Waters
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Oasis Cartridges and 96-Well Plates

CONTENTS

** The COA, contained in the Oasis package, displays results from stringent quality control tests on the batch of copolymer sorbent and the lot of packed plates.*

I. INTRODUCTION

The Oasis® family of solid-phase extraction (SPE) products are designed to simplify and improve your sample preparation by combining the right sorbent chemistry, device format and methodology. There are six available Oasis Sorbent Chemistries which are designed to meet all of your sample preparation needs. They are all built upon a unique, water-wettable Oasis HLB (Hydrophilic-Lipophilic Balance) copolymer and provide exceptional results.

A. OASIS HLB

Universal Sorbent for Acidic, Neutral, and Basic Compounds, Oasis HLB is a Hydrophilic-Lipophilic-Balanced, water-wettable, reversed-phase sorbent. 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, eliminating 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).

B. OASIS PRiME HLB

The best and first sorbent choice for sample cleanup in routine analysis using reversed-phase SPE for acidic, basic and neutral compounds. Oasis PRiME HLB was created to simplify solid phase extraction by enabling scientists to use SIMPLER protocols that produce CLEANER samples, while providing FASTER, more uniform flows across cartridges and plates with less sample plugging. The Oasis PRiME HLB Sorbent was created to take advantage of the high capacity and water-wettable nature of Oasis HLB, thus eliminating the need for condition and equilibration steps in the SPE protocol. Samples may be loaded directly onto the sorbent without these condition and equilibration steps, saving time and solvent expense. In addition, this water-wettable feature keeps the sorbent wet even under extended vacuum or positive pressure, producing higher and more reproducible recoveries than can be obtained on silica based or other polymeric materials that are not as water-wettable as Oasis sorbents. Oasis PRiME HLB is ideal for use with biological and food matrices.

C. OASIS PRiME MCX

Combining the simplicity and cleanliness of Oasis PRiME HLB with the specificity of a cation exchanger for compounds with basic characteristics, Oasis PRiME MCX provides the perfect solution for targeted sample clean up. Traditional mixed-mode SPE protocols require six steps and still may not remove matrix interferences such as phospholipids. Oasis PRiME MCX is a highly efficient, orthogonal (reversed-phase and ion-exchange) solid phase extraction product based on Oasis MCX Technology. Sample cleanup is achieved with a simple and fast **three step protocol** which removes **99% of phospholipids** from complex sample matrices while providing high and reproducible target analyte recoveries. Oasis PRiME MCX is QC tested for performance using analyte recovery and phospholipid removal as benchmarks. In addition, cartridges and plates are designed with a manufacturing optimization to increase flow reproducibility across wells and devices, making sample processing time more predictable.

D. OASIS MIXED-MODE REVERSED-PHASE ION-EXCHANGERS

The sulfonic acid MCX (Mixed-Mode Cation-eXchange), and quaternary amine MAX (Mixed-Mode Anion-eXchange) derivatives of Oasis HLB provide dual modes of retention (e.g. both reversed-phase and ion-exchange retention modes available), enabling greater cleanup selectivity and sensitivity for basic and acidic compounds respectively – even if the sorbent in the wells runs dry. The carboxylic acid WCX (Weak Cation-eXchange) and the piperazine WAX (Weak Anion eXchange) are also derivatives of Oasis HLB and provide dual modes of retention. These sorbents are specifically designed to offer the same benefits and features as HLB with the ability to retain and release strong bases (e.g. quaternary amines) and strong acids (e.g. sulfonates) respectively.

All of the six patented Oasis Chemistries are available in several device formats (e.g. cartridges, 96-well plates, and µElution plates) to fit your specific needs.

Note: Because the Oasis HLB Sorbent wets as well with water as with methanol, there is no need to keep the sorbent in the wells wet prior to sample loading. This benefit not only saves you time but provides higher and more reproducible recoveries than those obtained on traditional sorbents.

