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Characterization of Surface Organic Components of Human Hair by On-Line Supercritical Fluid Extraction–Gas Chromatography/Mass Spectrometry: A Feasibility Study and Comparison with Human Identification Using Mitochondrial DNA Sequences*

ABSTRACT: This paper discusses results of a supercritical fluid extraction–gas chromatography/mass spectrometry (SFE-GC/MS) study of small samples (100 μg to 1 mg) of human scalp hair. The method offers a number of benefits including greater sensitivity than liquid extraction methods because the entire extractable mass is transferred to the analytical system, compared with only a few percent from a conventional liquid extraction/injection. The project's goals were to determine if SFE-GC/MS analyses of the surface-extractable components of an individual's hair yield consistent chemical profiles and to investigate if the profiles are sufficiently different to distinguish them from those of other individuals. In addition, the mtDNA sequences from ten of the same individuals used in the SFE-GC/MS study from four family units were determined, and, while the families were distinguishable, the maternal relations yielded identical sequences. In tandem, SFE-GC/MS and mtDNA techniques may provide valuable complementary data from forensic hair samples.

KEYWORDS: forensic science, hair, supercritical fluid extraction, gas chromatography/mass spectrometry, surface organic analysis, human identification, mitochondrial DNA

Hair is routinely collected at crime scenes for subsequent forensic analysis, which typically involves inspection by microscope techniques to determine color, thickness, shape, and any other distinguishing characteristics that might help associate the sample collected at the scene with an individual. If the hair sample has a root (live cells), nuclear DNA techniques may be employed using the polymerase chain reaction (PCR) to generate short tandem repeat (STR) profiles for comparison of crime scene samples with those obtained from victims and suspected perpetrators. Recent advances in sequencing mitochondrial DNA (mtDNA) enable genetic characterizations of small hair samples (2 cm in length) with or without root cells (1–3). Since mtDNA is inherited through the maternal lineage, the mtDNA from maternal relations generally have identical sequences. This has the advantage of allowing samples from maternal relatives to confirm the identity of missing suspects or unknown crime victims, abducted children, and unknown soldiers whose bodies are recovered after many years. MtDNA is currently being used to identify the World Trade Center victims (4,5). Presently, no other chemical analysis is routinely performed on forensic hair samples. Nuclear and mitochondrial DNA characterization along with a chemical analysis of hair could provide valuable complementary information to microscopic forensic analysis of hair.

The main limitation in applying any chemical analytical technique to forensic hair samples is that these samples are often quite small, perhaps a length of hair or a few short segments. On-line supercritical fluid extraction–gas chromatography/mass spectrometry (SFE-GC/MS) has been proposed as a method for characterizing small samples (μg to mg of sample material) (6–12). The method offers a number of benefits including greater sensitivity than liquid extraction methods because the entire extractable mass is transferred to the analytical system, compared with only a few percent from a conventional liquid extraction/injection. Another benefit of the on-line technique is higher recoveries of volatile species, components that would be lost during a multi-step liquid extraction and concentration. In some sample-limited cases, SFE-GC/MS may be the only way of obtaining qualitative/semiquantitative chemical information from a sample. This paper discusses the results of an SFE-GC/MS study of small samples (100 μg to 1

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mg) of human scalp hair to determine if SFE-GC/MS analyses of the surface-extractable components of an individual's hair yield consistent chemical profiles, yet are sufficiently different from those of other individuals to distinguish them. MtDNA sequences of some of the same individuals (ten samples from four family units) used for the SFE-GC/MS study were also determined to compare the advantages and disadvantages of the two techniques for human identification. We conclude that the results of both analyses add valuable and complementary data to each other and to the results obtainable by microscopic forensic hair analyses.

Methods and Materials

Samples

Hair samples were collected from volunteers by a procedure approved by the National Institute of Standards and Technology (NIST) Institutional Review Board. Volunteers were asked to follow instructions included in a sampling kit, which detailed how the hair samples were to be collected so as to minimize contamination. Briefly, volunteers were asked to wear nylon gloves prior to handling hair, cutting hair with stainless-steel scissors (solvent-cleaned) and then transferring the hair samples to clean 4-mL amber vials (all items included in sampling kit). A total of 20 volunteers participated in the study, including eight adults (five female, three male, 30 to 40 years old) and twelve children (eight female, four male, four months to eight years old; see Table 1). Volunteers were not asked to specify the area on the head from which the hair was sampled, so this information is not available. Three of the samples represented mixtures of multiple samplings from the same individuals taken over a period of three years (composite Samples 5, 15, and 16). A blind study was administered by a third party who transferred hair from three vials from the set of 20 samples to pre-cleaned vials labeled "Blind A," "Blind B," and "Blind C."

TABLE 1—*Sample roster for on-line SFE-GC/MS of human hair study.*

Sample Identification No.	Gender and Age
1	male, 4.5 m
2	male, 2.25 y
3	female, 2.5 y
4	male, 2.5 y
5	female, composite 3–6 y
6	female, 5 y
7	female, 5.5 y
8	male, 6.5 y
9	female, 8 y
10	female, 8 y
11	female, 8 y
12	female, 8 y
13	female, 32 y
14	female, 35 y
15	female, composite 36–39 y
16	male, composite 36–39 y
17	female, 39 y
18	male, 39 y
19	male, 39 y
20	female, 42

NOTE: m = month; y = year.

