Improved Recoveries of Polar Acidic Compounds on Strata™-X-A versus a Weak Anion Exchange Sorbent

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The cleanup of weak acids from complex matrices is often hindered by weak SPE sorbent-analyte interactions, resulting in low recoveries. This technical note describes a new polymeric strong anion exchange sorbent that dramatically improves the recoveries and cleanup of weak acids as compared to a weak anion exchange SPE sorbent.

Introduction

The study of weakly acidic compounds, such as those containing carboxylic acids, is often a challenge due to the limited number of SPE sorbents that are ideal for these analyses. Traditional silica-based strong anion exchange sorbents are often used despite their limited pH stability of 1 to 8 which minimizes the workable pH. A second popular material used for the cleanup of weak acids is a polymer-based weak anion exchange sorbent. This sorbent provides a wide workable pH range of 1 to 14, but the analyte-sorbent interaction is not as strong as a strong anion exchange sorbent, which can inhibit the amount of organic tolerable in the wash, subsequently compromising the cleanliness and recoveries of weak acids of interest in the eluent. The objective of this work was to develop a product that provided the retention of a strong anion exchange SPE sorbent on a polymer base to provide high recoveries without the pH limitations of traditional silica-based material.

Experimental Conditions

500 µL of human plasma (Na_2EDTA) was spiked at 5 µg/mL with indole-3-carboxylic acid (pK_a~4). The spiked sample was diluted 1:1 with 500 µL of water that had been acidified with 2 % phosphoric acid. The diluted plasma samples were then simultaneously extracted using two different 30 mg/3 mL SPE tubes: a polymeric weak anion exchange sorbent and a polymeric strong anion exchange sorbent (Strata-X-A, Phenomenex, Torrance, CA, USA). The two SPE sorbents were evaluated by following the methods specified in **Table 1**. Extraction methods were chosen based on optimal conditions for each sorbent. Because Strata-X-A contains a quaternary amine ligand, it was necessary to perform an acidic elution which neutralized the analytes of interest in order to release them from the SPE sorbent. The polymeric weak anion exchange sorbent contains a weaker di-amino ligand and therefore necessitated a basic elution in order to ensure that the sorbent was completely neutralized. This neutralization, in turn, released acidic analytes that were retained in the sorbent.

The extract was then analyzed for recovery and cleanup by LC/UV.

Table 1.

Strata-X-A Polymeric Strong Anion Exchange SPE Sorbent (30 mg/3 mL, Part Number 8B-S123-TBJ)	Polymeric Weak Anion Exchange Sorbent (30 mg/3 mL)
1. Condition - 1 mL of Methanol - 1 mL Water	**1. Condition** - 1 mL of Methanol - 1 mL Water
2. Load - 500 µL human plasma (Na_2EDTA) spiked with Indole-3-carboxylic acid at 5 µg/mL, diluted with 500 µL Water acidified with 2 % Phosphoric acid	**2. Load** - 500 µL human plasma (Na_2EDTA) spiked with Indole-3-carboxylic acid at 5 µg/mL, diluted with 500 µL Water acidified with 2 % Phosphoric acid
3. Wash - 1 mL 50 mM Sodium acetate, pH 7.0 - 1 mL Methanol	**3. Wash** - 1 mL 50 mM Sodium acetate, pH 7.0 - 1 mL Methanol
4. Dry - 2 minutes at 10 inches of Mercury	**4. Dry** - 2 minutes at 10 inches of Mercury
5. Elute - 2x 500 µL of 5 % Formic acid in Methanol - Evaporate to dryness at 45 °C	**5. Elute** - 2x 500 µL of 5 % Ammonium hydroxide in Methanol - Evaporate to dryness at 45 °C
6. Reconstitute - Reconstitute with 500 µL initial mobile phase spiked with 50 µg/mL Salicylic acid (IS)	**6. Reconstitute** - Reconstitute with 500 µL initial mobile phase spiked with 50 µg/mL Salicylic acid (IS)

Results and Discussion

From the LC/UV analysis, it is clear that the method utilizing the polymeric strong anion exchange sorbent yielded higher recoveries and cleaner extracts than that with the polymeric weak anion exchange sorbent. This is most likely due to the di-methyl butyl quaternary amine ligand that acts as the functional group on the Strata™-X-A strong anion exchange sorbent. This ligand was able to tightly retain indole-3-carboxylic acid which allowed for a 100 % organic wash to remove more matrix interferences while the weak anion exchange sorbent resulted in analyte loss under the same harsh wash conditions (**Figures 1 and 2**).

