GAS CHROMATOGRAPHIC STUDIES
OF DRUGS OF ABUSE,

by

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INTRODUCTION

The excessive use of drugs is not peculiar to our time and our society. Suffering, lonely, or bewildered man has long sought to ease his pain or grief or to find meaning in his life by ingesting plant or animal substances which alter his consciousness and his perception of his environment.

Some drugs have been known to man for centuries for example those of natural origin like opiates and other hallucinogens. However the addiction problem did not appear until the eighteenth century when an epidemic of opium smoking spread throughout China under the influence of profiteers from the West. In modern times many synthetic drugs have aggravated this problem.

A drug is defined as a chemical compound capable of producing in a man a predictable series of physiological, biochemical, and behavioral changes (1). Addiction is a state of periodic or chronic intoxication produced by natural or synthetic drugs. Addictive drugs are harmful both to the individual and society. Habituation, on the other hand, is a state of psychological dependence upon the rewarding or pleasurable
aspects of drug use. Drugs causing habituation can impair the individual, but rarely have harmful effects upon society.

**Classification of Drugs**

There are many types of drug dependence, but they can be categorized physiologically in the following manner.

**Narcotics.** Dependence upon drugs of this generic type can be initiated by a therapeutic dose. An intense craving is usually associated with morphine type dependence, together with a need to rapidly increase the dose. Drugs of this type include: morphine, opiates, heroin, codeine, and the synthetic compounds like dilaudid and demerol. Addiction to this type of drugs may develop in two or three weeks.

**Depressants.** Barbiturates and alcohol are very similar in their physiological effects and their depressant action upon the central nervous system. A pleasant, euphoric feeling is achieved but at the expense of slurred speech, difficulty in walking, mental confusion, and emotional lability. Addiction to the barbiturates is the single, most serious form of drug abuse (1).
**Stimulants.** Among drugs of this type we find cocaine and amphetamines. Cocaine induces a state of ecstatic excitement with feelings of power and invulnerability. The over assessment of physical strength when combined with auditory or visual hallucinations may elicit dangerous assaultive and even homicidal impulses. No physical dependence is developed.

Amphetamines ("speed", or "pep pills") are widely used as stimulants and as appetite depressants. Their easy availability contributes to their enormous abuse, perpetuated by the elation and sense of well being they produce. This is followed, however, by a period of depression and fatigue when the dose is exhausted. Prolonged administration also results in disturbances in behavior such as irritability and aggressiveness. Amphetamines are not addictive although increasing doses are required to bring about the desired effect.

**Hallucinogens.** Marihuana is a mild hallucinogen; it alters perception and induces visual hallucinations. Smoking it evokes a feeling of reflective calm, facilitates social communication, and eases anxiety, with a distortion of the sense of space and time. There is also a disturbance of judgement and impairment in memory and coordination. Marihuana is habituating but not addictive.
Lysergic acid diethylamide (LSD) is among the most powerful hallucinogens. The ability of the brain to process sensory input is grossly disturbed by LSD, particularly the visual system. Colors become extraordinarily vivid, and there is a sense of union with the surroundings. However, suicidal behavior, impaired judgement, psychosis, panic and biological changes which might predispose one to malignancy make LSD an extraordinarily dangerous drug. Other hallucinogens are psilocybin and mescaline. These drugs are not addicting although some users become psychologically dependent upon them.

**Extent of Drug Dependence**

The extent and intensity of drug use is difficult to determine. There are in this country perhaps some 50,000 to 100,000 opiate addicts.

Without considering the 80 million social drinkers, there are 6 million alcoholics and the number of barbiturate users is estimated to be 10 million. There may in fact be no more than 200,000 true barbiturate addicts. Cocaine abuse is fairly limited. Amphetamines are widely abused and no good estimate of the number of users exists.

Hallucinogens are the most intriguing and satisfying type of drugs. Between 2 to 4 million Americans have had a marihuana experience. LSD was widely abused
between 1963-1964; now its use is considerably reduced.

Analytical Problems

Drug analysis at the present time is involved with the following subject areas:

1) Methadone clinic patients
2) New industrial personnel
3) Military personnel
4) Street-arrested users
5) Comatose/overdose patients
6) Autopsy subjects

All of these areas may require different analytical techniques. Although many methods, including thin layer chromatography, gas chromatography, ultraviolet and visible spectroscopy, and gas chromatography-mass spectrometry are available for the detection of large samples of drugs, no single method meets all of the requirements for analysis of drugs in serum or urine samples.

These requirements include:

1) Specificity. Ideally the analytical method would be so specific that little sample handling is required. In practice the drugs must be separated and concentrated by groups (narcotics, barbiturates, amphet-
amines, etc.) and then individually identified. In some legal situations, three independent physical methods of analysis are required for identification.

2) Rapidity of analysis. The entire procedure from the acquisition of the biological sample to recording of the results should not exceed 24 hours. In some cases analysis times of one hour or quicker are required.

3) Sensitivity. The method must be able to detect the presence of the administered drug or its metabolites in biological material for at least 24 to 48 hours following drug usage.

4) Simplicity. The method should not require skills or training beyond that normally achieved by a laboratory technician.

5) Cost. The cost must be kept to a minimum and therefore all procedures, capital equipment, depreciation, supplies and labor must be viewed from the cost per sample point of view.

The analytical problems involved in drug analysis are more complicated than they appear to be at first sight (2). Part of the confusion is due to a poor definition of the problem. The analyst is in the difficult position of analyzing blood and urine samples for drugs and narcotics and must report or interpret the results
to nurses, physicians, policemen and judges who do not appreciate the limitations or validity of chemical methods. The analyst cannot develop a method, much less perform a useful analysis and give a professional opinion without a rational and explicit purpose to each analysis.

The following information must be considered before an analytical scheme can be developed.

1) Drugs to be detected
2) Sensitivity limits
3) Sample type and size
4) Precision required
5) Intended use of the results
6) Costs
7) Available laboratory equipment and personnel

Although a great number of drugs can be abused, as described by B. S. Finkle (3, 10) a surprisingly small number will account for over 75% of all requested analyses, and less than 20 drugs will cover practically 100% of all cases.

Reliable and meaningful results can be obtained on biological samples for the following common drugs: morphine, codeine, methadone, cocaine, amphetamine, methamphetamine, amobarbital, pentobarbital, secobarbital, phenobarbital. Intensive research is being conducted, but at the present time there are no analytical
means to detect LSD or marihuana in biological fluids.

Minimum detectable quantities are not well defined. They are a complex function of sample size available, concentration technique, sensitivity of the analytical method and the interferences from other compounds present.

It is not possible to talk about sensitivity limits without discussing the sample size and type. Urine is the sample of preference because it is easier to obtain than blood, larger quantities are available, and current extraction and concentration procedures are better suited for urine samples (3).

Without a knowledge of interfering substances and a clear analytical appreciation of other therapeutic agents likely to be present, and the ability to recognize urine metabolic profiles of some drugs, the analyst will be unable to obtain and interpret meaningful results.

