Interest of high resolution mass spectrometry in analytical toxicology: focus on pharmaceuticals.

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Abstract

In the last few decades, access to high-resolution mass spectrometry (HRMS) technology has expanded, especially in analytical toxicology where tandem mass spectrometry was considered the "Gold Standard". Given its selectivity, sensitivity and versatility, HRMS represents a technique of choice for pharmaceuticals analysis. Indeed, specific applications such as pharmacokinetic and toxicokinetic studies or metabolomics studies (in vitro and in vivo drug metabolism studies) have expanded with the development of the technology. HRMS may also be suited for screening and quantification of pharmaceuticals and in development drugs. The aim of this work was to obtain a clear overview about the interests of HRMS technology applied to the field of analytical toxicology, in particular to the analysis of medicinal drugs. Throughout this review we explored the potential of HRMS on specific applications such as pharmacokinetic and toxicokinetic studies, metabolomics studies (in vitro and in vivo drug metabolism studies), screening, quantification of medicinal and in the development of drugs. Multiple studies supported the suitability of HRMS for the mentioned applications bringing forward the selectivity, sensitivity and versatility of this technology. On the other side, we stressed the need for the development of new analysis tools allowing for a better processing of the large amount of data generated through HRMS analysis.

1. Introduction

If prescribed drugs such as lithium or digoxin are measured in blood since the 1960s [1], tremendous progresses have been made in drug quantification because of mass spectrometry (MS), among other detectors. At first coupled to gas chromatography (GC), and then to liquid chromatography (LC) in the 1970s, MS has been a real catalyst for the development of analytical methods for the quantification of drugs. With the advent of high-resolution mass spectrometry (HRMS) in toxicology laboratories, a large panel of applications have emerged. Although tandem mass spectrometry (MS/MS) is still the cornerstone of many laboratories, HRMS has the potential to replace MS/MS in specific applications. Indeed, HRMS can be an interesting tool for prescribed drug analysis, especially in specific applications such as pharmacokinetic and toxicokinetic studies or metabolomics studies (in vitro and in vivo drug metabolism studies). HRMS is also well suited for quantification and has become a new standard for screening of prescription and in development drugs.

The objective of this literature review is to provide a clear overview about the interests of HRMS in analytical toxicology, with a special focus on its recent applications on prescribed drugs analysis.

2. Applications in clinical and forensic toxicology

2.1. Screening for detection of pharmaceuticals

Drug screening is a widely used approach for several clinical and forensic applications such as accidental or voluntary intoxications, medication compliance, substance abuse detection, drug abstinence monitoring, drug facilitated crimes, and post-mortem toxicological analysis [2].

In the last few decades, HRMS has gained attention in many areas as evidenced by the increasing number of publications and reviews on this subject [3–6]. This approach combines

high selectivity and robustness with high mass accuracy, which gives a powerful identification tool even for unknown or unexpected substances [4].

The two most popular HRMS platforms in clinical and forensic toxicology are quadrupole time-of-flight (QTOF) and Orbitrap instruments coupled with GC or LC, even if GC-HRMS seems to be less prominent in toxicology screening. For example, Grapp and his team presented a comprehensive method for systematic toxicological analysis (STA) in serum by LC-QTOF-MS and compared it to GC-MS. On 100 serum samples, their LC-QTOF-MS technique was able to detect 240% (335 versus 141) more drugs than their routine GC-MS procedure. Serum concentrations of identified substances ranged from traces to therapeutic or toxic values. The difference between the two methods could be explained by the sensitivity of the instrumentation and the physicochemical characteristics of the compounds [7]. Indeed, GC techniques show some limitations for the analysis of highly polar, non-volatile or thermolabile compounds. Despite this, Pan et al. developed a sensitive GC-Orbitrap MS screening method for 288 compounds (drugs of abuse, sedative-hypnotics, antidepressants, non-steroidal anti-inflammatory drugs, cardiovascular agents and pesticides) in blood and demonstrated its applicability on 136 forensic cases [8]. GC-HRMS approaches are particularly interesting for pesticides and pollutants screening in poisoning casework and environmental toxicology [9–11].

