APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 220

Determination of Hormones in Water by Back Extraction of TF-SPME-SBSE and LC-MS/MS

Fred D. Foster and John R. Stuff

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA

Keywords

Thin Film Solid Phase Microextraction (TF-SPME), Stir Bar Sorptive Extraction (SBSE), LC-MS/MS, Water Analysis Proof of Concept.

Abstract

Laboratory samples are typically extracted prior to analysis by sensitive GC-MS or LC-MS/MS methods to separate the analytes of interest from the bulk of matrix components that could interfere with the analysis. Typical extraction methods include liquid-liquid extraction or solid phase extraction strategies; however, these can involve the use of large volumes of hazardous chemicals and may require the evaporation of solvents to concentrate the final extract and/or exchange the solvent system to one more amenable to the analytical technique being used. Extraction techniques that avoid hazardous chemicals while still being able to concentrate and separate the analytes of interest would be of great benefit to those analytical laboratories hoping to achieve safer and less labor-intensive methods of preparing samples for analysis.

In this study, Thin Film Solid Phase Micro-Extraction (TF-SPME) devices with hydrophilic lipophilic balanced/polydimethylsiloxane (HLB/PDMS) coatings were investigated for their usefulness with LC-MS/MS analysis in combination with Stir Bar Sorptive Extraction (SBSE) using the GERSTEL Twister®. Six hormone compounds were extracted from water using TF-SPME-SBSE and then back extracted from the devices using methanol. The GERSTEL MPS was then used to inject the extracts into an LC-MS/MS system for subsequent analysis.

Introduction

The TF-SPME device is a 20 mm x 4.75 mm carbon mesh sheet, which is impregnated with a sorptive phase. A TF-SPME device can be used in either headspace or immersion mode. In immersion mode, the device is placed directly in a liquid sample and the sample is agitated by stirring. The TF-SPME devices work by the same principle as liquid-liquid extractions where the low amount of immobilized extraction solvent, in our case HLB/PDMS, is mixed with the aqueous sample and the extraction is based on sorption. Depending on the sample, TF-SPME devices have the added benefit of being reused 50 to 100 times.

In a recent publication [1], Y. Huang, et al., showed that the combination of TF-SPME and SBSE could be optimized to enable improvements in sensitivity and recovery of volatile compounds when compared to results of SBSE-only or TF-SPME-only extractions analyzed by thermal desorption. It is also possible to desorb these extraction devices using solvent back extraction. In the work presented here, TF-SPME-SBSE with solvent back extraction enabled us to reduce the volume of sample needed to an 18 mL aliquot while eliminating the need for evaporative concentration of the extraction solvent before detection using an Agilent Ultivo LC-MS/MS.

GERSTEL AppNote 220

Experimental

Materials

A reference standard containing testosterone, androstenedione, 17β-estradiol, 17α-ethynylestradiol, estrone, and equilin was purchased from Restek®. Deuterated internal standards D_3-testosterone and D_5-17β-estradiol were purchased from Cerilliant. All other reagents and solvents used were reagent grade. The LC-MS/MS system was first optimized using dilutions of the stocks to verify that sensitivity and linearity for the hormone compounds are adequate for this proof of concept study. Water samples containing the hormones were prepared at concentrations of 0.1 ng/mL.

Optimized Sample Preparation

1. An 18 mL water sample was prepared in a glass 20 mL vial.
2. Six grams of sodium chloride was added to each sample vial.
3. A thermally conditioned TF-SPME (HLB/PDMS) device and a Twister stir bar (10 mm x 0.50 mm, PDMS) were added to each sample vial. The TF-SPME devices were suspended into the water samples by use of metal holders. The metal holder allows TF-SPME device and the Twister bar to be used together within a single extraction vial.
4. The vials were stirred at 1000 rpm and 45 °C for 4 hours using the GERSTEL stirred agitator.
5. Following extraction, the TF-SPME devices and Twisters were removed, dipped in water, blotted dry, and then transferred together to a 4 mL glass vial with an insert.
6. A 400 µL aliquot of methanol was pipetted into each vial.
7. The vials were sonicated for 1 hour in an ultrasonic bath.
8. An aliquot of the methanol extract was pipetted into a 2 mL autosampler vial with a microliter insert and placed onto the GERSTEL MPS for injection into the LC-MS/MS system.

