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High Throughput Extraction of Basic and Polar Drug Compounds From Biological Matrices With a Novel Polymeric Solid-Phase Sorbent in a 96-Well Format

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ABSTRACT

Historically, sample preparation has been the bottleneck in laboratory productivity, especially with the onslaught of LC/MS/MS. Rapid and reproducible quantitation of classically difficult, basic and polar drug compounds from biological matrices has, in the past, been a challenging, time-consuming endeavor. Necessary sample preparation techniques used to concentrate or clean-up irreproducible. Presently, the most commonly used technique for high throughput sample preparation is reversed-phase solid phase extraction in a 96-Well format. The sorbent generally utilized is porous silica surface-bonded with C18. The major disadvantage to employing this type of system with basic compounds is that the silanols on the silica surface can deleteriously affect the recovery of the basic compounds. The surface silanols interact through ion exchange with basic compounds, such as doxepin. This interaction prevents complete elution of basic compounds and results in low, variable results. With polar compounds, such as procainamide and ranitidine, the analyte/sorbent interaction is weak resulting in breakthrough of the polar analyte. This, subsequently, yields poor, irreproducible recoveries. Another notable disadvantage to using traditional silica-based reversed-phase sorbents is that the wettability must be maintained through the tedious manipulation of stopcocks. The capacity of C18 is severely compromised if the sorbent runs dry.

In this paper we show that the sample preparation bottleneck has been eliminated. Highly reproducible and uncomplicated SPE methods were developed for these types of basic and polar drug compounds using a novel polymeric solid-phase sorbent, Oasis™ HLB, in a 96-Well format. Recoveries greater than 90% and reproducibilities less than 5% RSD (n=96) for basic antidepressant and polar drugs were realized. Most importantly, because Oasis™ HLB is a water wettable polymer, these results were achieved without concern as to the whether or not the sorbent ran dry.

What do You Want in a Sample Preparation Method?

- Meet requirements for fast HPLC analyses, i.e. achieve clean extracts to increase sensitivity and sample throughput
- High, reproducible recoveries for acidic, basic and neutral analytes with a broad range of polarities
- High sensitivity in complex matrices (plasma)
- Simple; Easy to use
- Rugged
- Fast and cost efficient

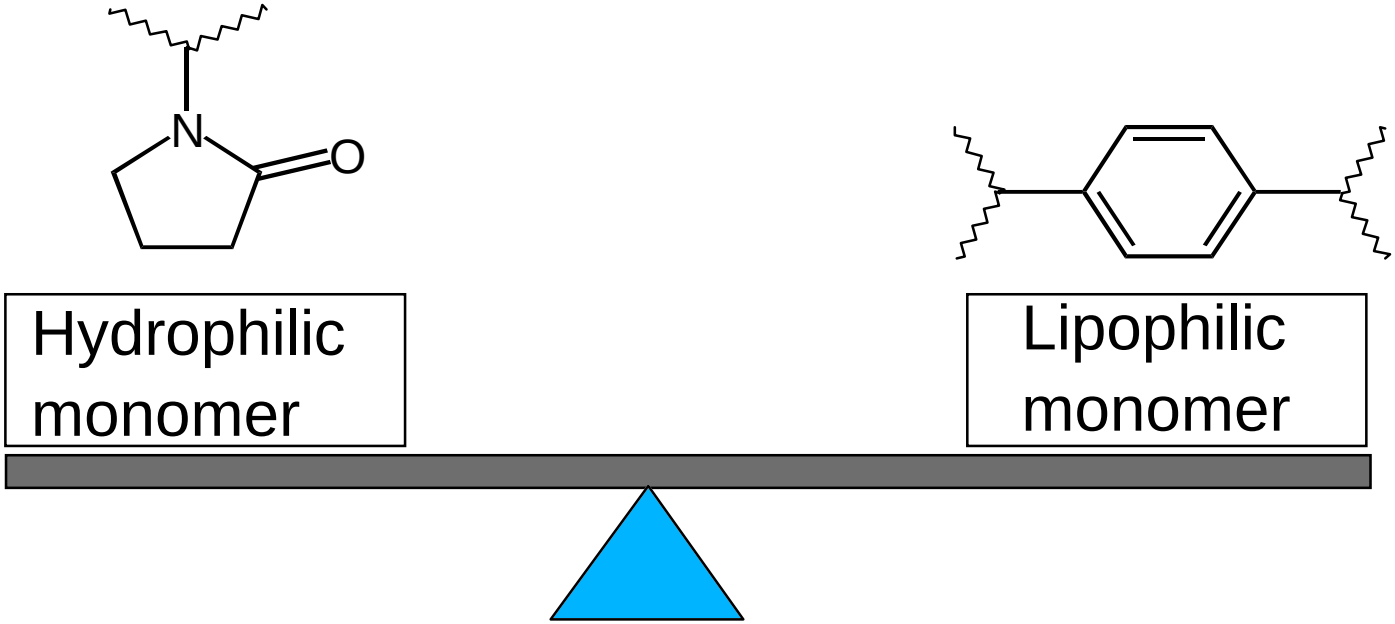
Disadvantages to Using Silica-Based SPE Sorbents

- Time consuming and complicated methods development resulting in a bottleneck for sample throughput

- Low Recovery
  - Breakthrough of polar compounds due to weak retention
  - Adsorption of basic compounds due to silanol interactions
- Tedious
  - Vacuum manifolds monitored closely so wells do not run dry

Why We Chose Oasis™ HLB as Our Extraction Sorbent...

