Journal of Chromatography B, 883–884 (2012) 189–197

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Online extraction toxicological MS(n) screening system for serum and heparinized plasma and comparison of screening results between plasma and urine in the context of clinical data☆

Daniel M. Mueller, Katharina M. Rentsch*

Institute for Clinical Chemistry, University Hospital Zurich, Raemistrasse, 100, 8091 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 25 May 2011
Accepted 17 August 2011
Available online 24 August 2011

Keywords:
Toxicological screening
Clinical toxicology
Online extraction
Turbulent flow chromatography
LC–MS

ABSTRACT

Background: The two main matrices for screening are urine or serum and heparinized plasma. Whereas urine has the advantage of usually higher concentrations and longer detection windows, serum or heparinized plasma represent the current systemic drug exposure of a patient.
Materials and methods: An online extraction LC–MS^n method using a MS^2 and MS^3 spectral library for the identification of substances has been developed and validated to screen serum and heparinized plasma. Extraction was performed by online turbulent flow chromatography under alkaline conditions. Chromatographic separation was achieved using a phenyl/hexyl column with acidic eluents. For detection, a linear ion trap, equipped with an APCI interface, was used and the different compounds were identified using a MS^2 and MS^3 spectral library containing 453 compounds.

From 47 patients, urine and heparinized plasma samples were analyzed and the results compared.
Results: The validation of the method gave satisfactory results. Only 3% of the compounds showed a matrix effect > 10% in serum. For all other substances and heparinized plasma, the quantitative matrix effect was <10%. 78% of the compounds where a therapeutic range was described in the literature had a limit of identification below the therapeutic range in heparinized plasma and 77% in serum, respectively.

In urine and heparinized plasma samples, a total of 168 substances (identified as 86 different compounds) could be identified. In 20 out of 47 cases (43%), the results were identical. On a substance level, the agreement between urine and heparinized plasma was in average 71% with a range of 0–100%.
Conclusions: The presented method allows a fast identification of 453 substances in serum and heparinized plasma. If plasma or serum is used for toxicological screening, the current systemic exposure of a patient can be monitored.

1. Introduction

In clinical toxicology, urine is often used as matrix for targeted or general unknown screening. Advantages of urine screening include the relatively easy availability and the usually higher concentrations of substances compared to serum or plasma, even though many substances are present mainly as metabolites. Furthermore, because of the reservoir function of the bladder, the detection windows in urine are usually longer as compared to serum and plasma.

However, urine is not always available. In a clinical setting, it is not unusual that intoxicated patients with acute renal failure do not produce urine in sufficient amounts, whereas serum or plasma can be easily withdrawn. Another argument for screening in serum or plasma is the better correlation of the identified substances to the clinical state of the patient as substances in the systemic circulation are thought to be active. Linder et al. [1] could show already several years ago that the predictive value of the presence of benzoylecgonine for an acute cocaine intoxication was significantly better in serum than in urine (53.4% in serum versus 17.8% in urine). Since blood is usually taken by medical staff, there is no risk for falsification, whereas urine sampling usually is not done under observation as long as the patient does not have a catheter.

Several toxicological screening methods by LC–MS for serum or plasma samples, sometimes even whole blood samples, have been published in recent years. Sturm et al. [2] recently presented a paper describing a targeted screening method for 365 substances using either 1 mL of urine or serum. Online solid-phase extraction (SPE) was chosen for sample preparation and the detection of

Abbreviations: SPE, solid phase extraction; LLE, liquid–liquid extraction; GC–MS, gas-chromatography–mass-spectrometry; HPLC-DAD, high-pressure liquid chromatography diode-array detection; APCI, atmospheric pressure chemical ionization; AU, arbitrary unites; LOI, limit of identification.

☆ This paper is part of the special issue "LC–MS/MS in Clinical Chemistry", Edited by Michael Vogeser and Christoph Seger.

* Corresponding author. Tel.: +41 44 255 22 90; fax: +41 44 255 45 90.
E-mail address: rentsch@access.uzh.ch (K.M. Rentsch).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.08.022

substances was performed using MS^2 spectra, acquired on an ion trap mass spectrometer in data dependent acquisition mode. Liu et al. [3] used a similar approach, using 1 mL of urine or blood. In this method, 800 drugs and toxic compounds could be detected after offline SPE or liquid–liquid extraction (LLE), also using an ion trap mass spectrometer.

Marquet et al. [4] in 2003 used a hybrid triple quadrupole-linear ion trap instrument in information dependent mode after offline SPE of 1 mL serum. An improved version of the method was published also in 2003 by Saint-Marcoux et al. [5], demonstrating the suitability of the method as complementary method for the established GC–MS and HPLC-DAD methods.

