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Law Enforcement and Corrections Standards and Testing Program

Trace Evidence Analysis of Human Hair by On-Line Supercritical Fluid Extraction – Gas Chromatography/Mass Spectrometry: A Feasibility Study

NIJ Report 600-99

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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, 100 Bureau Drive, Stop 8102, Gaithersburg, MD 20899-8102.

David G. Boyd, Director
Office of Science and Technology
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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	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	parts 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
dia	diameter	lbf·in	pound-force inch	rh	relative humidity
emf	electromotive force	lm	lumen	s	second
eq	equation	ln	logarithm (base e)	SD	standard deviation
F	farad	log	logarithm (base 10)	sec.	section
fc	footcandle	M	molar	SWR	standing wave ratio
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	miles 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)

0.30480 m =1ft	4.448222 N = lbf
2.54 cm = 1 in	1.355818 J =1 ft·lbf
0.4535924 kg = 1 lb	0.1129848 N m = lbf·in
0.06479891g = 1gr	14.59390 N/m =1 lbf/ft
0.9463529 L = 1 qt	6894.757 Pa = 1 lbf/in^2
3600000 J = 1 kW·hr	1.609344 km/h = mph

Temperature: $T°_C = (T°_F - 32)\times 5/9$

Temperature: $T°_F = (T°_C \times 9/5)+32$

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1. INTRODUCTION

Hair and fibers are routinely collected at crime scenes for subsequent forensic analysis. Hair is typically inspected by microscope techniques to determine color, thickness, and shape. If the hair sample has a root, genetic screening techniques may be employed to add these data to the evidence pool. Recent advances in determining mitochondrial DNA enable genetic characterizations of small hair samples without root cells. Maternal relations have indistinguishable mitochondrial DNA, so this type of genetic information has limitations. Mitochondrial DNA characterization along with a chemical analysis of hair could provide valuable complementary information to current forensic analysis of hair.

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) [1-3][1]. The method offers a number of benefits including increased sensitivity compared with 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 multistep 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.

[1] Numbers in brackets refer to suggested readings in section 4.

The SFE of hair discussed in the literature over the last few years has dealt mainly with off-line methods for extracting drugs-of-abuse from surface and internal regions of hair [4]. Only a few groups have attempted to extract and measure other surface components present on bulk hair (mg to g quantities), most focusing on residues from shampoo treatment [5,6]. Application of on-line SFE-GC/MS to small hair samples has yet to be discussed in the scientific literature, but might provide useful chemical information to complement morphological and genetic data.

This report discusses the results of an SFE-GC/MS study of small samples (100 μg to 1 mg) of human hair. The three goals of this study were:

1. To investigate the feasibility of distinguishing children's from adults' hair by the relative abundances of squalene and cholesterol;
2. To determine if SFE-GC/MS analyses of an individual's hair yields consistent chemical profiles, yet are sufficiently different from those of other individuals to distinguish them; and
3. To perform a study whereby "blind" samples from a pool of 20 previously characterized human hair samples are processed by SFE-GC/MS in order to match these unknown samples with the correct source hair samples.

2. EXPERIMENTAL METHODS[2]

2.1 Samples

Hair samples were collected from volunteers by a procedure approved by the NIST Human Ethics Research Board. Volunteers were asked to follow instructions included in the 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 and cutting hair with solvent-cleaned stainless-steel scissors and then to transfer the hair samples to clean 4 mL amber vials (all items included in sampling kit). Volunteers were also asked to note their age, gender, and time since last hair-washing on the sample label. A total of 20 volunteers participated in the study, including 8 adults (5 female, 3 male, 30 to 40 years old) and 12 children (8 female, 4 male, 4 months to 8 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. For the blind study, a third party (Dr. Michael J. Welch, NIST), transferred hair from sample vials from three volunteers to pre-cleaned vials labeled "Blind A," "Blind B," and "Blind C."

2.2 SFE-GC/MS

The SFE-GC/MS apparatus is shown in figure 1. A Hewlett-Packard 5890 GC and 5970 MS were used for this study, and the MS was tuned daily to ensure that the sensitivity and mass scale calibration were within desired operating specifications. Hair samples were weighed and transferred to an extraction vessel (40 μL internal volume), pressurized to 400 atm with SFE/SFC grade CO_2 using a syringe pump for a 10-min static extraction at 100 °C after which a 30 cm x 0.14 mm OD (25 μm ID) uncoated/deactivated linear restrictor was inserted in the GC column (60 m x 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, which was maintained at - 30 °C using N_2 (liq). After the dynamic SFE, the restrictor was removed from the injector and the column was 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 m/z 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 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 1,000 as the highest match score possible with the search

[2] Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the National Institute of Standards and Technology (NIST), nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

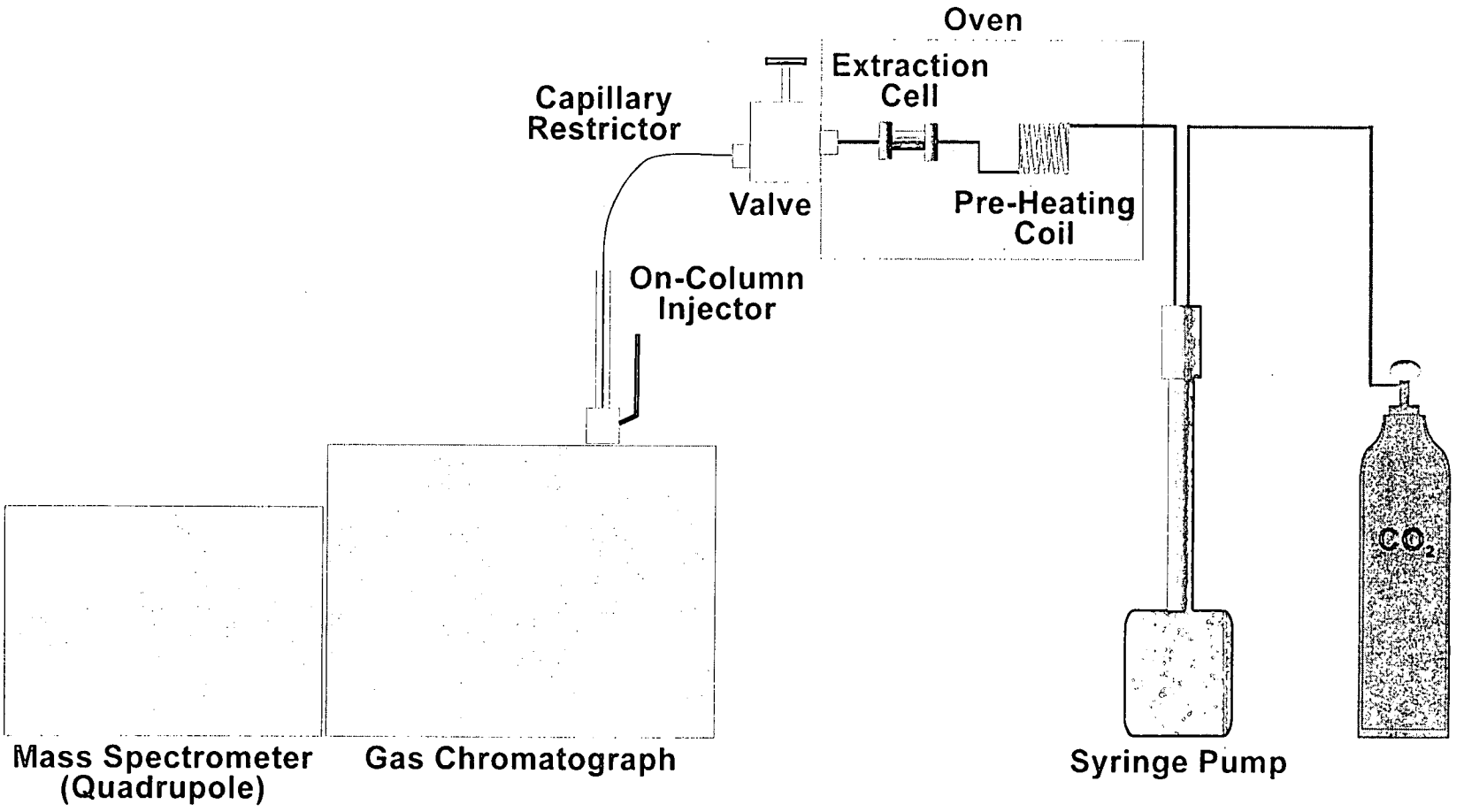

Figure 1. On-line SFE-GC/MS apparatus

software described above, documentation from this same software states that a score of ≥ 800 suggests a match 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 796 to 953. It should be noted that the match score 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).

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

Sample Identification No.	Gender and Age
1	Male, 39 yr
2	Female, 42 yr
3	Male, 6.5 yr
4	Female, 39 yr
5	Female, 5.5 yr
6	Female, 32 yr
7	Male, 28 mo
8	Male, 2.5 yr
9	Female, 35 yr
10	Male, 4.5 mo
11	Female, 5 yr
12	Female, 8 yr
13	Female, 8 yr
14	Female, 8 yr
15	Female, composite 3 to 6 yr
16	Female, 2.5 yr
17	Female, 8 yr
18	Male, 39 yr
19	Male, composite 36 to 39 yr
20	Female, composite 36 to 39 yr

Table 2. Identifications of components from on-line SFE-GC/MS of human hair (peak numbers indicated in figures in this report)

Peak No.	Identification[a]
1	tetradecanoic acid
2	pentadecanoic acid
3	pentadecanol
4	hexadecenoic acid
4a	hexadecanol
5	hexadecanoic acid
6	hexadecanoic acid ester
6a	2-hydroxy-3,3,5-trimethylcyclohexyl-benzoic acid ester (homosalate)[b]
6b	heptadecanol
7	octadecenoic acid
8	2-ethylhexylmethoxycinnamate[b]
9	octadecanoic acid
10	2-ethylhexymethoxycinnamate[b]
11	diisooctyl- or bis(2-ethylhexyl) phthalate
12	squalene
12a	octadecane
13	dodecanoic acid ester
14	cholesterol
15	hexadecanoic acid
16	tetradecanoic acid, C_{16} ester
17	hexadecenoic acid, C_{18} or C_{20} ester
18	hexadecanoic acid, C_{16} ester
18a	hexadecenoic acid, C_{18} ester
19	hexadecenoic acid, C_{18} ester
20	hexadecanoic acid, C_{18} ester

[a]Identifications based on results from standard runs and searches of the NIST/EPA/NIH Mass Spectral Library (version 1.6, December 1997).

[b]Compounds present in sunscreen formulations.

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 (typically one to two additional blank runs) so as to minimize any blank contribution to the subsequent hair analysis.

3. RESULTS AND DISCUSSION [3]

The work described in this report was designed to take advantage of the inherent sensitivity of on-line SFE-GC/MS as applied to small samples of 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, how reproducible were the chemical profiles from one individual, and if 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, possibly helping them narrow down the pool of suspects for a specific crime. Although the set of 20 samples used in this study is not a large number, we judged it to be sufficient to establish feasibility of the scientific approach.

The sources of residues for human hair include (see fig. 2) naturally-deposited components (from sebaceous and sweat glands), artificially-deposited species (from conditioners and treatments), and environmental contaminants (e.g., occupational exposure). 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 unique "chemical fingerprint" that could be used for distinguishing individuals (e.g., victims from suspects).

To check for completeness of extraction of surface components, samples from two individuals (samples #1 and #16) were processed by SFE-GC/MS, and the same samples were extracted and analyzed a second time. Figures 3 and 4 show that the first extraction removed the majority of the extractable components. Results of the second extraction for sample #16 (fig. 4) yielded small but detectable responses for five species. Our general conclusion was that the SFE conditions used for this study (see sec. 2.2) were sufficient to extract the bulk of the surface residue of a small hair sample (100 μg to 1 mg) in one extraction, providing a characteristic chemical profile of the residues.

