Waters Presents:

Solid-Phase Extraction with Oasis® HLB Sorbent:
Simple Procedures for Superior Sample Preparation

michael_s_young@waters.com

© 1998 Waters Corp.
Outline

• Introduction
• Oasis® HLB Sorbent
  – Physical properties
  – Advantages compared with traditional sorbents
• A Simple Strategy for Most Samples
  – Agrochemicals and metabolites
  – Pharmaceuticals
  – Environmental
• Advanced Strategies for Difficult Samples

© 1998 Waters Corp.
Introduction

Solid phase extraction (SPE) methods are widely used for organic analysis of aqueous samples in the agrochemical, pharmaceutical, and environmental industries.

Traditional silica based sorbents - effective for non-polar analytes

Polymeric sorbents - higher capacity than silica based sorbents

Oasis® HLB - more effective for polar analytes and metabolites
Problems of Traditional Sample Preparation Methods

- Liquid-Liquid Extraction
  - Cumbersome
  - Time consuming
  - Not easy to automate
  - Not good for polar drugs and metabolites

- Solid-Phase Extraction
  - Overcomes many problems of other methods, but .....
Disadvantages of Silica-Based SPE Sorbents

- Difficult to optimize for polar compounds
  - Inconsistant recovery
  - Lower capacity
- Tedious
  - Extractions require close attention so cartridges do not run dry

© 1998 Waters Corp.
The Ideal Sample Preparation Method:

- Removes interferences
- High and reproducible recoveries for acidic, basic and neutral analytes with a broad range of polarities
- Easy to automate for high sample throughput
- Easy to use and rugged
- Fast and cost efficient

© 1998 Waters Corp.
Oasis® HLB extraction products contain a resin made from a co-polymer of divinybenzene and vinyl pyrrolidinone. The pyrrolidinone functionality acts as an imbedded hydrophilic group. The pyrrolidinone functionality also provides enhanced retention for some polar analytes.

In this seminar, applications are presented for the agrochemical, pharmaceutical, and environmental industries.
Oasis® HLB Sorbent: A Hydrophilic-Lipophilic Balanced Copolymer

Hydrophilic monomer

Lipophilic monomer

Provides wetting properties
Reduces contact angle with water
Provides enhanced retention for polars

Provides reversed-phase property for analyte retention

© 1998 Waters Corp.
Physical Properties of Oasis® HLB Sorbent

- Specific Surface Area: 800 m²/g
- Average Pore Diameter: 80 Å
- Specific Pore Volume: 1.3 mL/g
- Average Particle Diameter: 30 µm *

*60 µm particles in products with more than 200 mg sorbent

© 1998 Waters Corp.
Effect of Drying on Recovery with Silica-based Cartridge

Bouvier, Capparella

Procainamide
Acetaminophen
Ranitidine
Propranolol
Doxepin

© 1998 Waters Corp.
Wetting:
Water vs Methanol on C<sub>18</sub>-Silica

**Water on C<sub>18</sub>**
- \(d = 100\text{Å}\)
- \(g = 72.8\text{ dynes/cm}\)
- \(\theta = 110.6^\circ\)
- \(P_c \approx 1500\text{ psi}\)

**Methanol on C<sub>18</sub>**
- \(d = 100\text{Å}\)
- \(g = 22\text{ dynes/cm}\)
- \(\theta = 39.9^\circ\)
- \(P_c \approx -1000\text{ psi}\)

Wetting: $C_{18}$ vs. Oasis® HLB

$C_{18}$

H = hydrophilic unit
L = lipophilic unit

HLB

© 1998 Waters Corp.
Drying Effect on Recovery: $C_{18}$ vs. Oasis® HLB Cartridges

Procainamide  Acetaminophen  Ranitidine  Propranolol  Doxepin

C18 Oasis™ HLB

© 1998 Waters Corp.
Advantages of Oasis® HLB Sorbent

• High Capacity - sample size of 75 - 250 mL on 60 mg cartridge, 1 L on 200 mg cartridge
• Low elution volumes (~1 mL for 60 mg cartridge)
• Higher recovery for polar metabolites such as hydroxyatrazine compared with silica-based sorbents
• Proprietary cleaning process - no significant cartridge interferences for GC/NPD:ECD or HPLC/PDA (200-360nm)
• Detection limits of 100 ng/L or better
• No stopcocks needed for routine analysis
Example: Sulfonylurea Herbicides

