

Comparison of Four Different Solid Phase Extraction Cartridges for Sample Clean-Up in the Analysis of Glufosinate Ammonium from Aqueous Samples

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Abstract: For the determination of glufosinate ammonium residue in water samples, the efficiency of different solid phase extraction (SPE) sorbents was studied. Four different SPE sorbents i.e. CROMABOND PS-H⁺, CROMABOND PS-OH, ISOLUTE ENV+, Water Sep-Pak and OASIS HLB were used. Sample clean-up performance was evaluated using a high performance liquid chromatograph (Agilent 1220 infinity LC) equipped with a fluorescence detector. Detection of FMO-derivatives was done at wavelengths $\lambda_{ex} = 260$ nm and $\lambda_{em} = 310$ nm. The OASIS HLB column was found to be the most suitable for the clean-up process in view of the overall feasibility of the analysis.

Keywords: Glufosinate ammonium, water samples, sorbents, clean-up.

Introduction

The IUPAC name for glufosinate ammonium is ammonium (3-amino-3-carboxypropyl) methyl phosphinate. Glufosinate, (GLUF) is the short name for glufosinate ammonium. The ammonium salt is a natural compound initially isolated from two species of *Streptomyces* fungi¹. GLUF 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) crops before harvest^{2,3}. The application of glufosinate leads to reduced glutamine and increased ammonium levels in plant tissues. This causes photosynthesis to stop and the plant dies within a few days^{4,5}. GLUF has become one of the main herbicides in the Malaysian oil palm industry⁶. Figure 1 shows the structural formula and polarity of glufosinate ammonium. Dissociation constant (P^{Ka}), water solubility, molecular mass and molecular formula of glufosinate ammonium were shown in Table 1.

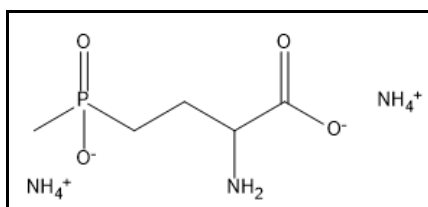


Figure 1: Structural formula of glufosinate ammonium

(Ref: <http://www.chemicalbook.com/Chemical product Property CB2697882>)

Table 1: Basic properties of glufosinate ammonium

Molecular formula	Molecular mass	Solubility in H ₂ O	P^{Ka}
C ₅ H ₁₅ N ₂ O ₄ P	198.19	>500g/L at 20°C	9.15±0.07

Source: Pesticide Action Network, North America. <http://www.pesticide.info.org>

The ammonium salt is soluble in water and insoluble in non-polar organic solvents⁷. The P-atom is surrounded by two, rather than one, carbon atoms, the positive charge on the molecule leads to a weak affinity towards coarser soils particles due to limited cation exchange sites on these particles^{8,9,10}. The amino and carbonyl groups that exist in the GLUF chemical structure may participate in hydrogen bonding. Hydrogen bonding appears to be the most important mechanism for adsorption of polar non-ionic organic molecules such as glufosinate ammonium on clay minerals^{11,12,13}. Most of the previous studies focused on the analysis of glufosinate ammonium content in different agricultural products (Corn, palm oil, wheat, fruits, and vegetables), but very little information of GLUF clean-up procedure from soil and water is known. Glufosinate ammonium is a very polar compound and it remains in the water and soil at ppb levels^{14,15}. Therefore, analysing the presence of glufosinate ammonium in soil and water is a complex issue. Solid phase extraction (SPE) is popular and the most commonly used among all the clean-up procedures^{16,17}. The sorbent is vital to acquire high efficiency in solid phase extraction techniques¹⁸. For the determination of glufosinate ammonium in water samples, additional set up is required in sample preparation procedure. The additional is needed for the separation of non-polar interferences from the highly polar glufosinate ammonium. In the present study water samples were used with reversed phase or ion exchange SPE cartridges.

