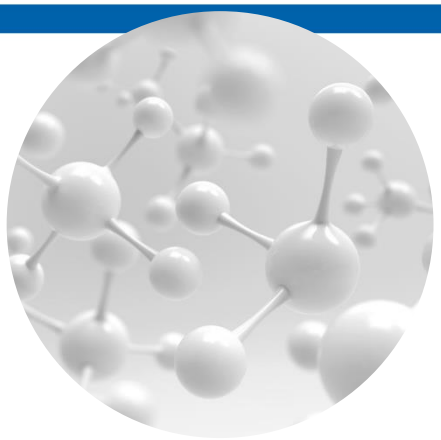

TN-0152

Solid Phase Extraction for Clean-up of Polar Molecules in Salty Aqueous Samples and Analysis with SFC-UV

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Introduction

Non-target screening (NTS) is a comprehensive analytical strategy which offers analysis of a wide range of complex samples containing organic molecules. Although NTS can detect a wide range of different organic compounds, the sample composition can be a challenge for the analysis. In that instance, RPLC-HILIC and SFC (polarity-extended chromatographic techniques) are often used for the separation of samples with non-polar, polar and/or very polar compounds.[1,2,3] The analysis of highly polar compounds is extremely difficult in samples high in inorganic salts due to negative effects in chromatography and mass spectrometric detection. Thus, sample preparation techniques are needed to remove salts from the solution without loss of polar organic molecules.

As part of the research project RIKovery (supported by the German Federal Ministry of Education and Research), samples with Sodium Chloride content up to 25 % were analyzed by NTS. Some of the samples are expected to contain phenolic compounds and will be treated with advanced oxidation processes. Therefore, the presence of polar and very polar molecules in the samples is very likely. The contained salts are not suitable for direct chromatographic separation and mass spectrometric analysis. Thus, a pre-treatment step of the sample is required. It should be capable of removing inorganic salts like Sodium Chloride but also show high recovery rates for polar and very polar organic compounds.

In this technical note, the use of solid phase extraction (SPE) for the removal of Sodium Chloride from aqueous samples was investigated. Four different SPE phases were tested, and SFC-UV was applied for polar molecule analysis.

Sample Preparation

	200 mg / 3 mL	500 mg / 6 mL
Sample Pre-treatment:	Sample solutions containing 0, 1, 5, 10, 15, 20, and 25 % Sodium Chloride were spiked with 100 mg/L each of the seven highly polar compounds described in **Table 1**	
Condition:	**Strata™ C18-E** (8B-S001-FBJ), **Strata CN** (8B-S007-FBJ), **Strata-X 33 µm** (8B-S100-FBJ), and **Strata-X PRO** (8B-S536-FBJ) 3 mL tubes with 1 wash of 3 mL Methanol	**Strata C18-E** (8B-S001-HCH), **Strata CN** (8B-S007-HCH), **Strata-X 33 µm** (8B-S100-HCH), and **Strata-X PRO** (8B-S536-HCH) 6 mL tubes with 1 wash of 6 mL Methanol
Equilibrate:	Tubes with 1 wash of 3 mL Water	Tubes with 1 wash of 6 mL Water
Load:	3 mL sample	6 mL sample
Wash:	Tubes with 3 washes of 3 mL Water	Tubes with 3 washes of 6 mL Water
Dry:	Tubes on -10 to -20 kPa vacuum for 2.5 min	Tubes on -10 to -20 kPa vacuum for 5 min
Elute:	With 2 washes of 1.5 mL Methanol / Water (80:20, v/v)	With 2 washes of 3.0 mL Methanol / Water (80:20, v/v)

Colorimetric Chloride Test

The Sodium Chloride content in SPE treated samples was determined using a colorimetric test based on Mercury (II) Thiocyanate and its reaction with Chloride. In the presence of Chloride, Iron (III) Thiocyanate is formed and results in a red color. The chloride concentration is determined semi-quantitatively through visual comparison of the color of the reaction solution with a reference. The influence of Methanol on the reaction was evaluated measuring different Sodium Chloride concentrations in Methanol / Water (80:20, v/v). For the determination of Sodium Chloride concentrations in SPE eluates, the solutions were compared visually with a 0.1 g/L Sodium Chloride solution in Methanol / Water (80:20, v/v).

