

Wide-Range Programmed Temperature Gas Chromatography in the Separation of Very Complex Mixtures

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► The range of programmed cryogenic temperature gas chromatography has been widened to include temperatures from -196° to over $+200^{\circ}$ C. Various and variable rates of temperature rise may be employed and automatic programming can be provided. The necessity for employing very low starting temperatures for mixtures containing very volatile components is demonstrated, and an example of a separation of a mixture of compounds having a boiling range from -161° to $+200^{\circ}$ C. is given. The technique has been applied to the total analysis of the carbon dioxide, center cut, and water fractions of the volatile compounds from irradiated beef. The efficacy of the separations enhances the use of a rapid scanning mass spectrometer for identification of the components in the eluate.

IN THE analysis of the volatile compounds isolated from natural products, an extremely complex variety of mixtures is encountered by the investigator who must separate and

identify the components of such mixtures. In this laboratory, for example, quantitative analyses are required of such diverse mixtures as the compounds isolated from fresh ground coffee, deteriorating fish, irradiated meat, oxidized fat, microbially decomposed fuels, insect secretions, and human sweat. Such mixtures have several features in common. They contain a great many components encompassing many different types of compounds. Some of the components are present in appreciable amount; others, only as traces. The relative volatility of the compounds may vary from substances which are fixed gases to compounds having boiling points above 200° C. The use of programmed temperature gas chromatography is essential for the separation of such mixtures.

Programmed temperature gas chromatography commencing at room temperature or above has been found inadequate for the effective separation of the low boiling constituents of many of the mixtures encountered. The development of a technique for separating multicomponent mixtures of highly volatile compounds by pro-

grammed temperature gas chromatography in the low temperature range—i.e., below room temperature—was described (4), its effectiveness was demonstrated, and the apparatus for programming from -80° C. to room temperature was described. The range of programmed temperature gas chromatography has now been extended to include temperatures from -196° to over $+200^{\circ}$ C. This paper shows the appreciable improvement in the separation of very complex mixtures when programming is carried out over a very wide range.

EXPERIMENTAL

Apparatus. Most of the data presented in this paper were obtained on modified commercial apparatus. Two different setups were employed.

The Barber-Colman Series 5000 modular system for subambient temperature programming was used without modification for programming from -65° to $+200^{\circ}$ C. Liquid nitrogen was used to cool the column chamber for chromatograms requiring starting temperatures below -65° C. Another