Instrumentation

All injections were performed using a MPS robotic^{PRO} sampler as shown in figure 1. All analyses were performed using an Agilent 1260 HPLC with a Phenomenex Gemini C18 column, (2.0 x 150 mm, 3 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet Stream Electrospray source. Sample injections were made using the GERSTEL LC-MS Tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop.

Figure 1: GERSTEL MPS robotic^{PRO} sampler with Agilent Ultivo LC-MS/MS.

LC Method Parameters

Mobile phase	A – 0.02% ammonium hydroxide in water B – 0.02% ammonium hydroxide in methanol

LC gradient

Time (min)	Flow (mL/min)	Pressure (bar)	% B
0	0.3	800	50
5	0.3	800	50
8	0.3	800	85
11	0.3	800	85
11.51	0.3	800	50

Run time	15 minutes.
Injection volume	10.0 µL (loop over-fill technique)
Column temperature	35 °C

Mass Spectrometer Parameters

Operation	electrospray with positive/negative switching
Gas temperature	350 °C
Gas flow ($N_{2)}$	5 L/min
Nebulizer pressure:	35 psi
Sheath gas flow (N_2)	11 L/min
Sheath gas temperature	400 °C
Capillary voltage	4000 V
Nozzle voltage	500 V

GERSTEL AppNote 220

The mass spectrometer acquisition parameters are shown in table 1 with qualifier ions.

Table 1: Mass spectrometer acquisition parameters.

Compound Name	Precursor Ion (m/Z)	Product Ion (m/z)		Frag (V)		CE (V)		Polarity	Ret. Time (min.)
D_3-Testosterone	292.2	109.1	97.1	130	130	25	25	+	9.901
Testosterone	289.1	109.1	97.1	100	100	25	25	+	9.919
Androstenedione	287.1	109.1	97.1	100	100	30	25	+	9.469
17α-Ethynylestradiol	295.2	159.1	145.1	120	120	45	45	-	9.842
D_5-17β-Estradiol	276.3	187.2	147.1	160	160	50	50	-	9.781
17β-Estradiol	271.2	183.1	145.1	120	120	55	55	-	9.831
Estrone	269.2	159.1	145.1	120	120	45	45	-	9.703
Equilin	267.1	223.2	143.1	120	120	35	35	-	9.544

Results & Discussion

Figure 2 shows representative mass chromatograms for all hormones and deuterated internal standards resulting from the analysis of a sample with hormone concentrations of 5 ng/mL.

