## "Evidence That Never Lies" Analytical Chemistry Applied to Human Hair

John V. Goodpaster, Ph.D.\*, Byron C. Drumheller, and Bruce A. Benner, Jr., Ph.D.

Analytical Chemistry Division
National Institute of Standards and Technology
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

Currently, methods for the forensic analysis of human hair rely upon microscopic examination or DNA comparisons. Chemical techniques, on the other hand, have only been used for extracting and identifying drugs of abuse that may be present in hair. Recent work in our laboratory has shown that naturally-occurring organic material on the hair's surface may yield valuable information to forensic scientists. Furthermore, the complex mixture of lipids present on scalp hair is easily accessible using conventional extraction techniques and solvents, readily analyzed using gas chromatography/mass spectrometry (GC/MS), and provides information that could be used for either forensic identifications or comparisons.

In this work, various extraction methods such as ultrasound, Soxhlet, pressurized solvent, and supercritical fluid extraction have been evaluated for their efficiency and selectivity. Using these procedures, the organic fraction of hair samples was isolated in methanol, acetone, dichloromethane, hexane, and supercritical carbon dioxide. In general, yields for all techniques decreased as solvent polarity decreased. In addition, yields for the various extraction techniques varied (i.e., from 0.9-1.8% (w/w) for ultrasonic extraction, 1.1-4.1% for Soxhlet extraction, and 2.5-3.6% for pressurized solvent extraction). Of the liquid solvent extraction techniques, pressurized fluid extraction combined high yields, convenience, and sample sizes as small as 50 mg. However, the most successful method to date is on-line supercritical fluid extraction where unmodified carbon dioxide is used in static and dynamic modes. The pressure, temperature, and time of extraction of this procedure have been optimized in order to maximize efficiency and hence sensitivity. As a result, samples as small as ~150 µg (representing approximately a 2 cm strand of hair) have been successfully analyzed. In this method, a restrictor is used to deposit the solutes as a narrow band on the stationary phase of the chromatographic column. Subsequent analysis time is on the order of 60 minutes.

Characterization of the liquid solvent and supercritical carbon dioxide extracts from various hair samples has reveled a complex mixture of components ranging in carbon number from  $C_{12}$  to  $C_{36}$ . This surface material largely consists of sebaceous excretions such as fatty alcohols, acids, esters, and other lipids including squalene and cholesterol. In some cases, anthropogenic material from consumer products such as shampoos or sunscreens was also found. Analysis of head hair from 20 subjects varying in age and gender has shown that the chromatograms obtained from human hair are suitably complex to provide some degree of individualization to a particular sample. Furthermore, there appears to be systematically higher levels of cholesterol in pre-pubescent versus post-pubescent individuals. Literature data suggests that further systematic differences in lipid composition according to gender and/or race may exist that would further increase the evidentiary value of chemical analysis. Finally, the chromatographic profiles obtained using this technique have proven to be stable under normal storage conditions and are reproducible for a single scalp location on an individual.

Future work will include evaluating other extraction techniques such as thermal desorption of the lipids from the hair surface. Plans are also underway to correlate the GC/MS results with demographic data from subjects such as age, race, and gender. An expanded sampling study is also needed to determine the amount of variation in the chromatographic profile of a single individual over time and scalp location. Lastly, chemometric pattern recognition algorithms will be used to quantitatively compare chromatographic profiles.