INFORMATION TO USERS

This reproduction was made from a copy of a manuscript sent to us for publication and microfilming. While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. Pages in any manuscript may have indistinct print. In all cases the best available copy has been filmed.

The following explanation of techniques is provided to help clarify notations which may appear on this reproduction.

1. Manuscripts may not always be complete. When it is not possible to obtain missing pages, a note appears to indicate this.

2. When copyrighted materials are removed from the manuscript, a note appears to indicate this.

3. Oversize materials (maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or in black and white paper format.*

4. Most photographs reproduce acceptably on positive microfilm or microfiche but lack clarity on xerographic copies made from the microfilm. For an additional charge, all photographs are available in black and white standard 35mm slide format.*

***For more information about black and white slides or enlarged paper reproductions, please contact the Dissertations Customer Services Department.**

U·M·I Dissertation Information Service

University Microfilms International
A Bell & Howell Information Company
300 N. Zeeb Road, Ann Arbor, Michigan 48106

Wilson, Linda Arney

THE CAPILLARY GAS CHROMATOGRAPHIC PROFILING OF HUMAN SKIN SURFACE LIPIDS FOR FORENSIC APPLICATION

Middle Tennessee State University D.A. 1986

THE CAPILLARY GAS CHROMATOGRAPHIC PROFILING OF HUMAN SKIN SURFACE LIPIDS FOR FORENSIC APPLICATION

by

Linda Arney Wilson

A dissertation presented to the
Graduate Faculty of Middle Tennessee State University
in partial fulfillment of the reqirements
for the degree Doctor of Arts

August 1986

THE CAPILLARY GAS CHROMATOGRAPHIC PROFILING
OF HUMAN SKIN SURFACE LIPIDS FOR
FORENSIC APPLICATION

APPROVED:

Graduate Committee:

A. Edwin Woodrow
Major Professor

Gale J. Clark
Committee Member

Jack D. Peters
Committee Member

Jon D. Swett
Chairman of the Department of Chemistry and Physics

Mary Martin
Dean of the Graduate School

ABSTRACT

THE CAPILLARY GAS CHROMATOGRAPHIC PROFILING OF HUMAN SKIN SURFACE LIPIDS FOR FORENSIC APPLICATION

By Linda Arney Wilson

Profile analysis has been applied to the study of human skin surface lipids (SSL) to determine their utility in forensic applications. SSL were collected from the forehead and cheek of 23 volunteers. A pentane extract was analyzed by split injection capillary gas chromatography (GC). A 25-meter long, 0.2-mm-I.D. BP-5 fused silica capillary column was programed from 80°C to 260°C at 10°C/min and then to 290°C at 3°C/min. The temperature was held at 290°C for 47 minutes for a total run time of 75 minutes.

This technique produced very reproducible profiles. The SSL profiles varied from individual to individual and there was also some variation of an individual's profile over time. Profiling was also used to demonstrate the effects of weathering on a SSL sample. Upon exposure to ambient laboratory conditions, the sample showed signs of degradation with the appearance of many components eluting early in the chromatogram. The use of cosmetics was shown

to influence the SSL profile with the appearance of many peaks early in the profile.

Transevaporator extraction/thermal desorption was also applied to SSL samples with promising results.

ACKNOWLEDGEMENTS

I wish to express my heartfelt thanks to Dr. A.E. Woods for his continued support and encouragment. My thanks also to Dr. Dan Scott, Dr. Gale Clark, Dr. Gary Wulfsberg, Dr. Jim Howard and the other faculty and staff of the Department of Chemistry and Physics for the encouragement, consultations and loan of equipment. I also wish to thank Dr. Jack Arters for the professional advice and helpful discussions over the years.

Finally, I want to express my deep appreciation to Dick, my husband, and Jennifer, my daughter, for their encouragement, patience and support, without which this project could never have been completed. My thanks also to Dick for his help in the preparation of this manuscript and to my mother and sister for their moral support.

TABLE OF CONTENTS

FIGURES

CHAPTER 1

INTRODUCTION

Gas chromatographic profile analysis is a versatile analytical technique that has been applied to a wide variety of samples ranging from environmental pollutants to the investigation of Reye's syndrome. Both packed column and capillary column gas chromatography (GC) have been used. Although capillary GC often yields a chromatogram of a hundred peaks or more, identifying every component is a laborious task that is often unnecessary. Each chromatogram is often uniquely characteristic of the material being analyzed. A detailed comparison of chromatograms is possible using retention times and either areas or heights of the various peaks. This comparison of the "fingerprint" patterns of a chromatogram has been called "profile analysis" and frequently is sufficient to differentiate between control (known) and suspect (unknown) samples. Only if detailed information about the samples is desired, is it necessary to identify the peaks responsible for the differences in the pattern.

The volatile components of certain foods have been profiled to yield information on various flavors and fragrances (1-25). Many comparisons of organoleptic (sensory) vs. instrumental evaluations of flavors have been made in an attempt to provide a more objective

approach to flavor analysis. This is most easily done by splitting the effluent from the GC so that the aroma contribution of each component can be determined by the use of the human olfactory system as a detector in conjunction with a conventional detector(1). Obviously, since the appreciation of flavor and aroma is a human trait, the professional "taster" is not likely to be completely replaced by instrumental techniques. Rapp et al. (2) discussed the important aspects of flavor analysis. One very important consideration is that sensory responses vary over several orders of magnitude for different compounds, meaning that trace components may make a far greater contribution to the flavor or aroma than major constituents. The flavors and aromas are complex mixtures of compounds of a wide range of polarities which complicates the analytical process. Finally, the sampling method must yield a true representation of the flavor or aroma without the development of artifacts. Vitzthum and Werkhoff (3) ratioed three components of coffee aroma to provide an index of freshness of whole or ground roast coffee beans. Parliment and Scarpellino (4) examined blueberry essence profiles to identify some of the major components responsible for its characteristic flavor. This is useful in the preparation of an artificial flavor with a more natural taste. Profiling is also useful in determining the cause of "off-flavors" (5,6). Moshonas and Shaw (6) collected

essence from oranges that were harvested before and after the January, 1982 freeze in the southeastern United States. GC and organoleptic data were compared. The gas chromatographic data clearly showed the detrimental effect that the freeze had on the flavor of the orange essence. They also used the technique to judge the effects of variety , temperature of storage and seasonal blending. Nordby and Nagy (7) used the hydrocarbon profile of sweet oranges as an indication of maturity.

Profiling has also been used in the evaluation of the flavors of wines and other alcoholic beverages (1,2,8,9) including classification by variety (1,10) and geographical origin (10,11,12). This information is then applied to the rating of quality and the improvement of flavor. Schreier and Reiner (12) used GC profiling to distinguish cognac from brandies and to distinguish between German grape brandy and French grape brandy.

Varietal differences in mango (13,14), apple (15), coffee (16) and other fruits and vegetables (17,18) have been investigated. It is important to be able to distinguish between different varieties of certain foods because often one variety is much cheaper and less desirable than the other. Profiling yields less subjective evaluations of product quality and variety. Engel and Tressl (13) used chromatographic profiling of the volatile components of two different varieties of mango to distinguish between them

and to identify 114 flavor components. Biggers, et al. (16) were able to differentiate between the two major varieties of coffee on the basis of their GC profiles. This is important in the task of blending various coffees to produce the best product at the lowest cost. Flavor profiles have also been used to determine the effects of aging on flue-cured tobacco (26).

Forensic applications of profile analysis are many. Lee, et al. (27) obtained profiles of engine oils such that they were able to differentiate one engine from another. This would be of value in the investigation of stolen automobile parts or "hit and run" accidents in which oil on the clothing of a victim could be matched to the oil on a suspect vehicle. Levy and Wampler (28) profiled toner materials from photocopiers for use in questioned document examinations. They were able to easily differentiate between 9 different toner systems. Other forensic applications include the characterization of automotive paints (29), volatile accelerants in arson cases (30) and correlation of samples of marihuana (31,32), opium and morphine (33) and heroin (34) with their respective origins. Saxberg, et al. (35) applied profile analysis to the detection of "counterfeit" whiskies. Some of the less reputable bars and restaurants cut costs by putting cheap non-Scotch whiskey in expensive whiskey bottles such as Chivas Regal. These authors were able to distinguish

between whiskies of different quality but not between whiskies of the same quality.

Profile analysis has been applied to the identification of microorganisms with varying degrees of success (36-40). Blomquist, et al. (36) were able to differentiate between three varities of the mold Penicillum but warned that variables such as cultivating medium, drying technique and strain of microorganism must be strictly controlled. Zechman, et al. (38) profiled the volatile metabolites of four bacterial species and found that they could differentiate two species from each other and from the other two. The profiles of the other two species were much alike.

Brill, et al. (41,42) profiled the cuticular hydrocarbons of the black imported fire ant in order to determine whether there were significant differences between the profiles of different colonies. Distinct differences between the profiles of different colonies led the authors to speculate that these compounds may be involved in colony recognition since they are known to be involved in insect communication.

The study of pollution in its many forms has involved profile analysis. Dunn, et al. (43) have investigated polychlorinated biphenyls (PCB's) in environmental samples. Dreisch and Munson (44) presented extensive information on the use of purge and trap sampling of certain priority

pollutant organic compounds coupled with profile analysis and gas chromatography/mass spectrometry (GC/MS). In a similar application, Dowty, et al. (45) found 13 halogenated hydrocarbons in New Orleans drinking water and five in blood plasma. Bertsch, et al. (46) identified more than one hundred organic compounds in their study of air pollution and found that the air pollution profiles varied only in quantitative amounts over an eighteen-month period. The fingerprinting of hydrocarbon pollutants and crude oil spills is a complex task, primarily due to weathering (degradation of the sample due to exposure to the environment). Jeltes (47) used the higher boiling fraction to limit the effects of weathering. He was able to identify the basic type of pollutant such as mineral oil gasoline, fatty oil, etc. Kawahara (48) used a combination of GC profiles and infrared spectroscopy to identify the type and source of pollution of residual fuel oils. Other workers (49,50,51) were able to classify crude oils according to their geographical origin. Trace quantities of petroleum type pollutants have been investigated in marine sediments (52) and in oyster and mussel (53), the latter being used as an index of oil pollution. Other applications of profiling include the characterization of cigarette smoke (54), mouth odor (55) and polymers (56).

Profiling has been extensively applied to the study and diagnosis of diseases and metabolic disorders which has

collectively been called "metabolic profiling" (57-130). The area of metabolic profiling was pioneered by Horning (57,65), Zlatkis and Liebich (58), Jellum (60) and Pauling (61). Jellum (131) and Gates and Sweeley (132) have written excellent reviews on metabolic profiling. Liebich (133) has extensively reviewed the use of GC/MS in clinical chemistry. Jellum has made major contributions in the area of metabolic profiling and he effectively sums up the operating principles involved in this area of medicine and research:

> One of the trends in modern biomedicine is the increasing understanding that many, if not all, diseases may be linked in some way with deviations from, or alterations in, one or more of the several thousand chemical reactions that normally take place inside the cells and body. It does not seem unreasonable to assume that if one were able to identify and determine the concentrations of all compounds inside the human body, including both high- and low-molecular-weight substances, one would probably find that almost every known disease would result in characteristic changes in the biochemical composition of the cells and of the body fluids. Such a "total" analysis is, of course, impossible to carry out at present. However, the promising results achieved during the last few years have substantiated the importance of multicomponent analyses. Chromatographic profiling of the body fluids in particular has been useful both for diagnostic purposes and for obtaining new information about the biochemical reactions that take place inside the body in healthy and diseased states (131).

Since it is currently impossible to obtain a profile of all constituents in a biological fluid or tissue, most analysts choose to profile a select class of substances,

such as organic acids (57,59,63,65,66,67,76,78,80,84, 86,87,95, 101,103,104,105,107,109,111, 114, 116-120,129), alcohols (62,88,107,115, 119), steroids (59,65,69,71, 83,92,94,97,100,128), ketones (90,98,107,115), carbohydrates (65,72,107,111,116), amines (85,107), or volatiles (58,61,62,68,70,73,74,75,77,81,82,91,93,96,98, 102,106,107,108,115,121-127). Much work has been done on diabetes (62,74,84,86,88,96,98,105,106,115,121-124,126, 127). In addition, the technique has been applied to maple syrup urine disease (111,114), bacterial or viral infections (79,91,96,113,122), schistosomiasis (130), rheumatoid arthritis (66), cataracts (72), renal insufficiency (73), Alzheimer's disease (85), Reye's syndrome (87), cancer (92, 98,116), stroke (104), cystic fibrosis (110), hereditary progressive loss of hearing (111), male infertility (111), cirrhosis of the liver (118) and many others.

In metabolic profiling, the identification of abnormal peaks (or absence of a normal peak) may lead to a better understanding of the disorder and perhaps to improved methods of treatment. It may also be used in the diagnosis of particular disorders. Jellum, et al. (107,112) have established a routine screening procedure involving several extractions and derivatizations, eight different gas chromatographic systems, mass spectrometry and a small computer. With this system they are able to diagnose about

40 metabolic disorders and have discovered at least four new ones (107). They investigated the urinary organic acids of a large family in Norway suffering from hereditary progressive loss of hearing. This disorder results in deafness at the age of 30-40 years. They found that the patients had accumulated large amounts of 3-methylcrotonyl-glycine and 3-hydroxyisovaleric acid which are intermediates in the metabolism of leucine, suggesting impaired activity of the enzyme, 3-methylcrotonyl-CoA carboxylase (111). Incidentally, the patients had no other clinical symptoms. The authors had not at that time proven that this enzyme defect is the cause of deafness.

Just as Jellum's research group have investigated organic acids, Zlatkis and his group have studied the relationship between organic volatile compounds and various disorders. In one study (122), they were able to use serum volatiles in the classification of normal and virus infected serum at a success rate of more than 85%. They also indicate that the same method may be a possible means of assessing virus susceptibility.

Ng, et al. (87) report the identification of long chain dicarboxylic acids not previously reported in association with Reye's Syndrome. They state that further investigation is necessary to determine whether this is specific for Reye's Syndrome or accompanies severe mitochondrial disease.

Jellum, et al. (116) profiled tissue extracts from brain biopsies in order to classify the tissue as normal brain tissue, pituitary tumors or "brain" tumors. The components profiled include fatty acids, carbohydrates, amino acids and cholesterol. They were able to distinguish between normal tissue and tumors but were not able to classify the tumors according to type.

Jakobs, et al. (114) utilized capillary GC for the first time in the investigation of maple syrup urine disease (MSUD). This is a thoroughly investigated metabolic disorder which is characterized by the patients having a distinctive odor of maple syrup and increased urinary excretion of certain 2-keto and 2-hydroxy acids as well as many other organic acids. These authors report the identification of a previously unreported metabolite, 2-hydroxyisobutyric acid, in association with MSUD.

Sample preparation techniques vary according to the class of component under study and the type of sample. Extraction with organic solvents and injection of the concentrate is a frequently used method (8,20,30,31,34, 36,45,48,53,64,73,75,90). Some workers have used direct injection of headspace samples in studying the more volatile components (13,14,15,17,22,32,38,62,71,79), others have concentrated the headspace components on glass wool or on a porous polymer such as Tenax or Porapak Q with subsequent thermal desorption into a cold trap precolumn

and then into the analytical column (18,21,25,44,45,46, 55,68,70,73,81,82,88,106,115,121,123,124). Brettell and Grob have written an excellent review on cryofocusing and cryogenic trapping (135). The analysis of organic acids or steroids require protein precipitation, extraction, derivatization, concentration and then injection onto the analytical column (59,60,65,66,67,69,71,76,78,80,83,87, 92,94,95,97,100,101,103-105,107,109,111,113,114,116-118). Pyrolysis is often used to study macro-molecules such as polymers (56) the classification of fungi (36,37) bacteria (136), the detection of cystic fibrosis heterozygotes (110) the forensic examination of photocopier toner (28) and automotive paints (29). Most of these methods require samples of 5 to 50 mL. Zlatkis and co-workers, (62,74,91, 96,98,102,122,125) have developed a method for headspace and/or extraction of samples of 50-100 microliters using an apparatus that they developed and call a "transevaporator." They have also developed a method of on-column injection of large liquid samples (64,99). Jennings, et al. (137) reviewed sample injection in GC.

Analysis of the data takes many forms. The simplest is the visual examination of the chromatogram, comparing peak heights and retention times. For obvious and major differences in the profiles, manual pattern analysis is sufficient. However, for subtle differences in the patterns, particularly in metabolic profiling, the

application of pattern recognition is necessary. Several pattern recognition computer programs have been developed and utilized (41,42,43,49,110.121,138-145). Varmuza (139) and Albano, et al. (145) have included a discussion and review of pattern recognition techniques.

Human skin oils have been extensively analyzed. Generally referred to as human skin surface lipids (SSL), they are a very complex mixture which is composed of 11-13% squalene, 20-22% wax esters, 2-3% sterol esters, 30-33% free fatty acids, 1-2% sterols and 29-32% glycerides (146). SSL are synthesized in the skin, the major part originating in the sebaceous glands with a small quantity being produced by the stratum corneum cells of the epidermis. Sixty percent of human sebum is composed of triacyl glycerols, part of which are then hydrolyzed in the gland ducts and on the skin surface to produce free fatty acids, glycerol, and mono- and diacyl glycerols. The fatty acids and glycerides exist in great variety in the SSL; some 200 species more than in the body tissues (147).

Downing (148) and Gloor (149) have reviewed the analysis of sebum and SSL. Thin-layer chromatography (TLC) has been used to fractionate and quantitate the major classes of components of SSL. The fractions obtained by preparative TLC have been studied by GC (146,150,151,152). Boniforti, et al. (152) examined the free fatty acid fraction and the fatty acids of the triglyceride fraction

for two subjects over a 6-7 week period. They identified the fatty acids by the number of carbon atoms and found that the percent of certain fatty acids varied from week to week for each of the subjects. Green, et al.(150) analyzed the fatty acids of the wax ester fraction taken from ten subjects over an eight-week period. They found that the subjects maintained essentially the same fatty acid composition over the test period and that large differences were seen between subjects. They feel that this difference may be the basis for olfactory recognition of individuals by animals with a keen sense of smell. Investigators usually prepare derivatives of the lipid fractions before GC; but, Goetz, et al. (146) demonstrated that the lipid extract could be analyzed without prior derivatization on an OV-1 capillary column. This yielded a complex chromatogram in which the authors showed the elution ranges of the different fractions. Wolff (151) investigated the use of SSL as a noninvasive technique to estimate the human body burden of persistent halogenated hydrocarbons. For 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (p,p'-DDE), this technique yielded results comparable to adipose tissue or serum. It was less successful for other compounds existing at lower concentrations.

Sampling techniques vary from absorbing the lipids onto cigarette paper (151) to placing a solvent in contact with the skin (152), rinsing the hair with ethanol (150) or

deposition on ground glass platelets (146). The composition of the resulting sample depends upon the sampling method due to the differing extractions of lipids from the epidermis, the skin surface and from the sebaceous gland secretory ducts (149). The quantity of SSL varies over the human body, with the greater amounts being found on the forehead and scalp (149).

Analysis of SSL could be of value in forensic applications. A general premise in forensic science is that ". . . every contact leaves its trace" (153). This statement is known as the Locard exchange principle from the contributions of Edmond Locard (1877-1966) (154). Paul L. Kirk (1902-1970)(154) has stated "It is virtually impossible for a criminal to commit a crime without leaving evidence behind and carrying evidence away with him" (153). Evidence of this nature is called trace evidence. As its name implies, this evidence exists in small, often microscopic, quantities.

In a case in which a victim is assaulted by an attacker, there is the opportunity for the exchange of trace evidence that may later be used to link the assailant to the scene of the crime. In such a situation, there may be the opportunity for SSL to be transferred from the face of one to the clothing of the other.

The author's background in forensic science coupled with an interest in profile analysis and the results of

Green (150) led to the hypothesis that SSL may produce a profile that is characteristic of an individual. No reference of the profiling of SSL was found in the current literature. To this end, samples from 23 subjects were examined. This work focused on developing a sampling method that would most nearly approximate that which would occur during the commission of a crime and on determining whether a simple solvent extract with no derivatization or fractionation could yield suitable individual profiles. In addition, the use of the transevaporator in conjunction with thermal desorption was investigated with the intent of increasing the sensitivity of the method to be able to analyze the trace quantities often encountered in forensic cases.

CHAPTER 2

METHODS AND MATERIALS

For clarity, the discussion of methodology is divided into four sections: A. sample collection, B. analysis by split injection, C. computer analysis of data and D. transevaporator sampling method.

Sample Collection

Skin surface lipid (SSL) samples were collected from 23 volunteers. First, the volunteers were given an alcohol swab to remove any contaminants from the touching surfaces of their fingers. Next, they were provided with one-inch squares of "Kimwipe" (trademark of Kimberly-Clark) tissue and instructed to wipe their forehead (or cheek) so as to saturate the tissue with as much skin oil as possible. They then placed the samples in individual, labeled, two-dram glass vials with foil lined screw caps. The samples were stored at 6°C in a refrigerator until analyzed. Samples were taken from these volunteers on two occasions over a time period of eight days in order to establish the short term variability of an individual's SSL profile.

Analysis by Split Injection

Instrumentation and Conditions

Analyses were conducted utilizing a Varian Vista 6000 capillary gas chromatograph (GC) which was fitted with a "split/splitless" capillary injector and a flame ionization detector (FID). Separations were carried out on a BP-5 (Scientific Glass and Engineering--SGE) fused silica capillary column, 25 m x 0.2 mm I.D. Helium was used as the carrier gas with a head pressure of 32 psig and a flow of 1.6 mL/min. Nitrogen was used as the detector make-up gas to obtain a flow of 30 mL/min through the detector. Air and hydrogen were used as the flame gases with flows of 300 and 30 mL/min respectively. The FID temperature was 340°C with a range of 10^{-12} and an attenuation of 64. The baffle glass insert was used in the injector in the split mode. The injector temperature was 250°C and the split ratio was 42:1. The initial column temperature was 80°C. Upon injection, the temperature was programed to 260°C at 10°C/min and then to 290°C at 3°C/min the temperature was held at 290°C for 47 minutes for a total run time of 75 minutes. After the completion of the run the column temperature was held at 290°C for an additional 18 minutes to purge the remaining sample from the column.

Data collection was performed by a Perkin-Elmer Model LCI-100 integrator which transmitted the data to a Perkin-Elmer Model 7500 laboratory computer where the data

was analyzed and stored. The 7500 was loaded with Chromatographics III (Perkin-Elmer, Version 2.0) software for data acquisition, analysis, and storage. Figure 1 is a computer printout of the parameters of the method used to acquire data. An appropriate method can be generated for each type of analysis.

Adsorbents, Reagents, and Materials

"Kimwipe" was chosen as the collecting medium because a pentane extract of "Kimwipe" yielded a very clean blank (Figure 2). Whatman #1 filter paper was also investigated but it showed the presence of contaminants at the concentration level being studied. Pentane (Burdick and Jackson, high purity) was used as received. Two-dram glass vials with foil-lined screw caps (Wheaton, Supelco) did not show the presence of contaminants and were not subjected to further cleaning.

Sample Preparation

The one inch-squares of "Kimwipe" containing the SSL samples were cut into several strips approximately 4 mm x 2.5 cm. Two strips were placed in two-dram glass vials to which was added 200 uL of pentane and the foil lined cap screwed on tightly. After approximately 15 minutes of occasional agitation the samples were ready for analysis.

Figure 1. Computer Printout of Data Acqiusition Parameters

METHOD# 26 : Wilson skoil I
: LCI-100/BP5/
CREATED : Sat Apr 19 16:14:42 1986
AUTHOR : L. Wilson
LAST MODIFIED : Sat Apr 26 10:53:32 1986
COLUMN : BP-5 25m .2mmid

This method is designed for the acquisition of data from the Varian Vista 6000 GC via the LCI-100. The LCI-100 will provide a real time plot only if one enters the necessary items on the plotter control page.

DATA HANDLING CHANNEL A

Method Type	Calculation Type	Peak Data	Calibration Sequence#
GC	NORMAL	AREA	0

DATA STORAGE

Start Time	End Time	Peak Width at Base
0.000	75.000	2.5 - 6.5

PEAK INTEGRATION

Lockout Time	Area Sens	Base Sens	Skim Sens	Baseline Treatment
0.000	200	70	10.000	B -to- B

QUANTITATION

Order of Fit	Unknown Resp Factor
0	1.00000e+000

PEAK IDENTIFICATION

Unret Peak Time	Comp Tol Fixed	Comp Tol Percent	Ref Peak Ret Time	Ref Peak Tolerance
0.000	0.020	0.500	0.000	0.100

REPORT

Device	Report to Disk	Print Tolerance	Peak Rej Area/Hgt
SYS PR	NO	0.00000e+000	1.50000e+004

PLOTTER CONTROL CHANNEL A

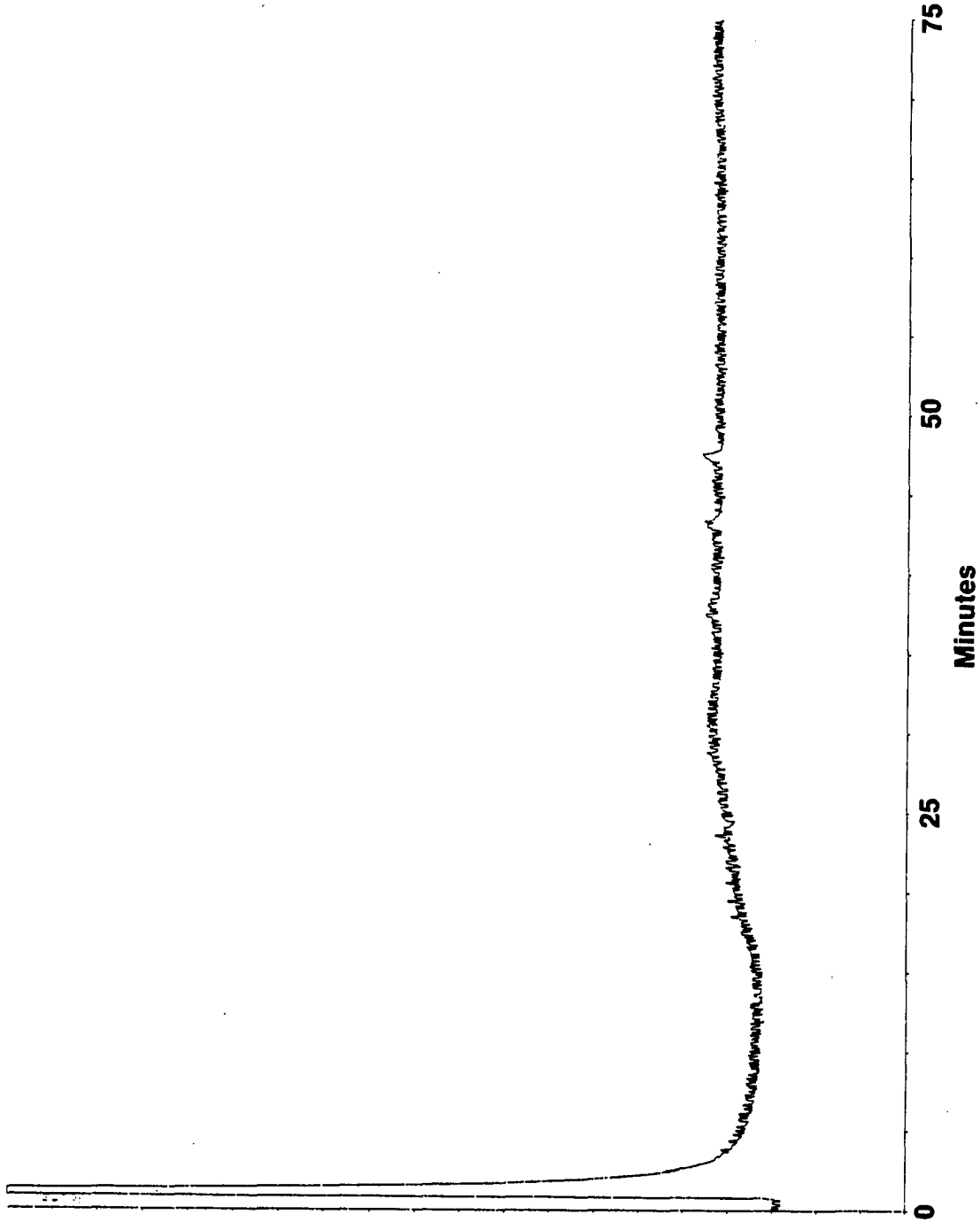
INITIAL VALUES

Chart Speed	Signal Attenuation	Pen Zero Offset
5	32	5

ANNOTATIONS

Tick Marks	Retention Times	Event Legends
NO	YES	YES

Figure 2. Pentane Extract of Kimwipe as Blank Run



Sample Analysis

Three uL of the pentane extract of the samples were injected into the GC without further sample preparation. The GC is fitted with a switch that starts the temperature program automatically. This switch was also connected to the external start terminals of the data system so that the data collection also begins simultaneously.

