# Combination Gas Chromatography-Mass Spectrometry

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## Abstract

The direct combination of gas-liquid chromatography with mass spectrometry represents one of the most powerful techniques available for the analysis of complex mixtures of lipids. Principal advantages are the extremely small amounts of material required, the relative speed of analysis, and the wealth of molecular structural information available. The ability to record rapidly several mass spectra of one emerging chromatographic peak may also frequently be of use in detecting impurities, unresolved mixtures, or thermal decomposition of the sample. In the development of new gas chromatographic analytical procedures, the combination instrument may frequently prove highly useful for following chemical reactions in the preparation of new types of derivatives for GLC.


## Introduction

FOR A NUMBER OF YEARS, both gas chromatography and spectrometry have followed separate but parallel roles in the analytical methodology of lipids. Gas chromatography has evolved as a routine tool employed primarily for the separation of complex mixtures, while mass spectrometry has developed into a powerful technique for structural characterization of lipids (1–3) and other natural products (3–5). Both techniques, however, share two important characteristics, in that each deals with samples in the vapor phase (which are thus "volatile," though not in the conventional sense) and in microgram quantities. Direct combination of gas chromatography-mass spectrometry thus provides an extraordinarily powerful and versatile tool for the characterization or structure elucidation of components of complex mixtures.

Uses of the combined instrument may be considered as falling into three categories. In the first, the mass spectrometer serves primarily as a unique type of detector for a gas chromatograph, with identification of components based mainly on comparison with files of reference spectra. In the second, the combination of the two instruments results in broad, flexible usage, relying equally on both instruments. In the third, the gas chromatograph serves as a specialized type of inlet system for the mass spectrometer, in which case the separation of components other than from solvents is not usually required. Emphasis may be placed on any or all three categories, depending on the objectives and types of problems under investigation.

The combination technique offers a number of distinct advantages. Samples need not be isolated or rigorously purified. Compounds which are subject to decomposition (hydrolysis, oxidation, etc.) when in contact with the atmosphere may be studied without difficulty. In routine work dealing with characterization of known compounds, more rapid identification with a greater degree of confidence may be obtained by comparison of reference mass spectra, as opposed to reliance on retention times alone. For components of unknown structure or class, information ranging from molecular weights to total structures may frequently be realized from samples in the microgram range. In addition, high resolution (double-focusing) mass spectrometers are capable of mass measurement of sufficient accuracy to establish empirical composition (6). Mass spectrometery further offers unique advantages in sample size for the determination and location of stable isotopes in studies of lipid biosynthesis (7–9). In laboratories in which new gas chromatographic separation and derivatization procedures are developed, the combination instrument becomes a highly useful tool with which to follow reactions on a micro scale, confirm and determine structures of new derivatives (10), and locate known and unknown components of a mixture when comparing results from columns with different liquid phases, independent of retention times.

A unique advantage of the combination instrument is the ability to obtain detailed information on any section of a gas chromatographic peak which is not homogeneous, due to the presence of impurities, unresolved major components, or decomposition, as will be discussed.

Since the first attempt at direct combination of gas chromatography and mass spectrometry in 1957 (11), increasingly more sophisticated approaches have been reported, including oscilloscope photography, using both time-of-flight (12) and magnetic deflection (13) instruments, rapid scanning with oscillographic recorders (13–17), use of capillary columns (14,17–20) and the direct coupling of gas chromatographs to high resolution (double-focusing) mass spectrometers, using photographic recording (6) and conventional scanning onto magnetic tape (21). (No attempt is made to refer to all variations or applications of the combination technique. See references 6,12–21 and 22–24 for more complete data.)

As interest in applying the combination technique grew to include compounds of greater molecular weight, greater dependence on higher mass spectral resolution resulted. Since the introduction of carrier gas directly into the ion source results in "pressure-broadening" of peaks in the mass spectrum, i.e., lower resolution, a means of preferentially removing carrier gas while removing as little sample as possible becomes more necessary as resolution requirements increase. (The "pressure broadening" effect is largely dependent on the mean pressure over the entire ion path, so that for instance, double-focusing instruments which employ differentially pumped analyzer systems are capable of maintaining high resolution even with ion source pressures in the $10^{-4}$–$10^{-5}$ mm Hg region (6,20). Two somewhat different types of carrier gas separators, were reported independently by three groups in 1964 (22–24) and have since gained wide acceptance. The operating principles of both types will be discussed later. Although improved resolution may be the primary reason for operating at lower ion source pressures, two additional factors are also involved. Higher pressures may result in lower sensitivity on an absolute basis,

