

# **Analysis of Drugs and Metabolites by Gas Chromatography- Mass Spectrometry**

Volume 1

Respiratory Gases, Volatile Anesthetics,  
Ethyl Alcohol, and Related Toxicological Materials

Benjamin J. Gudzinowicz

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# **ANALYSIS OF DRUGS AND METABOLITES BY GAS CHROMATOGRAPHY— MASS SPECTROMETRY**

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Alcohol, and Related Toxicological Materials

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## PREFACE

In the past two decades, remarkable progress has been made in the analysis of drugs, pharmaceuticals, and related toxicological materials. In great measure, these notable advances can be attributed to technological advancements in two specific types or areas of analytical instrumentation; namely, gas chromatography and integrated gas chromatography-mass spectrometry.

Since James and Martin revealed to the scientific community their gas chromatographic technique which permitted the separation of fatty acid mixtures into their individual components, the rapid growth of gas chromatography has been very evident. This remarkable progress can be directly correlated with the improvements that we have witnessed over the years in gas chromatographic stationary phase, carrier gas, column, and temperature- and pressure-controlling technology. Furthermore, it has assumed a position of even greater analytical significance since the advent of highly specific, rapid, sensitive detection systems.

On the other hand, the integrated GC-MS analytical system is rather unique and exceptional in that it combines the mass spectrometer's unexcelled identification potential with the gas chromatograph's separation capabilities. Although the integration of GC and MS was first reported in 1957 by Holmes and Morrell, it nevertheless remained a dormant, costly, and seemingly unappreciated technique until 1970. Since then, with improved instrumentation at a more reasonable price and newly developed operating techniques, numerous publications have appeared in the literature showing its applicability to a wide variety of difficult analytical problems, thus opening up new horizons for analytical research in toxicology, biochemistry, pharmacology, forensics, medicine, etc. To be able to monitor a drug, its persistence and metabolic fate in biological fluids of man via mass fragmentography at picogram concentration levels provides the researcher with a tool of immeasurable significance.

Because much has been written over the years about the analysis of drugs and their metabolites by either or both techniques, the objectives of these volumes are several-fold: (1) to compile from existing literature in a chronological manner the various GC and/or GC-MS procedures available for the analysis of specific drugs and their metabolites, (2) to describe with as much detail as possible all procedures (qualitative and quantitative) in order that they might be reproduced faithfully in one's laboratory, and (3) to indicate, wherever possible, not only the results, precision, accuracy, and limits of detection achieved by a given procedure, but also its applicability to pharmacokinetic studies. For this reason, in addition to the text, which is well referenced in each section, many illustrations of actual applications and tables of data for each instrumental technique are included as aids to the analyst for his greater appreciation and understanding of the limitations as well as potentials ascribed to each method. As stated in the past, from an analytical chemist's point of view, it is hoped that this deliberately combined visual and factual approach will find acceptance by the reader who would otherwise rely only on his interpretation of the written word relative to some published procedure.

Without wishing to be repetitious, in retrospect it must be again stated that this volume really represents the end result of many tedious and arduous investigations by numerous eminent scientists whose research efforts have appeared in the literature throughout the world. I am indeed humbly indebted to them, and to those journals, publishers, and organizations that granted special copyright permission to the authors.

Benjamin J. Gudzinowicz

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## Chapter 1

### RESPIRATORY GASES, VOLATILE ANESTHETICS, AND RELATED TOXICOLOGICAL MATERIALS

#### I. INTRODUCTION

Because gas chromatography's greatest attraction lies in its economy and versatility, this type of instrumentation has been given considerable attention in the past decade as a means of monitoring the concentration of anesthetics in mixtures expired by anesthetized patients during the course of an operation [1] and has been applied to the determination of the absorption, distribution, and elimination of such compounds as diethyl ether [2-4]. Under usual conditions the gases likely to be encountered are oxygen, nitrogen, nitrous oxide, diethyl ether, carbon dioxide, cyclopropane, chloroform, trichloroethylene, and fluothane (halothane), in addition to other chloro-fluoro-organics.

Using columns of dimethyl sulfoxide and dinonylphthalate connected in parallel with a thermistor detector, Adlard and Hill [5] showed in 1960 that ether, fluothane, oxygen, nitrous oxide, carbon dioxide, and cyclopropane could be resolved in 4.5 min.

