

Evaluation of Factors Influencing Recovery of Herbicide 2,4-D from Drinking Water

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ABSTRACT: *Many modern analytical methods deal with the trace-level determination of compounds of interest in highly complex environmental samples by means of chromatographic techniques. The introduction of a "clean" sample into an analytical instrument can make analyses easier and prolongs the equipment life. The use of solid-phase extraction (SPE) has grown and is a fertile technique of sample preparation as it provides better results than those produced by liquid-liquid extraction (LLE). The application of SPE can give selectivity of extraction providing a purified and concentrated extract. Through this study, optimization of trace enrichment and sample clean-up method via the use of bonded silica cartridges is discussed. SPE using bonded silica has been optimized with respect of sample pH, sample concentration, elution solvent strength, sample volume, and elution volume. In this investigation a variety of non-polar sorbent cartridges were also screened. During this study, the octadecyl bonded silica cartridge (C18) has proven successful in simplifying sample preparation. The present approach proved that 2,4-D could be retained on C18 based on specific interaction. Further study employed methanol to extract the analyte from spiked water and gave a clean sample for high pressure liquid chromatography equipped with ultra violet detection system. The optimized method was validated with three different pools of spiked samples and showed good reproducibility over six consecutive days as well as six within-day experiments.*

KEY WORDS: *Solid phase extraction, Sample preparation, Herbicide, Bonded silica, Analyte, Chromatography*

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INTRODUCTION

Due to increasing concern about toxic substances such as pesticides in the environment and workplace, it is becoming more important to monitor such chemicals in order to evaluate risk hazards and potential problems caused by exposure to toxic compounds [1-5]. In general, samples obtained from environmental sources are often too dilute, too complex, or are incompatible with the detection system to permit analysis by direct sample introduction [6,7]. Therefore, an essential need for sensitive and selective techniques for the analysis of trace pesticides in environmental matrices has been clearly recognized [8,9]. The use of detection system has also improved the selectivity of the analytical procedures. As these sensitive and selective methods require extensive equipment, they may not be available in most laboratories [10,11]. Consequently, sample pre-treatment procedures which can be performed in any laboratory have been developed to simplify analytical approaches as reduce expenses [12]. Although derivatization reactions performed either before or after analytical techniques, can enhance the sensitivity of the assay, this extra performance is not often a favorite stage in sample preparation followed by analysis.

Many analytical methods use liquid-liquid extraction (LLE) to perform sample clean-up [13]. In this procedure, large volume of solvents, having undesirable environmental concerns is used as well as problems associated with the technique to be automated. In addition, the recovery obtained from LLE is not often suitable and reproducible. Solid-phase extraction (SPE) methods using silica or bonded silica have proven useful in simplifying sample preparation [14]. Isolation and purification of the analyte can be achieved in a short time and only low volumes of solvents are used during the application of the method. The use of commercially available low cost vacuum manifolds allows many samples to be processed simultaneously. Furthermore, complete automation of procedures based on SPE is now possible using commercially available instrumentation. A wide range of phases from many suppliers based on silicas are available including reversed phase, normal phase, ion exchange and mixed-mode phases.

This paper explains how the factors can affect to achieve an optimized procedure for the herbicide 2,4-D (Fig. 1) to develop a simple SPE method.

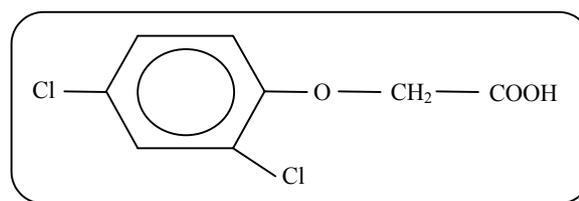


Fig. 1: Structure of 2,4 -D.

