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THE PREPARATION AND STORAGE OF ULTRA HIGH PURITY HEXANE FOR SOLVENT EFFECT GAS CHROMATOGRAPHIC ANALYSIS

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The preparation and storage of ultra high purity hexane for solvent effect gas chromatographic analysis

by

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TO MY PARENTS

If you use words to explain
there is bound to be ambiguities.

The most complete answer is
in the question itself.

The preparation and storage of ultra high purity hexane

for

solvent effect gas chromatographic analysis

By

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SUMMARY

The newly developed dynamic solvent effect (DSE) sampling technique for capillary gas chromatography requires ultra pure solvents for optimum performance. Hexane, the most popular solvent for this concentration technique, is not available commercially in a grade of the required purity. Trace impurities present in the freshly purified solvent and those originating from laboratory glassware, storage vials and decomposition of the hexane itself, can lead to grave errors in analytical results after concentration.

A goal was set to remove flame ionization detectable impurities less volatile than heptane to levels of 1 pp10^9 (m/v) per component and to maximize the shelf life of the purified hexane.

Methods investigated for purification include fractional distillation (with simple packed columns and with spinning band columns) as well as frontal elution gas and liquid chromatography. The storage of purified hexane was studied in borosilicate glass flasks with ground glass stoppers and borosilicate glass vials with various cap liner materials, under different conditions of temperature and light.

A cheap, simple, and reliable technique for preparing and storing ultra high purity hexane was developed and tested. The results could help to introduce the DSE technique to other laboratories specializing in trace analysis. Other analytical techniques that rely on achieving high sensitivity by evaporative concentration of hexane solutions could also benefit from the findings.

OPSOMMING

Die nuut ontwikkelde, dinamiese-oplosmiddeleffek-(DSE)-inlaattegniek vir kapillêre gaschromatografie vereis uiters suiwer oplosmiddels vir optimum doeltreffendheid. Heksaan is die mees geskikte oplosmiddel vir hierdie konsentrasie-tegniek maar is nie in die handel beskikbaar in die vereiste suiwerheidsgraad nie. Spore onsuiwerhede oorspronklik teenwoordig, asook díe afkomstig vanaf laboratorium-glasware, stoorflessies en van die ontbinding van die heksaan self, kan tot verkeerde analiseresultate lei na selektiewe verdamping van die heksaan.

Die verwydering van onsuiwerhede (wat minder vlugtig is as heptaan en wat met die vlamionisasiedetektor waarneembaar is) tot vlakke van 1 deel per 10^9 is as teiken gestel. Verder moes die rakleeftyd van gesuiwerde heksaan verbeter word.

Suiweringsmetodes wat ondersoek is, het fraksionele distillasie (met gewone gepakte kolomme en met die roterende band kolom) asook frontaaleluering gas- en vloeistofchromatografie ingesluit. Die berging van gesuiwerde heksaan is bestudeer in borosilikaat-glashouers met slypstuk-afdigting asook in klein flessies wat met 'n verskeidenheid van materiale afgeseël is. Die invloed van lig en temperatuur op rakleeftyd is bestudeer.

'n Goedkoop, eenvoudige en betroubare metode vir die vervaardiging en berging van ultra-hoësuiwerheid heksaan is ontwikkel en getoets. Die resultate kan meehelp om die DSE tegniek in ander laboratoria bekend te stel wat ook in spooranalise spesialiseer. Ander analisetegnieke wat staat maak op verdampingskonsentrering van heksaanoplossings om hoë sensitiwiteit te bereik, kan ook baat vind by die bevindinge.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

AtmosA = Atmospheric air

Chr = Chromatogram

(Chromatograms for all the analyses can be found in the appendix.)

HPN_2 = High purity nitrogen gas

$P\text{-}PH_2$ = Palladium-purified hydrogen gas

$pp10^9$ = 1 : 10^9 (m/v)

PTFE = Polytetrafluoroethylene

TNRS = Teflon-faced natural rubber septum

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Organic solvents have lots of uses in the chemical and allied industries and in scientific research such as for solvent extraction and separation by distillation, or for the preparation of solutions for chromatographic and spectroscopic examination. In many experiments, the factor which limits the accuracy of the results is the purity of the materials used rather than the refinement of the measurements, so the need for solvents of high purity is great.

The degree of purity required depends on the materials to be investigated, the use which is to be made of it, and the nature of the impurities. In addition to any accidental contaminants such as dust, scraps of paper, cork, etc., that may have been united into the sample during manufacture, all commercial solvents are in some measure impure. Distilled-in-glass grade solvents have been recommended as the solvent of choice for trace organic analysis because they generally have the smallest amount of impurities [1]. Among organic solvents there is a range of qualities varying from laboratory chemical to spectroscopic, chromatographic and electronic grades. Although many of these are usually satisfactory, as supplied, for most purposes in scientific and technological

work, it's also true that further purification is necessary for many applications.

Anyone who has undertaken the analysis of trace amounts of organic materials knows the importance of pure solvents, and needs pure enough solvents to produce an acceptable blank in his analytical procedures. When doing solvent extraction followed by concentration of the extract, or sampling by the dynamic solvent effect (DSE) [2], impurities originally present in the solvents are also concentrated. The background levels in the solvents after extraction and/or concentration can be significant sources of error in gas chromatographic analysis. There are many limitations with respect to inconvenience, expenditure, purity, and yield in the purification methods of solvents; moreover, commercial high-purity solvents are not available for more demanding applications like ultratrace gas chromatographic analysis [2] and are very expensive.

Impurities and contamination in general is definitely one of the most serious problems in trace analysis. A worldwide questionnaire survey on contamination problems in trace analysis revealed that in 96 laboratories the main sources of contamination were considered to be: laboratory air, reagents, equipment, and the investigator [3]. Solvent deterioration presents serious practical difficulties, the more obvious sources of contamination of solvents arise from storage in containers and from contact with cap liners. Organic compounds may change during storage, contamination from the walls

of containers rivals that from contact with laboratory air [4]. But alkanes are subject to atmospheric oxidation at room temperature by reactions which are free radical initiated, catalyzed by metals and reaction products themselves (autocatalysis) [5]. The deterioration of pure hexane was consistent with such a mechanism. Metal ions are present in higher concentration in soda-lime glass than in borosilicate glass and are absent in amorphous silica [6].

The abovementioned has prompted us to investigate the purification of commercially available solvents, and the recovery of previously used high-purity solvents and solvents which have only been partially used and stored. The first objective of this work is to simplify the procedure of purifying hexane in order to share the benefits of the DSE sampling with other researchers. Secondly, we are studying the stability of purified hexane on storage.

1.2 SOLVENT PURIFICATION IN GENERAL

There are a number of purification methods that rely on chemical and/or physical processes in the literature [7-12]. The chemical steps are characteristic of the compounds involved. Greater selectivity in purification can often be achieved by making use of differences in chemical properties between the substance to be purified and its contaminants. Many classes of organic chemicals can be purified by conversion into suitable derivatives, followed by regeneration. There are many physical techniques for purifying materials such as absorption, adsorption, countercurrent

distribution, distillation, etc.. A simple but effective method of purification for liquid chromatography is frontal analysis which has been modified by Buszewski [13]. This method could give satisfactory results, but it does have the drawbacks of high cost and small quantitative yield. Katusz [14] purified previously used HPLC-grade solvents by spinning band distillation and recovered solvents with purity at or near the specifications of commercially available HPLC-grade solvents. The hydrocarbons were subjected to purification in a number of ways [7, 8], e.g. fractional distillation, chromatography, selective photolysis followed by treatment with sulfuric acid, etc. [15], nevertheless, solvents specified as pure for a particular purpose may be quite impure for other uses.

Because of its outstanding chemical and physical properties hexane is one of the most used solvents in gas chromatography, specially in the DSE sampling. Ultraviolet-grade hexane was found to contain ethylene glycol diacetate, glycerol acetate, C_{10} acid, octyl adipate, and diethyl phthalate [16]. Hexane was subjected to purification in a number of ways [7, pp. 824-825; 13-15] applicable to the hydrocarbons. Apps [17] purified hexane for the DSE sampling by frontal elution gas adsorption chromatography on alumina, yielding hexane with impurities detectable by flame ionization detection only at the ppt ($1:10^{12}$) level.

However, the important question is not whether a substance is pure but whether a given sample is sufficiently pure for the intended purpose. Namely, are the contaminants likely to interfere in the process or measurement that is to be studied. It is possible to reduce levels of impurities to acceptable limits by suitable manipulation, but absolute purity is an ideal which can never be obtained.

Dry, noncorrosive solids usually do not react with container materials. However, fluids such as solvents frequently interact with their immediate environment. Extreme care must always be taken to avoid contacting the samples with any material of unknown composition or purity, since trace quantities of impurities are frequently leached from collecting devices which could be made of glass, plastics, and aluminium, etc.. On the other hand, a limiting factor in trace analysis of many substances is their tendency to become adsorbed onto glassware. It is a little-known fact that the type of glass from which a container is made may have a major influence on the degree of adsorption of some compounds [18].

The universal material of container in the chemical laboratory is glass. Glass is usually considered to be an " inert " material. For most glasses this is true relative to other materials. However, where the highest purity is to be maintained by solvents or solutions contained in glass, one must consider that glass can be

chemically active. There are three types of glass commonly used in the laboratory:

Type I, borosilicate, Class A (33 expansion glass)

Type I, borosilicate, Class B (51 expansion glass)

Type II, soda-lime (93 expansion glass)

Both Type I (borosilicate) glasses are much more resistant to chemical attack than Type II (soda-lime) glass. Soda-lime glass is used for the least demanding laboratory applications. Table 1.1 [19] compares the chemical composition of soda-lime and

Table1.1 Chemical composition of glasses.

Property	Type I, Class A	Type I, Class B	Type II
SiO_2	81 %	73 %	68 %
B_2O_3	13 %	10 %	2 %
Al_2O_3	2 %	7 %	3 %
BaO	tr.	tr.	2 %
CaO	tr.	1 %	5 %
Na_2O	tr.	6 %	15 %
K_2O	tr.	1 %	1 %
As_2O_3 plus Sb_2O_3, max.	tr.	tr.	tr.
PbO, max.	tr.	tr.	4 %
ZnO, max.	tr.	tr.	tr.
All Other Constituents, max.	0.2 %	0.4 %	-

tr.= maximum trace levels of 0.1 %

borosilicate glasses. For more demanding applications, as for containers of high purity liquids, borosilicate glass becomes necessary, specially Type I, Class A which indicates higher chemical resistance than Type I, Class B. This study focused on **Type I, Class A glass.**

Another source of contamination is from the seals of containers, and Teflon is the preferred material to avoid contamination problems. Metal foil liners are generally more resistant to organic solvents while Teflon liners are inert to most chemicals.

1.3 AIM AND APPROACH

Trace analysis has demonstrated that the purity attainable is affected by the methods of handling raw materials and intermediates as well as by the materials of construction for apparatus and storage containers. Sensitive methods have been developed for the detection and elimination of progressively lower levels of impurities. The need to eliminate trace impurities at the nanogram per ml level has placed greater emphasis on ultrapurification techniques. To meet this demand the range of purities of laboratory solvents has become correspondingly extended. Purification of individual solvents thus depends more critically on the answers to two questions --- purification from what, and to what permissible level of contamination. Where these questions can be specifically answered, suitable methods of purification can usually be found in the literature, or improved by applying more or less conventional

procedures. A knowledge of the origin of the starting material is important, since it will influence the procedure adopted [20; 21, pp. 300-302].

The aim of hexane purification in this study was to achieve a degree of purity according to two different concentration limits --- **50 pp10⁹ and 1 pp10⁹ (m/v)**, per component of hydrocarbon impurities, less volatile than heptane. These concentration limits are required for optimal use of the high volume DSE inlet technique for gas chromatography [2], relating to two distinct modes of operation when 20 µl (liquid capacity of concentrator) and a practical maximum of 1000 µl (concentrated off-line to 20 µl) of solvent are injected respectively.

To achieve these objectives we are studying the physical procedures (without involving chemical pretreatment) such as column chromatography, fractional distillation (simple packed and spinning band columns), and frontal-analysis chromatography [22]. They are the more useful methods for the purification of pure solvents, and less time consuming. For the purpose we obtained and repaired the auto annular Teflon spinning band distillation equipment (Console Model 251, Perkin Elmer), constructed another simple packed (Raschig rings) insulated fractionating distillation apparatus and used gas chromatography for testing the efficiency of the fractionating column as well as determining the purity levels of hexane.

The storage of purified hexane was studied in borosilicate glass (Type I, Class A) flasks with ground-glass stoppers and borosilicate glass (Type I, Class A) vials with various cap liner materials. This involved monitoring the increase in impurity levels with time under various conditions of temperature and light.

CHAPTER 2

INSTRUMENTATION AND EXPERIMENTAL PROCEDURES

2.1 CLEANING GLASSWARE AND SAMPLING PLUMBING

Glassware exposed to ordinary room air became detectably contaminated within minutes. Laboratory glassware can be cleaned satisfactorily for most purposes by treating initially with a solution of sodium dichromate in concentrated sulphuric acid, draining, rinsing with distilled water, and oven drying (and storage to prevent recontamination) at 250 °C. Where traces of Chromium (adsorbed on glass) must be avoided, a 1:1 mixture of concentrated sulfuric acid and nitric acid is a useful alternative. For most applications, washing glassware with hot detergent solution, using tap water, followed by rinsing with distilled water and acetone, and heating to 200-300 °C overnight, is adequate. However, volumetric apparatus should not be heated: after washing it is rinsed with acetone, then hexane, and air-dried. Prior to use, equipment can be rinsed with acetone, then with petroleum ether or hexane, to remove the last traces of contaminants. Calibrated glassware, e.g. pipettes and measuring cylinders that are damaged by heating to 450 °C were cleaned with a 1:1 mixture of concentrated sulfuric acid and nitric acid and held at 250 °C. A more convenient method for non-volumetric glassware was to rinse with distilled water and acetone, bake at 450 °C to oxidize organic contaminants, and maintain that temperature until use.

All black plastic screw caps with liners for the sample vials were ultrasonicated with acetone in a beaker. These were dried on the slightly heated surface on top of a laboratory oven.

Glassware were allowed to cool to ambient temperature in a desiccator. These were used immediately.

2.2 FRACTIONAL DISTILLATION

Distillation is the process of separating a mixture into its component parts, or preparing pure solvents by utilizing differences in the boiling points or partial pressures of the constituents. It is among the cheapest methods, since even the most expensive stills are less costly than the preparative scale chromatographic devices that are frequently used to isolate pure compounds. The distillation process is almost always used to separate mixtures that are liquids at ambient temperatures, because these generally distill in the temperature ranges and at the pressures most easily accessible in laboratories. Distillation has several possible roles in the purification procedures. It may be employed to purify starting materials and solvents, to remove solvents or secondary products introduced in preceding steps, or to remove impurities indigenous to the sample. There are two methods of distillation used: continuous or batch. It has been necessary to omit any description of continuous distillation [21]; although it is of importance on the commercial scale, it can scarcely be of much use in an analytical laboratory.

Conventional distillation processes can be divided into simple and fractional distillations. The goal is to obtain the components of the mixture in pure form or to recover at least one of the components virtually pure. The first and frequently the only step of solvent purification is fractional distillation, also referred to as fractionation or rectification. Fractional distillation at atmospheric or sub-atmospheric pressure is the most widely applicable and most commonly used methods of purification, especially of organic chemicals. Almost without exception, this method can be assumed to be suitable for all organic liquids and most of the low-melting organic solids [8, p. 4]. There are a number of fractionating columns, we are focusing on the cheap and simple packed (Raschig rings) insulated column and the auto annular Teflon spinning band distillation column (Console Model 251, Perkin Elmer).

Fractional distillation consists of passing the vapours from the boiling mixture through a vertical column, usually containing a packing of high surface area, so that ascending vapour and descending liquid are brought into intimate contact throughout its length. As a result of heat exchange between vapour and liquid all along the column, a whole series of vaporizations and condensations is effected continuously. The resultant vapour-to-liquid contact leads to the progressive concentration of the more volatile component at the head of the column. The fractional process may be

conducted batchwise, in which the main purpose is to obtain precise cuts between components and the maximum purity of each.

The following discussion is just a practical knowledge of fractional distillation as an aid in purifying hexane in our laboratory. For a detailed study of the theory of distillation, there are some recommended readings [21, 23-25; 26, pp. 235-317].

2.2.1 FACTORS TO BE CONSIDERED IN DESIGNING DISTILLATION EQUIPMENT [26, pp. 238-239]

The design of column, still heads, and kettles for general laboratory distillations depends largely on the following four points.

(1) Efficiency of separation desired

In choosing a column to perform a given separation, it is desirable to know the approximate number of plates required to effect the desired separation. The efficiency of separation is determined by the number of theoretical plates a column possesses at an infinite reflux ratio (total reflux). This is merely a comparison between stills and does not necessarily represent the efficiency under actual operating conditions. However, we can assume that with two columns the one showing the highest theoretical plate value at total reflux will probably have the most theoretical plates under operating conditions. Other considerations being equal, the

rectifying column showing the greatest enrichment per unit length is the most desirable.

(2) Rate of distillation

With any given column there is an optimum rate, above which there will be poor separation, and below which the increased separation will not warrant the extra time required. Naturally the ideal column would have a large number of theoretical plates, small holdup, low pressure drop, and a high throughput. Actually it is necessary to compromise to obtain the maximum efficiency. Usual distillation procedure is to keep the distillation rate constant.

(3) Temperature range to be covered

The temperature range over which the still will be operated will partly determine the design of the still. When the temperatures of the distillations are high, precautions must be taken to prevent, or compensate for, excessive heat loss. If this is neglected, fluctuation in the reflux results, causing flooding in the column or lack of reflux at the still head. A silver-plated, vacuum-jacketed system up to the reflux condenser is the best arrangement for solving this problem.

(4) Material to be fractionated

This is of importance in designing a column because of such physical and chemical properties as viscosity, surface tension, latent heat, tendency to foam, and corrosiveness. These factors

also determine, in part, the type of packing to be used in the finished column. Hydroperoxides and peroxides should be removed from mixtures before distillation, in order to avoid the risk of an explosion and to prevent possible peroxide-initiated polymerisations [21, pp. 300-302].

2.2.2 APPARATUS

Generally a batch distillation unit consists essentially of a still pot, a distillation column and a still head together with the auxiliary equipment to secure maximum fractionation.

The still pot contains the charge and heat is transmitted to it to supply vapour to the column. Spherical still pots are most commonly used for distillations. They may be equipped with standard joints for connection to the column. Electric heating is almost universally used for this purpose, since it permits easy control of boil-up which must be maintained at a constant rate.

The most important part of the whole unit is the distillation column, which can be classified as being of the film type, or of the plate type. The film type of column can be, for example, a simple vertical open tube or such a tube filled with "packing". The ideal packing is one that has a large surface for contact between the liquid and the vapour. It should pack evenly in the column, thus assuring uniformly distributed interstices, or free space, and have enough free space for the required throughput. A good packing

ensures efficient contact between vapour and liquid and high throughputs without excessive hold-up and pressure drop (Separation efficiencies generally increase by pre-flooding.). Columns of low H.E.T.P. (height equivalent of a theoretical plate)are usually preferred, but other factors, notably the throughput or capacity of the column and its hold-up and pressure drop, may determine the choice. The column must operate under adiabatic conditions, because any gain or loss of heat by the column will upset the vapour-to-liquid equilibrium and decrease column efficiency. Silvered vacuum jackets provide the most efficient insulation.

In most distillations, some losses occur, either through joints or through the condenser. To minimize losses, the number of joints should be as few as possible, and they should be precision ground or Clearfit clear glass joints.

The still head is to receive the vapour from the top of the distillation column, condense part or all of it, and return liquid reflux to the column. A still head, like the column, should be properly insulated and designed for the temperature and material to be distilled. Generally, no fractionation takes place in the still head. Column heads may be divided into general types: total condensation - variable takeoff and partial condensation - variable takeoff. Variations of these types have been developed to control reflux ratio automatically or semiautomatically, or facilitate the removal of distillate. If a total condenser is used, the still head

should contain a liquid partitioning device for controlling the reflux ratio. Two general methods are available for controlling the reflux ratio: vapour and liquid partitionings. The first consists of a flow-regulating device, which divides the condensate stream between the product receiver and the rectifying section; the second consists of a mechanism operated by a timing device, which intermittently diverts all the condensate to the product receiver or to the rectifying section of the column for controlled periods. It is essential to use a distillate receiver that can be withdrawn and replaced without producing disturbances in the system.

2.2.3 COLUMN CONSTRUCTION

In order to be in a position to decide on the type of column to use to carry out a given fractionation, we must know what properties make one column more suitable than another. The following characteristics of a column are usually considered in evaluating its efficiency: (i) holdup; (ii) pressure drop; (iii) throughput or boil-up; (iv) number of theoretical plates or H.E.T.P..

A good laboratory still should have low holdup to reduce cross-contamination between fractions and minimize sample volume requirements, low pressure drop to insure that the pot charge need not be superheated simply to achieve boil-up, high throughput to allow rapid collected of purified fractions, and, of course, high efficiency (low H.E.T.P.) so that maximum purity can be attained in the shortest amount of time [27].

Many different types of column have been devised. There are two factors to be considered: first, ease of construction and/or cheapness of the constructed unit, and, second, generality of usefulness. In this study a packed (Raschig rings) fractionating column was constructed. This equipment together with a commercial auto annular Teflon spinning band distillation column were evaluated for solvent purification to our stated requirements.

2.2.3.1 PACKED COLUMNS [28]

Packed columns are by far the most important type of laboratory distillation columns. The basic advantage of these is low initial cost. A practical method for increasing vapour-to-liquid contact inside the column is to fill the column with a material that offers a high surface area on which condensed liquid can form a layer that the ascending vapours contact. Note that the separating efficiency of a distillation system depends entirely on the amount of contact which occurs between the ascending vapour and descending refluxed liquid. Commonly, glass beads, broken glass, short lengths of tubing, etc. are used as packing. The primary objective is the formation of an extremely tortuous vapour path to provide a maximum amount of rectification. Although high efficiency can be achieved in this way, the holdup is very large. The high pot temperatures that are required to overcome the pressure drop of the tightly-packed column can be destructive to many compounds. The amount of throughput, or material taken off, is normally very small, since

the major part of condensate must be returned to assure coating of the large packing surface.

2.2.3.2 ANNULAR TEFLON SPINNING BANDS [28]

Spinning band stills, in which a motor-driven band is rotated inside the column, combine efficiencies exceeding those of the best packed columns, with the low holdup and low pressure drop of the most basic stills. The Teflon spinning bands are constructed of a spirally wound Teflon, permanently fastened to a central shaft, which is connected to a motor (Figure 2.1). As the tightly-fitted band is rotated, the exposed Teflon at the side of the band acts as teeth , which " comb " through the layer of liquid on the still walls. This results in the highest possible vapour-to-liquid contact and rectification.

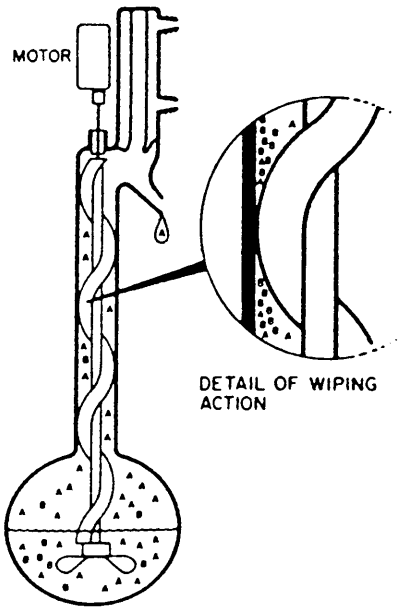

Fig. 2.1 Annular Teflon spinning band distillation column

The spinning band is constantly whirling through the ascending vapours, eliminating any tendency to form vapour channels, and the band is fitted with a stirrer that extends into the pot. The constant stirring eliminates thermal layering in the pot. Constant exposure of the total liquid charge to the heated area allows use of lower temperatures. This serves to reduce or prevent thermal decomposition of thermally labile materials.

A major advantage of low holdup is the ability to collect maximum amounts of pure components, with only minor portions contaminated by cross-mixing when higher boilers move up through the still, as all of the low boiler is removed. Pressure drop remains low since, even though it is spinning, the band offers several channels through which the vapours can travel. Throughputs, too, are high with Teflon band stills, since rapid boiling rates can be used, allowing significant amounts to be refluxed while useful portions of material are collected.

2.2.4 OPERATION

The major factors affecting the operating characteristics of a column may be divided roughly into two classes: those which are a part of the column construction, such as height, diameter, and packing, etc.; those which are a part of the operation, such as reflux ratio, throughput, etc.. There is no sharp line of demarcation between the effects of factors in one group and those in another.

After the pot has been charged, the cooling liquid is allowed to flow through the condenser and the system is adjusted to the operating pressure (atmospheric pressure), then, the still pot should be well insulated and evenly heated by an electrical heating unit to give the desired boil-up rate.

The column requires preflooding at the start to give the best and most reproducible performance. Preflooding is accomplished by setting the heat to the still pot high and bring the sample to the boil in the shortest time possible. As soon as boiling begins, or when the boiling point is nearly reached as shown by a thermometer in the boiling flask, the voltage used in heating the flask should be reduced to provide sufficient heat for the required boil-up rate only. The pot heat on the still pot is thereby reduced to allow the column to drain, and as the liquid returns to the still pot it thoroughly wets the packing.

In the case of the spinning band column, the still is first operated under conditions of total reflux to allow the establishment of primary equilibrium (frequently until the still-head temperature is constant). The reflux ratio is then set to the value decided, a sample of the material is taken from the top of the column. The early effluent is discarded, collection of the product then may be started after this period. In the case of the packed column, the still head of the packed column has a vapour-partitioning device (does not allow 100% reflux), which causes some

of the vapour to condense and reflux. The product is then collected after the first portion of the effluent is discarded.

After finishing the collection of the distillate, the system must be closed until the next distillation.

2.2.5 TESTING THE PACKED COLUMN

A satisfactory test for evaluating the efficiency of a fractionating column is to determine the number of theoretical plates associated with the column. For this determination, a binary mixture is distilled until the first distillate is collected under equilibrium condition. By simultaneously withdrawing and analyzing samples of the distillate and of the liquid from the still pot, the equivalent number of theoretical plates can be calculated with the following version of the Fenske equation [28]:

$$n = \frac{\log \left(\frac{Xa}{Xb}\right) x \left(\frac{Yb}{Ya}\right)}{\log \alpha} - 1$$

where X_a= % low boiler in head

X_b= % low boiler in pot

Y_a= % high boiler in head

Y_b= % high boiler in pot

α(alpha)= ratio of vapour pressure or relative volatility

Procedure. Choosing a test mixture to determine theoretical plates. The test mixture chosen for evaluating columns should meet at least the following conditions:

1. The relative volatility should be known.
2. The mixture should not be separable to give a pure component at head because, in that case, the formula used for calculating the number of plates does not apply.

A 1:3 mixture of n-heptane and methylcyclohexane was placed in a flask of convenient size (it should be at least one-half full, a few fragments of porous porcelain added), which is attached to the packed (Raschig rings) column (52 mm i.d. x 36 inches long) with a silvered vacuum jacket (Figure 2.2). There is a vapour partition takeoff head above the jacket. A thick layer of cotton tape was used to thermally insulate the head above the jacket. A drying tube was mounted on to the right condenser to protect hexane vapour from atmospheric moisture. A towel was placed around all remaining open areas of the pot flask to give more even control. The distilling pot was heated by an electric heating mantle. When boiling occurred the heat was reduced to maintain boiling and distillation (This setting on the heating mantle was obtained by trial and error.). After the vapours reached the top of the column, the electric heater was turned off and the first three drops were discarded. The liquid was withdrawn from both the head and still pot as nearly simultaneously as possible. The sample from the still pot is

withdrawn from a tube extending below the surface of the liquid (Figure 2.3), while the sample of distillate is collected slowly.

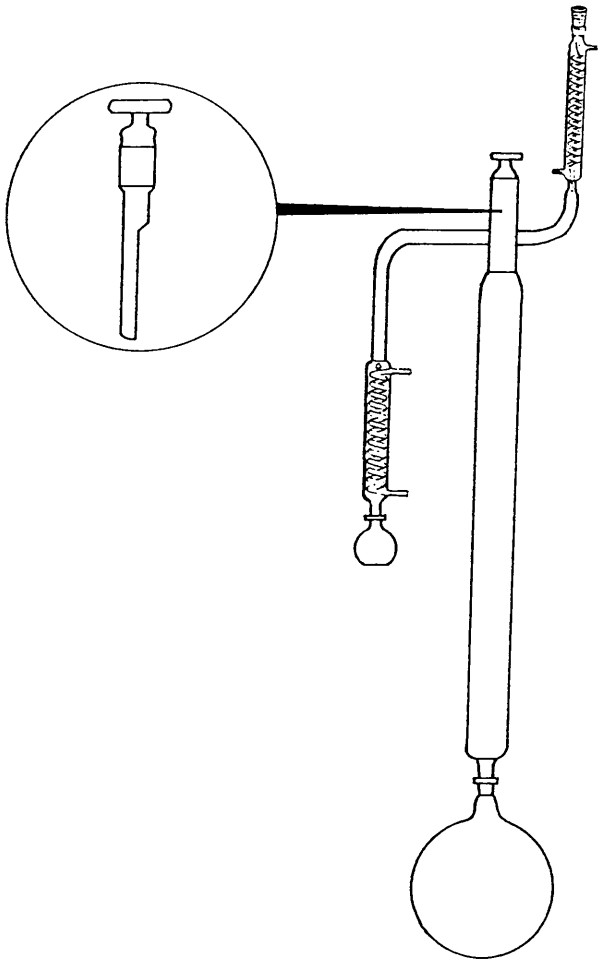

Fig. 2.2 The packed column. The vapour partitioner is a device for the partitioning of hot vapour between two condensers. The long end almost covers the inlet to one condenser while allowing most vapours to rise to the other.

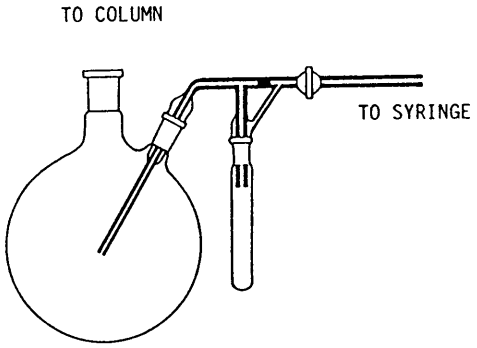

Fig. 2.3 Still pot for the removal of samples

Each of the samples was analysed by gas chromatography using conditions listed in Table 2.1 to determine their percentage composition. Using this data (Table 2.2) five theoretical plates was calculated according to the Fenske equation.

Table 2.1 Gas chromatographic conditions.

Column	: 25 m x 0.3 mm x 0.4 µm OV 101
Carrier gas	: H_2
Dead Time	: 50 sec.
Detector	: FID
Sensitivity	: 64 x 10^{-10} A mv^{-1}
Temperature	
Detector	: 250 °C
Injector	: 220 °C
Column Initial	: 80 °C
Rate	: 4 °C
Final	: 100 °C
Integrator	: Chromatopac C-R3A (Shimadzu)

Table 2.2 Theoretical plate calculation for the packed column.

Exp #	Percentage Composition				Theoretical Plates
	Head		Pot		
	Low[1] Boiler (X_a)	High[2] Boiler (Y_a)	Low[1] Boiler (X_b)	High[2] Boiler (Y_b)	
1	33.5	66.5	22.8	77.2	6.2
2	31.8	68.2	24.9	75.1	3.6
3	34.0	66.0	25.2	74.8	4.8
4	32.0	68.0	22.3	77.7	5.7
5	31.7	68.3	23.3	76.7	4.8
6	31.3	68.7	22.7	77.3	5.0
7	31.7	68.3	23.5	76.5	4.6
8	32.2	67.8	23.6	76.4	4.8
9	32.5	67.5	23.8	76.2	4.9

1. Lower boiler - n-heptane
2. Higher boiler - methylcyclohexane

α=1.076 [22, p.39]

2.2.6 EXPERIMENTAL CONDITIONS FOR HEXANE DISTILLATION

Hexane was purified without chemical pretreatment by fractional distillation and spinning band distillation.

Fractional distillation was carried out at atmospheric pressure by running technical grade hexane through a, 52 mm i.d. x 36 inches long, Raschig ring insulated column generating about 5 plates, and the take-off rate was ca. 60 drops/min.. This procedure is the same as that described in Sec. 2.1.5 above, only the first minute fraction of distillate was discarded.

Spinning band distillation was carried out on a Perkin-Elmer Corporation Console Model 251 using an 8 mm diameter x 36 inches long column with theoretical plate efficiency of 200 at atmospheric pressure. Technical grade hexane was placed in the 100 ml flask so that it was at least half full (> 50 ml). This was attached to the spinning band column. On the REFLUX RATIO module, The RATIO/100% FEFLUX switch was set to 100% REFLUX to close the reflux valve. On the POT TEMPERATURE module, the VOLTAGE regulator was set to 70 with the CONTROL/MANUAL switch on MANUAL. The SPEED regulator was set to 15 on the MOTOR CONTROL module. After the vapour reached the top of the column and condensation occurred freely at the blue drip tip, the SPEED regulator was adjusted to 25 (ca. 3600 rpm). The CONTROL/MANUAL switch was set to CONTROL and the SET POINT potentiometer was set to 345. The hexane was refluxed for 3 hours to ensure that equilibrium was reached [29]. The RATIO regulator was then set to 200:1 with the RATIO/100% REFLUX switch set to RATIO. The first minute portion of distillation was discarded. Under the above conditions the reflux ratio was calculated to be 2.85 and the take-off rate was 26 drops/min..

Solvent purity was analysed by gas chromatography (Sec. 2.4).

2.3 ADSORPTION CHROMATOGRAPHY

Chromatography is often used with advantage for the purification of small amounts of complex organic mixtures. This section reviews two simple chromatographic procedures for purification --- frontal

elution gas adsorption chromatography and column liquid adsorption chromatography.

2.3.1 FRONTAL ELUTION GAS ADSORPTION CHROMATOGRAPHY

In frontal elution gas adsorption chromatography [22] the sample is added to the top of the column. If a solution is used, the solvent selected should be adsorbed only very weakly in the column. During development the more polar components are adsorbed more tenaciously and are retained longer in the column. The most weakly adsorbed component of the mixture emerges at the end of the column first, only this component can be obtained in a pure form. The frontal-analysis method efficiently separates weakly or moderately adsorbed organic and inorganic compounds from very polar or strongly adsorbed impurities. The amount of sample that can be purified depends on the activity of the adsorbent and the nature of the impurities present in the sample.

Experimental

Frontal elution gas adsorption chromatography was carried out by running fractionally distilled hexane through a 1 m x 17 mm column of W 200 aluminium oxide. The carrier gas was charcoal-purified nitrogen which bubbled through 12 ml of hexane at 40 °C and passed through the column at a flow rate of 250 ml/min and a temperature 180 °C. Between runs the column was backflushed at the same flow at 270 °C [17, p. 173]. The eluting hexane vapour was condensed

directly into a storage container immersed in liquid nitrogen. The first 8 ml of hexane was collected.

Solvent purity was analysed by gas chromatography (Sec. 2.4).

2.3.2 COLUMN LIQUID ADSORPTION CHROMATOGRAPHY

In column liquid adsorption chromatography the theory is the same as that described in Sec. 2.3.1 above, but the sample is swept continuously onto the column without additional mobile phase. The adsorbent was dry-packed, the solvent being added after packing. If a good separation is achieved, pure solvent emerges from the column leaving more polar impurities on the column.

Experimental

Three chromatographic columns were dry-packed as follows:

1. 5g of aluminium oxide W 200 neutral
2. 5g of silica gel (30-70 mesh ASTM)
3. 5g of aluminium oxide W 200 basic and 5g of silica gel (70-230 mesh ASTM) were packed as separate layers.

Ten ml of impure hexane was chromatographed by frontal elution on the columns 1 and 2. A 25 ml aliquot of the hexane taken from the first distillate by fractional distillation was chromatographed on the column 3.

Solvent purity was analysed by gas chromatography (Sec. 2.4).

2.4 GAS CHROMATOGRAPHY

The analytical procedures used in the purity study must be capable of detecting traces of contaminants. Gas chromatography is the simplest and usually the fastest means of analysing a solvent and is a convenient and rapid means of monitoring purification. The sensitivity obtained from a gas chromatograph for a given solvent system depends on the characteristics of the instrument and on the operating conditions. Such factors as the sensitivity of the electrometer-amplifier, integrator, or computer, the length of column, the kind of stationary phase, inlet technique used and column temperatures, and the carrier gas and carrier gas pressure play important roles.

Irrespective of the grade of material to be purified, it is essential that some criteria exist for assessing the degree of purity of the final product. This assessment is best done using a procedure that closely resembles the intended application. All the impurities were therefore identified and quantitatively determined by GC analysis.

As a warning, it should be noted that " GC assays " are highly dependent on instrumental conditions, they are blind to any impurities that are not detected or resolved.

Many methods for sample introduction into a capillary gas-liquid chromatography column are currently available. However, it was

clear that the large amounts of sample loaded onto capillary columns could not be tolerated as either volume overloading, concentration overloading or both [30] would result and cause band distortion and loss of resolution. This lack of capacity is a serious problem when trace analysis is being considered. To overcome this problem a new inlet, the solvent effect inlet, has been designed, constructed, and tested in the Institute for Chromatography. It can be reproducibly injected with liquid samples up to a volume of 20 µl by the DSE concentrator. This sampling method can also concentrate a 1000 µl sample to 20 µl by off-line concentration before injection.

The DSE sampling is carried out on sintered glass beds, deactivated by a novel method using silicon and ethene [17, chapter 4], and held in tubes 120 mm long and 1.4 mm in internal diameter which are the DSE concentrators. Transfer of the sample to a capillary column is by desorption in a stainless steel and silica inlet (the solvent effect inlet). When sampling from solvent, gaseous or aqueous specimens the precision, sensitivity and freedom from artifacts of the DSE are as good as, or better than, other sampling systems [17, summary].

The following precautions were taken to ensure artifact-free DSE sampling.

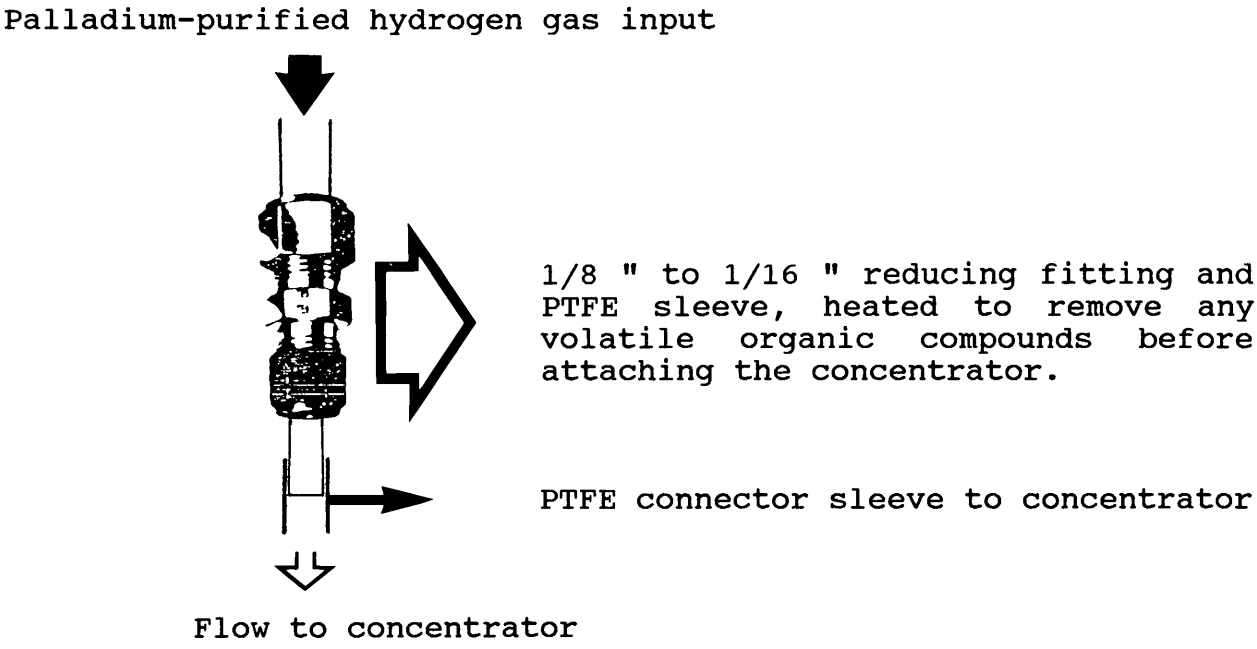

Fig. 2.4 The Swagelok fitting used for the DSE sampling

The purity of hydrogen purified by passage through a specially constructed palladium cell was tested. The flow of palladium purified hydrogen was controlled by the temperature of the palladium cell [17, p. 172]. Prior to attaching a connector with PTFE sleeve to the H_2 outlet, the other connector (Fig. 2.4) was heated to remove any volatile organic compounds that might be present. Special care was taken not to touch the PTFE connector sleeve after baking. About 10 ml/min of hydrogen was blown through an empty concentrator tube in a "plumbing" configuration [17, p. 165, Fig. 7.1, G] for the dynamic solvent effect sampling of hexane. Connectors with PTFE sleeves were kept at 250 °C and concentrators at 400 °C to precondition. They were allowed to cool to room temperature in a desiccator immediately before use.

