

Analyzing Phenoxyacid Herbicides, Using Liquid Chromatography

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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.

Because liquid chromatography does not require sample derivatization, it is an attractive option to the GC procedure. Several HPLC methods have been published for the analysis of phenoxyacid herbicides. EPA Method 555 discusses the analysis of the phenoxyacid herbicides, as the acids, in drinking water samples. The compound list is divided into two separate mixes using this method (Table I). EPA Method 8321 is a general LC/MS method for semivolatile compounds. An optimized method for analyzing phenoxyacid herbicides will be presented, as will a solid phase extraction procedure for recovering these compounds from water samples. A gradient procedure is useful when analyzing a range of herbicides; for the analysis of 2,4-D and Silvex alone, an isocratic method can be used.

Table I. Chlorophenoxyacid herbicides included in this study.

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

Column Selection

Selecting the proper stationary phase for analyzing phenoxyacid herbicides can be challenging. While a C18 stationary phase is specified in EPA method 555, C18 columns from different manufacturers can yield vastly different separations. In addition, many C18 columns rapidly lose efficiency, resulting in poor resolution of the herbicides. Since the phenoxyacid herbicides are weak acids, analysis should be done at a low pH. This will ensure that the herbicides are present in their protonated (non-ionic) form.

Figures 1 and 2 show the separations of phenoxyacid herbicides on an Ultra Aqueous C18 column (analytical conditions are given in Table II). The Ultra Aqueous stationary phase contains a polar embedded group, which provides enhanced selectivity for the target herbicides. By comparison, Figure 3 shows the analysis of mix A on two standard C18 columns. Resolution of the herbicides is poor, and tailing peaks indicate interactions between the herbicides and the stationary phase. To analyze just 2,4-D and/or Silvex, an isocratic method can be used, as shown in Figure 4.

Table II. Chromatographic conditions for separating phenoxyacid herbicides.

	Mix A	Mix B	Isocratic Mix
HPLC Column	Ultra Aqueous C18, 150x4.6mm, 5 μ m	Same	Same
Mobile Phase	A: 0.05% H ₃ PO ₄ B: acetonitrile	A: 0.05% H ₃ PO ₄ B: acetonitrile	A: 0.05% H ₃ PO ₄ B: acetonitrile
Gradient Program	0 min 20%B 15 min 80%B 20 min 80%B 21 min 20%B	0 min 10%B 10 min 45%B 16 min 45%B 22 min 90%B 24 min 90%B 25 min 10%B	60%A : 40%B isocratic
Flow Rate	1.0 mL/min	1.0 mL/min	1.0 mL/min
Detection	UV @ 225nm	UV @ 225nm	UV @ 225nm
Injection	10 μ L	10 μ L	10 μ L
Concentration	10 ppm each	10 ppm each	10 ppm each

Figure 1. Baseline separation of phenoxyacid herbicide mix A, using an Ultra Aqueous C18 column and the conditions in Table II.

