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Recent advances of liquid chromatography–(tandem) mass spectrometry in clinical and forensic toxicology — An update

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ABSTRACT

Liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) is a well-established and widely used technique in clinical and forensic toxicology as well as doping control especially for quantitative analysis. In recent years, many applications for so-called multi-target screening and/or quantification of drugs, poisons, and or their metabolites in biological matrices have been developed. Such methods have proven particularly useful for analysis of so-called new psychoactive substances that have appeared on recreational drug markets throughout the world. Moreover, the evolvement of high resolution MS techniques and the development of data-independent detection modes have opened new possibilities for applications of LC–(MS/MS) in systematic toxicological screening analysis in the so called general unknown setting. The present paper will provide an overview and discuss these recent developments focusing on the literature published after 2010.

1. Introduction

Liquid chromatography (LC) coupled to single stage mass spectrometry (MS) or tandem mass spectrometry (MS/MS) is nowadays widely used in clinical and forensic toxicology as well as doping control, especially for analysis of hydrophilic and thermolabile compounds. From an experimental technique in the 1990, it first evolved as a complimentary methodology to the gold standard technique gas chromatography–mass spectrometry (GC–MS). Being originally used primarily for targeted qualitative and quantitative analysis of a limited number of analytes, more recently the measurement modes of modern LC–MS(/MS) have opened new possibilities for its application in the so-called general unknown screening performed as a part of systematic toxicological analysis (STA) particularly in clinical and forensic toxicology.

Moreover, LC–MS(/MS) is the primarily employed technique for analysis of the so-called new psychoactive substances (NPS), hundreds of which have appeared on the recreational drug markets in Europe and other parts of the world [1]. It is further used for analysis of evolving matrices for toxicological analysis such das dried blood spots.

In 2011, Peters reviewed the literature on applications of LC–MS(/MS) in clinical and forensic toxicology as well as doping control published after 2006 focusing on general unknown screening methods and multi-analyte procedures [2]. Meanwhile several other review articles have become available dealing with more specific aspects of LC–MS(/MS) in these fields such as applications in clinical toxicology and forensics [3–10], analysis of NPS [11–13], alternative matrices [14,15], and doping control [16–20].

This review provides an overview on methods for untargeted STA and targeted multi-analyte procedures using LC–MS(/MS)-based bioanalysis in the field of clinical toxicology, forensic toxicology and doping control. A strict stratification of untargeted and targeted analysis was not always possible, as combined approaches were used in several of the reviewed articles. Papers listed in PubMed since 2011 were the basis for this overview. The following key words were used solely or in combination for stratification of the search results: LC–MS and/ clinical toxicology, /forensic toxicology, /forensics, /doping, /screening.

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; GC–MS, gas chromatography–mass spectrometry; STA, systematic toxicological analysis; NPS, new psychoactive substances; DUID, driving under the influence of drugs; DFC, drug-facilitated crime; ME, matrix effects; PP, protein precipitation; LLE, liquid liquid extraction; SPE, solid phase extraction; HILIC, hydrophilic interaction liquid chromatography; SALLE, salting out assisted liquid liquid extraction; RP, reverse phase; HR, high resolution; FS, fullscan; QTOF, quadrupole time of flight; APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; MFLC, microflow LC; LR, low resolution; DDA, data dependent acquisition; MRM, multiple reaction monitoring; PIS, collision cell induced information rich product ion spectra; QQQ, triple quadrupole; DIA, data independent acquisition; SWATH, sequential window acquisition of all theoretical fragment-ion spectra; AIF, all ion fragmentation; MS E, mass spectrometry using elevated collision energy; bbCID, broad band collision induced dissociation; HCD, higher energy collisional dissociation; CID, collision induced dissociation; QTrap, hybride triple quadrupole-linear ion trap; LOD, limit of detection; RE, recovery; PE, process efficiency; DBS, dried blood spots; QuEChERS, quick, easy, cheap, effective, rugged, safe; TOF, time of flight; IS, internal standard.

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2. LC–MS(/MS) applications in untargeted STA

In clinical and forensic toxicology, so-called STA is performed whenever no or no reliable information on the drugs and/or poisons involved in a particular case is available. It aims at covering and unambiguously identifying as many toxicologically relevant compounds as possible, which is a challenge even with modern instrumentation. Generally, a combination of analytical techniques is used for STA, most importantly GC–MS and LC–MS(/MS) as they combine high separation power with high selectivity and sensitivity [21–23]. LC–MS(/MS) methods used for the purpose of STA should ideally be untargeted, i.e., not involve any pre-selection of analytes. Various methods for untargeted LC–MS(/MS)-based STA have been developed in recent years. Screening concepts, covered analytes, ionization techniques, chromatographic systems as well sample preparation techniques of methods published from 2011 to 2016 using untargeted analytical concepts are summarized in Table 1.

