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Drug Monitoring and Toxicology

Screening of Drugs and Toxic Compounds with Liquid Chromatography-Linear Ion Trap Tandem Mass Spectrometry

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Background: In clinical and forensic toxicology, general unknown screening is used to detect and identify exogenous compounds. In this study, we aimed to develop a comprehensive general unknown screening method based on liquid chromatography coupled with a hybrid triple-quadrupole linear ion trap mass spectrometer.
Methods: After solid-phase extraction, separation was performed using gradient reversed-phase chromatography. The mass spectrometer was operated in the information-dependent acquisition mode, switching between a survey scan acquired in the Enhanced Mass Spectrometry mode with dynamic subtraction of background noise and a dependent scan obtained in the enhanced product ion scan mode. The complete cycle time was 1.36 s. A library of 1000 enhanced product ion–tandem mass spectrometry spectra in positive mode and 250 in negative mode, generated using 3 alternated collision tensions during each scan, was created by injecting pure solutions of drugs and toxic compounds.
Results: Comparison with HPLC-diode array detection and gas chromatography-mass spectrometry for the analysis of 36 clinical samples showed that linear ion trap tandem mass spectrometry could identify most of the compounds (94% of the total). Some compounds were detected only by 1 of the other 2 techniques. Specific clinical cases highlighted the advantages and limitations of the method.
Conclusion: A unique combination of new operating modes provided by hybrid triple-quadrupole linear ion trap mass spectrometers and new software features allowed development of a comprehensive and efficient method for the general unknown screening of drugs and toxic compounds in blood or urine.
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Liquid chromatography (LC)-mass spectrometry (MS)[5] has been a useful complement to immunoassays, HPLC-diode array detection (DAD) and gas chromatography (GC)-MS for the general unknown screening (GUS) of drugs and toxic compounds *(1–4)*. LC-MS is not affected by the limitation of GC-MS to volatile compounds. However, for single MS, the fragments obtained by in-source collision-induced dissociation at various orifice voltages *(5)* are less informative and reliable than the spectra obtained with electron ionization in GC-MS. Recent methods have used Data- or Information-Dependent Acquisition (IDA), an artificial intelligence program *(6)* aimed at improving compound identification with tandem MS. IDA detects the most abundant ions in each scan obtained in the single-stage MS mode (survey scan mode), and automatically and immediately switches the instrument to the product-ion scan mode (dependent scan mode), in which these ions are selectively transmitted by the first quadrupole to the collision cell, and the resulting fragments are analyzed in the 3rd quadrupole. Subsequently, the instrument is switched back to the survey scan. With a closed ion trap instrument, Fitzgerald et al. *(7)* used this procedure to identify 17 basic drugs. Decaestecker et al.

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[5] Nonstandard abbreviations: LC-MS, liquid chromatography-mass spectrometry; DAD, diode array detection; GC-MS, gas chromatography-mass spectrometry; GUS, general unknown screening; IDA, information-dependent acquisition; EMS, enhanced mass spectrometry; EPI, enhanced product ion; LIT, linear ion trap; DBS, dynamic background subtraction; IS, internal standard.

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used different IDA approaches with a hybrid quadrupole time-of-flight instrument operated in the positive mode *(6, 8)*. Müller et al. *(9)* recently developed a multitarget screening of 301 compounds with multiple reaction monitoring as the survey mode and enhanced product ion (EPI) scan mode as the dependent mode on a hybrid triple-quadrupole linear ion trap system. IDA has also been evaluated for the GUS of drugs and toxicants with the same instrument model *(10)*, whose 2nd MS stage is equipped with entrance and exit lenses, giving it the properties of an ion trap (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue9) *(11)*. The enhanced MS (EMS) full-scan mode, in which all the molecular or pseudomolecular ions produced in the source in the specified *m/z* range are trapped in the linear ion trap before being detected, was used as the survey mode. The EPI scan mode, in which the fragment ions produced in the collision cell are trapped in the linear ion trap before being detected or refragmented, was used as the dependent mode. A reconstituted spectrum was generated by adding up the EPI mass spectra obtained separately at 3 different collision energies *(10)*. EPI spectra generated with the same instrument were similar to the corresponding "classic" daughter-ion spectra and robust with concentration and time over long periods; also, IDA conditions allowing identification of a wide range of compounds were determined *(12)*.

