ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

Methods for Sample Preparation and Analysis of Residual Levels of Selected Herbicides at Various Production Stages of Sugar from Industries in Ethiopia, Based on Literature Review

GRADUATE PROJECT (CHEM. 774)

Amenu Asefa G/Kiros

July 2006

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By

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A Project Presented to the Department of Chemistry,

Addis Ababa University

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Acknowledgments

I want to express my sincere gratitude to my advisor Dr. Negussie Megersa for excellent scientific guidance, fruitful ideas, and interest on my work. My unreserved great fullness is due to Ato Tarekegn Birhanu for sharing his long experiences and for extensive scientific discussions. Thanks for the time you spent with us sailing; I owe you many thanks for editing and correcting my work. Dr. Mesfin Redi, Dr. Tewodros Asefa and Ato Ahmed Hussen are thanked for assistants and nice suggestions.

All colleagues and friends of the members and HPLC groups are greatly thanked for the cheerful working atmosphere. I appreciate Dr. Ademe Adenew, Ato Girum Asfaw and Ato Kassa Matelbo who are the member of Ethiopian Sugar Industry Support Center Sh, Co. Research and Training Service Division for their great friendship and fruitful co-operations.

It is my greatest pleasure to thank Ato Tewodros Yirgashewa, Ato Workineh Shewangizaw, Ato Tekaling Degafe, Ato Mekonnen Belete Ato Seyoum Bekele and Ato Anteneh Girma for excellent friendship, useful first hand advice and concern for my education and my family.

My respectfully thank my father, brothers, sister, brother- and sisters-in-law for their encouragement, support and morals.

Finally my heartfelt gratefulness goes to my wife, W/ro Kelemua Debebe, for your understanding love, patience and shouldering all the responsibilities. It is your greatest encouragement and sacrifices that brought this work to a successful end.

Above all, I praise the LORD!

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ABSTRACT

Methods for Sample Preparation and Analysis of Residual Levels of Selected Herbicides at Various Production Stages of Sugar from Industries in Ethiopia, Based on Literature Review

By

Amenu Asefa

Project Advisor: Dr. Negussie Megersa

Sampling, sample preparation and analysis methods based on supported liquid membrane atrazine and ametryn and phenoxyacid herbicides; 2,4-D. In SLM extraction, neutral herbicides could be extracted from a flowing aqueous solution to a stagnant acidic acceptor solution for the two weakly basic *s*-triazines and to stagnant basic acceptor solution for the weakly acidic phenoxyacid herbicide. Specifically, a liquid membrane containing 0.1 mol/L sulphuric acid for the triazines and 0.1 mol/L sodium hydroxide for the 2,4-D was suggested. The uncharged herbicide molecules from the flowing aqueous donor solution diffused through a liquid membrane impregnated in a porous poly(tetrafluoroethylene) membrane, immobilized organic solvent, and then will be trapped in a stagnant acceptor phase. The efficiency of the extraction process will be found to depend on the donor solution pH, the amount of the mobile carrier added to the liquid membrane and the concentration of the counter ion in the acceptor solution. From detailed studies, the optimum values of these parameters for the efficient extraction of the herbicides will be determined. The applicability of the method for (SLM) have been proposed for selective extraction of two s-triazine herbicides, namely extraction of these selected herbicides at different stages of production of sugar will be also investigated by spiking analyte sample solutions in sugar-cane juice, syrups, and molasses and sugar crystal samples. The suggested method will be utilized for trace enrichment of the analytes from samples containing matrices of various types and concentrations. A 20 minutes of sample enrichment at a flow rate of 1.0 mL/min, was suggested to determine the concentrations of atrazine, ametryn and 2,4-D in sugar samples at various stages of production.

1. INTRODUCTION

Commercial sugar cane cultivation for the production of sugar in Ethiopia started by Dutch Handles Vereening Amsterdam (HVA) company in 1951 [1] in the upper Awash River Basin of Wonji on 5,000 hectares of sugar cane plantation. Since then the sugar estates in Ethiopia have expanded to two more sites at Metahara and Finchaa and currently a total area of about 24,000 hectares of sugar cane plantation is covered. These three estates produce about 250,000 tons of sugar annually. These sugar estates solely use sugar cane plantations for their respective sugar factories, which produce sugar and molasses that are mainly used for domestic market. Since a few years ago, significant portion of this production is being exported to assist the country's economy through foreign currency. Ethiopia shipped about 14 thousand tons of raw sugar to EU in 2001-2002 and nearly 15 thousand tons during 2002-2003 periods under the Everything But Arms (EBA) initiative [2]. Despite the exports to international markets, the present annual consumption of sugar in the country, which is estimated at 3.5 Kg per capita, remains to be low. In fact, it is considered to be low even by the Africans standard, which is estimated to be an average of 20 Kg per capita. To increase this and satisfy the current total demand of sugar consumption in the country, Ethiopia needs to produce additional 80,000 metric tons per year [2]. All efforts that have been made to increase sugar production exist, but they have been hampered by several problems including sugar cane diseases, weeds and pests that are widely spread during cultivation of the sugar cane plants [3]. On the other hand controlling weeds in sugar cane plantations have been an ongoing major effort, in order to increase the production of sugar. Mechanical and chemical means of weed control have been among the major methods employed currently at the three estates of sugar cane plantation. Despite these efforts, the scarcity of labor and unfavorable weather conditions makes extensive application of herbicides to be the practical alternative choice more frequently.

1.1 Herbicides

1.1.1 History and Uses of Herbicides

Herbicides are chemicals with a capacity to kill certain plants selectively or non-selectively. Herbicide, in the broadest sense, is defined as any chemical compound that is 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 [4]. According to the general definition, weed is any plant, either a wild or cultivated variety that is undesired in a particular place at a particular time [5].

Herbicides show a wide range of beneficial effects such as improving plant health, maintains agro-ecosystems, food supply, economical advantage, etc [1, 2]. Furthermore, their use results the decrease in costs associated with labor and machinery for weed control. The removal of weeds can also save livestock from poisonous plants. Irrigation costs can be reduced and insect and disease control costs can easily be controlled by removal of host weeds.

Herbicides have been in use since the mid-nineteenth century. Up to Word War II, a variety of inorganic acids and salts, e.g., iron sulphate, sulphuric acid, sodium chlorate, arsenicals and copper sulphate were applied to control unwanted weeds. In 1930s, the first organic chemical herbicide, 4,6-dinitro-o-cresol (DNOC) was introduced [6]. DNOC was used as insecticide before its herbicidal effect was discovered. The introduction of DNOC was followed by the appearance, in the 1940s, of the substituted phenoxyacids, e.g., chlorinated phenoxyalkanoic acids, and the substituted ureas in 1951. The acidic herbicides are still among the most commonly used herbicides, with an estimated production of 34×10^6 Kg/year in USA in the mid 1980s.The triazine family of herbicides, which are the second largest group used today, have also appeared in the 1950s, and since then many other classes of compounds have been added for use as herbicides [7].

1.1.2 Major Classification of Herbicides

Herbicides can be classified in several ways. The major one are the following:

A) Selective and Non-Selective (Total Killer) Herbicides

As the name indicates, selective herbicides are those that kill some members of plants population with little or no injury to others. An example is alachlor, which can be used to kill grassy and some broad-leafed weeds in corn, soybeans and other crops. On the other hand, non-selective herbicides are those that kill all vegetation to which they are applied. Examples are paraquat and glyphosate that can be used to keep roadsides, ditch banks and rights-of-way open and weed-free [4, 8].