The challenge in STA, also called general unknown screening (GUS), is to cover the widest possible range of drugs and poisons. Therefore, it seems important to work both in positive and negative ionization mode. As example, Stephenson et al. recently showed a LC-Orbitrap-MS screening method to detect approximately 200 drugs in whole blood samples with a limit of identification (LOI) of 2 µg/L for most analytes. Sample preparation relied on a precipitation procedure and the supernatant was divided in two fractions for analysis in positive ion mode and in positive/negative polarity switching mode. It allowed the detection

of both basic and slightly acidic drugs like barbiturates. This screening approach was validated and controlled by a concordance study on 1000 samples [12]. For urine toxicology screening, another group proposed a LC-QTOF method with electrospray ionization in both positive (ESI+) and negative (ESI-) mode. This dilute and shoot method has a higher detection rate than the routine GC-MS procedure on non-hydrolysed urine (6.6 drugs/sample versus 2.5 drugs/sample) notably through the ability of the LC-QTOF mass spectrometer to detect metabolites and positive and negative ionizing compounds [13].

The HRMS full scan mode also contributed to the success story of HRMS in clinical and forensic toxicology, by allowing data acquisition in an unbiased or non-targeted manner. The data-dependent acquisition (DDA) mode and the data-independent acquisition (DIA) mode are the two main strategies designed to acquire data in this way [2]. In DDA, only selected precursor ions undergo fragmentation following predetermined criteria (e.g. signal intensity). Many authors used this DDA mode for screening purposes [14–17]. In DDA, the selection of the precursor is based on user-defined criteria and is always a compromise. The risk of missing relevant ions is present in DDA experiments. Moreover, as shown in a 2014 article by Roemmelt et al., DDA might miss ions when several compounds coelute. In fact, suppressing effects of coeluting ions might lead to decreasing the signal of relevant substances below the trigger threshold of DDA. According to the authors, SWATH seems to be the best approach for an actual comprehensive screening since this technique has the advantage of achieving fragmentation of all potential precursor [18]. In DIA, fragmentation occurs for every potential precursor ion without predefined criteria. As co-eluting analytes and endogenous substances are also fragmented, complex spectra are generated and interpretation of the results can sometimes be complicated [16]. Several solutions for DIA have been developed such as Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH) [19] and MS^E [7]. In 2019, Whitman et al. compared DDA to SWATH analysis in

clinical toxicology. They proposed a new variable window SWATH model with a variable and customized mass window ranging from 6 to 59 Da and showed that this method was adoptable in clinical toxicology workflows [20]. One of the common features of DDA and DIA is that they both generate a significant amount of data that can exceed a gigabyte per sample injection, which is an obstacle for many laboratories [2]. Moreover, retrospective data analysis for unknown or newly discovered compounds is possible with these two untargeted data acquisition modes [16].

Once untargeted full scan data are acquired, different data analysis strategies can be used: targeted, suspect or untargeted analysis [21]. For targeted analysis, identification of compounds is made on the basis of accurate mass, isotope pattern, chromatographic retention time and product ion spectrum. Accurate mass and isotope pattern can be extrapolated from the empirical formula of the targeted compound, but product ion spectrum and retention time should be verified on the instrument to allow very high confidence identification. For this purpose, the toxicology laboratory must purchase an analytical reference standard for each analyte of interest [21]. Thoren et al. demonstrated that LC-QTOF-MS identification performances using targeted analysis were comparable to LC-MS/MS operating in SRM-mode [15]. Suspect analysis can be performed using only accurate mass and isotope pattern to identify possible hits. Unfortunately, this method can lead to many false positive results. Nevertheless, suspect screen performances can be improved by including product ion library match [22]. No a priori information is needed for the untargeted analysis, but this procedure is very complex and requires experimented analysts [21]. Both suspect and untargeted analysis are attractive for GUS as they allow a tentative identification of unknown or unexpected compounds. Once a hit is preliminarily identified, laboratories must purchase a reference standard in order to confirm that the product ion spectrum and the retention time

match the analyte present in the sample [21]. Suspect screen has been proposed as a valuable and complementary method to targeted screening in routine laboratories [22].

