Correspondence

Commentary on Wu AHB, Hill DW, Crouch D, Hodnett CN, McCurdy HH. Minimal standards for the performance and interpretation of toxicology test in legal proceedings. J Forensic Sci 1999;44(3):516–522.

Sir:

Because our legal system relies on forensic scientists for dependable analysis and interpretations, the articulation of minimal standards in forensic toxicology is long overdue. In our experience, some of the minimal standards presented by Wu et al. are insufficient.

Wu et al. state that, "Chemical ionization (CI)-GC/MS assays using single ion monitoring can be employed if the laboratory is experienced in this methodology, and there is tight control over the extraction and analysis conditions. . . . Single ion monitoring may be acceptable if there are other corroborating analytic data to substantiate the analysis, such as positive results . . . of other biological fluids collected at or near the same time (e.g., urine)." The following example illustrates why this is not always correct.

In this laboratory's research, samples of saliva and skin wipes were collected from random automobile drivers to evaluate the suitability of these matrices as screening tests for DUI. The specimens were tested with a published procedure (1). Briefly, the skin wipes and saliva samples were extracted with dilute acid, the analytes concentrated by automated SPE, derivatized, and analyzed by CI-GC/MS on a Varian ion trap. Of the 153 matched samples tested, two sets of samples had retention times and single ions corresponding to MDMA in both the skin wipes and saliva samples (2). Full scan spectra were taken, as ion traps can be operated in this mode without much sensitivity loss. The full scan spectra were uninformative because isobutane chemical ionization tends to produce only protonated molecular ions; nevertheless, no co-eluting interferences were indicated. MS/MS analysis of the specimen identified the compound as N-methyltyramine (confirmed with an authentic standard), not MDMA, showing that single ion Cl spectra would lead to erroneous conclusions (Fig. 1).

This example illustrates why procedures, including those that test unusual matrices, must be continually evaluated. The reliance on pooled blanks from multiple individuals to validate a method and/or determine LOD/LOQ is not necessarily a good idea. Blanks from multiple individuals (rather than pooled blanks) must be tested to give some probability of finding unusual, interfering substances, especially as detection limits are lowered, because pooled blanks dilute individual aberrations to the point of being unobservable (3).

While more than eight million compounds are known in the literature, the number found in the human body is not known. Capillary GC can distinguish perhaps several thousand compounds, with MS providing added identification power. Information the-

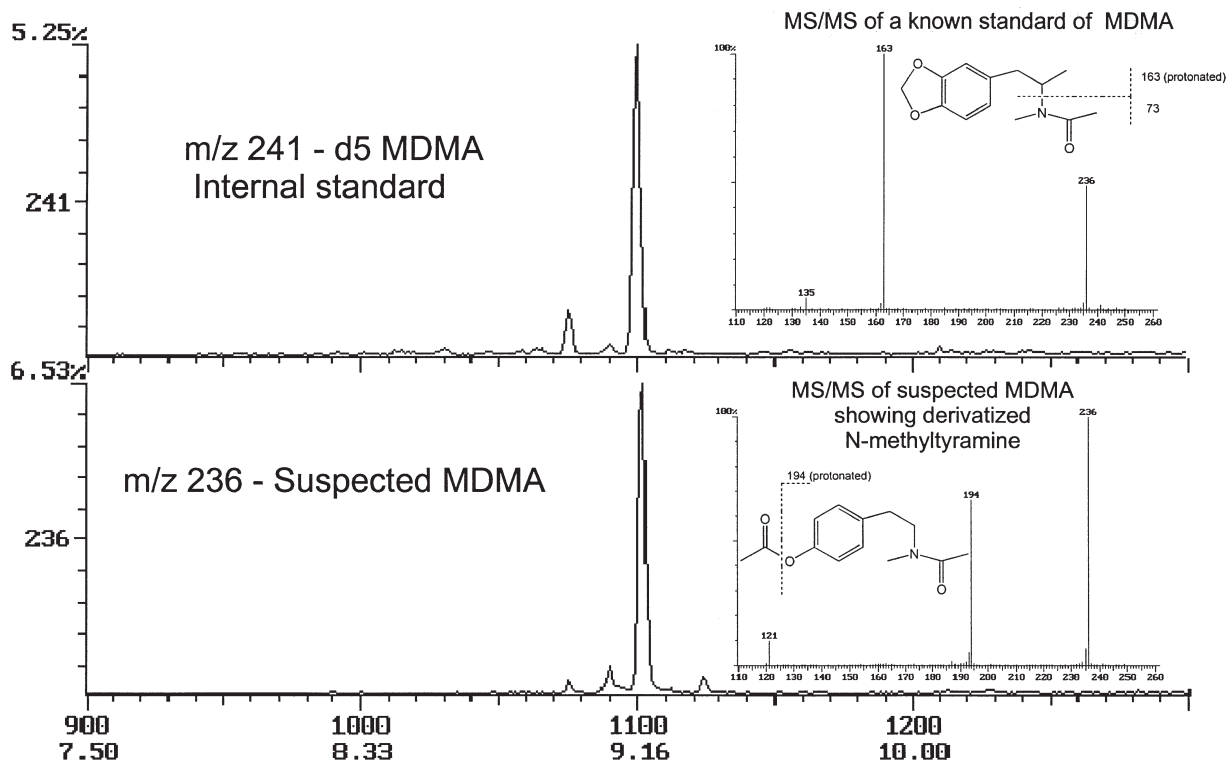

FIG. 1—*Ion traces for d5-MDMA and misidentified MDMA. Insets show that MS/MS spectra of authentic MDMA and the specimen unknown are different. Authentic standards identified the compound in the specimen as derivatized N-methyltyramine.*

