

METHOD 3535

SOLID-PHASE EXTRACTION (SPE)

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating target organic analytes from aqueous samples using solid-phase extraction media. The method describes conditions for extracting organochlorine pesticides and phthalate esters from aqueous matrices including groundwater, wastewater, and TCLP leachates using disk extraction media. Performance data for these extractions are provided in Method 8081 (organochlorine pesticides) and Method 8061 (phthalate esters). The technique may also be applicable to semivolatiles and other extractable compounds. Other solid-phase extraction media configurations, e.g., SPE cartridges, may be employed provided that the laboratory demonstrates adequate performance for the analytes of interest.

1.2 This method also provides procedures for concentrating extracts and for solvent exchange.

1.3 The method may be used for the extraction of additional target analytes or other solid-phase media if the analyst demonstrates adequate performance (e.g., recovery of 70 - 130%) using spiked sample matrices and an appropriate determinative method from Chapter Four (Sec. 4.3). Organic-free reagent water is not considered appropriate for conducting such performance studies. Specifically, many non-polar organic contaminants present in an aqueous sample are likely to be bound to particulate matter and extraction efficiencies are expected to be less than those determined from simply spiking organic-free reagent water.

1.4 Solid-phase extraction is called liquid-solid extraction (LSE) in EPA Drinking Water methods.

1.5 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample is adjusted to a specified pH (see Table 1) and then extracted using a Solid-phase Extraction (SPE) device.

2.2 Target analytes are eluted from the solid-phase media using methylene chloride or other appropriate solvent. The resulting solvent extract is dried using sodium sulfate and concentrated.

2.3 The concentrated extract may be exchanged into a solvent compatible with subsequent cleanup procedures (Chapter Four, Sec. 4.2) or determinative procedures (Chapter Four, Sec. 4.3) employed for the measurement of the target analytes.

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 The decomposition of some analytes has been demonstrated under basic extraction conditions. Organochlorine pesticides may dechlorinate and phthalate esters may hydrolyze. The rates of these reactions increase with increasing pH and reaction times.

3.3 Bonded phase silicas (e.g., C₁₈) will hydrolyze on prolonged exposure to aqueous samples with pH less than 2 or greater than 9. Hydrolysis will increase at the extremes of this pH range and with longer contact times. Hydrolysis may reduce extraction efficiency or cause baseline irregularities. Styrene divinylbenzene (SDB) extraction disks should be considered when hydrolysis is a problem.

3.4 Phthalates are a ubiquitous laboratory contaminant. All glass extraction apparatus should be used for this method because phthalates are used as release agents when molding rigid plastic (e.g., PVC). A method blank as described in Chapter One should be analyzed, demonstrating that there is no phthalate contamination of the sodium sulfate or other reagents specified in this method.

3.5 Sample particulates may clog the solid-phase media and result in extremely slow sample extractions. Use of an appropriate filter aid will result in shorter extractions without loss of method performance if clogging is a problem. Even when a filter aid is employed, this method may not be appropriate for aqueous samples with high levels of suspended solids (>1%), as the extraction efficiency may not be sufficient, given the small volumes of solvents employed and the short contact time.

4.0 APPARATUS AND MATERIALS

The apparatus and materials described here are based on data provided to EPA for disk-type solid-phase extraction materials. Other solid-phase extraction media configurations, e.g., SPE cartridges, may be employed provided that the laboratory demonstrates adequate performance for the analytes of interest. The use of other SPE configurations will require modifications to the procedure described in Sec. 7.0. Consult the manufacturer's instructions regarding such modifications.

4.1 Solid-phase extraction system - Empore™ manifold with 3-90 mm or 6-47 mm standard filter apparatus, or equivalent. Automatic or robotic sample preparation systems designed for solid-phase media may be utilized for this method if adequate performance is achieved and all quality control requirements are satisfied.

4.1.1 Manifold station - (Fisher Scientific 14-378-1B [3-place], 14-378-1A [6-place], or equivalent).

4.1.2 Standard Filter Apparatus - (Fisher Scientific 14-378-2A [47-mm], 14-378-2B [90-mm], or equivalent), consisting of a sample reservoir, clamp, fritted disk and filtration head with drip tip.

