

Sample Preparation and Confirmation Column for Phenoxyacid Herbicides by HPLC

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Introduction

Phenoxyacid herbicides, such as 2,4-D, dicamba, picloram, and Silvex, are used to control agricultural and aquatic weeds. These compounds can be found in the acid form, or as the salts or esters. While not considered highly toxic, phenoxyacid herbicides are monitored in agricultural monitoring wells and drinking water sources. Traditionally, these compounds have been analyzed by gas chromatography (GC), for example by US EPA Method 8151. To make these compounds amenable to GC, they must first be converted to the methyl esters using a derivatizing agent such as diazomethane, a time-consuming procedure.

An optimized HPLC method for analyzing phenoxyacid herbicides using a C18 column and gradient procedure was previously presented. In order to perform confirmation analysis, a second column producing retention time and elution order changes is needed. A confirmation column has been developed that has a different selectivity for the phenoxyacid herbicides than a C18 stationary phase using the same gradient procedure.

A solid phase extraction (SPE) method has been verified to extract and concentrate water samples up to 1L volume and to quantitatively recover at least 16 of the common underivatized herbicides in Table 1. This simple sample preparation step provides cleaner analytical samples and lower HPLC detection limits, making this a suitable replacement for GC analysis.

Table I. Chlorophenoxyacid Herbicides included in this study.

Mix A	Mix B	
picloram	4-nitrophenol	1,4-dichlorobenzene (IS)
chloramben	MCPA	2,4-dichlorophenyl acetic acid (surrogate)
dicamba	3,5-dichlorobenzoic acid	
bentazon	MCPP	
2,4-D	2,4,5-T	
dichlorprop	2,4-DB	
2,4,5-TP (Silvex)	dinoseb	
acifluorfen	pentachlorophenol	

Confirmation Column Selection

Previous work indicated that the Ultra Aqueous C18 stationary phase provided enhanced selectivity for the target herbicides. Many different types of HPLC stationary phases were evaluated as possible confirmation columns; such as the Ultra PFP (pentafluorophenyl), and IBD (intrinsically base deactivated), as well as the Pinnacle II™ PAH and Phenyl phases. These were all tested to establish if they provided adequate separation, rapid analysis times and sufficient elution order changes to qualify as a reliable confirmation column. Results of these studies indicated that the Allure™ Basix phase met all the necessary requirements.

Figure 1 demonstrates the problems when using either a standard C18 or even a base deactivated C18 phase for the analysis of these acidic herbicides. Figure 2 shows the significant analytical improvement when using the Ultra Aqueous C18 and the HPLC conditions listed in Table II. The chromatograms for Mix A and B are overlaid for clarity. Figure 3 chromatograms were generated using these same analytical conditions and the Allure Basix column. All of the runs were completed in less than 17 minutes and still provided adequate separations. The Ultra Aqueous stationary phase contains a polar embedded group in addition to the C18 functionality, while the Allure's highly retentive propyl cyano phase demonstrates a very different selectivity for the target herbicides under the same analytical conditions. Table III highlights both the differences in elution order and the shift in retention times that are critical to obtaining good confirmation of the identified underivatized analytes.

Figure 1. Analysis of phenoxyacid herbicides using standard C18 columns: non-base deactivated (upper) and base deactivated stationary phases (lower).