Gas chromatography-flame ionization detector (GC-FID) analyses were carried out on a Varian Aerograph Series 2700 gas chromatograph fitted with a solvent effect inlet and a 25 m x 0.3 mm x 0.4 µl methyl silicone column. The system was interfaced with a personal computer which collected gas chromatographic data by the Data Acquisition Plotting & Analysis (DAPA) chromatography software. The detector sensitivity was 1 x 10^{-12} A mv^{-1} full scale deflection. The carrier gas was hydrogen with a linear velocity of 50 cm s^{-1}. Hexane solvents were sampled by dipping the concentrator directly into the solvent and taking it out to shake one or two times lightly to remove the excess solvent, or by the dynamic solvent effect sampling. The concentrator was dropped into a solvent effect inlet. The starting temperature of both inlet and column was 40 °C, the inlet was heated ballistically to 220 °C. The procedure used to determine the correct heating time for a dynamic solvent inlet was as follows: The width in time of the top of the solvent peak was measured and a second injection was made with the heater switched on at 60% of the solvent peak width after injection [17, p. 55]. The column temperature was programmed at 10 °C/min.

Solvent purity was determined with about (a) 20 µl samples by dipping into hexane and (b) 1000 µl specimens of hexane sampled by the dynamic solvent effect with ca. 10 ml/min of palladium cell purified hydrogen at room temperature. In every method of instrumental analysis there exists a certain background interference, or noise level, that limits the sensitivity of the

method. If the instrument background is not constant with time, these fluctuations can " contaminate " a series of measurements made near the detection sensitivity of the instrument, since the data would be biased by the instrument's response. In any trace analysis a blank determination is important. Blank determinations consist of performing identical analyses, either without a sample or with a sample known to contain none of the elements being measured. Test mixture 1 (Table 2.3) in hexane was used for frequent testing of the gas chromatograph and its solvent effect inlet to ensure satisfactory, artifact-free, analyses {(Chr. 2.5); Chromatograms for all the analyses can be found in the appendix.}. The more complete test mixture in hexane to be able to satisfy requirements [17, p. 44] is the test mixture 2 (Table 2.3) in hexane.

The following commercial hexane samples were also tested without prior purification.

SAARchem, for UV Spectroscopy

Merck, GR

Merck, extra pure

Table 2.3 Test mixture[1] in hexane.

Test Mixture 1	Test Mixture 2[2]
A. 2,6-dimethyl-4-heptanone	n-octane
B. n-decane	n-nonane
C. p-cresol	dimethylheptanone
D. 2,4-dimethylaniline	n-decane
E. n-dodecane	p-cresol
F. n-tetradecane	linalool
G. n-pentadecane	dimethylaniline
	n-dodecane
	methyl decanoate
	n-tetradecane
	n-pentadecane

[1] All compounds at 4 ng/ 20 µl injection to give 4 ng per peak.

[2] [17, p. 114]

CHAPTER 3

RESULTS OBTAINED FOR PURIFICATION METHODS

3.1 COMMERCIAL HEXANE SAMPLES

All commercial hexanes revealed unacceptable levels of impurities {Chrs. 3.1. (a), (b), (c), (d)}. These results are comparable to previous results obtained in Apps's work [17].

3.2 PURITY RESULTS ACHIEVED WITH PACKED COLUMN DISTILLATION

Freshly distilled hexane was less contaminated than that produced by Apps [17, p. 178] and pure enough for both the 50 $pp10^9$ {Chr. 3.2 (a)} and the 1 $pp10^9$ {Chr. 3.2 (b)} purity levels of the DSE applications.

3.3 PURITY RESULTS ACHIEVED WITH SPINNING BAND DISTILLATION

With a 20 µl injection of purified hexane there was a contaminant peak (less than 1 ng) at ca. 14 minutes {Chr. 3.3, (a)}. With a 20 µl injection of the dynamic solvent effect concentration of 1000 µl of hexane there was less contamination occurring closely after the solvent peak and the main contaminant was seen more clearly {Chr. 3.3, (b)}.

Discussion

This purification method is better than the fractional distillation described in Sec. 3.2 above. Because of the higher theoretical plate efficiency of the spinning band column, with a 20 µl

concentrate injection the rear of the hexane solvent peak was sharper, with less contamination after the solvent peak {Chr. 3.3 (b)}. However, there was a main contaminant peak which might be from the spinning band column. The peak was still there even if the column was cleaned with acetone, chloroform, dichloromethane, and then distilled hexane. This contaminant in the spinning band column might be from a previously distilled sample.

3.4 PURITY RESULTS ACHIEVED WITH COLUMN LIQUID ADSORPTION CHROMATOGRAPHY

After liquid-solid frontal elution on the columns 1 and 2, the hexane was as impure {Chrs. 3.4-1, (a), (b)} as the commercial starting material {Chr. 3.1, (a)}. When applying the liquid adsorption process to fractionally distilled hexane {Chr. 3.4-2, (a)} on the column 3, it was clear that impurities were added in the " purification " process {Chr. 3.4.2, (b)}.

DISCUSSION

Its lack of success in this case may have been due to some defects in the procedure.It is perhaps significant that the liquid-solid chromatographic apparatus was not automatically shielded from atmospheric contamination as is its gas-solid counterpart [17, p. 184]. The purification of organic solvents by adsorption of the impurities can be improved if warming of the column during moistening is completely avoided [31].

3.5 PURITY RESULTS ACHIEVED WITH FRONTAL ELUTION GAS ADSORPTION CHROMATOGRAPHY

A batch (≤ 20 ml) of hexane was freshly prepared each day, and the column was backflushed overnight to be ready for purification the following morning. Production of a day's batch of hexane took about ninety minutes. About twenty five minutes was required to cool the column. The hexane was then introduced and tapping off of the solvent could occur after a further twenty minutes. It is the same (Chr. 3.5) as the previous results obtained in Apps's work [17], but more labour intensive than the packed column distillation.

CHAPTER 4

STORAGE OF PURIFIED HEXANE

The following remarks are found in the literature: " Believe it or not you could be experiencing ghost peaks from the seals in your vials. The solvent you use can have an effect on the seal which will cause it to degrade. " [19, p. 1] " When performing high sensitivity analysis, chromatographers often experience the appearance of unexplainable peaks. ... . One possible source of these peaks is the seals on the vials from which the samples are taken. Silicone oils, phthalate plasticizers, rubber crosslinking catalysts, and other impurities can be extracted from the seals by the solvent when the sample comes in contact with the seal. " [19, p. 17]

Denny [32] observed that the chromatograms of trace amounts of organic compounds in a methanol sample which were re-chromatographed after several weeks storage showed striking changes compared with the original chromatogram obtained under the same analytical conditions. Screw caps with metal foil liners are generally considered to be sufficiently inert to produce no sample contamination, but the study concluded that the contaminants originated from the cork backing of the metal foil and apparently reached the methanol by permeation at room temperature. It was found that the contamination was eliminated by inserting a PTFE-rubber laminated disc into the foil-lined screw cap with the

PTFE side facing the solution. However, many solvents may be obtained in containers which have screw caps with metal-foil liners and the impurity levels for these solvents may increase significantly with time.

Asakawa and Gejida [33] detected phthalates in the plastic apparatus used in their laboratory, and in four organic solvents purchased as extra-pure grade materials (benzene, chloroform, n-hexane and light petroleum ether). Three aluminium foils of different brands were found to contain phthalates, and all phthalates were removed when the aluminium foils were heated at 350-400 °C for 14 hours.

Most analysts prefer to use Teflon cap liners to avoid the contamination problems associated with rubber liners. However, Teflon is not entirely innocuous. Teflon is chemically inert, but it has been reported that electron-capturing materials leach out of it when it comes into contact with reactive reagents used for electron-capture GC [18, p. 548].

Anyway, contamination could be the leaching of constituents from container and seal materials into the sample during storage or chemical manipulations, from the introduction of airborne contaminants during the handling of uncovered samples, and from reaction products of the chemicals themselves, producing impurities

which increase with time, temperature, and light. Particularly organic molecules may undergo changes on storage.

A wide variety of containers and seals are manufactured to solve virtually every type of laboratory liquid handling application. Continued care is necessary in handling the distillate fractions from collection through storage and use. Containers can be hermetically sealed for preserving sample purity and extending sample shelf life. In this study all containers for collection and storage are made from Type I, Class A, borosilicate glass, because of its higher chemical inertness [19].

To assess the effect of different storage conditions on the deterioration of the solvent purity, pure distilled hexane was stored in the following containers:

1. Borosilicate glass flat bottom flasks (50 ml) with ground-glass stoppers
2. Borosilicate glass screw cap vials (4 ml) with white (styrene-butadiene) rubber cap liners (A)
3. Borosilicate glass screw cap vials (4 ml) which were covered with aluminium foil prior to capping with white-rubber-lined screw caps (B)
4. Borosilicate glass screw cap vials (4 ml) with metal foil cap liners (C)
5. Borosilicate glass screw cap vials (4 ml) with PTFE (polytetrafluoroethylene) cap liners (D)

6. Borosilicate glass autosampler vials (1 ml) with , Teflon-faced natural rubber septa (TNRS), aluminium crimp-on caps (E)

To assess the effect of oxidation on the degeneration of the hexane solvent after purification, distilled hexane was immediately sealed and stored after the following procedures:

a. purged (bubbled through) with a 10 ml/min. flow of high purity nitrogen gas (with HPN_2)

b. purged (bubbled through) with a 10 ml/min. flow of palladium-purified hydrogen gas (with $P\text{-}PH_2$)

c. stored with atmospheric air (with AtmosA)

Storage was at room temperature in daylight and darkness, and at 8 °C in the refrigerator.

In the following experiments (experiments 1-8), which were designed to study the increase in impurity levels with time, a 20 µl injection was used in part A and a 20 µl concentrate (original size 1000 µl, concentrated by off-line dynamic solvent effect, see pp. 2-23f.) injection in part B of the experiments. As previous results from storage experiments in our laboratory were extremely variable, we hoped to find (a) the main source(s) of contamination and (b) the shelf life for our set purity standards, under optimal conditions. With the number of variables in mind, a semi-systematic study seemed appropriate. The pure distilled hexane was prepared by packed column distillation (see Sec. 2.2.6).

The criterion used to determine the level of contamination in aged samples was arbitrarily taken as the concentration, in pp10^9, associated with the largest impurity peak, found in the chromatogram beyond the solvent peak tail. The concentration was determined by comparing peak areas to those of an external standard, test mixture 1 (p. 2-26), without response factor correction.

Experimental

PART A --- FOR A 20 µL INJECTION

4.1 EXPERIMENT 1 -- The effect of storing pure hexane with various gases in screw vials using different cap liners at 8 °C in the refrigerator.

As tests did not show differences between purging hexane with HPN_2 or P-PH_2 (see Exp. 4.7), HPN_2 was taken as purging gas for oxidation prevention because it is easily accessible in laboratories. This experiment studied the increase in impurity levels of hexane with time in screw vials using different types of cap liner. Two milliliters of pure distilled hexane (Chr. 4.1-1) was dispensed into eight borosilicate glass screw vials. They were divided into two groups. One group had air displaced with a 10 ml/min. flow of HPN_2 for one minute; another was kept with AtmosA. They were immediately sealed with four different liner screw caps [(A),(B),(C),(D); for identity see p. 4-3] in each group after displacing air, and then all of them were stored at 8 °C in the refrigerator. Each hexane sample was analysed by GC-FID with a 20 µl injection after nine days storage (Chrs. 4.1-2 ~ 4.1-9).

RESULTS AND DISCUSSION

A comparison of the data from the gas chromatograms is presented in Table 4.1. Both samples with cap liner A (white rubber) are

contaminated. Concentration levels of impurities in Chr. 4.1-6 (AtmosA) were greater than concentration levels of similar

Table 4.1 Contamination after nine-day storage of pure fractionally distilled hexane in borosilicate glass screw vials with various cap liner materials at 8 °C in the refrigerator. Figures quoted refer to the concentration, in pp10⁹, of the largest impurity found beyond the solvent peak tail.

Cap liner material	With HPN_2	With AtmosA
White rubber (A)	≤ 200 (Chr. 4.1-2)	>> 200 (Chr. 4.1-6)
White rubber plus Al foil (B)	< 50 (Chr. 4.1-3)	≤ 50 (Chr. 4.1-7)
Metal foil (C)	< 50 (Chr. 4.1-4)	≤ 50 (Chr. 4.1-8)
PTFE (D)	< 50 (Chr. 4.1-5)	≤ 50 (Chr. 4.1-9)

impurities in Chr. 4.1-2 (HPN_2). The other hexane samples still remained pure. Due to poor electric connections Chromatogram 4.1-5 shows a large amount of electric noise. The results also indicate that displacing air with HPN_2 seems to be a safe method to reduce the amount of oxygen in contact with hexane.

4.2 EXPERIMENT 2 -- The effect of storing pure hexane with HPN_2 in vials using various cap liners under different temperature and light conditions.

This experiment studied the storage of hexane with HPN_2 under different temperatures and light conditions. Two milliliters of pure distilled hexane (Chr. 4.2-1) was dispensed into twelve borosilicate glass screw vials and one milliliter in three autosampler vials. All of these were divided into three groups. Four vials had air displaced with HPN_2 for one minute and a autosampler vial was kept with AtmosA in each group. They were immediately sealed with five different liner caps [(A),(B),(C),(D),(E)] in each group. Then one group was stored at room temperature in daylight, another in darkness, the other at 8 °C in the refrigerator. Each of the hexane samples was analysed by GC-FID with a 20 µl injection (The hexane samples in autosampler vials were injected with a microsyringe.) after ten days storage (Chrs. 4.2-2 ~ 4.2-16).

RESULTS AND DISCUSSION

A comparison of the data from the gas chromatograms is shown in Table 4.2-1. In order to establish the validity of these results, the test was repeated with another batch of pure distilled hexane (Chr. 4.2-17). Each sample was analysed by GC-FID with a 20 µl injection after ten days (Chrs. 4.2-18 ~ 4.2-32). Table 4.2-2 lists the results of the duplicate experiment. The only systematic

observation that could be made, in these two similar experiments, is that using white rubber liner resulted in more contaminant peaks and higher concentration levels of impurities than the others under the same conditions for storing pure hexane. Another more subtle observation is that a group of peaks appeared at about 15 minutes retention time in all samples that were kept at room temperature in daylight.

Table 4.2-1 Contamination in pure fractionally distilled hexane* stored in borosilicate glass vials with various cap liner materials under various conditions after a ten-day storage period. Figures quoted refer to the concentration, in $pp10^9$, of the largest impurity found beyond the solvent peak tail.

	At room temperature		At 8 °C
Cap liner material	Daylight	Darkness	(refrigerator)
White rubber (A)	> 200 (Chr. 4.2-2)	> 200 (Chr. 4.2-7)	>> 200 (Chr. 4.2-12)
White rubber plus Al foil (B)	≤ 50 (Chr. 4.2-3)	≤ 50 (Chr. 4.2-8)	≤ 200 (Chr. 4.2-13)
Metal foil (C)	< 50 (Chr. 4.2-4)	< 200 (Chr. 4.2-9)	≤ 200 (Chr. 4.2-14)
PTFE (D)	≤ 50 (Chr. 4.2-5)	< 200 (Chr. 4.2-10)	≤ 200 (Chr. 4.2-15)
TNRS (E)	≤ 50 (Chr. 4.2-6)	< 50 (Chr. 4.2-11)	≤ 200 (Chr. 4.2-16)

* had air displaced with HPN_2 for one minute before storing

The serious contamination in Chr. 4.2-27 arose from a leaking seal, allowing impurities from the atmosphere to enter. The impurities found in the first batch of refrigerated samples, are probably due to vapour phase carry over from inside the refrigerator, indicating either permeation through the liner or leaks that occur due to contraction (with cooling) of plastic liner material. Contamination from four different sources, which are light sensitivity, refrigerator air, laboratory air, and white rubber, could be identified only due to the number of samples analysed. This indicates the absolute necessity of dealing with a large enough number of samples to gain statistically valid information.

In general the hexane sample stored in darkness remained pure longer than those stored in other conditions. The hexane solvent stored at 8 °C in the refrigerator stays pure as long as that stored at room temperature in darkness if the vial is sealed firmly. As a source of contamination, liner material under these storage conditions could be rated as follows:

$$(A) > (E) \geq (B) \geq (C) \geq (D)$$

Table 4.2-2 Contamination in pure fractionally distilled hexane* stored in borosilicate glass vials with various cap liner materials under various conditions after a ten-day storage period. Figures quoted refer to the concentration, in $pp10^9$, of the largest impurity found beyond the solvent peak tail.

	At room temperature		At 8 °C
Cap liner material	Daylight	Darkness	(refrigerator)
White rubber (A)	>> 200 (Chr. 4.2-18)	≤ 50 (Chr. 4.2-23)	>> 200 (Chr. 4.2-28)
White rubber plus Al foil (B)	≤ 50 (Chr. 4.2-19)	< 50 (Chr. 4.2-24)	≤ 50 (Chr. 4.2-29)
Metal foil (C)	≤ 50 (Chr. 4.2-20)	< 50 (Chr. 4.2-25)	< 50 (Chr. 4.2-30)
PTFE (D)	≤ 50 (Chr. 4.2-21)	< 50 (Chr. 4.2-26)	< 50 (Chr. 4.2-31)
TNRS (E)	≤ 50 (Chr. 4.2-22)	>> 200† (Chr. 4.2-27)	< 100 (Chr. 4.2-32)

* had air displaced with HPN_2 for one minute before storing
† leaking seal

4.3 EXPERIMENT 3 -- The effect of direct liquid contact of pure hexane with various cap liners.

As the previous work involved no liquid hexane contact with the liner, experiment 3 was designed to see how this would effect solvent purity. Two milliliters of pure distilled hexane (Chr. 4.3-1) was dispensed into four borosilicate glass screw cap vials and one milliliter in a autosampler vial. They were sealed with five different liner caps. Each of them was shaken five times,

and instantly analysed by GC-FID with a 20 µl injection (The hexane samples in autosampler vials were injected with a microsyringe.) (Chrs. 4.3-2 ~ 4.3-11). After the first injection each of them was sealed and laid on its side to allow the hexane sample to stay in contact with the liner at room temperature in daylight. After one day a sample of hexane from each vial was analysed by GC-FID to determine if any impurities existed (Chrs. 4.3-7 ~ 4.3-11).

RESULTS AND DISCUSSION

A comparison of the data from the gas chromatograms is displayed in Table 4.3. No contamination is found from any sample directly after shaking. This indicates that the surfaces of the liners were not contaminated. The gas chromatogram in Chr. 4.3-7 for the hexane sample, which had come in contact with white rubber liner (A) for a day, indicated impurities consisting primarily of low-molecular-weight compounds; components with higher concentrations and longer elution temperatures were found in Chr. 4.3-11 for the hexane sample which had come in contact with Teflon-faced natural rubber septum liner (E) for a day. These compounds all seem to be the result of extraction of impurities from the liner materials. The liner materials (B), (C) and (D) were good for storing pure hexane in this case, although peaks start to appear in (B) and (C) that are probably the result of liner extraction.

Table 4.3 Experiments conducted on hexane after contact with various cap liner materials. Figures quoted refer to the concentration, in pp10⁹, of the largest impurity found beyond the solvent peak tail.

Cap liner material	Samples shaken up five times	
	After a minute	After a day*
White rubber (A)	< 50 (Chr. 4.3-2)	>> 200 (Chr. 4.3-7)
White rubber plus Al foil (B)	< 50 (Chr. 4.3-3)	< 100 (Chr. 4.3-8)
Metal foil (C)	< 50 (Chr. 4.3-4)	< 100 (Chr. 4.3-9)
PTFE (D)	< 50 (Chr. 4.3-5)	< 50 (Chr. 4.3-10)
TNRS (E)	< 200 (Chr. 4.3-6)	>> 200 (Chr. 4.3-11)

* in contact with cap liner at room temperature in daylight

4.4 EXPERIMENT 4 -- The effect of storing hexane with various gases in flasks with ground-glass stoppers at room temperature under different light conditions. Hexane from each flask was rechromatographed at intervals to note the increase of impurities with time.

The former experiments were concerned with storing hexane in screw cap vials after collection of the pure distilled hexane in flasks. This experiment studied the effect of storing hexane in flasks with ground-glass stoppers after collection, without transferring hexane

to screw cap vials. This procedure should eliminate all foreign material except the glass, and additionally minimize the effect of the glass by working under low contact surface / hexane volume ratios. In borosilicate glass flat bottom flasks with ground-glass stoppers there were three fractions of freshly distilled hexane. One fraction (F_3) was purged with HPN_2 for five minutes and then sealed with a ground-glass stopper at once, another (F_2) with $P\text{-}PH_2$ also for five minutes, and the other (F_1) was sealed with AtmosA. Each of them was stored at room temperature in daylight. Sample F_3 (Chr. 4.4-7) was analysed after four days, sample F_2 (Chr. 4.4-4) after a day, and sample F_1 (Chr. 4.4-1) immediately after collection. All samples were analysed initially by GC-FID with a 20 µl injection and again at ten-day intervals over twenty days (Chrs. 4.4-1 ~ 4.4-9). After each analysis the samples were purged and/or sealed with the respective gases, prior to storage. An important feature of the aging study with repetitive analyses of hexane from the same container, is the possibility to clearly differentiate between impurities that were introduced into the container at the start of the experiment and those that increase in the container with time.

RESULTS AND DISCUSSION

Table 4.4-1 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass flat bottom flasks with ground-glass stoppers at room temperature in daylight, to determine the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in pp10^9, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	9 - 11 Days	23 - 25 Days
F_1 with AtmosA	< 50 (Chr. 4.4-1)	< 50 (Chr. 4.4-2)	< 200 (Chr. 4.4-3)
F_2 with P-PH_2	< 50 (Chr. 4.4-4)	< 100 (Chr. 4.4-5)	≤ 200 (Chr. 4.4-6)
F_3 with HPN_2	≤ 50 (Chr. 4.4-7)	< 100 (Chr. 4.4-8)	< 200 (Chr. 4.4-9)

A comparison of the data from the gas chromatograms is shown in Table 4.4-1. A cluster of contaminant peaks of the hexane sample appeared after a ten-day storage period. In each fraction sample, the concentration levels of the impurities increased with time. In this case it didn't make any difference whether the hexane in the flask was purged with HPN_2 or P-PH_2 and immediately sealed with ground-glass stoppers, or sealed with AtmosA, after twenty-day daylight storage. Another three fractions (f_2 ~ f_4) of freshly distilled hexane were also prepared by the abovementioned procedure, but stored in darkness (Chrs. 4.4-10 ~ 4.4-18). Table 4.4-2 presents a comparison of the data from the resulting gas chromatograms. The hexane solvent stored at room temperature in

darkness clearly stayed pure longer than that stored in daylight. Chromatogram 4.4-9 shows a small amount of electric noise.

Table 4.4-2 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass flat bottom flasks with ground-glass stoppers at room temperature in darkness, to determine the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in pp10^9, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	12 - 13 Days	21 - 22 Days
f_2 with AtmosA	< 50 (Chr. 4.4-10)	< 50 (Chr. 4.4-11)	< 50 (Chr. 4.4-12)
f_3 with P-PH_2	< 50 (Chr. 4.4-13)	< 50 (Chr. 4.4-14)	< 50 (Chr. 4.4-15)
f_4 with HPN_2	< 50 (Chr. 4.4-16)	< 50 (Chr. 4.4-17)	≤ 50 (Chr. 4.4-18)

4.5 EXPERIMENT 5 -- The effect of storing hexane with various gases in screw vials with PTFE liners under different temperature conditions in the absence of daylight.

From previous experiments the screw vial with PTFE liner (D) is regarded as optimum for storing pure hexane. This experiment was performed to recheck its reproducibility under different temperature conditions. Two milliliters of fresh fractionally distilled hexane (F_1) was dispensed into six borosilicate glass vials. They were divided two groups. In one group, one vial was purged with HPN_2 for one minute and then immediately sealed with a

PTFE-lined cap, another with P-PH_2 also for one minute, and the other was sealed with AtmosA. One group was stored at room temperature in darkness, and another at 8 °C in the refrigerator. Each hexane sample was analysed after six days. All samples were analysed initially by GC-FID with a 20 µl injection and again at ten-day intervals over twenty days (Chrs. 4.5-1. ~ 4.5-18). After each analysis the samples were purged and/or sealed with the respective gases, prior to the respective storage.

RESULTS AND DISCUSSION

Table 4.5-1 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass screw vials with PTFE liners at room temperature in darkness, to test the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in $pp10^9$, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	20 Days	27 Days
F_1 with AtmosA	< 50 (Chr. 4.5-1)	< 50 (Chr. 4.5-2)	< 50 (Chr. 4.5-3)
F_1 with P-PH_2	< 50 (Chr. 4.5-4)	< 50 (Chr. 4.5-5)	< 50 (Chr. 4.5-6)
F_1 with HPN_2	< 50 (Chr. 4.5-7)	< 50 (Chr. 4.5-8)	< 50 (Chr. 4.5-9)

Two tables (Tables 4.5-1 & 4.5-2) present a comparison of the data from the gas chromatograms. These results confirmed previous indications that purging with various gases does not influence the

rate of deterioration of hexane. Each of them kept the same purity as the corresponding starting sample after three weeks storage in the absence of daylight. Chromatograms 4.5-6, 4.5-9, 4.5-12, 4.5-15, and 4.5-17 show excessive electric noise.

Table 4.5-2 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass screw vials with PTFE liners at 8 °C in the refrigerator, to test the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in pp10^9, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	21 Days	28 Days
F_1 with AtmosA	< 50 (Chr. 4.5-10)	< 50 (Chr. 4.5-11)	< 50 (Chr. 4.5-12)
F_1 with P-PH_2	< 50 (Chr. 4.5-13)	< 50 (Chr. 4.5-14)	< 50 (Chr. 4.5-15)
F_1 with HPN_2	< 50 (Chr. 4.5-16)	< 50 (Chr. 4.5-17)	< 50 (Chr. 4.5-18)

PART B --- FOR A 20 µL CONCENTRATE INJECTION

4.6 EXPERIMENT 6 -- The effect of purging pure hexane with various gases.

Oxidation prevention by purging with a gas stream could introduce impurities in the gas stream to the sample. One batch of freshly distilled hexane (Chr. 4.6-1) was purged with P-PH_2 for five minutes and another fresh one (Chr. 4.6-3) with HPN_2 also for five

minutes. Each hexane sample was then immediately analysed by GC-FID with a 20 µl concentrate injection to test if any foreign impurities were introduced by the purging procedure (Chrs. 4.6-2 & 4.6-4).

RESULTS AND DISCUSSION

The hexane samples were not contaminated after purging with P-PH_2 or HPN_2. The slight increase in levels of impurities (All contaminants are less than or equal to 1 pp10^9.) can be ascribed to a preferential evaporation of the more volatile hexane during purging. The pure hexane solvent could be purged with a gas stream without contamination if the procedure is carried out carefully.

4.7 EXPERIMENT 7 -- The effect of storing hexane with various gases in flasks with ground-glass stoppers at room temperature in darkness.

From experiment 4.4 it is known that the storage of hexane is better at room temperature in darkness in flasks with ground-glass stoppers, and this experiment was designed to study the effect of storing pure hexane with various gases under these storing conditions --- this time with great sensitivity and with longer storage times. There were six fractions of fresh hexane distillate in borosilicate glass flat bottom flasks with ground-glass stoppers. They were divided two groups. In each group, one fraction was purged with HPN_2 for five minutes and immediately sealed with

a ground-glass stopper, another with P-PH_2, and the other was sealed with AtmosA. All of them were stored at room temperature in darkness. Each hexane sample was analysed by GC-FID with a 20 µl concentrate injection after three weeks in one group (Chrs. 4.7-1 ~ 4.7-3), and after a month in another group (Chrs. 4.7-4 ~ 4.7-6).

RESULTS AND DISCUSSION

In this case the rate of deterioration was very variable --- one fraction of the hexane with P-PH_2 stayed pure for three weeks (Chr. 4.7-2), another with AtmosA after a month (Chr. 4.7-4), and the other with HPN_2 after a month (Chr. 4.7-6) whereas the others developed impurities above a four-nanogram per milliliter level. After the storage period each hexane sample still had sufficient purity for the analysis of substances with boiling points higher than that of n-decane. Chromatogram 4.7-6 presents excessive electric noise.

4.8 EXPERIMENT 8 -- The effect of storing hexane with various gases in flasks with ground-glass stoppers at room temperature under different light conditions. Hexane was rechromatographed at intervals.

This experiment had the same objective of experiment 4.4, but the testing conditions were fifty times more sensitive than that in experiment 4.4. In borosilicate glass flat bottom flasks with

ground-glass stoppers there were three fractions (F_1 ~ F_3) of freshly distilled hexane. One fraction (F_3) was purged with HPN_2 for five minutes and then sealed with a ground-glass stopper at once, another (F_2) with $P\text{-}PH_2$ also for five minutes, and the other (F_1) was sealed with AtmosA. each of them was stored at room temperature in daylight. Sample F_3 (Chr. 4.8-7) was analysed after four days, sample F_2 (Chr. 4.8-4) after a day, and sample F_1 (Chr. 4.8-1) immediately after collection. All samples were analysed initially by GC-FID with a 20 µl concentrate injection and again at ten-day intervals over twenty days (Chrs. 4.8-1 ~ 4.8-9). After each analysis the samples were purged and/or sealed with the respective gases, prior to storage. There were another three fractions (f_2 ~ f_4) of freshly distilled hexane which were experimented with the similar procedures mentioned above, but stored in darkness (Chrs.4.8-10 ~ 4.8-18).

RESULTS AND DISCUSSION

Two tables (Tables 4.8-1 & 4.8-2) present a comparison of the data from the gas chromatograms. In borosilicate glass flat bottom flasks with ground-glass stoppers, at room temperature in daylight or darkness, a cluster of contaminant peaks in hexane appeared after one or a few days storage with different gases, but the hexane was still purer than Merck's GR hexane under the same analytical conditions. In each fraction sample the concentration levels of the impurities increased with time. Purging did not have any influence on the aging. In the absence of light, there is a

consistent decrease in the rate of formation of impurities. In each case the hexane solvent stored at room temperature in darkness stayed pure longer than that stored in daylight. After the storage period each hexane sample still had sufficient purity for the analysis of substances with boiling points higher than that of n-decane.

Table 4.8-1 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass flat bottom flasks with ground-glass stoppers at room temperature in daylight, to determine the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in pp10^9, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	9 - 11 Days	23 - 25 Days
F_1 with AtmosA	< 4 (Chr. 4.8-1)	>>> 4 (Chr. 4.8-2)	>>> 4 (Chr. 4.8-3)
F_2 with P-PH_2	> 4 (Chr. 4.8-4)	>>> 4 (Chr. 4.8-5)	>>> 4 (Chr. 4.8-6)
F_3 with HPN_2	>> 4 (Chr. 4.8-7)	>>> 4 (Chr. 4.8-8)	>>> 4 (Chr. 4.8-9)

A series of 5 major contaminant peaks, occurring at retention time between 15 and 20 minutes in the included chromatograms (Chrs. 4.8-1 ~ 4.8-18) , were chosen for the study of peak area vs time. These peaks were marked A, B, C, D and E as illustrated in the accompanying example (Fig. 4.8-1) that is the same figure as Chr. 4.8-4. From the graphs (Figs. 4.8-2 ~ 4.8-6) it is possible to estimate the purity level of the hexane sample after a certain

period of storage and the increase in contamination of the hexane samples stored at room temperature in darkness was found to be much lower than that of the hexane samples stored at room temperature in daylight. The increase of impurities over time was about four times greater for the hexane samples stored at room temperature in daylight when compared to the samples stored in darkness.

Table 4.8-2 Experiments conducted on pure hexane distillates, which were stored in borosilicate glass flat bottom flasks with ground-glass stoppers at room temperature in darkness, to determine the effect of the various storage conditions on the quality of the hexane sample. Figures quoted refer to the concentration, in $pp10^9$, of the largest impurity found beyond the solvent peak tail.

Sample	Starting	12 - 13 Days	21 - 22 Days
f_2 with AtmosA	< 4 (Chr. 4.8-10)	>> 4 (Chr. 4.8-11)	>> 4 (Chr. 4.8-12)
f_3 with P-PH_2	< 4 (Chr. 4.8-13)	>> 4 (Chr. 4.8-14)	>> 4 (Chr. 4.8-15)
f_4 with HPN_2	< 4 (Chr. 4.8-16)	>> 4 (Chr. 4.8-17)	>> 4 (Chr. 4.4-18)

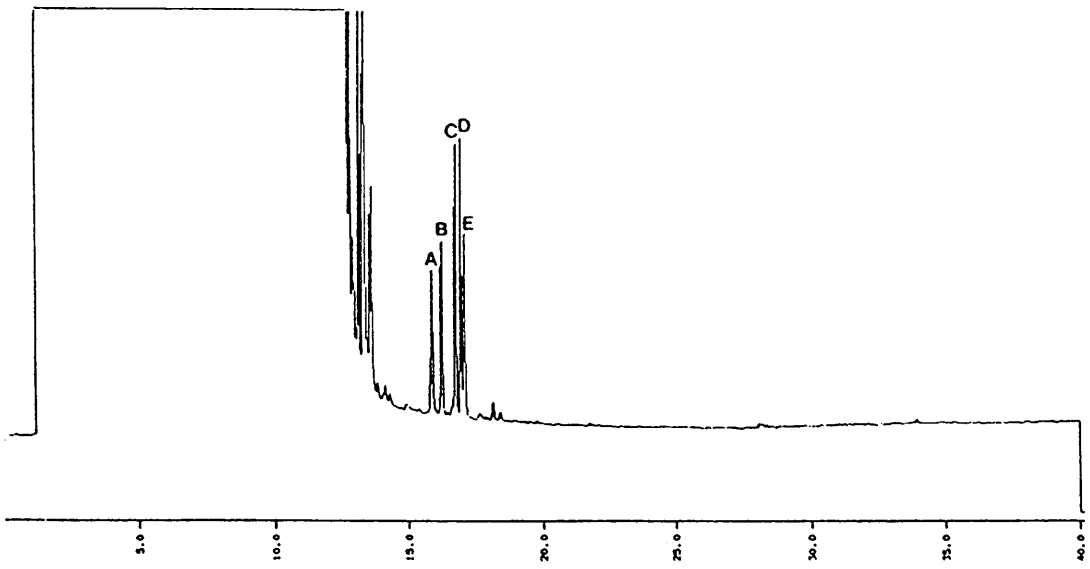

Fig. 4.8-1 The same chromatogram as Chr. 4.8-4 --- 20 µl concentrate (1000 µl) injection.

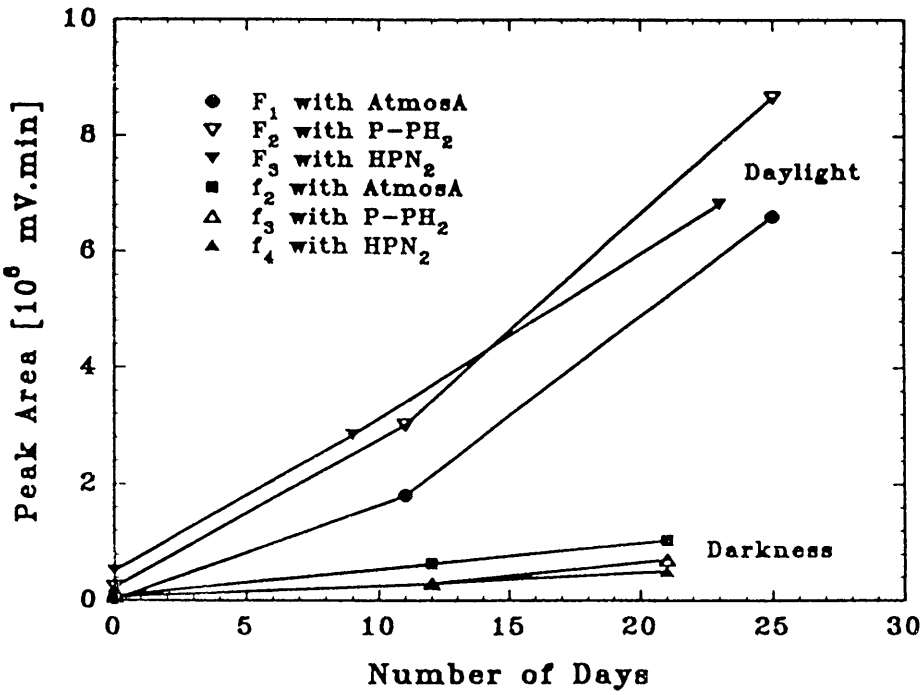

Fig. 4.8-2 Rate of production of impurity A in the presence and absence of daylight. Data from Chromatograms 4.8-1 ~ 4.8-18. See Fig. 4.8-1 for impurity peak identification.

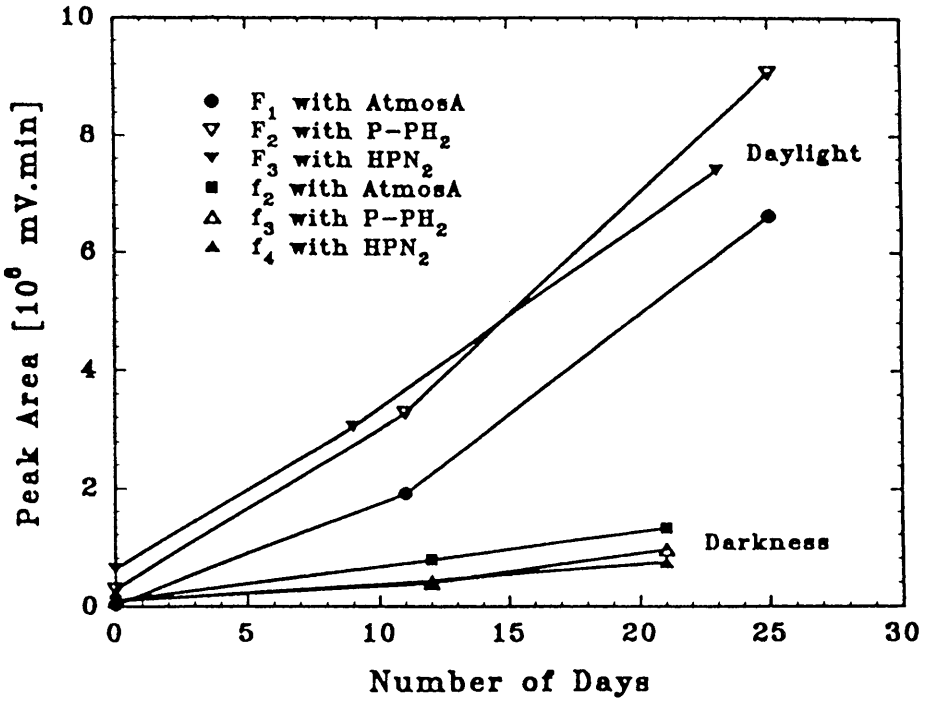

Fig. 4.8-3 Rate of production of impurity B in the presence and absence of daylight. Data from Chromatograms 4.8-1 ~ 4.8-18. See Fig. 4.8-1 for impurity peak identification.

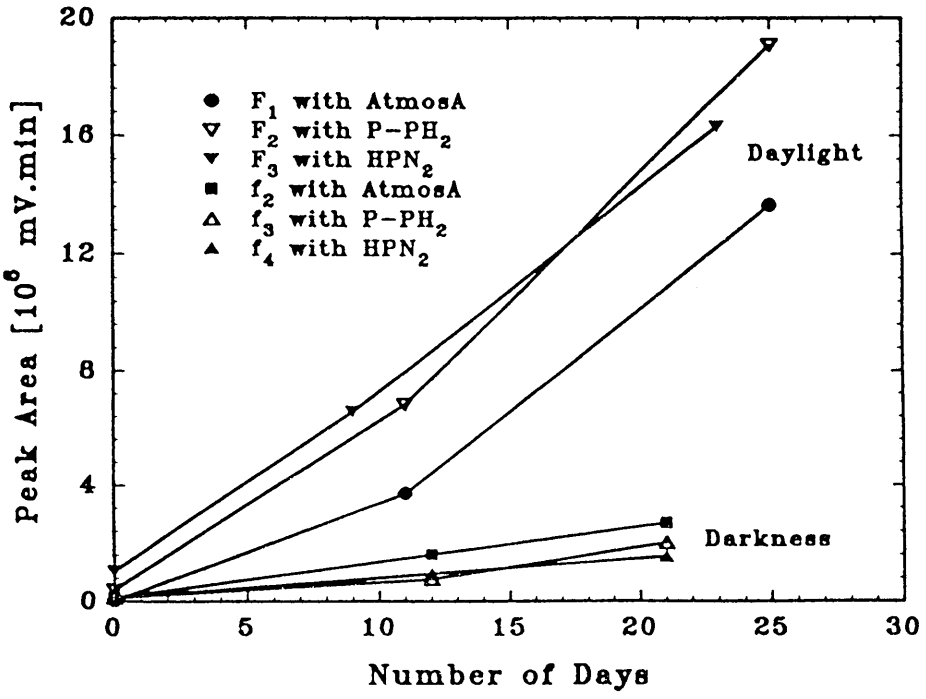

Fig. 4.8-4 Rate of production of impurity C in the presence and absence of daylight. Data from Chromatograms 4.8-1 ~ 4.8-18. See Fig. 4.8-1 for impurity peak identification.

