CHROMATOGRAPHY

Thermo Scientific HyperSep Solid Phase Extraction Method Development Guide

The following guide provides considerations, tips and general guidelines for developing SPE methods using the Thermo Scientific HyperSep family of Solid Phase Extraction (SPE) products.

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Considerations for SPE Method Development

Determine Appropriate Column Volume

The column volume indicated in the reference table within each method represents the maximum volume of sample or solvent that can be applied to the SPE tube or well per aliquot. The most appropriate column volume for a specific method allows for easy sample application and closely matches the necessary volume of wash solvent.

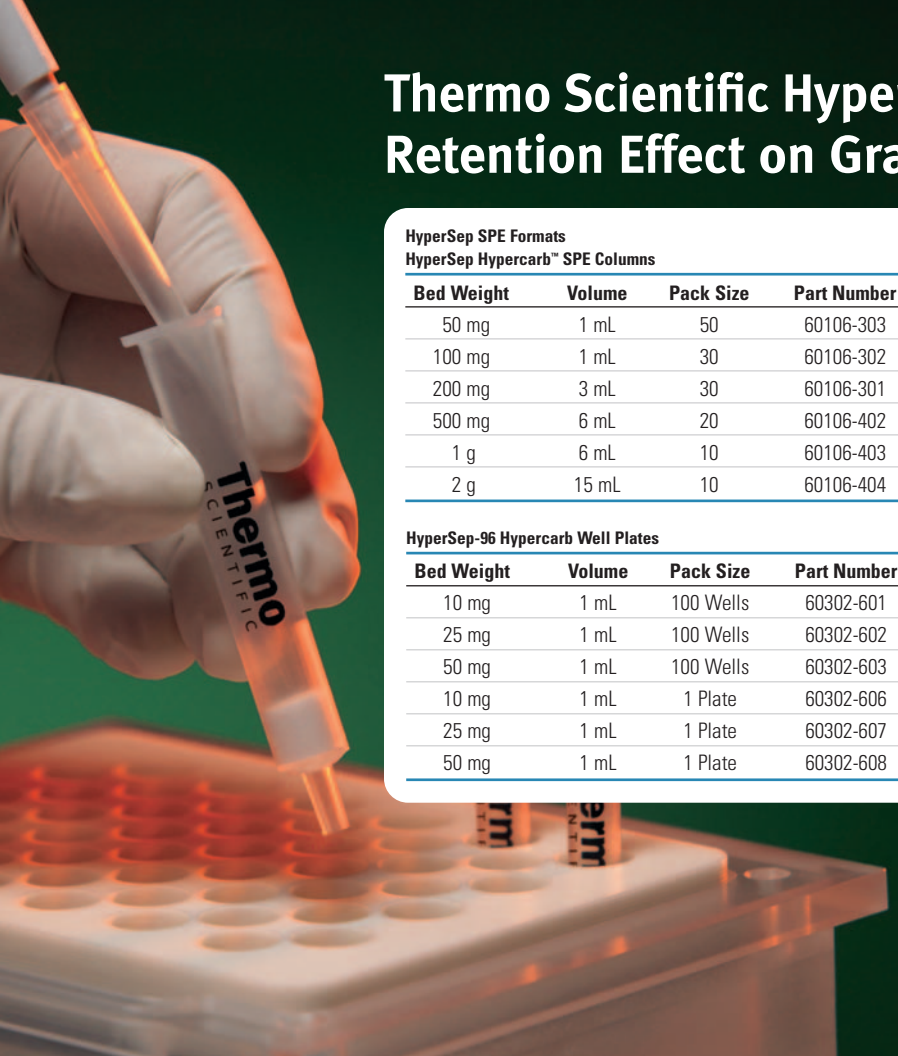
Determine the Appropriate Bed Weight

The bed weight defines the maximum amount of analyte that a sorbent can retain. Bed weights should be chosen based on the sample size, taking into consideration the approximate amount of contaminants present and the separation mode. For normal and reversed phase modes, typical bed capacity is 1-5% of the bed weight, up to 100 mg solute/g packing for strongly retained compounds. For example, a 500 mg bed weight can retain up to 25 mg of solute mass. For less retained compounds, if the bed weight is too small relative to the sample matrix volume, breakthrough (early elution) during sample loading can occur. For ion exchange sorbents, the number of charged sites available for interaction with the analyte determines the capacity. This ionic capacity of an ion exchange sorbent is given in milliequivalents/gram (mEq/g). The mean ionic capacity values range from 0.072 to 0.320 mEq/g. The bed weights for the various HyperSep columns and wells are provided in the tables with each method.

