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Introduction of Gas-Chromatographic Samples to a Mass Spectrometer*,†

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Summary

Of the various physical and chemical methods commonly used for qualitative analysis of gas-chromatographic fractions, only mass spectrometry can be routinely used with samples as small as 10^{-8} to 10^{-10} g. However, full utilization of this sensitivity is possible only by proper selection of the method of introducing the samples, and the methods generally employed vary in their minimal identification requirement from 10^{-4} to 10^{-10} g.

Each sampling technique has different merits. The advantages of the various collection methods are compared with the various direct-coupling methods. Examples are presented to illustrate the types of sample advantageously analyzed with each system.

Mass spectral investigation of gas-chromatographic fractions is generally performed for qualitiative analysis of unknown compounds or for confirmation of the identity of suspected compounds. These investigations can be accomplished by a variety of sampling techniques, including methods in which the sample is trapped

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and introduced batchwise or methods in which the chromatograph is connected directly to the mass spectrometer. For the most part, each of the various methods available for GC-sample introduction is applicable to a wide range of samples, but occasionally a special technique must be applied for analysis of a particularly difficult GC system. For these systems, the researcher must be aware of the merits of each sample technique and accordingly choose the most suitable one.

The development of increasingly sophisticated handling, separating, and detection methods for small GC samples has made sensitivity one of the most important consideration for subsequent qualitative analysis. Most of the chemical and physical techniques commonly used for qualitative analysis require from 10^{-5} to 10^{-3} g of sample for a reasonable identification. In contrast, suitable choice of mass-spectrometric sampling techniques can permit analysis on less than 10^{-10} g in favorable cases.

This contrast is illustrated in Table 1, which gives approximate detection and identification limits for a few chemical and spectral methods, and compares these with detection limits frequently attained in GC analysis. For the most part, the GC detectors are far more sensitive that the identification techniques, but because retention-time data can give an identification only if additional functional-group information is available, the GC signal is considered only a detection. Mass spectrometry offers no advantage when a standard batch inlet is used for collected samples. However, direct introduction of the sample from the GC column, or use of a direct probe with collected samples, results in a significant reduction of sample amount required.

In Table 1 an arbitrary 1 to 2 orders of magnitude increase above the detection limit was assumed necessary for an identification. In actual practice, it is not possible to assign such a factor. For some cases, a strong mass spectral pattern may be obtained which, in the absence of spectral data from authentic standards, does not give an identification without support from other techniques. On the other hand, certain favorable cases may be encountered in which the detection limit and identification limit are the same. One interesting example occurred during the GC-MS analysis of an extract from orange juice (*1*). The first peak eluted from the column was less than 1 part in 10^4, and the amount separated was estimated to be about 10^{-11} g. The mass spectrum

TABLE 1

	Detection limit, g	Identification limit, g
Gas chromatography		
Thermal detector	10^{-6}	—
Flame ionization	10^{-12}	—
Electron capture	10^{-13}	—
Chemical analysis		
Selective reagents	10^{-5}	10^{-5}–10^{-4}
Microreactor	10^{-6}	10^{-6}–10^{-5}
Infrared	10^{-7}–10^{-6}	10^{-6}–10^{-5}
NMR	10^{-4}	3×10^{-3}
Computer-averaged (24 hr)		10^{-5}
Mass spectrometry		
Standard batch inlet	10^{-7}–10^{-6}	10^{-5}–10^{-4}
Direct probe	10^{-11}–10^{-10}	10^{-9}–10^{-8}
Direct GC coupling	10^{-12}–10^{-11}	10^{-10}–10^{-9}

obtained (Fig. 1) easily identified the compound as methyl chloride, primarily because the two isotopes ^{35}Cl and ^{37}Cl give rise to peaks at masses 50 and 52, which can only be interpreted as CH_3Cl. A few other simple compounds can be identified at their detection limit (benzene, toluene, carbon disulfide, etc.), but in general a more intense spectrum is necessary.

Consideration of the amount of material present at each instrumental stage during a mass spectral analysis emphasizes the importance of giving careful consideration to the method of sample introduction. As is indicated in Fig. 2, it is not uncommon in modern mass spectrometry to measure an ion signal of less than 30 ions (2), corresponding to 10^{-19} to 10^{-20} g. However, though this small limit is impressive, it is not realistic, and factors due to scan time, dispersion of ions, and transmission efficiency necessitate that around 10^{-8} to 10^{-12} g of sample per sec must enter the mass spectrometer. This amount, then, exemplifies the sample consumption of a modern mass spectrometer for a minimal intensity mass spectrum. (The range of 4 orders of magnitude is principally due to the range of mass resolution that may be employed. A low-resolution spectrum, $M/\Delta M = 300$, requires much less sample than a high-resolution spectrum, $M/\Delta M = 10{,}000$.) In actual practice, sample-inlet devices may vary in their *total* requirement