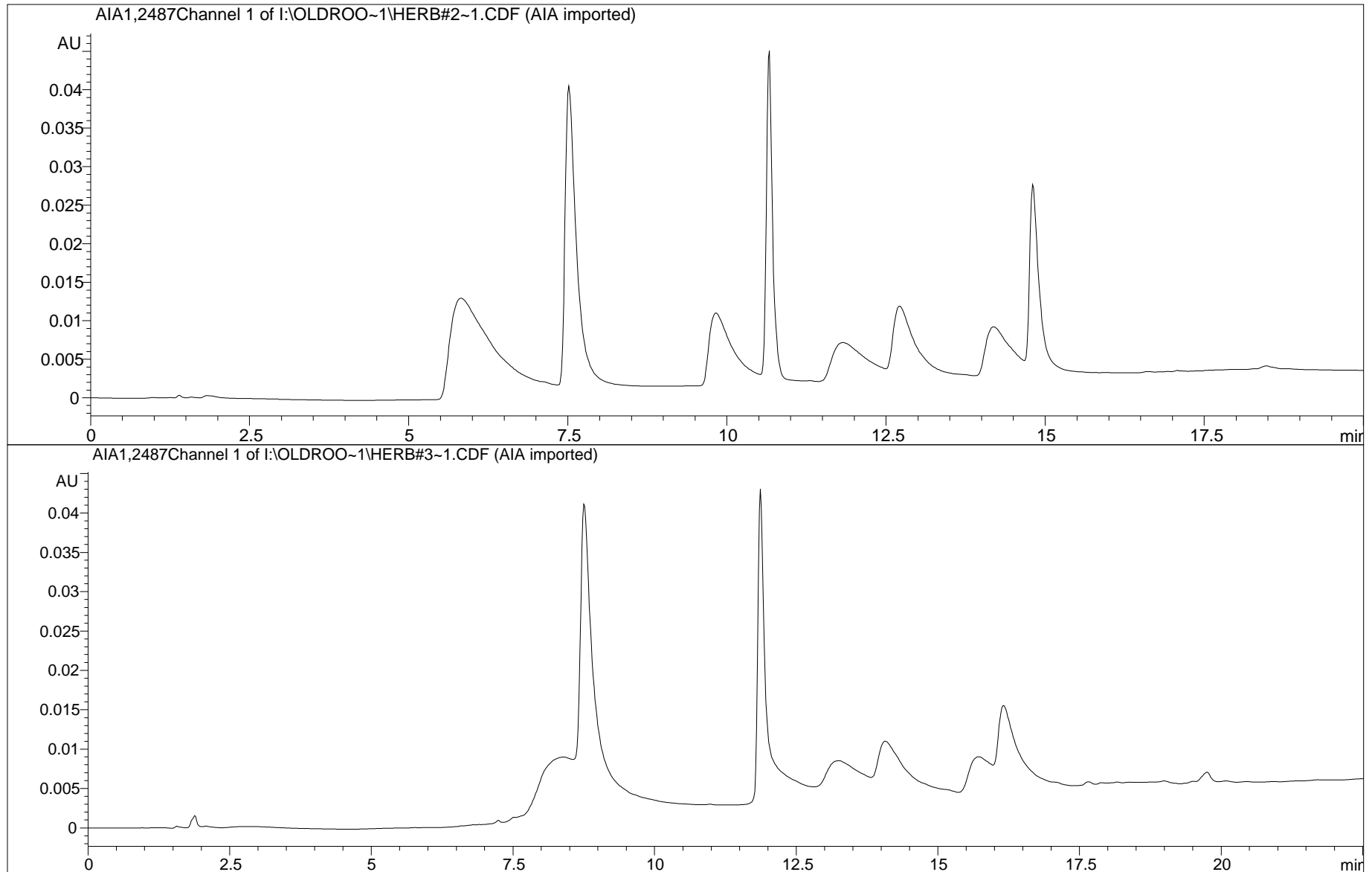


Table II. Improved chromatographic conditions for separation and confirmation of phenoxyacid herbicides.

HPLC Columns	Ultra Aqueous C18, 150x4.6mm, 5 μ m Allure™ Basix, 150x4.6mm, 5 μ m
Mobile Phase	A: 0.05% H ₃ PO ₄ B: acetonitrile
Gradient Program	0 min 20%B 28 min 80%B 33 min 90%B 34 min 20%B
Flow Rate	1.0 mL/min
Detection	UV @ 225nm
Injection	10 μ L
Concentration	10 ppm each

Figure 2. Separation of phenoxyacid herbicide mix A and B, using an Ultra Aqueous C18 column and the conditions in Table II.

