

Toxicology News

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Oral Fluid Testing An Alternative Approach to Monitoring Pain Management

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With the aging population and recognition that treatment of a patient's pain is a key healthcare objective, more patients are taking pain medications that must be taken consistently to be effective. But at the same time, many of these same medications are subject to abuse. These benefits and dangers drive the need to test for patient compliance.

Although urine has historically been the specimen of choice for this testing, oral fluid is an increasingly attractive alternative. A growing body of literature documents its scientific basis, and advances in liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis are now enabling routine use of oral fluid.

Researchers prefer the term oral fluid over saliva because oral specimens include a mixture of saliva and other constituents in the mouth. In addition to the three major salivary glands, other minor glands in the oral mucosa also produce fluids (1). Salivary glands are highly perfused with nutrients from blood, and drugs primarily enter saliva by passive diffusion through cell membranes; thus, drugs in oral fluid reflect free drug circulating in blood.

For diffusion to occur, the drugs must be non-ionized and have some degree of both lipid and water solubility. Conditions that affect a drug's excretion into oral fluid include the extent of its plasma-protein binding (bound drug cannot readily diffuse into oral fluid), its dissociation constant (pKa), and the pH of the oral fluid and blood (1,2). Oral fluid is typically more acidic than blood; consequently, weakly basic drugs (such as amphetamines, opioids, and cocaine) may be detected at higher concentrations in oral fluid than blood due to an ion-trapping effect (2).

Drug Detection and Prevalence in Oral Fluid

The drugs used in pain management are readily detectable in oral fluid (2), and they appear more quickly in oral fluid than in urine, where drugs or their metabolites may not appear until 2 to 8 hours after ingestion. In fact, many drugs can be detected in oral fluid within minutes after a user smokes or injects them (2,3). The detection window also is typically shorter in oral fluid than urine. Time courses for detection depend on the drug's pharmacokinetics, whether the use is acute or chronic, and the testing threshold used. Most drugs are detectable for up to 24–48 hours, although longer detection times occur in chronic users (2).

Oral fluid generally contains more parent drug than metabolites, which is the reverse of urine (3). Studies of acute dosing suggest that in many cases, oral fluid may contain no metabolites. For example, a study of codeine dosing detected codeine but no morphine (2). But because dosing tends to be chronic in pain management, metabolites are more commonly detected in these patients. A study of oral fluid specimens from pain patients reported many combinations of drugs, including parent drugs and metabolites (4).

Positive Rate Comparisons

Comparisons of test results from oral fluid and urine specimens have found similar positive rates, including a 2002 study of non-regulated workplace drug testing that compared the rates from 77,000 oral fluid specimens with the Quest Diagnostics' Drug Testing Index for urine. The positive rate in

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Oral Fluid Testing

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oral fluid was notably higher for some drugs, such as amphetamines and cocaine (5).

In a more recent study, researchers compared test results from 6,441 oral fluid specimens with published data from 13,948 urine results, both from chronic pain patients. They found similar positive rates for a large menu of licit and illicit drugs tested in the chronic pain population. The overall positive rate was 82.7% in oral fluid versus 78.3% in urine (4,6). Table 1 contains the comparisons of positive rates for individual drug classes. Differences in the populations and the years of specimen collection (2006 for urine and 2010 for oral fluid) could account for some of the differences in the positive rates. As in the 2002 workplace study, the positive rates for amphetamines and cocaine were higher in oral fluid than in urine. Even drugs that are difficult to detect in oral fluid, such as benzodiazepines and marijuana, exhibited positive rates similar to those in urine. The authors concluded that their results support the use of oral fluid testing in pain management.

A comparison of paired oral fluid and urine specimens taken from chronic pain patients at the same time found overall agreement of 85%, although the different time courses for drugs in the two matrices could account for some of the difference (7).

Collection Procedures and Analytical Concerns

As in all testing, accurate results depend on proper sample collection. An insufficient volume of oral fluid can lead to a specimen comprised primarily of buffer solution and a false-negative result. The use of a volume adequacy indicator during collection can help (8).

Attention to adequate volume is particularly important in patients with xerostomia (dry mouth), which is a side effect of several pain medications. Drugs that cause dry mouth include nicotine, cannabis, opioids, amphetamines, antipsychotics, antidepressants (particularly tricyclic antidepressants), and antihistamines. It is important to note that opioids and tricyclic antidepressants are commonly prescribed individually and together for chronic pain patients (9). Collections in these patients can take several minutes or fail entirely.

Some practitioners attempt to overcome xerostomia by stimulating saliva production, but this attempt can backfire. Increasing salivary flow with sour candies or citric acid can lower some drug concentrations below the testing threshold. In addition, stimulation can increase bicarbonate levels, thereby

increasing salivary pH. A neutral to basic pH decreases ionization of drugs, which reduces the ion trapping effect and concentrations of some drugs (1). For these reasons, salivary stimulation can lead to false-negative results.

