

AN ABSTRACT OF THE THESIS OF

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The volatile flavor fraction of lightly milled wheat grown in eastern Oregon was isolated for study by two methods: steam distillation under vacuum and steam distillation at atmospheric pressure. The sample obtained under vacuum was extracted with ether which was then evaporated to yield the desired wheat essence. Steam distillation at atmospheric pressure provided the headspace vapors that were used for a portion of the gas chromatographic analysis.

Identification of the organic substances contained in the volatile flavor fraction was made on the basis of chemical tests, paper chromatography, gas-liquid chromatography, and mass spectrometry.

Preliminary testing for amines and carbonyls indicated the presence of both of these functional groups, although in subsequent research amines were not detected. Additional information as to

the functional groups present was obtained by subjecting headspace vapor samples to gas chromatography and directing the column effluent into vials containing reagents selected to indicate the presence of alcohols, amines, esters, carbonyls, and mercaptans. A separate set of reagents was used for each eluting peak.

Formation of 2,4-dinitrophenylhydrazone derivatives and determination of their melting points provided one means of tentative identification of carbonyl compounds.

Ascending paper chromatography was used to separate the 2,4-dinitrophenylhydrazone derivatives of the carbonyls into classes and to ultimately assist in the identification of these compounds.

A Barber-Colman gas-liquid chromatograph equipped with a flame ionization detector was used as a means of separating the components in the samples. Identification of some of the compounds was possible through comparisons of retention times of the peaks on these columns with retention times of known compounds. Column packings used for gas-liquid chromatography were six percent Apiezon M, six percent Diethylene Glycol Succinate, and three percent Free Fatty Acid Phase, all on a solid support of Anakrom ABS, 90 to 100 mesh. The column was heated isothermally at 75° C with nitrogen as the carrier gas flowing at a pressure of five pounds per square inch.

Mass spectra of the wheat essence compounds were obtained

by use of an Atlas-MAT Ch-4 mass spectrometer using a two second scan, coupled with a gas-liquid chromatograph.

Twelve compounds were identified and six were tentatively identified in the headspace aroma or in the wheat flavor essence. Those identified were acetaldehyde, isobutyraldehyde, butyraldehyde, valeraldehyde, hexanal, heptanal, octanal, crotonaldehyde, 3-methyl-2-butanone, 2,2-dimethyl-3-pentanone, diacetyl, and ethyl acetate. The compounds tentatively identified included isoamyl alcohol, amyl alcohol, butanone, isovaleraldehyde, cyclopentanone, and phenylacetaldehyde.

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# SOME FLAVOR COMPONENTS OF WHEAT

## INTRODUCTION

The flavor of food has been of interest to the consumer for centuries and has been the focal point for research projects for many years. Methodology for the use of sensory panels has been carefully developed. Considerations that are important in the selection of a panel member, adaptability of various means for presenting samples to be judged, and commercial applications of results from sensory panel studies have all been the subjects of considerable research.

Although interest in flavor and its influence on consumer acceptance of food has not waned, the study of flavor is now frequently being approached from the analytical viewpoint. This shift in emphasis can be traced directly to the advent of gas chromatography. The present refinements in gas chromatographs enable the researcher to separate the extremely small quantities of volatiles that combine to give the total flavor impression of a food. Prior to the development of this powerful research tool, separation and identification of many flavor components of foods were virtual impossibilities.

Components of the flavor fractions of dairy products, wines, vegetables, meats, poultry, fish, and many other foods have been the subjects of intensive research. With the incentive of the commercial food industry's demand for extracts and concentrates of



fruit flavors for synthetic fruit drinks, gum, candy and other products, much of the current research on components of food flavors has been focused also on fruits.

Paradoxically, the realm of cereal flavors has been practically untouched despite the fact that cereal grains constitute the basis of the food supply for many peoples of the world.

Although hunger and even starvation have been with us since time began, the current rate of population increase and its concomitant problem of more serious food shortages have combined to give a sense of urgency to research geared toward developing new food sources to meet the approaching emergency. Potential food sources that have not been exploited previously are under study, and some products such as spun protein fibers from soybeans are being developed from these unconventional sources. Undeniably such new foods as these will help meet the world's nutritional needs, yet it is imperative that the overall palatability of the products as well as their nutritive merits be considered. If the appearance, texture, aroma, or taste of a new food is unfamiliar or distasteful, acceptance and use will be limited. A new product with some characteristics similar to a well-liked food will usually be accepted readily and frequently served.

Cereals form the backbone of the diets of many people, particularly in some underdeveloped countries where food shortages

are especially critical. If new simulated cereal products can be developed with flavors identical or closely akin to accepted cereals, their acceptance presumably should be excellent and an important nutritional contribution will be possible.

Since World War II, India has been plagued by famine, which the United States has helped to alleviate by the shipment of large quantities of wheat to the Indians. Consumer acceptance of wheat in India, as well as in the rice-eating country of Japan, suggests that the flavor of wheat is likely to be popular in newly-developed food products.

If the significant components in the cereal volatiles can be identified, then the avenue will be opened for synthesizing these important flavors. This task is complicated by the mild flavors of cereals, an indication of the extremely small quantity of material available for study.

This research project will be directed toward the identification of some of the important volatile constituents in wheat that contribute to its total flavor profile.

## REVIEW OF LITERATURE

### Flavor

Flavor may be defined as the total sensation compounded from the senses of taste and smell. The perception of taste is considered to be closely related to the chemical components of the material being sampled. The taste of a food becomes manifest when the various substances present are dissolved, hence, water-solubility is a requirement if a substance is to be tasted on the tongue (Moncrieff, 1951). There are four main types of taste receptors on the tongue, and these are regionally distributed to provide greater sensitivity to one type of taste in one region and to another taste in another area. The geographical concentrations of these taste receptors are: bitter taste at the back of the tongue, sweet taste at the tip, sour taste at both sides of the tongue, and salt taste toward the tip and along the edges. Taste can be perceived without benefit of olfactory response-- a fact often observed by persons with a severe cold that blocks the nasal passages.

Aroma or odor is detected by millions of olfactory receptor cells in contrast to the thousands of taste cells. Odor-containing air passing over the small area of yellow epithelium at the top and back of the nose stimulates the olfactory cells and causes electric

impulses to be transmitted to the brain. There is still considerable question about the mechanisms involved in the total perception of odor, but the importance of this aspect of sensation is evident.

The total impression of taste and odor is interpreted as the flavor of a food. It is apparent that any consideration of components contributing to flavor must be concerned with both volatility and solubility characteristics.

### Role of Gas Chromatography in Food Flavor Research

#### Theory

According to Patton (1959), the history of chromatography can be traced to 1903 when Tswett separated an extract of green leaves into several colored bands by pouring his solution onto an adsorbent in a column, followed by passage of fresh solvent. Gas chromatography principles were first described by Martin and Synge in 1941, but it was not until 1952 that the first practical application of this method was published.

Basically, gas chromatography is a means of separating or distributing a mobile mixture between two phases that move with respect to each other. One phase, referred to as the stationary phase, is fixed or immobile; the second phase is the mobile one. In the type of chromatography commonly being used for flavor

analysis today, the stationary phase is a liquid and the mobile phase is a gas, an arrangement that logically led to the designation, gas-liquid chromatography (GLC). The stationary liquid in such systems may be fixed either on the interior walls of a capillary column or as a thin film on a solid support of fine particle size for use in packed columns.

In gas-liquid chromatography the sample, which is injected either as a gas or liquid, is swept along the column by a carrier gas. The components in the sample mixture become distributed along the column as a function of their relative affinities for the fixed phase and their volatility. Ideally, complete separation of the components will occur and each one will emerge as a separate elution peak from the apparatus.

The retention time on the column of the various peaks is a sensitive, extremely useful means of helping to identify unknown chemical components in a mixture. It is of particular value when working with the infinitesimally small quantities of flavor components in foods. The matching of retention times of unknowns with known compounds under standardized conditions is a most useful qualitative tool in the identification of unknown mixtures. Since the peak height or the area under the peak is proportional to the amount of substance it represents, gas-liquid chromatography is also a useful quantitative tool.

To achieve optimum over-all separation, two factors must be considered: the number of theoretical plates and the separation achieved per plate. The section of a column in which the vapor leaving the column section is of a composition that would be in equilibrium with the average concentration of liquid solution in that section is defined as a theoretical plate. During the passage through one theoretical plate, one equilibration between the liquid and vapor phase will theoretically take place. It is desirable to maximize the number of theoretical plates in a column to optimize separation. This can be done by increasing the column length. An increase in column diameter causes a slight decrease in the number of theoretical plates. The separation achieved per plate is influenced by the choice of liquid to be used in the column, the nature of the mixture to be resolved, and the temperature of operation.

Careful selection of the column liquid is important to achieve good separation of components. A column liquid containing aromatic rings is a good choice when working with aromatic mixtures because such a liquid selectively detains the aromatics. In work with polar compounds, such as are common in food flavor mixtures, the use of a polar liquid in the column is recommended because of the greater affinity between the polar components in the sample and the stationary phase. Diethylene Glycol Succinate (DEGS) is a polar liquid well suited to effective separation of food flavor components.

One of the goals in the operation of a gas chromatograph is to reduce band width as much as possible and obtain a sharp, reasonably symmetrical peak. Chromatographic bands tend to broaden as they move down the column as a result of eddy diffusion, molecular diffusion, and resistance to mass transfer. Eddy diffusion is a result of the irregularities in the path the gas follows as it moves down the column. This phenomenon can be minimized by using uniformly small particles for the support and carefully packing the column. Molecular diffusion also needs to be controlled to minimize band broadening. The use of nitrogen, argon, and carbon dioxide is recommended for the carrier gas, rather than hydrogen or helium, as a means of minimizing molecular diffusion. However, hydrogen, helium, and nitrogen are generally the gases selected as the carrier for analysis of organic vapors because they perform satisfactorily without harming filaments and are reasonably priced. Increasing the carrier gas pressure and changing the temperature are also suggested as means of controlling band width. Resistance to mass transfer is the third factor influencing band broadening in gas-liquid chromatography. For best performance the liquid phase should be selected for its ability to effect high solubility of the injected sample components. This liquid film should then be kept very thin although it is essential that the support be completely covered. Sometimes an increase in column temperature will improve separation by

decreasing the viscosity of the column liquid, but other factors influenced by operating temperature may actually cause poorer efficiency of separation.

### Developments in Flavor Research

Although gas chromatography is conceded to be less sensitive to odorous substances than is the human nose, this analytical device is being put to practical use in laboratories conducting flavor and odor research throughout the country. Man is capable of detecting as little as  $10^{-10}$   $\mu\text{g}$  in 1 milliliter of ionone or sulfur compounds (Bayer, 1966). The threshold level for methyl acetate has been determined to be  $10^{-6}$   $\mu\text{g}$  and menthol has the still larger threshold of  $10^{-4}$   $\mu\text{g}$ . From these figures it is apparent that some substances appearing in very small concentrations, but which are readily detected by man, may be very important constituents in a flavor although they may appear to be insignificant because of small peak size in a chromatogram. Because of the possibility of overlooking trace substances when doing flavor research, some attention is appropriately given to the use of the human nose as well as to the gas chromatograph when attempting flavor analysis.

Weurman (1963) also emphasized the importance of checking odors to determine the possibility of changes in the odorous compounds in a sample. He pointed out that enzymes in a macerated



sample may cause new odorous compounds to form or existing ones to change. Thus, at all stages in the research, it is necessary to use one's sense of smell as an analytical tool (Flath, 1967). Finally, the components should be recombined as they are eluted from the gas chromatograph and the aroma checked for comparison with the original sample.

Weurman (1963) recommends the combination of headspace vapor analysis combined with the more traditional analysis of flavor concentrates. A flame-ionization detector is a very sensitive device that makes practical the analysis of the miniscule quantities of volatile flavoring compounds found in a headspace sample. In fact, the flame-ionization detector can detect as little as  $10^{-12}$  g of a compound passing through it in one second. The advantage of this sampling method (i. e., headspace) is the reduction of experimental artifacts. The obvious limitation is the need for more material to aid in establishing positive identification of components. The more traditional methods of obtaining samples for identification purposes are discussed later in this chapter.

Weurman (1963) points out that compounds occurring in the vaporous headspace may not appear in the sample prepared from the food itself. Evidence of this phenomenon is presented by Ng, Reed, and Pence (1960). These workers found two aldehydes in the oven vapors from baking white bread which were not also

present in the sample prepared from the baked bread. However, the other components identified were found in both the vapor and the bread itself.

Gas chromatography alone has been used by some workers to identify components of a mixture, but more rigorous evidence can be obtained by combining this technique with other methods. A fairly recent development has been the use of the mass spectrometer in conjunction with gas-liquid chromatography. Briefly, mass spectrometry may be described as follows. Electrons are used to bombard the molecules of sample in an ion source, a procedure that fragments the molecules into positively charged, and negatively charged or neutral ions. An electric field accelerates the positively-charged ions and a magnetic field imposed perpendicular to the ion stream causes the ions to go into a circular orbit. The ions with a constant proportion between mass and charge will focus on one spot. By maintaining a constant acceleration potential while varying the magnetic field, a full spectrum can be recorded with the aid of a detection device that has a fixed-focus slit.

Mass spectra give important structural information that aids in positive identification of unknowns. This instrument is gaining in importance in flavor analyses because it requires smaller sample size than does infrared analysis. The best results with the mass spectrometer are obtained when separation of components is effected

prior to mass spectral analysis (von Sydow (1963). When only small samples are available, as is true in food flavor analyses, good results have been obtained by combining the gas chromatograph and mass spectrometer. In this case the mass spectrometer is used in conjunction with a gas chromatograph. This arrangement has been used by several teams of investigators (von Sydow, 1963; Teranishi et al., 1963; Watson and Biemann, 1965; Day and Libbey, 1964; and Day and Anderson, 1965). It is generally agreed that positive identification of a compound is achieved when gas chromatographic and mass spectral data for a compound are in agreement with comparable data for the known material (Teranishi, Lundin and Scherer, 1967).

A potentially useful combination of research tools would be the coupling of a gas chromatograph with a sensitive infrared spectrophotometer. The separation achieved with the gas chromatograph is theoretically capable of producing an excellent infrared spectrum which would be extremely valuable in identifying compounds. To date, the linkage of these two instruments has not been refined to a useful degree. Poor resolution of detail in the infrared spectra attempted is the chief drawback at present (Teranishi, Lundin and Scherer, 1967). When the instrumentation-coupling problems have been overcome, this will doubtless prove to be a popular combination among research workers in the flavor field.

At the present time satisfactory infrared analyses require separate collection of peak components as they are eluted from the gas chromatograph. Several methods of trapping have been devised (Juvet and Dal Nogare, 1964) for use in large-scale preparative work. Hoffman and Silveira (1964) trapped 1 to 10 mg quantities in glass capillary melting point tubing inserted in the outlet of the gas chromatograph. The ends of the tube were sealed with a small torch and then the condensed material was centrifuged to one end where it could be removed with a microsyringe. Multiple collection using Teflon tubing with glass collectors was described by Teranishi et al. (1965). Such a method is suitable for collection of a large enough sample for infrared analysis, a not inconsiderable problem heretofore. Since most of the samples of concern in aroma analysis are liquids soluble in carbon tetrachloride and carbon disulfide, these solvents are ideal to use as diluents and carriers for samples trapped from gas chromatographic separation. It is still a problem to obtain enough sample of some components for infrared analysis, but devices are now available (Chrenko, 1964) that enable one to obtain a usable spectrum with between 0.1 and 0.5  $\mu$ l of gas-chromatographed purified sample. However, even these small quantities are difficult to obtain when they are being derived from bland foods.

### Isolation and Concentration of Flavor Fractions

The four means generally accepted as practical ways of obtaining a concentrate of flavor components are distillation, extraction, freezing out, and adsorption. In addition, headspace analysis gives useful information. The first two methods with variations in actual technique are most commonly used.

At the Western Regional Research Laboratory headspace gas was used in the development of their aromagram technique (Teranishi, Buttery and Lunden, 1962). In this method the food to be tested was placed in a covered Erlenmeyer flask for a short time prior to removal of some of the headspace vapors by means of a syringe. The vapor sample was immediately injected into the gas chromatograph for separation. Boiling water was added to samples with low vapor pressure before the vapor sample was collected. The use of relatively moist headspace vapors can be condoned because flame ionization detectors respond little, if any, to the water vapor present (Bernhard, 1966; and Johnson, Rooney and Salem, 1966). Other workers reporting utilization of headspace vapors for flavor analysis include Wolford et al. (1963), Bassette, Ozeris and Whitnah (1962), and Ozeris and Bassette (1963).

The most accurate ratio of volatiles is obtained by gas chromatographic analysis of headspace vapor (Weurman, 1963; Heins

et al., 1966. However, this method of isolating flavor components has some limitations that can be overcome by combining headspace analysis with analysis of sample obtained by other means.

Numerous distillation devices with varying degrees of sophistication have been devised in an attempt to remove the elusive, discouragingly minute quantities of flavoring substances from foods. Mabbitt (1963), Self (1963), Herz and Chang (1966), Coffman (1967), Reymond et al. (1966), and numerous other workers have reported on various modifications of their distillation apparatus designed to overcome the problems inherent in the particular type of food they were analyzing. Perhaps the design with the most universal application is the train of traps designed by Herz and Chang (1966). In this system, steam is bubbled through the sample and the vapors then move through a series of traps to ensure maximum recovery of highly volatile substances. The train of traps consists of five traps surrounded by solid carbon dioxide followed by three more traps immersed in liquid nitrogen. The entire system is under vacuum to reduce the temperatures needed for removal of the volatiles to be studied. The use of such an apparatus has the advantage over headspace vapor analysis in that some of the less volatile, but still significant flavor components that may have been impounded in the mass of material are more likely to be released and collected when steam vacuum distillation is used (Bernhard, 1966).

Reymond and coworkers (1966) used a relatively simple apparatus for extracting the volatiles from tea, coffee, and cocoa. They simply bubbled a controlled flow of nitrogen gas through the beverage maintained at  $80^{\circ}\text{C}$  during the 30-minute collection period. The volatiles were condensed in a trap held at  $-80^{\circ}\text{C}$ . At the end of the collection period the trap was heated to  $+80^{\circ}\text{C}$  for one minute and the sample was chromatographed immediately.

Coffman (1967) describes an apparatus somewhat less sophisticated in design than that of Herz and Chang, but nonetheless apparently quite effective for trapping oven vapors. In his system the oven vapors were first passed through an ice-chilled condenser before being passed into a train of six traps. In the first two traps, which were ice-chilled, a condensate collected that had a very objectionable odor until it was sprayed through an atomizer, at which time it exhibited a bread-like aroma. The condensate in the next two traps which were cooled by dry ice and in the following two liquid nitrogen-chilled traps had a fruity odor. The concentration of aroma constituents in bread was calculated by Coffman to be 50 to 100 parts per million. He preferred the above distillation method to use of ether extraction for obtaining his sample because the ether extraction process resulted in an odor change. The condensate obtained from distillation was then extracted with ethyl chloride followed by distillation in a water bath maintained between  $15$  and  $18^{\circ}\text{C}$  to remove

the ethyl chloride. Final yield was about one milliliter of concentrate from 48 loaves of baking bread.

Merory (1960) outlined four extraction methods considered to be suitable for removal of flavor components. Maceration was done by placing the food material in a cloth bag large enough to allow for expansion. Then alcohol was added (approximately 40 to 60 percent by volume) and the mixture was agitated twice a day for 5 to 8 days at which time the extract was drawn off. Water then was added to the residue and the fluid that was expressed from this mixture was added to the alcohol extract.

Digestion involved heating the food mixed with alcohol and water in a closed system at 140° F for 24 hours, but this was not considered to be as effective as maceration.

Percolation was accomplished by placing the food in a cloth bag resting in a cone-shaped colander and then covering the bag completely with alcohol throughout a three-day extraction period. On the fourth day percolation began. Percolation was accomplished by dripping alcohol through the bag of food at the rate of approximately 2 ml per minute. This 10-hour percolation was followed by a 24-hour extraction period in water after which the percolation procedure was repeated, this time using water. The alcohol and water collected from the two percolation periods were combined.

His fourth method of extraction was to soak the food with a



mixture of alcohol and water for 24 to 48 hours. The liquids collected from any of these four methods were then distilled.

Weurman (1963) has added a note of caution to the use of macerated samples because the enzymes present may cause formation of new flavoring compounds. The problem of flavor change must always be considered in any work on food flavors.

### Identification of Flavor Components

#### Preliminary Functional Group Tests

Weurman (1963) outlined simple preliminary tests to begin the identification process. These tests were conducted on a small amount of the press juice rather than on the concentrated final sample. If no odor change could be detected after dilute sulfuric acid was added to the press juice, it was concluded that there were probably no amines or bases present. An odor change following reaction of 2,4-dinitrophenylhydrazine with the press juice, was an indication that carbonyl groups were present. The addition of mercuric chloride to the press juice would cause an odor change if sulfides or mercaptans were present. The presence of esters or lactones would be detected by a loss of odor when the press juice was refluxed with alkali.

Walsh and Merritt (1960) developed a unique method for analyzing the functional group of each peak as it emerged from gas

chromatographic separation. Their technique required inexpensive equipment to be used in conjunction with a gas chromatograph equipped with effluent splitter. The exiting gas was directed through a three-way stopcock into a stream-splitting device consisting of 0.25 mm o.d. tubing equipped with a rubber serum cap through which five hypodermic needles of the same gauge had been inserted until their tips were exposed within the tubing. These needles effected a 5-way effluent split. In turn the base of each needle was immersed in a selected reagent contained in a vial. For convenience the five vials were held in a simply-designed cork platform. A separate set of reagents was prepared for each peak to be analyzed. By use of the three-way stopcock the next set of reagents could be positioned at the other outlet while a peak was passing and then the gas flow could be immediately directed to the fresh set of reagents at the appropriate moment. If more than five reagents were tested, it was necessary to do a second run rather than adding more reagents to the group. The reagents and functional groups tested were:

Alcohols: Nitrochromic acid (10 drops of 7.5 N nitric acid plus

1 drop of 1% potassium dichromate. Turns from yellow to blue. Good for primary and secondary alcohols. The ceric nitrate was also recommended as a test for all aliphatic alcohols.

Aldehydes: 2,4-Dinitrophenylhydrazine reagent (10 drops).

Yellow or orange precipitate forms. Schiff's reagent may be used to distinguish aldehydes.

**Ketones:** 2,4-Dinitrophenylhydrazine reagent (10 drops).

Yellow or orange precipitate.

**Esters:** Ferric hydroxamate reagent (10 drops of 1 N hydroxylaminehydrochloride in methanol plus 3 to 4 drops of 2 N alcoholic potassium hydroxide. After passing sample through, 5 to 6 drops of 2 N hydrochloric acid were added until solution was clear and colorless and then 1 to 2 drops of 10% ferric chloride were added. Esters caused a red color.

**Alkyl halides:** Mercurous nitrate solution (10 drops of 7.5 N nitric acid - 5% mercurous nitrate solution). Iodides, yellow to orange precipitate; chlorides, white precipitate; bromides, white or gray precipitate.

**Amines:** Rimini and Simon test for primary and secondary amines (10 drops of water, 2 drops of acetone, and 1 drop of 1% sodium nitroprusside). Primary amine gives red color. Add 1 to 2 drops of acetaldehyde. Secondary amine gives blue color.

**Mercaptans:** Sodium nitroprusside reagent (10 drops of 95% ethyl alcohol plus 2 drops of 5% potassium cyanide - sodium hydroxide solution). Two to three minutes after

sample passes add 5 drops of 1% sodium nitroprusside solution. Red color results. This test also indicates alkyl sulfides and disulfides.

**Aromatic nucleus and aliphatic unsaturation: LeRosen test**

(10 drops of concentrated sulfuric acid plus 1 drop of 37% formaldehyde. Wine color results.

Bassette, Ozeris and Whitnah (1962) used a somewhat different approach to the identification of functional groups. A dilute aqueous sample of selected aldehydes, ketones, esters, and sulfides was treated with acid hydroxylamine reagent in a serum vial, capped, mixed at room temperature for one hour and then treated with sodium sulfate. The headspace gas was chromatographed and the chromatogram compared with one made from the sample headspace gas prior to the hydroxylamine treatment. The peaks that were removed by this treatment were identified as those representing carbonyl or ester compounds. In a similar manner, sulfide compounds were detected by comparing chromatograms made before and after the sample was reacted with mercuric chloride. In tests that were a part of this same group of experiments it was demonstrated that the use of sodium sulfate was efficacious in increasing peak heights in comparison with untreated samples.

### Identification of Carbonyl Compounds

Carbonyl compounds have been identified by numerous workers and are considered to be significant components of food flavors. One of the most useful reactions employed in the identification of aldehydes and ketones is that of 2,4-dinitrophenylhydrazine with the unknown mixture. The aldehydes and ketones form 2,4-dinitrophenylhydrazones which can be separated. The melting point of the individual types of crystals provides one useful means of identifying these compounds (Cheronis and Entrikin, 1963; Shriner, Fuson and Curtin, 1964).

Resolution of mixtures of carbonyls into classes and then into homologs in a series is very useful in the identification of aldehydes and ketones. This is most often done by a combination of column and paper chromatography. Particular stress has been placed on the importance of using carbonyl-free reagents in these separations (Hornstein and Crowe, 1962; Schwartz and Parks, 1961). Schwartz, Parks and Keeney (1962) used column chromatography to separate a mixture of carbonyls into four classes: methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals. Separation was accomplished on a chromatographic column, 3 cm i.d.  $\times$  30 cm, packed with 10 g Celite 545 (dried 24 hours at 150° C) and 10 g magnesia made into a slurry with 90 ml of 15% solution of chloroform in high purity,

redistilled n-hexane. The mixture of 2,4-dinitrophenylhydrazone crystals was dissolved in chloroform and hexane before being applied to the column. Development of the column was accomplished with 150 ml of 15% chloroform in hexane, 150 ml of 30% chloroform in hexane, 100 ml of 60% chloroform in hexane, and finally with chloroform alone. The eluent was collected in 5 ml fractions and the percent transmission was measured. Methyl ketones exhibited maximum transmission at 365, saturated aldehydes at 355, 2-enals at 373, and 2,4-dienals at 390 m $\mu$ .

Corbin, Schwartz and Keeney (1960) have used a similar method to separate homologs of a series. They used Celite, hexane, and acetonitrile for the column and employed equilibrated hexane (hexane decanted from an acetonitrile-hexane mixture) as the developer. Again, a Beckman Model B spectrophotometer was used to read optical density.

Bidmead (1963) recommended the use of a column containing alumina and magnesium sulfate for separation of saturated and unsaturated aldehydes, ketones, keto-esters, and dicarbonyl compounds. Miller, Johnson and Robinson (1961) used a column of Hyflo Super Cel followed by descending paper chromatography and elution with chloroform to separate carbonyl compounds occurring in pre-ferments.

Alternative methods using paper chromatography have also

served as effective means of separating classes and homologs within a series. Ascending paper chromatography was the technique used by Ellis and Gaddis (1959) to resolve a mixture of 2-alkanones, alk-2-enals, alkanals, and alk-2,4-dienals. The 2,4-dinitrophenylhydrazones were dissolved in carbon tetrachloride for this method of chromatography. The strips used were Whatman #3 paper treated with petroleum ether. The homologous classes derived from this technique could then be separated into their individual components by using strips impregnated with 20% propylene glycol-80% methanol by volume and air dried for one hour (Ellis, Gaddis and Currie, 1958). The paper strips were spotted with the 2,4-dinitrophenylhydrazones and placed in a tube containing 5 ml of a 96% Skellysolve-4% methanol mixture. This procedure was effective in separating homologs having one to six carbon atoms; strips dipped in 7% vaseline in Skellysolve and developed in 6 ml of 89% methanol and 11% water were used to separate homologs having seven to 14 carbon atoms.

Bayer (1966) identified carbonyl compounds in an unknown mixture by first subjecting the sample to gas chromatography. Then he reacted the sample with 2,4-dinitrophenylhydrazine to remove the carbonyl peaks and compared this chromatogram with the one from the untreated sample. Ryder (1966) reacted a sample with sodium bisulfite to obtain a carbonyl-free neutral fraction. Gas chromatography of this sample then showed a pattern of alcohols and esters

with the aldehydes and ketones removed.

Miller, Johnson and Robinson (1961) regenerated the carbonyls from the 2,4-dinitrophenylhydrazones by heating them in alpha-ketoglutaric acid. The regenerated carbonyls then could be injected into the gas chromatograph. Regeneration of carbonyls from 2,4-dinitrophenylhydrazones was also reported by Keeney (1957). He used levulinic acid as the source of carbonyl groups. After heating the 2,4-dinitrophenylhydrazones in levulinic acid over a steam bath for five minutes, the volatile carbonyls were recovered by steam distillation or extraction with petroleum ether followed by washing of the extract with dilute sodium carbonate and evaporation of the ether.

The comparison of retention times of carbonyl peaks with retention times of known carbonyl compounds, together with chemical verification, provides highly reliable information for the identification of unknowns. This is a procedure commonly used by workers in flavor research. However, Soukup, Scarpellino and Danielczik (1964) have developed an excellent supplemental means of identification of carbonyl compounds that is more rapid and has fewer limitations than column or paper chromatographic procedures. These workers subjected the 2,4-dinitrophenylhydrazone carbonyl derivatives to gas chromatography. They dissolved the derivatives in benzene for injection on a column packed with 60 to 80-mesh acid



washed Chromosorb W coated with 10% General Electric silicone SF-96. This column was operated at 250° C, injection temperature 275° C, and helium flow at 86 ml per minute. Under these conditions effective separation of the 2,4-dinitrophenylhydrazones was accomplished and the unknown could then be compared with chromatograms of known 2,4-dinitrophenylhydrazones as standards run under the same conditions. This provided another means of verifying the identification of compounds present in the flavor concentrate.

#### Identification of Other Compounds

Although the chemical identification of compounds having functional groups other than the carbonyl has received less attention than has the carbonyl group, some work has been done with chemical reactions of esters, alcohols, and aromatic acids. Bidmead (1963) has reported the conversion of alcohols to 3,5-dinitrobenzoates for identification purposes. These 3,5-dinitrobenzoate derivatives were then subjected to paper chromatography. Bayer (1966) studied ester fractions by adding hydroxylamine to yield the hydroxamic acids and these were then separated as iron chelates by paper chromatography. This procedure permitted estimation of the number of carbon atoms in the acid portion of the ester. The number of carbon atoms in the alcohol moiety could then be determined by subtracting this number from the total carbon atoms in the ester. He

detected aromatic acids by examining the ultraviolet spectrum of the hydroxamic acid.

Ryder (1966) outlined a method for identifying the acid and alcohol components of the esters remaining in an unknown flavor mixture after removal of the carbonyl compounds had been effected. Reduction with lithium aluminum hydride broke the ester linkage, resulting in an increase either in the size or in the number of peaks representing the alcohols in the chromatogram. Any unsaturation in the released alcohols was not altered by the chemical reaction. The acids resulting from the cleavage of the esters were then esterified with diazomethane and studied as their methyl esters.

The use of melting points of derivatives presents another possible means of identification of unknowns (Cheronis and Entrikin, 1963; Shriner, Fuson and Curtin, 1964). Formation of 3,5-dinitrobenzoates and p-nitrobenzoates is a feasible means of identifying unknown alcohols. Carboxylic acids derived from esters may undergo reaction to form either the p-toluidide or the anilide. Useful derivatives of the amines are the benzene-sulfonamides and the benzamides. Mercaptans and thiophenols may be converted to 2,4-dinitrophenylthioethers or 3,5-dinitrothiobenzoate.

#### Flavor Components Identified in Various Foods

Identification of numerous flavor components has been reported

for several fruits and vegetables, milk and milk products, beverages, and flesh foods (Stevens, Bomben and McFadden, 1967; Wick et al., 1966; Day, 1966; Hornstein, 1967; Lindsay, 1967; Konigsbacher and Donworth, 1966; Tarr, 1966; Self, 1967; Jennings, 1967; Gianturco, 1967; and Silverstein, 1967).

In comparison with the work in these food categories there has been little work done on flavors of cereals and cereal products. The most activity in this realm has been in the analysis of bread flavors and flavor components of pre-ferments. Ng, Reed and Pence (1960) identified acetaldehyde, ethyl pyruvate, and furfural as constituents of fresh white bread. These workers also identified isobutyraldehyde and n-valeraldehyde in samples of the oven vapors, but were unable to find these two aldehydes in the baked sample. Wiseblatt and Kohn (1960) identified acetaldehyde, acetone, 2-ethyl hexanal, 2-hexanone, 3-heptanone, crotonaldehyde, diacetyl, pyruvaldehyde, and furfural aldehyde in fresh bread.

A review article by Johnson, Rooney and Salem (1966) summarized the organic acids found in pre-ferments, doughs, and bread by various workers and listed the following acids as flavor components: formic, acetic, propionic, n-butyric, isobutyric, valeric, isovaleric, caproic, isocaproic, heptanoic, caprylic, pelargonic, capric, lauric, myristic, palmitic, lactic, succinic, crotonic, pyruvic, hydrocinnamic, benzilic, itaconic, and levulinic. The

alcohols, in summary, from pre-ferments, oven vapors, and bread include: ethyl, n-propyl, isobutyl, amyl, isoamyl, 2,3-butanediols, and 2-phenylethyl. The carbonyl compounds isolated from pre-ferments, doughs, oven vapors, and bread were: formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde, isobutyraldehyde, n-valeraldehyde, isovaleraldehyde, 2-methylbutanal, n-hexaldehyde, crotonaldehyde, benzaldehyde, phenylacetaldehyde, pyruvaldehyde, furfural, hydroxymethylfurfural, methional, acetone, 2-butanone, 2-hexanone, 3-heptanone, diacetyl, and acetoin. Organic esters from pre-ferments and bread were: ethyl formate, ethyl acetate, ethyl lactate, ethyl pyruvate, ethyl levulinate, ethyl succinate, ethyl hydrocinnamate, ethyl benzilate, ethyl itaconate, and 1,3-propanediol monoacetate. Other compounds identified have been methyl mercaptan, hydrogen sulfide, isomaltol, maltol, and melanoidins. The significance for bread flavor of the melanoidins, brown polymers formed during browning, has not been determined.

Hodge (1967), as have other authors (Herz and Shallenberger, 1960; Kiely, Nowlin and Moriarty, 1960), observed that the oxidative degradation of alpha-amino acids to aldehydes or ketones with one less carbon atom occurred in some foods with simple heat treatment. This reaction is called the Strecker degradation reaction and is one of the pathways in the Maillard reaction. Practical applications of this degradation have been made in the synthesis of food flavors.

The Quartermaster Corps' Instant Bread Mix owes its bread-like aroma to the addition of leucine which reacts with the glucose present in the formula to produce the desired aroma. General Foods Corporation has autoclaved wheat bran and peanut flour with sugar and water briefly at 200 ° C to produce flavors resembling chocolate, tea, or coffee.

Linko, Johnson, and Miller (1962) report the following aldehydes formed from alpha-amino acids via Strecker degradation:

Isoleucine	2-Methylbutanal
Leucine	Isovaleraldehyde
Valine	Isobutyraldehyde
Methionine	Methional
Alanine	Acetaldehyde
Phenylalanine	Phenylacetaldehyde

Hodge (1967) pointed out that isobutyraldehyde, derived from valine, has an aroma of rye bread and isovaleraldehyde, derived from leucine, has a toasted, bready aroma. It was also noted that furfuraldehyde exhibited a fresh bread aroma when it was in very dilute concentration. Maltol was also mentioned as a compound contributing to a caramel-like aroma in cereal products.

Meister (1957) lists the following amino acid composition of wheat flour and whole wheat, calculated to 16 g of nitrogen.

<u>Amino acid</u>	<u>Wheat flour</u>	<u>Whole wheat</u>
Arginine	3.9	4.3
Histidine	2.2	2.1
Lysine	1.9	2.7
Tyrosine	3.8	4.0
Tryptophan	0.8	1.2
Phenylalanine	5.5	5.1
Cystine	1.9	1.8
Methionine	2.0	2.5
Threonine	2.7	3.3
Serine	4.3	4.3
Leucine	7.0	7.0
Isoleucine	4.2	4.0
Valine	4.1	4.3

It can be seen from these data that the parent compounds for some of the volatile substances found in bread are present in substantial amounts in the flour.

Yasumatsu, Moritaka and Wada (1966b) positively identified acetaldehyde and n-caproaldehyde as two of the five carbonyl compounds they found in the vapor of cooked rice. The other three peaks were tentatively identified as methylethylketone, n-valeraldehyde, and either propionaldehyde or acetone. The stale flavor of rice stored two months was subsequently studied and three peaks

were identified: propionaldehyde or acetone, n-valeraldehyde, and n-caproaldehyde (Yasumatsu et al., 1966a). These workers added n-caproaldehyde to fresh rice and most of their taste panel members stated that this sample had the flavor of stored rice. N-caproaldehyde (n-hexanal) has been found to be the main compound responsible for off-flavor in potato granules (Buttery, Hendel and Boggs, 1961; Boggs et al., 1964, peas (Bengtsson and Bosund, 1964), and potato chips (Mookherjee, Deck and Chang, 1961).

A possible source of some of the carbonyls in wheat may be traced to the presence of oils in the germ of the grain. According to Eckey (1954), two percent of the whole wheat grain is fat, and of this fat approximately 44 to 65 percent is linoleic acid, 8 to 30 percent is oleic acid, and 4 to 10 percent is linolenic acid. Most of the fat is contained in the germ. Gaddis, Ellis and Currie (1961) studied the carbonyls in oxidizing fat that was unheated and also in samples heated at 165°C for 15 minutes. In their work it was found that hexanal was present in significant amounts in the trilinoleate sample and in measurable amounts in trioleate. This aldehyde might therefore be expected in wheat samples containing the germ. Dec-2,4-dienal was also identified in the trilinoleate sample, and undec-2-enal, nonanal, and octanal were components of the trioleate sample. The two major aldehydes from linolenate were hept-2,4-dienal and propanal. In addition several other aldehydes were contained in very small amounts.

