Sample Preparation Techniques and Method Development

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Sep-Pak®
Sample Extraction Products

Sep-Pak CERTIFIED

Sample Preparation

DisQuE™
Dispersive Sample Preparation

Waters OASIS®
SAMPLE EXTRACTION PRODUCTS

OSStro™
SAMPLE PREPARATION PRODUCTS

Sirocco™
Protein Precipitation Plate

PoraPak™ Rxn
Post Synthesis Cleanup

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Our Approach to Method Development

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- No "one size fits all"
- Different segments of development process
  - Scientific and business drivers may be different
  - Drivers may be the same but with varying degrees of risk tolerance
- Use of scientifically appropriate criteria for final method choice

Overview

- Food en Environmental

- Bioanalysis

- Synthesis

Dispersive Sample Preparation
"QuEChERS" Method

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- "**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, **S**afe"
- Popular approach to performing sample cleanup
  - Low material costs
  - Method gaining world-wide acceptance
- High throughput sample preparation and screening analysis
- Combination of salting-out LLE and matrix dispersion extraction

DisQuE™
Dispersive Sample Preparation

Dispersive Sample Preparation
Benefits

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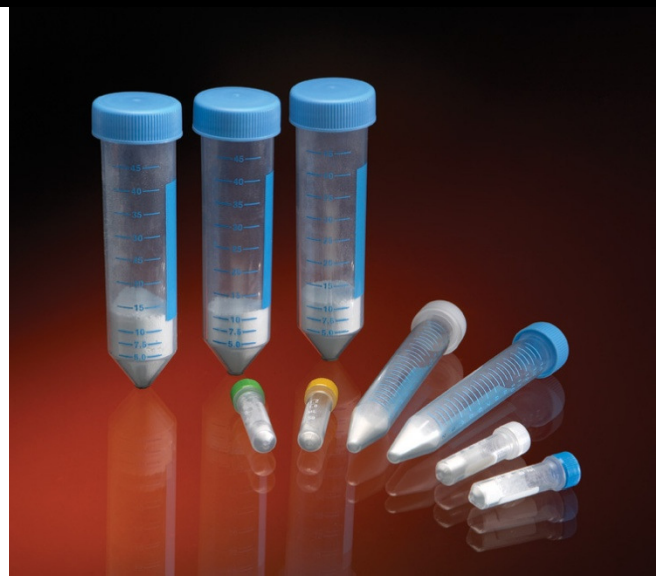

- Low cost
- Minimum specialist skill
- Reduced sample sizes
- Low development and troubleshooting

DisQuE™
Dispersive Sample Preparation

Typical Laboratory Workflow

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- Most "QuEChERS" and dispersive SPE applications require the user to source the individual components including vials, sorbents and buffers.
  - DisQuE Dispersive Sample Preparation Kits eliminate this lab outsourcing
    - Improves consistency in extraction product
    - Improves lab efficiency and workflow
    - Single Vendor source improves quality

- DisQuE offers:
    - Cleaner devices with lower extractables
    - Method and product support to enable customer success

DisQuE Product Line

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Extraction Tubes (Tube 1)

AOAC Configuration	*CEN Configuration*
1.5 g Sodium Acetate 6 g Magnesium Sulphate	4 g Magnesium Sulphate 1 g Sodium Chloride 1 g Trisodium Citrate 0.5 g Disodium Citrate

Clean Up Tubes (Tube 2 - 2 mL Option)

AOAC Configuration		*CEN Configuration*	
150mg Magnesium Sulphate 50mg PSA	150mg Magnesium Sulphate 50mg PSA 50mg C_{18}	150mg Magnesium Sulphate 25mg of PSA	150mg Magnesium Sulphate 25mg of PSA 25mg C_{18}

Clean Up Tubes (Tube 2 - 15 mL Option)

900 mg Magnesium Sulphate 150 mg of PSA	900 mg Magnesium Sulphate 150 mg of PSA 150 mg C_{18}

Extraction Tube 1

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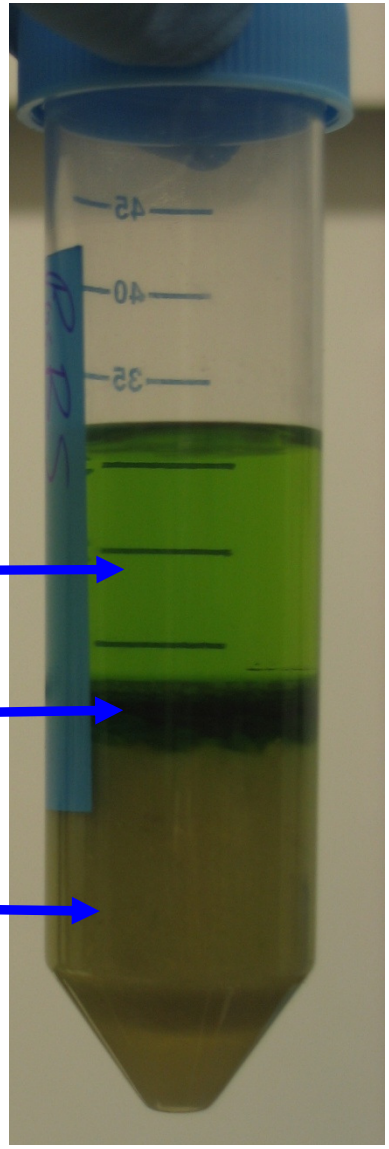
- Water is IMPORTANT!!
  - Removes unwanted matrix
  - Acetonitrile extraction more effective

Acetonitrile Layer **(ANALYTES ARE HERE)**

Water Layer **(POLAR INTERFERENCES)**

Solids Layer **(BUFFER SALTS AND SAMPLE)**



Cleanup Tube 2

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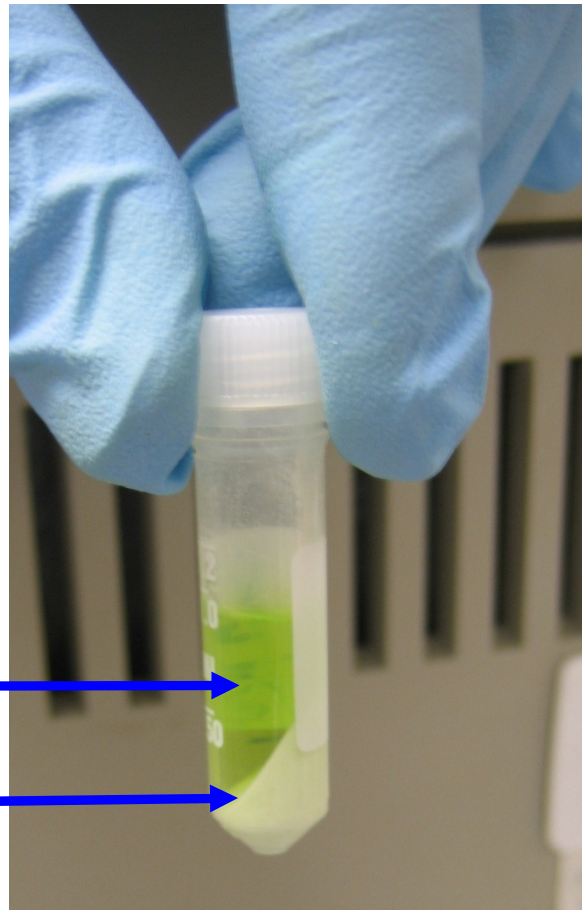
- Provides additional cleanup
- Sorbent choices
  - PSA
  - Graphitized Carbon Black (GCB)
  - C18



Acetonitrile Layer **(ANALYTES ARE HERE)**

Sorbent **(INTERFERENCES)**

DisQuE™ Performance
Highlights from Application Note

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- DisQuE method tested against 402 pesticide residues
  - Application Note: 720002628en
- Approved AOAC Method
  - Ref. Lehotay, *JAOAC Int.* **90**(2) 185-520 (2007).
- Excellent recovery
- Consistent results from matrix to matrix

[APPLICATION NOTE]

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A RAPID METHOD FOR THE SCREENING AND CONFIRMATION OF OVER 400 PESTICIDE RESIDUES IN FOOD

James Morphet and Peter Hancock, Waters Corporation, Manchester, UK

AIM

To utilize the power of UltraPerformance Liquid Chromatography (UPLC®) combined with fast MS acquisition rates, to give a rapid method for the screening of 402 pesticide residues in a single 10 minute run. A second injection, for confirmatory purposes, will meet SANCO Analytical Quality Control procedures for pesticide residue analysis (SANCO/2007/3131).

INTRODUCTION

Pesticides are widely used in the production of foodstuffs to meet consumer demand for plentiful food at reasonable prices, all year round. However, continued growth in the use of pesticides, poor agricultural practices, and illegal use can pose significant risks to human health through the presence of pesticide and metabolite residues in food products. Most countries have strict regulations governing pesticides. Legislation imposes Maximum Residue Limits[2] (MRLs) for pesticide residues in food products requiring analytical techniques that are sensitive, selective, and robust.

Multi-residue pesticide analysis is challenging due to the low levels present, the wide variety of pesticides, and the very different chemical classes they represent. As there are currently well over 1,000 pesticides in use, laboratories are under increasing pressure to broaden the range of pesticides determined in a single analysis over a shortened run time.

