new developments in

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New Developments in Gas Chromatography

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INTRODUCTION TO THE SERIES

The scope and even the purpose of analytical chemistry is growing so amazingly that even the dedicated scientist with time on his hands cannot follow the significant developments which appear now in every increasing numbers. Analytical chemistry has its new and wider roles and these new developments must become everyday working knowledge and be translated into practice. At present a serious time lag still exists between evolution and practice. This new venture aims to bridge the hiatus by presenting a continuing series of volumes whose chapters deal not only with significant new developments in ideas and techniques, but also with critical evaluations and the present status of important, but more classical, methods and approaches. The chapters will be contributed by outstanding workers having intimate knowledge and experience with their subject.

It is the hope and belief that *Advances in Analytical Chemistry and Instrumentation* will offer a new medium for the exchange of ideas and will help assist effective, fruitful communication between the various disciplines of analytical chemistry.

Additionally, articles of this series will discuss developments in other fields, such as biology, physics, electronics, and mathematics, which are within the scope of analytical chemistry in this broader context. These would include, for example, applications of kinetics, isotopic tracers, computers, and modern physical tools to studies of complex molecules, short-lived species, ultraclean systems, continuous plant streams, and living organisms.

These volumes contain articles covering a variety of topics presented from the standpoint of the nonspecialist but retaining a scholarly level of treatment. Although a reasonably complete review of recent developments is given, a dry and terse cataloguing of the literature without description or evaluation is avoided. The scope of the *Advances* is flexible and broad, hoping to be of service to the modern analytical chemist whose profession each day demands broader perspectives and solution of problems with increased complexity. The periodical literature is inherently specialized and the appearance of suitable monographs takes place only after many years. Reviews are frequently directed to the specialist and often lack adequate description or evaluation. *Advances* hope to fill in the resulting need for critical comprehensive articles surveying various topics on a high level satisfying the specialist and nonspecialist alike. Comments and suggestions from readers are heartily welcome.

THE EDITORS

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PREFACE

For those of us who have been involved in gas chromatography more or less since its inception, it is a chastening thought that our once-exciting infant has come of age. Each month, the journals of the world used to contain accounts of dramatic new developments in theory and technique. These days are far behind us now, and progress is more placid, with a staidness perhaps more befitting to a technique entered into adulthood. Nevertheless, it is salutory to think that, for the newest class of college graduates, gas chromatography always was.

Five years ago in an earlier volume in this series, I described my editorial approach as an attempt to find a balance between established areas of work deserving of review and topics that would be likely to develop. This volume is constructed on the same basis; it must be confessed, however, that it now proves difficult to visualize more than a few areas on the analytical side where great advance can be hoped for in the short term. The most striking growth in gas chromatographic application in recent years—and, it may be anticipated, for the immediate future—is on the nonanalytical applications side. For this reason, such topics take up a major part of this book.

As before, I have been fortunate in securing the services of authors who have both the authority and distinction to make the editor's work minimal. Again, what success this volume may enjoy is theirs.

HOWARD PURNELL

Swansea, Wales
January 1973

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Forensic Applications of Gas Chromatography

D. A. PATTERSON, *Home Office Central Research Establishment, Aldermaston, Reading, Berkshire, England*

I. INTRODUCTION

A eulogy of the gas chromatographic technique would not be within the scope of this chapter; but it would also be improper if the impact that its development has made on forensic science were not adequately acknowledged. First used in forensic science in 1955(1), gas chromatography is now employed in more analyses, numerically, than any other technique. Moreover, it is doubtful whether any analytical procedure has applicability to such a wide range of problems.

In the United Kingdom, when the Road Safety Act (1967) brought British law into line with that of a number of other countries, it became an offense to drive with more than the prescribed level of alcohol in the blood. Gas chromatography was adopted then as the standard method of analysis, and by 1970 more than 30,000 samples were being analyzed annually. But apart from this routine application, the inherent sensitivity, specificity, and versatility of the technique make it an indispensable part of the analytical armory of the forensic scientist, and it finds applications in such diverse fields as toxicology, drug abuse, and arson investigation, and in the identification of paints and fibers.

A review such as this cannot be comprehensive, but it is hoped that by reference to the works cited here, an analyst interested in a particular aspect will be able, at least indirectly, to trace the material or method that is relevant to his requirements.

II. CARBON MONOXIDE

Poisoning by carbon monoxide must have presented a problem for the toxicologist since man discovered fire, and a vast amount of literature has appeared on the subject. Generally, methods for its determination are divided into those for the gas itself and those for carboxyhemoglobin. Within these divisions, physical, chemical, and biochemical methods have been described and admirable reviews are available (2, 3).