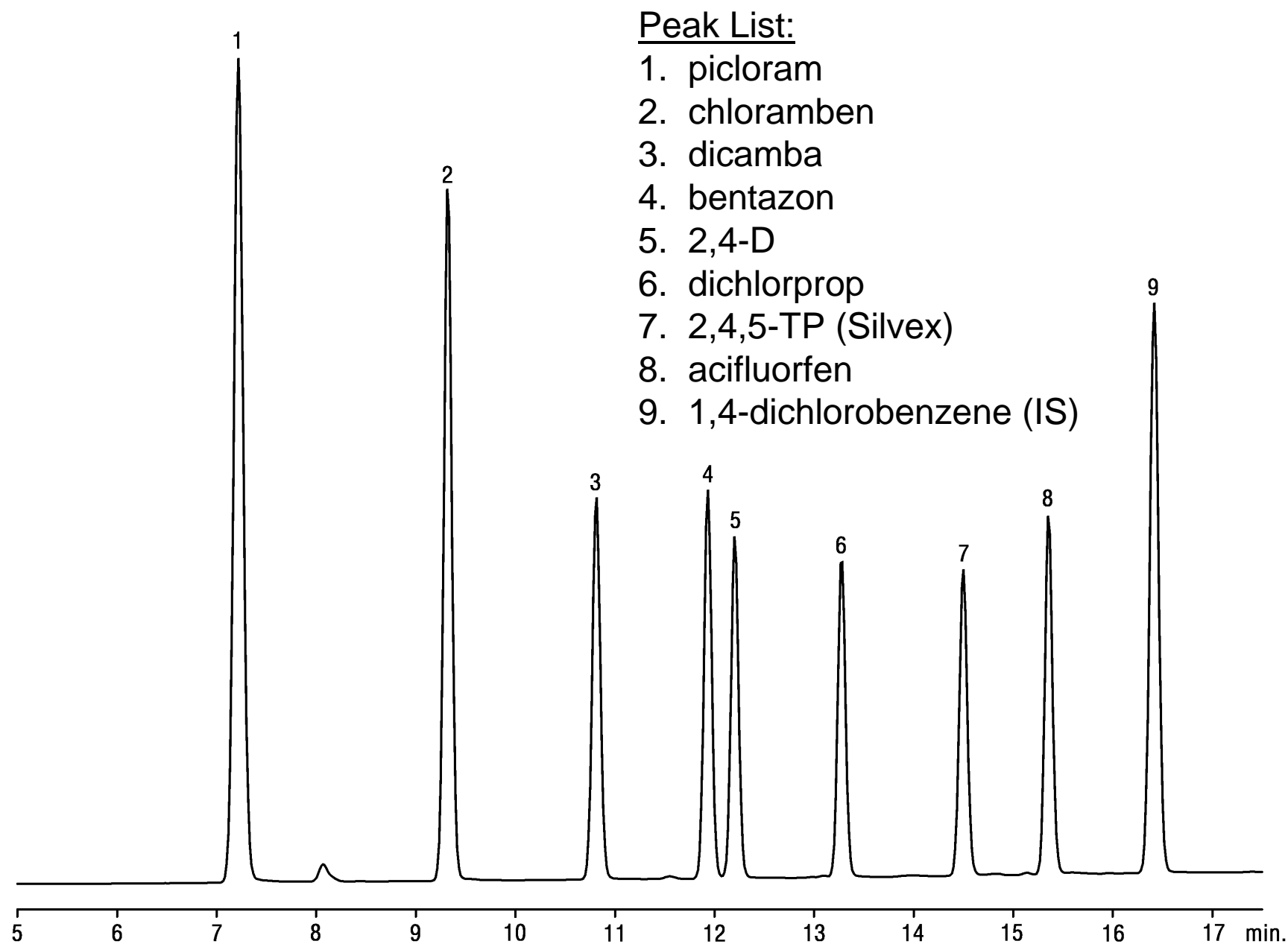


Figure 2. Separation of phenoxyacid herbicide mix B, using an Ultra Aqueous C18 column and the conditions in Table II.