In this study, we present a method aimed at development of a comprehensive and optimized GUS procedure based on linear ion trap MS/MS, using new computer program capabilities and at application of this procedure to clinical and forensic samples, to evaluate its performance.

Materials and Methods

standards and reagents

Organic solvents and reagents were analytical grade. We obtained acetonitrile and methanol from Carlo Erba, dichloromethane and isopropanol from Prolabo, and formic acid and ammonium formate from Sigma. Deionized water was used. The internal standard Glafenine (IS) was purchased from Sigma. Oasis MCX and HLB cartridges were purchased from Waters. We prepared stock solutions of standard compounds at 1 g/L in methanol for most compounds, with acetonitrile or water used for certain molecules because of solubility issues, and the solutions were kept at 4 °C or −20 °C (e.g., for benzodiazepines), depending on their stability.

liquid chromatography

The chromatographic system consisted of a Perkin-Elmer Series 200LC high-pressure gradient pumping system and a Rheodyne Model 7725 injection valve equipped with a 5-μL internal loop. A Waters XTerra MS C18, 3.5 μm (100 × 2.1 mm) column, maintained at 25 °C, was used with a linear gradient of mobile phase A (pH 3.0, 0.5 mmol/L ammonium formate) and mobile phase B [acetonitrile:pH 3.0, 10 mmol/L ammonium formate (90:10, by volume)], flow rate of 200 μL/min, programmed as follows: 0–2.5 min, 3% B; 2.5–21.5 min, 3% to 90% B; 21.5–23 min, 90% B; 23–23.5 min, decrease from 90% to 3% B; 23.5–25.5 min, equilibration with 3% B.

diode array detector (dad)

Ultraviolet (UV) detection was performed using an Agilent 1100 Series DAD, 200−400-nm scan range and 1-nm resolution. The UV trace and spectra were acquired continuously with Analyst® 1.4 Software.

mass spectrometry

Detection was carried out with an Applied Biosystems QTRAP™ LC-MS/MS System equipped with a TurboIonSpray™ ionization source and controlled by Analyst 1.4 Software. We optimized settings with the quantitative optimization function in the Analyst Software, by infusing at 5 μL/min a 1 mg/L-mixture of 11 compounds [glafenine, dextropropoxyphene, trimeprazine, omeprazole, lorazepam, quinine, ranitidine, nandrolone, lidocaine, ritonavir, and theophylline in acetonitrile pH 3.0, 2 mmol/L ammonium formate (30:70, by volume)]. Positive ionization was performed with the following settings: ion spray voltage, 5000 V; curtain gas, 20; ion source gas flow rates 1 and 2 at 15 and 35 units, respectively; declustering potential, 50 V; and temperature 350 °C. In the negative mode, the ion spray voltage was set to −4500 V, declustering potential at −50 V, and source temperature at 350 °C.

enhanced ms conditions

In the survey scan mode, the dynamic fill-time option was chosen, and *m/z* ratios were scanned from 100 to 1100 amu at a rate of 4000 amu/s. The EMS total scan time was 0.49 s. A new feature of the Analyst 1.4 Software, called dynamic background subtraction (DBS) *(13)* was also used.

information-dependent acquisition conditions

The 3 most intense ions in each background-subtracted EMS spectrum were selected as parent ions without applying any intensity threshold; each ion could be selected for a maximum of 4 occurrences; and the ions previously selected for 4 occurrences were excluded for 15 s.

enhanced product ion conditions

Collision energy was set at 40 V and −40 V and collision energy spread at 25 V and −25 V, in the positive and negative modes, respectively. Consequently, fragments generated at +/−15, +/−40, and +/−65 V were accumulated in the linear ion trap and analyzed altogether by scanning the +/−50–1100 *m/z* range at a rate of 4000 amu/s, leading to a scan time of 0.87 s, so that a complete cycle (EMS, IDA, and EPI) lasted 1.36 s. The dynamic

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fill-time option was also used in the EPI mode, and the source variables were kept unchanged.

libraries of enhanced product ion ms/ms spectra

A library of EPI MS/MS spectra was created by injecting mixtures of freshly prepared stock solutions of compounds (~10 compounds per solution including the IS glafenine) at 10 mg/L in acetonitrile:pH 3.0, 2 mmol/L ammonium formate (30:70, by volume), in both polarities successively. After we checked the fragments obtained against the LC-MS/MS literature or using Mass Frontier 4.0 (HighChem Ltd.), we entered the pertinent EPI spectra in the library. The relative retention time was added manually to each library entry, together with the compound chemical structure and Chemical Abstracts Services number (Merck Index, http://chem.sis.nlm.nih.gov/chemidplus/ or drawn using ChemSketch 7.0 (Advanced Chemistry Development)).

