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Pyrolysis-Capillary Gas Chromatography
of Human Hair for Forensic Application

presented by

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Jay A. Siegel
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Pyrolysis-Capillary Gas Chromatography of Human Hair for Forensic Application

By

Cynthia Jean Schutt-Aronson

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Abstract

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Human Hair for Forensic Application

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Human head and pubic hair from 27 different donors is examined using pyrolysis-capillary gas chromatography (Py-GC^2). Differences in the Py-GC^2 chromatograms are shown to exist between hair of different persons. However, significant variances in the Py-GC^2 peaks from hair of the same person are also observed. Possible forensic applications of the Py-GC^2 technique are discussed. Included is a review of the biological development and function of hair structural components.

Dedication

to Carl Lawrence

My Loving Husband,

Who Makes Each Day Interesting

Acknowledgments

I would like to thank my advisor, Professor Dr. Jay A. Siegel, for helping to define this project and encouraging me to continue working when I felt disheartened. I am also grateful for the opportunity to be a forensic science teaching assistant under Dr. Siegel. This experience provided much of the my initial excitement about the field. I wish to thank Dr. Laurence R. Simson and Richard Slocum of Sparrow Hospital, Lansing, MI for providing many of my hair samples.

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1. Introduction

Forensic Science is the application of science to compare, analyze, or identify matter for presentation in the courts of law (1). As the crime rate increases and the technology used in committing crimes becomes more advanced, there is a greater need for science in the court system (2). Advances in science and technology are already being utilized by many criminals. Therefore science and technology must also be used in the forensic science to analyze physical evidence in the criminal justice system (3). It is now common for the courts and law enforcement officers to seek the services of the criminalistics laboratory whenever a question concerning physical evidence arises. No longer is the scene of a crime viewed as a base for questioning witnesses. The scene of a crime is now considered as a field laboratory site where traces of physical evidence can be discovered and used as the basis of vital testimony at the trial (4).

Evidence is defined as anything admissible in court that will aid a criminal proceeding in establishing guilt or innocence of a person (5). It is important that law enforcement officers, court officials, and expert witnesses understand the equal importance of establishing innocence as well as guilt. Evidence can be the testimony of a person or the presence of an inanimate object. The testimony of an individual is only as credible as the memory of that person and the credibility of the testifying witness. Inanimate objects are a purer form of testimony of what happened because objects do not have the fallibility of humanity (i.e. loss of memory, perjury, ulterior motives) (3). This type of inanimate object evidence is known as physical or real evidence. The usual objectives of the scientific examination of physical evidence is to determine the manner in which a crime was committed, to connect a suspect with a crime, or to aid in establishing the identity of the criminal (4). However, the activities of the criminalistics lab are not solely confined to

these objectives. Not all objects found at a scene of crime or involved in a criminal act should be considered physical evidence. In most cases the criminalistics laboratory determines if an object has value. The value of an object is derived from its ability to either help prove a case or clear a suspect (1). Sometimes a piece of physical evidence may be so conclusive that it will sustain a conviction standing alone. However, at other times the summation of many articles of physical evidence is required for a case to be proven in court (1).

Some forensic evidence may serve as invaluable and concrete testimony for establishing if a crime was committed and how a crime was committed. In addition, this evidence can also serve to identify participants involved in the criminal act. There are not always eyewitnesses to a crime and even when they exist they have been known to conflict. In some cases physical evidence can function as the only tangible evidence of the commission of a crime. Science occupies an important and unique role in the criminal justice system. The scientist has the ability to supply accurate and objective information that reflects the events that have occurred at a crime (2). Human hair is an important type of physical evidence. Pubic hair is found and used as evidence in many sexual assault cases. Crimes of violence such as murder often involve hair evidence produced through struggle. Every crime scene in which a person was present has a potential of containing hair evidence since hair continually falls from the body of each individual. Contact between persons also allows for transfer of hair to clothing where it is not readily lost.

The current method used in forensic hair examinations is morphological comparisons. For additional information on hair morphology the reader can refer to the appendix of this thesis. Using optical microscopy, the forensic examiner compares the structural characteristics of the hairs from known and unknown origin. Standard morphological characteristics are used to compare two different hairs. However, it should also be noted that the morphology of hair from one individual can also vary greatly. Based on

experience, the hair examiner determines whether the unknown hair specimens fall within the morphological variations of the hair of known origin. The examiner then gives an unbiased opinion based on his/her examination whether the hair of unknown origin could or could not have come from the suspect. Hair is class evidence and can not be individualized or determined to come from a specific person by the current methodologies (i.e. morphological analysis). Analytical techniques that can be used in addition to microscopic morphological examinations have the potential of providing more quantitative and concrete data. These additional data could also provide an additional perspective and allow the examiner to give a more detailed opinion.

Research is currently underway using scanning electron microscopy (SEM), pyrolysis-capillary gas chromatographic (Py-GC^2), fourier transfer (FT-IR), and infrared (IR) spectroscopic analysis on different hair types. The different hair types found on the human body are known to possess different morphological characteristics. Pubic hair has a coarse diameter with significant diameter variation, a broad medulla, and an asymmetrical cross section. In comparison head hair can be much longer, with only slight variations in diameter, round to flat cross sections and cut tips.

The purpose of this research was two fold. First, to investigate the ability of distinguishing head and pubic hair from different individuals using Py-GC^2 techniques. And second, to determine if structural differences between pubic and head hair are reflected in the chemical composition of the protein chains. Differences in amino acid distribution between hairs might be detected by gas chromatographic separation.

Analysis of Hair

Several authors have examined the chemical content of hair specimens to determine if consistent differences exist between individual persons. Optical microscopy has been used

to analyze a host of different hair characteristics including color, density, shape of pigment granules, shape of root bulb, medulla characteristics, diameter of the hair shaft and diameter of the medulla to name a few (6) These characteristics are helpful in forensic identification of a hair. However this method of analysis is very time consuming, and is only valuable if performed by an experienced person who handles many cases a year. Because optical microscopical analysis is subjective and based only on the analyst's expertise, any disputes between forensic scientists can confuse a jury and depreciate or nullify the hair evidence. It is well known that hair specimens cannot be individualized or identified with enough assurance as to assign the sample to a certain person at this date. However the chemical content and composition of hair contains a wealth of information which could aid forensic scientists in eliminating possible sources of a hair sample. Also information concerning the chemical content and composition of different hair samples would provide more objective data to help courtroom participants understand the conclusions of hair experts. However due to the general stability of hair, it is necessary to degradate a hair sample and examine the pyrolyzate in order to get chemical composition information.

Pyrolysis

Pyrolysis is an extremely useful sample preparation technique which can expand the use of other instruments when working with thermally stable solids. Pyrolysis is a fairly flexible technique. The technique can be used on varying amounts of sample as well as diverse solids such as films, hairs, powders, pellets, fibers, and paint chips (7). The pyrolysis technique uses thermal energy to break a molecule's chemical bonds. The sample is broken into thermally stable fragments. The structure and size of the fragments is based on the relative strengths of the constituent chemical bonds. Therefore these fragments

reveal information about the atomic arrangement of the original molecule. During the pyrolysis of a substance, chemical bonds dissociate and free radicals are produced (8). The free radicals initially produced react together to form stable covalently bonded structures. Therefore the stability of the initial free radicals produced and the final product molecules determine the composition of the pyrolyzate (8). The original molecular bonds must be broken apart by pyrolysis at the same atoms in identical experiments to get acceptable reproducibility. Therefore, the same amount of thermal energy must be introduced to samples in repetitive experiments. This can be achieved by controlling the heating rate and final temperature.

There are three types of pyrolysis devices: the resistively-heated, the inductively-heated and the microfurnace. The Pyroprobe is a self-sensing, resistively heated pyrolysis filament device. An electrical current passes through a metal filament to produce the needed heat. The use of the electrical current provides a facile way to control the heating rate and maximum final temperature (9). The metal filament is either a wire, ribbon or foil made of platinum metal. The Curie Point pyrolyzer is an inductively heated device. The sample wire is heated by an electromagnetic field. The curie point of the metal alloy used to make the wire determines the temperature of pyrolysis. Usually alloys of iron, nickel and cobalt are used for the Curie Point wire. The relative percent of these metals in the alloy specifies the curie point temperature. A limitation of the Curie Point pyrolyzer is that the device can be used to heat only at its Curie Point temperature determined by the composition of the wire (9). The microfurnace, although much less prevalent, has been used to produce accurate data for pyrolysis of pure polymers. The microfurnace is a tubular furnace maintained at a constant set-point temperature. Small samples of polymeric materials are dropped into the furnace tube. The sample is pyrolyzed while it falls through the tube. The volatiles that are produced are normally analyzed by gas-chromatography (9).

Pyrolysis is a powerful sample preparation technique due to the ease in which it can be interfaced to other chemical characterization instruments such as gas chromatographs, mass spectrometers and FT-IR spectrometers. As a result, the use of these instruments can be expanded to include chemical characterization of thermally resistant solids, opaque and multi-component materials (8).

Pyrolysis-Capillary Gas Chromatographic Analysis of Hair

Keratin, the major component of cortical cells formed after disulfide crosslinking (see appendix), is a hard protein which is extremely resistant to solvent or enzymatic attack. Due to the stability of hair including heat resistance, the hair must be degraded in order to obtain quantitative chemical composition data. Keratin can be solubilized by strong acid or strong base and isolated by subsequent extraction. However, chemical degradation of the protein chains by such caustic solvents will occur. Chemical degradation has been used to analyze hair. Limited information including the location of keratinization zones has been obtained (10). Researchers have also used redox techniques to solubilize keratin.

Thus, thermal analysis has been the major investigative pathway used to quantitatively analyze the chemical components of hair. Pyrolysis or complete degradation of the protein by flash has been used by several research teams. Munson and Vick (11) initially used pyrolysis (Py) followed by capillary gas chromatography (GC^2) on human head hair. Initial experiments using different segments along the hair shaft resulted in only minor chromatogram differences. Experimentation thereafter involved the entire hair. The gas chromatogram showed peaks which corresponded to 185 chemical components which were also quantitated (11). These authors found that a few major components differed among hair of different individuals.

Munson and Vick (11) further analyzed the amino acid composition of hair samples by pyrolysis-capillary gas chromotographic separation followed by mass spectrometric (MS) analysis. Using Py-GC^2-MS techniques Munson and Vick (11) observed several components in the mass spectrum that varied in hair from different individuals. The differences among individuals in Py-GC^2-MS paralleled the findings from Py-GC^2. Six major components in the mass spectrum were either present or absent in different combinations in hair from different persons. While the varying components were not all identifiable chemically, a search of mass spectrum libraries yielded the proposed pyrolysis products of some of the MS peaks. These components were identified as a variety of residual organic molecules (toluene, styrene, phenol, cresol and more) resulting from bond cleavage and amino acid condensation upon pyrolysis.

Munson and Feterolf (12) used Py-GC^2-MS techniques to analyze head hair samples from different individuals. Some of the peaks found were small molecular weight aromatic organic compounds easily identified as products from the cleavage of amino acids. To determine the compounds of other spectrometric peaks, Munson and Feterolf (12) used electron impact and chemical ionization collisionally activated dissociation experiments. These experiments provided insight into a possible degradation mechanism. The authors then used pyrolysis-gas chromatography-tandem mass spectrometry (Py-GC-MS-MS) which provided data in support of their degradation mechanism. Both 5-substituted 2,4-imazoleidinediones as well as 5-substituted pyrolidino[1,2a]-3,6-piperazinediones were found to form during anaerobic human hair pyrolysis. Munson and Feterolf (12) believe that there may be genetically linked differences in the amino acid sequence of hair protein. They proposed that pyrolysis fragments can provide portions of the amino acid sequence that will show these genetic differences (12). However their current data showed that the 5-substituted 2,4-imazoleidinediones and 5-substituted pyrolidino[1,2a]-3,6-piperazinediones occurred in similar amounts for the five donors. Because these

compounds are derived from amino acid sequences less than three units long, it was not expected that these compounds would show measurable differences between head hair from different sources. Munson and Feterolf (12) plan to use Py-MS and Py-MS-MS to examine hair pyrolyzate of larger amino acid sequence fragments.

Ishizawa and Misawa (13) have also investigated the analysis of head hair by Py-GC^2-MS methods. These researchers used head hair from 18 different donors. Several of the components in the gas chromatograms were observed to vary between individuals. These components were similar to the aromatic compounds (benzene, toluene, styrene, etc.) identified by Munson and Fetterolf (12). In order to establish the origin of these aromatic compounds, Ishizawa and Misawa (13) performed Py-GC^2-MS experiments on 18 amino acids. Pyrolysis of the phenylalanine amino acid is known to yield aromatic compounds. However benzene, toluene and styrene were also obtained after pyrolysis of non-aromatic containing amino acids such as valine and leucine. Ishizawa and Misawa (13) attribute this to possible condensation cyclization of one or more amino acids. These authors state that the differences in amounts of toluene, styrene, phenol, cresol, etc. were due to different amounts of particular amino acids in the hair of different individuals. When the hair of an adult and a child were tested 18 months later, the peak ratios in gas chromatograms of benzene, toluene and styrene did not change although the values were consistently lower for the child (13). It is not known whether the composition of amino acids in the hair changes with aging.

2. Experimental

Hair Preparation

Human hair samples from the head and pubic area of twenty-seven persons were used. Eleven of these sets of pubic and head hair were obtained from postmortem donors aged 47 to 82 years. The origin and characteristics of all sets of donated hair are included in Table 1. Both head and pubic hairs were removed forcibly from postmortem individuals, and kept at room temperature in plastic bags until cleaned. Living donors between the ages of 20 and 25 years donated the remaining sixteen hair sample sets. The head hair from these samples were obtained by combings and the pubic hair by cutting hairs close to the skin surface. The hairs were stored in separate white envelopes.

The cleaning process involved washing each sample 3 times in a mixture of ethyl ether-ethanol (1:1 vol/vol) solvents. Ethyl alcohol was spectro (USI Division of Quantum Chemical Corporation). The ethyl ether used was ACS anhydrous from Columbus Chemical Industries Inc. The samples were then air dried. Clean forceps were used to place the dried hair samples in the bottom of 10 mm x 75 mm glass tubes taking care to avoid contact with the plastic caps.

For the final preparation of a sample, the hair was removed from a glass tube and placed on a clean surface and cut using a razor blade, into 12 to 25 pieces of approximately 6 mm in length. Root bulbs were cut off hairs and discarded. A representative sample of the hair segments was inserted into a quartz tube which had been previously cleaned with Manostat chromerge. The quartz tubes were previously cleaned by immersing them in chromerge for 24 hours, then thoroughly rinsed in deionized water, and finally dried for 24 hours in a 60^0C oven. After the hair samples were inserted into the clean quartz tube, both ends were plugged with silanized glass wool. The total hair sample mass used in each analysis ranged from 15 to 25 μg. The hair samples were stored in the quartz tubes until used for pyrolysis.

Table 1. Sources of Hair

Living Donors				Postmortem			
Number	Sex	Race	Age	Number	Sex	Race	Age
001	Male	Cauc.*	24	003	Male	Cauc.	68
002	Female	Cauc.	25	004	Male	Cauc.	70
014	Male	Cauc.	22	005	Male	Negr.**	66
015	Male	Cauc.	25	006	Male	Cauc.	72
016	Male	Cauc.	22	007	Female	Cauc.	80
017	Male	Cauc.	21	008	Male	Cauc.	60
018	Female	Cauc.	21	009	Male	Cauc.	50
019	Male	Cauc.	21	010	Female	Cauc.	82
020	Male	Cauc.	20	011	Male	Cauc.	47
021	Female	Cauc.	20	012	Female	Cauc.	72
022	Male	Cauc.	22	013	Male	Negr.	69
023	Female	Cauc.	21				
024	Female	Cauc.	22				
025	Male	Cauc.	21				
026	Female	Cauc.	21				
027	Female	Cauc.	22				

Key: * = Caucasian
** = Negroid

Although sex, race and age characteristics are listed for the samples shown here, trends in these traits were not analyzed. There was not a large enough distribution of each individual characteristic in order to draw conclusions. For example almost all persons were caucasian.

Pyrolysis-Capillary Gas Chromatography

Pyrolysis was carried out using a CDS pyroprobe model 100 (CDS, Oxford, PA, USA). The probe was inserted directly into the injection port of the gas chromatograph (Varian, Model 3300, Walnut Creek, CA, USA) equipped with an FID detector. The pyrolysis and GC conditions were as follows:

Pyrolysis conditions:

Approximately 1000°C for 20 seconds. Interface and ramp were off.

GC conditions:

Injector temperature: 200°C

Detector temperature: 250°C

Temperature program: 60°C for 5 minutes, then 10°C per minute to 250°C and then held for 5 minutes.

Column: DB-1 (J & W Scientific, Folsom, CA) 30 M in length and 25 μm diameter.

Split ratio: 100 to 1

Carrier gas: hydrogen at 40 psi

Chromatographic data was processed using an Area Manager Chromatography Integration System, (The Epsilon Company, Round Rock, Tx, USA) version 2.20 and a Compuadd Model 325 personal computer.

3. Results and Discussion

Table 2. Py-GC[2] Chromatograms of Human Hair

Head Hair			Pubic Hair		
Head 007	Run 01	Figure 6	Pubic 007	Run 01	Figure 19
Head 008	Run 01	Figure 7	Pubic 008	Run 01	Figure 20
Head 009	Run 01	Figure 8	Pubic 009	Run 01	Figure 21
Head 010	Run 01	Figure 9	Pubic 011	Run 01	Figure 22
Head 011	Run 02	Figure 10	Pubic 012	Run 01	Figure 23
Head 012	Run 02	Figure 11	Pubic 016	Run 01	Figure 24
Head 012	Run 01	Figure 12	Pubic 017	Run 01	Figure 25
Head 013	Run 01	Figure 13	Pubic 018	Run 01	Figure 26
Controls					
Head Hair			Pubic Hair		
Head 002	Run 02	Figure 1	Pubic 002	Run 02	Figure 14
Head 002	Run 03	Figure 2	Pubic 002	Run 03	Figure 15
Head 002	Run 04	Figure 3	Pubic 002	Run 04	Figure 16
Head 002	Run 05	Figure 4	Pubic 002	Run 05	Figure 17
Head 002	Run 06	Figure 5	Pubic 002	Run 06	Figure 18

Head Hair Controls

Due to experimental variations in the Py-GC[2], the only chromatograms that could be compared to each other were hairs run on the same day. The sensitivity of both the pyrolysis process and of capillary-gas chromatography resulted in daily fluctuations ultimately producing changes in the chromatograms. Figures 11 and 12, which are chromatograms of the same hair run on different days, illustrate the typical variation between runs of the same hair on different days. Absolute percent areas provided by the chromatography integrator could not be used. Integration values of the labelled peaks were consistently small due to a large unused peak in the beginning of each chromatograph. In addition, so many values were shown for each peak, shoulder, and even rising baseline it was impossible to differentiate which integration values corresponded to the labelled peaks. In order to establish the magnitude of the baseline of variation found in multiple runs of the same hair, five samples of head hair of the same person were run on the same day. The baseline of variation for each individual peak was taken to be the difference between the peak areas for the two runs that gave the maximum and minimum values. Samples run on the same day should show chromatographic separation differences inherent in the hair samples since experimental conditions are as similar as possible. Peak variation was established through multiple runs of hair from head 002. These chromatograms served as the baseline of variation and are shown in Figures 1-5.

