APPLICATION OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROPLET IN THE ANALYSIS OF ANTI-DEPRESSANT DRUGS

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ABSTRACT

A new simple and rapid sample preparation method based on dispersive liquid-liquid microextraction-solidification of floating organic drop (DLLME-SFO) combined with gas chromatograph-mass spectrometry (GC-MS) has been developed for the extraction and analysis of anti-depressant drugs in water samples. The DLLME-SFO method uses organic solvent with low density and less toxicity. In the method, the disperser solvent (0.5 mL acetonitrile) containing 30 μL n-hexadecane was rapidly injected by a syringe into the 5.0 mL water in a glass tube. Upon centrifugation for 7 min at 3500 rpm, the glass tube was transferred into a beaker containing crushed ice for cooling step. After 5 min, the solidified n-hexadecane solvent was transferred into a conical vial, where its melts quickly at room temperature and 2 μL of it is injected into a gas chromatograph for analysis. Several DLLME-SFO parameters were optimized, including the type and volume of the extraction solvent and disperser solvent, extraction time and salt effect. Under optimum conditions, the method showed good linearity in the range of 0.04 to 0.12 μg/mL for amitriptyline and chlorpromazine, with correlation of determination (r^2) in the range of 0.992 to 0.995. The limits of detections (LODs) were in the range 0.0085 to 0.0285 μg/mL. The extraction recoveries of amitriptyline and chlorpromazine from water samples at spiking level of 0.08 μg/mL were 71.34 to 73.52% and 73.83 to 91.09%, respectively. The relative standard deviations (RSDs) were in the range of 4.97 to 6.85% for amitriptyline and 4.84 to 7.49% for chlorpromazine. The method was successfully applied to the determination of anti-depressant drugs in drinking water, lake water and tap water samples.

ABSTRAK

Satu kaedah baru 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 pengekstrakan dan analisis dadah anti-kemurungan dalam sampel air. Kaedah DLLME-SFO menggunakan pelarut organik dengan ketumpatan yang rendah dan kurang toksik. 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. Selepas diemparkan selama 7 min pada 3500 rpm, tiub kaca dipindahkan ke dalam bikar mengandungi ais untuk langkah penyejukan. Selepas 5 min, pelarut pepejal n-heksadekana dipindahkan ke dalam tiub berbentuk kon, di mana ia akan melebur dengan cepat pada suhu bilik and 2 μL cecair itu disuntik ke dalam kromatografi gas untuk dianalisis. Beberapa parameter DLLME-SFO dikenalpasti, termasuk jenis dan isipadu pelarut pengekstrakan dan pelarut penyebar, masa pengekstrakan dan kesan garam. Dalam keadaan optimum, kaedah ini menunjukkan kelinearan yang baik dalam julat 0.04 ke 0.12 μg/mL untuk amitriptilina dan klorpromazina, dengan kolerasi penentuan (r^2) dalam julat 0.992 ke 0.995. Had pengesanan (LODs) adalah dalam julat 0.0085 ke 0.0285 μg/mL. Keboleh-pulangan pengekstrakan untuk amitriptilina dan klorpromazina dari sampel air pada tahap campuran 0.08 μg/mL adalah masing-masing 71.34 ke 73.52% dan 73.83 ke 91.09%. Sisihan piawai relatif (RSDs) adalah dalam julat 4.97 ke 6.85% untuk amitriptilina dan 4.84 ke 7.49% untuk klorpromazina. Kaedah ini berjaya diaplikasikan bagi pengesanan dadah anti kemurungan dalam sampel air minuman, air tasik dan air paip.

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LIST OF ABBREVIATIONS

AAS	-	Atomic Absorption Spectroscopy
CNS	-	Central Nervous System
DAD	-	Diode Array Detector
DLLME	-	Dispersive Liquid-Liquid Microextraction
DLLME-SFO	-	Dispersive liquid liquid microextraction based on solidification of floating organic droplet
DSPE	-	Dispersive solid phase extraction
ECD	-	Electron Capture Detection
ER	-	Extraction recovery
ETAAS	-	Electrothermal Atomic Absorption Spectrometry
FAAS	-	Flame Atomic Absorption Spectrometry
GC	-	Gas Chromatography
GC-ECD	-	Gas Chromatography-Electron Capture Detector
GC-FID	-	Gas Chromatography- Flame Ionization Detector
GC-MS	-	Gas Chromatography-Mass Spectrometry
GFAAS	-	Graphite Furnace Atomic Absorption Spectroscopy
g	-	Gram
HF-LPME	-	Hollow Fibre - Liquid Phase Microextraction
HPLC	-	High Performance Liquid Chromatography
ICP-OES	-	Inductive Couple Plasma-Optical Emission Spectrometry
ID	-	Internal Diameter
LC	-	Liquid Chromatography
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
LLE	-	Liquid Liquid Extraction
LPME	-	Liquid Phase Microextraction