SFE-GC/MS

The SFE-GC/MS apparatus used in this study is shown in Fig. 1, including a Model 5890 GC interfaced with a Model 5970 MS (both Hewlett-Packard, Wilmington, DE). The MS was tuned daily to ensure that the sensitivity and mass scale calibration were within desired operating specifications. Hair samples, typically three to ten segments of 1.5-cm lengths (portions of a single hair), were weighed and transferred to an extraction vessel (liquid chromatographic pre-column, 40-μL internal volume, Upchurch Scientific, Oak Harbor, WA), pressurized to 40.5 MPa (400 atm) with SFE/SFC grade CO_2 (Air Products, Allentown, PA) using a syringe pump (Isco, Lincoln, NE) for a 10-min static extraction at 100°C after which a 30-cm by 0.14-mm OD (25-μm ID) uncoated/deactivated linear restrictor was inserted in the GC column (60 m by 0.25 mm, 25 μm, DB-5ms, J&W Scientific) through a manual on-column injector. A shut-off valve was then opened, beginning a 10-min dynamic SFE whereby the extracted components of the hair's surface were transferred and deposited in the GC column, maintained at −30°C using N_2 (liquid). After the dynamic SFE, the restrictor was removed from the injector, and the MS was allowed to

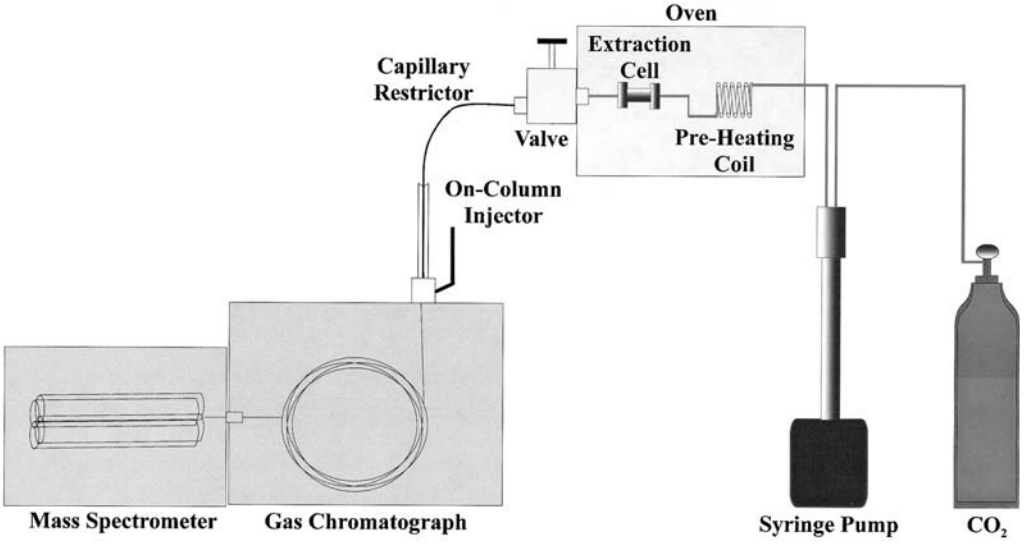

FIG. 1—*On-line supercritical fluid extraction-gas chromatography/mass spectrometry (SFE-GC/MS) apparatus.*

pump down for 2 min to remove residual CO_2 from the source. The column was then heated at 60°C/min to 50° C, then heated more gradually at 4°C/min to 300°C during which time the MS was scanned from 50 to 550 m/z (0.8 scans/s). Peak identifications were based on results from processing a standard solution containing a number of fatty alcohols, free fatty acids, squalene, and cholesterol through the SFE-GC/MS procedure, as well as from performing automated searches of mass spectra of individual chromatographic peaks using the NIST/EPA/NIH Mass Spectral Library (version 1.6, December 1997) linked with the GC/MS data station software (Enhanced Chem Station version A.0300, Hewlett-Packard). Considering 1000 as the highest match score possible with the search software described above, documentation from this same software states that a score of ≥800 suggests an association with a relatively high confidence level. Results from processing the standard solution (described above) through the SFE-GC/MS analysis and mass spectral search program yielded match scores ranging from 700 to >900. It should be noted that the match scores can be affected significantly by such factors as the peak's signal-to-noise ratio, co-eluting components (including background contributions), instrumental factors (high mass sensitivity and mass calibration), as well as the quality of the spectra in the library. Considering these points and experience from many mass spectral searches, peaks in sample hair extractions were tentatively identified when the peak's match score was ≥650 and after careful comparison of the unknown and matched spectra (see Table 2 for peak identifications).

Given the high sensitivity of this on-line method and to ensure that the analytical system was not contaminated prior to processing a hair sample, a blank run was performed between hair sample analyses. The blank run resulted from processing the empty SFE extraction vessel and associated plumbing through the same SFE-GC/MS method as the individual hair samples. If there were significant peaks detected in the resulting SFE-GC/MS chromatogram of the blank, the empty SFE extraction vessel was processed additional times so as to minimize any blank contribution to the subsequent hair analysis.

TABLE 2—*Identification of components from on-line SFE-GC/MS of human scalp hair.*

Peak No	Identification
1.	tetradecanoic acid
2.	pentadecanoic acid
3.	pentadecanol
4.	hexadecenoic acid
5.	hexadecanol
6.	hexadecanoic acid
7.	hexadecanoic acid ester
8.	2-hydroxy-3,3,5-trimethylcyclohexyl-benzoic acid ester (homosalate)
9.	heptadecanol
10.	octadecenoic acid
11.	2-ethylhexylmethoxycinnamate/salicylate
12.	octadecanoic acid
13.	2-ethylhexymethoxycinnamate/salicylate
14.	hexadecanoic acid ester
15.	diisooctyl- or bis(2-ethylhexyl) phthalate
16.	octadecanoic acid ester
17.	squalene
18.	N,N-dimethyl-1-dodecanamine
19.	octadecane
20.	dodecanoic acid ester
21.	cholesterol
22.	hexadecanoic acid ester
23.	tetradecanoic acid, C_{16} ester
24.	hexadecenoic acid, C_{18} or C_{20} ester
25.	hexadecanoic acid, C_{16} ester
26.	octadecanoic acid, C_{16} ester
27.	hexadecenoic acid, C_{18} ester
28.	hexadecanoic acid, C_{18} ester

