DOT HS-820 253

INSTRUMENTATION DEVELOPMENT FOR DRUG DETECTION ON THE BREATH

Contract No. DOT-HS-302
September 1972
Final Report

PREPARED FOR:
U. S. DEPARTMENT OF TRANSPORTATION
NATIONAL HIGHWAY TRAFFIC SAFETY ADMINISTRATION
WASHINGTON, D. C. 20590

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/HS-820 253		

4. Title and Subtitle	5. Report Date
INSTRUMENTATION DEVELOPMENT FOR DRUG DETECTION ON THE BREATH	September 1972
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
J.R. Hobbs and A.E. Barrington	DOT-TSC-NHTSA-72-9

9. Performing Organization Name and Address	10. Work Unit No.
Department of Transportation Transportation Systems Center Kendall Square Cambridge, Ma. 02142	R-3405
	11. Contract or Grant No. HS-302
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address U.S. Department of Transportation National Highway Traffic Safety Admin Washington, D.C. 20590	Final Report July 1971-April 1972
	14. Sponsoring Agency Code

15. Supplementary Notes

16. Abstract

Based on a survey of candidate analytical methods, mass spectrometry was identified as a promising technique for drug detection on the breath. To demonstrate its capabilities, an existing laboratory mass spectrometer was modified by the addition of a membrane separator and a field-ionization source.

Fourteen drugs were selected for investigation and it was possible to identify the signatures (mass spectra) of ten of these drugs with the modified instrument. Some drugs have been detected by direct sniffing, others first had to be dissolved in a suitable solvent and evaporated. The mass spectra presented in the report indicate the basic simplicity of field ionization as compared with ionization by the conventional method of electron impact. The report concludes with a description of the ease and rapidity of the new technique for clinical analysis.

January 1973

17. Key Words	18. Distribution Statement
Mass Spectrometry Breath Analysis Drug Signatures	unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	38	

Form DOT F 1700.7 (8-69)

PREFACE

The work described herein was performed in the context of an overall program at the Transportation Systems Center (TSC). The program is designed to establish techniques for measuring alcohol and drugs on the breath. This program is sponsored by the National Highway Traffic Safety Administration, Research Institute.

The program supports government activities designed to promote traffic safety through improving instrumentation and measurement techniques, which can assess whether a motorist is incapacitated by alcohol or drugs.

The primary objectives of the program at TSC are (1) to investigate promising candidate-measurement techniques, and (2) to develop improved instrumentation.

The authors acknowledge the assistance of Dr. A.L. Flores who designed and participated in the assembly of the ion source, and thereby substantially contributed to the success of the experimental program.

CONTENTS

ILLUSTRATIONS

1. INTRODUCTION

The work described herein is a preliminary step in a study to determine the feasibility of drug detection by chemical analysis of the breath. Drug detectability depends on the presence in the breath of measurable signatures of drugs or drug metabolites.

A survey of candidate analytical techniques indicated that mass spectrometry offers a reasonable chance of success, but that several major improvements in the instrumentation must be accomplished. Specifically, these improvements consist of (a) equipping an existing laboratory mass spectrometer with a membrane separator, and (b) providing a field-ionization source of novel design.

The expectations that these modifications would substantially reduce the instrumental background and also would simplify the drug signatures, are borne out by the experimental results.

A group of 14 drugs have been investigated, and one clinical sample of urine. The data are sufficiently encouraging to lead to an expansion of the program, where the feasibility of breath analysis of selected patients will be studied under clinical supervision.

2. PRINCIPLES OF MASS SPECTROMETRY

In the mass-spectrometer technique,[1] molecules of a sample containing a mixture of gases are charged electrically (ionized). Then the charged molecules (ions) are identified according to their mass to charge (m/e) ratio. The quantity of ions of a specific constituent of the sample is a measure of the concentration of this constituent.

The principal building blocks of a mass spectrometer are shown in figure 1. They include a sample introduction system, an ion source, a mass analyzer, and an ion detector. The instrument

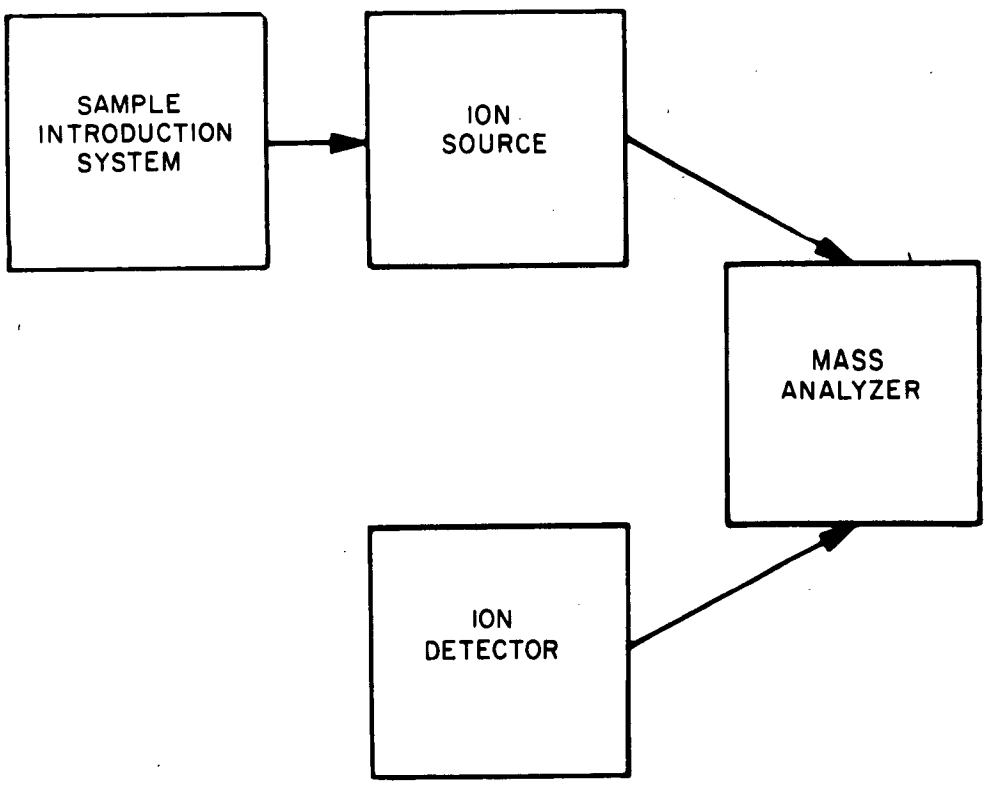


Figure 1. Mass-Spectrometer Schematic

must be evacuated to a pressure below 10^{-4} torr, but the necessary vacuum pumps and accessories are not shown in the figure.

The design of the sample introduction system depends on the type of sampling to be performed. It can be engineered to accept gaseous, liquid, or solid samples, and also to perform continuous or batch sampling.

The ion source is a region in which positive molecular or atomic ions are produced by detaching one or more electrons from a neutral atom or molecule. The magnitude of the ionic charge is equal to the charge of the electron (1.6×10^{-19} coulomb) or an integral multiple thereof. Under certain conditions, negative ions may also be formed by adding an electron to a neutral particle, but this process is not of concern here.

By far, the most widely used method for the ionization of gases is electron impact. Electrons which have been produced at a heated filament are accelerated so that their energy exceeds the ionization potential of the gas molecules. Other methods for the ionization of gases are photo-ionization and field ionization. Photo-ionization is effected by quanta of light whose energy exceeds the ionization potential of the gas molecules. Field ionization (Fig 2) occurs purely by the action of an electric field, and requires potential gradients of the order of a few volts per angstrom. Such fields can be generated by applying potentials of several kilovolts to fine metal tips, fine wires, or sharp metal edges. In the vicinity of a metal surface at a high-positive potential, an electron from the outer shell of a gas molecule is attracted into the metal, thus producing a positively charged ion.

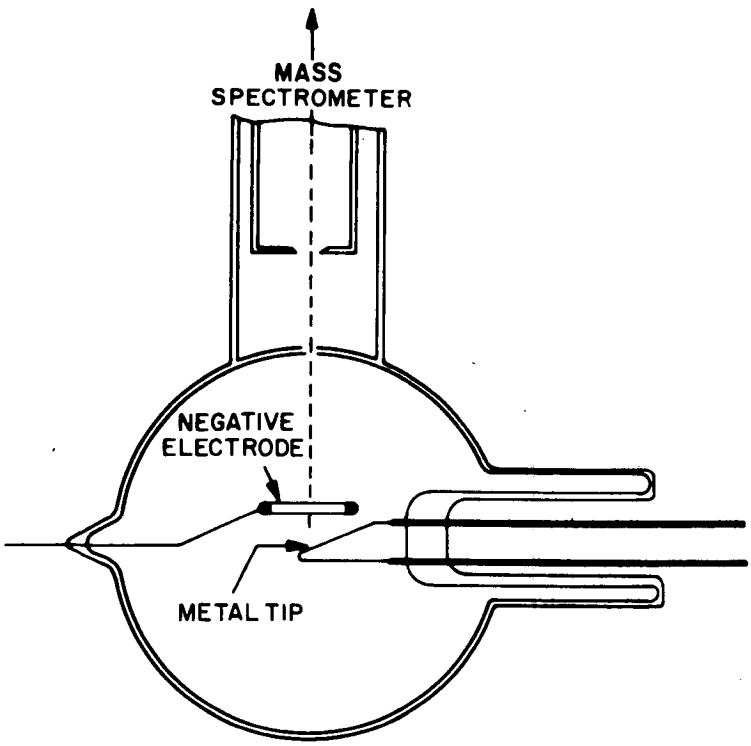


Figure 2. Field-Ionization Source

Regardless of the specific ionization process, ions and ionized fragments of the various molecular constituents of a gas sample are produced in the ion source. The ions are accelerated by an electric field into the analyzer where they are separated according to their m/e ratio in a region of electric or magnetic field. The relative value of m/e is proportional to the ionic mass.

