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Triclopyr 3, 5, 6-Trichloro-2-Pyridinyl Clean-Up Procedure from Soil, Sediment and Water Samples Using SPE-HPLC-VWD

(Tatacara Pembersihan Triklopir 3, 5, 6-Trikloro-2-Piridinil daripada Sampel Tanah, Sedimen dan Air Menggunakan SPE-HPLC-VWD)

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ABSTRACT

Triclopyr is a post emergence herbicide used to control woody plants. After application, the excess amount will enter the soil and water bodies and it is present in ppb level thus making extraction very difficult. The extraction of triclopyr 3, 5, 6-trichloro-2-pyridinyl residue from soil, sediment and water samples under different solid phase extraction (SPE) sorbent efficiency was studied for better recovery. Four different SPE sorbents i.e.: Oasis HLB, Water Sep-Pak, Cromabond (cation/anion PS-H⁺/OH⁻), Isolute ENV+ and a series of solvent i.e. potassium dihydrogen phosphate (KH₂PO₄ 0.1M), sodium hydroxide (NaOH 0.2M), potassium hydroxide (KOH 0.5 & 0.6M), ammonium acetate, methanol and water were used as extraction solution. Sample clean-up performance was evaluated using high performance liquid chromatography (HPLC, Agilent 1220 infinity LC) with variable wavelength detector (VWD) 290 nm. Cromabond[®]H⁺/OH⁻ column with 0.6 M KOH was the most suitable for the clean-up in view of the overall feasibility of the analysis. The highest recovery was 89.32%.

Keywords: Clean-up; sorbents; triclopyr; water samples

ABSTRAK

Triklopir adalah herbisid selepas percambahan (herbisid sistemik) yang digunakan untuk pengawalan tumbuhan berkayu. Selepas penyemburan, lebihan herbisid akan masuk ke dalam tanah dan badan air dan hadir pada aras ppb yang menjadikannya sukar untuk diekstrak. Pengekstrakan triklopir 3,5,6-trikloro-2-piridinil daripada sampel tanah, sedimen dan air dengan menggunakan kecekapan penjerapan pengekstrakan fasa pepejal (SPE) berbeza telah dikaji untuk perolehan yang lebih baik. Empat penjerap SPE berbeza iaitu Oasis HLB, Water Sep-Pak, Cromabond (kation/anion PS-H⁺/OH⁻), Isolute ENV+ dan beberapa siri pelarut seperti kalium dihidrogen fosfat (KH₂PO₄ 0.1M), natrium hidroksida (NaOH 0.2M), kalium hidroksida (KOH 0.5 & 0.6M), ammonium asetat, metanol dan air telah digunakan sebagai larutan pengekstrakan. Prestasi pembersihan dinilai menggunakan kromatografi cecair berprestasi tinggi (HPLC), (Agilent 1220 infinity LC) dengan pengesanan panjang gelombang pemboleh ubah (VWD) 290 nm. Turus Cromabond[®]H⁺/OH⁻ dengan 0.6 M KOH adalah yang paling sesuai bagi kaedah pembersihan dengan melihat keseluruhan analisis. Perolehan tertinggi bagi triklopir adalah 89.32%.

Kata kunci: Pembersihan; penjerap; sampel air; triklopir

INTRODUCTION

Triclopyr is a natural compound that was first isolated from two species of *Streptomyces* fungi (Ismail et al. 2015a). It is a broad-spectrum contact herbicide and is used to control a wide range of weeds after crop emergence and it is also used to desiccate (dry off) before harvest. The application of triclopyr leads to reduced glutamine and increased ammonia levels in the plant tissues. This causes photosynthesis to stop and the plant dies within a few days (Tayeb et al. 2015a). It has become one of the main herbicides in the Malaysian oil palm industry (Ismail et al. 2013). Triclopyr is soluble in both, water and organic solvents. Triclopyr is degraded by sunlight, microbial metabolism and hydrolysis. In soils, both the ester and amine formulations will degrade rapidly to the parent compound, triclopyr acid. The acid and ester formulations bind well with soils and therefore, are not likely to be mobile in the environment. The salt

however, does not readily adsorb and can be mobile (Ismail et al. 2015b). The ester can be highly volatile. Most of the previous studies focused on the analysis of triclopyr used in different agricultural products (corn, palm oil, wheat, fruits and vegetables), but very little information of triclopyr clean-up procedure from soil and water was known. Triclopyr remains in the water and soil at ppb level (Xiang et al. 2006).

