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What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening?

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ABSTRACT

This paper reviews critically LC–MS approaches for toxicological drug screening using (ultra) high performance liquid chromatography (UHPLC) coupled to low and high resolution mass spectrometry (HRMS) published since 2010. A concluding discussion focuses on progress and current status of sample workup, separation by HPLC vs. UHPLC, MS detection modes and their specificity, universality of LC–MS libraries, and validation necessary of LC–MS for screening methods. Finally, a discussion on what the future holds for LC–MS drug screening in clinical and forensic toxicology completes this review article.

1. Introduction

In clinical and forensic toxicology, workplace drug testing, and doping control, the presence or absence of drugs, drugs of abuse, poisons, and/or their metabolites must be definitely confirmed by unequivocal toxicological analyses. They may include qualitative analysis, so-called drug screening (yes/no decision, e.g. concerning defined cut-off values) and/or quantification if assessment of the pharmacological/toxicological effect is requested. Only a reliable analytical result can be the basis of a competent toxicological judgment, consultation and expertise [1]. In clinical and forensic toxicology, the compounds to be quantified must be identified first as they are often not known in advance. Today, mass spectrometric (MS) techniques hyphenated with gas (GC–MS) or liquid chromatography (LC–MS) are the gold standards for toxicological analyses providing high sensitivity, specificity, and universality. This is of particular importance for the analysis of complex biological matrices such as ante- or post-mortem blood (whole blood, plasma, serum, dried blood spots), urine, gastric content, tissues or alternative matrices such as hair, sweat and oral fluid, meconium, or nails. Depending on the request, all these body samples can be used for screening, but urine and blood samples are the samples of choice and taken into consideration for this review.

The drug screening strategy depends on the different tasks. If only a single drug or drug class has to be monitored, immunoassays can be used for preliminary screening in order to differentiate between negative and presumptively positive samples. However, positive results must be confirmed by a second independent method that is at least as sensitive as the screening test and that provides the highest level of confidence in the result [2–5]. Series of confirmation assays using GC–MS or LC–MS techniques were reviewed elsewhere [6–11] and not mentioned here. This two-step strategy is employed if only those drugs or poisons have to be determined, which are scheduled, e.g. by law or by international sport organizations, and for which immunoassays are commercially available. If these demands are not met, the screening strategy must be more extensive, because several thousands of drugs or poisons are on the market worldwide. The screening strategies can be classified into target or comprehensive non-target screening approaches. The latter are also named general unknown screening (GUS) or systematic toxicological analysis (STA). Today, mostly so-called multi-analyte procedures were developed for (multi) target screening, which are a compromise between the development/use of as few procedures as necessary for as many analytes as possible [6–11]. They allow screening for a certain number of analytes using selected-ion monitoring (SIM) or selected reaction monitoring (SRM), whereas analytes that are not included/selected a priori cannot be detected. For broad GUS, MS approaches are preferable, which use full scan acquisition and reference libraries for identification of a wide range of drugs, poisons, and/or their metabolites.

Although GC–MS is still widely used in routine for all these purposes, particularly for GUS [12,13], single-stage or tandem LC–MS (MS/MS) with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) coupled to classic or modern ultra-high performance liquid chromatography (UHPLC) is more and more established in clinical and forensic laboratories also for routine

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case work. The various sample workup options, LC columns, ion sources, mass analyzers have recently been discussed elsewhere [9,11,14]. High-resolution (HR) mass spectrometers have evolved in the last decades, from double focusing mass spectrometers over time-of-flight (TOF) apparatus to Orbitrap (OT) mass analyzers. Since such techniques are getting affordable to many laboratories, accurate mass measurements are more and more integrated in drug screening [10,14–18], which are shortly discussed in a separate chapter here. In the following, papers describing target as well as untargeted procedures using any type of LC–MS couplings will critically be reviewed and perspectives for further developments discussed. English written papers published after 2010 have been taken into consideration.

2. Target screening approaches using LC–MS with low mass resolution

Most LC–MS screening procedures focused on multi-target screening procedures rather than on GUS. They allow screening for several important compounds with one workup and injection, saving time and resources. Most of them can also be used for confirmation of immunoassay pre-screening results and some, particularly blood screening approaches, for validated quantification [6–10]. Even if a single analyte has to be determined only occasionally, which is often the case in clinical and forensic toxicology, such procedures allow a reliable quantification.

