International Journal of Anatomy and Physiology ISSN: 2326-7275 Vol. 8 (7), pp. 001-015, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Rapid screening for 61 central nervous system drugs in plasma using weak cation exchange solid-phase extraction and high performance liquid chromatography with diode array detection

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Accepted 8 March, 2011

Rapid and accurate screening for toxicants/chemicals in a broad range is an important element in systematic toxicological analysis (STA). Herein, we report a novel method for the rapid screening of 61 central nervous system (CNS) drugs in plasma, using a solid-phase extraction (SPE) column termed, weak cation exchange (WCX) and high performance liquid chromatography with a diode array detector (HPLC- DAD). The SPE column was preconditioned sequentially with 3 ml of acetonitrile, 1 ml of water and, 2 ml of buffer solution. The pretreated plasma was loaded onto the column, which was then washed with 2 ml of water, followed by 2 ml of acetonitrile, and the acetonitrile elution was collected as the neutral/acid fraction. Subsequently, 3 ml of trifluoroacetic acid-acetonitrile (2+98) was then used to elute the column and the elution was collected as basic fraction. The collected fractions were evaporated at 60°C under a nitrogen stream until about 100 l of solvent remained. The final volume was then adjusted to 1 ml with 5% of acetonitrile. The HPLC separation was accomplished on an Agilent TC -C18 column (250 × 4.6 mm, 5 µm) with acetonitrile and phosphate buffer solution as mobile phase, by gradient elution at a flow rate of 1.5 ml/min. The detection wavelength was 210 nm, and the full spectra were recorded from 200 to 364 nm. The absolute recoveries of 55 drugs tested, exceeded 50%; 42 of them exceeded 80%. In conclusion, the WCX SPE preparation combined with HPLC-DAD, is suitable for a broad drug screening for CNS drugs.

Key words: Solid phase extraction (SPE), weak cation exchange (WCX), high performance liquid chromatography with diode array detection (HPLC-DAD), systematic toxicological analysis (STA), drug screening.

INTRODUCTION

Systematic toxicological analysis (STA) is defined as a logical, systematic chemical-analytical search for potentially harmful substances whose presence is uncertain and whose identity is unknown (de, 1997). Acute poisoning is a leading cause of hospital admission; thus, STA is an impending task in emergency medicine. For STA, the sample preparation procedure plays a major role, because in most cases, the actual determination of the compounds of interest cannot be carried out before

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its isolation from biological specimens, even with the most sophisticated technology. One of the most crucial points in STA is that, its extraction step must be able to extract a wide variety of substances, ranging from the very lipophilic, to the moderately polar, with acidic, neutral, basic or zwitterion properties. Meanwhile, the extraction method must be rapid, reproducible, and accurate, and has a highly stable recovery rate (de, 1997).

Although the liquid-liquid extraction (LLE) method is still frequently used in STA (Tracqui et al., 1995; Elliott and Hale, 1997, 1998; Gaillard and Pepin, 1997; Lo et al., 1997; Herzler et al., 2003), a number of drawbacks associated with the methodology, limit its utility. For example, a distribution of the solute occurs between two non-mixable liquids. The isolation has to be performed at a pH at which the analyte is uncharged. For acidic drugs, the aqueous phase has to be acidified, whereas for basic drugs, the aqueous phase has to be basified. Therefore, it is difficult to establish a general LLE method that can extract a broad spectrum of chemicals and drugs of interest in STA. Pragst et al. (2004) developed a LLE method for sample preparation in STA. The acidic and basic compounds were pretreated by different procedures separately, while some drugs with higher hydrophilicity were pretreated by protein precipitation (PP). Emulsion formation and poor reproducibility are additional problems of LLE. Furthermore, LLE is labor intensive, time consuming, and difficult to automate (Yawney et al., 2002) . Protein precipitation (PP), a conventional method of sample pretreatment, is a fast, easy-to-handle procedure. Furthermore, it can be applied to the sample preparation for a wide range of analytes. A common PP procedure is to add a reagent (organic solvent, acids or salts) to biological samples; up to 98% of the protein in human plasma can be eliminated with an appropriate reagent (Polson et al., 2003; Souverain et al., 2004) . However, its limits of detection (LOD) are often poor, owing to the lack of a concentration step.

The use of solid-phase extraction (SPE) in biological sample preparation has recently become more accepted in STA (Lai et al., 1997; Franke and de, 1998; Soriano et al., 2001; Yawney et al., 2002; Alabdalla, 2005). SPE has a number of advantages over LLE, such as cleaner extracts, no emulation formation, increased selectivity for the compounds of interest, and easy to be automated (Yawney et al., 2002). Various SPE columns such as HLB (Hydrophilic—Lipophilic Balance) (Sturm, 2005), MCX (Mix Cation Exchange) (Yawney et al., 2002), and Bond Elut Certify column (Lai et al., 1997; Soriano et al., 2001) have been widely used in STA. A new SPE column, named Weak Cation Exchange (WCX) column, contains a mixed-mode cation-exchange sorbent. WCX was based on a polymer backbone of poly (divinyl- benzene-co- N-vinylpyrrolidone) with the addition of acetic acid groups to enable it retain cations. While several reports have demonstrated the utility of WCX SPE for extraction of individual drugs or a group of related drugs (Margout et al., 2009; Zhou et al., 2009), its application in screening a wide variety of drugs ranging from highly lipophilic to moderate polar compounds from biological samples has not been established.

The success of STA also depends on the quality of the analytical system used. Gas chromatography mass spectrometry (GC-MS) is widely used as the “gold-standard” for STA (Saint-Marcoux et al., 2003). However, GC-MS has some shortcomings. For instance, drugs can be detected in their native form only if they are thermally stable, volatile, and mildly or not polar; derivatization is therefore required to increase the volatility of many polar analytes, which would not otherwise be amenable to analysis by GC. The derivatization step is costly, time consuming, and susceptible to errors that affect the quality of the analytical results (Alabdalla, 2005; Stoll et al., 2006). Liquid chromatography, coupled with mass spectrometry (LC-MS) has gained attention in recent years, since it is better suited to the analysis of very polar and thermally labile analytes. Perhaps, the most significant problem currently facing LC-MS users, particularly those using electrospray ionization (ESI) to generate analyte ions from the liquid eluent, is the phenomenon of ionization suppression. Briefly, compounds that co-elute from the liquid chromatography column with the analyte of interest have a potential to suppress the ionization of the target analyte, thereby, altering the analytical results (Stoll et al., 2006).