- Old Method, $C_{18}$ Silica
  - 1000 mg cartridge
  - 500 mL sample, 50 minutes
  - final volume 4 mL
  - recovery > 85%
  - LOQ ~ 50 ng/L
  
  *if bed runs dry during conditioning or loading, start over*

- New Method, Oasis® HLB
  - 60 mg cartridge
  - 100 mL sample, 15 minutes
  - final volume 1 mL
  - recovery > 90%
  - LOQ ~ 50 ng/L
  
  *do not worry if bed runs dry during conditioning or loading*
## Major Industries Using SPE

<table>
<thead>
<tr>
<th>Sample Types</th>
<th>Typical Analytes</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical</td>
<td>drugs, metabolites</td>
<td>1-10mL</td>
</tr>
<tr>
<td></td>
<td><em>plasma, urine</em></td>
<td></td>
</tr>
<tr>
<td>Forensic</td>
<td>drugs of abuse</td>
<td>1-25mL</td>
</tr>
<tr>
<td></td>
<td><em>urine, tissue extracts</em></td>
<td></td>
</tr>
<tr>
<td>Environmental</td>
<td>organic pollutants</td>
<td>25mL-1L</td>
</tr>
<tr>
<td></td>
<td><em>water, soil</em></td>
<td></td>
</tr>
<tr>
<td>Agrochemical</td>
<td>pesticides, metabolites</td>
<td>1mL-1L</td>
</tr>
<tr>
<td></td>
<td><em>water, soil, tissue, plasma, urine</em></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>nutrients, pesticides</td>
<td>1-100mL</td>
</tr>
<tr>
<td></td>
<td><em>beverages</em></td>
<td></td>
</tr>
</tbody>
</table>
## Choice of Cartridge Based on Sample Size

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Cartridge Size (clean samples)</th>
<th>Cartridge Size (dirty samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-25 mL</td>
<td>1 cc, 30 mg</td>
<td>3 cc, 60 mg</td>
</tr>
<tr>
<td>25-200 mL</td>
<td>3 cc, 60 mg</td>
<td>6 cc, 200 mg</td>
</tr>
<tr>
<td>200-500 mL</td>
<td>6 cc, 200 mg</td>
<td>6 cc, 500 mg (LP)</td>
</tr>
<tr>
<td>500-1000 mL</td>
<td>6 cc, 500 mg (LP)</td>
<td>12 cc, 1g (LP)</td>
</tr>
</tbody>
</table>

© 1998 Waters Corp.
General SPE Method for HPLC Analysis
(conditions for 3 cc, 60 mg Oasis® HLB cartridge)

Prepare Sample

Condition/Equilibrate
1 mL methanol/1 mL water

Load
up to 200 mL sample

Wash
1 mL 5% methanol in water

Elute
1 mL methanol

Evaporate and Reconstitute

When analyzing for basic compounds, do not use high organic content wash. There are no silanols on Oasis® HLB sorbent. Use the general method first. Use 2-D method if interference reduction is required.
General SPE Method for GC Analysis
(conditions for 6 cc, 200 mg Oasis® HLB cartridge)

- Prepare Sample
- Condition/Equilibrate: 3 mL solvent*/3 mL methanol/3 mL water
- Load: up to 500 mL sample
- Wash: 3 mL 5% methanol in water
- Elute: 6 mL solvent*
- Dry (Na₂SO₄), Adjust to Final Volume

*typical solvents: ethyl acetate, MTBE, methylene chloride

© 1998 Waters Corp.
Applications

• Agrochemical, Environmental
  – triazine herbicides and metabolites
  – PAHs
  – acidic herbicides and phenols

• Pharmaceutical
  – tetracyclines
  – basic drugs (1-D and 2-D)

• Food
  – tetracyclines
  – pesticides
Applications

First, we will look at some applications using the general methods. The general methods are simple and one dimensional (1-D, no pH adjustment in wash/elute steps).

