

Performance Evaluation of a Thermal Desorption System for Detection of Basic Drugs in Forensic Samples by GC/MS

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KEYWORDS

SBSE, basic drugs, forensic

ABSTRACT

Stir bar sorptive extraction (SBSE) is an innovative sample extraction technique that can be used to process blood, urine, and tissue samples for routine drug screening in the forensic toxicology laboratory. The GERSTEL TDU System is a multifunctional thermal desorption unit capable of determining the presence of analytes from liquid samples after extraction using the TwisterTM stir bar. The TDU desorption system was evaluated for use in combination with GC/MS for determining the presence of basic drugs in forensic samples. Human blood fortified with known quantities of drugs was used to evaluate sample diluents, extraction time, injection parameters and recovery. Case specimens containing drugs typically encountered in forensic samples were evaluated using the desorption method and compared with a liquidliquid extraction method followed by GC/MS analysis. This evaluation demonstrated that the TDU desorptive method worked equally as well as the routine extraction method for the detection of basic drugs in screening forensic samples.

In addition, the described technique avoids the use of extraction solvents and the subsequent centrifugation,

transfer, and concentration steps required of liquidliquid and solid phase extraction methods.

INTRODUCTION

The GCMS analysis of basic drugs in biological samples is normally performed after isolation of the drug from the biological matrix. Most routine sample preparation methods are based on liquid-liquid extraction, solid phase extraction, or protein precipitation. These methods require the use of organic solvents, centrifuges, rotators, and concentrators.

The stir bar sorptive extraction method (SBSE) was designed to extract organic compounds from any aqueous matrix. Commercially available from GERSTEL, the TwisterTM is a small magnetic stirring rod encased in glass and coated with a layer of Polydimethylsiloxane (PDMS). The Twister Desorption Unit (TDU) was specially designed and optimized for use with the Twister and SBSE. The use of PDMS-coated stir bars in combination with state of the art thermal desorption instrumentation and GC/MS avoids the use of solvents for extraction, reduces sample preparation time, and minimizes equipment needed for sample preparation.

Previous evaluations using SBSE have included detecting specific drugs in urine, water, saliva, and bovine serum, along with analysis of pesticides in wine, contamination in lakes, and impurities and/or preservatives in food and beverage products [1-5].

The purpose of this evaluation was to examine the usefulness of TwisterTM bar extraction and thermal desorption for basic drug screening of forensic samples by GC/MS in a forensic toxicology laboratory. The intent of this contribution was also to provide a platform for future investigations involving screening and/or specific target analysis by means of Twister bar extraction.

EXPERIMENTAL

Instrumentation. Sample analysis was performed on two Agilent 6890 Gas Chromatographs interfaced with Agilent 5973 mass selective detectors. For the analysis of Twister samples, the 6890 GC was equipped with a PTV inlet (CIS 4) and manual Twister Desorption Unit (TDU).

For the analysis of liquid-liquid extractions, the second GCMS was equipped with the Agilent split/splitless inlet and the 7673 ALS. The column used, oven temperature program, column flow, and MS conditions were the same as those described for the

TDU application.

Analysis conditions.

Column: 35 m Rtx-5Sil (Restek)

5 m Integra-guard (Restek) di = 0.20 mm $df = 0.335 \mu m$

Pneumatics: He; Pi =23.6 psi;

constant flow = 1.3 mL

Oven: 70° C (1 min); 25° C/min;

310°C (19.4 min)

MSD: Scan, 40 - 550 amu

Thermal desorption conditions.

TDU: splitless

30°C; 60°C/min; 270°C (5 min)

PTV: solvent vent (50 mL/min)

split 20:1

-120°C; 12°C/s; 280°C (5 min)

Liquid injection conditions.

S/SL: 250°C, splitless

Inj.-volume: 2 μl

Standard Preparation. The methanolic stock standards were appropriately diluted with methanol to prepare working drug standards at a concentration of 20 mg/L. The working standards were then used to spike blood samples to the desired concentrations. The stock internal standard, SKF, was prepared at 1 mg/mL in methanol and diluted to 100 mg/L for the working IS solution.