Computer Analysis of Data

The acquisition and storage of data by computer lends flexibility to the analysis. In this manner, data can be collected under one method and later reprocessed under another, perhaps more suitable, method. Stored data can be rescaled, subtracted or submitted to an applications program for further analysis. This is very useful in a situation in which the data is collected over a period of months or longer, because data acquired early in the project can be reprocessed. This allows the analyst to take advantage of many of the improvements made in the course of the study without having to re-analyze the samples.

The Chromatographics III system is a multi-tasking, multi-channel data collection and processing system. The software is divided up into six environments including: SYSTEM, OPERATE, METHOD, QUEUE ("queue" is a British term meaning "to form in a line." Here it is used

to line up several methods in succession.), DATA ANALYSIS and APPLICATION.

SYSTEM includes SELECT, CONFIGURATION, MONITOR, UTILITIES and DIRECTORY. SELECT allows one to choose which of the four "chromatographs" one wants to work in. CONFIGURATION is used to set up the system configuration composed of integrators, printers and plotters. MONITOR generates a screen display of all data currently being collected. UTILITIES includes various operations such as archiving data, formatting floppy disks, setting the system clock, retrieving data, etc. DIRECTORY is a function that will permit the operator to access the directory of method files, peak files, data files or queue files at any time.

The OPERATE environment includes SETUP, METHOD, QUEUE, PLOT, and STATUS. SETUP sets up a remote integrator for data collection using a particular method. METHOD will display the method in use and allow certain modifications. QUEUE will permit the display and modification of queue entries if it is being utilized. PLOT allows one to view the real-time acquisition of data in plot format. STATUS will show the current status of data collection including any reasons for not ready status, etc.

The METHOD environment is used to generate a method that is tailored to the particular analysis including those involving quantitation. Methods may also be modified, linked, calibrated and stored.

QUEUE is used to process data from a series of samples using different methods or perform automatic calibration of a method, etc.

DATA ANALYSIS permits the re-analysis of data from previously collected files. Options are: REPORT, METHOD REPROCESS, METHOD DEVELOPMENT, INTERACTIVE REINTEGRATE, REPLOT, and PRINT PLOTS. REPORT permits the generation of a new report using a selected method. METHOD REPROCESS will generate new peak files using either the same or a different method. The METHOD DEVELOPMENT process is used to customize a method to a stored chromatogram. INTERACTIVE REINTEGRATION can be used to manually reintegrate individual plots. REPLOT permits the display of one or more plots by split screen or overlay, the subtraction of one plot from another and the labeling of certain features. PRINT PLOTS permits the plotting of one or more chromatograms.

APPLICATIONS permits the generation of Basic (or Fortran) programs and the processing of data using them.

For the comparison of two chromatograms, the REPLOT environment was used. First, the two plots of interest were brought up in the "overlay" mode. Next, "scale X" and "scale Y" were used to put the peak of interest on scale. The baseline of the secondary plot was adjusted to that of the primary plot and then the peak height of the secondary plot was adjusted to that of the primary plot for the peak

of interest. This is a way to normalize one plot to another using a common peak. In addition, the retention time of this peak in the secondary plot was adjusted to match that of the primary plot. These changes were "accepted" and then a "scaled data subtract" was performed on the plots. The subtracted chromatogram was stored for later replotting. The subtracted chromatograms very effectively pointed out even the minor differences between chromatograms and was an important tool in the visual comparison of chromatograms. Frequently, the latter part of the chromatograms displayed some slight retention time differences which showed up on the subtracted chromatograms as a combination of a positive and a negative peak. If two peaks of approximately the same height (one near the beginning of the chromatogram and one later in the chromatogram) had been chosen, these retention time differences could have been eliminated in the manipulation of the chromatograms before the subtraction was done. This was not done in this case because the peak chosen to scale the data was far larger than other peaks in the chromatogram and because both peaks must be on the screen at the same time, thus yielding little visible retention time difference for capillary chromatograms of 75 minutes. The reference peak was chosen because it appeared in each chromatogram and it seemed appropriate to scale other peaks to it as a natural internal standard. It was not practical

to add an internal standard to the samples because of the uncertainty in the quantity of skin oils on each piece of tissue.

Transevaporator Extraction, Thermal Desorption and Cryofocusing

Adsorbents, Reagents, and Materials

Grade five helium was used as the carrier gas in the transevaporator extraction. A dessicant/molecular sieve filter (Foxboro/Analabs) and an activated carbon filter (Foxboro/Analabs) were used in line to further purify the helium. All glassware was washed in Micro (International Products Corp.) solution, hot water, distilled water, acetone and pentane and then baked in a muffle furnace at 500°C. Pyrex brand glass wool (Corning) was heated in a muffle furnace at 500°C. Untreated glass beads, 80/100 mesh (Alltech) were washed with Micro solution, nitric acid, distilled water, acetone and ethyl ether and then heated in a stream of helium at 280°C for 24 hours. The pentane, Kimwipe and glass vials were used as described in a previous section. The glass tubing used for sample tubes and traps were 1/4" O.D. and 2 mm or 4 mm I.D. Pyrex (Ace Glass). The glass tubing was cleaned with Micro solution, hot water, distilled water, acetone and pentane and then heated in a muffle furnace at 500°C. Nylon gloves (American Scientific Products) were used when handling the extraction glassware. The glassware for the

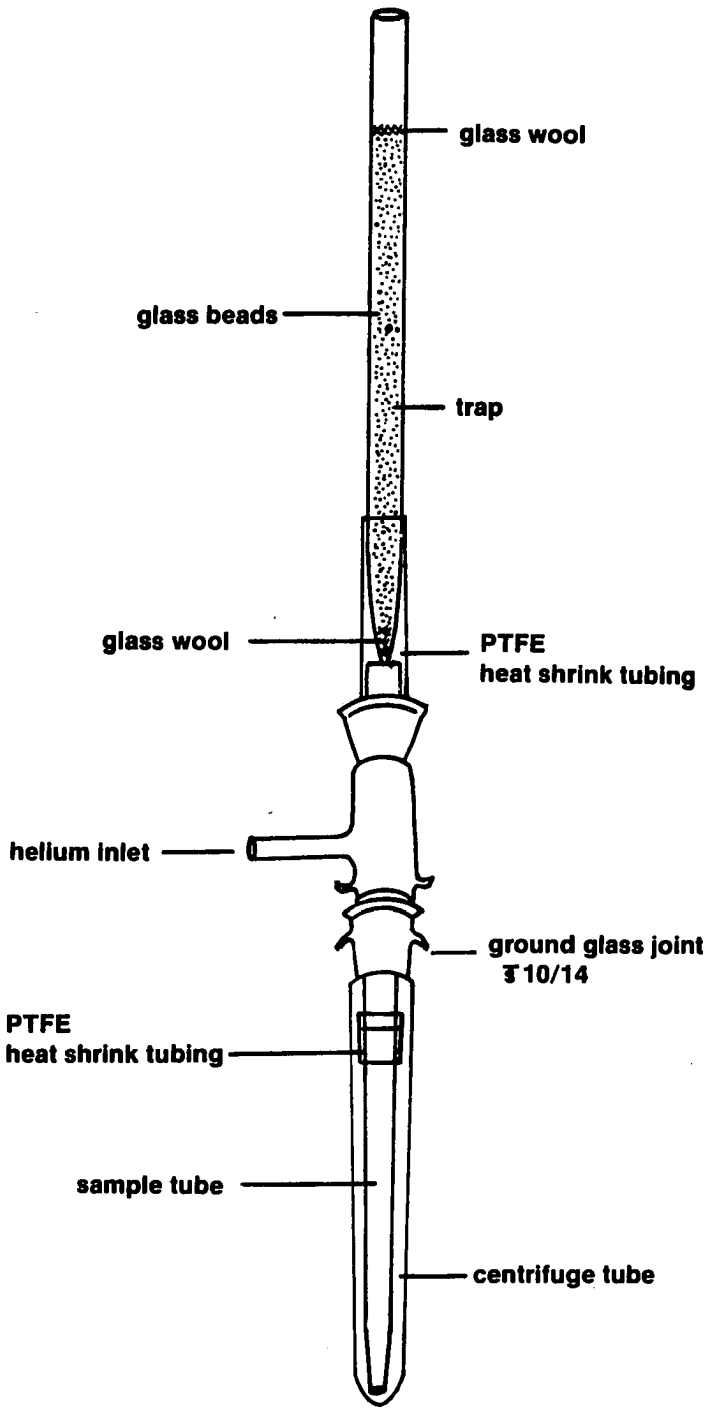
transevaporator was made by Southeastern Laboratory Apparatus.

Apparatus Design and Instrument Modification

The transevaporator design (see Figure 3) was basically that of Zlatkis, et al. as reported in Brazell's dissertation (98) with a few modifications. The glass bead trap was reduced in size from 8 mm I.D. to 4 mm I.D. and increased in length from 11 cm to 17 cm. It was felt that a reduction in diameter would make the thermal desorption more effective. The increase in length was done to retain trapping effectiveness. The trap was tapered on one end to a diameter of approximately 3 mm to coincide with the exit of the trap furnace. The small end of the trap was packed with a plug of glass wool then with glass beads and another plug of glass wool such that 4 cm of the large end of the trap was left empty. This assured that the packed portion would be positioned in the heated area of the furnace (Figure 4,5). The sample tube was 8.4 cm long with 1/4" O.D. and 2 mm I.D. and tapered on one end so that it would reach to the bottom of the conical tip of the centrifuge tube. It was not packed with adsorbent for these analyses.

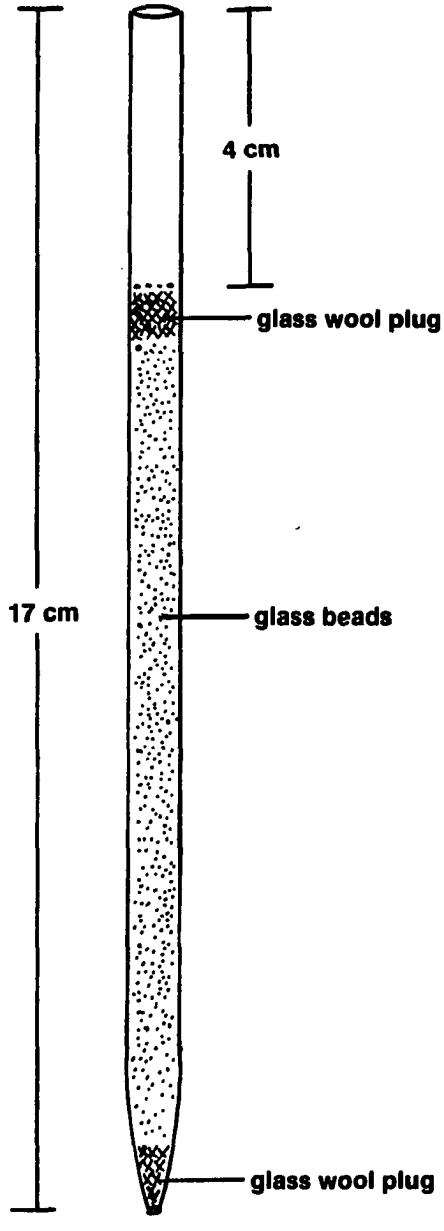
Empty fused silica capillary tubing, 0.32 mm I.D. (SGE) was used as the precolumn. Before use, it was silylated by treating with "Glass Treat" (Chrompack) and

Figure 3. Transevaporator Sampling Apparatus



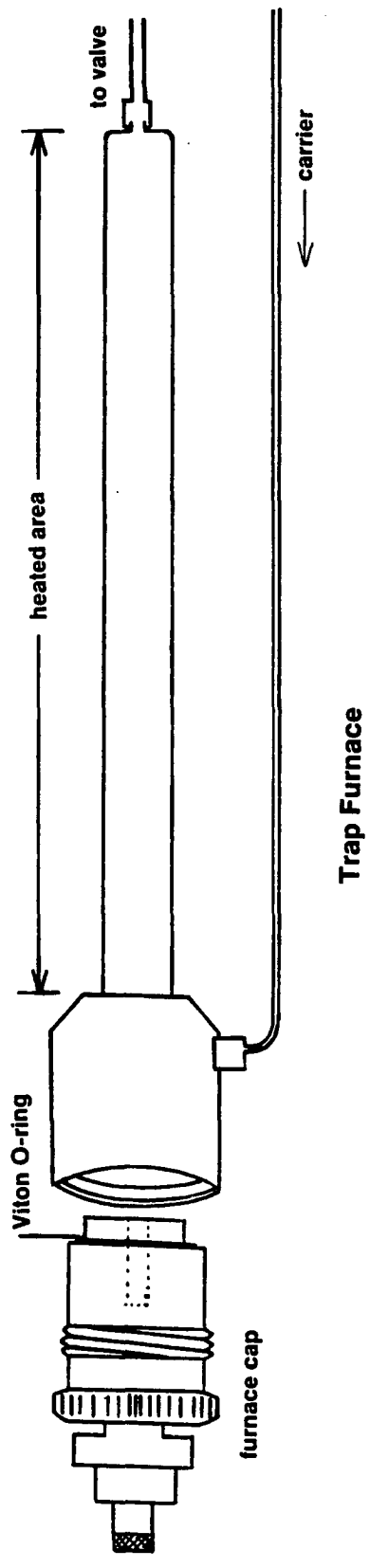
Transevaporator

Figure 4. Trap



Trap

Figure 5. Trap Desorption Furnace

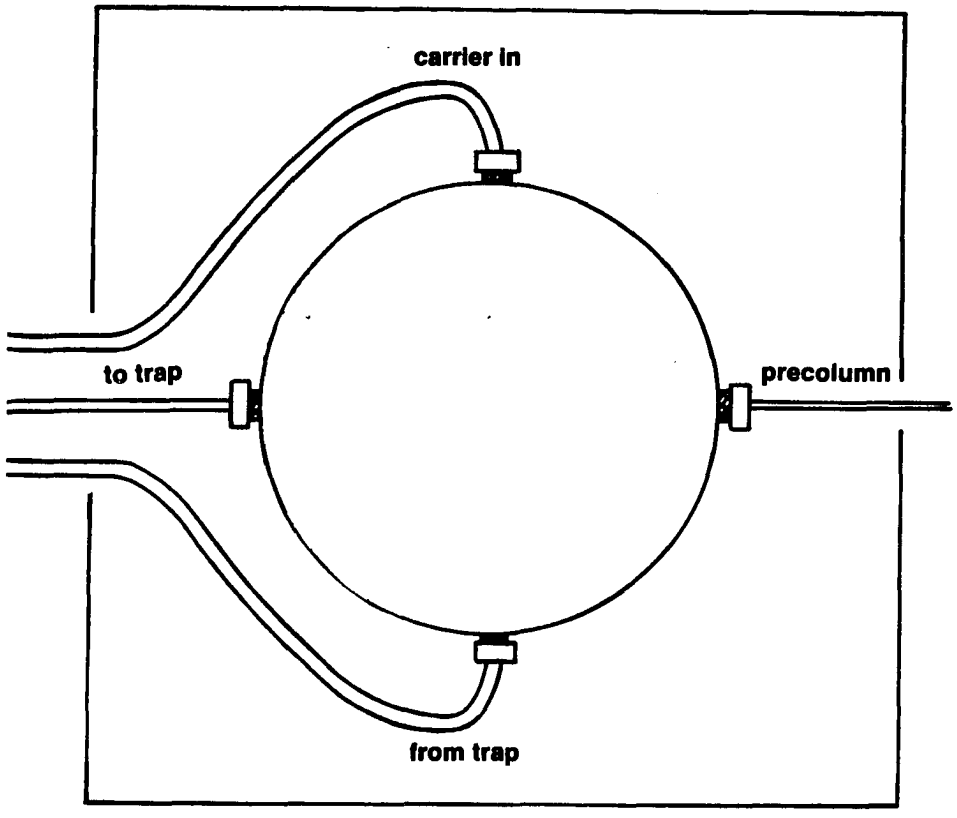


rinsing with methanol then flushing with dry helium. The fused silica tubing was very brittle after being exposed to the air for only an hour or so (whether silylated or not). It was subsequently determined that it should be installed as quickly as possible and heat treated immediately. If this was done no problems arose due to the breakage of the precolumn.

The trap furnace (Figure 5) was the 1/4" furnace designed for use in Tekmar's Model 5000 Thermal Desorber. It was fitted with Viton O-rings and attached to a Valco 4-port, high temperature, Hastalloy C 1/32" valve (Figure 6) via Hastalloy C tubing. Other connections to the valve were for carrier gas in to valve, carrier out to furnace, and flow out to the precolumn.

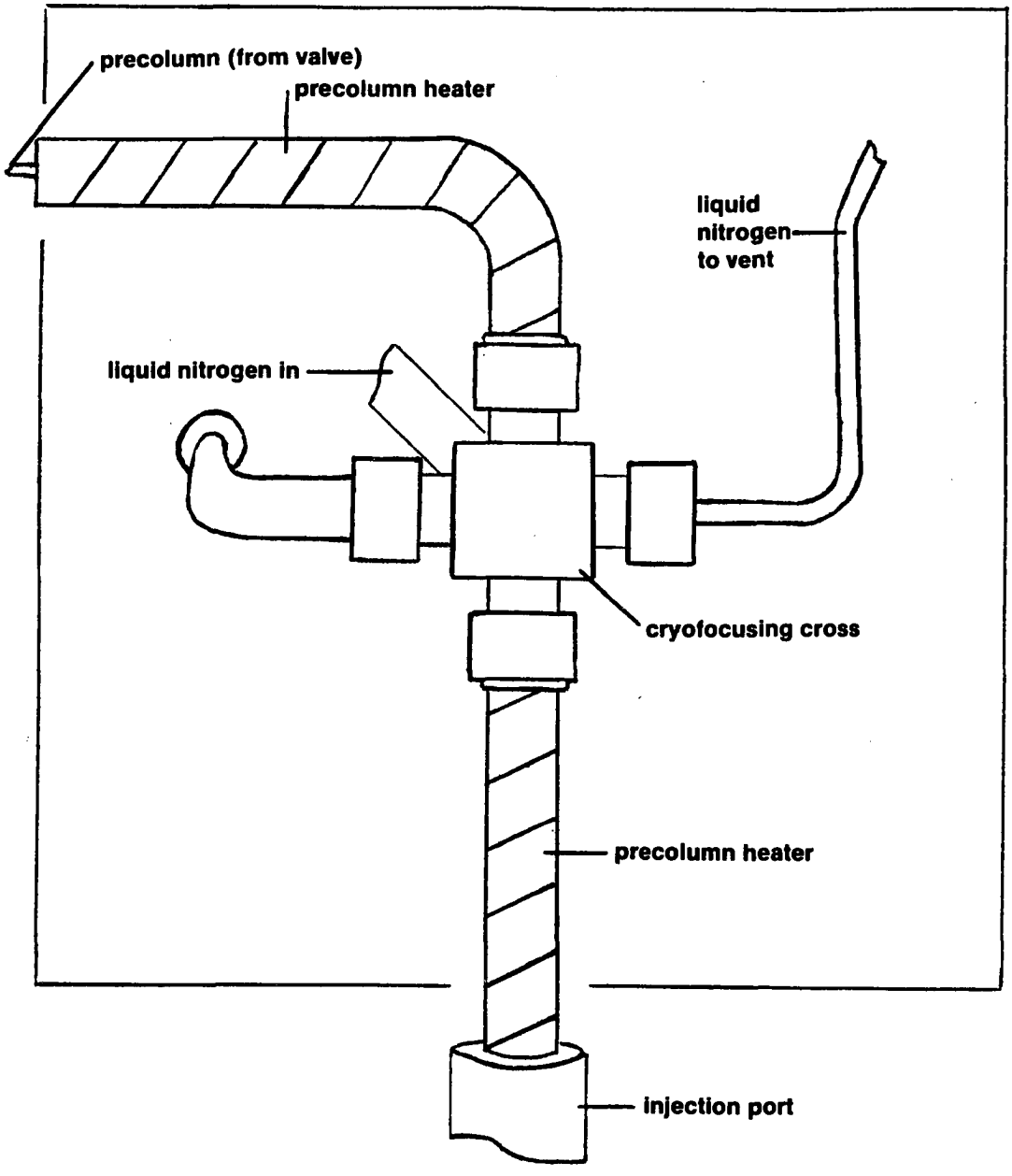
Tekmar's Model 1000 Capillary Interface (Figure 7)(hereafter referred to as the cryofocusing unit) was modified such that it could handle somewhat less volatile compounds than the purgeable halocarbons for which it was designed. This was accomplished by surrounding all exposed precolumn with 1/4" O.D., 2 mm I.D. glass tubing which was wrapped with a heat tape (Glas-Col) connected to a Variac transformer. This prevented the less volatile compounds from condensing in these areas of the precolumn, thus leading to better focusing of the sample. The cryofocusing unit was mounted over the injection port of the GC and the precolumn passed up through the top of the column oven,

Figure 6. Switching Valve



Valve

Figure 7. Tekmar Capillary Interface (Cryofocusing Unit)

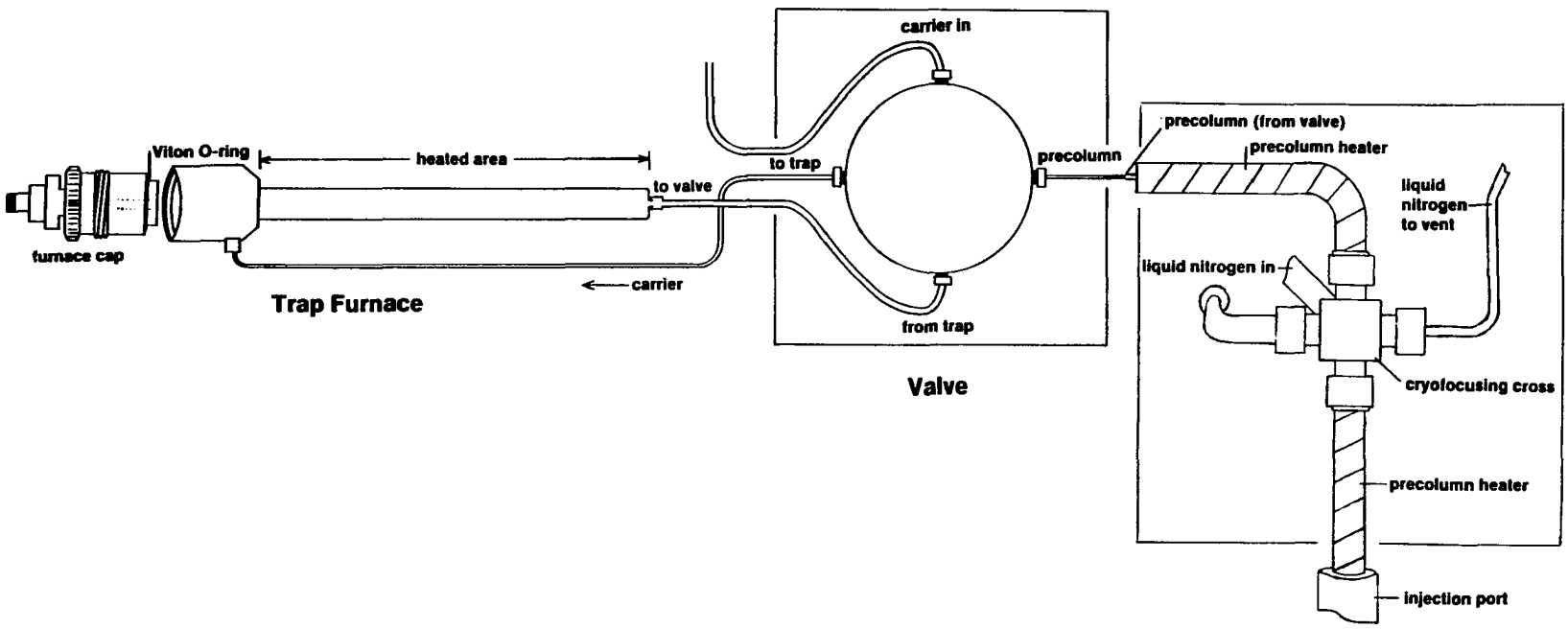


Tekmar Capillary Interface

through the injector and septum and up through the cryofocusing unit to the valve. This meant that the injector could be put back into service easily without dismantling. Figure 8 illustrates the entire combination of trap furnace, valve and cryofocusing unit.

The cryofocusing unit was designed such that, when used with the proper electronic interface, it could receive a ready signal from the GC and send a start run signal to the GC. The interface was purchased from Varian but after installation did not produce the correct communication between the two instruments. The interface was found to function properly only if the GC was interfaced to the Varian 401 data station which was not the case in our system. The electronic interface was discarded and two modifications were made. To obtain a pseudo-ready signal from the GC, a jumper wire was placed across the two "start cool" terminals on the back of the Model 1000 controller. Since there is a temperature probe in the GC oven that is connected to the Tekmar LSC-2 (not used in this project) the Tekmar sees a pseudo-ready signal when the GC oven is at or below the setpoint temperature for the GC oven. This feature was not utilized in this work. Instead, the desorption of the cryofocusing unit was started manually when the oven reached the proper temperature. A connection was made from "heat reed 1" on the back of the Model 1000 controller to the start run activator on the injection

Figure 8. The Combined Trap Desorption Apparatus



port. This would begin the column oven temperature program at the same time as the desorption of the cryofocusing unit. As mentioned previously, a connection was also made from the start run switch on the GC to the "external start" terminals on the integrator so that data collection would start simultaneously with the GC run.

Sampling Procedure and Conditions

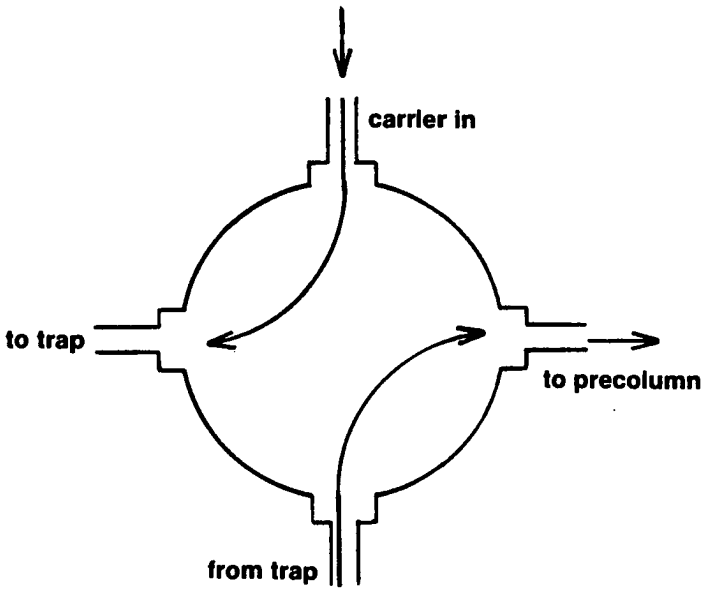
GC conditions and data collection methods were as described under the split injection mode. Other conditions will be described in the procedure detailed below.

