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# Comparative Analysis of Pesticide Extraction Methods

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# COMPARATIVE ANALYSIS OF PESTICIDE EXTRACTION METHODS

A THESIS

The Honors Program

College of St. Benedict/ St. John's University

In Partial Fulfillment

of the Requirements for the Distinction "All College Honors"

and the Degree Bachelor of Arts

In the Department of Chemistry

by Julie A. Lapos

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**Comparative Analysis of Pesticide Extraction Methods**

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## ACKNOWLEDGMENTS

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## ABSTRACT

Liquid-Liquid Extraction using methylene chloride, Liquid-Liquid extraction using 75:25 hexanes: ethyl acetate, and Solid Phase Extraction using C-18 disks were compared. Aliquots of water spiked with alachlor, atrazine, and trifluralin were extracted using each of the extraction techniques. The extracts were concentrated and analyzed using Gas Chromatography/ Mass Spectrometer with Selected Ion Monitoring. A surrogate, 2-nitro-*m*-xylene, and an internal standard, phenanthrene-$d_{10}$, were used. At spiking levels of 5-60 µg/L, there was not a significant statistical difference between the three methods when the liquid extractors were run for 24 hours. There was however a difference between the solid phase extraction and the liquid-liquid extractions when the liquid extractors were run for only 3 hours.

## INTRODUCTION

**Background.** At least once a year, Minnesota farmers apply 40-45 million pounds of pesticide onto more than 96% of their acreage for better quality and higher yield crops.[1,2] The widespread pesticide use, along with Minnesota's contiguous water resource has lead to the contamination of the water supply. Prior to the enactment of the Federal Environmental Pesticide Control Act (FEPCA) in 1972 which called for an improvement in pesticide control and usage, the ground water was assumed to be protected from pesticide contamination.[2,3] Monitoring of water since that time has indicated contamination by organic chemicals including pesticides.[4]

Pesticide contamination of ground water by agricultural practices is referred to as "non-point source" pollution. This type of pollution does not have a single, concentrated source and is typified by wide-spread low level contamination. Over the last ten years, nonpoint source contamination of water resources from agriculturally applied pesticides has been a topic of increased environmental concern.[5]

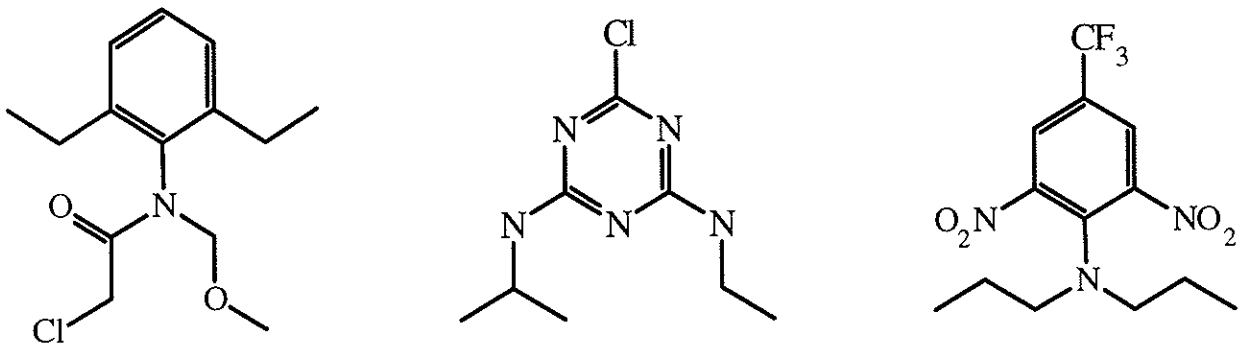
Because of the potential toxicity of pesticides to humans due to long term exposure, government organizations have set health guidelines detailing maximum exposure levels. Recommended allowable limits (RALs) for pesticide concentrations in drinking water are the maximum levels that a person can be exposed to with no adverse effects. Since the individual concentrations must not exceed 1 μg/L (part per billion or ppb), highly accurate and sensitive analytical methods are required.[6]

**Contamination of the Water Supply.** Herbicides, which consist of organophosphorous, organonitrogen or organohalogen compounds, are one type of pesticide applied to fields to provide resistance against unwanted plants. Upon application, the herbicide, which is usually not very water soluble due to its organic nature, is taken up by the plant through the root system. The molecule interferes with the plant's ability to grow and thus the target plant dies. Following application the pesticide starts to decompose due to microorganisms in the soil, sun and/or thermal exposure. Each pesticide has a known half-life, varying from 5-500 days which is the time it takes for the pesticide to decompose to half of its original concentration.

The characteristics of the compound all have an effect on the persistence of the pesticide and how the pesticide will travel into the water resource. Three values which are important in pesticide movement are the solubility of the analyte, its leaching potential, and its absorbance onto sediment. The soil composition and the pesticide both have an effect on the extent to which these three phenomena will occur and how the pesticide will enter the water supply.

Pesticides can enter the water system by adhering to soils such as clay and carried into surface waters such as lakes and rivers or by leaching through soils such as sand. Once in the water supply, the pesticide may then filter into ground wells and contaminate people's drinking supply. Because of the intimate relationship between surface water quality and ground water quality, detection of pesticides in surface water 'grab' samples can be used to monitor the distribution and movement of the pesticides.

**Pesticides Monitored.** The pesticides monitored were chosen based on their behavior during analysis, their commercial availability, and most importantly, their frequency of use by local farmers and appliers. The pesticides selected were alachlor, atrazine, and trifluralin (Figure 1).




a b c

**Figure 1.** Structures of the three herbicides tested; (a) atrazine, (b) alachlor, and (c) trifluralin.

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Alachlor is an aniline herbicide used to control annual grasses and broadleaf weeds in corn and soybeans. It is often applied in conjunction with other herbicides such as atrazine and trifluralin for more effective results. Alachlor can be purchased under the commercial names of Lasso, Lariat, and Crop Star. It is absorbed by germinating roots and shoots and interferes with protein synthesis and root elongation. Alachlor has a half-life of only 15 days depending on the soil type and climate. The main form of degradation is by soil microbes. Alachlor has a medium potential for movement by both surface runoff and leaching. In monitoring of wells in the

Midwest, the presence of alachlor was undetected, most likely due to its short half-life. The extent of human risk from prolonged exposure to alachlor is unknown at this time, but distinct health problems, such as tumors and cancer have been found in laboratory animals exposed to low doses.[7] The potential carcinogenicity of alachlor has caused its restricted use and the RAL to be set at 6 ppb.[6]

Atrazine is a pre-emergent herbicide, applied after the planting of the crop. As a selective triazine herbicide, atrazine is used to control broadleaf and grassy weeds in corn fields. Atrazine is the active ingredient in herbicides such as Aatrex, Alazine, Atred, Atrazine, and Atranex. Upon application atrazine is absorbed by the plants through the root system and through the foliage. The molecule accumulates in the growing tips and new leaves of the plant where it inhibits photosynthesis. Atrazine has been classified as a Restricted Use Pesticide due to its potential for ground water contamination. Trace quantities have been found in drinking and ground water samples in the Midwest.[8] Atrazine has a large potential for leaching especially in soils which have low clay or organic matter to which the pesticides adhere. The long soil half-life of atrazine, 60 days, during which time the pesticide is still active, causes a high potential for ground water contamination. Atrazine has a medium potential for surface runoff due to the low absorbance onto soils and is moderately soluble in water (33 ppm). Long-term consumption or exposure to atrazine has caused adverse health problems in animals including tumors.[8] Atrazine is a slightly toxic suspected human carcinogen with a RAL of 3 ppb.[6]

Trifluralin is a selective, pre-emergent dinitroaniline herbicide used to control annual grasses and broadleaf weeds in soybeans and small grains. It is sold under various names including Treflan, Tri-4, and Trust. Trifluralin is absorbed by the roots of germinating seedlings

and inhibits the growth of roots and shoots. Because of the strong absorbance of trifluralin with soils and its low solubility in water (0.3 ppm), leaching and ground water contamination are not expected to occur.[9] Trifluralin is sensitive to sunlight and the half-life in soils is 45 to 60 days. Consumption of high levels of trifluralin has caused liver and kidney damage and increased fetal problems such as low weights and miscarriages in animals. [9] The RAL established by the Minnesota Department of Health for trifluralin in drinking water has been set at 2 ppb.[6]

**Analysis-Experimental Design.** The long-term objective of this project was to analyze and monitor pesticide concentrations in the Sauk River Watershed District. To obtain low detection levels, the pesticides have to be extracted from the water, concentrated, and then analyzed using a sensitive technique. This study was directed toward the examination of pesticide extraction techniques for eventual application. The two types of extraction methods which have been used to extract the pesticides from water samples are Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE).