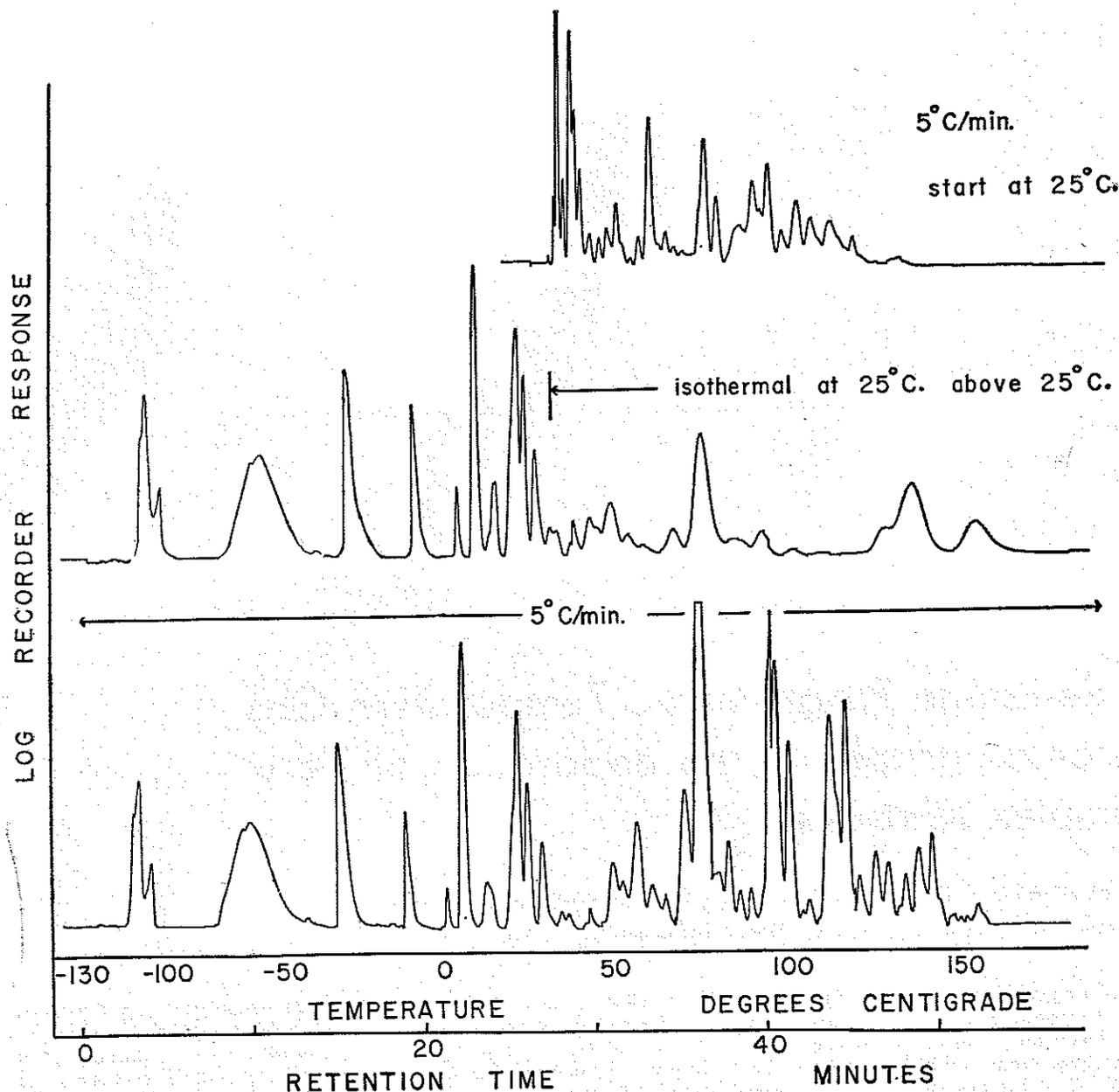


Figure 1. Chromatograms of "hydrocarbon mixture" obtained using different temperature programs

Column. 5% SE-30 on 80- to 100-mesh Chromosorb W, Barber-Colman apparatus. Log recorder response used to provide attenuation for more abundant components

apparatus used the Wilkens Instrument Co. Aerograph Model 500B Hy-Fi column oven as the column chamber and an F & M Scientific Co. Model 240 programmer, modified for subambient programming as described by Robertson, Issenberg, and Merritt (5). Liquid nitrogen or powdered dry ice was used to cool the column chamber to the appropriate starting temperature. Spontaneous warming of the column chamber (4) was used in the liquid nitrogen to dry ice temperature range and the modified F & M 240 programmer was used to control the temperature of the column chamber above -80°C . A conventional Aerograph Hy-Fi gas chromatograph was used to provide a flame detector, with the column oven serving to preheat the eluate from the column before entering the detector.

Procedures. Details concerning the columns employed, operating parameters, and nature of the samples studied are given mainly with the accompanying figures. Some procedures, however, were generally used throughout. All columns were stainless steel, 10 feet \times $\frac{1}{8}$ inch. All samples were swept onto the column from gas-sampling devices described in detail by Bazinet and Walsh (1) and by Forss, Bazinet, and Swift (2).

Lab gas used for very low temperature studies was obtained from the bench tap, but to ensure uniform composition, a single large volume was collected, the methane content was reduced by vacuum distillation at -196°C ., and small aliquots were taken. The gas was described by the local supplier as "natural gas, fortified with cracked

petroleum." The various samples of volatile compounds from irradiated beef were obtained according to procedures described by Merritt *et al.* (3). The "hydrocarbon mixture" was prepared by combining a commercial white gasoline with a petroleum ether (b.p. 30° to 60°C .), in which a mixture of light gases, including methane, ethane, and propane, was dissolved. This mixture was prepared to provide a widely boiling mixture.

