Solvent Microextraction of Chlorinated Pesticides

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Key Words

Gas chromatography
Solvent microextration
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Summary

This paper is a preliminary report on a fast method for the extraction of organochlorine pesticides from river water which is inexpensive, fast and has little to no waste that has been developed. Pesticide extraction was achieved using the relatively new technique of solvent microextraction. In this technique a 2 μL drop of organic solvent is suspended on the tip of a microsyringe in a stirred aqueous sample solution. After the prescribed extraction time the drop is drawn back into the syringe. The syringe is then removed and the contents are injected into a gas chromatograph for analysis. Extraction of eleven organochlorinated pesticides from aqueous solutions with concentrations down to 1 ng mL^{-1} was achieved. The developed procedure was tested as a screening method for pesticides with spiked river water samples and was found to be linear over the concentration range of interest.

Introduction

Harmful environmental contaminants in aqueous samples such as organochlorine pesticides (OCPs) are of particular concern and their analysis can be achieved using a variety of methods [1]. Sample preparation is often the most time consuming step in analysis. Liquid-liquid extraction is the most widely used method of sample pre-treatment for extraction of OCPs from aqueous samples but has many disadvantages including extraction times as long as 12 hours [1]. Recently our laboratory developed a method that coupled solid phase microextraction (SPME) to a fast GC system in order to yield a fast screening method for the analysis of OCPs in water [2]. This has many advantages but suffers from the drawback that SPME fibres are expensive and when reused there is the potential for sample carry over between runs which could invalidate the results [3–5].

Solvent microextraction is a method of sample preparation, which is quick, inexpensive, and has the potential to be easily automated. Solvent microextraction is a fairly new technique and has been recently described in several papers [6–10]. Two relatively simple techniques were described by Jennot and Cantwell [6, 7]. In their methods a microdrop of toluene was suspended on the tip of either a Teflon rod or microsyringe which was immersed in the stirred aqueous sample solution.

In the present study, extraction occurs by suspending a 2 μL drop of solvent from the tip of a microsyringe that is immersed in a stirred aqueous sample for a set extraction time. The drop remains on the tip of the microsyringe throughout the extraction time and is then retracted back into the needle and injected into a gas chromatograph (GC) for preliminary identification and quantification of the extracted solute. Preliminary method development of factors such as sampling volume, extraction time, drop size, stirring speed, extraction time, organic solvent, and use of an internal standard were all carried out using DDT as a representative of the class of organochlorine pesticides. The optimised conditions were then applied to distilled water and river water solutions of ten other pesticides in this class.

Experimental Data

Reagents

The pesticides *cis*-chlordane, *trans*-chlordane, *p-p'*-DDE, DDT (Supelco, Belefonte, PA, USA) and *p-p'*-DDD (Radian International, Austin TX, USA) were all purchased as the pure compound. Stock solutions of these solids were made by dissolving 2.5 mg in 25 mL of methanol for a final concentration of 100 μg mL^{-1}. Endrin, endrin ketone, endosulfan sulphate, α-benzene

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hexachloride (BHC), δ-BHC (all 1000 μg mL^{-1} in methanol), β-BHC (1000 μg mL^{-1} in acetone) and endrin aldehyde (1000 μg mL^{-1} in hexane) were all purchased from Radian. Decachlorobiphenyl was also purchased from Radian as the pure compound. All solvents used were HPLC grade unless otherwise stated. Solvents used were methanol, hexane, benzene (Fisher Chemicals, Fairlawn NJ, USA), toluene (Spectrum, New Brunswick, NJ, USA) and ultrapure, distilled, deionised (18.2 MΩ) water obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). Glassware was deactivated using dimethyldichlorosilane (Supelco) as described by the manufacturer and extraction vials were bought pre-silanised (Supelco). All gases were supplied by Pallini Industries (Athens, OH, USA)

Instrumentation

A Hewlett Packard model 6890 gas chromatograph (Hewlett Packard, San Fernando, CA) equipped with a split/splitless injector, an electron capture detector and connected to a Hewlett Packard Vetra XM2 4/100i desktop computer equipped with HPChem Station software (version 4.01) was used throughout the method development. Separation was carried out on a HP5 30 m × 320 μm × 0.25 μm (5 %)-diphenyl-(95 %)-dimethylsiloxane copolymer column (Hewlett Packard). Ultrapure grade helium was used as the carrier gas at a constant flow rate of 2.0 mL min^{-1}. Injections were made in the split mode with the split ratio set to 10:1. The injector port temperature was set constant at 250 °C. The oven temperature began at 70 °C and was programmed at 40 °C min^{-1} to 170 °C and then 5 °C min^{-1} to 240 °C, then 30 °C min^{-1} to 300 °C with a final hold of 1.5 min. The electron capture detector was held constant at 325 °C with a make up nitrogen flow of 60 mL min^{-1}. Quantification of the pesticides was based upon the ratio of the integrated area of the pesticide peak to the integrated area of the decachlorobiphenyl which was used as the internal standard in the extraction solvent hexane.