MAE	-	Microwave Assited Extraction
MS	-	Mass Spectrometer
Min	-	Minute
MOX	-	Malaysian Oxygen
NaCl	-	Sodium Chloride
NP	-	Nonylphenol
NSAIDs	-	Non-steroidal anti inflammatory drugs
OP	-	Octylphenol
OPPs	-	Organophosphorus pesticides
OCPs	-	Organochlorine pesticides
PCBs	-	Polychlorinated biphenyls
PHE	-	Phenothiazine
ppb	-	Part Per Billion
ppm	-	Part Per Million
RSD	-	Relative Standard Deviation
rpm	-	Rotation per Minute
r^2	-	Correlation of Determination
SD	-	Standard Deviation
SFE	-	Supercritical Fluid Extraction
SIM	-	Selected ion monitoring
SPE	-	Solid Phase Extraction
SPME	-	Solid Phase Microextraction
SDME	-	Single Drop Microextraction
TCA	-	Tricyclic anti-depressants
UHPLC	-	Ultra High Performance Liquid Chromatography
UTM	-	Universiti Teknologi Malaysia
UV	-	Ultra-violet
µm	-	Micrometer
µL	-	Microlitre
mL	-	Mililitre

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

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 [1]. Furthermore, pharmaceutical drugs are now being found in drinking water. A study was conducted in England that looked into 12 pharmaceuticals thought to pose an environmental threat, including painkillers, antibiotics, and antidepressants, and it found traces of these pharmaceuticals in both sewage waters and drinking water [2]. The study also found traces in the rivers downstream from the sewage treatment plants.

According to the latest reports in United States, there are a lot of things such as medicines antibiotics, sex hormones and many others that are mixed in the drinking water. This water is damaging the lives of the people and in many cases deaths have also occurred due to this poisoned tap water. Recently, government of United States announced to the people to apply immediate solutions to these problems.

1.2 Statement of Problem

Poisoned tap water, lake water and drinking water by pharmaceutical drugs have become the most searched topic on the internet and this calamity is being increased day by day which has to be dealt with immediate care. Anti-depressant 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. Exposure to this pharmaceutical drug in drinking water poses a hazard especially for pregnant women and their fetuses. This also could be one of the reasons why ocean life is continuing to decline around the world, since anti-depressant drugs such as amitriptyline and chlorpromazine have been blamed for altering sperm levels and spawning patterns in marine life. Sadly, we are increasingly living in a world that is increasingly polluted not only by heavy metals, polychlorinated biphenyls and emissions from gasoline engines but even our water is polluted with pharmaceutical chemicals.

In order to resolve the problems related to drug-contaminated water, it is important to determine and monitor the concentrations of these compounds in water. In recently published study, at least one pharmaceutical was detected in tests of treated water samples for 24 major metropolitan areas even in low level. The result of the study also indicates some drugs cause cellular effects at scant concentration that strangely cannot be seen at higher levels. Thus, it is necessary to develop new methods for extraction, separation and determination of drugs in water samples.

1.3 Objectives of study

This study embarks on three objectives. The first objective is to develop dispersive liquid liquid microextraction with solidification of floating organic method for the analysis of drug. The second objective is to apply the method for the analysis of drugs (amitriptyline and chlorpromazine) in water samples. The last

objective is to validate the method by using different parameters such as type and volume of disperser solvent and extraction solvent, extraction time and salt addition.

1.4 Significance of Study

There is an increasing need of knowledge about the presence of pharmaceuticals in the environment in many countries. This study will contribute significantly to the enhancement of this knowledge. This research also introduces a rapid and sensitive method for determination of pharmaceutical drugs in water samples. This method is both simple and inexpensive and thus could be used even in the less developed areas.