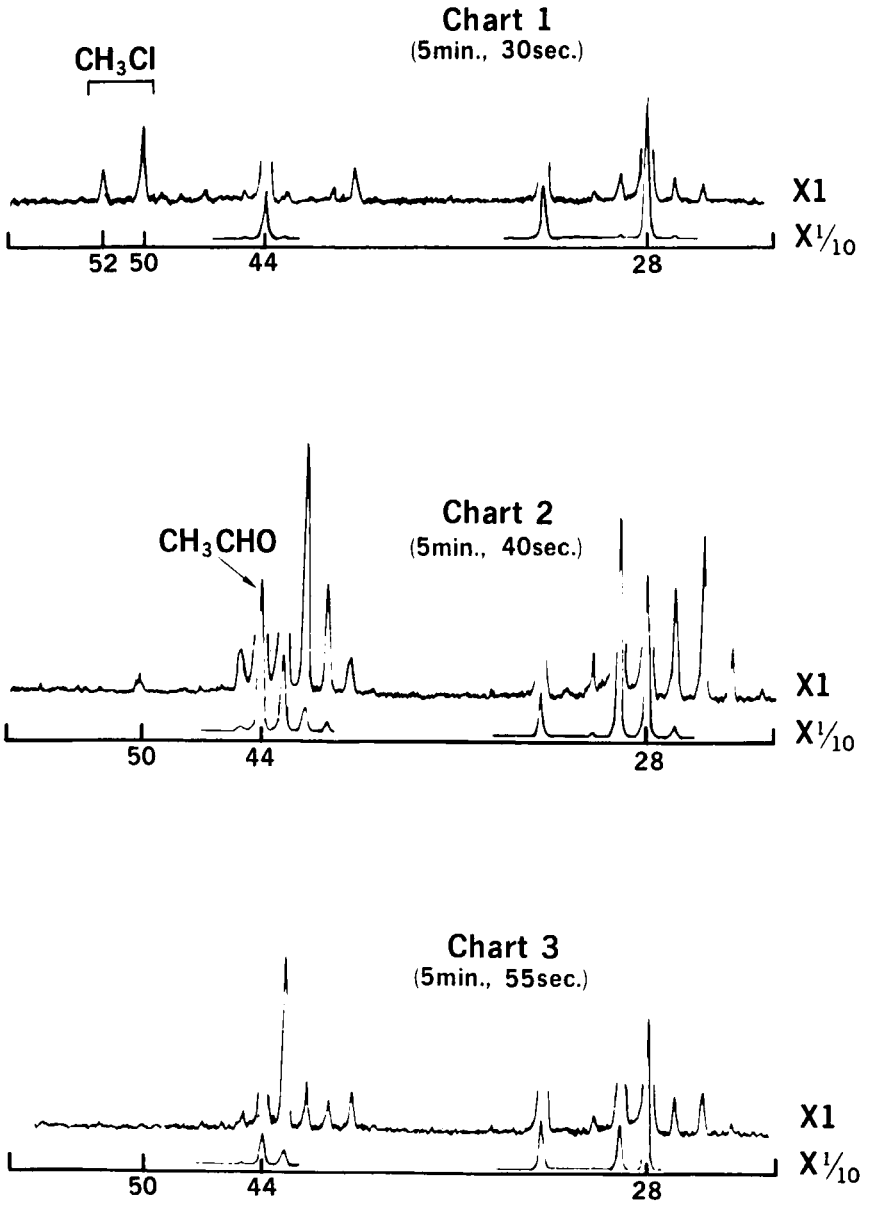

FIG. 1. Mass spectrum of CH_3Cl separated from an orange juice extract (approx. 10^{-11} g) (*1*).

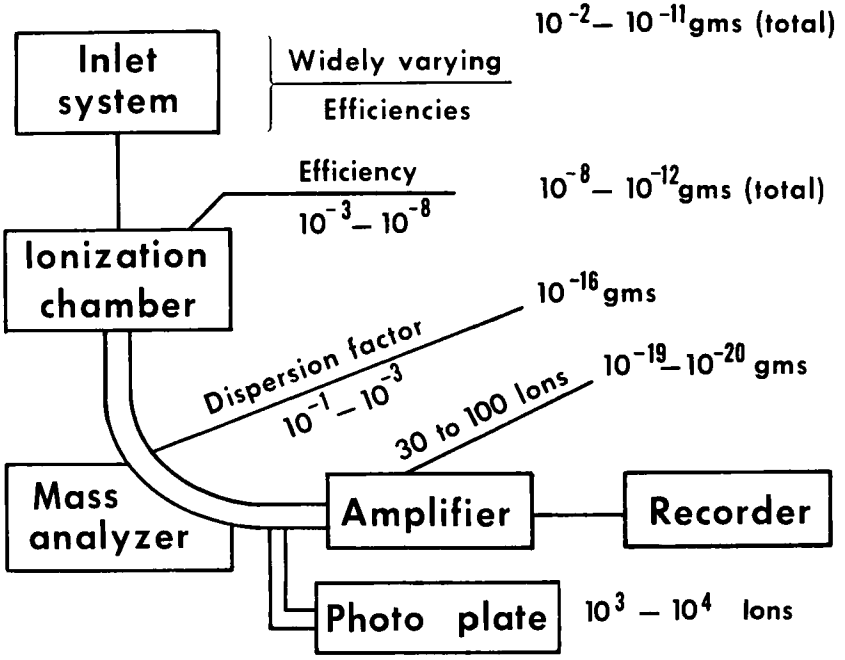

FIG. 2. Quantities of sample in use at various stages of a typical mass spectrometer.

from 10^{-3} to 10^{-11} g, and a careful choice of introduction techniques is important if the sample is available only in small quantities.

INTRODUCTION OF COLLECTED SAMPLES

A wide variety of batch methods is available for introduction of gas-chromatographic samples to a mass spectrometer. In general these could be classified into four methods or systems which are schematically indicated in Fig. 3. Approximate minimum amounts of sample are suggested for each system, consistent with a given time of sample introduction.

Standard batch-inlet systems of one form or another have been in use since the beginning of organic mass spectrometry (four or five decades). The sample is vaporized into a large expansion volume and effuses through a capillary or molecular leak into the ion chamber. For quantitative analysis, this type of system is essential. For qualitative analysis, its use is generally restricted

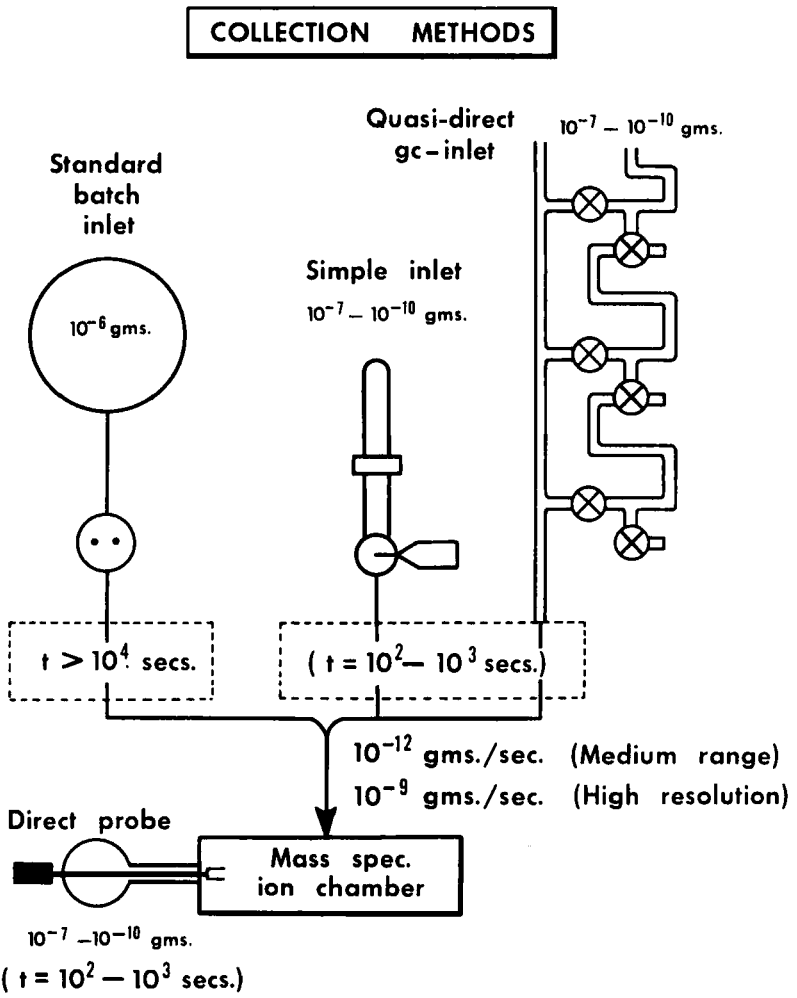

FIG. 3. Methods for introduction of collected samples to a mass spectrometer.

to sample sizes that exceed 0.1 μl. However, when the required amount of sample is available, this method is considered to be the easiest to use and the most reliable. In current practice, batch-inlet systems are constructed of stainless steel for use up to 150°C, or of glass for use up to 350°C.