The quality of the analytical results will vary for each laboratory and will be conditioned by the intended use of the results. For example quantitative analysis and unequivocal qualitative specificity, is often mandatory in forensic work, but it is not necessary in surveillance or employment screening programs. Similarly, programs monitoring methadone clinic patients and analysis of samples for emergency medical diagnosis of overdosed
patients rarely require quantitative work.

Most of the analytical methods that have been developed are for drug screening programs where simple "present" or "absent" answers are sufficient. Unfortunately the reliability of many of these methods are so poor as to question their over-all usefulness.

Analytical Methods

The techniques used for the determination and identification of drugs are essentially those routinely used in analytical chemistry for the characterization of chemical structure. Unfortunately, many of these techniques are effective only with pure materials. This is seldom the situation with drugs or drug metabolites. Although many analytical techniques are available, none fulfill the requirements of all the various groups demanding drug analysis.

At the present time, there are no standard methods for drug analysis. Some methods, however, have become widely recognized. These include thin layer chromatography, gas chromatography, fluorescence and gas chromatography/mass spectrometry. Less common, but still useful methods include: nuclear magnetic resonance (4); infra-red spectroscopy (5); electron spin resonance spectroscopy (FRAT) (6); and liquid chromatography (7).
Most current methods for analysis of body fluids involve an extraction and concentration step followed by thin layer chromatography (TLC). TLC is a rapid and convenient method for screening large numbers of samples. Positives are confirmed by gas chromatography, or less commonly by gas chromatography/mass spectrometry (GC/MS) (8, 9) or electron spin resonance spectroscopy (6).

Several extraction procedures have been used:

1) Extraction with ion-exchange paper (10)
2) Extraction in a charcoal column (11-12)
3) Extraction from Florisil (13)
4) Extraction with a nonionic resin (14, 15)
5) Selective solvent extraction at different pH (2, 16, 17)
6) Solvent extraction at only one pH (18, 19)

The most popular and most promising extraction procedures are the last three.

The use of a short column, with a nonionic resin (Rohm and Haas, XAD-2) to extract drugs is perhaps the most popular method at the present time. Good recoveries for many drugs have been reported (20) and there is no emulsion formation, a problem frequently encountered in solvent-extraction techniques. The extraction and
recovery efficiency is, however, dependent upon column preparation, solvent flow, solvent composition, and urine pH (15). Recently several modifications and improvements to this extraction technique were reported (21).

Solvent extraction at three different pH values is time consuming, but has been used with success (16): pH 2--4 for barbiturates; pH 9 for alkaloids and pH 11 for amphetamines. Solvent extraction has greater selectivity and greater recovery efficiencies than the resin adsorption column. The extraction technique at a single pH of 9.6 is perhaps the best approach for a screening program; reasonable recoveries have been reported (15) and the procedure is simple and rapid.

Thin Layer Chromatography

Most frequently TLC plates with a 250 micron layer of silica gel are used. Many solvent systems have been reported (2, 22); those reported by Davidow (19) and Cochran and Daly (20) are, however, the most common.

TLC provides good separation of the basic drugs in less than an hour. The barbiturates, however, cannot be separated on the same plate. An aliquot of the urine extract must be run on a separate plate under different conditions.

Spray reagent systems are numerous, and in some cases
sequential spraying is performed to improve the specificity of the test (23). Recognition of TLC patterns of commonly occurring metabolites, such as those from methadone (sometimes appearing without the parent drug), chloroquin (an antimalarial drug), phenothiazines and nicotine is mandatory for a correct evaluation of the plate. Operator skill and experience is required in TLC analysis of drugs. TLC is the preferred screening technique because it is cheap; it does not require specialized skills and it is well suited for mass screening. Some of its disadvantages include lack of specificity, lack of sensitivity, non-quantitative results, and difficulty in automating.

The powers of TLC do not lie in identification, but rather in separation. It is a method for rapidly and economically indicating those samples which are negative. It also lends itself well to processing large numbers of samples simultaneously.

**Gas Chromatography**

Gas liquid chromatography is currently used as a confirmatory technique however, some labs have adopted it for routine mass screening. As this thesis will show it is a rapid, sensitive technique with high resolution. It does require expensive instrumentation, skilled opera-
tors and care and patience in obtaining quantitative results.

GC/MS offers the best available method for unequivocal identification of drugs and their metabolites. Reference spectra are now published (7) which make identification easier. GC/MS is the only technique which will avoid false positive results so socially damaging to the life of the subject. Unfortunately the cost and operator skill demanded for GC/MS systems limits it to a small number of laboratories.

Quantitative Analysis

Quantitative analysis is rarely needed for mass screening and should be discouraged unless a need for quantitative results is demonstrated. Drug concentrations measured in biological samples cannot be correlated with the dosage ingested. The only unequivocal interpretation is that the subject did, in fact, ingest the drug.

Whereas drug concentrations in urine are frequently higher than therapeutic levels, it is impossible to correlate urine levels with original dosages.

Quality control and proficiency tests by means of blind samples containing drugs at realistic levels is
mandatory. Finally we should understand that accurate analysis of trace materials in complex matrices like urine or blood is very difficult and in any laboratory processing large numbers of samples will make mistakes.
HISTORICAL REVIEW

Very few reports discuss the problems commonly encountered when applying GC to drug analysis (29, 35, 36, 37, 39), and although some reports mention the analysis of numerous drugs (25, 26, 42) none gives a critical discussion of GC capabilities and limitations. In this section a short critical review of GC applications is included followed by a review of the work previously done on the three major types of drugs: barbiturates, alkaloids, and amphetamines.

Gas Chromatography Applications

Gas chromatography has become a cornerstone in many laboratories because it offers excellent resolution of many drugs together with high sensitivity and high accuracy. Sensitivity to submicrogram amounts of materials and the ability to separate drugs and drug metabolites from a matrix of biological origin have made the technique almost indispensable.

It is extremely important that the limitations of gas chromatography be appreciated. Qualitative analysis by gas chromatography depends upon the comparison of retention times of standards and unknowns. Retention times are influenced by many factors including column
temperature, flow rate, sample size, age of the column, temperature, and even in some cases the presence of other compounds in the sample. Sensitivity of GC detectors under favorable conditions can be for nanogram, even picogram quantities of matter. However, on the other hand, injection of microgram quantities can result in no peaks, due to reasons too numerous to mention here. These limitations need to be appreciated by persons using or considering the use of GC.

As early as 1961 the first applications of GC to toxicology were reported (24-25). Specialized studies on barbiturates (33-34), amphetamines (26-28), and alkaloids (26-29) followed rapidly thereafter. However, sensitivity and reliability were poor, mainly due to poor column technology.

Even in these early days several attempts were made to extend the use of GC to qualitative analysis by means of specialized techniques, like "peak shift" used for alkaloids, morphine and codeine (30); formation of derivatives for barbiturates (31); and "on-column" acid-base reactions for determination of free amphetamines (28). Later more "on-column" reactions were reported for barbiturates (35).

