

# **HPLC Determination of Antidepressants and their Metabolites in Plasma**

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Edouard S. P. Bouvier, Mark Capparella,  
Pamela Iraneta, Andy Jarrell, Uwe D. Neue,  
Dorothy J. Phillips, and Doug Wittmer**

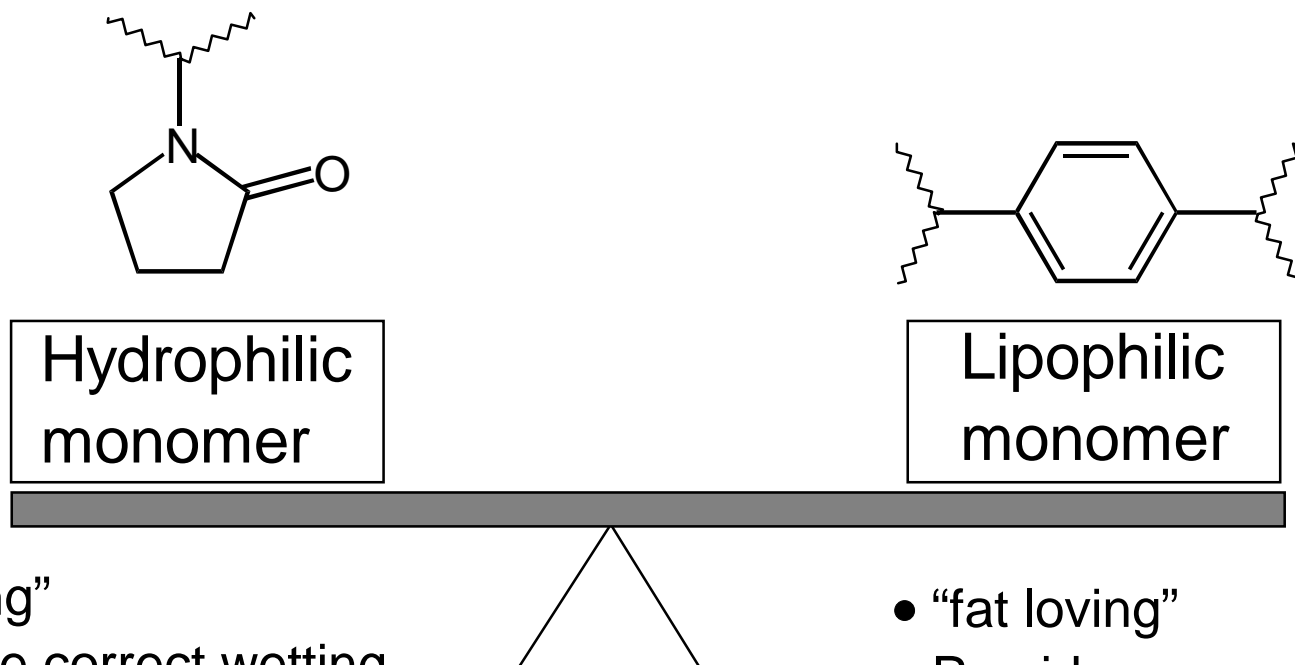
**Waters Corporation, 34 Maple Street,  
Milford, MA 01757**

# Abstract

- Using the **one-dimensional SPE method**, we were able to obtain very **high recoveries** (all greater than 86%) and **excellent reproducibility** (all less than 5.0%) for **a wide range of drugs**. These compounds included acids, neutrals, and bases
- Using the **two-dimensional SPE method**, we were able to **selectively isolate** analytes of interest from a complex sample matrix while **eliminating almost all interferences**.

# Oasis™ HLB Sorbent

## Hydrophilic-Lipophilic Balanced



- “water loving”
- Provides the correct wetting property to keep the surface of the packing solvated even when the cartridges run dry

- “fat loving”
- Provides reversed-phase property for analyte retention

**Two Unique Properties Are Carefully  
Balanced**

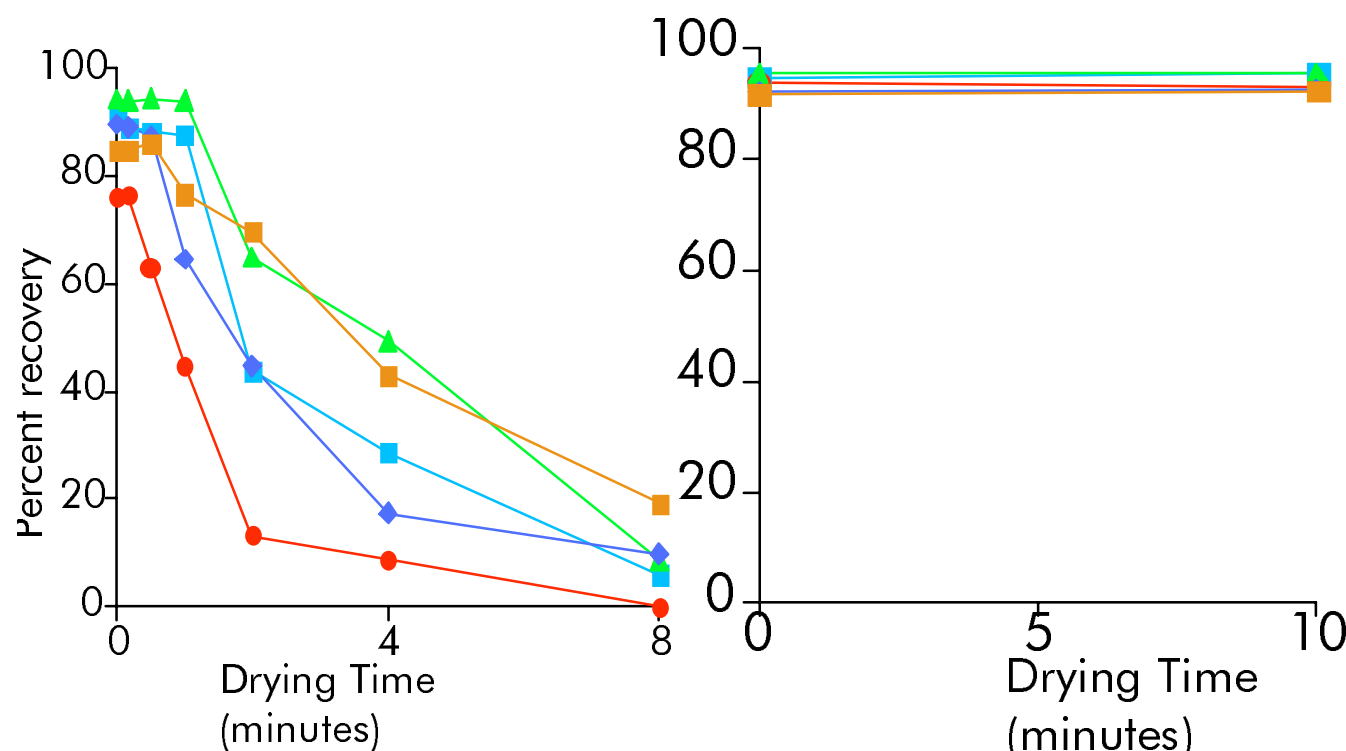
Reference: "A Novel Polymeric Reversed-Phase Sorbent for Solid-Phase Extraction," *LC•GC Int.* **10** (9)  
E.S.P. Bouvier, D.M. Martin, P.C. Iraneta, M. Capparella, Y. Cheng, and D.J. Phillips

# Drying Effect on Recovery:

## C<sub>18</sub> vs. Oasis™ HLB Cartridges

C<sub>18</sub> (1cc/100mg)

HLB (1cc/30mg)