The Oasis™ HLB Sorbent: A Hydrophilic-Lipophilic Balanced Copolymer



- "water loving"
- Provides wetting properties
- Reduces contact angle with water

- "fat loving"
- Provides reversed-phase property for analyte retention

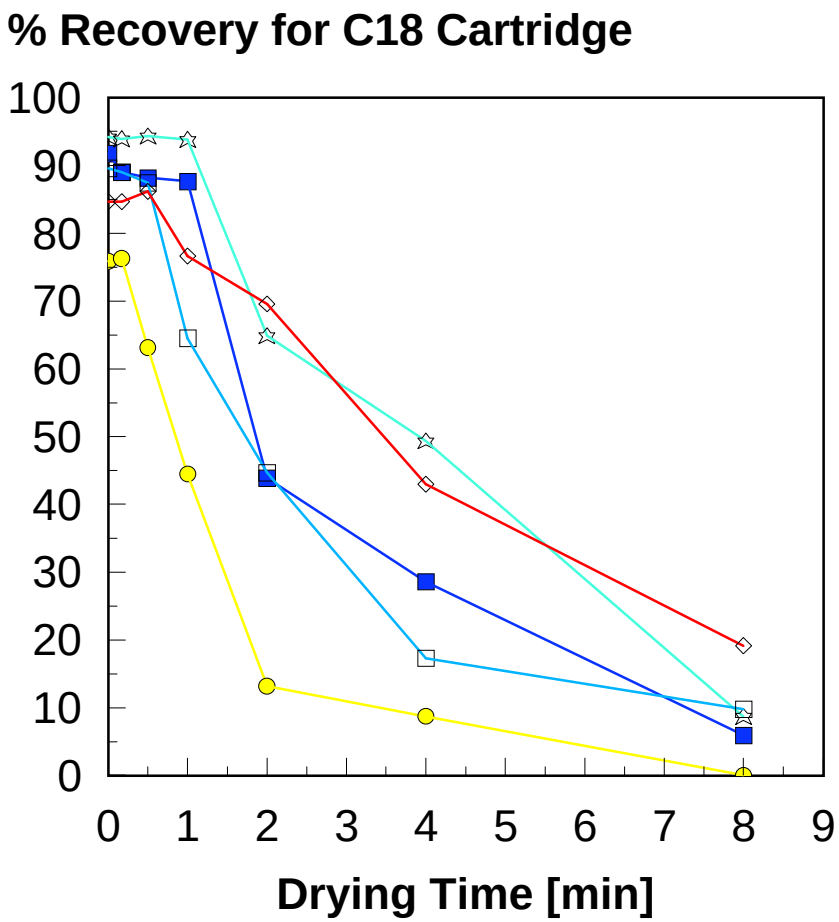
Advantages of the Oasis™ HLB 96-Well Extraction Plates:

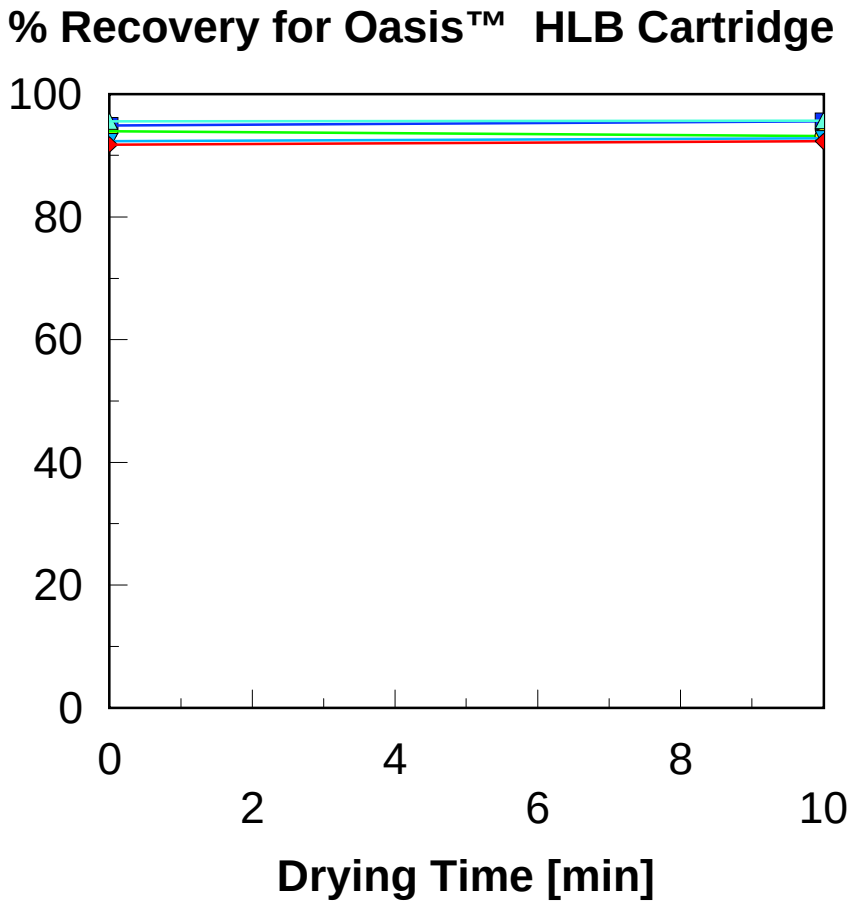
- General method is applicable to most analytes
  - better retention of polar compounds

