

# Automating Method 1664B With Solid Phase Extraction


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August 6<sup>th</sup>, 2020

# US EPA Method 1664B



United States Office of Water  
Environmental Protection Agency (4303) February 2010

 **Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry**

February 2010

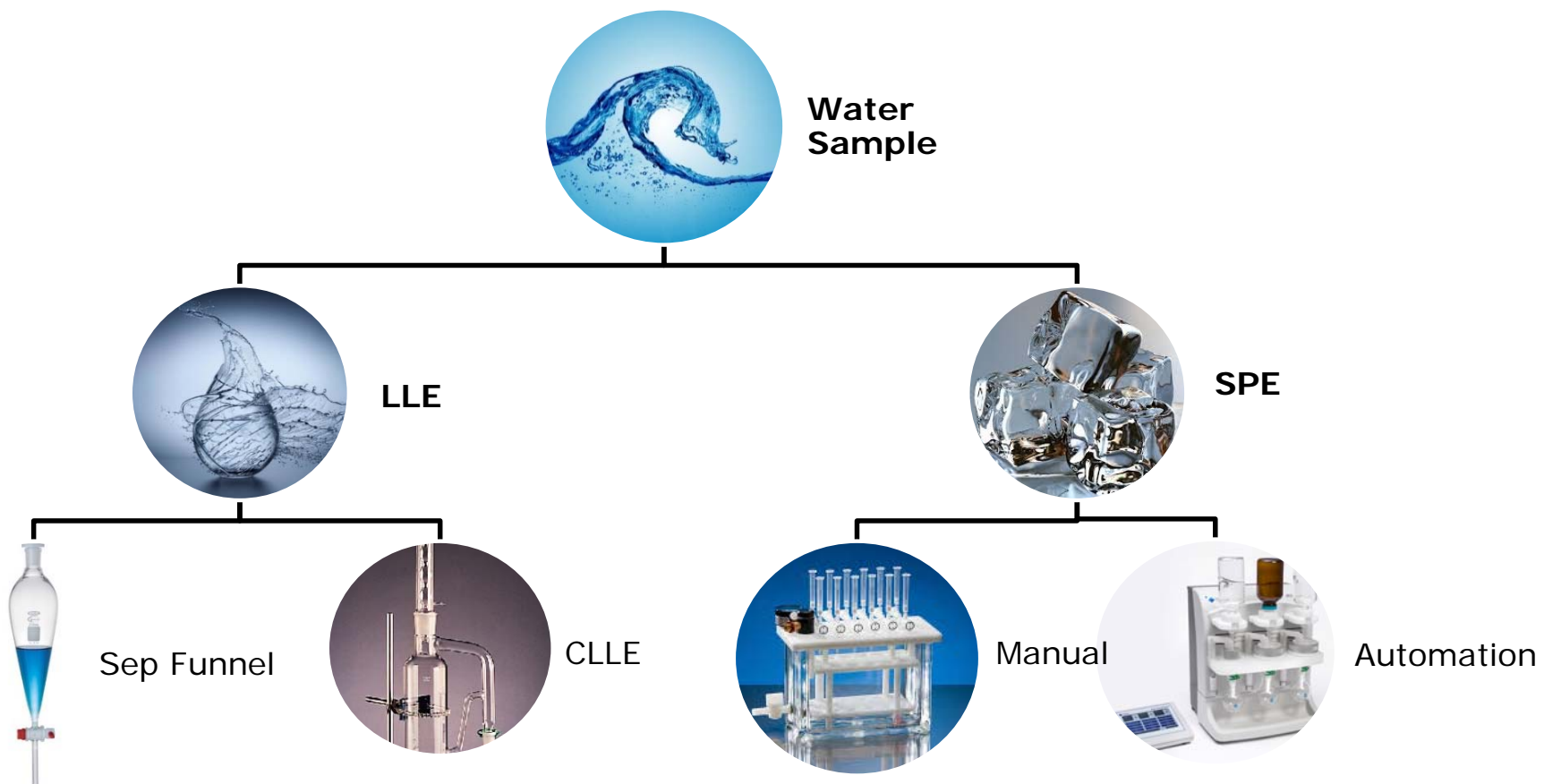


## Key Aspects of EPA Method 1664B

### Sample Preparation Techniques:

- » Written for LLE
- » SPE is an accepted alternative
- » 1.7 The laboratory is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met
- » The requirements for establishing method equivalency are given in Sections 9.1.2 and 9.2.3
- » Performance based allows for SPE technique to be automated and optimized