B) Pre-Emergence and Post-Emergence Herbicides

These are application methods and the classification here is based on the properties of the herbicide chemicals. Some herbicides are effective only when applied to the soil and adsorbed into the germinating seedlings, and therefore are used as pre-emergence herbicides, eg, trifluralin [4]. Others such as diquat, exert their herbicidal effects only on contact with plant foliage and are strongly inactivated when placed in contact with soil [7, 8]. However, the distinction between pre- and post- emergences is not always clear. For example, atrazine can exert its herbicidal action either following root absorption from pre-emergence application or after leaf absorption from a post emergence treatment, and thus it can be used with either application methods. This may be an advantage in high rainfall areas where a post-emergence treatment can be washed off the leaf onto the soil and nevertheless can provide effective weed control.

C) Chemical Classification of Herbicides

Based on their chemical structures herbicides can also be classified into the following ten groups [9, 10]. These are: i) phenoxyacid herbicides, ii) substituted urea and uracil herbicides, iii) bypiridilium herbicides, iv) dinitroaniline herbicides, v) amides, vi) carboxylic and benzoic acid herbicides, vii) carbamate herbicides, viii) phenols and biphenyl ether herbicides, ix) hetrocyclic nitrogen containing herbicides and x) miscellaneous herbicides.

1.1.3 Herbicide Uses in Sugar Plantations in Ethiopia

The first use of herbicide in Ethiopia at the sugar cane plantation estates dates back to 1968 [1] with the use of mixture of 2.4-D and trichloracetic acid (TCA). Since then, there is an increase in the use of herbicides at these sugar cane plantations. In the early 1970, a mixture of 2,4-D and Gesapax 80WP was introduced at these sugar estates, but due to the clogging problem of this mixture, the Gesapax 80WP was immediately replaced by its stereotype Gesapax combi 500FW (the combined form of Atrazine and Ametryn). This mixture has been under intensive use in all sugar cane plantation estates since then. Gesapax combi 500 FW is used as a pre- and post-emergence [11] treatment applied at 6-8 L/ha and 8-10 L/ha in light or medium and heavy soils, respectively.

The control of weeds by means of herbicides has provided many benefits. Freeing agricultural crops, including sugar cane plants, from weed competition results in higher production, lowering processing and harvesting costs, and improved quality of production, contributing to an abundant supply of less costly process of high quality agricultural products. Today there are more than 1,500 chemical substances in use as pesticides all over the world [8, 12]. Of these, compounds of the s-triazine family are among the most heavily used herbicides over the last 30 years as effective weed killers. The mode of actions of herbicides may vary but for the common compound atrazine, one of the s-triazine herbicides, its action as a weed killer originates from its inhibition of the plant's photosynthesis.

Despite the effectiveness of controlling weeds using these and other herbicides in order to increase the production of sugar, the intensive application of herbicides has resulted in the polluting the atmosphere, ground waters, and agricultural and food products such as wheat, corn, fruits, vegetables, etc. and biological systems either directly or indirectly [13]. As herbicide molecules are more or less toxic, they represent not only an environmental risk but also a health hazard [14]. The practice of using herbicides often leaves potentially harmful chemicals and herbicide residues in the products. Furthermore, some of these herbicides and their degradation products are suspected to be carcinogens and can potentially result in harmful effects to humans and other biological species [8, 15]. Consequently, the release and concentration of these pollutants in the environment should be monitored.

1.1.4 Symmetrical -Triazine Herbicides (s-Triazine Herbicides)

Among the chemical compounds grouped under the *s*-triazine class of herbicides, atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and ametryn (2-ethylamino-4-isopropylamino-6-methylthio-1, 3, 5-triazine) are most important due to their longer time application. These herbicides are available commercially as Gesapax combi 500 FW (the combined form of Atrazine and Ametryn). Symmetrical triazines (*s*-triazines) are one of the largest classes of agrochemicals produced and the most commonly used herbicides for pre- and post- emergence weed control for variety of crops including green vegetable. A report on world market [16] indicates that about 30% of all herbicides produced are triazines.

Their properties are determined mainly by the substituents at the-2-position, generally chlorine (the general name ending with –azine), methoxy- (ending-tone) and methyl (alkyl) thio- (ending-tryn). The 4- and 6- positions are usually substituted with various aminoalkyl groups [17]. They are all synthesized by substituting 1,3,5-triazine (or symmetrical *s*-triazines) in the 2-, 4- and 6-positions. The synthesis pathway of atrazine and ametryn is shown in figure 1.3.

i) Atrazine

Atrazine has been classified as a Restricted Use Pesticide (RUP) due to its potential for ground water contamination [18]. RUPs may be purchased and used only by certified applicators. Atrazine is slightly to moderately toxic to humans and other animals. It can be absorbed orally, dermally and by inhalation. Symptoms of poisoning include abdominal pain, diarrhea and vomiting, eye irritation, irritation of mucous membranes and skin reactions. Atrazine is available commercially as trade and other name as Aatre, Aktikon, Alazin, Atred, Atranex, Atrataf, Atratol and Azinotox etc [18].

Atrazine is selective *s*-triazine herbicides used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, pineapple, christmas tree and in conifer reforestation plantings. It is also used as a non-selective herbicide on a non-cropped industrial lands and on fallow lands. It is available as dry flowable, flowable liquid, liquid, water dispersible granular, and wettable powder formulations. The structure of atrazine is shown in Figure 1.1.

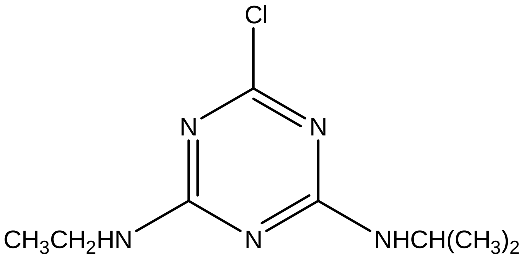


Figure 1.1 Chemical structure of Atrazine.

ii) Ametryn

Ametryn is an unrestricted or General Use Pesticide (GUP) [19]. Ametryn is slightly toxic to human. Symptoms of acute exposure to high doses include nausea, vomiting, diarrhea, muscle weakness and salivation. Ametryn is moderately irritating to eyes, skin, and respiratory tract. Ametryn are available commercially as trade and other name as Evik, Ametryn, Ametryx, Gesapax, G34162, Trinatox-D (a combination with 2,4-D), Crisazina-Crisatrina Kombi (a combination with atrazine), Doruplant, Mebatryne, and Amephyte etc [19].

Ametryn, a number of the triazine chemicals family, is an herbicide, which inhibits photosynthesis and other enzymatic processes. It is used to control broadleaf weeds and annual grasses in pineapple, sugar cane and bananas. It is used on corn and potato crops for general weed control. Ametryn is available as an emulsifiable concentrate, flowable wettable powder and a wettable powder. The structure of ametryn is shown in figure 1.2.