Agrochemical: Organonitrogen herbicides and metabolites

Pharmaceutical and Food: Tetracyclines

Environmental: Organophosphorous Pesticides
**HPLC Method**

- **Column:** SymmetryShield™ RP8
- **Mobile Phase:**
  - A: 15% Acetonitrile in pH 6.7 phosphate buffer (5 mM)
  - B: Acetonitrile
- **Gradient:** 100% A for 2 min, then linear gradient to 70% B in 25 min
- **Flow Rate:** 1.0 mL/min
- **Detection:** UV @ 214 nm (0.02 AUFS)
- **Sample:** 75 mL
- **Injection:** 75 µL

**SPE:** 3 cc, 60 mg Oasis® HLB

- Spiked well water (200 ng/L)
- N\(_2\)N\(_2\)N\(_2\)Cl\(\text{N} \cdot \text{NHCH(\text{CH}_3)_2} \cdot \text{N} \cdot \text{C}_2\text{H}_5\text{NH} \cdot \text{Cl} \)
- Atrazine
- Nonspiked sample
- Blank cartridge

**Triazine Herbicides and Metabolites - HPLC**

1: Desisopropylatrazine
2: Hydroxyatrazine
3: Desethylatrazine
4: Simazine
5: Cyanazine
6: Atrazine

© 1998 Waters Corp.
## Triazine Herbicides Results

Results are given as % recovery with % RSD in parenthesis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tap Water</th>
<th>Well Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spike level 500 ng/L</td>
<td>Spike level 200 ng/L</td>
</tr>
<tr>
<td></td>
<td>5 replicates</td>
<td>7 replicates</td>
</tr>
<tr>
<td>Desisopropylatrazine</td>
<td>98.4 (5.0)</td>
<td>95.6 (5.8)</td>
</tr>
<tr>
<td>Hydroxyatrazine</td>
<td>132 (1.3)</td>
<td>109 (11)</td>
</tr>
<tr>
<td>Desethylatrazine</td>
<td>106 (5.1)</td>
<td>104 (4.0)</td>
</tr>
<tr>
<td>Simazine</td>
<td>n.a.*</td>
<td>97.7 (3.9)</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>n.a.*</td>
<td>93.1 (3.7)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>101 (5.0)</td>
<td>101 (4.4)</td>
</tr>
</tbody>
</table>

*Simazine and cyanazine were not included in the tap water spike experiment*
Acetamide Herbicides and Metabolites

HPLC

SPE: 3 cc, 60 mg Oasis® HLB

HPLC Method

Column: Symmetry® C₈

Mobile Phase:
A: 30% Acetonitrile in pH 6.8 phosphate buffer
B: Acetonitrile

Gradient: 100% A initial, then linear gradient to 60% B in 20 min

Flow Rate: 1.2 mL/min

Detection: UV @ 214 nm (0.02 AUFS)

Injection: 80 µL

1: 2-[(2-ethyl-6-methylphenyl)amino]-1-propanol
2: 2-chloro-2’,6’-diethylacetanilide
3: 2,6-diethylaniline
4: alachlor
5: metolachlor

© 1998 Waters Corp.
# Acetamide Herbicides - Results

Results are given as % recovery with % RSD in parenthesis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tap Water</th>
<th>Tap Water</th>
<th>Well Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 µg/L</td>
<td>200 ng/L</td>
<td>200 ng/L</td>
</tr>
<tr>
<td></td>
<td>5 replicates</td>
<td>5 replicates</td>
<td>5 replicates</td>
</tr>
<tr>
<td>metolachlor-metabolite&lt;sup&gt;1&lt;/sup&gt;</td>
<td>99.1 (10)</td>
<td>78.9 (22)</td>
<td>88.3 (3.3)</td>
</tr>
<tr>
<td>alachlor-metabolite&lt;sup&gt;1&lt;/sup&gt;</td>
<td>98.5 (3.8)</td>
<td>109* (34)</td>
<td>105 (24)</td>
</tr>
<tr>
<td>alachlor-metabolite&lt;sup&gt;2&lt;/sup&gt;</td>
<td>93.3 (11)</td>
<td>80.3 (23)</td>
<td>89.2 (7.8)</td>
</tr>
<tr>
<td>alachlor</td>
<td>93.9 (3.2)</td>
<td>90.1 (21)</td>
<td>89.9 (1.2)</td>
</tr>
<tr>
<td>metolachlor</td>
<td>97.1 (6.6)</td>
<td>93.0 (16)</td>
<td>112 (3.3)</td>
</tr>
</tbody>
</table>

