Sains Malaysiana 42(2)(2013): 149–157

Application of Dispersive Liquid-liquid Microextraction Based on Solidification of Floating Organic Droplet to the Analysis of Antidepressant Drugs in Water Samples

(Aplikasi Pengekstrakan Cecair-cecair Serakan Berdasarkan Pemejalan Titisan Organik Terapung dalam Analisis Dadah Anti-Kemurungan di dalam Sampel Air)

MOHD. MARSIN SANAGI*, SITI UMAIRAH MOKHTAR, WAN AINI WAN IBRAHIM & HASSAN Y. ABOUL-ENEIN

ABSTRACT

A simple and rapid sample preparation method based on dispersive liquid-liquid microextraction-solidification of floating organic drop (DLLME-SFO) combined with gas chromatography-mass spectrometry (GC-MS) method was developed for the analysis of antidepressant drugs in water samples. This method uses organic solvent with low density and less toxicity. In the method, the disperser solvent (0.5 mL acetonitrile) containing 30 µL of n-hexadecane was rapidly injected using a syringe into 5.0 mL of water sample in a glass tube. After centrifugation for 7 min at 3,500 rpm, the mixture was cooled in ice bath for 5 min. The solidified n-hexadecane was transferred into a conical vial, where it melted rapidly at room temperature and 2 µL of it was injected into a gas chromatograph for analysis. Under optimized conditions, the method showed good linearity in the range of 0.04 - 0.12 µg mL^{-1} for amitriptyline and chlorpromazine with correlation of determination (r^2) in the range of 0.992 - 0.995. The limits of detections (LODs) were in the range 0.0085 - 0.0285 µg mL^{-1}. The extraction recoveries of amitriptyline and chlorpromazine from water samples at spiking level of 0.08 µg mL^{-1} were 71.34 - 73.52% and 73.83 - 91.09%, respectively, with relative standard deviations (RSDs) in the range of 4.97 - 6.85% for amitriptyline and 4.84 - 7.49% for chlorpromazine. The method was successfully applied to the determination of the analytes in drinking water, lake water and tap water samples.

Keywords: Antidepressant drugs; dispersive liquid-liquid microextraction-solidification of floating organic; gas chromatography-mass spectrometry; water samples

ABSTRAK

Satu kaedah penyediaan sampel yang ringkas dan cepat berdasarkan pengekstrakan mikro cecair-cecair penyerakan pemejalan titisan organik terapung (DLLME-SFO) bergabung dengan kromatografi gas-spektrometri jisim (GC-MS) telah dibangunkan untuk analisis dadah anti-kemurungan di dalam sampel air. Dalam kaedah ini, pelarut penyebar (0.5 mL asetonitril) yang mengandungi 30 µL n-heksadekana disuntik dengan cepat menggunakan picagari ke dalam 5.0 mL air dalam tiub kaca. Larutan diemparkan selama 7 min pada 3,500 rpm, tiub kaca direndam di dalam kukus ais untuk langkah penyejukan selama 5 min, pelarut pepejal n-heksadekana dipindahkan ke dalam tiub berbentuk kon dan ia akan melebur dengan cepat pada suhu bilik dan 2 µL cecair itu disuntik ke dalam kromatografi gas untuk dianalisis. Beberapa parameter DLLME-SFO dikenal pasti, termasuk jenis dan isi padu pelarut pengekstrakan dan pelarut penyebar, masa pengekstrakan dan kesan garam. Dalam keadaan optimum, kaedah ini menunjukkan kelinearan yang baik dalam julat 0.04 - 0.12 µg mL^{-1} untuk amitriptilina dan klorpromazina dengan kolerasi penentuan (r^2) dalam julat 0.992 - 0.995. Had pengesanan (LODs) adalah dalam julat 0.0085 - 0.0285 µg mL^{-1}. Keboleh-pulangan pengekstrakan untuk amitriptilina dan klorpromazina daripada sampel air pada tahap campuran 0.08 µg mL^{-1} adalah masing-masing 71.34 - 73.52% dan 73.83 - 91.09% dengan sisihan piawai relatif (RSDs) dalam julat 4.97 - 6.85% untuk amitriptilina dan 4.84 - 7.49% untuk klorpromazina. Kaedah ini berjaya diaplikasikan bagi pengesanan dadah anti kemurungan dalam sampel air minuman, air tasik dan air paip.

Kata kunci: Dadah anti-kemurungan; kromatografi gas-spektrometri jisim; pengekstrakan mikro cecair-cecair penyerakan pemejalan titisan organik terapung; sampel air

INTRODUCTION

A drug is any substance that when absorbed into the body of a living organism, alters normal bodily function. It is also a chemical substance used in the treatment, cure, prevention or diagnosis of disease or used to otherwise enhance physical or mental well-being. Pharmaceuticals are produced and used in great annual increasing volumes. This growth leads to a drastic fear about the effects of these compounds on the environment (Es'haghi 2009). Nowadays, certain pharmaceuticals are attracting attention

as a potentially new class of water pollutants. Such drugs as antibiotics, antidepressants, birth control pills, seizure medication, cancer treatments, pain killers, tranquilizers and cholesterol-lowering compounds have been detected in various water sources. They were found in trace amounts in sewage water, drinking water and also in the rivers downstream from the sewage treatment plants (Adams 2004). These drugs are synthetically produced, highly toxic chemicals that not only impact the health of human beings, but also potentially compromise the health of fish and creatures in our oceans.

