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The Gas Chromatograph-Mass Spectrometer as a New and Important Tool in Forensic Toxicology

The growing use of drugs in this country has caused the work of the toxicological chemist to become increasingly difficult. More and more, the courts, police departments, and coroners have had to rely on the chemist for evidence in respect to identification of drugs. In many cases testing procedures commonly used today do not, in fact, establish the identity of a substance of interest with a desirable degree of certainty and the presumptive identification needs confirmation by other techniques.

Gas chromatography and thin-layer chromatography are the two most popular techniques presently employed in drug screening. They have the ability to rapidly separate and presumptively identify compounds in a complex mixture. The virtue of these two approaches lies in their simplicity and the rapidity with which they can be applied as well as their excellent sensitivity. They are also supported by a large data base, making the interpretation of results obtained considerably easier for the chemist. However, this large amount of data also makes it apparent that for a given parameter several compounds may give a common positive result [*1–4*]. Both gas chromatography and thin-layer chromatography suffer from the fact that they determine only a few properties of a given compound; for example, Rf or retention time and a given reaction to a color reagent or other detector. The actual, positive identification of compounds by thin-layer chromatography is complicated by the fact that many compounds have the same Rf's in a given system and give the same reactions to a variety of locating reagents. A similar problem exists in gas chromatography for a given column and a single set of operating conditions. Gas chromatography and thin-layer chromatography are also prone to confusion because of the appearance of unrecognized peaks or spots, which often appear, particularly when the chemist is dealing with a wide variety of biological samples from a number of sources.

Thus, these two techniques are most valuable when they provide a negative rather than a positive answer. Several cases of misidentification of drugs have been documented by Goldbaum [*5*] and English [*6*]. A method with adequate sensitivity which can provide a sufficient number of physical parameters to positively identify drugs present in a biological matrix would minimize this problem.

The combination of gas chromatography and mass spectrometry has been successfully applied to drug and drug metabolite analysis [*7–19*]. This paper presents a systematic

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approach which uses the large amount of gas chromatotraphic retention time data available [*1–4, 20–26*] to tentatively identify a compound and to eliminate false positives and verify true positives with the mass spectrometer. Using this approach, a laboratory can make good use of the mass spectrometer without the need to initially accumulate a large library of spectra; and standard spectra can be acquired as needed to verify results obtained.

A quadrupole type mass spectrometer was chosen for this study because of its capability for rapid scanning, necessary when monitoring rapidly eluting gas chromatographic peaks, its ability to monitor a single mass peak, or any series of single mass peaks, its relatively low cost, and its simplicity and easy maintenance. The latter make it especially suitable for heavy routine use.

The majority of analyses presented in this paper were done on blood; however, the techniques have also been successfully applied to liver and urine extracts.

For the purposes of discussion, the compounds will be divided into the classifications of organic volatiles, acidic drugs, neutral drugs, basic drugs, and amphoteric drugs. The method for determining a drug's classification will be discussed further when dealing with each classification.

Apparatus

A Finnigan Model 3000 gas chromatograph peak identifier and a Finnigan Model 1015C gas chromatotraph-mass spectrometer equipped with a Finnigan System 150 computer were used to obtain the data. Both instruments were equipped with a Gohlke all-glass separator interface. A 5 ft by 2 mm inside diameter column packed with 3 percent OV-1 on 100/120 mesh Gas Chrom Q was used for the acidic, neutral, basic, and amphoteric drugs. A 3 ft by 2 mm inside diameter column packed with 80/100 mesh Porapak Q was used for the organic volatiles. Temperature programmed runs were from 120 to 220 C at 8 C/min. Conditions for other runs were as noted.

Procedures

Organic Volatiles

The organic volatiles are characteristically low molecular weight hydrocarbons or halogenated hydrocarbons which have an appreciable vapor pressure at room temperature. Compounds generally included in this class are the low molecular weight alcohols, ketones, aldehydes, hydrocarbons, and chlorinated hydrocarbons. To determine these compounds, 1 μl of a blood or urine:H_2O (1:5) solution was injected onto a glass wool packed inlet on a Porapak Q column at 160 C.

