[CARE AND USE MANUAL]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Oasis HLB Cartridges and 96-Well Plates

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I. OASIS HLB

Universal Sorbent for Acidic, Neutral, and Basic Compounds

Oasis® HLB is a Hydrophilic-Lipophilic-Balanced, water-wettable, reversed-phase sorbent for all your SPE needs. It is made from a specific ratio of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene. It provides superior reversed-phase capacity with a neutral polar 'hook' for enhanced retention of polar analytes.

Waters® has built a family of SPE sorbents which inherit some key features of this unique substrate: stability at pH extremes and in a wide range of solvents, extraordinary retention of polar compounds, and a relative hydrophobic retention capacity 3X higher than that of traditional silica-based SPE sorbents like C_{18}.

Water-wettable Oasis Sorbents exhibit excellent retention capacity for a wider polarity spectrum of analytes, even if the sorbent bed runs dry during conditioning or sample loading. This means that your SPE methods will be more rugged and robust, obviating the need for repeat preparation.

The advantage of having higher retention capacity [k] is that more analytes are retained with less breakthrough, improving the recovery and overall reproducibility of your SPE method.

Available in five particle sizes [60 µm, 30 µm, 25 µm, 15 µm, and 5 µm], Oasis HLB sorbent, in cartridge, plate, or column format, allows you to select the appropriate product based on the volume, viscosity, and turbidity of your sample.

III. SAMPLE PRE-TREATMENT

a. Biological Samples

This section contains recommendations for preparing your biological samples (plasma, serum, urine, etc.) prior to solid-phase extraction.

1. Prepare acidified or basified water diluents.

Note: (To prepare 4% phosphoric acid, Dilute 4.7 mL of 85% phosphoric acid (the most common available formulation) to 100 mL final volume with water. Or 11.76 mL to 250 mL). To prepare 5% concentrated ammonia, dilute 5 mL of concentrated ammonia solution to 100 mL with water. Users will need to prepare large volumes 100 -250 mL) of pretreatement buffer to accommodate multiple samples and ensure consistency in the buffer composition.)

2. Dilute plasma or urine, 1:1 with acidified or basified water. Add 10 to 50 µL of internal standard.

Note: Final concentration should be no more than 10% organic otherwise protein precipitation can occur and polar compound retention may be compromised.

3. If necessary, clarify samples by centrifugation at 8,000 x g for 10-30 minutes.

b. Solid Samples: Soil, Whole Foods, Tissue

1. Homogenize the sample with an appropriate solvent to obtain an aqueous based or an organic solvent based extract of the sample. Initial extraction conditions are chosen to maximize analyte recovery, while minimizing matrix interference. In many cases, it may be beneficial to add buffers, dispersive salts, or co-solvents to improve extraction efficiency.
2. Adjust the initial extract to optimize analyte retention onto the SPE sorbent. This can include pH adjustment, solvent adjustment, or solvent exchange through evaporation and reconstitution (refer to Sections IV and V). It may be necessary to centrifuge or filter the sample prior to loading.

c. Aqueous Samples: Water, Beverages

Adjust pH to maximize analyte retention on the SPE sorbent. Buffer salts and dispersive agents may be used to increase partitioning onto the SPE sorbent. Pretreatment to remove suspended matter prior to SPE treatment may include filtration or centrifugation.

d. Non-Aqueous Liquid

When appropriate the sample may be diluted with aqueous buffers and organic co-solvents for reversed-phased or mix-mode SPE. If sufficient dilution has occurred, the sample may be treated in a manner similar to an aqueous sample.

Note: If necessary, filter samples for suspended solids (Eg; Environmental/Waste water/Water analysis/Food, etc.).

