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Journal of Chromatography A, 1031 (2004) 203–211
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Screening for central nervous system-stimulating drugs in human plasma by liquid chromatography with mass spectrometric detection

Jan Leo Egge Reubsaet*, Stig Pedersen-Bjergaard

Department of Pharmaceutical Analysis, School of Pharmacy, Universtiy of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway

Abstract

Liquid chromatography and electrospray mass spectrometry was evaluated for screening of more than 70 central nervous system-stimulating drugs in human plasma. Protein precipitation was utilized as a simple sample preparation procedure, and the subsequent screening procedure involved two injections in a liquid chromatography–mass spectrometry system for each sample; a first screening without source induced dissociation to maximize sensitivity where potential positive identifications were based on retention time and molecular ion masses, and secondly a source induced dissociation confirmation based on retention time, molecular ions, and one or two fragment ions for each target generated by a 25 V fragmentation energy. The majority of central nerve system stimulating drugs were possible to identify within the actual therapeutic ranges. Experiences with 175 real samples supported this and strongly indicated that information reported by patients on their consumption of central nerve system stimulating drugs is highly unreliable. Thus, protein precipitation and liquid chromatography–mass spectrometry may be a valuable tool for broad drug screening in human plasma in the future.

Keywords: Screening; Drugs

1. Introduction

During recent years, substantial research has been focused on the development of identification and quantification procedures for drugs in human plasma based on liquid chromatography–mass spectrometry (LC–MS). In most of these cases, attention has been focused on a single compound or a few closely related compounds, where the conditions for both sample preparation and LC–MS have been carefully optimized to provide maximum sensitivity. On the other hand, relatively little has been published on more universal LC–MS screening methods for both urine and blood covering one or more groups of drugs [1–8]. Among these neuroleptics [1], antihistamines [2], β-blockers [3,8], some illicit drugs [5], barbiturates [7], and diuretics [7]. Since this screening is done on selected groups the chemical properties of the various analytes have been quite similar. Thus, as a consequence, selective sample preparation methods like solid-phase extraction have been applied, and LC–MS conditions have been optimized based on the common chemical properties of the targets.

In some cases, it is of interest to screen human plasma samples for a broad range of drugs, related to scientific investigations of certain types of accidents or to medical problems. In these cases, the analytes of interest may cover a very broad range in terms of chemical characteristics (polar, non-polar, neutral, basic, or acid), and consequently the analytical problem becomes much more complex. Normally, this is solved by utilizing different techniques for the screening like LC–MS, gas chromatography–mass spectrometry (GC–MS) or immunological methods [9,10]. In this work, we have evaluated the use of LC–MS as the only technique for a very broad screening for central nervous system (CNS)-stimulating drugs in human plasma. We included 72 different compounds with highly different chemical properties, and selected protein precipitation as a simple and universal sample preparation method. Both potential and limitations related to such a broad screening are discussed, and the potential was investigated during analysis of 175 human plasma samples from patients with severe hypoglycaemia, where information about their medication was available from interviews of the patients.

* Corresponding author. Tel.: +47-22-85-66-13; fax: +47-22-85-44-02.
E-mail address: leon.reubsaet@farmasi.uio.no (J.L.E. Reubsaet).

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doi:10.1016/j.chroma.2003.10.057

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. However, some drugs were extracted from tablets. These drugs are marked with an asterisk in Table 1. The drugs were extracted with ethanol, and the amount of ethanol was in each case adjusted to give a final drug concentration of 1 mg/ml assuming 100% recovery.

