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REVIEW

The improvements in forensic toxicology and its role in the forensic process (I)☆

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Abstract The scope of forensic toxicology services has currently become technically and intellectually so demanding. As our society progresses, it becomes more complex, and crime takes place in different ways. The development of highly sensitive detection techniques and appropriate data processing has enabled the analysis of a wide range of compounds, now in a wider range of matrices. Multiple associated technological innovations such as artificial intelligence or chemometric techniques, provide forensic toxicologists with different tools to facilitate the management of a multitude of generated data, or to devise a more effective analytical strategy.
The role of forensic toxicology in the forensic process is reliable and relevant for the resolution of criminal cases but it is still in development to minimize or overcome its deficiencies. In this context, it is necessary to work with a qualified system able to ensure the reliability of the results and that guarantees security to the judicial system and therefore to the citizen.

PALABRAS CLAVE
Toxicología forense;
Inteligencia artificial;
Quimiometría;
Automatización;
Estandarización;
Estrategia analítica

Avances en toxicología forense y su papel en el proceso forense (I)

Resumen En la actualidad, el alcance de los laboratorios de toxicología forense se ha vuelto técnica e intelectualmente muy exigente. A medida que la sociedad avanza, se torna más compleja y el delito se presenta de diferentes formas. Esto requiere el empleo de técnicas analíticas sofisticadas que permiten trabajar más y mejor con procedimientos automatizados proporcionando resultados para cientos de compuestos en diferentes matrices clásicas, alternativas y novedosas.
El uso de estos equipos genera multitud de datos y son las innovaciones tecnológicas asociadas como la inteligencia artificial o la quimiometría, las que ofrecen herramientas para facilitar al

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toxicólogo forense la clasificación y el estudio de los datos o bien son útiles para diseñar la estrategia analítica más rentable.
Aunque se trabaja para evitar o minimizar las deficiencias en algunos puntos, el papel de la toxicología forense en el proceso forense es fiable y relevante para la resolución de casos penales. Para ello se necesita trabajar con un sistema de calidad que garantice la fiabilidad de los resultados y confiera seguridad al sistema judicial y por tanto al ciudadano.

Introduction

The ready availability of deadly poisons in England, Western Europe, and the United States in the 19th century caused public anxiety about the extent of murder by poisoning, highlighting a type of homicide that was particularly difficult to prevent or detect. These poisoning crimes prompted the creation of the first medico-legal specialty, forensic toxicology, to which some of the best-known experts of the Victorian era contributed, such as Matthew J. B. Orfila, Alfred Swaine Taylor, Thomas Stevenson, and Theodore Wormley.

In the absence of irrefutable chemical evidence of poisoning, convictions were generally based on circumstantial evidence or the occasional confession. However, this changed with the investigations of M. J. B. Orfila (1787-1853) into the detection of poisons, and as increasingly more poisonings were discovered, toxicology became a key aspect of forensic practice.[1]

The particular challenges of toxicology as a forensic discipline[2] have resulted in analytical methods that are constantly updated to keep up with new analytical trends.[3] These trends require consistent development of novel analytical tools, including efficient sampling procedures, appropriate sample preparation protocols, and suitable methods, to optimise the detection of compounds even at trace levels.

Advances in forensic toxicology laboratories have enabled the detection of increasingly more substances with increased sensitivity in multiple matrices.[4] Advances in instrumental techniques have been coupled with greater use of information technology and automation, which are the keys to streamlining workload and improving quality.[5] This combination provides a wealth of data with evidential value, which also means that laboratories must develop strategies and adopt new methodologies to address the challenges of managing and interpreting these data.[6]

Following the growth in the utility of forensic toxicology in recent decades, the caseload of many laboratories has doubled or tripled and the increasing demands on toxicologists to produce more results in shorter timeframes has increased the risk of failure.

Internationally, management of this risk has led to the introduction of quality assurance systems and accreditation by appropriate external bodies. Many laboratories have realised that with the increasing demand and size of laboratories, proficiency assurance systems are necessary. The European Network of Forensic Science Institutes (ENFSI) is one of the most important platforms for international cooperation in this regard.

