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- Feeding Forensics: Novel Instruments for Advanced Drug Screening Analysis

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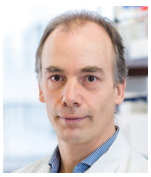
Mass Spectrometry

Feeding Forensics: Novel Instruments for Advanced Drug Screening Analysis

How ultra-fast, robust drug screening data is obtained at the Institute of Forensic Medicine, University of Freiburg using Bruker's Toxtyper™ solution



Professor Dr. Volker Auwärter's laboratory at the Institute of Forensic Medicine, University of Freiburg, benefits from specifically developed toxicological solutions, to further their research and services in forensics.

"With Bruker, we have a special kind of relationship – it's a great working atmosphere. Our co-development of the Toxtyper™ solution has provided us with ultrafast identification of drugs and toxins using LC-MSn, for our research and service needs."

The Institute of Forensic Medicine, Freiburg

The Institute of Forensic Medicine, based in Freiburg, Germany, is an academic laboratory renowned for its specialist capabilities in toxicology research and analysis, and is a leading national institute in forensic toxicology. The Institute makes up a department within the Medical Center at the University of Freiburg, which itself is organized into three sub-departments: Forensic Medicine, Forensic Molecular Biology, and Forensic Toxicology, with a total of 45 employees. 25 of these staff work in the forensic toxicology laboratory, and include four scientists, eight PhD students and four apprentices.

Professor Dr. Volker Auwärter, Head of the Forensic Toxicology department, has worked with the Institute for 11 years, and has experienced the progression of technological development and the evolution of techniques used to undertake both routine and uncommon drug screening cases. Prof. Dr. Auwärter leads the team and oversees the public and private sector activity of the Institute and is supported by Dr. Jürgen Kempf, a graduate engineer at the Institute, whose role is to coordinate the laboratory's research and routine case work.

As an academic laboratory, the key priorities of the Institute's work lie in research and analytical services, often for requests dealing with legal cases. The role of the Institute is to conduct toxicological analysis on cases where drugs or drugs of abuse (DOA) are involved. This primarily involves testing blood, urine and hair samples and in post-mortem cases, stomach contents, vitreous humor and occasionally organs such as liver, kidney, and muscle tissue. In some instances, analysis of powders, liquids, paper or other materials is also requested. Due to the nature of samples analyzed, semi- or broad-targeted approaches are needed to screen for a vast array of analytes.

The Institute undertakes work for a range of clients, including forensic psychiatric clinics, police/legal authorities and clinics such as ER, Children's Hospitals and the Poisons Information Centre. Depending on the case, the laboratory's work ranges from simple blood alcohol analysis to full post-mortem toxicology. In addition to drugs testing and death case analyses, the Institute carries out European Union (EU)-led projects, with a primary focus on drugs market monitoring. With their world-renowned expertise in the field of forensic toxicology, the Institute is placed at the forefront of novel technological approaches to drug and DOA screening.

Prof. Dr. Auwärter comments on the Institute in Freiburg's role in other countries:

"We conduct analysis for many surrounding countries, such as Luxembourg, Belgium and Poland. Although we'd like to, it's hard to send samples to us from further away as shipping biological material is complicated. We do cooperate with a laboratory in the US, where we contribute to some of their method development. Within Germany, most of the samples requiring

comprehensive new psychoactive substance (NPS) analysis are sent to us, because we're known for having the best methods to detect those substances."

Collaboration

With a history of collaboration with industrial partners, the Institute benefits from the instruments and expertise that such relationships offer, and in return their partners benefit from the participation of knowledge acquired from the laboratory's research. The laboratory has developed a close partnership with its vendors, and has been working with Bruker Daltonics since 2010. This two-way collaboration has seen the co-development of the Toxtyper™ and Toxtyper™ screening library, which are crucial to the Institute's work. The instrument was developed specifically to meet their needs, and therefore has a strong forensic focus. The Institute first bought the Toxtyper™ in 2013 for analysis of serum, urine and vitreous humor from post-mortem samples, roadside-testing and intoxication cases. A grant for a second Toxtyper™, installed earlier this year, was provided by the Medical Center at the University, and the instrument is used for analyzing drug powders and pills. It is necessary to analyze high concentration drug samples and trace-analysis biological samples on different systems to avoid contamination.