Preliminary results from a small number of hair samples (n = 5) processed 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 and Appendix A for SFE-GC/MS chromatograms), squalene was not detected in 4 of the 12 children's hair samples, but was detected in all of the adult samples. Eight of the 12 children's samples yielded relatively large cholesterol response, and 4 of 12 children's samples yielded detectable but small cholesterol peaks. On the other hand, only 1 of 8 of the adult hair samples yielded a relatively large cholesterol peak, and the remaining 7 adult samples yielded a detectable but small cholesterol peak. Since squalene was not always detected in the children's hair samples in the pool of 20 volunteers used in this study, the squalene-to-cholesterol ratio would not be a viable way of distinguishing children's hair from adults' hair.

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[3] For all GC/MS chromatograms presented in this report, abundance (y-axis title) represents the output of the ion counting device of the mass spectrometric detector (proportional to mass).

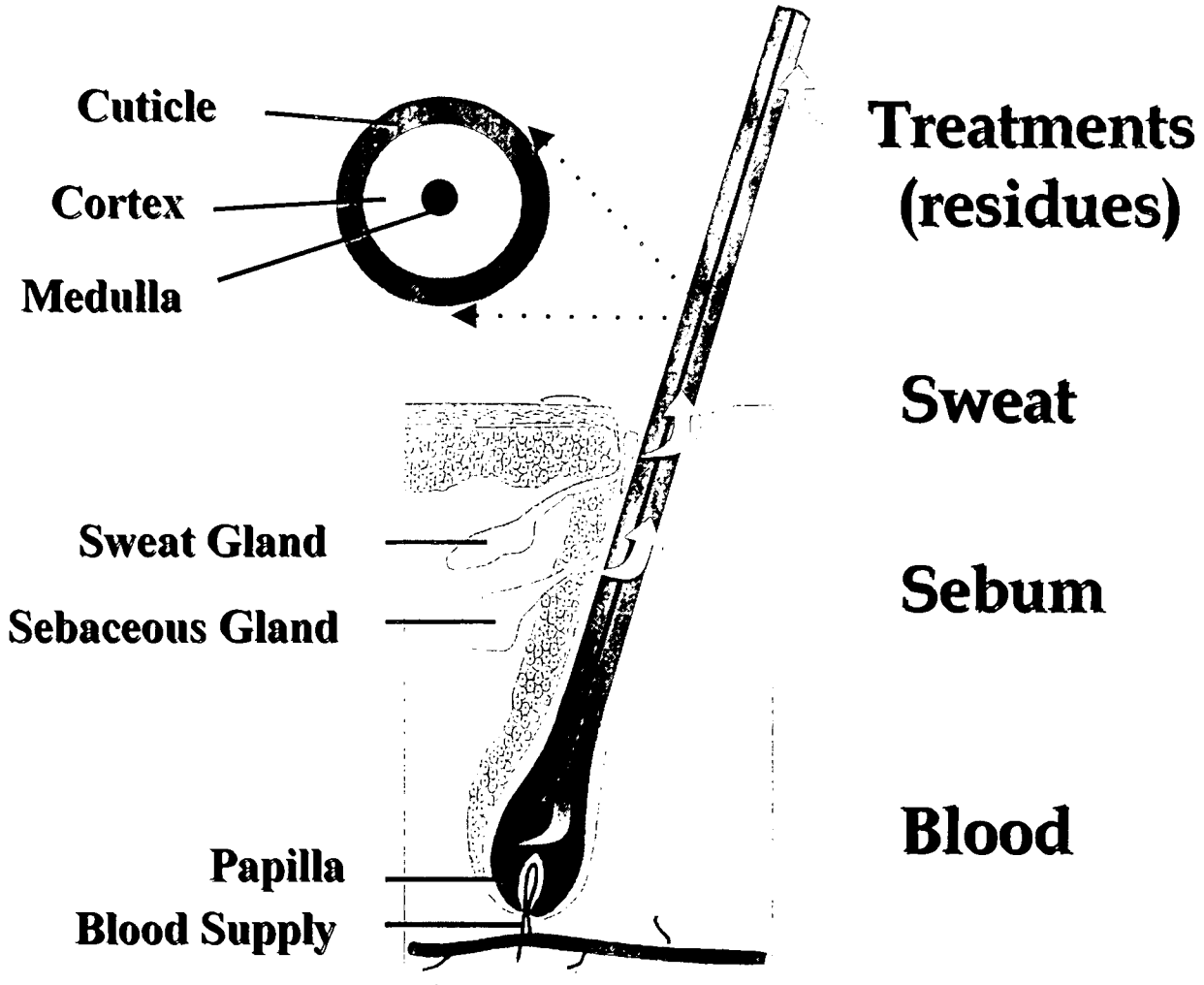

Figure 2. Hair cross section

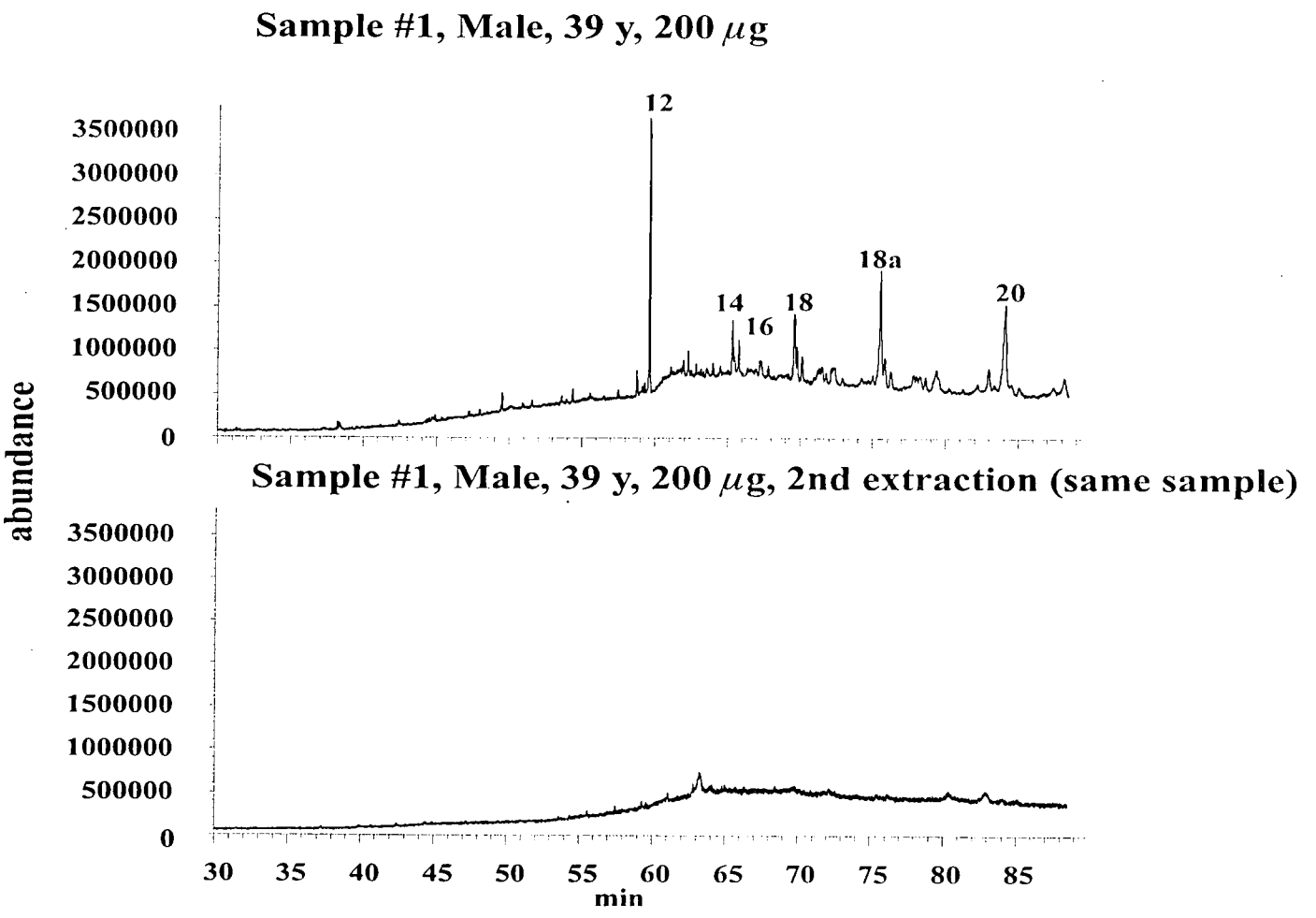

Figure 3. SFE-GC/MS of human hair: completeness of extraction

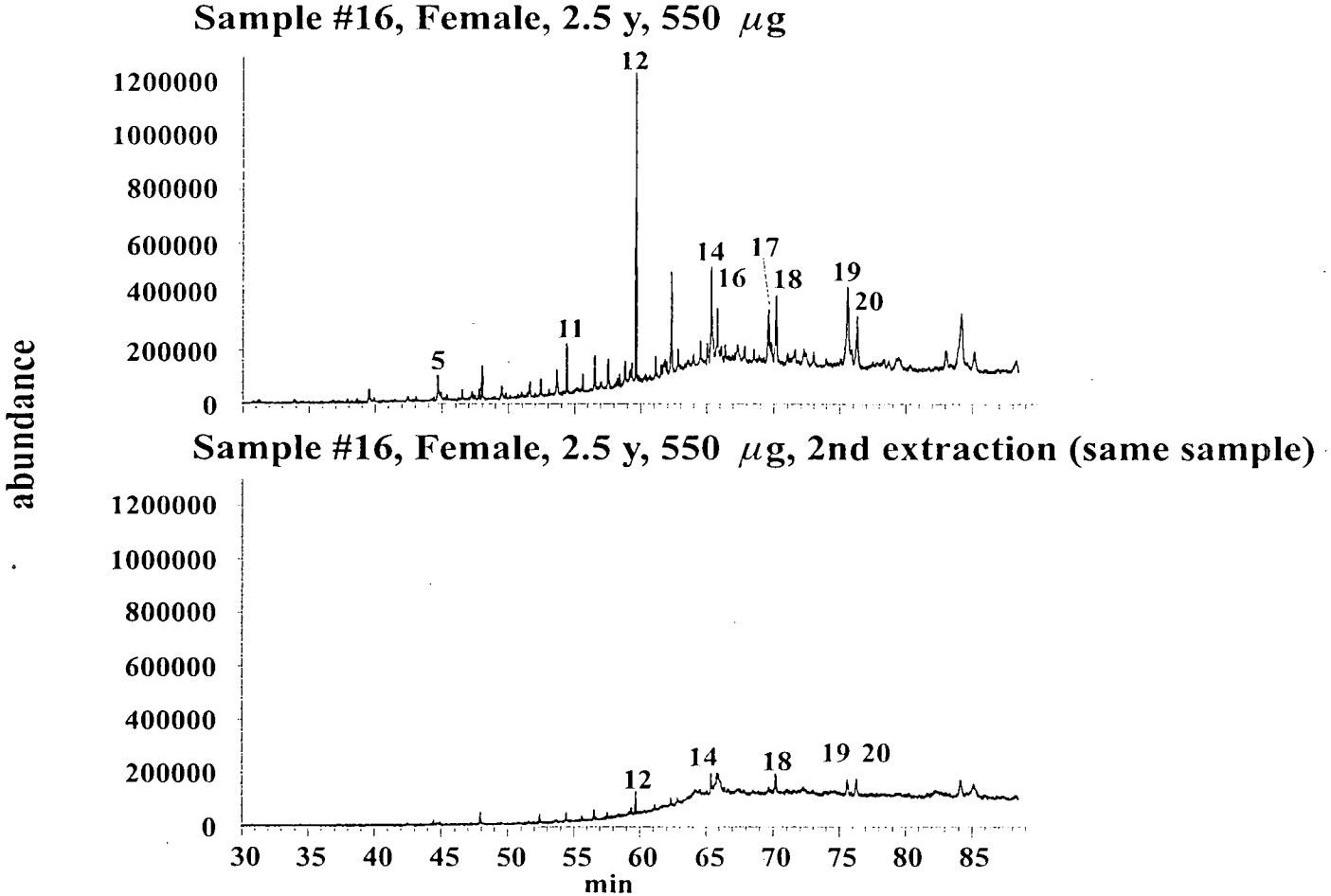

Figure 4. SFE-GC/MS of human hair: completeness of extraction

Cholesterol was a prominent peak in the majority of the hair samples from children, and may be used as a way of distinguishing children's from adults' hair.