Based on the assumption that the forensic toxicology laboratory is a part of the investigative process in the judicial setting, namely, the forensic process starting at the scene of the crime and ending in court, advances in forensic toxicology research have brought indisputable benefits, considerably increasing the speed and efficiency of the criminal justice system. However, these benefits are only effective when the quality of the process can be guaranteed to produce reliable results.[7]

This article presents the advances in the tools available to forensic toxicology laboratories to perform their assigned role in the forensic process.

Application of technological developments to forensic toxicology

Forensic toxicology has used highly sophisticated technology in recent years. Since its recorded inception in 1814, what was once a description of the effects of toxicants on the body has advanced in every area through modern forensic toxicology, and technological advances have enabled toxicologists to identify how different substances are absorbed and distributed in the body.[8]

In forensic toxicological analysis, chromatographic techniques have evolved enormously over the decades with different types of chromatographic separation columns, specific stationary phases, processing software, and detectors, including mass spectrometry, which has developed significantly over the last 20 years.[4]

The most remarkable improvements in forensic toxicology are clearly down to instrumental development. The field of forensic toxicology was revolutionised by the development of immunoassay, portable gas chromatograph-mass spectrometry (GC-MS) in the 1980s, and later liquid chromatography tandem mass spectrometry (LC-MS/MS) in the first decade of the 2000s. LC-MS/MS has provided picogram-level detection in a time-efficient manner, as it requires less sample preparation, avoiding cumbersome derivatizations, among other things.

The recent trend started with hyphenated high resolution mass spectrometry (HRMS), mainly with liquid chromatography in analytical toxicology.[9] The current detection of trace

analytes has enabled the analysis of new samples, such as hair or oral fluid, together with blood and urine.

It is a remarkable advance that it is now possible to test the single administration of certain intoxicants in drug-facilitated crime, to detect a completely new type of drug, or to estimate the chronology of drug use with a strand of hair.

It is also noteworthy that new psychoactive substances (NPS) with similar chemical structures, sometimes even structural isomers, stereoisomers, or "optical isomers", can be distinguished by the detection of single metabolites in biological fluids. This development of analytical technology helps in identifying cause of death and provides toxicological criteria for the development of international standards on NPS.[10]

This instrumental development has been accompanied by new technologies that have, as in other areas, played an essential role in forensic toxicology. Technology can be viewed as a major catalyst for the transition from scientific discoveries and knowledge to innovations and it is not surprising that these new technologies lead to growth; when any new investigative technique is introduced, there is always pressure to put it into practice. The 3 disciplines that have benefited most from the introduction of new technologies are forensic toxicology, DNA analysis in forensic genetics, and forensic information technology.[11]

For many years after colorimetric testing, the discipline of analytical toxicology has developed in line with the technology of new equipment, incorporating digital transformation and accompanying innovations.[4]

New equipment with new technologies

In terms of technological advances for compound screening, LC-MS was increasingly used in toxicological analytical systematics, which for a long time was dominated by GC-MS.

Soon after, the common feature of LC-MS/MS screening methods overcame the major drawback of early LC-MS mass spectra, which often contained relatively little spectral information, because compound identification is based on information-rich MS^n mass spectra.

In recent years, LC-MS/MS in Selected Reaction Monitoring mode has become the "gold standard" for targeted screening of multiple analytes, often combined with quantification. The identification power depends on the selectivity and the number of monitored transitions.[9] Modern LC-MS/MS allows the detection and quantification of a large number of molecules in samples of unknown composition, often frequent in forensic toxicology,[12,13] but while LC-MS/MS can determine the characteristics of a given compound, its lack of mass accuracy sometimes does not allow it to distinguish 2 molecules of the same mass and with the same fragments. Therefore a more accurate technology is needed.[14]

Therefore, forensic toxicology laboratories now use chromatography equipment hyphenated with HRMS for the detection, identification, confirmation, and quantification of organic compounds in samples of various kinds. The great advantage of HRMS is its greater precision and mass accuracy due to the better performance of the analysers, which allows the exact mass of a compound to be identified unequivocally. The ability to identify the exact mass of a compound is the first step in the structural elucidation of a compound by reducing the number of targets.