Table 1
List of compounds included in the present study

Opioids
Ketobemidone*
Hydromorphone
Methadone
Morphine
Oxycodone
Petidine
Tramadol
Buprenorphine
Pentazocine*
Dextropropoxyphene*
Benzodiazepines
Nitrazepam
Clobazam
Clonazepam
Chlordiazepoxide
Diazepam
Flunitrazepam
Estazolam
Bromazepam
Alprazolam
Lorazepam
Lormetazepam*
Oxazepam
Temazepam
Triazolam
Brotizolam*
Antidepressants
Citalopram
Paroxetine
Sertraline*
Neuroleptics
Chlorpromazine
Chlorprothixene
Levomepromazine*
Melperone*
Pipamperone*
Promazine
Thioridazine
Clozapine
Loxapine*
Periciazine*
Perphenazine
Prochlorperazine
Zuclopenthixol
Fluphentixol
Fluphenazine
Haloperidol
Pimozide
Risperidone
Olanzapine
Hydroxyzine
Antihistaminics
Promethazine
Chlorcyclizine
Alimemazine*
Cinnarizine*
Clemastine
Cyclizine*
Cyproheptadine
Dexchlorpheniramine*
Meclozine*
Mepyramine
Methdilazine*
Others
Phenobarbital
Primidone
Zopiclone*
Zolpidem*
Phenytoin
Oxcarbazepin*
Carbamazepine
Illicit drugs
Amphetamine
MDMA (Ecstacy)
Mescaline
Phencyclidine
Legal stimulants
Caffeine
Nicotine (cotinine)

Compounds marked with asterisk symbol (*) were extracted from tablets as discussed in Section 2.1.

2.2. Protein precipitation

Patient plasma (500 μl) was vortex-mixed with 1 ml cold acetonitrile (stored at −18 °C and used immediately after storage). After 10 min each sample was centrifuged for 10 min at 14000 rpm min^{-1}. A 750 μl aliquot of the supernatant was transferred to a new vial. The content was dried under a stream of N_2. The residue was resolved in 100 μl LC mobile phase A (see below) and vortex-mixed for 10 s. The sample was centrifuged again for 10 min at 14000 rpm min^{-1}. A 60 μl aliquot of the supernatant was transferred to a sample vial and placed into the autosampler for LC–MS analysis.

2.3. LC system

The LC system consisted of a TSP SCM1000 vacuum degasser, TSP SpectraSystem P4000 quaternary gradient pump and a TSP SpectraSystem AS3000 autosampler. Detection was carried out by a TSP SpectraSystem UV6000LP photodiode array detection (DAD) system coupled in-line to a Finnigan LCQ^{duo} ion trap mass spectrometer (MS). Xcalibur version 1.0 software was used to control this system and to

perform data acquisition (all Instrument-Teknikk, Østerås, Norway).

Separations were performed on a 50 mm × 2.0 mm (100 Å, 3 μm) Intersil ODS-3 column from Varian (Holger, Oslo, Norway) at a flow rate of 200 μl/min. To the analytical column a BDS-C_8 10 mm × 2.0 mm Javelin guard column from Thermo Hypersil-Keystone (Holger, Oslo, Norway) was attached. Both the guard and the analytical column were equilibrated with LC mobile phase A consisting of 5% acetonitrile in 10 mM ammoniumacetate, adjusted to pH 5.0 with acetic acid. After injection of 20 μl sample, the following gradient program was carried out using 90% acetontrile in 10 mM ammonium acetate pH 5.0 as mobile phase B: from $t = 0.0$ to 2.0 min, the composition of the mobile phase was changed from 100% mobile phase A to 80% mobile phase A. From $t = 2.0$ to 12.0 min the composition was changed to 40% mobile phase A. From $t = 12$ to 14 min the composition was changed to 100% mobile phase B. This composition was kept constant for 2 min (until $t = 16.0$ min). Within 1 min (from $t = 16.0$ to 17.0 min) the conditions were returned to the starting conditions again (100% mobile phase A). The column was re-equilibrated with more than 10 column volumes after $t = 21.0$ min at a flow of 500 μl/min.

After LC–MS analysis of each sample, the system was effectively rinsed to avoid carry-over from sample to sample. This rinse started with a washing step consisting of 100 μl 50% methanol in 1% acetic acid was injected over the 20 μl loop. Both the loop and the rotor-seal were washed including the incoming and outgoing ports. After injection a short LC-gradient was run. The rinse was finished with a re-equilbrating step by 10 column volumes of mobile phase A at a flow of 500 μl/min.

To avoid contamination of the mass spectrometer during sample analysis, the first 1.7 min of the flow was directed to waste.

2.4. DAD

In the first screening step DAD spectra between 230 and 300 nm were recorded during the whole run. The rise time was 1.0 s, the scan rate was 1 Hz and both scan band width and scan step were set at 1 nm.