Although ions of particular compounds ($CO^+, N_2^+ \longrightarrow m/e=28$), or ionic fragments, can have nearly identical values of m/e, the ionic pattern or signature of a given compound is usually specific and reproducible. The numerical value of m/e is expressed in atomic mass units (AMU); it is based on the value of m/e for H^+ equal to 1 AMU.

The signature or mass spectrum is recorded by the detector, a device which measures a current or voltage. For convenience, it may include a strip-chart recorder or oscilloscope. An analysis of complex mixtures of compounds may also require access to electronic data-processing equipment.

3. EXPERIMENTAL APPARATUS

The mass spectrometer available at TSC for this study is a Hitachi-Perkin Elmer RMU-6E, 90-degree magnetic sector mass spectrometer (Fig. 3). The ion source of the instrument is of the electron-bombardment type (Hitachi T-2N), which is operated at an electron energy of 80 volts and an ion-acceleration potential of 2.4 or 3.6 kilovolts. The source can be heated to a temperature of 250°C.

The analyzer is a magnetic sector, radius of 20 centimeters; the magnetic field can be varied to a value of 7.5 kilogauss.

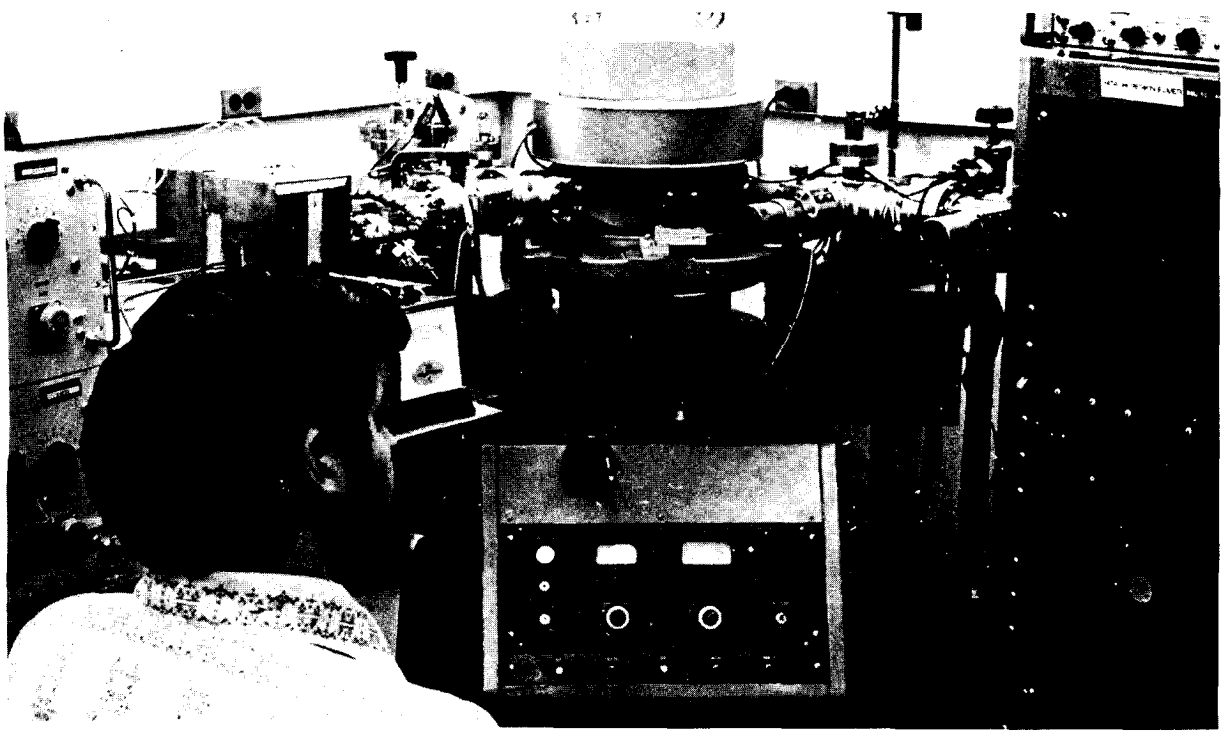


Figure 3. Laboratory Mass-Spectrometer Installation

The analyzer is equipped with a liquid-nitrogen trap, and a 120 liters per second diffusion pump, operating with Monsanto Santovac pump fluid. At the junction between analyzer and detector, a 50 liters per second sputter ion pump provides additional pumping capacity. The detector utilizes a ten-stage Allen-type electron multiplier with silver-magnesium electrodes. The gain is about 10^4 at an operating voltage of 3 kilovolts. To maximize the sensitivity (minimum detectable concentration) of this instrument, the following additional components have been designed and fabricated: (a) a membrane separator, and (b) a field-ionization source.

3.1 MEMBRANE SEPARATOR

The arrangement of the three-stage Llywellyn-Arnold[2] separator is shown in figure 4. The structural components which have been designed and fabricated for the investigation are shown in figure 5.