2.1. Target screening approaches using SRM

Series of target screening approaches using SRM for urine, blood, or alternative matrices were published over the last years. As Peters [9] reviewed them 2011 in detail, only more recent papers are mentioned here. For urine screening of fourteen stimulants relevant to doping control, Lu et al. compared GC–MS results after liquid–liquid extraction (LLE) with those obtained with a classic, new developed and validated LC–MS/MS method after solid-phase extraction (SPE) using SRM with three transitions each [19]. The validation results fulfilled the criteria set for doping control. From the LC and MS point of view, this is a classic “me too” application. The authors concluded, not surprisingly, that the combined use of both assays would provide more accurate and reliable information. Based on dilute and shoot methods described by the group of Beck [20–23], Bell et al. [24] developed a UHPLC–MS/MS dilute and shoot screening method for emerging drugs of abuse in urine. The authors state that each transition conferred sufficient specificity, although not all compounds were completely resolved by ultra-high performance LC. Guddat et al. [25] described a validated multi-target approach to screen for various classes of substances prohibited in sports using direct injection of urine specimens. This high-throughput method allowed also monitoring conjugates of phthalate metabolites as a marker for illicit blood transfusion. In the meantime, Beck and coworkers described another very promising drugs of abuse screening approach, the exhale and shoot concept [26–30]. The exhaled air was collected with a particular device and analyzed by LC–MS/MS using SRM with two transitions.

For blood screening, Oiestad et al. [31] developed a UHPLC–MS/MS after LLE detecting various drugs of abuse with only one transition per SRM. As this is not enough for a confirmed result [2,32], the authors state that they use this method in the same manner as an immunoassay to decide which samples will be reanalyzed with a confirmation method (GC–MS or LC–MS/MS). This is in fact essential because interferences cannot be excluded [2,32,33]. Another screening approach allowed detection of 31 newer designer drugs in serum after SPE by LC–MS/MS using SRM with two transitions each [34]. In cases of isobaric compounds, three transitions had to be used. As these substances show often nearly the same MS/MS spectra they could be differentiated only by considering the retention time and relative ion abundances of identical transitions. In case of such small basic drugs, GC–MS in the electron-ionization mode is much more selective, particularly after acetylation [17,35,36]. As already mentioned, multi-analyte procedures for drug quantification in blood, serum, or plasma can also be used for target screening. For example, Dresen et al. [37] described a validated LC–MS/MS method using SRM with three transitions each after LLE for synthetic cannabinoids, which play a major role in current drugs of abuse testing. The authors could proof the applicability analyzing 100 authentic samples successfully. Simonsen et al. [38] developed and validated a UHPLC–MS/MS approach with SRM and two transitions each for simultaneous screening and quantification of benzodiazepines in whole blood after LLE. Another “me too” UHPLC–MS/MS approach for benzodiazepines in urine was published by Ming and Heathcote [39]. Finally, Remane et al. [40–42] developed and validated a multi-analyte approach for screening and quantification of over 130 drugs of different relevant drug classes in plasma after LLE. Although using UHPLC separation, not all of the over 100 analytes and standards could sufficiently be separated chromatographically. Therefore, the authors tested intensively the impact of the various deuterated internal standards and of overlapping analytes on ion suppression or enhancement for ESI and APCI [43,44]. As expected, APCI showed much less suppression or enhancement effects than ESI so that the authors decided to use APCI although the sensitivity was lower for a few analytes. Two SRM transitions were chosen monitoring structurally specific fragments with high intensity. For analytes without specific fragments and/or sufficient sensitivity, a third transition was added.