Liquid-liquid extraction techniques are some of the most common techniques used for liquid sample preparation. In these extractions, the analytes are partitioned between two immiscible liquids, usually one being aqueous and the other organic. The choice of the two immiscible liquids effects the selectivity and efficiency of the extraction. When aqueous and organic solvents are used, the more hydrophobic compounds, such as the organic herbicides, reside in the organic phase while the more hydrophilic compounds reside in the aqueous phase. Extracting the analytes into the organic phase is usually preferred due to the easy removal of the solvent, allowing the analytes to be concentrated.[10] Continuous liquid-liquid extraction provides

a practical way of performing multiple extractions unattended and without the problem of emulsions. In a continuous liquid-liquid extraction with an organic solvent more dense than water (Figure 2), the solvent is distilled from the solvent reservoir, condensed above the liquid to be extracted, and then dripped through the immiscible aqueous layer, extracting the analytes. The solvent is returned through the solvent return tube to the reservoir and later concentrated. Fresh organic solvent is continuously cycled through the extractor for a determined amount of time. Figure 3 shows the extractor with a solvent less dense than water. The solvent is distilled from the reservoir and condensed above the aqueous sample. Because the solvent is less dense than water, the condensate is collected into a funnel. The solvent then percolates through the aqueous layer extracting the analytes and returns to the reservoir by overflowing the side arm. Continuous liquid-liquid extractions have excellent extraction efficiency; however, volatile compounds can be lost during distillation and thermally sensitive compounds can decompose.[10]

Solid Phase Extraction has increasingly been used in sample preparation due to the short extraction time, the small extraction volume, and the ease of coupling to on-line analyzing systems.[11,12] In SPE, a liquid is passed through a solid phase membrane or cartridge and the analytes are selectively removed from the liquid and retained on the solid phase (Figure 4). By using polar solvents, the analytes can be eluted from the solid phase and collected. SPE minimizes the amount of organic solvent used for extraction, which is also a pollution concern. Another advantage to SPE is its versatility. One may change the composition and type of solid phase and the solvents to maximize the extraction efficiency.

Both of these extraction techniques, LLE and SPE, have been used for the isolation of pesticides from water samples.[13-17] LLE methods using methylene chloride, also known as dichloromethane, with a density greater than water (d=1.362), have been the standard protocol for extracting pesticides from water samples.[11] The Minnesota Department of Agriculture has had success in the extraction of surface water using LLE with methylene chloride. The method is ideal for surface samples which have high amounts of particulate matter. The major disadvantage to this method is the use of large volumes of methylene chloride which is a potential environmental and health concern; therefore, other extraction methods are desired.

A mixture of hexane and ethyl acetate was shown to produce slightly better percent recoveries than methylene chloride in the extraction of organic substances and is considered more environmentally friendly.[18] Therefore, a solution of 75:25 (v/v) hexanes: ethyl acetate was used in place of methylene chloride in the liquid-liquid extractor. The solvent is less dense than water, so a slightly different LLE apparatus was used as shown in Figure 3.

SPE has increasingly been used in the last five years as an alternative to liquid-liquid extractions for the concentration of various pesticides in water samples.[17] The standard protocol for pesticide determination in ground water is the EPA protocol (Method 525.2 revision 1) which is a liquid-solid extraction, using either C-18 cartridges or disks. Methods have been developed which use disks and cartridges made of C-8 or C-18 bonded silica, styrene-divinylbenzene copolymer, graphitized carbon black, and Amberlite XAD resins to analyze pesticides.[11, 19-20] C-18 disks were chosen for this study because of their recent popularity in SPE. Figure 4 shows the liquid-solid apparatus for use with a C-18 disk. Just as in LLE, the organic pesticides will preferentially reside in the organic C-18 solid phase and be extracted from the water. The

pesticides retained on the disk, are then extracted using organic solvents and concentrated. When the analysis is applied to surface water samples, the C-18 filter is easily clogged by particulate matter. Large particles in surface samples can be pre-filtered using cellulose pads, but clogging of the C-18 disk still occurs. The presence of the particulate matter causes liter samples normally requiring twenty minutes per extraction to last for more than two days. Graded density glass micro-fiber filters have been used on top of the C-18 disk during the filtration to decrease the extraction time. The filters gradually filter the particulate matter in the sample preventing clogging.

Despite increasing availability of High Performance Liquid Chromatography procedures, High Resolution Gas Chromatography (GC) is the analytical technique most often applied for determining low pesticide levels.[11, 13, 20] The GC is coupled to selective nitrogen-phosphorus detectors and electron capture detectors or a low resolution Mass Spectrometer (MS) operating in Selected Ion Monitoring mode (SIM).[21] GC/MS SIM was chosen for pesticide analysis in this study.

The GC column is a glass capillary cross-linked with phenyl methyl groups creating a nonpolar internal surface. The interaction of the compounds with the column separates the analytes and determines their eluting order. Compounds which have little interaction will elute first while compounds which are more like the nonpolar column will interact more and have a longer retention time. By adjusting the ramping parameters of the column, maximum peak resolution with minimum run time can be accomplished. In this procedure, the column is quickly ramped for the first four minutes to elute the low boiling solvent. The column is then slowly ramped for the next 12 minutes to resolve the analyte peaks. After the analytes have been eluted,

the column is ramped to a high temperature to eliminate any impurities which may have adhered to the column.

A mass spectrometer located at the end of the column analyzes the separated compounds. Electrons bombard the analytes eluting from the column causing ionization and fragmentation. An analyzer is then used to determine the mass to charge ratio of the ions and the detected signal is amplified. In this method, known fragment masses of the analytes are specifically scanned using SIM to achieve an order of magnitude greater detection.

The limit of detection on the GC/MS used in this research was around 5 ppm. This final concentration translates to a concentration of 10 ppb in the initial water sample, a substantially greater amount than the sub-part per billion level which may be present in real samples. Because of the high limit of detection on the instrument, water samples were spiked with alachlor, atrazine, and trifluralin. A surrogate, 2-nitro-*m*-xylene (Figure 5) was added to the spiked water sample to monitor the efficiency of the extraction. An internal standard, phenanthrene-$d_{10}$, (Figure 6) was injected into the concentrated sample prior to analysis to correct for any variance in the analysis and to measure the relative responses of the analytes.