Patients should refrain from ingesting food or chewing gum for 10–15 minutes prior to specimen collection. Noncompliant patients who do not want to be caught sometimes chew a drug tablet immediately prior to presenting for a specimen collection, which can lead to very high drug concentrations. Some authors recommend an oral rinsing step prior to collection to reduce the effects of purposeful oral contamination (9).

Testing thresholds need to be lower than those used for urine because drug concentrations are lower, but many laboratories already have the technology for testing oral fluid. LC-MS/MS technology can achieve the desired level of sensitivity and is preferred over gas chromatography-mass spectrometry for testing small specimen volumes (8,9). Enzyme-linked immunosorbent assays (ELISA) can be used for screening at the thresholds used for oral fluid, but point-of-care immunoassays cannot yet achieve the needed sensitivity (1,8).

Interpretation

Because urine drug concentrations do not correlate to blood concentrations or to dose, urine test results cannot be used to check on compliance with dosage. Oral fluid might offer the advantage of dosage estimates in the future, pending further studies of the relationship between oral fluid and blood concentrations over time (1).

Taking a drug by smoking, snorting, or sublingual ingestion can greatly elevate oral fluid concentrations. In these cases, oral concentrations may start out much higher than blood concentrations, decline rapidly, then fall at a rate mirroring drug clearance from blood. Oral concentrations can be particularly high if a drug is deposited in the oral cavity and steadily released, such as when a sublingual buprenorphine tablet leads to collection of the drug in surrounding tissue. This pattern has also been shown to be true for cocaine and is thought to be true of THC after cannabis use (2).

The correlation of positive rates between urine and oral fluid indicates that oral fluid is a viable alternative, but the shorter detection period is still a consideration. Shorter detection periods may not matter in chronic pain patients who take medications every day. However, patients who take medications on an infrequent, as-needed basis or who have run out of their medication early might test negative for a short-acting drug they have not ingested for a day or

Table 1. Comparison of Positive Rates for Oral Fluid and Urine Specimens Tested in Pain Management

Drug Class	Urine (N=13,948)		Oral Fluid (N=6,441)	
	Specific Drugs Tested for, (Limit of Quantitation, ng/mL)	Positive Rate	Specific Drugs Tested for, (Limit of Quantitation, ng/mL)	Positive Rate
Amphetamines	Amphetamine (100); methamphetamine (100); MDMA (100); MDEA (100); MDA (100); p-methoxyamphetamine (100); phentermine (100)	1.1%	Amphetamine (5); methamphetamine (8); MDMA (8); MDA (8)	3.2%
Barbiturates	Amobarbital (100); butobarbital (100); butalbital (100); pentobarbital (100); secobarbital (100)	2.2%	Butalbital (25)	2.0%
Benzodiazepines	α -Hydroxyalprazolam (50); clonazepam (50); lorazepam (50); nordiazepam (50); oxazepam (50); temazepam (50)	17.2%	Alprazolam (0.5); clonazepam (1); diazepam (1); flurazepam (1); lorazepam (1); nordiazepam (2); oxazepam (0.5); temazepam (0.5)	15.5%
Carisoprodol	Carisoprodol (200); meprobamate (200)	4.4 %	Carisoprodol (10); meprobamate (10)	9.7%
Cocaine	Benzoyllecgonine (40)	2.2%	Cocaine (2); benzoyllecgonine (2)	5.6%
Fentanyl	Fentanyl (1)	3.3%	Fentanyl (0.1); norfentanyl (0.5)	6.8%
Marijuana	THC-COOH (2)	6.9%	THC (2); THC-COOH (2)	6.1%
Methadone	Methadone (100); EDDP (100)	8.7%	Methadone (2); EDDP (1)	9.7%
Opiates	Codeine (100); dihydrocodeine (100); hydrocodone (100); hydromorphone (100); morphine (100); oxycodone (100); oxymorphone (100)	64.5%	Codeine (1); dihydrocodeine (1); hydrocodone (1); hydromorphone (1); morphine (1); norcodeine (1); norhydrocodone (1); noroxycodone (1); oxycodone (1); oxymorphone (1)	64.6%
Propoxyphene	Propoxyphene (100); norpropoxyphene (100)	2.8%	Propoxyphene (5); norpropoxyphene (1)	2.6%

Source: 4,6

two (2). Practitioners should be advised of the detection periods when oral fluid is introduced.

In some situations, one matrix is clearly better than the other. For example, some medications are difficult-to-impossible to detect in oral fluid. Opioids administered intrathecally are present in sub-nanogram concentrations in blood, so their oral fluid concentrations probably fall below typical thresholds. Likewise, buprenorphine administration through the Butrans transdermal patch typically results in plasma concentrations in the picogram range, making oral fluid detection unlikely. Practitioners should use urine tests in these patients.