Sauvage et al. [6] and Dresen et al. [7] both used a similar methodology. Sauvage et al. [6] demonstrated the suitability of the method using a hybrid triple quadrupole-linear ion trap instrument for the detection of substances in serum, plasma, urine, gastric content, and also in whole blood. Whereas whole blood was first precipitated with zinc sulphate and methanol, all other matrices were directly extracted using offline SPE. Dresen et al. [7] used for a first overview only 100 μL of urine, whereas for lower concentrated samples, either 1 mL of urine or serum was extracted using alkaline LLE.

Viette et al. recently published two papers [8,9] using a similar instrumentation as Marquet et al. [4], Saint-Marcoux et al. [5], Sauvage et al. [6] and Dresen et al. [7]. The method concentrates on serum and was validated only for this matrix; it is not intended to be used for urine. Extraction of 1 mL serum was performed on an automated SPE system.

In this paper, the adaptation and validation of our previously published urine screening method using online extraction by turbulent flow chromatography [10] to serum and heparinized plasma samples are described. Additionally, a comparison between urine and heparinized plasma samples in patients entering the emergency station is presented to test the performance of the method under realistic conditions and to compare the information gained by using the different matrices.

2. Materials and methods

2.1. Chemicals and reagents

All solvents used were HPLC gradient grade. Acetonitrile was obtained from Romil (Cambridge, Great Britain), acetone from Merck (Darmstadt, Germany), and methanol and 2-propanol from Seelze (Seelze, Germany). Purified water was produced in-house using a central water purification installation (Burkhalter AG, Worblaufen, Switzerland).

Ammonium acetate and ammonium carbonate (both HPLC grade) were purchased from Scharlau (Taegerig, Switzerland). Formic acid and ammonia (both analytical grade) were obtained from Merck (Darmstadt, Germany).

The 453 compounds used to build the MS^2 and MS^3 library have been obtained as reference compounds either by commercial suppliers (e.g. Cerillant, Round Rock, TX, USA; Lipomed, Arlesheim, Switzerland) or by the manufacturers of the marketed drug.

2.2. LC–MS analysis

2.2.1. LC–MS system

The used Transcend TLX-1 HPLC System consisted of two Allegro pumps, an HTC PAL autosampler and a valve interface module with built-in six-port switching valves, controlled by Aria Software (version 1.6.3). As mass spectrometer, a LXQ linear ion trap, controlled by XCalibur 2.0.7 SP1 Software (all Thermo Fisher Scientific, Basel, Switzerland), was used. Ionization was performed under atmospheric pressure chemical ionization (APCI) conditions.

2.2.2. Spectral library and detection of compounds

As already described [10], the library of reference spectra was built in-house by direct infusion of a solution containing the reference compounds (10,000 ng/mL in solvent) into the MS. MS^2 as well as MS^3 spectra were acquired with normalized collision energy of 35% and stored in the library. Retention times of the compounds were determined by injection of spiked urine samples.

Chromatograms were processed using ToxID 2.1.1 (Thermo Fisher Scientific, Basel, Switzerland). For the detection of compounds, both, MS^2 and MS^3 spectra as well as the retention time were used concomitantly. The minimal search fit for the detection of compounds was set to 600, the minimal reverse search fit to 700 for both, MS^2 and MS^3 spectra. Retention time tolerance was set to 1.5 min.

2.2.3. Sample pre-treatment

To 100 μL of serum or heparinized plasma samples, 10 μL of a methanolic internal standard solution containing 10,000 ng/mL of each haloperidol-d4, morphine-d3 and temazepam-d5 were added. Samples were precipitated by the addition of 100 μL acetonitrile. After vigorous vortexing and centrifugation for 5 min at 11,700 × *g* and 10 °C, the supernatant was transferred into an autosampler vial. Samples were stored at 10 °C until injection.

2.2.4. LC–MS method

100 μL of the sample was injected into the LC system. Two columns (a Cyclone and a C18XL extraction column, both Thermo Fisher Scientific, Basel, Switzerland) in series were used for the extraction. As analytical column, a 3 μm Betasil phenyl/hexyl column, 100 mm × 3 mm, (Thermo Fisher Scientific, Basel, Switzerland) was utilized. Four different mobile phases were used. Online extraction was performed under alkaline conditions using 10 mM ammonium carbonate buffer in water as loading buffer and a mixture of 2-propanol, acetone, acetonitrile 1/1/1 (v/v/v) for cleaning. The analytical chromatography was performed using acidic mobile phases, with 5 mM ammonium acetate in water with 0.1% formic acid and 5 mM ammonium acetate in methanol with 0.5% formic acid. For cleaning, also a mixture of 2-propanol, acetone, acetonitrile 1/1/1 (v/v/v) was used. Chromatography was performed at room temperature (approximately 24 °C), and the LC flow was diverted to waste between 0 and 0.8 min, and 27 and 32.35 min, respectively, using a divert valve.