1. The trap is conditioned at 350°C at a helium flow of 14 mL/min for 30 min. The glassware is heated in a muffle furnace at 500°C for one hour. The trap furnace and valve (desorb mode) (Figure 9A) are purged at operating temperature and helium flow of approximately 200 mL/min for 30 minutes. The GC column is conditioned at maximum operating temperature for at least 30 minutes and normal flow.

2. A small piece of Kimwipe containing the sample (usually 3 mm x 2.5 cm but dictated by the sample concentration) is inserted in a sample tube. The sample tube is then attached to the transevaporator by a pre-shrunk PTFE sleeve. The cool trap tube is attached, tapered-end down, to the top in the same manner (Figure 3).

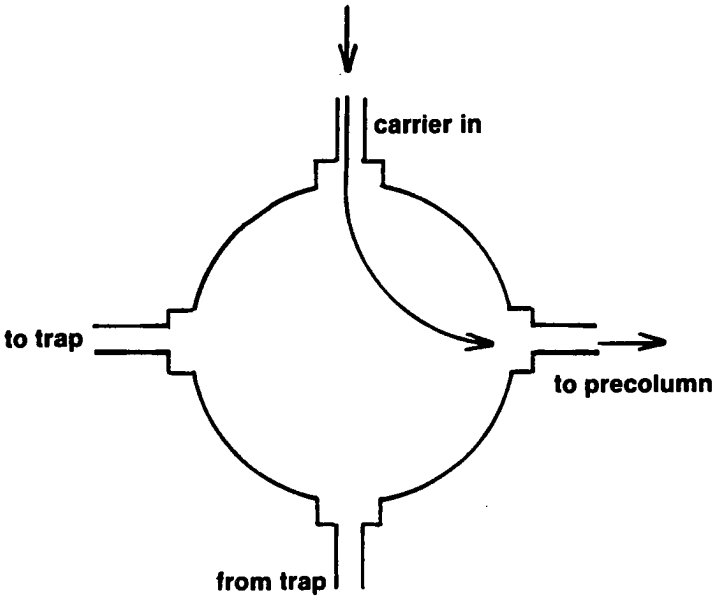
Figure 9. Valve in Desorb Mode (A) and Bypass Mode (B)

A



Valve in Desorb Mode

B



Valve in Bypass Mode

3. 300 uL of pentane is placed in the tip of the centrifuge tube which is then attached to the transevaporator body by a spring.

4. Helium at a flow of 7 mL/min is passed through the apparatus to push the pentane up through the sample tube and onto the trap. The helium flow is then increased to 14 mL/min to evaporate the excess solvent. This whole procedure takes five minutes.

5. The cryofocusing unit is cooled to -100°C with liquid nitrogen. When this is accomplished, the trap is quickly inserted into the pre-heated (280°C) trap furnace and the valve switched to the "desorb" mode (Figure 9A). The desorption of the trap occurs over a 7 minute period during which the flow through the trap is approximately 200 mL/min. This is done by opening the tee fitting that joins the precolumn to the analytical column. This high flow rate reduces the trap desorption time but also results in a partial loss of the more volatile constituents of the sample.

6. At the end of the trap desorption period, the tee fitting is closed and the valve is switched to the "bypass" mode (Figure 9B). A time of 2.5 minutes is allowed for the oven temperature to stabilize then the "heat-up" button on the controller is pressed. The cryofocusing unit is heated for 11 seconds to desorb the sample and the GC temperature program and data collection begin simultaneously.

7. The trap is removed from the furnace to avoid overheating the O-rings and to set up for the conditioning procedure mentioned in step 1. The glassware is placed in the furnace to prepare it for the next analysis.

CHAPTER 3

RESULTS AND DISCUSSION

Frequently during the commission of crimes of violence, there is contact between assailant and victim. This yields the opportunity for trace evidence to be transferred from one to the other. Evidence of this type usually includes hairs, fibers, soil and certain body fluids as well as many other types of evidence. Methodology for the analysis of these types of evidence is abundant; however, no reference to the profiling of skin surface lipid (SSL) samples was found in the current literature. SSL samples from 23 volunteers were examined to determine whether they would provide an individual specific "fingerprint" pattern upon visual examination of the chromatogram. In addition to determining whether the SSL profile is characteristic of an individual, the variability of individual profiles over time was determined. Other factors such as method of sample collection, exposure to ambient conditions, use of cosmetics and comparison of samples from different parts of the face were also considered.

Sample Collection

SSL samples were collected by having the subjects use a portion of a Kimwipe to wipe their forehead or other area under study. This method was chosen because it most nearly represented the way in which SSL would be transferred from assailant to victim or from victim to assailant. That is, during the crime, the skin lipids would be transferred by the rubbing of the face on clothing or other material and not by more elaborate chemical or mechanical extraction techniques. Gloor (149) states that the composition of the SSL is dependent upon the sampling technique. The SSL composition could be affected by environmental conditions such as food intake, frequency and style of bathing and contaminants. To investigate this point, samples were obtained from the subjects on two occasions eight days apart. This gave information on the short term variability of the profile. The only sample preparation performed was the extraction of the SSL from the Kimwipe with pentane. This method was selected to minimize the possibility of contamination. This is always an important consideration but it becomes of critical importance in forensic cases in which the guilt or innocence of the accused may depend upon the result of the analyses.

Reproducibility of the Technique

Figure 10 shows replicate injections of the same sample. Comparison of the two chromatograms show that the only difference between them are three small peak height differences between 13 and 19 minutes. That is three inconsistencies out of 114 points of comparison. The differences are pointed out on the figure with small arrows. Part C of Figure 10 is a subtracted chromatogram showing the differences between the two. In viewing the subtracted chromatogram, both here and in subsequent figures, note that the differences will be represented by either positive or negative peaks. The areas in the subtracted chromatogram that show both a positive and negative peak at essentially the same place represent a situation in which the retention times of a peak were slightly different, see Figure 10C at 27 minutes. This does not represent a true difference and these peaks should essentially be ignored when examining the subtracted chromatogram. Reproducibility was very good once the temperature program was extended to purge any sample remaining at the end of a run. Figure 11 shows replicate injections of a different sample. Out of 110 points of comparison, two small peak height differences can be seen at 23 and 48 minutes. The subtracted chromatogram is shown at the bottom of the figure.

Figure 10. Duplicate Injections of the Same Sample (A,B) and Subtracted Chromatogram (C)

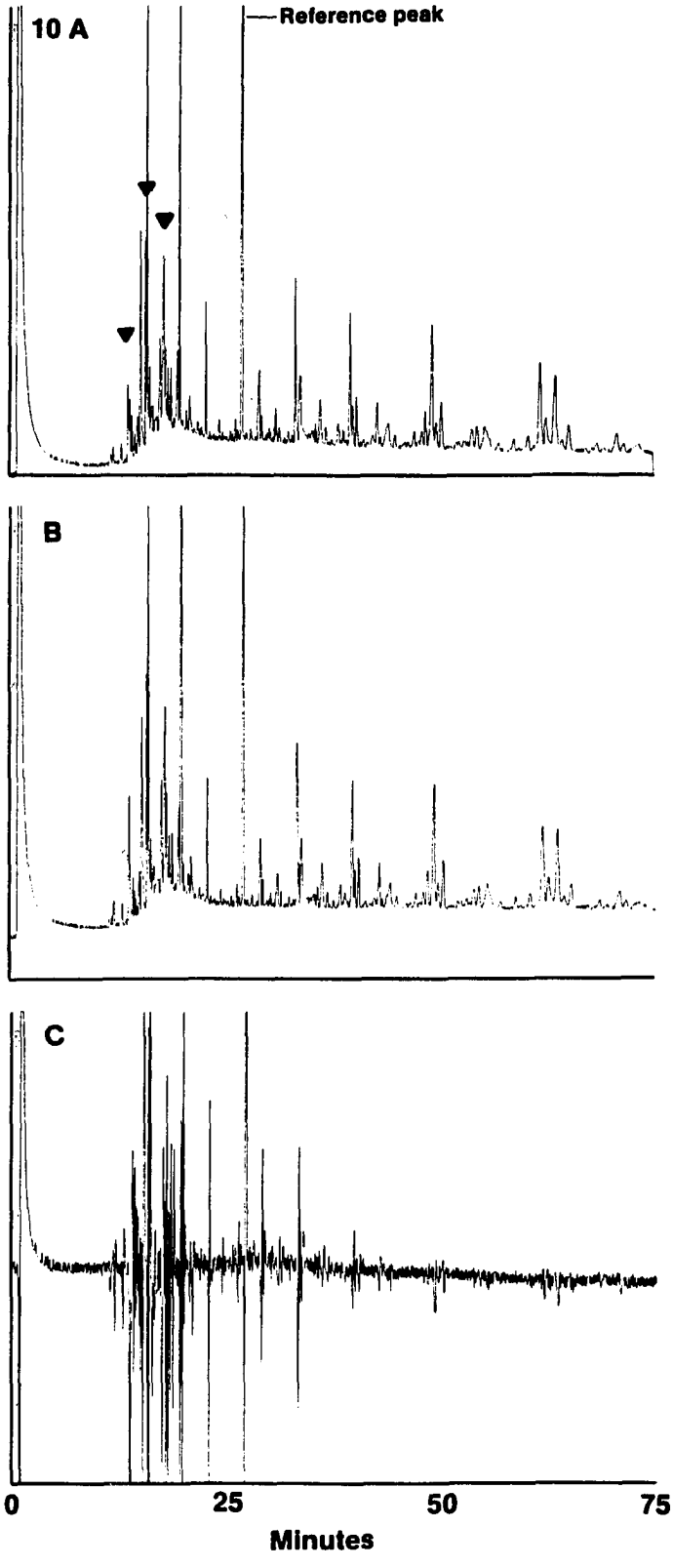
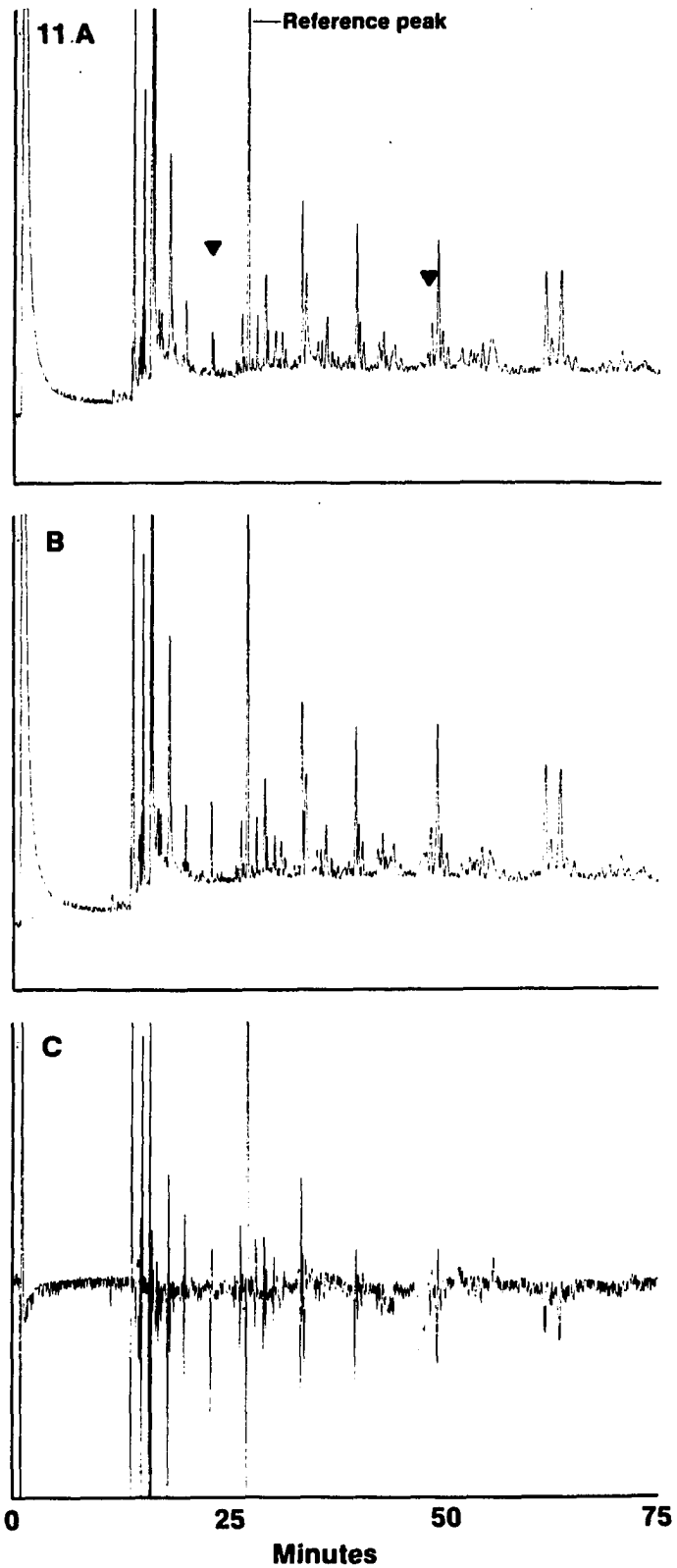


Figure 11. Duplicate Injections of the Same Sample (A,B) and Subtracted Chromatogram (C)



The Variability of an Individual's Profile Over Time

Two samples were taken over an eight-day period from each subject. Figure 12 is a comparison of the profiles from the individual that showed the least amount of variation over this period. Of 123 points of comparison, 6 minor differences can be seen at 16.8, 19.9, 20.7, 26.3, 43 and 47 minutes. Four major differences are in the region of 13.9, 14.6, 15.1 and 18 minutes. Minor differences are defined as being a change in peak height of less than 50%. Major differences are defined as being peak height changes of greater than 50% or the appearance of a new peak. The differences may be due to environmental or dietary changes. Figure 12C is a subtracted chromatogram of the two. Note that it is most useful in pointing out the larger differences. Figure 13 is a comparison of profiles from the individual that showed the greatest amount of variation over the same period. Of 125 points of comparison, minor differences may be noted at 12.2, 12.5, 17, 19.5, 20.7, 21.9, 25.7, 29.1, 30, 31, 36, and 47.1, minutes. There were major differences at 11.5, 13.7, 16.5 and 18.6 minutes. Figure 13C points out some of these differences. Basically, the differences between the profiles of the person showing little difference and those of the individual showing the most difference over time is that the former had 6 minor and 4 major differences and the

Figure 12. Profiles of the Same Person Taken 8 Days Apart Showing Few Differences (A,B) and Subtracted Chromatogram (C)

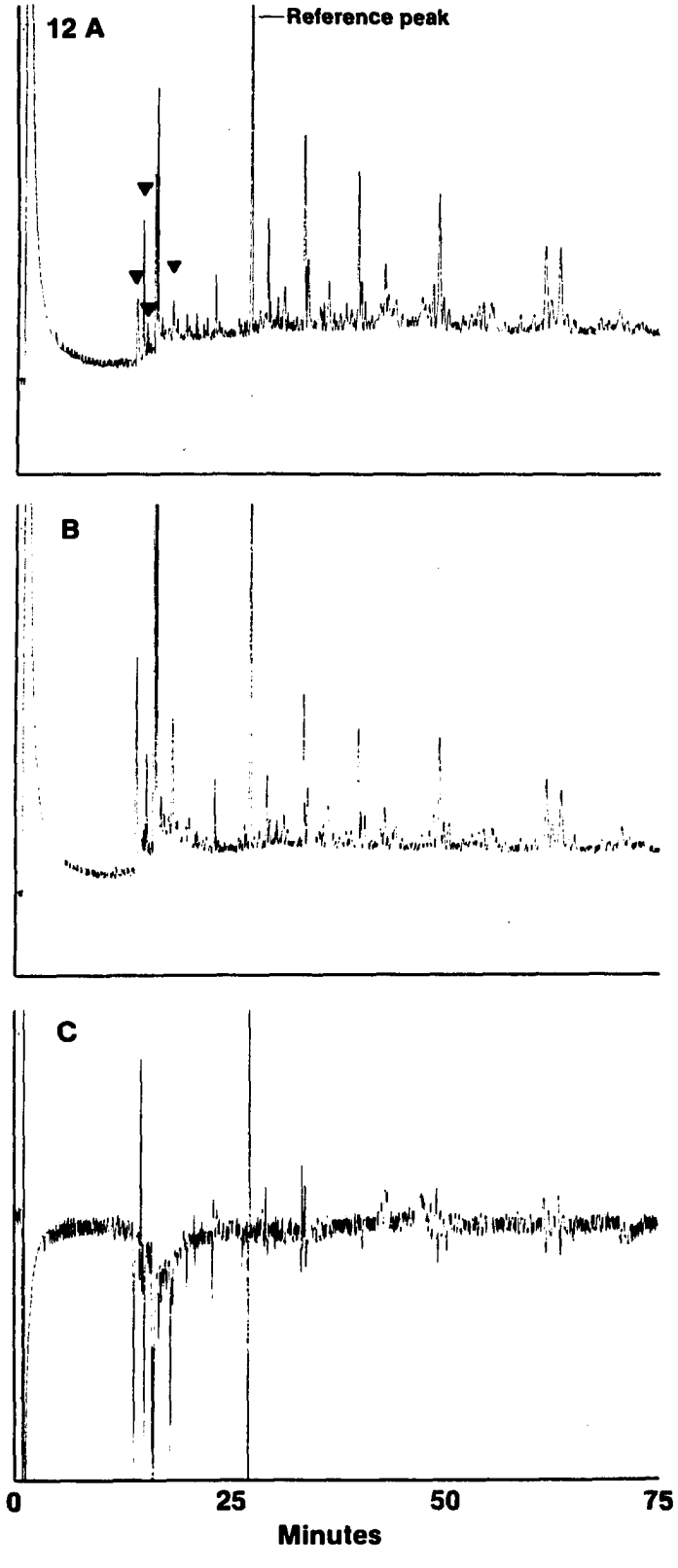
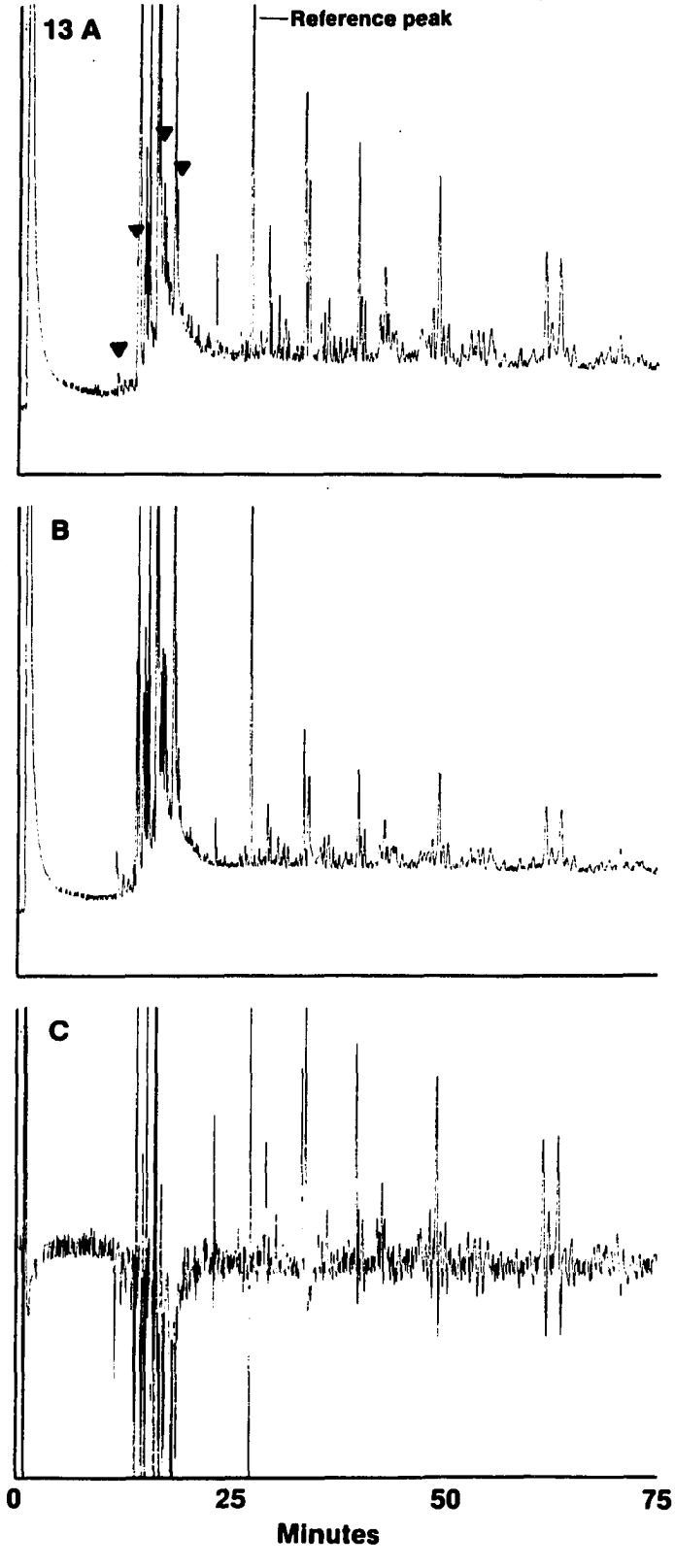


Figure 13. Profiles of the Same Person Taken 8 Days Apart Showing Many Differences (A,B) and Subtracted Chromatogram (C)

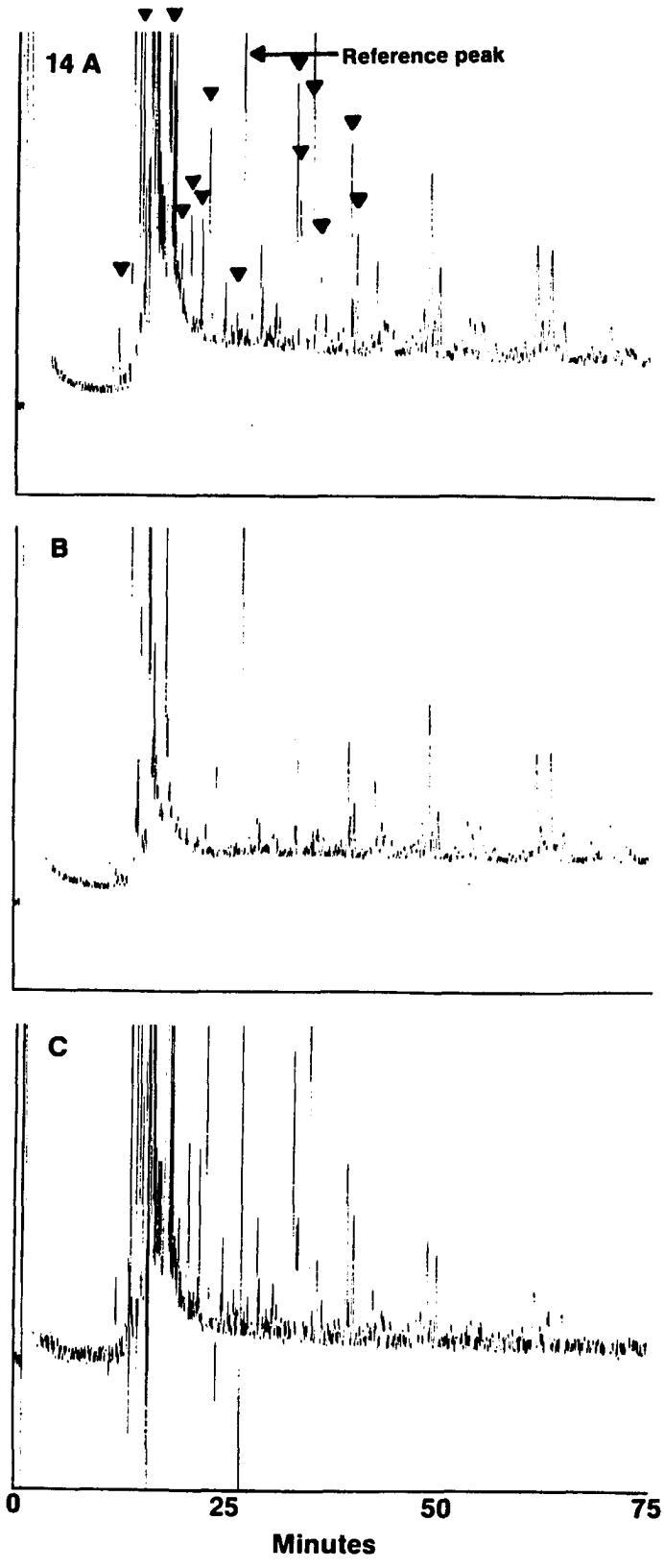


latter had 12 minor and 4 major differences. The consistency of the profile varied from individual to individual. Some subjects showed very little variation while others showed much more variation. One explanation is the possibility of inconsistent bathing habits and variations in diet as well as the fact that the samples were not obtained at the same time of day (one was taken at 9:30 a.m. while the other was taken at 12:00 noon). In addition, some small variations in retention time can be attributed to the fact that the profiles were obtained on different days thus leading to slight variations in instrument conditions. This is shown by the subtracted chromatogram showing a positive and negative peak at the same place. It is apparent that the control and questioned samples should be analyzed on the same day to minimize the influence of instrumental variations.

