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A Unique, New, Multipurpose Sorbent Engineered for Reversed-Phase SPE

Authors

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Abstract

In the last thirty-five years, reversed-phase extraction on a solid support has gone through several stages of evolution. First, a low surface area, high pore volume, hydrophilic support matrix such as diatomaceous earth or kieselguhr, packed in large glass columns, was used to absorb an aqueous matrix, followed by elution of important analytes with an immiscible organic solvent [1]. In 1968, a new, hydrophobic, macroporous, large particle, lightly cross-linked, polystyrene polymer was used for the extraction of steroids and their metabolites from 24-hour urine samples [2]. A major advance in miniaturization and efficacy occurred in 1977, with the invention of a small, pre-packed cartridge for SPE [3] containing an octadecylsilyl-bonded silica sorbent optimized for higher efficiency and recovery. Prototypes filled with a small-particle, highly cross-linked, macroporous, divinylbenzene polymer [4] presaged introduction in the last five years of devices using similar polymer sorbents.

Abstract continued

While the use of more efficient, hydrophobic, silica- and polymer-based sorbents has been a major advance in the practice of reversed-phase SPE, there are still problems with the use of these materials. A major cause of difficulty has been the need to pre-wet these sorbents with a water-miscible organic solvent followed by conditioning with water or an aqueous buffer solution, before applying the aqueous sample matrix. If the sorbent dries out, even partially, in between the wetting/conditioning and sample loading steps, retention of the analytes falls precipitously, capacity is severely compromised, and sample breakthrough may approach 100%.

A "next generation" polymeric sorbent (patent pending) has been designed and synthesized to solve these problems. It has a unique balance of lipophilicity, needed to effect reversed-phase, extractive adsorption, and hydrophilicity, required for water-wettability. This sorbent exhibits stronger retention and excellent recoveries

Abstract concluded

of a wider spectrum of analytes, even under conditions which cause phase-collapse of traditional C_{18}-bonded silicas or "drying" of hydrophobic polymers. The improved properties and dramatic performance advantages of this new replacement for traditional reversed-phase packings will be illustrated with examples, including the isolation of both highly polar and non-polar drugs from serum.

References:

1. P. K. Siiteri, *Steroids*, **2**, 687-712 (1963).
2. H. L. Bradlow, "Extraction of Steroid Conjugates with a Neutral Resin," *Steroids*, **11(3)**, 265-272 (1968).
3. P.D. McDonald, R.V. Vivilecchia, and D.R. Lorenz, "Triaxially Compressed Beds," U.S. Patent #4,211,658.
4. D.J. Mackey and H.W. Higgins, *J. Chromatography*, **436**, 243-257 (1988).

Problems in Reversed-Phase SPE

- Poor reproducibility
- Low recovery,
  often a result of breakthrough,
  esp., of polar compounds
- Complicated methods
  development
- Tedious manipulations

A Cause of Poor Reproducibility/Recovery

A hydrophobic sorbent , once properly conditioned & equilibrated, must remain wet in order to retain analytes from an aqueous sample matrix.

"Wetting the sorbent. First, a solvent capable of wetting the alkyl chains is brought in contact with the sorbent. In the dry state, the bonded alkyl chains are twisted and collapsed on the surface; on contact with a suitable solvent, they are solvated so that they spread open to form a bristle. This ensures a good contact between the analyte and the bonded phase in the adsorption step. It is important that the sorbent remains wet in the following two steps [conditioning/equilibration & sample loading]. Failure to perform this stage will result in poor recoveries of the analyte."

p. 283 in: "*Analyte Isolation by Solid Phase Extraction (SPE) on Silica-Bonded Phases, Classification and Recommended Practices, Technical Report, International Union of Pure and Applied Chemistry, Analytical Chemistry Division, Commission of General Aspects of Analytical Chemistry*," M. Moors, D.L. Massart, and R.D. McDowall, *Pure & Appl. Chem.*, 66(2), 277-304 (1994)

Oasis™ HLB Polymer* – a Unique Solution

HLB = Hydrophilic – Lipophilic Balance

With a proper ratio of hydrophilic & lipophilic monomers, we have designed a porous polymer which not only retains a broad polarity spectrum of compounds, but also, once wetted with water, remains wet, even if the bulk fluid is removed by vacuum-assisted flow.