Mitochondrial DNA Sequence Analysis

Mitochondrial DNA came from two to four hairs with roots from each of ten individuals in four families (Table 1). Family One consisted of: the father, age 39 years, Sample 18; the mother, age 42 years, Sample 20; and a male child, age 6.5 years, Sample 8. Family Two consisted of: the mother, age 39 years, Sample 17; and a female child, age 5.5 years, Sample 7. Family 3 consisted of: the mother, age 32 years, Sample 13; and a male child, age 2.25 years, Sample 2. Family 4 consisted of: the mother, age 35 years, Sample 14; a male child, age 2.5 years, Sample 4; and a male child, age 4.5 months, Sample 1.

DNA Extraction

Two to four hairs with roots from each individual were combined and placed into 200-μL hair lysis buffer (10 mmol/L Tris, pH 8.0; 100 mmol/L NaCl; 50 mmol/L EDTA, pH 8.0; 0.5% SDS) containing 40 mmol/L dithiothreitol (DTT) and 50 μg Proteinase K. Following overnight incubation at 56°C, the genomic DNA was purified by phenol/chloroform/isoamyl alcohol (25:24:1) extraction followed by recovery using Centricon 100® concentrators (Amicon, Inc., Beverly, MA).

PCR Amplification

Hypervariable Region 1 (HV1) was amplified using two overlapping primer sets: F15989 (5′CCCAAAGCTAAGATTCTAAT3′)/R16258 (5′TGGCTTTGGAGTTGCAGTTG3′ and F16144 (TGACCACCTGTAGTACATAA3')/R16410 5′GAGGATGGTGGTCAAGGGAC3′), whereas hypervariable Region 2 (HV2) was amplified using a single primer set (F15/R484) (13). Five microliters of DNA template were added to each PCR reaction, along with 1X *Pfu* reaction buffer (Stratagene, La Jolla, CA) [20 mmol/L Tris-HCl, pH 8.8; 2 mmol/L $MgSO_4$; 10 mmol/L KCl; 10 mmol/L $(NH_4)_2SO_4$; 0.1% Triton X-100; 0.1 mg/mL nuclease-free bovine serum albumin (BSA)], 200 μmol/L each dNTP, 0.2 μmol/L of the forward and reverse primers, 8 μg of BSA, 5 units of *Pfu* polymerase, and sufficient sterile distilled water to bring the final volume to 50 μL.

Following an initial denaturation of 95°C for 30 s, 35 PCR cycles were performed, consisting of 94°C for 20 s (denaturation), 56°C for 10 s (primer annealing), and 72°C for 30 s (extension). This was followed by a final extension period of 72°C for 7 min. The purity and integrity of the PCR product was determined using a 2% (w/v) agarose gel containing ethidium bromide.

DNA Sequencing

DNA sequencing was performed using the ABI PRISM™ BigDye™ Terminator CycleSequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) according to the protocol. The reaction product was purified and analyzed using the ABI 310 Capillary Electrophoresis Analyzer. Sequence Navigator software was used to examine, edit, and compare the sequence data to the published human reference sequence (14). Sequence differences between the hair samples and the Cambridge Reference Sequence were noted (14).

Results and Discussion

The inherent sensitivity of on-line SFE-GC/MS made it directly applicable to small samples of scalp hair—a sample type important in forensic analysis. The project's general goals were to investigate what chemical species were detectable in small samples of hair, the reproducibility of the chemical profiles from one individual, and whether these chemical profiles could be used to distinguish individuals from one another. Such data could be used in guiding the investigations of law enforcement officials, for example, possibly helping investigators decrease the number of suspects for a specific crime. Although the set of 20 samples used in this study is a modest number, it was judged to be sufficient to evaluate the feasibility of the scientific approaches.

The sources of surface residues of human scalp hair include (see Fig. 2) naturally deposited components (from sebaceous and sweat glands), artificially deposited species (from shampoos, conditioners, and other hair treatments), and environmentally deposited contaminants (e.g., occupational exposure). The natural lipid materials that coat the hair are well known (15,16) and include fatty alcohols, fatty acids, squalene, cholesterol, and large fatty acid esters. As opposed to integral lipids incorporated into the hair from both sebaceous gland and bloodstream involvement (17,18), the high density of sebaceous glands in the scalp (16) strongly suggests that surface lipids of the hair are mainly from secretions from those glands. An early study by Nicolaides (16) found that individual differences in enzyme concentrations, pH, body temperature, and other parameters could significantly affect the relative concentrations of fatty acids and related compounds and therefore yield a unique chemical signature for each person. Although one might expect sebum and natural surface components of the hair to be identical in composition, one could also envision chemical changes to these surface lipids by microbial action, air oxidation, and even hydrolysis by the solvent used for extraction (19). A recent paper (20) described the chemical characterizations of components emanating from the skin, with the ultimate purpose of identifying natural mosquito attractants. The majority of the compounds identified were saturated and unsaturated fatty acids and fatty acid esters. The workers did not report the measurement of fatty alcohols, squalene, or cholesterol, prominent components detected in hair samples in this work (see below). This is perhaps due to the heavier influence of perspiration than sebum to the skin emanations reported by Bernier and co-workers (20), who sampled from the palms of individuals, a body part with few (if any) sebaceous glands. Buchanan et al. (21) described the characterization of skin lipids extracted with isopropanol from the fingers of 50 volunteers. They determined that samples collected from children had higher levels of volatile free fatty acids than samples from adults. Adult samples had higher levels of less volatile fatty acid esters than those from children, possibly explaining the experiences of forensic investigators who have found that fingerprints from children do not remain on a surface as long as do those from adults. Finally, Auwärter et al. (22) found correlations between the concentrations of fatty acid ethyl esters (FAEE) in hair and the drinking habits of the donors, with the hair from heavy drinkers yielding significantly higher levels of FAEE than non-drinkers.

