

Forensic Toxicology Widens Net for Drugs of Abuse

The Rise of LC/MS/MS for Toxicology Testing

Tania A. Sasaki, Ph.D.

Everyone outside the toxicology field seems to believe the forensic toxicology laboratory can identify any drug or compound of interest with a simple push of a button.

Popular belief is that a lab can obtain an unknown sample, analyze it, and have a report printed out – complete with pictures, graphs, chemical structures, and compound identifications – in a matter of minutes.

However, those of us in the field know that the challenges many forensic toxicology labs include: limited resources, increasing demands, emergence of new “designer” drugs, and an increasingly larger number of samples being submitted for analysis.

Toxicology results and scientific evidence are getting more scrutiny than ever before. The criminal justice system increasingly relies on the output of forensic toxicology laboratories to provide evidence relevant to forensic cases, including homicides, sexual assaults, and impaired driving linked to fatalities.

Defense attorneys are quick to jump on any uncertainty that can be considered “reasonable doubt,” which is combined with a perception and expectation that the evidence should leave no unanswered questions.

All these issues merge and place a heavy burden on forensic toxicology labs

to analyze an increasingly larger number of samples in a shorter time, while maintaining accurate results that can survive legal scrutiny.

Fortunately, there is a fundamental shift occurring in the forensic toxicology field, and this article delves into the dynamics of the changes. ➤



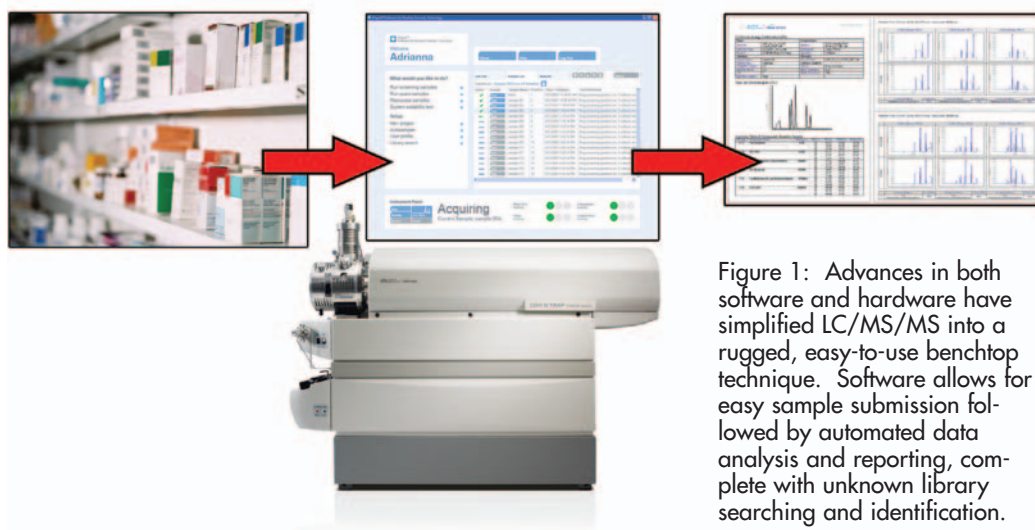


Figure 1: Advances in both software and hardware have simplified LC/MS/MS into a rugged, easy-to-use benchtop technique. Software allows for easy sample submission followed by automated data analysis and reporting, complete with unknown library searching and identification.

Affecting virtually all forensic toxicology laboratories one way or another, the changes occurring in forensic toxicology are due largely to advances in technology. Although there is a comfort level with the way toxicology testing has traditionally been done, the transition to newer, easier-to-use, more powerful technologies is helping improve the way analyses are performed.

As forensic toxicologists assess their own needs to improve and map out the direction their laboratories are headed, it is useful to review the issues that, despite being somewhat daunting, need to be addressed. The main issues include:

- New derivatives of drugs of abuse continue to come onto the market, and methods to test for these drugs must be quickly developed.
- A shortage of licensed scientists persists.
- A regulatory compliance paradox must be addressed by government entities.
- Detection of low concentration, potent drugs in the presence of high concentrations of therapeutic drugs.

INCREASING VARIETY OF DRUGS OF ABUSE

New drugs continue to emerge at an alarming rate. Just when a toxicology laboratory director thinks his or her database of drug information is up-to-date, new drugs or new derivatives of drugs emerge and create new identification challenges.

An analytical technique should be able to detect trace levels of drugs, even if present along with large concentrations of other drugs. Sensitivity and selectivity are crucial.

