

Simplified Method Development for the Extraction of Acidic, Basic and Neutral Drugs with a Single SPE Sorbent - strata™ X

Shahana Huq, Art Dixon and James Teuscher

Phenomenex, Inc. Torrance, CA USA

Solid Phase Extraction (SPE) has become a popular sample preparation technique that bridges the gap between specimen collection and instrumental analysis. SPE is very effective in concentrating and purifying the target analyte(s). The analyst's challenge is to optimize the conditions for each step of the reversed-phase SPE method (**Table 1**) to consistently achieve high recoveries of the target analyte(s). Method development consists of screening various sorbent chemistries along with determining the best solvents to use. This is usually accomplished through "trial and error", which is time consuming and expensive.

strata-X, a revolutionary polymeric SPE sorbent (patent pending), streamlines the method development process for reversed-phase SPE. The unique surface properties of strata-X offer numerous retention mechanisms, including hydrophobic, hydrogen bonding and π - π , providing "near universal" selectivity for acidic, neutral and basic drugs. With *one* sorbent using *one* simple SPE method, a wide range of compounds can be reproducibly extracted with high recoveries.

Table 1. Generic SPE procedure for reversed-phase sorbents.

Condition:	methanol
Equilibrate:	water or buffer
Load:	sample
Wash:	water or buffer
Elute:	methanol
	acetonitrile
(typical solvents of increasing strength)	acetone
	ethyl acetate
	chloroform
	acidic methanol
	basic methanol

NOTE: Exact solvent volume for each step depends on bed mass of sorbent.

Instrumentation/Equipment

Solid phase extraction

A 1mL syringe-barrel cartridge containing 30mg of polymer was used for the sample preparation of the target compounds. **Table 2** contains information on the physical and chemical properties of strata-X. The SPE tubes were processed with a 12-position SPE vacuum manifold supplied by Phenomenex.

Liquid Chromatography

All analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with quaternary pump, in-line degasser, multi-wavelength detector and autosampler. HP Chemstation software was used to analyze the data. The HPLC column was a Luna 5 μ m, C8(2) 150 x 4.6 mm from Phenomenex (order number: 00F-4249-E0-TN).

Table 2. strata-X particle characteristics

Phase:	Surface modified styrene-divinylbenzene polymer
Average particle diameter:	33 μ m
Nominal pore size:	85Å
Surface area:	800m ² /g

Experimental Conditions

Table 3 shows the simple strata-X method that serves as a starting point for method development. This procedure was successfully used to extract a variety of compounds from serum: acidic nonsteroidal anti-inflammatory drugs (NSAID), basic tricyclic antidepressants (TCA) and neutral bronchodilators. For each extraction, the strata-X tube was conditioned with 1mL methanol followed by 1mL water. A 1mL porcine serum sample spiked with an analyte probe was then loaded. (For the extractions of acidic and basic compounds, 2% concentrated phosphoric acid was also added to the sample prior to loading.) A slight vacuum was used to pull the conditioning solvents and sample through the column at a rate of approximately 1mL/min. To remove weakly bound interferences, the sorbent was washed with 1mL of water containing 5% methanol and then dried under vacuum (10 in. Hg) for 1 minute. The analytes were eluted with 1mL of methanol.

To prepare for HPLC injection, the extract was spiked with an internal standard, dried under nitrogen at room temperature and reconstituted in 200 μ L of 20mM phosphate buffer (pH = 7). The specific HPLC conditions for each class of compounds are given in **Figures 1-3**. The extraction procedure was repeated in triplicate for each of the acidic, basic and neutral probes.

Table 3. The simple strata-X method

Condition:	1mL methanol
Equilibrate:	1mL water
Load:	sample
Wash:	1mL 5% methanol in water
Elute:	1mL methanol*

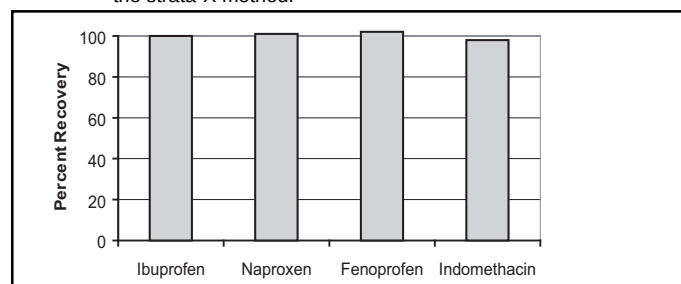
NOTE: Solvent volumes are recommended for 30 mg of sorbent.

*TIP: Use acetonitrile/methanol (50:50) to elute aromatic compounds.

Results

Acidic NSAID Compounds: Four analgesic and anti-inflammatory compounds were successfully extracted from porcine serum using strata-X. As shown in **Figure 1**, the average recovery for each acidic probe was greater than 90% (RSD <8% for Ibuprofen and Naproxen and <3% for Fenoprofen and Indomethacin).

Figure 1. Average recoveries for NSAID compounds using the strata-X method.



HPLC conditions: Mobile phase A was 0.05% H₃PO₄ and B was Acetonitrile. The flow rate was 1.5 mL/min. The gradient program began with 50:50 (A:B) for 8 min, followed by a linear gradient to 15:85.