Variation of Profiles Taken from Different Individuals

The magnitude of difference in profiles of two different individuals varied greatly, depending on the two individuals that were compared. Two individual profiles that showed the greatest difference (of those selected for comparison) are shown in Figure 14. A subtracted chromatogram is shown at the bottom of this figure. Minor differences may be noted at 20, 25.5, 26, 43.5, 44, 51, 52, 58, and 60 minutes. Major differences are seen at 12,

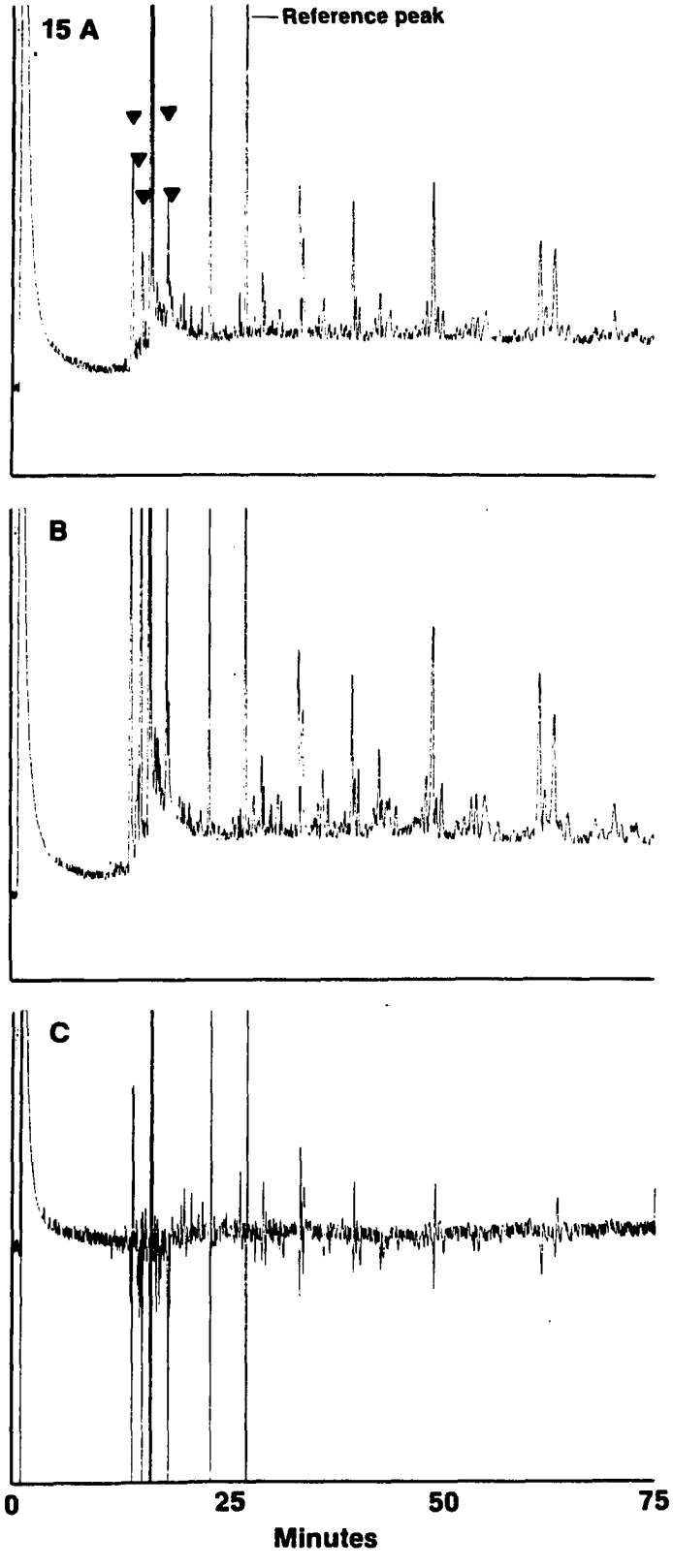
Figure 14. Profiles of Two People Showing Many Differences (A,B) and Subtracted Chromatogram (C)



14.7, 15, 15.2, 15.4, 16.6, 16.7, 16.8, 16.9, 17, 17.8, 18, 18.8, 19, 20.5, 22.1, 22.9, 23.5, 24.1, 25, 33.3, 33.7, 35.2, 36, 36.1, 36.2, 39.6, and 40.3. Of 140 points of comparison, there were 9 minor differences and 28 major differences. To minimize the contribution of instrumental effects, samples of each individual were taken from the same group of samples. All the profiles from this group were obtained within approximately a 36-hour period. The Appendix is a collection of one profile from each volunteer in this study. Note the similarities at 27, 33.5, 39.7, 49.1, 61.6, and 63.3 minutes. The area at 14-20 minutes is very similar for some and very different for others. The very weak profiles could be due to very recent bathing or very dry skin. Of the profiles shown in the Appendix, all are from caucasians; #17-21 are from females wearing cosmetics. Profiles 11 and 22 are from females wearing no makeup and all the rest are from males. The profiles from females wearing cosmetics were not used in any of the comparisons except one comparison of a profile without cosmetics to a profile of the same individual with cosmetics which is discussed later in this chapter.

The profiles shown in Figure 15 are profiles of two different individuals that show very similar profiles. Figure 15C is a subtracted chromatogram of the two. Minor differences may be noted at 19.5, 19.9, 20.3, 20.7, 26.3, 28.1, 36.8, 40.4, 42.7, 44.8, 50.2, and 61.6 minutes.

Figure 15. Profiles of Two People Showing Few Differences (A,B) and Subtracted Chromatogram (C)



Major differences are seen at 13.9, 14.6, 15, 18 and 18.2 minutes. Of 122 points of comparison, there were 12 minor differences and 5 major differences.

It seems logical that if the SSL profile is to be considered as an individual characteristic, the minimum difference between profiles of two different people (Figure 15) must be significantly greater than the maximum variation in profiles of a single individual over time (Figure 13). In consideration of the information yielded by these two figures, we see a comparison of 12 minor and 5 major differences in Figure 15 as compared to 12 minor and 4 major differences for Figure 13.

Variation Due to Weathering

Weathering is a term borrowed from geologists, originally, and then from those investigating oil spills and other similar events. It means exposure of the sample to environmental conditions which may cause degradation of the sample. It is not possible for actual forensic samples to be placed in airtight glass vials and refrigerated immediately. Envision a situation in which the suspect is not apprehended for several days. The investigating officer obtains some of the suspects clothing that meets the description of that worn by the assailant. Usually these are then placed in paper bags to maintain the integrity of the evidence and forwarded to the crime

laboratory. Many times the samples do not reach the laboratory for several days. In addition, the laboratory may have a backlog of cases such that it is just not possible to examine the evidence from this case immediately. All of these delays could contribute to the exposure of the sample to various conditions which may cause degradation of the sample (to simplify matters, let's assume that no contamination took place). The most common types of degradation include evaporation of the more volatile constituents and oxidation and/or hydrolysis of the more sensitive components. To illustrate the effect of weathering (exposure to ambient environmental conditions) on a sample, profiles were obtained at times that represented different lengths of exposure of the sample to ambient conditions. Figure 16A represents a profile that was obtained as soon as the sample was collected. Figure 16B is a profile of the same sample after it was stored at 6°C for 126 hours. Figure 16C is a subtracted chromatogram of the two. Minor differences may be noted at 19.5, 20.5, 34, and 35 minutes, with no major differences being noted. These minor differences may be due in part to slight fluctuations in instrumental conditions over the time period. Figure 17 illustrates the profiles obtained from the same sample at 8, 26.5 and 124 hours of exposure to ambient conditions in the laboratory. Note the increasing peak heights in the 0-30 minute region of the chromatogram

Figure 16. Profile Taken Immediately After Sample Collection (A), Profile Taken After Sample Was Stored at 6 °C for 126 Hours (B), and Subtracted Chromatogram (C)

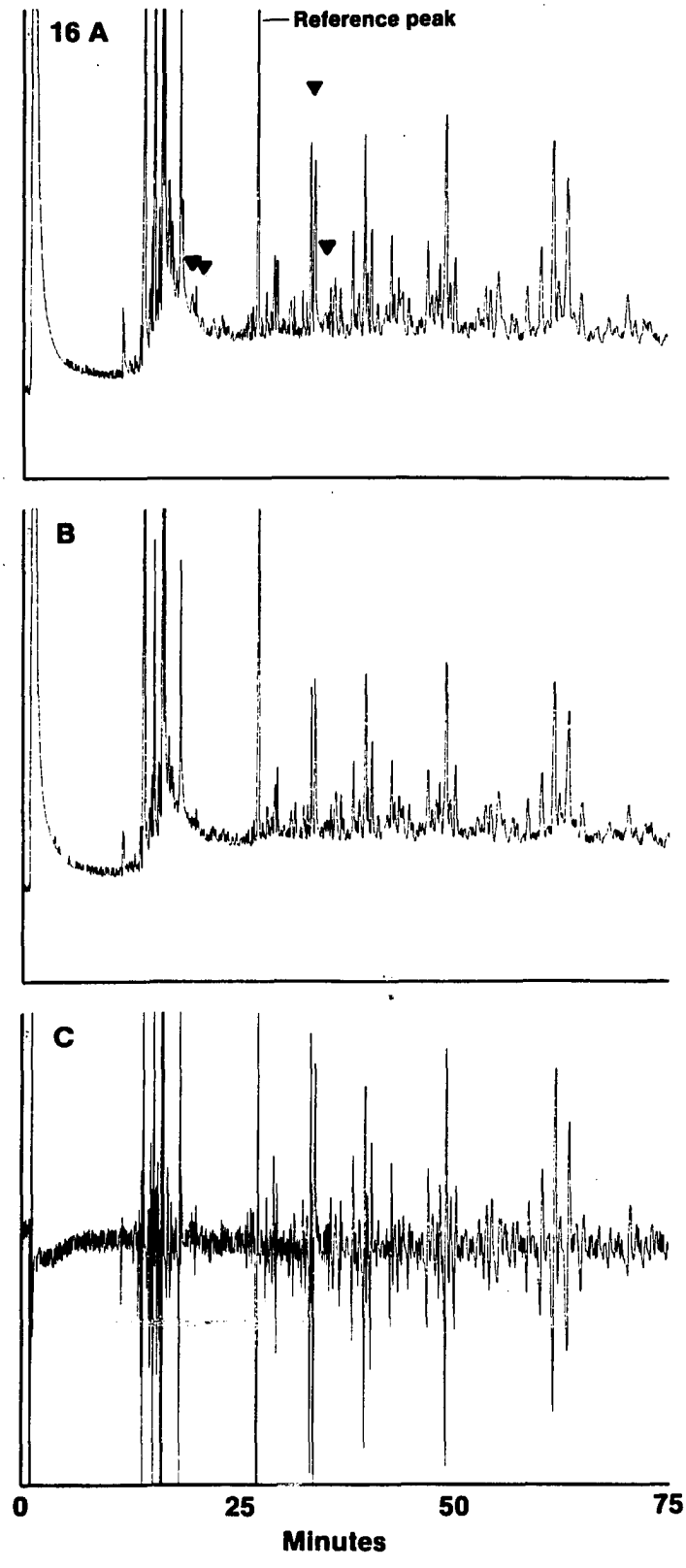
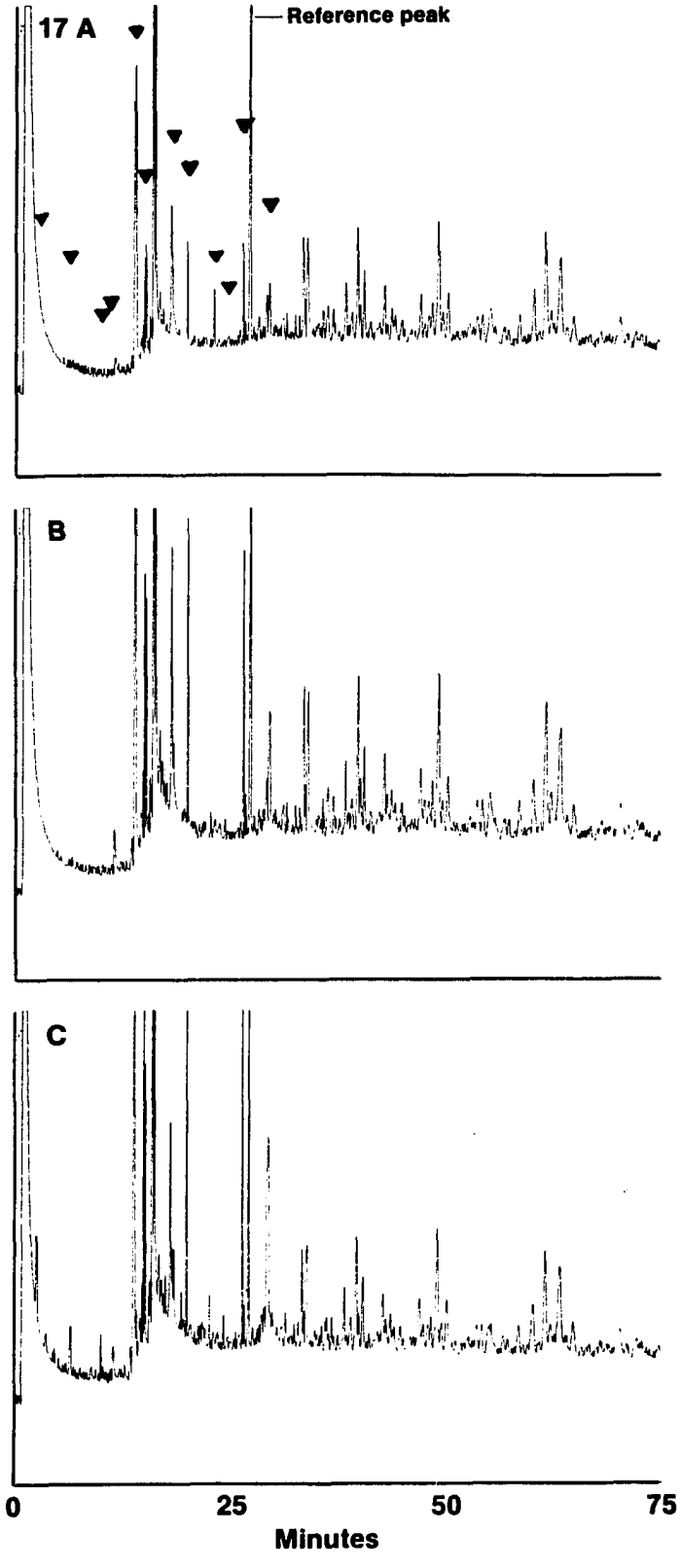


Figure 17. Profiles That Were Obtained After Sample Was Left Open to Ambient Conditions for 8 (A), 26.5 (B), and 124 Hours (C) Respectively



as the length of time of exposure increases. Major differences may be seen at 2.6, 6.6, 10.1, 11.5, 14, 14.9, 19.9, 22.6, 23.5, 26.3 and 29.2 minutes. Figure 18 is a comparison of the profile of the sample that was kept in the refrigerator at 6°C for 126 hours (A) and the same sample kept in the open under ambient laboratory conditions for 124 hours (B). Figure 18C is a subtracted chromatogram of the two. These two profiles were obtained within two hours of each other; therefore instrumental fluctuations should be minimized. Minor differences may be noted at 36.1, and 48 minutes. Major differences are seen at 2.6, 6.6, 10.1, 14.1, 14.9, 19.9, 22.6, 23.5, 26.3, and 29.2 minutes. Of 137 points of comparison, there were 2 minor differences, and 10 somewhat larger differences.

A Comparison of Forehead and Cheek SSL Samples

Comparisons of the SSL profiles from the cheek and forehead were made in order to determine whether different parts of the face produced different profiles. In any case, the competent investigator in a forensic case should obtain samples from the different parts of the face so that further comparisons could be made if necessary. Figure 19 is a comparison of profiles of SSL from the forehead (A) and the cheek (B) of the same individual. Minor differences can be seen at 21, 24.5, 26.3, 34.5, 40.8, and 52 minutes. Major differences can be seen at 13.9, 15.4,

Figure 18. Profile of Sample That Was Stored at 6 °C for 126 Hours (A), Profile of Sample That Was Stored Open Under Ambient Conditions for 124 Hours (B), and Subtracted Chromatogram (C)

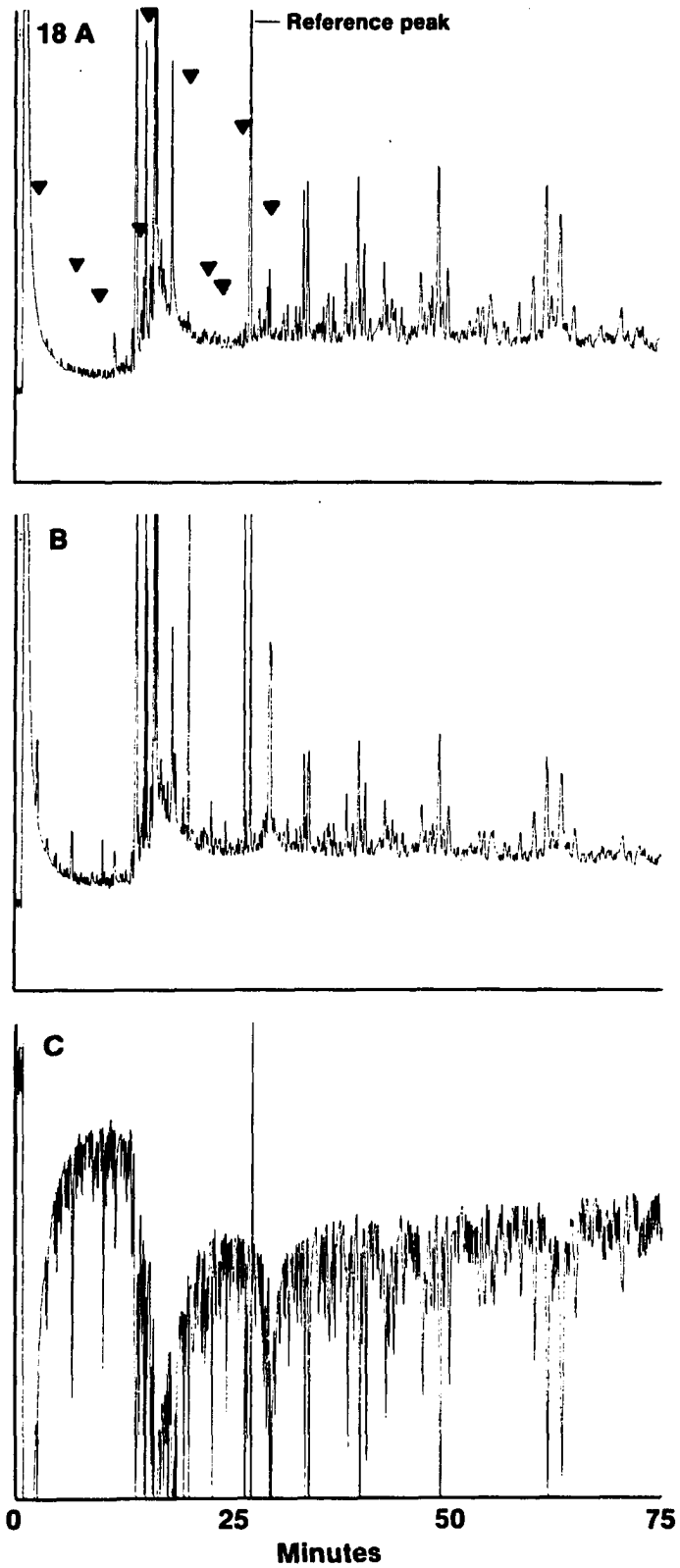
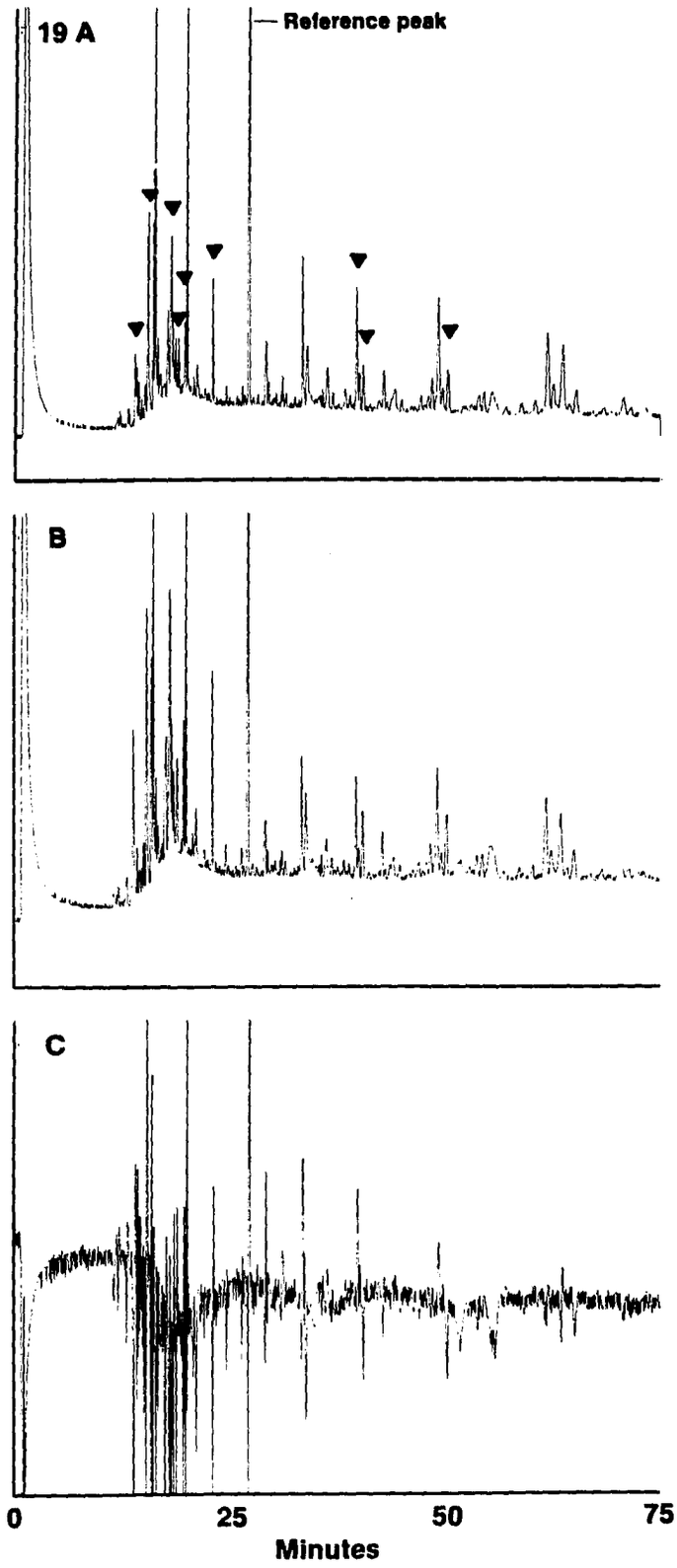


Figure 19. Profile of Forehead (A) and Cheek (B) SSL From Same Individual and Subtracted Chromatogram (C)



18, 18.2, 18.5, 18.8, 19.6, 23, 39.7, 40.5 and 50.3. Of 127 points of comparison, there were 6 minor differences and 11 major differences. A similar comparison of another individual is made in Figure 20. Of 123 points of comparison, two minor differences can be seen at 33.7 and 42.7 minutes and one major difference at 15.5 minutes. The difference in forehead and cheek profiles for each of these subjects was less than the variation of SSL profile for a single individual over time. The smallest variation in profile of a single individual over time (Figure 12) was 6 minor differences and four major differences.

Comparison of SSL Profiles Involving Cosmetics

Frequently, the most notable trace evidence of a crime is a smear of makeup from the victim on the clothing of the assailant. Figure 21A is a SSL profile of an individual without cosmetics. Figure 21B is a SSL profile of the same individual with cosmetics. The profile in Figure 21B was obtained 8 days earlier than the one in 21A. Figure 21C is a subtracted chromatogram of the two. Minor differences can be seen at 22.4 and 50 minutes. Major differences can be noted at 12.9, 13.7, 14.3, 14.9, 16.1, 16.7, 22.9, 23.3, 26.3, 28.9, 33.8, 36.8, 40.3, 43, and 47 minutes. Of 145 points of comparison, there were 2 minor differences and 15 major differences. Note that this difference in profiles includes that difference contributed by the presence of

Figure 20. Profile of Forehead (A) and Cheek (B) SSL From Same Individual and Subtracted Chromatogram (C)

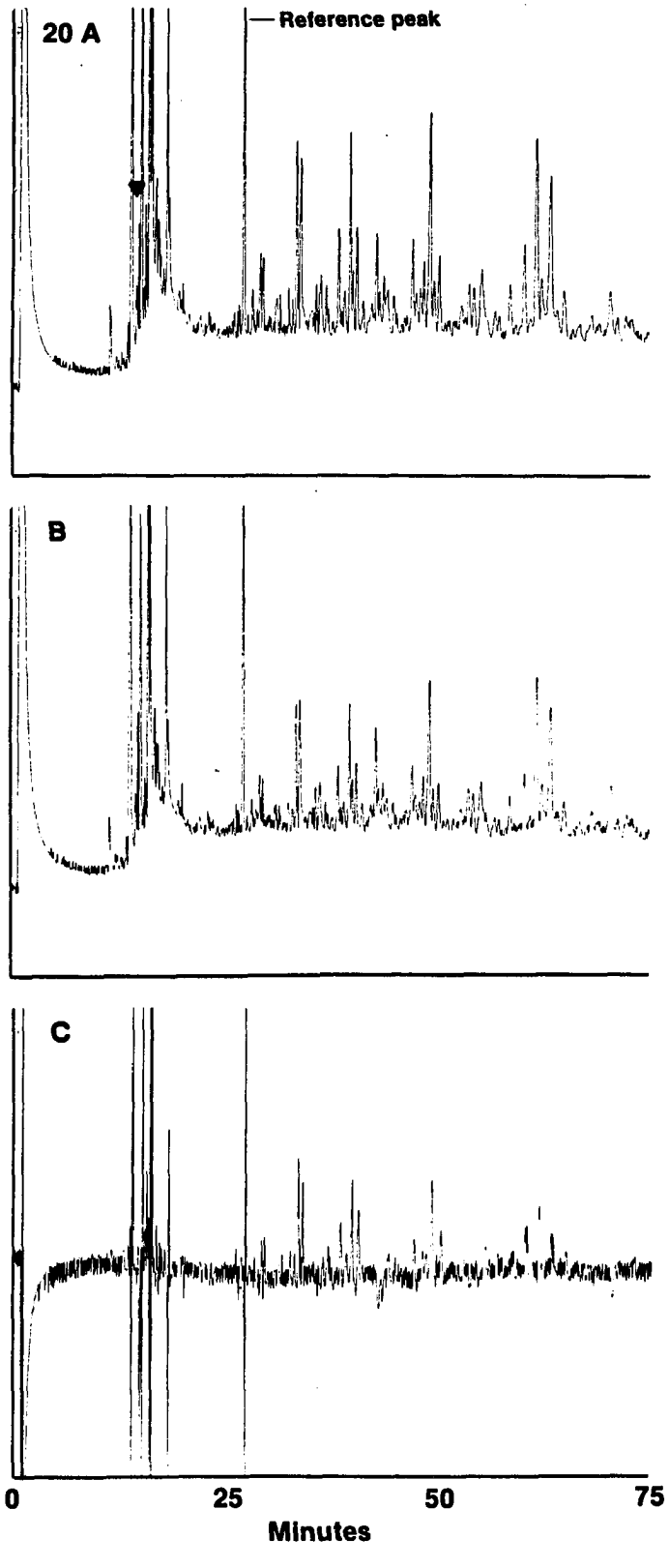
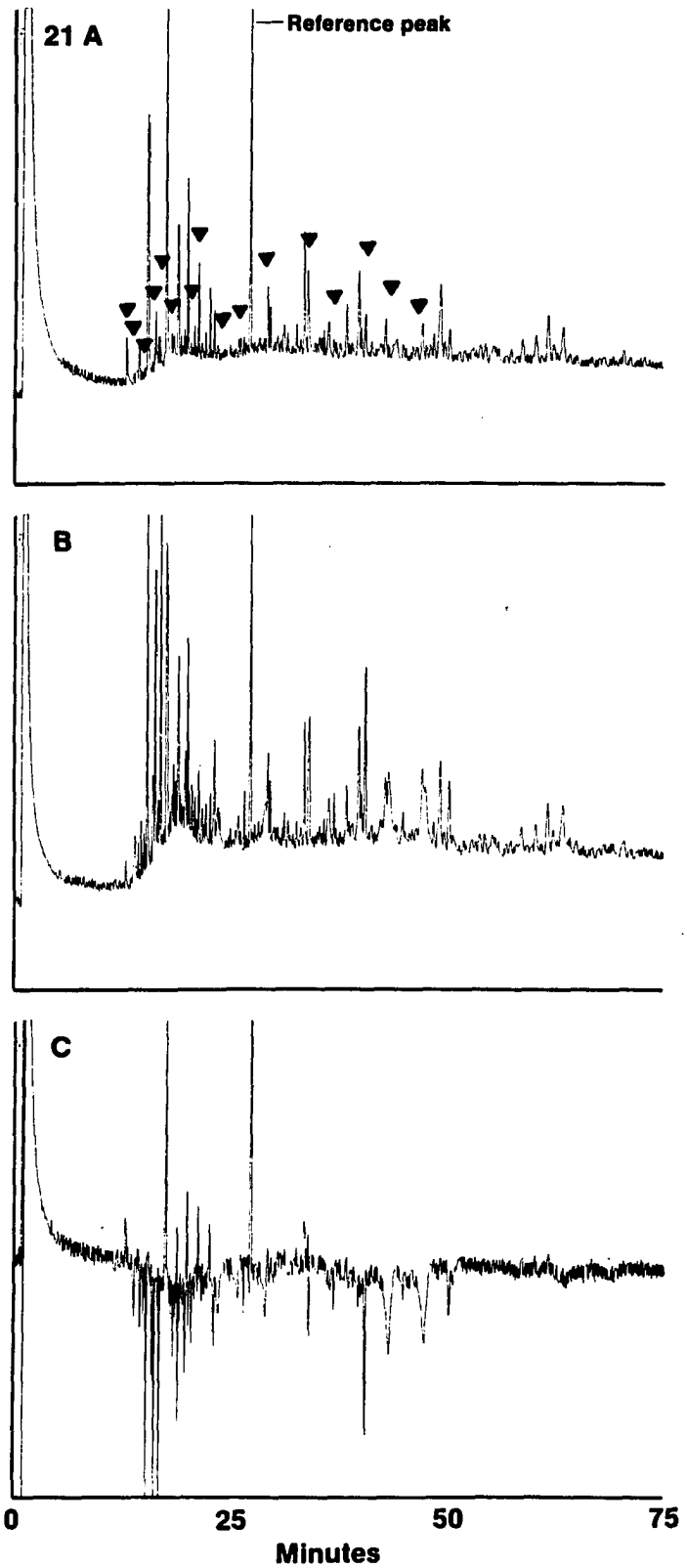


Figure 21. Profile of SSL From Individual With No Cosmetics (A) and With Cosmetics (B) and Subtracted Chromatogram (C)



cosmetics and the variation, if any, of an individual's profile over time. This difference was much greater than that of a single individual over time (Figure 13), 12 minor and 4 major differences and smaller than the difference between two individuals (Figure 14), 9 minor and 28 major. Note that most of the characteristic SSL peaks may be seen in both profiles.