Antidepressant drugs are medicines that relieve symptoms of depressive disorders. In the last few years, prescription of antidepressants has increased dramatically and these drugs are frequently encountered in emergency toxicology screening, drug-abuse testing and forensic medical examinations (Tatsuo et al. 2006). Depression is associated with an increased risk of suicidal thoughts, self-harm and suicide (Esrafili et al. 2007). The following are some other side effects that are known to be associated with this medicine such as dry mouth, drowsiness and drop in blood pressure when moving from a lying or sitting position to sitting or standing, causing dizziness and lightheadedness (postural hypotension).

Sample preparation plays an important role in the field of determination of pharmaceutical chemicals in various samples as a preconcentration step. The main aim of sample preparation is to clean up and concentrate the analytes of interest, while rendering them in a form that is compatible with the analytical system (Sobhi et al. 2007). Liquid–liquid extraction (LLE) has been a widely used and accepted sample preparation technique for the analysis of drugs. LPME is a type of microextraction that miniaturized LLE and it is a relatively recent technique. This type of extraction method as a novel sample preparation technique has attracted higher attention. Introduction of dispersive liquid-liquid microextraction (DLLME) has greatly contributed to meeting this objective, due to its simplicity, rapidity of operation and low consumption of solvents and reagents. Dispersive liquid–liquid microextraction (DLLME) was introduced by Assadi and co-workers in 2006 (Rezaee et al. 2006).

An alternative DLLME procedure named DLLME-SFO has been presented by Leong and Shang for organic compounds determination (Leong & Shang 2008). In this technique, the extractant with lower density than water, low toxicity and proper melting point near room temperature (in the range of 10-30°C) was used. The advantages of DLLME–SFO method are simplicity of operation, rapidity, low cost, high recovery, compatibility of the extraction solvent with the instruments analyses (Yamini et al. 2010). DLLME-SFO promises to have a wide application prospect in trace analysis area.

In this work, DLLME-SFO was used for the analysis of antidepressant drugs namely amitriptyline and chlorpromazine in water sample. The objectives of this study were to apply the method for the analysis of drugs in water samples and to validate the method by using different parameters such as type and volume of disperser solvent and extraction solvent, extraction time and salt addition.

Materials and Methods

Reagents and Samples

Antidepressant drug; amitriptyline (98%) and chlorpromazine were purchased from Sigma-Aldrich (St. Louis, USA). n-hexadecane purchased from Merck (Hohenbrunn, Germany) was used as extraction solvent while acetonitrile (HPLC grade) as disperser solvent was obtained from Caledone (Georgetown, Ont., Canada). Methanol (HPLC grade) was purchased from QRëC–Brightchem (Penang, Malaysia). The standard stock solutions of 1,000 ppm of the analytes were prepared in methanol and stored in freezer prior to use. The working standard solutions of lower concentrations were prepared by diluting standard stock solution with methanol. Each standard solution were prepared fresh every day in order to avoid any degradation. Tap, lake and drinking water were used as real samples in this work. Double-distilled deionized water of at least 18 MΩ was purified by Simplicity water purification system, Millipore (Molsheim, France).

Instrumentation

Experiments were carried out using an Agilent Technology 6870 N gas chromatography with split/splitless injector operated at 300°C in split mode (1 min), mass selective detector Agilent Technology 5973 inert (Palo Alto, CA, USA). A HP-5MS fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) used for the separation was obtained from Agilent Technology (Palo Alto, CA, USA). The column oven temperature was held at initial temperature of 220°C for 3 min. Then, it was raised to 270°C at 10°C min^{-1} and held at 270°C for 3 min. Total run time was 11 min. The carrier gas was helium (purity 99.9995%) supplied by Malaysian Oxygen (MOX) Sdn. Bhd. Malaysia; and it was further purified by passage through a helium gas purifier. A mass range of m/z 50-320 was scanned to confirm the retention time of analytes. GC-MS selected ion monitoring (SIM) mode was used for quantitation of target analytes with the following selected ions: amitriptyline (m/z 58 and 277) and chlorpromazine (m/z 58 and 318).

