ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Trace Enrichment and Selective Extraction of Residue of Atrazine and Its Major Metabolites in Environmental Waters and Human Urine Utilizing a Hollow Fiber Supported Liquid Membrane Extraction

Graduate Project

By

Tesfaye Tolessa

June 2010

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By

Tesfaye Tolessa

June 2010

DECLARATION

I, the undersigned, declare that this project is my original work, has not been presented for a degree in any other University and that all sources of material used for the project work have been duly acknowledged.

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The project has been submitted for examination with my approval as a university advisor.

_____

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Abbreviations	Description
K_{ow}	n-octanol-water partition Coefficient
MRL	Maximum residue limit
HPLC	High performance liquid chromatography
UV	Ultraviolet
DAD	Diode array detector
LLE	Liquid-Liquid extraction
LLME	Liquid-Liquid membrane extraction
SDME	Single drop microextraction
LPME	Liquid phase microextraction
HF-LPME	Hollow fiber based liquid phase microextraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
MIP	Molecularly imprinted polymer
MASE	Microwave-assisted extraction
SFE	Supercritical fluid extraction
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidenefluoride
PS	Polysulfone
RO	Reverse osmosis
UF	Ultrafiltration
MF	Microfiltration
ED	Electrodialysis

MMLLE	Microporous membrane liquid-liquid extraction
SLM	Supported liquid membrane
PME	Polymeric membrane extraction
SPMDs	Semipermeable membrane devices
MESI	Membrane extraction with sorbent interface
ELM	Emulsion liquid membrane
HF-SLM	Hollow fiber supported liquid extraction
LPME	Liquid phase microextraction
ATZN	Atrazine
DEA	Desethylatrazine
DIA	Desisopropylatrazine
DDA	Didealkylatrazine
ATOH	Hydroxyatrazine
RPM	Revolution per minute
E	Extraction efficiency
E_e	Enrichment factor
W/V	Weight by volume
ΔC	Change in concentration
ΔP	Change in pressure
ΔE	Change in electric potential
SD	Standard deviation
RSD	Relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantification

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Abstract

A simple sample preparation technique using a hollow fiber membrane in conjunction with high performance liquid chromatography has been developed for the extraction and determination of s-triazine herbicides and their major metabolites from environmental water and human urine samples. Optimum extraction conditions have been evaluated with respect to solvent type, acceptor and sample pH, extraction time, shaking speed and salt content as well as humic acid effect. The extraction method has been validated for matrices such as reagent water, tap water, river water and human urine samples, indicating that regent water can be selected as representative matrix for routine analysis of these environmental water samples and human urine samples. A high level of detection linearity with regression coefficients (r^2) ranged from 0.994 to 0.999 was obtained for herbicides over a range of analyte concentrations between 50 and 100 μg/L, using peak area as a response variable. The repeatability and reproducibility of the method were less than 11 and 17% for concentration of 50 μg/L, respectively. Limits of detection of the method ranged from 0.03 to 1.12 μg/L and the limits of quantification from 0.10 to 3.73 μg/L, which were low enough to determine all the herbicides residues except desisopropylatrazine at concentrations below or equal to the maximum residue levels (MRLs) specified by European Union. The method was finally applied to the determination of the herbicides in tap water, river water and human urine samples and the concentrations found in these samples were always lower than the MRLs except desisopropylatrazine in tap water.

1. INTRODUCTION

1.1 Herbicides

Pesticides can be defined as any substance or mixture of substances, natural or synthetic, intended for preventing, destroying, repelling or mitigating any pest. (The term *pest* includes harmful, destructive or troublesome animals, plants or microorganisms). A pesticide may also be described as any physical, chemical or biological agent that will kill an undesirable plant or animal pest. It is a generic name for a variety of agents that are classified more specifically on the basis of the pattern of use and organism killed. There are different classes of pesticides according to their type of use. The main pesticide groups are ***herbicides***, ***insecticides*** and ***fungicides*** [1]. On the other hand, pesticides comprise widely varying classes of pest-control agents under different categories according to target pests killed such as insecticides (kill insect pests), herbicides (kill weeds and other unwanted plants), fungicides (kill fungi), rodenticides (kill rodents), acaricides/miticides (kill round worm), molluscicides (kill mollusces), antimicrobials/biocides (kill microbes/bacteria) and Microbial pesticides (e.g., *Bacillus thuringiensis*). Some are persistent in the environment and others degrade readily into various types of transformation products, again varying in the degree of toxicity and distribution in a similar manner as their parent compounds [2-5].

Herbicides, in the broadest sense, are any substance or cultured biological organism that are capable of either killing or severely injuring weeds and may thus be used for elimination of unwanted plant growth or the killing of plant parts. They have been in use since the mid-nineteenth century. For a long time, until the Word War II, a variety of inorganic acids and salts (e.g., iron sulphate, sulphuric acid, sodium chlorate, arsenicals and copper sulphate) were dominating in the control of unwanted weeds. In 1930s the first organic chemical herbicide, 4, 6-dinitro-*o*-cresol (DNOC) was introduced. DNOC was used as an insecticide before its herbicidal effect was discovered. The introduction of DNOC was followed by the appearance, in the 1940s, of the substituted phenoxy acids, e.g., chlorinated phenoxyalkanoic acids and the substituted ureas in 1951. Introduction of the phenoxy carboxylic acids and organic herbicides made a

decisive breakthrough into selective chemical weed control. Today they are dominant in all areas where the control of undesired plant growth is practical [3].

1.2 Uses of Herbicides

Herbicides are the main group of pesticides used worldwide, followed by insecticides and fungicides. Use of herbicides has brought stable crop production and is labor saving, they protect crops from undue competition from weeds and enhance the nutritional quality of food. Herbicides are generally used as pre- and post-emergence for the control of weeds in agricultural crops [6]. One recent estimate indicate that only 0.1 - 5% of the herbicides applied reach the weeds targeted, and that only 0.003% of the insecticides used are consumed by the insects targeted [7]. The intensive application of herbicides has resulted in the contamination of the atmosphere, ground and surface waters, agricultural products; consequently, resulted in the direct or indirect pollution of biological systems, food and food products. Knowledge of physicochemical properties; that is, vapor pressure, octanol/water partition coefficient (log K_{ow}) and solubility in water allows the prediction of the fate and behavior of such chemicals in the environment [6- 7].

1.3 Classification of Herbicides

Herbicides can be classified in several ways and the major ones are briefly discussed below.

Soil and Foliage Herbicides. A rough classification of herbicides can be made on the basis of the manner of their absorption to the plant material [8]. A herbicidal agent taken by the plant through the roots is termed as soil herbicide, in contrast to foliage herbicides, which enters through the green, aerial plant parts.

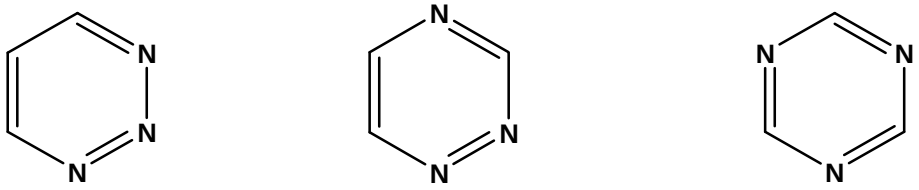
Contact and Translocated Herbicides. Foliage herbicides that exert their effect directly on the plant parts that have been contacted with are called contact herbicides. All agents that are translocated within the plant after absorption, that is, for which site of absorption and site of action are not identical, are known as symmetric or translocated herbicides [8].

Selective and Non-selective Herbicides. Those herbicidal agents that kill some members of a plant population with little or no injury to others are known as selective herbicides. Non-selective herbicides are those that kill all vegetation to which they are applied [8].

Chemical Classification of Herbicides. Herbicides can also be classified based on their chemical structure into the following ten groups. These are phenoxy acid herbicides, substituted urea, uracil herbicides, bypiridilium herbibides, dinitroaniline herbicides, amides, carboxylic and benzoic acid herbicides, carbamate herbicides, phenols and diphenyl ether herbicides, hetrocyclic nitrogen containing herbicides and miscellaneous herbicides [9, 10].

1.3.1 Triazine Herbicides

Triazines are aromatic six-membered heterocyclic ring compounds with three nitrogen atoms replacing carbon-hydrogen units in the benzene ring structure that give the name to these compounds: the prefix tri- means "three" and azine indicate a nitrogen-containing ring. The names of the three isomers indicate which of the carbon-hydrogen units on the benzene ring positions have been replaced by nitrogen, called 1,2,3-triazine, 1,2,4-triazine, and 1,3,5-triazine respectively, as shown in Fig. 1. Despite the fact that 1,3,5-triazines (also known as s-triazines or symmetrical triazines) are the most common and one of the oldest known classes of organic molecules, synthetic methods for the preparation of the analogs containing different substituents at each carbon are limited [11].



1, 2, 3-triazine 1, 2, 4-triazine 1, 3, 5-triazine

Figure 1. The common triazines

All of the three structures of triazines may be deduced from experimental observations of the C-C and C-N bond lengths, which have been found to be intermediate in length between the values expected for the characteristics single and double bonds.

1.3.1.1 General Properties of s-Triazines and Their Metabolites

s-triazine family of herbicides, introduced in 1950s, are one of the largest classes of agrochemicals produced and they are among the most commonly used herbicides. A report based on world market indicated that about 30% of herbicides produced are triazines [12]. The herbicidal properties of the *s*-triazines were discovered in 1952 by a research group of J. R. Geigy in Basel, Switzerland. The first patent application was made in 1954 covering 2-chloro-4, 6- bis (alkyl-amino)-*s*- triazines and their influence on the growth of plants [13]. It is being used as selective pre-and post- emergence control of leafy and grassy weeds in many agricultural crops like corn, soybeans, wheat, maize, sugar cane and barley [14, 15].

s-Triazines are strong inhibitors of photosynthetic electron transport and are very selective herbicides. Most of the traizine herbicides have a structure of N-substituted benzene ring with R-groups attached to the carbon atoms (Fig. 2). They are the derivatives of symmetrical *s*-triazines [15]. With few exceptions, the *s*-triazines used as selective or general herbicides are substituted diamino *s*-triazines which have commonly chlorine (the common name ending with –azine), methoxy (ending with-tone), methylthio or azido (ending with- etryn) groups attached to the third ring carbon atom [14, 15]. Different substitutions at the 2- , 4-, and 6-positions on the triazine ring have produced a wide range of physical, chemical, and biological properties of the compounds [16]. The chemical properties of *s*-triazine derivative compounds can be determined primarily by the constituents in position -2 such as chlorine, methoxy and methylthio groups [14, 15]. Various alkylamino groups [16] usually substitute at position 4 and 6.

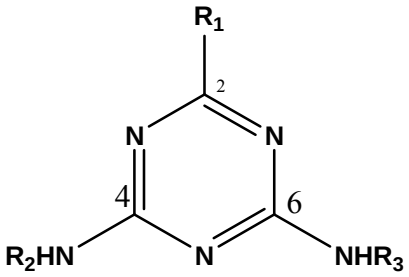


Figure 2. General structure of s-triazine

However, essential difference exists in electronic configuration between *s*-triazine and benzene as a consequence of the greater electronegativity of the nitrogen atom compared to that of the carbon atoms. Thus, the π-electrons in the *s*-triazine ring are localized in the vicinity of nitrogen

atoms rather than being evenly distributed over the whole ring. The delocalization effect in combination with inductive and mesomeric effects exerted by the substituents at C-2, C-3 and C-6 greatly influences the chemical behavior and physical properties of the *s*-triazine derivatives [15].

The remarkable stability of *s*-traizine derivatives can be explained by the electronic configuration of the heterocyclic ring, which resembles that of benzene to a certain extent. The ring system is stabilized by delocalization of its π-electrons, which are spread over all six-ring atoms as shown in Fig. 3. Therefore, it is important to understand that introduction of heteroatoms to the benzene structure allows for more canonical forms in the resonance hybrid and the electronegativity of the heteroatom localizes negative charge.

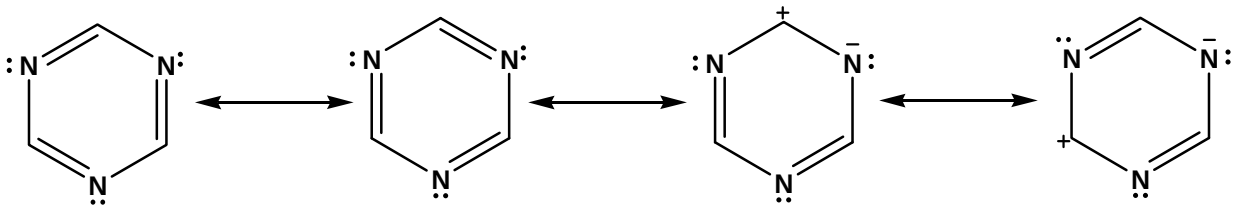


Figure 3. Resonance forms of 1,3,5-triazine

Generally, the water solubility and other physico-chemical properties of s-triazines are determined mainly by the substituent at 2-position. They are solids, with a low vapour pressure at room temperature and have water solubilities in the range 5 - 750 mg L^{-1} which is mostly pH independent. However, a pronounced increase in solubility is observed at pH values where strong protonation occurs, e.g., between pH 5.0 and 3.0 or lower for 2-methoxy and 2-methylthio-*s*-triazines and at pH 2.0 or lower for 2-chloro *s*-triazine. S-Triazines are relatively polar and have a log K_{ow} between 1.6 and 3.7 [12, 15].

All triazine herbicides are considered somewhat persistent in water and mobile in soil. The physico-chemical properties of triazines make them especially susceptible to leaching into ground water and runoff from the site of application to surface waters [16] especially during heavy rains. Because of their high water solubility, triazines have a large potential for movement into a solution and only a moderate potential for soil sorption. These properties have resulted in the contamination of surface and ground waters [17]. Therefore, the extent of contamination of

surface water should be monitored frequently to study the environmental impacts of these pesticides.

s-Triazine herbicides are slightly basic, with high solubility and moderate persistence in the environment. Attentions have been devoted to the determination of these pesticides, as they are toxic and rather persistent in living systems, soil and aquatic media. These herbicides, particularly simazine and atrazine, are widely used as pre- and postemergent weed control agents to enhance crop yields. Their half-lives vary from few weeks to several months and they are usually degraded/transformed into their metabolites which are more polar compounds with more tendencies to stay in aquatic media and the organic matter of soil [18]. The degradation is due to different biochemical processes like dealkylation, dechlorination, hydroxylation, deamination and ring cleavage of the parent compounds [19].

Triazine herbicides degrade by various pathways to a series of metabolites. For example, atrazine degrades in soil through both biotic and abiotic reactions to the dealkylated metabolites; DEA and DIA, and the hydroxylated metabolite, HA (see Fig. 4). DEA may further degrade to the dealkylated hydroxymetabolites of didealkylatrazine (DDA), hydroxydeethylatrazine (HDEA) and hydroxideisopropylatrazine (HDIA). DIA may further degrade to the hydroxy-metabolites of DDA and HDIA. HA may degrade to dealkylated HDIA and HDEA. The atrazine degradation pathway includes further dealkylation of DEA, DIA and HA to the opening of the triazine ring and eventual mineralization to carbon dioxide and ammonia [20].

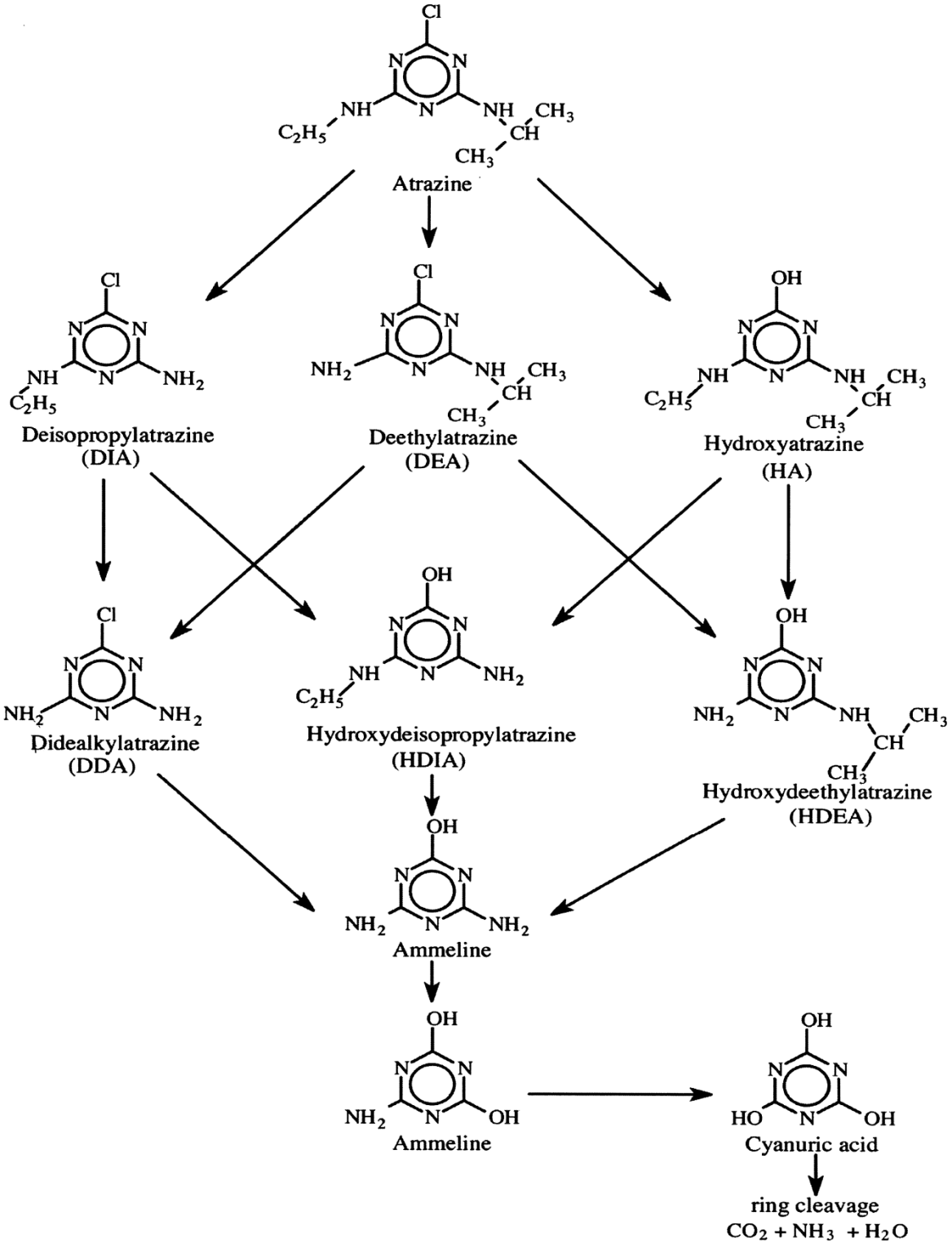


Figure 4. Pathways for degradation of atrazine [20].