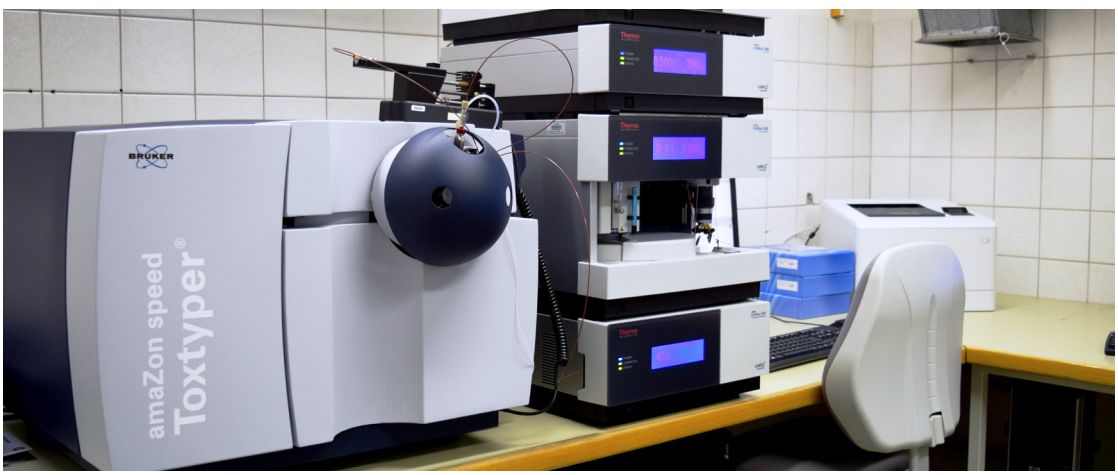
Besides using the Toxtyper™ and for a routine screening approach, the Institute is constantly developing new methods, for example for new compound classes, semi-quantitative screenings or libraries consisting of smaller subsets of compounds. When they see potential for a new technique, they substitute existing methods with automatic reporting (Toxtyper) or high resolution mass spectrometry with the ToxScreener™.

"A major advantage of working with Bruker is their size."

"Hardware, software and application development are all situated in the same building, so the expert for every problem is available personally or via WebEx" explains Dr. Jürgen Kempf.

Funding and Grants

Due to the Institute's focus on research, the majority of funding received by the laboratory is for this purpose. *"The laboratory stands on two legs: one is academic, where we have funding for teaching and research, and the other is the service we provide to private facilities and investigative authorities. The two elements support each other – if we're good in research, we can offer new, specialized methods to our customers, and if we provide a good service, this generates revenue to channel into innovative research. We're not a high-throughput laboratory, so we always have to keep on top of the latest developments in order to offer specialized services private laboratories with high-throughput machines are not able to provide"* explains Prof. Dr. Auwärter.



The Institute works closely with the EU, conducting EU-funded projects since 2011, which maintain a core focus on drugs market monitoring and development of methods to detect NPS in biological matrices. Every two years, the laboratory must apply for new funding, and over time the nature of the project has changed depending on the EU commission's goals. Prof. Dr. Auwärter explains the process:

"We have to assess what the EU commission wants, and which project would fit in with our purpose."