The low concentration of drugs and the complex biological matrix make the use of the flame ionization de-
tector (FID) mandatory. Some reports of the more selective electron capture (EC) detector are found (32). Recently a sophisticated approach to GC identification of drugs was reported using a dual column system (12). This means the injection of a single sample into a common injection port; here the sample is split and passed into two different columns containing different liquid phases. The retention times ratio is used as a qualitative aid for identification.

With the advances achieved in recent years in the areas of column technology, detector and electronics design, the detection limits and column efficiencies are much better than there were six or seven years ago. At the present time all glass systems with low dead volumes, highly improved non-adsorbent solid supports, and high temperature, stable, pure liquid phases as well as advanced design FID detectors are used in the analysis of drugs.

In the early reports there was a common problem: a non-quantitative relationship between detector response and sample size injected, even for quantities as large as ten micrograms (33). This problem arises from irreversible adsorption of sample onto the column packing.

In general the resolution of drugs has not been a problem, but in some specific cases the resolution of one
or two closely related compounds has been always difficult (27). Amobarbital, pentobarbital and secobarbital are good examples of closely related drugs difficult to separate by GC.

Solvent peak tailing is a problem when working at very low levels because high detector sensitivities must be used. This increases the solvent front signal and this makes detection and quantitation of trace quantities difficult. Solvent free injection has been used to overcome this problem (12).

Reviewing some of the early chromatograms, analyses were frequently in excess of 20 or 30 minutes (27, 28, 30, 31). These long times result in most cases from improper column technology and column selection. As the retention time increases the peaks become flatter and smaller even to the point where it is impossible to distinguish the drug peak from the base line. In order to increase sensitivity, the analysis time should be kept to a minimum. This demands very efficient columns usually of small diameter tubing with small amounts of liquid phase (1 - 3% by weight) and fast flow rates.

Only a few reports discuss the frequently encountered
problems in GC (35, 36, 37). These are principally:

1) Adsorption and or decomposition on the solid support or column tubing

2) Lack of resolution for some closely related drugs

3) Interferences due to the solvent peak tail

4) Excessive analysis time

5) Inadequate sensitivity

6) Interferences due to drug metabolites and other substances

7) Column contamination and lifetime

Adsorption on the solid support surface is the main problem encountered. The interested reader is referred to an excellent reference describing the surface properties of common solid supports (38). Deactivation methods are now commonly used to reduce the adsorption problem (39). Nevertheless the problem remains important, particularly for strongly basic drugs like amphetamines, which require a special deactivation of the solid support (38-39).

The tubing material can create adsorption problems with some active drugs such as morphine, amphetamines and barbiturates. Only "inert" materials like stainless steel or glass are recommended for drug analysis. A special deactivation process for stainless steel tubing has been reported (36).
Inadequate sensitivity can result from both detector and column problems. High sensitivity ionization detectors are required for biological samples; the thermal conductivity detector is not sensitive enough to be useful. The FID is used almost exclusively, but it is essential to optimize the hydrogen, air and carrier flow rates for maximum sensitivity. To achieve submicrogram detection by FID, a good detector design and careful adjustment of operational parameters are required (40). Non-detector problems such as contamination due to column bleeding, and adsorption of sample may result in inadequate sensitivity.

Several interferences due to metabolites have been reported (16); interferences due to extraneous substances are also known (3).

Column contamination and lifetime depend on many factors such as column temperature, liquid phase stability, non-volatile contaminants in the sample, column conditioning procedures, etc.

In spite of all the problems mentioned above, GC is a reliable and accurate analytical tool, which can provide valuable information when employed properly. In recent years toxicologists have become more interested in using GC to solve forensic problems, and although some misconceptions exist about GC capability, like
detection limits, long range reliability and reproducibility, etc., it is clear that GC will play a more important role in drug analysis in years to come.

**Barbiturates.** Before 1966 the work done in this area was mainly with pure compounds. Attempts were made to identify barbiturates according to their pyrolysis patterns (43). They were reacted with diazomethane and analyzed as their methyl derivatives (31). The separation as free acids was achieved in 1961 (24) and although retention times were listed for 23 barbiturates, it was not stated whether any mixture could be resolved. Some commonly encountered problems were: tailing peaks, excessive retention time, and strong adsorption in the column.

Different liquid phases were evaluated (44); usually with little improvement in results. Additives like dimeric acid (45), polyethylene glycol (26), and tristearin (37), were used to deactivate the solid support. This deactivation procedure, however, limits the use of the column to specific substances. A general deactivation process is the silanization of the solid support. Parker et al (26) introduced this process utilizing HMDS.

H. V. Street, using stainless steel columns and tristearin as an additive, reported detection at levels as low as 10 nm (37). The analysis time, however, was
excessive and column efficiency was poor, especially with the dipir amobarbital and pentobarbital.

The use of highly sensitive detectors other than flame ionization has been limited. Gudzinowicz (32) reported detection of 100 ng of barbiturates with an electron capture detector, but the peaks showed excessive tailing and retention times were not reproducible.

After 1966 more inert solid supports like Gas Chrom Q became available and were immediately applied to barbiturate analysis (33). Nevertheless, as mentioned by Martin and Driscoll, (27) some barbiturates were not resolved. Other workers like M. W. Anders (33) reported that column presaturation with large amounts of drugs was essential in order to obtain reproducible results with very small sample sizes.

The use of derivatives formed "on-column" was reported by Stevenson (46) as an aid to identification. This same procedure was recently improved by Barret (47) using a more refined system.

At the present time the determination of barbiturates as free compounds is possible, using very inert solid supports and highly purified liquid phases. Nevertheless, many results are not reproducible. In 1973 a special deactivation process using formic acid vapor constantly
diffusing into the carrier gas was reported for the analysis of phenobarbital (12).

A single step process for extraction, concentration and derivatization, using toluene and tetramethyl ammonium hydroxide, followed by GC analysis has been reported by McGee (48). An excellent article comparing different methods for barbiturate analysis is that by G. Kanamen (49).

Alkaloids. We find interesting early reports attempting the determination of alkaloids such as codeine, cocaine, and morphine as free compounds (22-26). Not surprisingly, very large sample sizes were used. Lack of reproducibility and the need to presaturate the column was observed.

The strong interaction between the phenolic groups and the solid supports, was minimized by preparing the acetate and propionate derivatives (30). This procedure was first reported by M. W. Anders et al as an aid for identification in the "peak shift" technique. This technique was later applied to the analysis of biological materials by Mulé (22).

The use of additives has been applied also to this group of drugs; specifically KOH (26) and polyvinylpyolidone (50). But in general, little improvement was
achieved and in some cases worse results were obtained.

Brochman and Hanssen (51) used several liquid phases, but they do not mention which was the best.

