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Forensic Science Identification of Drugs of Abuse

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ABSTRACT: The guidelines for forensic urine drug testing do not specify the gas chromatographic/mass spectrometric (GC/MS) procedure to be used for confirmation. Full mass spectral documentation together with a positive immunoassay and supporting data should insure the most certain identification. Preparation of specimens which test proficiency adequately is difficult, particularly for marijuana testing. The use of a full-spectrum GC/MS method should make the results admissible under the Frye Standard and the Federal Rules of Evidence, whereas ion monitoring methods could be challenged effectively. Duplicate specimens should be available, one for employer and the other for the employee. Precision suffers when determining low substance concentrations.

KEYWORDS: toxicology, workshop, drug use testing, urine

Urine testing for drugs of abuse has become a multimillion dollar business. Computer-controlled procedures and evaluations increase the number of specimens processed and reduce the costs. Most gas chromatographic-mass spectrometric (GC/MS) procedures depend on automated evaluation of retention times plus one to three mass ions for confirmation.

Confirmation of the identification of drugs following positive results of presumptive, initial, or "screening" tests for drugs of abuse requires the use of GC/MS methods. However, the actual GC/MS methods to be used are not described in the Federal Guidelines [*1*]. The College of American Pathologists [*2*] Standards for Accreditation do not require GC/MS confirmation.

Total Mass Spectrum

Classically a full mass spectrum obtained by electron impact ionization has been used for forensic analyte identification. The probability of identification is great when there are enough mass ions of the proper intensities produced from a scan at the proper retention time and when these data are matched with similar data obtained from a standard. This is considered the best use of GC/MS for compound identification. The retention time or scan number can be obtained from the total ion chromatogram (TIC). Potential interference can be detected using both the TIC and the number and amplitude of extraneous mass ions in full mass spectra. Analyte concentrations may be determined by comparing

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the areas of mass ions to those of the corresponding mass ions of the standard. In the absence of detectable interferences, quantitation of analytes may also be performed using the areas of TIC peaks. A deuterated analyte is preferred as an internal standard, whenever one is available.

Selected Ion Monitoring

In this procedure, the energy of a mass spectrometer is programmed to detect a small number of selected mass ions. Several of the more intense ions are selected, preferably those that are not too far apart. Advantages of the procedure are that time and expense can be saved, the sensitivity of detection can be increased, and the method can be automated. When three mass ions are selected the presumptive identification depends on the relative retention times and, in most instances, on the relative intensities of the three mass ions compared with those of the standard. The ratio obtained by dividing the area of the secondary mass ion by the area of the primary mass ion when compared to a ratio similarly obtained from a standard has been used if the coefficient of variance (CV) was less than $\pm 20\%$. The same CV in the ratio of the areas for tertiary mass ions versus the primary mass ions has been used.

What is the probability that the identification is positive? Since quantitation is based on comparisons of the areas of mass ions in the unknown with areas of the respective mass ions in the standard, does this mean that the quantitation using other mass ions could vary by $\pm 35\%$ or more? Information is not obtained which could allow for the recognition of interferences. The lower the concentration of analyte, the less certain are the identifications and quantitations.

This procedure is useful for eliminating specimens testing negative before proceeding with forensic identification obtained by matching the full mass spectrum of the unknown with that of a known standard.

Single Ion Monitoring

In another procedure, the sensitivity of the GC/MS method can be increased further by programming the instrument to select only a single mass ion, a technique used in negative ion chemical ionization GC/MS. The presumptive identification depends on the relative retention time for the analyte versus that for a standard. The area of the selected mass ion is used for quantitation. The retention time and the presence and intensity of a single mass ion are inadequate evidence on which to base the identification and quantitation of an analyte. Interferences would be impossible to recognize if this was the only method used. It is difficult to standardize this procedure.

In addition, some instruments are limited so that they cannot be used in the negative ion chemical ionization mode or do not have increased sensitivity when a small number of ions are selected (ion trap detectors and mass selective detectors).

It is not possible to establish the probability that a drug will be correctly or incorrectly identified using one of the various confirmatory methods of analyzing urine for the drugs of abuse. Most urine determinations depend on the identification of the drug metabolites, hydrolysis products, or chemically altered derivatives of the drug. Most proficiency or performance testing is based on urine which has been found by analysis to be free of interfering compounds before the analyte in question is added. Specimens used for such testing may not be representative of specimens obtained in either the clinical or forensic science setting. "Synthetic urine" has been used as the matrix for testing specimens. These specimens do not reveal what effect the many compounds that may appear in urine have on methods of analysis. For example, at least 30 metabolites of delta-9-tetrahydrocannabinol (THC) have been identified in urine. They all have the 3 rings of THC and

most are mono- and di-hydroxy THC compounds, or mono- and di-carboxy THC compounds. Added to these are other cannabinoids in *Cannabis*, other metabolites of cannabinoids, other plant constituents, and/or their pyrolysis products or metabolic products as well as of other xenobiotic or natural substances and their metabolites which might be present in urine. These substances which are not readily available are not put into proficiency testing specimens.