library searching

We performed the library search with the Library Search tool in the Analyst 1.4 Software. We sorted search results by 3 criteria: (i) purity, measured as the unmatched peaks between the unknown and known spectra; (ii) fit, measured by how well a library spectrum matches the unknown spectrum; and (iii) reverse fit, which measures how well the unknown spectrum matches a library spectrum. All these criteria range from 0% to 100%.

automatic data analysis and reporting

A specific program called "AutoSearch" was developed for this study by Applied Biosystems/MDS Sciex. This program allows an automatic detection of chromatographic peaks in the EMS survey scan and a comparison of the underlying EPI spectra with those in the library. Excel reports list peak intensities, the name(s) of the identified compound(s), identification criteria (purity, fit, and reverse fit), relative retention time, and peak height and area on the EMS survey trace. The similarity of the EPI spectra obtained with library entries is evaluated primarily with the purity factor. Search results with purity higher than a predefined value (here 60%) appear in bold characters, and the 1 to 3 best hits for each unknown spectrum can be visualized (Fig. 2 in the online Data Supplement). The relative retention times were compared with those in the library with an acceptability threshold of $+/-5\%$.

Only peaks above a fixed intensity threshold and a signal-to-noise ratio >3 were tagged for automatic MS/MS library searching.

sample preparation

To 1 mL serum, plasma, urine, or gastric content, we added 100 μL of a 10 mg/L aqueous solution of glafenine (IS). After vortex-mixing, we loaded the mixture on an Oasis HLB cartridge, previously conditioned with 2 mL methanol and 2 mL water. After rinsing the cartridge with 3 mL water and 3 mL of water mixed with methanol at a ratio of 90:10, by volume, we performed elution with 3 mL dichloromethane:isopropanol (75:25, by volume), containing 2% formic acid. Extracts were evaporated to dry and reconstituted in 100 μL acetonitrile: pH 3.0, 2 mmol/L ammonium formate (30:70, by volume). Whole blood samples were precipitated with a saturated mixture of zinc sulfate:methanol (70:30, by volume), centrifuged at 3400*g* for 5 min, and the supernatant diluted 1/3 with water before extraction.

ion suppression and extraction recovery

We checked ion suppression by infusing at 50 μL/min into the ion source a mixture of 20 compounds at 100 μg/L in acetonitrile: pH 3.0, 2 mmol/L ammonium formate (30:70, by volume), while injecting into the chromatographic system extracts of blank serum, urine, or whole blood in parallel, the 2 flows being merged by means of a peek tee at source entrance.

Extraction recovery in whole blood and plasma was determined for 54 compounds by comparing the peak areas of extracted compounds (3 replicates of blank matrix added at 100 μg/L) with those of extracts of the blank matrix reconstituted with a 1 mg/L solution of the same compounds in the above mentioned solvent.

We set up another acquisition method to achieve these tests by introducing the following modifications: (i) the linear ion trap (LIT) fill-time was fixed at 100 ms in the EMS mode; (ii) no exclusion of previously selected ions was applied; and (iii) no background subtraction was applied.

Results and Discussion

The different steps of the acquisition process are presented in Fig. 1, which corresponds to the injection of a mixture of 10 antipsychotics at 100 μg/L in the positive ion mode. The *arrow* in Fig. 1A (total ion current of the survey scan mode) shows the retention time considered in this example. Fig. 1B presents the underlying survey MS, and Fig. 1C the extracted ion chromatogram at *m/z* 370. Fig. 1D presents the rich and clean EPI spectrum of amisulpride resulting from the fragmentation of *m/z* 370 in the collision cell at 3 alternated fragmentation voltages. The protonated molecules were always present in the EMS survey scan, while a fragment of higher intensity than the pseudomolecular ion was found in only 1 of the 11 above-mentioned compounds. Because the concentrations of compounds in biological samples can vary, the dynamic fill-time option was chosen in the survey scan mode to automatically adjust the LIT fill time as a function of ion density in the ion trap and to decrease the risks of missing coeluting or low-intensity peaks. By use of DBS, the best results in terms of detection of compounds of interest were obtained with 3-point Savitsky-Golay smoothing of mass spectra and subtraction of the mean of the 2 spectra (defined as background noise)