Similar separations can be accomplished with parallel columns of 20% (w/w) Narcoil on 60-80 mesh Chromosorb P (3 ft x 1/8 in. SS (stainless steel) column maintained at 70°C) and 25% (w/w) dimethyl sulfoxide on 70-80 mesh Anakrom AS (20 ft x 1/4 in. copper column held at room temperature) using either a thermal conductivity (Fig. 1.1) or cross-sectional ionization (Fig. 1.2) detection system [6].



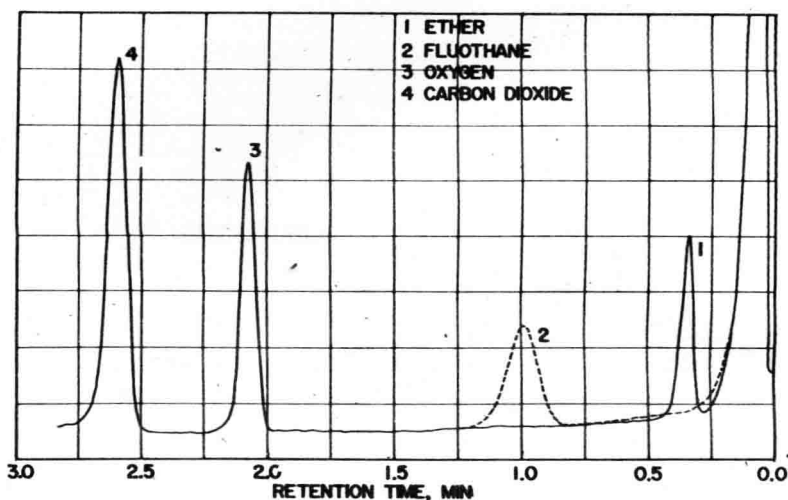


Figure 1.1. Separation of ether, fluothane, oxygen, and carbon dioxide (thermal conductivity cell). From Gudzinowicz [6].

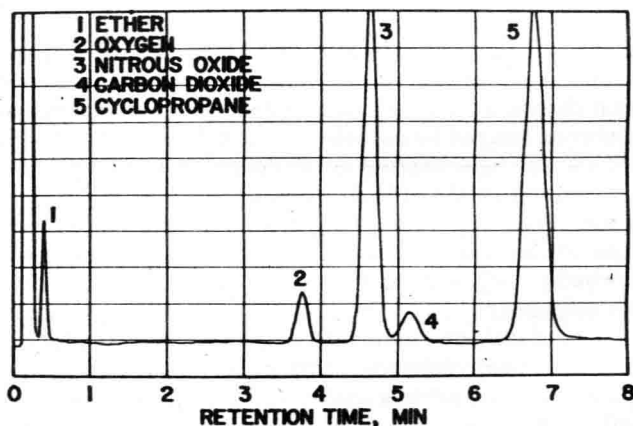


Figure 1.2. Separation of ether, oxygen, nitrous oxide, carbon dioxide, and cyclopropane (cross-section detector). From Gudzinowicz [6].

The basic method of analysis of dissolved gases ( $O_2$ ,  $N_2$ ,  $CH_4$ ,  $CO$ ,  $CO_2$ , etc.) in aqueous systems at concentration levels as low as 0.3 ppm in 0.5-liter samples [7] has been applied to the determination of fluothane tension in blood [8], toxic gases in tissues by direct sampling of air taken in a sealed container [4], and rapid screening of blood samples for volatile

substances such as ether, fluothane, chloroform, carbon tetrachloride, ethanol, acetone, trichloroethylene, and paraldehyde [9]. The gas chromatographic approach has also been used for the determination of partition coefficients of volatile anesthetics in blood, tissues, and lipids [10], the apparent volume of distribution, pulmonary clearance, and bioavailability of volatile or gaseous compounds after peripheral intravenous dosing [11], the protein binding of volatile and gaseous compounds such as fluorocarbon aerosol propellants, halothane (fluothane), and cyclopropane [12], sterilizing agents [13,14], common organic solvents in blood [15], and irritant compounds, some of which have been used by military and law enforcement agencies [16-18]. Some of the many methods appearing in the literature will be discussed in this chapter, thus showing the applicability of gas chromatography (hence, integrated gas chromatography-mass spectrometry) to anesthetic and related areas of research.

## II. RESPIRATORY AND BLOOD GASES

The approach to respiratory and blood gas analysis is very similar to the determination of the constituents of air important in nitrogen fixation, photosynthesis, as well as respiration--namely, nitrogen, oxygen, carbon dioxide, and water vapor.