HRMS has proven its usefulness on several biological matrices [23]. Improvements in terms of selectivity and sensibility allow to work with minimal analytical volumes and to detect low analyte concentrations. Dried Blood Spot (DBS) is a microsampling procedure requiring a small volume of blood (5-10 µL). These samples are safe to handle, convenient to store and the sampling can be easily performed. Joye et al. validated a LC-Orbitrap-MS broad-spectrum drug screening on 104 DBS from real toxicology cases. They compared the results with two other routine screening procedures using a QTrap (in MRM-DDA) and a Toxtyper (low-resolution ion trap technology-DDA). A total of 271 identifications were performed and the full-scan-DDA LC-HRMS method was able to detect 99% of the analytes using only two 10 µL spots. Moreover, 23% of the compounds in the low concentration ranges were only detected by this method. The analytes belonged to six classes of illicit drugs and 14 classes of pharmaceuticals, including benzodiazepines, neuroleptics, antidepressants, anaesthetics/analgesics, antidiabetics, antihistaminic, beta-blockers, proton-pump inhibitors, antifungals, and antimalarials [14].

Dried Urine Spot (DUS) has also been studied as an alternative sampling technique. Urine samples are commonly used in clinical toxicology due to the relative ease of collection and the wider detection window for drugs and metabolites than blood specimens [24]. For drug testing in urine, Michely and his team investigated the performance of LC-HRMS with or without conjugate cleavage or using DUS in comparison to LC-MS^n and GC-MS methods [25]. They applied and compared four sample preparation ways on 100 human urines: urine precipitation (UP), urine precipitation with conjugate cleavage (UglucP), DUS with on-spot cleavage followed by liquid extraction (DUSglucE), and lastly acid Hydrolysis, liquid liquid extraction followed by acetylation prior to GC-MS analysis. The results were

expressed as a percentage of the total number of hits, all techniques included. Not surprisingly, the LC-Orbitrap mass spectrometer was able to detect more compounds than the LC-MS^n procedure (+37% on average). Regarding sample preparation, the best detection rate for LC-HRMS was achieved with urine precipitation after conjugate cleavage (UglucP, 89%) followed by UP (80%), DUSglucE (77%) versus 56% for the GC-MS screening [25].

Finally, HRMS has proven itself in the analysis of samples with low concentrations, as illustrated by the recent study of Vincenti et al. [26]. They managed to detect picogram amounts of benzodiazepines in cups, glasses, cutlery surfaces, sometimes washed very quickly after a drug facilitated crime (DFC), with a LC-Orbitrap MS procedure. They employed a dispersive liquid-liquid microextraction (dLLME) method in order to improve sensitivity. Hair is another biological matrix potentially useful in DFC investigations. With a LC-Orbitrap mass spectrometer, Odoardi's team developed a rapid method for the screening of over 170 drugs of abuse and pharmaceuticals in hair samples. Detecting a single drug administration can be a challenge but they were able to obtain appropriate limits of detection (LOD) with their technique [27].

2.2. Quantification of pharmaceuticals

Well-known for its ability to screen a wide variety of substances, HRMS can also be used for quantitative analyses, including those of prescribed drugs. This is of particular interest in the field of forensic toxicology, since pharmaceuticals can be implicated in cases of driving under influence, fatal drug overdose, death by poisoning or drug-facilitated crimes. The quantification of prescribed drugs can also be used for clinical applications such as drug compliance monitoring to treatments and therapeutic drug monitoring (TDM). Because of its high sensitivity, the standard approach for pharmaceutical quantitation used to be LC-MS/MS

operating in selected reaction monitoring mode (SRM). However, as described below, some papers demonstrate that LC-HRMS technologies are interesting alternatives to this technique for the simultaneous quantification of multiple analytes, including pharmaceuticals.

