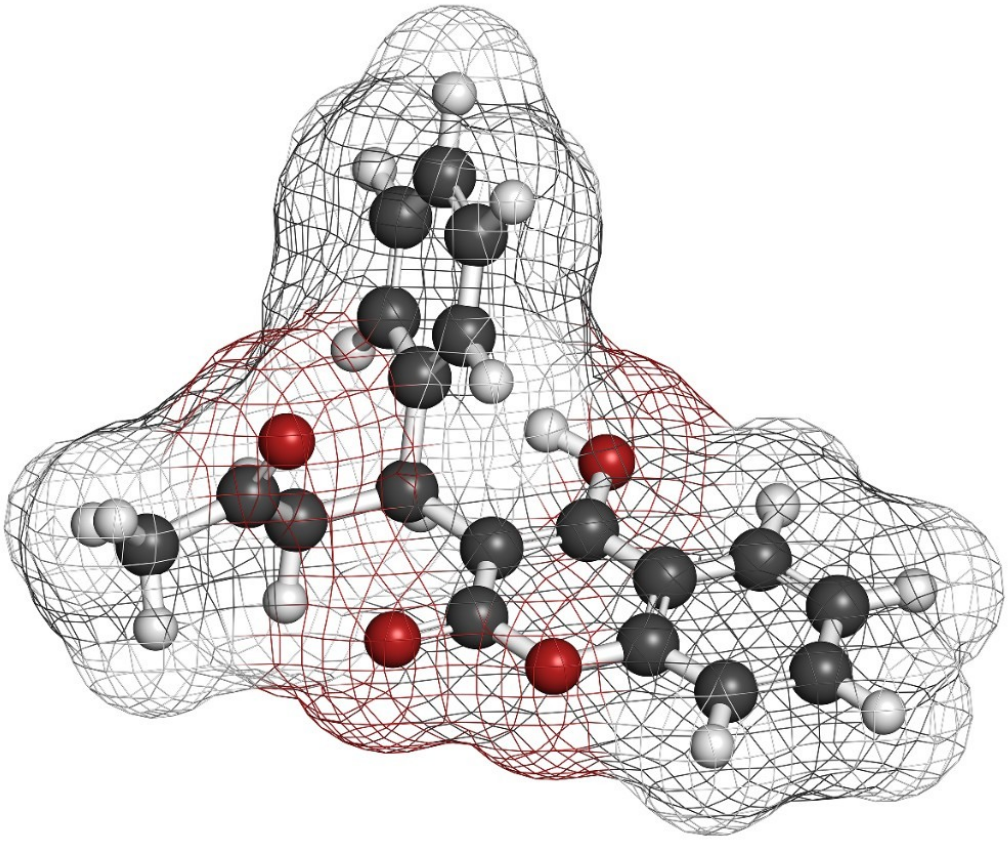
Waters™

應用手冊

# Oasis Sample Extraction Products: Methodology

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

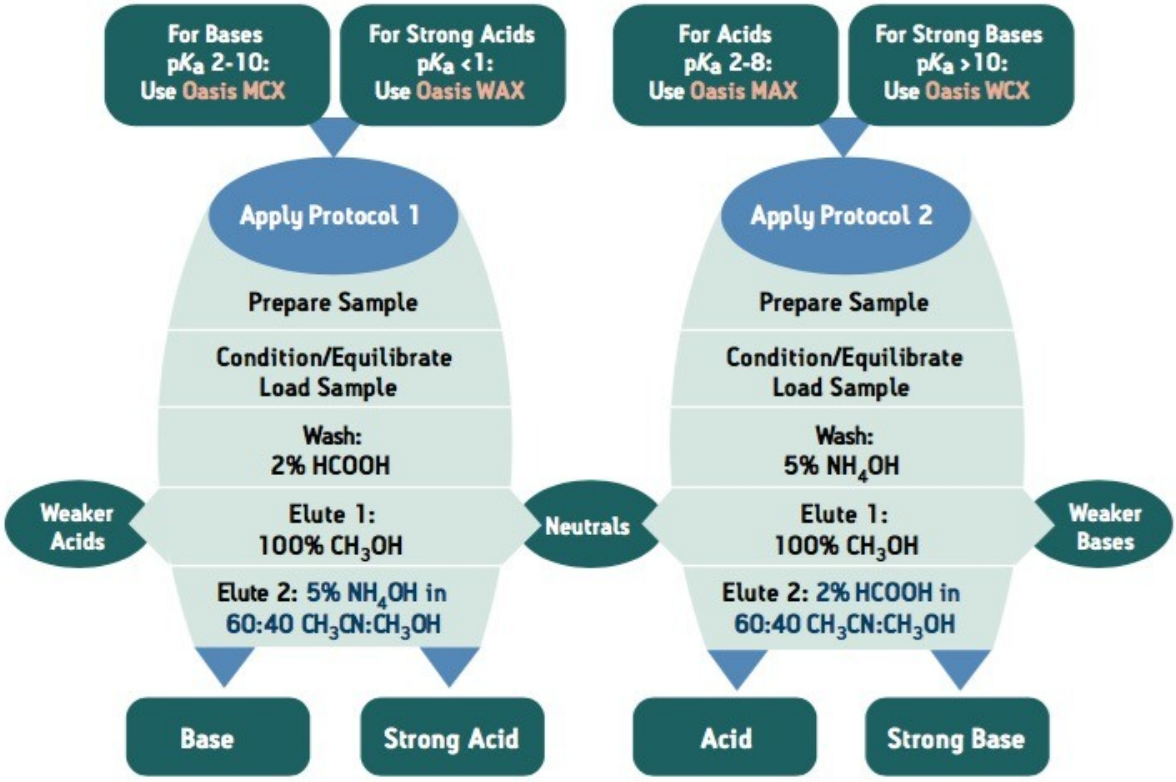
This application brief describes that the Oasis 2x4 Method is a simple, logical approach to the selection of a mixed-mode SPE sorbent and protocol. Two protocols and four sorbents provide the flexibility to extract acids, bases, and neutrals with high SPE recoveries while removing matrix components that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

## Introduction

The Oasis methodology provides a high degree of selectivity for the extraction of analytes from complex biological matrices. By selecting the appropriate Oasis sorbent and protocol, the bioanalytical scientist is able to develop sensitive, reproducible,and robust methods.

