon dioxide (CO_2), which has an easily attainable critical temperature (31 °C) and pressure (7.60 MPa). Because CO_2 is a gas at ambient conditions, there is no hazardous solvent waste generated following an extraction procedure. For this reason, SFE is often referred to as an environmentally "friendly" alternative to liquid solvent extraction. SFs possess gas-like matrix-penetrating power while maintaining liquid-like solvating power; consequently, SFs are considered attractive alternatives to conventional liquid solvent extraction for rapid, efficient recoveries of target analytes.

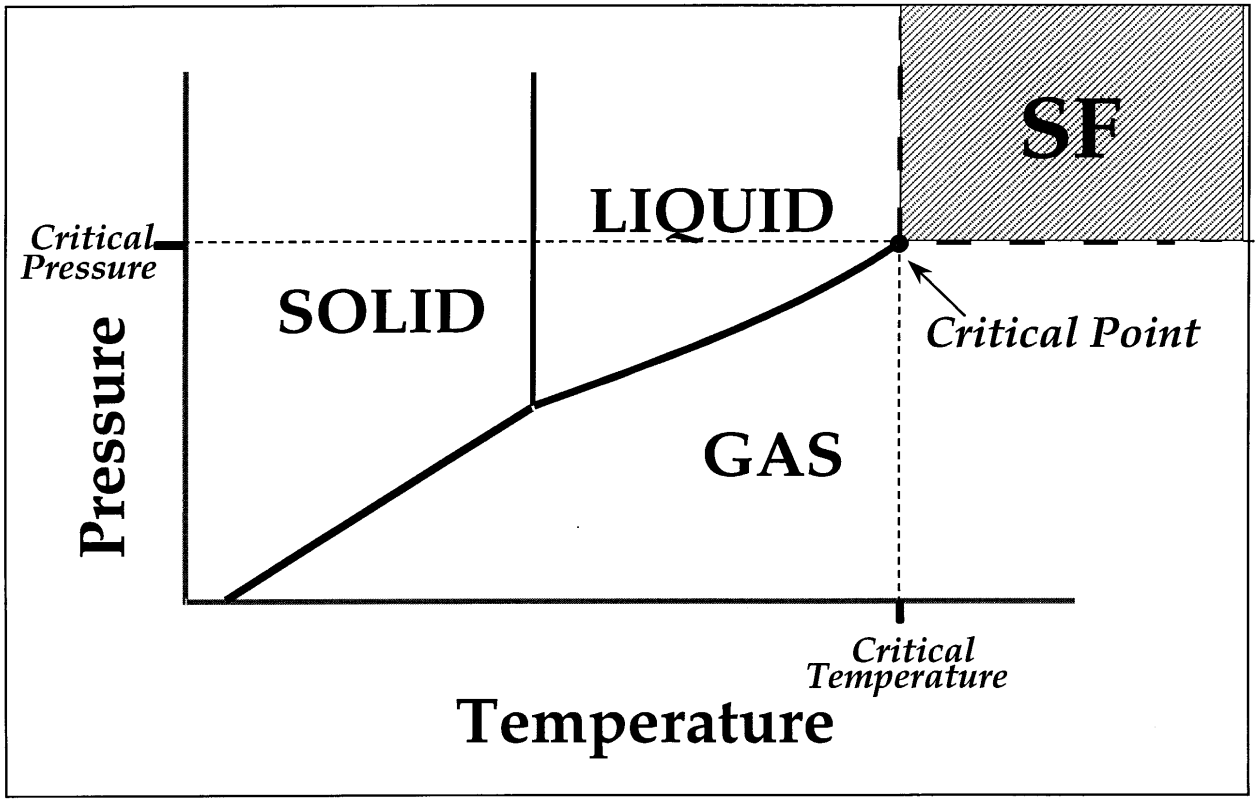

FIGURE 1. *Temperature-pressure phase diagram for a substance.*

Automated equipment for SFE is commercially available and is finding routine use in environmental and food analysis. Unlike liquid extraction techniques, a variety of parameters may be controlled in SFE (e.g., temperature, pressure, fluid composition) to provide selectivity. For more detailed treatment of the fundamental principles, theoretical aspects, and survey of applications of SFE, the reader is referred to several recent reviews [8–15].

Preliminary work in our laboratory [16] demonstrated rapid, efficient recoveries of cocaine from drug-fortified hair using CO_2 with addition of a modifier mixture of triethylamine (TEA) and water to the hair sample. An off-line SFE-radioimmunoassay (SFE-RIA) method was subsequently developed for the rapid screening of cocaine residues in drug user hair [17]. Edder et al. [18] reported an SFE method for the recovery of opiates from drug-fortified and drug user hair using CO_2 modified with a ternary mixture of methanol, TEA, and water under subcritical conditions. Cirimele et al. [19] have adapted the procedures of Edder et al., and Morrison et al., for use on a commercial SFE instrument, demonstrating recoveries of opiates, cocaine, and cannabinoids from drug user hair under a single set of SFE conditions.

The goal of the present study was to investigate SFE as a convenient alternative to currently used "wet" chemical methods for the extraction of cocaine (Fig. 2A) and its principal metabolite benzoylecgonine (BZE) (Fig. 2B) from hair and to evaluate the reliablity of the technique for distinguishing environmental contamination (both vapor and sweat-deposited drug) from active drug use. Underlying these practical goals were the more fundamental goals of elucidating the mechanisms of hair-drug binding by studying the influence of the hair matrix on drug extractability as well as investigating the role of modifiers in improving extractability by SFs.

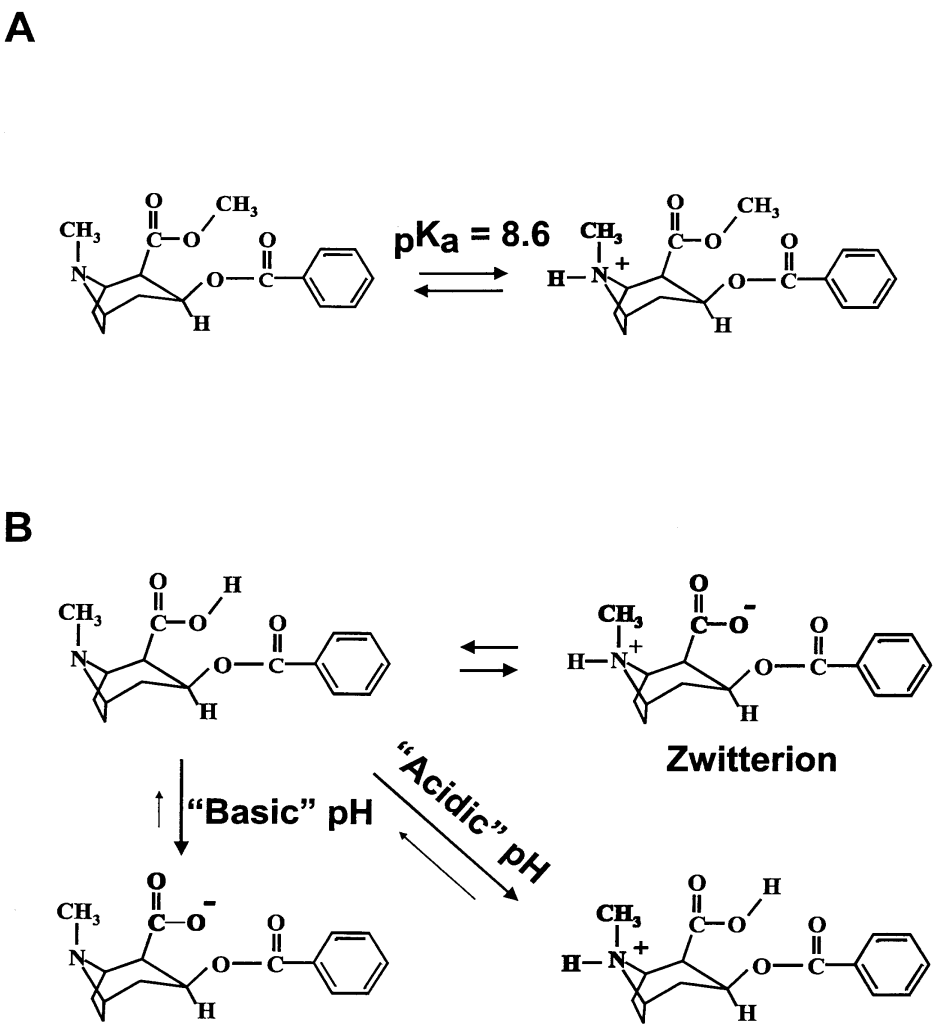

FIGURE 2. *Structures and potential charge states of (A) cocaine, and (B) benzoylecgonine in aqueous solution.*

2. EXPERIMENTAL METHODS[5]

2.1 Chemicals and Reagents

Pure crystalline cocaine hydrochloride and free base cocaine were obtained from Mallinckrodt (St. Louis, MO) and Sigma Chemical Company (St. Louis, MO), respectively. Benzoylecgonine tetrahydrate was purchased from ADRI Technam (Park Forest, IL). Methanolic solutions of cocaine-d_3 hydrochloride (0.098 mg/mL as free base) and benzoylecgonine-d_3 (0.1 mg/mL) were obtained from MSD Isotopes (Montreal, Canada) and Sigma Chemical Company, respectively, for use as internal standards for quantification. The silylation derivatization reagent *N*,*O*-bis(trimethylsilyl)acetamide (BSA) was supplied in 1 mL ampules from Pierce (Rockford, IL). HPLC-grade methanol and water were obtained from J. T. Baker (Phillipsburg, NJ). Triethylamine (99+ %) was obtained from Aldrich (Milwaukee, WI). USP Normal Saline (0.9 % NaCl for Irrigation) used in the sweat contamination experiments was obtained from McGraw, Inc. (Irvine, CA). A commercially available solid-phase ^{125}I radioimmunoassay kit (Coat-A-Count Cocaine Metabolite, Diagnostic Products Corporation, Los Angeles, CA) was employed for detection of cocaine in SF extracts during the temperature optimization experiments performed on vapor-contaminated hair in the decontamination studies.

