

## Application Note

# A Simplified Approach to Optimizing the Oasis PRiME HLB 2 Step Protocol to Maximize Recoveries

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## Abstract

Oasis™ PRiME HLB SPE removes >95% phospholipids from biological matrices, while maintaining the reversed-phase selectivity of Oasis HLB for your analyte of interest. With an easy, fast protocol, Oasis PRiME HLB allows scientists to get clean samples, fast and can be an excellent tool in bioanalytical laboratories.

Previous work described in technology brief ([720008684 <https://www.waters.com/nextgen/global/library/application-notes/2025/oasis-prime-hlb-the-fastest-way-to-clean-samples.html>](#)) shows how a 2 step Oasis PRiME protocol provides % recoveries very similar to the 3 step protocol across a diverse set of analytes.

In this work, we look at strategies to optimize the Oasis PRiME HLB 2 step protocol to improve recoveries for analytes using a single experiment allowing you to achieve the sensitivities needed for your assay.

## Benefits

- Oasis PRiME HLB SPE removes >95% phospholipids from samples
  - A quick, easy 2 step protocol is easily deployable across a diverse set of analytes
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- The 2 step protocol can be optimized in a simple, single experiment to enhance recoveries further, if desired

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## Introduction

Bioanalytical scientists globally are being required to do more with less to support a wide range of therapeutic modalities across multiple programs. In discovery ADME/DMPK laboratories, the emphasis is on efficiency. There is a need to have a single, simplified workflow to support the variety of programs they support. Development laboratories usually try to maximize the sensitivity of their assays to enable them to do more with less samples. For LC-MS/MS based workflows routinely deployed in both discovery and development laboratories, solid phase extraction (SPE) is a common technique used to clean up matrix interferences and selectively extract analytes of interest from the matrices. Oasis sample extraction products provide a wide range of options to fit the needs of the different phases of the journey of a molecule from discovery, through development to the clinic.

Oasis PRiME HLB sets the performance standard for solid phase extraction cleanup in routine analysis. This sorbent will provide reversed-phase cleanup of acidic, basic and neutral compounds from complex sample matrices. Oasis PRiME HLB has been designed to simplify SPE with fast, easy protocols. It also produces cleaner samples by removing greater than 95% of common matrix interferences such as salts, proteins and phospholipids. Based on the Oasis HLB sorbent technology, this water-wettable sorbent does not require conditioning and equilibration to provide excellent recoveries.

We have previously described how Oasis PRiME SPE can be deployed using a simple 2 step protocol to achieve acceptable recoveries across a diverse set of analytes. This quick and easy 2 step protocol saves time and reduces solvent use, making laboratory workflows more efficient. In this work, we examine strategies that can be utilized to optimize the Oasis PRiME 2 step protocol to enhance recoveries even further in assays where additional sensitivity may be required.

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## Experimental

### Chemicals, Reagents, Materials and Solvents

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All analytes used in the panel were procured from Fisher Scientific (Hampton, NH). All solvents, acids and bases used were purchased from Millipore Sigma (St. Louis, MO). Oasis PRiME HLB  $\mu$  elution plates, ACQUITY™ Premier Columns, ACQUITY Premier UPLC System and Xevo TQ Absolute Mass Spectrometer were all obtained from Waters Corporation (Milford, MA).

Compound	Molecular weight	logP
Amiloride	229.67	-0.48
Atenolol	266.33	0.57
o-toluamide	135.16	0.8
Morphine	285.33	0.99
o-toluidine	107.15	1.3
Prednisone	358.42	1.66
Cortisone	360.44	1.98
Corticosterone	346.46	2.09
Cortisone-21-acetate	402.48	2.35
Warfarin	308.32	2.41
Betamethasone-21-acetate	435.51	2.8
17-OH progesterone	330.54	3.2
Imipramine	280.42	4.53
Tamoxifen	371.51	7.10




Table 1. Analyte panel used for study.