Sample Preparation SBSE. Three milliliters of blood, urine, bile, vitreous, gastric contents, or tissue homogenate was pipetted into a 20 mL GC headspace vial. A 1:3 dilution was used for urine and bile. Tissue homogenates were prepared at a 1:4 dilution, and gastric contents were diluted 1:100 or 1:1000. All dilutions were made with deionized water. Exactly 50µL of the 100 mg/L IS solution was added, and a 10 mm stir bar (TwisterTM, GERSTEL) coated with 24 μL PDMS was added to the vial. The remaining headspace vial volume was filled with the carbonatebicarbonate buffer. The vial was sealed and the contents stirred for approximately 16 hours at room temperature. The TwisterTM bar was then removed with a pair of forceps, rinsed with deionized water, blotted dry with a KimwipeTM, and inserted into a clean TDU liner. Liquid Injection. A 3 mL aliquot of blood, urine, bile or tissue homogenate, was extracted using a standard multi-step liquid-liquid extraction method described

elsewhere [6].

RESULTS AND DISCUSSION

The purpose of this evaluation was to determine the usefulness of SBSE with the TwisterTM bar and the Twister Desorption Unit in the routine analysis of biological samples typically analyzed in the Forensic toxicology laboratory.

The Twister was desorbed using the TDU splitless mode with cryofocusing at -120°C in the inlet. A 20: 1 inlet split ratio gave excellent peak shape and good analyte responses and was therefore used for analysis of all subsequent standards and sample extracts. On a few occasions, the first blank run of the day showed trace amounts of drugs from previous injections. Repeating the blank run, or routine CIS 4 inlet maintenance (changing the liner every few weeks and solvent cleaning the inlet body as needed) eliminated carry over in the inlet.

Reconditioned TwisterTM bars were randomly desorbed and compared to new TwisterTM bars to monitor their extraction efficiency and to check for cleanliness prior to reuse. Reconditioned TwisterTM bars did not retain drugs from previous extractions when reconditioned as recommended.

A 3 milliliter sample size was used in the TwisterTM extraction to match the sample amount routinely used in our laboratory's liquid-liquid basic extraction method for ease of method comparison.

Several different diluents, including water, saline (pH 5.5), 30% sodium chloride (pH 8.25), and carbonate/bicarbonate buffer (pH 9.6) were evaluated for sample preparation in the TwisterTM method. The carbonate/bicarbonate buffer gave the best overall results in terms of number of drugs detected and the area response of the target compounds used to evaluate the diluents. Extraction times of 2-hr, 4-hr and 16-hr (overnight) were evaluated and 16-hr extractions were found to give the best recovery for the largest number of drugs (data not shown).

The recovery of several drugs was determined in blood which was spiked to a 0.3 mg/L concentration, extracted overnight, and desorbed with the described TwisterTM method. The area response of the extracted drug was compared to the area response of the same amount of drug dried directly onto a TwisterTM bar in a TDU liner. Recoveries ranged from 23%-99%. (Table 1).

Table 1. Drug recovery determined for several analytes spiked at 0.3 mg/L into blood

Compound	Recovery [%]	Compound	Recovery [%]
Fluoxetine	65.1	Propoxyphene	48.5
Chlorpheniramine	98.9	Promethazine	88.8
Sertraline	70.9	Diazepam	22.7
Methamphetamine	50.7	Meperidine	78.5
Diphenhydramine	86.5	Methadone	84.2
Amitriptyline	84.6	Tramadol	38.0
Lidocaine	59.0	Doxylamine	83.0
Venlafaxine	77.0	Dextromethorphan	94.0
Citalopram	82.0	Mirtazepine	77.4
Chlorpromazine	70.4	Trazadone	38.7
Verapamil	71.0		

To determine the scope of the TwisterTM extraction method, 3mL of blood was fortified with 145 different basic drugs at 1mg/L and extracted with the described method (Table 2). The predicted TwisterTM recovery for each drug was calculated using the GERSTEL® TwisterCalc software. As seen in Table 2, most drugs with low predicted recoveries were not detected after Twister extraction. However, several drugs with low calculated recoveries, such as Venlafaxine, Oxycodone, Nicotine, and Methylphenidate, were detected. Some drugs with high predicted recoveries such as Mesoridazine, Nifedipine, Noscapine, Salicylic acid and Temazepam were not detected. Under the pH 9.6 extraction conditions used, drugs that may contain ionized acidic groups may not be extracted.