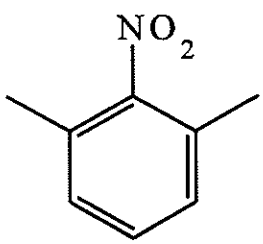


**Figure 5.** Structure of surrogate, 2-nitro-*m*-xylene.

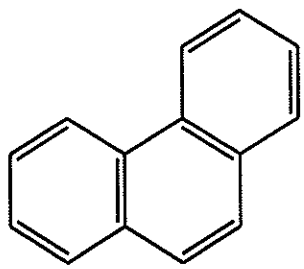

**Figure 6.** Structure of internal standard, phenanthrene-$d_{10}$.

**Purpose**. The purpose of this study was to compare three extraction methods: Solid Phase Extraction, Liquid-Liquid Extraction with methylene chloride, and Liquid-Liquid Extraction with 75:25 hexanes: ethyl acetate; to determine which method was the most efficient for pesticide extraction. The comparison was accomplished by spiking water samples from 5 ppb to 60 ppb with alachlor, atrazine, trifluralin, and 2-nitro-*m*-xylene. After extraction, the samples were analyzed using GC/MS SIM and the percent recoveries were calculated.

## EXPERIMENTAL SECTION

**Apparatus.** Kontes continuous liquid-liquid extractor solvent more dense than water (584550), Kontes liquid-liquid extractor solvent less dense than water (581250), were used for LLE extraction. Supelco solvent filtration apparatus (5-8061, 5-8062, and 5-8079) with a 47 mm Empore extraction disk Octadecyl (C18) purchased from Fisher Scientific was used. The samples were analyzed on a Hewlett Packard 5988A gas chromatography/mass spectrometer (GC/MS) equipped with a HP 7673A automatic sampling system. The capillary column was a Hewlett Packard HP-5 cross-linked 5% phenyl methyl silicone 30 m x 0.25 mm x 0.25 μm film thickness.

**Standard Preparation.** Phenanthrene-$d_{10}$ standard solution, 1000 ηg/μL, was purchased from Ultra Scientific and diluted by half with pesticide grade acetone (Fisher Scientific). Neat standards of alachlor, atrazine, and trifluralin were purchased from Ultra Scientific. Surrogate, 2-Nitro-*m*-xylene, 99%, was purchased from Aldrich. Stock standard solutions of 5000 ηg/μL were prepared using the neat standards dissolved in pesticide grade acetone (Fisher Scientific). A primary dilution standard solution, 50 ηg/μL, was prepared from the stock standard solutions. Calibration standards of 1, 5, 10, 20, 30, and 40 ηg/μL were prepared from the primary standard solution and 20 μL of phenanthrene-$d_{10}$ stock standard solution was injected into each sample.

**Procedure.** Two liters of purified B-pure water, 175 kilohm-cm resistance with removal of ions, and E-pure water, 18 megaohm-cm resistance with removal of ions and organics, (Barnstead) were spiked with primary standard solution to yield concentrations of 5 ηg/mL to 60

ηg/mL. Reagent blanks, containing no standards were also run to monitor for contamination during the extraction process. The water sample was divided into 500 mL aliquots to be used for each extraction. Glassware was cleaned with detergent and water, rinsed three times using E-pure water (Barnstead) and rinsed three times with pesticide grade methanol (Fisher Scientific).

LLE *Methylene Chloride*[22] The round bottom flask was filled with 150 mL of pesticide grade methylene chloride (Fisher Scientific) and boiling chips were added. The column was filled with 600 mL of methylene chloride and the water sample was added. Additional methylene chloride was added to the extractor column until the solvent level in the solvent return tube was nearly filled. Cold water was run through the condenser and a hot sand bath was used to heat the methylene chloride at a distillation rate of 5.8 mL/min.

LLE *Hexanes: Ethyl Acetate* A 75:25 (v/v) solution of hexanes: ethyl acetate was prepared using pesticide grade solvents obtained from Fisher Scientific. The round bottom flask was filled with 200 mL of 75:25 (v/v) hexanes: ethyl acetate solution and boiling chips were added. The water sample was poured through the interior funnel of the column. Approximately 35 mL of solvent was then added through the funnel so that the solvent filled the interior piece, percolated through the water, and formed a layer on top of the water which could then overflow the sidearm. Cold water was circulated through the condenser and a sand bath was used to heat the solvent in the round bottom flask at a distillation rate of 4.5 mL/min. Glass wool and aluminum foil were placed around the round bottom flask and mid-way up the extracting column to allow the solvent to condense above the funnel. The LLE extractors were run for a specified amount of time, three or twenty-four hours, and then disassembled. The round bottom flask was then evaporated at reduced pressure to approximately 2 mL. A solution of 40% toluene in iso-

octane (1 mL) and petroleum ether (25 mL) was added to the flask and evaporated at reduced pressure to near dryness. The extract was then transferred into an amber crimp top vial using three 0.5 mL hexanes washes. The mass of the contents was determined and the vial was stored at 4°C until analysis.

*Solid Phase Extraction*[23] A C-18 extraction disk was placed in a suction filtration system. The disk was washed with 5 mL of 1:1 ethyl acetate: methylene chloride prepared from pesticide grade solvents (Fisher Scientific). The disk was not allowed to go dry throughout the remainder of the experiment which would effect the recovery. The disk was wetted with 5 mL of pesticide grade methanol (Fisher Scientific) followed by 5 mL of E-pure water. Methanol was added to the sample (5 mL/L sample) and the sample was extracted. A full vacuum aspirator (-80 kPa) was used to pull the system at a flow rate of 100 mL/minute. The disk was dried by maintaining the vacuum for 10 minutes. The filtrate was removed and a new collection flask was used to collect the eluent. Ethyl acetate (5 mL) purchased from Fisher Scientific was used to rinse the sample bottle, then added to the filtration reservoir. The disk was soaked with the solvent for 1 minute and the vacuum was used to draw the solvent through the disk. This process was repeated with 5 mL of methylene chloride (Fisher Scientific pesticide grade). A 1:1 solution of ethyl acetate: methylene chloride (3 mL) was used to rinse the filtration reservoir twice and drawn into the collection flask. A drying tube containing anhydrous sodium sulfate (reagent grade, Fisher Scientific) and a clean filter flask were used to dry the collected extracts and three 3 mL portions of 1:1 ethyl acetate: methylene chloride were used to rinse the drying tube. The collected extract and washings were concentrated to 0.5 mL in a warm water bath under a stream of nitrogen. Ethyl acetate was used to transfer the concentrate into a 1.5 mL amber crimp top

vial and the mass of the concentrate was determined. The vial was stored in the refrigerator at 4°C until analysis.

**Gas Chromatography/Mass Spectrometer**. The automatic sampler was used to run the samples. The sequence consisted of the calibration standards run two times, followed by the samples run in duplicate, repeated by the calibration standards again. High purity Helium gas with a flow rate of 5 psi was used as the carrier gas and 2 μL of sample was injected into the column. Ramping conditions: Initial 45°C for 1 minute, ramp 45°C/min to 180°C, ramp 5°C/min to 240°C, ramp 45°C/min to 300°C, hold 3 minutes. Injection port at 250°C and ion source at 200°C. SIM ions 58, 77, 132, 134, 151, 160, 188, 200, 215, 264, 269 m/z were chosen based on the fragmentation patterns of the analyte standards.

## RESULTS AND DISCUSSION

**Chromatogram.** The GC/MS analysis is reported in a chromatogram (Figure 7).

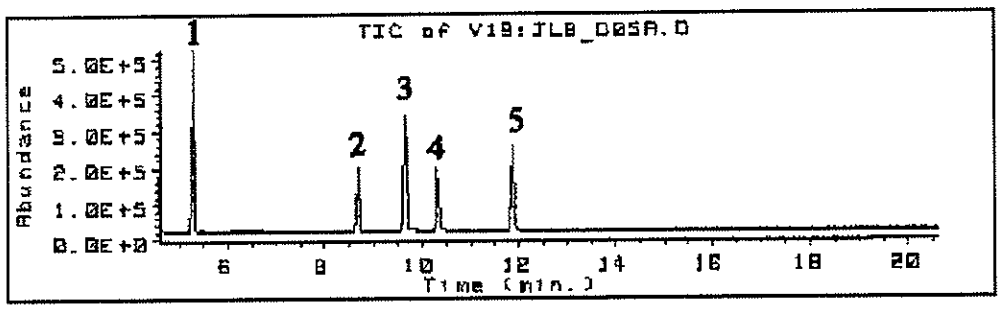


**Figure 7.** Chromatogram of calibration standard using GC/MS. The abscissa is in minutes of elution from column and the ordinate is the abundance of compound detected by mass spectrometer. The peaks correspond to (1) 2-nitro-*m*-xylene, (2) trifluralin, (3) atrazine, (4) phenanthrene-$d_{10}$, (5) alachlor.

