

OH100R^(a)**Direct Analysis of TCLP Acidic Semivolatile Compounds in Radioactive Liquid Wastes or Leachates Using HPLC with Ultraviolet Absorbance Detection**

The decision to use this method should be made during project planning (see Chapter 1). Project personnel, together with stakeholders, should determine the activities that need to be performed to qualify the method for use in the project (see Appendix B).

1.0 Scope and Application

- 1.1** This method describes the direct determination by high performance liquid chromatography (HPLC) of the toxicity characteristic leaching procedure (TCLP) acidic semivolatile compounds in leachates and aqueous liquid-waste samples. It is based upon U.S. Environmental Protection Agency (EPA) SW-846 method 8321 (EPA 1992), but incorporates different reverse-phase conditions and reagents for use in the analysis of TCLP acidic semivolatiles. No sample preparation is required beyond filtration (or pH adjustment, as needed); thus operator exposure to sample radioactivity and laboratory waste generation are minimized compared to methods based upon solvent extraction and gas chromatography. Compounds that can be determined using this method are found in Table 1.

Table 1. Compounds Determined by HPLC

<u>Compound Name</u>	<u>Cas No.*</u>
o-Cresol	95-48-7
m-Cresol	108-39-4
p-Cresol	106-44-5
2,4-Dichlorophenoxyacetic acid (2,4-D)	94-75-7
Pentachlorophenol (PCP)	87-86-5
2,4,5-Trichlorophenol (2,4,5-TCP)	95-95-4
2,4,6-Trichlorophenol (2,4,6-TCP)	88-06-2
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	93-76-5
2-(2,4,5-Trichlorophenoxy)propionic acid (Silvex or 2,4,5-TP)	93-72-1

* Chemical Abstracts Service registry number.

(a) This method was supplied by R. L. Schenley, J. E. Caton, W. H. Griest, and C-h. Ho (Oak Ridge National Laboratory, Analytical Chemistry Division, Oak Ridge, Tennessee).

- 1.2 This method may be applicable to the determination of other semivolatile or nonvolatile organic compounds that are present in aqueous samples or aqueous extracts of solid samples and that possess a chromophore sufficient for absorbance detection.
- 1.3 This method is restricted to use by or under the supervision of analysts experienced in HPLC.

2.0 Summary of Method

This method provides conditions for the determination of TCLP acidic semivolatile compounds using a mobile-phase composition gradient and for the determination of only the herbicides using an isocratic mobile-phase version. Aqueous liquid wastes and TCLP leachates are directly injected into a reverse-phase HPLC column and are eluted and detected using ultraviolet (UV) absorbance. Sample filtration and/or pH adjustment may be required before injection. Components are identified by their retention time on the primary column and are confirmed by either their ultraviolet-visible spectrum or their retention time on the secondary column. The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which is not a TCLP toxicity characteristic compound, may be used as a surrogate standard.

3.0 Interferences

- 3.1 Interferences may be encountered from other UV-absorbing compounds that co-elute with the target analytes from the reverse-phase column.
- 3.2 Highly alkaline samples may degrade some of the target analytes or may interfere with their chromatographic separation.
- 3.3 Mass spectroscopy may be used as an option for more selective detection or structural confirmation.
- 3.4 Organic sample matrices are not appropriate for this procedure and may require other HPLC conditions.

4.0 Safety

When handling radioactive samples, all applicable radiochemical handling procedures and health physics monitoring practices should be followed.

5.0 Apparatus and Methods

5.1 High Performance Liquid Chromatography System

5.1.1 **HPLC:** Binary or ternary gradient HPLC system with UV absorbance detector (280 nm), injector with at least a 25- μ L volume, mobile-phase degassing system, and chromatography data system. A Hewlett-Packard model 1090 with diode array UV absorbance detector, automatic sample injector, and ChemStation data acquisition and analysis software system (*HPLC ChemStation*, 1st Edition, Hewlett-Packard, Federal Republic of Germany, November 1987) were used in method development and performance evaluation.

5.1.2 Columns

- Primary column: Poly(styrene-divinylbenzene) HPLC column, 150 mm long x 4.1-mm ID, 5- μ m particles, 75 Å pores (Hamilton PRP-1 or equivalent) with matching guard column.
- Confirmatory columns:
 - C-8 bonded silica HPLC column, 150 mm long x 4.6-mm ID, 5- μ m particles (Econosphere C-8 or equivalent with 8% or greater carbon load) with matching guard column.
 - C-18 bonded silica column, 150 mm long x 2.1-mm ID, 5- μ m particles (ZORBAX RX-C-18 or equivalent) with matching guard column.