2.5. MS

The LC system was connected to the MS detector using an electrospray (ESI) interface which was operated in the positive mode. The spray voltage was 5 kV, sheath and auxillary gas were 40 and 5 arbitrary units, respectively. The other MS settings were: capillary temperature 250 °C, capillary voltage 15 V, tube lens offset was 0 V, octapole 1 offset—4.75 V, lens voltage—20 V, octapole 2 offset—8 V and octapole radio frequency amplitude 400 V. The number of microscans was set to 2, the maximum injection time 200 ms.

In the first screening step, four mass-time segments were used: from $t = 1.7$ to 6.7 min the scanned mass range was m/z 120–375, from $t = 6.7$ to 8.0 min the scanned mass range was m/z 245–500, from $t = 8.0$ to 11.5 min the scanned mass range was m/z 200–450 and from $t = 11.5$ to 23.0 min the scanned mass range was m/z 215–475. In this first screening step the molecular ions of the compounds were monitored. Instead of one segment ranging from m/z 120 to 500 various segments with a narrower mass range were chosen to ensure higher sensitivity. The limits of the various segments were chosen to ensure that all compounds of interest could be detected within the time frame of the specific segments.

In the second (confirmation) step only one segment was used were the scanned mass range was m/z 50–500. The final source induced dissociation (SID) voltage was set at 25.0 V. In this second screening step, both molecular ions as well as fragment ions were monitored.

3. Results and discussion

The CNS stimulating drugs included in the present work are summarized in Table 1. The targets included both some of the most popular drugs of abuse, as well as the most abundant drugs in the Nordic countries belonging to the opioids, benzodiazepines, antidepressants, neuroleptics, and antihistaminics. In addition, caffeine and cotinine were included to semi-quantitatively monitor the extent of caffeine intake and smoking. From a chemical point of view, the compounds varied substantially in terms of hydrophobicity. The majority of the drugs were basic, but also a few neutral (caffeine and carbamazepine) and acidic drugs (phenobarbital) were included. Attention was focused on their identification in human plasma samples.

3.1. Method development

Since the chemical nature of the targets varied substantially and because a single relatively simple procedure was considered most relevant, protein precipitation was selected as the sample preparation method. This method includes a drying and reconstitution step. The reconstitution step was required in order to reduce the level of acetonitrile in the sample solution; this was crucial in order to ensure sufficient focusing conditions and acceptable chromatographic performance for the early eluting targets. In addition, the reconstitution step served to pre-concentrate the targets by a factor of 2.5; as discussed below, this was important in order to match the therapeutic levels for several of the drugs.

The LC method included a gradient elution as mentioned in the experimental section to cover all the targets in a single run. The retention times for the analytes are summarised in Table 2. The retention times ranged between 2.42 min (morphine) and 16.55 min (primidone). The repeatability ($n = 8$) of the retention times, which is crucial for identification purposes, were within 0.07 to 1.28% for retention times >8 min, whereas the repeatability ($n = 8$) was between 0.37

Table 2
Molecular ions, major SID fragment (in order of decreasing intensity), and retention time data