In 1958, Smith, Swinehart, and Lesnini [19] showed that gas mixtures containing nitrogen, nitrous oxide, nitric oxide, carbon monoxide, and carbon dioxide could be resolved using a column packed with two layers of silica gel separated by iodine pentoxide. A 10-ft x 1/4-in. length of copper tubing was half-filled with silica gel followed by nearly 8 in. of iodine pentoxide powder and about 1/2 in. of silver metal powder. Additional silica gel was added to fill the remaining length of tubing. At elevated temperatures, the carbon monoxide and nitric oxide were totally oxidized, to carbon dioxide [20] and nitrogen dioxide [21], respectively, by iodine pentoxide with the liberation of iodine. Using helium as carrier gas at a flow rate of 30 ml/min, a column temperature of 115°C and a thermal conductivity detector, no separation of carbon monoxide, nitric oxide, and nitrogen occurred in the first section of silica gel, but the carbon dioxide was preferentially retarded. On the other hand, nitrogen was unaffected by iodine pentoxide, but carbon monoxide, when converted to carbon dioxide, lagged behind and was thus separated. The iodine and nitrogen dioxide produced at the iodine pentoxide layer remained in the column; it was postulated that the iodine was apparently removed by the copper tube or the silver metal whereas the nitrogen dioxide was irreversibly adsorbed by the silica gel. The concentrations of nitrogen, carbon monoxide (as carbon dioxide), and carbon dioxide were established from peak height measurements while the nitric oxide was determined by difference. At these

specified GC conditions, nitrogen, carbon monoxide, nitrous oxide, and carbon dioxide had retention times of 2.8, 6.7, 9.3, and 10.3 min, respectively, but, as noted by the authors, gas samples containing both nitrous oxide and carbon dioxide could not be adequately analyzed with a column of this length. However, if either one was present alone with the other gases, the method proved satisfactory.

A charcoal adsorption column is inadequate for this type of mixture, because  $\text{CO}_2$  is completely adsorbed, and further complications arise due to the reactivity of the packing with nitric oxide, which produces some nitrogen and carbon-oxygen surface complexes [22]. Also, charcoal does not separate nitric oxide and carbon monoxide. Szulczewski and Higuchi [23] described a similar GC separation of these same gases using a dehydrated silica gel column. In their method, after the emergence of carbon monoxide, the column temperature is raised from Dry Ice temperature to room temperature for the elution of nitrous oxide and carbon dioxide. Of greater importance is the fact that nitric oxide emerges as a peak, but several disadvantages are inherent with this method: the long time required for analysis and the rather diffuse separation of the gases which have long retention times. In contrast to these mixtures, the separation of respiratory gases is less complicated because these normally contain inert materials.

For anesthetic research, Hill [1] found it convenient to divide the components to be analyzed into two groups: (1) oxygen, nitrous oxide, and carbon dioxide; and (2) ether, halothane, chloroform, and trichloroethylene. For the oxygen-nitrous oxide-carbon dioxide mixture, this could be separated by a partition column 20 ft long x 1/2 in. i.d. packed with 52-60 B.S. mesh Silocel firebrick impregnated with 20% by weight of dimethyl sulfoxide. In contrast to the majority of gases, including oxygen and nitrogen, which have insignificant solubility in dimethyl sulfoxide (DMSO), certain gases such as  $\text{SO}_2$ ,  $\text{NH}_3$ , acetylene,  $\text{CO}_2$  and  $\text{N}_2$  exhibit high DMSO solubilities. With the column maintained at  $20^\circ\text{C}$  (room temperature) and using either hydrogen or helium (the latter preferred in the operating theater to reduce explosion risks) as carrier gas at a flow rate of 30 ml/min, a mixture consisting of 3% (v/v) carbon dioxide, 20% (v/v) oxygen, and 77% (v/v) nitrous oxide can be resolved.

To separate the second group of compounds, a 2-ft x 1/2-in. i.d. column packed with 52-60 B.S. mesh Silocel firebrick coated with 15% (w/w) dinonyl phthalate was used with hydrogen as carrier gas (30 ml/min flow rate). At a  $20^\circ\text{C}$  column temperature, cyclopropane samples could be injected at 30-sec intervals to obtain its "washout" curve. Operated in this fashion, the washout curve provides a measure of the speed with which a gas or vapor is transported by the blood from the tissues to the lungs and excreted in the breath.