Pesticide Separation

A mixture of α-BHC, β-BHC, δ-BHC, *cis*-chlordane, *trans*-chlordane, *p,p'*-DDD, *p,p'*-DDE, DDT, endosulfan sulfate, endrin, endrin ketone, and the internal standard decachlorobiphenyl were prepared and separated using the temperature program outlined in the experimental section above. From these chromatograms the retention times of each of the compounds were determined.

Extraction Procedure

The microextraction apparatus consists of a 5 μL microsyringe fitted with a Chaney adapter (Model 7105 KH, Hamilton Co., Reno, NV, USA), a magnetic stir bar and stir plate, a syringe stand (constructed inhouse) and a vial with a silicone rubber septum (Supelco, Bellfonte PA, USA). Using the Chaney adapter, the maximum syringe volume was set to 2.9 μL and the delivery volume was set to 2.0 μL. For the extraction, 2.9 μL of the extraction solvent was drawn into the syringe and the plunger is then depressed with the stop button activated expelling 0.9 μL of the solvent. The microsyringe was then placed above the extraction vial in such a way that the syringe needle passes through the rubber septum and the needle tip protrudes to a depth of about 8 mm below the surface of a stirred aqueous sample. Depression of the syringe plunger caused the remaining 2 μL of the extraction solvent to be suspended from the needle tip in the sample solution. After the solution had been extracted for the predetermined amount of time, the plunger was withdrawn to the maximum volume of 2.9 μL while the needle of the syringe was still immersed in the solution. The syringe was then removed from the syringe holder and the needle passed back through the septa. The extraction solvent with the extracted analytes was then injected into the gas chromatograph and analysed. In all cases the analytical signal measured was peak area.

Results

Method Development

For method development, DDT was chosen to represent the class of organochlorine pesticides. In the method development stage of the research a univariate optimisation approach was used with all extractions repeated five times and the mean, standard deviation and relative standard deviation (RSD) values calculated. The means were used to determine the optimum value of each parameter.

The effect of extraction time on amount extracted was examined on 1 mL aqueous samples in silanized vials using a 2 μL extraction drop. Although the amount extracted did increase with increased extraction time, it was found that at extractions times longer than 5 minutes the solvent drop fell off the tip of the syringe more than 50 percent of the time (Figure 1). Because of this an extraction time of 5 minutes was chosen.

The next experimental parameter to be investigated was the choice of the organic solvent. Extractions using hexane, chloroform, benzene, and toluene were compared and there was no apparent advantage offered by any of the solvents. Hexane is inexpensive, less toxic than the other solvents tested and commonly used in pesticide extraction, so it was chosen as the extraction solvent.

After the extraction solvent was chosen the effect of stirring rate on extraction was investigated. Stir settings of 0, 3, 5, 7, and 9 were tested (Figure 2). The stir position of 5, corresponding to approximately 240 r.p.m. was chosen because it had the lowest RSD (relative standard deviation), the highest average peak

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Table I. Comparison of RSD values and R^2 values for aqueous solutions with concentrations of 2 ng mL^{-1} and 20 ng mL^{-1} without using the syringe stand and with the syringe stand. 2 μL injection; five extractions at each concentration

Pesticide	2 ng mL^{-1} RSD		20 ng mL^{-1} RSD		R^2	
	before stand	after stand	before stand	after stand	before stand	after stand
α-BHC	48.1	10.6	50.2	42.2	0.967	0.969
β-BHC	44.6	10.2	18.6	28.3	0.982	0.947
δ-BHC	39.6	29.4	41.3	31.4	0.984	0.925
trans-chlordane	29.9	35.2	28.7	55.8	0.980	0.993
cis-chlordane	49.9	34.1	27.6	49.1	0.995	0.996
DDE	44.6	15.3	36.4	24.5	0.902	0.999
endrin	28.1	26.8	41.8	22.1	0.990	0.993
DDD	30.7	29.4	29.5	42.7	0.991	0.996
endosulfan sulfate	34.0	22.6	74.1	33.9	0.992	0.991
DDT	16.2	37.3	18.2	42.5	0.132	0.992
endrin ketone	26.4	13.3	54.7	35.6	0.998	0.991