# Extraction Techniques

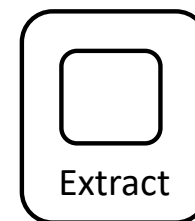
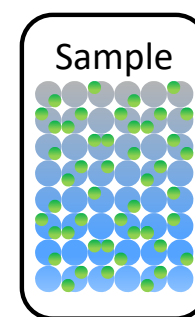


# Extraction

Extracting the analytes (green) from the matrix (blue)

Common types of extraction:

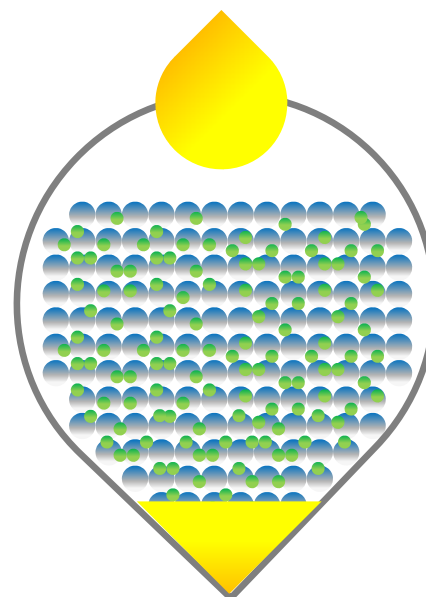
- » Liquid-Liquid Extraction
- » Solid Phase Extraction
- » Cartridge
- » Disk



Analyzed by GC or LC

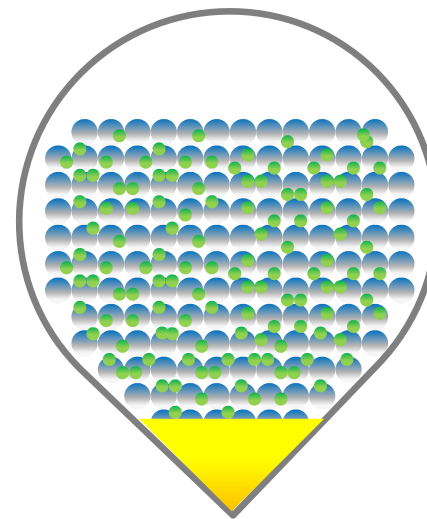
## Liquid-Liquid Extractions Separatory Funnel

- » Simple Process – solubility
  - » Yellow is **Solvent**
  - » Blue is water – **Sample**
  - » Green - **Analytes** of interest
  - » Analytes are soluble in the **Solvent**



## Liquid-Liquid Extractions Separatory Funnel

- » Shaking increases exposure of water and compounds of interest to the solvent
- » Goal of extraction is to partition all of the solvent soluble material out of the water



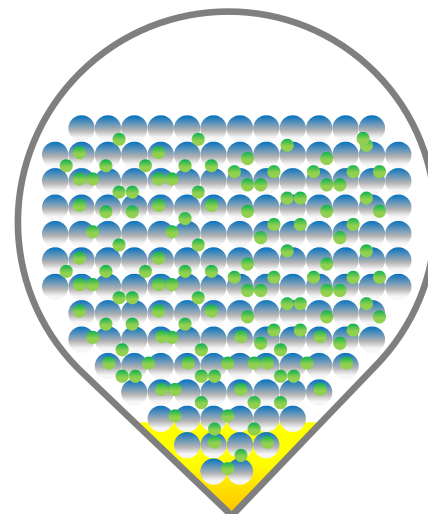
## Liquid-Liquid Extractions Separatory Funnel

### Strengths

- » Simple process
- » Relatively inexpensive
- » Easy & well understood

### Weaknesses

- » Labor intensive
  - » Best case – 4 man-hours to extract 20 samples
- » Solvent intensive
- » Incomplete extraction in some cases
- » Emulsions; low recoveries
- » Safety - exposure to solvent, repetitive movements, and glassware
- » Quality - subject to human error