5.1.3 Other Equipment

- Syringe: 1-mL gas tight (Hamilton 1001-LT or equivalent).
- Filter: Nylon filter, 13-mm OD, 0.22 μ m (Gelman or equivalent).

6.0 Reagents

- Water, HPLC grade
- Methanol, HPLC grade
- Acetonitrile, HPLC grade

- Glacial acetic acid, American Chemical Society (ACS) grade
- Sulfuric acid, ACS grade

7.0 Sample Collection, Preservation, and Handling

Sample collection, preservation, and handling should be addressed in the planning process.

8.0 Procedure

8.1 High Performance Liquid Chromatography Conditions

8.1.1 Primary Column 1

- TCLP acidic semivolatile compounds

Mobile phase: (see Table 2)
 Flow rate: 1 mL/min
 Injection volume: 25 μ L
 Temperature: room temperature (ca 21°C)
 Monitored wavelengths: 280 and/or 235 nm
 Reference wavelength: 450 nm
 Equilibration between runs: 9 min

Table 2. Mobile Phase Gradient for TCLP Acidic Semivolatile Compounds on Column 1

Step or Run Time (min)	Volume %	
	0.1 M Acetic Acid in 10/90 (v/v)	
	Acetonitrile/Water	Acetonitrile
Initial	62	38
4.5	62	38
10.5	3.0	97
13.0	3.0	97
14.0	62	38
16.0	62	38

- TCLP herbicides only

Mobile phase: isocratic 55 vol% of 0.0 M acetic acid in 10/90 (v/v) acetonitrile/water and 45% acetonitrile

Flow rate: 1 mL/min

Injection volume: 25 μ L

Temperature: room temperature (ca. 21°C)

Monitored wavelengths: 280 and/or 235 nm

Reference wavelength: 450 nm

8.1.2 Confirmatory Columns

- C-8 confirmatory column

Mobile phase: see Table 3

Flow rate: 1 mL/min

Injection volume: 25 μ L

Temperature: room temperature (ca. 21°C)

Monitored wavelengths: 280 and/or 235 nm

Reference wavelength: 450 nm

Equilibration between runs: 10 min

Table 3. Mobile Phase Gradient for TCLP Acidic Semivolatile Compounds on C-8 Confirmatory Column

Step or Run Time, (min)	Volume %		
	0.1 M Acetic Acid in 10/90 (v/v) Acetonitrile/Water	0.1 M Acetic Acid in Methanol	Acetonitrile
Initial	75	15	10
1.0	75	15	10
3.0	55	35	10
9.0	55	35	30
10.0	35	55	10
15.0	0	10	90
20	0	10	90
21	75	15	10
24	75	15	10

- C-18 confirmatory column

Mobile phase:	see Table 4
Flow rate:	0.57 mL/min
Injection volume:	2.5 μ L
Temperature:	40°C
Monitored wavelengths:	235 and/or 280 nm
Reference wavelength:	450 nm
Equilibration between runs:	6 min

Table 4. Mobile Phase Gradient for TCLP Acidic Semivolatile Organic Compounds on C-18 Confirmatory Column

Step or Run Time, (Min)	Volume %	
	Acetonitrile	Water
Initial	6	94
2.0	6	94
7.0	20	80
10.0	20	80
12.0	6	94
14.0	6	94

8.2 Calibration. Standard solutions of each analyte should be prepared in methanol over the range of concentrations expected in the sample, at least one of which must be at or below the TCLP Regulatory Limit. At least a 5-point calibration curve is recommended. Twenty-five microliter volumes of each standard should be injected. External standardization per SW-846 method 8000 should be performed.

8.3 Chromatographic Analysis

8.3.1 The samples should be prepared for analysis by acidifying alkaline samples to a pH of < 3 using concentrated sulfuric acid and filtering out particles using the syringe and nylon filter assembly. Acidic samples or leachates should be adjusted to pH 12 with NaOH, allowed to stand for 1 h, and then adjusted to pH 3 before filtration and analysis (EPA Method 515.1, Rev. 4). A known mass of 2,4,5-T (final concentration of 5.0 to 7.5 μ g/mL) also may be added to the sample to serve as a surrogate standard. Other compounds may be used as a surrogate standard if their performance with respect to the analytes is documented.