## EXPERIMENTAL PROCEDURE

### Isolation of Wheat Flavor Fraction

#### Preliminary Work

The isolation of flavor components from various foods and food mixtures has been accomplished in several research laboratories using a variety of techniques. The one finding common to all investigations was that the desired flavor fraction was always obtained in discouragingly small quantities, usually in amounts of only a few parts per million. Extreme care was required to retain these minute quantities of readily vaporized materials.

In the study of wheat flour, herein reported, several procedures were followed to isolate the flavor fraction of wheat. Preliminary trials included a prolonged extraction and percolation with ethanol, a similar extraction and percolation utilizing distilled water, a freezing out procedure, steam distillation, and vacuum distillation.

Ethanol Extraction and Percolation. Two pounds of 100 percent unbleached whole wheat flour were placed in a large glass jar and thoroughly mixed with two liters of 50 percent ethanol. This mixture was covered with aluminum foil and allowed to remain at ambient temperature for three days. The botanicals were stirred three times the first day, once on the second day, and one time on the third



to optimize contact of the ethanol with all of the wheat flour. At the end of three days the botanicals were stirred and then poured into a heavy muslin bag suspended above a battery jar preparatory to percolation as outlined by Merory (1960). The filtrate was collected in this large battery jar. Then the muslin bag was placed in a perforated metal cone suspended above a smaller battery jar. A separatory funnel containing the previously obtained filtrate was positioned just above the muslin bag and the flow rate was adjusted so that the dropwise delivery from the separatory funnel was approximately equal to the flow of filtrate from the bag into the battery jar. This percolation procedure was continued until all of the original filtrate had passed into the wheat flour sample. The muslin bag was firmly pressed to complete the removal of as much of the alcoholic extract as possible. The sample obtained from this alcohol extraction and percolation was a clear amber color with a wheaty aroma of moderate intensity.

Water Soaking and Percolation. The efficacy of an aqueous extraction was also tested following the same general procedure as outlined above, but using water rather than alcohol as the extraction medium. To control fermentation, the sample was refrigerated for a one-day soaking period and then percolated to obtain the desired filtrate. This sample had a distinctly wheat-like aroma.

In an attempt to recover some additional quantities of the

aromatic substances that obviously were still retained in the wheat residue from both the alcoholic and aqueous extractions, the botanicals were pressed by means of a Carver Hydraulic Press. The press fluids obtained did not have much odor, but the press cake smelled distinctly wheat-like. It was apparent that much of the flavor had not been stripped from the wheat flour by either of the above methods. However, there was a sufficiently strong odor in the aqueous sample to encourage further concentration of the flavor components present in it.

The flavor fraction in the aqueous extract of the unbleached whole wheat flour was extracted with ethyl chloride. The aqueous extract was first saturated with sodium chloride to facilitate removal of the desired volatile substances into the ethyl chloride phase. Three extractions with ethyl chloride were carried out to remove the organic flavor substances from the water phase. Then approximately half of the ethyl chloride was evaporated and the sample was injected into the gas chromatograph. The results of the chromatographic analysis indicated that a greater concentration of the desired flavor substances was needed if progress were to be made in this realm of flavor research on wheat.

Freezing Out. The freezing out of flavor components to achieve a much greater concentration of these substances has been developed by Bidmead (1963). His method was deemed a reasonable approach

to obtaining a sample sufficiently concentrated to test in the gas chromatograph. In this method a large pan was lined on the bottom and sides with dry ice and a smaller pan containing the aqueous extract was placed on this bed of dry ice. The space remaining around the inner pan was filled with crushed ice. Five hundred twenty-five milliliters of a saturated solution of calcium chloride were poured over the crushed ice. The aqueous mixture was agitated slowly by using one beater blade in an electric mixer set on speed one. The purpose of this procedure was to separate the water from the desired organic substances on the basis of difference in freezing points, but the method was extremely slow and the results in these preliminary tests appeared to be of questionable utility.

Steam Distillation. Since the aqueous extract had the true whole wheat aroma, a steam distillation process offered the possibility of collecting volatiles having the wheat aroma. Whole wheat flour in water was placed in a round-bottomed flask fitted with a condenser. Steam was introduced at the bottom of the wheat. The condenser was packed in ice and the receiving flask in dry ice, as recommended by Coffman (1967). This method resulted in collection of a small quantity of distillate that had a relatively intense aroma of wheat. To further concentrate the volatiles, the aqueous distillate was extracted with ethyl chloride. Subsequently, most of the ethyl chloride was distilled off by placing the sample flask in a

water bath maintained with the addition of ice within the temperature range of 15 to 18°C. The yield from the laboratory-scale distillation was too small to provide even the micro samples needed for gas chromatography. This method showed considerable promise if it could be implemented on a commercial-sized operation. Of course, this situation was not available for the project.

Vacuum Distillation. Vacuum distillation using the actual wheat flour sample boiling in water offered a practical possibility for obtaining a suitable sample. This was shown by a preliminary small-scale experiment in which 200 grams of wheat were heated under a moderate vacuum and the distillate collected in a trap chilled with chipped ice. The distillate had a strong wheat odor.

#### Preparation of the Wheat Flavor Essence

For the actual research, steam distillation of a fresh, lightly milled soft wheat (Moro) from eastern Oregon was accomplished utilizing facilities available at the Department of Food Science and Technology (Figure 1). A sample consisting of 1500 grams of the wheat carefully mixed with 6.5 liters of distilled water saturated with sodium chloride was introduced into an 18-liter, two-necked flask resting in a heating mantle. The sodium chloride was used to eliminate any possible yeast activity and to facilitate removal of the flavor components. Steam was led into this flask via a piece of glass tubing fitted with a special head that split the steam flow into six streams. This device introduced steam

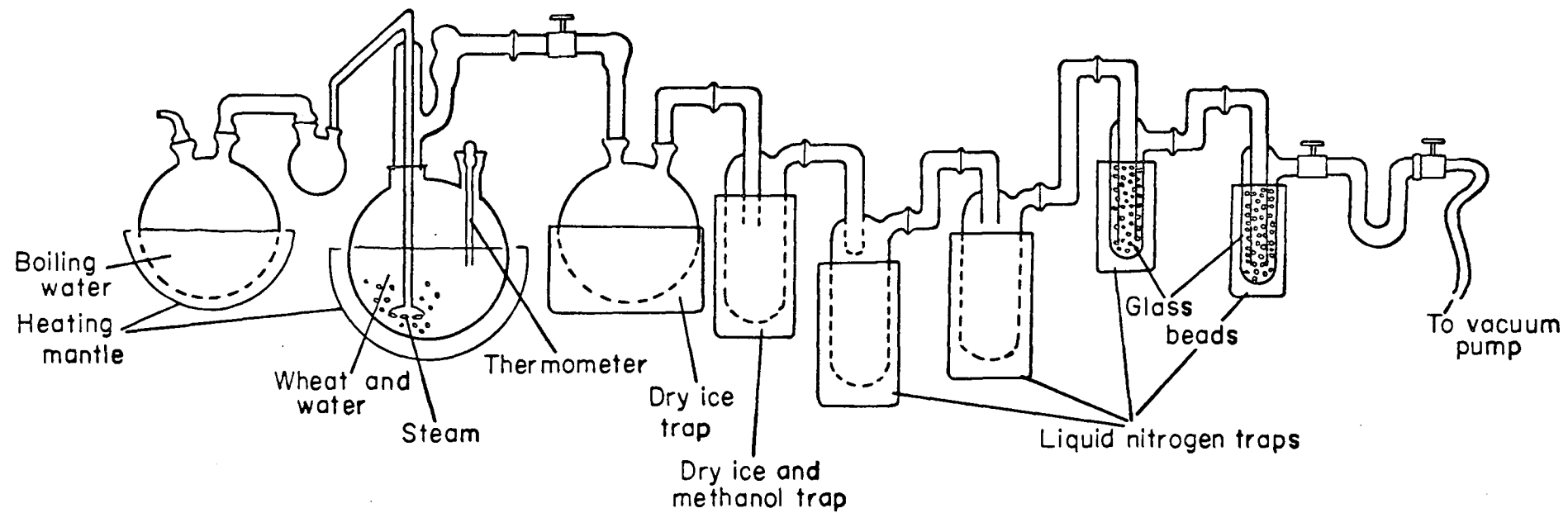


Figure 1. Apparatus for vacuum distillation of wheat flavor volatiles

beneath the surface of the sample and provided considerable agitation of the wheat mixture. Such action permitted effective extraction of all parts of the sample and prevented the caking that may present a problem with starch and cereal samples. A glass tube equipped with a thermometer was sealed into the second neck of the sample flask. The thermometer extended into the sample to permit continuous monitoring of the distillation temperature. The vapors from the agitated water suspension of wheat were led through glass tubing to the traps used for capturing the volatile components.

The first trap was a large, round-bottomed flask completely encased in dry ice. The majority of the water vapor from the sample and some of the flavor volatiles were condensed in this trap. The second trap in the train was a large pear-shaped flask suspended in a Dewar containing a mixture of dry ice and ethanol. A Dewar containing liquid nitrogen housed the third trap, another large pear-shaped flask. The fourth trap was identical to the third trap in all details. The last two traps were smaller, cylindrical traps containing glass beads. Liquid nitrogen was used to chill both of these.

The entire system was under vacuum (approximately three millimeters of mercury). At this reduced pressure it was possible to accomplish an effective distillation while maintaining the sample at approximately 30° C, with the temperature never exceeding 35° C. This low temperature helped protect the wheat volatiles from chemical

change during the prolonged distillation period. Distillation was continued for four hours.

At the end of the distillation period the traps were quickly warmed to free the frozen condensate from the walls before the super-cooled ice could expand and crack the traps. All of the traps had a similar odor although the first traps had a stronger odor than did the last ones. Because of the similarity of aroma in the traps, it was decided to combine the melted condensate from all the traps into the first and largest trap. Careful attention was given to removing all traces of stopcock grease from the neck joints of each trap before removing the condensate. Anhydrous diethyl ether was used for this task. After removal of the condensate, each of the flasks was rinsed with anhydrous diethyl ether to remove all traces of wheat-like odor from the trap. Rinsing was repeated until no odor could be detected. Sodium sulfate was then added to saturate the total distillate as an aid in the extraction of the volatiles. Sodium bicarbonate was added to neutralize acids present in the distillate.

Extraction was effected with anhydrous diethyl ether which was vigorously shaken with the sample in a tightly-closed container. The aqueous-ether mixture was then allowed to stand until the two phases separated, at which time the ether fraction was carefully decanted into a round-bottomed collection flask. The procedure was repeated several times with the addition of fresh ether until

the aqueous portion had only a mild wheaty aroma. The ether fraction collected had a strong wheat-like aroma that could be detected by dipping a tissue in it and then smelling it after the ether had evaporated into the air.

At this stage the volatiles were dissolved in approximately 1800 milliliters of ether. Ether was removed by distillation until the volume was reduced to approximately 125 milliliters. The distillation apparatus for this step was equipped with a column one meter long packed with glass helices. This column was traversed by the ether before entering the condenser. This precaution was taken to minimize loss of the flavor volatiles during the distillation of the ether. The round-bottomed flask was sealed to the column with a layer of calcium sulfate to reduce loss of volatiles at the connection junction.

When a volume of 125 milliliters was reached, the sample was transferred to a 250 milliliter round-bottomed flask and distillation was continued in a distillation apparatus equipped with a fractionating head adjusted to give a ratio of 1 to 3, that is, with one drop passing out through the condenser for every three drops that were passed back into the system. This ratio was maintained by setting the off cycle of the regulator at three and the on cycle at two. The heating mantle was turned to a power setting of 35. Again, a slurry of calcium sulfate was used to seal the sample flask to the



column. This fractional distillation was continued until only 15 milliliters of sample remained. The system was then turned off and the column drained completely before removal of the sample flask.

Final concentration of the sample was accomplished at room temperature. A portion of the remaining 15-milliliter sample was carefully poured into a two milliliter vial especially designed for holding the extremely small samples that must be so carefully conserved in flavor analysis work. This vial had a needle-shaped base with a capacity of 0.5 milliliter extruding from the upper portion, which had a diameter adequate to accommodate the barrel of a syringe. The vial, equipped with a ground glass stopper, had a total capacity of two milliliters. . Evaporation to final concentration was achieved by inserting a small piece of aluminum foil over the lip of the vial and replacing the stopper loosely. As volume was reduced, additional sample was added until all the ether-containing sample had been added to the vial. This final sample was also evaporated until only a very small amount of ether remained. At this point, 0.15 milliliters of sample remained. The aluminum foil strip was removed, the vial tightly stoppered, and the stopper joint tightly covered with aluminum foil. Freezer storage further curtailed loss of the volatile sample.

### Headspace Vapor Analysis

Headspace vapor analysis was accomplished by distillation at atmospheric pressure (Figure 2). The sample was prepared by combining and stirring 200 grams of lightly milled, fresh wheat from eastern Oregon with 600 milliliters of distilled water. This wheat suspension was then placed in a three liter round-bottomed flask resting in an oil bath. The flask was equipped with a distillation apparatus. One arm of this glassware housed a glass tube to carry steam from its source and introduce it underneath the surface of the sample. The vapors from the sample were passed through the sidearm of the distilling apparatus and into the condenser. Just before entering the condenser the sample was passed over the bulb of a removable thermometer that was introduced into the system through a ground glass fitting.

To operate this system water was first heated in an Erlenmeyer flask until steam began to pass across to the sample flask. Then the oil bath was quickly heated. When the sample began to boil actively, the heating element was placed on the low setting which maintained an active boil. As soon as the thermometer registered 95° C, a 10-milliliter syringe was held right next to the thermometer and quickly inserted in the thermometer hole as the thermometer was removed. The barrel of the syringe completely covered the

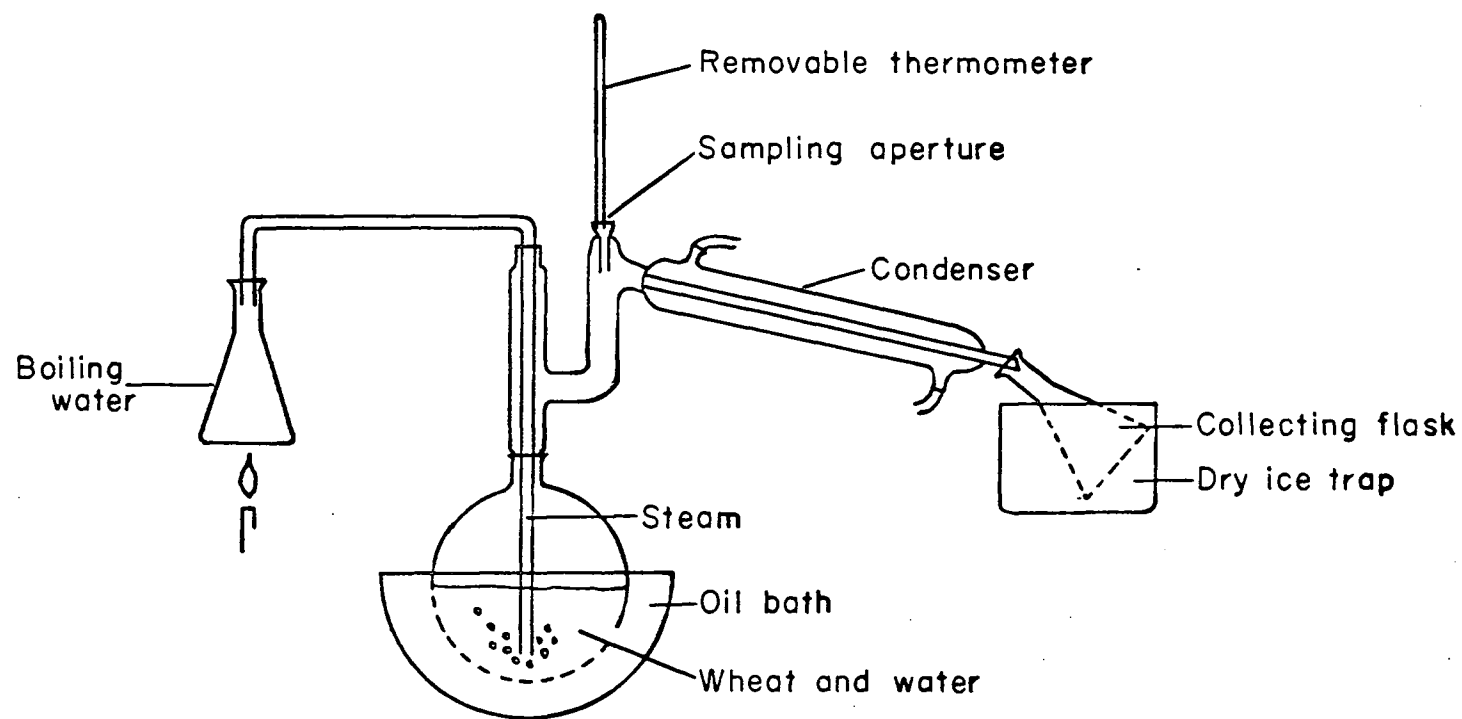


Figure 2. Apparatus for collection of head space vapor sample

hole and thus prevented excessive loss of volatiles while the sample was being taken. In this position the open end of the sampling needle was close to the center of the main stream of volatiles and an adequate sample, smelling strongly of wheat could readily be drawn into the syringe. The extremely volatile nature of this headspace vapor sample made it imperative to immediately inject this sample into the gas chromatograph. To minimize transit time from sampling to injection the distillation was carried out in a hood immediately adjacent to the gas chromatograph.

### Instrumental Description

Two instruments were used to analyze the volatiles from wheat. A gas-liquid chromatograph was employed to separate the components present in both the wheat essence and in the headspace vapors and to provide data on their retention times. The mass spectrometric analysis supplemented the information obtained from the gas chromatograms and confirmed the identification of certain compounds.

#### Gas Chromatography

The Gas-Liquid Chromatograph. The gas-liquid chromatograph used in this study, a Barber-Colman Model 10 equipped with a flame ionization detector, is shown in Figure 3. The sweep gas used for the column was nitrogen and the flame for the detector was supplied



Figure 3. The Barber-Colman gas-liquid chromatograph.

with a mixture of hydrogen and compressed air.

The sample was injected by means of a syringe into the injection port into which a tight-fitting septum had been inserted. Immediately the sample was vaporized by the flash heater and swept onto the column by the flow of nitrogen gas. As the sample components emerged from the column they could proceed to the flame ionization detector and ultimately be recorded on the chromatogram or they could be diverted outside the machine for olfactory or chemical analyses.

U-shaped glass columns six feet long with an inner diameter of one-fourth inch were suitably housed in the gas-liquid chromatograph.

Column Preparation. Column packings were prepared by dissolving the weighed, desired amount of stationary phase in a large excess of methylene chloride. This liquid was then stirred thoroughly into the support that had been selected. Additional methylene chloride was stirred in until quite a thin slurry resulted. This mixture, which was spread into a thin layer in a rectangular Pyrex container, was placed in a hood overnight or until the mixture was completely dry and all the methylene chloride had evaporated.

This mixture was then funneled into both arms of the U-shaped column, which was suspended from a ring stand. To facilitate tight packing of the column, a Vibra-graver was applied to the column

frequently during the packing operation. Filling of the column was continued until the packed material was within four inches of the exit port and within six inches of the injection port. A small quantity of glass wool was inserted in the sidearm, in the injection port, and the exit port when packing was completed. A thru-hole septum to accommodate the capillary tubing carrying the nitrogen was securely positioned at the entrance to the sidearm of the column. A similar thru-hole septum was placed in the exit port, and a half-hole septum was securely wedged into the injection port.

All of the column packings used in this research utilized Anakrom ABS (acid and base washed and vacuum siliconized) as the solid support. The Anakrom ABS was produced by Analabs of Hamden, Connecticut. The percentage and type of stationary phase were varied.

### Mass Spectrometer

An Atlas-MAT CH-4 mass spectrometer (9-inch, 60-degree sector, single-focusing instrument) was used in conjunction with a Perkin-Elmer Model 226 gas chromatograph to obtain a mass spectral analysis of the wheat flavor essence. The spectra of the eluting peaks were recorded by a Honeywell Model 1508 Visicorder. The rapid magnetic scan from  $m/e$  25 to 200 required two seconds.

## Instrumental Analysis

### Chromatography of the Wheat Flavor Essence

For gas chromatography of the wheat essence sample obtained by vacuum distillation, the following conditions were found to give the best results. The sample size used was 0.2 microliters. Nitrogen pressure to the column was five pounds per square inch; compressed air was maintained at a pressure of 49 pounds per square inch and hydrogen pressure to the flame detector was 19 pounds per square inch. Column temperatures were isothermal at 75° C for most of the analyses, but some trial runs were made using column temperatures of 50° C and 100° C. A voltage setting of 54 percent maintained the cell bath at approximately 225° C and the same setting on the flash heater maintained the temperature of this heater between 150 and 160° C.

The recorder was set with the attenuator at 64 and the sensitivity at 1000 when these samples were being chromatographed. Chart speed was one inch per minute.

Samples were chromatographed on the following stationary phases: 6 percent Apiezon M, 20 percent Apiezon M, 3 percent Free Fatty Acid Phase (FFAP), and 6 percent Diethylene Glycol Succinate (DEGS).



The Free Fatty Acid Phase was obtained from Varian Aerograph of Walnut Creek, California, and the other two stationary phases were procured from Wilken Instrument and Research Company, also of Walnut Creek, California.

### Chromatography of the Headspace Vapor

Chromatograms of the headspace vapor were obtained using the same operating conditions as were used to analyze the wheat essence. However, since the sample was gaseous rather than in the form of the liquid essence, it was necessary to use a considerably larger sample. A syringe with a 10-milliliter capacity was used to collect the vaporous 5,000 microliter (5-milliliter) headspace sample and inject it into the gas chromatograph.

Chromatograms of headspace vapors were obtained at 75 and 100° C on 3 percent FFAP, at 50, 75, and 100° C on 6 percent Apiezon, at 75° C on 6 percent DEGS, and at 75° C on 2 percent SE-30 (Wilken Instrument and Research Company, Walnut Creek, California).

### Chromatography of Standard Compounds

To assist in identification of the various compounds present in the flavor fraction it was necessary to chromatograph known compounds to serve as standards against which the retention times of the unknowns could be checked. To obtain a chromatogram which

showed the apex of the peak it was necessary to set the sensitivity indicator at 100 rather than the 1000 setting used for analysis of the unknowns, and reduce the sample size to the small amount that was retained only in the needle of the syringe (about 0.7  $\mu$ l). All other operating conditions were identical with those used in operating the gas chromatograph during the analysis of the unknown. These two modifications did not in any way alter retention time.

### Retention Times

Retention times were determined for each observable peak by measuring the distance on the base line of the chromatogram from the point of injection until the point of maximum peak height. Measurements were then converted to retention time in seconds. One inch on the chromatogram equaled 60 seconds of retention time. The retention times for the unknowns were compared with those of the standards run under comparable conditions.

### Mass Spectrometry of the Wheat Flavor Essence

Parameters for analyzing the wheat essence were established by preliminary work with the sample in a Perkin-Elmer Model 226 gas chromatograph using a 300 foot by 0.01-inch i.d. stainless steel capillary column coated with Butanediol Succinate as the liquid stationary phase. The carrier gas employed was helium. On the

basis of the work with this gas chromatograph the operating conditions for the mass spectral analysis were established.

A one microliter sample of the wheat essence was injected into the gas chromatograph which was operated isothermally at 60° C for the first 15 minutes and then was programmed to heat at the rate of 4° C per minute to a final hold temperature of 182° C. The effluent from the gas chromatograph was introduced directly into the mass spectrometer. During the run, 46 spectra were taken. The automatic shut-off switch had to be overridden during the first part of the analysis because of the massive ether peak. With this equipment interpretable spectra can be obtained on compounds present in amounts of 0.1 µg or more.

### Paper and Column Chromatography

The relative merits of column and paper chromatography for the separation of carbonyl compounds were explored. Paper chromatography to resolve 2,4-dinitrophenylhydrazone derivatives of unknowns into classes was done according to the procedure outlined by Gaddis and Ellis (1959). For these tests Whatman #3 filter paper was cut into strips  $10\frac{1}{2}$  by  $1\frac{3}{8}$  which were tapered just enough to fit into a 250 milliliter graduated cylinder. The 2,4-dinitrophenylhydrazone derivatives obtained from the above-mentioned aqueous extract were dissolved in methanol and methyl acetate. The

strips were spotted with 30 drops of the dissolved 2,4-dinitrophenylhydrazones and then carefully placed vertically in graduated cylinders containing five milliliters of redistilled petroleum ether. A rubber stopper was quickly applied to seal the cylinder. The development continued until the solvent front had moved 8 1/4 inches. Usually the various bands could be distinguished fairly easily, but if they were too faint, they could be made apparent by quickly dipping the strip in 10 percent potassium hydroxide solution. The bands were then cut out and reserved for further chromatography to separate the compounds within a class.

The classes of carbonyls into which the unknowns could be separated were the 2-alkanones, the n-alkanals, the alk-2-enals, and the alk-2,4-dienals. The 2-alkanone band could be identified at the top of the chromatogram as a beige-colored area, followed by the yellow alkanal band. Next came the yellow-orange band of the alk-2-enals, and the brown alk-2,4-dienal band was last. Some of the material remained at the origin. Formaldehyde and acetaldehyde traveled with the alk-2-enals rather than with the n-alkanals.

The various members of a class were chromatographed according to the method developed by Ellis, Gaddis and Currie (1958). The samples used for these analyses were obtained by eluting a class of compounds from the paper on which the previous chromatographic class separation had been achieved.

Elution was accomplished by placing the sample-containing paper in a small beaker and barely covering the paper with petroleum ether. The freed 2,4-dinitrophenylhydrazones were then spotted 1 1/4 inches from the bottom of a paper strip that had been impregnated with a 20 percent glycol-80 percent methanol (by volume) solution and then air-dried for one hour. The strip was then placed in a 250-milliliter graduated cylinder containing 5 milliliters of a 96 percent Skellysolve-4 percent methanol solution, and the cylinder was quickly closed with a rubber stopper. Further steps were the same as those used for separating the unknowns into classes. This treatment was designed to separate carbonyls within a class if the compounds contained not more than six carbon atoms.

A third paper chromatographic procedure was used to separate carbonyls within a class if the unknowns contained seven or more carbon atoms. The paper strips were prepared by dipping in a 7 percent solution of vaseline in Skellysolve and then air drying for 15 minutes. The samples used for spotting in this phase of the chromatographic separation were the same as those used to separate the carbonyls containing six or fewer carbons, as described in the preceding paragraph. The strips were developed in 6 milliliters of an 89 percent methanol-11 percent water solution. The approximate time required for the solvent front to move 8 1/4 inches in the stoppered cylinder was six hours, in contrast to the two-hour

development period for the other two separation procedures.

The 2,4-dinitrophenylhydrazone derivatives prepared by reaction of known carbonyl compounds with 2,4-dinitrophenylhydrazine were recrystallized from ethanol. These known derivatives were then dissolved in methanol and methyl acetate and chromatographed alongside the appropriate band obtained from the separation of unknowns by classes. The appropriate solvent system was used, depending upon whether the known was a carbonyl containing more or fewer than 6 carbons. Identification of a member of a class was considered to be positive if its location on the strip was coincident with the derivative of the known compound of the same class.

Column chromatography was done according to the method of Schwartz, Parks and Keeney (1962). A column 20 millimeters in diameter and 180 millimeters long equipped with a coarse fritted glass disc and clamped at the outlet was used for chromatographically separating 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes. The column was prepared by first filling the column with 15 percent chloroform in redistilled hexane and then slowly pouring in a mixture of 10 grams of magnesia and 10 grams of Celite 545 which previously had been slurried with 90 milliliters of 15 percent chloroform (ACS grade) in redistilled hexane and dried for 24 hours at 150° C. The Celite and magnesia were permitted to settle by gravity into a uniform packing.

Crystals of 2,4-dinitrophenylhydrazones, prepared by reacting equal quantities of the aqueous condensate from the vacuum distillation with the 2,4-dinitrophenylhydrazine reagent, were dissolved with limited success in 0.3 milliliters of chloroform and then 1.7 milliliters of hexane were added. This solution was carefully applied to the column. Undissolved 2,4-dinitrophenylhydrazones were not placed on the column. A small amount of glass wool was placed above the bed of the column. The column was developed by application of 150 milliliters of 15 percent chloroform in hexane, followed in order, by 150 milliliters of 30 percent chloroform in hexane, 100 milliliters of 60 percent chloroform in hexane, and finally by pure chloroform.

### Chemical Analyses

#### Functional Group Identification

Two chemical tests suggested by Weurman (1963) were used to determine the possibility of the presence of amines or carbonyl compounds in the total mixture. To some of the water extract a small amount of dilute sulfuric acid was added and the odor compared with the unreacted aqueous material. Subsequently this acidified mixture was then reacted with a few crystals of powdered 2,4-dinitrophenylhydrazine and the odor again noted.

Tentative identification of the functional groups of the unknown

compounds of the headspace vapors was made according to a method devised by Walsh and Merritt (1960). The first step was a careful programming of the time of emergence of each peak on the chromatogram. The gas chromatograph was then modified to direct the column effluent through capillary tubing to the outside of the machine. The gas flow was channeled into a three-way stopcock. The other two outlets of the stopcock were fitted with Tygon tubing which was terminated by a tight-fitting serum bottle cap through which needles could be inserted into the center of the tubing. Five hypodermic needles were then inserted an equal distance through the septum into the Tygon tubing. Each of these needles was directed into a sample vial just large enough to accommodate the Luer-lok end of the needle. The five vials holding the needles were secured in a large thermos cork, in which holes were bored to hold each of the vials (Figure 4). A cork platform equipped with five vials was prepared for each of the peaks to be collected. Each group of vials was filled with the following set of reagents:

Vial 1. Ten drops of 7.5 N nitric acid and one drop of one percent potassium dichromate.

Vial 2. Ten drops of 2,4-dinitrophenylhydrazine reagent.

Vial 3. Ten drops of 1 N hydroxylamine hydrochloride in methanol and three drops of 2 N alcoholic potassium hydroxide.



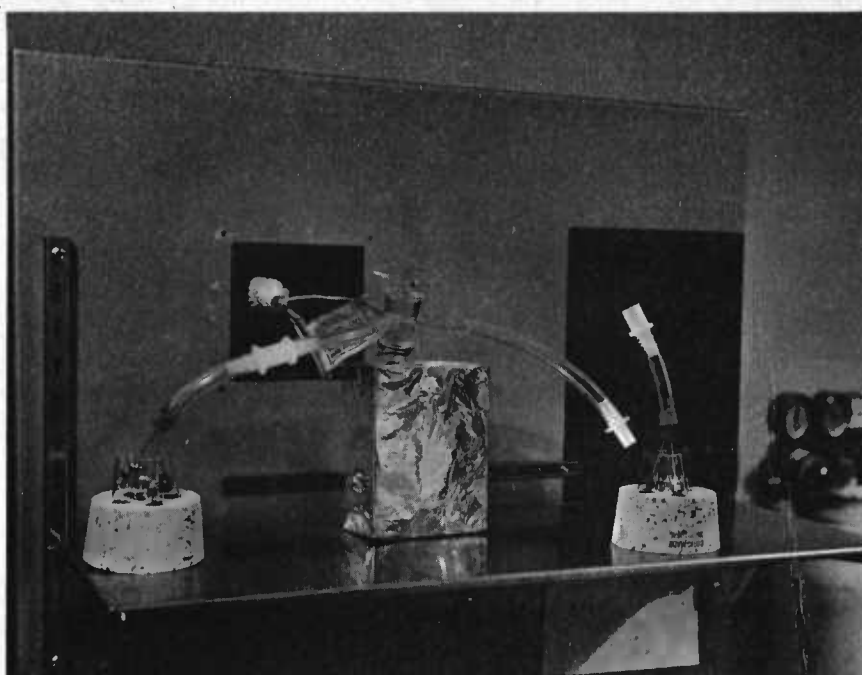


Figure 4. Apparatus for functional group analysis of the volatile components as they are eluted from the chromatographic column.

Vial 4. Ten drops of distilled water, two drops of acetone, and one drop of one percent sodium nitroprusside solution.

Vial 5. Ten drops of 95 percent ethyl alcohol plus two drops of five percent potassium cyanide-one percent sodium hydroxide solution.

The prepared vials were then lined up on a shelf at the front of the chromatograph ready for quick connection with the needle-containing apparatus.

A ten-milliliter sample of the headspace vapor was then injected into the gas chromatograph housing a DEGS-packed column at an isothermal temperature of 75° C. Immediately, timing was begun. The gas flow between 50 and 66 seconds was diverted into the first set of vials, the flow between 67 and 78 seconds into the second set, and that between 79 and 100 seconds was bubbled into the third set of vials. The last three peaks were collected at 105 to 130 seconds, 133 to 160 seconds, and 160 to 180 seconds. The total elapsed time from injection to completion of the sixth peak collection was three minutes. To insure accuracy in the critical timing and collection process, two operators were required: one announced the time and the other replaced the sets of vials and operated the stopcock.

As soon as possible after the sample was collected, five to six drops of 2 N hydrochloric acid were added to the third vial of

each set. Then two drops of ten percent ferric chloride were also added to this vial. To vial five, five drops of one percent sodium nitroprusside solution were added two to three minutes after collection of the sample. The reactions in each vial from each peak were noted. After the fourth vial had been examined, one to two drops of acetaldehyde were added and the color again noted.

### 2,4-Dinitrophenylhydrazone Derivatives

Preparation of 2,4-Dinitrophenylhydrazone Derivatives. One well-recognized means of identifying unknown carbonyl compounds is to form the 2,4-dinitrophenylhydrazone derivatives of the unknowns and establish identification on the basis of the melting points of the derivatives obtained.

The aqueous material that remained after the condensate from the vacuum distillation had been extracted with ether was reacted in equal quantities with the 2,4-dinitrophenylhydrazine reagent. This reagent was prepared by completely dissolving three grams of 2,4-dinitrophenylhydrazine in 15 milliliters of concentrated sulfuric acid and then slowly stirring this solution into a mixture of 20 milliliters of water and 70 milliliters of 95 percent ethanol. After thorough mixing, the solution was filtered and was ready for use (Shriner, Fuson and Curtin, 1964). After reaction of the 2,4-dinitrophenylhydrazine reagent with the sample, the crystals that formed

immediately were removed by filtration. Crystals continued to form in the filtrate when it was allowed to stand. Three additional filtrations were made at half-hour intervals. One final filtration was made approximately two days later. Recrystallization was done from ethanol.

#### Melting Points of 2,4-Dinitrophenylhydrazones Derivatives.

With the aid of a magnifying glass the various types of crystals were separated into groups and the melting points of individual crystals were determined. The melting point was obtained by inserting the sample into a capillary tube that had been heat-sealed at one end. This tube was then fastened to a thermometer; care was taken to be certain that the sample was positioned adjacent to the bulb of the thermometer with the sample clearly visible. The sample and the thermometer were then suspended in a Thiele tube containing a clear, light oil. The bulb and sample were centered near the outlet of the upper arm of the Thiele tube by use of a cork through which the thermometer was mounted. The oil was then slowly heated with a Bunsen burner until the sample melted. The temperature at which the sample melted was recorded. Slow heating was necessary to achieve an accurate melting point determination.

The melting points of the 2,4-dinitrophenylhydrazones were then compared with standard tables of the melting points of known derivatives (Shriner, Fuson and Curtin, 1964).

## RESULTS AND DISCUSSION

### The Headspace Vapor and Wheat Flavor Essence

One of the pitfalls of the flavor researcher is the possibility of creating or introducing experimental artifacts during the concentration process and ultimately endowing them with a natural existence. To avoid this problem it is necessary to continually check for any change in aroma from the time the product enters the laboratory until the final analysis is completed. This precaution was in the forefront throughout this research project. The wheat odor was carefully observed when the shipment arrived; the aroma of the heated sample for headspace vapor analysis was compared with the original aroma as were the odors at the various stages of concentration during the processing of the wheat essence.

#### Headspace Vapor

The aroma issuing from the headspace vapor apparatus when the thermometer was removed from the system was identifiable as strongly wheat-like. Subjectively, the only apparent way in which the sample differed from the unheated wheat was in intensity. The aroma from the boiling wheat was distinctly stronger than that from the original wheat sample. The method of headspace sampling

minimized the possibility of artifact formation. No stopcock grease was used and the distilled water used in preparation of the wheat slurry passed through only one meter of Tygon tubing.

The headspace vapor apparatus was in close proximity to the gas chromatograph. This was important in helping to reduce the possibility of loss of some of the lower boiling compounds en route from the sampling point to the injection port of the chromatograph. All of these factors were considered and controlled to optimize authenticity of the sample and eliminate artifacts. It may be concluded that the vapor sample obtained by this technique was a highly reliable sample of the low-boiling wheat volatiles and that the presence of artifacts was unlikely.

#### Wheat Flavor Essence

As each trap was removed from the vacuum steam-distillation train, each was smelled and the aroma of wheat was distinctly present in all of them. The odor at that stage of sample procurement was reasonably intense and wheat-like. The wheat essence that resulted after extraction with anhydrous diethyl ether and distillation of the ether was quite intense and easily recognized as wheat-like. This wheat essence had the most intense wheat-like odor of the various distillates tested, which was not surprising since the flavor volatiles were far more concentrated in this product than in any other sample

of wheat volatiles prepared in the laboratory.

It should be borne in mind that this method of isolating wheat volatiles did present the possibility of some artifact formation. The distilled water used in the preparation traveled a considerable distance (more than 15 meters) through Tygon tubing. The use of stop-cock grease, although necessary in the vacuum apparatus, presented another potential source of chemical compounds foreign to the wheat volatiles. When the final evaporation of the ether was being done, it was necessary to have the wheat essence under a hood in the laboratory where it could be observed at all times. Although there was only a small aperture to permit the ether to escape, there was still the possibility that volatile chemicals in the laboratory might contaminate the sample. The compounds identified in the wheat essence needed to be reviewed with these possible sources of artifacts in mind.