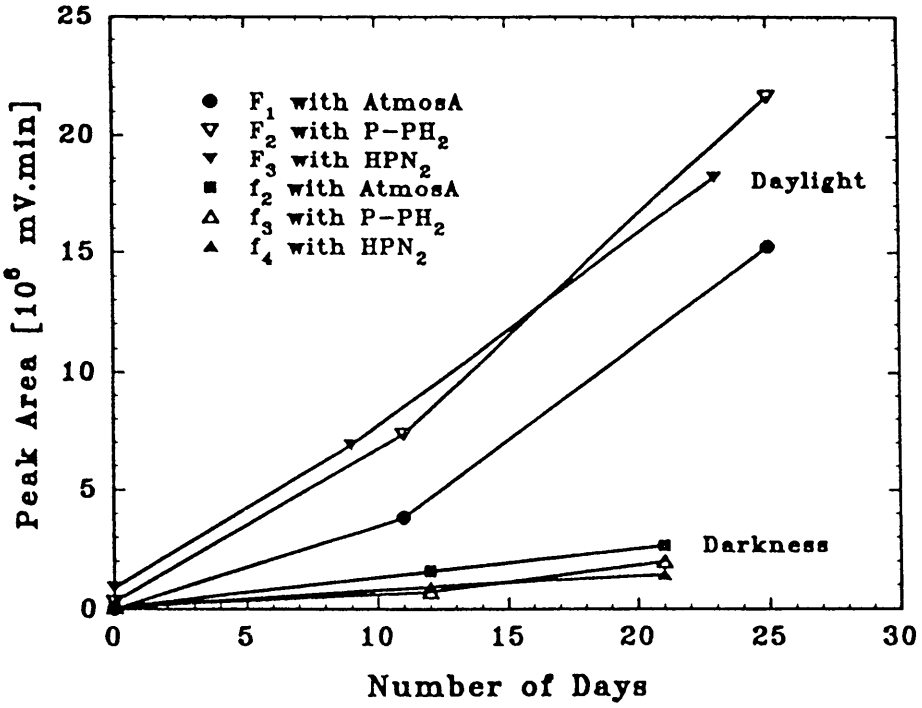

Fig. 4.8-5 Rate of production of impurity D in the presence and absence of daylight. Data from Chromatograms 4.8-1 ~ 4.8-18. See Fig. 4.8-1 for impurity peak identification.

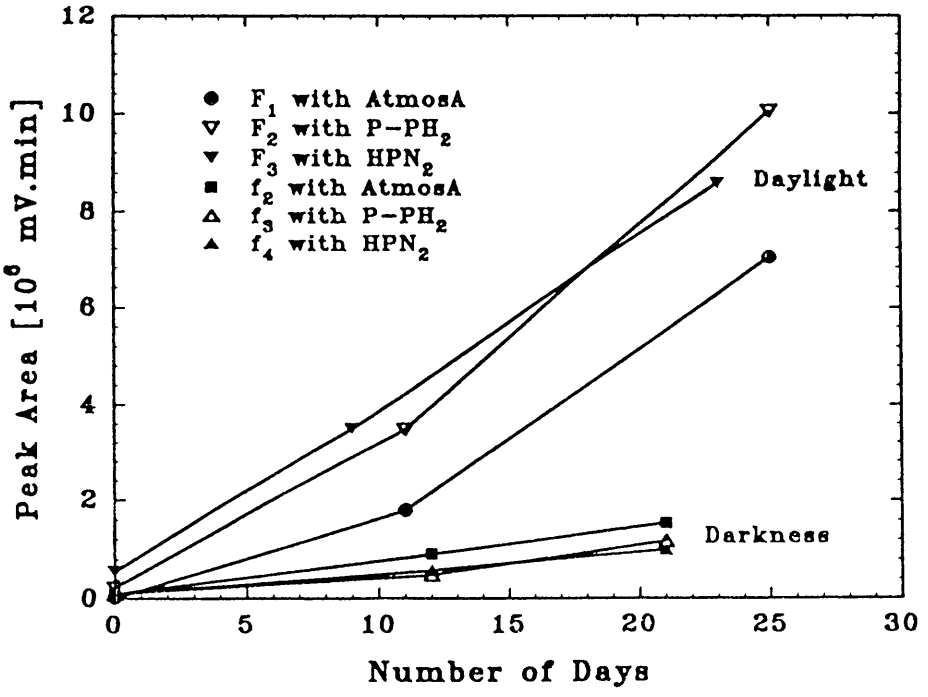

Fig. 4.8-6 Rate of production of impurity E in the presence and absence of daylight. Data from Chromatograms 4.8-1 ~ 4.8-18. See Fig. 4.8-1 for impurity peak identification.

CHAPTER 5

CONCLUSIONS

Hexane purification, using the packed column distillation or frontal elution gas adsorption chromatography, was successfully achieved to the degree of purity specified in the introduction (p. 1-8). Although marginally better results were obtained by frontal elution gas adsorption chromatography, the purity achieved with the packed column distillation is high enough for all DSE sampling applications. Packed column distillation at atmospheric pressure is a cheap and convenient technique, readily accessible to most laboratories. Spinning band distillation could give more efficient purification, except for a compound that - despite all efforts - consistantly appeared in the distillate and was obviously the result of previous contamination of the equipment. The spinning band technique has the disadvantage of high capital cost and not being readily available. The column liquid adsorption chromatography did not improve the hexane purity. In routine work it is economical to dedicate the packed column distillation apparatus specifically to hexane to prevent the type of contamination experienced with the spinning band distillation apparatus.

As far as the storage of purified hexane is concerned, different sources of contamination could be clearly distiguished. Impurities were found from laboratory and refrigerator air as well as from gas

phase and liquid phase contact with different cap liner materials. The remaining peaks that consistantly appeared and increased with storage time were associated with decomposition of the solvent itself in all glass containers. The formation of these peaks with time were studied in greater detail in an attempt to increase the shelf life of the ultra pure hexane. As it was assumed that the degradation involved air oxidation of hexane (- This was subsequently verified by GC-MS analysis. -), an attempt was made to remove air by purging the liquid solvent with high purity nitrogen or hydrogen gas. This procedure, as well as attempts to reduce the rate of formation of the products by refrigeration, were not effective. It was, however, found that the absence of light clearly prolonged shelf life of purified hexane. The influence of laboratory light was studied on the formation of pp10^9 levels of oxidation products in hexane. Studies of this kind have not been documented before, due to the difficulty in reliably handling and analysing these low levels of impurities.

We have obtained an optimum method for the shelf life of pure hexane used for GC-FID: The pure hexane is stored in Type I, Class A, borosilicate glass flasks with ground-glass stoppers or screw vials with PTFE liners, with atmospheric air, in a cool, dark place. For a 20 µl GC analysis, the hexane purity (individual components less than 50 pp10^9) can be maintained for at least three weeks; for a 20 µl concentrate (1000 µl) GC analysis, the maximum contamination of about 4 pp10^9 per component in the hexane can be

maintained for three days, and the hexane will have sufficient purity for the analysis of substances with boiling points higher than that of n-decane for at least three weeks. The storage of pure hexane in a refrigerator (also in the absence of light) is not recommended because of increased risk of contamination due to failure of sealing devices at the lower temperature.

The solvent effect inlet has already been shown to outperform other inlet systems designed for trace analysis in gas or liquid samples [34, 35]. A cheap, simple, and reliable technique for preparing and storing ultra high purity hexane was developed and tested in this thesis. Together with the commercial availability of various programmed-temperature vaporizer (PTV) inlets for gas chromatography, this may help to introduce the DSE inlet technique to other laboratories specializing in trace analysis.

Subsequent GC-MS work in our laboratory has tentatively identified a number of the peaks that consistantly appear in aged hexane purified by packed column distillation. In the first group of peaks, immediately following the solvent peak, cyclohexane, dimethyl pentane and pentanol have been identified. These arise from insufficient separation during distillation and do not seem to increase greatly with time. The second group (peaks A ~ E, Figure 4.8-1), the formation of which was shown to be dependant on time and light intensity, seem to consist of a number of C_6 and C_7 hydroperoxides. The mass spectra of these compounds are not well

documented as they are labile and, as recently has come to our notice [36], decompose during normal split injection. They are known to be the first reaction products of the autoxidation [37] or photooxidation [38] of branched alkanes. Their presence is thus consistent with our findings on the effect of light on decomposition rate and with the presence of dimethyl pentane in the first group of peaks (Branched C_6 compounds will elute with the n-hexane, and are thus not detectable.). Hunchak and Suffet [16] identified hydroperoxides in acetone-hexane solvent blanks, amongst a number of impurities extracted from soxhlet filters, but not in hexane solvent blanks.

Further study should clear up the exact structures of the indicated peaks from their mass spectra. The removal of branched alkanes from n-hexane could possibly be accomplished by the sharper separation with spinning band distillation or improved packed column distillation with more plates. Normal alkanes are known to be more stable towards autoxidation and photooxidation. The more efficient removal of oxygen should also be investigated, for example, by reaction with thallium metal shavings as successfully done by Forster [39] in the case of benzene or by additives that will not produce oxigenated products less volatile than hexane.

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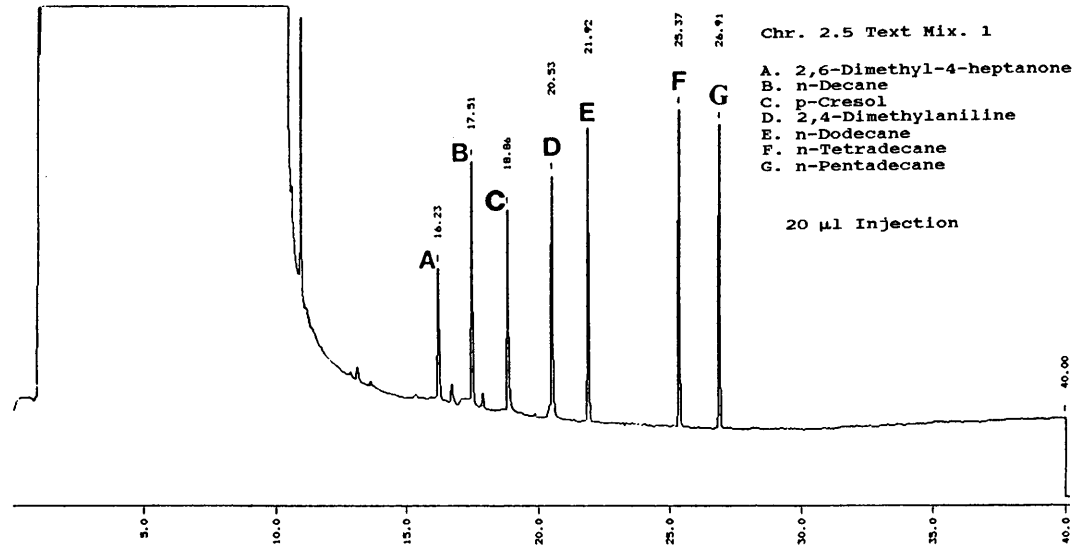
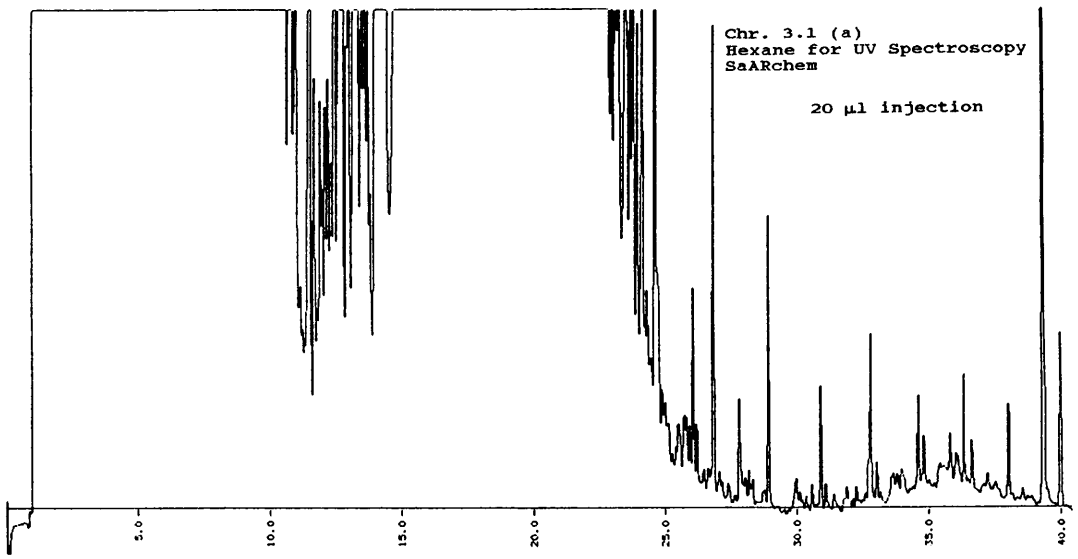
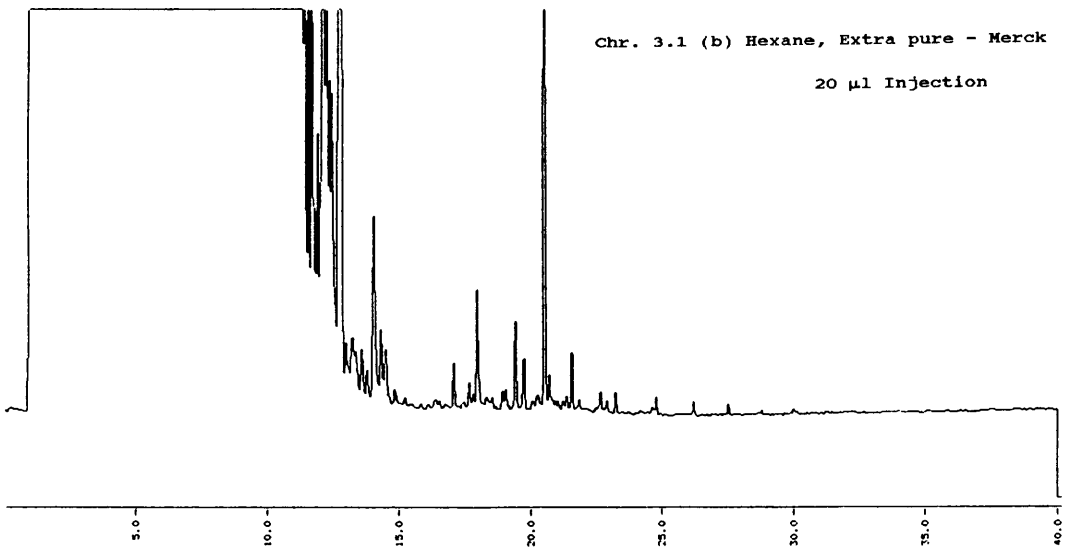
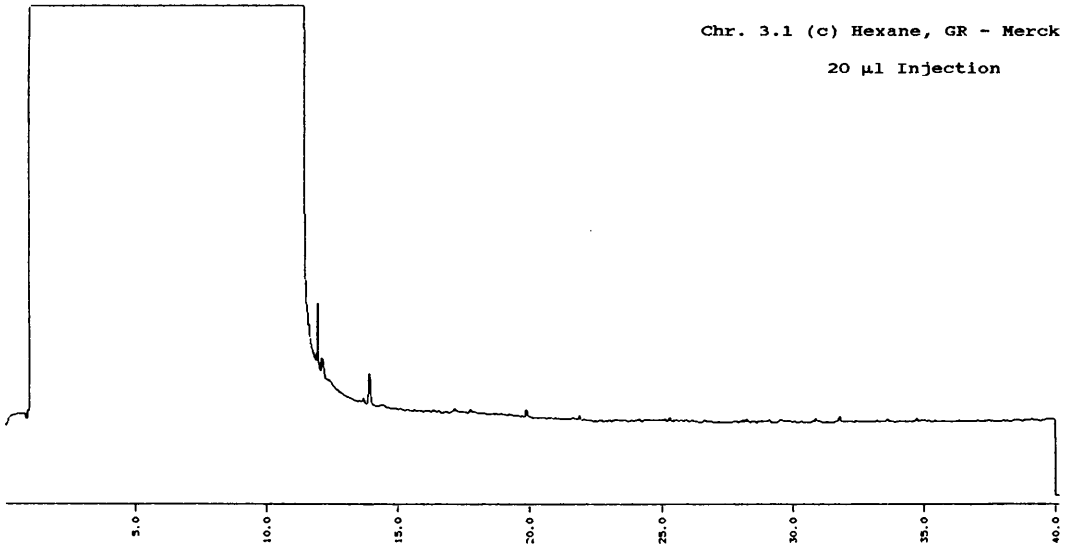
Appendix



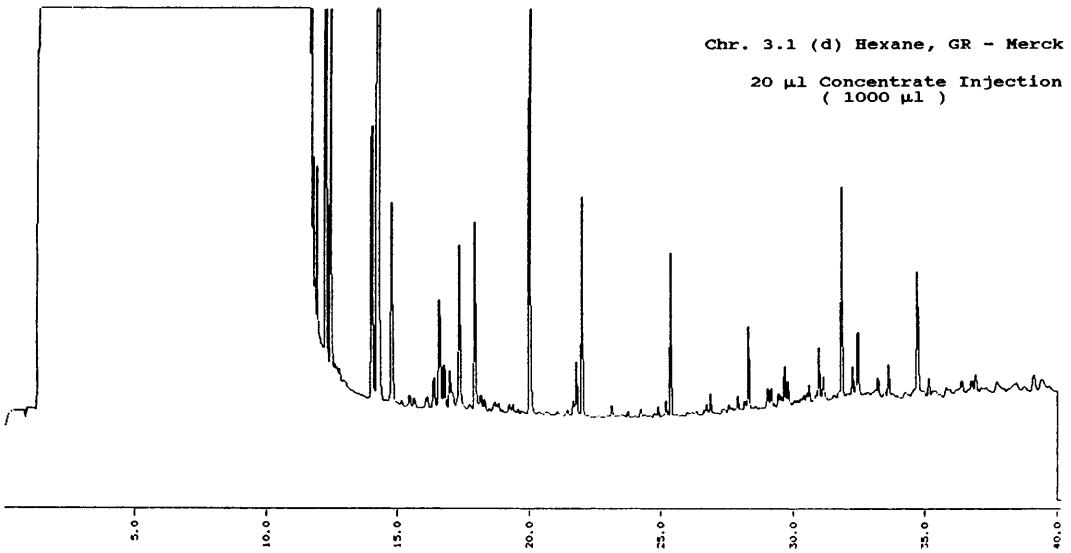






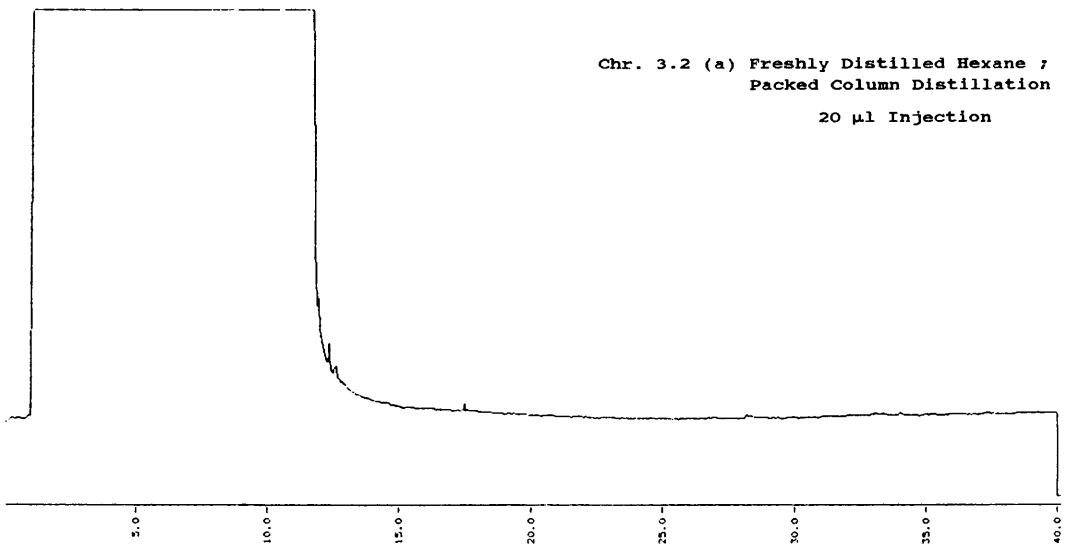
Chr. 3.1 (c) Hexane, GR - Merck

20 µl Injection



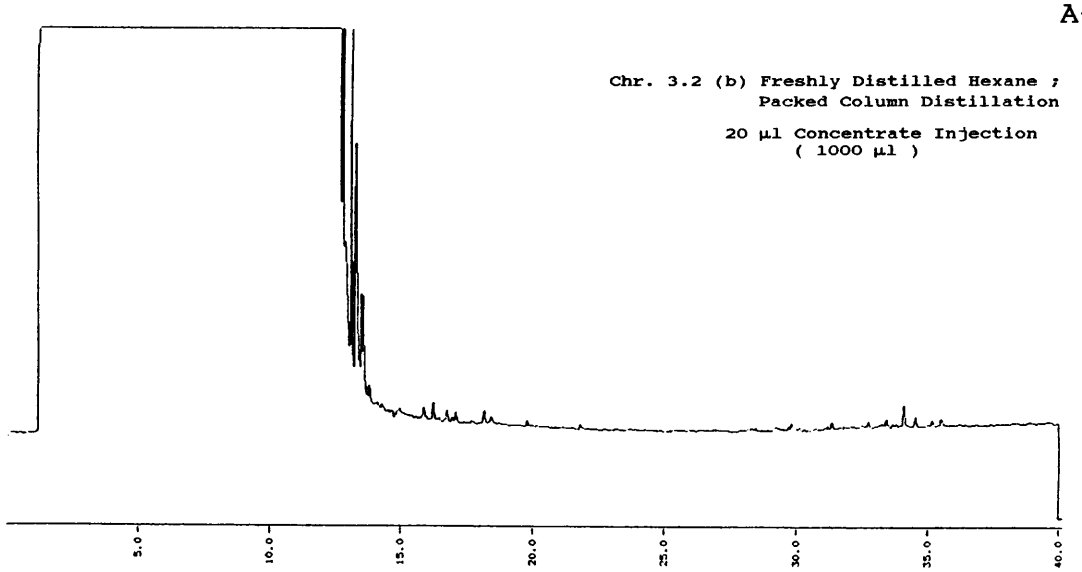
Chr. 3.1 (d) Hexane, GR - Merck

20 µl Concentrate Injection
(1000 µl)



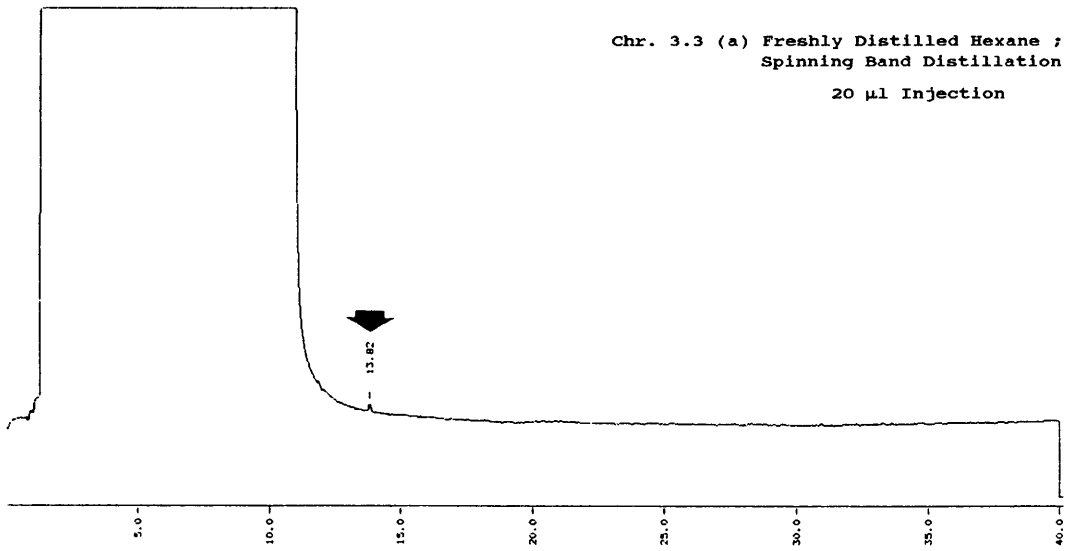
Chr. 3.2 (a) Freshly Distilled Hexane ;
Packed Column Distillation

20 µl Injection



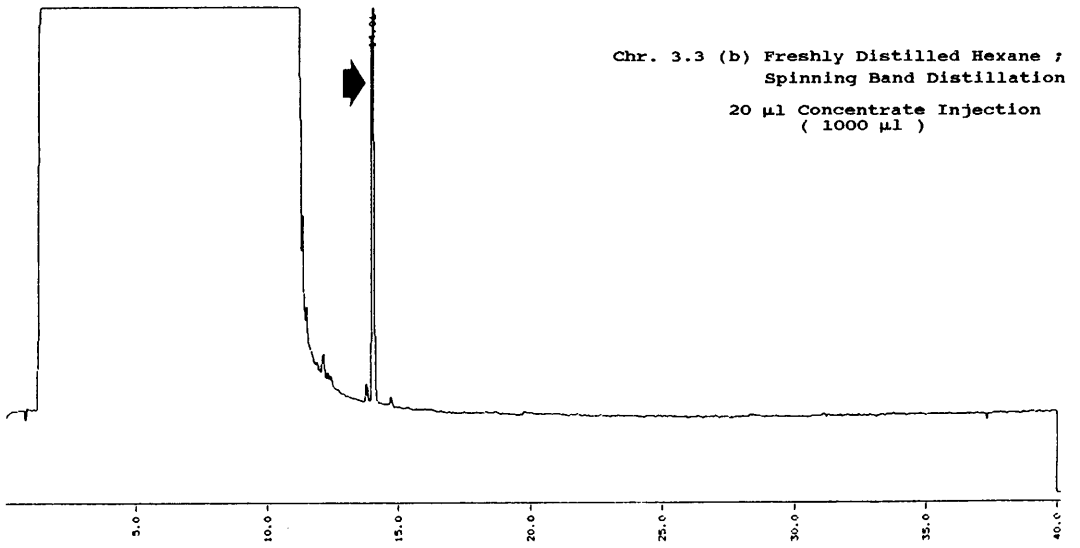
Chr. 3.2 (b) Freshly Distilled Hexane ;
Packed Column Distillation

20 µl Concentrate Injection
(1000 µl)



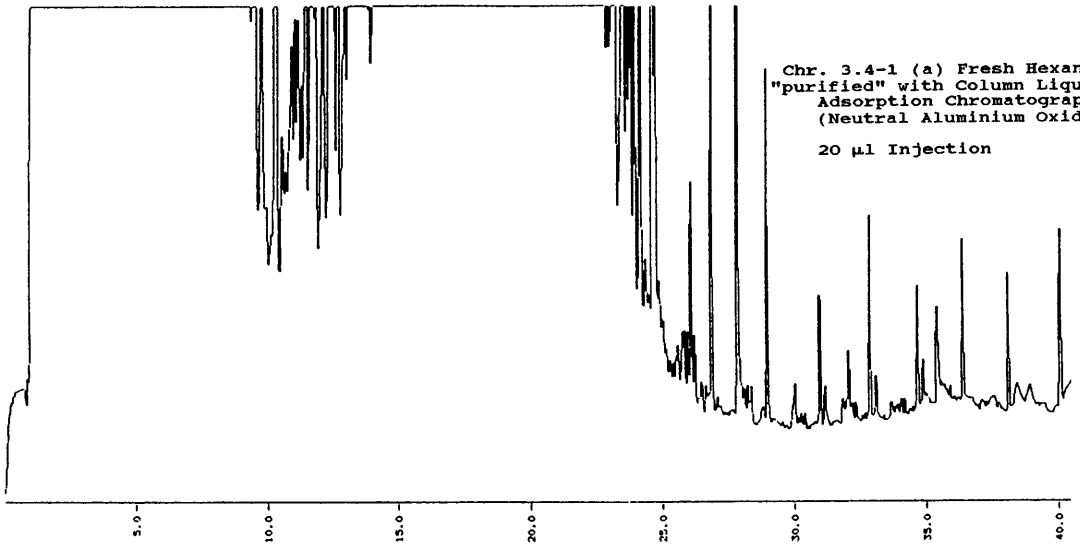
Chr. 3.3 (a) Freshly Distilled Hexane ;
Spinning Band Distillation

20 µl Injection



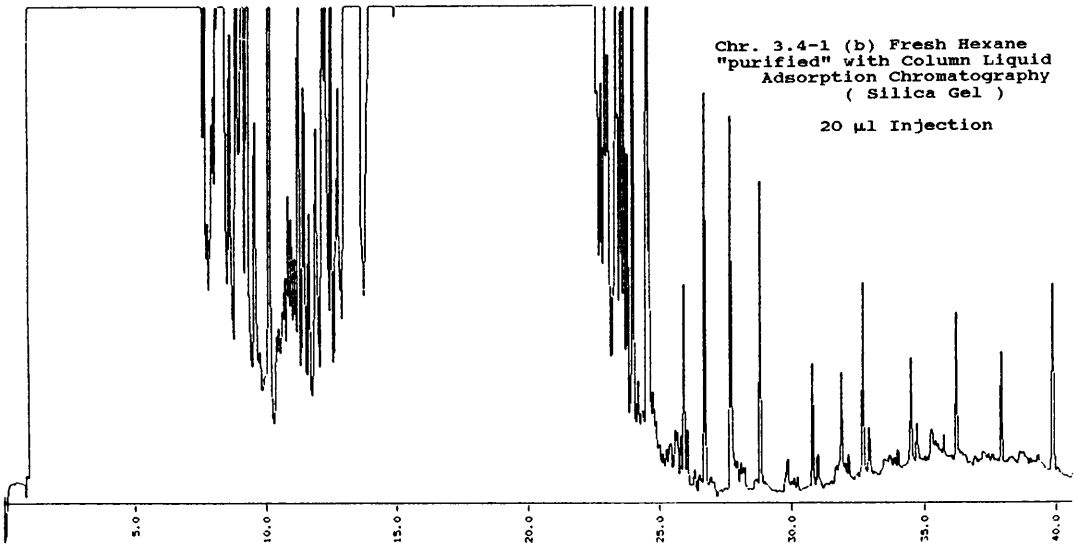
Chr. 3.3 (b) Freshly Distilled Hexane ;
Spinning Band Distillation

20 µl Concentrate Injection
(1000 µl)



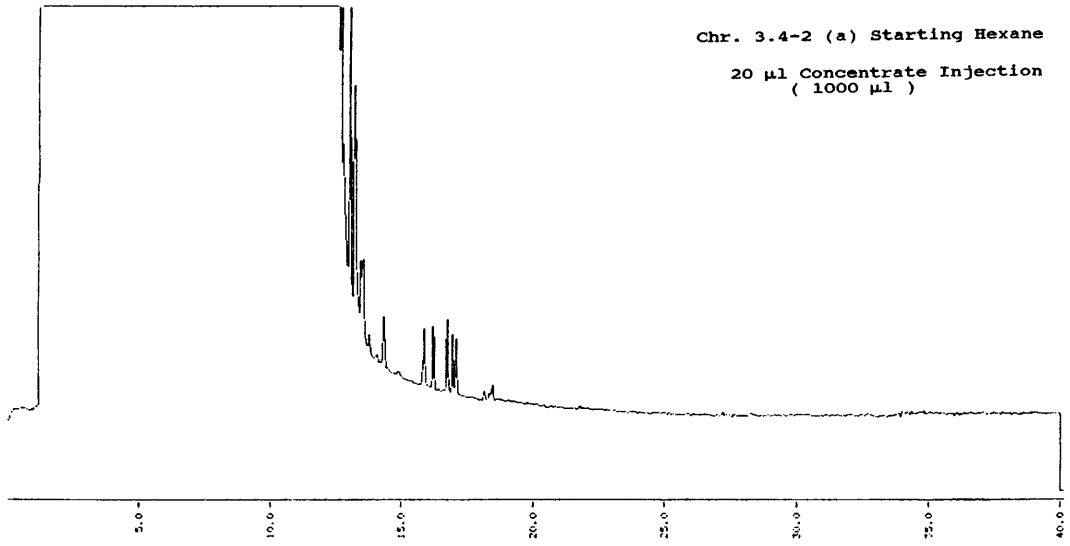
Chr. 3.4-1 (a) Fresh Hexane "purified" with Column Liquid Adsorption Chromatography (Neutral Aluminium Oxide)

20 µl Injection



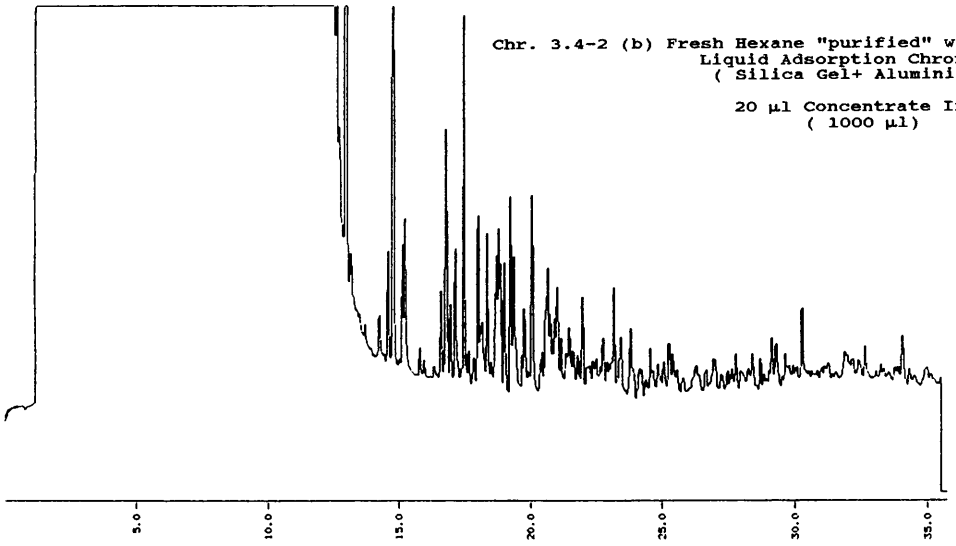
Chr. 3.4-1 (b) Fresh Hexane "purified" with Column Liquid Adsorption Chromatography (Silica Gel)

20 µl Injection



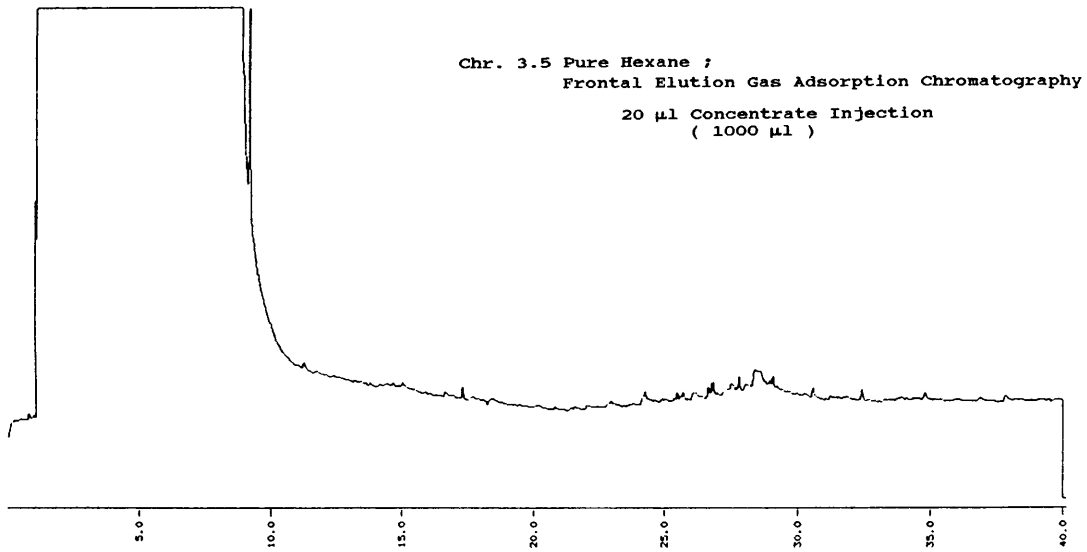
Chr. 3.4-2 (a) Starting Hexane

20 µl Concentrate Injection (1000 µl)



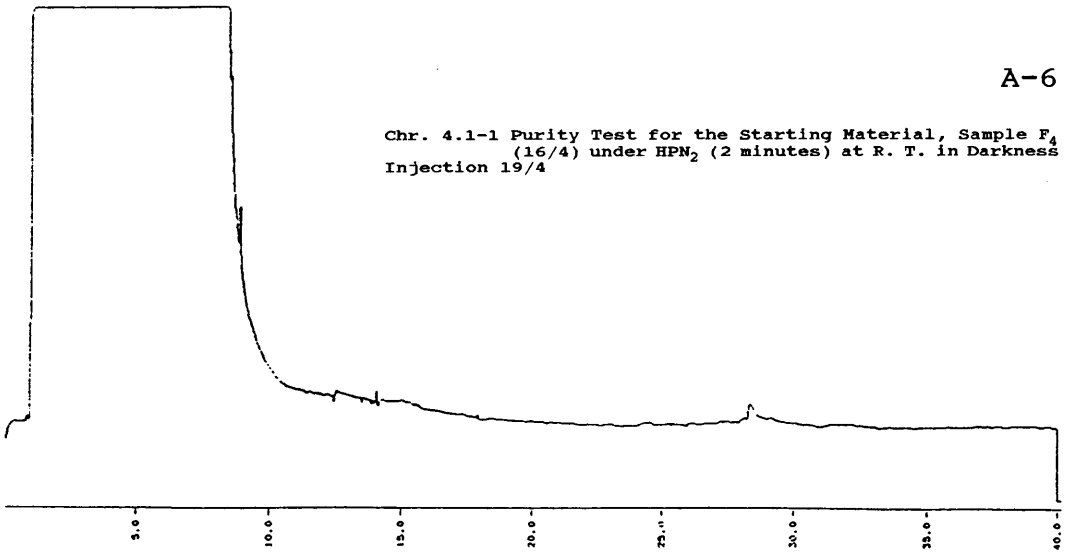
Chr. 3.4-2 (b) Fresh Hexane "purified" with Column
Liquid Adsorption Chromatography
(Silica Gel+ Aluminium Oxide)

20 µl Concentrate Injection
(1000 µl)



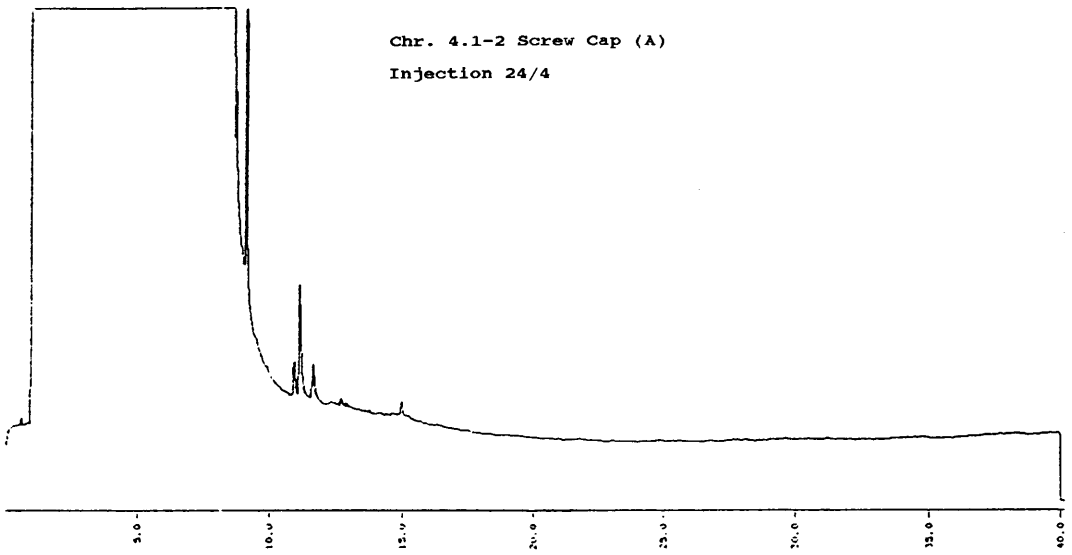
Chr. 3.5 Pure Hexane ;
Frontal Elution Gas Adsorption Chromatography