In the majority of cases, the forensic scientist is required to measure the amount of carboxyhemoglobin in a blood sample; if the blood is fresh and uncontaminated, a spectrophotometric method such as that described by Kampen and Klouwen (4) is eminently satisfactory. During the process of putrefaction, however, products that interfere with spectrophotometric procedures are formed, and a method allowing the specific estimation of carbon monoxide in the sample is necessary. The use of gas chromatography, together with subsidiary determinations for hemoglobin and iron (to calculate the total hemoglobin content) gives more reliable results in these cases (3,5–7, 11).

Usually, carbon monoxide is liberated from the blood sample by addition of potassium ferricyanide plus a detergent, although the use of sulfuric, hydrochloric, or lactic acid has also been reported (5, 8). A mixture containing 2.5% potassium ferricyanide, Teepol L or Triton X-100, and buffered to pH 9, liberates all carbon monoxide from a 1-ml blood sample within 10 minutes (3). The liberated gas is then transferred by way of a gas loop and sampling valve to the column. Blackmore (3) used a stainless steel column, 5 ft long and packed with 60-80 mesh molecular

sieve 5A from which the fines had been removed, whereas Goldbaum et al. (5, 11) used the same material in the form of 1/16-in pellets in a 2-m column which was run at 75 to 100°C, with helium as the carrier gas. Detection was by means of a katharometer and a typical chromatogram (3) is shown in Figure 1. Dominguez and his co-workers (9) and McCredie and Jose (7) have also described procedures using aluminosilicate sieves.

An alternative, indirect method of detection is provided by the incorporation of a nickel-catalyzed reduction stage; here a flame ionization detector serves to detect the resulting methane. This system is more sensitive and has been used (6, 10, 12) for accurate measurements of the carbon monoxide content of the blood of smokers. Less than 100 μl of blood suffices for the analysis.

For purposes of calibration, investigators commonly use both pure carbon monoxide gas and commercially available gas mixtures, as well as prepared mixtures of fully carboxylated and fully oxygenated blood

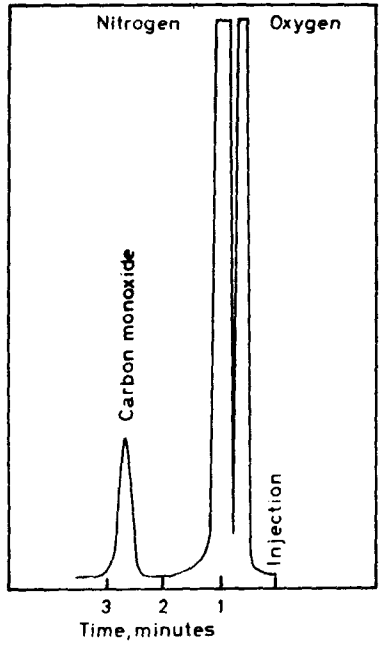

Fig. 1. Gas chromatogram (3) of a mixture of air and carbon monoxide from blood containing carboxyhemoglobin.

By courtesy of *Analyst*.

samples, peak height measurements being generally considered to give sufficient precision. Great care is necessary if mixed blood samples are to be used for calibration; for it has been shown (3) that blood stored at 20°C can lose 25% of its carbon monoxide combining power within a seven-week period. Treatment of fresh blood, with sodium dithionite, followed by carboxylation with pure carbon monoxide in a Lerquin apparatus and then flushing with oxygen-free nitrogen for 10 min, gives a 100% carboxylated sample. Figure 2 demonstrates the effect of sodium dithionite in achieving full carboxylation of fresh and stored blood.

III. VOLATILE SOLVENTS AND ANESTHETICS

The need to analyze for volatile low-molecular-weight compounds most commonly arises when deaths occur during general anesthesia or in connection with suicides involving the ingestion of household cleaners. Accidental deaths from "glue-sniffing" and from the injudicious use of cleaning solvents are not uncommon. Various methods for introducing these volatile compounds to the gas chromatograph have been recommended. Generally, a preliminary separation procedure, such as solvent extraction or steam distillation, is followed by injection; some workers, however, advocate direct injection of blood or urine samples (16). Bonnichsen and Maehly, in very comprehensive reports of poisonings by aromatic hydrocarbons (13) and chlorinated aliphatic hydrocarbons (14), favored preliminary steam distillation of biological samples, the steam distillate