- Polymeric sorbent
  - no silanols
  - water-wettable
  - can be used over a wide pH range (pH 0 to pH 14)
  - universal sorbent, leading to generic method for LC/MS/MS

- Wells can dry out with no effect on recovery
  - flow through wells do not have to be watched carefully to avoid drying of the sorbent
  - **no more dry wells and loss of valuable samples!!!**

Drying Effect on Recovery: C_{18} vs. Oasis™ HLB Cartridges



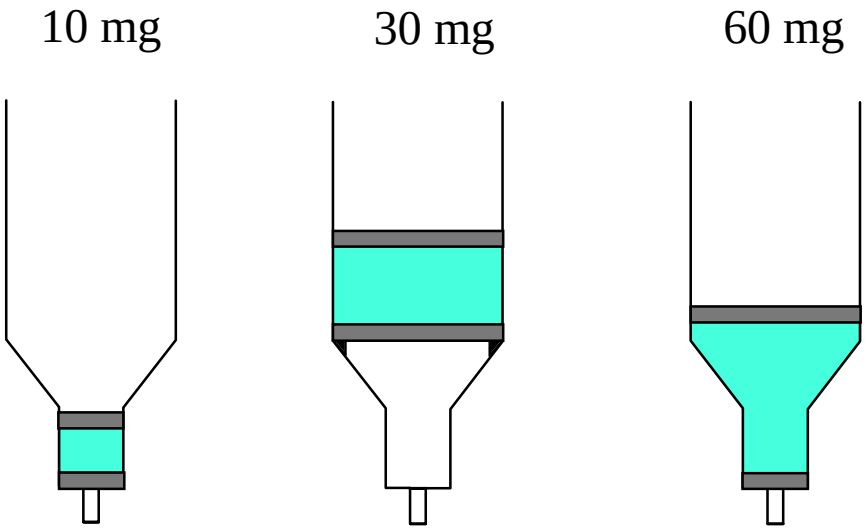


Procainamide Acetaminophen Ranitidine Propranolol Doxepin

Bouvier, Capparella

Waters 96-well Plate*
Two-Stage Well Design

- Enabling technology for 96-well plates - Oasis™ sorbent in the amount required to meet your capacity and elution volume needs.

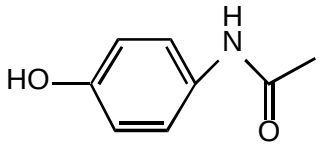


* patent pending

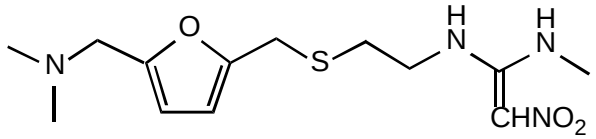
Three different sorbent amounts in Waters 96-well plates.