II. GENERIC SAMPLE PRE-TREATMENT

A. BIOLOGICAL SAMPLES

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

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. Add 11.76 mL of the acid and adjust to final volume of 250 mL with water. 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 accomodate multiple samples and ensure consistency in the buffer composition.

1. 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 will occur. 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 V and VI). 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 (Ex. Environmental/waste water/water analysis/food, etc.).

III. OASIS PRIME HLB PRE-TREATMENT FOR MULTIRESIDUE CONTAMINANT ANALYSIS IN FOOD SAMPLES

A. VETERINARY DRUG ANALYSIS OF SOLID SAMPLES: MEAT TISSUES, INTERNAL ORGANS, SEAFOOD, EGGS

Homogenize the sample with appropriate blenders or grinders. Extract 1–5 g of the homogenized sample with 10 mL extraction solvent.* After shaking for at least 30 minutes, centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the supernatant for next clean-up step.

Recommended extraction solvent composition: 80:20 acetonitrile/water with 0.2% formic acid.

** 8 mL extraction solvent is recommended for eggs.*

B. VETERINARY DRUG ANALYSIS OF DRY POWDER: MILK POWDER, INFANT FORMULA

Place 0.5–1.0 g of powder sample into a 50 mL centrifugation tube. Add 3 mL extraction solvent. After shaking for at least 30 minutes, centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the supernatant for next clean-up step.

Recommended extraction solvent composition: 70:30 acetonitrile/water with 0.2% formic acid.

C. VETERINARY DRUG ANALYSIS OF AQUEOUS SAMPLE: MILK, LIQUID INFANT FORMULA, DAIRY BEVERAGES

Pipet 1 mL of liquid sample into a 15 mL centrifugation tube. Add 4 mL acetonitrile with 0.2% formic acid. Shake the tube vigorously for 1 minute by hand, and then centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the top acetonitrile layer for next clean-up step.

D. PESTICIDE ANALYSIS OF FATTY FRUITS: AVOCADO

Follow the recommended QuEChERS protocol for sample extraction. Place 1–5 g sample into a 50 mL centrifugation tube. Add appropriate volumes of water and acetonitrile (or acetonitrile acidified by 1% acetic acid for AOAC method). After the vortex and shaking steps, add the QuEChERS extraction salts to the tube. Shake the tube vigorously for 1 minute by hand, and then centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the top acetonitrile layer for next clean-up step.

E. PESTICIDE ANALYSIS OF OILS: OLIVE OIL, PEANUT OIL, VEGETABLE OIL

Pipet 1 mL oil sample into 15 mL centrifugation tube. Add 1 mL hexane to the tube, and mix well. Add 5 mL acetonitrile saturated with hexane to the tube. Shake the tube vigorously for 1 minute by hand, and then centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the top acetonitrile layer for next clean-up step.

F. MYCOTOXIN ANALYSIS OF GRAINS: MAIZE, WHEAT, RICE

Follow QuEChERS protocol for sample extraction using the modified extraction solvent:

Place 1–5 g homogenized sample into a 50 mL centrifugation tube. Add 10 mL water and 10 mL 10:90 formic acid/acetonitrile to the tube. After the vortex and shaking steps, add the QuEChERS extraction salts to the tube. Shake the tube vigorously for 1 minute by hand, and then centrifuge the sample at approximately 2500 rcf for a minimum of 5 minutes. Remove the top acetonitrile layer for next clean-up step.

IV. SUGGESTED VOLUMES

Table 1. Recommended Volume for Generic Methods (assuming 1:1 dilution)

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

Table 2. Load Volumes for Large Volume Water Analysis

Cartridge size/ Sorbent mass	1 cc	3 cc	6cc (200 mg, 30 µm)	6cc (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

Table 3. Recommended Pass-Through Volume of Sample Extract for Multi-residue Contaminant Analysis Using Oasis PRiME HLB Cartridges

		Cartridges					
Cartridge size/Sorbent mass		3cc/60mg	3cc/150 mg	6cc/200 mg	6cc/500 mg	Plus light/ 100 mg	Plus short/ 335 mg
Sample Extract Volume	Typical	0.5 mL	1.3 mL	1.7 mL	4.2 mL	0.8 mL	2.8 mL
	Not more than	1.0 mL	2.5 mL	3.3 mL	8.0 mL	1.7 mL	5.7 mL

Note: Above listed volume are for food samples extracted by acetonitrile or mixture with aqueous buffer solutions.