The final essence obtained from the steam distillation of wheat under vacuum was a yellow, viscous liquid with a volume of only 0.15 milliliters. This small volume underlines the dismayingly small quantity of flavor volatiles in wheat, for a portion of this volume was contributed by ether still present with the sample. It is estimated that the flavor volatiles in wheat do not exceed ten parts per million and may actually be less than that figure.

## Identification of Components of Wheat Headspace Vapor and Wheat Flavor Essence

### Chemical Tests

Chemical tests were conducted to identify functional groups present in the headspace vapor and in the wheat flavor essence. Additional information regarding the actual carbonyl compounds present in the unknown mixture was obtained by determination of the melting points of 2,4-dinitrophenylhydrazone derivatives.

Functional Group Identification. After ether extraction, the aqueous condensate obtained from the vacuum distillation retained sufficient aroma to make it suitable for use in preliminary chemical tests. When dilute sulfuric acid was added to a portion of this material, the odor changed from that of wheat to one reminiscent of apple essence. According to Weurman (1963), such a change was considered to be indicative of the presence of an amine or amines in the sample.

The addition of a few crystals of powdered 2,4-dinitrophenylhydrazine to the mixture previously acidified with dilute sulfuric acid caused a noticeable aromatic change. The liquid assumed an aroma very similar to that of gingerale. This odor change was interpreted to be a definite indication of the presence of carbonyl compounds.



Additional insight into the complex array of substances contained in the headspace vapor was obtained by functional group analysis. Headspace vapors were injected into the chromatograph for identifying the functional groups emerging during the first 180 seconds. Diethylene Glycol Succinate was the stationary phase used. The effluent stream from the chromatograph was diverted into five vials containing selected reagents as the various peaks emerged from the column.

The first peak, collected between 50 and 66 seconds, resulted in the formation of a precipitate in the vial containing 2,4-dinitrophenylhydrazine. This provided confirmation of the possible presence of acetaldehyde and isobutyraldehyde.

Some precipitate also formed in the vial containing 2,4-dinitrophenylhydrazine during the collection period between 67 and 78 seconds. Butyraldehyde, if present, would have peaked at 73 seconds and the presence of the precipitate during this collection period would indicate that indeed butyraldehyde was a constituent of wheat volatiles.

The presence of 3-methyl-2-butanone, isovaleraldehyde, and possibly butanone was indicated by the formation of a precipitate in the vial containing 2,4-dinitrophenylhydrazine during the collection period ranging from 79 to 100 seconds. The retention times for the compounds were, respectively, 84, 81, and 79 seconds under the operating conditions used in this test. No detectable change

occurred in the ester reagent vial. It is likely that ethyl acetate and butanone, both with retention times of 79 seconds, emerged before the effluent was flowing freely into the vials after the shift from the previous collection system.

A precipitate formed in the vial containing 2,4-dinitrophenylhydrazine during the collection period between 105 and 130 seconds. This span of time would have covered a part of the 2,2-dimethyl-3-pentanone peak, all of the valeraldehyde peak, and diacetyl. On the basis of this functional group analysis test it appeared that these carbonyls were present in the headspace vapor of wheat.

Two changes were apparent in the vials during the collection period from 133 to 160 seconds. The vial containing the ester-detecting reagent, ferric hydroxamate, contained a flocculent, rusty precipitate and the one with the 2,4-dinitrophenylhydrazine reagent also contained a precipitate. Crotonaldehyde, if present, would be expected to peak at 148 seconds and thus should have been eluted in this period. The precipitate in the 2,4-dinitrophenylhydrazine reagent was not unexpected, but the reaction in the ester reagent was somewhat surprising. To determine whether this might be an indication of a second compound being eluted during this collection period the crotonaldehyde standard was reacted with the ester reagent. The same rusty-colored precipitate also formed when this known compound was run. On this basis it may be stated that it

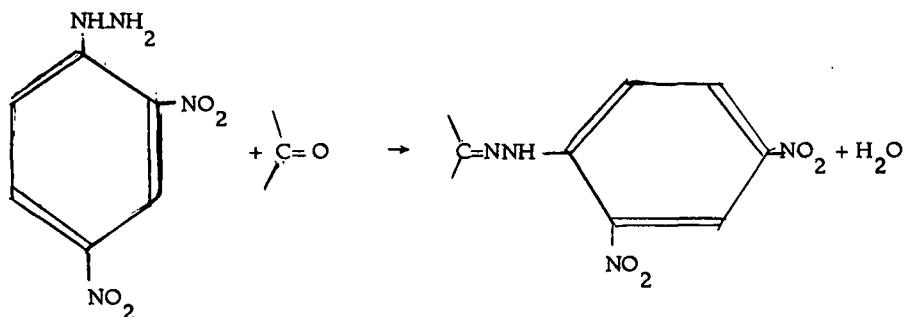
appears that crotonaldehyde is a component in the unknown vapor sample.

Once again, the 2,4-dinitrophenylhydrazine reagent was the only one showing a reaction during the collection period between 160 and 180 seconds. This reaction apparently was due to the presence of hexanal, the aldehyde that peaks at 164 seconds.

Melting Points of 2,4-Dinitrophenylhydrazone Derivatives. The determination of the melting points of 2,4-dinitrophenylhydrazone derivatives was a particularly useful tool in beginning the identification of the flavor components in wheat. These derivatives were obtained by reacting the aqueous sample (remaining after the condensate obtained by vacuum distillation of wheat had been extracted with ether) with 2,4-dinitrophenylhydrazine reagent, filtering to obtain the desired crystals, and then recrystallizing from ethyl alcohol.

Identification of unknown organic compounds by formation of crystalline derivatives and subsequent determination of their melting points is a well-established and accepted research technique. In the identification of carbonyl compounds the formation of 2,4-dinitrophenylhydrazones has proven to be particularly useful because so many of these aldehydes and ketones form crystalline derivatives with melting points within the temperature range where such

assessments can be accurately made. The reaction for the formation of these derivatives is (Shriner, Fuson and Curtin, 1964)



With cautious heating and the aid of a magnifying glass it was possible to determine accurately the melting point of the various crystals obtained in this derivative preparation. Although several aldehydes and ketones were identified by this method, two compounds found through this technique are of particular interest because they have not been previously reported in the studies on flavor. These were 3-methyl-2-butanone and 2,2-dimethyl-3-pentanone. The carbonyl compounds identified in this manner, along with the melting points obtained in this laboratory and the melting points reported in the literature, are summarized in Table 1.

The presence of acetaldehyde, isobutyraldehyde, crotonaldehyde, and 2,2-dimethyl-3-pentanone was clearly demonstrated because the

melting points of their respective 2,4-dinitrophenylhydrazine derivatives agreed closely with values reported in the literature. Ample evidence of the melting points reported in Table 1 was obtained, but only tentative identification of valeraldehyde, hexanal, heptanal, and octanal could be assigned because of the proximity of their melting points. For this same reason only tentative identification of 3-methyl-2-butanone and/or butanone and butyraldehyde and/or isovaleraldehyde must be reported. These findings were used as a guide in the subsequent work with paper chromatography and gas liquid chromatography.

Table 1. 2,4-Dinitrophenylhydrazone derivatives identified from wheat.

Carbonyl	Melting point of 2,4-dinitrophenylhydrazone (° C)	
	Laboratory	Literature <sup>a</sup>
Acetaldehyde	168	168
Isobutyraldehyde	182	182
Butyraldehyde	125	122
Crotonaldehyde	190	190
3-Methyl-2-butanone	119	117
Valeraldehyde	110	107
Hexanal	104	104
2,2-Dimethyl-3-pentanone	178	175
Heptanal	110	108
Octanal	109	106
Butanone	116	114
Isovaleraldehyde	125	122

<sup>a</sup>Values as reported in Shriner, Fuson and Curtin (1964) except those for valeraldehyde, 3-methyl-2-butanone, and 2,2-dimethyl-3-pentanone which were reported in Heilbron (1953).

## Paper Chromatography

In this study, paper chromatography proved more effective in separating carbonyl compounds into classes than did column chromatography. Therefore, paper chromatographic methods, as outlined by Gaddis and Ellis (1959) and Ellis, Gaddis and Currie (1958), were used to identify carbonyl compounds in the unknown mixture.

A satisfactory, readily discernible separation of carbonyl classes was accomplished by spotting 30 drops of 2,4-dinitrophenylhydrazones, dissolved in methanol and methyl acetate, on chromatographic strips ( $10\frac{1}{2} \times 1\frac{3}{8} \times 1$  inch) cut from Whatman #3 filter paper. During the course of the development in petroleum ether, the 2-alkanone carbonyls moved out fastest and formed a beige colored area a short distance behind the solvent front. A short distance behind the first class moved a yellow region, the n-alkanals. Directly behind the n-alkanals was the alk-2-enal class, as evidenced by a yellow-orange region. There was a brown area that moved only a short distance from the origin. This was interpreted to be the alk-2,4-dienal class although no evidence of such compounds had been found during the melting point determinations of 2,4-dinitrophenylhydrazone derivatives.

Further evidence for the presence of 3-methyl-2-butanone in wheat flavor was obtained when the chromatogram spotted with the

eluted sample of the first band, the 2-alkanone region, and a dissolved 2,4-dinitrophenylhydrazone derivative of 3-methyl-2-butanone was examined. The two spots traveled at the same rate and exhibited a similar appearance on the chromatogram.

According to Ellis, Gaddis and Currie (1958), acetaldehyde separated in the class separations with the alk-2-enals rather than with the expected n-alkanals. Therefore, the portion of the chromatogram containing the alk-2-enals in the present study might be expected to contain acetaldehyde as well as crotonaldehyde.

A sample eluted from the third band (the alk-2-enals) obtained in the paper chromatographic separation by classes was spotted on one side of a chromatogram and a mixture of the 2,4-dinitrophenylhydrazone derivatives of acetaldehyde and crotonaldehyde was dissolved and spotted on the other side of the chromatogram to serve as standards. This chromatogram, developed in 96 percent Skellysolve-4 percent methanol, did show two areas on both the left and right sides of the chromatogram that were a more intense yellow than the remainder of the area traversed by the samples, but there was some overlapping and the regions were not as clearly delineated as they were in the class separation chromatograms. However, the two more intensely colored areas on each side did travel at the same rate. This evidence gave additional support to the melting point findings that both acetaldehyde and crotonaldehyde were present in

wheat flavor.

The results of chromatographing the n-alkanals eluted from the second band of the class separation chromatograms in comparison with the 2,4-dinitrophenylhydrazone derivatives of the known n-alkanals were not conclusive because of the blurred appearance of the chromatograms.

### Gas-Liquid Chromatography

Operating Conditions. It was deemed advisable to use more than one type of chromatographic column for the purpose of determining retention times. If the retention time of a known standard has been shown to be coincident with the retention time of the unknown compound suspected to be the same as the standard, this has usually been considered to be excellent evidence of the identity of the unknown substance. However, it would surely be far more conclusive to match retention times on three columns than simply on one. Following this rationale, it was decided to use three stationary phases with somewhat different retention characteristics that should bring out any subtleties in retention times that might not be clearly revealed on only one column. The three columns selected, Diethylene Glycol Succinate, Apiezon M, and Free Fatty Acid Phase, proved to fulfill the demands imposed upon them. A fourth stationary phase, Methyl Silicone Rubber, did not produce



a chromatogram with the practical utility for identification purposes that was achieved with the other three column packings. Therefore, this stationary phase was not used in the actual analysis of the flavor components in the wheat samples.

The amount of liquid stationary phase to use needed to be determined. Apiezon M was the material used in testing the merits of a heavy coating versus a light coating. The 20 percent level was tested because this had been reported in other studies and was used as the basis for some retention volume data presented by McReynolds (1966). The 6 percent level was tried because several other stationary supports used in this laboratory previously had performed well when used at this level with the Barber-Colman chromatograph. An examination of Figures 5 and 6 revealed that resolution of the various flavor constituents was more effectively accomplished with the lighter coating of the stationary phase. Therefore the Apiezon M and Diethylene Glycol Succinate were used at the 6 percent level. The 3 percent level used for the Free Fatty Acid Phase was selected on the basis of manufacturer's recommendations (Varian Aerograph of Walnut Creek, California).

In all columns the solid support used was Anakrom ABS 90/100 mesh. The relatively small size of the particles provided a large surface area for uniform distribution of the liquid stationary phase. Anakrom ABS (produced by Analabs of Hamden, Connecticut) is

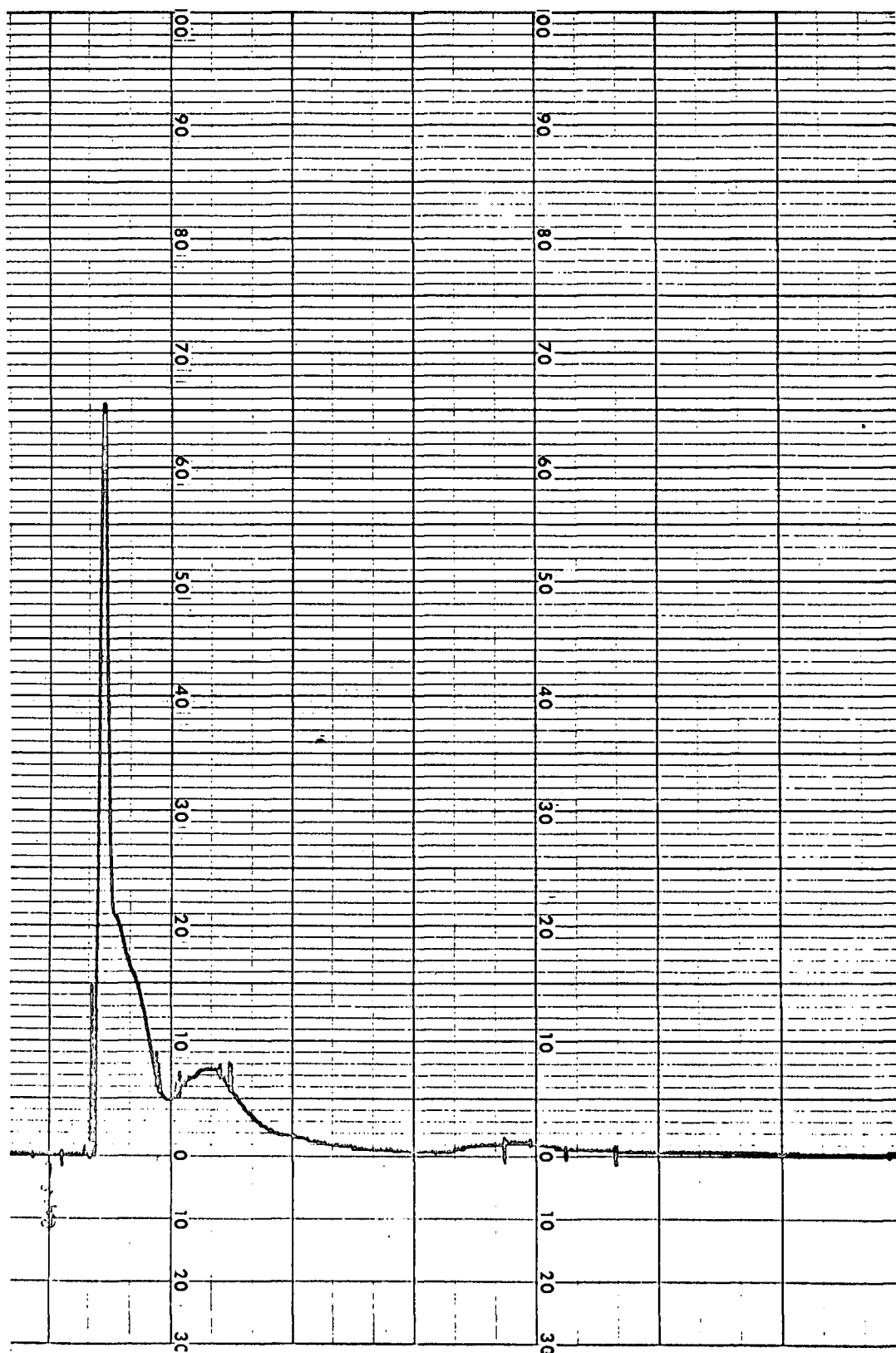


Figure 5. Chromatogram of wheat headspace vapor  
20% Apiezon M on Anakrom ABS 90-100, 75° C

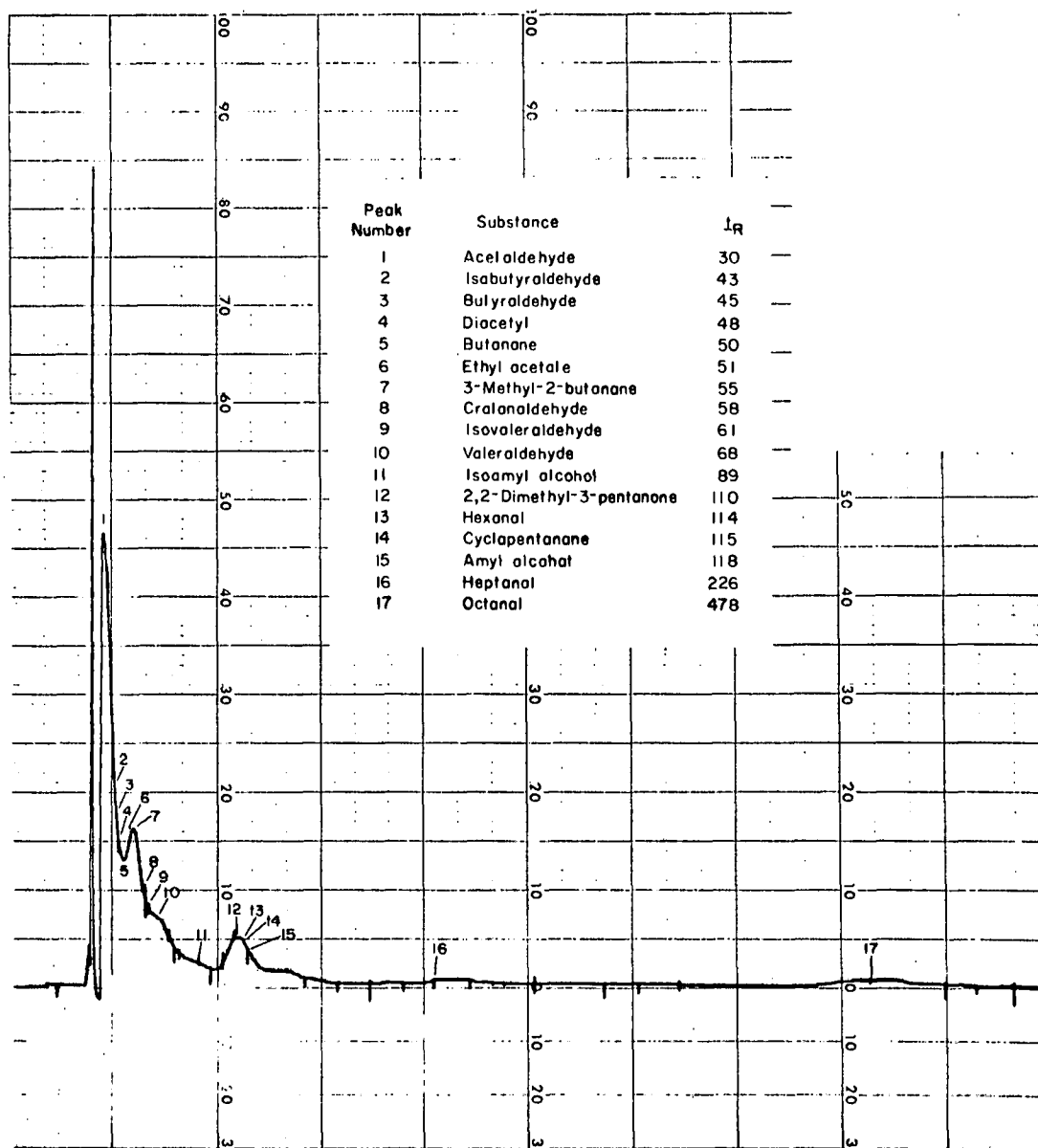


Figure 6. Chromatogram of wheat headspace vapor  
6% Apiezon M on Anakrom ABS, 90-100, 75° C.

acid washed to remove trace amounts of aluminum, iron, magnesium, and calcium. Following the acid washing with concentrated hydrochloric acid and rinsing with deionized water, the solid support is washed in alcoholic potassium hydroxide, a process designed to reduce tailing phenomena. After the column has been vacuum silicized, it is considered to be a high quality, all purpose solid support.

Isothermal column temperatures of 50° C, 75° C, and 100° C were compared during the analysis of headspace vapors on the 6 percent Apiezon M column. Examination of the three chromatograms (Figures 6, 7, and 8) revealed that 75° C gave the most detail for analysis of unknowns. The effect of temperature was also illustrated clearly when the chromatograms of the 3 percent Free Fatty Acid Phase column run at 75° C and 100° C were compared. Although both temperatures on this column packing gave excellent resolution, it was apparent that more detail was discernible in the chromatogram run at 75° C than in the one at 100° C. On the basis of these comparisons 75° C was selected for gas chromatographic column temperatures.

#### Retention Times of Components of Wheat Headspace Vapor.

Retention times of known compounds suspected to be present in the unknown headspace vapor sample were determined on columns containing the following stationary phases: 6 percent Apiezon M, 3

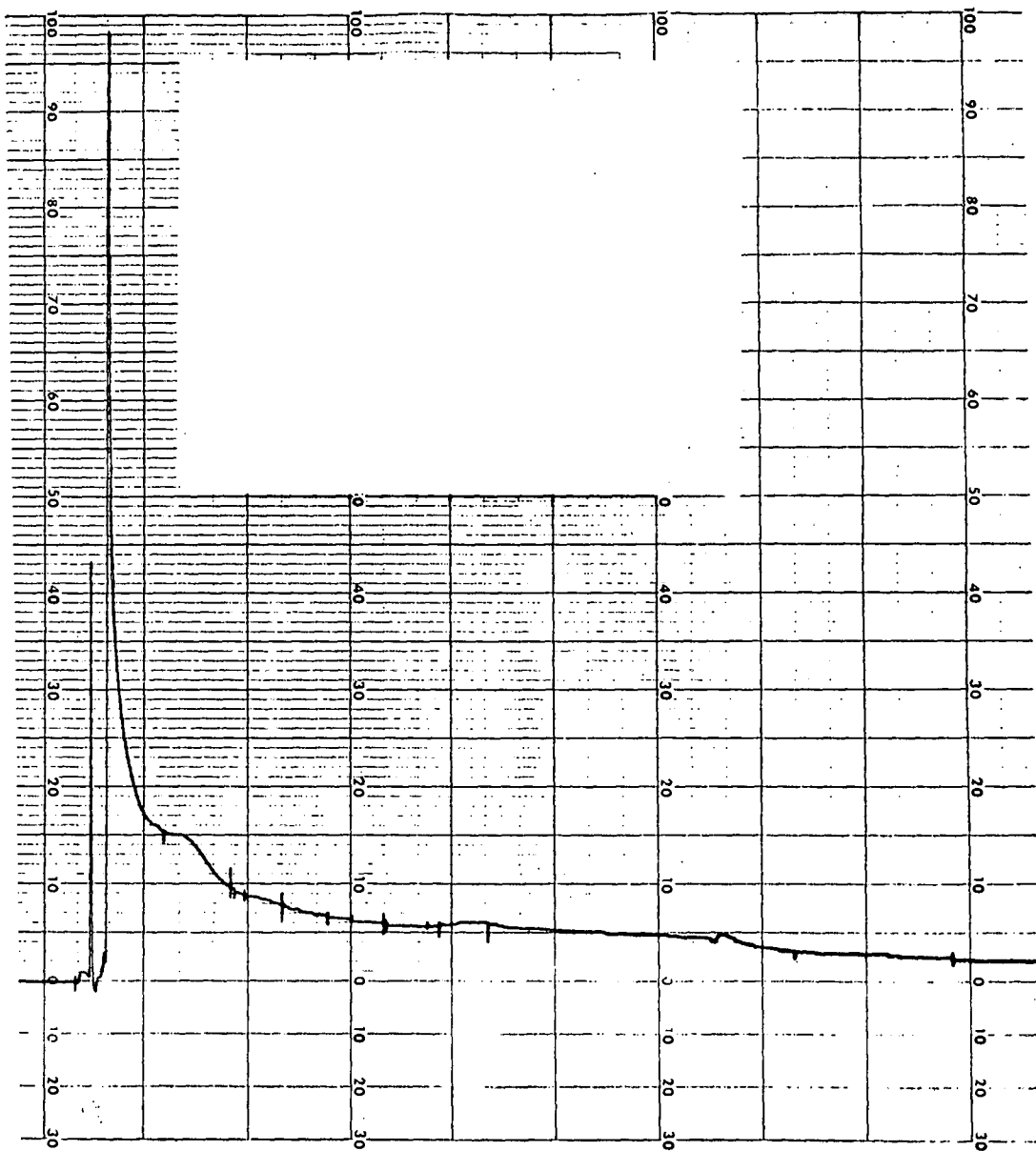


Figure 7. Chromatogram of wheat headspace vapor  
6% Apiezon M on Anakrom ABS, 90-100, 50°C

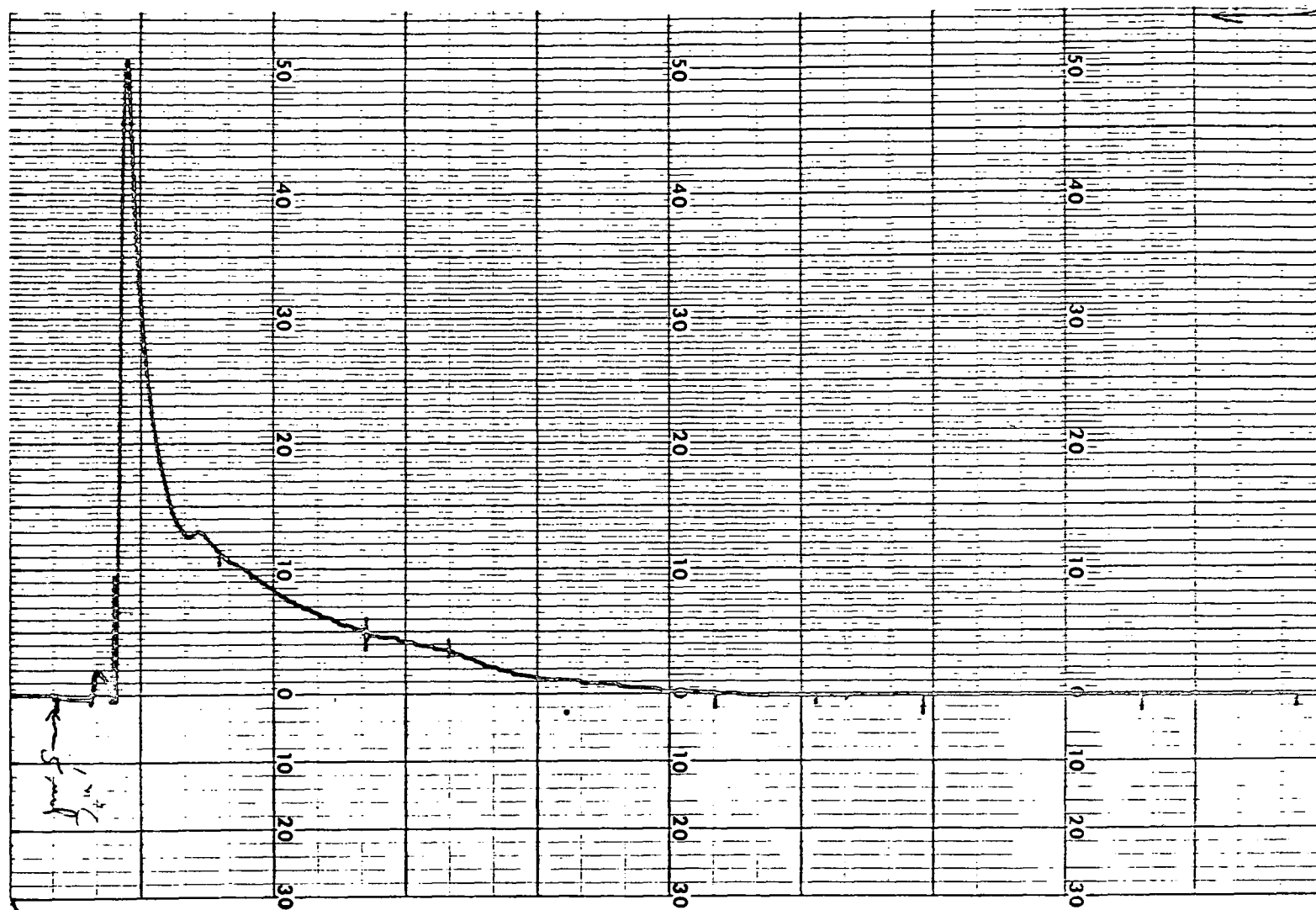


Figure 8. Chromatogram of wheat headspace vapor  
6% Apiezon M on Anakrom ABS, 90-100. 100° C.

percent Free Fatty Acid Phase, and 6 percent Diethylene Glycol Succinate. These standards were chromatographed under the same operating conditions as those used to chromatograph the headspace vapor sample, that is hydrogen, compressed air, and nitrogen at 19, 49, and 5 pounds per square inch, respectively, column temperature isothermal at 75° C, and attenuation at 64. In contrast to the sensitivity required for the headspace vapor analysis, it was necessary to reduce the sensitivity to 100 for the known samples to satisfactorily retain the complete height of the peak on the chart paper. This change, however, did not influence the retention time. These retention time values established for the standard compounds could then be used to compare with the retention time of various peaks on the chromatogram of the unknown sample. Identification of the various peaks discernible in the chromatograms of the wheat headspace vapor (Figures 6, 9, and 10) were made on the basis of the retention times as listed in Tables 2, 3, and 4 and as illustrated in the Appendix.

Acetaldehyde was clearly present in the chromatograms of the headspace vapors run on the 6 percent Diethylene Glycol Succinate and the 3 percent Free Fatty Acid Phase columns and appeared to be a probable compound on the 6 percent Apiezon M column.

The formulas and boiling points of substances studied with the aid of the gas chromatograph and mass spectrometer are presented in Table 5.

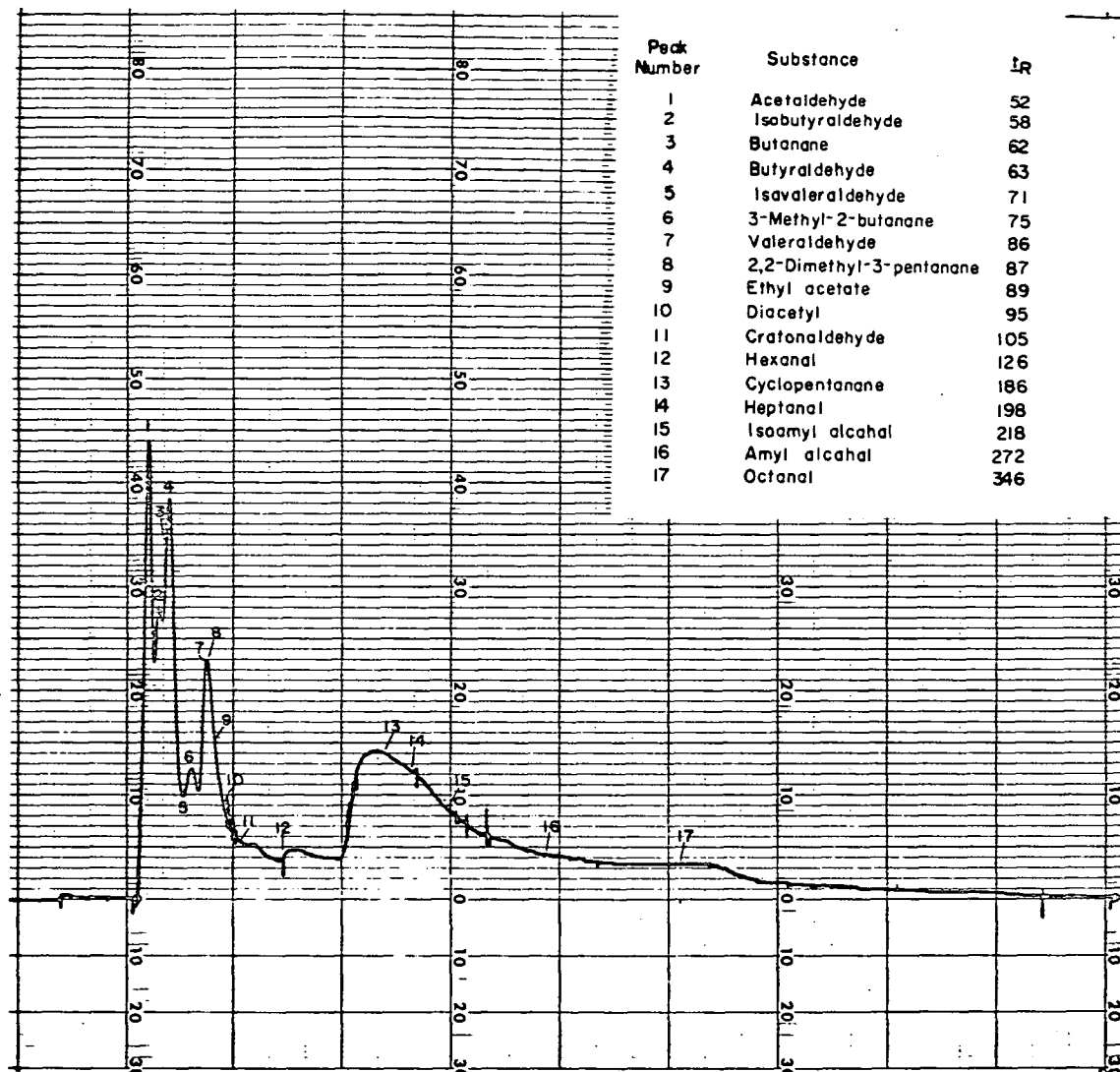


Figure 9. Chromatogram of wheat headspace vapor  
3% Free Fatty Acid Phase on Anakrom ABS, 90-100, 75°C.



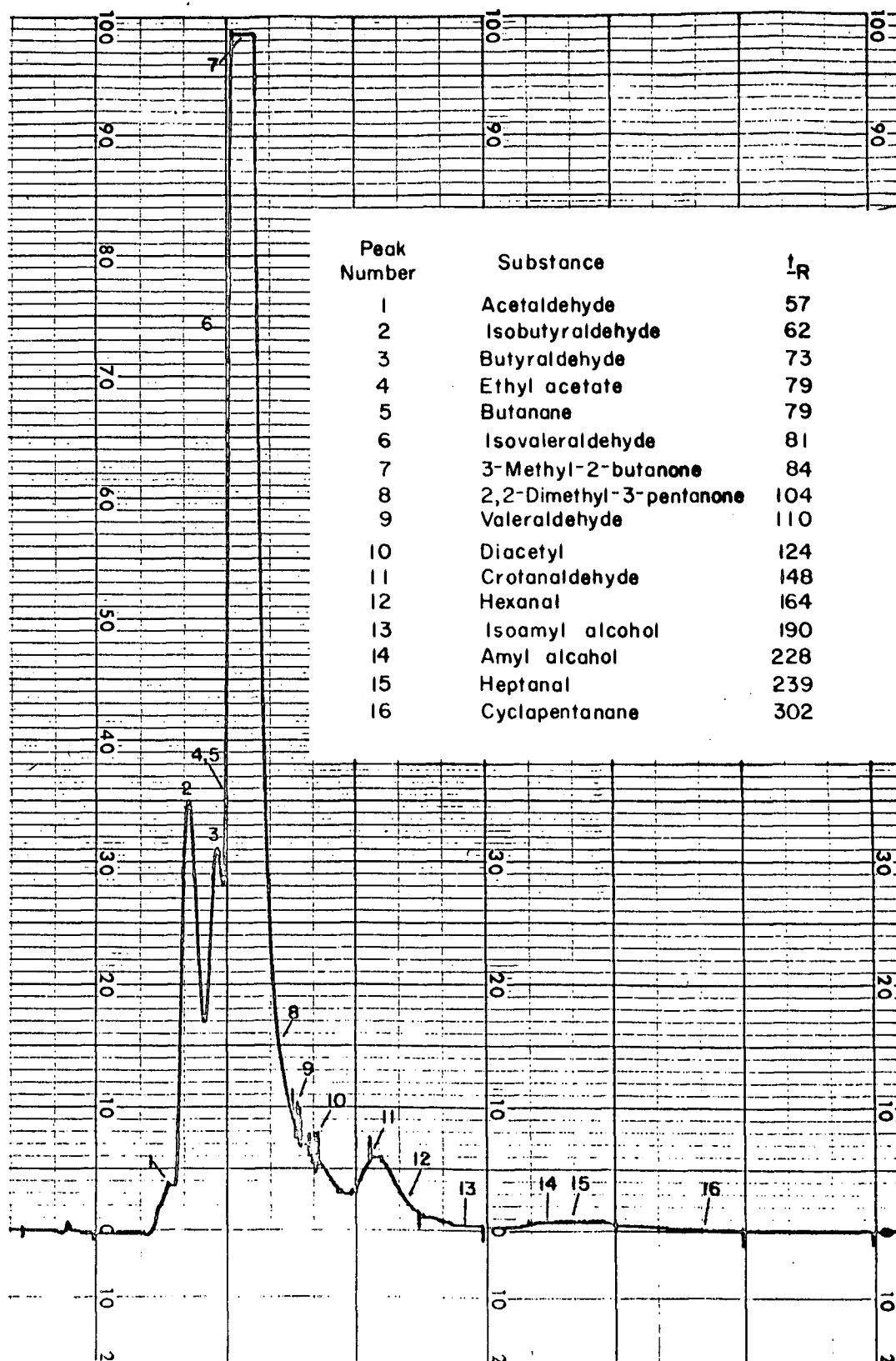


Figure 10. Chromatogram of wheat headspace vapor  
6% Diethylene Glycol Succinate on Anakrom ABS, 90-100, 75° C

Table 2. Retention times ( $t_R$ ) of standard compounds on 6 percent Apiezon M at 75°C.