2.2.1. Simultaneous multi-analyte quantitation

While the early-generations of HRMS technologies did not show good quantification performance in comparison with the well-established LC-MS/MS techniques, more and more studies now demonstrate comparable properties between both technologies. For example, at the beginning of the century, Zhang et al. developed a method for simultaneous quantification of 5 tricyclic antidepressants in human serum using a TOF technology [28]. The linear dynamic range of the method was limited due to detector saturation induced by an excessively large mass extraction window. Almost ten years later, the same research team developed another method for multi-drug quantification in plasma, including clozapine (antipsychotic), propafenone and ticlopidine (cardiovascular drugs) and antihistamines, this time using a Q Orbitrap technology operating in full scan mode [29]. The quantitative performances of this new method were equivalent to those of a triple quadrupole MS/MS in SRM mode, including the linear dynamic range, precision and accuracy, but with a better sensitivity of SRM mode.

The data-independent acquisition modes of HRMS, such as full scan or MS^E modes (^{E} denotes the collision energy, alternating between low and elevated), present advantages for the development of simultaneous multi-analyte quantification methods, in comparison with techniques based on SRM acquisition mode. These advantages are due to the possibility to perform post-acquisition selection of the quantifier and qualifier ions.

The post-acquisition processing of data eliminates the need of a pre-run optimization of the MS parameters, making possible the use of universal parameters to quickly and easily develop accurate quantitative methods. For example, Gish et al. were able to adapt in only

three hours a screening method that uses QTOF technology into a method able to quantitate two cardiotropic drugs: amlodipine and atenolol [30].

On the contrary of techniques based on SRM mode, known to present duty cycle limitations, the total cycle time of HRMS methods based on untargeted acquisition mode is not a function of the number of compounds analyzed, because no dedicated scanning times are needed. Therefore, it results in no change in the chromatographic peak resolution or in the number of data points when a large number of compounds are quantified [29,31]. For example, QTOF-MS^E technology was used in three papers for simultaneous quantification of 16 to 30 compounds, including common opioids and opiates (buprenorphine, morphine, codeine, fentanyl, methadone, oxycodone), analgesics (tramadol, dextromorphan, meperidine) antidepressants (citalopram), benzodiazepines and z-drugs, in human plasma, whole blood and post-mortem blood [31–33]. The developed methods present comparable accuracy and precision with MS/MS methods, with some variability of their performance depending on the compounds to be analyzed, their concentrations and the parameters used. QTOF-MS^E technology was also used, coupled with UPLC, in a rapid semi-quantitative method for the analysis of tadalafil, dextromorphan and their metabolites in bone tissues, which may be useful in the field of forensic toxicology when no other biological matrices are available [34].

Finally, the acquisition of multiplex full mass spectra allows the use and process of all data collected during the run at any time post-analysis. Therefore, it allows to simultaneously perform quantitative analysis of selected ions and qualitative analyses of untargeted compounds, which can present an interest in the field of analytical toxicology. For example, the multidrug quantitative method developed by Zhang et al. was also used in a preclinical study to follow the evolution of the plasma concentration of a new component MK-C, and the additional data collected was also used to identify the predominantly circulating metabolite of this in-development drug [29]. The method developed by Dalsgaard et al. also allows the

screening of hundreds of compounds in parallel with the quantitative analysis of 30 compounds [33]. However, using different techniques is strongly recommended by most laboratory guidelines for forensic toxicology to perform the initial identification test and then confirmation analysis. In this way, methods which allow simultaneous screening and quantification should be used with caution [35].

HRMS technology with parallel reaction monitoring (PRM) mode can also be used for quantitative applications, but this mode requires an optimization of MS parameters prior to the analysis. Q Orbitrap technology in PRM mode was used by Feliu et al. and Joye et al. for the quantification of drugs and pharmaceuticals in whole blood [36,37]. One method specifically targets opioids, opiates and derivatives, including morphine, codeine, buprenorphine, methadone and naloxone, while the other was developed to quantitate the main 22 psychoactive substances involved in impaired-driving cases, including prescribed opioids, benzodiazepines and z-drug. Both methods were fully validated : linearity, limits of detection (LOD) and quantification (LOQ), accuracy (precision and trueness), selectivity, matrix effect, carry-over and stability.