*Patent pending

Physicochemical Properties

This new polymer, now available packed in Oasis™ HLB Cartridges [1cc, 30 mg, or 3 cc, 60 mg], has the following certified, typical properties:

Specific Surface Area:	833 m^2/g
Average Pore Diameter:	82 Å
Total Pore Volume:	1.34 cm^3/g
Average Particle Diameter:	31 µm
Fines Content <10 µm:	0.1%
Extractable Impurities:	≤ ppm level

NOTE: A detailed Certificate of Analysis [COA] accompanies each package.

Effect of Drying - SPE Method

Condition
1 mL methanol

↓

Drying Time at 10" Hg
as shown on graph

↓

Equilibrate
1 mL water

↓

Load
1 mL spiked porcine serum

↓

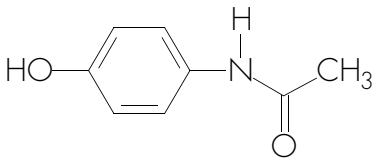
Wash
1 mL water

↓

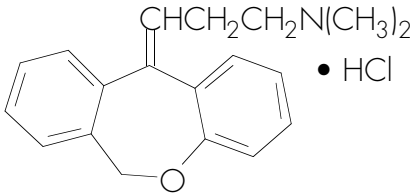
Elute
1 mL methanol

Test Compounds

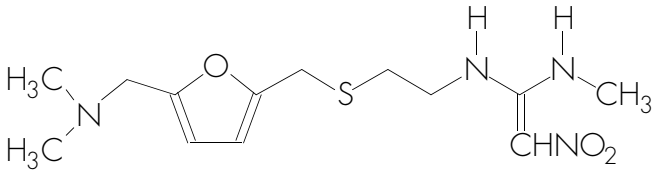
Acetaminophen



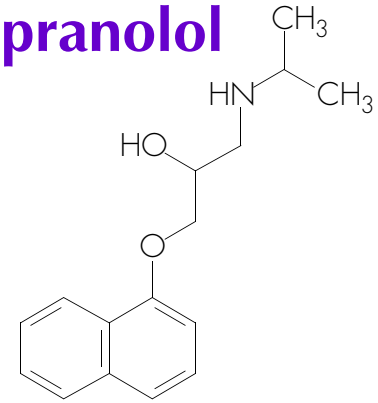
Doxepin



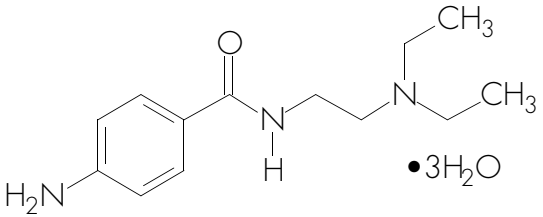
Ranitidine



Propranolol



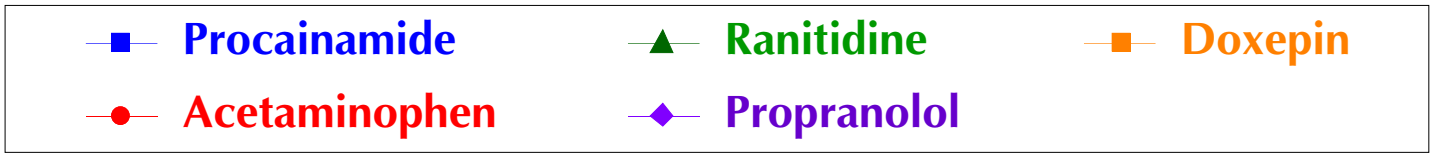
Procainamide

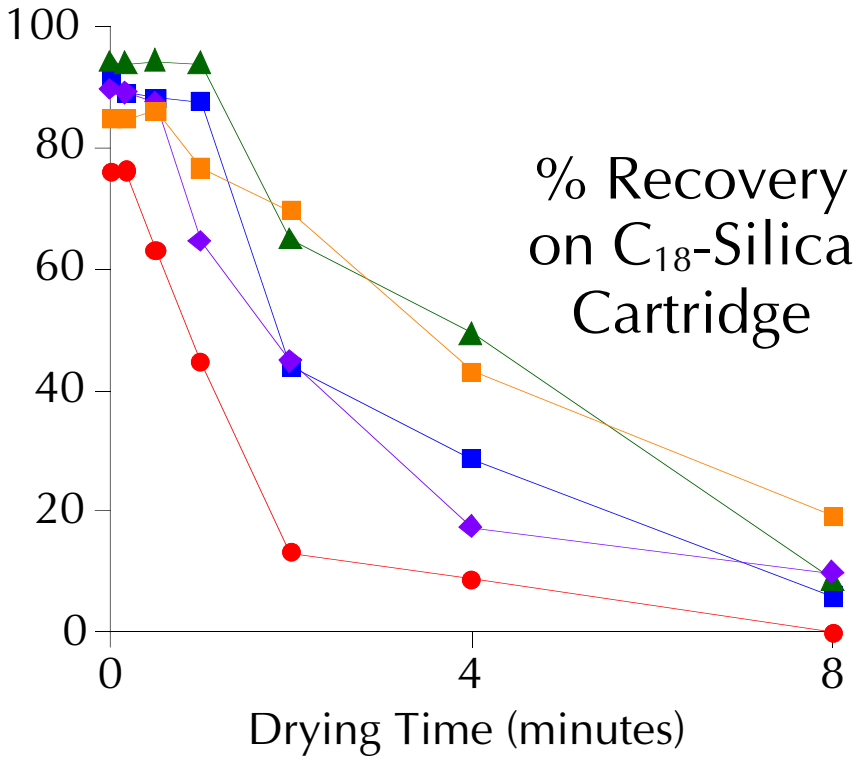


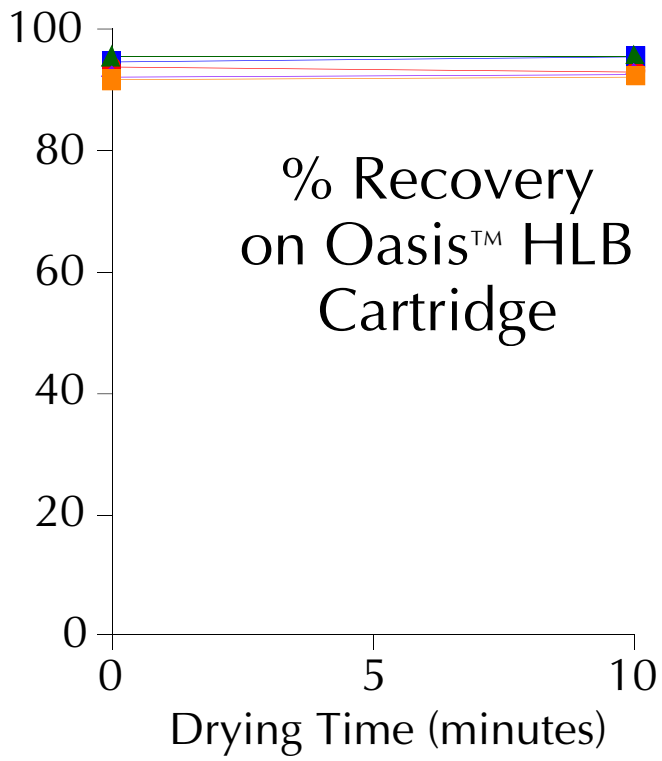
Drying Effect: HLB vs. C_{18}

		% Recovery			
		C_{18} 1cc, 100 mg		Oasis™ HLB Sorbent 1cc, 30 mg	
Compound	Concentration µg/mL	Wet	Dried 2 min	Wet	Dried 10 min