EXPERIMENTAL

Reagents

2-methyl,4-chlorophenoxyacetic acid (99%) (2,4-D) as standard, was obtained from Greyhound, Birkenhead, UK. Methanol, ethanol, acetonitrile, n-hexane, acetic acid (all HPLC grade), deionized water, and standard buffered solution at three pH values (4.00 ± 0.02 , 7.00 ± 0.02 , and 9.00 ± 0.02) were purchased from BDH-Merck, Poole, UK. Non-polar silica cartridges, octadecyl (C18), octyl (C8), ethyl (C2), cyclohexyl (CH), phenyl (PH), were obtained from Technicol (Cheshire, UK) and used for SPE.

Apparatus

A 10-place Vac-Elute Vacuum Elution Manifold from Varian (Harbor City, USA) was used for elution of the Bond Elute silica cartridges. A PTI-15 digital pH meter with a glass electrode from EDT Instrument (Dover, Kent, UK) was used for pH adjustment. The amount of reagents were measured, using a Satorius 2024 MP balance (Satorius Ltd, Bellmont, Surrey, UK) for milligram quantities or less. Quantitative liquid transfers were performed with Gilson Pipetman (Gilson Medical Electronics, Villiers-leBil, France). Vortex Genie from Scientific Industries, INC. (Bohemia NY, USA) was used for mixing solutions in the test tubes. The HPLC apparatus used in this study included the following equipments, a Beckman 110B single piston HPLC pump, (Beckman RIIC Limited, High Wycombe, Bucks, UK), the analytical column was a bondclone 10 C18 (30 cm \times 3.9 mm) (Phenomenex, Macclesfield, UK). The detector was a Pye LC-UV spectrophotometer (Unicham, Cambridge, UK), combined with a JJ Lloyd CR 652 Chart Recorder (JJ Lloyd Instrument Ltd., Southampton, UK). Because the reagents used in this study were HPLC-grade, there was no need to filter them. However, the analytical column in HPLC system was equipped with

a filter on the top. Solvents and mobile phase used in HPLC analysis were degassed by an on-line degasser attached to the solvent delivery system.

Optimized sample preparation procedure

The cartridges (200 mg) were conditioned with 2×3 ml of methanol followed by 2×3 ml of 0.01 M phosphate buffer/methanol 80:20 (v/v) with pH 2. Care was taken to prevent the cartridges from drying. The samples were then passed through the columns at a flow-rate of 6-8 ml/min. The cartridges were then washed with 6 ml of the same buffer solution. Finally, the herbicide 2,4-D was eluted from the column with 1 ml (2×0.5 ml) methanol. The extract was then analyzed by HPLC-UV.

Chromatographic conditions

The pump was operated at 1.0 ml/min, detection was by UV at 280 nm, the mobile phase consisted of methanol/water, 75:25 (v/v) containing 0.01 M acetic acid, flow rate, 1 ml/min injection volume was 100 µl, the analytical column was C18 (30 cm×3.9 mm i.d.), and the ambient temperature was used for the chromatographic system.

In this study, peak height was used as detector response and extraction recoveries were calculated by comparison of the peak height in the chromatogram of extracts with those in the chromatogram of standard solutions prepared in the same solvent as following:

$$\text{Recovery(\%)} = \frac{\text{Peak height (sample)}}{\text{Peak height (standard)}} \times 100$$

RESULT AND DISCUSSION

In order to achieve the optimum chromatographic condition for analysis of 2,4-D, variables including mobile phase composition and UV wavelength were optimized. Analytical column widely used for such compound analysis is generally reversed phase [15]. C18 was preferred due to its frequent use and efficient results in the trace analysis of phenoxyacetic acids [16]. The wavelength of 280 nm was more sensitive for determination of 2,4-D. Using these conditions, the compound was eluted in 4 minutes as shown in Fig. 2. The retention time of 2,4-D decrease with increasing concentration of organic modifier in the mobile phase. Therefore, retention time (k' value) can be varied by changing the composition of the mobile phase in order to

isolate the analyte from interferences contained in the sample.