Acidic Drugs

The acidic drugs are extractable from acid solutions by organic solvents, from which they can be extracted by aqueous or organic bases. The most commonly found drugs in this category are diphenylhydantoin, glutethimide, salicylates, and the barbiturates. These drugs were determined by the method of Gallaher [*29*]. In this method, trimethylanilinium hydroxide is used both to extract and to methylate the drugs. Elution temperatures relative to phenobarbital are given in Table 1.

Neutral Drugs

The neutral organic drugs are generally characterized by the fact that they remain in the organic solvent used to extract the biological specimen after the solvent has been washed

TABLE 1—*Chromatographic properties of methylated derivatives of acid drugs.*

Compound[a]	m.w.[b]	RET[c]	Comments
Barbital	212.2	0.790	
Probarbital	226.2	0.814	
Aprobarbital	238.2	0.833	
Butabarbital	240.2	0.854	These two compounds can be distinguished by examining the 210–220 a.m.u. portions of their spectra.
Butethal	240.2	0.855	
Amobarbital	254.3	0.876	
Pentobarbital	254.3	0.890	These three compounds can be distinguished by examining the 220–230 a.m.u. portions of their spectra.
Vinbarbital	252.2	0.904	
Secobarbital	266.3	0.912	
Cyclopal	262.2	0.951	These two compounds can be distinguished by examining the 220–230 a.m.u. portions of their spectra.
Hexethal	268.3	0.953	
Glutethimide	245.3	0.983	These two compounds can be distinguished by Doridens large 203 a.m.u. mass fragment.
Hexobarbital	264.3	0.984	
Phenobarbital	260.2	1.000	
Heptabarbital	278.3	1.060	
Diphenylhydantoin	280.3	1.183	

[a] Merck Index, 8th ed., names used for all compounds in this paper.
[b] m.w. = molecular weight of methylated derivative.
[c] Relative Elution Temperature (RET) = T_{ec} (elution temperature in C of drug)/T_{ep} (elution temperature in C of phenobarbital). RET provides a reliable retention index for temperature programmed gas chromatography [*32*].

with aqueous acid and aqueous base. The drugs most commonly found in this fraction are the carbamates, methyprylon, and phenacetin. The method used to extract these drugs was modified from that of Goldbaum [*30*]. Two ml of blood or urine and 2.0 ml of pH 9.2 Borate Buffer are extracted with 5.0 ml of ether in a 12-ml screw cap centrifuge tube for five minutes on a Fisher Roto Rack at about 60 rpm. The tube is centrifuged for 2 min at 2000 rpm, and a 4.0-ml aliquot of the ether is withdrawn and placed in a similar tube. The ether is then washed with aqueous 0.5*N* HCl. The acidic aqueous phase is saved for the analysis of basic drugs. The ether is transferred to a third tube and evaporated under a stream of dry nitrogen. To remove interfering fats, the residue is dissolved in 5.0 ml of a solution of ethanol:cyclohexane (2:5). To this solution 0.5 ml of water is added, the tube shaken, and, after phase separation the upper cyclohexane layer is aspirated. The ethanol: water solution is evaporated to dryness and the residue redissolved in 100 μl of ethanol. One μl of this solution is then injected. The carbamates have a tendency to decompose while being chromatographed. Even with pure standards, two or more peaks are often obtained. This can be avoided by basic hydrolysis to the corresponding alcohol, followed by chromatographic identification of the alcohol formed [*31*]; however, for the purpose of identification, it is better to obtain a spectrum of the intact compound. Decomposition can be minimized by maintaining an injection port temperature of 200 C or less. Elution temperatures relative to meprobamate are given in Table 2.