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The peaks of the chromatogram can be identified by the elution time and the fragmentation pattern of the peak. The elution time of the peak maximum is specific for each of the individual analytes and varies little with time. Standard solutions were run to confirm the retention times. The ion spectrum which shows the fragmentation patterns of the peak, can also be used for peak identification. Figures 8 and 9 show the elution time of the peak and the fragmentation patterns of the individual analyte standards. For each analyte, the molecular ion peak, the ion which has the same mass as the original molecule, and the base peak, the largest peak in the spectrum, were two of the ions monitored in SIM. The mass to charge fragments monitored were; 151, 134, and 77 m/z for 2-nitro-*m*-xylene; 215, 200, and 58 m/z for atrazine; 269, 188, 160, and 132 m/z for alachlor; 264 and 188 m/z for trifluralin; and 188 and 160 m/z for phenanthrene-$d_{10}$. SIM acquisition was used because the sensitivity of the instrument was increased by monitoring

specific mass to charge ratios. SIM acquisition, however, prevented the use of the fragmentation spectrum to confirm peak identity. Thus, the peaks were identified based only on retention time. This was problematic in the determination of peak area for a couple of reasons. First, the total elution times for the peaks varied between samples and second, based on the integration parameters chosen, sometimes the integrated peak was split into two separate areas.

**Concentration Determination.** On the chromatogram, the peaks of the calibration standards were integrated to determine their area. In each spectra, the analyte peak area was divided by the internal standard (IS) peak area to account for variations in sample volumes and/or the amount of sample injected onto the column. The relative peak areas were plotted versus the concentration of each calibration standard and a calibration line for each analyte was generated (Figure 10). A least squares line was fit to the data and showed a linear relationship between concentration and area with correlations ranging from 0.998 to 0.99991. The concentration of the unknown sample was derived by using the point slope formula

Area relative to IS= (slope)(concentration) + intercept

with the slope and intercept calculated from the calibration line. By knowing the relative peak areas of the extracted sample, the concentration of the analytes can then be determined. The theoretical concentration of the analytes in the initial two liter water sample was calculated and compared to the experimental result to yield a percent recovery for each analyte.

**Results of Extractions.** Twelve different spiked water samples were extracted by each of the three methods. The results are shown in Table 1.

**Table 1. Percent Recoveries of Extraction Methods**

| Spiking Concentration | Extraction Method [a, b] | Analytes [c, d] | | | |
|---|---|---|---|---|---|
| | | 2-nitro-m-xylene | Trifluralin | Atrazine | Alachlor |
| Extraction A 5.0 ppb | SPE | NA | 100±30 | 110±40 | 80±20 |
| | LLE MeCl2* | 90±30 | 80±20 | 80±30 | 90±20 |
| | LLE 75:25* | 70±20 | 80±20 | 80±30 | 90±20 |
| Extraction B 6.25 ppb | SPE | 80±20 | 70±20 | 100±40 | 110±30 |
| | LLE MeCl2* | 140±40 | 80±20 | 100±40 | 130±40 |
| | LLE 75:25* | 40±10 | 50±10 | 60±20 | 150±40 |
| Extraction C 6.25 ppb | SPE | NA | 60±20 | 80±30 | 90±20 |
| | LLE MeCl2* | 120±40 | 90±30 | 100±40 | 120±30 |
| | LLE 75:25* | 80±25 | 70±20 | 70±30 | 160±40 |
| Extraction D 7.5 ppb | SPE | 130±30 | 60±20 | 120±30 | 140±40 |
| | LLE MeCl2* | 90±30 | 70±20 | 120±30 | 150±40 |
| | LLE 75:25* | 60±20 | 80±20 | 130±50 | 140±40 |
| Extraction E 10.0 ppb | SPE | negative | 50±10 | 80±30 | 70±20 |
| | LLE MeCl2* | 120±40 | 70±20 | 80±30 | 90±20 |
| | LLE 75:25* | 80±30 | 40±10 | 80±30 | 80±20 |
| Extraction AA 10.0 ppb | SPE | 70±20 | 80±20 | 100±40 | 100±30 |
| | LLE MeCl2 | 50±20 | 50±10 | 50±20 | 50±10 |
| | LLE 75:25 | 18±5 | 40±10 | 50±20 | 50±10 |
| Extraction BB 20.0 ppb | SPE | NA | 50±10 | 90±30 | 80±20 |
| | LLE MeCl2 | 40±10 | 40±10 | 40±10 | 40±10 |
| | LLE 75:25 | 21±6 | 50±10 | 40±10 | 40±10 |
| Extraction CC 20.0 ppb | SPE | 70±22 | 70±20 | 90±30 | 90±20 |
| | LLE MeCl2 | 60±20 | 30±10 | 30±10 | 30±10 |
| | LLE 75:25 | 50±10 | 40±10 | 40±10 | 40±10 |
| Extraction DD 25.0 ppb | SPE | 11±3 | 60±20 | 70±30 | 90±20 |
| | LLE MeCl2 | 60±20 | 60±20 | 60±20 | 90±20 |
| | LLE 75:25 | 40±10 | 50±10 | 60±20 | 80±20 |
| Extraction EE 30.0 ppb | SPE | 80±30 | 70±20 | 90±30 | 80±20 |
| | LLE MeCl2 | 60±20 | 60±20 | 40±10 | 40±10 |
| | LLE 75:25 | 40±10 | 50±10 | 60±20 | 90±20 |
| Extraction FF 40.0 ppb | SPE | 8±2 | 60±20 | 80±30 | 70±20 |
| | LLE MeCl2 | 70±20 | 60±20 | 60±20 | 60±20 |
| | LLE 75:25 | 80±20 | 50±10 | 60±20 | 50±10 |
| Extraction GG 60.0 ppb | SPE | 110±20 | 80±20 | 100±40 | 90±20 |
| | LLE MeCl2 | 90±30 | 60±20 | 70±30 | 100±30 |
| | LLE 75:25 | 90±30 | 50±10 | 60±20 | 50±10 |

(a) MeCl2 refers to methylene chloride solvent, 75:25 refers to hexanes:ethyl acetate solvent. (b) * notes liquid extractors run for 24 hours, all unmarked LLEs were run for 3 hours. (c) NA, no integrated area for analyte. (d) negative, concentration calculated from calibration line was negative.

Note that the percent recoveries, especially in Extractions A-E, were at times greater than 100%. Throughout this research there was a continuous problem with large recoveries. Recoveries as large as 102 to 130% reported in literature and by the Minnesota Department of Agriculture were explained by gross error.[21] A substantial source of error in the results of this research was due to the error associated with the calibration curve. All four calibration curves (Figure 10) had an average error of 30%. This corresponds to the large absolute error given in Table 1.