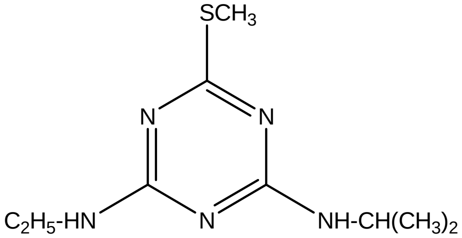
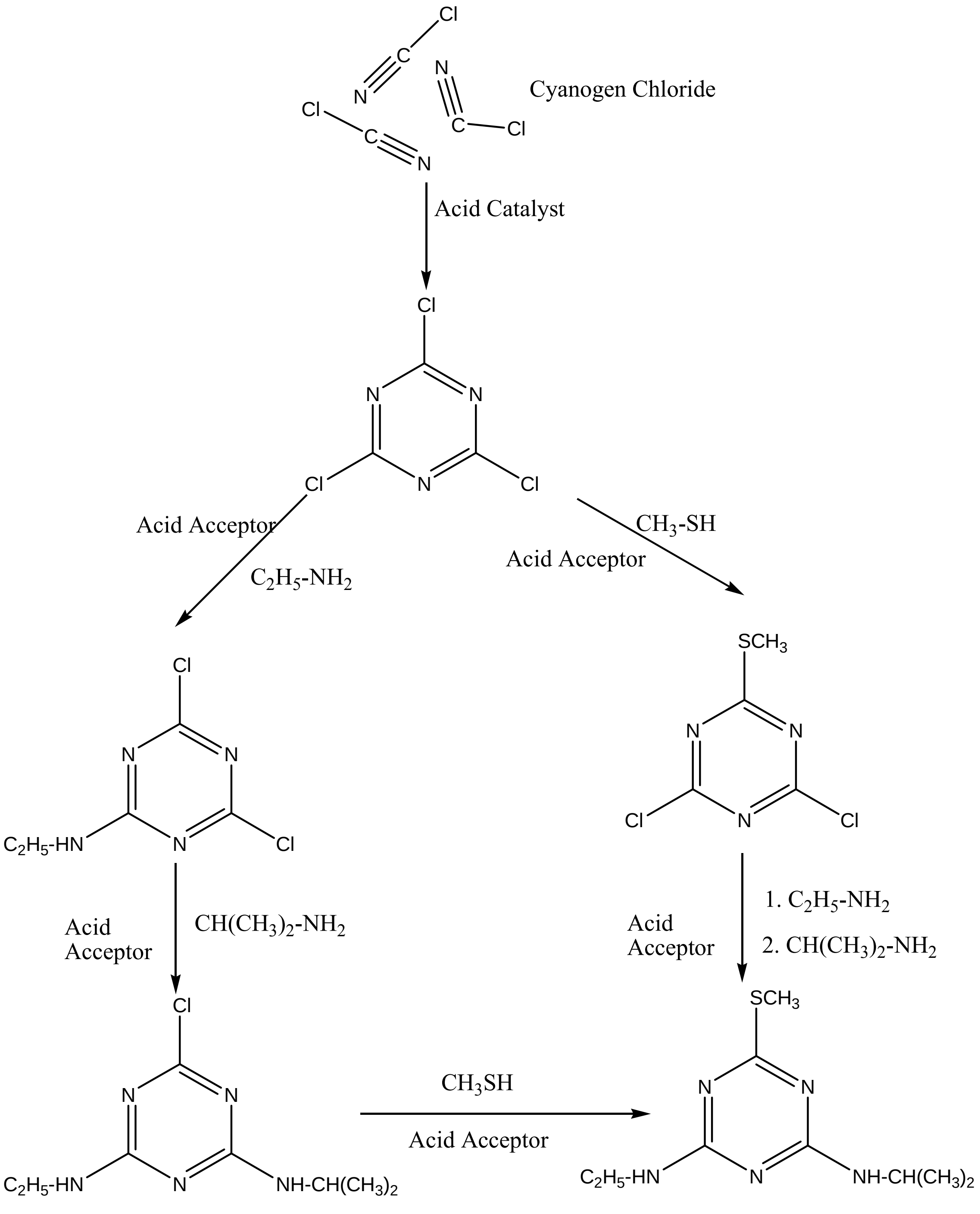


Figure 1.2 Chemical structure of Ametryn.

The synthesis pathway of atrazine and ametryn is shown in the figure below.



Atrazine **Ametryn**

Figure 1.3 Synthesis pathways of Atrazine and Ametryn.

1.1.5 Phenoxyacide Herbicides

Acidic herbicides, especially phenoxyacids are a group of herbicides that have been used worldwide for a long time. The phenoxyacids were first used as weed killer around 1940 [20] and they are still very useful. As it was found in the 70's that dioxins might a by-product in the production of the phenoxyacids 2,4,5-T, this compound was forbidden from use in many countries.

The chlorine substituted phenoxyacetic acids 2,4-D, MCPA [(4-chloro-2-methylphenoxy) aceticacid)] and 2,4,5-T [(2,4,5- trichlorophenoxy) aceticacid] were introduced as selective weed killers at the end of World War II, and most herbicides of this family are commonly in use today. They show high activity against many broad-leaved weeds and in particular, 2,4-D and MCPA are commonly used for control of weeds in cereal crops and grass pastures, while many woody and broad-leaved plants are effectively controlled by 2,4,5-T [21, 22].

2,4-D

Among the chemical compounds grouped under the phenoxyacid class of herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) is one and a member of the chlorophenoxyacid herbicides. Its amine ester formulations have been used for cereals, crops, sugar cane, fruit trees, fields and forest control [23]. The chemical structure of 2,4-D is shown in Figure 1.4.

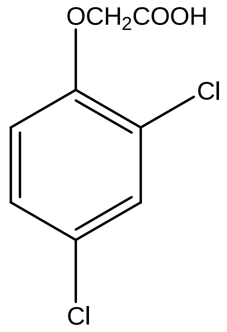


Figure 1.4 Chemical structure of 2,4-D.

2,4-D is used in many commercial products. A commercial name for products containing 2,4-D includes Aqua-Kleen, Barrage, Lawn-Keen, Malerbane, Planotox, Plantgard, Salvo, Weedone, and Weedtrine-II. 2,4-D is General Use Pesticides (GUP) in the U.S. In humans, prolonged breathing of 2,4-D causes coughing, burning, dizziness and temporarily loss of muscle coordination. Other symptoms of poisoning can be fatigue and weakness with possible nausea. It may be found in emulsion form, in aqueous solutions (salts), and as a dry compound [23]. The synthesis pathway of 2,4-D is shown in Figure 1.5.