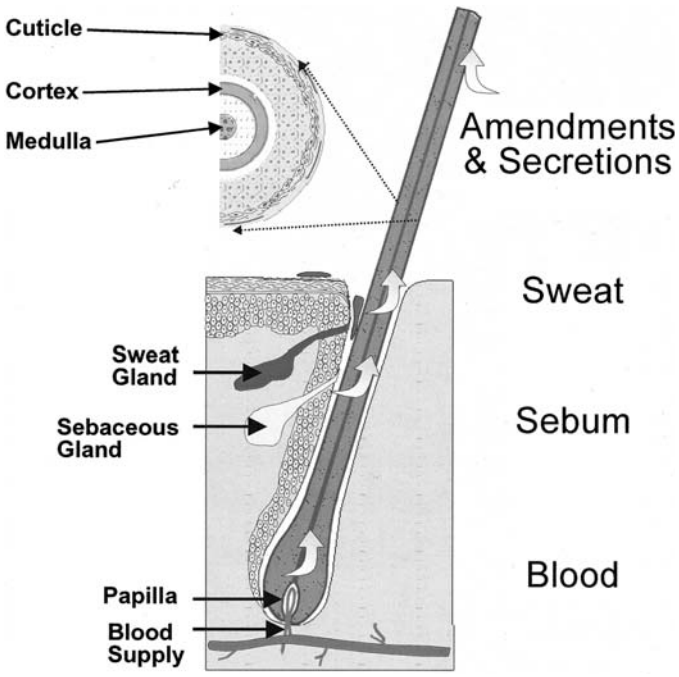

FIG. 2—*Hair cross section.*

Preliminary results from a small number of hair samples ($n = 5$) processed in an earlier study in our laboratory in 1993 suggested that squalene was a consistent surface component of hair from both adults and children and that cholesterol was a prominent component in surface extracts of children's hair. From these preliminary conclusions, it was thought that the ratio of squalene-to-cholesterol might distinguish children's from adults' hair. For this latest study involving hair samples from 20 volunteers (see Table 1 for sample roster), squalene was not detected in four of the twelve children's hair samples, but was detected in all of the adult samples. Eight of the twelve children's samples yielded relatively large cholesterol response, and four of twelve children's samples yielded detectable but small cholesterol peaks. On the other hand, only one of eight of the adult hair samples yielded a relatively large cholesterol peak, and the remaining seven adult samples yielded a detectable but small cholesterol peak. This difference in the cholesterol content may be a way of distinguishing children's from adults' hair.

To check for completeness of extraction of surface components, samples from two individuals (Samples 3 and 18) were processed by SFE-GC/MS, and the same samples were extracted and analyzed a second time. Figure 3 shows the first extraction removed the majority of the extractable components. Results of the second extraction for Sample 3 yielded small but detectable responses for five species. Our general conclusion was that the SFE conditions used for this study were sufficient to extract the bulk of the surface residue of a small hair sample (100 μg to 1 mg) in a single extraction.

Most of the 20 individual hair samples (17 of 20) were sub-sampled and processed at least twice, and ten of the individual samples were analyzed three times or more by SFE-GC/MS to determine the reproducibility of the resulting chemical profiles. In general, multiple trials of the same individual's hair yielded similar chemical profiles based on the identifications of specific peaks and their relative magnitudes. Some differences were observed in the relative abundances of the individual peaks as shown with the results of four runs of Sample 6 (see Fig. 4). Peak 19 in Sample 6, tentatively identified as octadecane, was the prominent peak observed in the 260 and 840 μg hair extractions, but absent from the 800 and 900 μg extractions. The results for the 800 through 900 μg extractions did yield remarkably similar chemical profiles, except for the aforementioned predominance of Peak 19 in the 840 μg extraction.