The need to meet mandated detection limits, develop generic sample preparation techniques for complex matrixes, and the desire to increase sample throughput are the main challenges facing food safety testing laboratories today. The use of a single multi-residue method per instrument can dramatically improve return on investment by removing the need to change method parameters. This is often the case when analysing a wide variety of commodities with differing lists of legislated pesticides.

Advances in chromatographic separation and detection technologies have enabled analysts to increase the number of analytes determined in a single run. Tandem quadrupole mass spectrometry offers a highly specific and selective detection technique that has become the technique of choice within the laboratory.[3]

The following method describes a solution for the rapid analysis of pesticides in mango, avocado, and fruit based baby food extracts that is able to exceed current worldwide legislation.

EXPERIMENTAL

A QuEChERS extraction was utilized for this multi-residue method by homogenizing a food sample, adding organic solvent, salts, and sorbent. Once mixed, the pesticide residues were partitioned into the organic solvent, which was then subjected to further clean up. The supernatant was collected, diluted, and injected onto the LC/MS/MS system as described below:

Extraction Procedure[4]:

1. Add 15 g homogenized sample to a 50 mL extraction tube containing 1.5 g sodium acetate and 6 g magnesium sulfate. Add 15 mL 1% acetic acid in acetonitrile.
2. Add any pre-extraction internal standards.
3. Shake vigorously for one minute and centrifuge > 1500 rcf for one minute.
4. Transfer 1 mL of the acetonitrile extract in to the 2 mL centrifuge tube containing 50 mg PSA and 150 mg of magnesium sulphate.
5. Shake for 30 seconds and centrifuge >1500 rcf for one minute.
6. Transfer 100 µL of final extract into an autosampler vial. Add any post-extraction internal standards. Dilute with 900 µL water.

Overview

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- Quechers

DisQuE™
Dispersive Sample Preparation

- Bioanalysis

Sirocco™
Protein Precipitation Plate

Ostro™
SAMPLE PREPARATION PRODUCTS

Waters OASIS®
SAMPLE EXTRACTION PRODUCTS

- Synthesis

PoraPak™ Rxn
Post Synthesis Cleanup

Different Methods for Different Purposes: Decision Making Process

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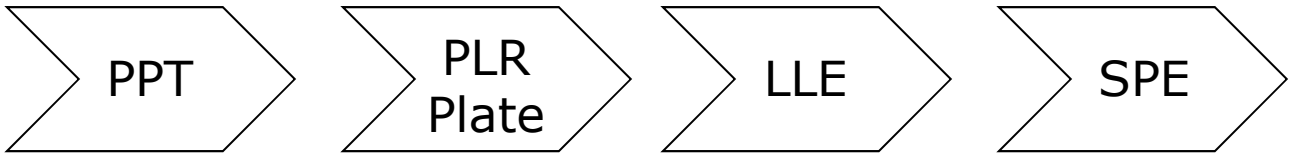

Highest Selectivity

Direct Inject

Simple

High throughput

PL Removal

Highest Sensitivity

"Clean" Extracts

Typical Sample Preparation Selection Process

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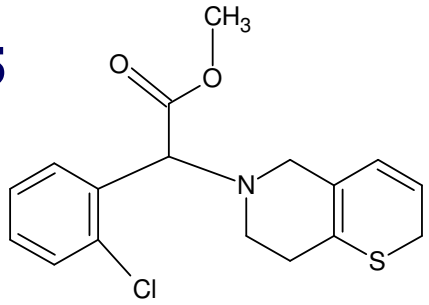
1. The simplest method which meets the assay needs is usually chosen.

2. For very challenging assays (low detection limits, closely related endogenous constituents, inhalation products, peptides, etc), SPE is often the first choice.

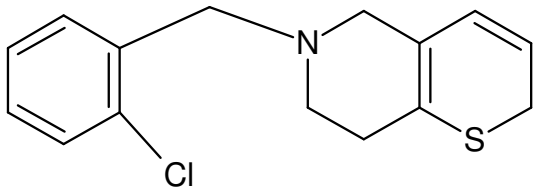
3. Exact technique chosen will depend on outcome of study and how much risk can be tolerated.

Method 1: Clopidogrel and Ticlopidine (IS) in Plasma

Clopidogrel
MW 321.8
pKa = 4.5



Ticlopidine (IS)
MW 263.8
pKa = 7.6



Assay Use:

- Routine analysis of patient samples, GLP or clinical lab

Assay Requirements:

- LLOQ 10-25 pg/mL
- Simple method
- Must transfer across lab with varying levels of expertise
- Concerns about build up of phospholipids on LC columns and in MS source

Clopidogrel and IS: Chromatographic Screening Results

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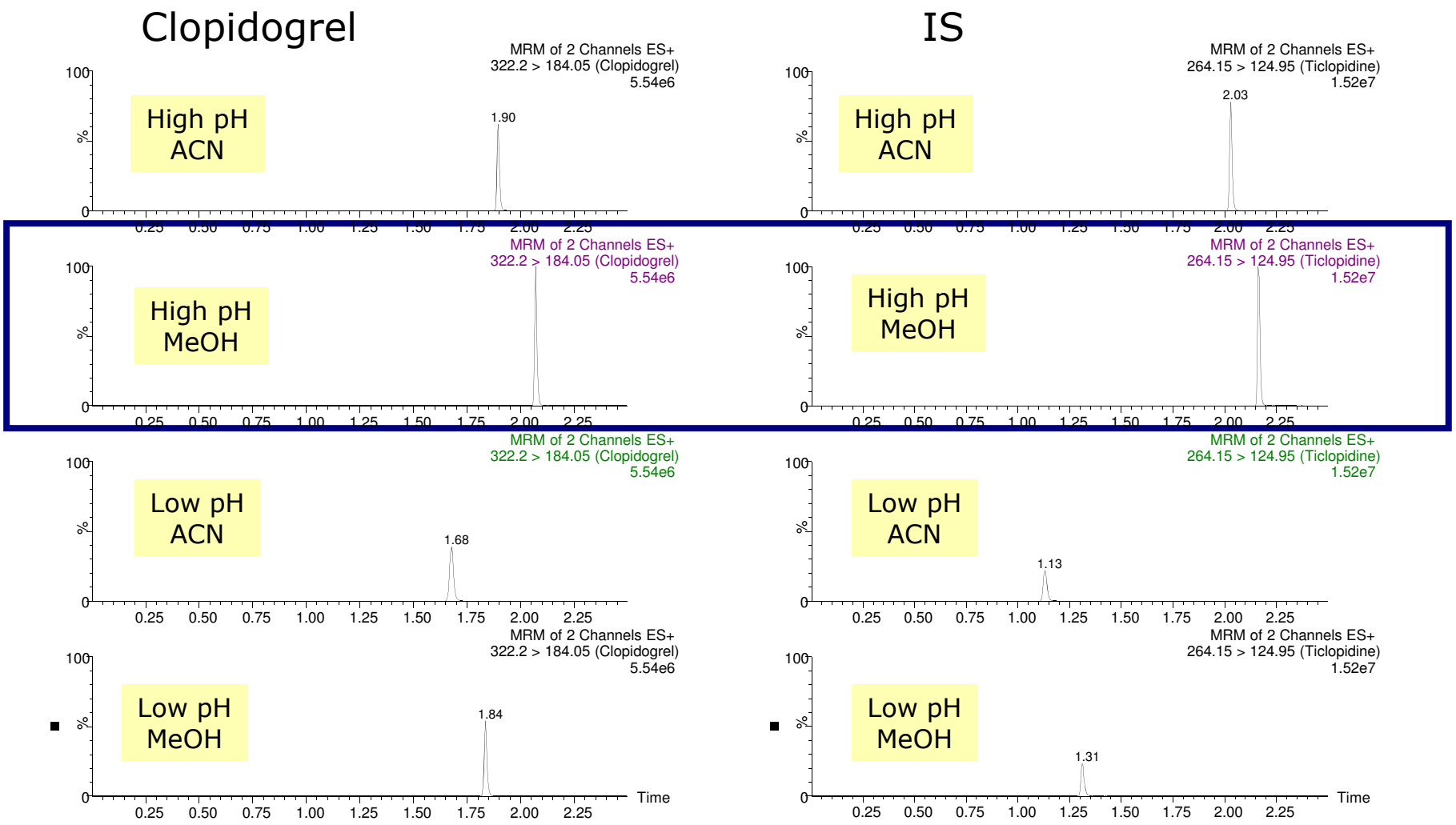
ACQUITY UPLC® BEH C_{18} Column