Compound	$t_R$ (seconds)
Acetaldehyde	30
Isobutyraldehyde	43
Butyraldehyde	45
Diacetyl	48
Butanone	50
Ethyl acetate	51
3-Methyl-2-butanone	55
Crotonaldehyde	58
Isovaleraldehyde	61
Valeraldehyde	68
Isoamyl alcohol	89
2,2-Dimethyl-3-pentanone	110
Hexanal	114
Cyclopentanone	115
Amyl alcohol	118
Heptanal	226
Octanal	478

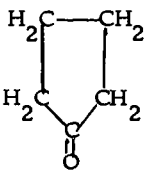
Table 3. Retention times ( $t_R$ ) of standard compounds on 6 percent Diethylene Glycol Succinate at 75° C.

Compound	$t_R$ (seconds)
Acetaldehyde	57
Isobutyraldehyde	62
Butyraldehyde	73
Ethyl acetate	79
Butanone	79
Isovaleraldehyde	81
3-Methyl-2-butanone	84
2,2-Dimethyl-3-pentanone	104
Valeraldehyde	110
Diacetyl	124
Crotonaldehyde	148
Hexanal	164
Isoamyl alcohol	190
Amyl alcohol	228
Heptanal	239
Cyclopentanone	302
Octanal	406

Table 4. Retention times ( $t_R$ ) of standard compounds on 3 percent Free Fatty Acid Phase at 75° C.

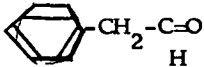
Compound	$t_R$ (seconds)
Acetaldehyde	52
Isobutyraldehyde	58
Butanone	62
Butyraldehyde	63
Isovaleraldehyde	71
3-Methyl-2-butanone	75
Valeraldehyde	86
2,2-Dimethyl-3-pentanone	87
Ethyl acetate	89
Diacetyl	95
Crotonaldehyde	105
Hexanal	126
Cyclopentanone	186
Heptanal	198
Isoamyl alcohol	218
Amyl alcohol	272
Octanal	346

Table 5. Compounds studied in the gas chromatograph; and the mass spectrometer.

Compound	Formula	Boiling point
Acetaldehyde	$\text{H}_3\text{C}-\underset{\text{H}}{\underset{ }{\text{C}}}=\text{O}$	21°C
Isobutyraldehyde	$\text{H}_3\text{C}-\underset{\text{CH}_3}{\underset{ }{\text{CH}}}-\underset{\text{H}}{\underset{ }{\text{C}}}=\text{O}$	64°C
Butyraldehyde	$\text{H}_3\text{C}-\text{CH}_2-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\text{C}=\text{O}$	74°C
Diacetyl	$\text{H}_3\text{C}-\underset{\text{O}}{\underset{  }{\text{C}}}-\underset{\text{O}}{\underset{  }{\text{C}}}-\text{CH}_3$	87-88°C
Butanone	$\text{H}_3\text{C}-\text{CH}_2-\underset{\text{O}}{\underset{  }{\text{C}}}-\text{CH}_3$	80°C
Ethyl acetate	$\text{H}_3\text{C}-\underset{\text{O}-\text{CH}_2-\text{CH}_3}{\underset{ }{\text{C}}}=\text{O}$	77°C
3-Methyl-2-butanone	$\text{H}_3\text{C}-\underset{\text{CH}_3}{\underset{ }{\text{CH}}}-\underset{\text{O}}{\underset{  }{\text{C}}}-\text{CH}_3$	94°C
Crotonaldehyde	$\text{H}_3\text{C}-\text{CH}=\underset{\text{H}}{\underset{ }{\text{CH}}}-\text{C}=\text{O}$	103°C
Isovaleraldehyde	$\text{H}_3\text{C}-\underset{\text{CH}_3}{\underset{ }{\text{CH}}}-\text{CH}_2-\underset{\text{H}}{\underset{ }{\text{C}}}=\text{O}$	92°C
Valeraldehyde	$\text{H}_3\text{C}-\text{CH}_2-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\text{C}=\text{O}$	103°C
Isoamyl alcohol	$\text{H}_3\text{C}-\underset{\text{CH}_3}{\underset{ }{\text{CH}}}-\text{CH}_2-\text{CH}_2\text{OH}$	130°C
2,2-Dimethyl-3-pentanone	$\text{H}_3\text{C}-\underset{\text{CH}_3}{\underset{ }{\text{C}}}-\underset{\text{CH}_3}{\underset{ }{\text{C}}}-\underset{\text{O}}{\underset{  }{\text{C}}}-\text{CH}_2-\text{CH}_3$	125-126°C
Hexanal	$\text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\text{C}=\text{O}$	128°C
Cyclopentanone		131°C
Amyl alcohol	$\text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\underset{\text{H}}{\underset{ }{\text{CH}_2}}-\text{OH}$	138°C

Continued on next page

Table 5. Continued

Compound	Formula	Boiling point
Heptanal	$\text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\underset{\text{H}}{\text{C}}=\text{O}$	156°C
Octanal	$\text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\underset{\text{H}}{\text{C}}=\text{O}$	171°C
Phenylacetaldehyde		194°C

Isobutyraldehyde is clearly indicated in the chromatograms of headspace vapor run on the 6 percent Diethylene Glycol Succinate and 3 percent Free Fatty Acid Phase columns. There is no clear-cut peak in evidence for the sample run on the 6 percent Apiezon M column. However, there obviously is some compound emerging from the column when isobutyraldehyde should be present. It may be concluded that the Apiezon M column is simply not providing a separation of the compounds.

The peak of butyraldehyde is sharply defined on both the Diethylene Glycol Succinate and Free Fatty Acid Phase columns,

but once again there is no clean separation of compounds on the Apiezon M. Butyraldehyde apparently is a part of the large peak that is passing at the correct retention time.

Diacetyl is readily identified on both the Free Fatty Acid Phase and the Diethylene Glycol Succinate columns. On the Apiezon M column the peak is not clearly differentiated, making positive identification uncertain. The yellow color of the diacetyl standard appeared to be the same as the color of the wheat essence.

The retention times of butanone and ethyl acetate are too close on Apiezon M and Diethylene Glycol Succinate to distinguish between the two compounds. There is a large difference in retention time of these two substances on the Free Fatty Acid Phase, but other suspected compounds, namely butyraldehyde and 2,2-dimethyl-3-pentanone, are close enough to make positive identification difficult. It does appear, however, that both of these compounds are present in wheat volatiles.

The first truly clean separation of compounds appearing on the 6 percent Apiezon M column is that of 3-methyl-2-butanone. This peak is also readily observed on the other two columns used.

Particularly strong evidence of the presence of crotonaldehyde is available on the Diethylene Glycol Succinate column where this peak is clearly separated from the rest of the chromatogram. This aldehyde can also be seen clearly on the other two columns. Crotonaldehyde is of particular interest because of its variable position between the different columns. On the Diethylene Glycol Succinate and Free Fatty Acid Phase columns it appears between diacetyl and hexanal and on the Apiezon M column between 3-methyl-2-butanone and valeraldehyde.

Isovaleraldehyde is not clearly distinguished on any of the columns although it is apparent that some substance is being eluted at the correct time. If it is present, it is not nearly as abundant as the preceding compounds. Only tentative identification of this aldehyde can be made from the existing data.

Valeraldehyde can be observed on all three columns, and it is most easily and clearly detected on the Apiezon M column. On the Free Fatty Acid Phase it appears as a bit of a shoulder on the front of the 2,2-dimethyl-3-pentanone peak. On the Diethylene Glycol Succinate chromatogram valeraldehyde is a shoulder showing up toward the end of the peak from 3-methyl-2-butanone.

Particularly strong evidence for the presence of 2,2-dimethyl-3-pentanone was seen on the chromatogram of headspace vapors run on Apiezon M. This compound was also identified on the Free



Fatty Acid Phase column. This ketone was probably present in the chromatogram from the Diethylene Glycol Succinate column, but the peak was not clearly differentiated.

A small amount of isoamyl alcohol appears to be present in wheat volatiles. A barely discernible peak may be seen on the chromatograms from Apiezon M and the Free Fatty Acid Phase columns. There is only a very slight indication of a peak at the correct retention time on Diethylene Glycol Succinate.

Hexanal is clearly seen in the chromatograms of the headspace vapor run on the FFAP and AM columns, although in rather small quantity. The next homolog in the n-alkanal series, heptanal, shows clearly in the Apiezon M and the Diethylene Glycol Succinate chromatograms and also appears to be a likely compound in the one from the Free Fatty Acid Phase column. There is not a clear peak showing on this latter column, but this seven-carbon aldehyde appears as a shoulder on a rather broad peak.

Cyclopentanone appeared to be present on the chromatogram from both the Apiezon M and the Free Fatty Acid Phase columns. The analysis of headspace vapors on Diethylene Glycol Succinate failed to differentiate this substance.

A small shoulder on the main peak at the correct retention time for amyl alcohol can be seen on the chromatogram of headspace vapors analyzed on Apiezon M. The headspace vapors

depicted on the Free Fatty Acid Phase chromatogram provide for the possibility of the presence of amyl alcohol, but do not afford conclusive proof. On Diethylene Glycol Succinate there is a flat peak at the correct retention time for amyl alcohol.

The clearest demonstration of the presence of octanal is on the Apiezon M column. This compound is also in evidence on the Free Fatty Acid Phase column. It appears that this higher boiling compound was not picked up in the sampling of the headspace vapor used for analysis on the Diethylene Glycol Succinate column.

This method of sampling utilizing the headspace vapors of lightly milled wheat boiling in distilled water produced excellent chromatograms of the lower boiling volatiles present in wheat without creating any apparent experimental artifacts. In no test was this method suitable for sampling of compounds with a higher boiling point than octanal, that is  $171^{\circ}\text{C}$ .

#### Retention Times of the Components of the Wheat Flavor

Essence. Chromatograms of the wheat flavor essence (Figures 11, 12, and 13) support the observations noted in the preceding section. On all three columns, the fastest-moving compounds were obscured in one large peak. Operating conditions were maintained to optimize the detection and recording of the slower-moving compounds since headspace vapor analyses previously had satisfactorily differentiated the early eluting compounds. No contradiction in the

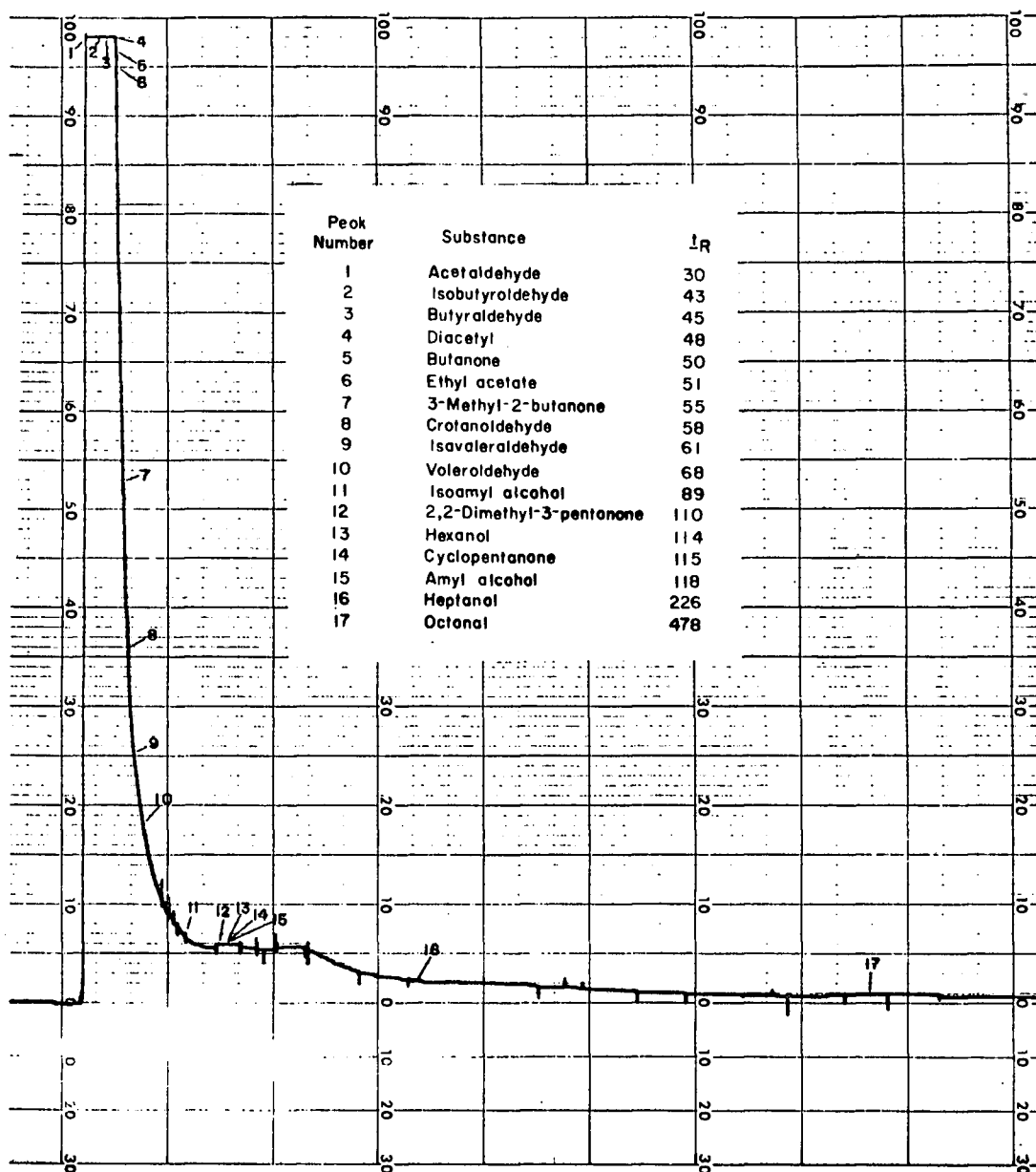


Figure 11. Chromatogram of wheat flavor essence  
6% Apiezon M on Anakrom ABS, 90-100, 75° C

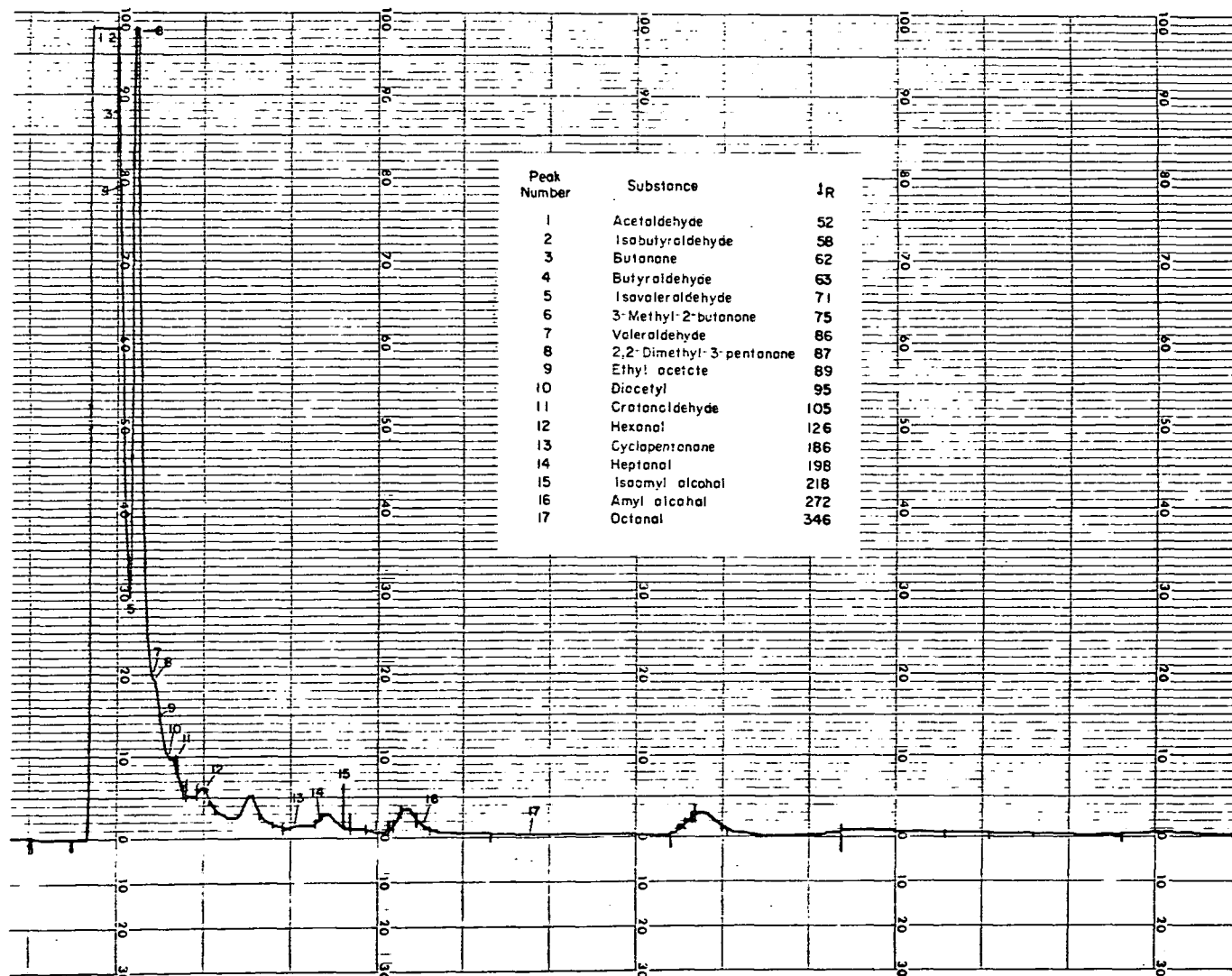


Figure 12. Chromatogram of wheat flavor essence  
3% Free Fatty Acid Phase on Anakrom ABS, 90-100, 75° C

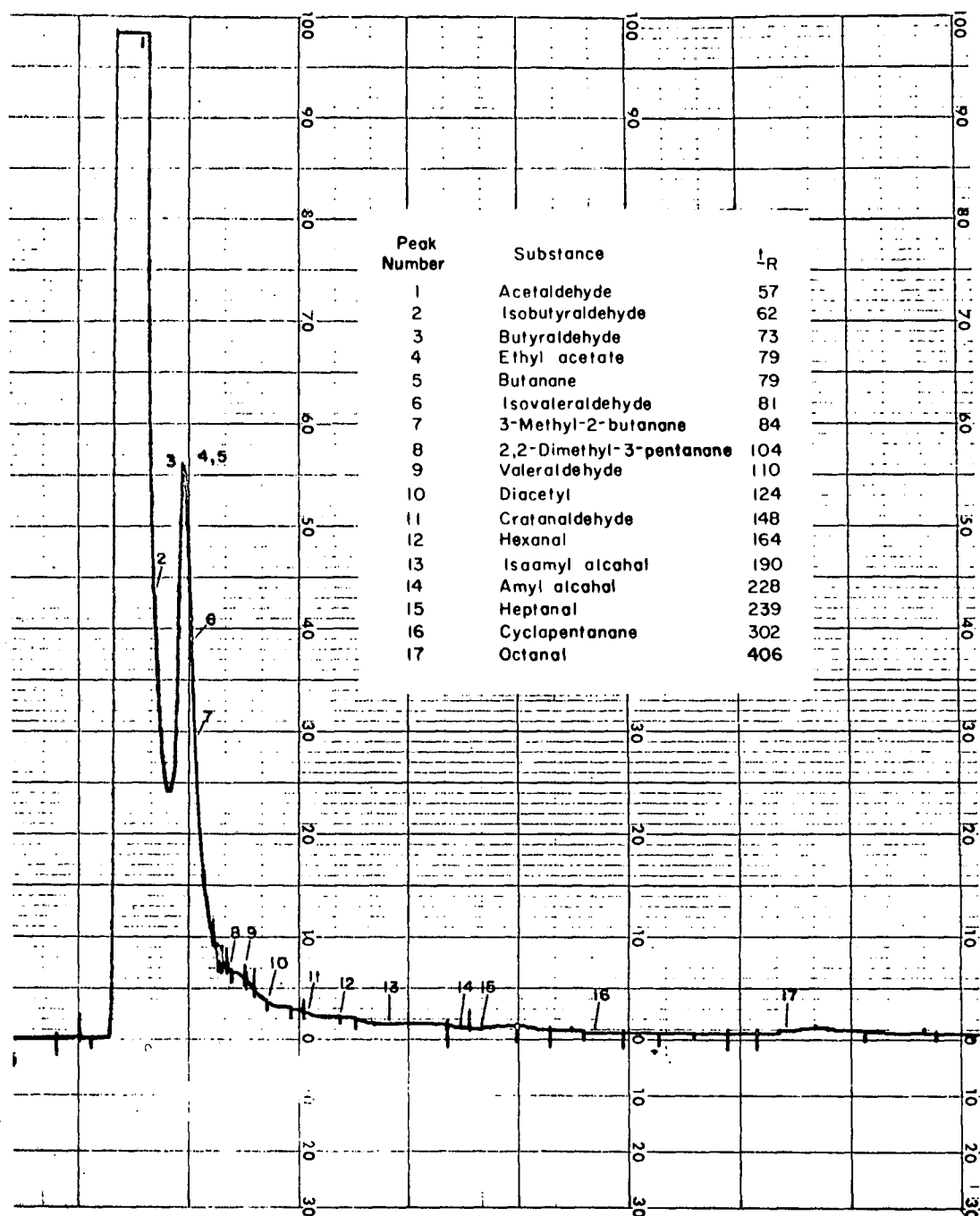


Figure 13. Chromatogram of wheat flavor essence  
6% Diethylene Glycol Succinate on Anakrom ABS, 90-100, 75° C

evidence was apparent between the headspace and essence samples. It should be pointed out that the best evidence for the presence of cyclopentanone was given in the chromatogram of the wheat flavor essence run on the Free Fatty Acid Phase column.

The wheat flavor essence chromatograms were especially valuable in determining the presence of compounds with retention times equal to or greater than that of 2, 2-dimethyl-3-pentanone. Hexanal showed more clearly on the chromatograms from the essence than from the headspace vapors. This was also true for 2, 2-dimethyl-3-pentanone, cyclopentanone, heptanal, amyl alcohol and octanal.

### Mass Spectral Analysis

Analysis of the mass spectral data reaffirmed the importance of being aware of possible contaminants. The first compound identified was chloroform, a compound readily apparent because of its chlorine peak at 35. This substance could have infiltrated the sample in the laboratory where ether extraction was done or in the area where the final evaporation of solvent took place. Chloroform was present in both situations in tightly capped reagent bottles. BHT was also recognized as another familiar compound in mass spectral analysis. The apparent source of this contaminant was Tygon tubing. Stopcock grease was also detected despite the care with which the

necks of the flasks were wiped clean before removal of the condensate. No doubt this material in some measure contributed to the viscosity of the sample obtained. These three substances were positively identified by analysis of the mass spectra, but they must be viewed as artifacts rather than as actual components of wheat volatiles.

Due to the extremely viscous nature of the essence, it was impossible to eliminate the bulk of the remaining ether from the essence and still draw the sample into the syringe for injection into the combined gas chromatograph-mass spectrometer. This limitation meant that by far the largest fraction of the sample was, of necessity, ether. As was expected, most of the early mass spectra that were run as the sample passed through clearly showed the overwhelming presence of ether. Mass spectra are in Table 6.

The spectrum of the scan just prior to the beginning of the ether peak clearly showed that acetaldehyde was a component of the sample. Ethyl acetate, followed in turn by chloroform and diacetyl, appeared in the mass spectra taken after the ether.

Temperature programming at the rate of  $4^{\circ}\text{C}$  per minute was initiated after these compounds emerged. At approximately  $120^{\circ}\text{C}$  phenylacetaldehyde was tentatively identified as one of the peaks.

With the exception of acetaldehyde and diacetyl, the mass spectrometer did not reveal the presence of the aldehydes and

ketones previously found by use of the gas chromatograph alone. This was probably due to the presence of the large proportion of ether and the resultant extremely small quantities of the desired volatiles. Not only were these compounds probably present in the injected sample in too small a quantity for detection, but also some of them may have been masked by the massive ether peak.

### Volatile Flavor Compounds and Possible Precursors

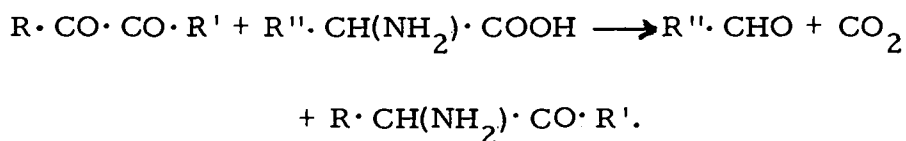
Several of the compounds identified in this study have been identified in various cereal products by other workers. Ng, Reed and Pence (1960) identified acetaldehyde in fresh white bread and isobutyraldehyde and valeraldehyde in the oven vapors. Acetaldehyde, crotonaldehyde, and diacetyl were found in fresh bread by Wiseblatt and Kohn (1960). Johnson, Rooney, and Salem (1966) summarized the findings of several research groups working with pre-ferments, doughs, oven vapors, and bread as follows: amyl alcohol, isoamyl alcohol, acetaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, hexanal, crotonaldehyde, phenylacetaldehyde, butanone, diacetyl, and ethyl acetate. In their studies on cooked rice, Yasumatsu, Moritaka and Wada (1966b) positively identified hexanal (caproaldehyde) and acetaldehyde and tentatively identified methylethylketone (butanone) and valeraldehyde.

Hexanal has been found to contribute to the stale flavor of



rice (Yasumatsu, Moritaka and Wada, 1966a), to off-flavor in potato granules (Buttery, Hendel and Boggs, 1961; Boggs et al., 1964) and to some other foods. It is probable, on the basis of these reports, that the level of hexanal in wheat volatiles would be greater in a sample that had been stored longer.

Strecker degradation reactions are thought to give rise to some of the carbonyl compounds identified in the flavor fractions of some foods. This reaction is an oxidative degradation of alpha-amino acids to form the aldehyde or ketone with one less carbon and is represented according to the following equation:



By this reaction it is possible to form acetaldehyde from alanine, phenylacetaldehyde from phenylalanine, isobutyraldehyde from valine, and isovaleraldehyde from leucine. Phenylalanine, valine, and leucine are present in wheat.

The presence of hexanal in the wheat volatiles may stem from the fat contained in the wheat germ, which was still retained in the wheat used for analysis. Linoleic acid and oleic acid are both contained in wheat; Gaddis, Ellis and Currie (1961) reported the formation of hexanal from both of these precursors. These same workers

also reported the formation of some octanal from trioleate. This aldehyde was identified in the wheat volatiles isolated in the present study.

## SUMMARY AND CONCLUSIONS

The volatile flavor fraction of lightly milled wheat grown in eastern Oregon was isolated for study by two methods: steam distillation under vacuum and steam distillation at atmospheric pressure. The sample obtained under vacuum was extracted with ether which was then evaporated to yield the desired wheat essence. Steam distillation at atmospheric pressure provided the headspace vapors that were used for a portion of the gas chromatographic analysis.

Identification of the volatile organic substances contained in the flavor fraction was made on the basis of chemical tests, paper chromatography, gas-liquid chromatography, and mass spectrometry.

Preliminary testing for amines and carbonyls indicated the presence of both of these functional groups, although in subsequent research amines were not detected. Formation of 2,4-dinitrophenylhydrazones and determination of their melting points provided one means of tentative identification of carbonyl compounds contained in the sample. Additional information as to the functional groups present was obtained by subjecting the samples to gas chromatography and directing the column effluent into vials containing reagents selected to indicate the presence of alcohols, amines, esters, carbonyls, and mercaptans. A separate set of reagents was used for each eluting peak.

Ascending paper chromatography was used to separate the 2,4-dinitrophenylhydrazone derivatives of the carbonyls into classes and to ultimately assist in the identification of these compounds.

Gas-liquid chromatography on columns packed with 6 percent Apiezon M, 6 percent Diethylene Glycol Succinate, and 3 percent Free Fatty Acid Phase was used as a means of separating the components in the samples. Identification of some of the compounds was possible through comparisons of retention times of the peaks on these columns with retention times of known compounds.

Mass spectra of the wheat essence compounds were obtained by use of an Atlas-MAT CH-4 mass spectrometer using a two-second scan, coupled with a gas-liquid chromatograph.

Twelve compounds were identified and six were tentatively identified in the headspace aroma or in the wheat flavor essence. The compounds and their bases for identification were as follows.

Acetaldehyde: identified by mass spectral analysis; retention time in chromatograms of the headspace vapors run on three columns, Free Fatty Acid Phase (FFAP), Diethylene Glycol Succinate (DEGS), and Apiezon M (AM); paper chromatography; and melting point of its 2,4-dinitrophenylhydrazone derivative.

Isobutyraldehyde: identified by retention time in chromatograms of the headspace vapors run on FFAP and DEGS columns; and by the melting point of its 2,4-dinitrophenylhydrazone derivative.

Butyraldehyde: identified by retention time in chromatograms of the headspace vapors run on FFAP and DEGS columns; retention time in the chromatogram of the wheat essence run on the DEGS column; and by the melting point of its 2,4-dinitrophenylhydrazone derivative.

3-Methyl-2-butanone: identified by paper chromatography; melting point of its 2,4-dinitrophenylhydrazone derivative; retention times in chromatograms of the headspace vapors run on three columns (FFAP, DEGS, AM); and retention time in the chromatogram of the wheat essence run on the FFAP column.

Crotonaldehyde: identified by paper chromatography; retention time in the chromatogram of the wheat essence on the FFAP and DEGS column; retention time in the chromatograms of headspace vapors run on three columns (FFAP, DEGS, and AM); and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

2,2-Dimethyl-3-pentanone: identified by retention times in the chromatograms of the headspace vapors on three columns (FFAP, DEGS, and AM); retention time in the chromatogram of the wheat essence on three columns (FFAP, DEGS, and AM); and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

Valeraldehyde: identified by retention times in the chromatograms of the headspace vapors on three columns (FFAP, DEGS, and AM); retention times in the chromatograms of the wheat essence on

three columns (FFAP, DEGS, and AM); and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

Hexanal: identified by retention times in the chromatogram of the headspace vapors on the FFAP and AM columns; retention times in the chromatograms of the wheat essence on the FFAP and DEGS columns; and by the melting point of the 2,4-dinitrophenylhydrazone derivative. The closeness of the melting point values reported for the 2,4-dinitrophenylhydrazone derivatives of valeraldehyde, hexanal, heptanal, and octanal make it necessary to interpret the results of melting point determinations of unknown derivatives falling within the range of 104° C to 110° C with some degree of caution. However, the occurrence of 2,4-dinitrophenylhydrazone derivatives within this range provides supporting evidence for the information derived from the gas chromatographic data.

Heptanal: identified by retention times in the chromatograms of the headspace vapors on three columns (FFAP, DEGS, and AM); retention times in the chromatograms of the wheat essence on three columns (FFAP, DEGS, and AM); and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

Octanal: identified by retention times in the chromatograms of the headspace vapors on the AM and FFAP columns; retention time in the chromatograms of the wheat essence on the AM and DEGS columns; and by the melting point of the 2,4-dinitrophenylhydrazone

derivative.

Diacetyl: identified by mass spectral analysis; retention times in the chromatograms of the headspace vapors on the FFAP and DEGS columns; and by retention times in the chromatograms of the wheat essence on the FFAP and DEGS columns.

Ethyl acetate: identified by mass spectral analysis; retention times in the chromatograms of the headspace vapors on the AM column; and by retention times in the chromatograms of the wheat essence on three columns (FFAP, DEGS, and AM).

Tentative identifications of the following compounds were also made.

Isoamyl alcohol: tentatively identified by retention times in the chromatograms of the headspace vapors on three columns (FFAP, DEGS, and AM); and by retention times in the chromatograms of the wheat essence on three columns (FFAP, DEGS, and AM).

Amyl alcohol: tentatively identified by retention times in the chromatograms of the headspace vapors on three columns (FFAP, DEGS, and AM); and by retention times in the chromatograms of the wheat essence on three columns (FFAP, DEGS, and AM).

Cyclopentanone: tentatively identified by retention times in the chromatograms of the headspace vapors on the FFAP and AM columns; and by retention times in the chromatograms of the wheat essence on the FFAP and AM columns.

Butanone: tentatively identified by retention time in the chromatogram of the headspace vapors on the DEGS column; retention time in the chromatogram of the wheat essence on the DEGS column; and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

Isovaleraldehyde: tentatively identified by retention time in the chromatogram of the headspace vapors on the DEGS column; retention time in the chromatogram of the wheat essence on the DEGS column; and by the melting point of the 2,4-dinitrophenylhydrazone derivative.

Phenylacetaldehyde: tentatively identified by mass spectral analysis.



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## APPENDIX

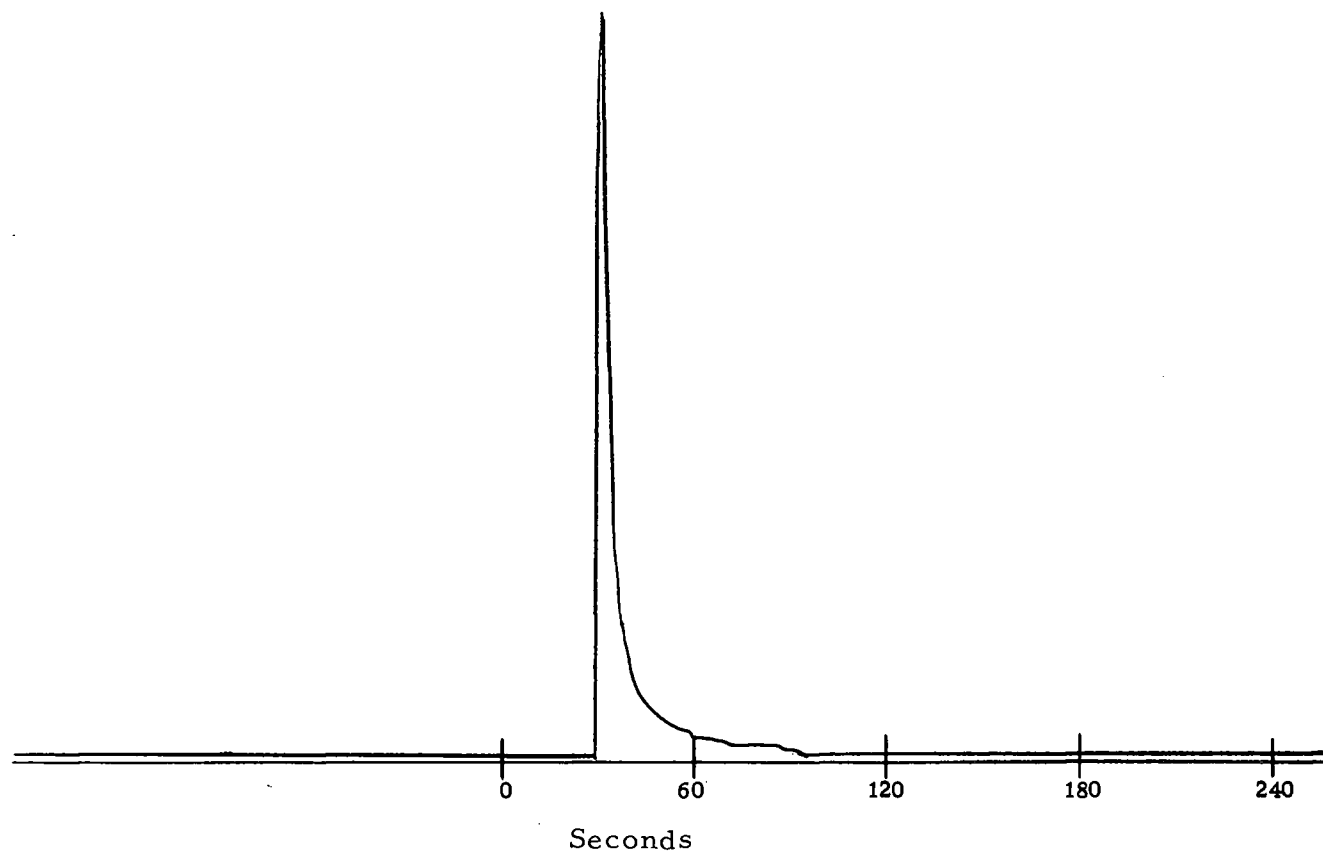


Figure 14. Retention time of known compounds.  
Acetaldehyde,  $t_R = 30$  seconds on 6% Apiezon M at 75° C.