4.1.3 Tube, collection - 60-mL (Kimble 609-58-A16, or equivalent). The collection tube should be of appropriate I.D. and length for the drip tip of the standard filter apparatus to be positioned well into the neck of the tube to prevent splattering.

4.1.4 Filter flask - 2-L with a ground glass receiver joint (Kontes K-953828-0000, or equivalent) (optional). May be used to carry out individual disk extractions with the standard filter apparatus and collection vial in an ALL GLASS SYSTEM.

4.2 Solid-phase extraction disks - Empore™, or equivalent, C₁₈ disks. 47-mm and 90-mm disks are available. Solid-phases other than C₁₈ may be employed, provided that adequate performance is demonstrated for the analytes of interest.

4.3 Filtration aid (optional).

4.3.1 Filter Aid 400 - (Fisher Scientific 14-378-3, or equivalent).

4.3.2 In-situ glass micro-fiber prefilter - (Whatman GMF 150, 1 micron pore size, or equivalent).

4.4 Drying column - 22-mm ID Pyrex® chromatographic column with a polytetrafluoroethylene (PTFE) stopcock (Kontes K-420530-0242, or equivalent).

NOTE: Fritted glass discs used to retain sodium sulfate in some columns are difficult to decontaminate after contact with highly contaminated or viscous extracts. Columns suitable for this method use a small pad of Pyrex® glass wool to retain the drying agent.

4.5 Kuderna-Danish (K-D) apparatus.

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

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

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

4.5.4 Snyder column - Two-ball micro- (Kontes K-569001-0219, or equivalent) (optional).

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

NOTE: The glassware in Sec. 4.6 is recommended for the purpose of solvent recovery during the concentration procedures (Secs. 7.13 and 7.14.1) requiring the use of Kuderna-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. The EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.6 Solvent Vapor Recovery System (Kontes 545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent).

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

4.8 Water bath - Heated, with concentric ring cover, capable of temperature control to $\pm 5^{\circ}\text{C}$. The bath should be used in a hood.

4.9 N-Evap - Nitrogen blowdown apparatus, 12- or 24-position (Organomation Model 112, or equivalent) (optional).

4.10 Vials, glass - Sizes as appropriate, e.g., 2-mL or 10-mL with PTFE-fluorocarbon-lined screw caps or crimp tops for storage of extracts.

4.11 pH indicator paper - Wide pH range (Fisher Scientific 14-850-13B, or equivalent).

4.12 Vacuum system - Capable of maintaining a vacuum of approximately 66 cm (26 inches) of mercury.

4.13 Graduated cylinder - Sizes as appropriate.

4.14 Pipets, disposable (Fisher Scientific 13-678-20C, or equivalent).

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 decreasing 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 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.

5.4 Solutions for adjusting the pH of samples before extraction.

5.4.1 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.

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

5.5 Extraction, washing, and exchange solvents - All solvents must be pesticide quality or equivalent.

5.5.1 Methylene chloride, CH_2Cl_2 .

5.5.2 Hexane, C_6H_{14} .

5.5.3 Ethyl acetate, $\text{CH}_3\text{COOC}_2\text{H}_5$.

5.5.4 Acetonitrile, CH_3CN .

5.5.5 Methanol, CH_3OH .

5.5.6 Acetone, (CH₃)₂CO.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

7.1 Using a graduated cylinder, measure a 1-liter sample. Take care to minimize any loss of sample particulates during this step. This method may not be appropriate for aqueous samples with greater than 1% suspended solids, as such samples can be difficult to filter and the extraction efficiency may be reduced as a result of the small volumes of solvents employed and the short contact time. If the particulate load significantly slows or prevents filtration, it may be more appropriate to employ an alternative extraction procedure.

7.1.1 Add 5.0 mL of methanol and any surrogate standards listed in the determinative method to all samples and blanks.

7.1.2 Prepare matrix spikes by adding appropriate matrix spike standards to representative sample replicates. The frequency with which matrix spikes are prepared and analyzed is described in Chapter One or as part of the determinative method.