If an instrument or technique used to test for the drugs is not selective or sensitive enough, it is possible to miss the fact that four or five drugs are actually present in a person's blood stream. This error is often caused because the current database cannot detect and identify the foreign substances or the concentrations of certain drugs are so low that they are below the detection limits of many techniques.

Toxicologists are continually thinking about how to expand their databases with more compounds. Or they dream of a built-in library to screen for hundreds of drugs of abuse, drug metabolites, and poisons.

The more automated the search of compounds within a sufficiently comprehensive library can be, the more efficiently the lab technicians can obtain the most accurate results that can be used to substantiate finds in a court of law.

ARE CURRENT SYSTEMS DETECTING ALL DRUGS?

Part of the re-evaluation of forensic toxicology includes an analysis of whether the most commonly used analytical techniques, including immunoassay, GC/MS, GC/FID, GC/NPD and/or LC/UV, are detecting all the drugs of abuse in a person.

The old assumption is that EMIT with GC/MS or LC/UV is the most proven in toxicology screening. However, the change that has been occurring in many forensic labs across the world is the implementation of LC/MS/MS. Unfamiliarity with LC/MS/MS has left many forensic toxicologists with misconceptions about this analytical technique. They believe it is too complex, too expensive, and requires extensive training.

These statements may have been true years ago, but technological advancements, combined with new software applications (such as Cliquant™ Drug Screen and Quant Software for Routine Forensic Toxicology from Applied Biosystems/MDS SCIEX) have emerged to make it easier for forensic toxicologists to utilize the power of LC/MS/MS in a benchtop instrument. Toxicologists can now benefit from a simple, step-by-step process with a lower cost per unit analysis. (Figure 1)

The suitability of LC/MS/MS as the basis for testing methods in toxicology laboratories may be eye-opening for many professionals in the forensic field. They may not have even entertained the thought of tapping into the performance of LC/MS/MS technology, a standardized analytical technique already heavily used in other fields, such as pharmaceutical drug discovery and development.

LC/MS/MS systems can detect lower concentrations of drugs than existing or traditional systems. As new drugs are identified, they can also be easily added to the LC/MS/MS screening method.

Greg Ohlson, a criminalist at the Arizona Department of Public Safety, explained, "When used as a screening system, LC/MS/MS may potentially replace immunoassay in the ➤

ease of development. In areas where immunoassay products are not available and high sensitivity is needed, the laboratory is forced to use GC/MS as a screen. Many of these compounds of interest require derivatization to be detectable at appropriate levels. LC/MS/MS excels at detecting a wider range of analytes without derivatization.”

A limitation has existed in forensic toxicology. Certain drugs were falling below the detection limit of older systems. Another problem was the issue of sorting out the amount of drugs when a mixture of several drugs was present in a body.

H. Chip Walls, the technical director at the University of Miami Toxicology Laboratory, said: “The reason we were interested in implementing LC/MS/MS in our lab is because of the enhanced screening capabilities. Immunoassay screening is very limited and some compounds just can't be analyzed easily – or at all – by GC/MS and, therefore, require LC/MS/MS. In addition, sample preparation is simplified. The less a sample is handled, the less chance for introduction of error or contamination, which is very important.”

The use of GC/MS as a primary technique entails a great amount of sample preparation and requires longer run times. What may not be as commonly known is that GC/MS also lacks sensitivity for certain drugs. As such, although GC/MS is perceived as “proven,” it no longer has an advantage over LC/MS/MS when the interface of LC/MS/MS is an easy-to-use software application.

‘HELP WANTED’

One potential consideration to address all these issues *without* changing technologies would be to hire and assign more scientists to stay on top of the emergence of new drugs and re-test over and over with current systems until they are fairly certain the results are accurate. However, even if you put aside the inherent technological limitations of existing technology, there is a growing shortage of certified forensic toxicologists. On

average, more toxicologists are retiring or leaving the field each year than the number of graduates who are entering this field. The reasons for this trend vary, but part of it could be that forensic toxicology is a misunderstood field.

Nonetheless, forensic toxicology is changing. The impact of toxicology testing is being recognized as greater than ever. People who enjoy science can appreciate the intricate challenges that give scientists new opportunities to redefine toxicology testing for the future.

But, as toxicology laboratory directors lay awake at night wondering about personnel changes, the issue won't be solved overnight. Even if hiring managers obtained more budget to pay more people to work in toxicology testing environments, it is not easy to find qualified professionals with the proper certifications. Labor costs can be high, and workload can fluctuate.