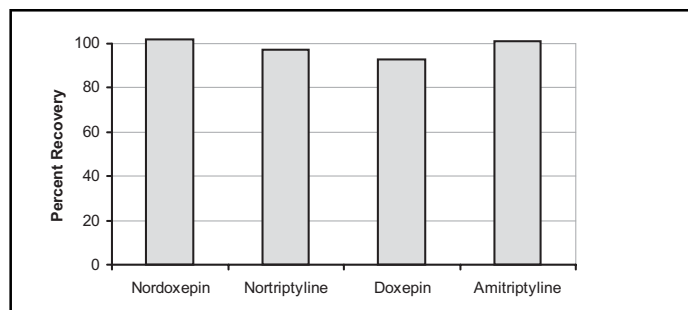
www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department by telephone, fax or e-mail: international@phenomenex.com.

phenomenex®
...breaking with tradition

Basic TCA Compounds: strata-X was used to extract four tricyclic antidepressant drugs from porcine serum. As shown in **Figure 2**, the average recovery for each TCA probe was greater than 90% (RSD <2% for Nordoxepin, Amitriptyline and Nortriptyline and <5% for Doxepin).

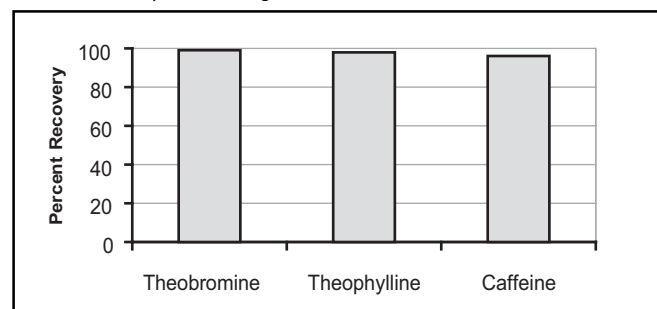
Figure 2. Average recoveries for tricyclic antidepressants using the strata-X method.



HPLC conditions: Mobile phase consisted of three components: A = KH_2PO_4 (pH = 7), B = Acetonitrile and C = Methanol. The flow rate was 2.0 mL/min. The gradient program began with 40:30:30 mixture (A:B:C) for 10 min. After 10 min. the gradient was changed to 10:45:45 for the remainder of the run time.

Neutral Bronchodilator Compounds: Three bronchodilator compounds showed high recoveries with the strata-X method. As shown in **Figure 3**, the average recovery for each neutral probe was greater than 95% (These compounds showed the best reproducibility with a RSD <1.5%).

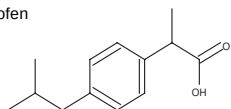
Figure 3. Average recoveries for neutral bronchodilator compounds using the strata-X method.



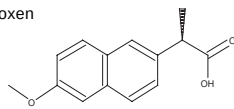
HPLC conditions: Isocratic mobile phase consisting of 20mM KH_2PO_4 (pH = 2.5) and Acetonitrile (95:5). The flow rate was 1.5 mL/min.

Acidic compounds

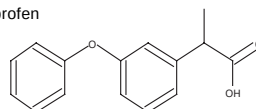
Ibuprofen



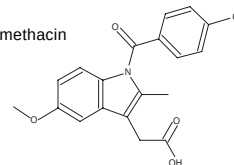
Naproxen



Fenoprofen

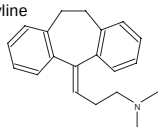


Indomethacin

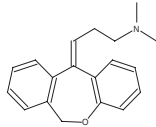


Basic compounds

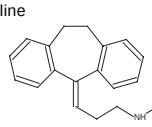
Amitriptyline



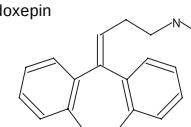
Doxepin



Nortriptyline

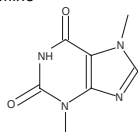


Nordoxepin

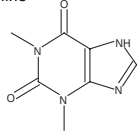


Neutral compounds

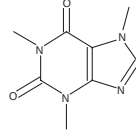
Theobromine



Theophylline



Caffeine



Conclusions

The strata-X method uses one polymeric sorbent to extract four acidic nonsteroidal anti-inflammatory drugs, four tricyclic antidepressants and three neutral bronchodilators. This extraction method consistently delivers recoveries >90% for all these classes of compounds from a serum matrix. The extraction is accomplished using simple procedure that eliminates the standard "trial and error" method development process and reduces solvent. In contrast, to extract the same compounds, several conventional SPE methods screening several different silica-based sorbent chemistries would have been required. Thus, the time and cost associated with "standard" SPE method development can now be significantly reduced.

Acknowledgments

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

Ordering Information

Strata-X is available in syringe-barrel tubes and 96-well plates.

Part Number	Description	Units (Box)
8B-S100-TAK-TN	strata-X 30mg/1mL Tubes	100
8B-S100-UBJ-TN	strata-X 60mg/3mL Tubes	50
8B-S100-FBJ-TN	strata-X 200mg/3mL Tubes	50
8B-S100-HBJ-TN	strata-X 500mg/3mL Tubes	50
8B-S100-ECH-TN	strata-X 100mg/6mL Tubes	30
8B-S100-FCH-TN	strata-X 200mg/6mL Tubes	30
8B-S100-HCH-TN	strata-X 500mg/6mL Tubes	30
8B-S100-HDG-TN	strata-X 500mg/12mL Giga Tubes	20
8B-S100-JEG-TN	strata-X 1g/20mL Giga Tubes	20
8E-S100-NGB-TN	strata-X 96 Well Plates (5mg/well)	2
8E-S100-AGB-TN	strata-X 96 Well Plates (10mg/well)	2
8E-S100-TGB-TN	strata-X 96 Well Plates (30mg/well)	2
8E-S100-UGB-TN	strata-X 96-Well Plates (60mg/well)	2
00M-S033-B0-CB-TN	strata-X 25µm On-Line Extraction Cartridge	each
CH0-5845-TN	20mm Cartridge Holder	each