* significant blank value subtracted

<sup>1</sup> 2-[(2-ethyl-6-methylphenyl)amino]1-propanol
<sup>2</sup> 2-chloro-2',6'-diethylacetanilide
<sup>3</sup> 2,6-diethylaniline
Tetracyclines in Serum

Sample: 1 mL spiked porcine serum
2% phosphoric acid
(demecycline as IS)

SPE: 1 cc, 30 mg Oasis® HLB

HPLC Method
Column: SymmetryShield™ RP₈
Mobile Phase: 0.1% TFA in water:
acetonitrile:methanol
(91:7:2)
Flow Rate: 0.9 mL/min
Detection: UV @ 279 nm
Injection: 20 µL

1. Minocycline
2. Tetracycline
3. Demeclocycline (IS)

© 1998 Waters Corp.
Tetracyclines - Serum Results

Results from analysis of 6 replicates, spike level 2.5 µg/mL

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocycline</td>
<td>94.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>104</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Compare with results obtained using C18 cartridges:

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocycline</td>
<td>40.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>67.4</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Tetracyclines in Meat

Sample: 5 g homogenized meat, extracted with 2 x 20 mL of McIlvaine buffer (mixed citrate/phosphate, pH 4.1 with EDTA). The combined extracts were filtered before SPE.

SPE: 6 cc, 200 mg Oasis® HLB

HPLC Method
Column: Nova-Pak® C₈
Mobile Phase: 20% acetonitrile in 50 mM oxalic acid/water
Flow Rate: 0.8 mL/min
Detection: UV @ 365 nm
Injection: 60 µL

© 1998 Waters Corp.
Tetracyclines in Milk

**Sample:** The milk sample (15 mL) was diluted with 25 mL of McIlvaine buffer (mixed citrate/phosphate, pH 4.1) with added EDTA. The diluted sample was centrifuged at 8000 x g for 10 minutes at 5°C before SPE.

**SPE:** 3 cc, 60 mg Oasis® HLB

**HPLC Method**
- **Column:** Nova-Pak® C₈
- **Mobile Phase:** 13% acetonitrile, 13% methanol in 50 mM oxalic acid/water
- **Flow Rate:** 0.8 mL/min
- **Detection:** UV @ 365 nm
- **Injection:** 60 µL
## Tetracyclines - Food Results

Results are given as % recovery with % RSD in parenthesis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Beef</th>
<th>Pork</th>
<th>Milk 25 µg/L</th>
<th>Milk 50 µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>96.2 (5.7)</td>
<td>103 (5.0)</td>
<td>70.7 (3.5)</td>
<td>67.7 (5.8)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>91.4 (5.5)</td>
<td>99.8 (6.1)</td>
<td>73.7 (7.3)</td>
<td>68.5 (5.1)</td>
</tr>
<tr>
<td>Chlortetramycin</td>
<td>80.6 (6.8)</td>
<td>83.4 (6.1)</td>
<td>76.7 (2.9)</td>
<td>67.3 (1.8)</td>
</tr>
</tbody>
</table>

© 1998 Waters Corp.
Organophosphorous Pesticides - GC

GC Conditions
- **Column:** SGE BPX5 capillary, 30 meters, 0.32 mm ID, 0.25 µm film
- **Carrier Gas:** Helium @ 30 cm/sec
- **Temp Program:** 50°C initial, 30°C/min to 150°C, then 6°C/min to 300°C
- **Detection:** NPD
- **Injection:** 2 µL

**Chemicals:**
1. dichlorvos
2. mevinphos
3. naled
4. ethoprop
5. phorate
6. demeton
7. diazinon
8. disulfoton
9. parathion
10. ronnel
11. chlorpyrifos
12. fenthion
13. trichloronate
14. tetrachlorovinphos
15. tokuthion
16. merphos
17. fensulfothion
18. bolstar
19. azinphos methyl
20. coumaphos

© 1998 Waters Corp.
Organophosphorous Pesticides
Oasis® HLB Method

• Water samples were analyzed using the general (1-D) GC based method. The elution solvent was 10% methanol in methyl t-butyl ether (MTBE).
• Apple samples (5g) were homogenized, spiked with pesticides and were extracted with 20 mL acetonitrile (1hr on shaker).
A 5 mL aliquot of extract was diluted to 100 mL with water and SPE was then performed using the same protocol as the water samples
Organophosphorous Pesticides Results

