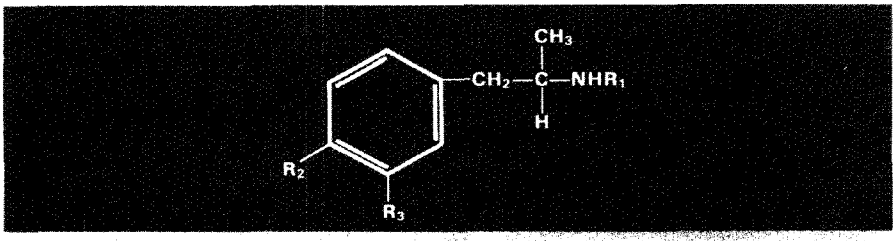
Larry S. Eichmeier and Michael E. Caplis
Northwest Indiana Criminal Toxicology Laboratory
540 Tyler Street
Gary, Ind. 46402

Figure 1. Structurally related amphetamines



	R_1	R_2	R_3	MW
Amphetamine	H	H	H	135
Methamphetamine	CH_3	H	H	149
3,4-Dimethoxyamphetamine (DMA)	H	OCH_3	OCH_3	195
3,4-Methylenedioxyamphetamine (MDA)	H	O, CH_2, O		179
3,4-Methylenedioxymethamphetamine (MDM)	CH_3	O, CH_2, O		193

The Forensic Chemist An "Analytical Detective"

The forensic chemist has the acute problem of constantly being required to make a rapid assessment of the nature of suspected illicit materials or to demonstrate the presence of poisons, drugs, and toxic chemicals in biological samples. Society demands that samples be completely analyzed prior to accusation and prosecution of any violation of its laws. Thus, the forensic chemist must be both swift and absolutely reliable in his assessment. To this end, it is his responsibility to identify and determine, without a reasonable doubt, illicit or toxic substances within a relevant time.

By virtue of this responsibility, the forensic chemist becomes an "analytical detective" who must deal with complex mediums from pharmaceutical and illicit preparations to samples of body fluids and tissues. Determination of toxic compounds in a biological matrix is complicated by the low concentration levels of the compounds and by their possible conversion to metabolites. Complete analysis of such materials requires a multitechnique approach. Techniques utilized include thin-layer chromatography, gas chromatography, high-pressure liquid chromatography, fluorospectrophotometry, gas chromatography–mass spectrometry, UV–VIS spectrophotometry, and infrared spectrophotometry, as well as chemical methods. Equipped with this armamentarium, there can be little justification for an incorrect analysis from the forensic laboratory.

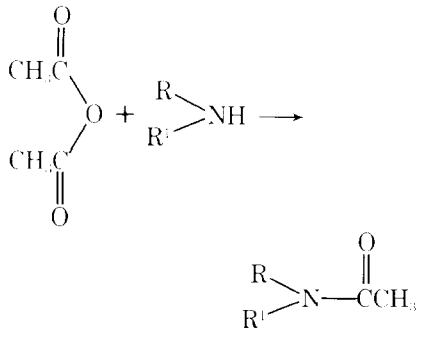
Two examples in which a judicial combination of techniques was utilized are cited. One involved the identification of a new street drug closely resembling a known illicit preparation, and the other involved a drug overdose which led to a homicide investigation.

Identification of Suspected Illicit Drug

A major abused class of drugs is amphetamine and its derivatives. In an attempt to combat this problem, undercover narcotic agents are constantly striving to determine the source and trafficking route of these drugs. In one particular instance, agents purchased capsules suspected to contain amphetamine or methamphetamine from a major distributor (Figure 1). These capsules containing a pink powder were submitted to the forensic laboratory for analysis. Preliminary screening of a 1*M* sulfuric acid solution of the powder by UV spectrophotometry indicated a dioxyamphetamine derivative, such as 3,4-dimethoxyamphetamine (DMA) or 3,4-methylenedioxyamphetamine (MDA). Amphetamine and methamphetamine were ruled out since the indicative pattern for monosubstituted benzenes (252, 257, 263 nm) was absent. Thin-layer and gas chromatographic screening after chloroform extraction from a basic solution showed chromatographic properties similar to 3,4-methylenedioxyamphetamine (MDA).

Differential chemical visualization following thin-layer chromatographic separation was used to determine if the substance was a primary, secondary, or tertiary amine (Table I). The material did not react with ninhydrin–acetone, but reacted with ninhydrin–isopropanol–acetic acid and iodoplatinate, indicative of a secondary or tertiary amine.

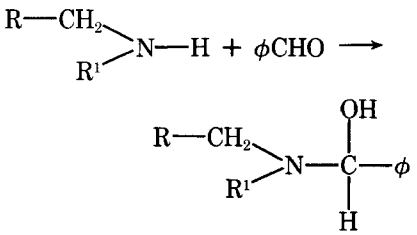
On-column gas chromatographic derivatization with acetic anhydride and benzaldehyde was employed to confirm the amine structure. The on-column derivatization of the amine with acetic anhydride gave a change in peak retention time indicative of the acid amide formation expected with a primary or secondary amine.