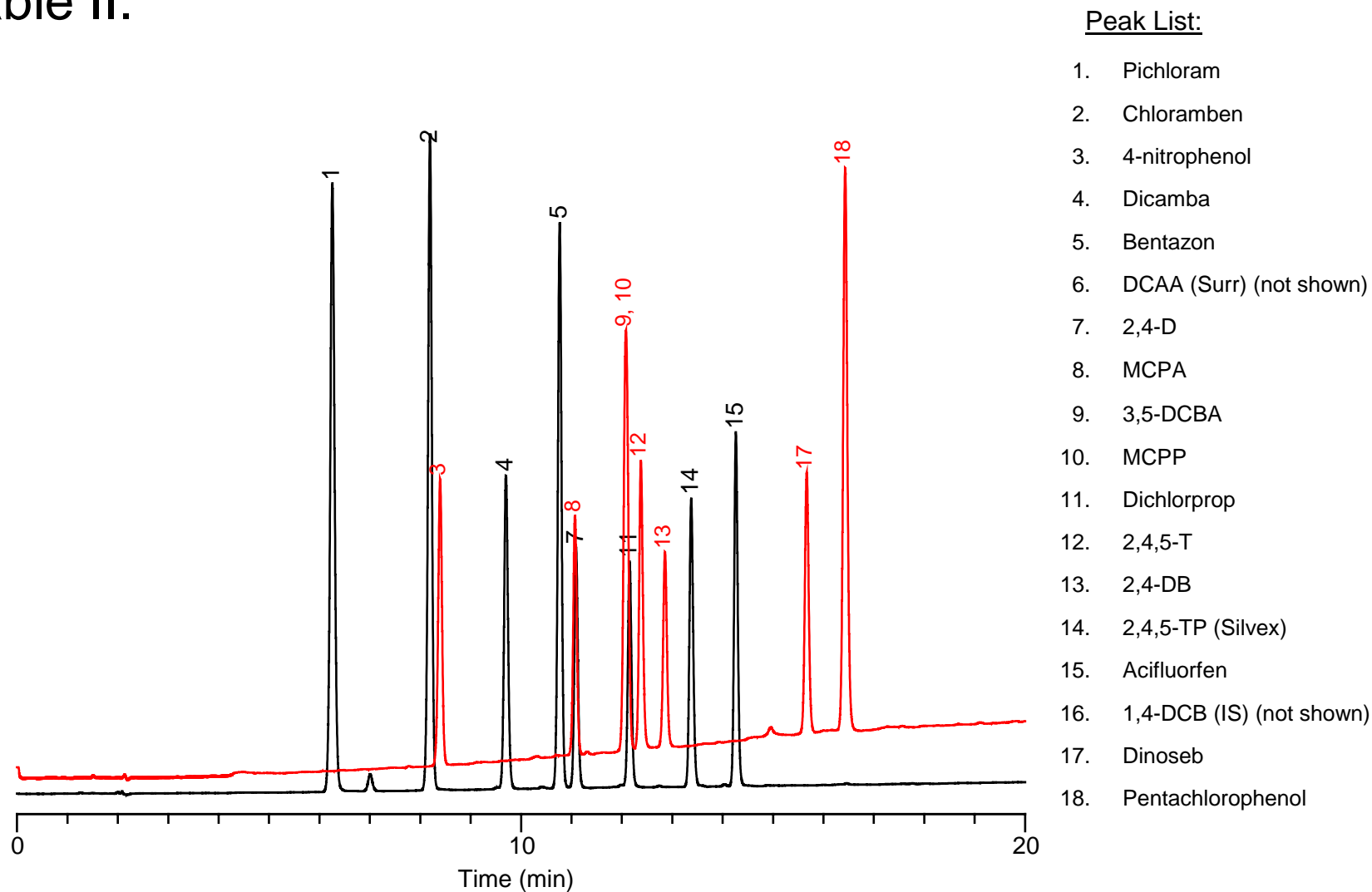


Figure 3. Separation of phenoxyacid herbicide mix A and B, using an Allure Basix column and the conditions in Table II.