**Error and Analysis.** The sequence method used to run the calibration standards and the samples can last for over 24 hours. There was concern as to whether or not the calibration line changed from the start to the end of the sequence. Figure 11 shows the two calibration lines analyzed before and after the extracted samples. The difference in the slope between the two calibration lines was not statistically different within the assumptions of a probability test performed using linear regression and a weighting of the data. A p-value of $<0.05$ indicated that the hypothesis which stated that there was not a difference between the two calibrations, should be rejected. Values from the test gave 2-nitro-*m*-xylene p=0.105, trifluralin p=0.497, atrazine p=0.142, alachlor p=0.274. This same statistical test was performed for calibration standards run on two separate occasions. The hypothesis was rejected in the second case because p values of 2-nitro-*m*-xylene and trifluralin calibrations were 0.019 and 0.045 respectively. The results concluded that the calibration line is valid for the sequence, but cannot be used to determine concentrations in samples analyzed during a different sequence.

The low concentration of the extracted sample (3 ppm), was assumed to be another explanation for the greater than one hundred percent recovery. The concentration was on the extreme end of the calibration line which cannot be trusted for accurate results. The low concentration also created a problem because the limit of detection of the instrument was being reached. In Table 1, Extraction E had a noticeable peak on the chromatogram for 2-nitro-*m*-xylene, but linear analysis showed a negative concentration.

Low concentrations (5 ppb) were also more prone to high recoveries when any type of contamination was present. Unspiked water samples, reagent blanks, were extracted and the results are given in Table 2.

**Table 2. Calculated Analyte Concentrations of Reagent Blank in ppm**

| Extraction Method [a] | Analytes [b] | | | |
|---|---|---|---|---|
| | 2nitromxylene | Trifluralin | Atrazine | Alachlor |
| SPE | 0.7±0.2 | NA | 0.4±0.1 | 0.6±0.2 |
| LLE MeCl2 | 1.7±0.5 | 1.5±0.4 | 0.4±0.1 | 0.7±0.2 |
| LLE 75:25 | 6±2 | NA | 0.5±0.2 | 0.7±0.2 |

Results of unspiked water sample extraction, LLEs run for 3 hours. (a) MeCl2 refers to methylene chloride solvent, 75:25 refes to hexanes: ethyl acetate solvent. (b) NA, no integrated area for analyte.

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The presence of alachlor and atrazine in all three extractors at approximately identical concentrations suggested contamination in an early step. Because these concentrations were at the low end of the calibration line there was a possibility for inconsistency within the results.

The water samples were spiked as high as 60 ppb to give final extracted concentrations in the middle of the calibration curve (10-25 ppm). Due to time constraints, the liquid-liquid extractors were cycled continuously for 3 hours which was cited in Marcomini et al. to produce recoveries similar to SPE.[22] The results are shown in Extractions AA-GG in Table 1. The shorter extraction time showed a noticeable decrease in analyte recovery in the liquid-liquid extractions. A Student's Standard t Test was performed on the individual differences between each method for the percent recovery of analytes (Tables 3 and 4). Comparisons of the 24 hour liquid-liquid extractions versus solid phase extraction showed a statistical difference (95% confidence level) of alachlor recovery between SPE and LLE methylene chloride. However, when SPE was compared to the 3 hour LLE methylene chloride and LLE 75:25, there was a significant difference in the extraction of most of the pesticides. There was a significant difference between the two LLEs in the extraction of the surrogate in both the 3 and 24 extractions and there was also a significant difference for the extraction of trifluralin between both of the 24 hour LLEs.

**Table 3. Student's t Analysis of SPE and LLEs Run for 24 Hours**

| Extraction Methods [a] | Analytes [b] | | | |
|---|---|---|---|---|
| | 2nitromxylene | Trifluralin | Atrazine | Alachlor |
| SPE/MeCl2 | 2.5 | 2.2 | 0.17 | **5.0** |
| SPE/75:25 | 0.81 | 0.90 | 1.5 | 2.1 |
| MeCl2/75:25 | **3.8** | **3.0** | 1.5 | 0.92 |

(a) MeCl2 refers to methylene chlroide extraction, 75:25 refers to hexanes: ethyl acetate extraction.
(b) Bold numbers indicate t-values that are greater than the standard t (2.132 d.f.=4) at 95% confidence.

**Table 4. Student's t Analysis of SPE and LLEs Run for 3 Hours**

| Extraction Methods [a] | Analytes [b] | | | |
|---|---|---|---|---|
| | 2nitromxylene | Trifluralin | Atrazine | Alachlor |
| SPE/MeCl2 | 0.79 | **2.5** | **4.9** | **2.6** |
| SPE/75:25 | 0.015 | **3.3** | 2.3 | **4.7** |
| MeCl2/75:25 | **2.5** | 1.0 | 0.81 | 1.2 |

(a) MeCl2 refers to methylene chlroide extraction, 75:25 refers to hexanes: ethyl acetate extraction.
(b) Bold numbers indicate t-values that are greater than the standard t (2.447 d.f.=6) at 95% confidence.

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**Explanation.** The results showed that the SPE, LLE methylene chloride, and LLE 75:25 hexanes: ethyl acetate have comparable extraction recoveries when the liquid extractors were run for a long period of time (24 hours). This was not the case when the liquid extractors were run for only three hours. The decrease in the liquid extractors' efficiencies can be explained by the extraction efficiency. The efficiency of an extraction is related to the number of extractions by the formula

$$E=1-[1/(1+K_DV)]^n$$

where $K_D$ is the distribution coefficient, V is the volume and n is the number of extractions.[10] Since an efficiency of 1 is ideal, the more extractions, n, performed the smaller the fraction will become. This equation shows that more extractions, i.e. the longer the extractor is run, increase the efficiency. Thus, more analyte will be recovered when the extraction is longer, such as 24 hours, and explains the significant difference that occurs when the liquid extractor is run for only three hours.

**Comparison.** The insignificant difference between the 24 hour LLEs compared to SPE concur with similar studies in the literature. Marcomini et al. reported efficiencies between 84 and 102% regardless of the extraction procedure, SPE C-18 and LLE methylene chloride, used.[21] Taylor et al., after extensively comparing LLE and SPE also concluded that the two methods were statistically equivalent.[12]

Since there was not a significant statistical difference between the methods when performed over a 24 hour extraction time, other factors can be examined when choosing a pesticide extraction method. LLE methylene chloride is a standard protocol, which has been used for numerous years and has been shown to yield consistent accurate results. The liquid extractors also are able to extract pesticides from samples high in particulate matter. However, the large volume of methylene chloride used (700 mL) not only poses a cost issue, but also a health and environmental concern. The 75:25 hexanes: ethyl acetate LLE does reduce that large volumes of potentially hazardous solvent. However, the long extraction time (3 and 24 hours) and high temperatures needed to boil the solvent in LLE may cause analyte loss due to thermal and photo decomposition. SPE is relatively fast, requiring an hour and a half per extraction which allows for more sample analyses to be performed. Analytes can be very sensitive to photo and thermal decomposition, therefore, the minimal temperature exposure and fast extraction time of SPE may allow for better recoveries. Other advantages to SPE include the use of low solvent amounts (50 mL) and water sample volumes can be varied. Disadvantages to SPE include; pesticide loss due to the multiple transfer steps and the saturation of active sites on the C-18 disk when samples that have high pesticide levels are extracted. Another problem with SPE is that the disks tend to clog when samples, containing high amounts of particulate matter are filtered.

## CONCLUSION

In summary, there was not a significant statistical difference in the extraction of alachlor, atrazine, trifluralin, and 2-nitro-*m*-xylene using SPE, LLE methylene chloride and LLE 75:25 hexanes: ethyl acetate when extracted for 24 hours. There was however, a difference when the liquid extractors were run for only 3 hours. Further research can be conducted to determine a minimum extraction time for the liquid extractor which yields similar results to SPE. Surface water samples may also be extracted to determine how matrix interferences effect the percent recoveries. Although there still appears to be difficulty in a number of different places in the analysis, such as the accuracy and sensitivity of the GC/MS, with continued dedication and work these problems may be solved.