In the present study, the SPE sorbents used were CROMABOND PS-H⁺, CROMABOND PS-OH⁻, ISOLUTE ENV+, Water Sep-Pak and OASIS HLB and they were compared to a wide range of packing materials available for obtaining high efficiency in the determination process.

Experimental

Chemicals and reagents

Standard analytical glufosinate ammonium (99% purity) was purchased from the laboratories of Dr. Ehrenstorfer Co., Germany. This GLUF was used in the preparation of the stock solution to obtain the calibration curve. Acetonitrile, acetone and diethyl ether were purchased from Scharlau Science (Barcelona, Spain). Analytical grade reagents such as disodium tetraborate decahydrate, potassium dihydrogen phosphate, 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 > 18 MΩ cm).

The SPE columns were CROMABOND PS-H⁺, CHROMABOND PS-OH⁻, 3ml/500mg, Macherey-nagel (Germany); ISOLUTE ENV+, 6ml/200mg, Biotage (Japan); Water Sep-Pak, 6ml/500mg, Waters (U.S.A); OASIS HLB, 6ml/500mg, Waters (U.S.A). Table 2 gives a short description of the SPE sorbents used for the clean-up process.

Table 2: Main characteristics of the SPE columns used^{16,18}

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	Water-wettable	Reverse phase
Water Sep-Pak	Hydrophobic	Silica based bonded phase	Reverse phase
CHROMABOND PS-H ⁺ CHROMABOND PS-OH ⁻	Resin based polystyrene-divinylbenzene	Beige powder	Cation & anion exchange

Apparatus

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

Methods

For the CROMABOND® PS-H⁺ column, conditioning was carried out with 2 × 2 ml distilled water to open the pores. For the CHROMABOND® PS-OH⁻ column, conditioning was undertaken with 2 × 2 ml distilled water, 2 × 2 ml 1 M NaHCO₃ solution, 2 × 2 ml distilled water. For the ISOLUTE ENV+column, conditioning was done with 2 × 2 ml distilled water. For the OASIS HLB and Water Sep-Pak columns, 3ml of ultra-pure water and 3ml methanol were used as conditioning agents¹⁹. After conditioning, 150ml of the aqueous solution was first passed through the PS-H⁺ and then through the PS-OH⁻ column at the optimized flow rate controlled by a vacuum pump. The cation exchanger column was disposed of the anion exchanger was dried with air or nitrogen. Finally, the elute was blown to near dryness and the residue reconstituted in 1ml of the mobile phase for HPLC determination. Before the HPLC analysis the following derivatization steps needed to be followed as shown in Figure 2. The clear supernatants were derivatised by adding 0.8 ml of borate buffer (0.025M) and 0.8 ml of acetone together with 0.2 ml of FMOC-Cl (0.01 M) solutions into 1 ml of sample. The mixture was swirled and left at room temperature for 30 minutes. After the reaction, the samples were washed with 1 ml of diethyl ether and ready for determination using high performance liquid chromatography (HPLC) equipped with a fluorescence detector²⁰.

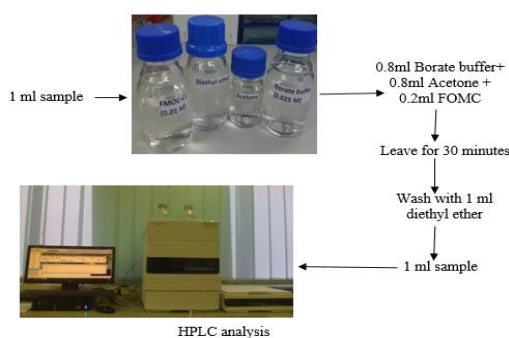


Figure 2: Derivatization process

The HPLC was performed on a NUCLEODUR® C18 Gravity chromatographic column with a mobile phase of (A) acetonitrile-(B) H₃PO₄, pH 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 min²¹ other specifications include: Flow rate-0.5 ml/min, injection volume 10µl and temperature 30°C. The fluorescence detector was set at λ_{ex} =263nm and λ_{em} =317nm.

