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Drug Detection in Urine by Chemical Ionization Mass Spectrometry

The analytical methods of thin-layer chromatography (TLC), gas chromatography (GC), and mass spectrometry (MS) have gained general acceptance as basic tools of the clinical and forensic toxicologist. The utility of these techniques resides in their ability to separate and identify a wide range of organic materials at relatively low detection limits. Assuming one has access to all of these procedures, when and how they are used will often depend on the degree of specificity one wishes to attach to the analytical data generated. In forensic laboratories, a thorough confirmation of all results is necessary if the analytical conclusions are to withstand the close scrutiny normally encountered during a legal hearing. On the other hand, clinical laboratories are often interested in rapid, simple, and economic testing procedures that can be executed on vast numbers of specimens. These tests are intended to provide data that are clinically reliable and have diagnostic value; however, they may not always yield data that are indisputable in a court of law. Of course, ideally, both the forensic and clinical toxicologists are continually striving for procedures that combine speed, versatility, and specificity. Undoubtedly, under these circumstances the mass spectrometer can be expected to play a far more important role in both types of laboratories in the future years.

Normally, optimum use of the mass spectrometer requires interfacing it to a GC and a computer. Under normal electron impact (EI) conditions, a mass spectrum is sufficiently complex to provide a "fingerprint" pattern for the chromatographed effluent. With a computer, a search can then be made of a comprehensive spectral library to complete the identification. However, this approach does have some drawbacks. The equipment required is costly and the analysis time can be lengthy. Furthermore, the identification of organic components is restricted to those that will pass through the GC column. This is a particularly important consideration for those drug metabolites that do not chromatograph well unless derivatized. Another complicating factor is that the EI mass spectra of drug metabolites, both underivatized and derivatized, may not be readily accessible to most toxicologists, and, for the most part, they are not available in commercial mass spectral drug libraries.

One approach for overcoming these drawbacks and enhancing the versatility of the mass spectrometer is to employ the direct probe of the mass spectrometer for analyzing the extracts of biological fluids and organs for drug content. This approach was first tried by De and Umberger [*1*] in 1968. They reported identifying the presence of some acidic

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and neutral drugs in tissue extracts. Unfortunately, their ability to resolve multicomponent drug mixtures was severely limited by the presence of impurities and metabolites in tissue extracts. Boerner et al [*2*] extended this method by using a three-stage Llewellyn membrane separator as a direct sampling inlet. Extracts of blood, urine, and gastric lavage were analyzed for drug content by direct injection into the mass spectrometer; however, they found it necessary to use a computer for processing the voluminous data generated during the analysis.

The introduction of chemical ionization mass spectrometry (CIMS) by Munson and Field [*3*] opened a new era in mass spectrometry. Whereas conventional EI mass spectra are produced by the direct impact of high energy electrons with the sample molecules, the CI process allows the high energy electrons to first ionize a reagent gas. The ionized reagent gas in turn reacts with the sample molecules to produce a CI spectrum. The selection of the reagent gas will determine the complexity of the spectrum [*4*]. It has been shown that with most commonly abused drugs isobutane reagent gas will yield the simplest CI spectrum [*5*]. The isobutane CI mass spectra for most drugs show intense protonated molecular ions (MH^+) and few, if any, significant fragmentation ions. Ordinarily such a spectrum can be expected to show four or less ions in abundances of 10% or greater. The simplicity of isobutane CI spectra means that many organic compounds can tentatively be identified from their MH^+.

Fales et al [*6*] and Milne et al [*7*] first took advantage of CIMS to identify tentatively the presence of barbiturates and other drugs in gastric contents and serum. Their procedure called for the insertion of the questioned specimens into the CI source with a direct probe; no prior GC separation was employed. Others have since demonstrated the feasibility of this approach, selecting as their reagent gas of choice either isobutane [*5,8–10*] or methane [*11–13*]. Because of its lower proton affinity, methane can be expected to yield slightly more fragmentation when compared to isobutane CI. Foltz et al [*14*] demonstrated the applicability of methane CIMS for the rapid detection of drugs in the body fluids of suspected overdose cases. Comprehensive methane and isobutane CI spectra libraries have been compiled for numerous drugs and drug metabolites [*5,15*].

