

Sample Preparation for Gas Chromatographic Analysis

**Walter G. Jennings and
Adolf Rapp**

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with 37 Figures and 5 Tables

Dr. Alfred Hühlig Verl
Heidelberg · Basel ·

Walter G. Jennings
Department of Food
Science and Technology
University of California,
Davis CA 95616, USA

Adolf Rapp
Department of Biochemistry
Federal Institute for
Viticulture and Enology Research
Geilweilerhof, Landau/Pfalz,
Federal Republic of Germany

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Jennings, Walter G.

Sample Preparation for Gas Chromatographic Analysis /

by Walter G. Jennings and Adolf Rapp. -

Heidelberg; Basel; New York: Hüthig, 1983.

(Chromatographic methods)

ISBN 3-7785-0858-X

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Printed in Germany

Typesetting: Druckerei Bitsch KG · Birkenau

Printing: Druckerei Bitsch KG · Birkenau

Preface

This offering is concerned with methods of preparing samples suitable for gas chromatographic analysis. It begins with considerations of the various injection modes, and explores in some detail direct headspace injections, with and without on-column cold trapping. Adsorption, absorption, extraction, distillation, co-condensation, freeze concentration and zone refining are then explored as isolation and concentration techniques. Special problems associated with the application of these methods to the analysis of air; biological matrices (including the isolation of fatty acids, bile acids, and amino acids from fluids and tissues), foods and food flavors, essential oils, forensic samples, sediments and water are also considered.

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1 General Considerations in Sample Preparation

The necessity for some type of sample preparation treatment, and decisions as to which routes should be employed to attain that goal, are dictated by the nature of the sample and the sample matrix, the type of information required, the time available for the analysis, and qualitative and quantitative considerations.

The analyst who is concerned with establishing compositional comparisons between some of the essential oils or light petroleum distillates is, in one respect, in a highly enviable position, because an aliquot of the parent substance can often be injected for gas chromatographic analysis directly, without the need of preliminary sample preparation. In other cases, this simple and direct approach may be precluded, sometimes by the nature of the sample itself, and sometimes by the results desired. The individual interested in the determination of pesticide residues, as they occur in sediments from settling ponds or on lettuce leaves destined for salad use, must normally first separate the materials for analysis from those sample matrices; the qualitative and quantitative validities of the results will be strongly dependent on how effectively this was accomplished, *i.e.*, the recovery efficiencies obtained. The flavor chemist may be interested in comparing, quantitatively and qualitatively, odorous constituents of fruit stored in an ethylene-carbon dioxide-rich atmosphere with those of a control stored in air. Good quantitation demands that the procedures used for isolation be stringent and exhaustive, but if the sample is treated too harshly, the possibility of qualitative changes in the sample is introduced. This, of course, would also affect quantitation.

Fermented beverages such as wine, beer, and distilled liquors pose other analytical problems: the major

volatile is water, and the next most abundant is ethyl alcohol. At normal sensitivities, the latter tends to produce an immense, tailing peak that obliterates much of the chromatogram and effectively masks a large number of compounds that may be critically important. For the analysis of these products, it becomes desirable not only to achieve a preliminary exclusion of any non-volatile materials, but to remove most of the ethyl alcohol without affecting the relative concentrations of the other volatile compounds. Other fields raise difficulties that can be even more challenging, and whose solution may be more critical. *Jellum et al.* (1981) reported that, for over 100 metabolic diseases, GC/MS patterns from appropriate body fluids or other tissues can produce typical patterns that can be readily distinguished from patterns obtained on fluids from non-diseased control patients. This offers the clinician a very powerful diagnostic tool but it can also require superior micro methods of sample preparation. High recovery efficiencies of fatty acids that are distributed through, and occasionally bound with fractions of a fragile biological fluid may pose a distinct challenge — and the amount of that fluid available to the analyst may be severely limited, as in the case, for example, on an infant delivered several month prematurely. The removal of any significant quantity of plasma from such a patient can be harmful and may even result in death, and while the results of a post mortem analysis may be interesting and informative to the attendant physician, they are not of great value to the deceased patient.

Equipment and methodology have undergone improvements to such a degree that new demands must be made on sampling methodology; some active compounds that failed to survive the chromatographic process can now be detected, even at low levels. This has triggered an interest in sample preparation procedures that ensure the recovery of these active solutes.

