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An automated screening method for drugs and toxic compounds in human serum and urine using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A fully automated screening using liquid chromatography–mass spectrometric method applying data-dependent acquisition was developed to identify toxicologically relevant substances in serum and urine. A library including more than 405 spectra of about 365 compounds (main drugs and important metabolites) was established. An easy to use program was created to automate and accelerate library search. Drugs were identified based on their relative retention times, molecular ions and fragment ions. Limits of detection were tested with 100 of the 365 compounds the majority of these were lower than 100 μg/l (67%). The developed LC–MS–MS system seems to be a valuable alternative to other general unknown screening methods allowing fast and specific identification of drugs in serum and urine samples.

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1. Introduction

A non-target screening for detection of drugs and toxic compounds is called general unknown screening (GUS) procedure or systematic toxicological analysis (STA). This procedure is an analytical method designed to detect and identify xenobiotics in biological fluids, which is necessary for confirmation of the diagnosis of an acute poisoning with drugs or other exogenous compounds. Rapid and comprehensive screening procedures are therefore necessary.

For current STA procedures in clinical and forensic toxicology, automated immunoassays for the most common drugs of abuse in combination with chromatographic techniques coupled to specific detectors are often used. Gas chromatography–mass spectrometry is so far the gold standard for this purpose [1–4]. Advantages of this method are the large number of compounds in the library (>8000) and the transferability of the library to GC–MS systems of different manufacturers.

Its application however is limited to non-polar, volatile and thermally stable compounds. In addition, derivatization is necessary for detection of polar compounds such as metabolites, which complicates the screening procedure, but enables to detect compounds with different pK_a values to be analyzed in one GC run [1].

HPLC coupled to UV diode array detection (DAD) overcomes these limitations [5–8]. However it shows reduced separation efficiency and the detection of compounds is not as specific and reliable as compared to GC–MS [1].

Therefore, in the last years, the combination of mass spectrometry with liquid chromatography has been evaluated for screening analysis. It was shown to be very sensitive, precise, specific, universal and very fast, when coupled to an automated extraction system [9–16].

Several authors described screening methods with LC–MS that apply in-source collision induced dissociation (CID), LC–MS–MS in the multiple-reaction monitoring mode, and LC–MS–MS using data-dependent acquisition (DDA) [9–18].

With single MS, the mass spectrometer operates in the scan mode and applies in-source CID. The sample is screened at variable orifice voltages [10,11,16,17]. Reconstructed spectra can be obtained and be compared with in-source CID spectra libraries.

MS–MS data have the advantage of providing a higher specificity and selectivity and more structural information than single MS. This mode has been shown to be helpful when an unknown substance has to be identified. Although LC–MS–MS in the multiple-reaction monitoring mode can be applied to a high number of previously selected compounds, but this number is limited [12].

LC–MS–MS using DDA seems to be the best procedure for simultaneous screening and identification of unknown compounds using

Abbreviations: GUS, general unknown screening; APCI, atmospheric pressure chemical ionization; MS–MS, tandem mass spectrometry; nd, not detected; LOD, limit of detection; DAD, diode array detection; CID, collision induced dissociation; DDA, data-dependent acquisition; SPE, solid-phase extraction; IS, internal standard; PE%, process efficiency in percent; RRT, relative retention time; RT, retention time.

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a single chromatographic run. In this procedure, ions that exceed a preset threshold are fragmented by CID and the resulting fragments are measured in the product-ion scan mode [13–15]. This technique is highly specific and selective. Spectra result from a single ion and the origin of the spectra is registered.

This study presents an approach of an automated screening procedure with a specially created library search program to perform compound identification. The chosen procedure consisted of on-line solid-phase extraction (SPE) and LC–MS–MS using DDA.

2. Experimental

2.1. Materials

Test substances obtained from various pharmaceutical companies were of pharmaceutical purity. Organic solvents and reagents were of analytical grade. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), ammonium formate from Aldrich (Steinheim, Germany) and formic acid from Fluka (Buchs, Switzerland). De-ionized water was generated with a Milli-Q water purification system from Millipore (Kloten, Switzerland). Drug free serum was purchased from Biorad, (Reinach Switzerland). Serum and Urine samples were used without any subject information for the comparison between different methods (this procedure was accepted by verbal agreement of the local State Ethic Commission (EKBB)).