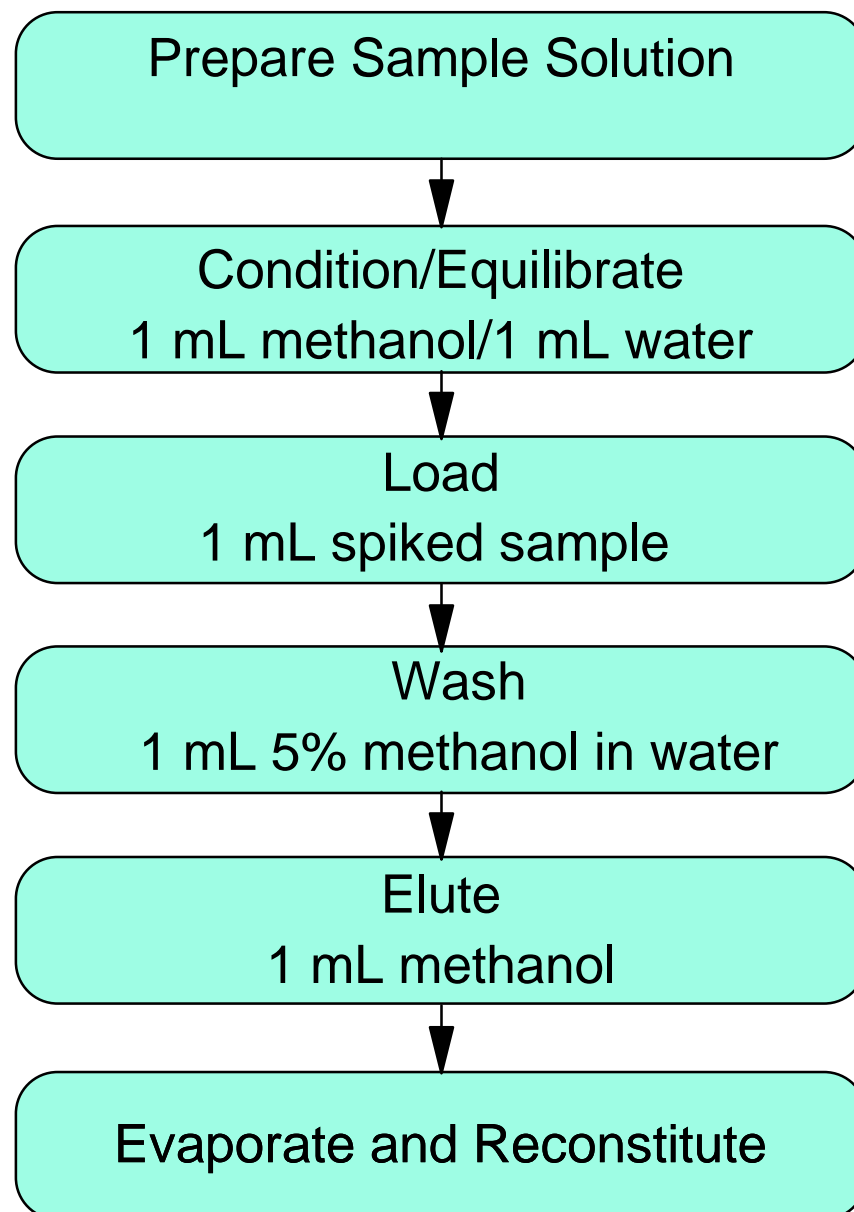
- \* No Impact of Sorbent Drying
- \* No Silanol Interaction
- \* No Breakthrough of Polar Analyte

Procainamide      Ranitidine      Doxepin  
Acetaminophen      Propranolol

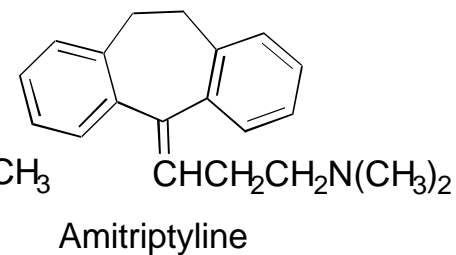
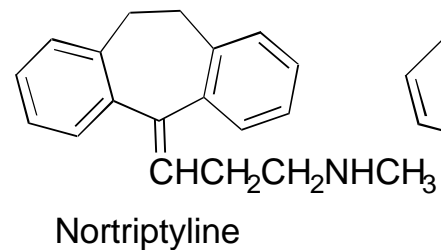
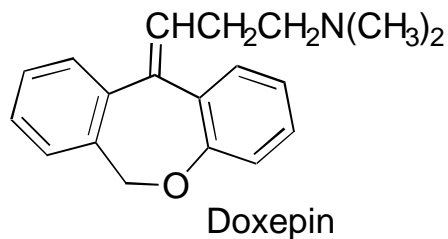
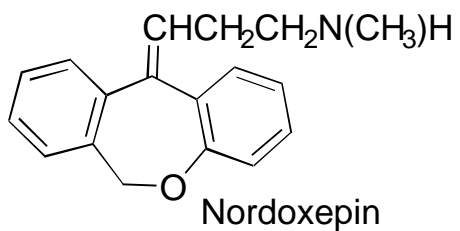
# One-Dimensional SPE Method

Simple, Rugged, and  
Highly Reproducible

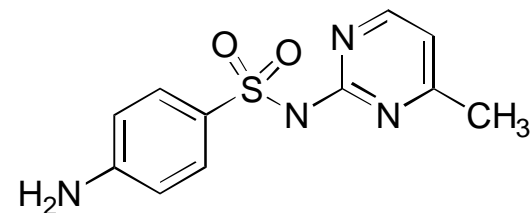
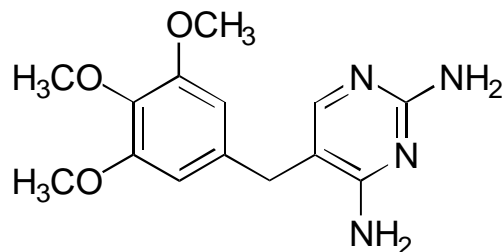
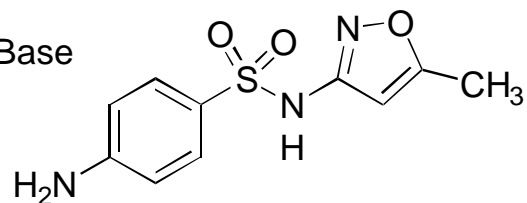
One General SPE  
Method for a Wide  
Range of Compounds



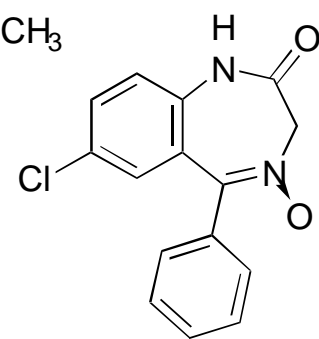
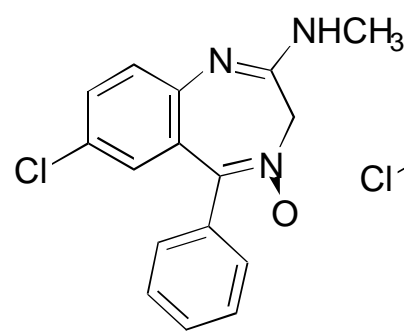
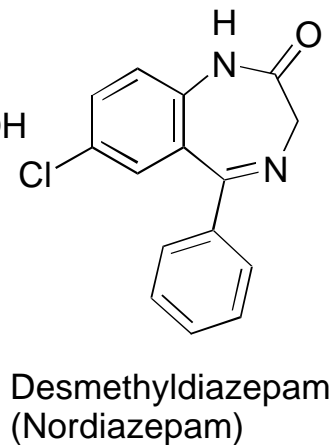
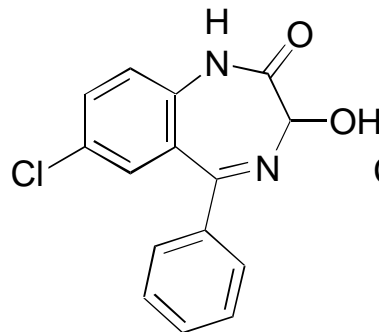
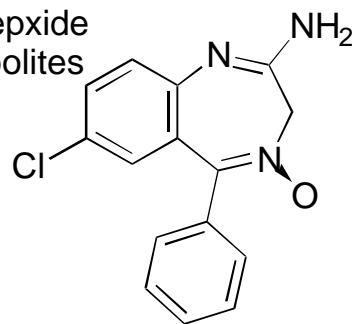
Base



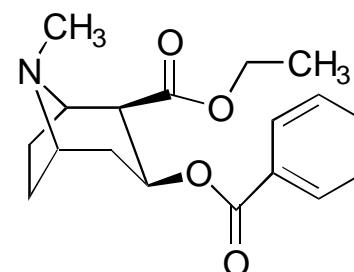
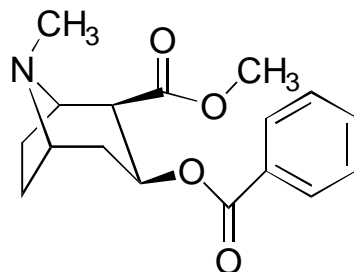
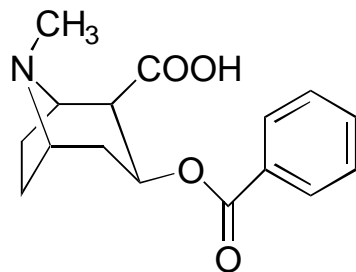
Acid and Base