The environmental analyst may be interested in the detection and measurement of trace contaminants in samples of soil, in water, or in air. In some samples, these contaminants may occur at dilutions so great that appreciable volumes of bulk sample may be required, and their isolation and concentration may necessitate several steps. In some cases, the contaminants may exist in the vapor state, and in other

cases they are present as aerosols; they may be adsorbed to airborne particulate matter, or contained in slimes and sediments. Again, both the qualitative and quantitative aspects are important; the selection of a representative sample, the removal of the components of interest from that sample, and the recovery of those components, even from the concentration apparatus, all threaten the validity with which the final analyte represents the composition of the original substance.

The past few years have seen notable progress in the development of capillary columns whose increased inertness makes the prospects of total or partial abstraction of sensitive solutes much less likely (e.g. *Jennings*, 1980a). Improved and more mild methods of sample injection, based largely on the work of *Grob* (1978) and *Grob and Grob* (1978, 1979) have had a very positive impact on the quantitative and qualitative aspects of sample introduction; later developments that employ a standard microliter syringe fitted with a flexible fused silica needle through which the unheated sample is deposited directly into a fused silica column coated with a chemically bonded and non-extractable film of liquid phase have succeeded in removing the stainless steel syringe needle from the analytical system (*Jenkins and Wohleb*, 1980). Metal syringe needles can exercise catalytic effects on some samples; these effects can be exacerbated by injection modes that heat the needle. Other metallic passages can also be detrimental to the validity of the analytical results. The dangers of solute abstraction occurring even in a very restricted length of column-detector interface (the flame jet) were pointed out by *Freeman* (1980), who recommended that the fine-diameter fused silica column be extended through the flame jet to terminate just below the base of the flame itself.

These developments have combined to offer increased assurance that the materials originally drawn into sampling syringe survive sampling, injection, and column transit, and are ultimately delivered unchanged to the sensing point of the detector; this prospect was previously subject to much greater uncertainty.

No single procedure of sample preparation is suitable for all samples in all situations.

While these increased possibilities of solute survival are indeed welcome, they emphasize to an even greater

degree the necessity for sample preparation techniques that complement these enhanced analytical capabilities. Many sample isolation processes involve distillation, extraction and/or adsorption, which may be followed by derivatization techniques; the analyst is faced with abundant opportunities to commit sins of omission and sins of commission. There is, unfortunately, no single sample preparation procedure that is best suited for all samples under all conditions. Today's chromatographer must instead be sufficiently familiar with a wide variety of techniques so that reasoned judgments as to which best suits a particular set of circumstances are possible. It is our hope that this offering will help in the attainment of that goal.

2 Direct Sample Injection

2.1 Introduction

At the beginning of the chromatographic process, the sample band must be as narrow as possible.

A basic requirement of the chromatographic process is that the sample, as it is introduced to the column, must occupy the shortest possible segment of column length. As the sample band traverses the column, broadening processes take place, and the degree to which any two substances are ultimately separated is a function of the extent to which these broadening processes can be limited (column efficiency), and the narrowness of the starting band (injection efficiency). Obviously, while band breadth has no effect on the amount of material in the band, it has an inverse relationship with the concentration of solutes in that band. Consequently, both the efficiency of the injection and the efficiency of the column affect the concentrations at which solutes are delivered to the detector; hence both also influence sensitivity.

A variety of injection modes are compatible with capillary chromatography. The most widely used include split, "splitless", and on-column. In addition, encapsulated injections, a falling needle injector and thermally focused inlets have been proposed for use with capillary columns. The former are discussed briefly below, and all have been discussed in greater detail elsewhere (*Jennings, 1980b*).

2.2 Split Injection

Split injections are popular because the equipment... as supplied or retrofitted... is relatively inexpensive, and good chromatographic results are relatively easy to obtain. Splitters can yield excellent results if the injection is properly executed on suitable samples.

In this mode, a syringe is used to inject the sample into a heated chamber, which is swept by a relatively large flow volume of gas (usually 30 to 200 cc/min). The major portion of the gas is discharged to the atmosphere

via some type of restriction, while a minor portion is directed through the column as the carrier gas. Because vaporization must in the ideal case occur instantaneously, inlet splitters should possess vaporization surfaces with high heat capacity, and in consequence may subject the sample to considerable thermal shock; hence this inlet mode may be unsatisfactory for thermally labile substances. Splitters also demand good syringe technique and rapidly executed injections.

