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Chemical Ionization Mass Spectrometry: A Rapid Technique for Forensic Analyses

The need for a rapid and accurate method for drug analysis has increased substantially in this country over the past few years. This has been due in large part to the rise in drug abuse and overdose cases now being handled by toxicologists and analytical chemists. In an effort to meet this need, recent applications of the gas chromatograph/mass spectrometer (GC/MS) to drug and drug metabolite analysis has been successfully accomplished [*1,2*]. This application has recently been enhanced by the development of a new technique—chemical ionization (CI). The CI technique provides simpler spectra with increased sensitivity, while allowing the optional deletion of the gas chromatograph.

With this method, the characteristic ionization of the materials for mass analysis is produced by ionic chemical reaction in the gas phase rather than by electron impact. Electron impact ionization (EI) is a physical process involving an electron-molecule collision to produce ions, while CI is a phenomenon resulting from ion-molecule chemical interactions.

CI requires a mixture of a reagent gas with the sample vapor such that the reagent gas is in excess by several thousandfold. The total pressure in the source region is 1 torr, while the pressure outside the source is 10^{-4} torr and the analyzer pressure is 10^{-5} torr (Fig. 1). Primary ionization of the reagent gas occurs via electron impact. The reagent gas then reacts with itself to form a stable set of ions (Fig. 2) which will not react further with the reagent molecules. Thus, these ions are available for ionization of the sample via ion-molecule reactions.

When a reagent ion encounters a sample molecule, one of several chemical interactions may occur, depending upon the nature of the reagent-sample pair as shown in Fig. 3. These interactions include transferring a proton, a hydride ion, another ion fragment, or simply a charge. In most cases the proton exchange reaction predominates. The number of sample ions produced is a large fraction of the sample molecules introduced into the system.

The ions produced by chemical ionization are different in several analytically advantageous ways from ions produced by electron ionization. The average energy transferred to the sample molecule by the reagent ion during the ionization is many times smaller than that transferred by electron impact ionization. In addition, the hydride ion, proton, or other ion fragment transferred produces sample ions with even numbers of electrons. Even electron ions are inherently more stable than the odd electron ions produced by electron impact. The sample ions are also stabilized by the

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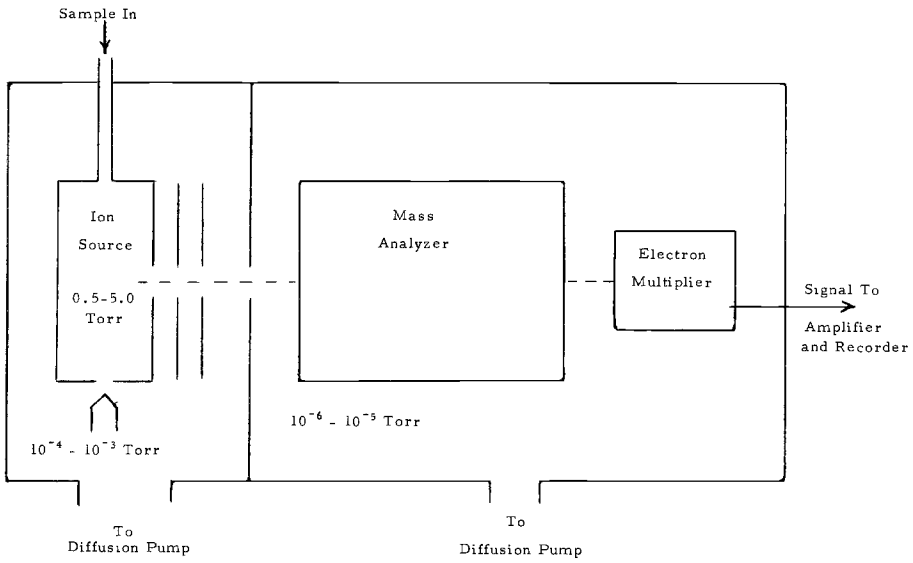