Chlordiazepoxide and Metabolites

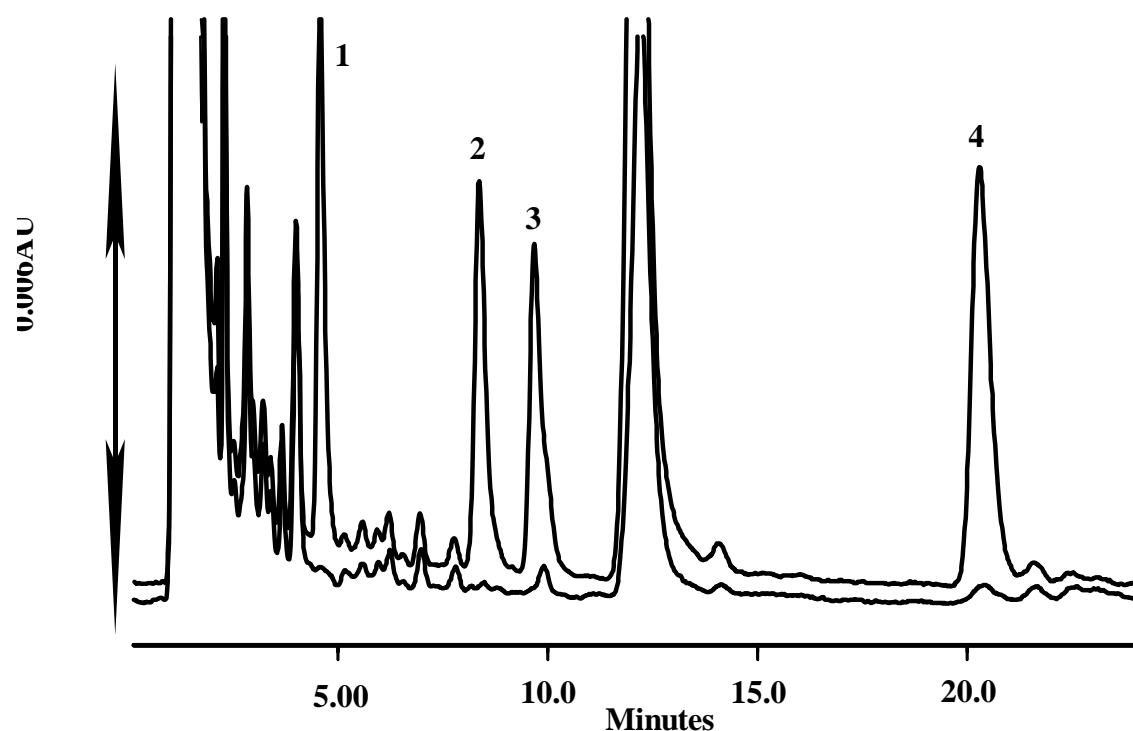


Cocaine and Metabolites



# Bases: Tricyclic Antidepressants

Compound	Concentration	% Recovery	% RSD
Nortriptyline	0.5 µg/mL	99.7	2.3
Doxepin	0.5 µg/mL	94.0	1.3
Amitriptyline	1.0 µg/mL	102	2.5



Column: Symmetry® C18, 5 µm,  
3.9 x 150 mm

Temperature: 35°C

Mobile Phase: 20 mM Phosphate pH 7:  
Methanol (30:70)

Detection: UV at 254 nm

Flow Rate: 1.0 mL/min.

Injection Volume: 20 µL

Sample Identification:

Peak 1: Nordoxepin (I.S.)

Peak 2: Nortriptyline

Peak 3: Doxepin

Peak 4: Amitriptyline

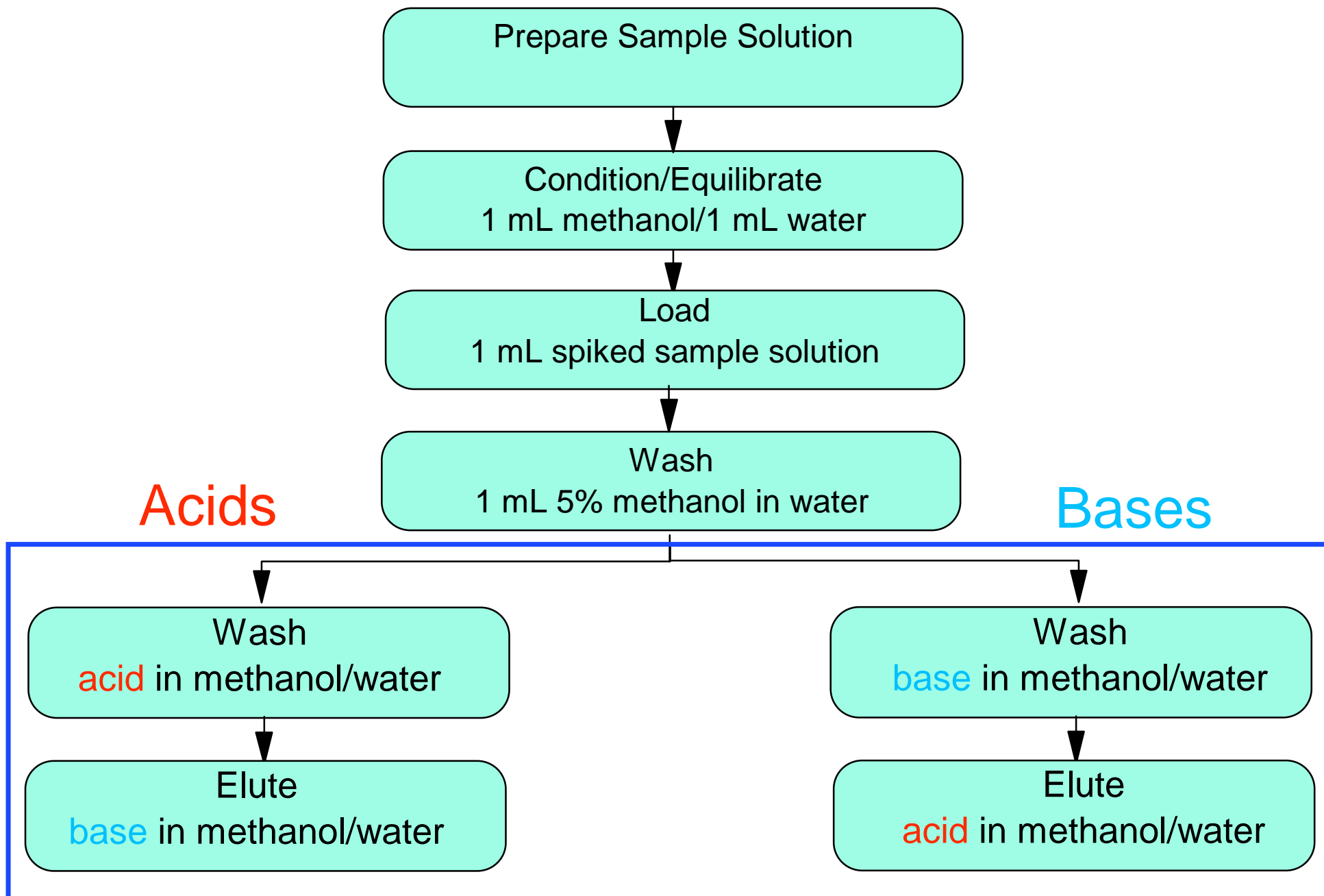
# Results from the One-Dimensional SPE Method

Using the **one-dimensional SPE method**, we were able to obtain excellent results for a wide range of compounds (concentration ranging between 40 ng/mL and 4.0 µg/mL) from a serum matrix:

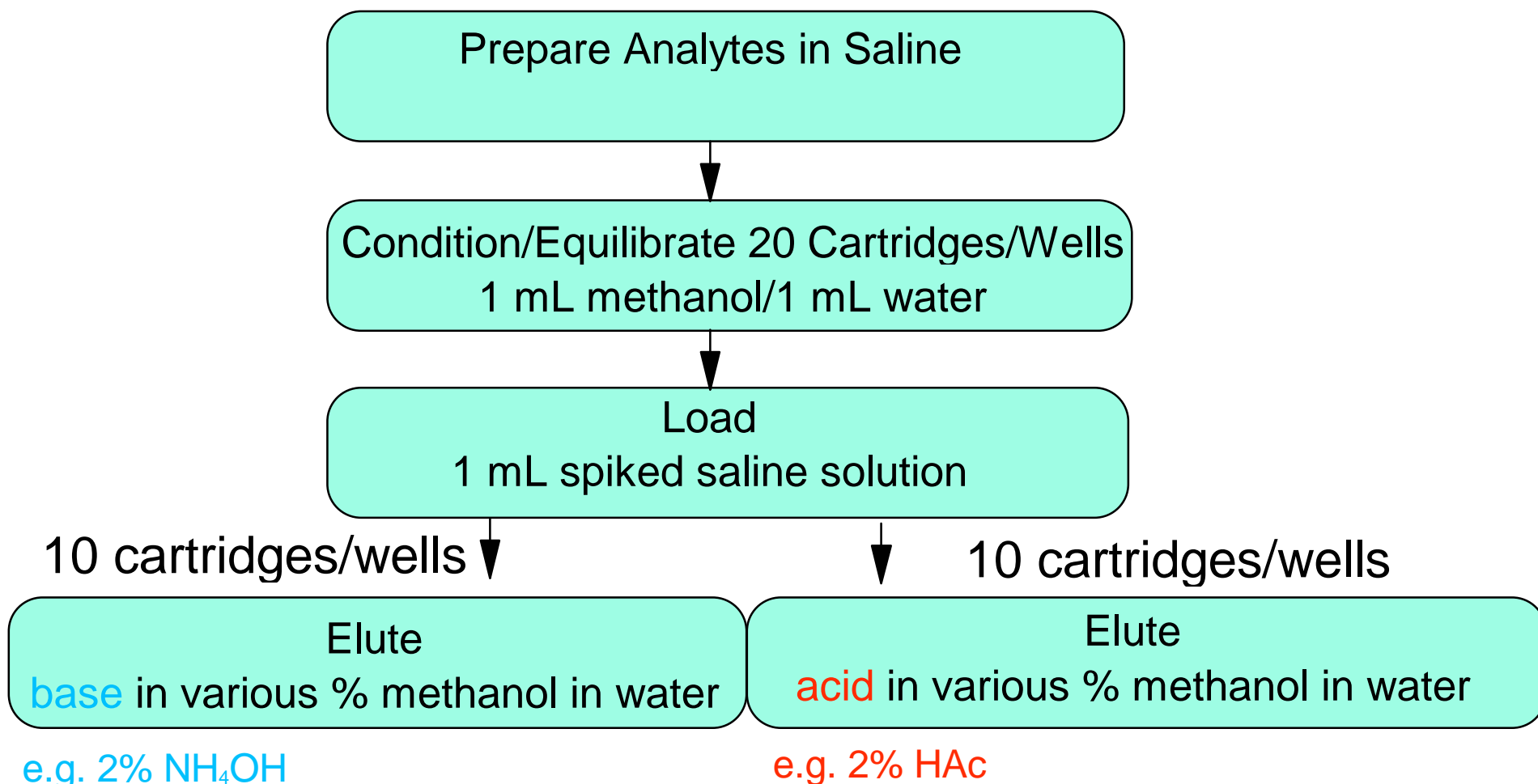
- Recoveries all greater than 86%
- Reproducibility all less than 5.0%



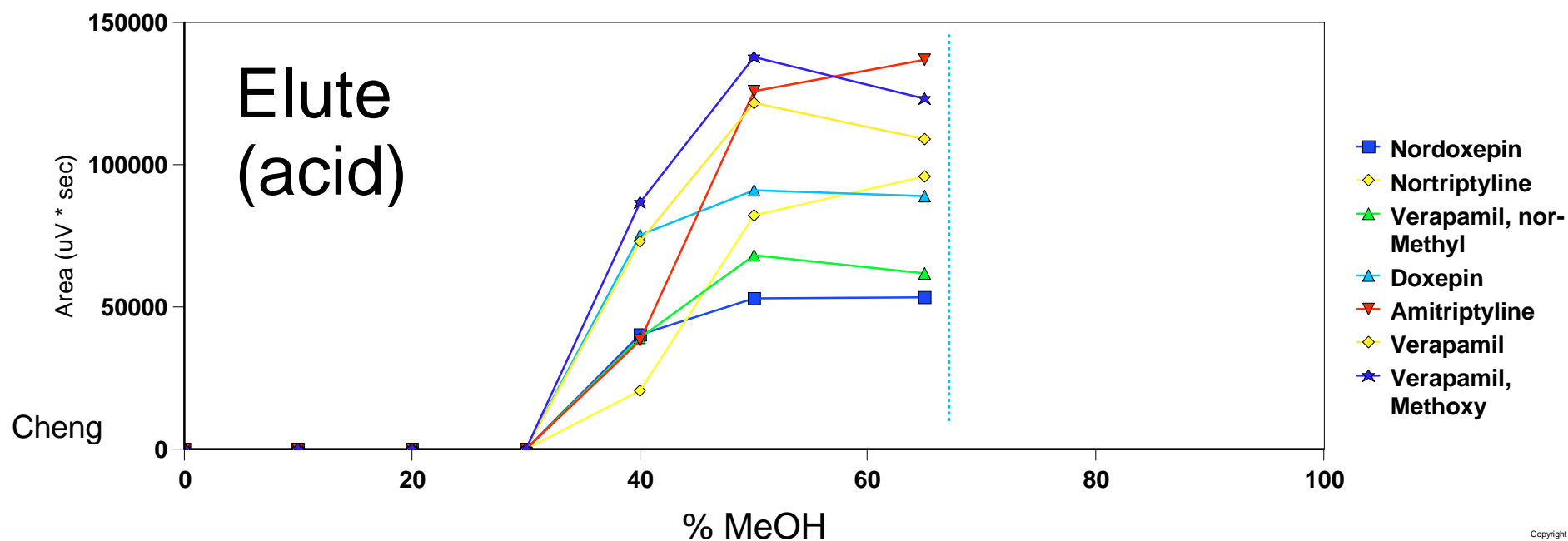
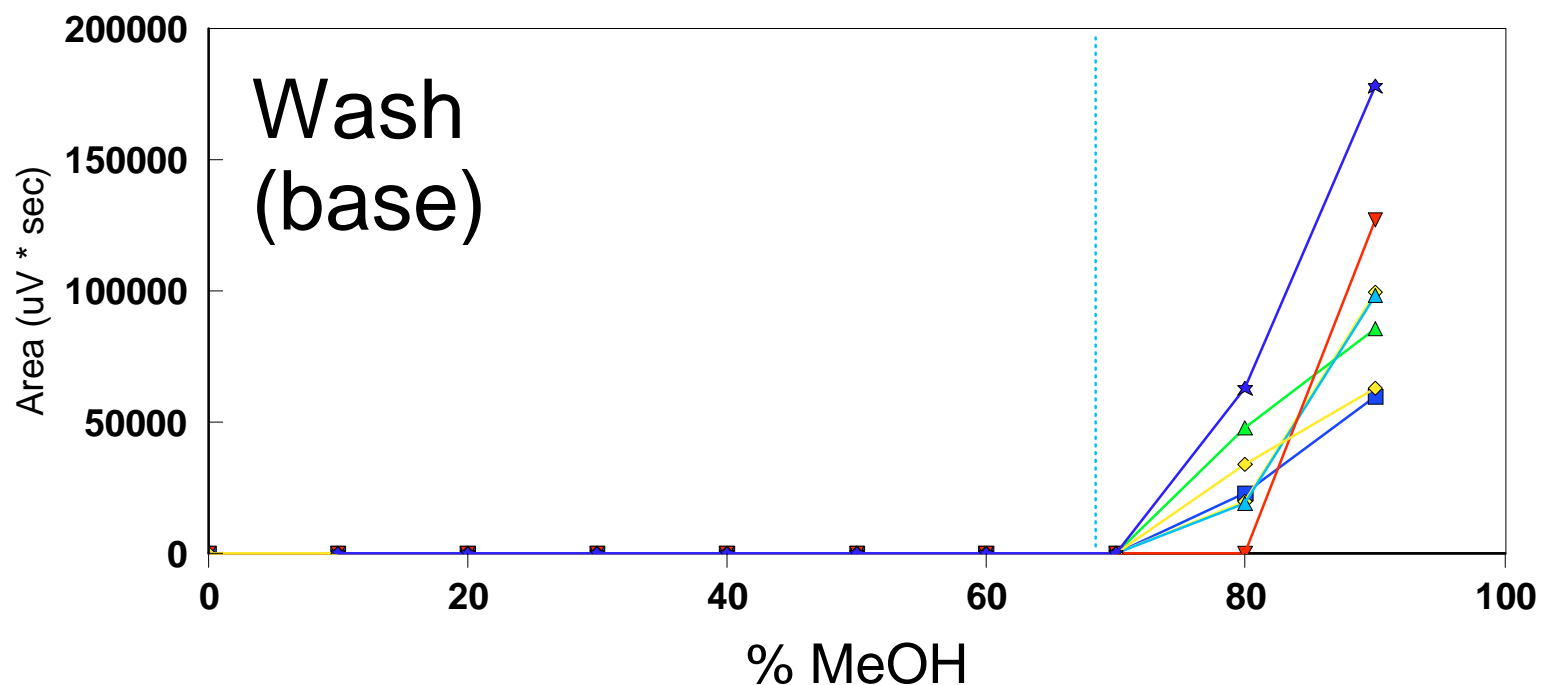
# Two-Dimensional SPE Method