In order to optimize SPE, there were several factors by which retention and elution could be altered. First, different sorbents including C18, C8, C2, CH, and PH, containing 200 mg/3 ml of bonded silica were evaluated for extraction recovery of herbicide 2,4-D. After conditioning the column with 6 ml methanol followed by the same volume of deionized water, 1 ml of 2,4-D standard at concentration of 10 µg/ml was applied. Retained analyte was washed with 6 ml of deionized water followed by elution with 1 ml methanol. From the result given in Table 1, it was deduced that, C18, C8, and C2 cartridges were more satisfactory for efficient recovery of the herbicide 2,4-D. It seems that, the non-polarity of the sorbents as well as the hydrophobicity of the compound can be the major factors for the mechanisms occurred. Although similar interaction mechanisms are taken place with the non-polar sorbents, C18 and C8 efficiently retained the herbicide 2,4-D. The quantity of the sorbent was not screened in this study, however, the greater quantity of the sorbent, the greater the sample breakthrough volume, and greater the elution solvent volume [17]. Due to the type of interaction, providing an efficient recovery, non-polar cartridges of C18, C8, and C2 were selected for further optimization steps.

The non-polar sorbents can be used over a pH range of 2-8. The 200 mg C18, C8, and C2 cartridges were activated and conditioned according to the method explained. 1 ml of sample at different pH, 2, 4, 6, and 8 were applied. The columns were then washed and retained analyte was eluted using the same procedure as explained beforehand. Fig. 3 shows the influence of sample pH on extraction recovery for 2,4-D. The results show that efficient recovery was obtained from C18 using sample pH 2 for the compound. However, the amount of analyte recovered from C18 and C8 at sample pH 4 were efficient. In comparison with C18 and C8, the recovery obtained from C2 was low.

This investigation showed that the pH of the sample should be adjusted according to the chemistry of the compound of interest. 2,4-D is ionizable compound (pK_a is 2.64) [18]. Therefore, it was necessary to adjust the pH of the sample in order to suppress the ionization of the 2,4-D and ensure that the compound was in appropriate