Acetaminophen	**10**	**75.4**	**13.2**	**93.9**	**93.2**
Ranitidine	**10**	**94.2**	**64.9**	**95.6**	**95.7**
Procainamide	**10**	**91.8**	**43.9**	**96.6**	**96.8**
Propranolol	**20**	**89.6**	**44.7**	**92.3**	**92.8**
Doxepin	**5**	**84.7**	**69.6**	**91.8**	**92.3**
	% RSD (n=3)	**0.10-0.80**	***6.0-54***	**0.55-1.5**	**0.18-2.0**

Drying: Oasis™ HLB Sorbent Unaffected







Ease of Use – Simple Procedure

With Waters Oasis™ HLB Cartridges:

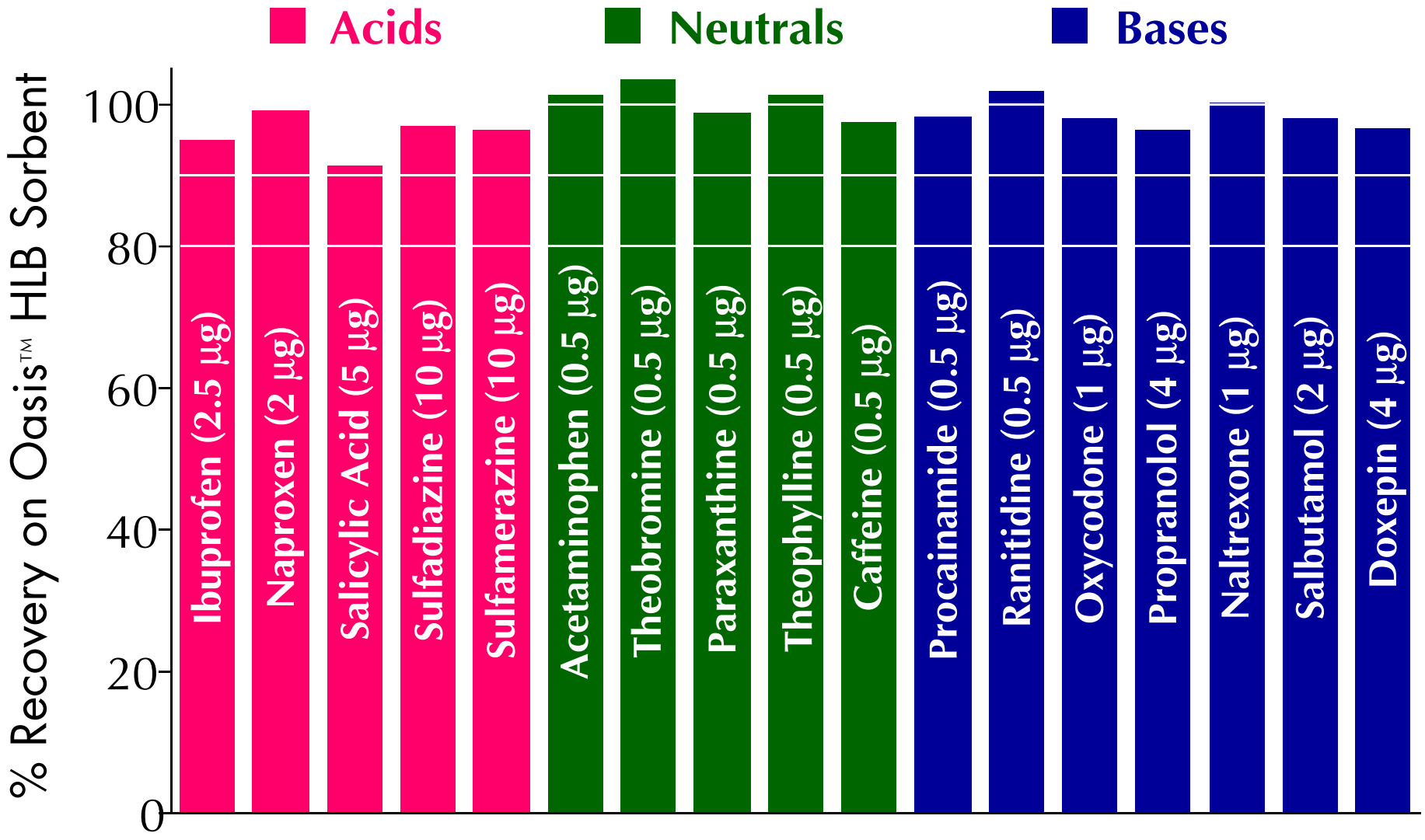
One sorbent/one protocol for many compound types – acids, bases, neutrals – makes methods development straightforward.