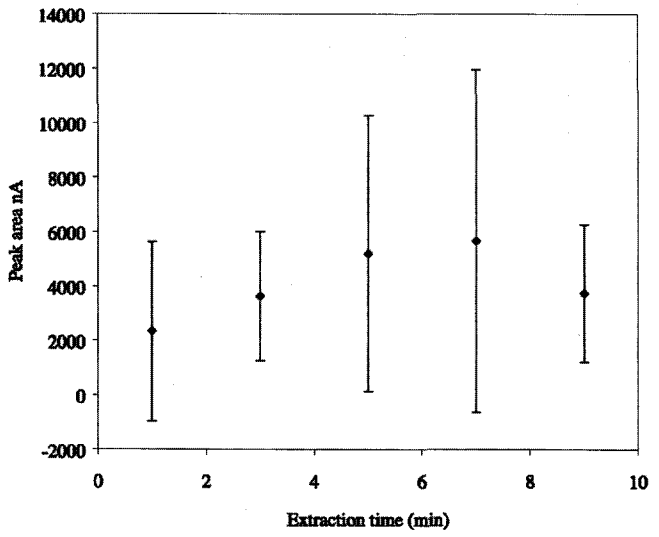

Figure 1
Plot of relationship between peak area extraction time for DDT. Each ◆ represents the average of five extractions. Error bars represent 95 % confidence interval using the Student t-test.

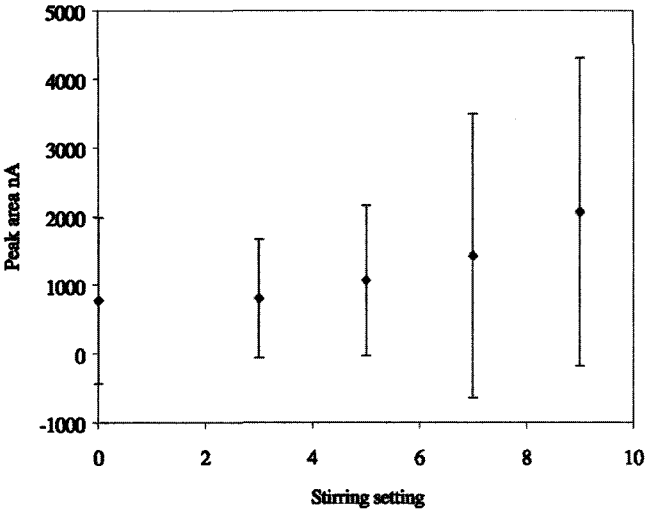

Figure 2
Plot of relationship between peak area and position of stir rate setting for DDT. Each ◆ represents the average of five extractions. Error bars represent 95 % confidence interval using the Students t-test.

area, and had a low incidence of drop loss during the extraction (0 drops lost in 5 extractions)

Since all extractions had been done on one-mL aqueous samples the next stage of method development investigated the effect of sample volume on extraction. Extractions from samples having volumes of 1, 2, 5, 10, and 20 mL were completed and a sample volume of 5 mL was found to give the optimum extraction (Figure 3).

Solvent Microextraction results

Extractions of aqueous pesticide solutions with concentrations between 50 to 0.5 ng mL^{-1} were performed using solutions prepared via serial dilutions. Internal standard was dissolved in the hexane extraction solvent and concentration was kept constant at 1 μg mL^{-1}. After extraction, the sample was injected into the GC for analysis (Figure 4). Calibration curves were made using the ratio of analyte peak area to internal standard peak area and concentration of the aqueous solutions. The data obtained was promising with high R^2 values but there were problems with large RSD values and a low R^2 value for DDT.