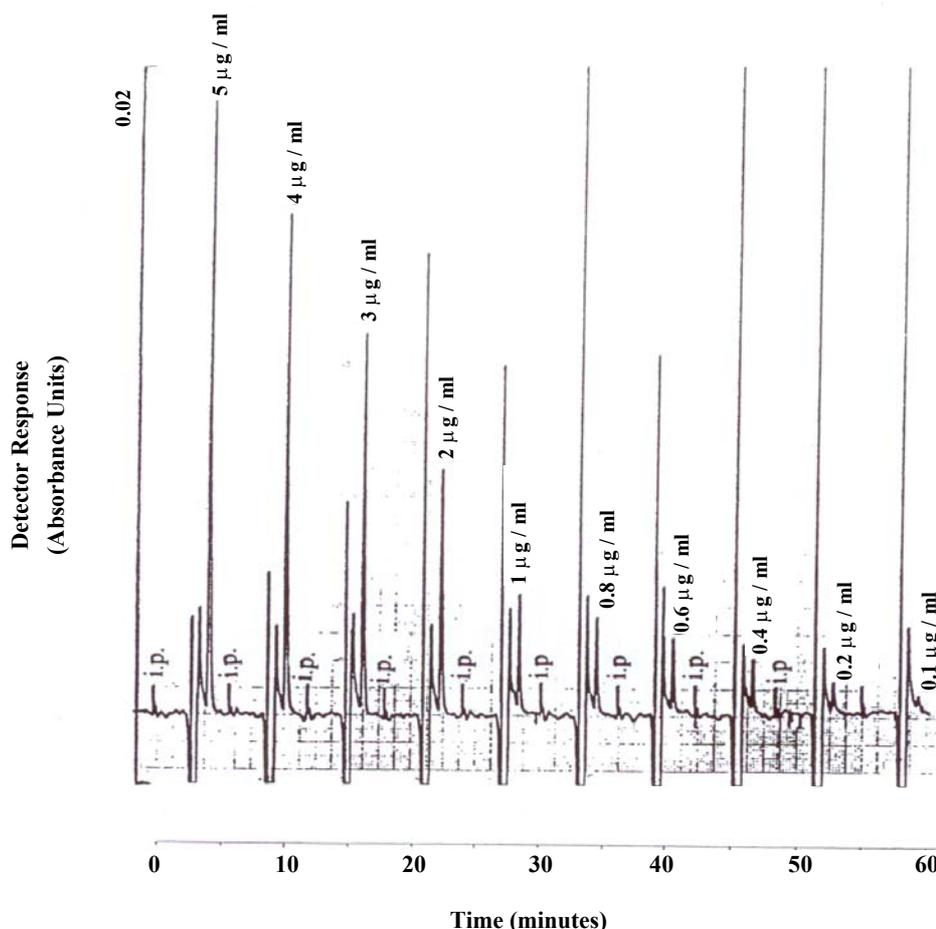


Fig. 2: HPLC chromatograms of 2,4-D at different concentrations. Mobile phase, methanol/water 75:25 (v/v) containing 0.01 M acetic acid; flow rate, 1 ml/min; analytical column C18 (30 cm \times 3.9 mm i.d.) UV detection at 280 nm, 0.02 a.u.f.s; injection volume. 100 μ l; ambient temperature.

non-ionic or weakly dissociated form to achieve efficient retention by the solid phase using non-polar interaction mechanism.

As the Fig. 3 shows, efficient recovery was achieved at sample pH 2. The extraction recovery obtained from C2 was relatively low, Therefore, C18 and C8 were selected for further optimization. Moreover, the 0.01 M phosphate buffer/methanol 80:20 (v/v) pH 2 provided appropriate column conditioning at the sample pH 2, giving an efficient retention and high extraction recovery for the analyte.

In order to evaluate the effect of sample concentration on SPE performance, different concentrations of 2,4-D ranged from 0.1 to 200 μ g/ml as mentioned in Table 2 were prepared using deionized water. Ideally, the extraction recovery should not be sample concentration

dependent. In other words, for the method to be useful, there should be no significant difference in recovery over the expected concentration range of the compound to be analyzed. Table 2 gives the recovery obtained after passing 1 ml sample at different sample concentration followed by elution with 1 ml methanol. As can be seen, the recovery is independent of sample concentration over the concentration range studied. However, the recoveries gained at some concentrations were poor. During this experiment, the breakthrough (B) fraction was also analyzed and no breakthrough of the compound was detected. With the C8 phase, low recoveries were obtained around the target concentration of <1 μ g/ml, so the C18 cartridge was chosen as appropriate to continue the study.

Table 1: The recovery of 2,4-D obtained from non-polar sorbents.

Sorbent types (200 mg)	Recovery of 2,4-D(%)
C18	105
C8	89
C2	61
CH	56
PH	51

Table 2: The recovery of herbicide 2,4-D from C18 and C8 at different sample concentrations.

Sample concentration ($\mu\text{g}/\text{mg}$)	Recovery of MCPA from C18	Recovery of MCPA from C8
0.1	85	30
1	81	83
10	87	69
30	74	60
50	78	74
70	74	63
90	80	64
130	88	74
170	91	71
200	94	70

Conditions: 1 ml of sample at pH 2 passed through 200 mg C18 and C8 conditioning with 6 ml of methanol followed by 0.01 M phosphate buffer/methanol 80:20 (v/v) pH 2, eluted by 1 ml of methanol.