FIG. 1—*Schematic of chemical ionization mass spectrometer showing different pressure regions.*

$$CH_4 + e^- \rightarrow CH_4^+ + 2e^-$$
$$\rightarrow CH_3^+ + H + 2e^-$$
$$\rightarrow CH_2^+ + H_2 + 2e^-$$

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

$$CH_2^+ + 2CH_4 \rightarrow C_3H_5^+ + 2H_2 + H$$

FIG. 2—*Ion-molecule reactions of methane at 1 torr.*

$$CH_5^+ + MH \rightarrow MH_2^+ + CH_4$$

$$C_2H_5^+ + MH \rightarrow MH_2^+ + C_2H_4$$

$$C_2H_5^+ + MH \rightarrow M^+ + C_2H_6$$

FIG. 3—*Reactions of "high pressure" methane ions with sample molecules.*

relatively high source pressures. These effects combine to cause less fragmentation of the sample ions than is observed in EI mass spectrometry.

Most biochemical compounds accept a proton from the reagent gas upon ionization, which results in a peak at a mass that is one unit greater than the molecular weight of the drug (Fig. 4). Some fragmentation may also occur. The amount of fragmentation is proportional to the amount of excess energy and thus depends upon the nature of the reagent gas. Isobutane has a high proton affinity and, therefore, does not transfer much excess energy and causes little or no fragmentation. Methane provides a small amount of fragmentation due to its moderate proton affinity. Hydrogen has a low proton affinity, transfers more excess energy, and causes substantial fragmentation. Figure 5 shows the