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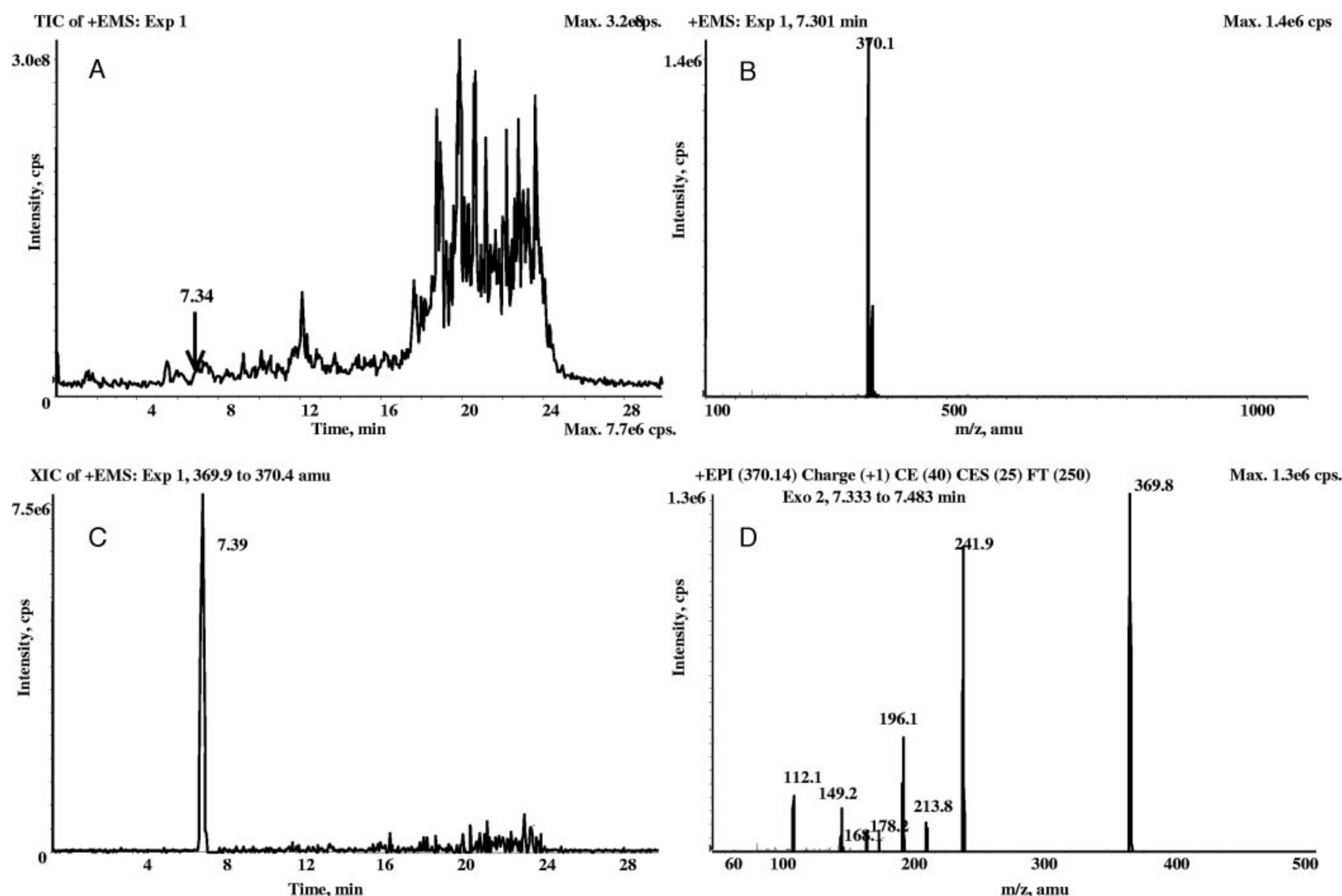

Fig. 1. *(A)*, Total ion chromatogram of a solution of 10 antipsychotics at 100 μg/L in methanol; *(B)*, underlying amisulpride Q3-only enhanced mass spectrum (EMS); *(C)*, corresponding extracted ion chromatogram; and *(D)*, enhanced production (EPI) spectrum of amisulpride.