Table 2. Drugs extracted at 1 mg/L concentration in blood using TwisterTM. Relative retention time (RRT), TwisterTM Recovery Calculation and the Log Octanol-water partition coefficient.

Compound	RRT	Twister Calc. [%]	log Ko/wr ²
Acepromazine	1.185	61.5	2.3
Alprazolam	1.350	51.3	2.12
Amantidine	0.547	68.8	-0.4
Amitriptyline	0.968	99.8	4.94
Amoxapine	1.154	nf	nf
Amphetamine	0.474	31.5	1.8
Atropine	0.971	35.1	1.8
Baclofen	nd	0.1	-1
Benztropine	1.012	0.4	0.4
Brompheniramine	0.935	99.0	4.1
Bupivicaine	0.996	95.4	3.4
Bupropion	0.718	nf	nf

Buspirone 1.729 77.3 nf Caffeine 0.838 0.7 -0.07 Captopril nd 1.7 0.34 Carbinoxamine 0.893 69.3 2.45 Carbinoxamine 0.923 76.1 2.6 Carbofuran 0.566 nf nf Carisoprodol 0.835 64.7 2.36 Chlordpheniramine 0.897 95.0 3.38 Chlordpheniramine 0.897 95.0 3.38 Chlorpromazine 1.09 100.0 3.4 Cimetidine nd 2.0 0.4 Cimetidine nd 2.0 0.4 Cimpramine 1.042 97.8 3.74 Clomidine 0.931 23.7 1.59 Clozapine 1.298 93.1 3.23 Clozapine 0.988 nf nf Cocaethylene 0.988 nf nf Cocaethylene 0.988 61.5 2.3	Compound	RRT	Twister Calc. [%]	log Ko/wr ²
Captopril nd 1.7 0.34 Carbamazepine 0.893 69.3 2.45 Carbinoxamine 0.923 76.1 2.6 Carbofuran 0.566 nf nf Carisoprodol 0.835 64.7 2.36 Chlorpheniramine 0.897 95.0 3.38 Chlorpheniramine 1.099 100.0 3.4 Chiorpromazine 1.09 100.0 3.4 Cimetidine nd 2.0 0.4 Cimetidine nd 2.0 0.4 Cimetidine nd 2.0 0.4 Cimetidine nd 2.0 0.4 Cimetidine 0.931 23.7 1.59 Clomipramine 1.046 99.9 5.2 Clondine 0.931 23.7 1.59 Cloaline 0.988 nf nf Cocaine 0.988 61.5 2.3 Codeline 1.047 11.0 0.6	Buspirone	1.729	77.3	nf
Carbamazepine 0.893 69.3 2.45 Carbinoxamine 0.923 76.1 2.6 Carbofuran 0.566 nf nf Carisoprodol 0.835 64.7 2.36 Chlordiazepoxide 1.092 68.8 2.44 Chlorpheniramine 0.897 95.0 3.38 Chlorpheniramine 1.09 100.0 3.4 Chlorpheniramine 1.09 100.0 3.4 Chlorpheniramine 1.09 100.0 3.4 Chlorpheniramine 0.94 2.0 0.4 Chlorpheniramine 1.042 97.8 3.74 Chloridine 0.91 2.0 0.4 Chloridine 1.042 97.8 3.74 Clomidine 0.931 23.7 1.59 Clorapine 1.298 93.1 3.23 Cocaethylene 0.988 nf nf nf Cocaine 1.047 11.0 0.6 1.0 0.6 1.0	Caffeine	0.838	0.7	-0.07
Carbinoxamine 0.923 76.1 2.6 Carbofuran 0.566 nf nf Carisoprodol 0.835 64.7 2.36 Chlordiazepoxide 1.092 68.8 2.44 Chlorpheniramine 0.897 95.0 3.38 Chlorpheniramine 1.09 100.0 3.4 Cimetidine nd 2.0 0.4 Citalopram 1.042 97.8 3.74 Clomidine 0.931 23.7 1.59 Clonidine 0.931 23.7 1.59 Clorapine 1.298 93.1 3.23 Cocaethylene 0.988 nf nf Cocaethylene 0.988 nf nf <td>Captopril</td> <td>nd</td> <td>1.7</td> <td>0.34</td>	Captopril	nd	1.7	0.34
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Citalopram 1.042 97.8 3.74 Clomipramine 1.046 99.9 5.2 Clonidine 0.931 23.7 1.59 Clozapine 1.298 93.1 3.23 Cocaethylene 0.988 nf nf Cocaine 0.988 61.5 2.3 Codeine 1.047 11.0 0.6 Colchicine nd 13.8 1.3 Codinine 0.770 nf nf Cotinine 0.770 nf nf Cyclobenzaprine 0.986 99.8 4.8 Cyproheptadine 1.033 99.7 3.2 Desipramine 0.986 99.8 1.4 Desmethyclomimpramine 1.058 nf nf Desmethyclomimpramine 1.058 nf nf Desmethyclomimpramine 1.058 nf nf Desmethyclomimpramine 1.058 nf nf Diltazepam 1.061 84.1 2.7<	Chlorpromazine	1.09	100.0	3.4
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Clozapine 1.298 93.1 3.23 Cocaethylene 0.988 nf nf Cocaine 0.968 61.5 2.3 Codeine 1.047 11.0 0.6 Colchicine nd 13.8 1.3 Cotinine 0.770 nf nf Cyclizine 0.906 88.2 2.97 Cyclobenzaprine 0.986 99.8 4.8 Cyproheptadine 1.033 99.7 3.2 Desipramine 0.988 99.8 1.4 Desipramine 0.988 99.8 1.4 Desmethyclomimpramine 1.058 nf nf Destromethorphan 0.986 nf nf Dijazepam 1.061 84.1 2.7 Ditliazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4	Clomipramine	1.046	99.9	5.2