Historically, gradient elution HPLC-DAD has been a common method for the screening and identification of unknown drugs in biological samples. HPLC-DAD has some advantages; for instance, the technique is relatively inexpensive compared with GC- MS and LC- MS. Furthermore, it has a broad chemical selectivity, and can produce precise retention time data under well controlled conditions. Herzler et al. (2003) had shown the selectivity of substance identification in STA by using a UV Spectra library of 2682 compounds. Furthermore, with up-to-date DADs, UV spectra can be measured with high sensitivity and reproducibility.

The objective of the present study was to develop a general procedure of SPE for plasma sample preparation using a WCX column for STA, coping with a high performance liquid chromatography-diode array detection (HPLC-DAD) method for sample analysis. The drugs we tested included, analgesics, benzodiazepines, barbiturates, anti-allergic drugs, anti-epileptics, antipsychotics, antidepressants, anesthetics, central stimulants, amphetamine-type stimulants, anti-asthmatics, anticholinergic agents and antidiarrheals, among others. It is hoped that the WCX SPE preparation, combined with HPLC -DAD, would be suitable for the screening of a broad spectrum of drugs. Since drugs affecting central nervous system are leading causes of poisoning and often seen in emergency clinics and poisoning control units, we developed our methods for

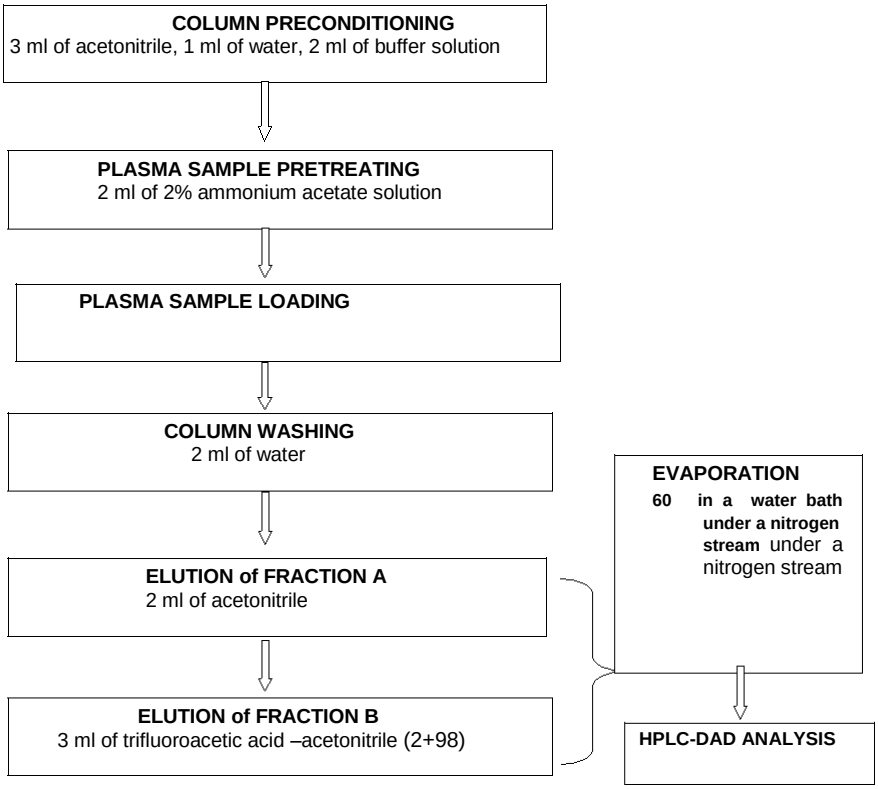

Figure 1. Flow chart of WCX SPE step.

this class of agents to provide proof-of-principle data for its utility in STA.

MATERIALS AND METHODS

Regents

Acetonitrile and methanol were HPLC grade and purchased from TEDIA Corporation (Fairfield, Chio, USA). Potassium dihydrogen phosphate (KH_2PO_4) and phosphoric acid were of analytical grade. 1-nitrobutane was GC grade and purchased from Sigma-Aldrich (Buchs, Switzerland). The water was filtered through the Millipore Milli-Q system (Billerica, MA, USA). A phosphate buffer solution (PBS, 20 mM, pH = 3.0) was prepared by 20 mM/l potassium dihydrogen phosphate solution, adjusted to the desired pH by appropriate addition of phosphoric acid.

Drug standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), except bucinnazine and nikethamide, which were purchased from KingYork group Co., Ltd (Tianjin, China). The stock solution of each drug was first prepared in 50% methanol/water stock solution, and then, diluted with water or methanol to a set of concentrations. An aliquot of these diluted drug standards was then added to healthy human plasma to prepare a set of standard concentrations. The concentration of methanol in these spiking solutions was 5%. OASIS WCX columns (3 cc/60 mg) and OASIS MCX (3 cc/60 mg) columns were purchased from Waters (Milford, MA, USA). Real plasma samples were collected from drug poisoning patients from the intensive care unit of the Second Affiliated Hospital of Fujian Medical University.

Instrumentation

The HPLC system (Beckman Coulter Inc., Fullerton, California, USA) consisted of a Series 508E auto sampler, a Series 125 pump, a Series 168 diode array detector (DAD), and a Series 32Karat chromatographic working station. Separation of analytes was performed using an Agilent TC- C18 column (5 m, 250 × 4 .6 mm, Agilent Technologies Inc, CA, USA). The following acetonitrile-phosphate buffer elution gradient was applied; the proportion of acetonitrile was linearly increased from 5 to 50% in 30 min at a rate of 1.5% /min, which then increased linearly to 80% in 5 min at a rate of 6% /min. The flow rate of mobile phase was 1.5 ml/min and the injection volume was 50 l. The injection loop volume was 100 l. The temperature of the column was set at 35°C. The UV Detection wavelength was set at 210 nm, and the full spectra were recorded from 200 to 364 nm. The equilibration time between two consecutive samples analyzed in series was set at 15 min.