2.2 Hair Samples

2.2.1 Hair Samples for Matrix and Modifier Studies

Drug-free (blank) hair, drug-fortified hair, and drug user hair were employed in this study. Drug-free hair was pooled from volunteers at NIST and was cryogenically ground [20] to a fine powder to produce a homogeneous blank control. A high level cocaine-fortified segmented hair (FH-S) and mid-level cocaine- and BZE-fortified powdered hair (FH-P) were prepared by soaking segmented hair in a dimethylsulfoxide (DMSO) solution of the drugs (cocaine hydrochloride and BZE-tetrahydrate) for a one-month period. Following removal of the DMSO, the hair was rinsed with methanol, allowed to air dry, and, for the FH-S samples, extracted with no further pretreatment. For the FH-P samples, the fortified hair batch was cryogenically ground to a fine powder. Hair samples demonstrated as cocaine-positive based on previous tests were provided by private commercial drug testing laboratories. Pooled cocaine-positive control hair (NMS-PPC) from drug users was obtained in powdered form from National Medical Services, Inc. (NMS, Willow Grove, PA). This pooled positive control hair had been rinsed prior to homogenization using methanol and pH 7 phosphate buffer and was extracted with no further pretreatment. Typical hair sample masses for SFE were 30 mg to 40 mg.

2.2.2 Preparation of Hair Samples Contaminated with "Vapor-Deposited" Drug

Drug-free black Asian hair was employed in the contamination experiments. Four samples (approximately 1 g each) of segmented hair were suspended in wire mesh baskets from the corners of a sealed vapor deposition box (80 cm × 71 cm × 56 cm (H × L × W)). Approximately 2 mg of cocaine free base was placed in a small beaker and heated to approximately 220 °C in a heating mantle positioned centrally at a height of approximately 30.5 cm (12 in) from the bottom of the vaporization chamber. The hair was exposed to the vapors for a period of 2 h. Following vapor deposition, the wire baskets containing the hair were removed from the vapor chamber and the hair was transferred using tweezers to a glass jar, which was capped tightly for later studies. The cocaine contamination level of the hair was determined following acid incubation and solid phase extraction (see below) with subsequent gas chromatography-mass spectrometry (GC-MS) quantification of the cocaine in the extracts (see below). The contamination level was found to be 5.21 ng/mg ± 0.05 ng/mg (mean ± one standard deviation, n = 6).

2.2.3 Preparation of Hair Samples Contaminated with Pseudo-"Sweat-Deposited" Drug

Five grams of drug-free Asian hair (cut) was soaked for 6 h at 37 °C in a drug-saline solution containing 1.45 mg cocaine hydrochloride, 33 mL saline (USP Normal Saline, 0.9 % NaCl, pH 5; McGraw, Inc., Irvine, CA), and 66 mL distilled water. Following exposure, the hair was removed from solution, blotted with paper towels, allowed to air dry, and stored in a capped glass jar for later studies. It should be noted that the hair employed in these studies was *cut* hair,

[5] Certain commercial equipment, instruments, or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), nor does it imply that the equipment, instruments, or materials are necessarily the best available for the purpose.

suggesting that drug contamination could result from “wicking” (i.e., drawing up as through a straw) of the solution into the hair segments in addition to surface contamination with subsequent diffusion into the hair. The cocaine contamination level in the hair was determined in the manner described above for vapor-contaminated hair and was found to be 3.36 ng/mg ± 0.30 ng/mg (mean ± one standard deviation).

2.3 Samples for Spike-Recovery SFE Experiments

Preliminary SFE experiments designed to optimize fluid composition and collection conditions for cocaine were performed using filter paper and Teflon™ wool (Alltech Associates, Deerfield, IL) as spiking matrixes. Filter paper disks (1 cm diameter) were cut from Whatman No. 42 filter paper and placed in the extraction vessel to produce a bed about 0.5 cm thick. A gas-tight syringe was used to deliver a weighed portion of a gravimetrically-prepared stock methanolic cocaine free base or cocaine hydrochloride solution to the filter paper bed, resulting in a total spike amount of 6 μg cocaine. Following evaporation of the spiking solvent, additional filter paper disks were placed in the extraction vessel to produce a total bed thickness of about 1 cm and the vessel was sealed and subjected to SFE. For Teflon™ wool spiking experiments, strands of Teflon™ wool were pre-cut into short segments (3 mm to 5 mm), loosely packed in the extraction vessel, and spiked in the manner described above.

2.4 SFE Instrumentation

2.4.1 Laboratory-Assembled Instrumentation

Experiments designed to optimize cocaine extraction and investigate the influence of the hair matrix and SFE modifier on drug extractability were performed using laboratory-assembled instrumentation shown schematically in Figure 3. An ISCO Model 100D syringe pump (ISCO, Inc., Lincoln, NE) was used for constant pressure delivery of carbon dioxide (SFE/SFC Grade, Air Products, Inc., Allentown, PA) to the extraction system. For introducing modifier to the sample, a Rheodyne Model 7125 rotary valve equipped with a 100 μL sample loop was mounted outside the extraction oven and connected to the entrance end of the extraction vessel. The modifier or modifier mixture was introduced directly to the sample by injection into the CO_2 stream upon initiation of the static extraction step. Extraction vessels (1 cm long × 1 cm i.d.; 780 μL internal volume) were constructed from conventional guard cartridge hardware (Upchurch Scientific, Oak Harbor, WA). A GOW-MAC Series 580 gas chromatographic oven (GOW-MAC Instrument Co., Lehigh Valley, PA) was employed for temperature control. A two-way shut-off valve was installed through the oven wall and connected to the exit end of the extraction vessel to allow operation in either the static (no flow) or dynamic (flowing) extraction modes. SFE was performed at 40.5 MPa and 110 °C with CO_2 as the primary fluid; modifiers and static/dynamic extraction times are specified in the Results and Discussion section. In all cases, a 10 min thermal equilibration period was employed prior to SFE (i.e., no CO_2 present) to allow the extraction vessel to pre-equilibrate to the operating temperature. A linear restrictor (90 cm long × 50 μm i.d.) constructed from aluminum-clad fused silica capillary tubing (Scientific Glass Engineering, Austin, TX)[5] was connected downstream of the shut-off valve for flow control. With this restrictor, fluid flow rates (measured at the pump) during dynamic extraction were reproducible at 1.1 mL/min to 1.2 mL/min (40.5 MPa, 110 °C). Extracted components were collected by immersing the restrictor tip in a test tube containing about 3 mL of methanol. The test tube was capped with a Mininert™[5] valve (Pierce, Rockford, IL) which had its rubber gasket removed to allow insertion of the restrictor.

The valve position on the cap was adjusted to a partially closed position sufficient to hold the restrictor in place while allowing adequate venting of CO_2. Appropriate deuterated internal standards were spiked into the collection test tubes prior to SFE. The collection tube was held in a heating block maintained at approximately 35 °C to prevent freezing or plugging at the restrictor tip.

2.4.2 Commercial Instrumentation

SFE decontamination experiments designed to evaluate the efficiency of supercritical carbon dioxide for the removal of vapor- and sweat-deposited cocaine from hair were performed using a commercially available extraction system. An ISCO Model SFX 3560[5] supercritical fluid extraction unit (fully automated sequential extractor with 24 sample capacity) was employed with pure carbon dioxide as the extraction fluid. Hair samples (30 mg to 40 mg) were placed in extraction cartridges (9 mL volume) composed of a high-temperature crystalline polymer. Glass beads were used to take up the dead volume in the sample cartridge. The samples were extracted at an extraction pressure of 40.5 MPa, employing a 10 min static extraction step followed by a 30 min dynamic extraction step. Extraction temperatures of 40 °C, 75 °C and 110 °C were investigated to optimize recovery in the vapor contamination experiments.