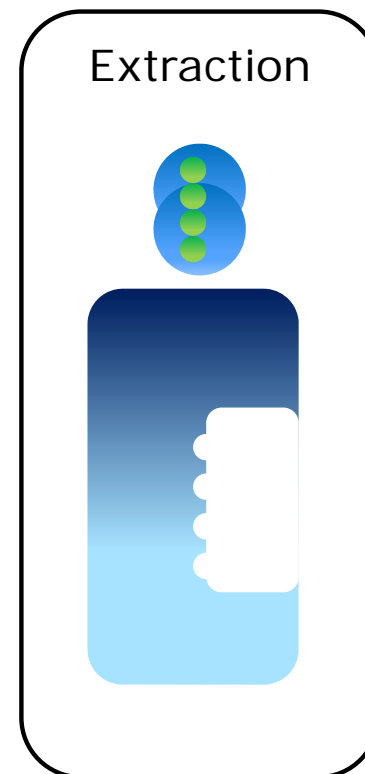
## Solid Phase Extraction

The goal remains the same

- » Separate the analytes from matrix

Process is slightly different

- » Adsorb analytes from matrix onto solid sorbent material (disk, cartridge, fiber)
- » Elute (extract) with small amount of solvent
- » Can be done manually or automated



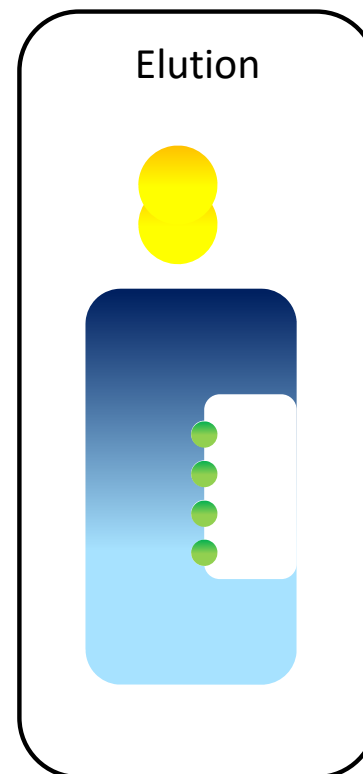
## Solid Phase Extraction

### Strengths

- » Can be automated
- » Little solvent used
- » Limited exposure to solvent, glassware, repetitive movement
- » Automation will remove much of the human error

### Weaknesses

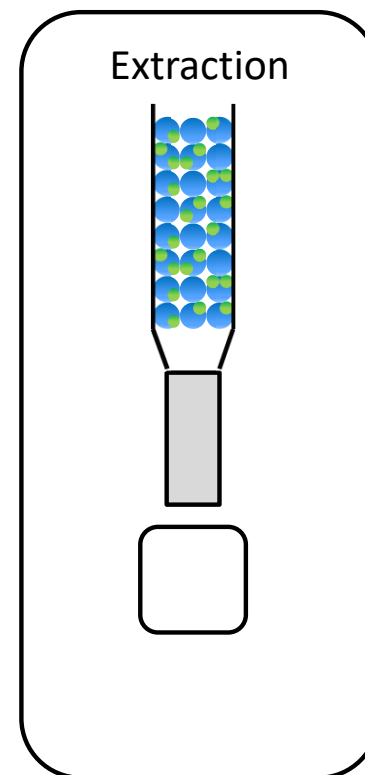
- » Perceived higher costs
- » Training is required
- » Different – some labs fear change



## Cartridge SPE

### Strengths

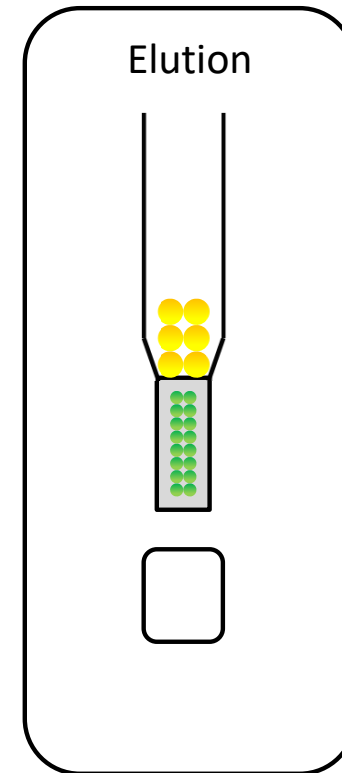
- » Commonly used
- » Easily automated
- » Removes human error
- » Very effective for small samples
- » Little solvent used
- » Limited exposure to solvent, glassware, repetitive movement



