APPENDIX A:
OASIS® HLB GLASS CARTRIDGE INSTRUCTION SHEET

SECTION 1: INTRODUCTION

Waters Oasis® HLB glass cartridges are available in **5 cc (200 mg)** configuration with **Teflon® frits**. The clean glass cartridge is designed for trace analysis at parts per trillion level including monitoring endocrine disruptors, such as phenols and phthalates.

Each lot of glass cartridges and Teflon® frits are tested for the presence of bisphenol A, 4-n-nonyl phenol, Dimethyl phthalate, Diethyl phthalate, Benzyl butyl phthalate, Di-n-butyl phthalate, Bis (2-ethylhexyl) phthalate and Di-n-octyl phthalate (≤ 20 ng per cartridge, per compound) before packing. These tests assure that endocrine disruptors, in water samples, can be analyzed to part per trillion levels.

The Certificate of Analysis [COA] reports recoveries, with RSDs, for three polar pharmaceutical compounds. The COA displays results from stringent quality control tests on the batch of polymer sorbent and the lot of packed cartridges.

SECTION 2: QUICK START SPE PROCEDURE FOR ENDOCRINE DISRUPTORS

1. If desired, add / mix 10 to 50 µL of internal standard to the sample (soil, food and other solid samples require pretreatment before SPE)

2. Adjust the sample to pH 3.

3. Place Oasis® HLB extraction cartridges on vacuum manifold and set vacuum to approximately 5" Hg. **The extraction procedure can also be done by positive pressure using the 5 cc Teflon® adaptors (part number 405000934)**.

   No individual stopcocks are necessary.

4. Solid-Phase Extraction Procedure: The following simple protocol should be used in preparing and using the cartridges for the isolation of a wide spectrum of acidic, basic, and neutral analytes especially classes of endocrine disruptors.

   No step should be omitted.

Procedure optimization is discussed in *Section 3*.

   Note: Once the HLB sorbent has been conditioned and equilibrated, there is no need to keep the cartridges wet prior to sample loading. Maintain a continuous vacuum on all cartridges throughout steps 4a-4d. This convenience will save you time.

   Note: For the load and elute steps, the recommended flow rate is 10 mL/min for 5 cc cartridges. You may need to momentarily increase the vacuum to start the flow of aqueous solutions.

4a. Condition: Add to and draw through each cartridge 5-10 mL 10% methanol in **methyl tertbutyl ether (MtBE*)** and then 3 mL methanol.

4b. Equilibrate: 3 mL water

4c. Load: Draw sample through the cartridge. The maximum recommended sample volume is 1 L for 5 cc cartridges.

4d. Wash: Add to and draw through each cartridge 3 mL of 5% methanol in water (v/v).
Release vacuum, remove manifold cover, and discard waste fluids. Insert rack containing collection vessels, replace cover, and turn on vacuum.

4e. Elute: Add to and draw through each cartridge 6 mL 10% methanol in MtBE*.
If desired, evaporate eluates to dryness.

5. Reconstitute in acetonitrile and adjust to the mobile phase concentration for LC analysis.

6. For GC analysis dry extract over sodium sulfate and reconstitute to 1 mL.

** Dethyl ether can be used as an alternative to MBTE*

[1] U.S. Patent No. 5,882,521

SECTION 3: ADJUSTMENTS TO OPTIMIZE RECOVERIES (TABLE 6)

Spike an appropriate volume of reagent water (for general analysis) or PBS (for biological fluids analysis) with all analytes and internal/surrogate standards. For preparation of PBS solution see Section 4. Follow steps 4a-4e in Section 2, but use a rack to collect the eluates in the Load (4c), Wash (4d), and Elute (4e) steps in separate collection vessels. In addition, repeat step 4e with a second portion of elution solvent and collect the eluate. Analyze all four collected fractions. Use the table to determine adjustments, if necessary, to optimize sample recovery.

TABLE 6

If the fraction from this step contains the analyte	Make this adjustment for optimum sample recovery
LOAD (4c)	The Oasis® HLB sorbent has been found to retain ionized analytes more strongly than silica-based reversed-phased sorbents. However, recoveries may be enhanced when analyte ionization is suppressed. For acidic analytes, adjust the sample pH to at least two pH units below the pK_a of the acid. For basic analytes, adjust the pH to at least two pH units above the pK_a of the conjugate acid.
WASH (4d)	Recoveries of very polar analytes can be increased by using only 1 mL of water (not 5% methanol in water) as the wash solution.
FIRST ELUTION (4e)	If an acceptable recovery of analyte(s) is obtained in this fraction (usually > 90%), no adjustements are necessary
SECOND ELUTION (4e repeated)	For very nonpolar analytes, stronger solvents such as acetonitrile, methylene chloride or ethyl acetate may be substituted, or used in sequence. In addition, for ionizable analytes, methanol may needed to be modified with the addition of 2% acid or 2% base, as appropriate. If solvents stronger than methanol or acetonitrile are used for the elution, then a preliminary conditioning step (see step 4a, Section 2) should be performed prior to the methanol conditioning step. For example, if ethyl acetate is to be used as an eluent, condition the cartridge with 1 mL of ethyl acetate, followed by 1 mL of methanol and 1 mL of water.

SECTION 4: PREPARATION OF PHOSPHATE-BUFFERED SALINE (PBS)

(PBS required only when analyzing analytes in serum, plasma, or urine, not required for water, soil, or food samples)

To make phosphate-buffered saline solution:

1. To a 1-liter flask, add the following anhydrous salts:

    a. 200 mg KCl
    b. 8000 mg NaCl
    c. 200 mg KH_2PO_4
    d. 1150 mg Na_2HPO_4

2. Add 1 liter of deionized water. Stir to dissolve.

3. Adjust pH to 7.0 with 10% phosphoric acid.

ORDERING INFORMATION:

Description	Part Number
Oasis® HLB Glass Cartridges 5 cc /200 mg 30/box	186000683
Adaptor, 5cc, Teflon® 10/pkg	405000934
Sep-Pak® Connector Kit	WAT011400