Transevaporator Extraction, Thermal Desorption and Cryofocusing

Frequently, forensic case evidence exists only in trace quantities. The simple extraction and injection method lacks the sensitivity for these small quantities. Therefore, the need for a simple extraction and concentration method is apparent. The transevaporator method seemed ideally suited to this application; however, some modifications were needed to decrease the amount of physical manipulation of the precolumn and analytical column. Brazell (98) reported a method in which the volatiles were transferred to a precolumn outside the gas chromatograph (GC). The precolumn was then physically attached to the analytical column. In the present investigation, steps were taken to eliminate this. A precolumn was installed in the instrument along with a commercial cryofocusing module. A valve was installed between the trap desorption furnace and then the precolumn was routed through the cryofocusing unit and then attached

to the valve. When desorption of a trap containing sample was attempted, it became apparent that the flow through the trap was insufficient to complete the desorption. Even with a desorption time of 15 minutes, the resulting chromatogram showed very small peak heights for some of the early eluting components and no peaks at all for some of the later eluting components. The flow during desorption was only 1-2 mL/min because it was limited by the flow through the analytical column. A low dead volume tee fitting was installed between the precolumn and the analytical column. Opening the third port of this tee increased the flow to 200 mL/min. This greatly improved the desorption as evidenced by larger early peaks and the appearance of the later sample peaks. This port could then be closed at the end of the desorption period and normal flow through the column resumed. This modification allowed the complete desorption of the trap to be accomplished in approximately seven minutes. A reduction in desorption time reduces the total analysis time. One further modification was the addition of small heaters around the exposed parts of the precolumn (Figure 5) in the cryofocusing unit. This served to prevent the condensation of the sample in parts of the precolumn other than in the cryofocusing area. Evidence of this was seen in chromatograms taken before and after this modification. After this modification, the peaks were much sharper and

the profiles much more reproducible in terms of retention times and relative peak heights.

Application of the transevaporator/thermal desorption method to the SSL samples produced profiles that were inconsistent with those produced by the split injection of pentane extract. Many of the early peaks were reduced in height and many of the latter peaks did not show up on the chromatogram. Various combinations of desorption time and sample quantities were utilized but no improvement in the profile was seen. From the profile that was obtained; however, it was apparent that this method did increase sensitivity over the split injection method. The reason for this is that the solvent extraction/split injection method results in a dilution of the sample. It is limited by the volume of sample that can be injected into the chromatograph, approximately 3 uL. If this extract is concentrated further, the possibility of contamination increases because minor contaminants (in the solvent, for example) now become more concentrated. The transevaporator method results in a concentration of the sample, without concentrating any trace contaminants. All of the sample that is placed in the transevaporator is transferred to the GC.

CHAPTER 4

CONCLUSIONS

Gas chromatographic profiling techniques have been used to investigate the individuality of skin surface lipid (SSL) profiles. No references to the use of SSL profiles in forensic cases have been found in the current literature. Of the 23 subjects studied, it was found that the SSL profile varied from one subject to another. However, the minimum variation found between two subjects was found to be approximately equal to the maximum variation of the profile of one subject over time. This indicates that it is very probable that SSL profiles are individual in nature but the sample preparation technique may need to be more selective in order to obtain these characteristic profiles. Variation in the individual profiles over time may be reduced by limiting the study to a particular fraction of the lipids. In addition it would be much easier to utilize a much less complex profile in the application of pattern recognition techniques because of improved peak resolution.

The effects of weathering on a sample were demonstrated. The major changes occurred in the first 25 minutes of the chromatogram. The generation of new peaks in this area indicates the degradation of of the sample when exposed to ambient conditions. Degradation may include the

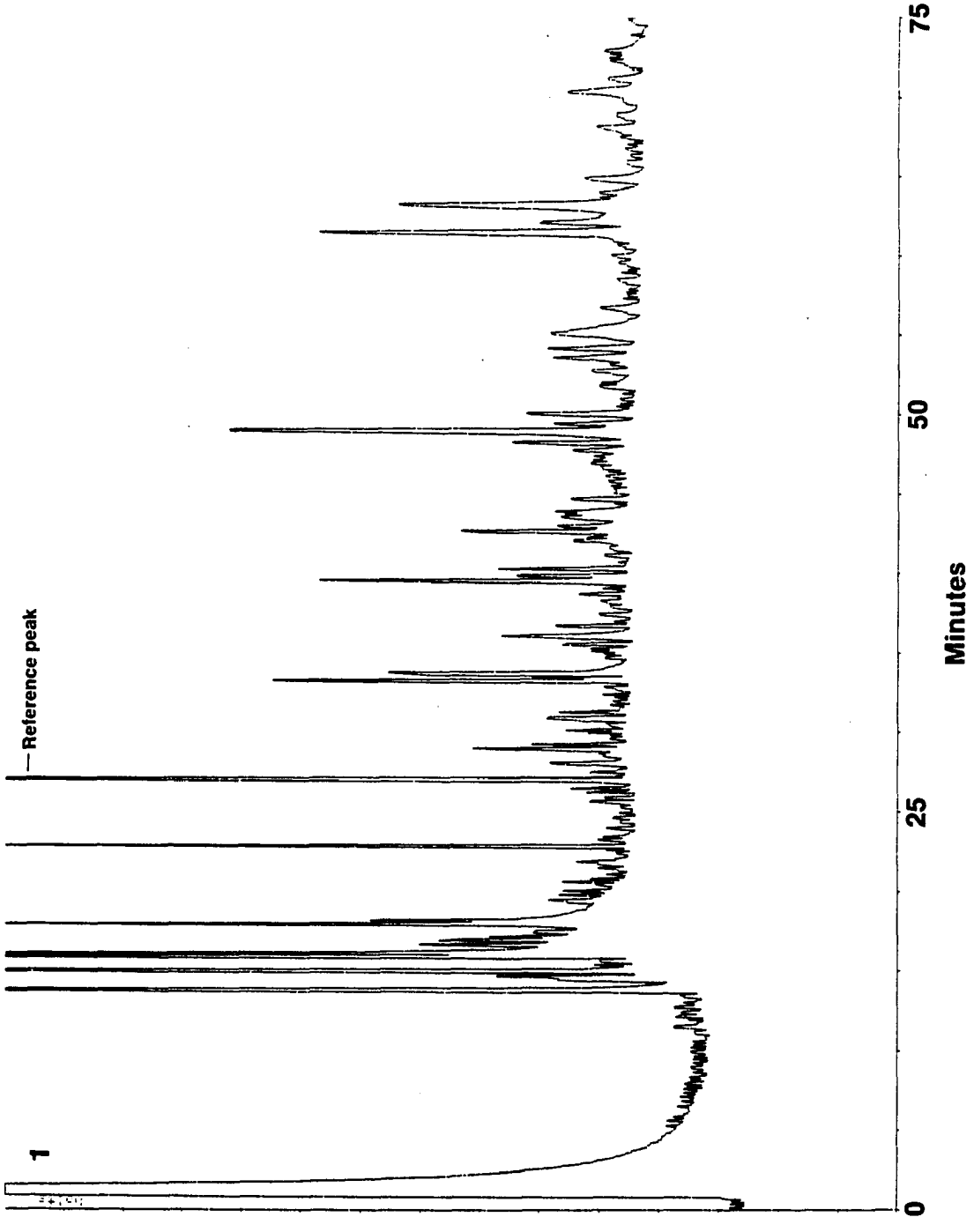
hydrolysis of the triacylglycerols to fatty acids and glycerol or mono- and diglycerides. Oxidation of unsaturates may produce aldehydes, ketones, and carboxylic acids. The more complex compounds would elute late in the chromatogram and its smaller degradation products would elute earlier in the chromatogram. It was shown that storage at 6°C in a closed vial prevented most major changes in the sample. This indicates that moist air and the presence of microorganisms speed up the degradation. Storage in a cool location slows the reactions. Future work should include the fractionation of the SSL and the investigation of which fraction is least affected by weathering. It is this fraction that will be of most interest in forensic work, since samples of this nature will likely have been affected to some extent by weathering.

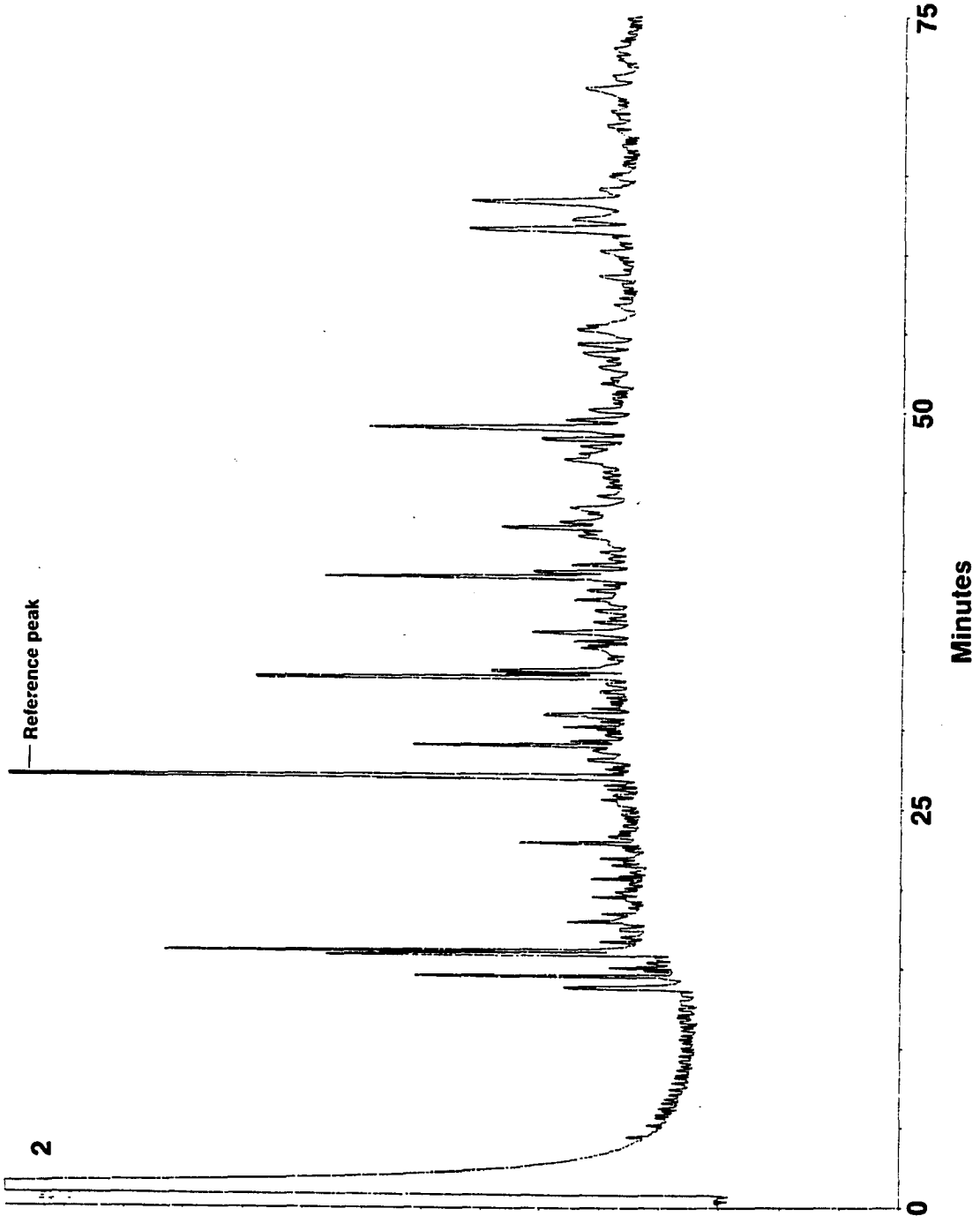
SSL from the forehead and cheek were compared and it was found that only very minor differences were noted in the profiles. The use of cosmetics markedly changed the profile; however, this may prove to be even more useful in the investigation of crimes of violence. The combined SSL/cosmetic profile should lend more individuality than either alone. In addition, it may be possible to identify the class and perhaps the brand of cosmetic in order to contribute another point of evidence to the investigation. Future work may investigate the possibility of differentiating the profiles by sex and race. Certain

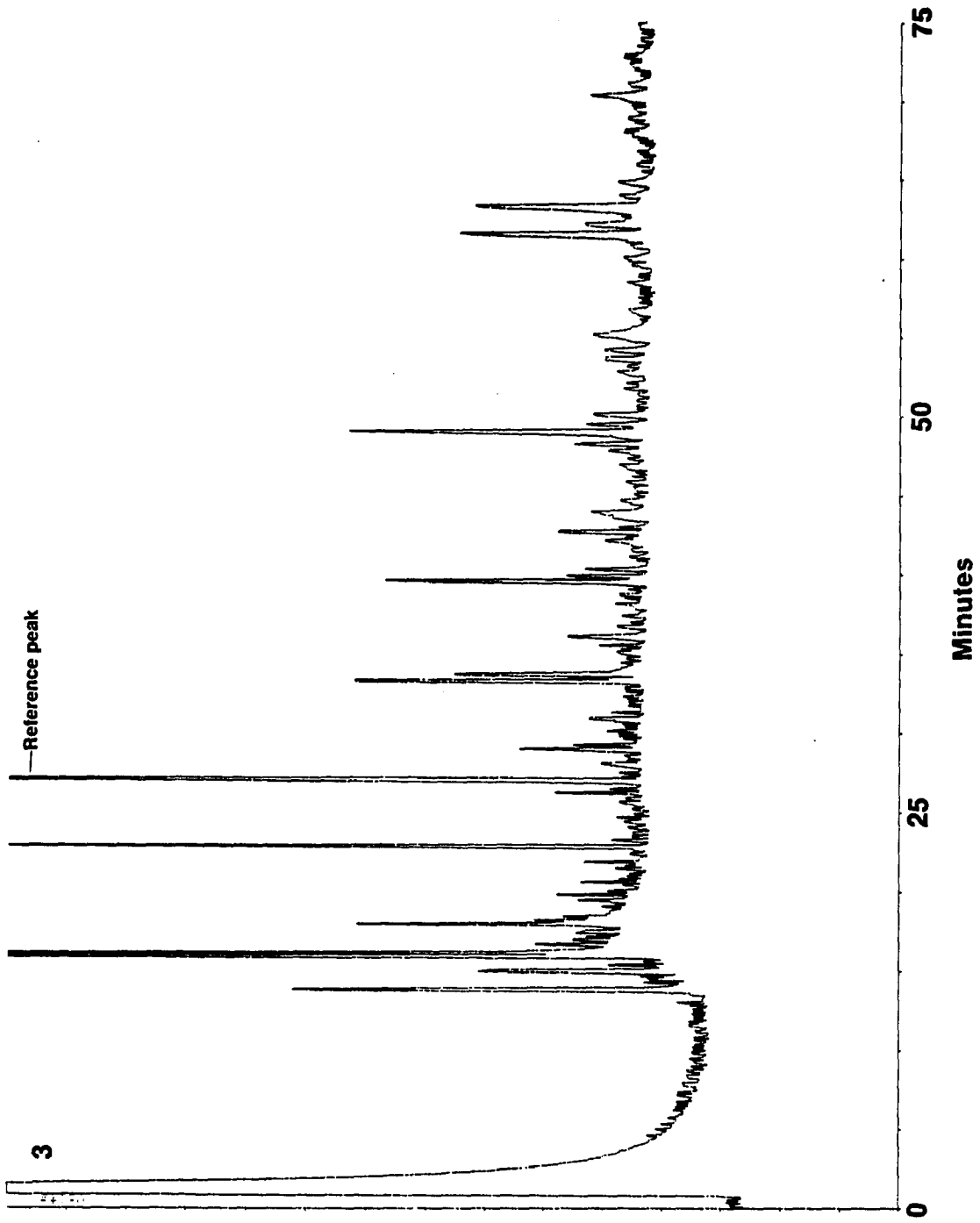
occupations may also be identified, particularly those involving volatile organics in the workplace air or skin contact with organic substances.

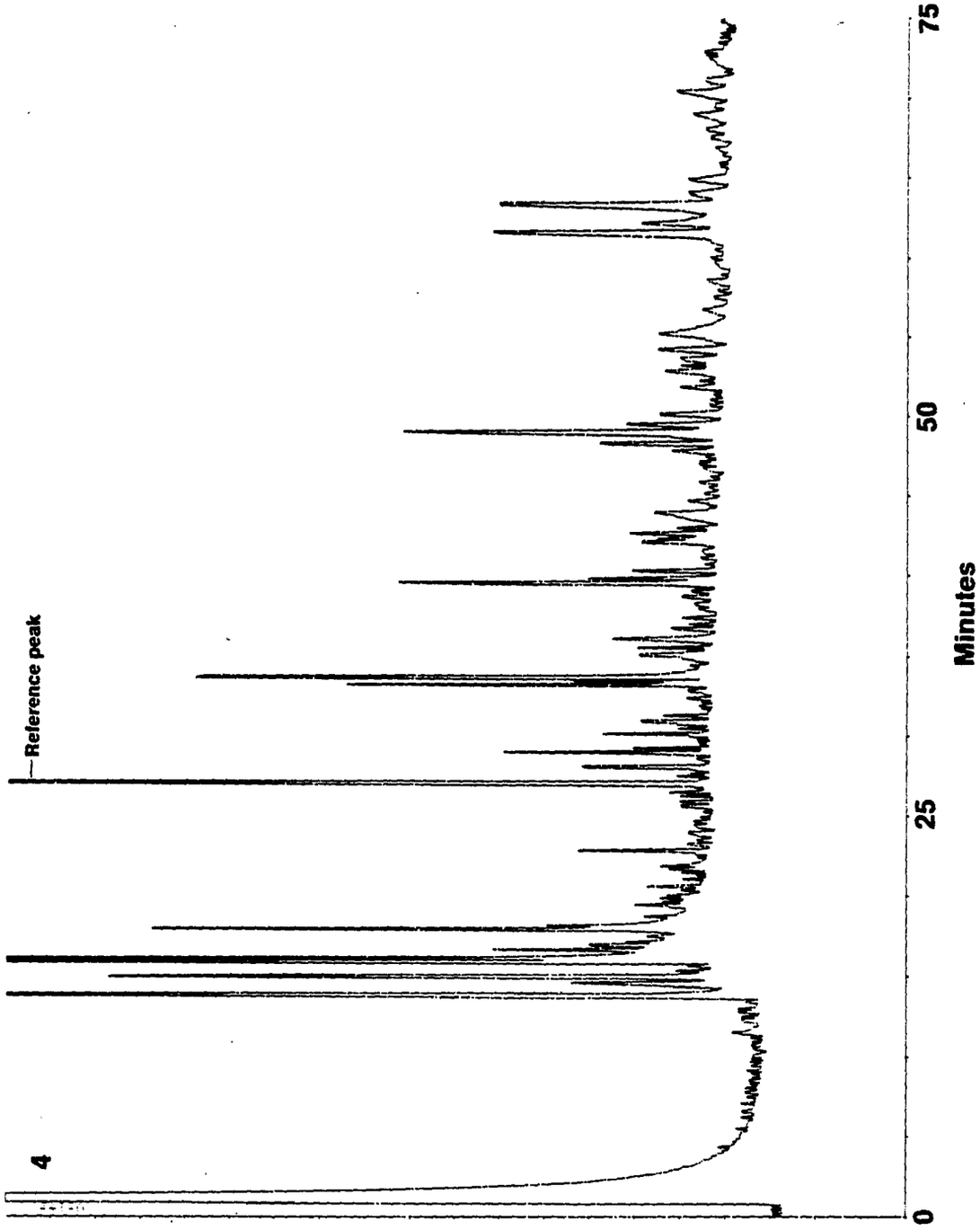
The modifications to the transevaporator and the cryofocusing unit improved this sampling method by eliminating the manipulation of the precolumn. The results of the use of the transevaporator indicate that it can yield the needed sensitivity. However, more experimentation is needed in the area of trap desorption before the efficacy of its use can be completely established.