Solid Phase Extraction Method Tips

1. Do not allow the sorbent to dry between conditioning steps or before sample application. To ensure that columns are properly solvated, apply each solvent immediately after the previous solvent.
2. Prior to elution, fully dried cartridges will ensure optimal analyte recovery. To confirm column dryness, press the sides of the cartridge at the sorbent level at full vacuum. Columns should not feel cool to the touch. If the column feels cool, water is likely to be present and the column needs further drying.
3. Always use fresh NH_4OH when preparing basic elution solvents. NH_4OH rapidly loses its strength when exposed to air and may lead to erratic recoveries. Proper elution pH (11-12) is also critical to achieve optimal recovery of basic drugs with high pK_as (i.e., amphetamines, some tricyclics, morphine) when using mixed mode sorbents such as Verify-CX.
4. Condition your column with an appropriate solvent to ensure optimal retentions are achieved.
5. Analytes that are not in their correct ionization state (i.e., neutral or charged) will not effectively bind to the sorbent and may result in erratic recoveries. To avoid, always verify the sample application pH. If using ion exchange, use a mobile phase that will ensure that the analyte is charged. If mechanism is reversed-phase, maximum retention is maintained by ensuring that the mobile phase pH encourages ion suppression.

For more technical tips, guides and applications, visit our Chromatography Resource Center at www.thermo.com/columns.



Thermo Scientific HyperSep Reversed Phase/Polar Retention Effect on Graphite Method (Hypercarb)

HyperSep SPE Formats
HyperSep Hypercarb™ SPE Columns

Bed Weight	Volume	Pack Size	Part Number
50 mg	1 mL	50	60106-303
100 mg	1 mL	30	60106-302
200 mg	3 mL	30	60106-301
500 mg	6 mL	20	60106-402
1 g	6 mL	10	60106-403
2 g	15 mL	10	60106-404

HyperSep-96 Hypercarb Well Plates

Bed Weight	Volume	Pack Size	Part Number
10 mg	1 mL	100 Wells	60302-601
25 mg	1 mL	100 Wells	60302-602
50 mg	1 mL	100 Wells	60302-603
10 mg	1 mL	1 Plate	60302-606
25 mg	1 mL	1 Plate	60302-607
50 mg	1 mL	1 Plate	60302-608

1. **Analyte Properties –** Polar, uncharged species – can also retain some ionic/charged species
2. **Sample Preparation/Application (Polar, aqueous) –** Add internal standard to the sample if quantification is desired. Optimize sample application by removing particulates if necessary (centrifugation or filtration) and/or diluting viscous matrices with water or buffer to ensure proper pH for desired interactions. This will require some experimentation.
3. **Column Conditioning –** 2-5 column volumes of a strong eluting solvent followed by 2-5 column volumes water.
4. **Column Wash –** Use water or a weak eluting solvent which will wash most interferences from the sorbent without loss of analytes. Wash pH may greatly affect cleanup and/or recovery.
5. **Elution –** Use a strong eluting solvent to elute the analytes of interest. Addition of 0.1% TFA to the solvent will increase its elution strength for polar analytes.

Suggested Elution Solvents

- *MeOH*
- *THF**
- *Ethyl Acetate**
- *Dichloromethane**
- *Chloroform**

Increasing Elution Strength

**Elution strength will depend on the nature of the analytes.*

Thermo Scientific HyperSep Reversed Phase Method for C18, C8, Phenyl

For HyperSep C18, C8, Phenyl SPE Columns

Bed Weight	Volume	Pack Size	C18 Part Number	C8 Part Number	Phenyl Part Number
50 mg	1 mL	100	60108-390	60108-391	60108-516
100 mg	1 mL	100	60108-302	60108-392	60108-386
200 mg	3 mL	50	60108-303	60108-393	60108-387
500 mg	3 mL	50	60108-304	60108-309	60108-388
500 mg	6 mL	30	60108-305	60108-394	60108-389
1 g	6 mL	30	60108-301	60108-427	60108-517
2 g	15 mL	20	60108-701	60108-704	60108-707
5 g	25 mL	20	60108-702	60108-705	60108-708
10 g	75 mL	10	60108-703	60108-706	60108-709