Sample pretreatment

The SPE column was preconditioned with 3 ml of acetonitrile, 1 ml of water, and 2 ml of buffer solution sequentially. The plasma sample was pretreated with 2 ml of 2% ammonium acetate solution and loaded onto the column, which was then washed with 2 ml of water and eluted with 2 ml of acetonitrile, and the final elution was collected as the neutral/acid fraction (fraction A). 3 ml of trifluoroacetic acid-acetonitrile (2+98) was added into the column and the elution was collected as basic fraction (named fraction B). The collected fractions were evaporated at 60°C in a water bath under a nitrogen stream until about 100 l of the solvent remained (Figure 1). The final volume of the elute was adjusted to 1 ml with

5% of acetonitrile. The extract was filtrated through a microporous membrane (0.45 m) into the sample vials, and 50 l of the filtrates was injected onto the HPLC system with an auto sampler.

Internal standard

For the quality control, 1-nitrobutane was chosen as internal standard. Briefly, 10 l of 1-nitrobutane (1.0 mg/ml) was added to each biological sample. Its retention time was used to calculate relative retention time (RRT) of each compound, using the formula: RRT = (the retention time of interest) / (the retention time of 1-nitrobutane). The retention also serves as an indicator of the reproducibility of gradient conditions.

Determination of recovery rate and limits of detection (LOD)

We divided 61 drugs into 7 groups; drugs with similar retention times were assigned to different groups so that maximum number of drugs could be eluted with a complete separation in a single chromatographic analysis, while their retention times were distributed evenly within a single run. We analyzed them in fractions A and B, respectively, so that we would know which drugs were retained in fraction A and which drugs were retained in fraction B. The extraction recovery of the drugs of interest was determined by comparing peak areas obtained after SPE procedure with those obtained from direct injections of standard solutions (two concentrations and three times for each compound).

%recovery = (peak area of extracted samples/peak area of unextracted samples) × 100. %RSD = (SD/mean) × 100,

Where RSD and SD are the relative standard deviation and standard deviation, respectively. The LOD for each drug was calculated at a signal -to-noise ratio of 3. Both recovery and LOD were determined from the peak areas at 210 nm.

Identification and quantification

Upon the completion of the chromatographic procedure, the RRTs of peaks to 1-nitrobutane were obtained. A drug database including the RRTs and UV spectra of 61 drugs was then set up. Peaks in biological samples were tentatively identified based on their RRTs. The identity and purity of each peak were then confirmed by comparing the UV spectra of the peak against the library entries of drugs with similar RRTs. An estimate of the similarity index (SI) was provided by the Karat working station. Concentrations of drugs were calculated by comparing the area ratios of the analyte with the corresponding standards containing a known amount of the analyte.

RESULTS

Retention behavior

The RRTs of 61 drugs are listed in Table 1. Figure 2 illustrates the representative chromatograms of 15 drugs (2 g/ml for each drug, with 10 g/ml being, the internal standard) by gradient elution in standard solution (5% acetonitrile-buffer). Figure 3a is the chromatogram of neutral/acid elution fraction of 15 drugs spiked plasma and blank plasma using WCX SPE and there were 6 drugs at the neutral/acid fraction. Figure 3b is the chromatogram of basic elution fraction of 15 drugs spiked plasma and blank plasma using WCX SPE, respectively and there were 10 drugs at the neutral/acid fraction. Bucinnazine was eluted both in neutral/acid fraction and basic fraction.

Recovery

Of the 61 drugs tested, most of them were successfully extracted (Table 1, two concentrations, three analyses for each). The absolute recoveries of 55 tested drugs exceeded 50%, and 42 of them exceeded 80%, with RSD ranging from 0.02 to 23.81 %.

LOD

Table 1 illustrates the LODs of all the 61 tested drugs. The LODs of 59 drugs were less than 0.10 g/ml, with those of 26 drugs being 0.01 g/ml and only 2 drugs being greater than, 0.1 g/ml.

Application to real plasma samples

This method has been used in our laboratory to screen about 30 plasma specimens in which 23 specimens were successfully detected. Figure 3 shows the results of the drug screen, which confirms the presence of clozapine in plasma; the similarity index (SI) was 0.9999 when compared with the drug standard of clozapine. We further calculated the concentration of clozapine, which was, 15.3 g/ml.

DISCUSSION

Chromatography separation

Reversed-phase HPLC with acidic pH mobile phase and gradient elution is the most commonly adopted strategy in STA owing to the ability of separating a wide variety of compounds with different physico-chemical properties within a single run (Polettini, 1999). Therefore, we adopted gradient elution, gradually increasing the proportion of acetonitrile from 5 to 80%, eluting polar to highly lipophilic drugs in succession. The duration of a chromatographic run was 35 min, eluting one drug each 0.5 min on average, so, some drugs were eluted at the same time, which was unavoidable in STA. However, we may further identify the majority of them, based on their characteristic UV spectrograms. There are few phenomena in which there are more than five drugs poisonings at the same time in STA. As shown in Figures 2 and 3, the drugs eluted as sharp symmetrical peaks and plasma matrix compound didn't interfere with the analysis. The peaks were relatively equally distributed

Table 1. Recoveries of drugs by WCX SPE (*n*=3) and their relative retention times (RRT) [1].

Drug	Batch number	RRT	Concentration (mg/L)	Recovery %	RSD	Elution fraction [2]	LOD/ (mg/L)	Therapeutic Concentration [4] (mg/L)	Toxic Concentration [5] (mg/L)
Benzodiazepines									
Alprazolam	171218-200603	1.069	1 10	90.67 91.96	11.15 11.70	a	0.02	[0.005-0.05(-0.1)][6]	[0.1-0.4]
Estazolam	1219-0102	1.025	1 10	82.91 87.16	7.29 1.77	a	0.01	[0.055-0.2]	
Diazepam	171225-200302	1.251	1 10	58.00 63.51	14.03 9.95	a	0.01	[anxiolytic 0.125-0.25; anti-epileptic 0.25 – 0.5; eclampsia, tetanus, strychnine-poisoning 1-1.5]	[1.5; L 5]
Oxazepam	171229-200302	1.008	1 10	90.92 88.13	6.23 5.26	a	0.01	[(0.15)0.5-2]	[2; L 3-5]
Lorazepam	171253-200401	1.039	1 10	78.31 73.42	6.74 13.27	a	0.01	[0.02-0.25]	[0.3-0.6]
Chlordiazepoxide	171248-200301	0.762	1 10	97.98 94.52	1.37 3.28	a	0.04	[0.4-4]	[3-10; L 20]
Clonazepam	171227-200302	1.065	1 10	84.41 89.06	9.03 4.78	a	0.01	[0.02-0.07]	[0.1]
Midazolam	171250-200401	0.857	1 10	54.80 62.30	6.51 3.50	a	0.06	[(postoperative awake 0.1-0.04)0.08-0.25]	[1-1.5]
Triazolam	1230-9701	1.089	1 10	83.15 79.62	13.46 9.26	a	0.02	[0.002-0.02]	[0.04]
Nitrazepam	171217-200402	1.025	1 10	50.42 57.33	5.87 7.15	a	0.01	[0.03-0.12]	[0.2-0.5]
Barbiturates									
Phenobarbital	171222-200504	0.804	1 10	71.15 85.24	11.47 10.50	a	0.01	[2-30(-40)]	[30-40; L 45-120]

Table 1. Contd.