The two features that adapt the standard batch inlet for quanti-

tative analysis are the large expansion volume and the slow leak rate through the molecular leak. These result in sample consumption of a few per cent per hour, and if it is valuable, the milligrams of sample left in the expansion volume can be reclaimed after mass analysis. However, if less-than-microgram quantities are available, the total sample requirement can be greatly reduced by decreasing the volume of the inlet and increasing the size of the leak, preferably with variable leak. Thus, at a sample-consumption rate of 10^{-10} g/sec, a good mass spectrum can easily be obtained with 10^{-7} to 10^{-8} g.

The simplicity of such a system is illustrated in Fig. 4. It may be constructed effectively of only three parts, a vacuum valve B, a quick-fit vacuum connector C, and a micrometer valve E. When heating is necessary the unit is easily wrapped with a small heating tape.

For all its simplicity, this inlet has many advantages. The sample can be in almost any form: an invisible layer in a GC collection tube, a submicrogram residue in a reaction flask (to be washed

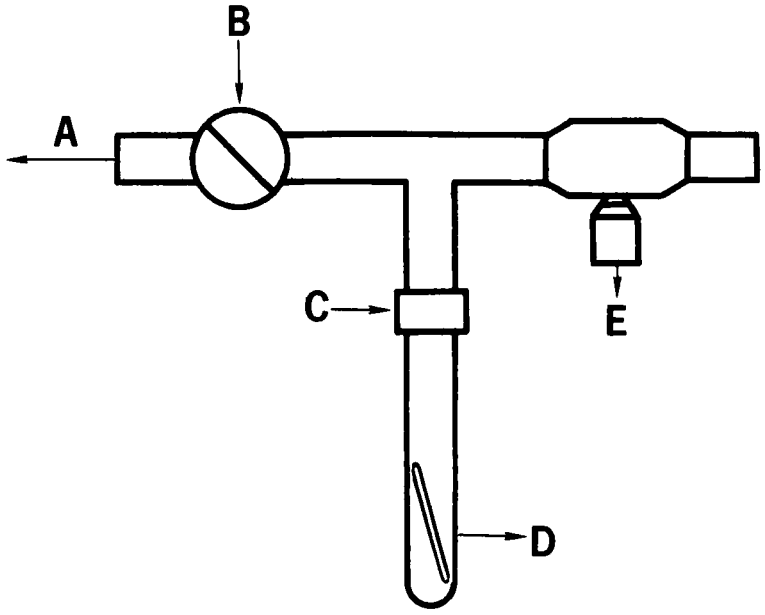

FIG. 4. Simple inlet for versatile sample introduction.

out with volatile solvent), a small sample from a paper or thin-layer chromatogram, etc. Often the paper or silica matrix can be put into a tube at D and the sample analyzed directly. If the sample is modestly volatile, it can be frozen before being pumped out by the auxiliary vacuum at A. If it is thermally unstable, the micrometer valve may be opened until sufficient sample effuses into the ion chamber, and thus the sample may be analyzed at room temperature. Excellent sample control can be obtained with small nonvolatile samples by first heating the sample to a desired temperature, and then opening the micrometer valve to obtain sufficient sample in the ion chamber. With the micrometer valve open wide, this inlet simulates the function generally accomplished by a direct probe (to be described). The quick-fit connector can also be used as a port for directly connecting the GC unit to the mass spectrometer. All in all, this assembly is possibly the most versatile inlet available for *qualitative* analytical work but, curiously, in modern practice it is the least common. Apparently, it lacks elegance.

The quasi-direct GC inlet system (*3,4*) is a series of traps connected directly to the mass spectrometer, as illustrated in Fig. 3. The effluent from the chromatographic column passes through each trap, and the various peaks of interest are sequentially frozen out. At a convenient time, the carrier gas is pumped away, and the evacuated sample is thawed and admitted to the mass spectrometer. With careful control of the admittance valve, the sample can be made to enter over a convenient time period (1 to 2 min) and the operator thus avails himself of the maximum sensitivity of the mass spectrometer. The necessity for fast scans (1 to 5 sec) is eliminated.

The obvious advantage of this system over the direct-introduction systems is the removal of carrier gas, thus permitting operation of the mass spectrometer at more suitable pressures. This advantage is considerably vitiated by the recent application of enriching devices as applied to direct-introduction systems. However, for identification of very dilute impurities (less than parts per million) the quasi-direct inlet still has advantages. In extreme situations, it may be necessary to use a fairly large diameter column to separate enough of a dilute impurity for mass analysis. The construction and efficiency of separators in current use limit their use to approximately $\frac{1}{8}$-in. columns.

An example of this type of analysis was shown by Ebert (3) using a quasi-direct inlet and Bendix Time-of-Flight mass spectrometer. Carbon disufide, present at a 0.1 ppm level, was separated from cyclohexane and an identifiable mass spectrum obtained. Although this sample could be identified easily at a lower level, the ion signal obtained (as shown in Fig. 5) would generally be considered minimal for most substances.

Use of a direct probe for introduction of samples right at the ion chamber has been widely used for samples of low volatility (Fig. 6). Recently, Amy et al. have shown that this technique is also convenient for introduction of collected GC samples (5). The sample-collection tube is a small capillary or melting-point tube packed with a suitable column packing. A GC sample emerging from a hot column is quantitatively collected on the cold column packing, and can be conveniently inserted in the probe and introduced to the mass spectrometer. In some modifications, the probe can also be cooled, thereby adapting the technique to more volatile samples.

Figure 7 presents the mass spectrum obtained from 0.1 g of pregnanediol diacetate collected in a small capillary packed with column packing (5). The ion signals of the more-abundant peaks were full scale on the most sensitive galvanometer, and a steady

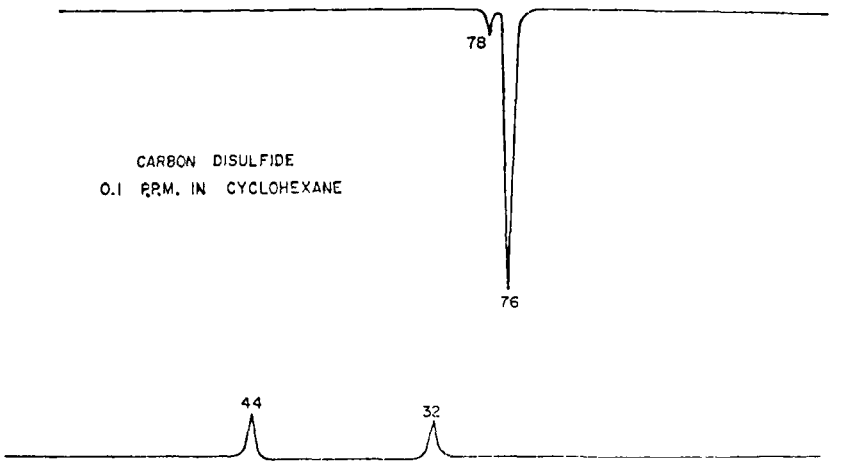

FIG. 5. Identification of 0.1 ppm of carbon disulfide in cyclohexane (3).