In other cases, oral fluid offers an advantage. Dronabinol (Marinol), or synthetic THC, is used as an antiemetic for chemotherapy regimens and as an appetite stimulant for patients with acquired immunodeficiency syndrome (AIDS). Some patients use their dronabinol prescription to conceal the ingestion of illicit marijuana. Patients who ingest dronabinol test positive for the metabolite THC-COOH in their

urine, but not for THC or THC-COOH in their oral fluid at the typical thresholds used. So oral fluid testing can differentiate the use of dronabinol from marijuana (2).

Passive exposure to marijuana is unlikely to cause a positive result in oral fluid. Some passive inhalation studies have found positive results for THC in oral fluid, but only for short time courses. Most subjects were negative 12 to 22 hours after exposure and some did not test positive at all (10,11).

Morphine positives from food with poppy seeds should also be rare. Subjects who ingested poppy seed bagels tested negative for morphine at a 3 ng/mL threshold. Subjects who ingested a very large number of poppy seeds (unlikely in a non-research setting) tested positive for morphine up to 205 ng/mL, but fell below a 40 ng/mL threshold 1 hour later, and below a 5 ng/mL threshold 8 hours later. In contrast, their urine specimens were positive for morphine above a typical clinical threshold (100 ng/mL) for up to 48 hours following ingestion (12).

Adulteration Issues

One of the primary advantages of oral fluid testing is the opportunity for easily observed collection. Patients using illicit or non-prescribed drugs may attempt to adulterate or substitute urine specimens, and clinicians may not be trained in proper specimen collection techniques, such as having test subjects empty their pockets and adding bluing agent to the toilet. Patients sometimes attempt to dilute their urine by ingesting enough water to drive drug concentrations below detection thresholds. Some patients adulterate their specimens with home products such as bleach, concentrated lemon juice, vinegar, table salt, or eye drops, or commercial oxidizing products such as Urine Luck, Stealth, Klear, or Instant Clean (9).

Patients who are selling their medications instead of taking them may add crushed tablets to their urine specimens in an effort to appear compliant. Although some patients may excrete parent drug instead of metabolites for a variety of reasons, a high concentration of parent drug in a urine specimen is a warning sign of adulteration. In cases of suspected urine specimen adulteration or substitution, oral fluid can provide an additional check.

Although some oral fluid adulterants are available, most function as a simple mouth wash and fail to destroy drugs or significantly alter pH. Sterilizing tablets containing sodium dichloroisocyanurate do not destroy drugs in oral fluid. The higher oral fluid positive rates of illicit drugs such as heroin and cocaine fluid may be related to the resistance of this matrix to tampering (9).

Oral fluid also provides an avenue for testing in small clinics that do not have the staff or facilities for urine collections. Patients with shy bladder or end-stage renal disease who cannot provide a urine specimen can submit an oral fluid sample. Pain practitioners should be advised that if patients are unable to provide a sufficient oral fluid specimen due to xerostomia, an alternative matrix should be tested.

Although there are differences in testing methodology and interpretation between oral fluid and urine, both are viable testing approaches in the clinical setting. Many practitioners, particularly in primary care, who prescribe controlled substances have not embraced urine drug testing, but they may be attracted by the ease of oral fluid collection to increase the use of compliance testing.

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Immunosuppressive Drug Monitoring An Update

By Anthony W. Butch

Clinicians must monitor immunosuppressive drug levels in organ transplant patients to optimize their effectiveness and minimize adverse events. As organ transplantation has become more successful and widespread, laboratories that support transplant programs perform therapeutic drug monitoring (TDM) of many immunosuppressive drugs, including cyclosporine A, tacrolimus, sirolimus, mycophenolic acid, and everolimus.

TDM of these drugs is important for a number of reasons. Most importantly, research has shown that blood concentrations of immunosuppressive drugs and clinical responses are closely related. Furthermore, the drugs have narrow therapeutic indices and wide inter- and intra-patient variability, and pharmacological responses can be difficult to differentiate from unwanted side effects. TDM also allows clinicians to assess patient compliance, which is essential for survival of the graft and patient. Finally, patients frequently take more than one immunosuppressive drug, leading to unpredictable drug levels.

Which Technique?

Although liquid chromatographic (LC) separation coupled with mass spectrometric (MS) detection offers unparalleled sensitivity, specificity, versatility, and lack of interference from drug metabolites, most U.S. laboratories use immunoassays to measure immunosuppressive drugs. In fact, 83% of the laboratories in the 2012 College of American Pathologists Immunosuppressive Drug Monitoring Survey measured the most widely used immunosuppressive drug, tacrolimus, by immunoassay. This statistic is somewhat surprising given the well-documented cross-reactivity the assays have for metabolites of immunosuppressants. This cross-reactivity varies dramatically depending on the organ transplanted, as well as the time post-transplant.

