Method ~~3550~~ 3535
Solid Phase Extraction Disk Method (SPE)

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating organic compounds from aqueous samples. The method also describes concentration techniques suitable for preparing the extract for the appropriate determinative methods described in Section 4.3 of Chapter Four.

1.2 This method is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample, usually 1 liter, at a specified pH (see Table 1), is extracted with a C_{18} Solid Phase Extraction (SPE) disk and eluted with methylene chloride. The methylene chloride is dried, concentrated (if necessary), and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method to be used (see Table 1 for appropriate exchange solvents).

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 Under basic extraction conditions required to separate analytes for the packed columns of Method 8250, the decomposition of some analytes has been demonstrated. Organochlorine pesticides may dechlorinate, phthalate esters may exchange, and phenols may react to form tannates. These reactions increase with increasing pH, and are decreased by the shorter reaction times available in Method 3510. Methods 3520/8270, 3510/8270, and 3510/8250, respectively, are preferred over Method 3520/8250 for the analysis of these classes of compounds.

4.0 APPARATUS AND MATERIALS

4.1 Solid phase extraction system. Empore™, 3-90 mm extraction glassware sets, 6 - station manifold (J. T. Baker, Fisher Scientific, Varian Associates, or equivalent). Automatic or robotic commercially available sample preparation systems designed for disks may be utilized in this method if all quality control requirements are met.

4.1.1 Standard Filter Apparatus, ALL GLASS, 90 mm. These should be used to carry out disk extractions when no manifold or automatic system is available.

4.2 Drying column - 20 mm ID Pyrex chromatographic column with Pyrex glass wool at bottom and a Teflon stopcock.

NOTE: Fritted glass discs are difficult to decontaminate after highly contaminated extracts have been passed through. Columns without frits may be purchased. Use a small pad of Pyrex glass wool to retain the adsorbent. Prewash the glass wool pad with 50 mL of acetone followed by 50 mL of elution solvent prior to packing the column with adsorbent.

4.3 Kuderna-Danish (K-D) apparatus.

4.3.1 Concentrator tube - 10 mL, graduated (Kontes K-570050-1025 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

4.3.2 Evaporation flask - 500 mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.3.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent).

4.3.4 Snyder column - Two ball micro (Kontes K-569001-0219 or equivalent).

4.3.5 Springs - 1/2 inch (Kontes K-662750 or equivalent).

4.4 Boiling chips - solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent).

4.5 Water bath - Heated, with concentric ring cover, capable of temperature control ±5°C). The bath should be used in a hood.

4.6 Vials, glass

4.6.1 Two (2) mL with TFE-fluorocarbon lined screw cap or crimp tops.

4.6.2 Fifty (50) mL with TFE-fluorocarbon lined screw cap for solvent collection when using Empore™ manifold for extractions. When using standard filtration glassware, the collection tube should be of appropriate I.D. and length for the funnel tip of the glassware base to be positioned well into the neck of the tube to prevent splattering.

4.7 pH indicator paper - pH range including the desired extraction pH.

4.8 Vacuum system capable of maintaining a vacuum of approximately 66 cm (26 inches) of mercury for use with disks.

4.9 Syringe - 5 mL

4.10 Graduated cylinder - 1 liter.

4.11 Solid Phase Extraction Disks, Empore™ (J. T. Baker, Varian Associates, Fisher Scientific, or equivalent), 90 mm, C_{18}.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Sodium hydroxide solution (10N), NaOH. Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL.

5.4 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride. If the sodium sulfate is precleaned with methylene chloride, a method blank must be analyzed, demonstrating that there is no interference from the sodium sulfate.

5.5 Sulfuric acid solution (1:1 v/v), H_2SO_4. Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of organic-free reagent water.

5.6 Extraction/exchange solvents

5.6.1 Methylene chloride, CH_2Cl_2 - Pesticide quality or equivalent.

5.6.2 Hexane, C_6H_{14} - Pesticide quality or equivalent.

5.6.3 2-Propanol, $CH_3CH(OH)CH_3$ - Pesticide quality or equivalent.

5.6.4 Cyclohexane, C_6H_{12} - Pesticide quality or equivalent.

5.6.5 Acetonitrile, CH_3CN - Pesticide quality or equivalent.

5.6.6 Methanol, CH_3OH - Pesticide quality or equivalent.

5.6.7 Acetone, $(CH_3)_2CO$ - Pesticide quality or equivalent.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Section 4.1.