Secobarbital	171223-200402	1.038	1 10	72.96 92.85	2.62 3.83	a	0.02	[2-10]	[>8; L (4-)10-50]
Amobarbital	171221-200402	0.980	1 10	79.08 89.28	7.08 11.60	a	0.01	[2-12]	[>9; L 13-96]
Other types of sedative-hypnotic									
Zolpidem	171258-200601	0.724	1 10	90.94 103.59	0.80 0.08	a	0.02	[0.08-0.15(-0.2)]	[0.5; L 2-4]
Amphetamine-type stimulants									
Benzedrine	171211-200502	0.482	1 10	96.88 100.40	1.30 0.46	b	0.05		
Pseudoephedrine	171237-200505	0.431	1 10	92.44 102.38	5.97 4.11	b	0.03	[0.5-0.8]	[L 19]
Addicted analgesics									
Buprenorphine	171244-200503	0.911	1 10	90.20 91.28	0.95 0.02	b	0.02	[0.001-0.01]	[0.2; L [4-13]
Fentanyl	171204-200303	0.860	1 10	94.40 100.98	8.06 1.40	b	0.10	[0.001-0.002]	[0.002-0.02]
Codeine	171203-200303	0.442	1 10	89.66 91.92	2.40 3.80	b	0.02	[T 0.01-0.05; P 0.05-0.250]	[0.3-1; L 1.6]
Morphine	171201-200521	0.310	1 10	76.50 75.22	4.42 5.73	b	0.04	[0.01-0.12; neonates under artif. breathing 0.08-0.12]	[0.15-0.5; L 0.05-4;
Pethidine	171213-200302	0.712	1 10	101.22 93.55	8.43 3.91	b	0.05	[0.1-0.8]	[(1-)2 L >5]
Bucinnazine	0511141[3)]	0.710	1 10	102.13[a b] 108.13	8.01 4.96		0.01		
Tramadol	171242-200503	0.652	1 10	88.34 89.35	2.66 1.22	b	0.03	[0.1-0.8(-1)]	[1; L 2]

Table 1. Contd.

Weight-reducing aid									
Fenfluramine	10073-0002	0.802	1 10	95.84 99.21	2.05 8.71	b	0.05	[0.05-0.15]	[0.5-0.7; L 6]
Antipyretic analgesics									
Ibuprofen	100179-200303	1.436	1 10	56.62 58.68	12.01 12.99	a	0.01	[15-30(5-50)]	[100]
Acetaminophen	100018-200408	0.409	1 10	70.38 58.29	5.46 6.74	a	0.01	[10-20(2.5-25)]	[T 75-100; P 100-150; L 160]
Nimesulide	100555-200501	1.305	1 10	34.86 57.88	13.97 3.35	a	0.01	[10-75]	
Diclofenac	100880-200601	1.404	1 10	72.60 83.18	3.51 5.49	a	0.01	[T 0.05-0.5; P 0.1-2.2]	[50;60]
Salicylic acid	100106-200303	0.752	1 10	38.60 38.18	12.64 1.36	a	0.01	[rheumatism (child 150)- 200-300; anticoagulant 50-125]	[400-500 child 300; L 500-900]
Indometacin	100258-200403	1.412	1 10	50.70 50.32	6.43 6.90	a	0.01		
Anticholinergic drugs									
Atropine	100040-200510	0.567	1 10	100.79 102.30	1.72 2.29	b	0.05	[0.002-0.025]	[0.03-0.1 L0.2]
Scopolamine	100049-200308	0.730	1 10	102.38 104.08	4.76 2.09	b	0.07	[0.0001-0.0003(-0.001)]	
Anisodamine	0249-9501	0.489	1 10	88.83 100.22	8.68 6.62	b	0.17		
Antiepileptic drugs									
Phenytoin	100210-200401	0.976	1 10	72.62 103.76	8.85 11.13	a	0.01	[10-20; baby 6-14]	[20-40 baby 15; L 70]
Carbamazepine	100142-199503	0.966	1 1089.82	87.04	3.18 1.39	a	0.01	[4-9(12)]	[(12)-15 L>25]

Table 1. Contd.

Antipsychotic drugs									
Perphenazine	100133-200602e	1.028	1 10	91.85 106.47	6.17 2.11	b	0.03	[0.0004-0.03]	[0.05-0.1]
Fluphenazine	100162-200302	1.101	1 10	90.84 95.42	2.31 1.53	b	0.01	[(0.0002-)0.001-0.017]	[0.05-0.1]
Haloperidol	100313-200301	0.923	1 10	96.97 104.37	2.25 3.36	b	0.03	[0.005- 0.015 (0.04)]	0.05-0.1; L 0.5]
Risperidone	100570-200401	0.744	1 10	88.58 90.47	1.13 3.29	b	0.03		
Chlorpromazine	100460-200501	1.070	1 10	0.00 69.98	3.02	b	0.03	[0.03-0.5; child 0.04-0.1]	[0.5-2; L2]
Clozapine	100323-200201	0.808	1 10	110.24 109.22	5.08 7.56	b	0.01	[0.1-0.6(0.8)]	0.8-1.3; L 3]
Chlorprothixene	0043-9701	1.093	1 10	104.32 105.48	4.89 2.01	b	0.01	[0.03-0.3]	[0.4-0.7; L 0.8]
Sulpiride	100203-200503	0.411	1 10	99.83 107.32	2.55 3.76	b	0.02	0.04-0.6	[L 3.8]
Antidepressants									
Amitriptyline	100518-200401	1.018	1 10	96.32 97.68	4.36 3.89	b	0.01	[0.05-0.3]	
Doxepin	0069-9702	0.909	1 10	80.06 108.80	10.55 7.56	b	0.01	[0.02-0.15]	[0.1; L 1-18]
Clomipramine	100843-200501	1.106	1 10	72.73 77.56	8.63 10.37	b	0.02	[(0.02)0.09-0.25]	
Paroxetine	100357-200301	0.955	1 10	103.29 101.38	4.40 8.15	b	0.03	[0.01-0.075(-0.1)	[0.35-0.4]

Table 1. Contd.