Sample Preparation Comparison

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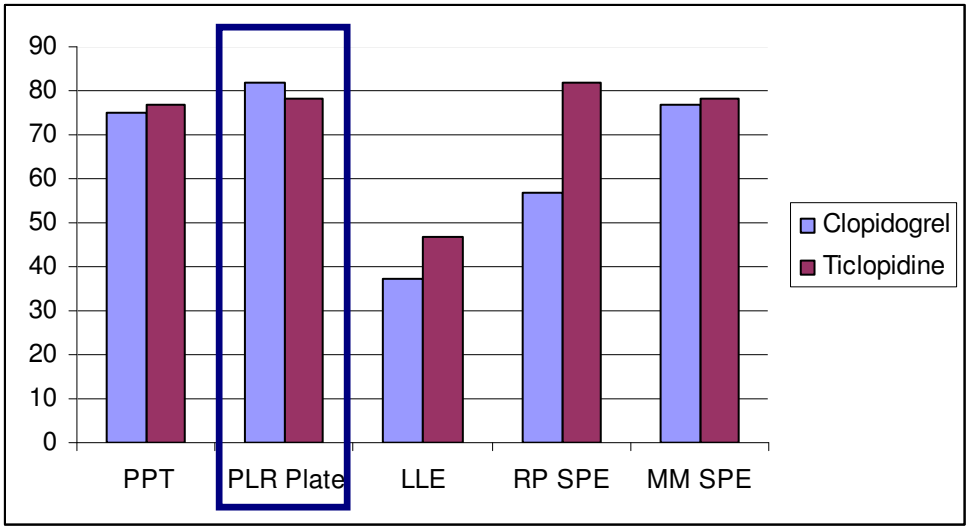
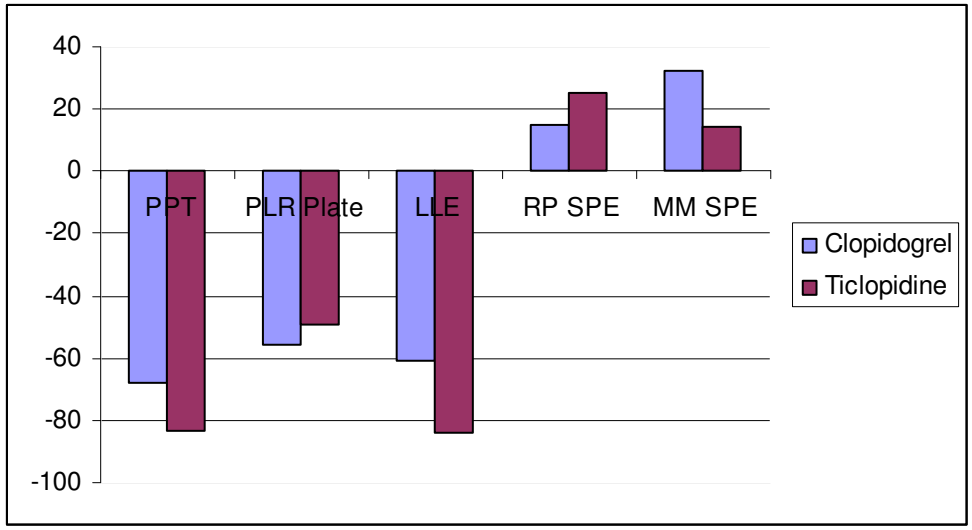
% Analyte Recovery



1. Adequate recovery?
2. Very simple method?
3. Phospholipid removal?

% Matrix Effects



Benefits of Phospholipid Removal Plates for this Application

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- Very simple protocol
  - Easily transferred from lab to lab
- Removes vast majority of phospholipids
  - Improved instrument uptime
  - More robust methods
- High recovery
  - Helps meet detection limits

Representative Validation Results: Ostro™ Sample Preparation Plate

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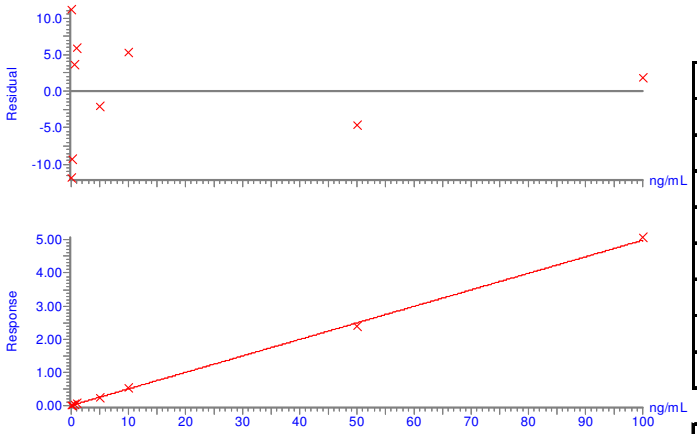
Compound name: Clopidogrel
Correlation coefficient: r = 0.999471, r^2 = 0.998943
Calibration curve: 0.049871 * x + 0.000843056
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



	Standard conc. ng/mL	Clopidogrel Area	IS Area	Response	Calc. conc. ng/mL	%Dev
Standard	0.01	157.3	122606.4	0.001	0.009	-11.8
Standard	0.05	426.5	118007.4	0.004	0.056	11.1
Standard	0.1	560.7	104434.5	0.005	0.091	-9.2
Standard	0.5	3013.1	112846.8	0.027	0.518	3.7
Standard	1	6155.2	114711.6	0.054	1.059	5.9
Standard	5	28044.7	114521.0	0.245	4.894	-2.1
Standard	10	60742.4	115491.1	0.526	10.529	5.3
Standard	50	274997.2	115583.9	2.379	47.690	-4.6
Standard	100	590079.4	116193.5	5.078	101.814	1.8
QC	0.075	432.0	109556.5	0.004	0.062	-17.1
QC	0.75	4382.0	113846.2	0.038	0.755	0.7
QC	7.5	42802.8	113869.8	0.376	7.520	0.3
QC	75	447395.8	110303.7	4.056	81.314	8.4
QC	0.075	355.6	112084.7	0.003	0.047	-37.7
QC	0.75	4944.7	121039.7	0.041	0.802	7
QC	7.5	39973.1	110003.2	0.363	7.270	-3.1
QC	75	403244.8	108186.3	3.727	74.722	-0.4
QC	0.075	534.3	123902.3	0.004	0.070	-7.2
QC	0.75	5479.0	126105.7	0.043	0.854	13.9
QC	7.5	46733.5	118830.3	0.393	7.869	4.9
QC	75	317429.4	93421.1	3.398	68.116	-9.2

Fit for Purpose?

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Assay Requirements:

- LLOQ 10-25 pg/mL

- Simple method

- Must transfer across lab with varying levels of expertise

- Lab is very concerned about build up of phospholipids on LC columns and in MS source

Sample Preparation Options

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NEW Ostro™ Sample Preparation Plate

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Simple Pass-through Method

Ostro™ SAMPLE PREPARATION PRODUCTS

Basic Protocol

1. Add plasma
2. Add organic
3. Aspirate to mix
4. Apply vacuum
5. Analyze

Ostro™ Sample Preparation Plate: Analyte Recoveries

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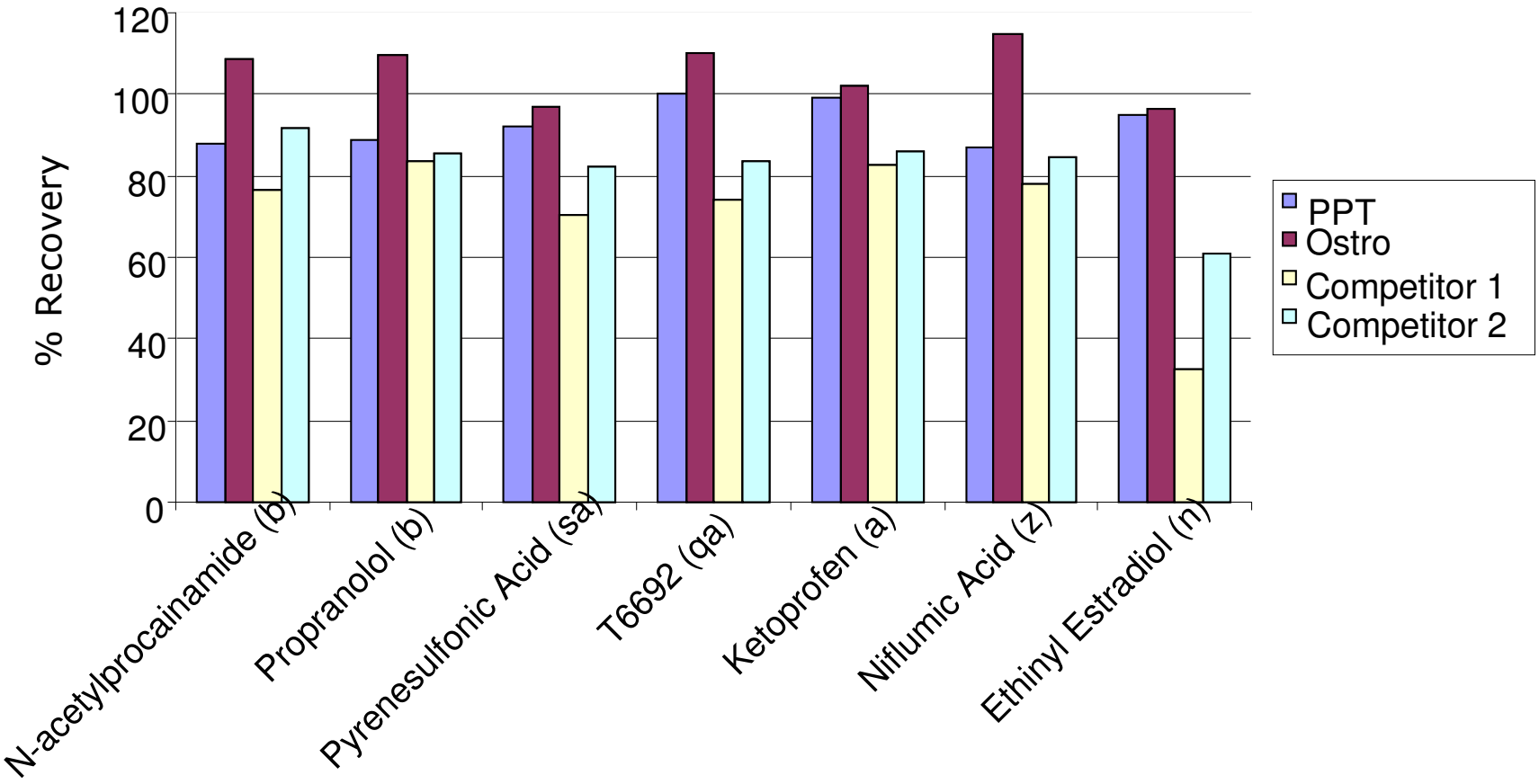
Best Recovery for Diverse Analytes



