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Beyond resolution: gaining confidence in targeted screening and quantitation in toxicology with Thermo Scientific Orbitrap technology

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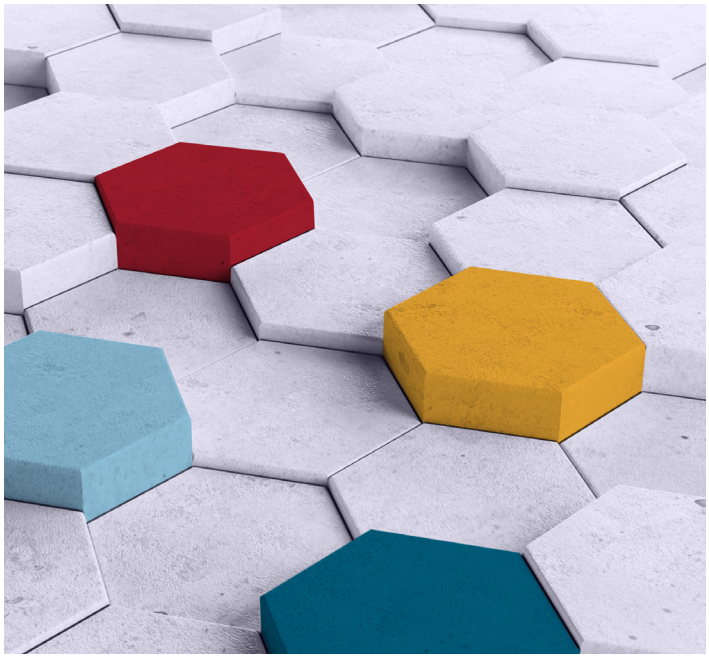

Most of us love watching the edge-of-the-seat crime thriller shows and movies where law enforcement is working to determine cause of death. Any biological sample available at the scene of the crime, whether it is blood, urine, or tissue, is collected and sent to a state-of-the-art laboratory for analysis. While the unsuspected criminal plans for his escape, it is the science in the laboratory that makes it possible to identify the criminal—beyond reasonable doubt. While the above-mentioned situation belongs to a forensic crime scene, there are several other areas where a drug (or mixture of drugs), when consumed, can have serious consequences. From deleterious drugs of abuse, to athletic performance enhancers and from employee monitoring to determining the contents to determining the contents of the 'unknown white powder' found in the trunk of a car—the discipline of toxicology can be divided into four major segments:

- **Clinical toxicology**—Discipline of detection and identification of drugs or other xenobiotics and their metabolites in human specimens for research. The number and type of compounds can vary significantly and range from prescribed drugs to drugs of abuse comprising novel psychoactive substances and novel psychoactive substances (NPS)
- **Forensic toxicology**—Uses a host of analytical chemistry, pharmacology and clinical chemistry-based technology to aid medical or legal investigation of death, poisoning, and drug use. Determining or identifying the known and unknown substances ingested is often complicated by the body's natural processes, as it is rare for a chemical to remain in its original form once in the body.
- **Sports anti-doping**—Consumption of banned substances as performance enhancers is a common concern in every sporting event. The term 'doping' is used extensively by organizations responsible for

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regulating human and animal sporting competitions. From determining and quantifying trace amounts of performance enhancers in athletes to optimizing biological passports for both humans and horses as a part of doping—scientists in the world of sports anti-doping seek high efficiency workflows further developed and optimized by LC-MS technology. Ensure confidence in identification to quantitation of performance enhancers and critical metabolites in any biological matrix.

- **Workplace testing**—A growing list of prohibited drugs that are commonly consumed has redefined the landscape of workplace testing to determine if employees or job applicants are under the influence or have taken drugs. From amphetamines, cocaine, marijuana, to opiates and alcohol markers—LC-MS assays used for workplace testing in today's world seek wider coverage of analytes and sensitivity for monitoring trace analytes, compared to immunoassays. Gain confidence with high resolution screening with an extensive library and ensure sensitivity requirements are met with this optimal LC-MS platform solution.

Considering the diverse nature of toxicology, and continuous challenges around analyte chemistry and matrix complexity—analysis of these samples requires an optimal combination of efficient analytical instruments and productive workflows that offer robust, reliable, reproducible, and sensitive assays. These assays may include numerous chromatographic methods, enzymatic tests, immunoassays and trace elemental analysis by spectroscopy. In everyday clinical chemistry, physicians usually ask for a specific compound allowing laboratories to use a very specific method for determination for this analyte. However, in clinical toxicology research, the analytical strategy usually begins with fast and unspecific methods in broad the case of an intoxicated subject in order not to miss substances present in blood or urine. After narrowing down the compound list, specific methods are applied with the hope to ID the target substance(s). In comparison, in the world of sports anti-doping, use of LC-MS has become prevalent to meet the rising demands of fast, robust, sensitive, and specific detection methods. Although GC-MS held a superior position in addressing various analytical challenges, such as those presented by volatile or polar target analytes, analyzing every analyte type can be challenging. The demands with regard to time and manpower, as well as the necessity to use hazardous derivatizing reagents in some applications, have further strengthened the position of LC-MS in sports anti-doping arena.[1] On a similar note, with increasing illicit drug consumption, many countries have already adopted drug testing as an essential tool to assess drug exposure in work places. From pre-employment drug testing to random testing of safety/security-sensitive personnel in designated positions to universal testing of all personnel on a random selection basis, LC-MS techniques have shown tremendous value and utility in workplace testing—from detection and quantification of drugs of abuse to determination of biomarkers of alcohol consumption.[2]

Immunoassays

The introduction of immunoassays into clinical and forensic toxicology in the early 1970s has had a major impact on the speed and efficiency at which biological samples could be screened for the presence of certain drug classes. Interpretation of immunoassay results has to take into consideration the limits of detection of the assay, the cross-reactivity of the antibody(ies) and the potential for interference.[3] (See Figure 1.)