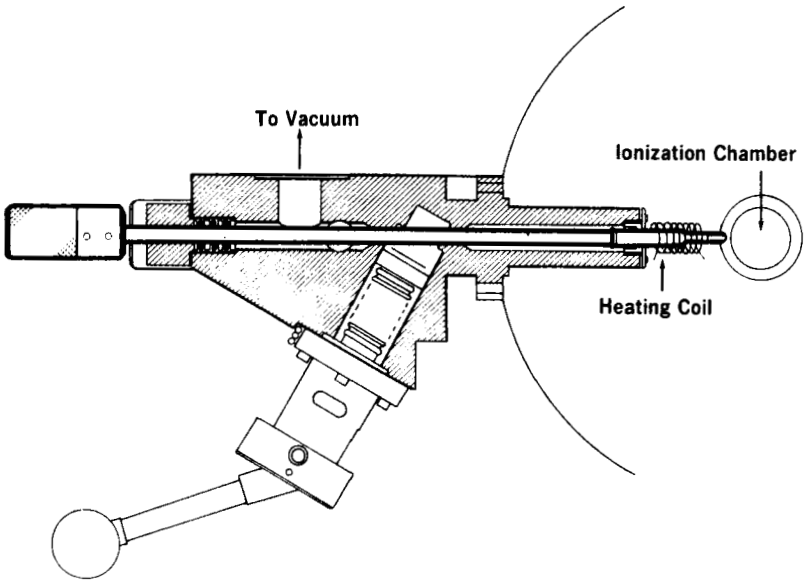

FIG. 6. Probe for introduction of sample directly to the ion chamber. (Courtesy of Consolidated Electrodynamics Corp., Monrovia, Calif.)

current was obtained for about 10 min. Extrapolation of the data suggests that a good high-resolution spectrum could be obtained on less than 10^{-8} g of material using a photoplate detection system (Mattauch-Herzog mass spectrometer).

DIRECT COUPLING OF MASS SPECTROMETER AND GAS CHROMATOGRAPH

When applicable, collection methods are generally considered to be more convenient and give more reliable data. The operator is able to inspect the spectra and repeat a run with modified conditions if necessary. However, many samples and chromatographic systems do not permit the leisure of collection and subsequent introduction to the mass spectrometer. Sometimes low collection efficiencies are obtained; sometimes the pure collected sample will polymerize or oxidize; sometimes the mixture may contain 150 or more components, and collection techniques are inconvenient. In addition, as has been emphasized, the time taken

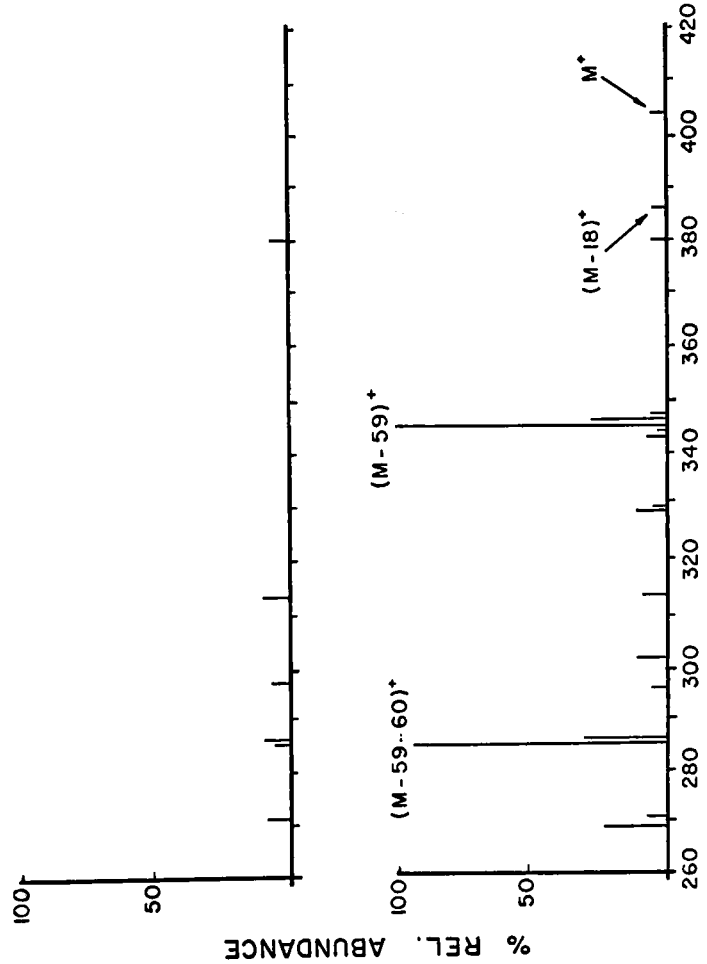

FIG. 7. High-resolution mass spectrum obtained from 10^{-7} g of pregnanediol acetate collected on Chromosorb P (5).

to introduce the complete sample is an important sensitivity parameter, and at least an order of magnitude lower sample size can be accomplished by certain tandem arrangements.

Four systems in general use for tandem introduction are shown schematically in Fig. 8. The commonest method for connecting a packed column is to split the effluent at the column exit and take approximately 0.5% of the total into the mass spectrometer. Because the pressure in the mass spectrometer cannot exceed 10^{-5} to 10^{-4}

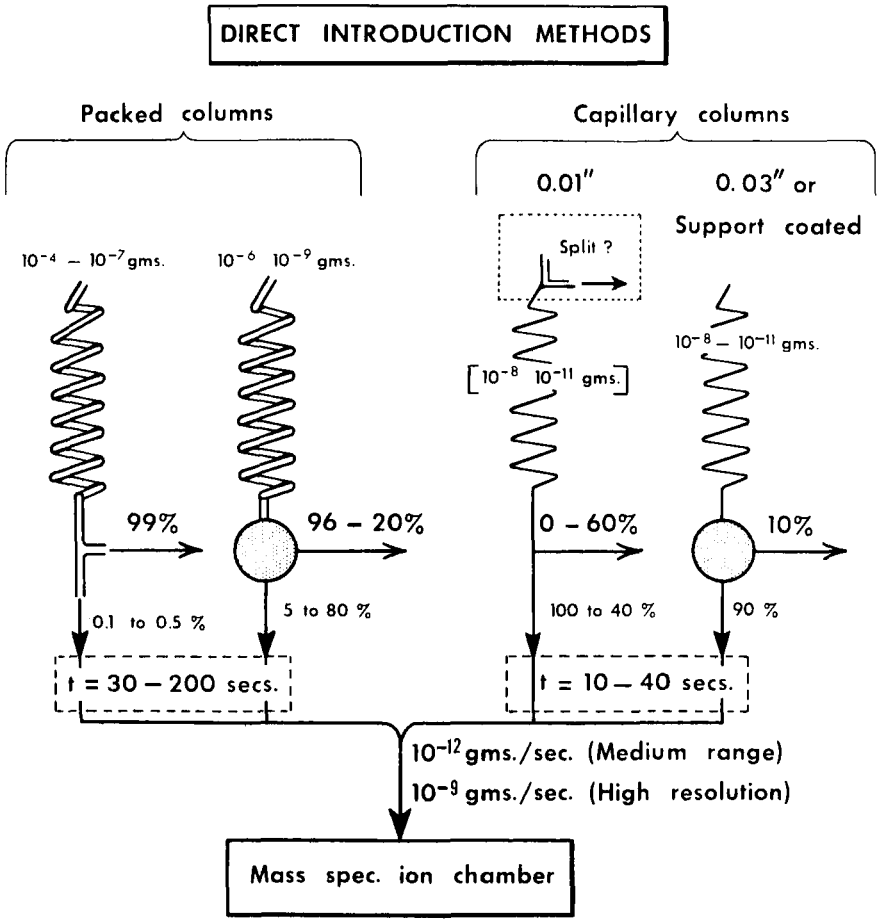

FIG. 8. Methods for direct introduction of gas-chromatographic samples to a mass spectrometer.