## LC Conditions

LC system:	ACQUITY™ Premier UPLC I-Class System
Vials:	96-well Sample Collection Plate, 700 $\mu$ L Round well
Columns:	ACQUITY Premier HSS T3 Column 1.8 $\mu$ m, 2.1 x 50 mm

Column temperature:	55 °C
Sample temperature:	10 °C
Injection volume:	5 µL
Flow rate:	500 µL/min
Mobile phase A:	0.1% Formic acid in 100% Water
Mobile phase B:	0.1% Formic acid in 100% Acetonitrile
Gradient:	5–95% B over 2 minutes. Total run time 3 minutes

## MS Conditions

MS system:	Xevo™ TQ Absolute
Ionization mode:	ESI positive
Acquisition mode:	MRM
Capillary voltage(kV):	2
Desolvation gas flow (L/Hr):	1000
Desolvation temperature (°C):	600
Cone gas flow (L/Hr):	150
Collision gas flow (L/Hr):	0.2
Nebulizer (bar):	7

## Data Management

Instrument control software: MassLynx™ (v4.2)

Quantification software: TargetLynx™ (v4.2)

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## Results and Discussion

Bioanalytical laboratories routinely encounter analytes with diverse physico-chemical properties. For this study, we have picked a panel of analytes spread across the chemical space. As shown in table 1, these analytes have a wide polarity range, from Amiloride with logP of -0.48 to Tamoxifen, with a logP of 7.10. Using this analyte panel, we performed SPE using the Oasis PRiME HLB 3 step and 2 step process. As shown in Figure 1, the % recoveries observed for both the 3 step and the 2 step process were comparable. It was also noted that using the default protocol, the % recoveries observed for the most polar analyte on the panel, Amiloride (logP -0.48) and the most non-polar analyte, Tamoxifen (logP 7.10) were both >80%, highlighting the broad utility of the Oasis PRiME HLB SPE sorbent. For some of the other analytes highlighted in the blue box in Figure 1, the analyte recoveries were moderate (60–80%) and for some other analytes highlighted in red, the analyte recoveries were lower (<60%). Depending on the assay requirements, these recoveries may be adequate to achieve the required levels of sensitivity. In cases where sensitivity levels cannot be reached using the starting protocol, there is an opportunity to optimize the 2 step protocol.

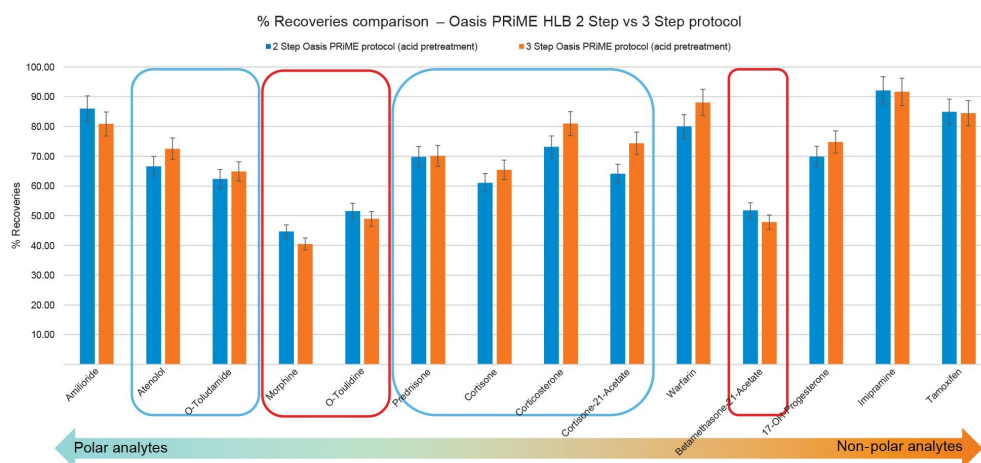


Figure 1. Overview of % recoveries observed for analyte panel.

Taking a closer look at the Oasis PRiME HLB 2 step protocol (Figure 2), there are 2 main areas which can be optimized to improve recoveries. As highlighted, the sample pre-treatment step can be optimized to pre-treat the samples with either 4% phosphoric acid, or 5% ammonium hydroxide. The solvent composition of the elution step can also be fine-tuned to maximize recoveries. Figure 3 shows a plate map for an experimental design that allows for the optimization of both these variables in a single quick experiment. Rows A–D are acid pre-treated pre and post spike samples, whereas rows E–H are base treated. Columns 1–11 use different elution solvents for the entire row, from 100% aqueous to 100% organic.