Dispersive Liquid Liquid Microextraction Based on Solidification of Floating Organic Droplet Method

An aqueous sample of double-distilled deionized water was placed in a screw cap glass test tube and spike with selected drug. Acetonitrile was used as the disperser solvent; it contained n-hexadecane for GC/MS as the extraction solvent. The acetonitrile-n-hexadecane mixture was rapidly injected into the sample solution with a 1 mL syringe. A cloudy solution, resulting from the dispersion

of fine n-hexadecane droplets in the aqueous solution was formed in the test tube. After centrifugation for 7 min at 3,500 rpm, the glass tube was transferred into a beaker containing crushed ice; the organic solvent solidified in 5 min. After 5 min, the solidified solvent was transferred to a conical vial; it melted quickly at room temperature and 2 μL was injected into the GC-MS for analysis.

Results and Discussion

Optimization of extraction conditions

In order to optimize the dispersive liquid-liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) of antidepressant drug from water samples, the analytical factors that potentially affect sample were studied. The parameters involved were the selection of extraction and disperser solvents, volume of extraction and disperser solvent, extraction time and salt addition.

Selection of extraction solvent

Selection of the extraction solvent is a key step in the optimization of DLLME conditions. The selected extraction solvent must satisfy several requirements. First, it should be immiscible with water, have low volatility, low density and be able to extract the desired analytes as well. Moreover, its peaks in the chromatogram must be well-separated from those of the analytes. Finally, it should have a melting point near the room temperature (in the range of 10-30°C). According to these considerations, several extracting solvents, including 1-undecanol and n-hexadecane were investigated (Table 1). Several extraction solvents with densities below 1 g mL^{-1}, low water solubilities and various polarities were initially considered for the DLLME-SFO of drugs. In addition, their chromatographic peaks could be easily distinguished from those of the selected drugs. In previous study (Asadollah et al. 2010), 1-undecanol was found to be the best extraction solvent. However, in this study, n-hexadecane resulted in the best extraction efficiency (Figure 1), and its chromatographic peaks could be easily distinguished from those of the other analytes. Consequently, n-hexadecane was used in subsequent experiments.

Selection of disperser solvent

Miscibility of the disperser solvent with extraction solvent and sample solution is one of the most important criteria for selection of disperser solvent. Thereby, acetone, acetonitrile and methanol were selected as candidates of disperser solvents. Acetonitrile was found to give the best efficiency (Figure 2). This may be due to the synergic effect of good compatibility of acetonitrile with aqueous solution and low distributive ratio of analytes in the mixed solution of acetonitrile and water (Mirzaei et al. 2011). Hence, acetonitrile was chosen as the dispersive solvent for the following experiments.

Effect of extraction solvent volume

The volume of the extraction solvent influences the extraction recovery and enrichment factor of the analytes. In order to examine the effect of extraction solvent

TABLE 1. Properties of extraction solvent for the DLLME-SFO method (Leong & Shang 2008)

Extraction solvent	Density (g mL^{-1})	Boiling point (°C)	Melting point (°C)
n-Hexadecane	0.77	287	18
1-Undecanol	0.83	243	13 - 15

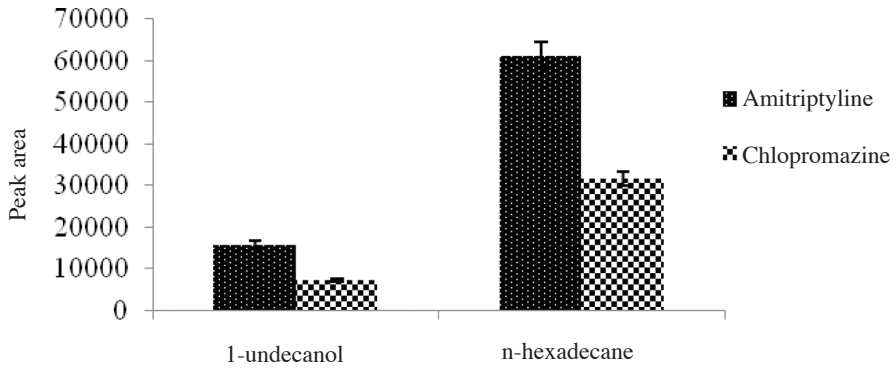

FIGURE 1. Effect of extraction solvent type on the DLLME-SFO technique. Samples spiked with 1 ppm of two drugs. Extraction conditions: aqueous sample volume 5 mL; extracted with each extraction solvent and 1.0 mL acetonitrile; centrifugation time: 7 min; salt addition (NaCl): 0.5 g

volume on the extraction efficiency, solutions containing different volumes of n-hexadecane with a fixed volume of disperser solvent were used with the same DLLME-SFO procedure. The experimental conditions were fixed, which included the use of 1.0 mL acetonitrile containing different volumes of n-hexadecane (20, 30 and 40 µL). Figure 3 shows peak area versus extraction solvent volume. It is clear that increasing the volume of n-hexadecane from 20 µL to 30 µL resulted in increased peak area. However, on further increasing the extraction solvent volume to 40 µL, the peak area was slightly decreased probably because of the dilution effect. Therefore, 30 µL was selected as the extraction solvent volume.