Most of the 20 hair samples (17 of 20) were processed at least twice, and 10 of the 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. Some differences were observed in the relative abundances of the individual peaks as shown with the results of four runs of Sample #11 (see chromatogram in Appendix I). Peak 12a in sample #11, tentatively identified as octadecane, was the prominent peak observed in the first two runs, but absent from the last two trials. The results for trials 2 through 4 did yield similar chemical profiles, except for the predominance of peak 12a in trial 2. One would expect that some of the materials coating the surface of hair would not be homogeneously distributed over the hair, 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.

Commonly observed components in the on-line hair extracts were squalene, cholesterol, fatty alcohols, fatty acids, and fatty acid methyl esters (FAMEs), all of which are present in secretions of the sebaceous gland (sebum). Other components extracted from hair include phthalates (peak #11), possibly from residues of shampoo stored in plastic bottles, and three peaks identified as sunscreen components (peak #'s 6a, 8, and 10), whose source could be either a sunscreen and/or hair treatment product. It is likely that a number of the components present in sebum are also ingredients in hair care products used by both adults and children. As a simple summary, table 3 shows specific components detected in the 20 samples of this study. Considering the seven volunteers whose hair samples yield the simplest chromatograms ($\leq$ 7 peaks), five were children (4.5 mo to 8 yr), and two were adults (male, 39 yr and female, 32 yr). The six hair samples yielding the most complicated chromatograms ($\geq$ 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 (peak #'s 6a, 8, and 10) 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. Regardless of the sources of the sunscreen components, their presence in hair may serve to help link a crime-scene sample with that removed from a victim or suspect.

In contrast to the chemical characterization technique described above, state-of-the-art mitochondrial DNA (mtDNA) characterization techniques have been discussed in some detail in the scientific and popular literature and provide confirmation of maternal relationships, since these mtDNA sequences are indistinguishable. 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 8 through 10 include a mother

Table 3. Summary of qualitative results of SFE-GC/MS of human hair samples (see table 2 for identifications)

Sample	gender, age	Peak No. 1	2	3	4	4a	5	6	6a	6b	7	8	9	10	10a	11	12	12a	13	14	15	16	17	18	18a	19	20
1	M, 39 y																+			+		+		+	+		+
2	F, 42 y	+	+		+		+	+				+	+	+			+			+		+	+	+		+	+
3	M, 6.5 y				+							+					+			+							
4	F, 39 y	+	+		+	+					+		+				+			+		+	+	+		+	+
5	F, 5.5 y								+			+		+			+			+			+	+			
6	F, 32 y					+	+			+							+			+		+		+			
7	M,28m					+	+			+							+			+		+		+			+
8	M,2.5 y						+						+			+				+							
9	F, 35 y	+	+	+	+		+	+			+	+	+	+		+	+		+	+	+	+	+	+		+	+
10	M,4.5m				+		+				+		+			+	+			+							
11	F, 5 y								+			+		+	+	+	+	+		+		+		+	+		+
12	F, 8 y			+			+			+		+		+			+			+				+			+
13	F, 8 y			+			+			+		+		+			+			+		+		+			
14	F, 8 y					+	+						+							+					+		+
15	F,3-6 y			+								+		+					+	+		+	+		+		+
16	F, 2.5 y						+									+	+			+		+	+	+		+	+
17	F, 8 y			+						+						+	+			+		+		+		+	+
18	M, 39 y	+			+		+			+							+			+		+	+	+		+	+
19	M,36-39 y	+	+		+		+				+		+				+			+	+	+	+	+		+	+
20	F,36-39 y	+	+	+			+						+				+			+	+		+	+		+	+

"+" indicates detection of the peak from the sample number noted.

(sample #9) and her two young sons (samples #8 and #10). The chemical profiles of the sons are similar yet distinguishable from each other, and easily distinguishable from their mother, whereas these three samples are likely to have indistinguishable mtDNA sequences.

It should be noted that from knowledge of the instrument detection limit and results of standard runs, that the components observed in an SFE-GC/MS analysis of a small hair sample are typically present at concentrations of approximately 1 ng/μg (1 part-per-thousand). This suggests that the total extractable mass from hair may be 2 percent to 5 percent (mass fraction). For measuring individual trace components on the hair's surface using the same instrument, the GC/MS system would need to be operated in the selected-ion mode, instead of the scan mode used for this study. Associated work for this study compared the results of SFE-GC/MS of the same subject's hair processed using both a GC/quadrupole MS (previous to this, solely used for on-line hair analysis) and a relatively new GC/ion trap MS. The experimental conditions used for both analyses were identical with the same type of GC column used in both instruments (60 m x 0.25 mm DB-5ms, 0.25 μm phase thickness). Comparison of the results of on-line SFE-GC/MS performed on the two instruments is shown in figure 5, and a similar pattern in the detected peaks and their relative amplitudes is observed. Although the sample size used for the SFE-GC/MS on the ion trap instrument was 37 percent less than that of the quadrupole MS, the signal-to-noise ratios observed for the ion trap run were significantly greater than those of the quadrupole MS. Comparisons of the mass spectra of the corresponding peaks in the two chromatograms show greater relative abundances of higher mass ions in spectra from the ion trap MS. This enhancement has also been observed during calibration of the ion trap detector and results from the design and operation of this MS. Such biases in mass spectra from this instrument could affect the analyte identification by spectra-search algorithms. In addition, we have observed a trend of lower relative response for later eluting species on the ion trap MS, attributed to losses in the transfer line (observed in fig. 5). Once these potential problems are addressed, the benefits of absolute and high mass sensitivities of the ion trap MS described above suggest that it could be suitable for on-line SFE-GC/MS of samples of human hair as small as 10 μg (hair segment ≈ 1 mm to 2 mm long).

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 (see fig. 6) was **incorrectly** identified as sample #5 (female, 5.5 yr); instead the correct match was sample #8 (male, 2.5 yr). The chemical profiles of sample #5 and #8 were similar; however, the sunscreen component 2-ethylhexylmethoxycinnamate was not detected in the one run performed on sample #8, although it was present as one of the prominent peaks in blind sample A. Blind sample B (see fig. 7) was **correctly** matched with sample #6, mainly by the prominence of peaks identified as hexadecanol and heptadecanol. Blind sample C (see fig. 8) was **correctly** identified as sample #14, 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

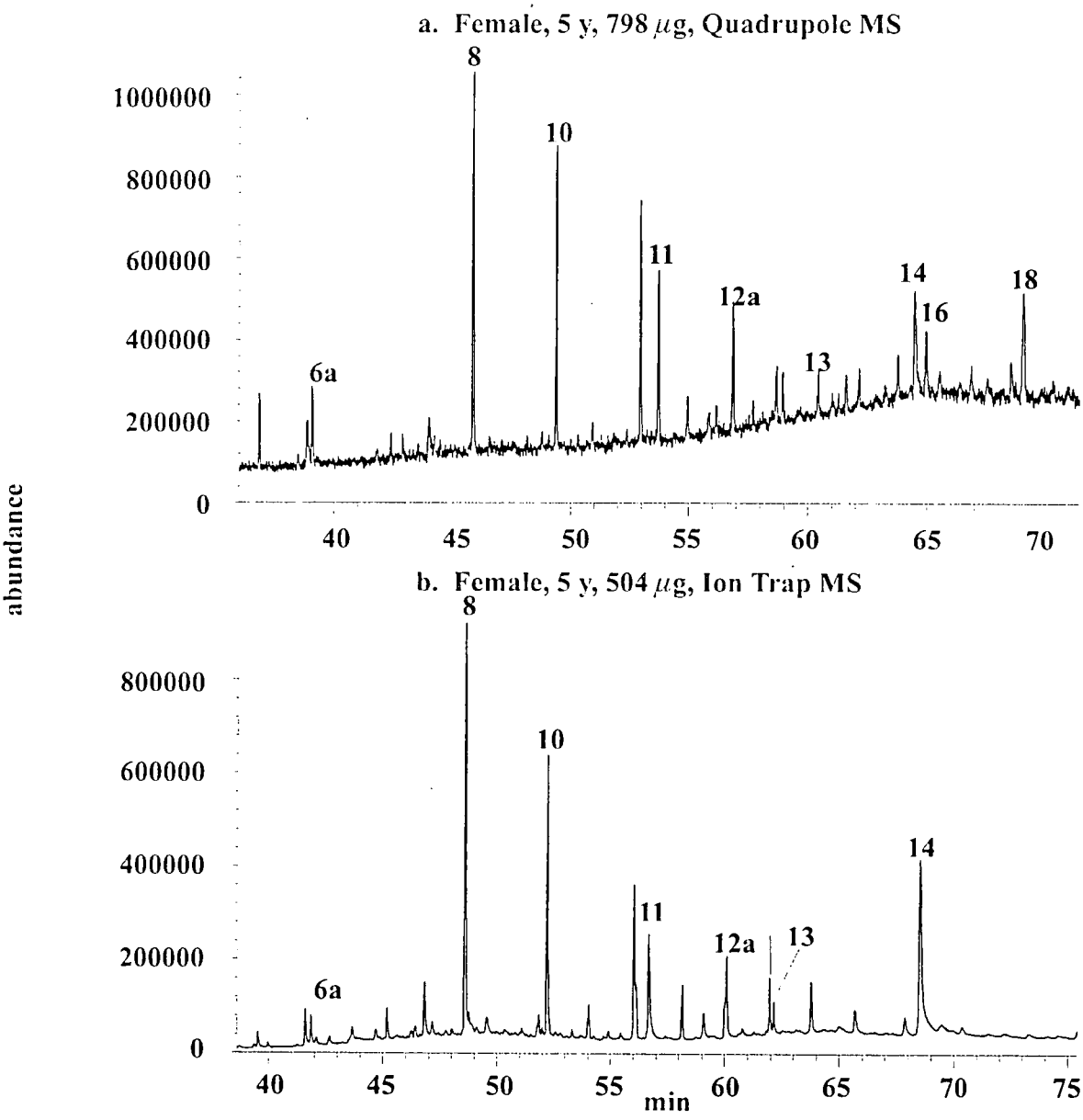

Figure 5. SFE-GC/MS of hair from same subject using quadrupole and ion trap mass spectrometers