Linearity — with splitters, the word represents the fidelity with which that portion of the injection directed into the column represents the true composition of the sample — is influenced by many factors. As the solvent and lower-boiling solutes flash into the vapor state, they absorb heat from the vaporization surface. Some higher boiling solutes may then resist vaporization on the cooled surface, and can be entrained as micro droplets to form an aerosol. Aerosol formation can cause severe problems in discrimination — *i.e.*, a lack of linearity. To minimize the possibility of aerosol formation, a small amount of packing such as Chromosorb W., lightly loaded with an apolar phase such as SE30, is sometimes substituted for the glass beads usually used for the vaporization surface. The inlet then functions as an extremely short packed chromatographic column, and ensures that materials proceeding to the split point are in the vapor phase. Band broadening, caused by the slightly retarded solute introduction is usually so slight that it causes no problems. This may relate to the fact that it is the higher boiling solutes that are most retarded, and these probably cold trap at the beginning of the column and enter into the chromatographic process only later in the temperature program. Samples whose constituents represent a wide range of volatilities are difficult to quantitate with split injections, because the higher boiling components usually vaporize more slowly (and may, in extreme cases, resist vaporization altogether), which may well lead to discrimination.

The split ratio (flow through the column/flow discharged through the vent) may be varied from as low as (for example) 1:5 to as high as 1:1000, but values of 1:50, to 1:200 are more usual. Hence, only 0.5 to 2% of each microliter injected is directed to the column. Assuming that all components have a density of 1.0, it can be ap-

proximated that a 1.0 μl injection, split 1:100, deposits on the column *ca.* 1.0 μg of a solute occurring at the 10% level, and *ca.* 1.0 μl of a solute occurring at the 0.01% level. The former is in the overload range of columns with film thicknesses of 0.1 to 0.25 μm (i.e., conventional columns), and the latter approaches the detection limit of the conventional FID. Hence, under normal conditions of use, splitters function best for solutes that occur at levels of 0.01 to 10% of the sample.

A good splitter should meet certain design criteria.

A good splitter is designed to expose only inert surfaces to the sample stream until that sample has progressed beyond the split point, to vaporize the sample rapidly and completely, to achieve thorough mixing of the sample with the gas stream, and to include a sample expansion area where the actual split occurs. A downstream buffer volume helps dampen, but does not eliminate, pressure surges that accompany solvent vaporization, and which can affect stream velocity, split ratio, and linearity.

A major advantage of the split injection is that non-volatile sample components are not deposited on the column, but remain in the splitter, whence they can be removed by periodic cleaning. The major disadvantages are that the sample is subjected to a relatively severe thermal shock, and discrimination may be observed, particularly with higher boiling solutes.

Splitters offer most chromatographers three primary advantages: they are relatively inexpensive, they are simple to use, and they can be designed so that non-volatile components in the sample remain in the splitter and are denied access to the column. They suffer from the primary disadvantages that the split may be non-linear (especially if the sample includes higher-boiling components), that the sample is subjected to a relatively severe thermal shock (they require more rapid vaporization of the sample than do the other inlet modes), and (particularly with short columns) they demand good injection technique. **Figures 1 to 3** show typical split injections of samples that required no special preparation.

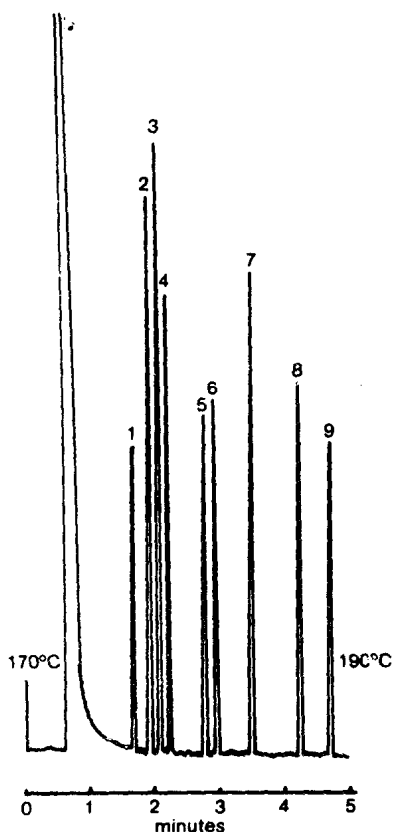


Figure 1

Split Injection of some N-methylcarbamate insecticides; FID chromatogram on 18 m x 0.31 mm ID fused silica capillary coated with SE 52; 170 to 190°C at 4°C/min and held.