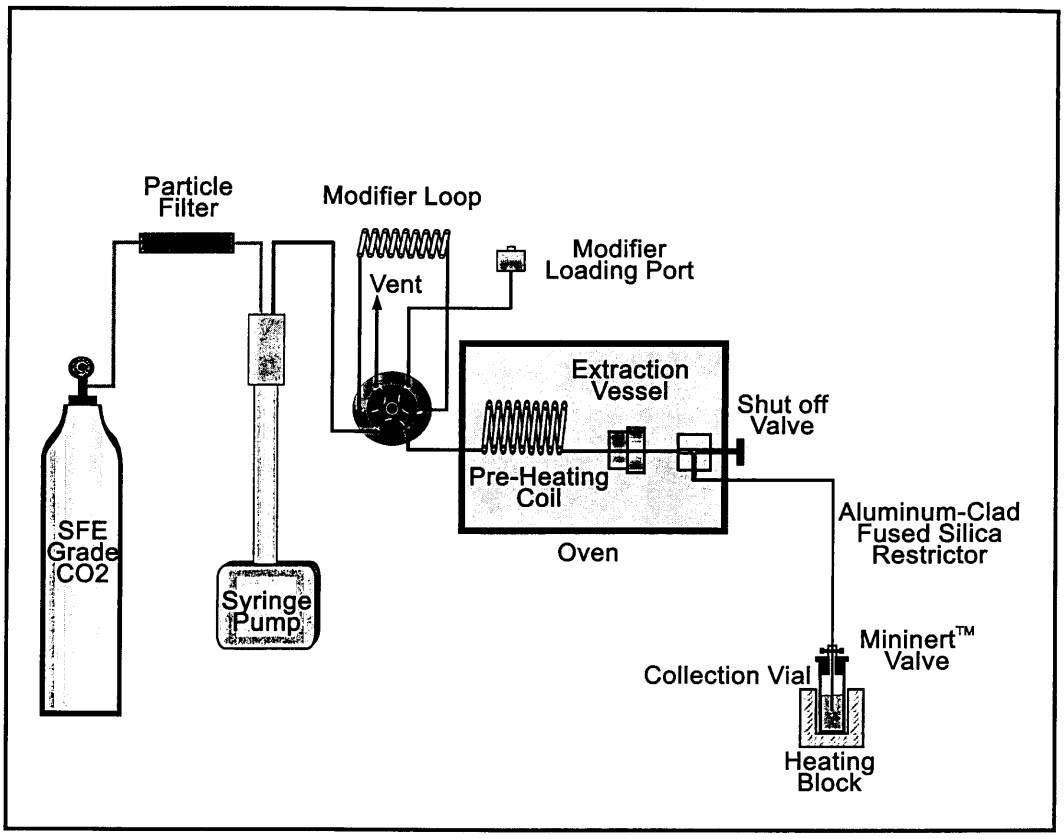

FIGURE 3. *Schematic illustration of laboratory-assembled SFE apparatus.*

Restrictor temperature and fluid flow rate were maintained at 80 °C and 1.2 mL/min, respectively. Extracted analytes were collected by bubbling the CO_2 into collection test tubes containing 7 mL methanol or acetonitrile.

2.5 Acid Incubation/Solid-Phase Extraction

For comparison with SFE results in the matrix and modifier studies, selected samples of drug-fortified hair were subjected to acid incubation followed by solid phase extraction (SPE). In the decontamination studies, initial cocaine contamination levels in the hair were determined following acid incubation/SPE and GC-MS quantification (see below). Additionally, following application of the decontamination procedure (either SFE, methanol rinsing, ethanol rinsing, or a modification of a procedure reported by Baumgartner and Hill [21]), each hair sample was subsequently subjected to acid incubation/SPE in order to determine how much drug remained in the hair following the decontamination protocol.

Hair samples (30 mg to 40 mg) were weighed into vials with Teflon™-lined screw caps, spiked with deuterated internal standards, and incubated with 2 mL of 0.1 mol/L HCl at 45 °C for approximately 24 h. The acid extract was subsequently adjusted to pH 6 with 1.0 mol/L KOH, followed by addition of 2 mL of 0.1 mol/L phosphate buffer (pH 6.0). Cocaine and BZE were isolated from the crude extracts using Bond Elut Certify™ SPE cartridges (Varian, Harbor City, CA) according to the manufacturer's instructions for urine samples.

2.6 GC-MS Analysis of Extracts

SFE and SPE extracts (containing deuterated internal standards) were transferred to conical test tubes and concentrated under a gentle stream of nitrogen to a volume of approximately 300 μL. This volume was transferred to a 0.3 mL Reacti-Vial™, evaporated to dryness, and reconstituted with approximately 30 μL of the silylation reagent BSA, which reacts with the carboxylic acid group of BZE (Fig. 2B) to form a volatile trimethylsilyl (TMS) derivative. The vials were tightly capped with Mininert™ valves and mixed on a vortex mixer prior to analysis.

Quantification of cocaine and BZE in extracts was performed by GC-MS using internal standard calibration with a four-point linear calibration plot. Calibration standards were prepared (by mass) in methanol from gravimetrically-prepared cocaine hydrochloride and BZE tetrahydrate stock solutions and spiked (by mass) with the deuterated internal standard solutions (cocaine-d_3 hydrochloride and BZE-d_3). The calibration standards were evaporated to dryness and reconstituted in BSA prior to analysis. Drug concentrations in the calibration standards were chosen to bracket the expected drug concentrations in the extracts.

GC-MS was performed using a Hewlett Packard (HP) 5890 gas chromatograph interfaced to a HP 5971 mass selective detector. Manual injections (1 μL to 2 μL) of derivatized extracts and calibration standards were made in the splitless injection mode onto a 30 m × 0.25 mm i.d. DB-5ms capillary column with a 0.25 μm film thickness (J & W Scientific, Folsom, CA). Helium was employed as the carrier gas at a head pressure of 103 kPa (15 psi). The oven temperature was programmed from 150 °C (1 min hold) to 250 °C at a rate of 25 °C/min, and then increased to 290 °C at a rate of 10 °C/min (3 min final hold). The injector and transfer line were maintained at 250 °C and 280 °C, respectively. Selected ion monitoring was performed at *m/z* 182 and 185 for cocaine and cocaine-d_3, respectively, and *m/z* 240 and 243 for BZE and BZE-d_3 (as the TMS derivatives), respectively.

2.7 Decontamination Methods for Removal of Vapor- and Sweat-Deposited Cocaine in Hair: SFE and Wet Rinsing Methods

SFE with pure carbon dioxide was evaluated as a decontamination method for the removal of vapor- and sweat-deposited cocaine from hair (see preparation methods for contaminated hair, Sec. 2.5) and the decontamination efficiency was compared with wet rinsing methods. For SFE, the ISCO SFX 3560 instrument was employed with pure SF-CO_2 under the conditions described in the *"Commercial Instrumentation"* (Sec. 2.4.2). Comparison wet rinsing methods included methanol rinsing, ethanol rinsing, and a modification of the rinsing protocol employed by Baumgartner and Hill [21]. The alcohol rinse protocols were performed on a 20 mg to 30 mg sample of contaminated hair and consisted of six sequential 30 min rinses using 15 mL of methanol or ethanol. The rinses were performed at room temperature with shaking on a shaker table. For the modified Baumgartner decontamination method, a 20 mg to 30 mg sample of contaminated hair was rinsed with 3 mL ethanol for 15 min, followed by three 30 min rinses with 3 mL 0.01 mol/L phosphate buffer (pH 6). All rinses were performed at 37 °C with shaking at 100 rpm.

Following application of rinsing protocols, all hair samples were subjected to acid incubation/SPE and GC-MS quantification to determine how much drug remained in the hair (i.e., to determine the efficiency of the decontamination method).

3. RESULTS AND DISCUSSION

3.1 SFE for the Recovery of Cocaine from Drug-User and Drug-Fortified Hair: Influence of Matrix and Modifiers

Initial studies were performed using Teflon™ wool as a model for a truly nonadsorptive, inert spiking matrix in order to investigate cocaine extraction behavior *in the absence of hair matrix effects*. The extraction profile data shown in Figure 4 demonstrates the efficient recovery of cocaine from Teflon™ wool using pure SF-CO_2, confirming the solubility of μg amounts of the drug in the unmodified fluid. In contrast, extraction profile data shown in the same figure for the recovery of cocaine from drug-fortified hair segments (FH-S) under identical SFE conditions demonstrate poor extraction efficiency, suggesting that cocaine is specifically adsorbed to binding sites on the hair and that desorption of cocaine from hair binding sites is a rate-limiting step in the SFE process. These data illustrate how matrix effects have a profound influence on extractability with SF-CO_2.

