A Simple Approach to Automated Solid Phase Extraction (SPE) with Strata™-X Polymeric Sorbents

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As more emphasis is being placed on higher throughput, many labs are turning to automation to help in the sample preparation process. Automation not only saves time but can also reduce costs associated with labor and solvents and can also increase reproducibility within a lab as well as within a network of labs. This document is intended for laboratories who are moving towards automated solid phase extraction (SPE) to guide them in setting up and optimizing their automated SPE capable system for unattended or semi-attended SPE extraction.

Sorbent Bed Mass Selection

The amount of sorbent within a well of an SPE 96-well plate will determine how much target analyte can be extracted. Polymeric sorbents typically have a retention capacity of 10-15 % of their bed mass, so 30 mg of a polymeric sorbent would be able to retain roughly 3 to 4.5 mg of target analyte. Because most bioanalytical analyses involve very low concentrations of target analyte and are not at risk of exceeding the sorbent capacity, the bed mass can be chosen in consideration with the sample volume to be extracted. Keep in mind that the concentration of endogenous components are in million fold excess as compared to the analyte of interest. Therefore, it is not just the analyte but also the presence of impurities that needs to be taken into consideration. Volumes of 500 µL and less are most suitable for 30 mg of sorbent while sample volumes of 150 µL and less are most suitable for a 10 mg bed mass.

Strata-X polymeric sorbents are available in 2 different particle sizes; a traditional 33 µm particle which is ideal for all sample matrices and a 100 µm particle which is designed for faster flow and gravity flow based applications. The 100 µm Strata-XL particles can also be used for large volume and viscous samples however the loading capacity of the larger particles is lower than the traditional Strata-X 33 µm particles due to a lower surface area.

Strata-X 33 µm Polymeric Sorbents: ideal for all sample matrices

Sample Matrix	Sample Volume	Recommended Sorbent Mass
Blood, serum, plasma	≤ 500 µL	30 mg
Urine	≤ 1 mL	30 mg
Filtered tissue homogenates	≤ 100 mg	60 mg

Strata-XL 100 µm Polymeric Sorbents: designed for faster flow or gravity flow

Sample Matrix	Sample Volume	Recommended Sorbent Mass
Blood, serum, plasma	≤ 250 µL	30 mg
Urine	≤ 500 µL	30 mg
Filtered tissue homogenates	≤ 50 mg	60 mg

Sample Pre-treatment

Bioanalytical samples often require a pre-treatment step prior to SPE analysis. The pre-treatment step helps to improve the flow rate and reproducibility of the SPE procedure. Large particles are often present in bioanalytical samples, especially after freeze and thaw cycles. These particles may cause precision and accuracy issues when using a robotic liquid handler and may also plug the top frit causing blockage. When optimizing unattended SPE extractions, sample pre-treatment should be implemented to minimize these problems. Sample pre-treatment may include, but is not limited to:

- Centrifugation of the sample before aspirating using the liquid handler. Centrifugation at 458 g (or 1,000 RPM on a 41 cm radius centrifuge) for 3 minutes is often enough to bring the solid particles to the bottom without decanting the component of plasma.
- Aspirate the sample near the surface without agitating particilates on the bottom of the tube.
- When using a large amount of matrix to be fortified for Standard and QC, the matrix may be pre-filtered using a syringe filter or medical gauze. In either case, consider possible interferences.