Basic Drugs

The basic organic drugs remain in the aqueous HCl after the neutral drug determination and are determined by making the HCl basic with NaOH and re-extracting with ether. The ether is evaporated to dryness and the residue, containing the nonvolatile basic drugs, is redissolved in 50 μl of ethanol, of which one μl is injected into the gas chromatograph-

TABLE 2—*Chromatographic properties of neutral drugs.*[a]

Compound	m.w.	RET	Comments
Bromisovalum	223.1	0.785	Keeping the injection port below 220 C decreases amount of breakdown.
Bromisovalum breakdown product		0.868	
Ethinamate	167.2	0.834	
Methyprylon	183.2	0.887	
Meprobamate	218.2	1.000	Injection port temperature affects breakdown.
Meprobamate breakdown product		0.865	
Carisoprodol	260.3	1.032	

[a] OV-1 Column

mass spectrometer. Mass fragmentography is often necessary to determine the drugs in this fraction. (See Table 3 for chromatographic conditions.)

Amphoteric Drugs

The amphoteric drugs are determined by hydrolyzing 5 ml of blood or urine with 0.5 ml of concentrated HCl. The mixture is then made basic with NH_4OH, saturated with $NaHCO_3$, and extracted with chloroform:isopropanol (4:1). The solvent is evaporated and the residue redissolved in 50 μl of 0.2*M* trimethylanilinium hydroxide in methanol. One μl of this solution is chromatographed and the drugs are determined as their methylated derivatives. The drugs most commonly found in this fraction are the morphine derivatives; however, the phenothiazines are also found. (See Table 3 for chromatographic conditions.) Using mass fragmentography morphine can be determined to be present at concentrations of 10 ng/ml in the original sample. Concentrations of 1 μg/ml in the original sample are sufficient to yield a complete mass spectrum of morphine.

TABLE 3—*Chromatographic properties of basic drugs.*

Compound	m.w.	RRT[a]	Mass Monitored	Sensitivity[b]
Meperidine	247.3	0.16	247	4
Methadone	309.4	0.46	165	4
Atropine	289.4	0.53	124	2
Dextroproxyphene	339.5	0.53	193	4
Amitriptyline	277.4	0.54	247	10
Nortriptyline	263.4	0.55	263	6
Imipramine	280.4	0.56	220	4
Carbamezapine	236.3	0.69	193	10
Hydromorphone[c]	299.3	0.72	297	6
Morphine[c]	313.3	0.89	313	4
Diazepam	284.8	1.00	256	2
Chlorpromazine[c]	333.9	1.22	232	4
Chlordiazepoxide	299.7	1.75	282	2

[a] RRT = Relative retention time to diazepam on OV-1 column at 200 C.
[b] Sensitivity = ng required for a full scale response on a 1-V recorder with 1% baseline noise.
[c] Methylated derivative.

Results and Discussion

Porapak Q was chosen for the analysis of the organic volatile compounds because of the large amount of retention data available for this packing [*20,21*] which makes it possible for the chemist to tentatively identify the compound in question. The identification can then be confirmed by comparison of the mass spectrum with a standard, or by examining available references [*27*]. Compounds in this class are easily identified because they have simple, easily recognizable mass spectra. This technique has been used to identify ingested isopropanol, suicidally ingested paint thinner, and homicidally administered chloroform.

The use of the acidic drugs is so widespread that cases of overdose involving them have become commonplace. Knowledge of the particular derivative in the case of structurally similar drugs may be necessary for proper treatment, as the potencies of these drugs may vary widely at a given concentration. In the case of barbiturates, a large number of photometric procedures available provide quantitative data but fail to distinguish between the various barbituric acid derivatives. Gas chromatography can distinguish nearly all of these compounds in their pure state; however, substances may be present in biological extracts which give rise to misleading peaks. The combination of gas chromatography and mass spectrometry has proven satisfactory in providing rapid and positive identification of these drugs. Using the method of Gallaher [*29*], drugs in this category can be identified both quantitatively and qualitatively within fifteen minutes of the receipt of the sample in the laboratory, providing the instrumentation is on a standby basis. Figure 1 shows a chromatogram obtained from a mixed barbiturate standard. Spectra obtained from this standard were compared to those obtained from a chromatogram of a blood extract (Fig. 2) to verify the presence of amobarbital (Fig. 3) and secobarbital (Fig. 4) in a case of overdose.