Nevertheless, immunoassays for immunosuppressive drugs dominate the market because they are automated and easy to perform without specialized training. They also have low start-up costs and rapid turnaround times. In contrast, LC-MS/MS systems are expensive and require staff with experience in chromatographic separation techniques and MS. These requirements limit the use of LC-MS/MS for TDM of immunosuppressive drugs to reference laboratories and hospital laboratories that support large solid-organ transplant programs.

Immunoassays

Erythrocytes contain the majority of cyclosporine A, tacrolimus, sirolimus, and everolimus, making EDTA-anticoagulated whole blood the specimen of choice for extracting the drugs for quantitation. Most commercially available immunoassays use whole blood specimens, although only one fully automated immunoassay allows whole-blood samples to be placed directly onto the instrument for testing, the ACMIA, by Siemens. Six semi-automated immunoassays require an off-line manual pretreatment step prior to analysis, and one manufacturer now offers an assay cleared by the Food and Drug Administration for everolimus, the QMS particle-enhanced turbidometric assay (Table 1).

Unlike the other immunosuppressive drugs, mycophenolic acid is not present in erythrocytes; therefore a serum or plasma sample is required. Consensus guidelines recommend monitoring total mycophenolic acid but not its metabolites or free mycophenolic acid. Only 47 U.S. laboratories currently measure this drug, one-tenth the number that measure tacrolimus. Eleven of these laboratories use either the EMIT or the Roche enzymatic immunoassay, while 36 use LC with ultraviolet detection (LC-UV), LC-MS, or LC-MS/MS.

LC-MS and LC-MS/MS

Before MS detection systems became widely available, LC-UV was the gold standard method for cyclosporine A, and laboratorians used it to evaluate new immunoassays. However, LC-UV analysis requires extensive sample clean-up to minimize interfering substances, and LC-MS has replaced it. LC separation followed by MS/MS detection not only greatly improves sensitivity and specificity, it also decreases run times and has turnaround times comparable to those of immunoassay.

However, laboratories need to take care in the sample clean-up steps. Blood is rich in proteins that adsorb to chromatographic columns, which reduces efficiency and increases column back-pressure, ultimately resulting in poor chromatographic separation. Matrix components not removed during sample clean-up also can induce inaccurate measurements by altering the ionization efficiency of the target compound and/or internal standard. The sample clean-up procedures require time and labor, slowing down sample throughput and increasing testing time.

Laboratories can perform the clean-up procedures manually off-line or fully automated as a component of the LC-MS/MS system. Off-line liquid-liquid extraction procedures yield the cleanest extracts but are labor-intensive and more time-consuming than other methods. The advantages of the

Table 1. Immunoassays for Measuring Immunosuppressant Drugs in Whole Blood

Immunoassay	Drug(s) Measured	Manufacturer
ACMIA	Cyclosporine A, tacrolimus, sirolimus	Siemens Healthcare
CMIA	Cyclosporine A, tacrolimus, sirolimus	Abbott Laboratories
CEDIA Plus	Cyclosporine A, tacrolimus, sirolimus	Thermo Fisher
Syva EMIT 2000	Cyclosporine A, tacrolimus	Siemens Healthcare
ADVIA Centaur	Cyclosporine A	Siemens Healthcare
FPIA	Cyclosporine A	Abbott Laboratories
QMS	Everolimus	Thermo Fisher

Abbreviations: ACMIA=antibody-conjugated magnetic immunoassay; CMIA=chemiluminescent microparticle immunoassay; CEDIA=cloned enzyme donor immunoassay; EMIT=enzyme-multiplied immunoassay technique; FPIA=fluorescence polarization immunoassay; QMS=quantitative microsphere system.

off-line solid-phase extraction procedure are that it produces a concentrated extract and is easy to automate.

For large sample volumes, LC-MS/MS systems with automated, on-line solid-phase extraction are the most practical. On-line sample extraction systems have an additional column for sample clean-up that alternates between on-line and off-line by automated switches. If the same column is used for numerous samples, column clogging and sample carryover can occur. Single-use cartridges can eliminate these problems.

On-line solid-phase sample extraction columns require introducing an internal standard into the samples before the clean-up step to adjust for variable drug recovery. Researchers have shown that the methods for automated injection of this internal standard are as accurate and precise as off-line sample extraction procedures. Despite their potential for reducing labor costs and turnaround times, LC-MS and LC-MS/MS systems with on-line sample preparation are not widely used for monitoring immunosuppressive drugs, possibly because of their high equipment costs.