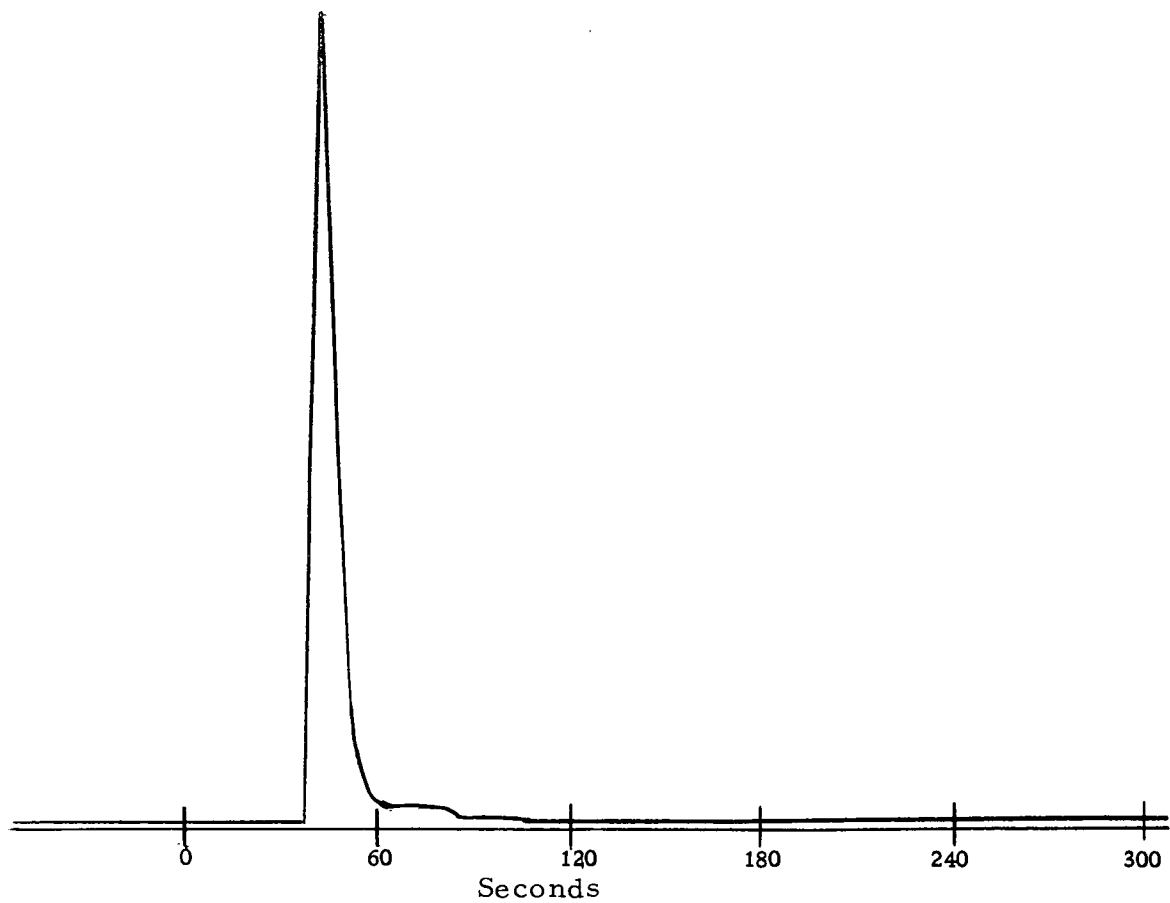


Figure 15. Retention time of known compounds.  
Isobutyraldehyde,  $t_R = 43$  seconds on 6% Apiezon M at 75° C.

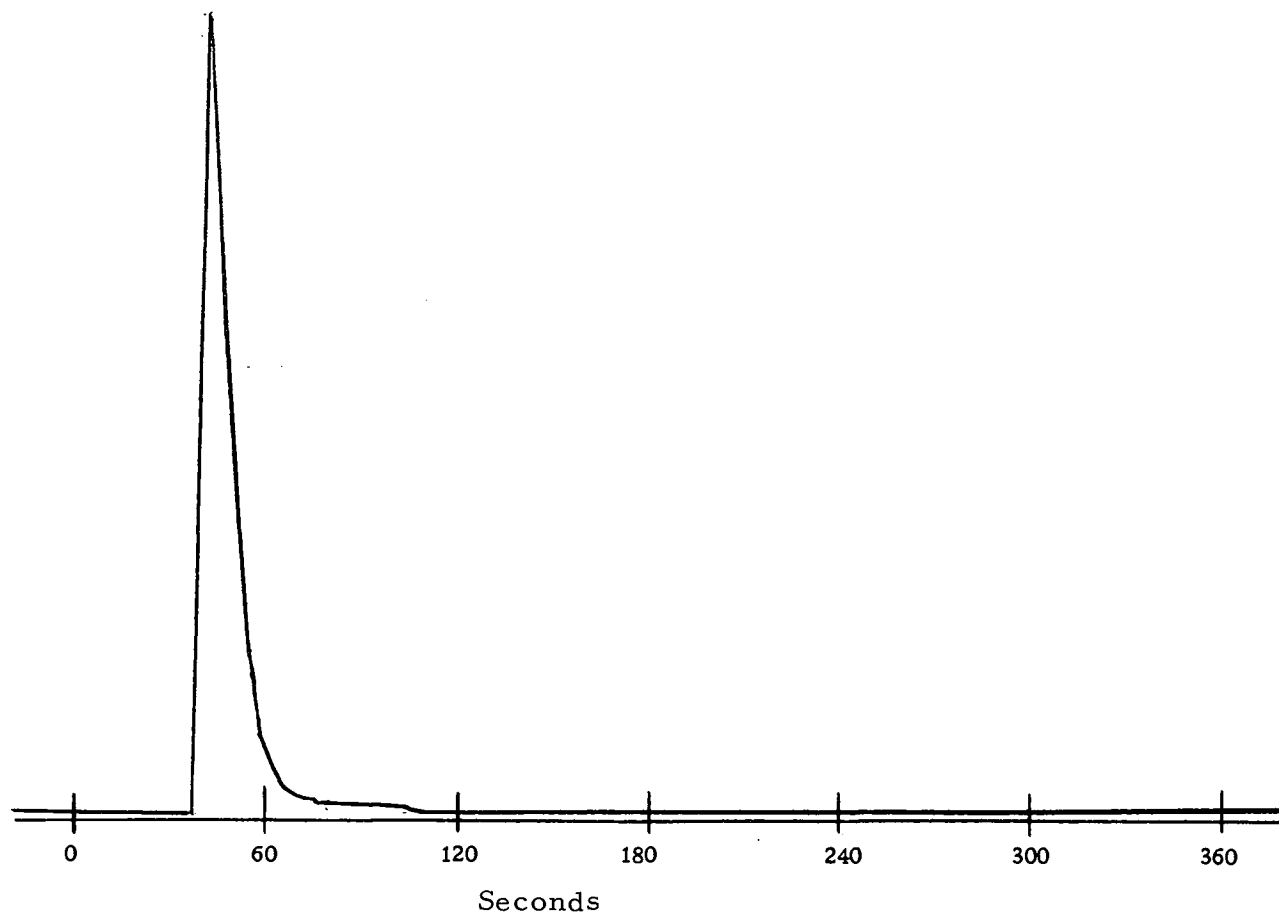


Figure 16. Retention time of known compounds.  
Butyraldehyde,  $t_R = 45$  seconds on 6% Apiezon M at 75° C.

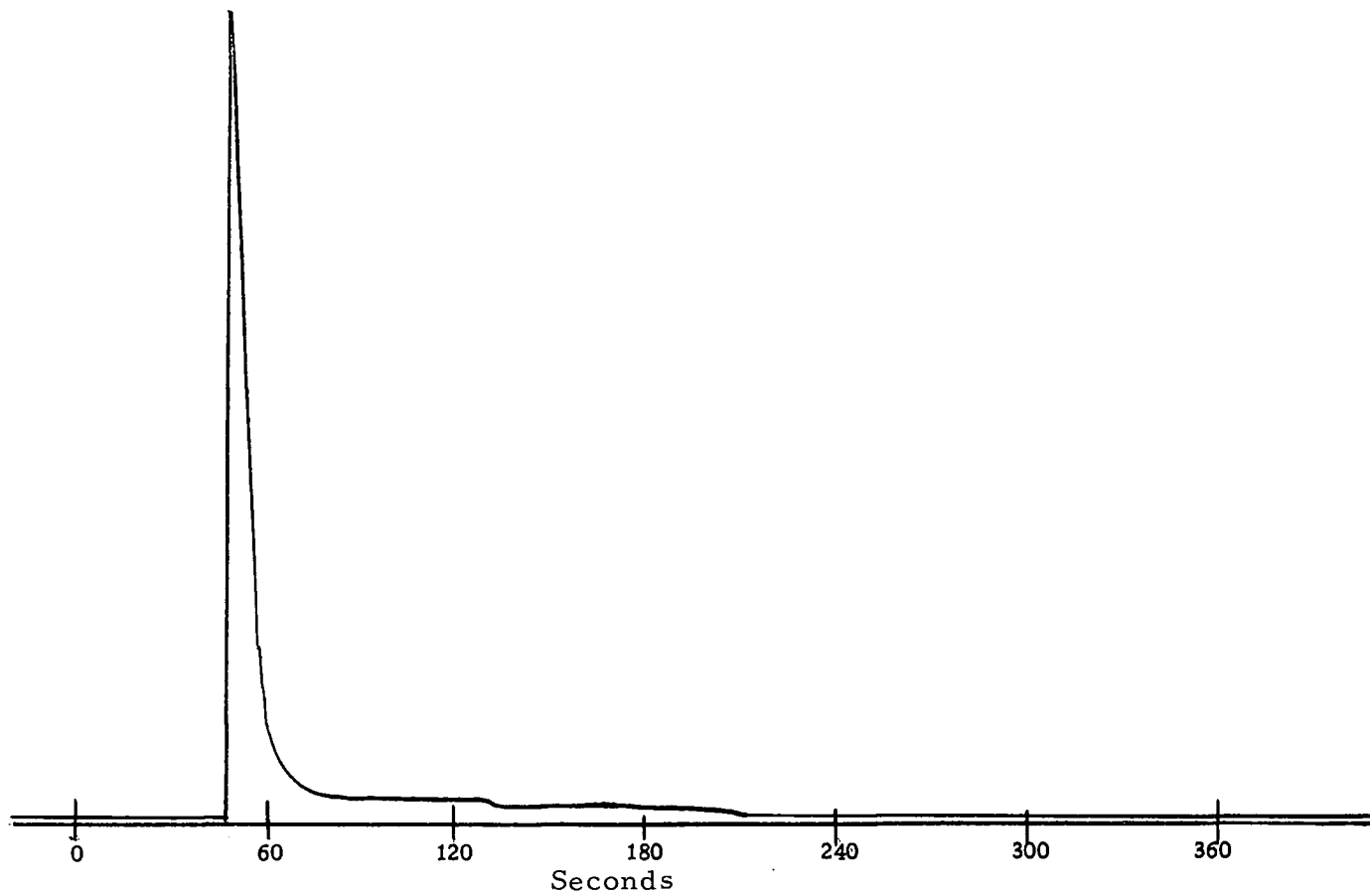


Figure 17. Retention time of known compounds.  
Diacetyl,  $t_R = 48$  seconds on 6% Apiezon M at 75° C.

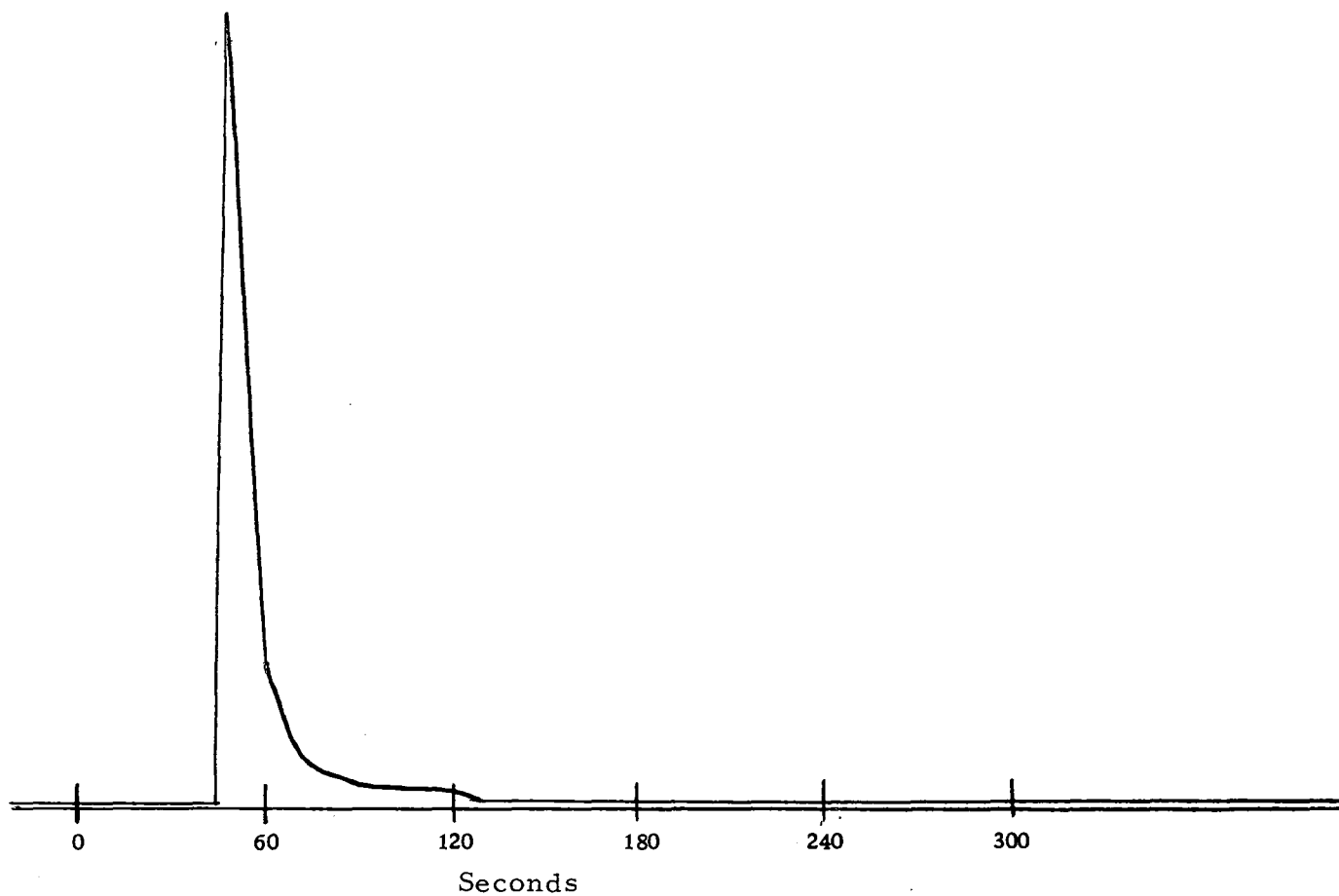


Figure 18. Retention time of known compounds.  
Butanone,  $t_R = 50$  seconds on 6% Apiezon M at 75° C.

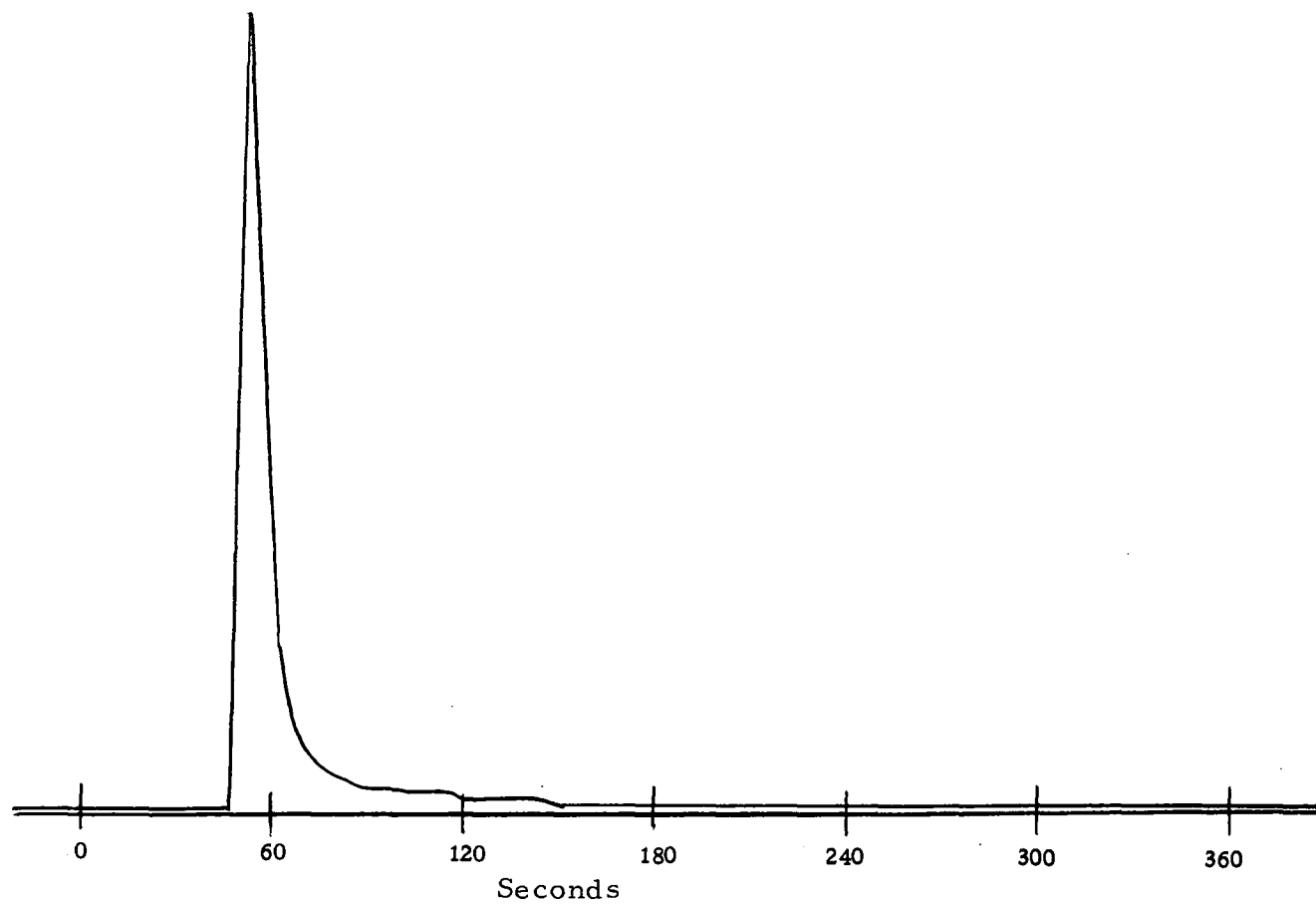


Figure 19. Retention time of known compounds.  
Ethyl acetate,  $t_R = 51$  seconds on 6% Apiezon M at 75° C.



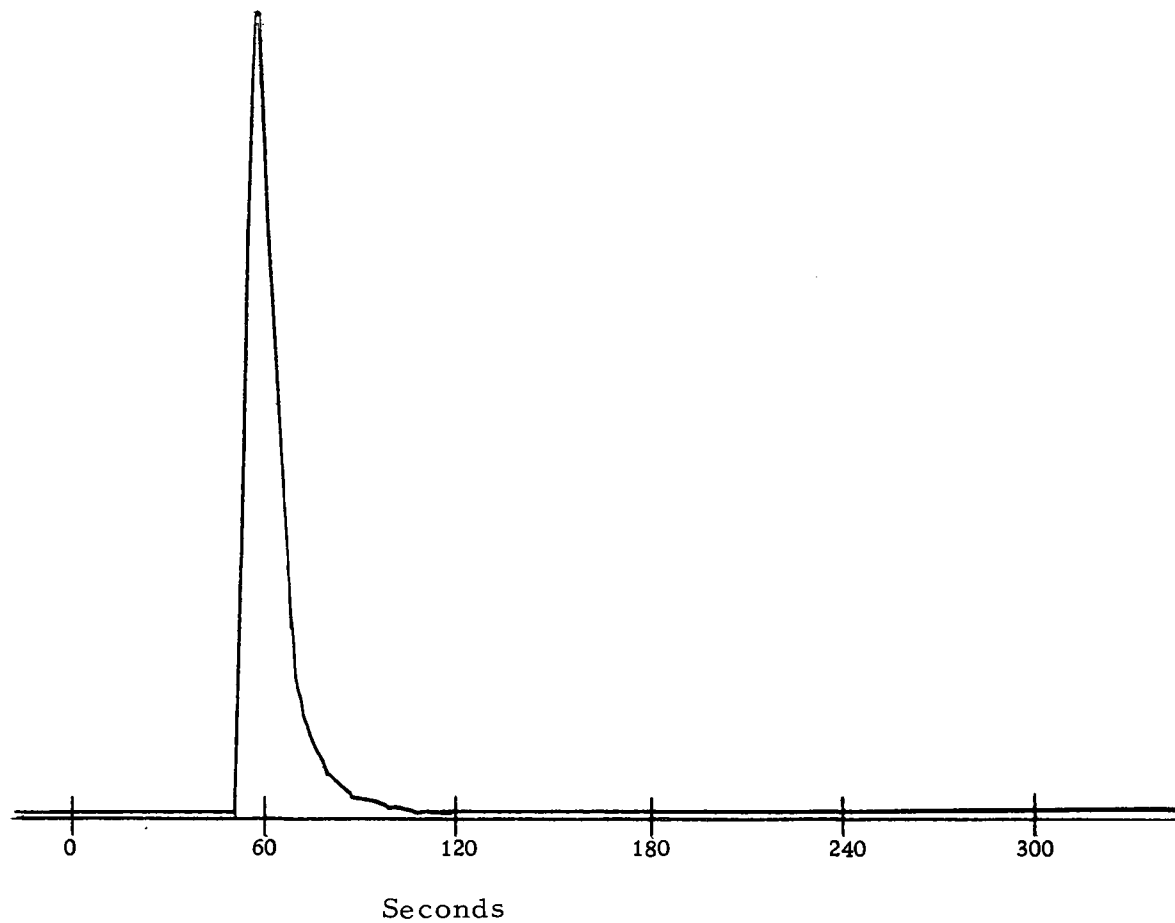


Figure 20. Retention time of known compounds.

3-Methyl-2-butanone,  $t_R = 55$  seconds on 6% of Apiezon M at 75° C.

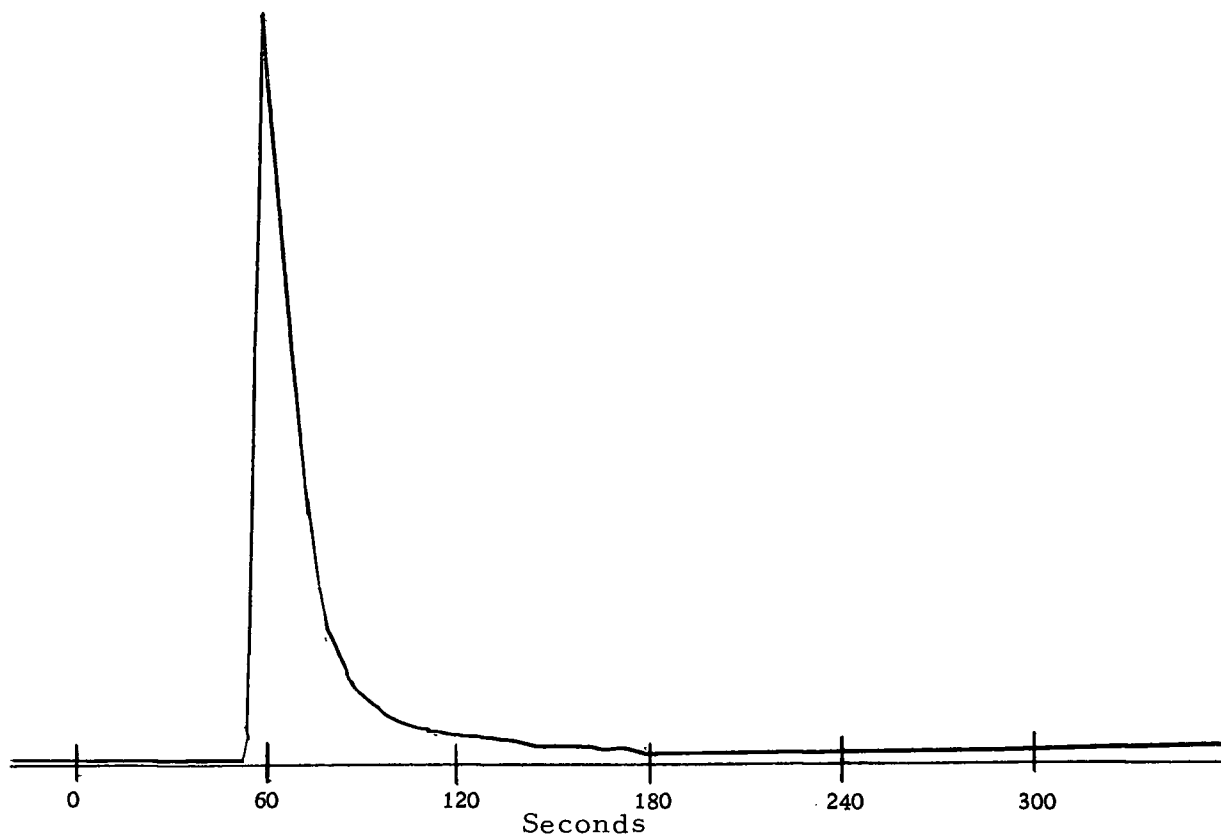


Figure 21. Retention time of known compounds.  
Crotonaldehyde,  $t_R = 58$  seconds on 6% Apiezon M at 75° C.

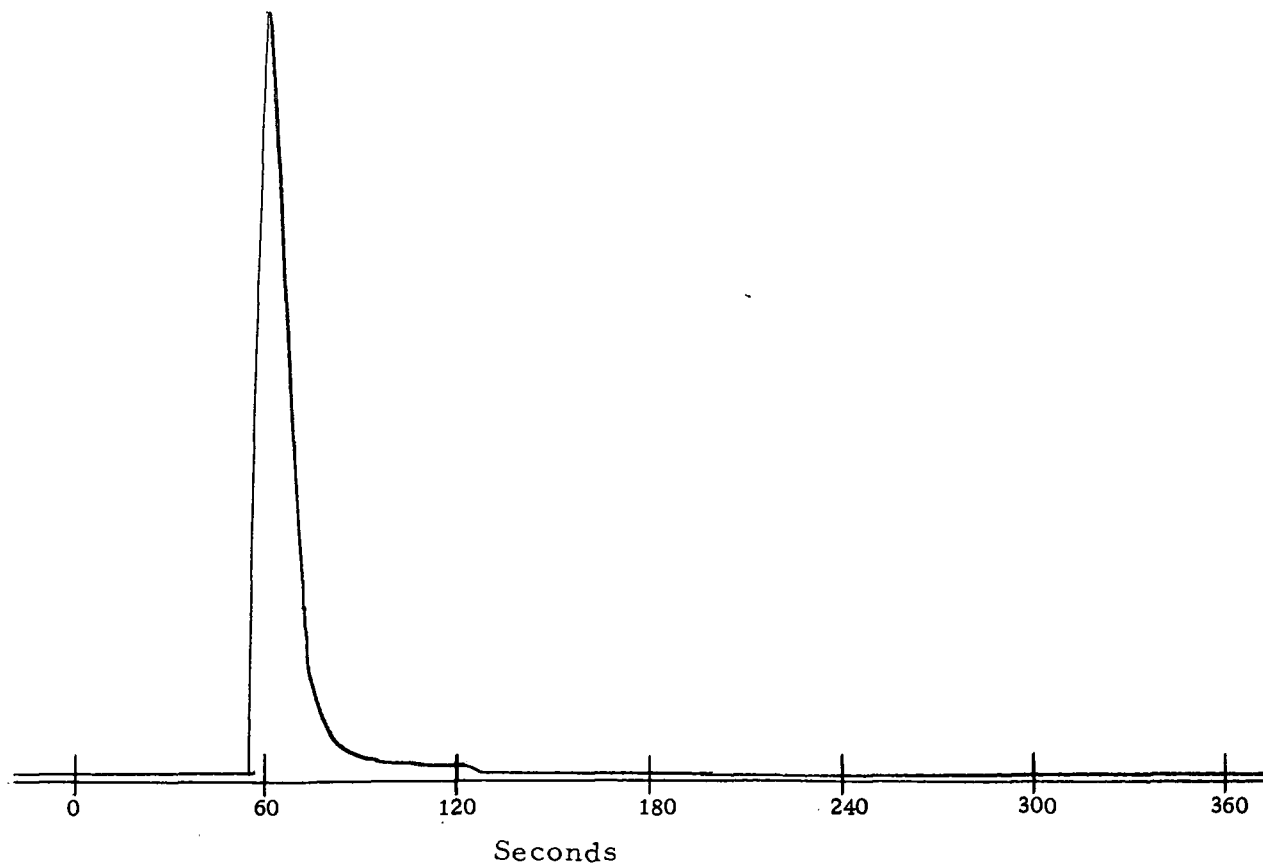


Figure 22. Retention time of known compounds.  
Isovaleraldehyde,  $t_R = 61$  seconds on 6% Apiezon M at 75° C.

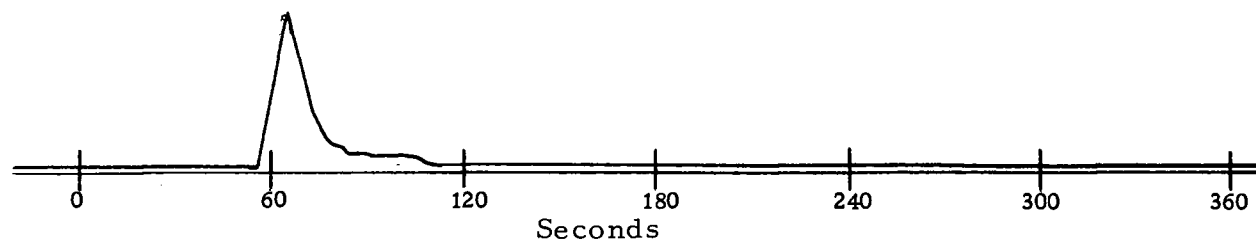


Figure 23. Retention time of known compounds.

Valeraldehyde,  $t_R = 68$  seconds on 6% Apiezon M at 75° C.

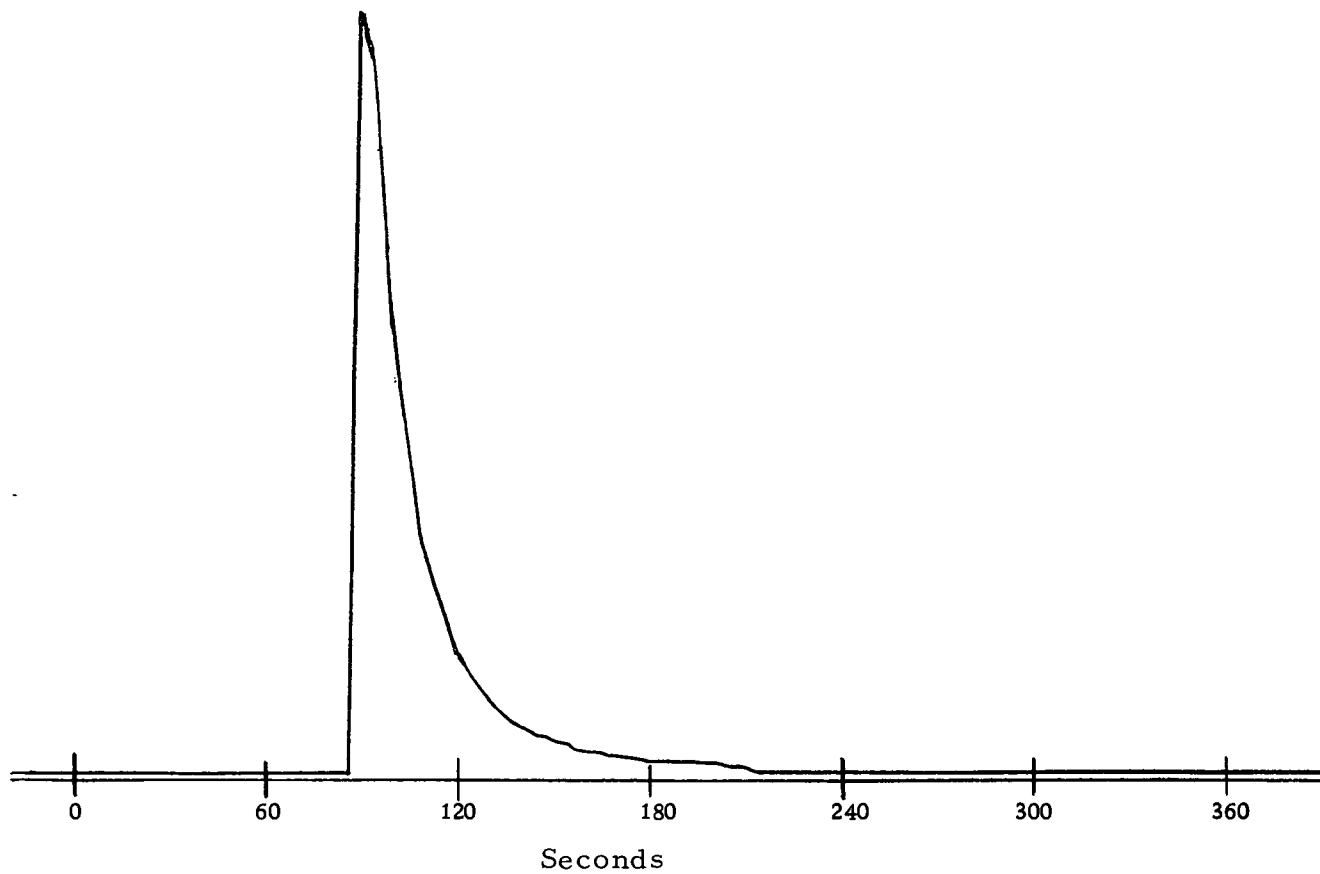


Figure 24. Retention time of known compounds.

Isoamyl alcohol,  $t_R = 89$  seconds on 6% Apiezon M at 75° C.

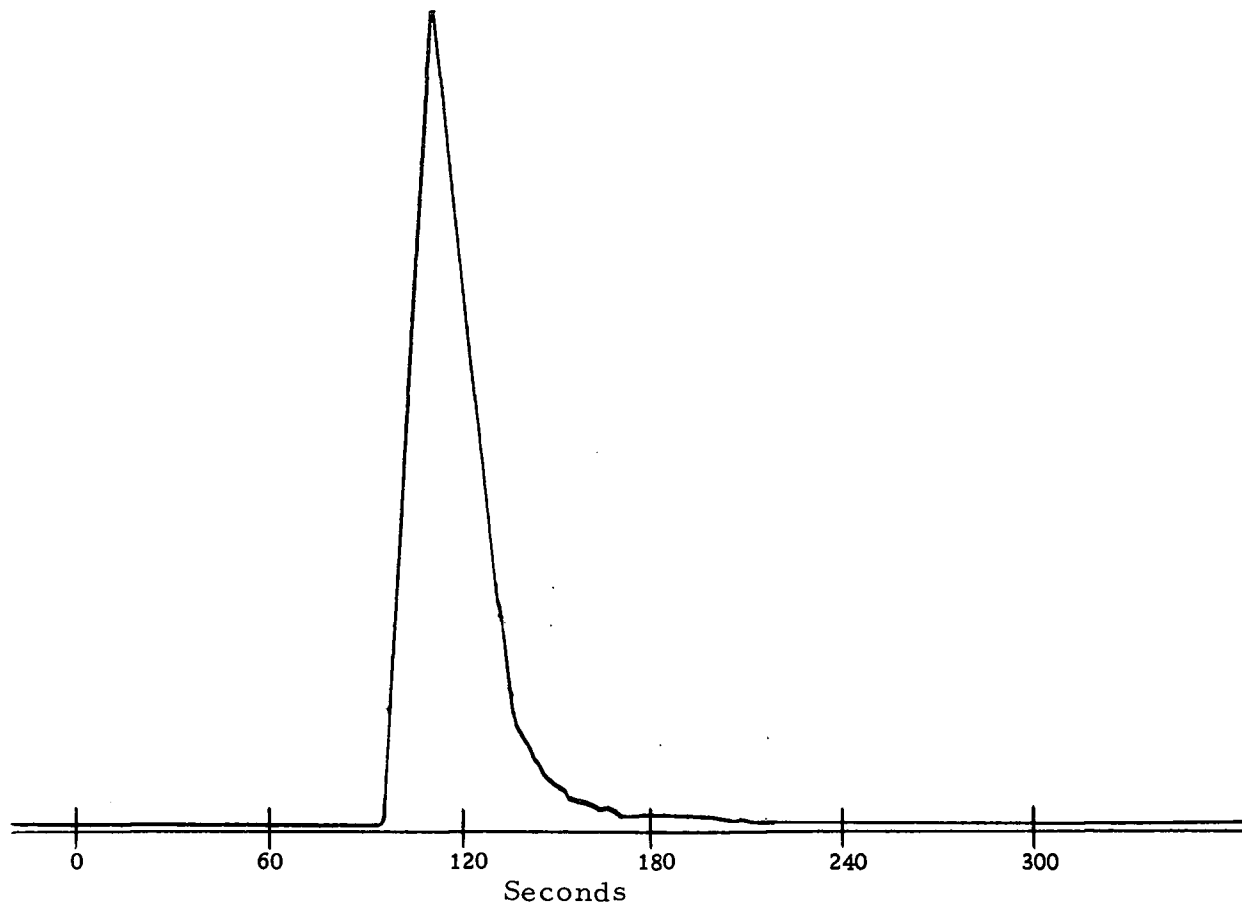


Figure 25. Retention time of known compounds;  
2, 2-Dimethyl-3-pentanone,  $t_R = 110$  seconds on 6% Apiezon M at 75° C.

