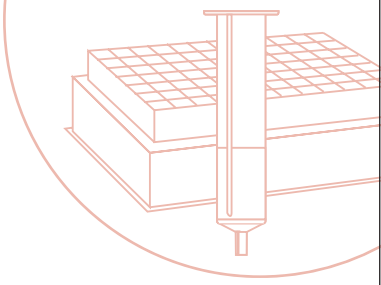
Fast, Effective & Convenient SPE Sorbent Selection using the Reversed Phase Strata™ Method Development 96-Well Plate



Shahana Huq, Art Dixon and James Teuscher
Phenomenex Inc., Torrance, CA, USA

Sample preparation can be a bottleneck in the analytical laboratory, occupying more than 60% of the analyst's time.[1] In the case of solid phase extraction (SPE), a common sample preparation technique, the 96-well plate format has become popular due to the enhanced productivity from the high-throughput of samples. However, selection of the proper sorbent from the wide array of commercially available SPE sorbents, a key step in method development, requires considerable cost, time and labor, even when using the 96-well plate.

The Strata Method Development 96-Well Plate was created to provide optimum sorbent selection with one simple generic SPE experiment. The plate consists of twelve wells of eight reversed-phase sorbents (six silica-based and two polymeric phases). The selectivity of each sorbent is distinct due to their different physical and chemical characteristics, as shown in ***Table 1***. With *one* 96-well plate, the retention and elution characteristics of *eight* different sorbents toward a particular analyte(s) of interest can be quickly evaluated. In this application, a simple generic SPE protocol was used to extract five analyte probes differing in polarity (log P = 0.46 - 4.29) on each sorbent. We demonstrate that parallel screening of the eight sorbents using the Strata Method Development Plate offers fast, effective and convenient sorbent selection for automated, high-throughput SPE.

Instrumentation and Equipment

Solid Phase Extraction

The Strata Method Development 96-Well Plate, consisting of six silica-based sorbents (50mg/well) and two polymeric resins (30mg/well), was processed with a 96-Well Plate Manifold. The elution solvent containing the analytes of interest was collected in a 96-Well Collection Plate (2mL/well). Both accessories were supplied by Phenomenex.

HPLC

The samples were analyzed using an HP 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a variable wavelength detector. A Phenomenex Luna C8(2), 5µ, 150 x 4.6mm was used. The data was analyzed with HP ChemStation software.

Experimental Conditions

Specimen Preparation

Phosphate Buffer Saline solution (PBS) was spiked with five analyte probes (5.0µg/mL). The probes were selected as representative of a wide polarity range, as shown in ***Table 2***.

Table 2. Log P values for the probe compounds

Compound	Log P
acetaminophen	0.46
salicylic acid	2.26
diazepam	2.82
naproxen	3.18
doxepin	4.29

SPE Method

Condition: 1mL methanol followed by 1mL deionized water.
Load: 1mL spiked sample.
Wash: 1mL deionized water with 5% methanol. Allow the plate to dry for 1-2 min.
Elution: 1mL methanol.

To further reduce extraction time, the method was run under continuous flow of vacuum.

Analysis

The eluate was spiked with an external standard (ranitidine, 5.0µg/mL) and then evaporated to dryness under a slow stream of nitrogen. The sample was reconstituted to 0.2mL using the HPLC mobile phase buffer (pH 7.0).

HPLC Conditions: A sample of reconstituted solution (50µL) was injected into the HPLC system with the UV detector set at 236nm. The gradient conditions, as shown in ***Table 3***, consisted of mobile phases A = 20mM KH_2PO_4, pH 7.0 and B = methanol.

Table 3. HPLC gradient conditions for mobile phase

Time	% B	Flow rate (mL/min)
0	10	1
3	10	1
13	70	2
18	70	2
19	10	1

Table 1. Physical and chemical characteristics of the phases in the Strata Method Development 96-Well Plate

	C18-E	C18-U	C18-M	C18-T	C8	Phenyl	strata-X	SDB-L
Silica Bonding	Trifunctional	Trifunctional	Monofunctional	Trifunctional	Trifunctional	Trifunctional	-	-
Endcapped?	Yes	No	No	Yes	Yes	Yes	-	-
% Carbon Load	17	18	15	15	10.5	10.5	-	-
Surface Area (m^2/g)	500	500	300	300	500	500	800	500
Particle Size (µm)	55	55	55	55	55	55	33	100
Pore Size (Å)	70	70	140	140	70	70	85	260

Fast, Effective & Convenient SPE Sorbent Selection using the Reversed Phase Strata™ Method Development 96-Well Plate

Results

Figure 1 shows the recoveries of the five target probes for each of the different phases in the Strata Method Development 96-Well Plate. As expected, the recoveries of the probes varied due to the different selectivity of each sorbent toward polar and nonpolar compounds. The unique hydrophobic and hydrophilic retention mechanism of the patent pending sorbent strata-X, significantly enhanced the recoveries of the polar analytes, as compared to the other seven sorbents. For the nonpolar compounds, strata-X and Strata C18-T showed consistently higher recoveries than the other sorbents.