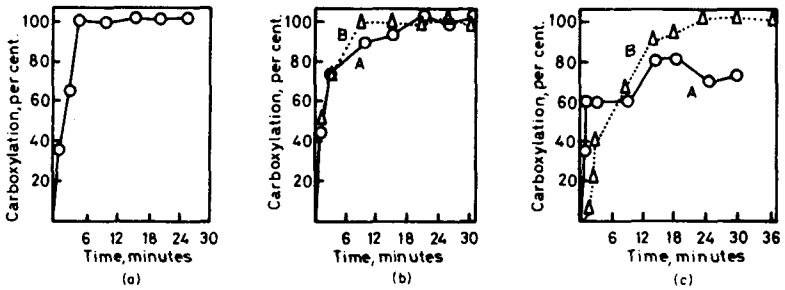

Fig. 2. Data of Blackmore (3) giving: (*a*) time required to carboxylate a sample of fresh blood diluted 1 : 4 with distilled water; (*b*) time required to carboxylate whole blood without *A* and with *B*, sodium dithionite; (*c*) time required to carboxylate a sample of blood stored at 37°C for 11 days and diluted with water before gassing: curve *A*, whole blood; curve *B*, whole blood and sodium dithionite.

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being absorbed on to ammonium sulfate in a flask that is subsequently closed with a rubber cap. A headspace sample is then injected on to the column.

When the presence of chlorinated hydrocarbons is suspected, the very sensitive Fujiwara reaction can be used as a qualitative screening test prior to chromatography. Bonnichsen and Maehly (13) stressed the danger in relying too heavily on this test, however, and pointed out that a number of compounds give negative reactions, including hexachloroethane, ethyl chloride, monochloroethylene, *sym*-dichloroethylene, and monochloroethanol. Trichloroethanol, the metabolite of both trichloroethylene and chloral hydrate, gives a weak response.

Bonnichsen and Maehly used a 6-ft, 20% Apiezon column both for aliphatic hydrocarbons (125°C), and for aromatic hydrocarbons (190°C). A 2.5-m, 25% Carbowax 1540 column was used, in addition, for confirmation of the identity of the aliphatic chlorinated hydrocarbons. Retention data were given for benzene, toluene, *o*-, *m*-, and *p*-xylenes, indene, naphthalene, methylene chloride, chloroform, dichloroethane, carbon tetrachloride, trichloroethylene, and perchloroethylene. Analyses for carbon tetrachloride and methylene chloride in accidental poisoning cases have also been the subjects of monographs by other workers (17, 18), and Nelms and his co-workers (19) have demonstrated the presence of hydrocarbons in blood and tissue in two cases of gasoline poisoning. Hall and Hine (20) have reported two cases of trichloroethane intoxication.

Recently (14), a scheme of analysis based upon the gas chromatographic technique has been described for the identification of almost 600 drugs and metabolites in human viscera; of these, a large number are volatile, low-molecular-weight compounds. A solvent extraction procedure in the initial stages is followed by analysis with seven gas chromatographic systems. Confirmation of structure is by infrared spectrophotometry and by mass spectrometry.

Special attention has been paid (15, 16) to the identification of aerosol propellants. These are composed of hydrocarbons, chlorinated hydrocarbons, fluorinated hydrocarbons, and inert inorganic gases; mixed propellants are commonly encountered. In one study (15), a 22-ft column packed with 20% silicone grease on firebrick was used; the column, which was at ambient temperature, allowed separation of eight common components in 15 min. Carbon dioxide and nitrous oxide were not resolved under these conditions, however, and where either gas was present, it was necessary to use an alternative, 6-ft porous polymer (Poropak Q) column for identification.

Fluorinated hydrocarbons, often present as propellants in pressurized aerosols used in the treatment of asthma, have been implicated in sudden

deaths of patients using them. Blood levels of the fluorocarbons in volunteers inhaling from placebo inhalers, have been measured (16), analysis being conducted with a 7-ft, 20% Carbowax 20M column at 70°C, in conjunction with an electron capture detector. Maximum blood concentrations of 1.7 μg/ml were observed. However, it is the author's opinion that whether the fluorocarbons are directly or indirectly causative of the cardiac crises in cases of this type has not been unequivocally established.

Of the volatile sedatives and general anesthetics, paraldehyde (21), ethchlorvynol (21, 22), ether (23), and halothane (24–26) have received most attention. In one report (26), in which a rapid, sensitive method for determination of halothane in blood was described, it was claimed that it was possible to monitor the halothane blood levels during the course of anesthesia. If this were indeed possible, the procedure could become a valuable adjunct to surgical techniques.

IV. ETHANOL

A number of comprehensive reviews of the early literature on alcohol analysis have appeared (27–30), although of necessity these described gas chromatographic methods only in passing. More recently, Walls and Brownlie (31) have dealt with alcohol and drugs in relation to driving and driving offenses, and this book, as an introduction and background to the subject, provides excellent reading.