A more strategic approach is needed, and each forensic toxicology team needs to judge for themselves. New technology plays its vital role in facilitating and justifying improvements.

LOWER TOTAL COST OF OWNERSHIP

One way to measure efficiency is cost. The total overall cost of ownership is lower for LC/MS/MS, both in time and money.

Warren Walsh, the senior technologist in charge of the toxicology laboratory at Sick Kids Hospital in Toronto, Canada calculated that each sample costs approximately \$19.50 on an LC/UV-based system, while each sample on an LC/MS/MS system costs approximately \$2.50 (after the initial cost of an LC/MS/MS system).

Walls at the University of Miami Toxicology Laboratory agreed. “As we move assays from GC/MS to LC/MS/MS, we were able to not only simplify sample preparation, but eliminate costly derivatization steps.”

The toxicology laboratory at the Arizona Department of Public Safety not only saved time and money, but also the amount of sample required to perform an analysis was reduced without sacrificing – and even improving – the detection limits of the assay.

Since the implementation of LC/MS/MS, a significant increase in sensitivity in comparison to GC/MS for analysis of benzodiazepines and zolpidem from urine has been noted. This has allowed for a reduction in the sample size used. In turn, it reduced the expense of hydrolysis reagents needed to detect drugs that are extensively metabolized, according to Ohlson. Relatively simple one hour hydrolysis to analyze 14 compounds is being used. Analytical run time is six minutes at a conservative cutoff of 25 ng/mL using a 0.5 mL sample size. This analytical technique has allowed a reduction in cutoff by half using half of the original sample size.

The fact that LC/MS/MS now requires minimal training also adds to the lower total cost of ownership. With new software emerging to make LC/MS/MS easier to use for technician-level chemists (or scientists) without graduate degrees in mass spectrometry, users gain the benefit of the functionality of automated library search and identification of peaks on a quantitative basis, which gives higher confidence in results. (Figure 2)



Figure 2: User interface of Applied Biosystems/MDS SCIEX's Cliquant™ Drug Screen and Quant Software for Routine Forensic Toxicology with four-step work flow, 1,200 compound library, new enhanced library search algorithm with automatic library search and reporting function, plus customizable pre-configured report templates.

CONFIRMATION PARADOX

A paradox exists in the area of compliance. When a result is obtained, it is necessary to confirm with another orthogonal analytical technique.

Suppose a good quality screening result from analysis of a blood sample using LC/MS/MS identified five different drugs, including cocaine and amphetamines, as well as two additional drugs present at extremely low concentrations. You are confident in the results, but now you have to perform the confirmatory test.

GC/MS analysis is used for confirmation and, subsequently, the results show that only two drugs are present; the other three drugs fall below the detection limits of the GC/MS system because of inherent limitations in the system. The expected confirmation is not achieved.

Taken at face value, it might be likely to conclude that the LC/MS/MS system must have been wrong and that only cocaine and amphetamines were present – and those are the only drugs of concern.

What should be the new policy when there is a discrepancy between more sensitive LC/MS/MS results and the results of an established and accepted GC/MS system? This is an important question that the whole field needs to address. Keep in mind that LC/MS/MS inherently has an internal confirmation step.

CONCLUSION

Popular belief and old assumptions, as well as a failure to re-think the policy-level issue of re-confirming results, threaten to hold back progress in the field of toxicology. Despite these challenges, the transition from a popular but limited technology to a new more sensitive technology is happening. Instead of being a cause of worry, this change is a unique opportunity for significant advancements in the field of forensic toxicology.

Ohlson at the Arizona Department of Public Safety summed up the future of LC/MS/MS and its applicability and utility in the forensic toxicology lab by saying, “Although GC/MS is currently considered the ‘Gold Standard’ of identification, LC/MS/MS for routine use in the forensic environment is on the way to developing into a new ‘Platinum Standard’ for toxicology testing.”

Tania Sasaki has 15 years experience in mass spectrometry and has spent the last four years working at Applied Biosystems, where she is currently a Sr. Product Applications Specialist and Small Molecule Group Leader in the Demo/Applications Lab in Foster City, CA. During the last three years, her focus has been on forensic toxicology applications. She received her B.A. in chemistry from Pomona College and her Ph.D. in analytical chemistry from the University of California, Riverside.

Disclaimer: The quotes expressed in this article are the views of those people quoted and do not necessarily represent those of their agency of employment.



stock number: #114AR14-01