Venlafaxin	100543-200401	0.757	1 10	104.18 105.30	5.39 6.74	b	0.07	[sum with metabolite 0.25-0.75]	[sum 1-1.5; L 6.6]
Antiparkinsonism drug									
Trihexyphenidyl	100067-200602	0.994	1 10	65.09 68.89	4.75 5.20	b	0.07	[0.05-0.2]	[0.5]
Antihistamines									
Diphenhydramine	0066-9705	0.881	1 10	86.10 83.04	9.52 2.66	b	0.01	[0.1-1]	[1; L 5]
Chlorpheniramine	100047-200305	0.741	1 10	102.10 100.92	6.05 8.13	b	0.05	[0.01-0.017]	[20-30; 1.1]
Promethazine	100422-200501	0.940	1 10	20.54 40.65	23.81 8.49	b	0.03	[(0.05)0.1-0.4]	[1; L 2.4]
Anesthetics									
Propofol	100806-200601	1.573	1 10	99.80 104.80	9.68 9.06	a	0.03	[narcose 2-4(8)]	220
Tetracaine	100456-200301	0.875	1 10	0.00 62.18	3.92	b	0.03		
Lidocaine	100341-200301	0.574	1 10	87.92 103.15	11.04 2.81	a	0.01	[(1-)1.5-5]	[6-10; L 10-25]
Ketamine	171257-200501	0.579	1 10	82.58 96.05	6.07 0.95	a	0.03	[0.5-6.5]	[7(abuse)]
Anti-asthmatics									
Aminophylline	100121-199903	0.410	1 10	74.60 86.05	1.53 5.46	a	0.02	[8-20; baby 5-10]	[25-30; baby 15; L 50-250]
Doxofylline	100625-200301	0.561	1 1094.366.76	89.390.74		a	0.01		

Table 1. Contd.

Central stimulants									
Caffeine	1215-9503	0.494	1 10	79.86 80.96	13.15 7.89	a	0.02	[8-20]	[30-50); L>80-100]
Nikethamide	0601051[3)]	0.540	1 10	85.71 76.70	13.83 9.36	a	0.02		
Antidiarrheal									
Diphenoxylate	171202-200303	1.265	1 10	38.63 35.20	9.84 10.65	b	0.01	[appr. 0.01]	

1) The retention time of internal standard (1-nitrobutane) is 21.767 min. 2) a: the neutral/acid fraction; b: the basic fraction. 3) Injection (KingYork group Co., Ltd, Tianjin). 4) [Ref. Concentration Therapeutic mg/L (T=trough; P=peak)]. 5) [Ref. Concentration Toxic mg/l (T=trough; L=lethal)]. 6) [0.005-0.05(-0.1)]: means: normally between 0.005-0.05 mg/L, but some authors or clinicians are using ranges between 0.005-0.1 mg/l.

and entirely separated. Neutral or acid drugs such as chlordiazepoxide, phenytoin, oxazepam, alprazolam and diclofenac were eluted in neutral/acid fraction. While basic drugs were eluted in basic fraction, examples were sulpiride, Benzedrine, atropine, clozapine. Bucinnazine was eluted both in neutral/acid fraction and basic fraction, we supposed it was because it stands cation and molecule state simultaneously in pH = 6 circumstance at our experiment condition.

UV detection wavelength

While some drugs such as, chlorpromazine, chlorprothixene, fluphenazine, and acetaminophen, have chrematistic UV absorption, and 210 nm is not their maximum absorption wavelength, all the 61 drugs tested have absorption at 210 nm. Considering the generality of our detection methods, we set 210 nm as our UV detection wavelength.

Selection of SPE column

Firstly, some of the drugs were pretreated by MCX column. We found that several drugs were poorly extracted such as Benzedrine, buprenorphine, nikethamide, triazolam, clonazeam, and paroxetine with their recoveries being 26.90 to 64.50%, and for the majority of the drugs, the figure was below 50%. We suggest that, this may be because these drugs were unstable at basic circumstance. The unsatisfactory results made us switch to WCX columns.

WCX columns contain a mixed-mode cation-exchange sorbent based on a polymer backbone of poly (divinyl-benzene-co-N-vinylpyrrolidone) with the addition of acetic acid groups to enable it to retain cations. Its mechanism was mixed mode, including weak cation exchange and hydrophobic retention. The acidic and neutral drugs were in un-ionized forms, and therefore, were absorbed on the column by hydrophobic functional groups of the mixed-mode sorbent, while the strong and weak basic drugs were retained by both ionic and hydrophobic interactions. We tested 61 drugs using WCX column and the results are shown on Table 1. Their absolute recoveries were better than those with MCX column for most drugs.

Recovery

The crucial point in STA was that the extraction step must be able to extract a very wide variety of substances, ranging from very lipophilic to moderately polar, and exhibiting acidic, neutral, basic or zwitterions properties. In addition, the most important was that, the extraction method must give good recoveries, not missing some of toxic substances. Of the 61 drugs tested, most of them were successfully extracted, which meant the extraction method had good recoveries for most drugs.