20 µl Concentrate Injection
(1000 µl)

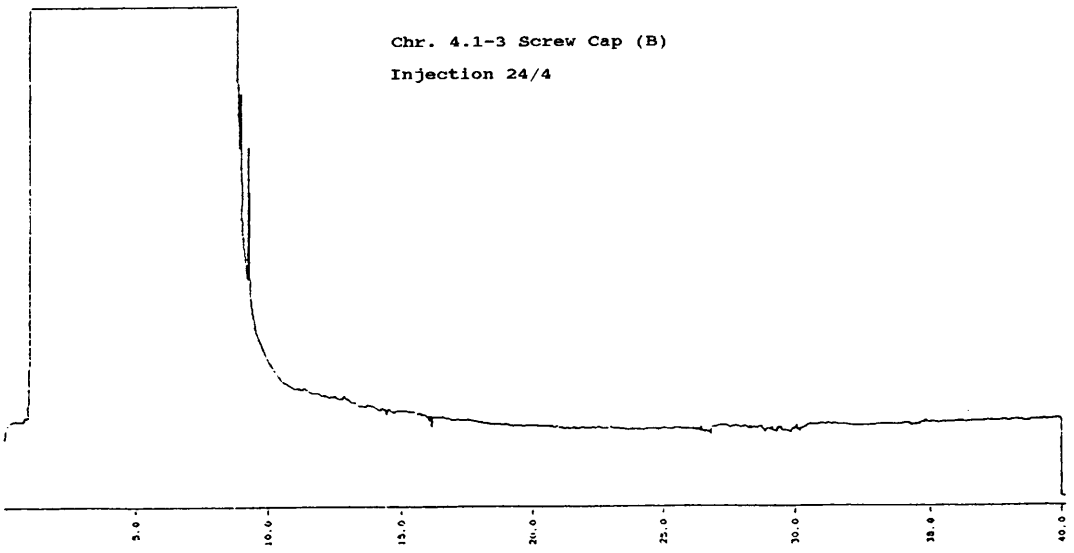


Chr. 4.1-1 Purity Test for the Starting Material, Sample F_4 (16/4) under HPN_2 (2 minutes) at R. T. in Darkness
Injection 19/4

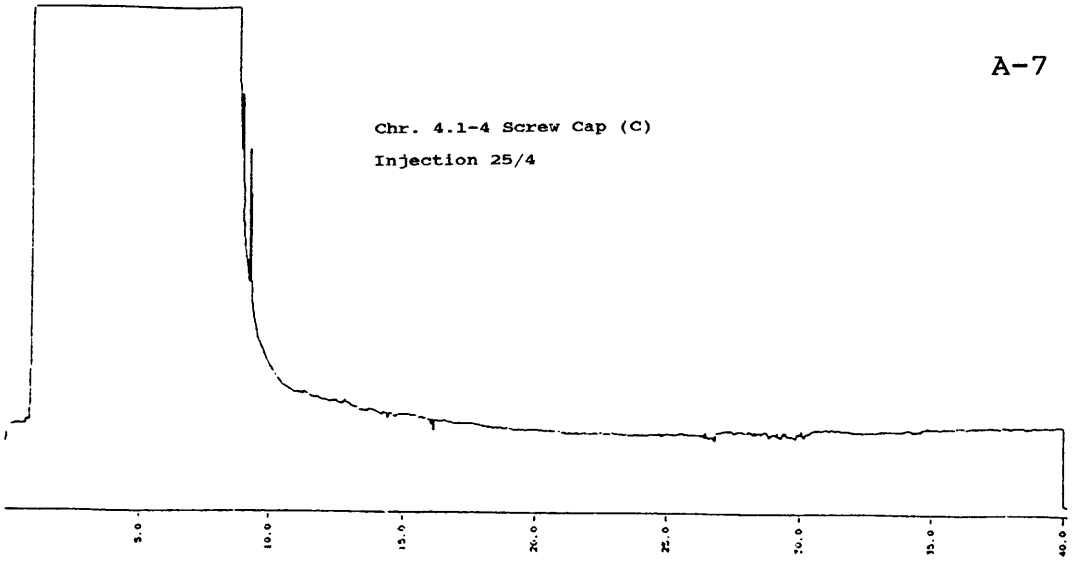
At 8 °C in the Refrigerator:
1. Under HPN_2 (1 minute)

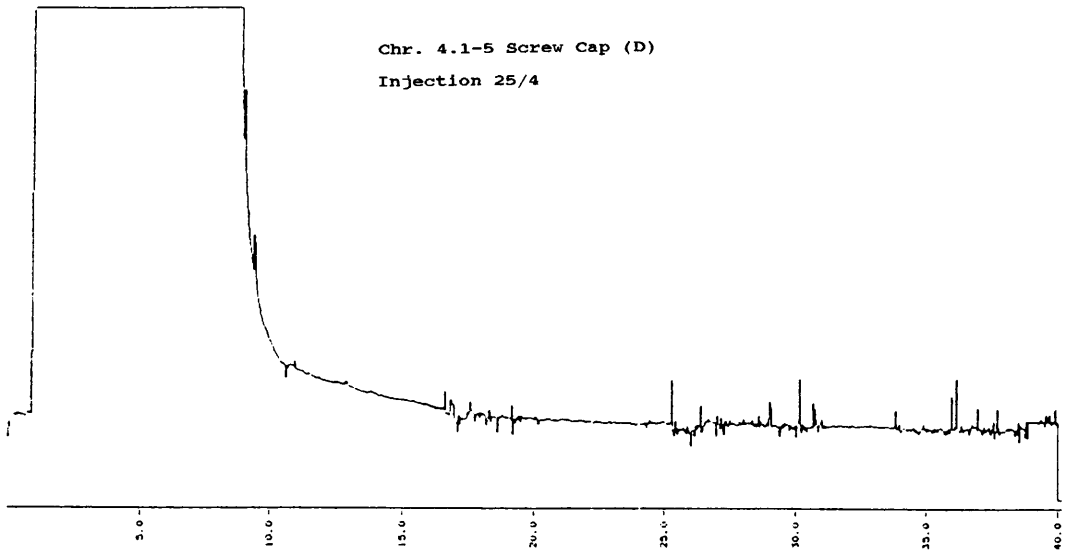


Chr. 4.1-2 Screw Cap (A)
Injection 24/4

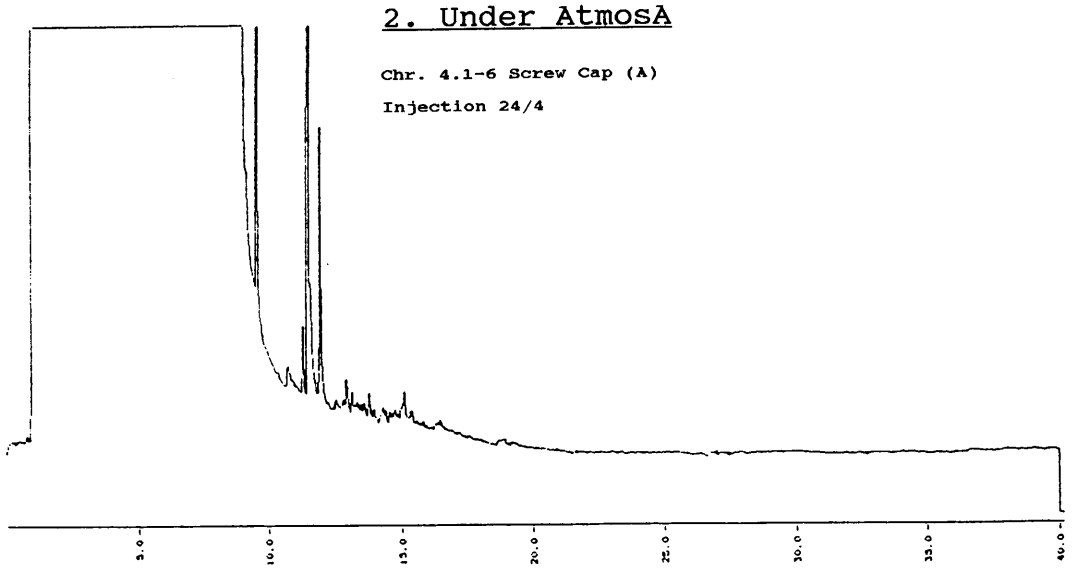


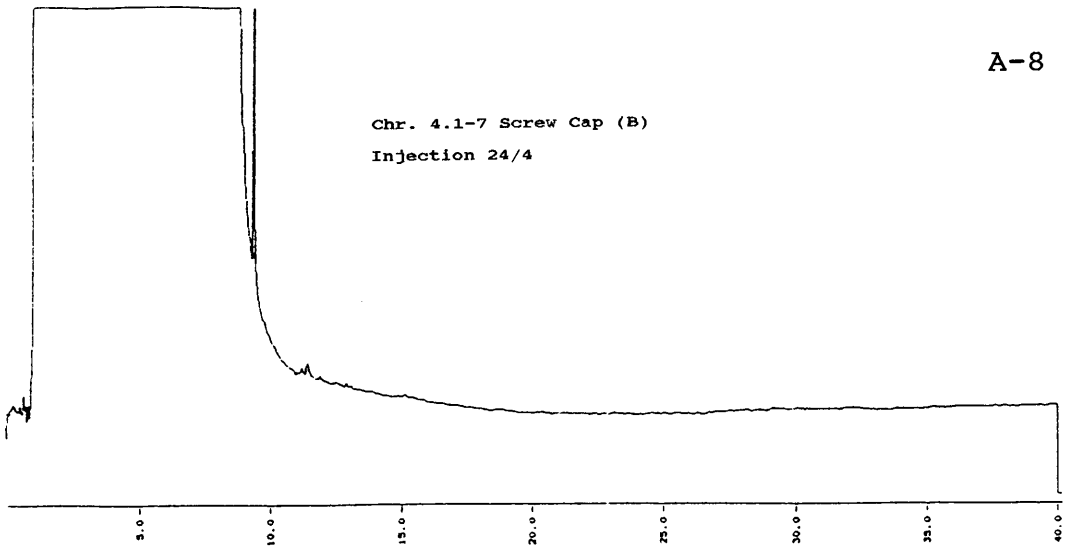
Chr. 4.1-3 Screw Cap (B)
Injection 24/4

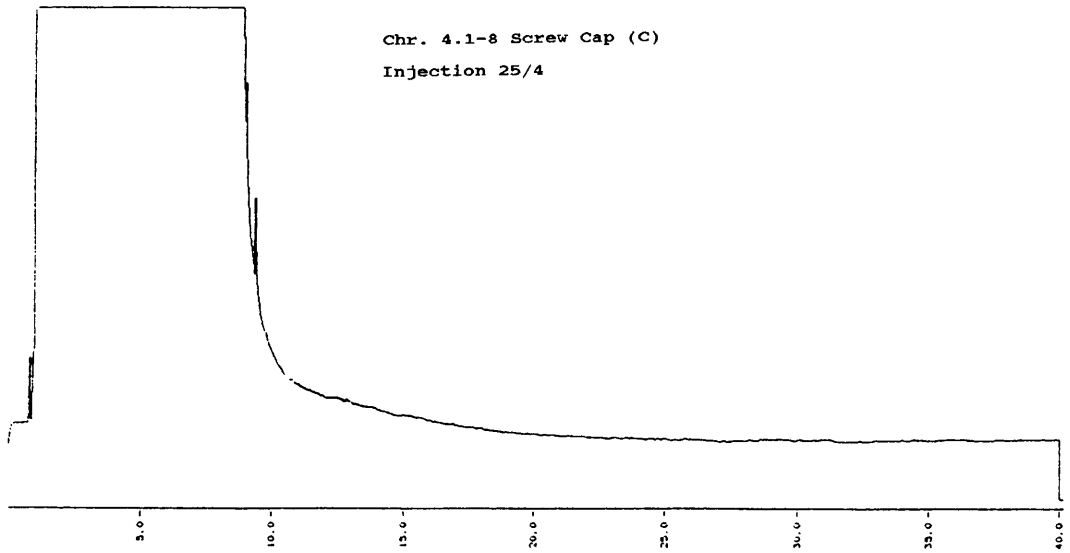


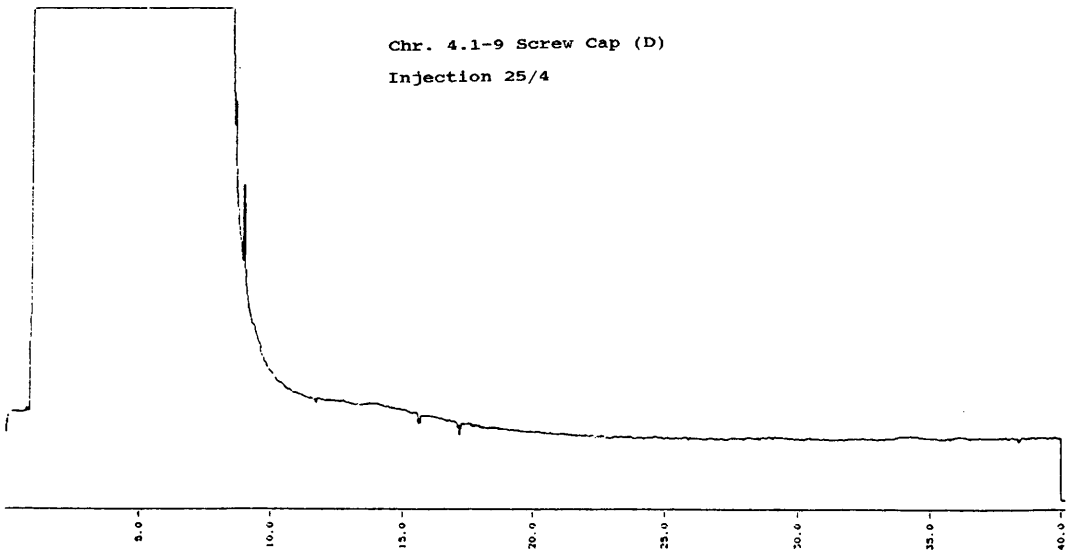


2. Under AtmosA



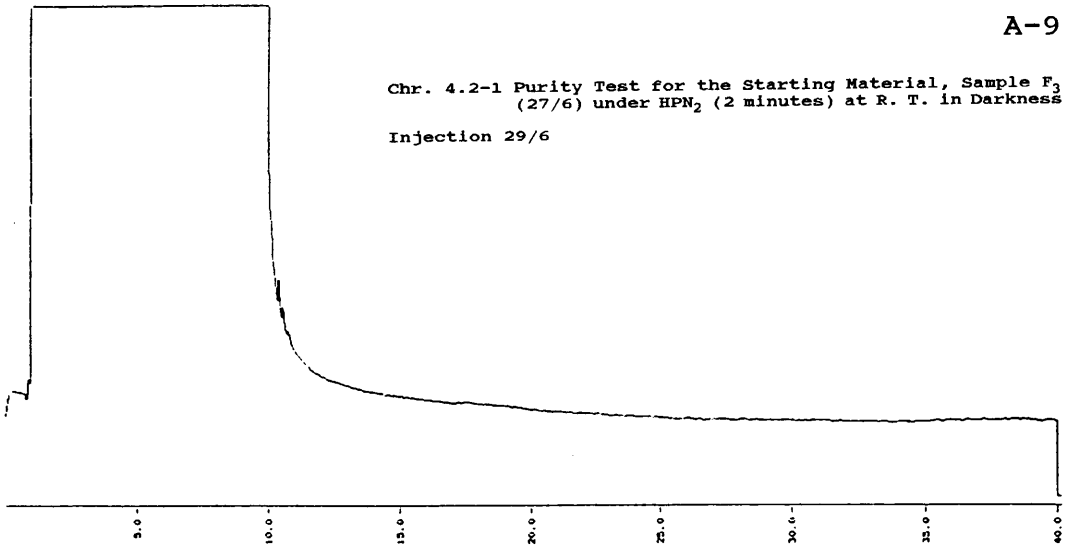






Chr. 4.2-1 Purity Test for the Starting Material, Sample F_3 (27/6) under HPN_2 (2 minutes) at R. T. in Darkness

Injection 29/6

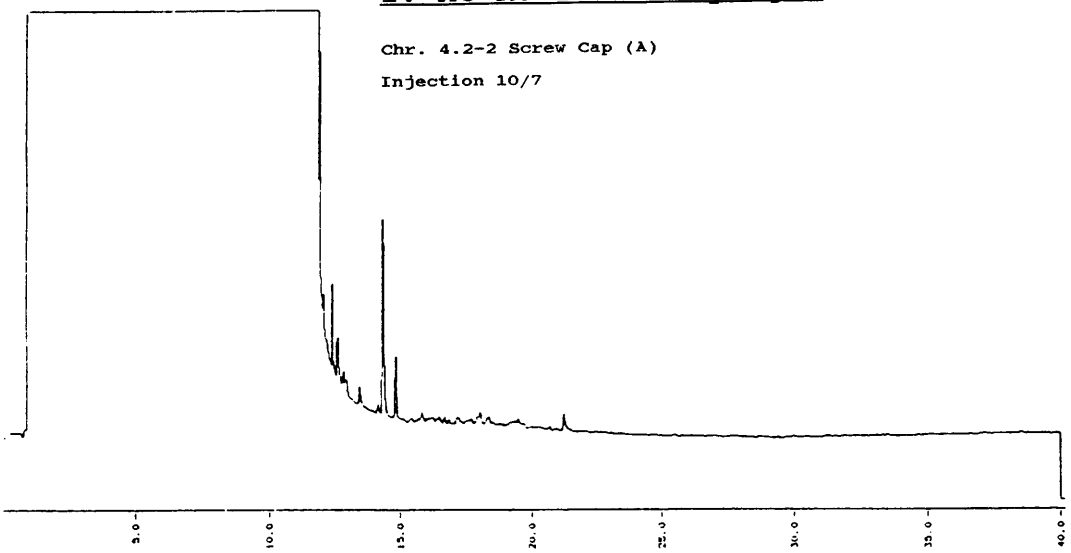


Under HPN_2 (1 minute):

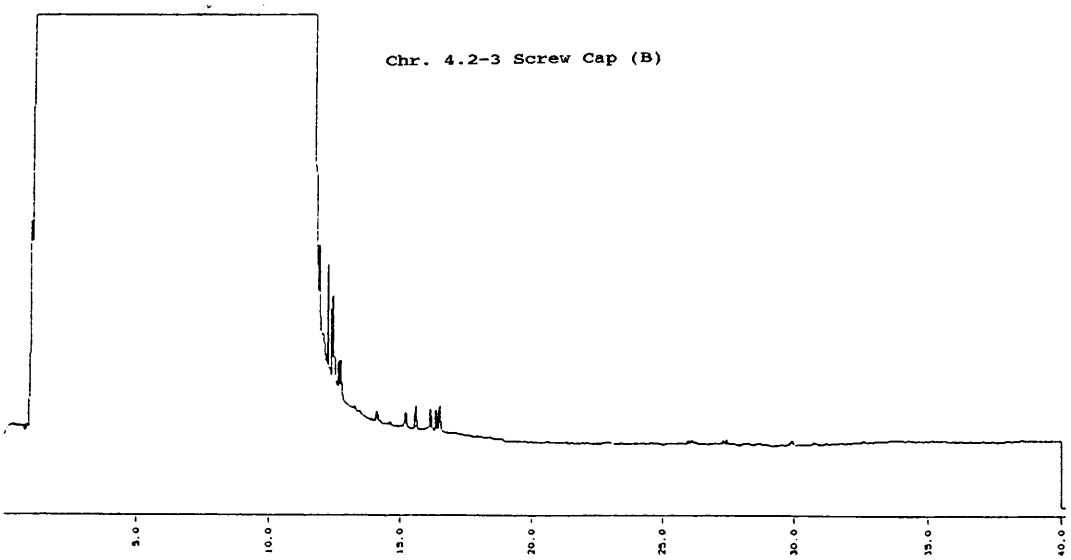
<u>1. At R. T. in Daylight</u>

Chr. 4.2-2 Screw Cap (A)

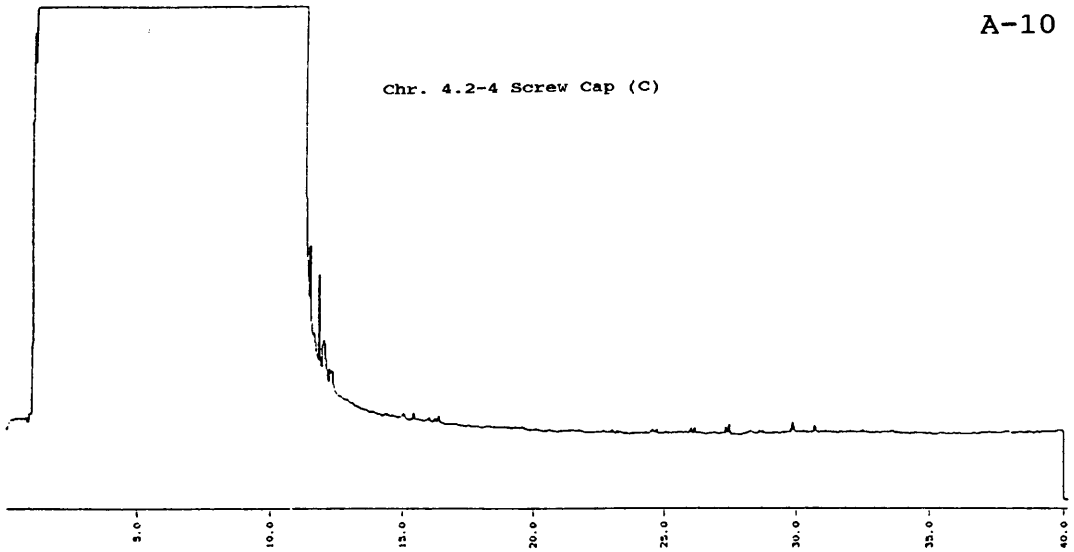
Injection 10/7



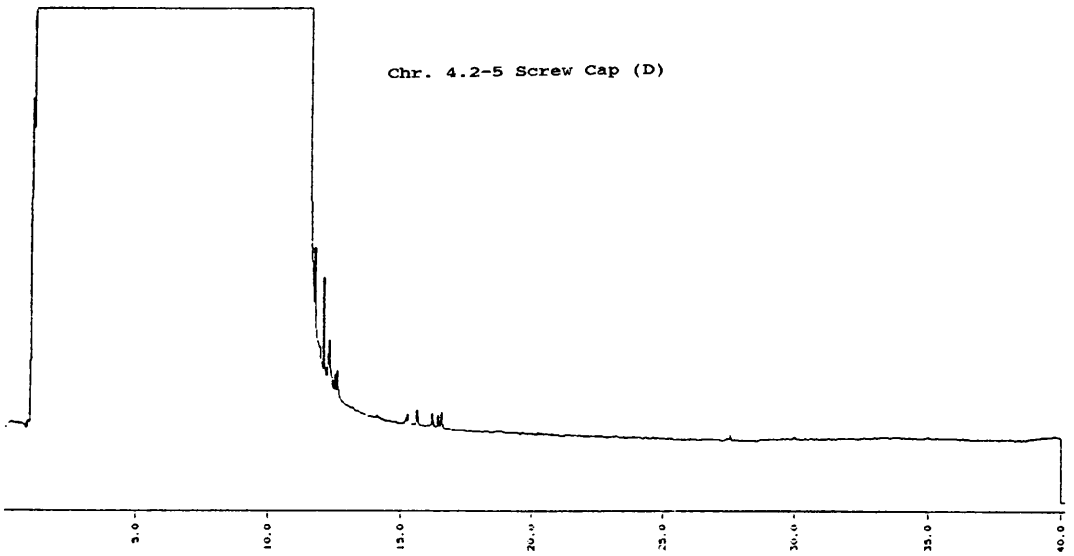
Chr. 4.2-3 Screw Cap (B)



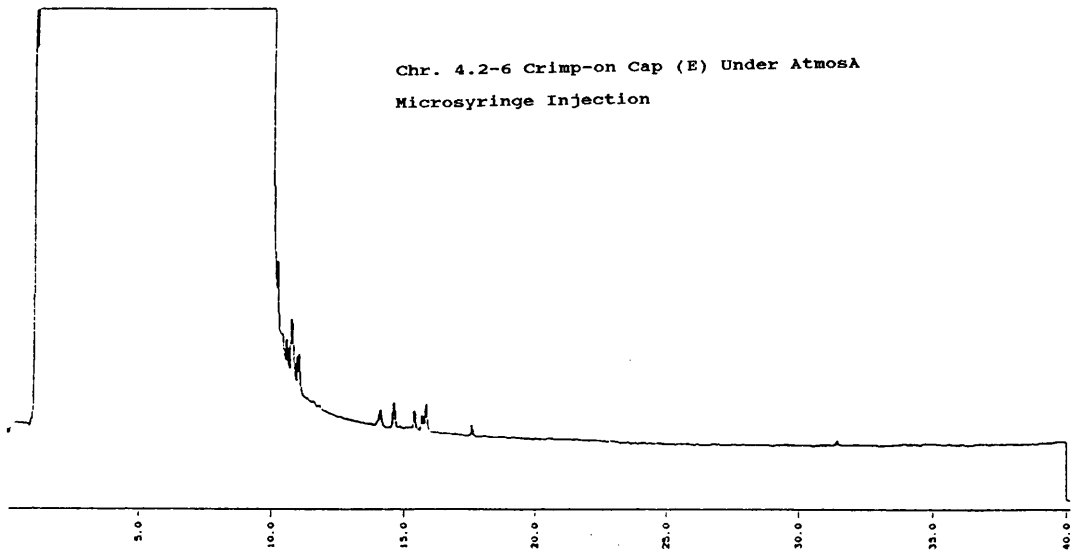
Chr. 4.2-4 Screw Cap (C)



Chr. 4.2-5 Screw Cap (D)

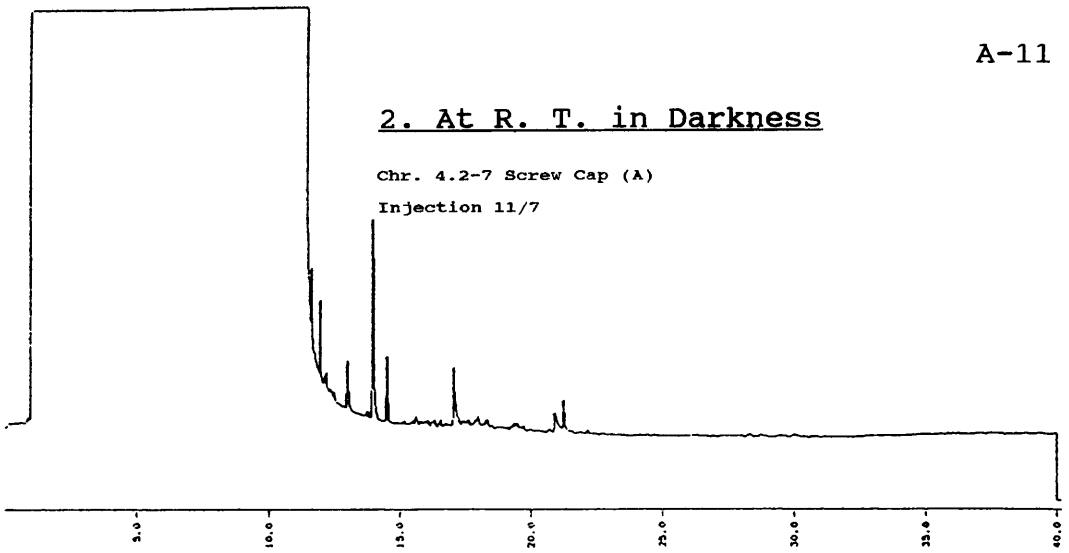


Chr. 4.2-6 Crimp-on Cap (E) Under AtmosA
Microsyringe Injection

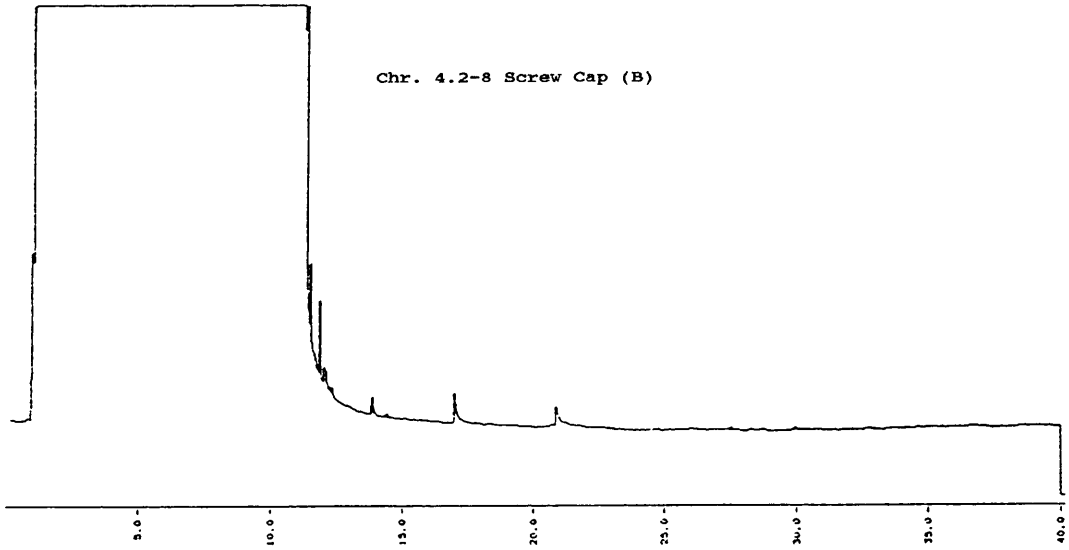


2. At R. T. in Darkness

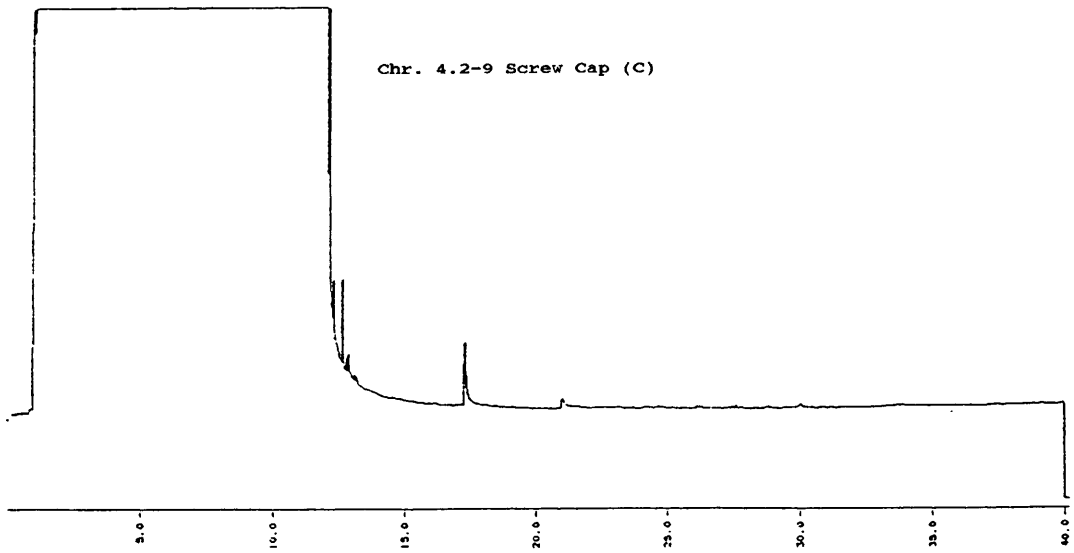
Chr. 4.2-7 Screw Cap (A)
Injection 11/7

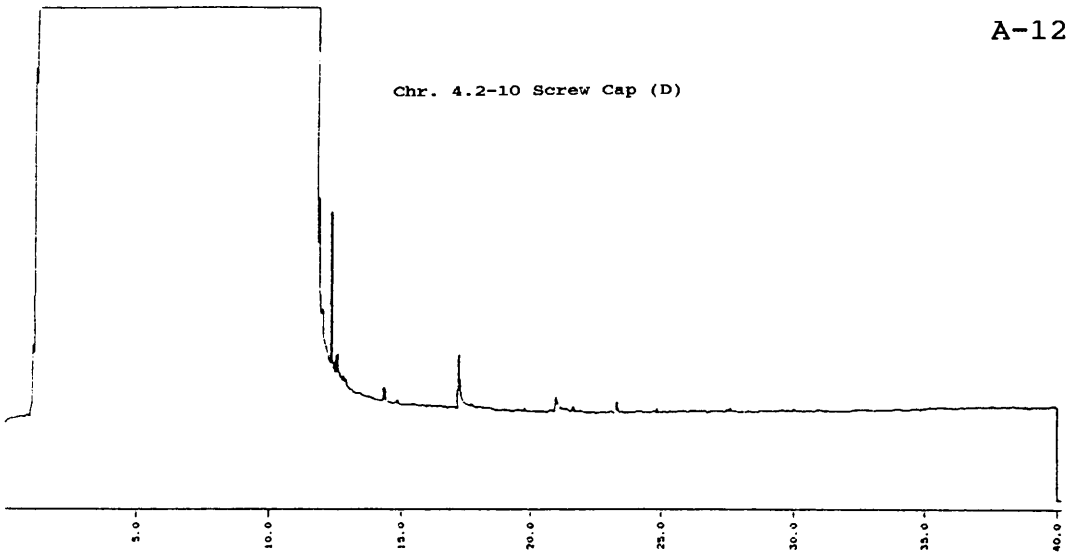


Chr. 4.2-8 Screw Cap (B)

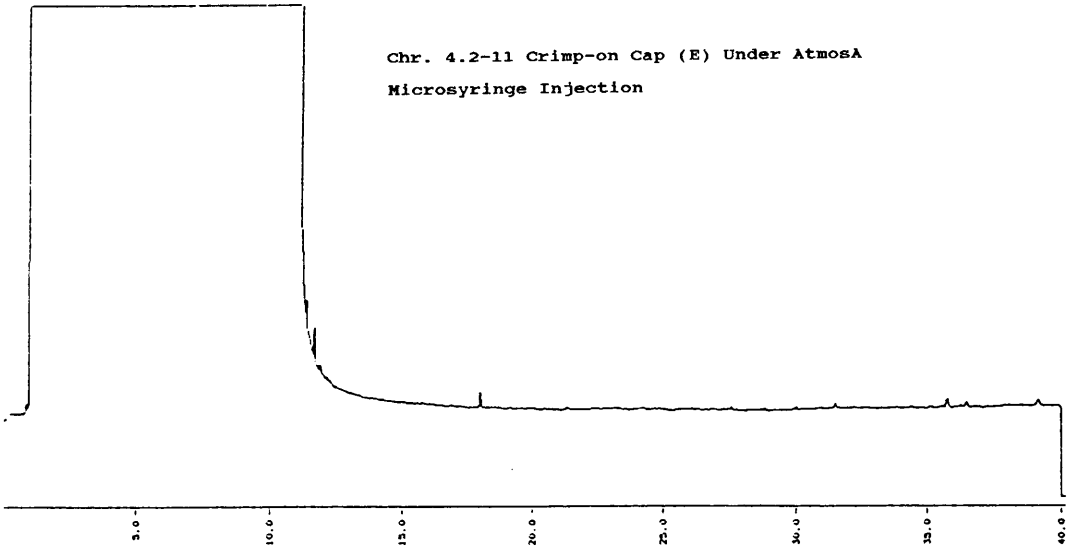


Chr. 4.2-9 Screw Cap (C)



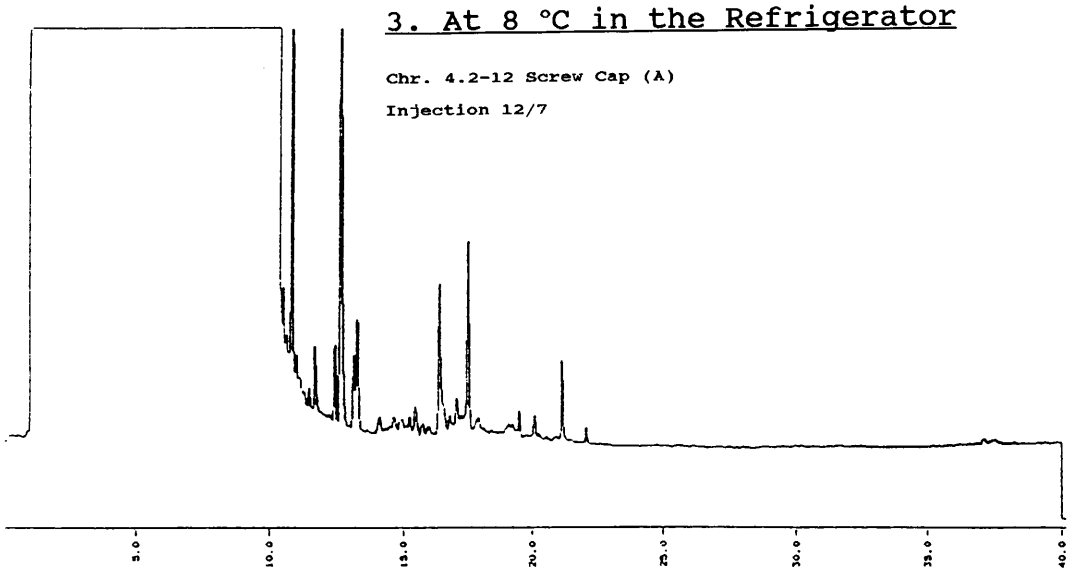


Chr. 4.2-10 Screw Cap (D)

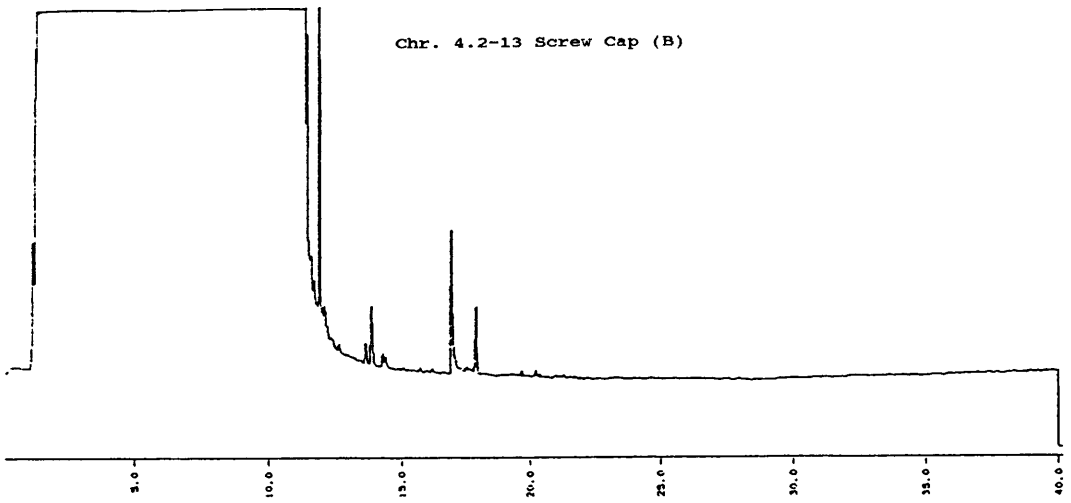


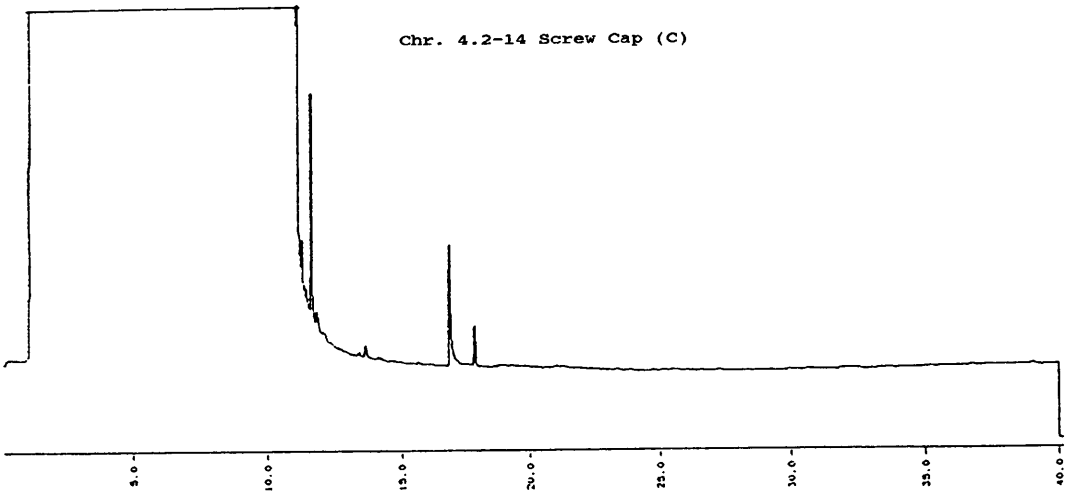
Chr. 4.2-11 Crimp-on Cap (E) Under AtmosA
Microsyringe Injection