7.1.3 If cleanup procedures are to be employed that result in the loss of extract, adjust the amount of surrogate and spiking cocktail(s) accordingly. In the case of Method 3640, Gel Permeation Cleanup, double the amount of standards to compensate for the loss of one half of the extract concentrate when loading the GPC column.

7.1.4 If high concentrations of target analytes are anticipated to be present in samples, a smaller volume may be extracted.

7.2 Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH range listed in Table 1.

CAUTION: The adjustment of the sample pH may lead to precipitation or flocculation reactions that may remove analytes from the aqueous portion of the sample. The analyst should note the formation of precipitates or floc and take care to transfer any such material to the extraction device, rinsing the graduated cylinder with organic-free reagent water, and adding the rinse to the extraction device.

7.3 Assemble a manifold for multiple extractions (Figure 1) using 47-mm or 90-mm Empore™ disks. Use a filter flask with the standard filter apparatus for single extractions. If samples contain significant quantities of particulates, the use of a filter aid or prefilter is advisable. Empore™ Filter Aid 400 or Whatman GMF 150 prefilters are recommended.

7.3.1 Pour about 40 g of Filter Aid 400 onto the surface of the disk after assembling the standard filter apparatus.

7.3.2 Place the Whatman GMF 150 on top of the Empore™ disk prior to clamping the glass reservoir into the standard filter apparatus.

7.4 Wash the extraction apparatus and disk with 20 mL of methylene chloride introduced by rinsing down the sides of the glass reservoir. Pull a small amount of solvent 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.

7.4.1 When using a filtration aid, adjust the volume of all wash solvents so the entire filtration bed is submerged.

7.4.2 In subsequent conditioning steps, volumes should be adjusted so that a level of solvent is always maintained above the entire filter bed.

7.5 Continue to wash the extraction apparatus and disk by adding 10 mL of acetone down the sides of the glass reservoir. Pull a small amount of solvent 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. When using a filtration aid, adjust the volume of acetone so that the entire filtration bed is submerged.

7.6 Pre-wet (condition) the disk by adding 20 mL of methanol to the reservoir, pulling a small amount through the disk and then letting it soak for about one minute. Pull most of the remaining methanol through the disk, leaving 3 - 5 mm of methanol above the surface of the disk. From this point until the sample extraction has been completed, the surface of the disk should not be allowed to go dry. THIS IS A CRITICAL STEP FOR A UNIFORM FLOW AND GOOD RECOVERY.

7.6.1 The disk is composed of hydrophobic materials which will not pass water unless they are pre-wetted with a water-miscible solvent. Should a disk accidentally go dry during the conditioning step, the methanol pre-wetting and water washing steps must be repeated prior to adding the sample.

7.6.2 When using a filtration aid, adjust the volume of conditioning solvents so that the entire filtration bed remains submerged until the extraction is completed.

7.7 Rinse the disk by adding 20 mL of organic-free reagent water to the disk and drawing most through, leaving 3 - 5 mm of water above the surface of the disk.

7.8 Add a water sample, blank or matrix spike (Sec. 7.1) to the reservoir and, under full vacuum, filter as quickly as the vacuum will allow (at least 10 minutes). Transfer as much of the measured volume of water as possible. After the sample has passed through the solid-phase media, dry the disk by maintaining vacuum for about 3 minutes.

NOTE: If the sample contains particulate matter or sediment that is considered part of the sample, allow the sample to settle and decant as much of the liquid as practical into the reservoir. After most of the aqueous portion of the sample has passed through the disk, swirl the remaining portion of the sample to suspend the particulate matter or sediment and transfer it to the reservoir. Use additional portions of organic-free reagent water to complete the transfer. The particulates must be transferred to the reservoir before all of the aqueous sample has passed through the disk. If the particulate matter or sediment is not considered part of the sample, allow the sample to settle before measuring the aliquot in Sec. 7.1.

7.9 Remove the entire standard filter assembly (do not disassemble) from the manifold and insert a collection tube. The collection tube should have sufficient capacity to hold all of the elution solvents. The drip tip of the filtration apparatus should be seated sufficiently below the neck of the

collection tube to prevent analyte loss due to splattering when vacuum is applied. When using a filter flask for single extractions, empty the water from the flask before inserting the collection tube.

