Pushing the Limits of Speed and Sensitivity in Drug Screening – an LC-MS solution

General unknown analysis of drugs in biological samples is made possible at the specialised laboratory of Dr Michael Böttcher, MVZ Labor Dessau GmbH

Author: Rohan Thakur, Executive Vice President, Bruker Daltonics

Comprehensive screening of biological samples in forensic toxicology and clinical research is not possible without powerful, highly sensitive instruments, which allow researchers to focus on the unambiguous identification of parent drugs and their corresponding metabolites. Gas chromatography mass spectrometry (GC-MS) is the traditional approach to toxicological drug screening using general unknown analysis – a broad screening method which screens for over 4000 substances – but has steadily been overshadowed by the rapid turnaround time, ease of use and high sensitivity of innovative LC-MS methods.

Forensic toxicology laboratories receive wide varieties of sample type, ranging from urine and blood, to hair and oral fluid, with some matrices posing more challenges to screening than others. Oral fluid testing has more recently been optimised for general unknown analysis, enabling laboratories to offer such a service to health clinics, psychiatric hospitals, prisons, specialist psychiatric prisons, as well as private physicians working in addiction medicine, for example.

Toxicology Case Study - Dr Michael Böttcher's Laboratory

Dr Michael Böttcher's laboratory at the MVZ Laboratory based in Dessau, Germany, is renowned for its specialist capabilities in drug screening analysis and toxicological studies, and offers a complete analysis spectrum for a wide range of biological matrices.

As a comprehensive analytical facility, the laboratory receives samples from a vast range of sources: "At Lab-Dessau we do all kinds of drug testing: therapeutic drug monitoring, drugs of abuse testing, workplace drug testing, intoxication cases, clinical drugs testing, especially for addiction medicine. We work for a large number of addiction clinics in large cities like Berlin. We monitor therapeutic drugs in addition to looking for drugs of abuse," explained Dr Böttcher, "we work for many institutions which are involved with addiction or drugs of abuse."

Dr Böttcher's laboratory services customers all over Europe, including in Ireland, the Netherlands, Sweden, Poland and Austria, and are renowned for their unique routine capabilities and rapid turnaround time.

"We are very specialised in numerous rare drugs tests. We also conduct forensic testing, as we're forensic accredited we work for a number of Forensic Institutes. A lot of customers need new psychoactive substance testing which is less routine, so they send the samples to us because of our renowned specialist capabilities," commented Dr Böttcher. Samples are sent to the laboratory frequently for synthetic cannabinoid testing, as well as synthetic opioids and internet drugs, so called 'legal highs'. Modern 'designer drugs' are sent to the laboratory, in addition to those which have been on the market for decades. Dr Böttcher's laboratory is capable of analysing drugs from capillary blood samples, urine, hair, and oral fluid - the latter of which has been most recently optimised and is particularly unique to this laboratory, as Dr Böttcher explained:

"We are quite specialised in oral fluid testing. We receive and test approximately 300-400 oral fluid samples per day and unlike many other laboratories which use immunoassay techniques, we use liquid chromatography-tandem mass spectrometry (LC-MS/MS). Using this method, we routinely look for 68 substances in one run, which is done very sensitively. From the beginning this has been a highly sophisticated testing method."

The laboratory relies on dependable instruments which operate with minimal downtime, in order to service customers with time-sensitive samples. The ability to run general unknown analysis depends on the comprehensive library of drug compounds available to the laboratory, so maintaining this as well as updating methods is critical to ongoing research and service development.

GC-MS vs. LC-MS

Gas chromatography mass spectrometry (GC-MS) is the traditional approach to toxicological drug screening using general unknown analysis, but has steadily been overshadowed by the rapid turnaround time, ease of use and high sensitivity of innovative LC-MS methods. Despite the increased popularity of LC-MS, Dr Böttcher's laboratory still uses GC-MS for screening certain samples, as some substances are not visible using LC-MS. For routine work the laboratory now uses the Toxtyper™ - an LC-MS^n library-based solution from Bruker Daltonics.

"For routine general unknown analysis, we don't use GC-MS anymore but in some cases, particularly in post-mortems where the matrix may be decomposed, it is still a useful backup technique. Post-mortems produce a lot of ion suppression in LC-MS, and the results are less honest than GC-MS results. The Toxtyper™ is a better way to handle the samples and get more satisfied customers – our customers have learned that we're quicker now, and have more sensitive techniques for most substances," explained Dr Böttcher.

Before switching to LC-MS, general unknown analysis using GC-MS on oral fluid was not cost-effective, and therefore not offered to customers. If customers are unaware that a certain type of screening is possible, they will not generate demand for it. The introduction of the Toxtyper™ allowed the laboratory to lower the cost of general unknown analysis, and therefore offer this service. To herald this, last year at the German Addiction Medicine Conference, the group's presentation won the first prize – an unusual accomplishment for a laboratory topic in addiction medicine. "They were convinced that this was a very nice approach, because with one shot you can see so many substances," commented Dr Böttcher.