Compound	MH^+ (*m*/*z*)	Principal SID fragment (*m*/*z*)	Optimal SID energy (V)	Retention time	
				Mean (min)	R.S.D. (%)
Alimemazine	299.1	100.0	25	10.96	0.52
Alprazolam	309.2	281.1	40	11.47	0.27
Amphetamine	136.0	118.9	16	5.46	1.15
Bromazepam	315.9	288.0	35	9.64	0.55
Brotizolam	395.0	314.1	30	12.10	0.36
Buprenorphine	468.3	414.3	35	12.88	1.28
Caffeine	195.0	–	–	5.51	1.57
Carbamazepine	237.2	194.1	26	10.44	0.09
Chlorcyclizine	301.1	201.0	20	11.43	0.52
Chlordiazepoxide	300.1	283.0	23	11.45	0.42
Chlorpromazine	319.1	273.9	31	11.82	0.22
Chlorprothixene	316.0	271.0	30	12.18	0.47
Cinnarizine	369.1	167.0	20	16.95	0.42
Citalopram	325.2	262.2	–	9.47	–
Clemastine	344.0	130.0	17	12.98	0.63
Clobazam	301.5	259.0	25	12.86	0.32
Clonazepam	317.8	301.0	10		
Clozapine	327.0	270.2	29	10.66	0.79
Cotinine	177.0	–	–	5.50	–
Cyclizine	266.9	167.1	18	9.70	0.38
Cyproheptadine	288.1	242.1	36	10.96	0.68
Dexchlorpheniramine	274.9	230.1	20	8.56	0.33
Dextropropoxyphene	340.0	266.0	15	11.10	0.46
Diazepam	285.1	257.1	41	14.03	0.18
Estazolam	295.1	267.1	39	11.07	0.23
Flunitrazepam	314.2	268.1	40	12.49	0.38
Flupenthixol	435.1	307.0	33	13.16	0.69
Fluphenazine	438.1	171.1	31	12.81	0.46
Haloperidol	376.1	165.0	30	10.25	0.46
Hydromorphone	286.2	185.0	35	3.60	4.05
Hydroxyzine	375.1	20.0	23	11.03	0.43
Ketobemidone	248.2	230.1	30	6.24	1.40
Levomepromazine	329.2	242.0	–	11.20	0.49
Lorazepam	321.1	302.9	22	11.48	0.22
Lormetazepam	335.1	316.9	20	13.00	0.33
Loxapine	329.2	271.0	–	11.57	0.71
MDMA	193.9	162.9	19	5.97	0.77
Meclozine	390.9	201.0	22		
Melperone	264.1	165.0	27	8.00	0.36
Mepyramine	286.1	241.1	25	8.82	0.35
Mescaline	212.0	195.0	16	5.56	1.02
Methadone	310.1	265.1	23	10.98	0.51
Methdilazine	297.1	266.1	27	10.73	0.51
Morphine	286.1	218.1	35	2.42	2.26
Nitrazepam	282.1	236.0	44	11.38	0.22
Olanzapine	313.1	256.1	30	7.96	1.34
Oxcarbazepine	253.1	236.0	20	9.20	0.34
Oxazepam	287.1	268.9	24	11.09	0.19
Oxycodone	316.1	298.1	20	5.69	1.53
Paroxetine	330.1	192.1	–	10.45	–
Pentazocine	286.1	218.0	27	8.24	0.67
Periciazine	366.1	142.1	30	10.03	0.56
Perphenazine	404.2	171.0	–	11.95	0.67
Pethidine	248.2	220.1	33	7.58	0.75
Phencyclidine	244.1	86.0	15	8.55	0.36
Phenobarbital	UV-detection			8.42	1.28
Phenytoin	253.8	236.7	20	10.43	0.45
Pimozide	462.3	430.7	30	13.12	0.66
Pipamperone	376.1	291.0	28	8.48	1.09
Primidone	219.0	–	–	16.55	
Prochlorperazine	374.1	141.1	28	12.93	0.61

Table 2 (*Continued*)

Compound	MH^+ (*m/z*)	Principal SID fragment (*m/z*)	Optimal SID energy (V)	Retention time	
				Mean (min)	R.S.D. (%)
Promazine	285.0	85.9	21	10.40	0.22
Risperidone	411.1	191.1	30	8.35	0.99
Sertraline	305.8	275.0	–	11.55	–
Temazepam	301.1	282.9	–	12.43	0.38
Thioridazine	371.1	126.0	30	12.95	0.38
Tramadol	264.1	245.9	25	6.88	0.81
Triazolam	343.1	308.1	40	11.68	0.19
Zolpidem	308.2	263.2	43	10.03	1.04
Zopiclone	388.9	344.9	15	7.47	0.97
Zuclopenthixol	401.1	277.9	33	12.99	0.69

and 4.05% for the early eluting targets. Careful washing of both the syringe and the injector was accomplished between each analysis in order to avoid carry-over problems. In addition, between each new sample, a rapid gradient elution was accomplished. This was performed to effectively rinse the column and to ensure that no carry-over occurred.