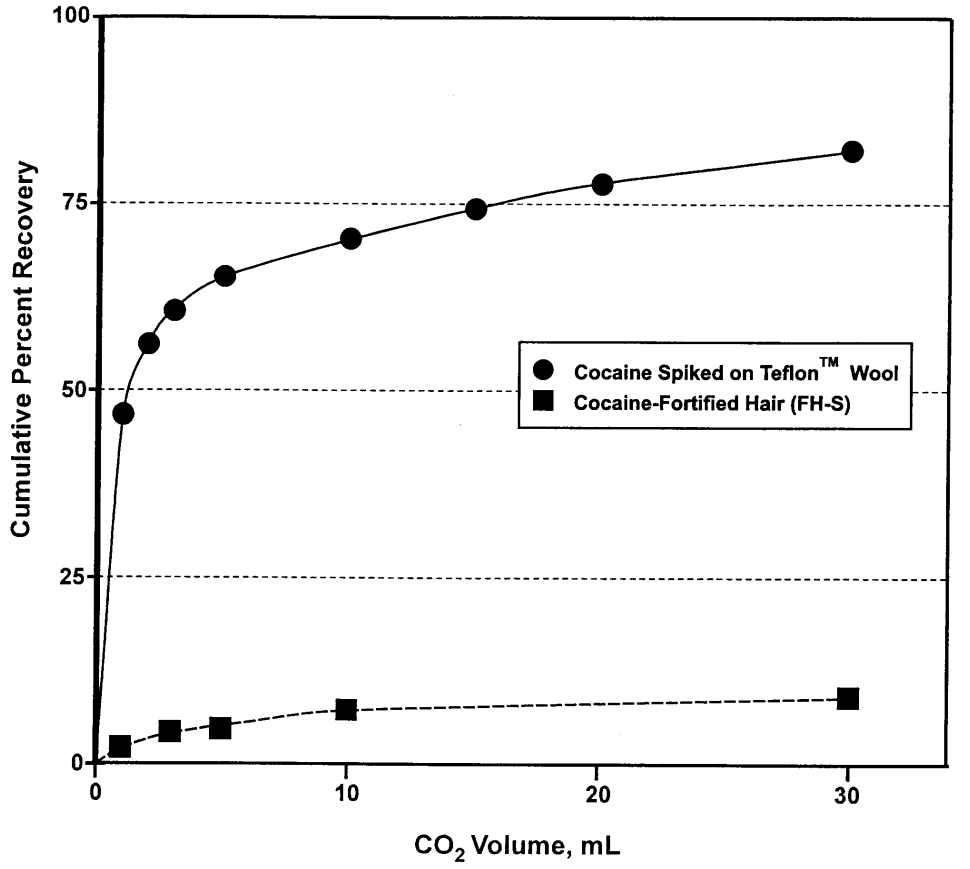

FIGURE 4. *Comparison of SF extraction profiles for recovery of cocaine from Teflon™ wool and drug-fortified hair (FH-S). SFE conditions: 100 % CO_2, 40.5 MPa, 110 °C. For Teflon™ wool, percent recovery is graphed relative to the absolute spike amount (5.8 μg). For FH-S, percent recovery is graphed relative to the cocaine concentration obtained following acid incubation/SPE (99.9 ng/mg). FH-S hair mass = 42 mg. Each point represents a single iteration.*

Amine additives have previously been used in SFE to improve recoveries of tightly bound basic amine analytes from retentive matrixes. Oostdyk and coworkers [22] successfully used 1,6-hexanediamine as an additive for the extraction of amine analytes from soil, and proposed that the addition of the basic amine additive masked active matrix sites and effected release of the target analytes through an exchange interaction. Alexandrou et al. [23] suggested that amine additives lower the energy barrier of desorption of basic chemisorbed analytes through interaction of the additive with the analyte/matrix binding complex. Edder et al. [24] used TEA as an additive in the SFE of basic drugs of abuse, including morphine and cocaine, after adsorption of aqueous solutions of the drugs onto solid-phase sorbents. Drugs were eluted from the sorbents using CO_2/methanol/TEA (90:8.5:1 v/v) at 25 MPa and 40 °C and the presence of water in the extraction vessel was found to be necessary for efficient extraction. Consistent with the findings of Edder for sorbents, in the current study dramatic improvements were observed in the recovery of cocaine from a filter paper matrix (see Fig. 5) upon the addition of aqueous or methanolic solutions of TEA, though TEA was ineffective as a modifier when used alone. It is possible that TEA (pK_a = 10.75) effects release of the weaker base cocaine (pK_a = 8.60) from cellulose binding sites through a competitive displacement interaction.

To more thoroughly evaluate the influence of the matrix on the SF extractability of cocaine, SFE results were compared for the recovery of cocaine from a series of matrixes representing a range of adsorptivity and different "degrees" of analyte/matrix interaction. Figure 6 compares the SFE recoveries of cocaine from spikes on Teflon™ wool (nonadsorptive; inert; no significant time period for interaction), spikes on drug-free powdered hair (surface adsorption; no significant time period for interaction), cocaine-fortified hair (FH-P) (penetration of drug into hair matrix; one month interaction period), and drug user hair (genuine samples) using pure unmodified CO_2, CO_2 + 100 μL methanol, and CO_2 + 100 μL TEA/water (15:85 v/v).

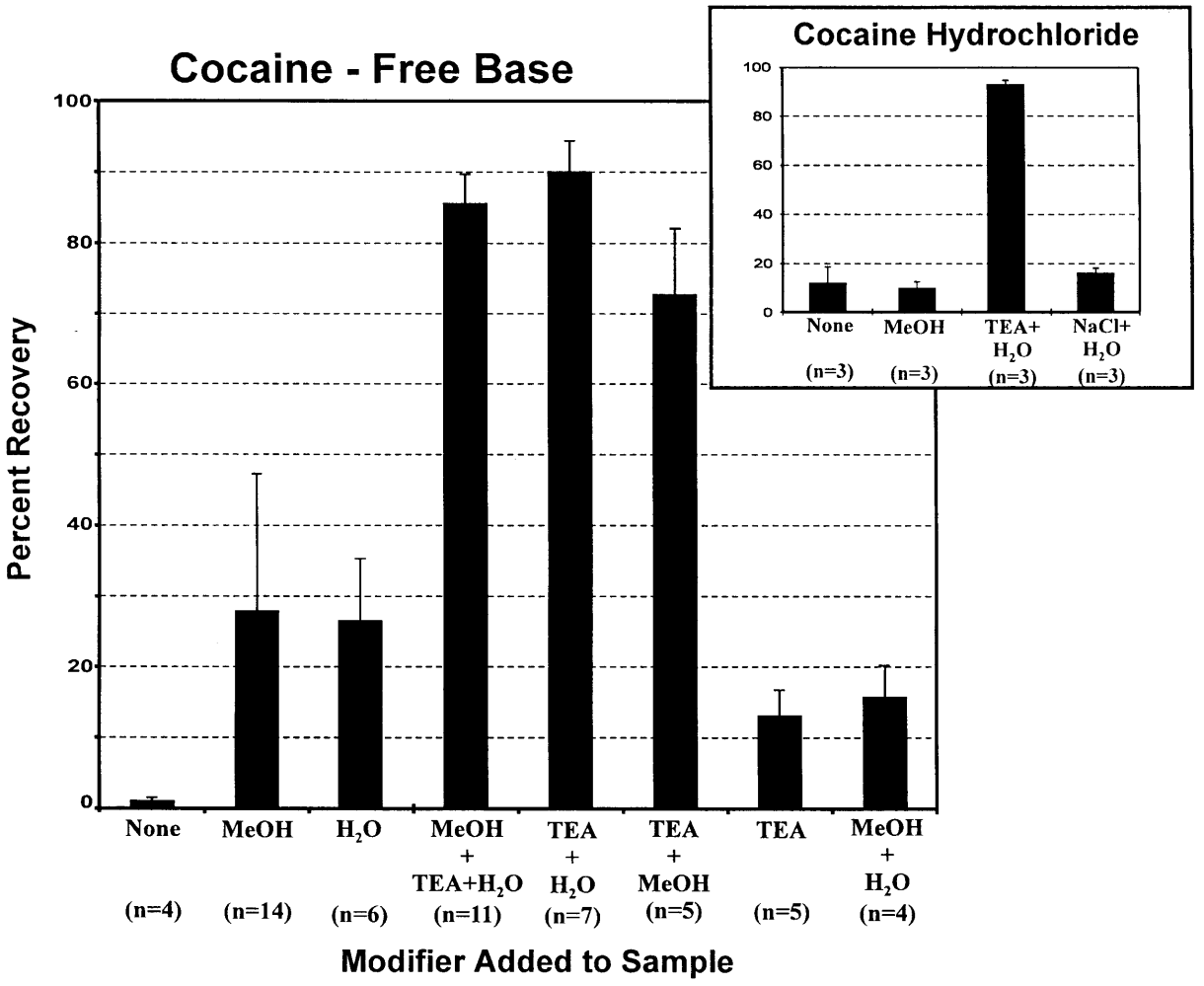

FIGURE 5. *Recoveries of free base cocaine and cocaine hydrochloride (inset) from filter paper spikes (6 μg) using a variety of modifiers. 100 μL of modifier or modifier mixture was added directly to the sample vessel by injection into the CO_2 stream upon initiation of the static step, except in the case of TEA, for which only 15 μL was injected. Error bars represent one standard deviation about the mean (iterations shown in parentheses). SFE conditions: 40.5 MPa, 110 °C, 10 min static, 15 mL CO_2 through extraction vessel during dynamic step. MeOH = methanol; TEA = triethylamine. Modifier mixture concentrations: MeOH/TEA/H_2O 76:14:10 v/v; TEA/H_2O 15:85 v/v; TEA/MeOH 15:85 v/v; MeOH/H_2O 85:15 v/v; NaCl/H_2O (inset) 1.0 M.*

Comparison of the results in Figure 6 reveals marked differences in cocaine extraction behavior between the spiked samples (Teflon™ wool and powdered hair) and the non-spiked fortified and drug user hair samples. As expected with the non-retentive Teflon™ wool substrate, no matrix effect was observed and 80 % to 90 % recoveries of cocaine were obtained regardless of the fluid composition employed. While powdered hair exhibited slightly more retention for cocaine spiked on its surface, the analyte could still be recovered with greater than 50 % extraction efficiency using pure SF-CO_2 under the given (non-optimized) SFE conditions. Addition of methanol or TEA/water to the CO_2 increased cocaine recoveries to greater than 85 %, with no significant difference in extraction efficiency observed between the two modifiers.