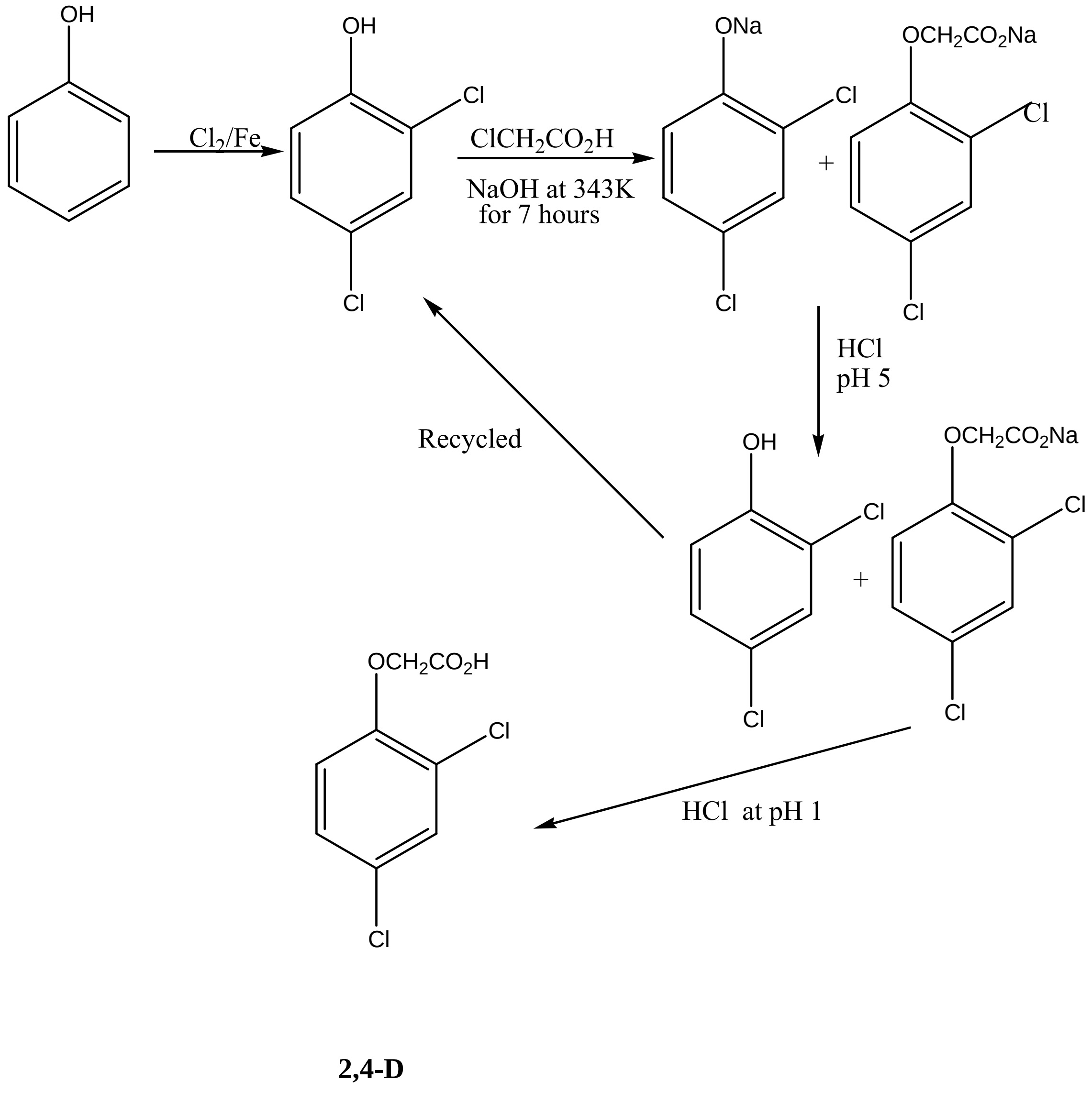


Figure 1.5 Synthesis pathways of 2,4-D.

1.1.6 Environmental Distribution and Transformation of s-Triazine Herbicides

General Properties of *s*-Triazines.

The water solubility and other physico-chemical properties of *s*-triazines derivatives are primarily determined by substituents in the 2-position [24, 25]. The properties of chloro-*s*-triazines considerably differ from those of the other groups, where as the methylthio- and methoxy-derivatives behave very similarly. Substituentes in positions 4- and 6- exert substantially smaller effects on the derivatives properties. *s*-triazine herbicides are weakly basic substances. The basicity increases with the order of substituenes in 2-position: Cl < SCH_3 < OCH_3 [26]. Other substituents have less effect on basicity.

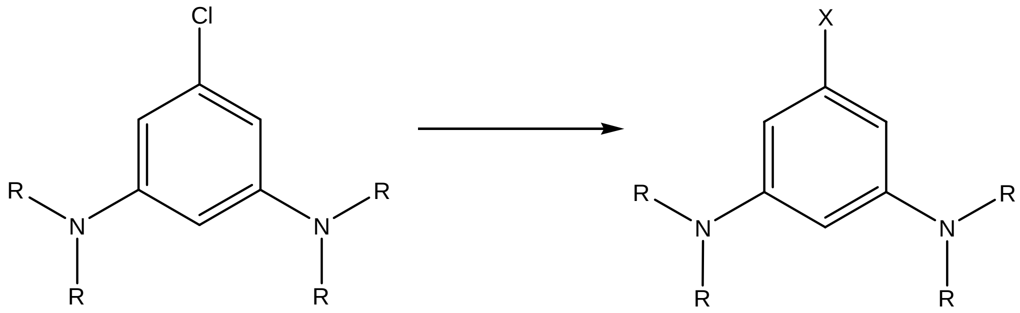
A remarkable stability of the *s*-triazine derivatives can be explained by the electronic configuration of the heterocyclic ring, which resembles that of benzene to a certain extent. Both ring systems are stabilized by delocalization of their π-electrons, which are spread over all the six atoms in the ring system. A partial localization of electrons in the vicinity of nitrogen atoms is due to its greater electronegativity, as a result, the aromatic character of *s*-triazine is less pronounced than that of benzene.

Degradation Products of *s*-Triazine Herbicides

The *s*-triazine herbicides undergo different kinds of reactions such as hydrolysis at C-2 position, N-dealkylation at C-4 and C-6 and photochemical degradation at all the ring carbons. The processes governing these degradations can be either chemical, photolytic or microbial or a combination of them.

The C-2 position, in particular, is a susceptible site for nucleophilic attack, and this effect is much pronounced for the 2-chloro-*s*-triazine family of herbicides. A number of chemical systems that can readily replace chlorine are summarized in Table 1.1.

Table 1.1 Nucleophilic reactions affecting the chlorine atom of 2-chloro-4, 6-bis(alkylamino)-*s*-triazines.



R can be H or alkyl group

Attacking reagent	**Resultant Substituent**
H^+ or OH^-	$-OH$
SH^-	$-SH$
Alkyl – OH, OH^-	–O – alkyl
Alkyl – SH, OH^-	–S – alkyl
$N(CH_3)_3$	$-(N(CH_3)_3)^+Cl^-$
NH_2NH_2	$-NHNH_2$
KCN	–CN
NaN_3	$-N_3$

Degradation in Higher Plants

The *s*-triazines are deactivated in tolerant plants by any one of or all of the following three different biotransformation pathways, benzoxazinone-mediated hydrolysis, N-dealkylation and glutathione conjugation.

1.1.7 Environmental Distribution and Transformation of Phenoxyacid Herbicides

The chlorine substituted phenoxyacids; 2,4-D, MCPA and 2,4,5-T were introduced as selective weed killers at the end of World War II. They showed high activity against many broad-leaved weeds, and in particular, 2,4-D and MCPA are commonly used for the control of weeds in cereal crops and grass pastures, while many woody and broad-leaved plants are effectively controlled by 2,4,5-T [21, 22].

2, 4-D has low soil persistence. The half-life in soil is less than 7 days. Soil microbes are primarily responsible for its disappearance. Despite its short half-life in soil and in aquatic environments, the compound has been detected in groundwater. Very low concentrations have also been detected in surface waters.

In aquatic environments, microorganisms readily degrade 2,4-D. Rates of breakdown increase with increased nutrients, sediment load, and dissolved organic carbon. Under oxygenated conditions the half-life is one week to several weeks.

2,4-D interferes with normal plant growth processes. Uptake of the compound is through leaves, stems, and roots. Breakdown in plants is by a variety of biological and chemical pathways. 2,4-D is toxic to most broad leaf crops; especially cotton, tomatoes, beets, and fruit trees.

1.2 Sampling of Sugar Cane Product at Various Stages

Samples of sugar at different stages were collected from Wonji (107 Km), Metehara (200 Km) southeast and Finchaa (358 Km) west of Addis Ababa. A preliminary trial sampling was performed in April 2006. A grab sampling procedure, i.e., taking a single sample increment at some intervals in a specific collection site [27], was followed to obtain representative samples and to average the variation of sample composition. Samples were collected in reddish brown glass bottles at five different stages in the production of sugar; namely Stage A called primary juice, Stage B condensed water, Stage C called Filter Cake, Stage D Molasses and the final Stage E sugar crystal. The general flow diagram

(Flow Chart) of juice clarification process for the production of plantation white sugar in all the three estates is shown in Figure 1.6.

Samples at different stages were brought to the laboratory with icebox in less than 24 h and kept in refrigerator below 0^oC. Before liquid membrane extraction, the samples at different stages should be diluted and filtered to remove the suspended impurities and particulate matters. The diluted and filtered samples should then keep in the refrigerator when they were not extracted immediately.

