Jan Andrasko,[1] *Ph.D. and Bengt Stocklassa,*[1] *M.Sc.*

Shampoo Residue Profiles in Human Head Hair

REFERENCE: Andrasko, J. and Stocklassa, B., **"Shampoo Residue Profiles in Human Head Hair,"** *Journal of Forensic Sciences,* JFSCA, Vol. 35, No. 3, May 1990, pp. 569–579.

ABSTRACT: Washing hair with shampoo results in an accumulation of shampoo components in the hair. Hair of individuals using different shampoos can be distinguished by analysis of shampoo residues.

A method for extraction and analysis of such residues is presented. The hair is extracted using a methanol/water mixture, and the extract is analyzed by reverse-phase high-pressure liquid chromatography (HPLC). The detector system consists of two ultraviolet (UV) detectors connected in series. The method is nondestructive to hair and is sensitive enough to be applied to a single hair 5 to 10 cm in length. Residues from hair balsams are analyzed by this technique as well.

The use of this method in forensic science examination of human head hair is demonstrated.

KEYWORDS: forensic science, hair, chromatographic analysis, shampoo, liquid chromatography, shampoo residue

Forensic science examination of human head hair has been dominated by morphological methods. Additional methods have been introduced, including blood grouping, element analysis, and protein analysis.

Most of the methods for forensic science comparison of hair are based on some property of hair itself as a biological specimen—for example, color, length, protein composition, morphology, or elements from the body accumulated in hair. Only a few attempts have been made to distinguish between human head hairs by using some property resulting from modification of hair by external treatment. So far, these attempts have been limited to hair treated cosmetically by bleaching, dyeing, and permanent-waving [*1–3*]. Not all individuals treat their hair in this manner, however. In fact, most individuals wash their hair regularly and usually use hair shampoos. Some persons treat their hair with hair balsam after washing it. Since the number of commercial shampoos and balsams is very large, the analysis of the residues from these preparations in hair should be valuable in distinguishing between head hairs from different individuals.

The possibility of detecting the residues of surface-active compounds in hair arose in previous work by the authors of this paper on permanent-waved hair [*2,3*]. To remove calcium from such hair, it was washed using diluted hydrochloric acid (HCl) in water. Afterwards, the uptake of Ca^{2+} or other metal ions from water was studied [*3*]. In some experiments, cetyltrimethylammonium bromide (CTAB) was added to the solution to improve the wetting of the hair. After the hair was dried, bromine was detected in it by X-ray microanalysis with the scanning electron microscope. Since the concentration of the surfactant was low, this result indicated enrichment of the hair with CTAB.

Received for publication 17 Nov. 1988; revised manuscript received 12 June 1989; accepted for publication 19 June 1989.

[1]Research associates, National Laboratory of Forensic Science, Linköping, Sweden.

In general, shower and hair shampoos contain as main components anionic, nonionic, and sometimes amphoteric detergents. A combination of fatty acid monoethanolamides and diethanolamides, sodium alkyl sulfates, and polyoxyethylene sodium alkyl sulfates have been most widely used [*4–6*]. Recently, other surfactants, for example, alkyldimethylaminoacetic acid, betaine, sodium *N*-acyl-*N*-glutamates, and alkyldimethylamine oxide, have been introduced in these products. In addition to surfactants, many other chemical compounds may be found in commercial shampoos, such as perfumes, colorants, buffers, pearl-lustre agents, protein hydrolyzates, thickening agents, and preservatives [*7*].

It is, of course, hardly possible to analyze so many chemically different compounds in samples available for forensic science examination (such as a single hair) in a single analytical step. The authors have developed a simple method for analysis of shampoo (or balsam) residues in hair. This method is based on reverse-phase high-pressure liquid chromatography (HPLC) of hair extracts, using ultraviolet (UV) detectors connected in series. The method is sufficiently sensitive to analyze a single hair and is nondestructive, thus permitting additional examinations of the same hair by other techniques. Some preliminary results have been reported elsewhere [*8*].