RESULTS AND DISCUSSION

Although the improved separation obtained with subambient programming was demonstrated for the analysis of complex mixtures of volatile compounds isolated from coffee and ir-

radiated beef (4), the necessity for wide-range programming of the temperature is not readily apparent. Comparison of the three chromatograms in Figure 1 demonstrates the obvious advantage of starting the program below -80°C . and completing it at a temperature above the elution temperature of the component having the highest boiling point. The sample is the "hydrocarbon mixture" whose most volatile component is methane and whose least volatile component has a boiling point above 200°C . The chromatogram in the upper portion of the figure was obtained using a conventional program of 5° per minute with a starting temperature of 25°C . The entire mixture was very rapidly eluted

from the column and very little separation was obtained. The chromatogram in the central portion was obtained by programming from -130° to 25°C . at a program rate of 5° per minute, and then holding at 25°C . The lower boiling, more volatile constituents of the mixture are well separated, but the higher boiling members tend to be eluted with poor efficiency and very marked loss of resolution at the isothermal end of the chromatogram. In the lowest chromatogram the column was heated from -130° to $+150^{\circ}\text{C}$. at 5° per minute. More than 55 components can easily be discerned in this mixture. Although on the whole the separation of the mixture on this SE-30 column is good, the very low boiling

members of the mixture—e.g., the first two components—are resolved poorly. The third peak is very broad. More effective separation may be obtained on polar columns such as diethylene glycol succinate or 1,2,3-tris(2-cyanoethoxy)propane.