8.3.2 Twenty-five microliters of the sample should be injected into the primary column, and analyte concentrations should be calculated using the method of

external standards. Where TCLP acidic semivolatile compounds are tentatively identified by retention time on the primary column, their identifications are confirmed using either their UV-absorbance spectra or their retention times on either of the confirmatory columns.

9.0 Quality Control

The use of this method should be supported by appropriate quality control (QC) procedures (e.g., as outlined in Chapter 3).

10.0 Method Performance

- 10.1** This method complies with requirements in Appendix A for a verified method except that no real world samples are used; surrogate samples are used in spiking experiments.
- 10.2 Chromatographic Performance and Spectra of Analytes.** Table 5 lists the chromatographic performance of the primary column. The PRP-1 column was chosen as the primary column because of its good efficiency, resolution of all analytes (except for m+p-cresols, which is permitted by the TCLP), and resistance to hydrolysis. The polystyrene packing is stable over the pH range of 1 to 13, versus the pH range of 2 to 8 for silica-based packings. A chromatogram of the analytes on the primary column is shown in Figure 1. Table 6 gives the retention times of the analytes on the confirmatory columns. Two columns are listed: a conventional C-8 column, and a 2.1-mm ID C-18 column. The latter reverses the relative retention times of the analytes and also reduces solvent consumption relative to the primary column and the C-8 confirmatory column. Figure 2 shows the separation of the TCLP acidic semivolatiles with the C-18 column. The UV absorbance spectrum of each analyte is included as Figure 3 (at the end of the method).
- 10.3 Detection Limits, Quantitation Limits, and Linear Range.** Table 7 lists the instrument detection and quantitation limits calculated according to Hubaux and Vos (1970). The TCLP regulatory limits for each analyte can be met by using the appropriate detector wavelength. The linear ranges are only approximate, and probably extend above the upper limits listed. Two wavelengths can be used with this procedure (280 and/or 235). The 280-nm wavelength is preferred because of its generally superior sensitivity and the lower interference from solvent and sample impurities.