Pre-treatment recommendations for common bioanalytical matrices

Plasma/Serum	Plasma and serum pre-treatments are analyte dependent. If the analyte of interest is an acid, 2 % phosphoric acid can be used (20 µL 85 % H_3PO_4 to 1 mL of plasma or serum) to disrupt the drug-protein interaction. If the analyte of interest is basic, 0.1 M sodium hydroxide can be used to disrupt the drug-protein interaction. After addition of acid or base, the sample should be vortexed for 20-30 seconds followed by centrifugation. The supernatant is now ready for further analysis.
Whole Blood	There are several pre-treatment strategies that can be followed for whole blood. If the target analyte is present in red blood cells, a hemolysis step is necessary. **a.** Hemolysis: To 0.2 mL whole blood (spiked with analytes and internal standard) in a 1.2 mL centrifuge tube, add 400 µL of 2 % zinc sulfate/80 % methanol. Vortex for 10-20 seconds followed by centrifugation at 14,000 rpm for 10 minutes. Collect the supernatant for further analysis. *Preparation of zinc sulfate/methanol:* Into a 100 mL volumetric flask add 20 mL water and 3.6 g $ZnSO_4$, $7H_2O$. After the solution is clear and the salt crystals have dissolved, add 100 % methanol. Refrigerate the solution at 2-8 °C for 7 days. **b.** Osmotic breakdown: To 1 mL of whole blood add internal standard and 4 mL of distilled water. Mix/vortex and let stand for 5 minutes. Centrifuge at 670 g for 10 minutes and discard the pellet. Adjust the pH of the supernatant accordingly with the addition of a buffer solution. **c.** Sonication: Sonicate 1 mL whole blood for 15 minutes at room temperature. Add 3-6 mL of an appropriate pH buffer (such as potassium phosphate buffer). Mix/vortex. Let stand for 5 minutes. Centrifuge (670 g) for 15 minutes. Analyze supernatant. ***Note:*** A comparison of the above pre-treatment techniques for whole blood was performed for acidic, basic, and neutral drugs. Recoveries were generally the highest when the whole blood sample was diluted with buffer and subjected to physical denaturing (sonication) rather than chemical means. In fact, the sonication process disrupts the cell membranes to the extent that no clogging was observed when the procedure listed above was followed.[1]
Urine	Enzymatic hydrolysis is necessary in case of conjugated forms (sulfated or glucuronide form) of the analye present. Enzymatic hydrolysis requires specific pH (pH 4-5) and temperature ranges. An acid or base hydrolysis can be advanced as well, depending on the stability of the compound. **a.** Enzymatic hydrolysis: To 500 µL sample (spiked with analyte and internal standard) add 100 µL acidic buffer (see below) and 20 µL beta-glucuronidase. Vortex 5-6 seconds. Incubate in a water bath at 63 °C for 30 minutes. Transfer sample to a 96-well collection plate or autosampler vial. Seal and centrifuge for 10 minutes at 2000 rpm. *Preparation of acidic buffer (1.0M acetate buffer, pH 4.0):* Dissolve 3.0 g of glacial acetic acid and 4.1 g of sodium acetate in a 1 L volumetric flask. **b.** Base hydrolysis: To 1 mL urine (spiked with analyte and internal standard) add 100 µL 10 N KOH. Mix, vortex, and hydrolyze for 20 minutes at 60 °C. Cool and adjust pH to 3.5-4.0 (by adding 200 µL glacial acetic acid). **c.** Acid hydrolysis: To 1 mL urine add 0.25 mL HCl in a screw capped test tube. Screw the tube top on loosely and heat in a boiling water bath for 60 minutes. Adjust to pH 7 (or as needed) with 1.0 N NaOH.
Saliva	No hydrolysis is required for oral fluids and the generic protocol used for plasma/serum pre-treatment may be followed.
Tissue	Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant, centrifuge or filter supernatant. Perform direct Matrix Solid Phase Dispersion (MSPD) extraction on tissue.

Dilution of Sample

There are several reasons to dilute a sample. One is to ensure that the sample is at the proper pH before loading. When considering unattended SPE extraction, dilution can reduce the viscosity inherent to each matrix to make the sample automation friendly. The unique design of Strata™-X 96-well plates allows you to dilute your sample up to 1.5 mL. This will allow you to use the same vacuum settings for various sample types (human plasma, serum, urine, rat plasma or serum, etc.) without having to change your sample load vacuum settings. Even different volumes (from 100 to 500 µL) can be successfully adapted to the same sample load vacuum settings using this technique. The smaller sample volume will of course load slightly faster, but the actual amount of crude sample loaded on the sorbent per second will remain the same.

The sample could be diluted in a collection plate or a tube prior to sample loading but in order not to be limited to the small tip capacity of some liquid handlers and their capacity to dispense reagent directly from bottles to the collection plate, sample can be diluted directly in the Strata™-X 96-well plate. To perform this technique, dispense a diluent volume over the SPE sorbent, then dispense the undiluted sample into the diluent and mix by aspiration and dispense cycles to ensure homogeneity with the diluents. If choosing to perform dilution within the wells of the Strata-X 96-well plate, special attention must be made to prevent the diluent from leaking. Refer to the Equilibration section for more information.

Vacuum Setting Tuning

Strata-X sorbents are automation friendly and are therefore wonderful sorbents to perform unattended SPE extraction. Unlike traditional silica-based sorbents, no special attention needs to be given to the Strata-X polymeric sorbents to prevent the sorbent from going dry after conditioning. In addition, Strata-X polymeric sorbents can accommodate various loading speeds without negatively affecting the performance of the sorbent. Unless otherwise mentioned, the recommended flow rate should be around 1 to 3 drops per second. As an example, 4 drops per second starts to be a continuous stream and is therefore too fast.