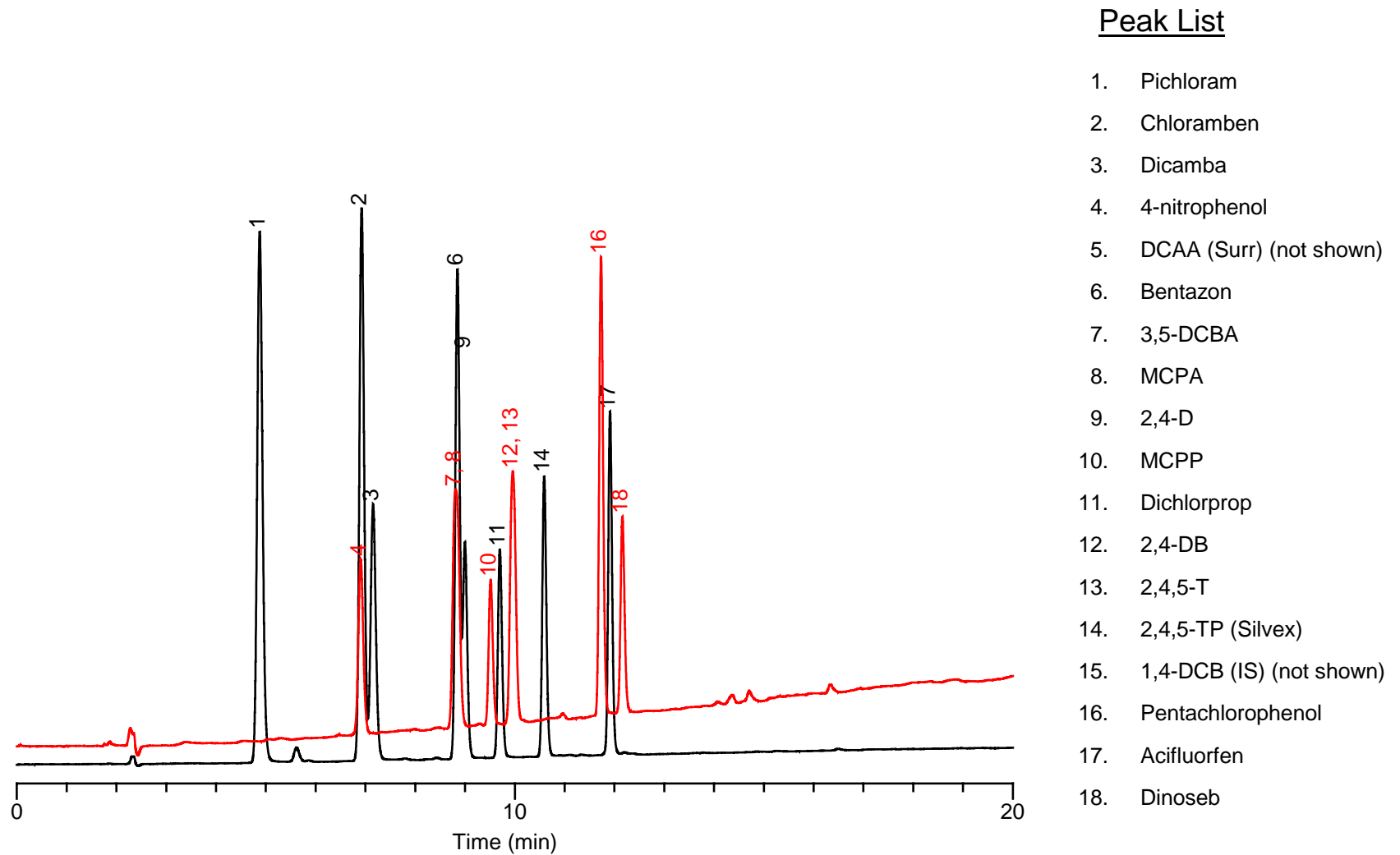


Table III. Comparison of retention times and elution orders for chlorophenoxy herbicides on Ultra Aqueous C18 and Allure Basix columns. Coelution groups are highlighted.

Compound	Ultra Aqueous C18
Pichloram	6.24
Chloramben	8.16
4-nitrophenol	8.38
Dicamba	9.64
Bentazon	10.69
DCAA (Surr)	10.83
2,4-D	11.03
MCPA	11.04
3,5-DCBA	12.05
MCPP	12.07
Dichlorprop	12.09
2,4,5-T	12.34
2,4-DB	12.82
2,4,5-TP (Silvex)	13.30
Acifluorfen	14.05
1,4-DCB (IS)	15.23
Dinoseb	15.62
Pentachlorophenol	16.39

Compound	Allure Basix
Pichloram	5.93
Chloramben	8.54
Dicamba	9.02
4-nitrophenol	9.08
DCAA (Surr)	10.62
Bentazon	11.07
3,5-DCBA	11.07
MCPA	11.19
2,4-D	11.32
MCPP	12.03
Dichlorprop	12.18
2,4-DB	12.55
2,4,5-T	12.65
2,4,5-TP (Silvex)	13.32
1,4-DCB (IS)	13.61
Pentachlorophenol	15.11
Acifluorfen	15.19
Dinoseb	15.84

Solid Phase Extraction

To meet required detection limits for these herbicides, a concentration step is necessary. Solid phase extraction (SPE) can be used to extract the herbicides as free acids from a water matrix, before elution with suitable solvents. Several types of beds were previously tested for this application, including C18, and graphitized carbon. We have confirmed that a specialty divinylbenzene material, the Resprep AH SPE 6mL/500mg tube, gave the best overall recoveries.