Results and Discussion

A model mixture was eluted through the CROMABOND PS-H⁺, CROMABOND PS-OH, ISOLUTE ENV+, Water Sep-Pak, and OASIS HLB columns. While retaining glufosinate ammonium and allowing other substances/components to elute, the SPE sorbents thus served as chemical filters.

pH of samples

In different pH solutions, glufosinate ammonium appears in different forms, which can affect the absorption and recovery rates. Hydrochloric acid and sodium hydroxide were used as the pH regulators. The recovery from acidic, neutral and alkaline samples is listed in Figure 3. From Figure 3, OASIS HLB which has pH close to neutral showed higher absorption rate²².

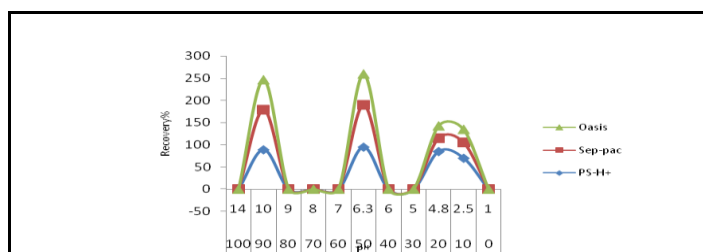
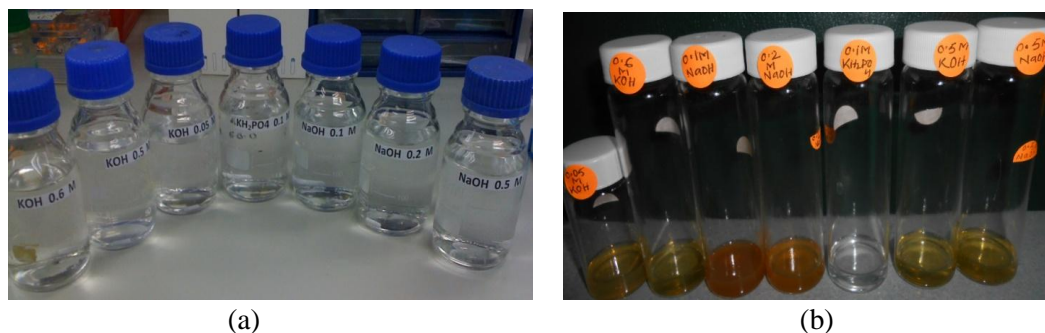


Figure 3: Relationship between the pH of the sample and the amount of glufosinate ammonium residue present in different SPE Cartridges.

Eluent component

For the CROMABOND[®] PS-H⁺ and CHROMABOND[®] PS-OH⁻ column, methanol and ammonium acetate, 0.1 M KH₂PO₄, 0.6 M KOH were chosen as eluents. In the case of ISOLUTE ENV+ and OASIS HLB columns, water, ammonium acetate, 0.2M NaOH, 0.5M KOH and 0.6 M KOH were used as eluents. For Water Sep-Pak, the selected eluents were water, ammonium acetate, 0.5M KOH and 0.6 M KOH.

Figure 4(a) showed that six different eluents and 4(b) display the derivatised supernatants before inject the HPLC. From Table 3, it can be seen that ammonium acetate exhibited a higher recovery rate with the CHROMABOND PS-H+CHROMABOND PS-OH- column. The ISOLUTE ENV+, Water Sep-Pak and OASIS HLB columns, 0.5M KOH showed better results than the other eluents.