Results are given as % recovery with % RSD in parenthesis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tap Water</th>
<th>Apples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250ng/L spike</td>
<td>400ng/g spike</td>
</tr>
<tr>
<td></td>
<td>4 replicates</td>
<td>4 replicates</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>86.7 (7.9)</td>
<td>104 (8.8)</td>
</tr>
<tr>
<td>Mevinphos</td>
<td>99.6 (7.8)</td>
<td>51.0 (3.5)</td>
</tr>
<tr>
<td>Naled</td>
<td>112 (8.0)</td>
<td>95.6 (9.6)</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>110 (8.9)</td>
<td>121 (5.8)</td>
</tr>
<tr>
<td>Phorate</td>
<td>88.2 (14)</td>
<td>84.0 (10)</td>
</tr>
<tr>
<td>Demeton</td>
<td>110 (9.9)</td>
<td>104 (10)</td>
</tr>
<tr>
<td>Diazinon</td>
<td>113 (11)</td>
<td>108 (11)</td>
</tr>
<tr>
<td>Disulfoton</td>
<td>115 (12)</td>
<td>102 (12)</td>
</tr>
<tr>
<td>Parathion Methyl</td>
<td>112 (9.8)</td>
<td>101 (12)</td>
</tr>
<tr>
<td>Ronnel</td>
<td>111 (11)</td>
<td>89.5 (13)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>120 (11)</td>
<td>84.7 (12)</td>
</tr>
<tr>
<td>Fenthion</td>
<td>112 (8.5)</td>
<td>88.4 (12)</td>
</tr>
<tr>
<td>Trichloronate</td>
<td>118 (7.9)</td>
<td>81.3 (11)</td>
</tr>
<tr>
<td>Tetrachlorovinphos</td>
<td>111 (9.8)</td>
<td>87.0 (12)</td>
</tr>
<tr>
<td>Tokuthion</td>
<td>112 (10)</td>
<td>73.8 (8.3)</td>
</tr>
<tr>
<td>Merphos</td>
<td>107 (9.9)</td>
<td>139 (5.2)</td>
</tr>
<tr>
<td>Fensulfothion</td>
<td>108 (9.4)</td>
<td>101 (10)</td>
</tr>
<tr>
<td>Bolstar</td>
<td>111 (7.8)</td>
<td>72.3 (9.8)</td>
</tr>
<tr>
<td>Azinphos Methyl</td>
<td>113 (9.3)</td>
<td>82.3 (2.7)</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>113 (9.0)</td>
<td>77.0 (9.6)</td>
</tr>
</tbody>
</table>

© 1998 Waters Corp.
Modifications to the General Methods

• For combined GC and LC analysis
  – Highly non-polar analytes (PAH)
• For reduction of interferences
  – Solvent selectivity method: retain interference, elute analytes (acidic herbicides and phenols)
  – Two dimensional SPE (2-D): retain analytes, wash off interference (drugs in plasma, base/neutrals in environmental samples)
For combined GC and LC analysis

• Use the general GC method
• Use an elution solvent which is compatible with GC but is volatile and simple to remove by evaporation
• For LC, exchange the volatile solvent for an LC solvent compatible with mobile phase
• For GC, dry the extract and adjust final volume

Example: PAHs are too non-polar for elution with methanol. Use methylene chloride (DCM) for elution and exchange to acetonitrile for LC.
PAH In Drinking Water

Sample: 1L tap water spiked with 16 PAH compounds

SPE: 6 cc, 200 mg Oasis® HLB general GC method, DCM elution

HPLC Method
Column: SepServe PAH, 125 x 4.6 mm
Mobile Phase: A: water
             B: Acetonitrile
Gradient: 60% A for 1 min, then linear gradient to 100% B in 15 min
Flow Rate: 1.2 mL/min
Detection: UV @ 254 nm (0.02 AUFS)
Sample: 1 L tap water spiked @ 1µg/L
Injection: 20 µL