2.2. Target screening approaches using DDA and library search of PIS

Another, more selective, target screening concept for urine and plasma after LLE or SPE was developed years ago by the group of Weinmann [45] using a hybrid triple quadrupole instrument (QTRAP) in which the third quadrupole can be used as a linear ion trap (LIT). This was based on tandem MS data-dependent acquisition (DDA) using SRM as a survey scan and an enhanced product ion (EPI) scan. Identification of the obtained product ion spectra (PIS) was conducted by library search using the authors’ EPI spectra libraries. As these spectra contain in most cases several fragment ions, these approaches provide better identification power than those using only 1–3 transitions [32]. The possibilities and limitations of this screening concept were already discussed in detail by Peters [9]. In 2010, Dresen et al. [46] extended the former assay for 300 drugs [45] to 700 drugs using timed SRM, which considerably increased the number of transitions and reduced the cycle time. Even using timed SRM as survey scan this method is still a limited target screening approach. Viette et al. [47] described another multi-target screening for about 100 drugs in plasma based on approach of the Weinmann group [45]. For data evaluation, they used SmileMS, a new mass spectrometry identification software platform for small molecules, which considers all MS/MS data points different from the background noise. This software was also applied successfully for non-targeted screening [48–50]. In addition, the authors compared the LC–MS results with those obtained by a standardized LC–UV method and concluded that the LC–MS approach is a reliable alternative to the no more supported LC–UV system [51].

A dilute and shoot approach for urine screening of 264 drugs was published recently by Stone [52]. The MS part is very similar to the above-mentioned with an in-house EPI library. A serious drawback is however, that she used the therapeutic drug promazine as

internal standard. This should always be avoided, because in analytical toxicology it can never be excluded that the patient to be monitored has taken this drug even if not available on the regional market.

Besides QTRAP apparatus, LIT apparatus have also been applied for target screening with DDA and library search. However, as they are also suitable for untargeted screening they are discussed in Section 3.

3. Non-targeted screening approaches (GUS, STA) using LC–MS with low mass resolution

GC–MS in full scan mode using comprehensive reference libraries with electron impact spectra and sophisticated search algorithms [13,53,54] still provides excellent non-targeted screening results with the limitation of very volatile and apolar as well as very low dosed compounds. LC–MS overcomes these disadvantages, so that several LC–MS, LC–MS/MS, or LC–MS^n screening procedures using library search were described. Different mass analyzers were used such as triple quadrupoles, ion traps, hybrids of both techniques, or HR TOF analyzers. Procedures based on full scan mode and library search can be used for target and particularly for untargeted screening.

Years ago, the group of Marquet [55] developed a non-targeted screening concept for urine and plasma after SPE similar to that of the Weinmann group [45]. The major difference was, however, the use of enhanced mass scan mode in the survey scan not limiting to pre-defined SRM transitions. Recently, Rosano et al. [56] described a postmortem drug screening by non-targeted GC–MS and UHPLC–MS as well as targeted UHPLC–MS/MS after SPE and compared the results obtained from case work. Unfortunately, it has to be criticized again that these authors also selected marketed drugs as internal standard. UHPLC–MS/MS with SRM selection showed higher detection rates thanks to higher sensitivity. In the cases where UHPLC–MS with in-source collision-induced dissociation (CID) was better, the UHPLC–MS/MS transition ions were present and abundant, but the ion-ratio criteria were not met. Finally, full scan GC–MS showed better results for barbiturates because of their poor electrospray ionization at the low pH conditions of the used mobile phase. As expected, the non-targeted GC–MS and UHPLC–MS methods identified a more comprehensive range of drugs and metabolites than the UHPLC–MS/MS. This study showed again that LC–MS(/MS) is not a full alternative, but a helpful supplement to GC–MS. Lynch et al. [57] came to the same conclusion when comparing screening results obtained by vendor-supplied screening methods using LC–UV, full scan GC–MS, LC–MS, LC–QTRAP–MS, and LC–LIT–MS.

Humbert et al. [58] described a comprehensive UHPLC–MS/MS screening method in serum after LLE operated in full scan mode under multiple fragmentation conditions. Thus, each molecule could be characterized by a combination of retention time and up to 12 individual spectra leading to a library containing 2975 spectra of about 500 compounds. It is surprising that the authors performed classic in-source CID based on a simple full scan mode although using a modern triple quadrupole instrument. As already discussed by Peters and Wissenbach [14], Humbert et al. overestimated the advantage of their full scan method with several spectra per analyte concerning selectivity. MS/MS procedures using QTRAP [46,55] or LIT [48,49,59,60] technologies with precursor selection and PIS comparison provide much better identification power.

Dulaurent et al. [59] published an LIT screening procedure for over 300 pesticides and metabolites in blood after SPE using DDA and library search of the PIS. In this scan type, the MS2 and MS3 spectra were generated by using the base peak of the parent scan as the precursor ion. From this base peak, an MS2 spectrum was generated and an MS3 spectrum from the base peak of the MS2 spectrum. These MS2 and MS3 libraries contained about 450 each and unknowns were searched herein using the NIST algorithm.