2.2.2. Drug compliance monitoring and therapeutic drug monitoring

Analyses of pharmaceuticals in biological matrices are not only performed to give clinicians valuable information about cases of intoxication, but they can also be used to monitor the compliance of patients to their prescribed medication. These analyses are generally performed on whole blood or plasma, but the standard venous sampling method is invasive and needs trained staff. Since it can be easily recovered, dried blood spot sample (DBS) is an alternative matrix that can be more suitable for drug compliance monitoring analyses in some specific contexts, for example with psychiatric patients [38]. In addition to

the common parameters to be validated for the quantitative analysis of traditional biological matrices (liquid blood, serum and plasma), extra validation parameters are needed for DBS-based methods : volume effect, hematocrit effect and volcano effect [39].

Various papers described the use of QTOF technology associated with LC for the analysis of cardiovascular drugs in DBS for compliance monitoring. In 2012, Lawson and al. used this technology for the determination of atenolol only [40]. Five years later, the same team developed a similar method able to quantify 10 cardiovascular drugs in DBS for drug compliance monitoring, thanks to the optimization of the MS conditions to obtain better sensitivity and selectivity [41]. However, calibration of the HRMS instrument must be performed regularly to maintain the quality of the mass resolution and accuracy. The same LC-HRMS conditions were later used with samples obtained by volumetric absorptive microsampling (VAMS) for the quantification of four targeted cardiovascular drugs [42]. This alternate sampling method to conventional DBS cards eliminates the altering effects on the accuracy associated with haematocrit and give therefore more repeatable results.

VAMS microsampling was also used by Jacobs et al. with an Orbitrap Q technology for the quantification of 13 antipsychotics on their whole therapeutic range in DBS. The method was developed for the compliance monitoring to drug therapy in populations with psychiatric disorders. The VAMS associated with LC-HRMS method was successfully developed and validated (selectivity, lower and upper LOQ, carry-over, linearity, accuracy at different concentrations, dilution integrity, matrix effect, recovery, stability), except for cyamemazine which failed the validation [38].

HRMS can also be used for TDM, mainly coupled with LC. The main technology used for TDM is Orbitrap. Several methods have been published for anti-infective drugs such as antibiotics [43], antimycotics [44,45] or antiepileptics [46]. TDM of hydroxychloroquine has also been described using HRMS [47].

Finally, the use of HRMS technique for drug compliance monitoring and TDM is also interesting because, as said in the previous chapter, it can be used to identify and / or quantify non-targeted substances as metabolites if necessary with a post-acquisition review of the registered data.

2.2.3. Focus on peptide therapeutics testing

Peptide therapeutics, such as therapeutic monoclonal antibodies (mAbs), have emerged as one of the major sources of innovative medicines in the past few years. As mAbs have a complex peptidic structure and a very high molecular weight compared to usual xenobiotics (greater than 100 kDa), their analysis can be challenging. The most common approach for quantifying these peptides is ligand-binding based assays such as ELISA. Recently, LC-MS/MS methods have been developed for the analysis of proteotypic peptides after sample purification and protein digestion [48,49]. More recently, quantification based on LC-HRMS emerged to offer direct measurement of intact proteins, allowing the study of isoforms and biotransformations, and easier sample handling [50]. Kellie et al developed a method to quantify liraglutide in LC-HRMS and compared it with a LC-MS/MS method. LC-HRMS had an increased selectivity for differentiation of interfering endogenous peaks and offered a great advantage for method development [51].

As an example, Millet et al developed a series of methods for quantitative analysis of oncology mAbs such as pembrolizumab, cetuximab, nivolumab or rituximab using Orbitrap technology and sample preparation based on IgG capture or albumin depletion followed by trypsin digestion [52–55]. Other teams developed methods for sacituzumab govitecan or rituximab monitoring [56,57].