Figure 2 compares the separations on three types of liquid phase. The samples are equal aliquots of lab gas. The top chromatogram shows an excellent separation obtained on tris(2-cyanoethoxy)propane. There is a loss of column efficiency on SE-30 at low temperature, as seen in the center chromatogram, but its overall resolving power is better. There is loss of both efficiency and resolution on the cyanoethyl silicone-diethylene glycol suc-

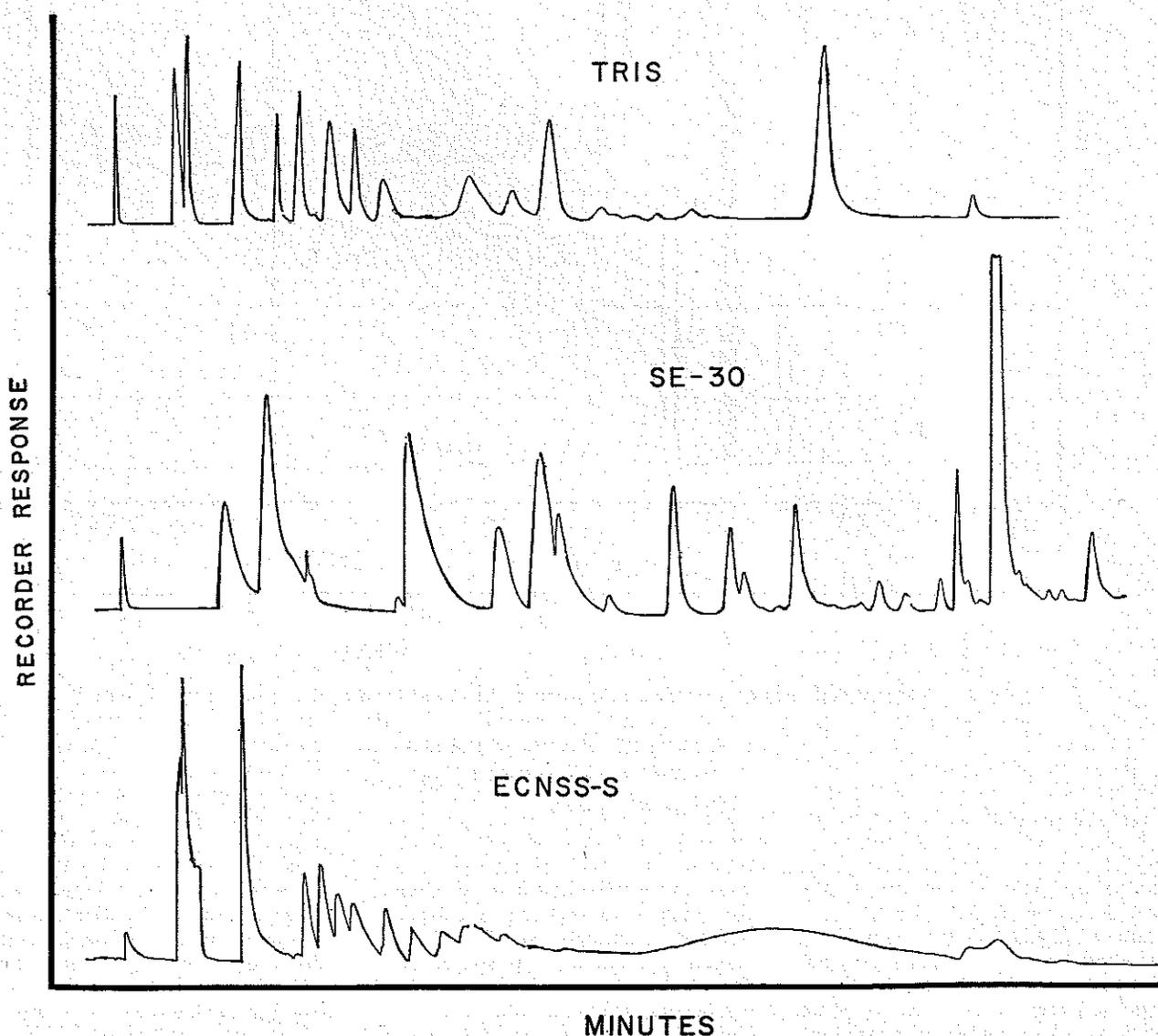


Figure 2. Behavior of lab gas aliquots on three columns

TRIS. 5% 1,2,3-tris(2-cyanoethoxy)propane on 60- to 80-mesh firebrick

SE-30. 5% methyl silicone polymer on 80- to 100-mesh Chromosorb W

ECNSS-S. 3% cyanoethyl silicone-ethylene glycol succinate copolymer on 80- to 100-mesh Gas Chrom P