The neutral drugs are usually present in such high quantity that their spectra are easy to obtain and identify. The extraction procedure of Goldbaum [*30*] eliminates interfering compounds of biological origin quite effectively. In a case of death from overdose of meprobamate, it was even possible to obtain a complete mass spectrum of meprobamate from the injection of one μl of urine without any extraction or purification.

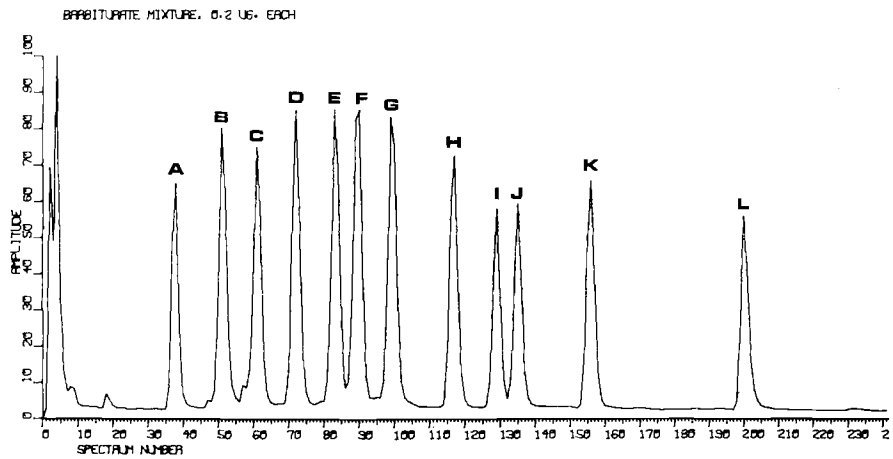


FIG. 1—*Reconstructed gas chromatogram of methylated barbiturate standards;* A = *barbital,* B = *probarbital,* C = *aprobarbital,* D = *butabarbital,* E = *amobarbital,* F = *pentobarbital,* G = *secobarbital,* H = *hexethal,* I = *hexobarbital,* J = *phenobarbital,* K = *heptabarbital,* L = *diphenylhydantoin.*