2.2. Apparatus

The chromatographic system consisted of a Rheos 2000 Micro HPLC pump from ThermoFinnigan (ThermoFisher, Reinach BL, Switzerland) and a Midas Symbiosis Autosampler from Spark Holland (Emmen, Netherlands) using a 100 µl loop. The detector was a ThermoFinnigan LCQ Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) device and the Xcalibur software. Automated solid-phase extraction was performed using a Prospekt 2 from Spark Holland consisting of an automatic cartridge-exchange module, dual cartridge clamps and a solvent delivery unit. HySphere Resin GP cartridges were purchased from Spark Holland.

2.3. Methods

2.3.1. Standard solutions

Separate stock solutions were prepared in methanol–water (1:1, v/v) at a concentration of 100 mg/l. Serum standards were prepared by spiking with stock solutions of drug mixtures to get concentrations ranging from 0.005 to 4 mg/l, resulting in a set of standards with the following concentrations: 0.005, 0.010, 0.025, 0.050, 0.100, 0.250, 0.500, 1.000, 2.000 and 4.000 mg/l.

d_3-Benzoylecgonine was prepared as internal standard (IS) at a concentration of 5 mg/l.

The results of the new method were compared to immunoassays, to analysis performed on a Remedi Systems from Biorad (Remedi HS and Remedi Benzodiazepine [28]), to an in-house developed LC–MS method (ThermoFinnigan Navigator) following the procedure described by Bogusz et al. [19] and to a full-scan GC–MS screening method [20] applied in the Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Germany.

2.3.2. Extraction procedure

The IS solution (100 µl) was pipetted into each sample of 1 ml of serum or urine. Thereafter the sample was acidified by addition of 20 µl concentrated formic acid (cleaving possible protein binding of drugs).

Table 1
Data dependent and global data dependent settings.

Setting	Value
Data dependent settings	
Default charge state	1
Default isolation width (m/z)	4.0
Normalized collision energy (% pos/neg))	40.0 resp. 35.0
Minimal signal required	20,000
Global data dependent settings	
Exclusion mass width (m/z)	0.5
Reject mass width (m/z)	1.0
Dynamic exclusion	enabled
Repeat count	1
Repeat duration (min)	0.5
Exclusion list size	25
Exclusion duration (min)	0.5
Exclusion mass width (m/z)	0.5

On-line SPE and elution were performed using the Prospekt 2 system. The HySphere Resin GP (Spark Holland) cartridge was conditioned with 1 ml of methanol (5 ml/min) and with 1 ml of water (5 ml/min). A 100-µl-aliquot of the serum or urine was loaded on the cartridge. The sorbent was washed with 1 ml of water (2 ml/min), and eluted directly with the mobile phase over 15 min.

2.3.3. Evaluation of matrix effects and process efficiency

Possible influences by matrix effects were studied with three different methods. In the first test, process efficiency was determined [29].

For calculation of the process efficiency expressed in percent (PE%), the peak area ratio (i.e. the peak area of the drug of interest was divided by the peak area of the IS) obtained after the on-line extraction of a serum or urine sample and compared to the peak area ratio obtained after direct injection of the same amount of an aqueous solution into the LC–MS–MS system. (The PE% of the IS (84%) was also considered in the calculations).

$$\mathrm{PE\,\%} = \frac{\text{Peak area ratio of a serum sample spiked before extraction}}{\text{Peak area ratio of an aqueous solution}} \times 100$$

The second procedure is based on the post-column infusion of an analyte in a chromatographic run of blank serum or urine. The signal was compared to the signal obtained with post-column infusion of the analyte into the eluent of the corresponding blank matrix extract [29]. In the last experiment, blank samples (serum and urine) used as negative controls were analyzed.

2.3.4. Liquid chromatography

The chromatographic separation was performed on a CC Nucleodur C18 Gravity 3 µm column (4 mm × 125 mm) with an integrated guard column 3 µm (4 mm × 8 mm) from Macherey-Nagel (Oensingen, Switzerland). The mobile phase was delivered at a flow rate of 400 µl/min. Each chromatographic run was performed with a binary, linear A/B gradient (solvent A was 10 mmol/l ammonium formate, pH 3.0. Solvent B was 90% acetonitrile, 10% 10 mmol/l ammonium formate, pH 3.0.). The program was as follows: 0–1 min, 6% B; 1–8 min, 6–100% B; 8–20 min 100% B; 20–23 min column equilibration with 6% B.

