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**Page S1**

Analysis of Androgenic Steroids in Environmental Waters by Large-volume Injection Liquid Chromatography Tandem Mass Spectrometry

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## 14 **Results**

15 **Inter and Intra Day RSD.** The inter and intra-day RSDs in wastewater influent ranged from 1.6 to 6.8 and  
16 3.7 to 6.8 %, respectively, while the combined RSDs ranged from 4.1 to 8.2 % for analytes with stability  
17 regression slopes that are statistically equivalent to zero (**Table S2**). Combining intra and inter-day RSD  
18 describes the overall precision of the method within and between days.<sup>1</sup> Combined RSDs are rarely  
19 calculated, but the inter-day RSDs presented here are slightly lower than what has been presented for  
20 androgens, although the sample matrix of that study was effluent.<sup>2</sup>

**Table S1)** Comparison of concentration values ( $\pm$  95% CI) obtained by standard addition and internal standard calibration for analytes in wastewater influent.

Analyte	Standard Addition Concentration (ng/L)	Internal Standard Calibration Concentration (ng/L)
<b>Test</b>	102 $\pm$ 3.9	97.2 $\pm$ 4.9
<b>Ando</b>	54.5 $\pm$ 3.0	52.6 $\pm$ 3.7
<b>Bold</b>	61.6 $\pm$ 7.0	59.0 $\pm$ 3.8
<b>5-Andro</b>	1620 $\pm$ 40	1260 $\pm$ 120*
<b>Meta</b>	215 $\pm$ 9.0	216 $\pm$ 9.0
<b>6-Meta</b>	1630 $\pm$ 130	997 $\pm$ 130*
<b>Stan</b>	218 $\pm$ 6.7	215 $\pm$ 15
<b>16-Stan</b>	209 $\pm$ 11	202 $\pm$ 12
<b>Tren</b>	203 $\pm$ 15	188 $\pm$ 7.5
<b>Epi Tren</b>	196 $\pm$ 11	188 $\pm$ 4.5
<b>Metete</b>	191 $\pm$ 6.7	189 $\pm$ 17
<b>CH<sub>3</sub>-Test</b>	209 $\pm$ 6.6	204 $\pm$ 5.7
<b>Nand</b>	214 $\pm$ 11	208 $\pm$ 17
<b>THG</b>	199 $\pm$ 9.4	204 $\pm$ 20
<b>Ox</b>	790 $\pm$ 33	775 $\pm$ 11
<b>Epi-Ox</b>	1580 $\pm$ 77	1360 $\pm$ 120*

\*indicates statistical difference between concentrations values at the 95% CI.

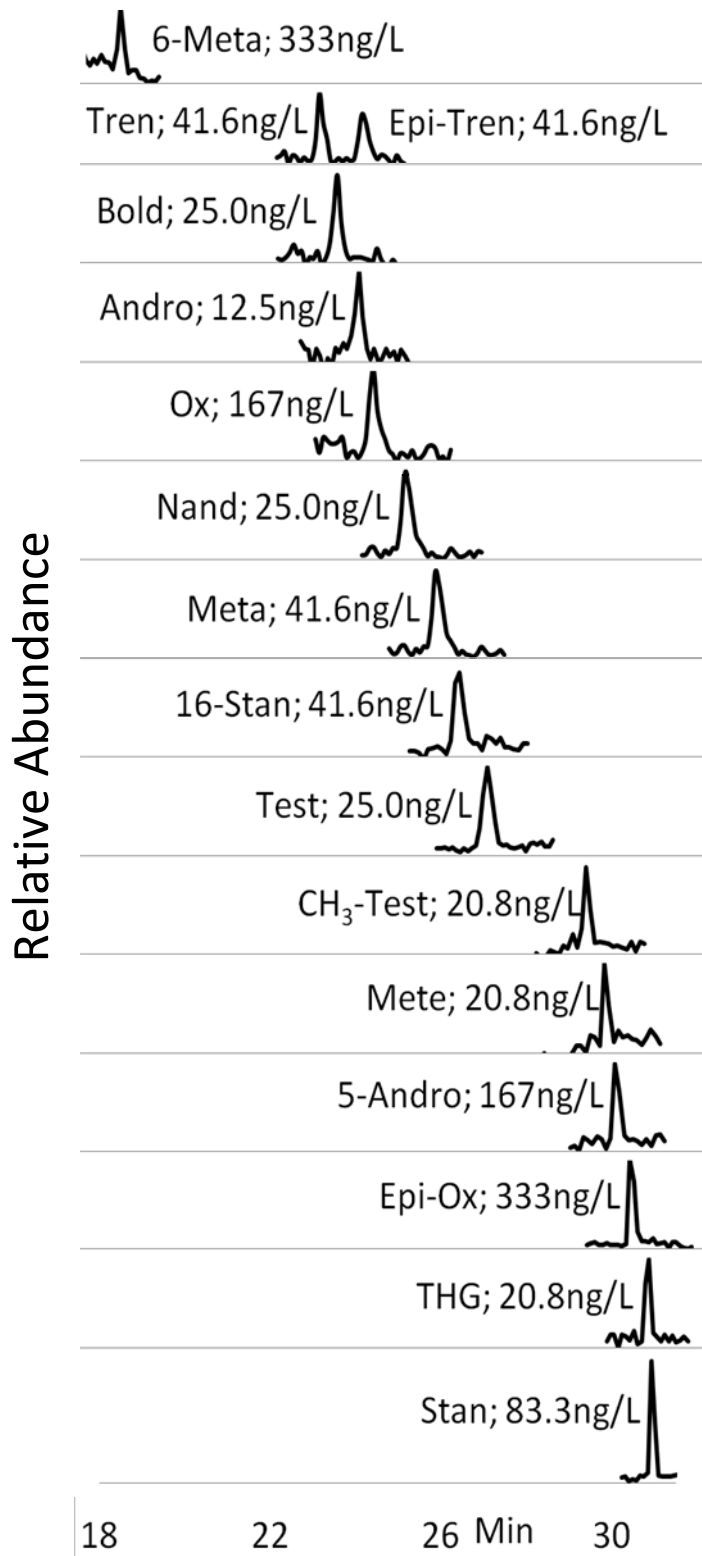
**Table S2)** RSDs calculated from a one-way ANOVA and stability study data.<sup>3</sup>