Acidic, Basic, and Neutral Test Compounds

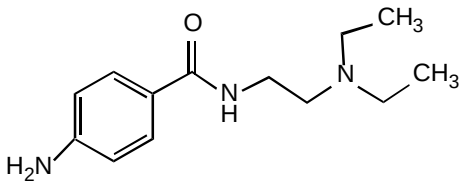
Polar Test Mix



Acetaminophen

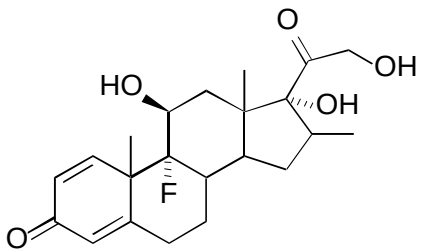


Procainamide

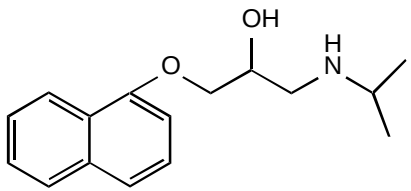


Ranitidine

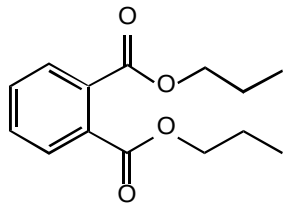
Non-Polar Test Mix



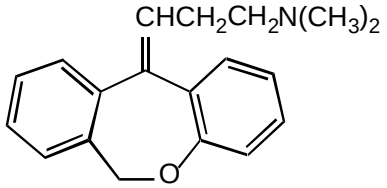
Betamethasone



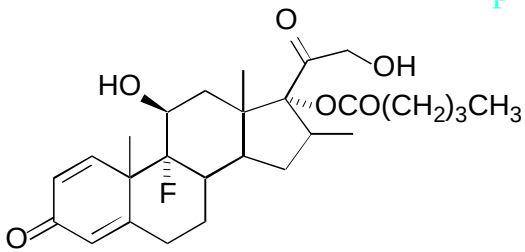
