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To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

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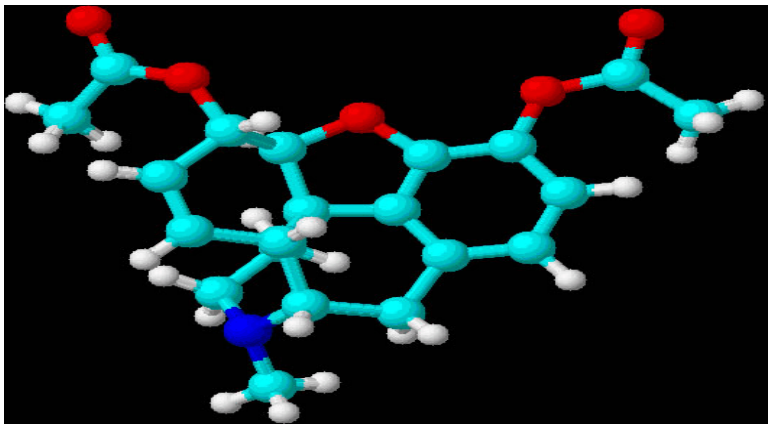

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Cover Art: "Ball and Stick" Model of Heroin (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA)

2003 Information and Instructions for *Microgram Journal*

[Editor's Preface: The following information and instructions are derived from the *Microgram* website < http://www.dea.gov/programs/forensicsci/microgram/index.html >, and are provided here for the convenience of those subscribers who do not have access to the Internet. Updates of this material will henceforth be published only in the respective January issues for each year.]

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Cover Letter - Provide the Author Contact Information and pertinent correspondence (if any) for the Editor.

Title - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

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Abstract - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under "Titles". Note that the abstract will be provided to *Chemical Abstracts*.

Keyword List - A minimum of five (maximum ten) abstracting keywords should be included.

Introduction - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid "Personal Communications").

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Results and Discussion - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

Conclusions - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or a summary paragraph in the Results and Discussion section.

Acknowledgments - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

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Osmolality - A Novel and Sensitive Tool for Detection of Tampering of Beverages Adulterated with Ethanol, γ-Butyrolactone, and 1,4-Butanediol, and for Detection of Dilution-Tampered Demerol Syringes

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ABSTRACT: Freezing point osmometry, an analytical tool used by clinical hospital laboratories and the consumer product and food industries, is investigated for its utility as a forensic screening method for detection of adulteration of commercial beverages with ethanol, γ-butyrolactone, or1,4-butanediol, and for detection of dilution of Demerol® syringes. A comprehensive list of baseline osmolality values for various commercially available beverages, eye drops, and mouthwashes is provided. Additional potential forensic applications are discussed.

KEYWORDS: Osmolality, Forensic Chemistry, Product Tampering, γ-Butyrolactone, GBL, 1,4-Butanediol, BD, Demerol

Introduction

Forensic drug testing laboratories have validated procedures in place for dealing with solid dosage samples and are well versed in the analysis of these types of cases. However, liquid samples containing relatively small percentages of low molecular weight substances can present analytical challenges - particularly if the supporting liquid matrix is itself a complex mixture (e.g., soda or beer). In the past, the only liquid samples submitted to this laboratory were small dropper bottles usually found to contain dilute solutions of LSD - a relatively trivial forensic challenge. More recently, however, the explosion of the "Rave/Club Drug" culture has resulted in the introduction of several different drugs and/or industrial chemicals which are also delivered in liquid form, including γ-hydroxybutyric acid (GHB) or butyrate (GHB^-), γ-butyrolactone (GBL), and 1,4-butanediol (BD). These may be submitted either as dilute solutions in commercial beverages or as concentrated or pure solutions in "dosing" bottles. In addition, laboratories may receive soda-type beverages, fruit drinks, or even mouthwashes seized from students and suspected of having ethanol added to them. Finally, recent terrorist events have increased public anxiety and suspicion, resulting in increased submissions of beverages suspected of having been adulterated with unknown poisons.

Many laboratories have already developed specific and robust methods for detection and identification of a few of the more commonly encountered compounds, e.g., GHB. However, there are no general methods in widespread use in forensic laboratories that are capable of rapidly and reliability detecting the presence of *any* soluble, low molecular weight compound (including novel compounds) in aqueous solutions. For example, the GHB substitutes 4-hydroxyvalerate (4-methyl-GHB), γ-hydroxybutyraldehyde, tetrahydrofuran (THF), and γ-aminobutyric acid (GABA) are already in use in illicit circles, but are not being tested for by most forensic laboratories. Future drug seizure cases and so-called Drug Facilitated Sexual Assault (DFSA) cases will undoubtedly involve these and still other compounds, and it is therefore important that forensic and toxicology laboratories be able to quickly detect their presence. A rapid screening method which could quickly identify "like" solutions would make it easier to separate exhibits into groups for statistical sampling and (where implicated) more advanced analytical testing. Osmolality offers the basis for such a technique.

Principles of Freezing Point Osmometry [1]

When a solute is dissolved in a pure solvent (e.g., water), the physical/chemical properties of the solvent are changed. The freezing point is depressed, the boiling point is elevated, the vapor pressure is lowered, and the osmotic pressure is increased [these are the so-called colligative properties.] In actual practice, therefore, one mole [gram-molecular weight] of a non-dissociating solute dissolved in 1 kg of water decreases the freezing point by 1.86ºC while exerting an osmotic pressure of about 17,000 mm Hg. There is no practical method for measuring osmotic pressure, however, freezing point depression is easily measured and has thus been a clinical and analytical tool for over 50 years. A solution with a measured freezing point depression of 1.86ºC would be said to have an osmolality of 1 Osmol/kg or 1000 milliosmols/kg, expressed as 1000 mOsm/kg.

An osmometer is a device for extremely accurate and precise determinations of the concentration of homogeneous solutions by means of freezing-point measurement. This is typically done by supercooling the target solution to several degrees below its presumed freezing point and then mechanically inducing the sample to freeze. The heat of fusion liberated during the freezing process causes the sample temperature to rise to a temporary plateau where a liquid/solid equilibrium is briefly maintained. This equilibrium temperature is, by definition, the freezing point of the solution. Osmometers include a highly accurate and precise electronic thermometer to continuously determine sample temperature and measure the freezing point of the sample.

The most common current use of osmometry is in hospital toxicology laboratories, for testing serum and urine to determine electrolyte balance, diabetic acidosis, lactic acidosis, shock, stroke, and intoxication from ethanol, methanol, isopropanol, and ethylene glycol. Osmometry is also useful for monitoring rehydration therapy for treatment of severe diarrhea or to assist in recovery after collapse from over-strenuous, dehydrating exercise (such as marathons).

An Advanced 3D3 Osmometer was utilized in the present study (see additional information under Experimental). In a typical analysis, 0.25 mL of a homogeneous liquid sample is pipetted into a disposable sample cup, which is then placed into the freezing chamber maintained at -7ºC. At the start of the experiment, a probe containing a thermistor and stir wire descends into the sample. Over the next minute, the sample is supercooled below its freezing point. The stir wire then vibrates, causing rapid freezing. The equilibrium temperature (i.e., the freezing point) is measured, and a microprocessor converts the freezing point to osmolality and displays the result in mOsm/kg.

Since the increase in osmolality is proportional to the molality of the solution, small molecular weight substances (i.e., with molecular weights less than 100), even when present in relatively low concentrations (1 - 5 percent) will detectably alter the osmolality. This makes osmometry an ideal general screening technique for substances such as GHB, GHB^-, GBL, and BD. However, “classical” drugs of abuse (cocaine, heroin, LSD, etc.) have molecular weights that are too large to noticeably effect the osmolality of typical solutions.

Experimental

An Advanced 3D3 Osmometer was utilized for all osmolality experiments. Osmolality calibration standard solutions of 100 mOsm/kg and 1500 mOm/kg were utilized this study. An American Optical T/S [Total Solids] Meter was used to measure the specific gravity of the solutions in the Demerol theft case. This (hand-held) instrument measures the refractive index of a liquid and provides a visual scale for conversion to specific gravity. It has a working measurement range of 1.000 to 1.035, which is adequate to measure dilute aqueous solutions. Commercial beverages, alcoholic beverages, mouthwashes, eye drops, and breath drops were purchased locally and used without any modification. Controlled substances and other abused substances were from laboratory stocks or seized exhibits.

Advanced 3D3 Osmometer Evaluation: [2]

Because most forensic chemists are unfamiliar with osmometry, the following details on the Advanced 3D3 Osmometer utilized in this study are provided as background. This instrument occupies approximately one square foot of counter space and weighs 25 lbs. It is solid state, consumes 150 watts an hour during operation, and has a small volume cooling bath design that allows for calibration and analysis within 15 minutes after powering up. The calibration is stored in RAM if power is disconnected.

The usable measurement range is 0 - 4000 mOsm/kg (more concentrated solutions can be measured after dilution). A full range of calibration standard solutions of known osmolality are supplied and validated by the manufacturer.

The instrument uses disposable 0.25 mL cuvettes (reusable cuvettes are also available). There is no auto-carousel on this model, but higher level models and other manufacturers provide this feature (some can handle up to 30 samples per hour). A typical experiment takes 2-3 minutes start to finish, and uses 0.25 mL sample. The sample is not destroyed by the osmolality analysis, and can be thawed and reanalyzed.

Results and Discussion

Linearity

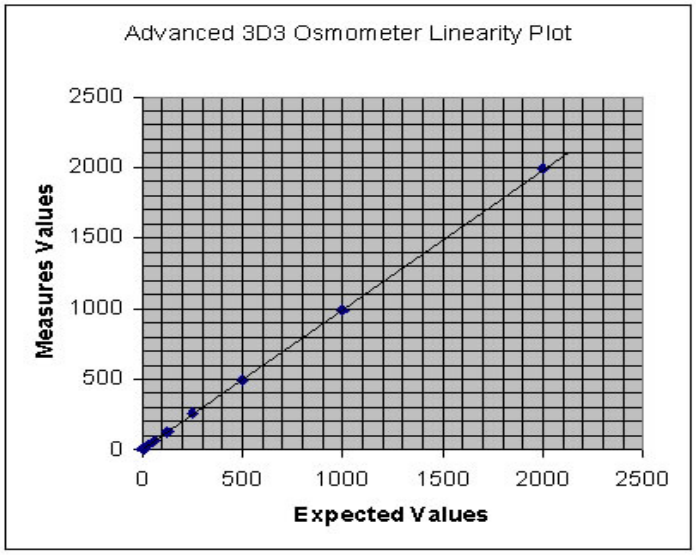
A linearity study was completed using the calibration standards; results are reported in Table 1 and Figure 1.

Table 1 - Linearity

Expected	Measured
2000	1991
1000	988
500	494
250	261
125	128
62.5	67
31.25	36
15.62	20
7.81	11
3.90	7
1.95	5
0.98	4

R = 0.9999
Slope: 0.9916
Intercept: 3.89

Figure 1



In-Run Precision [2]

Ten same lot samples of Mountain Dew and Diet Mountain Dew were run, alternating between the two types to check precision as well as carry-over. Results are given in mOsm/kg (see Table 2, next page). The low Coefficient of Variation (C.V.) values at both ends of the measurement range demonstrate excellent reproducibility.

Table 2 - Within-Run Precision

Advanced 3D3 Osmometer Within Run Precision		
Sample #	Diet Mt. Dew	Mt. Dew
1	27	804
2	27	801
3	27	808
4	27	807
5	27	806
6	26	806
7	26	805
8	27	805
9	26	808
10	27	809
CV	**1.7%**	**0.3%**

Beverage Baseline Database [2,3,4]

A comprehensive osmolality beverage database was needed as the first step in investigating beverage tampering with low molecular weight psychoactive substances. 146 beverages were tested. Whenever possible, 16 - 20 oz plastic, screw cap beverages were selected, as these are the most likely to be adulterated for illicit purposes. 8 oz "energy drinks" in non-resealing metal cans were also tested. [Note: The full database of results is available as an Excel Spreadsheet for download (contact the author if interested).]

Sports beverage results were interesting. Although producers of sports beverages claim their products are "isotonic" (approximately equal to serum values of 275 - 295 mOsm/kg), none of the tested beverages were actually in this “physiological range”. One sports beverage had a value of 190 mOsm/kg. The remaining eleven ranged from 361 - 428 mOsm/kg. Summarized results are reported in Table 3.

Table 3 - Beverage Osmolality Database [mOsm/kg]			
Beverage Type	Range	Average	Number
Water; Purified, Mineral, Tap	0-28	7	10
Diet; Sodas, Teas	13-44	29	19
Fruit Waters	24-39	36	5
Brewed Coffee [Black]	28-53	39	7
Sports Beverages	190-428	390	12
Sugar Containing Sodas, Fruit Drinks	537-1112	760	95
Energy Drinks; Red Bull, etc	673-1030	878	5

Most commercial beverages are produced at multiple locations across the country - and in some cases, across the world. To determine the validity of using baseline data across the U.S., several different lots of each beverage from different bottling locations were checked. Data for Pepsi and Diet Pepsi are reported in Table 4 (next page). The results show some variability, but good overall consistency. However, when possible, using a control beverage in order of preference: Same lot number / same bottling location / same country is (slightly) preferred when analyzing a specific beverage tampering cases. [Note: International variability was not checked in this study, and may be significant due to different formulations in use outside the U.S.]

Table 4 - Beverage Osmolality Database [mOsm/kg]							
Beverage	Osmol	Date	City, State	Beverage	Osmol	Date	City, State
Pepsi [2]	711	10/01	Augusta, ME	Diet Pepsi [2]	13	10/01	Buffalo, NY
Pepsi [2]	713	11/01	Rochester, NY	Diet Pepsi [2]	14	11/01	Rochester, NY
Pepsi [4]	726	6/01	Rochester, NY	Diet Pepsi [2]	15	11/01	Rochester, NY
Pepsi [2]	726	11/01	Buffalo, NY	Diet Pepsi [2]	20	10/01	Augusta, ME
Pepsi [2]	737	10/01	Portland, ME	Diet Pepsi [4]	27	6/01	Rochester, NY
				Diet Pepsi [2]	32	10/01	Portland, ME

Consumer Products Database [2]

A sampling of mouthwashes, breath drops, and eye drops were tested to determine if osmolality might be useful for forensic cases. LSD is often dosed from small dropper bottles that originally contained eye drops or breath drops. Results are reported in Table 5. Because LSD (a very high molecular weight substance) would have minimal osmolality, the finding of a very low osmolality value for a submitted exhibit of these products would indicate probable possible substitution of a water-based fluid containing LSD for the original product. Note that to prevent swelling or shrinking of the eye, eye drops are formulated to match the osmolality of natural tears; this explains their relatively low average osmolality value versus mouthwashes and breath drops. However, even this low value is much higher than a dilute aqueous solution of LSD.

Table 5 - Consumer Products Osmolality Database [mOsm/kg]			
Type	Range	Average	Number
Mouthwash	2660-4900	3683	6
Breath Drops	13950-14130	14040	2
Eye Drops	270-293	285	3

Estimated Osmolality Increases from Substances of Forensic Interest

The osmolality of an adulterated beverage will be increased above its baseline in proportion to the concentration of the agent used and that agent's molecular weight. Estimated osmolality values are reported in Table 6 (next page). Note that (where applicable) the presented results apply only to the free acid form of the material. Because of the dissociation of salt forms in solution, their actual osmolality values would be expected to be higher, in proportion to the molecular weight and concentration of each of the components. For example, a 10 percent solution of sodium γ-hydroxybutyrate, MW=126.1, completely dissociated in an aqueous solution, produces 18.2 grams of sodium cation and 81.8 grams of γ-hydroxybutyrate anion per liter. The resulting expected osmolality would therefore be 1577 mOsm/kg.

Beverage Tampering with GBL, BD [2]

To determine the effect of GBL and BD on the osmolality of beverages, 20 percent V/V solutions of GBL and BD in distilled water were prepared. Using Mountain Dew and Diet Mountain Dew as test beverages, each was spiked with concentrations of GBL and BD to give final solutions ranging from 0.5 - 10 percent. The osmolalities were measured and compared to the average beverage baseline measurements. The results are reported in Tables 7 and 8 (both on next page).

Table 6 - Estimated Osmolality Values [mOsm/kg]			
Substance	MW	1% Solution	10% Solution
Methanol	32.04	312	3121
Ethanol	46.07	217	2170
Acetone	58.08	172	1722
Isopropanol	60.09	164	1664
Ethylene Glycol	62.07	161	1611
GBL [γ-butyrolactone]	86.09	116	1161
GHB-Aldehyde [γ- hydroxybutyraldehyde]	88.11	113	1135
1,4-BD [1,4-Butanediol]	90.12	110	1110
GABA [γ-Aminobutyric Acid]	103.12	97	970
GHB [γ-hydroxybutyrate]	104.11	96	961
Methyl-GHB [4-hydroxyvalerate]	118.13	85	846

Table 7 - Mountain Dew				
GBL Spike	mOsm/kg		1,4-BD Spike	mOsm/kg
10%	1856		10%	Over-range
5%	1378		5%	1376
2%	1042		2%	1036
1%	930		1%	925
0.5%	868		0.5%	867
Baseline	805		Baseline	805

Table 8 - Diet Mountain Dew				
GBL Spike	mOsm/kg		1,4-BD Spike	mOsm/kg
10%	1332		10%	1358
5%	699		5%	671
2%	299		2%	279
1%	168		1%	152
0.5%	100		0.5%	91
Baseline	33		Baseline	33

Illicit use of these chemicals for recreation or for facilitation of sexual assault typically involves ingestion of 1 - 3 grams. “Dosing bottles” are usually diluted to about 30 percent of the psychoactive material; thus, a 6 mL "capful" from a "dosing bottle” contains one dosage unit. At this concentration, the “dosing bottle” solution would need to be diluted 1:5 with distilled water for testing purposes, as a 30 percent solution would exceed the osmometer’s upper measurement limit. At the lower concentrations, however, the results verify that adulterating a beverage with GBL or BD even at a level of only 0.5 percent will cause a measurable increase in the osmolality. This verifies that addition of one “dose” (1 - 3 grams) from a “dosing bottle” to a 16 - 20 oz. beverage will be detectable. This is important, because dilution into beverages is a typical route of administration for purposes of sexual assault, as the beverage flavor tends to disguise the “plastic” taste of the chemical (which has been described as akin to the taste of water from a garden hose left out on a hot day).

Ethanol in Soda-Type Beverages [2]

Numerous reports have indicated that some high school students occasionally "spike" their lunch beverages with alcoholic beverages. We therefore investigated the effect of ethanol on beverage osmolality. One oz [30 mL] of 80 proof vodka was added to 20 oz [590 mL] bottles of Mountain Dew and Diet Mountain Dew. Vodka was selected because it has almost no odor, and it is therefore the alcoholic beverage of choice for surreptitious adulteration by underage drinkers. One oz was selected as the minimum amount of alcohol that would probably be used, as being equivalent to one mixed bar drink. Actual adulteration amounts would likely be higher. The results are as follows:

Mountain Dew:	Baseline - 807 mOsm/kg,	With Vodka Spike - 1174 mOsm/kg
Diet Mountain Dew:	Baseline - 26 mOsm/kg,	With Vodka Spike - 332 mOsm/kg

The Case of the Missing Demerol [3]

Theft of Demerol and other controlled substances by health care professionals is a recurring problem across the U.S. In June 1989, the author (working at the toxicology lab of St. Mary's Hospital in Rochester, New York) received a call from the Drug Enforcement Administration (DEA) regarding a Demerol theft investigation. A number of patients at a local hospital were complaining that they still had pain even after receiving their Demerol injections. Toxicology studies suggested that they had not in fact received any Demerol, implying diversion/theft by a nurse or other health-care professional. Hundreds of nurses were working at any one time, and they often worked on different nursing stations. To identify a suspect, the case agent systematically switched all nurses' floor schedules over several days. This process demonstrated that the patient complaints only occurred when a certain nurse was on duty. The case involved 75 mg Demerol syringes. The agent reasoned that the Demerol was being removed and used by the nurse, and a unknown liquid placed back in the syringe for patient injection. Because no patient became ill, it was felt that the nurse was using one of four sterile solutions as the replacement. The agent wanted to know exactly which of the four solutions was being used so that he could confront the suspect from a basis of fact and thereby elicit a confession. The available solutions included two normal salines and two sterile waters. Osmolality and specific gravity testing were performed on a control (untampered) Demerol syringe solution, on a suspect (tampered) Demerol syringe solution, and on all four sterile solutions. An independent quantitative analysis on the suspect Demerol solution confirmed that it only had 3.9 mg of Demerol remaining - consistent with a single plunger removal of Demerol and refill with one of the sterile solutions. The osmolality and specific gravity results are reported in Table 8.

Table 8 - Osmolality and Specific Gravity Measurements in the Missing Demerol Case

Sample	Osmolality [mOsm/kg]	Spec. Gravity
75 mg Demerol Control Syringe	429	1.037
75 mg Demerol Suspect Syringe	**381**	1.011
Abbott Bacteriostatic Saline	**374**	1.010
Lyphomed Saline	291	1.004
Quad Bacteriostatic Water	93	1.005
Abbott Sterile Water	1	1.000

As the results show, the specific gravity testing had limited usefulness because it could not unambiguously differentiate between all solutions. However, the osmolality testing demonstrated that Abbott Bacteriostatic Saline was most likely used to refill the syringe. The observed 381 mOsm/kg result in the suspect syringe (slightly higher than the Abbott solution), was probably due to the slight effect of the 3.9 mg of Demerol still remaining in the solution. Upon confrontation with the evidence, the nurse admitted her guilt. With the

exception of osmolality, no other laboratory method available at that time could have been employed to differentiate between different brands of saline and water. Osmolality would clearly be a useful technique for similar, current cases of controlled substance thefts from hospitals, pharmacies, doctors' offices, and similar stocks.

Additional Potential Forensic Applications

Identification of Sugar-Based Beverages Substituted for Diet Beverages [2,4]

The accidental or purposeful substitution of a sugar-based beverage for a diet (sugarless) beverage can be harmful to a diabetic individual. Several different lots of Pepsi and Diet Pepsi were tested to determine if it would be possible to differentiate the sugar based beverage from the diet beverage. The results are as follows:

Pepsi:	711-737 mOsm/kg	(n=5)
Diet Pepsi:	13-32 mOsm/kg	(n=6)

Although only 11 different lots were tested, there is clearly enough difference between the two types of beverages to allow a reasonable determination of diet versus sugar-based.

Poisoning of Domestic Pets' Water with Ethylene Glycol

Dogs and cats are very sensitive to the poisonous effects of antifreeze (which contains ethylene glycol). Fatal amounts are 1.4 mL/kg for cats and 6.6 mL/kg for dogs [5]. The sweet odor and taste of ethylene glycol makes it very attractive to animals, and it is therefore a particularly insidious poison. Osmolality is a very useful initial screen for suspect solutions in that it will detect the presence of ethylene glycol (and also other alcohols) at very low levels in water. Based on ethylene glycol's molecular weight of 62.02, a 1 percent solution in water would read 161 mOsm/kg, versus a typical tap water value of approximately 3 mOsm/kg.

Identification of Water [2,3,4]

Water is submitted on occasion to crime laboratories. Although osmolality cannot detect the presence of large molecular weight compounds in water at low concentrations [i.e., most "classic" street drugs], it is an excellent tool to identify that a submitted solution is water. Most waters tested ranged from 0 - 8 mOsm/kg. Only high-mineral content spring waters had higher values, up to 28 mOsm/kg. Non-water solvents will not freeze and no result will be obtained. Any polar solvent mixed into water will greatly increase its osmolality. Acids and bases that have been added to the water will increase the osmolality and also give a pH change. For example, a solution of 1 mL of Chlorox [5 percent hypochlorite] in 100 mL of distilled water, has a pH of 10.5 and an osmolality of 43 mOsm/kg. A solution of 1 mL of 12N HCl in 100 mL of distilled water has a pH of 1.0 and an osmolality of 243 mOsm/kg. A 1 percent solution of ethanol in distilled water has a osmolality of 158 mOsm/kg.

Field Testing

With results available within 15 minutes after plug-in, on only 0.25 mL of sample, the Advanced 3D3 Osmometer instrument used in this study (or any equivalent osmometer) can be easily adapted for field testing at large concert events from police D.U.I. vans. This would allow rapid beverage screening before submission of case samples to the crime lab.

Limitations

"Date-Rape" Benzodiazepines in Solution [2]

As previously mentioned, the high molecular weight of common "classic" street drugs, and their low concentration in submitted solutions, makes osmolality an ineffective screening tool for their identification. For example, a single methylphenidate (Ritalin) tablet containing 5 mg of active drug and weighing 91 mg, produced a measured osmolality of only 11 mOsm/kg when dissolved in 30 mL distilled water. Therefore, osmolality is not viable for detection of drink tampering with, e.g., flunitrazepam (Rohypnol) or other sedative benzodiazepines that are employed for drug facilitated sexual assault.

Urine in Beverages [6]

Beverages are occasionally maliciously adulterated with urine. The osmolality of an individual's urine varies widely [50 - 1400 mOsm/kg] and greatly depends on the person's degree of hydration. Urea, the compound of highest concentration in the urine, varies from 0.7 - 3.3 g/100 mL, and is a better indicator of tampering than osmolality. Although a typical random urine volume of 4 - 8 oz [118 - 237 mL] may be produced, let us assume 1 oz [30 mL] was introduced into a 50 oz pot of coffee[1480 mL]. The resulting urea levels would be 14 - 67 mg/100 mL. This is easily measured with a typical urea analysis method, which usually have a dynamic range of 2 - 212 mg/100 mL.

Saliva in Beverages [3]

Similarly, beverages are occasionally maliciously adulterated with saliva. Amylase, which is present in very high levels in saliva [20,000 units/100 mL], is a better indicator of beverage adulteration with saliva versus osmolality. A typical 0.5 mL "spit" volume in an 8 oz [237 mL] cup of coffee would result in a measured amylase of 422 units/100 mL. This is easily measured with an amylase method having a dynamic range of 1-200 units/100 mL.

Conclusions

With ever increasing case loads and limited personnel resources, crime laboratories need efficient new tools to process the disturbing increases in liquid sample submissions. Osmolality, an effective analytical tool of the hospital laboratory and food and consumer products industries, is a low cost, rapid, facile, and non-destructive screening tool for forensic chemists and toxicologists.