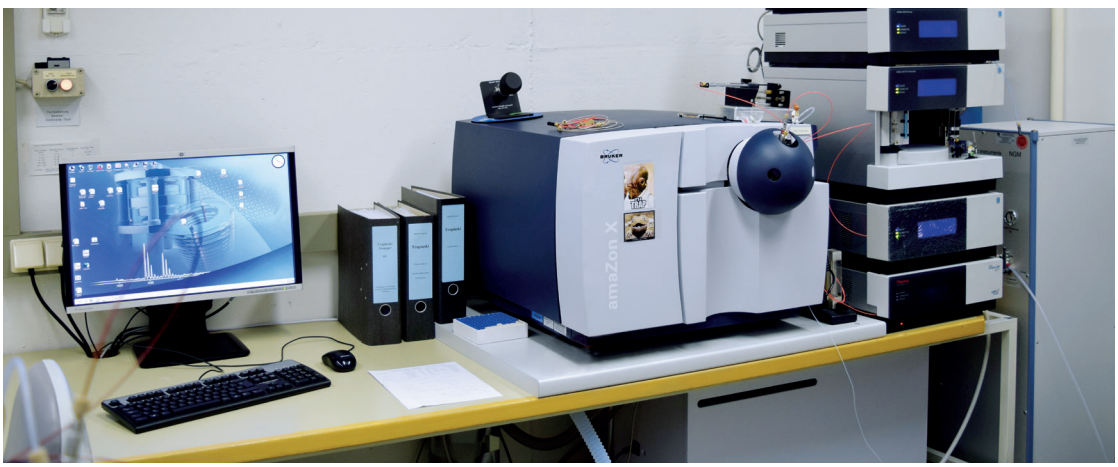
"Previously we have applied for a security research project, as well as the Drug Information and Prevention Program – you always have to look out for those which fit, and often need to adapt to them. In the beginning, the projects were more constructed around questions of prevention, whereas now, the projects are covered more by the criminal investigation area. At the moment, together with the Federal Criminal Police Office, we're contributing to some profiling work to see which kinds of impurities are found in drugs, to learn about their routes of synthesis."

The Toxtyper™ plays a key role in the EU monitoring projects: drug samples are purchased online by the laboratory to be analyzed for the principle active compounds. The Toxtyper™ quickly identifies these samples and where drugs have already been screened before, the instrument is used to identify specific groups of drugs, for example synthetic cannabinoids, and simultaneously look for unknown compound spectra. These compounds can then be selected, purified and then have their structures analyzed with other instruments.

Challenges and Solutions

Liquid chromatography-mass spectrometry (LC-MS) is a valuable tool for qualitative and quantitative analysis of biological samples in clinical and forensic toxicology. Before the development of the Toxtyper™, a major set back for toxicology research was the speed and reliability of drug identification. Previously used techniques such as gas chromatography-mass spectrometry (GC-MS) and immunoassays cannot provide the same high level of detail and specificity of information, and the range of compounds covered is far broader using LC-MS. Immunoassays, despite their popularity, are unable to cope with the structural diversity of substances such as synthetic cannabinoids and yield unreliable results. This has fueled the need for comprehensive screening method for detecting drugs and DOA in biological specimens.

Prof. Dr. Auwärter explains a key challenge facing the industry: *"As you may know, there are approximately 100 new substances appearing on the drugs market each year. You really need to be very close to market developments to keep pace with your methods. I'm quite sure that many countries keep their eyes closed on*



many types of substance, since a lot of effort is required to maintain your methods, and therefore a lot of cases go unresolved as there are not enough facilities."

The key analytes detected by the Toxtyper™ come under the groups of psychotropics and synthetic cannabinoids. Synthetic cannabinoids first emerged onto the recreational drug scene in 2004, and were first detected as an ingredient in the 'legal' cannabis substitute, *"Spice,"* in 2008. Since then, an increasing number of herbal mixtures containing changing synthetic cannabinoids have dominated the drugs market.

These so-called 'legal highs' are able to bypass current legislation and drugs testing, and stay ahead of the evolving prohibition laws by containing a slightly modified variant to the previously banned version. Due to the broad structural diversity of synthetic cannabinoids, and the constant emergence of new derivatives, comprehensive screening capabilities are demanded of toxicology laboratories. The Toxtyper™ - an LC-MSn library-based solution – can be applied to address these analytical challenges. The approach can be easily customized by adding new compounds to the library as they emerge, therefore keeping screening methods up-to-date.