EFFECT OF DISPERSER SOLVENT VOLUME

In order to investigate the effect of disperser solvent volume on the extraction efficiency, various volumes of acetonitrile (0.5, 1.0 and 1.5 mL) containing 30 µL of extraction solvent (n-hexadecane) were tested (Figure 4). Increasing the volume of acetonitrile from 0.5 to 1.5 mL resulted in decreased peak area and extraction efficiency. This was probably due to the increased solubility of the analytes in water as the volume of acetonitrile was increased. On the other hand, decreasing the volume of acetonitrile to a value of much lower than 0.5 mL apparently decreased the extraction efficiency (results not shown) because the extraction of the floated phase was unable to be formed efficiently. It appeared that, with small volumes of acetonitrile, the cloudy state was not formed well; there may not be sufficient disperser solvent to disperse the extraction solvent in the sample solution, which would decrease the contact surface between the aqueous sample and extraction solvent. Thus, 0.5 mL of acetonitrile was chosen as the optimum volume of the disperser solvent.

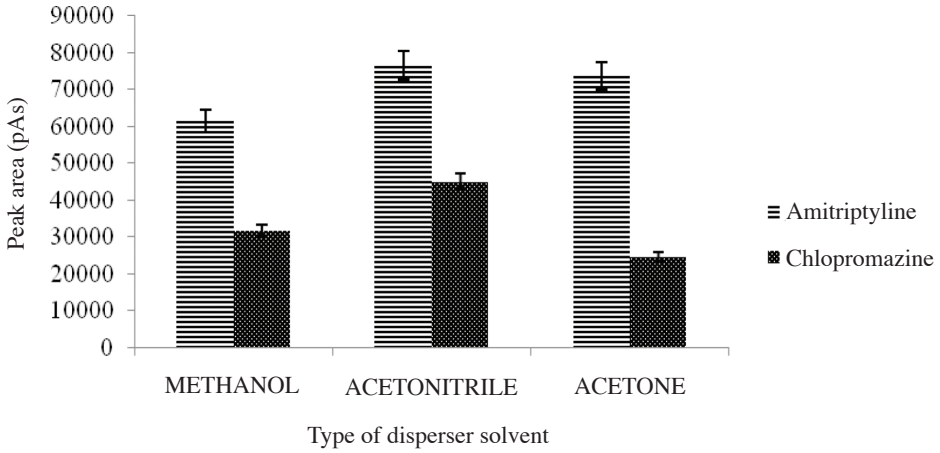

FIGURE 2. Effect of disperser solvent type on the DLLME-SFO technique. Samples spiked with 1 ppm of two drugs. Extraction conditions: aqueous sample volume, 5 mL; extracted with 20 µL n-hexadecane and 1.0 mL of each disperser solvent; centrifugation time: 7 min; salt addition (NaCl): 0.5 g

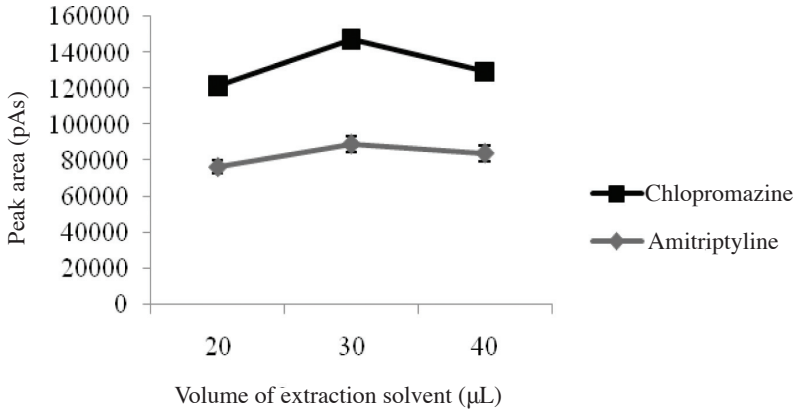

FIGURE 3. Effect of the volume of extraction solvent (n-hexadecane) on the DLLME-SFO technique. Samples spiked with 1 ppm of two drugs. Extraction conditions: aqueous sample volume 5 mL; extracted with different volumes of n-hexadecane and 1.0 mL acetonitrile; centrifugation time: 7 min; salt addition (NaCl): 0.5 g

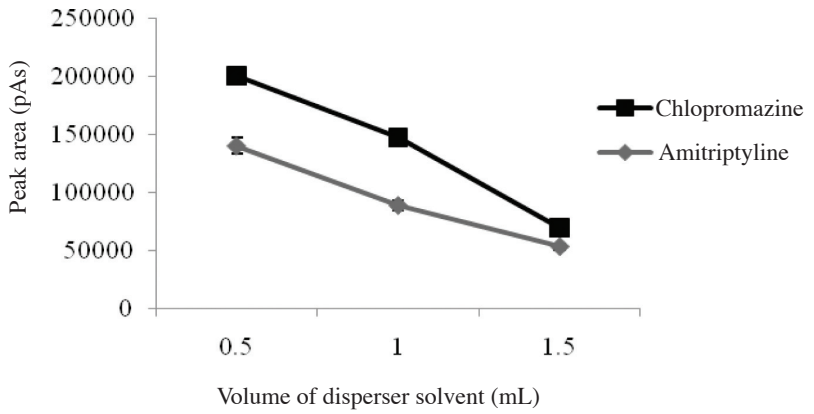

FIGURE 4. Effect of the volume of disperser solvent (acetonitrile) on the DLLME-SFO technique. Samples spiked with 1 ppm of two drugs. Extraction conditions: aqueous sample volume 5 mL; extracted with 30 μL of n-hexadecane and different volumes of acetonitrile; centrifugation time: 7 min; salt addition (NaCl): 0.5 g

