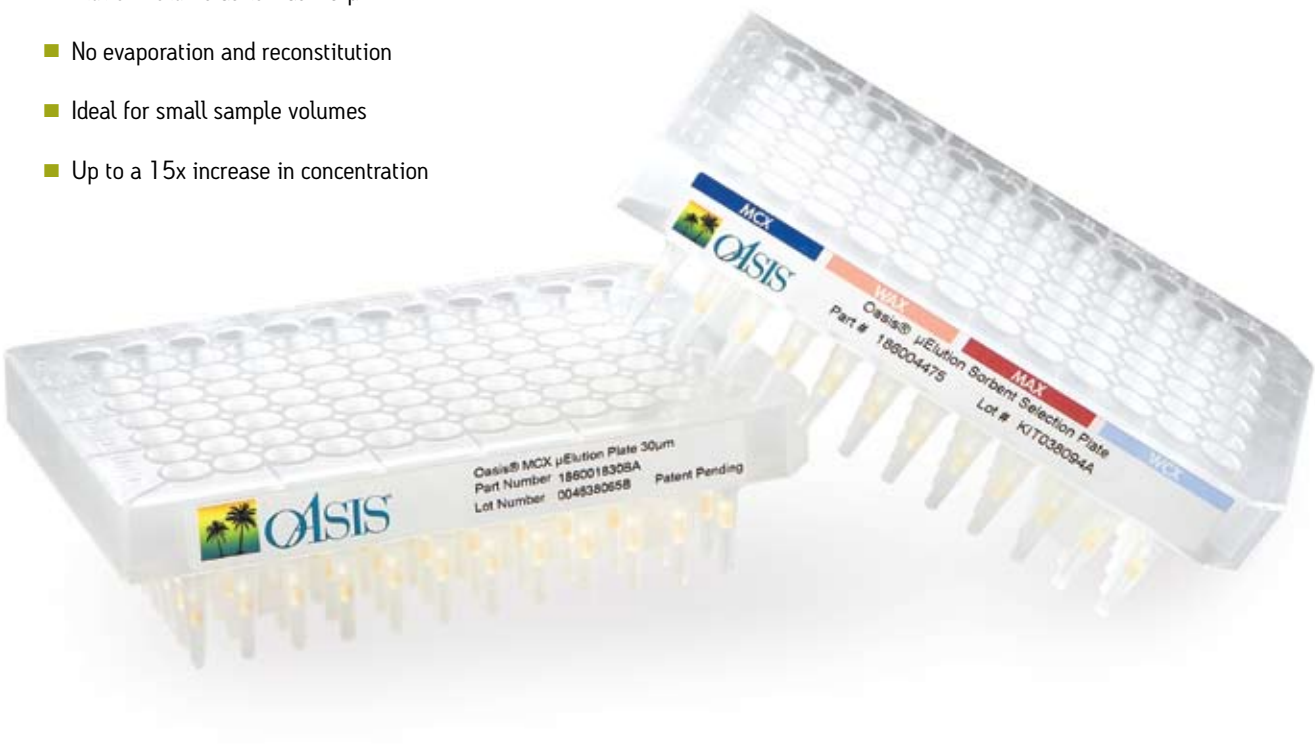
WATERS OASIS µELUTION PLATES

- Elution volume as low as 25 µL
- No evaporation and reconstitution
- Ideal for small sample volumes
- Up to a 15x increase in concentration



PATENTED INNOVATION

Now you can confidently perform SPE cleanup and analyte enrichment of very small sample volumes (10-25 µL) up to a maximum of 375 µL. The Waters Oasis® µElution plate combines patented* plate design, proven Oasis chemistries**, and straightforward protocols that deliver high analyte recovery and clean extracts in elution volumes as low as 25 µL. Using the Oasis µElution plate achieves superior results compared to other conventional SPE formats in less time. This plate format produces concentrated extracts that can be directly injected into your LC/MS/MS, eliminating the need for the time-consuming evaporation step.

Eluting in 25 µL without evaporation provides up to a 15–fold increase in analyte concentration, enabling sensitive, robust, and reproducible SPE results. Scientists in both pharmaceutical drug discovery and drug development can prepare the cleanest biological sample extracts for more sensitive LC/MS/MS analysis.

*U.S. Patent 6,723,236

** U.S. patents 5,882,521 (1996), 5,976,376 (1998), 6,106,721 (1999), 6,254,780 (2001), 6,322,695 (2001), 6,468,422 (2002), 6,726,842 (2004), 6,773,583 (2004), 6,723,236 (2004), additional patents pending.