Another reason LC-MS is increasingly favoured over GC-MS is the reduction in sample preparation time. Dr Böttcher explained how these time savings occur:

"With GC-MS, you have to complete three sample preparation steps – hydrolysis, extraction and derivatisation – before you can run the samples, all of which are very time consuming. All these steps are very selective and this is why we have a loss of substances. In addition, the samples must be run in batches as sample prep is so cumbersome, so if an urgent sample arrives late it is uneconomical to add to the routine. This is disadvantageous for general unknown analysis, as you don't know what you are searching for in the first place. The Toxtyper system does not require these selective sample preparation steps and therefore, random access is possible. Post-run time is also greatly reduced compared to GC-MS and data-mining is more efficient, and you don't need as much experience."

Screening Capabilities

In some cases, customers might know which drug they want to screen for, in which instance a multi-targeted screening approach can be used. The sensitivity of this method is much higher than general unknown analysis (untargeted approach), but in cases of intoxication where the drug group involved is not known, general unknown analysis is required (*Table 1*).

Table 1: LC-MS drug screening capabilities at the MVZ Laboratory Dessau, Limbach Group.

	General unknown screening	Multi-target screening (LC-MS/MS)
Number of substances	>4000	68
Sample type	Oral fluid, urine, blood, vitreous humor, gastric contents	Oral fluid, urine, blood, vitreous humor, hair, meconium
Screening capabilities	**General Unknown Screening** Screening for the complete broad range of drugs including: Opiates/opioids Benzodiazepines 145 Synthetic cannabinoids Amphetamines Antidepressants and many more	**Dedicated methods** ø Opiates/opioids = 65 substances ø Benzodiazepines and set substances = 75 substances ø Synthetic cannabinoids = 100 substances ø Amphetamines/designer drugs = 70 substances
Sensitivity	1-25 ng/ml	0.1-2 ng/ml

Multi-target analysis is conducted and embedded in the laboratory's multi-target analytical system with LC-MS/MS. This can be modified and adapted to the customer's individual needs, and currently holds the capability to screen for 68 different substances. The laboratory's dedicated multi-target methods, for example an opiate method or a benzodiazepine method, are comprehensive for that specific substance class.

"The customer usually decides when we conduct multi-target analysis or general unknown analysis, but this can depend on the case. For example, if a certain case cannot be solved using one dedicated analysis, general unknown analysis is implemented. But if a customer requests an opioid test, we would use the dedicated opioid method. For the opioid method we can test for over 60 substances, the benzodiazepine method, 50 substances and with the synthetic cannabinoid method we can test for approximately 100 substances. This can be applied to oral fluid, but also other body fluids," commented Dr Böttcher.

In addition to ultra-high performance liquid chromatography (UHPLC), the LC-MS solution draws upon a comprehensive drug library for toxicological analysis. Dr Böttcher describes how the availability of such a library facilitates their work:

"When we heard that a library is available, which is at least the same size as the GC-MS library, but with the addition of glucuronides, we were immediately interested. If the library contains all the important glucuronides, hydrolysing isn't necessary. To establish such a library on your own is very complicated, because you can't buy these glucuronide molecules. Hydrolysing cleaves the glucuronides, which is time-consuming and selective because some substances are not cleaved 100%, and you can't buy these substances to verify your methods. This is not widespread knowledge, and is an important issue in urine toxicology. The combination of the library containing the glucuronides and the Toxtyper™ instrument is a big success, especially for urine analysis."

Dr Böttcher's laboratory uses two drug libraries for their toxicology work; one developed by Bruker and one by an external scientific institute: the Maurer/Wissenbach/Weber (MWW) Library (Wiley-VCH, Weinheim, Germany, 2014, TT-M2). The laboratory uses Bruker's library, which contains approximately 1000 parent substances, for scheduled purposes: there is a narrow detection time window where the substance must be found if it is present. This leads to increased sensitivity as the software is directed to the specific substance. The MWW library contains 1500 substances as well as 3000 metabolites (including glucuronides) and unlike the Bruker library, it is not indexed on retention time. This means that sensitivity is lower, but the library is extremely comprehensive and contains the necessary metabolites in addition.

Toxicology in the Future

Due to the rapidly changing industry in which laboratories such as MVZ Labor Dessau conduct their work, new methods must constantly be developed to keep pace with the drugs market. To facilitate this, instrumentation and software must also remain up-to-date. Extensions of external drug libraries are usually a top priority for future developments. Many new psychoactive substances and synthetic cannabinoids break into the market at a rapid pace, so are not all included in the current libraries.

Laboratories are able to add substances to the Toxtyper™ library themselves but for urine samples, metabolites are required and cannot be bought like the parent substances. Updates to urine metabolite libraries are, therefore critical to the continued success of toxicology laboratories.

About The Author

Rohan Thakur, Executive Vice President at Bruker Daltonics. Dr Thakur has over 20 years of experience in mass spectrometry, including 14 years in MS development and has several patents in the field of ion optics. During his career he held positions as Director Global Marketing for mass spectrometry solutions at Thermo and was Director of Drug Discovery at a Pharma CRO for 2 years before joining Bruker. Dr Thakur received his PhD in Chemistry from Kansas State University and did post-doctoral studies at Rutgers University, where his work involved using high-resolution MS analysis to prove the formation of ring-opened benzene metabolite-DNA and protein adducts.