If the anesthetic mixture contains air, oxygen and nitrogen can be separated by gas-solid chromatography with a 12-in. column packed with Linde-type 5A molecular sieve. At a hydrogen flow rate of 60 ml/min, oxygen and nitrogen can be fully resolved in 30 sec. If used in parallel with a 6-ft-long column of 52-60 B.S. mesh Davison grade 70 silica gel (average pore diameter 140 Å), one obtains a chromatogram such as that shown in Figure 1.3 for a mixture of oxygen, carbon dioxide, and cyclopropane. The peaks observed in the chromatogram are deflected in opposite directions since the gas emerging from each of the two columns passes over a thermistor in opposite arms of the Wheatstone bridge.

Figure 1.4 results from monitoring the output of a standard Boyle-type anesthetic machine using an automatic sampling valve. Using a simple ionization chamber detector as suggested by Boer [24], Hill was able to determine nitrogen washout curves with samples injected at 10-sec intervals.

Some practical applications of gas chromatography in the analysis of respiratory gas include a method for measuring carbon monoxide diffusing capacity and lung capillary blood flow [25]. To measure diffusion capacity, the neon, oxygen, and carbon monoxide content of expired air must be analyzed; whereas lung capillary blood flow is based on concentration measurements of neon and acetylene. The analysis of fixed gases at the following concentrations,

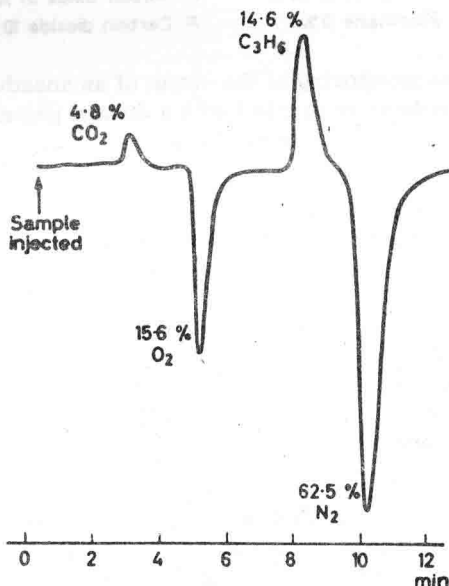


Figure 1.3. Analysis of the mixed expired gas of a dog using a molecular sieve column in parallel with a silica gel column. From Hill [1], courtesy of Butterworths.

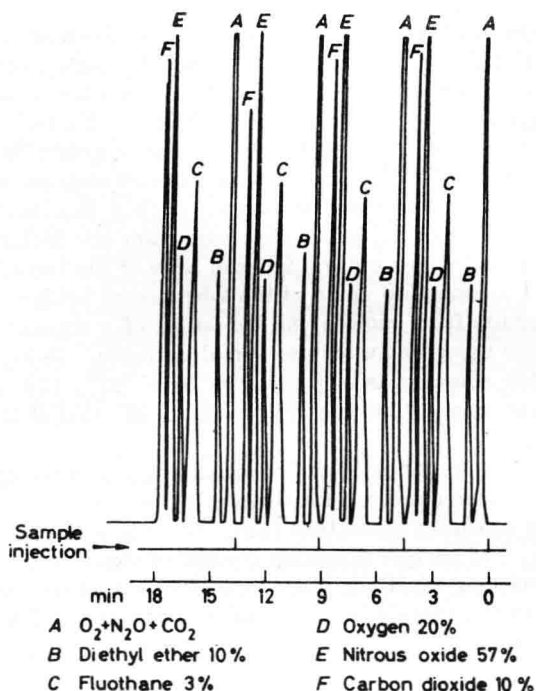


Figure 1.4. Routine monitoring of the output of an anesthetic machine. Dimethyl sulfoxide column in parallel with a dinonyl phthalate column. From Hill [1], courtesy of Butterworths.

Gases	Concentration (%)
Neon	0.1-0.30
Oxygen	20-95
Nitrogen	0-75
Carbon dioxide	0-10
Carbon monoxide	0.03-0.30
Acetylene	0.03-0.03

was performed without the necessity of temperature programming using a 4-ft x 1/2-in. SS column packed with 60-100 mesh silica gel for separation of acetylene and carbon dioxide and a 9-ft x 1/2-in. SS column packed with 40-60 mesh 5A molecular sieve for separation of neon, oxygen, nitrogen, and carbon monoxide. By appropriate positioning of a double four-way dual-column valve, the arrangement of the molecular sieve and silica gel columns

is selective; that is, it may be either in series or in parallel as indicated in Figure 1.5. A typical gas chromatogram showing separation of neon, oxygen, nitrogen, carbon dioxide, carbon monoxide, and acetylene is shown in Figure 1.6.