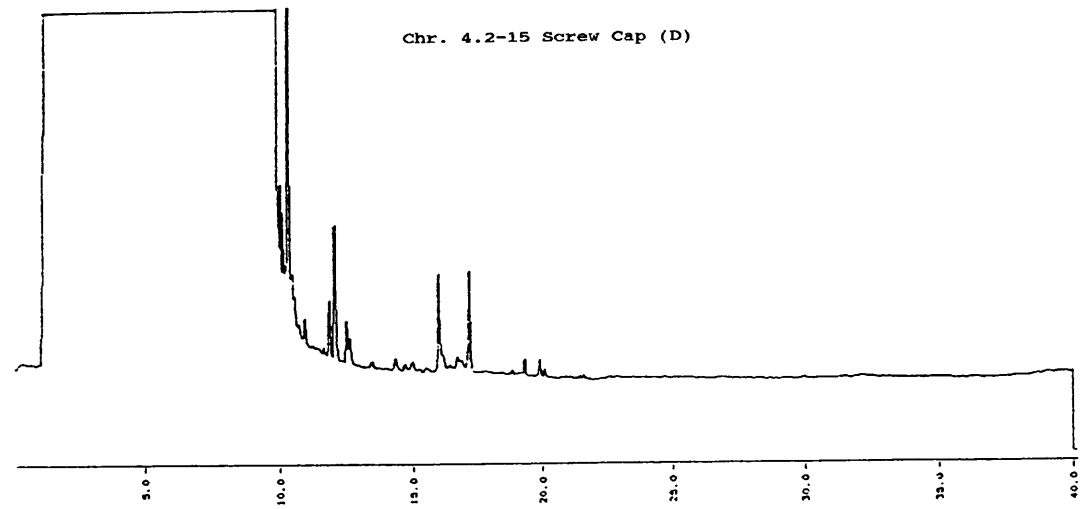
3. At 8 °C in the Refrigerator

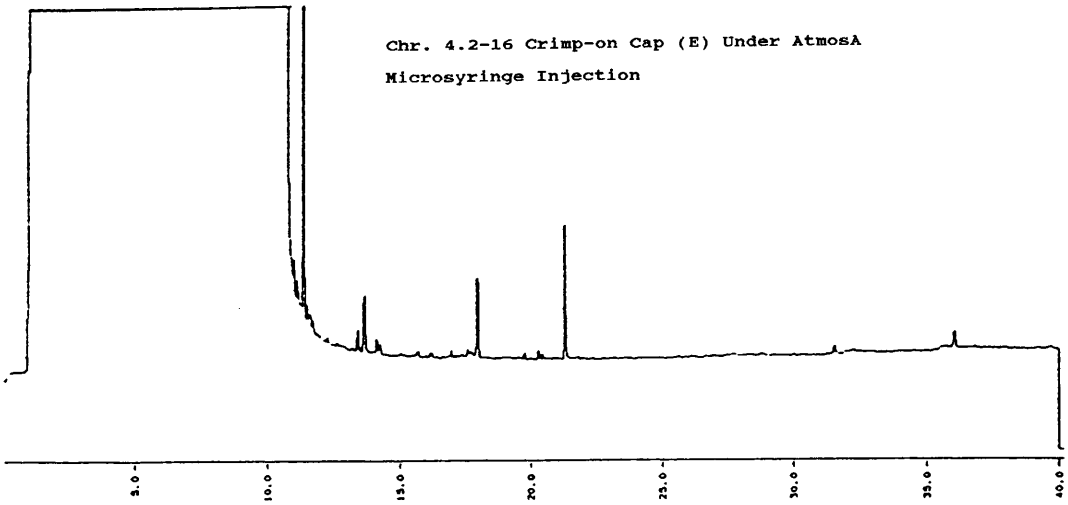


Chr. 4.2-12 Screw Cap (A)
Injection 12/7

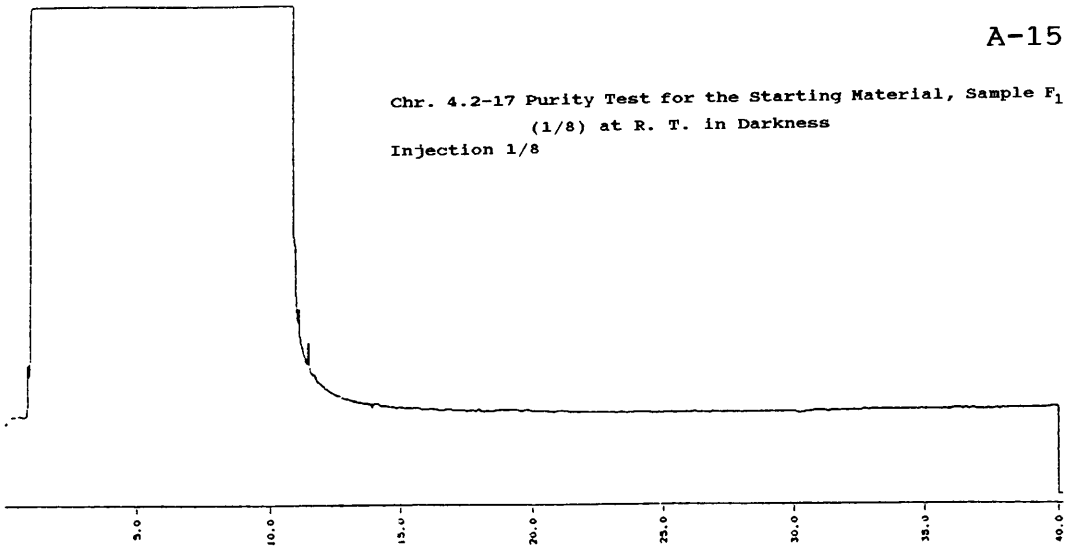






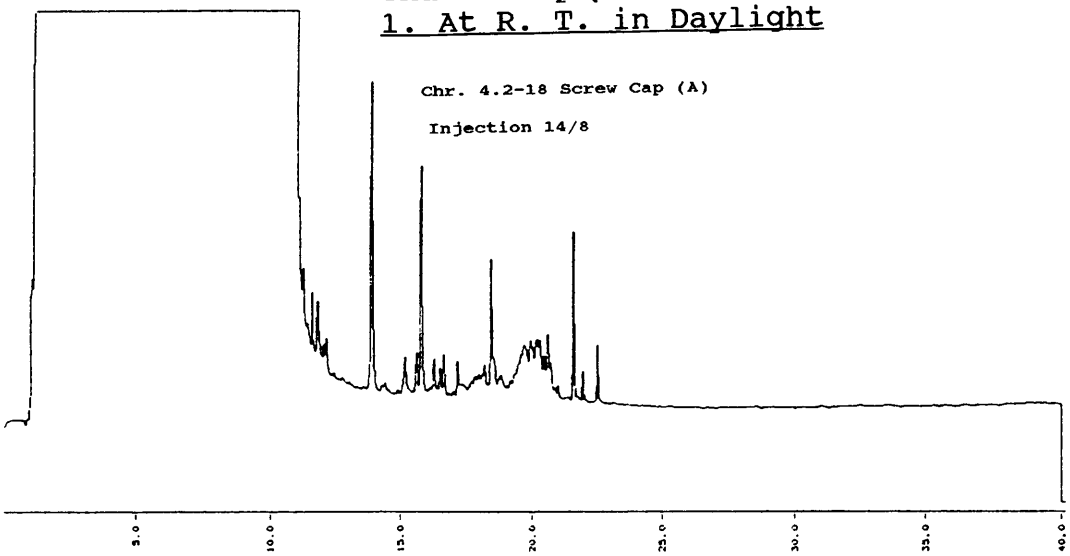


Chr. 4.2-17 Purity Test for the Starting Material, Sample F_1 (1/8) at R. T. in Darkness
Injection 1/8

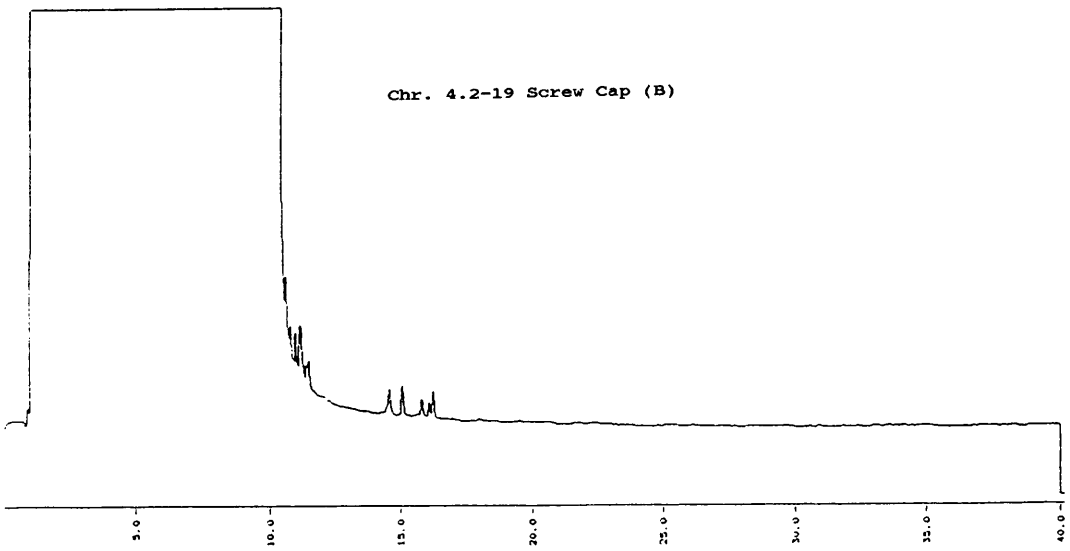


Under HPN_2 (1 minute):
1. At R. T. in Daylight

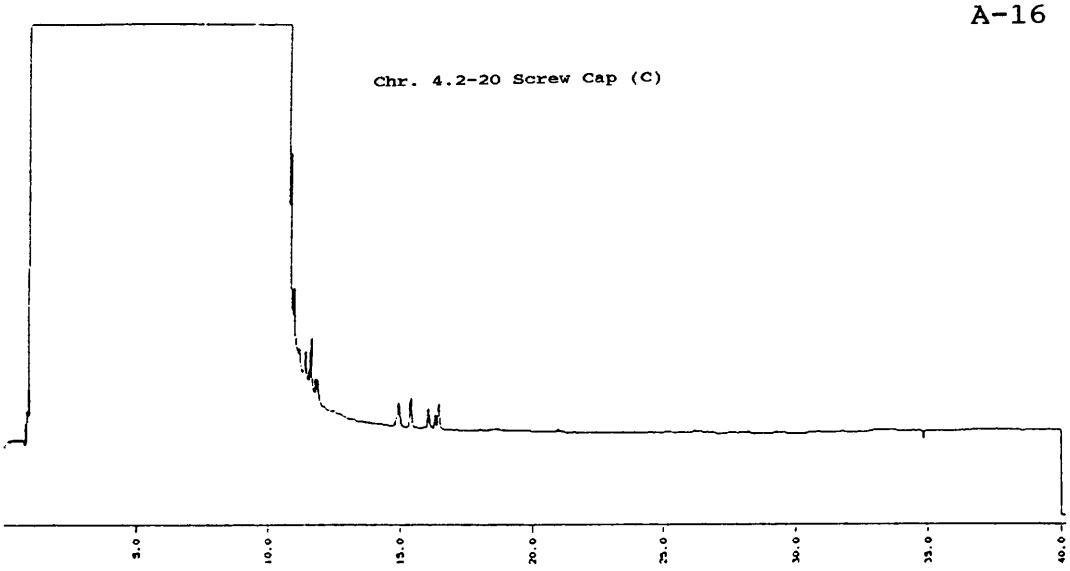
Chr. 4.2-18 Screw Cap (A)
Injection 14/8



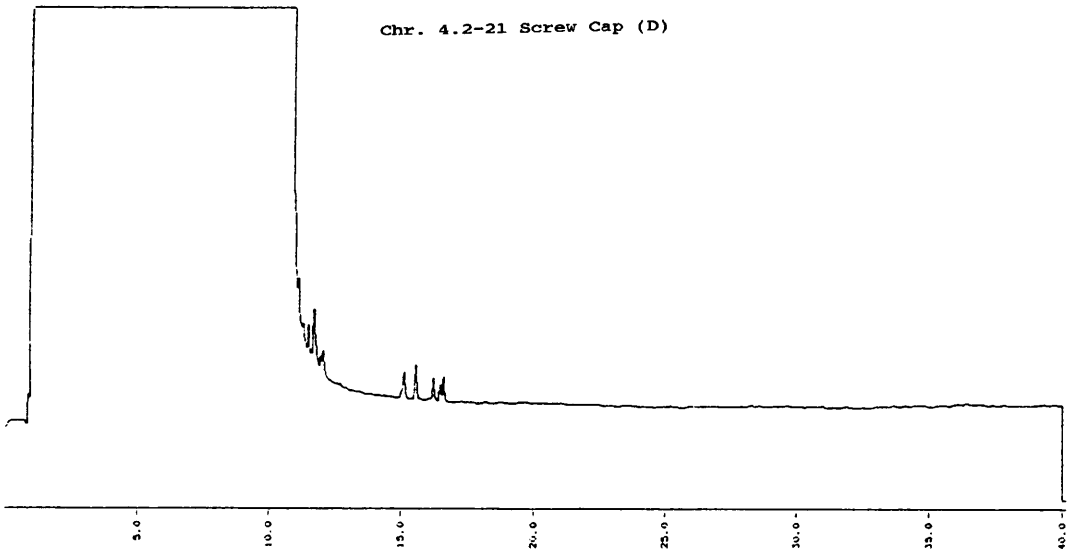
Chr. 4.2-19 Screw Cap (B)



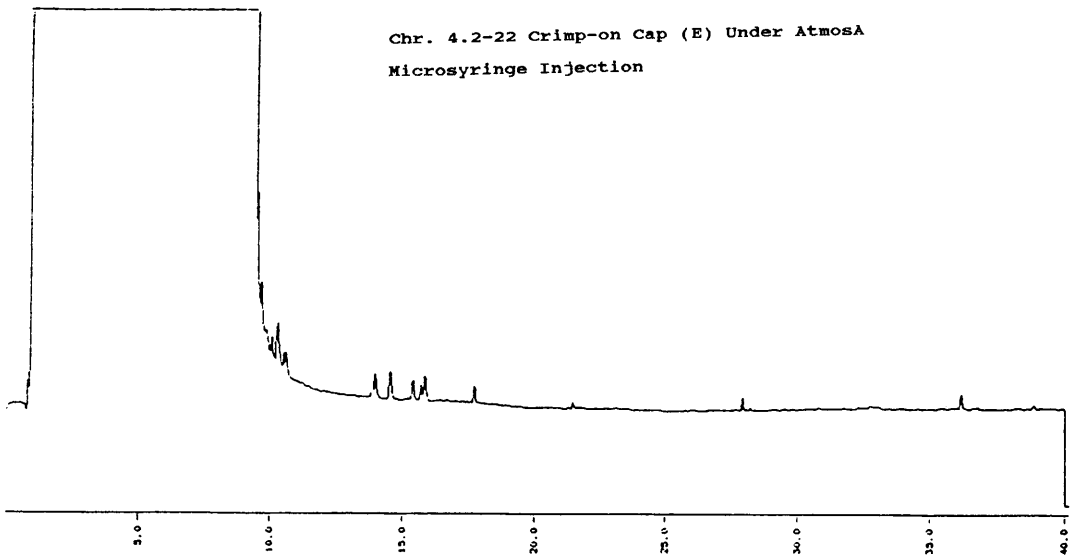
Chr. 4.2-20 Screw Cap (C)



Chr. 4.2-21 Screw Cap (D)



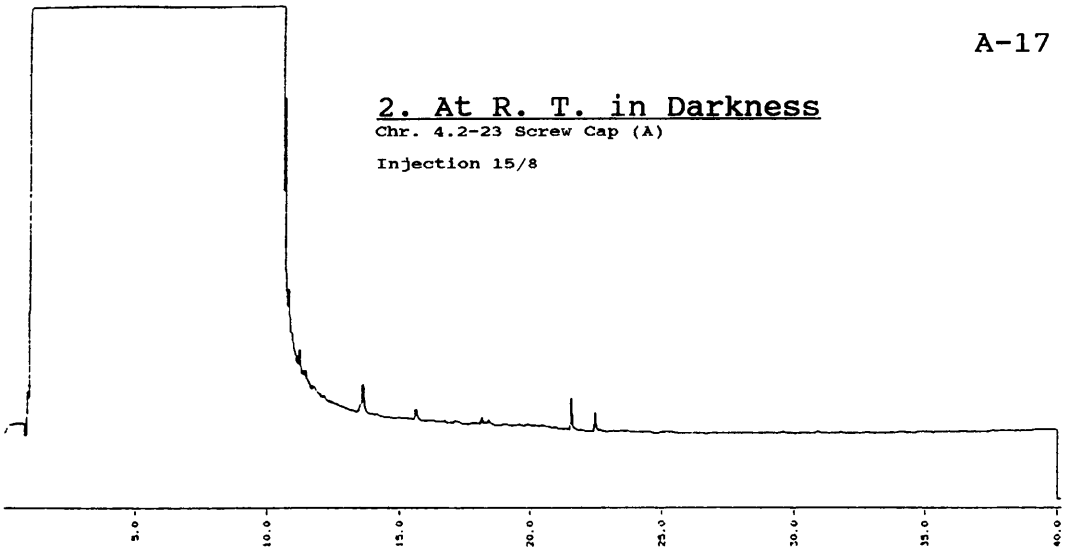
Chr. 4.2-22 Crimp-on Cap (E) Under AtmosA
Microsyringe Injection



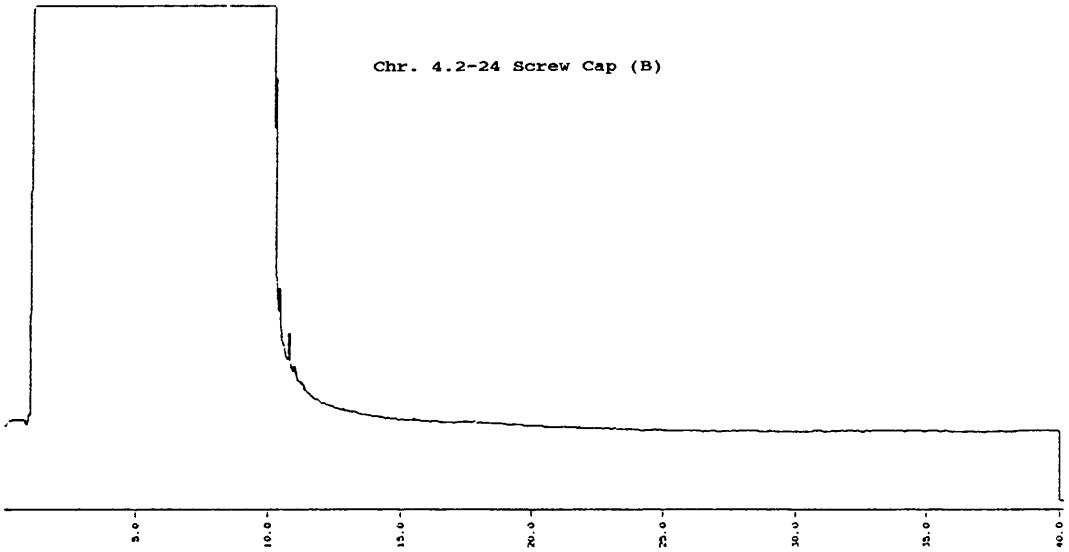
2. At R. T. in Darkness

Chr. 4.2-23 Screw Cap (A)

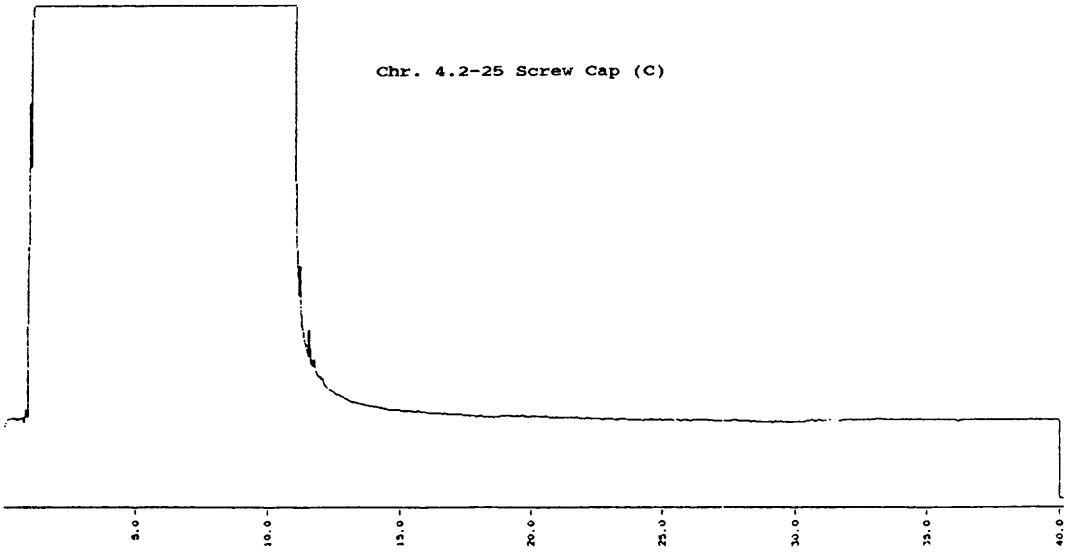
Injection 15/8

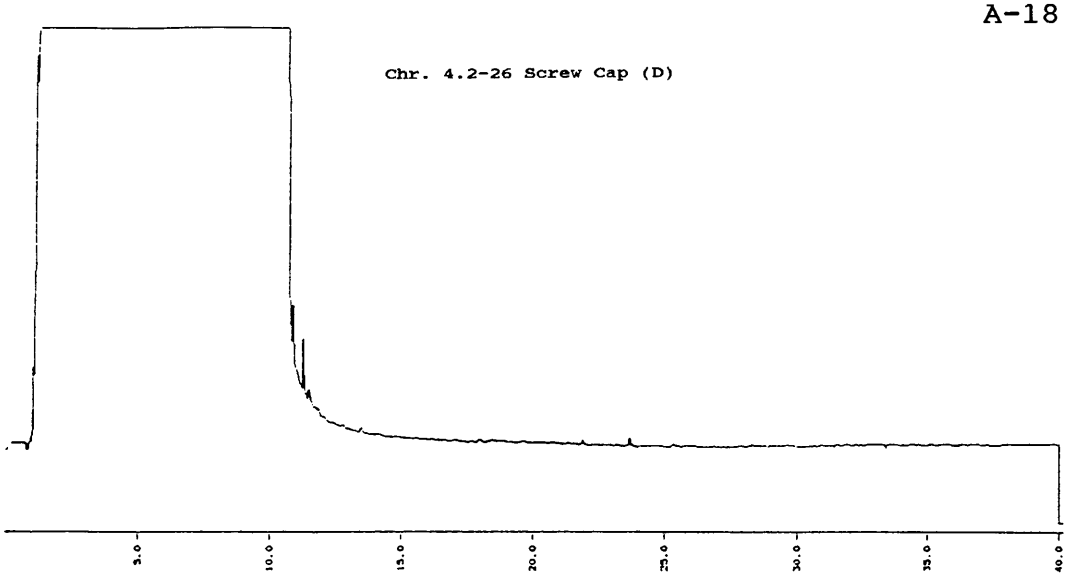


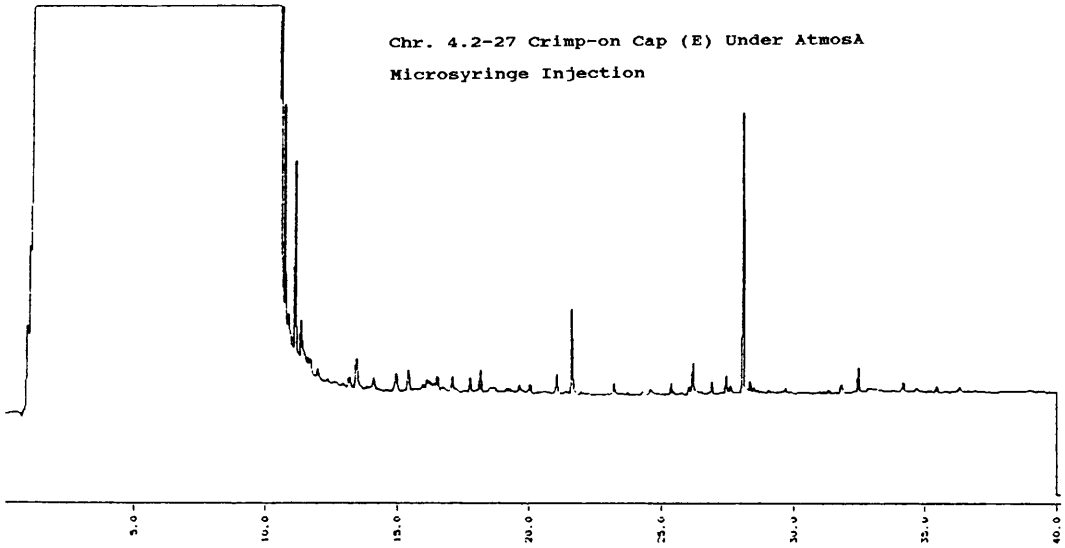
Chr. 4.2-24 Screw Cap (B)



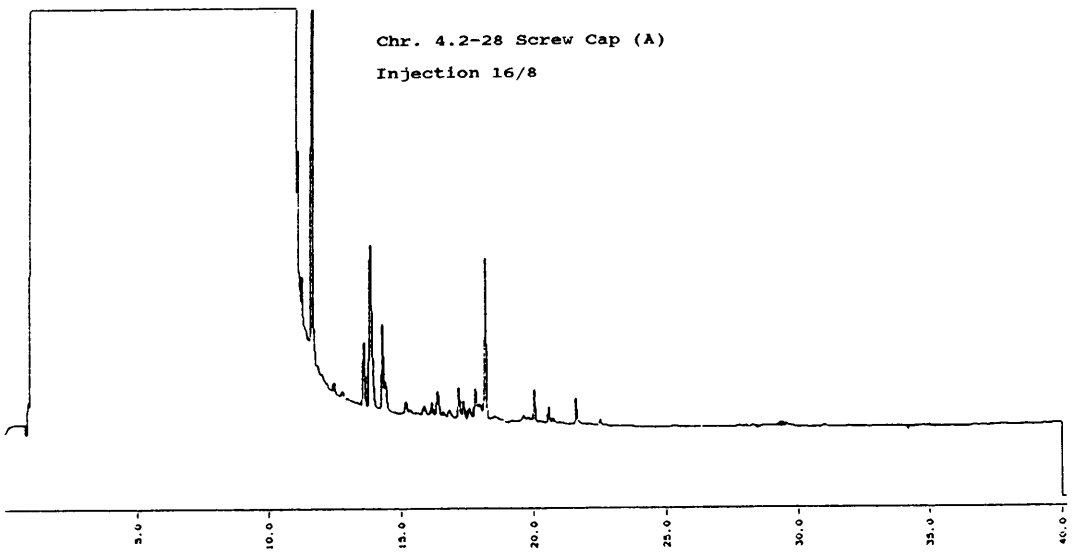
Chr. 4.2-25 Screw Cap (C)

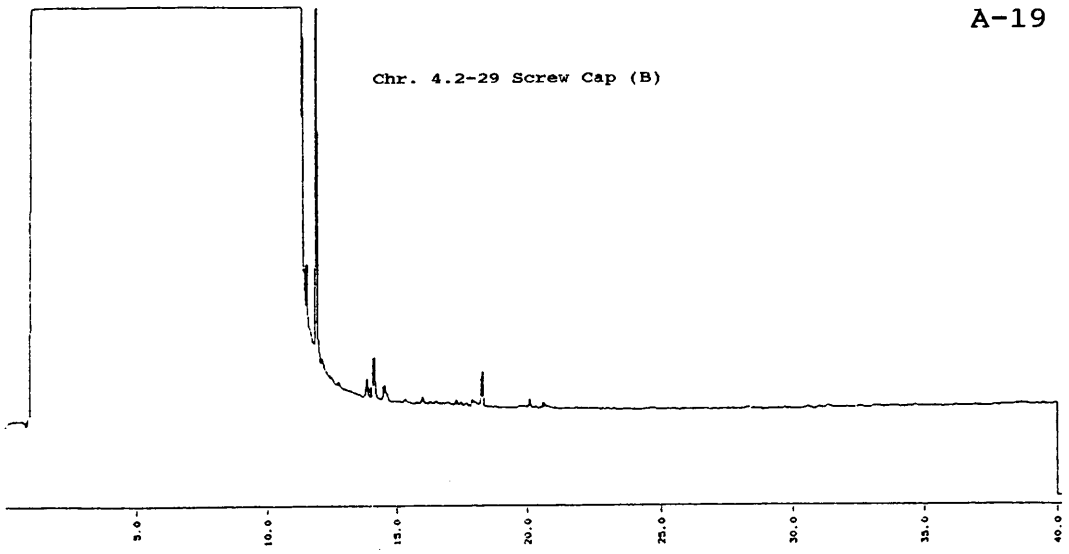


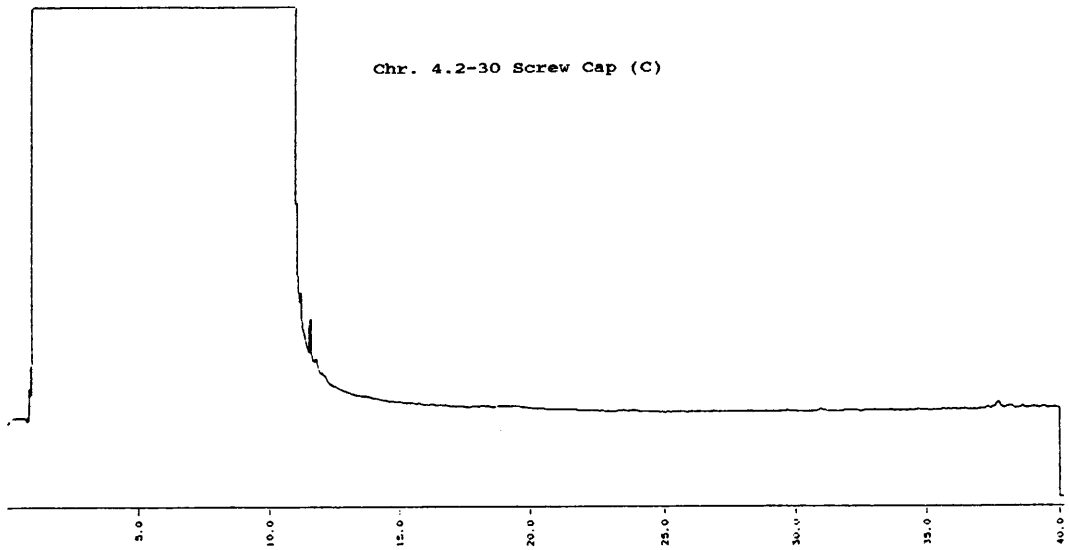


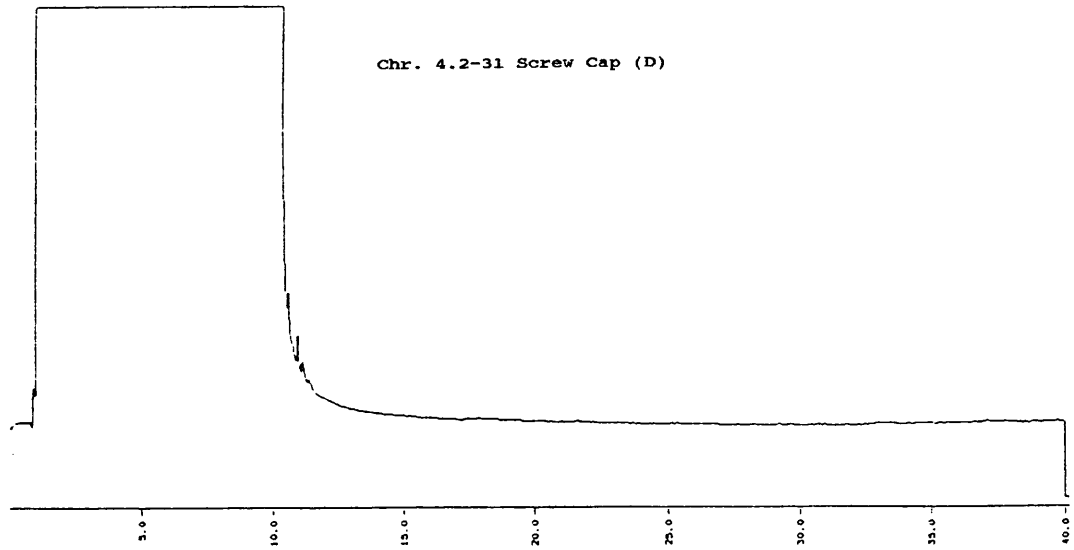


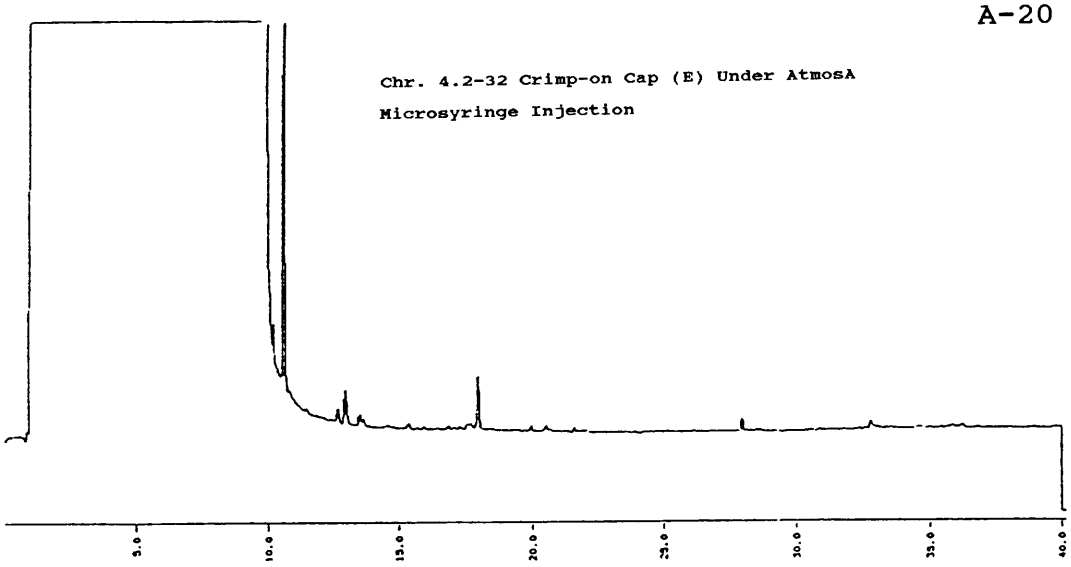
3. At 8 °C in the Refrigerator

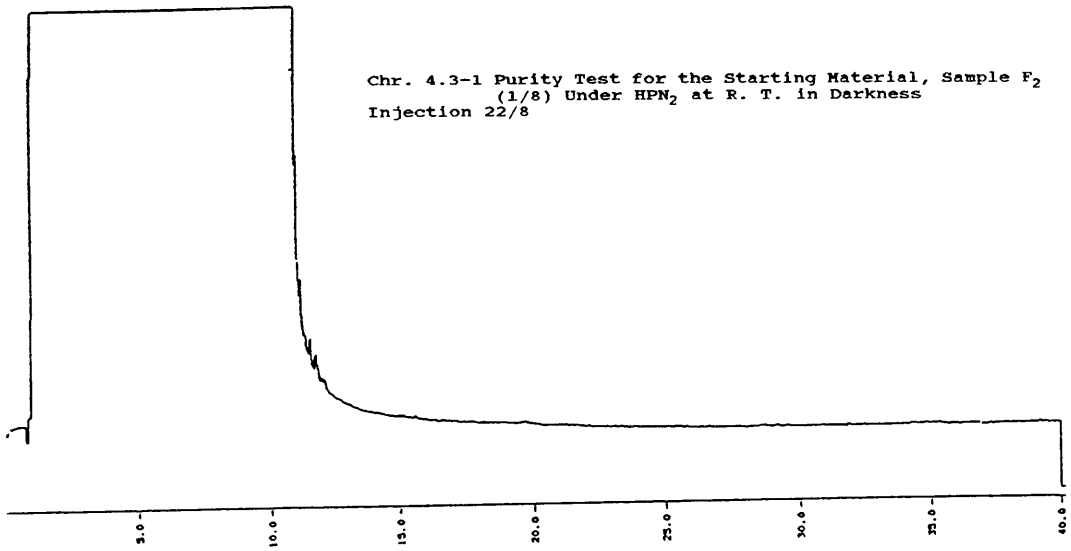






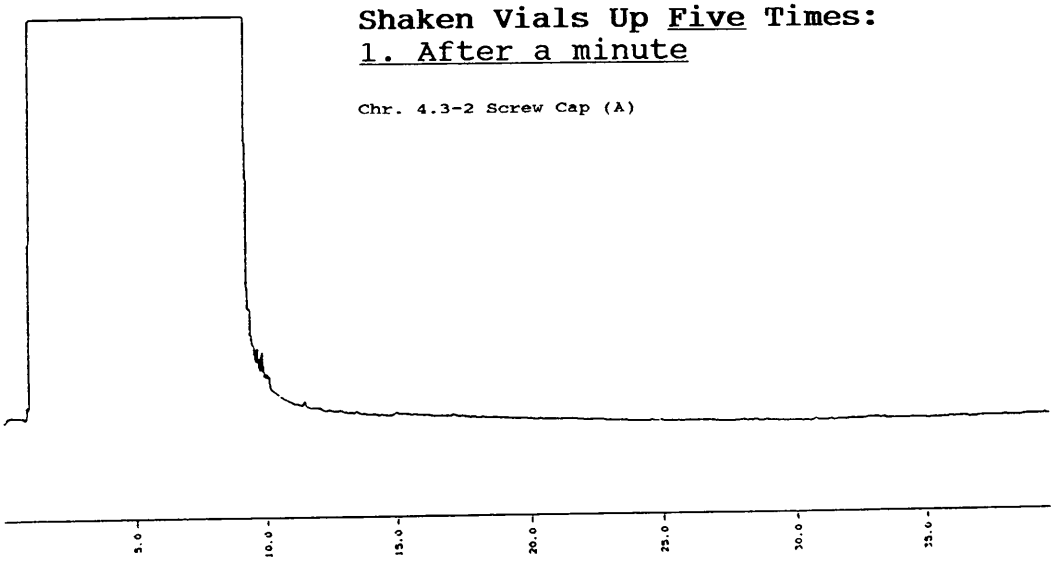




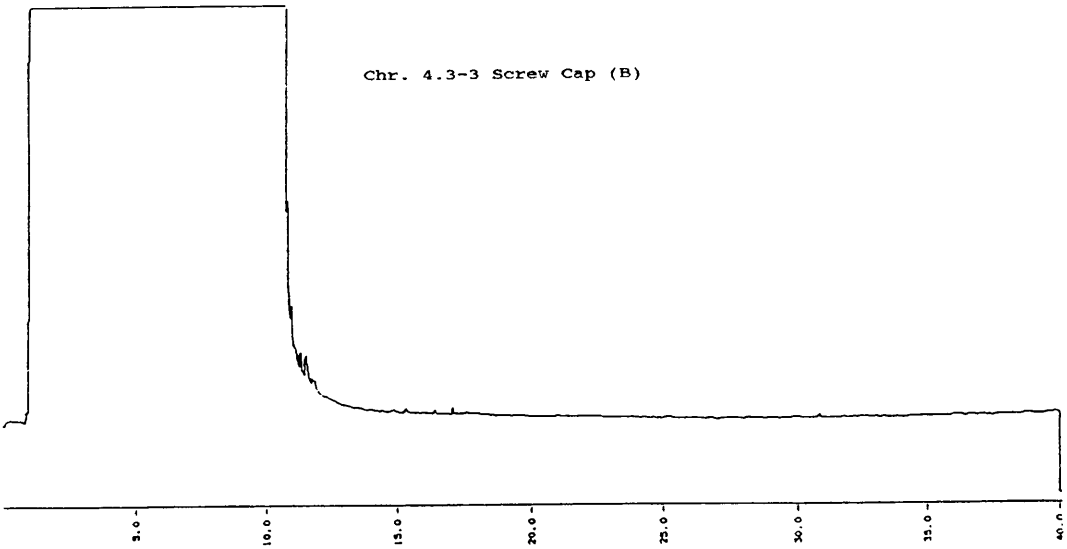


Chr. 4.3-1 Purity Test for the Starting Material, Sample F_2 (1/8) Under HPN_2 at R. T. in Darkness
Injection 22/8