When pulsed vacuum is used, it is known that two stronger short pulses used during the first second of vacuum allow the liquid to soak through the sorbent. After having initiated the flow, a lower intensity of vacuum may be used to continue. If the strong pulses are omitted, the vacuum is not strong enough to break the initial resistance to flow.

Solvent Volumes

The condition/equilibration, wash, and elution volumes used should be in consideration with the sorbent bed mass. Modification to these volumes without further tests may result in poor well-to-well flow reproducibility and/or blockage. The below selection chart outlines recommended solvent volumes which have been optimized for unattended SPE extraction.

Sorbent Mass	Strata-X (33 µm Particle) Minimum Recommended Solvent Volumes		Strata-XL (100 µm Particle) Minimum Recommended Solvent Volumes	
	Wash	Elution	Wash	Elution
10 mg	300 µL	300 µL	--	--
30 mg	800 µL	400 µL	1 mL	600 µL
60 mg	1.5 mL	800 µL	--	--

* Performing 2 aliquots during the elution step can boost recoveries. For example, instead of a single 300 µL elution, perform 2x 150 µL elutions and combine the two aliquots.

Conditioning the Sorbent

Conditioning the sorbent is an important step because it prepares the chemistry of the sorbent to interact with your target analytes. The conditioning step is typically performed with the same solvents that will be used during the elution step. This is the easiest part to set up. The vacuum at this step has to be intense enough so that the conditioning occurs quickly. Vacuum should also be long enough to ensure that there is no remaining volume either on top of the sorbent or in the sorbent before the next step.

Equilibrating the Sorbent

Equilibration of the sorbent prepares the conditioned sorbent for the sample. The equilibration step is typically performed with a solvent that is similar to the sample. For example, bioanalytical analysis often involves using water or buffer for the equilibration step. This step could be carried out the in the same way that the conditioning step was performed. If you intend to dilute your sample within the wells of the Strata-X 96-well plate like described in the Dilution of Sample section, a few extra pulses must be inserted in order to gently dry the sorbent. This will cause the top frit to repel the diluent and prevent the diluent from leaking through before the sample is added.

Load Diluted Sample

The load step should be set so that the flow rate is around 1 to 3 drops per second. Sample loading can be followed by a stronger vacuum that ensures that there is no sample diluent remaining in the well before the next step. No special care needs to be taken to load slower than 2 drops per second or to prevent empty (unused) wells from going dry when other wells are not completed.

Wash

The wash step removes unwanted interferences without disrupting the analyte-sorbent interaction. When performing reversed phase extractions, the wash often consists of a water or buffer solution with 5-50 % organic solvent. The concentration of organic solvent can be optimized to find a balance between cleanup and recovery by performing several washes of different strengths then analyzing the eluent for analyte breakthrough. The ideal wash will be strong enough to wash away interferences but weak enough that it will not disrupt the analyte-sorbent interaction. When performing ion-exchange methods, a 100 % organic wash can be perfomed as long as the wash is kept at a pH that does not disrupt the ionic interaction of the analyte and sorbent.

The wash step should have a flow rate of 1 to 3 drops per second. Extra vacuum should be included to make sure no volume remains in the wells before the next step. The last wash should be ended with a dry-down period of 1 minute at a high vacuum setting in order to remove as much moisture from the sorbent as

possible. This will greatly reduce the time required to dry down the sample eluent and will maximize the potency of your elution solution.

Elution

The elution step breaks the analyte-sorbent interaction so that the target analyte can be collected and further analyzed. For reversed phase methods, this step often involves using a 100 % organic solvent solution. For ion-exchange methods, this step involves a 100 % organic solvent solution at a pH that neutralizes either the sorbent or the analyte.

The elution step should be slow enough so that the elution solution contact time with the sorbent is sufficient and does not channel through the sorbent without interacting with it. The flow rate should not exceed 2 drops per second. Furthermore, since the elution solution is highly concentrated in analyte, special care should be taken to prevent cross-well contamination. The collection plate should be as close as possible to the SPE plate without interfering with the free compression of the vacuum seals between the various parts of the manifold and SPE plate. It is also recommended to wipe the top of the collection plate by applying a lint free cloth, without rubbing, to remove any solvent that may promote movement of liquid across the top of the well plate when a sealing mat is used.

Special Considerations

During initial set up for unattended SPE, a pause should be inserted in the program between each step to allow the user to confirm that the liquid is cleared from each well before proceeding to the next step. Observing ten plates is generally sufficient to confirm that the program will work unattended. During this verification, if liquids remain in the wells, increase the duration for that step. If there is blockage, verify if this blockage is due to large particles in the sample. If not, then verify the conditioning volumes. If these are okay, contact Phenomenex for additional technical support.

