Multi-analyte procedures for screening for and quantification of drugs in blood, plasma, or serum by liquid chromatography-single stage or tandem mass spectrometry (LC-MS or LC-MS/MS) relevant to clinical and forensic toxicology

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Abstract

This paper reviews multi-analyte procedures for screening and quantification of drugs in blood, plasma, or serum using liquid chromatography coupled with a single stage or tandem mass spectrometer (LC-MS, LC-MS/MS). These procedures are relevant tools in clinical and forensic toxicology, and cover analysis of amphetamines, cocaine, hallucinogens, opioids, anesthetics, hypnotics, benzodiazepines, antidepressants, neuroleptics, antihistamines, sulfonylurea-type antidiabetics, beta-blockers, and other cardiac drugs. Basic information on the procedures is given in two tables and multi-analyte screening, identification, and quantification are illustrated in three figures. A critical discussion on the pros and cons of such LC-MS procedures is also included.

Keywords: Screening; Quantification; Drugs; Liquid chromatography; Mass spectrometry

Introduction

Efficient toxicological analysis is the basis of a competent toxicological judgement and consultation in clinical and forensic toxicology. Multi-analyte procedures for screening and quantification of drugs in blood, plasma, or serum are relevant tools in these fields because they allow analysis of several important compounds with a single sample extract injection, thus saving time and resources. In pharmaceutical research and development, liquid chromatography coupled with a single stage and, especially, tandem mass spectrometers (LC-MS, LC-MS/MS) is increasingly...
used because of its versatility for high-throughput determinations of drug concentrations in biological samples obtained from pharmacokinetic or toxicological studies [1–4]. In such studies, the analytes are known and they only have to be quantified. In clinical and forensic toxicology, the compounds, which have to be determined, are, mostly, unknown. Therefore, the first step, before quantification, is to screen for and identify any compounds of interest. High-throughput procedures in analytical toxicology mean that numerous relevant intoxicants can be screened for simultaneously using one single procedure (so-called systematic toxicological analysis, STA).

The choice of method in analytical toxicology depends on the problems to be solved. The analytical strategy often includes a screening test and a confirmatory test before quantification. If only a single drug or drug class has to be monitored, immunosays can be used for preliminary screening in order to differentiate between negative and presumptively positive samples. 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. Up to now, gas chromatography-mass spectrometry (GC-MS) has been the most widely used reference method in analytical toxicology [5]. 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 sports organizations, and for which immunosays are commercially available. If these demands are not met, the screening strategy must be more extensive, because several thousand drugs or pesticides are on the market worldwide [6]. For such high-throughput screening in routine analysis, thin-layer chromatography, gas chromatography with common detectors, electrokinetic techniques, and high-performance liquid chromatography (HPLC) coupled with a diode-array detector (DAD) are principally applicable, but GC-MS is by far the most widely used method in this context. The position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control has been reviewed recently [5].

LC-MS or LC-MS/MS still plays only a limited role for comprehensive screening, but some applications are looking promising [7–19]. In the following, procedures are reviewed for screening and/or quantification of drugs in blood, plasma, or serum relevant to clinical and forensic toxicology. Papers concerning urinalysis have already been reviewed elsewhere [5] and are not dealt with here. Only papers written in English were taken into consideration.

Biosamples and their preparation

Although urine is still the sample of choice for non-target comprehensive screening for and identification of unknown drugs or poisons [5], blood, plasma, or serum can and sometimes must be used for at least a limited screening especially for target analytes within multi-analyte procedures. This is especially advantageous, if only blood samples are available and/or if the procedures allow screening and subsequent quantification [8,11,12,14,16, 19–21]. In driving under the influence of drugs (DUID) cases, blood analysis is even mandatory in some countries [22].

LC-MS has been claimed to be less demanding concerning sample preparation. One procedure reviewed here required only deproteinization [23]. All others required a more or less selective extraction especially in order to avoid ion suppression effects [24–30]. In contrast to urinalysis, cleavage of conjugates is mostly not necessary for blood analysis, because the conjugates, with the exception of morphine-6-glucuronide, are pharmacologically inactive and only present at low concentration due to lack of tubular reabsorption.