Materials and Methods

Extraction Procedure

Hair samples were extracted by using a mixture of methanol and water (70:30) in 1-mL glass vials. A single hair was coiled on the bottom of a glass vial. Fifty μL of the extraction solvent was added, and the vial was closed and left to stand overnight. Care was taken to check that the hair sample was completely immersed in the solvent. For longer hair, or when several hairs were available, the amount of solvent added was 50 μL per 10-cm length of hair.

HPLC Profiles of Shampoo Residues

The chromatographic system used consisted of a Varian Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Model 100). The detector wavelength was set to 205 nm. This wavelength was found to be optimal considering the number of detectable components and the background absorption of the mobile phase. In series with this detector, a fixed-wavelength detector (Waters Model 440) operating at 254 nm was connected. The HPLC column used was a 20-cm 5-μm Nucleosil C_{18}, 4.6 mm in inside diameter (Skandinaviska GeneTec AB). The temperature was at ambient level. The injections were made by means of a valve injector (Rheodyne Type 7125) with 50-μL loop. The first detector was connected to an HP 3390 A integrator, and the 254-nm detector was connected to an ordinary chart recorder. The mobile phase consisted of 72% methanol and 28% water, and the flow rate was 1 mL/min. In our preliminary report [*8*] and in some of the experiments reported here, a mobile phase consisting of 50% acetonitrile and 50% water, with a flow rate of 1.5 mL, was used. The analyses were carried out under isocratic conditions, since the baseline drift resulting from gradient elution was unacceptably high at the sensitivities used. The methanol was of ordinary HPLC grade.

The eluting solvents were filtered and degassed before analysis. The samples of commercial shampoos were diluted with methanol and filtered. The samples of hair balsam were prepared in a similar way, except that a small amount of water was also added.

The chromatograms obtained for extracts from hair exhibited a peak with a retention time of about 18 min (the mobile phase was a methanol/water mixture). The peak was

identified as dibutyl phthalate. Since this compound is not found in shampoos, its origin is unknown. Dibutyl phthalate is frequently used as a softener in plastics and is presumably an environmental contamination in hair. It is also possible that this plasticizer is leached out by shampoo from the shampoo container. We made use of this compound for monitoring fluctuations of retention times.

Results and Discussion

Analysis of Commercial Shampoos

Many surfactants present in shampoos (for example, alkyl sulfates) do not exhibit strong UV absorption, the detection mode used in this study. Nevertheless, the simple analytical procedure used can distinguish between many types of commercial shampoos and balsams. The use of dual detection wavelengths provides complementary information about the sample composition. Monoethanolamides and diethanolamides of fatty acids (C_{12} to C_{18}), frequently present in shampoos, are not detected at 254 nm but represent many of the chromatographic peaks at 205 nm.

In total, samples of more than 20 commercial shampoos were analyzed and all the profiles were distinguishable. Some profiles are shown in Fig. 1. To reduce the total number of figures, only the profiles obtained at 205 nm are shown.

Profiles from a Single Human Head Hair—Profile Stability

Analyses of extracts from single scalp hairs from various individuals revealed that the amount of shampoo/balsam residue in a single hair 5 to 10 cm in length is sufficient for one HPLC analysis. When hair balsam was used after the hair had been washed with shampoo, more intense profiles were obtained, presumably because balsam is not rinsed off from hair to the same degree as shampoo.

The shampoo components detected by HPLC analysis of hair extracts did not appear to be volatile. Profiles obtained after long storage of hair (up to one year) did not differ from those of "fresh" samples.

Profile Variation Among Individuals Using Different Shampoos or Balsams

To investigate the ability of this method to differentiate between different individuals, hair samples from ten persons were collected and analyzed. All these individuals used different hair-washing preparations (shampoo/balsam). All the analyses resulted in clearly distinguishable profiles [*8*]; the discrepancies were of the same order of magnitude as those shown in Fig. 2.

Profiles from Persons Who Wash Their Hair Repeatedly with the Same Kind of Shampoo

As expected, individuals who kept to the same brand of shampoo during a long time period (for at least ten hair washings) were the most suitable group for forensic science examination of shampoo residues in hair.