RAPID AND SENSITIVE METHOD FOR THE DETERMINATION OF ROPINIROLE IN HUMAN PLASMA

Ropinirole is a potent drug marketed under the name Requip® in the U.S. and Adartrel® in Europe. It is prescribed for both restless leg syndrome and Parkinson's disease. A bioanalytical method was developed and validated using Oasis MCX µElution plates for sample preparation and concentration, and an ACQUITY UPLC®/MS/MS for highest sensitivity. The required LLOQ (lower limit of quantitation) of 0.005 ng/mL (5 pg/mL) for ropinirole in human plasma was easily achieved. The response at 0.005 ng/mL is more than 10x the level found in the blank sample, surpassing the FDA guidelines for determining the limit of quantitation in bioanalytical methods.

This example dramatically demonstrates both the powerful extraction capability and capacity of Oasis MCX mixed-mode cation-exchange sorbent in the µElution plate format and the high sensitivity, speed, and specificity capabilities of the combination of Waters ACQUITY UPLC and Quattro Premier™ XE MS systems.

Oasis MCX µElution 96-well Plate Procedure

Condition:
200 µL CH_3OH

Equilibrate:
200 µL H_2O

Load:
600 µL diluted human plasma (300 µL plasma diluted 1:1 with 4% H_3PO_4 in H_2O)

Wash 1:
200 µL 2% CHOOH in H_2O

Wash 2:
200 µL CH_3OH

Elute:
1 x 25 µL 5% NH_4OH in CH_3OH

ACQUITY UPLC Conditions

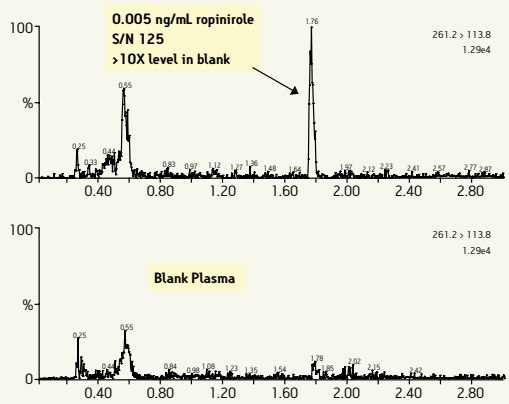
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column part number:	186002350
Mobile phase A:	10 mM NH_4COOH, pH 9
Mobile phase B:	CH_3OH
Flow rate:	0.5 mL/min

Gradient:

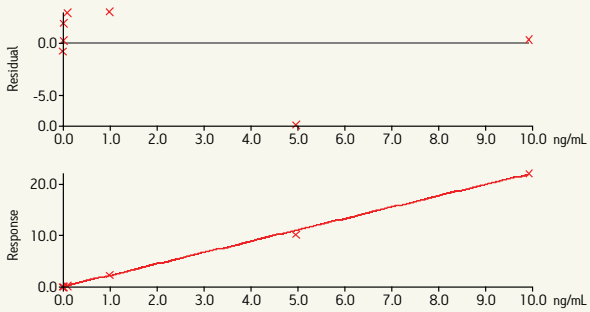
Time (min)	Profile A (%)	Curve B (%)	
0.0	95	5	6
2.0	2	98	6
2.5	2	98	6
2.6	95	5	6
3.0	95	5	6

Injection volume:	8 µL
Column temperature:	45 °C
Sample temperature:	15 °C
Sample diluent:	CH_3OH + 5% NH_4OH
Strong needle wash:	60:40 CH_3CN:IPA + 0.5% HCOOH (1200 µL)
Weak needle wash:	95:5 H_2O:CH_3OH (500 µL)
96-well collection plate part number:	186002481

UPLC®/MS/MS Chromatogram of the XIC for a Sample of Ropinirole in Human Plasma at LLOQ of 0.005 ng/mL