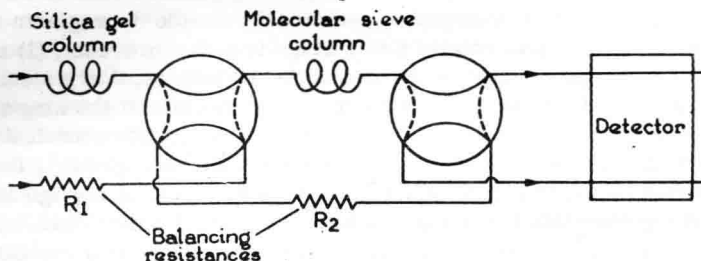


Figure 1.5. Column arrangement: 4-ft silica gel column and 9-ft molecular sieve column may be in series or parallel by appropriate positioning of double four-way dual-column valve. From De Graff [25], courtesy of Journal of Chromatographic Science.

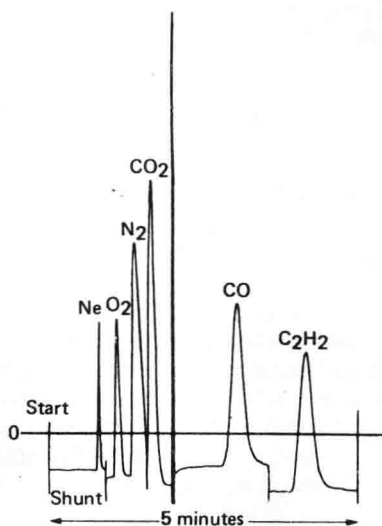


Figure 1.6. Gas chromatogram showing separation of neon, oxygen, nitrogen, carbon dioxide, carbon monoxide, and acetylene, as indicated. From De Graff [25], courtesy of Journal of Chromatographic Science.

As noted by De Graff [25], one of the major applications of gas chromatography in a cardiopulmonary laboratory is in the measurement of the diffusing capacity of the lung for carbon monoxide or, expressed another way, the transfer capacity of carbon monoxide from alveolar gas to intracorporeal red cell hemoglobin. The apparent diffusing capacity of the lung for carbon monoxide may be defined as the transfer rate of CO from alveolar air to red cell hemoglobin per mm Hg alveolo-hemoglobin carbon monoxide gradient. Essentially a two-stage transfer process [(1) the passive diffusion of carbon monoxide across the alveolo-capillary membrane where gas transfer takes place between the small units of the lung and the blood vessels in contact with these lung areas, and (2) the chemical association or combination of CO with intracorporeal hemoglobin], the pressure exerted by CO gas in combination with hemoglobin is negligible because of the strong chemical bonds formed between CO and hemoglobin. Under these conditions, the alveolo-capillary CO gradient is nearly the CO partial pressure within the alveoli. Consequently, the diffusion capacity calculation is rather simple, since only the CO partial pressure in the gas phase must be considered. As pointed out by De Graff, the apparent diffusing capacity is therefore equal to the instantaneous rate of disappearance of CO at any given time divided by the partial pressure of CO within the alveoli:

$$D_{LCO} = \frac{V_A}{P_{ACO}} \left[ \frac{dF_{ACO}}{dt} \right] \quad (1.1)$$

where

$D_{LCO}$  = apparent diffusing capacity of the lung for CO

$V_A$  = alveolar volume

$P_{ACO}$  = partial pressure of alveolar CO

$F_{ACO}$  = fractional concentration of alveolar CO

$t$  = time

The CO concentration at the start and end of breath holding is measurable, and if the differential equation is integrated, the diffusing capacity is defined by the change in fractional concentration of CO during breath holding. Since the rate of diffusion of CO across the alveolo-capillary membrane is predictably related to the  $O_2$  diffusion rate across the same membrane boundary, the resistance to CO transfer offered by the alveolo-capillary membrane and by the chemical combination of CO with hemoglobin can be assessed independently of one another by varying the concentration of inspired oxygen (the CO-hemoglobin rate of combination varying with the partial pressure of oxygen in contact with red cell hemoglobin).

