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Determination of Endocrine Disrupting Hormones in Drinking and Wastewater

APPLICATION NOTE

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Key Words

Endocrine Disrupting Hormones, drinking water, SPE, LC/MS/MS

Introduction

Hormones in drinking water are a growing concern, since the presence of these compounds can potentially be linked to serious health hazards such as human developmental and reproductive side-effects. Hormones are introduced into our environment in several ways. Natural and synthetic hormones that are given to livestock, pass though the animals, runoff into surface water, and leach into underground water supplies. Humans also produce and excrete natural hormone waste every day. An example is the natural and synthetic estrogens and androgens that are used in large quantities for medical purposes, such as oral contraceptives and hormone replacement. Human waste water flows through to sewage treatment plants, but these plants are not designed to handle the removal of hormones, so the hormones filter though the treatment process, and can ultimately end up in drinking water.

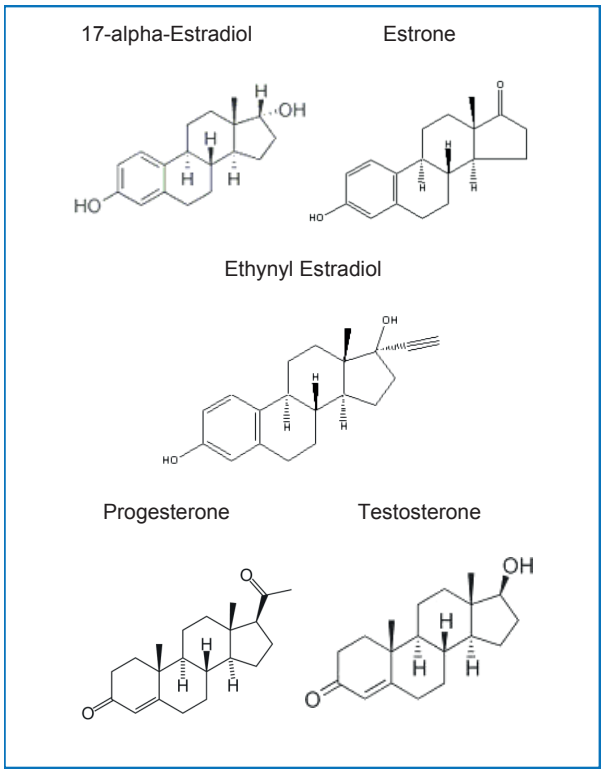

Figure 1: Chemical Structures of Hormones used in this analysis.

The Atlantic® HLB (Hydrophilic-Lipophilic Balanced) solid-phase extraction disk is able to retain the listed organic semi-volatile compounds through its unique ratio of hydrophilic N-vinylpyrrolidone and lipophilic divinylbenezene sorbent. The Atlantic HLB sorbent has a number of key features that enable it to perform the extraction, including stability at pH extremes and in a wide range of solvents, extraordinary retention of polar compounds, and a hydrophobic retention capacity three times greater than that of traditional silica-based SPE sorbents.

The SPE-DEX® 4790 provides automatic extraction of liquid samples by solid phase extraction methods, and can handle samples ranging from 20 mL to 4 L. The Envision® Platform Controller provides a user-friendly, web-based controller capable of interacting with up to eight extractors via one PC. The DryVap® Concentrator System provides automatic sample drying with patented DryDisk® separation membrane technology, automatically concentrating each dried extract by applying heat, vacuum, and sparge flow for up to six samples simultaneously. The Reclaimer™ Solvent Recovery System (SRS) (now replaced by the SolventTrap $_{SVOC}$) is designed to handle the volume of solvent vapor generated from the DryVap Concentrator System while in operation.

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Instrumentation

- Horizon Technology SPE-DEX 4790 Automated Extraction System
- Envision Controller
- DryVap Concentrator
- DryDisk Separation Membranes
- Reclaimer (Solvent Recovery System)
- Atlantic HLB SPE Disks (47mm)
- Agilent 1200SL HPLC
- Sciex API 4000 MS/MS

Shown above: SPE-DEX 4790 Automated Extraction System, Envision Controller and DryVap Drying and Concentration System

Method Summary

1. A 500 mL aliquot of sample is used.
2. Samples are preserved with 25 mg of ascorbic acid.
3. Agitate the bottles by hand until preservative is dissolved.
4. Verify the pH is between 5-7.
5. Spike the compound standard into samples (samples spiked at 50 ppt).
6. Start extraction method shown in Table 2 and programmed into the Envision Controller.
7. Collect the extract at high vacuum at –25 in. Hg.
8. Extract volume should be approximately 15 mL.
9. Add the extract to the DryDisk holder and start the concentration process on the DryVap using the conditions in Table 1.
10. Concentrate the extract to 0.5 mL.
11. Add 2 mL of MTBE into the concentrator tube and mix for solvent exchange.
12. Concentrate to 0.5 mL
13. Transfer the extract to a GC vial.
14. Analyze by LC/MS/MS.