- ❑ Step 1: Wet w/ MeOH
- ❑ Step 2: Condition w/ water or buffer
- ❑ Step 3: Load sample (acidify, if needed)
- ❑ Step 4: Rinse w/ water or 5% MeOH
- ❑ Step 5: Elute w/ MeOH

Operation under continuous vacuum eliminates fiddling with stopcocks & worry about work interruptions!

Versatility + Uniformly High Recoveries

Wide variety of pharmaceutical compounds spiked in porcine serum at indicated number of µg/mL were recovered by extraction with 1cc, 30 mg, Oasis™ HLB Cartridges using procedure described above. [All % RSD's < 4, n = 6.]



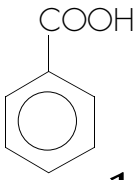
Increased Retention for Polar Compounds

Oasis™ HLB sorbent vs. trifunctional C_{18}-bonded silica

Mobile Phase: 20 mM potassium phosphate, pH 7.0/ MeOH (95/5 v/v)

Temp: 30° C. Flow: 1.0 mL/min Detection: UV@254 nm

		Retention Factor (*k*)		
	Compound	HLB	C_{18}	Ratio
1	Benzoic acid	7.36	2.05	3.6
2	Salicylic acid	34.0	4.97	6.8
3	Pyrogallol	32.8	2.60	12.6
4	Theobromine	35.9	15.5	2.4
5	Acetaminophen	69.0	9.93	6.9
6	Catechol	149	8.33	17.9



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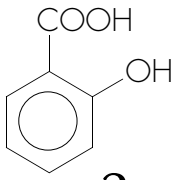

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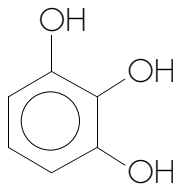

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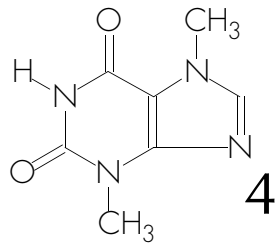

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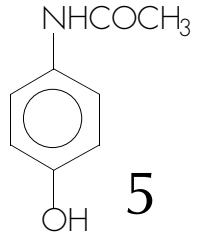

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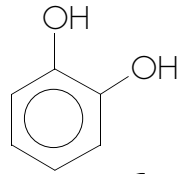

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Tricyclic Antidepressants

A simple method, without amine modifiers, achieves high recoveries.