1) Methomyl; 2) Propoxur; 3) Azobenzene; 4) Landrin; 5) Carbofuran; 6) Matacil; 7) 3-Ketocarbofuran; 8) Carbaryl; 9) Mesurol. From Wehner and Selber (1981).

2.3 Splitless Injection

Splitless injection is best applied to dilute solutions of higher boiling solutes. The boiling point relationships of the solvent and solutes with respect to the temperature of the inlet and the column are critically important.

In this process, the sample, diluted in solvent, is injected into a heated chamber under conditions where that chamber is swept with a relatively low flow

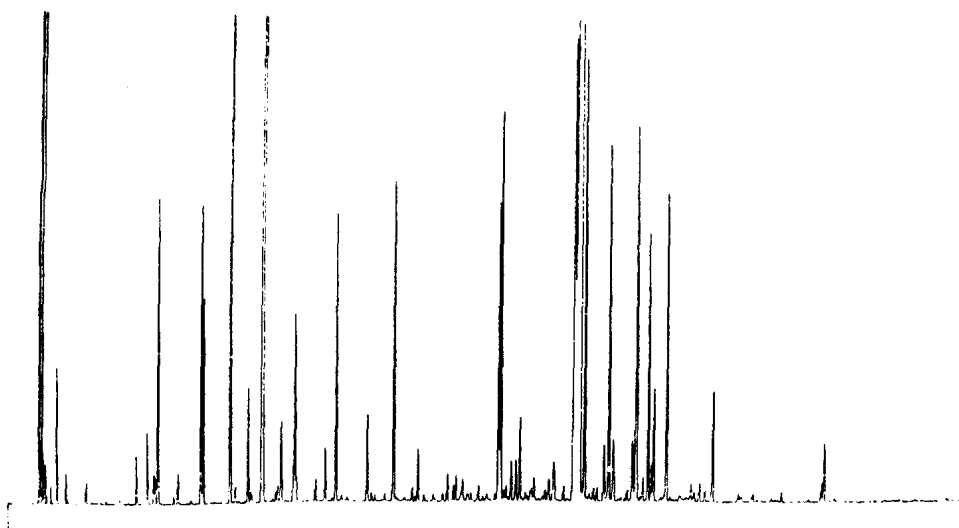


Figure 2

Split injection of 1 μ l (split 1:100) of midwest natural spearmint oil; 30 m X 0.25 mm ID fused silica capillary coated with 50/50 SE 30 and Carbowax; 50°C 5 min, 4°C/min. to 200°C. Courtesy of Rand Jenkins, J & W Scientific, Inc.

volume of carrier gas — typically 1 to 3 cc/min — all of which then flows through the column. The solvent must be high boiling with respect to the column temperature, and low boiling with respect to the components to be analyzed. Under these conditions, the vaporized solvent is carried to the cooler column, where it condenses and serves as an alternative "liquid phase", depressing the phase ratio of the column in this locale so that the solutes which now enter the column experience massive increases in their partition ratios (*Grob and Grob, 1972, 1974, 1978; Jennings et al., 1978*). After some 1 1/2 volumes of gas have passed through the heated inlet and into the column, a purge function is activated by redirecting the gas flow to enter the bottom of the inlet, where the flow stream is divided, one portion continuing forward through the column to serve as carrier gas, while the other sweeps residual volatiles from the chamber to atmosphere via a restrictor. Hence, a "split" does occur during "splitless" injection, and that split takes