Due to the large number of targets, MS with selected ion monitoring (SIM) was not convenient. However, it was desired to operate the MS in retention time segments, where mass spectral data were collected in as narrow mass ranges as possible. The actual ranges and times are summarised in the experimental section. Initial experiences with human plasma samples revealed that an identification criterion based on a positive mass signal at a correct retention time was insufficient for reliable identification, and most samples resulted in some false positive identifications based on these criteria. Thus, a mass spectral confirmation procedure was required to improve the reliability. Tandem MS was not an alternative in this work due to the large number of targets eluting in a relatively short time window. Therefore, SID [11] was tested, and the idea was to expose all samples to a confirmational LC–MS run based on SID. It was desired to perform the SID confirmation with a fragmentation energy providing strong signals for one or more fragments ions while maintaining a substantial signal also for the molecular ion. Therefore, the fragmentation of each analyte was studied as function of fragmentation energy in the range 5–50 V. The fragmentation energy resulting in fragment ions of similar abundance as for the molecular ion (optimal fragmentation energy) was determined and the values are included in Table 2. As expected, the optimal fragmentation energy varied substantially from compound to compound, and 25 V was selected as a compromise. The mass of the major fragments observed are also included in Table 2. In conclusion, each sample was subjected to two injections in the LC–MS system; a first screening with no SID to maximize sensitivity where potential positive identifications were based on retention times and molecular ions, and secondly, a SID confirmation based on retention times, molecular ions, and one or two fragment ions for each target. Retention times and mass spectral data were loaded into an identification library (optional in Xcalibur software) of the instrument and provided a rapid tool for target identification.

3.2. Detectability and identification potential

An important part of the present work was to evaluate how detection limits obtained with the general screening procedure matched concentration levels typical for therapeutic drugs and for drugs of abuse. Therefore, detection limits are summarized in Table 3 based on the initial LC–MS screening (without SID). In this table, typical concentration levels in plasma for most of the compounds are included [12–16]. These detection limits were obtained by spiking the samples with varying concentrations of each compound (0.5, 1, 5, 10 and 50 ng/ml). The concentration of compound at which a signal was obtained with a S/N ratio $\geq$3 was identified as detection limit. For the opioids, which included 10 candidates, the screening method easily matched the levels for seven of the compounds, whereas the sensitivity was not sufficient for plasma detection of hydromorphone and buprenorphine. In both cases, concentrations in human plasma are normally low, and especially for hydromorphone, the signal-to-noise characteristics were relatively poor caused by a low analyte signal on mass 286.2. For morphine, the detection limit was close to the lower actual concentration range, and problems may be experienced during the identification of this compound. For the benzodiazepines, which included 15 different compounds in this study, 10 of the compounds were easily detected within the therapeutical range. For clonazepam, the sensitivity of the mass spectrometer was poor, and this particular compound was not covered by the screen. For the low-dose benzodiazepines flunitrazepam, lormetazepam, triazolam, and brotizolam, the detection limits matched the reported therapeutical ranges, but sensitivity problems may potentially occur because the detection limits were close to the lower actual ranges. For the three antidepressants included, the detection limits matched typical therapeutic levels. For the group of neuroleptics (20 compounds), 15 of the drugs were easily detected by the screen, whereas for prochlorperazine, perphenazine, flupenthixol, fluphenazine, and pimozide, the detectability was too poor. For the four former