Ostro™ Phospholipid Removal: Reproducibility

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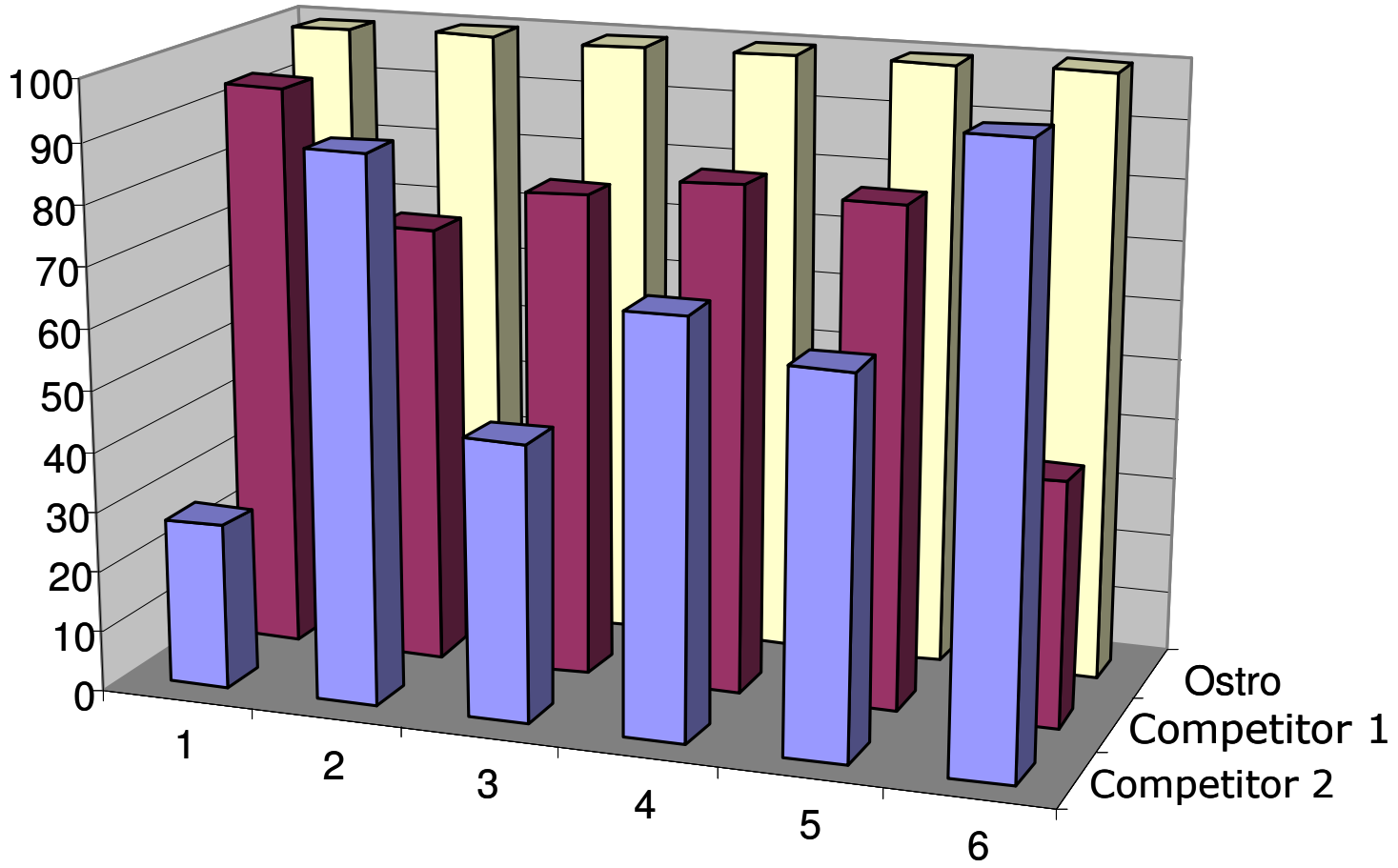

Reproducibility of Phospholipid Removal in 6 Lots of Human Plasma

Method 2: Bioequivalence Study

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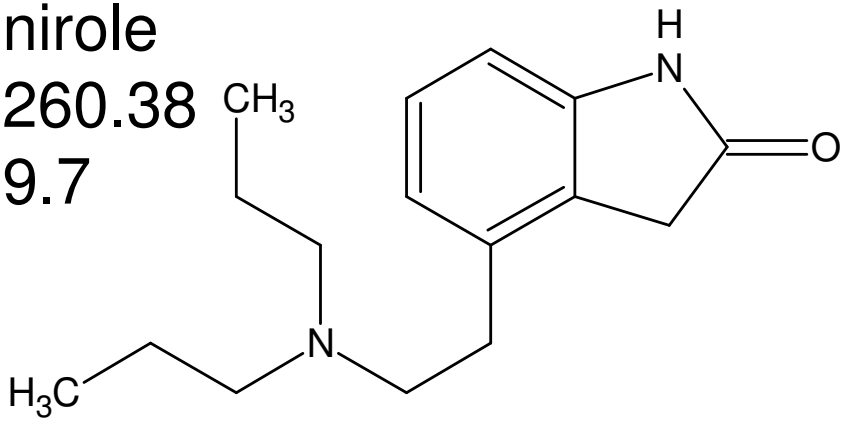
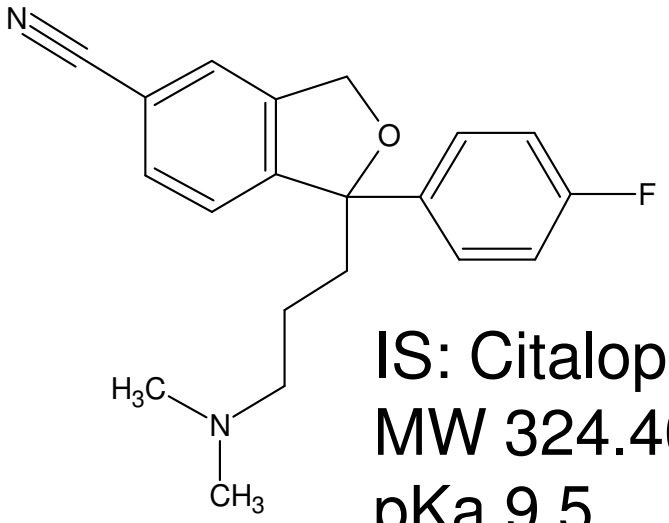
Ropinirole
MW 260.38
pKa 9.7





IS: Citalopram
MW 324.40
pKa 9.5

Assay Use:

- Routine analysis of patient samples, GLP or clinical lab

Assay Requirements:

- LLOQ 5 pg/mL
- Concerned with matrix effects
- Meeting FDA requirements
- Fast method development

Oasis® Solid-Phase Extraction (SPE)