It is now common to have instruments with time-of-flight (TOF) or Orbitrap (OT) mass analysers coupled to an extra quadruple (QTOF, QOT) that allow selection of the precursor ion and work in MS^2 or MS^n mode, respectively. They are very useful when the compounds of interest are unknown and not in libraries (unknown-unknown). A quadrupole at the front (QTOF, Q-OT) allows MS^2 or MS^n fragmentation into reproducible spectra[15] if fragmentation conditions are maintained. The high resolution helps to resolve the differentiation of isobaric compounds with the same nominal mass but different elemental compositions.[16]

With both techniques, different compound screening strategies can be applied that are essentially based on measuring the mass of a compound or fragment with sufficiently high accuracy that its elemental composition can be directly determined.[17,18]

HRMS establishes the mass of analytes and their fragments, elucidating their molecular formulae. The list of possible candidates can be narrowed down to one or a limited number of compounds, which can be further investigated in the available databases. In other words, the analysis can initially be performed without reference standards, because the general information provided by HRMS allows the identification of compounds.[19] The combined use of high resolution with more classical detection techniques such as infrared spectrometry, Raman, or even different specific capillary columns in gas chromatography allows the identification of almost all compounds.[18] Even so, final analysis by MRI is essential for identification if no standards are available in case legislation is required.

HRMS is currently the most powerful and flexible technique in analytical toxicology and is used for various applications, such as targeted and non-targeted detection, quantification, drug metabolism, and metabolomics. HRMS is now the only technique that meets the criteria of an all-in-one device for the various applications needed in analytical toxicology and HRMS can be expected to become the gold standard, and replace most assays with other techniques in the future, considering suitable separation and/or ionisation techniques such as GC with EI, or LC with ESI, APCI, or APPI.[9]

From HRMS, data acquisition can be performed using either data-dependent (usually MS^2) or data-independent mode. In the former case, compounds are quantified by the exact mass of the precursor ion and confirmed by matching the library of product ion spectra, taking into account the exact mass and intensities of these ions.[20] A limitation of this approach is that it does not allow retrospective data on new compounds if they were not subjected to MS/MS, therefore, samples would need to be extracted and re-analysed, which may not be possible. This problem is overcome with stand-alone data acquisition

that offers the possibility of retrospective data acquisition of all spectra from both MS and MS/MS. The main limitation in this case is multiple precursor ions simultaneously undergoing ionisation in the same MS/MS event, spectra containing product ions are generated for all precursor ions[21] and their sensitivity is reduced.

One of the most advantageous applications of high-resolution instrumentation is the targeting of NPS in forensic laboratories for qualitative detection, either by gas chromatography-mass spectrometry or high-performance liquid chromatography-mass spectrometry.[22] The proliferation of NPS has sparked considerable interest in the development of so-called "non-targeted" screening strategies to detect and identify putative new compounds without using certified reference materials (CRMs) or mass spectral libraries,[21] which is a clear example of the abovementioned "unknown-unknown".

Alternatives have been developed for quantitative analysis without CRMs, such as the use of quantitative nuclear magnetic resonance spectroscopy (1HqNMR) with internal standard, which allows quantification in seized drugs, based on the fact that each signal in the spectrum is proportional to the number of hydrogen atoms generating the signal.[23] The use of CRMs with similar structures or from the same family of compounds is accepted for certain quantifications where CRMs are not available or do not exist.

Along these line, other authors have proposed the use of new equipment consisting of gas chromatography (GC) coupled to nitrogen chemiluminescence detection (NCD) and atmospheric pressure chemical ionisation quadrupole time-of-flight mass spectrometry (APCI-QTOFMS). In this concept, the GC stream is split in appropriate ratios between NCD for single-calibrant quantification using the equimolar response of the nitrogen detector and QTOFMS for accurate mass-based identification, offering a possibility for qualitative and quantitative analysis in the absence of reference standards.[24] The use of these lines of work in the field of forensic toxicology is still in its infancy and remains to be evaluated in practice.