Profiles from hair samples collected on different occasions do not change noticeably for these persons. This conclusion is based on the results of the study on ten individuals. One hair from each of these ten persons was collected on different occasions, the time period between subsequent collections being at least one week. The different persons employed different shampoos and did not change the brand of shampoo during the study period. Two of these individuals washed their hair only once a week, the others more

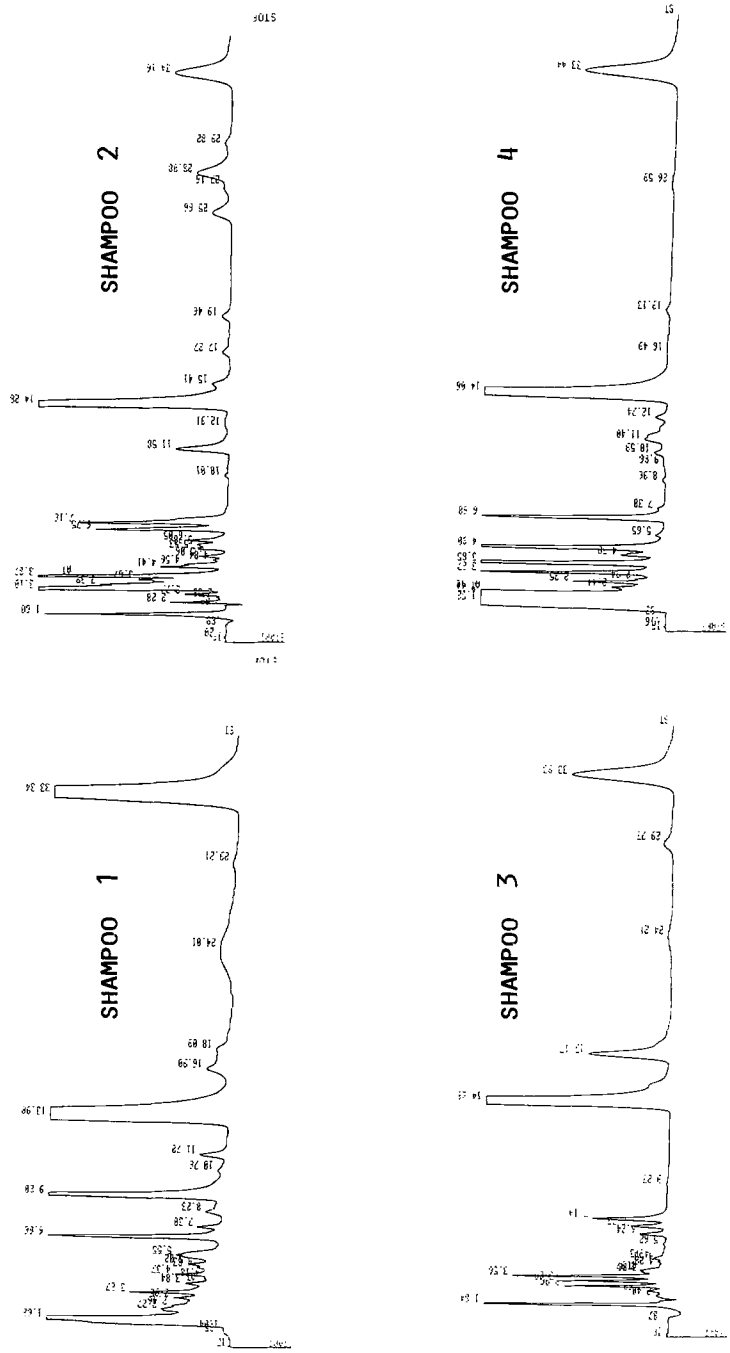

FIG. 1—*Profiles of four commercial hair shampoos. Analytical conditions: mobile phase, methanol/water (72 : 28); flow rate 1 mL/min; and detection wavelength, 205 nm.*