7.10 Add 5.0 mL of acetone to the disk. Allow the acetone to spread out evenly across the disk (or inert filter) then quickly turn the vacuum on and off to pull the first drops of acetone through the disk. Allow the disk to soak for 15 to 20 seconds before proceeding to Sec. 7.11.

7.10.1 The initial elution with a water-miscible solvent, i.e., acetone, improves the recovery of analytes trapped in water-filled pores of the sorbent. Use of a water-miscible solvent is particularly critical when methylene chloride is used as the second elution solvent.

7.10.2 When using a filtration aid, adjust the volume of eluting solvent so that the entire filtration bed is initially submerged.

7.11 Add 15 mL of methylene chloride (or other suitable elution solvent, see Table 1) to the sample bottle. Rinse the bottle thoroughly and, with the initial portion of acetone still on the disk, transfer the solvent to the disk with a disposable pipette, rinsing down the sides of the filtration reservoir in the process. Draw about half of the solvent through the disk and then release the vacuum. Allow the remaining elution solvent to soak the disk and particulate for about one minute before drawing the remaining solvent through the disk under vacuum. When using a filtration aid, adjust the volume of elution solvent so that the entire filtration bed is initially submerged.

7.12 Repeat Sec. 7.11 with a second 15-mL aliquot of elution solvent (see Table 1).

7.13 K-D concentration technique.

7.13.1 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporation flask.

7.13.2 Dry the combined extracts in the collection tube (Secs. 7.10-7.12) by passing them through a drying column containing about 10 g of anhydrous sodium sulfate. Collect the dried extract in the K-D concentrator. Use acidified sodium sulfate (Method 8151) if acidic analytes are to be measured.

7.13.3 Rinse the collection tube and drying column into the K-D flask with an additional 20-mL portion of solvent in order to achieve a quantitative transfer.

7.13.4 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Attach the solvent vapor recovery glassware (condenser and collection device, see Sec. 4.6) to the Snyder column of the K-D apparatus, following manufacturer's instructions. Prewet the Snyder column by adding about 1 mL of 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.

7.13.4.1 If a solvent exchange is required (as indicated in Table 1), momentarily remove the Snyder column, add 50 mL of the exchange solvent and a new boiling chip.

7.13.4.2 Reattach the Snyder column. Concentrate the extract, raising the temperature of the water bath, if necessary, to maintain a proper distillation rate.

7.13.5 Remove the Snyder column. Rinse the K-D flask and the lower joints of the Snyder column into the concentrator tube with 1 - 2 mL of solvent. The extract may be further concentrated by using a technique outlined in Sec. 7.14 or adjusted to a final volume of 5.0 - 10.0 mL using an appropriate solvent (Table 1).

7.14 If further concentration is required, use either the micro-Snyder column technique (7.14.1) or nitrogen blowdown technique (7.14.2).

7.14.1 Micro-Snyder column technique.

7.14.1.1 Add a fresh clean boiling chip to the concentrator tube and attach a two-ball micro-Snyder column directly to the concentrator tube. Attach the solvent vapor recovery glassware (condenser and collection device) to the micro-Snyder column of the K-D apparatus, following manufacturer's instructions. Prewet the Snyder column by adding 0.5 mL of methylene chloride or the exchange solvent to the top of the column. Place the micro-concentration 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.

7.14.1.2 When the apparent volume of liquid reaches 0.5 mL, remove the apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse its lower joints into the concentrator tube with 0.2 mL of solvent. Adjust the final extract volume to 1.0 - 2.0 mL.

7.14.2 Nitrogen blowdown technique.

7.14.2.1 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 phthalate interferences.

7.14.2.2 Rinse down the internal wall of the concentrator tube several times with solvent during the nitrogen blowdown. During evaporation, position the concentrator tube to avoid condensing water into the extract. Under normal procedures, the extract must not be allowed to become dry.

CAUTION: When the volume of solvent is reduced below 1 mL, some semivolatile analytes such as cresols may be lost.

7.15 The extract may now be subjected to cleanup procedures or analyzed for the target analytes using the appropriate determinative technique(s). If further handling of the extract will not be performed immediately, stopper the concentrator tube and store in a refrigerator. If the extract will be stored longer than 2 days, it should be transferred to a vial with a PTFE-lined screw-cap, and labeled appropriately. In no case should the recommended holding times for analytical procedures provided in Chapter Four, Table 4-1 be exceeded.