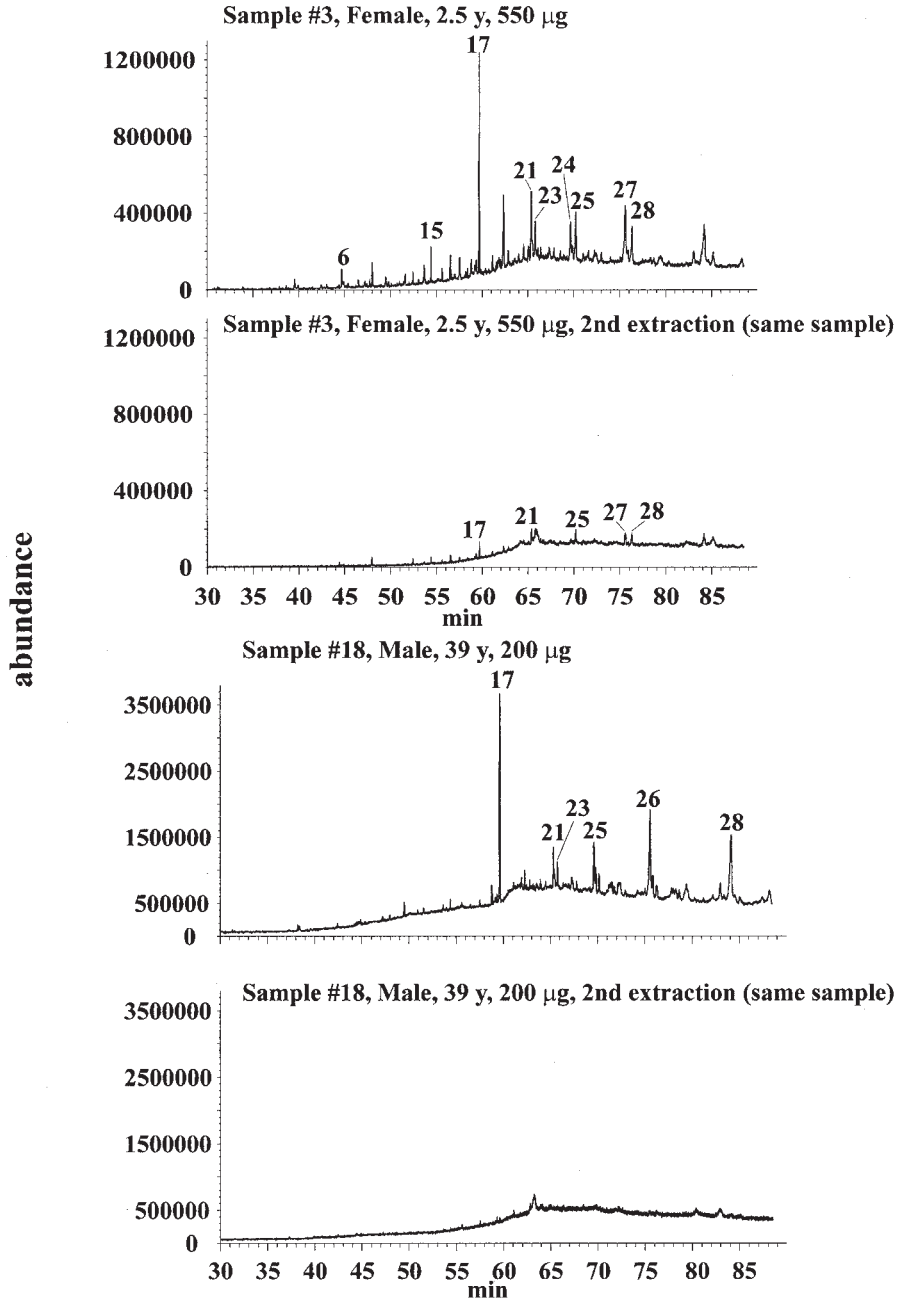

FIG. 3—*SFE-GC/MS of human hair: completeness of extraction (see Table 2 for peak identifications).*

One would expect that some of the materials coating the surface of hair would not be homogeneously distributed over the hair, possibly explaining the obvious differences in the chemical profiles of the 260 μg extraction with respect to the three extractions at ≥800 μg. This is because the sources of the materials (natural, artificial, and environmental), by their nature, would deposit components in isolated locations and might not be smoothed uniformly throughout the hair on the individual. The small samples of hair processed by this technique might also increase the magnitude of sample heterogeneity. Larger samples probably would reduce heterogeneity in the analytical results; however, samples collected at crime scenes are often of sizes similar to those used in this study.

Commonly observed components in the on-line hair extracts were squalene, cholesterol, fatty alcohols, fatty acids, and fatty acid esters, all of which are present in secretions of the sebaceous gland (sebum) (16). Other components extracted from hair include phthalates (Peak 15), possibly from residues of shampoo stored in plastic bottles, three peaks identified as sunscreen components (Peaks 8, 11, and 13), whose source could be either a sunscreen and/or hair treatment product, and an amine antioxidant (tentatively identified as N,N-dimethyl-1-dodecanamine), also likely in the formulation of a hair care product. It is quite possible that a number of the components present in sebum are also ingredients in hair care products used by both adults and children. As a simplified summary, Table 3 shows specific components detected as major (>25% of the largest peak) or minor (<25% of the largest peak) components in the 20 samples of this study. Except from an absence of Peaks 1 and 2 (tetradecanoic acid and pentadecanoic acid, respectively) in any of