Probably the most difficult specimen to obtain for proficiency or performance testing is a cannabinoid specimen. Large volumes of urine or synthetic urine are needed which contain about 100 ng/mL of cross-reacting cannabinoids which respond positively and equally to all of the immunoassays and also contain about 15 ng/mL of THC-carboxylic acid (COOH). Since it appears to be impossible to prepare such a specimen, it will probably be necessary to pool many urine specimens to obtain the proper concentrations for both the initial and confirmatory assays. The final product should then be assayed by all of the immunoassays and GC/MS procedures by the best reference laboratories to establish reasonable expected concentration or target value. The specimens could then be sent as blind specimens. To test performance, it has been suggested that laboratories be told to test certain specimens by GC/MS only for THC-COOH. Unless these specimens contain other naturally occurring cannabinoids and there is some way of submitting them blind, they will not truly test the system. A far simpler and more forensically acceptable procedure would be to delete the meaningless concentrations in the confirmatory test but to require full mass spectra for forensic science identification.

Assume that in analyzing a urine specimen for marijuana metabolites, one of the methods that is used for presumptive testing is an immunoassay, thin-layer chromatography (TLC), gas chromatography (GC), or high performance liquid chromatography (HPLC), followed by a mass spectral confirmation. What could be present in urine that will bind with an antibody, alter an enzyme reaction, produce a positive TLC response, give one or more electronic responses at the right times, have single or three mass ions in the right places, or produce the proper combination of the above? The more parameters that are correct, the more certain the identification. Problems with drug analysis technology have been reviewed recently [*3*].

Some idea of the probability of misidentifications might be obtained by comparing full mass spectra from all of the positive specimens obtained from any of the above procedures with those of their respective reported analytes' standard spectra. If it was shown that the reported positives were confirmed or rarely failed to be confirmed, then there would be high probability that the procedure was valid.

Admissibility of Test Results

Courts will have to decide whether to accept urine test results. The decisions will be based on one or both of the standards for the admissibility of scientific evidence: the Frye Standard [*4*] and the Federal Rules of Evidence [*5*].

The Frye Standard requires that scientific evidence be admitted only if the scientific technique has been generally accepted in the relevant scientific community. The courts must decide that the procedures used are accepted by that portion of the scientific community with expertise in the area of forensic toxicology, who are familiar with both the scientific theory and equipment used in the particular types of tests, that the tests identify the drug, and that identification indicates use [*6*].

Under the Federal Rules of Evidence, the court must balance the relevancy of the evidence against prejudice to the defendant in determining the admissibility of test results. Although all of the tests used for urine drug testing might be considered relevant evidence, the tests will be given different weights when balanced against considerations of prejudice to the defendant because of their varying certainty of positive identification. There is not

agreement on the reliability of the identification based on most of the procedures used in the identifications and quantitations. Reliability affects the weight of both relevancy and prejudice to the defendant. The court must evaluate the potential rate of error, the quality of a particular test, and the expert's stature in the community and qualifications. In the absence of improper laboratory procedures, the results of properly interpreted full mass spectra should be admissible as evidence under either standard because of the high probability of relevancy and the low probability of prejudice to the defendant. In most cases, the test results are the only evidence on which innocence or guilt must be determined. If it is necessary to establish that a drug was used at a certain time or that performance was adversely affected, positive results of urine testing including drug concentration should have little relevancy.

Documentation [*1*] should be complete enough to allow a qualified expert to judge the quality of the analysis. An attorney should be able to follow the chain of custody to establish that it is properly documented. It is unlikely that he could evaluate the analytical documentation such as procedures and worksheets which are used in the presumptive testing and the confirmatory forensic science identification. Chemists and other scientists without special knowledge would be unable to evaluate the reliability of the forensic science identification by mass spectrometry.

Duplicate Specimens

Most of the workers whose urines are tested have no signs, symptoms, or other evidence of drug use or impairment. The sole evidence is a positive finding of a substance in a single urine specimen allegedly obtained from the employee. All decisions are based on the results of tests on one specimen obtained, controlled, tested, and saved for the employer. Control of the chain of custody is a critical part of the testing program, but it can be simplified and safeguarded by using split specimens. Testing programs should require that the original specimen be split into duplicate specimens. One specimen could be sent to the laboratory by the employer. The other specimen should be labeled, sealed, and preserved by freezing, if necessary. When the first specimen tests positive and the worker challenges the results, the second specimen could be thawed and divided equally and put into two containers, one for the employer, the other for the employee or his or her representative. Both the employer and employee can have aliquots of the specimen tested independently by qualified analysts. This procedure could solve many of the problems that arise from testing a single specimen, such as chain of custody; mislabeling; specimen switching; contamination; carryover; and instrumental, technician, and reporting errors.