After the injection, the substances were transferred onto the extraction column using 100% of the ammonium carbonate loading buffer and were extracted for 50 s using a high flow-rate of 2 mL/min. Afterwards, the analytical column was switched in-line, and the analytical chromatography was performed with all columns in-line using the two acidic buffers (ammonium acetate in water or methanol with formic acid). The gradient started with 1% of the organic phase, which was held until 1:35 min. Afterwards, the composition was gradually changed until 7:15 min to 45% organic. Then, the gradient was slowed down and changed to 60% organic until 14:45 min. Until 23:05 min, the composition was changed to 98% organic, which was held constant until 27:05 min. Thereafter, both columns were washed separately using the mixture of 2-propanol, acetone and acetonitrile, and reconditioned to the initial conditions until 32:35 min. More details of the applied gradient are described in our previous publication [10].

The vaporizer temperature of the APCI interface was set to 450 °C, the sheath gas to 30 arbitrary units (AU) and the auxiliary gas to 5 AU. The discharge current was fixed at 5 μA and the capillary temperature was maintained at 275 °C. Polarity was switched constantly from positive to negative mode.

The mass spectrometer was operated in data dependent acquisition mode with constant polarity switching. The maximal cycle

time of the mass spectrometer was 2.5 s. If a precursor mass from a predefined list was detected with intensity above 100 counts per second, a data dependent scan was triggered. The isolation width was set to a window of 1 amu.

The scan range was set to 100 up to 1000 amu in MS^1. The scan ranges for MS^2 and MS^3 spectra were automatically chosen by the instrument when performing data dependent acquisition: the upper mass value was set 10–15 amu above the mass of the precursor ion and the lower mass at the low-mass cut-off of the instrument (about (1/4) of the precursor mass).

Further details of the method are presented in the previous publication of our method [10].

2.3. Method validation

In order to prove the specificity of the method, serum as well as heparinized plasma samples from 6 different healthy volunteers not taking any medications were analyzed and searched against the spectral library.

Matrix effects on the MS detection were evaluated qualitatively according to the method described by Bonfiglio et al. [11]. 6 serum samples and 6 heparinized plasma samples from different patients not taking any medications were pre-treated as described. As representative sample out of the 453 substances in the library, 47 substances were chosen (Table 1). The substances have been selected to cover the whole retention time and mass range. The 47 substances (each 1000 ng/mL) were introduced in groups of 8–10 substances by post-column infusion via a T-valve with a flow rate of 3 μL/min during the injection of the 6 serum or heparinized plasma samples.

For a quantitative estimate of matrix effects, an approach according to the one described by Matuszewski et al. [12] was adapted. The same serum samples and heparinized plasma samples as used in the qualitative approach were spiked with the same mixtures of 47 substances at 100 ng/mL. The resulting peak areas were compared to the peak areas of solvent standards.

To check the recovery, the same subset of 47 substances already used for the matrix effect experiments was used at 100 ng/mL. Recovery was calculated in analogy to the approach of Matuszewski et al. [12] by dividing the peak area of the solvent standards injected onto the extraction columns with subsequent analytical chromatography by the peak area of solvent standards injected directly onto the analytical column (without the online extraction step).

For the determination of the limits of identification (LOI), aliquots of pooled drug-free serum and heparinized plasma from different healthy volunteers were spiked with all 453 substances to achieve a concentration of 1, 10, 100, 1000 and 10,000 ng/mL of each substance. The lowest concentration where a substance was identified by ToxID was considered as the LOI.

2.4. Patient samples

Urine and heparinized plasma samples of 47 patients were collected out of the archive of the routine laboratory. Patients being treated in the emergency station or in the intensive care unit were selected when urine and heparinized plasma samples were drawn at a similar time point.

The urine samples used for this comparison were analyzed twice, once enzymatically hydrolyzed using glucuronidase/arylsulfatase from *Helix pomatia* to cleave off the glucuronides and once natively, as described in our previous paper [10].

Table 1
The 47 substances used for the evaluation of matrix effects and recovery selected 47 representative substances with the corresponding values for recovery.