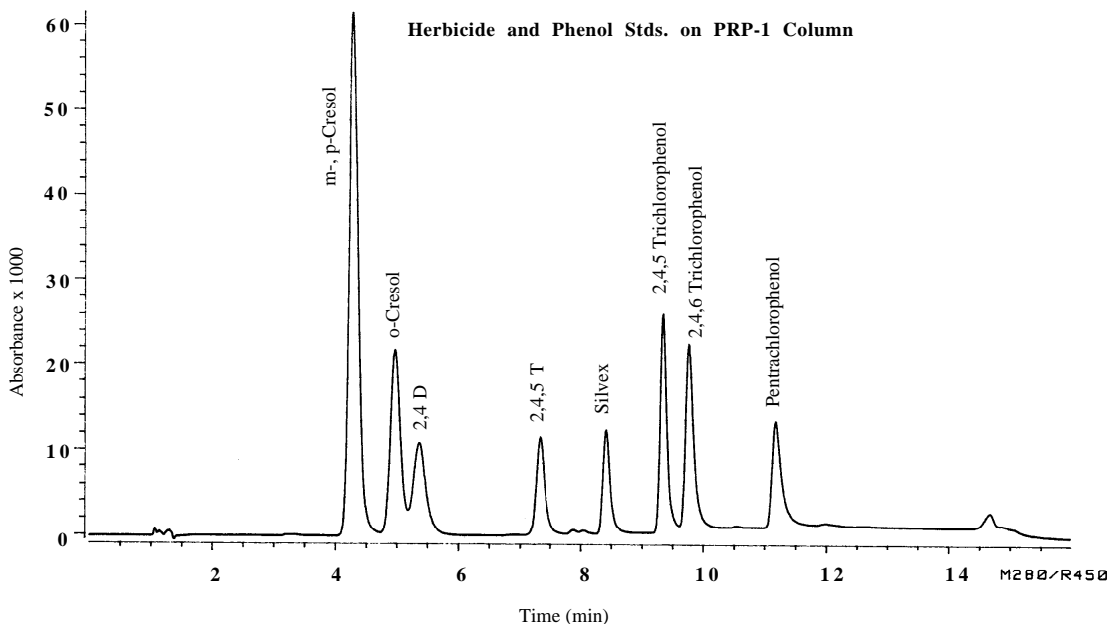


Figure 1. Chromatogram of TCLP Acidic Semivolatiles on Primary Column 1

Table 5. Chromatographic Performance of the Primary Column 1

<u>Compound</u>	<u>RT</u> ^(a) <u>(Min.)</u>	<u>Sym</u> ^(b)	<u>Width</u> ^(c) <u>(Min.)</u>	<u>K</u> ^(d)	<u>N</u> ^(e)
p-Cresol	4.235	0.84	0.152	2.682	3105
m-Cresol	4.271	0.85	0.155	2.713	3037
o-Cresol	4.926	0.82	0.182	3.283	2930
2,4-D	5.375	0.75	0.207	3.673	2697
2,4,5-T	7.334	0.75	0.142	5.377	10670
Silvex	8.4111	0.71	0.119	6.313	19983
2,4,5-TCP	9.333	0.66	0.115	7.115	26346
2,4,6-TCP	9.753	0.60	0.136	7.480	20571
PCP	11.175	0.53	0.162	8.717	19034

(a) Retention time

(b) Peak symmetry = a complex function reflecting peak area before apex divided by peak area after apex^(f)

(c) Peak width = $0.3T + 0.7 A/H$, where T = time between peak inflection points; A = peak area; H = peak height^(f)

(d) Capacity factor

(e) Number of theoretical plates

(f) *HPLC Chemstation*, 1st Edition, Hewlett-Packard, Federal Republic of Germany, November 1987

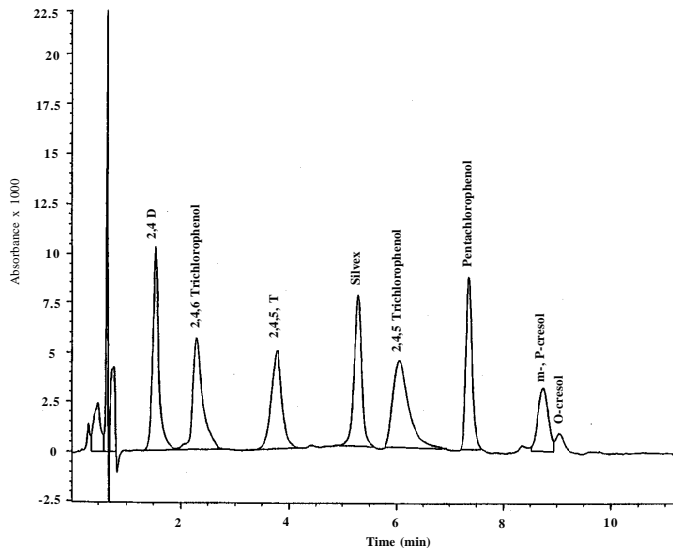


Figure 2 Chromatogram of TCLP Acidic Semivolatile Organic Compounds on C-18 Confirmatory Column

Table 6. Analyte Retention Times on the Confirmatory Columns

<u>Compound</u>	<u>Retention Time (min)</u>	
	<u>C-8</u>	<u>C-18</u>
o-Cresol	—	8.995
m-Cresol	4.659	—
m + p Cresols	—	8.756
o + p-Cresols	4.676	—
2,4,-D	6.354	1.551
2,4,5-T	7.991	3.785
Silvex	8.961	5.295
2,4,5-TCP	10.021	6.060
2,4,6-TCP	10.321	2.300
PCP	13.542	7.366

Table 7. Detection Limits, Quantitation Limits, and Approximate Linear Range for TCLP Acidic Semivolatile Organics by HPLC-UV Detection on Column 1^(a)

Compound	Detection Limit $\mu\text{g/mL}$		Quantitation Limit $\mu\text{g/mL}$		Approximate Linear Range $\mu\text{g/mL}$
	235 nm	280 nm	235 nm	280 nm	
o-Cresol	4.6	2.6	8.9	5.2	2 - 100
m-Cresol	2.2	0.9	4.3	1.8	1 - 100
p-Cresol	2.5	2.1	5.0	4.2	2 - 100
m + p-Cresols	3.6	5.7	6.9	11.2	4 - 100
2,4-D	0.59	0.93	1.2	1.8	0.5 - 150
2,4,5-T	1.8	1.3	3.6	2.7	1 - 175
Silvex	0.97	0.81	1.9	1.6	1 - 250
2,4,5-Trichlorophenol	1.2	2.2	2.4	4.4	1 - 125
2,4,6-Trichlorophenol	0.74	0.46	1.5	0.91	0.5 - 125
Pentachlorophenol	7.3	3.7	14.3	7.4	4 - 350

(a) Detection limit and quantitation limit determined according to Hubaux and Vos (1970).