Various types of chemical analysis can monitor the determination of concentrations chemical pollutants over a course of time in the environment. Since the herbicide residues and their degradation products are found in various complex matrices at very low concentration levels, preconcentration of the chemical substances is highly required, prior to their analysis [24, 28, 29]. The detection level of the residues, especially in environmental samples, seems to depend more on the isolation and enrichment procedure chosen than on the types of method used in the final determination [30]. To this end, numerous sample preparation techniques, that make use of various means of separation of the analytes of interest from the sample matrix, have been developed.

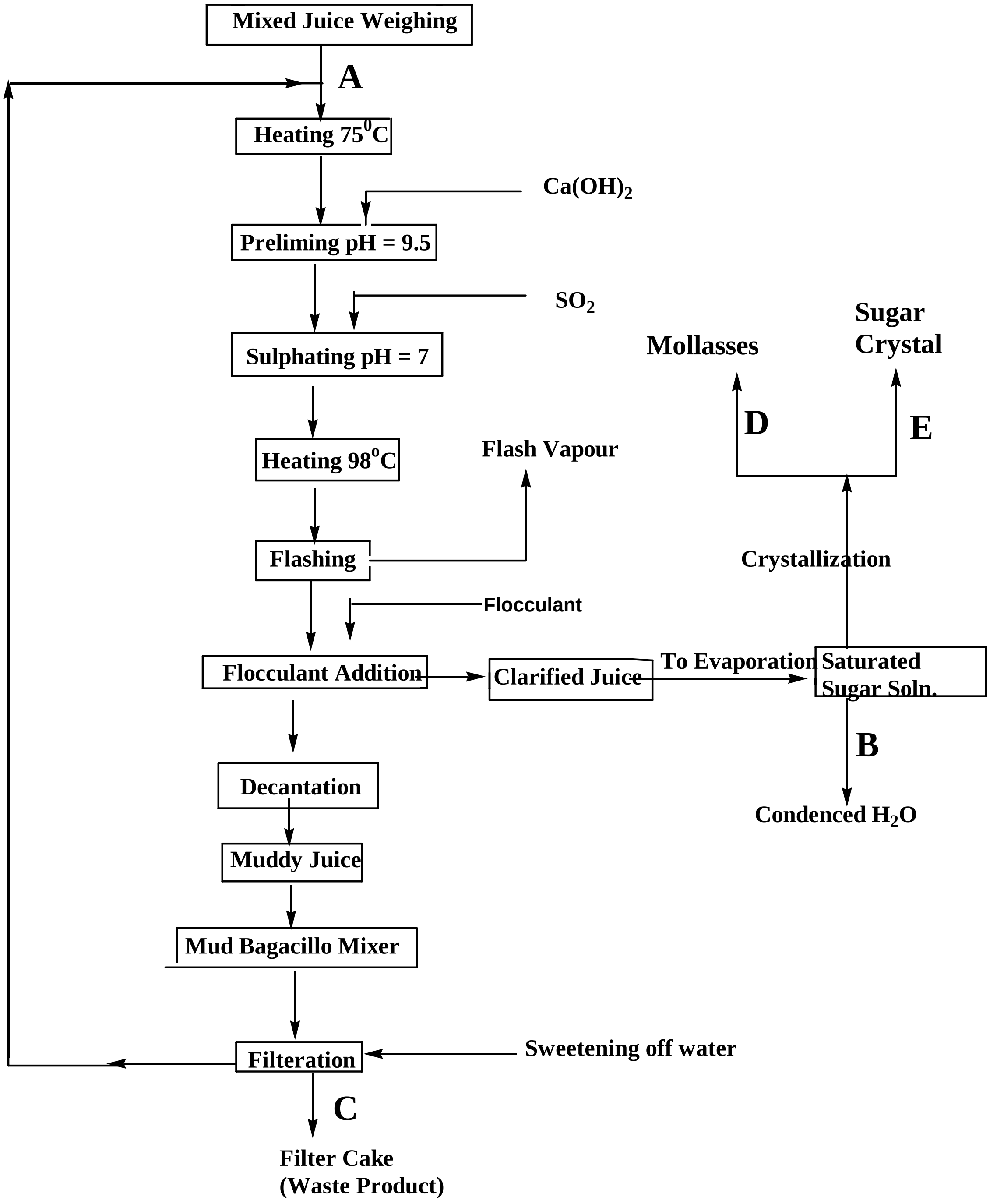


Figure 1.6 Juice Clarification Process for the Production of Plantation White Sugar

1.3 Sample Preparation Techniques for Extraction of s-Triazine Herbicides

1.3.1 Membrane Based Sample Preparation Technique for s-Triazine Herbicides

A membrane is a selective barrier between two phases, which restricts or enhances the movement of molecules across it in a very specific manner. The separation process is accomplished by differences on how various components in the sample respond to a driving force such as concentration gradient, pressure gradient or electrical flux. A wide variety of membrane materials are used for sample preparation [31-34], and all are grouped into two categories, as porous and non-porous membranes, based on their morphology or structure.

In porous membranes, any compound of appropriate size can be transported by diffusion through the membrane pores. Due to this, sample preparation techniques that make use of these membranes are known to suffer from less selectivity. The important structural parameters for the selectivity of porous membranes include size, shape, geometry and distribution of the pores and surface porosity.

In non-porous membranes separation is accomplished when the molecules to be separated are dissolved in the membrane. The transport of molecules is determined by the physical and chemical properties of the compounds in the membrane phase, which includes their solubility and diffusion coefficient. Hence, the extent of interaction between the membrane and the compounds is a more important factor to be considered than the membrane pore size and molecular size of the analyte. Consequently, non-porous membranes can be regarded as relatively selective membranes.

Non-porous membranes can further be sub-divided into solid (polymeric) and liquid membranes. The solid membranes are often made of polymers which may have different physical properties, i.e., crystalline, amorphous, glassy or rubber. In polymeric membrane extraction (PME), like other membrane-based extraction techniques, the partitioning of the analytes into the polymer, diffusion through it and partitioning into the receiving phase are important factors that influence the overall mass transfer coefficient.

The use of liquid membranes has recently gained much attention for developing new, selective and efficient extraction processes and to construct automated sample handling setups. Liquid membrane systems involve an organic liquid that serves as a semi-permeable barrier between two liquids or gases (or a liquid and a gas) [35]. The transport of analytes across a liquid membrane is driven by the concentration difference over the two phases other than the membrane liquid. The theory and applications of liquid membranes have been reported in a number of publications [36, 37].

Based on their configuration, liquid membranes can be divided into three types:

Bulk Liquid Membranes (BLM*)*: BLM's consist of source and receiving phases, usually aqueous, separated by a bulk membrane phase, organic liquid, in a stirred container or U-tube [38]. Fluxes through BLM's are low due to the thickness of the membrane and hence their utility for practical analytical applications is limited. Their analytical use has mainly been restricted to the studies of the transport mechanisms through liquid membranes [39].

Emulsion Liquid Membranes (ELM*)*: Vigorous mixing of two immiscible phases, for example, water and oil prepare ELM's. This permits in the formation of emulsion droplets, which are stabilized by addition of a surfactant. The emulsions are then dispersed in a continuous aqueous solution (the donor phase) from which the analytes are to be extracted [36, 40]. Mass transfer takes place between the donor phase and the inner acceptor phase through the immiscible membrane liquid. Emulsion instability, secondary emulsion formation and necessity of controlling a number of parameters are some of the common problems encountered in the use of ELM's for analytical purposes [41].

Supported Liquid Membrane (SLM): Supported liquid membrane (SLM), which is also called immobilized liquid membrane, is the most utilized format of the liquid membranes and the most flexible for structural modifications according to various needs. It is commonly prepared by immobilizing a water immiscible organic solvent within a microporous film.