# Cartridge SPE

## Weaknesses

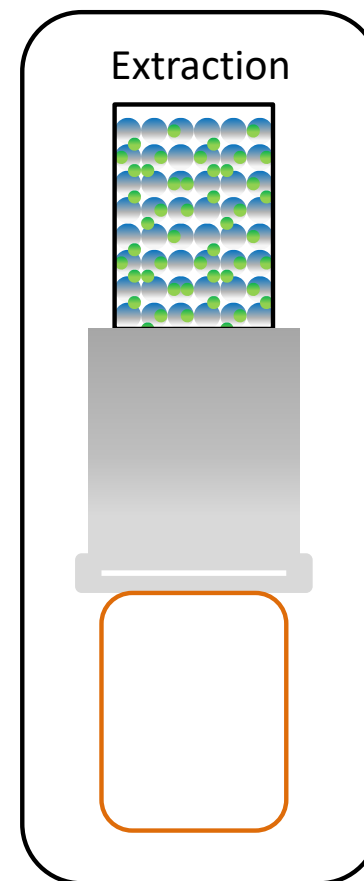
- » Limited sample size
- » Difficult to process
  - » Particle-laden(dirty) samples
- » Training is required



## Disk SPE

### Strengths

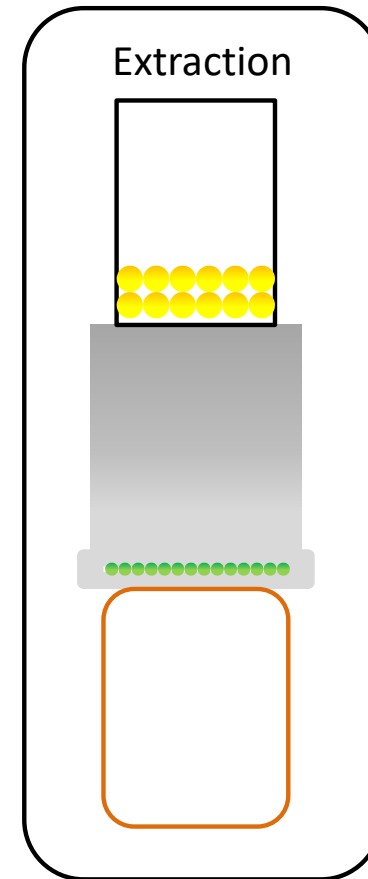
- » Designed to handle large sample volumes
- » Designed to completely extract all samples even those with high particulates (dirty)
- » Rapid sample flow rates ( > 100 mL per minute)
- » Easily Automated – remove human error
- » Limited exposure to solvent, glassware, repetitive movement



# Disk SPE

## Weaknesses

- » Costs – Higher up front with Automation
- » Training - Some training is required
- » 'New' 'Different' – some labs fear change



## Use of Methanol

### EPA Method 1664B

#### 1.7.2 Unacceptable Modifications

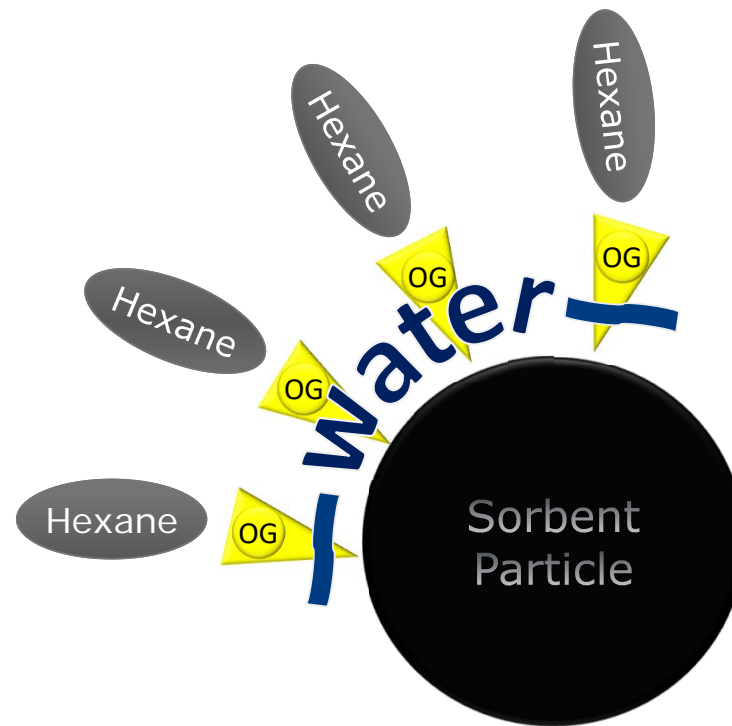
- 1.7.2.1** The extraction solvent must be n-hexane (85% minimum purity, 99.0% min. saturated C<sub>6</sub> isomers, residue less than 1 mg/L – see Section 7.3). Alternate extraction solvents or co-solvents including methanol, acetone and others that react with or introduce the target pollutant into the sample are not allowed.