10.4 Analyte Recoveries. Two standard matrices (TCLP leaching fluid, nitrate liquid) were spiked with known concentrations of the analytes. The spikes were prepared at a concentration at or below the TCLP regulatory limit (“low spike”), and a concentration that was generally a factor of four higher than the regulatory limit (“high spike”). The measurement of each spiked sample was the average of triplicate injections on each of three days. The determined concentrations were compared with the prepared values. Table 8 shows the results for TCLP leaching fluid #2 (SW-846 Method 1311) (EPA 1992). A graphical representation of the recoveries is included in Figure 4 (at the end of the method). The recoveries were 87% or better for this sample matrix. Corresponding results for analysis in nitrate liquid (5 M NaNO_3) are shown in Table 9. The latter solution emulates the conditions found in many nuclear waste tanks. This matrix required acidification before analysis by HPLC. It is not surprising that spike recoveries are much lower for this hostile matrix than from the TCLP leaching fluid, and the results suggest that many of the TCLP acidic semivolatile compounds will not survive in some of the waste matrices. A small peak that disappeared during a relatively short period of storage time (less than 24 h) was observed in early range-finding analyses for pentachlorophenol.

Table 8. Average Recoveries of TCLP Acidic Semivolatile Organic Compounds and Their 95% Confidence Intervals from TCLP Leaching Fluid

Compound (Wavelength)	Low Spike		High Spike	
	Conc. ($\mu\text{g/mL}$)	Recy. ^(a) (%)	Conc. ($\mu\text{g/mL}$)	Recy. ^(a) (%)
m+p-Cresols (280)	10	102.0 \pm 1.0	40	111.0 \pm 2.2
o-Cresols (280)	5	88.6 \pm 6.2	20	88.9 \pm 8.7
2,4-D (235)	5	91.1 \pm 7.7	20	106.8 \pm 3.2
2,4,5-T (280)	5	89.4 \pm 1.7	20	100.9 \pm 2.0
Silvex (280)	1	97.5 \pm 11.4	20	117.0 \pm 4.0
2,4,5-Trichlorophenol (280)	5	96.2 \pm 6.5	20	95.6 \pm 3.5
2,4,6-Trichlorophenol (280)	2	128.2 \pm 11.2	20	101.3 \pm 4.0
Pentachlorophenol (280)	10	96.0 \pm 8.9	40	103.9 \pm 4.0

(a) Average statistics and confidence intervals are based on three replicates (days 1, 2, and 3).

10.5 Surrogate Standard Recoveries. The isocratic mobile-phase method has been used extensively for the determination of 2,4-D and silvex in TCLP leachates of wastes. A surrogate standard, 2,4,5-T, was spiked at concentrations of 5.0 to 7.5 $\mu\text{g/mL}$. The 95% confidence interval for the average recovery for 61 samples analyzed over the course of 12 months was $96.7 \pm 3.8\%$. The standard deviation for an individual measurement was 14.8%.

Table 9. Average Recoveries of TCLP Acidic Semivolatile Organic Compounds and Their 95% Confidence Limits from Surrogate Nuclear Waste Tank Liquid (5 M NaNO_3)

Compound (Wavelength)	Low Spike		High Spike	
	Conc. ($\mu\text{g/mL}$)	Recy. ^(a) (%)	Conc. ($\mu\text{g/mL}$)	Recy. ^(a) (%)
m + p-Cresols (280)	10	91.2 \pm 2.2	40	101.9 \pm 0.7
o-Cresol (280)	5	77.4 \pm 4.7	20	86.5 \pm 5.5
2,4-D (235)	5	58.1 \pm 2.5	20	66.5 \pm 13.7
2,4-D (280)	5	41.5 \pm 5.2	20	90.4 \pm 5.0
2,4,5-T (280)	5	ND	20	63.6 \pm 2.7
Silvex (280)	1	ND	20	33.4 \pm 0.5
2,4,5-Trichlorophenol (280)	5	ND	20	28.7 \pm 1.0
2,4,6-Trichlorophenol (280)	2	ND	20	66.0 \pm 3.7
Pentachlorophenol (280)	10	ND	40	ND

(a) Average statistics and confidence intervals are based on three replicates (days 1, 2, and 3).
ND = not detected.