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

- Characterize your analyte (Neutral, Acid or Base, pKa).
- Select one of the four Oasis sorbents.
- Apply the indicated Protocol (1 or 2).
- Determine SPE recoveries by LC analysis.




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.

## Experimental

### Recovery Study:

To demonstrate the logic, simplicity, and effectiveness of the Oasis 2x4 method, five samples of rat plasma were prepared, each spiked with one of the previously characterized test analytes shown below:

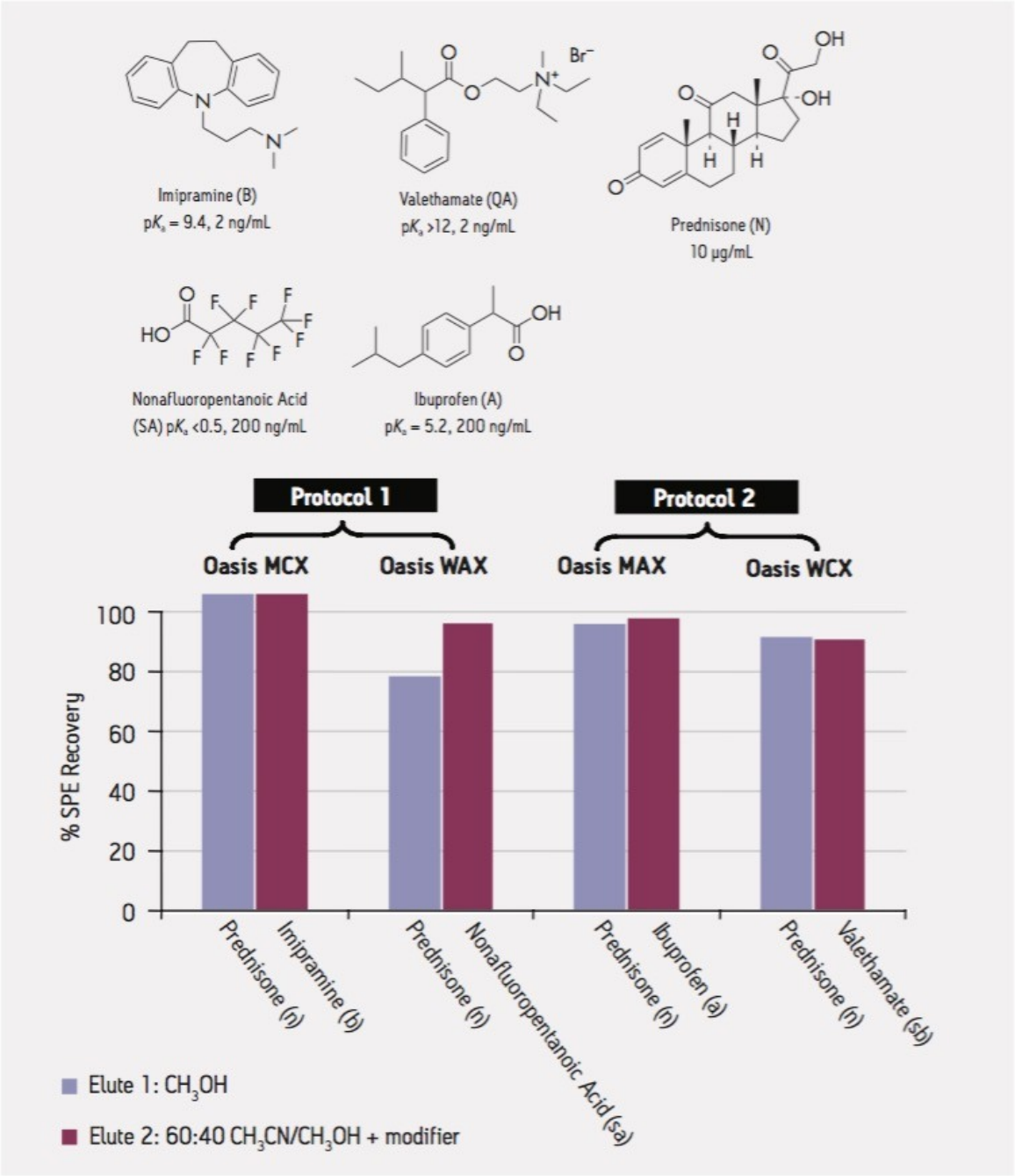
- Imipramine: pKa = 9.4 (Base).
- Ibuprofen: pKa = 5.2 (Acid).