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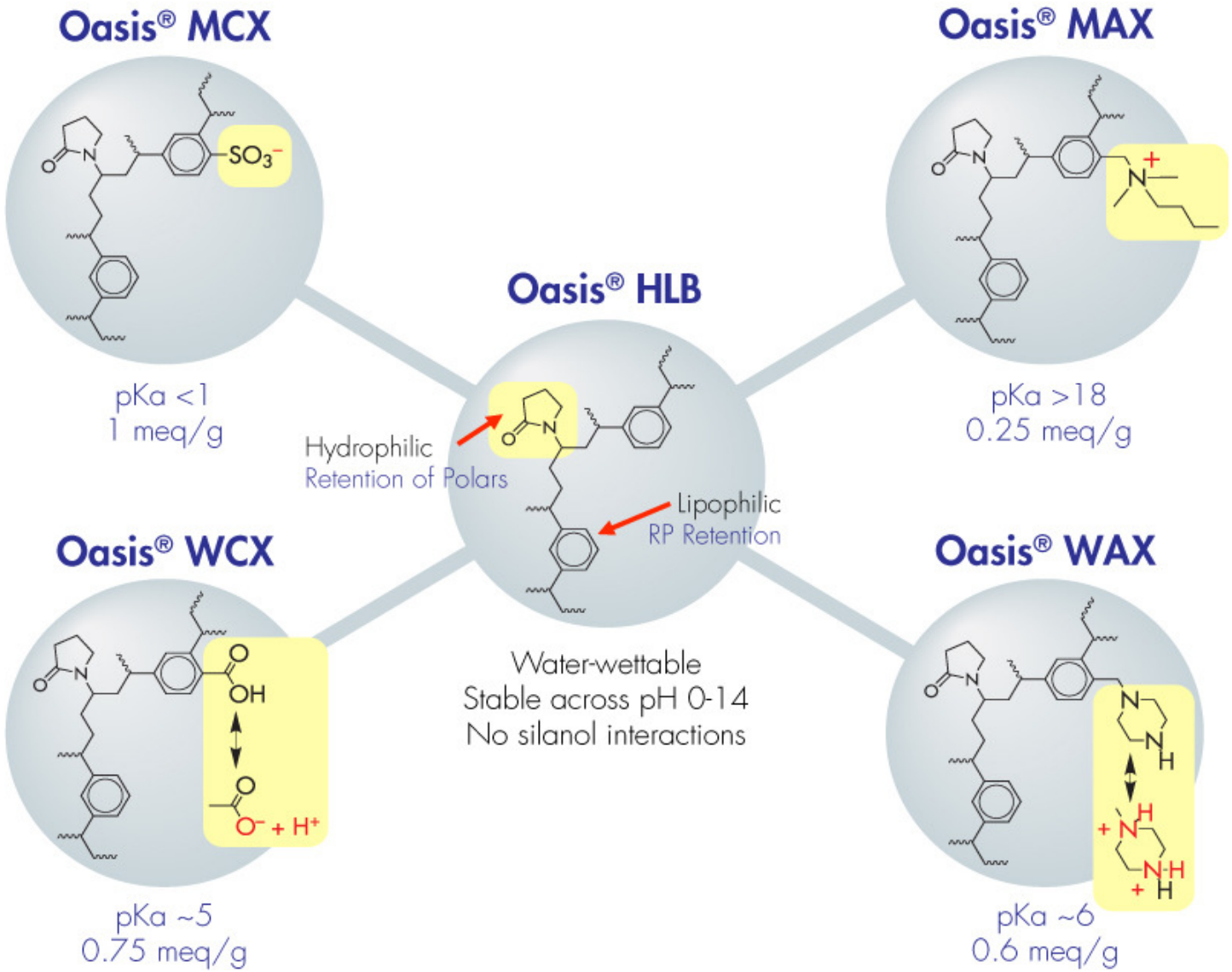
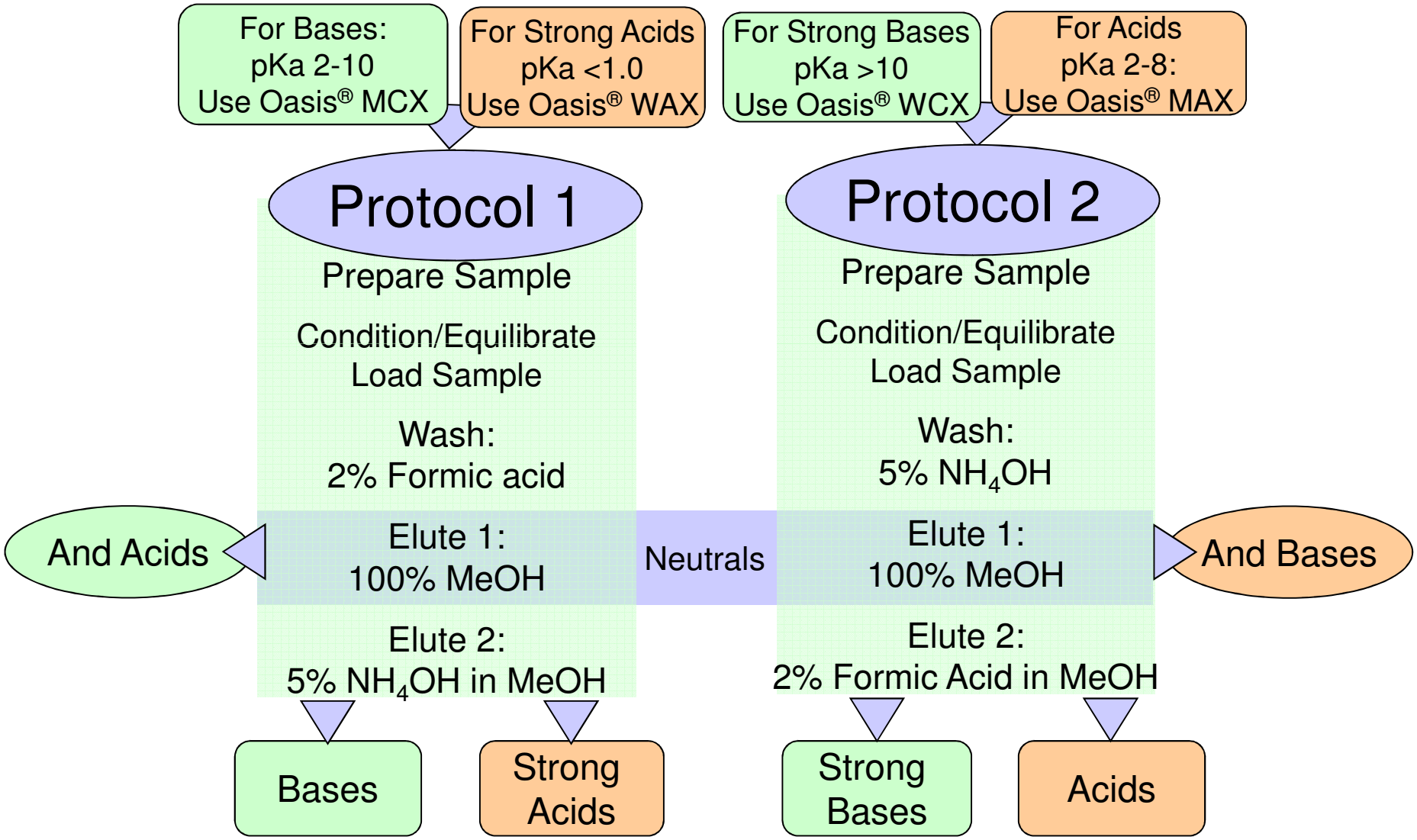


Oasis® 2x4 Method



SPE 96-Well Plate Format: Oasis® µElution Plates

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- Novel plate technology enables 25 µL SPE elution
  - Allows loading sample volumes from 25 to a maximum of 375 µL
    - 50 to 750 µL 1:1 diluted sample, 750 µL is the well volume
  - Elution volume in as little as 25 µL
- No evaporation means higher throughput and sensitivity
  - Sensitive and selective SPE for bioanalytical clinical samples
  - Increased sensitivity: up to 15x concentration factor (through format change)
- SPE without an evaporation step

Sample Enrichment: Up to a 15X Concentration Factor

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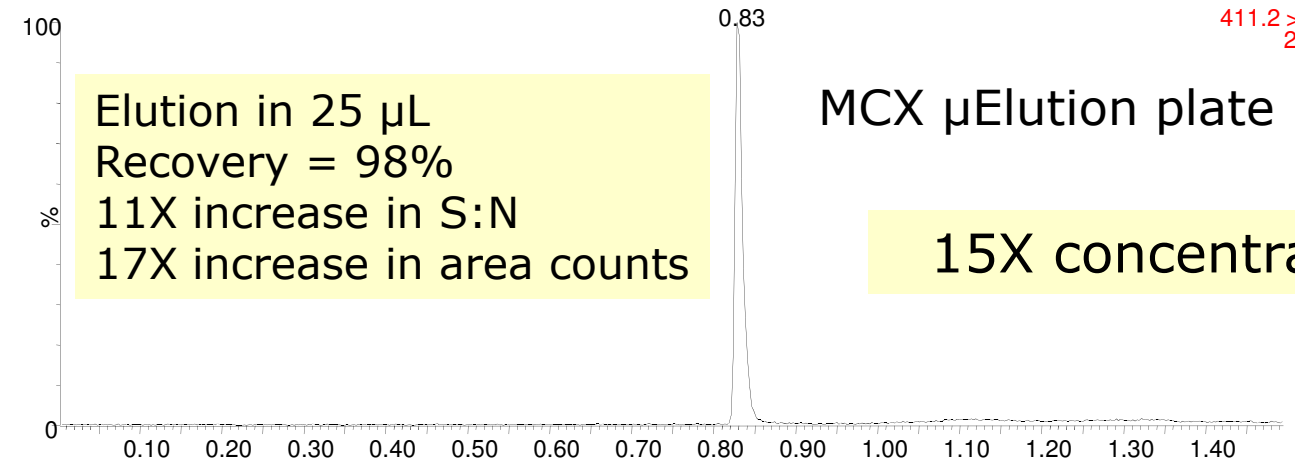
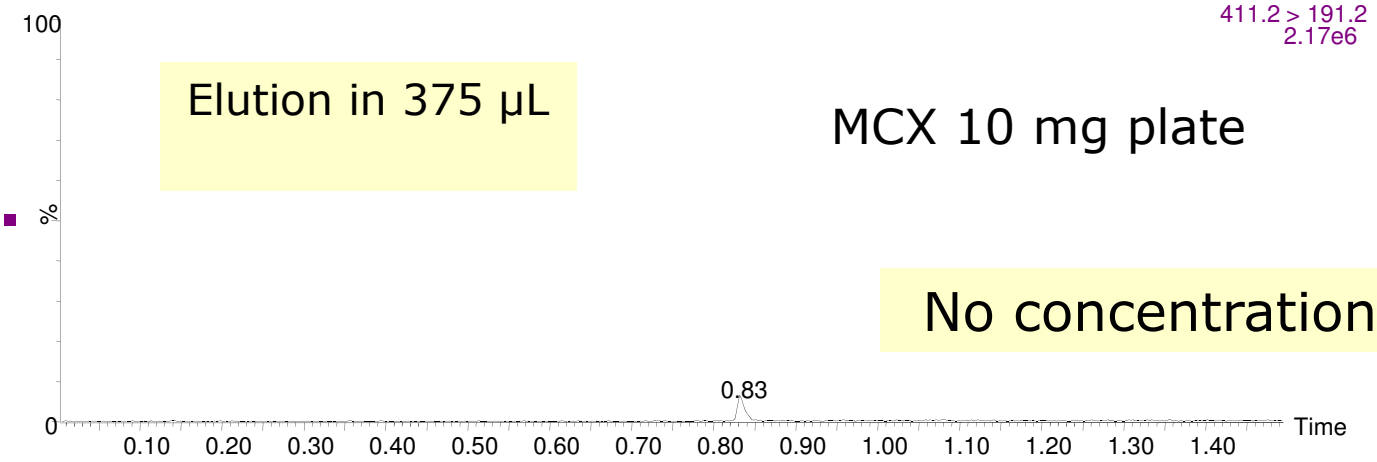
0.5 ng/mL risperidone
375 µL sample diluted with 375 µL 4% H_3PO_4 loaded onto Oasis® MCX µElution plate



