METHOD 3665

SULFURIC ACID/PERMANGANATE CLEANUP

1.0 SCOPE AND APPLICATION

1.1 This method is suitable for the rigorous cleanup of sample extracts prior to analysis for polychlorinated biphenyls. This method should be used whenever elevated baselines or overly complex chromatograms prevent accurate quantitation of PCBs. This method cannot be used to cleanup extracts for other target analytes, as it will destroy most organic chemicals including the pesticides Aldrin, Dieldrin, Endrin, Endosulfan (I and II), and Endosulfan sulfate.

2.0 SUMMARY OF METHOD

2.1 An extract is solvent exchanged to hexane, then the hexane is sequentially treated with (1) concentrated sulfuric acid and, if necessary, (2) 5% aqueous potassium permanganate. Appropriate caution must be taken with these corrosive reagents.

2.2 Blanks and replicate analysis samples must be subjected to the same cleanup as the samples associated with them.

2.3 It is important that all the extracts be exchanged to hexane before initiating the following treatments.

3.0 INTERFERENCES

3.1 This technique will not destroy chlorinated benzenes, chlorinated naphthalenes (Halowaxes), and a number of chlorinated pesticides.

4.0 APPARATUS

4.1 Syringe or Class A volumetric pipet, glass; 1.0, 2.0 and 5.0 mL.

4.2 Vials - 1, 2 and 10 mL, glass with Teflon lined screw caps or crimp tops.

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 Vortex mixer.

5.0 REAGENTS

5.1 Reagent grade inorganic 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.

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 Sulfuric acid/Water, H_2SO_4/H_2O, (1:1, v/v).

5.4 Hexane, C_6H_{14} - Pesticide grade or equivalent.

5.5 Potassium permanganate, $KMnO_4$, 5 percent aqueous solution (w/v).

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

7.1 Sulfuric acid cleanup

7.1.1 Using a syringe or a volumetric pipet, transfer 1.0 or 2.0 mL of the hexane extract to a 10 mL vial and, in a fume hood, carefully add 5 mL of the 1:1 sulfuric acid/water solution.

7.1.2 The volume of hexane extract used depends on the requirements of the GC autosampler used by the laboratory. If the autosampler functions reliably with 1 mL of sample volume, 1.0 mL of extract should be used. If the autosampler requires more than 1 mL of sample volume, 2.0 mL of extract should be used.

CAUTION: Make sure that there is no exothermic reaction nor evolution of gas prior to proceeding.

7.1.3 Cap the vial tightly and vortex for one minute. A vortex must be visible in the vial.

CAUTION: Stop the vortexing immediately if the vial leaks, AVOID SKIN CONTACT, SULFURIC ACID BURNS.

7.1.4 Allow the phases to separate for at least 1 minute. Examine the top (hexane) layer; it should not be highly colored nor should it have a visible emulsion or cloudiness.

7.1.5 If a clean phase separation is achieved, proceed to Sec. 7.1.8.

7.1.6 If the hexane layer is colored or the emulsion persists for several minutes, remove the sulfuric acid layer from the vial and dispose of it properly. Add another 5 mL of the clean 1:1 sulfuric acid/water.

NOTE: Do not remove any hexane at this stage of the procedure.

7.1.7 Vortex the sample for one minute and allow the phases to separate.

7.1.8 Transfer the hexane layer to a clean 10 mL vial.

7.1.9 Add an additional 1 mL of hexane to the sulfuric acid layer, cap and shake. This second extraction is done to ensure quantitative transfer of the PCBs and Toxaphene.

7.1.10 Remove the second hexane layer and combine with the hexane from Sec. 7.1.8.

7.2 Permanganate cleanup

7.2.1 Add 5 mL of the 5 percent aqueous potassium permanganate solution to the combined hexane fractions from 7.1.10.

CAUTION: Make sure that there is no exothermic reaction nor evolution of gas prior to proceeding.