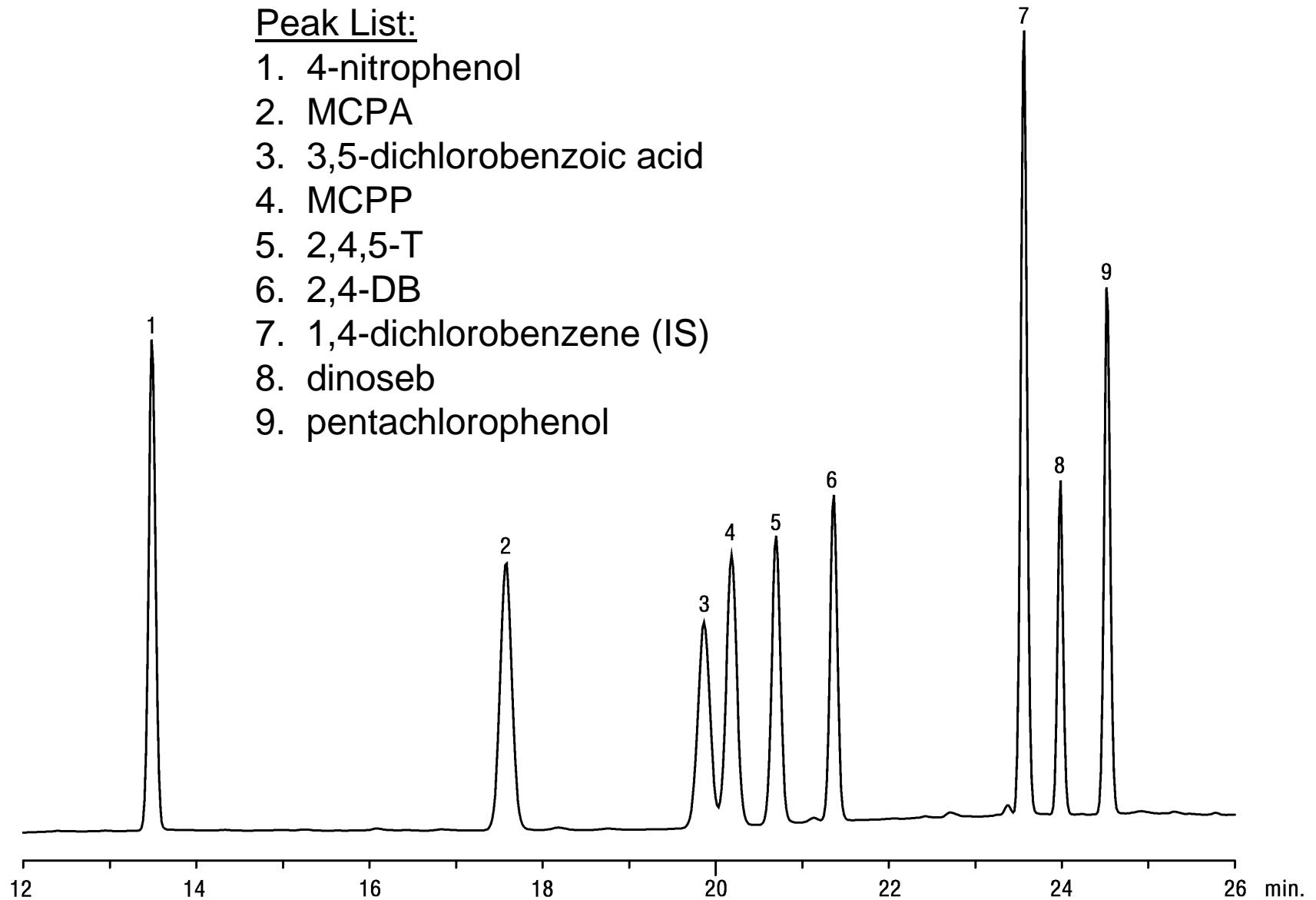


Figure 3. Analysis of phenoxyacid herbicides using standard C18 columns: non-base deactivated (upper) and base deactivated stationary phases (lower).