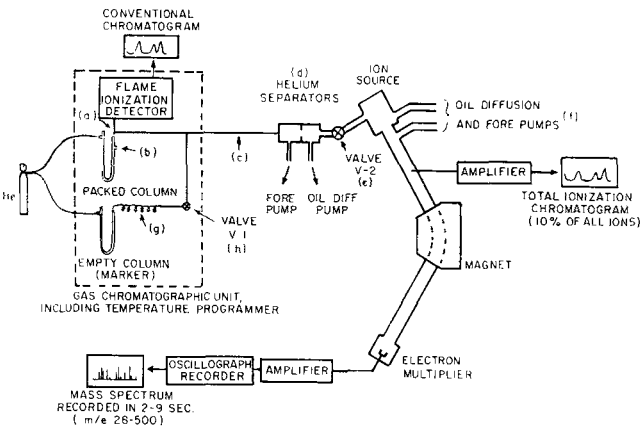


FIG. 1. Schematic diagram of the combination gas chromatograph-mass spectrometer used in the author's laboratory. The gas chromatographic unit consists of a Barber-Colman Model 5000 gas chromatograph, with a two-column oven and Model 5084 electronic temperature programmer. A portion of the effluent is monitored by a hydrogen flame ionization detector with a Keithley Model 417 electrometer. The mass spectrometer is an Atlas CH-4 modified for rapid scanning (22).

*(a)* Splitting device (shown in Fig. 3) is used to control the time difference between flame and total ionization detectors. *(b)* Early column exit for a "prevue" chromatogram (see Fig. 4). *(c)* Stainless steel capillary of 70 cm total length: 40 cm 1.0 mm I.D. plus 30 cm 0.25 mm I.D. *(d)* Carrier gas separators (22), see also Fig. 5b. *(e)* Atlas high temperature valve *(f)* Hg-diffusion pumps have been replaced by Edwards EO-2 oil diffusion pumps, which do not require liquid nitrogen coolant. *(g)* Pressure drop through the marker line between the helium source and mass spectrometer is effected by 9 meters of 0.25 mm I.D. stainless steel capillary. *(h)* Toggle valve (No. 1GS2, Whitey Research Tool Co., Emeryville, Calif.) for simultaneous admission of mass marker (Fig. 8).

and may also produce spectra which exhibit considerable bias against ions of higher mass. For example, we have observed that if n-octacosane is introduced through a conventional inlet and the ion source pressure is varied by helium flow through the gas chromatographic inlet, the molecular ion virtually disappears (relative to the lower mass peaks) as the pressure approaches approximately $10^{-3}$ mm Hg. We have no explanation for this phenomenon; helium ions are apparently not involved, since similar results are obtained at both 20 and 70 ev. Teeter and coworkers have made similar observations (25).

## Experimental Procedures and Discussion

The combination instrument currently in use in our laboratory is represented in Figure 1, and may be seen to consist of essentially three sections: gas chromatograph, carrier gas separators, and mass spectrometer. In principle almost any gas chromatographic instrument may be coupled with a mass spectrometer. However, in laboratories in which gas chromatography is used extensively, greater flexibility will result from use of a commercially available gas chromatograph with the combination instrument. For instance, columns may be easily and rapidly interchanged and conditions (especially temperature programming) used on other instruments more accurately reproduced on the combination instrument.

Injection of the sample into the gas chromatograph is made with valve V-2 closed (see Fig. 1), so that the solvent front does not either actuate the automatic protection circuit (filament, pumps, high voltage, etc.), or if that circuit is turned off, cause high