Substance	Recovery [%]
Acetaminophen	98.2
Amiodarone	100
Amphetamine	61.4
Amprenavir	100
Bisacodyl	100
Buprenorphine	100
Cilazapril	100
Citalopram	100
Clarithromycin	100
Clonidine	100
Cocaine	100
Colchizine	100
Darunavir	100
Dextrorphan	100
Diacetylmorphine	100
Diazepam	100
Digitoxine	100
Domperidone	100
EDDP	100
Ergotamine	100
Fexofenadine	100
Flufenamic acid	98.0
Glimepiride	100
Haloperidol	100
Irbesartan	100
Itraconazole	100
Ketoconazole	100
Levomepromazine	100
Lopinavir	100
Mepivacaine	83.7
Methylecgonine	99.3
Morphine	85.2
Morphine-3-glucuronide	89.8
Nadolol	100
Nelfinavir	100
Nicardipine	100
Nicotine	100
Octodrine	88.0
Ondansetron	100
Penfluridol	100
Pindolol	89.6
Ritonavir	100
Roxithromycin	100
Sildenafil	100
Tranylcypromine	100
Triazolam	100
Trimipramine	100

3. Results

3.1. Method validation

In the 6 heparinized plasma and the 6 serum samples, only nicotine and cotinine in samples from smokers and caffeine in samples from coffee consumers could be identified. In samples from non-smokers and non-coffee drinkers, no substances could be identified.

Using the qualitative approach, ion suppression was observed at 0–2 min and at 24–30 min of the chromatographic run, where no target compounds elute.

In the quantitative matrix effects experiments, for serum, 97% of the tested substances showed a suppression < 10%. Maximum suppression was 15% (morphine). For heparinized plasma, no substance showed a suppression > 10%.

Recovery was >90% for 95% of the tested substances and >60% for all substances.

The LOIs are graphically depicted in Fig. 1. At 1 ng/mL, 98 substances (22%) in serum and 78 substances (17%) in heparinized plasma could be identified out of the 453 substances. At 10 ng/mL, 313 (69%) substances in serum and 332 (73%) substances in

Fig. 1. Percentage of all tested substances found at each concentration. The results for serum and heparinized plasma are shown separately.

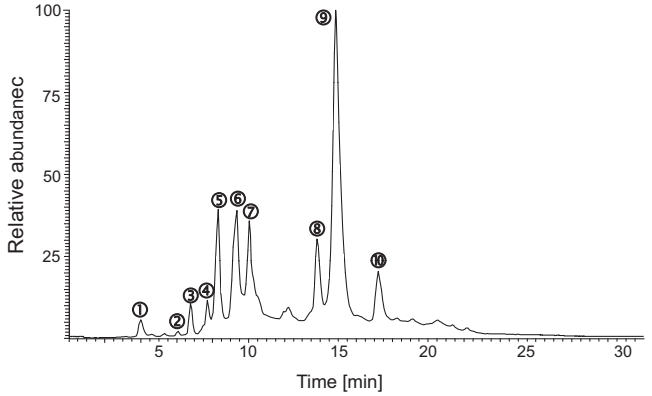

Fig. 2. Example of a chromatogram of a patient's plasma sample. Displayed is the total ion chromatogram in positive mode. 1: methylecgonine, 2: morphine, 3: codeine, 4: metabolite of metamizol, 5: zolpidem, 6: caffeine, 7: benzoylecgonine, 8: EDDP, 9: oxazepam, 10: methadone.

heparinized plasma could be identified. At a concentration of 100 ng/mL, 410 (90%) of all substances could be identified in serum and 418 (92%) in heparinized plasma. At 1000 ng/mL, 444 substances (98%) could be identified in serum and 448 (99%) in heparinized plasma, respectively. At a concentration of 10,000 ng/mL, all substances could be identified in both materials.

The achieved limits of identification have been compared to the lower and the upper limit of the therapeutic range as well as the lower toxic concentration as described in the literature [13–16] (Supplementing Material, Table 1). Out of the 453 substances, a lower therapeutic limit could be retrieved in the literature for 288 substances and an upper therapeutic limit for 256 substances. Toxic ranges could be found in the literature only for 168 substances. At concentrations lower than the therapeutic range, 226/288 substances (78%) could be identified in heparinized plasma and 221/288 in serum (77%). At the upper limit of the therapeutic range, in both, serum and heparinized plasma, 234/256 substances (91%) could be successfully identified. At the lower limit of toxicity, 159/168 substances (95%) could be identified in heparinized plasma and 158/168 substances in serum (94%) (Table 2).

3.2. Patient samples

An example of a chromatogram of a patient's plasma sample is displayed in Fig. 2. The results obtained from the patient samples comparison are shown in Table 3. Included are also details on the drugs applied and drugs of abuse, according to the case history. The results for urine include the information from both, the native and the hydrolyzed run.

In 45 patient samples, a total of 168 substances (identified as 86 different compounds) could be identified. In 2 cases no substances could be identified in both matrices. They were excluded from the statistical analysis of the results. In 20 cases (44%), identical results have been obtained in both matrices. On a substance level, the agreement between urine and heparinized plasma was in average 71% with a range of 0–100%. Of the compounds which have been taken either by the patient (anamnestic information) or given in the hospital, 86% (range 0–100%) could be identified in average in urine and 77% (range 0–100%) in heparinized plasma.

