Troy T. Handlovic

Fairleigh Dickinson University, Class of 2020
Major: **Chemistry**
Minor: **Biology**

Faculty Advisor: **Mohammed R. Elshaer, Ph.D.,** Assistant Professor
Department of Chemistry and Biochemistry

Quantification of Estrogenic Compounds by SPE-GC-MS in New Jersey Natural and Wastewater Sources

Estrogens are known endocrine-disrupting agents which have emerged as a class of pollutants that pose danger to aquatic organisms at low concentrations. Among their negative impacts on aquatic life is their effect on reproductive behavior, which can ultimately move up trophic levels of the ecosystem resulting in a compounding environmental effect. Estrogenic compounds detected in wastewater effluents and surface water at nanogram to picogram per liter concentrations include estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), and estriol (E3). Despite their known risks, concentrations in the Northeastern United States have remained largely unknown. We have developed and validated an SPE-GC-MS method for the quantification of these estrogenic compounds in the Whippany River and Loantaka Brook (NJ, USA). Estrogens extracted from water samples via SPE are subsequently derivatized using BSFTA + 2% TMCS and successfully separated and quantified at ppb levels using this GC-MS method.

Independent Colleges Undergraduate Research Award Recipient 2020

Independent Colleges Scholarship Award: ***Schering-Plough Undergraduate Research Scholarship, 2019-20***

Additional Funding: ***Becton College of Arts and Sciences; Novo Nordisk Pharmaceutical Company***

Acknowledgments: ***Fairleigh Dickinson Department of Chemistry and Biochemistry; Morris Township water pollution control utility staff***

Quantification of Estrogenic Compounds by SPE-GC-MS in New Jersey Natural and Wastewater Sources

Troy T. Handlovic,[a] James D. Salierno, Ph.D.,[b] and Mohammed R. Elshaer, Ph.D.[a]

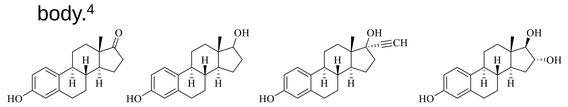
[a] *Department of Chemistry and Biochemistry, Fairleigh Dickinson University- Florham, Madison NJ 07940*

[b] *Department of Biological and Allied Health Sciences, Fairleigh Dickinson University- Florham, Madison NJ 07940*

Becton College of Arts and Sciences
ELSHAER RESEARCH GROUP

Introduction

- Estrogenic hormones are known **strong endocrine disrupting compounds (EDC).**
- These compounds have proven to disrupt the reproductive behavior of both vertebrate and invertebrate aquatic organisms.[1,2]
- The synthetic estrogen of interest is **17α-ethinyl estradiol (EE2).**
- EE2 is medicinally **used by over 100 million people**, in combination with progesterone, as the oral contraceptive commonly known as "the birth control pill".[3]
- Computational models tell us that around **40% of all ingested EE2 from birth control is excreted** by the body.[4]



Estrone (E1)	β-Estradiol (E2)	17α-Ethinylestradiol (EE2)	Estriol (E3)
MW: 270.37 g/mol	MW: 272.39 g/mol	MW: 296.41 g/mol	MW: 288.39 g/mol
MP: 304.29 °C	MP: 292.65 °C	MP: 386.06 °C	MP:454.74 °C
BP: 542.72 °C	BP: 562.41°C	BP: 598.53 °C	BP: 719.28 °C
pKa: 9.574	pKa: 9.577, 14.656	pKa: 9.570, 13.362	pKa: 9.442, 14.243, 17.95
LogS: -3.907	LogS: -4.287	Log: -4.393	LogS:-3.999

Figure 1: Molecular structure of the estrogenic compounds and their associated chemical physical properties.

Solid Phase Extraction

- All samples were extracted via solid phase extraction (SPE) using Water's Oasis Hydrophobic Lipophilic Balance (HLB) SPE cartridges.

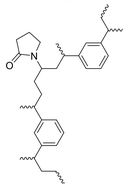


Figure 2: Structure of Water's Oasis HLB SPE sorbent.