SFC Conditions

Column: 3 µm HILIC
Dimensions: 150 x 3.0 mm
Mobile Phase: A: CO_2
B: Methanol + 20 mM Ammonium Acetate

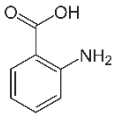
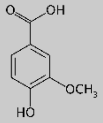
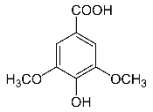
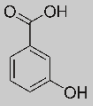
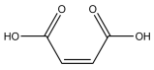
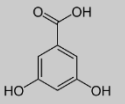
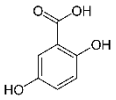
Gradient:

Time (min)	%B
0	5
1	5
7	60
11	60
11.1	5
12	5
13	5

Flow Rate: 2.0 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detection: UV @ 210 nm

Revision: 2
PHEN-RUO-00173

Table 1. Applied Compounds with Study Relevant Information.

Analytes	Retention Time (min)	logD (pH 7)	InChiKey[1]	Structure
2-Aminobenzoic Acid (2-AB)	4.5	-0.8	RWZYAGGXGHYGMB-UHFFFAOYSA-N	
Vanillic Acid (VAN)	4.7	-1.6	WKOLLVMJNQIZCI-UHFFFAOYSA-N	
Syringic Acid (SYR)	4.9	-1.9	JMSVCTWVEWCHDZ-UHFFFAOYSA-N	
3-Hydroxybenzoic Acid (3- HB)	5.7	-1.7	IJFXRHURBJZNAO-UHFFFAOYSA-N	
Maleic Acid (Mal)	6.5	-4.8	VZCYOOQTPOCHFL-UPHRSURJSA-N	
3,5-Dihydroxybenzoic Acid (3,5-DHB)	7.9	-2.1	UYEMGAFJOZZIFP-UHFFFAOYSA-N	
2,5-Dihydroxybenzoic Acid (2,5-DHB)	9.0	-1.8	WXTMDXOMEHJXQO-UHFFFAOYSA-N	

[1]https://water.for-ident.org and https://pubchem.ncbi.nlm.nih.gov/

Results and Discussion

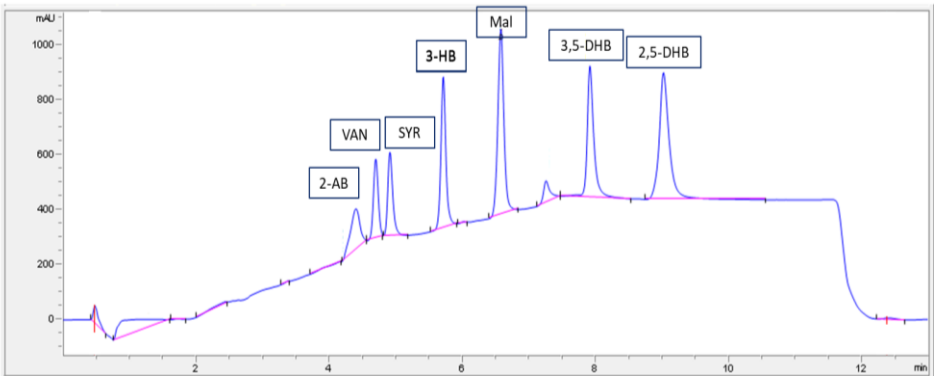
The seven compounds (with logD values in the range from -0.8 to -4.8; details see **Table 1**) were separated well with high k values and baseline separation (**Figure 1**) using SFC. Consequently, RTs were observed with relative standard deviations in the calibration measurements below 0.7 % over the total study period. The compounds in real samples (following the desalting step) behaved the same. Further, the compounds could be monitored with robust UV detection at 210 nm wavelength. The recovery studies for the applied compounds were performed in a calibration range. The relative standard deviations of the signal area were 1 % (Mal and 3,5-DHB), 3 % (2-AB and 2,5-DHB), 6 % (3-HB), or 9 % (VAN and SYR) in the lowest concentration, and below 4 % at the higher concentrations. The linearity of the six UV values of each compound calibration was given with R^2 values better then 0.999 (only 2-AB with 0.998). Thus, the analysis and calibration were robust and reproducible and further used for the recovery study.