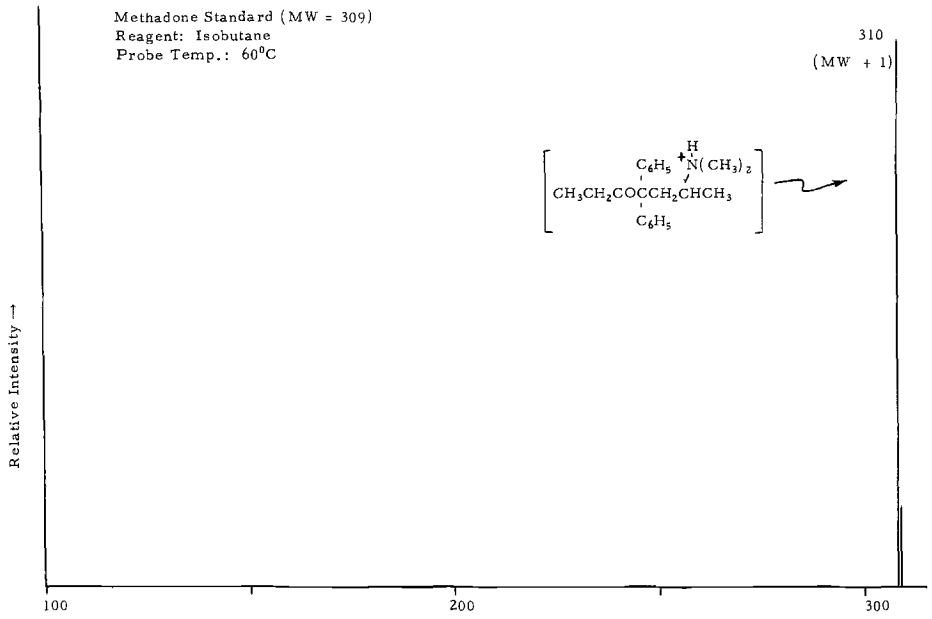

FIG. 4—*Chemical ionization spectrum of methadone.*

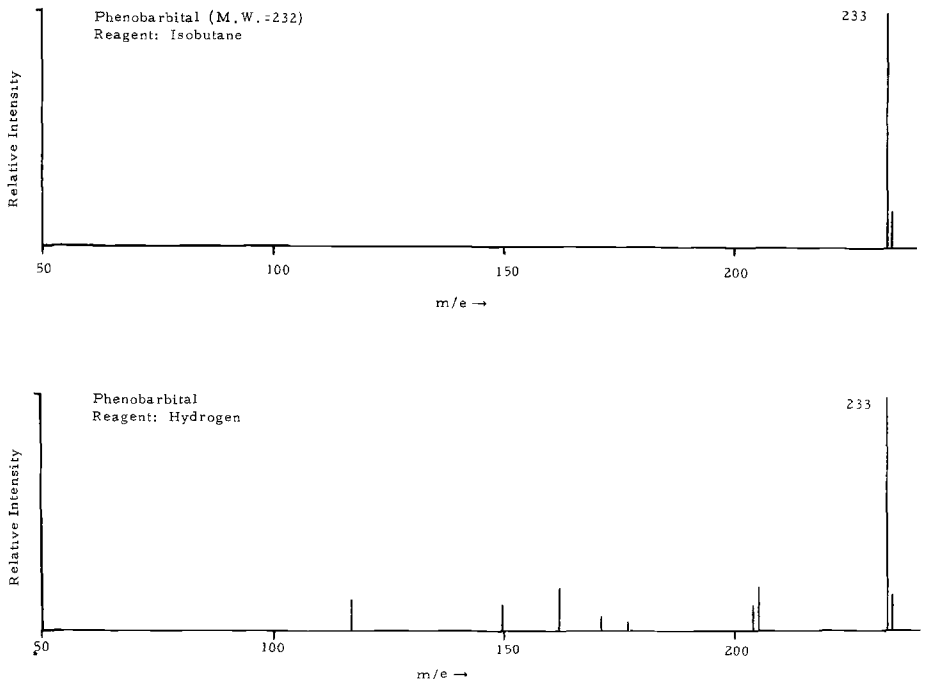

FIG. 5—*Chemical ionization spectrum of phenobarbital with isobutane and hydrogen.*

increase in fragmentation of phenobarbital with a change in reagent gas.

No fragmentation is desirable if one wishes only to know the molecular weight of the sample; minor fragmentation is helpful as confirmatory identification, while moderate fragmentation can provide positive identification of an unknown. Little or no fragmentation is desirable when analysis of a mixture of compounds is needed, and the list of possible components is limited to several hundred items.

CI lends itself well to the analysis of biochemical compounds, since all of them contain a nitrogen or oxygen atom. These are invariably nucleophilic sites, ideal for the acceptance of a donated proton. The nucleophilic nature of these compounds increases the reaction cross section and enhances the sensitivity. Presently, chemical ionization instrumentation is being used for analysis of drugs, pesticides, air pollutants, steroids, lipids, amino acids, and autacoids.

A particularly appropriate application of CI is its use in drug analyses. It may be used as a routine screening device, with isobutane as the reagent gas, providing only a single peak for each component of a mixture. It may also be used for positive confirmatory analyses by using an appropriate reagent gas (for increased fragmentation) with or without a gas chromatograph, depending upon the complexity of the sample.

Instruments for the specific application of chemical ionization mass spectrometry to the analysis of drugs are commercially available. In the system[2] used by the authors, the use of isobutane as the reagent gas provides a simple, reliable, and efficient method for each drug or drug metabolite. Table 1 lists some of the common drugs which can be identified by this technique. Any drug listed in the table may be positively identified in body fluids at concentrations down to 0.1 μg/ml (0.1 ppm). In general, the chemical structures of all drugs are such that they will yield easily interpreted chemical ionization mass spectra at a concentration level similar to that stated. The identification of an unknown drug may be verified in a simple fashion by comparison of its spectra with that of an authentic sample. If any two compounds are found to give the same spectrum via

TABLE 1—*Partial list of important drugs identified by chemical ionization mass spectrometry* [1].