	Intra Day RSD %	Inter Day RSD %	Combined RSD %
Test	5.1	6.3	8.1
Ando	4.9	6.2	7.9
Bold	4.8	1.7	5.1
5-Andro	5.9	3.1	6.7
Meta	5.4	4.1	6.8
6-Meta	6.2	5.3	8.2
Stan	4.7	3.4	5.8
16-Stan	5.8	5.8	8.2
Tren	5.5	3.1	6.3
Epi-Tren	5.7	1.6	6.0
Mete	5.4	4.1	6.8
CH3-Test	3.7	1.9	4.1
Nand	4.8	3.7	6.0
THG	6.8	4.5	8.1
Ox	4.6	6.8	8.2
Epi-Ox	6.0	11	12

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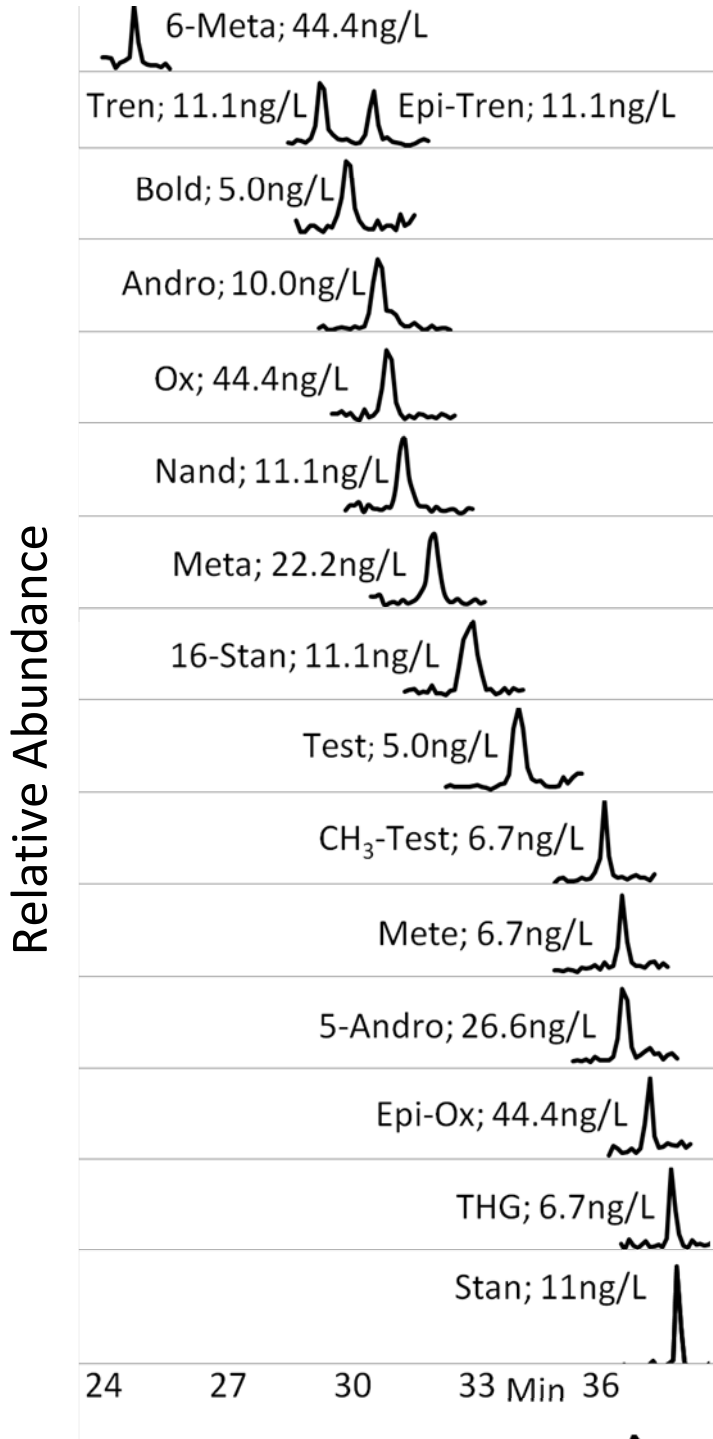
**Figure S1)** Chromatograms of analytes spiked into wastewater effluent matrices near their LOD or LOQ.