Figure 1.
LC/UV Analysis of Spiked Samples Extracted on Strata-X-A

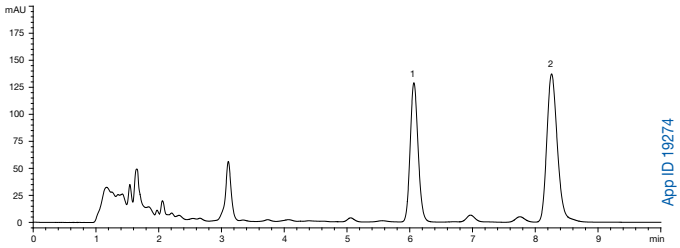
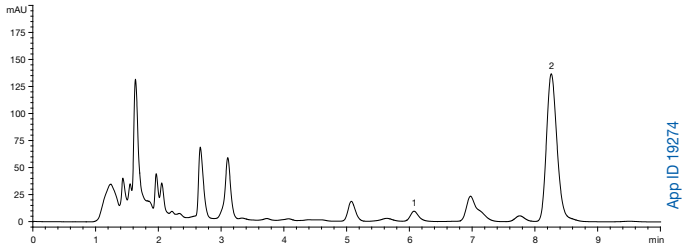


Figure 2.
LC/UV Analysis of Spiked Samples Extracted on a Polymeric Weak Anion Exchange Sorbent



Conditions for both columns:

Column: Gemini® 5 µm C18
Dimensions: 150 x 4.6 mm
Part No.: 00F-4435-E0
Mobile Phase: 20 mm Monobasic potassium phosphate, pH 2.5 / Acetonitrile (75:25)
Flow Rate: 1.0 mL/min
Temperature: Ambient
Detection: UV @ 220 nm
Sample: 1. Indole-3-carboxylic acid
2. Salicylic acid (IS)

It was also determined that the strong retention of weak acids by Strata-X-A resulted in more consistent results, with a 3.1 % CV as compared to the weak anion exchange sorbent which produced a 24.5 % CV (**Figures 3 and 4**).

Figure 3.
% Recovery of Indole-3-Carboxylic Acid

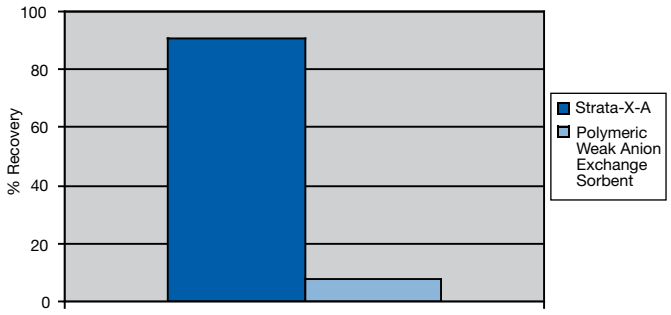


Figure 4.
% Recovery and % CV of Indole-3-Carboxylic Acid

	Spiked Concentration (µg/mL)	Strata-X-A		Polymeric Weak Anion Exchange Sorbent	
		% Recovery	% CV	% Recovery	% CV
(1) Indole-3-Carboxylic Acid	5.0	91 %	3.1	8.0 %	24.5
(2) Salicylic Acid	50	(IS)		(IS)	

Conclusion

When extracting weak acids (especially those containing carboxylic acids) from complex matrices, it is important to choose an SPE sorbent that provides a strong analyte-sorbent interaction such as Strata-X-A in order to achieve the highest recoveries, cleanest extracts, and most consistent results.

APPLICATIONS

Ordering Information

Strata™-X-A

Sorbent Mass	Part No.	Unit
Tube		
30 mg	8B-S123-TAK	1 mL (100/box)
30 mg	8B-S123-TBJ	3 mL (50/box)
60 mg	8B-S123-UBJ	3 mL (50/box)
100 mg	8B-S123-EBJ	3 mL (50/box)
100 mg	8B-S123-ECH	6 mL (30/box)
200 mg	8B-S123-FBJ	3 mL (50/box)
200 mg	8B-S123-FCH	6 mL (30/box)
500 mg	8B-S123-HBJ	3 mL (50/box)
500 mg	8B-S123-HCH	6 mL (30/box)
Giga™ Tube		
500 mg	8B-S123-HDG	12 mL (20/box)
1 g	8B-S123-JDG	12 mL (20/box)
1 g	8B-S123-JEG	20 mL (20/box)
2 g	8B-S123-KEG	20 mL (20/box)
5 g	8B-S123-LFF	60 mL (16/box)
96-Well Plate		
10 mg	8E-S123-AGB	2 Plates/Box
30 mg	8E-S123-TGB	2 Plates/Box
60 mg	8E-S123-UGB	2 Plates/Box

Gemini®

Part No.	Description	Dimensions (mm)	Unit
00F-4435-E0	Gemini 5 µm C18	150 x 4.6	ea

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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