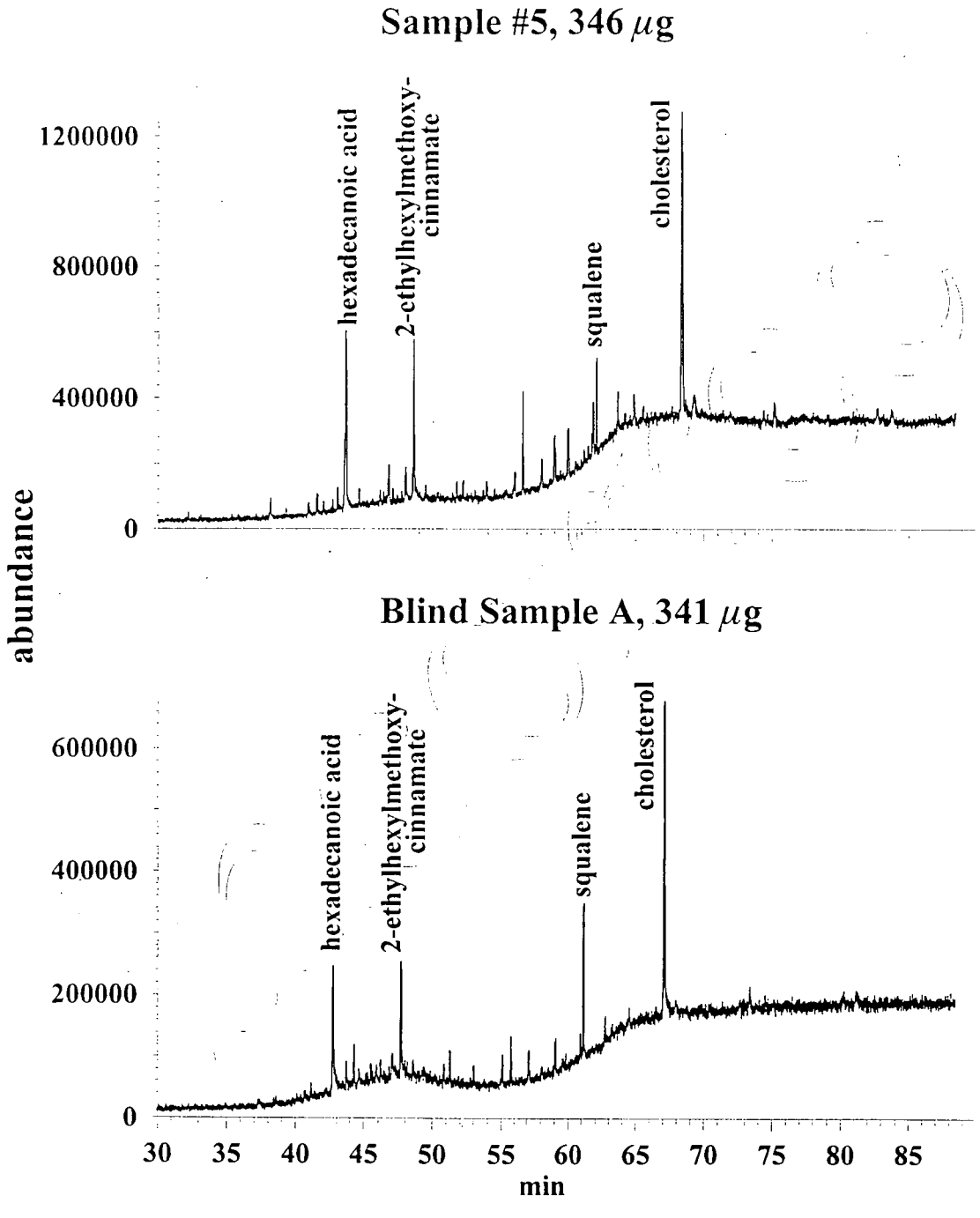

Figure 6. SFE-GC/MS match for Blind Sample A
(incorrectly identified)

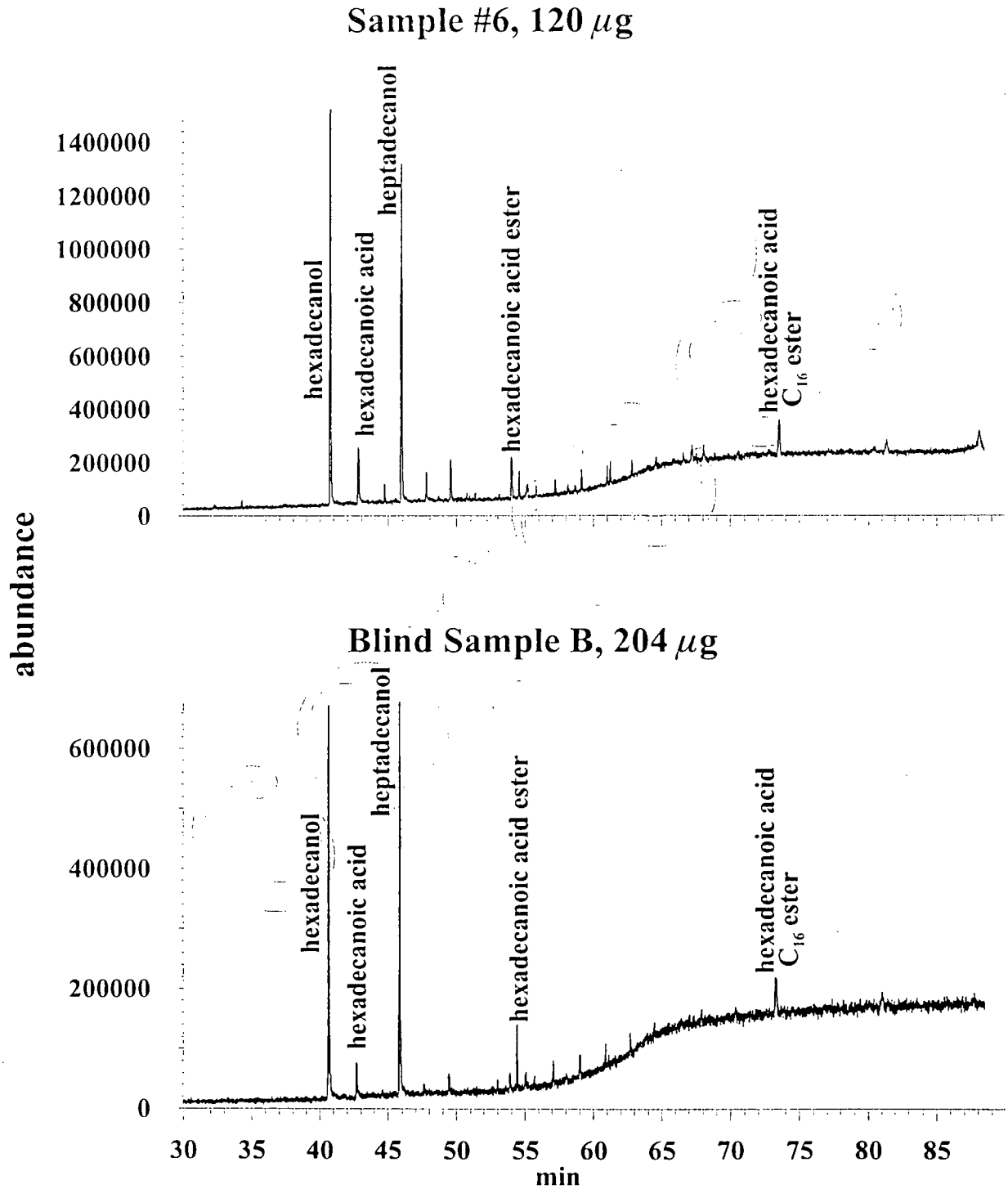

Figure 7. SFE-GC/MS match for Blind Sample B
(correctly identified)

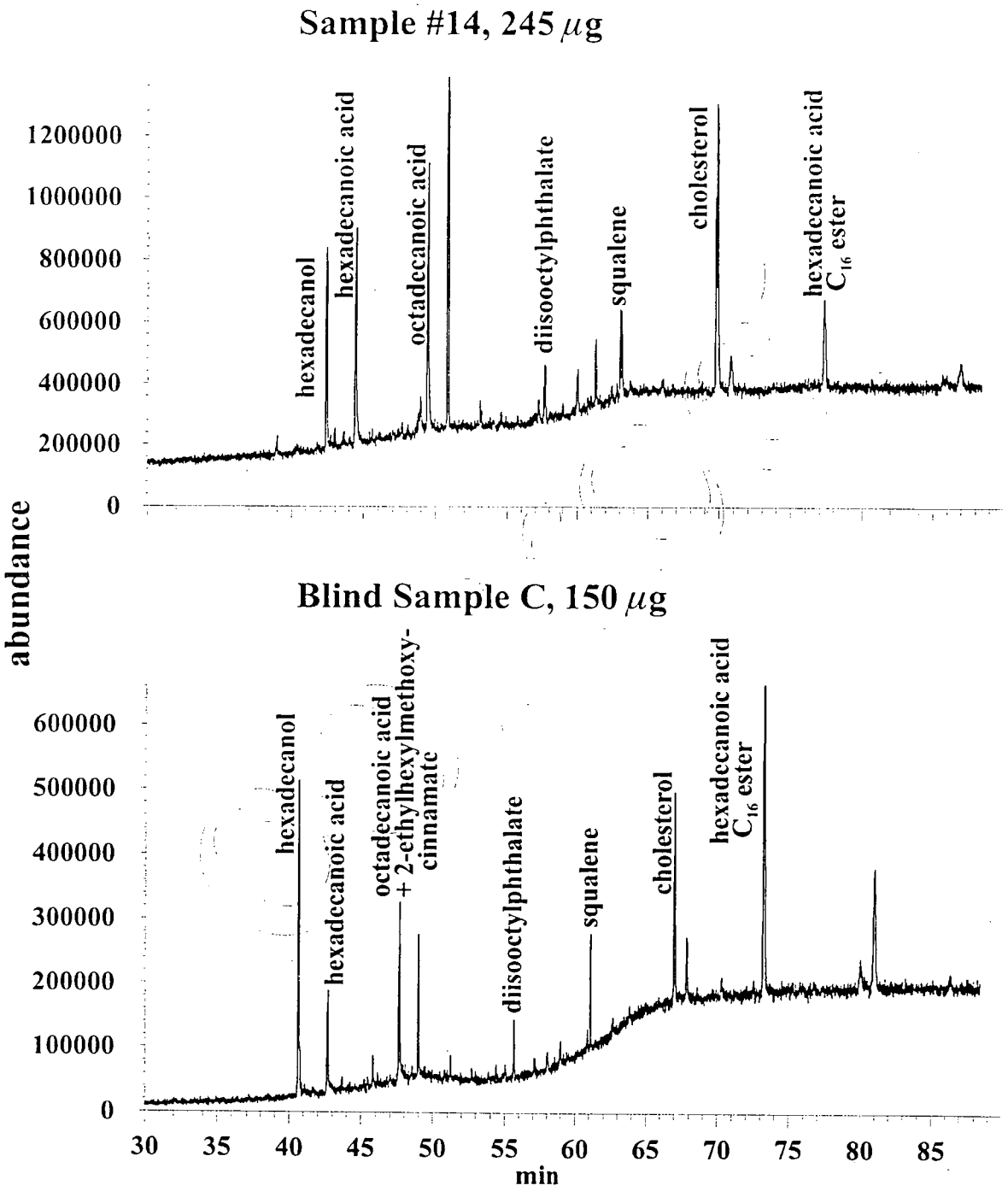

Figure 8. SFE-GC/MS match for Blind Sample C
(correctly identified)

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.

Success in matching "unknown" hair samples with those from known individuals depends on the consistency of the chemical profiles from a specific individual's hair, the distinguishing characteristics in chemical profiles between individuals, and the model or method used in establishing a data match. 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 match 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). Additional considerations of a subsequent study might be the consistency of chemical profiles of hair from areas other than the head as well as more specific detection of trace level species that might better serve to distinguish an individual than the higher level components discussed in this report.

4. SUGGESTED READINGS

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[4] Morrison, J.F., S.N. Chesler, W.J. Yoo, and C.M. Selavka, "Matrix and Modifier Effects in the Supercritical Fluid Extraction of Cocaine and Benzoylecgonine From Human Hair," *Anal. Chem.*, 70/1 (1998): 163-172.