2-chloro-*s*-triazines herbicides, atrazine, simazine, and propazine can also metabolise through various routes to several common dealkylated metabolites as shown in Fig. 6. Each of the three dealkylated metabolites can be further dechlorinated via 2-hydroxylation.

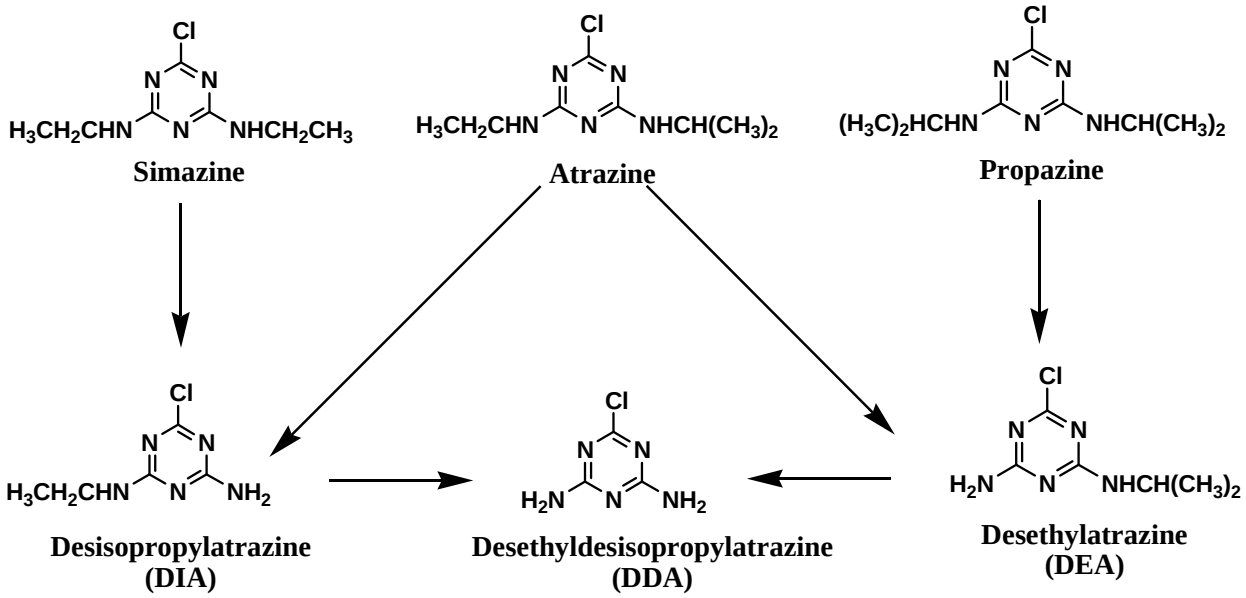


Figure 5. The various routes of metabolism of the 2-chloro-s-triazines herbicides atrazine, simazine, and propazine to several common dealkylated metabolites. Each of the three dealkylated metabolites can be further dechlorinated via 2-hydroxylation [21].

The concentration of metabolites in water commonly may be equal to or even exceed concentrations of parent compounds. It has been found that metabolite concentrations in ground water often exceed parent compound concentrations for both triazine and chloroacetanilide herbicides, whereas in surface water the parent compound is most abundant after the application of herbicides in the spring and is replaced gradually with metabolites throughout the growing season. Metabolites are generally more water soluble than parent compounds and are leached more rapidly through the soil profile into ground water. This leaching process causes metabolite concentrations to be higher in ground water [20]. The formation of these metabolites in several cases can produce compounds not always less phytotoxic than the parent compound; for example, deethylatrazine (DEA) has the same toxicity as atrazine [19, 20].

In general, herbicide transformation products (TPs) can be more or less mobile and toxic as their parent herbicides. Battaglin *et. al*. [22] in their recent report indicated that of 89 pesticide metabolites arising from 37 source pesticides, 70 percent were equally toxic as or less toxic than their source compounds, and 30 percent were more toxic.

1.3.1.2 Exposure to Triazine Herbicides

Triazines do not readily enter the body via dermal exposure, with the actual amount absorbed being one percent. The most significant route of exposure for humans and animals to triazine herbicides is through drinking water contamination and the routes of excretion are through bile, urine, and feces, with urine as the major route [23].

The physio-chemical properties of triazines make them especially susceptible to leaching and surface runoff, especially during heavy rains. Triazines have a large potential for movement into a solution and only a moderate potential for soil sorption. Example, the moderate water solubility and small partition coefficients favor movement of atrazine in the dissolved state from treated soils into surface or subsurface waters. These properties have resulted in the contamination of surface and ground waters [23].

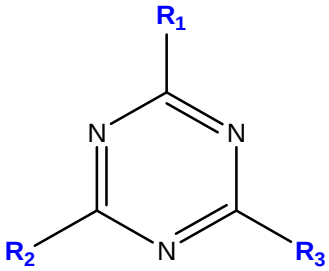
1.3.1.3 Toxicity of Triazine Herbicides

In 2002, USEPA published a report that evaluated a series of structurally related triazine pesticides for inclusion in a common mechanism group (i.e., atrazine, simazine, propazine and their chlorodegradates) based on their ability to induce similar toxic effect through a common mechanism of toxicity [24]. These herbicides are determined to cause neuro-endocrine related developmental and reproductive effect, through their ability to induce aromatase activity in human adrenocortical carcinoma cell line at concentrations of submicro-molar range [25-27].

Epidemiologic studies have been documented long term exposures to triazine herbicides with increased risk of ovarian cancer in female farm workers in Italy and of breast cancer in the USA [25]. Upon chronic exposure, atrazine may cause weight loss, cardiovascular damage, retinal degeneration, and mamary tumors in rats [28]. Ametryn is classified as toxicity class III of EPA, which is slightly toxic. Ametryn is moderately toxic to fish, highly toxic to crustaceans and moderately to highly toxic to mollusks [29].

In this project work, emphasis will be given to some s-triazine herbicides and their major metabolites as these pesticides are widely used in Ethiopian agricultural sector that are shown in Table 1. Thus, a hollow fiber supported liquid membrane extraction technique has been employed for sample preparation and enrichment of selected s-triazine herbicide residues in particular attention to their metabolites from environmental water and human urine samples because the determination of these transformation products in environmental water systems and human urine samples is, therefore, equally important as their parent compounds.

Table 1. General structure and some properties of triazine herbicides and metabolites considered in this work [30, 31].



Compounds	**R_1**	**R_2**	**R_3**	**Log K_{ow}[a]**	**pK_a**
Atrazine (ATRN)	-Cl	-NH-CH(CH_3)$_2$	-NH-CH_2-CH_3	2.5	1.68
Hydroxyatrazine (ATOH)	-OH	-NH-CH(CH_3)$_2$	-NH-CH_2-CH_3	1.4	5.15
Deethylatrazine (DEA)	-Cl	-NH-CH(CH_3)$_2$	-NH_2	1.52	1.30-1.65
Deisopropylatrazine (DIA)	-Cl	-NH_2	-NH-CH_2-CH_3	1.15	1.30-1.58
Didealkylatrazine (DDA)	-Cl	-NH_2	-NH_2	0	1.5

[a] log*K*ow: *n*-octanol–water partition coefficients, defined as the ratio of the equilibrium concentrations of a dissolved substance in two immiscible solvents.

1.4 Modern Sample Preparation Methods for the Analysis of Environmental Samples

Sample preparation is a step meant to isolate components of interest from a sample matrix into a sample suitable for analysis. The analytes of interest are separated from the matrix and are pre-concentrated to improve the selectivity, sensitivity, reliability, accuracy and reproducibility of the analysis. Some of the commonly encountered steps in sample preparation are described in figure 6. However, they depend on the sample, the matrix, the concentration level, and the analytical technique to be employed [32, 33].

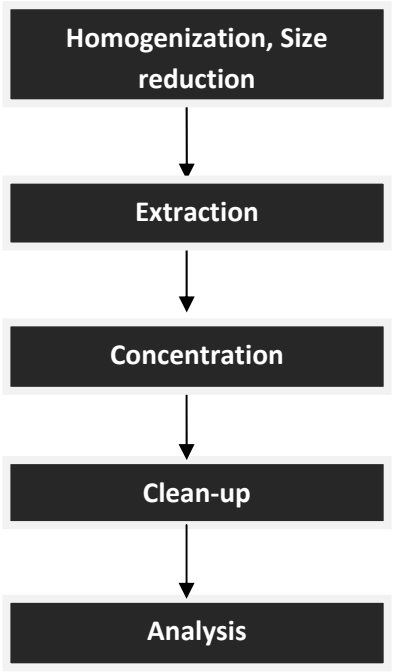


Figure 6. Steps in sample preparation [33]

The principles and applications of some modern sample preparation techniques in particular attention to membrane based extraction techniques that are commonly and frequently used in environmental analysis will be discussed as follows.

Major advances have made during the last two decades in the area of trace analysis in environmental sample. The major work was mainly in the development of analytical instruments. Despite the fact that these instruments are so sophisticated, they cannot handle environmental samples directly. Hence analysis by these instruments should be proceeded by appropriate sample-preparation or sample pre-treatment steps that concentrate analytes and clean up

interferences.[34] The pre-treatment may either involve simple procedures, such as filtration, precipitation or it may consist of very laborious multiple extractions, followed by evaporation to dryness and re-dissolution in a solvent compatible with the selected analytical techniques.

In most cases, sample preparation is necessary for one of the following reasons:

a. to remove the coexisting interferences since discriminating power of the analytical technique between analytes and other sample constituents is limited,
b. to improve deterioration of the analytical systems, and
c. to improve the detection limit.

Classical solvent extraction or liquid-l1quid extraction (LLE) is still among the most popular in routine sample preparation. Most chemists remember well their college experiments in the organic chemistry laboratory, continuously shaking their large separatory funnels attempting to isolate a pure fraction from a synthesis. It is one of the oldest and most widely used sample-preparation techniques. It involves the distribution of the sample components between two immiscible liquid phases and used for qualitative and quantitative analysis. As for any sample preparation technique, sample clean-up and/or analyte preconcentration are the most common goals of LLE and it is a versatile sample-preparation technique, prescribed in many standard analytical methods. Despite its widespread use, it has got many limitations [35, 36]. The main limitations of LLE are:

- the use of large amounts of high purity solvents which are expensive and toxic,
- its labor and time intensive procedure,
- its tendency to form emulsion,
- its poor potential for automation, and
- its multi-step nature which leads to analyte loss.

As a result, there is a great need for change in analytical sample preparation procedures. This need led to the development of new methods which are fast, consume negligible volume of solvents and allow trace level detections. In many cases, the new methods provide more accurate results in less time and at a lower cost than older techniques, so deciding which method to use is critical to analytical success.

The analytical community responded to this challenge by trying to develop newer sample preparation techniques which can give higher yields, perform better sample clean-up, cost effective, convenient for workers safety and environmentally friendly. These methods of sample preparation techniques are classified in several ways; one of these classifications is based upon the mode of extraction [37]. Accordingly, ***fluid-phase partitioning extraction***, ***sorptive extraction*** and ***membrane based extraction*** methods are the major ones. Among fluid-phase partitioning methods, single drop and liquid microextraction are applied to aqueous analysis, and supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction methods are more frequently applied to the extraction of solid samples.

1.4.1 Liquid-Phase Microextraction (LPME)

By definition, microextraction is an extraction technique where the volume of the extracting phase is very small in relation to the volume of the sample. It usually requires the use of 200µL or less of organic solvent to extract analytes from moderate amounts of aqueous matrices [38]. In microextraction, extraction yields hinge on the partitioning (or more strictly on the partitioning coefficient) of analyte(s) between the sample bulk phase and the extraction deprived-phase.

In recent years, solvent microextraction has been shown to be a fairly new and an alternative sample preparation method to conventional LLE. Miniaturized LLE or liquid phase microextraction (LPME), was introduced simultaneously by Liu and Dasgupta [39], and Jeannot and Catwell [40] in 1996, and involved the extraction of analytes into a droplet. Single-drop microextraction (SDME) and hollow fiber based liquid phase microextraction (LPME) are sample preparation methodologies that utilizes solvent microextraction principle developed recently. These methods are simple, inexpensive, environmentally friendly, fast and virtually solvent-free sample preparation techniques, and results in more efficient sample enrichment and easier automation compared to classical LLE

Single-drop Microextraction (SDME)

It involves a droplet of organic solvent hanging at the end of a PTFE rod or a microsyringe needle. This organic microdroplets (microextract) is placed in an aqueous sample, and the analytes present in the aqueous sample are extracted into the organic microdroplets. Subsequently, the organic microdroplet is withdrawn into the syringe and the microextract is transferred or injected into capillary or gas chromatography [41-44].

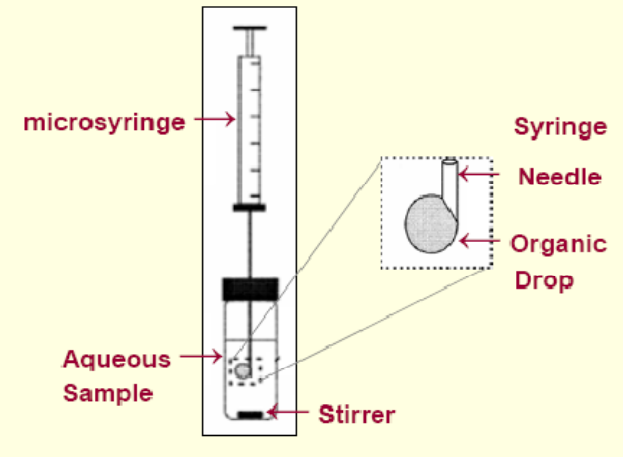


Figure 7. A schematic diagram of the single-drop microextraction

Two alternative single-drop microextraction modes have been described:

1. Static SDME, where the organic drop is exposed to an aqueous sample solution and the analytes are transferred to the organic drop by diffusion until thermodynamic equilibrium is attained or the extraction is stopped, and
2. Dynamic SDME, which is performed between microliters of aqueous sample and microliters of extraction agent-solvent by repetitively pulling and pushing the plunger within the glass barrel of a microsyringe. Dynamic mode achieves higher enrichment factors within shorter extraction time but relatively poorer precision.

Single-drop microextraction which based on hanging droplets is not very robust [45], and the droplets may be lost from the tip of needle of syringe during extraction. This is especially the case when samples are stirred vigorously to speed up the extraction process. In addition biological samples, such as, plasma may emulsify substantial amount of organic solvents, and this may also affect the stability of hanging droplets during extraction. However, problems with drop stability have been found, and novel microextration techniques that involve the protection of the organic solvent have been introduced. Among them, the use of a hollow fiber provides

mechanical stability to the organic solvent, which is termed as hollow fiber supported liquid membrane (HFSLM) or "liquid-phase microextraction using hollow fiber membranes (LPME-HF)" [45].

1.4.3 Solid Phase Extraction (SPE)

The SPE technique was first introduced in the mid-1970s. It became commercially available in 1978, and now SPE cartridges and disks are available from many suppliers. Conventional SPE is generally performed by passing aqueous samples through a solid sorbent in a column. The principle of SPE is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. One highly important aspect in SPE is the selection of the sorbent. The selection of an appropriate SPE extraction sorbent depends on understanding the mechanism(s) of interaction between the sorbent and analyte of interest. That understanding in turn depends on knowledge of the hydrophobic, polar and ionogenic properties of both the solute and the sorbent. The most common retention mechanisms in SPE are based on van der Waals forces ("non-polar interactions"), hydrogen bonding, dipole-dipole forces ("polar" interactions) and cation-anion interactions ("ionic" interactions) [46, 47]. SPE is an established method, in which a liquid sample is passed through a specific sorbent, similar to the stationary phases used in HPLC, where the analytes are retained.

1.4.4 Microwave-Assisted Extraction (MAE)

The use of microwave energy to aid organic extraction was first achieved using conventional household systems in the late 1980s. The emergence of commercial microwave systems which are specifically designed for extraction is rather recent, and has encouraged renewed interest in the technique. Microwave-assisted extraction utilizes microwave energy to desorb pollutants from their solid matrices. Microwave energy is a non-ionizing radiation (frequency 300-300 000 MHz) that causes molecular motion by migration of ions and rotation of dipoles (Dipole rotation refers to the alignment, owing to the electric field, of molecules in the solvent and samples that have dipole moments). Principle of the extraction consists of heating the extractant (mostly liquid organic solvents) in contact with the sample with microwave energy. The partitioning of

the analytes of interest from the sample matrix to the extractant depends on the temperature and the nature of the extractant [48].

To model the extraction process, the matrix can be compared to a grain constituted of an impermeable core covered by a porous organic layer. The extraction and recovery of a solute from a solid matrix can be regarded as a five-stage process: the desorption of the compound from the active sites of the matrix, diffusion into the matrix itself, solubilization of the analyte in the extractant, diffusion of the compound in the extractant and collection of the extracted solutes. In order to obtain quantitative and reproducible recoveries, careful control and optimization of each step are required. A quantitative extraction must overcome interactions between analytes and matrix. These interactions depend partly on the composition of the matrix [49].

