OASIS® HLB PRODUCT AND GENERIC METHOD INFORMATION

I. INTRODUCTION

Considerable time and effort is consumed in choosing an appropriate solid-phase extraction (SPE) sorbent and extraction protocol. The limitations of today's sorbents require the analyst to watch carefully and control closely the extraction procedure. Even then it is difficult and time-consuming to achieve high, reproducible recoveries for analysis of important polar drugs and metabolites. A new polymeric SPE sorbent has been developed that allows more samples to be processed in one batch, resulting in a significant increase in throughput. The bottleneck at the sample preparation step can now be significantly reduced or eliminated.

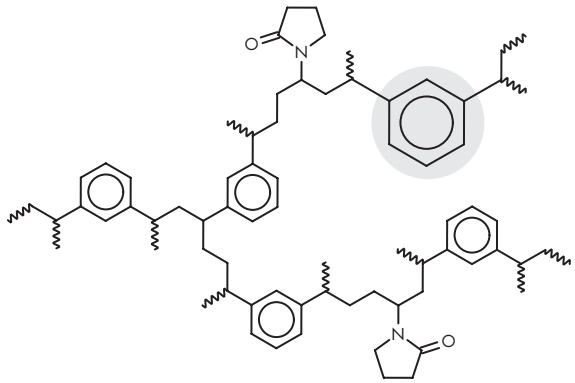
TRADITIONAL METHODS FOR SOLID-PHASE EXTRACTION

The most commonly used sorbents are porous silica particles surface-bonded with C_{18} or other hydrophobic alkyl groups. Prior to use, the sorbent must first be conditioned with a water-miscible organic solvent to solvate the alkyl chains, and then equilibrated with water or buffer solution. Because these sorbents are not hydrophilic, or water-wettable, care must be taken to ensure that the sorbent stays wet before loading the aqueous sample. Failure to do so prevents proper sample-sorbent-contact and is the major cause of low analyte recoveries and poor assay-to-assay reproducibility.

Waters has designed Oasis® HLB sample extraction products to overcome these limitations of reversed-phase SPE and to streamline the sample preparation process. The key to this advancement has been the development of a novel patented polymeric reversed-phase sorbent. This macroporous copolymer [poly(divinylbenzene-co-N-vinylpyrrolidone)] exhibits both hydrophilic and lipophilic retention characteristics. HLB is an acronym for hydrophilic-lipophilic balance which describes two major features of this sorbent: the unique abilities to (1) remain wetted with water, and (2) to retain a wide spectrum of both polar and nonpolar compounds. Oasis® HLB extraction plates and cartridges deliver higher, more reproducible recoveries for a wide range of analytes using rapid, straightforward extraction protocols. These results can be achieved without having to worry about watching your samples as they are processed, because the sorbent can be allowed to dry out during the extraction

Oasis® HLB sample extraction products are available in various cartridge, plates, and column configurations. A Certificate of Analysis (COA) in each product box reports recoveries, with RSDs, for three polar pharmaceutical compounds. The Certificate of Analysis displays results from stringent quality control tests on the batch of polymer sorbent and the lot of packed cartridges.

FIGURE 1: Structure of Oasis® HLB solid-phase extraction sorbent [poly(divinylbenzene-co-N-vinylpyrrolidone)] and physical characteristics.



Average Pore Diameter (Å)	*82*
Specific Surface Area (m^2/g)	*831*
Total Pore Volume (cc/g)	*1.4*
Mean Particle Diameter (µm)	*31.4*
Percent Fines < 10 µm	*0.1%*

II. EFFECT OF DRYING TIME ON RECOVERY

The effect of recovery on cartridge drying time for pharmaceutical compounds in porcine serum is shown for Oasis® HLB extraction cartridges and for Bond Elut® C_{18} cartridges. The Waters HPLC system used in this experiment consists of a 600 Multi-Solvent Delivery System, a 486 Tunable Absorbance Detector and a 717plus Autosampler. Data acquisition was performed using 860 ExpertEase™ software installed on an 845 workstation. A 20-position vacuum manifold (MSE, Torrance, CA) with a vacuum pump was used to process solid-phase extraction cartridges.

Freshly thawed porcine serum was spiked with either a polar solution containing 10 µg/mL each of procainamide, acetaminophen and ranitidine (Solution 1) or a non-polar solution containing 10 µg/mL each of doxepin and propranolol (Solution 2). All analyses were performed in triplicate. 1 cc, 100 mg Bond Elut® C_{18} and 1 cc, 30 mg Oasis® HLB extraction cartridges were conditioned with 1 mL of methanol. When the methanol level reached the top frit of the cartridge, the vacuum was maintained for 0, 10, 30, 60, 120, 240, or 480 seconds to vary the cartridge drying time. l mL of water was then applied to the cartridge followed by 1.0 mL of porcine serum spiked with Solution 1 or Solution 2.