Step 1: Condition Oasis™ HLB Cartridge (1cc, 30 mg) with 1 mL MeOH

Step 2: Equilibrate with 1 mL water

Step 3: Load 1 mL acidified (20 µL H_3PO_4) & spiked porcine serum

Step 4: Wash with 1 mL MeOH/water, 5/95 v/v

Step 5: Elute cartridge with 1 mL MeOH

Step 6: Evaporate; reconstitute in 200 µL 20/80 MeOH/buffer; inject 20 µL

Tricyclic Antidepressants

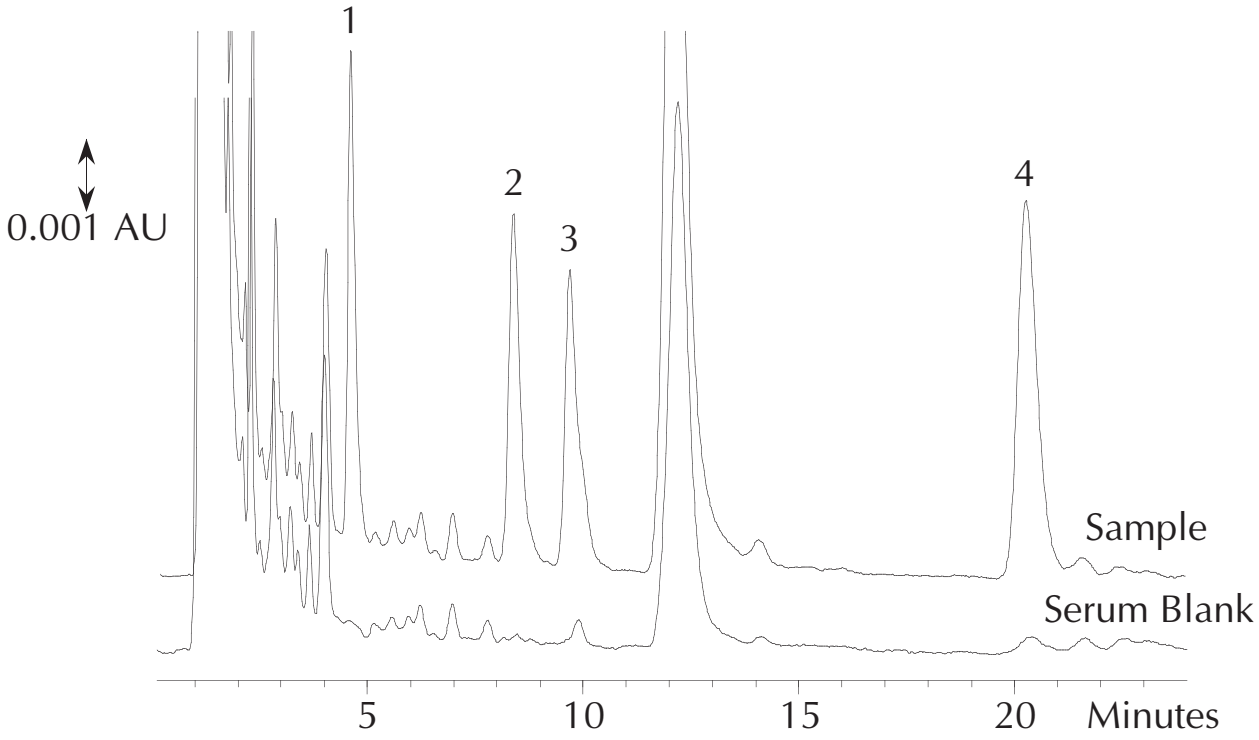
Recovery Data Comparison: HLB vs. C_{18}

		% Recovery (% RSD)	
Compound	Concentration (in porcine serum)	Oasis™ HLB Cartridge 1cc 30 mg (n=5)	C_{18} Cartridge 1cc 100 mg (n=3)
Nortriptyline	0.5 µg/mL	99.7 (2.3)	22.0 (2.3)
Doxepin	0.5 µg/mL	94.0 (1.3)	36.6 (3.9)
Amitriptyline	1.0 µg/mL	102.0 (2.5)	29.8 (8.4)

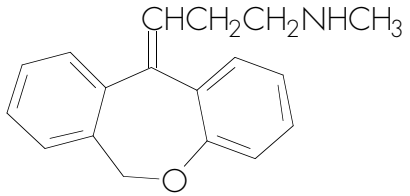
Tricyclic Antidepressants

HPLC Analysis:

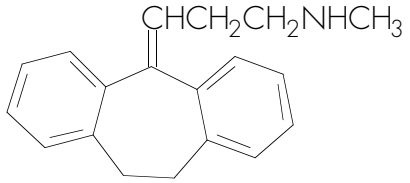
Column: Waters Symmetry® C_{18} Column, 3.9 x 150 mm
Mobile Phase: 20 mM phosphate, pH 7/MeOH (30/70 v/v)
Temp.: 35° C.; UV Detection: 254 nm; Flow rate: 1.0 mL/min
Upper Curve - Sample; Lower Curve - Serum Blank



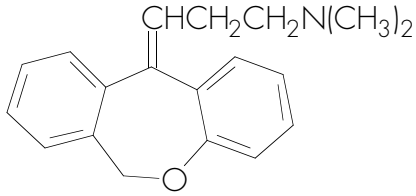
Peak 1: Nordoxepin [I.S.]