In the past century, immunoassays have been used as the initial screen to assess compliance. They are also extensively used in most hospital laboratories for routine tests in clinical chemistry. Immunological kits have been defined as "the nine sisters" referring to the classes of psychotropic substances they detected: Amphetamine (AMP), Methamphetamine (MET), Barbiturates (BARB), Benzodiazepines (BZD), Cannabinoids (CNB), Cocaine (benzoylecgonine; BE), Morphine-specific (MOR), Opiates (class; OPI), Phencyclidine (PCP) and Lysergic acid diethylamide (LSD).[4] These tests are intended to provide quick information on the drug content of the sample that has been collected. For samples testing negative, no additional tests are necessary. However, samples that test positive, will have to be tested again using a confirmatory method for increased accuracy in results. This is where the selectivity and versatility of mass spectrometry comes in.[5] The process of confirmation is fundamental across many areas of toxicology, such as: fatalities or road accidents suspected to be due to drug intoxication, workplace drug testing, driving license regranting in former drug consumers, child custody and even cases of firearms license investigations—essentially, all these processes require a legal decision generated by a true positive result.[6]

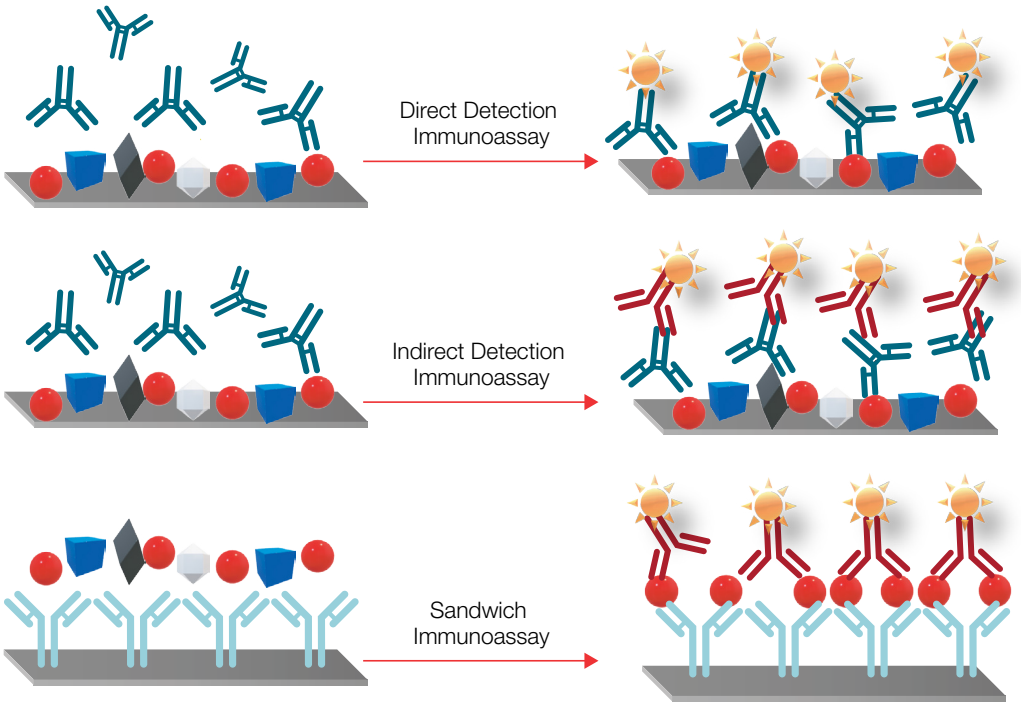


Figure 1. Different formats of Immunoassay typically followed in analytical laboratories.

Typically, an LC-MS/MS assay requires a clear understanding of the desired approach (e.g., qualitative vs. quantitative) and method development to include both choice of analytes and the best suited clean up approach applicable to the type of sample matrix involved. It is well known that immunoassays can only cover a small portion of drugs and their metabolites found in toxicology samples. Their methods run the risk of missing compounds that do not bind and/or cross-reacting with many other compounds that are not relevant. Immunoassays lack specificity and selectivity, which are typical strong points offered by LC-MS. Since immunoassays can cause false positive or false negative results due to lack of specificity or cross-reactivity, and immunoassays may not be available for a number of drugs, MS has been used for confirmation of immunoassay results[7] and sometimes used directly as screening methods. Recently, many developments have taken place in new fields of study, particularly endocrinology and hormone testing in clinical research labs.[8-10]