Acetaminophen	Cyclobarbital	Methapyrilene
Acetophenetidin	Dextropropoxyphene	Methaqualone
Acetyl salicylic acid	Diazepam	Methyl phenidate
Alphaprodine	Diphenyl hydantoin	Methyprylon
Allylbarbituric acid	Diphenoxylate	Morphine
Amobarbital	Ephedrine	Nalorphine
Amphetamine	Epinephrine	Nicotine
Antipyrine	Ethinamate	Normorphine
Aprobarbital	Glutethimide	Pentobarbital
Barbital	Heptabarbital	Phenazocine
Butabarbital	Hexobarbital	Phencyclidine
Butethal	Hydromorphone	Phenobarbital
Caffeine	Levorphanol	Probenecid
Carbromal	Lignocaine	Procaine
Chlordiazepoxide	Meperidine	Quinine
Chloroquine	Meprobamate	Secobarbital
Chlorothiazide	Mescaline	Scopalamine
Chlorpromazine	Methadone	Thebain
Cocaine	Methamphetamine	THC (Δ^9)
Codeine		

[2] BIOSPECT®, Scientific Research Instruments Corp., Baltimore, Md.

isobutane chemical ionization, these compounds may be differentiated by the use of other reagent gases or by simple extraction techniques.

In addition, the EI spectrum of any compound may be obtained to provide information which is compatible with presently available mass spectral libraries [*3,4*]. The EI spectra may be obtained by turning off the reagent gas or by using dry helium as the reagent gas. The latter method is more effective because it results in increased sensitivity. Figure 6 shows how well the helium CI spectrum of aspirin matches the EI spectrum of the same compound. This multidimensional aspect of obtaining mass spectra is a powerful means of identifying the more difficult unknown organic compounds because it combines the molecular weight determination ability of chemical ionization with the extensive "fingerprinting" aspect of the electron impact ionization. This aspect may also be obtained through the use of mixtures of reagent gases to provide "mixed" EI/CI spectra [*5,6*]. These mixed spectra provide a strong indication of the molecular weight, along with the desired amount of fragmentation for positive identification.

A block diagram of a chemical ionization mass spectrometer is shown in Fig. 7. The basic unit is the CI quadrupole mass spectrometer with differential pumping. An optional gas chromatograph is also shown in the diagram. The reagent gas passes either through the GC or the solids probe inlet, carrying the sample with it into the ion source. After ionization of the sample occurs, the ion beam is mass analyzed and the resulting signal is amplified and appears on an oscilloscope display for easy recognition. A hard copy of any spectra appearing on the oscilloscope can be made by the oscillographic recorder. Also, a total ion monitor (TIM) recorder is available to provide a chromatogram of all the peaks eluting from the GC.

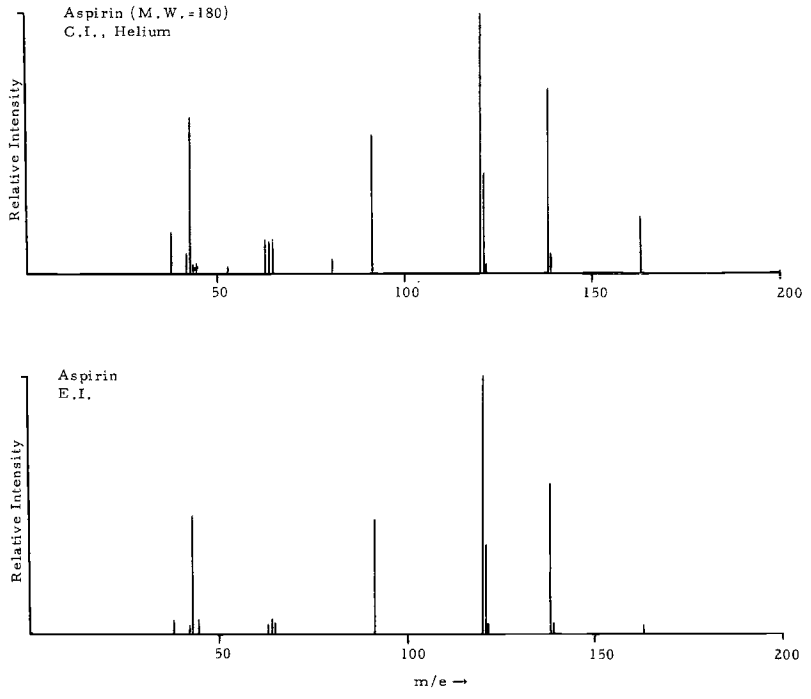

FIG. 6—*Comparison of electron impact and chemical ionization (helium) spectra of aspirin.*