Interestingly, Reinders et al developed an analytical method to assess the occupational health risk of airborne mAbs (rituximab, trastuzumab and daratumumab) using LC-HRMS

and air sampling [58]. Yao et al used Q-TOF technology and immunocapture to study the biotransformation of therapeutic peptides. Their approach was untargeted, allowing to systematically study the universal biotransformation of therapeutic biologics [59]. Finally, the use of HRMS for the quantification of therapeutic proteins is promising. Given the fast growth of therapeutic proteins, applications in forensic toxicology will expand.

3. Specific applications

3.1. In vitro and in vivo drug metabolism studies

HRMS has been widely used for *in vitro* and *in vivo* drug metabolism studies. It can be applied to analyze drug metabolite profiles, to elucidate the metabolic pathways of drugs and more generally to understand the human metabolism of specific medical compounds. During drug discovery, drug development, and pre-clinical and clinical studies, HRMS techniques are also a valuable tool in the identification of potentially toxic metabolites. The current challenge in the detection of drug metabolites, i.e. the existence of unknown metabolites and the detection at low concentration in complex matrices, can be taken up when using HRMS technologies [60]. Literature shows that both Orbitrap Q technology and QTOF technologies have been used to explore the metabolism of a wide variety of drugs, in vitro and in vivo.

The Orbitrap Q technology has been used for several *in vitro* studies on liver cells. For example, Le Daré et al. studied the antipsychotic drug quetiapine, by investigating its metabolism in human liver cell models incubated with the compound of interest [61]. They also demonstrated the potential of a molecular networking approach on the analysis of HRMS data [62,63]. Jeong et al. tried to elucidate *in vitro* the metabolic pathways of evogliptin, an anti-diabetic drug, using human hepatocytes, liver microsomes and S9 liver fractions and tried to identify the enzymes responsible for its metabolite formation [64]. Wang et Wang

characterized *in vitro* the interspecies variability for the metabolism of the anti-cancer drug olaparib using human and animal liver microsomes [65].

The Orbitrap Q technology is also found in studies aiming to identify the metabolites of the antimalarial artemisinin-based combination therapy (ACT) for which the metabolism is still not completely understood. For example, one of the compounds used for this type of therapy, piperaquine, was investigated *in vivo* in humans and in rats [66]. Another compound, naphthoquine was analyzed *in vitro* using liver microsomal incubates (human, cynomolgus monkey, beagle dog, mini pig, rat and CD1 mouse), CYP enzymes and *in vivo* analyzing rat plasma, urine, bile and feces [67].

In the literature, a variety of studies on drug metabolism also report the usage of a QTOF mass spectrometer. For example Ni et al. published an *in vivo* study on the complex metabolism of *Glechoma longituba* in rats, a remedy coming from the traditional Chinese medicine used to treat various diseases [68]. QTOF technology has also been employed for clinical studies, in order to investigate *in vivo,* on human and rats subjects*,* the metabolite profile of new drugs such as imigliptin, a novel DPP-4 inhibitor used in the treatment of type 2 Diabetes Mellitus [69]. Vishnuvardhan et al. published an *in vivo* study on rats, investigating the metabolism of silodosin, an α1-adrenoceptor antagonist and a new treatment for prostatic hyperplasia [70]. Chavan et al. used the QTOF technology in order to study the drug development of palbociclib, a CDK4/6 inhibitor. Metabolite identification was performed *in vitro* using human and rat microsomes and S9 fractions and *in vivo* in rat biological matrices [71]. A similar in vitro and in vivo study was performed by the same team on riociguat, a guanyl cyclase inhibitor, in order to identify the metabolites of this drug used for the treatment of pulmonary conditions [72].