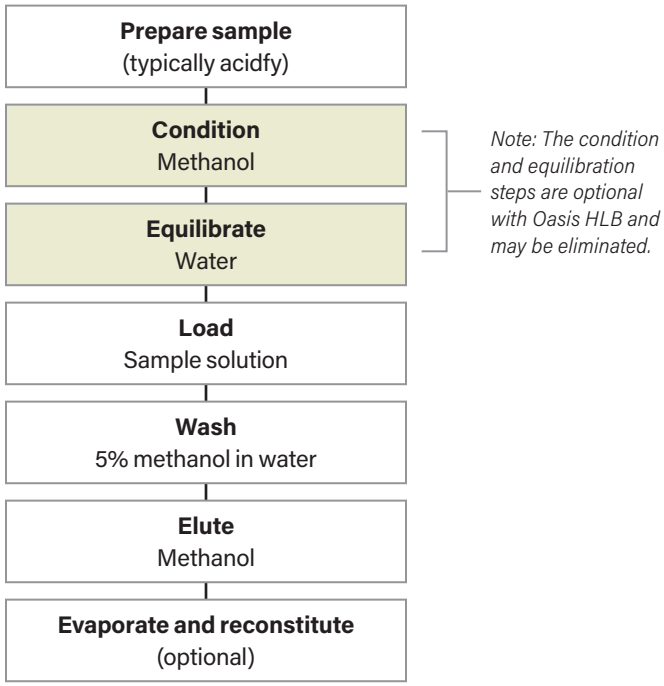
V. SOLID-PHASE EXTRACTION PROCEDURE FOR ACIDIC, NEUTRAL, AND BASIC COMPOUNDS USING OASIS HLB

1. Place Oasis HLB Cartridge or plate on the vacuum manifold and set the vacuum to 5" Hg.
2. Condition with methanol (This step is optional and not necessary when using Oasis HLB).
3. Equilibrate with water (This step is optional and not necessary when using Oasis HLB).
   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 you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

A. GENERIC METHOD OASIS HLB SPE



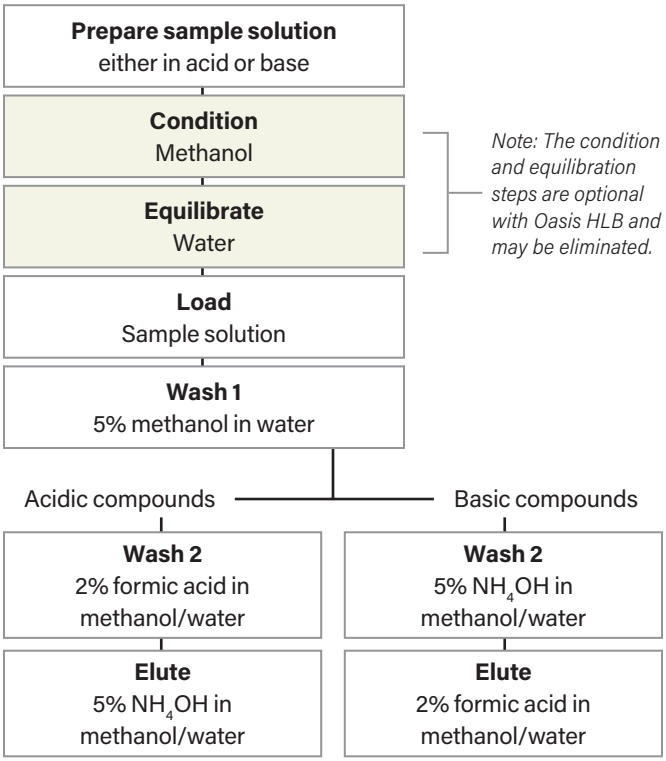
Avoid using methods developed for C_{18} or other silica-based cartidges (see note below).