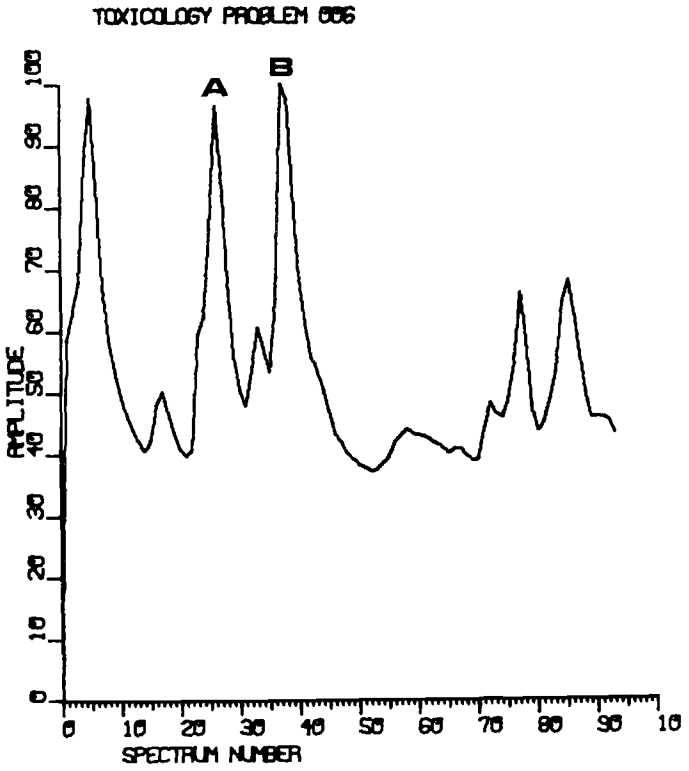
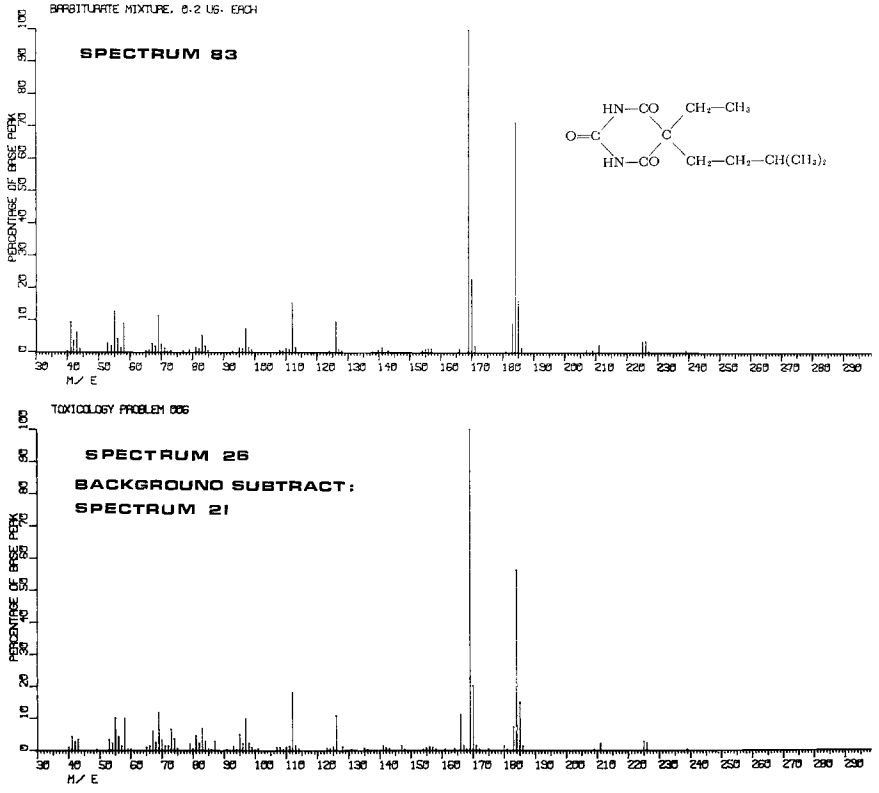


FIG. 2—*Reconstructed gas chromatogram of blood extract from Tuinal, amobarbital* (A) *and secobarbital* (B), *overdose.*

The basic and amphoteric drugs present the toxicological chemist with his greatest challenge. The low concentrations at which these drugs are usually found and the thousand or more available drugs in this category make them difficult to determine both quantitatively and qualitatively in biological specimens. Thin-layer chromatography using any of the well documented methods available [*1–4*] is undoubtedly the best method of screening for these drugs at the present time. The difficulty in this technique, as previously mentioned, lies in the fact that many compounds may have the same Rf in more than one chromatographic system, and also the same reaction to locating reagents. Furthermore, thin-layer chromatography in itself yields only roughly quantitative results.

Gas chromatography-mass spectrometry serves to complement thin-layer chromatography by providing positive identification and suitable quantitation of drugs whose presence is suspected as a result of thin-layer chromatographic screening. Conditions for gas chromatography of the suspected drug can be found in the large number of available references [*1–4,23–26*]. In some cases, usually with urine and liver extracts, there is a sufficient quantity of the drug, approximately 100 ng, to obtain a complete mass spectrum. Figure 5 shows a comparison of a spectrum of morphine extracted from a urine sample obtained from an automobile driver in comparison with a spectrum obtained from a standard. In cases where it is not possible to obtain a complete spectrum, a dramatic