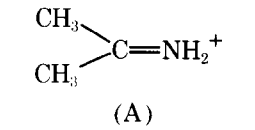
However, on-column derivatization with benzaldehyde resulted in loss of any gas chromatographic activity, indicating formation of the chromatographically inactive substituted secondary amine.

Table I. Summary of Tests and Conclusions for Suspected Illicit Drug

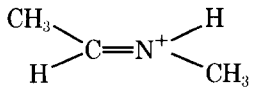
Examination	Results	Conclusion
UV (1*M* H_2SO_4)	252 nm, 257 nm, 263 nm: Absent	No monosubstituted benzene present
	287 nm, 233 (max): Present	Dioxyamphetamine-like derivative
TLC		
Ninhydrin–acetone	No reaction	Secondary or tertiary amine
Ninhydrin–isopropanol–acetic acid	Reaction	
Iodoplatinate	Reaction	
GC		
Acetic anhydride	Peak shift	Secondary amine
Benzaldehyde	No peak	
Mass spectrometer		
193 *m/e*	Weak—recognizable (molecular ion)	Secondary amine
135 *m/e*	Strong	Aryl methylenedioxy substituent
58 *m/e*	Intense (base peak)	Alkyl amine
151 *m/e*	Absent	No aryl dimethoxy substituent present
91 *m/e*	Absent	No monosubstituted benzene present



Mass spectral analysis showed fragments at 58, 135, and 193 *m/e*. Even though the ion 193 *m/e* was recognizably weak, it was determined to be the molecular ion. Characteristically, a weak parent ion is common to aryl-substituted secondary amphetamine-like derivatives. The base peak at 58 *m/e* can be attributed to either of two fragments:

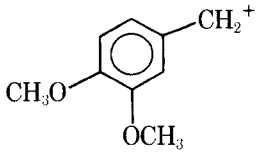


(A)

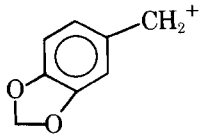


(B)

Greater significance can be assigned to fragment B because previous data indicated a secondary amine. Additionally, the absence of any ion at 151 *m/e* negated the possibility of a dimethoxy substituent, whereas the presence of the 135 *m/e* ion indicated a methylenedioxy substituent:



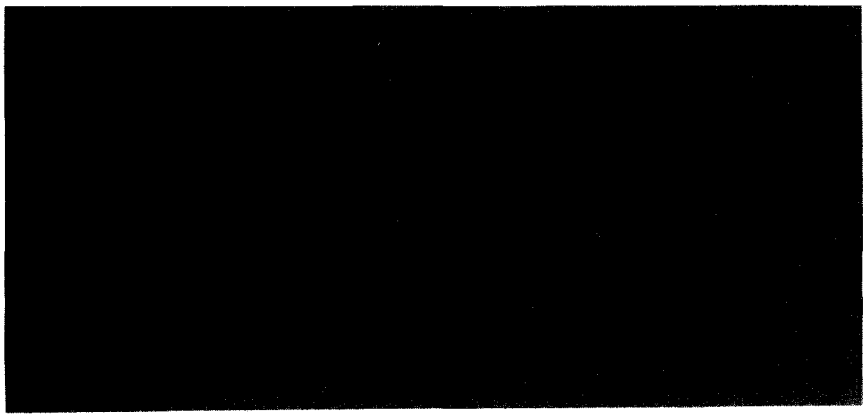
(151 *m/e*)



(135 *m/e*)

Inspection of the analytical data allowed identification of the material as 3,4-methylenedioxymethamphetamine (MDM). The data and conclusions are summarized in Table I. This substance, similar in structure and general analytical properties to MDA, is exempt from federal control, whereas MDA is controlled. Thus, the rapid utilization of general and specific techniques provided valuable information on a suspected illicit preparation and averted embarrassment and unwarranted prosecution.

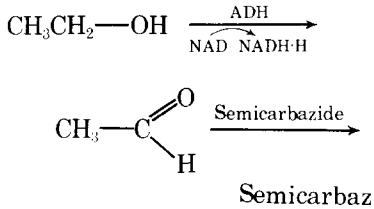
Figure 2. Isolation and identification scheme for volatiles and nonvolatiles in blood