In contrast to the results observed for spiked substrates, large differences in the recovery of cocaine were observed from fortified and drug user hair for the different fluid compositions studied. In both cases, recoveries were poor with neat SF-CO_2, the addition of methanol offered improved efficiency, and incorporation of TEA/water resulted in dramatic increases in cocaine recovery. Three conclusions can be drawn from the results shown in Figure 6: (1) SF extractability of cocaine is not limited by analyte solubility, since cocaine has sufficient solubility in neat CO_2; (2) the conditions required for recovery of cocaine are highly dependent on the nature of the matrix and the manner of analyte incorporation in the matrix, suggesting that desorption of cocaine from hair binding sites is a rate-limiting step in the extraction process; and (3) it may be possible to exploit this matrix effect to distinguish drug present in different discrete binding "domains" in hair based on differences in extraction behavior, an idea which is investigated below.

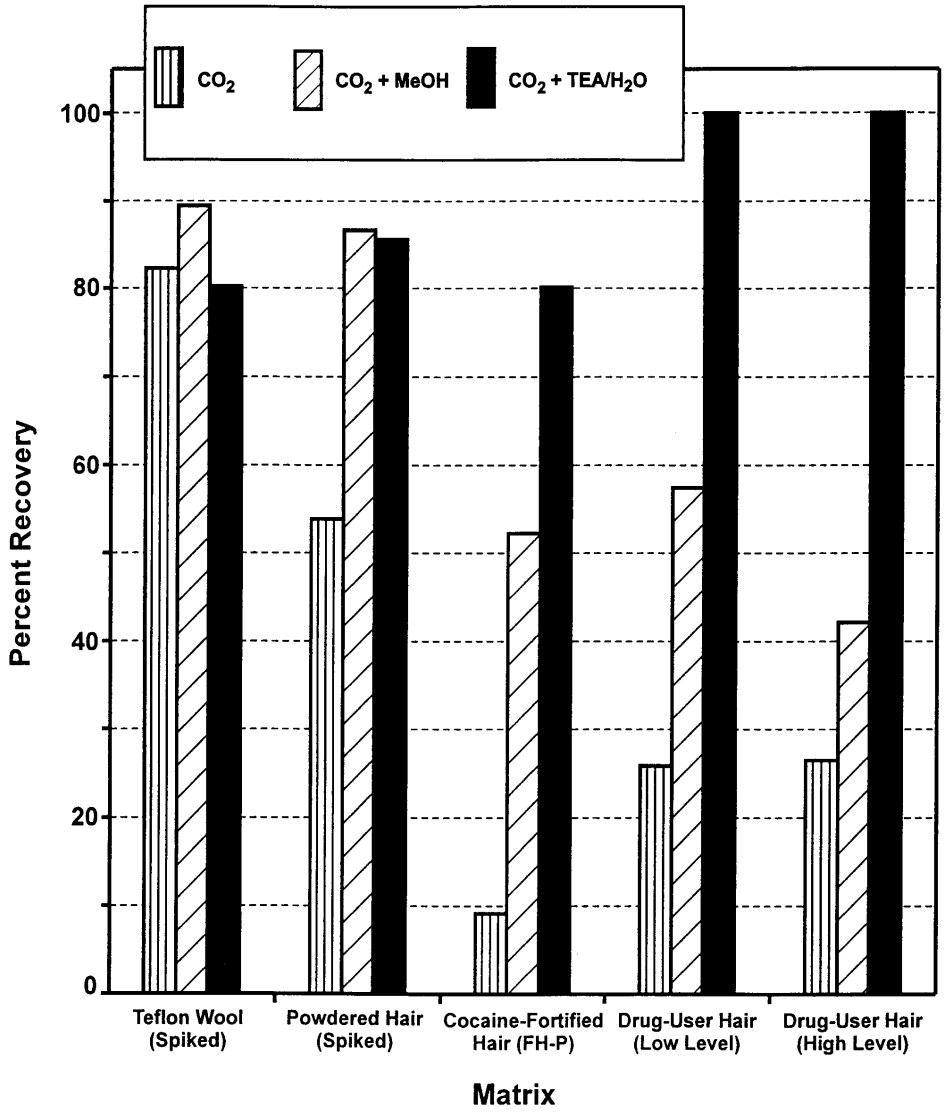

FIGURE 6. *Influence of matrix on the SF extractability of cocaine using CO_2, CO_2 + 100 μL MeOH, and CO_2 + 100 μL TEA/H_2O (15:85 v/v). SFE conditions: 40.5 MPa, 110 °C, 10 min static, 20 mL CO_2 through extraction vessel during dynamic step. Percent recoveries for the spiked matrices are graphed relative to the absolute spike amounts (6 μg). Percent recoveries for FH-P and low- and high-level drug user hair samples are graphed relative to the cocaine concentrations obtained following acid incubation/SPE (5.6, 0.81, and 19 ng/mg, respectively).*

3.2 Proposed Extraction Mechanism in the SFE of Cocaine from Hair: Support for the Kidwell-Blank Model for Drug Incorporation in Hair

It is probable that hydrophobic interactions, hydrogen bonding, and ionic interactions all play a role in the binding of drugs to the hair matrix. However, current models suggest that ionic interactions predominate for the majority of drugs detected in hair to date. One model for drug incorporation postulated by Kidwell and Blank [25] suggests that the predominant interaction mechanism involves cation exchange interactions with anionic side groups such as glutamic and aspartic acid residues in the keratin structure. Melanin, the primary pigment in hair, is also a suspected binding site for drugs in hair, and is believed to behave like a weak cation exchange polymer [26]. The Kidwell and Blank model was developed in part to explain the generally observed phenomenon that drugs with cationic properties (e.g., weak bases containing protonatable amine groups, such as cocaine) incorporate in hair to a much greater extent than anionic species (e.g., weak acids containing deprotonatable carboxylic acid groups, such as aspirin or the marijuana metabolite, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol) [1, 25, 26]. The model suggests that as pH increases above the isoelectric point of hair (about 4) [25], the carboxylic acid side chains become deprotonated and the hair becomes increasingly

negatively charged. As a result, positively charged drugs would be preferentially ion-exchanged over negatively charged drugs, which would tend to be repulsed. Changes in pH would affect both the charge state of the hair and the charge state of the drug.

The results of our SFE studies actually lend support to the Kidwell-Blank model for drug incorporation in hair, and, when considered within the framework of this model, allow us to propose a mechanism to explain the supercritical fluid extraction behavior of cocaine from hair. We propose that TEA, which is a stronger base than cocaine (pK_a = 10.75 and 8.60, respectively), exists largely as the triethylammonium cation at the hair/water/CO_2 interface (due to the local acidic environment created at this interface from the formation and dissociation of carbonic acid [27]) and that the TEA cation effectively displaces cocaine from negatively-charged hair binding sites through an ion exchange mechanism. A thorough discussion of this extraction mechanism, while beyond the scope of this report, has been recently published [28].

3.3 Quantitative SFE Recovery Data for Cocaine and BZE from Hair

Table 1 provides quantitative SFE recovery data for cocaine and BZE from samples of both drug-fortified hair (FH-P and FH-S) and genuine drug user hair (NMS-PPC and RR-8) and compares the SFE values with values obtained by a reference wet chemical extraction method. For the fortified hair samples, reference values were generated at NIST using the 24 h acid incubation/SPE method described in the Experimental section, followed by GC-MS quantification. Reference values for the NMS-PPC drug user hair were generated at National Medical Services using a previously described overnight acid incubation/LLE procedure with GC-MS quantification [29]. Reference values for the RR-8 drug user hair represent the mean results from a twelve-laboratory round robin study administered by NIST and are based upon a variety of sample extraction methods (i.e., acid incubation, buffer-enzyme dissolution, methanol extraction, or alkaline digestion followed by SPE or LLE) and quantification by GC-MS or liquid chromatography/mass spectrometry (LC-MS) [30].