In 1966 the formation of trimethylsilyl derivatives for morphine and codeine was reported (52). H. V. Street (29) in an excellent publication describing the preparation of inert support by silanization established minimum detectable quantities of some drugs, most of them in the submicrogram level. No chromatograms were shown however. The same authors in a later paper (36) and using metal columns reported analysis of many alkaloids at low levels in blood and urine showing chromatograms with concentrations in the microgram range.

Perhaps the only critical publication about GC problems in drug analysis is that of H. V. Street (39) in which he described the preparation of many inert and efficient columns. No chromatograms are shown, nevertheless very valuable information and good practical points are mentioned.

In the first report of the preparation of TMS derivatives for morphine and codeine (52) a mixture of hexamethyldisilizane (HMDS) and dimethylchlorosilane (DMCS) was used as a reagent. Later N. Ikekawa (11) and T. Fish and Wilson (53) using Bis-trimethylsilyl
acetamide and better columns applied this method to urine extracts. Particularly interesting is the work by Ikekawa who was able to detect morphine in urine 72 hours after the administration of therapeutic doses.

A somewhat ambiguous report (54) mentions the complete separation of codeine and morphine as TMS derivatives. More recently an interesting approach using two different columns and solvent free injection has been applied to alkaloids (12). An interesting and thorough review of different methods for alkaloids analysis has been published by Mulé (55).

Amphetamines. This group of drugs is difficult to analyze due to their strong basic character. Their basicity aggravates the common chromatographic problems of adsorption and tailing.

The first report on GC analysis of biologically important amines was by Fales and Pisano (56). The sample sizes were in the range of 100 micrograms.

Due to the strongly basic character of these compounds, the use of KOH or other strong bases as additives to reduce column adsorption is essential for an analysis of amphetamines as free compounds. This was first reported by Thompson in 1965 (28).

The use of two different columns was reported by
Cartoni et al in 1968 but they do not mention if any actual separations were made (57). Surprisingly, the use of derivatives was not reported until 1970: Schweitzer (58) and Lebish et al (59). The derivatives reported were acetates and propionates. Lebish et al (59) detected amphetamines in blood and urine by using different columns and KOH as additive.

Recent reports (55) describe standard methods and conditions, but in general, little information is available about the minimum detectable amounts.

Discussion and Proposal. Most people think that GC is a very efficient, rapid, reliable, and sensitive separation technique. Certainly this is true in many cases; but closer to reality for drug analysis is the statement that GC is highly dependent upon the operators' capability and experience. This applies particularly to the interpretation of results. Many published articles express their conclusions and results, with no data given for the weight of compound introduced into the chromatograph. At best, the volume but not the concentration of the solution injected is given.

Data reported in these ways may be completely misleading. For instance, there is no difficulty in obtaining reasonably good peaks when 30 micrograms of any drug
are injected. However, submicrogram quantities require more refined chromatographic conditions.

Another essential point is the efficiency of separation or resolution. In many cases only retention times are published, not mentioning if any actual separation was possible. Few papers show actual chromatograms.

Minimum detectable quantities are rarely mentioned. In many cases it is stated that it was possible to "recognize" down to a certain amount of sample. Analysis time has been of little concern to most researchers, and little mention is encountered of attempts to reduce the analysis time.

Although it has been known for a long time that adsorption is the major problem encountered in trace analysis of polar compounds few people have studied or defined the magnitude of this problem.

Among the reports published to date, little or no data has been reported dealing with the testing of new liquid phases. New liquids may improve column efficiency and separation.

The purpose of this research was to study the efficiency of several new liquid phases for the analysis of the commonly abused drugs; to determine the magnitude of the adsorption problem, particularly with submicro-
gram samples; and to improve in general the overall chromatographic system in terms of separation, analysis time and sensitivity. A final purpose was to study the use of derivatives.
EXPERIMENTAL

Apparatus. A biomedical gas chromatograph (Bendix Model 2600) equipped with a flame ionization detector and glass "U" shaped columns was used throughout this work. Column lengths of 3 feet and 6 feet were evaluated as were internal diameters of 2 and 4 mm. The carrier gas used was helium and hydrogen and air or oxygen were used to provide the flame.

The recorder employed was a Texas Instrument recorder (Model Servoriter II) with 1 mv span and variable chart speed. For the peak area integrations a Vidar Autolab Model 6300 digital integrator was used.

Columns and Materials. All liquid phases, solid supports as well as some pre-coated column packing materials were obtained from Supelco, Inc. (Bellefonte, Penn.). All reagents and solvents were analytical reagent or spectro-quality, obtained from Fisher Scientific Co.

Pure drug standards were obtained from Technicon Instrument Corp., Tarrytown, N. Y. and were used without further preparation unless otherwise specified. The drugs studied are shown in Table I.

Internal standards of pure n-hydrocarbons, $C_{22}$,
C\textsubscript{24}, C\textsubscript{26}, C\textsubscript{32}, and C\textsubscript{34} were obtained from Applied Science Lab, Bellefonte, Penn. Squalane was obtained from Eastman Kodak.

**Column Preparation.** The method employed to prepare the packing material was the "Funnel Coating" procedure (41). Several tests were made until reproducible coatings were obtained, usually in the range of 3% by weight liquid phase loading. Since volatile solvents were used (acetone or chloroform) the packings were dried at room temperature and packed into the glass U tube columns by vibrating with Burgess "vibro-graver" (Model V-73). For the quantitative studies only pre-coated packings from Supelco (Bellefonte, Pa.) were used to assure uniform quality.

Small silanized glass wool plugs were placed in both ends of the columns. The column conditioning procedures were slightly different for each column, but essentially they were as follows: conditioning with no carrier gas flow during 4 to 6 hours \textless{} 20\textdegree{}C. below the maximum expected operating temperature and then overnight 30\textdegree{}C. above the maximum operating temperature with a small helium flow of around 10-15 ml/min.

**Standards Preparation.** Standard solutions of drugs were prepared by weighing the calculated amount of drug on a Mettler Gram-matic balance capable of reading to
one tenth of a milligram, and then dissolving the sample in CHCl₃ and a few drops of methanol in a 25 ml volumetric flask. From these solutions several more standards were prepared by dilution. The range of concentrations was between 8 and 500 ng/microliter expressed as the free compound.

Standard solutions of known concentrations containing several drugs and/or internal standards were prepared in this way and kept in volumetric flasks at room temperature. Every month new standards solutions were prepared as required.