However, a methanol or other polar solvent rinse after sample filtration may be allowed to remove water residual when using SPE technology in a modified method provided:

1. The methanol rinse is immediately discarded to waste.
2. The SPE filter is sufficiently air dried with vacuum to remove any residual methanol remaining in the SPE filter to trace amounts so as to ensure residual methanol will not introduce the target analyte into the sample and at no time will residual methanol collocate with or be collected with the n-hexane extractions.
3. The laboratory must demonstrate and document the appropriate operating conditions (1 & 2) above to allow this use of methanol.

## Impact of Residual Water

1. **Oil and Grease** analytes attach to sorbent within disk
2. **Water** encapsulates analytes
3. **Hexane** cannot extract analytes from sorbent
4. **Hexane** elutes to collection vessel without all analytes





## Comparing Recoveries Influent WWTP Samples

### With Methanol

SPE Replicate	Begin Wt. (g)	Final Wt. (g)	HEM (mg)
1 – 1 Liter Influent	6.1831	6.2136	30.5
2 – 1 Liter Influent	6.2030	6.2359	32.9
3 – 1 Liter Influent	6.2015	6.2357	34.2
<b>Avg. Reported Value</b>			<b>32.5</b>

### Without Methanol

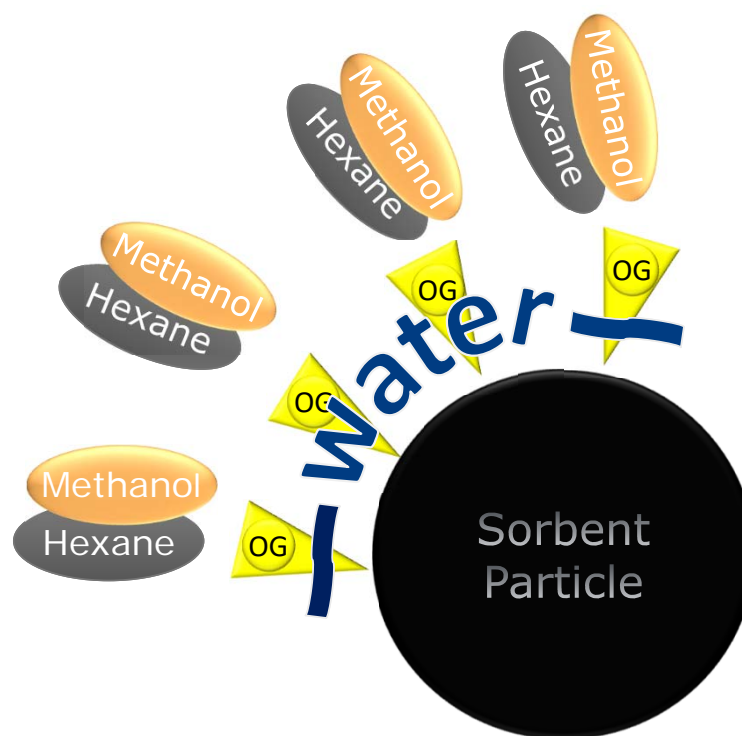
$$\% \text{ Loss} = (32.5 - 18.8) / 32.5$$

SPE Replicate	Begin Wt. (g)	Final Wt. (g)	HEM (mg)
1 – 1 Liter Influent	6.2473	6.2656	18.3
2 – 1 Liter Influent	6.1801	6.2045	24.4
3 – 1 Liter Influent	6.2506	6.2643	13.7
<b>Avg. Reported Value</b>			<b>18.8</b>

42.2% Loss!

## Using A Methanol Rinse

1. **Oil and Grease** analytes attach to sorbent within disk
2. **Water** encapsulates analytes
3. **Hexane** cannot extract analytes from sorbent
4. **Hexane** elutes to collection vessel without all analytes
5. **Methanol** removes residual water.
6. **Hexane** extracts remaining **Oil and Grease**



## Using A Methanol Rinse

A **methanol rinse is allowed** to remove residual water if:

1. The **methanol is immediately discarded to waste.**
2. The **SPE filter is sufficiently air dried** with vacuum to remove any traces of methanol

How do we ensure the Methanol is Removed?