In the last decade, hundreds of novel psychoactive substances (NPS) have entered both the illicit traditional trafficking and web-based trade. These molecules, including synthetic cannabinoids, synthetic cathinones, fentanyl analogs, triptamine and piperazine derivatives and others, have been introduced as legal derivatives or analogues of traditional psychoactive substances. Most have a very short existence,[11,12] being sold for the time they remain legal and/or if they meet consumers approval. Although limited scientific literature exists on the toxicity of these substances, many acute intoxications and fatalities have recently been reported.[12,13] Immunoassays for fast screening at emergency departments are available for only a very limited number of NPS, so LC-MS/MS methodologies have been developed to cover the highest possible number of these substances.[14,15] To address the challenges posed by the constantly evolving market of NPS and other associated drugs of abuse, the analytical techniques warrant frequent updates.[3]

Mass spectrometry

The general mass spectrometric analytical approach for identification, screening, monitoring, and quantitation of drug compounds and their metabolites in biological matrices typically involves i) extraction, ii) separation, iii) detection and iv) quantitation. From the mid-nineteenth century, the evolution of extraction techniques started with development of liquid-liquid and solid-phase extraction methods. This was followed by development and adoption of chromatographic separation techniques both gas and liquid-based followed by detection by mass spectrometry (MS). Continued evolution of MS, GC, and LC technologies also resulted in sophisticated methods that offered significant advantages. Mass spectrometry is a powerful tool for both qualitative and quantitative analysis that functions by ionizing an analyte (of interest) and determining its mass-to-charge (*m/z*) ratio. MS dates to over a century ago to 1918, when J.J. Thompson ionized neon and separated the ions using magnetic and electric fields. For many years after, mass spectrometry was used

to detect isotopes and simple elements. The advent of novel ionization techniques such as, electrospray and desorption techniques enabled researchers to develop the ability to detect biomolecules, which quickly escalated to the development of analytical methods for disease detection and therapeutic treatments.

The evolution in MS technologies has resulted in significant improvement of the ion source design, sensitivity, improvement of resolution, mass accuracy, and ease of use. A mass spectrometer contains three main elements—an ion source, an analyzer, and a detector. The ion source is what makes the analytes of interest ionize into gaseous form in order to be transmitted through the mass spectrometer. The analyzer (also termed as mass selector), transmits ions to the detector, based on mass to charge ratio of the analyte ions. There are different MS analyzers, some of the popular ones are—quadrupole mass filter, ion traps, orbitrap, sector, time-of-flight, and Fourier transform ion cyclotron resonance. Each of these analyzers offer certain benefits and increased selectivity and specificity, such as isolating ions of interest or having enough resolving power to separate analytes from close *m/z* interferences. A good understanding of the importance of mass spectrometry as an impactful technology can be obtained from Figure 2. In a little more than hundred years, several significant discoveries in the world of MS has immensely enabled both research and applied laboratories.

One important feature of a mass spectrometer is the ability to fragment molecules, which in turn enhances its selectivity and ultimately results in confident identification. There are multiple fragmenting methods, such as collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), higher energy collisional dissociation (HCD), and ultraviolet photo dissociation (UVPD).

As described above, mass spectrometers are usually coupled with analytical instruments that either separate analytes by liquid (high or ultra-high performance liquid chromatography) or gas (gas chromatography) phases. Chromatography is a very important analytical technique because the instrument separates the analytes of interest from the matrix based on hydrophobicity and/or polarities. When LC and MS are coupled together, they become a powerful technique to detect a wide range of analytes in complex biological matrices.