The study described herein is concerned with determining the sensitivity of isobutane CIMS for the detection of common drugs of abuse in urine. A simple and rapid extraction procedure was employed for removing drugs from urine. The extract was subjected to CI mass spectral analysis via the direct probe. Limits of detection for the drugs studied in this manner were determined. Furthermore, urines obtained from normal healthy adults were analyzed to establish whether normal urinary constituents could yield interferences coinciding with the CI spectra of drugs.

Experimental Procedure

A 10-ml sample of urine is adjusted to pH 2 with 10% hydrochloric acid. The urine is extracted with 15 ml and 10 ml of chloroform. Before use, the chloroform is washed with 1*N* sodium hydroxide, 1*N* hydrochloric acid, and water to minimize the presence of impurities. The combined chloroform extracts are washed with 5 ml of sodium bicarbonate that was adjusted to pH 7.8. The chloroform extract is again washed with a 5-ml portion of water, dried over sodium sulfate, decanted, and taken to dryness on a water bath set at 60°C. The residue is reconstituted with 10 μl of chloroform, of which 5 μl is transferred to a closed-end capillary tube and to dryness. The tube is then inserted into the source of the mass spectrometer via the direct probe.

All CIMS spectra were taken on a Du Pont 21-490 single focusing mass spectrometer. The instrument was fitted with a dual EI/CI source. The reagent gas was isobutane (99.9% pure) at a source pressure of 66 to 133 Pa (0.5 to 1.0 torr). The source temperature was kept at 200°C. The ionizing voltage was 420 eV in the CI mode. All mate-

rials analyzed were admitted into the source through the direct probe. Specta were recorded at probe temperatures of 150 and 200°C.

Results and Discussion

A simple chloroform extraction on urine adjusted to pH 2 recovers many of the drugs commonly abused in our region of the country. Table 1 lists some of these drugs along

TABLE 1—*Isobutane CIMS of selected drugs of abuse.*

Drug	Molecular Weight	Mass Spectral Peaks
Amobarbital	226	227 (100%)
Butabarbital	212	213 (100%)
Cocaine	303	304 (100%), 182 (33%)
Glutethimide	217	218 (100%)
Meprobamate	218	219 (100%), 158 (61%)
Methadone	309	310 (100%)
Methaqualone	250	251 (100%), 250 (30%), 235 (20%)
Pentobarbital	226	227 (100%)
Phenobarbital	232	233 (100%)
Secobarbital	238	239 (100%), 199 (36%)

with their most significant isobutane CI ions. In the course of our experiments normal urines were spiked with various concentrations of these drugs to determine the lower limits of detectability when the direct probe technique described in the Experimental Procedures section was used. Normally the isobutane CI spectra patterns for a typical urine extract will display few predominant peaks and will appear relatively simple. However, in the search for therapeutic drug concentrations it may be necessary to record the CI spectrum at the higher sensitivity settings of the instrument, thereby increasing the intensity of ions associated with normal urine components. Under these circumstances, ions may be observed at almost every mass unit between 100 and 400. The task of distinguishing the ions associated with normal urine constituents from those relating to the presence of drugs is necessarily an arbitrary one. We chose to approach the problem by designating two relative levels of ion intensities in the CI spectrum. To illustrate, a urine spike with 1.0 μl/ml of meprobamate is shown in Fig. 1. An intensity level, designated A, is selected above which there exist 10 to 15 ions between masses 150 to 250. A second intensity level, B, is drawn at twice the height of A. All ions up to mass 300 showing intensity values equal to or exceeding that of B are deemed to be significant. Because the CI mass spectra of urines consistently show a significant reduction in ion intensities above mass 300, all ions above this mass were considered significant when their intensities were equal to or exceeded that of the lower level A. This approach produced high levels of detection for the drugs selected while minimizing the number of interfering ions arising from normal urine constituents.

Sensitivity values for the drugs studied were calculated by monitoring the drug's major CI ion while adhering to the signal to background criterion described above. The values determined are listed in Table 2.