Elution in 25 µL
Recovery = 98%
11X increase in S:N
17X increase in area counts

MCX µElution plate

15X concentration



Elution in 375 µL

MCX 10 mg plate

No concentration

Improving Sensitivity When Sample Limited: Example

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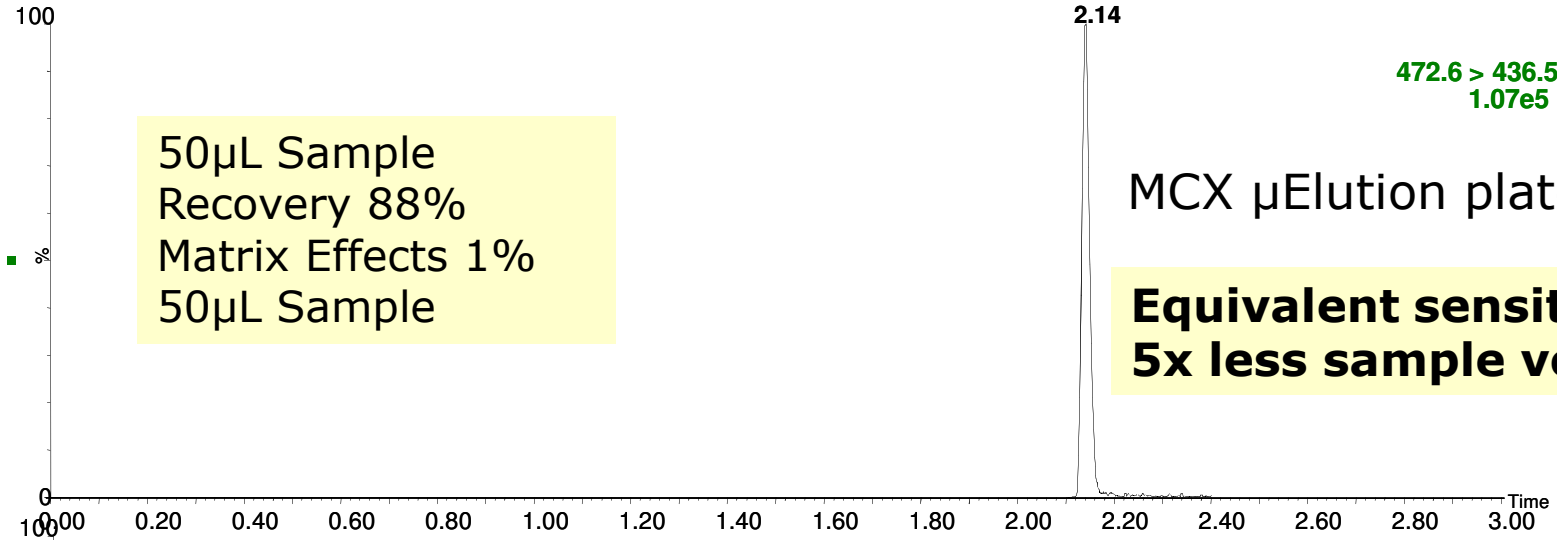
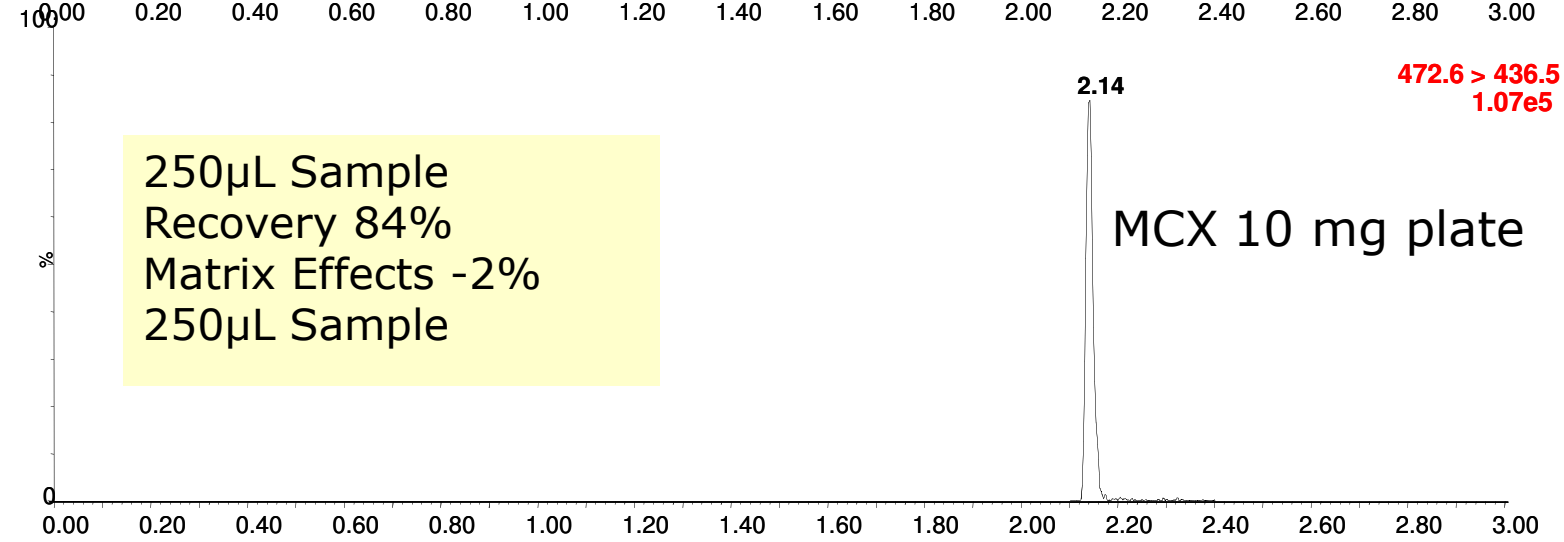
1 ng/mL Terfenadine in rat plasma, Oasis® MCX



50µL Sample
Recovery 88%
Matrix Effects 1%
50µL Sample

MCX µElution plate

Equivalent sensitivity with 5x less sample volume!



250µL Sample
Recovery 84%
Matrix Effects -2%
250µL Sample

MCX 10 mg plate

Why Eliminate Evaporation and Reconstitution?

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- Many general protocols include an evaporation and reconstitution step before injection
  - To obtain concentration necessary to reach desired limits of detection
- Certain small analytes such as ibuprofen and pseudoephedrine are susceptible to evaporative loss†
- Direct injection from the final eluate ensures maximum recovery for volatile analytes
- Direct injection saves analyst's time
- Direct injection eliminates an additional handling step
- Allows robots to be used to process more samples

†Naidong, W. *J Chromatog. B.* 796 (2003) 209-224

SPE Protocol: Oasis® MCX µElution 96-Well Plate

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Condition:	200 µL MeOH
Equilibrate:	200 µL H_2O
Load:	Various volumes of plasma and urine, diluted 1:1 with 4% H_3PO_4 in H_2O
Wash 1:	200 µL 2% CHOOH in H_2O
Wash 2:	200 µL MeOH
Elute:	2 x 25 µL 5% NH_4OH in 60:40 ACN:MeOH
Dilute:	50 µL H_2O
Inject:	5 µL