Acknowledgements

Special thanks to Don Wiggin from Advanced Instruments for the loan of the 3D3 osmometer, and to the Rochester Institute of Technology and Drug ID Systems for providing the samples for testing.

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Psychotria Viridis - A Botanical Source of Dimethyltryptamine (DMT)

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ABSTRACT: Dimethyltryptamine was identified by GC/MS in a sample of dried leafy material that was subsequently identified as *Psychotria viridis* (Rubiaceae), a tropical shrub native to Central and South America that has ethnobotanical use as a hallucinogen by many indigenous peoples of tropical South America. The botanical characteristics of *Psychotria viridis* are illustrated and described.

KEYWORDS: *Psychotria viridis*, Dimethyltryptamine, DMT, *Banisteriopsis caapi*, *Ayahuasca*

Introduction

The Naval Criminal Investigative Service Regional Forensic Laboratory (NCISRFL) in San Diego, California recently received several items that investigators had obtained from a U.S. Marine stationed in Yuma, Arizona. Item A (see Figure 1) consisted of a self-sealing plastic bag containing dried whole leaves mostly still attached

Figure 1 - A Portion of the Sample as Received

to stem pieces. Analysis by macro and microscopic examination indicated that the material clearly was not marijuana, nor were there any visible signs that anything had been added to the leaves.

Experimental

Approximately 1 gram of dried leaf material was placed in a glass beaker and covered with about 3 mLs of methanol. The beaker was then heated on a hot plate in a fume hood. When the methanol volume had been reduced to about 0.5 mL, the beaker was removed from the hot plate and 1µL of the remaining extract was injected into a Hewlett-Packard 5890 Gas Chromatograph (Palo Alto, CA) equipped with a 5971 Mass Selective Detector and fitted with an HP-1 capillary column (crosslinked methyl silicone, 20 m x 0.25 mm i.d. x 2.65 µm film thickness). The column oven temperature was programmed from an initial temperature of 70˚ C (held for 2 min) to 200˚ C at 10˚ C/min, then held at 200˚ C for the final 2 minutes.

Results

The total ion chromatogram revealed just one strong peak above the background, as shown in Figure 2. The mass spectrum of this peak is shown in Figure 3. A library search gave N,N – dimethyltryptamine (DMT) as the

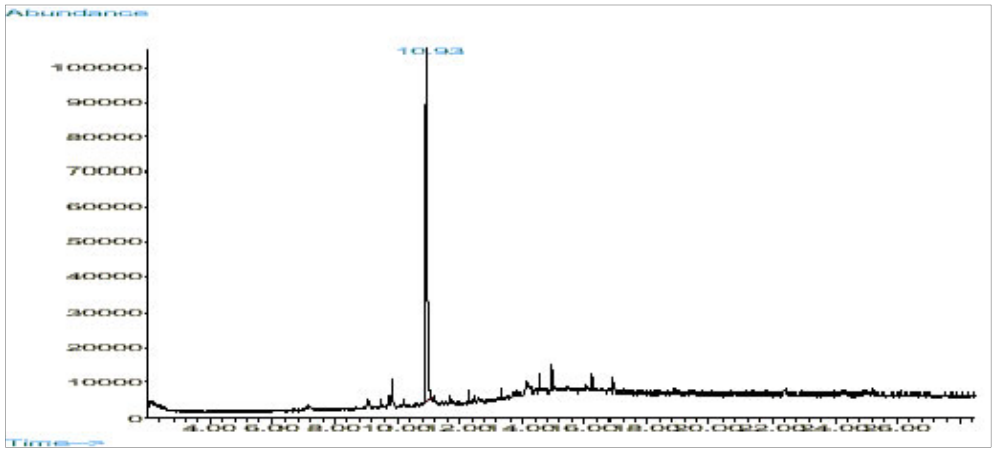

Figure 2 - Total Ion Chromatogram of a Methanol Extract

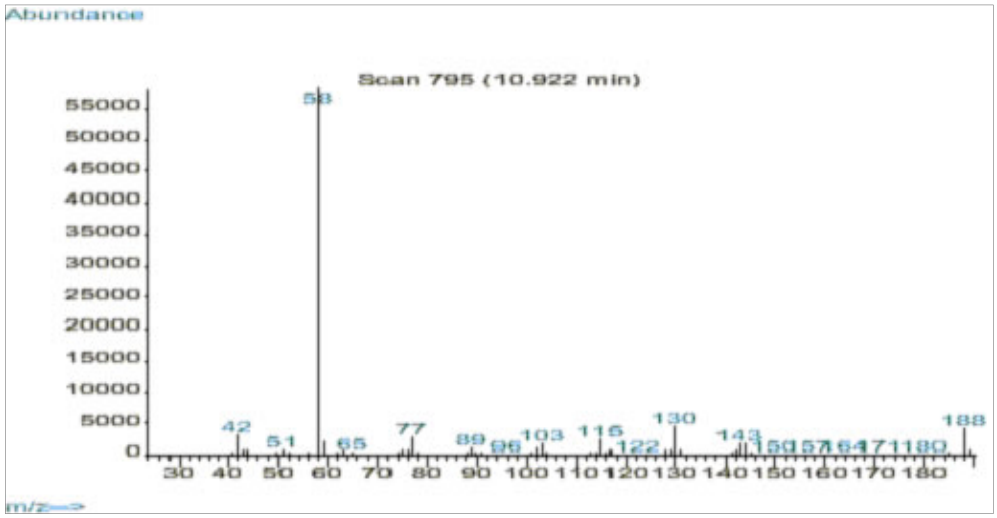

Figure 3 - Mass Spectrum of Dimethyltryptamine (Major Peak)

closest hit. The identification of DMT was confirmed when subsequent injection of a DMT standard produced a matching spectrum at the same retention time. DMT, an hallucinogen, is a Schedule I Controlled Substance. The dried leaves and stems were in good condition for botanical evaluation, and were matched to reference specimens of *Psychotria viridis* from Peru. DMT is known to be present in *Psychotria viridis* (1,2).

Ethnobotanical Use of Psychotria viridis

A narcotic drink often called *ayahuasca* or *caapi* is made from an infusion of the bark of the so-called "Spirit Vine", *Banisteriopsis caapi* [(Spruce ex Griseb.) C.V. Morton, Malpighiaceae] and related species of tropical rainforest lianas, by many indigenous peoples of the Amazon River basin and northwestern South America (2,3). *Ayahuasca* contains several hallucinogenic alkaloids, including harmine and harmaline, and is widely used in traditional medical rites and mystical and religious ceremonies as a purgative, a magic hallucinogen, and for prophecy, diagnosis, and telepathy. Other plants are frequently added to the infusion to alter and/or enhance the effects of the *Banisteriopsis* hallucinogens. A commonly used admixture is another plant containing DMT, which reportedly increases the intensity and duration of the *ayahuasca* intoxication. DMT is found in several plant species that grow in the same region as *Banisteriopsis*, including *Psychotria viridis*. Schultes and Hoffmann have detailed the botany, ethnobotany, and chemistry of *ayahuasca* and its common admixtures (3), and Casale and Koles have detailed the forensic analysis of a typical sample (4).

Botanical Identification

Psychotria is a large genus of shrubs and small trees found in tropical regions around the world (including about 1400 species, with perhaps 700 in the New World), and its taxonomy is somewhat complicated. Not surprisingly, several other New World tropical species are morphologically similar to *Psychotria viridis*, and at least some of these may also be used as admixtures in *ayahuasca* (3).

Psychotria viridis [Ruiz & Pav., Rubiaceae] can be recognized by a combination of features found on the vegetative portions of the plant, listed below and shown in Figure 1, although reproductive structures provide conclusive identification [see Figure 4 (next page) for illustrations of the reproductive characters]. *Psychotria viridis* grows naturally in wet lowland tropical forests in Cuba and northern Central America through western and central South America; it appears to be most common in Amazonian Peru and Bolivia. Because the genus *Psychotria* includes a large number of morphologically similar species, and there are other genera of the same plant family that are similar, the presence of all the characteristics listed below is needed to conclusively identify *Psychotria viridis*. Botanical identification of shredded or powdered material, or even leaves without stems, would be challenging.

· **Stems.** In the middle and lower parts of the stem, situated between the insertion points of the two opposite leaves there is a horizontal scar 0.3-1 mm wide that extends between the leaves (or leaf scars) and sometimes also connects over the tops of these scars, and along the top side of this scar there is a dense, usually furry line of fine trichomes (i.e., plant hairs) usually 0.5-1 mm long that are reddish brown when dried (Figure 4A). This combination of features is diagnostic for many species in the genus *Psychotria*, though not for any individual species [i.e., these features distinguish *Psychotria* L. Subg. *Psychotria*; other subgenera of *Psychotria* lack the well developed reddish brown trichomes inserted above the stipule scars]. On the upper stems of *Psychotria viridis* these features are obscured by a stipule (see below), which covers the trichomes; the scar actually marks the point where this structure has fallen off.

· **Stipules.** These are leafy structures that cover and protect the young developing leaves, then fall off leaving scars on the stem. The stipules are produced in pairs, and their form is distinctive for *Psychotria viridis*: They are 5-25 x 4-12 mm, elliptic in outline, sharply angled at the apex, papery to [continued on page 22]

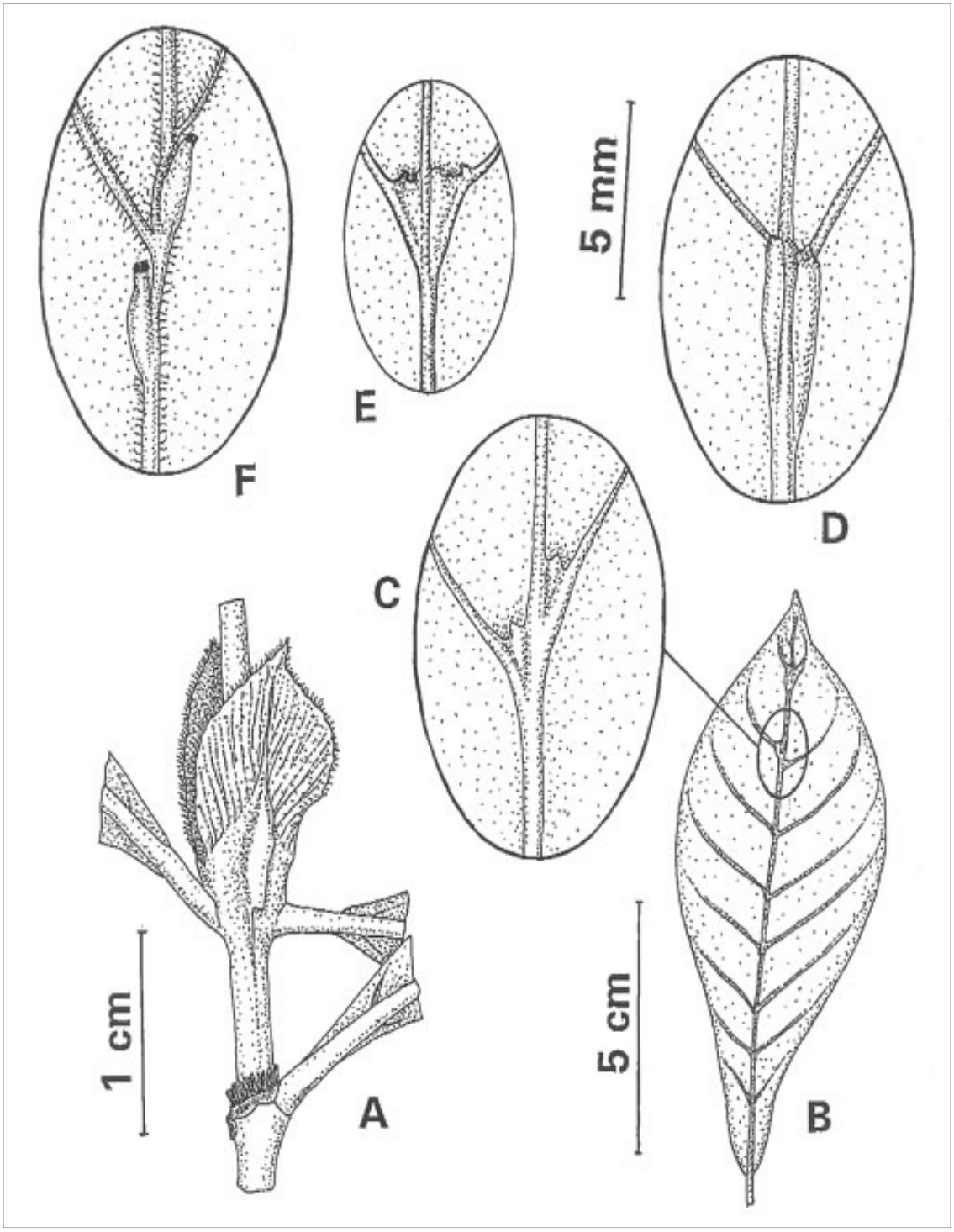

Figure 4 - Vegetative characters of *Psychotria viridis*. A, Portion of upper stem showing, from top, a pair of well developed stipules, the bases of a pair of leaves, a stipule scar with a fringe of trichomes above it, the base of another leaf, and the scar of this last leaf's pair that has fallen off. B, Leaf, underside view with a pair of foveolae circled. C, Enlarged view of foveolae from leaf shown in B. D, Enlarged view of foveolae from the forensic sample discussed in this article. E, Enlarged view of foveolae from a different botanical specimen of *Psychotria viridis*. F, Enlarged view of a different botanical specimen of *Psychotria viridis*. C, D, E, F to 5-mm scale. A, B, C based on *N. Ritter and Wood 3702* (MO), from Bolivia; E, *Gentry and Jaramillo 57585* (MO), Peru; and F, *Solomon and Urcullo 14103* (MO), Bolivia.

membranaceous in texture, ciliate (i.e., fringed) along the upper margins, and longitudinally flanged or winged along the middle (Figure 4A). However, stipule shape and size is quite variable among different plants, and also depends on the stipule's developmental stage and other factors such as whether the stem that produced it is reproductive or vegetative.

· **Leaves.** These (Figure 4B) are opposite in arrangement (i.e., produced in pairs along the stems), generally 5-15 x 2-6 cm, in outline generally elliptic or often widest above the middle, usually sharply angled at base and apex, papery in texture, overall smooth or infrequently with microscropic plant hairs on the lower surface, have 5-10 pairs of secondary veins, and on the lower surface usually have foveolae (see next item). The leaves are borne on petioles (i.e., leaf stalks) generally 1-10 mm long. When dry, the leaves of *Psychotria viridis* usually are gray or reddish brown. The leaves of *Psychotria viridis* are similar to a few other New World species of *Psychotria*.

· **Foveolae.** These are small pockets found on the lower leaf surface near the junction of the secondary (i.e., side) veins with the central vein. They function as shelter for tiny invertebrates such as mites that live on the plant leaf. These mites apparently often are symbiotic with the plant, taking shelter in these structures and eating fungi and herbivorous invertebrates that can damage the leaf. The foveolae (also called domatia) are distinctive for *Psychotria viridis* and a few related species: They are generally 1.5-5 mm long and 0.5-1 mm wide at the top, conical and tapered to a closed base, open and truncate or variously ornamented at the top, and situated along the sides of the central vein with the opening usually near a secondary vein (Figure 4C). These foveolae vary in shape among different plants (Figure 4C, 4D, 4E, 4F), and in number on individual leaves, and may not even be present on some leaves. Most often each leaf bears at least one pair of foveolae, which may be close to the apex; the foveolae are often more numerous on leaves from vegetative stems than on those from reproductive stems.

Conclusions

How does a U.S. Marine obtain plant material that grows in the Amazon basin? The suspect refused to cooperate, but an Internet sales contact was the most likely source. *Psychotria viridis* leaves in various forms (whole, broken, finely powdered, shredded) reportedly exported from Peru are offered for sale on the Internet.

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Evaluation of Ninhydrin Analogues and Other Electron-Deficient Compounds as Spray Reagents for Drugs on Thin Layer Chromatograms

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ABSTRACT: Twenty-four electron-deficient compounds were evaluated as potential spray color-reagents for basic drugs on TLC plates. Two of them, 4-chloro-7-nitro-2,1,3-benzoxadiazole and 5,6-dimethoxyninhydrin, were superior to ninhydrin with respect to sensitivity and selectivity, and offer considerable potential.

KEYWORDS: Thin Layer Chromatography, TLC, Spray Reagents, Ninhydrin, Illicit Drugs

Introduction

Since the discovery by Dutt and Teo[1] that spraying thin layer chromatographic (TLC) plates bearing drug spots with ninhydrin produces a variety of colors that can distinguish between many drugs, this reagent has been intensively used in this laboratory. The colors that are produced with ninhydrin, when correlated with the specific migration values (R_f) for each spot on specific TLC plates and using select solvent systems, greatly enhance the specificity of TLC for various drugs.

In forensic laboratories, the main use of ninhydrin as a spray reagent has been for detection of fingerprints, especially on porous surfaces such as paper and cardboard.[2-4] However, despite its great utility, research has continued to develop even more sensitive or selective reagents. Over the last two decades a significant number of ninhydrin analogous and similar, electron deficient compounds have been synthesized and evaluated as fingerprint reagents. Some of these new reagents have displayed superior properties versus ninhydrin in their sensitivity to amino acids and latent fingerprints, particularly in the fluorescence mode.[2-7]

The aim of the present study was to evaluate some of these new fingerprint detection reagents for drug detection on TLC plates. The development of new, more intense, or fluorescent colors for various drugs would increase the overall specificity and sensitivity of drug-screening TLC. Such reagents could also discriminate between drugs that produce the same color with ninhydrin.

Experimental

Drugs

The controlled substances examined in this study included the following pharmaceutical and illicit drugs: Cocaine HCl and morphine HCl (Merck, Germany), diazepam, flunitrazepam, codeine phosphate, and methadone

HCl (Teva, Israel), lysergic acid diethylamide (LSD) (Sigma, Israel), amphetamine (Assia Chem Laboratory, Israel), heroin base, opium, and 3,4-methylenedioxymethamphetamine (MDMA) HCl (from DIFS case files), and methamphetamine, 3,4-methylenedioxyamphetamine (MDA) HCl, and 3,4-methylenedioxyethylamphetamine (MDEA) HCl (synthesized at DIFS). Similar aliquots (same concentration) of each drug were deposited on TLC plates for comparison.

Imaging Reagents

Twenty-four potential imaging reagents were tested (see Table 1, on pages 25 - 27, for names, sources, and structural formulas). Like ninhydrin, all the compounds that were studied are molecules with electron-deficient cores. Also like ninhydrin, most of them possess the indane-dione skeleton; the remainder have quininoid or cyclobutenedione type structures. All reagents were dissolved in 95% ethanol to reach testing concentrations from 0.5 - 10%.

TLC, Elution Solvents, and Spray Reagents

TLC was carried out on standard silica gel plates (10 x 20 cm) containing a fluorescent indicator (254 nm) on aluminum support (Macherey-Nagel, Germany). A dioxane:xylenes:ethanol:ammonia (40:30:5:5) solvent mixture was used as the mobile phase in the developing tank. After the solvent elution, the plates were dried in an oven at 120ºC for 3 - 4 minutes, then cooled to room temperature. The plates were then sprayed with the reagent solution, then heated again for 10 minutes. The colors of the spots as well as background interferences were immediately recorded and photographed.

Methods

1st Stage

At the first evaluation stage, all 24 reagents were tested on TLC plates against five basic drugs: Heroin, cocaine, MDMA, diazepam, and flunitrazepam. At this stage the plates were not processed in the solvent system; rather, the drugs were spotted on the plates and the spots were treated with the reagents (5 - 10% w/v) via direct application using a pipette or cotton swab. When a color reaction was noted using these initial reagent concentrations, a lower concentration solution (0.5%) of the target reagent was attempted.

2nd Stage

At the second evaluation stage, only those reagents that had produced colored spots with at least one drug were investigated. At this stage, the selected color reagents were evaluated for all 14 of the above listed target drugs. In addition, in the second stage, each TLC plate bearing the drug spots was eluted using above specified the TLC solvent system, then sprayed with the reagent solution, then heated to 120ºC. The results were compared versus those obtained by the ninhydrin solution routinely used in the laboratory.

3rd Stage

In the third stage, experimental parameters were optimized for the successful color reagents identified at the second stage. The principal optimization parameters were reagent concentration and color development temperature. Ethanolic solutions of six concentrations (0.5, 1, 2, 3, 4 and 5% v/w) were prepared for each one of the successful reagents. Each successful reagent at each given concentration was tested against each drug that it had displayed a colored spot with in Stage 2, and after elution evaluated at different development temperatures (80, 100, 120, 130, 140, 160 and 200ºC). It was noted that while high reagent concentrations produced more intense colors, they also usually resulted in development of significant background colorations. High temperatures had a similar effect. Colors developed and background interferences were recorded for each set of experiments.

Table 1. Names, Sources, and Structural Formulas for Imaging Reagents
(continued on pages 21 - 22).

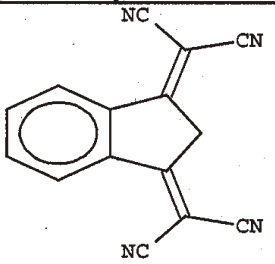
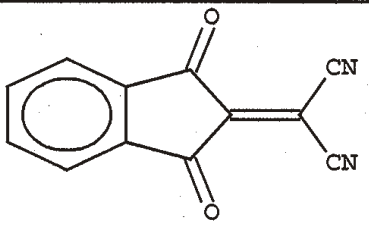
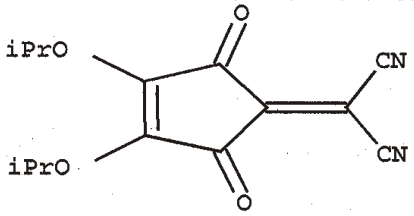
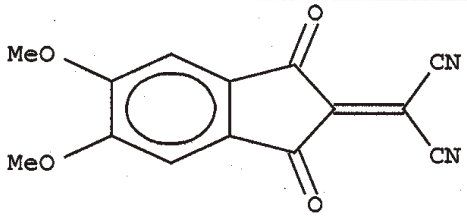
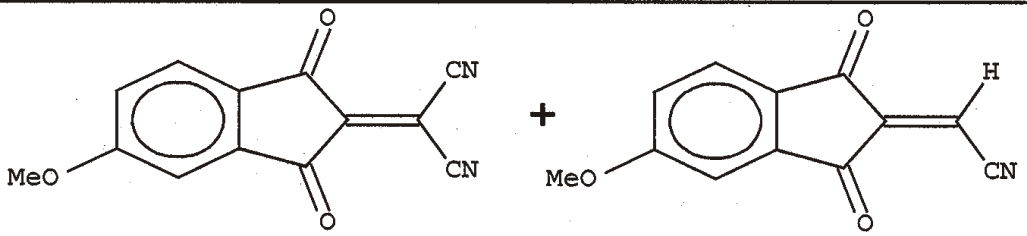
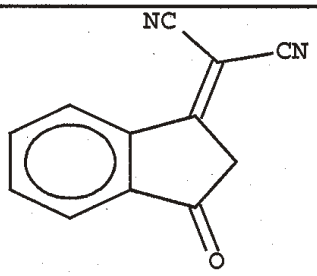
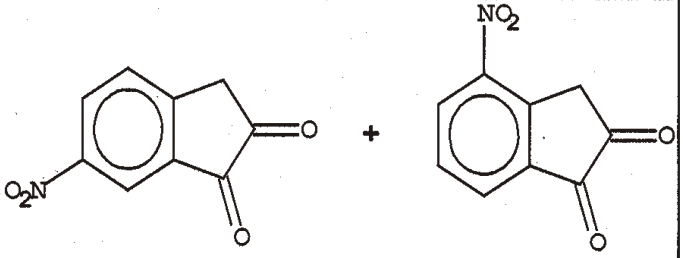
(A) [3-(dicyanomethylene)-2,3-dihydro-1H-inden-1-ylidene] malononitrile *source*: DIFS - synthesis[8]	**(B)** (1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile *source*: DIFS - synthesis[8]
	
(C) (3,4-dimethyl-2,5-dioxocyclopent-3- en-1-ylidene) malononitrile *source*: DIFS - synthesis[8]	**(D)** (5,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile *source*: DIFS - synthesis[8]
	
(E) (5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile + (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene) propanenitrile (mixture of 2 compounds) *source*: DIFS - synthesis[8]	
	
(F) (3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile *source*: DIFS - synthesis[8]	**(G)** A mixture of 4- and 6-nitro-1,2-indanediones *source*: DIFS – synthesis[12]
	

Table 1 (continued).