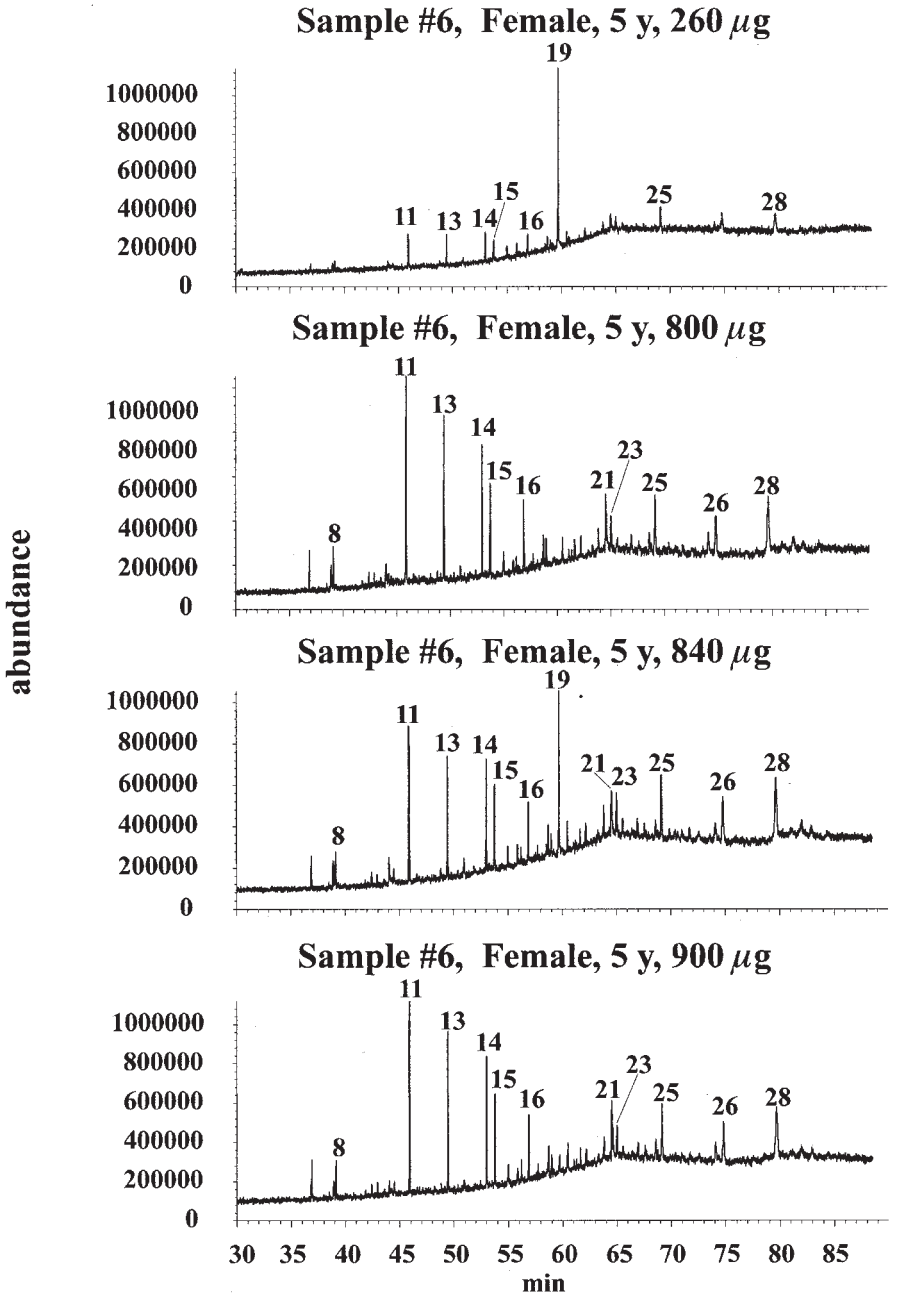

FIG. 4—*Replicate SFE-GC/MS measurements of hair samples from a volunteer (see Table 2 for peak identifications).*

the samples from children, with both peaks present in six of the eight adults, no obvious pattern is observed that groups individuals by either age or gender. Considering the seven volunteers whose hair samples yield the simplest chromatograms (≤7 peaks), five were children (4.5 months to 8 years), and two were adults (male, 39 years, and female, 32 years). The six hair samples yielding the most complicated chromatograms (≥12 peaks) included four from adult females, one from an adult male, and one from a juvenile female. The eight hair samples containing one, two, or all three of the sunscreen components (Peaks 8, 11, and 13) included five from juvenile females, two from adult females, and one from a juvenile male. Four of these juvenile female samples were collected during the summer when many parents apply sunscreen as a barrier against sunburn. It is quite possible that the sunscreen residue could be transferred to the hair by touch or even by deliberate application to the scalp. It is also possible that the presence of these sunscreen components in adult hair could be from an application of a hair treatment, as many cosmetic products include sunscreens in their formulations. Whether the source of the sunsceen components is a hair care product or a topical skin formulation, the identification of these species in a small hair sample may help link a crime-scene sample with that removed from a victim or suspect. It should be noted that, from knowledge of the instrument detection limit and results of standard runs, the components observed in an SFE-GC/MS analysis of a small hair sample are typically present at concentrations of approximately 1 mg/g (one part per thousand). This suggests that the total extractable mass from hair as determined by this on-line method may be 2 to 5% (mass fraction).

TABLE 3—*Summary of qualitative results of SFE-GC/MS of hair samples (see Table 2 for peak identifications).*

		Peak No.																									
Sample	sex, age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	19	20	21	22	23	24	25	26	27	28
1	M,4.5m				x		+				+		+			+	+			+							
2	M, 2 y					x	+			+							+			+		+		x			x
3	F, 2.5 y						x									x	+			+		x	+	+		+	+
4	M,2.5 y						+						+			+	x			+							
5	F,3-6 y			+								+		+					x	x		+	+		+		x
6	F, 5 y								x			+		+	+	+	+	+		+		x		+	x		+
7	F, 5.5 y								x			+		+			x			+			x	x			
8	M, 6.5 y				+							+				x	x			+							
9	F, 8 y			x			+			+		+		+			+			+				+			+
10	F, 8 y			+			+			x		+		x			x			+		x		+			
11	F, 8 y					+	+						+							+		x			+		x
12	F, 8 y			x						x						+	+			+		+	x	+		+	+
13	F, 32 y					+	+			+							x			x		x		x			
14	F, 35 y	+	+	+	+		+	+			+	+	+	+		x	+		+	+	x	+	+	+		+	x
15	F,36-39 y	+	+	x			+						+				+			+	+		x	+		+	+
16	M,36-39 y	+	+		x		+				x		+				+			+	+	+	x	x		+	x
17	F, 39 y	+	+		x	+					x		+				+			x		x	+	+		+	+
18	M, 39 y																+			x		x		x	+		+
19	M, 39 y	x			x		+			x							+			+		x	x	+		+	x
20	F, 42 y	x	x		x		+	x				+		x			+			x		x	x	x		x	x
	"+" indicates peak detection at > 25% of largest peak.																										
	"X" indicates peak detection at < 25% of largest peak.																										
	Empty cell indicates peak was not detected.																										

