Sample Preparation of Tricyclic Antidepressants for Drug Screens: The advantages of Strata™-X over mixed-mode sorbents

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Solid phase extraction (SPE) is a popular sample preparation technique in toxicological analysis for the screening of biological active compounds. A generic SPE method, commonly used for drug screening, is shown in **Table 1**. (For complete details refer to the article by de Zeeuw and Franke [1]). It uses a "mixed-mode" sorbent that typically consists of C8 silica mixed with silica functionalized with an ion exchange group. Utilizing both hydrophobic and ion exchange retention mechanisms, acidic, basic, and neutral drugs are extracted from biological matrices.

Mixed-mode sorbents have several disadvantages including the need to prepare fresh buffers and the dissolution of the silica in strongly alkaline solutions. In addition, halogenated solvents are typically used for elution. These harsh solvents require care when handling and have strict disposal requirements. These factors increase the time and cost of sample preparation.

strata-X, an innovative, patent pending polymeric resin developed by Phenomenex, is an attractive alternative to mixed-mode sorbents for sample preparation. The unique surface properties of this material provide almost universal reversed-phase selectivity, giving high recoveries for a wide range of compounds using a simple, fast extraction protocol.

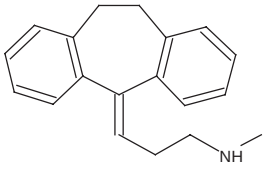
Table 1: Generic Sample Preparation Protocols

	Mixed-mode sorbent	strata-X
Sorbent bed mass:	150mg	30mg
Condition :	2mL methanol	1mL methanol
	2mL phosphate buffer (pH = 6)	1mL water
Load :	8mL diluted sample	sample
Wash :	1mL water	1mL 5% methanol in water
pH adjustment:	0.5mL 0.01M acetic acid	Not required
Elute:	4mL acetone/chloroform	1mL methanol
	2mL 2% ammonia in ethyl acetate	

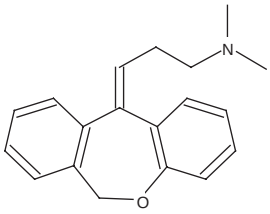
Instrumentation/Equipment

A syringe-barrel tube (1mL) containing 30mg of strata-X polymer was used for the sample preparation of the target compounds.

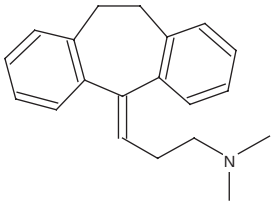
All analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA) equipped with quaternary pump, in-line degasser, multi-wavelength detector and autosampler. HP Chemstation software was used to analyze the data. The HPLC column was a Luna 5µ, C8(2), 150 x 4.6 mm (Phenomenex,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



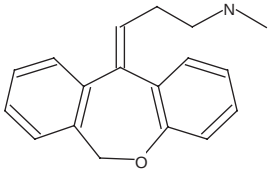
Nortriptyline (Log P = 4.74)



Doxepin (Log P = 4.29)



Amitriptyline (Log P = 4.92)



Nordoxepin (Log P = 3.83)

Experimental Method

Table 1 shows a simple generic method for the extraction of biologically active compounds using strata-X. The procedure was repeated in triplicate to extract tricyclic antidepressants (TCA) from serum. The target probes were Doxepin, Nordoxepin, Amitriptyline and Nortripyline.

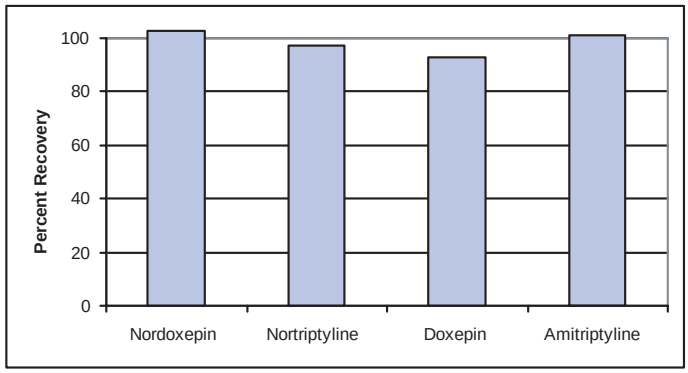
For each extraction, the strata-X tube was conditioned with 1mL methanol followed by 1mL water. A 1mL porcine serum sample spiked with the TCA analyte probes and 2% concentrated phosphoric acid was then loaded. A slight vacuum was used to pull the conditioning solvents and the sample through the column 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 inches Hg) for 1 minute. The analytes were then eluted with 1mL of methanol.