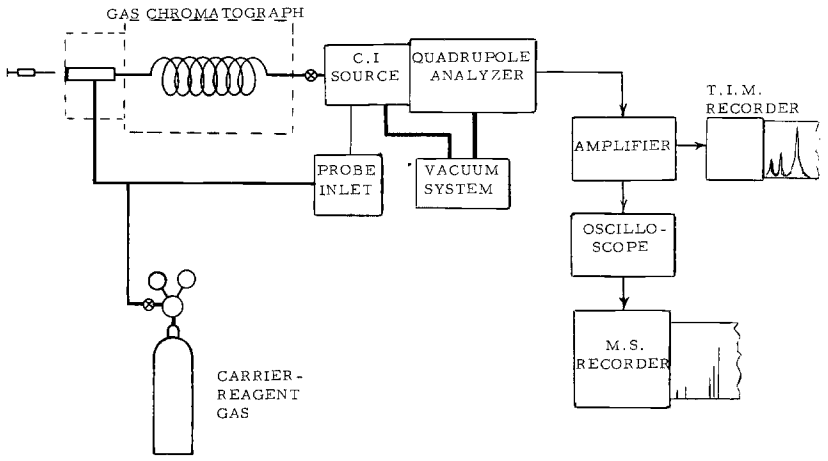

FIG. 7—*Block diagram of a chemical ionization mass spectrometer system (see Footnote 2).*

In an effort to demonstrate the sensitivity of this instrument, a control sample of urine was doped with 1 ppm of methadone and 2 ppm of dextropropoxphene. As can be seen in Fig. 8, a strong response was measured. The peak at m/e 279 represents butyl phthalate, a common plasticizer. The origin of this compound is attributed to contamination from a plastic container. This experiment, along with others, has shown this method capable of measuring 10 to 100 pg of sample.

During a methadone maintenance program at a local hospital, a chemical ionization mass spectrometer was used to monitor the urine samples of the patients. Those patients who were taking drugs outside the program were identified as well as the specific identity of the drug(s) of abuse. During the course of these analyses, it was noted that the progress of the metabolism of the methadone could be monitored. This was done by noting the ratio of the pure drug and one of the methadone metabolites in the urine. Figure 9 shows two spectra representative of different stages of metabolism. The metabolite at m/e 278 appears to be the *N*-demethylated metabolite identified by Beckett et al [7]. The relative intensity of the metabolite peak and methadone peak indicate the elapsed time between methadone ingestion and urine sampling.

Isobutane is a particularly useful reagent gas, since it tends to produce only one or two peaks in a spectra for each compound present. Thus, if a sample contains a mixture of several drugs, identification of the components may be made without prior separation if isobutane is the reagent gas. Examples of this technique are shown in Figs. 10 and 11. A

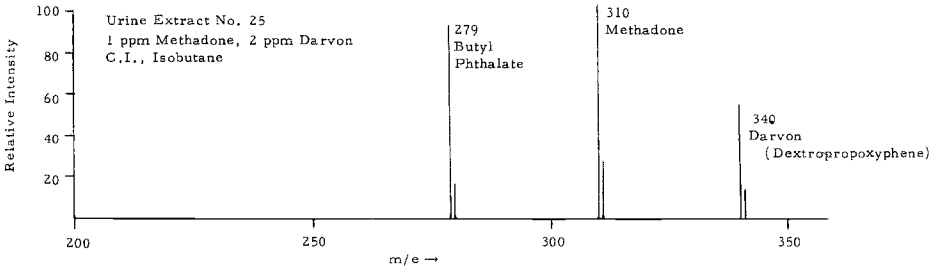

FIG. 8—*Chemical ionization spectrum of 1 ppm of methadone and 2 ppm of dextropropoxyphene.*