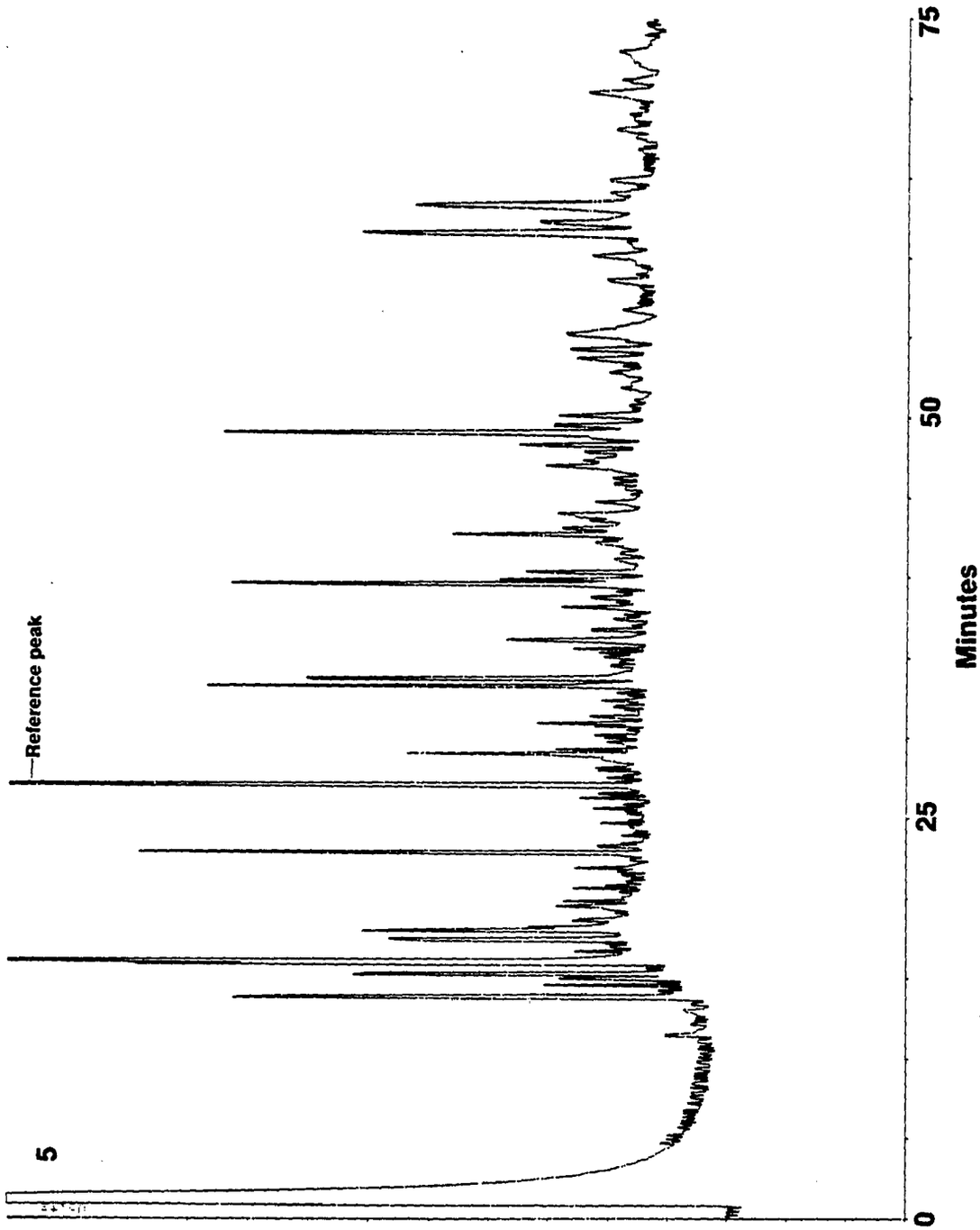
APPENDIX

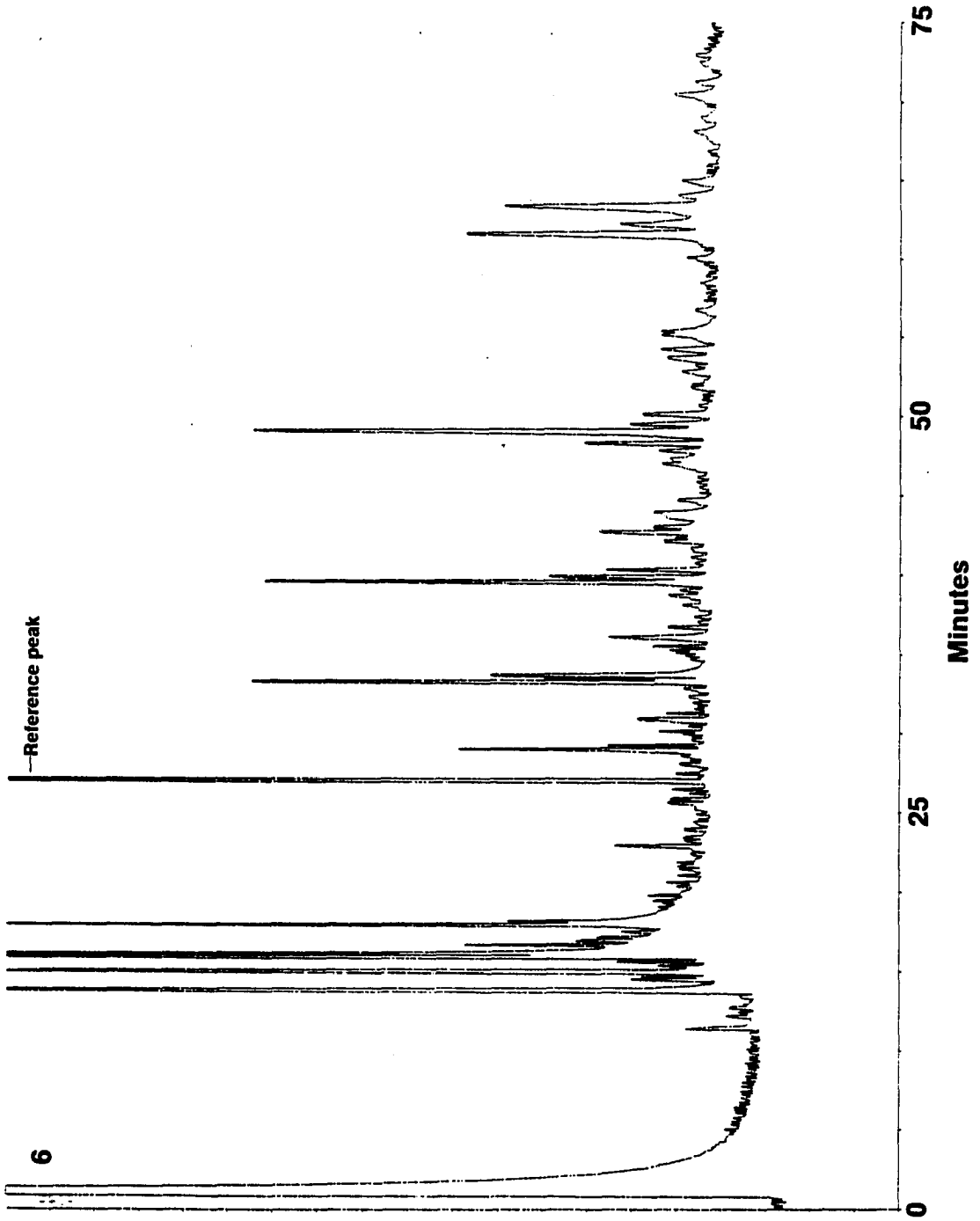


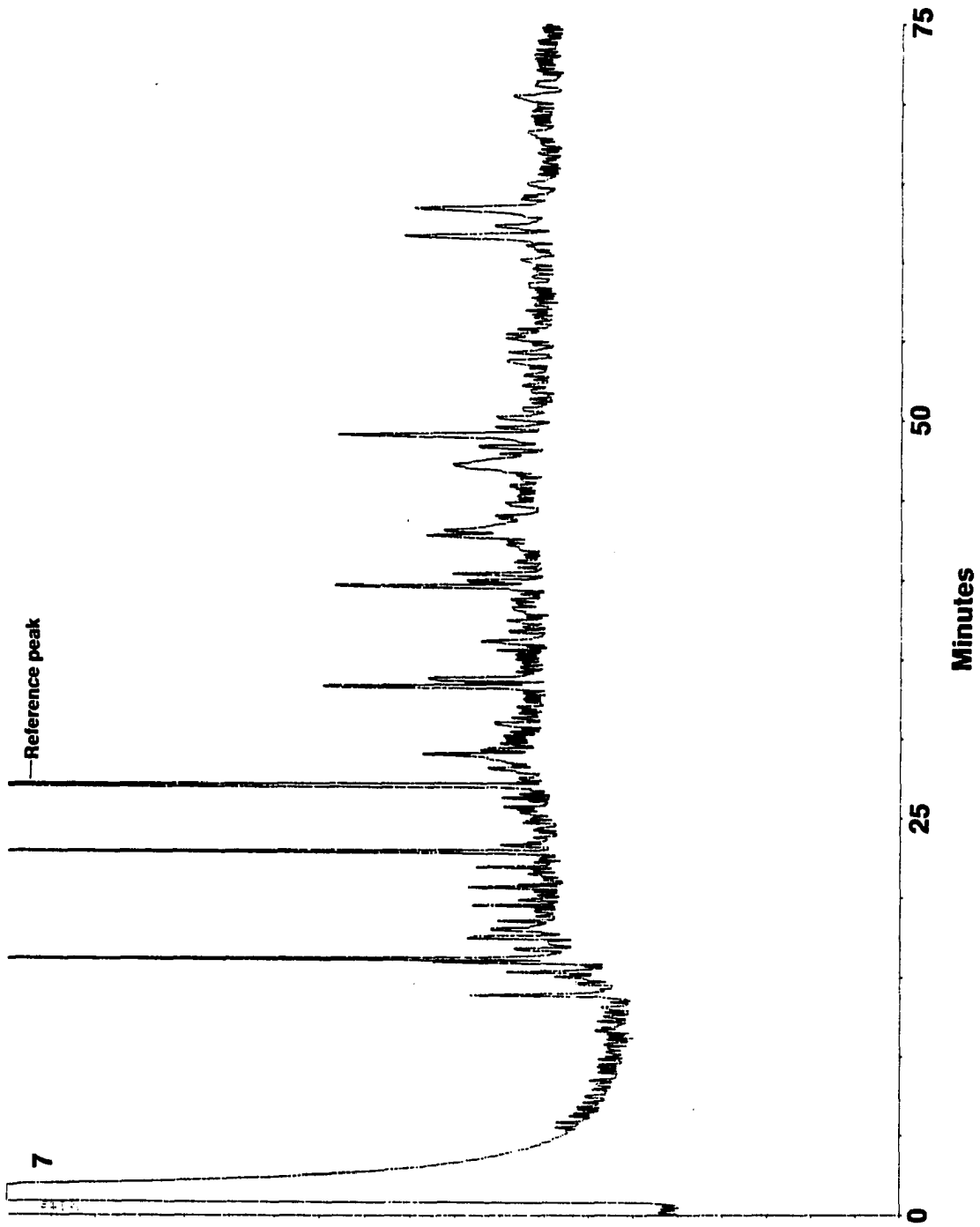


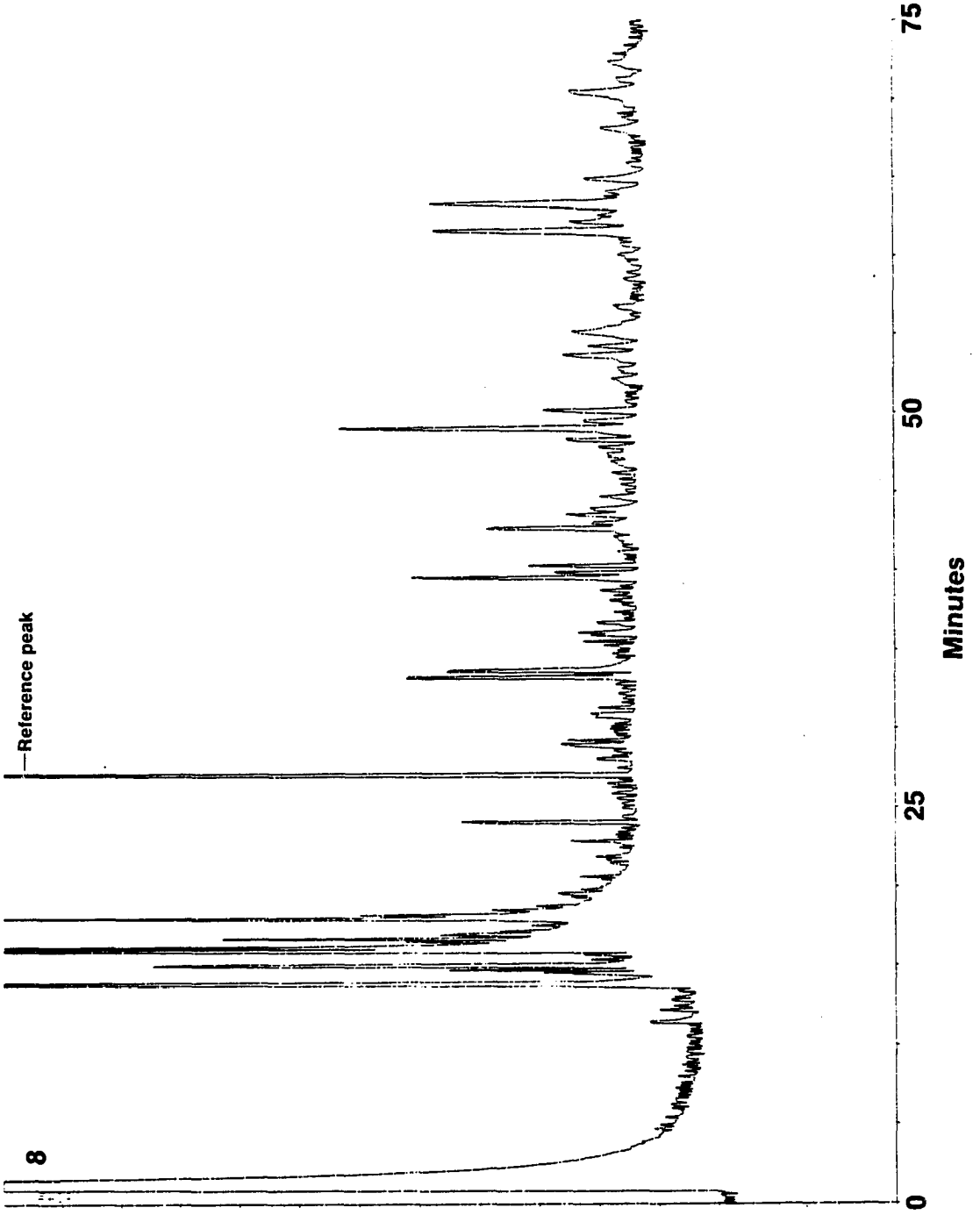


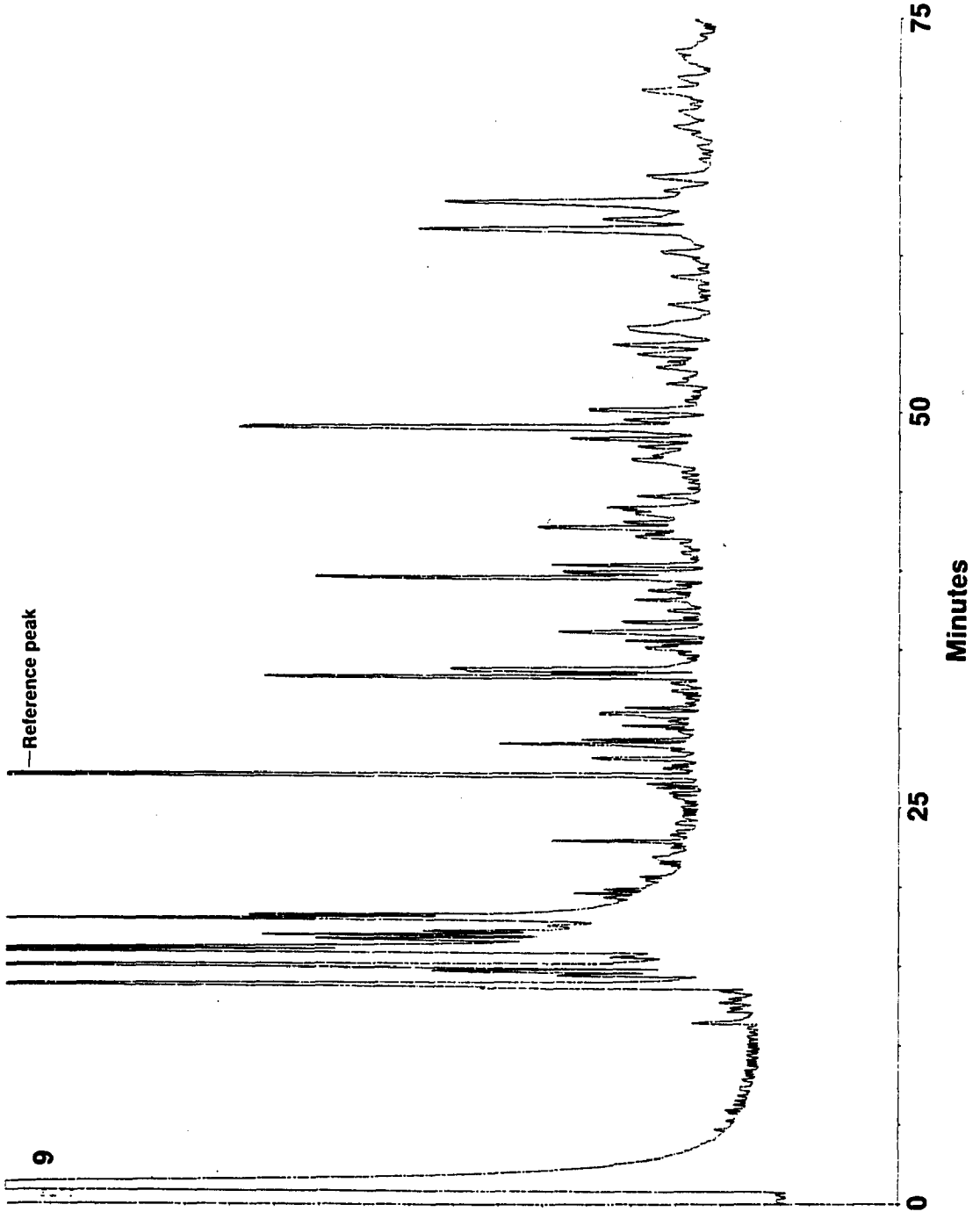


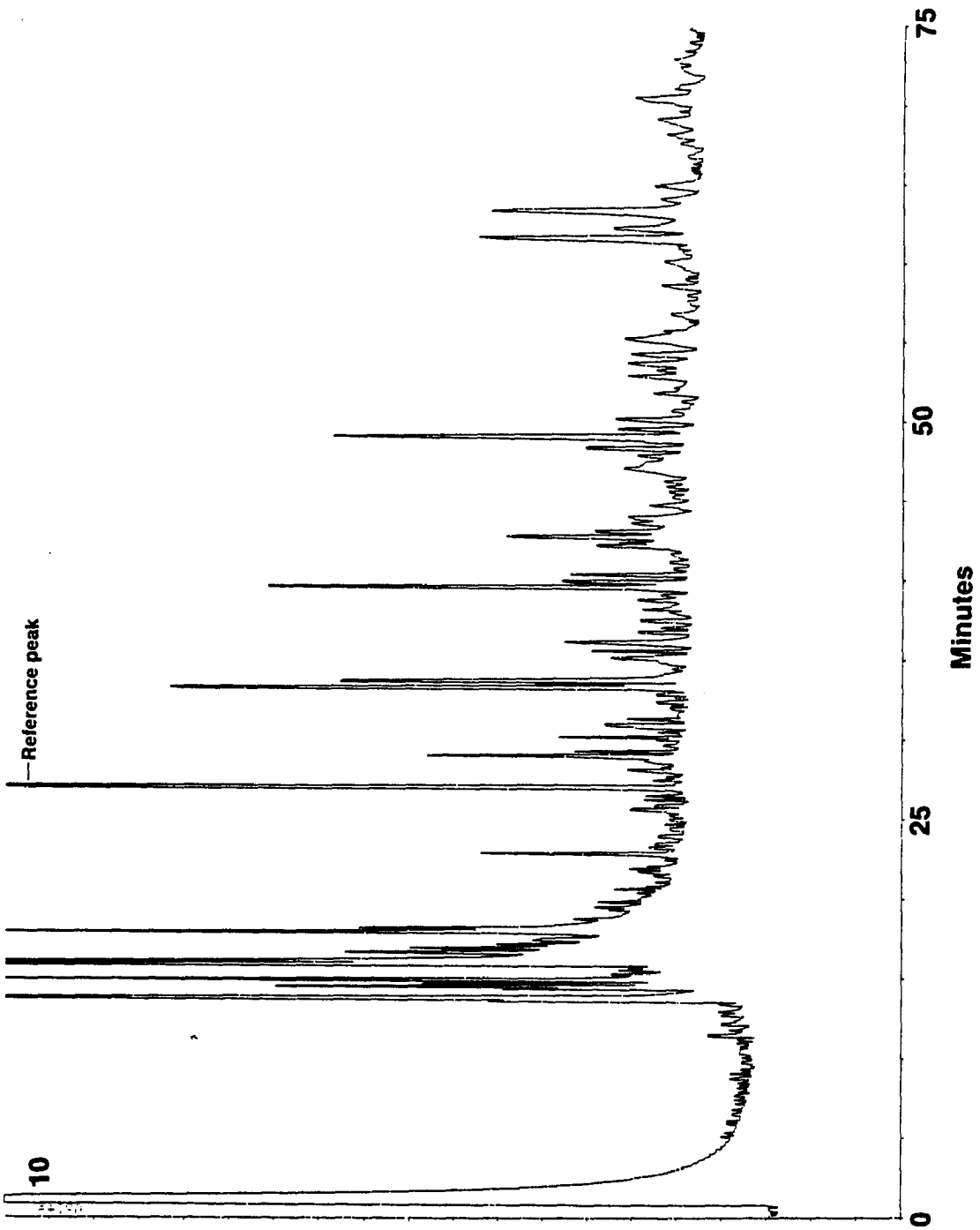


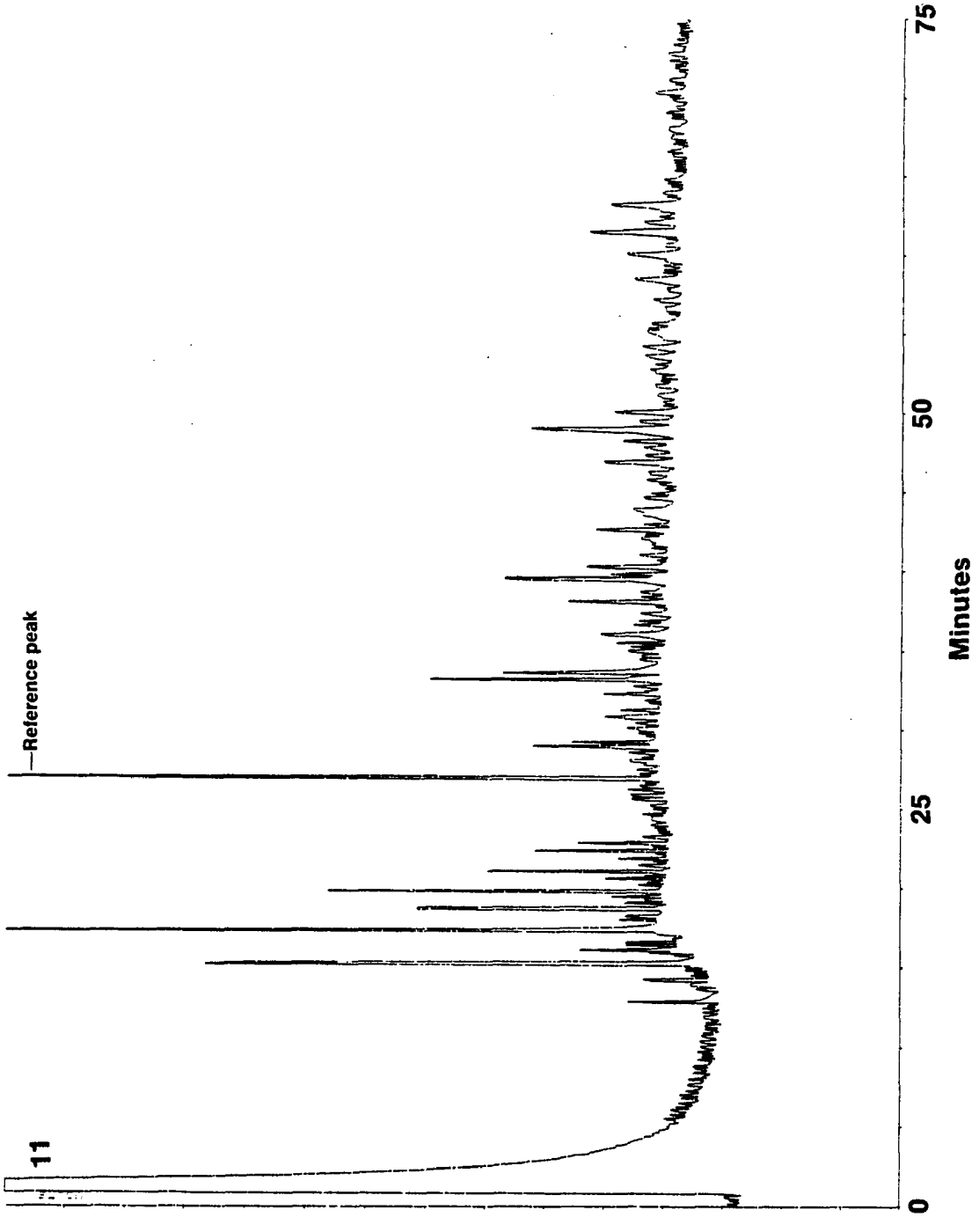


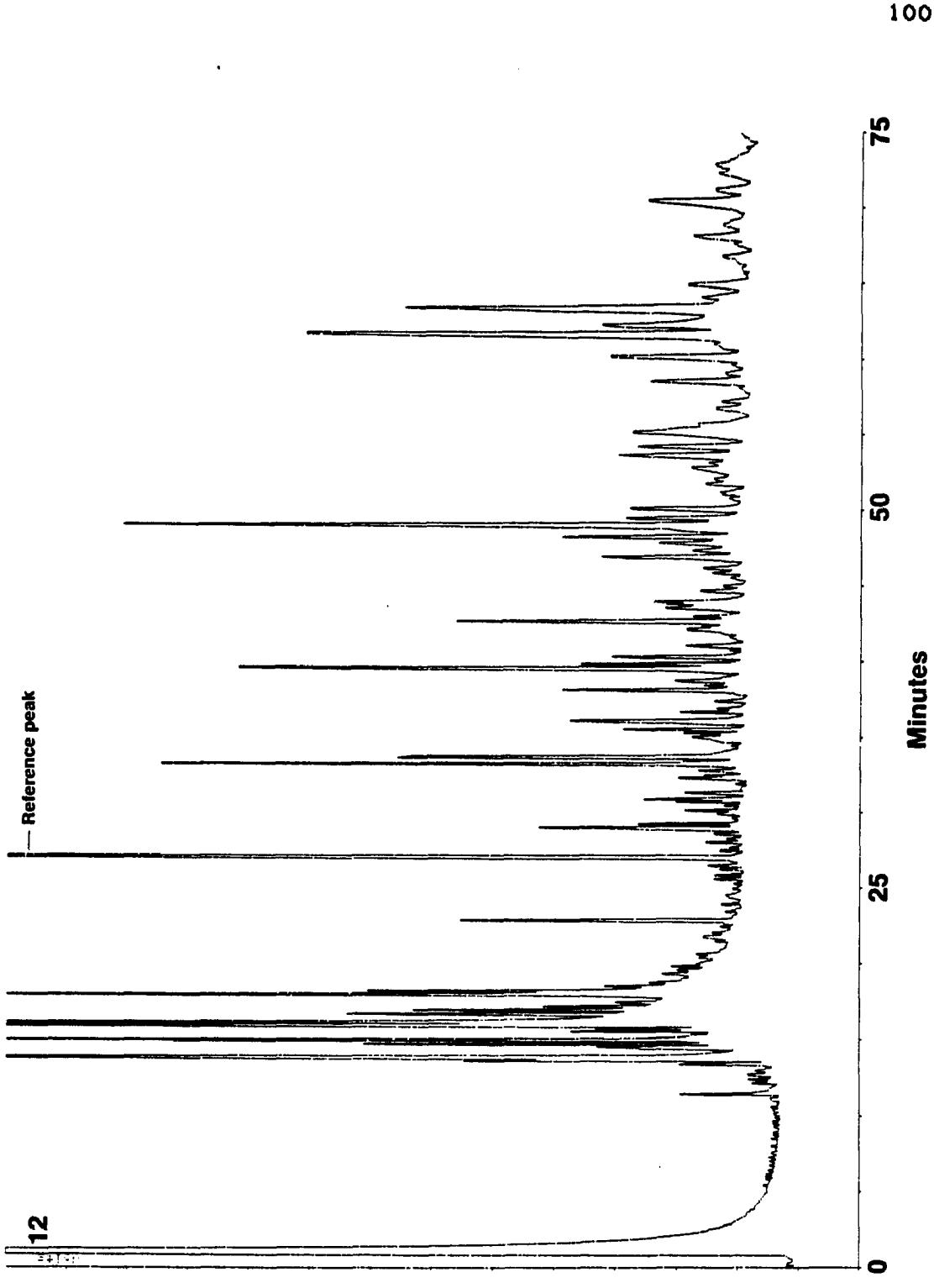


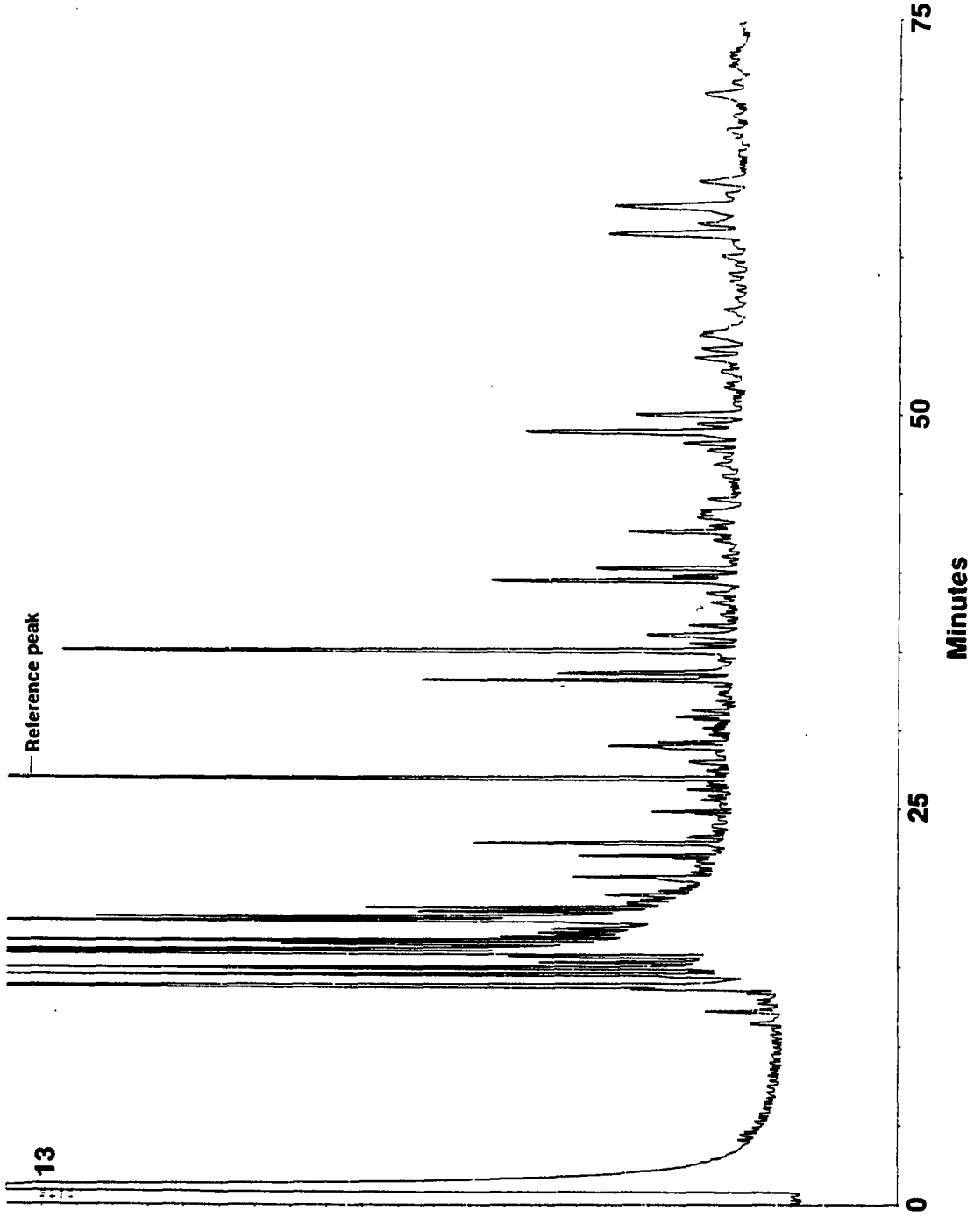


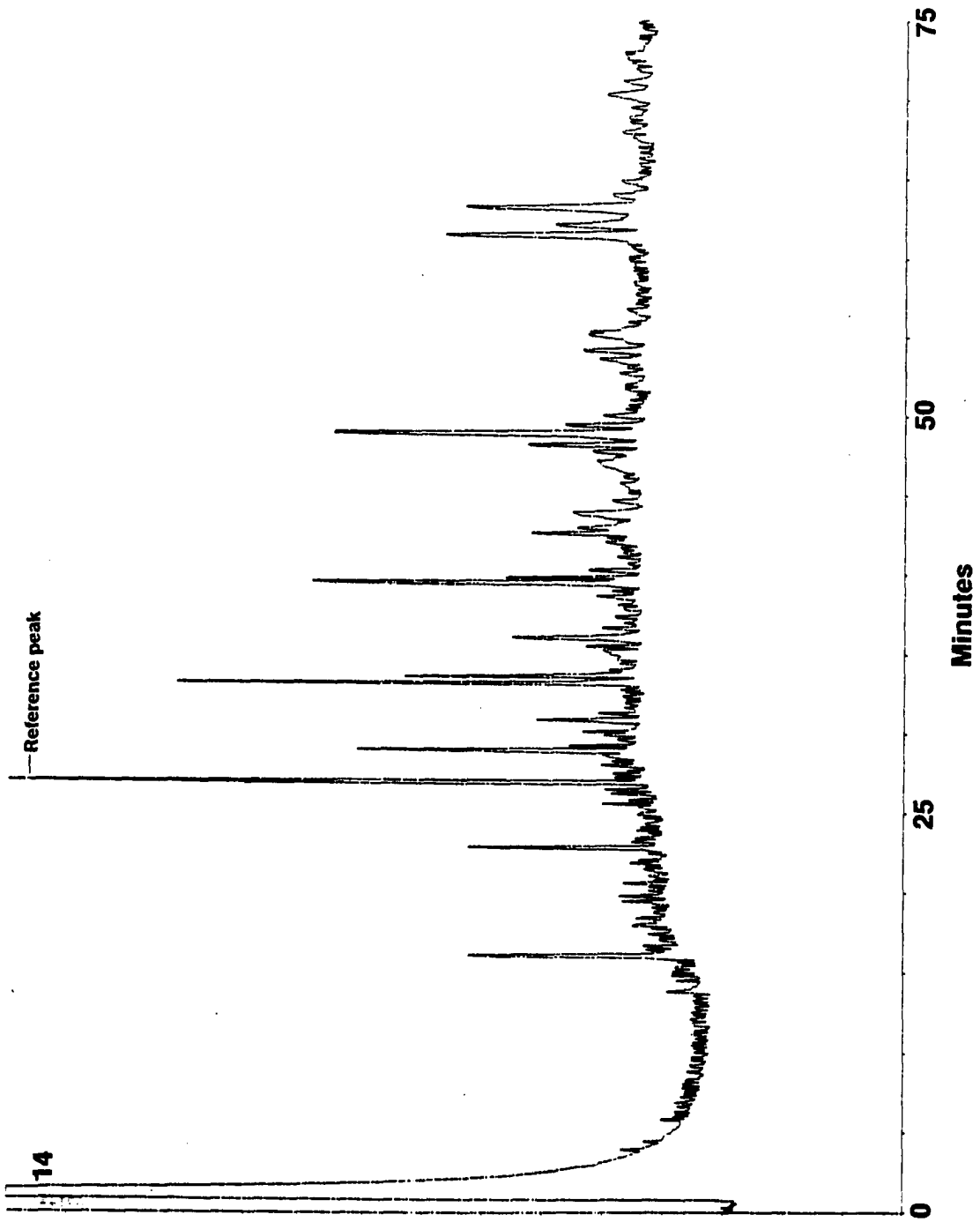


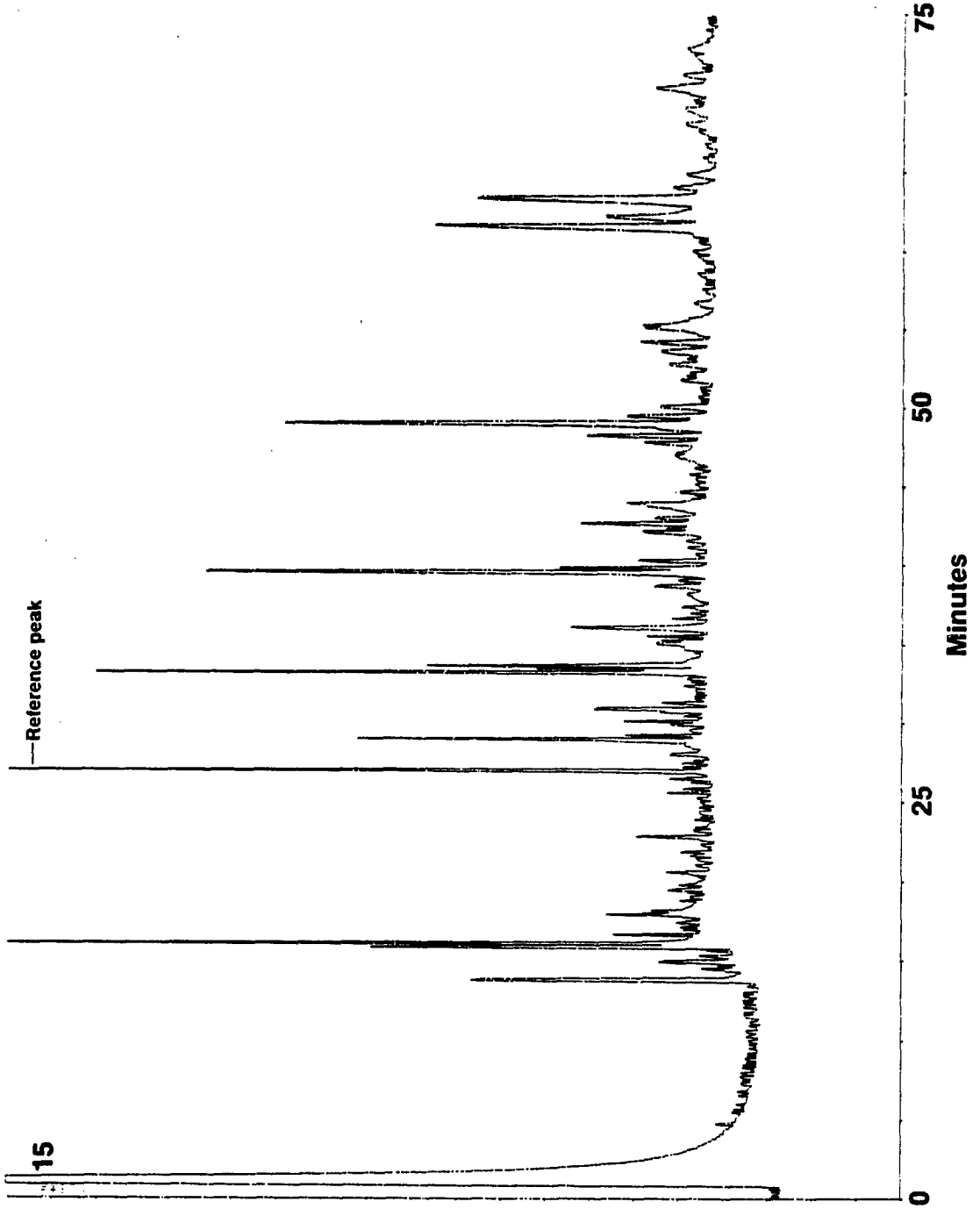


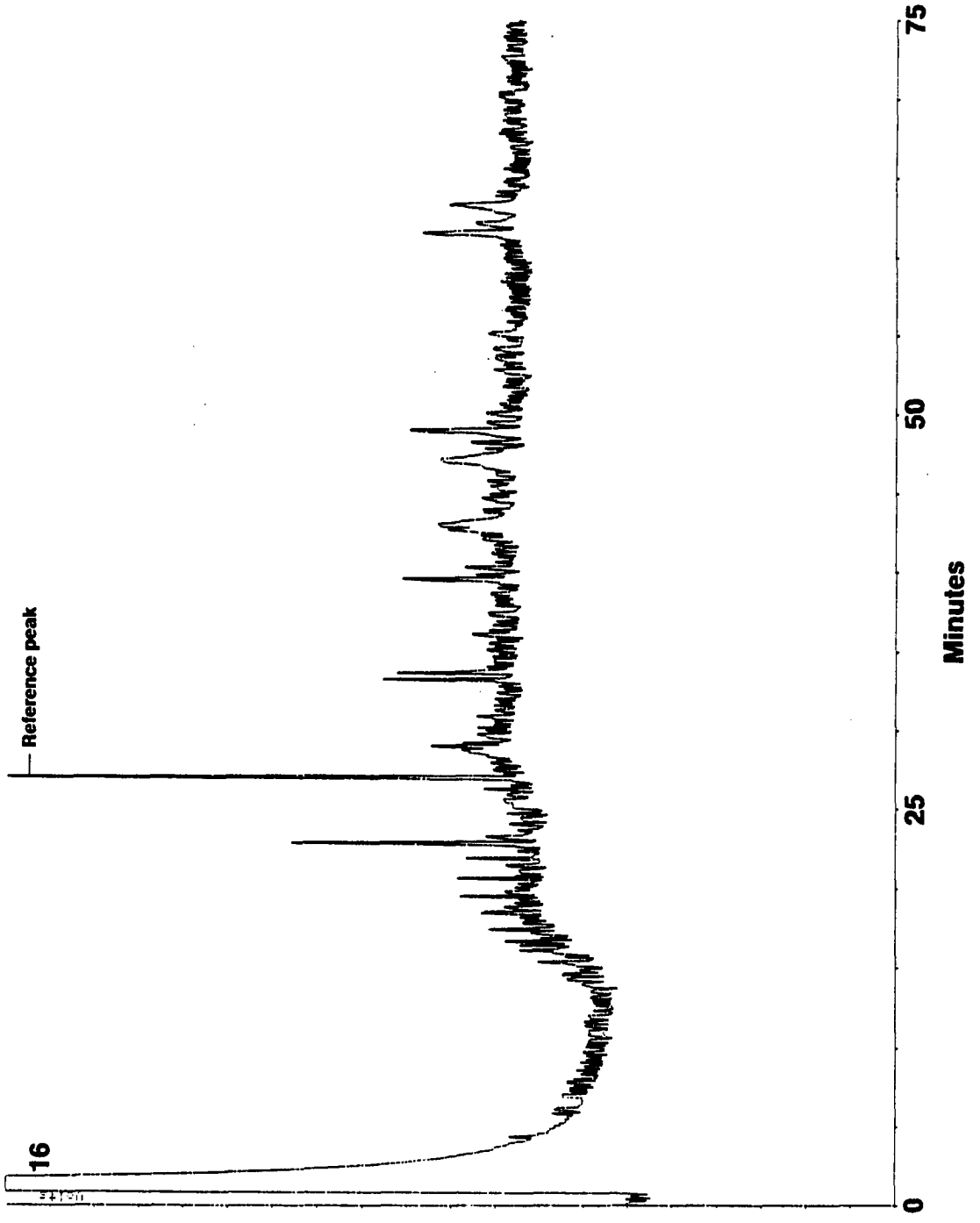


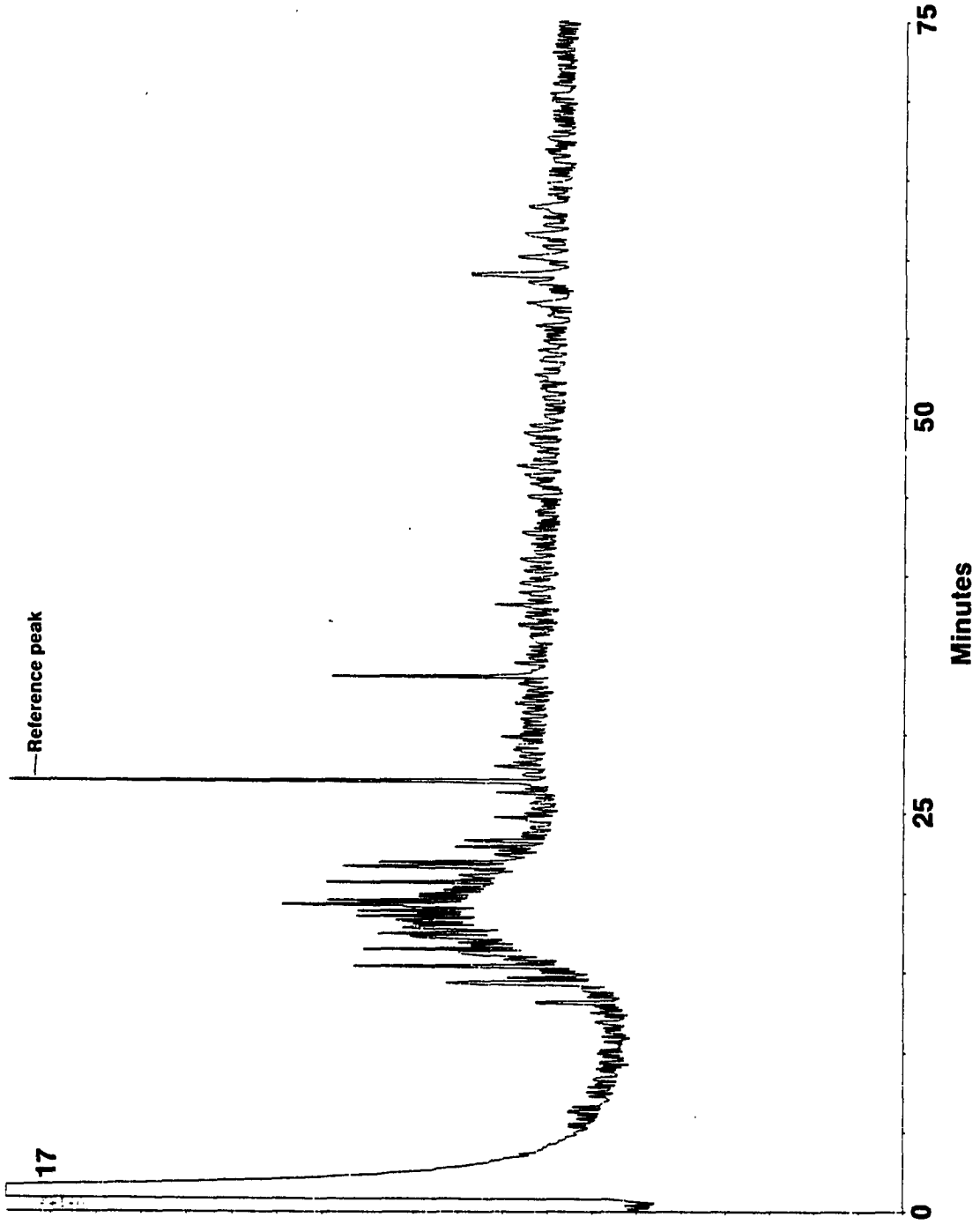


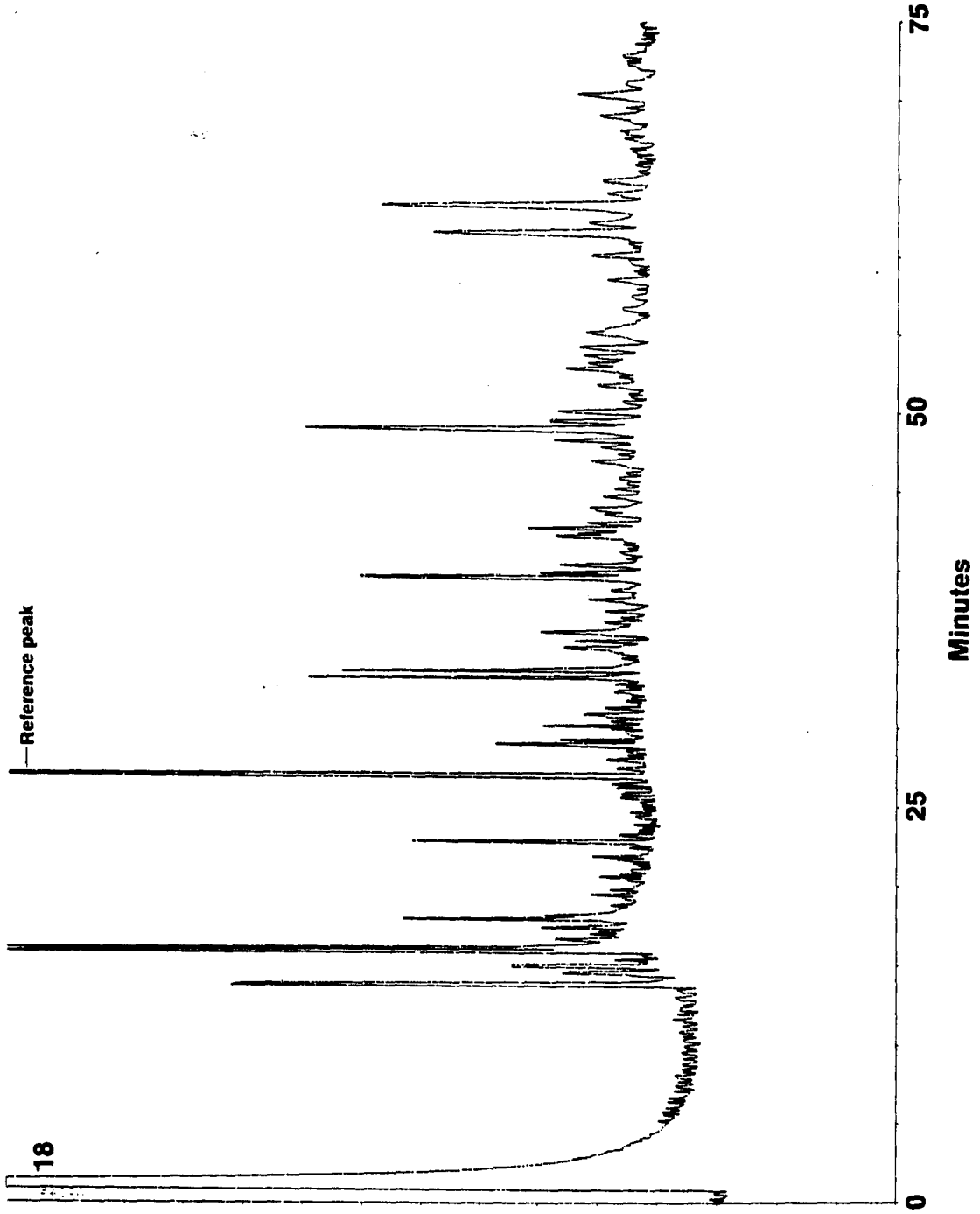


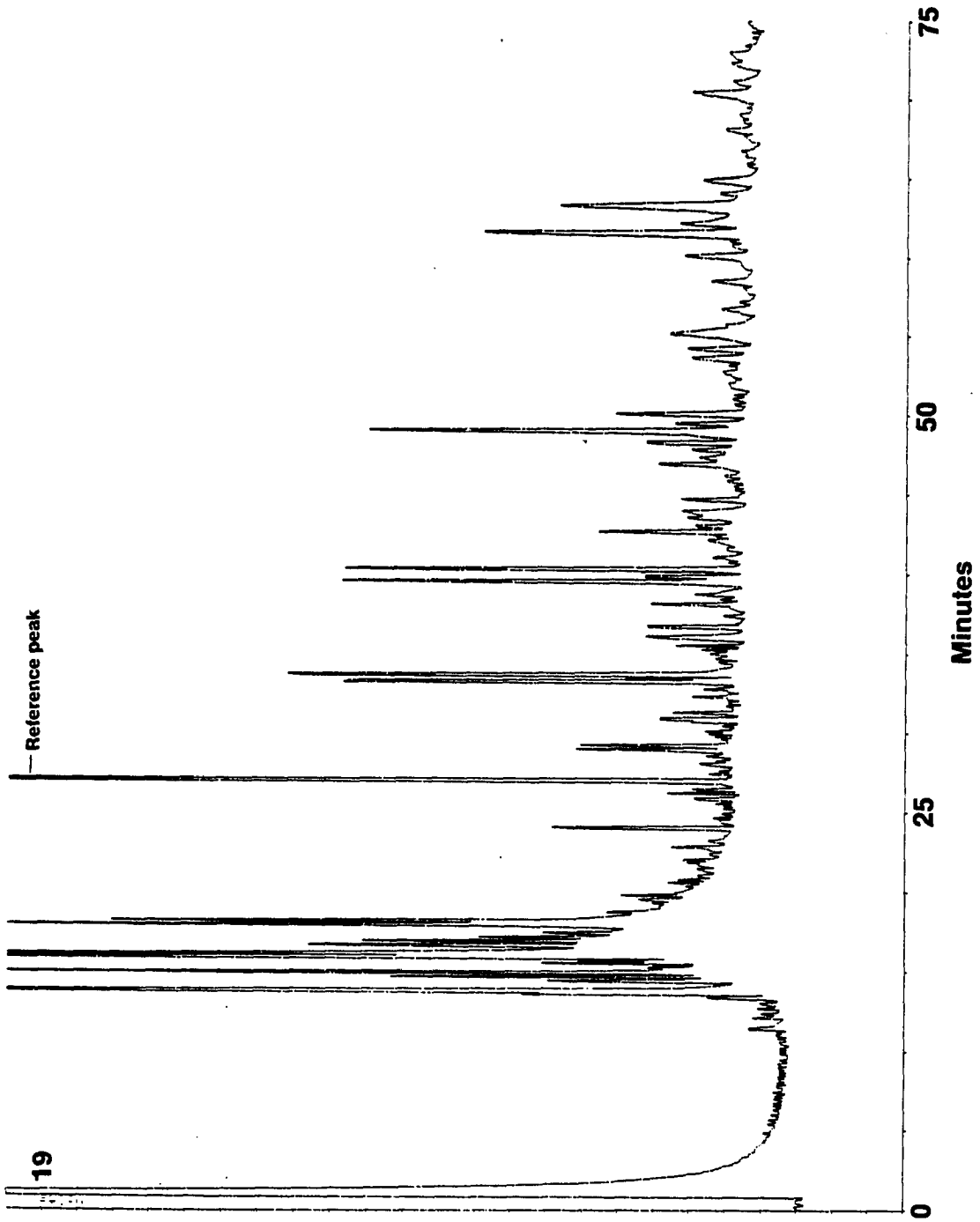


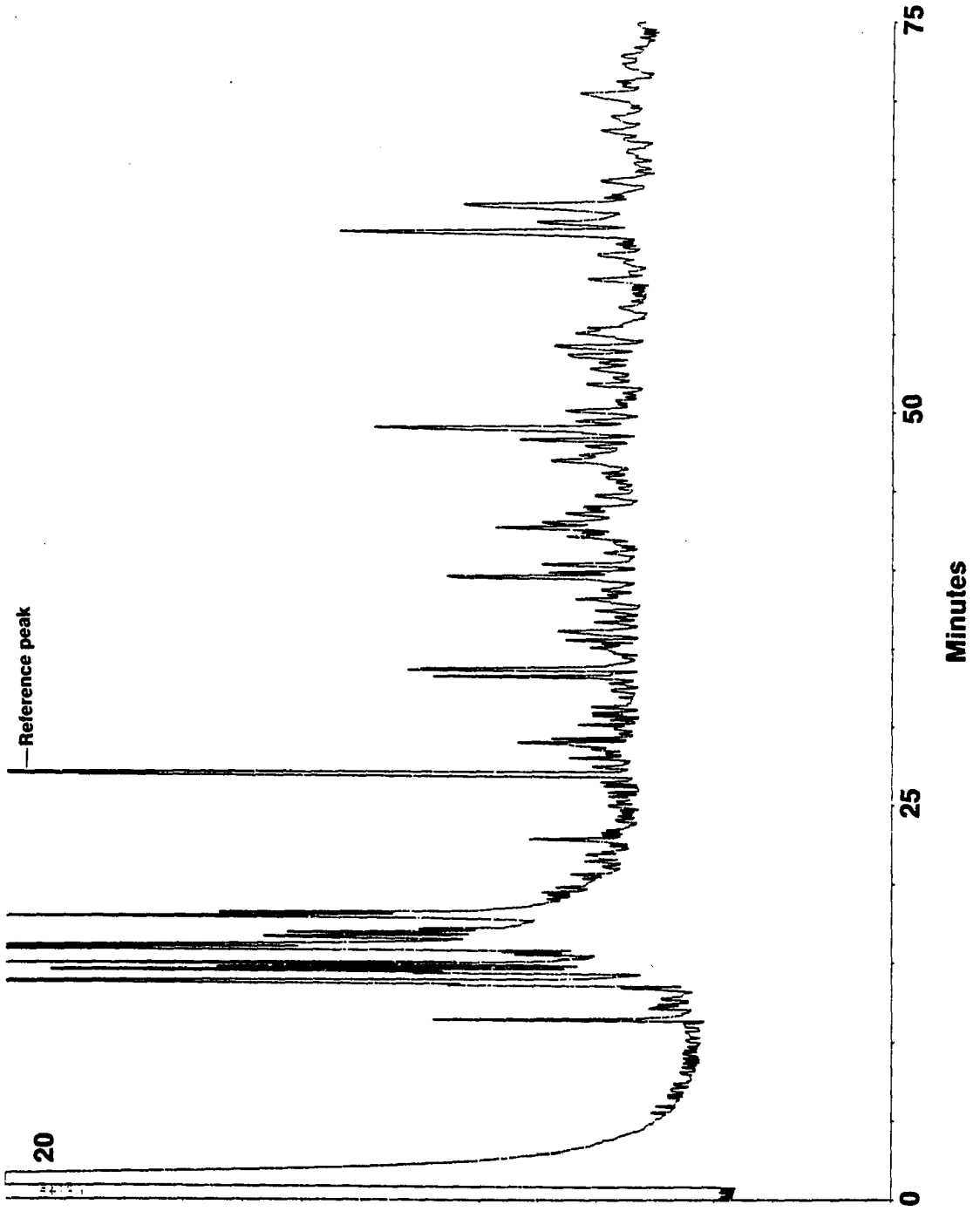


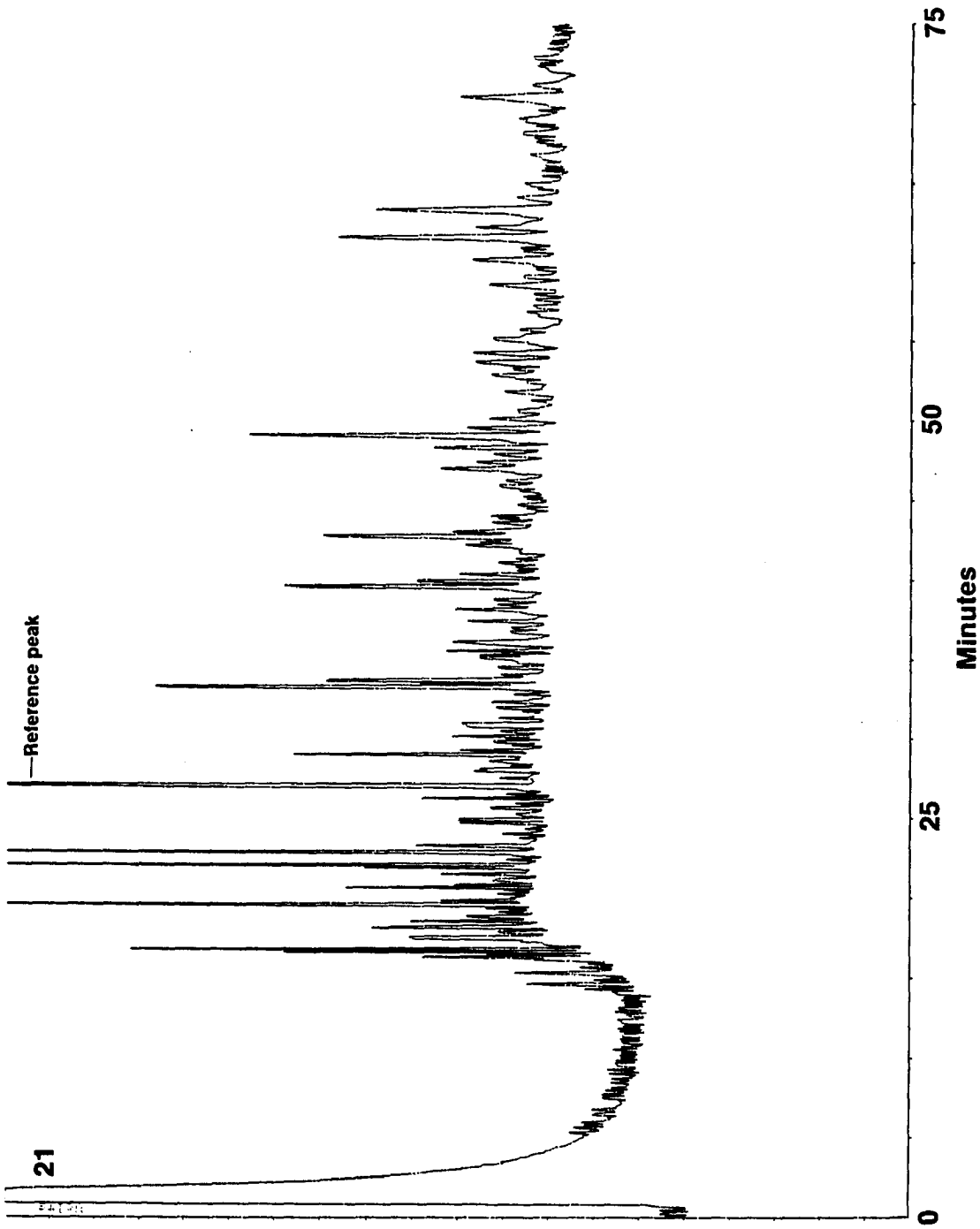


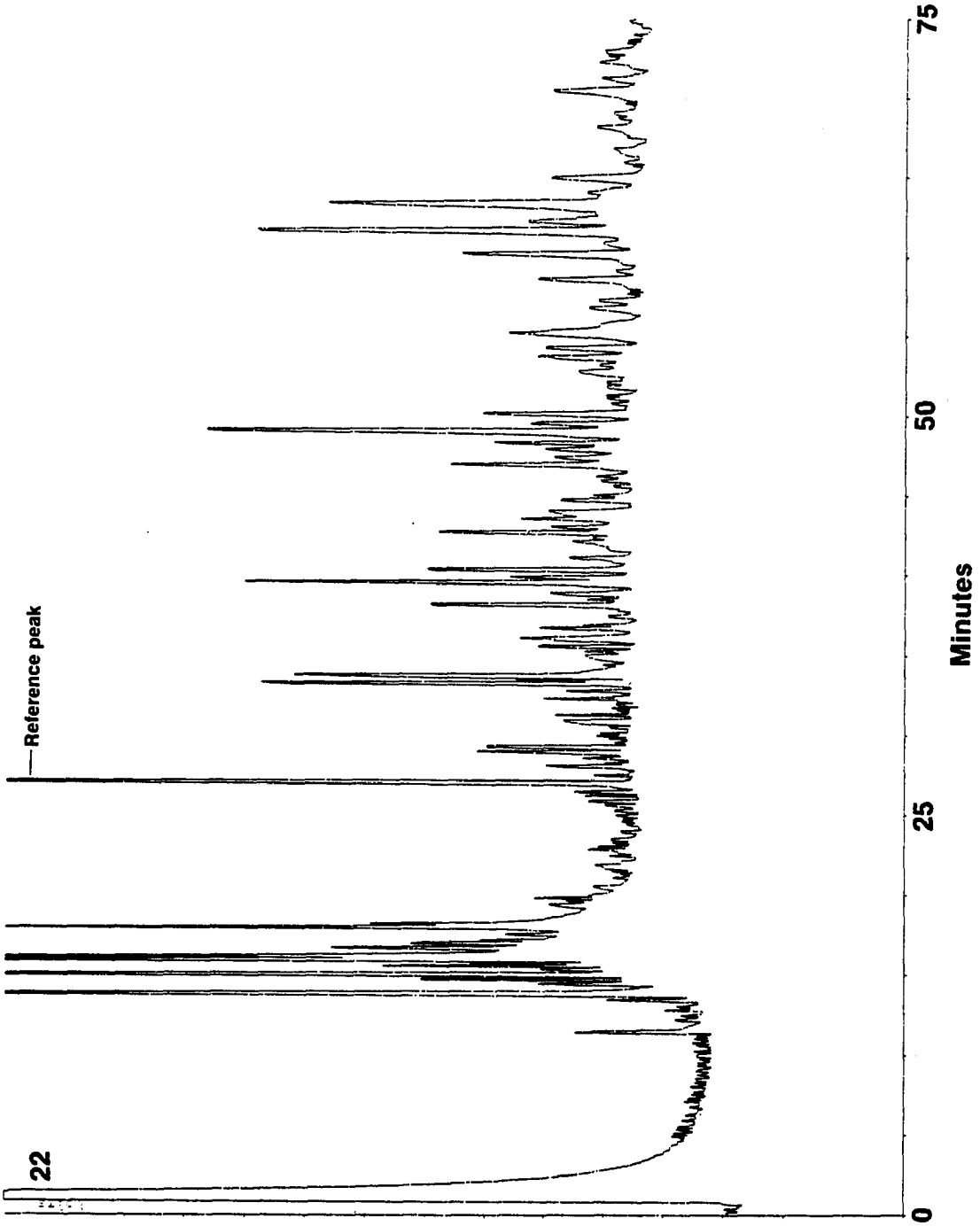


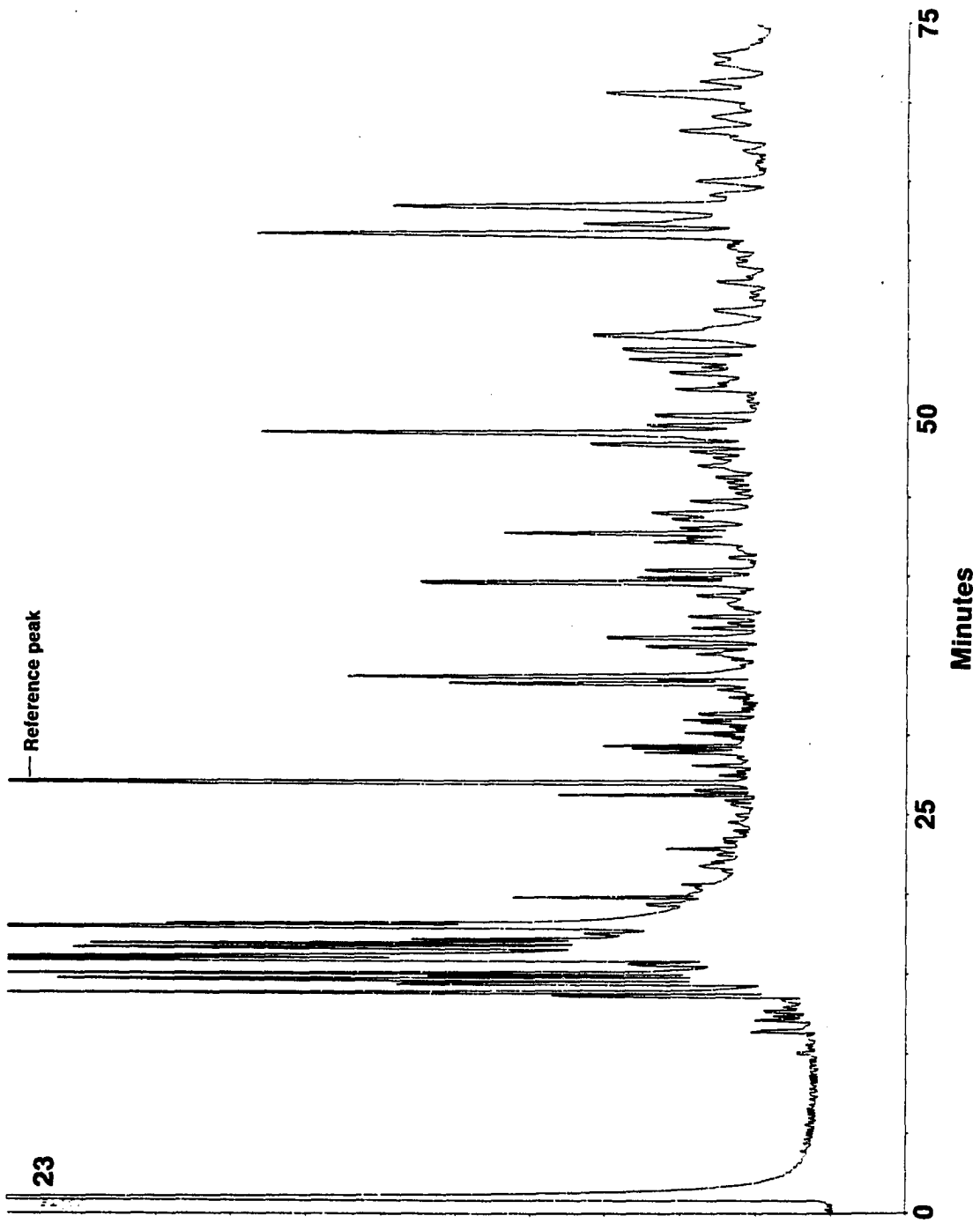












LITERATURE CITED

LITERATURE CITED

1. Noble, A.C., in Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp. 203-228 (1978).

2. Rapp, A., Hastrich, H., Engel, L., Knipser, W., in Flavor of Foods and Beverages: Chemistry and Technology, Charalambous, G., Inglett, G.E., eds., Academic Press, New York, pp. 391-417 (1978).

3. Vitzthum, O.G., Werkhoff, P., in Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp. 115-133 (1978).

4. Parliment, T.H. and Scarpellino, R., J. Agric. Food Chem. 25, 97 (1977).

5. Kimura, K., Nishimura, H., Iwata, I. and Mizutani J., J. Agric. Food Chem., 31, 801 (1983).

6. Moshonas, M.G. and Shaw, P.E., J. Agric. Food Chem., 32, 526 (1984).

7. Nordby, H.E. and Nagy, S., J. Agric. Food Chem., 25, 224 (1977).

8. ter Heide, R., de Valois, P.J., Visser, J., Jaegers, P.P. and Timmer, R., in Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp249-281 (1978).

9. Akiyama, H., Yoshizawa, K. and Ouchi, K. In Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp. 229-248 (1978).

10. Kwan, W. and Kowalski, B.R., Anal. Chim. Acta, 122, 215 (1980).

11. Kwan, W. and Kowalski, B.R., J. Agric. Food Chem., 28, 356 (1980).

12. Schreier, P. and Reiner, L., J. Sci. Food Agric., 30, 319 (1979).

13. Engel, K. and Tressl, R., J. Agric. Food Chem., 31, 796 (1983).

14. MacLeod, A.J. and Snyder, C., J. Agric. Food Chem., 33, 380 (1985).

15. Flath, R.A., Forrey, R.R. and Teranishi, R., J. Food Sci., 34, 382 (1969).

16. Biggers, R.E., Hilton, J.J. and Gianturco, M.A., J. Chromatogr. Sci., 7, 453 (1969).

17. Buttery, R.G. and Teranishi, R., Anal. Chem., 33, 1439 (1961).

18. Boyko, A.L., Morgan, M.E. and Libbey, L.M., in Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp. 57-80 (1978).

19. Jennings, W. and Shibamoto, T., Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press, New York, pp. 1-472, (1980).

20. Hawkes, S.J. and Wheaton, M.D., J. Food Sci., 32, 629 (1967).

21. Dirinck, P., Schreyen, L. and Schamp,N., J. Agric. Food Chem., 25, 759 (1977).

22. Buttery, R.G. and Teranishi, R., Agric. Food Chem., 11, 504 (1963).

23. Aishima, T., Agric. Biol. Chem. 45, 2847 (1981).

24. Aishima, T. and Nobuhara, A., Agric. Biol. Chem., 41, 1841 (1977).

25. del Rosario, R., de Lumen, B.O., Habu, T., Flath, R.A., Mon, T.R. and Teranishi, R., J. Agric. Food Chem., 32, 1011 (1984).

26. Sakaki, T., Fukuhara, K., Niino, K., Sakuma, H. and Sugawara, S., Agric. Biol. Chem., 49, 1785 (1985).

27. Lee, M.L., Bartle, K.D. and Novotny, M.V., Anal. Chem., 47, 540 (1975).

28. Levy, E.J. and Wampler, T.P., J. Forensic Sci., 31, 258 (1986).

29. McMinn, D.G., Carlson, T.L. and Munson, T.O., J. Forensic Sci., 30, 1064 (1985).

30. Kelly, R.L. and Martz, R.M., J. Forensic Sci., 29, 714 (1984).

31. Novotny, M., Lee, M.L., Low, C. and Raymond, A., Anal. Chem., 48, 24 (1976).

32. Hood, L.V.S. and Barry, G.T., J. Chromatogr., 166, 499 (1978).

33. Neumann, H., J. Chromatogr., 315, 404 (1984).

34. O'Neil, P.J., Baker, P.B. and Gough, T.A., J. Forensic Sci., 29, 889 (1984).

35. Saxberg, B.E.H., Duewer, D.L., Booker, J.L. and Kowalski, B.R., Anal. Chim. Acta, 103, 201(1978).

36. Blomquist, G., Johansson, E., Söderström, B. and Wold, S., J. Chromatogr., 173, 7 (1979).

37. Rosen, R.T., Rosen, J.D. and DiProssimo, V.P., J. Agric. Food Chem., 32, 276 (1984).

38. Zechman, J.M., Aldinger, S. and Labows, J.N., Jr., J. Chromatogr., 377, 49 (1986).

39. Moss, C.W. and Dees, S.B., J. Chromatogr., 112, 595-(1975).

40. Morris, N.M. and Brannan, M.A.F., J. Chromatogr., 374, 27 (1986).

41. Brill, J.H., Mayfield, H.T., Mar, T. and Bertsch, W., J. Chromatogr., 349, 31 (1985).

42. Brill, J.H., Mar, T., Mayfield, H.T. and Bertsch, W., J. Chromatogr., 349, 39 (1985).

43. Dunn, W.J.,III, Stalling, D.L., Schwartz, T.R., Hogan, J.W., Petty, J.D., Johansson, E. and Wold, S., Anal. Chem., 56, 1308 (1984).

44. Dreisch, F.A. and Munson, T.O., J. Chromatogr. Sci., 21, 111 (1983).

45. Dowty, B., Carisle, D., Laseter, J.L. and Storrer,J., Science, 187, 75 (1975).