LOD

LOD is an important parameter in quantitative

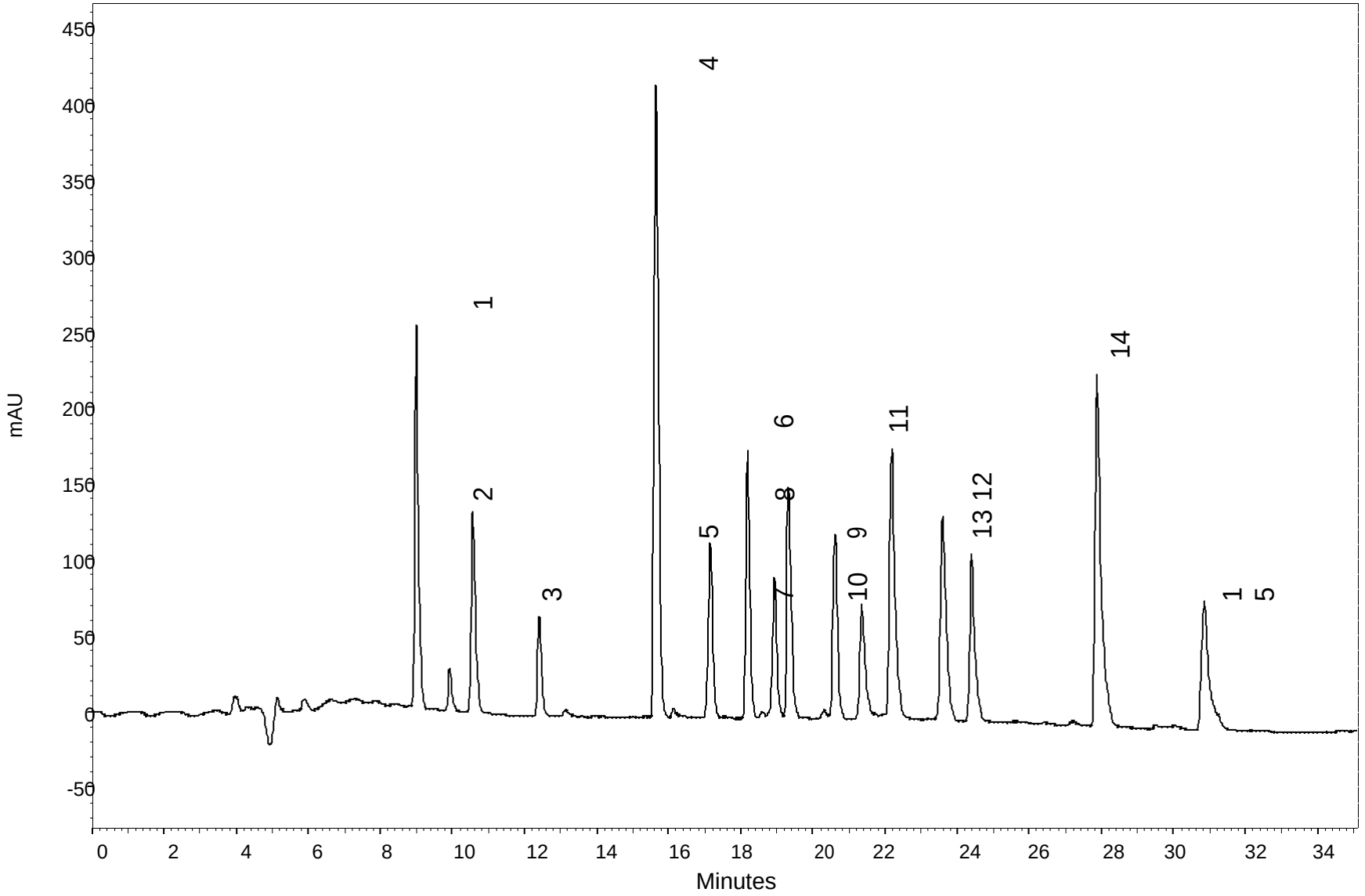

Figure 2. Chromatogram of 15 drugs standard solution (10 g·ml^{-1} each drug, detected at 210 nm). Conditions: Agilent TC-C18 column, 250 mm × 4.6 mm, 5 m; detection wavelength, 210 nm; injection volume; 50 l, mobile phase, 20 mmol/l KH_2PO4 buffer solution (pH 3.0) (A) and acetonitrile (B); flow rate, 1.5 ml/min; gradient elution: 0→30 min, 5% B→50% B; 30→35 min,50% B→80% B. Peak identification: 1; sulpiride, 2; benzedrine, 3; atropine, 4; bucinnazine, 5; chlordiazepoxide, 6; clozapine, 7; fentanyl, 8; tetracaine, 9; haloperidol, 10; phenytoin, 11; oxazepam, 12; alprazolam, 13; clomipramine, 14; diphenoxylate, 15; diclofenac.