Sturm et al. developed also an ion trap procedure for automated screening of about 300 drugs in serum and urine after on-line SPE without cleavage of conjugates using an in-house search algorithm and library [61]. The software considered match factor and reverse match factor, mass-to-charge ratio, relative retention time before reporting the result. In a comparison study with authentic clinical samples, they found 85% agreement with the GC–MS reference method [12] consisting of LLE, acetylation, full scan acquisition and library search [13]. A similar assay was developed by Mueller et al. [60,62]. They used a newer LIT, performed time-consuming cleavage of conjugates before turbulent flow on-line workup and added more compounds in their precursor list. Thanks to conjugate cleavage, several drugs could be detected more sensitively. The disadvantage of both procedures was the lack of metabolite spectra. Therefore, Mueller et al. [63] described recently an attractive approach to generate spectra of phase I metabolites by automated online metabolism method using human liver microsomes with subsequent MS identification. However, the spectra of only the major phase I metabolites could be recorded while Wissenbach et al. could record spectra of over 2700 phases I and II metabolites really present in rat or human urine samples [48–50].

Wissenbach et al. [48,49] developed also a LIT screening approach for drugs in urine after fast protein precipitation. This was the first metabolite-based LC–MS screening also using DDA and MS2 and MS3 reference spectra. Two software tools were used, ToxID and SmileMS, which were both suitable for target screening with some pros and cons, but only SmileMS was suitable for untargeted screening being not limited to precursor selection. Looking for mostly several metabolites per drug markedly increased the identification power. As urine matrix or overlapping compounds can hamper the ionization and DDA, it is advantageous to have several targets per drug. Furthermore, the detection of metabolites confirms the body passage. The library consisted of MS2 and MS3 data of more than 1000 parent drugs and about 2700 metabolites (including phase II metabolites) or artifacts recorded from authentic rat or human urine samples after administration of the corresponding compounds. Of course, the assay was validated according to recommendation for qualitative methods [64]. The same authors transferred their library and the screening procedure to a QTRAP system successfully with some limitations [50]. This study will further be discussed in Section 5.4.

4. Screening approaches using LC–MS with high mass resolution

LC–HRMS screening procedures have recently been reviewed in detail [10,14,18] and Ojanpera et al. [18] have also discussed the principles and pros and cons of HR mass analyzers. Therefore, only landmark procedures will be mentioned here. In recent years, HRMS came back to the toxicology laboratories. Already in early time of GC–MS, HRMS using double-focusing sector field mass analyzers were used for general drug screening. HRMS allowed determining the accurate mass and the corresponding empirical formula of an unknown compound in urine or blood so that the potential drug or poison could be selected from a list of accurate masses of several thousand potential drugs, poisons and pesticides [65]. Ten years ago, a similar concept was described by the group of Ojanpera for LC–MS using time-of-flight (TOF) mass analyzers [66–70]. Mass spectral identification was based on matching measured accurate mass and isotopic pattern of a sample component

with those in the database using a newly developed software for automated reporting of findings in an easily interpretable form [69]. Polettini et al. [71] created an accurate mass list with over 50,000 compounds taken from the PubMed Compound database also including lots of metabolites. However, comparing a measured accurate mass with lists of theoretic accurate masses provided only a screening result that had to be confirmed because there are several potential drugs with the same empirical formula and molecular mass (e.g. isomers like morphine, hydromorphone and the pepper ingredient chavicine) [65]. Looking for accurate masses of potential metabolites does not confirm because the metabolites of isomeric compounds will still have the same accurate mass. Liotta et al. [72] tried to overcome this limitation with a so-called metabolomics approach. They simply looked whether isomeric compounds are able or not to form particular observed metabolites. A better alternative to solve this problem was forming CID spectra with more or less selective fragmentation patterns, so that today, accurate mass measurement, relative retention time and spectra comparison may lead to a sufficient screening result [73]. Already in 2009, Lee et al. [74] described a similar approach but they used nominal mass spectra for initial candidate proposal. Ojanpera et al. [18] supposed that the reason was insufficient reproducibility of mass accuracy. Nevertheless, a comparison study with several laboratories showed promising results.

