

# Using Bonded Silica Solid Phase Microextraction Fibers as a Screening Tool for Pharmaceuticals and Personal Care Products in Drinking Water



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# Abstract

EPA Method 1694 is used in the determination of pharmaceuticals and personal care products (PPCPs) in environmental samples. This method uses solid phase extraction (SPE) and high performance liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) to quantitate PPCPs in a variety of environmental matrices. The sample extraction process is tedious with typical processing times of up to 90 minutes per sample.

SPME (Solid Phase Microextraction) fibers made from functionalized silica particles bound to a metal core were used to extract PPCPs directly from a small volume of water. The analytes were then desorbed from the fibers and the resulting extracts were evaporated and reconstituted with mobile phase. The SPME procedure required about half of the sample preparation time of the SPE procedure described in Method 1694.

This study compares SPE to the utility of SPME as a fast and efficient method of sample screening. Analyte recovery along with detection limits are discussed.

# Introduction

## SPE vs. SPME

The focus of this study was the Group 1 compounds listed in Method 1694. Using the procedure described in the method, 500 mL samples were processed by SPE. The extraction required the use of single-use extraction cartridges and pumps and a vacuum manifold.

The SPME process used a much smaller sample volume (1 mL) than SPE. Some concentration is achieved during the sample evaporation/reconstitution step, but it was not equivalent to that of SPE. For these reasons, it was expected that detection limits achievable by SPME would be higher than SPE.

# Experimental

## LC-MS-MS Conditions

<b>Instrument</b>	Applied Biosystems 3200QT			
<b>Column</b>	Ascentis <sup>®</sup> Express C18, 10 cm x 2.1 mm, I.D., 2.7 $\mu$ m			
<b>Mobile Phase A</b>	0.1% formic acid and 0.1% ammonium formate in water			
<b>Mobile Phase B</b>	50:50, methanol:acetonitrile			
<b>Gradient and Flow</b>	Time	Flow $\mu$ L/min.	%A	%B
	0.00	150	95.0	5.0
	4.00	250	95.0	5.0
	22.50	300	12.0	88.0
	23.00	300	0.0	100.0
	26.00	300	0.0	100.0
	26.50	150	95.0	5.0
	33.00	150	95.0	5.0
	43.00	150	95.0	5.0
<b>Temperature</b>	40 °C			
<b>Injection Volume</b>	5.0 $\mu$ L			
<b>Source Conditions</b>	Turbo ion spray ESI +, MRM			
<b>MS-MS Transitions</b>	See Table 1			
<b>Dwell time</b>	50 msec			

## Experimental (contd.)

### SPE Extraction Method

<b>Extraction Cartridge</b>	Supel™ Select HLB SPE Tube, 500 mg/6 mL
<b>Sample</b>	500 mL of drinking water spiked with Group 1 compounds, adjusted to pH=4 with 6M HCl
<b>Tube Conditioning</b>	20 mL of methanol, 6 mL of water, 6 mL of water at pH 2 (with 6 M HCl)
<b>Sample Extraction</b>	10 mL/min through tube
<b>Dry Time</b>	5 min.
<b>Elution</b>	12 mL, 50:50 methanol:acetonitrile
<b>Dry Down</b>	40 °C, under nitrogen stream
<b>Reconstitution</b>	To final volume of 2 mL using mobile phase