1.4.5 Supercritical Fluid Extraction (SFE)

The supercritical state of a substance is reached when it is above the critical point (i.e., when the pressure is above the critical pressure and the temperature is above the critical temperature). Beyond the critical point, the densities of the gas and liquid phases become identical and the distinction between the two phases disappears; the substance becomes a supercritical fluid. The fluid is neither a gas nor a liquid but possesses properties of both. Viscosity is low and diffusivity is high, as for the gas, while the solvating power is similar to that of liquids.

The most frequently used extraction fluid is supercritical carbondioxide, CO_2. It has inherent benefits such as low cost, availability in high purity, low toxicity and low reactivity, mild critical conditions and compatibility with detectors. CO_2 is an excellent extraction fluid for non-polar analytes, reasonably good for moderately polar analytes while the solubility of polar analytes is poor. It can be enhanced by addition of modifiers or co-solvents. The commonly used modifiers are ethanol, methanol, isopropanol, acetone and ethyl acetate. Addition of the modifier can be done either by mixing with the carbondioxide in a gas tank or by using a separate modifier pump. It can also be added directly to the sample in the extraction cell.

A number of compounds such as N_2O, NH_3, SF_6, CFC_S and recently water have also been used as supercritical fluid in SPE applications. Most of them have some kind of drawback that includes: being reactive, toxic, and explosive and having high critical temperature and/or pressure.

Supercritical fluid extraction is thus the technique that employs this fluid phase to effect solubilization of solutes. Several advantages have been gained by making use of the supercritical fluid because of the unique physical properties that these fluids possess. They allow more efficient mass transfer of the solutes from sample matrix than the liquid solvents, which can be attributed to the lower viscosity and higher diffusivities. Their solvent power can also be adjusted, by varying, i.e., the pressure and temperature, so as to accomplish selective extraction of the analytes, which are mainly free from any contaminating solvent.

1.5 Membrane Based Sample Preparation Methods in Environmental Samples

A membrane is a selective barrier through which different gases, vapors and liquids move at varying rates.

1.5.1 Typical Materials Used in Fabricating Membranes

Typical polymeric membranes used in analytical chemistry are polypropylene (mainly for hollow fibres), silicone, polytetrafluoroethylene (PTFE), polyvinylidenefluoride (PVDF) and polysulfone (PS) which are resistant to most acids, bases and other chemicals, thermally stable and hydrophobic. Membranes can also be made of metal. Common examples are Pd alloys, which have a high hydrogen flux, are chemically resistant and are typically used in hydrogen purification [50, 51].

1.5.2 Membrane Classification

Membrane techniques exist in various forms and may be classified into several groups depending on the mode of classification. For example, membrane techniques can be classified in to several groups depending on their function, morphology, geometry and structure. In all these configurations, the analyte molecules are selectively partitioned between two or three phases. For example, synthetic membranes may be classified based on a number of properties including but not limited to geometry, function, and morphology [51, 52]. The basic classification can be represented in fig. 8.

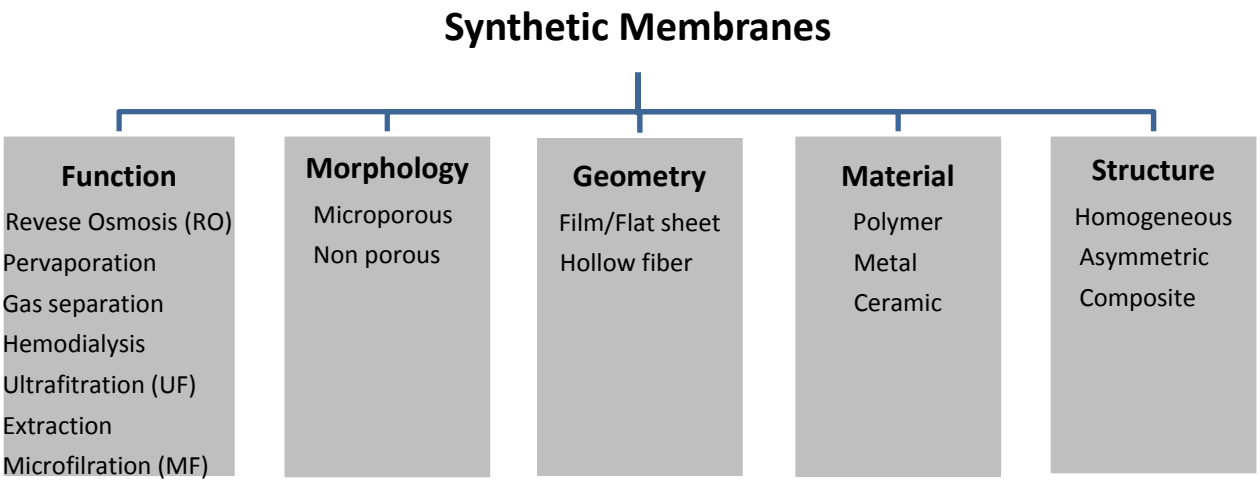


Figure 8. Classifications of membranes [51].

Function: Membranes can be used in various separation functions such as *reverse osmosis*, *pervaporation*, *gas separation*, *hemodialysis*, *ultrafiltration, extraction* and *microfiltration*. The method selected depends on the sample (e.g. aqueous, non-aqueous, biological, air, etc.,) and also the properties of the analytes, such as, size and presence/absence of a charge. Reverse osmosis (RO), ultrafiltration (UF) and microfiltration (MF) are all pressure driven water separation processes. They depend upon the pore size of the membranes used.

Microfiltration is the membrane process most closely related to filtration of suspensions in conventional filters. The pore sizes in membranes range from 0.1 μm to 10 μm. This ensures retention of molecules with diameters greater than 10 μm. Ultrafiltration is characterized by the use of membranes in which the pore sizes range from 2 to 100 nm. This allows separation of components with molecular masses ranging from 5×10^3 to 1×10^6 amu. At pressure differences of 0.2–1.0 MPa, only the solvent and molecules of those substances whose size is less than the molecular cut-off level can penetrate the membrane. Reverse osmosis is a membrane process in which the solvent is separated from low-molecular-weight inorganic and organic compounds at a pressure difference of 1–10 MPa. Separation here is based on the solubility and mobility of ions. Hence, the molecules being separated have sizes comparable to those of solvent molecules. Thus, the membranes used in RO must have considerably lower porosity (<1 nm) than the membranes used in UF or MF [51].

Gas separation is a membrane technique that is used to separate gas mixtures, for example, nitrogen removal from air, and the separation of CO_2 from methane in the energy industry.

Dialysis is the process of separation of solutes in liquid samples as a result of a concentration gradient. Separation of components is based on differences in diffusion rates of solutes in the membrane material, which in turn results from the differences in size of molecules. The material of a porous membrane is used to retain molecules of sizes exceeding the membrane coefficient (dependent on the kind of material), thus preventing them from passing across the membrane due to a steric effect.

Electrodialysis (ED) is a membrane process in which ionic components of a solution pass across the membrane driven by an external electric field. The membranes used in this process are ion exchange membranes capable of selective transfer of ions. An electrodialyzer of conventional design is equipped with alternating cation and anion exchange membranes. Anions present in a sample are passed through the anion-permeable membrane and stopped by the cation-permeable membrane. Similarly, cations moving in the opposite direction are passed through the cation-permeable membrane and stopped by the anion-permeable membrane. As a result, the adjacent compartments have alternating high and low concentrations of an ionic solution. Consequently, the application of this technique enables enrichment or depletion of electrolyte solutions [52].

Morphology: Membrane morphology refers to the quantity, size and distribution of pores throughout the membrane structure. Membranes which have no pores in their structure are known as non-porous, while those which possess pores are referred to as porous. The size, shape and distribution of pores in a membrane are largely dependent on the processes by which they are made and play a significant role in their mode of separation [51].

Structure: Structure refers to the degree of uniformity of the pores as well as the membrane material. For instance, homogenous (isotropic) membranes are uniform throughout while asymmetric (anisotropic) and composite thin-films are not. Isotropic membranes, the microporous membrane shown here illustrates pore uniformity while the non-porous the absence of pore structures (see Fig. 9). Anisotropic membranes refer to those in which the material, the porosity and pore size vary throughout the structure and include thin-film composites and Loeb–Sourirajan membranes. These membranes are heterogeneous in terms of pore size and pore

distribution. The composite membrane usually consists of different polymers where the surface layer determines selectivity, while the porous layer serves as a support (see Fig. 10).

Figure 9. Top view of isotropic membranes

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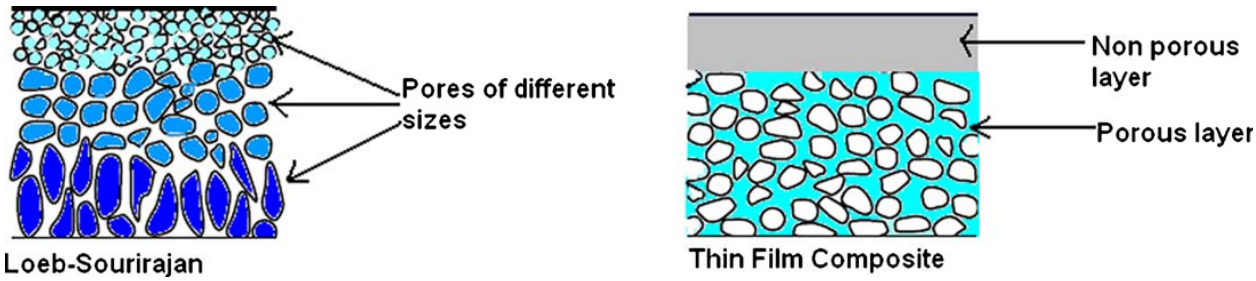


Figure 10. Top view of anisotropic membranes

As the name suggests, microporous membranes have openings through which selected molecules pass. Separation in microporous membranes (pore size between 101 and 104 nm) is a function of particle and pore size distribution, and movement through these membranes is therefore by size exclusion and the membranes are used in applications such as nanofiltration, microfiltration and dialysis. During extraction, two liquid phases meet at the pores and during pervaporation, the analytes vaporize at these sites. Non-porous membranes are solid (pore-free) structures and the molecules must move through them via diffusion and hence separation is influenced by partition coefficient as well as diffusivity of components in the membrane. These types of membranes are commonly used for extraction, reverse osmosis and pervaporation [51].

Geometry: In the context of membranes, geometry is synonymous with shape. The two main classes are flat sheet and hollow fiber (see Fig. 11). The latter have a tubular geometry. Each shape has its advantages and disadvantages.

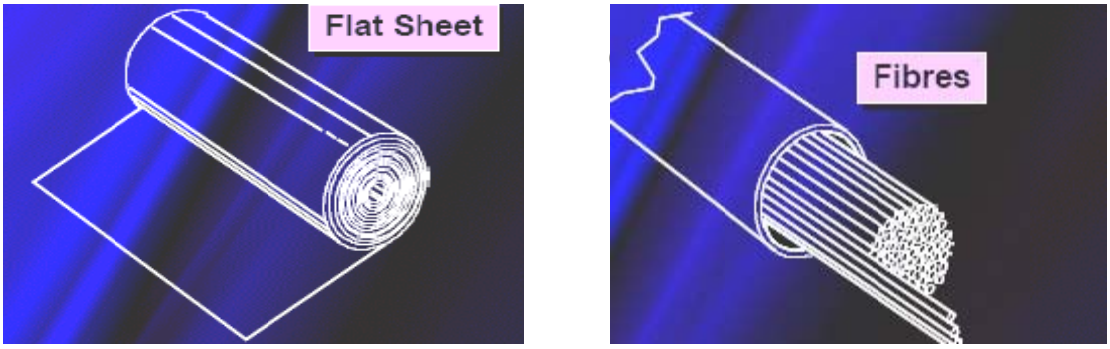


Figure 11. (a) Simple flat sheet membrane module and (b) Simple hollow fiber membrane module

There is also more complicated membrane module which is referred to as the plate and frame design (shell and tube, flat sheet and microfluidic) are based on the membrane geometry. Typical hollow fibers are made of glass, polyethylene or metal and 100–500 μm in diameter and may be porous, solid or composite.

1.5.3 Membrane Extraction Techniques

Over the last few decades, membranes have found many applications in various separation processes, such as, desalination, dialysis, ultrafiltration, gas separation, dehumidification, osmosis, reverse osmosis and electrodialysis. Thus, it is conceivable that they can be used to achieve extraction, concentration and cleanup in an analytical application. Being selective to a particular species, the membrane primarily functions as a separator of two bulk phases, and controls the mass transfer between them. This allows the enrichment of the species of interest and their removal from the sample matrix [51].

The application of membrane extraction as a sample preparation tool in analytical chemistry was pioneered by G. Audunsson, who was then at the department of analytical chemistry at Lund University (Lund,Sweden) [53].

In recent years, the use of membrane for sample preparation/extraction purpose has in many instances become a preferred option. This is largely due to the fact that they facilitate extraction without the mixing of two phases, thus eliminating problems such as emulsion formation and high solvent usage. A very important usage of the membrane processes is that the sample and extractant can be continuously brought into contact, thus providing the basis of a continuous, real-time process leading to automation and on-line connection to analytical instruments. These

techniques allow the simultaneous extraction and enrichment of analytes, and typically facilitate selective extraction at trace levels while consuming small amounts of organic solvents [51, 53].

Separation takes place when one species is diffused or tranported from the sample (donor) to the extractant (acceptor).The most common thermodynamic driving forces that applied across a membrane to effect transport of matter through membranes are: (1) Concentration difference, ΔC (mol.cm-3), (2) Electrical potential difference, ΔE (V) and (3) Pressure difference, ΔP (bar).
The process of diffusion in liquid membranes is governed by Fick's first law of diffusion [50]:

$$J = -D(\frac{\partial \Phi}{\partial \chi}) \quad (1)$$

Where J denotes the extent of flux of the analyte molecules per given area and time (e.g., $g \cdot cm^{-2}s^{-1}$ or $mol \cdot cm^{-2}s^{-1}$), D gives the diffusibility measure of the analyte across the membrane (diffusion coefficient), with units of cm^2s^{-1} and is proportional to the velocity of the diffusing particles, Φ is the concentration in dimensions of amount of substance per volume e.g., $mol \cdot cm^{-3}$; χ is the position e.g., cm or metre (m).

The concentration gradient is given by the ratio of the change in concentration (∂Φ) to the change in position (∂χ), that is$(\frac{\partial \Phi}{\partial \chi})$.The term D depends on the temperature, viscosity of the fluid and the size of the particles according to Stokes-Einstein equation:

$$D = \frac{K_\beta T}{6\pi r \eta} \quad (2)$$

Where D is the diffusion constant, r is the radius of solute, η is the solution viscosity = mobility of the particles, k_B is Boltzmann's constant and T is the absolute temperature. Integrating the Fick's equation:

$$J = D(\frac{C_{is} - C_{il}}{L}) \quad (3)$$

Where c_{is} is the concentration of analyte i at the outer membrane interface, c_{il} the concentration of analyte i in acceptor phase and L is the membrane thickness.

Differences in pressure produce a volume flow related to the Hagen-Poiseuille equation [54]:

$$J = KA(\frac{dP}{dx}) \quad (4)$$

Where K represents hydrodynamic permeability and A represents the area of the membrane available for diffusion.

And the difference in electrical potential is governed by Ohm's law:

$$J = RA(\frac{dE}{dx}) \quad (5)$$

Where R represents electrical resistance and A represents the area of the membrane available for diffusion.

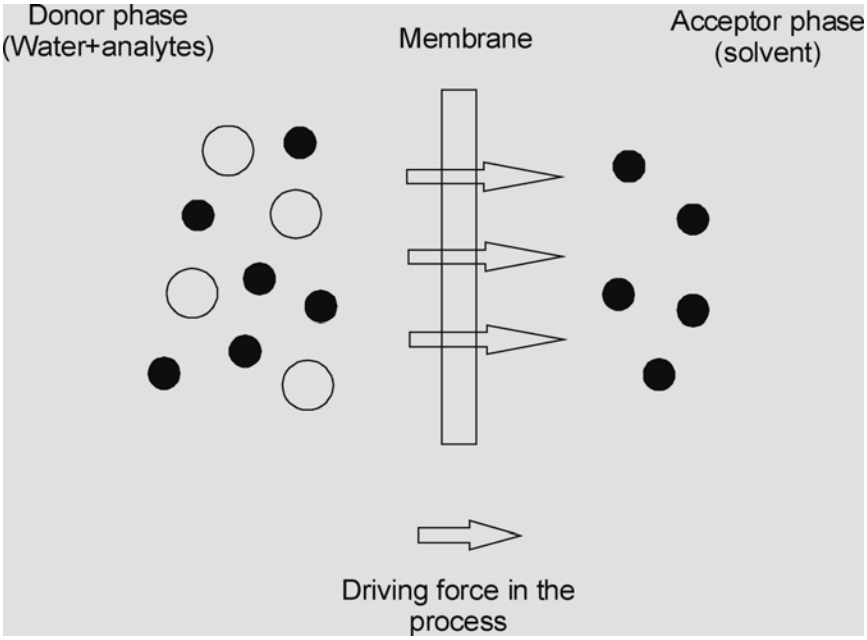


Figure 12. Schematic representation of the transport through membranes [52].

In all types of membrane extraction, the membrane separates the sample solution (called donor or feed solution) from the acceptor or strip solution and the analyte molecules pass through the membrane from the donor to the acceptor. This process is sometimes called pertraction (permeation extraction). Membrane extraction techniques can be divided into two main categories, porous and non-porous membrane techniques. Another distinction is between one-, two-, or three-phase membrane extraction techniques.