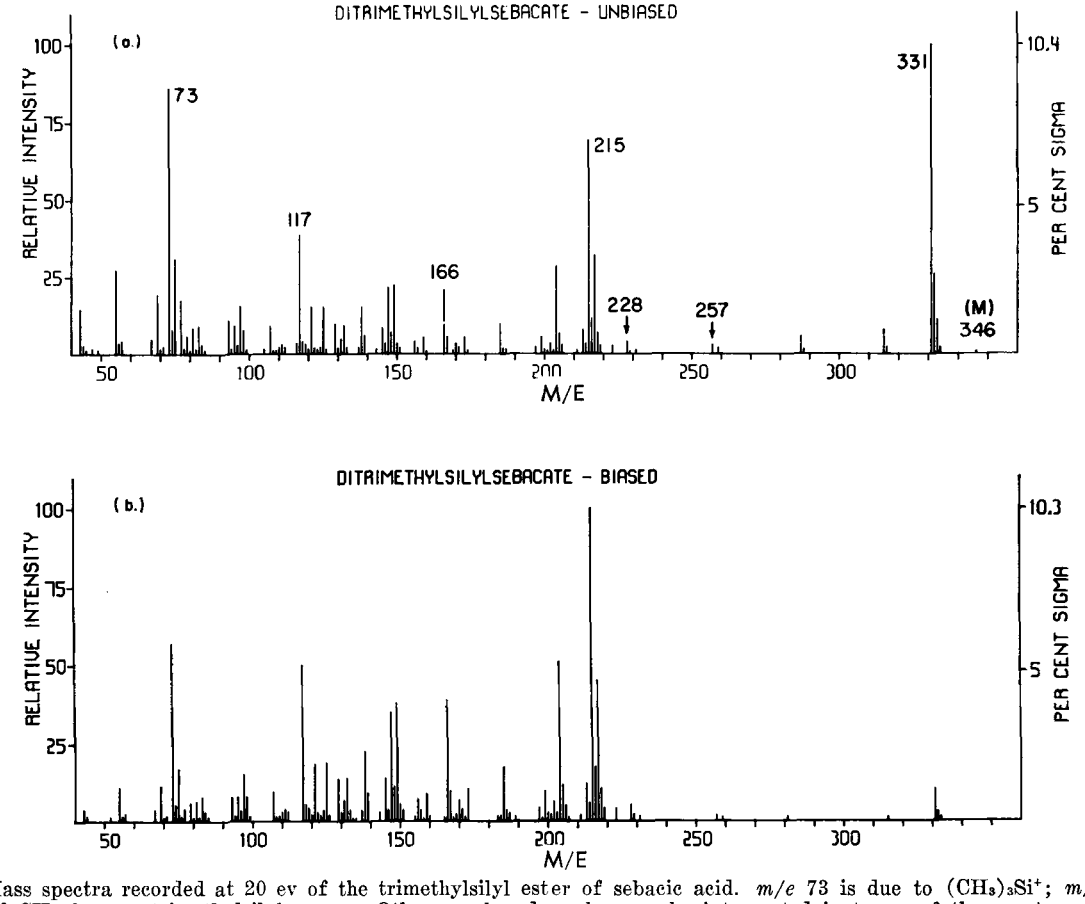


FIG. 2. Mass spectra recorded at 20 ev of the trimethylsilyl ester of sebacic acid. *m/e* 73 is due to $(CH_3)_3Si^+$; *m/e* 331 represents loss of $CH_3$ from a trimethylsilyl group. Other numbered peaks may be interpreted in terms of the spectrum of dimethylsebacate (39), with appropriate mass shifts. (a) Unbiased 1.7-sec scan recorded on the apex of the gas chromatographic peak. (b) Biased 44-sec scan recorded across a GLC peak 66 sec wide.

voltage arcing in the ion source. In some cases the solvent front could overload the ion source pumps, with the result that solvent would still be present (with a corresponding high total ion current, discussed below) during the elution of very early peaks from the gas chromatograph. Solvent front passage may be noted on the hydrogen flame ionization detector, which receives about 4% of the effluent, or on pressure gauges (not shown in Fig. 1) associated with the carrier gas separators (22). After V-2 is opened the mass spectrometer itself may function as a detector through the total ion current resulting from collection of approximately 10% of all ions after acceleration but before mass separation. The total ionization chromatogram is of considerable help in determining the exact portion of the emerging peak of which the mass spectrum is to be taken, since it instantaneously records the changing sample concentration in the ion source. As eluates emerge from the column, their presence is first recorded on the hydrogen flame ionization detector, which serves among other things to provide advance notice of peak shape and intensity. The time difference between the flame and total ionization chromatograms is controlled primarily by a splitting device (*a*, Fig. 1), as will be discussed. Most of the sample (>95%) passes into the carrier gas (helium) separators, where the sample is concentrated with respect to the helium, and passes into the ion source. Upon manual activation of the oscillographic recorder, the magnetic scan is commenced automatically at a preselected scan rate and the mass spectrum recorded. The rapidly changing magnetic field in the vicinity of the total ionization circuit induces a slight voltage drop in the total ionization signal. The resulting marks on the total ionization chromatogram (see for instance Fig. 7-a) provide a permanent record of the exact point on the chromatogram at which the scan was begun and stopped. The importance of scan rate and the position on the peak at which the spectrum was recorded is illustrated by a comparison of the spectra in Figure 2, a and b. The spectrum shown in Figure 2a was recorded in 1.7 sec on the apex of the emerging peak, and thus represents an unbiased spectrum which may be compared with reference spectra, spectra of homologs, etc. If the spectrum is scanned too slowly, as was the case in Figure 2b (44 sec), the spectrum will be biased due to changing sample concentration in the ion source and will not represent the true relative distribution of ion abundance which is inherent and characteristic in the mass spectrum of that compound.

Even with relatively fast scans, it may be difficult to obtain unbiased spectra from very sharp peaks which may result from efficient columns and temperature programming. For this reason, it may frequently be of help to be able to predict the moment at which a shoulder or apex of a peak appears on the total ionization chromatogram. We have constructed a simple splitting device, shown in Figure 3, which allows the flame ionization detector to record the emergence of a peak in advance of its passage into the ion source (which normally takes less than one second). A hypodermic needle (*a*, Fig. 3), silver-soldered to a piece of metal capillary (*b*) may be easily inserted through a silicone rubber septum cap in the exit of the column, and into the packing. The distance which the needle is inserted determines the time lead which the flame detector will hold over the total ionization detector, and will equal approximately (d/l) × RT, where d = the distance the needle is inserted, l = the length of the column, and RT = retention time of the sample. We have found that a lead of 4 to 6 sec provides a convenient time difference, and allows the recording of spectra with very little bias. The ratio of the amounts directed to the flame detector and mass spectrometer may be empirically adjusted by changing the internal diameter and length of the capillary which leads to the detector. Unlike the time delay, the ratio of the split is of a more permanent nature, so that once the adjustment of the ratio is made it may be left unchanged. Such a simple splitting device can be used without column modification. In some cases however, a greater time difference may be advantageous. We have frequently found useful a 6-ft column containing the flame detector exit at 5 ft (*b* in Fig. 1, and Fig. 4), thus giving a time difference of about one-sixth the retention time. Although the peaks emerging early will show somewhat poorer separation when compared with the total ionization detector, it may be convenient to have a "prevue" chromatogram when working with complex mixtures which may require many spectra, or in cases in which the operator may not wish to devote complete attention to the instrument while waiting for the sudden emergence of a component of interest.