Propranolol



Dipropyl Phthalate

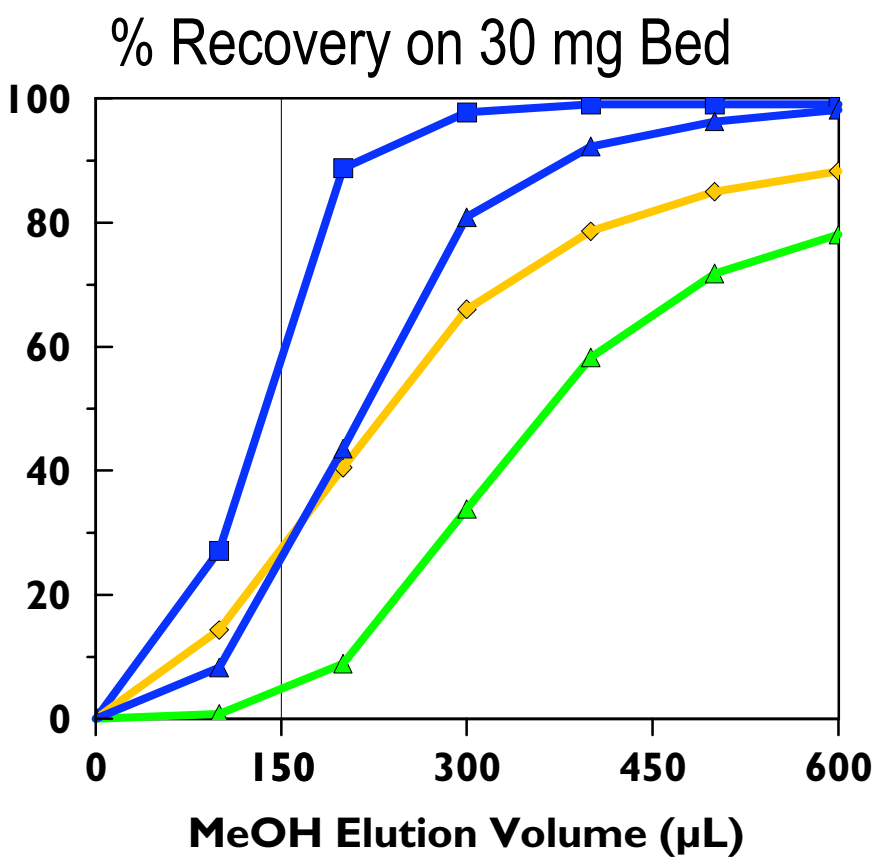


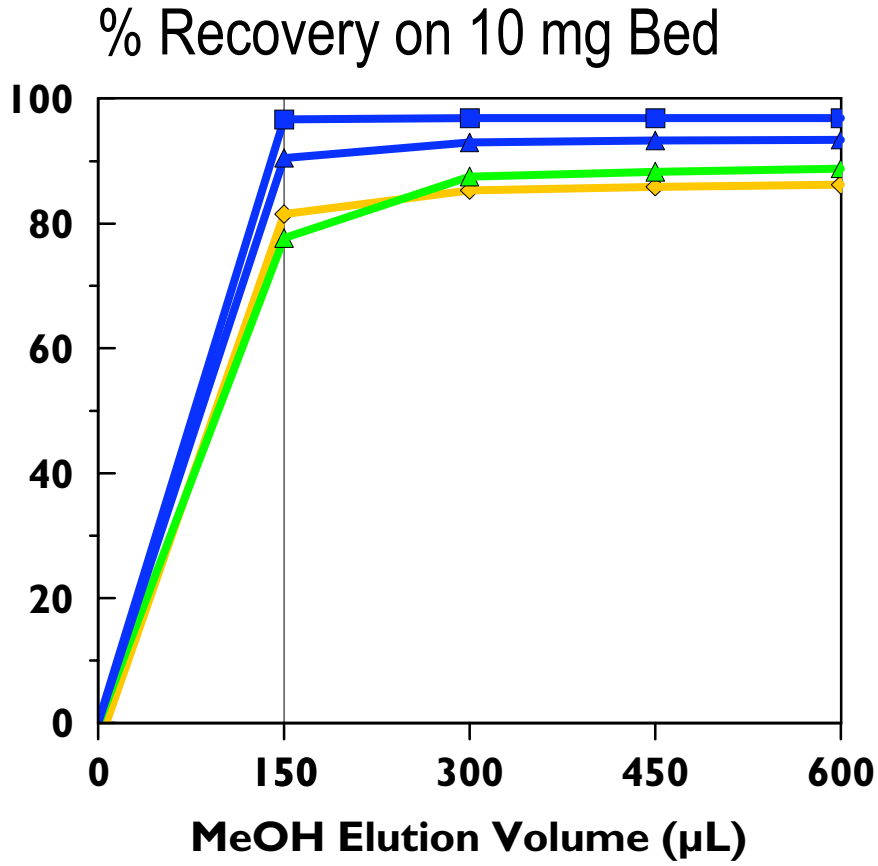
Doxepin



Betamethasone Valerate

Comparison of Elution Profiles on 30 mg vs.10 mg Oasis™ Plate

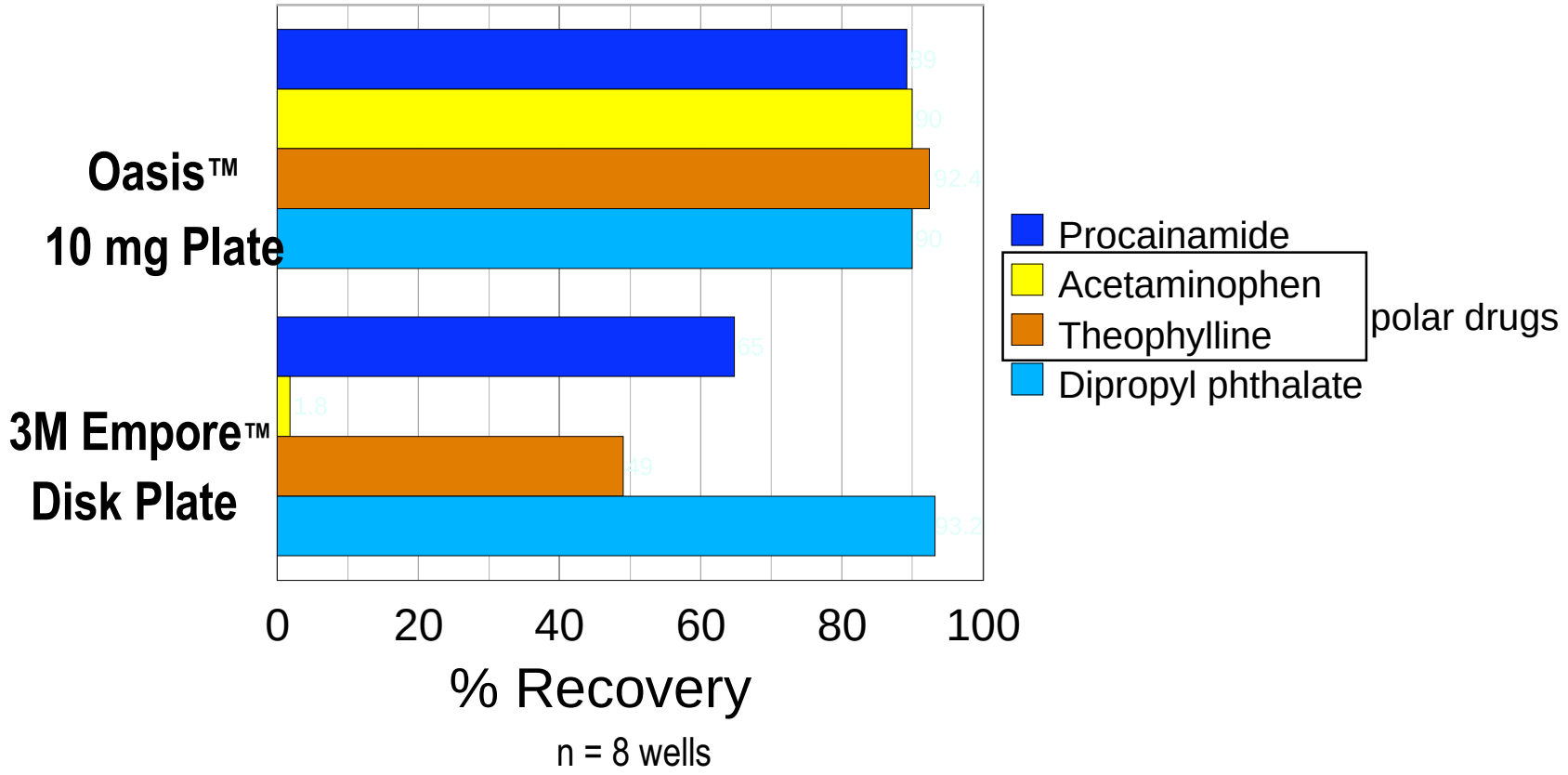




Ranitidine (polar base)
Propranolol (intermediate polarity base)
Doxepin (non-polar base)
Betamethasone valerate. (non-polar neutral)