torr, a larger amount cannot be utilized. This was the first tandem GC-MS system to be used (*6,7*), but, because more than 99% of the material is rejected, the minimum sample required is in the range of 10^{-7} g.

Recently, the use of enriching devices has permitted a considerable increase in the percentage of the total effluent sample that can be introduced into the mass spectrometer. Three types of molecular separators have been used or proposed which utilize either the difference in diffusion rate of sample and carrier gas (*8,9*), or the selective adsorption or solubility of sample over the carrier gas (*10*). As is indicated in Fig. 8, 5 to 80% of the total organic material can be utilized from a packed column using an enricher, and the minimum sample size required is thus reduced to about 10^{-9} g.

The most common enriching system in current use is that proposed by Watson and Biemann (Fig. 9). The sample and carrier gas enter the separator from the column at I through a pressure reducer G and into a cylindrical glass frit. The outer region of this frit is pumped at D, and sample and carrier that do not diffuse out continue on to the mass spectrometer at A. The greater diffusion rate of helium permits significant enrichment of sample, and enrichments have been reported in which the sample-to-carrier gas ratio is increased 50 to 100 times. However, as parameters are adjusted to increase the enrichment, it is necessary to pump more of the sample away.

The important parameter is not the enrichment attainable, but the total per cent of sample that can be directed into the mass spectrometer. This must be carefully considered in selecting valves for the restriction at B, for the frit size, porosity, etc. In general, it appears that 10 to 30% of the organic sample from a ⅛-in. column can enter the mass spectrometer.

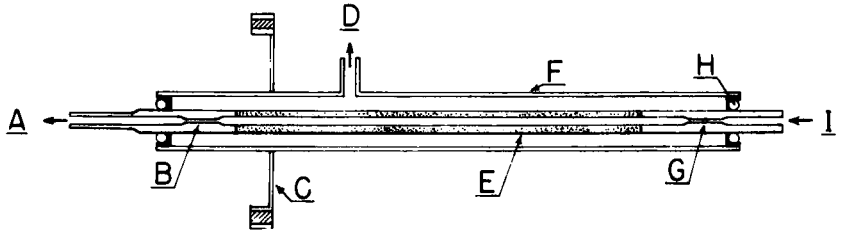

FIG. 9. Biemann-type molecular separator (*8*).

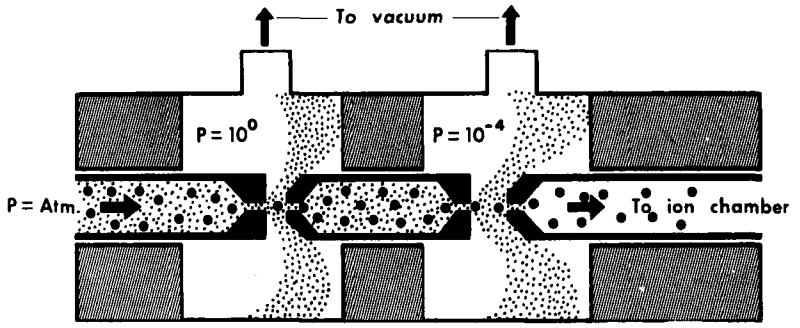

FIG. 10. Ryhage-type molecular separator.

Another enriching device that utilizes the difference in diffusion rates has been proposed by Ryhage (Fig. 10). The column effluent passes through a very fine orifice, which is carefully aligned close to a similar orifice. The light carrier gas (He) diffuses away from the line of sight more rapidly than the organic sample, thus effecting enrichment. Utilizing two stages of this system, it is again possible to get 10 to 30% of the total organic effluent into the mass spectrometer. Unfortunately, this type of molecular separator is not available commercially except as an accessory for one particular mass spectrometer.

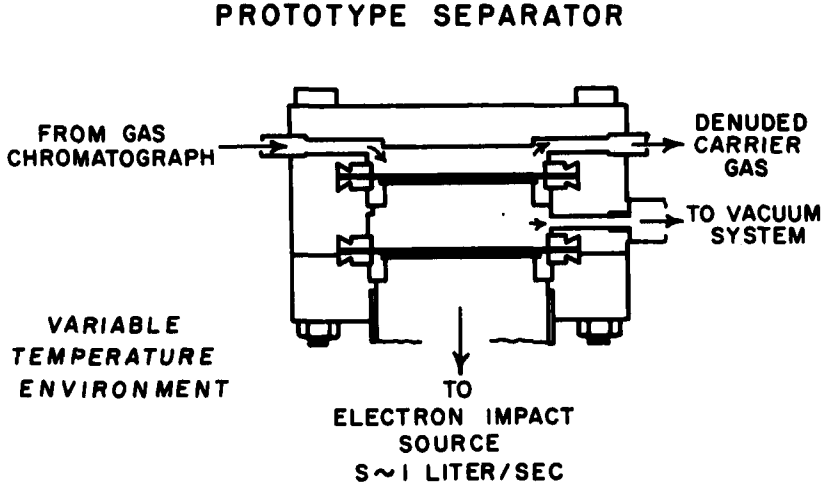

FIG. 11. Llewellyn-type molecular separator. (Courtesy of Varian Associates, Palo Alto, Calif.)

A third type of molecular separator has been proposed by Llewellyn (Fig. 11). In this system, sample is removed from the carrier by selective adsorption through a thin organic membrane. By suitable choice for rate of removal of the carrier gas at the two stages, it is reported that 90 to 95% of the organic material can enter the mass spectrometer, while the partial pressure of helium remains at about 10^{-7} torr. The time constant for passage through the diaphram is currently reported to be 2 to 3 sec.

Many complex mixtures (40 or more components) cannot be satisfactorily separated by conventional ⅛-in. packed columns, and capillary or support-coated capillary columns are required to obtain sufficient separating power. For four or five years now, 0.01-in. capillaries have been used in many applications for direct GC-MS analysis, particularly with complex petroleum fractions (*11*) or complex flavor and odor extracts (*12,13*). Because of the smaller amount of carrier gas used, it has been possible to permit 40 to 100% of the total sample into the mass spectrometer, and thus to utilize fully the small amounts of material (10^{-6} to 10^{-10} g) so effectively separated.