Analytical conditions are the same as in Table II. The SPE preparation method is detailed in Table IV. Water sample volumes from 500milliliters to 1liter must be adjusted to a pH of 2 for best recovery results. Results of a final study for all 16 herbicides, internal and surrogate standards are summarized in Tables V and VI. When using a 1L sample size, recoveries of the herbicides were 92-100%, with the exception of the internal standard 1,4 dichlorobenzene. This analyte is not well retained on the packing and reduced recovery is due to breakthrough during the sample extraction step. The 1L samples were spiked with 10ppb of each herbicide and the SPE method overall produced a 200-fold concentration of the analytes. The use of MTBE during elution is optional. Most recoveries improved only slightly, but it is required for quantitative recovery of pentachlorophenol.

Table IV. Conditions for the solid phase extraction of phenoxyacid herbicides.

SPE tube	6mL, 500mg Resprep AH tube, 6mL/500mg cat#26029
Tube conditioning	<ol style="list-style-type: none"> 1. 4mL acidified methanol (0.01% phosphoric acid) 2. 4mL deionized water (pH=2)
Sample	500mL or 1L water, pH adjusted to 2
Sample flow rate	Sample passed through tube at rapid vacuum flow rate
Wash	Inner surface of tube rinsed with small amount of acidified water, if necessary
Dry	Tube dried for less than 1 minute
Extraction	<ol style="list-style-type: none"> 1. 3 x 2mL acidified methanol 2. Additional 2mL methyl t-butyl ether (optional) 3. Sample concentrated to 5mL (ambient temperature, nitrogen purge)

Table V. Recoveries of phenoxyacid herbicides after solid phase extraction.

Analyte	% Recovery 500mL sample	%RSD n=4	% Recovery 1L sample
pichloram	89.9	5.8	97.5
chloramben	90.6	5.9	99.1
dicamba	89.2	5.5	98.4
bentazon	89.0	5.7	97.5
2,4-D	88.7	5.7	98.3
dichloroprop	91.9	6.9	100.4
2,4,5-TP	89.0	5.5	98.0
acifluorfen	82.9	5.6	91.9
1,4 dichlorobenzene (IS)	60.5	4.4	77.3

Table VI. Recoveries of additional phenoxyacid herbicides after solid phase extraction.

Analyte	% Recovery 500mL sample	% RSD n=3	% Recovery 1L sample	% RSD n=3
4-nitrophenol	97.2	2.4	92.3	0.9
2,4 DCAA (surrog)	100.7	6.3	97.4	0.9
MCPA	99.7	2.4	96.5	1.5
3,5 dichlorobenzoic acid	99.8	2.2	95.2	1.1
MCPP	100.6	2.8	96.6	1.5
2,4-T	96.2	2.3	96.2	1.5
2,4-DB	99.1	2.4	97.7	1.7
1,4 dichlorobenzene (IS)	73.2	1.3	72.0	3.0
Dinoseb	93.4	1.0	95.0	1.4
pentachlorophenol	93.0	1.3	93.5	2.5

Conclusions

Based on this study, HPLC analysis of phenoxyacid herbicides after solid phase extraction is a viable alternative to the gas chromatographic procedure. The chromatographic separation of the herbicides was optimized using an Ultra Aqueous C18 column, a C18 stationary phase with a polar embedded group, and confirmed using an Allure Basix column, a propyl cyano stationary phase. These phases have enhanced selectivity for the acidic compounds when analyzed at low pH. This HPLC technique does not require derivitization of the acidic analytes, a step which requires experienced laboratory personnel and a significant preparation time commitment for each sample.

In order to meet the required detection limits, a solid phase extraction method was developed which resulted in a 200-fold concentration of the analytes during cleanup. The SPE method, using a substituted divinylbenzene packing material, showed excellent recoveries for all 16 acid herbicides and 1 surrogate, even with 1 liter samples. The internal standard shows lower recoveries than the target herbicides; other options for the internal standard will be explored. The SPE method allows for rapid and quantitative recovery of these herbicides in their free acid form.