preceding the survey scan. DBS resulted in the IDA selection of ions with increasing intensity only, because the subtraction process canceled the *m/z* ratios with stable or decreasing intensity, enhancing the signal-to-noise ratio and facilitating the selection of pertinent parent ions. Because of this real-time background subtraction, the IDA intensity threshold could be set at 0. To decrease the risks of false-negative results in case of coelution, the 3 most intense ions were selected for a maximum of 4 occurrences, and then they were excluded for 15 s. As shown in Fig. 2, cyamemazine, trimeprazine, and methotrimeprazine had almost the same retention time but, with these IDA settings, each could be unambiguously identified at 10 μg/L. As previously described *(12)*, each EPI spectrum was composed of fragments generated from the same parent ion at 3 different collision energies within a single scan, resulting from the accumulating property of the LIT and the new collision energy spread feature of the software (see Fig. 3 in the online Data Supplement).

The current library is comprised of ~1000 EPI spectra in the positive mode and 250 EPI spectra in the negative mode. The EPI spectra contain at least 4 fragments with a relative abundance >10%, including that of the molecular or pseudomolecular ion. The robustness of the EPI spectra obtained in the positive mode was studied during 3 periods of 5 days over a period of 6 months, with 5 injections per day of a 10-mg/L mixture of 5 compounds (amisulpride, clozapine, metoclopramide, nordazepam, and tiapride, chosen with respect to their fragmentation properties; see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/vol52/issue9). The interassay CV of the relative intensity [calculated using 1-way ANOVA with occasion as grouping factor, following Krouwer and Rabinowitz *(18)*], was always <22% and intraassay CV% <15%.

During this period, the QTRAP instrument was regularly used for analyzing samples of various biological matrices, so preventative cleaning of the curtain plate, orifice, and cone was arbitrarily performed once a week. The source also had to be cleaned when pollution or dirtiness were detected by multiple reaction monitoring techniques, because sensitivity and signal-to-noise ratios are difficult to assess with the present procedure because of the autoadaptive LIT fill time. The chromatographic column used was stable for >1300 injections.

As previously reported *(4)*, solid-phase extraction with a mixed-mode phase (Oasis MCX) was a good compro-

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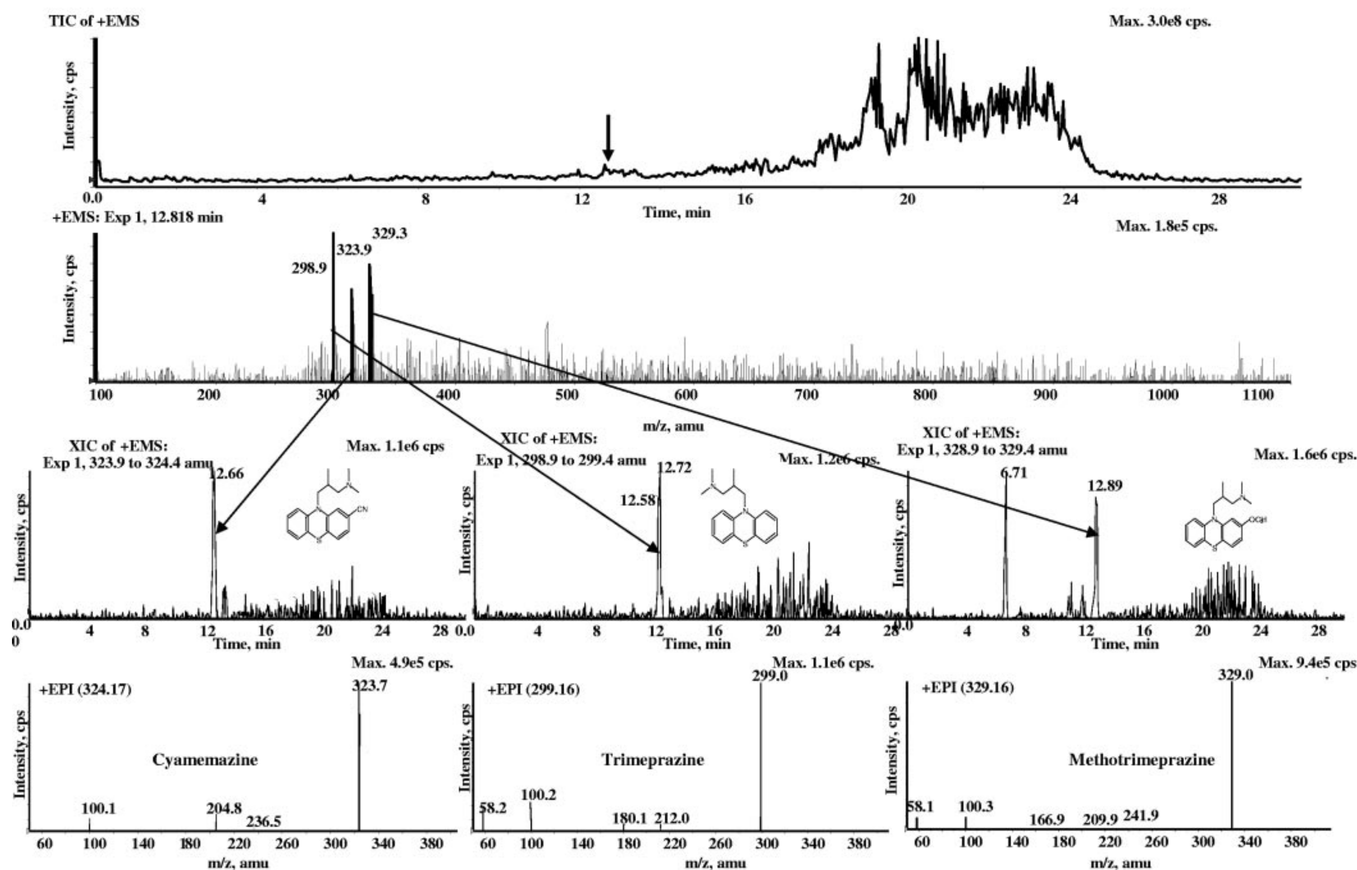