Two modestly different variations have been used for coupling the capillary column to the mass spectrometer. In one, the exit gas is casefully split into two streams (*11,14*). One stream continues to a flame detector or other detector for chromatographic monitoring, and the other stream goes through about 2 or 3 ft of 0.001-in. capillary tubing to the mass spectrometer. Adjustment of the length of the 0.001-in. capillary determines the per cent of sample used in the mass spectrometer.

The other variation used to couple a 0.01" capillary column to the mass spectrometer modifies the column operation by operating the exit directly in the vacuum system (*12*). This necessitates a reduction of 1 atm at the column inlet to maintain the same average linear velocity through the column. Studies have shown that there is no serious reduction of column efficiency (*15*). The advantage of this method is that, under the vacuum conditions at the column exit ($P \simeq 10^{-3}$ torr), the high gas velocities eliminate the possibility of eddy currents which might impair column separations. In addition, the reduced volume of gas corresponding to the same average linear velocity permits a larger fraction of the total to enter the mass spectrometer at a given pumping speed.

Direct coupling of 0.02- and 0.03-in. capillary columns to a mass

spectrometer can be accomplished in essentially the same fashion. However, the increased volume of carrier gas is now more than most mass-spectrometer pumping systems can accommodate, and only 5 to 25% of the total effluent can be utilized. It follows quite naturally that a separating device can increase this value to as high as 90%. With the greatly increased column load permitted by 0.03-in. columns, the dynamic range for such systems can be extended to about 1 part in 10^6 (10^{-4} g maximum to 10^{-10} g minimum). When separations require columns of greater that 100,000 plates, this system certainly offers the maximum elegance in GC-MS analyses.

One important point to emphasize is that, because of the sharper chromatographic peaks obtained by the capillary columns, the time taken to introduce the total sample is reduced. This naturally

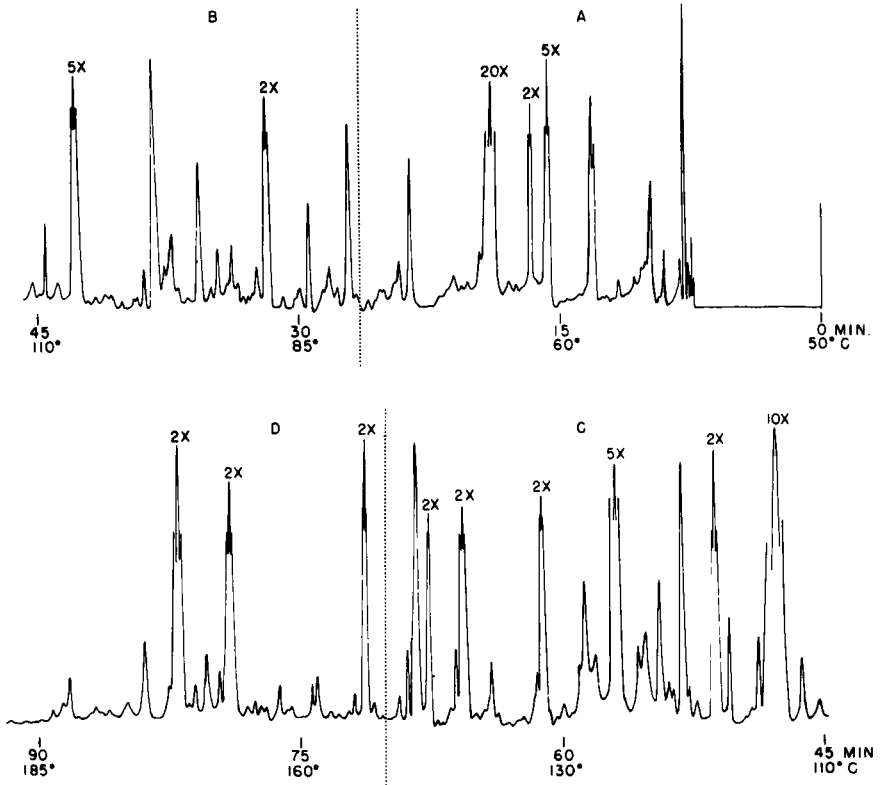

FIG. 12. Capillary column chromatogram of complex strawberry oil (*16,17*).

results in a smaller sample threshold and, as is noted in Fig. 8, minimal sample requirements approach 10^{-11} g.

Data obtained during analysis of a complex strawberry oil illustrate the advantages of tandem capillary GC-MS analyses (*16,17*). The capillary chromatogram, showing more than 150 components, is given in Fig. 12. Most of the major peaks were identified, and many were shown to contain more than one component even though they were quite symmetric. The final large peak at $T = 175°$ strikingly illustrates this point. The mass spectra obtained while this peak was being eluted are given in Fig. 13.

The first mass spectral chart, taken about half way up the peak, showed the presence of two components (149 and 150). The mass peaks at *m/e* 187, 143, 99, 82, and 67, which were essentially absent on the second chart, indicated a possible hexenyl hexanoate. Such preliminary identifications are always tentative and must be confirmed by comparison with authentic mass spectral and GC data. The next chart continued to show mass peaks at 150, 108, 79, and 65, which easily identified component 150 as benzyl acetate. In addition, a new set of peaks was developing due to component 151. The chart taken about half way down the GC peak gave mass peaks at 136, 121, 93, and 59 from component 151, and also at masses 198, 99, and 81 due to component 152. The experienced mass spectrometrist quickly recognizes component 151 to be α-terpineol, and component 152 to be another hexenyl hexanoate. Finally, at the base of the chromatographic peak, a mass spectral chart is obtained free of significant background from the previous components. The shoulder peak was identified as a pentyl octanoate.

This set of mass spectral charts indicates clearly that one cannot, a priori, assume from symmetry that a chromatographic peak contains only one or two components. Obviously, it is important to obtain a mass spectral chart at the optimum time. This is greatly aided by having an oscilloscope display of the mass spectra, which gives a visual monitor of the instantaneous condition in the ion chamber. The charts shown in Fig. 13 were taken at 7- to 10-sec intervals.