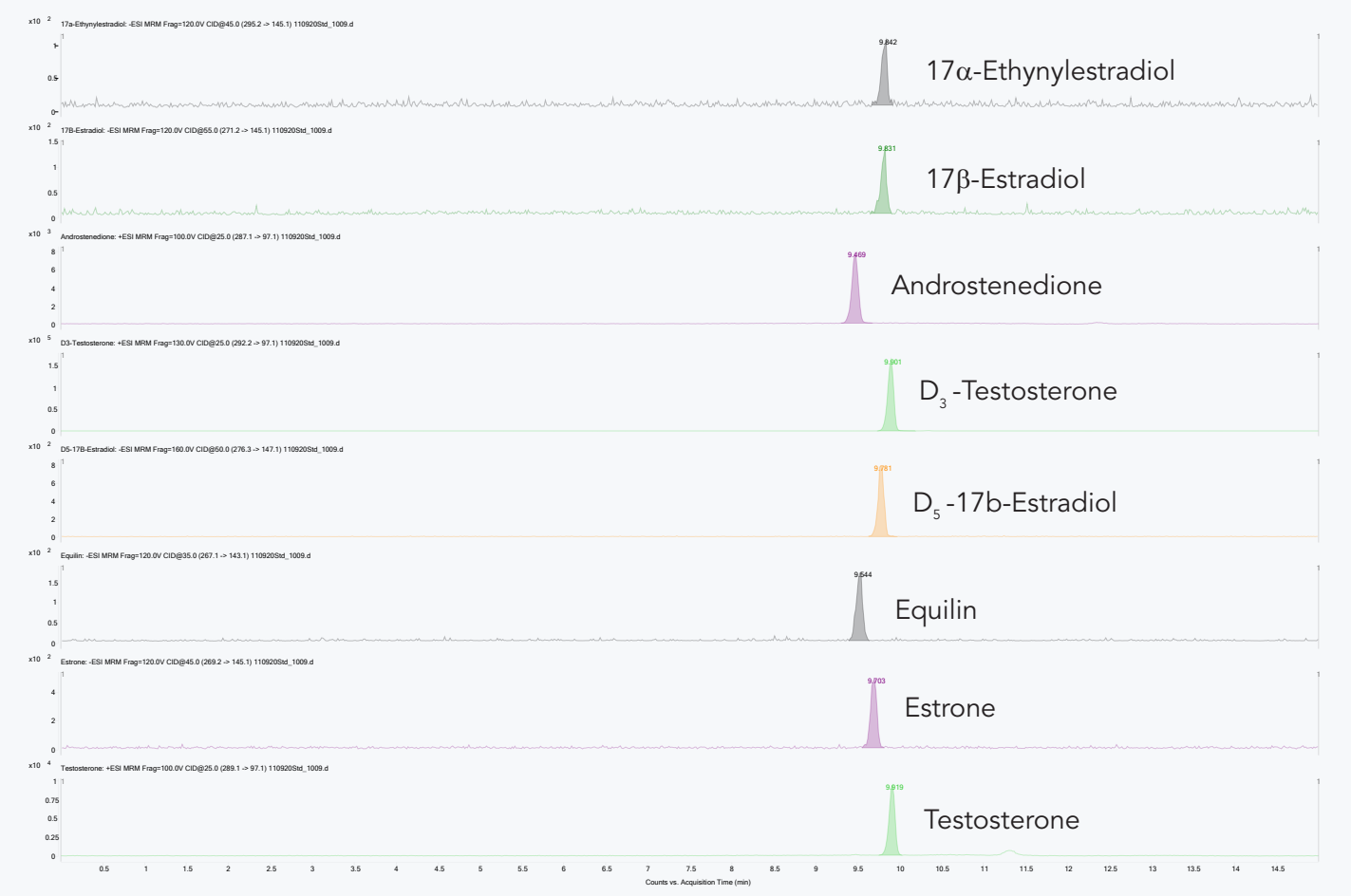

Figure 2: Representative stacked mass chromatograms of hormone compounds analyzed.

GERSTEL AppNote 220

The lower limits of quantitation for this method were 0.0500 ng/mL for testosterone and androstenedione, 0.100 ng/mL for equilin and estrone, 0.125 ng/mL for 17β-estradiol, and 0.175 ng/mL for 17α-ethynylestradiol. Representative calibration curves are shown in figures 3 A-B. Regression analysis for all hormones analyzed within this method resulted in R^2 values of 0.99 or greater.