Table 1: Conditions used for the DryVap Concentrator

Parameter	Setting
Dry Volume	20
Heat Power	5
Auto Rinse	Off
Heat Timer	Off

Table 2: Hormone Method Programmed into the Envision Controller

Step	Solvent	Soak Time	Dry Time
Prewet 1	MTBE	1:00 min	30 sec
Prewet 2	Methanol	1:00 min	2 sec
Prewet 3	Reagent Water	1:00 min	0 min
Prewet 4	Reagent Water	1:00 min	0 min
Process Sample			
Air Dry 10:00 min			
Rinse Step 1	MTBE	1:30 min	30 sec
Rinse Step 2	MTBE	1:30 min	30 sec
Rinse Step 3	MTBE	1:30 min	1:30 min
Rinse Step 4	MTBE	1:30 min	2:00 min

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Results

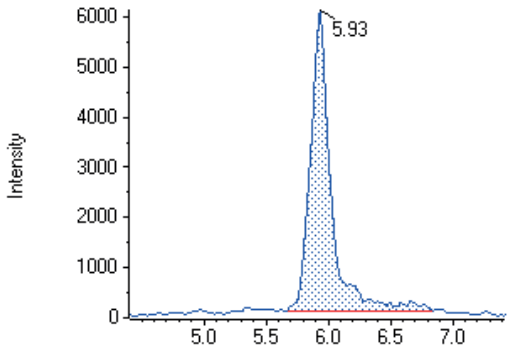
The results for the Atlantic HLB disk are listed in Table 3. The sample was spiked at 50 ppt and resulted in a concentration factor of 1000. The final extract concentration was 50 ppb. The table shows the compound name, amount recovered, and percent recovery. The Atlantic HLB SPE disk had a recovery range between 88-91% for the hormones. Table 4 shows the chromatograms of the selected hormones and their calibration curves.

Table 3: Recoveries of Selected Hormones

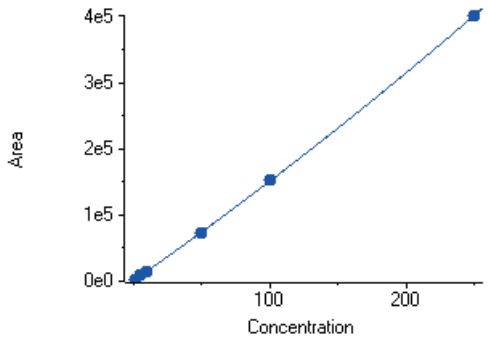
Compound	Final Concentration (ppb)	Recovery (%)
Alpha-Estradiol	45.4	91
Ethynyl Estradiol	44.2	88
Progesterone	44.3	89
Esterone	44.5	89
Testosterone	44.8	90

Table 4: Chromatograms and Calibration Curves for Selected Hormones

Analyte name: Estradiol (255.1 / 158.9) in blue



Analyte name: Estradiol (255.1 / 158.9)
Calibration Equation: y = 0.65732 x^2 + 1435.99399 x + 1233.29640
(r = 0.99992)



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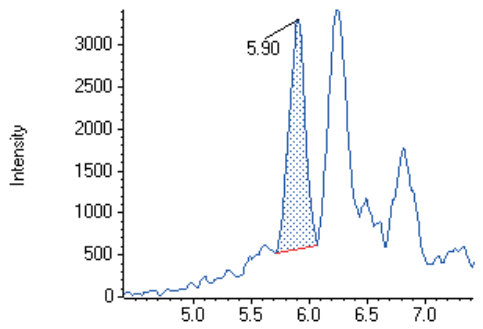
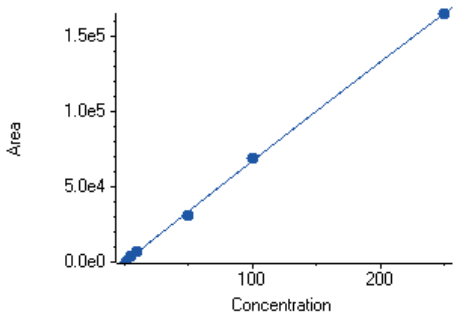
Analyte name: EthnylEstradiol (279.1 / 133.1) in blue

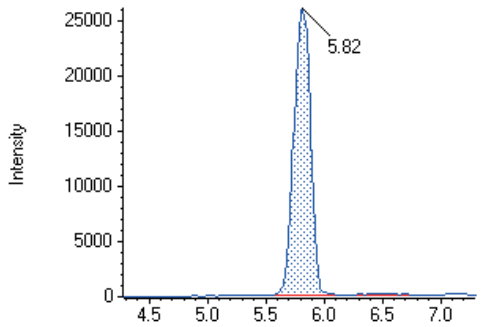


Analyte name: EthnylEstradiol (279.1 / 133.1)