1.5 Scope of the Study

This research introduces a relatively new method, namely, dispersive liquid liquid microextraction with solidification of floating organic (DLLME-SFO) for determination of pharmaceutical drugs in water samples. Two antidepressant drugs namely amitriptyline and chlorpromazine were selected drugs in this research. The extraction method was combined with gas chromatography (GC) coupled with mass spectrometry (MS). Acetonitrile was used as a disperser solvent while n-hexadecane was used as extraction solvent. Several parameters involved were investigated including type and volume of disperser solvent and extraction solvent, extraction time and salt addition.

CHAPTER II

LITERATURE REVIEW

2.1 Anti-Depressants Drug

Depression, a common mental disorder, is a chronic or recurrent illness that affects both economic and social functions of patients and can eventually lead to suicidal behavior [3]. 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 [4]. Depression is one of the most frequent of all major psychiatric illnesses. Clinically significant depressive symptoms are detectable in approximately 12–36% of geriatric patients with another nonpsychiatric general medical condition. The prevalence of major depression ranges from 10 to 27% in stroke patients, from 40 to 65% in victims of myocardial infarction, from 30 to 40% in patients with Alzheimer's disease, and from 20 to 25% in cancer patients [5].

The first agents to be used against depression and related disorders were the tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors; when compared with monoamine oxidase inhibitors, TCAs have the advantage of not interacting with monoamines present in food with potentially fatal effects. TCAs are still widely used in the psychiatric practice, due to their high activity: in several cases they seem to have higher efficacy than newer antidepressants, especially against the most severe forms of depression and in children and adolescents.

TCAs are relatively much cheap and their pharmacological and toxicological profiles are well known. They can be advantageous for patients who are non-responders to other classes of antidepressants. Most TCAs share similar pharmacokinetic and pharmacodynamic profile and their toxic effects, such as hypotension, tachycardia, arrhythmia and seizures, can be particularly dangerous and life-threatening. This is particularly worrisome since many depressed patients attempt suicide by drug overdose [4]. Moreover, in the clinical setting severely depressed patients are often treated with two or more TCAs, or with TCAs and other antidepressants. Of course, this kind of therapeutic regimen can often cause toxicity or pharmacological interactions [5].

2.1.1 Amitriptyline

Amitriptyline is a tricyclic antidepressant (TCA). As well as reducing depressive symptoms, these type of tricyclic also aid migraines, tension headaches, anxiety attacks and some schizophrenic symptoms. It is believed that in some patients with depression, abnormal levels of neurotransmitters (chemicals that nerves use to communicate with each other) may relate to their depression. Amitriptyline elevates mood by raising the level of neurotransmitters in nerves of the brain.

Depression is associated with an increased risk of suicidal thoughts, self-harm, and suicide [3]. A small number of children, teenagers, and young adults (up to 24 years of age) who took antidepressants ('mood elevators') such as amitriptyline during clinical studies became suicidal (thinking about harming or killing oneself or planning or trying to do so). Children, teenagers, and young adults who take antidepressants to treat depression or other mental illnesses may be more likely to become suicidal than children, teenagers, and young adults who do not take antidepressants to treat these conditions. However, experts are not sure about how great this risk is and how much it should be considered in deciding whether a child or teenager should take an antidepressant. Children younger than 18 years of age should not normally take amitriptyline, but in some cases, a doctor may decide that amitriptyline is the best medication to treat a child's condition.

Medicines and their possible side effects can affect individual people in different ways. The following are some other side effects that are known to be associated with this medicine such as dry mouth, drowsiness, blurred vision, constipation, nausea, difficulty in passing urine and drop in blood pressure when moving from a lying or sitting position to sitting or standing, causing dizziness and lightheadedness (postural hypotension). The therapeutic index for this drug is in the range of 80-200 ng/mL. Figure 2.1 shows the structure of amitriptyline while physical properties of this drug are shown in Table 2.1.