Drug Homicide

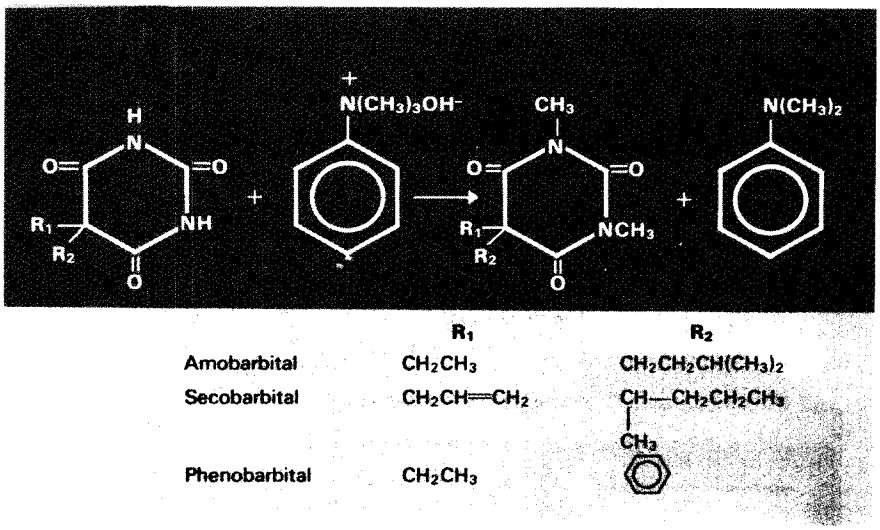
Blood from a woman found by a relative was submitted to the forensic laboratory for toxicological analyses. There was no apparent cause for death. The woman and two of her male friends had been drinking heavily outside her apartment complex earlier that evening. Police were called to quell a disturbance, and the trio had retired to her apartment. Later a witness testified that cocktails had been forcibly administered to the woman in an attempt to subdue her. Medical reports showed the woman was under a doctor's care with a prescription for phenobarbital.

Examination for blood volatiles involved gas chromatography and UV spectrophotometry (Figure 2). Screening was accomplished by injecting 10 μl of whole blood into a gas chromatograph. Results indicated the presence of ethyl alcohol. The ethanol concentration was determined by an enzymatic procedure with alcohol dehydrogenase (ADH).



In this procedure a mole of ethanol is oxidized to a mole of acetaldehyde, which is removed as the semicarbazone, with the concurrent reduction of a mole of nicotine adenine dinucleotide (NAD). Monitoring of the reduced NAD at 340 nm with a UV spectrophotometer gave an ethanol concentration of 0.23% (0.1% = intoxication).

Preliminary screening with a chloroform extract of an acidified blood sample (Figure 2) gave conflicting results. Gas liquid chromatography suggested the presence of three barbiturates: amobarbital, secobarbital, and phenobarbital. However, thin-layer chromatography indicated only the presence of amobarbital and secobarbital. Gas chromatography–mass spectrometry resolved the inconsistency with identification of amobarbital, secobarbital, and *n*-dibutylphthalate. Dibutylphthalate has the identical gas chromatographic properties of pheno-



	R_1	R_2
Amobarbital	CH_2CH_3	$CH_2CH_2CH(CH_3)_2$
Secobarbital	$CH_2CH{=}CH_2$	$CH(CH_3)CH_2CH_2CH_3$
Phenobarbital	CH_2CH_3	

Figure 3. Methylation of barbiturates

barbital but without the chemical visualization utilized in thin-layer chromatography.

The secobarbital and amobarbital were quantitated by gas chromatography as their dimethyl derivatives via an on-column reaction with trimethylanilinium hydroxide (Figure 3). This provides sharper chromatographic peaks with a minimum amount of tailing. The secobarbital and amobarbital levels determined were greater than each drug's minimum toxic level, 1.0 and 1.5 mg/100 ml, respectively.

Each of the drugs [alcohol (0.23%), secobarbital (1.1 mg/100 ml), and amobarbital (1.6 mg/100 ml)] was present at toxic levels and individually could have caused intoxication. The synergistic effects of alcohol and barbiturates are well documented, and in this case, an acute intoxication and overdose death were quite probable.

The forensic chemist's analysis indicating adverse blood barbiturate levels, to which the subject had no known access, prompted further investigation.

Apprehension of the male companions showed one to have traces of white powder in the pockets of the pants worn on the fatal evening. Preliminary screening of the trace powder with UV spectrophotometry indicated the presence of a barbiturate (Figure 4). Further examinations of the powder by gas chromatography, thin-layer chromatography, and gas chromatography–mass spectrometry showed the powder to contain both amobarbital and secobarbital.

Thus, a suspected overdose death was transformed into a drug homicide investigation and eventual criminal trial as a consequence of the forensic chemist's analyses.

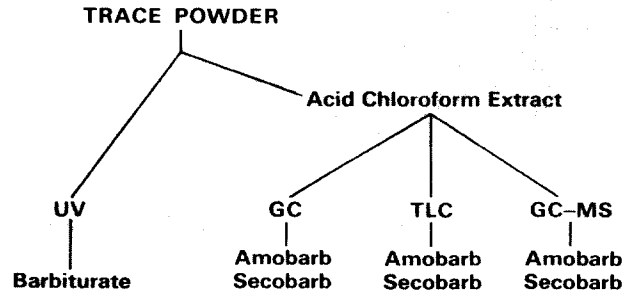


Figure 4. Identification scheme for unknown powder