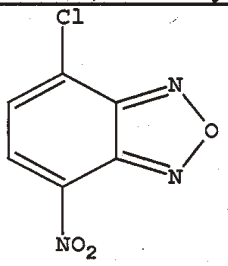
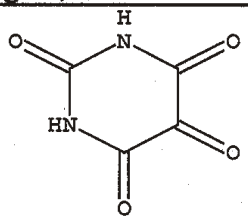
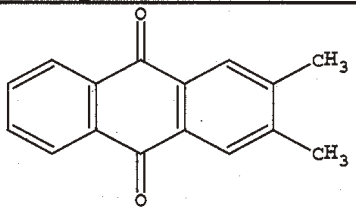
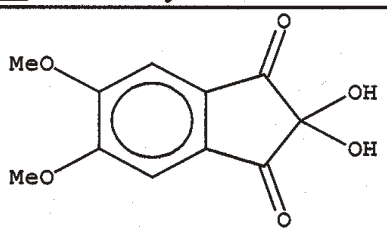
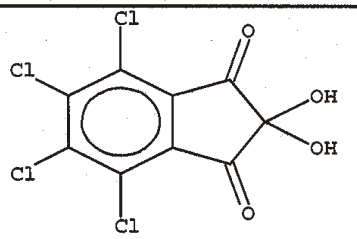
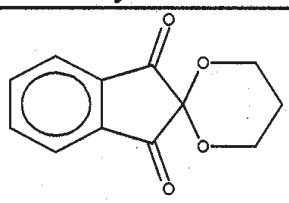
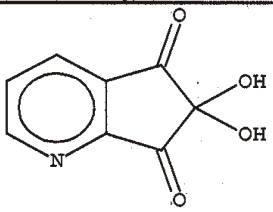
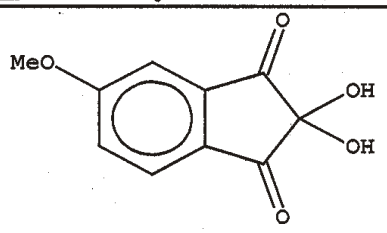
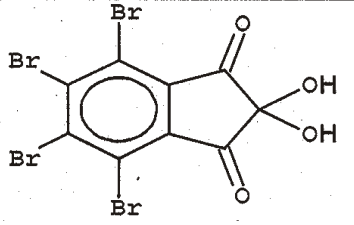
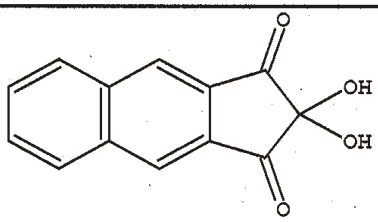
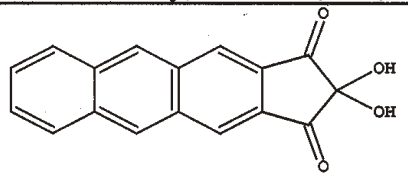
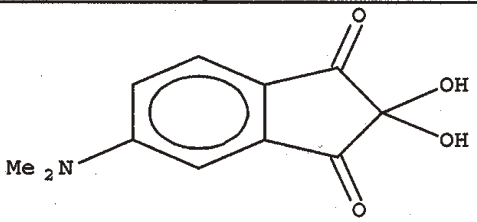
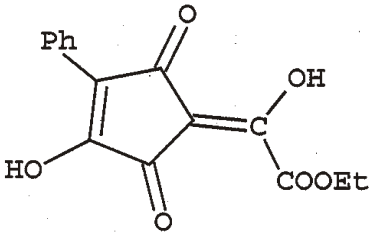
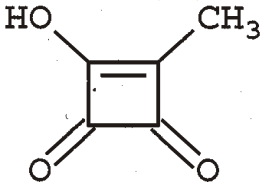
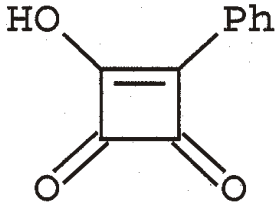
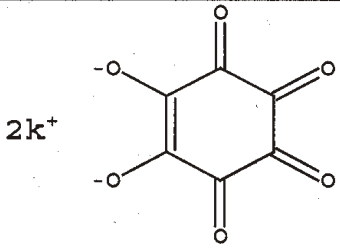
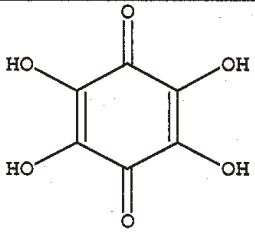
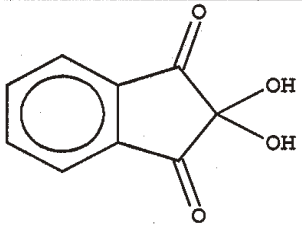
(H) 4-chloro-7-nitro-2,1,3-benzoxadiazole (*NBD-chloride*) *source*: Aldrich, Germany	**(I)** alloxan *source*: Sigma, Israel
	
(J) 2,3-dimethylanthraquinone *source*: Sigma, Israel	**(K)** 5,6-dimethoxyninhydrin *source:* DIFS - synthesis[9]
	
(L) 4,5,6,7-tetrachloroninhydrin *source*: Dr. A.A. Cantu, US Secret Service	**(M)** spiro[2,5-dioxacyclohexane-1,2'-indene]-1',3'-dione *(ninhydrin-2-trimethyleneketal)* *source*: DIFS - synthesis[10]
	
(N) pyridine analogue of ninhydrin *source*: DIFS – synthesis[12]	**(O)** 5-methoxyninhydrin *source*: DIFS - synthesis[11]
	
(P) 4,5,6,7-tetrabromoninhydrin *source*: Dr. A.A. Cantu, US Secret Service	**(Q)** benzo[f]ninhydrin *source*: DIFS - synthesis[12]
	

Table 1 (continued).

(R) naphtho[f]ninhydrin *source*: Prof. E.R. Menzel, Texas Tech. University, Lubbock, TX, USA	**(S)** 5-dimethylaminoninhydrin *source*: DIFS - synthesis[12]
	
(T) ethyl (2E)-hydroxy[3-hydroxy-2,5-dioxo-4-phenylcyclopent-3-en-1-ylidene]acetate *source*: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA	**(U)** 2-methyl-3,4- dioxocyclobut-1-en-1-ol *source*: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA
	
(V) 3,4-dioxo-2-phenylcyclobut -1-en-1-ol *source*: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA	**(W)** potassium rhodizonate *source*: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA
	
(X) 3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrol *source*: Prof. R.C. West, , Dept. of Chemistry, University of Wisconsin, Madison, USA	**(Y)** ninhydrin *source*: Spectrum, USA.
	

Results and Discussion

Five of the twenty-four reagents examined at the first evaluation stage yielded a color reaction with at least one drug (see Table 2, next page). These were reagents **E** (mixture of 5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile and (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene) propanenitrile, **F** (3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile, **H** (4-chloro-7-nitro-2,1,3-benzoxadiazole), **K** (5,6-dimethoxy-ninhydrin), and **O** (5-methoxyninhydrin). Seven of the twenty-four compounds gave no visible reaction, and the remainder were rejected because of the development of intense background coloration.

At the second stage, the five preliminarily successful reagents listed above were evaluated for all 14 drugs. The results are summarized in Table 3 (see page 30), and are detailed below:

Reagent E (a mixture of 5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene)malononitrile and (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene)propanenitrile), at working concentrations of 1 - 5% w/v: A yellow background is observed and the sensitivity is low; therefore, the colored spots are weak in comparison with the background.

Reagent F ((3-oxo-2,3-dihydro-1H-inden-1-ylidene)malononitrile), at a working concentration of 2% w/v: Intense brown-red spots are observed, mostly with amphetamines. In contrast, opiates (heroin, morphine) and cocaine produce only low intensity red colored spots. No reaction is observed with LSD. At low drug concentrations, the red colored background interferes with the colored spots.

Reagent H (4-chloro-7-nitro-2,1,3-benzoxadiazole), at working concentrations of 1.5 - 2% w/v: Intense brown-purple spots are formed with amphetamines, yellow spots with narcotine and papaverine in opium, blue spots with heroin, and brown spots with cocaine. An intense color reaction is also observed with LSD. In general, the colors obtained are very similar to the colors developed with ninhydrin, but the sensitivity of **H** is higher; therefore, a lower reagent concentration is required.

Reagent K (5,6-dimethoxyninhydrin), at a working concentration of 0.5% w/v: Very intense spots are formed with amphetamines, LSD and methadone. Opiates (heroin, morphine) produce weak purple spots. Strong purple spots are formed by MDMA and MDEA. Amphetamine and MDA yield milky-yellow spots.

Reagent O (5-methoxyninhydrin), at a working concentration of 2.5% w/v: Intense purple spots are formed with amphetamines, while opiates and LSD produce only very weak purple spots. In addition, a pink background discoloration is observed.

Of the five above reagents, **H** and **K** showed better performance versus the other three, and were therefore selected for further investigation. Optimization trials were carried out with both **H** and **K** at various concentrations and color development temperatures. The optimized parameters for **H** are: A 3% solution (w/v) with color development at 120ºC. Under these conditions, opiates (heroin, morphine) can also be detected. The optimized conditions for **K** are: A 0.5-1% solution (w/v) with color development at 120ºC. Under these conditions, only amphetamines show strong color reactions. It is noted for comparison that ninhydrin is typically utilized as a 10% solution.

Conclusions

4-Chloro-7-nitro-2,1,3-benzoxadiazole and 4,6-dimethoxyninhydrin both show good potential as spray reagents for drugs on chromatographic plates. Both reagents show some advantage over ninhydrin in their reactivity, developing more intense colors at lower reagent concentrations. Furthermore, 5,6-dimethoxyninhydrin also produces two different colors with different amphetamines: Purple spots are formed by (continued on page 30)

Table 2. Results Correlated Against Structures.

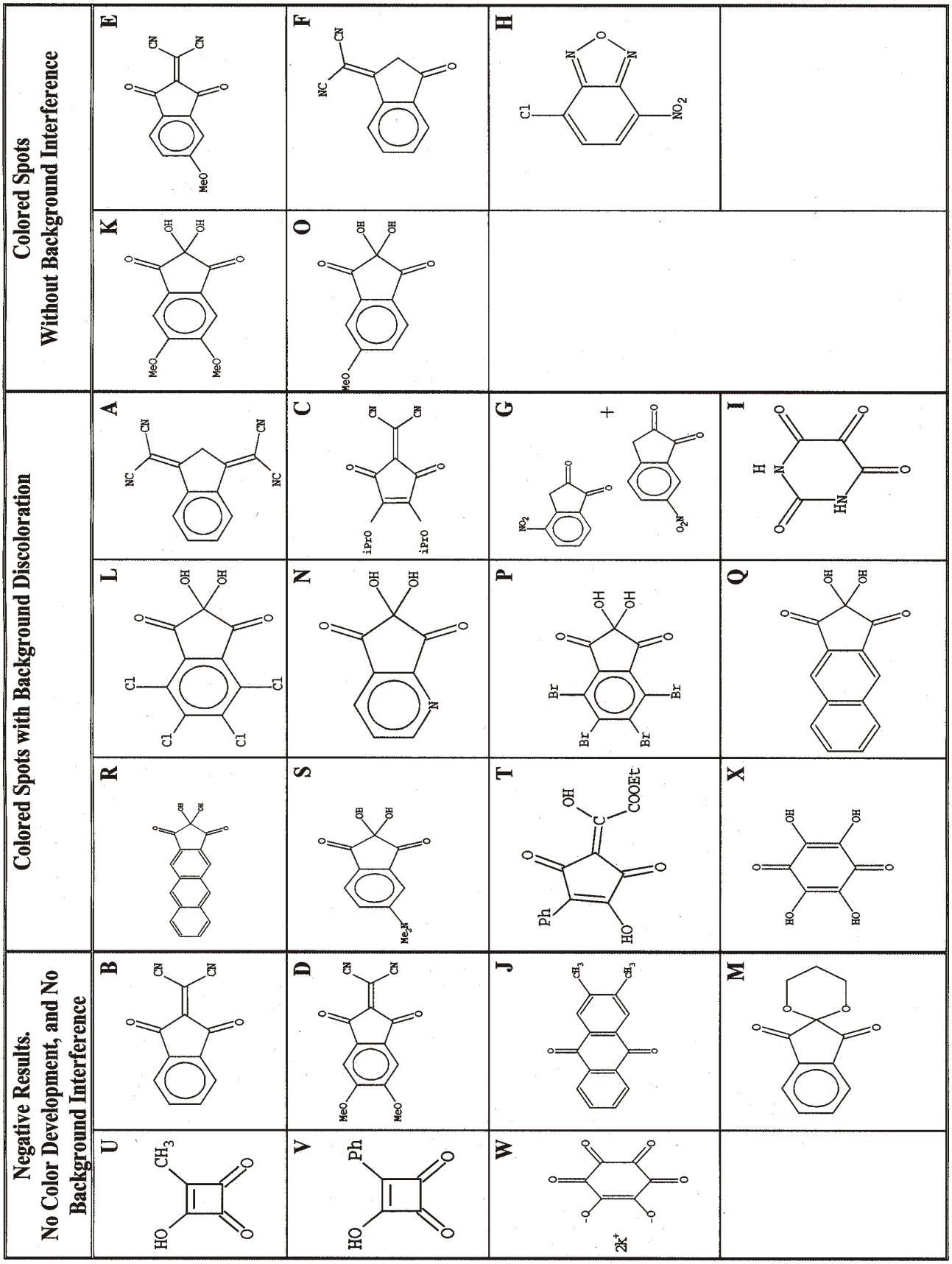


Table 3. Correlation of Results of Most Successful Reagents Against 14 Selected Drugs.

Drug	Reagent					
	E 1%	**F** 2%	**H** 1-2.5%	**K** 0.5%	**O** 2.5%	**ninhydrin** 10%
Heroin	-	Red	Blue	-	Purple	Blue
Cocaine	-	Red	Brown	-	Purple	Beige
Diazepam	-	-	-	-	-	-
Flunitrazepam	-	-	-	-	-	-
LSD	-	-	Blue	Purple	Purple	Blue
Morphine	-	Red	Brown-purple	Purple	Purple	Blue
Codeine	-	Red	Brown-purple	Purple	Purple	Blue
Opium	-	-	5 compounds yellow/purple	Undefined color	Undefined color	5 compounds yellow/purple
Methadone	-	Red	Brown-purple	Purple	Purple	Brown
Methamphetamine	-	Red	Brown-purple	Purple	Purple	purple
Amphetamine	-	Purple	Brown-purple	Milky yellow	Purple	Beige-brown
MDMA	-	Brown-red	Brown-purple	Purple	Purple	Purple
MDA	-	Brown-red	Brown-purple	Milky yellow	Purple	Purple
MDEA	-	Brown-red	Brown-purple	Purple	Purple	Purple

methamphetamine, MDMA, and MDEA, and milky yellow spots are formed by amphetamine and MDA. A mechanistic study of these color formation reaction may lead to a rational design of even better reagents of this family.

Acknowledgments: The authors are indebted to Dr. Antonio A. Cantu, Chief Chemist, US Secret Service, to Professor Robert C. West of the Chemistry Department, University of Wisconsin, Madison, and to Professor E. Roland Menzel, Director of the Center for Forensic Studies, Texas Tech University, Lubbock, for kindly providing them with some of the compounds for testing. The authors also gratefully acknowledge Ms. Lital Cohen for her technical assistance.

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12. Almog J, Hirshfeld A, Frank A, Sterling J, Leonov D. Aminoninhydrins: Fingerprint reagents with direct fluorogenic activity-preliminary studies. J Forensic Sci 1991;36(1):104.

* * * * *

Technical Note

Instrumental Separation of 3,4-Methylenedioxyamphetamine (MDA) from 1-(3,4-Methylenedioxyphenyl)-2-propanol, a Co-Eluting Compound

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ABSTRACT: Analysis of a set of mixed-component Ecstasy tablets by GC/MS indicated an apparent mixture of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylendioxyamphetamine (MDA); however, the mass spectrum for the MDA did not exactly match an MDA standard. Additional work confirmed that the presumed MDA was actually a co-eluting mixture of MDA and 1-(3,4-methylenedioxyphenyl)-2-propanol. The latter alcohol has a mass spectrum that is highly similar to MDA, but displays a molecular weight peak of 180 (versus 179 for MDA). Varying the temperature programming of the normal GC/MS run separated the alcohol.

KEYWORDS: 3,4-Methylenedioxymethamphetamine, MDMA, 3,4-Methylenedioxyamphetamine, MDA, 1-(3,4-Methylenedioxyphenyl)-2-propanol, Ecstasy, GC/MS, Co-Elution

Introduction

Over the past few years, so-called "Ecstasy" tablets have undergone a dramatic transition in their composition. Five years ago, most Ecstasy tablets contained either 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), or (less commonly), a mixture of MDMA and MDA. More recently, however, Ecstasy tablets have often contained complex mixtures of controlled substances, control substance analogues, alternate abused substances, adulterants, diluents, and manufacturing impurities and byproducts. These mixed component tablets can offer unusual analytical challenges.

In late 2001, this laboratory received an exhibit consisting of 11 white tablets with a three-point crown imprint on one side and unmarked on the other side (photo not available), total net mass 3.3 grams. The exhibit was seized just north of Tampa, Florida, but had no other associated source information. Analysis was conducted by color testing (Marquis, cobalt thiocyanate, and Dille-Koppanyi), thin layer chromatography (TLC) (Clarke's TB developer, visualized with acidified iodoplatinate), and gas chromatography/mass spectrometry (GC/MS). The color test results were consistent with typical (MDMA) type preparations. The Marquis test showed the usual purple, blue, and green colors, and the cobalt thiocyanate gave a slight blue reaction. After elution, spraying, and development, the TLC showed two spots consistent in both color and R_f to MDMA and MDA; however, a third spot was also noted. The first GC/MS run revealed four peaks, one with a retention time and mass spectrum corresponding to MDMA, a second with a retention time and mass spectrum very similar to MDA but with an apparent molecular ion at 180 instead of the expected 179 (for MDA), and two unknowns that did not correspond to any known controlled substances and were therefore not further analyzed. Closer examination of the "MDA" mass spectrum indicated that the fragment ion ratios at 135 relative to 136, and at 106 relative to 105, both appeared to be slightly higher than normally expected for MDA. The sample was then injected on a second GC/MS to determine if the anomalous results were an instrumental variation or a glitch of some type in the run. The second GC/MS run again revealed the same four peaks (see Figure 1, next page); the first compound (designated "A1" on Figure 1) had a retention time and mass spectrum very similar to MDA but still with the

apparent molecular ion at 180 instead of the expected 179. The second peak (designated "A2" on Figure 1) had a retention time and mass spectrum corresponding to MDMA. The two additional peaks ("A3" and "A4" on Figure 1) were also still present, but were not identified. The mass spectra of A1 through A4 are shown in Figures 2 - 5.

Figure 1.
Total Ion Chromatogram

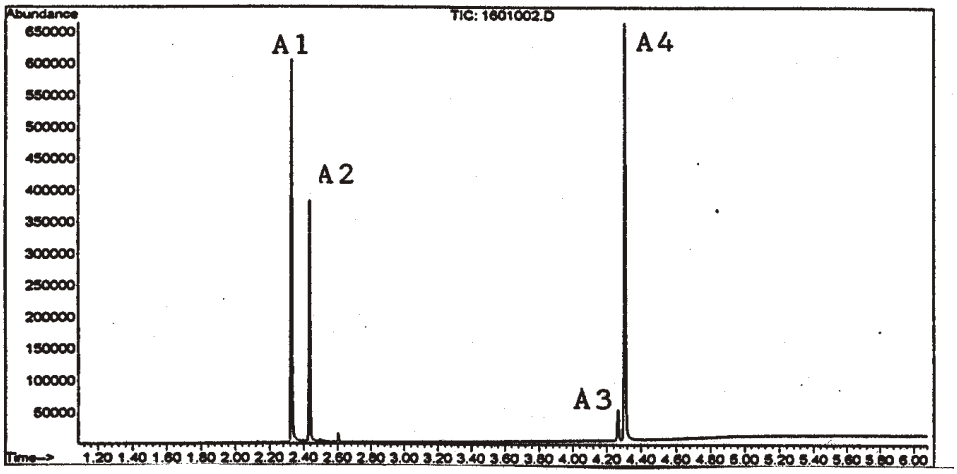


Figure 2.
Mass Spectrum of Compound A1 (Anomalous MDA).

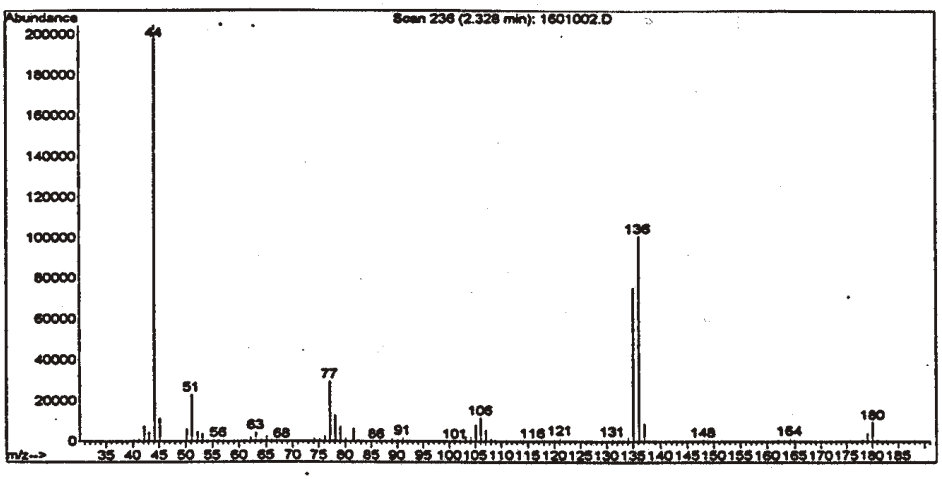


Figure 3.
Mass Spectrum of Compound A2 (MDMA)

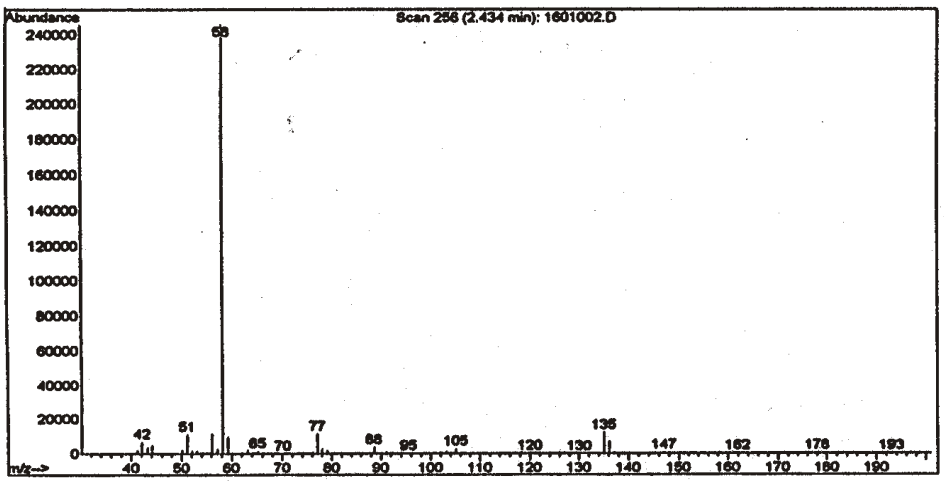


Figure 4.
Mass Spectrum of
Compound A3 (Unknown)

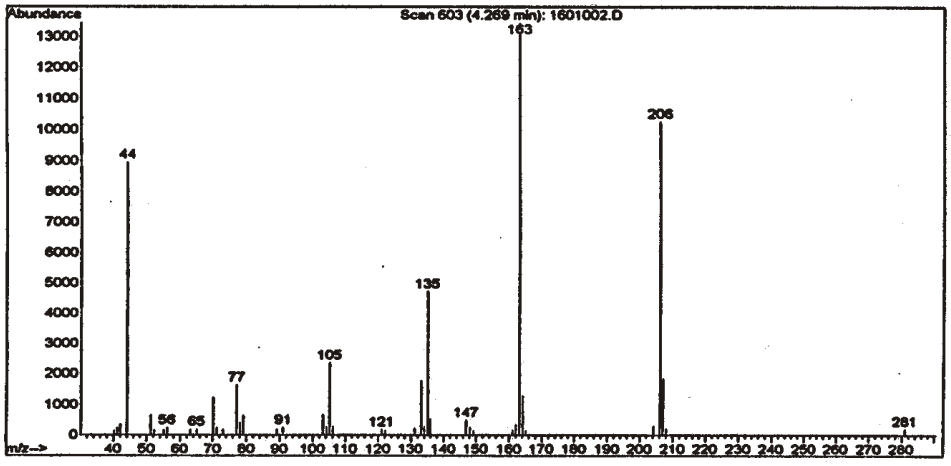
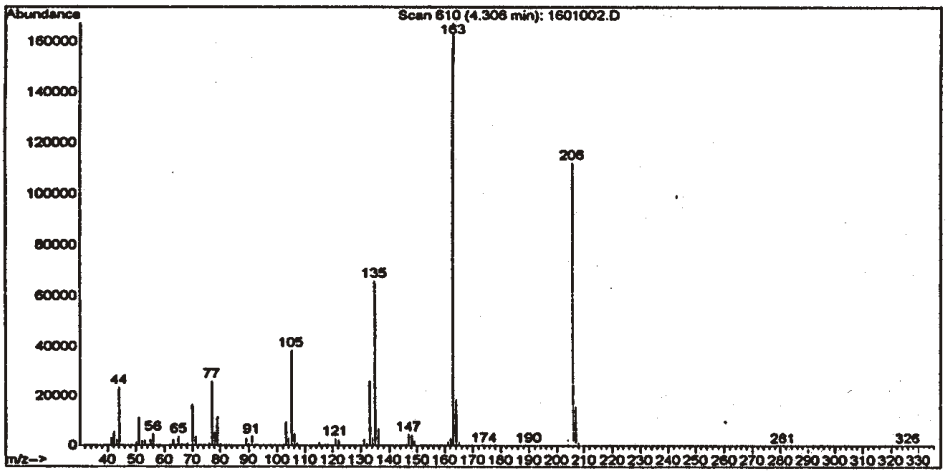
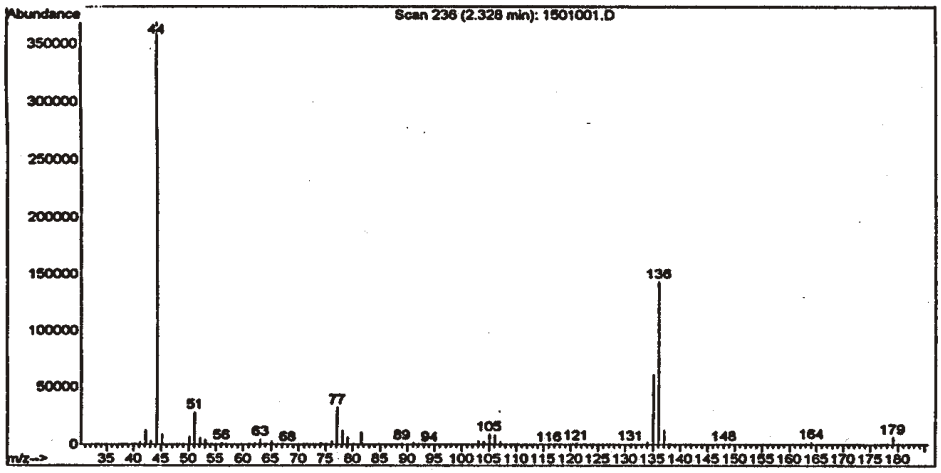


Figure 5.
Mass Spectrum of
Compound A4 (Unknown;
expanded to display possible
molecular ion at m/z = 326)



A standard of MDA was then run on the second GC/MS, and the resulting spectra was found to be normal (i.e., displaying a typical MDA spectrum with a "proper" 179 molecular ion (See Figure 6). Since the mass spectrum of standard MDA run on the same instruments in the same time frame did not match the unknown, it was clear

Figure 6.
Mass Spectrum of
MDA Standard Under
Identical Conditions



that this could not be a simple instrumental variation of the MDA spectrum. A literature search of mass spectra found no matches. The question then arose: Was the second component actually an unknown substance, or was the anomalous spectrum the result of a compound co-eluting with MDA? The presence of a third compound by TLC analysis suggested the possibility of a co-eluter.