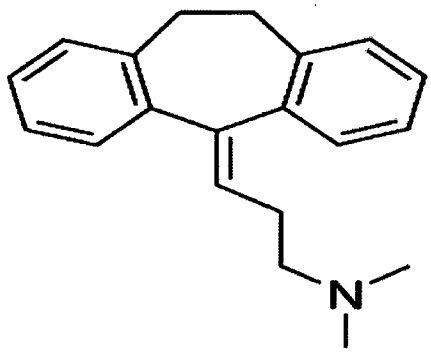

Figure 2.1: Structure of amitriptyline

Table 2.1: Physical properties of amitriptyline

Physical Properties	Info
Molecular formula	$C_{20}H_{23}N$
Molar mass	277.403 g/mol
Log $K_{o/w}$	4.9
Solubility in water	9.7 mg/L

2.1.2 Chlorpromazine

Chlorpromazine is one of the antipsychotic drugs in phenothiazine (PHE) derivatives, was discovered in the early 1950s. It is extensively used as a sedative hypnotic in veterinary application. However, improper and over usage of chlorpromazine poses a serious threat to human health and animal production. In

general, antipsychotics are administered in oral doses of only a few milligrams per day and they are extensively metabolized in the body [6]. Due to the difficulty in monitoring trace level of chlorpromazine residues in complicated biological matrices, specific adsorption materials for preconcentration and clean-up of chlorpromazine are indispensable for sample preparation before further determination [7].

Chlorpromazine controls excitement, agitation and other psychomotor disturbances in schizophrenic patients and reduces the manic phase of manic-depressive conditions. It is used to control hyperkinetic states and aggression and is sometimes given in other psychiatric conditions for the control of anxiety and tension. Their action mechanism is based on the blockade of nervous impulses from the central nervous system (CNS), because phenothiazine drugs are antagonists of dopamine receptors [8].

Chlorpromazine and other phenothiazines may cause many side effects. There are several common side effects such as decreases of blood pressure, especially on arising, which may cause dizziness or fainting, rapid heart rate, abnormal liver tests and blurred vision. The therapeutic index for chlorpromazine is in the range of 20-500 ng/mL. Figure 2.2 shows the structure of chlorpromazine and Table 2.2 are several physical properties of this drug.

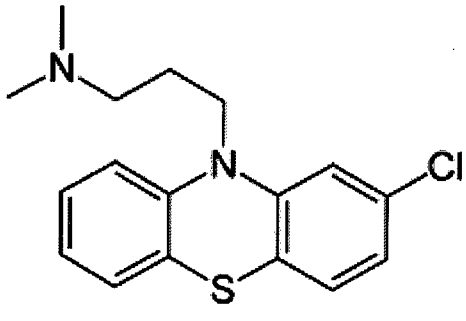

Figure 2.2: Structure of chlorpromazine

Table 2.2: Physical properties of chlorpromazine

Physical Properties	Info
Molecular formula	$C_{17}H_{19}ClN_2S$
Molar mass	318.86 g/mol
Log K $_{o/w}$	5.35
Solubity in water	50 mg/mL

2.2 Extraction Method

The analytical procedure usually comprises five steps: sampling, sample preparation, separation, detection, and data analysis. Although, each step is involved in obtaining correct results, sampling and sample preparation are the most important of the analytical process. Majority of the analysis time is spent on these two steps. It is also important to keep in mind that all five of these analytical steps are consecutive, and the next step cannot begin until the preceding one has been completed. Performance of the procedure would be poor and errors would be introduced if one of these steps is not followed properly [9].

Moreover, 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 [10]. For organic trace analysis, this step mainly comprises extractions, which serve to isolate compounds of interest from a sample matrix. The ultimate purpose, the concentration of target compounds is enhanced (enrichment) and the presence of matrix components is reduced (sample clean up). The analysis of trace level analytes involves the discreet steps of extraction, concentration and clean-up prior to detection by an instrument.

Traditional methods for sample preparation including liquid-liquid extraction, Soxhlet extraction, chromatography, distillation, and absorption, usually suffer from

the disadvantages of time-consuming and tedium, large amounts of toxic organic solvent to be used, and difficulty in automation to some extent. Therefore, a lot of research efforts in separation science and related fields have been focused on the development of new sample preparation techniques, which are less time -consuming, more effective, and require smaller amounts of organic solvents.

Liquid–liquid extraction (LLE) has been a widely used and accepted sample preparation technique for the analysis of drugs. Although LLE offers high reproducibility and high sample capacity, it is a time-consuming and labour-intensive procedure with a tendency for emulsion formation and with a poor potential for automation. In addition, LLE requires large amounts of high-purity solvents which are expensive and toxic which resulting in the production of hazardous laboratory wastes [10].