- Valethamate: pKa >12 (Quaternary Amine).
- Nonafluoropentanoic Acid: pKa <0.5 (Strong Acid).
- Prednisone: Neutral.

Each plasma sample was diluted [1:1, v:v] and acidified with phosphoric acid [4% in water]. Respective aliquots were then processed on Oasis µElution sorbent selection plate, using the protocol and the Oasis mixed-mode sorbent designated by the Oasis 2x4 Method for the corresponding sample type. LC-MS/MS analysis was used to determine SPE recoveries. The neutral analyte was processed on all four sorbents used.

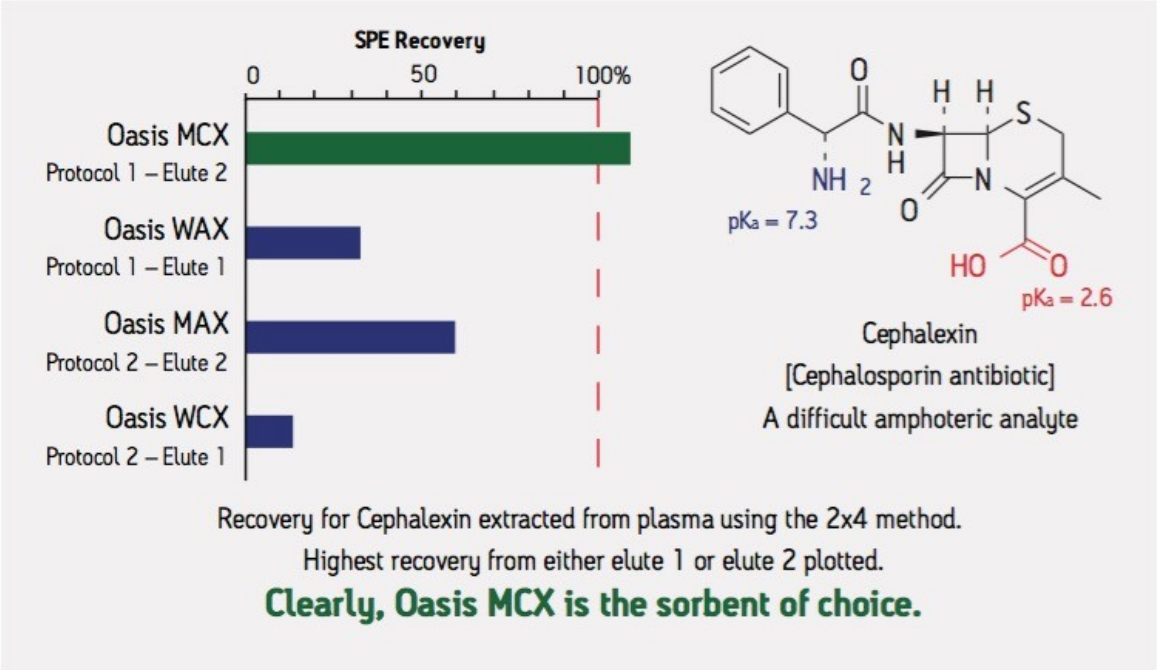
## Results and Discussion

## Analytes Spiked into Rat Plasma




## Oasis Sorbent Selection Plates and Cartridge Kit

The Oasis Sorbent Selection Plates and Cartridge Kit were developed to facilitate rapid development of SPE methods for LC-MS analysis. Having all four Oasis mixed-mode sorbents (MCX, MAX, WAX and WCX) in a single plate is convenient for scouting the best ways to accomplish efficient isolation of unknown, zwitterionic compounds, or mixtures of analytes with different retention/elutionproperties.



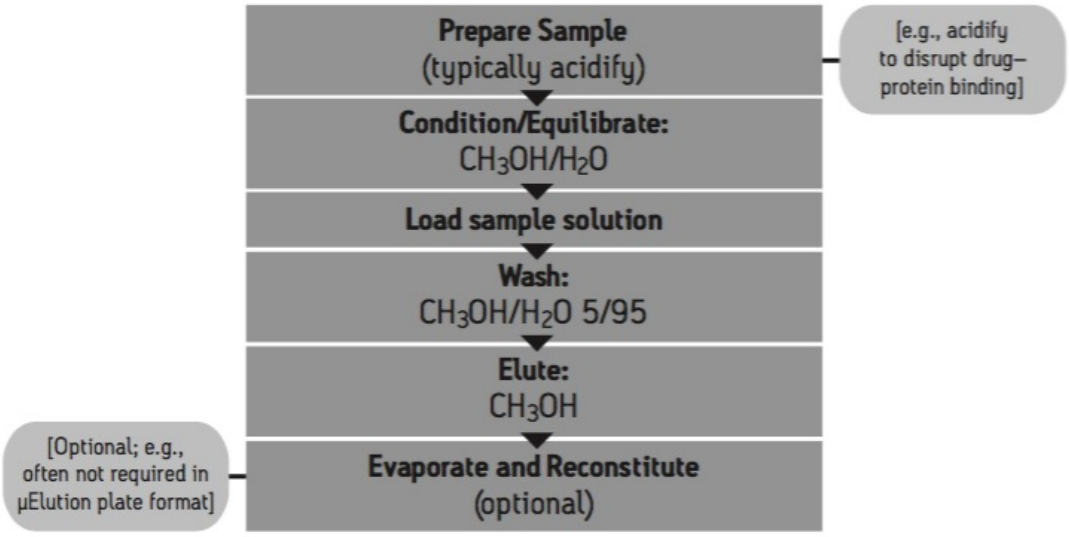

## Oasis HLB Generic Method

When isolating a range of acidic, basic, and neutral compounds, a generic method using Oasis HLB (reveresed-phase only sorbent) is recommended to remove unretained matrix constituents [e.g., salts, sugars, polar lipids, proteins) while reproducibly retaining, and subsequently eluting, a broad chromatographic polarity range of acidic, basic, and neutral analytes.

When transferring methods from C18-bonded-silica phases, consider these four unique advantages of Oasis HLB:

- Its higher capacity (2-3X more surface area) means correspondingly less sorbent weight is required. Bed and elution volumes can be reduced. Smaller-scale device formats are more effective.
- Its higher retention power (3-5X) reduces breakthrough, increases enrichment factors, and permits finer tuning of gradient steps for more selective washing and elution sequences. Especially hydrophobic analytes may require stronger elution solvents.
- Oasis sorbents are water-wettable, maintaining high retention and capacity for a wide spectrum of analytes even if the well or cartridge runs dry.
- The base co-polymer particle is stable from pH 0-14.

## Oasis HLB Generic Method for Acids/Neutrals/Bases




## Conclusion

The Oasis 2x4 Method is a simple, logical approach to the selection of a mixed-mode 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. The generic HLB method provides a simple, universal solution for simultaneously extracting a broad range of compounds from biological matrices.

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