In general, the detection limit for many of the drugs analyzed was less than 0.5 μm/ml, the only noteworthy exception being phenobarbital which was detectable at 3 μg/ml. Except for cocaine, which is rapidly metabolized to benzoylecgonine and, to a lesser extent, ecgonine, the drugs studied yielded sensitivities sufficient to detect therapeutic concen-

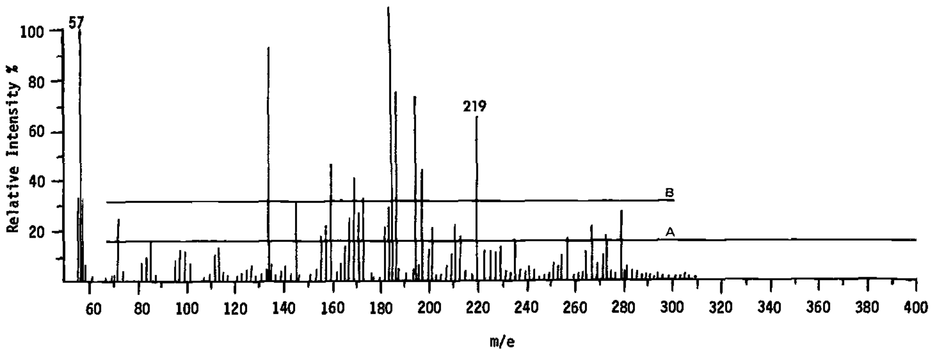

FIG. 1— *Direct probe isbutane CI spectrum of urine extract spiked with 1.0 μg/ml of meprobamate. Ions below* m/e *300 with a relative intensity equal to or greater than Level* B *are significant. Above* m/e *300, ions having a relative intensity equal to or greater than Level* A *are significant.*

TABLE 2—*Commonly abused drugs and their levels of detection in urine by direct-probe isobutane CIMS.* [a]

Drug	Detection Level, μg/ml
Amobarbital	0.15
Butabarbital	0.25
Cocaine	0.60
Glutethimide	0.30
Meprobamate	0.50
Methadone	0.50
Methaqualone	0.50
Pentobarbital	0.20
Phenobarbital	3.0
Secobarbital	0.25

[a] Sample volume 10 ml.

trations in urine. Furthermore, these values compare favorably to ones obtained in studies using GC for urine drug analysis [*16,17*].

When the direct probe insertion approach for urine drug analysis is evaluated, it must be emphasized that the resultant spectra will not provide a conclusive determination for confirming the presence of a drug. For example, isobutane CIMS does not distinguish amobarbital from pentobarbital. Both have the same molecular weight and will yield indistinguishable isobutane CI spectra. The "fingerprint" pattern of EI mass spectroscopy has been sacrificed for the simplicity of an isobutane CI spectrum; an unequivocal identification has been reduced to a tentative identification. However, direct probe of analysis does have the advantage of providing rapid low-level drug detection in urines that have been subjected to minimal sample preparation. The resultant data can be used for drug screening purposes or to complement other procedures, such as TLC and GC, in a toxicological analytical scheme.

Urine is a complex fluid containing many organic materials derived from normal and abnormal metabolic processes. Steroids, urinary acids, preservatives, food stuffs, inhalants such as nicotine, as well as drugs and their metabolites, including caffeine, are likely to be

encountered in urine. For these reasons it is always possible that some other component of urine will produce an MH^+ corresponding to one of the drugs in question. Hence, the effectiveness of direct probe procedure is inherently related to its ability to distinguish normal urine constituents from those of drugs of abuse.

A determination as to the number of possible interferences arising from the direct CI analysis of urine was made by testing 173 normal urines obtained from members of the 90th and 91st recruit classes of the New Jersey State Police. Each contributor was required to list all medications, prescription and nonprescription, taken up to one week prior to submitting urine. Anyone admitting any drug intake, with the exception of caffeine and nicotine, was eliminated from participation in the study. All participants except one were male. All the urines selected were extracted and analyzed by direct probe CIMS.

As with any screening procedure, the direct probe technique is subject to interferences; however, while many natural urine components are observable in spectra taken with a direct probe, for the most part they did not correspond to any of the drugs used in this study. A listing of the CI ions observed in normal urines along with their frequency of occurrence is shown in Table 3.