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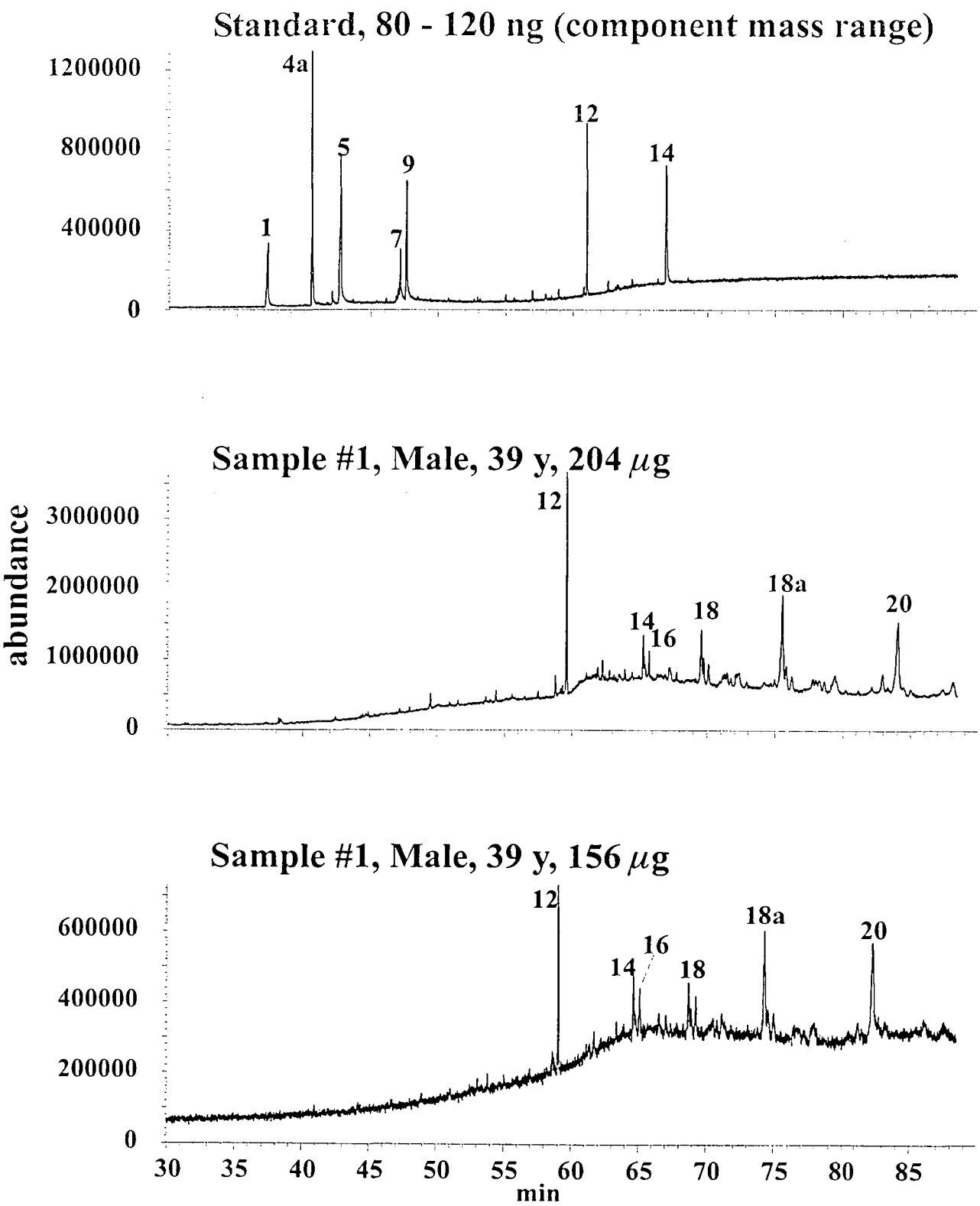
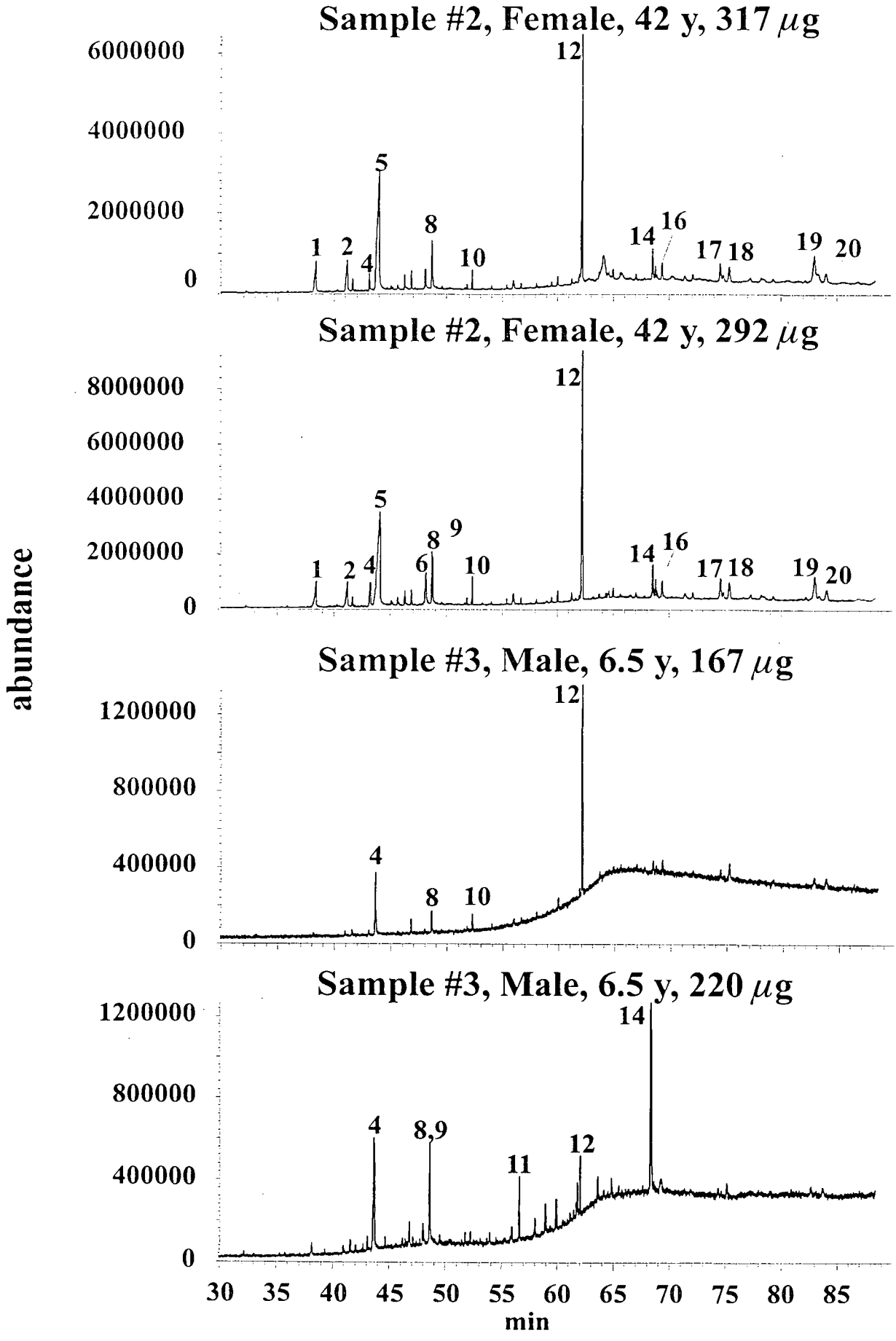
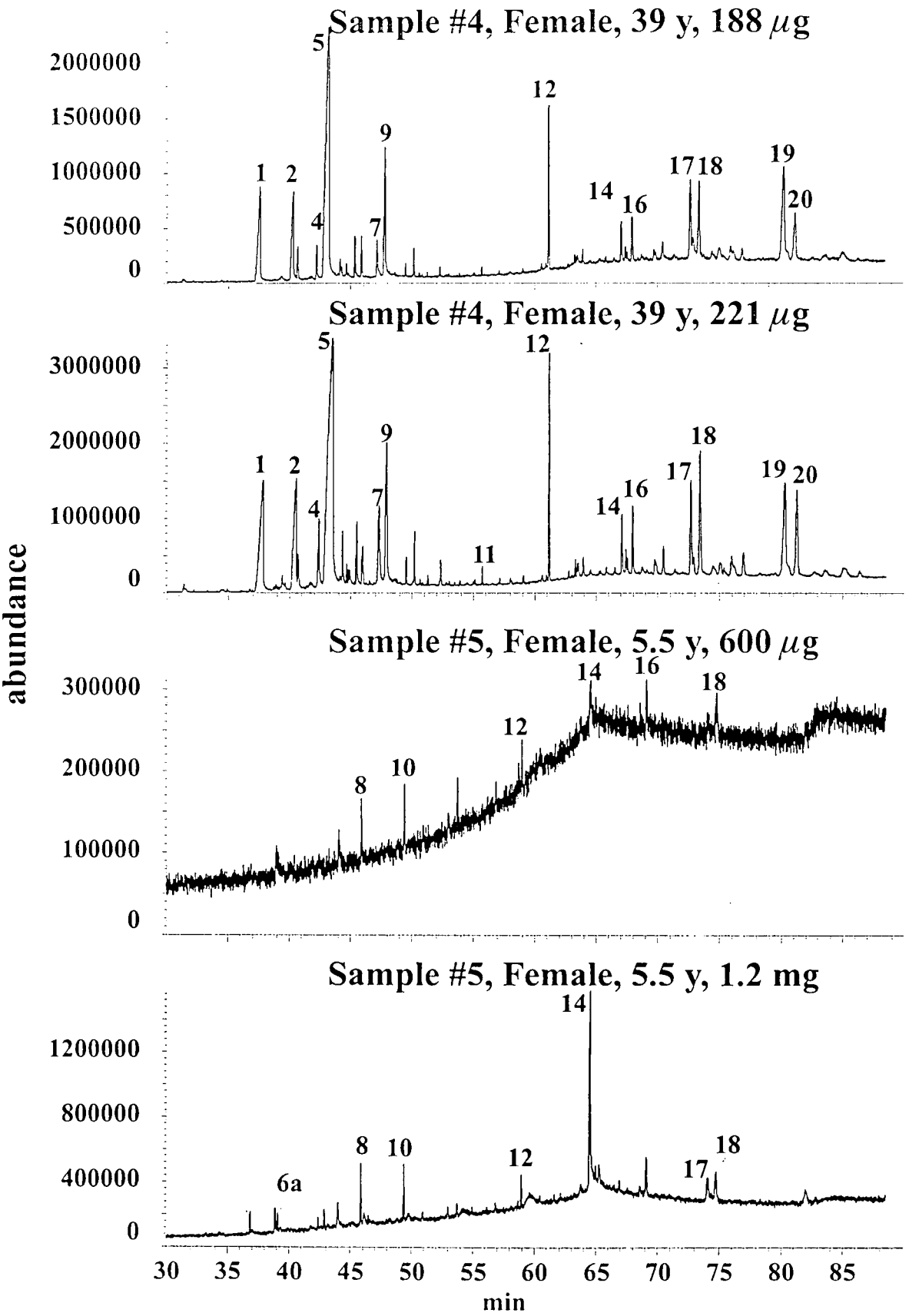
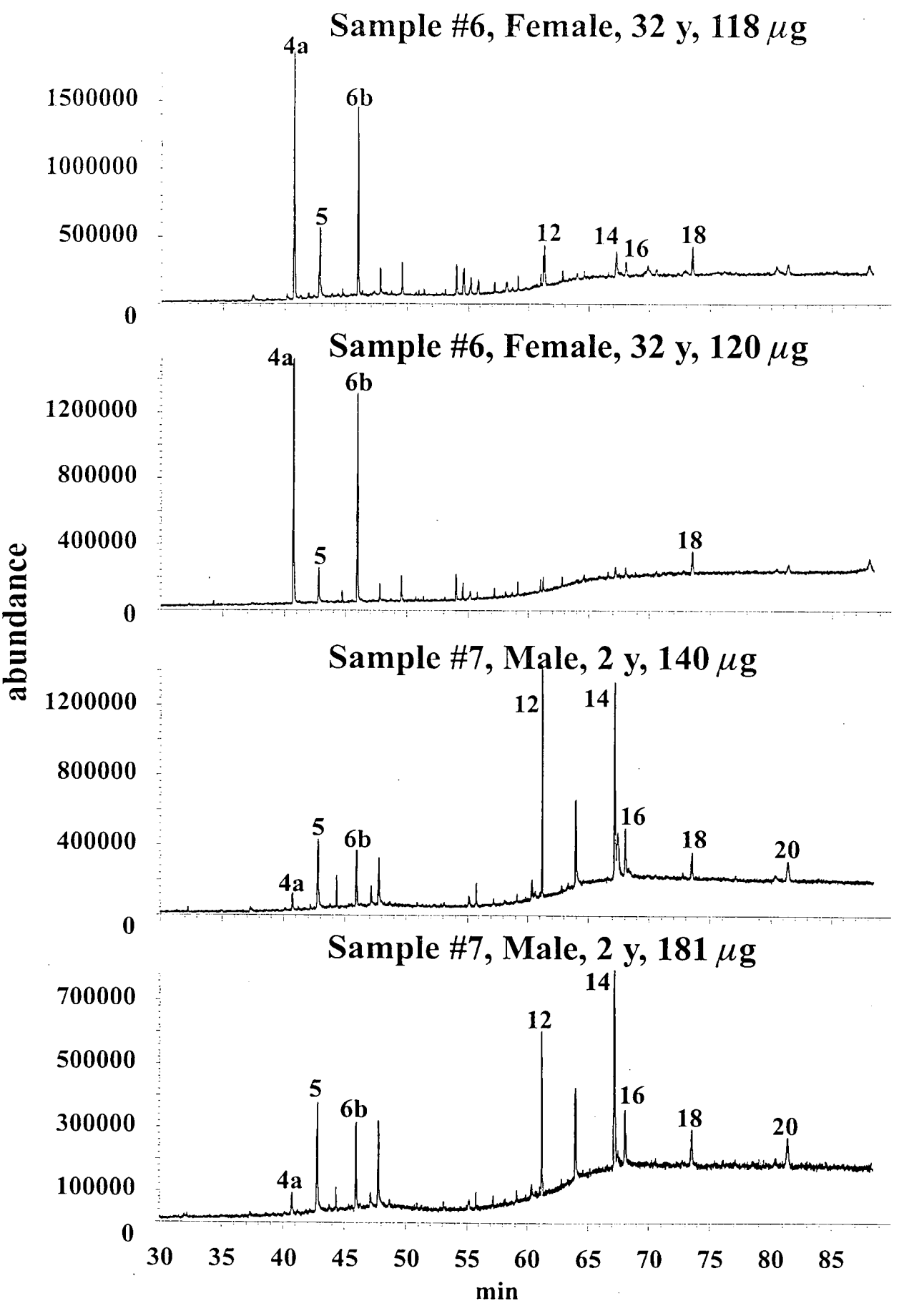
[6] Andrasko, J. and B. Stocklassa, "Shampoo Residue Profiles in Human Head Hair," *J. Forensic Sci.*, 35/3 (1990): 569-579.