1.3.2 Other Related Techniques Used in Sample Preparation of *s*-Triazine

In most instances, samples obtained from various origins, e.g., biological, environmental or industrial cannot be analyzed directly without passing them through some sample preparation processes. The main reason for this is that the samples are too complex, too dilute or incompatible with the analytical instruments. The overall goal of sample preparation is, therefore, to obtain a sub-fraction of the original sample enriched in all the substances of analytical interest, removing other components, which may interfere in the subsequent analysis. In honey analysis sonication was used in sample prefer eatenent for better dissolution of honey [42] and for extraction of volatile compounds [43].

Liquid-Liquid Extraction (LLE): LLE as a sample preparation technique for the s-triazine herbicides in the environmental water has been documented in various papers [44-46]. The degradation products of *s*-triazines are usually extracted from soils sediments in a mixture of water–methanol solution [47] that is followed by concentration of the aqueous extract prior to HPLC analysis [48]. In honey analysis LLE used for isolation of pesticides [49].

Solid-Phase Extraction (SPE): SPE in environmental sample analysis has been reviewed by several workers [30, 44, 50-52]. It is used for trace enrichment of s-triazine herbicides, off- and on-line; in various environmental, biological and food samples is also available in literatures [19, 48, 50]. The technique has become rather popular in providing quantitative recovery of s-triazine herbicides from aqueous samples and has been introduced into some standard methods [53].

Supercritical Fluid Extraction (SFE): SFE of s-triazines from solid samples has been described in several papers [54-58]. The efficiency of the extraction process depends on their solubility in the supercritical fluid, and the latter in turn depends on the fluid density, i.e., the *s*-triazine herbicide vapor pressure and on the kind and content of modifiers [59]. It has also been described in several reviews [25, 60-63] that, the recovery for the triazines with SFE increases with increasing pressure and methanol is added as a modifier, an increasing temperature somewhat decreases the recovery.

Microwave-Assisted Extraction (MAE): The MAE technique has also been employed for sample preparation of triazine herbicide compounds, particularly atrazine and the principal degradates from soil samples [64]. Molines et al. [65] extracted atrazine, deethylatrazine, deisopropylatrazine and simazine from 10 g soil with 40 mL of (9:1) methanol and dichloromethane at 115^{O}C for 20 min.

1.4 Sample Preparation Techniques for Extraction of Phenoxyacid Herbicides

In recent years quite a few new sample preparation methods have been developed, including microwave-assisted extraction (MAE), sonication, supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) [66-68]. In all the techniques smaller quantities of solvents are used, extraction time is shorter, and efficiency often increased. Quite recently applicability of subcritical water for the extraction of polar and non-polar trace organics from solid environmental samples has been shown. Lou et al. [69] applied such an approach to extract many acidic herbicides (Pentachlorophenol, dinoseb, 3,5-dichlorobenzoic acid, dicamba, 2,4-D, 2,4-DP, 2,4,5-TP, 2 4 5-T, 2,4-DB) from soil.

1.5 Sample Clean - Up Methods Applied to Herbicides Used in Sugar Plantation

The major goal of the sample preparation step is to isolate the target compound from the bulk matrix, thus facilitating the next step; the analyte determination. So, further purification of the extract is required before the analytes determination. This step is called the clean-up step and aims at the isolation of the target compound from potential contaminants as well as getting rid of the extraction solvent and preparing the target analyte in a chemical form appropriate for its characterization and quantification. The need for sample clean up depends on many factors such as the type of the sample, the concentration levels and the degree of selectivity in the detection mode.

Complex matrices from food and environmental samples such as sugar, molasses, honey, juice, water, etc can some times be removed by single extraction procedures [68]. In most cases clean up may not be necessary for sugar and condensed water because comparatively they are clean but if LLE and SPE are used for extraction, clean up steps are necessary as they are non-selective extraction methods. Various methods have been reported for the determination of pesticides in honey. Classical extraction techniques used in the determination of pesticides residues in honey has been partitioning with organic solvents, often followed by subsequent clean up procedures before GC determination. SFE [70], SPE with stationary phase packed in a cartridge or in disks [71], SPME [72] are different techniques that have been used with the aim in the analysis of pesticides. The conformation of the system for the general approach employed in a typical complex analysis is shown in Figure 1.7.

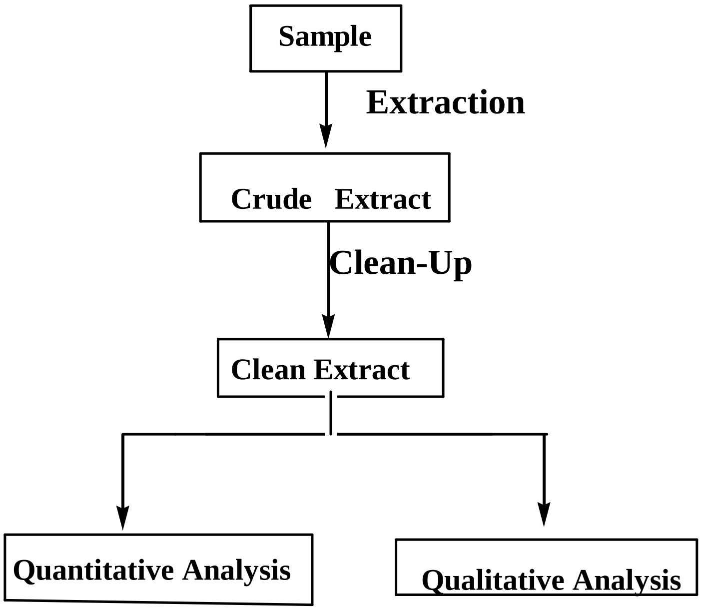


Figure1.7 General separation approach employed in a typical complex analysis.

1.5.1 Sample Clean-Up Methods Applied to s-Triazine Herbicides and Their Metabolites

Preconcentration and prepurification of herbicides residues are decisive steps in the determination of target compounds. The efficacy of the extraction determines the reliability of the subsequent separation and detection. The development of sorbents with higher adsorption capacity and enhanced selectivityis needed to increase the overall performance of the whole analytical system.

SPE with Non-Polar Sorbents: For determination of hydrophobic herbicides, the clean-up procedure can be included in the SPE sequence. Sample clean up of chloro-s-triazines has been reported [69] using two columns, the first being packed with non specific graphatized carbon black sorbent for trapping of many compounds, while only basic analytes being transferred and reconcenterated into a second cartridge packed with a more specific sorbent such as a cation exchanger.

Polar Sorbents (Adsorption Chromatography): Sample clean-up based on fractionation of the extracts using polar LC sorbents such as silica, alumina, Florisil (Synthetic magnesium silicate) or silica chemically modified with amino group are the widely applied method. The principle that governs this process is the difference in partitioning of the analytes and co-extractives in the polar solvents. Valls et al. [73] described the possibility of collection of seven fractions from the extract of urban waste and coastal waters. They indicated that most common sorbent, florisil, has successfully been used for the extracts containing various herbicides [74-76]. Silica gel and alumina have also been used for clean-up purpose; the former for non-polar herbicides [77-81], while application of the latter is limited to few herbicide compounds.

Size Exclusion Chromatography (SEC): Clean-up of the extracts using size exclusion (also called Gel Permeation Chromatography (GPC)) chromatography is based on separation by molecular weight. The technique primarily removes materials of higher molecular weight, leaving all the herbicides and other compounds of similar molecular weight [75]. Molecular separation occurs in the GPC column. Inside the column there are two phases:

a) The stationary phase consists of an inert gel of porous beads, so-called because it does not move in the column.

b) A mobile phase, which is the eluent or liquid that is run through the column.