# Determination of % MeOH in 2-D SPE Method: Wash-Elute Study



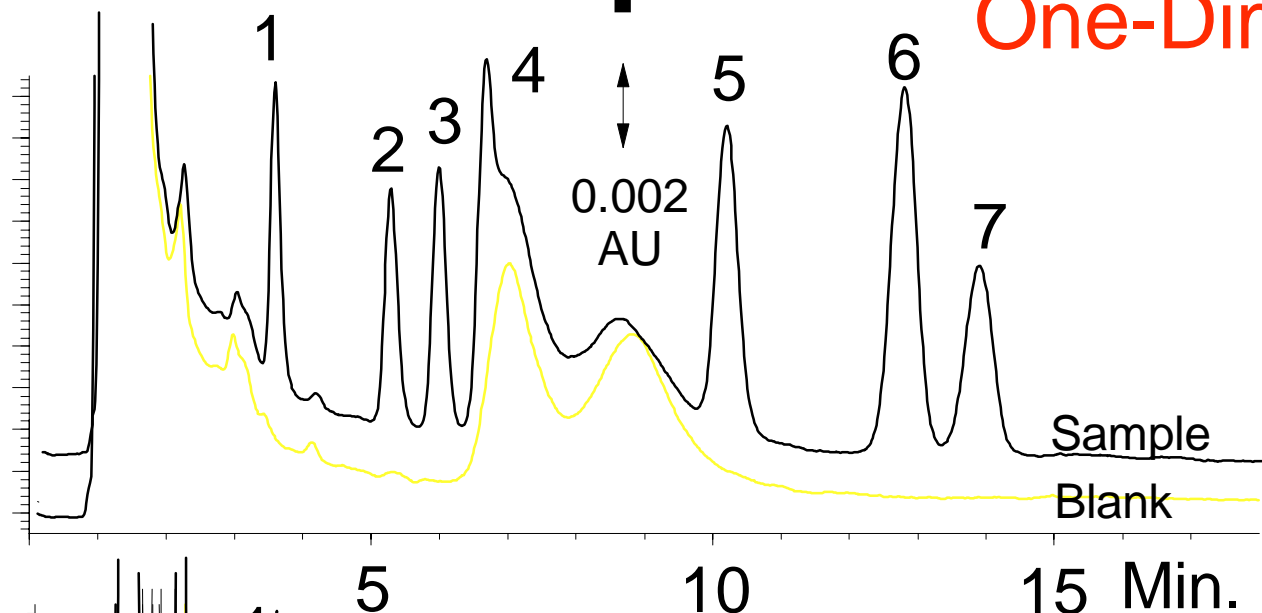
# Wash-Elute Study (Antidepressants):



# Comparison of SPE Methods:

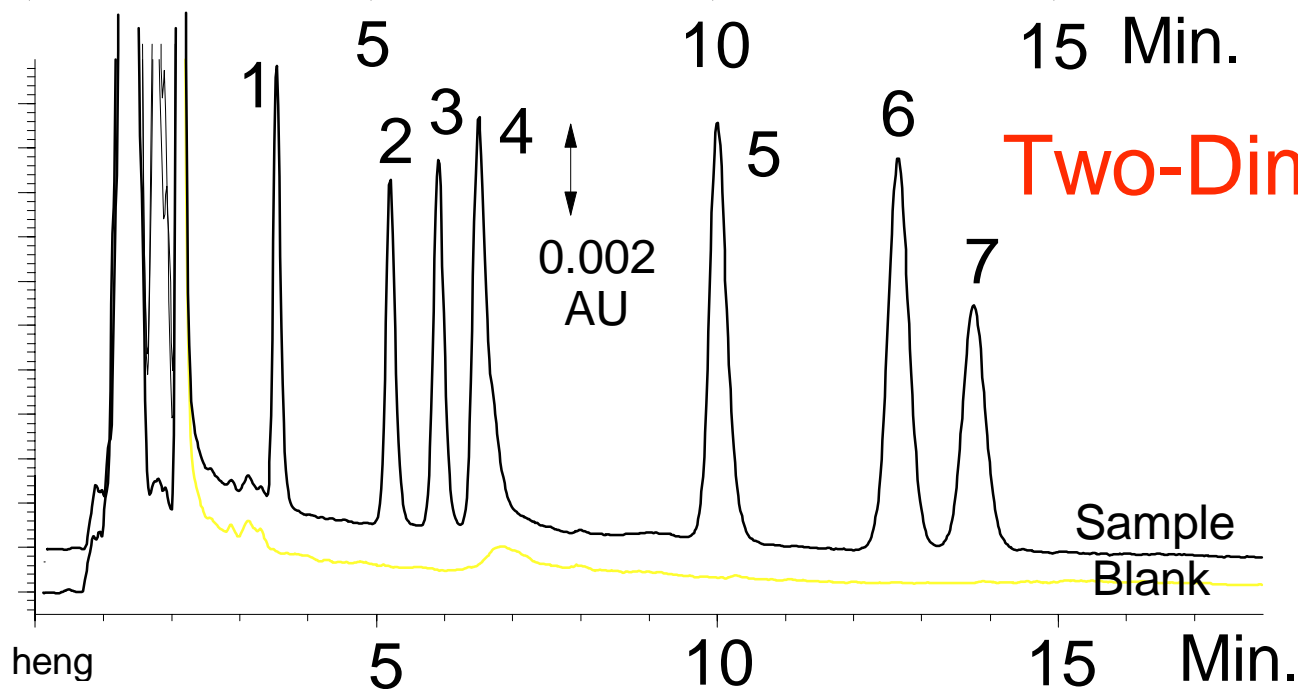
## Antidepressants in Plasma

One-Dimensional



Column: Symmetry® RP8, 5  $\mu$ m  
3.9 x 150 mm  
Temperature: 30°C  
Mobile Phase: 100 mM Phosphate pH  
acetonitrile:methanol  
(55:35:10)  
Detection: UV at 230 nm  
Flow Rate: 1.0 mL/min.  
Injection Volume: 50  $\mu$ L

Two-Dimensional



Sample Identification:

- Peak 1: Nordoxepin
- Peak 2: Nortriptyline
- Peak 3: Norverapamil
- Peak 4: Doxepin
- Peak 5: Amitriptyline
- Peak 6: Verapamil
- Peak 7: Methoxyverapamil (I.S.)