EFFECT OF EXTRACTION TIME

The extraction time is usually an important factor in most extraction procedures. In the DLLME–SFO, the extraction time was defined as the interval elapsed between the addition of the mixture of extraction solvent and dispersive solvent to the sample and the time before centrifugation. An optimum extraction time is the minimum time necessary to achieve equilibrium between the aqueous and the organic phase so that the extraction of the analyte, the sensitivity and the speed of extraction are maximized. For the present study, the extraction time was varied in the range between 1 and 10 min under constant experimental conditions. The results indicated that the extraction time has no significant effect on the extraction efficiency. This was due to the fact that after formation of the cloudy solution, the surface area between the extraction solvent and aqueous phase was infinitely large and therefore, transition of the complex from the aqueous phase to the extraction solvent was fast. Subsequently, equilibrium state is achieved quickly after injection of the extraction solvent into the sample solution. This is one of the considerable advantages of the DLLME-SFO method.

EFFECT OF SALT ADDITION

The addition of salt into the sample solution sometimes can improve the extraction efficiency with increasing analytes polarity due to salting out effect. However, the presence of higher concentrations of salt could change the physical properties of the extraction film and thus reducing the diffusion rates of the analytes into the organic phase. Therefore, the amount of salt should be optimized in DLLME-SFO. The effect of the addition of salt on the extraction efficiency was studied by adding NaCl (0–20%, w/v) into the aqueous solution containing 1 ppm of each drug. The results obtained (Figure 5) showed that increasing the amount of salt resulted in increased peak area and enhanced the extraction efficiency of

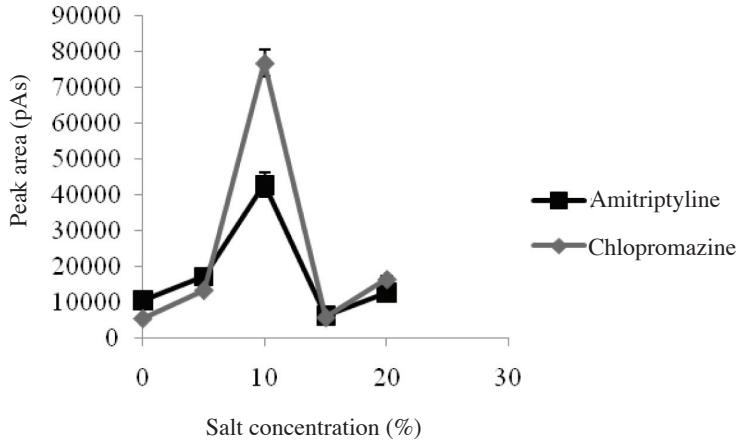

FIGURE 5. Effect of the salt addition on the DLLME-SFO technique. Samples spiked with 1 ppm of two drugs. Extraction conditions: aqueous sample volume 5 mL; extracted with 30 μL of n-hexadecane and 0.5 mL of acetonitrile; centrifugation time: 7 min; different percentage of salt (NaCl)

the extraction solvent in the aqueous phase. However, further addition of salt decreased the peak area while the extraction efficiency also decreased. Therefore, the salting out effect increases the enrichment factor up to 10% w/v of NaCl. Consequently, 0.5 g (10% w/v) NaCl was used in subsequent experiments.

METHOD VALIDATION

In order to validate the DLLME-SFO method, all optimized conditions were used for the extraction of the two selected antidepressant drug. The optimized conditions for DLLME-SFO of the selected drug were n-hexadecane as extraction solvent in 30 μL, 0.5 mL of acetonitrile as disperser solvent, 10% w/v salt addition and no extraction time counted in this work. These conditions were used in the quantitative analytical parameters validation. The quantitative results such as correlation of determination (r^2), the limit of detection (LOD) and limit of quantification (LOQ) of each drug are shown in Table 2.

REAL SAMPLE ANALYSIS

The developed extraction method (DLLME-SFO) was applied to the analysis of selected drinking water samples obtained commercially from local market and environmental water samples. The lake water samples were obtained from UTM Johor Bahru campus lake. The tap water samples were obtained from Analytical Chemistry Laboratory, Faculty of Science, UTM. Triplicate extractions were carried out for the water samples. Prior to DLLME-SFO, the samples were filtered through a Whatman 0.45 μm nylon membrane filter (Taiwan) to remove any solid particles.