- The fast Oasis HLB generic method is ideal for LC-MS/MS analysis.
- 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.

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.

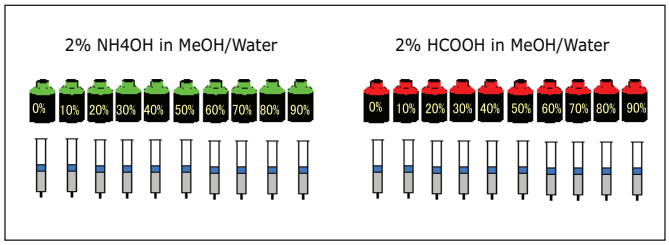
Wash/Elution Steps: 20 Bottle Optimization for Oasis HLB

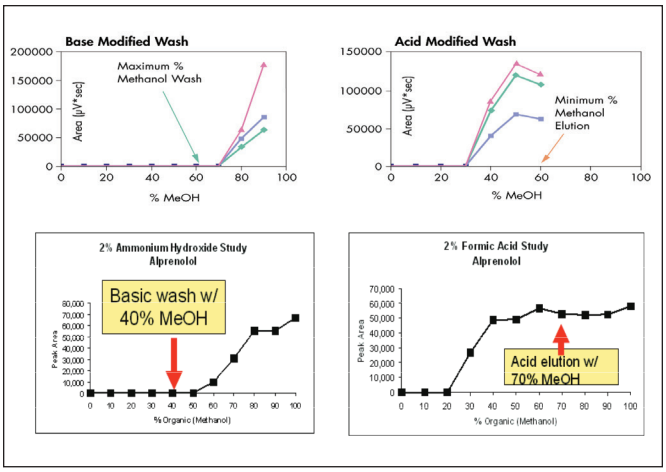
The 20 Bottle Optimization method for Oasis HLB is set up first by spiking the analyte into saline and loaded it into the wells of the 96-well plate or 20 cartridges of Oasis HLB. We prepare the 20 bottle of solvents as described bellow. First, we start with 5% MeOH with base wash step to remove proteins, 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.

C. TROUBLESHOOTING

a. Adjustment to Optimize Recoveries

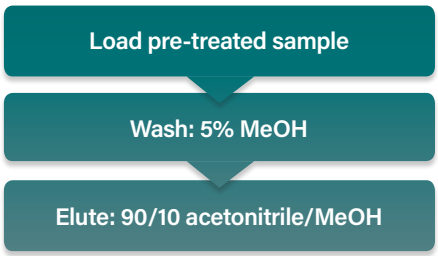
Spike an appropriate volume of reagent water with all analytes and internal/surrogate standards. Follow steps 4a–4e in Section II, but use a rack to collect the eluates in the Load (4c), Wash (4d), and Elute (4e) steps in separate collection vessels. In addition, repeat step 4e with a second portion 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 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 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).

For more information, download Oasis Sample Extraction Products brochure (literature number 720001692en) on www.waters.com.