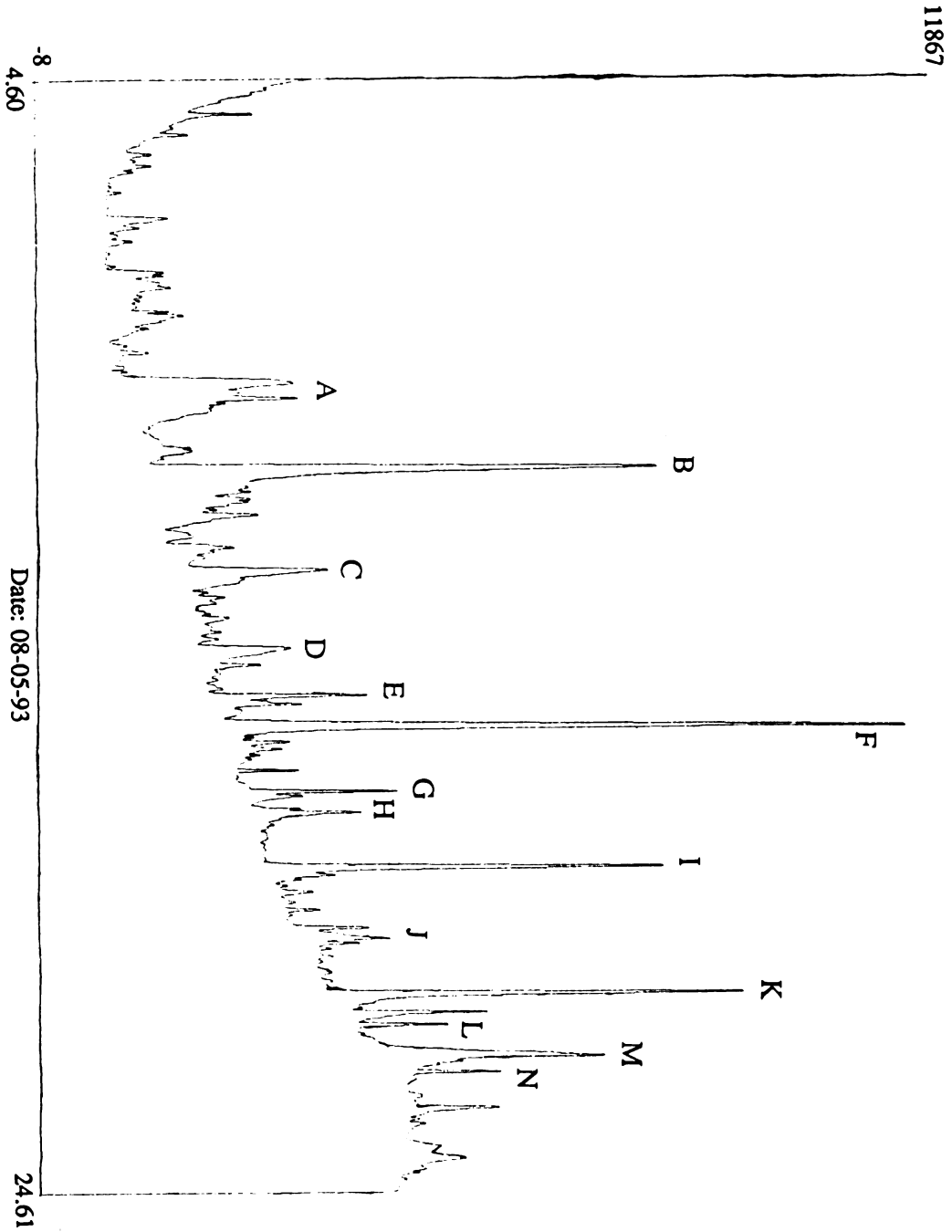

Figure 1. Py-GC^2 Chromatogram of Head Hair 002, Run 02

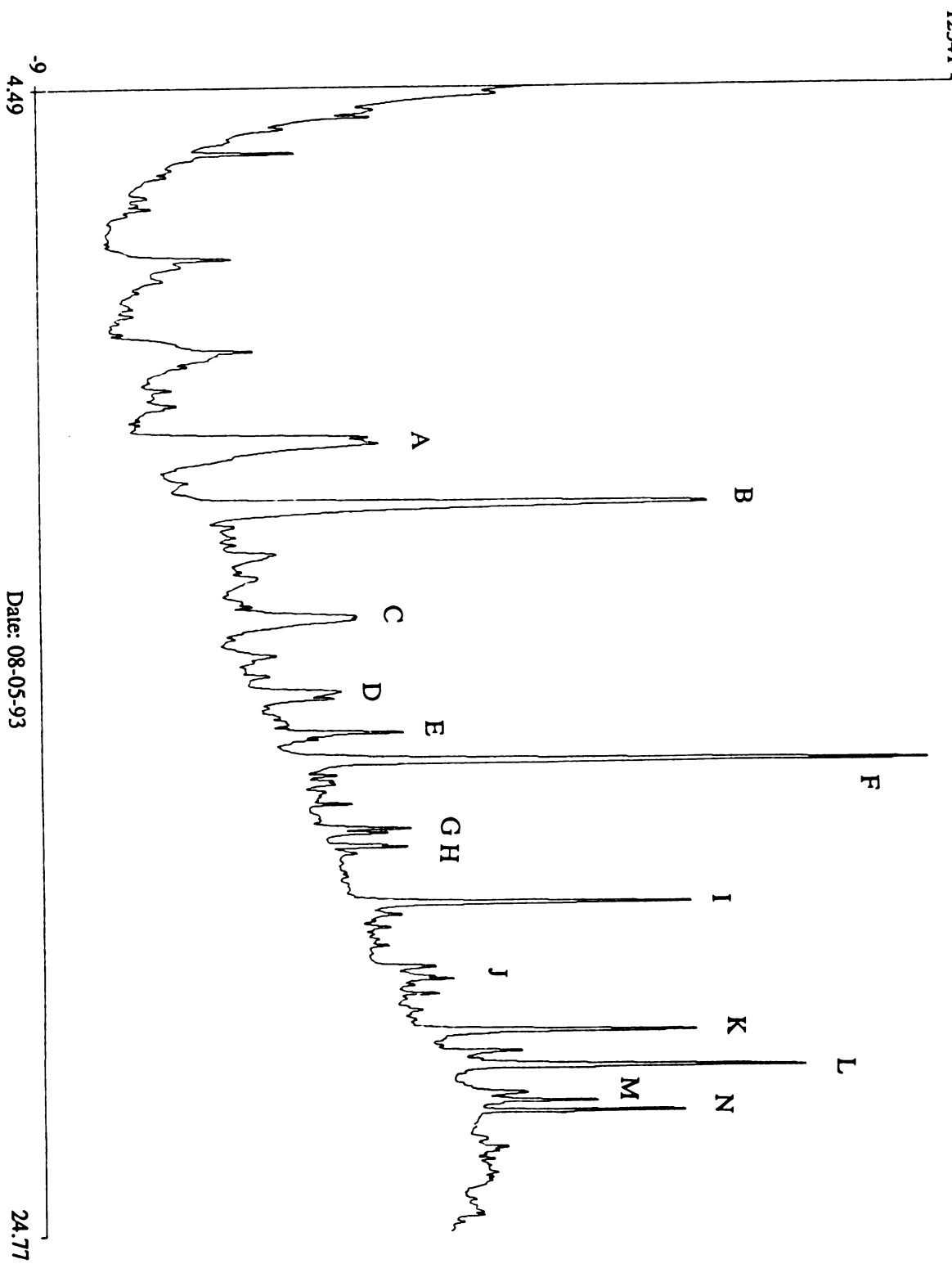

Figure 2. Py-GC^2 Chromatogram of Head Hair 002, Run 03

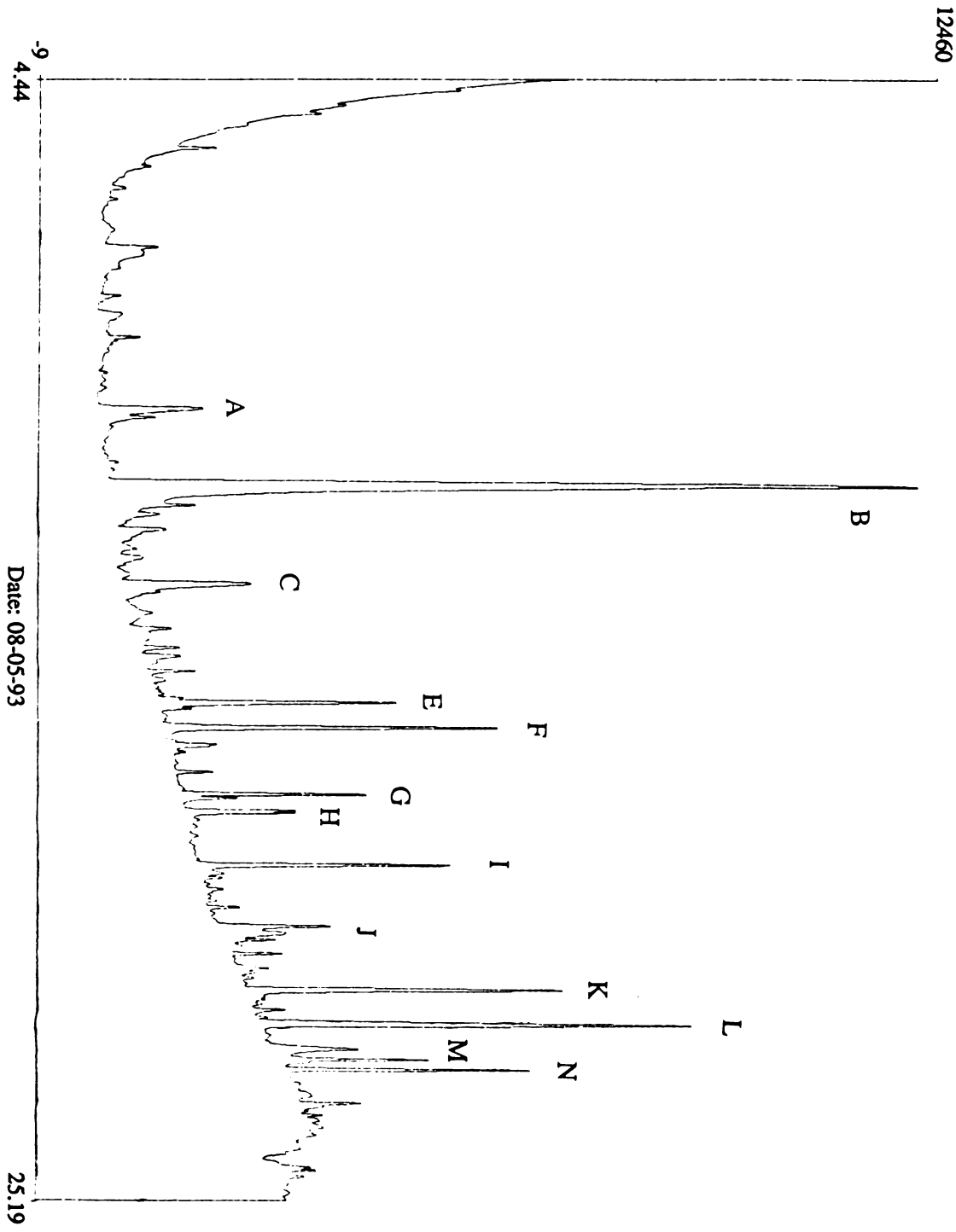

Figure 3. Py-GC^2 Chromatogram of Head Hair 002, Run 04

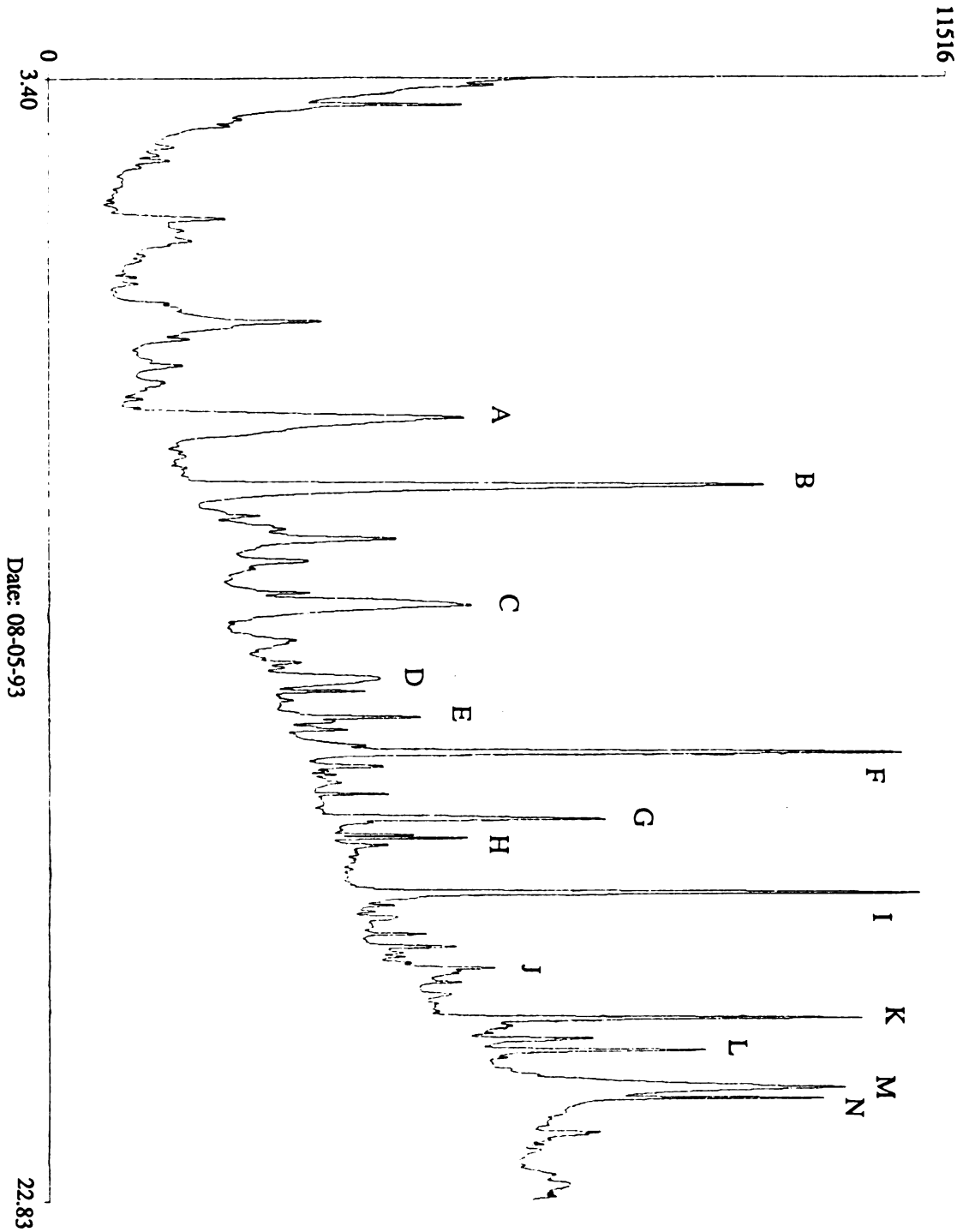

Figure 4. Py-GC^2 Chromatogram of Head Hair 002, Run 05

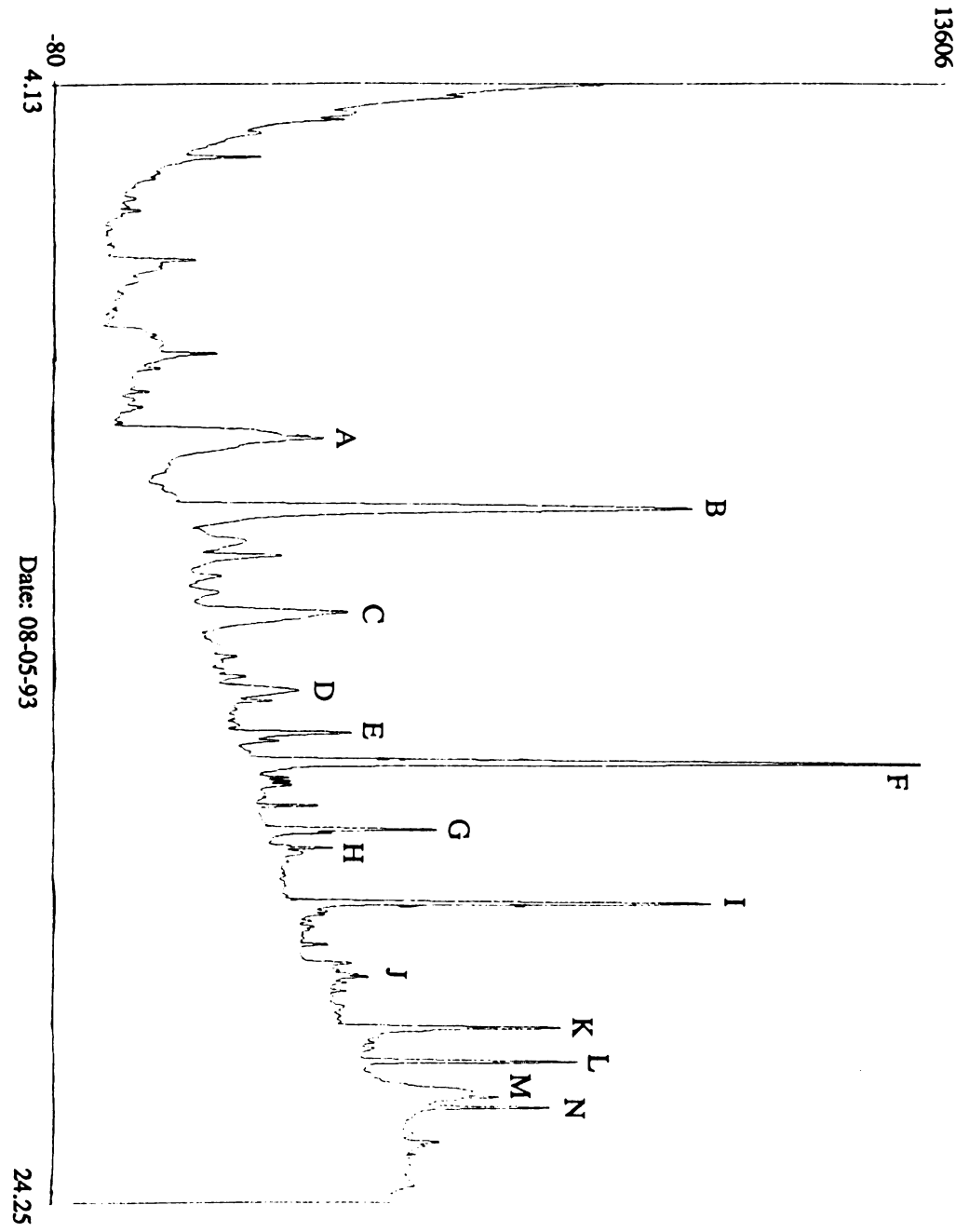

Figure 5. Py-GC^2 Chromatogram of Head Hair 002, Run 06

Head Hair of Different Individuals

Figures 6-13 contain head hair chromatograms from a representative sampling of different individuals. Chromatograms run on the same day were compared to find differences in peak height or peak area for a particular peak. Some of the largest peaks were identified by letter to aid in comparison. Peaks in different chromatograms labelled with the same letter are peaks corresponding to nearly the same retention time. Any variation found between corresponding peaks in Figures 6-13 was compared to the baseline of variation from the control head hair in Figures 1-5. Peaks that had more variation in area between the hair of different individuals than the baseline control runs were considered to possibly contain identifiable traits of head hair. On the contrary if a peak showed more variation in the control chromatograms (Figures 1-5) than in the head chromatograms of different people, variation was considered not significant and was probably the result of uncontrolable experimental conditions. Variation beyond the baseline could also be attributed to the hair sample itself.