Other instrumental techniques such as cyclic voltammetry, electrochemical detectors, or capillary electrophoresis are less common in forensic toxicology laboratories.[25] In other cases, Raman spectrometry or infrared spectrometry can be used for identification in drug seizures because they do not destroy the sample and can be used in special situations in identifying compounds in biological fluids.[26] These techniques are of great help and provide a great deal of information in the structural identification of compounds with isomerisms that are difficult to elucidate by HRMS.

Digital transformation in forensic toxicology

The sophisticated equipment discussed in the previous section operates using computer systems that collect and archive data which are processed by hardware and software with specialised algorithms that allow comparison with mass spectral libraries. Libraries are typically available from instrument suppliers or other commercial library developers that are regularly selected and updated for new compounds. Library development is generally based on information from primary sources, such as CRMs, and secondary sources, such as information published in the literature, which limits the databases to known compounds. In addition, commercial libraries are often updated with new analogues; however, it is unrealistic to believe that libraries are updated as soon as new analogues are detected and therefore there will be a delay in detection capabilities.[21]

Forensic laboratories are using these computerised systems for case management and for processing analyses and results.[27]

New digital tools also provide time-saving steps in analysis, interpretation, and reporting workflows so that key information can be identified, and problems solved more quickly. Some even allow integrated customised calculations that eliminate errors caused by exporting data. Centralised instrument management and system administration increases laboratory efficiency and instrument uptime.[28]

Although the increased reliance on digital technology creates risks for laboratories, the benefits such as traceability and data integrity, reliability, and reproducibility of results from extracted and stored information, and the use of artificial intelligence (AI) to support forensic analysis, outweigh the drawbacks. Digital transformation continues to advance and forensic laboratories require a robust strategy to manage the associated risks and seize the opportunities.[29]

Associated innovations

Artificial intelligence (machine learning, deep learning, expert systems)

AI is a discipline that combines computer science and data sets to enable problem solving. It also encompasses the subcategories machine learning and deep learning, which are often mentioned alongside AI. These disciplines comprise AI algorithms that seek to create expert systems that make predictions or classifications based on input data. These disciplines have made significant advances in the various domains of forensic science and play an important role in supporting the justice system as they can connect various databases with other sources of information in the investigative process and link data across disciplines to link current and past crimes.[30]

Machine learning is used to handle large data sets and offers new possibilities, such as artificial neural networks, which can be designed to classify large amounts of data. To review and interpret this data quickly and efficiently, machine learning consists of 4 steps: pre-processing of the input data, conditioning of the deep learning model, storage of the prepared learning model, and implementation of the model.[4]

Machine learning has addressed different problems in the field of LC-MS and has been used in different publications to predict chromatographic retention time and to implement metabolite fragment prediction. However, so far, it has not been tested for use on raw HRMS data sets, e.g. non-extracted mass spectra, to distinguish between blank samples and samples containing the analyte, but it shows enormous potential for data handling.[31]

The use of expert systems, as a result of the development of AI, is nothing new and has been in parallel with advances in computer hardware since the 1990s.[32] Expert systems can be described as a sophisticated computer programme for solving problems in a small sector by emulating expert thinking. They have been used to predict time and response in cases of amitriptyline deaths and for data pattern recognition.[33]

Recently, algorithms have been developed to prevent the development of new drugs. An example is DarkNPS, which can produce 8.9 million compounds that could be created by modifications of existing drug molecules. The authors used a neural network (a type of machine learning) to generate this number, that vaguely resembles the human brain and is often used to analyse human languages. It works like a human brain understanding a sentence, but the algorithm uses atoms and chemical bonds instead of words and grammar.[34]