VI. SOLID-PHASE EXTRACTION PROCEDURES FOR ACIDIC, NEUTRAL, AND BASIC COMPOUNDS USING OASIS PRiME HLB

3 Step (Catch and Release) Protocol

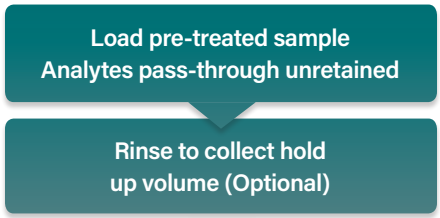


1. Place Oasis PRiME HLB Cartridge or Plate on the vacuum manifold and set the vacuum to 3" Hg.
2. Load your diluted sample.
3. Switch on or open valve at lowest possible vacuum and gradually increase as needed in order to load the entire sample onto the sorbent bed.
4. Switch off the vacuum pump or stop vacuum by closing the valve.
5. Apply 5% methanol in water as a wash solvent.
6. Switch on vacuum to 3" Hg (adjust/increase as needed).
7. Pull vacuum for another 30 sec to a minute to eliminate residual wash solvent.
8. 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).
9. Release vacuum and discard waste fluids, insert collection device and replace the cover.
10. Apply 90/10, ACN/MeOH elution solvent and let it flow through by gravity before switching on the vacuum pump.
11. Switch on or open valve at lowest possible vacuum and gradually increase as needed.
12. Pull vacuum for another 30 sec to a minute (to collect all elution solvent).
13. Remove collection device.
14. Evaporate/reconstitute or dilute as needed.
15. Transfer to vial or plate for analysis, if needed.
16. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

2 Step (Pass-Through) Protocol



Note: The 2 step protocol is ideal for high organic containing samples (such as meat or tissue extracts in ACN) that do not require salt removal and/or concentration.

1. Place Oasis PRiME HLB Cartridge or Plate on the vacuum manifold and set the vacuum to approximately 3" Hg. If using the Plus format cartridges, attach a appropriate reservoir on top of the plus cartridges.
2. Place an appropriate sample collection vessel underneath the device.
3. Load your high organic content sample.
4. Switch on or open valve at lowest possible vacuum (approximately 3" Hg, adjust as needed) and gradually increase as needed in order to pass the entire sample through the sorbent bed, collecting the eluate.
5. Add additional solvent (same as sample load) to the sorbent and pass-though, to rinse out the remaining hold up volume of the device. Collect this volume into the same collection vessel.
6. Pull vacuum for another 30 sec to a minute (to collect all solvent).
7. Remove collection device.
8. Evaporate/reconstitute or dilute as needed.
9. Transfer to vial or plate for analysis, if needed.
10. If using plates, cover prior to analysis.

Manual Operation Using Plus Cartridges

Notes: Plus Light/Plus Short cartridges can be used without a vacuum or positive pressure manifold. The Oasis PRiME HLB Cartridge is attached to the bottom of a syringe, then the sample is pushed through the cartridge.

1. Withdraw the sample extract from the sample container according to Table 3.
2. Attach the Plus cartridge to the tip of syringe. Push the piston and collect the cleaned up sample into a sample vial.
3. If desired, dilute the sample extract with appropriate solvent for LC or GC analysis.

Optimization

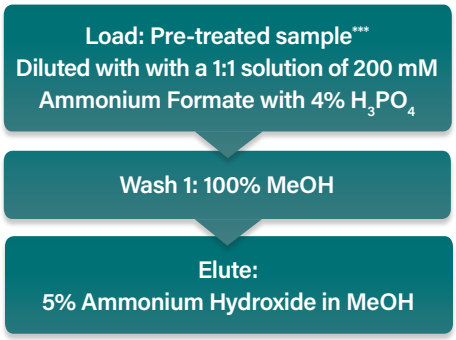
Wash step: The wash step may be optimized by increasing the concentration of MeOH in water, dependent upon the hydrophobicity of your target analytes. Choose the highest percentage of MeOH that will not elute the anaytes of interest while allowing less hydrophobic interferences to be washed away.

Elution step: The 90/10, ACN/MeOH elution solvent is optimal for allowing complete elution of most target analytes while keeping matrix interferences trapped on the sorbent. This step may be optimized to increase the MeOH concentration up to approximately 30% if a higher volume of a protic solvent is required for elution.

For more information, download Oasis Sample Extraction Products brochure (literature number 720001692en) on www.waters.com.