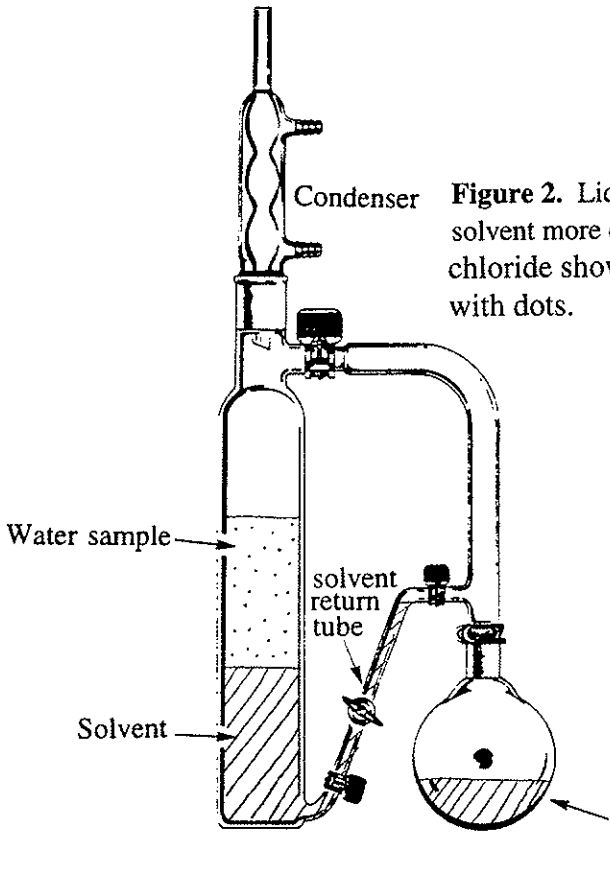



**Figure 2.** Liquid-Liquid Extraction apparatus, solvent more dense than aqueous. Methylene chloride shown with stripes, sample shown with dots.

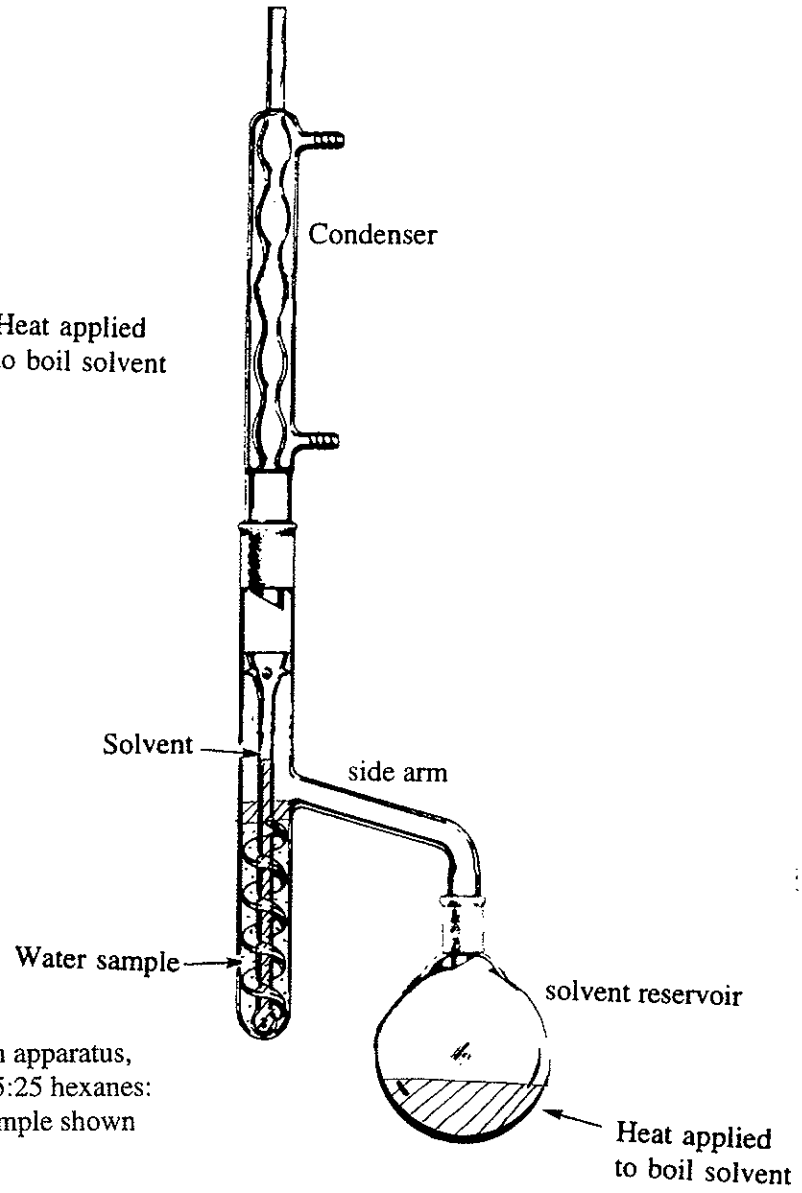



**Figure 3.** Liquid-Liquid Extraction apparatus, solvent less dense than aqueous. 75:25 hexanes: ethyl acetate shown with stripes, sample shown with dots.

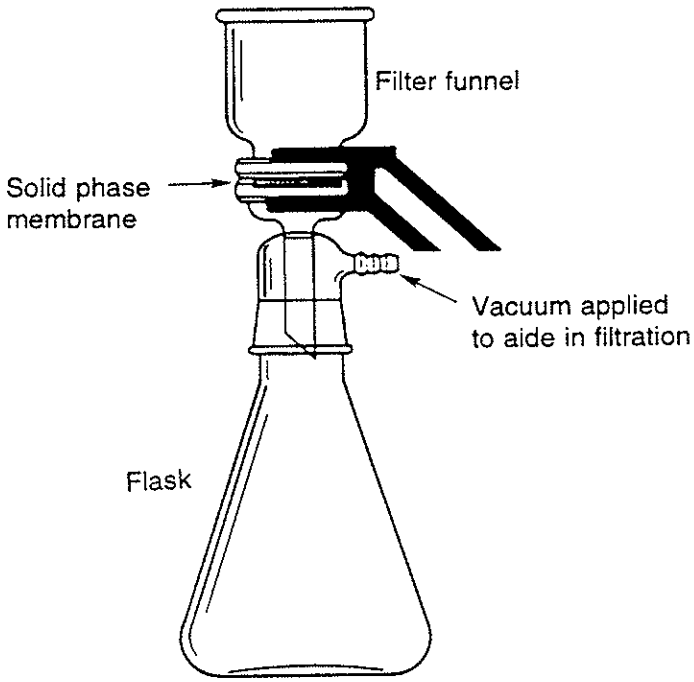
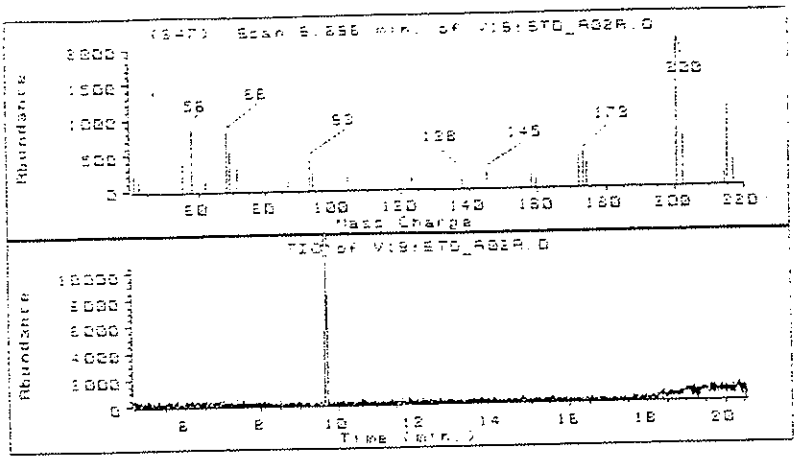
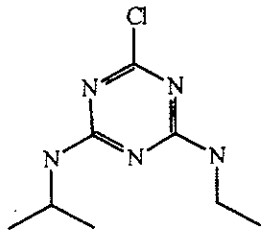