The first report of the gas chromatographic separation of alcohols appeared in 1956 (32); but it was almost ten years before the method became generally accepted as the method of choice for the quantitative determination of ethanol. Like most other forensic science laboratories, the Government Laboratory in Stockholm, until 1962, had used a chemical as well as an enzymatic method for the determination of alcohol in blood and in urine; in some cases, large discrepancies had been noted between the results obtained by the two methods. For example, by use of a mixed polyethyleneglycol–polypropyleneglycol column in a "gas fractometer" equipped with a flame ionization detector and by direct injection of urine, the presence of methanol, acetone, and acetaldehyde, as well as ethanol, was detected in the samples; but the amounts recorded differed (33). Calculating from direct comparison of peak heights, it was found that in all cases the results obtained from the gas chromatographic determination of ethanol agreed more closely with those obtained from the enzymatic method than did those obtained by the chemical method. However, this technique was not applicable to blood samples and, from the forensic point of view, determination of the ethanol content of blood was considered to

be more desirable, since this value was more likely to give a true reflection of the state of intoxication of the individual.

Subsequently, several approaches to the problem were adopted. These were: (*a*) direct injection of blood into the gas chromatographic column (34–36), (*b*) azeotropic distillation of the blood prior to chromatography (37), (*c*) preliminary separation of the ethanol by a solvent extraction procedure (38, 42), and (*d*) injection of head space samples (39–41). It was, however, the procedure described in 1966 by Curry, Walker, and Simpson (36) that first made it possible to perform the analysis with sufficient precision, accuracy, and speed that it became accepted for routine use in the United Kingdom. Furthermore, the procedure was applicable to small blood samples, in accordance with a later requirement of the Road Safety Act. The analysis was at 85°C with a 5-ft, 10% PEG 400 column connected to a flame ionization detector feeding an integrator. The blood (ca. 20 μl) was diluted with ten times its volume of aqueous *n*-propanol solution using a Griffin and George type 221 hemoglobin-type diluspence, the propanol serving as internal standard. Approximately 1 μl of the diluted blood sample was then injected directly into the column, and ratios of peak areas of ethanol to propanol were calculated. Calibration was achieved by using blood samples containing known amounts of ethanol and plotting the ratio of ethanol to propanol pulses from the integrator against the concentration of ethanol in the blood. The graph obtained was independent of the volume of blood injected; when 20 injections of the same solution were made (250 mg/100 ml), the error in the determined ethanol-to-propanol ratio had a standard deviation of less than 0.75, expressed as a percentage of the mean value. Chromatographic separation was complete in 4.5 min, and ether, acetaldehyde, acetone, methanol, and higher alcohols were all easily resolved from ethanol.

The foregoing procedure has been outlined in some detail because of its known excellence and also because it has been used widely without modification for more than five years now—a rare achievement in these days of rapid "progress." Figure 3 shows a typical chromatogram obtained using the procedure. If the method has a weakness, it is that isopropanol (which is sometimes used for swabbing the skin in the area from which blood is to be taken) is not completely resolved from ethanol by the PEG column. This difficulty is overcome in practice by duplication of the analysis using a column containing Poropak Q at 185°C when these two alcohols are easily resolved. Although the procedure described is based on direct injection of a diluted blood sample, contamination of the column is not a great problem. Some tailing of peaks occurs after about 1000 injections, but resolution can be restored by repacking the first few inches of the column. Even this necessity can be obviated, however, by choice of a

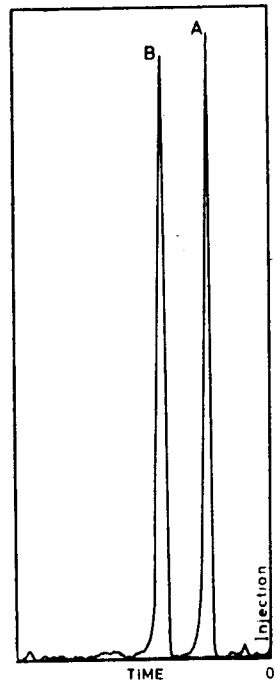

Fig. 3. Gas chromatogram of blood containing ethanol following procedure of Curry, Walker, and Simpson: curve *A*, ethanol; curve *B*, *n*-propanol.

gas chromatograph that allows the use of a glass insert near to the inlet end of the column. The blood is deposited within the insert, which can be replaced easily when necessary.