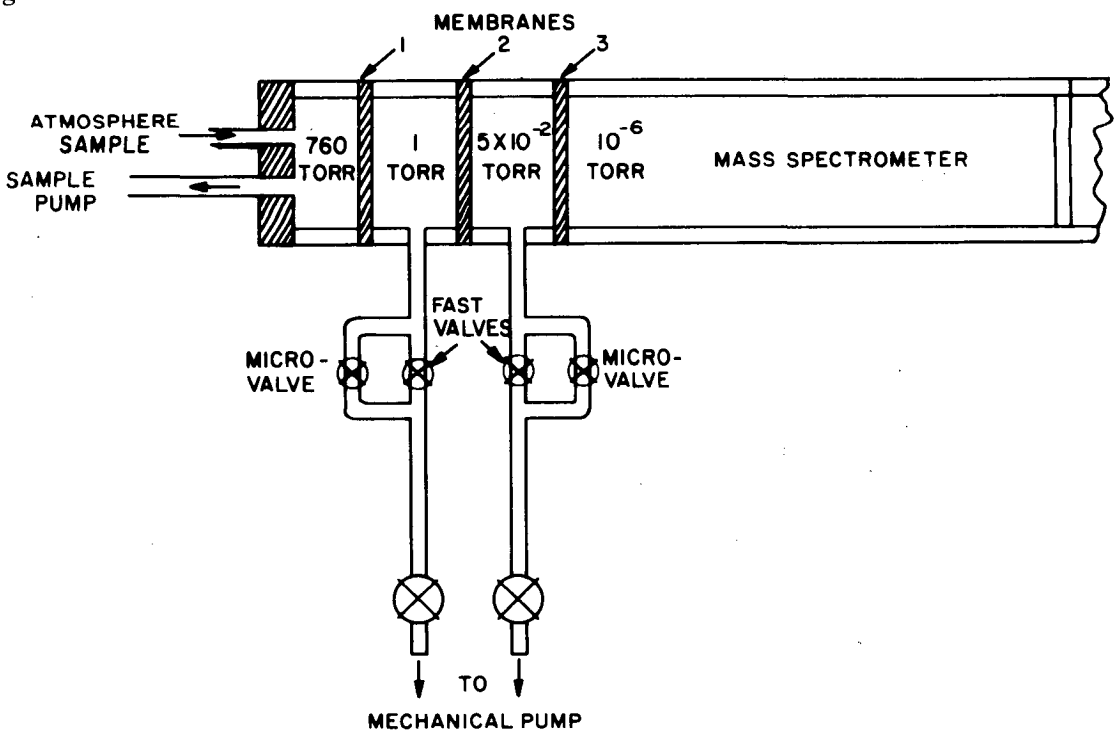


Figure 4. Mass-Spectrometer Schematic with Membrane Separator

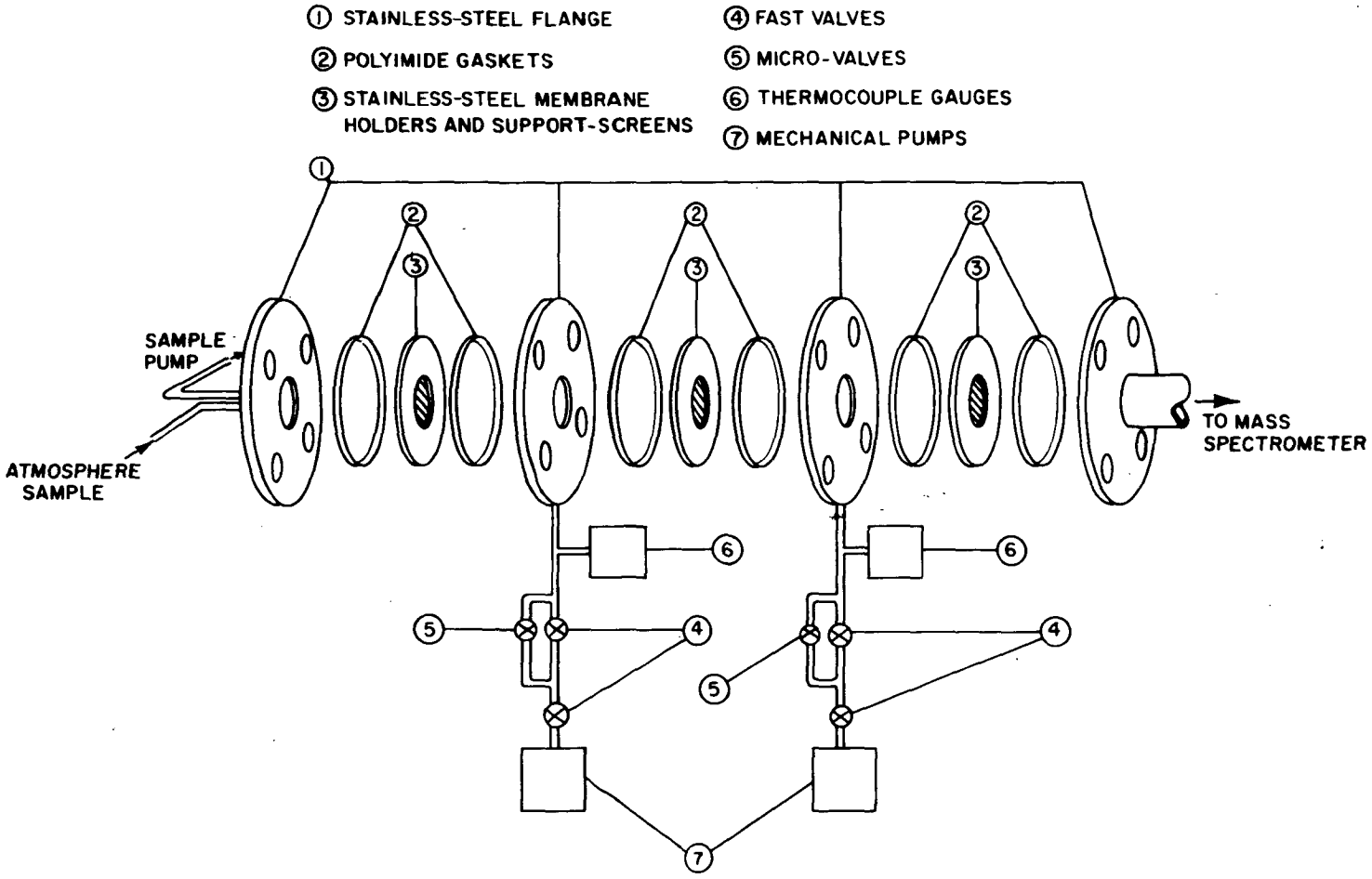


Figure 5. Components of Membrane Separator

The four vacuum flanges of the separator housing are made of stainless steel with gasket-face finish. The innermost flanges have a one-inch diameter hole in the center and a 1/4-inch diameter hole from the edge of the flange to the hole in the center. A length of 1/4-inch stainless-steel tubing forms a stem for interstage pumping. The inlet flange contains two 1/8-inch stainless-steel tubes which are welded to the center. One tube serves as the heated inlet tube, and the other connects to a flowmeter and an air pump to serve as the sample exit tube. Flow rates of 50 to 500 cubic centimeters per minute have been used. The final flange is welded to a 1/2-inch stainless-steel tube for connection to the mass spectrometer.

The membranes which have been used are made of 1-mil dimethylsilicone (General Electric). These membranes are attached to stainless-steel holders which contain a 1-inch diameter stainless-steel photo-etched support screen, 0.008-inch thick, and with a 19-percent open area. The support screen is arc welded into a recessed 3/4-inch-diameter hole in the center of the holder. The dimethylsilicone membranes are placed over the support screens and bonded to the holder with General Electric RTV-108 adhesive resin. After the adhesive has cured for 24 hours, the membranes and holders are conditioned for 8 hours at 300°C and 10^{-6} torr, to remove any uncured resin and free-radical initiator which may be left in the resin. The stainless-steel holders with the membranes are positioned between the vacuum flanges and are held in place, separately, by two 0.060-inch vacuum gaskets of Dupont "Polyimide." The properties of Polyimide are as follows:

a. Polyimide parts are able to withstand temperatures to 300°C for long periods of time,

b. Polyimide is inert to most aliphatic or aromatic solvents, ethers, alcohols, and tertiary amine componds,

c. The above compounds are not readily adsorbed on the polyimide surfaces,

d. The material has enough elasticity to deform under pressure and to make a vacuum seal.

The pumping system for each stage consists of a Welch 1402 roughing pump, which is isolated by a valve. Two valves in parallel are between the isolation valve and the separator flange; i.e., one for fast pumping, and the other (a bellows-sealed needle valve) for fine control of the interstage pumping. The complete assembly has been housed in an asbestos-insulated oven which can be heated to 300°C.

3.2 FIELD-IONIZATION SOURCE

A major drawback of ionization of a gas sample by electron impact is the fragmentation of gas molecules. As a result, the signatures of even simple molecules are complicated by the presence of ions other than those of the parent molecule.

Since fragmentation of molecular ions is greatly reduced if a field-ionization source is used rather than an electron-impact source,[3] the mass spectrometer has been equipped with a field-ionization source to be used interchangeably with the existing electron-impact source. The design of the source is based on previous work performed at the NASA Electronics Research Center.

The source consists of a matrix which has 2000 tips, one micron in diameter. This configuration is obtained by drawing a bundle of platinum wires which are embedded in a silver matrix. After drawing, about one-half inch of the matrix is cut off, and the wire tips are exposed by etching. The matrix is mounted on a high-voltage feedthrough, which is welded to a stainless-steel flange.

Another feedthrough, which also has been welded to this flange, is attached to a circular accelerating plate with a 1/4-inch hole in the center. The distance between tips and accelerating plate is adjustable. Typical operating potentials are 3.6 kilovolts positive on the tip and 10 kilovolts negative on the accelerating plate, from Fluke power supplies model Nos. 408B and 410B, respectively.

4. DRUGS

The drugs have been obtained from the following sources: (a) methadone-hydrochloride, morphine, cocaine, and codeine from Pennick Chemical, (b) chloral hydrate, d-amphetamine, and mephamphetamine from Aldrich Chemical, (c) chlorpromazine-hydrochloride in the form of Thorazine tablets (100 mg) from Smith, Kline, and French, (d) ethchlorvynol in the form of Placidyl capsules (100 mg) from Abbott Pharmaceuticals, (e) phenobarbital and secobarbital reference standards from U.S. Pharmacopeia, and (f) heroin-hydrochloride, LSD-tartrate, and mescaline sulfate from the U.S. Treasury Department, Bureau of Narcotics and Dangerous Drugs. All drugs have been used without further purification.

5. DRUG SIGNATURES

The drug signatures were obtained by two techniques. For those drugs with obvious odor, the vapor above the drug was "sniffed" directly. For this measurement, a vial containing the drug was placed close to the end of the heated inlet, and the sample pump was adjusted for flow rates of 50 to 500 cubic centimeters per minute, depending on the vapor pressure of the drug under study. The pressure and temperature of the membrane separator were adjusted for maximum throughput of material to the ion source.

Drugs which exhibited no noticeable odor were first checked to determine if they could be "sniffed," using the sample pump. If no results were obtained by the "sniffing" technique, a flash-evaporation technique was employed which did not require the air flow provided by the sample pump. First, a solution of the drug in a suitable solvent was prepared. For most drugs, the choice of solvent was ethyl alcohol and the solution concentrations were from 5 to 20 milligrams per milliliter. Second, an aliquot (1 to 5 microliters) of this solution was taken in a microliter syringe. This aliquot was injected into the inlet tube which was at room temperature. The temperature of the inlet was then raised to the desired temperature (200° to 320°) for vaporization and the mass spectrum was obtained.

6. EXPERIMENTAL RESULTS

The mass spectra which are presented in this section indicate the basic simplicity of field-ionization spectra as compared with electron-impact spectra. They also show that by means of the membrane separator the background of the major components of the ambient air can be kept sufficiently low to detect the presence of minute amounts of drug effluents. Thus, a new powerful analytical technique has been established which applies to many problems in chemical-trace analysis. The spectra shown in figures 6 to 16 illustrate this point (relative intensity versus mass-to-charge ratio).