One might envision these natural lipid materials sorbing to the hair shaft from direct contact with the scalp, with shampooing effectively removing some of the naturally applied species from the hair, while adding additional lipids and other compounds to the hair as conditioners. Andrasko and Stocklassa (23) reported a liquid chromatographic (LC) method characterizing the methanol/water extracts of hair. Differences in the profiles from individuals were believed due to the different shampoos used by the subjects, although no mention was made of natural components from sebum coating the hair. The main interest in environmental deposits to hair has been in discriminating drug users from those who encounter the smoke of those using drugs (e.g., crack cocaine, marijuana). Staub (24) reviewed the use of SFE as applied to hair analysis and concluded that polar modifiers should be added to the carbon dioxide (CO_2) to extract endogenous drugs from the hair, whereas SFE with CO_2 alone would extract exogenous drugs added to the hair through environmental contamination. Morrison and co-workers (25) discussed the use of SFE for distinguishing endogenous (incorporated) versus exogenous (external contamination) drugs associated with hair. Regardless of the sources of specific components detected in a hair sample, the extracted chemical profile of a person's hair sample might exhibit a type of "chemical fingerprint" that could be used for distinguishing individuals (e.g., victims from suspects).

In contrast to the chemical characterization technique described above, state-of-the-art mitochondrial DNA (mtDNA) sequence analysis techniques have been discussed in some detail in the scientific and popular literature and enable human identification especially when the nuclear DNA is degraded or available in limited amounts. The human identifications can be verified by the examination of maternal relatives, since, unless a somatic mutation has occurred since birth, the mtDNA sequences are inherited through the maternal lineage and are indistinguishable (1–3,14). MtDNA sequence analysis was performed on hair taken from ten of the 20 individuals representing four family units. The results of this study, summarized in Table 4, were consistent with the major tenet of the technique: that mtDNA sequences from different families are distinguishable, but mtDNA sequences from maternal relations are indistinguishable; thus, they are extremely useful for verifying these relations. The SFE-GC/MS chemical profiles from this work for maternal relations were typically quite different and could add important complementary information to that from mtDNA sequencing data. For example, Samples 1, 4, and 14 (see Fig. 5) include samples from a mother (No. 14) and her two young sons (Samples 1 and 4). The chemical profiles of the sons are similar yet distinguishable from each other and easily distinguishable from their mother, whereas these three samples have indistinguishable mtDNA sequences (see Table 4). Considering that this on-line SFE-GC/MS method does not appear to disrupt the hair structure or extract internal components from the sample, subsequent mtDNA analysis of the same hair sample may be feasible, thus allowing both a surface organic analysis plus an internal genetic characterization of the same hair sample.

As a challenge for the technique, a blind study was performed in which a third party selected hair samples from three of the 20 volunteers. The results of the blind study were mixed. The data analysis was kept as simple as possible, consisting of visual comparisons of the chemical profiles of the three blind samples with previous SFE-GC/MS runs of the hair from the 20 volunteers. Blind sample A was *incorrectly* identified as Sample 7 (female, 5.5 years); instead, the correct match was Sample 4 (male, 2.5 years). The chemical profiles of Samples 7 and 4 were similar; however, the sunscreen component 2-ethylhexylmethoxycinnamate was not detected in the one run performed on Sample 4, although it was present as one of the prominent peaks in blind Sample A. Blind Sample B was *correctly* matched with Sample 13, mainly by the promi-

TABLE 4—*Mitochondrial DNA sequence analysis of hair from ten individuals in four families.*

			Comparison with Cambridge Reference Sequence (CRS)*										
			CRS		Sample Number and Family Relationships								
Region	Region Amplified	Length of Target Region	#	Bp	18†	20	8	17	7	13	2	4	14	1
HV2	F15 - R484	430	73	A	G	...‡	...	G	G	...	...	...	...	...
			152	T	...	C	C	...	...	...	...	...	...	...
			239	T	...	...	...	...	...	C	C	...	...	...
			263	A	G	G	G	G	G	G	G	G	G	G
			309.1	...	C	...	...	...	...	C	C	...	...	...
			309.2	...	...	...	...	...	...	C	C	...	...	...
			315.1	...	C	C	C	C	C	C	C	C	C	C
			456	C	...	...	...	T	T	...	...	...	...	...
HV1	F15989-R16258	230	16189	T	C	...	...	...	...	...	...	...	...	...
	F16144-R16410	227	16256	C	T	...	...	...	...	...	...	T	T	T
			16269	A	G	...	...	...	...	...	...	...	...	...
			16270	C	T	...	...	...	...	...	...	...	...	...
			16291	C	T	...	...	...	...	...	...	...	...	...
			16304	T	...	...	...	C	C	...	...	...	...	...
			16362	T	...	...	...	...	...	C	C	...	...	...

Family relationships:
Family One: Father—Sample 18, Mother—Sample 20, Child—Sample 8
Family Two: Mother—Sample 17, Child—Sample 7
Family Three: Mother—Sample 13, Child—Sample 2
Family Four: Child—Sample 4, Mother—Sample 14, Child—Sample 1
*Cambridge reference sequence: see Ref 14.
†Sample number.
‡Same as CRS.