Due to consistent differences in chromatography output intensity, an individual peak area was often normalized with respect to another peak on the same chromatogram. This allowed comparison of relative differences between different hair. In the chromatograms of head hair it was found that control runs of head hair 002 produced a large baseline variance for peaks A and B with respect to each other. The area of peaks A and B varied more in the control runs than in the hair from different individuals. In the control runs of head hair 002 variation was largely due to severe changes in the area of peak A as opposed to peak B. There was also more variation in the control runs for peaks E and F than in chromatograms of different individuals. Peaks E and F were normalized for this comparison. There was more height and area variation in peak D within runs of the control group (Figures 1-5) than in chromatogram peaks from head hair of different people (Figures 6-13). Although it

was difficult to resolve the peaks at higher retention times due to a rising baseline, there were indeed differences in the relative peak heights of K and L in Figures 6-13. However, the variation for peaks K and L did not surpass the baseline of variation. Little variation in peak height was found when comparing peaks C, H, and I on different chromatograms. It was difficult to study peaks M and N, which were often cut off the chromatograms because of the steep rise in baseline toward the end of the run time.

There were a few peaks that significantly varied between individuals compared to the control chromatograms of head hair 002. In Figure 8, peak G had a greater peak height than the corresponding peak in Figure 9. This variation was greater than the baseline of variation for peak G. A similar increase in height for peak G was observed only in one other set of chromatograms: Figures 10 and 11. Peak J was also observed to vary significantly with respect to the baseline of variation. Figures 6-13 showed that peak J varied in height and which part of the multiplet had the greatest height or area. In the controls, peak J had a minimal height although the section of the multiplet peak with the greatest area did shift. Although this was a significant find, peak J alone does not provide enough information to identify hairs.