increase in sensitivity and specificity can be obtained by using the technique of mass fragmentography [*14,17,18*]. This technique involves selecting a mass at which the compound of interest has a relatively strong ion intensity and monitoring just the ions at that mass instead of scanning the entire range. In general, it is best to pick the highest mass fragment of reasonable intensity to avoid interference from lower molecular weight compounds. The combination of retention time coupled with the presence of a specific mass fragment makes the determination specific for the drug. The limit of detectability for this technique with a 2:1 signal-to-noise ratio is usually on the order of 50 picograms or less injected on the column (see Table 3). It is, of course, necessary to suspect the identity of the drug present to have correct chromatographic conditions and to monitor the correct mass fragment. Figure 6 shows a chromatogram of Valium (diazepam) extracted from blood which was obtained by monitoring mass 256. The lack of interference from other compounds in the extract and even from the solvent peak can be clearly seen. Drugs which have been so far determined in blood in this manner are: chlordiazepoxide, dextropropoxyphene, amitriptyline, nortriptyline, diazepam, meperidine, methadone, carbamazepine, imipramine, atropine, dilaudid, morphine, and chlorpromazine.



NOTE—Background subtraction in sample spectrum allows a good spectrum to be obtained from a relatively bad sample.

FIG. 3—*Standard spectrum and sample spectrum used to verify amobarbital in blood sample. Mass range = 40–199; 200–300, integration time = 8 ms/AMU; 12 ms/AMU, electron energy = 70 eV, emission current = 600 μA.*

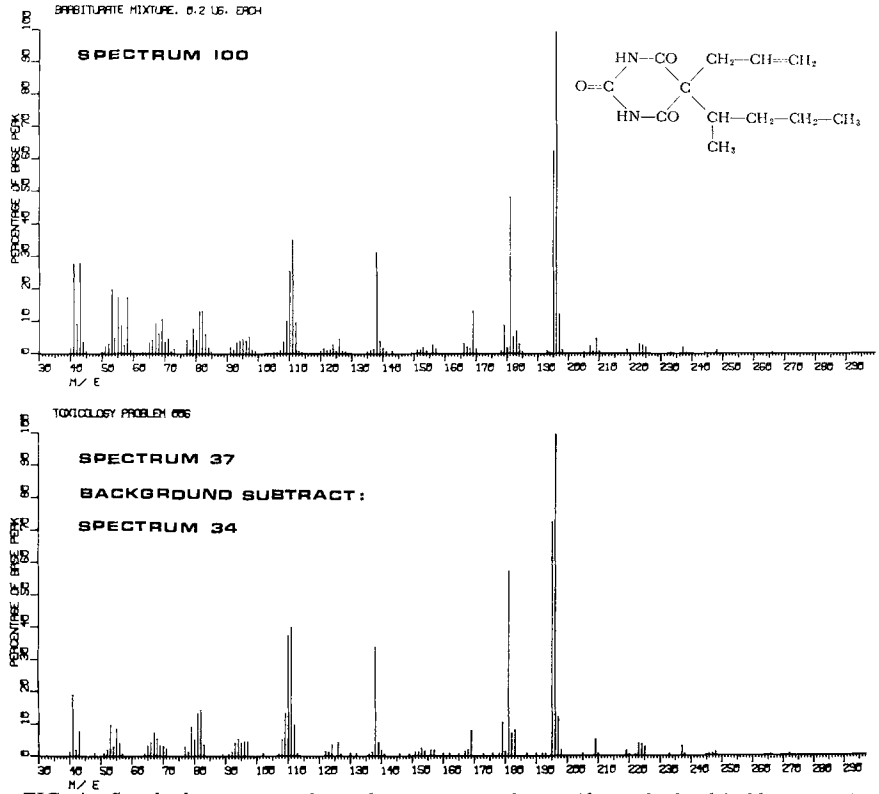


FIG. 4—*Standard spectrum and sample spectrum used to verify secobarbital in blood sample.*

Summary

Methodology has been presented for the practical application of combined gas chromatography-mass spectrometry to analytical toxicology. The technique provides the analyst with a reliable method for the sensitive and specific determination of many drugs. In addition, it complements simple gas chromatography, thin-layer chromatography, and other techniques already in use in many laboratories.