Porous membrane extraction techniques

In actual sense these techniques may not qualify fully as liquid membrane extraction techniques. In porous membrane techniques, the analyte molecules are partitioned from one phase which

forms the feed/donor through the porous hydrophobic membrane to the second phase which forms the stripping/acceptor. For example, porous membranes are frequently used in the filtration, dialysis and electrodialysis fields, where the membrane is directly used without any modifications so that the pores of the membrane are in direct contact with the liquid on both sides of the membrane. Therefore, the analytes are transported across the membrane merely by diffusion without giving any further selectivity of separation [55].

The driving forces in the porous membrane extraction include temperature, concentration, pressure, heat flux, volume flux, momentum flux and electrical flux. The selectivity in the extraction process is governed mainly by the membrane properties, as well as the physical-chemical properties that exist between the membrane and the permeate components [56].

Non-porous liquid membrane techniques

Non-porous membranes do not have pores as the name suggest, therefore analytes movement in these setups is controlled mostly by diffusion. Non-porous membrane systems exploit the advantage of the porous membranes to be implemented for analyte extraction by simply modifying the porous characteristic of the membrane. This could be easily achieved by filling the pores of the membrane with a suitable organic extraction phase to form two-phase (microporous membrane liquid–liquid extraction) or three phase (supported-liquid membrane extraction). A nonporous membrane can be either a porous membrane impregnated with a liquid or entirely a solid, like silicone rubber.

Table 2. Different major nonporous membrane extraction techniques used in analytical applications [57].

Name	Abbreviation	Type	Donor/membrane/acceptor combinations
Microporous membrane liquid-liquid extraction	MMLLE	Nonporous membrane	Aqueous/organic/organic
Supported-liquid membrane extractio	SLM	Nonporous membrane	Aqueous/Organic/aqueous
Polymeric membran extraction	PME	Nonporous membrane	Aqueous/polymer/aqueous, organic/polymer/aqueous or vice versa
Semipermeable membrane devices	SPMDs	Nonporous membrane	Aqueous/polymer/organic
Membrane extractio with sorbent interfac	MESI	Nonporous membrane	Gas/polymer/gas, liquid/polymer/gas

Although different configurations of non-porous membrane extractions are known to exist, some common approaches in analytical separations are:

- Microporous membrane liquid-liquid extraction
- Supported liquid membrane extraction
- Polymeric membrane extraction
- Semipermeable membrane devices
- Membrane extraction with sorbent interface

1.5.3.1 Microporous Membrane Liquid-Liquid Extraction (MMLLE)

In MMLLE extraction, the analyte is typically extracted from an aqueous solution in to an organic one and hence it is a two-phase system, organic-aqueous system where the organic phase is partly in the membrane pores and partly in the acceptor channel [51]. It essentially liquid-liquid extraction with the phases physically separates by a membrane, and in contact only at the membrane pores. The acceptor phase is an organic solvent, which also fills the pores of the hydrophobic membrane. The acceptor phase can be stagnant or flowing. If the acceptor is stagnant, the only driving force for the mass transfer is the attainment of a distribution

equilibrium between the aqueous and organic phases, and the efficiency will then be higher if the partition coefficient is large (i.e., the hydrophobicity of the analyte is large). The mass transfer can be improved if the acceptor phase is continuously or intermittently pumped, removing the extracted molecules successively from the acceptor [52].

1.5.3.2 Supported Liquid Membrane Extraction (SLM)

When a liquid is immobilized in the pores of porous material, via capillary action, that liquid can serve as the membrane and functions solely as a support. This is referred to as supported liquid membrane and can be prepared simply by immobilizing a porous membrane in the supporting solvent. The SLM technique was first initiated as a tool for analytical chemistry by Audunsson in 1986 and since that time, it has been successfully applied for extraction of different classes of organic and organometallic compounds and metal ions in environmental and biological aqueous samples [53].

SLM extraction has been applied in various configurations, like flat sheet, spiral wound and hollow fiber (tubular). Flat sheet and hollow fiber modules are most commonly used membranes (see Fig. 13 and 14). One important property among these configurations is the ratio between membrane surface area and its volume. This ratio should be high to get large enrichment factors especially for analytical purpose. It is highest for the tubular configuration (1000-10,000), followed by the spiral module (100-1000) and lowest for the flat configuration (10-100) [54]. The tubular configuration of SLM is sometimes referred to as hollow fiber based SLM (HFSLM) which is stable, very cheap and easy to prepare compared to the flat membrane configurations.

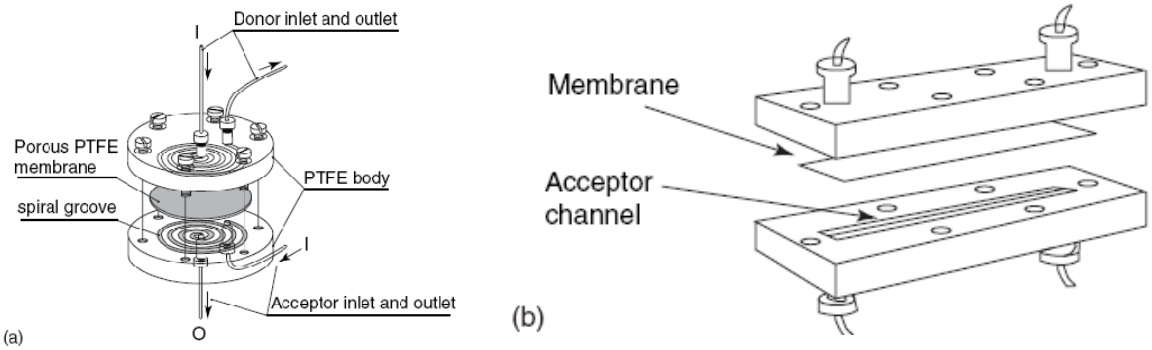


Figure 13. Flat-membrane extraction units that can be used in automated flowing systems. (a) Flat-membrane module with spiral channel of 1mL volume. (b) Flat-membrane module with 10 mL channel volume [55].

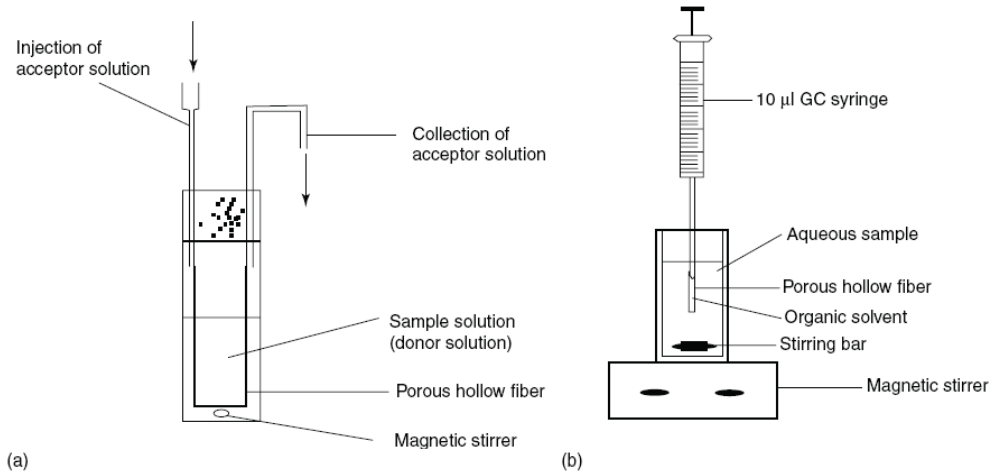


Figure 14. The principle of liquid–liquid–liquid microextraction with hollow fiber supported by (a) two needles. (b) by a 10 mL HPLC syringe fitted at one end of the hollow fiber and the other end is flame-sealed [55].

Principles of SLM Extraction

Supported liquid membrane (SLM) is a three-phase system in which an organic phase is sandwiched (interposed) between two aqueous phases. This liquid is immobilized in the pores of a supporting porous material and is held by the capillary action. One of the two aqueous phases is actually the test or sample solution containing the analyte of interest, while the other one forms the stripping solution to accept the extracted analyte (stripping/acceptor solution).The analytes are partitioned from the aqueous sample stream into the organic membrane and are then re-extracted into the aqueous acceptor phase. The driving force is the difference of the analyte concentration between the donor and acceptor phases. In order to maintain the concentration gradient across the two phases, the solutes must be able to exist in two forms: in a nonionic or uncharged form on the donor side to be extracted into the membrane and in an ionic form on the acceptor (which is usually stagnant) side in order to be irreversibly trapped. This is most simply achieved by pH adjustments in the two aqueous phases, and the method is, therefore, particularly well suited for polar and ionic compounds such as metals and medium-to-weak organic acids and bases [57, 58].

In the extraction of basic or acidic analytes, the pH of the donor solution must be adjusted such that the compounds are in their neutral or uncharged forms, thus allowing them to enter the membrane. The pH of the acceptor is maintained such that once in the membrane, the analytes

are extracted into the acceptor in a charged form and cannot be back extracted into the donor (see Fig. 15). The pH gradient therefore provides the driving force [59].

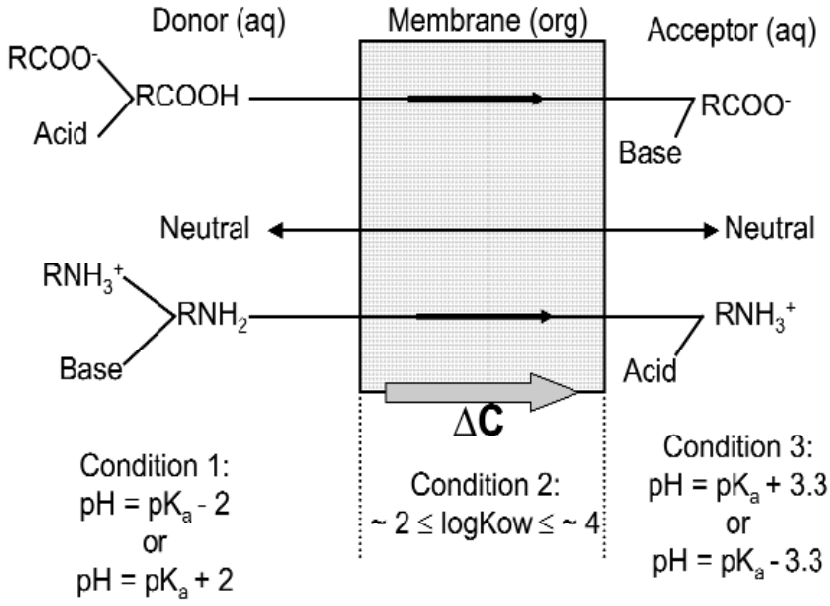


Figure 15. Principles of SLM extraction of ionizable organic compounds where the transport mechanism is simple Permeation **[59].**

Theory of Hollow Fiber Supported Liquid Membrane Extraction (HFSLM)

The hollow fiber is a thin microporous membrane that separates the sample (donor phase) and the accepter phase solution. The membrane is impregnated with a water immiscible solvent (organic phase), and the analytes are extracted from the donor phase, which is generally an aqueous phase, through the organic solvent immobilized in the pores of the hollow fiber into the acceptor phase present into the lumen of the hollow fiber. Depending on the acceptor phase, there are two sampling modes which can be used for this technique [56]:

a. Two-phase HFSLM sampling mode if the acceptor is the same organic solvent used to impregnate the hollow fiber,
b. Three-phase HFSLM sampling mode if the acceptor is an aqueous phase.

Thus, analytes are extracted from the aqueous sample, through the organic phase in the pores of the hollow fiber, and further into an acceptor solution inside the lumen of the hollow fiber. To speed up this process, extensive agitation or stirring of the sample is applied.

The acceptor solution may be the same organic solvent as immobilized in the pores, resulting in the extraction of the analyte (A) in a two phase system in which the analyte is collected in an organic phase. The two-phase system may be applied to most analytes more soluble in an organic solvent immiscible with water than in aqueous medium. The acceptor solution in this mode is directly compatible with GC, whereas evaporation of solvent and reconcentration in an aqueous solution is required for HPLC or CE.

Alternatively, the acceptor solution may be another aqueous phase providing a three-phase system, in which the analyte (A) are extracted from an aqueous sample, through the thin film of organic solvent, and into an aqueous acceptor solution. The three-phase mode is limited to basic or acidic analytes with ionizable functionalities. After extraction, the aqueous acceptor solution can be directly injected into HPLC or CE systems.

These two-phase and three-phase hollow fiber based liquid-phase microextraction systems are based on diffusion, in which extraction is promoted by high partition coefficients. However, the chemical nature of same analytes results in poor partition coefficients that prevent them from being extracted in systems based diffusion alone. This is especially so for very hydrophilic compounds. In this case, hollow fiber-based LPME may be accomplished in an active transport mode. The carrier which is relatively hydrophobic ion-pair reagent providing acceptable water solubility, forms ion pairs with the analytes of interest followed by extraction of the ion pair complexes into the organic phase in the pores of the hollow fiber [59, 60].

In the contact region of the organic phase and the acceptor solution, the analytes are released from the ion-pair complex into the acceptor solution, whereas counter ions present in excess in the acceptor solution forms an ion pair with the carrier in the contact area, and the new ion-pair complex is back-extracted in to the sample. In the sample again, the carrier releases the transported carrier ion, forms an ion pair with a new analyte molecule, and the cycle is repeated. For basic analytes, the carrier may typically be a carboxylic acid with an appropriate hydrophilic moiety (e,g octanoic acid) , PH in the sample is adjusted to ensure that the analytes are present in their ionized state, and PH in the acceptor solution is low to insure that the carrier is not trapped within this phase and sufficient protons are present to serve as counter ions.

Calculation of recovery and enrichment in HFSLM

Two-phase HFSLM

In two-phase HFSLM, the analytes are extracted from the aqueous sample solution (donor phase) and into the organic solvent (acceptor phase) present in the pores and inside the lumen of the hollow fiber. This process may be illustrated with the following equation:

$$A(donor) \longleftrightarrow A(accepter) \tag{6}$$

where A represents the analyte of interest. The partition coefficient $K_{org/d}$ is:

$$\mathrm{K_{org/d}} = \frac{\mathrm{C_{eq,org}}}{\mathrm{C_{eq,d}}} \tag{7}$$

where $C_{eq,org}$ is the concentration of A in the acceptor phase at equilibrium and $C_{eq,d}$ is the concentration of A in the donor phase at equilibrium. The initial amount of analyte n is equal to the sum of the individual amounts of analyte present in the two phases during the whole extraction process:

$$n_i = n_d + n_{org} \tag{8}$$

where n_d is the amount of analyte present in the donor phase and n_{org} is the amount of analyte present in the acceptor phase. At equilibrium, Eq. (6) can also be written as:

$$C_i V_d = C_{eq,d} V_d + C_{eq,org} V_{org} \tag{9}$$

where C_i is the initial analyte concentration in the sample, and V_d and V_org are the sample volume (donor phase) and acceptor phase volume, respectively. At equilibrium, the amount of analyte extracted into the acceptor phase $n_{eq,org}$ of the system can be expressed by:

$$n_{eq,org} = \frac{K_{org/d} V_{org} C_i V_d}{K_{org/d} V_{org} + V_d} \tag{10}$$

The recovery (R) of the analyte is calculated by the equation:

$$R = \frac{100 n_{eq,org}}{C_i V_d} = \frac{K_{org/d} V_{org}}{K_{org/d} V_{org} + V_d} \tag{11}$$

The enrichment (E) of the analyte can be calculated by the formula:

$$E = \frac{C_{org}}{C_i} = \frac{V_d R}{100 V_{org}} \tag{12}$$

C_{org} is the concentration of A in the acceptor phase at the end of extraction. Eqs. (10) and (11) may also be used for two-phase HFSLM. For two-phase HFSLM, the actual recovery is lower than calculated by Eq. (10) because the fraction of immobilized in the pores of the hollow fibre is not available for further analysis; only the fraction present in the lumen may be collected into a micro insert [61].

Three Phase HFSLM

In three-phase HFSLM, the analytes are extracted from the aqueous sample solution (donor phase), through the organic solvent immobilized in the pores of the hollow fiber (organic phase), and further into the acceptor solution (acceptor phase) present inside the lumen of the hollow fibre. This process may be illustrated with the following equation:

$$A(donor\ phase) \longleftrightarrow A(organic\ phase) \longleftrightarrow A(accepter\ phase) \quad (13)$$

In the three-phase system, the initial amount of analyte n_i is equal to the sum of the individual amounts of analyte present in the three phases during the whole extraction process:

$$n_i = n_d + n_{org} + n_a \quad (14)$$

n_d is the amount of analyte present in the donor phase (the sample), n_{org} is the amount of analyte present in the organic phase, and n_a is the amount of analyte present in the acceptor phase at any time during extraction process. At equilibrium, Eq. (14) can be expressed:

$$C_i V_d = C_{eq,d} V_d + C_{eq,org} V_{org} + C_{eq,a} V_a \quad (15)$$

C_i is the initial concentration in the sample. $C_{eq,d}$ $C_{eq,org}$ and $C_{eq,a}$ are the analyte concentrations in the donor phase, organic phase, and acceptor phase at equilibrium, respectively. V_d , V_{org} , and V_a are the volumes of sample (donor phase), organic phase, and acceptor phase, respectively.