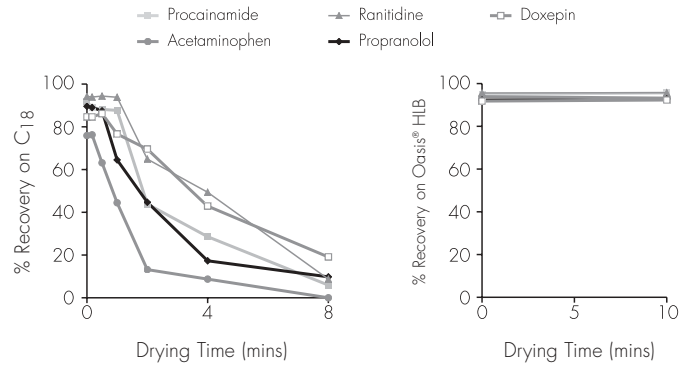
Cartridges were washed with either 1 mL deionized water for the C_{18} Bond Elut® cartridges, or 1 mL of 5% methanol in water for Oasis® HLB extraction cartridges. The analyte was eluted with 1 mL of methanol. Ten µL of 1000 µg/mL internal standard (sulfanilamide for Solution 1, and butyl paraben for Solution 2) was added. Samples were vortexed and analyzed by HPLC.

Figure 2 shows the percent recovery versus cartridge drying time for pharmaceutical compounds in porcine serum on Bond Elut® cartridges. The results show that recovery drops markedly for the most polar compounds even before the first minute of cartridge drying. The results are consistent with hydrophobic collapse of the C_{18}–alkyl chains on the silica surface when the conditioning solvent is lost. All reversed-phase silica sorbents behave similarly, regardless of the manufacturer.

The results in Figure 2 are most compelling when multiple samples are processed simultaneously on vacuum manifolds. If some of the cartridges are inadvertently dried out, poor recovery and irreproducibility result. For polar compounds such as drug metabolites, this drying has tremendous impact. When samples are processed using traditional hydrophobic (like C_{18} or PS-DVB) sorbents, every cartridge must be watched to keep it wet with the conditioning solvent until the sample is loaded. This becomes difficult when many solid-phase extraction cartridges are being processed simultaneously and virtually impossible when plates are processed. The results in Figure 2 emphasize that the Oasis® HLB sorbent is essential to the reliable use of the 96-well plate format.

The percent recovery for several drying times for Oasis® HLB extraction cartridges demonstrates the ability of the sorbent to maintain surface conditioning even if the cartridge is allowed to run dry. Reproducibility and recoveries are not affected and sample processing becomes easier and more efficient. In addition, samples can be processed faster since the vacuum can be run continuously instead of stopping and starting the vacuum or manipulating stopcocks to stop the flow of solvent before it reaches the sorbent bed.

FIGURE 2: Percent recovery versus cartridge drying time for pharmaceutical compounds in porcine serum using Bond Elut C_{18} and Oasis® HLB extraction cartridges.



III. GENERIC METHODS FOR OASIS® HLB EXTRACTION CARTRIDGES AND PLATES

SECTION A: GENERIC SPE PROCEDURE FOR OASIS® HLB SORBENT

The Quick Start SPE Procedure for Oasis® HLB (Table 1) sorbent is an excellent starting point for any method. Methods developed on the 1 cc/30 mg cartridges are directly transferable to the 30 mg/well 96-well extraction plates. The procedure also applies to the other 96-well plates as well as µElution plates (refer to recommended volume for generic method, pg 15).

TABLE 1

NOTE: If necessary, clarify the samples by centrifugation at 8000 X g for 20 minutes prior to loading on the cartridge.

STEPS FOR 1 cc/30 mg CARTRIDGES	PURPOSE
CONDITION/EQUILIBRATE 1 mL methanol/1 mL water	Prepares sorbent for use
LOAD 1 mL spiked and acidified plasma or urine	Acidic, neutral, and basic analytes are retained by reversed-phase mechanism.
WASH 1 mL 5% MeOH	Removes proteins and salts
ELUTE 1 mL MeOH	Elutes analytes of interest.
EVAPORATE AND RECONSTITUTE in 100 µL of an appropriate solvent or solution (optional)	Concentrates sample and/or changes solvent for analysis.