Temperature programmed from -170°C . at $\sim 3^{\circ}\text{C}/\text{minute}$

Aerograph, F & M apparatus

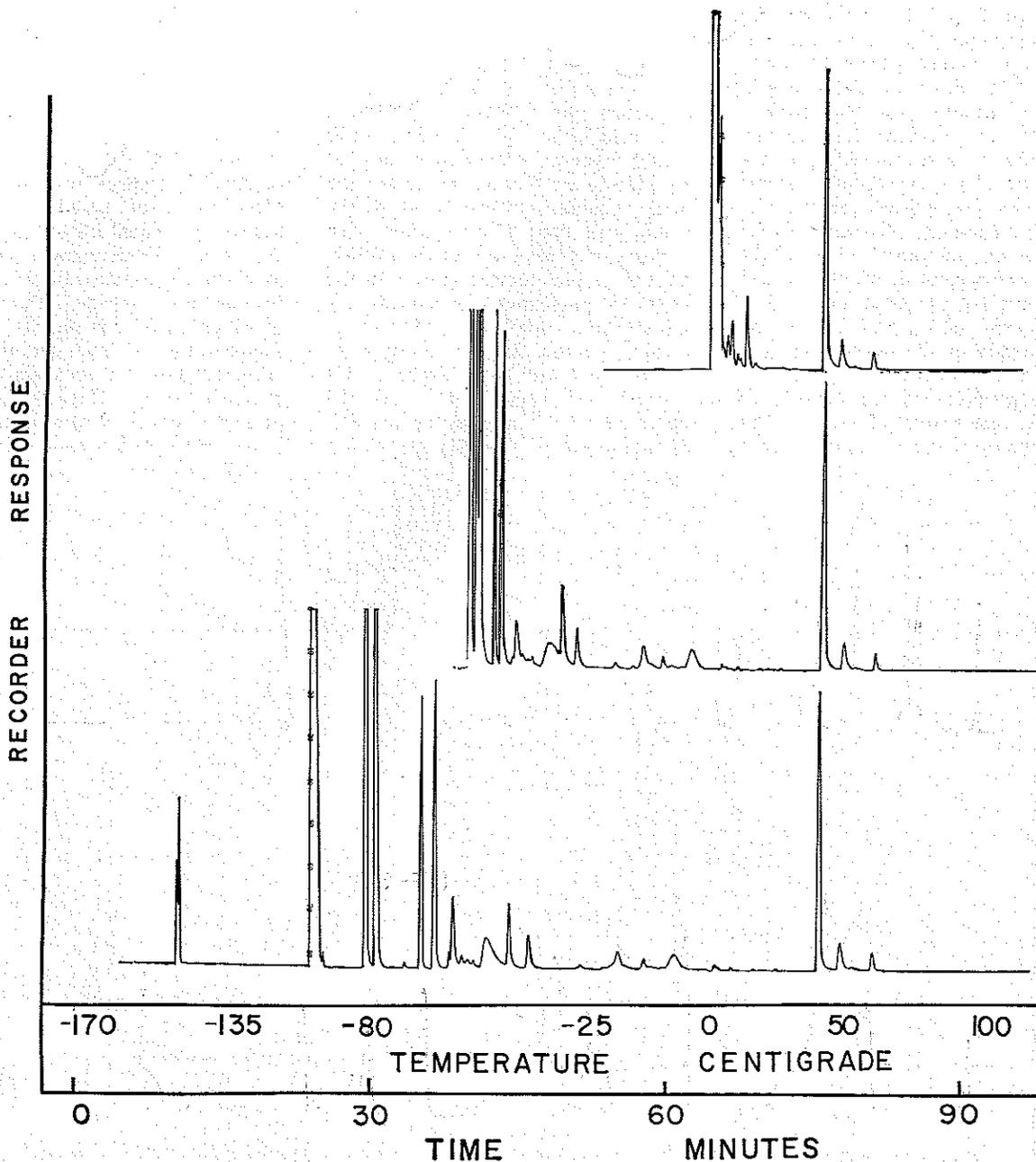


Figure 3. Programmed temperature chromatograms of lab gas starting at different temperatures

Column. 5% TRIS on 60- to 80-mesh firebrick

Temperature program. $\sim 5^\circ \text{C./minute}$

Aerograph, F & M apparatus

cinat copolymer, as shown in the bottom chromatogram.

