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

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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 **17 α -ethinyl estradiol (EE2)**.
- EE2 is medically **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

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: Silylation 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
Injection Details	SSL Injector in Splitless Mode 45 s Splitless Time, 250 °C
Temperature Program	95 °C (1 min hold) - 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 25.00 μ m
Mass Spectrometer	Full Scan Mode (40-450 AMU) Dwells Every 0.20 Seconds

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.

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).

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

Table 2: Species identification parameters.

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

N = 18

- All calibrators (5) were analyzed in triplicates for a concentration range between ≈ 50 ppb and ≈ 540 ppb.
- Calibration plots (Figure 8) were made by plotting the average $\frac{A_{\text{Estrogen}}}{A_{\text{Internal Standard}}}$ versus $\frac{[\text{Estrogen}]}{[\text{Internal Standard}]}$ 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 $\pm$ 0.010	-0.001 $\pm$ 0.002	0.996
β -Estradiol	0.336 $\pm$ 0.017	0.000 $\pm$ 0.004	0.992
17 α -Ethinylestradiol	0.114 $\pm$ 0.004	0.001 $\pm$ 0.001	0.996
Estriol	0.093 $\pm$ 0.002	0.000 $\pm$ 0.000	0.999

N = 5, K = 3

Conclusions / Further Studies

- The method was validated using 2L, ≈ 100 ppt ($N = 4$) 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.

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