Isolation can be performed by liquid–liquid extraction (LLE) at a pH value at which the analyte is not ionized [8,12–14,19,31] or by solid-phase extraction (SPE) [15,16,18,21,32,33]. Whole blood samples require deproteinization and centrifugation steps before being loaded onto the SPE column. Decaestecker et al. [34] tested the suitability of an entire series of SPE sorbents in order to select the best sorbent with regard to extraction yields of 18 neutral and basic compounds as well as to the cleanliness of the extracts. The authors found that an Isolute C(8) sorbent performs best in this sense. Whatever SPE column is used, the analyst should keep in mind that there are differences from batch-to-batch and that comparable sorbents from different manufacturers may also lead to different results [35]. Therefore, use of suitable internal standards (IS, e.g., deuterated analogues of the analytes) is recommended.

Solid-phase microextraction (SPME) is becoming an alternative to SPE and LLE, mainly for GC-MS analysis. SPME is a solvent-free and concentrating extraction technique especially for rather volatile analytes. It is based on the adsorption of the analyte on a stationary phase coating a fine rod of fused silica. SPME procedures for LC-MS/(MS) determinations in body fluids have been described for phenothiazines [20] or diazepam and its metabolites [36], but such procedures require special interfaces.

Multi-analyte LC-MS/(MS) procedures for screening for drugs in blood, plasma, or serum

LC-MS and LC-MS/MS are becoming increasingly routine pieces of apparatus, especially for blood and plasma/serum analysis [5,7,9,37]. However, when establishing LC-MS screening procedures in routine work, several limitations should be kept in mind, as stated by the majority of experts in this field [5,9,11,38–40]. The spectral information of electrospray ionization (ESI) and/or atmospheric pressure chemical ionization (APCI) spectra is
limited compared to electron ionization mass spectra. Collision-induced dissociation (CID) caused by increasing the orifice or fragmentor voltage leads to formation of structure-related fragments. Modern apparatus allows very fast switching between different voltages so that different traces can be recorded during LC separation. Thus, different compounds with different fragmentation properties can be analyzed in one run without loss of spectral information. However, the fragmentation can vary considerably between different instruments [39–42]. Weinmann et al. [39] could show that different types of apparatus may lead to rather reproducible ESI spectra formed by in-source CID if the apparatus had been tuned using certain test compounds, such as haloperidol, paracetamol, metronidazole, or metamizol. They concluded that mass spectral library searching of an ESI-CID-MS library set-up with one of the two instruments should be possible with the other instrument after adjusting the CID energies by means of at least two tuning compounds. Criteria for compound identification by single or multiple stage LC-MS were recently reviewed by Rivier [40]. The author of the present review agrees with Rivier’s final conclusion that the responsibility lies with the toxicologist to decide, depending on the case, how and when the minimum requirement for identity confirmation has been reached, and not to rely exclusively on match quality parameters.

Another important problem for ESI is the reduction of the ionization of an analyte by co-eluting compounds, the so-called ion suppression effect [24–30], because in these cases a relevant intoxicated might be overlooked, [43] perhaps compromising patient outcome. Dams et al. [27] evaluated the synergistic effect of ionization type, sample preparation technique, and bio-fluid (urine, oral fluid, and plasma) on the presence of matrix effects. They found that both ESI and APCI showed matrix effects, with ESI being much more susceptible than APCI, and that sample preparation could reduce (clean-up) or magnify (pre-concentrate) matrix effects. Mallet et al. [24] studied ion suppression effects in ESI from mobile phase additives and SPE, and concluded that a clean up of plasma extracts based on ion exchange leads to minimal ion suppression or enhancement. Liang et al. [26] showed that all the nine investigated target drugs and their co-eluting isotope-labeled ISs suppressed each other’s ionization response in the ESI mode. In contrast, APCI caused seven of the nine investigated target drugs, and their co-eluting isotope-labeled IS, to enhance the ionization responses of each other. The mutual ionization suppression or enhancement could possibly influence assay sensitivity, linearity, accuracy, and precision in quantitative LC-MS/(MS). Ion suppression results from the presence of compounds that can change the efficiency of droplet formation or droplet evaporation in the spray chamber which, in turn, affects the amount of charged ion in the gas phase that ultimately reaches the detector. Salts, ion-pairing agents, endogenous compounds, drugs, metabolites, and even isotope-labeled ISs have been shown to be responsible for ion suppression [24–26]. In the opinion of the author, papers dealing with bioanalytical procedures using ESI LC-MS/(MS) should only be accepted for publication by scientific journals if ion suppression studies have been performed. Such studies should, at least, include signal recovery studies using extracts of blank matrix to which analytes are added or, even better, a more comprehensive approach using post-column infusion of the analyte to evaluate protracted ionization effects [25]. If relevant ion suppression occurs, specimen clean-up, chromatographic changes, reagent modifications, and effective internal standardization should be performed. Nevertheless, LC-MS is an excellent complementary technique to GC-MS for screening, library-assisted identification, and quantification of thermolabile, low-dosed, high-molecular, and/or polar compounds, especially in plasma [5,7,9,37].