Experimental

Two GC/MS instruments were utilized in the study. The first was an Agilent 5973 Mass Spectrometer interfaced with an Agilent 6890 Gas Chromatograph equipped with a 12 meter capillary column of 0.20 mm i.d. and having a 0.33 µm film thickness of methyl silicone. The temperature program was 100ºC held for one minute, then ramped at 75ºC per minute to 200ºC, then ramped at 50ºC per minute to 325ºC, held for one minute. The second was a Hewlett Packard 5971A Mass Spectrometer interfaced with a Hewlett Packard 5890 Gas Chromatograph also equipped with a 12 meter capillary column of 0.20 mm i.d. and having a 0.33 µm film thickness of methyl silicone. The temperature program was 100ºC with no hold, ramped at 5ºC per minute to 200ºC, then ramped at 25ºC per minute to 325ºC, held for two minutes.

Results and Discussion

The color testing, TLC, and GC/MS results excluded common manufacturing byproducts or "mistakes" such as N-hydroxy-3,4-methylenedioxymethamphetamine or 1-(3,4-methylenedioxyphenyl)-2-propanone-oxime. However, an unusual impurity 1-(3,4-methylenedioxyphenyl)-2-propanol had been identified in another recent case seen in this laboratory. This compound can result from reduction of excess 1-(3,4-methylenedioxyphenyl)-2-propanone in botched clandestine syntheses. 1-(3,4-Methylenedioxyphenyl)-2-propanol has a molecular weight of 180, and a base peak of 135. The mass units for the remaining peaks are nearly identical to MDA, though their abundances vary. To determine if the MDA mass spectrum anomaly was in fact the result of a co-elution with 1-(3,4-methylenedioxyphenyl)-2-propanol, the sample was injected onto the Hewlett Packard 5971A mass spectrometer with a much slower temperature programming (i.e., 5ºC per minute from 100 to 200ºC, then ramped at 25ºC per minute to 325ºC, held for two minutes). There still appeared to be a single peak for the MDA area on the ion chromatogram (see Figure 7), but the mass spectrum of the suspected MDA (taken at the peak) was now normal. However, by expanding the peak on the computer screen, a shoulder became visible (see Figure 8). The mass spectrum of this shoulder (Figure 9) was that of 1-(3,4-methylenedioxyphenyl)-2-propanol, confirmed by comparison to a spectrum copy obtained from Drug Enforcement Administration's Southeast Laboratory (Miami, Florida). Though the peaks did not fully resolve even using the slower temperature programming, they were separated enough to obtain identifiable mass spectra for both MDA and 1-(3,4-methylenedioxyphenyl)-2-

Figure 7.
Total Ion Chromatogram at Slow Temperature Programming

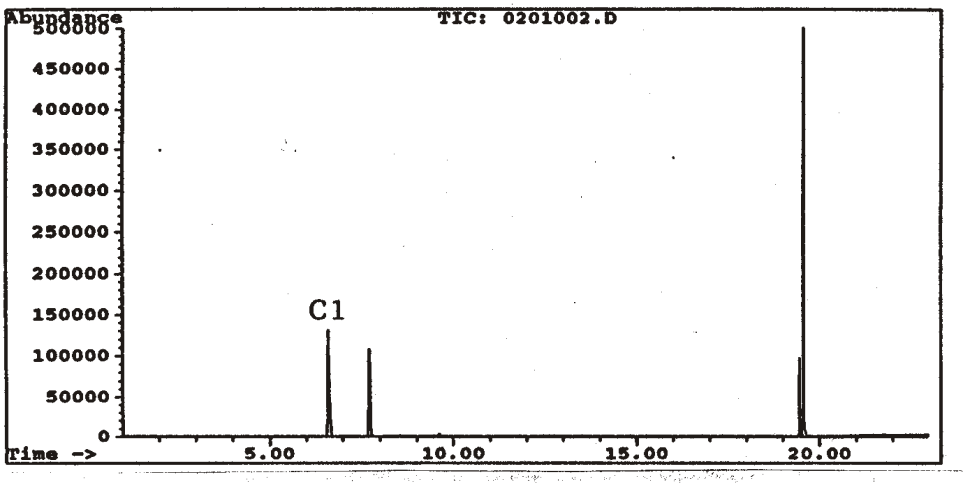


Figure 8.
Expanded Total Ion Chromatogram at at Slow Temperature Programming

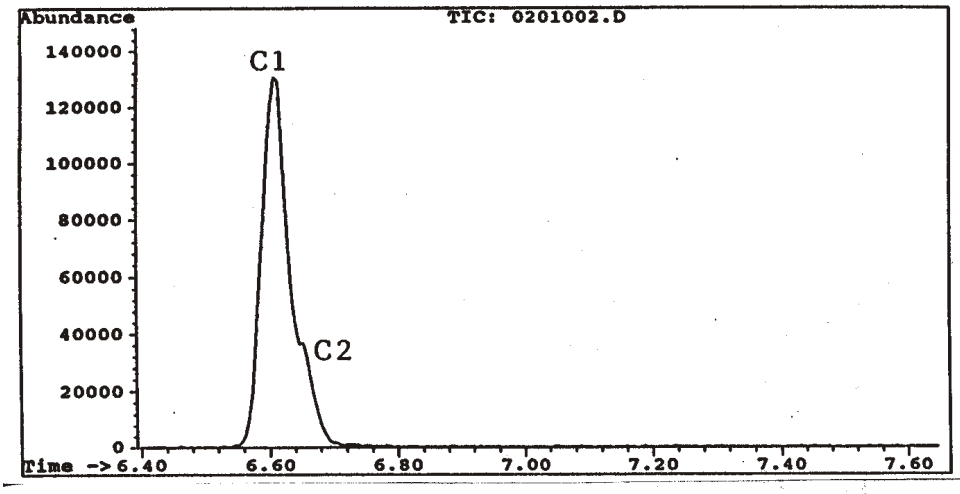
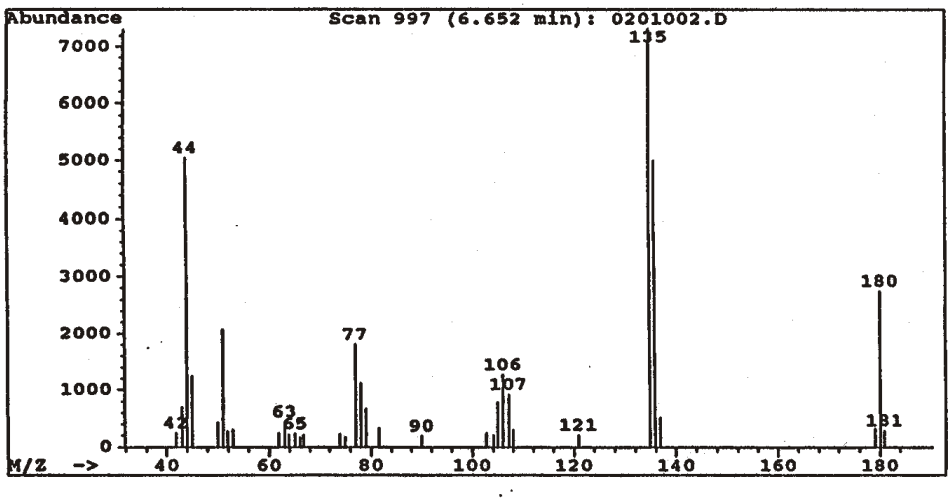


Figure 9.
Mass Spectrum of 1-(3,4-methylenedioxy-phenyl)-2-propanol



propanol, allowing for a positive identification of the controlled substance. Since the alcohol is not controlled, further analyses (e.g., acid/base shakeouts or derivatization studies) were not required; however, such procedures could be useful for other laboratories who encounter similar mixtures or who wish to more formally isolate and identify 1-(3,4-methylenedioxyphenyl)-2-propanol.

Based on the peak heights as measured by the Agilent 5973 (total ion chromatogram), the extracted components in the mixture were approximately 22% MDMA, 35% MDA, and 3% 1-(3,4-methylenedioxyphenyl)-2-propanol, and 40% other, unidentified components (there may also have been other components which did not extract). To date no other samples of this particular mixture have been encountered at this laboratory.

* * * * *

Technical Note

Potency of Cannabis Seized in Central Florida During June 2002

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ABSTRACT: The potency of cannabis seized in central Florida during the month of June, 2002, is reported. Δ^9-Tetrahydrocannabinol (Δ^9–THC) was extracted from cannabis seizures with a mixed methanol chloroform solution, and then analyzed with gas chromatography using an external standard. The average Δ^9–THC concentration was found to be 6.20%.

KEYWORDS: Δ^9-Tetrahydrocannabinol, Δ^9–THC, Marijuana, Cannabis, Gas Chromatography

Introduction

Cannabis remains one of the most frequently submitted substances for analysis to the Florida Department of Law Enforcement's Orlando Regional Crime Laboratory. Δ^9-Tetrahydrocannabinol (Δ^9–THC) is the substance responsible for most of the psychopharmacological effects that cannabis has on humans. According to the University of Mississippi's Potency Monitoring Project, the non-normalized average potency of cannabis seizures has steadily increased since measurement began in the 1970's. The average Δ^9–THC potencies were 0.90% in 1977, 2.93% in 1987, 4.53% in 1997, and 6.19% in 2002 (1). In this study, samples were collected from seizures made in central Florida and submitted for laboratory analysis during June 2002, and their respective Δ^9–THC contents determined by gas chromatography (GC) using an external standard.

Experimental

Instruments and Materials

A Hewlett Packard 5890 Gas Chromatograph (GC) with a flame ionization detector was used for all analyses. The GC was equipped with an Alltech (AT-1) fused silica 10-meter capillary column with an internal diameter of 0.25 mm and having a film thickness of 0.20 µm of methyl silicone. A Mettler AE260 DeltaRange electronic analytical balance was used for weighing the samples. The external Δ^9–THC standard employed was from Alltech (Lot Number 281). Methanol and chloroform (both Fisher Scientific) were used as received. A total of 36 cannabis samples obtained from 36 separate cases submitted to the laboratory in June 2002 were examined in this study. All samples were dry.

Analytical Protocol

After removing seeds and large stem pieces, the samples (roughly 200 mg) were weighed on an analytical balance (see Table 1, page 39, for exact dry weights), then covered and soaked overnight in 5 mL of methanol/chloroform 9:1 to exhaustively extract the Δ^9–THC from the plant material (2). Because of the small size of the autosampler vials used on the GC, a 1.5 mL aliquot of the extract of each sample was evaporated to dryness in an autosampler

vial, and another 1.5 mL aliquot of extract was added and the vials were sealed; this doubled the concentration of the extract. The Δ^9–THC external standard was prepared to a final concentration of 1.0 mg/mL.

The GC was operated at a split ratio of 50:1. The helium flow rate was 1 mL/minute. The temperature program started at 100˚C and was increased at a rate of 50˚C/minute to 325˚C, with a final hold for 2.25 minutes. The samples were bracketed between two standards in groups of ten. Each sample was injected in triplicate with a volume of 1 µL per injection, and the average of the three peak areas for each sample was used for quantitation. Five already extracted samples were chosen randomly and the extraction and analysis procedures were repeated on them to ensure that all of the samples had been exhaustively extracted (which they were).

Results and Discussion

The amount of Δ^9–THC found in the samples ranged from 1.41% to 12.62% by dry weight (see Table 1, next page). The average Δ^9–THC content was 6.20%, which is almost identical to the 2002 value reported by the University of Mississippi's Potency Monitoring Project. Since there have been no other known studies of this type for cannabis seizures in central Florida, these values cannot be compared with local data to show a trend in cannabis potency. However, the results clearly suggest that local cannabis potencies are closely tracking national averages.

Acknowledgments

Thanks to the members of the Chemistry Section of the Florida Department of Law Enforcement Orlando Regional Crime Laboratory for their assistance with sample collection for this project. Special thanks to Dr. Frank Davis, Orlando Regional Crime Laboratory, for assistance with formulation of experimental methods and interpretation of results.

References

1. ElSohly MA, Ross SA. Potency Monitoring Project, Quarterly Report #80. National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677.

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Table 1. Amount of Δ^9-THC found in Central Florida Cannabis Samples

Sample #	Sample Weight (grams)	Percent THC by Dry Weight
1	0.2095	6.59
2	0.2254	9.83
3	0.2154	3.79
4	0.2188	11.46
5	0.1609	6.64
6	0.1770	5.24
7	0.1447	6.02
8	0.1928	1.41
9	0.2079	2.20
10	0.1413	4.61
11	0.1549	4.46
12	0.2231	6.87
13	0.2185	8.59
14	0.2056	5.32
15	0.1585	4.74
16	0.2259	9.12
17	0.1230	3.57
18	0.1560	6.88
19	0.2315	3.94
20	0.1975	4.42
21	0.2168	7.81
22	0.1568	10.92
23	0.1685	9.82
24	0.1874	6.16
25	0.2202	6.77
26	0.1438	2.59
27	0.2159	8.69
28	0.2175	3.23
29	0.2219	12.62
30	0.1828	4.05
31	0.1990	8.56
32	0.1805	6.08
33	0.2217	5.86
34	0.2161	5.67
35	0.2226	2.23
36	0.1686	6.58
Mean THC Content (by Dry Weight) :		**6.20%**

Technical Note

A Study of Acids Used for the Acidified Cobalt Thiocyanate Test for Cocaine Base

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ABSTRACT: Four acids (hydrochloric, sulfuric, nitric, and acetic) were used as acidifying reagents in the "one well" cobalt thiocyanate test for cocaine base. Concentrated sulfuric, nitric, and acetic acids were found to be equally fast as concentrated hydrochloric acid (the standard acid used in the test). In addition, dilute (down to 0.1 N) hydrochloric acid was found to be as effective as concentrated hydrochloric acid. Only concentrated hydrochloric acid gave a transient blue color upon addition to the cobalt thiocyanate reagent. A number of other controlled substances, adulterants, and diluents were also tested and confirmed to not give false positives with sulfuric, nitric, acetic, or dilute hydrochloric acids.

KEYWORDS: Cocaine, Cobalt Thiocyanate, Acidified Cobalt Thiocyanate, Spot Tests, Color Tests

Introduction

The cobalt thiocyanate color test is widely used in forensic laboratories to determine the presence of cocaine salt, i.e., cocaine hydrochloride (1,2). However, the test requires a water soluble form of cocaine, and is ineffective for testing cocaine base. Therefore, a modified version of the test, the acidified cobalt thiocyanate test, is used to determine for the presence of cocaine base. The addition of an acid to the reagent allows the cocaine base to dissolve, and the color reaction can proceed. A sustained blue colored precipitate is a positive test.

There are two general procedures for running these tests. The first is to have two separate solutions prepared (one "normal" and the second acidified) and use them in two separate spot wells of a standard porcelain spot plate. The other is to run the normal (non-acidified) test first, observe for any color change, and if none then add a small amount of acid to the spot well, and again observe for any color change. This latter technique is referred to as the "one-well" method.

A literature search found that the only documented acid used for this "one well" test is concentrated hydrochloric acid (HCl). However, there is a complication when using this acid in that when it is first introduced to the cobalt thiocyanate solution, the color of the solution temporarily turns from pink to blue even if cocaine base is not present- and blue is also the characteristic color change observed for cocaine. Although this change is only temporary (as well as distinguishable to the trained eye), and there is no blue colored precipitate, it can be confusing to novices, and can potentially give ambiguous results with samples containing only trace amounts of cocaine. The latter problem can be an issue with commercial field test-kits.

In this study, a series of acids commonly utilized in most forensic/analytical laboratories were used to perform the "one well" test for cocaine base. A variety of other controlled and non-controlled substances were also studied using the same acids. In addition, the concentration of HCl used for the "one well" test was also studied to determine if the test would still be effective if a diluted version was used.

Experimental

Chemicals

Chemicals were purchased from the following vendors.

Benzocaine	Mallinckrodt	Lidocaine	K&K Loaboratories
Caffeine	Matheson Coleman and Bell	Mannitol	Mallinckrodt
Cobalt Thiocyanate	Sigma-Aldrich	Methamphetamine	(case sample)
Cocaine HCl and Base	Sigma-Aldrich	Nicotinamide	JT Baker Chemical Co.
Diphenhydramine HCl	Sigma-Aldrich	Nitric Acid	Fisher
Ephedrine	Sigma-Aldrich	Phencyclidine (PCP)	US Pharmacopeia
Glacial Acetic Acid	Fisher	Procaine	JT Baker Chemical Co.
Glucose	Mallinckrodt	Pseudoephedrine	Sigma-Aldrich
Heroin	(case sample)	Quinine HCl	Matheson Coleman and Bell
Hydrochloric Acid	Fisher	Sodium Bicarbonate	Fisher
Inositol	Eastman	Sulfuric Acid	Fisher
Lactose	Mallinckrodt	Tetracaine	K&K Loaboratories

Prepared Reagents

Cobalt thiocyanate reagent: 2 grams of cobalt thiocyanate were dissolved in 100 mL distilled water.

Acidified cobalt thiocyanate reagent: 2 mL of concentrated HCl were added to 98 mL of above cobalt thiocyanate reagent.

Procedure

Several controlled and non-controlled substances were studied, as well as numerous case samples of cocaine base. For each sample, the following procedure was followed:

1. Add a few drops of the cobalt thiocyanate reagent to five (A-E) wells on a spot plate.

2. Add the acidified cobalt thiocyanate reagent to one well (F).

3. Add a few micrograms of solid chemical to each spot well.

4. Observe color changes (if any).

5. Add one drop of each concentrated acid to each designated well (hydrochloric to (B), sulfuric to (C), nitric to (D), and acetic to (E)).

6. Observe any new color changes in wells (B) through (E).

The effect of the concentration of HCl added to the cobalt thiocyanate solution was separately studied. Two to three drops of the cobalt thiocyanate reagent were added to several wells of a spot plate. One drop of HCl (of varying concentrations) was added to each well.

Results and Discussion

It was found that all four acids (hydrochloric, sulfuric, nitric, and acetic) produced the same test results for cocaine base (see Table 1, next page). All four concentrated acids were equally fast. In addition, no false

positives were observed with any of the other controlled substances, adulterants, and diluents tested when sulfuric, nitric, or acetic acids were substituted for concentrated HCl. Notably, *only* concentrated HCl gave the transient blue-colored solution when added to the "normal" (non-acidified) cobalt thiocyanate reagent that did not contain cocaine.

1. Results of Cobalt Thiocyanate + Acid

	Cobalt Thiocyanate	Add HCl	Add H2SO4	Add HNO3	Add HOAc	Acidified Cobalt Thiocyanate (w/ HCl)
Standard Samples						
Cocaine HCl	Blue	Blue	Blue	Blue	Blue	Blue
Cocaine Free Base	NR	Blue	Blue	Blue	Blue	Blue
Lactose	NR	NR	NR	NR	NR	NR
Glucose	NR	NR	NR	NR	NR	NR
Mannitol	NR	NR	NR	NR	NR	NR
Inositol	NR	NR	NR	NR	NR	NR
Tetracaine	Blue	Some disappears	Most disappears	Most disappears/yellow	Blue	Blue
Benzocaine	NR	Slight Blue	Slight Blue	NR	NR	NR
Procaine	Blue	Disappears	Disappears	Disappears	Some disappears	Slight Blue
Lidocaine	NR	Blue	Blue	Blue	Blue	Blue
Caffeine	NR	NR	NR	NR	NR	NR
Diphenhydramine HCl	Deep Blue	Deep Blue	Deep Blue/Yellow	Disappears	Disappears	Deep Blue
Heroin	Blue/Green	Blue/Green	Blue/Green	Blue/Green	Blue/Green	Blue/Green
Methamphetamine	Dirty Blue	Fades	Fades	Fades	Fades	Dirty Blue
Nicotinamide	NR	NR	NR	NR	NR	NR
Sodium Bicarbonate	NR	Fizz	Fizz	Fizz	Fizz	NR
Phencyclidine (PCP)	Blue	Blue	Blue	Blue	Blue	Blue
Ephedrine HCl	Slight Blue (disappears)	Slight Blue	NR	NR	NR	NR
Pseudoephedrine	Slight Blue (disappears)	NR	NR	NR	NR	NR
Quinine Sulfate	NR	Blue	Blue	Blue (Disappears)	Blue	Blue at edges (insol.)
Case Samples						
Cocaine Base Samples						
Test Sample 1	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 2	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 3	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 4	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 5	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 6	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 7	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 8	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 9	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 10	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 11	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 12	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 13	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 14	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 15	NR	Blue	Blue	Blue	Blue	Blue
Test Sample 16	NR	Blue	Blue	Blue	Blue	Blue
Cocaine Salt Samples						
Test Sample 17	Blue	Blue	Blue	Blue	Blue	Blue
Test Sample 18	Blue	Blue	Blue	Blue	Blue	Blue
Test Sample 19	Blue	Blue	Blue	Blue	Blue	Blue

(NR = No Reaction)

Dilute HCl (from 1:1 down to 0.1 N) produced the same results as concentrated HCl, but also did not give the transient blue-colored solution when added to the "normal" (non-acidified) cobalt thiocyanate reagent that did not contain cocaine (see Table 2). When cocaine base was present, it was noted that the weaker the HCl solution, the slower the color reaction, but it never took more than a few seconds for the blue precipitate to form, and the overlaying solution did not turn blue even when cocaine was present. Thus, dilute HCl is as effective as concentrated HCl for the test. The collective results suggest that substituting an alternative acid or a diluted form of HCl for concentrated HCl for the acidified cobalt thiocyanate test would be advantageous.

Table 2. Effects of Hydrochloric Acid Dilution

Concentration of HCl (v/v)		Turns solution blue?	Proper reaction with Coc Base?
Concentrated	(12 N)	Yes	Yes
50%	(6 N)	No	Yes
40%	(4.8 N)	No	Yes
30%	(3.6 N)	No	Yes
20%	(2.4 N)	No	Yes
10%	(1.2 N)	No	Yes
0.80%	(0.1 N)	No	Yes

Acknowledgements

Thanks to Sandy Kassner and the members of the Chemistry Section at the Florida Department of Law Enforcement, Tampa Regional Crime Laboratory, for their help and contributions to this project.

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Velapoldi RA, Wicks MS. The use of chemical spot test kits for the presumptive identification of narcotics and drugs of abuse. Journal of Forensic Science 1974;19(3):636-656.

* * * * *

1,4-Butanediol (BD) - Forensic Profile

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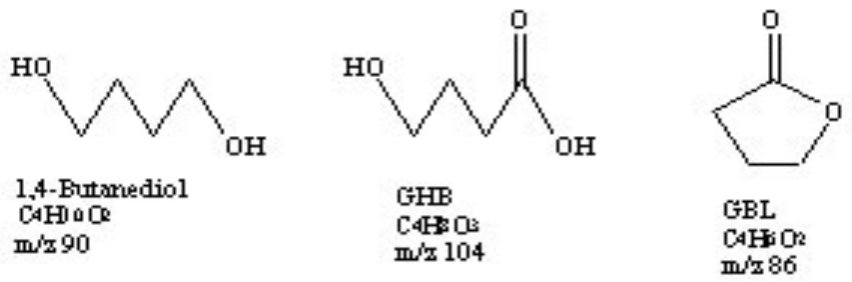
ABSTRACT: 1,4-butanediol (BD), an analog and "pro-drug" of *gamma*-hydroxybutyric acid (GHB), is increasingly being added to so-called dietary, health, sleep aid, or sports (bodybuilding) supplements, and is also being sold on the Internet and on underground markets for purposes of illicit abuse. When so intended for human consumption, BD meets the definition of a controlled substance analog under the Controlled Substances Act, Title 21, and can be prosecuted as a Schedule I substance. A comprehensive analytical profile for BD is presented, including GC/MS, FTIR, NMR, GC/IRD, and GC/FID. Analytical parameters for the quantitative analysis of BD are also presented, along with linearity and reproducibility data.

KEYWORDS: 1,4-Butanediol, *gamma*-Hydroxybutyric Acid, *gamma*-Butyrolactone, BD, 1,4-BD, GHB, GBL, Analogs, Pro-Drug

Introduction

The widespread, illicit abuse of *gamma*-hydroxybutyric acid (GHB) is due to its euphoric, sedative, hallucinogenic, and alleged steroidal effects (1). Recently, GHB abusers have been switching to related compounds in an attempt to circumvent the Federal controls on GHB (2,3,4,5,6). 1,4-Butanediol (BD) and *gamma*-butyrolactone (GBL) (Figure 1) are the two most commonly encountered such compounds, and are considered to be both analogs and "pro-drugs" of GHB, since their chemical structures are substantially similar to GHB and they are metabolized into GHB upon ingestion and therefore produce the same psychopharmacological effects as GHB (2,3,4,5,6).

Figure 1: Diagram of Structures.



BD is an important industrial solvent and precursor with numerous applications; for this reason, it is widely available. On the underground market, BD is most commonly seen in illicit dietary, health, sleep aid, or sports (bodybuilding) "supplements", and also as the primary ingredient or a major component in various "solvents" of nebulous makeup and dubious claimed applications. Some examples include "Dream On", "Soma" (Photo 1), and "Rejoov" (Photo 2). Soma, for example, is labeled as a dietary supplement and sold in 32 oz bottles, and is marketed as a "sleep aid". The label on the bottle itself states that 2.0 grams of BD have been added per 1 fluid oz. Although various warning and/or disclaimer labels are usually present on such products, there is no mention that BD is a Schedule I controlled substance if intended/sold for human consumption. All of these various supplements and solvents are commonly obtained through Internet (usually from foreign sources) and on the underground drug market, especially at "Raves" and concerts, but also at gymnasiums and similar sports/body-building venues. Not surprisingly, BD (like GHB and GBL) has also been implicated in drug facilitated sexual assaults.