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Cyproheptadine 1.033 99.7 3.2 Desipramine 0.988 99.8 1.4 Desmethyclomimpramine 1.058 nf nf Dextromethorphan 0.986 nf nf Diazepam 1.061 84.1 2.7 Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluxorazole nd 7.4 1 Fluxoxetine 0.830 98.9 4.05 Fluxoxamine nd 0.94 16.4 1.4 Hydrocodone 1.073 55.9 2.2	Cyclobenzaprine	0.986	99.8	4.8
Desipramine 0.988 99.8 1.4 Desmethyclomimpramine 1.058 nf nf Dextromethorphan 0.986 nf nf Diazepam 1.061 84.1 2.7 Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluintrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydromorphone nd nf -4		1.033	99.7	3.2
Desmethyclomimpramine 1.058 nf nf Dextromethorphan 0.986 nf nf Diazepam 1.061 84.1 2.7 Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4		0.988	99.8	1.4
Dextromethorphan 0.986 nf nf Diazepam 1.061 84.1 2.7 Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluoretine 0.830 98.9 4.05 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyzine 1.271 66.8 2.4 Ibup	-	1.058	nf	nf
Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Keta		0.986	nf	nf
Diltiazem 1.340 83.1 2.79 Diphenhydramine 0.837 93.7 3.3 Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluritrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Keta	Diazepam	1.061	84.1	2.7
Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluritrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidoca	·	1.340	83.1	2.79
Disopyramide 1.070 75.3 2.6 Doxepin 0.981 66.8 2.4 Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluritrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidoca	Diphenhydramine	0.837	93.7	3.3
Doxylamine 0.856 66.8 2.4 EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluconazole nd 7.4 1 Fluronazole nd 7.4 1 Fluroscetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4		1.070	75.3	2.6
EDDP nd nf nf Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Fluconazole nd 7.4 1 Fluritrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Doxepin	0.981	66.8	2.4
Etomidate 0.852 90.0 3 Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Doxylamine	0.856	66.8	2.4
Flecainide 0.962 98.0 3.8 Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	EDDP	nd	nf	nf
Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Etomidate	0.852	90.0	3
Fluconazole nd 7.4 1 Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4				
Flunitrazepam 1.155 47.9 2.1 Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Fluconazole	nd	7.4	1
Fluoxetine 0.830 98.9 4.05 Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4				2.1
Fluvoxamine nd 0.9 0.04 Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	-			4.05
Guaifenesin 0.744 16.4 1.4 Hydrocodone 1.073 55.9 2.2 Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Fluvoxamine	nd	0.9	0.04
Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Guaifenesin			
Hydromorphone nd nf -4 Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	Hydrocodone	1.073	55.9	2.2
Hydroxyamphetamine nd 13.8 1.3 Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4				
Hydroxyzine 1.271 66.8 2.4 Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	<u>'</u>	nd	13.8	1.3
Ibuprofen 0.665 98.7 4.0 Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4				
Imipramine 0.979 99.8 2.5 Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4	L			
Ketamine 0.841 91.0 3.1 Lidocaine 0.841 68.8 2.4				
Lidocaine 0.841 68.8 2.4	· ·			
Loxapine 1.124 97.0 3.6				