Several concepts have been developed for LC-MS screening and detection. Bogusz [32], Rittner et al. [33], Marquet and coworkers [18,38], and Maurer and coworkers [11,12,16,19,21,44] have developed screening procedures for blood analysis based on different single stage LC-MS apparatus. Gergov et al. [13] described a multi-analyte blood screening method using classical triple-quad LC-MS/MS, while Marquet et al. [17] compared, in a preliminary study, a new and more sensitive procedure using a quadrupole-linear ion-trap mass spectrometer with the above-mentioned single-stage procedure. The basic information on the multi-analyte procedures for screening for drugs in blood, plasma, or serum is summarized in Table 1 to simplify the rapid selection of a method suitable for an actual analytical problem.

In addition to these general procedures, some LC-MS (MS) procedures have been described for screening for single drug classes, such as antihistamines [8], serotoninergic drugs [14], phenothiazines [20], or neuroleptics [15]. At this center, we are developing universal single stage LC-MS procedures for screening, library-assisted identification, and, in contrast to other screening procedures, additional fully validated quantification of many drug classes in blood plasma. These drug classes include anesthetics, low-dosed hypnotics and opioids [44,45], benzodiazepines [19], sulfonylurea-type antidiabetics [12], neuroleptics [16], and beta-blockers [21]. This general LC-MS procedure is based on previously published standard LLE [12,19,46] or SPE [16,21,47,48] procedures also used for GC-MS analyses. The SPE procedure has proved to be especially useful for low-dosed basic drugs, but unfortunately, it has not proved applicable to the isolation of all studied analytes [19]. The standard separation is based on a fast gradient leading to analysis times of 5–10 min. The APCI mode was preferred over the ESI mode for the reasons given above. Moreover, the LC-MS system used showed higher sensitivity for the studied analytes when operated in the APCI mode. The mass spectra were recorded at two different fragmentor voltages (100 V and 200 V) with a very short cycle time. However, as already discussed above, it should be kept in mind that the same fragmentor voltages selected in different
<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample</th>
<th>Work-up</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detection mode</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines, Benzodiazepines, Hallucinogens, Opioids, Cocaine, Olanzapine</td>
<td>B, S (Urine)</td>
<td>SPE</td>
<td>Superspher RP 18</td>
<td>Aqueous ammonium formate and acetonitrile</td>
<td>APCI, positive mode, SIM screening</td>
<td>[32]</td>
</tr>
<tr>
<td>Amphetamines, Benzodiazepines, Hallucinogens, Opioids, Hypnotics, Neuroleptics</td>
<td>S</td>
<td>SPE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient acetonitrile, water, methanol</td>
<td>ESI, positive mode, scan</td>
<td>[33]</td>
</tr>
<tr>
<td>Antidepressants, Benzodiazepines, Cardiac drugs, Opioids, Hypnotics, Neuroleptics</td>
<td>S</td>
<td>SPE</td>
<td>Nucleosil C18</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>ESI, positive and negative mode, scan</td>
<td>[18,38]</td>
</tr>
<tr>
<td>238 Drugs</td>
<td>B</td>
<td>LLE</td>
<td>Purospher RP-18</td>
<td>Gradient with acetonitrile and aqueous ammonium acetate</td>
<td>Triple stage, ESI, positive mode, multiple-reaction monitoring (MRM)</td>
<td>[13]</td>
</tr>
<tr>
<td>18 Antihistamines</td>
<td>B</td>
<td>LLE</td>
<td>Purospher RP-18</td>
<td>Gradient with acetonitrile and aqueous ammonium acetate</td>
<td>Triple stage, ESI, positive mode, MRM, confirmation using product ion spectra</td>
<td>[8]</td>
</tr>
<tr>
<td>13 Anesthetics, low-dosed hypnotics and opioids</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, scan</td>