This approach was developed with an automated SPE vacuum system. Positive pressure can also be used with the same considerations. For more information about Strata™-X sorbents and method development tips, please visit www.phenomenex.com/StrataX.

Customized SPE Method Development in Under 1 Minute

www.phenomenex.com/MDTool

Phenomenex has developed an interactive SPE Method Development Tool which can customize an SPE method. Simply input the name of your target analyte or enter the analyte characteristics if proprietary, enter your sample matrix and volume, and instantly receive a customized SPE method that will serve as a starting point for your method development.

References:

1. Chen et al., *J. Anal. Toxicol*. 1992, v18, pages 352-355.

Strata™-X Sorbent Selection and Ordering Information

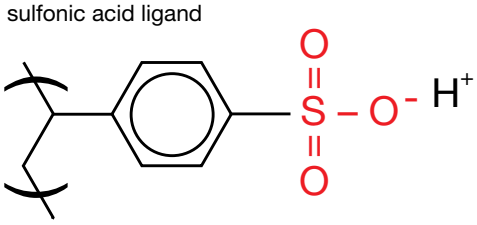
EXTRACTION of BASIC COMPOUNDS

Strata-X-C (33 µm) and Strata-XL-C (100 µm)

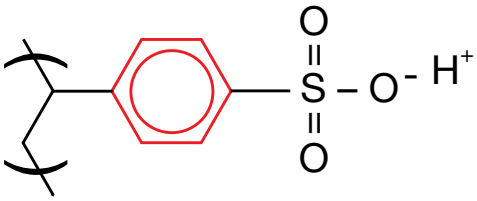
for cleanup of weak bases (*pK*a 8-10)

3 Mechanisms of Retention

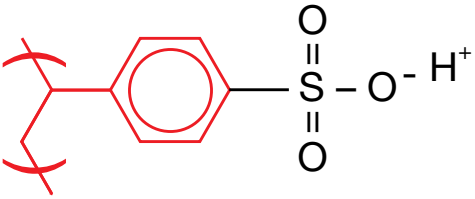
Strong Cation-Exchange:
sulfonic acid ligand



π-π Bonding



Hydrophobic Interaction



	10 mg/well	30 mg/well	60 mg/well
Strata-X-C	8E-S029-AGB	8E-S029-TGB	8E-S029-UGB
Strata-XL-C	--	8E-S044-TGB	--

Alternative to:

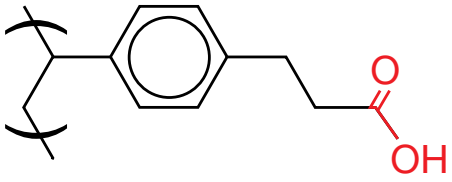
Agilent (formerly Varian) Bond Elut™ Plexa™ PCX and Waters Oasis® MCX

Strata-X-CW (33 µm) and Strata-XL-CW (100 µm)

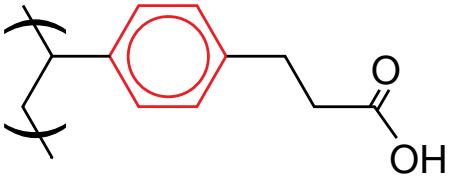
for cleanup of strong bases (*pK*a >10)

3 Mechanisms of Retention

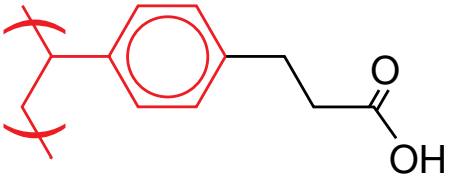
Weak Cation-Exchange:
carboxylic acid ligand



π-π Bonding



Hydrophobic Interaction



	10 mg/well	30 mg/well	60 mg/well
Strata-X-CW	8E-S035-AGB	8E-S035-TGB	8E-S035-UGB
Strata-XL-CW	--	8E-S052-TGB	--

Alternative to:

Waters Oasis® WCX

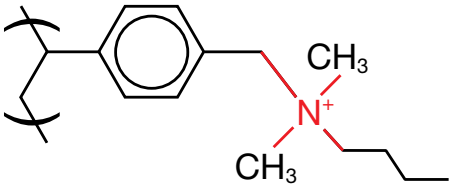
EXTRACTION of ACIDIC COMPOUNDS

Strata-X-A (33 µm) and Strata-XL-A (100 µm)

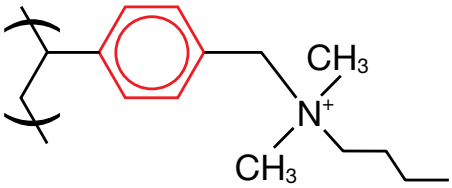
for cleanup of weak acids (*pK*a 2-4)