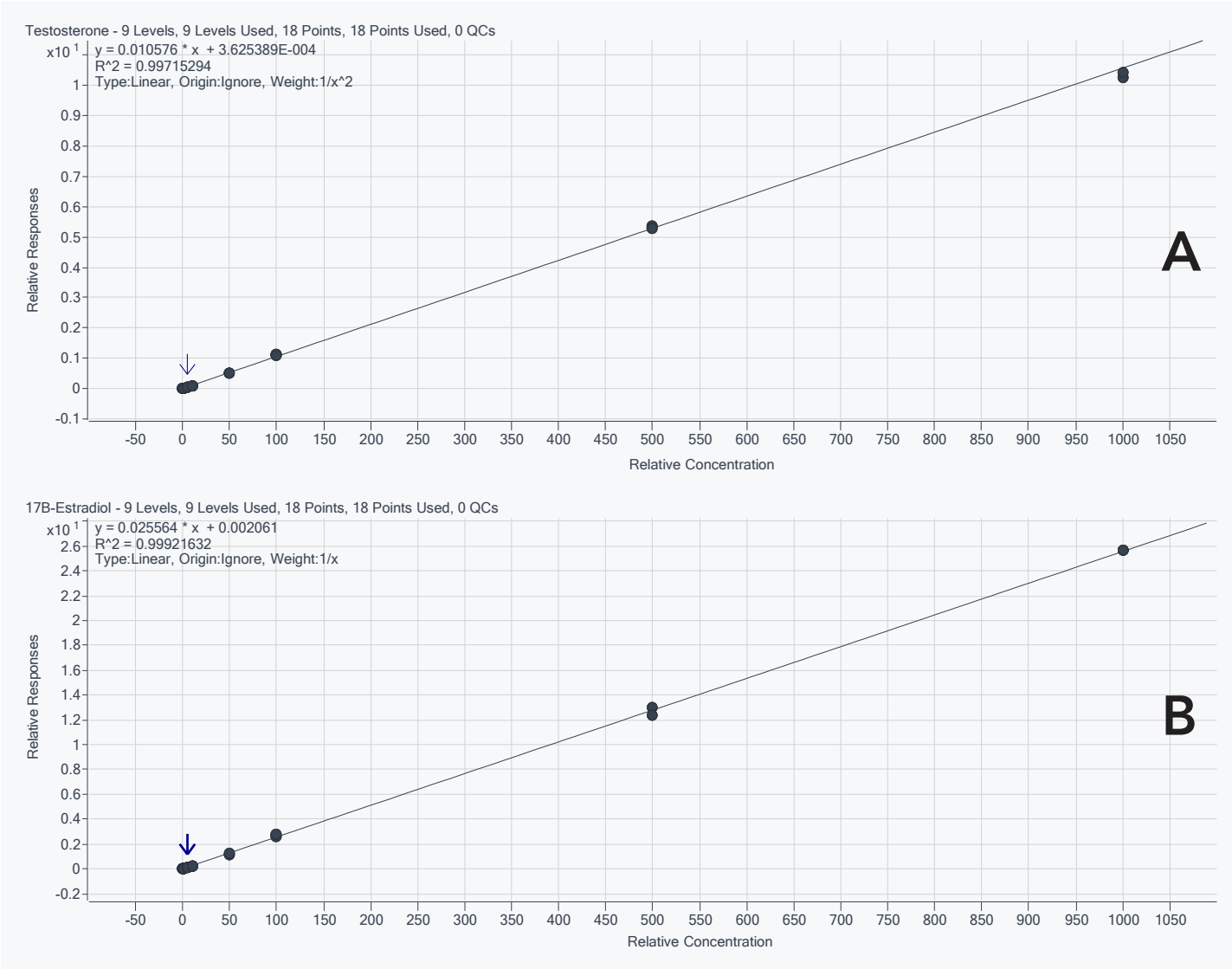

Figure 3 A-B: Resulting calibration curves from testosterone (A) and 17β-estradiol (B).

GERSTEL AppNote 220

Adding salt to an aqueous sample can aid the adsorption of organic compounds from aqueous solution onto TF-SPME devices, in effect improving the partitioning of the analytes from the aqueous sample into the TF-SPME and Twister devices. To examine whether the addition of salt to hormone spiked water samples improve extraction recoveries, we compared triplicate water samples extracted either with or without addition of sodium chloride. As shown in figure 3, the addition of sodium chloride resulted in improvements in the extraction recoveries of all hormones being monitored.