Oasis™ Low Elution Volume Plate Versus 3MEmpore™ Plate:

Recoveries in 150 µL

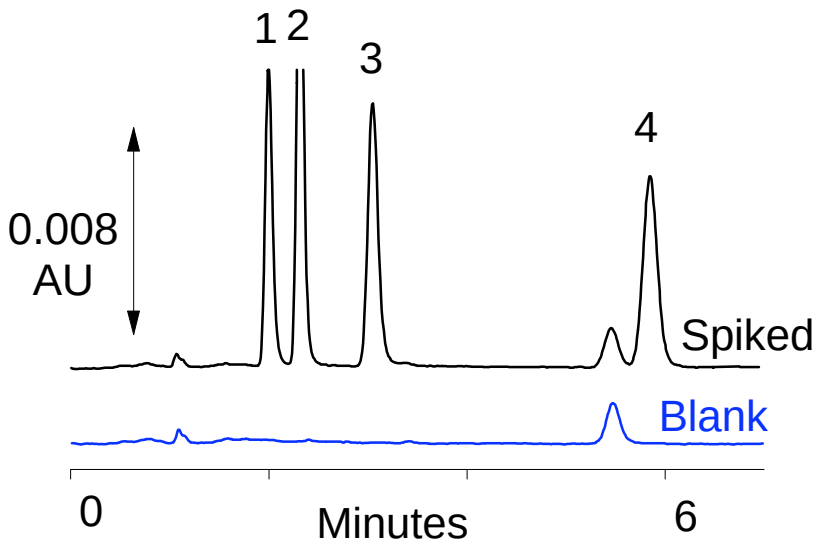


New Oasis™ plate gives low elution volumes and high recovery of polar drugs.

Low Elution Volume (150 µL) Results:

Plasma Extracted on Oasis™ HLB Extraction Plate, 10 mg per Well

Polar Compounds	Spike [µg/mL]	% Recovery n = 95	% RSD n = 95
1. Procainamide	10	92.5	2.0
3. Ranitidine	10	93.4	2.3
4. Acetaminophen	10	82.0	5.6



Column: SymmetryShield™ RP8 3.5 µm 4.6 mm x100 mm with Sentry™ SymmetryShield RP8 guard column 3.9 mm x 20 mm

Mobile Phase: 25 mM KH_2PO_4, pH3:Methanol (95:5 v/v)

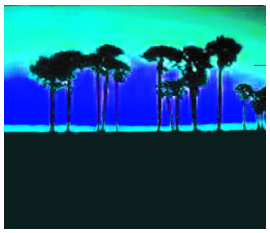
Flow Rate: 1.4 mL/min

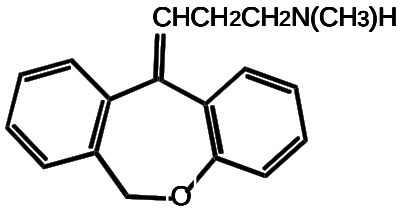
Detection: UV at 220 nm

Injection Volume: 5 µL

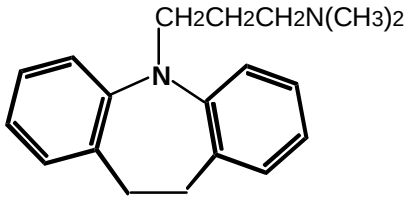
Note: Peak 2 is sulfanilamide, the internal standard

Structures and SPE Method: Tricyclic Antidepressants (TCAs)

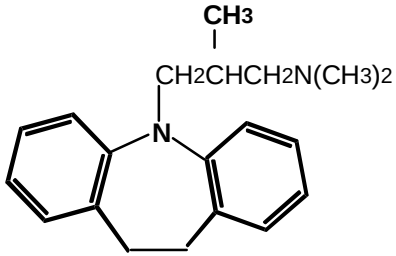




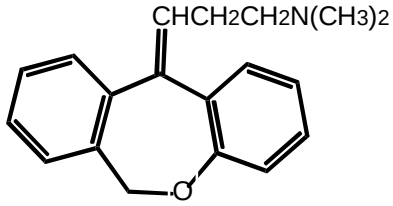
Nordoxepin (I.S.)