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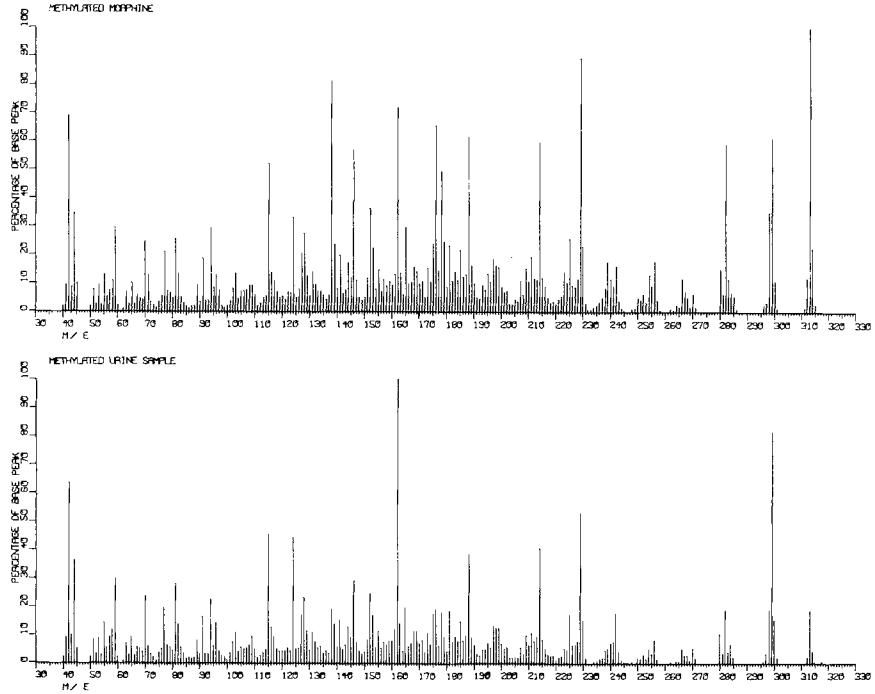

FIG. 5—*Methylated morphine standard spectrum and spectrum obtained from urine of driver convicted for driving under the influence of narcotics. Mass range = 40–199; 200–325, integration time = 8 ms/AMU; 14 ms/AMU, electron energy = 70 eV, emission current = 600 μA.*

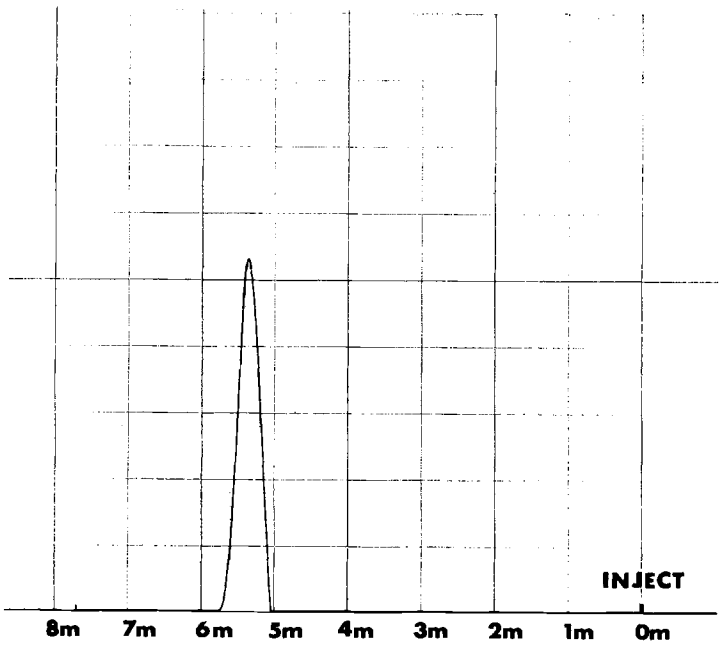

FIG. 6—*Chromatogram obtained by monitoring mass 256 on basic drug extract of blood in case of Valium overdose. Concentration = 0.3 mg%.*

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