Examination of the last two columns of Table 1 reveals that while SFE recoveries of cocaine were 80 % to 90 % relative to the wet chemical extraction methods, SFE recoveries of BZE were poor. The inefficiency of the reported SFE method for the isolation of BZE has been demonstrated in every hair sample that has been analyzed in our laboratory to date. To verify that BZE is not degraded under the SFE conditions employed, selected samples of drug-fortified and drug user hair which had been extracted by SFE were subjected to a subsequent acid incubation/SPE step to determine whether any residual drug could be extracted. The data shown in Figure 7A for a sample of drug-fortified hair (FH-P) illustrates that mass balance could be achieved for both cocaine and BZE (relative to the acid incubation method) when the post-SFE acid incubation was performed, confirming that BZE was not degraded during SFE, but rather was simply not extractable under the conditions employed. These findings demonstrate further the selectivity of the SFE method.

The poor SFE recoveries of BZE may be due to poor analyte solubility and/or a binding mechanism in hair which differs from that of cocaine and which renders the metabolite unextractable under the SFE conditions. Figure 7B compares BZE recoveries from Teflon™ wool and drug-fortified hair using CO_2, CO_2 + methanol, and CO_2 + TEA/water, and demonstrates that extraction efficiency is poor regardless of the matrix or fluid employed. The chemical composition of BZE differs from that of cocaine in the replacement of the methyl ester group of cocaine with a carboxylic acid group in BZE (Fig. 2B). The carboxylic acid moiety renders the BZE metabolite more polar and the amphoteric nature of the BZE molecule allows for the existence of a variety of charged states, as illustrated in Figure 2B. The combination of increased polarity and multiple charged states suggests that poor SFE recoveries of BZE may be explained by both decreased analyte solubility as well as inability to desorb BZE from matrix binding sites under the conditions employed. Various *in situ* ion-pairing and derivatization approaches to improve BZE recoveries from hair are currently under investigation.

3.4 SFE as a "Decontamination" Method: Potential for Distinguishing Endogenous from Exogenous Sources of Cocaine in Hair

The demonstration of a significant matrix effect in Figure 6 suggests the possibility of differentiating cocaine present at different binding domains in the hair matrix based on differences in SFE behavior. Recall that cocaine spiked on the surface of hair or an inert matrix could be recovered using pure CO_2, while isolation of cocaine from drug-fortified or non-contaminated drug user hair required the addition of the TEA/water modifier. These findings prompted an investigation to determine whether or not cocaine present on the surface of hair due to environmental contamination (e.g., vapors or particles) could be removed by pre-extraction with neat CO_2 prior to the TEA/water step.

TABLE 1. *Recoveries of cocaine and benzoylecgonine (BZE) from fortified and Drug User Hair by SFE and acid incubation.*

Sample	*SUPERCRITICAL FLUID EXTRACTION*[a]					*ACID INCUBATION*					*SFE EFFICIENCY RELATIVE TO ACID INCUBATION (%)*	
	Cocaine Recovered			*BZE Recovered*		*Cocaine Recovered*			*BZE Recovered*			
	n	*Mean (ng/mg)*	*RSD (%)*	*Mean (ng/mg)*	*RSD (%)*	*n*	*Mean (ng/mg)*	*RSD (%)*	*Mean (ng/mg)*	*RSD (%)*	*Cocaine*	*BZE*
Fortified Hair, Powder (FH-P)	8	4.49	11	0.56	53	3	5.60	2.4	4.84	7.3	80.1	11.6
Fortified Hair, Segments (FH-S)	8	89.9	21	NA[b]		3	99.9	6.2	NA[b]	NA[b]	90.0	NA[b]
Drug User Hair (NMS-PPC)	5	1.78	6.3	0.08	61	31	2.16	20	0.72	24	82.2	11.0
Drug User Hair (RR-8)	4	32.6	3.5	nd[c]		12[d]	36.6	36	14.6	56	89.1	nd[c]

[a] SFE conditions: CO_2 + 100 μL TEA/H_2O (15:85 v/v); 40.5 MPa, 110 °C, 10 min static, 30 mL CO_2 through extraction vessel during dynamic step. Sample mass = 30–40 mg.

[b] NA = not applicable [FH-S sample was not fortified with BZE]

[c] nd = none detected

[d] Reference results represent the mean values from a twelve-laboratory round robin study and are based on acid incubation, buffer-enzyme dissolution, methanol extraction, or alkaline digestion followed by GC-MS or LC-MS.

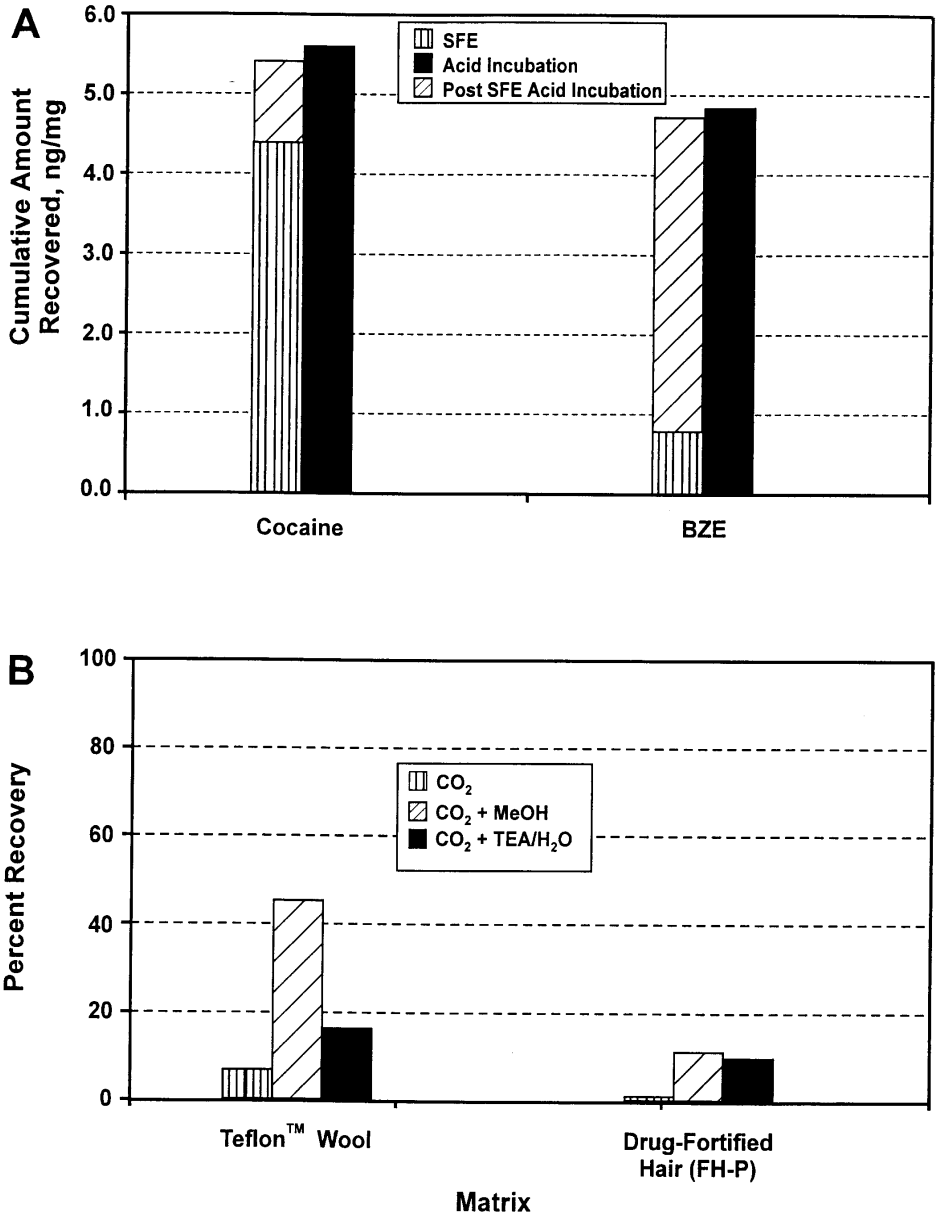

FIGURE 7. *(A) Comparison of SFE and acid incubation/SPE values for the recovery of cocaine and BZE from drug-fortified hair (FH-P) and deminstration of mass balance. (B) BZE recoveries from Teflon™ wool and drug-fortified hair (FH-P) using CO_2, CO_2 + MeOH, and CO_2 + TEA/H_2O (15:85 v/v). SFE conditions: 40.5 MPa, 110 °C, 10 min static, 30 mL CO_2 through extraction vessel during dynamic step.*

Figure 8A shows the GC-MS single ion chromatograms obtained for sequential SF extractions performed on a sample of a high level drug user's hair that was known to be additionally contaminated on its surface (based on rinse results reported by the contributing laboratory). The hair sample was extracted exhaustively with pure CO_2 (steps 1–3) followed by two sequential extractions with CO_2 modified with TEA/water (steps 4–5). It is hypothesized that the CO_2-extractable fraction represents physisorbed cocaine present on the hair surface due to environmental contamination, while the CO_2 + TEA/water fraction is believed to represent cocaine chemisorbed at active sites within the hair matrix. For comparison, a similar sequential SFE protocol was performed on a sample of *non-contaminated* hair (based on rinse results reported by the contributing laboratory) from a high level drug user (Fig. 8B). In this case, the chromatograms shown in steps 1–3 using neat CO_2 showed no recovery of cocaine, which was recovered only upon the addition of TEA/water (step 4). These results suggested the possibility of distinguishing exogenous from endogenous sources of drugs in hair based on differences in SF extraction behavior and prompted further studies (see below) to evaluate this potential fully.