10.6 Sources of Variation. Table 10 lists the standard deviations due to different experimental factors (i.e., sample matrices, spikes within the matrices, and replicates within spikes). Figure 5 (at the end of the method) is a graphical representation of the sources of experimental variation. It is clear that the instrumental method represented by the variation due to replicates within spikes is very reproducible, with standard deviations all less than 2.6%. Matrix effects are the main source of variation, and when hostile matrices such as concentrated sodium nitrate are encountered, variation can be large between different matrices.

10.7 Blanks. No peaks that could be quantitated were observed in reagent blanks of either sample matrix.

11.0 References

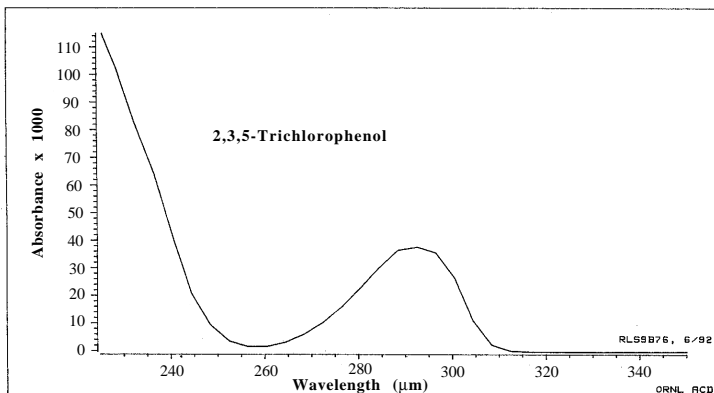
Hubaux, A., and G. Vos. 1970. "Decision and Detection Limits for Linear Calibration Curves." *Anal. Chem.*, 42:849-855.

U. S. Environmental Protection Agency (EPA). 1992. *Test Method for Evaluating Solid Waste. Physical/Chemical Methods*, SW-846. 3rd Edition, Final Update I. Office of Solid Waste and Emergency Response, Washington, DC. Available from the National Technical Information Service, Springfield, Virginia.

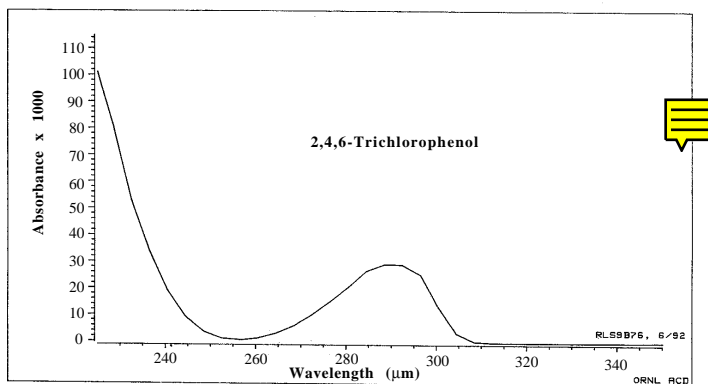
Table 10. Standard Deviations of Percent Recovery from Experimental Sources

<u>Compound</u>	<u>Experimental Sources</u>		
	<u>Between Matrices (%)</u>	<u>Spikes Within Matrices (%)</u>	<u>Replicates Within Spikes (%)</u>
o-Cresol	3.5	4.3	2.6
m,p-Cresol	5.0	7.0	0.7
2,4-D	12.0	17.9	2.0
PCP	70.6	3.8	2.0
2,4,5-TCP	56.8	14.3	1.5
2,4,6-TCP	52.0	35.6	2.5
2,4,5-T	38.5	32.3	0.8
Silvex	62.5	19.3	2.4

(a)



(b)



(c)