3 Mechanisms of Retention

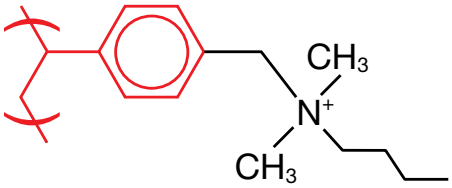
Strong Anion Exchange:
di-methylbutyl quaternary amine ligand



π-π Bonding



Hydrophobic Interaction



	10 mg/well	30 mg/well	60 mg/well
Strata-X-A	8E-S123-AGB	8E-S123-TGB	8E-S123-UGB
Strata-XL-A	--	8E-S053-TGB	--

Alternative to:

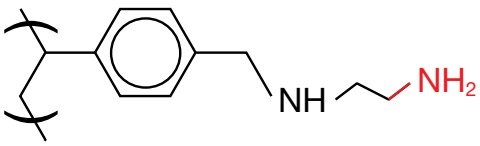
Agilent (formerly Varian) Bond Elut™ Plexa™ PAX and Waters Oasis® MAX

Strata-X-AW (33 µm) and Strata-XL-AW (100 µm)

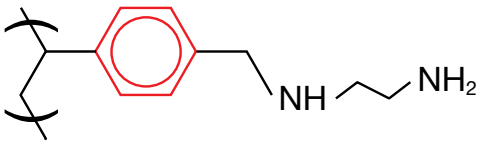
for cleanup of strong acids (*pK*a <2)

3 Mechanisms of Retention

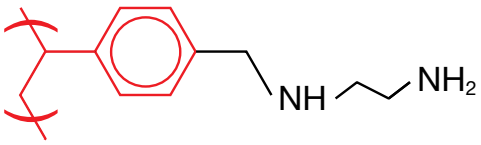
Weak Anion Exchange:
di-amino ligand



π-π Bonding



Hydrophobic Interaction



	10 mg/well	30 mg/well	60 mg/well
Strata-X-AW	8E-S038-AGB	8E-S038-TGB	8E-S038-UGB
Strata-XL-AW	--	8E-S051-TGB	--

Alternative to:

Waters Oasis WAX

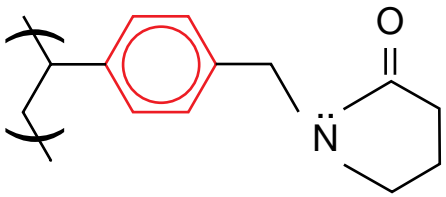
EXTRACTION of NEUTRAL COMPOUNDS

Strata-X (33 µm) and Strata-XL (100 µm)

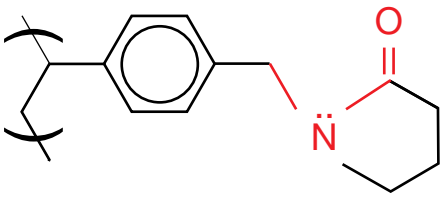
for cleanup of neutral compounds

3 Mechanisms of Retention

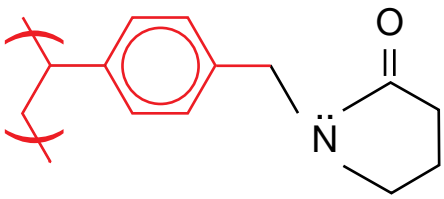
π-π Bonding



Hydrogen Bonding
Dipole-Dipole Interactions



Hydrophobic Interaction



	10 mg/well	30 mg/well	60 mg/well
Strata-X	8E-S100-AGB	8E-S100-TGB	8E-S100-UGB
Strata-XL	--	8E-S043-TGB	--

Alternative to:

Agilent (formerly Varian) Bond Elut™ Plexa™ and Waters Oasis® HLB

Accessories Ordering Information

Collection Plates (deep well, polypropylene)		
AH0-7192	Strata® 96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	Strata 96-Well Collection Plate, 1 mL/well	50/pk
AH0-7194	Strata 96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	Strata 96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	Strata 96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	Strata 96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
Sealing Mats		
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk
Vacuum Manifolds		
AH0-8950	Strata 96-Well Plate Manifold, Universal with Vacuum Gauge	ea

* Manifolds include: Vacuum-tight glass chamber, vacuum gauge assembly, polypropylene lid with gasket, male and female luers and yellow end plugs, stopcock valves, collection rack assemblies, polypropylene needles, lid support legs. Waste container included with 12-position manifold.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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