Adequate storage of blood samples pending analysis is of great importance, for it has been demonstrated (43) that many organisms commonly found in or on the body during life, or following post mortem contamination, are capable of producing significant quantities of ethanol from carbohydrate or tissue. In addition, of course, measures are necessary to prevent clotting of the samples, particularly if a direct injection procedure is to be used. In practice, stability and fluidity of the blood are readily achieved by addition of sodium fluoride and potassium oxalate, respectively, to the sample. In post mortem cases, bacterial formation of ethanol could well be underway by the time the blood sample is taken; indeed, it is

very doubtful whether any interpretation at all can be placed on a finding of ethanol in a single blood sample from such cases. It is perhaps reassuring to note, however, that under laboratory conditions where ethanol is known to have been produced by bacteria, *n*-butyric acid and isobutyric acid have been consistently detected also (43). Since these are resolved from one another and from ethanol on the Poropak Q column and are not normally detected in fresh samples, a good indication of bacterial action can be obtained. As a result of his investigations into the bacterial production of ethyl alcohol, Blackmore (43) suggested the following very useful analytical sequence for the determination of ethanol in post mortem samples.

1. All analyses should be undertaken by gas chromatography using two columns working in dissimilar operating conditions.
2. Urine from an intact bladder is the fluid of choice, since ethanol is unlikely to be produced by its bacterial contamination.
3. If urine is not available, blood sampling should be from left and right heart and one other peripheral source. The samples should be immediately preserved, and the presence of *n*-butyric acid or isobutyric acid should render the analysis suspect.
4. In the absence of urine and blood, muscle should be taken from three peripheral sites and stored at 0°C before homogenization with distilled water and treatment in a similar manner to blood.

Current aims of those concerned with blood alcohol analyses are in the development of a fully automated procedure, although, so far as the author is aware, this has not yet been achieved. The development of the Perkin-Elmer Multifract F40 Gas Chromatograph represents a significant advance in this direction, however. Using this apparatus it is still necessary to perform the dilution of the blood manually, but thereafter, up to 30 samples can be analyzed without attention at the rate of 10 to 15 samples per hour. Accuracy and reproducibility of results are comparable to those obtained using the method of Curry, Walker, and Simpson.

The F40 comprises a thermostatted sampling turntable, which holds the samples (previously prepared for analysis) in vials closed with a rubber septum cap. When equilibrium has been reached, samples of vapor above the diluted blood are withdrawn and successively injected into the column by means of an electropneumatic device. Each stage of the analysis can be programmed in advance; automated baseline correction is incorporated, and the chart is marked during each analysis to allow identification of samples that have been analyzed.

In some areas of the world, evidence of breath alcohol levels is per-

missible in drunk driving cases, and at least one device based on gas chromatography of a breath sample is available (44). The subject can blow directly into the instrument and analysis is claimed to be rapid and accurate. The major disadvantage of such an approach, however, appears to lie in the difficulties involved in the efficient collection and storage, for further confirmatory analysis in the laboratory, of additional samples.

Finally, in connection with ethanol, inquiries into the illicit production of alcoholic beverages should be mentioned. Comparative analyses are usually required, and both inorganic and organic congeners can be investigated. Of the latter, acids, esters, and higher alcohols, which are co-products of the fermentation process, have been shown to be present in different beverages in varying proportions (45–47), and whiskies, gins, rums, and brandies have been characterized by gas chromatography of these compounds (46, 48).

V. DRUGS

The literature dealing with gas chromatographic separation and identification of drugs is now voluminous, and the forensic scientist has at least a potential use for a large proportion of this material. Fortunately, Gudzinowicz has comprehensively reviewed the subject to 1967, and other workers (50, 51) have abstracted methods which they consider to be particularly applicable in the forensic context. Of the several thousand drug preparations available, it is possible to analyze for a very large proportion by gas chromatography; but whether this is necessarily the technique of choice in the routine laboratories is another matter. Aspirin and lysergic acid diethylamide (LSD), for example, are substances for which gas chromatographic methods have been described; in practice, however, other methods are usually more convenient. In view of this, no attempt has been made here to give comprehensive coverage; rather, selected classes of substances have been chosen for which the gas chromatographic approach has a particular advantage.

Identification may be required of the pure drug itself, of the drug compounded into dosage form, or of the drug in a body fluid taken from a live patient or from the organs and body fluids taken post mortem. In difficult cases, the process of putrefaction may be underway or even well advanced while, to complicate the picture further, there is sometimes strong circumstantial evidence that a particular drug has been ingested, whereas in other cases a general "screen" for drugs is required. Usually, a preliminary solvent extraction procedure is necessary prior to

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