Chemometric techniques

Chemometric techniques (multivariate analysis and other statistical methods) are recognised as powerful tools in forensic science to interpret and optimise analytical procedures.[35] Chemometrics is a chemical discipline that uses mathematics, statistics, and formal logic to design or select experimental procedures to provide the most relevant information by analysing data and gaining knowledge about chemical systems. The main application of chemometric methods is the design of experiments (DoE) that optimises the method and evaluates chemical analysis data with the minimum number of trials.[36]

In chromatography, DoE is used in method validation specifically to identify significant factors in robustness studies and then optimise a response to them. Plackett-Burman designs are used for validation studies, while the most popular optimisers are fractional factorial designs and their extensions, such as central composite designs.

Using design of experiments, Costa et al.[37] achieved the optimal combination of the values of the factors studied (temperature, time, and volume) in the application to the development of the method to test for morphine 3-glucuronide in urine. Taking into account the interactions between the factors, they obtained the best results while simultaneously optimising resources and saving time and costs.

Other authors have applied DoE by means of multifactorial analysis to test for ethyl glucuronide in hair samples taking into account the influence of factors such as extraction solvent, ultrasonication, temperature and incubation time, solvent amount, and hair particle size.[38] Along the same lines, Alladio et al.,[39] conducted a systematic evaluation of the conditions of ethyl glucuronide extraction from pulverised hair by DoE considering extraction time, temperature, pH, and solvent composition as possible influencing factors.

One field of application of chemometric techniques in toxicology is in the analysis of seized drugs, the results of which are used to identify and/or quantify active ingredients to support the judicial process.[40]

Multivariate analysis and other statistical methods include data processing with pre-screening of data, preprocessing of data, and calculation of similarity scores between samples.[41] For a significant number of samples, multivariate statistical analysis is advised due to its ease of interpretation of results, reliability, and speed and it is suggested the principal component analysis (PCA) method is used before developing any mathematical model, because it reduces the chances of error. The spectra or chromatograms obtained by the different analytical methods may vary between different types of samples and chemometric methods extract the information to individualise and classify sample classes, using what is called pattern recognition. These patterns are further divided into supervised and unsupervised pattern recognition. Unsupervised pattern recognition includes PCA, which is the most widely used in the analysis of seized drugs.[42]

A recent example is the application of this chemometric method (PCA) to exploratory analysis in the prior evaluation and subsequent development of an analysis protocol using another chemometric model, SIMCA (Soft Independent Modelling by Class Analogy), to classify the mixtures of the predominant solvents (dichloromethane, trichloroethene, and chloroform) in the composition of the profile of "loló", a very common inhalant in Brazil, using near-infrared spectroscopy.[43]

A recent review of the most common chemometric techniques for illicit drug profiling has also concluded that several configurations of chemometric techniques can assist in the interpretation of data, harnessing their ability to add value to research and provide insight into drug markets. In the case of illicit drug data, these patterns are related to batch trends, links between specimens, geographical location, distribution route, or synthesis route. Drug profiling has proven useful in confirming links originally posited by researchers and has also shown the potential to identify previously unconnected entities.[44]

Chemometric techniques such as HCA (hierarchical clustering), PCA, and k-NN (k-nearest neighbours) have been used to simulate the chain of distributors and check the influence (if any) of diluents on the analytical results of illicit drugs.[45]

In addition to its usefulness in the study of chemical-toxicological cases, chemometrics can also provide additional information in complex crime cases and improve productivity by enhancing data handling and interpretation processes in various applications. Large data sets from

different cases can be processed using these techniques, for tactical or intelligence policing, as well as for crime analysis and prevention purposes, by improving the usefulness of information in databases.[46]

Improvement resources. Automation

The mission of forensic laboratories is to maximise the value of evidence and because price and quality are relatively fixed, the primary measure of service effectiveness is timeliness.[47] The increase in demand for forensic services has far outstripped the resources allocated; laboratories are feeling the pressure of increased workloads and experiencing challenges in responding to these demands and to better serve justice.