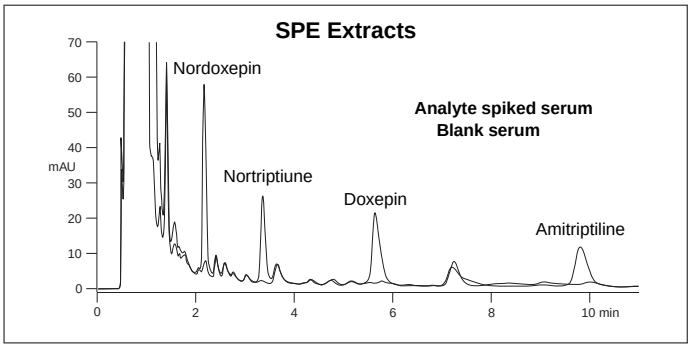
To prepare for HPLC injection, the extract was spiked with an internal standard, dried under nitrogen at room temperature and reconstituted in 200µL of 20mM phosphate buffer (pH 7).

Results

Due to the hydrophobic nature of the TCA probe compounds (high log P values), reversed phase SPE effectively extracted these compounds from serm. As shown in **Figure 1**, the average recovery for each TCA compound was greater than 90% (RSD <2% for Nordoxepin, Amitriptyline and Nortripylin and <5% for Doxepin), which is equal to, or better than, those obtained from mixed mode sorbents.

Figure 1: Average recoveries for TCA compounds using strata-X.





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 (A:B:C) for 10 min. After 10 min, the gradient was changed to 10:45:45 for the remainder of the run time. UV detection at 210nm.

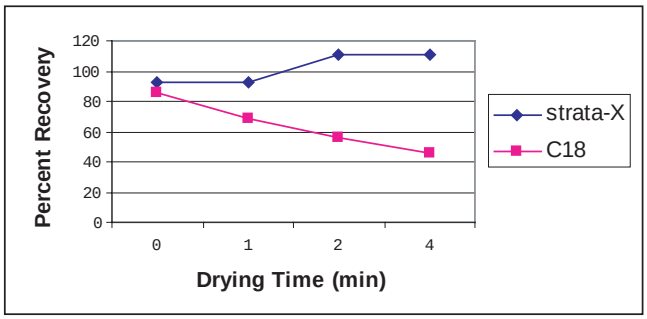
Discussion

strata-X offers several advantages over the mixed mode sorbents. The protocol is simplified, and no hazardous halogenated solvents are required for analyte desorption.

The 33µm particle has a surface area of 800m^2/g, resulting in a capacity that is significantly higher than that of silica-based sorbents. The strata-X bed mass can be reduced by as much as 2/3 the mass used in the silica-based tubes, resulting in a faster flow of the serum sample.

High recoveries are obtained even if the sorbent accidentally runs dry between conditioning and the loading of the sample. This effect significantly impairs the retention of silica-based sorbents. As shown in **Figure 2**, the percent recovery of Doxepin using the C18 sorbent dropped below 60% with as little as two minutes of drying time.

Figure 2. Effect of drying time on the recovery of doxepin: strata-X vs. silica-based C18



Conclusion

The versatility and selectivity of the polymeric strata-X resin makes it ideal for a simple generic extraction of a wide range of acidic, basic and neutral compounds from biological fluids, giving high and reproducible recoveries. The time, cost and potential hazards of preparing samples for drug screening analysis can now be significantly reduced over traditional mixed-mode methods.

Reference

1. R. de Zeeuw and J. Franke, Solid Phase Extraction Principles, Techniques and Applications, 2000 Marcel Dekker, Inc. pp.243-273.

Ordering Information:

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

Part Number	Description
8B-S100-TAK	30mg/1mL tubes
8B-S100-UBJ	60mg/3mL tubes
8B-S100-FBJ	200mg/3mL tubes
8B-S100-ECH	100mg/6mL tubes
8B-S100-FCH	200mg/6mL tubes
8B-S100-HCH	500mg/6mL tubes
8B-S100-HDG	500mg/12mL Giga tubes
8B-S100-JEG	1g/20mL Giga tubes
8E-S100-AGB	10mg 96 well-plates
8E-S100-TGB	30mg 96-well plates