© 1998 Waters Corp.
## PAH Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spike level 1.0 µg/L</th>
<th>Spike level 200 ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 replicates</td>
<td>4 replicates</td>
</tr>
<tr>
<td>naphthalene</td>
<td>66.5</td>
<td>55</td>
</tr>
<tr>
<td>acenaphthylene</td>
<td>99.2</td>
<td>78</td>
</tr>
<tr>
<td>acenaphthene</td>
<td>98.4</td>
<td>77</td>
</tr>
<tr>
<td>fluorene</td>
<td>105</td>
<td>82.4</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>114</td>
<td>94.4</td>
</tr>
<tr>
<td>anthracene</td>
<td>103</td>
<td>83.0</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>115</td>
<td>91.9</td>
</tr>
<tr>
<td>pyrene</td>
<td>117</td>
<td>98.9</td>
</tr>
<tr>
<td>benzo(a)anthracene</td>
<td>102</td>
<td>85.9</td>
</tr>
<tr>
<td>chrysene</td>
<td>105</td>
<td>90.3</td>
</tr>
<tr>
<td>benzo(b)fluoranthene</td>
<td>104</td>
<td>80.1</td>
</tr>
<tr>
<td>benzo(k)fluoranthene</td>
<td>90.9</td>
<td>77.4</td>
</tr>
<tr>
<td>benzo(a)pyrene</td>
<td>94.3</td>
<td>69.8</td>
</tr>
<tr>
<td>dibenzo(g,h,i)perylene</td>
<td>92.1</td>
<td>59.5</td>
</tr>
<tr>
<td>benzoperylene</td>
<td>92.0</td>
<td>65.9</td>
</tr>
<tr>
<td>indenopyrene</td>
<td>92.8</td>
<td>62.3</td>
</tr>
</tbody>
</table>

RSDs < 5 %                  RSDs < 10 %
Solvent Selectivity Method for Reduction of Humic Interferences

- Useful for surface water or soil extracts
- Use an elution solvent which is polar enough to effectively elute analytes but retains humic material on sorbent (10% Methanol in MTBE is a very good choice for many analytes)
- Evaporate the MTBE from the eluent and take up the residue in HPLC mobile phase

Example: Acidic herbicides and phenols
  Sample: 40-150 mL acidified to pH 2 with H₃PO₄
  SPE: 3 cc, 60 mg Oasis® HLB Cartridge
Reduction of Humic Interference

A: Elution with methanol
B: Elution with 10% methanol in methyl t-butyl ether

HPLC
Columns: SymmetryShield™ RP8
Mobile Phase: A: pH 3.0 phosphate buffer (20mM)
B: Acetonitrile
Gradient: 85% A linear to 20% A in 15 min
Flow Rate: 1.0 mL/min
Detection: UV @ 215 nm (0.3 AUFS)
Injection: 50 µL
Sample: 150 mL of surface water

1. picloram
2. dicamba
3. chloramben
Acidic Herbicides - 15 Components

Column: SymmetryShield™ RP₈
Mobile Phase:
A: pH 3.4 phosphate buffer (13 mM)
B: Acetonitrile
Gradient: 85% A linear to 70% A in 8 min, hold until 15 min, then linear to 40% A in 30 min, then linear to 10% A in 35 min.
Flow Rate: 1.0 mL/min
Detection: UV @ 230 nm (0.015 AUFS)
Injection: 75 µL

1: picloram
2: dicamba
3: chloramben
4: 4-nitrophenol
5: bentazon
6: 2,4-D
7: MCPA
8: dichlorprop
9: 2,4,5-T
10: MCPP
11: 3,5-dichlorobenzoic
12: 2,4-DB
13: 2,4,5-TP
14: acifluorfen
15: dinoseb