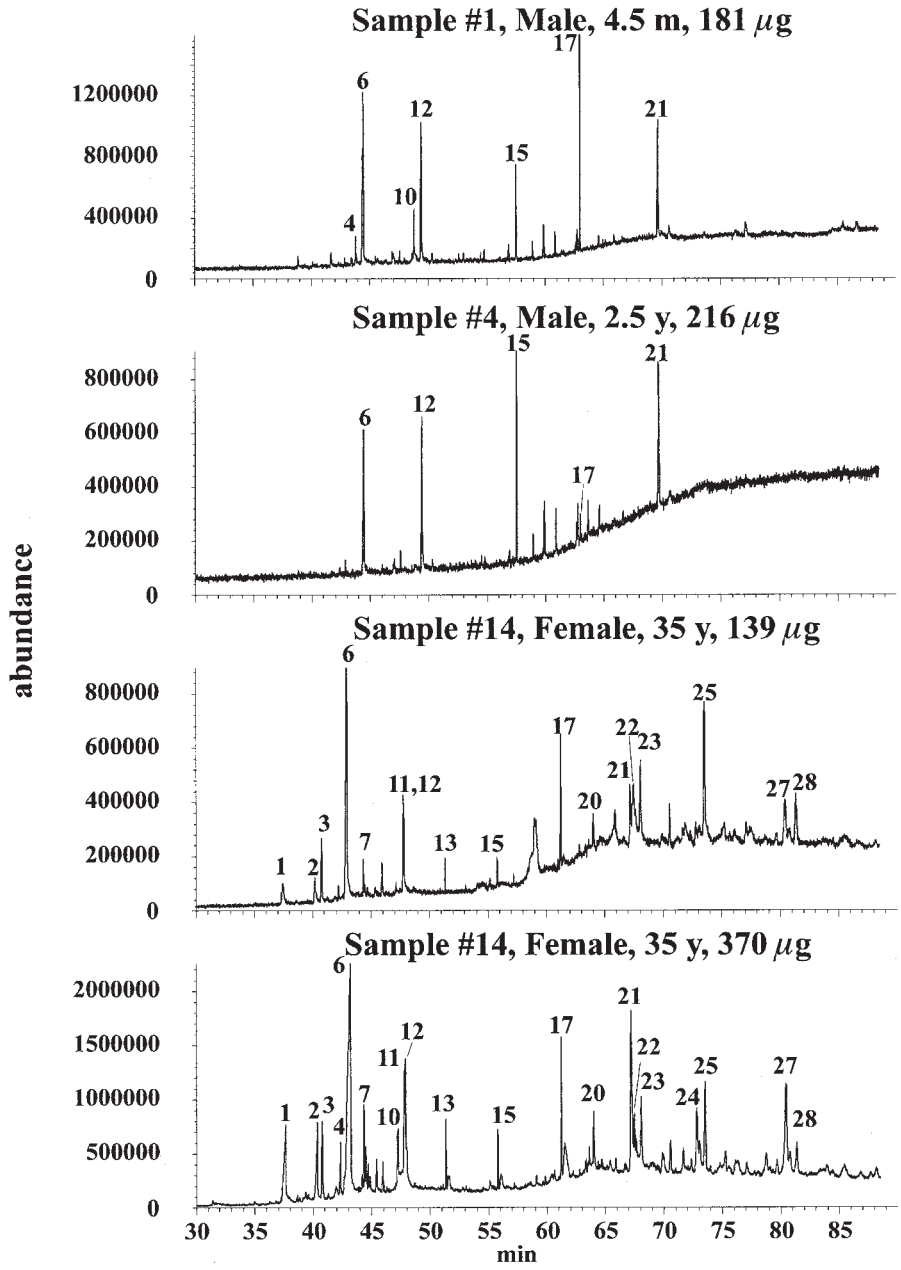

FIG. 5—*SFE-GC/MS of hair from maternal relations (see Table 2 for peak identifications).*

nence of peaks identified as hexadecanol and heptadecanol. Blind sample C was *correctly* identified as Sample 11, since it included a rather unique suite of eight peaks, including one that could not be identified after searching the NIST/EPA/NIH Mass Spectral Library. In general, correctly matching two of three unknown samples from a pool of 20 samples is encouraging but also raises some concerns of the consistency of the chemical profile from an individual's hair, concerns that could be either confirmed or minimized by additional sampling and analyses.

Nine of the 20 hair samples that were processed by SFE-GC/MS in 1997 and 1998 were again analyzed in 2000 to determine if the initial chemical profiles would be preserved by storage of the samples in sealed vials at room temperature. Five of the nine samples, from four adults and one child, yielded similar chemical profiles from fresh and archived samples. Four of the nine samples, from four children, provided significantly different profiles between fresh and archived samples, with the archived sample analyses noticeably depleted in the more volatile species observed in the fresh sample runs. These data are consistent with experiences of forensic investigators (21), as described earlier, who have observed that children's fingerprints do not remain on a surface as long as those from adults. Refrigerating archived hair samples may preserve the volatile components typically observed in children's hair samples, but children's samples collected after an extended period in the field could be expected to have significantly different chemical profiles than fresh samples.

Summary

Success in linking "unknown" hair samples with those from known individuals depends on the consistency of the chemical profiles and mtDNA sequence analysis from a specific individual's

hair, the distinguishing characteristics in chemical profiles between individuals, and the model or method used in establishing a data association. This preliminary study, while considering a modest number of head hair samples ($n = 20$), suggests that analysis of the surface components of small hair samples (100 μg to 1 mg) by SFE-GC/MS may help associate hair samples taken at a crime scene with those of specific individuals. The conclusions of this feasibility study generally encourage further investigation of a number of factors that could influence the chemical profile of the surface components from an individual's hair, as well as the reproducibility of that chemical profile. These factors include, but are not limited to, the location from where the sample was taken, any treatments applied to the hair by the individual, and the age of the hair sample (fresh or archived). Subsequent studies to the one detailed in this report will investigate differences in the chemical profiles of hair from adults, adolescents, and children, as well as individuals of different genders and heritages.

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