The rapid screening method allows you to immediately choose the sorbent that has the highest selectivity and fits your overall SPE goals. For instance, if naproxen was the only compound of interest, strata-X or Strata C18-T might be selected for further optimization. If the SPE goal were to develop a general screening method for many compounds varying in polarity, strata-X would be the best choice, as it showed high recoveries for all the probes under these experimental conditions. After selecting the proper SPE phase, the method conditions can then subsequently be adjusted to obtain optimal recoveries. As shown in ***Figure 2***, the relative recoveries of the five probes on strata-X increased to >90% after the method was optimized.

Figure 1. Percent recoveries from the initial screen of the eight reversed-phase sorbents

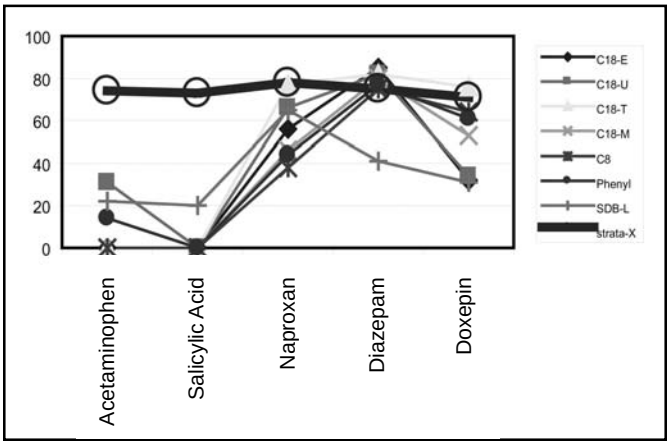
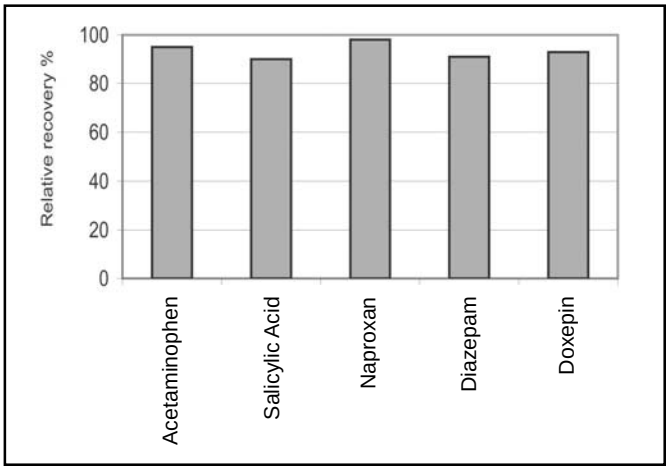


Figure 2. Percent recoveries on strata-X after optimizing extraction conditions



Conclusion

The Strata Method Development 96-Well Plate serves to streamline method development by simplifying and reducing the analysis time necessary to select the proper SPE sorbent. With one plate, the analyst can screen, in parallel, eight different reversed-phase SPE sorbents to quickly determine the best sorbent chemistry for the analytes of interest.

Acknowledgments

Special thanks to Dr. Phil Koerner and Dr. Stanley Lok for their critical review of this application note and helpful discussions.

Reference

1. *J. S. Fritz* ***in Analytical Solid Phase Extraction*** *(Wiley-VCH, New York, 1999 pg. 1)*

Ordering Information:

Order Number	Description
KS0-7293-TN	Strata Method Development 96-Well Plate
AH0-7284-TN	Acrylic 96-Well Plate Manifold
AH0-7192-TN	Polypropylene 96-Well Collection Plate 350µL/well
AH0-7193-TN	Polypropylene 96-Well Collection Plate 1mL/well
AH0-7194-TN	Polypropylene 96-Well Collection Plate 2mL/well
AH0-7195-TN	96-Well Sealing Mat
AH0-7363-TN	96-Well Sealing Tape
00F-4249-E0-TN	Luna C8(2) 5µ, 150 x 4.6mm

2590