© 1997 Waters Corp.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Tap Water 2.0 µg/L 5 replicates</th>
<th>Tap Water 400 ng/L 5 replicates</th>
<th>Well Water 2.0 µg/L 5 replicates</th>
<th>Well Water 400 ng/L 5 replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>picloram</td>
<td>90.9 (7.0)</td>
<td>126 (5.3)</td>
<td>97.5 (3.8)</td>
<td>106 (2.3)</td>
</tr>
<tr>
<td>dicamba</td>
<td>85.1 (7.2)</td>
<td>115 (4.4)</td>
<td>98.5 (3.8)</td>
<td>96.3 (8.3)</td>
</tr>
<tr>
<td>chloramben</td>
<td>86.7 (7.3)</td>
<td>99.2 (6.9)</td>
<td>95.1 (10)</td>
<td>90.6 (5.6)</td>
</tr>
<tr>
<td>4-nitrophenol</td>
<td>83.3 (6.1)</td>
<td>113 (6.0)</td>
<td>90.4 (1.7)</td>
<td>112 (13)</td>
</tr>
<tr>
<td>bentazon</td>
<td>89.3 (6.0)</td>
<td>114 (5.6)</td>
<td>91.2 (3.0)</td>
<td>104 (8.8)</td>
</tr>
<tr>
<td>2,4-D</td>
<td>92.3 (7.1)</td>
<td>107 (3.1)</td>
<td>86.5 (1.8)</td>
<td>122 (12)</td>
</tr>
<tr>
<td>MCPA</td>
<td>97.6 (8.2)</td>
<td>104 (4.5)</td>
<td>80.8 (3.6)</td>
<td>96.7 (5.5)</td>
</tr>
<tr>
<td>dichlorprop</td>
<td>96.4 (11)</td>
<td>107 (9.0)</td>
<td>87.4 (3.0)</td>
<td>103 (6.0)</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>106 (6.2)</td>
<td>116 (8.8)</td>
<td>95.1 (5.0)</td>
<td>96.6 (12)</td>
</tr>
<tr>
<td>MCPP</td>
<td>100 (7.7)</td>
<td>116 (6.6)</td>
<td>93.8 (3.0)</td>
<td>94.7 (2.9)</td>
</tr>
<tr>
<td>dichlorobenzoic</td>
<td>93.3 (6.3)</td>
<td>119 (9.7)</td>
<td>84.3 (2.7)</td>
<td>96.9 (5.9)</td>
</tr>
<tr>
<td>2,4-DB</td>
<td>95.4 (5.1)</td>
<td>110 (8.4)</td>
<td>83.7 (5.6)</td>
<td>83.3 (5.2)</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>89.3 (7.9)</td>
<td>92.5 (6.7)</td>
<td>87.7 (5.3)</td>
<td>82.7 (10)</td>
</tr>
<tr>
<td>acifluorfen</td>
<td>94.8 (8.3)</td>
<td>102 (8.5)</td>
<td>70.0 (17)</td>
<td>81.3 (8.2)</td>
</tr>
<tr>
<td>dinoseb</td>
<td>71.7 (7.1)</td>
<td>73.8 (6.8)</td>
<td>54.7 (5.2)</td>
<td>88.1 (1.9)</td>
</tr>
</tbody>
</table>

© 1998 Waters Corp.
Acidic Herbicides - Discussion

- The SPE procedure is based on general GC method
- Elution solvent is 10% MeOH/MTBE
  - compatible with derivatization for GC
  - the same sample prep procedure for either GC or LC
  - provides similar recovery to methanol for HPLC, but minimizes humic interference compared with methanol elution

- The SymmetryShield™ RP₈ column allows separation of all analytes in one HPLC run
Chlorinated Phenols - HPLC

- **Column:** SymmetryShield™ RP_8
- **Mobile Phase:**
  - A: pH 2.8 phosphate buffer
  - B: Acetonitrile
- **Gradient:** 30% B for 2 min, then linear gradient to 70% B in 25 min
- **Flow Rate:** 1.0 mL/min
- **Detection:** UV @ 210 nm (0.02 AUFS)
- **Sample:** 40 mL PCB site groundwater
- **Injection:** 75 µL

**Peak Identification:**
1: 2,4-dichlorophenol
2: dibromophenol (SS1)
3: trichlorophenol (a)
4: trichlorophenol (b)
5: 2,4,6-trichlorophenol
6: trichlorophenol (c)
7: trichlorophenol (d)
8: tribromophenol (SS2)
9: tetrachlorophenol (a)
10: tetrachlorophenol (b)
11: pentachlorophenol

(recovery > 85%)
Confirmation of Pentachlorophenol (PCP) at 400 ng/L Using Waters™ 996 PDA

PCP Reference Spectrum

Sample Spectrum

PCP Site Sample

Unknown Spectrum

© 1998 Waters Corp.
2-D SPE Method for HPLC Analysis
(conditions for 30 mg Oasis® HLB cartridge or 96 well plate)