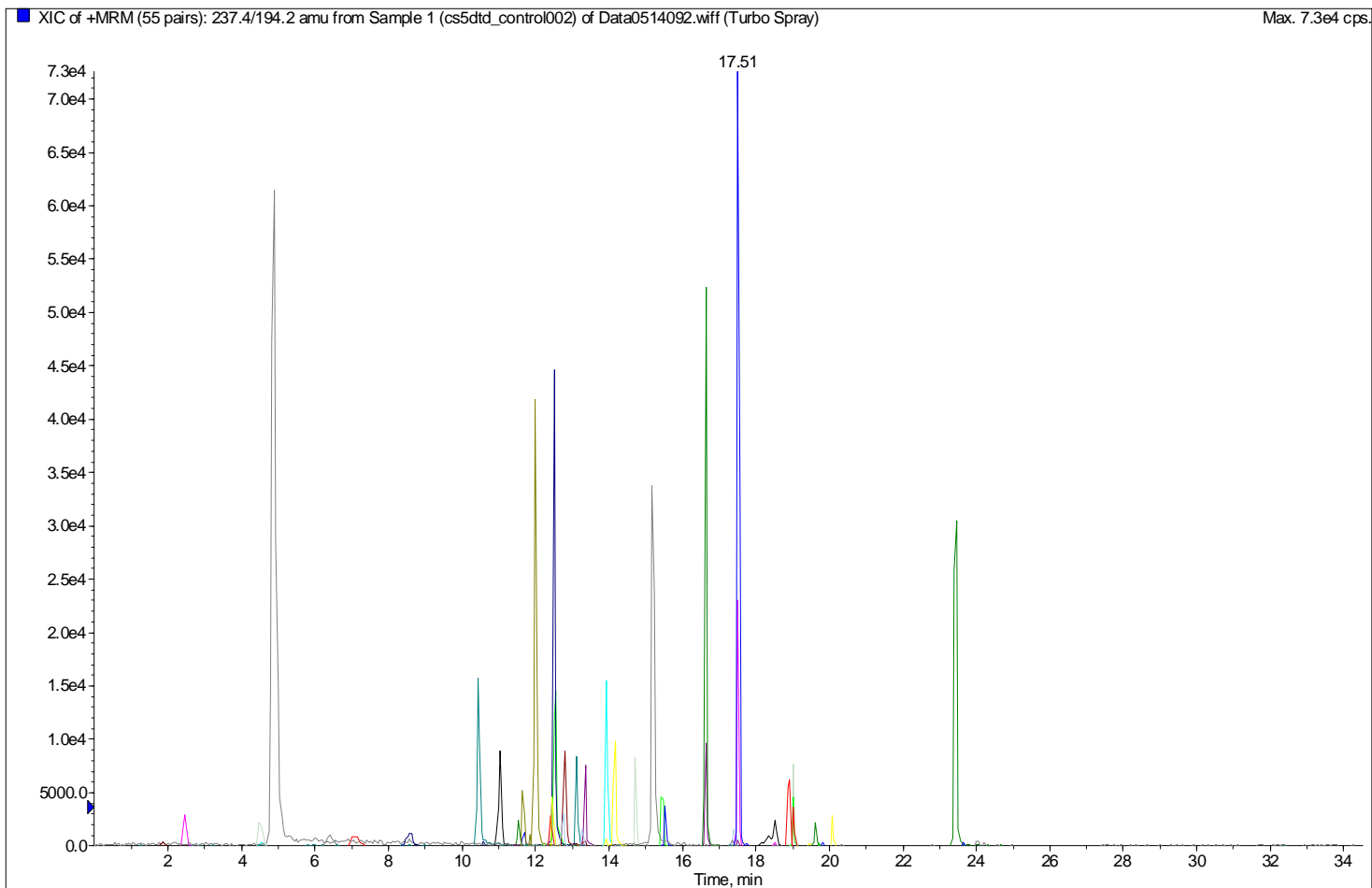
# Experimental (contd.)

## SPME Extraction Method

<b>Fiber Type</b>	Functionally Bonded C18
<b>Samples</b>	1. Potassium phosphate buffer, 20 mM, pH=7 2. Drinking water, buffered with potassium phosphate to pH=7
<b>Sample Size</b>	1 mL
<b>Fiber Conditioning</b>	15 min. in methanol
<b>Fiber Equilibration</b>	15 min. in water
<b>Extraction</b>	60 min., with rotation
<b>Desorption Solvent</b>	90:10, methanol: 20 mM NH <sub>4</sub> OAc , pH=4
<b>Desorption Process</b>	200 µL solvent, 30 min., with rotation
<b>Dry Down</b>	40 °C, 20 min., under nitrogen stream
<b>Reconstitution</b>	75 µL 95:5 (A): 0.1% formic acid and 0.1% ammonium formate in water; (B): 50:50 methanol:acetonitrile (HPLC mobile phase)

# Results

## Figure 1. LC-MS-MS Chromatogram of Group 1 Compounds




# Table 1. Recovery of Group 1 Compounds from Spiked Drinking Water

<b>Compound</b>	<b>MRM Transition</b>	<b>SPE Spiking Level (µg/L)</b>	<b>% Recovery</b>	<b>SPME Spiking Level (µg/L)</b>	<b>% Recovery</b>
Acetaminophen	152.2-126.2	40	199%	200	87%
Ampicillin	350.3-106.1	1	ND	50	56%
Azithromycin	749.9-591.6	1	95%	50	88%
Caffeine	195.0-138.0	10	129%	50	74%
Carbadox	263.2-231.2	1	123%	5	66%
Carbamazepine	237.4-194.2	1	42%	5	80%
Cefotaxime	456.4-396.1	4	ND	200	63%
Ciprofloxacin	332.2-314.2	3.5	105%	17.5	100%
Clinafloxacin	366.3-348.1	4	48%	20	105%
Cloxacillin	469.1-160.1	2	ND	100	72%
Codeine	300.0-152.0	2	15%	10	70%
Cotinine	177.0-98.0	1	18%	NA	
Dehydronifedipine	345.5-284.1	0.4	46%	2	102%
Digoxigenin	391.2-355.2	4	128%	20	105%
Digoxin	781.5-113.1	10	136%	500	84%
Diltiazem	415.5-178.0	0.2	27%	1	93%
1,7-Dimethylxanthine	181.2-124.0	100	58%	500	61%
Diphenhydramine	256.8-168.1	0.4	122%	100	79%
Enrofloxacin	360.0-316.0	2	83%	10	67%
Erythromycin	734.4-158.0	0.2	ND	10	19%
Erythromycin anhydrate	716.4-158.0	0.2	59%	10	89%
Flumequine	262.0-173.7	1	60%	50	73%
Fluoxetine	310.3-148.0	1	37%	5	70%
Lincomycin	407.5-126.0	2	ND	10	1%