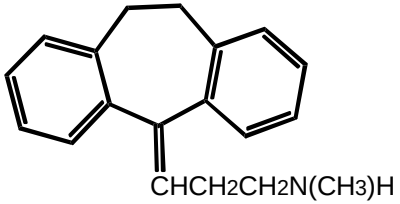
Imipramine



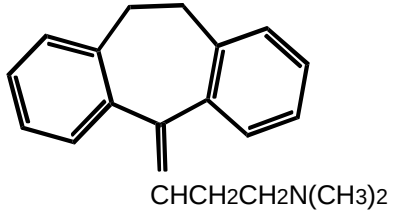
Trimipramine



Doxepin



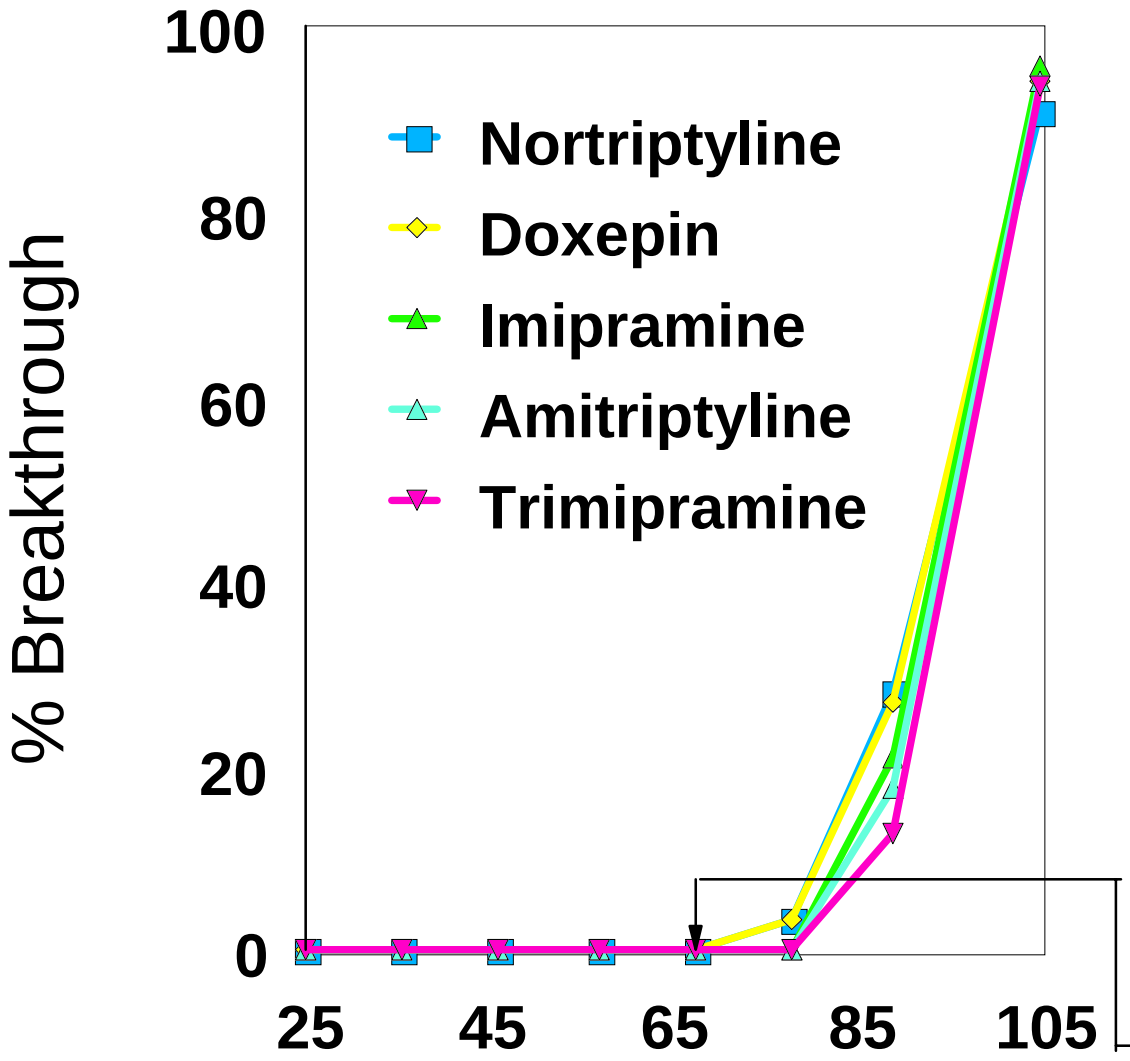
Nortriptyline



Amitriptyline

- Prepare Sample
  - 1 mL spiked porcine plasma plus 20 µL conc H3PO4
- Condition and Equilibrate
  - 1 mL methanol (MeOH) and 1 mL water
- Load Sample
- Wash (see methods development section)
  - A. 1 mL 2%NH4OH in 5% MeOH (to remove polar interferences)
  - B. 1 mL 2% NH4OH in 65% MeOH
  - C. 1 mL 2% CH3COOH in 5% MeOH (to ionize TCAs)
- Elute (see methods development section)
  - 600 µL 65% MeOH
- Add internal Standard
  - 60 µL 36 µg/mL Nordoxepin in 10 % NH4OH

