

OASIS HLB GLASS CARTRIDGES

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I. 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. The Oasis glass cartridge contains a unique (patented¹) sorbent, a copolymer designed to have a hydrophilic-lipophilic balance (HLB), that gives high and reproducible recoveries for acidic, basic, and neutral compounds - even if the cartridge runs dry.

Each lot of glass cartridges and Teflon frits are tested for the presence of bisphenol A and other phenols and phthalates before packing. These tests assure that endocrine disruptors, in water samples, can be analyzed to part per trillion levels.

This Instruction Sheet outlines in Section II the Quick Start Solid-Phase Extraction (SPE) Procedure for extracting endocrine disruptors and many other types of compounds. Section III gives a simple protocol to determine if any adjustments are necessary to optimize recovery. Section IV describes the preparation of phosphate-buffered saline (PBS) solution (required only for serum, plasma or urine matrix).

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.

OASIS®
SAMPLE EXTRACTION PRODUCTS

¹ US Patent # 5,882,521

II. QUICK START SPE PROCEDURE

1. If desired, add 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 many classes of endocrine disruptors.

No step should be omitted.

Procedure optimization is discussed in Section III.

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.

III. ADJUSTMENTS TO OPTIMIZE RECOVERIES

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 IV. Follow steps 4a-4e in Section II, 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.

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-phase 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 pKa of the acid. For basic analytes, adjust the pH to at least two pH units above the pKa 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 adjustments 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 need 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) 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.

IV. 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 KH₂PO₄
 - d. 150 mg Na₂HPO₄
2. Add 1 liter of deionized water. Stir to dissolve.
3. Adjust pH to 7.0 with 10% phosphoric acid.

V. ORDERING INFORMATION

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

Visit us at www.waters.com for more information and a complete list of part numbers for cartridges, plates, and columns.

Sales Offices:

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