Urine drug testing should be reserved for people who have signs and symptoms which might be due to the misuse of drugs. If testing was used only when there was a reasonable suspicion that the person's performance was impaired by drugs, the specific drugs that might be responsible for performance impairment could be included in the analytical procedure. This would be preferable to the present system of limited and random testing for a few analytes which may not be associated with impairment. The testing which would be more complete and more expensive per specimen could be of true forensic science quality. Because of the very small number of such tests that would be necessary, the cost of such a program would be a small fraction of the cost of the present random and not-for-cause testing programs. Part of the money saved could be used in assistance programs for those who need it and who could be rehabilitated. To be reasonably certain that employees are free of impairing amounts of drugs, it would be necessary to test their bloods immediately before they are to perform a safety-related task and to be able to correlate the drug concentration with the amount of impairment that has been shown to be produced by that concentration of drug. This will not prevent an employee from consuming a drug at work after he or she has been tested.

Laboratory certification will not completely eliminate misidentifications, and it will be necessary for competent forensic science experts to examine the work on which identifications are based if the results are to be used in adversarial proceedings.

Drug Concentrations

What do the urinary drug concentrations that are being reported mean? There is rarely a scientifically defensible, behaviorally linked justification for the number chosen as a concentration or cutoff for a specimen that is labeled positive. These assigned values might have been offered because those not familiar with the lack of meaning of urine drug concentrations were looking for a "per se" concentration for other drugs as there is for alcohol. Often these "cutoff" concentrations are based solely on analytical criteria such as a minimum detectable concentration, without regard to any behavioral impairment considerations. Effects on behavior have been related to alcohol concentrations in blood and can be related to urine alcohol concentrations, but it required many years of research to establish these correlations for this one drug. There is still controversy over interpretation of alcohol results. Scientific data which could be used for correlating concentration of most other drugs with behavior have not been found in the literature.

The loss of precision becomes a major factor as analyte concentration decreases into the lower nanogram/millilitre range [*7*]. The interlaboratory precision of chemical analyses was found to be independent of analyte, matrix, and method at low concentrations. It appeared that the variability was lowered by half for every two orders of magnitude increase in concentration scale. The interlaboratory coefficients of variation (CV%) are given in Table 1.

Results from about 120 laboratories had CVs of 21 to 40% for concentrations of 200 to 5000 ng/mL from a set of 10 urine proficiency test specimens which contained amphetamines, opiates, phencyclidine, cocaine, and marijuana metabolites [*8*].

The use of concentrations and cutoffs might be an attempt to obtain uniformity by holding everyone to the same standards. This is not possible even if every urine was tested in one laboratory using the same procedure for each specimen. The results of identical procedures vary greatly, not only from test to test and from person to person, but also between urine specimens obtained from the same person at two different times but under otherwise identical conditions (that is, between specimens collected the same time period after the ingestion of the identical drug dose by the same person at another time).

Setting a concentration high enough above the background noise of an analysis might lend more credence to a presumptive identification. The cutoff of the initial test must be high enough so that it will be possible to confirm the identification.

The armed services set concentration based on the lowest value that it is felt that the

TABLE 1—*Coefficient of variation versus concentration.*

CV, %	Concentration, ng/mL
16	1000
19	500
22	100
27	50
32	10
38	5
45	1

method will detect an analyte in spiked specimens. Their interest appears to be in detecting drug use and not in when the drug has been used or in what its possible effects are. The National Master Freight Agreement [9] specify uninterpretable and scientifically indefensible concentrations for blood cannabinoids and for no other drugs except alcohol. Sports testing does not appear to set concentrations.

Reasons are not stated in the Federal Guidelines [1] for the concentrations or cutoffs required for the initial or the confirmatory test. It is stated that they are "subject to change by U.S. Dept. H.H.S. [1] as advances in technology or other considerations warrant identification of these substances at other concentrations." It does not seem possible that it will ever be possible to equate urine drug concentrations with impairment.

If a forensic science identification is important and if use at some time is the only issue, then a proper full mass spectrum should be sufficient. Since for most drugs even blood concentrations cannot be correlated with performance, it is unscientific to attempt to relate urine drug concentrations with the time and amount ingested or to state that the drug caused impairment [10]. The panel members who reached this consensus have recently reaffirmed their opinions [11].

Challenging a single positive result in an adversarial proceeding is expensive and time-consuming. The large-scale testing that currently is being done and that appears likely to increase will produce many cases where positive results will be challenged by the accused. There are not enough judges, arbitrators, and qualified forensic science experts for even less than 1% of the positive results to be challenged. The unemployed non-union grievant cannot afford the costs of a challenge. There are many who could afford the expense of testing of a second specimen by a GC/MS full-spectrum procedure in a certified laboratory. This could fairly resolve the issue of forensic science identification.

Equal justice under law is not possible when judgments are based solely on the results of analyses of urine specimens.

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