For HyperSep-96 C18, C8, Phenyl Well Plates

Bed Weight	Volume	Pack Size	C18 Part Number	C8 Part Number	Phenyl Part Number
10 mg	1 mL	100 Wells	60300-421	60300-441	60300-681
25 mg	1 mL	100 Wells	60300-422	60300-442	60300-682
50 mg	1 mL	100 Wells	60300-423	60300-443	60300-683
100 mg	1 mL	100 Wells	60300-524	60300-444	60300-684
10 mg	1 mL	1 Plate	60300-425	60300-445	60300-685
25 mg	1 mL	1 Plate	60300-426	60300-446	60300-686
50 mg	1 mL	1 Plate	60300-427	60300-447	60300-687
100 mg	1 mL	1 Plate	60300-428	60300-448	60300-688

1. **Analyte Properties –** Moderately polar to non-polar, uncharged
2. **Sample Preparation/Application (Polar, aqueous) –** Add internal standard to the sample if quantification is desired. Optimize sample application by removing particulates if necessary (centrifugation or filtration) and/or diluting viscous matrices with water or buffer to ensure proper pH for optimum retention.
3. **Column Conditioning –** 2-5 column volumes of a strong solvent such as methanol at low vacuum (-3 inches Hg). Apply deionized or distilled water to remove excess solvent. Momentary high vacuum (5 to 8 inches Hg) may be necessary to restart flow. If the sorbent is accidentally dried, then resolvate and reflush column.
4. **Column Wash –** Use a solvent which will wash most interferences from the sorbent without loss of analyte. Wash pH may greatly affect cleanup and/or recovery.
5. **Elution –** Use a strong eluting solvent which will elute the analytes of interest.

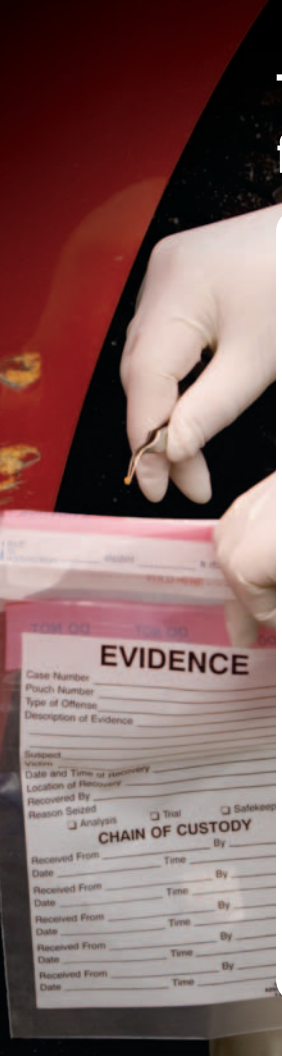
Suggested Elution Solvents

- *MeOH*
- *Acetonitrile*
- *THF*
- *Ethyl Acetate*
- *Dichloromethane**

Increasing Elution Strength

**If using dichloromethane the sorbent needs be dried by vacuum to remove any traces of water.*

Thermo Scientific HyperSep Normal Phase Method for Silica, Florisil, Aminopropyl



For HyperSep Silica, Florisil™ or Aminopropyl SPE Columns

Bed Weight	Volume	Pack Size	Silica Part Number	Florisil Part Number	Aminopropyl Part Number
50 mg	1 mL	100	60108-390	60108-391	60108-516
50 mg	1 mL	100	60108-409	60108-402	60108-424
100 mg	1 mL	100	60108-317	60108-403	60108-364
200 mg	3 mL	50	60108-410	60108-404	60108-425
500 mg	3 mL	50	60108-315	60108-405	60108-518
500 mg	6 mL	30	60108-411	60108-500	60108-519
1 g	6 mL	30	60108-426	60108-431	60108-432
2 g	15 mL	20	60108-710	60108-735	60108-738
5 g	25 mL	20	60108-711	60108-736	60108-739
10 g	75 mL	10	60108-712	60108-737	60108-740