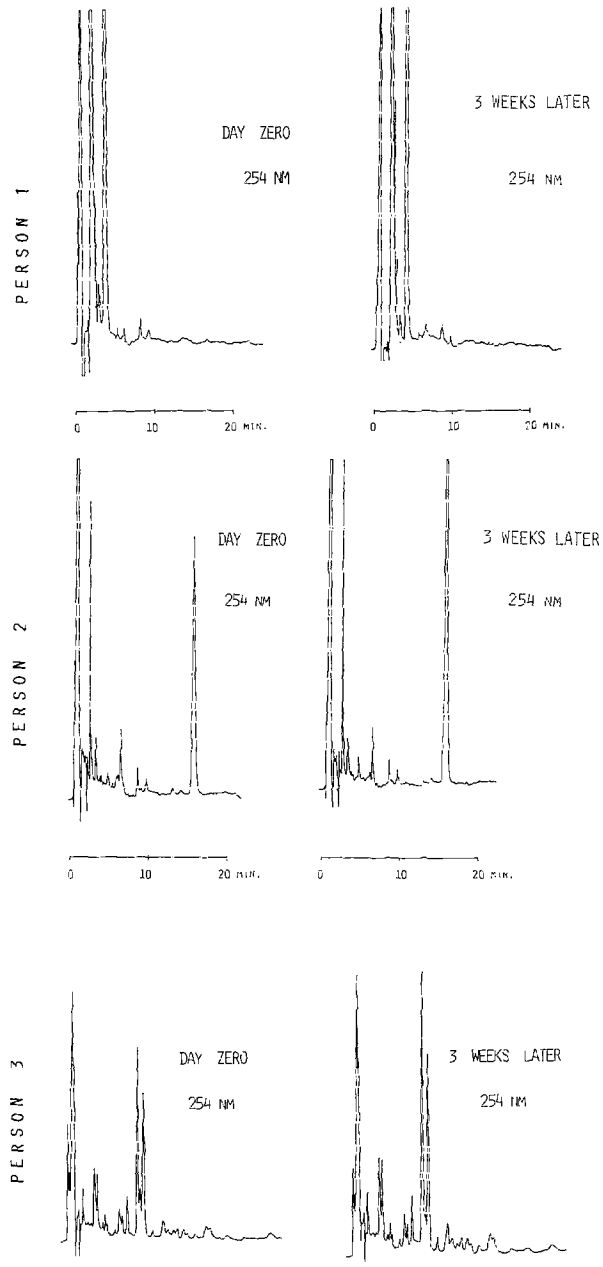

FIG. 2—*Profiles from persons keeping to the same brand of shampoo in subsequent hair washings. Three hairs of each individual were collected at different occasions. The analyses correspond to 10 cm of hair length. Person 1 used both shampoo and balsam for hair washing. Analytical conditions: mobile phase, acetonitrile/water (50:50); flow rate, 1.5 mL/min; UV detection at 254 nm; and 0.005 AUFS.*

often (two to four times a week). The hair samples from each individual were stored in plastic boxes at room temperature and were all extracted and analyzed after collection of the last sample. Some typical results are shown in Fig. 2.

We have also studied profile variation with the location of hair on the scalp. A serious drawback of many methods for forensic science examination of human head hair is the large variations in properties found among different hairs from the same individual. Washing of hair with the same shampoo is a procedure that probably does affect every single hair of the same individual in a similar manner. To verify this assumption five single hairs from three individuals were collected and analyzed. The hairs were obtained from different areas of the head by combing. The profiles for different hairs from the same scalp were found to be very similar. No qualitative differences were noted. The results are illustrated by Fig. 3. Some variations of total intensity were observed, presumably because the thickness and the length of each of the five hairs was not the same. The explanation for this may also be that the amount and the concentration of shampoo distributed among individual hairs is somewhat dependent on the location of the hairs on the head. Further rinsing of shampoo from hair is not necessarily equally effective across the whole head.

An interesting question is whether the profile obtained from the hair is similar to that obtained from the shampoo itself. Our experiments showed that the profiles were not always very similar [*8*] and were even quite different in some cases. Our observations may be explained by the different affinity of certain shampoo components to hair.