# Table 1. Recovery of Group 1 Compounds from Spiked Drinking Water (contd.)

<b>Compound</b>	<b>MRM Transition</b>	<b>SPE Spiking Level (µg/L)</b>	<b>% Recovery</b>	<b>SPME Spiking Level (µg/L)</b>	<b>% Recovery</b>
Lomefloxacin	352.2-308.1	2	106%	10	51%
Miconazole	417.0-161.0	1	74%	5	101%
Norfloxacin	320.0-302.0	10	75%	50	108%
Norgestimate	370.5-124.0	2	6%	100	42%
Ofloxacin	362.2-318.0	1	83%	5	106%
Ormetoprim	275.3-259.1	0.4	128%	2	80%
Oxacillin	402.2-160.2	2	ND	100	61%
Oxolinic acid	244.1-216.1	0.4	77%	20	110%
Penicillin G	335.1-160.1	2	ND	NA	
Penicillin V	373.2-182.2	4	17%	NA	
Roxithromycin	837.0-158.8	0.2	2%	10	55%
Sarafloxacin	386.0-299.0	9.12	100%	45.6	128%
Sulfachloropyridazine	285.0-156.0	1	21%	NA	
Sulfadiazine	251.2-156.1	1	22%	NA	
Sulfadimethoxine	311.0-156.0	0.2	1%	NA	
Sulfamerazine	265.0-156.0	0.4	18%	NA	
Sulfamethazine	279.0-156.0	0.4	7%	20	191%
Sulfamethizole	271.0-156.0	0.4	19%	20	90%
Sulfamethoxazole	254.0-156.0	0.4	13%	20	125%
Sulfanilamide	173.2-108.2	10	18%	NA	
Sulfathiazole	256.3-156.0	1	24%	50	56%
Thiabendazole	202.1-175.1	1	119%	5	144%
Trimethoprim	291.0-230.0	1	1%	5	78%
Tylosin	916.6-174.2	4	57%	20	60%
Virginiamycin	526.3-508.3	2	ND	100	13%



Both SPME and SPE were used to process a spiked sample of drinking water. The samples were then quantified using a single-point calibration in the case of SPE, and a multi-point extracted calibration for SPME. The absolute recovery of each compound was calculated (Table 1), and no internal standards were used. The spiking level used for the SPE samples was based on the concentration of the high calibration standard (CS5) described in Method 1694. The spiking level used for SPME was 5x-10x higher, depending on the compound.

SPME was found to be quantitative for the extraction of the majority of the Group 1 compounds, thus samples found to be positive for these compounds by SPME would require no further analysis. In addition, the SPME process was found to require less “hands-on” time than SPE, and allowed for the preparation of a greater number of samples at one time.

# Results

## SPE

- Group 1 compounds (standard) at the CS5 level shown in Figure 1.
- Good chromatographic resolution obtained with Fused-Core™ LC column.
- Table 1 shows recoveries using both extraction methods. An abnormally high recovery was obtained for acetaminophen, and this compound was not detected in an unspiked sample. The reason for this result is not apparent at this time, and will require further study.
- Most of the –cillin type compounds were not detected. Further experiments are needed to check retention characteristics on the SPE phase for these compounds.
- Differences between the published method MRM transitions and those used here were seen. All transitions used are listed in Table 1.