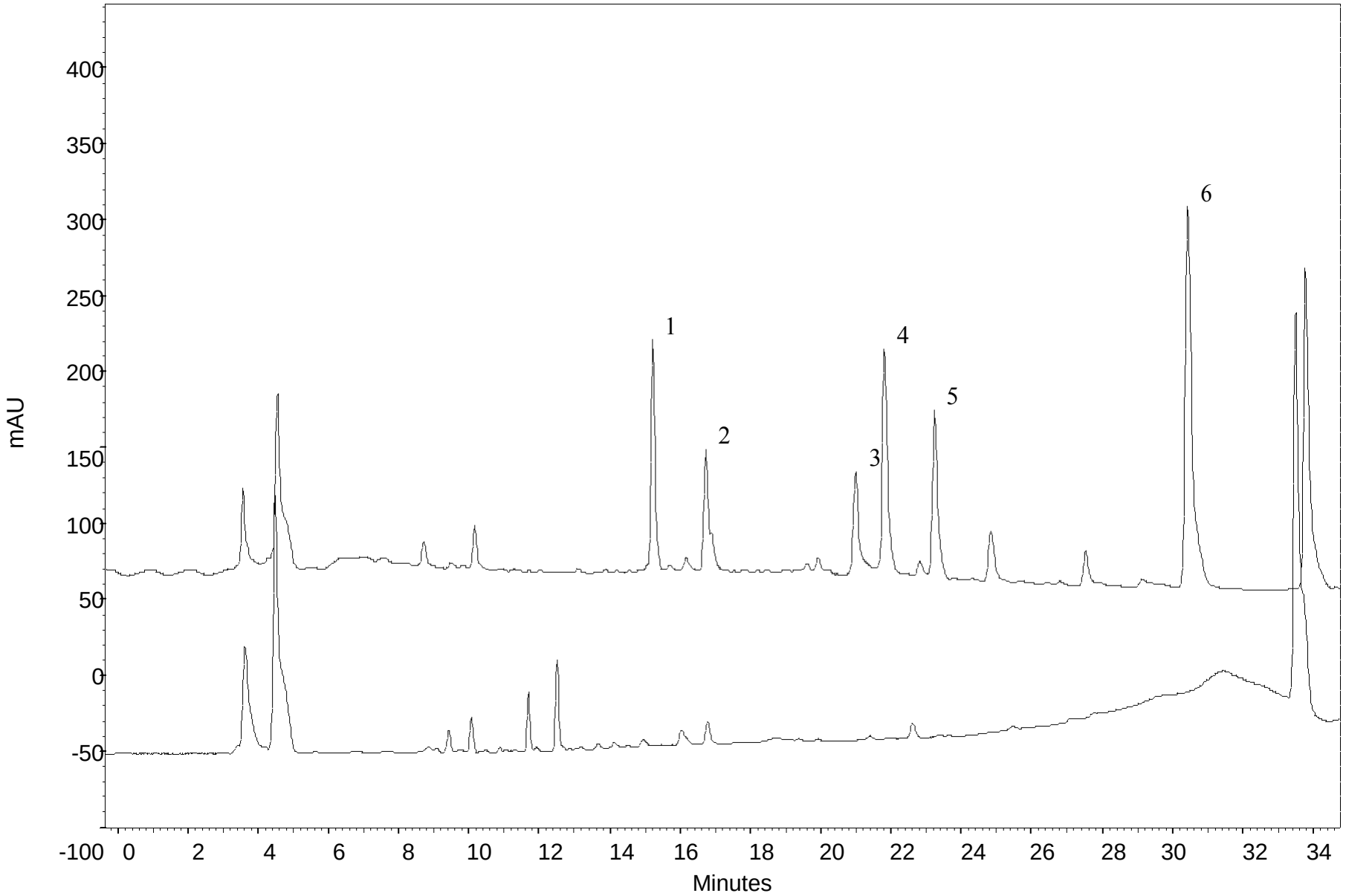

Figure 3a. Chromatogram of neutral/acid elution fraction of 15 drugs spiked plasma (upper) and blank plasma (lower) using WCX SPE (10 g•mL^{-1} each drug, detected at 210 nm). Conditions are as in Figure 1. Peak identification: 1; bucinnazine, 2; chlordiazepoxide, 3; phenytoin, 4; oxazepam, 5; alprazolam, 6; diclofenac.

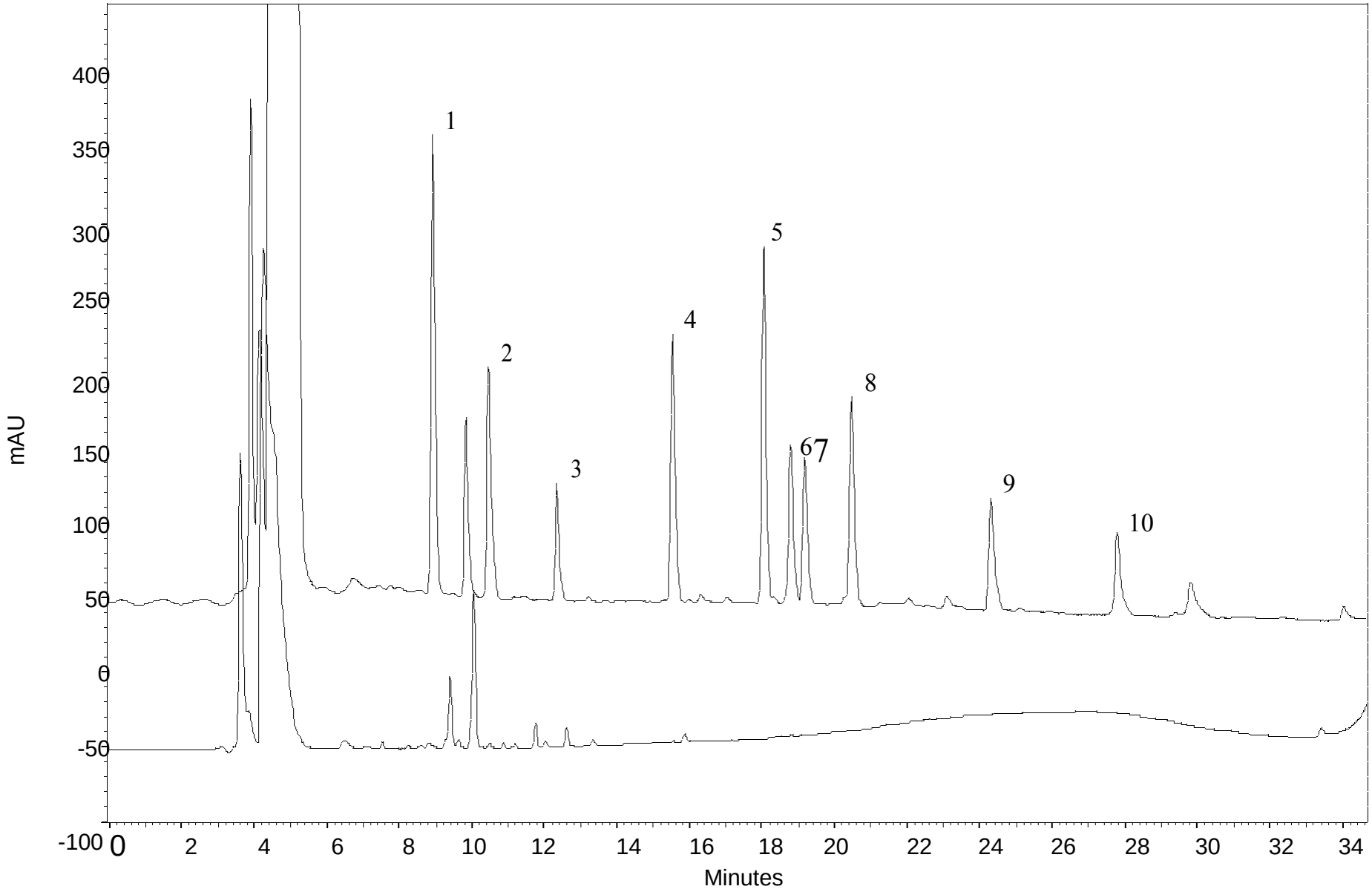

Figure 3b. Chromatogram of basic elution fraction of 15 drugs spiked plasma (upper) and blank plasma (lower) using WCX SPE (10 g•ml^{-1} each drug, detected at 210 nm). Conditions are as in Figure 1. Peak identification: 1; sulpiride, 2; benzedrine, 3; atropine, 4; bucinnazine, 5; clozapine, 6; fentanyl, 7; tetracaine, 8; haloperidol, 9; clomipramine, 10; diphenoxylate.