7.2.2 Cap the vial tightly and vortex for 1 minute. A vortex must be visible in the vial.

CAUTION: Stop the vortexing immediately if the vial leaks. AVOID SKIN CONTACT, POTASSIUM PERMANGANATE BURNS.

7.2.3 Allow the phases to separate for at least 1 minute. Examine the top (hexane) layer, it should not be highly colored nor should it have a visible emulsion or cloudiness.

7.2.4 If a clean phase separation is achieved, proceed to Sec. 7.2.7.

7.2.5 If the hexane layer is colored or the emulsion persists for several minutes, remove the permanganate solution from the vial via a glass pipette and dispose of it properly. Add another 5 mL of the clean aqueous permanganate solution.

NOTE: Do not remove any hexane at this stage of the procedure.

7.2.6 Vortex the sample and allow the phases to separate.

7.2.7 Transfer the hexane layer to a clean 10 mL vial.

7.2.8 Add an additional 1 mL of hexane to the permanganate layer, cap the vial securely and shake. This second extraction is done to ensure quantitative transfer of the PCBs and Toxaphene.

7.2.9 Remove the second hexane layer and combine with the hexane from Sec. 7.2.7.

7.3 Final preparation

7.3.1 Reduce the volume of the combined hexane layers to the original volume (1 or 2 mL) using the Kuderna-Danish Technique (Sec. 7.3.1.1).

7.3.1.1 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 of hexane 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 1-2 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.3.1.2 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1-2 mL of hexane. The extract may be further concentrated by using either the micro Snyder column technique (Sec. 7.3.2) or nitrogen blowdown technique (Sec. 7.3.3).

7.3.2 Micro Snyder Column Technique

7.3.2.1 Add another one or two clean boiling chips to the concentrator tube and attach a two ball micro Snyder column. Prewet the column by adding about 0.5 mL of hexane 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 with about 0.2 mL of hexane and add to the concentrator tube. Adjust the final volume to 1.0-2.0 mL, as required, with hexane.

7.3.3 Nitrogen Blowdown Technique

7.3.3.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

7.3.3.2 The internal wall of the tube must be rinsed down several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

7.3.4 Remove any remaining organochlorine pesticides from the extracts using Florisil Column Cleanup (Method 3620) or Silica Gel Cleanup (Method 3630).

7.3.5 The extracts obtained may now be analyzed for the target analytes using the appropriate organic technique(s) (see Sec. 4.3 of this Chapter). If analysis 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 Teflon lined screw cap or crimp top, and labeled appropriately.