Studies also show that the QTOF mass spectrometer can be used in metabolomics studies in order to assess the toxicity of drug metabolites. For example, a QTOF mass spectrometer was

used in order to study the metabolites of amiodarone. Characterization of the metabolites has been performed *in vitro*, on rat liver microsomes, on rat and human liver S9 fractions as well as *in vivo* on rat feces, urine and plasma, in order to determine the one supposed to cause hepatotoxicity [73]. HRMS technology can also be used in the study of metabolic disposition as it has been done by Gerisch et al, who aimed to study *in vitro* the antitumor drug regorafenib on human hepatocytes [74].

3.2. Pharmacokinetic and toxicokinetic studies

By definition, pharmacokinetics focuses on the study of drug absorption, distribution, metabolism, and excretion in the body over time. Toxicokinetics is similar to pharmacokinetics, except that studies are carried out with greater doses [75]. HRMS, as previously seen, is an interesting tool for drug metabolism studies. It can also be applied to pharmacokinetics and toxicokinetics studies in preclinical or clinical settings.

Several recent preclinical pharmacokinetics studies used HRMS techniques. Molloy et al. studied gefitinib (EGFR inhibitor used in certain cancers) pharmacokinetics in mice using QTOF technology [76]. Yi et al. used Orbitrap technology to study pharmacokinetics in rat plasma of TUG-891 (agonist of FFA4 receptor developed for type 2 diabetes and obesity) and its metabolites [77]. Sun et al. studied pharmacokinetics of trepibutone (used for functional gastrointestinal disorders) in rats using Orbitrap technology as well [78]. Other teams studied in rats the pharmacokinetics of anti-Alzheimer's drug candidates (7-MEOTA-tryptophan hybrids), piperine and artemisinin [79–81].

Recently, HRMS has been applied to clinical studies implying the pharmacokinetics and metabolism of drugs, especially in oncology. As an example, Machon et al. studied the intracellular anabolism of 5-fluorouracil and its incorporation in nucleic acids with Orbitrap technology [82]. Cardonick et al. studied paclitaxel and its metabolites in human meconium

from newborns with gestational chemotherapeutic exposure [83]. Dahmane at al. studied tamoxifen and 40 metabolites in human plasma with relative quantification [84]. HRMS can also be suited for pharmacokinetic studies of peptide drugs. Esposito et al. studied the distribution of subcutaneously administered therapeutic peptides and its impact on their pharmacokinetics [85]. Finally, David et al. identified novel metabolites suited for acetaminophen biomonitoring based on the study of the dynamic metabolism of acetaminophen with QTOF technology [86].

Few toxicokinetics studies based on HRMS have been published. Notably, Gish et al. used QTOF technology applied to real-time monitoring of blood concentrations in a case of severe cardiotropic drug poisoning [30]. Mohamed et al. developed and validated a method for the quantification of cannabinoids and their metabolites in human plasma, suited to therapeutic cannabinoid toxicokinetic studies [87]. Sun et al. studied the toxicokinetics of Zearalenone-14-Glucoside in rats [88]. All in all, toxicokinetics studies based on HRMS, especially after intoxication, are still lacking given the potential of the technology.

4. Conclusion

HRMS has become a powerful and versatile technique in analytical toxicology because it can be used for multiple applications: screenings (targeted and non-targeted), quantification, drug metabolism studies, and pharmacokinetics and toxicokinetics studies, in clinical or preclinical studies. Analysis of prescribed drugs and metabolites by HRMS have also given particularly interesting insights, specifically allowed by this technology. HRMS can be used in both research laboratories and routine laboratories.

In conclusion, HRMS could become the cornerstone technique of analytical toxicology, given its main qualities such as its selectivity, high sensitivity and flexibility. Nevertheless, several limitations to its widespread use persist: the cost of the instruments, the

storage capacity for the huge amount of data generated, the complexity of the data analysis requiring highly trained operators and the need for a powerful and user-friendly software. In the near future, artificial intelligence and machine learning approaches could handle these large datasets generated by HRMS. Indeed, as suggested in Streun's proof of concept study, artificial neural network may be a promising tool for substance identification and sample classification in toxicology [89].

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