IV. SOLID PHASE EXTRACTION FOR ACIDIC, NEUTRAL AND BASIC COMPOUNDS USING OASIS HLB

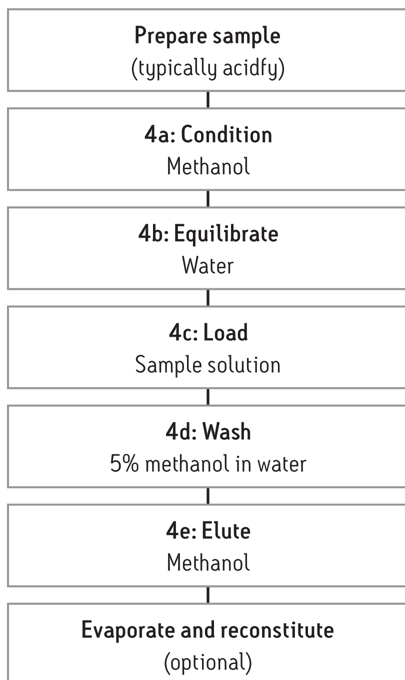
1. Place Oasis HLB cartridge or plate on the vacuum manifold and set the vacuum to 5" Hg.
2. Condition with Methanol.
3. Equilibrate with Water
   a. In each case (conditioning and equilibration) add the solvent before applying vacuum.
4. Switch off the vacuum pump or Stop vacuum by closing the valve (before switching off the vacuum pump, please reduce the vacuum to the lowest possible setting).
5. Load your diluted sample.
6. Switch on or open valve at lowest possible vacuum and gradually increase as needed in order to load the entire sample onto the sorbent bed.
7. Switch off the vacuum pump or stop vacuum by closing the valve.
8. Apply 5% methanol in water wash solvent.
9. Switch on vacuum to 5" Hg (adjust/increase as needed).
10. Pull vacuum for another 30 sec to a minute to eliminate residual wash solvent.
11. Switch off the vacuum pump or stop vacuum by closing the valve (before switching off the vacuum pump, please reduce the vacuum to the lowest possible).
12. Release vacuum and discard waste fluids, insert collection device and replace the cover.
13. Apply 100% organic elution solvent and let it flow through by gravity before switching on the vacuum pump.
14. Switch on or open valve at lowest possible vacuum and gradually increase as needed.
15. Pull vacuum for another 30 sec to a minute (to collect all elution solvent).
16. Remove collection device.
17. Evaporate / Reconstitute or Dilute as needed.
18. Transfer to vial or plate for analysis.
19. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that flow rates should not exceed 1.0 ml/min, In all other steps, flow rates up to 5 ml/min are acceptable.

Note: You may need to momentarily increase the vacuum to start the flow of aqueous solutions.

IV. OASIS HLB PROTOCOL CHARTS

a. Generic Method Oasis HLB SPE



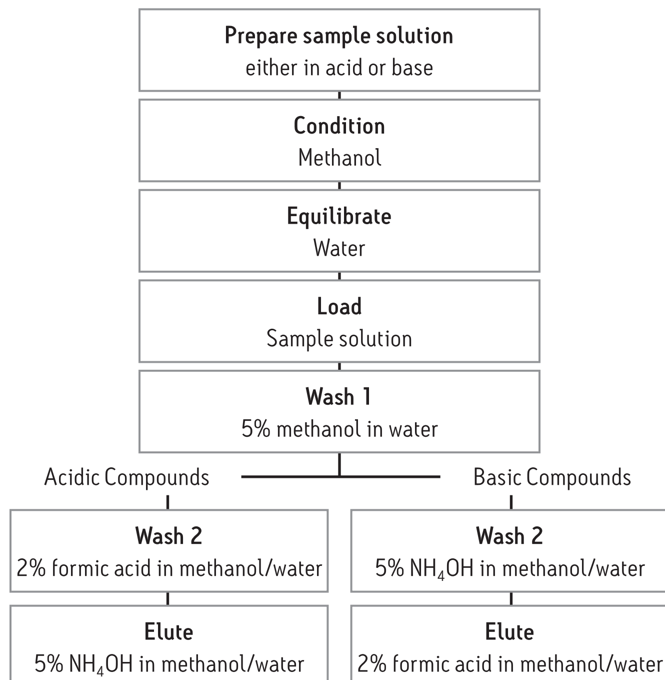
Avoid using methods developed for C_{18} or other silica-based cartidges (see note below)

- The fast Oasis HLB generic method is ideal for LC/MS/MS analysis.
- The generic method is an excellent starting point since it works for a wide range of compounds, yielding high recoveries (> 85%) and consistent results (< 5% RSD).
- When cleaner backgrounds are required for higher sensitivity or selectivity, the generic protocol can be optimized using a straighforward method development strategy.