The upper spectrum in these figures is that which has been determined with the electron-impact source; the lower spectrum, with the field-ionization source. Since the drug signatures are obtained in normal laboratory air, the signatures of this air background had to be established first (figure 6). These spectra demonstrate the selective properties of the membranes. Thus in the lower trace of figure 6, the peaks of water vapor (17, HO^+; 18, H_2O^+; 19, H_3O^+; and 37, $H(H_2O)_2^+$) exceed those of nitrogen (28, N_2^+) and oxygen (32, O_2^+). The additional peaks in this spectrum are argon (40, A^+; 41, AH^+) and carbon dioxide (44, CO_2^+). The peaks at 19 and 37 AMU are not present in the electron-impact spectrum (upper trace), and the peak at 41 AMU is greatly reduced. On the other hand, the electron-impact spectrum contains the fragmentation peaks at 14(N^+) and 16(O^+) AMU, which are absent in the field-ionization spectrum. Since there are essentially no background peaks beyond 50 AMU, the identification of the signatures of the drugs (molecular weight in excess of 135) is quite straightforward.

The major difference in the signatures which have been obtained by the two ion sources is the greater simplicity of the field-ionization spectra. Typically, the field-ionization spectra contain only a few characteristic peaks which serve as convenient means of qualitative identification. Except for ethchlorvynol, phenobarbital, cocaine, and codeine, all the spectra have ex-

hibited a "parent" peak; only in some cases, is this not also the largest major peak. Every drug, however, has one or more characteristic peaks which can be identified uniquely.

A list of the 14 drugs, their physical state, melting or boiling point, molecular weight, detection method, characteristic field-ionization mass peaks, and membrane temperature is presented on page twenty-eight. As shown, the solid drugs with melting points near 60°C and the liquid drugs with boiling points to 212°C exhibit sufficiently high-vapor pressures to be detected by direct sniffing. Solids with melting points above 60°C have to be dissolved in a suitable solvent to be detected by the flash-evaporation technique. The flash-evaporation technique has necessitated rapid scanning by the mass spectrometer to cover the entire mass range before the drug material is completely vaporized. These fast scans require a higher level of drug concentration to produce a signature. Once the signature has been determined, a narrow scan over the appropriate mass range of the signature is sufficient for detection of considerably smaller drug concentrations.

Morphine, heroin-hydrochloride, LSD-tartrate, and mescaline sulfate have been available only in 5-milligram quantities, and solutions of only 5 milligrams per milliliter have been prepared. A microliter aliquot of these solutions may not yield sufficient vaporized material to be detected. Morphine is not sufficiently soluble in alcohol, and it is not desirable to introduce water, the only other solvent available, into the mass spectrometer. The melting point of morphine is greater than 250°C, which is the maximum operating temperature of the membranes before the onset of membrane rupture. Heroin-hydrochloride also has a high melting point (243°C), which may limit its detectability. It is also possible that the vapor from these four drugs cannot penetrate the membranes in sufficient quantity to produce a measurable signal in the mass spectrometer.

The following experiment will illustrate the ease and rapidity of the flash-evaporation technique for clinical samples. Figure 17, upper trace, is the mass spectrum of urine; figure 17,