Internal Standards

Choosing an appropriate internal standard is important because it corrects for analyte recovery and matrix effects. Ideally, the internal standard should have the same physicochemical properties as the drug being measured. This means that laboratories should use stable-isotope-labeled immunosuppressive drugs whenever possible. The internal standard should co-elute with the analyte of interest and there should be no isotopic overlap between the target analyte and

the standard. The isotopic purity of the internal standard also should be high because contamination with unlabeled analyte can produce measurement bias. The internal standard should be used at concentrations that are above the limit of quantification but that do not introduce ion suppression. Examples of internal standards used for measuring cyclosporine A by LC-MS and LC-MS/MS include ascomycin, cyclosporine D, and deuterium-labeled cyclosporine A. Ascomycin is popular because it can be used for simultaneously monitoring cyclosporine

A and other immunosuppressive drugs in the same sample. However, it produces the highest imprecision compared with the other internal standards. Metabolites in blood can interfere with cyclosporine D, increasing the peak area of certain transitions, which can cause negative bias. These drawbacks illustrate the need for laboratories to carefully evaluate which standard to use.

Unlike immunoassay kits that contain all the necessary reagents, including calibrators and controls, LC-MS and LC-MS/MS methods typically require a laboratory to prepare its own calibrators, controls, and internal standards. While these methods are accurate, preparing in-house reagents can contribute to assay variability and bias. A few commercial suppliers now provide kits containing freeze-dried calibrators and internal standards that help standardize TDM of immunosuppressants by LC-MS and LC-MS/MS.

To look at the impact of assay variability and bias, we recently sent nine paired aliquots of whole-blood samples to two reference laboratories that use validated LC-MS/MS methods for measuring everolimus. Figure 1 shows that the results differed significantly, indicating that either one or both of these laboratories suffers from assay bias. Such results can potentially impact everolimus dosing decisions and highlight the need for standardized methods and reagents.

TDM Tomorrow

Despite significant cross-reactivity with drug metabolites, most laboratories today use immunoassays to measure immunosuppressive drug concentrations in whole blood. While immunoassays are a popular and familiar format for laboratories,

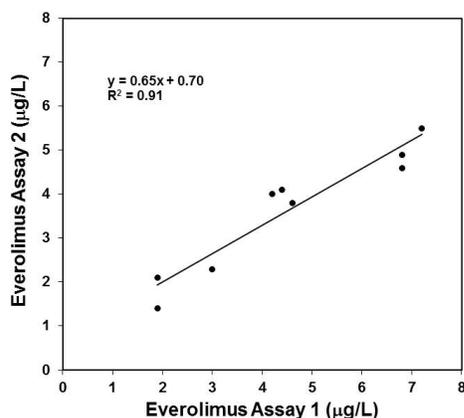


Figure 1. Reference Laboratories Report Different Results from Paired Aliquots

LC-MS/MS offers many significant advantages. It not only produces excellent sensitivity and specificity, but the method also lacks cross-reactivity problems and has the ability to measure multiple drugs simultaneously.

Unfortunately, the high cost of the instruments and the need for specialized training have prevented the widespread use of LC-MS/MS for TDM of immunosuppressive drugs. But as the cost of immunoassay reagents increases, LC-MS/MS systems will become a good investment for laboratories supporting large solid-organ transplant programs and elevate the need for improved standardization and harmonization of LC-MS/MS results across laboratories.

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Case History

Desoxypipradrol Intoxication

By Vijay Bhardwaj, MD, and John M. Wilson, PhD

The new “bath salts” and cannabinoid derivatives that have come on the market in recent years illustrate the need for laboratories to constantly update their procedures and databases. Our laboratory experienced this need when a 17-year-old Caucasian boy presented disoriented and hallucinating at our emergency department.

A rising high school senior who lived with his family, the boy was home alone in the early morning hours when he snorted Scarface bath salts he had purchased from a gas station. Six hours later he became agitated and delusional, imagining faces and hearing voices. Snorting bath salts was not out of character for the boy: he started smoking marijuana at age 13, had been in juvenile detention at least six times, and was on adult probation for a charge of possession of marijuana.

The boy’s family brought him to the emergency center of a local hospital at 5:30 p.m. He was: agitated; sweaty; hallucinating; exhibiting pressured, nonsensical, and slurred speech; and could not recognize his mother. Belligerent and combative, he tried to stand on his stretcher. His vital signs were: weight, 52 kg; heart rate, 98; respiration rate, 19; blood pressure, 146/94; and oxygen saturation, 99% in room air. His pupils were 5 mm and equal.

Initial lab studies showed an unremarkable CBC; Na, 141 mmol/L; K, 4.1 mmol/L; Cl, 103 mmol/L; CO₂, 20 mmol/L; BUN, 12 mg/dL; creatinine, 0.8 mg/dL; and glucose, 102 mg/dL. ALT was 29 U/L; albumin, 5.0 U/L; and creatine kinase (CK), 108 U/L. A urine drug panel was negative. A chest X-ray was normal.

Initial Treatment

The emergency center staff placed the boy in leather restraints and administered 4 L of normal saline. To control his agitation, they gave him 43 mg of lorazepam intravenously along with 10 mg of Zyrpexa (olanzapine) between 6 and 8:40 p.m. They also put him on an IV of the short-acting anesthetic propofol for transport to our facility.