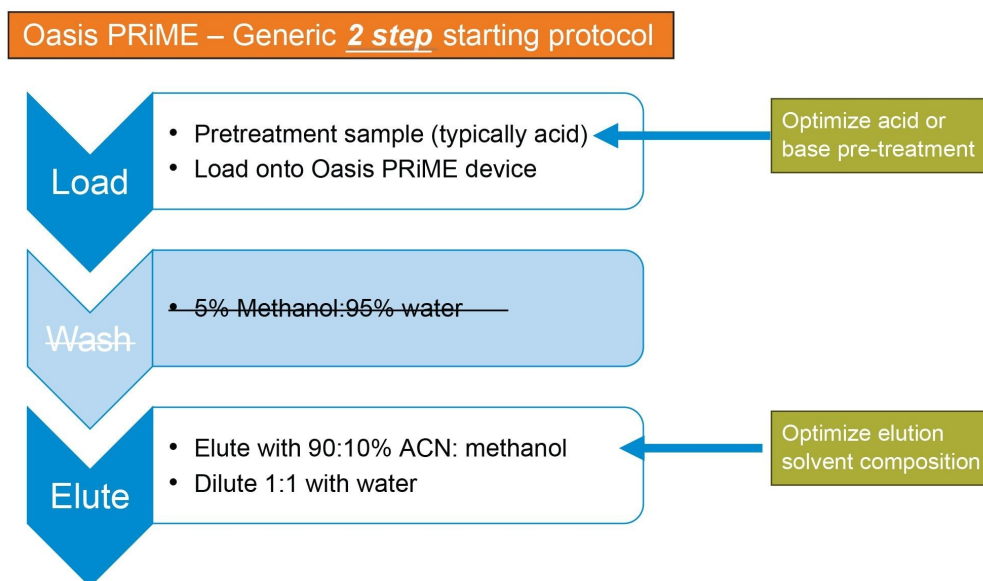


Figure 2. Strategy for protocol optimization.

	1	2	3	4	5	6	7	8	9	10	11	12		
A													Pre-spike	Acid pre-treatment
B														
C													Post-spike	
D														
E													Pre-Spike	Base pre-treatment
F														
G													Post-spike	
H														
Elution solvent	100% Aqueous	90:10	80:20	70:30	40:60	50:50	60:40	70:30	80:20	90:10	100% Organic			

Figure 3. Plate map for method optimization in a single experiment.

Using this experimental set up, we were able to increase the recoveries for the analytes mentioned above which previously had moderate to low recoveries. Figure 4 shows % recoveries plot for (A) Prednisone and (B) Atenolol. Based on the optimization results, % recoveries for prednisone can be maximized using a 20:80 aqueous:organic elution solvent composition. The pre-treatment step does not seem to affect the recoveries meaningfully. For Atenolol, base pre-treated samples eluted using 20:80 aqueous:organic solvent provide the highest recoveries.

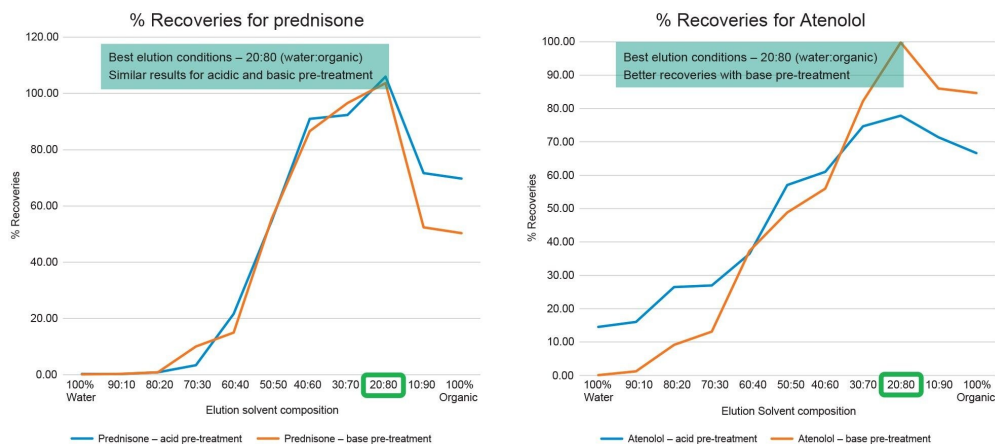


Figure 4. Method optimization results – (A) Prednisone and (B) Atenolol.

For O-Toluidine (Figure 5A) and Morphine (Figure 5B), base pre-treated samples showed better recoveries. Recoveries were highest using 20:80 aqueous:organic for O-Toluidine and 100% organic for Morphine.