Table 3
Detection limits and typical concentration levels in human plasma

Compound	Detection limit in plasma (ng/ml)	Typical concentration level in plasma (therapeutic concentration)	
		ng/ml	Reference
Alimemazine	0.5	50–400	[12]
Alprazolam	1	5–80	[12]
Amphetamine	5	20–100	[12]
Bromazepam	10	50–200	[12]
Brotizolam	1	1–20	[12]
Buprenorphine	1	0.5–5	[12]
Caffeine	10	2000–10000	[12]
Carbamazepine	1	2000–12000	[12]
Chlorcyclizine	0.5	–	
Chlordiazepoxide	5	400–3000	[12]
Chlorpromazine	1	30–500	[12]
Chlorprothixene	1	20–200	[12]
Cinnarizine	1	–	
Citalopram	10	≈300	[12]
Clemastine	1	–	
Clobazam	10	100–400	[12]
Clonazepam	50	4–80	[12]
Clozapine	0.5	100–600	[12]
Cotinine	–	–	
Cyclizine	1	100–250	[12]
Cyproheptadine	0.5	–	
Dexchlorpheniramine	1	3–17	[12]
Dextropropoxyphene	1	50–500	[12]
Diazepam	1	50–500	[12]
Estazolam	1	400–870	[12]
Flunitrazepam	5	5–15	[12]
Flupenthixol	1	0.5–1	[12]
Fluphenazine	1	0.2–4	[12]
Haloperidol	0.5	5–50	[12]
Hydromorphone	10	5–15	[12]
Hydroxyzine	0.5	50–100	[12]
Ketobemidone	1	≈25	[13]
Levomepromazine	0.5	5–25	[12]
Lorazepam	10	80–250	[12]
Lormetazepam	5	5–100	[12]
Loxapine	0.5	1–10	[14]
MDMA	0.5	20–300	[15]
Meclozine	>50	5–200	[16]
Melperone	0.5	–	
Mepyramine	0.5	–	
Mescaline	5	–	
Methadone	0.5	100–500	[12]
Methdilazine	0.5	–	
Morphine	5	10–100	[12]
Nitrazepam	5	30–100	[12]
Olanzapine	5	30	[12]
Oxcarbazepine	10	22000	[12]
Oxazepam	5	200–1500	[12]
Oxycodone	5	20–50	[12]
Paroxetine		10–50	[12]
Pentazocine	0.5	10–200	[12]
Periciazine	5	5–30	[12]
Perphenazine	5	1–20	[12]
Pethidine	0.5	100–800	[12]
Phencyclidine	1	–	
Phenobarbital	–	10000–30000	[12]
Phenytoin	50	5000–15000	[12]
Pimozide	50	4–10	[12]
Pipamperone	0.5	100–400	[12]
Primidone	>50	4000–12000	[12]
Prochlorperazine	5	10–40	[12]
Promazine	0.5	10–400	[12]
Risperidone	1	–	
Sertraline		–	
Temazepam	5	20–500	[12]
Thioridazine	0.5	100–2000	[12]
Tramadol	0.5	100–1000	[12]
Triazolam	1	2–20	[12]
Zolpidem	0.5	80–150	[12]
Zopiclone	>50	<100	[12]
Zuclopenthixol	1	5–100	[12]

drugs, the sensitivity problem was principally because they are administered at low dose, whereas for pimozide, the mass spectrometer signal was low resulting in poor signal-to-noise characteristics. Among the antihistaminics (11 compounds), all detection limits except for meclozine were low, and the method matched the levels relevant for this class of drugs. For meclozine however, the signal from the mass spectrometer was low. Among the remaining 13 compounds (illicit drugs, legal stimulants and others), all were easily detected except zopiclone, which provided a low signal on the mass spectrometer. In conclusion from this discussion, about 80% of the compounds were easily detected in their relevant concentrations, in 6% of the cases, the sensitivity only matched the upper concentration range, whereas for 14% of the compounds, the proposed scheme did not provide sufficiently low detection limits.

3.3. Experiences with real plasma samples

Plasma samples ($n = 175$) were collected from patients with severe hypoglycaemia. The patients were interviewed about their intake of drugs and legal stimulants included in this screening (see Table 1), and their samples were analyzed with the proposed screening and confirmation methods. A typical identification of a drug substance is illustrated in Fig. 1. This particular sample was found to contain citalopram, which was in agreement with the medical history of the patient. Citalopram was discovered in the first screening method based on the appearance of a significant peak (signal-to-noise ratio > 3) in the 325.2 mass chromatogram, which corresponded to the molecular ion, at retention time 9.47 ± 0.5 min. In the confirmational method utilizing SID, a significant signal was observed within the same retention window at mass 262.2, which corresponded to the major SID fragment of citalopram.

Among the 175 samples, 32 of the samples were expected to contain drugs belonging to Table 1 according either to the patient interviews or to the LC–MS analysis (or both).