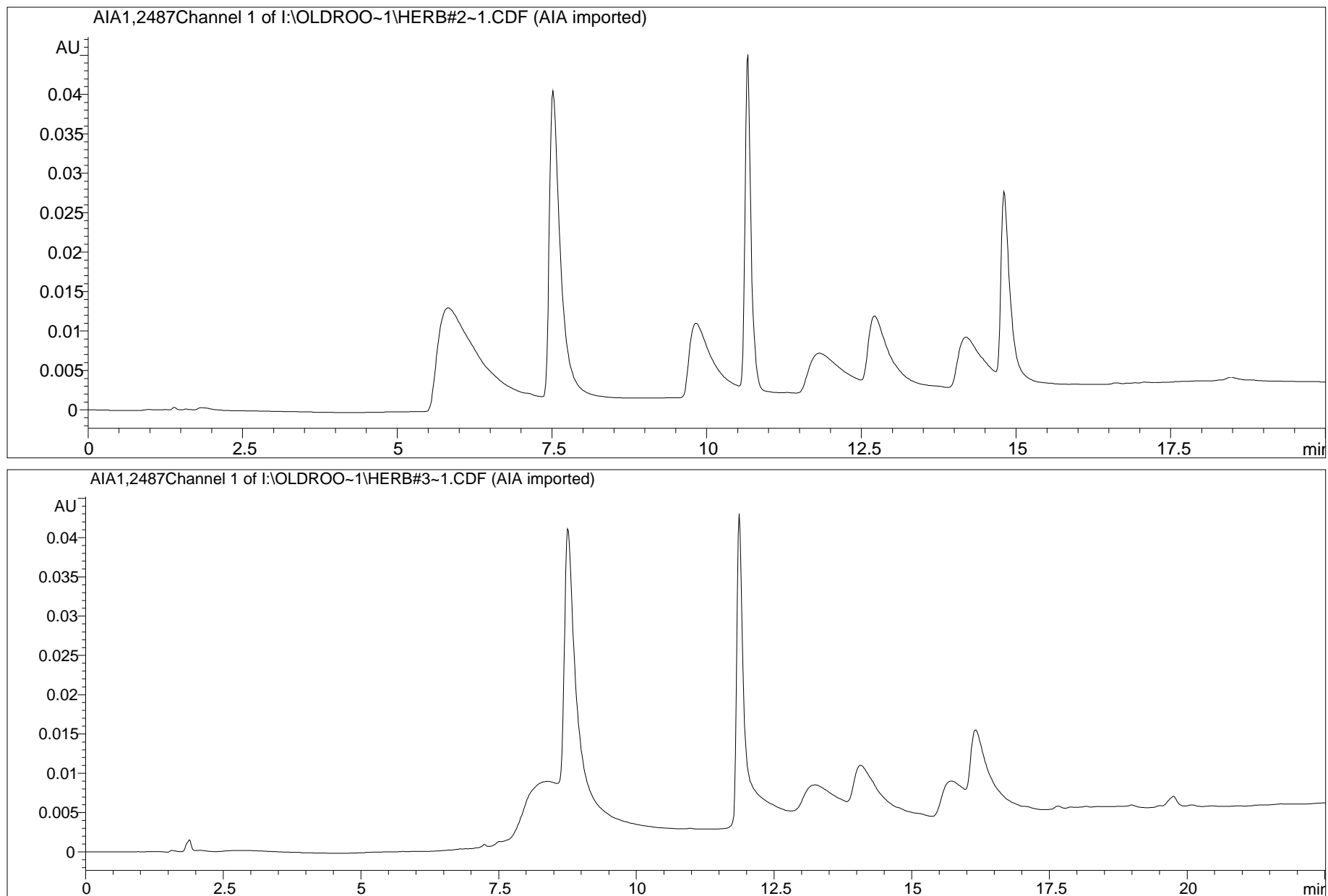
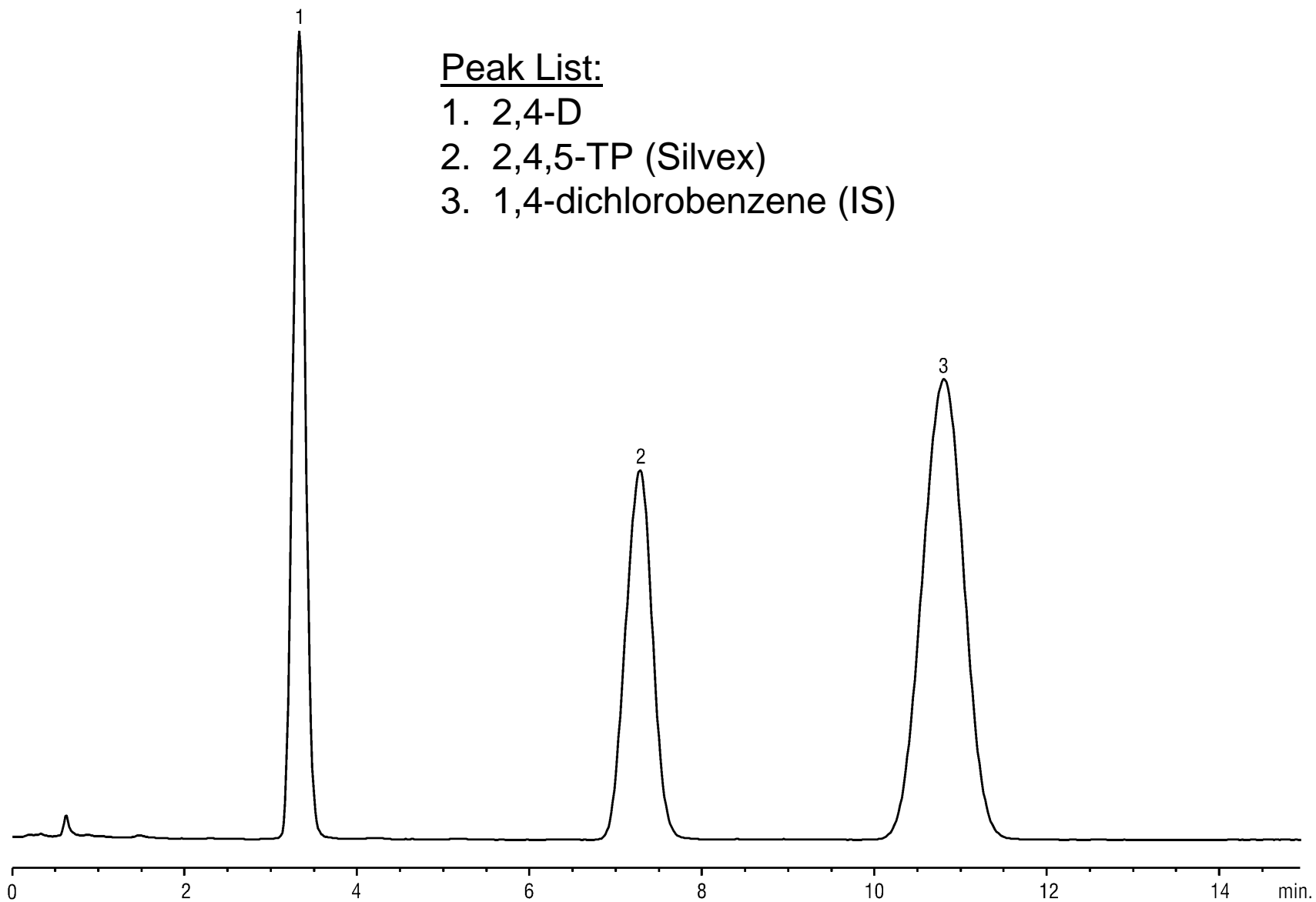