Calibration Equation: y = -0.08339 x^2 + 683.27710 x + -161.26060
(r = 0.99894)

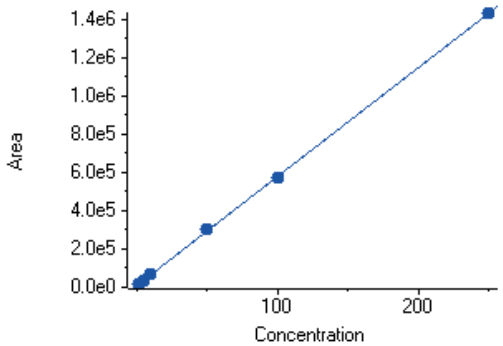


Analyte name: Progesterone (315.1 / 109.0) in blue

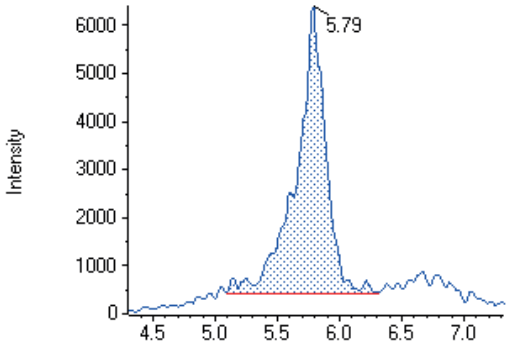


Analyte name: Progesterone (315.1 / 109.0)

Calibration Equation: y = -0.09310 x^2 + 5708.08805 x + 10034.34686
(r = 0.99994)

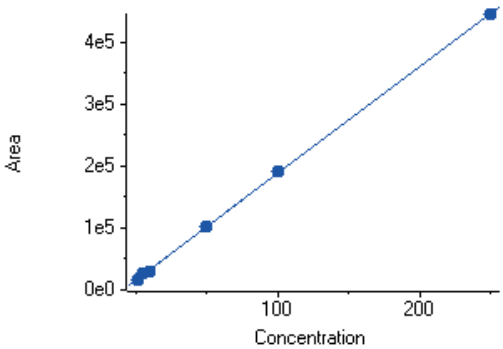


Analyte name: Esterone (271.2 / 253.0) in blue



Analyte name: Esterone (271.2 / 253.0)

Calibration Equation: y = -0.05881 x^2 + 1733.77152 x + 15864.79951
(r = 0.99942)



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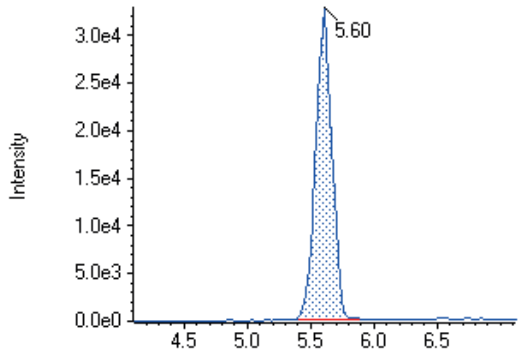
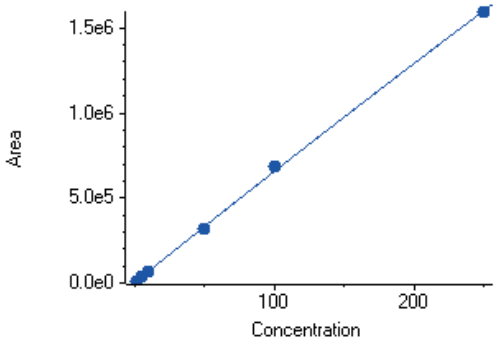
Analyte name: Testosterone (289.2 / 108.8) in blue



Analyte name: Testosterone (289.2 / 108.8)

Calibration Equation: y = -0.93283 x^2 + 6618.40123 x + 5051.50214
(r = 0.99958)



Conclusion

The resulting data from this study demonstrates that the Horizon Technology fully-automated extraction, drying and concentration systems used with the Atlantic HLB SPE disk are capable of the detection of selected hormones, resulting in data that is both accurate and precise. Extraction times were typically 30 minutes and drying and concentrating times were approximately 20 minutes.

The Horizon Technology SPE-DEX 4790 Automated Extractor System coupled with the Envision Platform, DryVap Concentrator System and the Reclaimer Solvent Recovery System reduces analyst labor, solvent usage, turn-around-time, and greatly improves accuracy and precision.

Acknowledgement
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