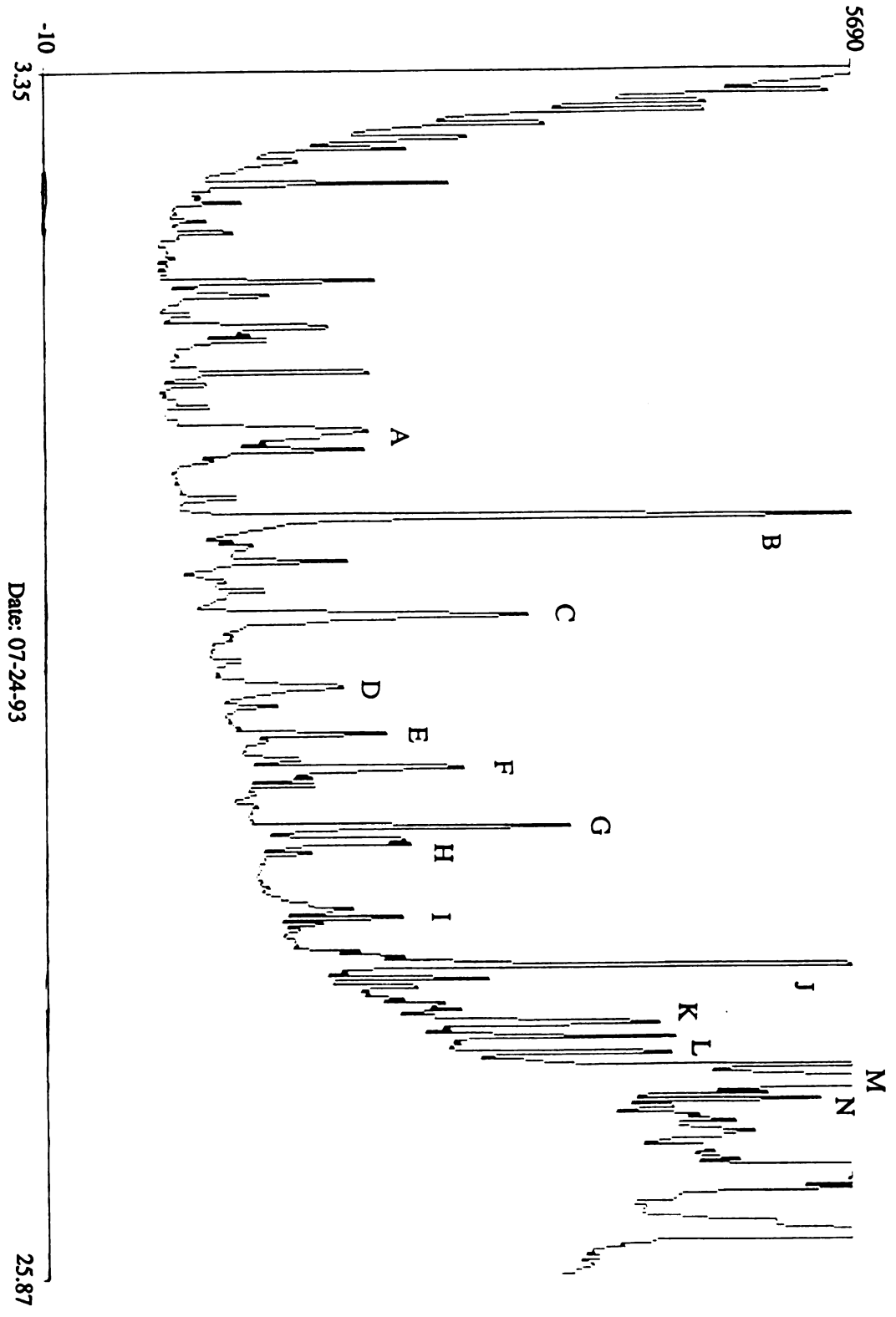

Figure 6. Py-GC^2 Chromatogram of Head Hair 007, Run 01

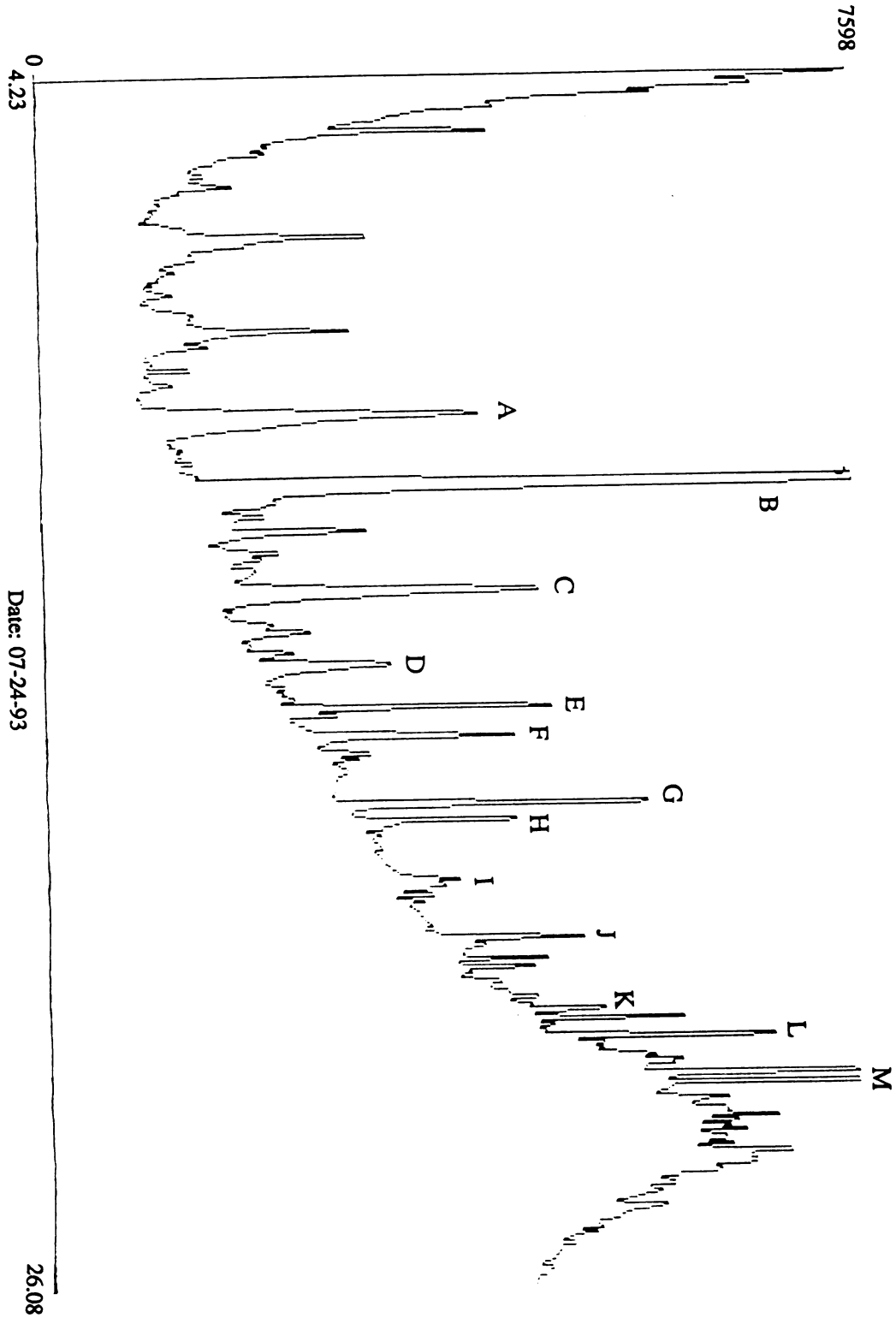

Figure 7. Py-GC^2 Chromatogram of Head Hair 008, Run 01

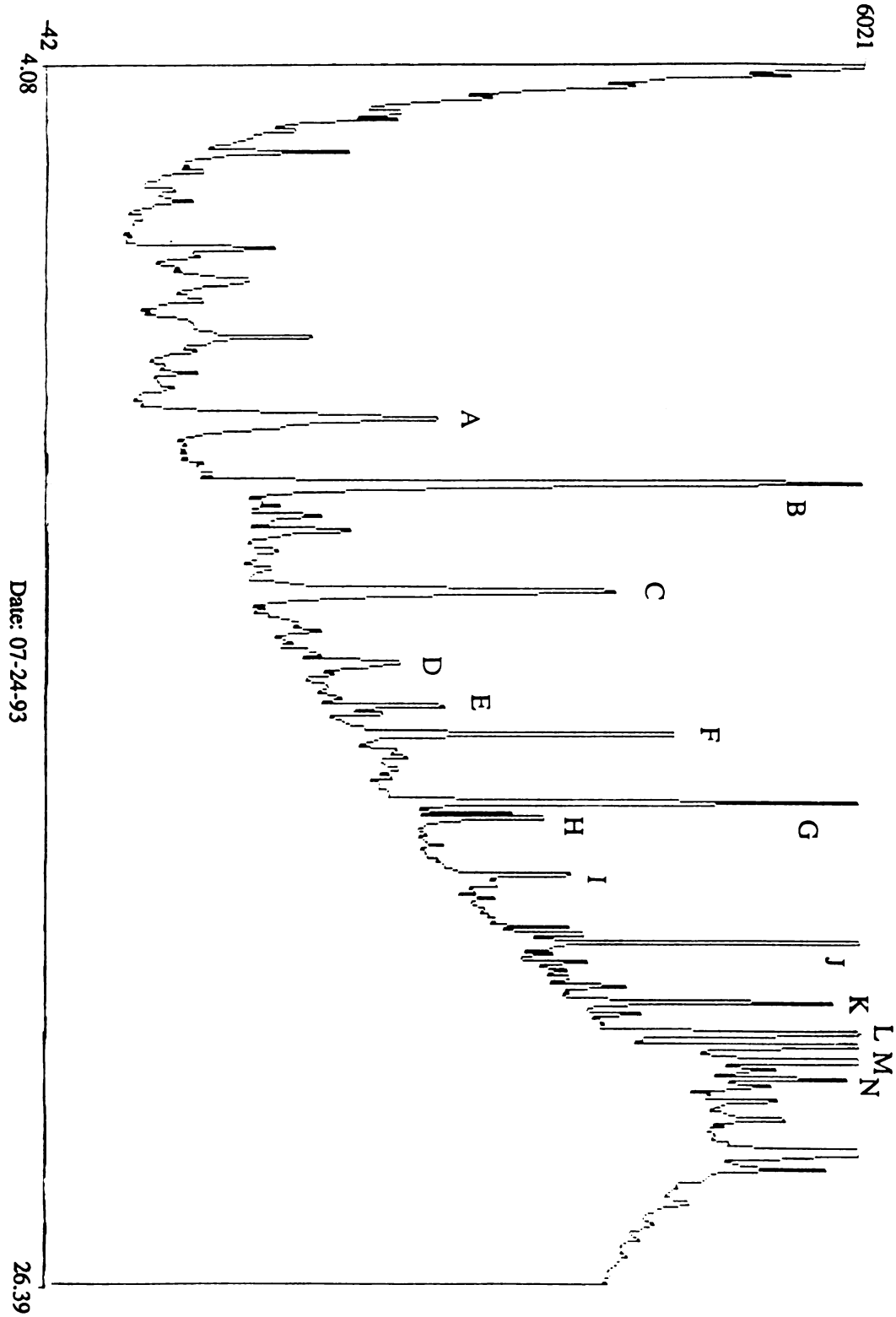

Figure 8. Py-GC^2 Chromatogram of Head Hair 009, Run 01

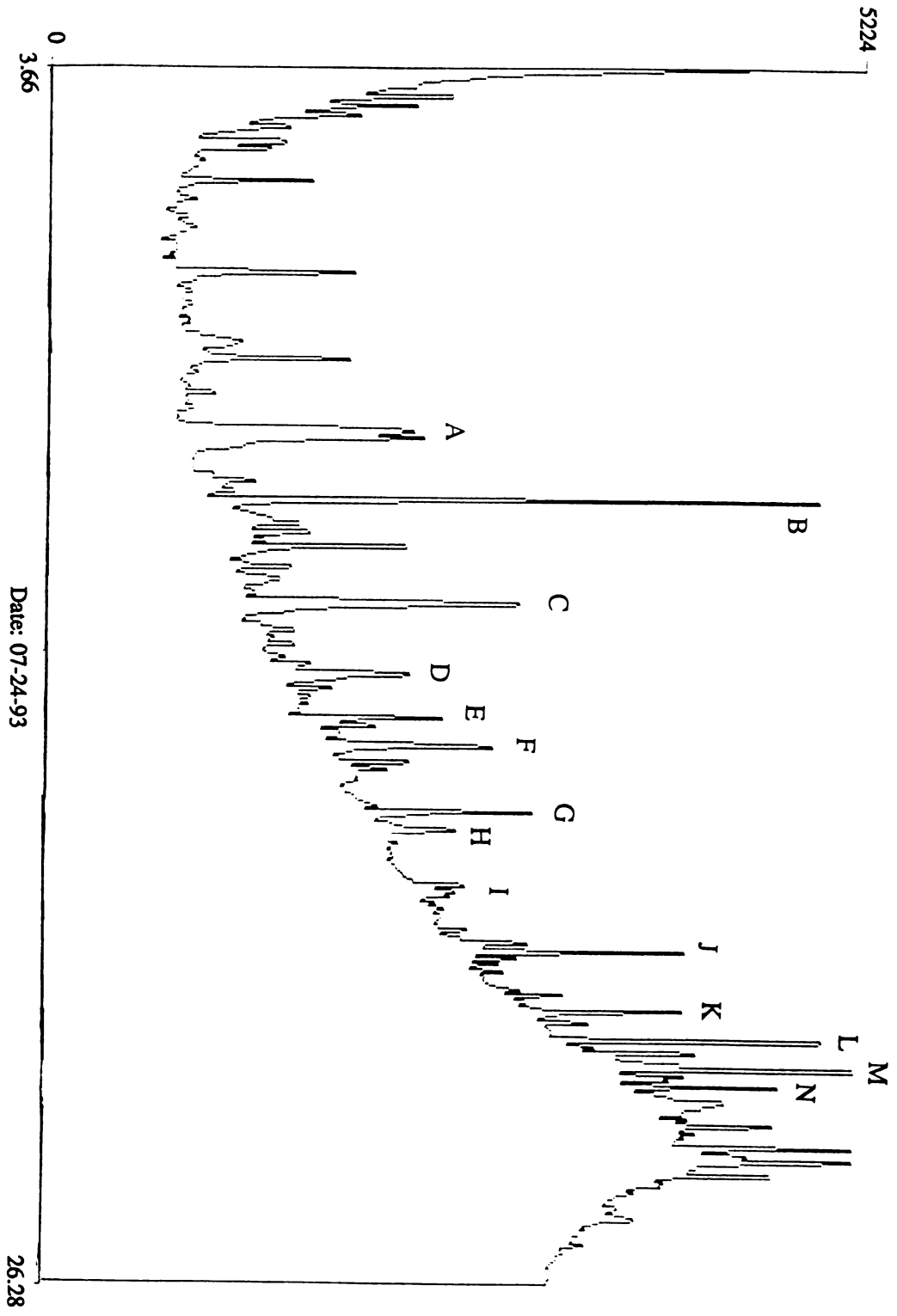

Figure 9. Py-GC^2 Chromatogram of Head Hair 010, Run 01

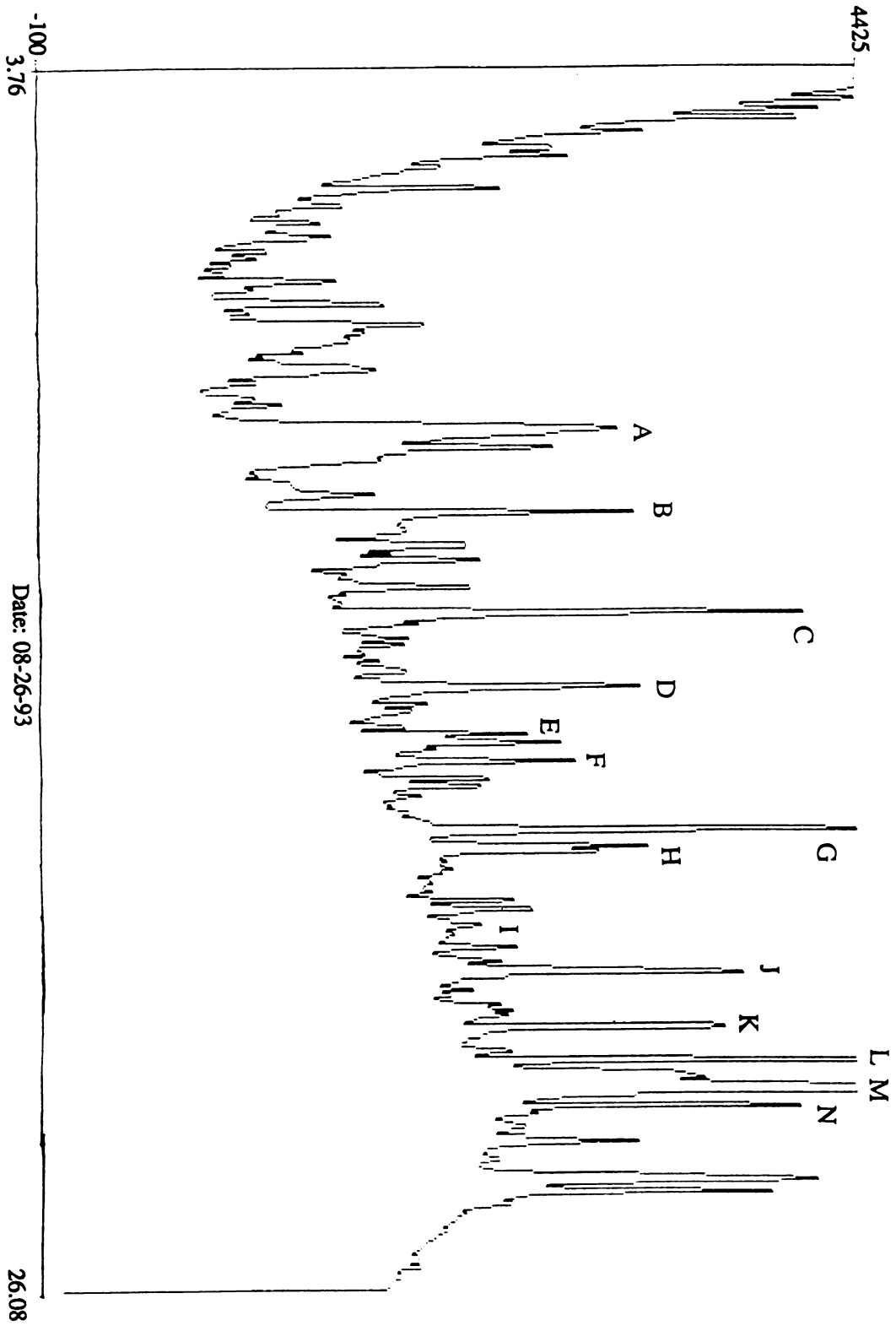

Figure 10. Py-GC^2 Chromatogram of Head Hair 011, Run 02

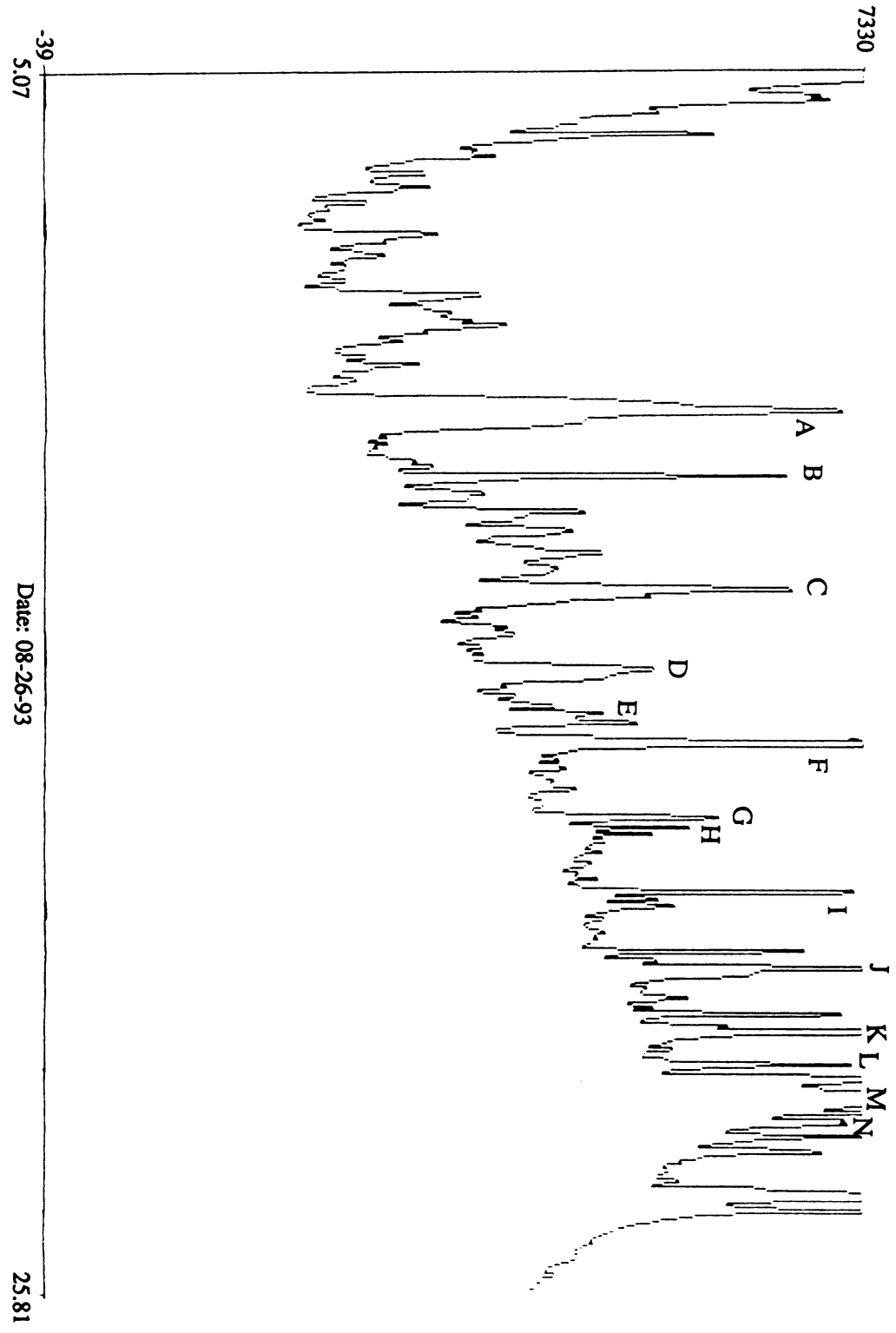

Figure 11. Py-GC^2 Chromatogram of Head Hair 012, Run 02

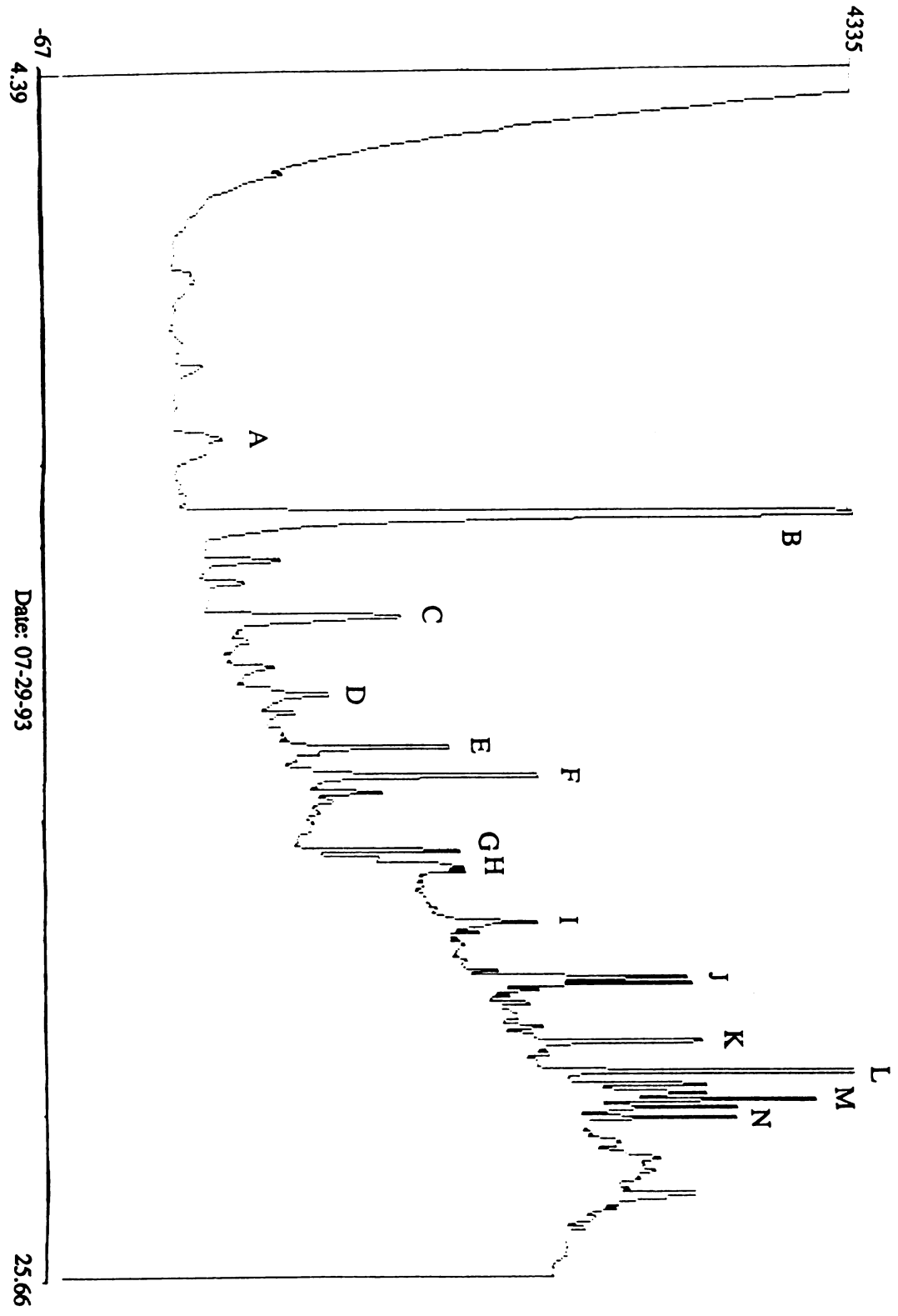

Figure 12. Py-GC^2 Chromatogram of Head Hair 012, Run 01

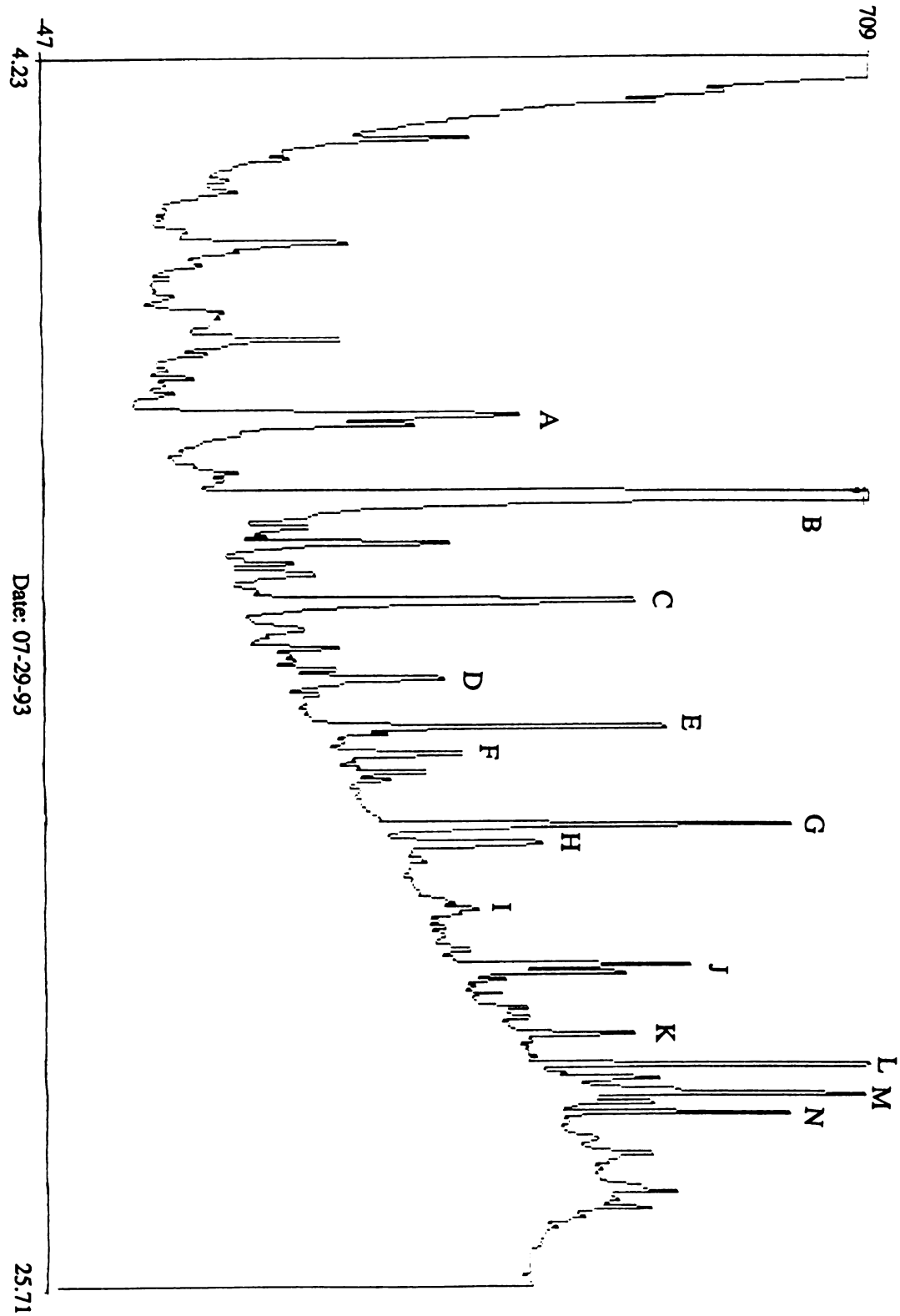

Figure 13. Py-GC^2 Chromatogram of Head Hair 013, Run 01

Pubic Hair Controls

The pubic hair 002 (same person as head 002) was run multiple times to establish baseline of variation found within the same hair. The baseline of variation for each individual peak was taken to be the two runs that gave the maximum and minimum values. Since these chromatograms were run on the same day, experimental conditions were as consistent as possible. Therefore any variation in peak height or area would be due to uncontrolable variations in the hair samples or in the pyrolysis-gas chromatography process. The maximum differences in peak area or height in the following runs established the baseline of variation inherent in the hair itself or the Py-GC^2 system. The significance of peak variation of pubic hair between different individuals will be based on its comparison to the baseline of variation of the following chromatograms. Chromatograms of the control experiments on pubic hair 002 are found in Figures 14-18.