Table 2
Limits of identification (LOI) for heparinized plasma and serum versus the lower and the upper therapeutic range and the toxic range.

	Heparinized plasma	Serum
LOI ≤ low therapeutic range	79%	77%
LOI ≤ high therapeutic range	91%	91%
LOI ≤ toxic concentration	95%	94%

4. Discussion

4.1.1. Sample pre-treatment

To reduce the loss of certain analytes due to protein binding (e.g. phenprocoumon), samples were first precipitated using 100 μL acetonitrile. Moreover, the lifetime of the extraction columns could be improved by injecting only these diluted, already deproteinized samples.

The method utilizes only 100 μL of sample, which is an advantage e.g. when analyzing samples of small children, where the sample volume is very limited. Most published methods need 1 mL of sample [2–4,6,7,9].

Since online extraction is used for sample extraction, no manual SPE or LLE step is required. This minimizes possibilities for handling errors. Also, time is an important factor. With the method presented, reports can be generated within 45 min.

The reason for the use of three internal standards was to get a reference for the retention times over the whole chromatographic run and to control the efficiency of the extraction on both extraction columns.

4.2. Method validation

The method did not show any problems in distinguishing between matrix components and substances in the library. The use of MS^2 and MS^3 spectra as well as the retention times added sufficient identification points to identify substances with a high specificity.

The observed ion suppression in the qualitative approach at the end of a chromatographic run (24–30 min) was most likely due to phospholipids eluting around this time, a major cause of ion suppression in LC–MS analysis of serum or plasma in general [18]. The ion suppression at the beginning of the chromatogram (0–2 min) could be caused by hydrophilic constituents of the serum present in high concentrations. However, at the very beginning, no compounds present in the library elute.

The results from the matrix effects experiments were regarded acceptable for a qualitative screening method. Heparinized plasma shows a slightly better performance concerning matrix effects than serum. There is nothing known about a possible reason for this

Table 3
Drugs identified in the patient urine and plasma samples. Δt = time difference of the withdrawal of the heparinized plasma sample relative to the withdrawal of the urine samples. For better readability, identified metabolites are not specifically mentioned.

#	Urine results	Plasma results	Agreement urine/plasma [%]	Δt [h]	Used drugs and drugs of abuse	% of applied drugs identified in urine	% of applied drugs identified in plasma	Discussion
1	Lorazepam Metamizol Metoprolol Venlafaxine	Lorazepam Metamizol Metoprolol Venlafaxine	100	−2	Lorazepam, venlafaxine	100	100	
2	Buprenorphine Diazepam Mirtazapine Salbutamol Venlafaxine	Diazepam Mirtazapine Venlafaxine	60	−5.75	Buprenorphin, mirtazapine, salbutamol, venlafaxine	100	66	Buprenorphine paused on day samples were withdrawn. Time point of salbutamol inhalation not documented
3	Negative	Negative	100	−1.75	Ethanol[a]	–	–	
4	Midazolam Pipamperone	Haloperidol Pipamperone	33	−2.25	Haloperidol, midazolam, pipamperone	50	100	Midazolam stopped before plasma sample, haloperidol given between plasma and urine sample
5	Haloperidol Metoclopramide Quetiapine Venlafaxine	Haloperidol Metoclopramide Quetiapine Venlafaxine	100	17	Haloperidol, quetiapine, venlafaxine; ethanol[a]	100	100	
6	Midazolam Tropisetrone	Diazepam Metamizol Midazolam	25	12	Diazepam, midazolam	50	100	Documentation incomplete – no time points of administration. 12 h between samples, substances could have been given in these hours
7	Acetaminophen Citalopram Morphine	Acetaminophen Citalopram	66	−9.5	None documented	–	–	Documentation incomplete – no time points of administration. 9.5 h between samples, substances could have been given in these hours
8	Citalopram Pindolol	Citalopram Pindolol	100	−2.5	Citalopram, pindolol	100	100	
9	Morphine Pethidine Tropisetrone	Morphine Pethidine Tropisetrone	100	0.25	Acetaminophen, metamizol, morphine	33	33	
10	Acetaminophen	Acetaminophen	100	−19	None documented	–	–	
11	Ephedrine Fentanyl Midazolam	Ephedrine Midazolam	66	−9	Ephedrine, fentanyl, midazolam	100	66	Fentanyl: given only as bolus during intubation
12	Acetaminophen Cocaine Codeine Metamizol Methadone Midazolam Morphine Oxazepam Zolpidem	Acetaminophen Cocaine Codeine Metamizol Methadone Midazolam Morphine Oxazepam Zolpidem	100	−2.5	Benzodiazepine (not specified), bupropion, cocaine, methadone, opiate (not specified), oxazepam, zolpidem	86	86	
13	Negative	Negative	100	0	None documented	–	–	
14	Lamotrigine	Lamotrigine	100	−1	Lamotrigine	100	100	
15	Metoclopramide	Diazepam	0	−2	Benzodiazepines (not specified), metoclopramide; ethanol[a]	50	50	Metoclopramide: given between plasma and urine sample
16	Acetaminophen Citalopram Metamizol Phenprocoumon	Acetaminophen Citalopram Metamizol Phenprocoumon	100	−9	Acetaminophen, citalopram, ciprofloxacine, metamizol, phenprocoumon	80	80	