In the three-phase HFSLM system, partition coefficients both between the organic phase and the donor phase as well as between the acceptor phase and the organic phase have to be considered:

$$\mathrm{K_{org/d}} = \frac{\mathrm{C_{eq,org}}}{\mathrm{C_{eq,d}}} \quad (16)$$

$$\mathrm{K_{a/org}} = \frac{\mathrm{C_{eq,a}}}{\mathrm{C_{eq,org}}} \quad (17)$$

The partition coefficient between the acceptor phase and the donor phase K can be written as:

$$\mathrm{K_{a/d}} = \frac{\mathrm{C_{eq,a}}}{\mathrm{C_{eq,d}}} = \mathrm{K_{org/d}K_{a/org}} \quad (18)$$

The amount of analyte extracted into the acceptor phase of the system can be calculated by substituting $K_{a/d}$ $C_{eq,d}$ for $C_{eq,a}$ and by rearrangement of Eq. (14). At equilibrium, the amount of analyte present in the acceptor phase n may be calculated by:

$$n_{eq,a} = \frac{K_{a/d} V_a \, C_i V_d}{K_{a/d} V_a + K_{org/d} V_{org} + V_d} \quad (19)$$

The recovery (R) can be expressed as:

$$R = \frac{100 n_{eq,a}}{C_i V_d} = \frac{100 K_{a/d} V_a}{K_{a/d} V_a + K_{org/d} V_{org} + V_d} \quad (20)$$

The enrichment (E) can be calculated by the formula:

$$E = \frac{C_a}{C_i} = \frac{V_d R}{100 V_a} \quad (21)$$

Eqs. (20) and (21) may be used to calculate recovery and enrichment for three-phase HFSLM. In the case of three-phase HFSLM, the whole volume of acceptor phase is available for further analysis, and the recovery is therefore directly calculated from Eq. (20) [62-64].

Eqs. (20) and (21) may also be used for calculation of recoveries and enrichment in three-phase LLE, where analytes in a first step are extracted from an aqueous sample into an organic solvent immiscible with water, and secondly back extracted in a separate step into a new aqueous phase [64].

1.5.3.3 Polymeric Membrane Extraction (PME)

In this case, instead of a porous membrane, an entirely solid membrane is used to separate the donor and the acceptor solutions. Silicone rubber is mostly used because it is hydrophobic and gives high permeability for small hydrophobic molecules [65]. The difference in the solubility and diffusion of various analytes into the polymer is the basis of selectivity. Changing conditions in the acceptor phase, such as making sure that analytes ionize, can enhance the selectivity [66]. This condition is a similar requirement as in supported-liquid membrane extraction technique as described above.

1.5.3.4 Membrane Extraction with a Sorbent Interface (MESI)

The technique is based on membrane extraction into a gas followed by trapping of the analytes on a solid sorbent (cryofocusing) and subsequent thermal desorption into a gas chromatographic system [67]. The technique is therefore suitable for volatile organic compounds either in air or aqueous samples. The receiving phase is always a carrier gas that continuously strips off and transports the analytes on the sorbent. The detailed theory of the technique and the type of sorbents that are used to trap the analytes have been described by Pawliszyn et al. and Harper, respectively. The basis of selectivity of the method is differences in solubility and diffusion of various analytes into the nonporous polymer. The main drawback of the technique is that it has a narrow application window for environmental analysis; only volatile organic compounds can be

extracted. Pawliszyn et al. recently developed a method for analysis of volatile breath components using membrane extraction with a sorbent interface [68].

1.5.3.5 Semipermeable Membrane Devices (SPMDs)

In SPMDs, hydrophobic organic analytes passively diffuse from aqueous donor phase through a polymeric membrane such as polyethylene into the acceptor phase filled with a thin film of a synthetic lipid such as triolein [69]. It is, therefore, used as a time-weighted passive field sampler. It is an important technique in exposure risk assessment of pollutants since it provides truly dissolved and bioavailable time-weighted average pollutant concentrations over longer periods. Generally, this technique is suitable for extraction of hydrophobic nonpolar compounds.

1.5.3.6 Other Membrane-based Extraction Techniques

Emulsion Liquid Membrane (ELM) Technique

The emulsion liquid membrane (ELM) is designed to have a large surface area per unit sample volume, which gives it an advantage of high flux rates across the membrane as well as high preconcentration factors. ELM technique has several disadvantages, all related to the formation of emulsion and these are:

- Controlling the factors that affect emulsion stability such as ionic strength, pH, etc.
- If for any reason, the membrane does not remain intact during use, the separation that has been achieved is destroyed
- In order to recover the receiving phase and also to replenish the carrier phase, one has to break the emulsion. This is a difficult task, since in order to make the emulsion stable; you have to work against the ease of breaking it back down. Despite the above cited drawbacks, several applications of ELMs have been reported both for speciation of metal ions [50].

Pervaporation

While LLME and SLM deal with the extraction of analytes from one liquid into another, volatile organics are better separated by extracting into a gas phase. Pervaporation refers to the separation of a liquid mixture by partial vaporization through a porous, or a non-porous membrane. The sample flows on one side of the membrane, and the volatile species permeate as

a gas to the other side, which is maintained under low pressure, or just purged with a stream of carrier gas. Theoretically, pervaporation can be viewed as a three-step process comprising of partitioning from the donor to the membrane, diffusion through the membrane followed by evaporation into the gas phase [51].

1.6 Analysis of s-Triazine Herbicides and Their metabolites in Environmental water samples

Among the different spheres of the environment (the atmosphere, the hydrosphere and the lithosphere), the hydrosphere is given special attention. This is because water is vital for life. We need water not only for drinking but also for different purposes. The water systems or the hydrosphere can be subdivided into natural waters and wastewaters. Natural waters consist of ground, surface and drinking waters. Wastewater, on the other hand, is the water which has had its composition and properties changed by human activities [70]. The effect of anthropogenic chemicals in the environment depends, among other parameters, the composition and concentration of the chemicals. As a result, each body of water to be used needs analysis for its chemical composition and concentration on a regular basis. One of these chemicals available in this regard are pesticides which are used to control weeds, herbs and insects that affect the germination, growth, ripening and harvest of various agricultural products. Today there are more than 1500 chemical substances in use as pesticides all over the world [71].

Pesticides are among various types of anthropogenic chemicals that contaminate any one of the natural water bodies. The cause of contamination of water systems by pesticides and their transformation products can be attributed to several sources. The pesticide pollution can generally arise from point source contamination; like effluent from pesticide pollution, direct introduction of pesticides. Considering the aquatic environment, for example, the chemical substances can reach surface water through runoff, leakage from nearby rivers or by wind and ground water by passing through the soil profile as a result of the action of percolating water Thus the water bodies inevitably contain a considerable quantity of the pesticide residues and their degradation products sufficient to affect their quality and thus harm the human and aquatic lives [72, 73].

Water quality, on the other hand, has received considerable attention in recent years. Owing to the environmental impact of pesticides, several priority lists have been published to protect the

quality of drinking and surface water [74]. For instance the current European Union (EU) directive dedicates the concentration of pesticides should not exceed a maximum admissible concentration of 0.1 µg/L for individual pesticides and at 0.5 µg/L for the total pesticide content in drinking water. The maximum residue levels of total herbicides ranging from 1–5 µg/L in pre-treatment potable water and 50–100 µg/L in river water. In surface water, these limits are about an order of magnitude higher (1-3 µg/L). Such strict limits imply lower limits of detection for analytical methods. Recommended limits of detection for drinking water are in the 0.01-0.02 µg/L range [75-77]. Therefore it is very important to determine the level to which the residues of pesticides that are accumulated in environmental water bodies.

Consequently, analytical separation techniques are needed which can detect pesticide residues and their transformation products (metabolites) at ultra-trace levels in various types of complex environmental samples. The detection level of the residues, especially in the environmental samples, seem to depend more on the isolation and enrichment procedure chosen than on the method used for final determination [78].

The current research work is based on the utilization of hollow fiber supported liquid membrane extraction for trace analysis of residues of atrazine and its major degradation products. Literature survey on the aspects of the indicated work revealed that no such analytical methodology and procedure has been developed for systematic extraction of the analytes in question. To this end, the main purpose of this research work centered at developing a new and relatively simpler technique based on hollow fiber supported liquid membrane extraction for selective isolation of the target analytes, indicated above, from matrices of environment waters and human urine, and the final analysis of which to be performed using high performance liquid chromatography.

1.7 Objective

1.7.1 General Objective

The general objective of this project is to enrich s-triazine herbicide residues and their metabolites in environmental water and human urine samples utilizing a hollow fiber liquid membrane extraction technique and determine their levels by high performance liquid chromatography.

1.7.2 Specific Objectives

1. To determine the linear calibration of selected s-triazine herbicides and their metabolites so as to determine their levels using liquid chromatographic method.
2. To establish optimum extraction parameters by performing blank extraction and standard sample extraction utilizing hollow fiber supported liquid membrane extraction methodology.
3. To perform method validation on spiked samples.
4. To collect water samples such as tap water from tap water used in Addis Ababa University, river water from Awash River and human urine samples from students learning at Addis Ababa University.
5. Finally, to apply the method of extraction as sample preparation and enrichment method for the determination of trace levels of selected s-triazine herbicides and their metabolites in environmental water samples and determine levels of the herbicides and their metabolites in selected water systems and compare the result with the maximum residue levels (MRLs) set by European Union.

2 EXPERIMENTAL

2.1 Chemicals and Materials

The s-triazine herbicides and their metabolites that were used in this study include atrazine (ATZN), desethylatrazine (DEA), desisopropylatrazine (DIA), didealkylatrazine (DDA), and hydroxyatrazine (ATOH). All the herbicides were reference materials for residue analysis purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The organic solvents used for immobilizing into the membrane were undecane, di-n-hexyl ether and 1-octanol all obtained from Sigma-Aldrich Chemie (steinheim, Germany). Humic acid (ALDRICH, Germany) and Sodium chloride (Labmerk Chemicals PVT Ltd., India) were used in optimization parameters. Other chemicals including phosphoric acid obtained from Sigma Chemical co. (st. Louis, USA), potassium dihydrogen phosphate obtained from Fluka Chemie AG (Bushs, Swizerlands), dipotassium hydrogen phosphate Alanar® BDH laboratory supplies (poole, Ingland), and HPLC-grade acetonitrile and methanol purchased from Techno Pharmchem (Haryana, India) were also used in the experiments. In addition, NaOH and HCl were used to adjust sample pH. All reagents and solvents used in this study were of analytical grade or HPLC grade.

Hollow fiber membrane, 50/280 Accurel® PP polypropylene hollow fiber tubing (50 μm wall thickness, 280 μm inner diameter, 0.1 μm pore size was obtained from Membrana GmbH (Wuppertal, Germany). BD MicroFine Syringes (with needle of 0.30mm outer diameter and 8mm length, 0.5 ml prepared for U-100 insulin injection) obtained from BD Consumer Healthcare (Franklin Lakes, USA) were used to fill the acceptor into the lumen of the hollow fiber for extraction and to flush out the acceptor into a small glass vial (200 μL) after extraction.

Other materials such as conical flasks (50, 100, 200 and 500 mL), volumetric flasks (5, 50, 200, 500 and 1000 mL), pipettes (0.5, 1, 2, 5 and 10 mL), oven, electronic analytical balance, sonicator, filtrating apparatus with Vacuum pump, cellulose acetate filter papers (0.22 and 0.45 μm Millipore, and Whitman no. 1 and 42), distiller, deionizer and vials (0.200 and 2 mL) were used in the process of sample preparation and analysis.

2.2 Instruments

The HPLC system used for the analysis was Agilent 1200 series equipped with Quaternary Pump, Agilent 1200 Series Vacuum Degasser, Agilent 1200 series Autosampler and Agilent 1200 Series Diode Array detector Purchased from Agilent Technlogies (Hewlett-Packard-Strasse Waldbronn, Germany). Chromatographic separation of the compounds was performed on a C_{18} analytical column (Techsphere 5ODS, 25 cm x 4.6 mm ID; HPLC Technology, Macclesfield, Cheshire, UK). The samples were shaken using HT Infors orbital shaker from Infors AG (Bottmingen, Switzerland). A pH meter, Hanna Instruments (Portugal) was used to adjust the sample and buffer solution pH and data manipulation was carried out by B.02.0x revision Agilent ChemStation software.

2.3 Standard Solution Preparation

100 mg/L standard stock solutions were prepared from standards of s-trizine herbicides and their degradation products as follows: Atrazine, desethylatrazine and desisopropylatrazine were dissolved in acetonitrile, and desethyldesisopropylatrazine was prepared by dissolving in 5 ml of reagent and 5 ml of acetonitrile. Hydroxy product of atrazine, hydroxyl-2-atrazine was first dissolved in 1 ml of 1.0 M HCl. All the resulting solutions were diluted to the final volume with acetonitrile. All stock standard solutions were stable and stored at 4^0C when not in use.

A mixed working standard solution of 20 mg/L, containing each of the herbicides was prepared every week by mixing appropriate amount of the stock solution and diluting with reagent water to a given volume. A series of concentrations of standard solutions for calibration were prepared in the concentration range of 10 to 500 μg/L at five points (10, 50, 100, 250, and 500 μg/L). A 0.5 mg/L mixture containing all herbicides was prepared from the 20mg/L standard solution for spiking. Evaluation of the precision was based on triplicate injections and peak area was taken as instrumental response which was finally converted to enrichment factor for comparison. Acceptor solution was prepared by taking appropriate amount of 37% HCl acid in 50 mL and diluted to the final volume with reagent water. The donor sample solution was prepared by taking appropriate amount of the mixture solution and diluting to the required volume and then pH was adjusted to 7.00 by phosphate buffer.

2.4 Chromatographic Conditions

Chromatographic separations were carried out using gradient elution with 3.5 mM phosphate buffer at pH = 7 as phase A and acetonitrile as phase B as the profile shown in Table 3. The gradient program was as follows: 10% acetonitrile increasing to 15% during 5 min, then constant until 10 min and further increasing to 70% until 33 min, constant until 37 min and thereafter restored to 10% during 1 min followed by a 5 min equilibration time. A 3.5 mM phosphate buffer solution of pH 7 was prepared by dissolving 118 mg of dipotassium hydrogen orthophosphate, K_2HPO_4 and 146 mg of potassium dihydrogen orthophosphate, KH_2PO_4, in a 500 mL volumetric flask and diluting the solution to the final volume with reagent water. Its pH was adjusted to 7 using diluted solution of potassium hydroxide and hydrochloric acid. The flow rate was 1mL/min and the column temperature was maintained at 25 ^{0}C. Five microlitres of extracted was injected and eluted using the gradient program for 28 minutes run time. The detection was performed at 235nm wavelength for each analytes.

Table 3. Gradient elusion program profile

Time (min.)	Phosphate buffer, A (%)	Acetonitrile, B (%)
0	90	10
5	85	15
10	85	15
33	30	70
37	30	70
38	90	10
43	90	10

2.5 Membrane Preparation and Extraction Procedure

The extraction procedure described by T. Berhanu et al. was followed in this study [79]. Briefly, the procedure was as follows: The hollow fiber was cut manually into approximately 20 cm length and the two ends were looped together, to give appropriate shape, leaving both ends free for subsequent use. Then, the lumen of a single hollow fiber was flushed and filled with the acceptor solution using the BD Micro-Fine syringe. Afterwards the fiber was dipped in organic solvent for 30 seconds to impregnate the pores of the hollow fiber wall forming the organic liquid membrane. Then, slowly the lumen of the fiber was flushed with more acceptor solution to

remove any organic solvent and air bubbles from the lumen and filling it completely. The two ends of the fiber were folded and enveloped with a piece of strip of aluminum foil and inserted into a small piece of glass tubing. Subsequently, the filled and sealed fiber was rinsed with reagent water and this ready hollow fiber supported liquid membrane device was transferred to 200mL sample solution. After shaking the whole setup using an orbital shaker at 150 RPM on an orbital shaker for 5 hours, the acceptor solution containing the analyte was collected into 200 μL vials with inserts. The collection of the sample was as follows: one of the ends of the sealed fiber was cut and connected to a retracted syringe needle and the other end then cut and put in the vial. Next, the syringe plunger was pushed in, to dispense the acceptor solution containing analyte into the vial. The acidic acceptor solution, 1M HCl, was neutralized by 7 M NaOH solution. After capping, the vial was put on the autosampler of the HPLC for injection. Approximately 10–12 μL solution was collected and 5 μL was injected into the HPLC system.

3 RESULTS AND DISCUSSION

3.1 Optimization of the HFSLM Extraction Parameters

Different parameters that influence the extraction of the pesticides between the blank standard solution and the acceptor solution were optimized. Conditions for the HFSLM device were tested using blank standard solution with 0.5 mg/L of each pesticide spiked in reagent water using parameters such as selection of membrane solvent, and effect of acceptor and sample solution pH, extraction time and shaking speed, salinity of sample solution and humic acid on enrichement factor. To obtain the optimized extraction conditions, enrichment factor was selected as response because the enrichment factor (E_e) is often used to evaluate the performance of the extraction. It is defined as the ratio of the concentration of analyte in the acceptor (C_A) after extraction divided by the concentration in the sample before extraction (C_S) [80]: $E_e = C_A/C_S$. In membrane extraction high concentration enrichment can be obtained at a low recovery because the acceptor volume is generally much lower than the sample volume and the analyte is irreversibly trapped in the acceptor solution.

3.1.1 Selection of Membrane Solvent

To study the composition of the organic solvent in HFSLM extraction, the selection is based on proper immobilization of pores of fiber, immiscibility of solvent with water and stability. Dihexyl ether, n-undecane, and 1-octanol were tested as organic solvents immobilized in the membrane. Extraction of the analytes was carried out from 0.5 mg/L concentrated standard solution of each pesticide using the organic solvents; 1-octanol, n-undecane and dihexyl ether using 1M HCl accepter solution for three hours at 100 rpm shaking speed. The pesticides were not extracted at detectable level when 1-octanol and n-undecane were tested, and they were only extracted when dihexyl ether was used.

3.1.2 Effect of Acceptor Solution pH and Sample Solution pH

In the theoretical treatment of the supported liquid membrane extraction technique for maximum enrichment factor, it has been shown that the acceptor pH is critical and should be at least 3.3 units below the lowest pKa value of basic compounds for best performance [81]. The pKa values of the compounds investigated ranges from 1.30 to 5.15 (see Table 1). This means that the

acceptor solution ideally should have a pH of least less than −2.0. To this effect, the effect of the pH of the acidic acceptor solution was studied using HCl consisting of 50 mM, 100 mM, 250 mM, 500 mM and 1000 mM HCl, obtaining the results shown in Fig. 16. It can be observed that the better results were obtained when 1000 mM of HCl was used. But the above theoretical consideration that is pH below 0.0 was not fully met for some of the compounds and these could therefore not be completely trapped. However, it has been shown in earlier studies [81] that even under incomplete trapping situation, useful maximum enrichment factors can be reached. Therefore, 1 M HCl solution was used as an acceptor solution. Since it was not desirable to inject 1 M HCl directly into the LC system, 7M NaOH was used to neutralize the collected acceptor solution before the chromatographic analysis, in order to obtain reproducible results.