The samples were spiked individually with amitriptyline and chlorpromazine at 80 ppb to determine the analyte recoveries of the method by adding known amounts of both analytes into the samples. By using EPA method, MRLs for amitriptyline and chlorpromazine are 18 ppb and 25 ppb, respectively (Kevin et al. 2010). Figures 6 - 8 show chromatograms for spiked and unspiked drinking water, tap water and lake water samples, respectively. Based on the unspiked sample chromatograms, it was found that chlorpromazine was detected in each sample while amitriptyline was detected only in lake water sample. In order to avoid any chances of cross contamination and carry-over, the samples were handled neatly by using three different syringes to inject the samples onto GC-MS. Each step was repeated triplicate for both unspiked and spiked samples.

The results obtained (Table 3) showed the concentration of amitriptyline and chlorpromazine for each samples. The

TABLE 2. Quantitative results of amitriptyline and chlorpromazine after DLLME-SFO by using optimum conditions

Analytes	Concentration range (μg mL^{-1})	Coefficient of determination (r^2)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Amitriptyline	0.04 – 0.12	0.992	0.0085	0.0067
Chlorpromazine	0.04 – 0.12	0.995	0.0285	0.0224

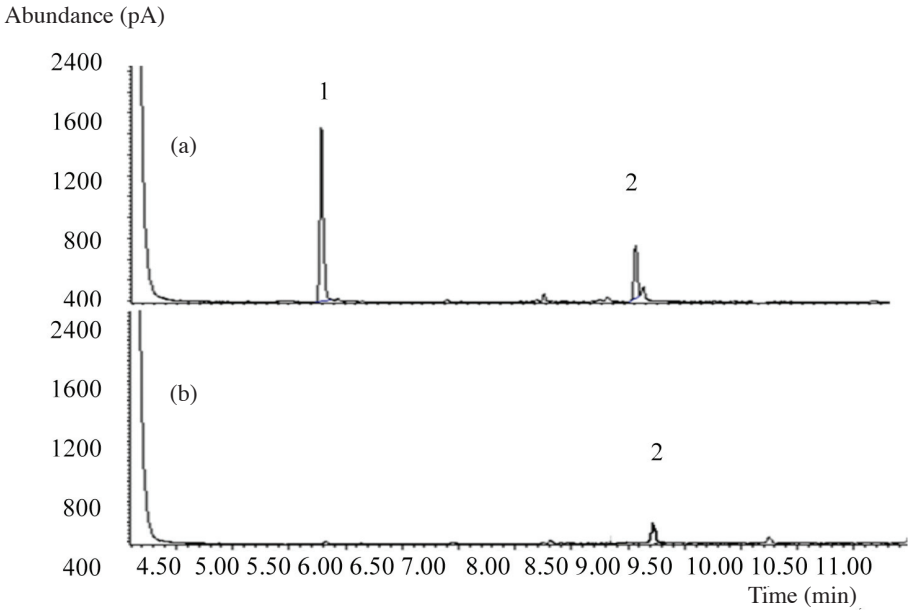

FIGURE 6. GC-MS (SIM mode) tracings of DLLME-SFO extract for (a) drinking water sample spiked with amitriptyline and chlorpromazine at 80 ppb and (b) unspiked drinking water. Peaks: 1. Amitriptyline and 2. Chlorpromazine

results for spiked water samples are shown in Table 4 with extraction recovery and standard deviation. The results showed that reasonably good analyte recoveries were obtained ranging from 71.34% to 73.52% for amitriptyline while 73.83% to 91.09% for chlorpromazine. As seen, the highest extraction recovery was shown by chlorpromazine in drinking water while the lowest extraction recovery was shown by amitriptyline in the same water. Extraction recovery (ER) was calculated from percentage of spiked sample concentration found divide by the concentration of standard drugs spiked. The RSDs were less than 10% for n=3 which proved the excellent repeatability of the analysis and chromatographic method with mass spectrometry.