Additional standard solutions were prepared for derivative studies. Known weights of morphine·SO₄, Codeine·HCl, amphetamine·SO₄ and metamphetamine·HCl were dissolved in 1 ml distilled water. Concentrated KOH (1 ml) was added to obtain a strongly basic solution (pH 12-14), and then the solution was extracted five times with 2 ml portions of a 4:1 CHCl₃/isopropanol solvent mixture. The organic phase was dried by filtration over glass wool and anhydrous Na₂SO₄. The volume was adjusted to 25 ml in a volumetric flask with CHCl₃. These solutions were employed in the derivatization experiments.
### TABLE I. DRUGS STUDIED

#### A. BARBITURATES

1. Amobarbital
2. Pentobarbital
3. Secobarbital
4. Phenobarbital

2. Cocaine $\text{SO}_4$
3. Codeine $\text{HCl} \cdot 2\text{H}_2\text{O}$
4. Morphine $\text{SO}_4 \cdot 5\text{H}_2\text{O}$

#### B. ALKALOIDS

1. Methadone $\text{HCl}$

#### C. AMPHETAMINES

1. Amphetamine $\text{SO}_4$
2. Metamphetamine $\text{HCl}$
**Derivatization.** Derivatization reactions were carried out in small glass sample vials (5 ml capacity) with phenolic screw caps. The reagents employed were "Regisil" (Regis Chemical Co.), acetic anhydride (Fisher Scientific Co.) and trimethylphenyl ammonium hydroxide, 0.1M in methanol (Fisher Scientific Co.). A known volume of the standard solution of the drug was deposited in the vial; the solvent was evaporated and a known volume of reagent added. A 4-5 fold excess or reagent was used.

The amount of reagent added was calculated to give the desired concentration of the drug. Trial experiments were performed to determine the time and temperature necessary for complete reaction.

When the reaction was complete, a small portion of the reaction mixture (1-5μl) was withdrawn and injected into the gas chromatograph. The range of drug sample sizes injected was between 50 nanogram and 1 microgram per microliter of solvent.

**Injection Technique.** A Glenco 10 microliter removable needle model 199I3 syringe was used throughout this work to introduce the samples. Unless otherwise specified, the double reading syringe technique was employed. To know accurately the volume injected, the total volume in the syringe was read before and after injection; the difference gives the volume injected.
RESULTS AND DISCUSSION

Gas chromatography is a technique that requires experience and knowledge in order to obtain and interpret good quantitative results. There are numerous factors and operational parameters which influence the chromatographic results. This helps explain why results at trace levels are always difficult if not impossible to reproduce.

Certainly it is difficult to speak of the "best" or "optimum conditions" for any analysis since in principle there are no instruments, columns, or conditions which could not be improved.

In any gas chromatographic method the most important part is the column. Gas chromatography is a separation technique and the separation occurs only in the column. The rest of the system provides the proper conditions for the separation and detection to occur. For this reason most of this work was devoted to studying the performance of different columns for the three types of drugs.

As mentioned before this work will cover four main aspects:

1) The study of different liquid phases in terms of solvent efficiency.

2) Linearity of response, covering the range from nanograms to micrograms in sample size.
3) Conditions for rapid analysis.

4) Study of derivatives for drug analysis.

In order to make an organized presentation of the results, a few comments about the general work will be offered followed by a detailed presentation of results for each of the three types of drugs.

**General.** Gas chromatographic analysis of reactive compounds and thermolabile or high molecular weight compounds is always demanding and requires attention to detail and experience. Of the three types of drugs studied, some chromatographic problems are present in each group. For example, amphetamines although relatively low in molecular weight are very reactive, requiring specially deactivated solid supports and columns. In addition some barbiturates are very similar structurally and difficult to resolve. Amobarbital and pentobarbital differ only in the position of one methyl group. Chromatographically barbiturates are the most difficult group of drugs to resolve. Alkaloids are the highest molecular weight compounds studied and they are also reactive. Morphine is such a difficult chromatographic problem that its determination as a free compound is ruled out. Adsorption on the
column prevents any quantitative analysis with trace levels.

With the exception of the amphetamines the drugs studied can be considered high molecular weight compounds and high column temperatures are needed in order to keep the analysis time short. The liquid phases used should have operating temperatures of at least 275°C. or higher in order to provide a stable long lived column.

Low liquid phase loadings and short columns are also required. Since the extent of column adsorption was to be studied, an all glass system was required to minimize adsorptive effects from the injection port detector and connections. The Bendix Model 2600 is a very good "all glass system" in this respect.

Throughout this study mostly 4mm I. D. glass columns were used. These were readily and cheaply available from the glass shop of the chemistry department. Some commercially available 2mm I. D. glass subing was also studied but few differences were noted. Column length was usually 3 feet and although this short length is not widely used, it was sufficient for the drugs studied.

In most columns a 3% by weight liquid phase loading was used. This amount of liquid phase is normal for biomedical work, and much data has been accumulated using 3%
liquid phase packings. For lighter liquid loadings the adsorption problem may become critical and it is more difficult to obtain an even coating of the solid support surface. Above 3% the retention time increases and higher column temperatures are required.

Among the liquid phases studied is a series of polysiloxanes specially prepared for gas chromatography work. These liquid phases are the OV series from the Ohio Valley Co. (see Table II).

**Alkaloids.** The efficiency of different liquid phases was measured by determining relative retention times. Relative retention times are the ratios of adjusted retention times (41). Relative retention times or $\alpha$ values are perhaps the most important parameters involved in any separation. High $\alpha$ values indicate high solvent selectivity. A temperature was chosen such that with all columns the analysis time was reasonable (between 3-8 minutes). It is correct to compare $\alpha$ values only when they are measured at the same temperature.

The temperature selected to measure the $\alpha$ values of different liquid phases for the alkaloids was $230^\circ$C. The values obtained are summarized in Table III.

The order of elution in all columns was: methadone, cocaine, codeine, and morphine. An ideal liquid phase would be one which gives $\alpha$ values of about 1.5. Values less
<table>
<thead>
<tr>
<th>Table II.</th>
<th>Liquid Phases Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE-30</td>
<td>Dimethyl-Silicone Gum</td>
</tr>
<tr>
<td>OV-7</td>
<td>Phenyl-methyl-Silicone (22% phenyl substitution)</td>
</tr>
<tr>
<td>OV-17 (Sp-2250)</td>
<td>&quot; &quot; &quot; (50% phenyl substitution)</td>
</tr>
<tr>
<td>OV-25</td>
<td>&quot; &quot; &quot; (75% phenyl substitution)</td>
</tr>
<tr>
<td>OV-210</td>
<td>Trifluoropropyl-methyl-Silicone</td>
</tr>
<tr>
<td>OV-225</td>
<td>Cyanopropyl-methyl-phenyl-methyl-Silicone</td>
</tr>
<tr>
<td>Carbowax 20M</td>
<td>Polyethylene Glycol</td>
</tr>
<tr>
<td>Amine 220</td>
<td>1 Hydroxy-2-Heptadecylimidazoline</td>
</tr>
<tr>
<td>Apiezon L</td>
<td>Hydrocarbon Grease</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>Octylphenoxypoly(ethyleneoxy)ethanol</td>
</tr>
<tr>
<td>Liquid phase</td>
<td>$\alpha_1$ (cocaine/methadone)</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>SE-30</td>
<td>1.12</td>
</tr>
<tr>
<td>OV-7</td>
<td>1.33</td>
</tr>
<tr>
<td>OV-17 (SP-2250)</td>
<td>1.48</td>
</tr>
<tr>
<td>OV-25</td>
<td>1.67</td>
</tr>
<tr>
<td>OV-210</td>
<td>1.72</td>
</tr>
<tr>
<td>OV-225</td>
<td>1.96</td>
</tr>
</tbody>
</table>
than 1.3 give poor resolution and overlapping peaks; values greater than 1.8 are excessive and require longer analysis times. We can see from Table III that the best liquid phase with respect to \( \alpha \) values is OV-210; unfortunately all peaks tail badly in this column and this liquid phase is not of general use.