Next, desalting was done using SPE on the reference standards and various concentrations of Sodium Chloride. The resulting eluates were analyzed with the validated quantitation method for recovery rates if the salt content was below 0.1 g/L. This was determined by comparing the results of the colorimetric assay of the eluates with a 0.1 g/L solution (**Figure 2**). The desalting procedure was applied to all four SPE phases. All could reduce the salt content below 0.1 g/L (**Table 2**). The Strata™ CN SPE phase, in both tube sizes, could not desalt the 25 % Sodium Chloride containing solution below the effectively needed value. Thus, this solution was not considered in the study further on.

The recoveries of studied analytes in desalted eluates were determined by SFC-UV analyses as described above. **Figures 3** and **4** represent the detailed recoveries (with standard deviation by triplicates) of all organic molecules in several salty solutions in both SPE tube sizes. Strata-X 33 µm and Strata-X PRO had excellent recovery rates among investigated phases, whereas Strata CN could not retain any organic compound and Strata C18-E only significantly reduced amounts. Both Strata-X materials reached recoveries between 60 % and 120 % for all phenolic compounds and 50 % for maleic acid.

Overall, Strata-X provides a significantly better recovery than Strata-X PRO in the 200 mg / 3 mL tube size. For the phenolic compounds, the observed recoveries after using Strata-X in the 200 mg / 3 mL tube size were closer to 100 %, whereas Strata-X PRO in the 200 mg / 3 mL tube size and both phases in the 500 mg / 6 mL tube size resulted significant overdeterminations.

Figure 1. Chromatogram of Seven Compounds Observed by SFC-UV Analysis.



Analytes	Retention Time (min)
2-aminobenzoic acid (2-AB)	4.40
vanillic acid (VAN)	4.69
syringic acid (SYR)	4.91
3-hydroxybenzoic acid (3-HB)	5.71
maleic acid (Mal)	6.57
3,5-dihydroxybenzoic acid (3,5-DHB)	7.91
2,5-dihydroxybenzoic acid (2,5-DHB)	9.01

Figure 2. Results of the Colorimetric Assay on the Eluates of Different Concentrated Sodium Chloride Solutions on the Strata™ Cyano (CN) SPE Phase Compared to a 0.1 g/L Solution (Right Side).

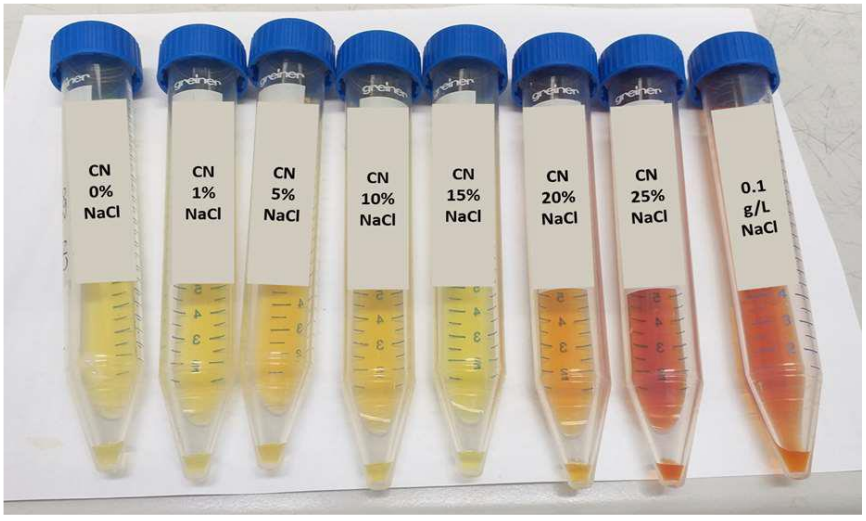


Table 2. Sodium Contents of the SPE Eluates for Both Tube Dimensions After the Cleaning Procedure.