Compound	RRT	Twister Calc. [%]	log Ko/wr ²
Maprotaline	1.028	99.6	4.5
MDA	0.663	25.9	1.64
MDMA	0.689	nf	nf
Meclizine	1.401	100.0	5.9
Meg X	0.813	nf	nf
Meperidine	0.790	80.8	2.7
Mepivacaine	0.921	41.6	1.9
Meprobamate	nd	3.9	0.7
Mescaline	nd	4.6	0.8
Mesoridazine	nd	100.0	5.6
Metaxalone	0.954	nf	nf
Methadone	0.943	98.6	3.93
Methamphetamine	0.385	48.5	2.1
Methaqualone	0.962	99.4	4.3
Methylecgonine	0.668	nf	nf
Methylphenidate	0.783	1.3	0.2
Metoclopramide	1.019	76.9	2.6
Metoprolol	0.902	37.8	1.9
Midazolam	1.125	99.4	4.3
Mirtazapine	0.996	100.0	7.1
6-monoacetylmorphine	nd	nf	nf
Morphine	nd	5.8	-0.1
Nicotine	0.599	10.6	1.2
Nifedipine	nd	55.9	2.2
Norcitalopram	1.051	nf	nf
Nordiazepam	1.091	87.2	2.9
Norfluoxetine	0.822	nf	nf
Nordoxepin	0.987	nf	nf
Normeperidine	0.806	nf	nf
Norpropoxyphene I	1.028	nf	nf
Norpropoxyphene II	1.038	nf	nf
Norpropoxyphene III	1.043	nf	nf
Norpropoxyphene Amide	1.099	nf	nf
Nortriptyline	0.975	28.6	1.7
Norvenlafaxine	0.948	nf	nf
Norverapamil	1.576	nf	nf
Noscapine	nd	44.4	2.0
Olanzapine	1.199	nf	nf
Oxycodone	1.108	3.9	0.7
Papaverine	1.238	87.7	0.5
Paroxetine	1.118	98.6	3.95
PCP	0.859	99.8	4.7
Pentazocine	0.996	99.7	4.6
Phenmetrazine	0.651	20.2	1.5
Phentermine	0.493	38.9	1.9
Phenylephrine	nd	0.4	-0.3
Phenyltoloxamine	0.917	98.1	3.8
Pimozide	nd	100.0	6.3
PMA	0.611	nf	nf

Compound	RRT	Twister Calc. [%]	log Ko/wr ²
PMMA	0.638	nf	nf
PPA	nd	3.6	0.7
Procainamide	nd	5.7	0.9
Promazine	1.036	99.6	2.5
Promethazine	1.001	86.4	2.9
Propofol	0.505	98.0	3.79
Propoxyphene	0.846	99.2	4.2
Propranolol	0.952	96.0	1.2
Propylamphetamine	0.579	96.2	3.5
Protriptyline	0.993	99.8	4.9
Pseudoephedrine	nd	5.8	0.9
Quinidine	1.243	95.7	3.4
Quinine	1.240	95.7	3.4
Quetiapine	nd	1.6	nf
Ranitidine	nd	1.5	0.3
Salicylic Acid	nd	59.3	2.3
Scopolamine	nd	7.1	1.2
Sertraline	1.035	99.9	5.29

Compound	RRT	Twister Calc.	log Ko/wr ²
		[%]	
SKF-525A	1.000	nf	nf
Strychnine	1.876	40.5	1.9
Temazepam	nd	55.3	2.19
Theophylline	nd	0.8	0
Thioridazine	1.763	100.0	5.9
Tiletamine	0.765	nf	nf
Tramadol	0.876	89.1	3.01
Tranylcypromine	0.530	24.2	1.6
Trazodone	1.817	92.7	3.2
Trimethobenzamide	1.708	61.5	2.3
Trimethoprim	nd	6.1	0.91
Trimipramine	0.974	100.0	5.43
Venlafaxine	0.923	2.1	0.43
Verapamil	1.503	98.0	3.8
Zaleplon	1.372	nf	nf
Zolazepam	1.005	nf	nf
Zolpidem	1.323	98.3	3.85

nd=none detected, nf=not found

Postmortem blood samples from previously tested case specimens were extracted with the TwisterTM method and compared to the described liquid-liquid extraction normally used prior to GC/MS analysis (Table 3). TwisterTM extractions compared well with the liquid-liquid extractions followed by GC/MS analysis. The liquid-liquid extraction did not include a back extraction, and a significant cholesterol peak was noted in blood and tissue samples extracted by this method. Little, if any, cholesterol was detected in the Twister extractions (Figure 1).