Photo 1

Photo 2

Abusers of BD indicate that its ingestion results in some unpleasant side effects, including a hangover (7). Therefore, some clandestine laboratories convert BD to GBL, which is the lactone of GHB and therefore a more direct pro-drug of GHB. Methods for conversion of BD to GBL have been published in various venues [Details and methodologies not provided, per *Journal* policy]. However, the most commonly seen clandestine laboratories working with BD are simple "re-packaging" operations. In these laboratories, clandestine chemists dilute industrial-grade BD with water and/or other components such as flavoring agents, coloring dyes, and/or sugars, then repackage the resulting mixtures in small bottles with homemade labels on them. Such laboratories usually consist of drums of BD, flavoring agents, coloring dyes, sugars, volume dispensing pumps, and various other chemicals (see Photos 3a - 3d, next page).

The forensic analysis of BD has been previously reported (2,4,8); however, these previous studies were published in law enforcement restricted venues. Herein is reported detailed procedures and techniques that can be utilized for the comprehensive analysis of BD.

Photo 3a - 55-Gallon Drum of Industrial BD

Photo 3b - Various Compounds Typically Added to BD

Photo 3c - Coloring Dyes

Photo 3d - Flavoring Agents

Experimental

Reagents

1,4-Butanediol standard and octane ($C_8H_{18,}$ used as an internal standard) were obtained from Aldrich. Other solvents (such as high purity methanol and chloroform) were obtained from Baxter.

GC/MS

Gas chromatography/mass spectrometry analyses were performed on a Hewlett Packard (HP) 6890 GC interfaced with a Hewlett Packard 5973 Mass Selective Detector (MSD), using a scan acquisition from 35 to 500 amu. A crosslinked 5% phenyl methyl siloxane column (HP-5), with 0.25 mm internal diameter x 30 m and 0.25 µm film thickness, was utilized. The injection port temperature was 260ºC and the detector and transfer line temperatures were 280ºC. The GC oven temperature was held at 50ºC initially for 2 minutes, then ramped at 35ºC/min to 290ºC, with a final hold of 4 minutes.

FTIR/ATR

A Nicolet Nexus 470 with a potassium bromide (KBr) beamsplitter and a deuterated triglycine sulfate (DTGS) KBr detector, equipped with a Durascope Dicomp ATR accessory with a 3-bounce diamond ATR element, was

utilized for attenuated total reflectance IR analyses. The resolution was set at 4.000 cm^{-1}, with 32 scans between 4000 cm^{-1} and 550 cm^{-1}. The mirror velocity was 0.6329 cm per second. BD (neat) was prepared on a KBr pellet and analyzed using the same parameters, except that the wavenumbers were set between 4000 cm^{-1} and 400 cm^{-1}.

Aqueous samples were easily analyzed by allowing a portion of the sample to evaporate at low heat on the heating plate of the ATR instrument. However, many BD-containing "supplements" also contain color dyes, flavoring agents, and sugars. These added components form a residue with BD during evaporation, thereby making it difficult to obtain clean IR spectra. A chloroform extraction is recommended for such samples.

GC/FTIRD

Vapor phase infrared spectra were obtained with a HP 6890 GC/BioRad IRD II Infrared Detector using a HP 5% phenyl methyl siloxane, 25 m x 0.32 mm x 0.52 μm (HP-5) column. The temperature program was set at 50˚C for 1.5 minutes, then ramped up at 35˚C/min to 290˚C , with a final hold of 3 minutes. Column flow was 1.5 mL per minute with an average velocity of 28 cm/sec. The inlet was set at a splitless mode with an initial temperature of 260˚C. The purge gas was nitrogen at 50.0 mL per minute.

NMR

FT-NMR spectra were obtained using a Varian Gemini 300 nuclear magnetic resonance spectrometer, operating at 300 MHz for proton. A standard ^{1}H-NMR was performed, with 64 transients. Deuterated water, deuterated methanol, or deuterated chloroform can be used as solvents for BD; however, only spectra in deuterated water and deuterated chloroform are presented in this study.

Quantitation by GC/FID

Serial dilutions of standard were prepared in methanol, ranging in concentration from 0.0869 mg/mL to 20.67 mg/mL. The internal standard solution was prepared by dissolving octane in methanol, for a final concentration of 2.00 mg/mL. For analysis, an aliquot of the standard solution was mixed with an equal amount of the internal standard solution.

GC analyses were performed on a Hewlett Packard 6890 Gas Chromatograph with a flame ionization detector, using a 0.25 mm internal diameter x 30 m HP-5 column with a 0.25 μm film thickness. An isothermal method (90ºC for 2.5 minutes) was used. One μL of the standard and each sample solution were injected using an autosampler. The injection port and detector temperatures were maintained at 260ºC and 270ºC, respectively.

Results and Discussion

GC/MS

The mass spectrum of BD is shown in Figure 2 (next page). Figures 3a-3g (next two pages) show a suggested fragmentation scheme. The mass spectrum has a base peak at m/z 42 and the [M-1] (molecular weight minus a hydrogen) ion at m/z 89 (Figures 3a, 3b). The peak at m/z 57 results from a loss of 32 from the M-1 fragment via a 1,3 hydride shift to form (+OH=CH-CH=CH_2) at m/z 57 (Figure 3c). The second most abundant fragment (at m/z 44) results from the cleaving of the BD molecule to form (·+OH-CH=CH_2) (Figure 3a). The loss of a water molecule from BD, followed by H-rearrangement leads to the formation of a ring (Figure 3d). When the ring cleaves, radical and charge stabilization become important (9). Thus, a second H-rearrangement occurs to yield a stable product (Figure 3e). Furthermore, the intense peak at m/z 71 is a result of several possible fragments (Figure 3f). Other peaks at m/z 42 and m/z 43 are shown below (Figures 3a, 3g).

Figure 2: The Mass Spectrum of BD Standard.

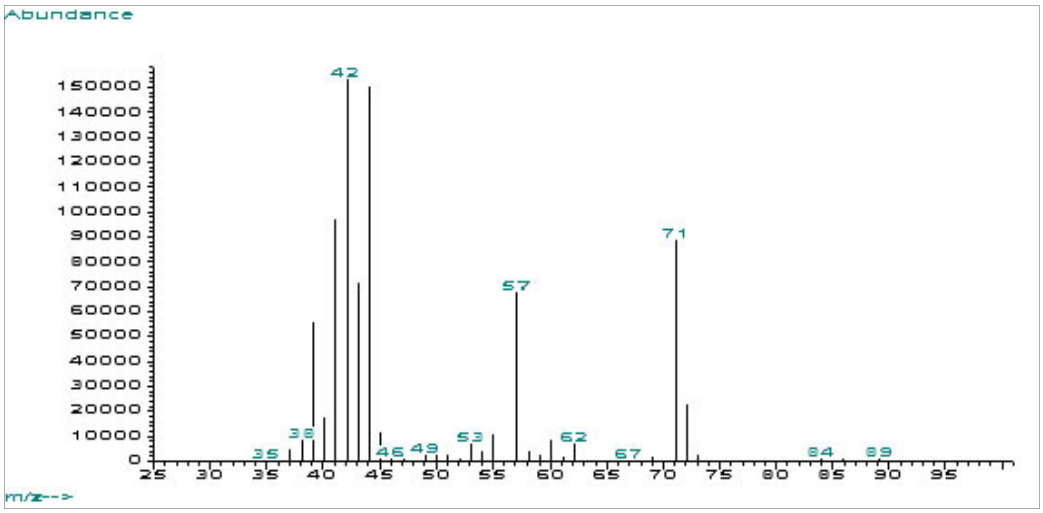


Figure 3a:

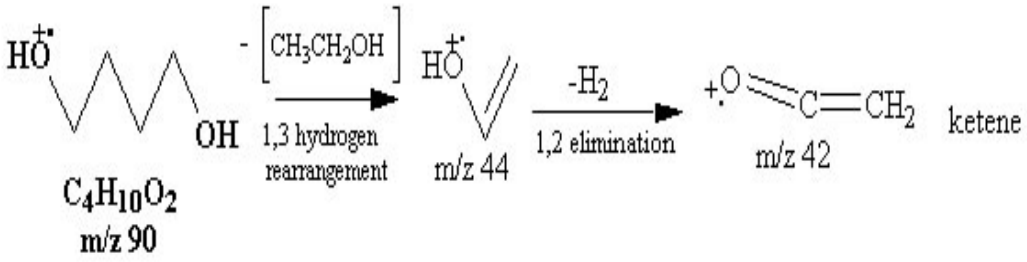


Figure 3b:

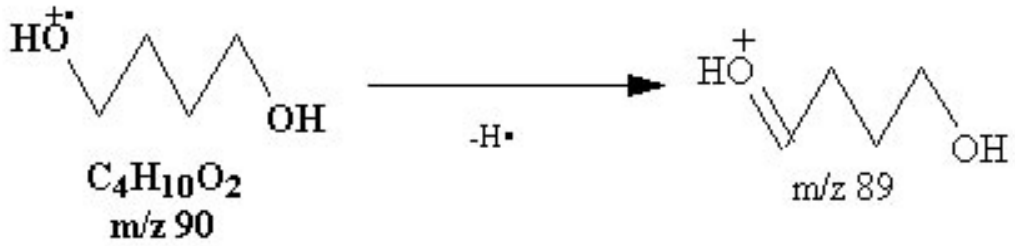


Figure 3c:

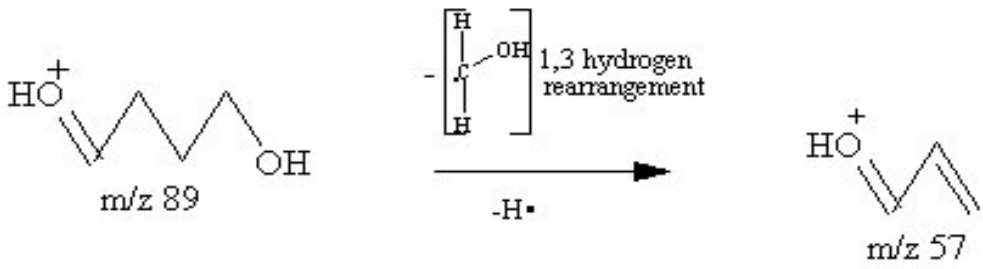


Figure 3d:

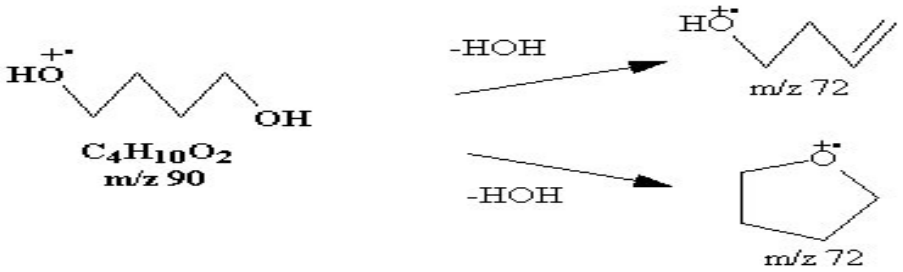


Figure 3e:

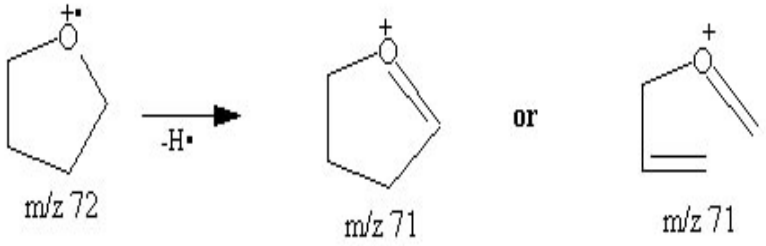


Figure 3f:

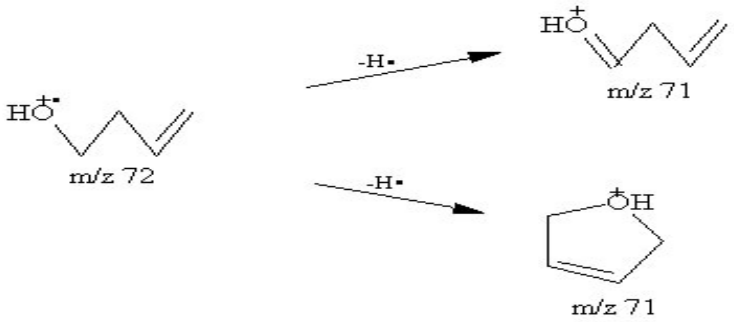


Figure 3g:

FTIR/ATR

The FTIR/ATR spectrum of BD (Figure 4a, next page) has significant bands at 2936 and 2867 cm^{-1}. The 2936 cm^{-1} peak is due to the asymmetric stretching of the methylene groups, while the symmetric stretching of the methylene groups causes the weaker 2867 cm^{-1} peak. Wagging of the methylene groups causes a series of bands from 1380 to 1150 cm^{-1}. The broad band at 3300 cm^{-1} is due to inter- and intramolecular hydrogen bonding. The most prominent peak is at 1048 cm^{-1} and is due to the two primary alcohol groups (10).

When utilizing an ATR, depth of penetration in the sample can affect peak intensities. The depth of penetration of the infrared beam in the sample is a function of wavelength, i.e., the longer wavelengths will show more absorbance. This is a characteristic in ATR analyses versus analyses using the traditional KBr matrices. A sample of BD was analyzed as a neat liquid on a KBr pellet to show this difference (Figure 4b).

Figure 4a: The FTIR/ATR Spectrum of BD Standard.

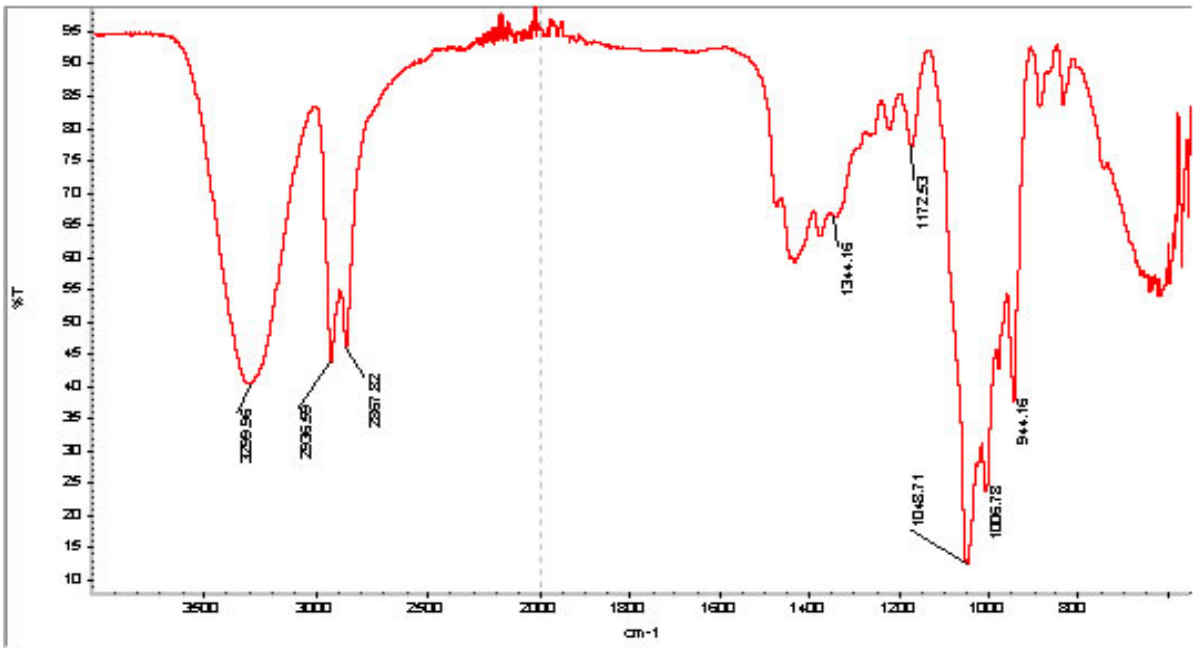
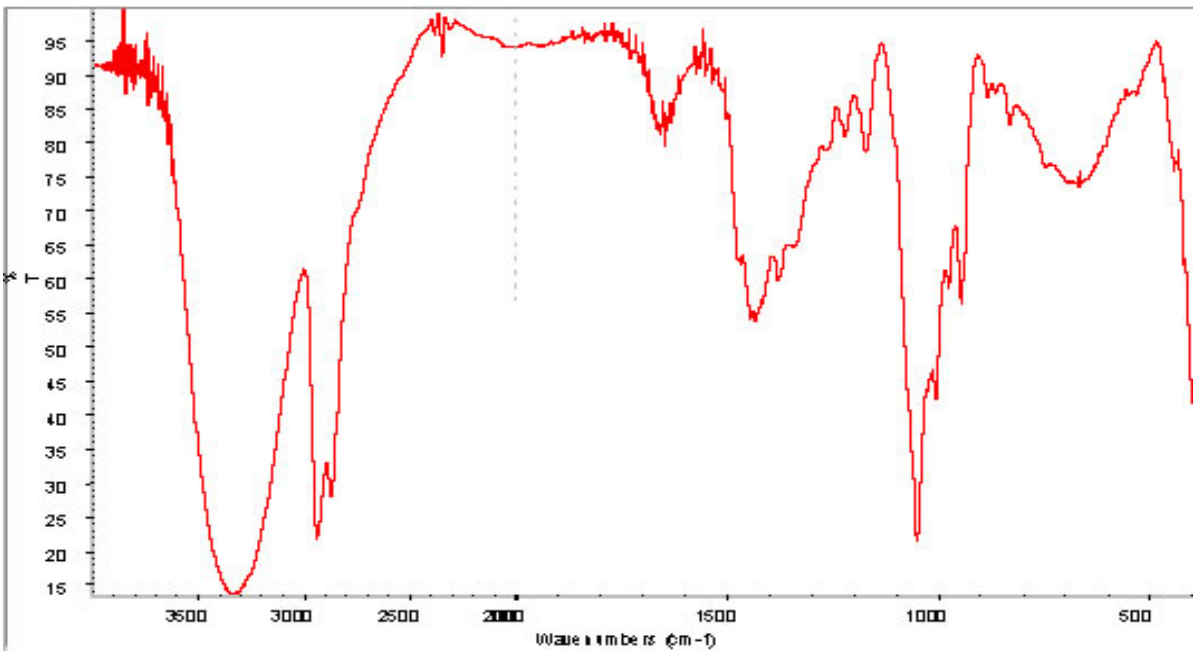


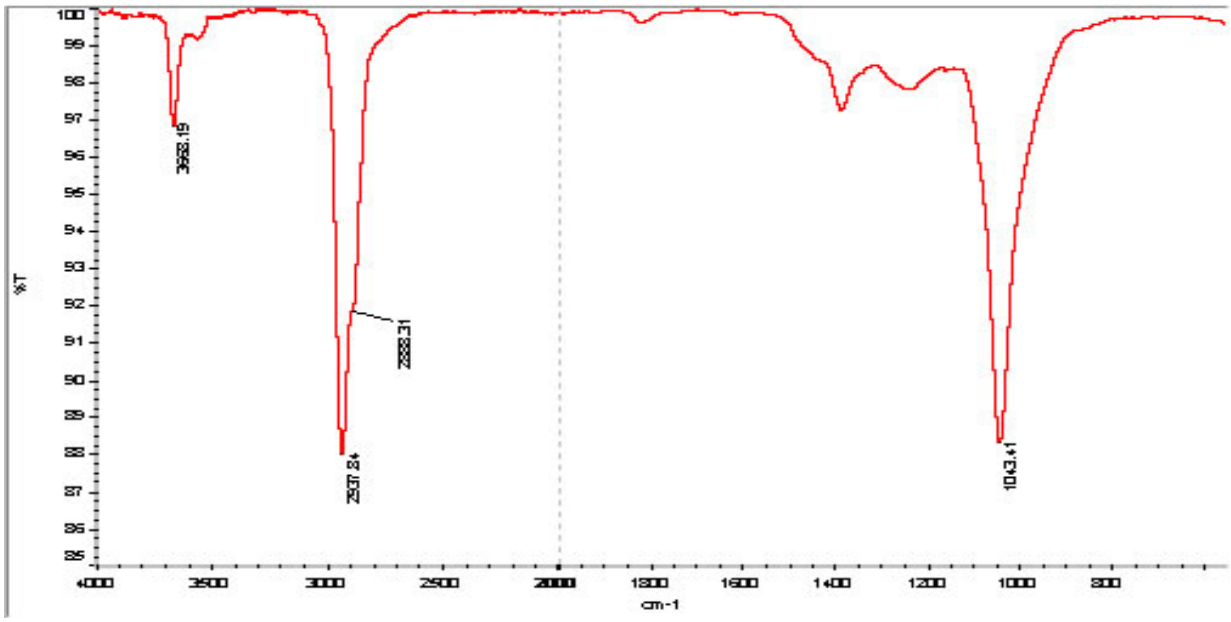
Figure 4b: The FTIR Spectra of BD as a Neat Liquid on KBr Plate



GC/FTIRD

The vapor phase infrared spectrum of BD is considerably simplified (Figure 5). The primary bands at 2938, 2888, and 1043 cm^{-1} represent the same bands seen at approximately the same wavelengths in the FTIR/ATR spectrum. However, the -O-H stretch at 3300 cm^{-1} in the FTIR/ATR spectrum shifts to 3668 cm^{-1} in the vapor phase, suggesting little or no hydrogen bonding (10).

Figure 5: The GC/FTIRD Spectra of BD Standard.



NMR

The proton spectra in $CDCl_3$ showed singlet peaks at 1.4 (C2/C3 methylenes), 3.4 (C1/C4 methylenes), and 4.7 (hydroxyl protons) ppm (Figure 6). When the sample was run in D_2O, chemical shift increases of 0.2 ppm were observed for all peaks, i.e., singlets were found at 1.6, 3.6, and 4.8 ppm, respectively (Figure 7, next page).

Figure 6: The Proton NMR spectra of BD standard in $CDCl_3$.

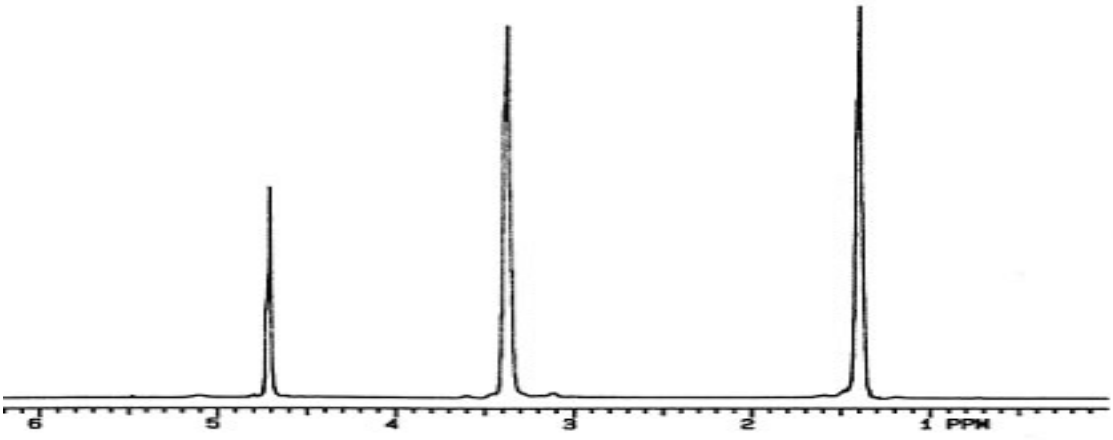
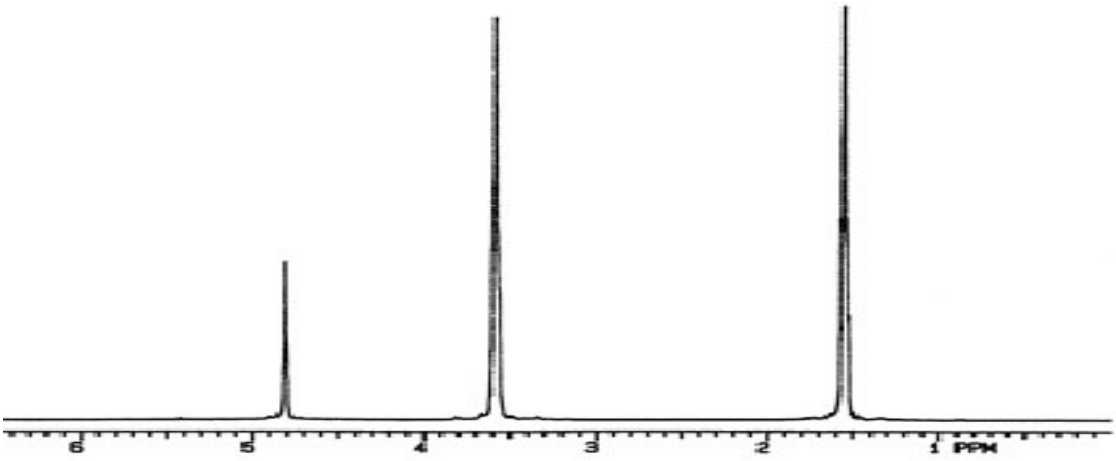


Figure 7: The Proton NMR spectra of BD in D_2O.



GC/FID

Using the specified GC/FID parameters, BD had a retention time of 1.862 minutes, while octane (I.S.) had a retention time 1.457 minutes (see Figure 8). Area ratios of the standard/internal standard were plotted against the corresponding BD concentrations. Linear responses for BD were found to be from 0.87 mg/mL to 10.64 mg/mL (Figure 9). The correlation coefficient was 0.9997, indicating a highly linear relationship. Reproducibility for both area counts and retention times were below 2.3% RSD (Table 1).

Samples of BD could contain GBL or GHB. GHB, however, converts to GBL in heated injection ports under standard GC operating conditions, so only a GBL peak would be observed for exhibits containing GBL and/or GHB. For this reason, GBL was added to a sample of BD (to ensure that they do not co-elute), and was found to have a retention time of 1.678 minutes.

Figure 8: The GC/FID Chromatogram of BD Standard.