The ability to process and analyse increasingly more forensic samples requires not only increased laboratory capabilities, but also the efficiency of the system to meet the demands and effectively address the backlog of cases to contribute to the judicial investigation. There are many ways to measure and track workload over time - requests received, tests performed, backlogged cases, and the length of time taken to process a case.[6]

Turnaround time is often used to assess the efficiency and performance of a laboratory. This metric has been a point of contention among stakeholders and is often misunderstood and misinterpreted. Turnaround time is generally defined as the number of days between the submission of a forensic analysis request to the laboratory and the delivery of test results or issuance of a report. If the analysis has not been completed within 30 days of receipt by the laboratory, it is considered a backlog in many laboratories.[48]

Some laboratories have implemented the Lean Six Sigma methodology (methodology aimed at improving processes) to increase profitability and productivity, make better use of resources, and improve their performance by working more efficiently to reduce backlog and turnaround time.[6,11] In other cases, innovative mechanisms for better use of resources and facilities have been established to increase efficiency, for example with the design of lean facilities.

Automated sample preparation and analysis is another resource used to reduce turnaround times of case samples in forensic toxicology laboratories, combining high-throughput procedures, while consistently producing laboratory data.[49] Automated methods provide improved accuracy, precision, and standardisation of results, and therefore, by implementing automated solutions in the laboratory workflow process, the efficiency of the lifecycle of the evidence sample can be maximised to reduce delays and turnaround times.[50]

Modern forensic toxicology laboratories are moving towards simplification, miniaturisation, high throughput, automation, online coupling with instrumental systems, small sample volumes, and a strong reduction or absence of organic solvents according to green analytical chemistry principles.[51]

Simplification is related to the sensitivity of high-resolution instruments and allows the replacement of tedious extraction methods with single incubation to confirm the presence of illicit drugs, even in complicated samples such as hair.[52]

Furthermore, automation (sample handling) has increased the throughput of laboratories requiring little or no interaction from the analyst. This has increased the number of samples per unit time, decreased human error, controlled resources, minimised sample volume, and integrated extraction with analytical tools.

In forensic toxicology, sample preparation is a fundamental prerequisite for the successful application of high-tech analytical tools for the qualitative and quantitative determination of substances. With technological advances and increasing analytical sensitivity, eliminating interferences in the matrix is essential to optimise the analytical method. For this purpose, different procedures are available such as liquid-liquid extraction, solid phase extraction, liquid supported extraction, phospholipid depletion, or direct injection into the system when the matrices can be diluted (dilute and shoot) to acceptable concentration levels for the technique used and the required detection limit. The latter is very useful in the forensic field with the introduction of HRMS.

Due to its high extraction efficiency and its possibility of miniaturisation (microextraction)[53] and automation, solid phase extraction (SPE) is of great importance in analytical toxicology. SPE automation is often driven by the need for laboratory accreditation, decrease in systematic errors, reduction of costs and time spent on each case, and finally improvement of technician safety. Different devices can be used for SPE automation, each with its own advantages and disadvantages.[54]

Although the "dilute and shoot" method comes with potential challenges and risks, such as the presence of matrix components or substances that interfere with the analytical system, it is a very popular method for doping control. Together with LC-MS, dilution can quickly and accurately detect and quantify stimulants and narcotics in urine samples.[49] Other equipment that avoids sample preparation, such as DART-MS (Direct Analysis in Real Time Mass Spectrometry) or DART-ToF (Direct Analysis in Real Time - Time of Flight), is restricted to use in forensic toxicology to analyse samples from drug caches due to the difficulty of interpretation, especially in cases of complex mixtures, requiring costly databases.[55] Nevertheless, by combining it with machine learning algorithms, very recent publications have used it for differentiation of positional NPS isomers.[56]

Regardless of the type of automation, the technology helps to increase the number of samples prepared for analysis within a given time period, to decrease human

error, closely control resources, track samples with the use of barcodes, and minimise sample usage by reducing the volume needed for subsequent analysis.[57]