Fig. 2. Total ion chromatogram, extracted ion chromatogram, and EPI spectra of cyamemazine, trimeprazine, and methotrimeprazine added at 10 μg/L in blank serum.

mise between nonselectiveness and high yield. However, the analysis of a Seronorm Pharmaca level 1 internal quality control by this extraction procedure and the present GUS method showed that some compounds were not detected, in particular nortriptyline and desipramine, although their presence had been confirmed by a quantitative LC-MS/MS technique in the multiple reaction monitoring mode developed for this purpose (Table 1). To improve the extraction conditions, enriched and internal quality-controlled samples were extracted in parallel with Oasis MCX or HLB cartridges. Although some peaks showed higher intensity with the MCX, some compounds were detected and identified only at therapeutic concentrations with the HLB extraction (Table 1). Moreover, despite the lower recovery, antidepressants present in 12 serum samples sent to our laboratory for therapeutic drug monitoring by LC-MS/MS *(17)* were detected with a purity ≥85% with the present GUS technique (see Table 2 in the online Data Supplement).

No ion suppression was observed at the retention time of the 20 compounds studied (see Fig. 4 in the online Data Supplement). Ion suppression cannot universally be appraised, but the 20 compounds used for this experiment present a wide range of retention times (5.5 to 18.5 min) and polarity.

The tests for ion suppression and extraction recovery were performed at different IDA settings from those of the GUS procedure, because the combined effects of automatic exclusion after selection, automatic background subtraction, and dynamic (i.e., variable) trap fill time did not allow computation of the peak area of a selected ion on the EMS survey trace. The chromatographic peaks would have been interrupted in their ascending part, 2 successive acquisition time periods would not necessarily have corresponded to the same trap fill time, and the horizontal signal expected with the continuous infusion of pure standards (in the absence of any ion suppression) would have been erratic.

The extraction recovery results for 54 compounds, presented in Table 2, show higher yields in plasma than in whole blood. However, all values were >40% (and in plasma most of them were >90%). Decaestecker et al. compared the extraction yields obtained for 18 basic and neutral compounds with 7 nonpolar, 3 mixed-mode, and 2 polymeric solid-phase extraction sorbents *(15)*. They found that the Isolute C_8 and OASIS MCX (mixed-mode) phases had the highest clean-up potential, and that the former gave the best extraction yields. In a second step, they optimized the extraction procedure on the C_8 sorbent, which led to a higher recovery (75% to 100%) for all

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Table 1. Analysis of commercial quality control of drugs at low therapeutic stages (Seronorm Pharmaca, Stage 1) by the present GUS technique.