When he was admitted at 10:00 p.m., our pediatric intensive care unit team stopped the propofol and did not give the boy benzodiazepines overnight. The next morning, the patient was less agitated but still hallucinating, with episodes of yelling, hitting, and talking to nonexistent companions. He showed no hypertonia or excessive sweatiness, and his pupils measured 4 mm. His serum CK was 584 U/L at admission

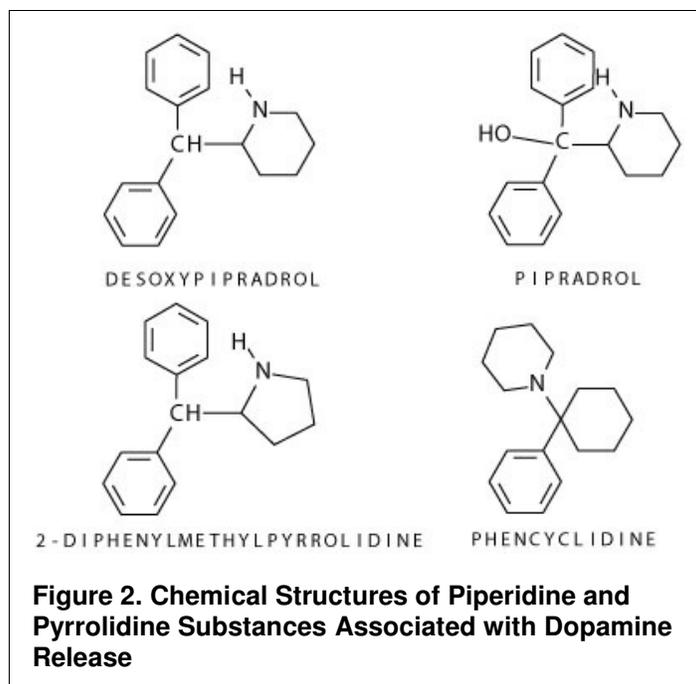
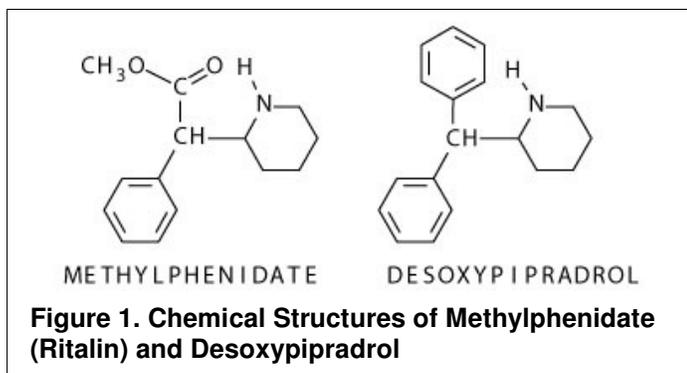
and 2456 U/L the next morning, peaking at 4004 U/L about 24 hours after admission. A comprehensive drug screen detected an unidentified substance in his urine that gas chromatography/mass spectrometry analysis subsequently identified as desoxyipipradrol.

After 48 hours, he still needed leather restraints, had bursts of excitement, and was too agitated for our psychiatrist to evaluate him. PICU gave him four doses of lorazepam overnight. His serum CK began to decline with IV hydration, and at about 60 hours post-admission, he calmed down enough for psychiatric evaluation. The next day, some 90 hours after admission, the psychiatrist advised that we transfer him to an inpatient facility with a substance-abuse program.

A New Stimulant

This case history illustrates use of a relatively new stimulant, desoxyipipradrol, that started showing up in patients brought to emergency departments within the past five years (1). One of a wave of designer hallucinogens, the drug is sold in convenience stores and other outlets in packets of white powder labeled “bath salts.” Although marketed as “legal highs,” the powders contain substituted phenethylamines, tryptamines, piperazines, and cathinone derivatives. Legislators and public health officials are now making a concerted effort to define these stimulants as substances of abuse and make them illegal. Some of them are so new that we have little information on their pharmacology, pharmacokinetics, and potential risks to users. Furthermore, we do not know how to accurately identify them analytically. Much of the initial information has come from experiences in the U.K. and Europe, although, as this case study shows, problems are occurring in the U.S., too.

Ciba developed desoxyipipradrol as part of the same research that led to the introduction of methylphenidate (Ritalin) in the 1950s for managing attention deficit hyperactivity disorder (ADHD) and narcolepsy (Figure 1). It was marketed as an anesthetic reversal agent in Germany under the brand name Weckamine (2). A number of countries licensed the



hydroxylated form, pipradrol (Figure 2), for treatment of ADHD, but the U.K. made pipradrol a controlled substance because of its abuse potential (2).