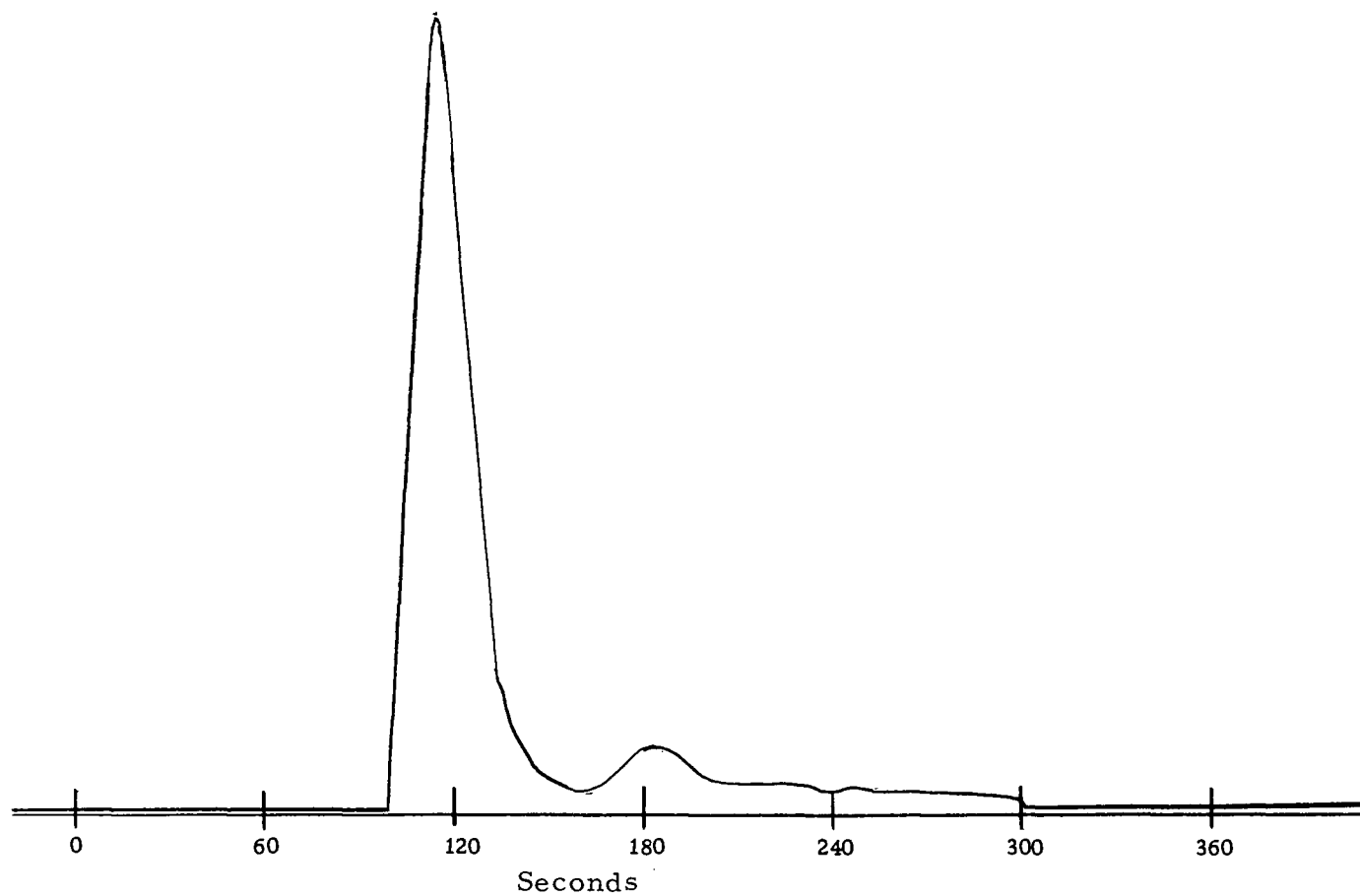


Figure 26. Retention time of known compounds.  
Hexanal,  $t_R = 114$  seconds on 6% Apiezon M at 75° C.

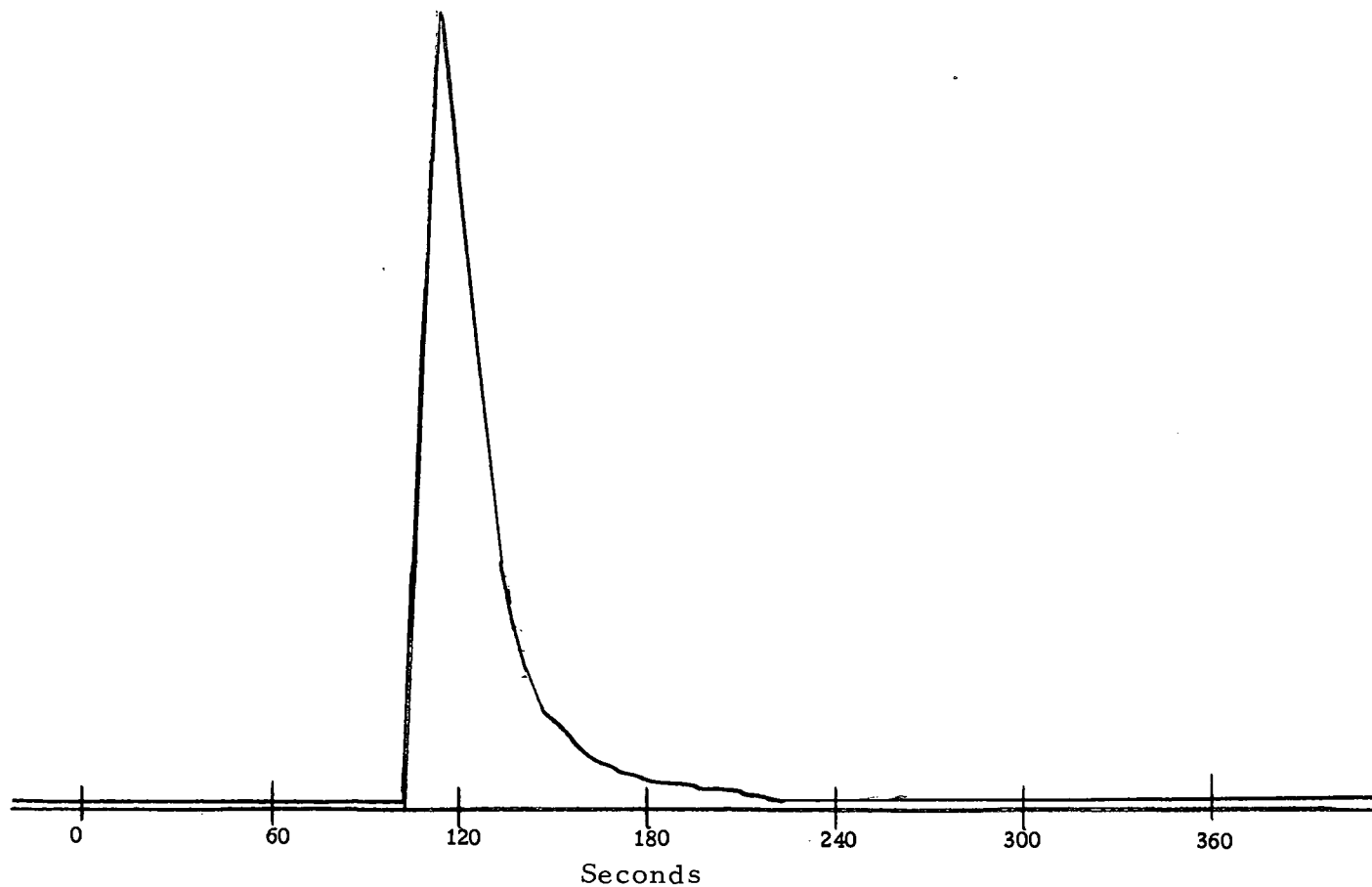


Figure 27. Retention time of known compounds.  
Cyclopentanone,  $t_R = 115$  seconds on 6% Apiezon M at 75° C.



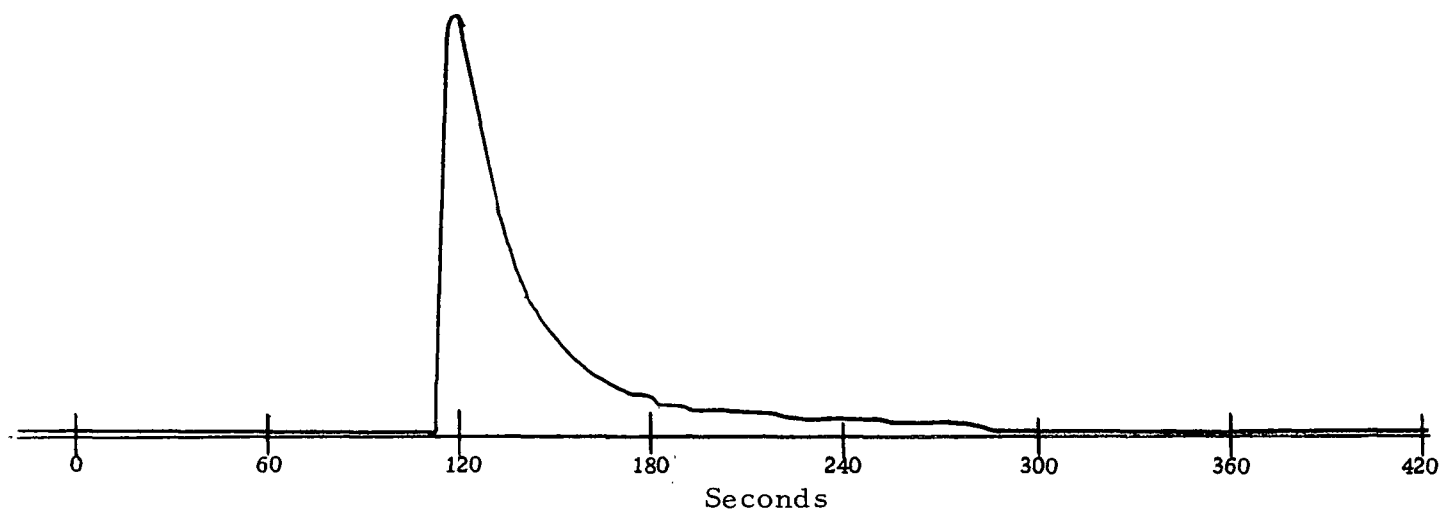


Figure 28. Retention time of known compounds.  
Amyl alcohol,  $t_R = 118$  seconds on 6% Apiezon M at 75° C.

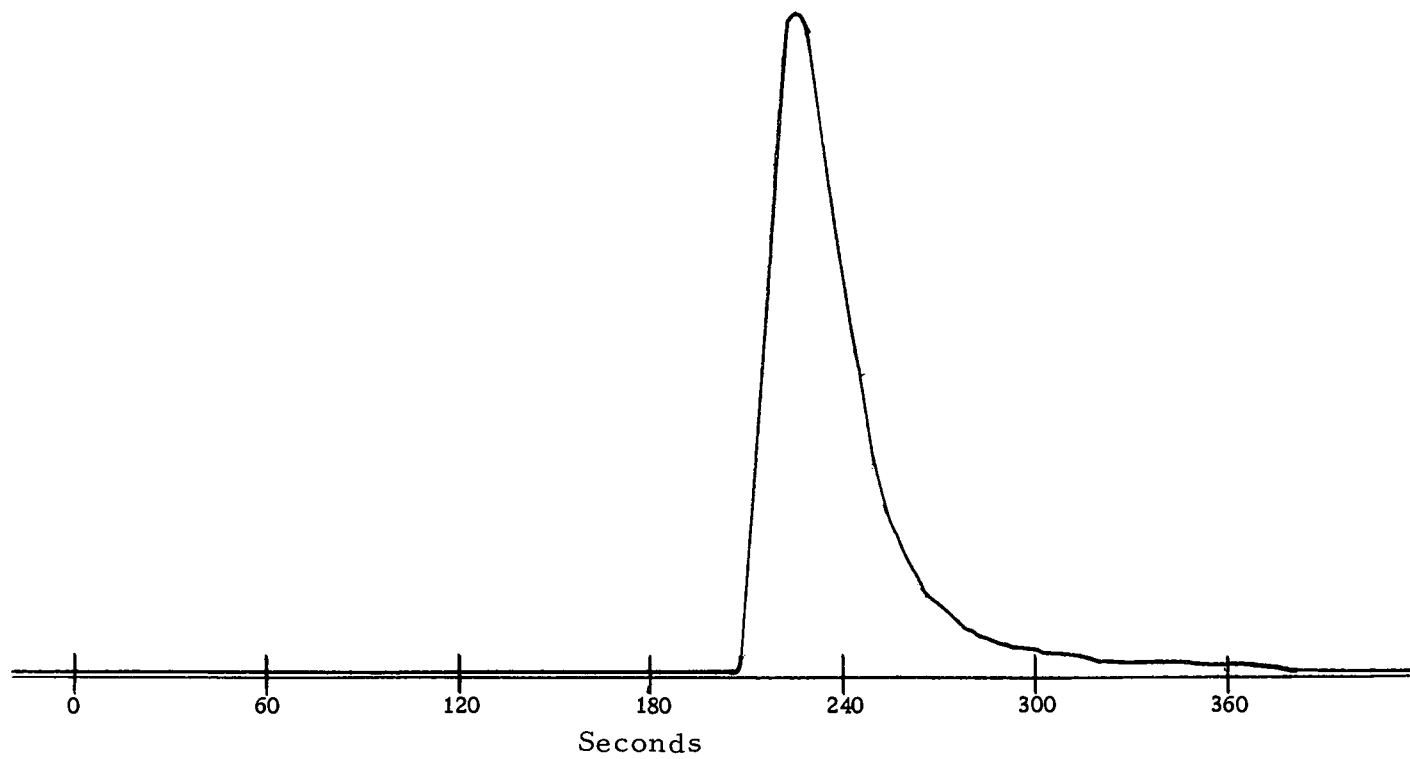


Figure 29. Retention time of known compounds.  
Heptanal,  $t_R = 226$  seconds on 6% Apiezon M at 75° C.

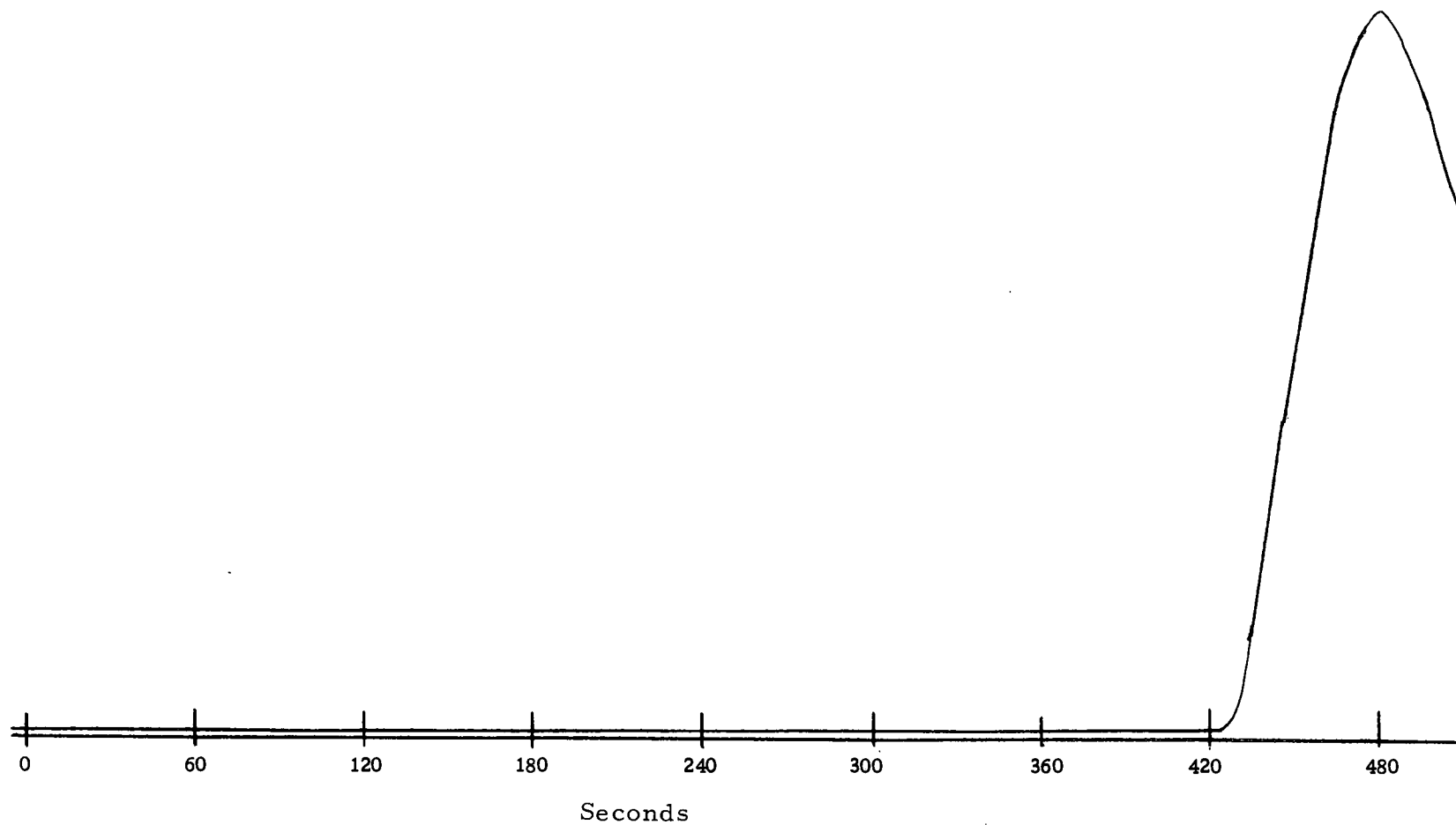


Figure 30. Retention time of known compounds.  
Octanal,  $t_R = 478$  seconds on 6% Apiezon M at 75° C.

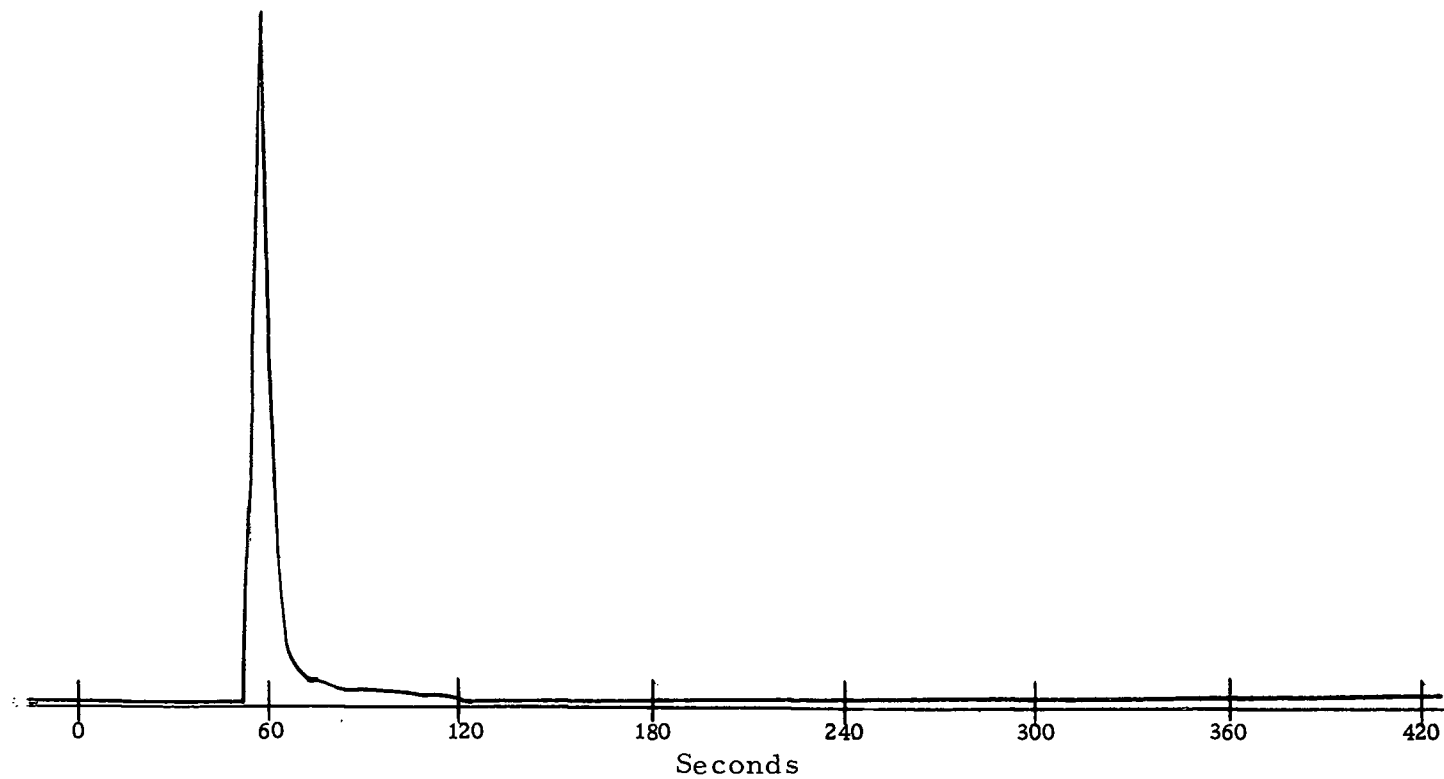


Figure 31. Retention time of known compounds.  
Acetaldehyde,  $t_R = 57$  seconds on Diethylene Glycol Succinate at 75° C.

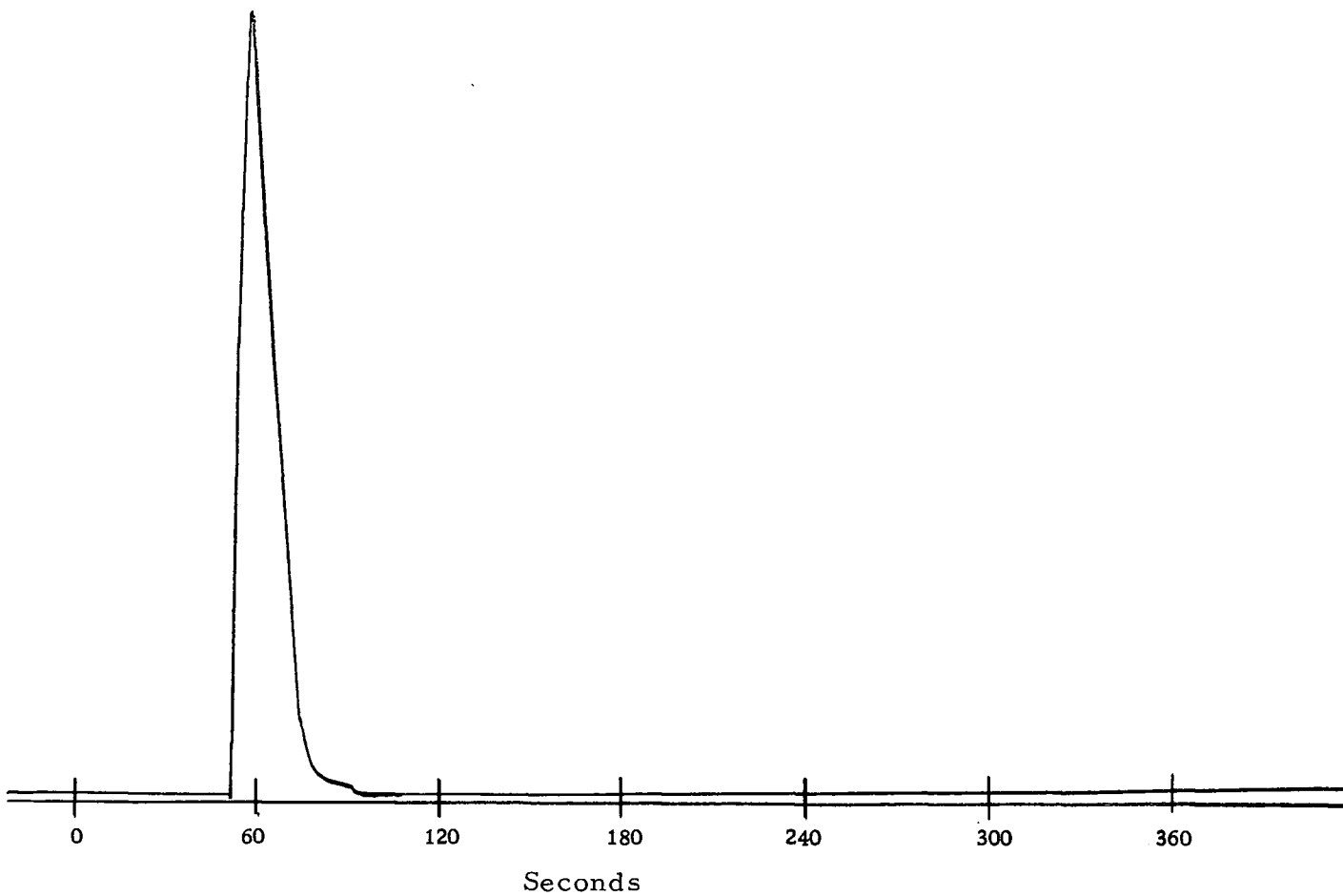


Figure 32. Retention time of known compounds.

Isobutyraldehyde,  $t_R = 62$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

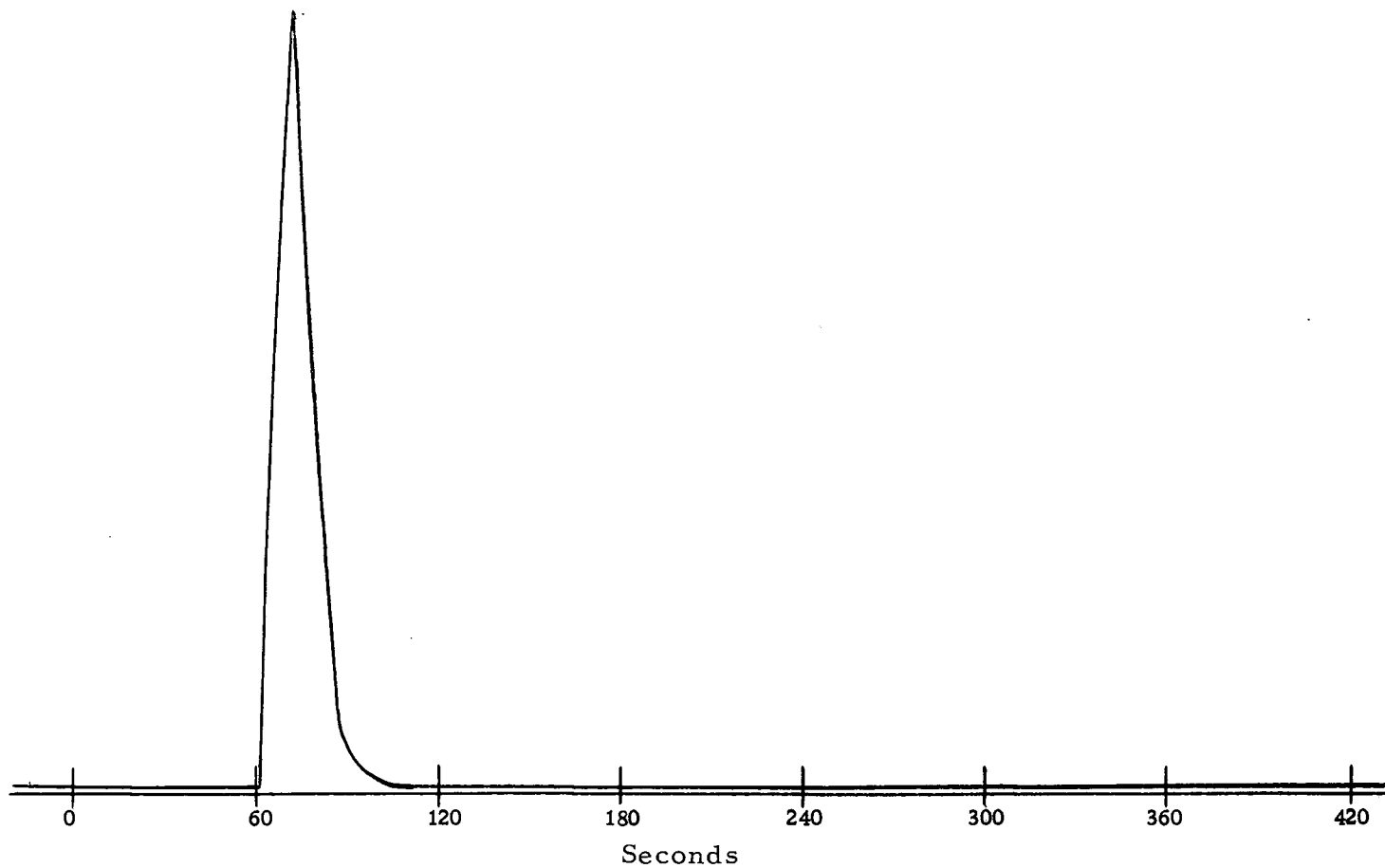


Figure 33. Retention time of known compounds.

Butyraldehyde,  $t_R = 73$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

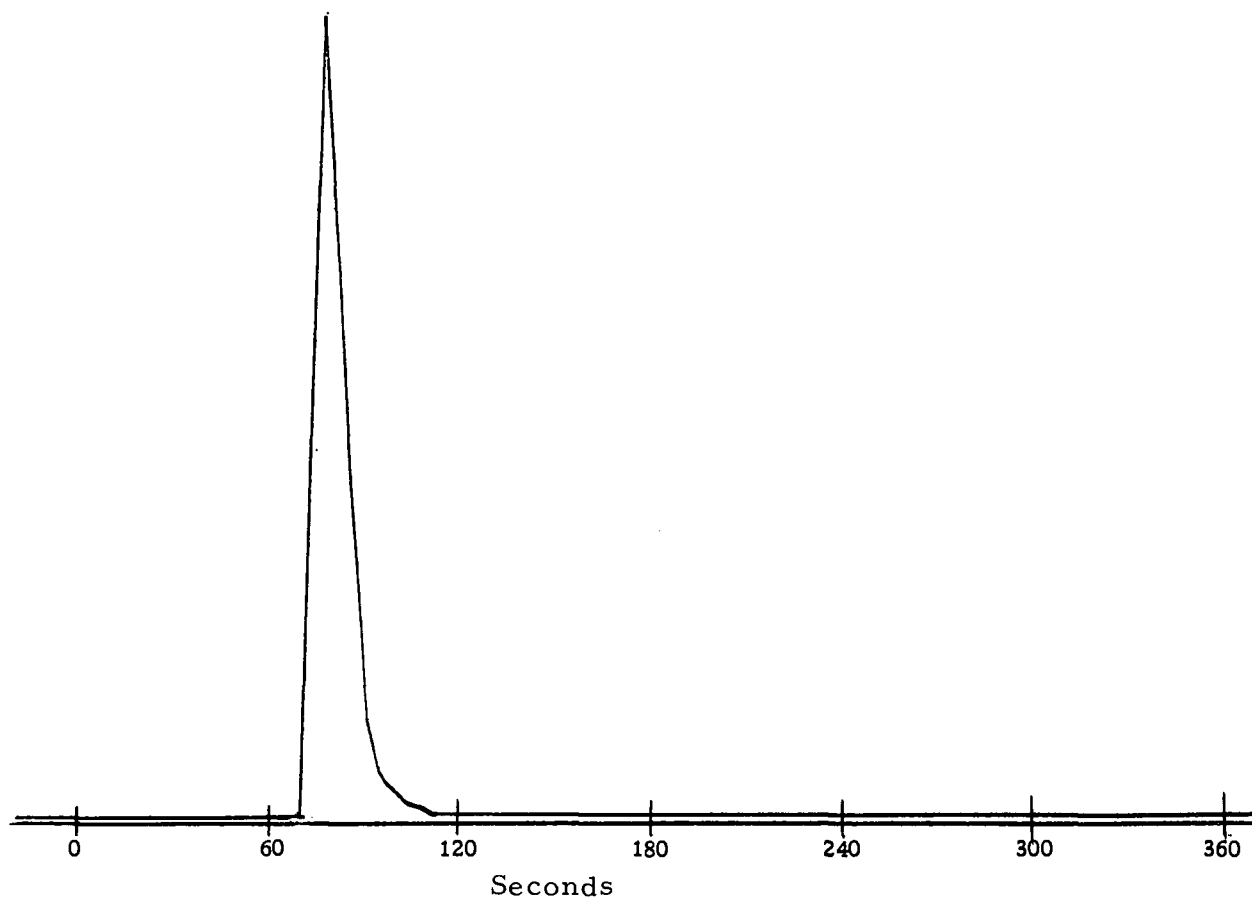


Figure 34. Retention time of known compounds.

Ethyl acetate,  $t_R = 79$  seconds on Diethylene Glycol Succinate at 75° C.

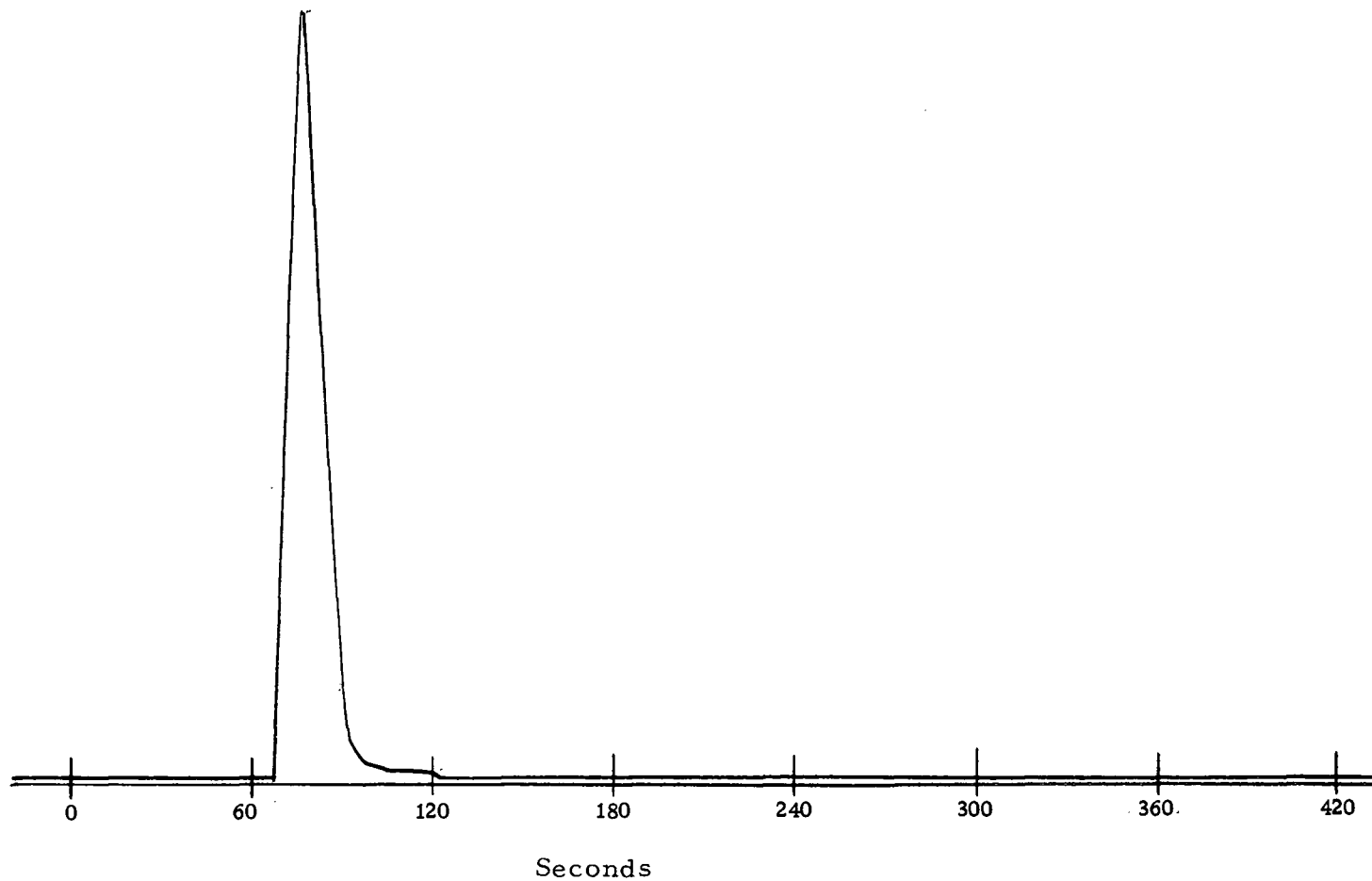


Figure 35. Retention time of known compounds.

Butanone,  $t_R = 79$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .



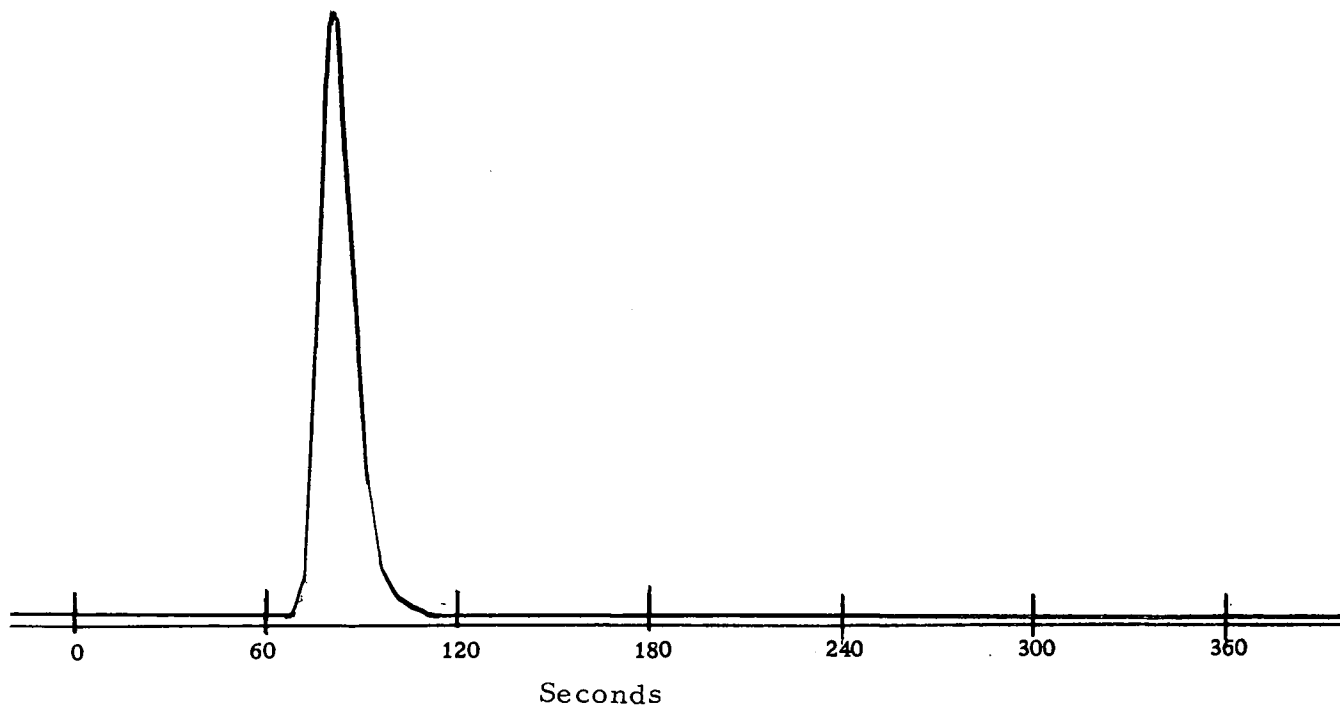


Figure 36. Retention time of known compounds.  
Isovaleraldehyde,  $t_R = 81$  seconds on Diethylene Glycol Succinate at 75° C.