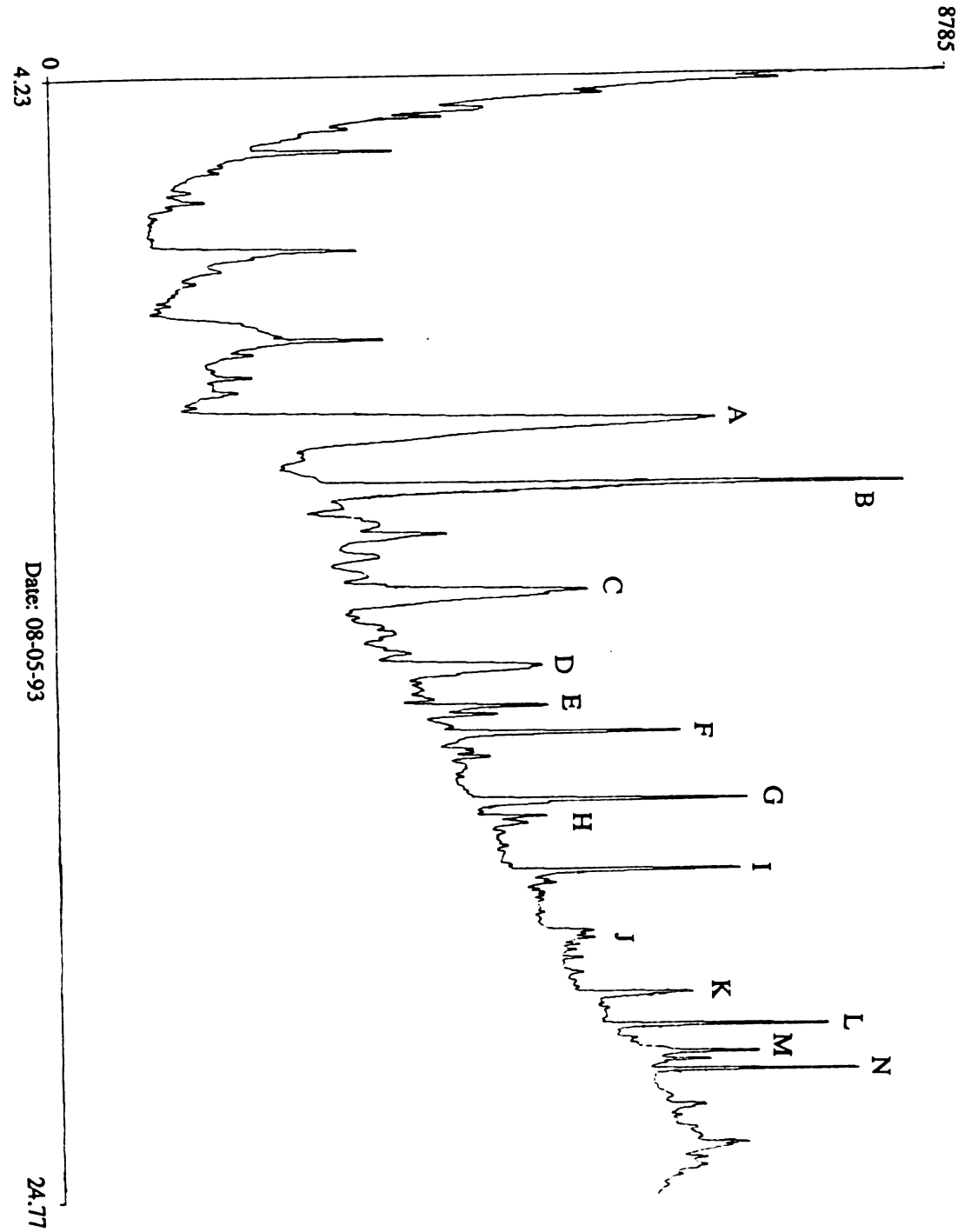

Figure 14. Py-GC^2 Chromatogram of Pubic Hair 002, Run 02

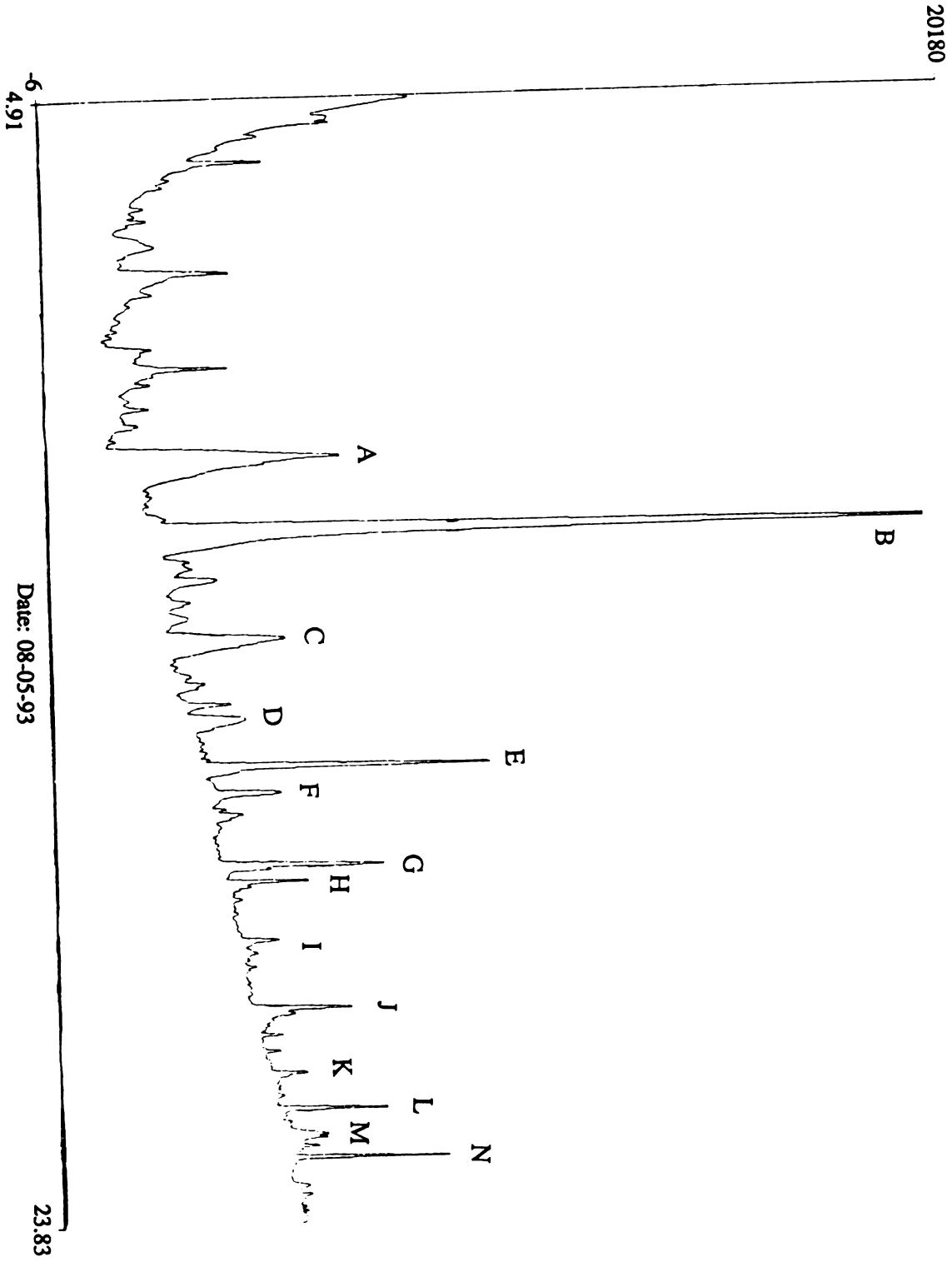

Figure 15. Py-GC^2 Chromatogram of Pubic Hair 002, Run 03

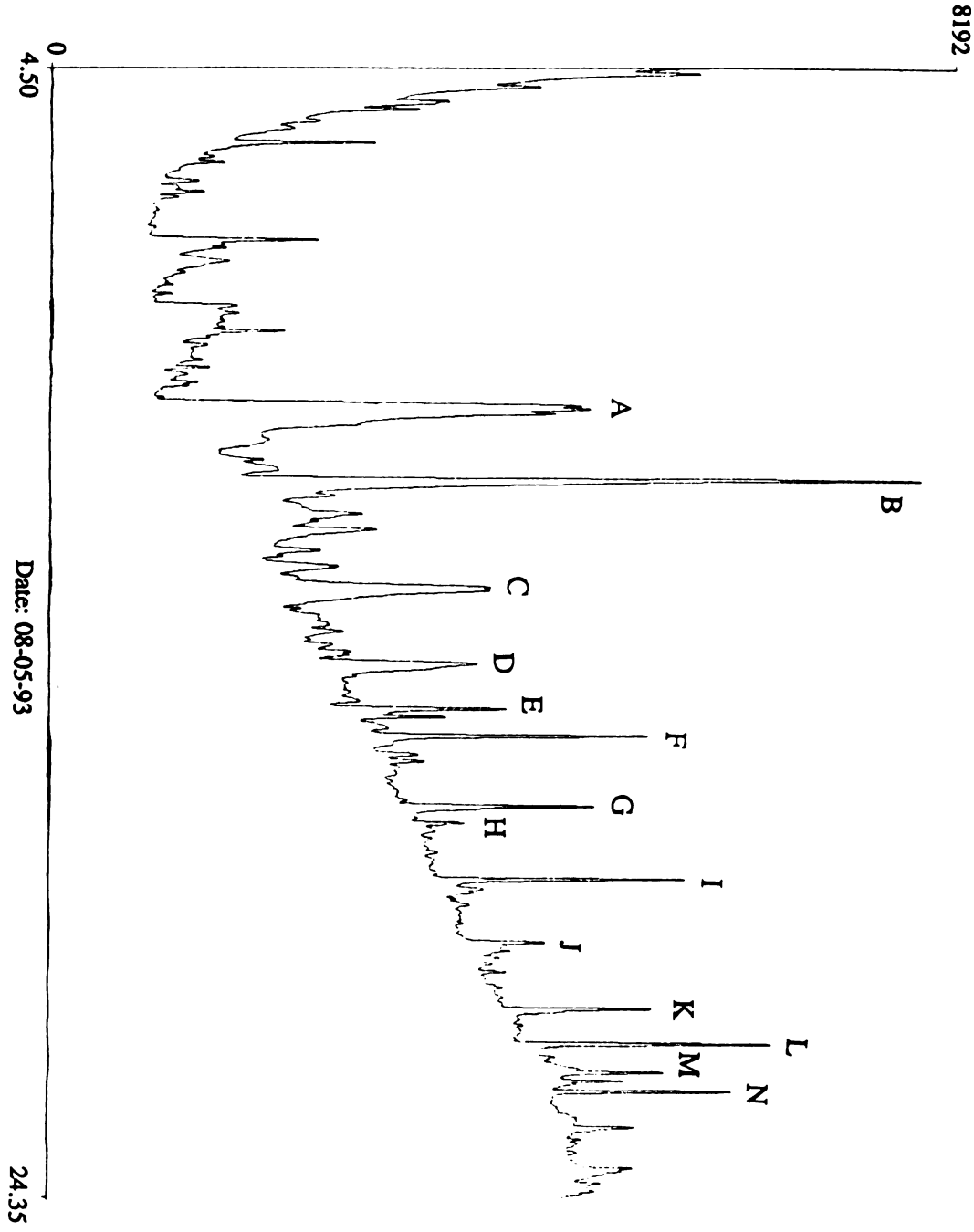

Figure 16. Py-GC^2 Chromatogram of Pubic Hair 002, Run 04

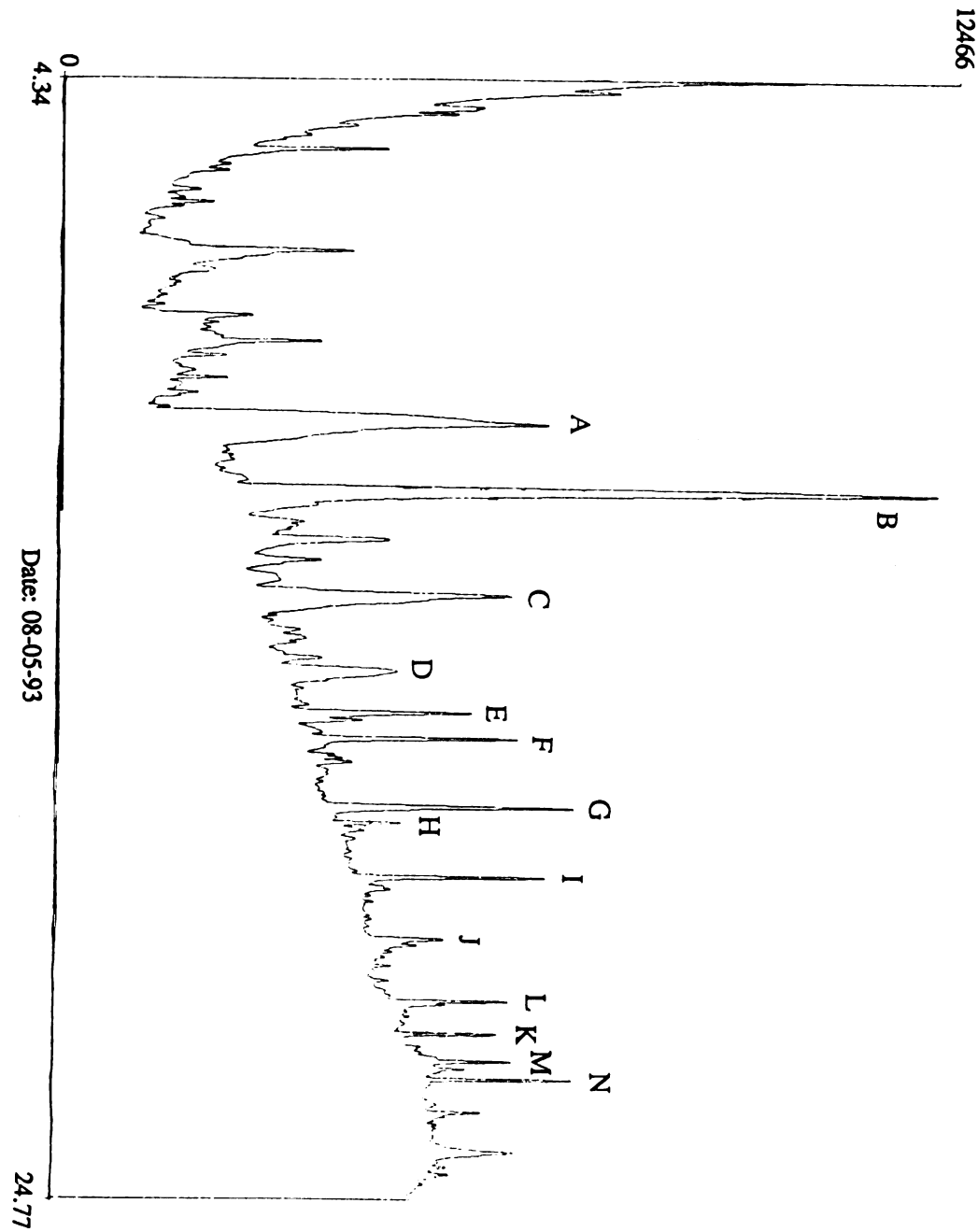

Figure 17. Py-GC^2 Chromatogram of Pubic Hair 002, Run 05

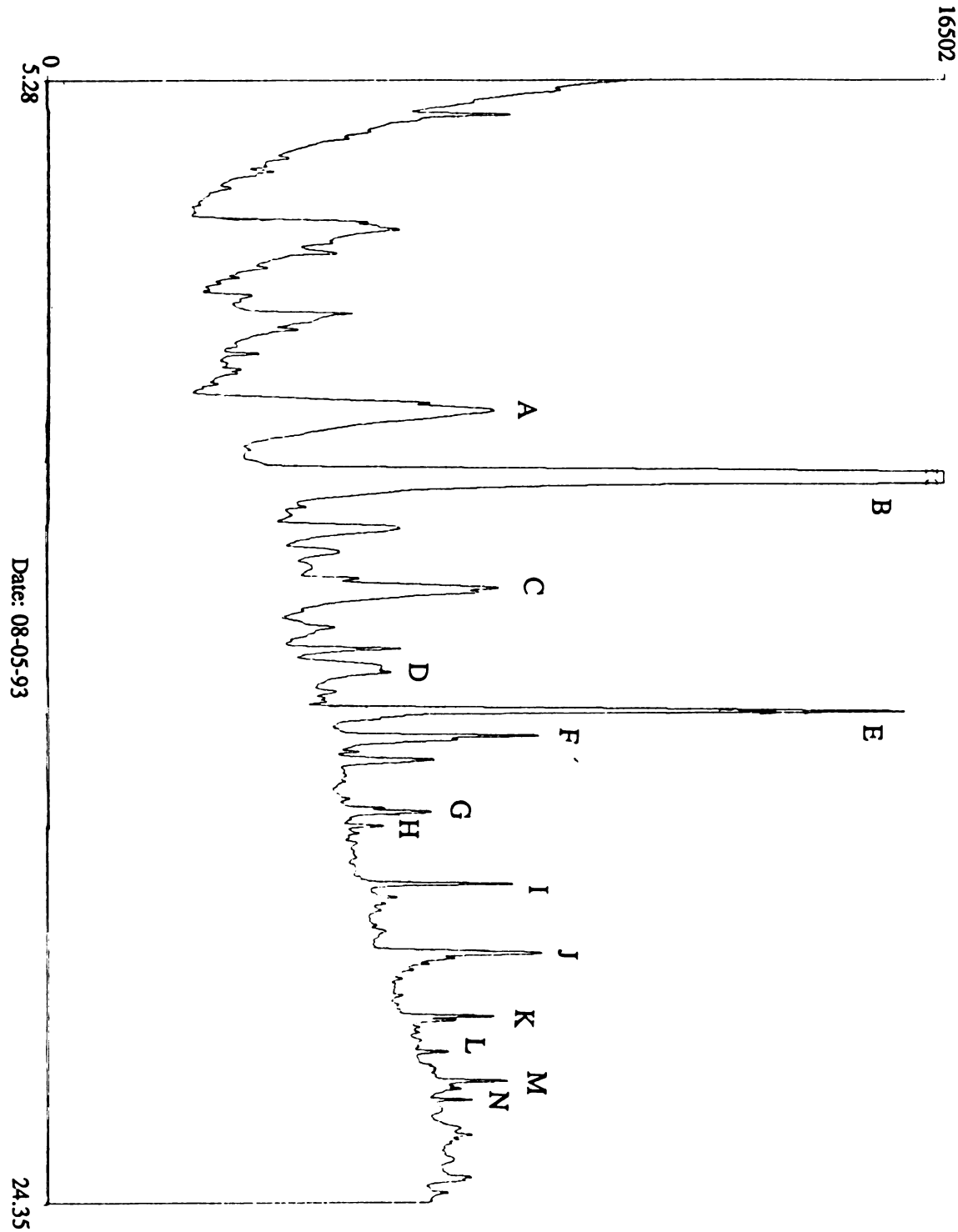

Figure 18. Py-GC^2 Chromatogram of Pubic Hair 002, Run 06

Pubic Hair of Different Individuals

A representative sampling of various individual's pubic hair is found in Figures 19-26. Peaks in chromatograms run on the same day were compared to look for differences in peak area or height. Corresponding peaks in all the figures are labelled with letters to aid in comparison. The amount of variation found between a peak in the chromatograms of pubic hair of different individuals was then compared to the baseline of variation found in the control runs of pubic hair 002. Peak areas with significant variation are defined to have more variation on chromatograms of different individuals than the baseline of variation of the control experiments in Figures 14-18.