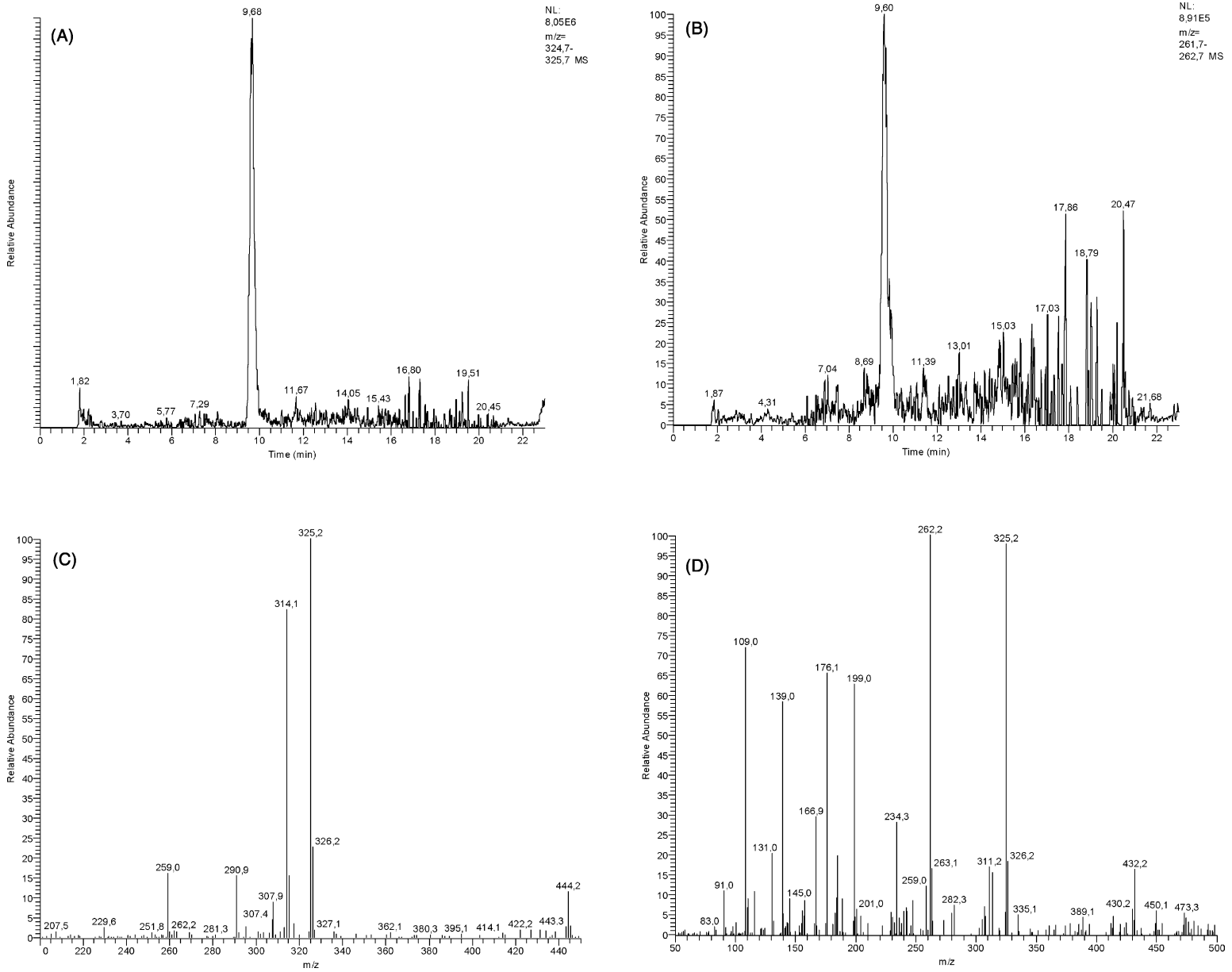

Fig. 1. (A) Screening: ion trace chromatogram of citalopram (*m/z* 325.2). Retention time 9.68 min. (B) Screening: mass spectrum obtained from signal at 9.68 min from chromatogram (A). The signal at *m/z* 314.1 is an artefact. (C) Confirmation: ion trace chromatogram of main fragment of citalopram (*m/z* 262.2). Retention time 9.60 min. (D) Confirmation: mass spectrum obtained from SID at retention time 9.60 min.

Table 4
Samples containing drugs based on interviews and/or LC–MS analysis

Sample	Content according to interview	Content according to LC–MS
1	Estazolam	Estalozam
2	Citalopram	Citalopram
3	Citalopram	Citalopram
4	Nitrazepam, tramadol	Tramadol, methadone
5	Morphine, zolpidem	–
6	Oxazepam	–
7	Sertraline	–
8	Citalopram, morphine, bromazepam	–
9	Lorazepam	–
10	Bromazepam	–
11	Tramadol	Tramadol
12	Zopiclone	–
13	Chlorprothixene	–
14	–	Haloperidol
15	Zopiclone	–
16	–	Diazepam
17	–	Diazepam
18	Citalopram	Citalopram
19	–	Tramadol
20	Citalopram, chlorprothixene, pimozide	Citalopram
21	–	Paroxetine
22	–	Diazepam
23	Tramadol	–
24	Sertraline	–
25	Ketobemidone	–
26	Morphine	–
27	Buprenorphine	–
28	Nitrazepam	Nitrazepam
29	Tramadol	–
30	–	Amphetamine
31	Sertraline	Sertraline
32	Sertraline, zopiclone	Sertraline