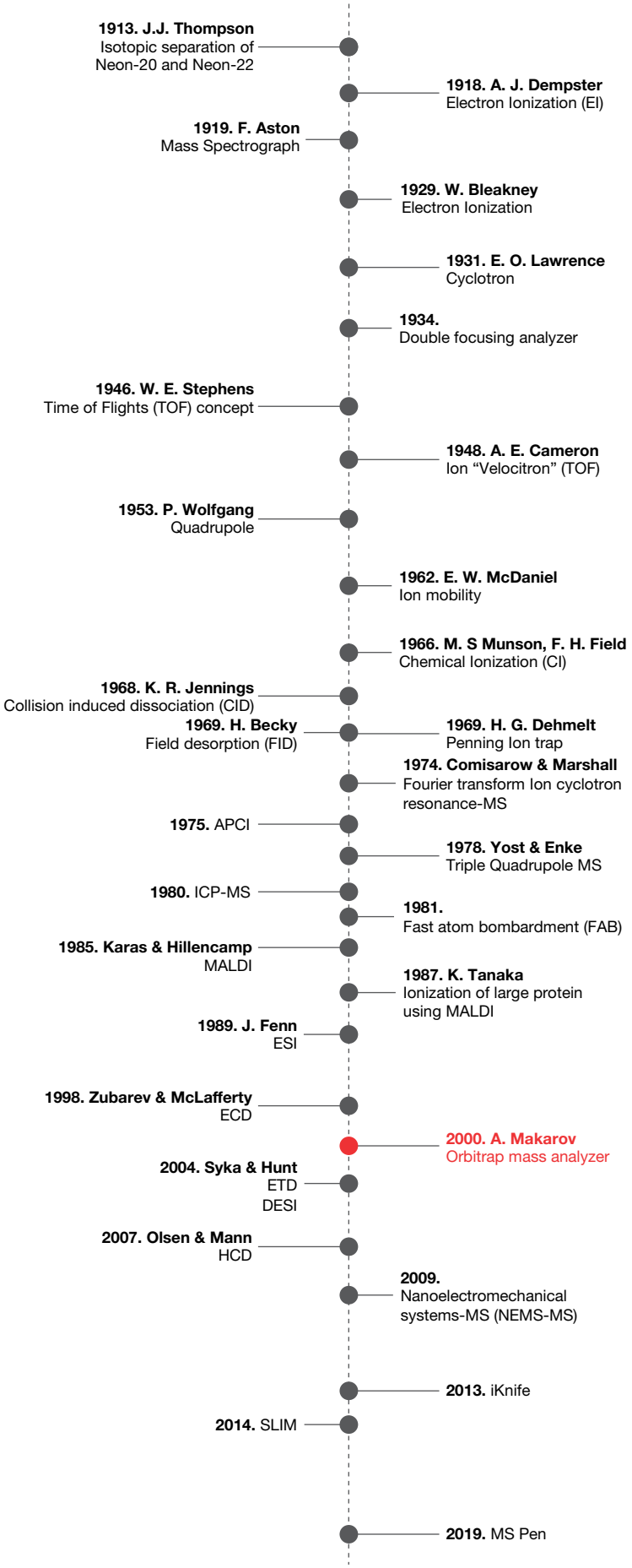


Figure 2. Evolution of mass spectrometry with timeline of ionization and fragmentation techniques.

GC-MS for toxicology

Advent of GC-MS revolutionized the analysis of trace amounts of toxic compounds in complex matrices such as, bio fluids. GC-MS based approaches were used in toxicology for almost 30 years, and is considered as the gold standard in the domain of toxicology. In order to improve detection and identification of compounds using GC-MS, negative and positive modes of analysis in MS have been integrated, taking advantage of the stability of the fragments after a positive or negative ionization.[16] In the recent past, GC-MS methodologies have been used to analyze heroin and cocaine.[17,18] However, if the analysis aims to identify target compounds, and if specific fragments of a molecule are known, it is possible to increase the S/N with the use of mass spectrometry in tandem (MS/MS). GC-MS/MS is commonly used in SRM and product ion scan modes with collision induced dissociation (CID).

Implementation of LC-MS in a toxicology laboratory

Reliable qualitative and quantitative toxicological analysis is the basis of a competent toxicological judgment and consultation in clinical and forensic toxicology. It is rather obvious that implementation of MS in a toxicology (Clinical or Forensic) laboratory is not an easy change—especially when the laboratory has been functioning with a different technology for a number of years.[19-27] While GC-MS has been widely recognized as the 'gold standard' in forensic testing, use of LC-MS is gaining popularity. LC-MS offers several advantages over GC-MS such as being faster, requiring less extensive extraction procedures, and being able to identify and quantify a broader array of analytes. Unlike GC based analyses, sample volatilization is not required for LC which thus avoids problems associated with chemical degradation and formation of new products that typically form under high heat conditions of a GC.[28]

The benefits of LC-MS—from achieving desired specificity and selectivity with the desired sensitivity to reaching the desired productivity goals—might have encouraged a transition to implementation of LC-MS, however, the technology comes as a major investment. From the perspective of both financial and human capital for any organization—this is an investment that requires careful planning and careful execution for a successful outcome. Some of the major steps that are to be considered in implementation of MS in a toxicology laboratory are summarized below in Table 1.

Table 1. Critical needs and considerations to be addressed by a laboratory for transitioning to LC-MS from other technologies.

Needs/considerations	Factors to consider
Analytical challenges	• Number and types of analytes to be identified, monitored and/or quantified • Reducing turnaround time, cost/sample • Controlling sample handling process and reducing handling errors • Developing robust, reliable, sensitive, and reproducible assays for one to a large panel of analytes • Building new methods for integration into laboratory workstream
Financial	• Acceptable return on investment • Bringing tests in house and reducing send-out costs • Investing in the instrument(s)—capital investment • Understanding other financial responsibilities – Service terms and conditions – Infrastructure and space requirement – Connectivity to existing LIS – Daily operational expenses (reagents, gas, etc.)
Optimal instrument selection	Clear understanding of analytical challenges (targeted or untargeted screening to sensitive, targeted quantitation) to be addressed
Assay selection	Depends on type of instrumentation, analytes, Laboratory staff experience and training