Similar to head hair, there were relatively significant height variances in peaks A and B in the pubic hair 002 runs. Pubic hair 002 control experiments revealed a greater height variation than differences in corresponding peaks in hair of different individuals shown in Figures 19-26. Peaks E and F tended to vary greatly in height relative to one another throughout the pubic hair chromatograms. However, a similar magnitude of variation was also observed in the control chromatograms of pubic hair 002. In Figures 24 and 25, peak G splits and is shorter than peak H. In all other pubic chromatograms, including the controls of pubic hair 002, peak G was taller than peak H. In pubic hair 002, Figures 14 and 15, peak G has a shoulder at the same position the split occurred in Figures 24 and 25. This may indicate that better chromatographic resolution was achieved on the day where the chromatograms show the splitting of peak G. However, peak G may contain valuable information and should be followed closely in further studies. Finally there are variations in peak areas of I and J in the hair of different people that surpass the baseline of variation of pubic hair 002. It is not a matter of peak height but instead the splitting of these two multiplet peaks. The major area of peaks I and J shifted among the different sections in the multiplet, see Figures 24-26 and Figures 19-21. However in the control runs in Figures

14-18, the major area of the peak was located in the portion with the shortest retention time. This splitting effect of peaks I and J has the potential of providing valuable information. The Py-GC^2 technique would have to be developed further to be able to consistently produce such desirable resolution before ultimate forensic laboratory use. To date, two major drawbacks exist with the data shown here. There are not enough differences between peaks in the hair of different people with respect to the baseline of variation and insufficient peak resolution exists thus not allowing conclusive identification based solely on Py-GC^2 output.