The availability of a conventional detector also

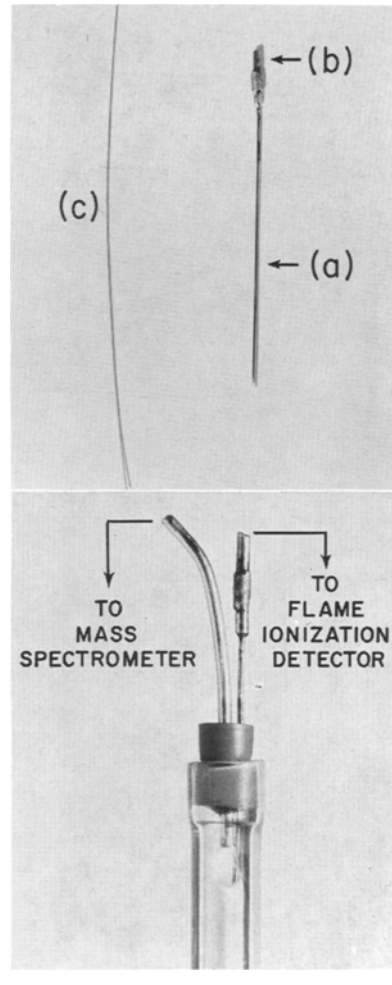


FIG. 3. End-of-column split used to control the time delay between flame and total ionization detectors. A 3 in. 22-gauge hypodermic needle (*a*), silver-soldered to a conventional piece of metal capillary (*b*) is inserted through a silicone rubber septum cap and into the column packing. Wire *c*, inserted in the needle to keep out packing material, is withdrawn after positioning of the needle.

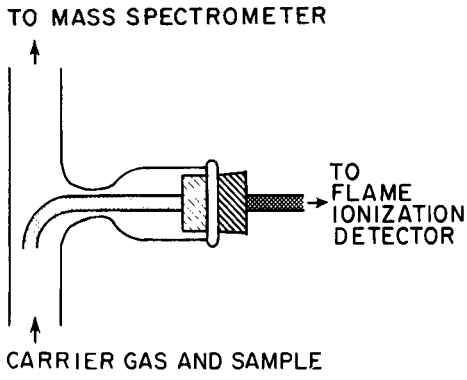


FIG. 4. Flame ionization detector exit at 5 ft on a 6 ft column, used for obtaining an early "prevue" chromatogram to determine peak shape and size well before its elution into the mass spectrometer.

provides a useful means of assuring that the total ionization chromatogram is a true representation of the separated components. For instance, decomposition or absorption of samples in the transfer system between the exit of the column and the ion source, which may result in poor sensitivity or tailing on the total ionization chromatogram, may be recognized by comparison of peak shapes and areas from the two chromatograms.

Operating temperatures are generally kept as low as possible to avoid thermal decomposition. However, for many large or polar molecules, high temperatures may be required, which in the case of the gas chromatograph may result in excessive column bleed and a high background spectrum. In cases in which gas chromatographic separation is not a major factor, e.g., use of the gas chromatograph simply as a convenient inlet system for the mass spectrometer, the use of very short columns results in lower temperatures, shorter retention times, and broader peaks which are more easily scanned. Table I shows the approximate temperature differences obtained from various 1% SE-30 columns.

Helium is the most widely used carrier gas for combination gas chromatograph-mass spectrometers, for two reasons. First, being lighter than any other gas except hydrogen, its greater mobility in the carrier gas separation process (discussed below) results in greater enrichment factors and lower ion source operating pressures. Second, it is helpful, though not necessary, to avoid ionization of the relatively large amount of carrier gas, which would lead to large base line variations on the total ionization chromatogram. Since helium has the highest ionization potential (~24.4 ev) of the rare or common gases, its ionization may be avoided by operating at an electron energy of 20 ev. Spectra obtained under these conditions do not usually differ significantly from 70 ev spectra. For a discussion of the effects of electron energy on relative ionic abundance, i.e., ionization efficiency curves, see: Biemann, K., "Mass Spectrometry," McGraw Hill, New York, 1962, Chapt. 3.