Oasis PRiME MCX 3 Step Protocol

3 Step Oasis PRiME MCX Protocol



****Note: The sample is diluted 1:1 with a solution containing 200 mM ammonium formate with 4% H_3PO_4, making a final concentration of 100 mm Ammonium formate and 2% H_3PO_4.*

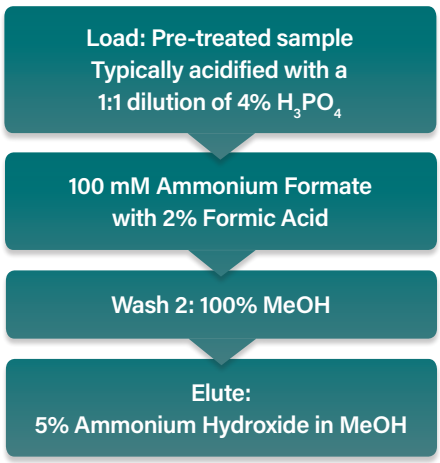
1. Place Oasis PRiME MCX Cartridge or Plate onto the vacuum manifold and set the vacuum to 3" Hg.
2. Load sample diluted as suggested in the protocol, or with another acidic solution appropriate for the disruption of protein binding and charging the basic compounds.
3. Open valve and/or switch on the vacuum at the lowest setting and gradually increase as needed to load the entire sample onto the sorbent bed.
4. Switch off the vacuum pump or stop the vacuum by closing the valve.
5. Apply 100% Methanol as a wash solvent.
6. Switch on vacuum to 3" Hg (adjust/increase as needed).
7. Pull vacuum for another 30 seconds up to 1 minute to eliminate residual wash solvent.
8. Switch off the vacuum pump or stop vacuum by closing the valve (before switching off the pump, reduce the vacuum to the lowest possible setting).
9. Release the vacuum and discard waste fluids, insert collection device and replace the cover.
10. Apply 5% Ammonium Hydroxide in 100% Methanol as the elution solvent and let it flow through by gravity before switching on the vacuum pump.
11. Switch on or open the valve at the lowest possible vacuum and gradually increase as needed.
12. Pull vacuum for an additional 30 seconds up to 1 minute (to collect all of the elution solvent).
13. Release the vacuum and remove the collection device.
14. Evaporate and reconstitute or dilute as needed prior to analysis.
15. Transfer to vial or plate if needed.
16. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

Oasis PRiME MCX 4 Step Protocol

4 Step Oasis PRiME MCX Protocol***



**** Contains an additional wash step that can be used to remove additional matrix interferences if needed*

1. Place Oasis PRiME MCX Cartridge or Plate onto the vacuum manifold and set the vacuum to 3" Hg.
2. Load sample diluted as suggested in the protocol, or with another acidic solution appropriate for the disruption of protein binding and charging the basic compounds.

3. Open valve and/or switch on the vacuum at the lowest setting and gradually increase as needed to load the entire sample onto the sorbent bed.
4. Switch off the vacuum pump or stop the vacuum by closing the valve.
5. Apply 100 mM Ammonium Formate with 2% Formic Acid as a wash solution
6. Switch on vacuum to 3" Hg (adjust/increase as needed)
7. Pull vacuum for another 30 seconds up to 1 minute to eliminate residual wash solvent.
8. Switch off the vacuum pump or stop vacuum by closing the valve (before switching off the pump, reduce the vacuum to the lowest possible setting).
9. Apply the 100% Methanol as a wash solution
10. Switch on vacuum to 3" Hg (adjust/increase as needed)
11. Pull vacuum for another 30 seconds up to 1 minute to eliminate residual wash solvent.
12. Switch off the vacuum pump or stop vacuum by closing the valve (before switching off the pump, reduce the vacuum to the lowest possible setting).
13. Release the vacuum and discard waste fluids, insert collection device, and replace the cover.
14. Apply 5% Ammonium Hydroxide in 100% Methanol as the elution solvent and let it flow through by gravity before switching on the vacuum pump.
15. Switch on or open the valve at the lowest possible vacuum and gradually increase as needed.
16. Pull vacuum for an additional 30 seconds up to 1 minute (to collect all of the elution solvent).
17. Release the vacuum and remove the collection device.
18. Evaporate and reconstitute or dilute as needed prior to analysis.
19. Transfer to vial or plate if needed.
20. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

VI. SOLID PHASE EXTRACTION FOR BASIC COMPOUNDS USING OASIS MCX AND STRONGLY ACIDIC COMPOUNDS USING OASIS WAX

1. Place Oasis MCX or Oasis WAX 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.

Note: It may be possible to eliminate the condition and equilibration steps due to the water-wettable nature of Oasis HLB. If desired, compare the results of the protocol with and without these steps to evaluate their impact on final analytical results.