Capacity: SPE Recovery for Polar and Non-Polar Analytes in Plasma Example

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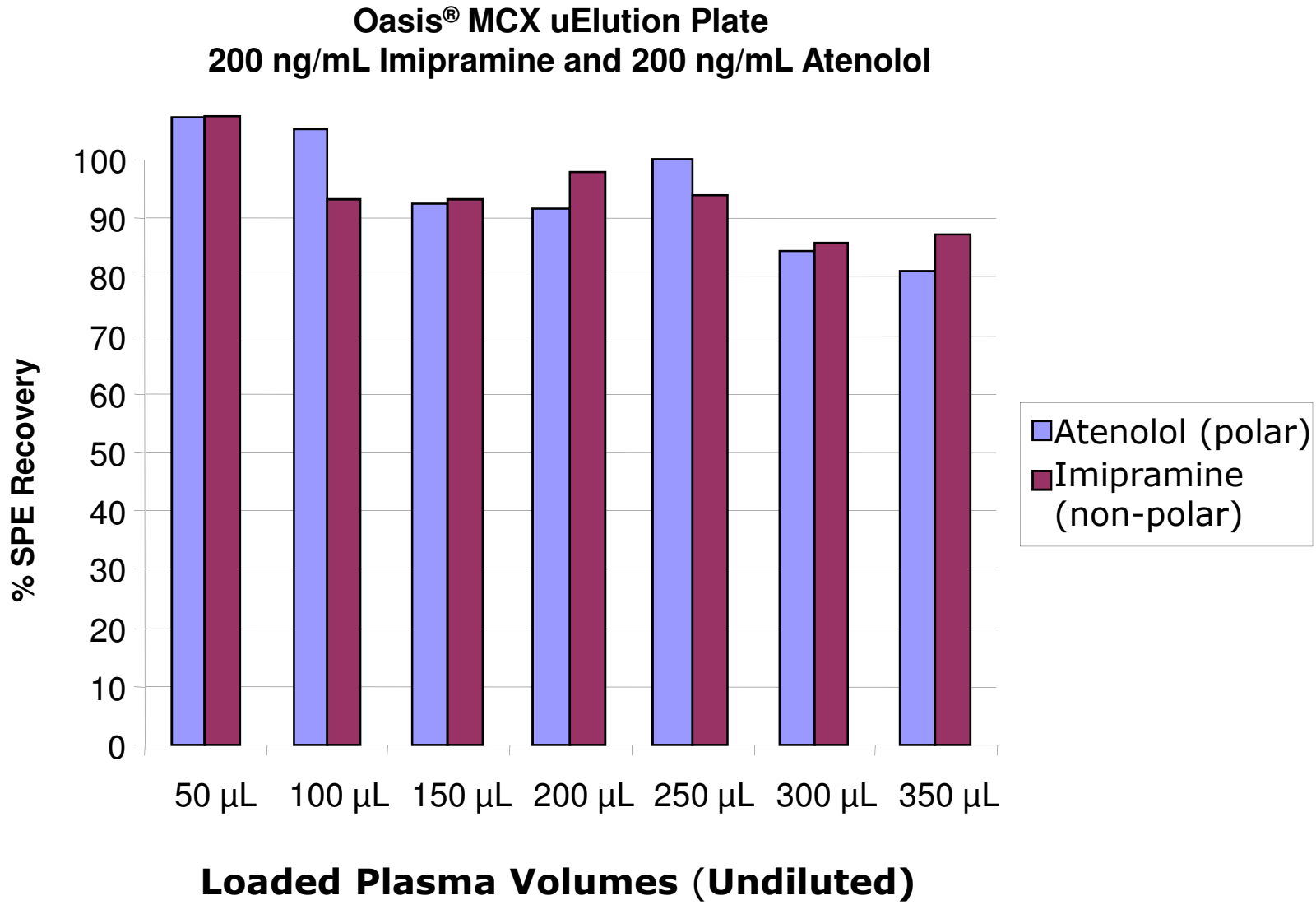

Capacity: SPE Recovery for Polar and Non-Polar Analytes in Urine Example

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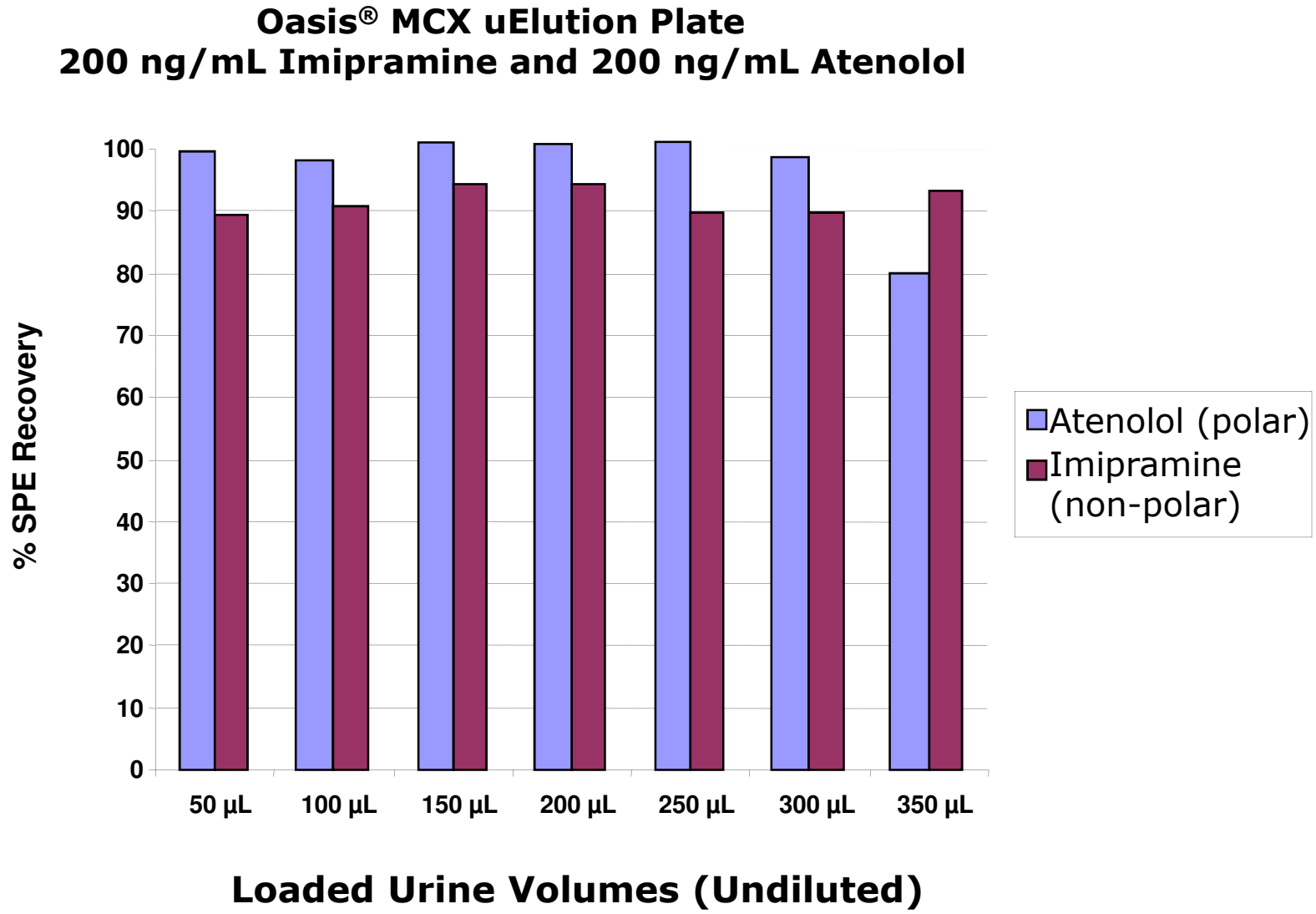

Matrix Effects for Polar and Non-Polar Analytes in Plasma

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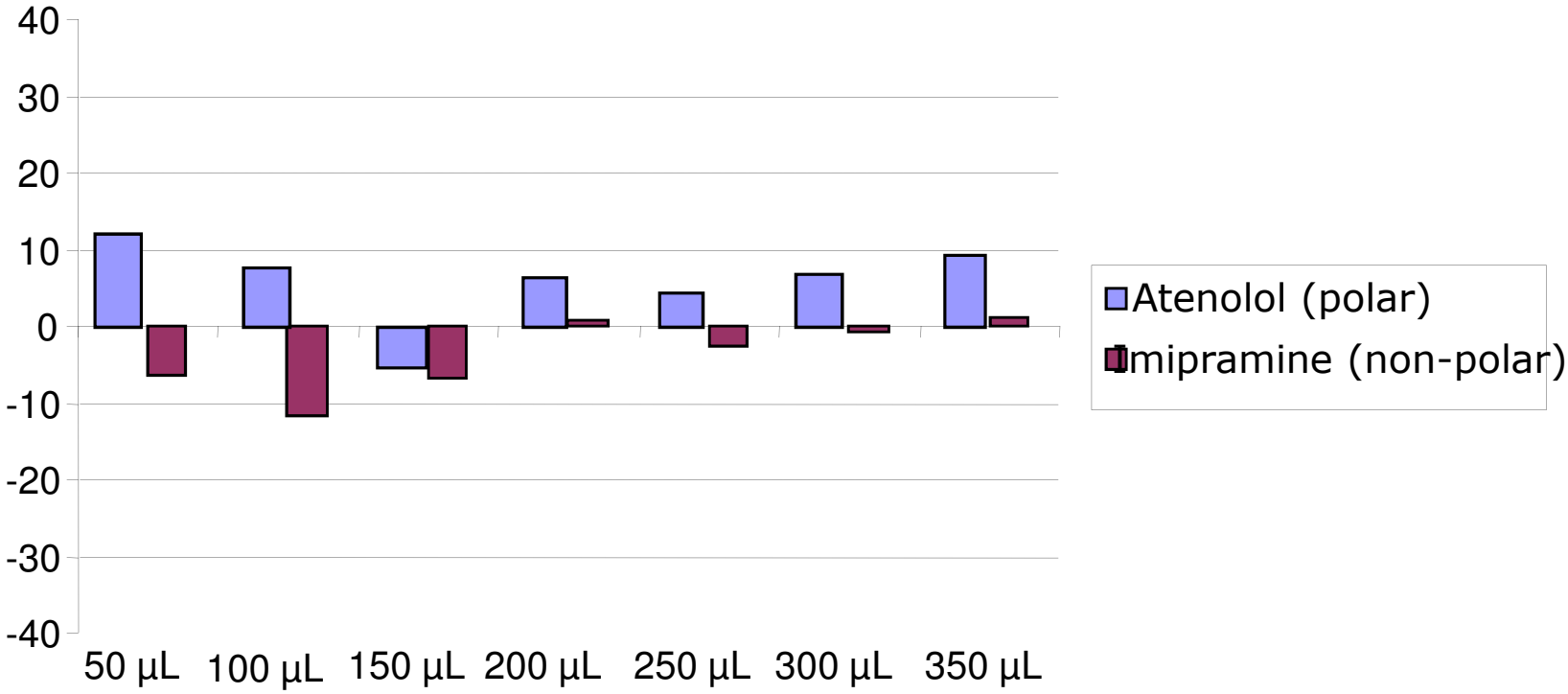
Oasis® MCX uElution Plate
200 ng/mL Imipramine and 200 ng/mL Atenolol