46. Bertsch, W., Chang, R.C. and Zlatkis, A., J. Chromatogr. Sci. 12, 175 (1977).

47. Jeltes, R., J. Chromatogr. Sci., 12, 599 (1974).

48. Kawahara, F.K., J. Chromatogr. Sci., 10, 629 (1972).

49. Clark, H.A. and Jurs, P.C., Anal. Chem., 51, 616 (1979).

50. Desideri, P.G., Lepri, L., Heimler, D., Checchini, L. and Giannessi, S., J. Chromatogr., 322, 107 (1985).

51. Adlard, E.R., Creaser, L.F. and Matthews, P.H.D., Anal. Chem, 44, 64 (1972).

52. Overton, E.B., Bracken, J. and Laseter, J.L., J. Chromatogr. Sci., 15, 169 (1977).

53. Ogata, M. and Fugisawa, K., J. Chromatogr. Sci., 21, 420 (1983).

54. Parrish, M.E., Good, B.W., Jeltema, M.A. and Hsu, F.S., Anal. Chim. Acta, 150, 163 (1983).

55. Mackay, D.A.M. and Hussein, M.M., in Analysis of Foods and Beverages: Headspace Techniques, Charalambous, G., ed., Academic Press, New York, pp. 283-357 (1978).

56. Kullik, E., Kaljurand, M. and Koel, M., J. Chromatogr., 126, 249 (1976).

57. Dalgliesh, C.E., Horning, E.C., Horning, M.G., Knox, K.L. and Yarger, K., Biochem. J., 101, 792 (1966).

58. Zlatkis, A. and Liebich, H., Clin. Chem., 17, 592 (1971).

59. Horning, E.C. and Horning, M.G., Clin. Chem., 17, 802 (1971).

60. Jellum, E., Stokke, O. and Eldjarn, L., Clin. Chem., 18, 800 (1972).

61. Teranishi, R., Mon, T.R., Robinson, A.B., Cary, P. and Pauling, L., Anal. Chem., 44, 18 (1972).

62. Liebich, H.M., Al-Babbili, O., Zlatkis, A. and Kim, K., Clin. Chem., 21, 1294 (1975).

63. Gates, S.C., Sweeley, C.C., Krivit, W., DeWitt, D. and Blaisdell, B.E., Clin. Chem., 24, 1680 (1978).

64. Zlatkis, A., Wang, F.S., Shanfield, H., Anal. Chem., 55, 1848 (1983).

65. Horning, E.C. and Horning, M.G., J. Chromatogr. Sci., 9, 129 (1971).

66. Heininger, J., Munthe, E., Pahle, J. and Jellum, E., J. Chromatogr., 158, 297 (1978).

67. Sweeley, C.C., Young, N.D., Holland, J.F. and Gates, S.C., J. Chromatogr., 99, 507 (1974).

68. Stafford, M., Horning, M.G. and Zlatkis, A., J. Chromatogr., 126, 495 (1976).

69. Reimendal, R. and Sjövall, J.B., Anal. Chem., 45, 1083 (1973).

70. Politzer, I.R., Githens, S., Dowty, B.J. and Laseter, J.L., J. Chromatogr. Sci., 13, 378 (1975).

71. Baty, J.D. and Wade, A.P., Anal. Biochem., 57, 27 (1974).

72. Paffenberger, C.D., Szafranek, J. and Horning, E.C., J. Chromatogr., 126, 535 (1976).

73. Liebich, H.M. and Woll, J., J. Chromatogr., 142, 505 (1977).

74. Zlatkis, A., Poole, C.F., Brazell, R., Lee, K.Y. and Singhawangcha, S., J. High Resolut. Chromatogr. Chromatogr. Commun., 2, 423 (1979).

75. Stoner, E., Cowburn, D. and Craig, L.C., Anal. Chem., 47,344 (1975).

76. Brown, G.K., Stokke, O. and Jellum, E., J. Chromatogr., 145, 177 (1978).

77. Zlatkis, A., Bertsch, W., Bafus, D.A. and Liebich, H.M., J. Chromatogr., 91, 379 (1974).

78. Liebich, H.M., Pickert, A., Stierle, U. and Woell, J., J. Chromatogr., 199, 181, (1980).

79. Hayward, N.J., J. Chromatogr., 274, 27 (1983).

80. Goodman, S.I., Helland, P., Stokke, O., Flatmark, A. and Jellum, E., J. Chromatogr., 142, 497 (1977).

81. McConnell, M.L. and Novotny, M., J. Chromatogr., 112, 559 (1975).

82. Matsumoto, K.E., Partridge, D.H., Robinson, A.B., Pauling, L., Flath, R.A., Mon, T.R. and Teranishi, R., J. Chromatogr.,85, 31 (1973).

83. Pfaffenberger, C.D. and Horning, E.C., J. Chromatogr., 112, 581 (1975).

84. Malcolm, R.D. and Leonardo, R., Clin. Chem., 22, 623 (1976).

85. Herranen, J., Huhtikangas, A., Tirronen, H., Halonen, T., Huuskonen, M., Reinikainen, K. and Riekkinen, P., J. Chromatogr., 307, 241 (1984).

86. Niwa, T., Maeda, K., Ohki, T., Saito, A. and Tsuchida, I., J. Chromatogr., 225, 1 (1981).

87. Ng, K.J., Andresen, B.D., Hilty, M.D. and Bianchine, J.R., J. Chromatogr., 276, 1 (1983).

88. Liebich, H.M., Al-Babbili, O., Zlatkis, A. and Kim, K., Clin. Chem., 21, 1294 (1975).

89. Maurer, H. and Pfleger, K., J. Chromatogr., 305, 309 (1984).

90. Liebich, H.M. and Huesgen, G., J. Chromatogr., 126, 465 (1976).

91. Lee, K.Y., Ph.D. Dissertation, University of Houston, Houston, TX (1978).

92. Pfaffenberger, C.D., Malinak, L.R. and Horning, E.C., J. Chromatogr.,158, 313 (1978).

93. Krotoszynski, B., Gabriel, G. and O'Neill, H., J. Chromatogr. Sci., 15, 239 (1977).

94. Luyten, J.A. and Rutten, G.A.F.M., J. Chromatogr., 91, 393 (1974).

95. Miyazaki, H., Ishibashi, M., Inoue, M., Itoh, M. and Kubodera, T., J. Chromatogr., 99, 553 (1974).

96. Lee, K.Y., Nurok, D. and Zlatkis, A., J. Chromatogr., 158, 377 (1978).

97. Axelson, M. and Sjövall, J., J., Chromatogr., 126, 705 (1976).

98. Brazell, R.S., Ph.D. Dissertation, University of Houston, Houston, TX, (1979).

99. Zlatkis, A., Wang, F.S. and Shanfield, H., Anal. Chem., 54, 2406 (1982).

100. Malya, P.A.G., Wright, J.R. and Nes, W.R., J. Chromatogr. Sci., 9, 700 (1971).