Another experiment performed during this study was evaluating of the eluent strength on 2,4-D recovery. Seven solvents were screened for their ability to produce optimum elution of the retained herbicide 2,4-D from the C18 sorbent (200 mg). They were 1M acetic acid (HOAc), methanol (MeOH), acetonitrile (AcN), ethanol (EtOH), ethanol/methanol (EtOH/MeOH) 50:50 (v/v), ethyl acetate (EtOAc), and hexane (Hex.). The same sequence of conditioning, washing, and elution were used as in previous section. The results of this process are shown in Fig. 4. To produce this data, aqueous samples (1 ml) at three different concentrations i.e. 0.1 $\mu\text{g}/\text{ml}$, 1 $\mu\text{g}/\text{ml}$, and 10 $\mu\text{g}/\text{ml}$, at pH 2 were used. The retained compound was then eluted with three fractions of

2 \times 0.5 ml, 0.5 ml, and 0.5 ml of each solvent separately. The result shows, 1 ml of methanol (2 \times 0.5 ml) recovered 2,4-D, so MeOH was chosen as ideal eluent for efficient extraction of analyte.

Understanding the chemistry of the compound under analysis such as its hydrophobicity or ionizability can be useful in designing appropriate conditions to obtain efficient extraction recovery. Highly hydrophobic compounds result in strongly retained analyte making elution difficult and subsequently giving poor recovery from non-polar sorbents. In this study, methanol was found to be superior to other solvents to break hydrophobic interaction between sorbent and analyte of interest. As acetic acid is a strongly polar solvent with low hydrophobicity and hexane is a strong hydrophobic non-polar solvent, no analyte was eluted using these eluents. There was also no considerable difference in recovery when different sample concentrations were applied using all eluents. As a consequence, the strength of eluents are high enough to elute sample concentration up to 10 $\mu\text{g}/\text{ml}$. Methanol is an optimum eluent as it increases the solubility of the analyte and minimizes physical losses on sample handling.

Enrichment of the analyte in SPE is achieved by applying large volumes of sample and eluting the analyte in a minimum volume of eluent. The eluent volume must be just sufficient to elute the compound of interest from the sorbent. The result obtained from an evaluation of elution volume showed that the smallest satisfactory volume for methanol, from 200 mg of sorbent, was 1 ml (2 \times 0.5 ml). The same results obtained with different eluents i.e. acetonitrile, ethanol, ethanol/methanol 50:50 (v/v), and ethyl acetate. As a consequence, the volume required to elute analyte from the sorbent, depends on two important parameters. First, the capacity factor (k') of the compound of interest, showing the strength of its retention. Solvent with greater elution strength can be used to elute an analyte in less volume but may incorporate undesirable contaminants into the eluted fraction, secondly, the sorbent mass used in SPE. Using a larger sorbent mass cartridges require an increased elution volume to be applied.

More experiments were performed on drinking water to valid the present method. Drinking water can be a suitable model as it may contain interfering constituents similar to natural water [19]. The spiked samples of 50 ml

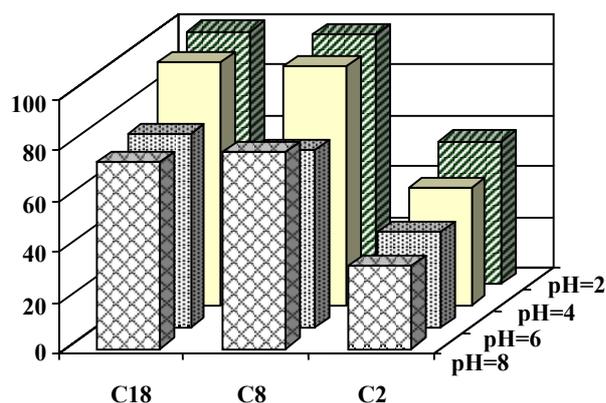


Fig 3: Effect of sample pH on recovery of 2,4-D obtained from non-polar sorbents (200 mg). 1 ml of sample (10 µg/ml) was extracted using 6 ml of 0.01 M phosphate buffer/methanol 80:20 (v/v) pH 2 as conditioning and wash solvent and 1 ml of methanol as eluent. C18: octadecyl, C8: octyl, C2: ethyl.