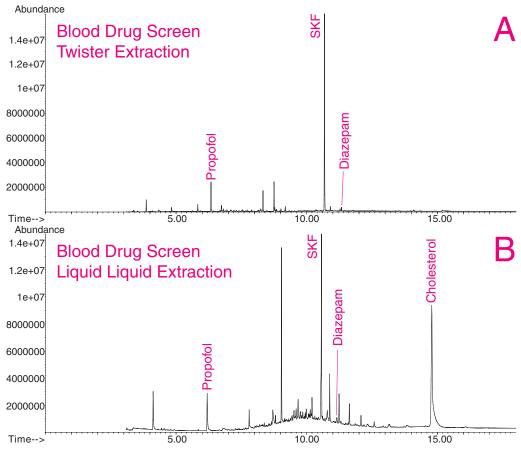


Figure 1. Comparison of the total ion chromatograms of TwisterTM (A) vs. Liquid-Liquid (B) Extraction of case sample. Whole blood sample was positive for Propofol at 0.5 mg/L and Diazepam at 0.08 mg/L.

Table 3. Comparison of Reported Case Findings and Liquid-Liquid Extraction vs. TwisterTM Extraction followed by GC/MS Analysis.

Sample	Reported Results (mg/L)	Liq-Liq GC/MS Results	Twister™ GC/MS Results
1	Phenobarbital (5.4)	Negative	Negative
2	Negative (Caffeine <0.1)	Negative	Negative
3	Fluoxetine (1.1) Caffeine (2.6)	Fluoxetine Caffeine	Fluoxetine Caffeine Nicotine
4	Methadone (0.75) Hydrocodone (<0.05) EDDP (<0.1) Acetaminophen (13)	Methadone	Methadone Hydrocodone Nicotine
5	Negative	Negative	Negative
6	Carisoprodal (4.3) Hydrocodone (0.79) Diazepam (0.26) Caffeine (3.4)	Carisoprodal Hydrocodone Diazepam Caffeine	Carisoprodal Hydrocodone Diazepam Caffeine
7	Venlafaxine (0.50) Diphenhydramine (0.17)	Venlafaxine Diphenhydramine	Venlafaxine Diphenhydramine
8	Citalopram (0.11)	Citalopram	Citalopram
9	Diazepam (1.9) Diphenhydramine (0.15) Chlorpheniramine (0.15) Hydrocodone (0.44) Dextromethorphan (<0.10) Acetaminophen (35)	Diazepam Diphenhydramine Chlorpheniramine Hydrocodone Dextromethorphan	Diazepam Diphenhydramine Chlorpheniramine Hydrocodone Dextromethorphan
10	Bupropion (0.26) Methadone (0.74) Caffeine (1.0)	Bupropion Methadone Caffeine	Bupropion Methadone Caffeine
11	Caffeine (6.8)	Caffeine	Caffeine
12	Lidocaine (2.2) Cocaine (0.2) Cocaethylene (<0.05)	Lidocaine Cocaine Cocaethylene	Lidocaine Cocaine Cocaethylene
13	Negative	Negative	Negative
14	Diazepam (0.28) Nordiazepam (0.66) Fentanyl (10µg/L) Temazepam (<0.05) Cocaine (<0.05)	Diazepam Nordiazepam	Diazepam Nordiazepam
15	Clozapine (12.0) Fluoxetine (1.5) Fentanyl (3 µg/L)	Clozapine Fluoxetine	Clozapine Fluoxetine
16	Amitriptyline (0.48) Alprazolam (56 µg/L)	Amitriptyline	Amitriptyline Nortriptyline
17	Propofol (0.50) Diazepam (0.08) Promethazine (0.17) Nordiazepam (0.11) Morphine (0.06) Hydrocodone (<0.05)	Propofol Diazepam Promethazine	Propofol Diazepam Promethazine Nordiazepam (tr)
18	Methadone (0.5) Caffeine (1.0)	Methadone Caffeine	Methadone Caffeine Nicotine
19	Diphenhydramine (0.14) Caffeine (2.6)	Diphenhydramine Caffeine	Diphenhydramine
20	Venlafaxine (0.73)	Venlafaxine	Venlafaxine Nicotine
21	Flecainide (9.6) Trazodone (1.0)	Flecainide Flecainide Artifact Trazodone Nicotine (tr)	Flecainide Flecainide Artifact Trazodone Nicotine