ory provides a mathematical method to estimate the identification power of various analytical procedures (4) and predicts that fewer criteria result in poorer confidence in the result. Mass spectrometric identification using selected ion monitoring should require three ions (such as used in regulated urine testing). The ions must contain a portion of the molecule targeted in the assay; fragment ions that contain only portions of the derivatization reagent must not be selected. In our opinion, ion ratios must be calculated, which cannot be done from a single ion. MS/MS analysis alone is not a substitute for these criteria. However, two ions and one ratio may be acceptable for MS/MS analysis as the third ion is provided by the parent ion and background from co-eluting materials, which could confuse the identification, is eliminated. Conclusions drawn from analyses of unconventional matrices and novel compounds (that do not have the legacy that urine testing has and frequently are accompanied by lower detection limits) have weaker reliability unless increased specificity is provided in the analysis.

Immunoassay screening adds some confidence to the procedure but even this can be insufficient. Generally, immunoassays are used to save cost by eliminating negative samples. The correlations of immunoassay results with GC/MS results are often poor due to cross-reacting materials interfering with the immunoassay. Because of this, when laboratories do not correlate concentrations for individual specimens, the potential increase in confidence in the procedure that the immunoassay could provide is diminished. Even so, not every laboratory uses a dual testing procedure (screening by immunoassay and confirmation by GC/MS for regulated testing) because immunoassays may not be available for the analyte in question or the matrix being tested. Over reliance on immunoassay can lead to false positives where the immunoassay identifies one compound and the GC/MS analysis another (5–7).

Another form of quality control is investigation of claimed innocence. No laboratory practitioner wants the appearance of making mistakes. When 99+% of the laboratory's testing is not questioned, the presumption that every result is correct seems to be reinforced. One may never know that a system is flawed until an innocent individual questions the analytical results. Innocent individuals may lack the resources or knowledge to challenge results. More often, the individual may suspect adulteration of their food or beverage rather than problems with an analytical result itself. Even if the analysis is questioned, the laboratory may dismiss that objection without sufficient consideration. When a result is questioned, the laboratory must have in place additional procedures to check the result, including sample retesting by a more specific technology. Finding errors and putting in place procedures to avoid those errors is a way to increase the confidence in a system, as information theory predicts.

Minimal standards need to exploit modern technology to its fullest practical extent to assure quality results. Single ion monitoring does not meet this goal.

References

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D.A. Kidwell, Ph.D.
Chemistry Division, Code 6177
US Naval Research Laboratory
Washington, DC 20375
Telephone: (202) 767-3575
Facsimile: (202) 767-3321
Electronic mail: kidwell@ccf.nrl.navy.mil

F.P. Smith, Ph.D.
Department of Justice Sciences
The University of Alabama at Birmingham
Birmingham, Alabama 35294-2060
Telephone: (205) 934-2069
Electronic mail: fsmith@uab.edu

Author's Response

Sir:

Thank you for the opportunity to respond to the letter of Drs. Kidwell and Smith. In our article, we presented several cases to make two major points: 1) That minimum standards are needed for medicolegal testing; and 2) All analytic methods used in medicolegal testing should be validated and vigorously challenged for sensitivity and selectivity in the laboratory before implementation. Their letter supports our belief about the need for validation.

We also believe that there are several common lessons from our cases and the single case they present. Foremost in any validation, the laboratory must determine if the method is suitable for analysis of the target drug(s)/metabolite(s) in the chosen specimen. This is a requirement of Good Laboratory Practice. Despite the technical developments in GC, HPLC, and mass spectrometry, there is no substitute for good chromatographic separation. Therefore, the validation experiments should ensure that there are no interferences from endogenous substances or other drugs and chemical agents. In addition to the formal validation, expertise in mass spectrometry and experience with the chosen method is a must.

We disagree, however, with the sweeping generalization that single ion monitoring using chemical ionization (CI) mass spectrometry (MS) is insufficient for forensic testing. The example provided by these toxicologists demonstrates our points about the need for testing standards. The success of single ion Cl-MS analysis depends on the extraction procedure, reagent gas(es) used, source temperature, chemical structure of the analyte, the derivative (if formed), the chromatographic technique, the chromatographic column, carrier gas and conditions, the scan function, whether positive or negative ions (or both) are detected, and many other related parameters. Condemnation of a time tested technique based on one example using a single set of analysis parameters is unwarranted. Doing so may pre-condemn other recent innovations that make use of Cl, such as Atmospheric Pressure Ionization for HPLC-MS and HPLC-MS/MS.

As scientists we should be cognizant of the fact that all methods have limitations. The advent of mass spectrometry made clear the limitations of gas chromatography with flame ionization, nitrogen phosphorus, and electron capture detectors. Mass spectrometry using ion ratio calculations is also not infallible. This was demonstrated in the early 1990s with the analysis of sympathomimetic amines (1). We now enter a time when GC-MS/MS, HPLC-MS, and HPLC-MS/MS is being introduced into our discipline. Although these are exciting and revealing technologies, they also have limitations. As these and other technologies develop, we urge forensic scientists to embrace them into their testing arsenal and to establish minimum standards for their forensic use.

Reference

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Dennis J. Crouch, B.S., MBA
Interim Director, Center for Human Toxicology
Research Assistant Professor, Pharmacology and Toxicology
University of Utah
Salt Lake City, UT 84112

Alan H.B. Wu, Ph.D.
Director, Clinical Chemistry and Toxicology
Hartford Hospital,
80 Seymour St.
Hartford, CT 06102