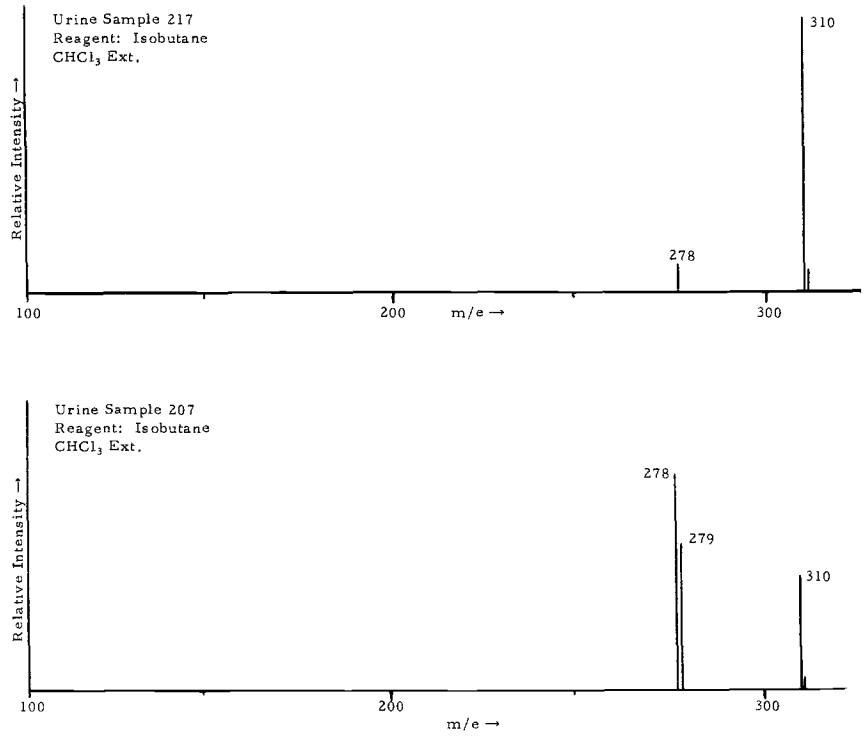

FIG. 9—*Comparison of urine extracts of different sampling times.*

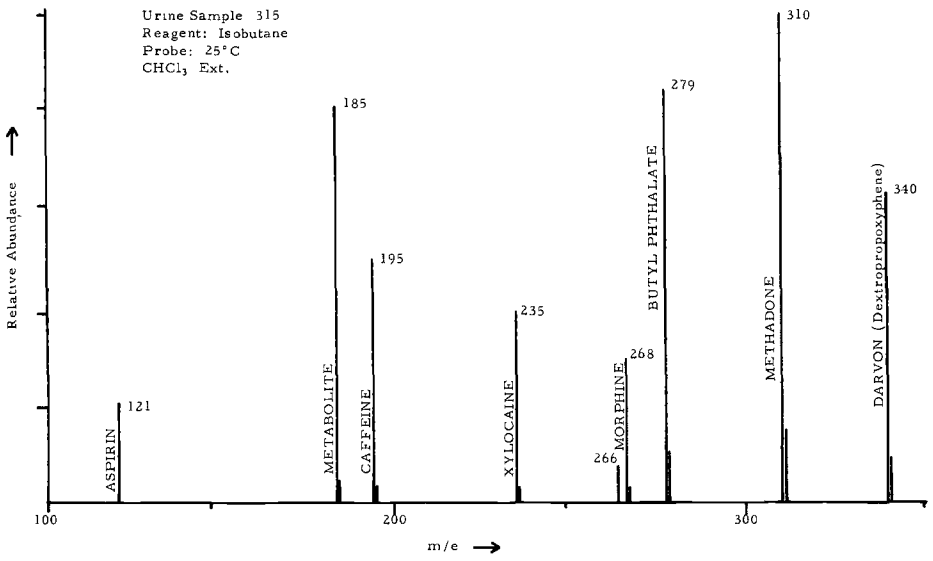

FIG. 10—*Chemical ionization (isobutane) spectrum of urine extract.*

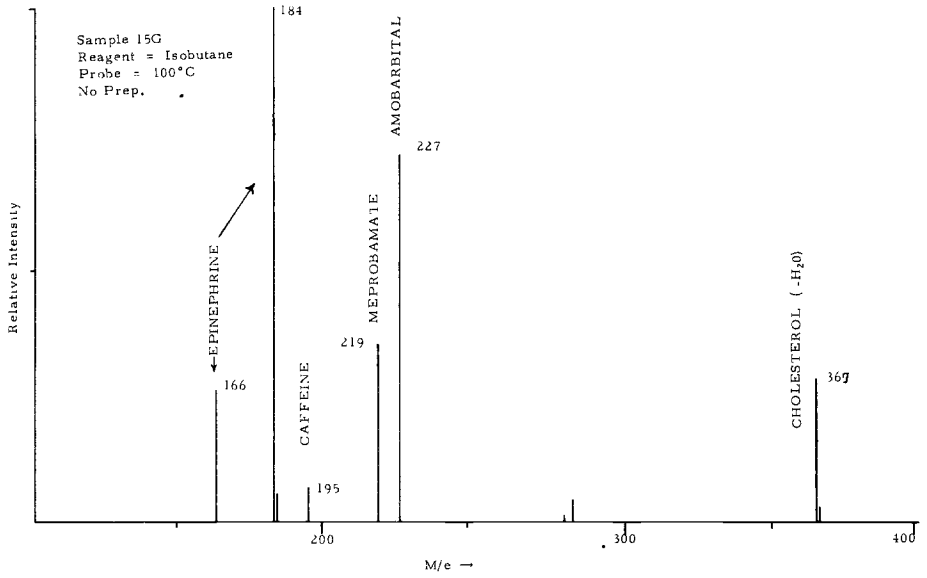

FIG. 11—*Chemical ionization (isobutane) spectrum of raw gastric lavage.*

simple chloroform extract of an urine sample was taken at a neutral pH. The spectrum of this extract reveals the presence of dextropropoxphene, methadone, butyl phthalate, morphine, xylocaine, caffeine, and aspirin. The peak at m/e 185 is an unidentified metabolite, while the peaks at m/e 286 and 266 represent morphine and dextropropoxphene, respectively. Similarly, a raw gastric sample injected directly into the mass spectrometer gave a spectrum (Fig. 11) identifying cholesterol, amobarbital, meprobamate, caffeine, and epinephrine.

The application of chemical ionization mass spectrometry to the analysis of a wide variety of compounds has demonstrated its versatility and general applicability. Longevialle et al [*8*] have studied a broad series of biochemical compounds using this technique. A good review of this subject has been written by Munson [*9*]. Applications of particular interest to the forensic scientist include the identification of drug metabolites [*10*], components of marihuana smoke [*11*], barbiturates [*12*], and dangerous drugs [*1*].

The application of chemical ionization mass spectrometry to the forensic sciences appears to provide a rapid new technique of analysis applicable to many different areas of interest. The simplicity and sensitivity of CI, along with its ability to use many different reagent gases to provide different effects, provides a powerful tool to aid the analytical chemist.

Summary

Chemical ionization mass spectrometry offers unique analytical capabilities. This analysis system provides a simple spectrum which allows easy identification of the molecular weight of the sample. Likewise, components of a mixture may be identified without prior separation. By changing the reagent gas, fragmentation of the sample can be effected for positive identification.

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