2.3.5. Mass spectrometry

The following APCI inlet conditions were applied. The heated vaporizer was kept at 465 °C. Both the sheath gas set at 60 relative units and the auxiliary gas set at 15 relative units were nitrogen. The capillary entrance to the ion trap was at an offset of 28 V in the positive mode, −4 V in the negative mode and was maintained at 220 °C. The corona current was 5 µA. Table 1 shows the data dependent and global data dependent settings.

DDA was used, generating a full-scan between 80 and 750 atomic mass units in the first mode. If ions exceeded the preset

threshold a MS–MS spectrum of the most intense ion of the previous full-scan was acquired in the second mode.

The maximum injection time was set to 50 ms, and three microscans were collected for each data point. Normalized collision energy was 40.0% in the positive mode and 35.0% in the negative mode. Dynamic exclusion was enabled meaning that a refractory period was applied to the last selected ion. The refractory period was 30 s.

2.3.6. Evaluation of the limit of detection (LOD)

For qualitative purposes only the LOD of each substance in the library of a GUS method is important in order to know the specific performance of the procedure. The LOD's were tested for 100 of the 365 compounds by analyzing each substance after spiking to drug free serum (or urine) with decreasing concentrations in the range of 4.000–0.005 mg/l (two times the same procedure with on-line extraction, chromatographic separation and defining the limit by the mean of the two results).

2.3.7. Mass spectral library

Standard solutions were prepared in methanol–water (1:1, v/v) at a concentration of 1–2 mg/l. Two mass spectral libraries were created, one for each ionization mode (positive and negative), by injecting 20 μl of these solutions directly without HPLC separation into the MS system. The obtained MS–MS spectra were added to the library. Relative retention times (RRT) were acquired by actual LC–MS (-MS) analysis running a mixture of each compound spiked to serum (and in a new trial in urine) with the IS. Obtained MS–MS data from a chromatographic run were compared to the MS–MS library using the NIST Mass Spectral Program 2.0 from ThermoFinnigan. A computer program (XcLibraryScreening) was created to automate the searching process and to include the RRT and the molecular ion in the identification of unknown compounds.

2.3.8. Mass spectral library search program (XcLibraryScreening)

To automate the search process and to combine the MS–MS library with the RRT and mass-to-charge ratio of each substance a Microsoft Windows application (XcLibraryScreening) was developed. It was written in Microsoft Visual Basic.NET and requires the Microsoft .NET Framework as well as a running copy of the NIST Mass Spectral Program 2.0 from ThermoFinnigan with the configured MS–MS library. RRTs and mass-to-charge ratios of each compound are stored on a file that can be edited.

Criteria to search with the XcLibraryScreening were configured (Supplementary material Fig. 5) by adding the retention time of the internal standard, and defining the accepted windows for the mass-to-charge ratios and RRTs and threshold for the match factors (*Match Factor* and *reverse Match Factor*). In a separated window (Fig. 1), the program listed the identified compounds matching the defined criteria.

3. Results and discussion

On-line SPE was chosen as an extraction technique because this procedure is universal, rapid and can be automated. Therefore, this method is becoming popular in bioanalytical analysis [24]. The Prospect SPE can be linked to the LC–MS–MS instrument [25].

This system couples and automates sample extraction and instrumental analysis. The method has a time saving advantage compared to other techniques because the evaporation of the liquid sample extract is not necessary. The extraction is an on-line procedure and the elution solvent is the mobile phase. In addition, the mobile phase consists of a gradient.

The PE% of 10 drugs from different substance classes (acidic, neutral, and basic) was determined to have an idea of the extraction recovery at a concentration of 1 mg/l for each substance (Table 2).

Fig. 1. In this result file the different hits are presented with their substance name, match factor, reverse match factor, relative retention time (RRT) and mass-to-charge relation compared to the corresponding parameters in the library. The same substance can be found several times with different tandem mass spectrometry (MS–MS) spectra.

Among the 10 substances, the PE% range was 80–119%. Due to this acceptable process efficiency of the 10 tested substances, it is supposed that a high extraction rate for most of the substances in the library was achieved and that our method does not seem to be considerable affected by suppression or enhancement of ionization due to sample matrix.