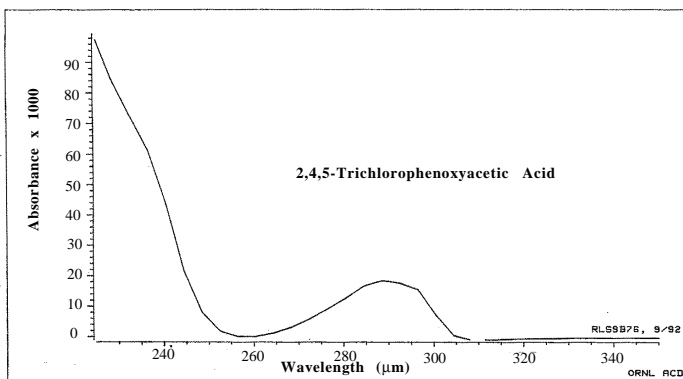
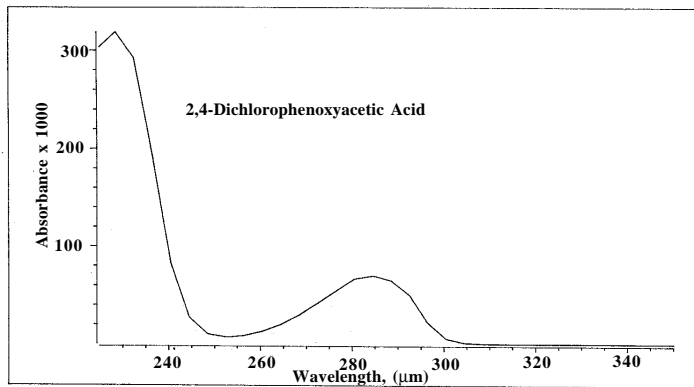
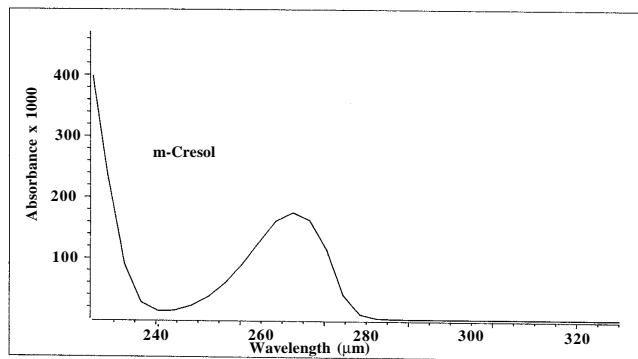


Figure 3. UV Absorbance Spectra of TCLP Acidic Semivolatile Compounds

(d)



(e)



(f)

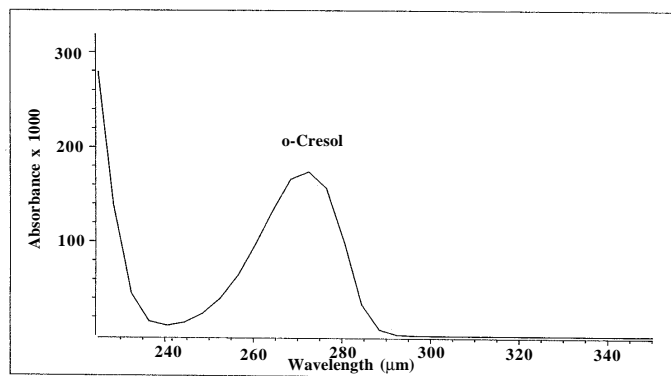
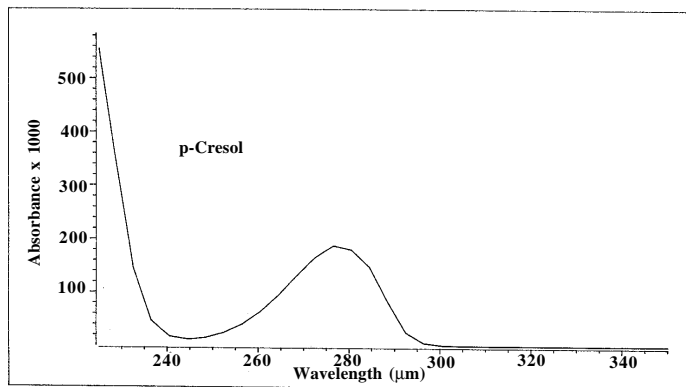
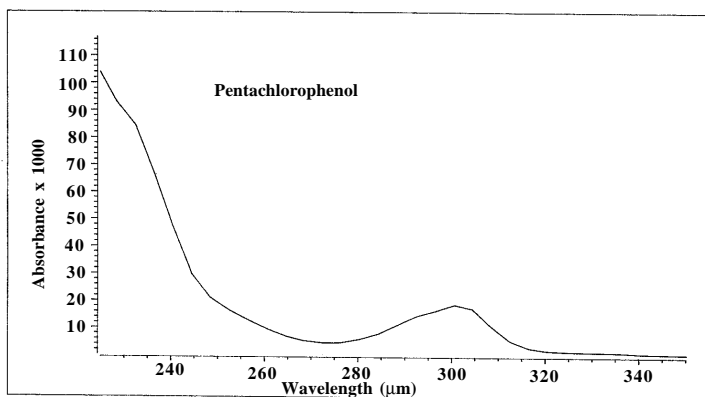