The applications of the techniques have been demonstrated by several workers [82, 83].

1.5.2 Sample Clean-Up Methods Applied to Phenoxyacid Herbicides and Their Metabolites

Matrix complexity and the fact that acidic herbicides should be monitored at low concentration make the analytical methods of high sensitivity; selectivity and resolution power must be applied for their determination in soil, sediments, water and other environmental samples [84-86]. Environmental samples must generally be subjected to deep treatment before the final chromatographic analysis. Preparation for chromatographic analysis generally consists of a few steps. The preparation is based on the isolation of acidic herbicides of interest, removal of interfering substances and transfer of the analytes to an appropriate solvent [87].

In recent years quite a few new sample preparation methods have been developed including Microwave Assisted Extraction (MAE), Sonication, Supercritical Fluid Extraction (SFE), and Accelerated Solvent Extraction (ASE)[66-68]. In all these techniques smaller quantities of solvents are used, extraction time is shorter, and efficiency often increased. Quite recently, applicability of subcritical water for the extraction of polar and nonpolar trace organics from solid environmental samples has been shown. Lou et al. [69] applied such an approach to extract many acidic herbicides from environmental samples. Ngan and Ikesaki [88] extracted nine acidic herbicides with ethyl ether and then esterified with diazomethane. A relatively new and fast method of organic pollutants isolation from soil samples is a Supercritical Fluid Extraction (SFE). For the isolation of acidic herbicides, it was first applied by Rochette et al. [27] for determination of 2,4-D in soil.

1.6 Scope of the Present Study

The use of pesticides has improved agricultural productivity by offering quick and convenient way to eliminate annoying or destructive organisms. At the same time, however, misuse of these compounds causes serious environmental problems, as they are highly toxic substances.

s-Trazine and Phenoxyacids herbicides are one of the commonly used chemicals for the control and management of terrestrial vegetation. These substances are potential contaminants of the environmental samples because of their persistence and polar characters. It is of paramount importance, therefore, to determine the level to which the residues of these pesticides are accumulated in the sugar for the well being of the human beings.

The knowledge of concentrations of herbicide residues in the sugar and molasses and the development of routine analytical methods are important to reliably control the quality of sugar and its raw materials. This is particularly becoming more important as the current trends of increasing use of herbicides in Ethiopian Sugar Estate continued. The control of potentially harmful substances and herbicides in food products are unquestionably required. Furthermore, in order to be competent on the global market for export sugar products and for the safety of the consumers both domestically and internationally, extensive research to develop methods for routinely analyzing herbicides at the various stages of production of sugar in Ethiopia is in dire need.

This calls for the use of certain analytical systems for sample processing and quantitation of their amount so as to enable one to study the various possible effects they may cause. Determination of pollutants in environmental matrices, on the other hand, demands both efficient and selective sample handling processes. The SLM extraction format provides these characteristics and the technique has been frequently utilized for the sample preparation of various compounds. So far no work has been reported on the preconcentration and isolation of s-triazine and phenoxyacid herbicides using this extraction methodology from sugar samples of any origin in Ethiopia.

The main objective of this study is thus, to propose a method, based on literature review for sample preparation and enrichment of s-triazine and phenoxyacid herbicides in various stage of production of sugar and sugar based on SLM technique and determination of the analytes using HPLC with UV-detection

2. EXPERIMENTAL - SUGGESTED SAMPLING, EXPERIMENTAL SET-UPS AND PROCEDURES

2.1 Sampling and Sample Pretreatments

Field sampling for the analysis of pesticides is usually performed using either grab sampling or continuous sampling techniques. In grab sampling, a single sample increment is taken at a specific site. Such samples represent only the conditions at the time of collection, and should not be used as the sole basis for a decision about pollution abatement. However; some sources are quite stable in composition, and may be represented well by single grab samples. The quality of grab sample can be improved by collecting a series of smaller samples in a single container. The mixing of the collected sample averages the variation in sample composition.

On the other hand continuous sampling is a collection procedure where samples are constantly withdrawn from source or parent population and accumulated for collection at a later time [27]. In principle, a continuous sampling is a technique that should give representative sample of the whole. Even though a continuous sampling is advantageous we suggest that the sugar samples: sugar, molasses, primary juice, filter cake and condensed water be sampled by grab sampling method based on the physical setting of the factories.

The strategy of the sample pretreatment is guided by the nature of the substances to be determined and the level of the analyte concentration required for analysis, in addition to the method of detection. The major requirements for such techniques are simplicity, speed, high recovery, precision and robustness. These and other aspects of sample preparation technology have been reviewed by several workers [29, 59, 89]. Based on these reviews and the major the requirements of sample pretreatment and preparation we suggest the use of supported liquid membrane (SLM) for the preparation and pretreatment of sugar and sugar products samples for the analysis of the residue of s-triazines and 2,4-D herbicides.

2.2 Chemicals, Reagents and Working Solutions

From literature it is possible to note that standard *s*-triazines and 2,4-D herbicides were used for method development. Therefore we suggest the use of high purity standard for method development and calibration curves. The organic solvents used for impregnation of the membrane were di-*n*-hexylether, undecane, 6-undecanone, 1-decanol, 1-dodecanol and 1-tetradecanol [11] were used as membrane solvents elsewhere, even though optimization of the choice of solvents is required we suggest the use of di-*n*-hexylether, undecane. Generally Sodiumdihydrogenphosphate monohydrate (NaH_2PO_4. H_2O, 0.02 mol/L) and disodiumhydrogenphosphate dihydrated (Na_2HPO_4 $2H_2O$, 0.01 mol /L) were used as a buffer [4] by scientists to give a pH of 7.0 as a result we also suggest the use of these buffer solution. For calibration it is required that a series of concentrations of herbicides be prepared. Henceforth, we suggest the preparation of stock solutions of 100 mg/L and a series of solutions ranging from the concentration of 0.1 – 2.0 mg/L for this work. All stock, standard and extracted solutions should be stored in a refrigerator when not in use.

2.3 Experimental Set-Up Based on SLM

The arrangement of the membrane unit used elsewhere for the analysis of triazine herbicides using the supported liquid membrane extraction technique application to the study of waters in Ethiopian lakes analysis is shown in Figure 2.1 [11]. We also suggest the use of this set up for the current analysis.

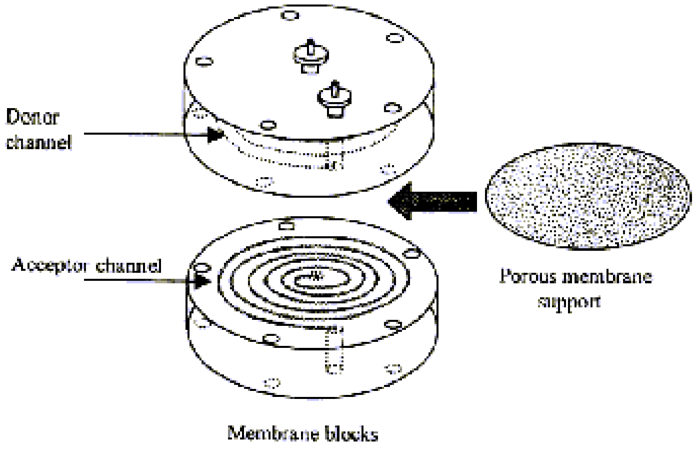


Figure 2.1 The membrane unit.

For the extraction of the analyte the two peristaltic pumps should pump the donor and the acceptor phases.