<td>[44,45]</td>
</tr>
<tr>
<td>23 Benzodiazepines</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, scan</td>
<td>[19]</td>
</tr>
<tr>
<td>9 Sulfonylurea-type antidiabetics</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, scan</td>
<td>[12]</td>
</tr>
<tr>
<td>15 Neuroleptics</td>
<td>P</td>
<td>SPE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, scan</td>
<td>[16]</td>
</tr>
<tr>
<td>23 Beta-blockers</td>
<td>P</td>
<td>SPE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, scan</td>
<td>[21]</td>
</tr>
<tr>
<td>12 Serotonergic drugs</td>
<td>B</td>
<td>LLE</td>
<td>Zorbax Extend-C18</td>
<td>Ammonia, methanol, tetrahydrofuran</td>
<td>APCI, positive mode, scan</td>
<td>[14]</td>
</tr>
<tr>
<td>11 Phenothiazines</td>
<td>B (Urine)</td>
<td>SPME</td>
<td>Capcell Pak C18</td>
<td>Gradient with aqueous ammonium acetate and acetonitrile</td>
<td>ESI, positive mode, MS in scan, MS/MS, selected reaction monitoring (SRM)</td>
<td>[20]</td>
</tr>
<tr>
<td>19 Neuroleptics</td>
<td>B, hair (Urine)</td>
<td>B and hair: SPE</td>
<td>Zorbax Stable Bond Cyano</td>
<td>Gradient with methanol, acetonitrile and aqueous ammonium formate</td>
<td>ESI, positive mode, MS/MS, MRM</td>
<td>[15]</td>
</tr>
</tbody>
</table>
apparatus may result in different abundances of the formed fragments [39–42]. Therefore, each user has to select the fragmentor voltage most suitable for their specific apparatus, which produces mass spectra comparable with those noted in the corresponding papers [12,16,19,21,49]. In the author’s experience with three different pieces of apparatus of the same type, this has allowed the successful use of the screening procedure. As an example of the screening and identification procedure, smoothed and merged mass chromatograms (scan mode, 100 V) of the ions \( m/z \) 450, 271, 446, 312, 298, 367, 494, 491, 528 of an authentic plasma extract indicating the presence of oral antidiabetics of the sulfonylurea-type are shown in the upper part of Fig. 1. The mass spectrum underlying the marked peak (lower spectrum), the reference spectrum (upper spectrum), the structure, and the hit list found by computer library search [50] are shown in the lower part of Fig. 1.

Multi-analyte LC-MS/(MS) procedures for quantification of drugs in blood or plasma

In general, after screening and identification, the relevant analytes must be quantified. All quantification assays should be fully validated according to international recommendations which were critically reviewed by Peters and Maurer [51]. The authors recommend testing the following parameters: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, accuracy, and precision, as well as stability if the sample on the bench top, as a processed sample, during freeze/thaw cycles and long-term stability and, finally, the applicability. Such validation data are the only objective basis for assessment of a procedure’s performance and quality.

The basic information of the multi-analyte procedures for quantification of drugs in blood or plasma is summarized in Table 2 to simplify the rapid selection of a method suitable for an actual analytical problem. Zhang et al. [31] described very fast quantification of five tricyclic antidepressants in plasma using a time-of-flight (TOF) mass spectrometer after LLE. Mortier et al. [52] developed and validated an LC-MS procedure for some amphetamine-related designer drugs in blood, urine, and postmortem tissues. However, for these analytes, LC-MS is not mandatory, as GC-MS procedures for four times the number of drugs have also been developed and successfully validated [48]. Sayer et al. [23] published an LC-MS assay for determination of five non-depolarizing neuromuscular blocking agents and their degradation products/metabolites in biosamples. The assay was validated for blood serum.