7.0 PROCEDURE

7.1 Using a 1 liter graduated cylinder, measure 1 liter (nominal) of sample. If high concentrations are anticipated, a smaller volume may be used. Add 5.0 mL methanol and 1.0 mL of the appropriate surrogate standards to all samples, spikes, and blanks (see Method 3500 and the determinative method to be used, for details on the surrogate standard solution and the matrix spike solution). For the sample in each analytical batch selected for spiking, add 1.0 mL of the matrix spiking standard. For base/neutral-acid analysis, the amount added of the surrogates and matrix spiking compounds should result in a final concentration of 100 ng/uL of each base/neutral analyte and 200 ng/uL of each acid analyte in the extract to be analyzed (assuming a 1 uL injection). If Method 3640, Gel-Permeation Cleanup, is to be used, add twice the volume of surrogates and matrix spiking compounds since half the extract is lost due to loading of the GPC column.

7.2 Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH to that indicated in Table 1 for the specific determinative method that will be used to analyze the extract.

7.3 Assemble an all glass filtration assembly (Figure 1) using a 90 mm C_{18} Empore™ disk. Use of a manifold for multiple extractions is acceptable.

7.3.1 If samples contain significant quantities of particulates, the use of an in-situ glass micro-fiber prefilter (Whatman GMF 150, 1 micron pore size or equivalent) and/or an inert depth filter such as Empore™ Filter Aid 400 is advisable. The glass fiber prefilter is placed on top of the Empore™ disk prior to placement of the glass reservoir and clamp. When using Filter Aid 400, assemble the glassware with the disk

(and glass fiber prefilter) and clamp in place. Pour about 40 grams of the Filter Aid onto the surface of the stacked filters.

7.4 Wash the extraction apparatus and disk by adding 20 mL of methylene chloride (MeCl2) to the reservoir washing down the sides of the glass reservoir in the process. Pull a small amount through the disk with a vacuum; turn off the vacuum and allow the disk to soak for about one minute. Pull the remaining solvent through the disk and allow the disk to dry. Repeat the wash step using 10 mL acetone.

7.4.1 When using Filter Aid, adjust the volume of all solvents so the volumes used are sufficient to submerge the bed. In subsequent steps volumes should be adjusted so the liquid level is maintained above the depth filter bed for ease of observation.

7.5 Pre-wet (condition) the disk by adding 20 mL methanol (MeOH) to the reservoir, pulling a small amount through the disk then letting it soak for about one minute. Pull most of the remaining methanol through the disk, leaving 3-5 mm of methanol on the surface of the disk, which should not be allowed to go dry from this point until the sample extraction has been completed. THIS IS A CRITICAL STEP FOR A UNIFORM FLOW AND GOOD RECOVERY. The disk is composed of hydrophobic materials. To make them amenable to a water solution, they must be pre-wet with a water miscible solvent (MeOH) or they will not allow water to pass through the materials. Should the material accidentally dry during the conditioning step, simply repeat the pre-wetting step prior to adding the sample.

7.6 Rinse the disk by adding 20 mL of reagent water to the disk and drawing most through, again leaving 3-5 mm of water on the surface of the disk.

7.7 Add the water sample to the reservoir and, under full vacuum, filter as quickly as the vacuum will allow. Drain as much water from sample bottle as possible. Particulate-free water may pass through the disk in as little as 10 minutes without reducing analyte recoveries. Allow the entire sample to pass through the disk then dry the disk by maintaining vacuum for about 3 minutes.

NOTE: With heavily particle-laden samples, allow the sediment to settle; decant as much liquid as is practical into the reservoir. Allow most of the liquid to filter then swirl the sediment portion and add it to the reservoir. Before the entire sample has filtered, rinse the sample bottle with reagent water and add to the reservoir to transfer any particulates remaining in the bottle. Drain as much water as possible from the sample bottle.

7.8 Remove the entire filter assembly (do not disassemble) and insert suitable sample tube for eluent collection. The sample tube should have sufficient capacity to contain the elution solvent volume and should fit around the drip tip of the extraction glassware base unit. The tube should be seated so that the drip tip is below the neck of the sample tube to prevent splattering (and subsequent analyte loss) when the vacuum is applied. If using a filter flask, empty the water filtrate before inserting the collection tube.

7.9 Add 5.0 mL acetone to the disk. Allow the acetone to spread out evenly across the disk then quickly turn the vacuum on and off to pull a fraction of a milliliter through the disk. Allow the disk to soak for 15 to 20 seconds. This water-miscible solvent penetrates the water-filled pores of the sorbent and improves the recovery of compounds adsorbed to intrastitial surfaces.