New samples

Technological innovations have enabled the detection of more substances with increasing sensitivity in a variety of matrices. The analytical focus has been on classical matrices such as blood and urine, but interest in other matrices could further increase, especially in post-mortem (PM) situations. In this context, the role of PM changes and possible drug redistribution requires further investigation and identification of the presence and extent of markers. While instrumentation has improved, in the future, nanotechnology may play a role in selective and sensitive analysis as well as bioassays.[4]

The increased sensitivity of analytical techniques enables the detection of compounds at increasingly lower concentrations, which not only allows the use of smaller volumes of traditional matrices (blood, urine), or complementary or alternative matrices (hair, saliva), but also enables the detection of compounds in less common matrices.

Fingerprints (sebum and sweat) are such a matrix. They are a non-invasive matrix, difficult to falsify, which can be traceable in a sampling procedure and ensure the chain of custody. Other studies explore the possibility of its use to determine from a fingerprint whether a person has used or touched a drug.[58]

Expired air is a new matrix for drug detection. Since the first demonstrations of its usefulness,[59] the new methods of sampling and application of mass spectrometry have enabled the detection of several drugs such as amphetamines, opioids, benzodiazepines, cocaine, and delta-9-tetrahydrocannabinol (THC) in breath samples in security-related cases.[60–62]

However, there remain key interpretative considerations such as pharmacogenomics and drug-drug interactions, as well as determination of tolerance and, in the future, analytical confirmation of an individual's metabolic profile that may support a personalised medicine and judicial approach.[4]

The need to work to quality standards

Traditionally, forensic services have remained largely unregulated by governments, with a reliance on voluntary standards and limited public investment in the development of specific forensic standards. However, over the last decade there has been a strong international call for quality forensic standards and there is now a conscious and real push to establish, where possible, standards applicable to forensic sciences.[63]

The successful implementation of new substance identification techniques has major impact on the reliability of the results and therefore for the criminal legal process. Applying quality standards to the toxicology laboratory provides evidence that the laboratory operates under a quality system, proves that the laboratory is technically competent, and demonstrates that it is capable of generating technically valid results. Therefore, standards related to method development and method validation have been paramount over the last decades.

Therefore, reporting reliable toxicological analytical data is a prerequisite for the development of forensic investigation and for the interpretation of toxicological findings. The international forensic scientific community requires results and data in case reports that are valid, reproducible, and comparable. Also, the quality of routine work must be guaranteed as the final results are used by judicial authorities to implement legal measures.[64]

Unlike other forensic disciplines, toxicology has benefitted from the experience of analytical chemistry, and from most of the standards or guidelines that standardise its application in specific fields such as the food industry,[65] or for general analytical procedures.[66]

However, different recognised bodies are now developing specific best practice guidelines that toxicology laboratories can incorporate in developing an effective quality system.

In general, the Standard Practice for Quality Assurance of Forensic Science Service Providers Performing Chemistry Analysis, which was last updated in January 2021, should be highlighted. This practice provides a quality framework for the processing of evidence.[67]

More directly, we can highlight the United Nations Recommended Guidelines for Quality Assurance and Good Laboratory Practice, the guidelines of the Society of Forensic Toxicologists, and the American Academy of Forensic Sciences, the European Laboratory guidelines for Legally Defensible Workplace Drug Testing, those of the United Kingdom and Ireland Association of Forensic Toxicologists,[68] the French Society of Toxicology,[69] the German Society of Toxicology and Forensic Chemistry,[70] and the International Association of Forensic Toxicologists.[71]

Other standards focus on specific parts of the analytical process such as method validation and are established by international organisations both in general terms[72] and targeting different forensic disciplines, such as toxicology.[73] There are also well-established articles published in peer-reviewed scientific journals.[64,74] All of them highlight validation as an essential part of the process in forensic toxicology to ensure the reliability of the results issued to the judicial bodies. Validation is the process that demonstrates the inherent quality of an analytical method by generating objective evidence that ensures a specific use. First the objective of the method must be established, then method development, and finally validation will assess "fitness for purpose" by meeting predefined requirements.[64]