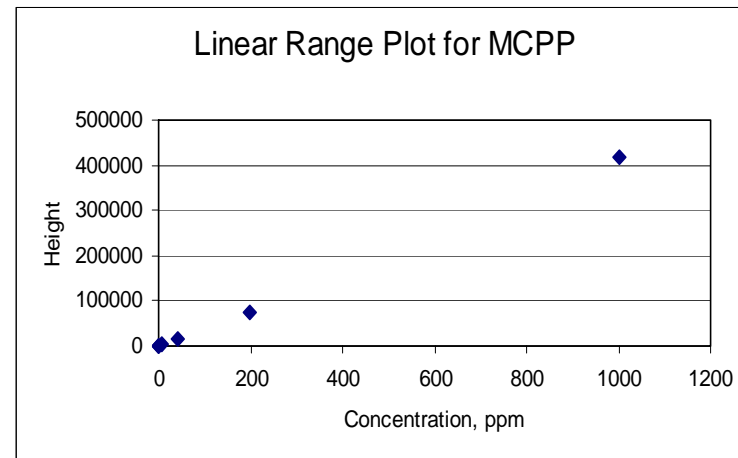
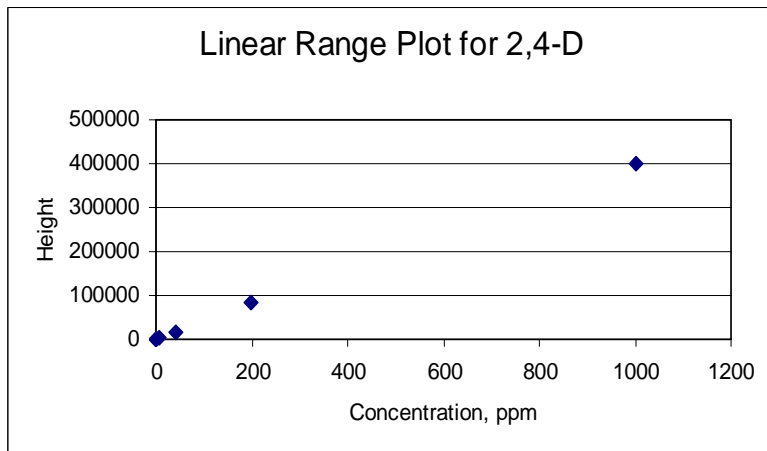