Recent studies and toxicological evaluations have confirmed that desoxyipipradrol affects the neuro-adrenergic system (2–5). Ferris and Tang demonstrated that it is a potent norepinephrine re-uptake inhibitor in several animal species (3). Davidson and Ramsey found that it is more potent than cocaine in causing neuro-cellular release of dopamine and inhibiting its re-uptake in rat nucleus accumbens tissue. In clinical settings, patients exposed to desoxyipipradrol have exhibited agitation, anxiety, aggression, insomnia, paranoia, increased blood pressure, sweating, tachycardia, chest pain, bruxism, loss of appetite, and palpitations, all of which are consistent with a neuro-stimulant similar to amphetamine and cocaine (1).

Related Substances

Laboratorians and emergency personnel should also be aware of an analogous substance, 2-diphenylmethylpyrrolidine (2-DPMPy) (1,2,5). The desoxy- product of diphenylprolinol, 2-DPMPy contains a pyrrolidine ring substituted for piperidine, and often occurs in the same toxicological context (Figure 2).

Figures 3 and 4 show representative mass spectra for desoxyipipradrol and 2-DPMPy. Additional documentation can be found at Forendex, a compilation of the Southern Association of Forensic Scientists (forendex.southernforensic.org), the Wiley Library of Psychoactive Substances (onlinelibrary).

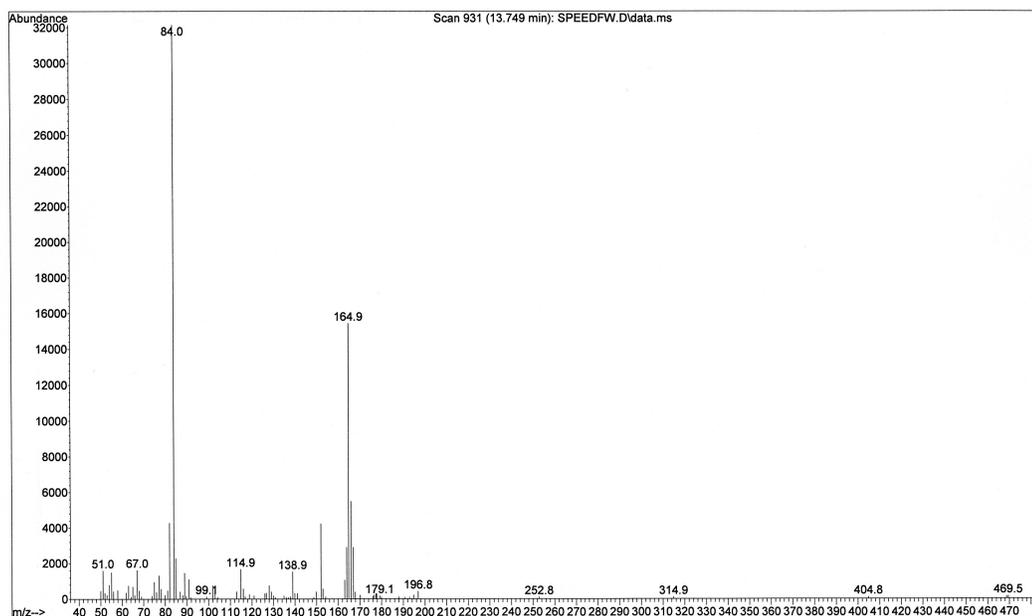


Figure 3. Mass Spectrum of Desoxypipradrol

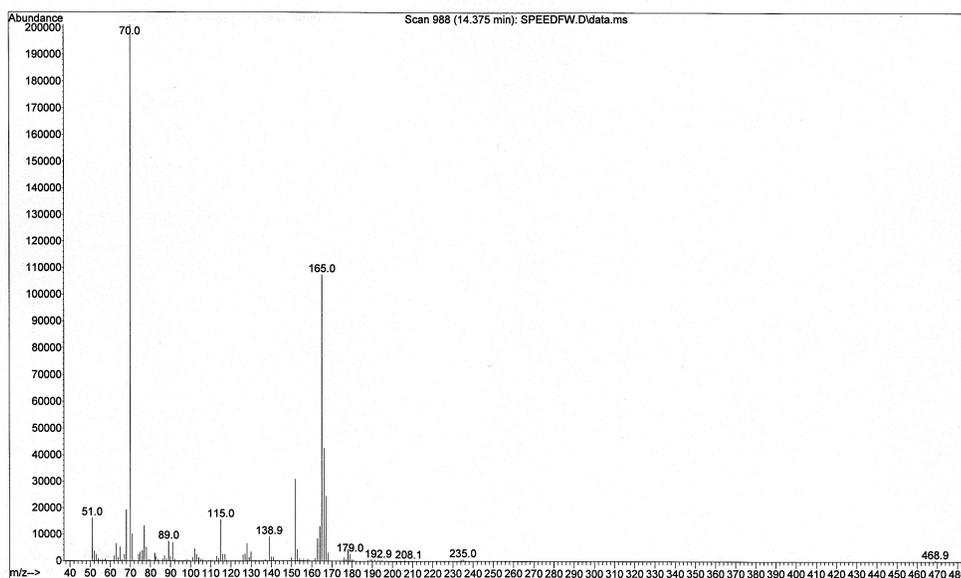


Figure 4. Mass Spectrum of 2-Diphenylmethylpyrrolidine

wiley.com), and the resource library of the Cayman Chemical Company (www.caymanchem.com).