The American National Standards Institute/American Standards Board (ANSI/ASB) published in 2021 the first version (still under revision) of 3 standards concerning the analytical scope and sensitivity of blood toxicology tests in

medico-legal investigations, in driving under the influence investigations, and a standard for the analytical scope and sensitivity of urinalysis in cases of drug-facilitated crime investigations.[75]

With specific guidelines for forensic toxicology laboratories and general guidelines for various purely metrological and analytical issues, we can meet the requirements of ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories,[76] which sets out requirements for generic aspects of a forensic laboratory, including management, training, and general processes, but does not provide specific guidance for any particular discipline.

International standards are used not only to implement a quality system in toxicology laboratories, but also to achieve and maintain accreditation, which is the recognition by an independent body of a laboratory's competence to deliver technically valid results.[77] The current trend for forensic laboratories is to obtain accreditation for the tests they perform, and it is even a requirement of the ENFSI that 50% of the tests are accredited for the laboratory to belong to the network.[78]

The number of accredited laboratories worldwide has grown considerably over the last two decades. Data from 2020 reveal that there are more than 50 accredited laboratories in England, 30 in Romania, and 19 in the Czech Republic, in addition to many others in various countries of the European Union to improve the mutual exchange of information in forensic sciences required by the ENFSI.[79] In Spain, official forensic laboratories belonging to the State Security Forces and the National Institute of Toxicology and Forensic Sciences, and private laboratories that issue forensic results are accredited for various tests.

Given the difficulties for forensic laboratories in complying with the requirements of international standards, in 2007, ILAC created an additional document that addresses the forensic science process as a whole and provides common guidance in areas where activities overlap or where insufficient instruction is provided (such as field testing). The resulting document, ILAC G19:08/2014 Modules in a Forensic Science Process, provides guidance for laboratories, crime scene investigation units, and other entities involved in examination and testing in the forensic science process.

Along the same lines, the ISO TC 272 technical committee, with representatives from 23 countries including Spain, is working on the preparation and publication of a new standard, ISO 21043 Forensic Sciences, with 5 parts: the first on terms and definitions, the second on the recognition, recording, collection, transport, and storage of items, the third on analysis, the fourth on interpretation, and the last on reporting. Parts 1 and 2 are currently approved and translated.

The abovementioned standard and other standards and guidelines can be used as guidance for forensic laboratories; however, they are not equivalent to competency-based standards (17025:2017) and cannot be used to substitute conformity assessment, in other words, they cannot be used for accreditation. However, they can be used within a quality framework or can be assessed independently in third party accreditation, within the scope of accreditation, against a competency-based standard.[62]

Considerations

The current situation and future outlook for forensic science in the ENFSI Strategic Plan 2020-2023 document "Trends in Forensic Science"[80] indicates that all activities included aim to provide forensic science services that are reliable, transparent, impartial, and robust from crime scene to courtroom.

The importance of toxicological analytical results in the forensic process means that they must be scientifically valid and that the methods and procedures used ensure their accuracy, precision, and specificity. In other words, they must be reliable.

Increased sensitivity with the new analytical equipment used in forensic toxicology laboratories and associated innovations in method design and data handling have enabled lower concentrations to be detected in traditional, alternative, and new matrices using automated workflows and validated methods within the framework of quality assurance.

These advances are the result of technological innovations in forensic toxicology in collaboration and multidisciplinary research with tools provided by other branches of science such as statistics or emerging technologies, to the benefit of our input to judicial decisions.

There is a broad panorama of possibilities, but it is also subject to the investment of personal and material resources which makes it difficult to have all the equipment mentioned in many cases. This should not be a problem as long as the laboratory knows its limits and has a quality system in place to guarantee the results it issues.

This article presents an overview of developments in forensic toxicology laboratories from a strictly analytical point of view. However, the role of the forensic toxicologist does not end with the issuing of results, they must interpret these results based on the information received and the scientific information available. We shall present the advances that have been made in the interpretation of forensic toxicological results in a second part.

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