Two types of carrier gas separators (22–24) have found extensive use and are now commercially avail-

TABLE I

Example of Decrease in Column Operating Temperatures (at constant retention time) Resulting from the Use of Shorter Columns, with data Determined Using Hydrocarbon Standards on 1% SE–30

| Column length ft | Temperature, C |
|---|---|
| 6 | T |
| 3 | T–14 |
| 1 | T–35 |
| 0.25 | T–59 |

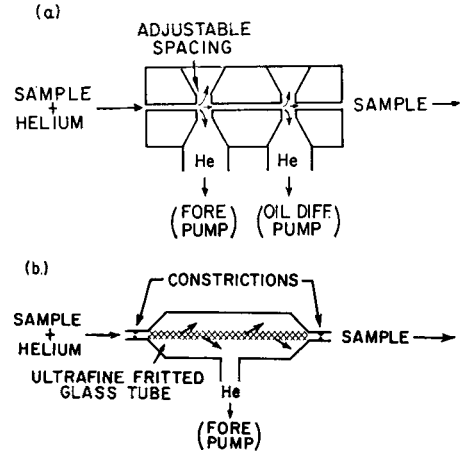


FIG. 5. Schematic diagrams of carrier gas separators. (a) jet-orifice type, developed by Ryhage (22) and Stenhagen (23) from the design of Becker (26,27); (b) porous glass frit type, designed by Watson and Biemann (6).

able. In our laboratory during the past two years we have been using separators of the jet-orifice type, developed for mass spectrometry by Ryhage (22) and Stenhagen (23) from the principles of Becker (26, 27). The principle of operation (Fig. 5a) relies on preferential pumping off of the relatively light carrier gas (helium) as the mixture flows at high velocity through a series of jet orifices. Helium is withdrawn in two stages: the first using a mechanical pump and the second an oil diffusion pump, ultimately removing over 99% of the carrier gas. The slower effusion of heavier sample molecules results in 1) a concentration effect, and 2) an overall reduction of pressure in the ion source. Empirical adjustment of the spacing between jets controls sensitivity and pressure. In our work we encounter relatively few situations in which our sensitivity is actually insufficient. Therefore our main concern is low operating pressure and thus good resolution, rather than higher sensitivity. The overall efficiency of the separation process requires the specification of three interrelated operating parameters: carrier gas flow rate, ion source operating pressure, and the relative amount of sample reaching the ion source (i.e., separator "yield"). The enrichment factor, which may also be a useful guide to separator efficiency, is incomplete in itself, since the corresponding separator yield could be any value, even approaching 0%. Other factors, such as residence time of the sample in the separator, which may effect remixing of adjacent GLC peaks, are also important but are not directly related to separator efficiency. Operating parameters in our instrument are adjusted to yield mass spectra of sufficient intensity from samples of 0.5 to 3 $\mu$g per component, with an overall separator yield of 20%, an operating pressure of 1–2 $\times$ $10^{-6}$ mm Hg measured by a Penning gauge calibrated for air) in the ion source, with a 35 ml/min flow rate. Yield calculations are based on comparison of total ionization peak areas obtained from identical samples, one introduced through the GLC inlet, and the other through a semiconventional system employing a heatable sample probe. Although the percent yield can be increased by changing the separator spacings, the result would be higher pressures and serious loss of resolution over mass 500 with fast scan rates.

The carrier gas separator of the glass frit-effusion type developed by Watson and Biemann (6,24) is schematically represented in Figure 5b. The sample and carrier gas are passed into a heated, jacketed glass

fritted tube. Under proper conditions (6), molecular flow is attained, and the relatively light carrier gas readily effuses through the (1 $\mu$ dia) pores and is pumped away. An experimentally determined enrichment factor of 50 for diethyl ether has been reported (6). This factor would of course increase for compounds of higher molecular weight. For both types of separators, the yield of the separation process and the operating pressure in the ion source are directly related. In the fritted glass separators, these parameters are controlled by adjustment of flow rates through constrictions at the entrance and exit of the unit (Fig. 5b).

In our laboratory we have very recently made a preliminary investigation of the glass frit separator with a single focusing mass spectrometer (Fig. 1), in preparation for construction of a gas chromatographic inlet on a double focusing (high resolution) instrument. The separator was of essentially the same dimensions as described by Watson and Biemann (6), and is available from Special Apparatus Sales, Corning Glass Works, Corning, N.Y., Corning Drawing No. 998020603. Pressure drop between the column and separator was effected by a length of metal capillary (*c*, Fig. 1), while valve V-2 (Fig. 1) provided a variable constriction at the exit of the separator. Manipulation of this valve provided a convenient means of varying the operating parameters of the system. Connections at either end of the separator were made by Kovar-to-Pyrex seals and stainless steel Swagelok fittings. Our initial results indicate that the percentage of sample reaching the ion source (separator yield) varies more with ion source pressure (actually, with residence time of the sample in the separator) than was observed with the jet-orifice type separator. The per cent yields were identical (20%) at $2 \times 10^{-6}$ mm Hg pressure as measured with the Penning gauge calibrated for air. However at $10^{-5}$ mm, the amount of sample reaching the ion source was approximately doubled. At these pressures, serious loss of resolution was observed, although with instruments which employ differential pumping over the entire analyzer region (e.g., ref. 6) pressures of this magnitude should present no problems. Although increasing the separator yield by a factor of two may be possible by changing separator design or dimensions, increasing the electron multiplier voltage is in general a more satisfactory and effective means of obtaining more intense spectra. In this way, the sample sizes mentioned previously can be decreased by at least a factor of 10 before encountering a base line noise level significantly higher than normal ($<$ 2 mm on the most sensitive trace of a three trace (1:10:100) oscillographic recorder). It should be realized that the required sample size is also a function of the nature of the information desired. The amount of material required to distinguish two compounds, for instance methyl linoleate from methyl linolenate, may be an order of magnitude less than that used for detailed structural work in which metastable transitions are studied.