For HyperSep-96 Silica, Florisil or Aminopropyl Well Plates

Bed Weight	Volume	Pack Size	Silica Part Number	Florisil Part Number	Aminopropyl Part Number
50 mg	1 mL	100	60108-390	60108-391	60108-516
10 mg	1 mL	100 Wells	60300-481	60300-721	60300-501
25 mg	1 mL	100 Wells	60300-482	60300-722	60300-502
50 mg	1 mL	100 Wells	60300-483	60300-723	60300-503
100 mg	1 mL	100 Wells	60300-484	60300-724	60300-504
10 mg	1 mL	1 Plate	60300-485	60300-725	60300-505
25 mg	1 mL	1 Plate	60300-486	60300-726	60300-506
50 mg	1 mL	1 Plate	60300-487	60300-727	60300-507
100 mg	1 mL	1 Plate	60300-488	60300-728	60300-508

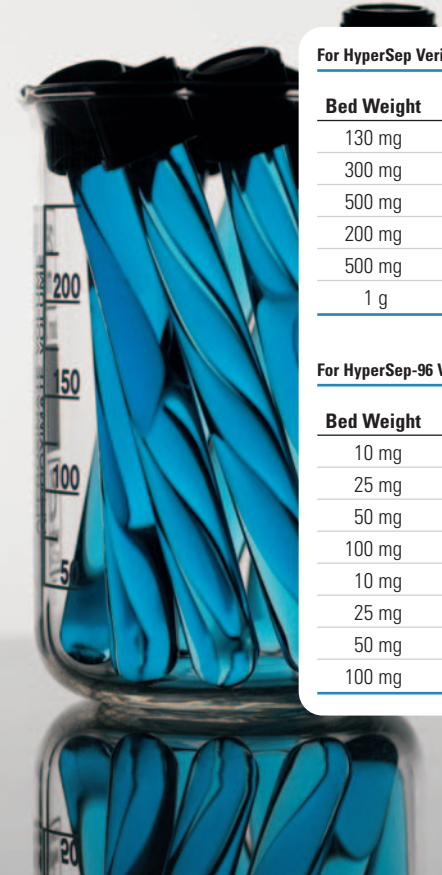
1. **Analyte Properties –** Moderately polar to polar compounds
2. **Sample Preparation/Application (Non-polar or Moderately Polar Organic Solvent) –** Add internal standard to the sample if quantification is desired. Optimize sample application by removing particulates if necessary (centrifugation or filtration).
3. **Column Conditioning –** 2-5 column volumes non-polar solvent such as heptane at low vacuum (-3 inches Hg). Release the vacuum or begin flushing immediately upon completion. The more air that passes through the column before sample loading, the less solvated the sorbent will be.
4. **Column Wash –** Use a solvent which will wash most interferences from the sorbent without loss of analyte.
5. **Elution –** Use a more polar solvent (e.g., introduction of more ethyl acetate, acetone or MeOH).

Suggested Elution Solvents

- *Heptane*
- *Toluene*
- *Dichloromethane*
- *Acetone*
- *Ethyl Acetate*
- *MeOH*

Increasing Elution Strength ↓

Thermo Scientific HyperSep Copolymeric Phase Method for Verify-CX, Verify-AX



For HyperSep Verify™-CX or Verify-AX SPE Columns

Bed Weight	Volume	Pack Size	Verify-CX Part Number	Verify-AX Part Number
130 mg	1 mL	100	60108-719	60108-727
300 mg	3 mL	50	60108-720	60108-728
500 mg	3 mL	50	60108-721	60108-729
200 mg	6 mL	50	60108-722	60108-730
500 mg	6 mL	30	60108-723	60108-731
1 g	6 mL	30	60108-724	60108-732

For HyperSep-96 Verify-CX or Verify-AX SPE Well Plates

Bed Weight	Volume	Pack Size	Verify-CX Part Number	Verify-AX Part Number
10 mg	1 mL	100 Wells	60300-801	60300-809
25 mg	1 mL	100 Wells	60300-802	60300-810
50 mg	1 mL	100 Wells	60300-803	60300-811
100 mg	1 mL	100 Wells	60300-804	60300-812
10 mg	1 mL	1 Pack	60300-805	60300-813
25 mg	1 mL	1 Pack	60300-806	60300-814
50 mg	1 mL	1 Pack	60300-807	60300-815
100 mg	1 mL	1 Pack	60300-808	60300-816