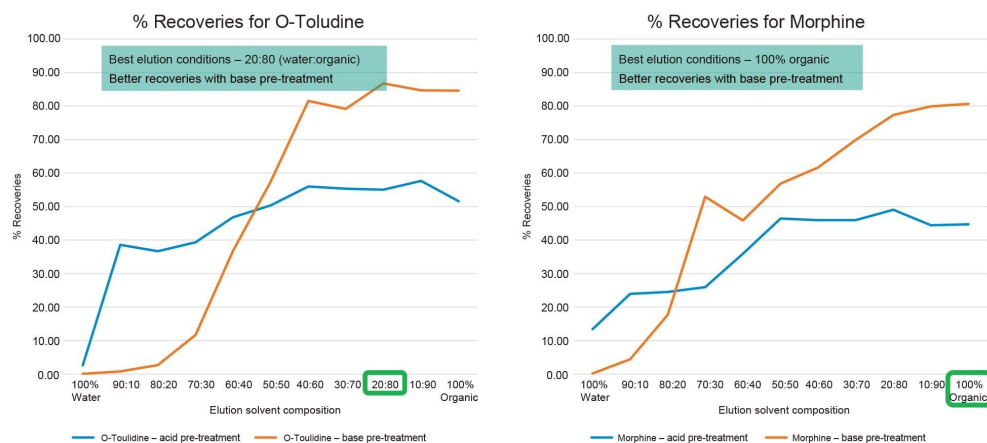


Figure 5. Method optimization results – (A) O-Toluidine and (B) Morphine.

As shown in Figure 6, Betamethasone-21-Acetate showed the highest recoveries when pre-treated with 4% phosphoric acid and eluted using 20:80 aqueous:organic solvent.



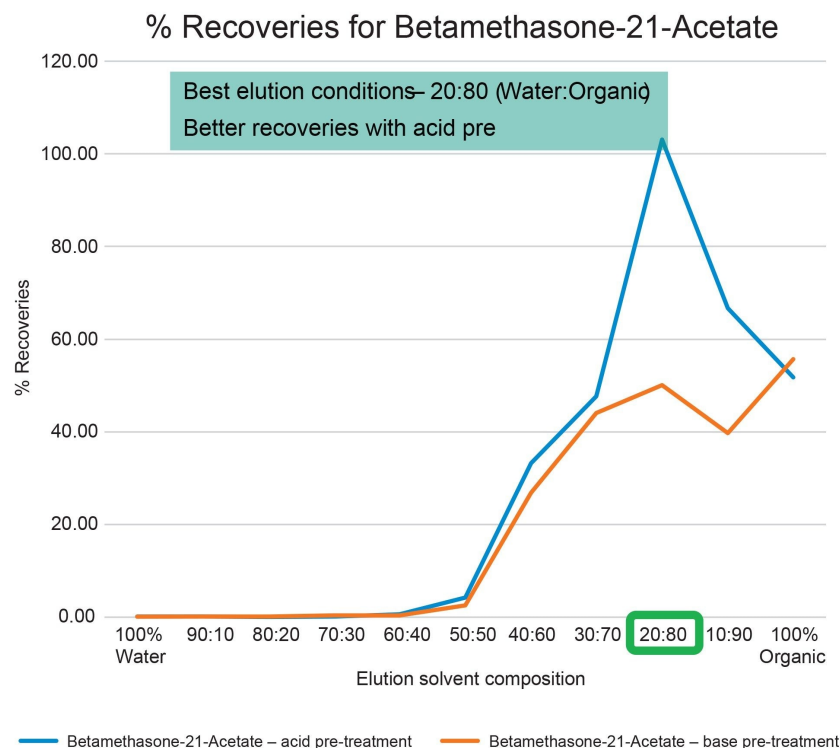


Figure 6. Betamethasone – Low recovery in initial experiment (<60%).

## Conclusion

Oasis PRiME HLB SPE is an easy, quick way to get clean samples fast. Using the default 2 step protocol, we can get adequate recoveries across a diverse panel of analytes. In cases where recoveries need to be optimized to reach desired levels of sensitivity, the 2 step protocol can be fine-tuned using the experimental design outlined to get the best conditions for a given analyte of interest in a single experiment, making method development quick and efficient.

## Featured Products

[ACQUITY Premier System <](#)

<https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-premier-system.html>>

[Xevo TQ Absolute Triple Quadrupole Mass Spectrometer <](#)

<https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html>>

[MassLynx Mass Spectrometry Software <](#)<https://www.waters.com/nextgen/global/products/informatics-and-software/mass-spectrometry-software/masslynx-mass-spectrometry-software.html>>

[ACQUITY Premier HSS T3 Column <](#)<https://www.waters.com/nextgen/global/shop/columns/186009467-acquity-premier-hss-t3-column-18--m-21-x-50-mm-1-pk.html>>

[Oasis PRiME HLB 96-well  \$\mu\$ Elution Plate <](#)<https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186008052-oasis-prime-hlb-96-well--elution-plate-3-mg-sorbent-per-well-1-p.html>>

[96-well Sample Collection Plate <](#)<https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186005837-96-well-sample-collection-plate-700--l-round-well-25-pk.html>>

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