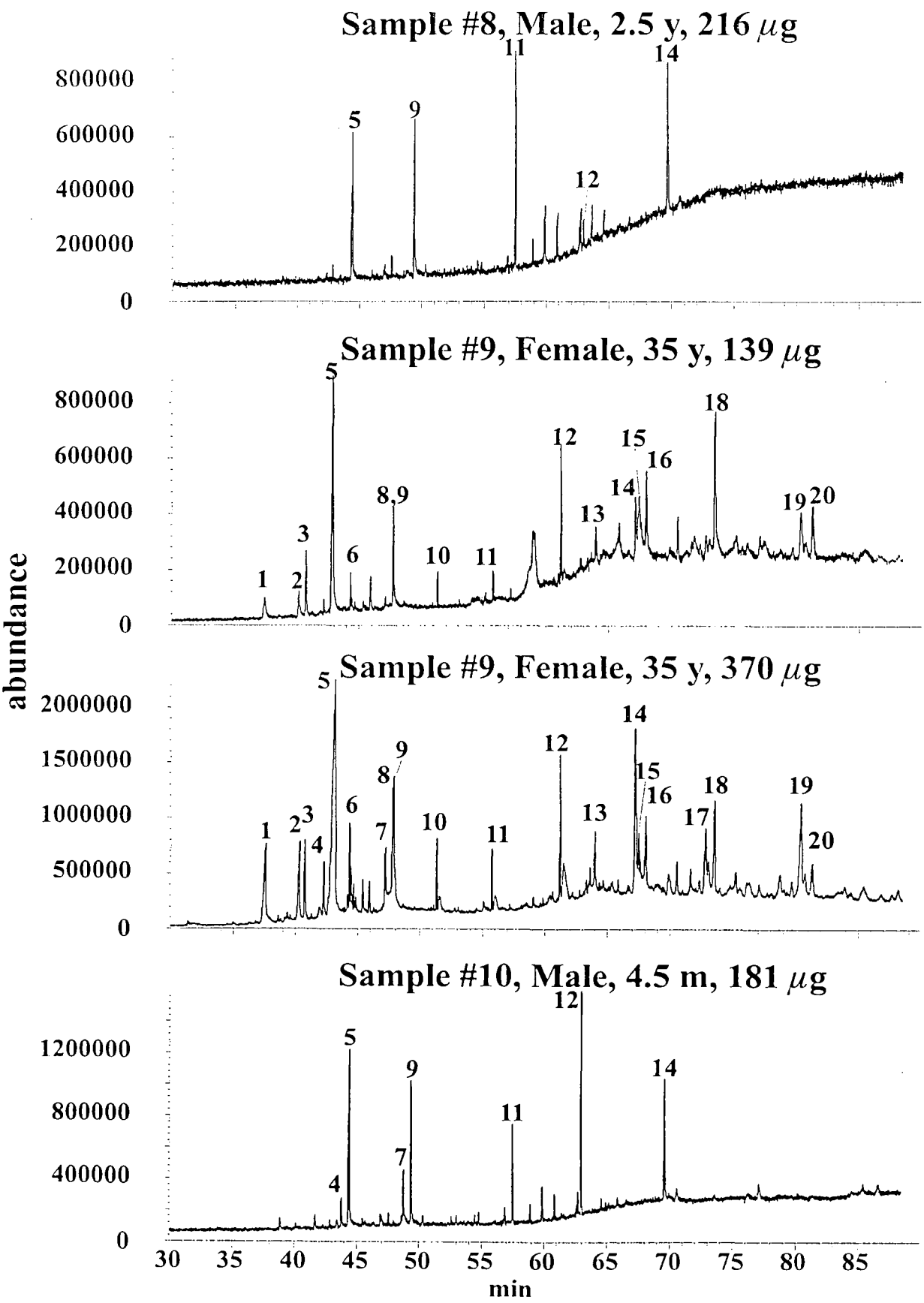
Appendix A. SFE-GC/MS Chromatograms of Hair Samples From 20 Volunteers Used in Trace Evidence Feasibility Study (See Table 2 for Peak Identifications)

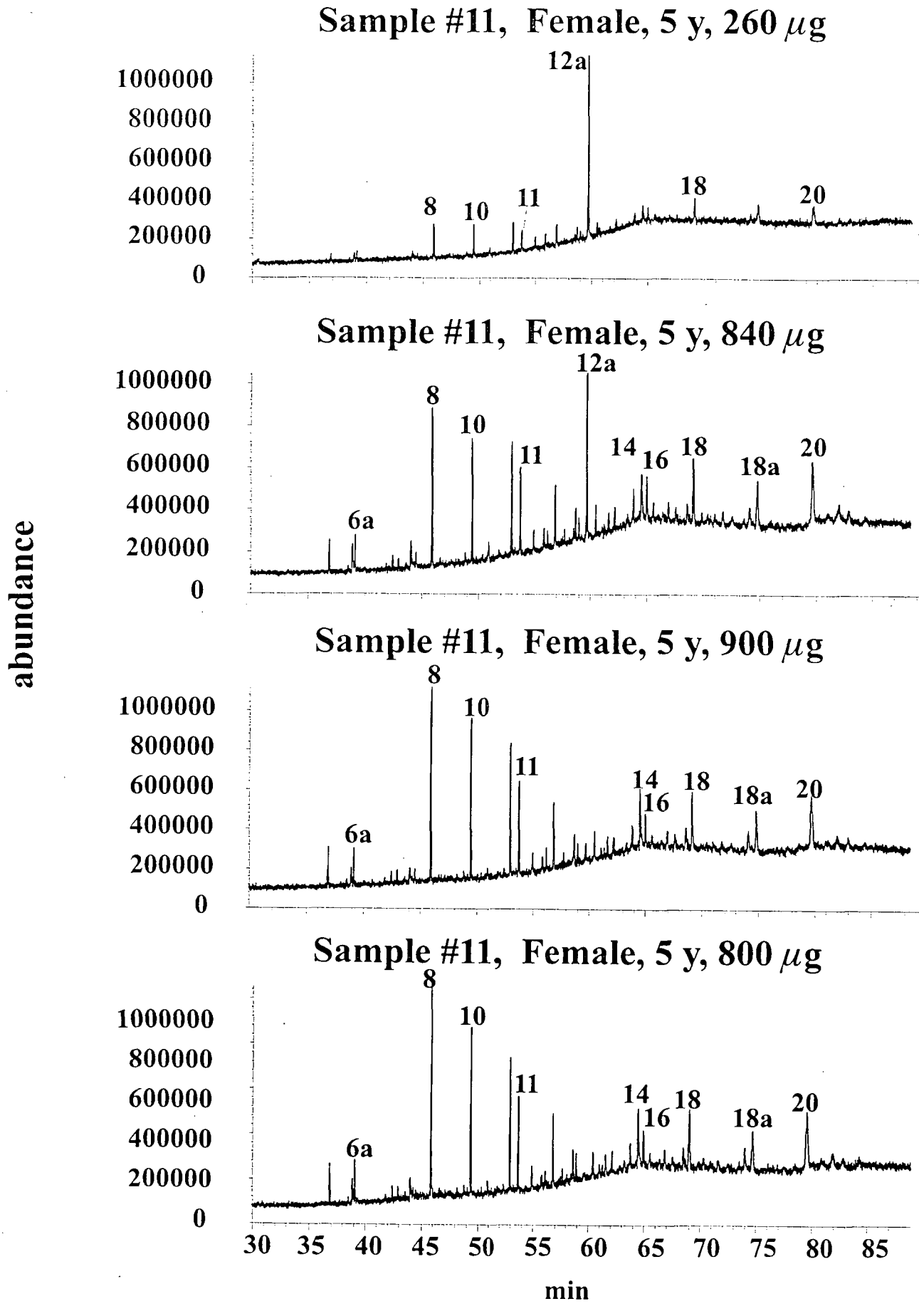

Standard, 80 - 120 ng (component mass range)
1200000
800000
400000
0
4a
5
9
12
14
1
7
Sample #1, Male, 39 y, 204 μg
abundance
3000000
2000000
1000000
0
12
14
16
18
18a
20
Sample #1, Male, 39 y, 156 μg
600000
400000
200000
0
12
14
16
18
18a
20
30
35
40
45
50
55
60
65
70
75
80
85
min