1. **Analyte Properties –** Moderately polar to non-polar and ionized and charged compounds
2. **Sample Preparation/Application –** Add internal standard to the sample if quantification is desired. Optimize sample application by removing particulates if necessary (centrifugation or filtration) and/or diluting viscous matrices with water or buffer to ensure proper pH for optimum retention. Neutral species are unaffected by pH. Charged species may show maximum retention under acidic or basic conditions and method development is required to establish this.
3. **Column Conditioning –** 2-5 column volumes polar solvent such as methanol at low vacuum (-3 inches Hg). Release the vacuum or begin flushing immediately upon completion. The more air that passes through the column before sample loading, the less solvated the sorbent will be. Apply deionized or distilled water to remove excess solvent. Momentary high vacuum (5 to 8 inches Hg) may be necessary to restart flow. If the sorbent is accidentally dried, then resolvate and reflush the column.
4. **Column Wash –** Use water to disrupt the hydrophilic interactions and methanol to disrupt residual hydrophobic and hydrophilic interactions.
5. **Elution –** Use an organic solvent, possibly with some water to elute hydrophobically bound analytes. Then use an aqueous buffer with a pH that would neutralize ionically bound analytes or an aqueous with high ionic strength or a solvent with a counter ion that would bind to a sorbent.

Thermo Scientific HyperSep Ion Exchange Phase Method for SCX, SAX

HyperSep SAX & SCX SPE Columns

Bed Weight	Column Volume (mL)	Quantity	SAX Part Number	SCX Part Number
50 mg	1	100	60108-417	60108-420
100 mg	1	100	60108-418	60108-421
200 mg	3	50	60108-419	60108-422
500 mg	3	50	60108-521	60108-423
500 mg	6	30	60108-360	60108-520
1 g	6	30	60108-434	60108-433
2 g	15	20	60108-713	60108-716
5 g	25	20	60108-714	60108-717
10 g	75	10	60108-715	60108-718

HyperSep-96 SAX Well plates and individual wells

Bed Weight	Column Volume (mL)	Quantity	SAX Part Number	SCX Part Number
10	1	100 Wells	60300-561	60300-581
25	1	100 Wells	60300-562	60300-582
50	1	100 Wells	60300-563	60300-583
100	1	100 Wells	60300-564	60300-584
10	1	1 Plate	60300-565	60300-585
25	1	1 Plate	60300-566	60300-586
50	1	1 Plate	60300-567	60300-587
100	1	1 Plate	60300-568	60300-588

1. **Analyte Properties –** Ionized and charged particles
2. **Sample Preparation/Application –** Add internal standard to the sample if quantification is desired. Optimize sample application by removing particulates if necessary (centrifugation or filtration) and/or diluting viscous matrices with water or buffer to ensure proper pH for optimum retention. Neutral species are unaffected by pH. Charged species may show maximum retention under acidic or basic conditions and method development is required to establish this.
3. **Column Conditioning –** 2-5 column volumes polar solvent such as methanol at low vacuum (-3 inches Hg). Release the vacuum or begin flushing immediately upon completion. The more air that passes through the column before sample loading, the less solvated the sorbent will be. Apply a low ionic strength buffer after flushing to ensure that the sorbent pH is optimal for the sorbent analyte interaction desired. Follow guidelines concerning pK_a, pH and ionic binding to ensure optimal retentions. Use the same vacuum guidelines as used for flushing.
4. **Column Wash –** Use an organic solvent or aqueous buffer at a pH that allows the ion to remain charged (2 pH units from the relevant pK_a of your analyte and sorbent).
5. **Elution –** Use an aqueous buffer with a pH that would neutralize ionically bound analytes or an aqueous with high ionic strength or a solvent with a counter ion that would bind to a sorbent.

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- Column selection and cross-reference tools to ensure the correct tool
- Interactive troubleshooting tools, with an e-mail connection to our columns technical support teams
- Tips and FAQs you can rely on daily
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