The Effect on the Profile of a Change of Shampoo

Many individuals change their brand of shampoo occasionally. For example, when 60 members of our laboratory staff were asked about their hair-washing habits, only 30% had kept to the same brand of shampoo for the past ten hair washings. The majority had used two brands, and 10% reported three or more brands of shampoo in their latest ten hair washings.

It seems likely that a change of shampoo in subsequent hair washings would cause problems for forensic science examination of hair by the method described. One might expect large variation in the profiles after each change of shampoo. However, in the majority of the cases we have investigated the profile did not change drastically. Figure 4 shows four profiles from a person who stopped using one brand of shampoo and started using a different one. Repeated washings with the new shampoo did not remove residues of the original one immediately. Gradually, the chromatograms contain mixed peaks for the two shampoos (Fig. 4). In contrast, medical shampoos strongly affected the composition of residue extracted from hair after a single washing. Such shampoos—that is, those used for treatment of skin diseases or dandruff—are applied to hair several times during each washing and their effects last much longer than those of ordinary shampoo.

The fact that many people alternate between two or more brands of shampoo may have some positive value for shampoo residue profiling. The mixed profiles that may result increase the possibility of distinguishing between different individuals. Thus, the number of distinguishable groups of hair is thus theoretically higher than the number of shampoos commercially available.

On the other hand, in some experiments, we have noted varying profiles from differently located hairs of persons who frequently changed the brand of shampoo for hair washing. Such observations may be explained as variations in the proportions between the different shampoos across the scalp.

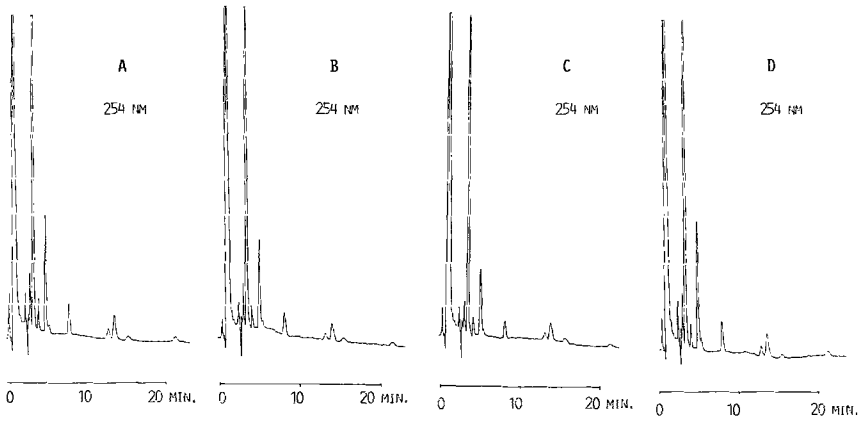

FIG. 3—*Profile variation with location of the hairs on the head. This person washed the hair repeatedly with the same shampoo. Four single hairs (5 to 8 cm in length) were analyzed. The analytical conditions were as in Fig. 2.*

Profile Variation with Time Among Individuals with Unknown Hair-Washing Habits

In this experiment, 3 hairs from each of 25 individuals were collected. The hair-washing habits of these persons were not known. Between 7 to 11 days later, new hair samples were collected. All the samples were analyzed (under identical analytical conditions) shortly after the second collection. Hairs from the same individual were extracted together to give "average" profiles representative for each scalp. The samples collected on the first occasion were intended for evaluation of the number of individuals within these 25 who could be distinguished by analysis of their hair extracts. The second sampling was intended for studying profile changes with time.

More than 30 different shampoo components were detected and used for distinguishing individuals in these investigations. Even for small quantitative differences in profile composition, all but 2 of the 25 profiles could be distinguished. However, assuming that the profile variation within a scalp was about the same as that discussed at the end of the previous paragraph, 19 of the 25 profiles could be distinguished. Within the remaining 6 profiles, 2 profiles of one group and 4 profiles of another group were judged to be matching.