In addition, a post-column infusion test after Bonfiglio et al. was conducted [29]. Post-column infusion test for possible ion suppression with the model substances codeine and benzoylecgonine in a chromatographic run of blank serum and urine were compared to eluent only. The chromatograms indicate that in general no change in the ionization process (enhancement, suppression of the ionization) of the two tested substances due to co-eluting compounds was observed (Fig. 2).

The separation of the drugs was carried out under acidic conditions (pH = 3). The first peak eluted at 5.9 min (morphine) and the last at 18.4 min (delta-8-THC).

Analysis of different plasma samples was performed on the same day and on different days to study the intra- and inter-assay precision of the IS retention times. The intra- and inter-assay time ranges of the two IS were 6.92 ± 0.02 min and 6.91 ± 0.03 min respectively ($N = 8$).

The extraction and analysis of a sample can be performed for both (positive and negative) modes in less than one hour including the library search. This is an acceptable analytical time period for a GUS.

Out of all tested compounds (about 365), only 13 (3.7% amobarbital, acetylsalicylic acid, butalbital, carbromal, coumaphos,

Table 2
Process efficiency (%) of 10 substances from different substance groups. Each substance in a concentration of 1 mg/l in a drug free serum.

Drug	Process efficiency (%)	Drug	Process efficiency (%)
Morphine	85	Torasemide	80
Olanzapine	119	Propranolol	95
Ephedrine	94	Bupivacaine	105
Gliclazide	91	Phenprocoumon	97
Citalopram	106	Phenolphtalein	118

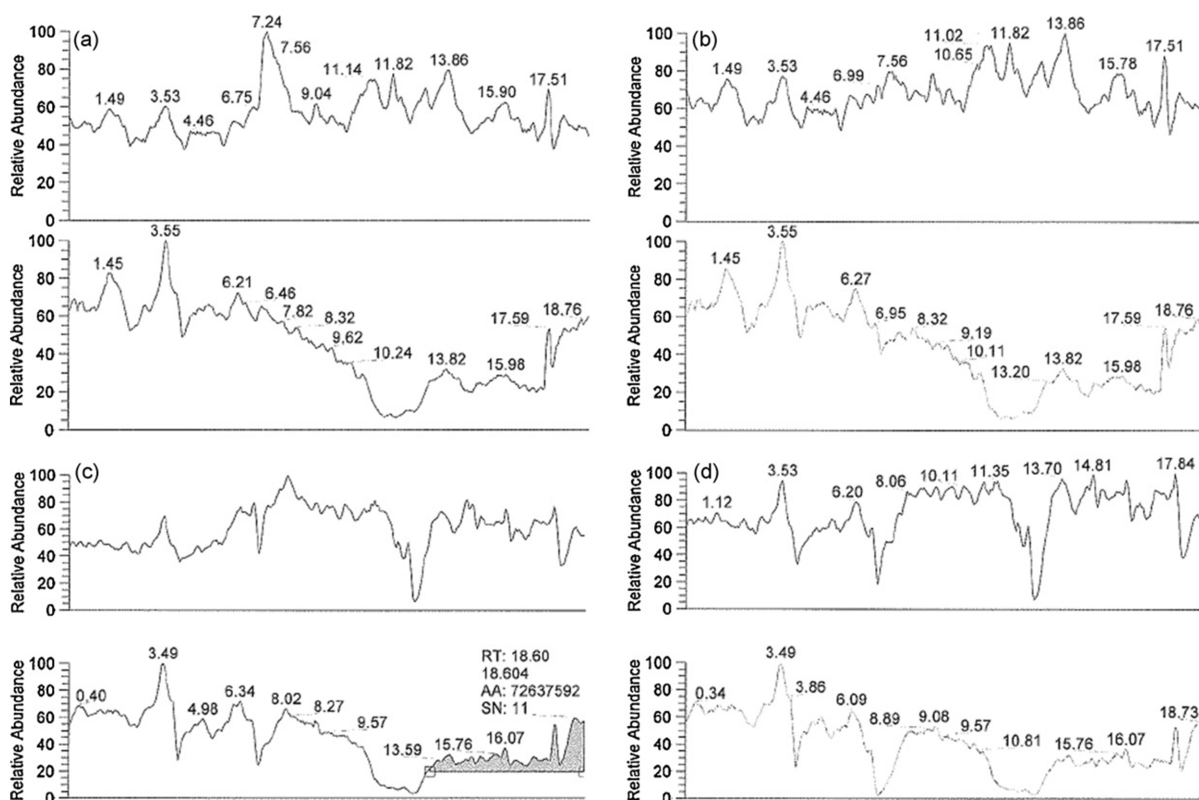

Fig. 2. The post-column infusion chromatogram of analyte d_3-benzoylecgonin (first scan) and codeine (second scan) from a mobile phase injection and an infusion chromatogram following injection of blank water (a) and (b) or plasma (c) and (d). (a) and (c) full scan, (b) and (d) scan of the corresponding MS–MS transitions.