These samples are summarized in Table 4. In only seven of the cases (corresponding to 21.9%), there were complete agreement between the medical history of the patients and the LC–MS results. For 18 of the samples, which corresponded to 56.3%, the medical history included drugs that we were not able to identify with the LC–MS screening. For morphine, zoplicone, pimozide, and buprenorphine, this disagreement probably occurred due to sensitivity problems as discussed above, and seven of the patient entries which was not found by LC–MS may be explained by this. However, for the remaining 14 entries in the medical histories (of 33 in total) which were not found by LC–MS, sensitivity problems should in principle not be the problem. In other words, up to 42.4% of the entries in the medical histories may have been wrong. Looking at this problem the opposite way, about 44.4% of the compounds found during the LC–MS screening were not reported by the patients. This strongly indicates that about 40% of the CNS-stimulating drugs reported by the patients either suffered from poor compliance or were wrong. In conclusion, the results from Table 4 indicated that a small number of drugs were not identified by the screening method due to sensitivity problems, and that investigations based only on medical histories without chemical screening may be highly unreliable for CNS-stimulating drugs. The latter aspect confirm earlier publications focused on illicit drugs where poor agreement between information from patients and analytical results from urine and plasma have been reported [17–19].

In addition to drugs utilized in clinical therapy and illicit drugs, we also screened the samples for caffeine. During the interview, their intake of caffeine-containing drinks was monitored with focus on coffee and tea. Among the 175 samples analyzed, 139 showed agreement between the patient information and the LC–MS results; 115 samples contained caffeine while 24 samples were negative. Among the remaining 36 samples, where disagreement was observed between patient information and LC–MS data, caffeine was not detected by the LC–MS in of the 27 samples, while 9 samples were positive. There may be several logical explanations to the deviations observed, but it is outside the focus of this paper. In conclusion however, the agreement between patient information and LC–MS results was substantially higher for caffeine than for the CNS-stimulating drugs, and this suggested that the inclusion of caffeine in the LC–MS screening may provide valuable information.

A similar study was conducted to verify smoking among the patients, and in this case we focused on the detection of cotinine which is a metabolite of nicotine. Among the 175 samples analyzed, 155 showed agreement between the patient information and the LC–MS results; 71 samples contained cotinine while 84 samples were negative. Among the remaining 20 samples, where disagreement was observed between patient information and LC–MS data, cotinine was not detected by the LC–MS in of the 17 samples, while 3 samples were positive. Thus, also for the monitoring of smokers, the LC–MS screening for cotinine may provide valuable information.

4. Conclusion

The present work has focused on potentials and limitations of a very broad screening method for CNS-stimulating drugs and some legal stimulants in human plasma based on protein precipitation and LC–MS. Although the simple sample preparation procedure provides low sample enrichment and clean-up, and in spite of the fact that the MS is operated with a single mass analyzing step, 62 of the 72 CNS stimulating drugs tested were easily detected within the therapeutic relevant concentration ranges using positive electrospray MS. For the remaining 10 compounds, this screening provided insufficient sensitivity; these were either compounds with plasma concentrations close to 1 ng/ml or compounds with a poor LC–MS signal. When operated in the single MS mode, a confirmational step was required to avoid false positive identifications, and this was effectively accomplished by SID. Experiences with 175 different

human plasma samples from patients with severe hypoglycaemia demonstrated that medical information from interview of patients may be highly unreliable, and that LC–MS screening may important to provide reliable information on medication and compliance. Caffeine and cotinine were included to monitor caffeine intake and smoking, and in both cases the LC–MS screening was found to provide valuable additional information with respect to both.

Acknowledgements

Dr. Ulrik Pedersen-Bjergaard (Department of Internal Medicine, Hillerød Hospital, Hillerød, Denmark) is acknowledged for the provision of human plasma samples and for the interview of patients regarding their intake of drugs. Finn Tønnesen (Department of Pharmaceutical Analysis, School of Pharmacy, University of Oslo, Norway) is acknowledged for the sample preparation of the human plasma samples.

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