While no effort was made to confirm the identity of the urine components observed, some of the more frequently occurring ions can be associated with substances previously reported in urine extracts [14]. For example, the ions at *m/e* 257 and 285 correspond to the MH^+ of palmitic and stearic acids, respectively; similiarly, *m/e* 279 and 391 correspond to

TABLE 3—*Ions observed in the chloroform extracts of acidified urine by direct probe isobutane CIMS.*[a]

m/e	Frequency of Occurrence, %	*m/e*	Frequency of Occurrence, %
101	2	211	69
109	4	213	7
111	2	219	3
112	14	220	5
113	30	221	5
125	6	235	10
127	1	254	1
129	2	255	2
149	29	257	39
154	9	268	13
160	1	279	5
167	6	282	3
169	40	285	3
177	8	299	34
179	2	301	1
180	6	304	1
181	90	305	2
182	16	323	1
183	2	333	1
185	90	335	13
186	6	355	5
193	3	361	3
194	38	369	4
195	96	387	1
196	32	391	54
197	89	399	100
198	2	400	48
205	3	439	2
209	5		

[a]One hundred seventy-three urines examined; ions having a frequency of occurrence of less than 1% are not listed; and ions below *m/e* 100 are not listed.

the MH^+ of dibutyl phthalate and dioctyl phthalate, respectively. Tri-2-butoxyethyl phosphate, found in the rubber tops of some types of containers, accounts for the presence of *m/e* 399 in all the specimens examined.

The most serious interference problem arose with butabarbital. Seven percent of the normal urines analyzed show a significant ion at *m/e* 213 coinciding with butabarbital's MH^+. Three percent of the urines had significant ions at *m/e* 219, corresponding with meprobamate's protonated molecular ion. However, none of the urine specimens showed ions at *m/e* 158, meprobamate's major CI fragmentation ion. Only 1% of the urines tested had an ion at *m/e* 304 corresponding to cocaine's MH^+.

One further advantage derived from the direct probe technique is the ability to identify multicomponent drug mixtures. Not only is it feasible to identify tentatively two or more drugs that may be simultaneously present in a urine extract, but CI is also capable of distinguishing accompanying nonconjugated drug metabolites. When accompanied by the parent drug, ions associated with major metabolites can provide corroborating spectral data to substantiate the presence of a particular drug, a situation that often compensates for the inherent simplicity of the isobutane CI spectra of most drugs.

Two examples that demonstrate the utility of metabolic data are shown in Figs. 2 and 3. In the former, intense ions at *m/e* 227 and 239 point to the presence of amobarbital and secobarbital, respectively. This tentative conclusion is further substantiated by the presence of ions corresponding to each drug's hydroxy metabolites. Major fragmentation ions from these compounds are accounted for by the loss of water from the metabolite's protonated molecular ion. Hence, *m/e* 255 and 237 corresponds to hydroxysecobarbital's MH^+ and $MH^+ - 18$, respectively, while *m/e* 243 and 225 correlates in the same manner with hydroxyamobarbital's CI ions. Figure 3 is a CI spectrum of a urine extract containing amobarbital and glutethimide. Again, amobarbital is accompanied by ions corresponding to its hydroxy metabolites. The presence of glutethimide is supported by ions corresponding to its major metabolites. As expected, the hydroxy metabolites of glutethimide show an intense MH^+ at *m/e* 234, while 2-phenylglutarimide (molecular weight 189), a metabolite of glutethimide, has an MH^+ at *m/e* 190. In this case, the simultaneous detection of five substances foreign to the normal urine extract amply demonstrates the potency of CI in