**Figure 4.** Solid Phase Extraction apparatus.

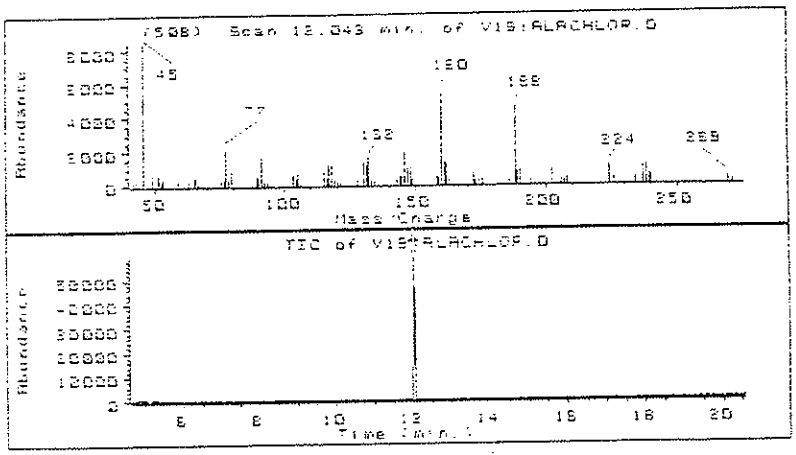
a

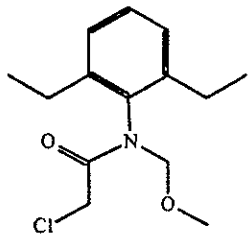




Atrazine
MW=215

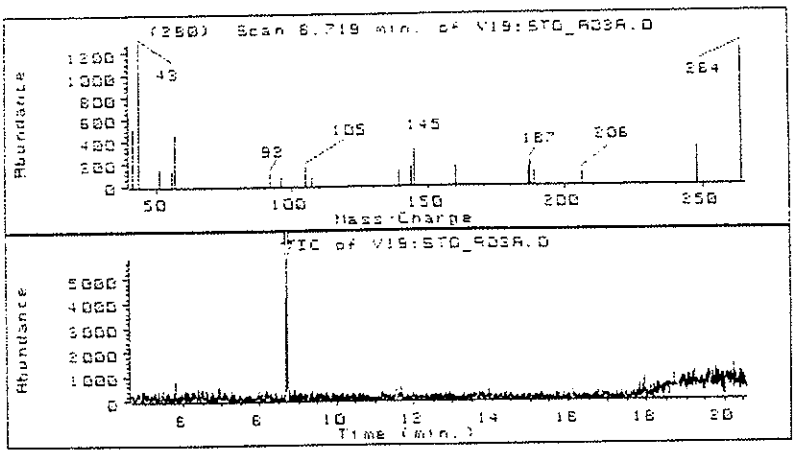
b

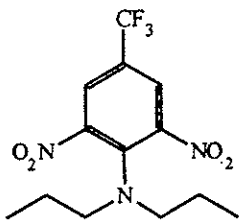




Alachlor
MW=269

c

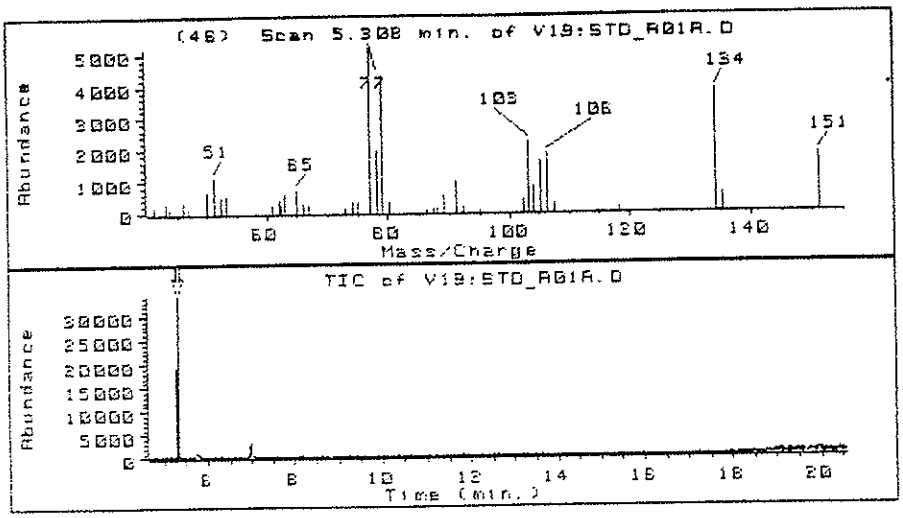


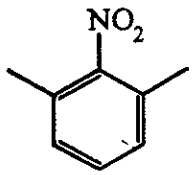


Trifluralin
MW=335

**Figure 8.** Total Ion Chromatogram and Spectra of standard solutions; (a) atrazine, (b) alachlor, (c) trifluralin. The structure and molecular weight are shown on the side.

**a**

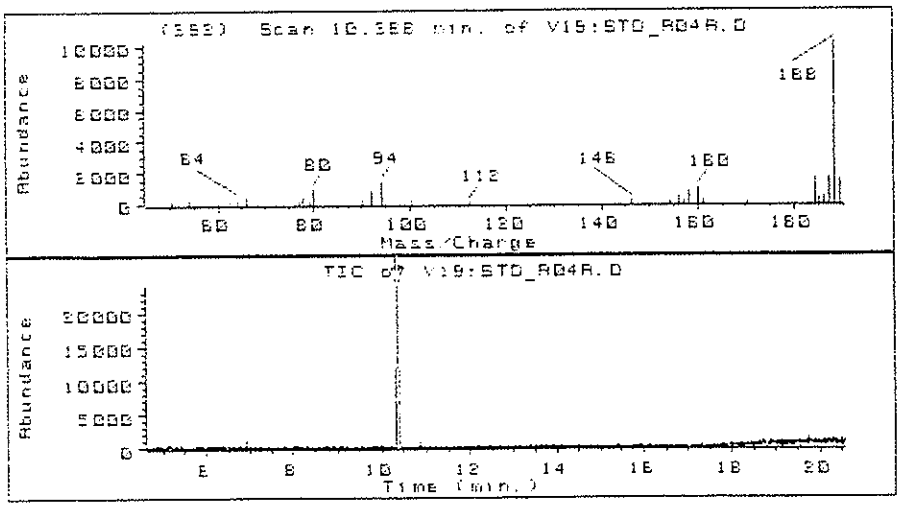


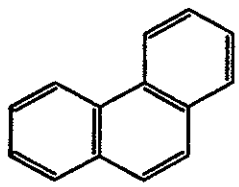




2-nitro-m-xylene

**MW=151**

**b**



$d_{10}$ Phenanthrene

**MW=188**

**Figure 9.** Total Ion Chromatogram and Spectra of standard solutions; (a) 2-nitro-m-xylene, (b) phenanthrene-$d_{10}$. The stucture and molecular weight are shown on the side.