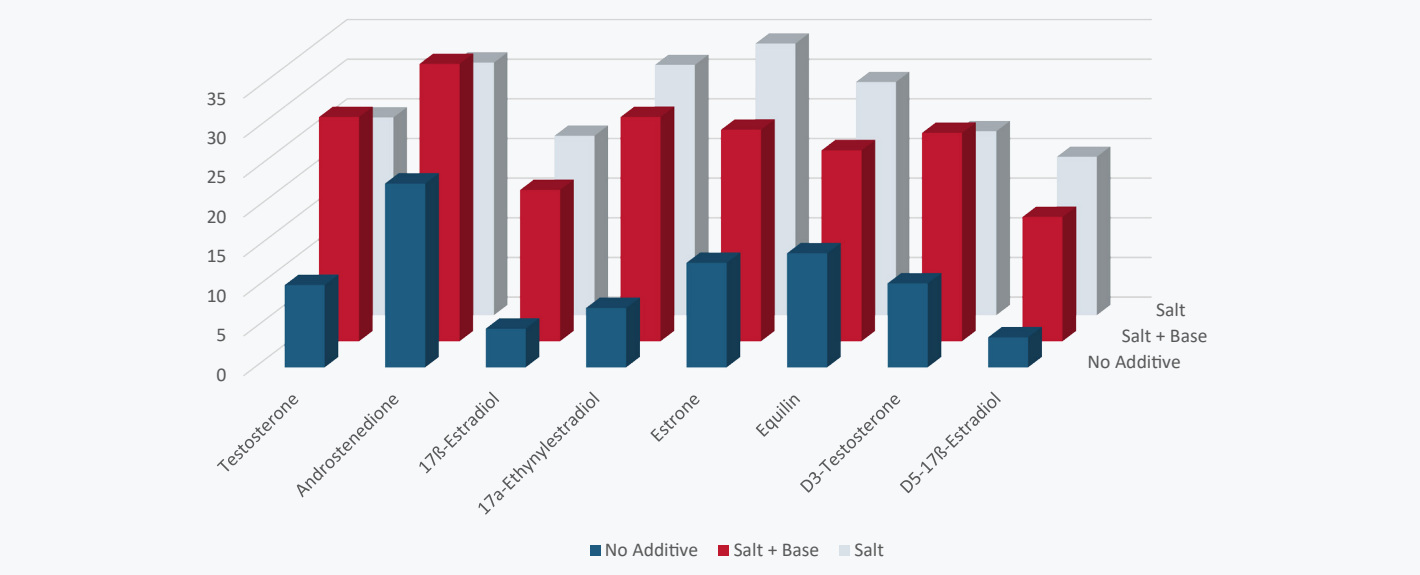

Figure 4: Results from examination of sample additives on % recovery of analytes.

Extracting samples for a longer time generally promotes partitioning of analytes from one phase to the other, improving the extraction recovery. To examine the effects of extraction time on the recovery of hormones, triplicate water samples were extracted for 4 hours and the results compared to those for triplicate water samples extracted for 24 hours. As shown, there was a significant improvement in extraction recovery of all hormones when increasing the time of extraction.

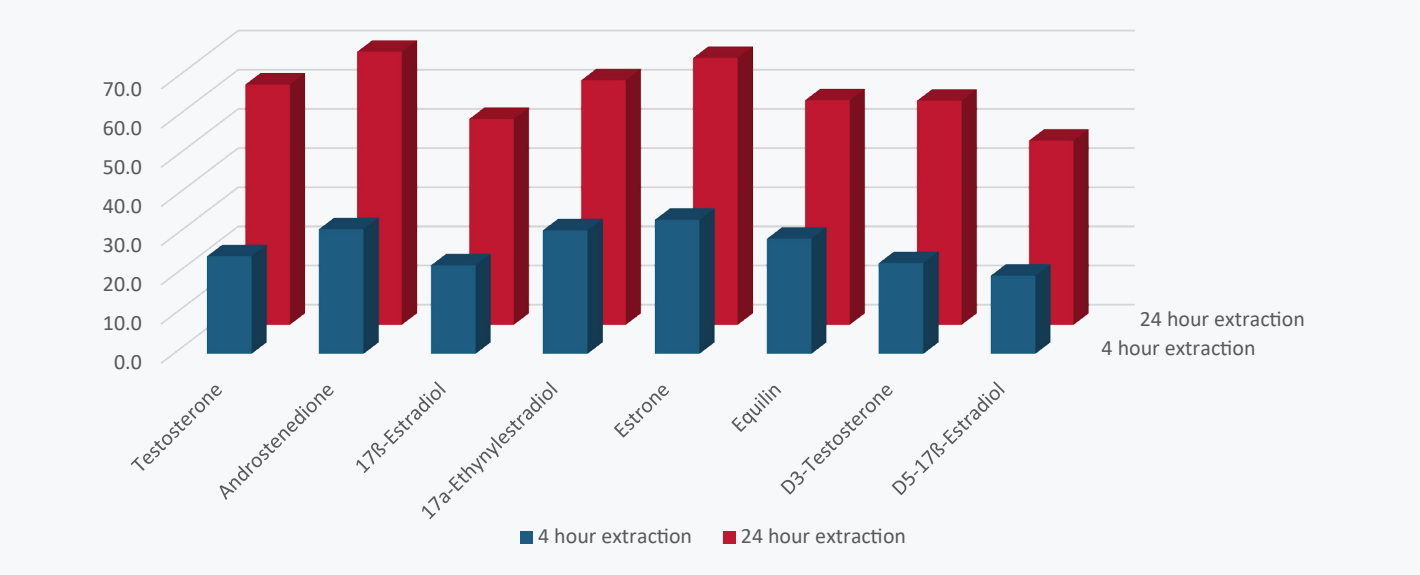

Figure 5: Results from examination of extraction time on % recovery of analytes.