Prepare Sample

Condition/Equilibrate
1 mL methanol/1 mL water

Load
1 to 5 mL sample

Acids
Wash
1 mL 5% methanol in water

Wash
acid in methanol/water

Elute
base in methanol/water

Bases
Wash
base in methanol/water

Wash
acid in methanol/water

Elute
base in methanol/water

© 1998 Waters Corp.
Wash-Elute Study:
Optimize % MeOH for Wash step 2 in 2-D SPE Method

1. Prepare Analytes in Saline
2. Condition 20 cartridges/wells
   - 1 mL methanol/1 mL water
3. Load
   - 1 mL spiked saline solution
4. Elute
   - base in various % methanol in water (e.g. 2% NH₄OH)
   - acid in various % methanol in water (e.g. 2% CH₃COOH)

© 1998 Waters Corp.
2-D Method Example 1
Reduction of Humic Interferences

• Useful for basic or neutral analytes in surface water or soil extracts
• Major humic interference is acidic (fulvic acid)
  – Conduct wash/elute study to determine the maximum methanol content for optimum recovery and optimum reduction of interference
  – Wash with basic wash (1% NH$_3$) with maximum methanolic content
Water Contaminants Neutrals

A: 1-D method, wash with 5% methanol
B: 2-D method, wash with 10 % methanol, 1% NH₃

HPLC
- Column: SymmetryShield™ RP₈
- Mobile Phase: A: water
  B: Acetonitrile
- Gradient: 50% B linear to 100% B in 10 min
- Flow Rate: 0.8 mL/min
- Detection: UV @ 225nm (0.03 AUFS)
- Injection: 20 µL
- Sample: 150 mL of surface water spikes @ 1 ng/L

1. dimethyl phthalate
2. diethyl phthalate
2-D SPE Method Example 2
Reduction of Interferences in biological fluids

- Useful for minimizing interferences for basic or acidic compounds in plasma, urine, or tissue extracts
- Start with the general (1-D) method
- Perform a second wash with solution which is acidic (for acids) or basic (for bases); *this wash solution is of organic content much higher than 5%*
- Elute with methanol/water plus acid (for bases)
- Elute with methanol/water plus base (for acids)
Verapamil and Metabolite (Bases)

Verapamil

Norverapam

Methoxyverapam (I.S.)
Wash-Elute Study (Verapamil)

Wash (base)

Elute (acid)

Verapamil, nor-Methyl
Verapamil
Verapamil, Methoxy

© 1998 Waters Corp.
Comparison of SPE Methods: Verapamil in Plasma

Column: SymmetryShield® RP8, 5 µm, 3.9 x 150 mm
Temperature: 30°C
Mobile Phase: 50 mM Phosphate pH 7:
acetonitrile:methanol (41:37:22); Alliance
Detection: UV at 230 nm
Flow Rate: 1.0 mL/min.
Injection Volume: 40 µL
(after evaporation and reconstitution in 200 µL water)

Peak Identification:
Peak 1: Norverapamil
Peak 2: Verapamil
Peak 3: Methoxyverapamil (I.S.)
# Results: Verapamil (Oasis™ HLB 96-Well Extraction Plate)

<table>
<thead>
<tr>
<th>Compound</th>
<th>1-D</th>
<th>2-D (2 washes)</th>
<th>2-D (3 Washes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. [µg/mL]</td>
<td>Recovery (%)</td>
<td>RSD (%) (n=12)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.68</td>
<td>100.4</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>101.5</td>
<td>3.39</td>
</tr>
<tr>
<td>Nor-Ver</td>
<td>0.46</td>
<td>117.6</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>0.092</td>
<td>164.1</td>
<td>10.8</td>
</tr>
</tbody>
</table>

© 1998 Waters Corp.
Conclusions

• Oasis® HLB Extraction Cartridges are superior to traditional SPE cartridges
  – higher capacity for polar compounds
  – clean background
  – fast analysis; 200:1 enrichment in 15 minutes
  – no stopcocks

• Methods have been developed for pesticides and metabolites, pharmaceuticals, and pollutants

• Methods have been developed for water, soil, food, plasma, urine, etc.
Acknowledgements

Yung-Fong Cheng
Pam Iraneta
Uwe D. Neue
Edouard S. P. Bouvier
Dorothy J. Phillips
and .... the Oasis® Team

© 1998 Waters Corp.