ibuprofen, methylphenidate, naproxen, pentobarbital, salicylic acid, secobarbital, spironolactone and thiopental) were not detectable with this LC–MS method. These compounds were identified neither at high therapeutic concentrations nor at low toxic concentrations. Methylphenidate could be detected by its metabolite ritalinic acid, acetylsalicylic acid and salicylic acid by its metabolite gentisic acid. Most of the undetectable drugs were acidic compounds belonging to the class of analgesics or barbiturates. These compounds were undetectable because the ionization efficiency was very low or the normalized collision energy was too high for fragmentation to occur. They might alternatively be detected with HPLC-DAD [19,21,28].

APCI was preferred to electrospray ionization in order to reduce the risk of ion suppression. This phenomenon affects the formation of the analyte ions. Sample matrix and co-eluting compounds can contribute to ion suppression. Although ion suppression can have effects on both electrospray ionization and APCI, evidence indicates that the electrospray interface is more impacted [22,23,26]. The method of choice in this study to detect and identify compounds was a DDA procedure. Compounds can elute at identical retention times. In this case, only the mass spectra of the ion with the highest intensity would be detected. In contrast, ions with low intensity would be lost. To overcome this problem, a refractory period was introduced. Based on the average peak width this period was set at 30 s. A refractory period longer than 30 s can result in a loss of identification of one or more of the compounds with the same molecular mass ion. With a shorter refractory period, the method can fail to detect substances eluting with a similar retention time.

Due to different chemical properties of the substances, mass spectra (established with pure drugs in aqueous solution) were recorded in the negative as well as in the positive mode. For both modes a library was created. At best, substances detected in both polarities could be identified in the two respective libraries.

The normalized collision energies of 40.0% in the positive and 35.0% in the negative mode were empirically chosen in order to obtain fragmentation of all compounds. A decrease in the normalized collision energy would yield less fragmentation. Applying higher normalized collision energy would result in lower peak intensities of the fragments because further fragmentation occurs in most cases.

The established library includes for each spectrum the name of the compound, the molecular formula and the molecular ion together with its relative retention time for all 365 substances (the MS–MS spectra with all its transitions are in an XCalibur library which is connected to the library search program). This mass spectral library comprises spectra of about 280 drugs and 85 metabolites (important metabolites for screening in urine) from a large diversity of substance classes. With this procedure, several acidic, neutral as well as basic drugs could be detected and identified.

A (see Section 2.3.8) application program was developed for the automated identification of unknown compounds with LC–MS.

In order to identify unknown compounds in a serum or urine sample, a chromatographic run was performed in each ionization mode. In the next step the developed application program compared each recorded MS–MS spectrum to the reference spectra in the library from the Xcalibur software. With the help of this application program, the number of best hits that the unknown spectrum should be compared to could be specified. In the procedure described in this paper, the 10 best hits were chosen.

The similarity defined as the grade of correlation between the library spectra and the unknown spectra is characterized by the match factor and the reverse match factor (comparison of transition ions and intensities). In our procedure, the threshold was set at 400–500 for both factors. With these thresholds the best results were obtained. A higher threshold resulted in a higher LOD together with more specificity of results. In contrast a lower