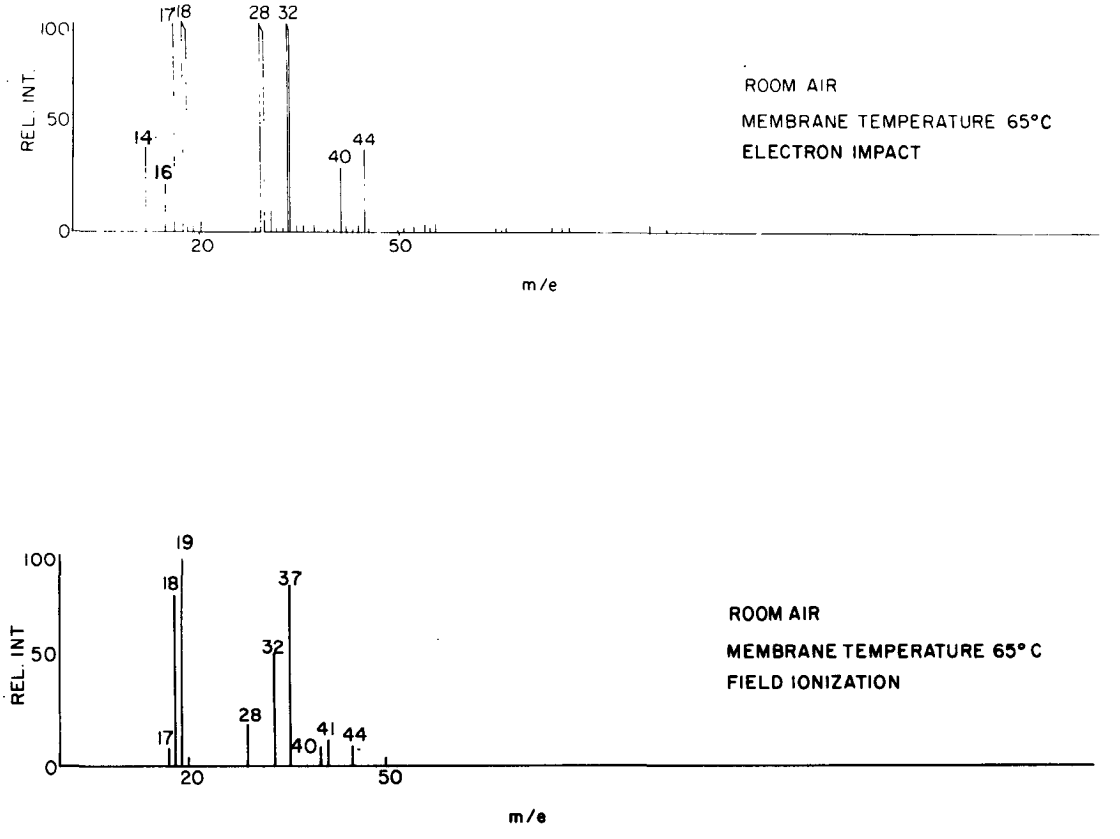


Figure 6. Mass Spectrum of Room Air

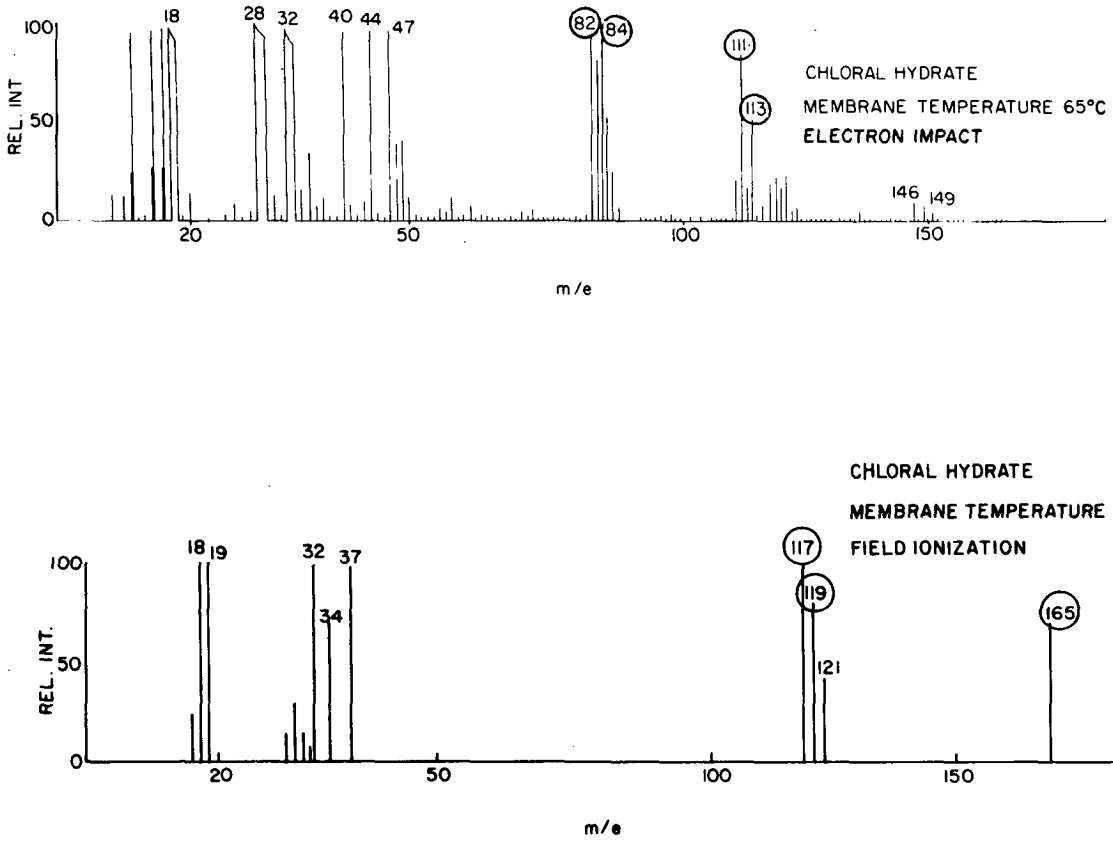


Figure 7. Mass Spectrum of Chloral Hydrate

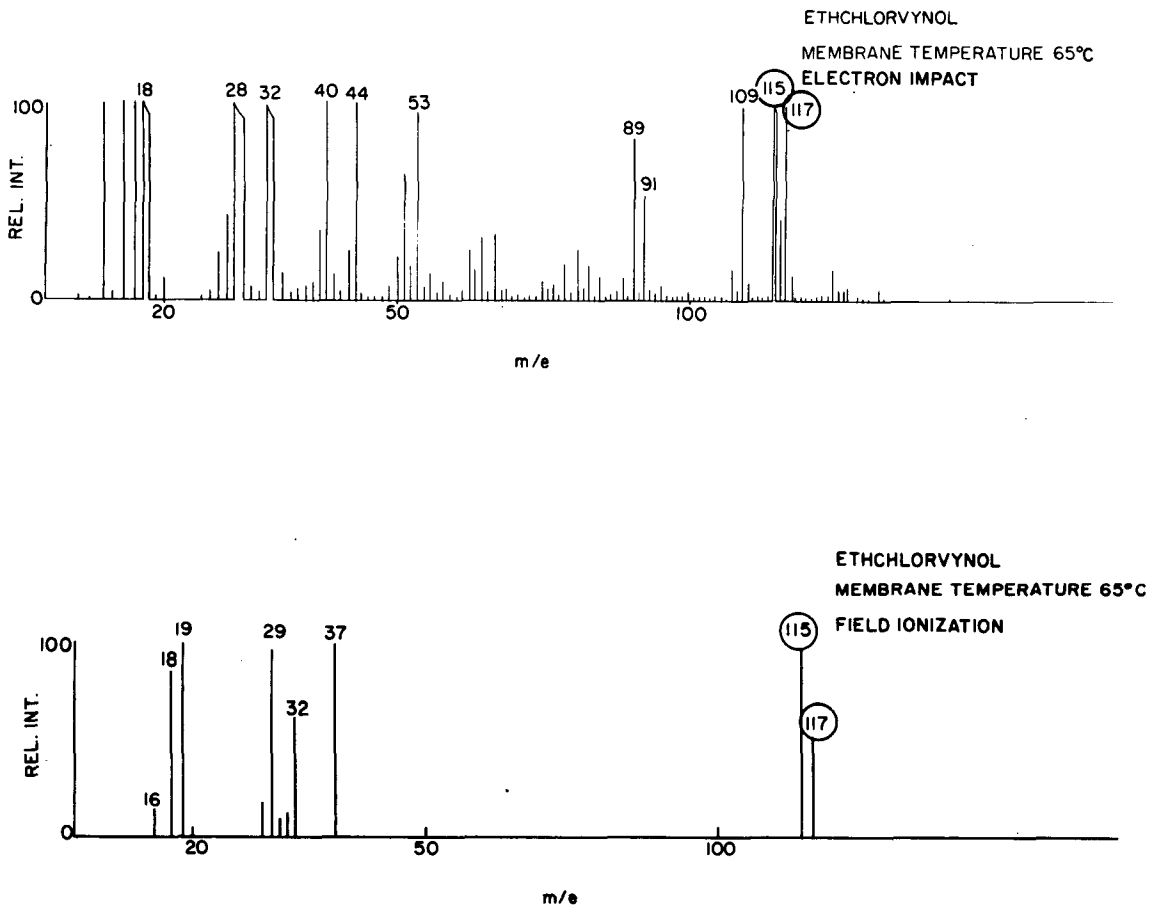
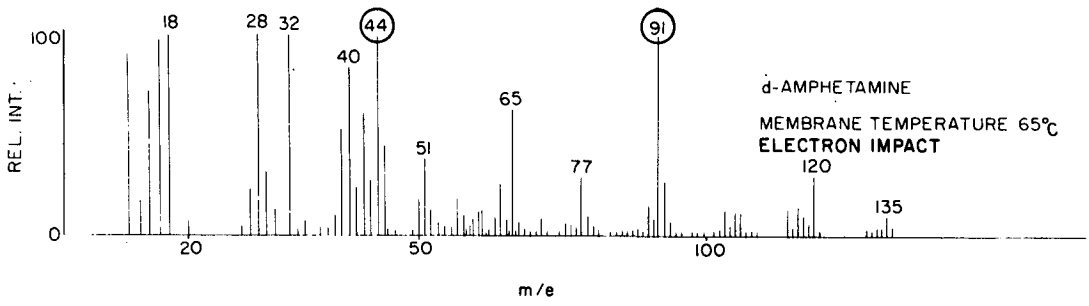


Figure 8. Mass Spectrum of Ethchlorvynol



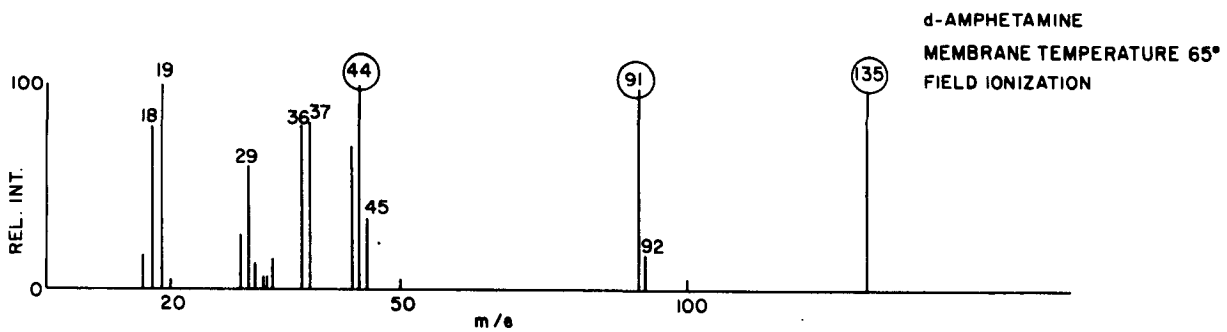


Figure 9. Mass Spectrum of d-Amphetamine

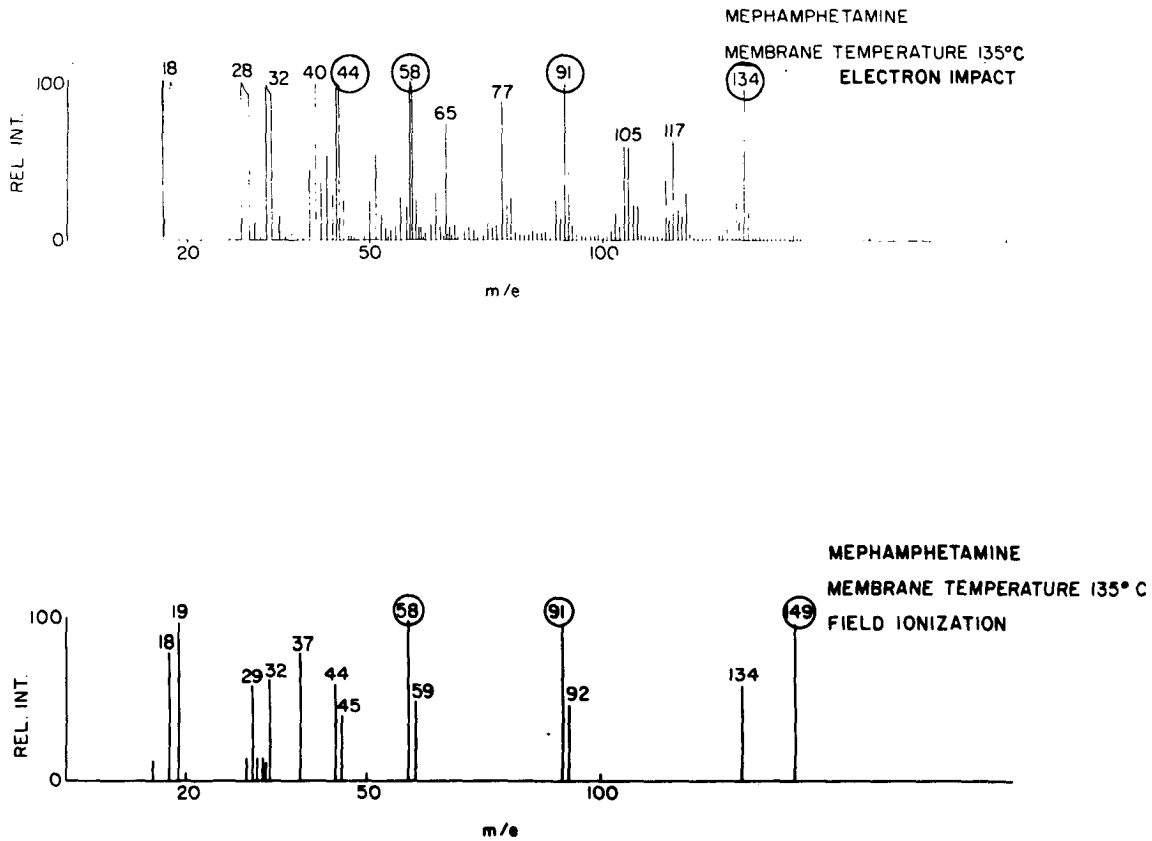
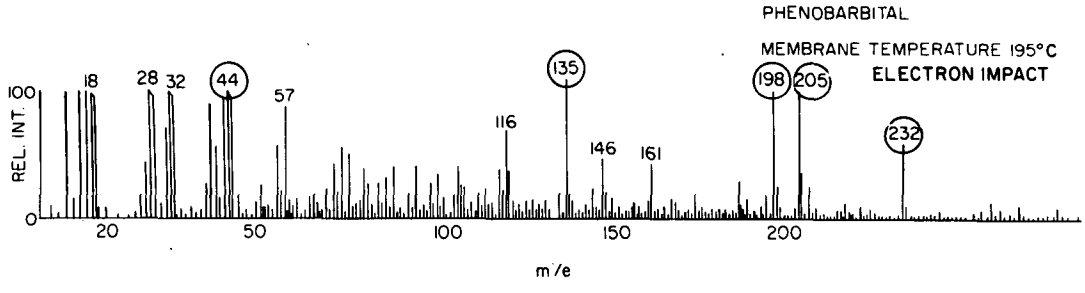