	0%	1%	5%	10%	15%	20%	25%
Strata C18-E, 200 mg / 3 mL	-	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1
Strata CN, 200 mg / 3 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	>0.1
Strata-X, 200 mg / 3 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Strata-X PRO, 200 mg / 3 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Strata C18-E, 500 mg / 6 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Strata CN, 500 mg / 6 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	>0.1
Strata-X, 500 mg / 6 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Strata-X PRO, 500 mg / 6 mL	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Figure 3 . Recovery Rate of Studied Compounds in the Eluates for Both Tube Dimensions.

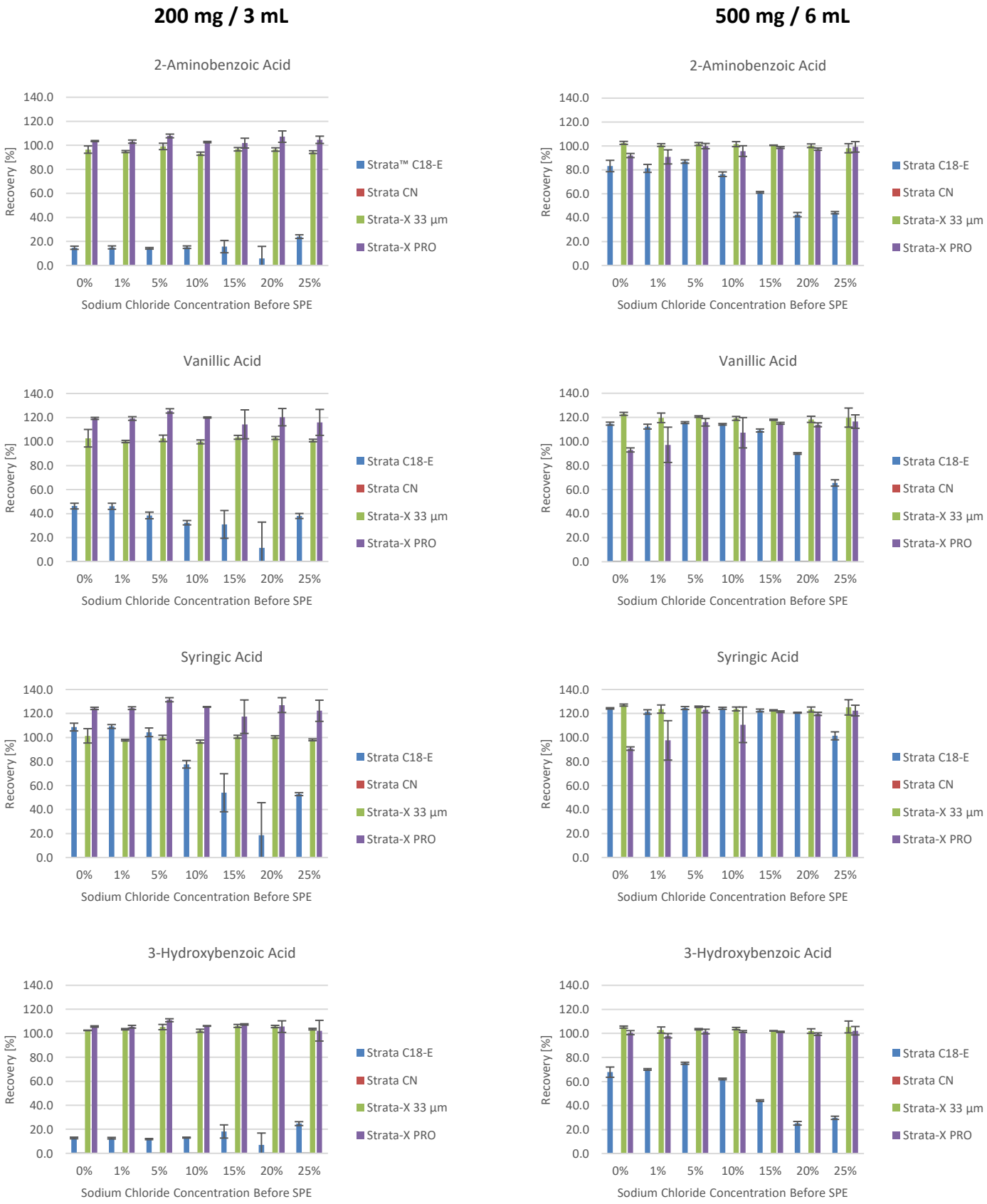
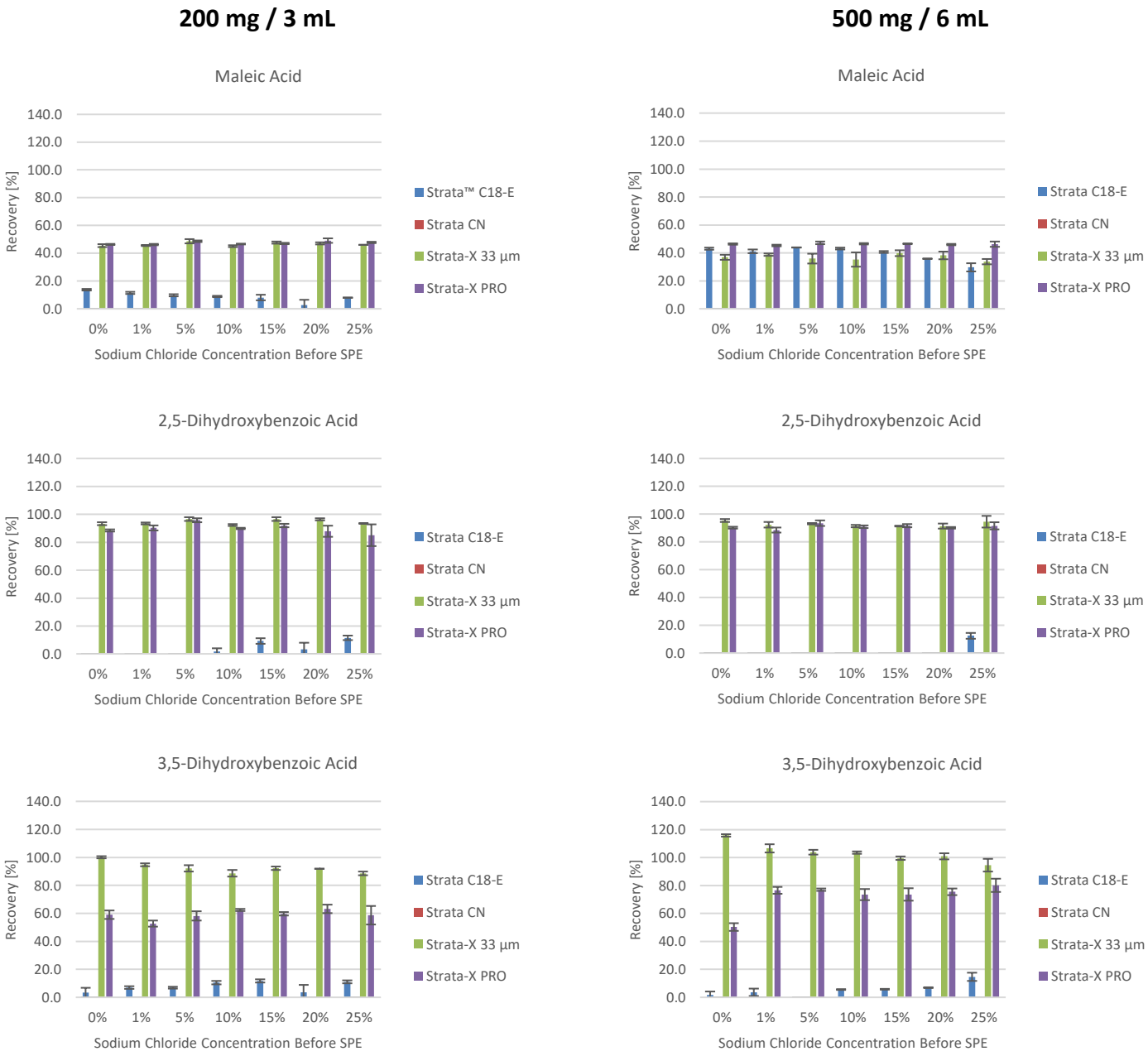


Figure 4. Recovery Rate of Studied Compounds in the Eluates for Both Tube Dimensions.