Table 3 (*Continued*)

#	Urine results	Plasma results	Agreement urine/plasma [%]	Δt [h]	Used drugs and drugs of abuse	% of applied drugs identified in urine	% of applied drugs identified in plasma	Discussion
17	Acetaminophen Buprenorphine Cocaine Codeine Dextromethorphan Methadon Clonazepam Diazepam Flunitrazepam Methylphenidate Midazolam Mirtazapine Morphine Naloxone Pipamperone	Diazepam Methadon Cocaine Midazolam Mirtazapine Pipamperon	40	−2	Buprenorphine, cocaine, diacetylmorphine, diazepam, flunitrazepam, naloxone	86	29	Detection windows in urine are usually longer
18	Diclofenac Ibuprofen Metoclopramide	Diclofenac Ibuprofen Metoclopramid	100	−0.75	Diclofenac, other analgesic (not specified); ethanol[a]	100	100	
19	Diazepam Naloxone Propranolol Spironolactone	Diazepam Propranolol Spironolactone	75	−2.5	Naloxone, propranolol, spironolactone, trimipramine	75	50	Naloxone: concentration in serum too low
20	Negative	Metamizol	0	−0.5	Analgesia (not further specified)	0	100	Metamizol as analgesic very likely due to prescribtion habits
21	Citalopram Midazolam Quetiapine Trazodone	Citalopram Midazolam Quetiapine Trazodone	100	−0.75	Citalopram, midazolam, quetiapine, trazodone; ethanol[a]	100	100	
22	Ephedrine Dexchlorphenamine Midazolam	Amiodarone Ephedrine Dexchlorphenamine Midazolam	75	−0.5	Amiodarone, fentanyl, ephedrine, midazolam	50	75	
23	MDMA	neg	0	0	None documented	–	–	Patient spent night one day before admission to the hospital at a party
24	Butylscopolamine Metamizol Pethidine	Metamizol Pethidine	66	−0.5	Butylscopolamine, metamizol, pethidine	100	66	Butylscopolamin: low dose, only 1 bolus before admission into hospital.
25	Amisulpride Cocaine Ephedrine Fentanyl Mianserine Midazolam Morphine Oxazepam	Amisulpride Cocaine Fentanyl Midazolam Oxazepam	63	0	Fentanyl, midazolam With unknown time of intake and dose: amisulpride, morphine, oxazepam Unknown antidepressant	100	66	Ephedrine: given during emergency treatment, most likely already cleared out of systemic circulation Intake time point of morphine and mianserine unknown
26	Metoprolol Morphine Midazolam	Morphine Midazolam	66	0	None documented	–	–	Documentation incomplete–no time points of administration
27	Olanzapine	Olanzapine	100	−1.25	None documented	–	–	
28	Cocaine Fentanyl Levamisol Methadone	Cocaine Levamisol Methadone	75	−5	Cocaine, methadone	100	100	Fentanyl: given only as bolus during intubation
29	Acetaminophen Ephedrine Fentanyl Midazolam	Acetaminophen Ephedrin Fentanyl Midazolam	100	−1	Ephedrine, fentanyl, midazolam	100	100	
30	Cocaine	Cocaine	100	−0.25	Cocaine	100	100	

Table 3 (*Continued*)