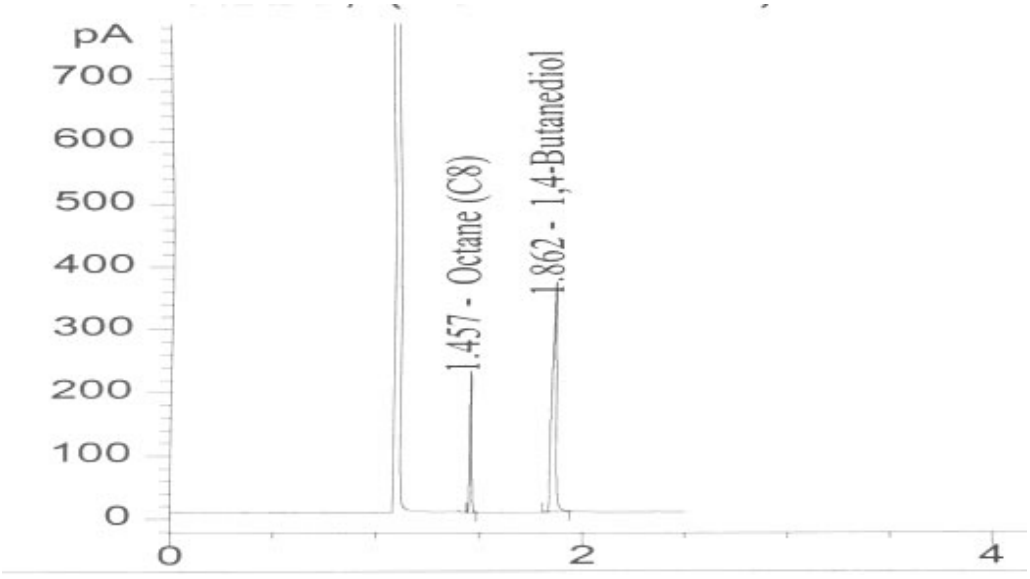


Figure 9: Linearity of BD.

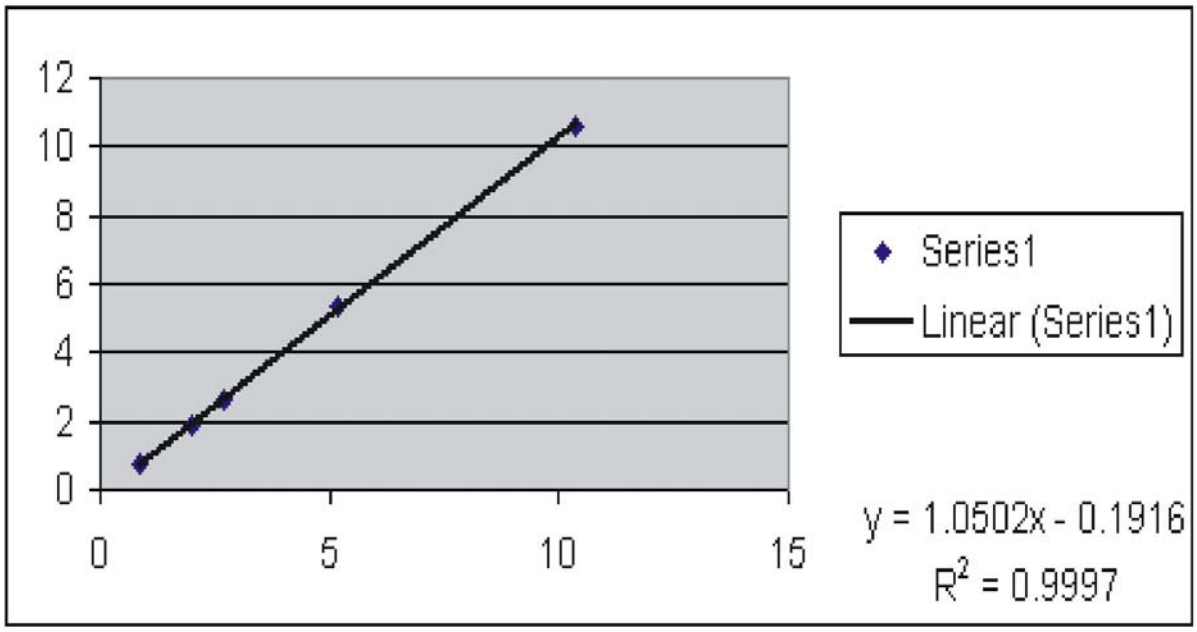


Table 1: Reproducibility Data of BD and Octane.

	Octane Retention Time (minutes)	Area	1,4-butanediol Retention Time (minutes)	Area	Area STD/ Area ISTD
	1.460	204.453	1.853	139.819	0.684
	1.460	208.700	1.854	139.204	0.667
	1.461	216.972	1.856	144.346	0.665
	1.460	212.846	1.854	144.476	0.679
	1.461	212.209	1.855	142.213	0.670
average	1.460	211.036	1.854	142.012	0.673
std. dev.	0.00	4.71	0.00	2.46	0.01
%RSD	0.04	2.23	0.06	1.73	1.19

Conclusions

A variety of techniques can be used for the analysis of BD, including GC/MS, FTIR, NMR, GC/IRD, and GC/FID. The more difficult BD samples to analyze are illicit dietary supplements and commercial solvents, due to the presence of additional components. In these instances, a chloroform extract is recommended.

Acknowledgements

The authors would like to thank Christine Sannerud (Deputy Chief, Drug and Chemical Evaluation Section, Drug Enforcement Administration (DEA)), for sharing her expertise in GHB and related samples. The authors would

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Detection and Analysis of Drugs of Forensic Interest, 1992 - 2001; A Literature Review

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ABSTRACT: The scientific literature of the detection and analysis of drugs of forensic interest, as published from 1992 through 2001, is reviewed. 1,377 references are included.

KEYWORDS: Forensic Chemistry, Analytical Chemistry, Illicit Drugs, Controlled Substances, Review

Introduction

This review presents a 10 year survey of the detection and analysis of drugs of forensic interest, as published in the mainstream scientific literature from 1992 through 2001. Analyses of drugs in post-ingestion biological matrices are not included, except for select studies which provide structural, spectral, and/or analytical data above and beyond routine toxicological "screening" techniques. In addition, due to their inherently transitory nature, Internet references are not included. Finally, forensic association newsletters and "underground" publications are not included.

Articles are first organized by overall focus, and subcategorized (where applicable) by specific drug or drug class, or instrumental technique. The focus categories are as follows:

* Previous Reviews and Overviews
* Analyses of Specific Drugs and Drug Groups
    - Illicit Drugs
    - Select Adulterants and Diluents
    - Occluded Solvents
* Simultaneous Analyses of Drugs in the Presence of Select Adulterants and Diluents
* Instrumentation Focus
* Analytical Artifacts
* Qualitative Tests
* Sampling Plans
* Source Determination/Impurity Profiling
* Source Determination/Stable Isotope Analyses
* Comparative Analyses
* Reference Standards

* Clandestine Laboratories
* Clandestine Laboratory Appraisals and Safety
* Portable Instrumentation/Trace Detection
* Surveys
* Miscellaneous Topics

Abbreviations

The authors have utilized common abbreviations throughout the review; however, all terms are defined in order to avoid ambiguity with some of the more crowded or obscure acronyms, as follows:

AA	Atomic Absorption
AP	Atmospheric Pressure
C-13	Carbon-13
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CGC	Capillary Gas Chromatography
CI	Chemical Ionization
CZE	Capillary Zone Electrophoresis
DAD	Diode Array Detection
DSC	Differential Scanning Calorimetry
ECD	Electron Capture Detection
EKC	Electrokinetic Chromatography
EI	Electron Impact
FID	Flame Ionization Detection
FT	Fourier Transform
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IMS	Ion Mobility Spectrometry
IR	Infrared Spectroscopy
IRD	Infrared Detector
IRMS	Isotopic Ratio Mass Spectrometry
ITD	Ion Trap Detector
LC	Liquid Chromatography
MECC	Micellar Electrokinetic Capillary Chromatography
MEKC	Micellar Electrokinetic Chromatography
MS	Mass Spectroscopy
MSD	Mass Selective Detector
MS-MS	Tandem Mass Spectroscopy
NMR	Nuclear Magnetic Resonance (Spectroscopy)
NPD	Nitrogen Phosphorus Detection
PDA	Photodiode Array
RP	Reverse Phase
SFC	Supercritical Fluid Chromatography
SHS	Static Headspace
SPME	Solid Phase Microextraction
TD	Thermal Desorption
TLC	Thin Layer Chromatography
UV	Ultraviolet (Spectroscopy)
Vis	Visible (Spectroscopy)

Previous Reviews and Overviews

The forensic analysis of illicit drugs has been the subject of a number of minor review articles and monographs over the past 10 years (1,2,3,4,5,6,7,8,9). In addition, several articles have given more general overviews of the field (10,11). Systematic approaches to substance identification have also been presented (12,13,14), and a number of scientific working groups are currently establishing national/international standards for forensic analysis of illicit drugs (15,16,17(see also:18)). Finally, forensic chemistry has been the subject of several textbooks and chapters in textbooks (19,20,21,22,23,24,25,26).

Analyses of Specific Drugs and Drug Groups

An immense amount of analytical data has been published over the past 10 years for drugs of abuse. "Comprehensive" data compilations (defined in this context as three or more analytical profiles in a specific study) have been previously provided for virtually all "traditional" drugs of abuse, but a number of updated compilations have been provided which reflect improvements in existing instrumentation and/or the advent of new instrumental techniques. In addition, "comprehensive" data compilations have been provided for a number of new drugs of abuse; these include previously unknown "designer," "analog," or "homolog" drugs, and also various pharmaceuticals or industrial chemicals which either had not been previously subject to abuse, or had been only rarely encountered in illicit settings. Furthermore, hundreds of studies which analyzed either specific drugs of abuse or groups of structurally related drugs of abuse, but only by one or two select analytical techniques, have also been provided. [Note that multiple citations are organized as follows: reviews, then overviews, then comprehensive studies, then by specific analytical techniques (alphabetized), and finally in reverse date order (most recent citation first).]

Controlled Substances: Over the past 10 years, the following substances were the subjects of moderate to comprehensive analytical profiling: **alkyl nitrites** (inhalants) (comprehensive) (27), by GC-IR (28), and by headspace GC/MS (29); ***Amanita Muscaria*** by ion-interaction HPLC (30); **amphetamine** by CE (31), by CE and LC (32), by GC-MS (role of self-protonation) (33), by HPLC (after acetylation) (34), by HPLC using chiral crown ether coated reversed-phase packing (35), and by HPLC using a two-dimensional column-switching chromatographic system with on-line derivatization (36); **amphetamine and methamphetamine** - (general review of the syntheses and analyses of phenylacetone, amphetamine, and methamphetamine) (37), in abuser's clothing by HPLC with UV and fluorescence detection (38), by CE with cyclodextrins to determine isomers (39), by GC and GC-MS versus internal standards (40), by GC-CI/MS (following derivatization with perfluorinated acid chlorides) (41), by headspace sampling and GC-MS (analysis of betel) (42), by HPLC (43,44), by HPLC for quantitation of clandestinely produced mixtures (45,46), by MS (47), by micro-Raman scattering (48), by surface enhanced Raman scattering detection after modification with 2-mercaptonicotinic acid (49), and by UV/Vis spectrophotometry after derivatization with 1,2-naphthoquinone-4-sulfonic acid (50); **amphetamines** (review of biological/forensic issues (includes amphetamine and methamphetamine analogues)) (51), by CE (52,53), by CE (chiral) (54), by CE with added anionic chiral selectors (55), by CE and LC (56), by color tests, TLC, GC/MS, GC/IR, plus GC/IR/MS of N-acetyl derivatives (differentiation of side chain isomers of ring-substituted amphetamines (4-Me, 4-OMe, 3,4-MD- amphetamine and methamphetamine)) (57), by CZE (58), by CZE with added cyclodextrins (59), by EI-MS (N-substituted amphetamines) (60), by GC (61), by GC, HPLC, and MS (62), by GC-FTIR (63), by GC/MS (64,65), by GC-MS (differentiation of acylated derivatives of methamphetamine and regioisomeric phenethylamines) (66,67), by HPLC with added cyclodextrins and using a chiral stationary phase (68), by HPLC with fluorimetric detection (69), by HPLC after derivatization with chloroformates (70), by LC with fluorimetric detection after precolumn derivatization with (+)-1-(9-fluorenyl)ethylchloroformate (71), by MECC (72), by negative-ion CI-MS (73), by carbon dioxide negative-ion CI-MS (74), by SFC (75), by SFC, HPLC, GC, and CZE (76), by TLC with diazonium salts as visualization reagents (77), and by GC/FID using n-alkanes and other indirect reference standards when no internal standard is available (78); **ayahuasca** by GC and GC/MSD (79); **barbiturates** by HPLC with PDA-UV detection (80), by HPLC using 3-(18-naphthalimido)-propyl-modified silyl silica gel as a stationary phase (81), by micro-HPLC with post-column photochemical derivatization (82), by ion-trap and quadrupole MS (83), by IR and MS (84), by mercurimetric potentiometric

determination using a solid-state iodide ion-selective electrode (85), by micellar LC (86,87), by SPME and CE (88), by SPME and ion-trap GC-MS (89), by a thermally tuned tandem column (separation of barbiturates and phenylthiohydantoin amino acids) (90), and by two-dimensional overpressured layer chromatography (91); **benzodiazepines** by CE (92), by electrospray probe/MS (93), by free zone electrophoresis (94), by FT-Raman and FT-IR (95), by HPLC (nitrazepam, diazepam, and medazepam) (96), by HPLC (clorazepate, diazepam, and diltiazem in pharmaceuticals) (97), by HPLC-DAD (98,99), by HPLC using diode-array, electrochemical, and thermospray MS detection (100), by HPLC/electrospray MS-MS (101), by HPTLC (diazepam, chlordiazepoxide, and midazolam) (102), by MECC (103,104), by SERS after adsorption on the Ag colloidal surface (diazepam and nitrazepam) (105), by LC (alprazolam) (106), by microcolumn LC using a cholesteryl-10-undecenoate bonded phase (107), by RP-LC (flurazepam) (108), and by automated SPME-LC/EI-MS (109); **1-benzyl-1-*n*-butyl-barbituric acid** (comprehensive) (110); **bromazepam** by flow injection stopped-flow kinetic determination (111); **4-bromo-2,5-dimethoxyphenethylamine** (2C-B or NEXUS) and related compounds (overview) (112), (comprehensive) (113,114), by GC/MS and HPLC (115), by GC-MS and NMR (including analysis of derivatized samples) (116), and by LC and GC/MS (117,118,119); **bufotenine** (comprehensive) (120,121), and by GC/MS after BSTFA derivatization (122); **bufotenine, psilocybin and related indole alkaloids** by CE (123), by GC/CI-MS (124), by GC/IRD (125,126), by GC/MS (includes general overview) (127), and by MS (128); [**"Love Stone"** (contains bufotenine) (overview) (129), (overview and GC/MS) (130), and by GC/MS (comparison with Chinese "Chan Su") (131)]; **cathinone** (*alpha*-aminopropiophenone) (review with 79 refs) (132), (comprehensive) (133), by NMR (enantiomeric determination of N-acetylcathinone) (134), and by spectrophotometric detection (135); **2-chloro-4,5-methylenedioxymethamphetamine** (comprehensive) (136); **clenbuterol** by CE (chiral analysis with added CD's) (137), by EKC (epinephrine, terbutaline, clenbuterol, and salbutamol) (138), by flow-injection fluorimetry (139), by GC after derivatization and electrospray MS (140), and by RP-HPLC (141); **cocaine** by CE (simultaneous analysis of coca alkaloids and sugars in illicit cocaine) (142), by free-zone CE (143), by DSC (and GC/FID) (144), by FT-IR and HPTLC (145), by flow injection analysis with amperometric detection (146), by FTIR, GC/MS, and quantitative GC (cocaine HCl in wax) (147), by GC (cocaine base) (148), by GC/MS after field testing (149), by HPLC (150,151), by HPLC and GLC (152), by an ISFET device, GC, and UV (153), by LC/AP-CI-MS (154), by MS (evaluation of fragmentation patterns (155), by collision induced dissociation MS (156), by MECC (157), by Raman microspectroscopy (158,159), by SPME/GC/MS (methylbenzoate from cocaine) (160), by TLC, GC, UV, and GC/MS (identification of cocaine in samples in the presence of other local anesthetics) (161), and by transmission and internal reflection IR (cocaine base versus HCl) (162,163); **cocaine analogs** by NMR (164), **cocaine base** (comprehensive) (165); **cocaine-N-oxide** by LC and MS (166); **coca tea** by solid-phase extraction followed by GC-MS (167); **codeine** by chemiluminescence (168), by LC and LC-MS/MS (169), and by NMR (170); **codeine pharmaceuticals** by CE (171), by free solution CE (172), and by HPLC (173); **creatine** (comprehensive) (174,175), and by TLC/densitometry (176); **cyclofenil** (comprehensive) (177); **cyclohexyl nitrite** (comprehensive) (178); **dexfenfluramine** by HPLC on a chiral column (179); **diazepam** by ion-trap and quadrupole mass spectroscopy (180), and by polarography (181); **dihydroetorphine** and **etorphine** (comprehensive) (182); **2,5-dimethoxy-4-ethylthiophenethylamine** (2CT-2) by IR, GC/IRD, and GC/MS (183); **2,5-dimethoxy-4-(N)-propylthio-phenethylamine** (2C-T-7) (comprehensive) (184); **dimethpramide** (comprehensive) (185,186,187); **dimethylaminorex** (comprehensive) (188); **dimethylamphetamine** by GC, IR, and UV/VIS (stability study) (189), by GC/MS, HS-GC/MS, and LC-ESI/MS (analysis of dimethylamphetamine pyrolysis products) (190); **dragon's blood incense** (overview) (191), and by GC/MS (192); **ergot alkaloids** (review) (193), determination and isolation by LC (194), by LC with fluorescence detection (195), and determination of ergonovine maleate by flow injection analysis with chemiluminescence detection (196); **etonitazene** (comprehensive) (197); **fenethylline** by IR, UV, and TLC (198), and by TLC, UV/VIS, and toolmarks (199); **fentanyl** (review) (200), and by cyclic voltametry (201); **fentanyl and fentanyl analogs** (review) (202), by RP-HPLC (203), and by GC, GC/MS, and IR (204); ***Flos Daturae*** by CE (205); **flunitrazepam** (Rohypnol) (overview) (206), (comprehensive) (207,208), by color testing (screening) (209), by derivatization followed by TLC with fluorescence detection (urine screening focus) (210), by FTIR, FT-Raman, and NMR (degradation study) (211), and by screening techniques (overview) (212); **4-fluorophenylacetone**, **4-fluoroamphetamine** and **4-fluoromethamphetamine** (comprehensive) (213); ***para*-fluorofentanyl** (comprehensive) (214), **heroin** by bioluminescent assay (215), by CZE (216), by rapid GC (217), by GC/MS, FTIR, and TLC (for determination of heroin base in heroin citrate) (218), by HPLC (degradation study) (219), by IR and TLC (220), by continuous flow IRMS (221), by MECC

(222,223), by NMR (224), and by TLC (225); **heroin and related morphine alkaloids** by HPCE (226); **heroin and amphetamine** by CE (227); **human chorionic gonadotropin** (*beta* subunit*)* by MS (228); ***gamma*-hydroxybutyric acid** (GHB) or ***gamma*-hydroxybutyrate** (review) (229), (overview) (230), (comprehensive) (231,232,233), by free zone CE with direct UV detection of GHB (234), by color testing (235), by FTIR and color testing (236), by GC/MS after extraction on a SPME fiber and derivatization with BSTFA (237), by ICP-atomic emission and MS (includes ephedrine) (238), by IR using a 3-bounce diamond ATR element (239), by microcrystal testing with cupric nitrate/silver nitrate solution (240), by NMR (241), and by SPME - GC/quadrupole ion trap spectrometry (242); ***gamma*-hydroxybutyric acid (GHB) and *gamma*-butyrolactone (GBL)** (interconversion study) (243,244), by CE and HPLC (245), by GC/MS with BSTFA derivatization (246), by HPLC (247), by HPLC/UV-VIS and HPLC/thermospray MS (248), ***gamma*-butyrolactone** in wine by GC/MS (249); ***gamma*-hydroxybutyric acid (GHB), *gamma*-butyrolactone (GBL), 1,4-butanediol (BD), tetrahydrofuran (THF), and/or GHB/GBL analogs** (overview, comprehensive) (250), (overview of analysis of GHB, GBL, and BD) (251), (overview of GHB, GABA, and various analogs) (252), by CE (253), (comprehensive) (BD in a liquid exhibit) (254), and by GC/MS and FT-IR (BD) (255); **N-(2-hydroxyethyl)-amphetamine** (comprehensive) (256,257); **N-hydroxy-3,4-methylenedioxyamphetamine** by GC-FTIR after derivatization (258); **N-hydroxy-3,4-methylenedioxymethamphetamine** (comprehensive) (259), and by GC and GC/MS (260); **imazalil** (comprehensive) (261); **imipramine** by flow-injection extraction - spectrophotometric determination with methyl orange (262); **jimson weed** (overview and analysis by GC/MS: 263); **ketamine** (comprehensive) (264), and by GC and GC/MS (265); **khat** (*Catha Edulis*) by GC and GC/MS after derivatization with (R)-(+)-*alpha*-methoxy-*alpha*-(trifluoromethyl)phenylacetic acid (266), by GC/MS (267,268,269), by GC/MS/MS (270), by HPLC and TLC (271), and by NMR (272); **lorazepam** by UV (273); **lysergic acid diethylamide** (LSD) (review) (274), (overview, comprehensive) (275), (comprehensive) (276), by enzyme immunoassay and immunoaffinity extraction and HPLC-MS (277), by GC (278), by GC/MS using electronic pressure controls and pulsed split injection (279), by GC/MS/FTIR (280), by GLC (281), by HPLC (282), by HPLC and GLC (283), by MECC (284), by NMR (285), and by automated TLC (286); **LSD and psilocin** - by GC/MS and GC/MS-MS (287); **marijuana and related cannabinoids** by botanical and microscopic examination and GC/FID (288), by CEC (289,290), by DNA analysis (291,292), by the Duquenois-Levine test (on charred marijuana residue) (293), by fluoroimmunoassay (294), by GC and GC-FTIR (includes ephedrine and pseudoephedrine) (295,296), by GC, HPLC, and TLC (297), by GC/FID (versus cannabidiol as a reference standard (298), (versus cannabinol as a reference standard) (299), by GC/MS (300,301), by HPLC (302,303), by HPLC (seeds) (304), by HPLC of neutral cannabinoids of marijuana and hashish after supercritical fluid extraction (305), by LC/MS and LC/MS-MS (306), by plant propagation (determination of viability of stem cuttings) (307), by SFC/AP-CI-MS (308), by TLC and botanical characterization of morphological features (309), by monoclonal antibody (against tetrahydrocannabinolic acid) (310), and by GC/MS (butyl cannabinoids in marijuana) (311); **mescaline** in hallucinogenic *Cactaccae* by ion-interaction HPLC (312), and in peyote by GC/MS (comparison of 6 different extraction procedures) (313); **methamphetamine** (and related compounds) comprehensive (314), by CE (chiral analysis) (315), by diasteriomeric salt formation (316), by FT-Raman (317), by GC, HPLC, and/or CE following derivatization (for enantiomer determination) (318), by full scan GC-ion trap EI- and CI-MS (319), by GC/MS (improving ion mass ratio performance at low concentrations through internal standard selection) (320), by HPLC with circular dichroism detection for determination of enantiomers (321), by IR (chiral analysis) (322,323), by NMR (chiral analysis) (324), by TD-IMS and SIMPLISMA (325), and by CE with UV and LIF detection (326); **methaqualone** (review) (327), and by color testing (includes mecloqualone) (328); **methcathinone** (ephedrone) (review) (329), (comprehensive) (330), by GC and GC/MS (methcathinone and some designer analogues) (331), by GC and GC/MS after chiral derivatization with S-(-)-(trifluoroacetyl)-prolyl chloride (332), by GC/MS (333), and by animal testing (potency comparison of enantiomers; includes syntheses) (334); **methcathinone** and **cathinone** (comprehensive) (335); **4-methoxyamphetamine** (PMA) and **4-methoxymethamphetamine** (PMMA) (comprehensive) (336), and **2-**, **3-**, **4-PMA, PMMA, and P2P** (comprehensive) (337); **3-methoxy-4,5-methylenedioxyamphetamine** (comprehensive) (338,339); **2,3-methylenedioxyamphetamines** (comprehensive) (340); **2,3- and 3,4-methylenedioxyamphetamines** (combined studies) by GC/MS-MS (341,342), by LC and MS (343,344); **3,4-methylenedioxyamphetamines** (MDA's) (general review of the syntheses and analyses of MDA's and precursors and related compounds) (345), (comprehensive) (346), by C-13 solid-state NMR (347), by CE (348,349), by field testing (350), bt FT-IR (for tablets) (351), by FT-IR microscope (352), by FTIR and GC/MS (353), by GC (tablets) (354), by GC and GC/MS