(a)

(b)

Figure 4: Different eluents

Table 3: Effects of seven different eluents for four different sorbents on the recovery analytes

Sorbents	Water	Methanol	Ammonium acetate	NaOH 0.2M	KH ₂ PO ₄ 0.1M	KOH 0.5M	KOH 0.6M
CROMABOND [®] PS-H ⁺ / PS-OH ⁻	-	72.57	73.42	-	46.28	-	68.44
ISOLUTE ENV+	16.99	-	36.73	40.13	-	71.78	61.47
Water Sep-Pak	33.30	-	48.20	-	-	78.50	64.23
OASIS HLB	18.30	-	79.16	54.77	-	98.36	88.19

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. 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. In the experiment, CROMABOND PS-H⁺ CROMABOND PS-OH⁻ was washed with 3ml AcOH/water (5:95, v/v) for the removal of impurities. It was washed again with 3ml methanol/water (20:80, v/v) to remove neutral and acidic compounds. Compounds were eluted with 2 × 2 ml methanol/acetone (1:1, v/v) +20% AcOH²³.

Other parameters

In the experiment, CROMABOND PS-H⁺ CROMABOND PS-OH⁻ was used to obtain 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⁺ CROMABOND PS-OH⁻ columns at five different flow rates (1.0, 3.0, 5.0, 7.0 and 10 ml/min). The eluents were then collected and determined using the HPLC Agilent 1220 Infinity (LC) to get the recovery. Figure 5 shows that by gradually increasing the sample flow rate, recovery could be decreased. The reason being the velocity of the sample flow was too fast to reach equilibrium in the sorbent and the targeted components did not get optimal absorption. Reducing the velocity of the flow rate can increase sample processing time. The flow rate of 1ml/min showed good recovery and it also reduced the processing time²⁴.

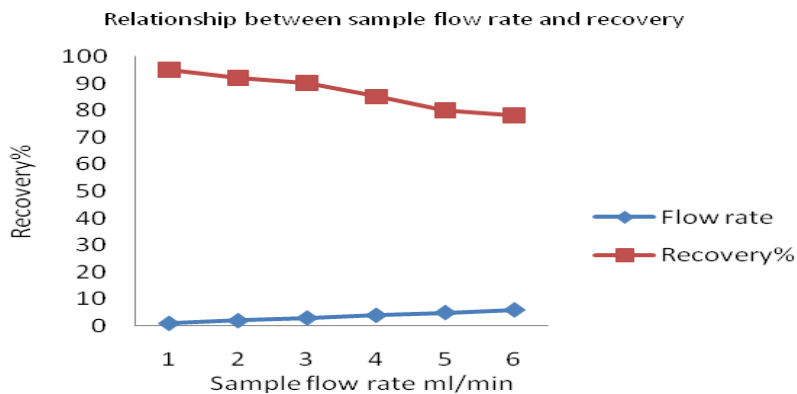


Figure 5:Relation between sample flow rate and recovery

Different volumes of treatmentsamples with the same amount of glufosinate ammonium solution in 1.0, 3.0, 5.0, 7.0 and 10ml of water were tested to get the approximate sample treatment volume.From Figure6 it can be seen that the recoveryrates did not have any significant difference from the ranges of 1.0-10.0 ml sample volumes. Comparing the processing time and the results, the 5ml sample was chosen as the volume for sample treatment²⁵.

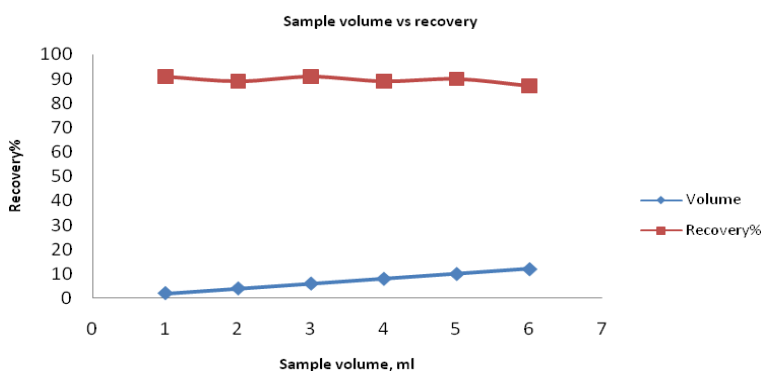


Figure 6:Relation between sample volume and recovery

Several flow rates of 1.0, 3.0, 5.0, 7.0, and 10.0 ml /min were tested to get higher recovery rates. From Figure7, it can be seen that lower elution rates can result in higher recovery rates but time taken was longer. For this reason, 0.5ml/min was chosen as the optimum elution rate²⁶.