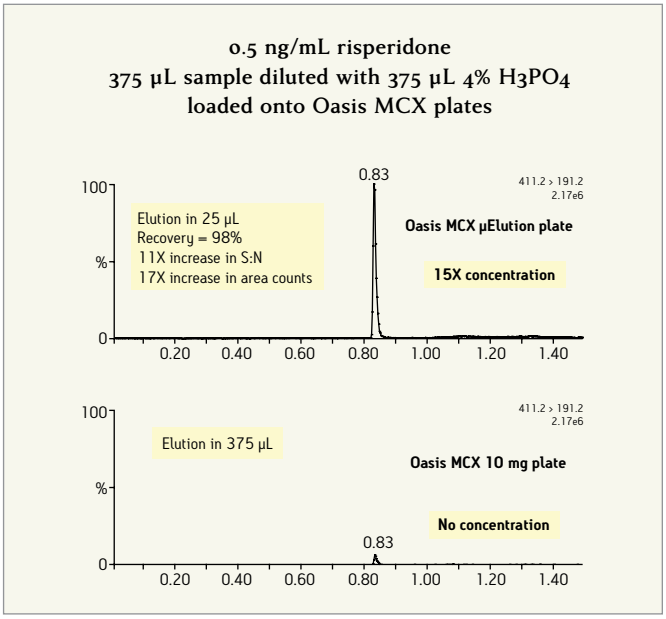


Ropinirole Standard Curve and Linearity Data



Ropinirole standard linear curve fit with $1/x^2$ weighting, from 0.005 ng/mL to 10 ng/mL over 3.5 orders of magnitude, Correlation Coefficient: $r = 0.999054$, $r^2 = 0.998108$, and
Calibration Curve Equation: 2.20969 * x + 0.00746661

SAMPLE ENRICHMENT: UP TO A 15X CONCENTRATION FACTOR



Oasis MCX µElution Plate Protocol

Condition: 200 µL CH_3OH
Equilibrate: 200 µL H_2O
Load: 750 µL diluted plasma (375 µL plasma diluted 1:1 with 4% H_3PO_4 in H_2O)
Wash 1: 200 µL 2% CHOOH in H_2O
Wash 2: 200 µL CH_3OH
Elute: 25 µL 5% NH_4OH in 60:40 CH_3CN:CH_3OH
Inject: 5 µL

Oasis MCX 10-mg Plate Protocol

Condition: 500 µL CH_3OH
Equilibrate: 500 µL H_2O
Load: 750 µL diluted plasma (375 µL plasma diluted 1:1with 4% H_3PO_4 in H_2O)
Wash 1: 500 µL 2% CHOOH in H_2O
Wash 2: 500 µL CH_3OH
Elute: 375 µL (125 µL x 3) 5% NH_4OH in 60:40 CH_3CN:CH_3OH
Inject: 5 µL

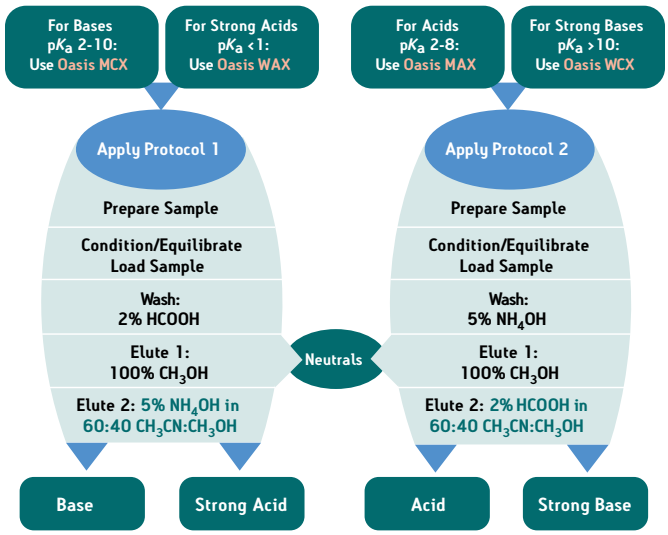
STRAIGHTFORWARD METHODOLOGY

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 the analysis.

This flow chart outlines the simple steps required to achieve high recovery 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.