## Results (contd.)

### SPME

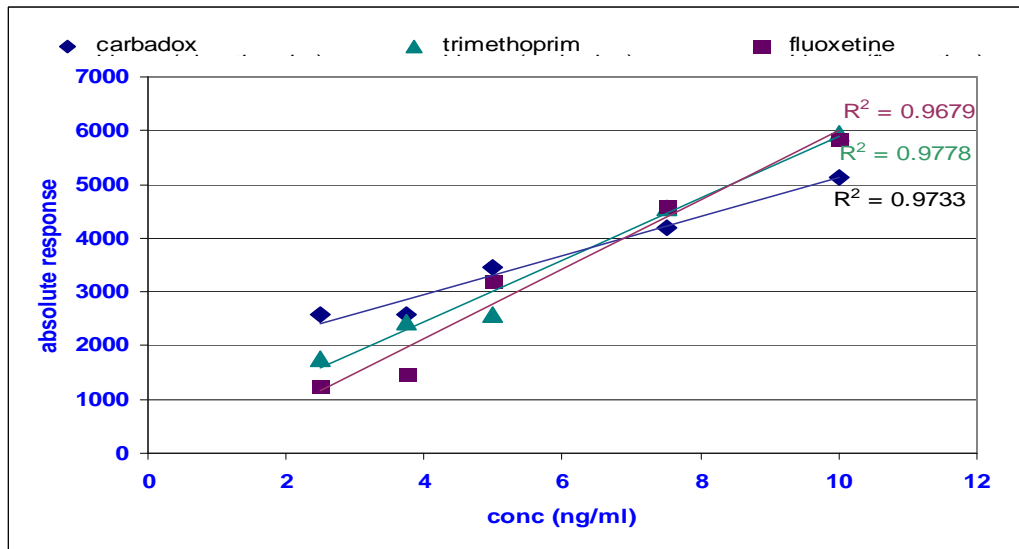
The SPME recoveries of the spiked drinking water sample were determined by comparing the known spiked value for each compound with a value determined experimentally. The experimental value was calculated using an external standard method and multi-point calibration curve. The calibration standards used for the SPME sample were extracted using the procedure previously described.

For the SPME recoveries, compounds indicated as “NA” could not be quantified due to low or no response. The sulfa drugs showed extremely low response in unextracted controls, and were not detected consistently in extracted samples. Lincomycin and virginiamycin showed very low recovery from the spiked water sample. It is possible that a modification in extraction conditions would increase recovery, however this would have to be confirmed with additional investigation.

# Quantitation Using SPME

The extraction of a multi-level calibration curve in buffered, deionized water allowed for a study of SPME as a quantitative technique for this application. The concentration range for calibration was similar to that described in Method 1694. In many cases, it was found that one or two points were affecting the overall linearity. This may be due to variability in the extraction performance of individual SPME fibers. The use of internal standards should compensate for this variability.

**Linearity of SPME for the Extraction of Pharmaceutical Compounds from Deionized Water**



## SPME: Limits of Detection

As discussed previously, SPME was not expected to reach the detection levels achievable with SPE. SPME limits of detection for the Method 1694 Group 1 compounds were estimated by comparing data from extracted samples of buffered, deionized water, spiked at different levels. The results (partial list) of this study are summarized in Table 2. Of the Group 1 compounds, nine were not detected with certainty from the highest level extracted. These compounds are indicated in the table. The difference in detection limits between the compounds is due to differences in extraction efficiency with the SPME fiber, and ionization efficiency in the LC-MS-MS system.

## Table 2. SPME Estimated Limits of Detection (Partial List)

Estimated detection level (µg/L)	0.2	0.5	1	5	10	20	25	50	75	100	250
Acetaminophen								X			
Ampicillin									X		
Azithromycin					X						
Caffeine								X			
Carbadox				X							
Carbamazepine		X									
Cefotaxime										X	
Ciprofloxacin							X				
Clinafloxacin				X							
Cloxacillin						X					
Codeine					X						
Cotinine					>						
Dehydronifedipine	X										
Digoxigenin				X							
Digoxin								X			
Diltiazem	X										
1,7-Dimethylxanthine											X
Diphenhydramine	X										
Enrofloxacin			X								
Erythromycin						>					
Erythromycin anhydrate	X										
Flumequine								X			
Fluoxetine				X							
Lincomycin				X							

> compound not detected at highest level extracted

# Discussion

SPME is:

- less labor intensive than SPE and can be an excellent screening method for detecting higher levels of these compounds in water.
- quantitative if analyte is in linear range of SPME
- a technique that can be used for simultaneous, multiple sample extraction and desorption, and constant monitoring is not required.
- an equilibrium process, and not an exhaustive extraction technique, resulting in higher method detection limits (MDL) compared to limits determined using SPE.

Method modifications to the SPME procedure presented here (such as adjustments to extraction pH and elution buffer composition) may increase recovery of some analytes. Additional data generated during the course of this study indicated that increasing the sample volume was ineffective in reducing detection levels. However, an increase in the extraction time from 30 to 60 minutes was found to increase response for most analytes.

# Conclusions

1. SPME was able to detect most of the analytes listed in the method, but the method detection limits (MDL) for many analytes are higher than the MDLs using SPE with a 0.5 L sample.
2. It has been demonstrated that SPME can be used to quantify analytes.
3. SPME is a simpler, less labor intensive method compared to SPE.
4. SPME shows promise as a method for routine monitoring, especially once the MALs (maximum allowable limits) of the analytes are determined.

# References

1. US EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by LC-MS/MS, Dec. 2007.
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