#	Urine results	Plasma results	Agreement urine/plasma [%]	Δt [h]	Used drugs and drugs of abuse	% of applied drugs identified in urine	% of applied drugs identified in plasma	Discussion
31	Bisoprolol Mirtazapine Phenprocoumon	Phenprocoumon	33	3	Bisoprolol, mirtazapine, phenprocoumon	100	33	Time point of intake unknown (patient was not seen for days) Phenprocoumon has long half-live (120 h [19])
32	4-Fluoroamphetamine Acetaminophen Amphetamine Cetirizine Cocaine Diltiazem Hydroxyzine MDMA Methamphetamine Metoclopramide Midazolam Phenacetine	Cocaine Hydroxyzine Levamisol Methamphetamine Metoclopramide Phenacetine	46	0.25	Amphetamines, cocaine	100	100	Detection windows in urine are usually longer
33	Fentanyl Midazolam	Fentanyl Midazolam	100	−1	Fentanyl, midazolam	100	100	
34	Acetaminophen Lamotrigine	Acetaminophen Lamotrigine	100	0.25	Lamotrigine; ethanol[a]	100	100	
35	Mefloquine Fentanyl Midazolam	Mefloquine Midazolam	66	0	Fentanyl, midazolam, mefloquine	100	66	Fentanyl: given only as bolus during intubation
36	Citalopram Fentanyl Lorazepam Midazolam Metoprolol Morphine Quetiapine	Citalopram Fentanyl Metoprolol Midazolam Quetiapine	71	−3.75	Citalopram, fentanyl, lorazepam, metoprolol, midazolam, quetiapine	100	83	Lorazepam: given between withdrawal of plasma and urine Morphine: nothing documented about time point of administration
37	Fentanyl Metamizol Midazolam	Amiodarone Metamizol Midazolam	75	−2	Amiodarone, fentanyl, midazolam	66	66	Fentanyl: given only as bolus during intubation
38	Acetaminophen Diclofenac Urapidil	Acetaminophen Diclofenac Urapidil	100	−2	None documented	–	–	Incomplete documentation
39	Fentanyl Midazolam	Midazolam	50	0	Fentanyl, midazolam; gamma-hydroxybutyrate[a]	100	50	Fentanyl: given only as bolus during intubation
40	Acetaminophen Metamizol Metoclopramide	Metoclopramide	33	−0.25	None documented	–	–	Documentation incomplete – no time points of administration
41	Negative	Negative	100	0	None documented	–	–	
42	Cetirizine Cocaine Metamizol	Cetirizine	33	−5	Metamizol	100	0	Metamizol: given between withdrawal of plasma and urine, cocaine: short half-live, presumably already cleared out of systemic circulation
43	Acetaminophen	Acetaminophen	100	−1	None documented	–	–	
44	Acetaminophen Levetiracetam Metamizol Midazolam Tropisetrone	Acetaminophen Levetiracetam Metamizol Midazolam	80	−9	Acetaminophen, levetiracetam, midazolam	100	100	Documentation incomplete–no time points of administration

Table 3 (*Continued*)

#	Urine results	Plasma results	Agreement urine/plasma [%]	Δt [h]	Used drugs and drugs of abuse	% of applied drugs identified in urine	% of applied drugs identified in plasma	Discussion
45	Acetaminophen Atropine Bupropion Cetirizine Cocaine Diazepam Fluconazole Levamisol Naproxen Phenacetine	Bupropion Cocaine Diazepam Fluconazole Levamisol Naproxen Phenacetine Thiopental	64	0	Atropine, bupropion, thiopental	66	66	In intoxication cases, urine offers the advantage of a more complete picture of the involved substances because of the longer detection windows Thiopental (administered in the hospital) was detected only in plasma. Combination of both, urine and plasma screening, is an advantage
46	Diazepam Methadone Mirtazapine Nevirapine Trimethoprim	Diazepam Methadon Mirtazapine Nevirapine	80	19	Methadone, mirtazapine, nevirapine, trimethoprim	100	75	Trimethoprim given only every second day. 19 h between urine and plasma sample, so most likely given on day of the urine sample
47	Metoclopramide	Negative	0	−0.5	Ethanol[a]	–	–	Incomplete documentation – metoclopramid perhaps given between plasma and urine sample

[a] Cannot be detected by our LC–MS^n screening method.

phenomenon. Also other authors testing for matrix effects observed matrix effects in their methods [2,9].

The recovery is good for the tested substances, selected to cover the whole retention time and mass range. This indicates that the online extraction procedure is able to extract different compounds having different polarities and masses with good recoveries. Compared with other published methods, the recovery is comparable to other online and offline extraction techniques [2,6,9].

The achieved limits of identification are comparable to already published methods [2–4,9].

Already at the lower limit of the therapeutic range, most of the substances can be identified using the described method.

4.3. Patient samples

The major problem when comparing urine and heparinized plasma samples in our patient cohort is the time between the withdrawals of the different samples. Plasma samples usually are withdrawn very early after the patient has come to the emergency station, whereas urine samples usually cannot be obtained at the same time. In average, the heparinized plasma samples were withdrawn 1.15 h earlier than the urine samples, with a maximal interval of 19 h. In 31 out of 47 cases, the plasma sample was obtained earlier; in 7 out of 47 cases the urine sample was first collected. The maximal time interval between the two sample types was as long as 19 h (cases 10 and 46). Nevertheless, the fact that plasma is usually withdrawn earlier than urine reflects the fact that for most clinical–chemical analyzes in emergency settings, heparinized plasma is used. If a toxicological screening in patients with a suspected intoxication could be performed out of plasma, the results would therefore also be much earlier available.