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).
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 2% fromic acid in water or other suitable acid (such as 0.1 N HCL) as 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. Apply 100% organic elution solvent and let it flow through by gravity before switching on the vacuum pump.
13. Switch on vacuum to 5" Hg (adjust/increase as needed).
14. Pull vacuum for another 30 sec to a minute to eliminate residual wash solvent.
15. 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).
16. Release vacuum and discard waste fluids, insert collection device, and replace the cover.
17. Apply 5% ammonium hydroxide in methanol as elution solvent and let it flow through by gravity before switching on the vacuum pump.

18. Switch on or open valve at lowest possible vacuum and gradually increase as needed.
19. Pull vacuum for another 30 sec to a minute (to collect all elution solvent).
20. Remove collection device.
21. Evaporate/reconstitute or dilute as needed.
22. Transfer to vial or plate for analysis.
23. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

VII. SOLID PHASE EXTRACTION FOR ACIDIC COMPOUNDS USING OASIS MAX AND STRONGLY BASIC COMPOUNDS USING OASIS WCX

1. Place Oasis MAX or Oasis WCX 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.

Note: It may be possible to eliminate the condition and equilibration steps due to the water-wettable nature of Oasis HLB. If desired, compare the results of the protocol with and without these steps to evaluate their impact on final analytical results.

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).
5. Load your diluted sample.
6. Switch on or open valve at lowest possible vacuum and gradually increase as needed.
7. Switch off the vacuum pump or stop vacuum by closing the valve.
8. Apply 5% ammonium hydroxide in water as 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. Apply 100% organic elution solvent and let it flow through by gravity before switching on the vacuum pump.
13. Switch on vacuum to 5” Hg (adjust/increase as needed).
14. Pull vacuum for another 30 sec to a minute to eliminate residual wash solvent.
15. 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).
16. Release vacuum and discard waste fluids, insert collection device and replace the cover.
17. Apply 2% formic acid in methanol as elution solvent and let it flow through by gravity before switching on the vacuum pump.
18. Switch on or open valve at lowest possible vacuum and gradually increase as needed.
19. Pull vacuum for another 30 sec to a minute (to collect all elution solvent).
20. Remove collection device.
21. Evaporate/reconstitute or dilute as needed.
22. Transfer to vial or plate for analysis.
23. If using plates, cover prior to analysis.

Note: For the Load and Elute steps, it is recommended that you should observe discrete drops eluting from the SPE cartridge or wells to ensure adequate interaction between the liquid, compounds and SPE sorbent.