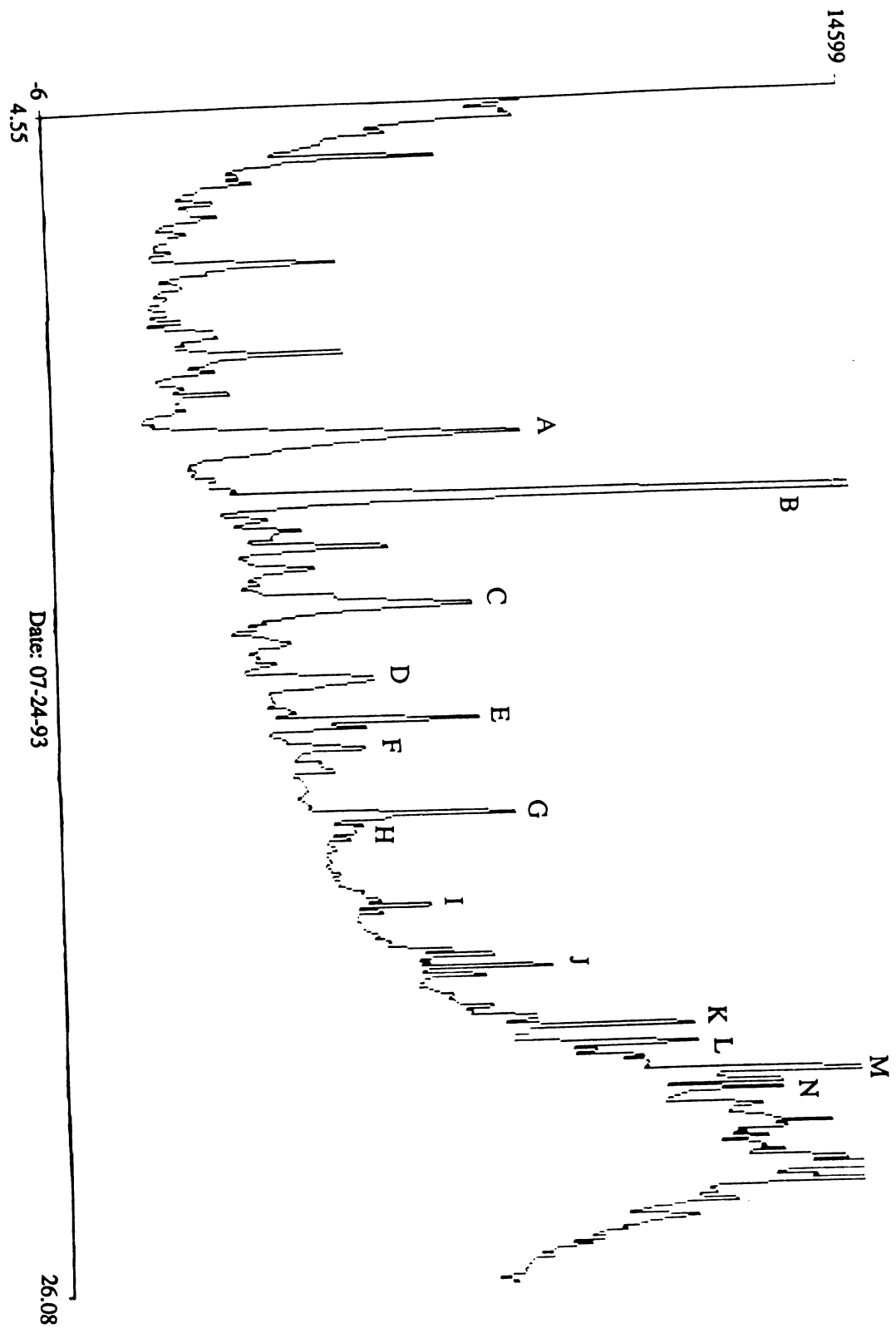

Figure 19 Py-GC^2 Chromatogram of Pubic Hair 007, Run 01

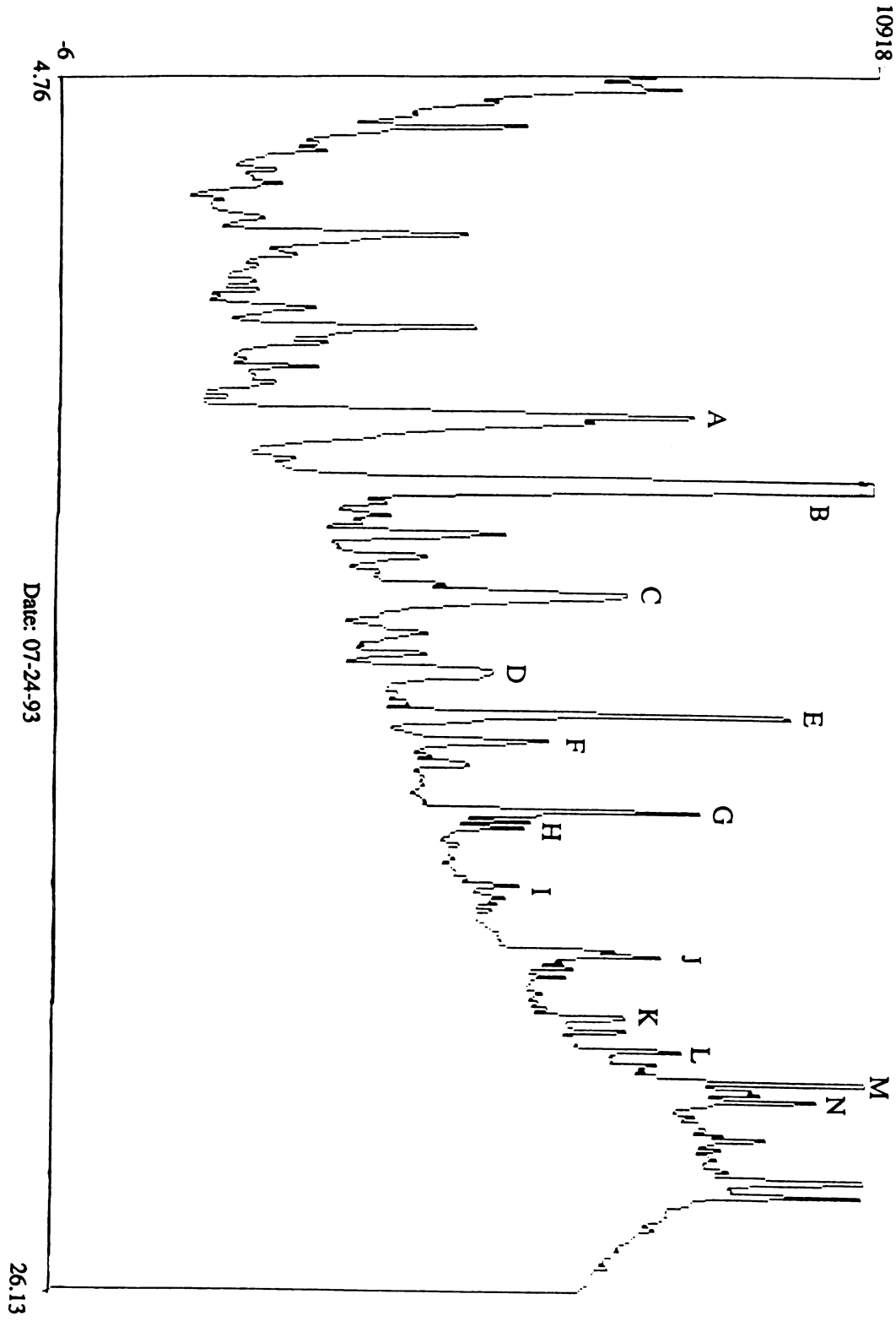

Figure 20. Py-GC^2 Chromatogram of Pubic Hair 008, Run 01

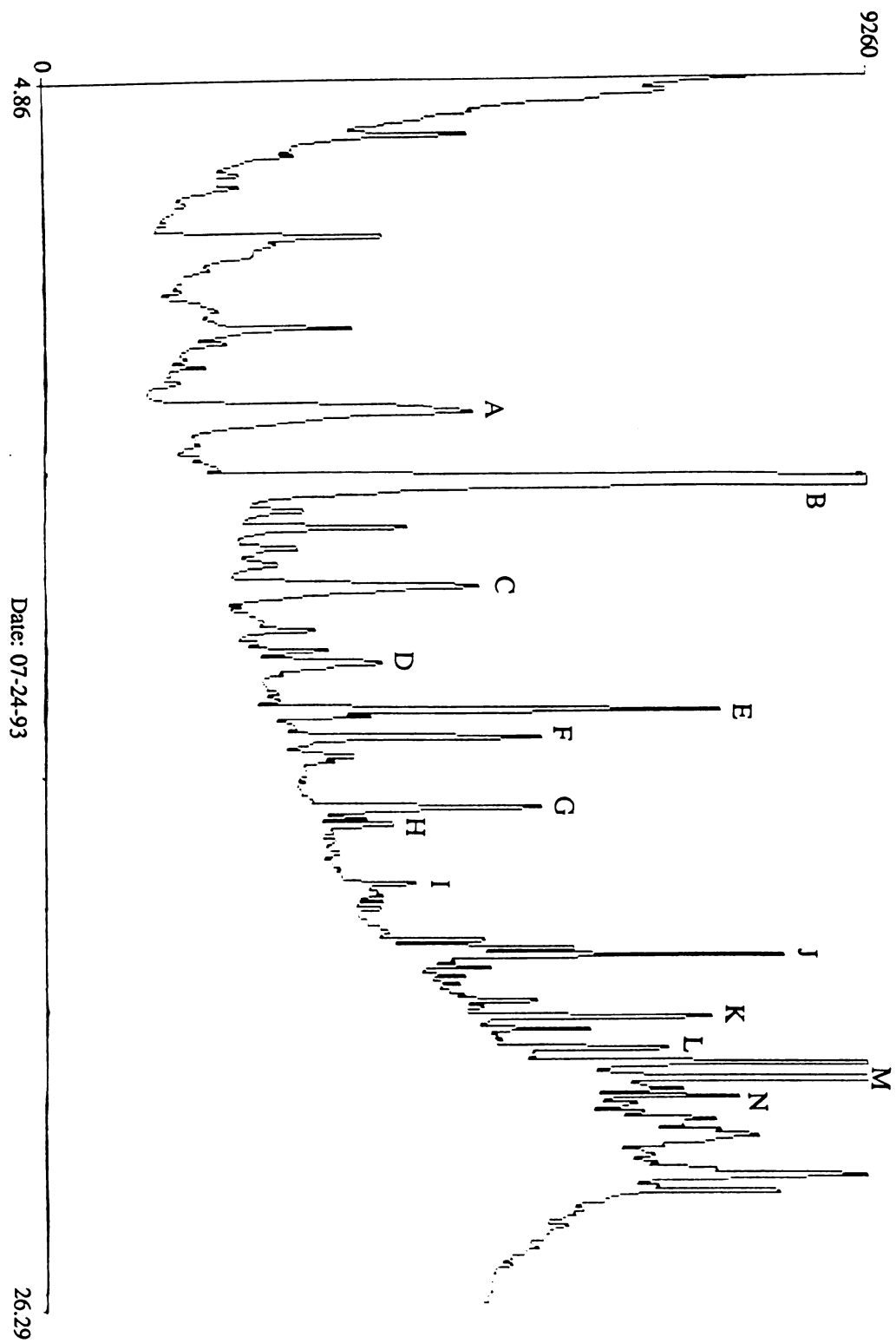

Figure 21. Py-GC^2 Chromatogram of Pubic Hair 009, Run 01

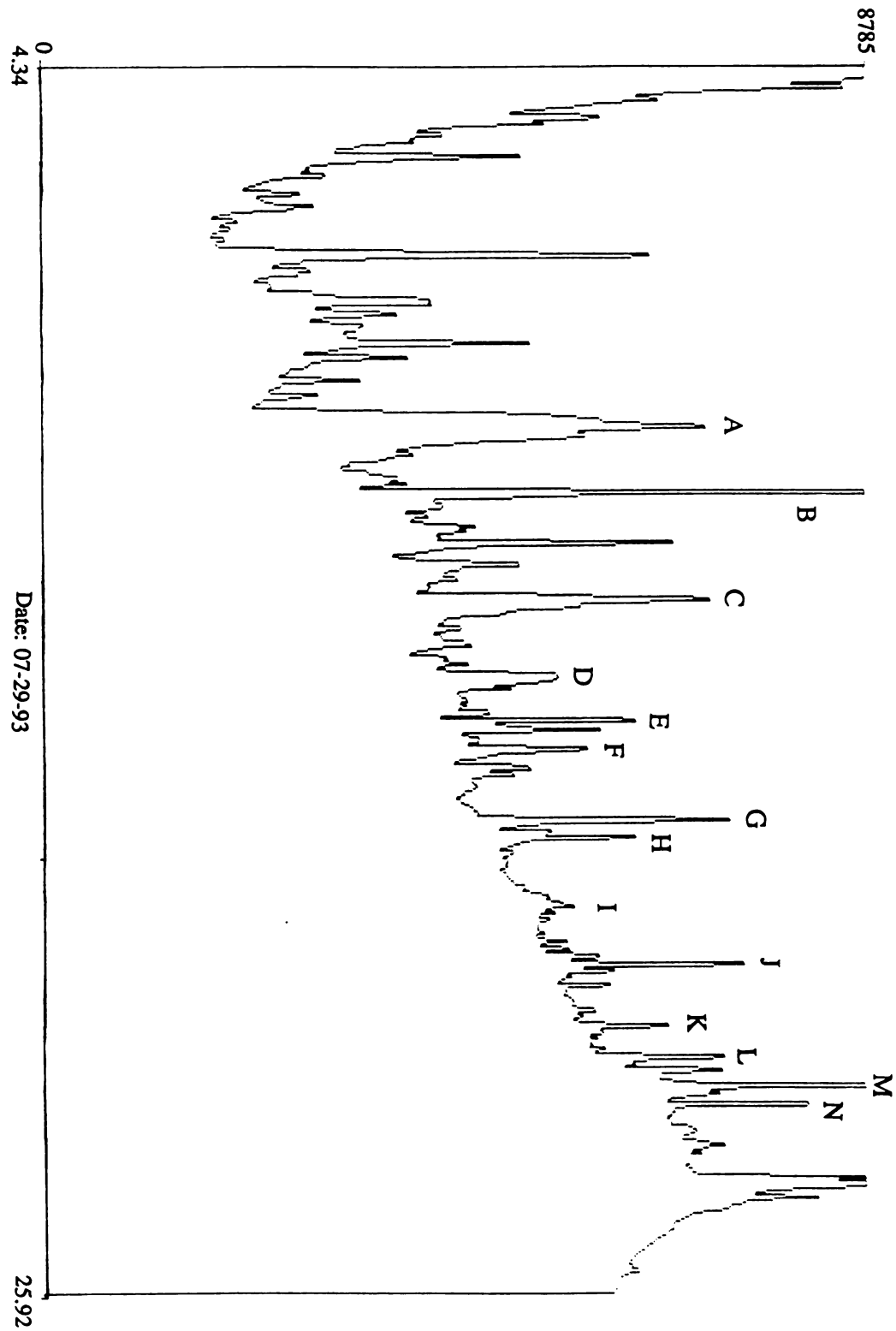

Figure 22. Py-GC^2 Chromatogram of Pubic Hair 011, Run 01

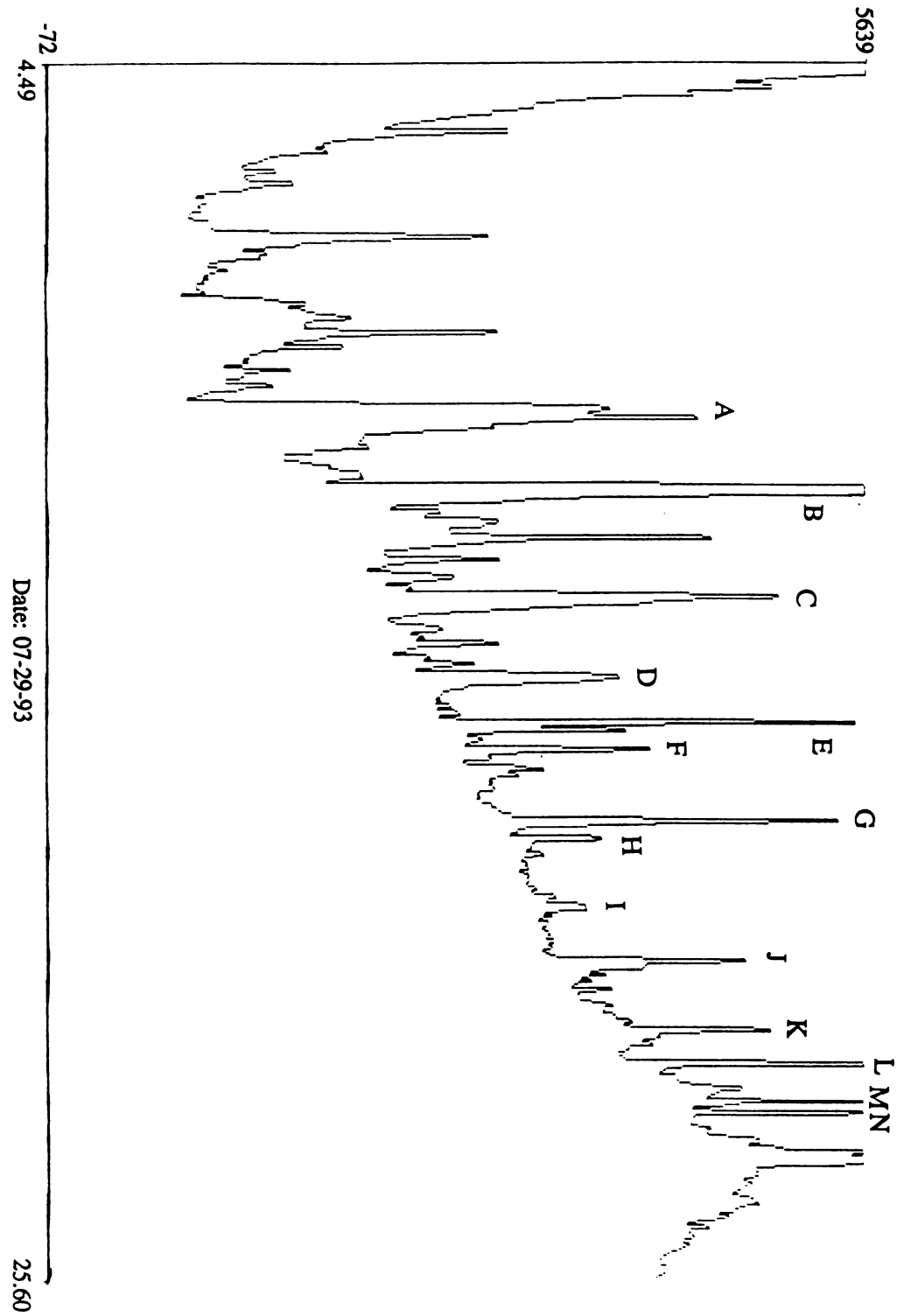

Figure 23. Py-GC^2 Chromatogram of Pubic Hair 012, Run 01

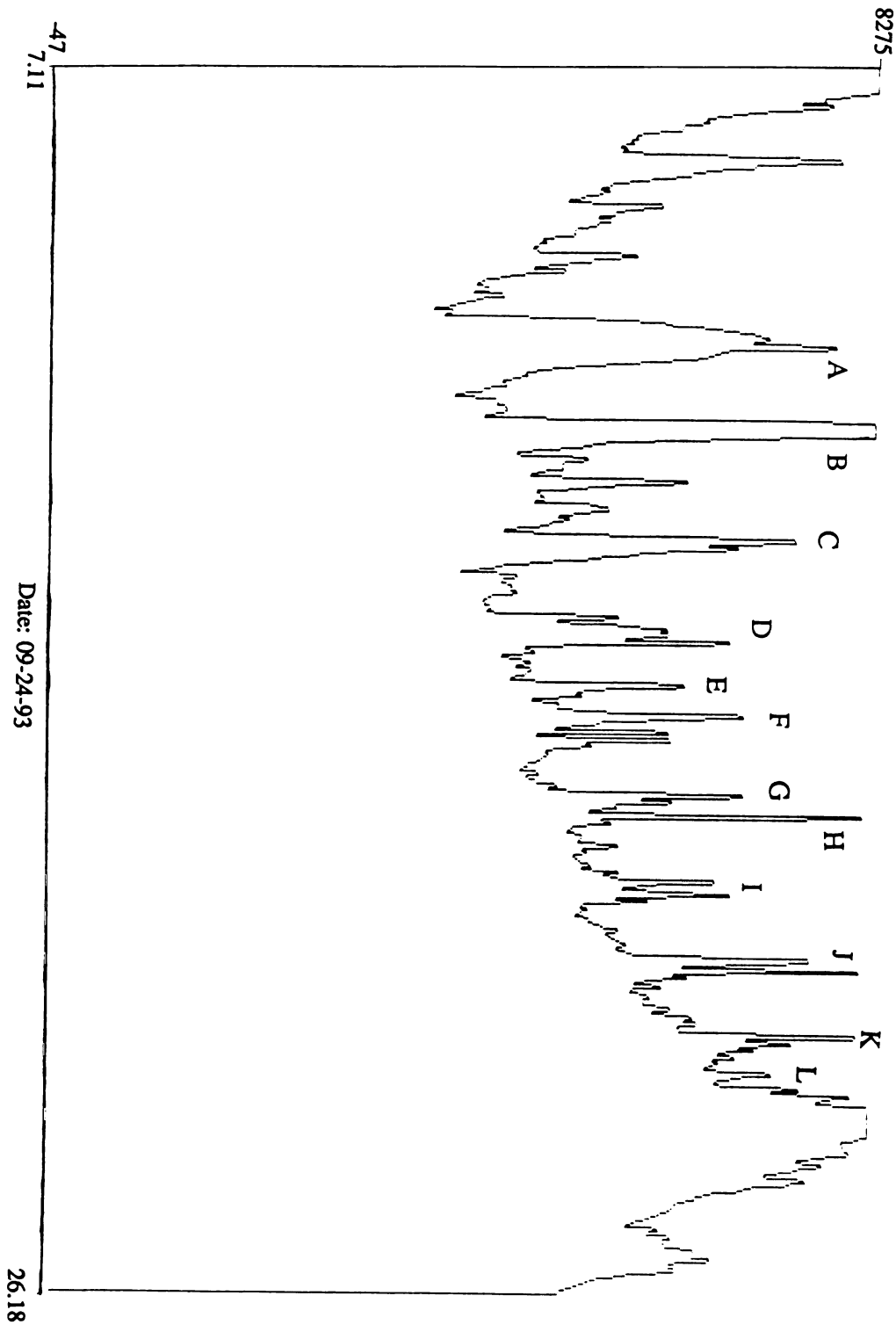

Figure 24. Py-GC^2 Chromatogram of Pubic Hair 016, Run 01

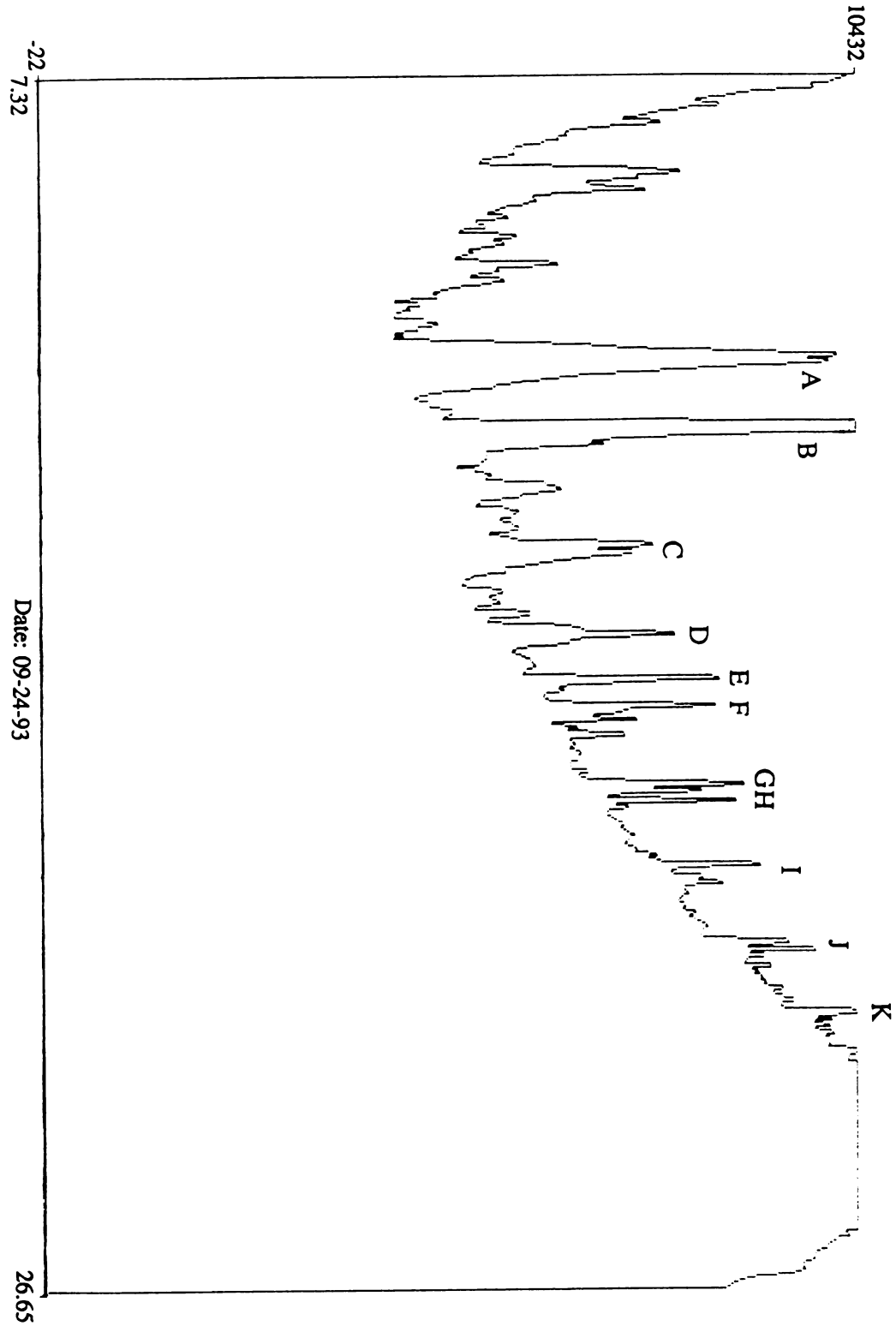

Figure 25. Py-GC^2 Chromatogram of Pubic Hair 017, Run 01

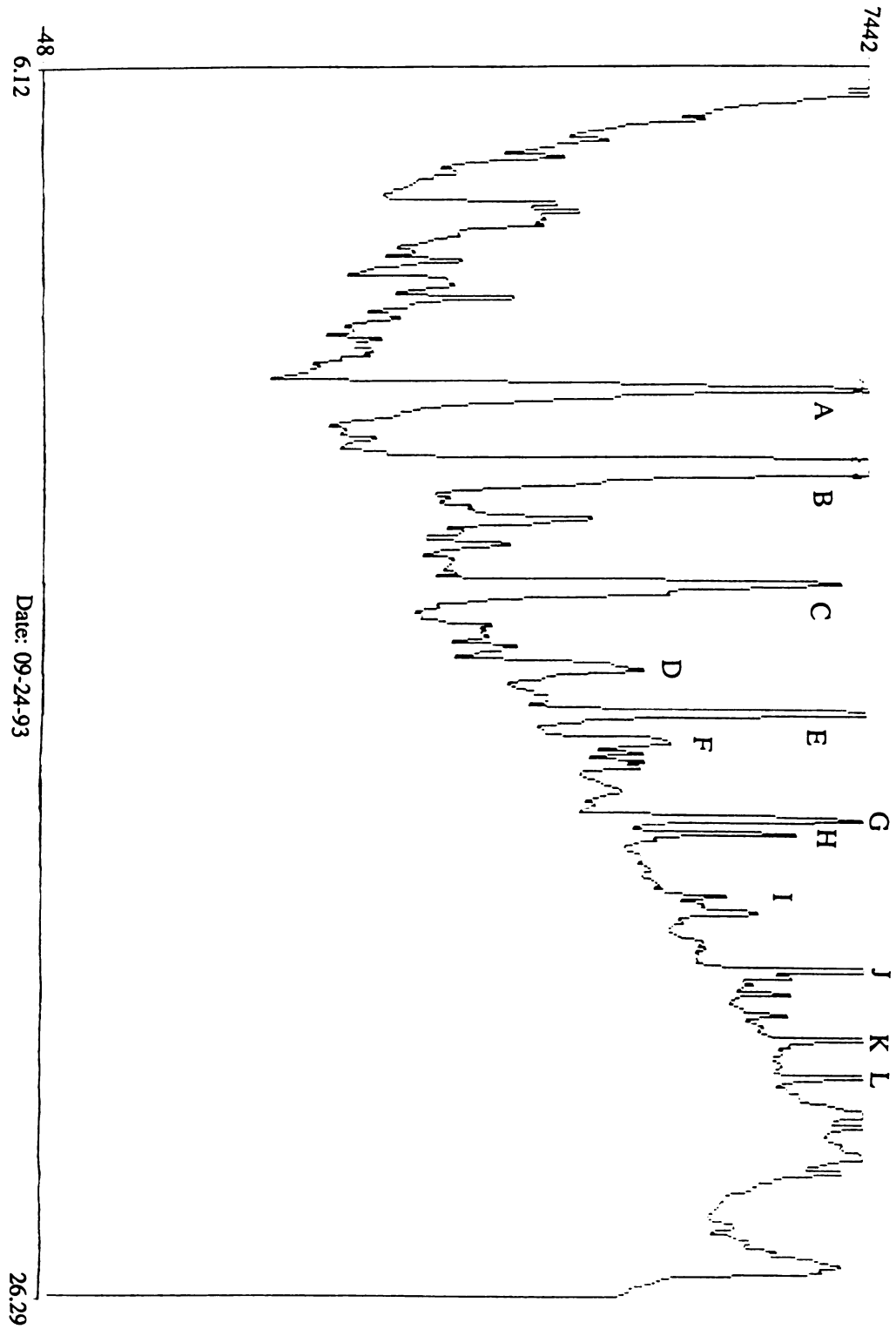

Figure 26. Py-GC^2 Chromatogram of Pubic Hair 018, Run 01

4. Conclusions and Recommendations

The pyrolysis-capillary gas chromatography (Py-GC^2) technique used here is not suggested as an alternative to morphological hair comparisons. It is proposed that this method for studying hair would provide information on the amino acid composition of hair. Py-GC^2 data in conjunction with morphological studies currently conducted by forensic scientists, could provide more information pertaining to the origin of hair samples. This additional information would allow the forensic expert to make a more educated conclusion. In order for the Py-GC^2 data to be useful, the variation in peaks and peak height or area in the chromatograms of various people must be greater than any variation found in the chromatograms of the same person's hair. Multiple runs of the same person's hair provide the baseline of variation in these experiments. It was found from this study that almost all chromatographic peak variations among hairs of different people were of similar magnitude to variations in corresponding chromatogram peaks of a single person's hair. The baseline of variation was therefore not surpassed. Thus, the Py-GC^2 technique has not yet been developed to the point where it could supply bias-free information in forensic hair comparisons based on this study. However, it should be noted that pyrolytic decomposition of the hair samples and subsequent chromatographic separation was successfully accomplished.

This study has shown that there is a high degree of variation in peak height on chromatograms run on the same hair sample on the same day. There are a number of possible explanations for the peak height variations. Most of the explanation lies in the first analytical step of this technique: pyrolysis of the hair sample. Pyrolysis is the procedure where the hair is broken down into smaller chemical fragments which are later observed as peaks in the gas chromatograms. Therefore any changes in the pyrolyzer will have

significant effects on the capillary gas chromatography peak areas and heights. There are a number of different pyrolyzer variables. (1) Slight changes in the temperature and heating rate of the pyrolyzer probe. (2) The hair sample may be situated differently within the quartz tube during different runs, thereby changing the overall surface area of the hair available to heat transfer. (3) The quartz tubes that hold the sample may not distribute the heat uniformly. (4) Each quartz tube is placed differently within the heating coils of the probe and therefore creates a unique contact with the heating surface. (5) In addition, the coils themselves probably do not uniformly radiate heat nor heat at exactly the same rate in each consecutive experiment.

There may be variations with regards to the hair itself. There are known morphological differences within the hair on one person's head. These morphological differences may result from amino acid differences which could be observed in peak height or area differences in the Py-GC^2 output. These differences may be discernable even though the samples are random mixtures of several hairs. Any one of these changes in experimental conditions could potentially explain the baseline variation found in runs of the same hair. It is unknown how many or which factors contributed to the baseline variation reported in this study.Further studies in our laboratory should attempt to remove the above five variables in a systematic manner in order to see the effect of each on Py-GC^2.

The first topic of this study, to examine the possibility of distinguishing head and pubic hair from different individuals by Py-GC^2 was deemed not possible using the data from experiments reported above. No overall peak differences exist in the chromatograms to enable distinguishing each chromatogram's origin as coming from pubic or head hair. In comparing chromatograms between head and pubic hair run on the the same day, structural differences reflected in the chemical composition of the protein chains, were observed as differences in peak areas on the chromatograms. However, there are two pubic hair peaks

(I and J), as well as one head hair peak (J), with height differences which surpassed the experimental baseline of variation for the controls.

It still remains to be seen if further development of this technique will enable chemical composition differences between hair of different individuals to be distinguishable. Until this is achieved, Py-GC^2 can not provide definitive information in forensic science hair comparisons. In future studies, this laboratory will try pyrolysis-capillary gas chromatography-mass spectrometry (Py-GC^2-MS) of hair. Identifying these peaks may provide insight into possible chemical composition differences between hair of different individuals. Another possible direction may be to look at fragments of hair protein in the attempt to find genetically-linked differences in the amino acid sequence. Munson and Fetterolf (6) have done some preliminary work using Py-GC-MS. Our laboratory should try Py-MS and Py-MS-MS experiments to see if genetically linkable amino acid fragments can be consistently isolated and observed.