Method says to weigh filter **before use** and **after** the methanol step

## SPE vs. LLE

### Equivalency Demonstration:

- » Automated SPE
- » 47 mm Pacific® Premium Disk
- » 2 L Separatory Funnel

**SPE HEM = 103% of LLE HEM**

Method Requirement: 78-114%

**SPE SGT-HEM = 108% of LLE SGT-HEM**

Method Requirement: 64-132%

Better accuracy and precision than LLE

HEM	LLE % Rec.	SPE % Rec.
1	96.3	100.5
2	97.5	100.3
3	96.5	100.3
4	99.8	99.3
Average % Rec.	97.5	100.1
Deviation	1.6	0.6
SGT-HEM	LLE % Rec.	SPE % Rec.
1	98.0	104.0
2	93.5	99.5
3	84.5	99.5
4	94.0	95.5
Average % Rec.	92.5	99.6
Deviation	5.7	3.5

# Automated Extraction Times for 90 mm SPE Disk



**Dirty Sample**

Sample Process Time: + 84 min  
 Total Extraction Time: ~101 min  
 Final Extract Volume: 75 mL

**Prewet:** 3:25 min  
**Air Dry:** 3:00 min  
**N-Hexane Rinse:** 3:32 min  
**Methanol Rinse:** 1:24 min  
**N-Hexane Rinse:** + 6:01 min  


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**17:22 min**

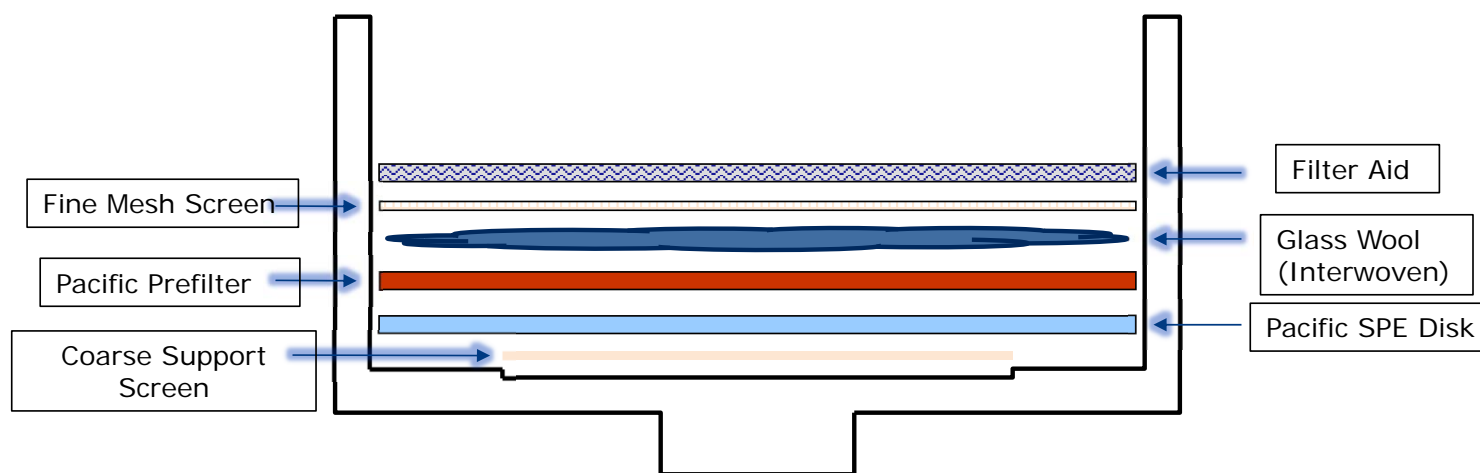
**Clean Sample**

+ 2 min  
~20 min  
75 mL



NOTE: Truly "clean" samples can use a 47 mm disk for a final volume of 25 mL!

# Dirty Samples It's All About Layering



## Key Points to Take Away

- » SPE is an accepted alternative
- » 1.7 The laboratory is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met
- » The requirements for establishing method equivalency are given in Sections 9.1.2 and 9.2.3
- » Automation reduces human error
- » Results in sample to sample consistency
- » Makes time for other tasks that require direct attention

Questions?



### Contact Information

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