Although the R^2 values we obtained for the calibration curves for extractions from aqueous solutions were generally good, very large and extremely variable RSD values were obtained. In order to overcome this problem a syringe holder was constructed which would fit on top of the stir plate and also hold the sample vial in position (Figure 5). This holder would ensure greater reproducibility of syringe tip depth and syringe position in the sample vial. Silane treated vials (15-mL) were also used

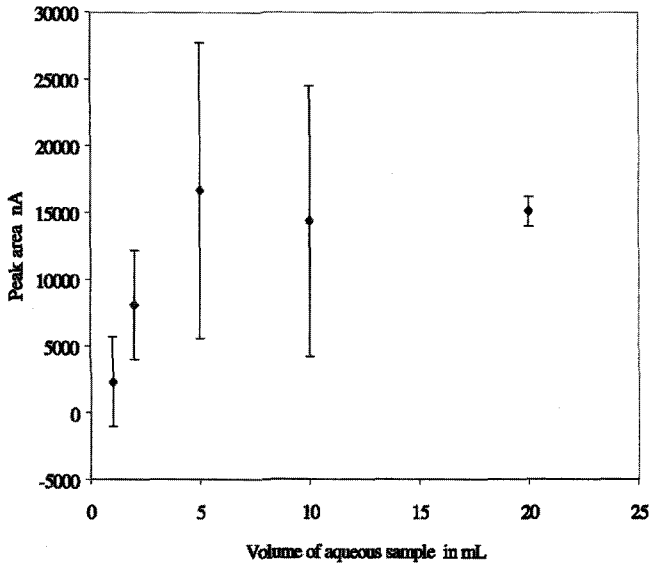

Figure 3

Plot of relationship between peak area and volume of aqueous solution for DDT. Each ◆ represents the average of five extractions. Error bars represent 95 % confidence interval using the Students t-test.

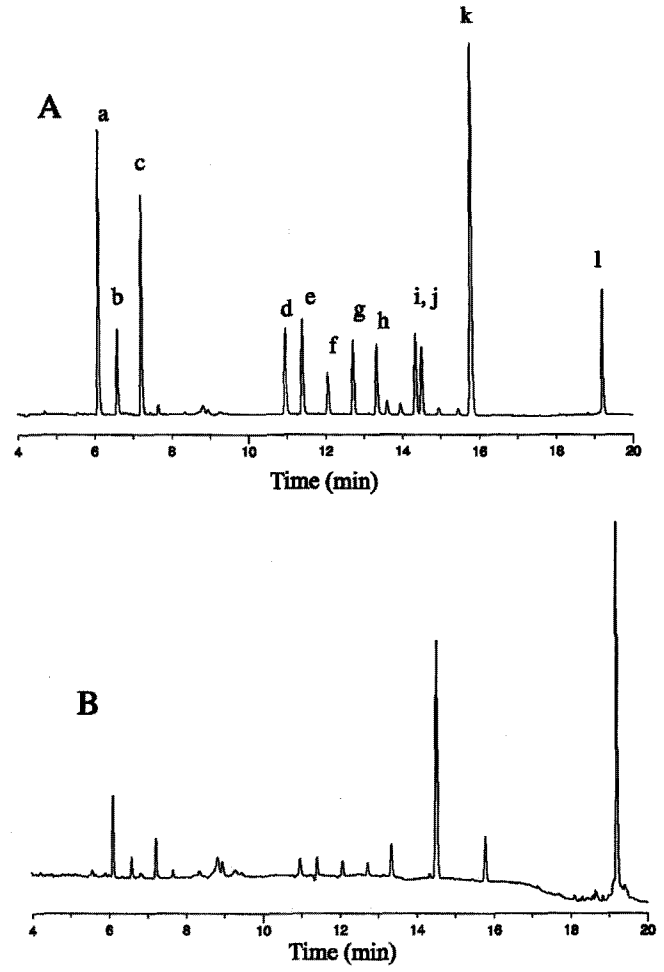

Figure 4

Chromatograms from extraction drops of aqueous solutions with concentrations of (A) 50 ng mL^{-1} and (B) 2 ng mL^{-1}. Chromatographic conditions as described in experimental section. **a** = α-BHC, **b** = β-BHC, **c** = δ-BHC, **d** = *trans*-chlordane, **e** = *cis*-chlordane, **f** = DDE, **g** = endrin, **h** = DDD, **i** = endosulfan sulphate, **j** = DDT, **k** = endrin ketone and **l** = decachlorobiphenyl