The analyses of hair samples from the first and the second sampling revealed that only 13 of the 25 individuals exhibited unchanged profiles. In 3 cases the profiles were quite different (at both wavelengths), and in 2 other cases the differences observed were too large to consider the samples to have originated from the same person. Of our laboratory staff, 60 members were questioned about their frequency of hair washing and reported a rate of between 1 and 6 times a week. Considering the frequency of hair washing and the fact that the majority of people frequently change their kind of shampoo, these results are not surprising. They indicate that a time delay of 1 week or more between the crime and the sampling of hair from the suspect will invalidate the results for forensic science comparison of hair.

Test Experiments

Before introducing this method in casework, we made a number of tests designed to imitate the typical situation of a low number of suspects. In each of these tests, three

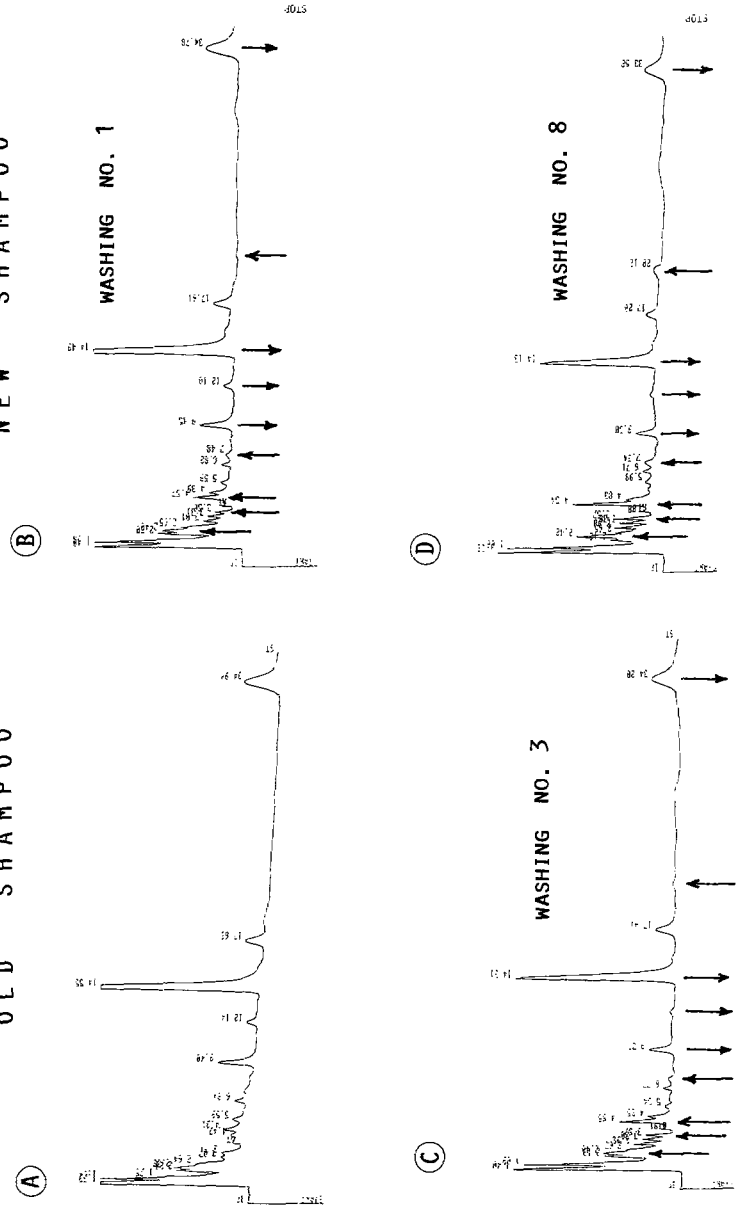

FIG. 4—*The effect of change of shampoo. This person stopped washing the hair with one brand of shampoo and started using another shampoo:* A—*last washing with the first brand of shampoo;* B—*first washing with the new shampoo;* C—*third washing with the new shampoo; and* D—*eighth washing with the new shampoo. The arrows indicate chromatographic peaks that increase or decrease after the change of shampoo. These peaks represent main components in the new and the old shampoo, respectively. The analytical conditions were as in Fig. 1.*