Many of the advantages of the combination instrument rely directly on the ability to scan and record the mass spectrum in a relatively short time. This is particularly true if one wishes to examine sections of an emerging gas chromatographic peak which is known or suspected to contain more than one component. Although there is an ultimate limit to the speed with which a spectrum may be scanned, due to the statistics of collecting ions for very short periods

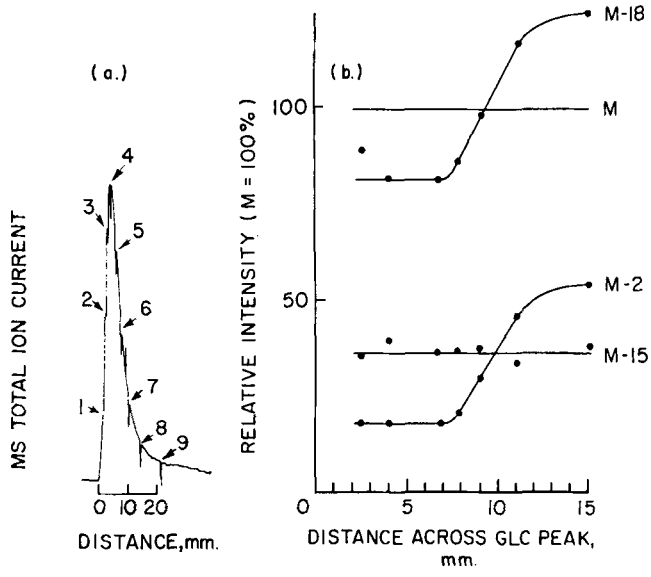


FIG. 6. Mass spectrometric data obtained from rapid scans across an emerging GLC peak of 2.2 $\mu$g of cholesterol (column: 1% SE-30; temperatures: GLC column 225C, transfer line and ion source 250C, separators 173C; mass spectrometer filament emission current 60 $\mu$a; ion source pressure $2 \times 10^{-6}$ mm Hg). (a) Total ionization chromatogram, showing points at which mass spectra were recorded. (b) Plots of relative intensities (M = 100%) of three mass spectral peaks as a function of distance across the GLC peak.

(28), we have found that scan times of from 2 to 9 sec (m/e 28 to 500) are sufficiently fast to obtain good spectra from all but the sharpest gas chromatographic peaks. Visual inspection of the spectra will usually provide the information needed. If more detailed information is required such as the relative amounts of each component, or to which component certain mass spectral peaks are to be assigned, the data may be plotted in several ways as a function of the distance across the peak. Consider as an example the total ionization chromatogram of cholesterol, Figure 6a. Cholesterol is useful as a convenient standard to adjust the temperature of the transfer system between the column exit and ion source. At too low temperatures it is easily adsorbed, while at higher temperatures it pyrolyzes by loss of $H_2O$ or $H_2$. The difficulty in avoiding thermal decomposition of cholesterol is illustrated by the wide range of relative intensities of the M-18 ion which have been reported: 106% (29), 95% (1), and 12% (30). Data from eight scans from the peak shown in Figure 6a are plotted in Figure 6b as distance across the peak vs. intensity of three ions relative to the molecular ion (arbitrarily = 100%). Recorded intensities from the ninth scan were too low for accurate measurement. The M-15 ion, due to loss of an angular methyl group, maintains a constant intensity in relation to M, which is expected since it is formed by electron impact only. On the other hand, M-2 (2H) and M-18 ($H_2O$) change in intensity in relation to M in the tail of the GLC peak, indicating pyrolysis. Plots of this type may be made for any number of peaks in the spectrum, using any major mass spectral peak as a reference peak, but preferably one representing an ion formed only by electron impact.