8.0 QUALITY CONTROL

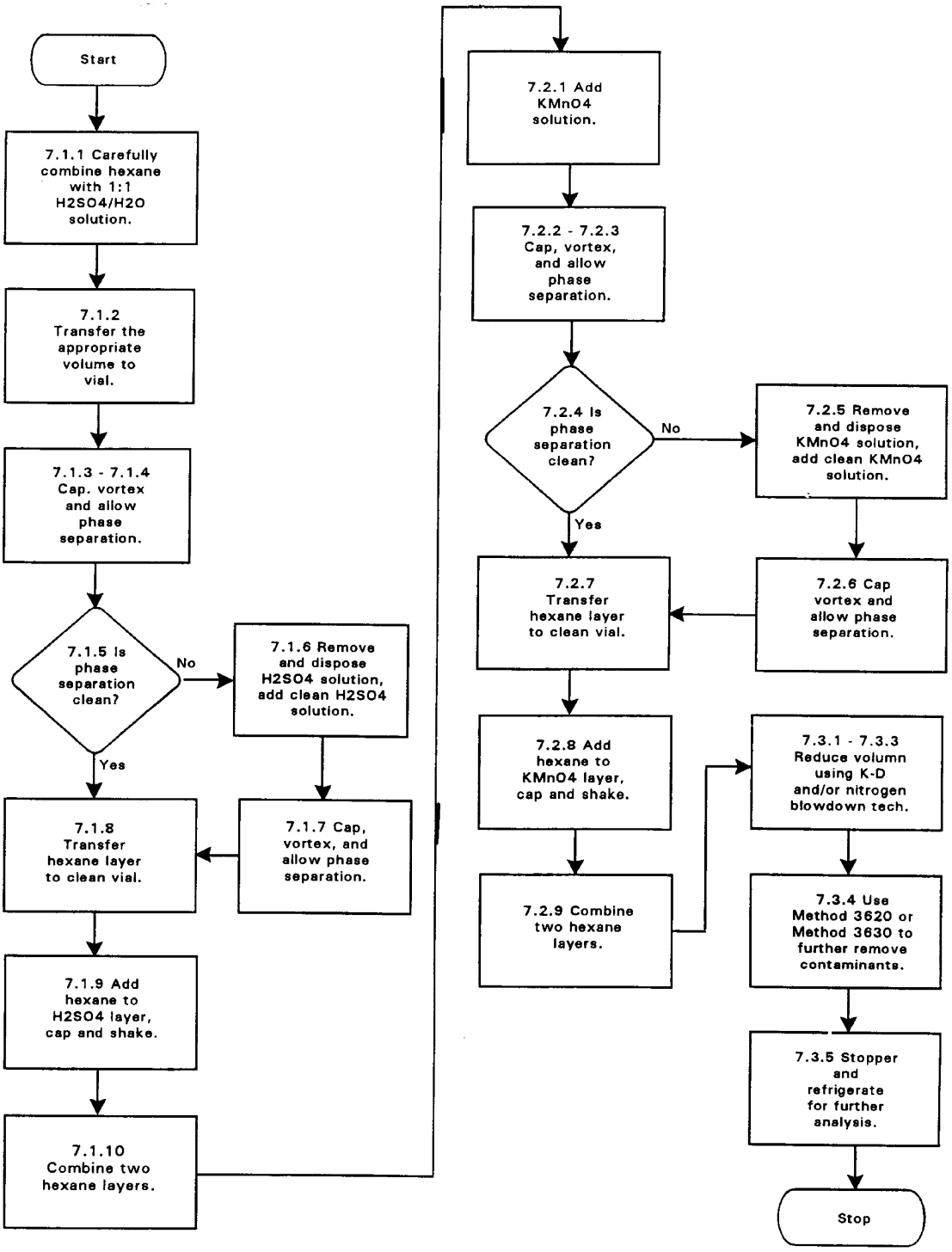
8.1 Refer to Chapter One for specific quality control procedures.

9.0 METHOD PERFORMANCE

9.1 No performance data are currently available.

10.0 REFERENCES

None required.


Start
7.1.1 Carefully combine hexane with 1:1 H2SO4/H2O solution.
7.1.2 Transfer the appropriate volume to vial.
7.1.3 - 7.1.4 Cap. vortex and allow phase separation.
7.1.5 Is phase separation clean?
No
7.1.6 Remove and dispose H2SO4 solution, add clean H2SO4 solution.
Yes
7.1.7 Cap, vortex, and allow phase separation.
7.1.8 Transfer hexane layer to clean vial.
7.1.9 Add hexane to H2SO4 layer, cap and shake.
7.1.10 Combine two hexane layers.
7.2.1 Add KMnO4 solution.
7.2.2 - 7.2.3 Cap, vortex, and allow phase separation.
7.2.4 Is phase separation clean?
No
7.2.5 Remove and dispose KMnO4 solution, add clean KMnO4 solution.
Yes
7.2.6 Cap vortex and allow phase separation.
7.2.7 Transfer hexane layer to clean vial.
7.2.8 Add hexane to KMnO4 layer, cap and shake.
7.2.9 Combine two hexane layers.
7.3.1 - 7.3.3 Reduce volumn using K-D and/or nitrogen blowdown tech.
7.3.4 Use Method 3620 or Method 3630 to further remove contaminants.
7.3.5 Stopper and refrigerate for further analysis.
Stop