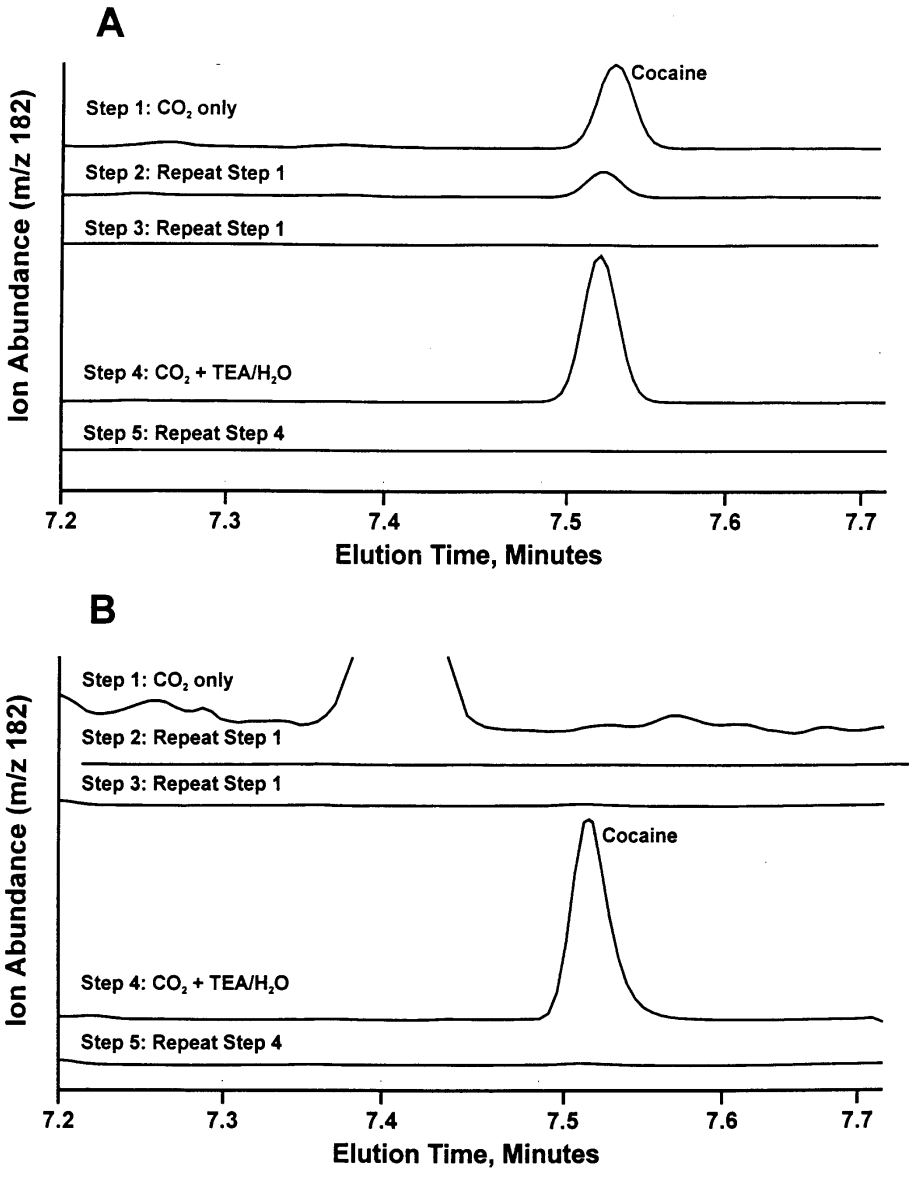

FIGURE 8. *GC-MS single ion chromatograms of sequential SF extracts obtained on (A) a high level drug user hair that was additionally contaminated on its surface (environmental contamination), and (B) a high level drug user hair that exhibited no environmental contamination. SFE conditions—Steps 1–3: 100 % CO_2, 40.5 MPa, 110 °C, 10 min static, 30 mL CO_2 through extraction vessel during dynamic step. Steps 4–5: CO_2 + 100 μL TEA/H_2O (15:85 v/v), 40.5 MPa, 110 °C, 10 min static, 30 mL CO_2 through extraction vessel during dynamic step. Cocaine concentrations in hair determined following acid incubation/SPE were 32 ng/mg (including environmental contamination) and 26 ng/mg for A and B, respectively.*

3.5 Comparison of SFE with Wet-Rinsing Methods for the Removal of "Vapor-Deposited" Cocaine from Hair

Hair that was exposed to cocaine free base vapor in an enclosed chamber for a period of 2 h was subsequently subjected to various decontamination procedures in order to compare the effectiveness of SFE using pure CO_2 with existing liquid rinsing procedures. The results of these experiments are summarized in Table 2 and Figure 9. The total level of cocaine in the hair following vapor exposure was determined by subjecting six aliquots to acid incubation/SPE with GC-MS quantification; the resulting contamination level was 5.21 ng/mg ± 0.05 ng/mg (mean ± standard deviation; n = 6). For decontamination, SFE with pure CO_2 at 110 °C and 400 atm was compared to methanol rinsing

TABLE 2. *Comparison of SFE with wet rinsing methods for the removal of cocaine from vapor-contaminated hair.*[a]

	AMOUNT OF COCAINE REMAINING IN HAIR FOLLOWING DECONTAMINATION (ng/mg)[b]			
TRIAL	*SFE*	*Ethanol Rinsing*	*Methanol Rinsing*	*Modified Baumgartner-Hill [21] Rinse Protocol*
1	0.699	2.02	1.21	1.92
2	0.934	2.36	1.17	1.90
3	0.925	2.17	1.30	2.11
4	0.826			
5	0.852			
6	0.773			
Mean Amount Remaining (ng/mg)	*0.835*	*2.18*	*1.23*	*1.98*
Standard Deviation	*0.090*	*0.17*	*0.07*	*0.12*
RSD[c] *(%)*	*10.8*	*7.8*	*5.4*	*6.0*
DECONTAMINATION[d] EFFICIENCY	84 %	58 %	76 %	62 %

[a] Target cocaine contamination level = 5.21 ng/mg.
[b] Amount remaining determined following application of acid incubation/SPE to hair that was decontaminated using the listed method. See Experimental Methods for details.
[c] Relative standard deviation.
[d] Decontamination efficiency = ((target contamination level—amount remaining)/target contamination level)) × 100.

(six sequential 30 min rinses at room temperature), ethanol rinsing (six sequential 30 min rinses at room temperature), and a modified version of the rinsing protocol previously reported by Baumgartner and Hill [21] (one 15 min rinse with 3 mL ethanol, followed by three sequential 30 min rinses with 3 mL 0.01 mol/L phosphate buffer at pH 6; all at 37 °C with shaking at 100 rpm). Under the conditions employed, SFE proved to be superior to all of the liquid rinsing methods studied in terms of decontamination efficiency (Table 2). Decontamination efficiency was evaluated by difference by first determining the percent of residual cocaine left in the hair after application of the particular decontamination procedure (compared to the initial contamination level of 5.21 ng/mg). The decontamination efficiency for SFE (40.5 MPa, 110 °C, 10 min static, 40 min dynamic) was 84 %, followed by 76 % efficiency for methanol rinsing, 62 % for Baumgartner's protocol, and 58 % for ethanol rinsing. These numbers suggest that 16 % contamination *remains* on the hair following SFE decontamination (24 % for methanol procedure; 38 % for Baumgartner's method; and 42 % for ethanol rinsing). Mass balance was demonstrated in the SFE case by analyzing *both* the cocaine content in the SF extracts and the residual cocaine content remaining in hair *following* SFE. Figure 9 illustrates that removal of vapor-deposited cocaine by SF-CO_2 was more effective at higher temperatures.

Subsequent work will be aimed at improving the effectiveness of CO_2 for removal of vapor deposited cocaine by incorporating sequential CO_2 steps (here, only a single 50 min extraction (10 min static, 40 min dynamic) was performed). Nonetheless, this preliminary investigation suggests the superior performance of SFE with CO_2 compared with wet rinsing methods for the removal of vapor-deposited cocaine from hair.