Another landmark in LC–HRMS screening was published by Broecker et al. [75]. They described a procedure similar to the above-mentioned QTRAP approaches consisting of full scan survey scan screening and PIS library search confirmation, but with the higher identification power of HR PIS spectra. After simple LLE or protein precipitation of blood or urine and common LC separation, the samples were analyzed using a quadrupole TOF (QTOF) instrument in the DDA mode. The compounds were identified by their PIS using a HR spectra library of 2500 compounds. It is obvious that this technique combines the advantages of selective screening by DDA and powerful confirmation by highly specific PIS. The same authors described recently hair testing using the same technique and an interesting option for semi-quantification [76]. In the meantime, quadrupole OT instruments are also available with similar potential and will be applied for a fully automated, comprehensive, and metabolite-based screening approach (A.G. Helfer, M.R. Meyer, A.A. Weber, H.H. Maurer, in preparation).

5. Concluding discussion

5.1. Sample workup

At the beginning of the LC–MS era, enthusiasts claimed that time-consuming sample preparation, mandatory for GC–MS, would no more be needed. Over the years, scientists had to learn that even for LC–MS, sample preparation is relevant in most cases. It improves sensitivity and removes interfering matrix compounds in the sample lowering the risk of matrix effects in LC–MS procedures [77,78]. Dams et al. described years ago in detail the impact of sample workup and risk of ion suppression in toxicological analysis [77]. Peters and Remane critically reviewed recently the impact of matrix effects in LC–MS applications in clinical and forensic toxicology and provided guidance on matrix effect studies for STA and postmortem toxicology [78].

It can consist of filtration or centrifugation followed by dilute and shoot [24,25,52], protein precipitation with or without enrichment [48,49,75], simple or complex LLE [31,37,38,40,58,74,75], SPE [19,34,56,59,73], as well as on-line SPE [61] or turbulent flow extraction [62]. The pros and cons were already discussed elsewhere [8,9,14].

5.2. Separation by HPLC or UHPLC

For electrospray ionization techniques, the selection of mobile phases is rather limited to a few volatile solvents or mixtures. The selection of the stationary phase is much broader from normal to reverse phase. Since a few years, stationary phases with sub-2 μm particle sizes are in use resulting in a much higher separation power as compared to conventional LC columns. Thanks to these columns and LC pumps with much higher pressure, the so-called UHPLC allowed reducing the retention time drastically with still good separation. However, it should be mentioned that peak widths could be too narrow for monitoring a sufficient number of data points per peak in the given cycle time. This is especially true for comprehensive DDA based screening methods and particularly for reliable quantification. Lee et al. [74] described that they had to retard the separation because recording TOF-MS spectra at two aperture voltages extended the MS cycle time so much that a sufficient number of points per peak could not be guaranteed. Nevertheless, UHPLC will replace conventional LC.

5.3. MS detection modes and their specificity

Various MS detection modes and apparatus were applied for screening such as single stage, triple stage, quadrupole, ion trap, TOF, OT analyzers, and hybrids of them. Most authors used ESI and only few APCI in order to minimize the risk or matrix effects [41–44,60,61]. According to an European guideline [32], the identification power depends on the number of monitored ions expressed as identification points. For single stage apparatus, one point is given per ion, for HR, two points per ion, and for MS/MS four points for one precursor and two transitions. Also from this point of view, MS/MS techniques coupled to HR mass analyzers provide the best identification power. Furthermore, they allow collecting and storing data for all ions in a sample and thus retrospective analysis if further case questions arise. These couplings could become the screening technique of the future if the apparatus costs will become reasonable.

5.4. Universality of LC–MS libraries

As documented above, LC–MS with various techniques has found its place also in drug screening. Particularly, the library-based assays providing the demanded identification power are established after years of development. However, one disadvantage that is still not yet overcome is the poor transferability of reference spectra (in-source, tandem in space, or tandem in time CID spectra) from one platform to the other [79]. In contrast, GC–MS providing highly reproducible electron ionization spectra allows screening approaches using huge reference libraries and sophisticated search algorithms [5,8,12,13,53,54]. However, standardization of reference spectra using tuning compounds and variation of collision parameters allowed an inter-laboratory and inter-instrument transfer of the reference spectra for a particular apparatus type [46,48,49,79–87]. In the meantime, sophisticated search algorithms have been developed to compensate some variations (e.g. ion abundance) in reference spectra recorded on different apparatus types [47,51,88–92]. In contrast to dot-product-derived algorithms (e.g. by NIST), such new search algorithms weight relative or absolute fragment intensities to no or a minor extent. Thus, such search algorithms allow compensating variations between spectra recorded using tandem in time or tandem in space fragmentation [89–93]. The group of Oberacher showed promising results comparing reference spectra recorded by QTRAP, QTOF, and LIT [92]. Wissenbach et al. [50] transferred successfully a recently developed linear ion trap (LIT) LC–MS^n screening approach and reference library [48,49] based on tandem in time fragmentation to an

LC–QTRAP system providing tandem in space fragmentation using the SmileMS search algorithm [88].