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 general quality control procedures and Method 3500 for specific QC procedures for extraction and sample preparation.

9.0 METHOD PERFORMANCE

Refer to the determinative methods listed in Table 1 for performance data.

10.0 REFERENCES

1. Lopez-Avila, V., Beckert, W., et. al., "Single Laboratory Evaluation of Method 8060 - Phthalate Esters", EPA/600/4-89/039.
2. Tomkins, B.A., Merriweather, R., et. al., "Determination of Eight Organochlorine Pesticides at Low Nanogram/Liter Concentrations in Groundwater Using Filter Disk Extraction and Gas Chromatography", JAOAC International, 75(6), pps. 1091-1099 (1992).

FIGURE 1
DISK EXTRACTION APPARATUS

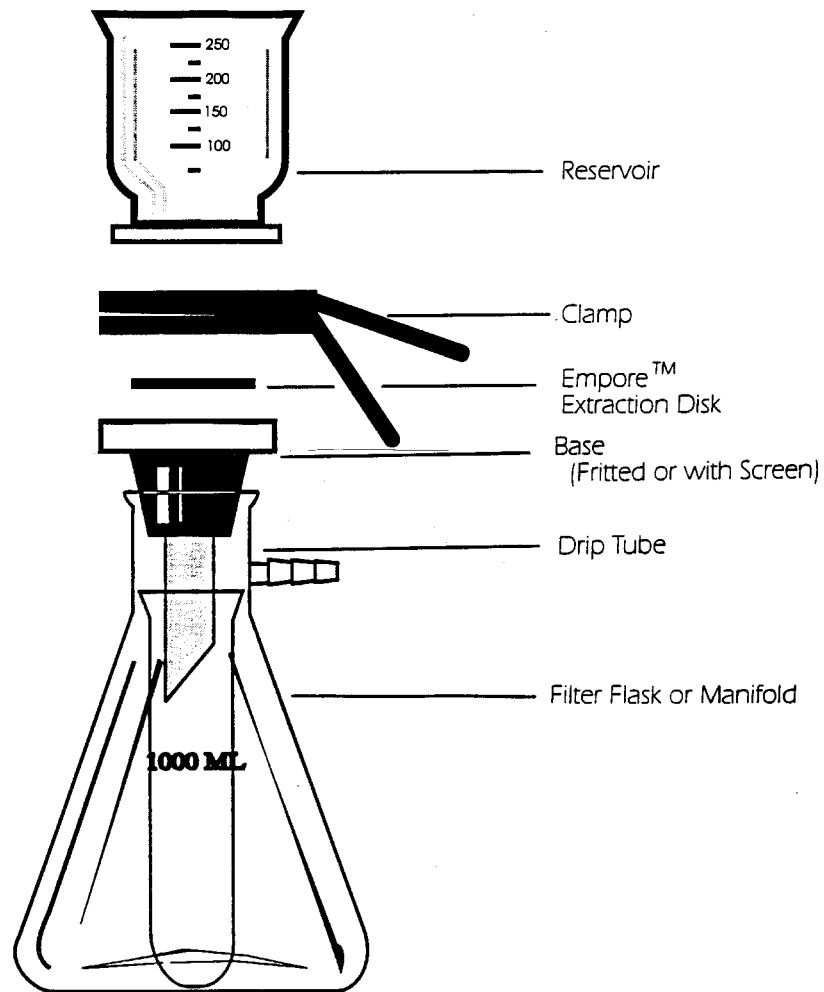
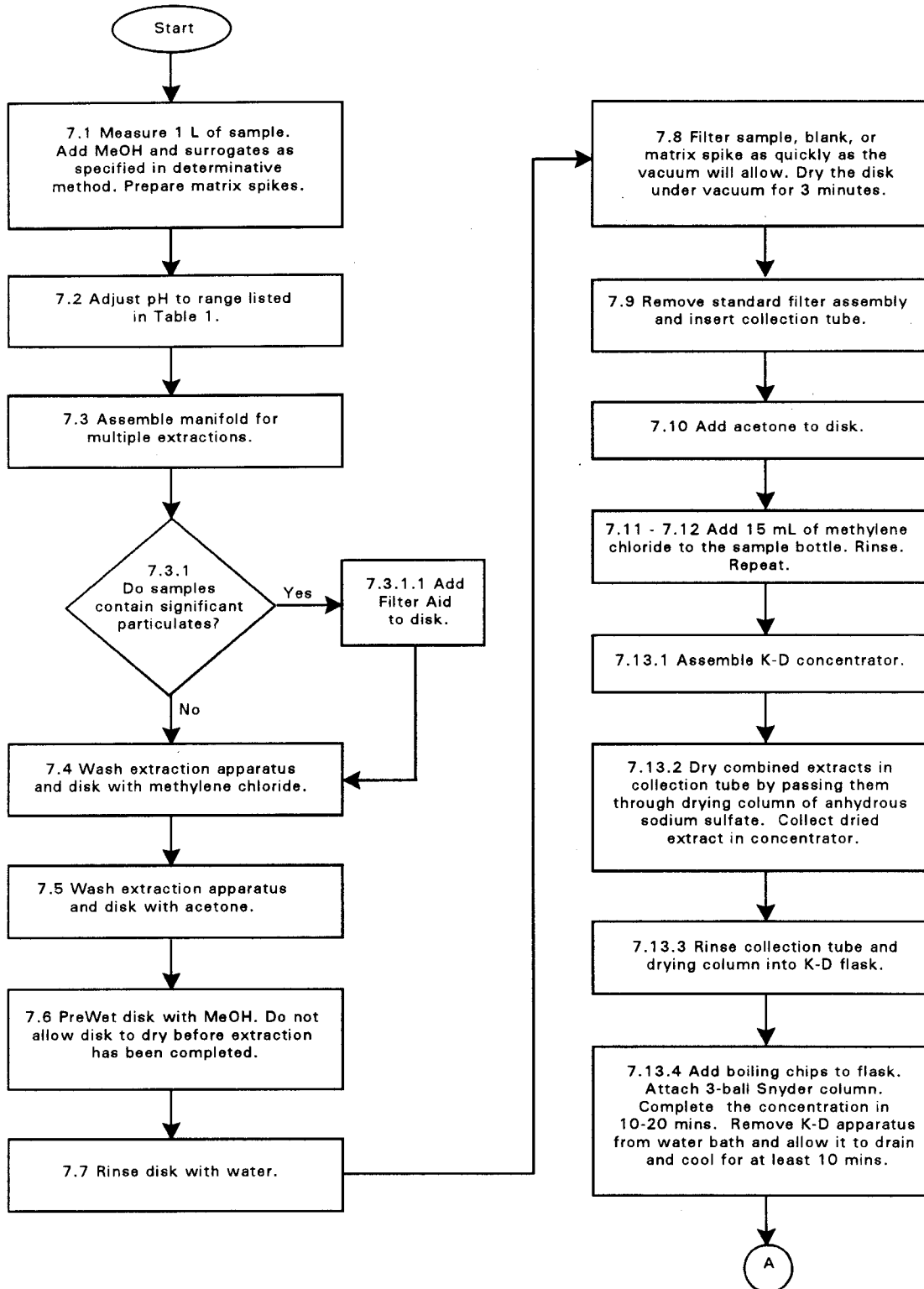


TABLE 1
SPECIFIC EXTRACTION CONDITIONS FOR VARIOUS DETERMINATIVE METHODS

Determinative Method	Extraction pH	Disk Medium	Elution Solvent	Exchange Solvent	Final Extract Volume for Analysis (mL) ^a
8061	5-7	C ₁₈	acetonitrile	hexane	10.0
8081	5-9	C ₁₈	methylene chloride	hexane	10.0
8325	7.0	C ₁₈	methanol or acetonitrile	methanol	1.0

^a For methods where the suggested final extract volume is 10.0 mL, the volume may be reduced to as low as 1.0 mL to achieve lower detection limits.

METHOD 3535
SOLID-PHASE EXTRACTION (SPE)



METHOD 3535
SOLID-PHASE EXTRACTION (SPE) (Continued)