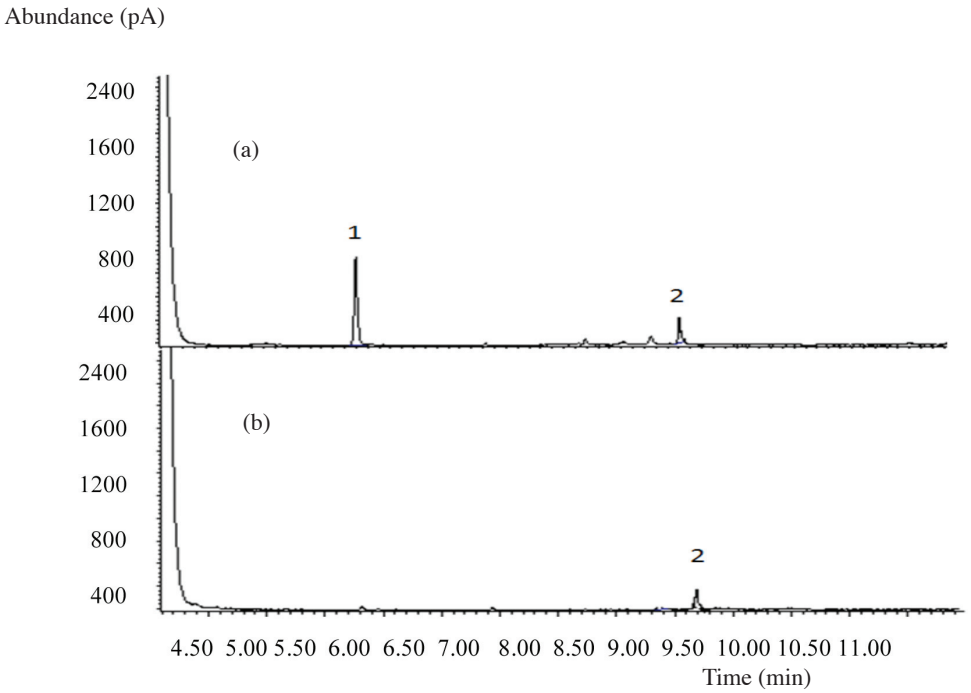

FIGURE 7. GC-MS (SIM mode) tracings of DLLME-SFO extract for (a) tap water sample spiked with amitriptyline and chlorpromazine at 80 ppb and (b) unspiked tap water. Peaks: 1. Amitriptyline and 2. Chlorpromazine

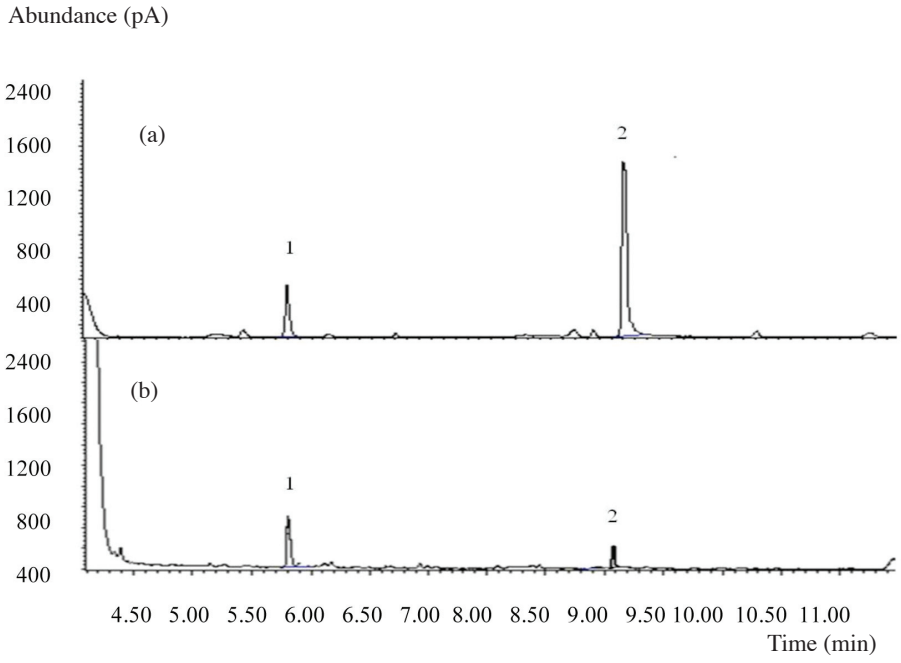

FIGURE 8. GC-MS (SIM mode) tracings of DLLME-SFO extract for (a) lake water sample spiked with amitriptyline and chlorpromazine at 80 ppb and (b) unspiked lake water. Peaks: 1. Amitriptyline 2. Chlorpromazine

TABLE 3. Concentration of amitriptyline and chlorpromazine detected after DLLME-SFO for unspiked and spiked sample at 80 ppb (n=3)

Analyte	Concentration in unspiked sample (ppb)			Concentration in spiked sample (ppb)		
	Drinking water	Tap water	Lake water	Drinking water	Tap water	Lake water
Amitriptyline	-	-	32.11 ±2.88	57.07 ±1.84	58.00 ±1.39	58.82 ±1.62
Chlorpromazine	38.33 ±2.24	32.38 ±2.69	37.03 ±3.48	72.87 ±3.44	59.06 ±1.55	67.05 ±2.39

TABLE 4. The extraction recovery (ER) and standard deviation of tap, drinking and lake water samples spiked with amitriptyline and chlorpromazine at 80 ppb

Analytes	Tap water (n=3)		Drinking water (n=3)		Lake water (n=3)	
	ER (%)	RSD	ER (%)	RSD	ER (%)	RSD
Amitriptyline	72.50 ±1.74	4.97	71.34 ±2.31	6.85	73.52 ±2.03	5.68
Chlorpromazine	73.83 ±1.94	4.84	91.09 ±4.29	7.49	83.81 ±2.99	5.99