# Results: Antidepressants

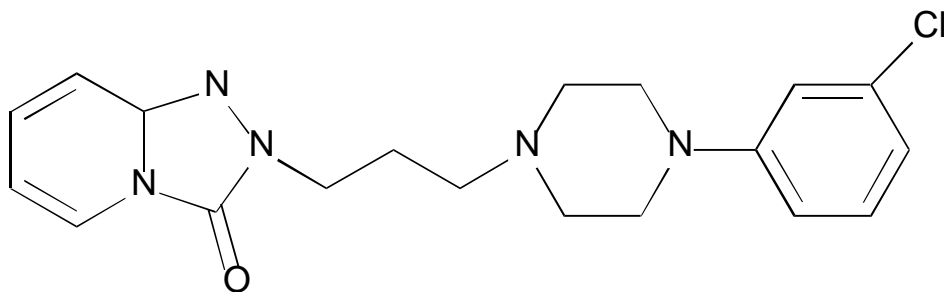
Compound		2-Dimensional		1-Dimensional	
	Concentration [µg/mL]	Recovery (%)	RSD (%) (n=6)	Recovery (%)	RSD (n=3)
Amitriptyline	1.46	99.9	1.1	100.7	0.9
Doxepin	1.0	98.4	0.7	114	3.3
Nordoxepin	0.5	92.8	0.9	94.8	1.1
Nortriptyline	0.62	95.3	1.3	97.7	0.2
Verapamil	2.5	96.7	0.6	118	0.6
Norverapamil	1.0	95.9	0.6	97.3	1.0

# Oasis™HLB

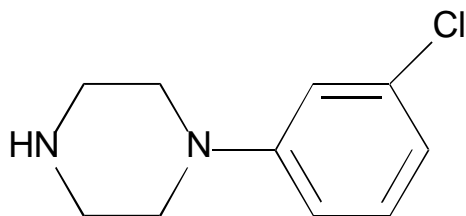
## Extraction Plates and Cartridges



# Oasis™ 96-Well Extraction Plate for High Throughput Analysis: Trazodone and Metabolite



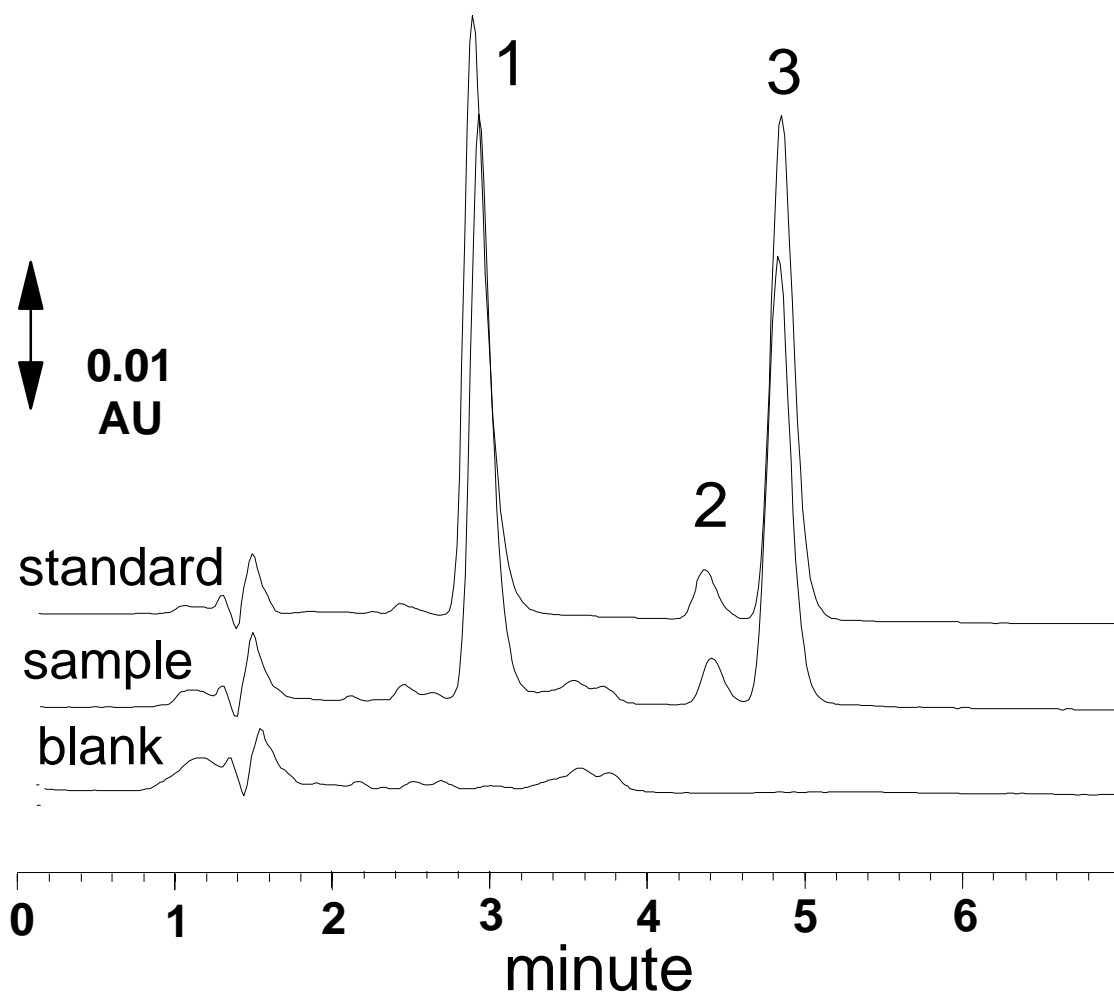
Trazodone



1-(3-Chlorophenyl)piperazine  
dihydrochloride

- **Prepare Sample**
  - 1 mL spiked porcine serum
- **Condition and equilibrate**
  - 1 mL methanol and 1 mL water
- **Load Sample**
- **Wash**
  - 1 mL 40% methanol containing 2% ammonium hydroxide
- **Elute**
  - 500 µL 80% methanol containing 2% acetic acid
- **Add 500 µL 2% ammonium hydroxide**

# Results from Oasis™ 96-Well Extraction Plate and Oasis™ Extraction Cartridges



Cheng

- Recoveries all greater than 88%
- Reproducibility all less than 5.6%

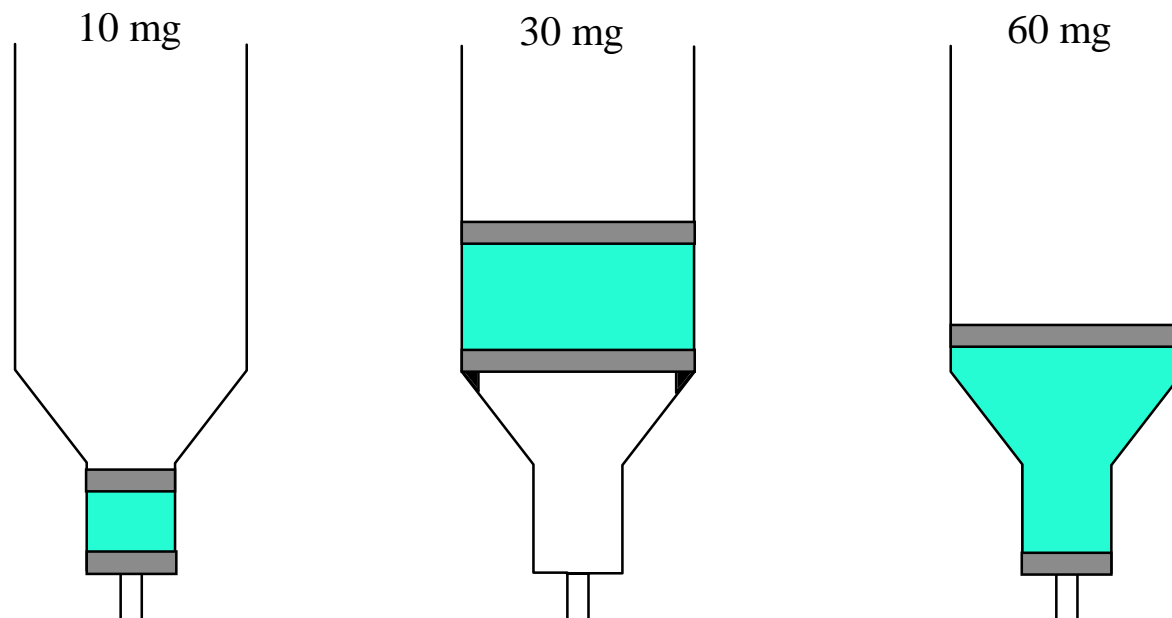
- Column: Symmetry® RP8, 5 µm, 3.9 x 150 mm
- Temperature: 30°C
- Mobile Phase: 50 mM Phosphate pH 7: :methanol (36:64)
- Detection: UV at 250 nm
- Flow Rate: 1.0 mL/min.
- Injection Volume: 25 µL
- Peak 1: 1-(3-chlorophenyl)peperazine
- Peak 2: Maprotiline (I.S.)
- Peak 3: Trazodone



# Low Elution Volume

## Waters Proprietary\* Two-Stage Well Design

- single mold
- smaller lower stage for low bed masses
- larger upper stage for high bed masses
- current bed configuration maintained