The strategy uses the full pH range (pH 1 to pH 14) and varies the organic solvent level.

Note: Wash and Elute steps developed for C_{18} or other silica-based sorbents may not be appropriate for the polymeric Oasis HLB sorbent.

b. Strategy for Optimizing the Generic SPE Method



The strategic procedure to obtain cleaner extracts start with the generic method through the first wash.

By adjusting the pH of the additional washes to increase analyte retention, higher concentrations of organic solvent may be applied to remove interferences.

- The pH is decreased for acidic analytes (below the pK_a of the compound) to increase retention.
- The pH is raised for basic compounds (above the pK_a of the compound) to increase retention.
- The pH is then changed to elute the analyte. The % solvent in the Wash 2 and Elute steps is determined by varying the % methanol in 10% increments at each pH.
- Analyze the Wash 2 and Elute samples to determine optimum % methanol.
- Select the highest % methanol in Wash 2 that does not remove any analytes.
- Select the lowest % methanol in the Elute step that elutes the analytes.

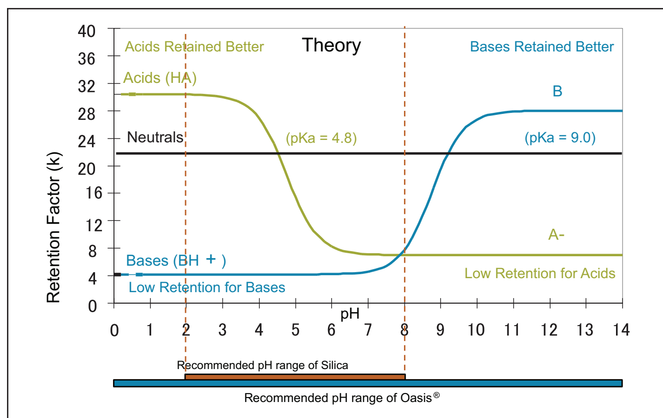


Figure 1. Retention Factor Versus pH For Acids, Bases, and Neutrals.

Summary

With the generic SPE method, one method yields good results for a wide range of compounds. With an optimized method, cleaner extracts can be obtained.

V. OASIS HLB 20 BOTTLE OPTIMIZATION APPROACH

Chemical and chromatographic principles may be applied to optimize methods on Oasis HLB. Selectivity is dramatically enhanced by tuning pH, as well as the ratio of organic solvent to water, in the mobile phase to manipulate retention.

If analytes or interferences are ionizable, then, as highly polar entities in their charged states, they may be eluted in weak mobile phases. If, by changing pH, they are converted to neutral form, they are retained primarily by the strength of their hydrophobic interaction with the sorbent surface. Stronger mobile phases, with higher organic solvent concentrations, will then be required for successful elution.

Published work by Waters chemists clearly demonstrates the benefits of such a 2-D [two-dimensional] process on Oasis HLB. The theory of retention, wash-elute studies for alprenolol, and successive selectivity improvements made by refining the Oasis HLB method are summarized in the figures below.

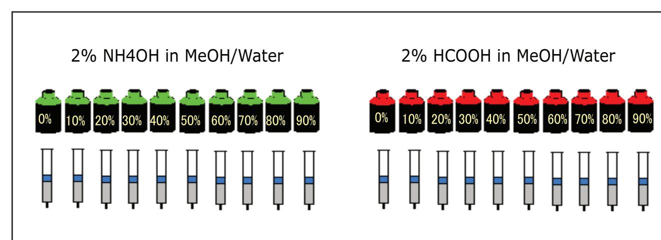
Wash/Elution Steps: 20 Bottle Optimization for Oasis HLB

The 20 Bottle Optimization method for Oasis HLB is set up first by spiking the analyte into saline and loaded it into the wells of the 96-well plate or 20 cartridges of Oasis HLB. We prepare the 20 bottle of solvents as described bellow. First, we start with 5% MeOH with base wash step to remove proteins, to prevent the wells from clogging, as well as putting bases in a neutral state for more retention by reverse phase.