(355), by GC/MS (356), by HPLC with fluorimetric detection (357), by HPLC with fluorometric detection, using added cyclodextrins (chiral analysis) (358), by HPTLC using 0-benzenesulfonamido-*p*-benzoquinone for detection (359), by LC (360), by ion trap MS (361), by LC-MS with thermospray, electrospray, and APCI interfaces (362), by RP-LC, GC, and EI-MS methods (363), by NIR and HPLC (of tablets, including MDEA and amphetamines) (364,365), by NMR (366,367), by Raman (368,369), and by SERS (370); **methylenedioxy-cathinones** (comprehensive) (371), and by melting points (372); **methylenedioxyethylamphetamine** (MDEA) (comprehensive) (373,374,375); **methylenedioxymethamphetamine** (MDMA) (comprehensive) ("crystal" MDMA) (376), by CE (377), by FTIR (for determination of hydration polymorphism) (378 and 379), by C-13 NMR (380), by FTIR (MDMA phosphate) (381), by HPLC (382), and by X-ray crystallography (383); **(3,4-methylenedioxyphenyl)-2-butanamines** (MBDB's) (comprehensive) (384), by GC/MS and LC (385,386,387,388), and a comparative study of N,N-dimethyl-3,4-methylenedioxyamphetamine and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (389); **3-methylfentanyl** (comprehensive) (390); **4-methylfentanyl** (comprehensive) (391); **methylmethaqualone** by NMR (392); **methylphenidate** by CE (393); **1-(4-methyl-phenyl)ethylamine** (comprehensive) (394); **N-methyl-1-phenylethylamine** (comprehensive) (395,396); **4-methylthioamphetamine** (comprehensive) (397,398), by GC/MS and FTIR (399), and by LC-MS/MS (400); **midazolam** by UV/Vis (401); **morphine** by aqueous and nonaqueous CE (for quantitative determination of morphine in pharmaceuticals) (402), by flow-injection analysis with spectrophotometric determination (403), by HPLC with chemiluminescence detection (404), with a fluoride-selective electrode following derivatization with 1-fluoro-2,4-dinitrobenzene (405), and by displacement TLC (406); **nandralone-*para*-hexyloxyphenyl-propionate** (comprehensive) (407); **nootropics/"smart drugs"** (overview) (408); **opiate alkaloids** by CEC (409), by high-resolution electrospray ionization – IMS/MS (evaluation of opiate separation) (410), by HPLC (hydrocodone in Tussionex) (411), and by HPTLC (oxycodone in pharmaceutical solutions) (412); **opium** (overview of characterization methodologies: 413), by CE (414), by non-aqueous CE (415), by CE-TOF-MS (416), by GC (quantitative determination of opium alkaloids in opium) (417), by GC/MS (narcotine and papaverine in seeds) (418), by HPLC (419), by RP-HPLC on a base-deactivated stationary phase (420), by HPLC of Sep-Pak C-18 cartridge extracts (421), by MECC (422), by pyrolysis-GC (423), by synchronous excitation spectrofluorimetry (424), by TLC-UV densitometric and GC-MSD methods (425), by UV/VIS (for morphine in opium) (426), and by GC/FID, GC/MS, and GC/FTIR (headspace constituents of opium) (427); **opium alkaloids** by CE with acidic potassium permanganate chemiluminescence detection (428), by CZE (429), by flow-injection analysis using soluble manganese(IV) for chemiluminescence detection (430), by GC/MS after oxime-TMS derivatization (431), by GC/MS (6-acetylmorphine) (432), by HPLC using porous and non-porous stationary phases (433), by HPLC-DAD (stability of morphine containing solutions) (434), by RP-HPLC (codeine and ethyl morphine HCl in pharmaceutical tablets) (435), by LC (for acetylsalicylic acid, caffeine, and codeine phosphate in pharmaceuticals) (436), (for acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine, and thiamine in pharmaceuticals) (437), (for paracetamol, caffeine, and codeine phosphate in pharmaceuticals) (438), by RP-LC (papaverine in tablets) (439), by spectrofluororimetric detection (acetylsalicylic acid and codeine in pharmaceuticals) (440), and by spectrophotometric detection (of noscapine with bromocresol green in chloroform) (441); **opium**, **morphine**, and **heroin** (combined study) (comprehensive) (442); **oxazepam** by automatic kinetic determination (443); **pentobarbital** by GC/MS (444), and by HPLC (445); **phencyclidine** (and **1-piperidinocyclohexane-carbonitrile**) (review) (446), by IMS (447), and by IR after liquid-liquid extraction from case samples (448); ***alpha*-phenethylamine** (comprehensive) (449,450), ***beta*-phenethylamine** (overview) (451,452), and by GC/MS, FTIR, and GC/IR (453); **phenobarbital** by CE (tablets) (454), by LC (for determination of scopalamine, hyoscyamine and phenobarbital in tablets) (455), by multivariate spectrophotometric calibration (for simultaneous determination of phenobarbital and phenytoin in tablets) (456), and by spectrophotometric determination (457); **phenylpropylmethylamine** (comprehensive) (458); **piperazines** (comprehensive) (459); ***psilocybe* mushrooms** by DNA (460,461), by IMS and GC/MS (462), by GC/MS and HPLC/UV (463), by morphological, microscopic, microchemical, and HPLC with 266 nm UV detection (464), by TLC, GC/MS, and LC/MS (465), and by TLC and GC/MS (psilocin and psilocybin in developmental mushrooms) (466); ***Salvia Divinorum*** by GC/MS (467); **secobarbital** using C-13 labelled secobarbital as an internal standard (468); **sibutramine** (comprehensive) (469); **steroids** (overview) (470), (overview, comprehensive) (471), (comprehensive) (472), by EI, CI, and CI/tandem MS (473), by GC (474), by GC/MS (475), by GC/MS after SFC isolation from aqueous matrices (476), by GC-MS and NMR after derivatization with N-methyl-N-alkylsilyltrifluoroacetamide-I-2 (477), by GLC (478), by HPLC with cyclodextrin coated columns (479), by

HPLC with UV/Vis-particle beam MS (480), by HPLC-FTIR (481), by HPTLC and HPLC (482), by MS (of *tert*-butyldimethylsilyl ether derivatives) (483), by MS/MS (484), by quadrupole ion trap tandem MS (485), by 13C - NMR (486), by MECC (487), by MECC, gradient HPLC, and capillary GC (488), by capillary SFC with FID and ECD (489), and by TLC (490); **telazol** (comprehensive) (491,492); **terbinafine** (comprehensive) (493), and by UV and nonacqueous voltametry (494); **triazolam** by TD-GC (495); **tricyclic antidepressants** - by electrogenerated chemiluminescence (496); and **tryptamines** by chemiluminescence (497).

Adulterants, Diluents and Precursors (general overviews of essential chemicals and precursors) (498,499), by GC, HPLC, and CE (500), by LC (for determination of non-UV detectable organic impurities) (501), by various analytical techniques (for determination of "secondary" drugs present in cocaine, heroin, marijuana and phencyclidine) (502); **acetaminophen** by Raman microprobe spectroscopy (503); **acetic acid** (in acetic anhydride) by NMR (504), **dextromethorphan** by MEKC (dextromethorphan, pseudoephedrine and guaifenesin) (505); **diethylaminoethylaniline** (comprehensive) (506), ***cis*- and *trans*-2,5-dimethoxy-4,*beta*-dimethyl-*beta*-nitrostyrenes** by FTIR/Raman (507); **dimethylsulfone** (overview) (508), (removal by sublimation) (509,510), by GC/MS, IR and GC/IR (in amphetamine and methamphetamine samples) (511); **dimethyl terephthalate** (and **dimethyl phthalate**) (comprehensive) (512), differentiation of **dimethylterephthalate** from **dimethyliso-phthalate** by GC/FTIR (513), identification of **dimethyl terephthalate** in cocaine samples (comprehensive) (514); **dipyrone** in pharmaceuticals with a flow cell containing gold electrodes (515); **ephedrine/pseudoephedrine** comprehensive (516), by CE, UV, NMR, and MS (for chiral recognition of the enantiomers of ephedrine derivatives) (517), by CE on a chip with amperometric detection (for chiral analysis) (518), by acetonitrile modified CZE (for ephedrine in *ephedra callus*) (519), by derivative spectrophotometry and ratio spectra derivative spectrophotometry (for simultaneous determination of pseudoephedrine, dexbrompheniramine, and loratadine) (520), by differential-derivative spectroscopy (assay of ephedrine/theophylline containing pharmaceuticals) (521), by an ephedrine based electrode (522), by a double-membrane ephedrine selective electrode (523), by flow injection - pulse amperometric detection (524), by GC (for determination of pseudoephedrine and diphenhydramine) (525), by GC/MS (for determination of ephedrine alkaloids and tetramethylpyrazine in *ephedra sinica* Stapf) (526), by HPLC (for determination of ephedrine, pseudoephedrine, norephedrine, and methylephedrine in Chinese folk medications) (527), by HPLC using chiral stationary phases (for separation of the enantiomers of ephedrine, norephedrine, and pseudoephedrine) (528), by HPLC and CE (discrimination of ephedrine and pseudoephedrine) (529), by HPTLC (for simultaneous determination of pseudoephedrine and cetirizine in pharmaceuticals) (530), by LC (for N-methylephedrine, after derivatization with 9-fluorenylmethyl chloroformate) (531), by LC (for determination of pseudoephedrine and carbinoxamine pharmaceuticals) (532), by proton NMR (for determination of ephedrine, pseudoephedrine, and norephedrine in bulk and dosage mixtures) (533), by RP-HPLC-UV (with data analysis to handle quantitation of overlapping peaks) (534), by SPE-LC/UV (for determination of 7 ephedrine alkaloids in herbal products) (535), by TLC and FTIR (for determination of pseudoephedrine in a pseudoephedrine/chlorpheniramine pharmaceutical) (536), and by impregnated TLC (for direct resolution of (+/-)-ephedrine and atropine) (537); **ethoxy-1-(2-nitro-1-propenzyl)benzenes** by FTIR and Raman (538); **guaifenesin** by HPLC (539); **3-hydroxy-N-phenyl-2-naphthalene carboxamide** (comprehensive) (540); **lactitol** in cocaine by NMR and IR (541); **4-(N-methyl-acetamido)-antipyrine** (comprehensive) (542); **paracetamol** by FTIR (543), by ion chromatography (544), by reflectance NIR spectroscopy (545), by NIR transmittance spectroscopy (546), and by simultaneous stopped-flow determination and FTIR (for paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations) (547); **pheniramines** by CZE (for pheniramine, chlorpheniramine, and brompheniramine) (548), and by an imprinted sensor (for chlorpheniramine) (549); **phenylpropanolamine** by HPLC in pharmaceutical preparations (using 4-dimethylaminobenzaldehyde) (550); **procaine** by spectrophotometry (using *p*-dimethylaminobenzaldehyde) (551); **quinine** by flow-injection chemiluminescence (552), and by HPLC with polarimetric detection (553); **safrole** by SFC extraction and GC/MS (for determination of safrole and related allylbenzenes in sassafras) (554); **sugars** by GC after derivatization with trimethylsilylimidazole (for quantitation of sugars in drug samples) (555), **theophylline** by adsorptive cathodic stripping voltammetry (556), by a flow fluoroimmunosensor (557), by micellar LC and spectrophotometric detection (558), and by UV and HPLC (559); **thiamine** by cathodic stripping voltametry (560), by cyclic voltametry and HPLC with amperometric detection (561), by flow injection turbidimetric determination using silicotungstic acid (562), and by spectrofluorometry (563); and **triprolidine** by a kinetic method based on oxidation w/ KMnO4, with spectrophotometric determination (564).

Occluded Solvents in **cocaine** by GC/MS (565), in **cocaine and heroin** by headspace - GC/FID and GC/MS (566), and by SHS-GC/MS (567); in **methamphetamine** by SPME/GC-MS (characterization of volatile components) (568); and in **pharmaceuticals** (overview, emphasizing headspace - CGC and impurity profiling) (569), by CGC (570), by wide-bore CGC (571), by CGC-ITD (572), and by automated SHS - CGC-MS (573).

Simultaneous Analyses of Drugs and Adulterants/Diluents

A number of studies have been reported which allow simultaneous identification and quantitation of mixtures of controlled substances and adulterants without separating them into individual components. In general, such techniques may be only employed in select geographical areas in which the submitted exhibits are reasonably consistent (that is, routinely the same adulterants and diluents, at or below a threshold percentage). In most cases, they allow much more rapid sample analysis and throughput without unduly compromising the identification of the controlled substance. Such techniques have been utilized for: **cocaine** by IR (574,575), by Raman (576,577), by spectrometric methods (578), and by sequential second-derivative spectroscopy (579); and **pharmaceuticals** by NIR and Raman (580), and by spectrophotometry in conjunction with PLS-1 and PLS-2 data processing methods (581); and for determination of interferences by common diluents in street-level drugs by micro-FTIR (582).

Instrumentation Focus

In addition to the above studies which concentrated on specific drugs or drug groups, there have been a large number of studies which focused on specific instrumental techniques, analyzing two or more unrelated drugs or drug types in order to illustrate the utility of the described methodology, including: **capillary electrophoresis:** (general reviews) (583,584,585,586,587,588,589,590,591, 592,593,594,595,596,597), (general overview and reviews) (598,599), (general overview, comparing various CE techniques) (600), (review for court admissibility) (601), for separation and permanganate chemiluminescence on-line detection of some alkaloids with *beta*-cyclodextrin as an additive (602), for on-chip separation of amphetamine and related compounds labeled with 4-fluoro-7-nitrobenzofurazane (603), for enantioselective separations of various amphetamines and methylenedioxyamphetamines using cyclodextrins (604,605), for analysis and confirmation of synthetic anorexics in adulterated traditional Chinese medicines (606), for chiral analysis of drugs (607), for chiral identification of drug isomers (608), for chiral analysis of basic drugs using oligosaccharides (609), for chiral separation of basic drug racemates using linear, neutral polysaccharides (610), for chiral resolution of cationic drugs of forensic interest with mixtures of neutral and anionic cyclodextrins (611), for chiral separation of enantiomers of drugs using *beta*-cyclodextrin (612), for chiral separation of drug stereoisomers with cyclodextrins (613), for chiral separation of basic drugs using ionic and neutral polysaccharides (614), for ultra-fast chiral separation of basic drugs (615), for illicit drug seizures (616), for CE-TOF/MS of drugs of abuse (617,618), for separation of enantiomers of basic drugs (by affinity CE using a partial filling technique and α1-acid glycoprotein as chiral selector) (619), for separation and identification of amphetamines, methadone, venlafaxine, and tropane alkaloids by CE-electrospray MS (620), for separation and identification of designer drugs with CE-ionspray MS (621), for determination of drug-related impurities (622), for analysis of heroin and amphetamine (623), for simultaneous chiral analysis of methamphetamine and related compounds (624), for routine analysis of methamphetamine, amphetamine, MDA, MDMA, MDEA and cocaine with dynamically coated capillaries (625), for analysis of basic pharmaceuticals by CE in coated capillaries with on-line MS detection (626), for quantitation of common illicit drugs (627,628), for chiral separation of selegiline, methamphetamine, and ephedrine using a neutral *beta*-cyclodextrin epichlorhydrin polymer (629), for tropane alkaloids in a plant extract (630), for CE-DAD-electrospray MS of tropane alkaloids, hyoscyamine, scopolamine, and plant extracts (631), CEC: (introductory overview) (632), for simultaneous separation of acidic, basic, and neutral organic compounds, including strong and moderate acids and bases (633), for analysis of drugs of forensic interest (634); CZE: characterization of drugs of forensic interest by CZE/electrospray ionization MS (635), for chiral separation of amphetamine and phenylephrine, using cyclodextrins (636), for chiral separation of basic drugs using cyclodextrins (637), and for chiral separation of some basic drugs (influence of the buffer organic cation) (638); EKC: for chiral separation of

drugs by electrokinetic chromatography (639), for separation of enantiomers and geometric isomers using a charged cyclodextrin (640), and for chiral separation of neutral and basic enantiomers using anionic cyclodextrins (641); MECC: (general review) (642), for chiral differentiation of pharmacologically active substances by cyclodextrin-modified MECC using a bile salt (643), for analysis of controlled substances, using different micelles (644), and for analysis of phenethylamines (645); MEKC: for separation of sympathomimetic amines of abuse and related compounds (646); non-aqueous CE: for analysis of drugs (647,648,649,650), for analysis of drugs by nonaqueous CE with electrochemical detection (651), and for analysis of tropane alkaloids and amphetamine derivatives (652); polymethod: characterisation of retention in micellar HPLC, in MEKC and in MEKC with reduced flow (653), complementary use of CZE and MECC for mutual confirmation of results in forensic drug analysis (654), and the study of the CZE behavior of selected drugs and its comparison with other analytical techniques for their formulation assay (655); general: CE using polyacrylamide-coated columns (656,657), effect of methanol in sample solution on an electropherogram (658), evaluation of the use of cyclodextrins in chiral separation of basic drug substances by CE (659), improved chiral separation of basic compounds using *beta*-cyclodextrin and tetraalkylammonium reagents (660), quantitative aspects of the application of CE to the analysis of pharmaceuticals and drug related impurities (661), separation selectivity in chiral and achiral CE with mixed cyclodextrins (662), and use of large-volume sample stacking for selected drugs of forensic significance (663); **fluorescence spectroscopy** (review) (664); **gas chromatography** and **gas chromatography/mass spectrometry:** (general reviews (books)) (665,666), to distinguish amphetamine, methamphetamine, and 3,4-methylenedioxymethamphet-amine from other sympathomimetic amines following derivatization with propyl chloroformate (GC/CI-MS) (667), to distinguish and quantify the enantiomers of amphetamines, phenol alkylamines, and hydroxyamines following stereospecific derivatization (CGC/MS) (668), for enhanced detection of trace-level controlled substances using GC/MS with pulsed splitless injections (669), for the quantitation of cocaine, heroin, diazepam, methaqualone, codeine, and oxycodone (GC) (670), forensic analysis by GC with dual MS and NPD detection (671), by GC with surface ionization detection (672), using isotopic analogues as internal standards (673,674,675), by MS and electrospray ionization MS (676), with a programmable temperature vaporizing injector and cold on-column injector (677) by rapid GC (678), by secondary electrospray IMS/MS (679), by TLC and GC/MS (680), by wide-bore column GC-NPD (681), a dual internal standard method for screening by GLC at the one percent level (682), internal quality control of a general GC drug screen in forensic toxicology (683), use of MSD's for identification of unknowns (684), normalization of residual ions after removal of the base peak in EI-MS (polydrug study) (685), practical determination of GC–MS limits of detection (686), sample concentrator for sensitivity enhancement in chromatographic analyses (687), SPME-GC (review) (688), and trace analysis by splitless GC/MS (689); **high-performance liquid chromatography** (and tandem HPLC techniques)**:** general overview (690), (recent progress in HPLC analyses for drugs of abuse) (691), for analyses of barbiturates, LSD, MDA, and psilocybin (HPLC using continuous on-line post-elution photoirradiation with diode-array UV or thermospray-MS detection) (692), to separate and identify cocaine, morphine, heroin, codeine, papaverine, benzocaine, procaine, and lidocaine (HPLC-DAD) (693), for the rapid analysis of illicit heroin and cocaine samples (HPLC-DAD and CGC/NPD) (694), for assaying morphine and hydromorphone in pharmaceuticals (695), for direct chiral resolution of phenylalkylamines (using a crown ether chiral stationary phase (includes amphetamine and cathinone)) (696), for the simultaneous determination of triprolidine, pseudoephedrine, paracetamol, and dextromethorphan (697), for drug screening (698), for analysis of drugs of forensic interest (RP-HPLC) (699), for analysis of alkaloid drugs of forensic interest (RP-HPLC-PDA) (700), for analysis of some alkaloids on unmodified silica gel with aqueous-organic solvent mixtures (701), for determination of alkaloids in foods (multi-detector HPLC) (702), for detection in the forensic sciences (LC-PDA) (703), to determine the enantiomeric composition of abused drugs (704), for determination of illicit drugs and related substances (HPLC with an electrochemical coulometric-array detector) (705), for forensic analyses (on-line HPLC/FAB-MS) (706), for purity testing for tropane alkaloids (707), for resolution of racemic drugs (using a new chiral column based on silica-immobilized cellobiohydrolase) (708), to study the effects of chromatographic conditions on the retention indices of forensically relevant substances (RP-HPLC) (709), and for analysis of pharmaceuticals and drugs (HPLC using unmodified silica and polar solvents) (710); **HPLC retention indices:** (711,712,713); **infrared and Raman spectroscopy**: (minor review of IR and Raman for detection of narcotics) (714), (general review of Raman of narcotics and explosives) (715), FTIR and microcrystal tests for rapid identification of drugs (716), FTIR microspectrophotometry of illicit drugs (sample preparation) (717), FT-NIR for validation of controlled substance identifications (718), NIR for identification of

drugs and various adulterants/diluents (719), NIR, FT-Raman, and DR-FTIR for non-destructive identification of Chinese traditional drugs (720), GC-FTIR for screening of hallucinogenic and stimulant amphetamines (721), vapor-phase FTIR for identification of novel illicit amphetamines (722), HPTLC-FTIR for identification of LSD, MBDB and atropine (723), use of a diamond anvil cell with a beam condenser and an FTIR microscope for analyses of some particulate drug mixtures (including cocaine, heroin, and methamphetamine) (724), internal reflectance spectra library (725), FT-Raman for nondestructive determination of raw plant medicinal drugs (726), filtered fiber optic Raman probes for analysis of illicit drugs (727), micro-Raman for identification of narcotics (including opium alkaloids) (728), SERRS for drug analysis (729), and evaluation of silver substrates for SERRS of cocaine and other stimulant drugs (730); **ion chromatography:** for determination of ionic compounds, exicipients, and contaminants in drug evidence (731); **microscopy:** (general overview) (732), and videomicroscopy (733); **nuclear magnetic resonance spectroscopy:** (general review) (734), for assessing drug enantiomeric composition (including amphetamine) (735), for chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine, and methcathinone (includes GC analyses) (736), to identify impurities in drug substances (by LC-NMR) (737), and for routine analyses (738,739); **phosphorimetry:** of barbital, codeine, morphine, and practolol after labelling with dansyl chloride (740); **robotics** and/or **specialized computer programs:** (overview and review) (741), for automated CGC heroin analysis (742), combined Rf and UV library search software for TLC and RPTLC (743), a computerized IR search system (744), for optimized analysis of heroin by RP-HPLC (745), to evaluate an HPLC column's performance (746), and to optimize gradient and isocratic HPLC analyses (747); **supercritical fluid chromatography:** (general reviews) (748,749), and for extraction of tropane alkaloids (including cocaine) from E. coca extracts (750); **thin layer chromatography:** (general review) (751), TLC/DAD for forensic analyses (752), overpressured TLC (for determination of morphine, codeine, heroin, opium alkaloids, nicotine, amphetamine, cocaine, and LSD) (753), TLC for separation of cocaine, pramocaine, fentanyl, and diphenhydramine (754), and TLC with a special visualization reagent for tertiary amines (including dimethylamphetamine, flunitrazepam, methamphetamine, methaqualone, nicotine, theophylline, triazolam, and others) (755); and **miscellaneous:** Comparison of IR and MS for drug analyses (756).

Analytical Artifacts

GC and GC/MS are the current methods of choice for routine screening, identification and quantitation of controlled substances. However, the use of high-temperature injectors can produce artifacts via unimolecular rearrangements of and/or intermolecular reactions between the various components (including even the injection solvent). Artifacts are also possible in other techniques. Over the past 10 years, artifacts have been reported for: **cannabinoids:** nitrites in cannabinoid analyses (urine testing focus) (757,758,759); **cocaine:** determination of ecgonidine methyl ester vapor pressure (760), identification of methyl esters of ecgonine as injection port produced artifacts from cocaine base (crack) exhibits (761); **heroin:** identification of a heroin/chloroform-impurity reaction product (762); **morphine and codeine:** hydromorphone and hydrocodone interference in GC/MS assays for morphine and codeine (763); **phenethylamines:** artifacts in the GC analysis of amphetamine and MDA (764), GC/MS identification of amine-solvent condensation products formed during analysis of drugs of abuse (from ethanol with amphetamine, MDA, and *beta*-phenethylamine) (765,766), conversion of ephedrine to methamphetamine and methamphetamine-like compounds during and prior to GC/MS analyses of heptafluorobutyrate and carbethoxyhexafluorobutyrate derivatives (urine testing focus) (767) identification of a GC/MS artifact peak as methamphetamine (768), matrix effects in the IR of methamphetamine salts (769,770), and a procedure for eliminating interferences from ephedrine and related compounds in the GC/MS analysis of amphetamine and methamphetamine (771); **piperonal:** an artifact in the GC analysis of piperonal (772); and **miscellaneous:** influence of large amounts of drugs on the peak areas of their coinjected deuterated analogues measured with APCI-LC-MS (773), and a simple software procedure to determine if a GC/MS blank injection is contaminated (774).

Qualitative Tests

Spot tests are a mainstay of forensic analysis of controlled substances, and offer a reliable means for very rapid screening of submitted exhibits. Over the past 10 years, the following qualitative testing studies were reported: (general overview) (775), (textbook) (776), (review of color comparisons in forensic science, including drug color tests) (777), for anhydrous ammonia (778), for cocaine (mechanistic study of the Scott Ruybal test) (779), for drugs of abuse (12 spot tests) (780), for lithium (781), for pemoline, fenozolone, and thozalinone (color tests) (782), and for red phosphorus (783,784,785).

Sampling Plans

Large drug seizures are almost invariably comprised of multiple units of a standard container size (for example, several thousand 1 kilogram packages of cocaine). Comprehensive analysis of such seizures is a daunting and prodigiously labor intensive task; therefore, statistically based sampling plans are utilized that enable valid assessment of an entire shipment based on analyses of a select number of representative, randomly selected exhibits. The classic study in this field (by Frank, Hinkley, and Hoffman) was reported in 1991, but is included here as critical background (786). Over the past 10 years, the following additional studies were reported: (overviews and general discussions) (787,788,789,790,791), a case studies of heroin (792,793,794).