If the data are plotted instead in terms of absolute peak heights (17,31), components of an unresolved GLC peak may be graphically separated, and an estimation made of their concentrations and degree of partial separation. Figure 7a shows the total ionization chromatogram from an unresolved mixture

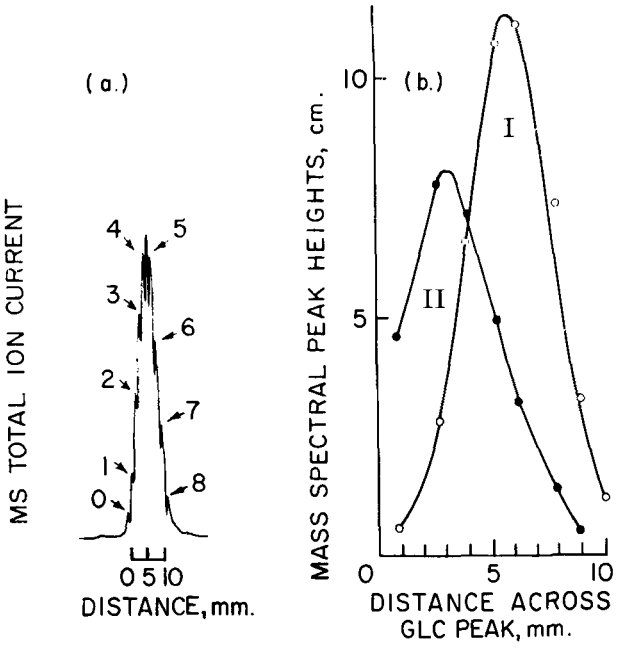


FIG. 7. Mass spectrometric data obtained from rapid scans across emerging unhomogeneous GLC peak consisting of (I) unlabeled and (II) labeled ($d_{12}$) di-O-isopropylidenes derived from 6,9-octadecadiene. (a) Total ionization chromatogram, showing points at which mass spectra were recorded. (b) Plots of the absolute peak heights of *m/e* 383 (M-$CH_3$, from I) and 392 (M-$CD_3$, from II) as a function of distance across the GLC peak.

of normal (I) and deuterium labeled (II) acetonides derived from 6,9-octadecadiene.

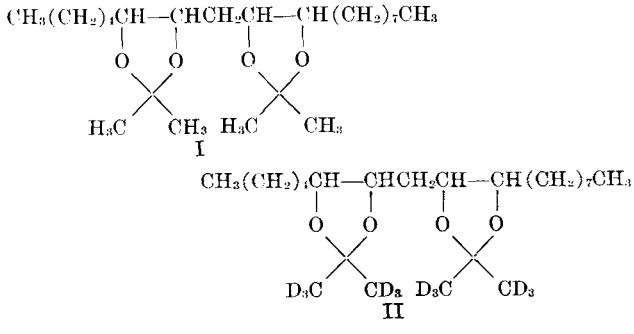


Figure 7b plots the absolute recorded intensities of *m/e* 383 and 392, due to loss of methyl groups ($CH_3$ and $CD_3$, respectively) from the 1,3 dioxolane rings of each component (32). The partial separation of the two components, the $d_{12}$ analog having the shorter retention time, is clearly indicated. Using either of these two methods of plotting the data, no a priori knowledge of the mass spectrum is necessary. A number of full mass spectral scans may be made in a very short time during the elution of a GLC peak, and the data examined and plotted at any later time.

It may be realized from the foregoing discussions that in most cases a correctly counted mass spectrum constitutes the most important piece of data provided by the combination instrument. It should be emphasized that an incorrectly counted mass spectrum, if relied upon, may be far worse than none at all. In most cases, counting begins at low mass numbers with some easily recognized peaks, such as those from air background, and proceeds to higher masses, until counting becomes difficult due to an unfavorable signal to noise ratio. This problem is usually approached by bleeding in a known "marker" compound during the time the spectrum is recorded. From the superimposed spectra thus obtained, nominal mass values of the peaks in question may be determined by direct counting, extrapolation or interpolation from marker peaks. For a discussion of the various aspects of mass identification, see ref. (4), Chapter 1.

Fluorocarbons, because of their great volatility, are the most extensively used mass markers for both low and high resolution (33) mass spectrometry. Perfluorokerosine, the spectrum of which has been published by Beynon (34) exhibits peaks past mass 1300 and is widely used. Because of the sometimes confusing recurring pattern of intensities in the upper mass ranges, a small added amount of perfluorotributylamine (35) may serve to provide several additional characteristic peaks. A 20 ev partial mass spectrum of this marker mixture is represented in Figure 8.

In working with the combination instrument the problem of mass marking may be more difficult than usual because, 1) rapid scanning and recording with an oscillographic recorder results in a higher noise level on the recorded trace, primarily due to stray 60 cps noise signals; 2) peaks from the mass marker may not always exhibit the proper range of intensities in relation to the wide range of sample concentrations found in complex mixtures; 3) if only a very small amount of material is available (i.e., enough for one run) it may be difficult to obtain marked and unmarked spectra, or spectra of some GLC peaks marked and others not, all in the same run. Our approach to this problem has been the construction of a simple marking system, shown in Figure 1. An empty U-tube column is placed in parallel with the conventional packed column. The pressure drop normally effected by the column packing is produced by a coil of wire capillary, *g*. Valve V-1, a simple toggle valve mounted on the face of the gas chromatograph is heated to ~ 60C and separates the marker column from the main line to the mass spectrometer. Several microliters of marker are injected