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

VIII. OASIS SORBENT SELECTION AND PROTOCOL CHART

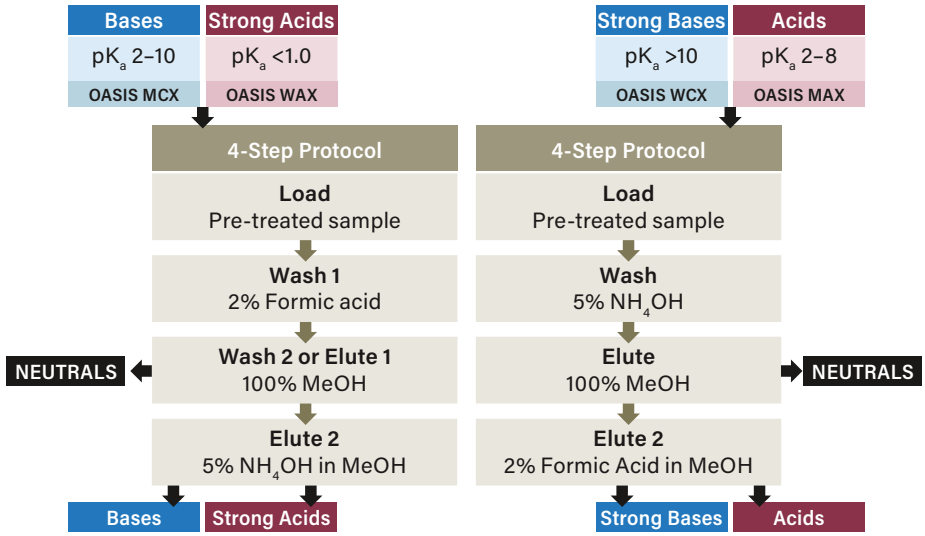
The Oasis 2x4 Method is a simple, logical approach to the selection of an SPE sorbent and protocol. Two protocols and four sorbents provide the flexibility to extract acids, bases, and neutrals with high SPE recoveries while removing matrix components that may interfere with analysis.

Follow the simple steps outlined in this flow chart to achieve high recoveries and the cleanest extracts:

- Characterize your analyte [Neutral, Acid or Base, pK_a]
- Select one of the four Oasis Sorbents
- Apply the indicated Protocol [1 or 2]
- Determine SPE recoveries by LC analysis

**Recoveries for all acids, bases, and neutral compounds aregreater than 85% with RSD's less than 5%.*

Oasis 2 × 4 Method Development Protocol



IX. OASIS 2X4 METHOD OPTIMIZED FOR µELUTION PLATE

The proven Oasis 2x4 Method elution solvent is optimized to accommodate the elutropic requirement of the small elution volume. Methanol is good as a generic elution solvent, but is often not strong enough for 25 µL elution volumes. The elution solvent recommended to be used with the µElution Plate must possess a high enough elutropic strength to fully elute analytes in small volumes, and be appropriate for a diverse set of analytes.

The recommended elution solvent for the Oasis 2x4 Method optimized for the µElution Plate format is 60% CH_3CN:40% CH_3OH with a modifier. This was chosen as a starting point as it meets all of the above criteria.

Note: In order to ensure optimal performance with plasma samples, a small amount of the equilibrium solution should remain on the top of the bed prior to loading.

For more information, download Oasis Sample Extraction Products brochure (literature number 720001692en) on www.waters.com.

X. OASIS PST µELUTION METHOD OPTIMIZED FOR THERAPEUTIC PEPTIDES BIOANALYSIS

The bioanalysis of therapeutic peptides <5,000 Dalton currently presents analytical challenges for the pharmaceutical industry. Typically, regulatory compliant bioanalytical methods are routinely required to achieve LLOQs in the low pg/mL range in biological matrices, and must demonstrate linear response over several orders of magnitude. Waters offers the new PST Therapeutic Peptide Method Development Kit and protocol that simplifies the process of sample preparation and LC method development for the analysis of therapeutic peptides in plasma. The kit contains an Oasis PST µElution Method Development Plate, a PST reversed-phase column and a detailed screening protocol. Kindly, refer to the detailed protocol in the PST Therapeutic Peptide Method Development Kits for the necessary details needed to screen for a bioanalytical method, that is capable of achieving the required sensitive and accurate quantification of therapeutic peptides <5,000 Dalton.

For more information, download Bioanalysis Method Development Strategy for Therapeutic Peptides (Literature number 720003055en) or, download the Oasis Sample Extraction Products brochure (literature number 720001692en) at www.waters.com.

XI. 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:

Oasis 96-Well Plates:

- 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 microplates.

XII. CAUTIONARY NOTE

Some products may be classified as hazardous during/after use and are intended for use by professional laboratory personnel trained in the competent handling of such materials. Responsibility for the safe use of products rests entirely with the purchaser and user. The Safety Data Sheet (SDS) for these products are available at www.waters.com.

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