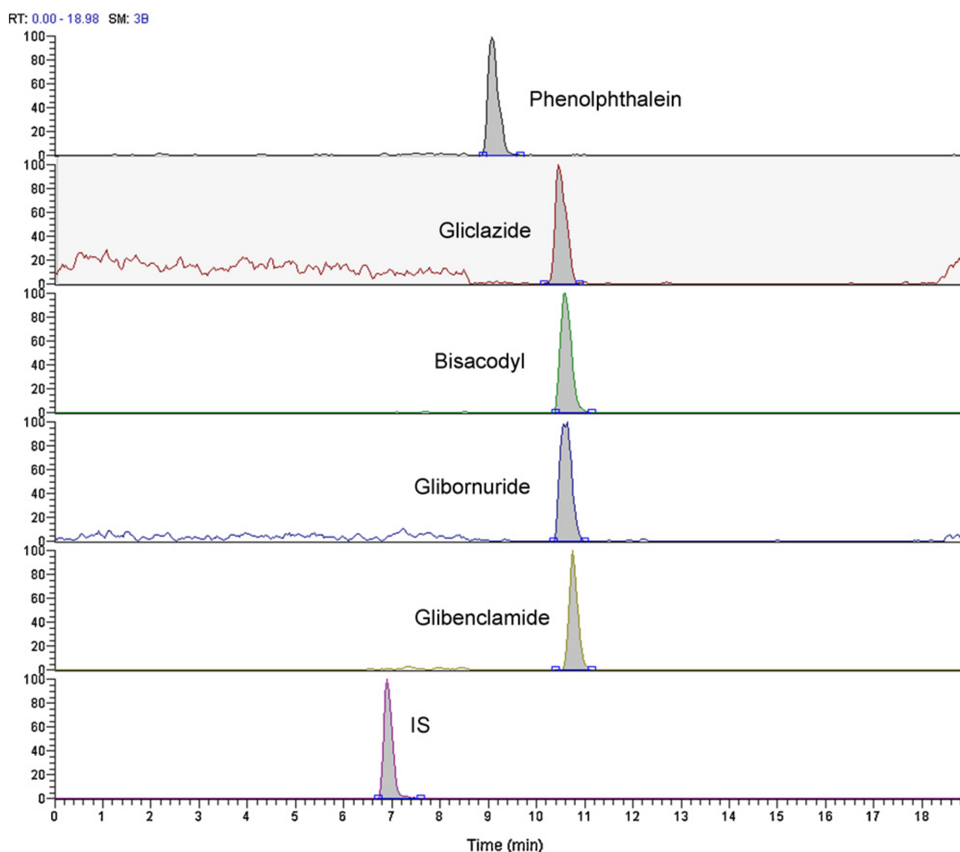

Fig. 3. Chromatogram of 5 substances and the internal standard (d_3-benzoylecgonine) all together spiked into a drug free serum (see Section 2.1) of a 1 mg/l IS 5 mg/l concentration.

threshold resulted in a lower LOD but also in a higher number of false positives.

The following example (Fig. 3) shows a run with a serum sample spiked with phenolphthalein, gliclazide, bisacodyl, glibornuride and glibenclamide at a concentration of 1 mg/l. The run was performed in the positive mode.

Each product ion mass spectrum was subjected to an automated library searching routine against the library spectra. Fig. 4 shows the MS–MS spectrum of glibornuride from spiked serum obtained with this procedure compared to the MS–MS spectrum of the library. The match factor of the presented mass spectra was 889, the reverse match factor was 989.

Compound identification took into account the mass-to-charge ratio of the unknown compound selected before fragmentation. The mass-to-charge ratio had to be within ±2 m/z of the reference mass-to charge ratio recorded in the library to be considered as a hit. This width of the mass-to-charge ratio window allowed the search for

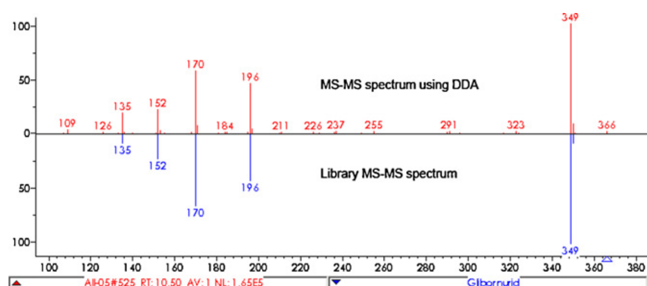

Fig. 4. Comparison of the positive tandem mass spectrometry (MS–MS) spectrum of glibornuride spiked at a concentration of 1 mg/l in a serum sample using data-dependent acquisition (DDA) to the MS–MS spectrum of the library.

possible isotopes of the compounds. With a larger window the risk for false positives was increased. A smaller window resulted in false negatives because of differences in mass related to isotopes of the element.

The pseudomolecular ion (usually, protonated in the positive mode, deprotonated in the negative mode) and its fragments were detected and compared to references in the library. Each MS–MS spectrum recorded was derived from one single mass-to-charge ratio (representing the most intense ion of the previous full scan).