Therefore, analysing the presence of triclopyr in soil and water is a complex issue. Solid phase extraction (SPE) is popular and most commonly used among all the clean-up procedures. The sorbent is vital to acquire high efficiency in solid phase extraction technique (Neue et al. 2001). For the determination of triclopyr in water samples, additional set up was required in sample preparation procedure. Water samples were used with reversed phase or ion exchange SPE cartridges. Extraction and clean-up of triclopyr

residues from soil, sediment and water are important in understanding its behaviour of persistence. This research investigated the efficiency of SPE cartridge in the extraction and clean-up of triclopyr from soil, sediment and water. Eight different extracting solvents, pH conditions and sorbents were tested. All the SPE cartridge sorbents were tested with the auto sampler HPLC. The objective of this experiment was to compare which SPE sorbent packing materials gave better recovery of triclopyr from soil, sediment and water.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Triclopyr analytical standard (99% purity) was purchased from the laboratories of Dr. Ehrenstorfer Co., Germany. This triclopyr was used in the preparation of stock solution to run the calibration curve. Acetonitrile, acetone and diethyl ether were purchased from Scharlau Science (Barcelona, Spain). Analytical grade reagents such as disodium tetraboratedecahydrate, potassium dihydrogenphosphate, and hydrochloric acid (37%), potassium hydroxide, sodium hydroxide and 9-Fluorenylmethyl Chloroformate (Fmoc-Cl) were purchased from Merck. The water used for solution preparation and analysis was obtained from a Milli-Q (Billerica, MA) system (resistivity >18MΩ cm). The SPE columns were Cromabond PS-H⁺/OH⁻, 3ml/500mg, Machererey- nagel (Germany); Isolute ENV+, 6 mL/200 mg, Biotage (Japan); Water Sep-Pak, 6 mL/500 mg, Waters (U.S.A); Oasis HLB, 6 mL/500 mg, Waters (U.S.A). Table 1 shows a short description of the SPE sorbents used for the clean-up experiment.

APPARATUS

Sigma-Aldrich SPE system with pump (Supelco, USA) was used for solid phase extraction while the HPLC system was Agilent 1220 Infinity LC equipped with variable wavelength detector.

METHODS

A model mixture was eluted through Cromabond PS-H⁺, Cromabond PS-OH⁻, Isolute ENV+, Water Sep-Pak, and

Oasis HLB columns. Retaining triclopyr while allowing others to elute, the SPE sorbents thus served as chemical filters. For Cromabond® PS-H⁺ column, conditioning was 22 mL distilled water to open the pores. For Chromabond® PS-OH column, conditioning was 2 mL distilled water, 2 mL 1 M NaHCO₃ solution, 2 mL distilled water. For Isolute ENV+ column, conditioning was 2 mL distilled water. For Oasis HLB and Water Sep-Pak columns, 3 mL ultra-pure water and 3 mL methanol were used as conditioning. After conditioning, 150 mL aqueous solution of the sample was passed first through the PS-H⁺ and then through the PS-OH⁻ column at the optimized flow rate controlled by a vacuum pump. Dispose off the cation exchanger column and dry the anion exchanger with air or nitrogen. Finally, the eluent was blown to near dryness and the residue reconstituted in 1 mL of mobile phase for HPLC determination. The HPLC determination was performed on a Nucleodur® C18 Gravity chromatographic column with a mobile phase of (A) acetonitrile-(B) H₃PO₄, P^H 1.2, 30-35% A for 27 min, 35-90% A for 3 min, 90% A for 6 min, 90-30% A for 2 min and 30% A for 7 min. The flow rate was at 0.5 mL/min while the injection volume was 10 µL and the temperature at 30°C. The variable wavelength detector was 290 nm while the method detection limit was 0.01 µg/g and percentage of recovery was 92%.