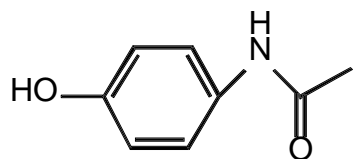


\* patent pending

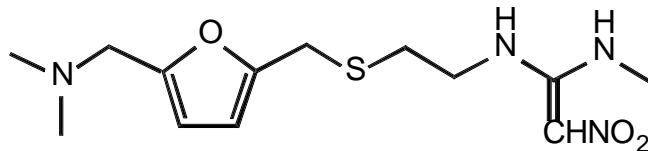
# Low Elution Volume Test Mixture:

## Acids, Neutrals, and Bases

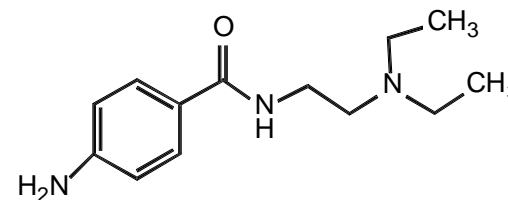
(Arranged from Most to Least Polar)



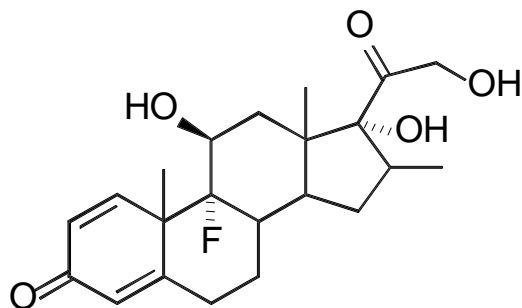
Acetaminophen



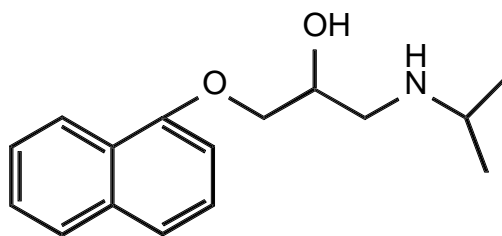
Ranitidine



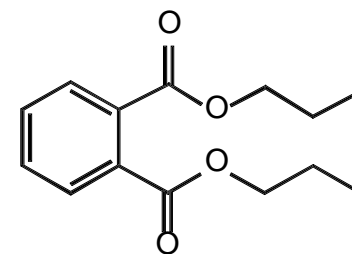
Procainamide



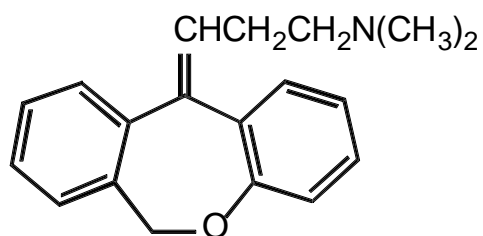
Betamethasone



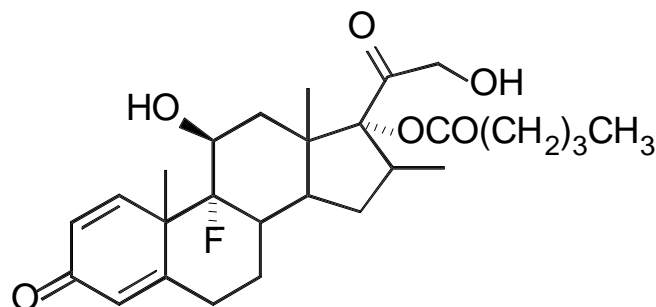
Propranolol



Dipropyl Phthalate

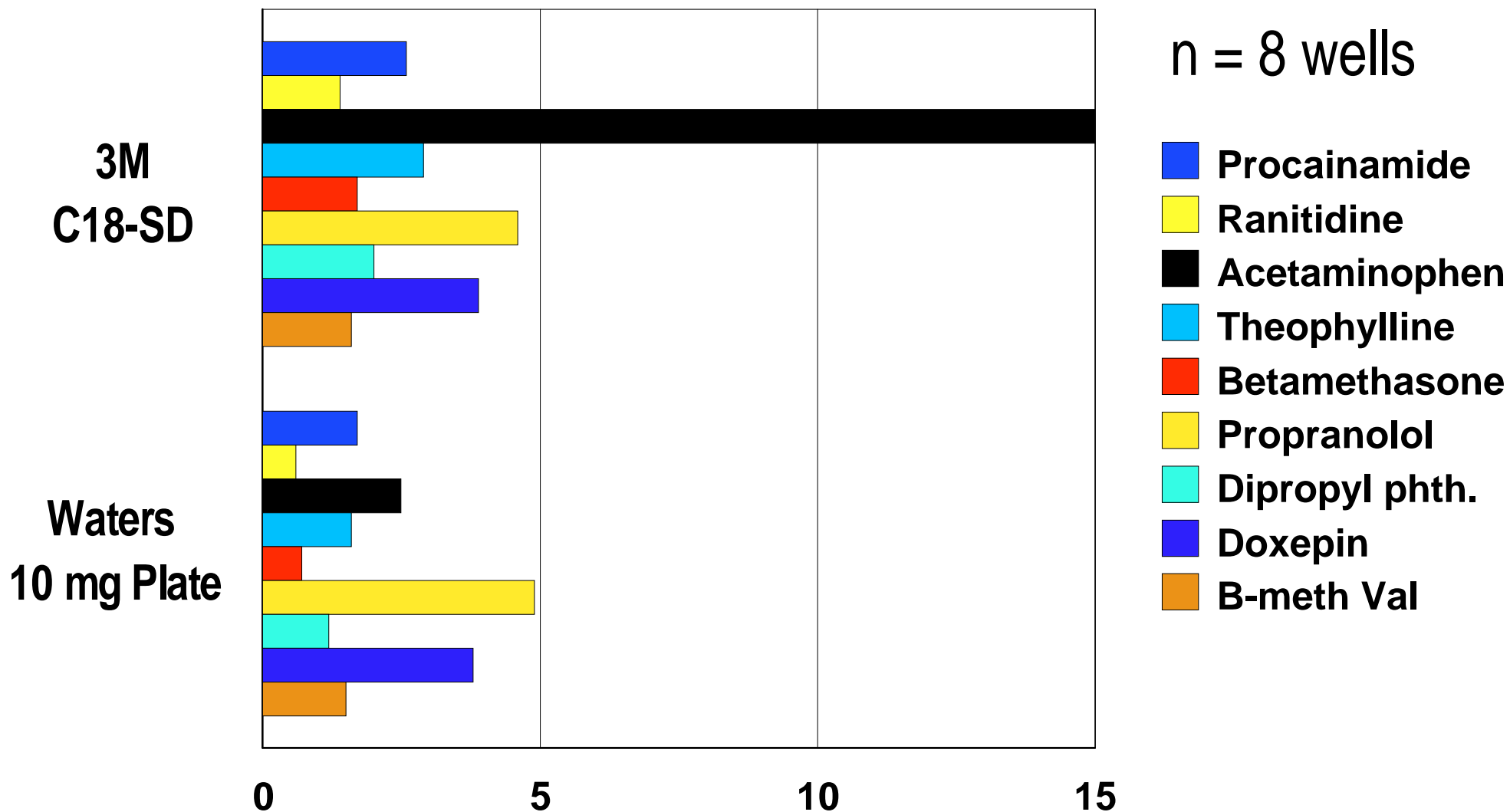


Doxepin



Betamethasone Valerate

# Waters 10 mg Extraction Plate: % RSDs in 150 $\mu$ L Elution Volume

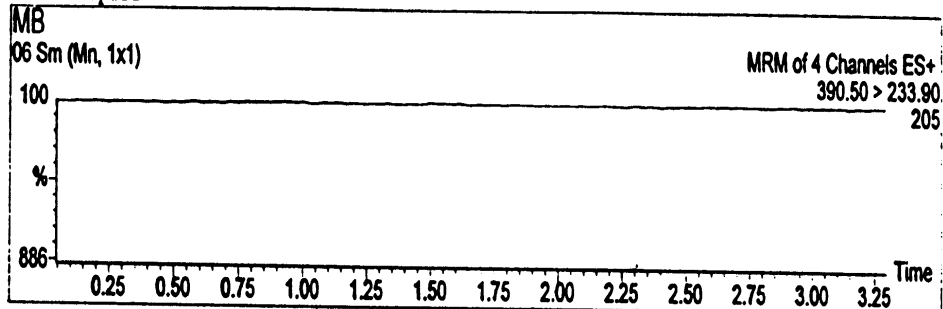


# High Sensitivity Detection

## Enalapril and Enalaprilat in Human Plasma (BLANKS)

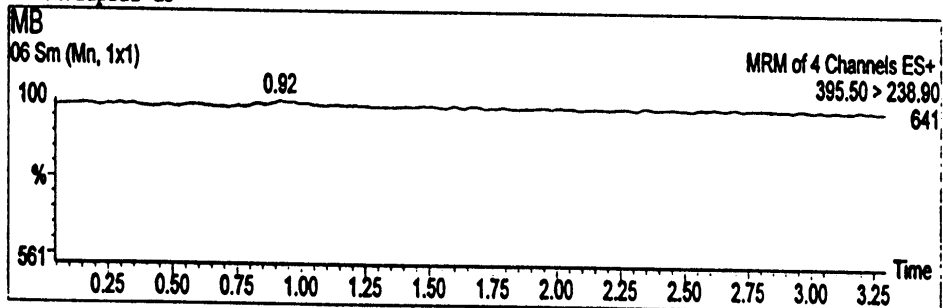
Name: 06 ID: MB Acquired: 19-Sep-1997 14:53:31  
Text:

1: enalapril



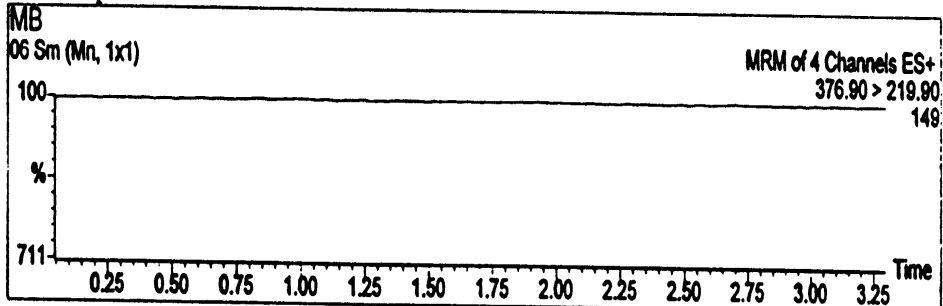
Enalapril

2: enalapril-d5



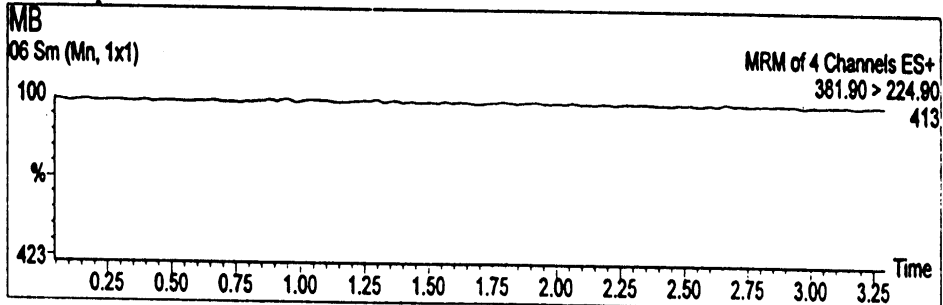
Enalapril -d<sub>5</sub>  
(IS)

3: enalaprilat



Enalaprilat

4: enalaprilat-d5



Enalaprilat -d<sub>5</sub>  
(IS)

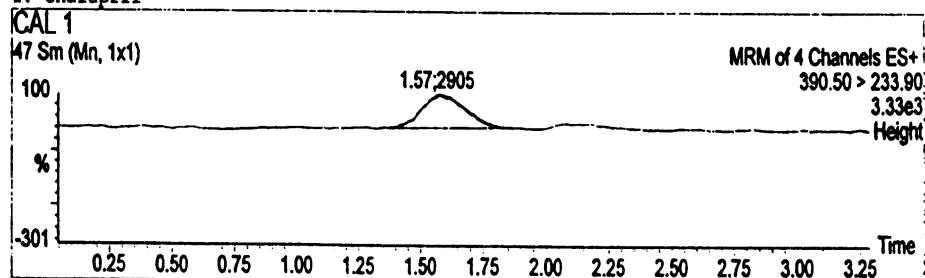
PPD Pharmaco