LC-MS workstream—components in a toxicology laboratory

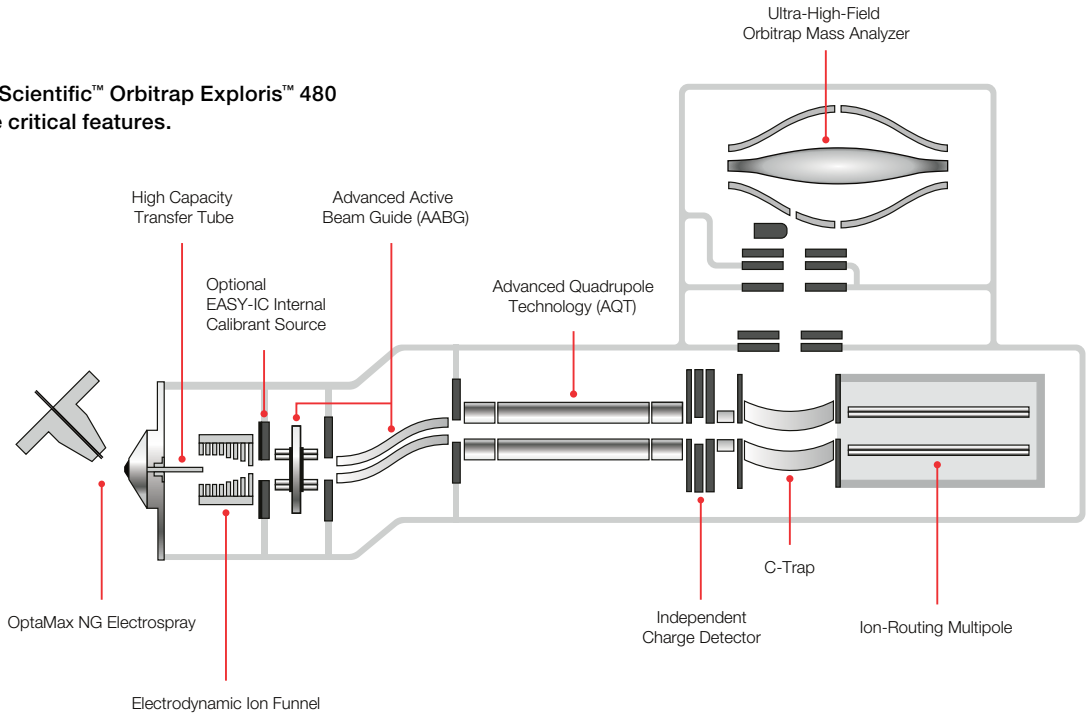
Clinical research laboratories are increasingly using LC-MS technology for detection and identification of compounds in biological samples. The most frequently used samples in toxicology are blood (plasma/serum), and urine with the main analytes of interest being therapeutic drugs, drugs of abuse and toxic compounds. Most LC-MS screening procedures analyzing smaller groups of compounds end in multi-target screening procedures as opposed to having a systematic toxicological analysis.[7] Such methods have helped to extend the spectrum of sensitive and specific MS-based methods to analytes, which are not amenable to traditional GC-MS analysis because of hydrophilic or thermolabile properties.

In general, LC-MS methods in toxicology can be divided in two categories: Targeted methods for identification and quantitation of a limited number of known compounds, and, methods intended for unknown screening aiming to identify every compound present in a sample. As discussed above, chromatographic methods coupled to mass spectrometry are the methods of choice for these confirmation assays. While GC-MS was the gold standard for this purpose[29,30], it has been replaced to a large extent by LC-MS in toxicology laboratories owing to the laborious sample preparation. However, for LC-MS methods, several factors are to be taken into consideration for efficient, robust, reliable development and implementation of targeted screening and quantitation assays using LC-MS technology:

Choice of mass spectrometer

Traditionally, for targeted screening and quantitation of a well-defined number of analytes, triple stage quadrupole (QqQ), ion traps (IT), or linear ion traps (LIT) are used every day in most toxicology laboratories.[31] Their speed sensitivity, reliability and reproducibility requirements allow every toxicology laboratory to use QqQs as the 'gold standard' for assays that screen for a limited targeted list of compounds, while also offering excellent quantitation of trace amounts of analytes of interest. The compounds that are typically monitored belong either to the drug class which has been positively pre-screened by the immunoassay or to drug classes which cannot be detected by immunoassay (e.g., opioids, antihypertonic drugs). The number of compounds belonging to one drug class may be huge (e.g., benzodiazepines, synthetic cathinones, cannabinoids, etc.), but there is a defined number of compounds which need to be included in the respective confirmation method. In samples with a positive result from the immunoassay, which cannot be confirmed by LC-MS, it might be helpful to elucidate which compound has led to the false positive result. It may, therefore, be useful to add compounds to the respective confirmation assay which are known to interfere with a certain immunoassay. However, as already discussed in this report, the continuously increasing number of NPS and the need to screen for more drugs/metabolites that may have been missed constantly challenges LC-MS technology. The recent advances in the high-resolution accurate-mass (HRAM) mass spectrometers powered by the revolutionary Thermo Scientific™ Orbitrap™ technology (Figure 3) enable every toxicology laboratory to now conduct targeted and untargeted screening using the same platform.

Figure 3. Schematic of the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer highlighting the critical features.



In contrast to the targeted analyses typically practiced, the screening approach should be untargeted for a comprehensive toxicological analysis, which is only possible with HRAMs.[32]

Data-dependent analyses with HRAMs do not perform a preselection of ions, allowing for additional analysis and therefore, compounds not being part of the initial database can be seen in the chromatogram. Using the exact mass of a yet unknown peak, the structural formula of the compound can be elucidated. With QqQ, IT or LIT instruments a toxicological screening can only be performed as a targeted method in order to ensure enough sensitivity using data dependent acquisition.[33] Due to the preselected ions either undergoing MS^n fragmentation or SRM, compounds having an *m/z* ratio not being part of the library in the respective time frame cannot be seen at all. While use of HRAM for screening is not new to the world of LC-MS, the present HRAM instruments now offer significant sensitivity allowing targeted quantitation of analytes along with qualitative screening identification. HRAM helps in addressing a lot of the critical issues (from providing a comprehensive high resolution data via full scan MS to enabling the researcher with the ability to conduct retrospective analysis without having to reinject the sample) typically faced when trying to screen for a large panel of drugs.