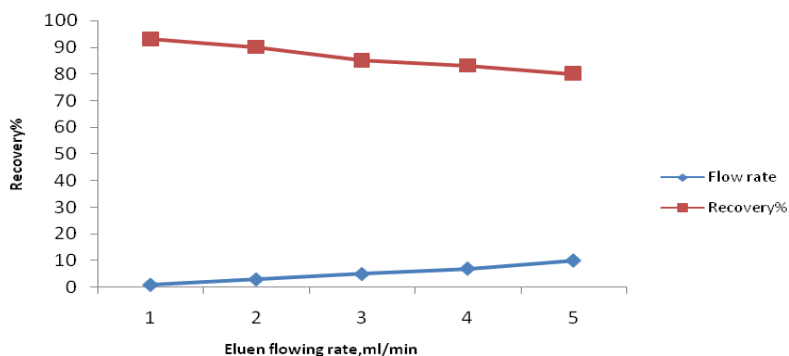


Figure 7: Relation between eluent flow rate and recovery

Five eluent volumes of 1.0, 3.0, 5.0, 7.0, and 10.0 ml of the same concentration were tested to determine the volume of elution. Figure8 shows that the recovery increased with more eluting solvent. However, more eluting solvent needed more time and had negative impact on the concentration. Therefore, the 7 ml sample was considered the best²⁷.

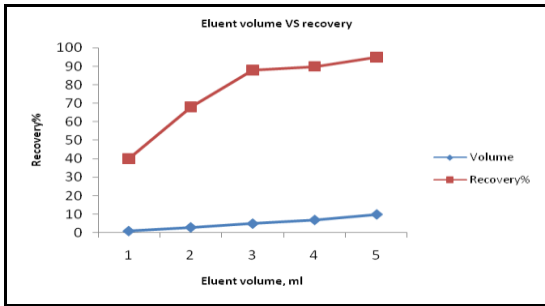


Figure 8: Relation between eluent volume and recovery

Comparison of HPLC chromatograms

Using high performance liquid chromatography with fluorescence detector $\lambda_{ex}=270$ nm and $\lambda_{em}=315$ nm, the clean-up performance was evaluated. Calibration of working standard solution was used to test the ability of the procedures and instruments for determination glufosinate ammonium. Linearity of the calibration was assessed from a linear regression of response area versus concentrations of glufosinate ammonium solution in ppm. Figure 9 shows that the procedures and instrument used had shown good ability in separation of glufosinate ammonium. The comparative HPLC chromatograms purified by the four solid phase extraction sorbents are shown in Figure 10.