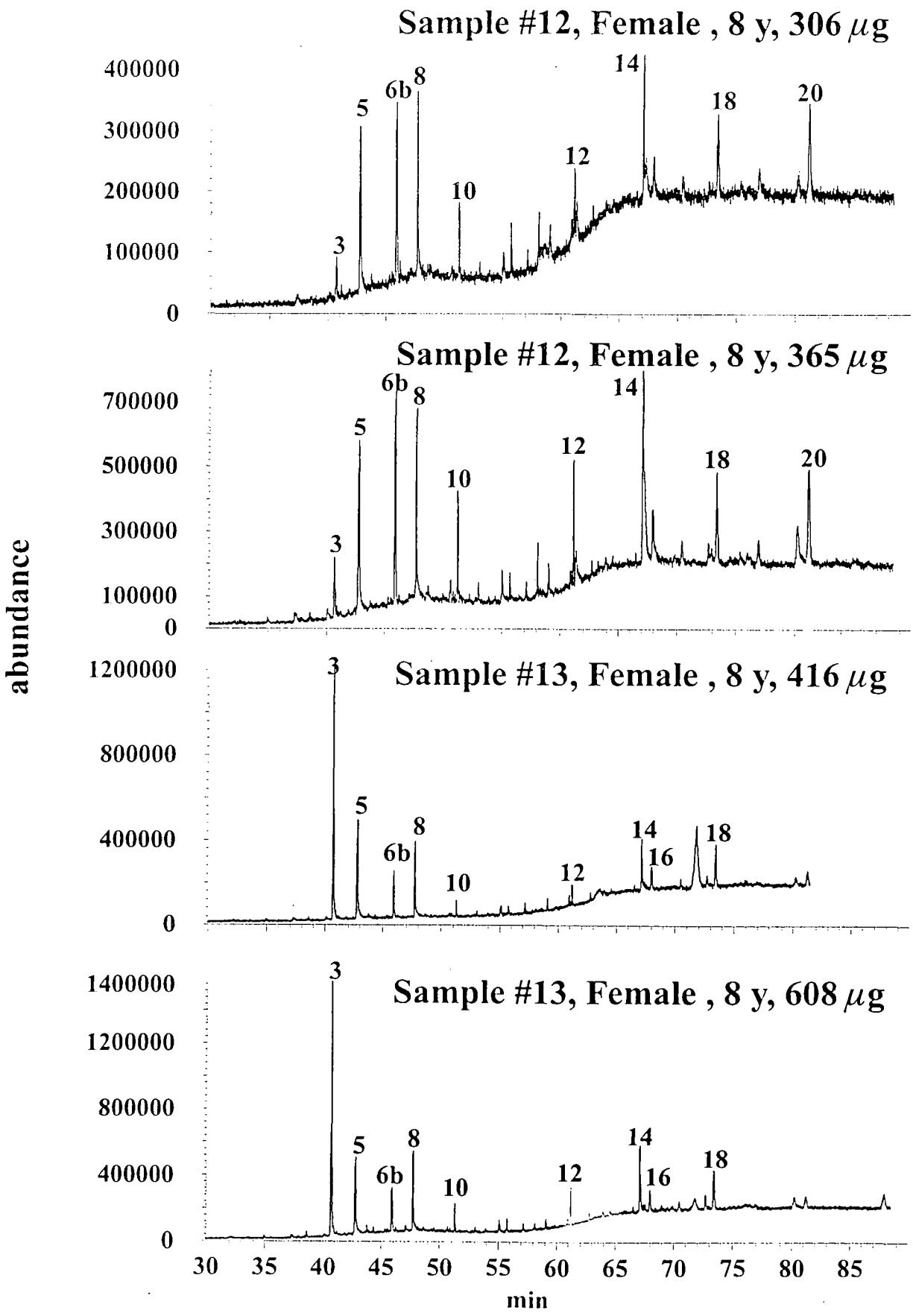

Sample #2, Female, 42 y, 317 μg
6000000
4000000
2000000
0
12
5
8
1
2
4
10
14
16
17
18
19
20
Sample #2, Female, 42 y, 292 μg
8000000
6000000
4000000
2000000
0
12
5
9
8
1
2
4
6
10
14
16
17
18
19
20
abundance
Sample #3, Male, 6.5 y, 167 μg
1200000
800000
400000
0
12
4
8
10
Sample #3, Male, 6.5 y, 220 μg
1200000
800000
400000
0
14
4
8,9
11
12
30
35
40
45
50
55
60
65
70
75
80
85
min

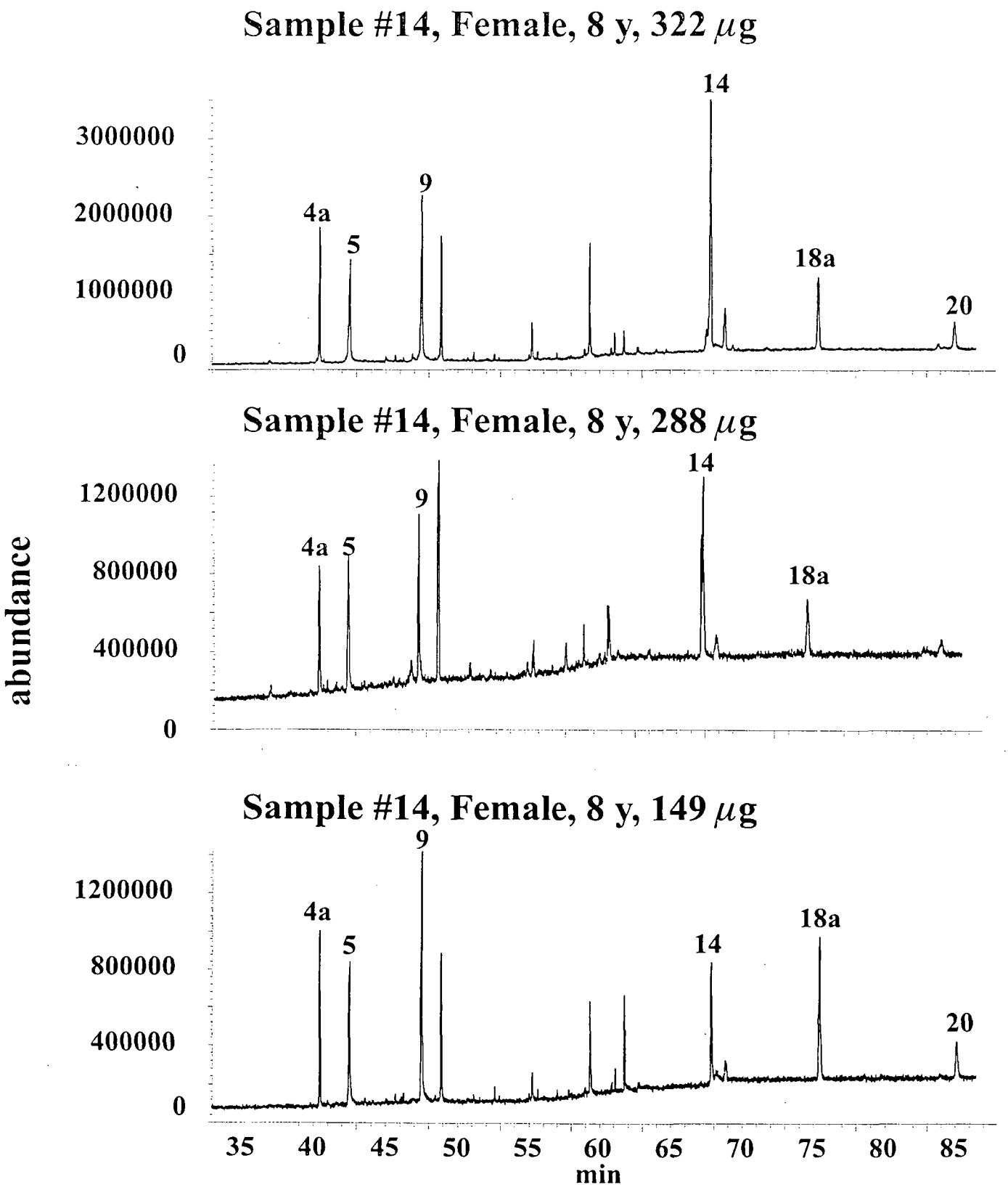

Sample #4, Female, 39 y, 188 μg
Sample #4, Female, 39 y, 221 μg
Sample #5, Female, 5.5 y, 600 μg
Sample #5, Female, 5.5 y, 1.2 mg
abundance
min
2000000
1500000
1000000
500000
0
3000000
300000
200000
100000
1200000
800000
400000
30
35
40
45
50
55
60
65
70
75
80
85
1
2
4
5
6a
7
8
9
10
11
12
14
16
17
18
19
20

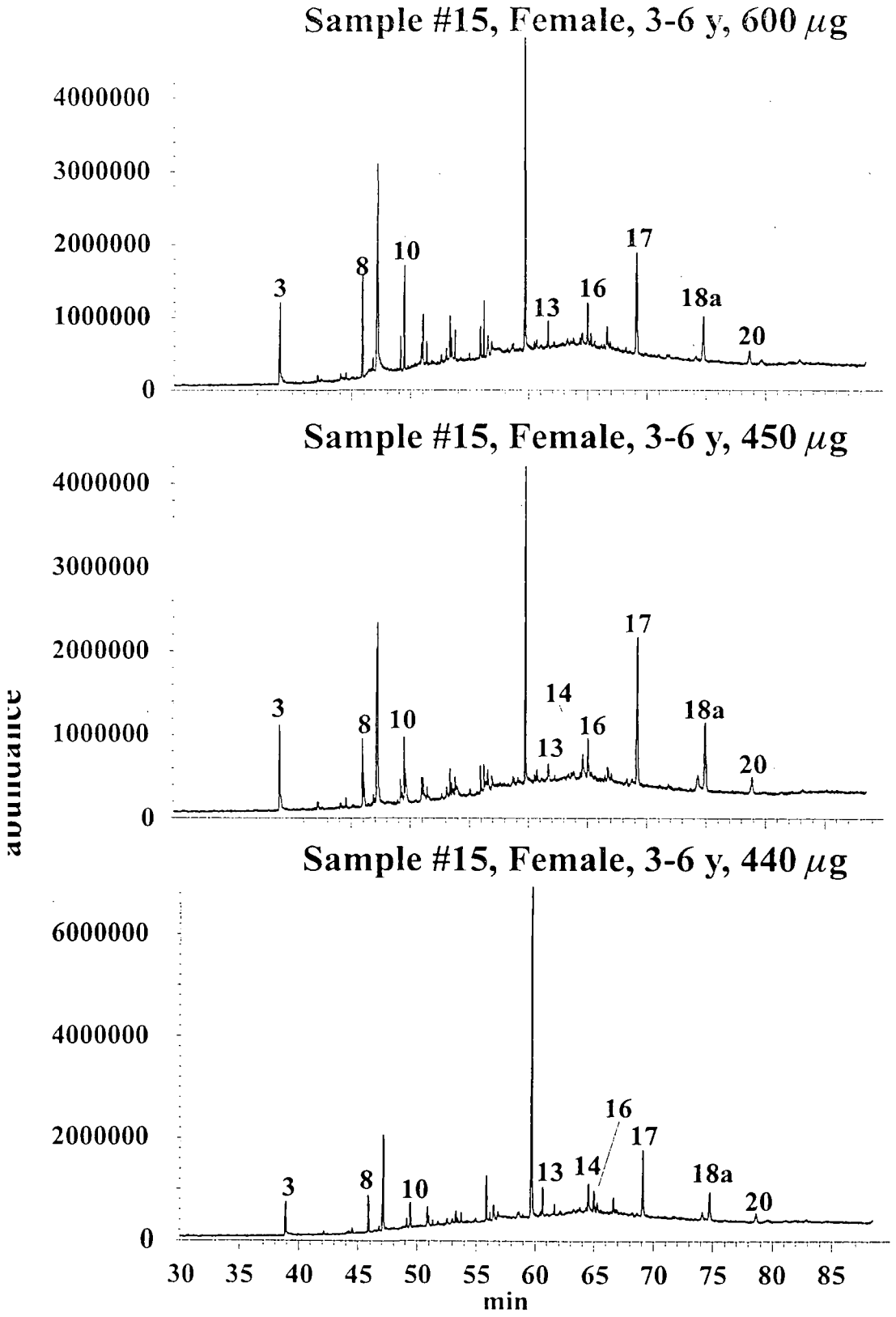

Sample #6, Female, 32 y, 118 μg
4a
6b
5
12
14
16
18
1500000
1000000
500000
0
Sample #6, Female, 32 y, 120 μg
4a
6b
5
18
1200000
800000
400000
0
abundance
Sample #7, Male, 2 y, 140 μg
12
14
16
5
6b
4a
18
20
1200000
800000
400000
0
Sample #7, Male, 2 y, 181 μg
14
12
5
16
6b
18
20
4a
700000
500000
300000
100000
0
30 35 40 45 50 55 60 65 70 75 80 85
min