Figure 4. Isocratic separation of 2,4-D and Silvex, using the conditions in Table II.



Linear Range Study

Herbicides in mixes A and B were analyzed over a concentration range of 320ppb - 1000ppm. All of the herbicides displayed a linear response over this range. For example, $R^2 = 0.99997$ for 2,4-D, and $R^2 = 0.9994$ for MCP. However, dalapon shows very low response when using UV detection, and could be detected only at concentrations greater than 100ppm. From this, the limit of quantitation was determined to be 100ppb for each herbicide, with the exception of dalapon (~100ppm).



Solid Phase Extraction

To meet the required detection limits for these herbicides, a concentration step is necessary. Solid phase extraction (SPE) can be used to extract the herbicides from a water matrix, before elution with acidic methanol. Several types of beds were tested for this application, including C18, graphitized carbon, and substituted divinylbenzene. We found the divinylbenzene material gave the best overall recoveries. Analytical conditions are given in Table III, and the results of a preliminary study for mix A herbicides are in Table IV and Figure 5. When using a 100mL sample size, recoveries of 82-92% were achieved, with the exception of the internal standard. The 1L sample size showed even higher recoveries, again with the exception of the internal standard. The 1L sample had a concentration of 10ppb each herbicide, with the exception of dicamba (20 ppb).