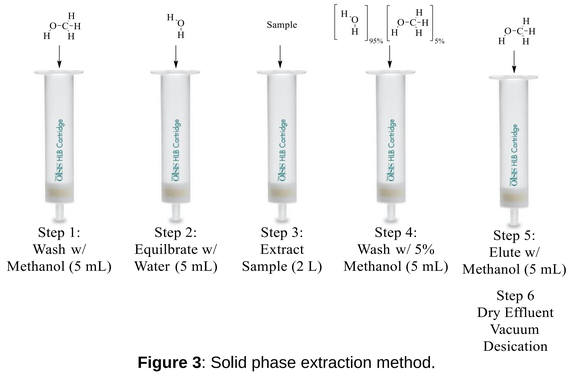
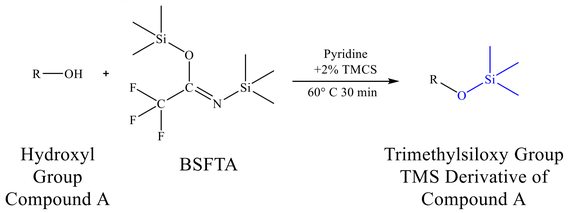


Figure 3: Solid phase extraction method.

- Vacuum up to 15 mm Hg was used to pull samples through the SPE cartridge.

Derivatization

- Due to the high boiling point, and polarity of the hydroxyl groups present in the estrogenic hormones, tri-methyl silyl (TMS) derivatization was performed using BSFTA + 2% TMCS.



Scheme 1: Silaytion reaction conditions showing the substitution of the hydrogen on the hydroxyl group with the trimethylsilyl group (TMS) (Blue) to form the trimethylsiloxy group.

- Resultantly, the TMS derivatives (Figure 4) of the compounds enter the GC-MS analysis.

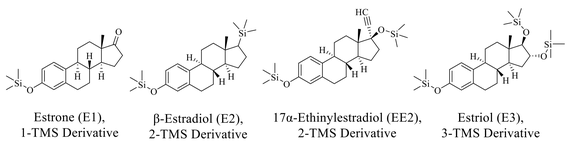


Figure 4: Molecular structures of the estrogenic hormones TMS derivatives.

Qualitative GC-MS Analysis

- Full scan GC-MS analysis was conducted to confirm the successful derivatization.

Table 1: GC-MS instrumental parameters.

	Conditions
Instrument Details	Thermo Scientific Trace 1300 GC Thermo Scientific ISQ Single Quadrupole MS Chromleon 7.2 Chromatography Software
Temperature Program	100°C-220°C @ 30°C·Min^{-1} 220°C-300°C @ 6°C·Min^{-1}
Carrier Gas	Ultra Pure Hydrogen Flow Rate: 1.4 mL·Min^{-1}
Column	RTX-5SIL MS 30.00m by 25.00mm by 25.00µm
Mass Spectrometer	Full Scan Mode (40-450 AMU)

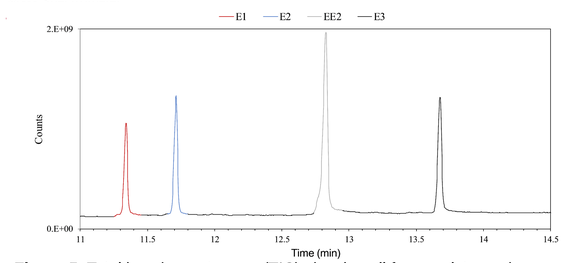


Figure 5: Total ion chromatogram (TIC) showing all four analyte peaks.

- The mass spectra fragmentation pattern for each peak in Figure 5 was compared to the NIST Webbook to confirm compound identity.

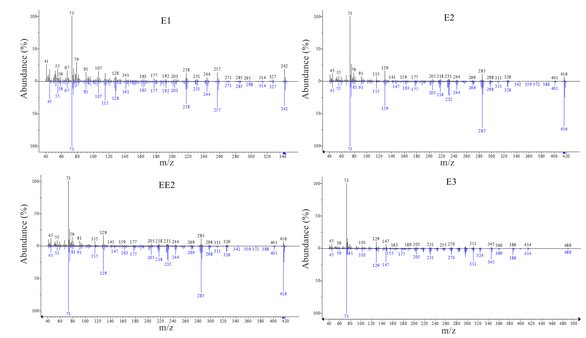
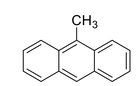


Figure 6: Head-to-tail plots showing recorded mass spectra fragmentation patterns (black) *versus* the NIST Webbooks match for each species (blue).