Other authors used collision-induced dissociation at different voltages to obtain the same information. Mass spectra were acquired by continuously switching between a low and a high orifice voltage throughout the run to obtain both protonated molecular ion (low-voltage scan) and mass spectral fragments (high-voltage scan) from the CID in the ion source [10,27]. With the procedure presented in this study it was not necessary to switch between different orifice voltages.

RRT was also considered in the identification procedure. The RRT of the unknown compound had to be within a range of ±5% of the reference RRT (RRTs showed a deviation of 1–2%, data not shown). The RT of the IS was registered in the positive mode with a value of approximately 6.9 min.

Only if all the parameters were within the fixed areas a hit was reported. In summary the match factor and the reverse match factor had to be above 400 or 500, the mass-to-charge ratio had to be ±2 m/z and the RRT had to be within 5% compared to the library parameters. Each MS–MS spectrum, which fulfilled these conditions, was reported.

The new program (see Section 2.3.8) automatically releases a report, which consists of different hits with the substance names together with the parameters mentioned above compared to the ones in the library.

In the above mentioned serum sample all 5 substances (phenolphthalein, gliclazide, bisacodyl, glibornuride and glibenclamide) were identified at a concentration of 1 mg/l with our procedure as an example (concentration in a toxic level for these substances). Fig. 1 shows the output generated by the application program and the instrument software. The substances were listed in the results file. Gliclazide, bisacodyl, glibornuride and glibenclamide all have similar retention times. However, this was no problem because the refractory period applied enabled to identify the compounds even though they were not chromatographically separated. Importantly, in contrast to single MS CID methods the co-eluting substances did not affect the MS–MS spectra in the presented procedure. Therefore, the analysis of unknown compounds was more rapid using MS–MS.

During routine screening the search program proposed some false positive substances because of similar ion transitions of substances with the same main mass. The comparison of the whole ion-scan spectra often resulted in exclusion of such misinterpretations e.g. demethylvenlafaxine and tramadol, moclobemide and doxylamine (Supplementary material Fig. 6).

In order to test the transferability spectra of 37 compounds were compared with different equipments from ThermoFinnigan (LCQ Deca and ThermoFinnigan LTQ). The most intensive transitions of all tested spectra were identical (Supplementary material Table 3).

Serum samples spiked with decreasing concentrations of the tested drugs were analyzed in order to determine the LODs. Each concentration of the drugs from 0.005 to 4 mg/l was extracted and analyzed two times. The LOD was defined as the lowest concentration where both runs of each substance fulfilled the mentioned requirements to be identified.

With our procedure, the 87 tested compounds could be detected at high therapeutic drug concentrations or at concentrations in the low toxic range (Supplementary material Table 4). For drug confirmation measurements in urine all substances like opiates, amphetamines, cocaine metabolites have LODs beyond the recommended cutoff values for workplace testing according to NIDA (National Institute for Drug Affairs, USA) (chromatographic confirmations). The LOD was $\leq$100 μg/l for 67% of the compounds. Most of the drugs were better detected in the positive mode. Exceptions were some molecules containing acidic sites like diclofenac.

The following examples illustrate the application of the DDA LC–MS–MS system to clinical investigations. The results of serum and urine sample analysis using SPE-LC–MS–MS were compared to the results obtained with a conventional STA strategy (including a combination of immunoassays, HPLC (Remedi) and LC–MS). Remedi is an HPLC-based broad-spectrum drug profiling system. It is used to detect and identify basic and neutral drugs and their metabolites in serum and urine samples of patients) [28]. To identify benzodiazepines an additional run has to be performed on a special Remedi System. This conventional STA strategy has been applied for several years in the laboratory of the University Hospital Basel.

In addition, the opioids, amphetamines, benzoylecgonine, cocaine, cocaethylene, buprenorphine, norbuprenorphine, and other opioids were analyzed on a validated LC–MS system with different methods applied for each drug class (LODs between 5 and 20ng/ml). These methods are also used for differentiation and quantification of the mentioned substances.

Twelve serum and 13 urine samples of expected intoxicated individuals and or drug addicts were analyzed (Supplementary material Table 5). With the new method, urine samples were treated like serum samples (automated extraction followed by LC–MS–MS).