With their acute toxicity, desoxypipradrol and its chemical analogs pose significant health risks. Before his release, our case patient said words to the effect that it was “the worst experience ever” and he would never do it again. But the easy access to bath salts and synthetic cannabinoid products via the Internet and in stores can expose unsuspecting users to potentially dangerous substances. Laboratories

must continually be on the watch to recognize patients affected by these new drugs.

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CAP Toxicology Programs News and Updates

By Glynnis Ingall, MD, PhD

New Proficiency Surveys Coming in 2013

Starting in 2013, the College of American Pathologists (CAP) will offer two new proficiency testing (PT) surveys for therapeutic drug monitoring (TDM) and toxicology—for everolimus and ethanol biomarkers.

Everolimus

The Everolimus (EV) Survey is being added because there is no PT provider in the U.S. for this immunosuppressant drug. CAP anticipates more laboratories will be offering everolimus testing because of the drug's increasing use. This survey will eliminate the need for laboratories to make arrangements for alternative proficiency testing.

The program will offer two shipments per year, with three 5.0-mL whole blood specimens per shipment.

Ethanol Biomarkers

The Ethanol Biomarkers (ETB) Survey is being offered in response to feedback from CAP proficiency survey participants who indicated that they provide or plan to add testing for ethanol biomarkers in urine. Laboratories may be adding this testing to their menus because of increasing demand from a number of sources, including alcohol rehabilitation, pain management, and maternal alcohol monitoring programs.

CAP is currently the only U.S. PT provider for these biomarkers. The targeted analytes will be ethyl glucuronide and ethyl sulfate. The program will offer two shipments per year, each consisting of three 10.0-mL synthetic urine specimens.

Toxicology Proficiency Survey Grading Changes

Starting in 2013, ethylene glycol challenges in the CAP/American Association for Clinical Chemistry (AACC) Serum Alcohol/Ethylene Glycol/Volatiles (AL2) Survey and the Whole Blood Alcohol/Ethylene Glycol/Volatiles (AL1) Survey will be graded using the criterion of $\pm 25\%$ of the all-method mean.

In the CAP/AACC Urine Drug Testing (Screening) (UDS) and Urine Drug Testing (Limited) (UDS6) surveys, fentanyl challenges will be graded beginning in 2013.

Clinical History as Guide to Toxicology Testing

Periodically, the CAP Toxicology Resource Committee adds supplemental questions to the proficiency surveys to query participants about their current laboratory practices and any new or planned toxicology or TDM test additions to their laboratory menus. The committee uses this feedback to improve the surveys to better meet the needs of the participants and to develop new products for emerging analytes of toxicological or therapeutic interest.

Recently, the following questions were included in the 2012 CAP Urine Toxicology (UT-B) and Toxicology (T-B) Survey mailings to learn about the role clinical histories play in toxicology laboratory practice:

- Do you find the clinical histories provided in this survey useful or relevant in deciding which specific drug tests to order?
- In your lab do you routinely receive clinical histories with your orders for toxicology testing?
- If so, do you use this clinical history to determine what toxicology testing is performed?

The responses indicated that most T Survey (72%) and half of the UT Survey respondents (50%) found that the clinical histories were useful in making decisions as to which toxicology tests to perform. The committee will continue to include clinical histories in these surveys because of the positive response.

Most respondents from both T (85%) and UT (73%) surveys indicated their laboratory used clinical histories, if available, to determine which toxicology tests should be performed. Unfortunately, less than half of the T Survey respondents (48%) and very few UT Survey respondents (13%) routinely receive this clinical information.

In conclusion, most laboratories would use clinical histories to optimize their testing protocols, but many laboratories do not routinely receive them with their lab orders. Without this information, many labs resort to standard testing protocols and miss any additional, non-routine tests a given patient may require. Efforts to encourage clinicians to provide this information would help the toxicology laboratory perform relevant, patient-specific testing.

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Learning objectives

After reading *Clinical & Forensic Toxicology News*, the reader should be able to:

- Describe emerging and changing trends in drug abuse, including new designer drugs, usage patterns, and contaminants/adulterants.
- Identify potential analytes (drugs, metabolites, biomarkers) of clinical and/or forensic significance.

- Evaluate methodologies for their utility and limitations relative to the needs of toxicology labs.
- Discuss relevant regulations, such as analytical performance requirements, or the legality of new drugs of abuse.
- Explain the analytical and regulatory issues unique to specific applications, including postmortem toxicology, workplace drug testing, and drug screening.
- Describe the medical implications of drug abuse, toxicity associated with therapeutic agents, and exposure to other toxicants.

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