Mass spectrometry, by itself, has undergone a paradigm shift in the last 10–15 years. Today, accurate mass data with unambiguous mass assignments are mandatory for MS and, increasingly, for MS/MS mass spectra.[34, 35] Critical benefits of HRAM, enable some critical benefits:

1. Screen, identify, confirm, and quantify—all with one instrument. Address multiple requirements in daily toxicology assays with one instrument
2. Confirmation of analyte structure—ensuring a confident start for both identification and confirmation that can ensure
   a. Analysis of unknown-unknowns to known-unknowns—identify every signal in every run
   b. Retrospective search for new compounds without additional experiments—resolution full scan data—from identifying every signal to data mining and looking for analytes that were missed
3. Utilize data-dependent acquisition for the identification of unknown structures via MS/MS spectra
4. Exceptional mass accuracy and stability within and between runs—even at low mass ranges

Matrix effects

Matrix effects (ME) are a common issue faced by most in the toxicology laboratory. ME are influenced by several conditions such as sample matrix complexities, sample preparation, chromatographic separation, and the type of ionization technique(s) that are implemented.[36] During validation of a confirmation method, the ME needs to be carefully tested using either a post-column infusion approach or a post-extraction addition approach, with at least five to six blank matrix sources for each type of matrix analyzed.[37, 38]

Chromatography

ME may have a huge influence on the detectability of different analytes in confirmation assays, and the conditions for chromatography have to be selected very carefully. In addition, isobaric compounds (compounds with same *m/z* ratio) cannot be separated by mass spectrometry and require chromatographic separation.[39] For targeted screening assays, isobaric compounds that are not part of the panel of analytes to be detected should be taken into account to prevent false-positive results. An optimal combination of chromatographic conditions, including that of the stationary and mobile phase, can address many ME challenges.

Since its invention in the mid-1970s, a steady development of HPLC techniques has occurred with respect to general performance parameters, such as separation efficiency (increasing plate number), sensitivity, reproducibility, robustness, automation, and applicability to a wide range of analytes and matrices, and to adaptation for special analytical problems. Ultra-high-performance liquid chromatography (UHPLC) columns are packed with porous sub-2 µm particles and are run under ultrahigh pressure conditions (Figure 4). The present generation of UHPLCs, such as Thermo Scientific™ Vanquish™ UHPLC system, enables outstanding resolution and separation under high pressure conditions, which in turn, ensures the fastest turnaround of samples meeting the productivity goals. Compared to columns of larger particles, columns of sub-2 µm particles, offer some distinct advantages, such as:

1. Efficient, fast separations that allow better separations without increasing the run times
2. Sharper signals that enable better higher detection sensitivity
3. Faster separations reduce solvent usage

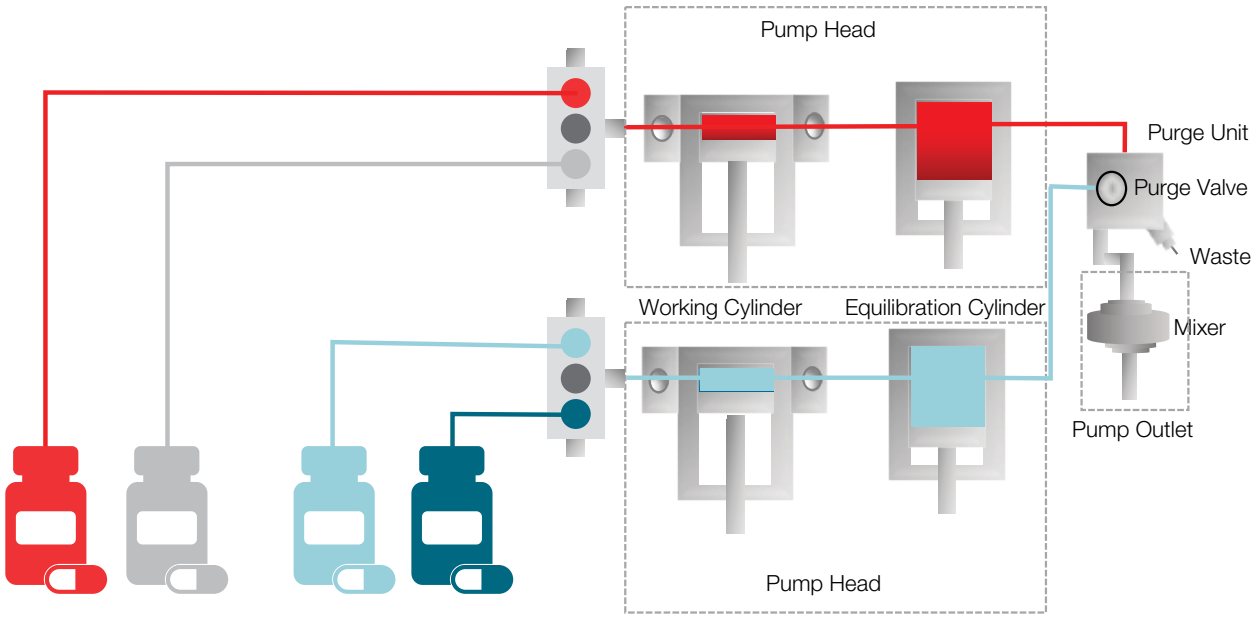


Figure 4.
Schematic setup of a high-pressure gradient pump.