For example, since alveolo-capillary membrane diffusion for CO is directly proportional to that for O<sub>2</sub>, disability resulting from impaired O<sub>2</sub> diffusion can be assessed from a knowledge of membrane-diffusing capacity for CO. In the measurement of diffusing capacity (Figs. 1.7 and 1.8) or lung blood flow, it is necessary to estimate the disappearance of one gas from the lung with reference to a second gas in the expired gas mixture which is not soluble in tissue or blood and hence does not disappear from the lung [25]. Consequently, any estimate of disappearance of reactive gas is therefore dependent on four separate gas analyses (that is, the concentration of reactive-nonreactive gases in the test mixture inspired and their concentrations in the expired gas mixture).

Because acetylene is a gas that is quite soluble and diffusible in blood, it is ideal for determining the measurement of lung capillary blood flow. As noted by De Graff, acetylene rapidly diffuses from alveolar air into the blood and traverses the capillaries lining the alveolar walls so that by the time blood leaves the capillary, virtual equilibrium has been established between the partial pressure of alveolar and blood acetylene. Since the rate of acetylene disappearance from alveolar air is a function of blood flow and Bunsen solubility of acetylene in blood (the solubility coefficient being constant and known), one is able to calculate blood flow. Since lung capillary blood flow is equal to cardiac output, its measurement is important in assessing disability of patients as a result of primary heart disease or heart disease secondary to lung disease.

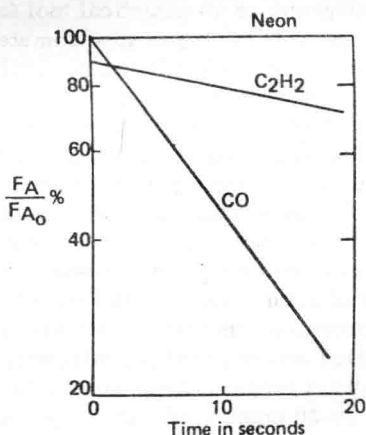


Figure 1.7. Change in lung carbon monoxide and acetylene concentration relative to neon during breath holding. Note depressed acetylene intercept indicating initial equilibration of acetylene in lung tissues. From De Graff [25], courtesy of *Journal of Chromatographic Science*.



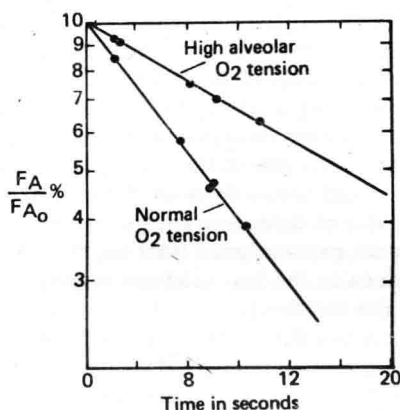


Figure 1.8. Disappearance of lung carbon monoxide relative to neon during breath holding in the presence of high and low lung partial pressures of oxygen. Membrane-diffusing capacity for carbon monoxide and lung capillary volume are calculated from the change in carbon monoxide disappearance rate resulting from altering oxygen concentration in the lung. From De Graff [25], courtesy of *Journal of Chromatographic Science*.

Jay et al. [26] and Hamilton and co-workers [27-29] also adapted the gas chromatographic technique for use in respiratory physiology. Jay et al. used the gas chromatograph as an analytical tool for measuring pulmonary functions, in contrast to the mass spectrometer and infrared analyzer used by Forster et al. [30] and Riley et al. [31] for determining the percentage composition of different mixtures of nitrogen, oxygen, carbon dioxide, helium, nitrous oxide, and carbon monoxide in the study of respiratory physiology. They noted that analytical problems are magnified spectrometrically if additional components, such as nitrous oxide or acetylene, are introduced into a gas mixture to measure pulmonary blood flow [32] and cardiac output [33]—especially when estimating pulmonary diffusing capacity [34,35] simultaneously with pulmonary capillary flow and volume. Using two sets of sample loops of different volumes to accommodate high and low gas concentrations with a Beckman GC-2 gas chromatograph, Jay et al. separated helium, oxygen, nitrogen, and carbon monoxide on a molecular sieve column prepared from copper tubing 12 ft x 1/2 in. i.d. packed tightly with 20-30 mesh Linde 5A molecular sieves. Via a switching valve assembly, nitrous oxide and carbon dioxide were analyzed by a second column (an 18-in. length of stainless steel, 1/2-in. i.d. tubing filed with equal 9-in. sections of 40-60 mesh charcoal and 20-30 mesh acid-washed firebrick). The area attributed to each component peak in the