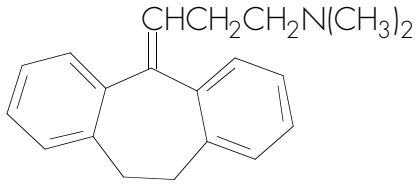
Peak 2: Nortriptyline



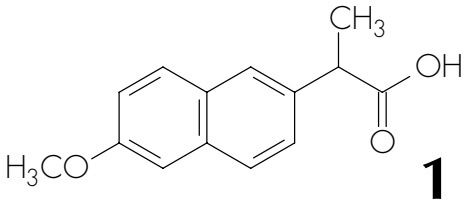
Peak 3: Doxepin



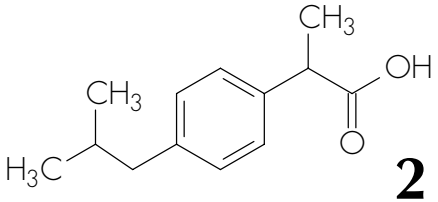
Peak 4: Amitriptyline



Analgesic/Antiinflammatory: Naproxen/Ibuprofen



1 Naproxen



2 Ibuprofen

Solid Phase Extraction Protocol: *Oasis™ HLB 1cc Extraction Cartridge*

Condition → **Load** → **Wash** → **Elute** → **Evaporate/Reconstitute**

Condition	Load	Wash	Elute	Evaporate/Reconstitute
1 mL MeOH, then 1 mL water	1 mL porcine serum acidified w/ 20 µL H_3PO_4	1 mL 5% MeOH in water	1 mL MeOH	40° C. under nitrogen stream 200 µL mobile phase

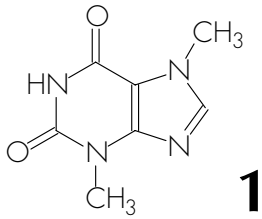
HPLC Analysis Conditions:

Column:	SymmetryShield™ RP_8, 3 x 150 mm
Sample:	20 µL of reconstituted porcine serum extract
Mobile Phase:	1.2% glacial acetic acid/ACN (50/50 v/v)
Flow Rate:	0.6 mL/min
Temperature:	25° C.
UV Detection:	254 nm

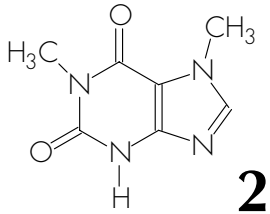
SPE Spiked Serum Results

Compound	[µg/mL]	%Recovery	%RSD (n=6)
1 Naproxen	0.100	90.%	4.0%
	0.500	98.7	1.9
	2.000	99.2	0.85
2 Ibuprofen	0.500	86.8	4.6
	2.50	95.0	3.7

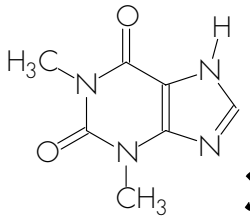
Bronchodilator: Theophylline and Other Caffeine Metabolites



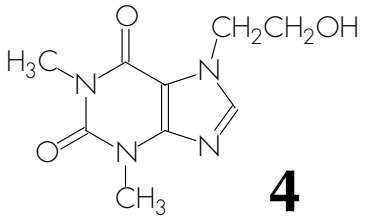
1 Theobromine



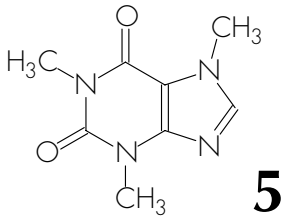
2 Paraxanthine



3 Theophylline



4 β-Hydroxyethyl-theophylline [I.S.]