RESULTS AND DISCUSSION

pH OF SAMPLES

In different pH solutions, triclopyr is in different forms, which can affect the absorption and recoveries. Hydrochloric acid and sodium hydroxide were used as the pH regulator. The recoveries in acidic, neutral and alkaline samples were listed in Figure 1. From Figure 1, it was observed that Oasis HLB which is close to neutral pH condition has a higher absorption rate.

ELUENT COMPONENT

From Table 2, potassium hydroxide 0.6 M has a higher recovery rate with Cromabond®H⁺/OH⁻ column. For Isolute ENV+ 0.5M KOH, Water Sep-Pak 0.6M KOH and Oasis HLB columns methanol has better results than the other eluents.

TABLE 1. Main characteristics of the SPE columns used

SPE columns	Symbols	Sorbent materials	Retention mechanism
Isolute ENV+	Hydroxylated polystyrene-divinyl benzene co-polymer	Highly retentive non polar SPE phase	Ion exchange
Oasis HLB	Universal polymeric, Hydrophobic-Lipophilic-Balanced, Water-wettable, mixed-mode sorbent (Kirkland et al. 1999)	Water-wettable	Reverse phase
Water Sep-Pak	Hydrophobic	Silica based bonded phase	Reverse phase
Chromabond PS-H ⁺ /OH ⁻	Resin based polystyrene-divinylbenzene	Beige powder (Tayeb et al. 2015b)	Cation & anion exchange

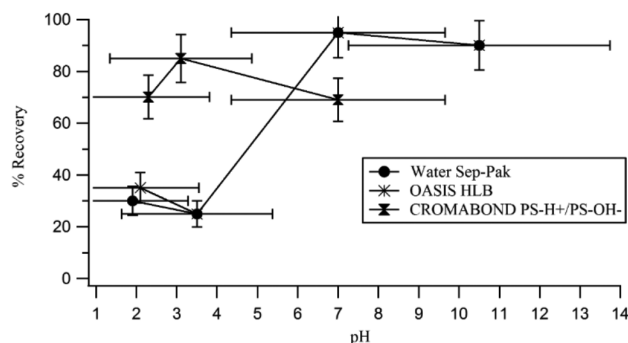


FIGURE 1. Relationship between the samples pH and the amount of triclopyr residues in different SPE cartridges

TABLE 2. Effects of eight different eluents for four different sorbents on the recovery analytes

Sorbents	Recovery%						
	Water	Methanol	Ammonium acetate	NaOH 0.2M	KH ₂ PO ₄ 0.1M	KOH 0.5M	KOH 0.6M
Oasis HLB	–	73.55	72.40	–	47.20	–	67.40
Isolute ENV+	15.91	–	37.74	41.30	–	72.77	62.45
Water Sep-Pak	33.50	–	49.50	–	–	77.56	76.30
Cromabond®H ⁺ /OH	19.31	–	80.16	55.74	–	65.24	89.32

ADSORBENT ELUENT

The solvent can be used as the appropriate adsorbent eluent to remove the interfering components while retaining the analyte in the column. The sample solvent strength should be equal or a little stronger than the solvent (Ismail et al. 2015c). The adsorbent eluents are usually organic solvents containing appropriate concentrations of buffer solution or aqueous solution for the reverse phase column. The adsorbent eluents for anion-exchange are usually ionic compounds (Ismail et al. 2015d). In the experiment, Cromabond PS-H⁺/OH⁻ was washed with 3 mL AcOH/water (5:95, v/v) for the removal of impurities. It was then washed again with 3 mL methanol/water (20:80, v/v) to remove neutral and acidic compounds. Compounds are eluted with 2 mL methanol/acetone (1:1, v/v) +20% AcOH.

OTHER PARAMETERS

In the experiment, Cromabond PS-H⁺ / OH⁻ was used to get the volume of sample treatment, appropriate sample flow rate, eluent volume and eluent flow rate.