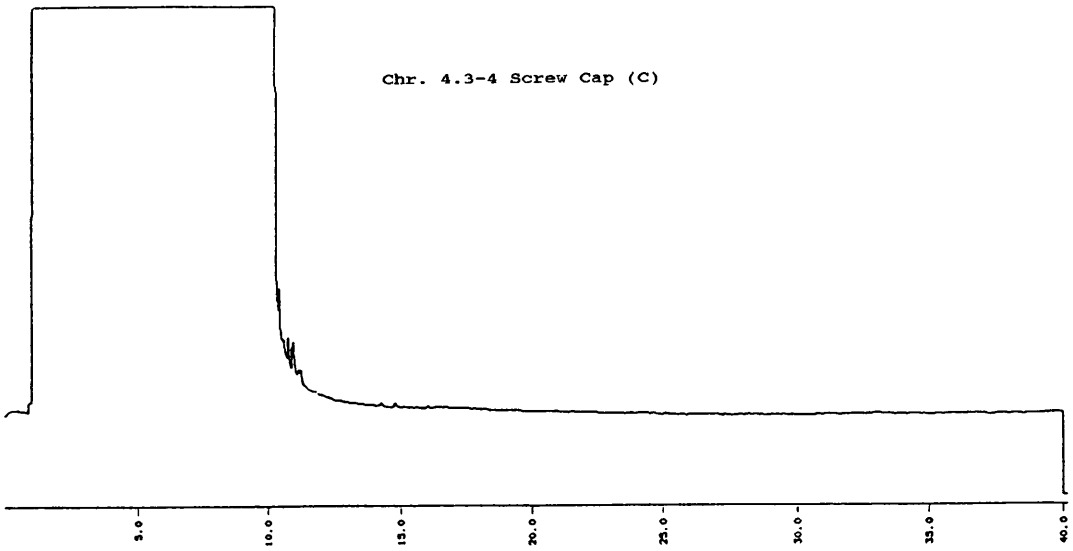
Shaken Vials Up <u>Five</u> Times:
<u>1. After a minute</u>

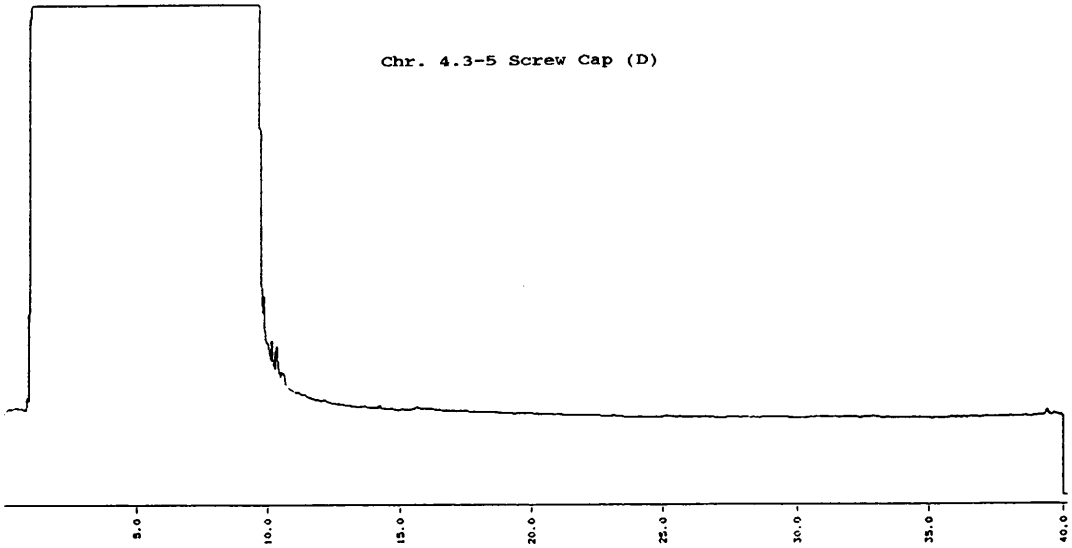


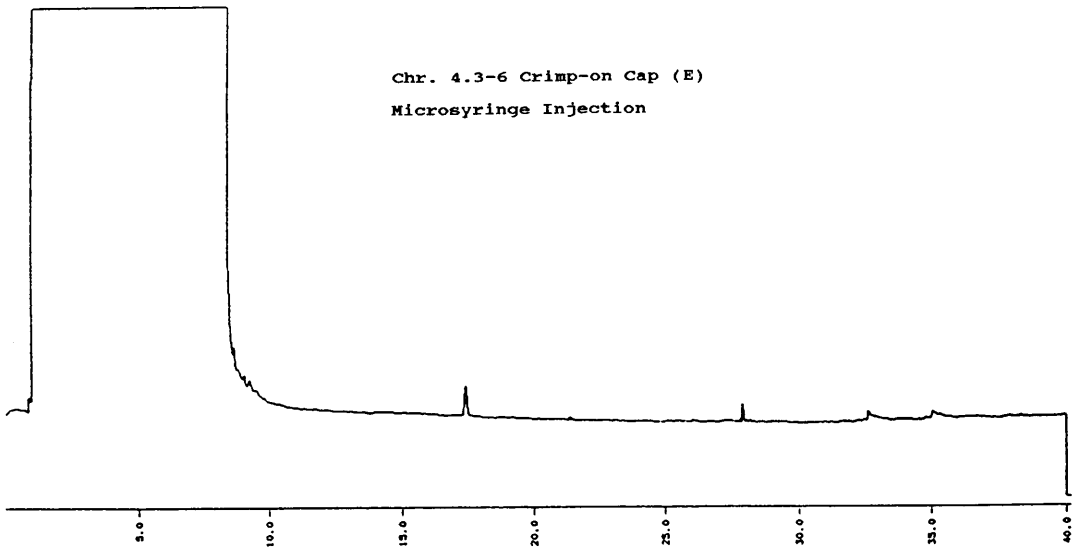
Chr. 4.3-2 Screw Cap (A)



Chr. 4.3-3 Screw Cap (B)



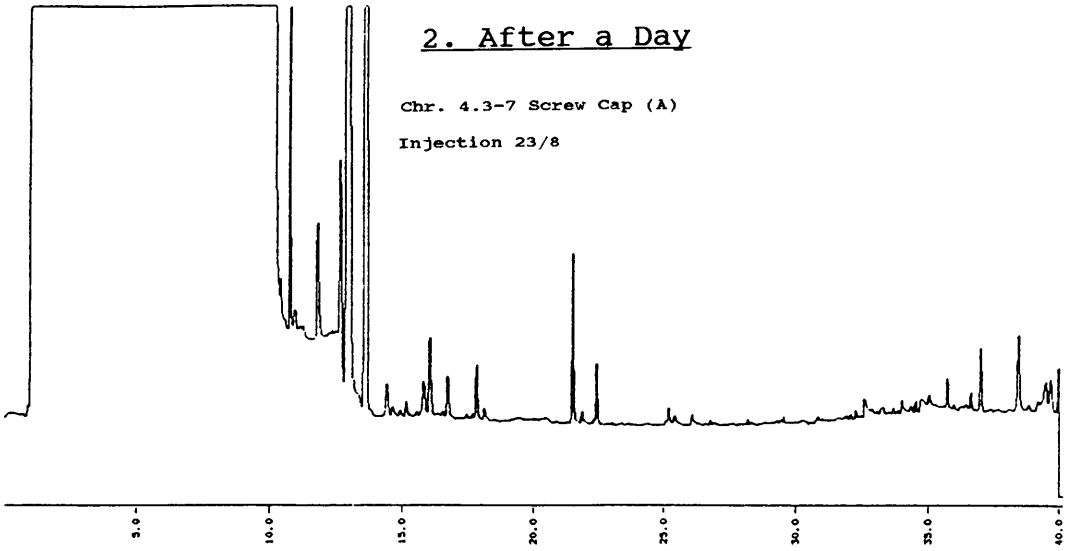




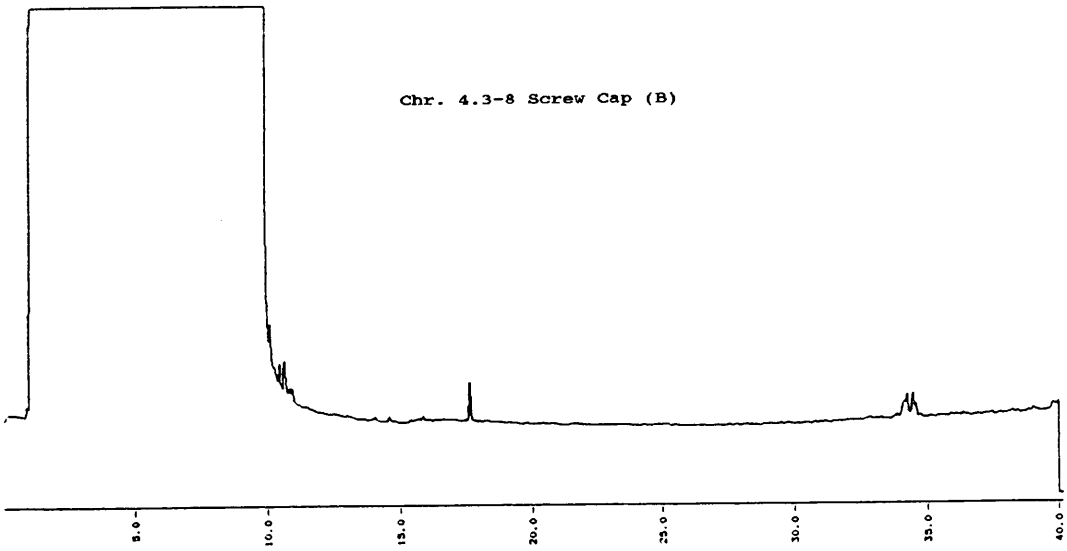
2. After a Day

Chr. 4.3-7 Screw Cap (A)

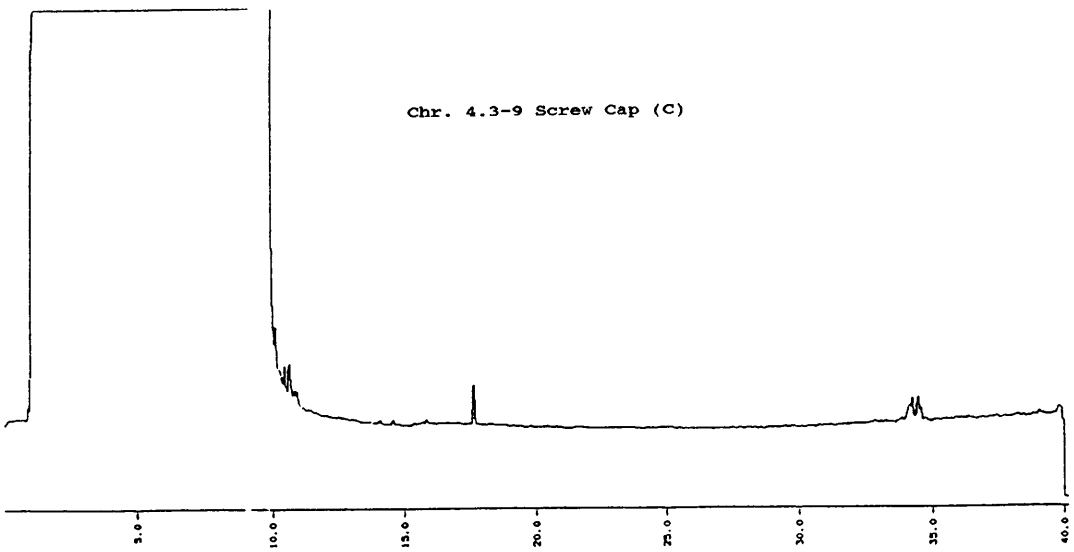
Injection 23/8

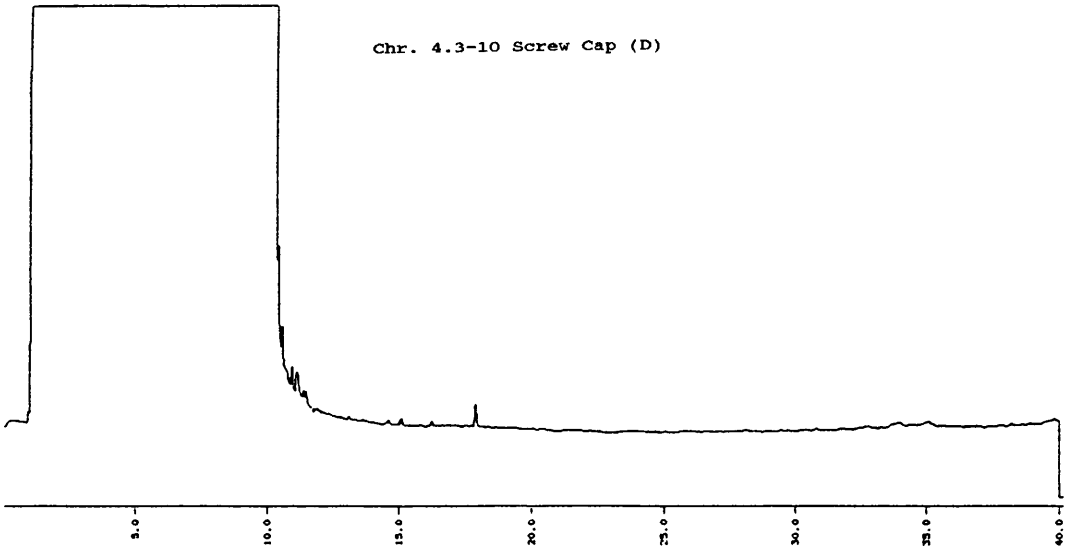


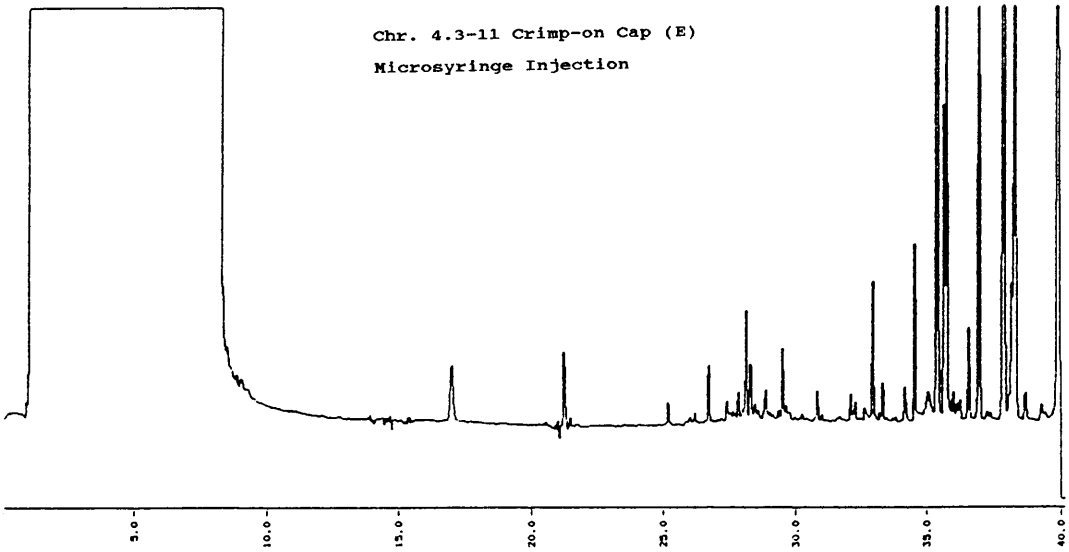
Chr. 4.3-8 Screw Cap (B)



Chr. 4.3-9 Screw Cap (C)

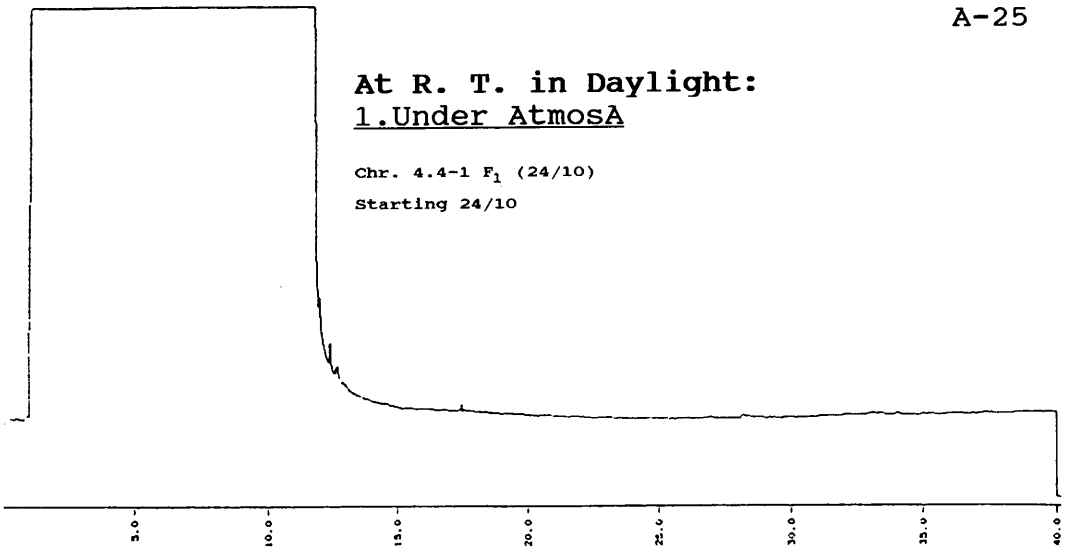




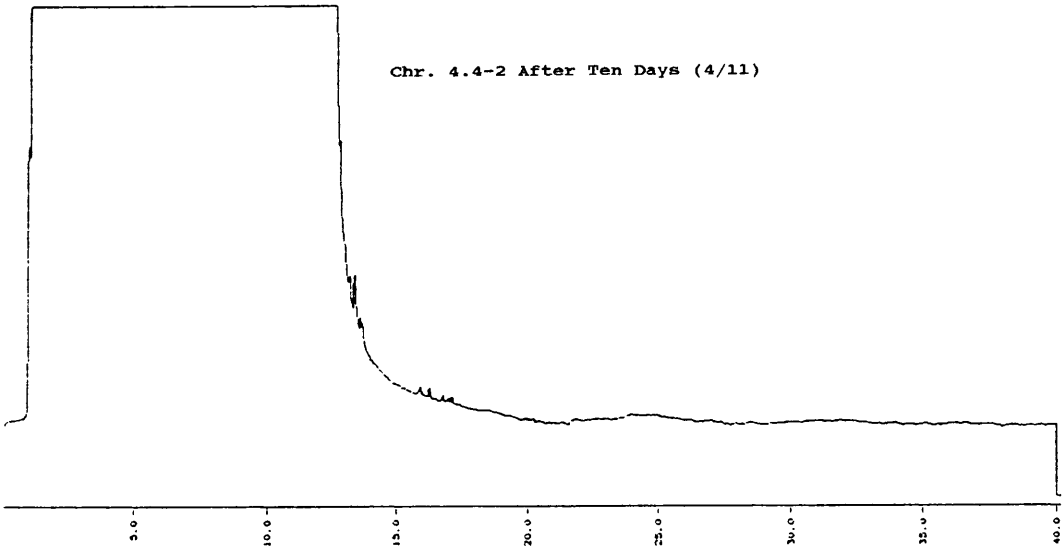


At R. T. in Daylight:
1.Under AtmosA

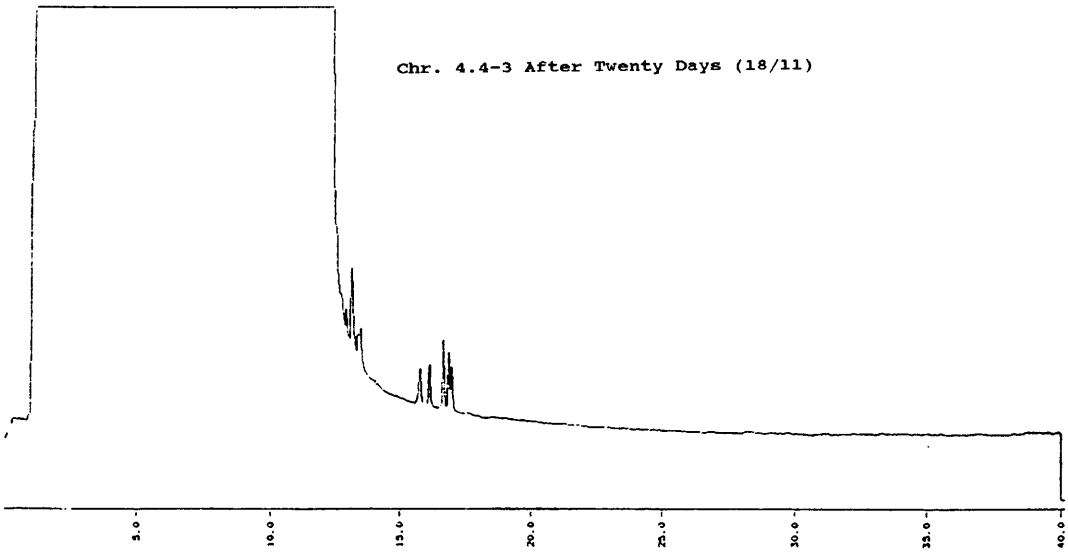
Chr. 4.4-1 F_1 (24/10)
Starting 24/10



Chr. 4.4-2 After Ten Days (4/11)

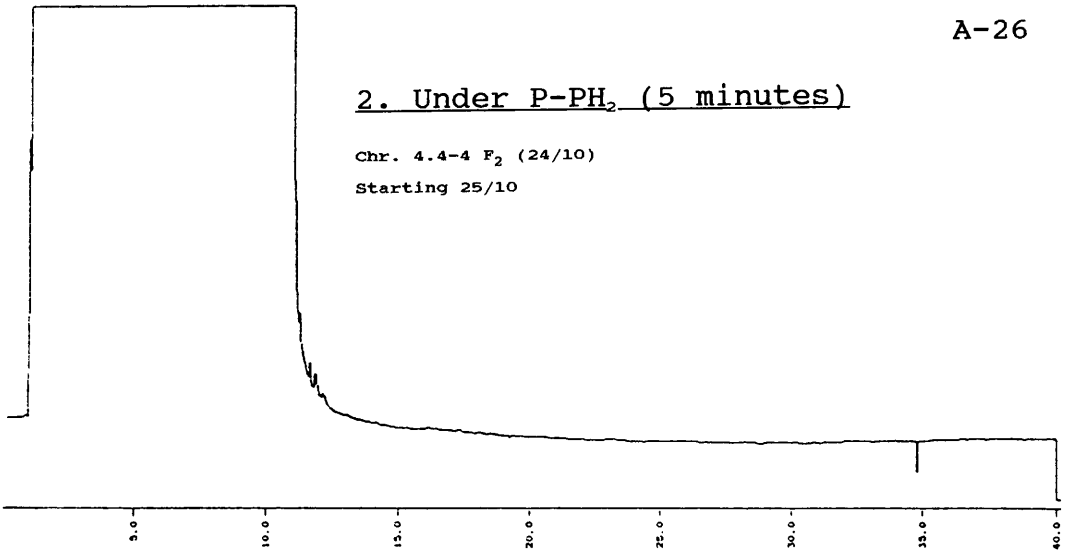


Chr. 4.4-3 After Twenty Days (18/11)

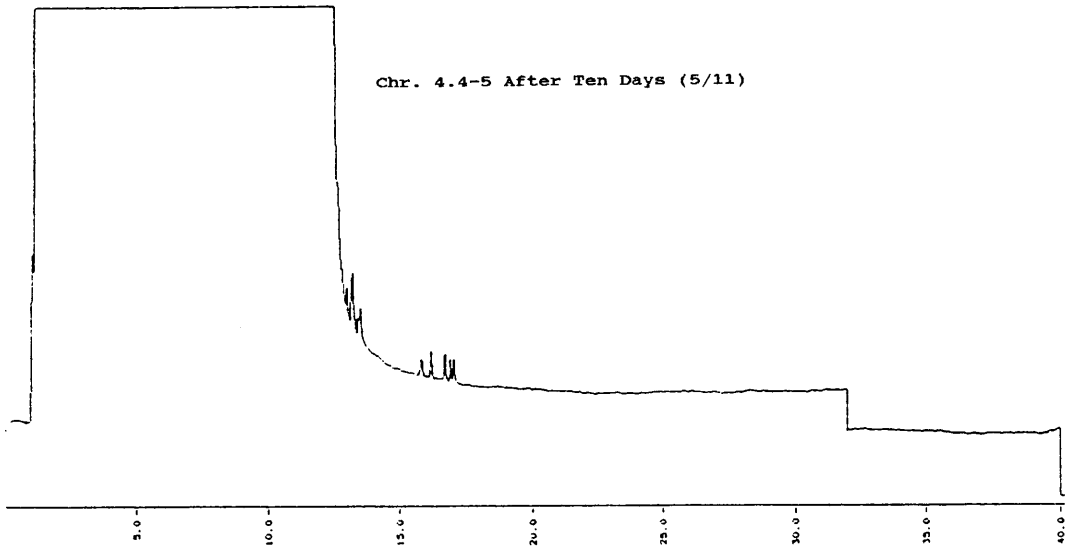


2. Under P-PH_2 (5 minutes)

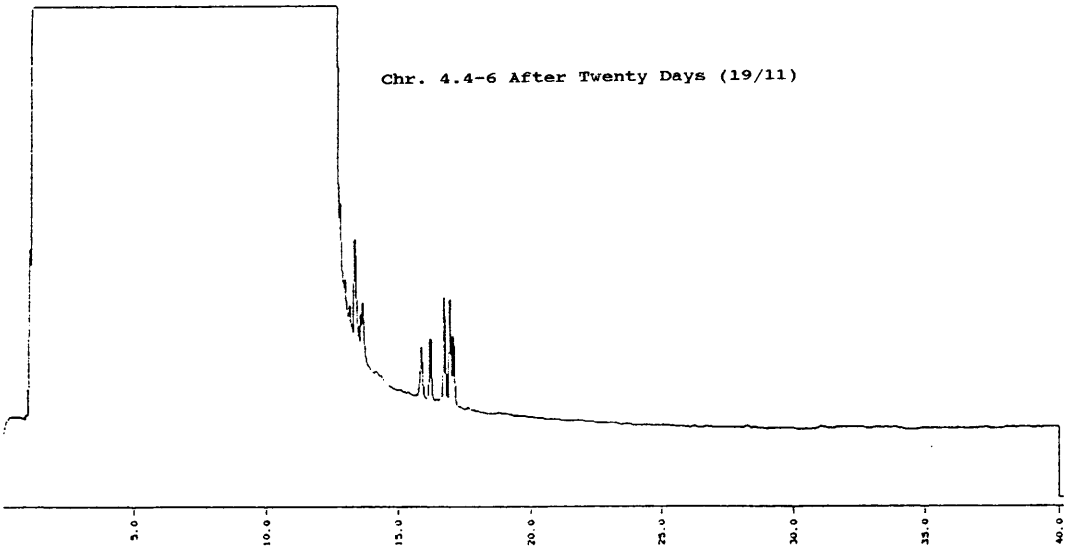
Chr. 4.4-4 F_2 (24/10)
Starting 25/10



Chr. 4.4-5 After Ten Days (5/11)

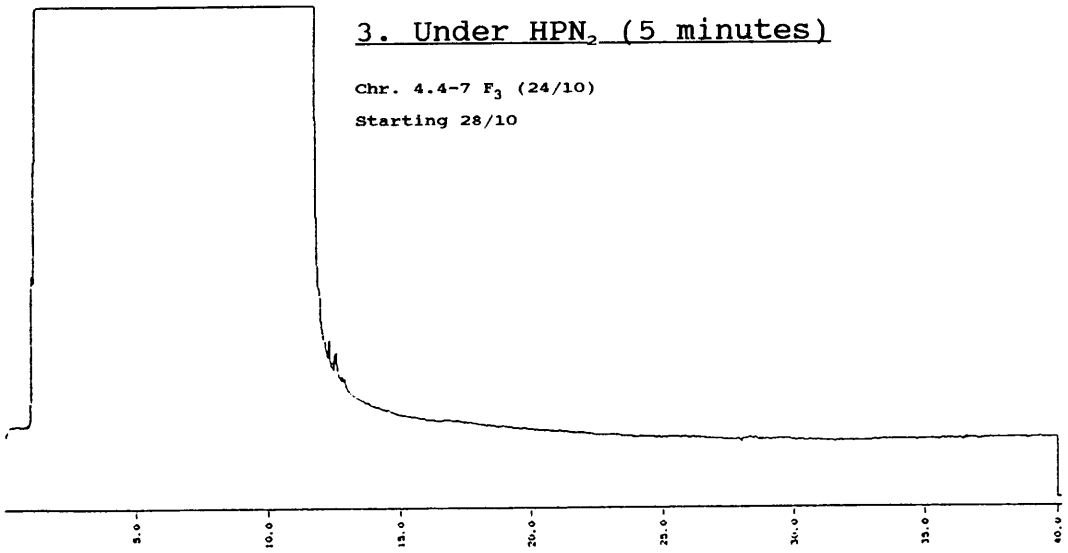


Chr. 4.4-6 After Twenty Days (19/11)

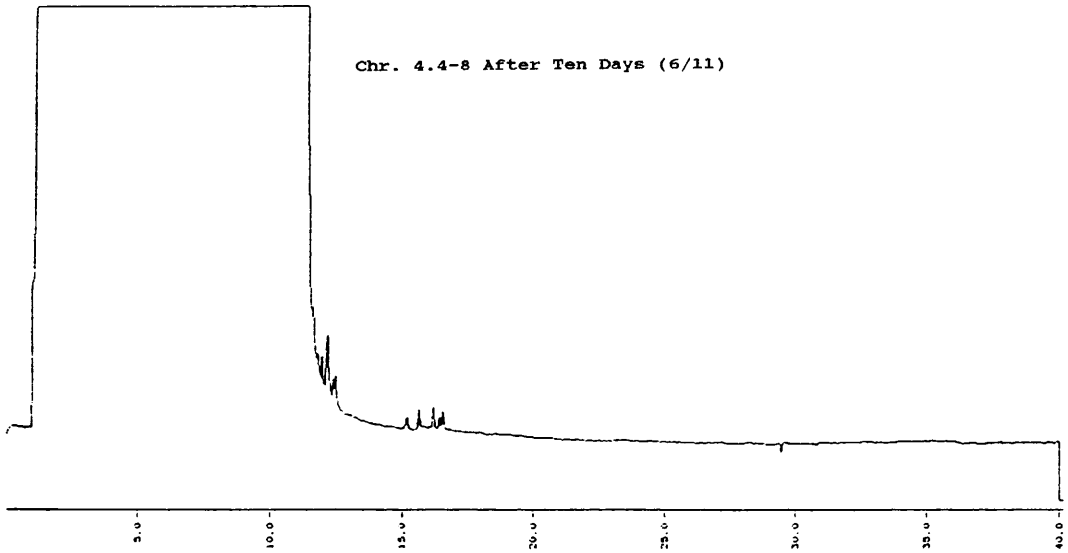


3. Under HPN_2 (5 minutes)

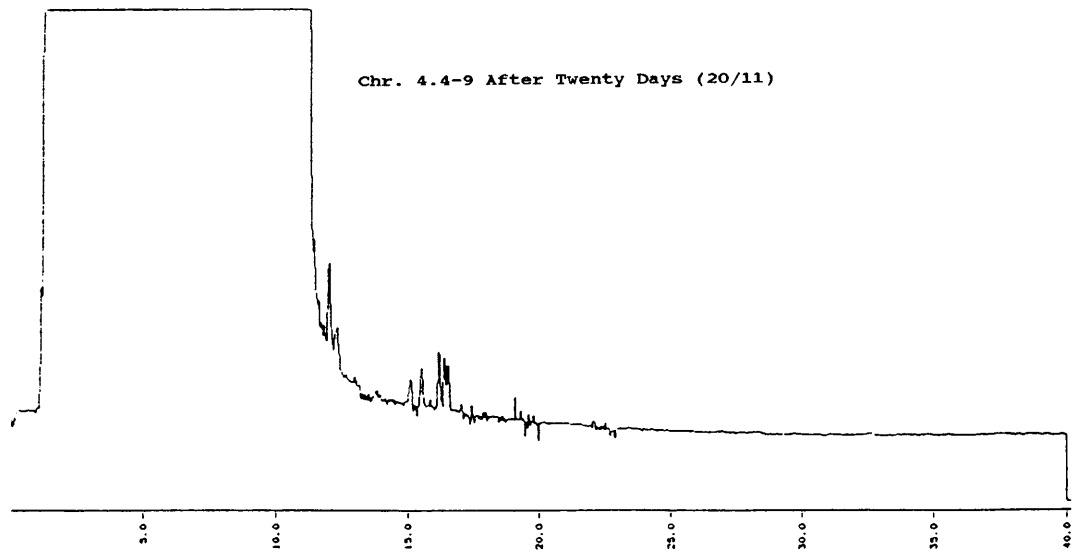
Chr. 4.4-7 F_3 (24/10)
Starting 28/10



Chr. 4.4-8 After Ten Days (6/11)

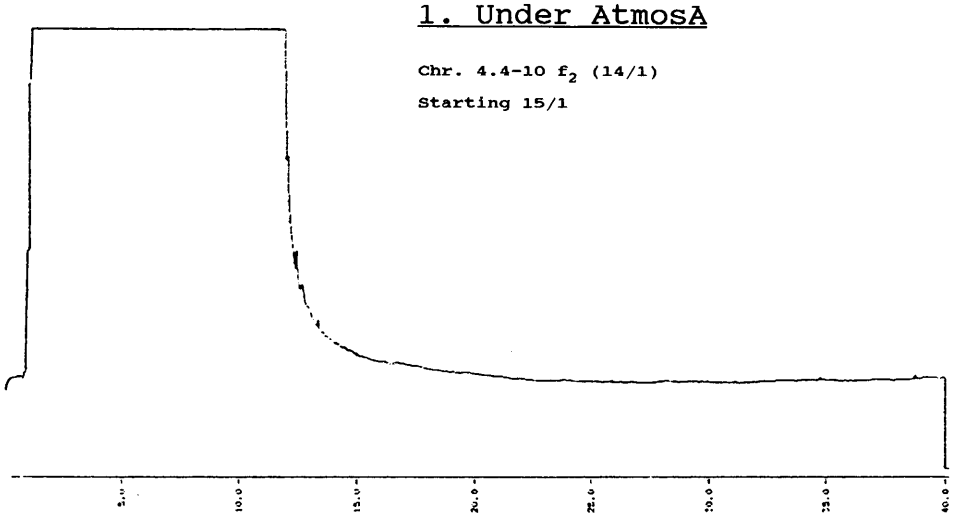


Chr. 4.4-9 After Twenty Days (20/11)

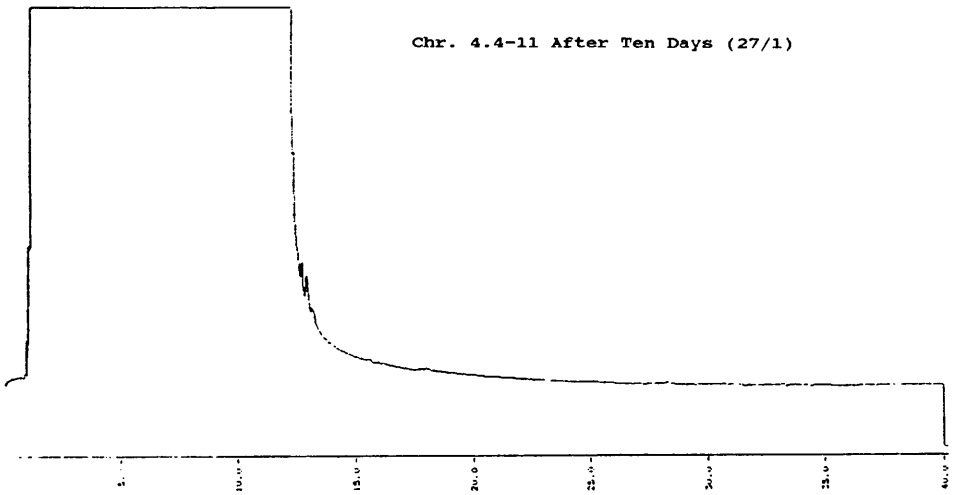


At R. T. in Darkness:
1. Under AtmosA

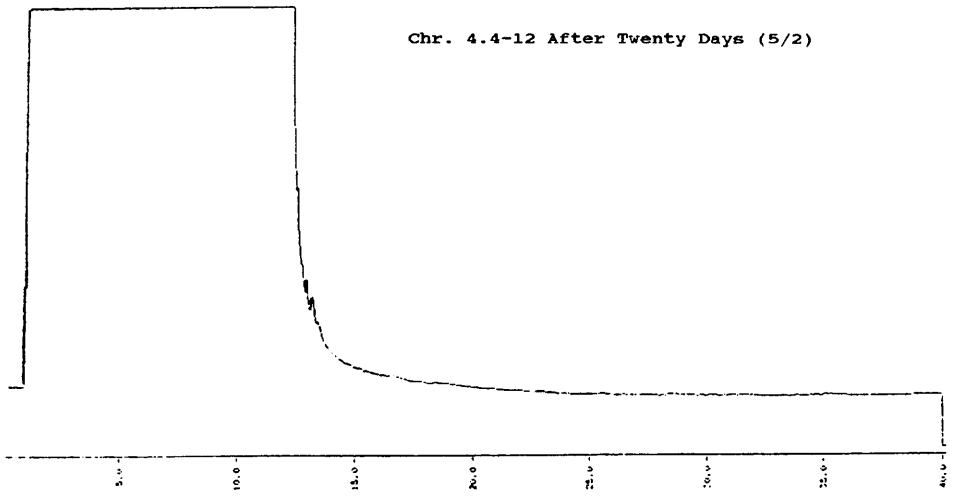
Chr. 4.4-10 f_2 (14/1)
Starting 15/1



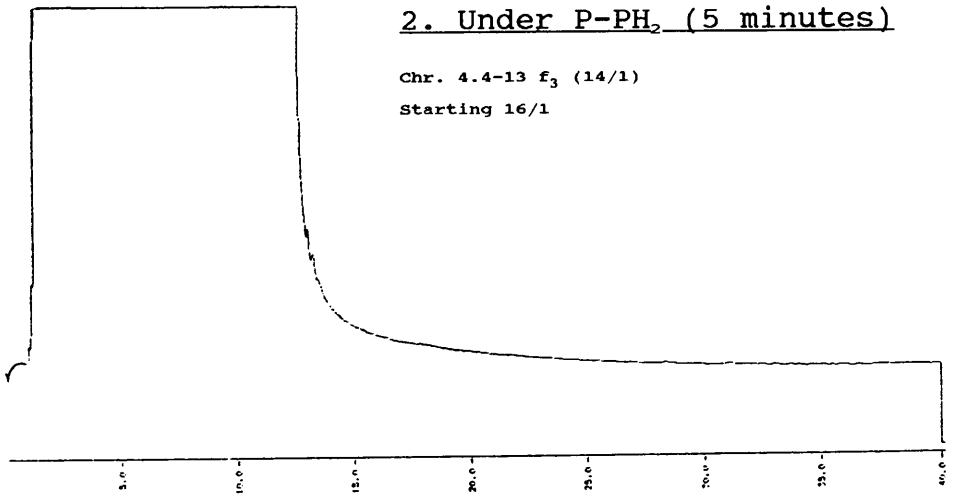
Chr. 4.4-11 After Ten Days (27/1)



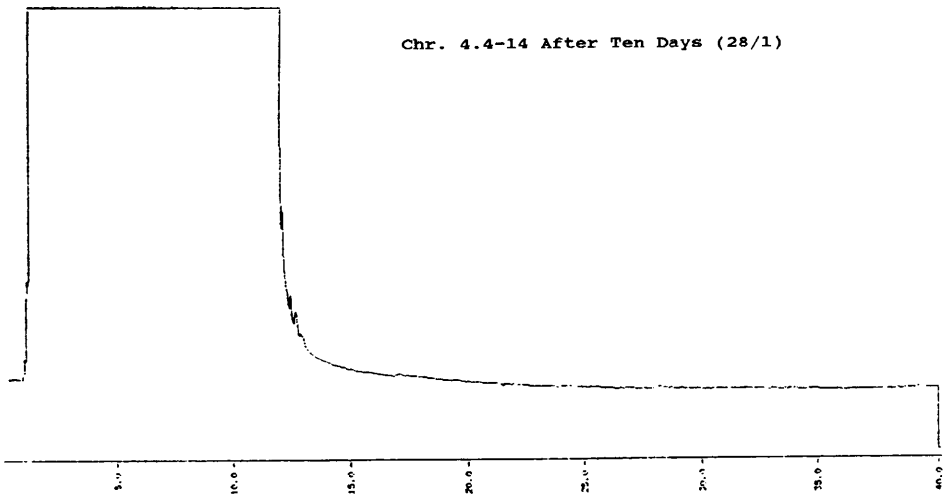
Chr. 4.4-12 After Twenty Days (5/2)