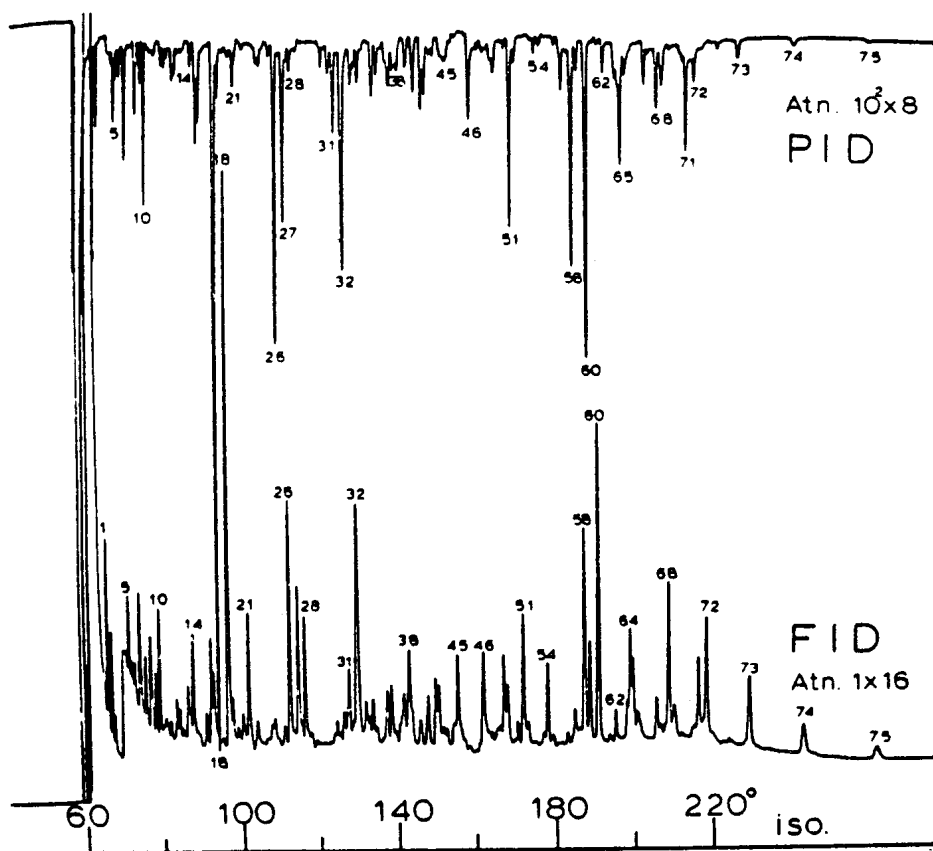


Figure 3

Split injection of the neutral fraction of a coal tar; 10 m fused silica capillary coated with SE 54; outlet split to miniature photolization detector (PID) and FID. From Kapila and Vogt (1981).

place under conditions that have no provision for safeguarding the linearity of the split.

Because a longer period is permitted for the vaporization of the sample and its transport to the column, the splitless inlet can be operated at somewhat lower temperatures than the split injector, which of course means that the sample can be subjected to less thermal shock. The primary use of "splitless" injection has been in the analysis of trace high boiling solutes that can be injected with a low-boiling solvent. Figures 4 to 6 show typical applications.

