

Extraction of Acidic and Neutral Compounds Using Strata Screen A Mixed Mode Solid Phase Extraction

SPE Cartridge:

Strata Screen A Mixed-Mode (Hydrophobic + SAX)

25 mg / well 96-well plate, Part number 8E-S019-CGB

50 mg / well 96-well plate, Part number 8E-S019-DGB

200 mg / 3 ml, Part number 8B-S019-FBJ

200 mg / 20 ml Funnel Shaped Tube, P/N 8C-S019-FEH

Introduction:

In this method, acidic/anionic and neutral compounds are extracted from plasma, urine or any other aqueous matrix using Strata Screen A. Strata Screen A is a mixed-mode SPE sorbent containing a proprietary mix of strong anion exchange (quaternary amine) and hydrophobic C8 functionality's.

Hydrophobic and anionic analytes are retained at the loading step. Very polar compounds, salts and large proteins (>30kD) are then removed from the sample via a wash step.

Hydrophobic / neutral analytes retained via the non-polar/C8 interaction are then eluted from the sorbent via a methanol elution.

Anionic/acidic analytes are subsequently eluted using a mixture of water miscible organic solvent with acid modifier in order to neutralize the charged functional group.

Specimen Preparation:

Dilute sample a minimum of 1:1 with D.I. water or appropriate buffer ("sample buffer"). Note: sample pH must be such that anionic groups are quantitatively charged (typically between pH 6.5- 8.0). Use low ionic strength buffer (< 0.1 M) of low counter ion selectivity. Typically safe are acetate, phosphate, formate. (See pg. 23 Phenomenex SPE Users Guide for more info).

Condition:

4 to 8 bed volumes of methanol (see table)

4 to 8 bed volumes of sample buffer

1. Load:

Apply the sample at a rate not to exceed 2 ml / minute.

Wash / Dry:

1. 1 – 2 column volumes of sample buffer or DI water
2. Dry column 0.5 to 1 minute at full vacuum to remove all traces of wash solvents. Release vacuum.

Elute Neutrals:

With the vacuum off, apply 4 –8 bed volumes of Methanol.
Allow to slowly percolate through cartridge for a few seconds before applying vacuum.

Elute Anions / Acids:

With the vacuum turned off, apply 4 to 8 bed volumes of Acidic Elution Solvent (see below). Allow to slowly percolate through cartridge for a few seconds before applying vacuum.

Acidic Elution Solvent: Methanol plus 1% concentrated HCl or glacial acetic acid. (e.g. 99 parts MeOH and 1 part acid).

Analysis:

The extract of this method is volatile making it easy evaporate and reconstitute in a solvent compatible with the chosen analytical system.

Please contact your Phenomenex Technical Representative for more information on analytical separation conditions.

Table 1. Suggested solvent processing volumes.

Note: Solvent volumes necessary for conditioning, washing and eluting are directly proportional to sorbent MASS and not the tube reservoir size (in ml or cc). More specifically, minimum solvent volumes are determined the cartridge bed dead volume which is proportional to sorbent mass.

Sorbent Mass	Minimum Wash and Elution Volume (2 bed volumes)	“Safe” Wash and Elution Volume (4 bed volumes)	Method Development Wash and Elution Volume (8 bed volumes)
25 mg	75 ul	125 ul	250 ul
50 mg	125 ul	250 ul	500 ul
100 mg	250 ul	500 ul	1 ml
150 mg	375 ul	750 ul	1.5 ml
200 mg	500 ul	1 ml	2 ml
360 mg	900 ul	1.8 ml	3.6 ml
500 mg	1.25 ml	2.5 ml	5 ml
1 g	2.5 ml	5 ml	10 ml
2 g	5.0 ml	10 ml	20 ml
5 g	12.5 ml	25 ml	50 ml
10 g	25.0 ml	50 ml	100 ml

This method is designed to start as a convenient starting point for further investigation. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.