The enrichment factor in liquid phase microextraction of a weak organic base and acid also depends on the pH of sample solution. The sample solutions were often adjusted to appropriate alkalinity to de-ionize atrazines and their metabolites for obtaining higher enrichment factor. In this case, solutions of the herbicides containing 0.5 mg/L of each of the herbicides under study were spiked with 5 mM phosphate buffer, ranging in pH from 2.0 to 8.0. The buffers were prepared from H_2PO_4/KH_2PO_4 (pH=2), KH_2PO_4 (pH=4) and KH_2PO_4/K_2HPO_4 (pH=6.0-8.0). Fig. 17 showed the profiles of the enrichment factor with varied pH, which indicates that, the enrichment factor increases with the increasing of pH in pH range between 2 and 7, and then decreases for each analytes. Therefore, pH 7.0 was chosen as the final sample solution pH value for subsequent studies.

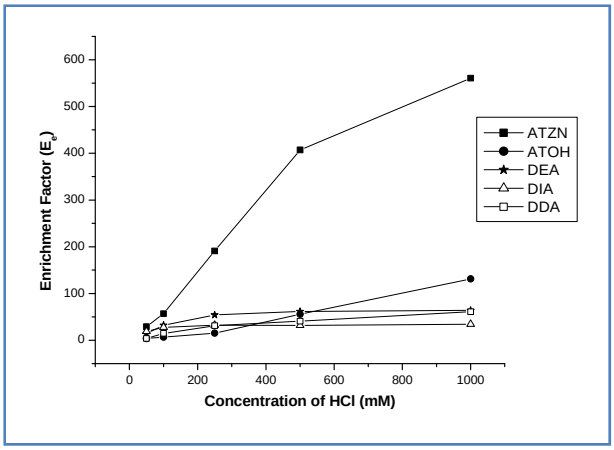


Figure 16. Effect of acceptor pH on the enrichment factor of the triazine compounds.

Conditions: spiked concentration of 0.5 mg/L for each triazines, shaking speed 150 rpm, sample volume 200 mL, time of extraction 3 hrs, extracted with various concentration HCl acceptor solutions.

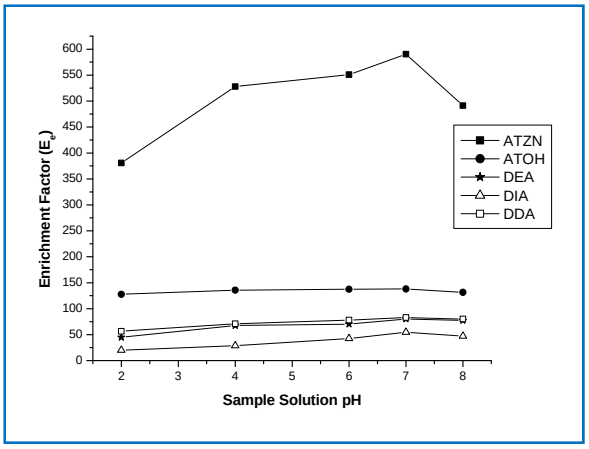


Figure 17. Effect of sample solution pH on the enrichment factor of the triazine compounds.

Conditions: spiked concentration of 0.5 mg/L for each triazines, shaking speed 150 rpm and 200 mL buffer solutions with various pH (pH adjusted by 5 mM phosphate buffer) spiked with the triazines extracted with 1.0 M HCl for 3 hrs.

3.1.3 Effect of Sample Volume

It is clear that both two-phase and three-phase LPME may provide very high analyte enrichments, since the V_D/V_A ratio is normally high. Thus, if, e.g., the volume of donor solution is 5 mL and the volume of acceptor solution is 2 µL, analytes may be enriched by a factor up to 2500. Thus, sample volume is one of the major factors making LPME very attractive, especially for relatively small sample volumes, as similar enrichment may not be obtained with SPE or LLE [82]. To this effect, effect of sample volume on enrichment factor was studied by taking 50, 100, 200 and 500 mL of buffer solutions (pH=7 adjusted by 5 mM phosphate buffer) spiked with the triazine herbicides extracted using 1.0 M HCl accepter solution. As can be seen from Fig. 18, the enrichment factor increases with increasing sample volume and begins to decrease after 200 mL of sample volume for each analytes. This is reasonable because as sample volume increases, the amount of analytes also increases, but the decreasing in the enrichment factor after 200 mL of sample solution is probably due to lower in the shaking speed that unable to mix large volume of sample solution. To keep the volume of the sample at certain constant value, 200 mL of sample volume has been chosen and used for subsequent experiments.

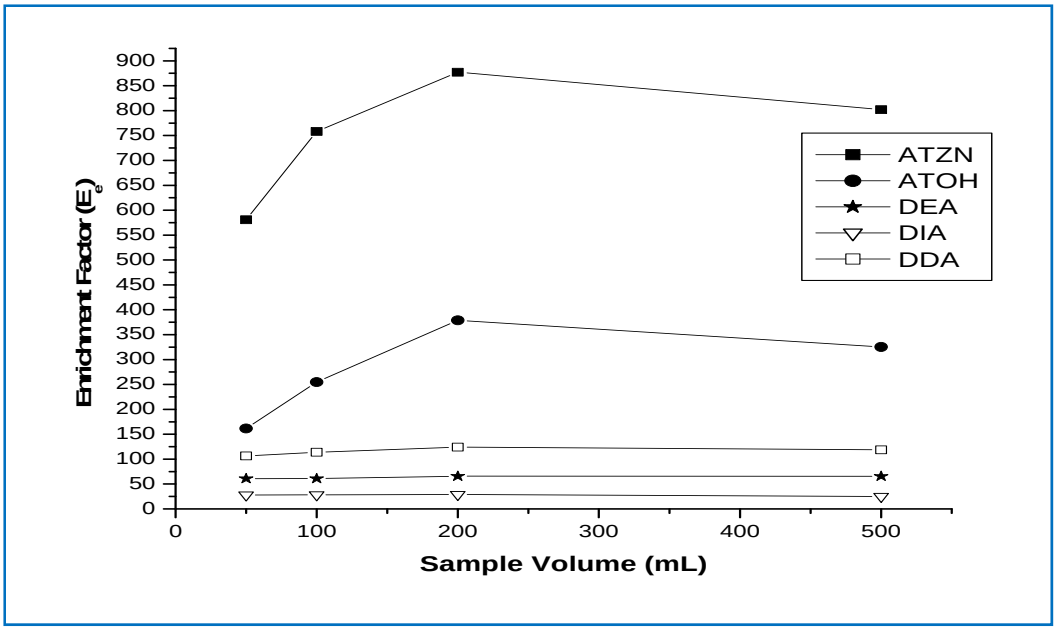


Figure 18. Effect of sample volume on the enrichment factor of the triazine compounds.

Conditions: buffer solutions with pH 7.0 (pH adjusted by 5 mM phosphate buffer) spiked with of 0.5 mg/L of each triazines, shaking speed 150 rpm, extracted using 1.0 M HCl acceptor solution for 3 hrs.

3.1.4 Effect Shaking Speed and Extraction Time

Once the donor and acceptor phases have been optimized, the influence of hydrodynamic conditions was studied. As the extraction process is done in kinetic uptake regime, the system is subjected to the effect of many dynamic processes (static, stirring and shaking). One dynamic process which will affect the enrichment factor of the extraction is the agitation of the sample. Agitation can be done either by stirring or shaking. A preliminary study was carried out comparing static, stirring and shaking conditions. Extraction efficiency is the highest when the sample is shaking. This may be explained by the fact that during shaking, the fiber itself moves and mixes the acceptor solution in the lumen of the fiber and facilitates the transfer of the analytes into the acceptor phase at faster rate. It should also be noted that when the static mode is used, very low E_e was obtained. As shaking gives the best enrichment within a given time, the effect of shaking speed was investigated and shown in fig. 19. It can be observed that with increasing shaking speed, the enrichment factor also increases up to a certain maximum value. This is so because the diffusion coefficient in the aqueous phase increases with increasing agitation rate, due to faster agitation rate decreases the diffusion layer in the aqueous phase

around the surface of the membrane. Thus, mass transfer increases and also it permits the continuous exposure of the extraction membrane surface to fresh aqueous sample. Sufficient E_e was obtained when shaking speed is 150 rpm. However, a higher E_e can be obtained by increasing extraction time, keeping shaking speed constant, because under diffusion controlled conditions, it is possible to reduce shaking speed by increasing extraction time or vice versa to obtain a maximum enrichment factor.

HFSLM is a three-phase extraction system with two liquid–liquid interfaces; as a result, the analyte molecules need to have enough time to diffuse through each phase and cross all interfaces to get into the acceptor phase. Therefore, controlling the extraction time is critical when working in the kinetic regime. The amount of analyte extracted increases with longer extraction time before any kind of equilibrium is attained until a maximum is obtained near the equilibrium. Hence, the influence of extraction time on the enrichment factor of the four analytes was studied. Fig. 20 shows the effect of extraction time on the enrichment factor of the compounds in HFSLM. For this work, 5 h extraction time, which is at the linear position of the curve, was chosen for subsequent experiments. Even though, the extraction time in this regard is relatively long, it is possible to do many parallel extractions within this time.

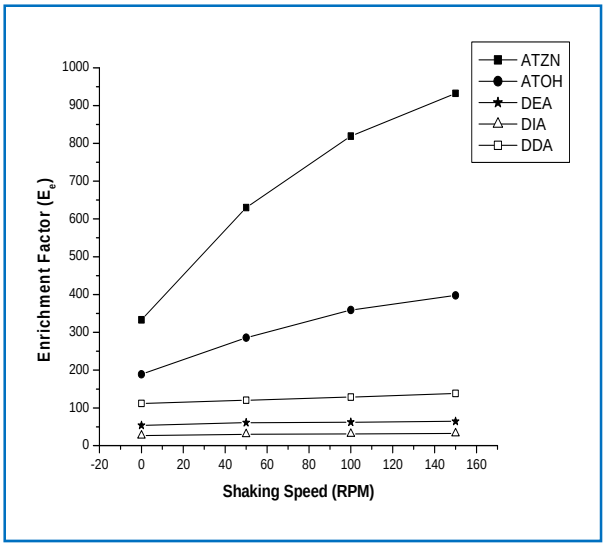


Figure 19. Effect shaking speed on enrichment factor of the triazine compounds.

Conditions: spiked concentration 0.5 mg/L for each triazines, 200ml buffer solutions (pH=7 adjusted by 5 mM phosphate buffer) spiked with the triazines extracted with 1.0 M HCl for 3 hrs for various shaking speeds.

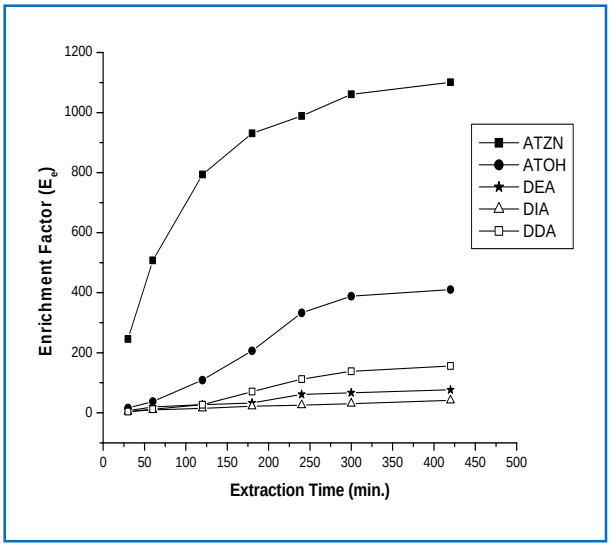


Figure 20. Effect of extraction time on the enrichment factor of the triazine compounds.

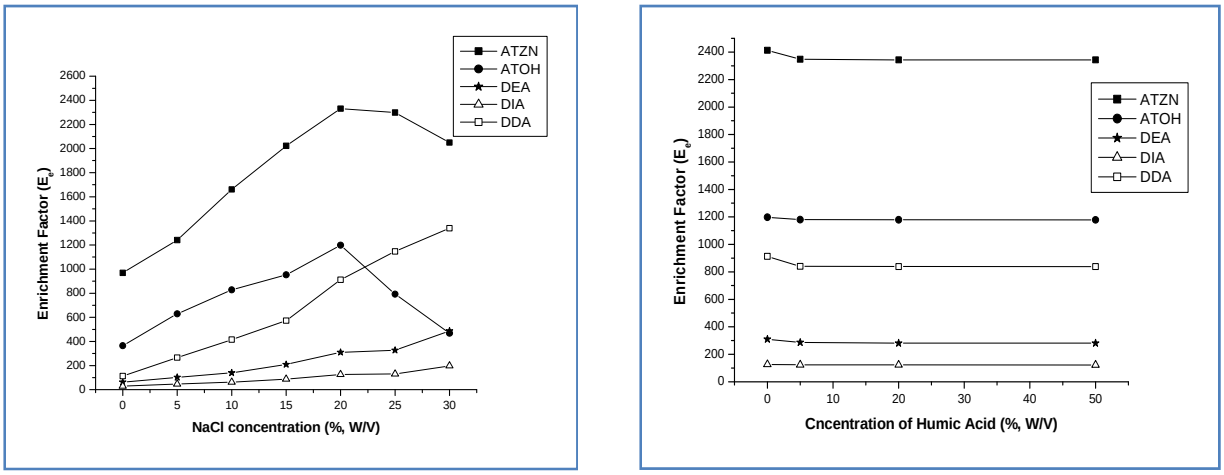
Conditions: spiked concentration of 0.5 mg/L for each triazines, shaking speed 150 rpm, 200 mL buffer solutions (pH=7 adjusted by 5 mM phosphate buffer) spiked with the triazines extracted with 1.0 M HCl, for various extraction time.

3.1.5 Effect of Salinity of Sample Solution and Humic Acid

In most traditional extraction processes, extraction of analytes can be enhanced or retarded by addition of salts depending on the nature of analytes [79]. In this study, various amounts of sodium chloride (NaCl) were added into the sample solution to investigate the effect of salinity on the extraction. As a result, the effect of the salinity of the sample was studied by adding 0, 5, 10, 15, 20, 25 and 30% (w/v) of NaCl to the sample solutions. Fig. 21(a) depicts the effect of concentration of NaCl solutions on the enrichment factors of the five herbicides in reagent water. The enrichment of more hydrophobic analytes (atrazine and atrazine-hydroxy) increased significantly initially and begins to decrease as more salt has been added. The decrease is possibly due to electrostatic interaction of salt molecules with analyte molecules may begin when salt concentration increased further.

Humic acids are dissolved organic carbon (DOC) often present in natural water at various concentrations mainly ranges from 0 to 50 mg/L [79]. The presence of such compounds might

complicate the extraction. The influence of four different concentrations of humic acid on the enrichment factors of the compounds was tested in the concentration range of 0–50% (w/v) as shown in Fig. 21(b). The experimental results shown indicate that the addition of humic acid did not significantly affect the enrichment factor because one way ANOVA test revealed that there is no statistically significant difference between the mean enrichment factors of each analyte from one level of humic acid concentration to another in the range tested at 95% confidence level. This is because humic acid (pKa = 5.5) [83] presented mainly in ionized form at sample pH 7.0 and thus prohibited its transfer through the hollow fiber supported liquid membrane device. The ionization of humic acid also decreased its binding to analytes that is helpful for extraction of analytes. Furthermore, there was no interfering peak at the retention times of the analytes with HPLC analysis upto 50% (w/v) concentration of humic acid.



(a) **(b)**

Figure 21. (a) Effect of the salinity of the environment on the enrichemnt factor and (b) effect of concentration of humic acid on the enrichemnt factor of the triazine compounds.

Conditions: spiked concentration of 0.5 mg/L for each triazines, shaking speed 150 rpm, 200 mL buffer solutions (pH=7 adjusted by 5 mM phosphate buffer) spiked with the triazines extracted with 1.0 M HCl for 5hrs.

3.2 Method Validation

3.2.1 Linearity, Limits of Detection and Quantification, Repeatability, and Reproducibility

Once the method had been optimised, its analytical performance was studied in reagent water because optimization for the HFSLM device was tested using reagent water. The following characteristics were investigated: linearity; limit of detection (LOD); repeatability; and reproducibility. The results are shown in Table 4.

Limit of detection is the concentration derived from the smallest response or signal that can be detected with reasonable certainty for a given analytical procedure [84]. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated by analyzing blank spiked samples, and they were determined as the lowest concentrations of the analytes that produce chromatographic peaks. The LOD and LOQ values were determined using peak height measurements, since accurate peak area measurements are difficult due to tailing or fronting of peaks at lower concentrations. In chromatographic analysis; LOD and LOQ are usually defined as concentrations that give a signal to noise ratio of 3:1 and 10:1 respectively, and are expressed as the concentration of the analyte [61]. The LOD and LOQ values were calculated accordingly for the triazines, in different matrices considered.

Linearity is determined by calculating the regression line using a mathematical treatment of the results (*i.e.*, least mean squares) versus analyte concentration. In this case, linear regression with proportional weighting was calculated for the plot of peak area versus concentrations of the analytes. Linearly proportional relationship between the amount of the extracted analyte and its initial concentration in the sample matrix is critical in developing any sample preparation technique. To this effect, linearity of the method was tested by running five extraction solutions containing analyte with concentrations between 10 and 500 µg/L (10, 50, 100, 250, 500 µg/L) in reagent water. All of the analytes exhibited good linearity with squared regression coefficients (r^2) ranged from 0.9944 to 0.9999. Limits of detection of the method ranged from 0.03 to 0.75 µg/L and the limits of quantification from 0.10 to 2.50 µg/L, (see Table 4.) which were low enough to determine all the herbicides residues except desisopropylatrazine at concentrations below or equal to the maximum residue levels (MRLs) specified by European Union.