An example of the use of an enriching device with packed columns is given in the work of Eneroth et al. on a study of fecal steroids (*18*). One fraction gave a chromatogram (Fig. 14) in which the retention time of certain components did not correspond to

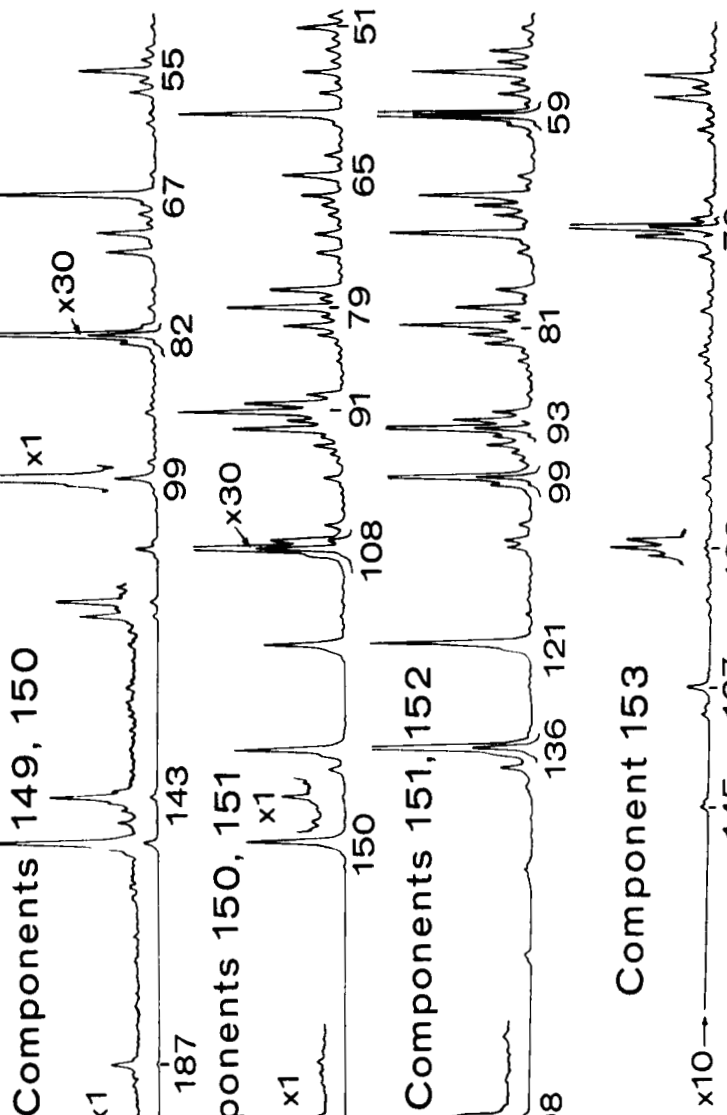

FIG. 13. Mass spectral charts of five components in one chromatographic peak of strawberry oil (*17*).

known metabolites. A solution containing about 1 μg of the steroidal trimethylsilyl ethers was chromatographed and introduced to the mass spectrometer using a Ryhage-type separator. Three of the mass spectra thus obtained are given Fig. 15. Compound A was confirmed as coprostanyl trimethylsilyl ether. The unknown compounds C and D were shown to be homologs of A. Thus all spectra have common family characteristics (peaks at masses 108, 215, 230, 257), but ion fragments in the higher-molecular-weight range differ from the spectra of A by 14 and 28 mass units [CH_2 and $(CH_2)_2$].

Techniques for direct introduction of chromatographic effluents to obtain high-resolution mass spectra have been demonstrated by Watson and Biemann (*19*). In a double-focusing mass spectrometer, the ion current to the intermediate beam monitor can be conveniently used to obtain a chromatographic trace. For a specific type of geometry (Mattauch-Herzog), the mass spectrum can be recorded

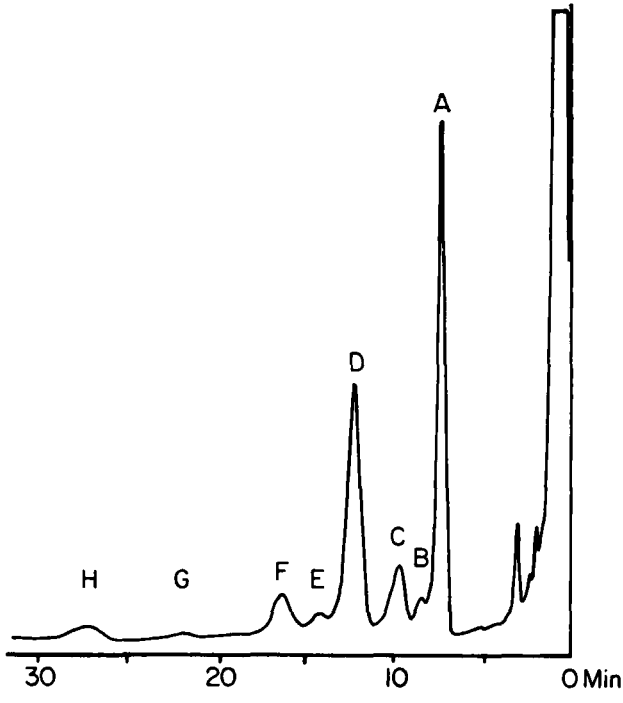

FIG. 14. Chromatogram of steroids from fecal samples (*18*).

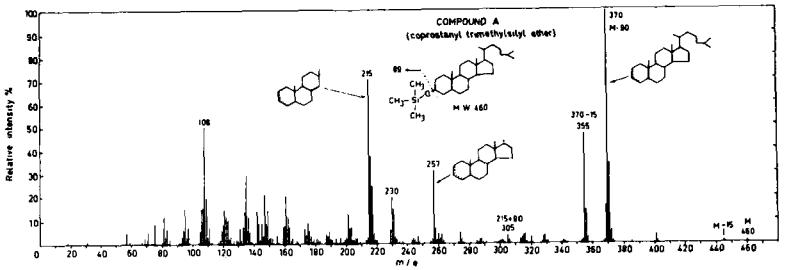

FIG. 10a

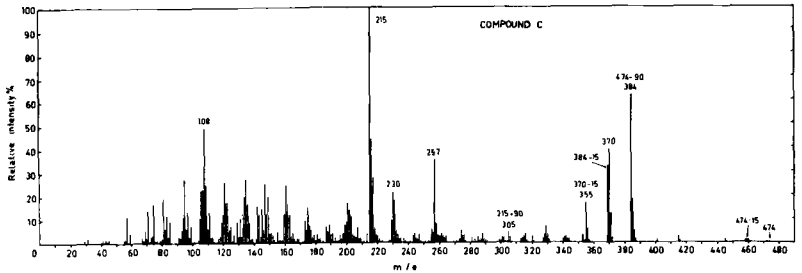

FIG. 10b

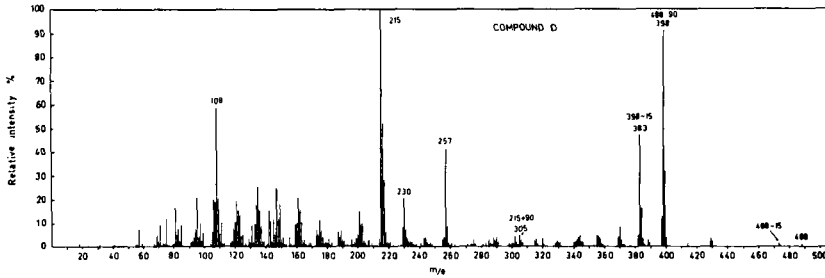

FIG. 15. Mass spectra of compounds A, C, and D in chromatogram of Fig. 14 (*18*).

on a photoplate placed at the focal plane, which thus eliminates the necessity for fast-scan amplifiers and recorders.