Conclusions

Solid Phase Extraction with Strata™-X 33 µm and Strata-X PRO materials has shown to be a very suitable method for the desalting of aqueous samples. Overall, the salt content does not influence the recovery rate of polar and very polar compounds for these phases. Strata-X 33 µm in the 200 mg / 3 mL tube size was the optimal SPE phase to extract small, slightly acidic and polar organic compounds out of salty solutions.

References

1. S. Bieber, G. Greco, S. Grosse, and T. Letzel: RPLC-HILIC and SFC with mass spectrometry: Polarityextended organic molecule screening in environmental (water) samples. Analytical Chemistry 2017, 89 (15), 7907-7914.
2. S. Bieber and T. Letzel: White Paper - Polarity-Extended Chromatography: A holistic solution analyzing organic molecules in the aqueous environment? AFIN-TS Forum 2020, February (1): 1-4.
3. S. Bieber and T. Letzel: Achiral SFC Separations - Gold Standard for the Next Generation of Non-Target Screening. Analytical Science Advances, 2021, 2: 43–46

Ordering Information

Strata™ Cyano (CN)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S007-EAK	1 mL (100/box)
	200 mg	8B-S007-FBJ	3 mL (50/box)
	500 mg	8B-S007-HBJ	3 mL (50/box)
	500 mg	8B-S007-HCH	6 mL (30/box)
	1 g	8B-S007-JCH	6 mL (30/box)
Giga™ Tube			
	2 g	8B-S007-KDG	12 mL (20/box)
96-Well Plate			
	50 mg	8E-S007-DGB	2 Plates/Box

Strata-X

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK**	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ**	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/box)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)
Giga Tube			
	500 mg	8B-S100-HDG	12 mL (20/box)
	1 g	8B-S100-JDG	12 mL (20/box)
	1 g	8B-S100-JEG	20 mL (20/box)
	2 g	8B-S100-KEG	20 mL (20/box)
	5 g	8B-S100-LFF	60 mL (16/box)
Teflon® Tube			
	200 mg	8B-S100-FBJ-T	3 mL (50/box)
	200 mg	8B-S100-FDG-T	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S100-AGB	2 Plates/Box
	30 mg	8E-S100-TGB	2 Plates/Box
	60 mg	8E-S100-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S100-4GA	ea

Strata C18-E

Format	Sorbent Mass	Part Number	Unit
Tube			
	50 mg	8B-S001-DAK	1 mL (100/box)
	100 mg	8B-S001-EAK**	1 mL (100/box)
	100 mg	8B-S001-EBJ	3 mL (50/box)
	200 mg	8B-S001-FBJ**	3 mL (50/box)
	200 mg	8B-S001-FCH	6 mL (30/box)
	500 mg	8B-S001-HBJ	3 mL (50/box)
	500 mg	8B-S001-HCH	6 mL (30/box)
	1 g	8B-S001-JEG	20 mL (20/box)
Giga Tube			
	500 mg	8B-S001-HDG	12 mL (20/box)
	2 g	8B-S001-KDG	12 mL (20/box)
	5 g	8B-S001-LEG	20 mL (20/box)
	10 g	8B-S001-MFF	60 mL (16/box)
	20 g	8B-S001-VFF	60 mL (16/box)
	50 g	8B-S001-YSN	150 mL (8/box)
	70 g	8B-S001-ZSN	150 mL (8/box)
96-Well Plate			
	25 mg	8E-S001-CGB	2 Plates/Box
	50 mg	8E-S001-DGB	2 Plates/Box
	100 mg	8E-S001-EGB	2 Plates/Box

Strata-X Pro

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK**	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ**	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/box)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
96-Well Plate			
	10 mg	8E-S536-AGA	ea
	30 mg	8E-S536-TGA	ea
	60 mg	8E-S536-UGA	ea
96-Well Microelution Plate			
	2 mg/well	8M-S536-4GA	ea

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