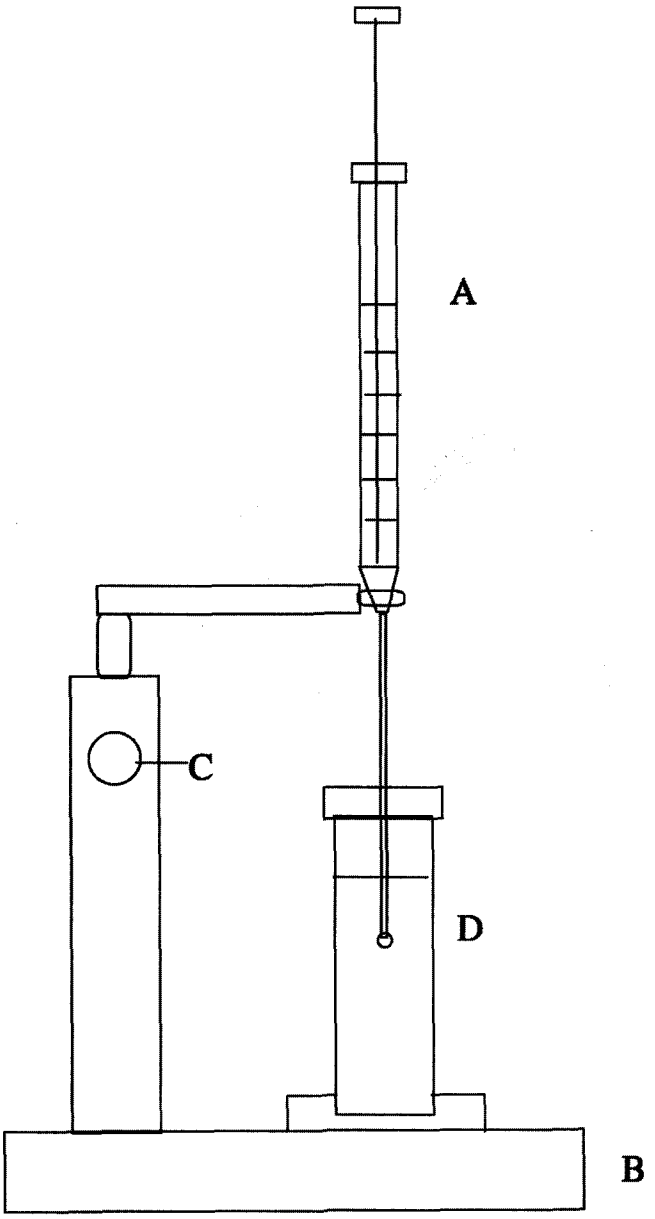

Figure 5

Schematic of syringe stand. A: syringe, B: stand, C: screw used to adjust the height of the syringe and D: extraction vial.

with neoprene septa (Supelco) to prevent analyte absorption on the surface of the glass. The extractions of aqueous solutions were then repeated yielding reduced RSD values. The R^2 values were found to be comparable to extractions made without the stand (Table I).

River water from the Hocking River in Athens, (OH, USA) was sampled. A calibration curve was prepared from extractions of river water samples spiked with pesticide so that the concentration of each pesticide in the river water was 20, 10, 5, 2 and 1 ng mL^{-1}. The results obtained were similar to those achieved from extraction of samples in distilled deionised water.

Discussion

In the development of the extraction procedure it was found that in order to completely draw up the 2 μL extraction drop into the syringe for injection into the GC, it was necessary to draw up a total of 2.9 μL. Because of this 0.9 μL of the aqueous solvent is also introduced into the GC. This may be one contribution to the high RSD values obtained. The injection of the aqueous portion of the drop had no apparent effect on the quality of the chromatogram and no major maintenance or column replacement had to be undertaken after over 350 injections. Several papers have been published on direct aqueous injection [11–13], which involves injection of aqueous solutions onto the column. These studies have found that column activation due to water is far more pronounced for condensed water than for water vapour. As we are injecting only a small amount of water in the larger hexane drop, we would expect rapid vaporisation of the aqueous portion of the drop in the injection port resulting in minimal condensed water being present in the column.

The surface area of the drop was estimated by calculating the radius using the equation $V=4/3\,\pi\,r^3$ (V=volume, r=radius) and using this value for SA=$4\,\pi\,r^2$ (SA=surface area). Using these equations the surface area was estimated to be 7.7 mm^2, which is comparable to those calculated for SPME fibres in previous studies (9.8, 5.4, 3.9 mm^2) [2]. This calculated drop surface area is probably larger than the actual surface area in contact with the solution because at least a portion of the drop is in contact with the syringe and thus not available for extraction. Also, this calculation assumes that the drop is perfectly spherical which it probably is not. Comparison of the amounts extracted in this study to those achieved in the previous SPME study further illustrates these differences. In the SPME study (with surface area of 9.8 mm^2) an over 100 fold concentration effect was achieved for all pesticides. Concentration factors in this study were found to be between 37 and 98 when extracting from an aqueous solution with pesticide concentrations at 1 ng mL^{-1}.