Loaded Plasma Volumes (Undiluted)

Method 2: Bioequivalence Study

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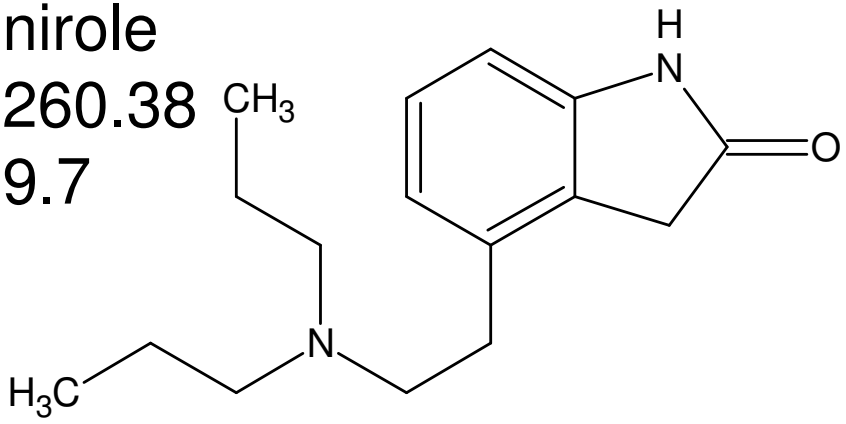
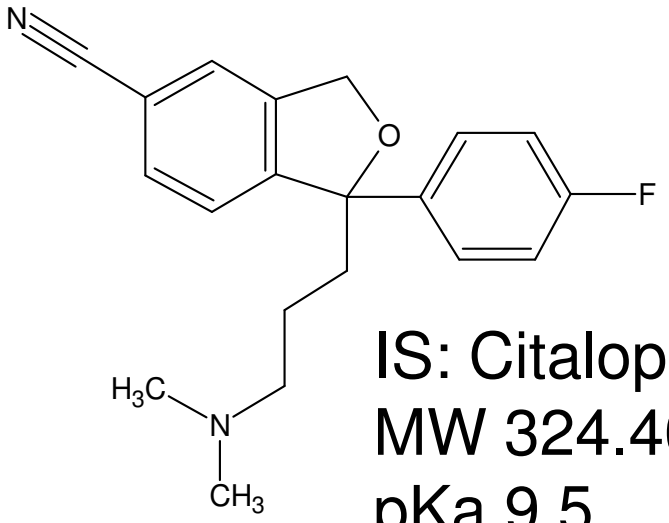
Ropinirole
MW 260.38
pKa 9.7





IS: Citalopram
MW 324.40
pKa 9.5

Assay Use:

- Routine analysis of patient samples, GLP or clinical lab

Assay Requirements:

- LLOQ 5 pg/mL
- Concerned with matrix effects
- Meeting FDA requirements
- Fast method development

SPE Protocol: Oasis® MCX µElution 96-Well Plate

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Condition:	200 µL MeOH
Equilibrate:	200 µL H_2O
Load:	600 µL diluted Plasma sample (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 MeOH
Elute:	25 µL 5% NH_4OH in MeOH*
Inject:	8 µL
LLOQ:	0.005 ng/mL

*During method development, it was determined that a 100% MeOH elution solvent was sufficient for good recovery

Ropinirole: Accuracy Data

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FDA Acceptance Criteria: ± 15%, LLOQ ± 20%

Name	RT	Area	IS Area	Response	ng/mL	%Dev
blank plasma	1.77	32.8	70.0	0.468		
0.005 ng/mL	1.76	348.6	18914.4	0.018	0.005	-0.8
0.010 ng/mL	1.76	755.8	25514.2	0.03	0.01	0.3
0.020 ng/mL	1.76	1502.8	28613.7	0.053	0.02	1.9
0.100 ng/mL	1.76	6850.6	29148.2	0.235	0.103	3
1 ng/mL	1.76	60103.1	26299.2	2.285	1.031	3.1
5 ng/mL	1.76	267985.3	26320.0	10.182	4.604	-7.9
10 ng/mL	1.76	551196.6	24835.3	22.194	10.041	0.4

Deviations from -7.9% to 3.1%

Fit for Purpose?

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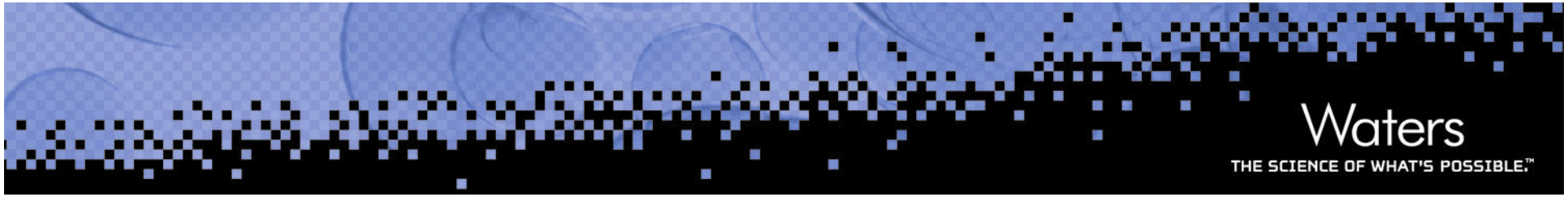
Assay Requirements:

- Rapid method developed using Oasis 2x4 approach and Oasis µElution Plate

- LLOQ 5 pg/mL

- Method easily meets the FDA criteria for acceptability
  - Linearity across 3.5 orders of magnitude
  - Excellent sensitivity: 0.005 ng/mL (5 pg/mL)
  - Accuracy for all points on standard curve

- Matrix effects for analyte and internal standard <15%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SIMPLY CLEAR
THE FASTER WAY TO CLEANER
SENSITIVITY IN ITS PUREST FORM
Sirocco™ Protein Precipitation Plate
P/N: 186002448
Lot No. 906801
Ostro™ 96-Well Plate
Mfg. Lot No.: 123456789B
Sorbent Batch No.: 101
Part No.: 186005518
Made in Ireland
Oasis® HLB µElution Plate
Part Number 186001828BA
Lot Number 057A35215A
Sirocco™
Protein Precipitation Plate
OASIS®
SAMPLE EXTRACTION PRODUCTS
Ostro™
SAMPLE PREPARATION PRODUCTS

Overview

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- Quechers

- Bioanalysis

- Synthesis

Cleanup Challenges

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- Convert from a high boiling point solvent before next reaction step
- Separate product (bases) from reactants and reaction solvents
- Remove TFA
- Convert from last solvent used in synthesis before Prep LC
- Remove water from fraction collected from Prep LC for faster dry down

Product - PoraPak™ Rxn

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Copolymer that exhibit the following properties:

- Hard material that does not develop increasing back pressure with flow. (Not compressing)
- Little swelling or shrinking across a range of solvents and pH extremes.
- Low hydraulic resistance enables flow by gravity.
- pH extreme tolerant without dissolution or hydrolysis, both limitations of silica-based sorbents.

This combination of physical and chemical properties makes Porapak Rxn an ideal material for synthesis cleanup.

Recommended Techniques

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	Technique	Recommended Product
Reaction Products	Bases → Catch and Elute	PoraPak Rxn CX
	Acids → Pass Through	PoraPak Rxn CX
	Neutrals → Adsorption	PoraPak Rxn RP
Reaction Components	TFA removal → Catch and Elute	PoraPak Rxn CX
	Convert from high boiling point solvent for bases → Catch and Elute	PoraPak Rxn CX
Pre-and-Post Preparative Column	Fractionate sample before Prep for bases → Catch and Elute	PoraPak Rxn CX
	Remove water from post column fraction for bases → Catch and Elute	PoraPak Rxn CX

Sample Preparation Techniques and Method Development – Information?

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Product information on our website:

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Oasis – Sample Extraction Product
http://www.waters.com/Oasis
Ostro – Sample Preparation Product
http://www.waters.com/Ostro
Sirocco – Protein Precipitation Plate
http://www.waters.com/Sirocco
Certified Sep-Pak – Critical Clean SPE
http://www.waters.com/waters/nav.htm?cid=10105548
Sep-Pak – SPE
http://www.waters.com/SepPak
DisQue - Quechers
http://www.waters.com/DisQue
PoraPak – Synthesis Clean-Up
http://www.waters.com/PoraPak
Envirogel GPC Clean-UP
http://www.waters.com/webassets/cms/support/docs/WAT036556.pdf