2.4 Membrane Preparation and Extraction Procedures

As described in several papers [90-95] there are many choices of membrane solvents. The liquid membrane should be prepared by immersing the porous polymeric membrane support in a given particular solvent. After installation of the membrane unit, its acceptor and donor channels should be flushed with distilled water to remove excess organic solvent from the surface of the support material. The acceptor channel then should be filled with the trapping (acceptor) solution and kept stagnant. Following this, samples of the herbicides and the buffer should be pumped into the donor channel of the membrane unit with a peristaltic pump, in a given volume (should be optimized) and appropriately mixed ratio. After the sample enrichment, the donor channel should be washed with appropriate buffer based on their structural properties. We suggest the use of acidic acceptor solution for *s*-triazine and basic acceptor phase for the 2,4-D extraction. The flow rate should be optimized in each case.

2.5 Optimization of the Extraction Parameters

The liquid membrane system for the extraction of the *s*-triazine and phenoxyacid herbicids should be optimized in terms of the amount of the extractant dissolved in the membrane solvent, membrane solvent and pH of the acceptor and sample solutions.

The effect of the amount of the mobile carrier, mixed with the membrane liquid, on the efficiency of the extraction should be investigated by extracting samples of the analytes mixture using liquid membranes containing appropriate solvent at different concentrations.

To study the effect of change in the concentration of hydrogen ion in the acceptor phase on the degree of extraction of the *s*-triazine herbicides, extractions should be made by varying the concentration of the acceptor solution (sulphuric acid solution) in the concentration range expected in a sample or as appropriate. On the other hand, in the study of the influence of donor solution pH on the extraction efficiency, sample solutions should be mixed with phosphate buffers, in the pH range chosen based on the optimization of the method before entering the donor channel of the extraction unit.

2.6 Further Recommendations

Many of samples in the production of sugar are sticky and may have influence in the extraction and detection; therefore stickiness can be reduced either by diluting the sample or by using the modifiers such as acetonitrile or methanol. The application of this study can be extended to the analysis of herbicide residues in fruits, vegetables and food samples. To draw a more general conclusion about the residues of these herbicides we suggest sampling steps to be extended to others like bagasse, soil, water used in the factories etc.

3. DISCUSSIONS BASED ON THE LITERATURE REVIEW

3.1 Determination of Experimental Variables

The theory of SLM extraction has been described by Audunsson [89] and thoroughly discussed by Jonsson *et al.* [90] with some additional aspects included recently [96, 97]. The result and discussion part can be dealt with based on the three important quantitative parameters frequently utilized to express the process of extraction in liquid membrane processes. These are extraction efficiency, enrichment factor and carry over effect (COE).

The extraction efficiency of the technique can be calculated from experimentally measured quantities using equation 3.1 [11, 96].

$$E = C_A V_A / C_I V_I \qquad (3.1)$$

Where C_I and C_A are the concentrations of the analyte molecules in the aqueous sample and enriched in the acceptor phase, and V_I and V_A are the volumes of the donor and acceptor solutions, respectively.

The enrichment factor of the SLM extraction process refers to the extent to which the extracted molecules are accumulated in the acceptor compartment. This can also be calculated from experimentally measured quantities. It is calculated by equation 3.2 [11, 96].

$$E_e = C_A / C_I \qquad (3.2)$$

The carry-over effect (COE) can be evaluated from the peak heights using the following equation [37];

$$COE = Pb / (Pb + Ps) \qquad (3.3)$$

Where Pb and Ps are peak heights of the blank extraction and sample mixture, respectively.

3.2 Effect of the Membrane Solvent

One of the major factors that determine the selectivity and efficiency of the SLM extraction is the type and composition of the organic liquid immobilized in the porous membrane support, as the transport rate of analytes is related to their partition coefficients between the aqueous and organic phases. The points that have to be considered when choosing the membrane liquid have been described elsewhere [54] and are summarized below.

The type of the porous support material in which the membrane liquid is immobilized is also equally important in SLM extraction processes. As it is reported in the literature [55], the ideal support should be very thin (<10 µm), should have high porosity (>50%), a mean pore size of less than 0.1 µm and a narrow pore size distribution. Furthermore, it should be stable and easy to handle. Membranes with PTFE materials are found to be suitable and used in most applications of SLM extraction. Therefore, we suggest the use of PTFE porous support material for the membrane liquid.

3.3 Study of pH on Extraction

Study of the donor and the acceptor pH

As has been theoretically predicted by Jöns on et al. [98], for basic compounds such as triazines, the pH of the flowing donor solution should be at least 2 pH units more than the highest pKa value to facilitate their dissolution into the membrane. On the other hand when the SLM technique is applied to either acidic or basic compounds, the pH of the stagnant acceptor phase plays an important role in controlling the degree of extraction of the target analytes. According to theoretical considerations [98] to achieve a nearly complete extraction for the basic compounds, the pH on the acceptor side should be at least 3.3 pH units below the pKa of the analytes in question. Therefore, the effect of donor and acceptor pH on the extraction efficiency or enrichment factors should be investigated in all of the matrices.

3.4 Donor Flow Rate and Life of the Membrane

One of the advantages of liquid membrane extraction is the possibility of increasing the amount of sample passing the donor channel per unit time, especially when large sample volumes are available, so as to increase the total amount of analyte accumulated in the stagnant acceptor solution. The influence of the donor flow rate on the extraction efficiency, and therefore, on the enrichment of the herbicide compounds, should be investigated over a wide range of flow rates. Previous investigation showed [94] that enrichment factor increases with increasing flow rate. This investigation also showed that problems associated with increasing the donor flow rate are the decrease in the lifetime of the membrane and reduced extraction efficiency for the subsequent extractions. The authors attributed the cause of these to the dissolution of the membrane liquid in to the flowing large volume of the aqueous donor phase. In fact, the donor flow rate can be increased to a certain value without causing pronounced effects on the lifetime of the membrane as a result the donor flow rate should be optimized.

3.5 Determination of the Method Detection Limit (MDL)

The method detection limit (MDL) is a statistical concept, based on the ability of a measurement method to determine an analyte in a sample matrix, regardless of its source of origin. The definition of method detection limit (MDL) for a particular method is important especially when traces analysis is involved [99]. The MDL for the extraction of *s*-triazine herbicides as described elsewhere [100] ranges from 40-70 $\mu g\ l^{-1}$ in water samples. We suggest the determination of MDL for the matrices under investigation

3.6 Application to the Real Samples

The method optimization and all investigation should be done on standard samples and ideal matrices like reagent water. The developed method should be applied to the analysis of real samples from sugar factories in the country. The possible sampling and sample preparation method for the sugar samples have been discussed above.

4. CONCLUSIONS

Various researches showed that the SLM extraction is a powerful analytical technique for selectively enriching trace herbicides in water [94], blood plasma [101], urine [102] and animal manure [103] samples. Based on these works the use of supported liquid membrane (SLM) extraction technique for enrichment of trace level of herbicides in samples of various stages of sugar product has been suggested. Optimization of the most important factors was also suggested. Ametryn and Atrazine members of symmetrical triazines and 2,4-D phenoxyacid herbicides should be selectively extracted using the suggested optimized method. The possibility of lowering the limit of detection of these compounds should be investigated by extracting lower concentrations at relatively higher flow rate.

To draw a more general conclusion about these herbicides and others in the sugar samples, it is desirable that a certain monitoring programmes be performed in all the three sugar estates in Ethiopia. Parameters such as sampling technique and time, collection site (stage), type of sugar cane used, and other physical and meteorological variables, etc should be taken into consideration.

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DECLARATION

I, the undersigned person, declare that this project is my work and that all sources of materials used for the thesis have been duly acknowledged.

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This project work has been submitted for examination with my approval as university advisor.

Dr. Negussie Megersa ________________________