Table 4. Validation characteristics of the HF-SLM for the five herbicides in reagent water

Analyte	Linear range (μg/L)	LOD (μg/L)	LOQ (μg/L)	Regression equation	r^2, [a]	Rept.[b], 50 μg/L, (%, n=3)	Repd.[c], 50 μg/L, (%, n=4)
ATZN	10-500	0.03	0.10	Y=18777.7x- 30.308	0.9944	4.59	11.31
ATOH	10-500	0.08	0.27	Y=13977.7x+5.428	0.9996	10.50	16.21
DEA	10-500	0.40	1.33	Y=2808.5x-11.537	0.9999	8.49	16.10
DIA	10-500	0.75	2.50	Y=1048.2x-11.537	0.9997	6.21	13.80
DDA	10-500	0.18	0.60	Y=2627.2x-24.600	0.9997	3.14	15.90

[a] regression coefficient, [b] repeatability, [c] reproducibity, LOD & LOQ: limit of detection and quantification, respectively.

Reproducibility and repeatability studies were conducted in order to evaluate the precision of the method. The repeatability (intra-day precision) of the method, expressed as relative standard deviation (RSD), for the compounds was investigated by analysing reagent water three samples spiked to a final concentration of 50 μg/L. The RSD values were below 11%, which is acceptable. The reproducibility of the method (inter-day precision) was studied by analysing four samples on four different days, with a final analyte concentration of 50 μg/L. The RSD values in this case were below 17% for reagent water. Deviations observed in the reproducibility test might be the result of differences in wall thickness and pore size as well as manual handling of the fibers. The method shows very good repeatability and reasonable reproducibility at such low concentration of analytes and thus shows the analytical applicability of HFSLM extraction for trace enrichment of the triazine herbicides and other related polar contaminants in environmental water samples.

3.3 Applications

The optimised and validated HF-SLM method was applied for extraction of parental triazine (atrazine) and triazine metabolites (deethylatrazine, desisopropylatrazine, didealkylatrazine and hydroxyatrazine) in environmental water samples (tap water and river water) and human urine samples. Tap water was collected using grab sampling at three points from tap water used in Addis Ababa University, Ethiopia and river water was collected different depths of Awash River which is located about 50km from Addis Ababa, Ethiopia. Human urine sample was collected from five students learning at Addis Ababa University, Ethiopia.

None of the analytes were detected in the tap water, river water and human urine samples. As a result, the applicability of the method was further investigated by extracting spiked tap water, river water and human urine samples with known different concentrations, and the linearity, limit of detection and limit of quantification of the method was compared with that of the reagent water. The linearity was studied in the analytes concentration range of 50–500 μg/L. As shown in table 5, all analytes exhibited good linearity with squared regression coefficients (r^2) ranging from 0.990 to 0.9998, using peak area as a response variable. The detection limit of the method for the analytes in the sample matrices considered ranges from 0.03 to 1.12 μg/L. That is of atrazine in tap water sample and desisopropylatrazine in human urine sample, respectively. The quantification limit ranges from 0.10 μg/L for atrazine in tap water samples to 3.73 μg/ L for desisopropylatrazine in human urine samples. The detection and quantification limits obtained are below the maximum residue limits set by EU and other regulatory bodies for all analytes in river water and all analytes except desisopropylatrazine in tap water.

Table 5. Linearity, limit of detection and quantification of the optimized analytical method for extraction of the triazine herbicides in tap water, river water and human urine samples

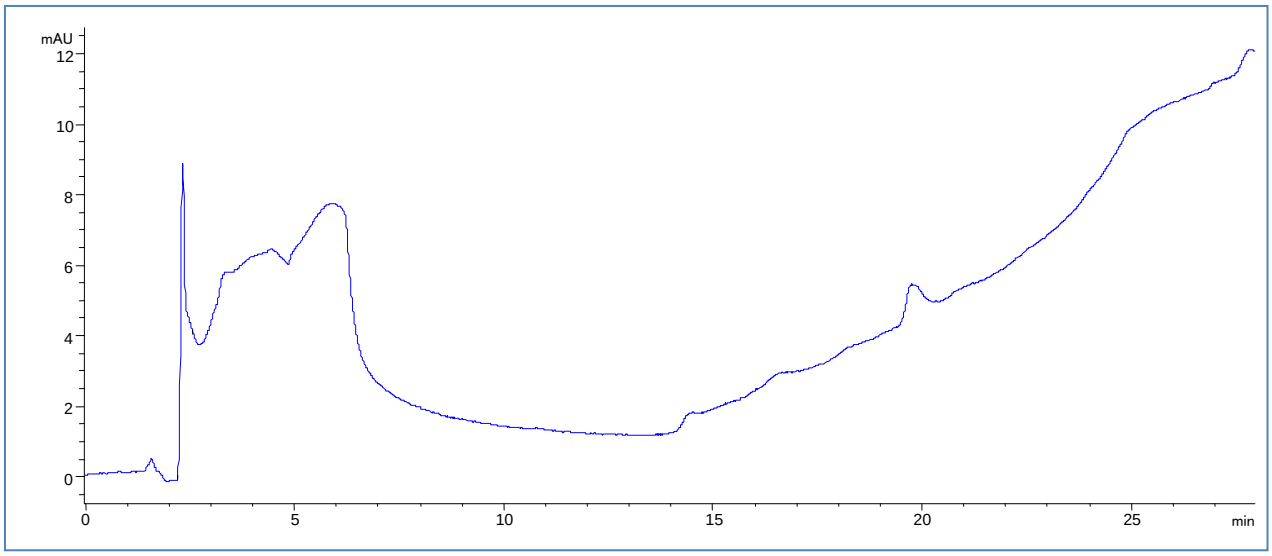
Matrices	Analytes	Conc. range (µg/L)	LOD (µg/L)	LOQ (µg/L)	Correlation coefficient	Enrichment Factor (E_e)	% RSD (n =3)
Tap water	ATZN	10-500	0.03	0.10	0.9924	2342.60	5. 21
	ATOH	10-500	0.09	0.30	0.9986	1260.57	11. 14
	DEA	10-500	0.44	1.47	0.9994	298.23	8. 61
	DIA	10-500	0.82	2.70	0.9990	106.13	9.10
	DDA	10-500	0.20	0.67	0.9991	981.86	5. 80
River water	ATZN	10-500	0.03	0.10	0.9950	2351.03	5. 89
	ATOH	10-500	0.09	0.30	0.9986	1255.75	7. 03
	DEA	10-500	0.45	1.50	0.9998	291. 32	10.47
	DIA	10-500	0.84	2.80	0.9991	101.36	6. 14
	DDA	10-500	0.20	0.67	0.9993	982.18	8. 88
Human urine	ATZN	10-500	0.05	0.17	0.9907	2351.03	6.09
	ATOH	10-500	0.12	0.40	0.9992	1265. 32	13.53
	DEA	10-500	0.48	1.60	0.9978	291. 05	9.93
	DIA	10-500	1.12	3.73	0.9961	103. 81	11.62
	DDA	10-500	0.27	0.90	0.9997	982. 63	13.42

3.4 Selectivity of the Analytical Method

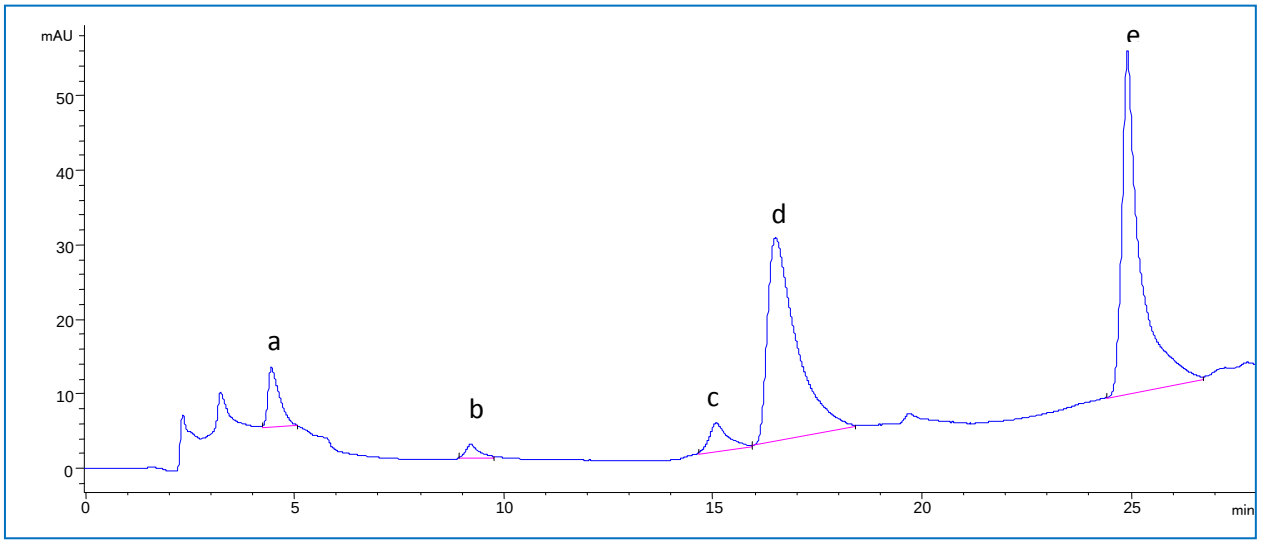
According to the latest IUPAC recommendation; 'selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other compounds of similar behavior' [85]. To obtain a high selectivity membrane extraction has a clear advantage over other sample preparation techniques, as all the compounds that reach the analytical instrument selectively cross the membrane to be enriched in the acceptor phase [86]. The term *selectivity* in membrane extraction techniques can be interpreted in two ways: first, as the ability to distinguish among molecules according to their size and, secondly, as the differentiation of compounds of the same molecular mass. The selectivity of membranes used in the membrane extraction techniques depends primarily on the membrane material (physical state, morphology, structure, and polarity), on the properties of the

donor and acceptor phases (pH value, polarity), and on the properties and concentration of analytes. By selecting proper values of the above parameters, the selectivity of a given membrane module can be adjusted [68].

By implementing the optimum sample treatment and sample storage conditions and then applying all the optimized liquid membrane extraction and HPLC separation conditions, the selectivity of the method can be enhanced. The selectivity of the optimized liquid membrane extraction method in the environmental water and human urine sample matrices can be evaluated from the final chromatograms obtained for the extracts. No interfering peaks were observed around the retention times of all the triazines, which can easily be compared from the chromatograms of the unspiked and spiked environmental water and human urine samples extract as shown in figures 22 to 24.

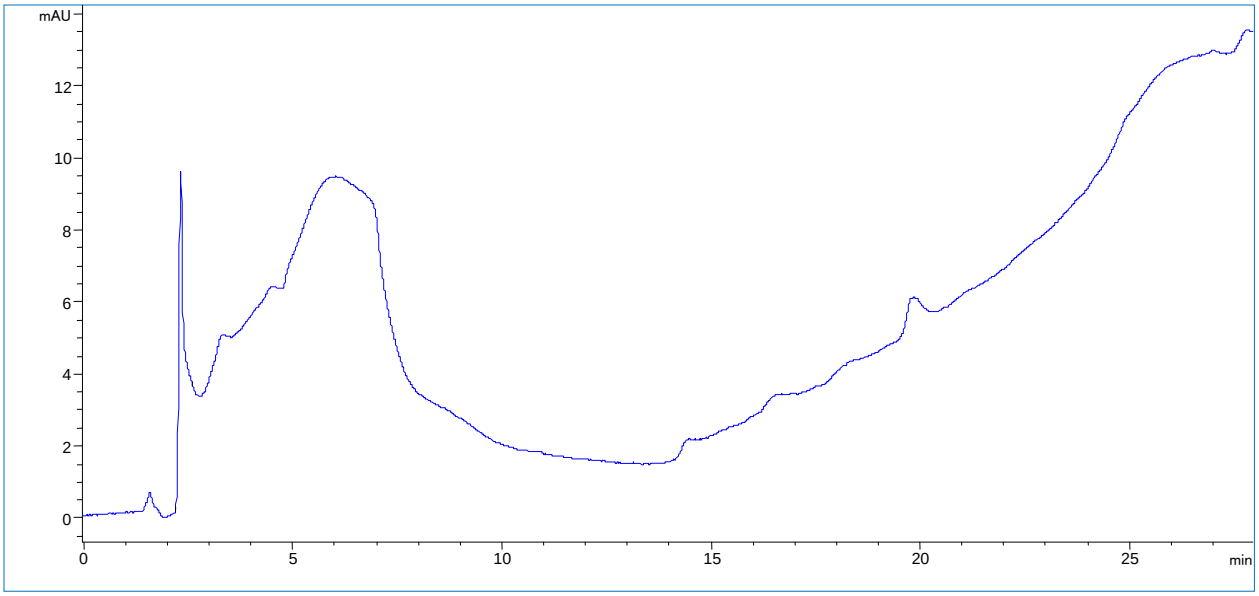


(i)

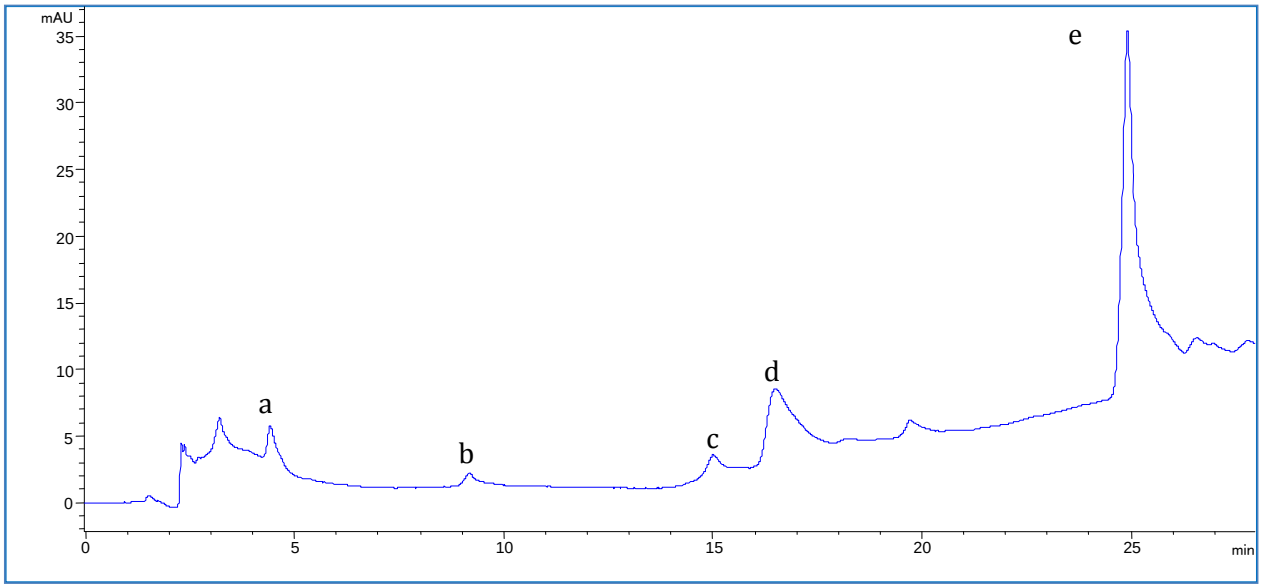


(ii)

Figure 22. HPLC-UV chromatograms for 5 µL injection of extracts obtained after 5 hrs enrichment of (i) unspiked tap water extract and (ii) 0.05 mg/L triazine standard mixture spiked tap water extract **(a: DDA, b: DIA, c: DEA, d: ATOH, and e: ATZN)**

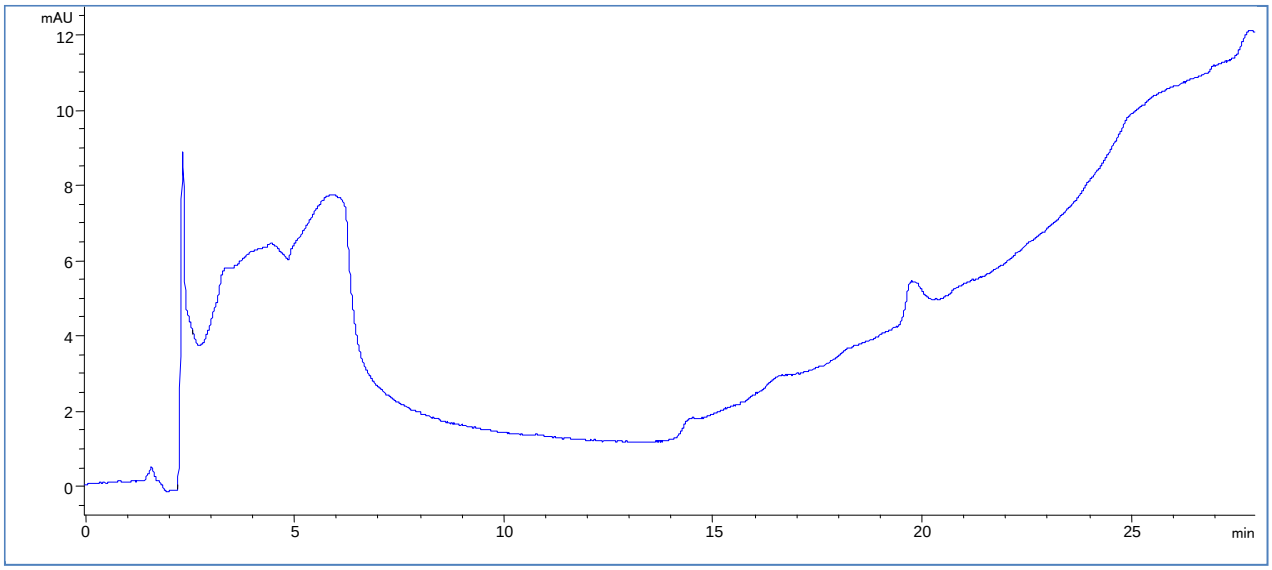


(i)