Spiked water samples were passed through the Cromabond PS-H⁺/OH⁻ columns in five different flow rates (1.0, 3.0, 5.0, 7.0 and 10 mL/min). The eluents were then collected and determined by HPLC Agilent 1220 Infinity (LC) to get the recovery. Figure 2 shows that by gradually increasing the sample flow rate, it could decrease the recovery. The reason being the velocity of the sample flow was too fast to reach equilibrium in the sorbent and the targeted components were not fully adsorbed. Reducing the velocity of the flow rate can increase sample processing

time. Flow rate of 1 mL/min has good recovery and it could also reduce the processing time (Ismail et al. 2015b).

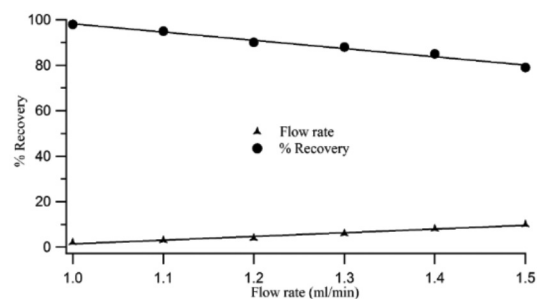


FIGURE 2. Relationship between spiked sample flow rate and recovery

Different volumes of sample treatment but adding the same amount of triclopyr solution in 1.0, 3.0, 5.0, 7.0 and 10 mL of water were tested to get the approximate sample treatment volume. From Figure 3, the recoveries did not have any significant difference between the ranges of 1.0-10.0 mL sample volumes (Tayeb et al. 2016). Comparing the processing time and the result, 6 mL sample was chosen as the volume for sample treatment.

Several flow rates of 1.0, 3.0, 5.0, 7.0, and 10.0 mL/min were tested to get higher recoveries. From Figure 4, it can be seen that lower elution rates can result in higher recoveries, but it took a longer time. For this reason, 0.5 mL/min was chosen as the optimum elution rate (Frank et al. 2002).

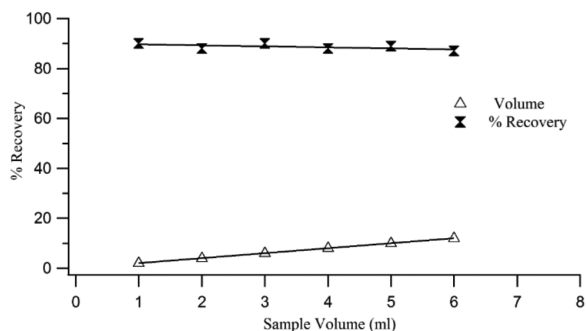


FIGURE 3. Relationship between sample volume and recovery

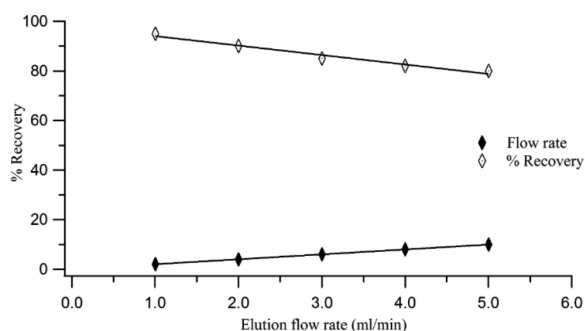


FIGURE 4. Eluent flowing rate vs recovery

Five eluent volumes of 1.0, 3.0, 5.0, 7.0 and 10.0 mL in the same concentration were tested to determine the volume of elution. Figure 5 shows that recovery was increased with more eluting solvent. However, more eluting solvent needs more time and has negative impact on the concentration. Therefore, 3 mL sample was considered the best (Ferenc & Biziuk 2006).