Sample	Reported Results (mg/L)	Liq-Liq GC/MS Results	Twister™ GC/MS Results
22	Chlorpheniramine (0.20) Hydrocodone (<0.05)	Chlorpheniramine	Chlorpheniramine
23	Citalopram (0.20)	Citalopram	Citalopram
24	Citalopram (1.2) Nordiazepam (4.7) Carbamazepine (7.8) Alprazolam (211µg/L)	Citalopram Nordiazepam Carbamazepine	Citalopram Nordiazepam Carbamazepine Nicotine
25	Amphetamine (0.10) Methamphetamine (0.68) Diazepam (0.10) Nordiazepam (0.09) Methadone (0.44) EDDP (<0.1) Benzoylecgonine (<0.05)	Methamphetamine Methadone Diazepam Nordiazepam	Methamphetamine Methadone Diazepam Nordiazepam (tr)
26	Alprazolam (27 µg/L) Methamphetamine (0.36) Diazepam (0.05) Nordiazepam (0.03) Amphetamine (<0.05)	Diazepam Methamphetamine	Diazepam Methamphetamine
27	Diazepam (0.11) Metaxalone (0.45) Nordiazepam (0.30) Fentanyl (21 µg/L) Promethazine (0.98)	Promethazine Promethazine Metabolite	Promethazine Promethazine Metabolite Norpropoxyphene (I, II, III, Amide)
28	MDMA (0.62) MDA (<0.10) Phencyclidine (< 25 μg/L) Methylecgonine (<0.05) Benzoylecgonine (0.12)	MDMA Phencyclidine	MDMA Phencyclidine Nicotine
29	Negative	Atropine	Negative
30	Amantadine (3.6) Citalopram (0.13)	Amantadine Citalopram	Amantadine Citalopram
31	Diazepam (0.26) Acetaminophen (31) Oxycodone (0.20) Oxymorphone (0.12) Acetaminophen (31)	Diazepam	Diazepam Methamphetamine Oxycodone (tr)
32	Negative	Negative	Negative
33	Citalopram (0.65) Fentanyl (8 µg/L) VPA (40) Cocaine (<0.05)	Citalopram	Citalopram Cocaine
34	Propoxyphene (0.88) Fentanyl (31 µg/L)	Propoxyphene Norpropoxyphene (I, II, III, Amide)	Propoxyphene Norpropoxyphene (I, II, III, Amide)
35	Dextromethorphan (0.19) Chlorpheniramine (0.46) Lorazepam (<10 μg/L) Hydrocodone (<0.05) Caffeine (4.5)	Dextromethorphan Chlorpheniramine Hydrocodone Caffeine	Dextromethorphan Chlorpheniramine Hydrocodone Caffeine
36	Diazepam (0.78) Nordiazepam (0.30) Oxycodone (0.22) Oxymorphone (<.05)	Diazepam Nordiazepam Caffeine	Diazepam Nordiazepam Oxycodone (tr) Nicotine
37	Cocaine (0.14) Methylecgonine (0.24) Benzoylecgonine (1.7)	Cocaine	Cocaine Nicotine
38	Cocaine (0.05) Cocaethylene (0.08) Benzoylecgonine (0.68) Methylecgonine (0.09)	Cocaine Cocaethylene	Cocaine Cocaethylene Nicotine
39	Venlafaxine (0.64)	Venlafaxine	Venlafaxine Nicotine
40	Amphetamine (0.05) Methamphetamine (0.22)	Methamphetamine (tr)	Methamphetamine