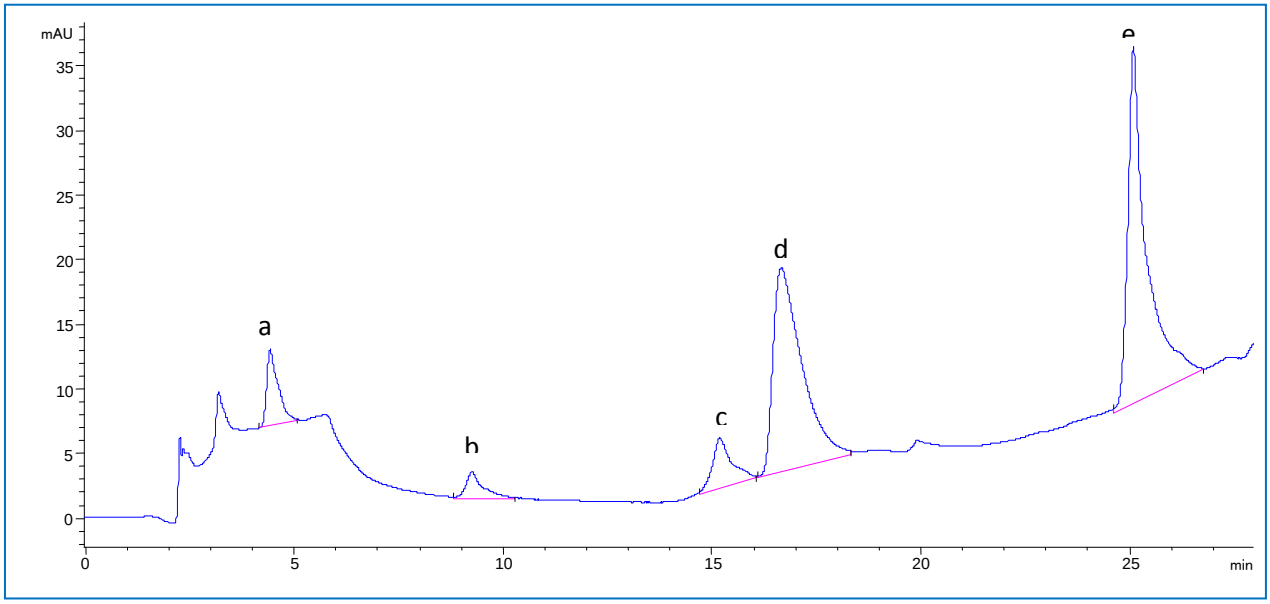


(ii)

Figure 23. HPLC-UV chromatograms for 5 µL injection of extracts obtained after 5 hrs enrichment of (i) unspiked river water extract and (ii) 0.01 mg/L triazine standard mixture spiked river water extract. **(a: DDA, b: DIA, c: DEA, d: ATOH, and e:ATZN)**



(i)



(ii)

Figure 24. HPLC-UV chromatograms for 5 µL injection of extracts obtained after 5 hrs enrichment of (i) unspiked human urine extract and (ii) 0.05 mg/L triazine standard mixture spiked human urine extract **(a: DDA, b: DIA, c: DEA, d: ATOH, and e: ATZ).**

4 Conclusion and Recommendations

The hollow fiber supported liquid membrane extraction methodology has the advantages of low organic solvent consumption, and low cost, and is simple to perform compared to other liquid membrane extraction techniques. The limitation of the method is the long extraction time as compared with other equilibrium extraction devices like SPME, but this is efficiently offset by the cheap materials and simple handling, permitting the extraction of many samples in a parallel way.

The proposed method was validated for three samples, selecting reagent water as a representative matrix for the analysis of tap water, river water and human urine samples and showed good linearity, limit of detection, limit of quantification and acceptable repeatability for triazines considered in the study. The method also showed better linearity for all the triazines, with linear curves of correlation coefficients greater than 0.990 and average percent relative standard deviation (% RSD) values less than 14% for all matrices considered in the study. The detection limit of the method for the analytes in the sample matrices considered ranges from 0.03 to 1.12 µg/L. That were of atrazine in tap water and river water samples, and desisopropylatrazine in human urine sample, respectively. The quantification limit ranges from 0.10 µg/L for atrazine in tap water and river water samples to 3.73 µg/ L for desisopropylatrazine in human urine samples. The detection and quantification limits obtained are below the maximum residue limits set by EU and other regulatory bodies for all analytes in river water and all analytes except desisopropylatrazine in tap water. Therefore, proposed HFSLM extraction technique appears to be suitable for determination of trace level triazines and their degradation products in environmental water and biological aqueous sample matrices. It is also suitable for individual or total extraction of analyte/s in environmental water samples with high enrichment factors under the optimized extraction conditions.

The method can be further optimized for the same analytes or other similar polar contaninants and successfully applied to other environmental water or biological aqueous sample matrices for extraction. By varying the same or other HF-SLM extraction parameters, it could be possible to obtain even better selective extraction with higher enrichment factor and lower detection and quantification limits. For example, higher enrichment and lower detection and quantification

limits might be obtained by processing large volume of samples if the sample volume is not a limiting factor and using automated-online HF-SLM extraction methods that are now days developing.

5 REFERENCES

[1]. T. Berhanu, *Ph.D. Thesis,* Addis Ababa University, Addis Ababa, Ethiopia, (2008).

[2]. Jose L., Tadeo, *Analysis of pesticides in Food & Environmental Samples.* CRC press, Boca Raton, (2008).

[3]. N. Megersa, *Ph.D. Thesis,* Addis Ababa University, Addis Ababa, Ethiopia, (2000).

[4]. *Food and Drug Administration;* Pesticide Program, Residue Monitoring, **(**1996).

[5]. http://www.agf.gov.bc.capesticidesc_2.htm (Accessed December, 2009).

[6]. K. H. Buchel, *Chemistry of pesticides*, Johns Wiley and Sons, New York, (1983).

[7]. D. Drożdżyński, *J. Plant Protect. Res*. **46,** 145, (2006).

[8]. G. Jager, *Chemistry of pesticides*, K. H. Buchel (Ed.), John Wiley & Sons, New York, (1983).

[9]. P. C. Keary, and D. D. Kaufman (Eds.), *Herbicides*, Chemistry, Degradation and Mode of Action, Vol. 1, Marcel Dekker, New York, (1975).

[10]. P. C. Keary, and D. D. Kaufman (Eds.), *Herbicides*: Chemistry, Degradation and Mode of Action, Vol. 2, Marcel Dekker, New York, (1976).

[11]. K. Bester, H. Hiihnerfuss, U. Brockmann, and H. J. Rick, *Arch. Environ. Contam. Toxicol.* **29**, 277, (1995).

[12]. *World Pesticide Market*, FC Special Report Farm Chemicals **138**, 45, (1985).

[13]. M. Hamada, and R. Wintersteiger, *J. Biochem. Biophys. Methods*, **53,** 229, (2002).

[14]. M. Gfrerer, D. Martens, B. M. Gawlik, T. Wenzi, A. Zhang, X. Quan, C. Sun, J. Chen, B. Plazer, E. Lankmayr, and A. Kettrup, *Chemosphere,* **47,** 455, (2002).

[15]. S. John, and B. S. Walther, *Surface Water Pesticide Contamination in the upper Terrebonne Basin of Louisiana,* Louisiana State University, Louisiana, (2003).

[16]. N. Megersa, T. Solomon, B. S. Chandravanshi, and J. Åke Jönsson, *Bull. Chem. Soc. Ethiop*. **14**, 9, (2000).

[17]. A. Abdulahi, *Pest. Mgt. J. Eth*. **1**, 57, (1997).

[18]. C. M. Aelion, and P. P. Mathur, *Environ. Toxicol. Chem.* **20,** 2411, (2001).

[19]. L. Guzzella, S. Rullo, F. Pozzoni, and G. Giuliano, *J. Environ. Quality,* **32**, 1089, (2003).

[20]. E. A. Scribner, E.M. Thurmana, and L. R. Zimmermanb, *The Science of the Total Environment,* **248**, 157, (2000).

[21]. J. T. Sanderson, R. J. Letcher, M. Heneweer, J. P.Giesy, and M. van den Berg, *Environmental Health Perspectives*, **109**, 10, (2001).

[22]. E. M. Thurman, M. T. Meyer, M. S. Mills, L. R. Zlmmerman, and C. A. Perry, Environ. *Sci. Technol.*, **28**, 2207, (1994).

[23]. W. A. Battaglin, E. M. Thurman, S. J. Kalkhoff, S. D. Porter, and J. Am. *Water Res*. *Ass.,* U.S.

[24]. G. A. Smith, B. V. Pepich, and D. J. Munch, *J. Chromatogr. A.*, **1202**, 138, (2008).

[25]. J. T. Sanderson, R. J. Letcher, M. Heneweer, J. P. Giesy, and M. Van den Berg, *Env. Health Prospective*, **109**, 1027, (2001).

[26]. J. T. Sanderson, W. Seinen, J. P. Giesy, and M. Van den Berg, *Toxicological Sciences*, **54**, 121, (2000).

[27]. S. V. Trentacoste, A. S. Friedmann, R. T. Youker, C. B. Breckenridge, and B. R. Zirkin, *Journal of Andrology*, **22**, 142, (2001).

[28]. P. B. Tchounwoul, B. Wilson, A. Ishaque, R. Ransome, Ming-Ju Huang, and J. Leszczynski, *Int. J. Mol. Sci.*, **1**, 63, (2000).

[29]. A. E. Jacomini, P. B. De Camargo, W. E. P. Avelar, and P. S. Bonato, *J. Braz. Chem. Soc.*, **20**, 107, (2009).

[30]. N. Papadopoulos, E. Gikas, G. Zalidis, and A. Tsarbopoulo, *J. Agric. Food Chem*., **55,** 7270, (2007).

[31]. N. Megersaa, L. Chimukaa, T. Solomon, and J. Åke Jönsson, *J. Sep. Sci*., **24**, 567, (2001).

[32]. M. Khrolenko, P. Dżygiel, and P. Wieczorek, *J. Chromatogr. A*, **975**, 219, (2002).

[33]. S. Mitra, *Sample Preparation Techniques in Analytical Chemistry,* John Willey & Sons, New Jersey, (2003).

[34]. M. Knutsson, *Sapling and Sample Preparation of Phenollic Pollutants in Complex Matrices Especially Using Supported Liquid Membrabe,* Ph.D Thesis, Lund University, Lund, (1995).

[35]. A. J. Handley, (ed), *Extraction Methods in Organic Analysis*, Sheffield Academic Press, Sheffield, (1999).

[36]. H. Prosen, and L. Zupancic-Krali, *Trends Anal. Chem*., **18**, 4**,** (1999).

[37]. D. E. Raynie, *Anal. Chem*., **78**, 3997, (2006).

[38]. H. Liu, and P. K. Dasgupta, *Anal. Chem*., **689,** 1817, (1996).

[39]. M. A. Jeannot, and F. Cantwell, *Anal. Chem*. **68,** 2236, (1996).

[40]. G. Shen, and H. K. Lee, *Anal. Chem*., **74**, 648, (2002).

[41]. M. A. Jeannot, and F. Cantwell, *Anal. Chem.*, **68,** 2236, (1996).

[42]. M. A. Jeannot, and F. Cantwell, *Anal. Chem*., **69,** 235, (1997).

[43]. V. He, and H. E. Lee, *Anal. Chem*., **69,** 4634, (1997).

[44]. L. Zhao, and H. K. Lee, *J. chromatogr. A*, **919,** 381, **(**2001).

[45]. K.E. Kramer, and A.R.J. Andrews*, J. chromatogr. B,* **760**(2001).

[46]. Y. Picó, M. Fernández, M. J. Ruiz, and G. Font, *J. Biochem. Biophys. Methods*, **70**, 117, (2007).

[47]. A. Żwir-Ferenc, and M. Biziuk, *Solid Phase Extraction Technique* – Trends, Opportunities and Applications, 80-952 Gdańsk, Poland, (2006).

[48]. J. R. Dean, and G. Xiong, *Trends Anal. Chem.,* **19**, 553, (2000).

[49]. D.A. Lambropoulou, and T. A. Albanis, *J. Biochem. Biophy. Methods*, **70**, 195, (2007).

[50]. T. Msagati, L. Chimuka, and E. Cukrowska, *Water SA*., **34**, 3, (2008).

[51]. K. Hylton, and S. Mitra, *J. Chromatogr. A*, **1152,** 199, (2007).

[52]. J. Åke Jönsson, and L. Mathiasson, *trends in analytical chemistry*, **18**, 5, (1999).

[53]. J. Åke Jönsson, and L. Mathiasson*, J. Sep. Sci.*, **24**, 495, (2001).

[54]. F. M. Lanças, and M. A. Ruggiero, *J. Microcolumn Sep.*, **12**, 61, (2008).

[55]. J. Åke Jönsson, and Th. Barri , *Supported-Liquid Membrane Extraction*, Lund University, Lund, Sweden, (2007).

[56]. G. Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaen, and K. E. Rasmusson, *J. Sep. Sci*. **26,** 1520, (2003).

[57]. S.D. Doig, A.T. Boam, A.G. Livingston, and D.C. Stuckey, *Journal of Membrane Science*, **154**, 127, (1999).

[58]. R. C. Martinez, E. R. Gonzalo, E. H. Fernhdez, and J. H. Mhdez, *Anal. Chim. Acta*, **304**, 323, (1995).

[59]. L. Chimuka1, E. Cukrowska and J. Åke Jönsson, *Pure Appl. Chem.*, **76**, 707, (2004).

[60]. M. H. El-Saeid, I. Kanu, E. C. Anyanwu, and M. A. Saleh, *the scientific world journal*, **5**, 11, (2005).

[61]. T. S. Ho, S. Pedersen-Bjergaard, and K. E. Rasmussen*, J. Chromatogr. A*, **963,** 3, (2002).

[62]. D. Louch, S. Motlagh, and J. Pawliszyn, *Anal. Chem*. **64**, 1187, (1992).

[63]. Z. Zhang, and J. Pawliszyn, *Anal. Chem*. **65,** 1843, (1993).

[64]. J. Pawliszyn, *Solid-Phase Microextraction*, Theory and Practice, Wiley, New York, (1997).

[65]. N. Jakubowska, Ż. Polkowska, J. Namienik, and A. Przyjazny, *Critical Reviews in Analytical Chemistry*, **35**, 217, (2005).

[66]. J. Åke Jönsson and L. Mathiasson, *lcgc north america*, **21**, 5 (2003).

[67]. K. F. Pratt, and J. Pawliszyn. *Anal. Chem*. **64**, 2101 (1992).

[68]. H. Lord, Y. Yu, A. Segal, and J. Pawliszyn. *Anal. Chem*. **74**, 5650 (2002).

[69]. W. A. Ockenden, A. J. Sweetman, H. F. Prest, E. Steinnes, and K. C. Jones. *Environ. Sci. Technol*. **32**, 2795 (1998).

[70]. http://npic.orst.edu/RMPP/rmpp_ch4.pdf (accessed Jan 2008/9).

[71]. C. Tomlin (Ed.), *The Pesticide Manual*, 7th ed., British crop protection Council, UK, 1997.

[72]. E. Björklund, C. von Holst, and E. Anklam, *Trends Anal. Chem.,* **21**, 39, (2002).

[73]. H. J. Hayes, and E. R. Laws, *Handbook of Pesticide Toxicology*, Acadamic press, San Dieogo, (1991).

[74]. D. Barcelo, *J. Chromatogr. A*, **643**, 117, (1993).

[75]. H. Prosen, L. Zupančič-Kralj, and J. Marsel, *J. Chromatogr. A*, **704**, 121, (1995).

[76]. H. Bagheri, and F. Khalilian *, Analytica Chimica Acta,* **537,** 81, (2005).

[77]. A. Laganà, A. Bacaloni, I. De Leva, A. Faberi, G. Fago, and A. Marino, *Analytica Chimica Acta,* **462**, 187, (2002).

[78]. T. Tekel, and J. Koracicoka, *J. Chromatogr. A*, **643**, 291, (1993).

[79]. T. Berhanu, J. Liu, R. Romero, N, Megersa, and J. Åke Jönsson, *J. Chromatogr. A*, **1103,**1, (2006).

[80]. S. Zorita, L. Mårtensson, and L. Mathiasson, *J. Sep. Sci.*, **30**, 2513, (2007).

[81]. N. Megersa, L. Chimuka, T. Solomon, and J. Åke Jönsson, *J. Sep. Sci.,* **24**, 567, (2001).

[82]. D. A. Lambropoulou, and T. A. Albanis, *J. Biochem. Biophys. Methods* **70,** 195, (2007).

[83]. J. Peng, J. Lü, X. Hu, J. Liu, and G. Jiang, *Microchim Acta*, **158**, 181, (2007).

[84]. James N. Miller, and Jane C. Miller, *Statistics and Chemometrics for Analytical Chemistry 5th edition*, Pearson Prentice Hall Ltd., Harlow, (2005).

[85]. J. Vessman, R. I. Stefan, J. F. van Staden, K. Danzer, W. Lindner, D. T. Burns, A. Fajgelj, and H. Müller, *Pure Appl. Chem*, **73**, 1381, (2001).

[86]. Jönsson, J.Å., *Membrane extraction in preconcentration, sampling and trace analysis. Handbook of Membrane Separations: Chemical, Pharmaceutical and Biotechnological Applications*, A.M. Sastre, A.K. Pabby, and S.S.H. Rizvi, Editors. Marcel Dekker Inc., New York, (2008)**.**