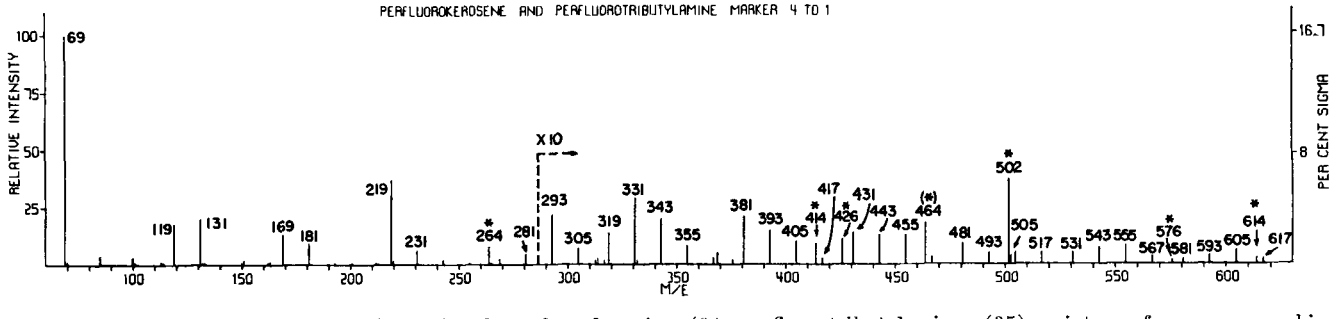


FIG. 8. Partial mass spectrum (20 ev) of perfluorokerosine (34)-perfluorotributylamine (35) mixture for mass marking. Peaks due primarily to perfluorotributylamine are indicated by asterisks. All peaks of relative intensity greater than 0.5% below *m/e* 283, and greater than 0.2% above *m/e* 283 are represented.

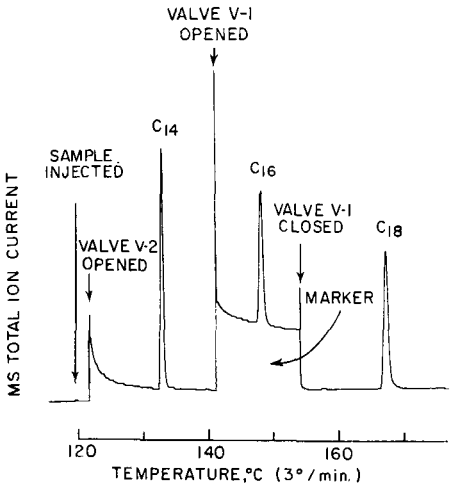


FIG. 9. Total ionization chromatogram of $C_{14}$, $C_{16}$ and $C_{18}$ normal hydrocarbons (12 ft, 1% SE-30) showing mass marker contribution to the total ion current from opening and closing of valve V-1 (*h*, Fig. 1).

into the marker column and the vapors allowed to diffuse through the column and capillary. With V-1 open and a slow flow of helium, marker is then available for continuous use after about 30 min. If the marker is injected in the evening, the column is ready for use the next morning. The empty column therefore serves as a reservoir of marker, easily maintained for instant use without tying up a conventional mass spectrometer inlet system, which of course can also be used for the same purpose. When marker is needed, V-1 is opened, and marker reaches the ion source and assumes an essentially constant flow rate in less than 2 seconds. Figure 9 shows the effect of opening and closing V-1 on the total ionization chromatogram. On opening, the base line rapidly assumes a new level, due to ionization of the steady flow of marker. When V-1 is closed, the base line assumes its original position, and spectra may be determined in the absence of marker. Unless the emerging gas chromatographic peak is very narrow, both marked and unmarked spectra may be recorded from the same peak, although perhaps at the expense of obtaining unbiased spectra, depending on scan rates and other factors.

It is evident that operation of the combination instrument represents automation in a real sense, allowing the acquisition of enormous amounts of data—hundreds of spectra per day in many cases. This may unfortunately lead to the incorrect conclusion that the information sought or problems which are approached may be acquired or solved with similar speed. It must be realized that although some operations such as plotting of spectra may be automated,[1] the interpretation of results, in particular the mass spectra,[2] may require considerably more time and effort. Nonetheless, the combination of gas chromatography and mass spectrometry, particularly in conjunction with the high resolution "element-mapping" approach (38), represents one of the most powerful instrumental techniques available to organic chemists for the study of natural products.

[1] Figures 2 and 8 were computer plotted (with the exception of "a" and "b" in Fig. 2, the "x 10" dotted arrow in Fig. 8, and individual peak numbering) and per cent Σ values calculated, from tabular intensity data. An IBM 1427 Plotter driven on line by an IBM 1410 Computer required about 3 min of plotting time and 1.8 min of computer time (for a Fortran IV program) per spectrum.

[2] Progress in the computer-aided interpretation of mass spectra has been reported by several groups; see for instance Refs. 36 and 37.

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