Several liquid phases have been evaluated for use at very low temperatures and, in general, most polar phases seem to work well. Among those which have been found satisfactory are tris(cyanoethoxy)propane, oxydipropionitrile, diethylene glycol succinate, Carbowax 20M, and Carbowax 4000. Among the nonpolar phases which have been found satisfactory are squalane, Apiezon M, silicone SF-96, and, to a limited extent, SE-30.

An important question is whether it

is necessary or desirable to go to extreme low temperatures; in particular, whether or not it is worth while programming below dry ice temperature. The separations obtained on a tris(cyanoethoxy)propane column of three identical aliquots of lab gas are shown in Figure 3. In each case the temperature program rate was 5° per minute, but the starting temperature at the top was 0° , in the center -80° , and at the bottom -170°C . The very low starting temperature is seen to be necessary for complete resolution of the mixture.

Low temperature gas chromatography is very important in the analysis of natural products where often the major portion of the volatile material isolated is water and carbon dioxide and only trace amounts of the flavor and odor compounds are present. Thus, in isolating the off-odor products in irradiated beef, it is necessary to carry out a preliminary separation to remove the gross amounts of water and carbon dioxide (3). This is usually done by low temperature-high vacuum distillation, followed by gas chromatographic separation of the components in the two

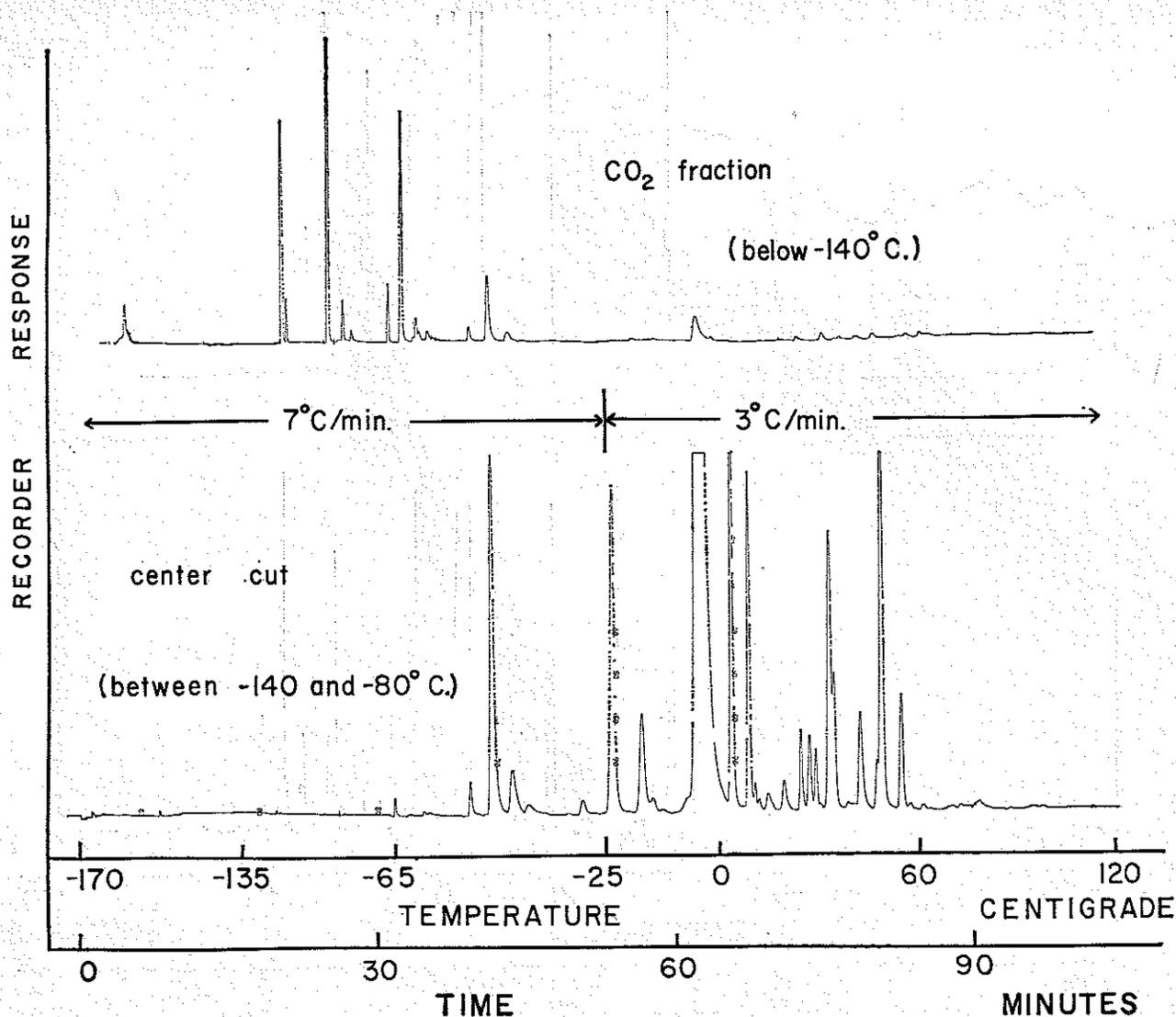


Figure 4. Programmed cryogenic temperature gas chromatograms of carbon dioxide and center cut fractions from irradiated beef volatiles

Column. 5% TRIS on 60- to 80-mesh firebrick
Aerograph, F & M apparatus

fractions. The choice of temperature range for these vacuum separations is vital. The situation is illustrated by chromatograms shown in Figure 4.

The upper chromatogram shows the separation obtained when a CO₂ fraction—i.e., the distillate obtained below -140° C.—is programmed from -170° C. In this case, although the sample is mainly carbon dioxide, the separation of the trace components may readily be observed. Since this fraction is separated by vacuum distillation at a temperature of -140° C. and a pressure of 10⁻³ torr, only the most volatile compounds of the initial sample are present here. On a low temperature program such as this they are all separated and eluted by the time the column temperature has reached -80° C. The lower chromatogram shows the separation of the center cut fraction—

that is, the material distilled between -140° and -80° C. at 10⁻³ torr. This fraction was programmed from -170° C. and no components, or very few components, were eluted below -80° C. The components of the center cut are separated and eluted above -80° C. Thus, while a very low temperature program is required to separate the compounds in the CO₂ fraction, a program starting at -80° C. would separate the compounds in the center cut. The amount of overlap between these two chromatograms indicates the effectiveness of the preliminary separation by vacuum distillation.

A new technique employing evaporation of a solvent extract onto a cold column has recently been developed for isolating and separating the volatile flavor compounds present in the very

dilute aqueous solutions obtained as water fractions (3). Although described elsewhere in detail (2), it should be noted here that since the solvent extract method now permits analysis of the water fraction and since the low boiling compounds in the CO₂ and center cut fractions can readily be separated from water by vacuum distillation at -80° C. and analyzed by low temperature gas chromatography, complete identification of flavor compounds in complex systems is now possible. When the system is not complicated by the presence of large excesses of water or carbon dioxide, wide-range temperature programming may be employed directly—for example, in the analysis of the rancid odor compounds present in oxidized butter oil.