TCA Methods Development: Selecting Methanol (MeOH) Concentration in Wash B

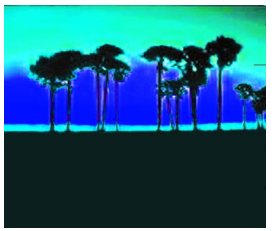


Load 1 mL TCAs
in Saline + 5µL H_3PO_4

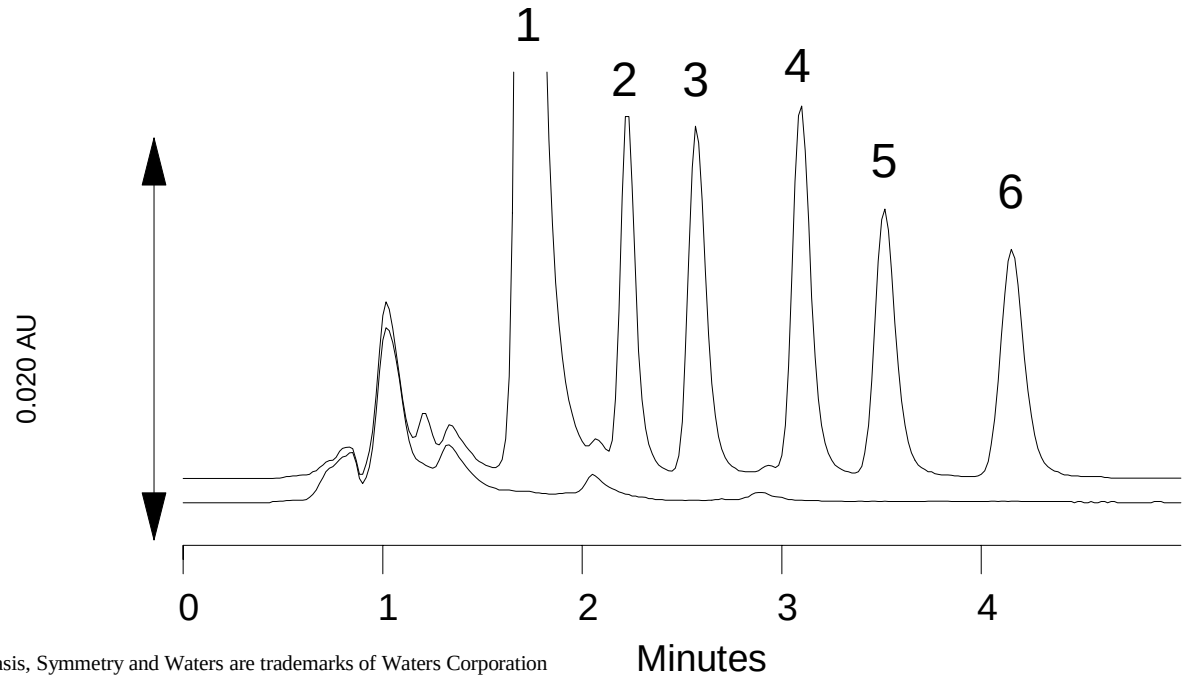
Wash B 1 mL 2% NH4OH
containing 25, 35, 45, 55,
65, 75, 85, 100 % MeOH

Collect and Analyze Wash B
select highest %MeOH
without breakthrough

HPLC Method: Tricyclic Antidepressants



Column:	SymmetryShield™ RP8, 3.5 µm, 4.6x75mm
Guard Column:	Sentry™ SymmetryShield RP8, 5 µm
Temperature:	29°C
Mobile Phase:	50 mM Phosphate, pH7:Methanol (26:74)
Detection:	UV at 254 nm
Flow Rate:	1.4 mL/min
Inj. Volume:	140 µL



Plasma Extracts:

Spiked at 500 ng/mL vs. Blank

1. Nordoxepin (IS)
2. Nortriptyline
3. Doxepin
4. Imipramine
5. Amitriptyline
6. Trimipramine

Extracted Plasma Results: Tricyclic Antidepressants

	500 ng/mL		100 ng/mL	
	% Recovery (n = 96)	% RSD (n = 96)	% Recovery (n = 95)	% RSD (n = 95)
Nortriptyline	92.3	1.4	90.8	5.7
Doxepin	90.6	1.4	90.4	4.7
Imipramine	92.2	1.7	86.4	5.3
Amitriptyline	90.2	1.6	85.3	5.8
Trimipramine	90.3	1.9	89.8	6.1

Conclusion:

- Able to achieve excellent recovery (>90%, >85%) and reproducibility (<5%, <6%) for both basic and polar compounds at low concentrations using low elution volumes (150 µL) with the 30 mg and the new 10 mg 96-well Oasis™ HLB extraction plate.
- Do not have to be concerned with drying out of wells.
- Opportunity for high sample throughput with use of Oasis™ HLB in the 96-well plate and column formats.