GERSTEL AppNote 220

Heating a sample during extraction can accelerate the mass transfer resulting in improved extraction recovery. To examine whether increased temperatures improved extraction recoveries, triplicate water samples spiked with hormones were extracted at room temperature, 45 °C, and 70 °C, respectively, and the results compared. As shown in figure 5, the maximum extraction recoveries for all hormones monitored were achieved at 45 °C.

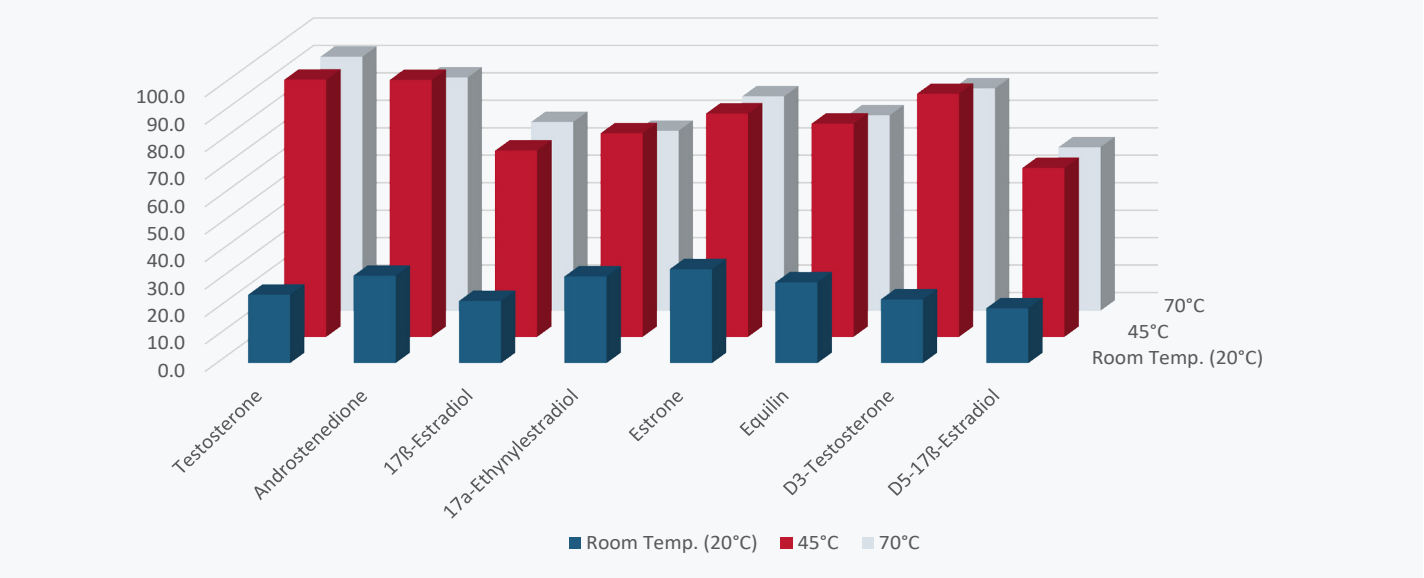

Figure 6: Results from examination of extraction temperature on % recovery of analytes.

Conclusions

As a result of this study, we were able to show:

- The combination of TF-SPME (HLB/PDMS) and SBSE (PDMS) was found to extract hormone compounds from an 18 mL sample of water with excellent recovery. Larger samples can be extracted for improved limits of determination.
- Solvent back extraction of hormones can be used for TF-SPME-SBSE enabling subsequent determination by LC-MS/MS without a solvent evaporation/analyte concentration step.
- The use of TF-SPME-SBSE with solvent back extraction greatly simplified the process by eliminating both the need for tedious, labor intensive liquid-liquid extraction or SPE steps, and the solvent evaporation/analyte concentration step.
- The GERSTEL MPS roboticpro sampler can efficiently inject from the small volume of methanol directly into the LC-MS/MS system, and if desired can be used to automate the solvent back extraction step of the method.

References

[1] Y. Huang, C.S.M. Liew, S.X.L. Goh, et al., "Enhanced extraction using a combination of stir bar Sorptive extraction and thin film-solid phase microextraction", J. Chrom. A., 2020, 461617.