CONCLUSION

Dispersive liquid-liquid microextraction based on solidification of floating organic droplets (DLLME-SFO) method coupled with GC-MS was successfully developed for the determination of antidepressant drugs in water samples. Excellent separations of analytes, namely, amitriptyline and chlorpromazine were achieved in less than 10 min. The developed method provides simple, rapid, low toxic, good repeatability and good analyte recovery. The limits of detection (LODs) were between 0.0085 and 0.0285 μg mL^{-1} while limits of quantification (LOQ) were between 0.0067 and 0.0224 μg mL^{-1}. Acceptable extraction recoveries were obtained in the range of 71.34% to 73.52% for amitriptyline with RSDs in the range of 4.97 to 5.68%. The extraction recoveries for chlorpromazine were in the range of 73.83% to 91.09% with RSD values in the range of 4.84 to 7.49%. Thus, from this work, it can be concluded that the developed method can be applied for the determination of antidepressant drugs in real water samples: drinking water, lake water and also tap water even at low ppb levels.

ACKNOWLEDGEMENT

The authors are grateful to Universiti Teknologi Malaysia for the facilitations and the Ministry of Higher Education (MOHE) Malaysia for financial support through Research University Research Grant vote number Q.J130000.2526-02H29 and Q.J130000.2426.00G04.

REFERENCES

Adams, M. 2004. Antidepressant drugs found in drinking water; pharmaceuticals have now become environmental pollutants. Accessed on line at: http://www.naturalnews.com/001891.html (27 July 2012).

Asadollah, T., Dadfarni, S. & Shabani, A.M.H. 2010. Separation/preconcentration and determination of vanadium with dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME-SFO) and electrothermal atomic absorption spectrometry. *Talanta* 82: 208-212.

Es'haghi, Z. 2009. Determination of widely used non-steroidal anti-inflammatory drugs in water samples by *in situ* derivatization, continuous hollow fiber liquid-phase microextraction and gas chromatography flame ionization detector. *Analytica Chimica Acta* 64: 83-88.

Esrafili, A., Yamini, Y. & Shariati, S. 2007. Hollow fiber-based liquid phase microextraction combined with high-performance liquid chromatography for extraction and determination of some antidepressant drugs in biological fluids. *Analytica Chimica Acta* 604: 127-133.

Kevin, J.B., Jim, T.Y., Mehmet, C., Edward, J.B. & Roberts, L. 2010. Trace determination of pharmaceuticals and other wastewater-derived micropollutants by solid phase extraction and gas chromatography/mass spectrometry. *Journal of Chromatography A* 1217: 558-564.

Leong, M.I. & Shang, D.H. 2008. Dispersive liquid–liquid microextraction method based on solidification of floating organic drop combined with gas chromatography with electron capture or mass spectrometry detection. *Journal of Chromatography A* 1211: 8-12.

Mirzaei, M., Behzadi, M., Abadi, N.M. & Beizaei, A. 2011. Simultaneous separation preconcentration of ultra trace heavy metals in industrial wastewaters by dispersive liquid–liquid microextraction based on solidification of floating organic drop prior to determination by graphite furnace atomic absorption spectrometry. *Journal of Hazardous Material* 186: 1739-1743.

Rezaee, M., Assadi, Y., Hosseini, M.R.M., Aghaee, E., Ahmadi, F. & Berijani, S. 2006. Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A* 1116: 1-9.

Sobhi, H.R., Yamini, Y. & Abadi, R.H.H.B. 2007. Extraction and determination of trace amounts of chlorpromazine in biological fluids using hollow fiber liquid phase microextraction followed by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* 45: 769-774.

Tatsuo, S., Masaru, T. & Einosuke, T. 2006. Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Science International* 162: 108-112.

Yamini, Y., Rezaee, M., Khanchi, A., Faraji, M. & Saleh, A. 2010. Dispersive liquid–liquid microextraction based on the solidification of floating organic drop followed by inductively coupled plasma-optical emission spectrometry as a fast technique for the simultaneous determination of heavy metals. *Journal of Chromatography A* 1217: 2358-2364.

Mohd Marsin Sanagi* & Wan Aini Wan Ibrahim
Ibnu Sina Institute for Fundamental Science Studies
Universiti Teknologi Malaysia
81310 Johor Bahru
Malaysia

Mohd Marsin Sanagi*, Siti Umairah Mokhtar
& Wan Aini Wan Ibrahim
Department of Chemistry
Faculty of Science
Universiti Teknologi Malaysia
81310 Johor Bahru
Malaysia

Hassan Y. Aboul-Enein
Department of Pharmaceutical and Medicinal Chemistry
National Research Centre
Dokki, 12311, Cairo
Egypt

*Corresponding author; email: marsin@kimia.fs.utm.my

Received: 11 May 2012
Accepted: 13 August 2012