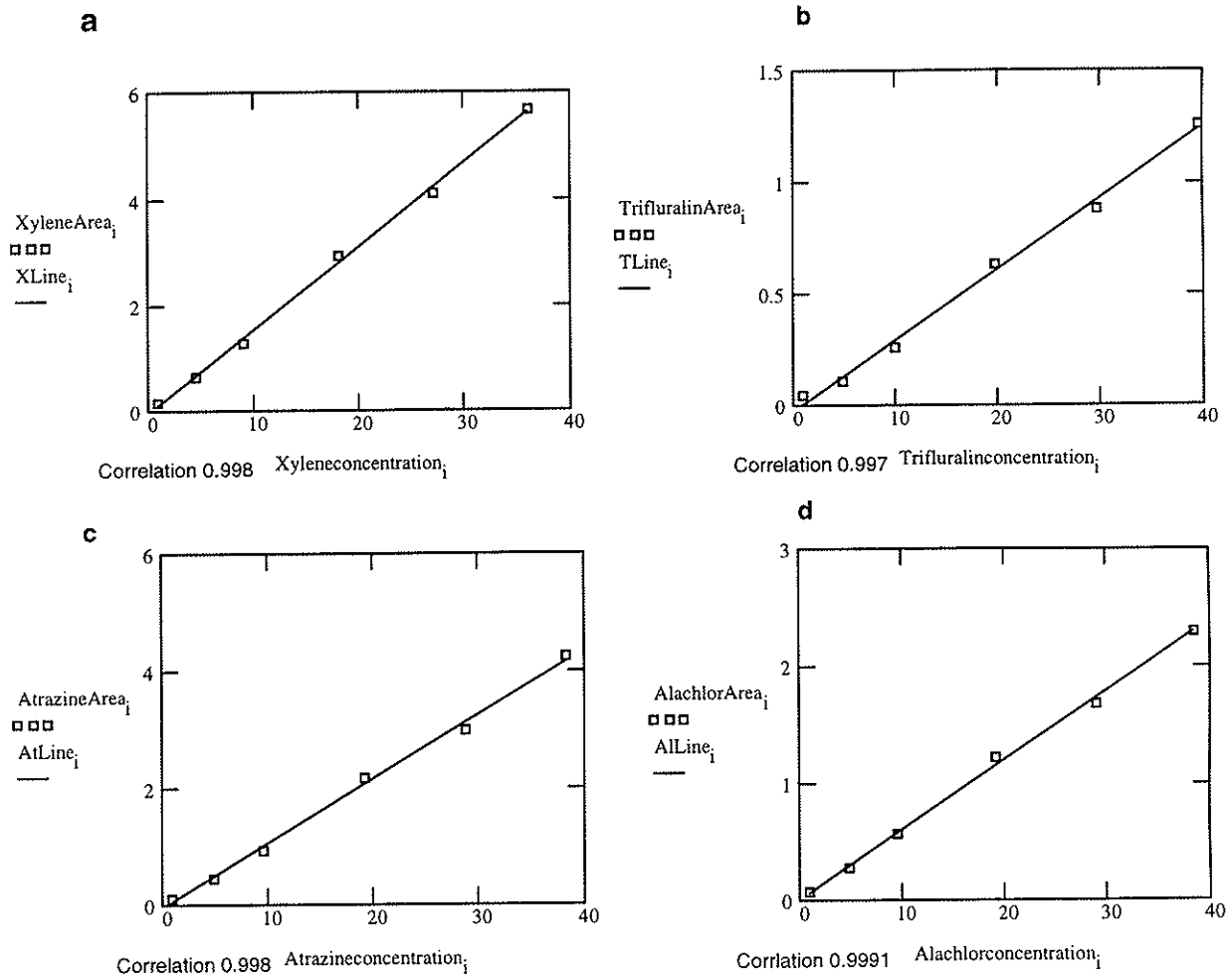



**Figure 10.** Calibration Line for (a) 2-nitro-m-xylene, (b) trifluralin, (c) atrazine, (d) alachlor showing the least squares line fitting the actual data represented by boxes.

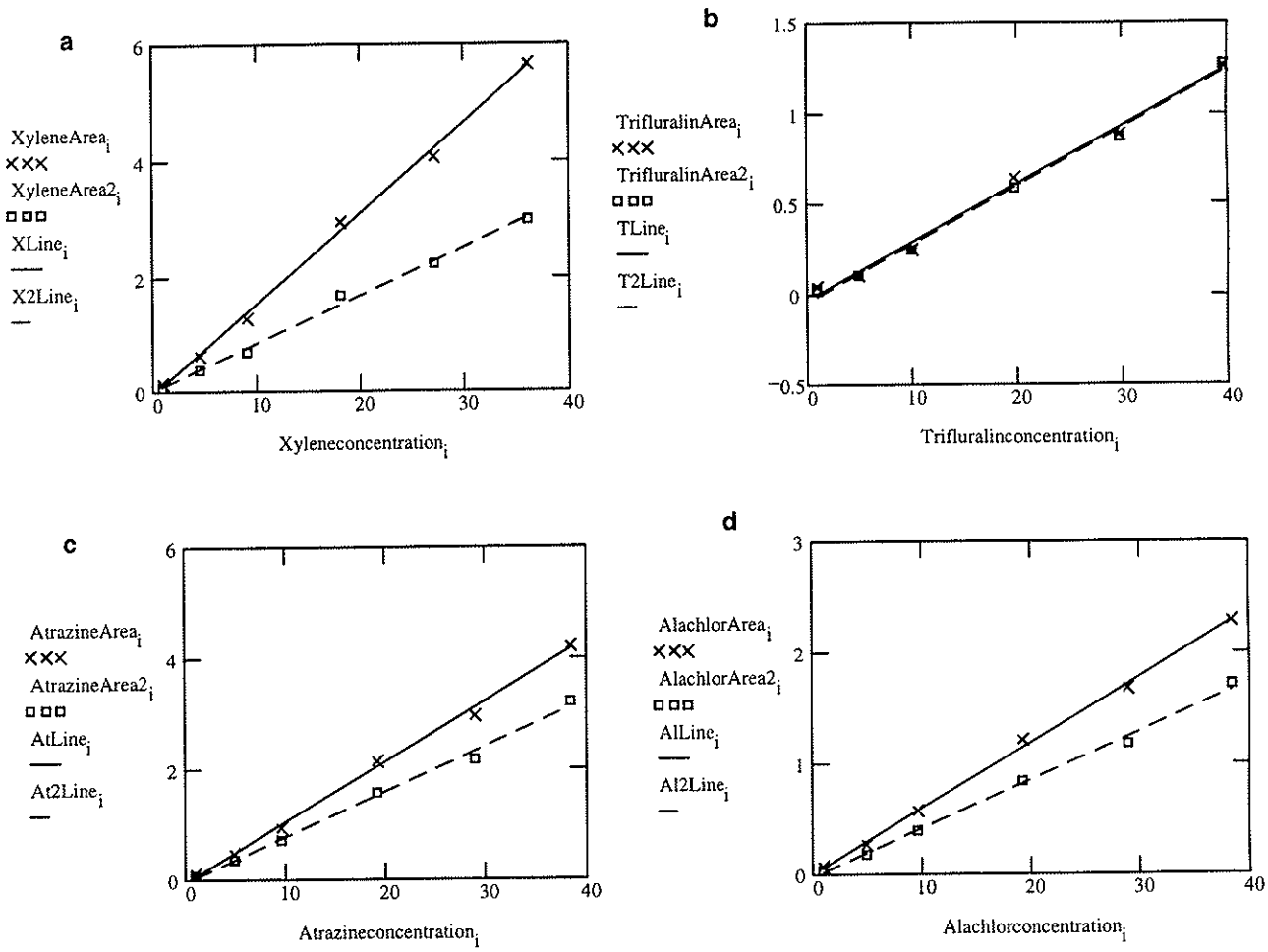



**Figure 11.** Overlay of two calibration lines and data. The solid line and x refer to calibration standards run before the extracted samples. The dashed line and boxes refer to the same standards run after the extracted samples (24 hours later). (a) 2-nitro-m-xylene, (b) trifluralin, (c) atrazine, (d) alachlor.

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