We also see that the separation between codeine and cocaine is always more than required, thus taking more analysis time. The next best liquid phases are OV-7, OV-17, and perhaps OV-25. But as we will see later, if the analysis of morphine on the same column is required we have a more difficult problem.

Since \( \alpha \) values decrease with temperature only liquid phases with relatively high \( \alpha \) values will give good resolution at the high temperatures required for alkaloid separations.

No chromatograms in the literature showed a separation of these three alkaloids in less than 5 minutes. Conditions for rapid analysis were studied and acceptable chromatograms at 200 nanogram levels were produced in 60 seconds (Figs. 1 and 2). These results have proven so attractive that the column packing used in Fig. 1 has now become commercially available as a special packing for rapid drug analysis (61).

Fig. 2 shows a higher per cent liquid phase loading (3\%) and therefore requires a higher temperature (30°C. more) to provide the same analysis time. The selectivity of
SP-2250 and OV-7 is too low to provide adequate resolution of alkaloids at 280°C. Either column, 1% SP-2250 at 250°C. or 3% OV-25 at 280°C. is possible for rapid analysis.

One minute analyses however are not important for manual chromatographic techniques since other steps involved determine the overall analysis time. A more realistic and practical approach is an analysis time of around 2-4 minutes.

Fig. 3 shows slightly longer retention times using 3% liquid phase loadings of OV-7 and SP-2250. The resolution of SP-2250 is better; however, both columns are more than adequate for rapid analysis of trace levels of alkaloids.

If the analysis time is too short, trace analysis is complicated by interference from the solvent peak (see fig. 4 and 5). These chromatograms show 40 and 8 nanograms respectively analyzed on 1% SP-2250 under identical conditions. Fig. 5 does not show an acceptable peak for 8 nanograms of codeine, but in addition, the resolution of methadone and cocaine from the chloroform solvent is not adequate for reliable identification.

To visualize the extent of adsorption and the possible effect on quantitative analysis, calibration curves were determined for the four alkaloids. In most cases a hydrocarbon was introduced as an internal standard to compare peak behavior at very low concentration levels.
Fig. 1. RAPID ANALYSIS OF ALKALOIDS
1% SP-2250 on 100/120 mesh Supelcoport;
250°C.; 200 nanograms/peak
Fig. 2. RAPID ANALYSIS OF ALKALOIDS
3% OV-25 on 100/120 mesh Supelcoport; 280°C.; 200 nanograms/peak.
Fig. 6-9 show the peak area vs sample size relationships obtained for these compounds. With the exception of methadone (Fig. 6) all show non-linear curves at low concentration levels. Fig. 6 shows methadone to behave almost as a hydrocarbon. A linear relationship exists down to 50 nanograms of sample. This level is considerably lower than that required to monitor methadone in most biological samples. Lower levels producing integrator counts of less than 1000 would not generate meaningful results.

Fig. 7 shows a non-linear, but useful calibration curve for codeine. Since the squalane response was linear, it is concluded that column adsorption effects produce the non-linear response with codeine. The smallest peak measured was produced by 50 nanograms.

Cocaine (Fig. 8) also shows a non-linear relationship; 50 nanograms was easily measured, however.

The adsorption problem is dramatically seen in the case of morphine (Fig. 9). The lowest concentration measured was 4 micrograms. Adsorption is so bad that sample sizes of one microgram are barely visible. Morphine is a very difficult compound to elute from a GC column. The phenolic group in the molecule strongly interacts with the solid support. The data for Fig. 9 was obtained using a 1% SP-2250 column in order to elute morphine as rapidly as possible. The 3%
Fig. 3. LESS RAPID ANALYSES OF ALKALOIDS
(a) 3% OV-7; (b) 3% SP-2250 both on 100/120 mesh Supelcoport; 260°C; 200 nanograms/peak
Fig. 4. ALKALOIDS
40 nanograms/peak on 1% SP-2250; 250°C.
Fig. 5. ALKALOIDS

8 nanograms/peak on 1% SP-2250; 250°C.

1. methadone
2. cocaine
3. codeine
Fig. 6. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR METHADONE AND C-24 HYDROCARBON

3% SP-2250 on 100/120 mesh Supelcoport;
240°C; FID
Fig. 7. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR CODEINE AND SQUALANE

3% SP-2250 on 100/120 mesh Supelcoport; 240°C.; FID.
Fig. 8. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR COCAINE AND C-24 HYDROCARBON

3% SP-2250 on 100/120 mesh Supelcoport; 240°C; FID.
Fig. 9. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR MORPHINE
1% SP-2250 on 100/120 mesh Supelcoport; 240°C.; FID.
liquid loaded columns used for the other alkaloids are not practical for the analysis of free morphine. It is almost mandatory to form derivatives for quantitative morphine analysis by gas chromatography.

Methadone obviously needs no derivative and for cocaine there is none which can be easily prepared. Codeine and morphine can be easily reacted to form either acetates or TMS ether derivatives.

Both derivatives were tested with the following results: (1) both reactions are simple and easy to carry out, especially TMS ether formation with "Regisil"; (2) both reactions are essentially quantitative and the derivatives show good peak shapes. The calibration curves for the TMS derivatives for codeine (Fig. 10) and morphine (Fig. 11) show linear response even at 50 nanogram levels. In the case of morphine there is a substantial increase in sensitivity. In the case of codeine there is not a great enhancement in sensitivity, but detection is easier due to improved peak shapes (see fig. 12). Obviously derivative formation improves both the peak shape and detectability.

The separation of morphine and codeine as TMS ethers is not complete on 3% SP-2250 (Fig. 13a). This problem can be resolved however by using a more polar liquid phase such as OV-25 (Fig. 13b).
Another solution to the resolution problem is to form the acetate derivatives. These are easily resolved on 3% SP-2250 at 230°C. (Fig. 14).

**Barbiturates.** The same liquid phases evaluated for the alkaloids were tried for the barbiturates. Alpha values were measured at 200°C, since the barbiturates elute more rapidly. Table IV summarizes the results.

The data in Table IV leads one to the following conclusions:

1. The amobarbital/pentobarbital separation is the most difficult;
2. The phenobarbital/secobarbital separation is usually excessive resulting in lost time;
3. OV-17 (SP-2250) is the best liquid phase, but it does retain phenobarbital too long.