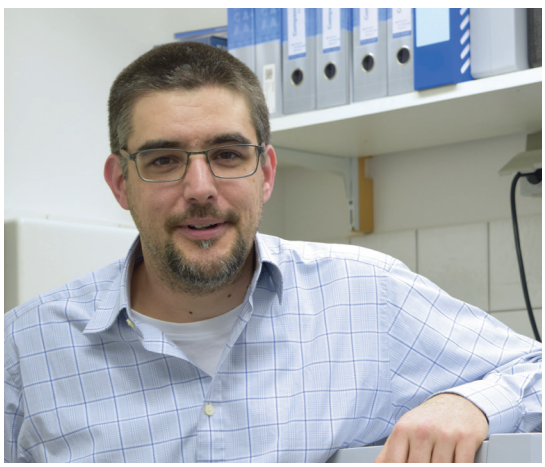
Staying ahead of Spice

Due to the nature of the Institute's work, certain trends in the drugs market will come and go. In 2008, the problem of detection of new psychoactive substances (NPS), and 'Spice' in particular, swept across the industry and as a result, a large proportion of the laboratory's work is involved with detecting this substance.

The initial EU Spice project (JUST/2009/DPIP/AG/0948) conducted by the laboratory ran between 2011-2012, with the overarching aim to strengthen prevention work by providing evidence-based reasons against the use of these drugs, thereby delaying first use in young people. Providing the law enforcement with quick information on new drugs on the market was also a key objective. Following this, the Spice II Plus project (JUST2011-2012/DPIP/AG/3597) (2013-2014) developed an integrated and innovative approach to tackling the issues of NPS, focusing on evaluating toxic potentials and elucidating health risks by characterizing the type of damage, the underlying mechanisms and activation/detoxification pathways. The current Spice Profiling project (2015-2017) studies the metabolism of selected NPS, and methods for the detection of such drugs in human biological samples have been developed.

The personal health dangers of Spice are often overlooked by users due to the 'legality' of the drug. The reality is, however, that intoxication cases involving Spice are increasingly requiring intensive care treatment, with recent reports of fatalities.

As specialists in NPS detection, the demand for this service from the Institute has been high, so the research effort to keep up to speed with rapid developments in identification of new compounds in NPS is huge. Since 2012, screening for NPS has become an issue even in standard cases, and is a constant factor in the laboratory's work:



"Every death case we complete will undergo the whole spectrum of NPS screening"

adds Prof. Dr. Auwärter, *"although this is a huge public health concern, it is good for us as the demand for our specialist services has risen."*

"In Germany, NPS detection cases often get sent to us. I'm quite sure that in many countries this type of substance goes undetected, but for us it's a constant factor of our research and services. As a subgroup of analytes, synthetic cannabinoids make up a large proportion of the work we use the Toxtyper™ for. For many years we have had one project going on with the EU involving NPS" comments Prof. Dr. Auwärter.

The Work

Due to the variety of cases presented to the laboratory, the samples used for drug screening will depend on the case. For example, urine and/or blood are the matrices of choice when conducting systematic toxicological analysis, but in many cases (such as in post-mortems), sufficient sample volumes are often unavailable. In such cases, stomach contents and organs such as liver, kidney, and muscle tissue are suitable, although the sample preparation process can be more laborious. Vitreous humor – the gel substance between the lens and retina of the eyeball – is a suitable alternative to using blood or urine, and is routinely analyzed in post-mortems in Freiburg.

Currently, the Institute receives approximately 11,000 cases to analyze per year, ranging from simple blood alcohol analysis to full post-mortem toxicology.

The source of the samples sent to the laboratory varies, and has changed over time:

"In the beginning, death cases and services for investigative authorities were the main source of work for us as a forensic laboratory. Over time, we have received more samples from the police, taken from offenders under the influence. Then it developed further and we now have more clinical samples, for example from prisons and forensic psychiatric clinics. Now this represents the larger portion of our work" explains Prof. Dr. Auwärter.

The process of analysis also depends on the source of the sample. For example, samples from the police usually come with an idea of the type of analyte to be detected: they have usually conducted pre-tests in urine so have a broad idea of the class of drug in question. In these cases, the laboratory only needs to screen for a few analytes. Prof. Dr. Auwärter explains the more complex cases:

"The death cases are less clear: if there is a suspected toxicological reason of death, we are sent the case but most of the time, we have no idea which poison we are looking for."