Cryogenic separations are reproducible. This is shown by the two

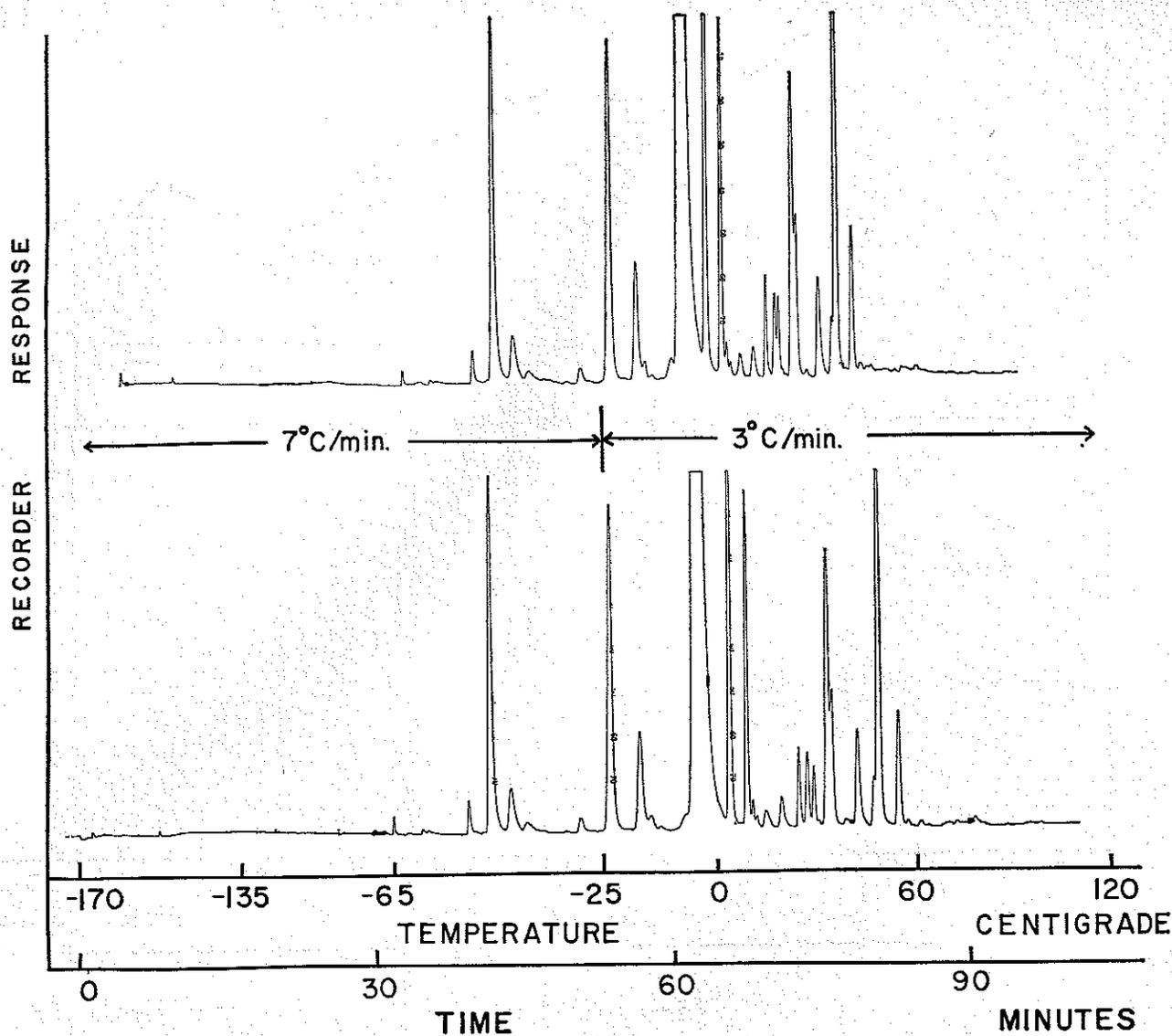


Figure 5. Duplicate programmed cryogenic temperature gas chromatograms of irradiated beef center cut fraction aliquots

Column. 5% TRIS on 60- to 80-mesh firebrick
Aerograph, F & M apparatus

chromatograms of aliquots of irradiated beef, center cut volatiles programmed from -170°C . on tris(cyanoethoxy)propane (Figure 5). The temperature rise rate for the upper chromatogram is slightly faster than the lower.

The ultimate goal of the analysis of natural products is usually to identify the compounds isolated. Gas chromatography separates the compounds. In this laboratory, identification is then accomplished by means of a rapid scanning mass spectrometer. The mass spectra of the components of the mixture are obtained as they are eluted from the gas chromatograph. The quality of the gas chromatographic separation, therefore, is reflected in the quality of the mass spectra obtained. The use of subambient and wide-range temperature programming has been of tremendous value in providing the type of separation required to get definitive mass spectra of well separated components.

Figure 6 shows some examples of mass spectra of compounds isolated from a center cut fraction of the volatiles in irradiated ground beef. At -60°C . and 2 minutes after the start of the chromatogram, the compound being eluted into the ion source of the mass spectrometer is *n*-butane. Half a minute later and at -58°C . the butane has disappeared and the spectrum seen is that of *n*-butene-1. This spectrum shows no residual interferences from butane and demonstrates a clear-cut separation. Another example is seen at 18.5 minutes, when the temperature has risen to -25°C . and the mass spectrum shows that the compound being eluted is *n*-heptane. A minute later, another peak is eluted which the mass spectrum indicates is a mixture of two compounds not separated on the chromatographic column. In this case, however, heptene-1 and dimethyl sulfide are both identified in the mixture from

their contributions to the mass spectrum. *n*-Heptane and *n*-octane are separated by more than 10 minutes. Moreover, it is interesting to note the relatively low temperature at which these hydrocarbons are eluted, *n*-heptane at -25°C . and *n*-octane at -8°C . Elution of compounds at lower temperatures reduces the possibility of decomposition on the column.