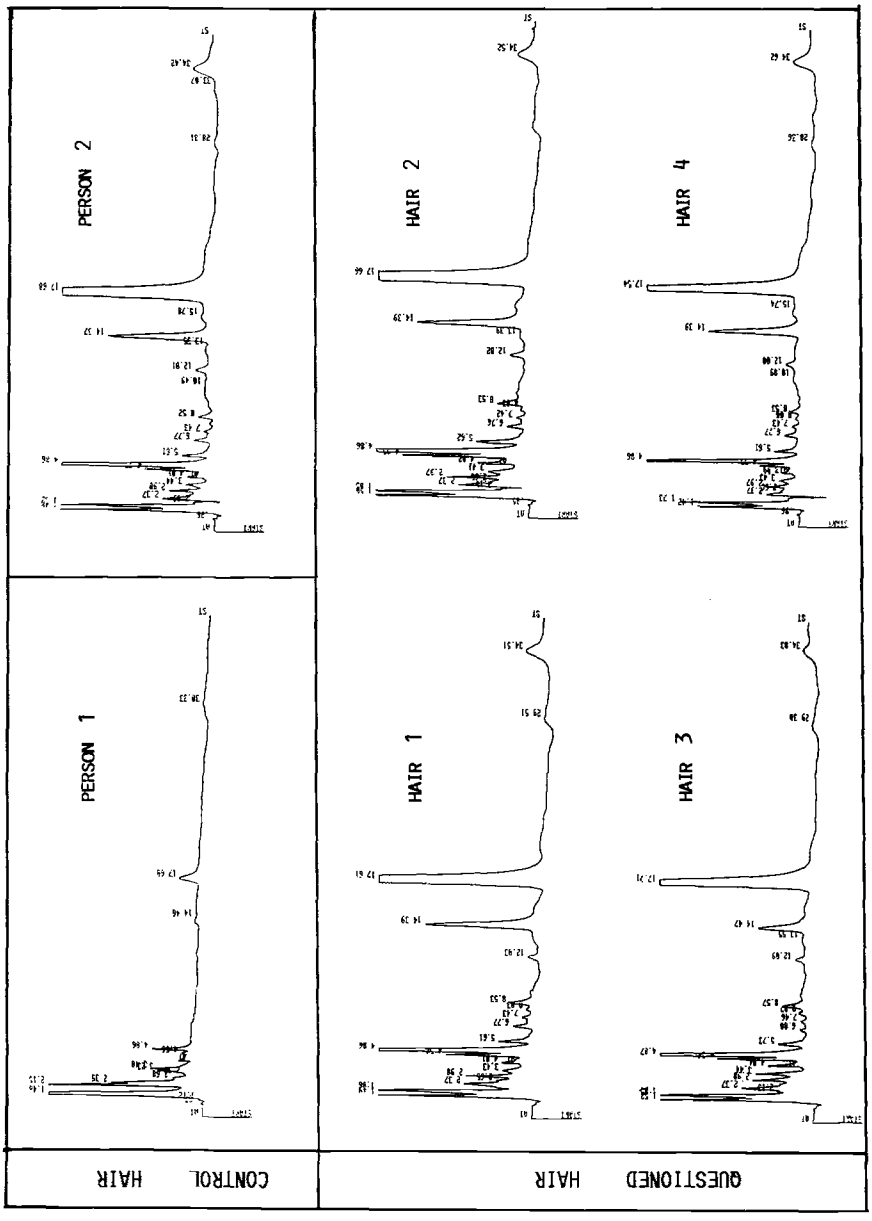

FIG. 5—*Comparison of profiles from four wrenched hairs (Hairs 1 through 4) found at the place of an assault with those from the hair of two persons (Persons 1 and 2). The analytical conditions were as in Fig. 1.*

individuals were chosen as "suspects." From each individual, three hairs were taken from different parts of the head as control samples. An extra single hair was taken from one individual as the "questioned" sample. The samples were given to one of the authors (J. A.), with the objective of excluding two individuals and thus linking the questioned hair to the third one. No morphological examination of the hair samples was performed, and the three individuals were chosen so that their hair color matched.