As already mentioned above, some multi-analyte procedures for screening also allow consecutive quantification, such as that of Gergov et al. for antihistamines [8], that of Goeringer et al. for serotonergic drugs [14], that of Kumazawa et al. for phenothiazines [20], or that of Josefsson et al. for neuroleptics [15]. However, in the last
<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample</th>
<th>Work-up</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Detection mode</th>
<th>Validation data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Amphetamine-related designer drugs</td>
<td>B</td>
<td>LLE</td>
<td>Hypersil BDS phenyl</td>
<td>Gradient with acetonitrile and aqueous formic acid</td>
<td>ESI, ion trap, positive mode</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, applicability</td>
<td>[52]</td>
</tr>
<tr>
<td>5 Antidepressants</td>
<td>P</td>
<td>LLE</td>
<td>SB-C18 Mac Mod</td>
<td>Gradient with acetonitrile and aqueous ammonium acetate</td>
<td>ESI, TOF, positive mode</td>
<td>Selectivity, linearity, LOQ, recovery, accuracy and precision</td>
<td>[31]</td>
</tr>
<tr>
<td>18 Antihistamines</td>
<td>B</td>
<td>LLE</td>
<td>Purospher RP-18</td>
<td>Gradient with acetonitrile and aqueous ammonium acetate</td>
<td>Triple stage, ESI, positive mode, MRM</td>
<td>Linearity, LOD, LOQ, recovery, accuracy and precision, applicability</td>
<td>[8]</td>
</tr>
<tr>
<td>5 Non-depolarizing neuromuscular blocking agents and their degradation products/metabolites</td>
<td>S</td>
<td>Deproteinization</td>
<td>X-Terra MS C18</td>
<td>Gradient with acetonitrile and ammonium formate</td>
<td>ESI, positive mode, SIM</td>
<td>Linearity, LOD, LOQ, recovery, accuracy and precision, applicability</td>
<td>[23]</td>
</tr>
<tr>
<td>12 Serotonergic drugs</td>
<td>B</td>
<td>LLE</td>
<td>Zorbax Extend-C18</td>
<td>Ammonia, methanol, tetrahydrofuran</td>
<td>APCI, positive mode, scan</td>
<td>Selectivity, linearity, recovery, accuracy and precision, applicability (Data given only as range of those measured for only selected drugs)</td>
<td>[14]</td>
</tr>
<tr>
<td>11 Phenothiazines</td>
<td>B</td>
<td>SPME</td>
<td>Capcell Pak C18</td>
<td>Gradient with aqueous ammonium acetate and acetonitrile</td>
<td>ESI, positive mode, MS/MS, SRM</td>
<td>Linearity, LOD, recovery, accuracy and precision, applicability</td>
<td>[20]</td>
</tr>
<tr>
<td>19 Neuroleptics</td>
<td>B</td>
<td>SPE</td>
<td>Zorbax Stable Bond Cyano</td>
<td>Gradient with methanol, acetonitrile and aqueous ammonium formate</td>
<td>ESI, positive mode, MS/MS, MRM mode</td>
<td>Linearity, LOQ, applicability</td>
<td>[15]</td>
</tr>
<tr>
<td>15 Neuroleptics</td>
<td>P</td>
<td>SPE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, SIM</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, stability (bench top, processed sample, freeze/thaw, long-term), applicability</td>
<td>[16]</td>
</tr>
<tr>
<td>23 Benzodiazepines</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, SIM</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, stability (bench top, processed sample, freeze/thaw, long-term), applicability</td>
<td>[19]</td>
</tr>
<tr>
<td>13 Anesthetics, low-dosed hypnotics and opioids</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, SIM</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, stability (bench top, processed sample, freeze/thaw, long-term), applicability</td>
<td>[44,45]</td>
</tr>
<tr>
<td>9 Sulfonylurea-type antidiabetics</td>
<td>P</td>
<td>LLE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, SIM</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, long-term stability, applicability</td>
<td>[12]</td>
</tr>
<tr>
<td>23 Beta-blockers</td>
<td>P</td>
<td>SPE</td>
<td>Superspher 60 RP Select B</td>
<td>Gradient with acetonitrile and aqueous ammonium formate</td>
<td>APCI, positive mode, SIM</td>
<td>Selectivity, linearity, LOD, LOQ, recovery, accuracy and precision, stability (bench top, processed sample, freeze/thaw, long-term), applicability</td>
<td>[21]</td>
</tr>
</tbody>
</table>
of these papers, no validation data were presented with the exception of the LOQ, so it is not clear whether this procedure fulfills forensic or clinical toxicological standards. Maurer and coworkers have also developed universal single stage LC-MS procedures for quantification after screening and library-assisted identification of many drug classes in plasma such as anesthetics, low-dosed hypnotics and opioids [44,45], benzodiazepines [19], sulfonylurea-type antidiabetics [12], neuroleptics [16], and beta-blockers [21]. The quantification assays were developed using the same extracts and standard LC separation as used for screening, but operated in the positive APCI selected-ion monitoring (SIM) mode. These procedures were fully validated and the acceptance criteria were fulfilled for the majority of analytes tested. These are the only published LC-MS multi-analyte procedures for which the different forms of stability have been tested. In the author’s opinion, such stability testing should be mandatory, especially in forensic toxicology, because in most cases the specimens are not analyzed directly after sampling. Repeated freezing and thawing is often the rule rather than the exception.