5 Caffeine

Solid Phase Extraction Protocol: *Oasis™ HLB 1cc Extraction Cartridge*

Condition	→	**Load**	→	**Wash**	→	**Elute**	→	**Evaporate/Reconstitute**
1 mL MeOH, then 1 mL water		1 mL porcine serum with 0.2 µg I.S.		1 mL water		1 mL MeOH		40° C. under nitrogen stream 200 µL mobile phase

HPLC Analysis Conditions:

Column:	SymmetryShield™ RP_8, 3 x 150 mm
Sample:	20 µL of reconstituted porcine serum extract
Mobile Phase:	20 mM ammonium acetate, pH 5/ACN, 95/5 (v/v)
Flow Rate:	0.6 mL/min
Temperature:	25° C.
UV Detection:	273 nm

SPE Spiked Serum Results

Compound	[µg/mL]	%Recovery	%RSD (n=6)
1 Theobromine	0.100	103.%	3.4%
	0.500	104.	2.4
2 Paraxanthine	0.100	101.	6.2
	0.500	98.9	2.3
3 Theophylline	0.100	100.	5.2
	0.500	101.	2.1
5 Caffeine	0.100	92.6	4.4
	0.500	97.5	3.2

Summary

A new polymer has been made with Hydrophilic-Lipophilic Balance to deliver unique benefits for SPE:

- It retains compounds with a wide range of polarities, even if the sorbent runs dry, for *high, reproducible recoveries*.
- A single, simple, rugged method for acidic, basic, and neutral analytes *streamlines methods development* dramatically.

Summary

- No longer are tedious, cumbersome, stopcock manipulations required to prevent cartridge drying. This *makes SPE rapid & easy to perform*, even on multiple vacuum manifolds operated in parallel *for high sample throughput*.

- Oasis™ HLB sorbent is chemically & mechanically stable, extraordinarily clean, free of fines & silanol interactions for *predictable, consistent performance,* making *methods rugged and reliable*.

Want More Information?

For more information on Oasis™ HLB Extraction Cartridges, please contact your nearest Waters office.

Note added in proof: Oasis™ HLB sorbent is now available in 96-well plate format (see next page).

Please visit our Internet site at (click on URL):
<http://www.waters.com/oasis/welcome.htm>

Try the Oasis™ Cartridge Challenge!

Sales Offices: Austria and Export (Central Europe, CIS, Middle East, India and Subcontinent) (43) 1 8771807, *Australia* (61) 2 9933 1777, Belgium and Luxembourg (32) 02 7261000, *Brazil* (55) 11 543 7788, *Canada* 800 252 4752, *CIS* 7 095 9 336 7000, *Czech Republic* (42) 2 472 8672, *Denmark* (45) 46 598080. *Finland* (358) 90 506 4140, *France* (33) 1 30487200, *Germany* (49) 06196 40 06 00, *Hong Kong* (852) 2964 1800, *Hungary* (36) 1 270 5086, *India* (91) 80 839 4799, *Italy* (39) 02 2500565, *Japan* (81) 3 3471 7191, *Malaysia* (603) 704 8600, *Mexico* (525) 524 7636, *The Netherlands* (31) 76 508 7200, *Norway* (47) 638 46 050, *Peoples Republic of China - Beijing* (86) 10 6506 0277, *Poland* (48) 22 33 4400, *Puerto Rico* (787) 747 8445, 790 2225, *Singapore* (65) 225 4855, *Spain* (34) 3 325 9616, *Sweden* (46) 8623 0090, *Switzerland* (41) 62 889 2030, *Taiwan* (886) 2 706 8766, *UK and Ireland* (44) 1 923 816700, *All other countries: Waters Corporation U.S.A.* 508 478 2000/800 252 4752

Oasis™ HLB Sorbent Enables High Performance RP-SPE in 96-Well Plates