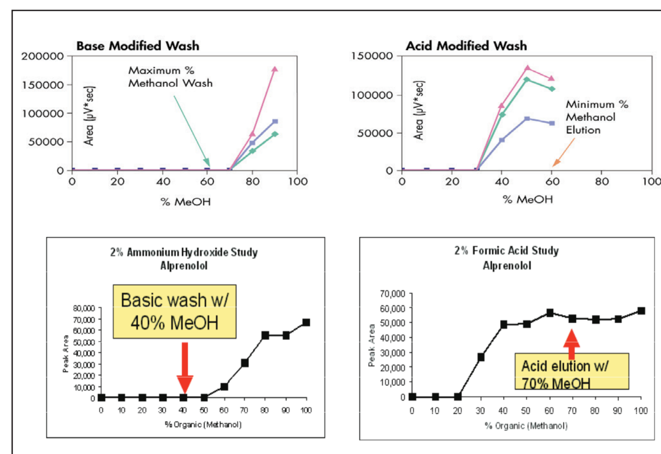
The 5% MeOH wash step (removes salts and proteins) in the generic Oasis HLB method is weak enough that the analyte should not wash off. Use the washes with increasing percentages of organic solvent on identical replicate samples.

Plot the response against the organic solvent ratio, and determine what percent organic to use for the wash and elution step After running the 2-D optimization ("20 Bottle Optimization"), it is important to select a wash step that is not too strong, or you may lose your analyte, and is the most effective in the removal of unwanted components.

It is also recommended to select the elution organic solvent ratio that is just strong enough to elute the analyte and retain the most hydrophobic interferences on the sorbent.



20 Bottle Optimization Oasis HLB: Example



- Wash 1: Base with 5% MeOH (remove proteins to prevent clogging of wells; bases are in neutral state for more retention).
- Wash 2: Base with 40% MeOH (removes hydrophilic bases and neutrals and all acids).
- Wash 3: 100% Water (removes residual ammonium hydroxide).
- Elute: Acid with 70% MeOH (101% recovery from rat plasma).

Note: Depending on matrix and sensitivity requirements, additional washes may be required.

VI. TROUBLESHOOTING

a. Adjustment to Optimize Recoveries

Spike an appropriate volume of reagent water with all analytes and internal/surrogate standards. 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 methanol and collect the eluate. Analyze all four collected fractions. Use the provided table to determine adjustments, if necessary, to optimize sample recovery.

If the fraction from this step contains the analyte:	Make this adjustment for optimum analyte 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 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 non-polar analytes, methanol may not have adequate elution strength. Stronger solvents such as acetonitrile 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 ethyl acetate, followed by methanol (4a) and then water (4b).

Recommended volume for generic methods (assuming 1:1 dilution)

	Cartridges						96 well Plate				µelution plate
Cartridges size (cc)/ Sorbent mass (mg)	1	3	6	12	20	35	5	10	30	60	2
Condition/ equilibration (ml)	1	2	3	5	10	50	0.2	0.5	0.5 -1.0	0.5 -2.0	0.2
Maximum load of matrix and dilution (ml)	1	2	5	15	30	100	0.5	1	1 - 2	1 -2	0.025 - 0.75
Wash (ml)	1	2	4	5	10	40	0.2	0.5	0.5 -1.0	1 - 2	0.2
Elute (ml)	1	2	4	5	10	60	0.05 - 0.2	0.15 - 0.3	0.4 -1.0	0.8 - 2.0	0.025-0.10

Note: The above listed sample volumes are for biological samples and for certain types of samples (i.e drinking water) up to 20 times of sample solution is possible.

Note: Solutions should be made fresh daily.

Load volumes for large volume water analysis

Cartridges size/ sorbent mass	1 cc	3 cc	6 cc (200 mg, 30 µm)	6 cc (200 mg, 60 µm)	12 cc	20 cc	35 cc
Load (ml water) total of matrix and dilution	50 ml	200 ml	500 ml	1000 ml	1000 ml	2000 ml	5000 ml

Oasis 96 Well Plates

All Oasis 96 Well Plates, including µelution plate are designed to work individually as well as on any automated robotics system.

The specific dimensions are:

96 well plate:

- Length = 5.030 inches
- Width = 3.365 inches
- Height = 2.015 inches

µelution plates:

- Length = 5.030 inches
- Width = 3.365 inches
- Height = 1.501 inches

All Waters Oasis 96 Well Plates and µelution plates meet the ANSI (American National Standards Institute) guidelines for dimensions of microplates.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

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