7.10 Add 15 mL of methylene chloride to the sample bottle. Rinse the bottle thoroughly and, with the acetone still on the disk, transfer the solvent to the disk with a dispo-pipette, rinsing down the sides of filtration reservoir in the process. Draw about half of the solvent through disk then release the vacuum. Allow the remaining methylene chloride to soak the disk for about one minute then draw remainder through under vacuum.

7.11 Repeat the bottle rinse and elution in step 7.10 with a second 15 mL aliquot of methylene chloride.

7.12 Dry the combined elutes by pouring through a drying column (Sect. 4.2) containing anhydrous sodium sulfate. Rinse the collection tube and sodium sulfate with 20 mL methylene chloride and place combined solvent into a 500 mL KD flask fitted with a 10 mL receiving tube. Add silicon carbide boiling chips and place a 3 ball Snyder column on the KD flask.

7.13 Perform the concentration (if necessary) using the Kuderna-Danish (K-D) Technique (Sections 7.14.1 through 7.14.4).

7.14 K-D Technique

7.14.1 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporation flask. Dry the extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a K-D concentrator. Rinse the elution vial, which contained the solvent extract, with 20 mL of methylene chloride and add it to the column to complete the quantitative transfer.

7.14.2 Add one or two clean boiling chips to the flask and attach a three ball Snyder column. Prewet the Snyder column by adding about 1 mL or methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (15-20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10-20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.14.3 If a solvent exchange is required (as indicated in Table 1), momentarily remove the Snyder column, add 50 mL of the exchange solvent, a new boiling chip, and reattach the Snyder column. Concentrate the extract, as described in Section 7.11, raising the temperature of the water bath, if necessary, to maintain proper distillation.

7.14.4 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1-2 mL of methylene chloride or exchange solvent. If sulfur crystals are a problem, proceed to Method 3660 for cleanup. The extract may be further concentrated by using the technique outlined in Section 7.15 or adjusted to 10.0 mL with the solvent last used.

7.15 If further concentration is indicated in Table 1, either the micro-Snyder column technique (7.15.1) or nitrogen blowdown technique (7.15.2) is used to adjust the extract to the final volume required.

7.15.1 Micro-Snyder Column Technique

7.15.1.1 If further concentration is indicated in Table 1, add another clean boiling chip to the concentrator tube and attach a two ball micro-Snyder column. Prewet the column by adding 0.5 mL of methylene chloride or exchange solvent to the top of the column. Place the K-D apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 5-10 minutes. At the proper rate

of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 0.2 mL of extraction solvent. Adjust the final volume to 1.0-2.0 mL, as indicated in Table 1, with solvent.

7.15.2 Nitrogen Blowdown Technique

7.15.2.1 Place the concentrator tube in a warm bath (30°C) and evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: New plastic tubing must not be used between the carbon trap and the sample, since it may introduce interferences.

7.15.2.2 The internal wall of the tube must be rinsed down several times with methylene chloride or appropriate solvent during the operation. During evaporation, the tube solvent level must be positioned to avoid water condensation. Under normal procedures, the extract must not be allowed to become dry.

CAUTION: When the volume of solvent is reduced below 1 ml, semivolatile analytes may be lost.

7.16 The extract may now be analyzed for the target analytes using the appropriate determinative technique(s) (see Section 4.3 of Chapter 4). If analysis of the extract will not be performed immediately, stopper the concentrator tube and store refrigerated. If the extract will be stored longer than 2 days, it should be transferred to a vial with a Teflon lined screw-cap or crimp top, and labeled appropriately.

8.0 QUALITY CONTROL

8.1 Any reagent blanks or matrix spike samples should be subjected to exactly the same analytical procedures as those used on actual samples.

8.2 Refer to Chapter One for specific quality control procedures and Method 3500 for extraction and sample preparation procedures.