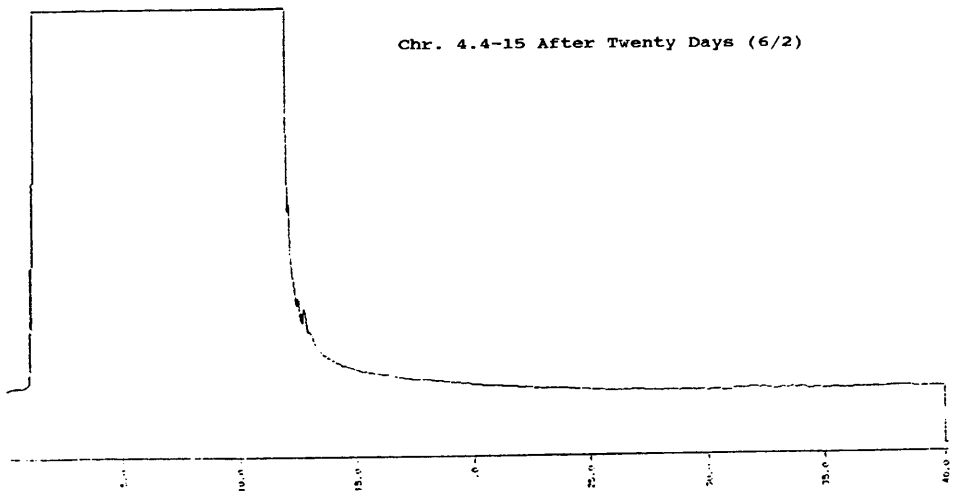
2. Under P-PH_2 (5 minutes)



Chr. 4.4-13 f_3 (14/1)
Starting 16/1



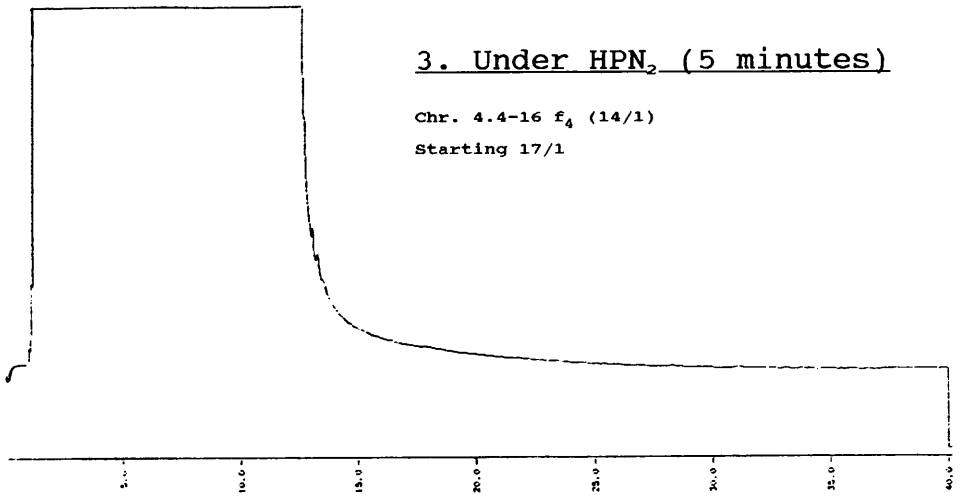
Chr. 4.4-14 After Ten Days (28/1)



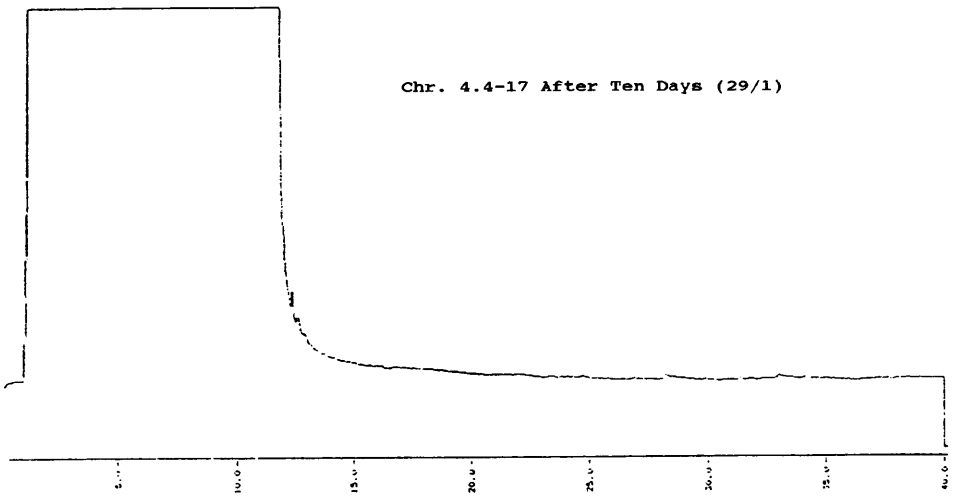
Chr. 4.4-15 After Twenty Days (6/2)

3. Under HPN_2 (5 minutes)

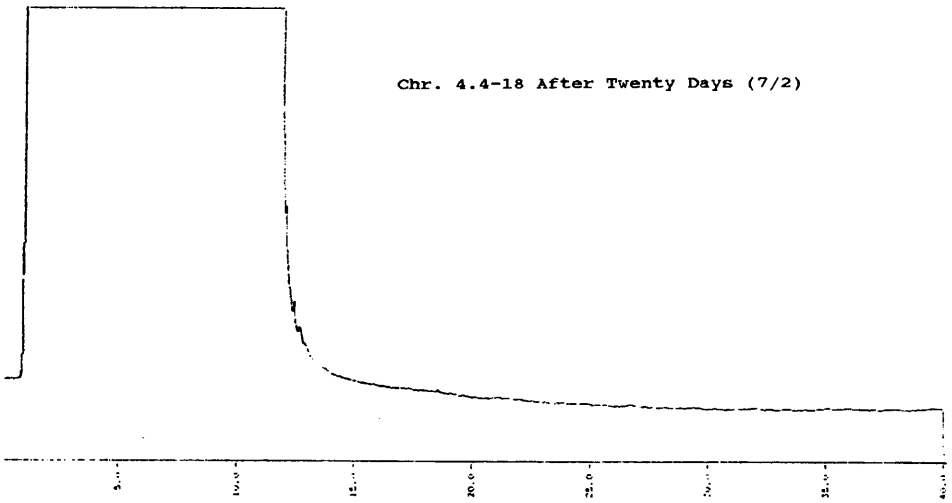
Chr. 4.4-16 f_4 (14/1)
Starting 17/1



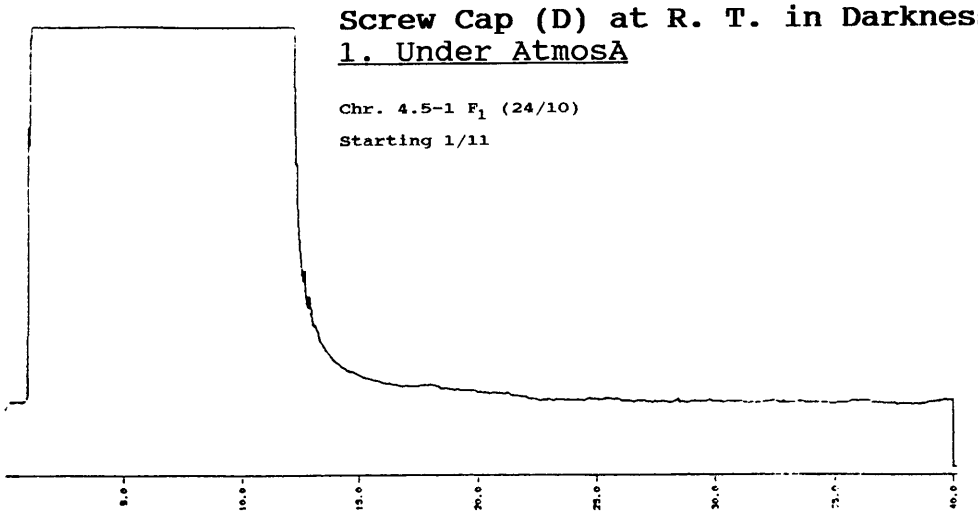
Chr. 4.4-17 After Ten Days (29/1)

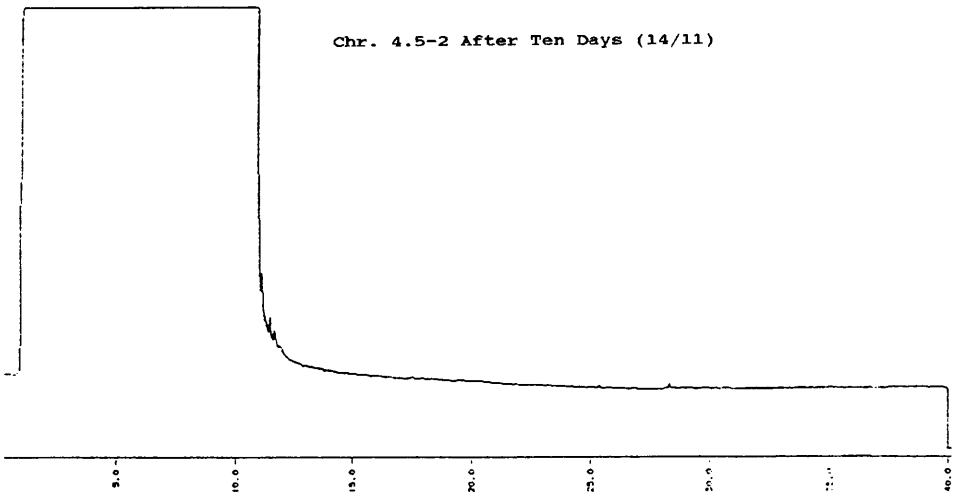


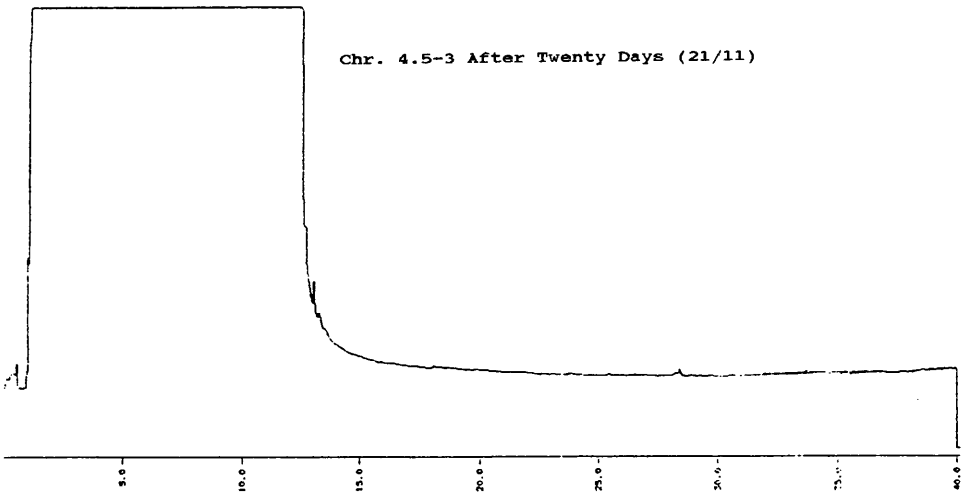
Chr. 4.4-18 After Twenty Days (7/2)



Screw Cap (D) at R. T. in Darkness:
1. Under AtmosA

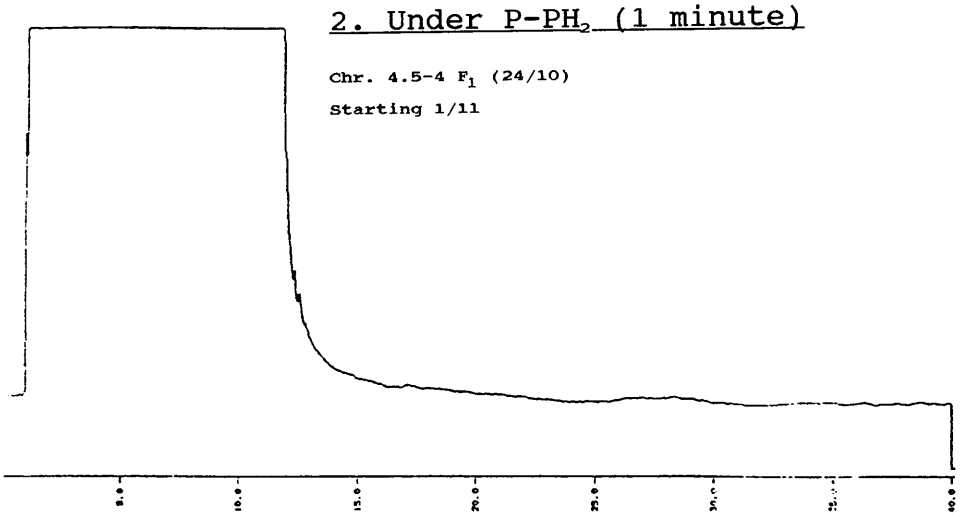




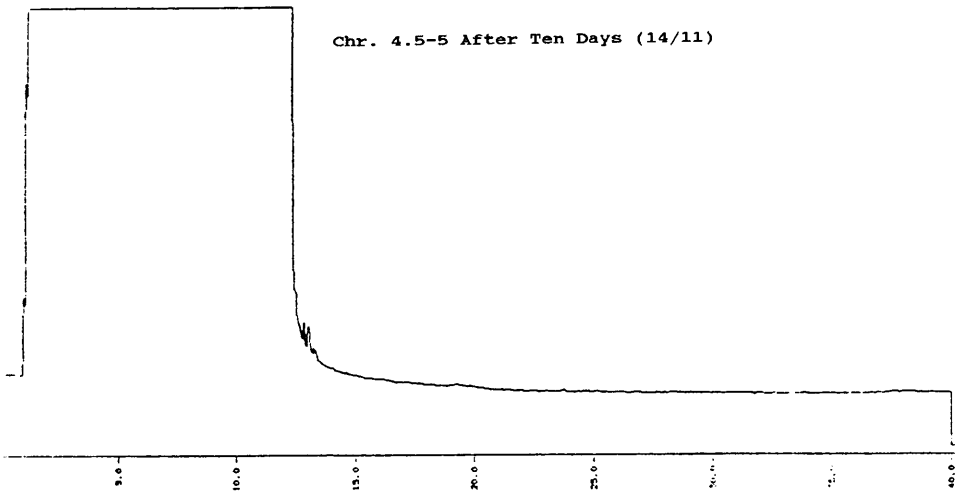


2. Under P-PH_2 (1 minute)

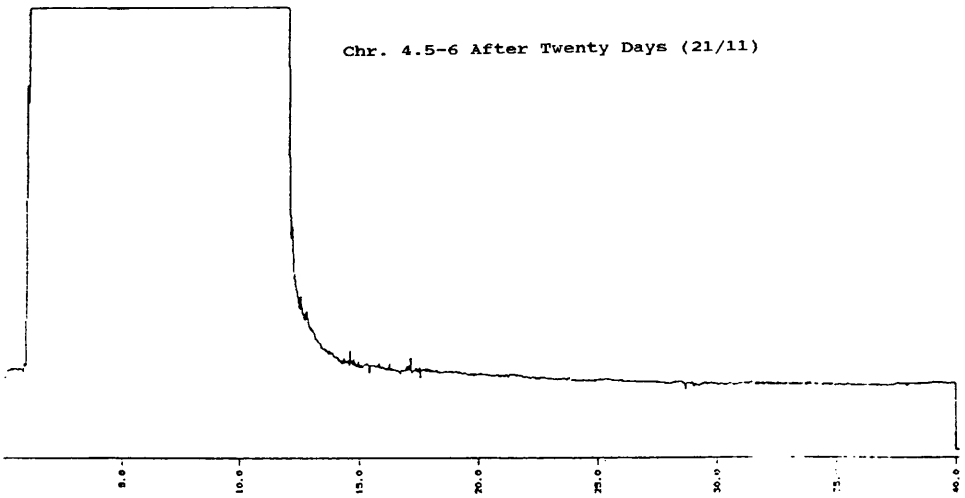
Chr. 4.5-4 F_1 (24/10)
Starting 1/11



Chr. 4.5-5 After Ten Days (14/11)

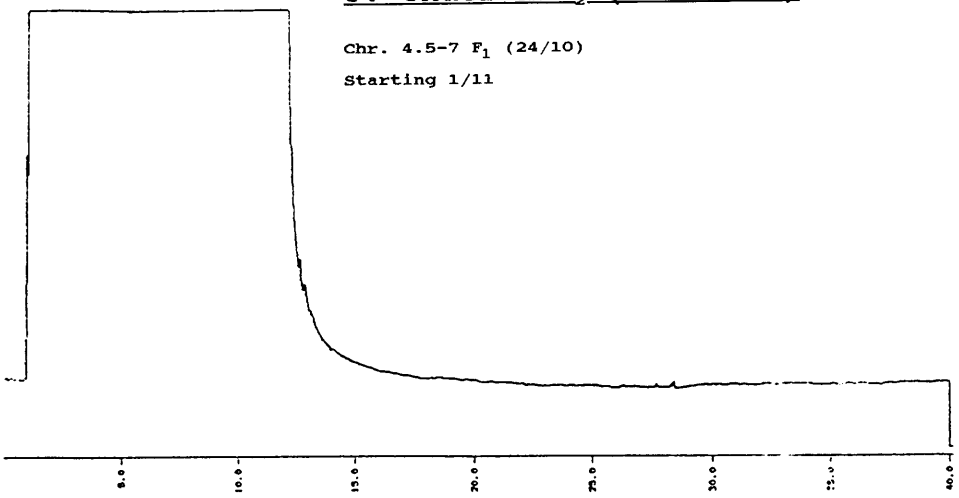


Chr. 4.5-6 After Twenty Days (21/11)

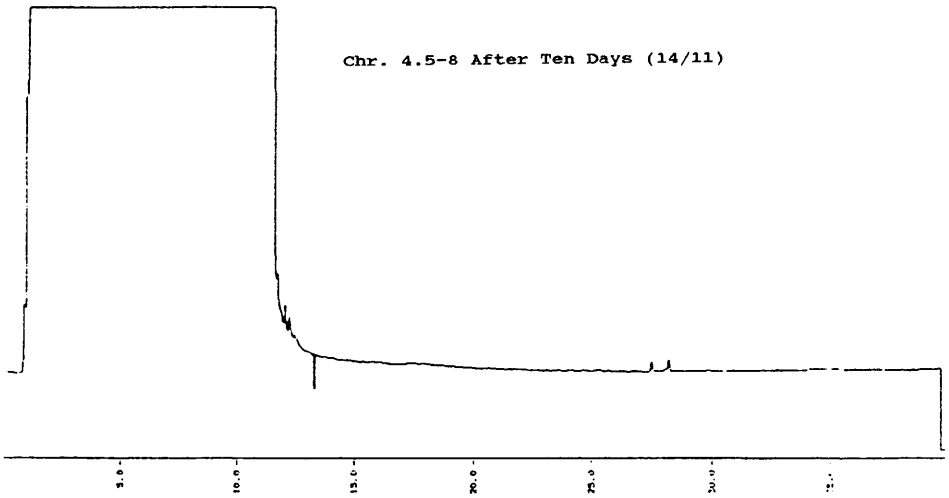


3. Under HPN_2 (1 minute)

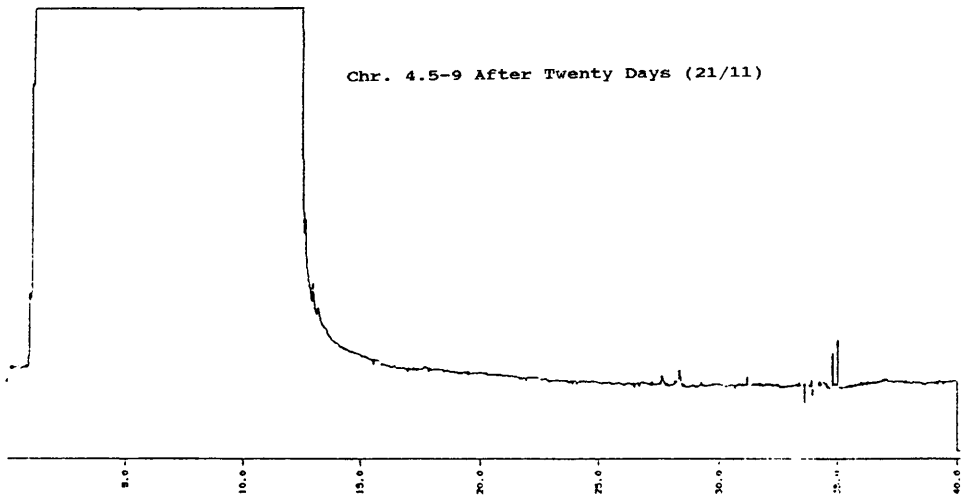
Chr. 4.5-7 F_1 (24/10)
Starting 1/11



Chr. 4.5-8 After Ten Days (14/11)

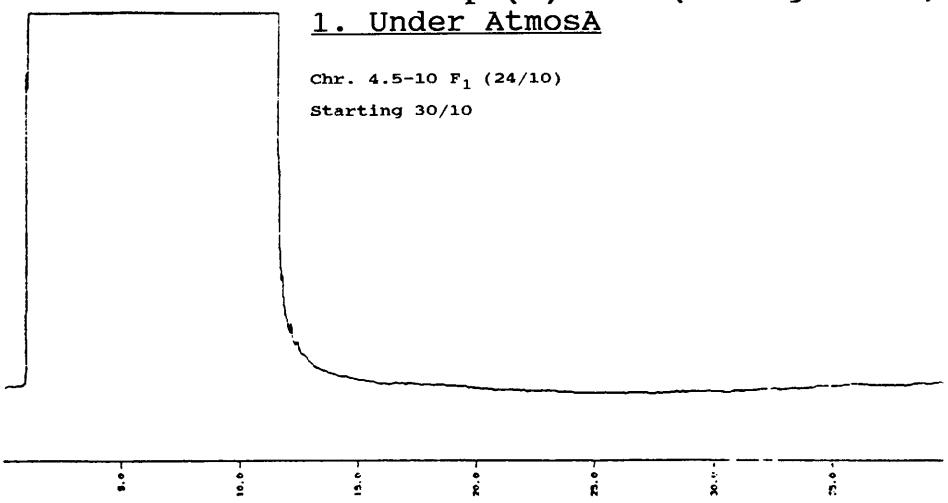


Chr. 4.5-9 After Twenty Days (21/11)

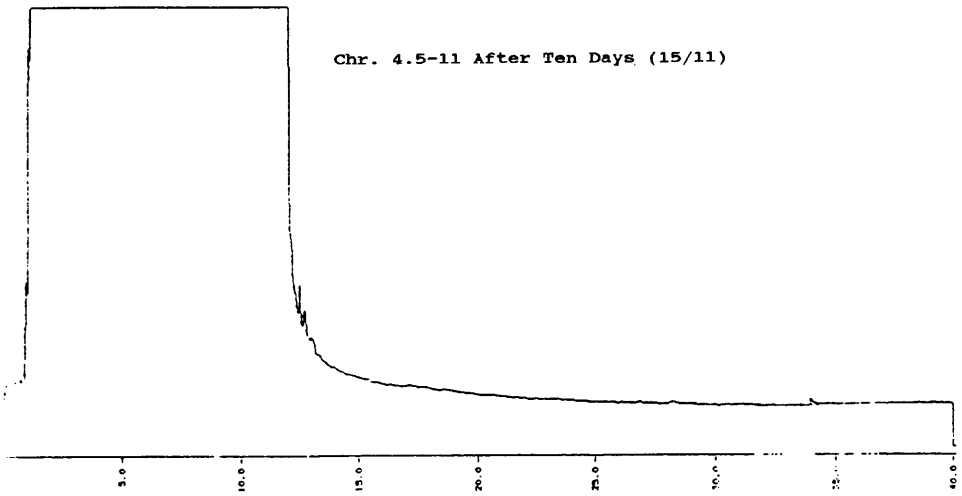


Screw Cap (D) 8 ℃ (Refrigerator):
1. Under AtmosA

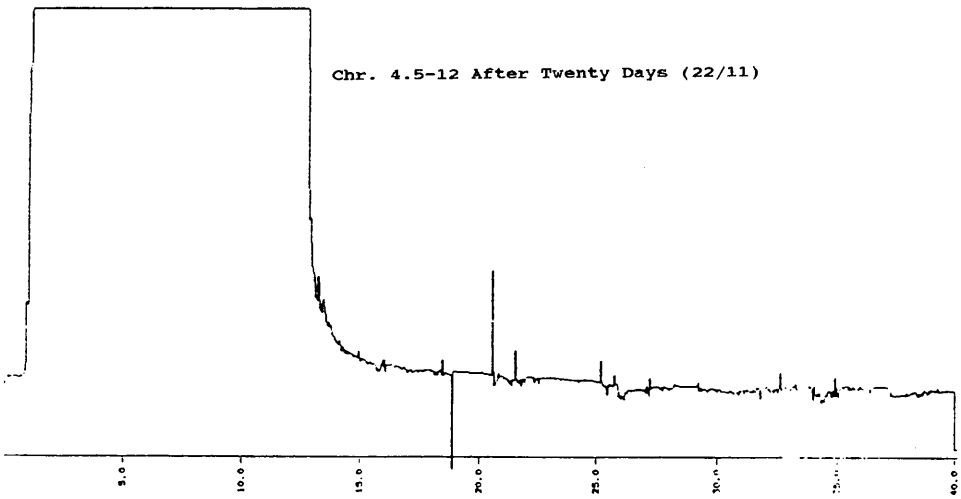
Chr. 4.5-10 F_1 (24/10)
Starting 30/10



Chr. 4.5-11 After Ten Days (15/11)

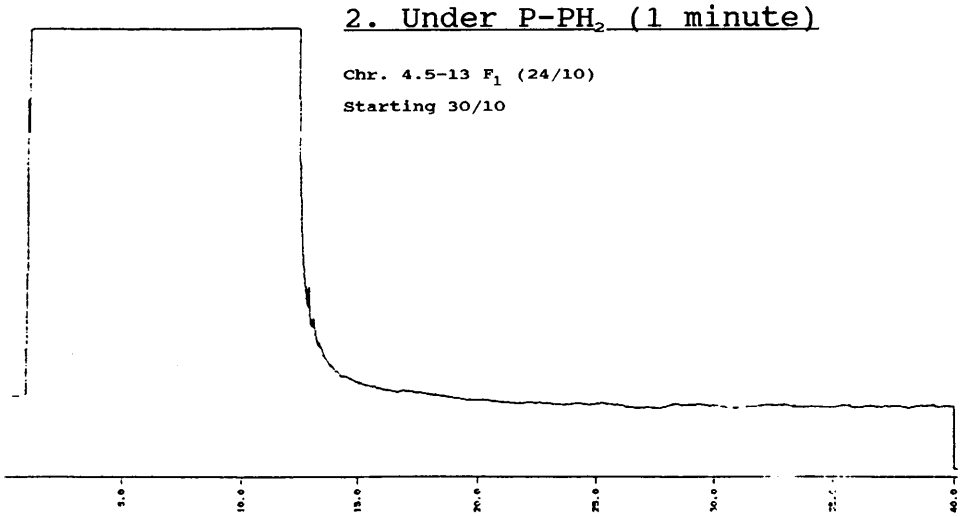


Chr. 4.5-12 After Twenty Days (22/11)

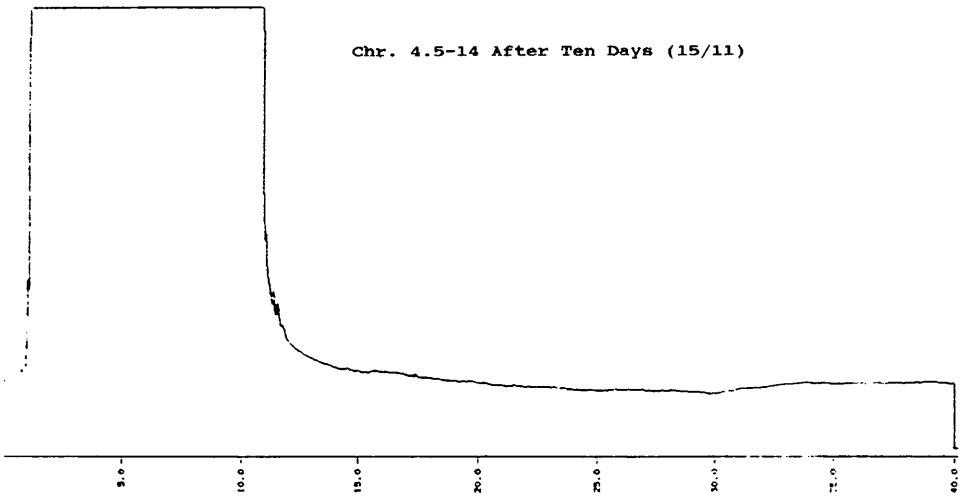


2. Under P-PH_2 (1 minute)

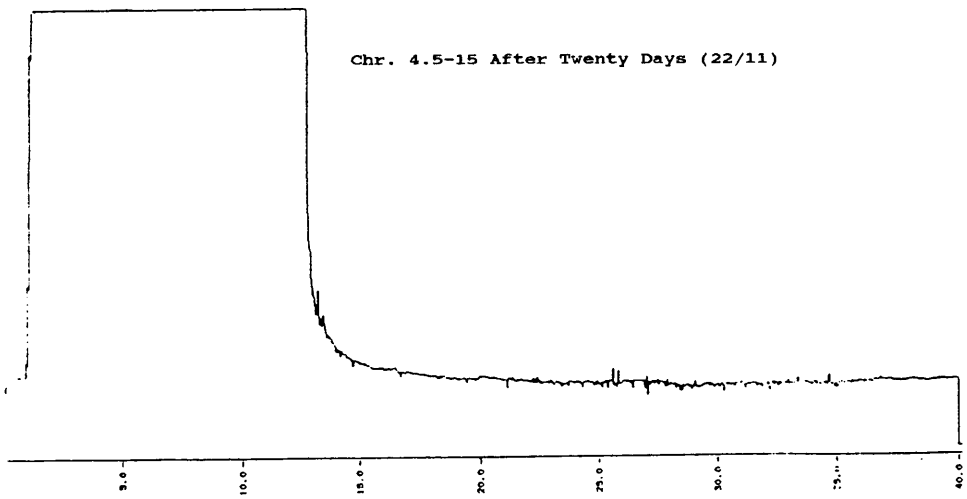
Chr. 4.5-13 F_1 (24/10)
Starting 30/10



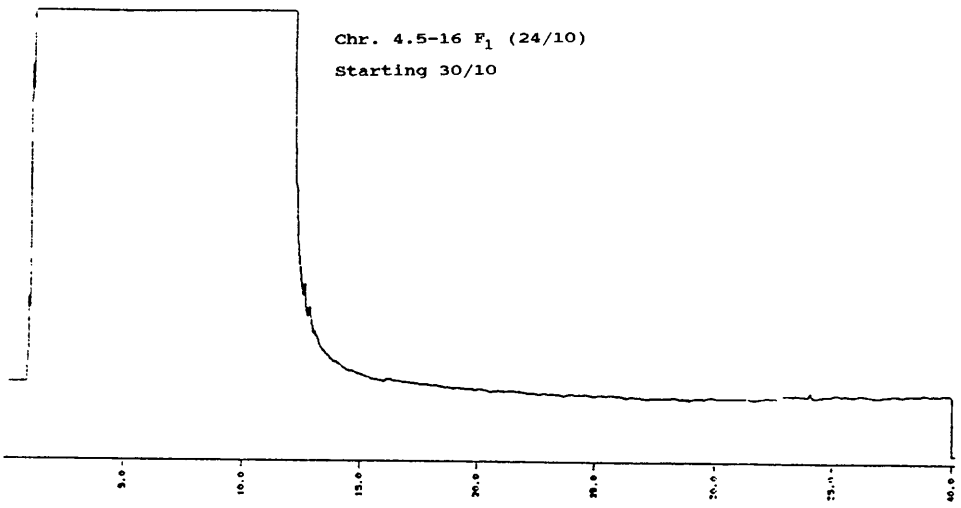
Chr. 4.5-14 After Ten Days (15/11)

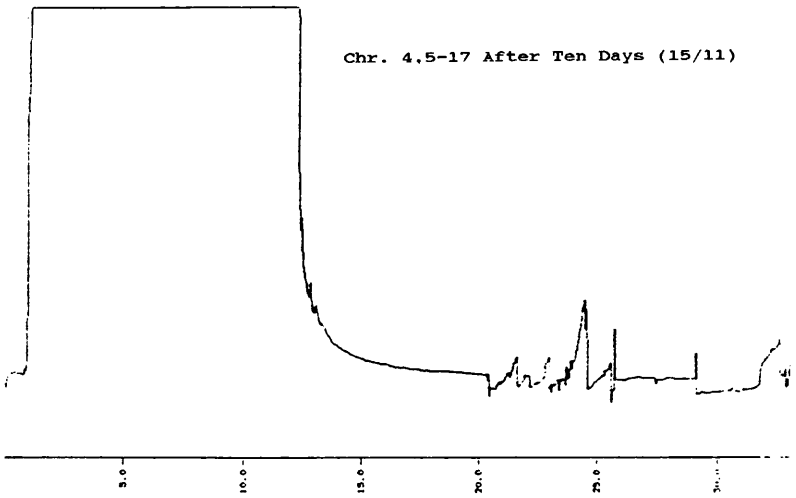


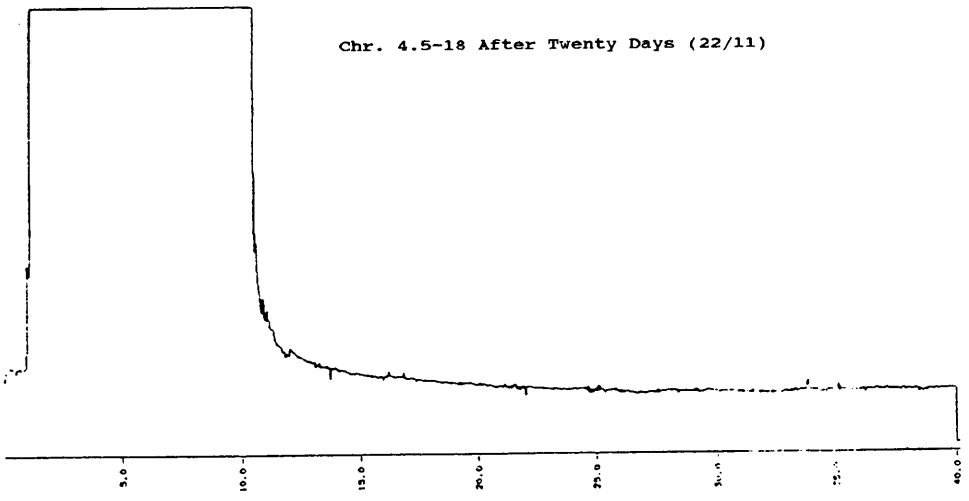
Chr. 4.5-15 After Twenty Days (22/11)

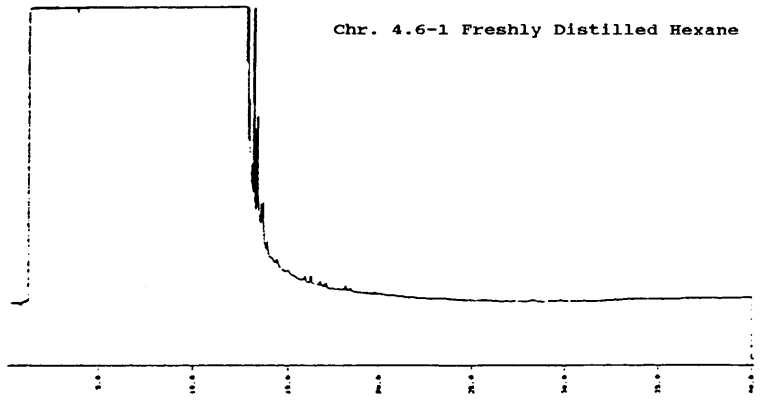


3. Under HPN_2 (1 minute)

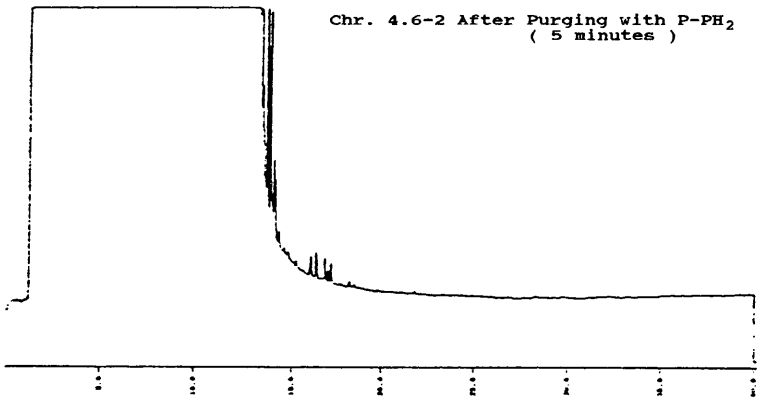




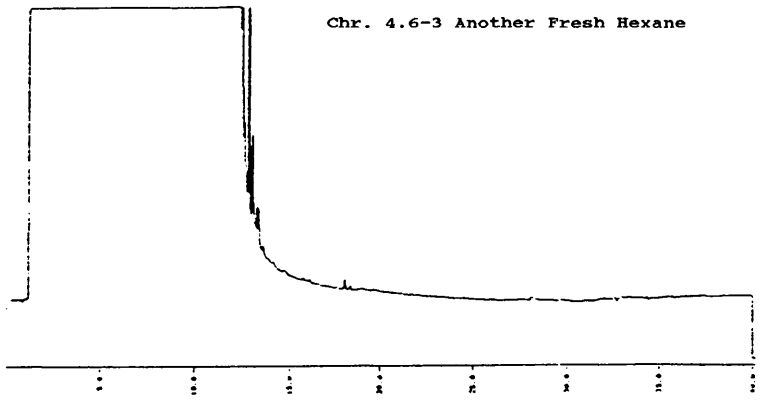




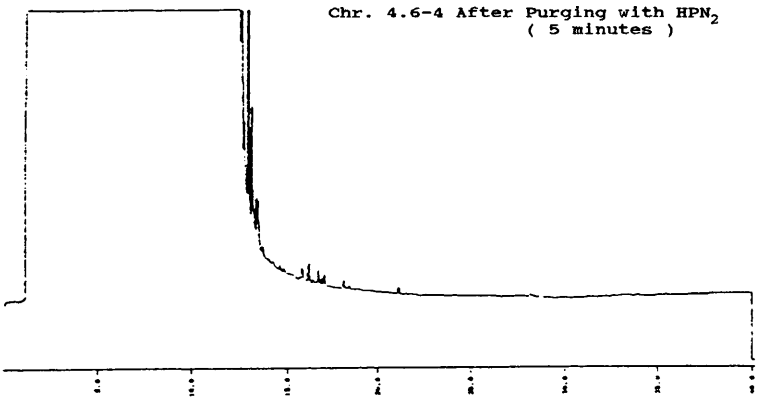
Chr. 4.6-1 Freshly Distilled Hexane



Chr. 4.6-2 After Purging with $P-PH_2$
(5 minutes)



Chr. 4.6-3 Another Fresh Hexane

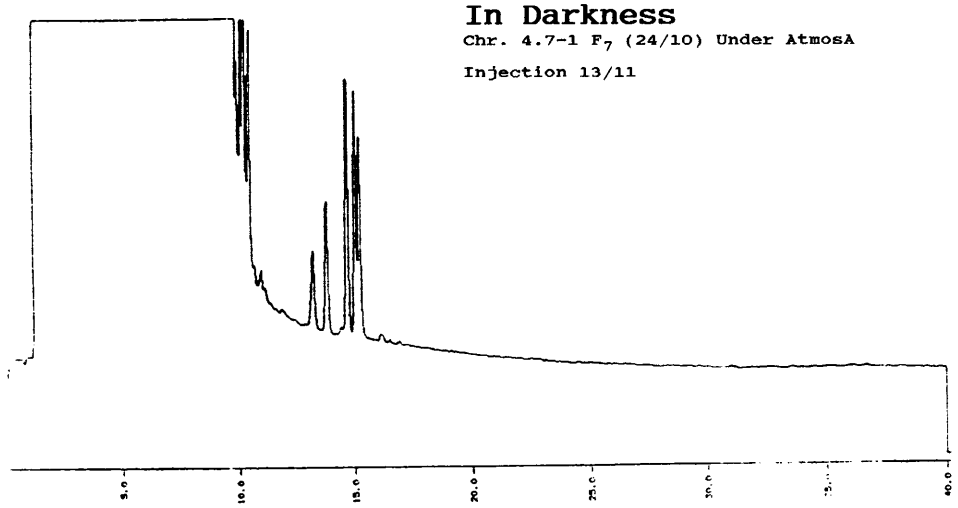


Chr. 4.6-4 After Purging with HPN_2
(5 minutes)