In total, five such tests were performed independently and correct results were obtained in all five tests.

Casework

Normally, we know nothing about the hair-washing habits of the suspect individual—the frequency of hair washing or the kinds of shampoo, balsam, or other hair preparations he or she uses. Since the profiles may be changed by changing the brand of shampoo for hair washing, the time between the crime and the collection of hair samples from the suspect should be short. These limitations do, of course, not apply to examination of hair from dead persons.

To examine whether the suspect exhibits profile variation within the scalp, three hairs from different parts of the head should be analyzed as control samples.

An extract from hairs of about 10 cm is normally sufficient for one analysis. Hairs shorter than 5 cm usually give few detectable peaks in the resulting chromatograms but may still be useful for investigation.

Figure 5 illustrates the results obtained in one of our first cases. Four wrenched hairs (Hairs 1 through 4) found at the place of an assault were submitted to our laboratory for analysis. Control hair samples from two individuals (Person 1 and Person 2) were also submitted. The police wondered if the wrenched hairs originated from the head of Person 1 or Person 2, or even from some other person. The results excluded Person 1 for good reasons and indicated that the questioned hairs came from Person 2. Figure 5 shows the chromatograms obtained at 205 nm, but similar agreement was observed at 254 nm, recorded simultaneously. Only one of three analyses of hair samples from Person 1 and Person 2 are shown since the profile variation within the scalps was negligable.

It is obvious that this method cannot individualize human head hair. In cases of agreement, the results would indicate that the questioned hair originated from the suspect. However, strong conclusions can be drawn from clear disagreements, provided the scalp variation is small and the sampling is done shortly after the event. Exclusion of one individual may be of great importance in the numerous cases in which the number of possible alternatives is low. Thus, although it has limitations, shampoo residue profiling seems to be a promising method for forensic science examination of human head hair.

Acknowledgment

The authors are grateful to Dr. L. Strömberg for helpful discussions and comments on the manuscript.

References

[*1*] Roe, G. M., "The Detection of Cosmetic Treatments on Hair," *Cosmetics and Toiletries,* Vol. 95, 1980, pp. 40–44.

[*2*] Andrasko, J. and Lundgren, B., "Forensic Examination of Permanent-Waved Hair," *Proceedings,* Second Scandinavian Conference on Forensic Science, Linköping, Sweden, August 1983, pp. 81–86.

[*3*] Andrasko, J., "Calcium Profiles in Permanent-Waved Hair and Its Application to Forensic Science," *Proceedings,* Tenth International Meeting of the Forensic Science Society, Oxford, England, October 1984.

[4] Reng, A. K., "Rezeptierung von Haarreinigungs-und nachbehandlungspräparaten mit speziellen Eigenschaften," *Seifen-Öle-Fette-Wachse,* Vol. 103, No. 9, 1977, pp. 215–217, 243–248, and 307–308.
[5] Fiedler, H. P., "Chemie der kosmetischen Mittel," *Deutsche Lebensmittel-Rundschau,* Vol. 74, No. 4, 1976, pp. 113–119.
[6] Roehl, E. L., "Shampoos—Eine Übersicht," *Seifen-Öle-Fette-Wachse,* Vol. 104, No. 15, 1978, pp. 424–427 and 485–488.
[7] König, H. and Walldorf, E., "Zur Analyse von Haut- und Haarwaschmitteln auf Basis synthetischer Tenside," *Frezenius Zeitschrift für Analytische Chemie,* Vol. 299, No. 1, 1979, pp. 1–18.
[8] Andrasko, J. and Stocklassa, B., "HPLC Analysis of Shampoo Residues in Human Head Hair," *Proceedings,* Eleventh International Meeting of the Forensic Science Society, Vancouver, Canada, August 1987.

Address requests for reprints or additional information to
J. Andrasko, Ph.D.
The National Laboratory of Forensic Science
S-581 01 Linköping
Sweden