Solid phase extraction (SPE) becomes the most common sample preparation technique in environmental areas and has gradually replaced classical liquid–liquid extraction (LLE). SPE has several advantages over LLE such as higher recoveries, improved selectivity, specificity and reproducibility, less organic solvent usage and shorter sample preparation time [9]. There were various studies on the pharmaceutical compound by SPE such as study on non-steroidal anti inflammatory drugs (NSAIDs) in waste water sample [12, 13]. Other than that, there was a study on drugs by using SPE with ultra high performance liquid chromatography (UHPLC) [14]. Since SPE column required pretreatment, it can be relatively expensive although use less solvent than LLE.

Solid phase microextraction (SPME) is a modern sampling or sample-preparation method used for isolating and pre-concentrating organic molecules from gaseous, liquid and solid samples. It is highly sensitive and can be used for polar and non-polar analytes with different types of matrix. The mechanism of SPME is similar to that of SPE because SPME is a miniature version of SPE, the only difference being the volume of sorbent. An SPME–LC–UV method for the determination of naproxen was developed for the first time [15]. The procedure requires simple sample pre-treatment and allows an easy quantification of naproxen within its typical

urinary concentration. SPME has become prominent as a sample-preparation technique for analyzing pharmaceuticals from environmental samples.

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 and has attracted increased attention. Normally, it is carried out using a membrane as an interface between the sample (donor) and the organic solvent (acceptor), as that avoids mixing the two phases and other problems encountered in classical LLE. There are different operating modes in LPME such as single drop microextraction (SDME), hollow fiber LPME (HF-LPME), dispersive liquid liquid microextraction (DLLME) and so on. In SDME, the microdrop is suspended on the microsyringe needle, through which the analyte can be extracted from the water sample. The advantages of this technique is cheap and has a minimal exposure to organic solvents but the major disadvantages are that a small organic solvent drop held at the tip of a needle is unstable, and may be dislodged during extraction. In addition, the technique is not suitable for dirty samples. SDME also involves a tedious manual operation, and the precision of this method is not so good [16].

Hollow-fiber LPME is an alternative to LPME based on a porous polypropylene hollow fiber, which is placed in an aqueous sample. HF-LPME utilizes a porous hollow fiber containing suitable organic solvents inside its pores to extract the analytes from aqueous samples. The problem of this method is the long analysis time [16]. The HF-LPME method was successfully developed for the extraction and analysis of trace amounts of chlorpromazine in biological fluids [17]. There was a study that showed the HF-LPME also able to determine NSAIDs in sewage sludge by LC-MS [18] and also determine organophosphorus pesticides in vegetables sample [19].

The other extraction methods can also have been developed in order to identify the pharmaceutical drug in real sample such as hollow fiber-based liquid–liquid–liquid microextraction (HF-LLLME) [20] and microwave-assisted extraction (MAE) [21].

2.3 Dispersive liquid liquid microextraction

One of the most important objectives of modern analytical chemistry is miniaturization, simplification and automation of the whole analytical procedure, especially to speed up sample treatment, which is currently the bottleneck of analysis. 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 [22]. DLLME has attracted much interest from scientists working in separation science. Since its introduction in 2006 for preconcentration of organic analytes from water samples, a good number of works have reported efficient, quick extraction of organic or inorganic analytes [23].

This method is based on dispersion of tiny droplets of the extraction solvent within the aqueous solution. For DLLME, water-immiscible extraction solvent dissolved in a water-miscible dispersive solvent was rapidly injected into an aqueous solution by a syringe. The mixture is then shaken and a cloudy dispersion solution (water/disperser solvent/extraction solvent) was formed in the test tube. The analytes in the sample were extracted into the fine droplets, which were further separated by centrifugation, and the enriched analytes in the sedimented phase were determined by either chromatographic or spectrometric methods [23]. DLLME has recently been introduced for the extraction of nonylphenol (NP) and octylphenol (OP) [24], triazole pesticides [25], parabens [26], triazines [27] and organophosphorus pesticides [28, 29].

In DLLME, the factors that affect extraction efficiency are as follows: (1) suitable extracting solvent, (2) suitable disperser solvent, (3) volume of extracting solvent and (4) volume of disperser solvent. Selection of an appropriate extracting solvent is the major parameter for DLLME process. Organic solvents are selected on the basis of their higher density rather than water, extraction capability of interested compounds and good chromatographic behavior. Halogenated hydrocarbons such as chlorobenzene, chloroform, carbon tetrachloride and tetrachloroethylene are usually