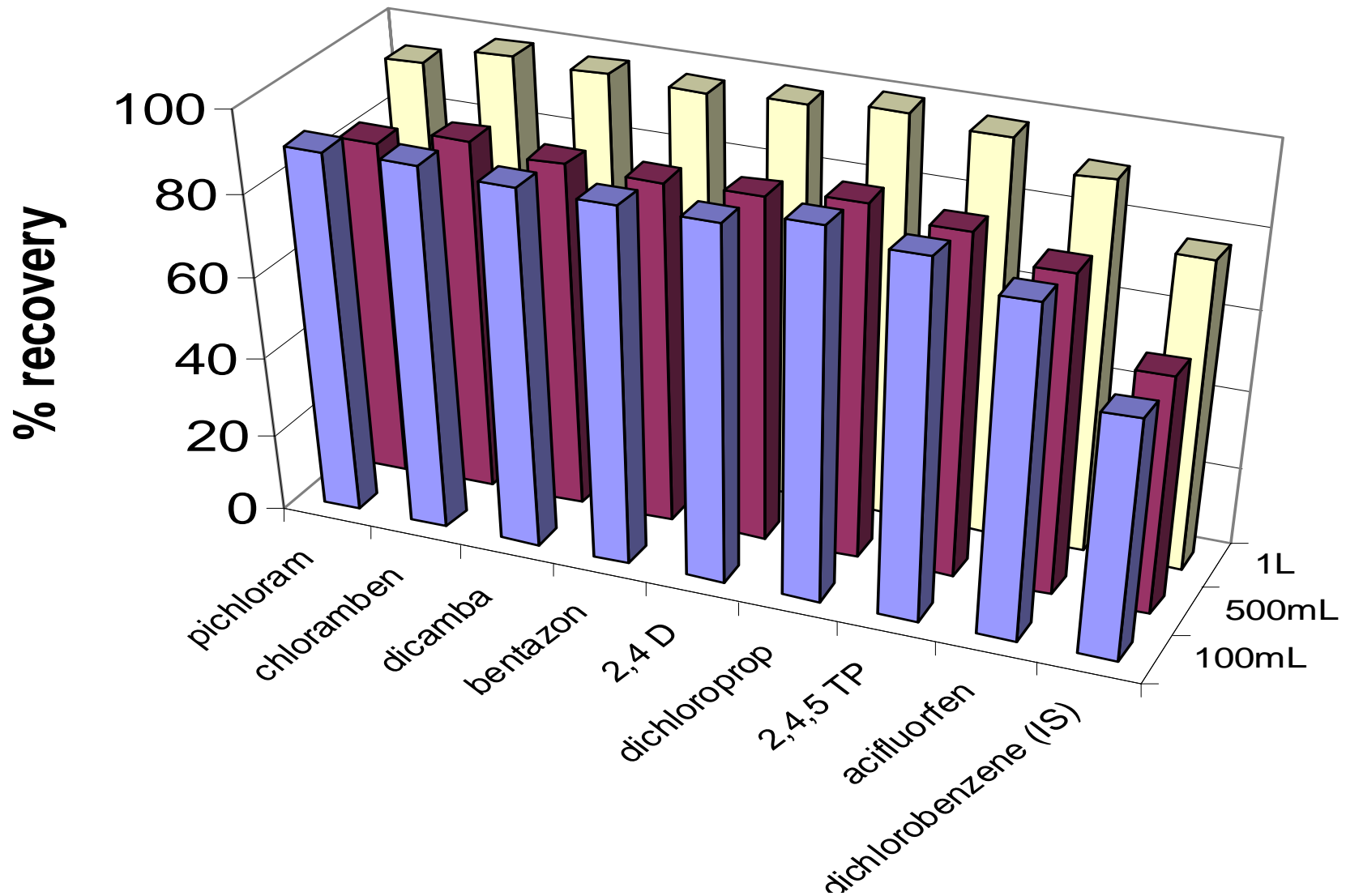
Table III. Conditions for the solid phase extraction of phenoxyacid herbicides.

SPE tube	6mL, 500mg substituted divinylbenzene
Tube conditioning	1. 4mL acidified methanol (0.01% phosphoric acid) 2. 4mL deionized water (pH=2)
Sample	100mL or 1L water, pH adjusted to 2
Sample flow rate	Sample passed through tube at slow flow rate or with gravity only
Wash	Inner surface of tube rinsed with small amount of acidified water
Dry	Tube dried for less than 30 seconds
Extraction	3 x 2mL acidified methanol; concentrated to 5mL

Table IV. Recoveries of phenoxyacid herbicides (mix A) after solid phase extraction.

Analyte	% Recovery 100mL 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

Figure 5. Recoveries for mix A herbicides using sample sizes of 100 mL, 500 mL, and 1 L.



Conclusions and Future Work

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 a C18 stationary phase with a polar embedded group. This phase has enhanced selectivity for the acidic compounds when analyzed at low pH. An isocratic method also was developed for use with 2,4-D and Silvex.

In order to meet the required detection limits, at least a 1000-fold concentration will be needed. Preliminary experiments with solid phase extraction using a substituted divinylbenzene material show excellent recoveries for mix A herbicides, even with 1 liter samples. The internal standard shows significantly lower recoveries than the target herbicides; other options for the internal standard will be explored. Future work also will include the application of this procedure to mix B herbicides, and determining the effects of concentrating and heating on herbicide recoveries.