Quantitative GC-MS Analysis

- For all quantitative analyses, the multipoint internal calibration method was applied using 9-methylanthracene as an internal standard.



9-Methylanthracene

MW: 192.26 g/mol
MP: 102.02 °C
BP: 587.19 °C
LogS: -4.987 °C

Figure 7: Molecular structure of the internal standard (IS) (9-Methylanthracene) and associated physical/ chemical properties.

- Quantitative GC-MS analysis was conducted using similar parameters shown in Table 1 apart from performing MS selected ion monitoring mode (SIM) with dwells every 0.05 seconds.
- A quantitative and conformational ion was selected for each species shown in Table 2. This allows for the calculation of an ion ratio for each species (Equation 1).

$$ion\ ratio = \frac{A_{quant.\,ion}\ (counts \times min.)}{A_{conf.\,ion}\ (counts\ \times min.)} \qquad \text{(Eq. 1)}$$

Table 2: Species identification parameters.

	$\bar{t_r} \pm s_{t_r}$ (min)	Quant. Ion (m/z)	Conf. Ion (m/z)	$\overline{ion\ ratio} \pm s_{ion\ ratio}$
9-Methylanthrancene	6.734 ± 0.004	165.00	176.00	8.35 ± 0.36
Estrone	11.256 ± 0.008	342.00	257.00	1.37 ± 0.06
β-Estradiol	11.632 ± 0.008	416.00	285.00	0.841 ± 0.037
17α-Ethinylestradiol	12.734 ± 0.009	425.00	440.00	3.96 ± 0.23
Estriol	13.579 ± 0.008	504.00	311.00	1.15 ± 0.11

N=18

- All calibrators (5) were analyzed in triplicates for a concentration range between $\approx 50\ ppb$ and $\approx 540\ ppb$.
- Calibration plots (Figure 8) were made by plotting the average $\frac{A_{Estrogen}}{A_{Internal\ Standard}}$ *versus* $\frac{[Estrogen]}{[Internal\ Standard]}$ for each calibrator.

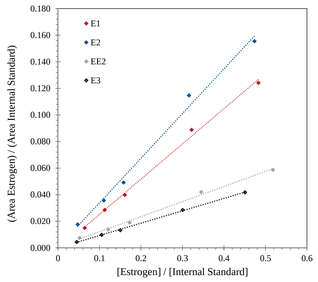


Figure 8: Calibration plots for each estrogen showing the ratio between the area of the estrogen peak and the area of the internal peak *versus* the ratio between the concentration of the estrogen and the concentration of the internal standard. (Note both axes are ratios and therefore are unitless).

Table 3: Statistics for the linear regression of the calibration plots.

	$m \pm s_m$	$b + s_b$	R^2
Estrone	0.265 ± 0.010	-0.001 ± 0.002	0.996
β-Estradiol	0.336 ± 0.017	0.000 ± 0.004	0.992
17α-Ethinylestradiol	0.114 ± 0.004	0.001 ± 0.001	0.996
Estriol	0.093 ± 0.002	0.000 ± 0.000	0.999

Conclusions / Further Studies

- The method was validated using 2L,≈ 100ppt (N=4) and showed recovery values of 67-74%.
- Initial field work was conducted at two sites and showed quantifiable estrogen concentrations in wastewater effluent prior to the lab being shut down for COVID-19.
- Upstream, downstream, and effluent samples will be collected this spring and quantified using this SPE-GC-MS(SIM) method.
- River flow will be measured, and the estrogen concentrations in the aquatic ecosystems will be modeled.
- The threat these components pose on the environment will be assessed.

References

1. Clubbs, R. L.; Brooks, B. W. *Ecotoxicology and Environmental Safety* **2007**, *67*(3), 385–398.
2. Goto, T.; Hiromi, J. *Marine Pollution Bulletin* **2003**, *47*(1-6), 139–142.
3. Barton, M.; Duby, R.; Traupe, T. *Expert Opinion on Investigational Drugs* **2002**, *11*, 329–332.
4. Johnson, A. C.; Williams, R. J. *Environmental Science & Technology* **2004**, *38*(13), 3649–3658.

Acknowledgments

- The Independent College Fund of NJ
- Novo Nordisk- Becton College Summer Research Program
- Becton College Grant-In-Aid Program