Dependent on the pharmacokinetic properties of the compounds taken by the patients before coming to the emergency station and on the drug treatment given during early medical care, many discrepancies between the results obtained in the urine and heparinized plasma samples can easily be explained.

As the urine is usually stored in the bladder, the detection window is longer and the concentrations usually are higher than in plasma. The major analytical problem with urine is the fact that in urine mostly metabolites are present which can only hardly be obtained as reference compounds, whereas in plasma usually the parent drug has a higher concentration. Additionally, the drugs being present in plasma reflect the drugs having an actual influence on the patient, whereas the urine describes a more retrospective situation. The longer detection window in urine samples is of advantage to obtain more complete information on all substances the patient has taken recently compared to plasma.

The evaluation of the patient data demonstrate that we were able to identify all compounds in heparinized plasma adding to the patient's clinical situation with our online extraction toxicological LC–MS^n screening method.

In conclusion, heparinized plasma is superior for the monitoring of the current systemic exposure of a patient to drugs at the time point of the withdrawal of the sample. If an unclear situation should be clarified retrospectively, urine has the advantage of longer detection windows. For very low dosed drugs, e.g. fentanyl, the presence of a higher concentration in urine may be of advantage. Of course, the most complete picture of the compounds having been taken by the patient can only be obtained if both, urine and heparinized plasma samples, are analyzed.

5. Conclusions

The presented method using online turbulent-flow extraction allows a fast identification of 453 substances in heparinized plasma

and serum and its performance was carefully evaluated. The comparison of the limits of identification with the therapeutic ranges in the literature showed that roughly 80% of all substances where a therapeutic range was described in the literature could be successfully identified at the lower limit of the therapeutic range.

The comparison of the toxicological analysis in patient urine and plasma samples resulted in 44% of the cases in identical results. In the sample pairs with different compounds identified, it could clearly be demonstrated that plasma reflects better the current exposure to drugs whereas urine has a longer detection window and therefore allows the detection of compounds being no more present in the systemic circulation.

Acknowledgement

The authors would like to thank Thermo Fisher Scientific for providing the LC–MS system and the online extraction instrument.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.08.022.

References

[1] M.W. Linder, G.M. Bosse, M.T. Henderson, G. Midkiff, R. Valdes, Clin. Chim. Acta 295 (2000) 179.
[2] S. Sturm, F. Hammann, J. Drewe, H.H. Maurer, A. Scholer, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 878 (2010) 2726.
[3] H.C. Liu, R.H. Liu, D.L. Lin, H.O. Ho, Rapid Commun. Mass Spectrom. 24 (2010) 75.
[4] P. Marquet, F. Saint-Marcoux, T.N. Gamble, J.C. Leblanc, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 789 (2003) 9.
[5] F. Saint-Marcoux, G. Lachatre, P. Marquet, J. Am. Soc. Mass Spectrom. 14 (2003) 14.
[6] F.L. Sauvage, F. Saint-Marcoux, B. Duretz, D. Deporte, G. Lachatre, P. Marquet, Clin. Chem. 52 (2006) 1735.
[7] S. Dresen, N. Ferreiros, H. Gnann, R. Zimmermann, W. Weinmann, Anal. Bioanal. Chem. 396 (2010) 2425.
[8] V. Viette, D. Guillarme, R. Mylonas, Y. Mauron, M. Fathi, S. Rudaz, D. Hochstrasser, J.L. Veuthey, Clin. Biochem. 44 (2011) 45.
[9] V. Viette, D. Guillarme, R. Mylonas, Y. Mauron, M. Fathi, S. Rudaz, D. Hochstrasser, J.L. Veuthey, Clin. Biochem. 44 (2011) 32.
[10] D.M. Mueller, B. Duretz, F.A. Espourteille, K.M. Rentsch, Anal. Bioanal. Chem. 400 (2010) 89.
[11] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[12] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[13] M. Schulz, A. Schmoldt, Pharmazie 58 (2003) 447.
[14] D. Uges, TIAFT reference blood level list of therapeutic and toxic substances, 2004.
[15] R. Baselt, Disposition of Toxic Drugs and Chemicals in Man, 8th ed., Biomedical Publications, Foster City, 2008.
[16] P. Baumann, C. Hiemke, S. Ulrich, G. Eckermann, I. Gaertner, M. Gerlach, H.J. Kuss, G. Laux, B. Muller-Oerlinghausen, M.L. Rao, P. Riederer, G. Zernig, Pharmacopsychiatry 37 (2004) 243.
[18] O.A. Ismaiel, M.S. Halquist, M.Y. Elmamly, A. Shalaby, H. Thomas Karnes, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 875 (2008) 333.
[19] A. Moffat, M. Osselton, B. Widdop, Clarke's Analysis of Drugs and Poisons, 3rd ed., Pharmaceutical Press, London, 2004.