Oasis 2x4 Method Optimized for µElution Plate



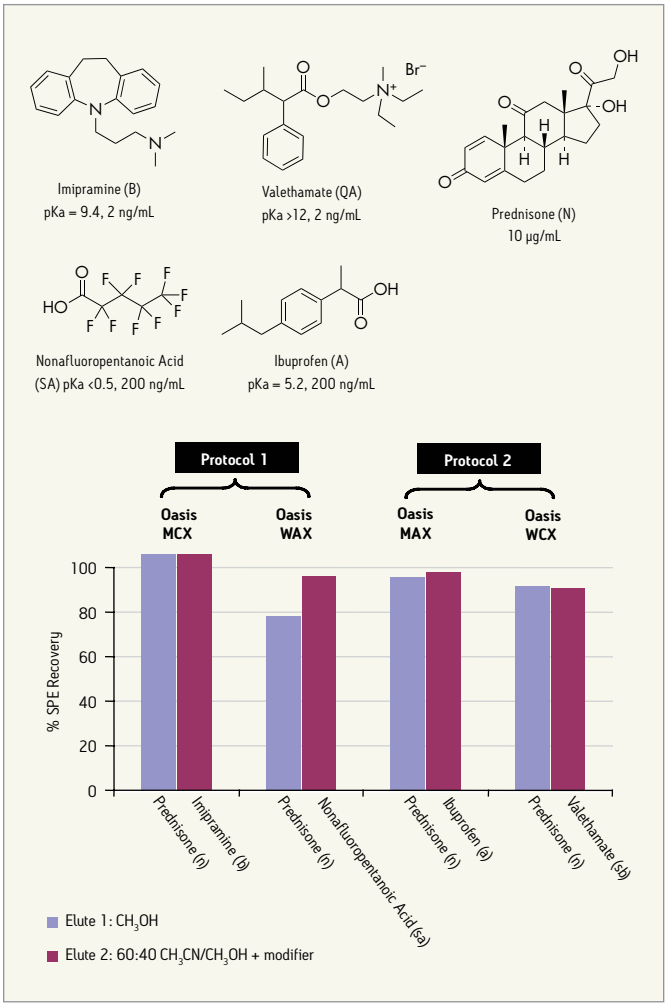
Those who have used the Oasis 2x4 method with cartridges will note that the elutropic strength of the final elution solvent in the above protocols has been increased to optimize recovery in minimal elution volumes. We recommend as a starting point, using a 60:40 ratio of acetonitrile to methanol, not 100% methanol; this provides the optimal elution strength, viscosity, and solubility properties appropriate for high analyte recovery of a diverse set of analytes in only 25 µL.

OASIS 2X4 METHOD PROOF OF CONCEPT

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:

Proof of Concept: Analytes Spiked into Rat Plasma



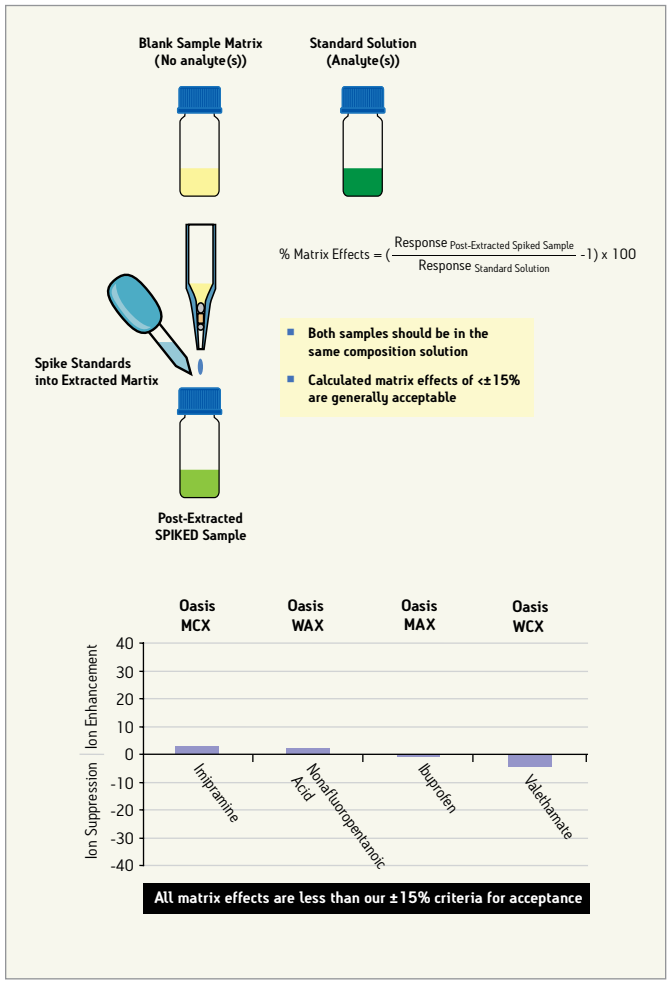
Each plasma sample was diluted [1:1, v:v] and acidified with phosphoric acid [4% in water]. Respective aliquots were then processed using the protocol and the Oasis mixed-mode ion-exchange sorbent designated by the Oasis 2x4 method for the corresponding sample type. SPE recovery was determined by LC/MS/MS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

Matrix Effects Study

Matrix effects* are an alteration in MS response caused by interfering components in a sample. They may cause a loss of signal, ion suppression or gain in signal, ion enhancement. Phospholipids or lysophopholipids have been identified and reported as a major contributor to matrix effects in plasma samples.** Matrix effects decrease analytical method robustness and reproducibility, raise limits of detection/quantitation, and may lead to spurious results.

SPE cleanup using Oasis 2x4 method with Oasis µElution plates effectively reduces plasma interferences and their corresponding matrix effects.

