High Recoveries and Aggressive Clean-up of Sulfa Drugs from Plasma using a Polymeric Ion Exchange Sorbent - strata™ X-C

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The discovery of sulfa drugs during the late 19th century laid the foundation for the concept of chemotherapy and led to unprecedented therapeutic triumphs during the 20th century. Paul Ehrlich, who was awarded the Nobel Prize in 1909 for this discovery, demonstrated that the growth of bacteria, responsible for infections in humans and animals, could be stopped by interference of these sulfa drugs with the synthesis of folic acid from 4-aminobenzoic acid. Besides a host of sulfa drugs which originated from the parent sulfanilamide structure, a number of modern sulfur-containing medicines evolved from the discovery that this same parent molecule inhibits carboanhydrase activity.[1] Examples of these drugs include the diuretics furosemide and hydrochlorothiazide and sulfonylureas such as tolbutamide which is used in the treatment of type II diabetes mellitus.

With the advent of the beta lactam antibiotics, the prescription of sulfa drugs (excluding the diuretics and antidiabetics) for treatment of human infections has considerably decreased. However, these drugs continue to be extensively used in the veterinary area. A large volume of literature has been documented on the analysis/determination of sulfa drugs from different sources, as for example, from bovine milk[2], waste waters[3], tissue samples of animals[4], food products of animal origin[5], residues in eggs[6] and biological fluids.[7] Instrumental techniques employed for these analyses include HPLC, GC or CE either alone or in tandem with mass spectrometry. Sample preparation methods predominantly consist of solid phase extraction (SPE), solvent extraction or matrix solid phase dispersion. Some reports also utilize on-line concentration/purification/analysis using the LC/MS technique for sulfa drugs from biological fluids or animal sources.[8] Most of the SPE methods for sulfa drugs utilized C18, although a few used polymeric sorbents as well. To the best of our knowledge, no SPE protocol for sulfa drugs with a cation exchange polymeric sorbent has been reported so far. In this bulletin, we demonstrate the effective extraction of sulfa drugs from plasma using strata-X-C.

strata-X-C is a revolutionary, patent pending polymeric resin that has been functionalized with polar and strong cation exchange groups. As a result, strata-X-C exhibits numerous retention mechanisms including hydrophobic, dipole-dipole, π-π and strong cation exchange. These mechanisms allow for the effective extraction of the sulfa drugs along with an aggressive organic wash to remove matrix interferences.

Instrument and Equipment

Solid phase extraction: A 1mL syringe-barrel tube containing 30mg of strata-X-C was used in this sample preparation method. ***Table 1*** lists the chemical and physical properties of this new polymeric ion exchange resin. The samples were processed using a 12-position SPE vacuum manifold, supplied by Phenomenex.

Liquid Chromatography: All analysis were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector and autosampler. HP Chemstation software was used to analyze the data. The HPLC column was a Synergi Max-RP, 4µm, 150 x 4.6mm from Phenomenex.

Table 1. Characteristics of the strata-X-C resin

Phase:	Surface modified styrene-divinylbenzene
Average particle diameter:	33µm
Nominal pore size:	85Å
Surface area:	800m²/g
Ionic capacity:	1meq/g
pH stability:	1-14

Experimental Conditions

Specimen preparation: The sulfa drug mixture (0.5µg/mL) was spiked into porcine plasma (diluted 1:1 with water containing 2% phosphoric acid). Sulfanilamide was used as the internal standard.

SPE method:

Condition:	1mL methanol
Equilibrate:	1mL deionized water
Load:	plasma sample containing sulfa drug probes
Wash #1:	1mL of 0.1N HCl
Wash #2:	1mL of methanol
Elution:	5% ammonia in methanol

Analysis: The eluate was evaporated to dryness and reconstituted in 200µL of 20mM potassium dihydrogen phosphate (pH 2.5).

Results and Discussion

The recoveries of these drugs using the strata-X-C method are shown in ***Table 2***. The strong retention capability of strata-X-C for these sulfa drugs arises from the ionic interactions of the sulfonic acid groups on strata-X-C with the primary amino function on these drug molecules. The results are high recoveries (> 88%) from plasma with excellent reproducibility.