As an example of the procedure, quantification, smoothed, and merged mass fragmentograms normalized in relation to the abundance of the IS (SIM mode, 100 V, ions m/z 450, 271, 446, 312, time window 0–4.8 min; 324, 298, 367, 494, 491, 528, time window 4.81–7 min) of a blank plasma sample (top) and of a blank plasma sample spiked with 1.0 mg/L of the IS (middle) and of a blank plasma sample spiked with IS and sulfonylurea-type antidiabetics (bottom). Taken from Ref. [12].

These fragmentograms show the normalized abundance of the ions at m/z 450, 271, 446, 312, 324, 298, 367, 494, 491, 528, indicating the absence of the analyte of interest in the blank sample and the presence of the analyte in the spiked sample. The corresponding fragmentograms

Fig. 2. Smoothed and merged mass fragmentograms normalized in relation to the abundance of the IS (SIM mode, 100 V, ions m/z 450, 271, 446, 312, time window 0–4.8 min; 324, 298, 367, 494, 491, 528, time window 4.81–7 min) of a blank plasma sample (top) and of a blank plasma sample spiked with 1.0 mg/L of the IS (middle) and of a blank plasma sample spiked with IS and sulfonylurea-type antidiabetics (bottom). Taken from Ref. [12].

These fragmentograms show the normalized abundance of the ions at m/z 450, 271, 446, 312, 324, 298, 367, 494, 491, 528, indicating the absence of the analyte of interest in the blank sample and the presence of the analyte in the spiked sample. The corresponding fragmentograms

Fig. 3. Smoothed and merged mass fragmentograms (SIM mode, 100 V) with the given ions of an authentic plasma extract (same extract as used in Fig. 1) indicating 0.15 mg/L of glibenclamide. Taken from Ref. [12].
recorded at 200 V were also free of interfering peaks. As the drugs were first identified in the full scan mode, the use of only the target ion for quantification, without qualifiers, was acceptable. Fig. 3 shows smoothed, normalized, and merged mass fragmentograms (SIM mode, 100 V) with the given ions of an authentic plasma extract indicating 0.15 mg/L of glibenclamide.

Conclusions and perspectives

LC-MS has been shown to be an ideal supplement to GC-MS, especially for detection and quantification of more polar, thermolabile, or low-dosed drugs, especially in blood plasma. It may become the gold standard in clinical and forensic toxicology and doping control if the costs of the apparatus become markedly reduced, and the current disadvantages, such as irreproducibility of fragmentation, reduction of ionization by matrix, etc., can be overcome. Finally, it would be an advantage if one of the increasing number of quite different techniques were to become the apparatus standard.

In conclusion, although urine is still the sample of choice for non-target comprehensive screening for and identification of unknown drugs or poisons [5], blood, plasma, or serum can and sometimes must be used for at least a limited screening especially for target analytes within multi-analyte procedures. Some of these procedures also allow validated multi-analyte quantification. In the future, such procedures will certainly help to solve many analytical and time-consuming problems in clinical and forensic toxicology.

Acknowledgments

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