Figure 3. (Contd)

(g)



(h)



(i)

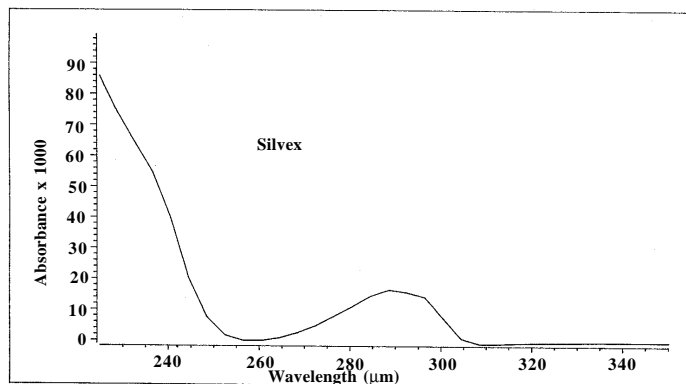


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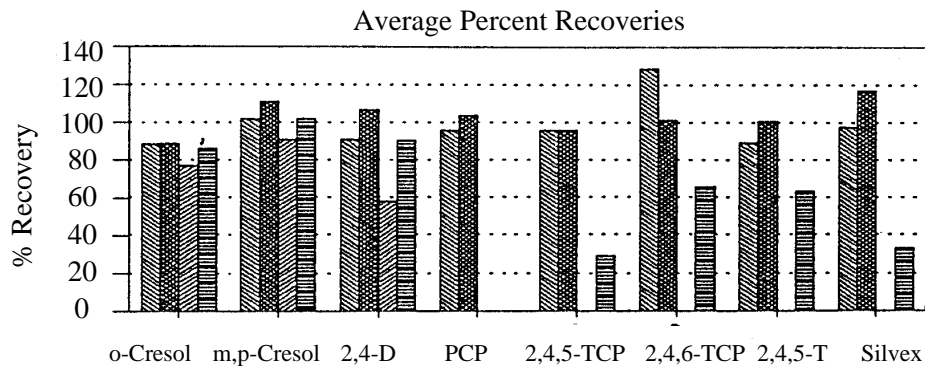


Figure 4. Average Percent Recoveries for Direct HPLC Analysis of TCLP Acidic Semivolatile Compounds

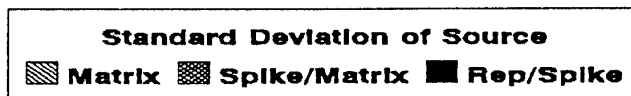
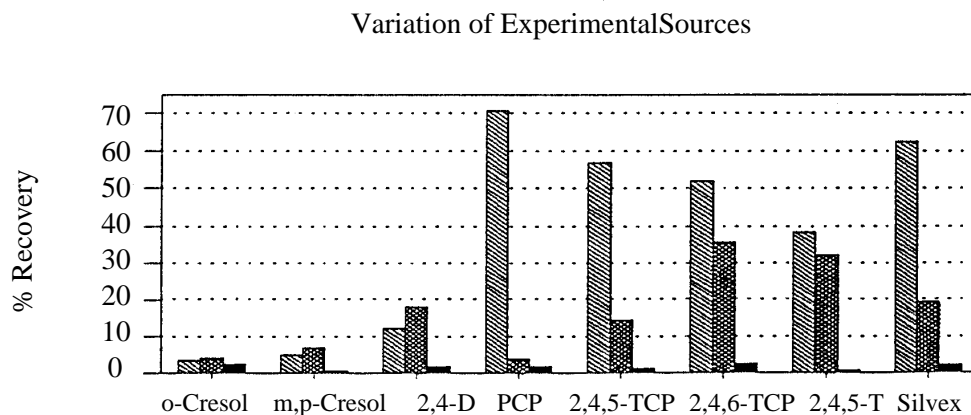


Figure 5. Standard Deviations of the Experimental Sources for Direct HPLC Analysis of TCLP Acidic Semivolatile Compounds