Figure 10. Mass Spectrum of Mephamphetamine



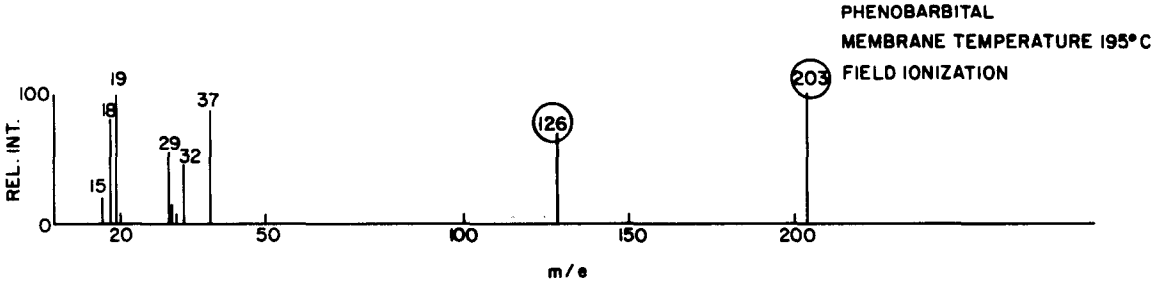


Figure 11. Mass Spectrum of Phenobarbital

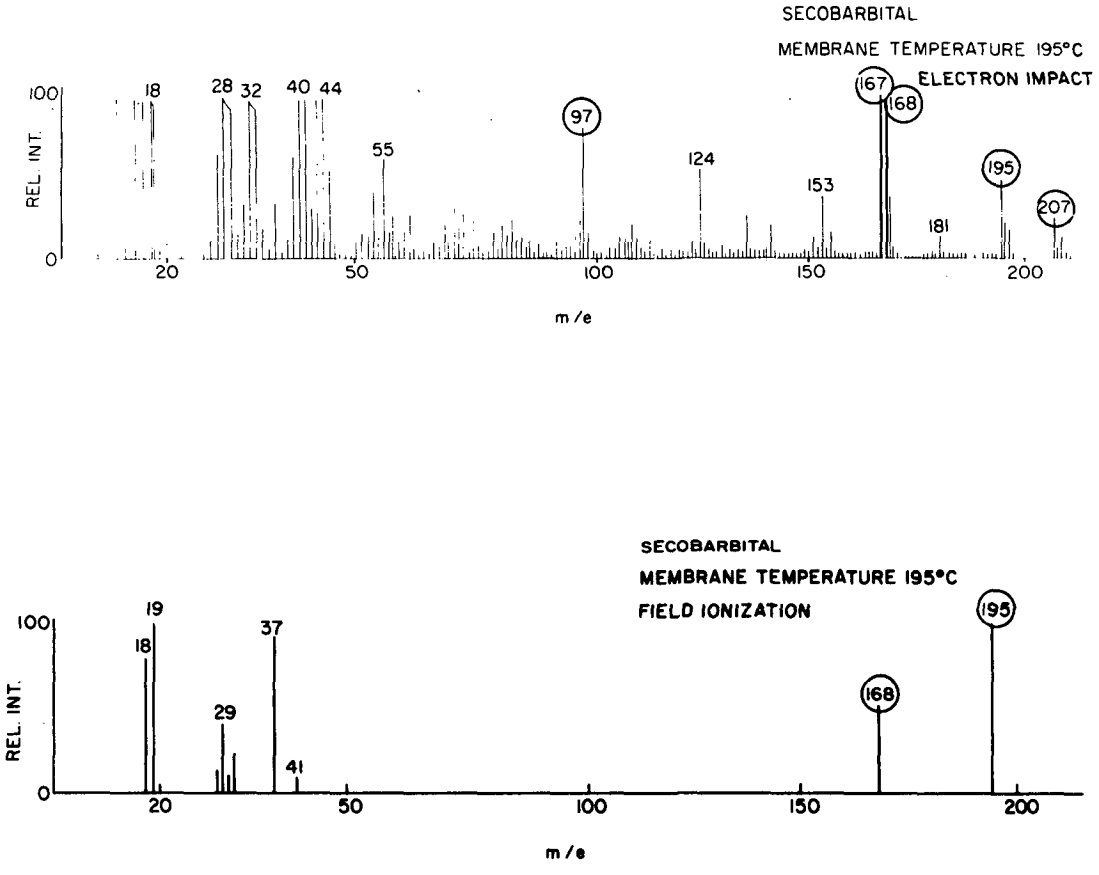


Figure 12. Mass Spectrum of Secobarbital

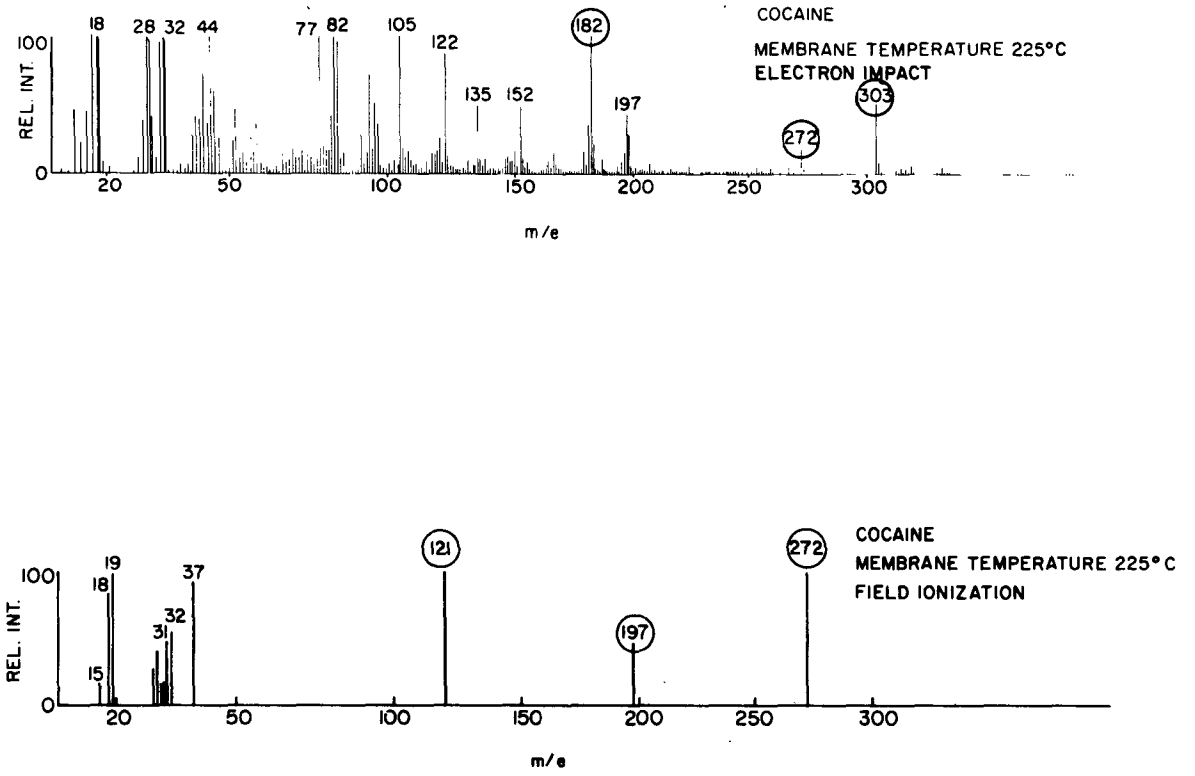


Figure 13. Mass Spectrum of Cocaine

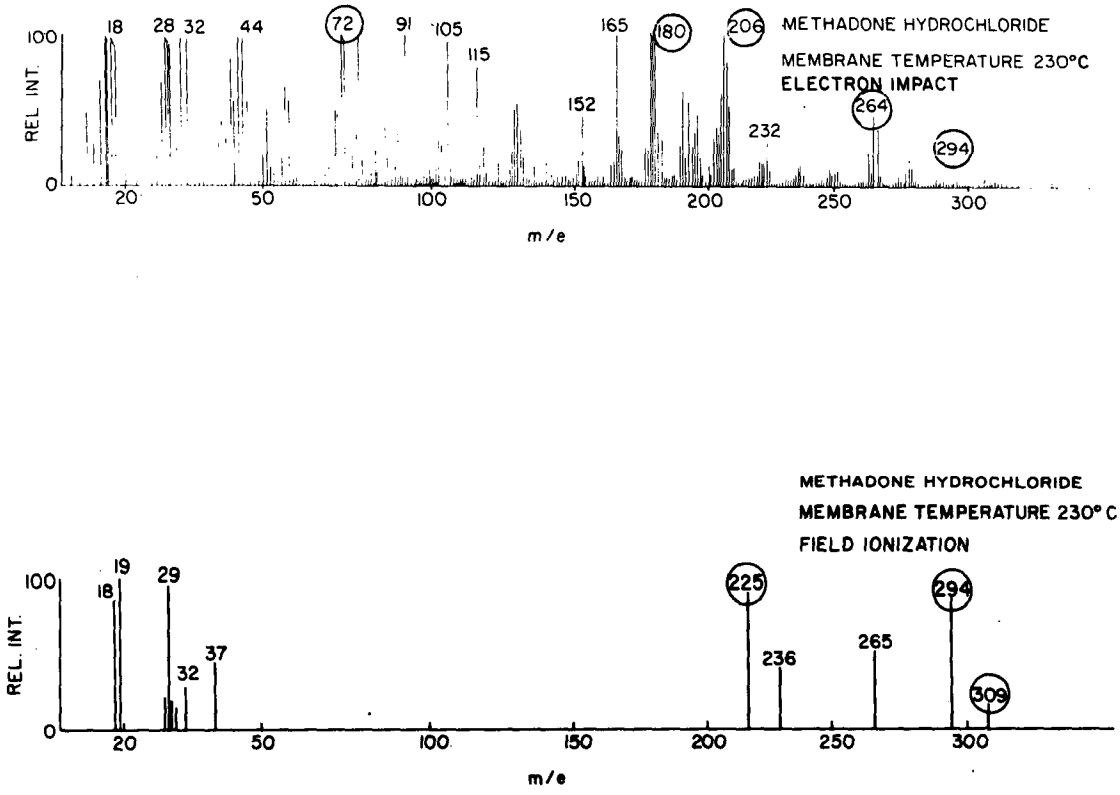


Figure 14. Mass Spectrum of Methadone Hydrochloride

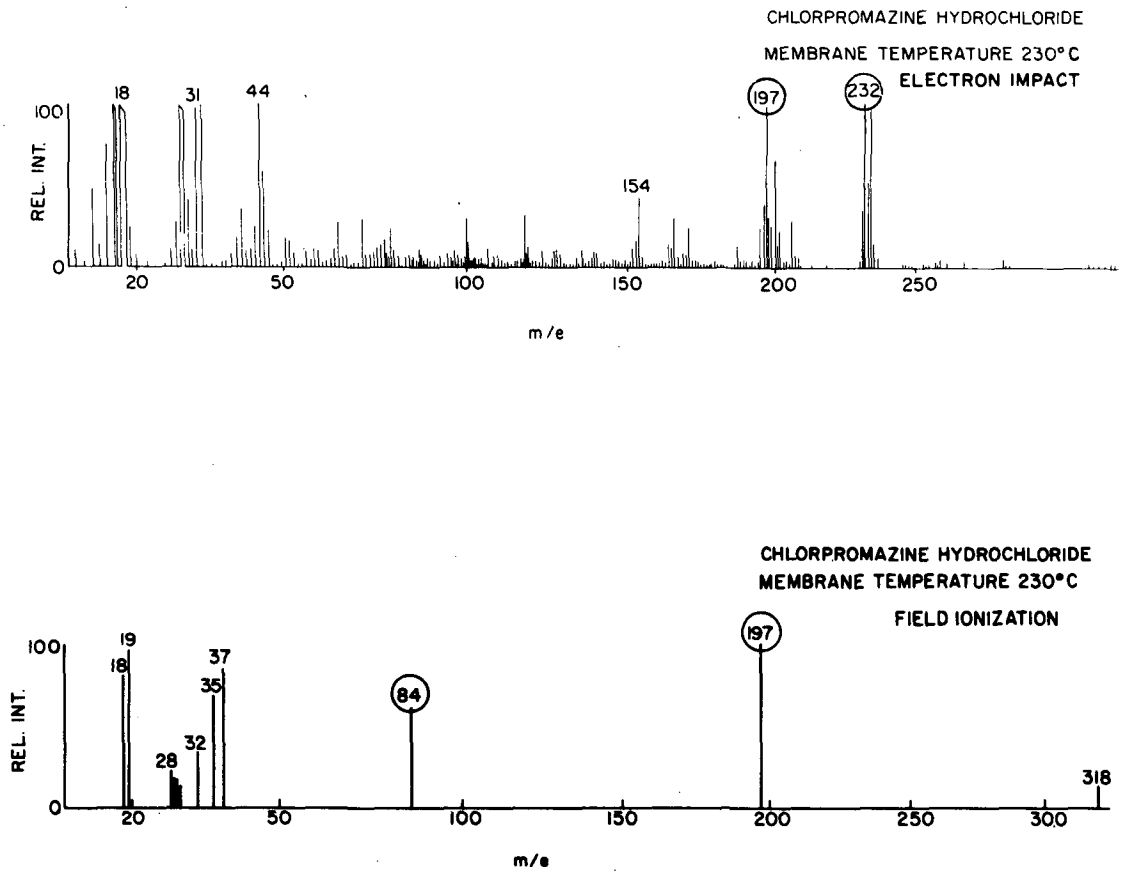


Figure 15. Mass Spectrum of Chlorpromazine Hydrochloride

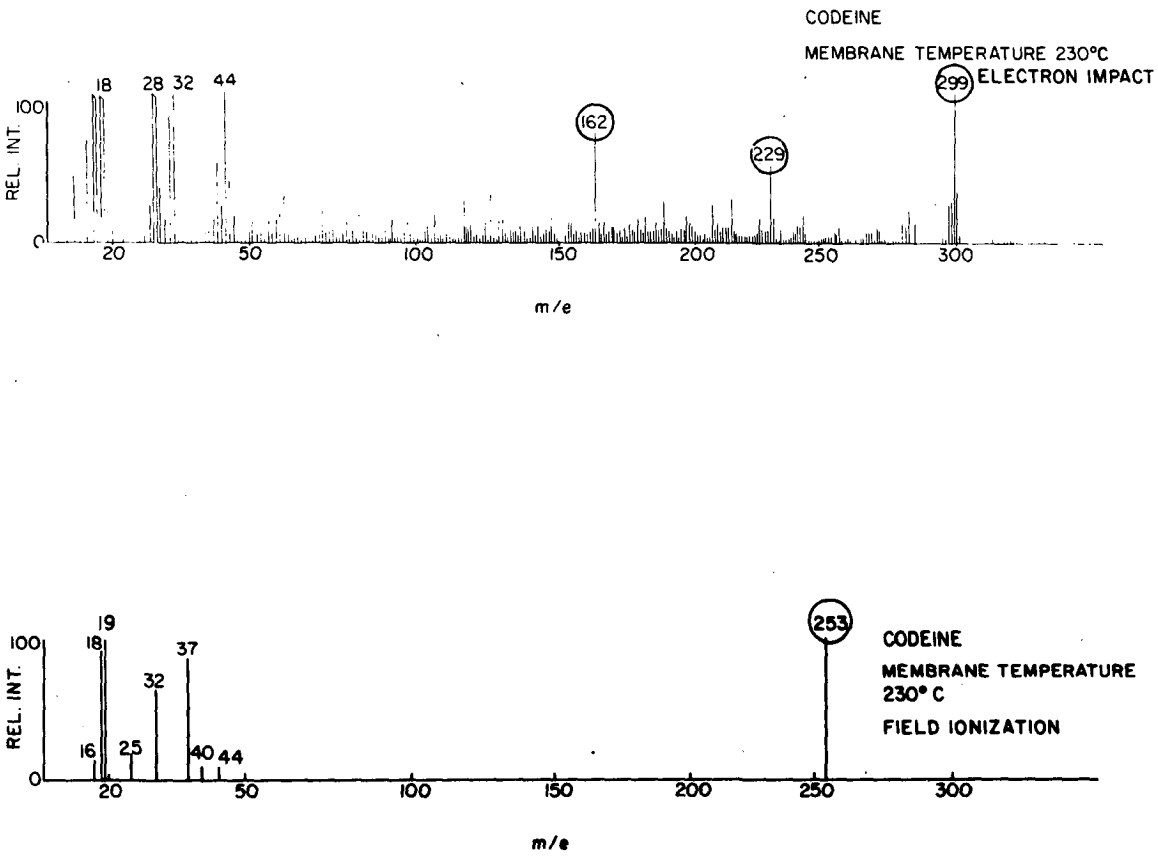


Figure 16. Mass Spectrum of Codeine

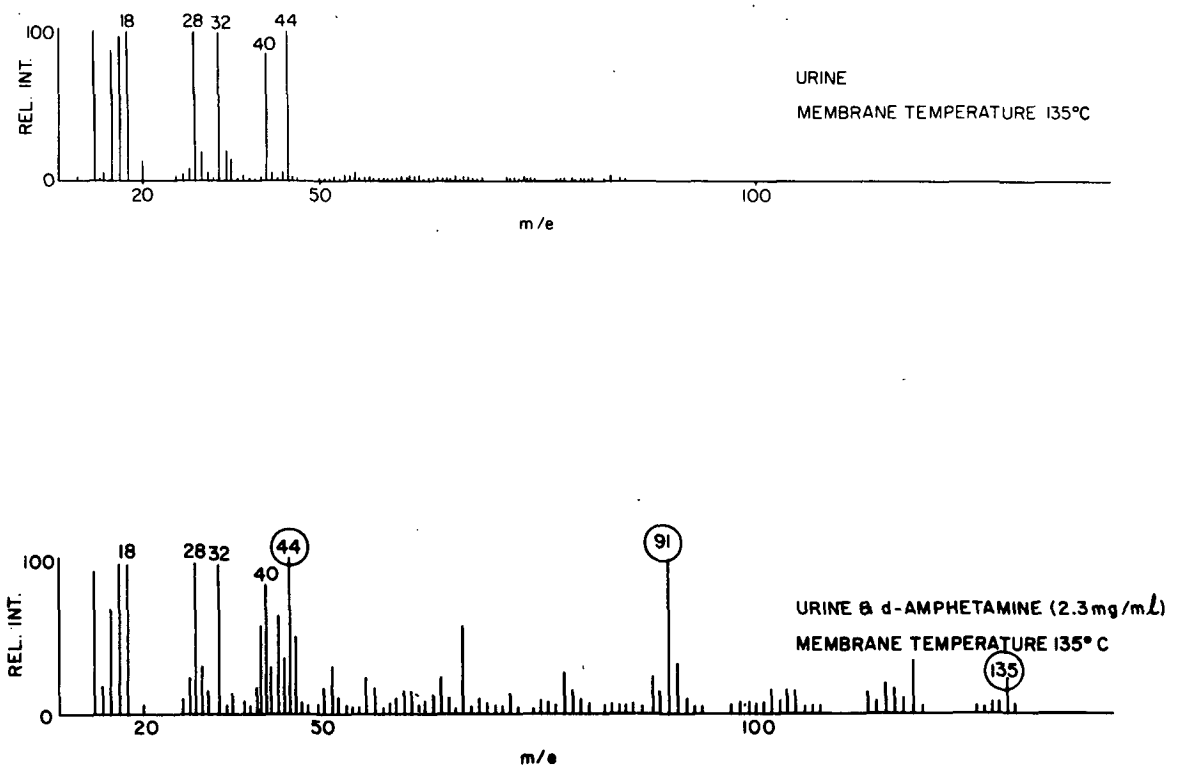


Figure 17. Mass Spectra of Urine (Upper Trace) and of a Standard Sample of Urine + d-Amphetamine (Lower Trace)

the lower trace, is that of a standard solution of d-amphetamine in urine (2.3 milligrams per milliter) from a 5-microliter aliquot. By comparison with the signature of d-amphetamine in figure 9 (upper trace), the characteristic peaks at 91 and 135 AMU can be clearly identified. Since the peak at 44 AMU is characteristic of urine as well as of the drug, it cannot be used for identification of this drug in urine.

Once the characteristic peak for a given situation is established, it is no longer necessary to perform a mass spectrometer scan. This is illustrated in figure 18, which shows the response of the mass spectrometer to an actual clinical sample of a d-amphetamine in urine (7 micrograms per milliter) (Leary