In Darkness

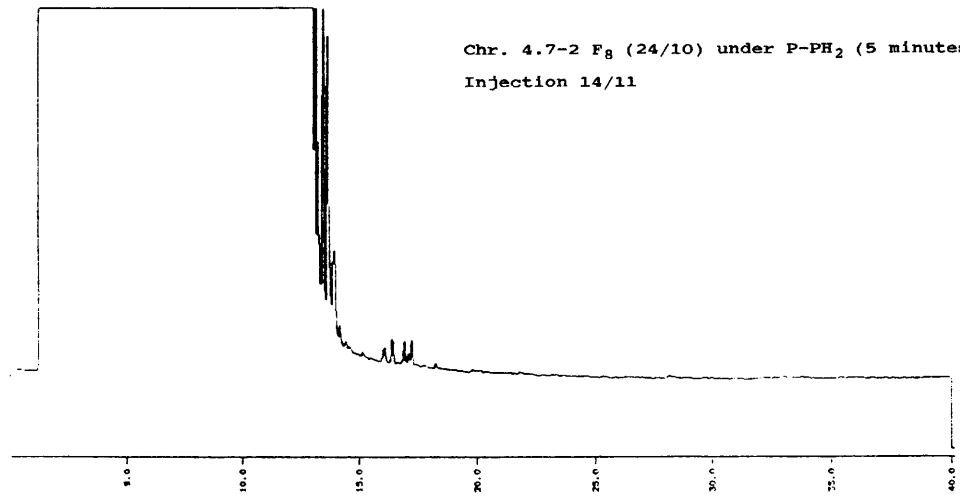
Chr. 4.7-1 F_7 (24/10) Under AtmosA

Injection 13/11



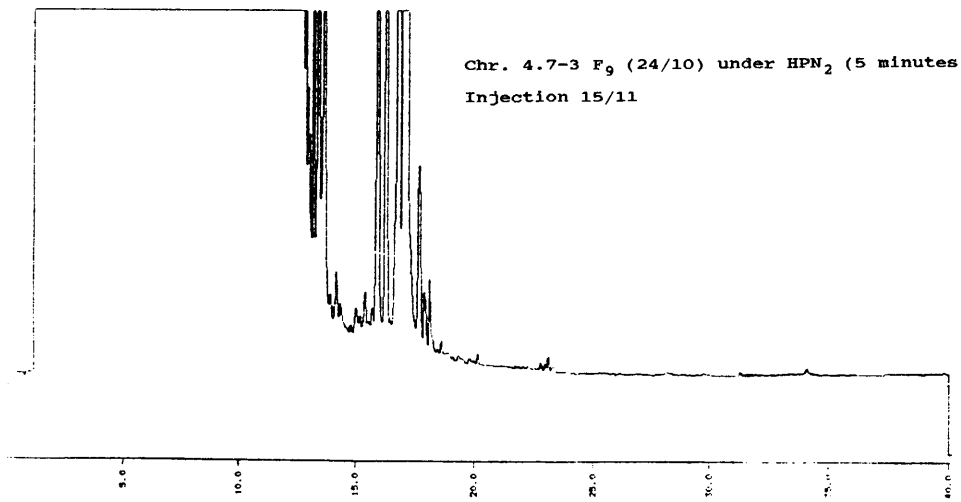
Chr. 4.7-2 F_8 (24/10) under P-PH_2 (5 minutes)

Injection 14/11



Chr. 4.7-3 F_9 (24/10) under HPN_2 (5 minutes)

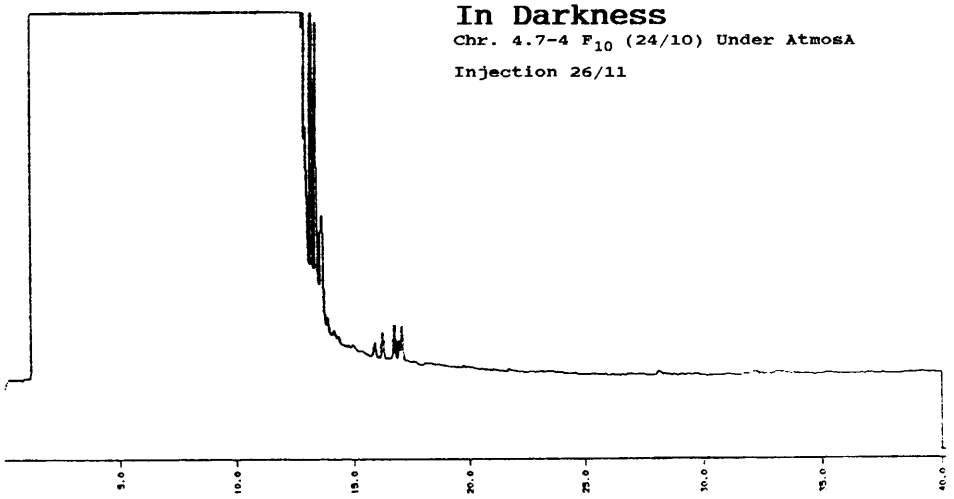
Injection 15/11



In Darkness

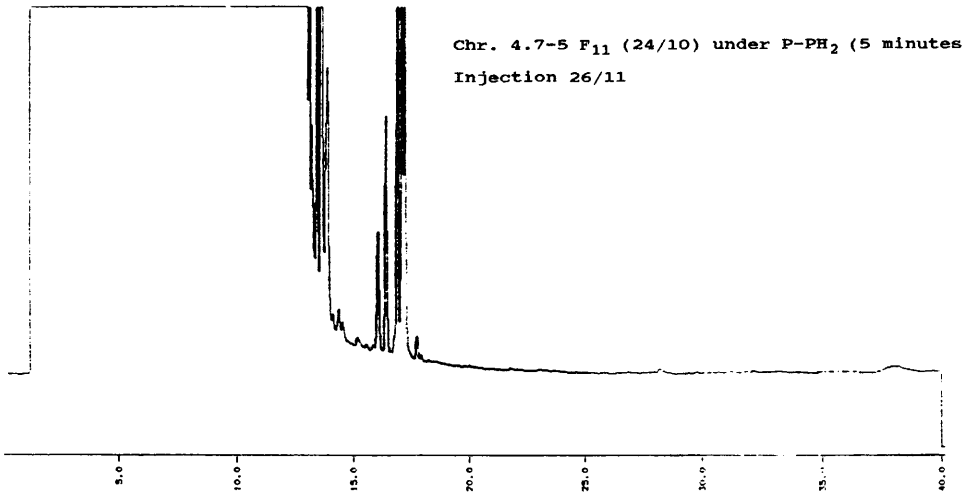
Chr. 4.7-4 F_{10} (24/10) Under AtmosA

Injection 26/11



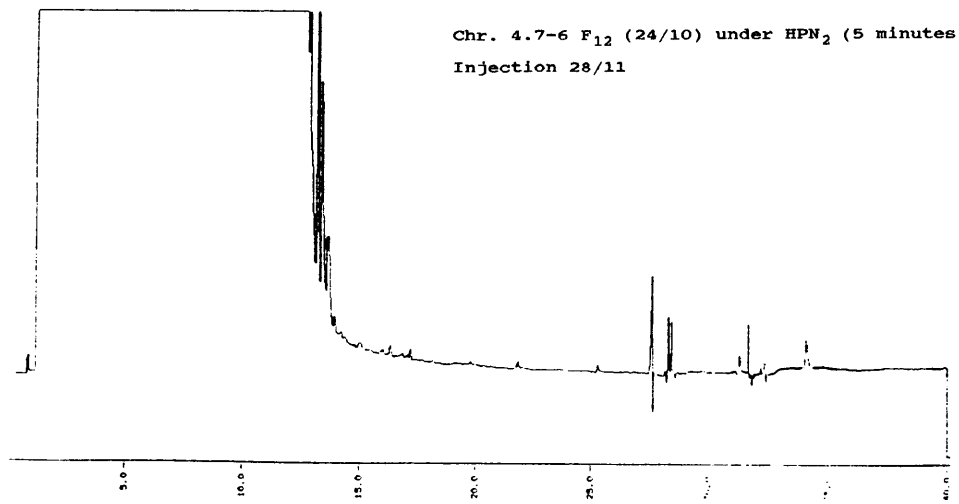
Chr. 4.7-5 F_{11} (24/10) under $P\text{-}PH_2$ (5 minutes)

Injection 26/11



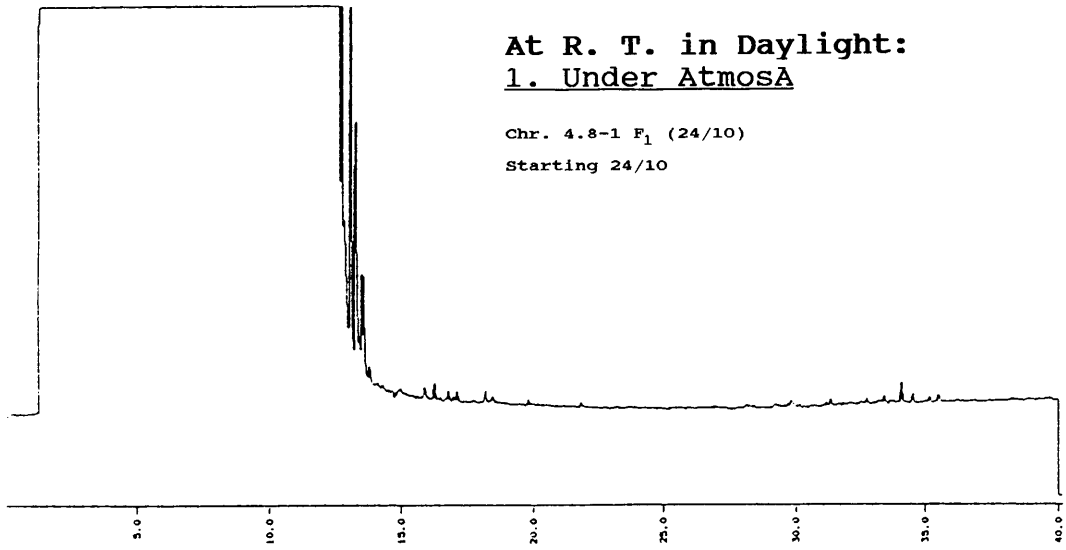
Chr. 4.7-6 F_{12} (24/10) under HPN_2 (5 minutes)

Injection 28/11

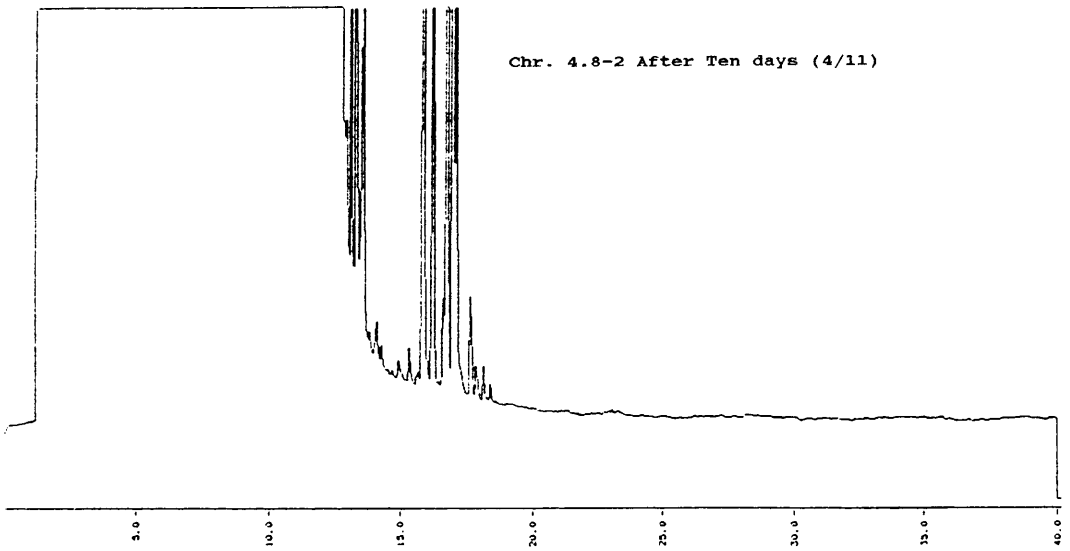


At R. T. in Daylight:
1. Under AtmosA

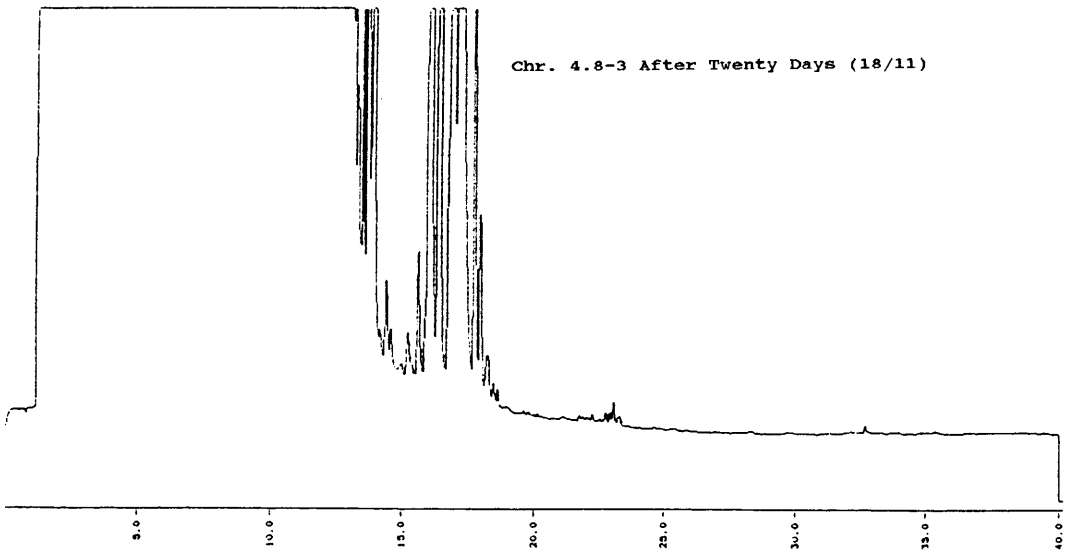
Chr. 4.8-1 F_1 (24/10)
Starting 24/10



Chr. 4.8-2 After Ten days (4/11)

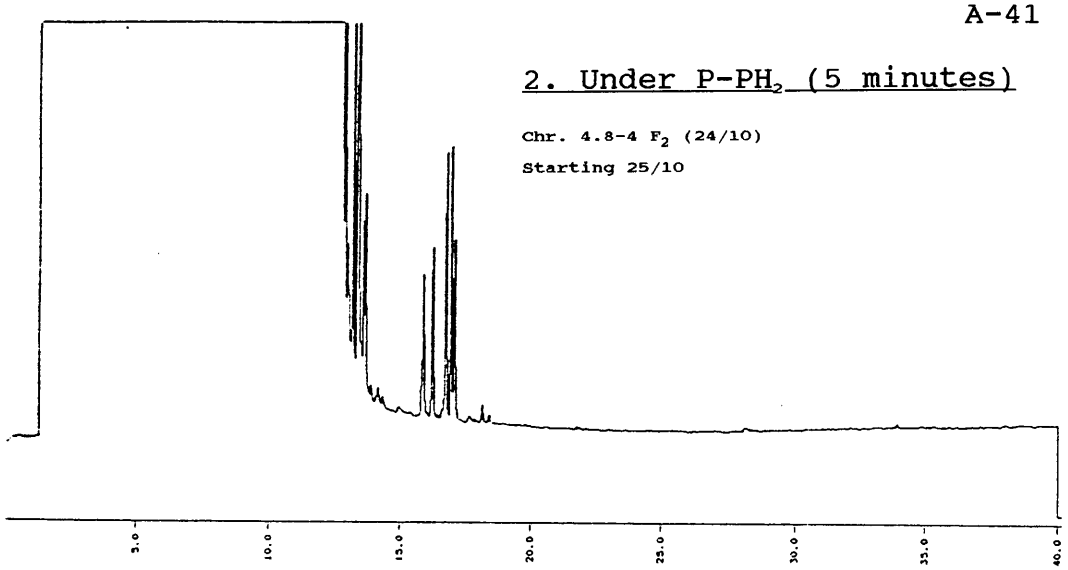


Chr. 4.8-3 After Twenty Days (18/11)

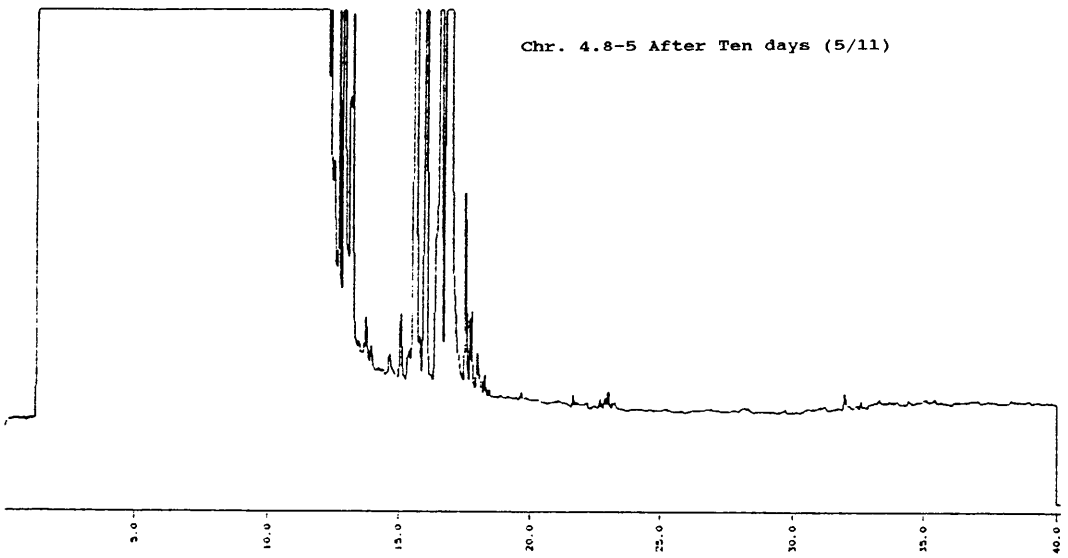


2. Under P-PH_2 (5 minutes)

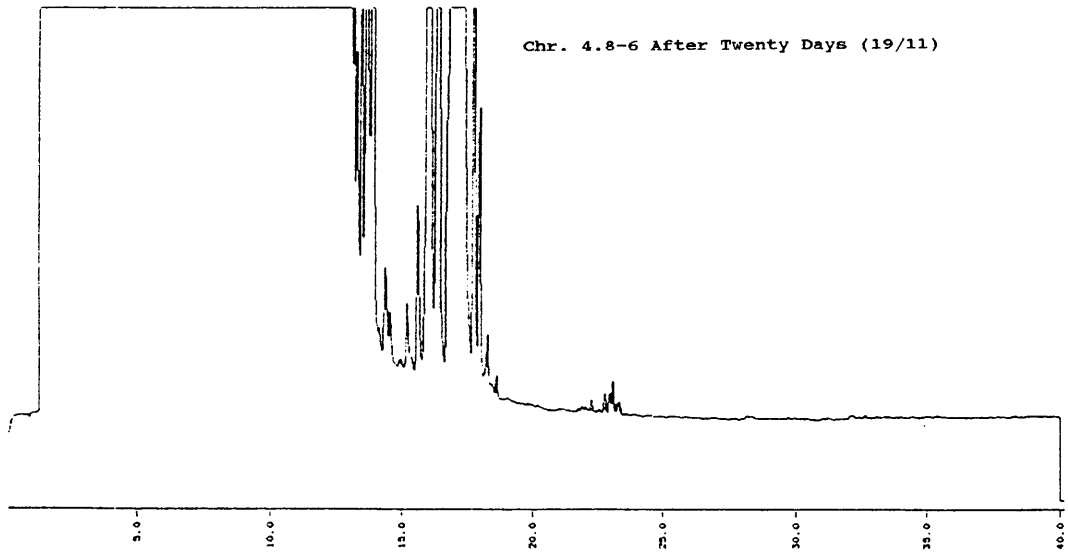
Chr. 4.8-4 F_2 (24/10)
Starting 25/10



Chr. 4.8-5 After Ten days (5/11)

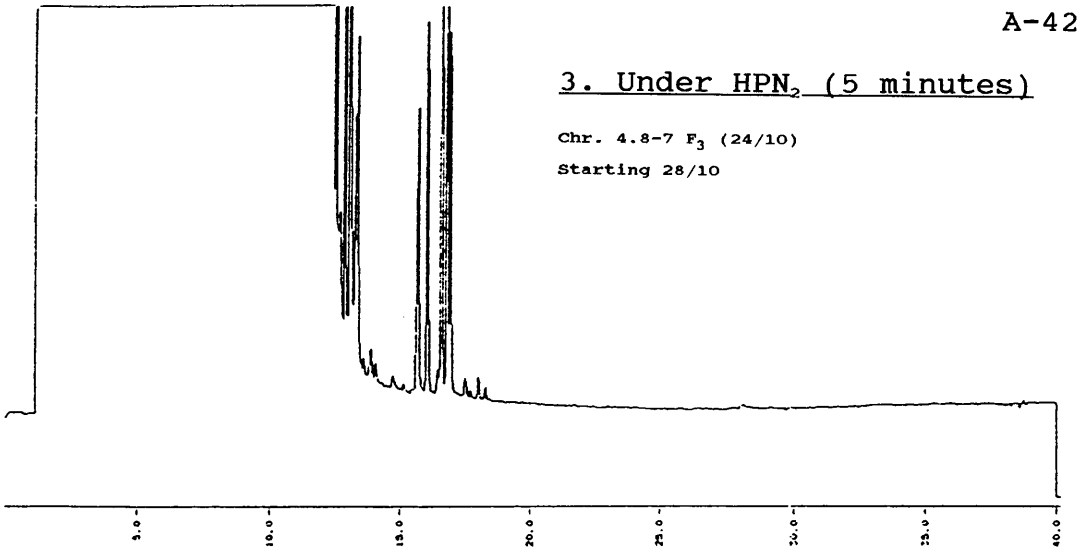


Chr. 4.8-6 After Twenty Days (19/11)

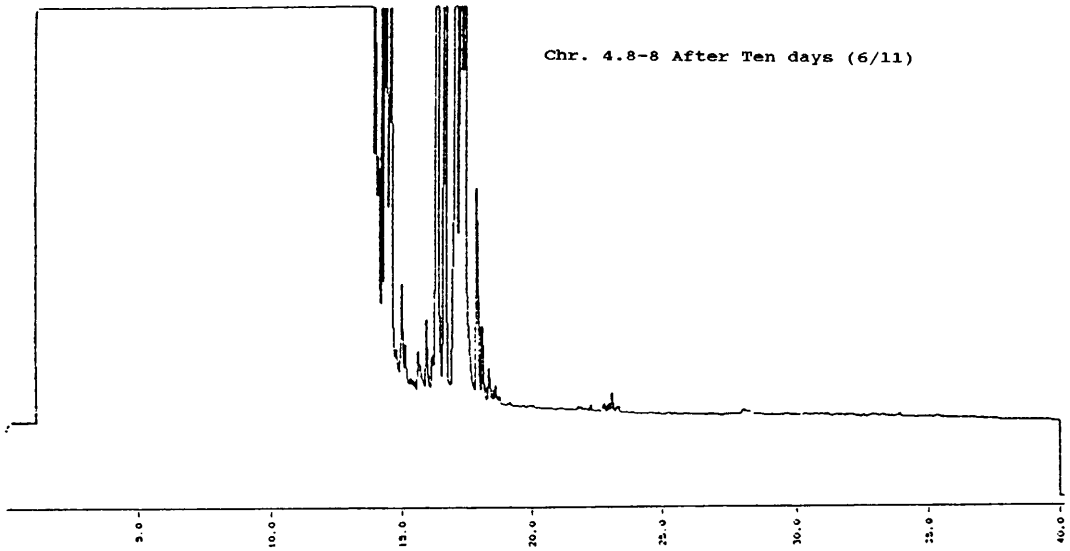


3. Under HPN_2 (5 minutes)

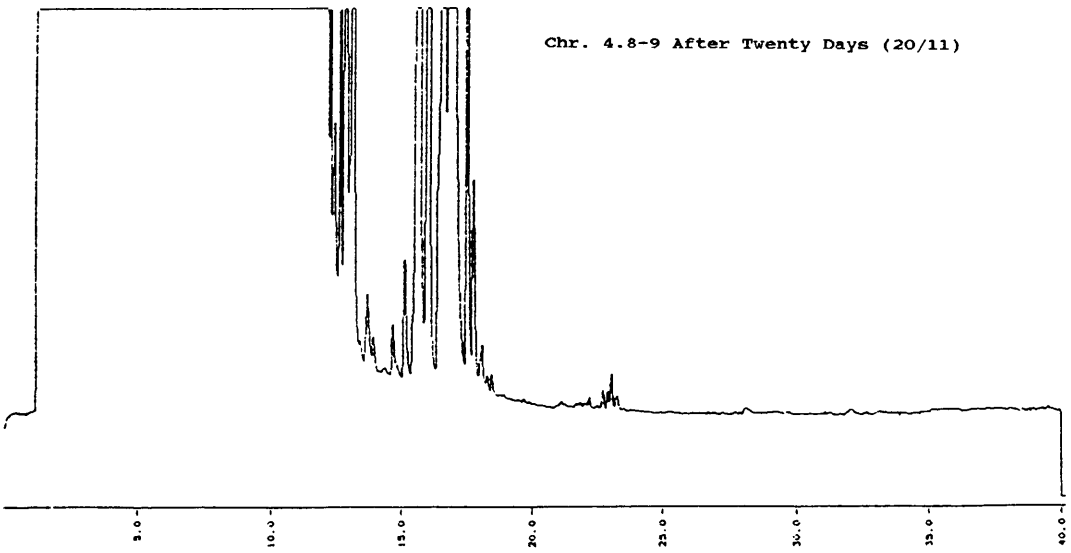
Chr. 4.8-7 F_3 (24/10)
Starting 28/10

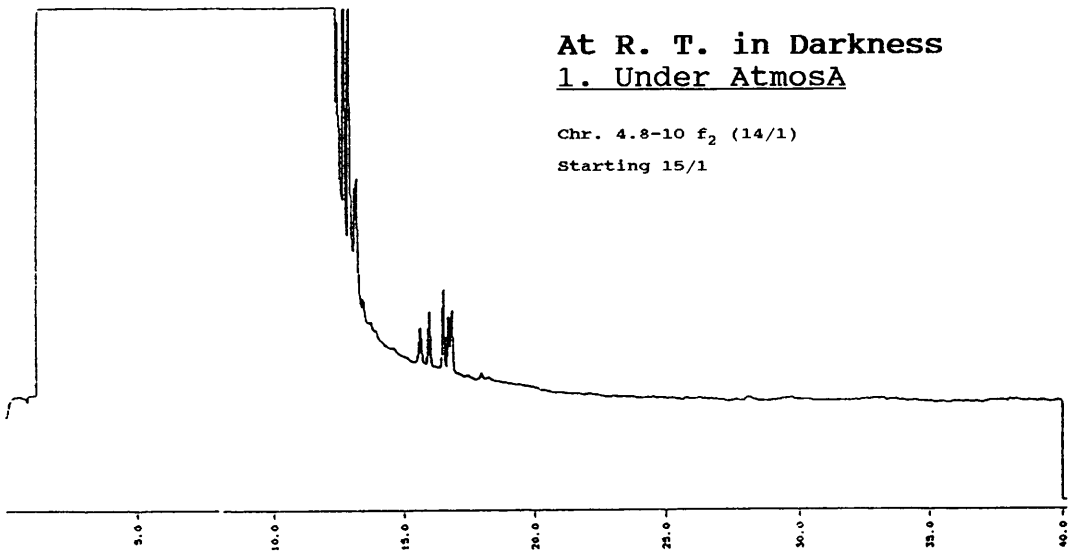


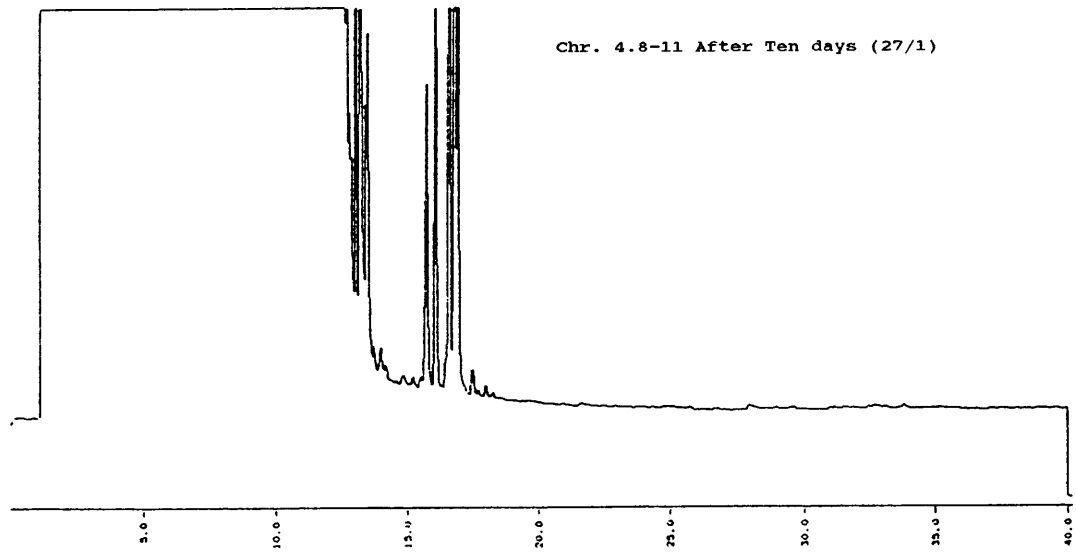
Chr. 4.8-8 After Ten days (6/11)

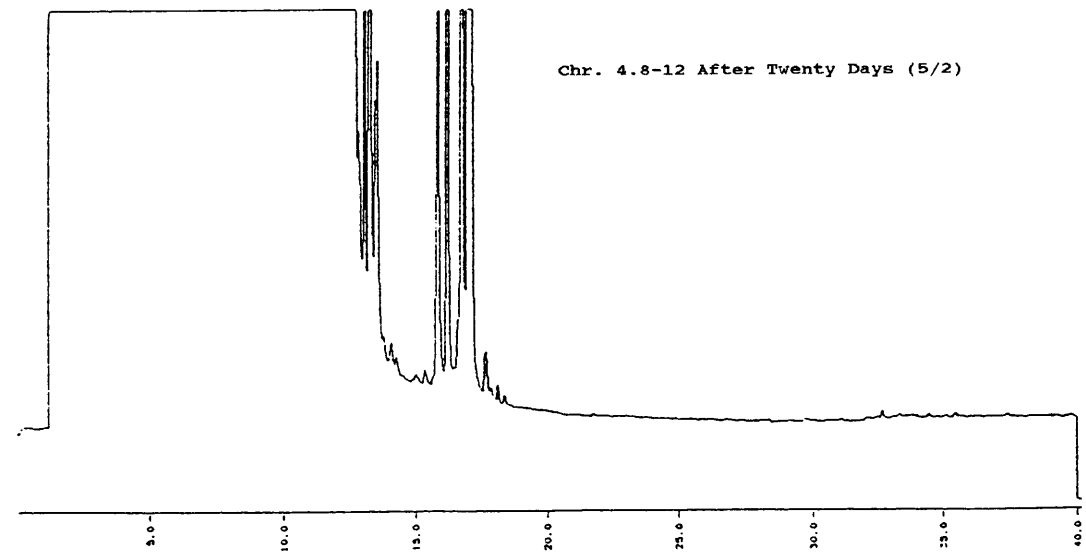


Chr. 4.8-9 After Twenty Days (20/11)



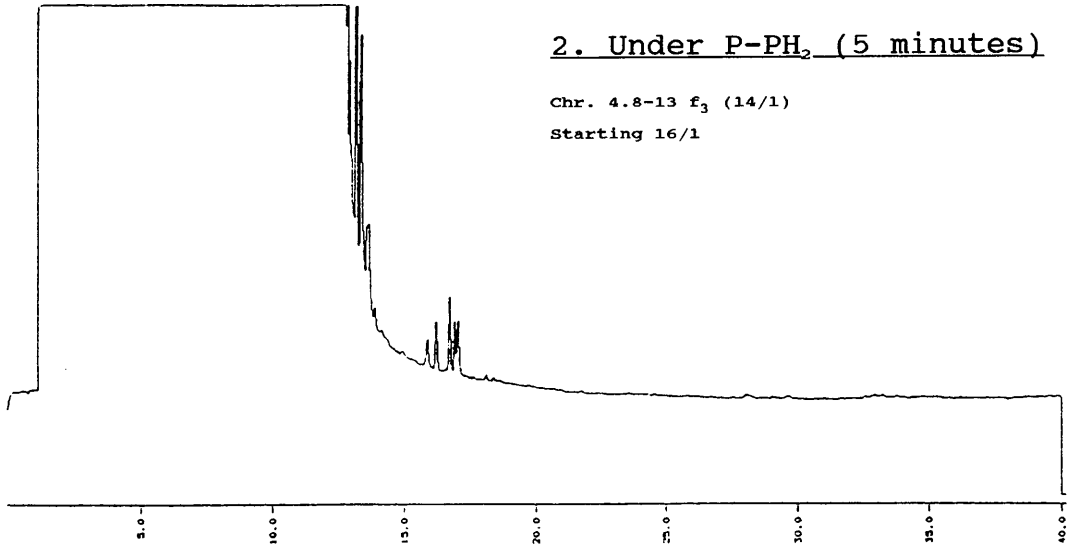




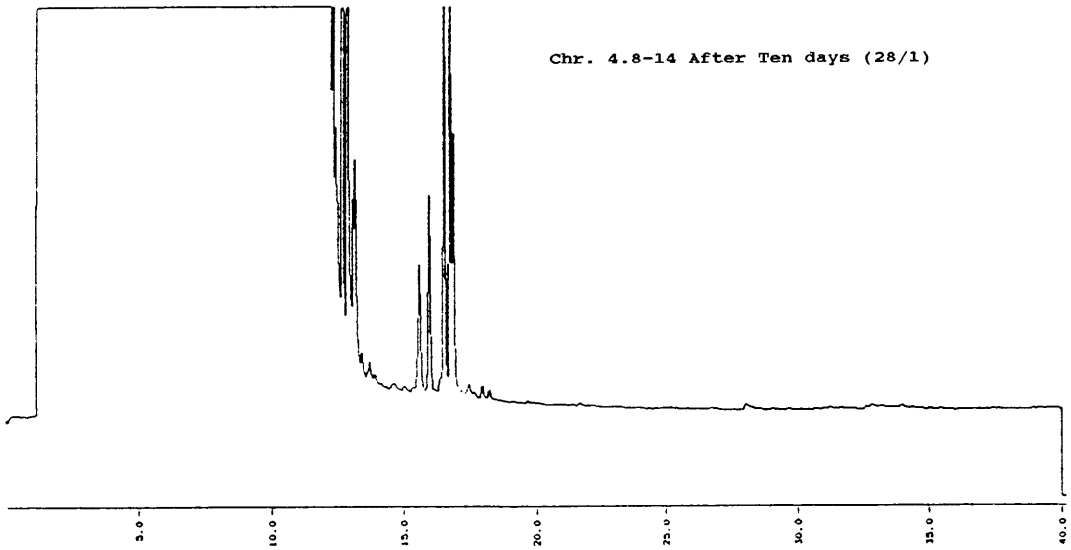


2. Under P-PH_2 (5 minutes)

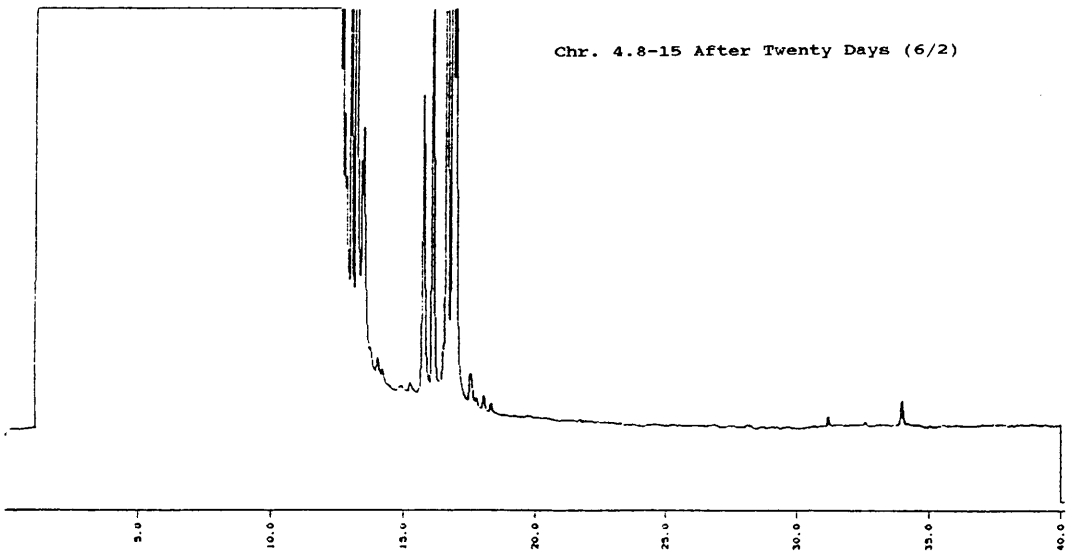
Chr. 4.8-13 f_3 (14/1)
Starting 16/1



Chr. 4.8-14 After Ten days (28/1)

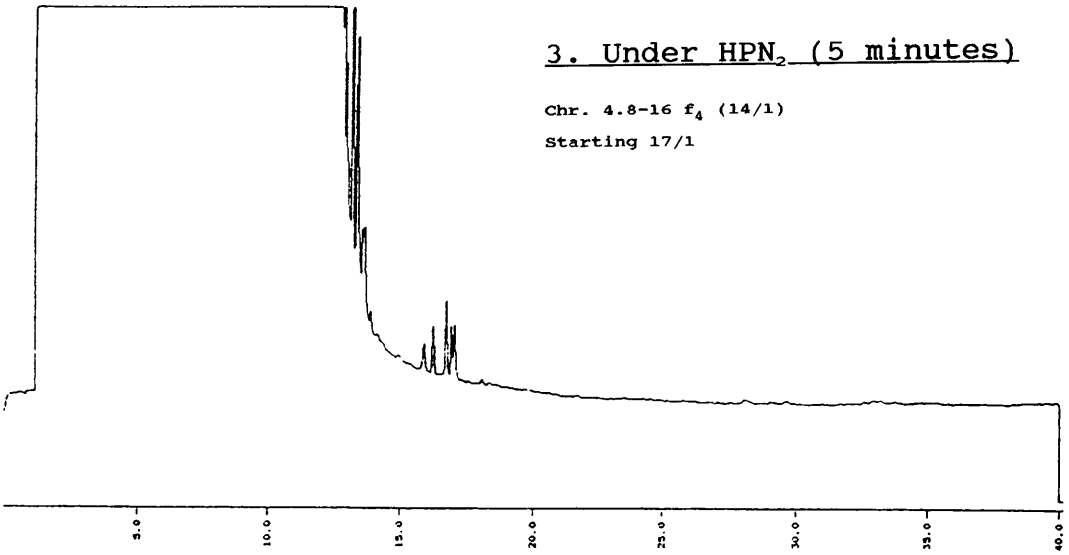


Chr. 4.8-15 After Twenty Days (6/2)

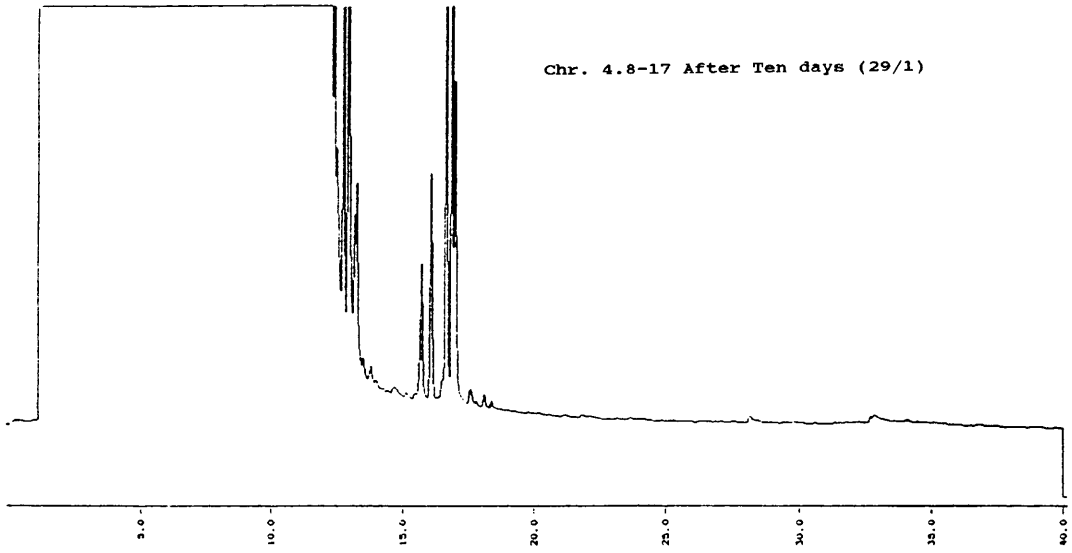


3. Under HPN_2 (5 minutes)

Chr. 4.8-16 f_4 (14/1)
Starting 17/1



Chr. 4.8-17 After Ten days (29/1)



Chr. 4.8-18 After Twenty Days (7/2)