Peters and Wissenbach [14] discussed recently the two main strategies for spectra transfer. The first strategy aims to achieve similar fragmentation patterns on different instruments by systematic change of the parameters until a fragmentation pattern comparable to the reference spectrum is recorded [80]. The second strategy aims to collect PIS recorded with several different collision energies hoping that the unknown spectrum fits at least with one of the library spectra. However, it must be kept in mind that the selectivity of the library search results may be lowered with the increasing number of spectra (of the same compound) [53]. Although these strategies were tested and used for years [14], there is still no universal and robust LC–MS library available providing results similar to those obtained by GC–MS. Nevertheless, the above-mentioned sophisticated search algorithms with no or minor focus on the relative ion abundance look promising for acceptable screening results [48,49,90]. And finally, the selectivity and specificity of fragment ions recorded in HR and present in spectra recorded on different HR apparatus will be sufficient once for unequivocal compound identification.

5.5. Validation of LC–MS screening methods

Validation of screening methods must not be as comprehensive as of quantitative methods, but should include tests for selectivity, matrix effect, recovery, detection limits, analyte carryover, and stability [14,64]. Peters and Wissenbach [14] recommended the paper of Mueller et al. [60] as a template for suitable validation. Fortunately, most papers reviewed here described more or less method validation. Matrix effect may markedly bias LC–MS procedures [9,78,94,95]. In case of drug screening, matrix or co-eluting compounds with high abundance can lead to false negative results if they hamper the ionization or DDA of a low abundant compound. Both risks can be overcome best by focusing on more than one target analyte per drug, e.g. by monitoring as many metabolites as possible [48,49].

6. Perspectives

The progress of LC–MS technologies over the last years, well documented in series of review articles, allowed the various LC–MS approaches to be established in many routine laboratories in clinical and forensic toxicology and doping control not only for high-throughput quantification but also for screening purposes. Of course, the screening power of all data-dependent scanning approaches is limited if the respective peaks do not trigger or the PIS quality is insufficient. After robust on-line sample workup or even without workup and fast separation with UHPLC methods, several screening strategies are available today for the different demands (single analyses, huge analysis series) in the various laboratories. Advances in separation sciences (e.g. chip technology) may further accelerate the analysis, but only if the MS acquisition is able to follow the speed. The major advances in MS technologies should be the availability of benchtop HRMS apparatus that are robust, easy-to-handle with excellent software options, suitable for use by less well trained staff in daily routine work, and for a reasonable price. Then, HRMS will become the gold standard in analytical toxicology.

As there is no trend that the vendors intend to produce universal standard apparatus like for GC–MS, the transfer of reference libraries to the various types of analyzers must be promoted. This is particularly important for metabolite spectra as they cannot be recorded on all types of apparatus because reference standards are mostly not available. Therefore, existing comprehensive metabolite libraries built up during time-consuming metabolism studies should be made available for the various apparatus. This is important for several reasons. Most lipophilic compounds can be detected particularly in urine only via their metabolites. Detection of several metabolites per drug markedly increased the identification power. As ion suppression by urine matrix or overlapping compounds can never be excluded, it is advantageous to have several targets per drug. Furthermore, the detection of metabolites confirms the body passage.

Most authors who compared their new LC–MS screening approach with well-established GC–MS assays concluded that LC–MS was not a full alternative, but a helpful supplement to GC–MS. Thus GC–MS will still have a place in laboratories of the future, not only in countries with lower budgets.

If once available, fully automated black box LC–MS systems that allow series of analyses, as current immunoassay-based multi-analyzers in clinical chemistry, will bring the end of the immunoassay era and start definitely the MS era in drug monitoring, clinical and forensic toxicology, workplace drug testing and doping control.

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