The $\alpha_1$ values are so low that very efficient columns are required for adequate resolution. Fig. 15 shows the separation of barbiturates on 3% OV-17 at 230°C and 200°C. It is difficult to choose optimum conditions for the simultaneous determination of all four barbiturates. At 230°C, the phenobarbital elutes in 3 minutes with a fair peak shape, however the amobarbital, pentobarbital and secobarbital are poorly resolved. At 200°C, the first 3 barbiturates are resolved better, however phenobarbital (200 nanograms) gives a poor peak shape.

An improved chromatogram is obtained with a 6 foot column (Fig. 16). Longer retention times are obtained, by at 215°C.
<table>
<thead>
<tr>
<th>Liquid phase</th>
<th>$\alpha_1$ Pento/Amo</th>
<th>$\alpha_2$ Seco/Pento</th>
<th>$\alpha_3$ Pheno/Seco</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE-30</td>
<td>1.06</td>
<td>1.170</td>
<td>1.710</td>
</tr>
<tr>
<td>OV-7</td>
<td>1.08</td>
<td>1.190</td>
<td>2.380</td>
</tr>
<tr>
<td>OV-17 (SP-2250)</td>
<td>1.12</td>
<td>1.180</td>
<td>2.870</td>
</tr>
<tr>
<td>OV-25</td>
<td>1.070</td>
<td>1.17</td>
<td>3.050</td>
</tr>
<tr>
<td>OV-210</td>
<td>1.00</td>
<td>1.00</td>
<td>2.26</td>
</tr>
<tr>
<td>OV-225</td>
<td>1.10</td>
<td>1.29</td>
<td>1.79</td>
</tr>
</tbody>
</table>
Fig. 10. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP CODEINE TMS
Fig. 11. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP MORPHINE TMS
Fig. 12. (A) CODEINE; (B) CODEINE TMS
Conditions same as fig. 7. Both 200 nanograms/peak
Fig. 13. (A) MORPHINE AND CODEINE TMS DERIVATIVES ON 3% SP-2250 at 230°C.
(B) Same conditions except 3% OV-25
Fig. 14. MORPHINE AND CODEINE ACETATE DERIVATIVES ON 3% SP-2250 at 230°C.
Fig. 15. BARBITURATE ANALYSIS ON 3% SP-2250
(a) 230°C; (b) 200°C.
the resolution of amobarbital, pentobarbital and secobarbital
is considerably better and the 200 nanogram peak of pheno-
barbital is acceptable. This same 6 foot column produces an
acceptable chromatogram for 40 and 20 nanogram levels of
phenobarbital at 240°C. (Fig. 17). This is the best column
and best chromatogram obtained for the barbiturate analysis.

Care must be exercised with long columns at lower tem-
peratures. Fig. 18 shows the same column as Fig. 17, except
at 215°C. No phenobarbital peak is observed for 40 nanograms
sample size. A combination of column adsorption and long
retention time prevent any observation of the peak.

One minute analysis of barbiturates is also possible
(Fig. 19). This chromatogram was obtained on a 3' x 4mm I. D.
glass column containing 3% SP-2250 on 100/120 mesh Supelcoport.
The conditions required for this analysis are difficult to
obtain and not easy to reproduce.

Calibration curves for all four barbiturates were ob-
tained on a 3 foot x 4mm I.D. glass column containing 3%
SP-2250 (Fig. 20-23). The chromatograms for amobarbital were made at 220°C.; the chromatogram for
phenobarbital at 230°C. If we compare these results with the
alkaloid curves (Figs. 6, 7, and 8) it is obvious that the
barbiturates present a more difficult analysis. The barbi-
turate peaks tail badly; the lowest measurable quantity is
about 100 nanograms; and the calibration curves do not pass
Fig. 16. BARBITURATE ANALYSIS, 6' COLUMN, 3% SP-2250; 215°C.; 200 nanograms.
Fig. 17. BARBITURATE ANALYSIS, 6' COLUMN, 3% SP-2250; 240°C.; A-40 nanograms; B-20 nanograms.
through the origin. Only pentobarbital (Fig. 21) and phenobarbital (Fig. 23) show nonlinear relationships. This is true even at high concentration levels. The quantitative analysis of phenobarbital is a more difficult case as observed earlier in the chromatograms.

From the preceding facts, we realize the difficulty in analyzing barbiturates at trace levels. Fortunately there are some ways to improve the chromatographic results. One way is to diffuse formic acid vapor in the carrier gas prior to the column to minimize adsorption, and thus improve peak shape (12). This approach although not tested in this work does not seem practical for routine analyses.

A better to improve the barbiturate resolution is the preparation of N-methyl derivatives using trimethylphenyl-ammonium hydroxide (0.2M in methanol). The technique used was as follows: one to two microliters of sample was withdrawn with a 10 microliter syringe and the total volume measured; then in the same syringe twice as much volume of the trimethylphenyl ammonium hydroxide solution was taken and the mixture injected onto the column. This procedure is called "On-Column-Derivatization".

Results obtained with the N-methyl derivatives are shown in Fig. 24. There is a great improvement in resolution, sensitivity and peak shape. These chromatograms were obtained
fig. 18. BARBITURATE ANALYSIS, 6' COLUMN 3% SP-2250, 215°C.; 40 nanograms.
Fig. 19. RAPID BARBITURATE ANALYSIS 3% SP-2250; 240°C; 200 nanograms.
Fig. 20. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR AMOBARBITAL.
Fig. 21. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR PENTOBARBITAL.
Fig. 22. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR SECOBARBITAL.
Fig. 23. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR PHENOBARBITAL AND SQUALANE.
at 215°C. with a 6 foot column containing 3% SP-2250 loading on Supelcoport 100/120 mesh. Both chromatograms were made under the same conditions. The free phenobarbital peak is not visible, but the N-methyl derivative is clearly seen with a greatly improved peak shape. The resolution among amobarbital, pentobarbital and secobarbital is also better as derivatives.

Fig. 25 shows the effect of reagent concentration on the "On-Column-Derivative" technique for barbiturate analysis. Chromatogram A resulted from 2 microliters of 0.2M reagent and chromatogram B resulted from 1 microliter of 0.2M reagent. The sample concentration was 500 nanograms of each barbiturate in a total volume of 1 microliter. Chromatogram B shows the effect of insufficient reagent.

Since only phenobarbital shows a critical adsorption problem, the calibration curve for the N-methyl phenobarbital derivative is the only one shown (Fig. 26). A good linear relationship is obtained by using the derivative. This should be compared with the same graph for free phenobarbital (Fig. 23). Obviously a derivative step is essential for either trace or reasonable quantitative analysis of phenobarbital.
Fig. 24. A DERIVATIZED AND B FREE BARBITURATES ON 6' COLUMN, 3% SP-2250, 215°C, 500 nanograms/peak.
Fig. 25. EFFECT OF AMOUNT OF DERIVATIVE REAGENT ON BARBITURATE ANALYSIS
A) $2 \times 10^{-6}$ M Reagent/500ng barbiturate
B) $1 \times 10^{-6}$ M Reagent/500ng barbiturate
Fig. 26. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR N-METHYL PHENOBARBITAL
Amphetamines. The fact that only two amphetamines are physiologically important simplifies their analysis. Their strong basic character and their relatively low molecular weight however, require different columns from those used for the barbiturate and alkaloid analyses. None of the silicon liquid phases give a good separation for the free amphetamines.