"We always perform broad screening methods, for example with the Toxtyper™. With this approach we then get untargeted peaks in the spectrum and can look further in depth at these."

Approaches to Screening

To analyze drugs and DOA in biological matrices, LC-MS and GC-MS are used, but when analyzing body fluids, LC-MS has become the key method of choice. An automated LC-MSn approach has been developed for the detection of drugs and DOA in a range of biological samples. The Toxtyper™ facilitates the broad screening methods required by the laboratory's various clients, and allows automated data evaluation, speeding up the process and allowing more samples to be evaluated in a shorter time frame. The open library concept of the Toxtyper™ allows rapid and simple generation of new qualitative methods for drug detection – ideal for an innovative laboratory such as that at the Institute of Forensic Medicine.

"It is often the case that, if they could, customers would always like to have the whole picture of xenobiotics present in the sample. But money is a restriction for them: if you want to conduct a full toxicological analysis, the price will be very high. This is why it's valuable to us to be able to offer broad screening with the Toxtyper™, covering almost all relevant analytes, at a lower price. It's a benefit to us and to them." explains Prof. Dr. Auwärter.

The Toxtyper™ was developed to simplify LC-MS drug screening, so that the instruments could be easily operated on a daily basis by every laboratory technician. Benefits of the Toxtyper™ are its speed, ease of use and relatively simple methods of use; it only takes a few hours to learn how to use. *"In my opinion, operating and maintaining the Toxtyper™ is quite easy, and can easily be taught to laboratory technicians or students. The new Toxtyper™ 2.0 software has improved the usability of the instrument, by concentrating on the main functions like running a sample, reviewing data and building a method in one neat browser-like software interface"* comments Dr. Jürgen Kempf, Graduate Engineer at the Institute.

The robustness, quality of data and ease of use have simplified and accelerated the mass spectrometry screening capabilities in the laboratory, shortening the overall turnaround time of a case. By reducing the time spent on routine sample analysis, more time can be spent on complex intoxication or post-mortem cases, which is very valuable.

The laboratory also uses the impact II (ToxScreener™), primarily for research projects such as identification of metabolites, or to screen samples (serum, urine, hair) if the results of routine methods are negative, or data from routine methods is affected by matrix compounds. For this, the use of high resolution MS enables acquisition of data, which is unavailable when using unit resolution LC-MS/MS or LC-MS^n.

Before purchasing the Toxtyper™, the laboratory used a completely different technical approach to screening, using a Triple Quad with ion trap function. GC-MS screening has also been used, but requires an experienced technician and takes approximately 30 minutes to completely evaluate the data file. Now with the Toxtyper™, an automated result can be obtained in approximately 10 minutes, and the method can be learned quickly. The laboratory still uses GC-MS for screening, as it offers a different range of analytes that cannot be seen with LC-MS, but the new approach allows the laboratory to make significant time savings and therefore optimized the price-performance ratio.

Moving forward

The designer drug testing arena is rapidly expanding and is unlikely to slow down in the near future. Therefore, laboratories such as that of the Institute of Forensic Medicine will need to rely more and more on the advancing technologies provided by companies such as Bruker, which are specifically designed with these research challenges in mind. The commitment of vendors such as Bruker to provide the innovative instrumentation required for research Institutes to carry out increasingly complex work paves the way for future discoveries.

"We have a very good relationship with the people from Bruker..."

Prof. Dr. Auwärter explains, *"we visit them in Bremen and they visit us in Freiburg, as well as meeting at conferences. It's a great working climate we have with them, which benefits both sides. Their expertise is invaluable to us and propels each aspect of our work in forensic toxicology."*

For more information about the Toxtyper™, please visit https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/ion-trap/toxtyper/overview.html. For more information about the Institute of Forensic Medicine, please visit https://www.uniklinik-freiburg.de/rechtsmedizin.html.

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com - www.bruker.com