High Throughput Sample Preparation of Acidic, Basic, and Neutral Drugs in Serum Using 96-Well SPE Plates

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Presentation Outline

• An overview of solid phase extraction
• The emergence of 96-well SPE
• Discovery SPE-96 Products
• Applications
• Conclusion
Solid Phase Extraction (SPE)

**Purpose of SPE:** extend chromatographic system’s lifetime & improve quality of analysis

- Digital chromatography
- Liquid sample (sample matrix, impurities, compounds of interest) is loaded
- Compounds of interest are “extracted” on to the sorbent
- Co-extracted impurities selectively washed off the sorbent
- Finally, desired analytes are recovered with an elution step
- **The Result:** simpler matrix, semi-purified, trace enriched, chromatography friendly.
- **Major Concerns:** recovery & reproducibility
The Power of SPE = 15 Years of Versatility

Extensive Range of Chemistries… each offering unique retention properties.

+ Multitude of plastic ware.

One can truly appreciate the scope of SPE.
SPE Extends to 96-Well Technology

- Historically, seasoned operators process 12-24 samples.
- Advances in CombiChem, robotics, automation, & LC-MS (1-3 min. analysis time).
- More drug candidates put through developmental pipeline. 1000s of samples can be generated routinely during bio-analytical analysis.
- Productivity, reproducibility, & throughput = critical factors in the pharma industry.
- Sample prep = 10x’s longer than the analyses itself.
Supelco Discovery SPE-96 Well Plate & PlatePrep Manifold
Discovery SPE Phase Profile

Base Silica: Irregularly shaped, acid washed
Mean Particle Size: 50\(\mu\)m
Mean Particle Diam: 70Å
Total Pore Volume: 0.9cm\(^3\)/g
Specific Surface Area: 480m\(^2\)/g

Bonding: polymeric, trifunctional octadecyl
% C Loading: 18%
Surface Coverage: 2.5 (-18) \(\mu\)mole/ m\(^2\)
Endcapped: Yes

*Narrower Particle Sz. & Pore Sz. Distribution.*
Process up to 96 Samples in < 40 Min.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Description</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Conditioning/Equilibration</td>
<td>Condition each well w/ 1-2mL MeOH &amp; DI Water.</td>
<td>Solvates alkyl chains &amp; ensures consistent interaction for maximal retention.</td>
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</table>
| Sample Load            | Load each well w/ 1mL serum sample diluted 1:1 w/ water.                    | **Basic compounds**: add 3-6 uL 10N KOH/mL sample.  
**Acidic compounds**: add 6-24 uL H₃PO₄/mL sample. |
| Wash                   | Wash each well w/ 2mL 5% MeOH                                               | Removes co-extracted impurities.                                     |
| Drying                 | Vacuum dry with manifold for 5-15 min.                                      | Removes excess aqueous residues on the sorbent.                      |
| Elution                | Elute compounds w/ 1-2 mL MeOH.                                            | Overcomes retention interactions & compounds of interest are recovered. |
| Drying/Reconstitution  | Dry eluate w/ nitrogen purge (40°C; 15-20 min.); reconstitute w/ mobile phase. | Concentration, and sample is compatible with chromatography system.   |
Pharmaceutical Compounds Analyzed

Naproxen
Cimetidine
Notriptyline
Procainamide
Theobromine
Ranitidine
Theophylline
Sulfamethazine
1mL porcine serum samples were spiked with 0.5µg of each compound, and were extracted with 100mg/ well DSC-18 SPE-96 well plate.

Extracts were quantitated via HPLC analyses against external standards.

N = 8 for each cmpd.
The Average Recovery of 16 Different Spike Concentrations Ranging Between 0.050-350µg Cimetidine per mL Serum was 95.56%!!

N = 2 for each concentration.
Discovery DSC-18 vs. Competitors’ Extraction Plates

- **Notriptyline**
- **Procainamide**
- **Panitidine**
- **Cimetidine**
- **Sulfamethizole**
- **Theophylline**

**Legend:**
- DSC-18 (100mg/well)
- Competitor W - Polymer phase (30 mg/well)
- Competitor V (100 mg/well)
- Competitor-I (100mg/well)
Discovery DSC-Si SPE-96 Well Plate CombiChem Application

Binding Capacity of 4-Flouro-3-Nitrobenzoic acid on DSC-Si (100mg/well).

<table>
<thead>
<tr>
<th>Load Amount (Sample Matrix = in 200 ul Methylene Chloride)</th>
<th>Break Through Amount</th>
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<tbody>
<tr>
<td>2.5 mg</td>
<td>No Break Through</td>
</tr>
<tr>
<td>5.0 mg</td>
<td>No Break Through</td>
</tr>
<tr>
<td>10.0 mg</td>
<td>No Break Through</td>
</tr>
<tr>
<td>12.5 mg</td>
<td>No Break Through</td>
</tr>
<tr>
<td>15.0 mg</td>
<td>0.10 % Break through</td>
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- Modified Flash Technique for reaction clean up & baseline impurity removal.
Conclusion

High Recoveries and Low RSDs attributed to:

- Narrower Particle Sz. & Pore Sz. Distribution
- Consistent bonding & Bed weights
- Higher Carbon Loading

Flash Technique: High Capacity demonstrated by increased surface area.