3.6 Comparison of SFE with Wet Rinsing Methods for the Removal of "Psuedo-Sweat-Deposited" Cocaine from Hair

Hair that was exposed for 6 h at 37 °C to a saline solution of cocaine hydrochloride was subjected to the aforementioned decontamination procedures in order to compare the effectiveness of SFE with existing wet rinsing methods. The results of these experiments are summarized in Table 3. These data suggest that when sweat is a vehicle for drug incorporation in hair, SFE with pure CO_2 is less effective than wet rinsing methods for decontamination; however, all of the methods tested were inadequate for removal of the "sweat-deposited" cocaine. In all cases, greater than 50 % of the target level of contamination (determined to be 2.75 ng/mg to 3.36 ng/mg based on controls run on the day of analysis) remained in the hair following the application of the decontamination procedure (i.e., the methods were less than 50 % effective for decontamination). Previous reports [1–3, 5] have also demonstrated the

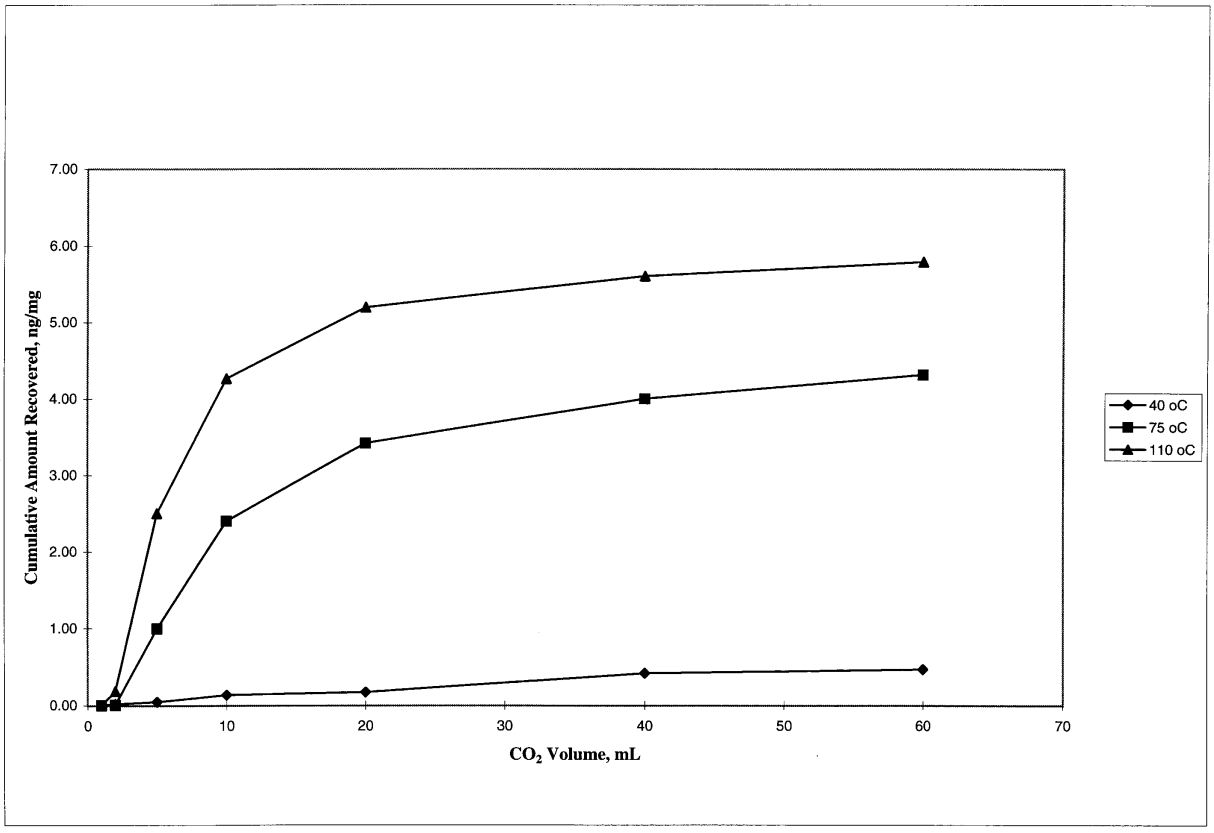

FIGURE 9. *Kinetics of removal of vapor-deposited cocaine from hair using SF-CO_2 at three different temperatures. X-axis represents the volume of CO_2 which has passed through the extraction vessel. Target cocaine contamination level in the hair was 5.21 ± 0.05 ng/mg.*

ineffectiveness of rinsing procedures for the removal of cocaine following contamination with aqueous solutions of drug (note, however, that the exposure conditions may not be realistic in these soaking studies, including the work presented here, in terms of exposure concentration, time, and potential for wicking). As our preliminary investigation evaluated only pure SF-CO_2 for its decontamination effectiveness, subsequent work will take advantage of the extraction "tuneability" of SFE by incorporating various modifiers into the CO_2 and evaluating the effectiveness of the modified fluids for *selectively* removing contamination while leaving physiologically incorporated drug intact. The goal is to establish a sequential extraction protocol (essentially a fractionation) in which contamination is removed in an initial extraction, followed by removal of the physiologically-incorporated cocaine using the CO_2/water/TEA mixture evaluated above. Future experiments will also be focused on developing a more realistic model for exposure of hair to "solutions" of drugs where sweat in the vehicle for drug deposition.

4. CONCLUSIONS AND FUTURE WORK

The current study demonstrates that the SF extractability of cocaine and BZE is highly dependent upon the chemical nature of the hair matrix and the manner in which the analytes are incorporated into or on that matrix. The results suggest that desorption of cocaine from hair binding sites is a rate-limiting step in the SFE of this analyte from hair. Addition of a TEA/water modifier mixture resulted in dramatic improvements in cocaine recovery. A displacement SFE mechanism is hypothesized, where TEA (as the triethylammonium cation) effectively competes with cocaine for negatively-charged hair binding sites. The presence of water facilitates the extraction process, possibly by swelling the hair matrix to allow better CO_2/TEA accessibility to those binding sites and/or by affecting pH equilibrium.

TABLE 3. *Comparison of SFE with wet rinsing methods for the removal of cocaine from "sweat" solution-contaminated hair.*[a]

	AMOUNT OF COCAINE REMAINING IN HAIR FOLLOWING DECONTAMINATION (ng/mg)[a]			
TRIAL	*SFE*	*Ethanol Rinsing*	*Methanol Rinsing*	*Modified Baumgartner-Hill [21] Rinse Protocol*
1	2.43	2.41	2.00	1.78
2	2.58	2.02	1.89	1.80
3	2.35	2.68	2.02	1.69
4	1.56			
5	1.70			
6	2.49			
Mean Amount Remaining (ng/mg)	*2.18*	*2.37*	*1.97*	*1.75*
Standard Deviation	*0.44*	*0.33*	*0.07*	*0.06*
RSD[b] *(%)*	*20.2*	*13.9*	*3.6*	*3.4*
Target Cocaine Contamination Level[c] (ng/mg)	2.75	3.36	3.36	3.36
DECONTAMINATION[d] EFFICIENCY	21 %	29 %	41 %	48 %

[a] Amount remaining determined following application of acid incubation/SPE to hair that was decontaminated using the listed method. See Experimental Methods for details.
[b] Relative standard deviation.
[c] Target cocaine contamination levels determined on unrinsed controls analyzed on the same day as the decontaminated samples.
[d] Decontamination efficiency = ((target contamination level—amount remaining)/target contamination level)) × 100.

The dependence of extractability on drug/hair binding interactions allows one to distinguish cocaine present at different domains in the hair based on differences in SF extraction behavior. These findings suggest the potential of SFE for distinguishing drug present in hair due to passive environmental exposure from drug present due to active drug use by taking advantage of this matrix effect. Model studies designed to mimic vapor-deposition of cocaine on hair demonstrated the superior performance of SFE compared with currently used liquid rinsing methods in terms of decontamination efficiency. Subsequent work will be aimed at optimizing this SFE decontamination efficiency by incorporating sequential CO_2 rinsing steps for the removal of vapor-deposited drug from the hair surface, and validating this approach by testing genuine drug user hair obtained from commercial testing laboratories.

In model studies designed to mimic "sweat deposition" of cocaine, SFE with pure CO_2 was less effective than liquid rinsing methods for decontamination; however, when drug exposure occurred by the saline solution soaking experiments performed here, all of the methods tested proved inadequate for the removal the cocaine incorporated in this manner. Subsequent work will be aimed at developing a more realistic experimental model to represent sweat as the vehicle for drug deposition. Future experiments will also evaluate various modifiers in CO_2 for selective removal of contamination while leaving drug due to active use intact.

Poor SFE recoveries of BZE from hair are likely due to both limited analyte solubility and failure to desorb this metabolite from hair binding sites under the conditions employed. The potential for multiple charged states and the presence of an additional ionic binding site in the BZE molecule provide a challenge for SFE. Studies are underway to evaluate various approaches to improve recoveries of this analyte, including in situ ion-pairing and derivatization approaches.

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