2.1. Sample matrices for untargeted screening analysis

Traditionally, urine has been the preferred biological matrix for STA because it can be non-invasively sampled, is generally available in comparatively large volumes and – most importantly – because excreted compounds and their metabolites are concentrated in urine [21,24]. As can be seen from Table 1, urine is still a widely used biological fluid in the more recently developed approaches to untargeted screening analysis employing LC–MS(/MS) [25–41]. In some applications, it was complemented by blood-derived matrices, i.e., whole blood, plasma or serum [42–47], which is advantageous when urine samples are not available as in most cases of driving under the influence of drugs (DUID). Many of the methods summarized in Table 1 are exclusively dedicated to analysis of whole blood [48–56], plasma [57,58], or serum [59,60], which requires higher sensitivity and is generally associated with shorter detection windows as compared to urinalysis. Only three methods have been published for untargeted screening of hair samples [44,61,62], one of which was primarily dedicated to benzodiazepines and Z-drugs [62]. While these methods are likely suitable for detection of drugs after chronic use, it seems questionable that such untargeted screenings methods are sufficiently sensitive to pick up single administration of all covered drugs as would be required for application in cases of drug-facilitated crimes (DFC). Two of the papers listed in Table 1 also included analysis of tissue samples [44,47] or gastric content [44].

When sample matrices with potentially very high analyte concentrations such as gastric content or urine are analyzed on the same LC–MS(/MS) instrument as matrices with generally much lower analyte concentrations such as blood or hair, it is important to take effective measures against carry-over (e.g. by rinsing/flushing of injection loop/needle, intermitting blank injections). Otherwise, there will be a considerable risk of false positive findings due to the high sensitivity of modern LC–MS(/MS) systems. For this reason, systematic carry-over experiments should always be included in method development and validation as also discussed in Section 2.9.

2.2. Sample preparation for untargeted screening analysis

An overview on recent developments for sample preparation in bioanalysis was published in 2011 by Kole et al. [63]. When analyzing urine samples, it must be considered that many analytes are primarily or even exclusively excreted in urine in form of phase I and/or phase II metabolites, mainly glucuronic acid and/or sulfuric acid conjugates. Such conjugates can either be directly analyzed simplifying sample preparation or cleaved prior to extraction yielding the respective phase I metabolites. For compounds being partly excreted in unconjugated form and partly as sulfate esters and/or glucuronic acid conjugates, this will lead to three distinct peaks whereas after cleavage of conjugates all three entities elute in a single peak of a respectively higher abundance increasing the sensitivity of detection. Only few methods summarized in Table 1 employed conjugate cleavage using beta-glucuronidase from red abalone [33,38], *Patella vulgata* [35] and *Escherichia coli* K12 [30,34,37]. Combinations of beta-glucuronidase and arylsulfatase from *Helix pomatia* have been used for additional cleavage of sulfuric acid conjugates [41,44,64]. Ahrens and coworkers used a high purified beta-glucuronidase to obtain cleaner extracts and thus minimizing matrix effects (ME) [36]. Chemical cleavage of phase II metabolites was performed by the workgroup of Oberacher [42,43]. Benefits of this approach is the short incubation time in addition to lower sample cost, but formation of artifacts may compromise the detection of certain compounds.

With the compounds present in a particular case being unknown, the sample work-up for untargeted analysis should be as unselective as possible. Simple dilution or protein precipitation (PP) therefore should be most suitable for LC–MS(/MS)-based STA. Both approaches are also quick and cheap. At first glance, it therefore seems remarkable that only comparatively few of the more recently published procedures employed dilution [31,35,36,45] or PP [26,39,40,54]. While dilution may decrease sensitivity and ME, the PP may provide higher sensitivity, but also be associated with stronger ME, as will be discussed later on.

Of those methods that employed liquid-liquid extraction (LLE) or solid-phase extraction (SPE), the used extraction conditions (solvent or sorbent, pH) were either selected to cover a wide range of physicochemical properties or chosen to achieve adequate recovery for the mainly targeted groups of drugs. The latter of course limits the applicability in the sense of untargeted STA. A number of authors used online-SPE for sample preparation. The extraction sorbents were mainly general purpose phases such as C18 and polydivinyl-benzene [49,64]. Gorgens et al. used a hydrophilic interaction liquid chromatography (HILIC) based online preparation for the detection of highly polar compounds by HILIC chromatography [25]. The importance of such methods may be expected to increase in the future, because they can be automated.

Salting out-assisted liquid-liquid extraction (SALLE) was used for screening of classic drugs of abuse and/or NPS [28,50]. After mixing urine with the water miscible solvent acetonitrile highly concentrated salt solutions were added to achieve phase separation with the lipophilic analytes present in the organic phase which was further used for analysis [28,50].

2.3. Chromatographic system

Most of the screening methods listed in Table 1 employed more or less generic reverse-phase (RP) C18 chromatography for separation of different matrices like ante mortem or post mortem blood, serum, urine, hair, organ tissues and gastric content. In a very recent study, Periat et al. confirmed the applicability of established RP chromatography for multi-target and non-targeted screening approaches and demonstrated less ME using unselective sample preparation for RP in comparison to HILIC. However the authors stated that both chromatographic systems provide complementary separation techniques [65].

In 2012, Croley and coworkers studied the influence of the chromatographic system on mass accuracy of non-targeted high resolution (HR) MS using single stage fullscan (FS) on Orbitrap and quadrupole time of flight (QTOF) instruments. They stated that a high chromatographic separation power is essential to obtain high quality HR MS data, as mass accuracy and thus numbers of detected compounds are highly influenced by co-eluting compounds [66]. This underlines that chromatographic separation must not be neglected, even when using highly selective and sensitive HR MS detection. In 2012, Fekete and Fekete described the role of core-shell and very fine particles for current fast liquid chromatograph and stated that the expected theoretical benefit of core-shell and very fine particle columns is sometimes