Compound	Molecular Weight	Detection mode	Extraction MCX	Extraction HLB	Concentration level (mg/L)	Plasma highest therapeutic concentration (mg/L) *(12)*	Plasma concentration leading to serious toxicity (mg/L) *(12)*
Procainamide	235.8	Positive	D	D	3	8	12
Acetaminophen	151.2	Positive	D	D	100	30	200
Theophylline	180.2	Negative	D	D	5	20	40
Lidocaine	234.4	Positive	D	D	2	5	8
Caffeine	194.2	Positive	D	D	20	15	60
Quinidine	324.4	Positive	D	D	2	5	8
Disopyramide	339.5	Positive	D	D	2	5	8
Primidone	218.3	Positive	D	D	8	12	40
Flecainide	414.4	Positive	D	D	0.4	0.7	1
Propranolol	259.4	Positive	ND	D	0.4	0.2	2
Imipramine	280.4	Positive	D	D	0.1	0.3	1
Desipramine	266.4	Positive	ND	D	0.1	0.3	1
Nortriptyline	263.4	Positive	ND	D	0.05	0.3	1
Salicylic acid	138.1	Negative	D	D	200	300	400
Phenobarbital	232.2	Negative	D	D	20	40	100
Chloramphenicol	323.1	Negative	D	D	5	25	40
Carbamazepine	236.3	Positive	D	D	4	12	30
Diazepam	284.7	Positive	D	D	0.3	2	5
Amiodarone	645.3	Positive	D	D	1	2	Not known

D, detected; ND, not detected

Table 2. Extraction recovery (n = 3) of 54 compounds in plasma and whole blood by use of the generic solid-phase extraction method.

Compound	Plasma, %	Whole blood, %	Compound	Plasma, %	Whole blood, %
Acebutolol	84.9	78.9	Lormetazepam	99.0	50.6
Alprazolam	95.2	58.3	Medifoxamine	68.4	69.8
Amisulpride	93.7	90.3	Metoclopramide	93.2	70.6
Amitriptyline	96.1	71.4	Mianserin	99.9	68.6
Atenolol	86.5	60.5	Mirtazapine	99.1	70.9
Bromazepam	97.0	46.1	Desmethylmirtazapine	99.7	62.1
Bupivacaine	99.0	89.3	Moclobemide	99.9	77.5
Carbamazepine	89.8	58.2	6–monoacetylmorphine	87.3	50.1
Chlordiazepoxide	79.2	58.5	Monodesmethylcitalopram	99.9	99.2
Cibenzoline	94.5	79.4	Naloxone	85.2	56.4
Citalopram	99.7	83.7	Naltrexone	85.5	64.2
Clomipramine	99.0	57.7	Norclozapine	95.9	48.1
Clozapine	99.4	64.2	Nordiazepam	93.8	91.3
Cocaethylene	95.4	71.8	Nortriptyline	99.0	61.5
Cocaine	81.8	75.3	Oxazepam	92.9	49.9
Codeine	88.7	68.9	Pipamperone	99.2	74.6
Domperidone	84.2	44.7	Propranolol	88.5	65.7
Desipramine	93.0	57.0	Reduced Haloperidol	98.5	67.9
Desmethylclomipramine	99.5	52.7	9–OH Risperidone	99.0	77.2
Dihydrocodeine	89.8	75.6	Risperidone	99.8	81.9
Disopyramide	70.8	77.7	Sulpiride	88.8	82.9
Doxepin	99.5	88.2	Sultopride	90.9	84.9
Etidocaine	86.8	64.2	Tianeptine	99.5	54.4
Haloperidol	99.9	67.1	Tiapride	89.7	86.9
Imipramine	99.7	67.3	Tramadol	86.8	71.1
Lidocaine	77.1	64.9	Venlafaxine	99.8	73.0
Loprazolam	98.6	58.7	Viloxazine	94.1	68.7

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drugs except for benzoylecgonine and morphine, whose recovery decreased by ~40% *(16)*. The present extraction procedure appears to be at least as efficient for a larger number of drugs tested.

This combination of improved extraction conditions and GUS technique improved the detection and identification of compounds compared with our previous results *(4, 5, 12)*. Although it has been shown herein that some antidepressants and antipsychotics could be identified at concentrations as low as at 10 μg/L, the detection limits of drugs from various therapeutic classes warrant further studies. Because only peaks above a fixed intensity threshold and a signal-to-noise ratio >3 were tagged for automatic MS/MS library searching, further manual analysis and inspection of each EPI trace by an experienced operator is mandatory, because some low-intensity or coeluting peaks might not be picked up by the automatic data processing program. Indeed, IDA and EPI are applied to each scan without intensity or signal-to-noise ratio conditions.