9.0 METHOD PERFORMANCE

9.1 Refer to the determinative methods for performance data.

10.0 REFERENCES

1. U.S. EPA 40 CFR Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," October 26, 1984.

p,p' - DDT

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	86.6	4.4	0.6	2.4
GW high	105.4	2.7	---	---
*TCLP low	95.1	3.5	0.52	2.1
TCLP high	90.9	22.1	---	---
*WW low	105	4.3	0.71	2.8
WW high	111	4.7	---	---

Aldrin

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	37.3	23.7	1.4	5.6
GW high	93.5	5.5	---	---
*TCLP low	51.0	16.8	1.3	5.4
TCLP high	82.3	22.2	---	---
*WW low	79.3	6.7	0.83	3.3
WW high	94.0	3.4	---	---

alpha Chlordane

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	75.4	12.8	1.5	6.1
GW high	112.3	2.7	---	---
*TCLP low	88.3	4.4	0.61	2.5
TCLP high	90.2	26.2	---	---
*WW low	78.9	4.7	0.58	2.3
WW high	89.5	2.4	---	---

beta BHC

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	89.2	6.5	0.91	3.7
GW high	107.8	2.5	---	---
*TCLP low	99.6	5.3	0.82	3.3
TCLP high	101.1	6.7	---	---
*WW low	79.7	1.6	0.20	0.78
WW high	82.3	4.2	---	---

* GW = Ground Water
TCLP = TCLP Leachate
WW = Waste Water

delta BHC

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	106.2	5.6	0.93	3.7
GW high	86.0	2.4	---	---
*TCLP low	116.0	4.6	0.84	3.4
TCLP high	105.9	10.2	---	---
*WW low	88.9	2.5	0.35	1.4
WW high	83.4	4.2	---	---

Dieldrin

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	83.4	7.1	0.9	3.8
GW high	96.1	2.3	---	---
*TCLP low	93.5	4.4	0.64	2.6
TCLP high	83.5	20.1	---	---
*WW low	81.2	3.8	0.49	2.0
WW high	93.3	3.6	---	---

Endosulfan I

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	79.6	10.6	1.3	5.3
GW high	99.1	2.3	---	---
*TCLP low	91.3	4.2	0.60	2.4
TCLP high	83.6	22.0	---	---
*WW low	79.6	4.1	0.51	2.0
WW high	87.9	3.8	---	---

Endosulfan II

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	94.5	5.8	0.9	3.4
GW high	101.6	2.8	---	---
*TCLP low	102.9	3.6	0.58	2.3
TCLP high	82.2	25.7	---	---
*WW low	82.7	4.2	0.54	2.2
WW high	93.5	4.1	---	---

Endrin

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	88.3	6.2	1.7	6.9
GW high	98.4	2.3	---	---
*TCLP low	96.8	4.5	1.4	5.5
TCLP high	81.4	22.8	---	---
*WW low	85.1	3.1	0.82	3.3
WW high	89.6	2.9	---	---

Endrin Aldehyde

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	87.5	6.0	0.8	3.3
GW high	99.9	4.0	---	---
*TCLP low	92.1	8.1	1.2	4.7
TCLP high	88.7	16.8	---	---
*WW low	69.0	3.3	0.36	1.4
WW high	80.2	5.9	---	---

gamma Chlordane

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	70.7	15.8	1.8	7.0
GW high	98.9	2.7	---	---
*TCLP low	84.3	4.0	0.53	2.1
TCLP high	84.7	21.7	---	---
*WW low	79.9	4.6	0.58	2.3
WW high	93.9	2.9	---	---

Heptachlor

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	43.1	19.2	1.3	5.2
GW high	95.4	3.9	---	---
*TCLP low	60.5	8.8	0.83	3.3
TCLP high	81.4	22.0	---	---
*WW low	71.8	5.0	0.56	2.3
WW high	78.6	2.8	---	---

Heptachlor Epoxide

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	76.4	12.1	1.5	5.8
GW high	97.6	2.4	---	---
*TCLP low	89.9	3.8	0.54	2.2
TCLP high	83.4	21.4	---	---
*WW low	75.3	2.9	0.34	1.4
WW high	83.4	3.3	---	---

Lindane

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	81.3	11.1	1.4	5.7
GW high	115.2	3.2	---	---
*TCLP low	98.4	5.1	0.79	3.2
TCLP high	106.0	12.5	---	---
*WW low	82.1	2.4	0.32	1.3
WW high	85.3	3.1	---	---

p,p' - DDE

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	80.3	8.3	1.0	4.2
GW high	96.0	2.5	---	---
*TCLP low	90.1	5.3	0.75	3.0
TCLP high	81.3	22.4	---	---
*WW low	85.1	4.4	0.59	2.4
WW high	97.9	2.4	---	---

p,p' - TDE (DDD)

	Bias (%)	Precision (%)	MDL (ug/L)	RQL (ug/L)
*GW low	90.5	4.8	1.4	5.4
GW high	101.1	2.4	---	---
*TCLP low	97.8	4.4	1.4	5.4
TCLP high	84.9	22.9	---	---
*WW low	74.9	4.6	0.85	3.4
WW high	79.6	2.9	---	---