# High Sensitivity Detection

## Enalapril and Enalaprilat in Human Plasma

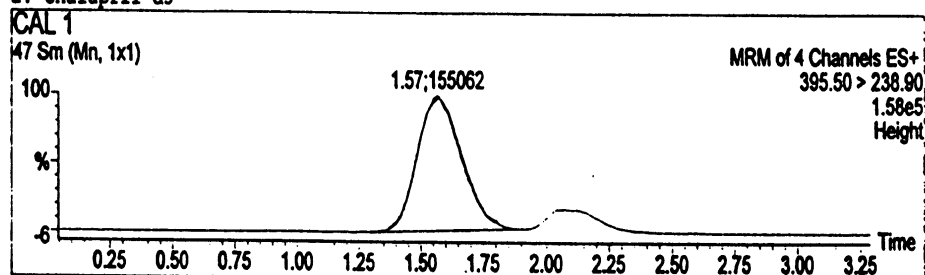
Name: 47 ID: CAL 1 Acquired: 19-Sep-1997 17:54:57  
Text: 0.25 ng/mL

1: enalapril



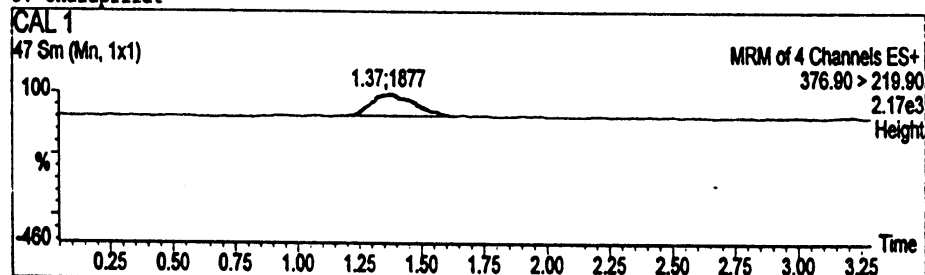
0.25 ng/mL  
Enalapril

2: enalapril-d5



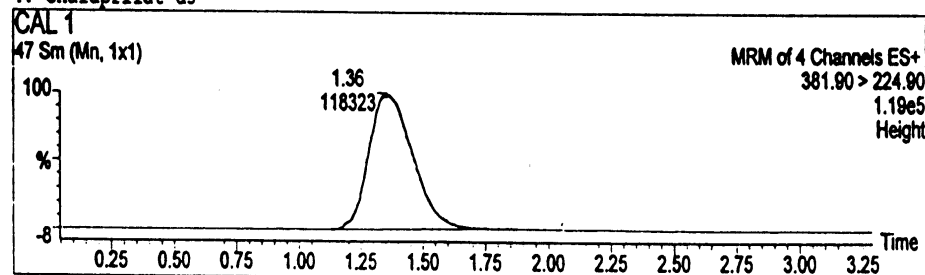
5 ng/mL  
Enalapril -d<sub>5</sub>  
(IS)

3: enalaprilat



0.25 ng/mL  
Enalaprilat

4: enalaprilat-d5



5 ng/mL  
Enalaprilat -d<sub>5</sub>  
(IS)

# Validated Method for Analysis of Enalapril in Human Plasma

Concentration	% Recovery n=6	Interday Precision, %CV n= 5 (x 2)	Intraday Precision %CV n=6
0.5 ng/mL	110.9	5.2	3.6
5 ng/mL	112.2	2.2	4.3
80 ng/mL	107.0	1.6	1.6
Internal Std.	91.1		

# Validated Method for Analysis of Enalaprilat in Human Plasma

Concentration	% Recovery n=6	Interday Precision, %CV n= 5 (x 2)	Intraday Precision %CV n=6
0.5 ng/mL	103.6	5.2	3.0
5 ng/mL	109.4	3.4	3.2
80 ng/mL	104.5	1.7	1.2
Internal Std.	94.3		

# Summary

- **Unique Sorbent**
  - No impact of drying
  - No silanols
  - Extended pH range
  - Universal sorbent
- **Fast and Easy Method Development**
  - One-dimensional method
    - Simple method
    - One method, One result
  - Two-dimensional method
    - Straightforward method
    - Cleaner extracts