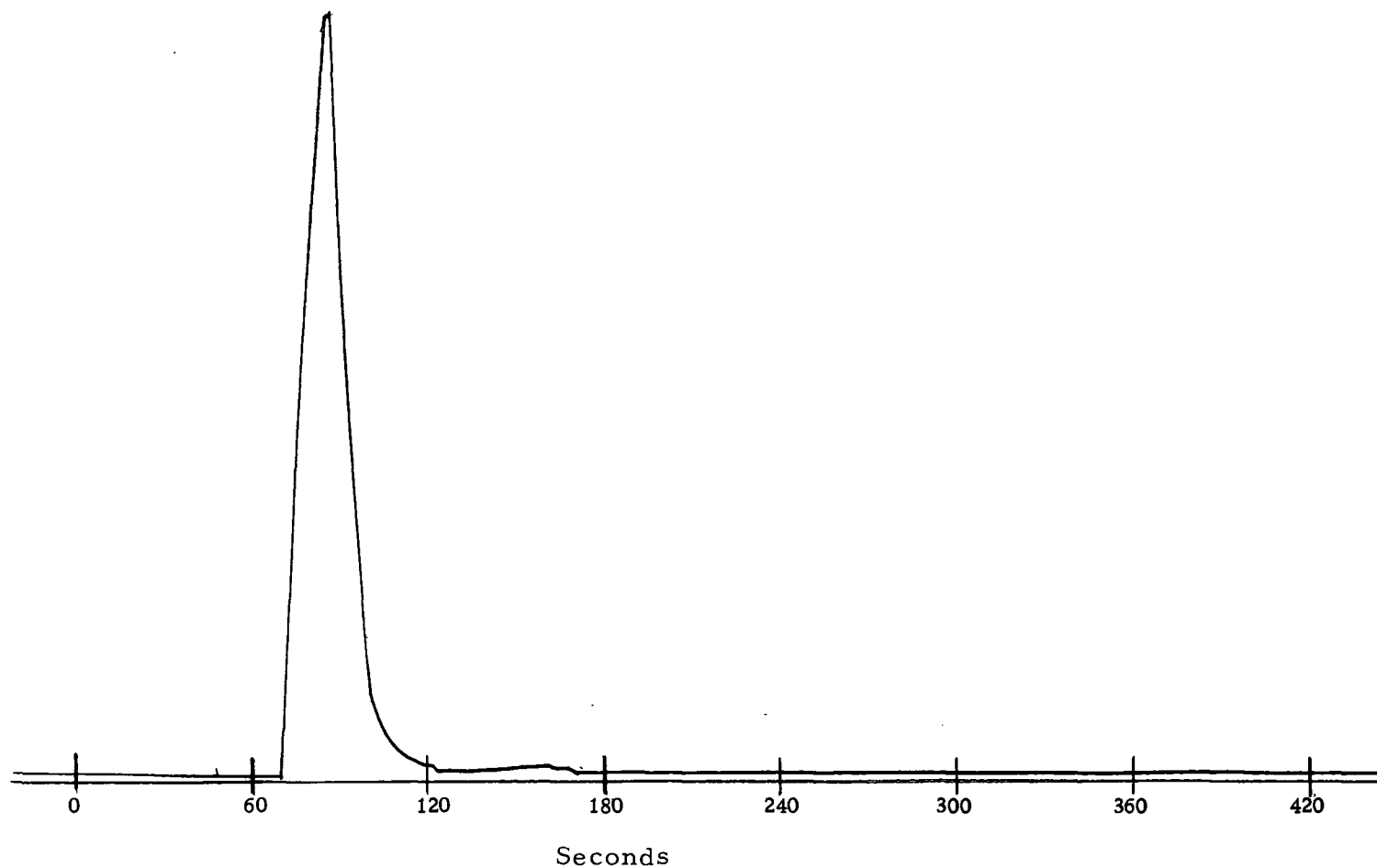


Figure 37. Retention time of known compounds.

3-Methyl-2-butanone,  $t_R = 84$  seconds on Diethylene Glycol Succinate at 75° C.

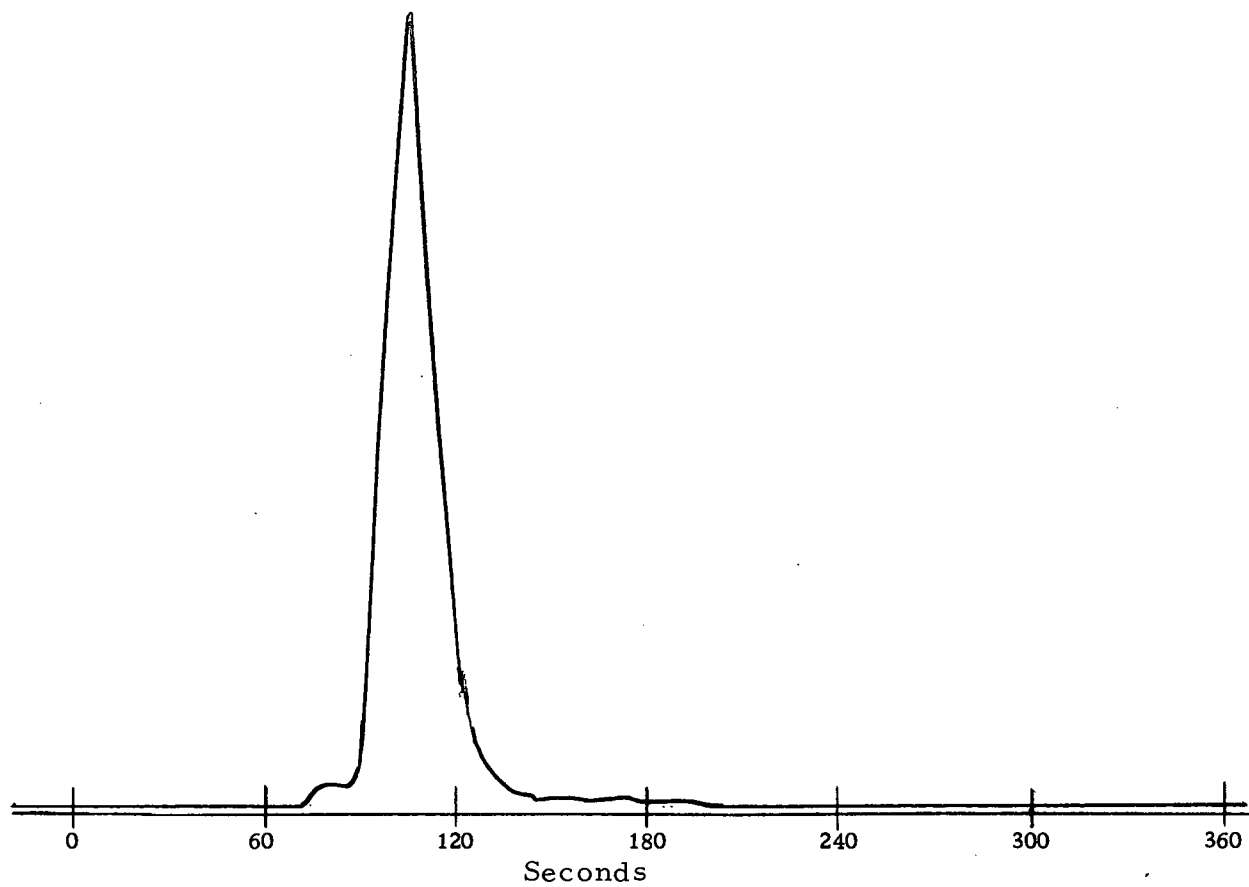


Figure 38. Retention time of known compounds.  
2,2-Dimethyl-3-pentanone,  $t_R = 104$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

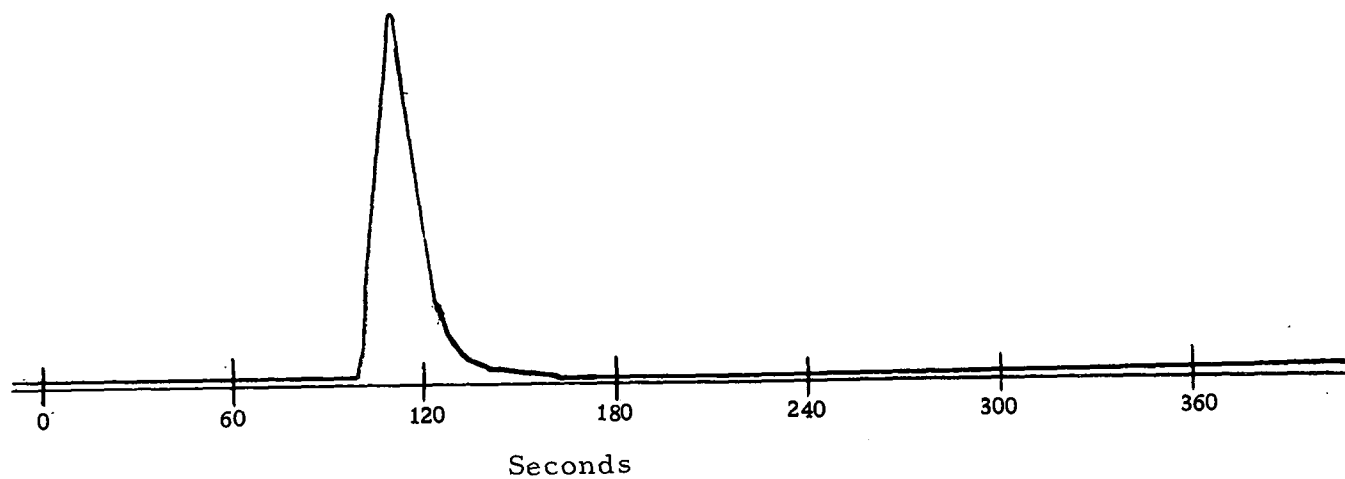


Figure 39. Retention time of known compounds.  
Valeraldehyde,  $t_R = 110$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

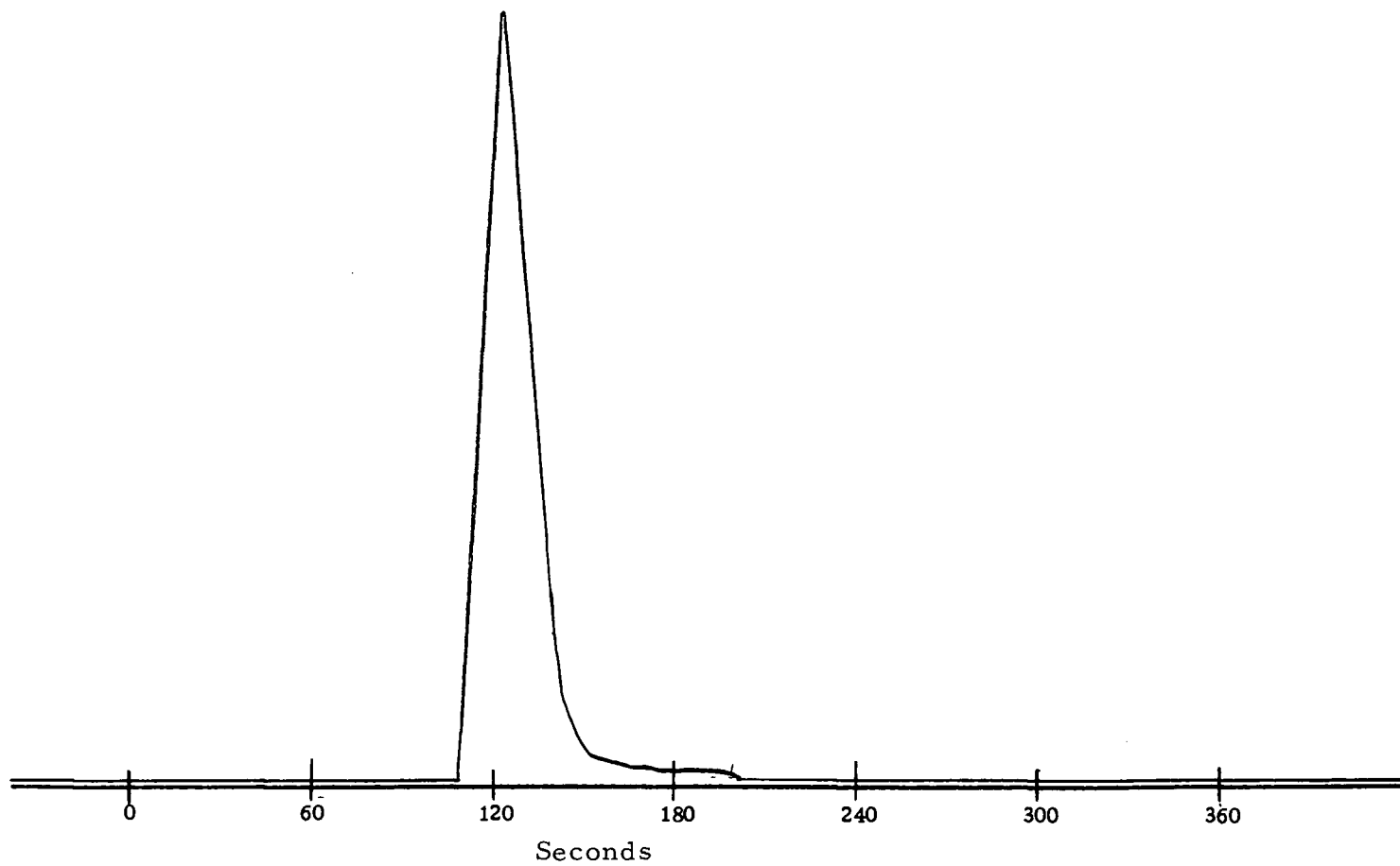


Figure 40. Retention time of known compounds.

Diacetyl,  $t_R = 124$  seconds on Diethylene Glycol Succinate at 75° C.

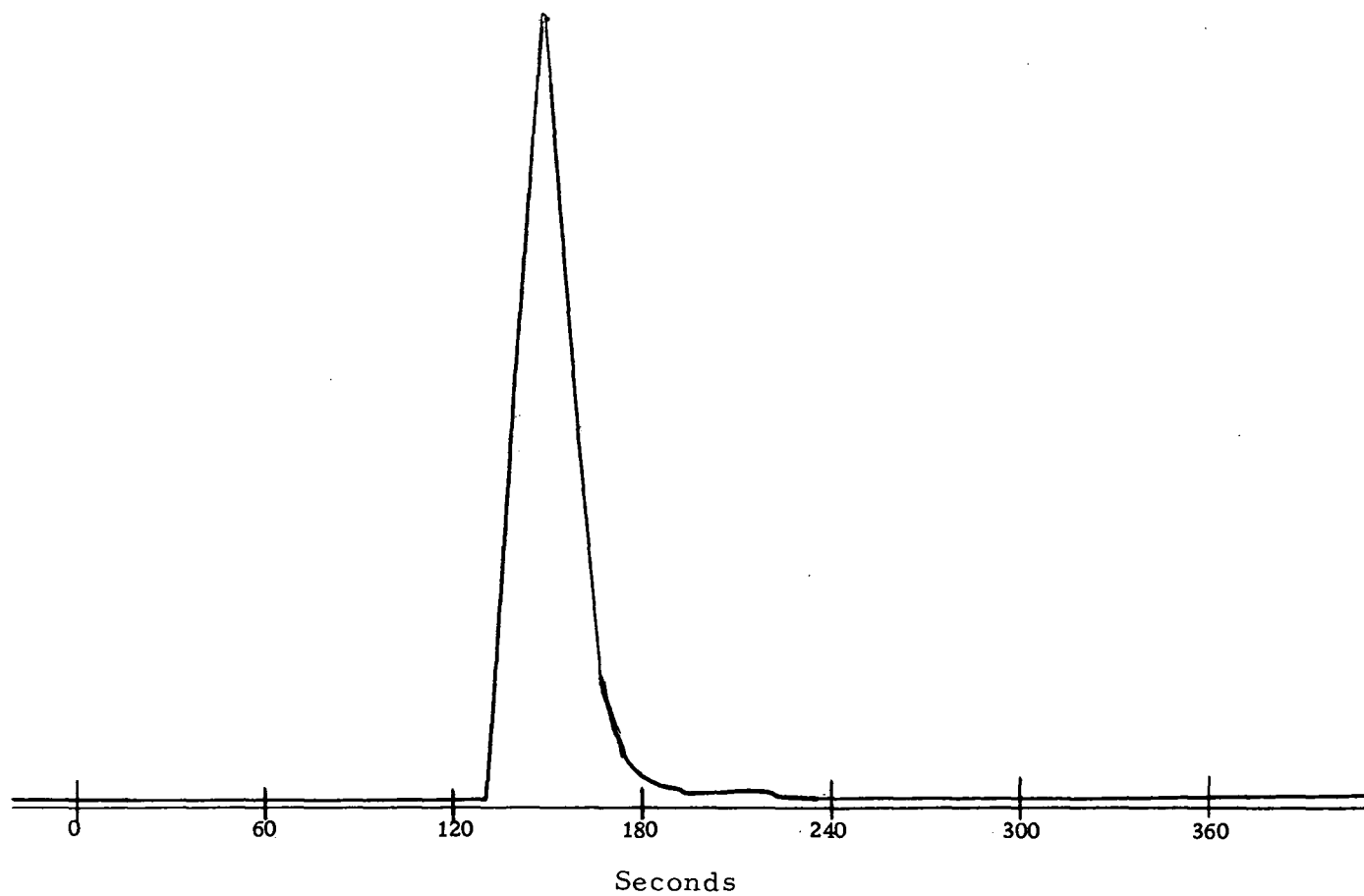


Figure 41. Retention time of known compounds.

Crotonaldehyde,  $t_R = 148$  seconds on Diethylene Glycol Succinate at 75° C.

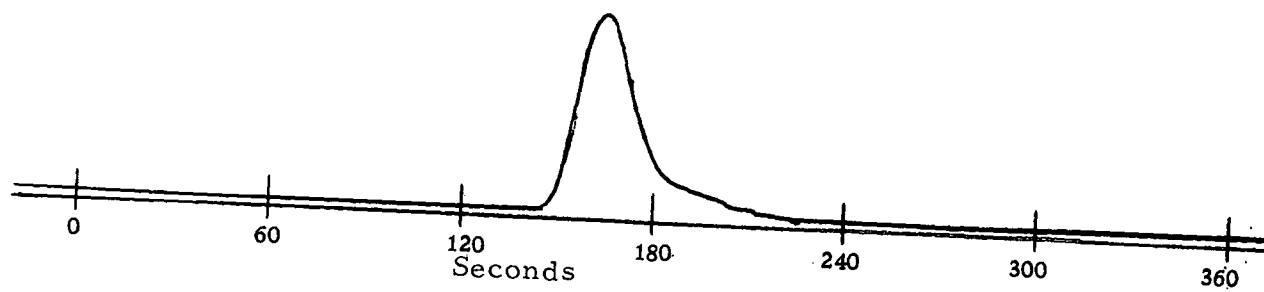


Figure 42. Retention time of known compounds.  
Hexanal,  $t_R = 164$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

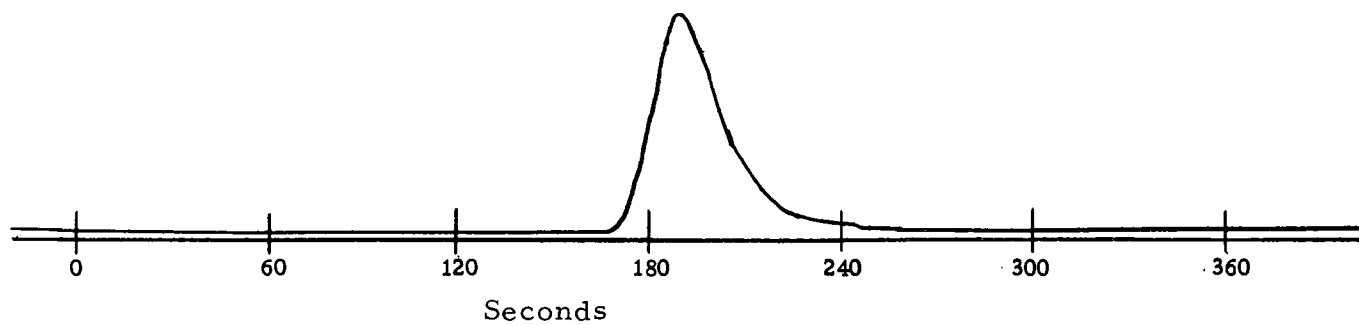


Figure 43. Retention time of known compounds.

Isoamyl alcohol,  $t_R = 190$  seconds on Diethylene Glycol Succinate at 75° C.



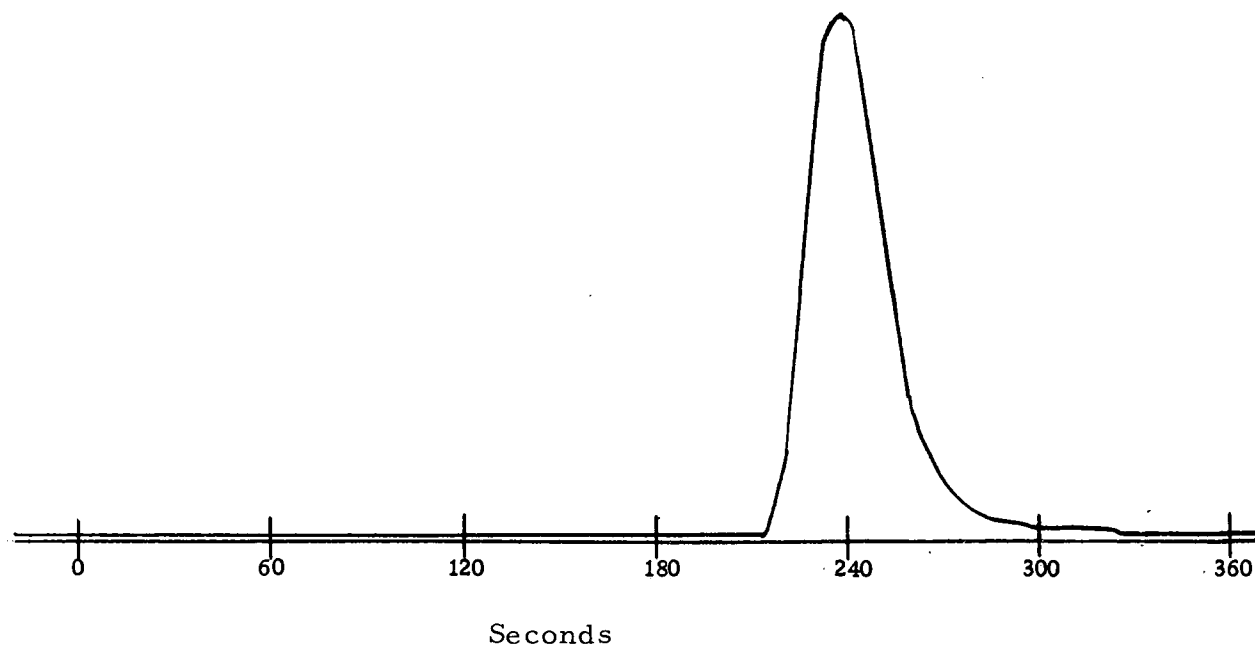


Figure 44. Retention time of known compounds.

Amyl alcohol,  $t_R = 228$  seconds on Diethylene Glycol Succinate at 75° C.

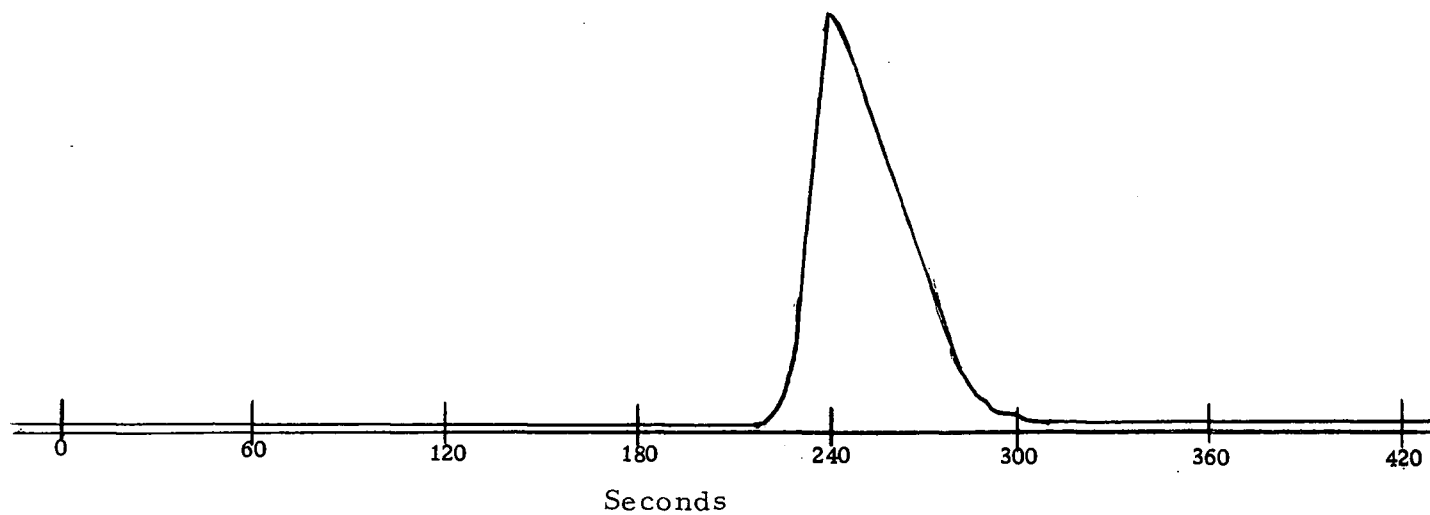


Figure 45. Retention time of known compounds.

Heptanal,  $t_R = 239$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

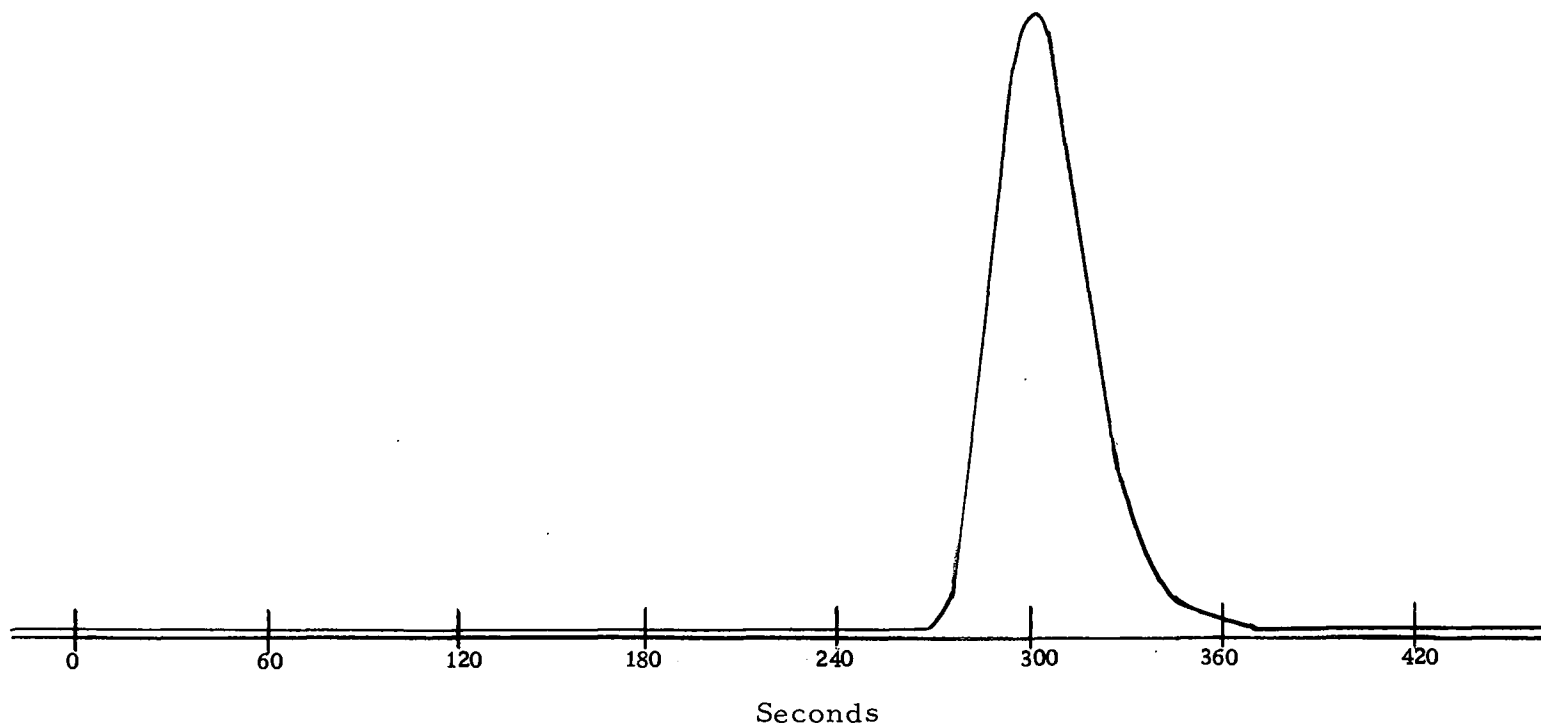


Figure 46. Retention time of known compounds.

Cyclopentanone,  $t_R = 302$  seconds on Diethylene Glycol Succinate at 75° C.

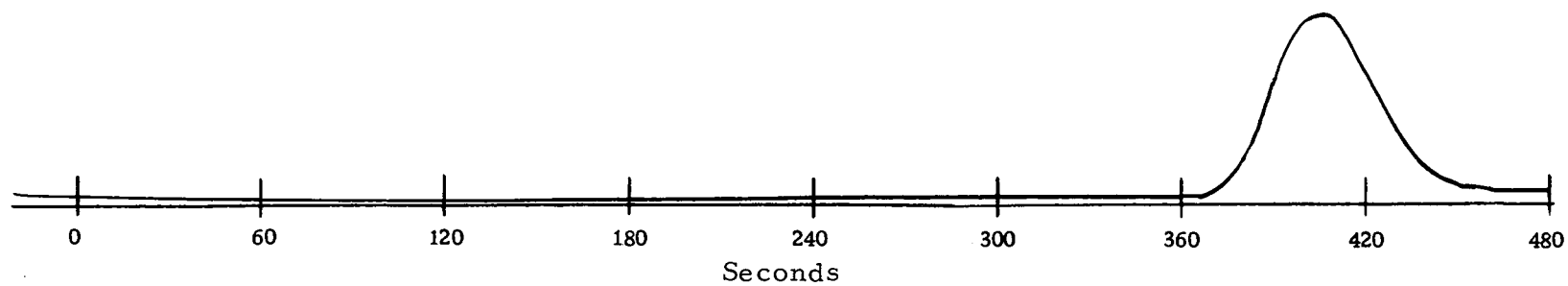


Figure 47. Retention time of known compounds.

Octanal,  $t_R = 406$  seconds on Diethylene Glycol Succinate at  $75^\circ \text{C}$ .

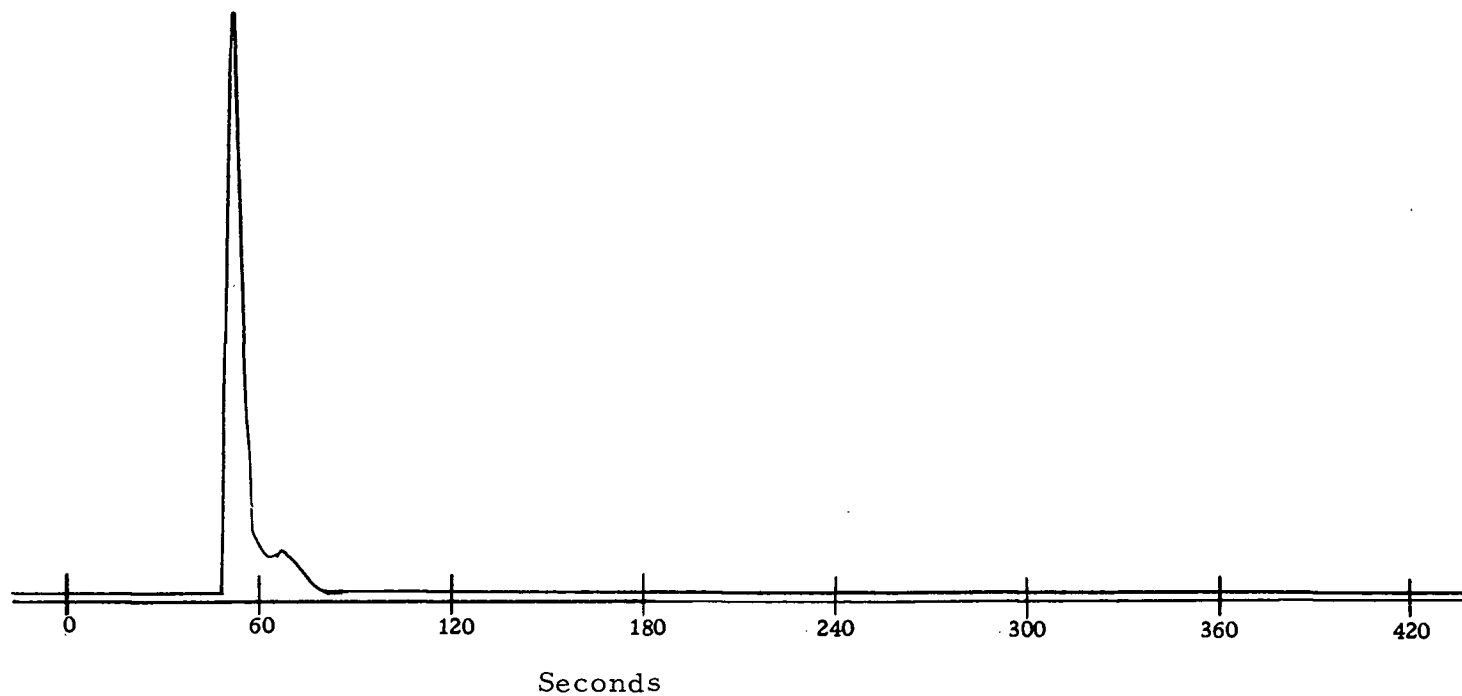


Figure 48. Retention time of known compounds.

Acetaldehyde,  $t_R = 52$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

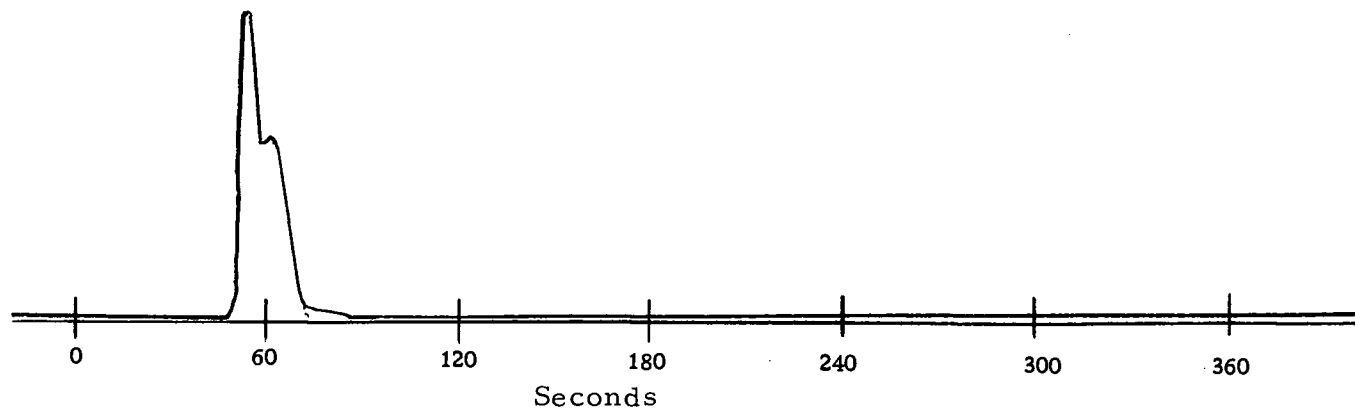


Figure 49. Retention time of known compounds.  
Isobutyraldehyde,  $t_R = 58$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

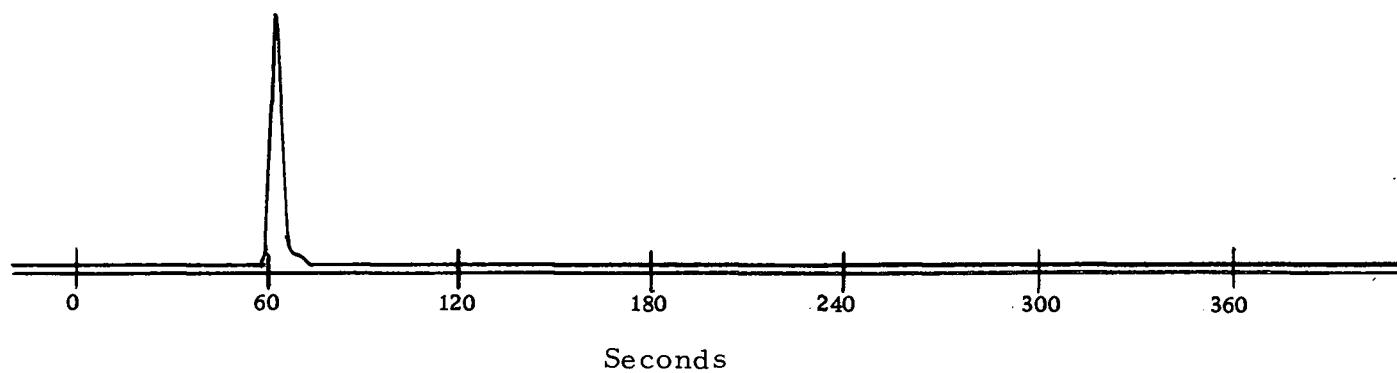


Figure 50. Retention time of known compounds.