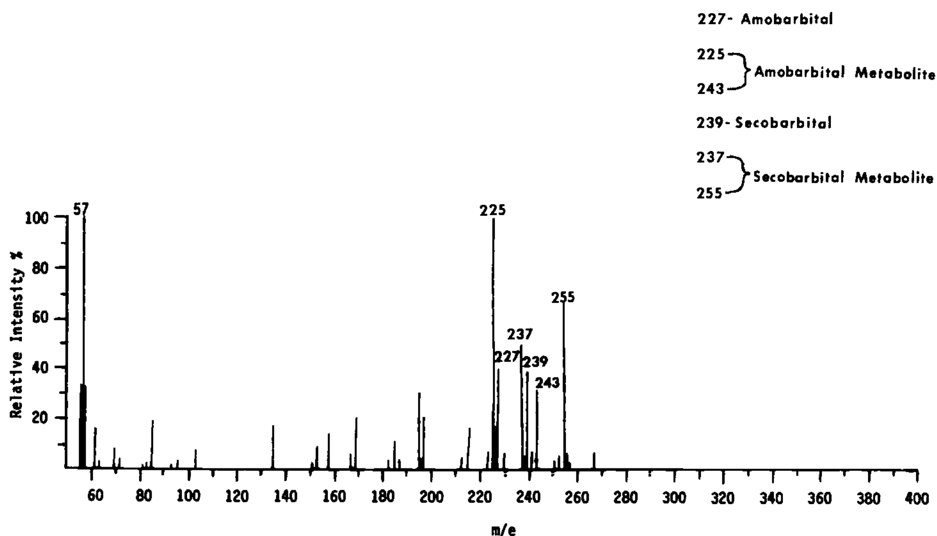

FIG. 2—*Direct probe isobutane CI spectrum of urine extract containing amobarbital and secobarbital.*

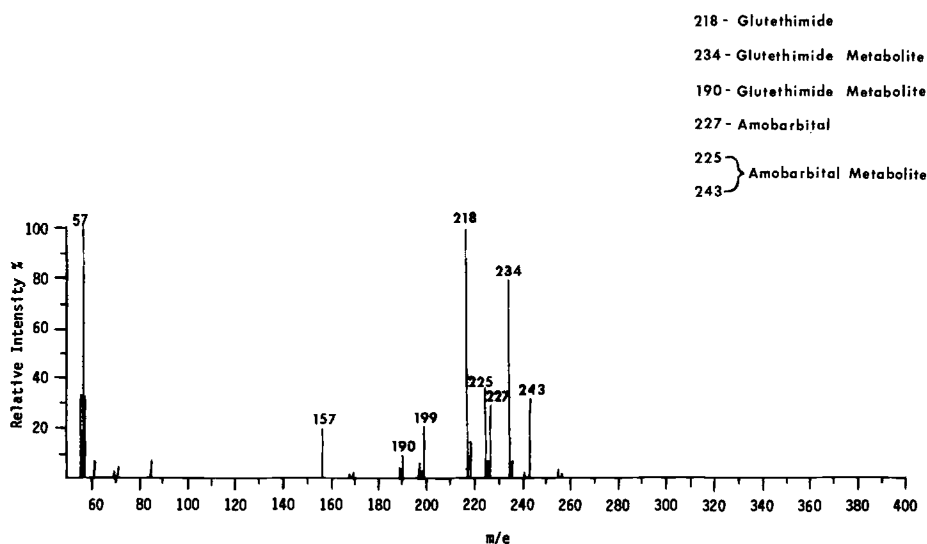

FIG. 3—*Direct probe isobutane CI spectrum of urine extract containing glutethimide and amobarbital.*

toxicological analyses. In the same manner, urine extracts containing methaqualone usually show a significant MH^+ ion at *m/e* 267 corresponding to its hydroxy metabolites.

The examination of urine extracts by the CI direct probe technique provides the toxicologist with a technique suitable for rapidly screening urines for drug content. The small amount of extract required for this technique means that sufficient sample remains to be tested by other confirmation procedures. Actual mass spectral analysis time is less than 3 min. The most time-consuming aspect of the analysis is the time involved in performing the sample extraction. A more extensive extraction procedure can be instituted to reduce or eliminate background ions associated with normal urine constituents, while similar efforts could be made at enhancing drug recoveries. However, such attempts would obviously increase the overall time of analysis.

While the data in this paper emphasize the detection of drugs and their metabolites present in the chloroform extracts of acidified urine, in actual practice the technique has been extended in our laboratory to the detection of all classes of organic drugs extracted from blood, urine, and tissues at various pH values. Since the concentration of drugs encountered in overdose cases is generally significantly greater than any compound normally present in biological extracts, the problem of interfering ions is not a major concern. The sensitivity of the mass spectrometer combined with CI's ability to assay urine rapidly without prior chromatographic separation makes the technique extremely useful for the tentative identification of drugs and drug metabolites.

Summary

The detection of drugs and their metabolites in urine by direct probe CIMS has been described. The technique is both rapid and sensitive. Common drugs of abuse can be detected at therapeutic concentration levels with minimal interferences from components present in normal urine. The simplicity of the CI spectrum readily permits the detection of multicomponent drug mixtures and their metabolites in urine extracts without prior chromatographic separation. The technique is useful for drug screening or for confirming data generated by other analytical procedures.

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