101. Gates, S.C., Smisko, M.J., Ashendel, C.L., Young, N.D., Holland, J.F. and Sweeley, C.C., Anal. Chem., 50, 433 (1978).

102. Zlatkis, A., Poole, C.F., Brazell, R., Lee, K.Y., Hsu, F. and Singhawangcha, S., Analyst, 106, 352 (1981).

103. Issachar, D., Holland, J.F. and Sweeley, C.C., Anal. Chem., 54, 29 (1982).

104. Lin, S. and Horning, E.C., J. Chromatogr., 112, 483 (1975).

105. Liebich, H.M., Pickert, A., Stierle, U. and Woll, J., J. Chromatogr., 199, 181 (1980).

106. Zlatkis, A., Bertsch, W., Lichtenstein, H.A., Tishbee, A., Shunbo, F., Liebich, H.M., Coscia, A.M. and Fleischer, N., Anal. Chem., 45, 763 (1973).

107. Jellum, E., Stokke, O. and Eldjarn, L., Anal. Chem., 45, 1099 (1973).

108. Robinson, A.B., Partridge, D., Turner, M., Teranishi, R. and Pauling, L., J. Chromatogr., 85, 19 (1973).

109. Gan, I., Korth, J. and Halpern, B., J. Chromatogr., 92, 435 (1974).

110. Pino, J.A., McMurry, J.E., Jurs, P.C., Lavine, B.K. and Harper, A.M., Anal. Chem., 57, 295 (1985).

111. Jellum, E., Storseth, P., Alexander, J., Helland, P., Stokke, O. and Teig, E., J. Chromatogr., 126, 487 (1976).

112. Eldjarn, L., Jellum, E. and Stokke, O., J. Chromatogr., 91, 353 (1974).

113. Edman, D.C. and Brooks, J.B., J. Chromatogr., 274, 1 (1983).

114. Jakobs, C., Solem, E., Ek, J., Halvorsen, K. and Jellum, E., J. Chromatogr., 143, 31 (1977).

115. Liebich, H.M., J. Chromatogr., 112, 551 (1975).

116. Jellum, E., Bjornson, I., Nesbakken, R., Johansson, E. and Wold, S., J. Chromatogr., 217, 231 (1981).

117. Heindl, A., Dietel, P. and Spiteller, G., J. Chromatogr., 377, 3 (1986)

118. Liebich, H.M. and Pickert, A., J. Chromatogr, 338, 25 (1985).

119. Niwa, T., Asada, H., Maeda, K., Yamada K., Ohki, T. and Saito, A., J. Chromatogr., 377, 15 (1986).

120. Burke, D.G., Hilliard, E., Watkins, K., Russ, V. and Scott, B.J., Anal. Biochem., 149, 421 (1985).

121. McConnell, M. L., Rhodes, G., Watson, U. and Novotny, M., J. Chromatogr., 162, 495 (1979).

122. Zlatkis, A., Lee, K.Y., Poole, C.F. and Holzer, G., J. Chromatogr., 163, 125 (1979).

123. Zlatkis, A. and Andrawes, F., J. Chromatogr., 112, 533 (1975).

124. Zlatkis, A., Lichtenstein, H.A., Tishbee, A., Bertsch, W., Shunbo, F. and Liebich, H.M., J. Chromatogr. Sci., 11, 299 (1973).

125. Zlatkis, A. and Kim, K., J. Chromatogr., 126, 475 (1976).

126. Liebich, H.M. and Al-Babbili, O., J. Chromatogr., 112, 539 (1975).

127. Rhodes, G., Miller, M., McConnell, M.L. and Novotny, M., Clin. Chem., 27, 580 (1981).

128. Vrbanac, J.J., Braselton, W.E., Jr., Holland, J.F. and Sweeley, C.C., J. Chromatogr., 239,265 (1982).

129. Tsai, M.Y., Oliphant, C. and Josephson, M.W., J. Chromatogr, 341, 1 (1985).

130. Brooks, J.B., Basta, M.T., Holler, J.S., Alley, C.C. and Kholy, A.M., J. Chromatogr., 339, 243 (1985).

131. Jellum, E., J., Chromatogr., 143, 427 (1977).

132. Gates, S.C. and Sweeley, C.C., Clin. Chem., 24, 1663 (1978).

133. Liebich, H.M., J. Chromatogr., 146, 185 (1978).

134. Zlatkis, A., Brazell, R.S. and Poole, C.F., Clin. Chem., 27, 789 (1981).

135. Bretell, T.A. and Grob, R.L., Am. Lab., 17, 50 (1985).

136. Windig, W., Haverkamp, J. and Kistemaker, P.G., Anal. Chem., 55, 81 (1983).

137. Jennings, W. and Mehran, M.F., J. Chromatogr. Sci, 24, 34 (1986).

138. Chien, M., Anal. Chem., 57, 348 (1985).

139. Varmuza, K., Anal. Chim. Acta, 122, 227 (1980).

140. McCown, S.M., Manos, C.G., Jr., Pitzer, D.R. and Earnest, C.M., Analyst, 107, 1393 (1982).

141. Massart, D.L., Kaufman, L. and Coomans, D., Anal. Chim. Acta, 122, 347 (1980).

142. Lea, R.E., Bramston-Cook, R. and Tschida, J., Anal. Chem., 55, 626 (1983).

143. Mayfield, H.T., Ph.D. Dissertation, University of Alabama, University, AL (1984).

144. Demirgian, J.C., J. Chromatogr. Sci., 22, 153 (1984).

145. Albano, C., Dunn, W., III, Edlund, U., Johansson, E., Norden, B., Sjostrom, M. and Wold, S., Anal. Chim. Acta, 103, 429 (1978).

146. Goetz, N., Kaba, G., and Bore, P., J. Chromatogr, 233, 19 (1982).

147. Nicolaides, N., Science, 186, 19 (1974).

148. Downing, D.T., in Thin Layer Chromatography: Quantitative Environmental and Clinical Applications, Touchstone, J.C. and Rogers, D., eds., Wiley, New York, pp. 495-516 (1980).

149. Gloor, M. in Cosmetic Science, Vol. 1, Breuer, M.M., ed., Academic Press, New York, pp. 217-273 (1978).

150. Green, S.C., Stewart, M.E. and Downing, D.T., J. Invest. Dermatol., 83, 114 (1984).

151. Wolff, M.S., Anal. Chem., 56, 1492 (1984).

152. Boniforti, L., Passi, S., Caprilli, F. and Nazzaro Porro, M., Clin. Chim. Acta, 47, 223 (1973).

153. De Forest, P.R., Gaensslen, R.E. and Lee, H.C., Forensic Science: An Introduction to Criminalistics, McGraw-Hill, New York, p. 149 (1983).

154. Saferstein, R., Criminalistics: An Introduction to Forensic Science, 2nd ed., Prentice-Hall, Englewood Cliffs, New Jersey, pp. 5-7 (1981).