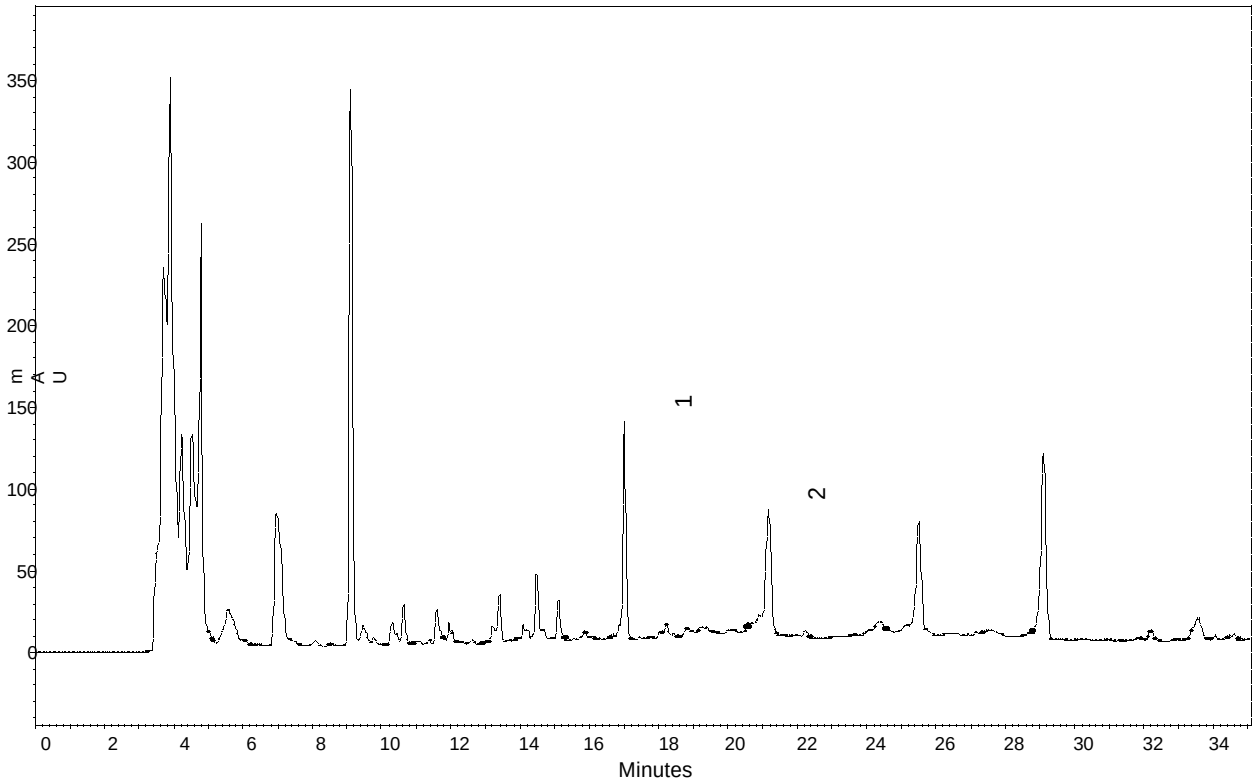

Figure 4. Chromatogram of drug screen on plasma sample of case study 1 (detected at 210 nm). Conditions are as in Figure 1. 1; clozapine, 2 ; 1-nitrobutane.

analysis. To a certain degree, whether a poison could be detected lies on the value of LOD. The LODs in STA should be lower than its toxic concentration so that it could be detected. The LODs of most drugs tested using the WCX column could meet the requirement of STA (TIAFT Reference Committee, 2004) (Table 1). Examples were alprazolam, diazepam, oxazepam, lorazepam, phenobar-bital, secobarbital, and pseudoephedrine. Furthermore, many drugs can be detected at therapeutic concentrations, including diazepam, oxazepam, phenobarbital, zolpidem, tramadol, ibuprofen, and acetaminophen. Only LOD of fentanyl, scopolamine and anisodamine could not meet the requirement of STA.

SI

Mathematical models for the assessment of spectral similarity, use the description of the spectrum as a vector in *n*-dimensional space, where *n* is the number of absorbance wavelength pairs measured. The SI is defined as cos and is calculated by its chromatographic working station (Herzler et al., 2003) . For two identical spectra, cos equals 1.0000. Because near 1.0000 the cosine function is relatively insensitive for changes of , already, small deviations from 1.0000 can indicate significant differences between the spectra. We defined SI = 0.9990 as a threshold value above which two spectra were regarded as identical. As a result of our real plasma sample (Figure 4), SI was 0.9999, which could be well-founded identified as clozapine.

Advantages and disadvantages of WCX column

There are several advantages with WCX column over other columns. Firstly, basic drugs can be eluted under either acid or basic conditions. Some drugs are not stable in basic circumstances, so, they could be eluted in acid circumstance in order to improve their recoveries, while MCX column should be eluted at basic circumstance. Secondly, WCX can extract acid, basic and neutral drugs simultaneously owing to its mix mechanisms of extraction, which meets the requirement of STA for various compounds with different properties. Thirdly, WCX has better sensibility owing to its concentration step compared with PP. Fourthly, the neutral/acid fraction and basic fraction could be collected in succession, so we could analyze only one fraction in therapeutic drug monitoring (TDM), which could avoid interference from the other fraction.

On the other hand, the extraction procedure with WCX column has some disadvantages. Firstly, certain drugs such as salicylic acid are not successfully extracted, presumably due to its pKa, which was, 3.12 at 25°C, and it was in the anion state in our experimental condition (pH = 6), so it could not be retained by cation exchange mechanism, although, it could be partly retained by hydrophobic retention mechanism. Secondly, for some heat-labile drugs, for instance, promethazine, chlorpromazine and tetracaine, the procedure of evaporating at 60°C could decrease their recoveries.

Conclusions

We established a rapid screening method for the quailification and quantification of 61 drugs in plasma by using WCX SPE column and HPLC-DAD analysis. The drugs we tested included, analgesics, benzodiazepines, barbiturates, anti-allergic drugs, anti-epileptics, antipsychotics, antidepressants, anesthetics, central stimulants, amphetamine-type stimulants, anti-asthmatics, anticholinergic agents and antidiarrheals. This method has been used in our laboratory to screen plasma specimens. The sample preparation was rapid and had good recoveries for most drugs.

ACKNOWLEDGEMENTS

The work was supported by the Research Fund of Fujian Public Health Bureau (2007-1-27) and Quanzhou Science and Technology Bureau (2008Z24).

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