In addition to its usefulness as a means of separating mixtures with a wide range of boiling points, wide-range temperature programming also permits a more general approach to the separation of a variety of types of mixtures. Thus, the same column may be used to separate a mixture of light hydrocarbons or fixed gases and for a mixture of high boiling fatty acid esters. Only the selection of an appropriate temperature program is required. This great versatility of the method not only greatly enhances the usefulness of gas chro-

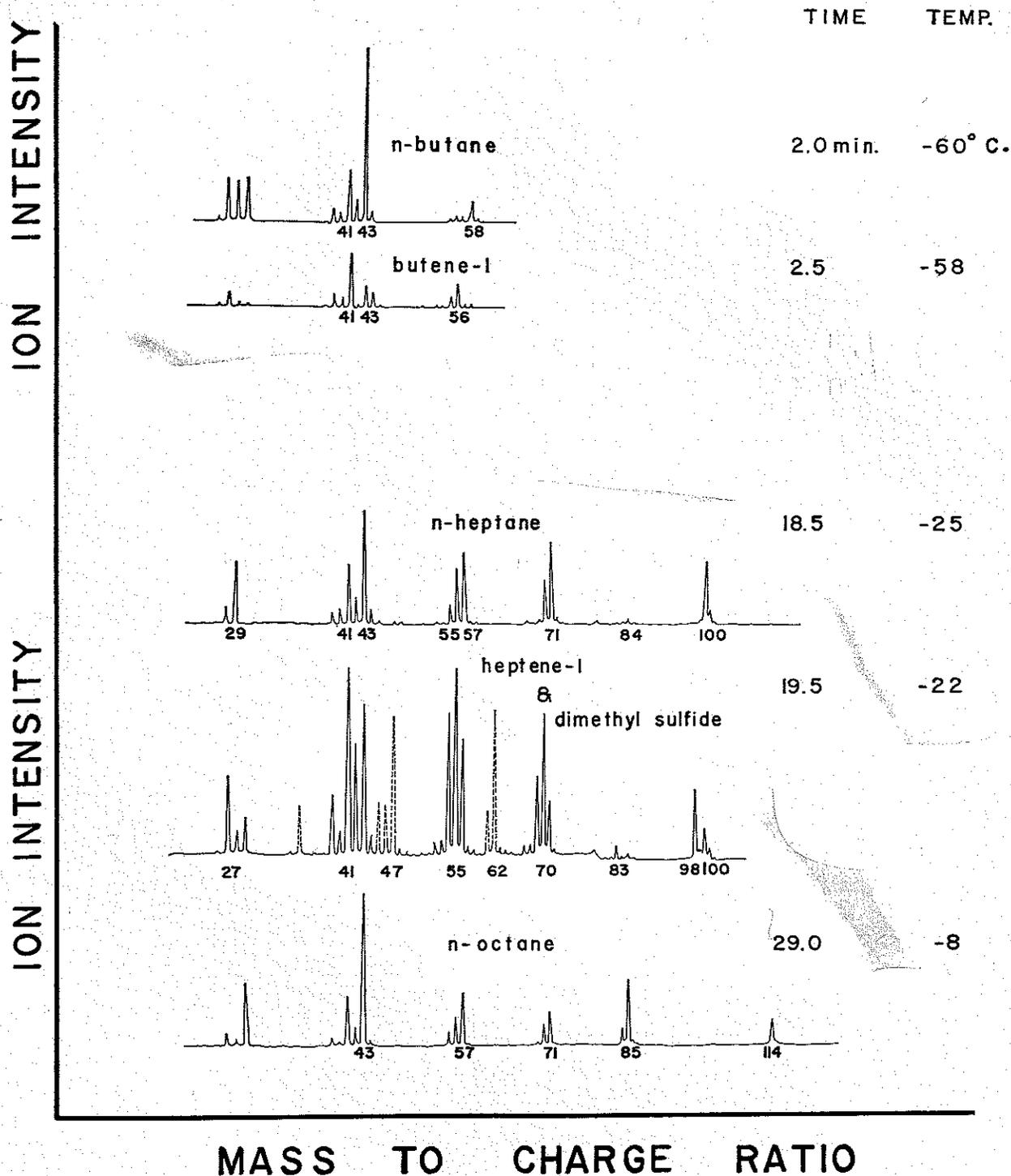


Figure 6. Time-of-flight mass spectra of some components eluted from programmed cryogenic temperature gas chromatogram of an irradiated beef center cut fraction

Column. 5% β,β -oxydipropionitrile on 80- to 100-mesh firebrick
 Temperature program. -65° to $+30^{\circ}$ C. at 3° /minute

matography itself, but also permits greater utilization of ancillary methods such as rapid scanning mass spectrometry.

ACKNOWLEDGMENT

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