Laboratories, Boston, Mass.). The mass spectrometer was preset to the peak at 91 AMU, characteristic of d-amphetamine. A 5-microliter aliquot of the sample was injected into the inlet, the inlet temperature was raised for evaporation and the change in the peak intensity was recorded with time. Within 30 seconds, the peak intensity rose to a maximum. This performance demonstrates the potential of the technqiue for rapid analysis.

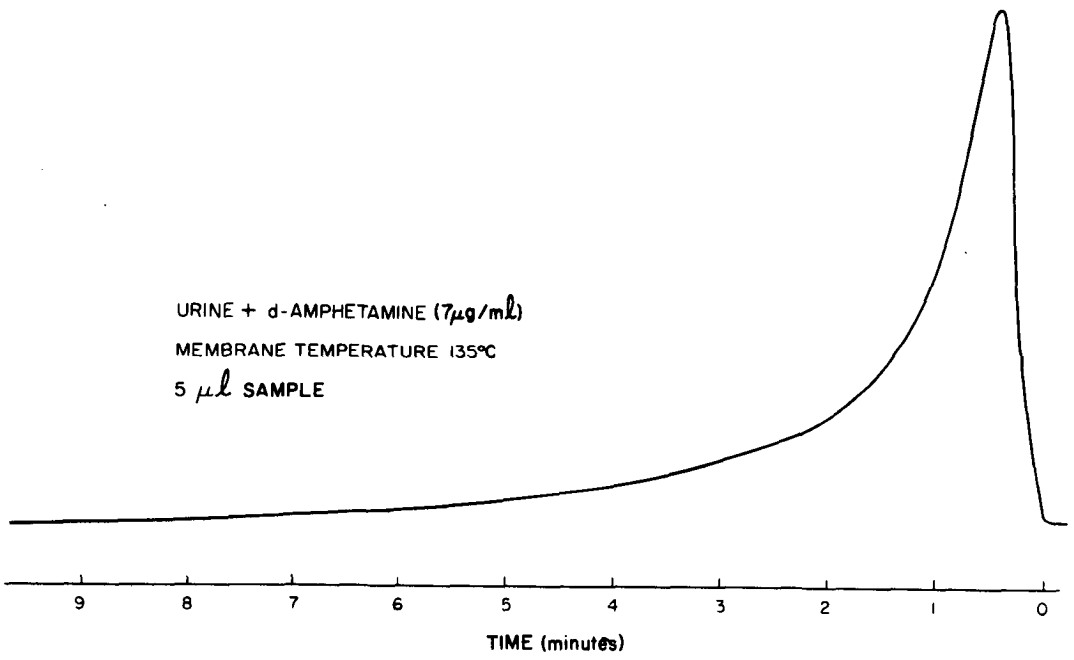


Figure 18. Response at 91 AMU to Clinical Sample of Urine + d-Amphetamine

Summary of Drug Characteristics

Drug	State	Melting point °C	Boiling point °C	Molecular weight	Method of detection	Characteristic peak m/e*	Membrane temperature °C
Chloral Hydrate	solid	50-58	--	165.4	sniffing	165, 117, 119	65
Ethchlorvynol	liquid	--	172-174	144.6	sniffing	115, 117, 29	65
d-amphetamine	liquid	--	200-203	135.2	sniffing	135, 91, 44	65
Mephamphetamine	liquid		212	149.2	sniffing	149, 58, 91	135
Phenobarbital	solid	174-178	--	232.2	flash evaporation	203, 126	195
Secobarbital	solid	100	--	238.0	flash evaporation	195, 168	190
Cocaine	solid	98	--	303.0	flash evaporation	272, 197	225
Methadone-HCl	solid	235	--	345.9	flash evaporation	294, 225	230
Chlorpromazine-HCl	solid	179-180	--	355.3	flash evaporation	197, 84	230
Codeine	solid	154-156	--	299.3	flash evaporation	253	230
Morphine	solid	254	--	285.3	-- --	--	--
Heroin-HCL	solid	243	--	423.9	-- --	--	--
LSD-Tartrate	solid	198-200	--	473.4	-- --	--	--