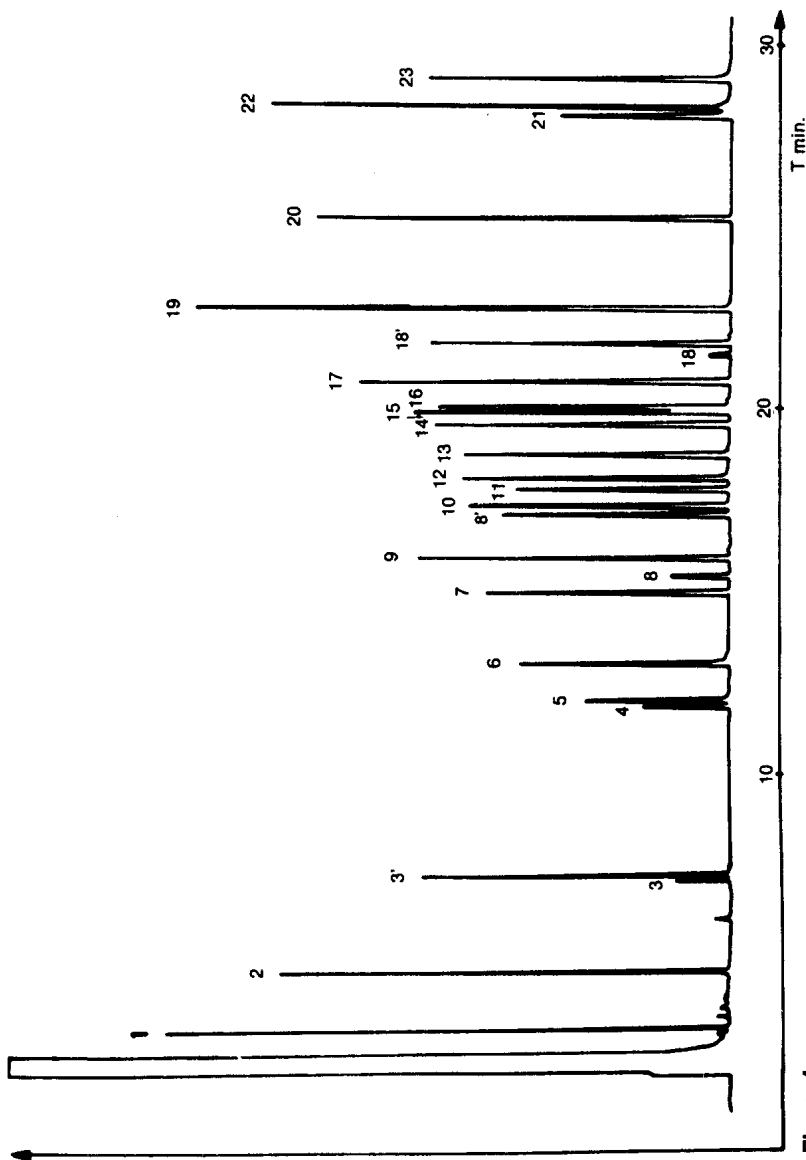


Figure 4

Splitless injection of a mixture of organophosphorus pesticides; 14 m x 0.25 mm ID glass capillary coated with OV 1; 115 to 190 °C at 4 °C/min. 1) Dimefox; 2) Dichlorvos; 3) 3'-Mevinphos; 4) Naled; 5) Dimethoate; 6) Foncosos; 7) Diazinon; 8) Parathion-methyl; 9) Malaoxon; 10) Parathion-methyl; 11) Malathion; 12) Fenitrothion; 13) Parathion-methyl; 14) Malathion; 15) Parathion-methyl; 16) Chlorpyrifos; 17) Bromophos; 18) Chlorfenvinphos; 19) Tetrachlorfenvinphos; 20) Ethion; 21) Phosalone; 22) Azinphos-methyl; 23) Azinphos-ethyl. From Wolf et al. (1981).

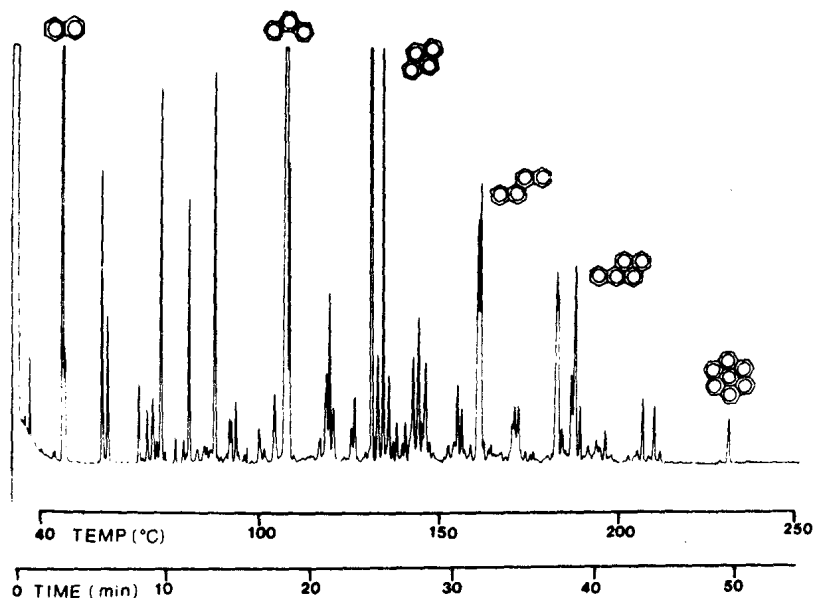


Figure 5

Splitless injection of coal tar on 4 m x 0.3 mm ID glass capillary coated with SE 52; 40 (2 min) to 250°C at 4°C/min. Hydrogen carrier at $u = 100$ cm/s. From Wright and Lee (1980).

2.4 On-Column Injection

On-column injections offer the best approach for the introduction of high boiling solutes, and should offer the ultimate in quantitation.

Both of the inlet modes discussed above are characterized by the fact that the sample is injected into a heated chamber, where it is converted into a vapor that is conducted to the column where it is re-condensed. *Desty* (1965) suggested that a distinct advantage could be realized if the sample were vaporized under the mildest conditions possible, i.e., those of the chromatographic process itself. Such on-column injections have long been used with packed columns, but their extension to capillary chromatography poses some special problems, especially with small-bore capillary columns. *Schomburg et al.* (1977) proposed that on-column injections might be accomplished by utilizing a sample-loaded capillary pipet within the inlet system; when the pipet was thrust forward by means of an external control, the carrier gas flow stream was redirected to flow