Simplified Method Development for the Extraction of Acidic, Basic and Neutral Compounds with a Single SPE Sorbent — Strata X™

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Introduction

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 (Figure 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, an innovative polymeric SPE sorbent (patent pending), streamlines the method development process for reversed-phase SPE. The unique surface properties of Strata X provide "near-universal" selectivity for acidic, neutral and basic drugs. With one sorbent and one simple SPE method, a wide range of compounds can be reproducibly extracted with high recoveries.

Figure 1: Generic SPE procedure for reversed-phase sorbents.

Condition:	Methanol
Equilibrate:	Water or buffer
Load:	Sample
Wash:	Water or buffer
Elute: (typical solvents of increasing strength)	Methanol, acetonitrile, acetone, ethyl acetate, chloroform, acidic methanol, basic methanol

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

Figure 2: The Strata X method for easy extraction of acidic, basic and neutral compounds.

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

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

Instrumentation/Equipment

1 mL Strata X syringe-barrel cartridge (Phenomenex, Torrance, CA, USA) containing 30 mg of 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, California, USA) equipped with quaternary pump, in-line degasser, multi-wavelength detector and autosampler. HP Chemstation software was used to analyse the data. The HPLC column was a Luna® 5µ, C8(2), 150 × 4.6 mm (Phenomenex, Torrance, California, USA).

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Experimental Conditions

Figure 2 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 cartridge was conditioned with 1 mL methanol followed by 1 mL water. A 1 mL 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 the sample through the column at a rate of approximately 1 mL/min. To

remove weakly bound interferences, the sorbent was washed with 1 mL of water containing 5% methanol and then dried under vacuum (10 in. Hg) for 1 minute. The analytes were then eluted with 1 mL 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 µL of 20 mM phosphate buffer (pH 7). The specific HPLC conditions for each class of compounds are provided in the results section. The extraction procedure was repeated in triplicate for each of the acidic, basic and neutral probes.

Figure 3: Average recoveries for NSAID compounds using the Strata X method. HPLC conditions: mobile phase A = 0.05% H_3PO_4 and B = 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.

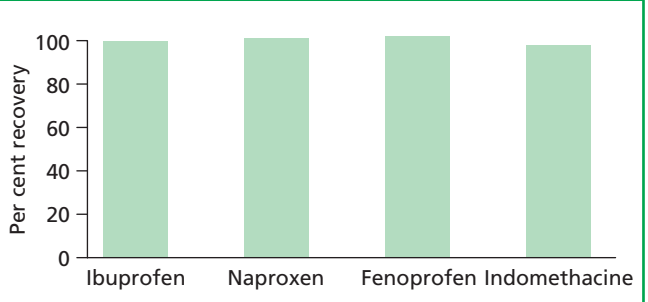


Figure 4: Average recoveries for tricyclic antidepressants using the Strata X method. HPLC conditions: mobile phase consisted of three components: A = KH_2PO_4 (pH = 7), B = acetonitrile and C = methanol. The flow-rate was 2.0 mL/min. The gradient program began with 40:30:30 mixture (A:B:C) for 10 min. After 10 min the gradient was changed to 10:45:45 for the remainder of the run time.

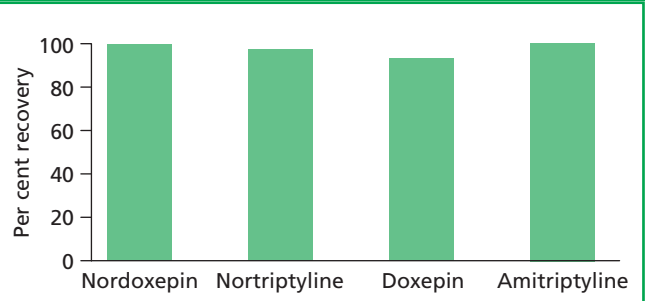
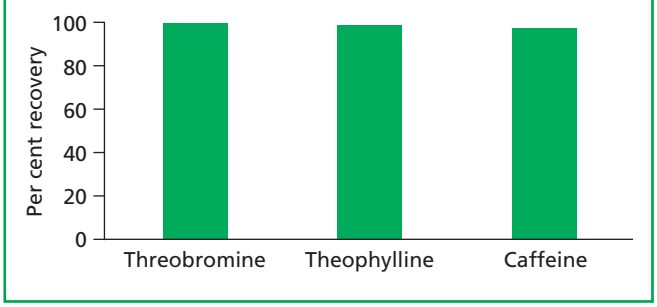


Figure 5: Average recoveries for neutral bronchodilator compounds using the Strata X method. HPLC conditions: isocratic mobile phase consisting of 20 mM KH_2PO_4 (pH = 2.5) and acetronitrile (95:5). The flow-rate was 1.5 mL/min.



Results

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

Basic TCA compounds: Strata X was used to extract four tricyclic antidepressant drugs from porcine serum. As shown in Figure 4, the average recovery for each TCA probe was greater than 90% (RSD <2% for Nordoxepin, Amitriptyline and Nortriptyline and <5% for Doxepin).

Neutral bronchodilator compounds: Three bronchodilator compounds showed high recoveries with the Strata™ X method. As shown in Figure 5, the average recovery for each neutral probe was greater than 95%. These compounds showed the best reproducibility with a RSD <1.5%.

This extraction method delivers recoveries greater than 90% for all these compounds from a serum matrix.

Conclusions

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

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