The present GUS technique was tested for different types of matrices by comparing the present LC-MS/MS with GC-MS and HPLC-DAD *(4)* for the analysis of 36 actual specimens (3 gastric content, 6 serum, 8 whole blood, and 19 urine samples). Of the 130 positive results (89 different compounds), 94% were obtained using the present GUS technique, of which 19% were identified only by the present GUS technique (Fig. 3). Moreover, each of the compounds identified by LC-MS/MS was the first hit from the search process with a purity factor always >75%. By comparison, GC-MS identified 64% of the compounds (1.5% not found by the other techniques) and HPLC-DAD identified 55% (1.5% unique findings). Some compounds, particularly antidepressants and antipsychotics, could be identified only with the present GUS technique because of high extraction recovery and high selectivity in the IDA mode with DBS. A few compounds, which are usually detectable by GC-MS and/or HPLC-DAD, were not detected in some specimens because of coelution with other compounds with larger peaks. Conversely, the absence of detection of 2 benzodiazepines by LC-MS/MS resulted from a poor extraction recovery of <50% for these compounds in whole blood.

In 1 case of acute intoxication, numerous compounds belonging to various therapeutic classes were detected in serum, urine and gastric content. Only LC-MS/MS was able to identify venlafaxine in serum and domperidone in urine, and nordiazepam was detected only by GC-MS (after manual search). All the other compounds were also identified by LC-MS/MS, but the other 2 techniques were unable to detect the urine metabolites of 6 of the 9 parent compounds (Table 3 in the online Data Supplement). Other examples are provided in Figs. 5–7 in the online Data Supplement).

The present GUS technique is efficient for a large range of compounds and complementary to the other classical screening techniques. Identification of metabolites is possible in certain cases because of their spectral similarities to their parent compound, even when the latter is not found. However, confirmation of the nature of putative metabolites is systematically performed by (i) injecting these metabolites as pure compounds, if available; (ii) comparison with MS/MS spectra in the literature; or (iii) performing in vitro metabolism experiments with a pool of human liver microsomes and analyzing the incubation supernatants with the present technique (e.g., zolpidem metabolite see Fig. 7 in the online Data Supplement). This allows continuous enrichment of our library.

Diode-array detection was also used upstream of MS/MS, but the computer program used was unable to process the UV spectra. Future software developments may allow this processing so that the number of screening techniques running in parallel can theoretically be decreased.

LC-MS/MS 93.8%
1-OH midazolam, amisulpride, cetirizine (2), descarbethoxyloflazepate, diazepam, diltiazem, domperidone (2), hydroxyzine (2), metoclopramide, mirtazapine (2), norfenfluramine, olanzapine (2), oxazepam, paroxetine (2), quinine, ranitidine, temazepam, tianeptine(2)
19.1%

GC-MS 63.8%
ecgonine-methyl-ester (EME), tetra-hydro-cannabinol (THC)
1.5%

acetaminophen, amitriptyline (2), benzoylecgonine, clotiazepam, cocaine, codeine, dextropropoxyphene, EDDP, etomidate, fluphenazine, ketoprofen, lidocaïne (3), loxapine, morphine, mepivacaine, meprobamate (4), methadone, methotrimeprazine, nalbuphine, norpropoxyphene, nortriptyline (2), tetrazepam (2), venlafaxine, zaleplon
24.6%

Aceprometazine (4), atropine, caffeine, citalopram (2), clomipramine (2), cyamemazine (6), demoxepam, diazepam, EDDP, laudanosine, methadone, metronidazole, mianserin (2), midazolam (3), Nordazepam (4), oxazepam (2), scopolamine, tramadol, trimebutine, trimeprazine, trimetazidine, trimipramine, tropatepine (2), verapamil, zolpidem (2), zopiclone
34.6%

acetaminophen, alprazolam, amiodarone, amprenavir, atenolol, bisoprolol, bromazepam, buflomedil, clobazam, clonazepam (3), clozapine, flurazepam, ketoprofen, lormetazepam, propranolol, risperidone, ritonavir, tiapride
15.4%

acetaminophen (2), ibuprofen, propofol
3.1%

lorazepam, lormetazepam
1.5%

HPLC-DAD 54.6%

Fig. 3. Comparison of LC-MS/MS, GC-MS, and HPLC-DAD GUS procedures for the analysis of 36 clinical samples (serum, whole blood, urine or gastric content).

The number of occurrences is in brackets.

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