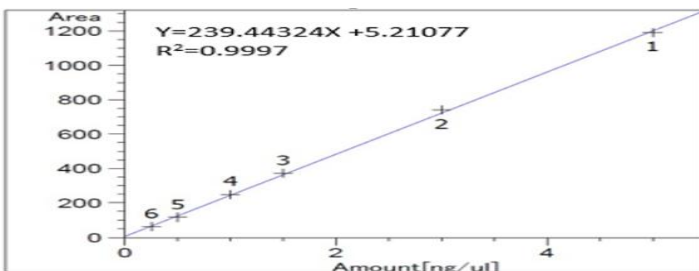
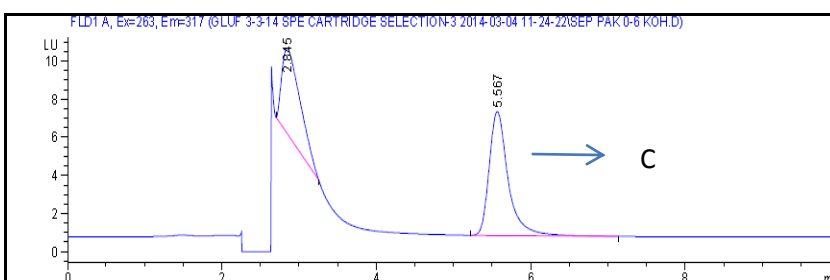
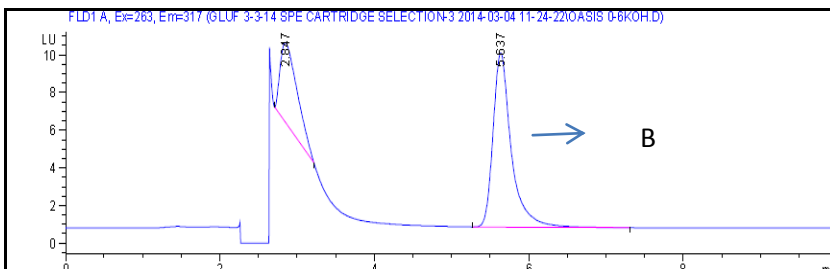
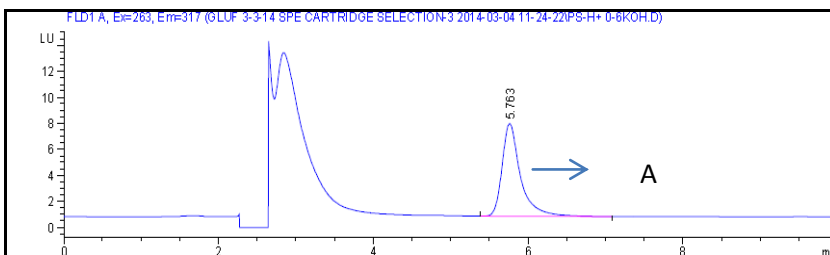


Figure 9: Linear regression of standard Glufosinate ammonium



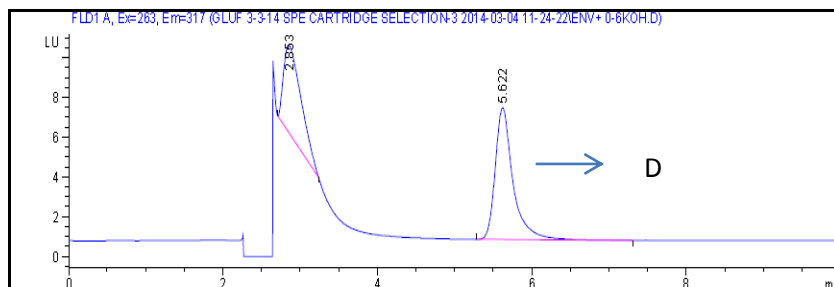


Figure 10: Comparison of four SPE sorbents namely CROMABOND®PS-H⁺ (A), CHROMABOND® PS-OH⁻ ISOLUTE ENV+ (B), Water Sep-Pak (C) and OASIS HLB (D) for HPLC chromatograms of glufosinate ammonium purified.

In the case of CROMABOND PS-H⁺ CROMABOND PS-OH⁻SPE column, although it has good recovery, the complex ionic behaviour of glufosinate ammonium made it difficult to adjust an appropriate pH for consistent and quantitative extraction. The other three SPE sorbents i.e.: ISOLUTE ENV+, Water Sep-Pak, and OASIS HLB are seen to be suitable for clean-up operations²⁸. At the same time, the OASIS HLB chromatograms were better than the Water Sep-Pak and ISOLUTE ENV+. The dimethyl butylamine group extracts acidic compounds with anion exchange groups and this is the speciality of OASIS HLB.

Conclusion

The comparative study of the four solid phase extraction (SPE) sorbents for sample clean-up in the analysis of glufosinate ammonium residue from water samples showed that the highest recovery of 98.36% and clear chromatograms came from OASIS HLB. So from the present study, OASIS HLB was found to be the most suitable for sample clean-up. However, the process can still be improved by finding a better way to reduce the cost and simplify the clean-up operation.

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