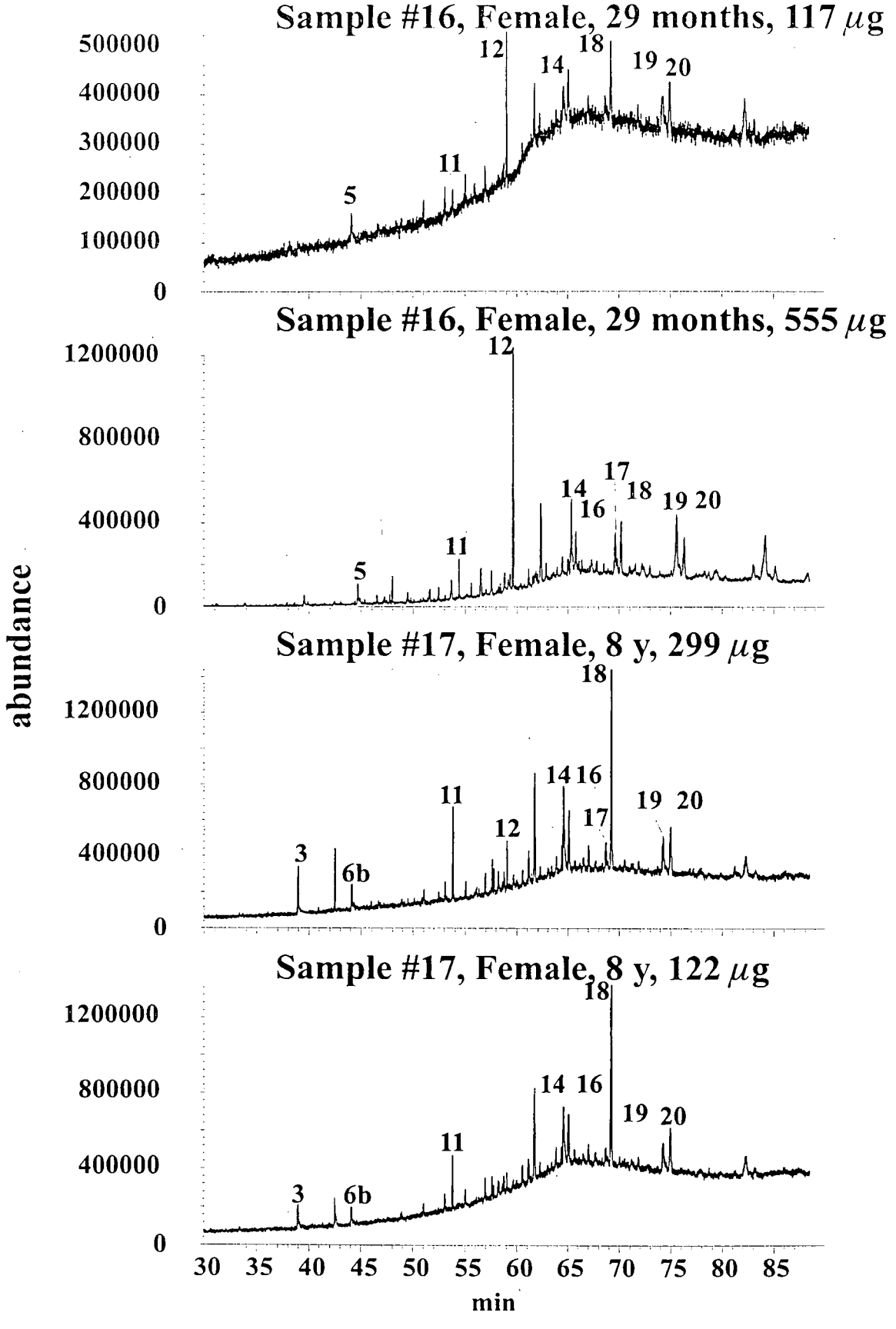

Sample #8, Male, 2.5 y, 216 μg
Sample #9, Female, 35 y, 139 μg
Sample #9, Female, 35 y, 370 μg
Sample #10, Male, 4.5 m, 181 μg
abundance
min

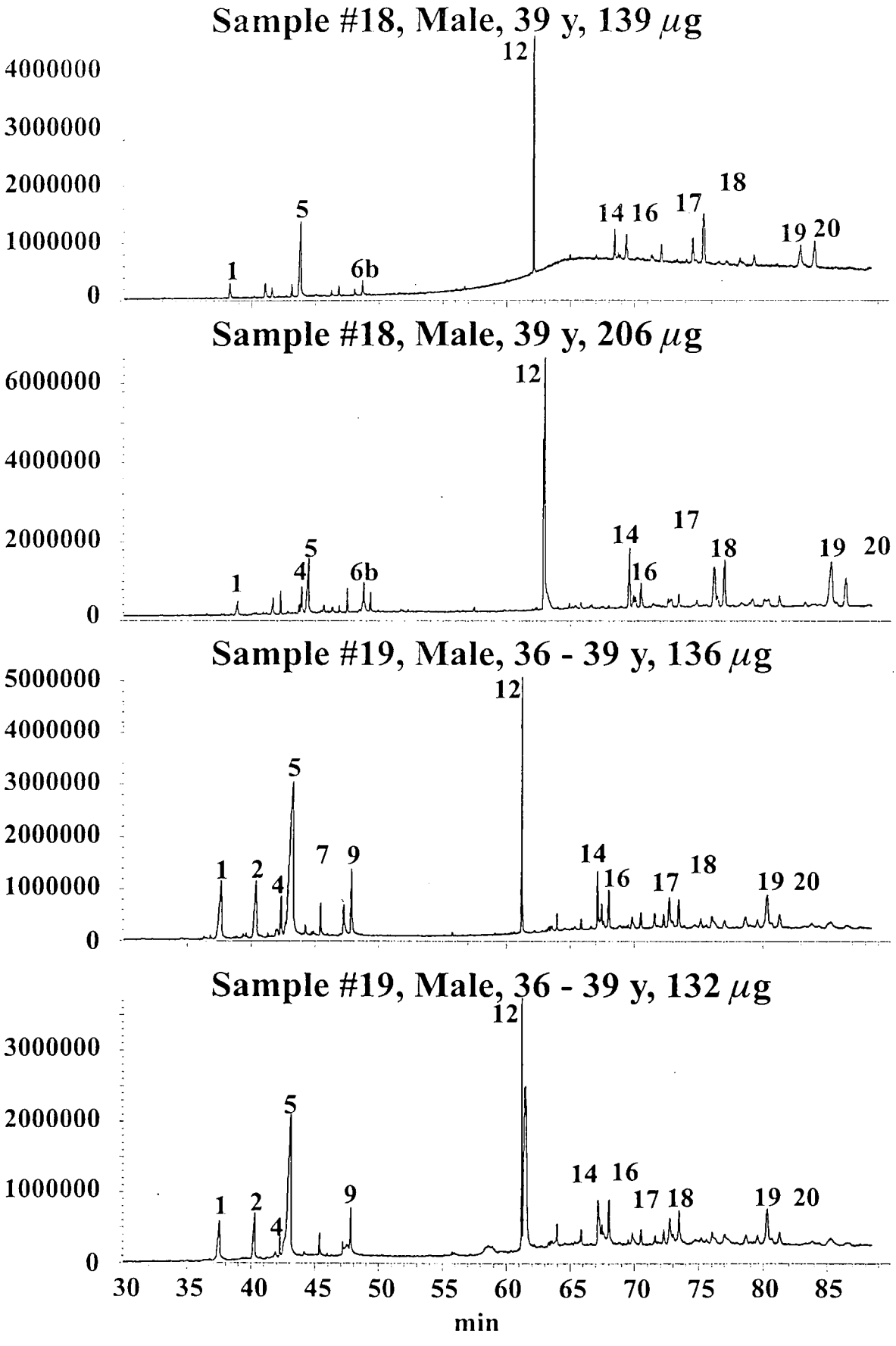

Sample #11, Female, 5 y, 260 μg
Sample #11, Female, 5 y, 840 μg
Sample #11, Female, 5 y, 900 μg
Sample #11, Female, 5 y, 800 μg
abundance
1000000
800000
600000
400000
200000
0
6a
8
10
11
12a
14
16
18
18a
20
30 35 40 45 50 55 60 65 70 75 80 85
min

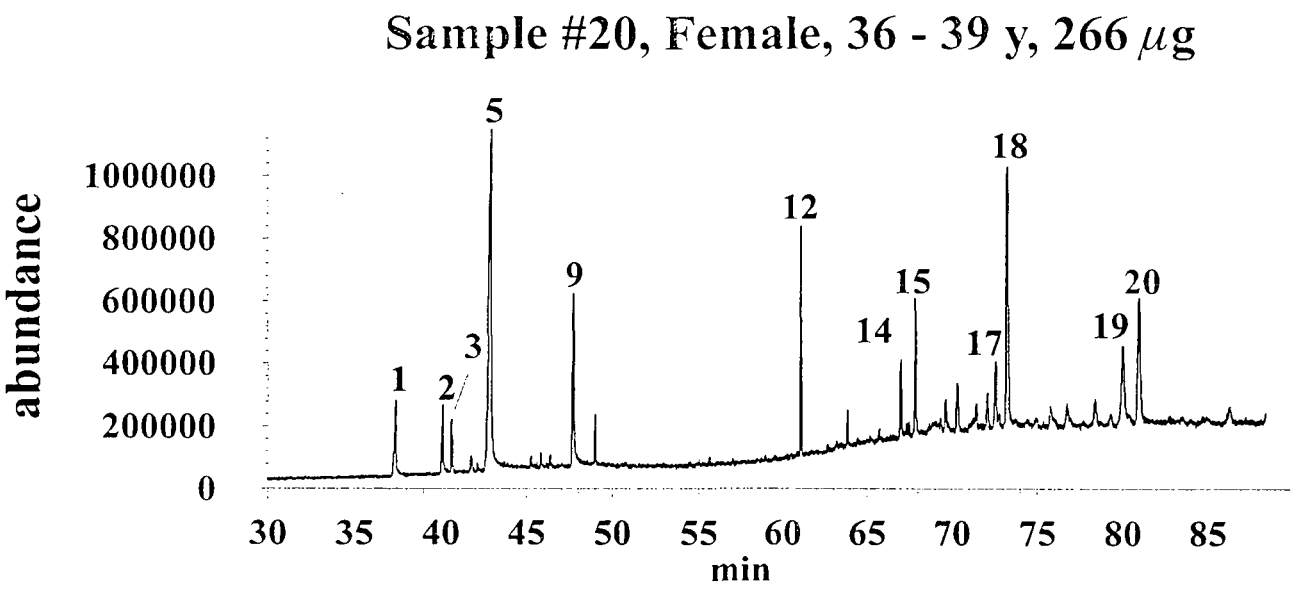

Sample #12, Female , 8 y, 306 μg
Sample #12, Female , 8 y, 365 μg
Sample #13, Female , 8 y, 416 μg
Sample #13, Female , 8 y, 608 μg
abundance
min
3
5
6b
8
10
12
14
16
18
20


Sample #14, Female, 8 y, 322 μg
3000000
2000000
1000000
0
4a
5
9
14
18a
20
Sample #14, Female, 8 y, 288 μg
1200000
800000
400000
0
4a
5
9
14
18a
Sample #14, Female, 8 y, 149 μg
1200000
800000
400000
0
4a
5
9
14
18a
20
35
40
45
50
55
60
65
70
75
80
85
min
abundance


Sample #15, Female, 3-6 y, 600 μg
4000000
3000000
2000000
1000000
0
3
8
10
13
16
17
18a
20
Sample #15, Female, 3-6 y, 450 μg
4000000
3000000
2000000
1000000
0
3
8
10
13
14
16
17
18a
20
Sample #15, Female, 3-6 y, 440 μg
6000000
4000000
2000000
0
3
8
10
13
14
16
17
18a
20
30 35 40 45 50 55 60 65 70 75 80 85
min
abundance


Sample #16, Female, 29 months, 117 μg
Sample #16, Female, 29 months, 555 μg
Sample #17, Female, 8 y, 299 μg
Sample #17, Female, 8 y, 122 μg
abundance
min
500000
400000
300000
200000
100000
0
1200000
800000
400000
0
30 35 40 45 50 55 60 65 70 75 80 85
3
5
6b
11
12
14
16
17
18
19
20


Sample #18, Male, 39 y, 139 μg
4000000
3000000
2000000
1000000
0
12
5
14 16
17
18
19 20
1
6b
Sample #18, Male, 39 y, 206 μg
6000000
4000000
2000000
0
12
14
17
5
18
19 20
4
6b
16
1
Sample #19, Male, 36 - 39 y, 136 μg
5000000
4000000
3000000
2000000
1000000
0
12
5
1
2
7
9
14
4
16
17
18
19 20
Sample #19, Male, 36 - 39 y, 132 μg
3000000
2000000
1000000
0
12
5
14 16
1
2
9
17 18
19 20
4
30 35 40 45 50 55 60 65 70 75 80 85
min


Sample #20, Female, 36 - 39 y, 266 μg
abundance
1000000
800000
600000
400000
200000
0
30
35
40
45
50
55
60
65
70
75
80
85
min
1
2
3
5
9
12
14
15
17
18
19
20