Sample preparation

The first step in the analytical process has a huge impact on the more detailed LC-MS based identification/screening/quantitation assay. Most targeted analyses are multi-target assays that are comprised of not only a large number of analytes, but analytes with different chemical attributes, such as polarity, size, hydrophobicity, etc. A thorough understanding of the target, and diligent optimization process of sample preparation protocols offer critical advantages.

The majority of the cases analyzed are blind, that is, the substances causing death are unknown. A wide variety of compounds can be found, ranging from highly lipophilic to moderately polar in nature, and exhibiting basic, acidic, or neutral properties. Extraction of analytes from biological matrices is one of the most tedious and time-consuming steps in systematic toxicological analysis. In addition, the extraction step is required for several reasons: to eliminate possible substance interference, to concentrate and stabilize the analytes that may be in the sample, and finally to take the sample to the optimal conditions for instrumental analysis. Some typical sample preparation procedures available are as follows:

Dilute and shoot

The simplest method of sample preparation of urine samples is the “dilute and shoot” approach. This method has been successfully used across several assay types including screening of antihypertensive and several other classes of drugs in urine. However, it has also been suggested that there is a need for careful method validation regarding the influence of the matrix on ionization especially for early eluting components.[41]

Protein precipitation

Protein precipitation is another commonly used sample preparation process.[39] It is typically conducted by addition of organic solvent to a serum, plasma or urine sample resulting in precipitation of proteins. The resulting clear supernatant can directly be injected into the HPLC system. This method allows a good recovery of most analytes and is widely used in most toxicology methods with LC-MS. However, one has to be aware that the endogenous phospholipids and other potentially interfering compounds are not removed and then can cause ME’s if an additional sample preparation step is not added. Due to the necessity of a centrifugation step, automation of protein precipitation can be complicated for some laboratories.

Liquid-liquid extraction (LLE)

This is a simple, robust and transferable approach which whereby the sample matrix is reduced by fractionating into solvents.[37,42] This process requires evaporation of the solvent and reconstitution of the residue in mobile phase—which adds multiple steps, requires additional time, and increases chances of error. For a single sample it may be better suited towards urgent analyses than solid-phase extraction (SPE), however, it may not be applicable for hydrophilic compounds as the formation of emulsions can make it difficult to isolate the extraction solvent. The other disadvantages include large amounts of organic solvents and the challenges to automate LLE when the solvent needs to be evaporated. Sensitivity is significantly compromised in the absence of this evaporation step.

Solid-phase extraction

SPE is a sample preparation technique that chromatographers use to remove interfering compounds, concentrate the analyte, and extract the anayte prior to performing LC-MS analysis. SPE offers different possibilities for a very selective extraction of the compounds to be analyzed as there are many different stationary phases available.[43]

Traditionally, LLE has been routinely used in most toxicology laboratories. However, in the past several years SPE has become a popular technique in the preparation of samples for analysis and has been increasingly used for extracting drugs from biological matrices. Among the several advantages that SPE offers over LLE are higher selectivity, cleaner extracts, more reproducibility, and the avoidance of emulsion formation.[44,45] SPE removes uncertainty and adds consistency to chromatographic results by reducing the adverse effects of the sample matrix and significantly improving robustness, reproducibility, and sensitivity of LC-MS analyses. Disadvantages of SPE include the cost of the SPE cartridges or plates and the need for specialized equipment to perform the extraction. Also, like LLE, the process is geared towards a specific group of analytes at the expense of others. This is not ideal for a large panel screening method containing analytes with differing properties.

Mass Spectral library

The most important aspect for screening a large panel of compounds with a HRAM MS methodology is the spectral library which is referred to for identification of peaks and their respective spectra. Traditionally, LC-MS libraries containing mass spectra could only be transferred from one type of instrument to another (with difficulties), even when the instruments came from the same manufacturer.[46] Different ionization pathways and associated technologies contribute towards the difficulties in flexibility and utility of the generated libraries. However, advances in modern technology allow seamless portability and transfer of libraries across HRAM technologies. Such libraries are expected to be a comprehensive collection of MS/MS data and multi-stage MS^n spectra acquired at various collision energies, and isolation widths using Collision-induced dissociation (CID) and Higher-energy collisional dissociation (HCD).