Source Determination/Impurity Profiling

Determination of synthetic route origin (including processing variants) and/or geographical origin is important for developing tactical and strategic intelligence. Historically, source determination has been conducted by in-depth impurity profiling; that is, determining discriminatory marker compounds and/or ratios of marker compounds which are characteristic of origin. More recently, trace element analyses and (especially) stable isotope analyses (*vide infra*) have increased the confidence of geographical sourcing; this aspect of source determination is rapidly expanding. Finally, increasingly sophisticated pattern recognition techniques (often neural network based) have been employed to handle the enormous databases generated by source determination programs. A large number of source determination studies have been reported over the past 10 years: (general discussions) (795,796), (pattern recognition techniques screening for drugs of abuse (illicit amphetamines) with GC-FTIR) (797); **amphetamines:** systematic approach to profiling amphetamines (798), automated GC method for amphetamine profiling (799), amphetamine profiling in the UK (800), from arylpropenes with acetonitrile and sulfuric acid (Ritter reaction) (801), of Leuckardt amphetamine (802,803,804,805), from 1-phenyl-2-nitropropene (806,807), improved data processing for amphetamine profiling (808), from phenylacetone synthesized from phenylacetic acid (Leukardt reaction) (809), and a pan-European method for profiling amphetamines (810); **amphetamines and marijuana:** of impurities (811); **cocaine:** reviews (812,813,814), comprehensive profiling (815,816,817,818), of 2-carbomethoxy-3-alkyloxy- and heteroaroyloxy substituted tropanes in cocaine (819), of 2-carbomethoxy-3-oxo analogs in cocaine (820,821), of chlorinated cocaines from cocaine treated with bleach (822, see also:823), of cuscohygrine in cocaine (824), of heteroaroyl analogs in cocaine (825), of 1-hydroxy-tropacocaine in cocaine (826), of hygrine in cocaine (827), of hygrine and cuscohygrine in cocaine (828), of norcocaine in cocaine (829), of occluded solvents in cocaine (effects of microwave radiation on solvent profiles) (830), of pharmaceutical cocaine (831), of pseudococaine in cocaine (832), of trace metals in cocaine (833), of trimethoxy analogs of cocaine, cinnamoylcocaine, and tropacocaine in cocaine (834), of truxillines in cocaine (835), of truxillines and similar high molecular weight impurities in cocaine (836), of illicit cocaine by X-ray Diffractometry (also GC and GC/MSD) (837); **cocaine and heroin**: (combined studies) of trace metals in cocaine and heroin (838,839,840,841,842,843,844,845,846), and by palynology (pollen analysis) (847); of occluded solvents in cocaine and heroin (848); **ephedrine:** by microscopic examination (849); **fentanyl:** prepared from 1-phenethyl-4-piperidone (850); **heroin:** overview (851), (review) (852), of acid and neutral impurities in heroin (853), of anions and cations in heroin (854,855), of basic byproducts and adulterants in heroin (856), of impurities in heroin (857,858,859,860,861), of metal contamination in heroin (862), of O6-monoacetylmorphine in "homebake" heroin (863), of trace elements in heroin by ICP-MS (864,865), of trace organic impurities (866);

marijuana: of cannabidiol and delta-9-THC in stored marijuana (867), of impurities in hashish (868,869), of impurities in marijuana (870,871,872), of natural constituents in marijuana (reviews) (873,874), and of marijuana DNA (875,876,877,878,879,880,881,882,883); **methamphetamine:** review (UNDCP) (884), overview (885), generic articles on impurity profiling (886,887), of N-acetylmethamphetamine in illicit methamphetamine (888), of chloroephedrine and aziridines in methamphetamine (889), of impurities in methamphetamine (890,891), of inorganic impurities in methamphetamine (892), of methamphetamine synthesized from allylbenzene (893,894,895), of impurities in methamphetamine synthesized via HI/red P (896), of methamphetamine synthesized via HI/red P (focusing on reaction byproducts of common cold tablet ingredients) (897,898), of methamphetamine containing a hydrocarbon wax (899), of methamphetamine synthesized from pseudoephedrine tablets (900), of trace elements in methamphetamine (901), of methamphetamine seized in Australia (overview and development of a national database) (902), of methamphetamine seized in Japan (903,904, and overview: 905), and of methamphetamine seized in Korea (906); **4-methoxyamphetamine:** of impurities (907); **methylenedioxyamphetamines:** overview of approach in Australia (908), of impurities in methylenedioxymethamphetamine (909,910), of precursors, intermediates, and reaction byproducts for methylenedioxymethamphetamine (911), of impurities in methylenedioxymethamphetamine and amphetamine (912), of impurities in methylenedioxyamphetamine and methylenedioxymethamphetamine (913,914), determination of synthetic route markers for methylenedioxyamphetamine and methylenedioxymethamphetamine (915), of methylenedioxymethamphetamine tablets by logo and headspace comparisons (916), of commercially available methylenedioxyphenylacetone (917), from the Ritter reaction (using safrole) (918), of methylenedioxyphenylacetone and methylenedioxyamphetamine synthesized from isosafrole (919,920), and of methylenedioxyamphetamine synthesized from nitoethane and piperonal (921); **nicotine:** (overview of tobacco smoke) (922); **opium:** of opium alkaloids (for origin determination) (923), of proteins in opium latex (924); **pharmaceuticals:** overview (925), of impurities (926,927,928); **phenyl-2-propanone:** of illicit phenylacetone synthesized from phenylacetic acid with acetic anhydride versus lead (II) acetate (929); **precursors:** of essential oils used as precursors in the synthesis of phenethylamine-type designer drugs (930), and **testosterone undecanoate:** of impurities (931).

Source Determination/Stable Isotope Analyses

Historically, processing origin could be reasonably correlated with geographical origin. However, the expansion of drug producing regions and the concommitant convergence of processing techniques, along with the international exchange or sale of precursors (for example, 3,4-methylenedioxyphenylacetone) or crudely refined controlled substances (for example, morphine or heroin base) across the world, have mandated more sophisticated analyses. Because the natural abundances of the stable isotopes of hydrogen, carbon, nitrogen, and oxygen vary across the world, and their incorporation into natural products is unaffected by subsequent illicit processing, stable isotope analyses offer a powerful tool for determining "true" geographic origin (that is, not indirectly inferred based on processing methodology). Recent advances in instrumentation (notably isotopic ratio mass spectrometry and high field nuclear magnetic resonance spectroscopy) have enabled the determination of the isotopic makeup of controlled substances with reasonable precision and accuracy. To date, only cocaine and heroin have been subjected to comprehensive studies; however, this field is expected to expand to other controlled substances over the next decade. Recent reports include: (overviews) (932,933,934); **cocaine:** by carbon-13 isotope analysis: (935,936), by IRMS and trace alkaloid analysis (937); **cocaine and heroin:** by IRMS (938), by site specific deuterium-NMR (939); and **heroin:** by GC/IRMS (940), and by GC/MS and GC/IRMS (941).

Comparative Analyses

Establishing commonality of origin between 2 or more exhibits requires systematic application of detailed impurity profiling. Comparative analysis does not require formal determination of synthetic, processing, or geographical origin, but rather determination of "degree of match" between profiles (usually trace-level chromatographic analyses; however, establishment of synthetic, processing, or geographical origin is a common spinoff of comparative analysis protocols). Studies reported over the past 10 years include: (general overviews)

(942,943); **amphetamine:** computerized comparisons of Leuckart amphetamine (944); **cocaine:** (overview of methodologies) (945), database for comparison (946), by CGC/ECD (947), by CGC/NPD (948), comparison of crack cocaine by matching fracture lines between pieces (949), cocaine comparison court case (950), by rapid GC (951), by HPLC-DAD (952), by a neural network (953); **hashish:** by HPLC, GC, and AA (954); **heroin**: (general overview) (955), by CGC (956), computerized comparison (957), predictive model (958), harmonization study for retrospective comparisons (959,960), of SWA heroin by GC (961); **marijuana:** comparison by RAPD and HPLC (962); **methaqualone:** tablets by NIR reflectance spectra (963); **methylenedioxymethamphetamine:** by natural isotope abundances (964); **opium:** by RAPD, HPLC, and ELISA (965), **pharmaceuticals:** evaluation of neural networks (966); and **tablets and capsules:** indices of physical characteristics (967,968).

Reference Standards

Accurate analyses of controlled substances and related analogs require high purity standards, including isotopically labelled analogs. Structurally related compounds are also needed as internal standards for chromatographic analyses. Reports over the past 10 years include: (general reviews) (969,970); **bufotenine** (and related tryptamines)**:** (971), **butalbital:** (972); **cannabinoids:** (973,974); **cocaine:** (975,976), aza analogs of cocaine (977), deuterium-labelled cocaine, cocaethylene and metabolites (978), cocaine by one step esterification of benzoylecgonine (979), 6- and 7-hydroxylated cocaines (980), C-3 alkyl analogs of cocaine (981); **lysergic acid diethylamide** (982,983); **d- and l-methamphetamine:** via optical resolution (984), "Ice" methamphetamine (985); **methcathinone:** (986); **methohexital:** (987); **morphine:** (988,989); **polydrug:** (N-ethylmethylenedioxyamphetamine, N-hydroxymethylenedioxyamphetamine, mecloqualone, 4-methylaminorex, phendimetrazine, and phenmetrazine) (990), (O6-monoacetylmorphine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine, and N-methyl-3,4-methylenedioxyphenyl-2-butanamine, from seized drugs) (991), and a reference garden of hallucinogenic and narcotic plants in Australia (992); **(1S,2S)-pseudoephedrine:** (993); and **psilocybin** and O-acetyl psilocybin (994).

Clandestine Laboratories

The illicit production of drugs is a dynamic and constantly changing field. Reports over the past 10 years included: (general review) (995), amphetamine (996,997,998), failed synthesis of amphetamine (999), amphetamine in methamphetamine (1000), analyses of inorganic components found in clandestine drug laboratory evidence (1001), arsenic oxide (potential reagent in methamphetamine synthesis) (1002), Birch reduction (general review) (1003) (overview of developments in the midwestern US (1004), cocaine (1005,1006), concealment and trafficking (1007), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) (1008), ephedra (1009,1010), ephedrine and/or pseudoephedrine (1011,1012), etonitazene (1013), fentanyl (1014), freons in methamphetamine production (1015,1016,1017), hash oil (1018,1019), heroin (acetylated opium) (1020), heroin (review) (1021), hydriodic acid for methamphetamine production (1022,1023,1024), hypophosphorus acid for methamphetamine production (1025), inorganic acids (1026,1027), iodine (GC/MS identification) (1028,1029), lysergic acid amide from morning glory seeds (1030), lysergic acid diethylamide (1031), marijuana (1032,1033), methadone (1034), methamphetamine (1035,1036,1037,1038,1039,1040,1041), methamphetamine (by dissolving metal reduction) (1042,1043,1044), methamphetamine in Taiwan (1045), methaqualone and analogs (1046), methcathinone (1047), methylenedioxymethamphetamine (1048), methylenedioxyphenethylamines (1049), morphine (by dealkylation of codeine) (1050), overview of illicit drug production in the Czech Republic from the 70's through the 90's (1051), phencyclidines (1052,1053,1054), phenylacetone and methylenedioxyphenylacetone (1055,1056), piperonal (1057), polydrug (methamphetamine, phenylacetone, methylenedioxyamphetamine, and methaqualone) (1058), steroids (1059), substitution of white phosphorus for red phosphorus in hydriodic acid reduction laboratories in Idaho (1060), *delta*-9-THC precursors (1061), *delta*-9-THC acetate (1062), unusual defense to charge of MDMA manufacture (1063), and an unusual designer drug laboratory (polydrug) (1064).

Clandestine Laboratory Appraisals and Safety

The rapid expansion of clandestine laboratories in the US over the past 15 years has resulted in a large number of studies concerning proper assessment and safe dismantling, including reports on: assessment and remediation of contaminated sites (1065), clandestine laboratory production capabilities (1066), confined space laboratories (1067,1068,1069,1070), decontamination of biohazardous evidence (1071), determination of occupational exposure to cocaine by crime lab personnel (1072), determination of volumes in clandestine laboratory reaction vessels (1073), environmental impact and adverse health effects of the clandestine manufacture of methamphetamine (1074), field methods to render safe pressurized tanks of ammonia at clandestine labs (1075,1076), hydrogen sulfide fatality (1077), OSHA and NIOSH regulations (1078,1079), phosphine gas exposure from a methamphetamine laboratory investigation (1080), phosphine gas fatalities (1081,1082), phosphine gas detection and monitoring instrumentation (1083), safety training for clandestine laboratory investigators (1084,1085), supplier of 22-liter flasks put on notice (1086), training (1087,1088), triacetonetriperoxide causes explosion during analysis (1089), and useful websites for personnel involved in forensic laboratories and/or clandestine laboratories (1090).

Portable Instrumentation/Trace Detection

New world trade agreements and the easing of formerly restrictive national and international borders have resulted in dramatic increases in cargo transshipping and personal travel, thereby complicating drug inspection and interdiction efforts at POE's. The need for rapid and accurate screening, and high sample throughput, requires on-site equipment capable of assessing humans, animals, and a vast array of shipping containers. In addition, on-site equipment is needed for proper assessment of clandestine laboratories. However, the typical size and operational requirements of most laboratory instrumentation preclude their use in field settings. This has resulted in a growing industry dedicated to development of man-portable, field rugged equipment for detection and identification of controlled substances. Many of the pertinent studies are proprietary, but a large number have nonetheless been reported over the past 10 years: **general:** (reviews) (1091,1092,1093,1094,1095), (general assessments) (1096,1097,1098,1099), appraisal of drug detection scenarios - operational analysis for drug detection (1100), and determination of high-risk cargo (1101); **amperometric assay:** for opiates (1102); **biosensor technologies** for the detection of illegal drugs (1103,1104,1105,1106,1107), antibody-based field kits for cocaine and heroin (1108), a fiber-optic cocaine biosensor (1109), an ISFET device for cocaine analysis (1110), the use of heroin esterase in the development of a biosensor (1111), and use of recombinant DNA in the design of a heroin sensor (1112); **calibration standards:** for narcotics detection devices (1113); **correlated column micro-GC:** for the detection of contraband drugs in cargo containers (1114); **field ion spectrometry:** (1115); **gamma ray detectors:** (1116,1117,1118); **gas sensor arrays** for drug "aroma" detection (1119); **immunoassay based detection systems:** (1120,1121) and similar technologies (1122); **DRUGWIPE:** (1123); **ion mobility spectrometers:** (1124,1125,1126,1127,1128), detection of cocaine and heroin by a custom built IMS (1129), detection of drugs of abuse in Customs scenarios using IMS (1130), use of fluorescence spotting to identify areas for IMS (1131), detection of methamphetamine and ephedrine in abandoned clandestine laboratories with IMS (1132), differentiation of methamphetamine versus nicotine using IMS (1133), DSP techniques for narcotic detection using IMS (1134), field applications of IMS (1135,1136), and use of SPME with IMS (1137); **ion trap mobility spectrometers:** (1138,1139,1140); **laser-based near- and mid-IR:** (1141); **neutron-based technologies:** (1142,1143,1144,1145,1146,1147,1148,1149,1150,1151), combined neutron and gamma ray detection (1152), evaluation of neutron techniques for illicit substance detection (1153,1154,1155), and pulsed fast neutron analysis (1156,1157); **N-14 nuclear quadrupole resonance:** (1158,1159,1160,1161); **particle detection:** cocaine phenomenology study (1162), confidence in the detection of cocaine particulates by IONSCAN and SENTOR systems (1163), particle generators for testing of particle detection equipment (1164), particle size distribution of cocaine HCl (1165), particle size analysis of six illicit heroin preparations seized in the UK (1166), use of methylene blue as a simulant for cocaine HCl and heroin HCl (1167), test material for narcotics detection equipment (1168), and voltametric determination of cocaine microparticles (1169); **piezoelectric ringing:** (1170); **portable GC/MS:** for clandestine laboratory investigations (1171,1172); **Raman spectroscopy:** (1173,1174,1175), minor review of applications (1176), near-IR Raman to identify illegal drugs in

solid mixtures (1177), and Raman microscopy for direct 2-D imaging of explosives and drugs (1178); **human screening:** of internal body packers by magnetic resonance (1179), of internal body packers by X-ray scanning (1180), of packages on persons by X-ray imaging (1181,1182), of prisoners in the Canadian Correctional Service by IMS and ion trap mobility spectroscopy (1183), and a survey of current portal technology for screening people for illicit substances (1184); **SENTOR:** recent developments (1185); **solid-state gas sensors:** (1186); **surface ionization detection:** (1187); **surface acoustic wave (SAW) detectors:** detection of taggants and volatiles by SAW/GC (1188), and portable detection system for illicit materials based on SAW resonators (1189); **surface sampling:** detection of drugs on vehicle surfaces (general study) (1190), and study of surface sampling procedures for improved sampling/detection protocols (1191); **tandem mass spectrometry (CONDOR):** (1192,1193); **testbeds:** chemical vapor test-beds (1194), and a nonintrusive inspection technology testbed (1195); **TOF-MALDI mass spectrometry:** (1196); **vapor detection:** analysis of volatiles from cocaine (1197,1198,1199), analysis of vapors from cocaine and heroin with the aid of SPME (1200), cocaine and heroin vapor pressures (1201), detection of cocaine in cargo containers by high volume vapor sampling (field test) (1202), and formation of methyl benzoate from cocaine HCl under different temperatures and relative humidities (1203); and **X-ray technologies** (1204,1205).

Surveys

Critical to total threat assessments and the monitoring the effectiveness of counter-narcotics efforts are surveys of drug use and related topics. Over the past 10 years, surveys have been reported for: **amphetamine:** of global amphetamine abuse (1206,1207), and of amphetamine type drugs used in Bulgaria (1208); **cocaine:** of adulterants in cocaine in Rome in 1996 and 1997 by GC and GC/MS (1209), of cocaine seized in Spain 1985-1993 (1210), of intralaboratory precision of cocaine analysis by CGC (1211), and of occluded solvents in cocaine 1986 - 1991 (1212); **designer drugs:** (review) (1213), of amphetamine-type designer drugs in Europe (1990-1996) (1214), of designer drugs in Canada (1215), of designer drugs in the European Union (1216,1217), and of designer drugs in Italy (1218); **drug use (general):** global trends - 2000 (1219), global trends - 1999 (1220), of drug abuse in Hungary (1221), of Irish drug seizures (1222), of drug usage in San Diego County 1990-1997 (1223), of drug contents of powders and other illicit preparations in the UK (1224), of drugs imported into the UK (1225), and of drug abuse in Western Denmark during the eighties (1226); **flunitrazepam:** of Rohypnol Tablets (1227); **heroin:** of heroin in Australia (in Sydney in 1997) (1228), of heroin in Denmark, 1981-1992 (1229), of heroin seized in France (1230), of heroin in Israel during 1992 (1231), of noscapine in heroin in Slovenia, 1997 - 1999 (1232), of heroin seized in Spain (1233), of heroin in Andaluza, Spain (1234), of heroin in the UK, 1984 to 1989 (1235), of retail level heroin purchases in the US during 1992 (1236), of the cutting of heroin in the US in the 1990's (1237), and of cutting of heroin in New York City (1238); **LSD:** of LSD blotter papers logos (1239); **marijuana:** of the THC content of cannabis cultivated in Austria (1240), of recent developments in Europe concerning licit cultivation of cannabis (1241), of the cannabinoid content of marijuana seized in Greece (1242), of *delta*-(9)-THC content in cannabis of Greek origin (1243), the potency of cannabis in New Zealand, 1976 to 1996 (1244), of cannibis resin and cannibis seized in the Republic of Ireland (1245), of trends in illicit cannabis cultivation in the UK and Northern Ireland (1246), of potency trends of Δ9-THC and other cannabinoids in confiscated marijuana from 1980-1997 in the US (1247), of the global situation of cannabis consumption, trafficking, and production (1248), and of recent developments in cultivation and quality of illicit cannabis (worldwide) (1249); **methylenedioxyamphetamines:** of MDMA, MDA, MDEA, NEXUS, and MBDB tablets seen in southwestern Spain (1250,1251), and of MDMA, MDEA, and MBDB tablets seen in the United States (1252,1253,1254,1255); **polydrug:** of heroin, cocaine, and cannabis from British Columbia (1256), and of heroin and cocaine seized in a Swiss town (1257); **UNDCP Reports** (by year)**:** World Drug Report - 2000 (1258), List of Narcotic Drugs under International Control (INCB "Yellow List") - 1999 (1259), (INCB "Green List") - 1999 (1260), (INCB "Red List") - 1999 (1261), Report of the International Narcotics Control Board - 1999 (1262), Manufacture of Narcotic Drugs, Psychotropic Substances, and their Precursors - 1999 (1263), Narcotic Drugs Estimated World Requirements - 1999 (1264), Precursors and Chemicals Frequently Used in the Illicit Manufacture of Narcotic Drugs and Psychotropic Substances - 1999 (1265), Psychotropic Substances - Statistics - 1999 (1266), Terminology and Information on Drugs - 1999 (1267), the World Drug Report - 1997 (1268), and Supply of and Trafficking in Narcotic Drugs and Psychotropic Substances - 1996 (1269);

miscellaneous: of adolescents' use of embalming fluid with marijuana and tobacco (1270), and of crime laboratory proficiency testing results 1978-1991 (1271).

Miscellaneous Topics

The following topics of peripheral interest to the analysis and detection of drugs of forensic interest were also reported from 1992 - 2001: **angel trumpet:** overview (1272); **amphetamine:** a review of U.S. statutes on methamphetamine and how they led to an increase in illicit amphetamine production (1273); **ayahuasca:** notes on an Ayahuasca court case in Holland (1274,1275); **barbiturates:** charge transfer complexes with phenytoin (1276); **canines:** use of activated charcoal to circumvent canine detection of concealed narcotics (1277), analysis of volatile drug components and their relevance to canine alerts (1278), characterization of the Auburn Olfactometer (1279), of cocaine on currency (1280), drug money and detection by canines (1281,1282), scientific protocol to evaluate and certify odor detection by canines (1283), and sensitivity of canines to cocaine HCl and methylbenzoate (1284); **(traditional) Chinese medications:** overview (1285), manufacturing flaws and misuse of Chinese herbal medicines (1286), determination of some active components in Chinese medicial preparations by CE (1287), screening of Chinese proprietary medications for undeclared therapeutic substances by HPLC and GC/MS (1288), identification of Western medicines as adulterants in Chinese herbal medicines by HPLC and GC/MS (includes diazepam) (1289), screening for chemical drugs used to adulterate in rheumatic and analgesic traditional Chinese medicine by HPLC-DAD (includes diazepam and phenylbutazone) (1290), determination of adulterated chemical drugs in rheumatic and analgesic traditional Chinese medicine by MEKC (includes diazepam and phenylbutazone) (1291), determination of clobenzorex HCl and diazepam adulterated in anorexiant traditional Chinese medicines by MECC (1292), and determination of fluoxymesterone, methyltestosterone and testosterone in adulterated Chinese herbal preparations by HPLC (1293); **cocaine:** alkaloid content in *Erythroxylum Coca* tissue during reproductive development (1294), the base-catalyzed C-2 exchange and epimerization of 3-*beta* substituted 8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylates (1295), biomass accumulation and alkaloid content in leaves of *Erythroxylum Coca* and *Erythroxylum Novogranatense* Var *Novogranatense* grown in soil with varying pH (1296), effects of cyanoacrylate processing (for fingerprinting) on cocaine HCl trace analysis (1297), gas phase detection of cocaine by means of immunoanalysis (1298), protest against cocaine base sentencing (1299), SFC extraction of cocaine from coca leaf (1300), solubility of cocaine in gasoline (1301), and stability of cocaine in *Agua Rica/Agua Madre* (1302); **(analysis for) controlled substances on currency:** comprehensive review (covers cocaine, heroin, THC, and phethylamines) (1303), cocaine on currency (1304,1305), cocaine on currency by GC-MS (1306), cocaine on currency by IONSCAN IMS and LC/MS with electrospray ionization or by GC-ITD/MS (1307), analysis of drugs on currency by GC/MS (1308), by MS/MS, TD-MS, and APCI-MS (1309), by tandem MS (CONDOR) (1310,1311), and screening of currency by TD/APCI-MS (1312); **designer drugs (unusual):** dihydrobenzofuran analogues of hallucinogens (1313), lactam analogs of fentanyl (1314), methylenedioxyisoquinolines (1315), synthesis and pharmacological evaluation of ring-methylated 3,4-methylenedioxyamphetamines (1316), reference directory of designer drugs (1317), 1,2,3,4-tetrahydro-isoquinoline analogs of phenylalkylamine stimulants and hallucinogens (1318,1319), and the texts by Ann and Alexander Shulgin (1320,1321); **dextromethorphan:** (overview) (1322); **dimethylamphetamine**: mechanistic study of preparation from methylephedrine (1323); **ephedrine:** extraction of ephedrine from ephedra by SFC (1324); **heroin:** homogenization of illicit heroin samples prior to analysis (1325); **inhalants:** general discussions of inhalants and solvent abuse (1326,1327,1328); **Internet:** discussion of internet resources for forensic science (1329,1330); **lysergic acid diethylamide:** detection of LSD on blotter papers after processing for fingerprints (1331), and stability of LSD under various storage conditions (1332); **marijuana:** botanical considerations for forensic investigation of marijuana (1333), embalming fluid-soaked marijuana (**compare to Elwood/Fry article)** (1334), filtering effects of various household fabrics on the pollen content of hash oil (1335), identification and quantitation of 11-nor-delta(9)-tetrahydrocannabivarin-9-carboxylic acid (1336), manufacture of *Cannabis Sativa* for legitimate applications (1337), mineral nutrition of *Cannabis Sativa L.* (1338), and comments on the naming of the Duquenois and related tests for cannabis (1339); **mass spectrometry:** archive of mass spectral data files on CD-ROM and a computerized database (1340), ion ratio instability of a GC/MS system (1341), and poor reproducibility of in-source collisional AP MS of drugs (1342); **methadone:** claim that DEA chemists erred in calculating quantity of methadone that could be synthesized from precursor chemicals (1343; and response: 1344); **nightshade alkaloids:** historical review (1345); **opium:** historical review (1346), biodiversity of *Papaver*

Somniferum L. (1347), and determination of loss on drying of opium samples using microwave ovens (1348); **oxycodone:** overview (1349); **phencyclidine:** ionic associates of phencyclidine with sulfophthaleins and azo dyes (1350); **polydrug:** hypnotics and sedatives not belonging to the classes of barbiturates and benzodiazepines (1351); **poppy tea:** case study/overview (1352); **thebaine:** synthesis from codeine methyl ether (1353); **other topics:** analysis of clandestine drug records (1354), analysis of drugs in unconventional samples (1355), analysis of false positives in drug proficiency testing (1356), analysis of a fruit juice extract that was suspected to be a narcotic beverage by GC/MS (1357), analysis of plastic packaging to trace the source of illicit drugs (1358), computerized management of a forensic analytical laboratory (1359,1360), considerations for planning and site preparation for modern laboratory instrumentation (1361,1362), development of a forensic evidence protection kit (1363), drug smuggling techniques and problems associated with analysis (1364), drug smuggling by internal body carries (1365), environmental impact of illicit narcotics cultivation and processing (1366,1367), expert evidence and forensic misconceptions of the nature of exact science (1368), GC/MS guide to ignitable liquids (1369), modification of an extraction procedure for acidic and neutral drugs (1370), neural networks in forensic science (overview/general discussion) (1371), protecting group chemistry (1372), separation and identification of drugs of abuse in drug cottons by HPLC coupled with electrochemical array detectors (1373), solid phase extraction for systematic toxicological analysis (1374), the UNDCP Dictionary of Narcotics (1375, and addendum: 1376); and an overview of the United Nations International Narcotics Control Board (1377).

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