Previous papers report that the distribution coefficient (K_D) for DDT between hexane and water is 91,000 while that of benzene and water can be calculated to be 3.4×10^7 [14, 15]. Because of these high K_D values, if extraction was allowed to proceed to equilibrium we would expect 97.3 % of the DDT in the aqueous phase to be extracted if hexane is the organic solvent and greater than 99.9 % to be extracted if benzene is the organic solvent. Although these differences in distribution coefficients should result in greater amount extracted in the benzene this was not observed.

The rate constant is related to both the volume of aqueous and organic phases V_{aq}, V_o, the interfacial area of contact between the organic and aqueous phases A_i, the overall mass transfer coefficient β_o, as well as the distribution coefficient K_D (Eq. (1)) [7].

$$k=(A_i/V_o)\,\beta_o\,[K_D(V_o/V_{aq})+1] \quad (1)$$

It is thought that although the higher K_D value should result in an increased rate of extraction, k, these differences are not evident due to the short extraction times and small aqueous phase volumes. These initial studies on organic solvent were carried out using an aqueous solution volume of one mL. Larger V_{aq} values would increase the difference in the extraction rates between hexane and benzene perhaps making the difference between the two solvents more experimentally apparent. Regardless of this hexane was found to give adequate extraction at concentrations of interest with short extraction times. The reduced toxicity of hexane in relation to the other solvents used is beneficial and contributes to our decision to use hexane as our extraction solvent.

The short extraction times result in approximately 8 % extracted when using a 5 minute extraction time on a 5 mL solution of 2 ng mL^{-1} DDT in hexane compared to 97.3 % if the extraction was allowed to proceed to equilibrium. Although we are not close to equilibrium, the amount extracted is sufficient for detection in this proposed screening method.

A theoretical model of mass transfer for solvent microextraction has been developed and is based on film theory of convective-diffusive mass transfer,

$$\beta_{aq}=D_{aq}/\delta_{aq} \quad (2)$$

where D_{aq} is the diffusion coefficient in the aqueous phase, β_{aq} is the mass transfer coefficient in the aqueous phase and δ_{aq} is the thickness of the diffusion film [7]. It was found that as stirring speed was increased the thickness of the diffusion film in the aqueous phase decreased linearly causing an increase in mass transfer coefficient. Our results correlate with this explanation.

The large and highly variable RSD values that were obtained initially in the study were partially attributed to adsorption onto the glass surface and the non-reproducible placement of the syringe into the stirred solution. This was greatly improved by the use of silanized glassware and the syringe stand. It is possible that controlling the temperature during the extraction could further reduce the RSD values. Heating of the sample by the stir plate may cause volatile components to be evaporated into the headspace and making them unavailable for extraction. Currently we have no data on the reproducibility of the drop formation. If drop formation at the syringe tip is irreproducible, this may be a cause of the high RSD values seen. Additionally, it is also possible that the internal standard present in the organic drop is partitioning between the organic phase and the aqueous phase during the extraction period. This possibility is presently being examined in our labs and is also thought to contribute to the large RSD values. It is important to note that our higher RSD values occurred at higher concentrations, well above the maximum contamination levels. This would be acceptable for a screening method because even with high RSD values,

the distribution of the response would always indicate a positive response for aqueous solutions with concentrations this high. At concentrations near the maximum contamination levels, the results are within EPA guidelines for α-BHC, β-BHC, δ-BHC, DDE, endrin, endrin sulphate and endrin ketone [16].

The detection limit of the detector and the proposed method was estimated using calibrant solutions as well as aqueous extractions. It was found that the detector could respond to extractions from aqueous solutions with concentrations as low as 0.25 ng mL^{-1}. These levels are sufficient to detect pesticides at the levels required by the EPA [16].

Conclusion

By using inexpensive equipment that is found in a typical analytical laboratory a screening method has been developed which is inexpensive, fast, requires little solvent and produces little to no waste. Combining this solvent microextraction method with Fast-GC should yield a screening system with a total analysis time of 10 minutes and we will report on this shortly. These studies suggest that at this stage solvent microextraction does not yet have the sensitivity of SPME but has the advantage of no carryover between extractions.

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