LC-MS software

Often overlooked, software holds the key to maximizing the value of LC-MS based workstream in every analytical environment. For most toxicology laboratories, the expertise to operate LC-MS systems is often found to be mixed, which results in increased expectations from the software. The software is expected to be intuitive, easy to use, and capable of conducting every aspect of the assay—from method development to data monitoring, review, analysis, and reporting.[47]

A comprehensive solution—the challenge continues

The continuous developments observed in the world of LC-MS technology, along with issues commonly faced whilst using techniques, such as immunoassays or GC-MS, point towards the most obvious direction—increased adoption of an LC-MS workstream for everyday assays in toxicology laboratories. However, it is fair to say that transitioning to the world of LC-MS offers some initial complexities, which in turn, pose challenges to every researcher in the toxicology laboratory. As indicated in Table 1, these challenges comprise:

- A robust, reliable UHPLC method for ideal separation
- True high-resolution accurate mass spectrometers that can offer sensitive data
- Ability to confidently develop robust, reliable, reproducible, sensitive targeted screening and quantitation assays with LC-MS
- Easy access to a comprehensive and extensive library to cater to any toxicology panels
- Availability of proven, tested methods for increased efficiency and productivity
- A comprehensive configuration with all the necessary components that enable any researcher to address a large panel of toxic analytes, regardless of type and matrix complexity
- Achieve organizational scientific and business goals
- Reduce cost/sample while achieving desired sensitivity
- Laboratory staff experience and training
- Building new methods for integration into laboratory workstream
- Staying up to date on constantly evolving novel and synthetic compounds

Unfortunately, while several LC and MS systems exist, it is difficult to find a one-stop shop that can offer a comprehensive solution, enabling any toxicology researcher to confidently address the above-mentioned challenges.

Tox Explorer Collection for confident, concise, and comprehensive drug analysis

The Thermo Scientific™ Tox Explorer™ Collection is an easily implementable optimal combination of sample preparation guidelines, UHPLC and MS technology, an extensive compound database and library to enable screening and quantitation, proven methods and powerful software with customizable reporting templates, alongside comprehensive training and support—all geared towards enabling you to address your critical challenges in toxicology.

With a proven, pre-tested method, a comprehensive library with more than 1,500 molecules, and customizable sample preparation options, our UHPLC configuration and MS technology enables every toxicology laboratory to step up their productivity and achieve high quality analysis of data with confidence. From accurate targeted screening to robust, reliable quantitation methods—every toxicology laboratory can now achieve their scientific and business goals and maximize efficiency and productivity, fully capitalizing on the instrument's versatility, without having to worry about cost/sample or cost/analysis, varying methods or guidelines, for analyzing a multitude of compound classes,ensuring solution and instrument capabilities are maximized. Critical features of Tox Explorer Collection include:

- **Starting with confidence**—Proven methods in the Tox Explorer Collection allow every toxicology laboratory to accelerate their efforts to achieve highly efficient assays. The tested method allows everyone to start from a higher ground when it comes to targeted screening workflows for screening and quantifying drugs of abuse in biological matrices.

- **Robust software**—As described above, toxicology laboratories in the present world yearn for a software that is not only intuitive, but can easily transit between high resolution and targeted quantitation data, while also enabling fast processing, and customized report generation. High-throughput screening and quantitation assays in toxicology demands rapid access to confident results where users may have different experience levels. Thermo Scientific™ TraceFinder™ software can boost productivity through acquiring and processing targeted screening and routine quantitative data from LC and GC (including multiplexed and third-party systems) coupled to triple quadrupole and HRAM-MS systems.[48]

- **Expansive library for targeted screening**—An extensive library comprising more than 1,500 molecules contains a host of valuable information including compound names, respective chemical structures, computationally and manually annotated fragments (peaks), identified adducts and multiply, charged ions, molecular formulas, predicted precursor structures, detailed experimental information, peak accuracies, mass resolution, InChi, InChiKey, and other identifiers. The intuitive software with our open library ensures automated assignment of analytes covering a large range of compound classes.

- **Customizability enables efficiency**—Every toxicology laboratory has its own set of challenges. While it is productivity and throughput for labs with large sample loads, it is sensitivity for another. While resolution is sought by some laboratories for efficient screening of a complex mixture of analytes, another toxicology laboratory might face budgetary constraints and would prefer an affordable platform solution for conducting targeted screening and quantitation daily. Tox Explorer Collection offers a suite of LC and MS capabilities that allows every laboratory to achieve their goals with ease—regardless of their challenges.

- **Easy access ensures high efficiency and real-time results**—The Thermo Scientific™ mzCloud™ mass spectral library offers critical benefits from offering confidence for unknown identification to ensuring the reduction of the number of unknowns during an analysis. From accurately predicting structure from a spectrum to having wide applicability across research and applied analytical environments, all the information can be accessed via internet—without the user having to be logged in to his/her workstation. The real-time nature of the database ensures that laboratories have access to the latest list of available compounds, including novel psychoactive substances and synthetic compounds.

Identification of a large panel of drugs of abuse in complex biological matrices is challenging. The constant addition of NPS, new active metabolites, and other targets that need regular monitoring add to the increasing demands of speed, sensitivity and selectivity. While LC-HRAM(MS) offers some significant advantages owing to its superior resolution capabilities and ability to provide accurate results, the challenges of addressing these assay requirements are best addressed with a comprehensive workstream suitable for confident toxicology analysis, regardless of user experience or matrix complexity. Tox Explorer Collection allows every toxicology researcher to capitalize on a comprehensive and concise collection of instruments, library, methods, application training, support, etc. to achieve the desired confidence in data.

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