Quantitative Assessment of Matrix Effects:
Matrix Effects Data for the Oasis 2x4 Method Proof of Concept



* Neue and McDonald, Waters Whitepaper

** Chambers et al., J. Chromatogr. B, 2007, Jun 1;852 (1-2): 22-34

µELUTION PLATE LOADING CAPACITY

SPE device capacity is defined as the total mass of analytes and endogenous sample components retained by the sorbent bed under loading conditions. Breakthrough will occur when the capacity of the sorbent bed is exceeded. The physicochemical properties of Oasis sorbents are designed to provide exceptionally high loading capacity, even though each well in a Waters Oasis µElution plate contains only 2 mg of Oasis sorbent.

To determine the Oasis µElution plate capacity, increasing volumes of plasma and urine samples (from 50 µL to 350 µL in 50 µL increments) were spiked with 200 ng/mL imipramine (non polar base) and 200 ng/mL atenolol (polar base). The plasma aliquots were diluted 1:1 with 4% aqueous H_3PO_4 and the urine aliquots were diluted 1:1 with H_2O and then loaded onto the µElution plate. SPE recovery was calculated and plotted for each loading level.

SPE Protocol for Oasis MCX µElution 96-well Plate

Condition:
200 µL CH_3OH

Equilibrate:
200 µL H_2O

Load:
Various volumes of:
- Plasma diluted 1:1 with 4% H_3PO_4 in H_2O
- Urine diluted 1:1 with H_2O

Wash 1:
200 µL 2% CHOOH in H_2O

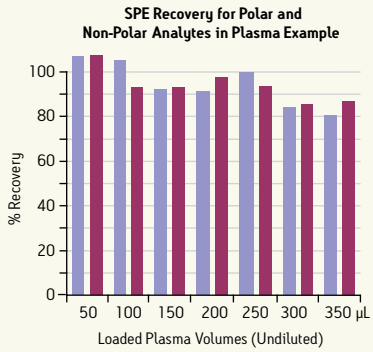
Wash 2:
200 µL CH_3OH

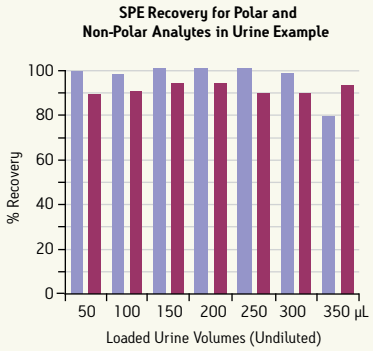
Elute:
2 x 25 µL 5% NH_4OH in 60:40 CH_3CN:CH_3OH

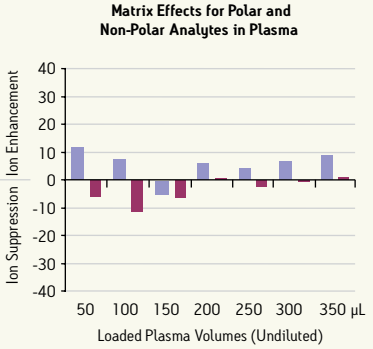
Dilute:
50 µL H_2O

Inject:
5 µL

SPE Recovery for 200 ng/mL Imipramine and 200 ng/mL Atenolol on Oasis MCX µElution Plate







OASIS 96-WELL µELUTION PLATES (1/pkg)

Description	Part Number
Oasis HLB 30 µm	186001828BA
Oasis MCX 30 µm	186001830BA
Oasis MAX 30 µm	186001829
Oasis WCX 30 µm	186002499
Oasis WAX 30 µm	186002500
Oasis Sorbent Selection Plate, 3 rows each MCX, WAX, MAX and WCX	186004475

Oasis µElution Plate

MANIFOLD FOR EXTRACTION PLATES

Description	Qty	Part Number
Extraction plate manifold for Oasis 96-well plates	1/box	186001831
Extraction plate manifold kit A (includes extraction plate manifold, reservoir tray, sealing cap and 350 µL sample collection plate)		WAT097944
Disposable reservoir tray	25/box	WAT058942
Sample collection plate, 350 µL	50/box	WAT058943
Sealing cap for 96-well collection plate	50/pkg	WAT058959
SPE vacuum pump 115 V, 60 Hz		725000417
SPE vacuum pump 240 V, 50 Hz		725000418
Vacuum box gasket kit Kit includes: 2 foam top gaskets 2 orange O-rings		186003522

SPE Vacuum Pump
(Includes two gauges and pressure regulator)

Vacuum Box Gasket Kit

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