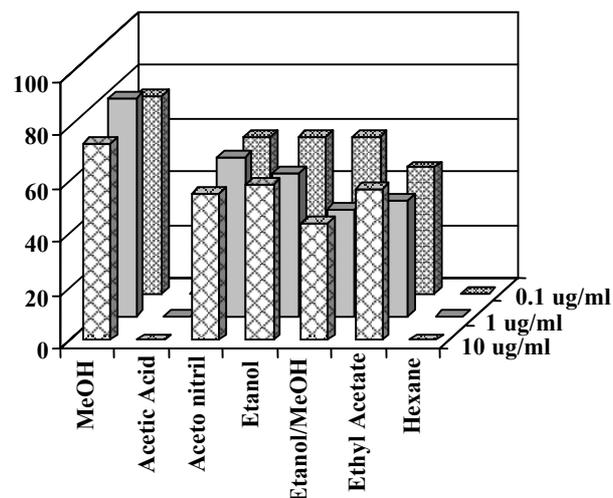


Fig. 4: The recovery of herbicide 2,4-D from C18 sorbent, using different elution solvents at different concentrations. 1 ml of samples pH 2 were passed through 200 mg cartridges conditioned with methanol followed by 0.01 M phosphate buffer/methanol 80:20 (v/v) pH 2, MeOH: methanol, HOAc: acetic acid, AcN: acetonitrile, EtOH: ethanol, EtOAc: ethylacetate, Hex.: Hexane.

Table 3: Day-to-day reproducibility of herbicide 2,4-D spiked in drinking water.

Days	Concentration added (µg/ml)		
	0.10	1.0	2.0
1	0.12	0.9	2.0
2	0.10	1.2	2.0
3	0.09	1.1	1.9
4	0.10	1.0	1.9
5	0.09	1.0	1.8
6	0.11	0.9	1.9
Mean	0.10	1.02	1.95
SD	0.01	0.12	0.11
%CV	11.69	11.46	5.38

Conditions: Sample volume: 50 ml.

of 2,4-D were used for extraction followed by HPLC-UV determination. Linear standard curve (for extracted samples) over the range 0.1-2.0 µg/ml were obtained each day (n=6) with correlation coefficient of 0.997 or greater. The extraction procedure was reliable and reproducible from day-to-day and within-day. The coefficient of

Table 4: Within-day reproducibility of herbicide 2,4-D spiked in drinking water.

Experiments	Concentration added (µg/ml)		
	0.10	1.0	2.0
1	0.10	1.1	1.8
2	0.11	1.0	2.0
3	0.12	1.2	1.2
4	0.10	1.0	2
5	0.09	1.2	1.9
6	0.08	0.90	2.0
Mean	0.10	1.07	1.97
SD	0.014	0.12	0.10
%CV	14.14	11.32	5.24

Conditions: Sample volume: 50 ml.

variation (%CV) of 11.69, 11.46, and 5.38 were obtained for 0.10, 1.0, and 2.0 µg/ml respectively for day-to-day and 14.14, 11.32, and 5.24, at the same concentrations, respectively for within-day, showing suitable accuracy and precision (Tables 3 and 4). The detection limit of the method (signal/noise: 3:1) using spiked sample volume of

50 ml was 0.02 µg/ml as well as reproducible and quantitative recoveries, ranging from 95% to 107% were possible.

CONCLUSIONS

The procedure developed during this study, has shown that solid phase extraction using silica-bonded is more advantageous than liquid-liquid extraction. Depending on chemical and physical properties of the analyte, manipulating of the factors involving sorbent types, sample pH, type and volume of eluent can play a main role in optimizing the method, providing reliable, easy to use, and cost effective procedure to overcome difficulties associated with other sample preparation techniques. Applicability of the method for treatment of different classes of pollutants such as pesticides and different hydrocarbons, can make the technique to be popular when a selective and sensitive trace residue analysis is required. SPE is a fertile area for sample preparation methods and based on the needs and facilities can be more developed in the near future.

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