Table 2: Recovery of sulfa drugs from porcine plasma using strata-X-C

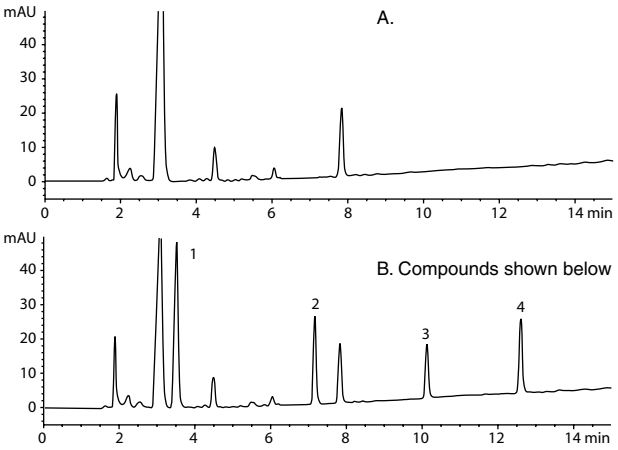
	strata-X-C	% RSD
Sulfathiazole	99	1.09
Sulfamethoxazole	89	1.42
Sulfaquinoxaline	88	1.23

The sulfa drugs studied are extremely polar molecules (see log P values) that are not adequately retained on any silica-based reversed phase sorbent. When C18 or cyclohexyl is used for the clean-up of sulfa drugs, either water alone is used as the washing agent or no washing is done at all.[2,9] This rinse step does not adequately remove all the matrix interferences. Adding a small amount of organic to the aqueous wash solvent (i.e., 5% methanol) can prematurely elute the analytes, resulting in low

recoveries. The strong interaction between the strata-X-C sorbent and sulfa drugs overcomes the problem of insufficient clean-up of contaminants. In fact, the sulfa drugs used in the current study are well retained on strata-X-C, even when subjected to 100% organic wash.

The cleanliness of the extracts from strata-X-C is presented in ***Figure 1*** and demonstrates the efficiency of this sorbent in removing interferences from porcine plasma samples. An aggressive wash series of the strata-X-C sorbent includes an acid rinse followed by 100% organic solvent, enabling complete removal of all impurities and interferences. The result is a clean extract that will not show any ion suppression under LC/MS/MS conditions.[10]

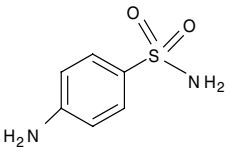
Figure 1. Chromatogram of plasma extracts from strata-X-C:
A) blank; B) spiked sample



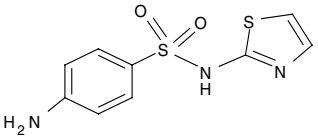
HPLC conditions: A Synergi Max-RP (150 x 4.6mm) column at a flow rate of 1mL/min and detector wavelength of 254nm, with 20µL injection volume. A gradient from 5% acetonitrile and 95% potassium dihydrogen phosphate (20mM) at 0 min to 80% acetonitrile in 15 min was employed.

Sample:

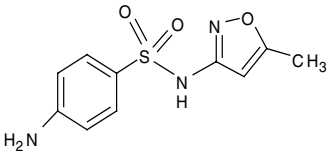
1. Sulfanilamide (log P = -0.62, pKa = 2.40, 10.40)



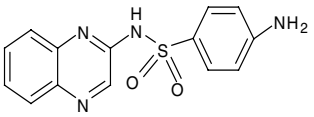
2. Sulfathiazole (log P = 0.09, pKa = 2.08, 7.07)



3. Sulfamethoxazole (log P= 1.58, pKa = 1.83, 5.65)



4. Sulfaquinoxaline (log P = 1.68, pKa = 1.86, 5.56)



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Acknowledgments

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Ordering Information

Order No.	Description
8B-S029-TAK-TN	strata-X-C 30mg/1mL Tubes (100/Box)
8B-S029-UBJ-TN	strata-X-C 60mg/3mL Tubes (50/Box)
8B-S029-FBJ-TN	strata-X-C 200mg/3mL Tubes (50/Box)
8B-S029-HBJ-TN	strata-X-C 500mg/3mL Tubes (50/Box)
8B-S029-ECH-TN	strata-X-C 100mg/6mL Tubes (30/Box)
8B-S029-FCH-TN	strata-X-C 200mg/6mL Tubes (30/Box)
8B-S029-HCH-TN	strata-X-C 500mg/6mL Tubes (30/Box)
8B-S029-EDG-TN	strata-X-C 100mg/12mL GigaTubes (20/Box)
8B-S029-HDG-TN	strata-X-C 500mg/12mL Giga Tubes (20/Box)
8B-S029-JDG-TN	strata-X-C 1000mg/12mL GigaTubes (20/Box)
8E-S029-AGB-TN	strata-X-C 96-Well Plate 10mg/well (2/Box)
8E-S029-TGB-TN	strata-X-C 96-Well Plate 30mg/well (2/Box)
00F-4337-E0-TN	Synergi Max-RP, 4µm, 150 x 4.6mm
AH0-6023-TN	12-Position SPE Vacuum Manifold

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