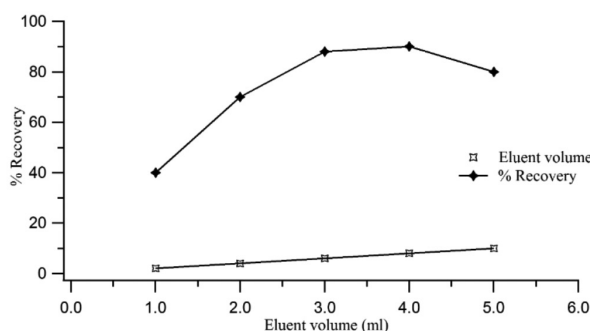


FIGURE 5. Eluent volume vs recovery

HPLC CHROMATOGRAMS

Using high performance liquid chromatography with variable wavelength detector with 290 nm, the clean-up performance was evaluated. The HPLC chromatogram and calibration curve are shown in Figures 6 and 7, respectively. In the case of Cromabond PS-H⁺, Cromabond PS-OH- SPE column, although it has good recovery, the complex ionic

behaviour of triclopyr made it difficult to adjust to an appropriate pH for consistent and quantitative extraction (Kuivinen & Bengtsson 2002). The other three SPE sorbents i.e. Isolute ENV+, Water Sep-Pak and Oasis HLB are also suitable for clean-up operations. At the same time, Water Sep-Pak chromatograms were slightly better than Oasis HLB and Isolute ENV+. Dimethyl butylamine group extracts acidic compounds with anion exchange groups which is the speciality of Water Sep-Pak.

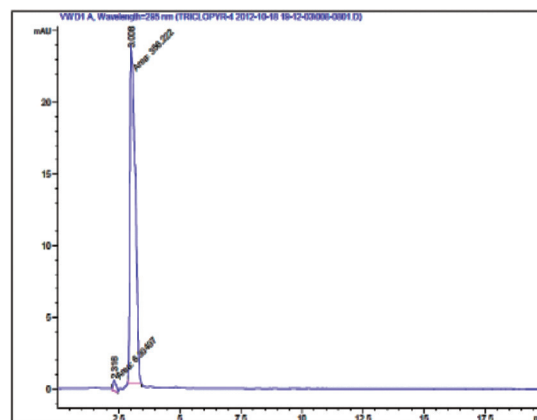


FIGURE 6. Chromatograms of triclopyr standard

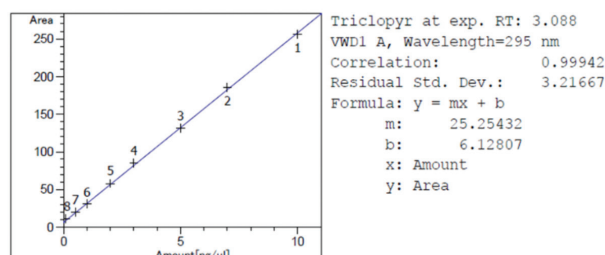


FIGURE 7. Linear regression of Triclopyr standard (HPLC-VWD)

CONCLUSION

Recovery is the most important part of the pesticide residue analysis. Among the whole recovery process sample, clean-up is the most important; otherwise it is not possible to detect the target compound in acceptable recovery range (80-120%). The comparative study of the four solid phase extraction (SPE) sorbents for sample clean-up in the analysis of triclopyr residue from soil, sediment and water samples have been accomplished. The main findings of the experiments were that near to neutral pH, spiked sample flow rate 1 mL/min, sample volume 6 mL, eluent flow rate 0.5 mL/min and eluent volume 3 mL showed better recovery. In terms of overall feasibility of analysis, Cromabond[®]H⁺/OH⁻ column with 0.6 M KOH was the most suitable for the sample clean-up and found the highest recovery 89.32%. The second highest result was found 80.16% at Cromabond[®]H⁺/OH⁻ with ammonium acetate. Lowest recovery 15.91% was found at Isolute ENV+ with water as an eluent. Triclopyr recovery was higher in tap

water than soil and sediment. In water sample, triclopyr recovery percentage was 92%. In soil and sediment sample triclopyr was adsorbed by soil particle and organic matter. Soil microorganism in organic matter also rapidly degrades triclopyr. That is why recovery percentage was comparatively less than water sample. Soil and sediment recovery results were almost similar. However, it can still be improved by finding a better way to reduce the cost and to simplify the clean-up operation.

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