The results of 8 of the 12 analyzed serum samples, were in complete agreement with the Remedi and immunoassay results. The newly developed DDA LC–MS–MS system also found metoclopramide, trimipramine, amisulpride and atenolol, amitriptyline metabolites inclusive nortriptyline, lamotrigine, mefenamic acid (all confirmed by HPLC analysis). In contrast, the following substances were not detected because the MS spectra of these substances were not included into the MS–MS library: lidocaine metabolite (MEGX), atracurium, dipyrone (metamizole), fluoxetine metabolites, pethidine metabolites, a quetiapine metabolite and salicylate. The LC–MS–MS procedure failed to detect THC (THC or THC-carbonic acid because they were at concentrations below the LOD of the new method).

Complete agreement was found in 9 of 13 urine samples. The new DDA method detected in contrast to the Remedi/Immunoassay STA method, oxazepam, mirtazapine, zolpidem, metoprolol, lidocaine, codeine, paroxetine, furosemide, and a bromazepam metabolite. On the other hand, amitriptyline in one sample, amphetamine, methamphetamine, MDA, methadone, benzodiazepines in an other sample, THC-carbonic acid, opiates, nordazepam, a quetiapine metabolite (7-hydroxy-quetiapine not included in the library) and a flurazepam metabolite were missed by the newly developed system because of different LODs or substances not included in the library.

The agreement of measurements in serum was acceptable. For urine another comparison was conducted (17% disagreement). This new study was performed by comparing the results of 20 urine samples using the newly developed method with those obtained using an established GC–MS screening method in an external laboratory (H.H. Maurer, Homburg [20,30]).

The comparison shows the following results (Supplementary material Table 6):

Overall, agreement was found in 85% of all GC–MS positive results and in 91% if the LOD of the LC–MS–MS was considered. 13 urine samples were in complete agreement if substances not included in the library or under the LOD of the new method were not considered. In 4 urine samples paracetamol was missed by the new method. However paracetamol metabolites (glucuronide, sulphate and others) are not included in the LCMSMS library. 7 samples were discrepant (positive by GCMS, negative by the new method) for one or two substances (torasemide, lorazepam and doxepine (both at low concentrations, lorazepam was found by the new method in other urines of this comparison), pseudoephedrine and norepinephrine (also not detected by Remedi), zolpidem and chlorprothixene (zolpidem in low concentrations), codeine (however a metabolite of morphine could be detected), mirtazapine (also not detected by Remedi), trazadone (metabolites are not included in the library). With the new method in addition glafeninic acid, xylometazoline, minoxidile and 17-methylmorphinane could be detected. All these substances are not detected by the GC–MS method because they are not included in its library. In one sample GC–MS was negative for venlafaxine and its metabolites, which were detected by LC–MS–MS and confirmed by REMEDI. A sample was discrepant for codeine, codeine glucuronide, norcodeine, 6-acetylmorphine, morphine and morphine glucuronides – all found by the new LC–MS–MS and confirmed by REMEDI.

With the developed LC–MS–MS procedure, basic, neutral as well as acidic substances can be identified within the same system. However, some acidic compounds should be analyzed with a different procedure.

Importantly, with the new method, drugs were identified in two runs and without hydrolysis of glucuronides. Furthermore, time-consuming dilutions of samples can be avoided. The presented approach was robust and the information content was high. Substances from different groups (amphetamine-derived designer

drugs, antidepressants, benzodiazepines, cocaine, opiates, antidiabetics, neuroleptics etc.) were detected and identified.

4. Conclusions

In this study, it was demonstrated that the DDA LC–MS–MS screening method seems suitable for routine screening of serum and urine samples. The described procedure is fully automated (from the extraction to the detection of a drug) and easy to handle. The method was highly specific because compounds were detected and identified by their retention times, the mass-to-charge ratio of their molecular ions and fragments. Rapid identification in screening experiments was achieved by the creation of the small application program. With the method presented here, the analysis and interpretation of serum or urine samples could be performed in less than one hour. The constructed library comprises more than 400 spectra with the corresponding relative retention times of more than 350 compounds. Most of the compounds were detected at therapeutic concentrations. The matrix effects appeared to be negligible.

In conclusion, for routine screening, the combination of SPE, LC and APCI-MS seems to be with the limitations discussed in results and discussion sections, an attractive alternative for the analysis of samples from suspected intoxicated individuals or drug addicts, if combined with HPLC-DAD.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.08.013.

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