Four different liquid phases were evaluated for amphetamine analysis. All are commonly used for amine analysis in gas chromatography: Triton X-100, Amine 220, Carbowax 20M and Apiezon-L. All liquid loadings were 10% by weight with 2% KOH as additive on Supelcon 80/100 mesh.

The Triton X-100 fails to give any separation between amphetamine and metamphetaime, even at 90°C., but the retention time is too long for routine analyses. Higher temperatures give insufficient resolution, and lower temperatures give even longer retention times.

The Carbowax 20M column at 190°C. shows no separation. At 130°C. the retention time is 5 minutes but only a partial separation is obtained. At 100°C. two peaks are visible but the retention time is in excess of 5 minutes.

The best and most convenient packing for amphetamines is 10% Apiezon-L and 2% KOH (Fig. 27). A one minute analysis is easily obtained even at levels as low as 10 nanograms. Symmetrical peak shapes are also obtained.

The additive (KOH) is essential to obtain good results. A 4% Apiezon-L loading on Chromosorb G NAW 100/120 mesh was
tried as a possible substitute for the 10% Apiezon-L and 2% KOH on Chromosorb W. Although the resolution was good, the peaks show too much tailing. In Fig. 28 an amphetamine analysis is shown using a 4% loading of Apiezon-L and 1% KOH on Chromosorb G NAW 100/120 mesh. This is essentially the same amount of liquid phase per unit of surface area and the results are essentially the same as with 10% Apiezon-L and 2% KOH on Chromosorb W. Obviously the KOH is essential for the amphetamine separation.

Amphetamines could also be analyzed as acetate derivatives. The acetylation reaction is easily carried out at 60°C for 10 minutes with acetic anhydride. A comparison of the separation as free drugs and as the acetate derivatives is shown in Fig. 29. The free amphetamines were analyzed on the 10% Apiezon-L and 2% KOH column. The acetates were analyzed on a 3% SP-2250 column, the same column employed for barbiturate and alkaloid analysis. Both separations were made at 170°C.

A small increase in sensitivity is obtained for the acetate derivatives and a more general purpose column is employed. However there is no real advantage in preparing the acetate derivatives. The resolution, time of analysis and quantitative results obtained as the free drugs is more than acceptable.
Fig. 30-33 show the response curves for amphetamine and methamphetamine as free compounds and as their acetate derivatives. Essentially no advantage is observed for the derivatives.
Fig. 27. RAPID AMPHETAMINE ANALYSIS
3', 10% Apiezon-L and 2% KOH on Supelcon, 170°C.
Fig. 28. RAPID AMPHETAMINE ANALYSIS
3', 4% Apiezon L + 1% KOH on Chromosorb G,
100/120 mesh, 170°C.
Fig. 29. AMPHETAMINE ANALYSIS

a) Free 10% Apiezon-L + 2% KOH 170°C.
b) Acetate Derivatives 3% SP-2250 180°C.
Fig. 30. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR AMPHETAMINE.
Fig. 31. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR METHAMPHETAMINE.
AMPHETAMINE ACETATE

Fig. 32. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR AMPHETAMINE ACETATE.
Fig. 33. PEAK AREA, SAMPLE CONCENTRATION RELATIONSHIP FOR METHAMPHETAMINE ACETATE.
CONCLUSIONS

1. The sensitivity obtained even with the free compounds (except morphine) make gas chromatography a good technique for trace analysis of drugs in biological samples.

2. For many of the drugs studied the detector response curves are not linear with sample concentration but the calibration curves are reproducible if care is excercised. This makes quantitative analysis feasible down to 50-100 nanogram levels with proper technique.

3. Long column life is needed for routine analyses. Those columns employed in this work were used continuously for over seven months with no appreciable deterioration.

4. Rapid chromatographic analysis is possible, usually in the order of 60 seconds for each major class, however these chromatographic conditions are difficult to obtain and difficult to reproduce. They are not recommended for routine analyses. Analysis times of 2-4 minutes are possible under conditions amenable to routine analyses.

5. The preparation of derivatives not only improves the sensitivity, the quantitative results, but can also be used to help identify the drug. Due to the
adsorption problem, derivatives should be considered in the case of phenobarbital, morphine and codeine.

6. Column selection procedures for gas chromatographic analysis is in most cases made by trial and error. No theoretical approach exists which assures success for a particular separation. Sufficient work has been done to enable one to "suggest" possible column systems which give good performance.

A. Alkaloid Analysis - If the analysis of free morphine is desired the 3 foot, 1% SP-2250 Supelcoport 100/120 mesh is the recommended column; this column also provides a good approach for fast analyses of the alkaloids.

For only methadone, cocaine and codeine; the 3% SP-2250 or 3% OV-25 are recommended. When TMS derivatives are prepared for morphine and codeine, only 3% OV-25 gives complete resolution. For the acetate derivatives either 3% SP-2250 or 3% OV-25 will give good results. The 1% SP-2250 is not recommended when derivatives are used. More details are provided on the chromatograms in the text.

B. Barbiturate Analysis - As free drugs the best column is 6 foot, 3% SP-2250 on Supelcoport 100/120 mesh. The same packing in a 3 foot column is recommended if a fast analysis is desired.

As N-methyl derivatives either column is a good selection if the right temperature is used. So many advantages are ob-
tained by using derivatives, that they are highly recommended.

C. Amphetamine Analysis. - The best column is the 3 foot, 10% Apiezon-L + 2% KOH on Supelcon A.W. 100/120 mesh for free amphetamines.

The 3 foot, 3% SP-2250 or 3% OV-25 on Supelcoport 100/120 mesh can be used for the acetate derivative, but as was mentioned before no great advantage is obtained.
REFERENCES

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Vita

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GAS CHROMATOGRAPHIC STUDIES
OF DRUGS OF ABUSE

by

Benjamin Esquivel Hernandez

ABSTRACT

Gas chromatographic methods have been applied for many years to the analysis of drugs of abuse. Unfortunately little data is available to evaluate the chromatographic conditions necessary for reproducible results, for the detection of trace quantities and for the precision available when trace quantities are analyzed. This study focused on choosing the best liquid phase or phases for the three major categories of drugs of abuse, (barbiturates, amphetamines and alkaloids). In addition the linearity of response using a flame ionization detector was measured in the range of nanogram to microgram quantities of the major drugs. Optimum conditions for rapid analysis, about two minutes per sample, were also studied. Chemical derivatives were formed of most of the drugs of abuse and the advantages of the derivatives were compared with the free drugs.