In this way, a mixture of alkaloids was chromatographed (*19*) (Fig. 16). Vertical dips occur when the ion beam is interrupted to shift to a new photoplate position, which thus records the position in the chromatogram corresponding to a photoplate line. A portion of the mass spectra obtained is shown in Fig. 17. A background spectrum is displayed at position 17 and shows ion traces due to a fluorocarbon introduced simultaneously for mass marking purposes. On line 18, new ion traces appear due to the

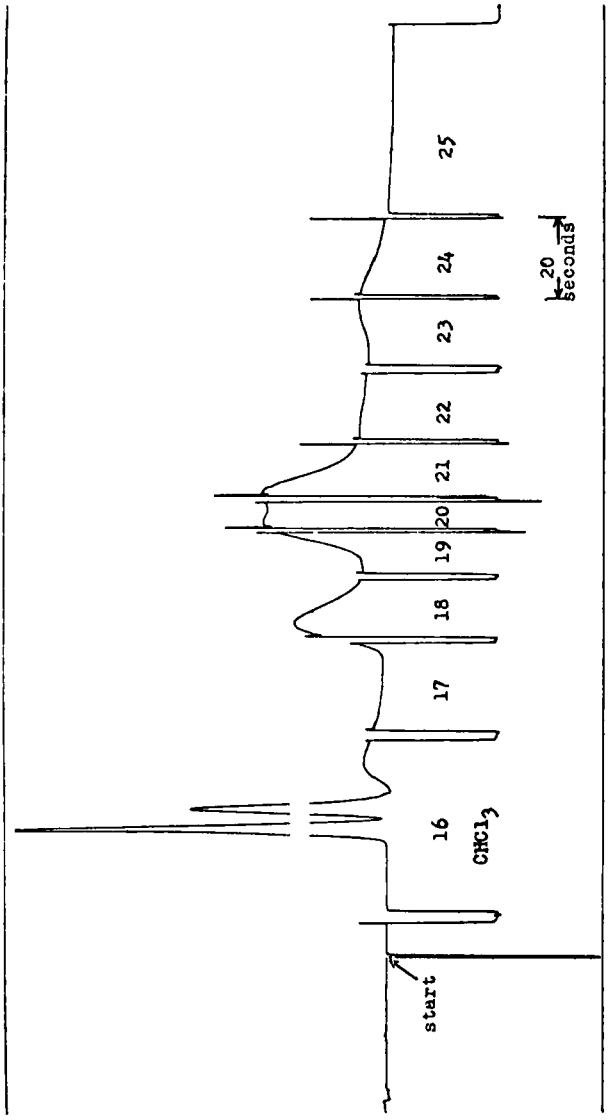

FIG. 16. Chromatogram of alkaloid extract obtained using ion beam monitor of CEC-21-110 mass spectrometer (*19*).

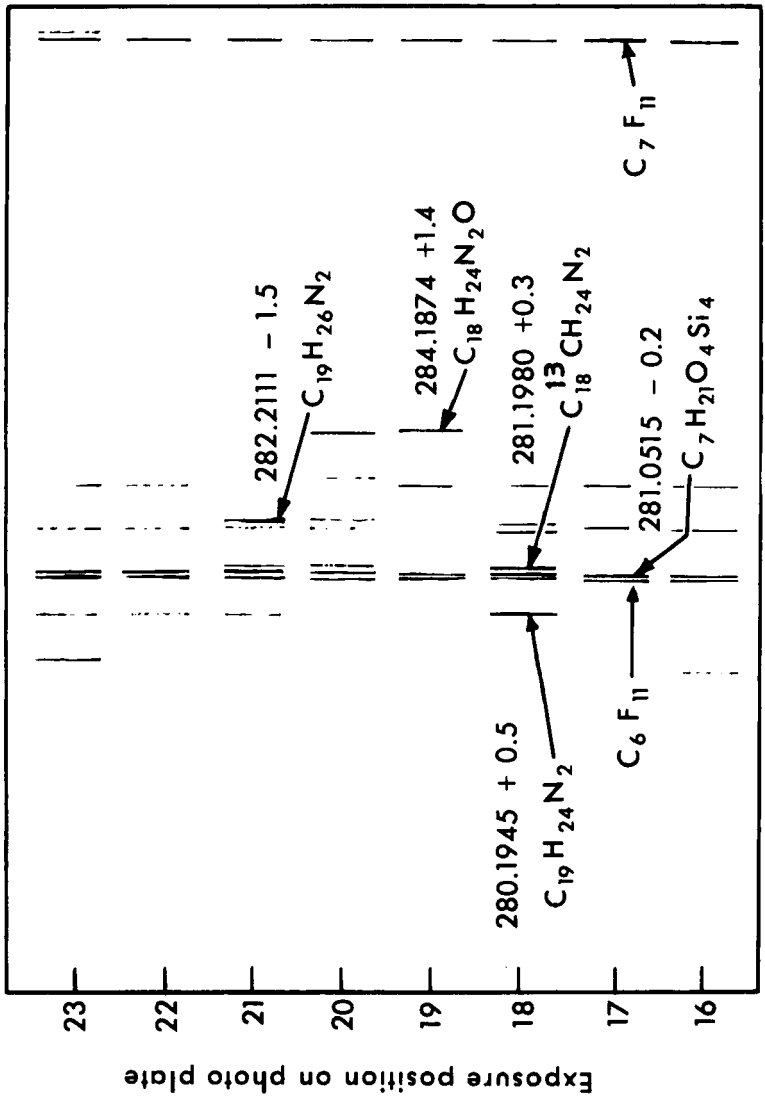

FIG. 17. Magnified portion of photoplate recorded during chromatographic run (*19*)

alkaloid. Accurate positional determination (to about 0.5 μ) of these lines with respect to the reference lines gives a molecular weight to approximately 10 ppm and permits assignment of a definite molecular formula. Thus, one determines the per cent nitrogen, oxygen, etc., in the parent molecule and its fragments, and interpretation of the spectrum is greatly facilitated.

If the double-focusing mass spectrometer is not of a type that gives focus of all ions in a plane, high-resolution mass spectra are obtained by using a high-speed amplifier and tape recorder (*20–22*). Because of the sharpness of the mass peak at resolution 10,000, a 10-sec scan for a mass decade (e.g., 24 to 240) requires a time constant of about 10^{-4} sec (*23*).

Tape recording of mass spectra provides a convenient means for computer processing of data (*22*), which may then readily be presented in the so-called element-map form introduced by Biemann for processing of photoplate data (*25*). In general, fast recording by tape recorder will be considered more convenient and elegant than the photoplate system. However, because of the rapid rate at which a mass peak is traversed in a high-resolution scan, a statistical limitation occurs that would seem to make the integrating photoplate system more desirable for small samples. Comparison of the data of Amy et al. (*5*) with those of McMurray and Lipsky (*22*) indicates that, in current practice, the photoplate system will give useful spectra with one or two orders of magnitude less sample.

Note added in proof: A recent article [Lipsky et al., *Anal. Chem.*, **38**, 1587 (1966)] describes the use of a thin Teflon tube as an enriching interface for GC-MS coupling. The system offers distinct advantages over the glass frit, but it must be operated in the temperature range 240 to 250°C to obtain effective diffusivity.

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