Sample	Reported Results (mg/L)	Liq-Liq GC/MS Results	Twister™ GC/MS Results
41	Diphenhydramine (0.34) Methylecgonine (0.62) Cocaine (0.07) Cocaethylene (0.05) Benzoylecgonine (2.3) Morphine (0.36)	Diphenhydramine Methylecgonine Cocaine Cocaethylene	Diphenhydramine Methylecgonine Cocaine Cocaethylene
42	Amitriptyline (0.61) Nortriptyline (0.59) Citalopram (0.46) Cyclobenzaprine (0.10) Temazepam (0.06) Oxycodone (0.12)	Amitriptyline Nortriptyline Citalopram Cyclobenzaprine	Amitriptyline Nortriptyline Citalopram Cyclobenzaprine Desmethylcyclo benzaprine
43	Carisoprodal (21) Metoprolol (0.9) Hydrocodone (0.6) Acetaminophen (47) Quetiapine (<0.5) Dihydrocodeine (0.05)	Carisoprodal Metoprolol Hydrocodone	Carisoprodal Metoprolol Hydrocodone
44	Venlafaxine (0.37)	Venlafaxine	Venlafaxine Dextromethorphan
45	Sertraline (0.14) Diphenhydramine (0.33) Acetaminophen (13)	Sertraline Diphenhydramine	Sertraline Diphenhydramine
46	Olanzapine (0.71) Morphine (0.14)	Olanzapine	Negative
47	Papaverine (not reported)	Papaverine	Papaverine
48	Diphenhydramine (0.22) Chlorpheniramine (0.48) Amitriptyline (1.4) Nortriptyline (2.1) Hydrocodone (<0.05) Oxycodone (0.13)	Diphenhydramine Chlorpheniramine Amitriptyline Nortriptyline	Diphenhydramine Chlorpheniramine Amitriptyline Nortriptyline
49	Methadone (0.47) Diazepam (0.24) Hydrocodone (0.09) Diltiazem (0.51) Zolpidem (0.13) Nordiazepam (<0.05) EDDP (<0.05) Hydrocodol (<0.05)	Methadone Diazepam Hydrocodone Diltiazem Zolpidem	Methadone Diazepam Hydrocodone
50	Fluoxetine (0.66) Venlafaxine (<0.1) Trazodone (0.89)	Fluoxetine	Fluoxetine Venlafaxine

⁽tr) = peak identified by ion search with poor chromatography but reasonable library match quality

In addition to blood samples, other sample matrices such as liver, kidney, brain, bile, urine, vitreous, and gastric contents, were successfully extracted and analyzed using the TwisterTM method (Figure 2).

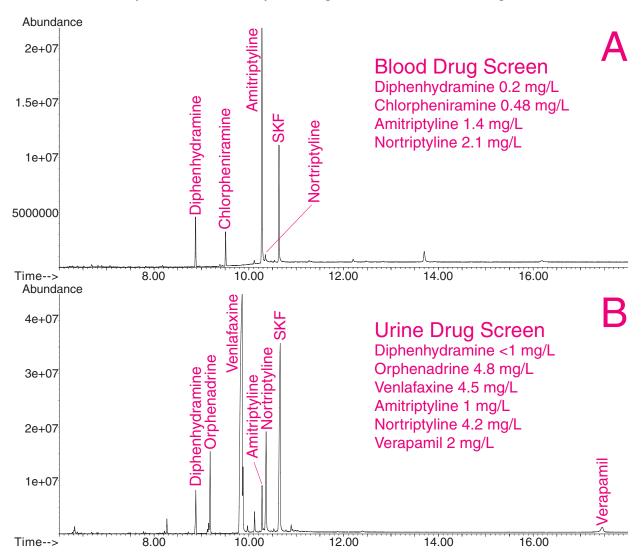


Figure 2. Total ion chromatograms of blood (A) and urine (B) after Twister extraction. Urine diluted 1:3 with deionized water.

Conclusions

The TwisterTM extraction method is a viable alternative to liquid-liquid or solid phase extractions for the screening of basic drugs in biological samples. This method:

- Simplifies the extraction compared to typical multi-step sample preparation.
- Eliminates the need for the equipment and the solvents usually needed for extractions.
- Twister stir bars are reusable and do not appear to carry over analytes from previous extractions once desorbed and reconditioned.

The use of the TwisterTM method for forensic samples would require that each TwisterTM bar be tested after reconditioning to document that it is negative for the analytes being tested.

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