Mescaline sulfate	solid	183-186	--	459.0	-- --	--	--

* The first peak tabulated is the most characteristic and the most abundant (relative intensity).

7. CONCLUSIONS

Although limited to only a small number of drugs and a single clinical sample, the results which have been described are considered significant and encouraging. Thus, under present conditions, the minimum detectable concentration of d-amphetamine in urine is about 1 microgram per milliliter. Further modifications of the laboratory instrument are now underway; namely, addition of an electron multiplier with a gain of 10^6 (as compared to the present gain of 10^4) and enlargement of the analyzer slits to optimize sensitivity without sacrifice of the necessary resolution. In this manner, the minimum detectable concentration level might be improved into the range of 1 nanogram per milliliter. The use of the technique for direct detection of the drugs on breath remains to be demonstrated. Further work is planned with human subjects under clinical supervision.

8. REFERENCES

1. Kiser, R. W., Introduction to Mass Spectrometry and Its Application, Prentice Hall, Englewood Cliffs, N. J., 1965, 350p.

2. Green, D. E., "Highly Sensitive, Adaptable Procedures for Mass Spectrometric Real-Time Monitoring of Drugs and Other Compounds," Intra-Science Chemistry Reports 4 (1970), 211-221.

3. Beckey, H. D., Field Ionization Mass Spectrometry, Pergamon Press, New York, N. Y., 1971, 344p.

78723