5. Appendix

Hair Follicle Structure and Development

Human hair follicles begin to develop eight weeks after conception (14). Areas in the primitive epidermal layer of the skin divide and push downward into the dermis layer forming cell plugs. Initially the base of the plug is flat, but soon the edges of the base grow downward and enclose a group of dermal cells. These dermal cells later specialize into the dermal papilla. Cells near the top of the hair plug divide laterally into the dermis and form the sebaceous gland. This gland produces sebum, a wax-like coating that protects the hair surface. The arrector pili is a muscle which controls the hair. It forms in the dermis on the same side of the hair that contains the sebaceous gland. The structure of the hair follicle is shown in Figure 27.

The developed hair follicle is composed of several specialized cell regions responsible for different areas of hair growth and development. Within the lower hair bulb is the basal cell layer which produces, through cell division, the cells that become the hair and the inner root sheath. Also contained in the lower hair bulb are melanocytes. Melanocytes produce the hair pigment melanin found in the hair cortex. In the upper bulb region of the hair follicle, cells produced by the basal layer begin differentiating into six cylinders of cells. The inner three cylinders become the hair proper, while the outer three cylinders become the inner root sheath layers.

The outer root sheath has a similar structure to the epidermis and the two run as a continuum. The main function of the outer root sheath is protection. The inner root sheath serves more complex functions compared to the outer sheath. As the developing hair grows and moves up the follicle shaft, the inner root sheath grows along with it. The inner

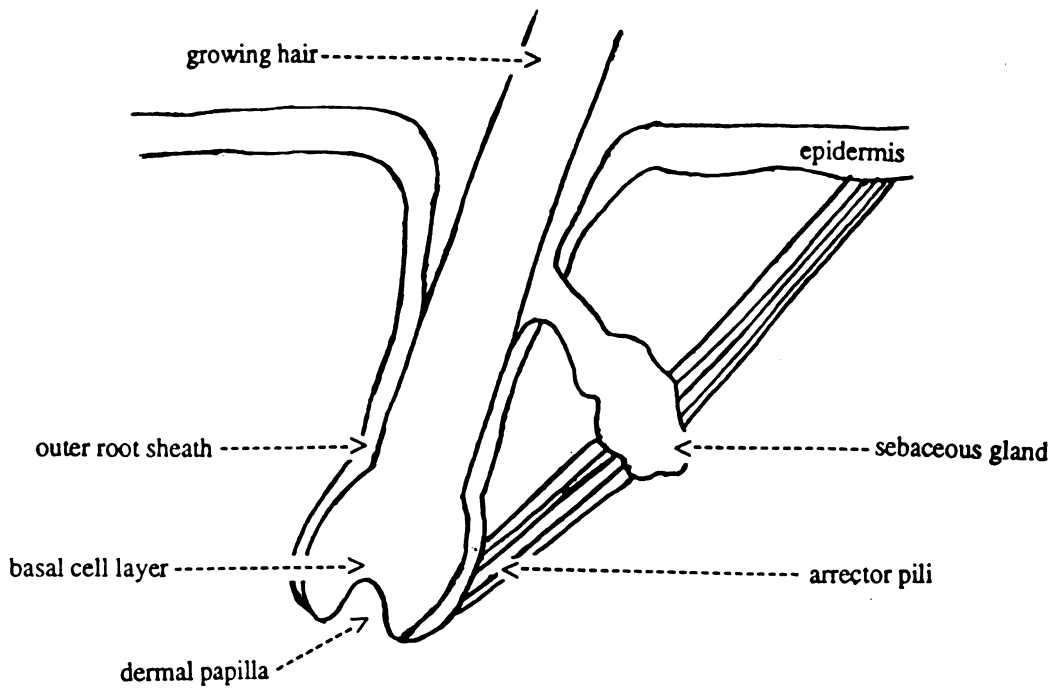

Figure 27. Structure of Hair Follicle

sheath controls the rate of movement of the hair cells as they travel from the upper bulb region into the follicle shaft. Nourishment for the developing hair is also provided by the inner root sheath. The inner sheath is supplied by an extensive capillary network. In addition, some of the physical features of the fully developed hair are produced by pressure from the root sheath (14).

A vital component of hair development is partially enclosed by root bulb and is located underneath the base of the hair follicle. The dermal papilla controls the hair follicle by setting the follicular hair growth cycle. Hair growth has the following three distinct stages.

1. Anagen stage: This stage begins with the development of a new hair follicle. It is the time of active hair production and growth. The anagen stage usually lasts between four and seven years.
2. Catagen stage: This stage lasts several weeks and is the transition between hair growth and hair bulb rest. The hair bulb activity begins to slow and the base of the hair follicle shrinks toward the epidermis.
3. Telogen stage: The resting stage where there is no new hair growth. The hair is shed during this stage and has a club root shape comprised of the old hair follicle attached to the hair. The anagen stage resumes with the production of a new hair follicle.

There are four types of hairs. Primordial hair is produced inutero and is lost before birth. This coarse hair grows during the third month of gestation on the eyebrows, upper lip, palms, and on the soles of the feet. The loss of primordial hair is followed by the growth of lanugo hair near the twentieth week of intrauterine life. Lanugo hair is fine, usually nonpigmented, relatively long and is eventually replaced by vellus and terminal hairs. Vellus hairs are relatively short (less than 1 cm), fine, and usually both nonpigmented and unmedullated. They are found over the entire body. For women 55% of all chest, trunk, shoulder, arm, and leg hair is vellus hair. In men only 10% of hair on

the same areas is vellus hair, whereas 90% is terminal hair (15). In contrast terminal hair are large, dark, pigmented and medullated. The primary terminal hair is found on the scalp, eyebrows and eyelashes. The secondary terminal hair develops from vellus hair during puberty as a result of hormone release. The pubic area, abdomen, beard, arms and legs are the general areas where the vellus hairs become secondary terminal hairs.

Structure of Hair

Cuticle

The translucent, non-pigmented outer most region of the hair is the cuticle. The cuticle is composed of imbricated flat scales which are stacked similar to roof tiles. Five-sixths of each scale is covered by other scales. On average, there are seven layers of scales found on human hairs near the root region. Scales are attached at the proximal or root end of the hair with the surface scale edges pointing to the hair tip.

The scale structure of the cuticle is formed in the follicle shaft. This happens before the keratinization of the cuticle cell protein. The hardened, keratinized inner sheath initially flattens the cuticle cells to a thickness of 0.5 to 1.0 mm and a length of about 45 mm. Then, as the inner sheath progresses up the hair follicle it drags the leading edge of each flattened cuticle cell over the cuticle cell directly above it in the follicle. This process is in agreement with the cuticle scales pointing to the distal end of the hair.

The number of scale layers found in a hair can be valuable in species identification. For example, although human hair typically has five to ten scale layers, whereas sheep have only one to two layers. There are two other scale measurements that greatly aid in species identification. The scale count is the number of scales per unit length. The ratio of scale length to hair diameter is the scale index. Although forensically these measurements help in

determining species, they are not very useful for eliminating individuals within a species. The scale number and scale count values vary almost as widely on the hairs of an individual as the entire distribution of the population for that species (6).

Each cuticle scale is comprised of several intracellular lamellar subcomponents. One of the outer cuticle layers, the A layer, illustrates how the cuticle serves effectively as protection for the hair. The proteins in the A layer contains over 30% cystine residues which form disulfide crosslinks. These crosslinks create biochemical stability in the A layer of each scale. Laminar scale structure as well as the layering of the cuticle scales creates an impermeable barrier.

Medulla

The medulla is the central section in hair. It appears under transmitted light as a dark core in the middle of the hair. In humans this dark color is due to air filled spaces. The medulla can also appear as a yellowish color when these spaces are filled with liquid. For animals the dark appearance of the medulla is a result of pigments.

There is also a difference between animal and human hair with regard to the medulla index. The medulla index is the ratio of the medulla's diameter to the diameter of the hair shaft. In humans the medulla index is one-third or less, while in coarse animal hairs the medulla index is greater than one-half. It is believed the medulla in animals serves an important function in thermoregulatory processes. However this is not an important requirement for humans (6).

During hair development in the hair follicle the medulla cells are loosely packed. After dehydration the cells shrivel up, leaving internal air spaces called vacuoles along the length of the hair. These air spaces appear dark under transmitted light. In human hair the dark medulla is observed to be continuous, absent, or somewhere in between. The

determination of the amount of medulla in hair is an important part of forensic science analysis. Scientists have numerous systems to describe the amount of medulla found in each hair but little is known about the medulla structure. The most likely function of hair medulla is to increase the protective properties of hair with internal air spaces. Beyond this function Menkart (16) states that the medulla contributes very little to chemical and mechanical properties of human hair.

Cortex

The cortex is both the most complex and widely studied, and hence well characterized region, of hair. One reason for this attention is the almost sole dependence of the mechanical properties on the cortex. The cortical cells are comprised of macrofibrils (macrofilaments) and a matrix which crosslink to form an intricate network. During keratinization these cells elongate longitudinally with respect to the axis of the hair to become 1 to 6 microns thick and approximately 100 microns long. The long macrofilaments develop within the cytoplasm during this time. After keratinization is complete, the diameter of the hair has been reduced by 25% due to contraction of the macrofilament-matrix complexation and from water loss (17). Dehydration results from an increase in plasma permeability of the cortical cell membranes (17).

Within the cortex structure are small air spaces called cortical fusi. These spaces are fluid filled during the formation of the cortex from the preliminary cells. No significant structural attribute can be assigned to these fusi, however they are valuable in forensic science hair comparisons. The size, shape, and distribution of the cortical fusi can vary widely between individuals (6).

Cortex pigment is also extensively used in forensic determinations. The location of pigment granules within the cortex is an indicator of whether the hair has a human or

animal origin. The pigment granules lie near the medulla in animal hairs but in humans are more evenly distributed throughout the cortex or are in greater intensity at the periphery. Pigment can also be used to determine racial origin in human hair. Negroid hair has clumped pigment granules, while granules are evenly dispersed through the cortex in Caucasian hair (6).

Melanocytes located in the basal layer of the hair bulb produce the pigment melanin. Melanin granules travel by the dendritic processes of melanocytes to developing cortical cells where they are ingested by phagocytosis. There are two types of melanin pigment. Eumelanin, the more common type, produces the ranges of black, brown, and yellow hair color. Red and "true" blond hair result from phaemelanin granules. Both types of melanin are synthesized identically except for the last step. Tyrosinase is the enzyme responsible for the first two steps in melanin formation. The most plausible and widely excepted explanation for the graying of hair is the inhibition of tyrosinase activity (14).

Macrofibrils and Matrix

Within cortical cells are spindle shaped structures known as macrofibrils. According to Randebrook (18), macrofilaments are approximately 0.1 to 0.4 microns in diameter. Within each macrofibril are smaller fibrillar units called microfilaments as well as a protein matrix situated around the microfibrils. The matrix is a relatively unordered area whereas the microfibrils are more highly ordered.

The matrix is often described as an amorphous region. Johnson and Sikorski (19), however, acknowledge that the matrix must have some degree of structural organization. Because the matrix is capable of contracting in parallel with the microfibrils, there must be some orientation of its protein chains (19). Matrix protein has a higher sulfur content than α-helical fibril protein due to an abundance of cystine residues. Therefore, matrix protein

γ-keratose is able to form many disulfide bonds with the fibrillar protein (14). In general, the matrix to microfibrillar ratio is about unity (14, 15, 20-22). Regardless of the relative amount of crosslinking between the matrix and microfilament, there exits a significant likelihood for formation of disulfide linkages between the matrix chains.

Microfibrils and Protofibrils

The radius of a macrofilament is approximately 4,000 angstrom units (15). Determination of a microfibril diameter is complicated due to the difficulty of discerning the matrix-microfibril boundary (23). However, there is general agreement that the microfibril diameter is on the order of 70 angstroms (15). Within the microfilament are smaller fibril units known as protofibrils. Although the exact number and arrangement of protofibrils within the microfibril has not been determined (24), there are two highly regarded models.

Filshie and Rogers (23) by selective staining and use of electron micrographs measured the diameter of the protofibril at approximately 20 angstrom units. This diameter value has since been verified separately by Johnson and Sikorski (24) and Dobb (25). Filshie and Rogers contend that the number and arrangement of protofilaments that best explain the electron micrographs and fit within the microfibril is a "9 + 2" arrangement (23). This arrangement would have a central core of two protofibrils surrounded by a circle of nine more protofibrils.

Another model called the "ring-core" structure by Fraser (15) is also highly regarded. Fraser believes the diameter of the microfibril allows up to nine protofibrils. One protofilament is the core and the remaining number form a circle surrounding it (15).

Macromolecular Structure

Within the protofibrils are polypeptide chains. These chains are approximately 10 angstroms in diameter including all side chains of the amino acid residues (15). Pauling and Corey (26, 27) propose that these polypeptide chains take the form of an α-helix. Studies from a decade earlier in wide-angle X-ray diffraction of human hair and other keratin fibers show equatorial spacing of 9.8 angstroms and meridional spacings of 5.1 and 1.5 angstom units (15). Pauling and Corey interpreted how these measurements correspond to the α-helical model. They determined the 1.5 angstrom spacing represents the distance between each amino acid residue and the 5.1 angstrom spacing as corresponding to the repeat distance for coiling, which is 3.6 amino acid residues (26, 27). The 9.8 angstrom unit spacing represents the center-to-center distance between two α-helix chains or approximately the diameter of one α-helix (15, 27).

Although the α-helix keratin protein is a good model for the protofibril, several authors have reported that the density of a perfect α-helix conflicts with the density of a protofibril (28). Pauling and Corey account for this discrepancy with a "coiled coil" model for a protofibril. The "coiled coil" model proposes that the protofibril consists of two or three strands of an α-helical protein coiled around each other like twisted rope (28, 29). Although several variations now exist for the "coiled coil", the three strand version proposed by Crick (29) is still the most widely accepted (15).

Molecular Structure

Human hair is 65% to 95% protein depending on its moisture content (15). The remaining portion is composed of water, lipids, pigments and trace elements. The trace

elements most frequently found are the metals Ca, Cd, Cr, Cu, Hg, Zn, Pb, Fe, As and Si (14). These trace metals are likely to complex with the pigments or with the fatty acid groups of the lipids. A great deal of study has been done in attempting to use trace elements in hair as indicators of specific diseases. In addition, trace metals in human hair are being used as potential monitors of exposure to environmental pollutants (30).

Hair is primarily composed of proteins. Proteins are condensed polymers of amino acid residues. Several research groups have determined the amount of amino acid residues found in human hair (15). The results of amino acid quantitation on whole human hairs by Deem and Rieger (31) are shown in Table 3.

Table 3. Amino Acid Composition of Human Hair

Amino Acid	Micromoles/Gram of Dry Hair
Alanine	362-384
Arginine	499-550
Aspartic acid	444-453
Cysteic acid	22-40
Glutamic acid	995-1036
Glycine	463-513
Half cystine	1407-1512
Histidine	64-86
Isoleucine	244-255
Leucine	502-529
Lysine	206-222
Methionine	50-56
Phenylalanine	132-149
Proline	646-708
Serine	1013-1091
Threonine	648-673
Tyrosine	177-195
Valine	477-513

Process of Keratinization

Fibrils begin forming in cortical cells as primitive hair moves into the zone of fibrillation, see Figure 28. Next the fibrils orient into the α-keratin pattern. This pattern is held or stabilized by hydrogen bonds between each filament chain, between the filament chains and the matrix as well as between the matrix chains (7). When the hair enters the zone of keratinization, the α-keratin structure is consolidated from oxidative linking of cysteine (-SH) side chains to form cystine bridges between fibril chains as well as within the matrix. Cells of the developing hair die during the keratinization process. The cell membranes degrade and nuclei lose their DNA. Mitochondria and ribosomes degenerate and their remnants along with nuclei can be found in the cytoplasm within the γ-matrix protein (17).

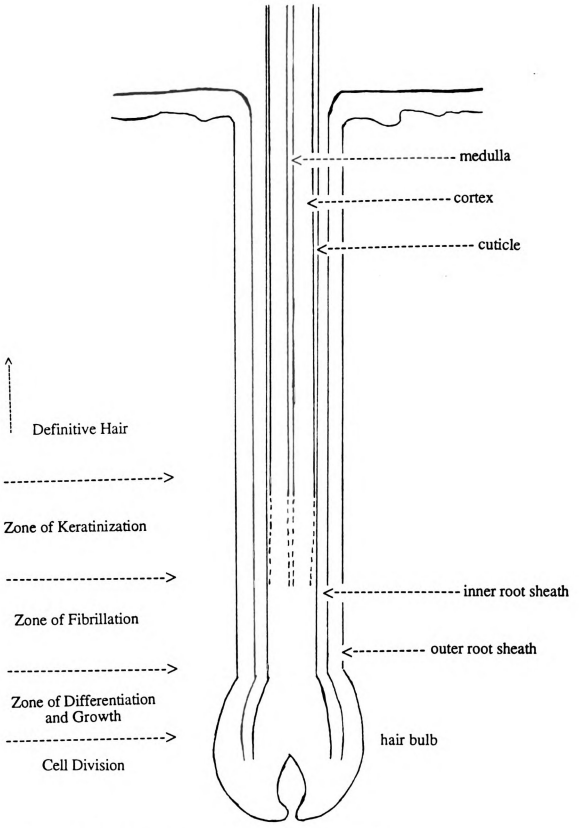

Figure 28. The Phases of Hair Growth and Development

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Vita

Cynthia Jean Schutt was born on December 7, 1967 in Hammond, Indiana USA to George Edward and Betty Francis Schutt.. She spent her early childhood in South Holland, Illinois although the majority of her schooling was obtained in Holland, Michigan. She graduated as Class Valedictorian at the West Ottawa Senior High School, Holland, Michigan in 1986. She attended Hope College in Holland from 1986-1990 and graduated Magna Cum Laude, a member of Phi Beta Kappa with a major in biology and a minor in biochemistry. Cynthia married Carl Lawrence Aronson in December 1991. She continued her education with graduate work in the Department of Criminal Justice at Michigan State University, East Lansing. During her tenure at Michigan State her advisor was Forensic Science Professor Dr. Jay A. Siegel. This thesis is a tribute to Cynthia's perseverance and scientific knowledge.