Butanone,  $t_R = 62$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

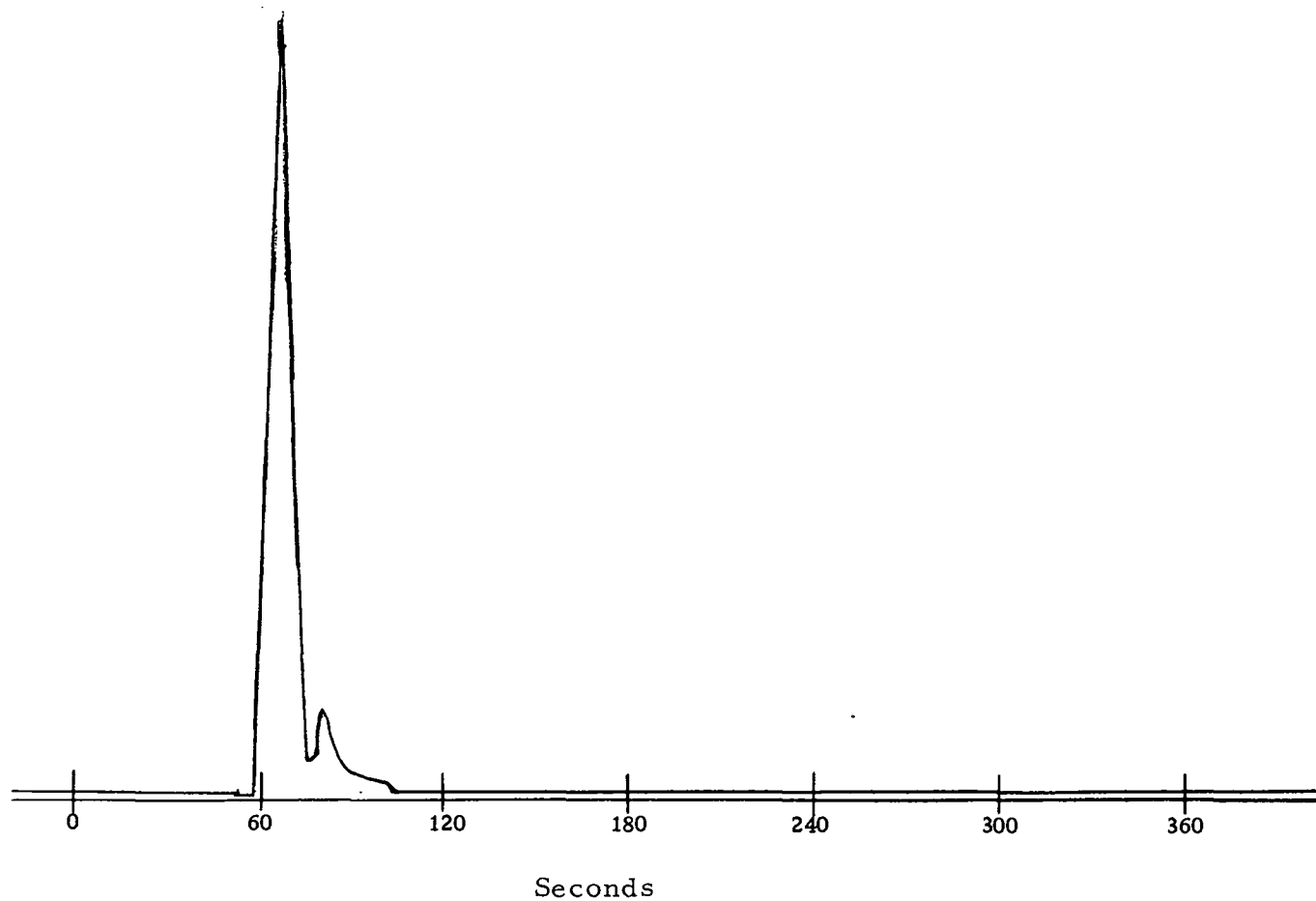


Figure 51. Retention time of known compounds.

Butyraldehyde,  $t_R = 63$  seconds on Free Fatty Acid Phase at 75° C.



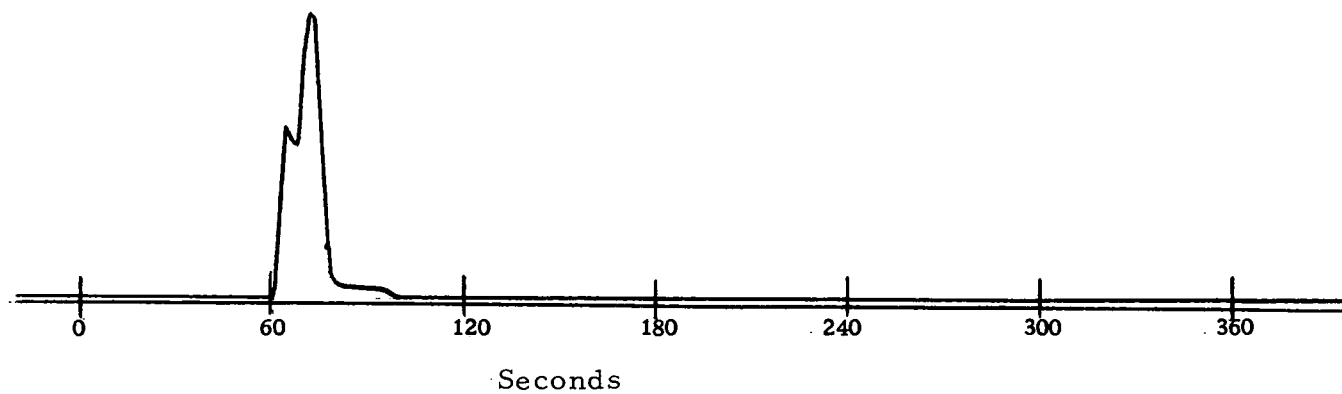


Figure 52. Retention time of known compounds,  
Isovaleraldehyde,  $t_R = 71$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

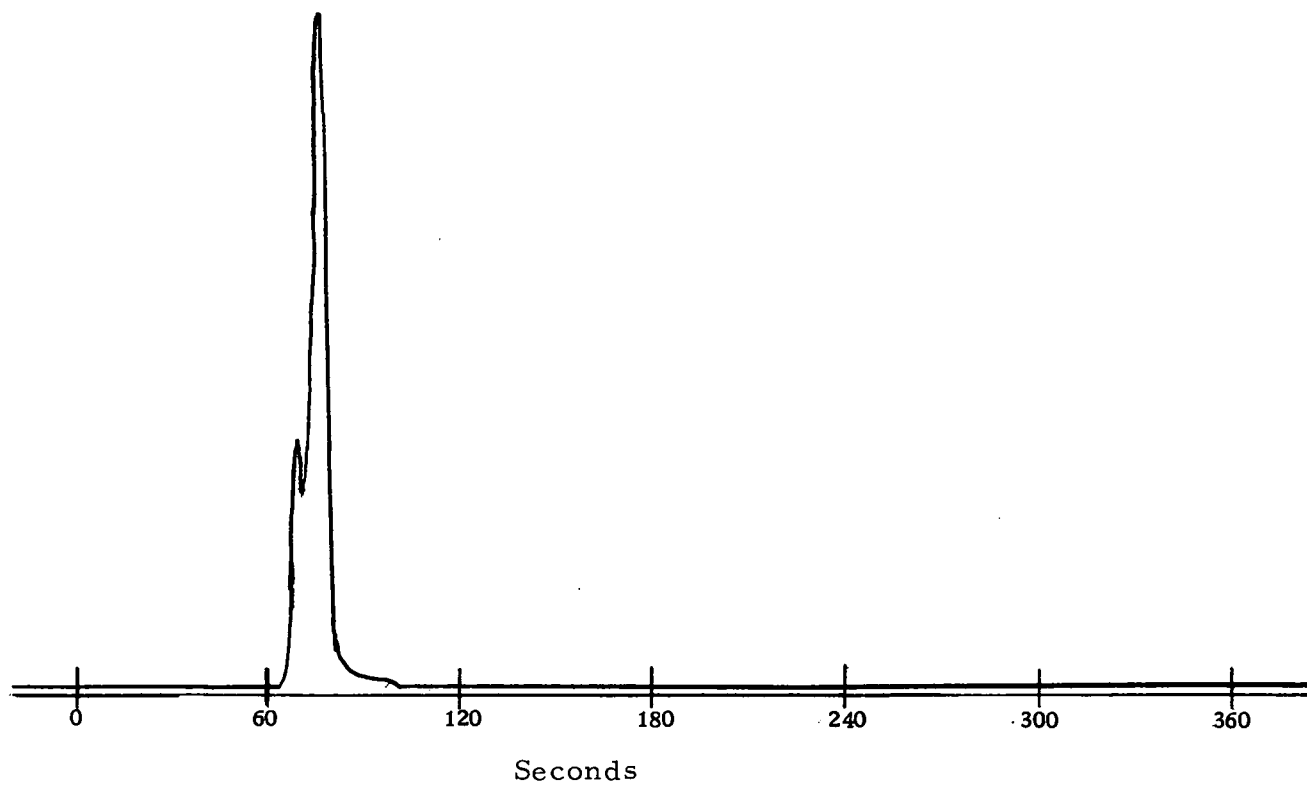


Figure 53. Retention time of known compounds,  
3-Methyl-2-butanone,  $t_R = 75$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

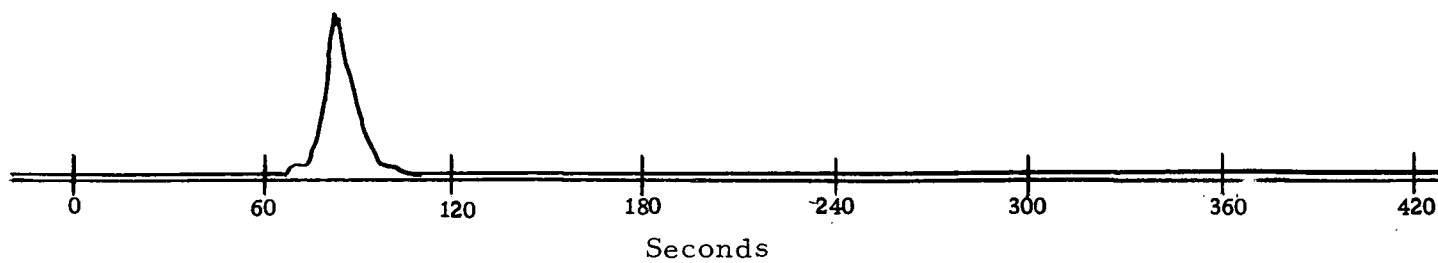


Figure 54. Retention time of known compounds.  
Valeraldehyde,  $t_R = 86$  seconds on Free Fatty Acid Phase at 75° C.

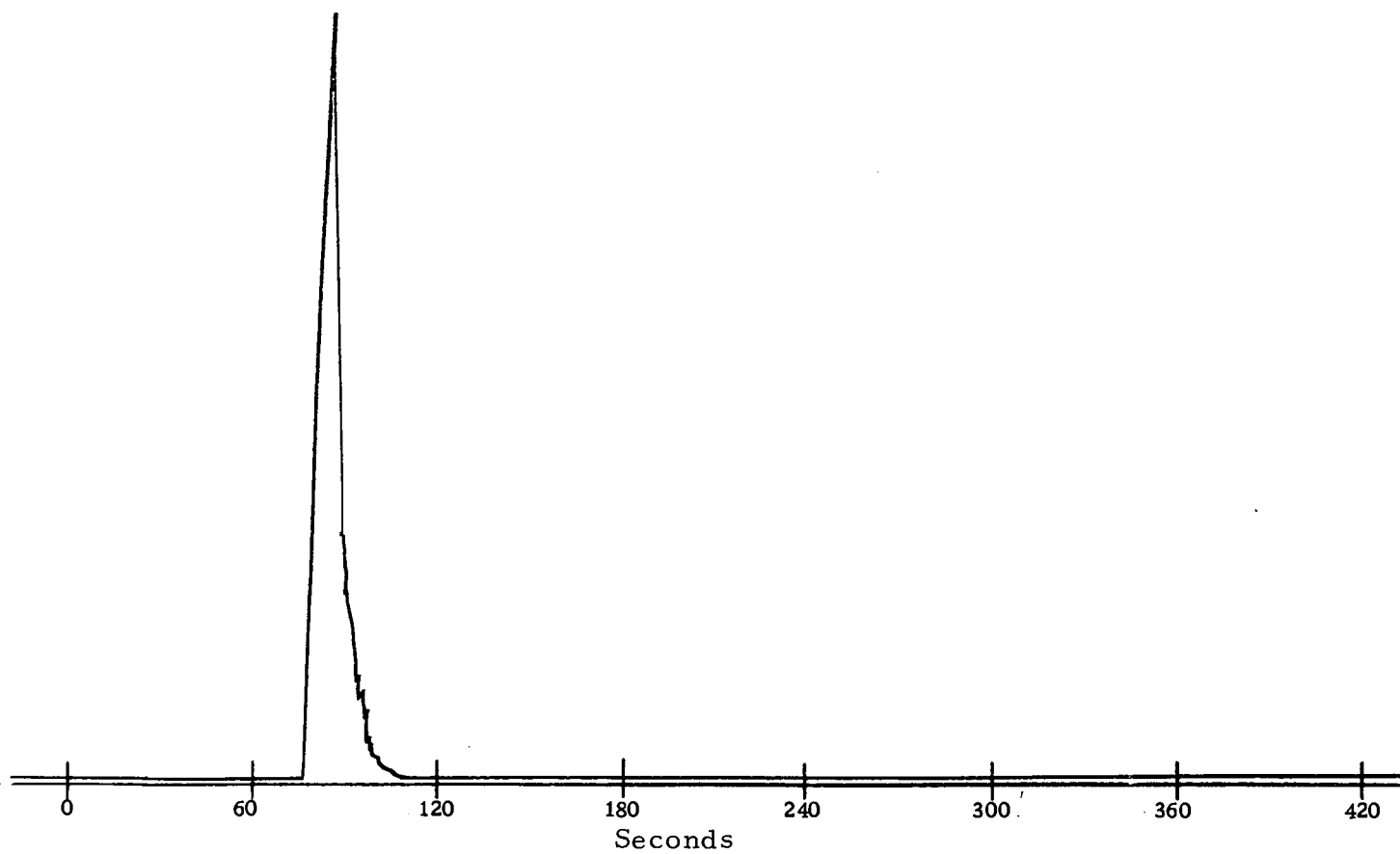


Figure 55. Retention time of known compounds.  
2,2-Dimethyl-3-pentanone,  $t_R = 87$  seconds on Free Fatty Acid Phase at 75° C.

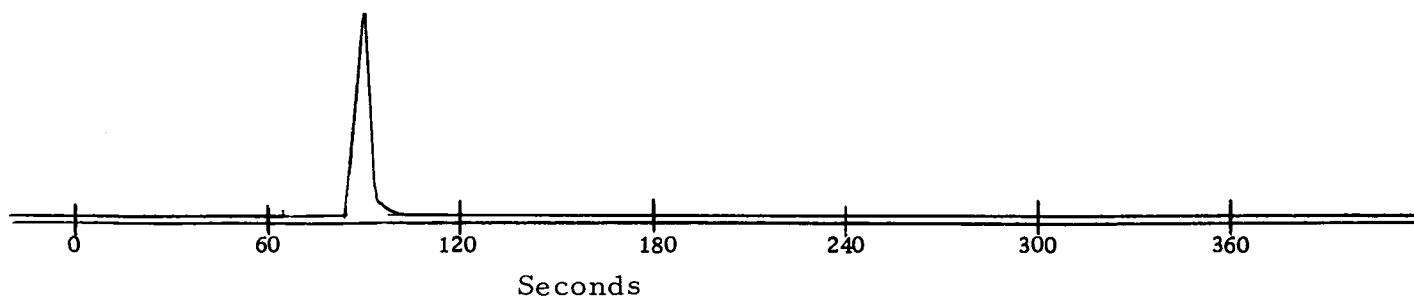


Figure 56. Retention time of known compounds.

Ethyl acetate,  $t_R = 89$  seconds on Free Fatty Acid Phase at 75° C.

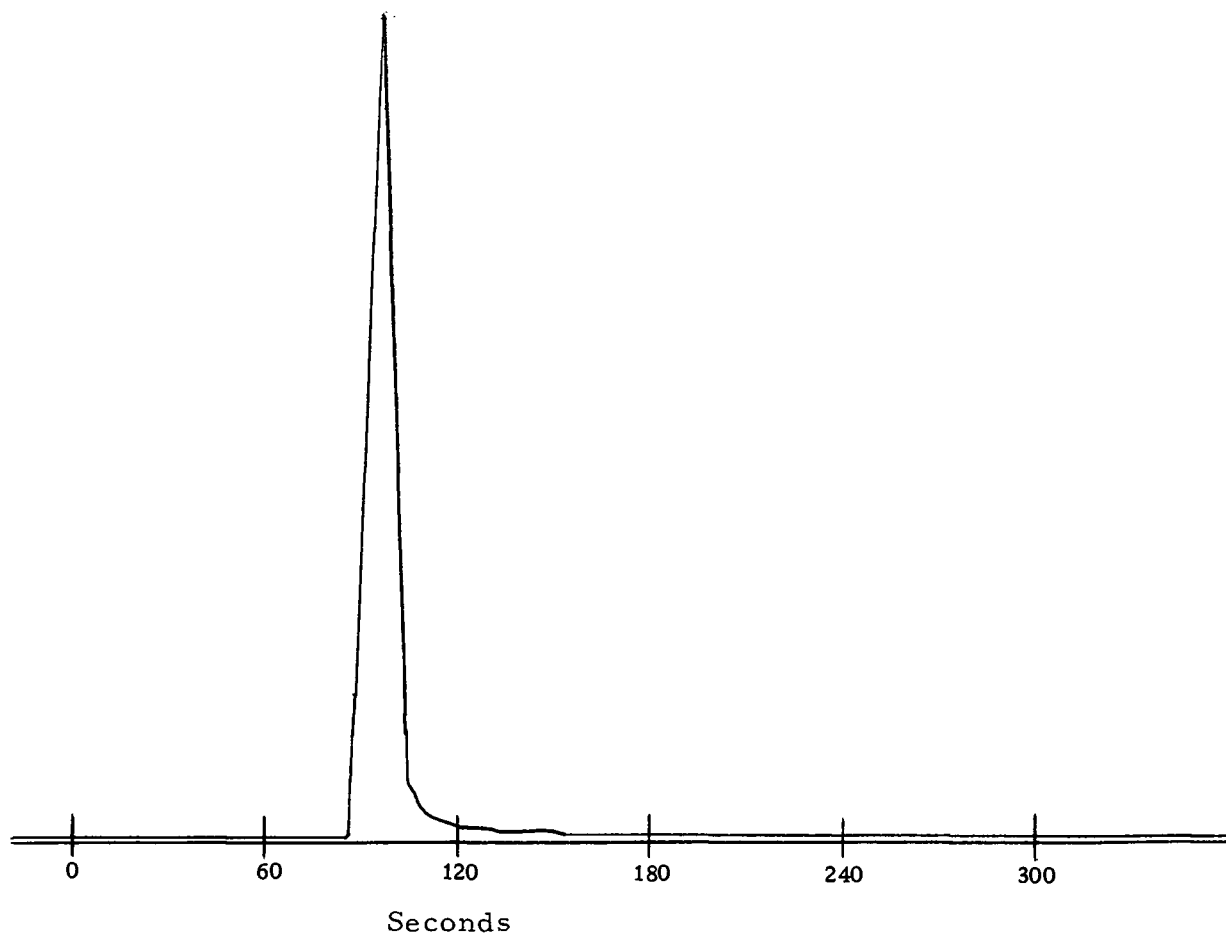


Figure 57. Retention time of known compounds.

Diacetyl,  $t_R = 95$  seconds on Free Fatty Acid Phase at 75° C.

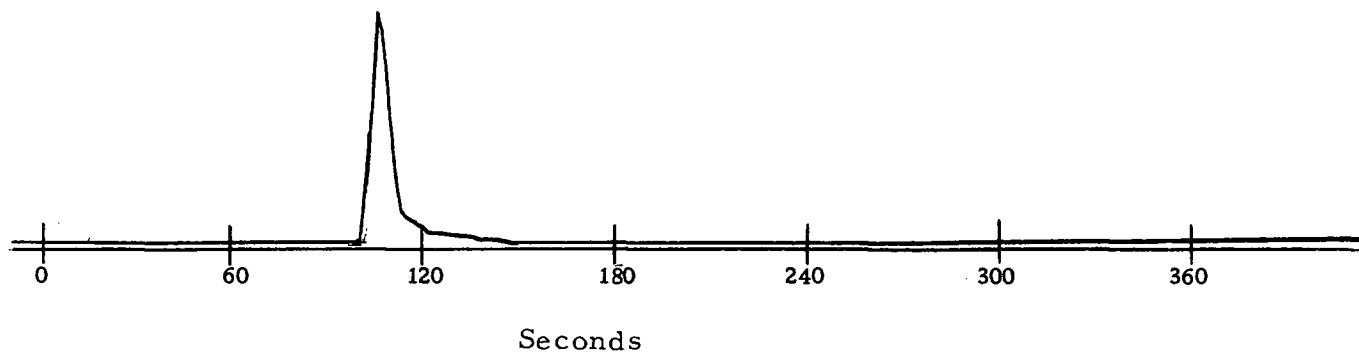


Figure 58. Retention time of known compounds.

Crotonaldehyde,  $t_R = 105$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

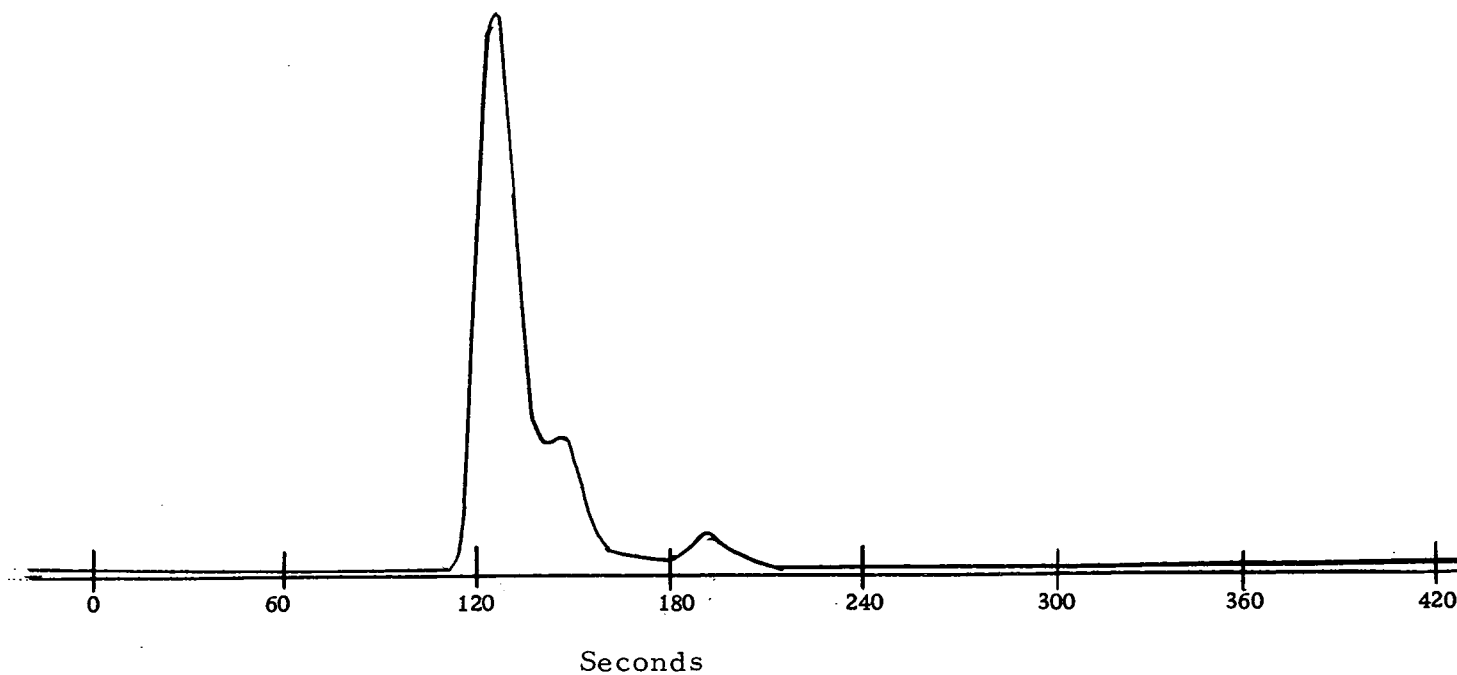


Figure 59. Retention time of known compounds.

Hexanal,  $t_R = 126$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .



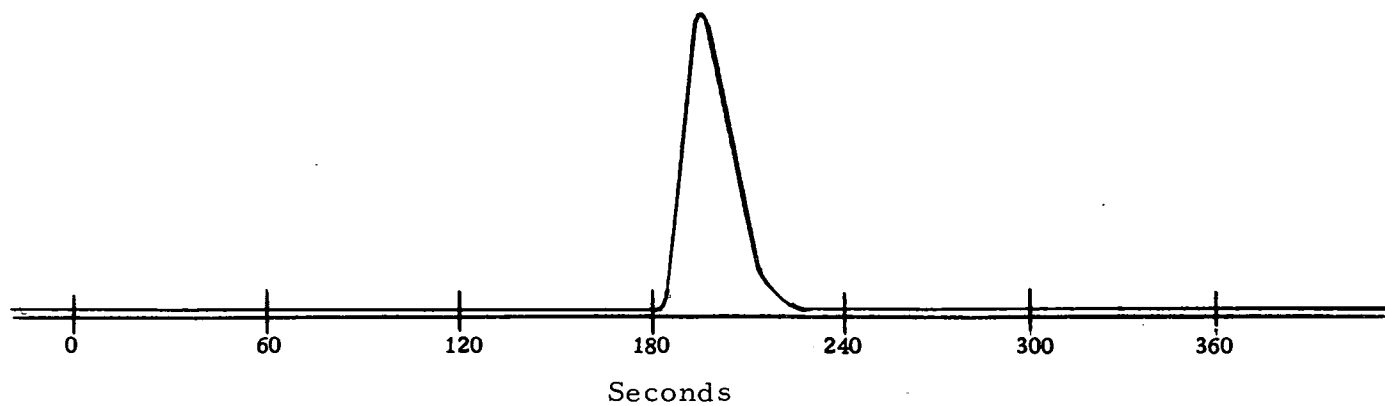


Figure 60. Retention time of known compounds.

Cyclopentanone,  $t_R = 186$  seconds on Free Fatty Acid Phase at 75° C.

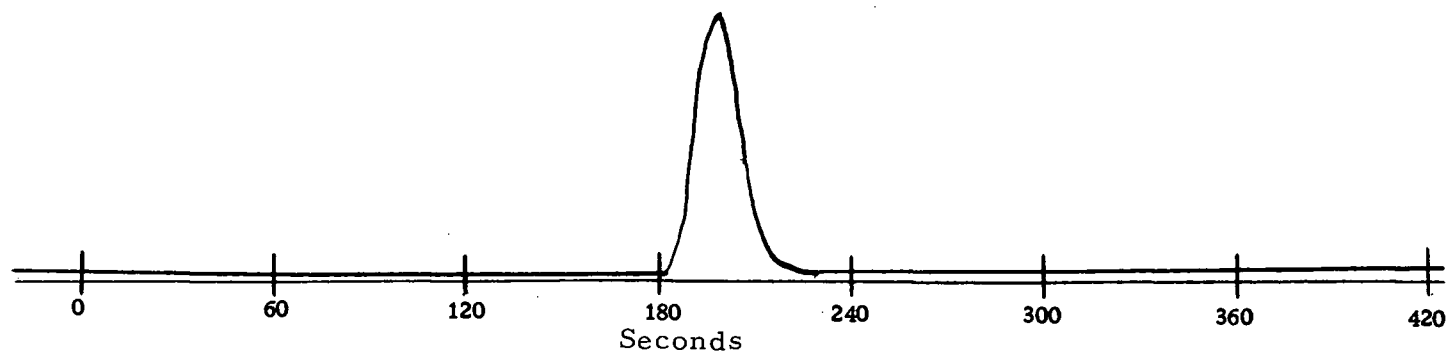


Figure 61. Retention time of known compounds.

Heptanal,  $t_R = 198$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

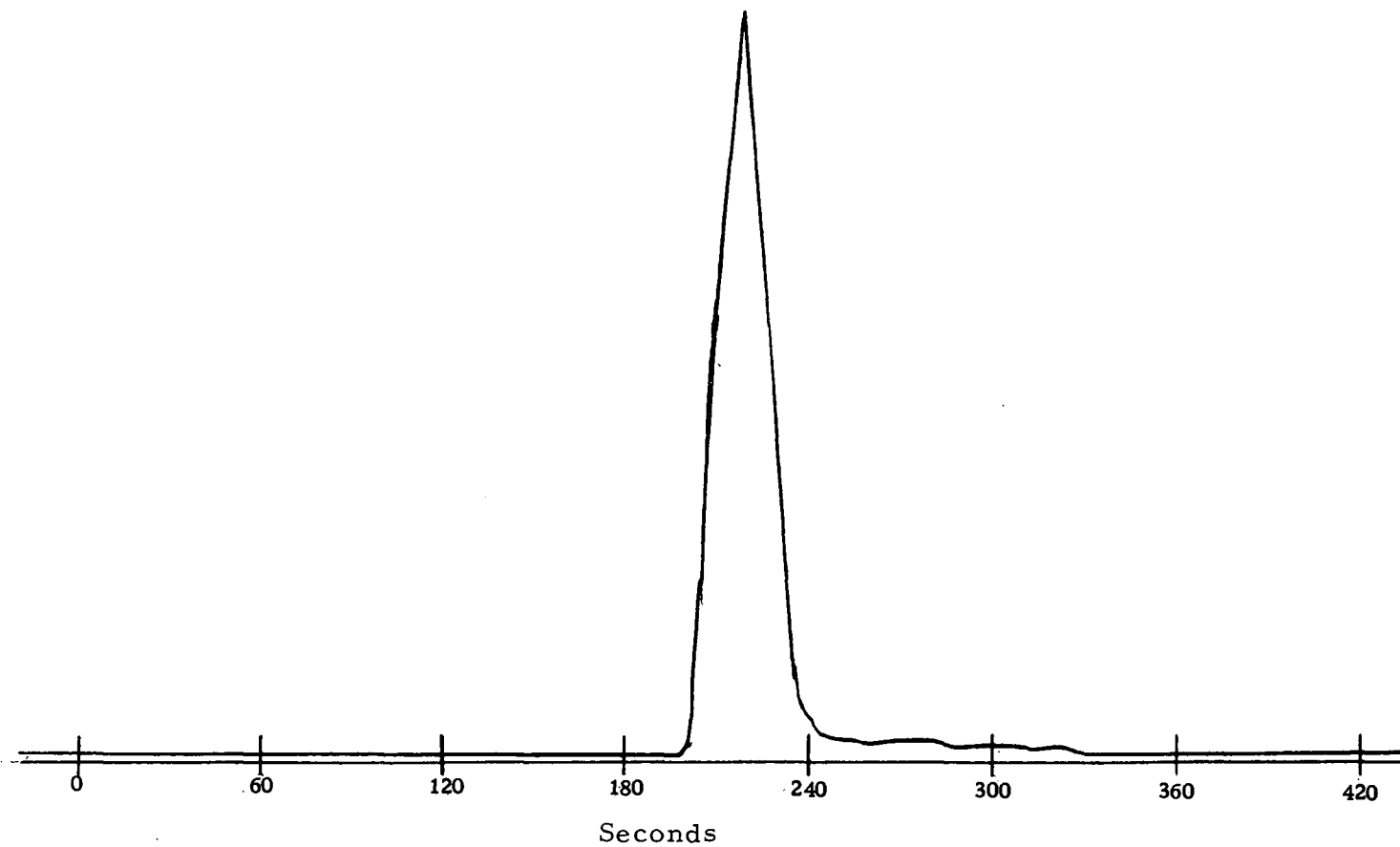


Figure 62. Retention time of known compounds.

Isoamyl alcohol,  $t_R = 218$  seconds on Free Fatty Acid Phase at 75° C.

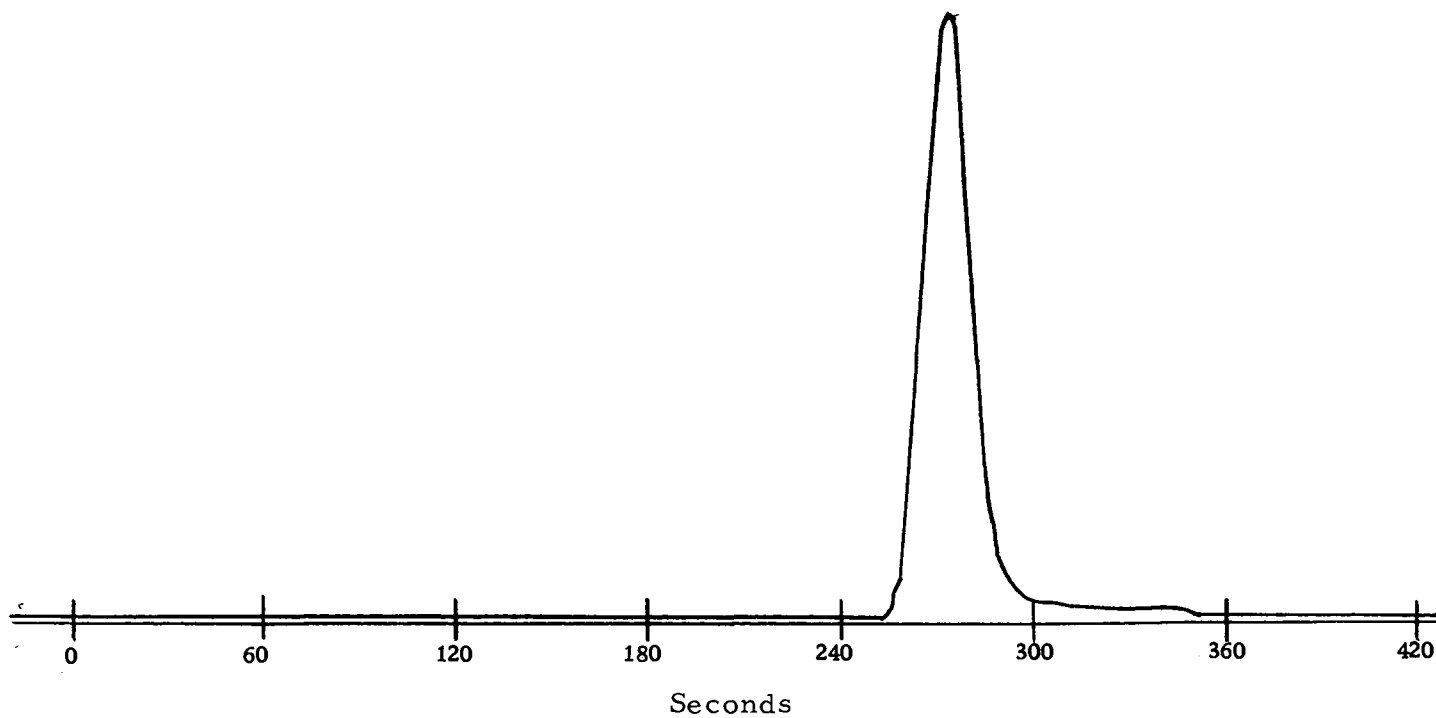


Figure 63. Retention time of known compounds.  
Amyl alcohol,  $t_R = 272$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

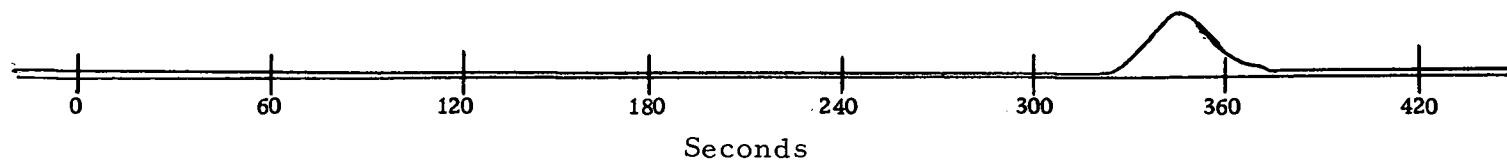


Figure 64. Retention time of known compounds.  
Octanal,  $t_R = 346$  seconds on Free Fatty Acid Phase at  $75^\circ \text{C}$ .

Table 6, Mass spectral data.<sup>a</sup>

m/e	Unknown %	Standard %	m/e	Unknown %	Standard %
Acetaldehyde			Diacetyl		
29			43		
44	90	88	15	--	34
43	46	50	86	7	11
28	12	9	14	--	10
41	8	6	42	12	7
Ethylacetate			Phenylacetaldehyde		
43			91		
61	14	16	120	29	25
45	18	15	92	25	23
29	30	13	65	21	19
70	10	10	39	14	16

<sup>a</sup>American Society for Testing and Materials. Committee E-14, Subcommittee IV. Uncertified mass spectra. Philadelphia, n. d. 14 vols.