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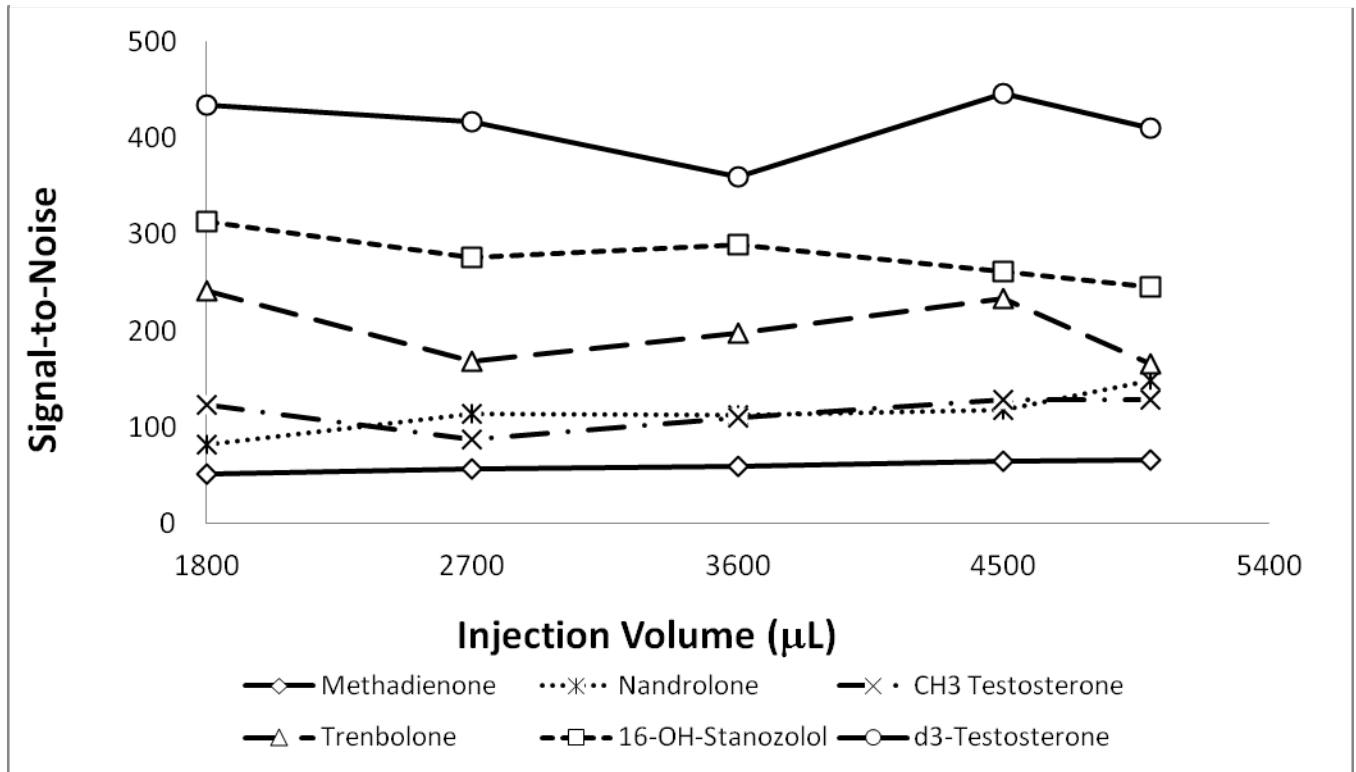
**Figure S2)** Analytes spiked into river water matrices near their LOD or LOQ.



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**Figure S3)** Signal-to-noise as a function of injection volume for selected analytes in wastewater influent. Analyte concentration was kept constant at 250 ng/L.



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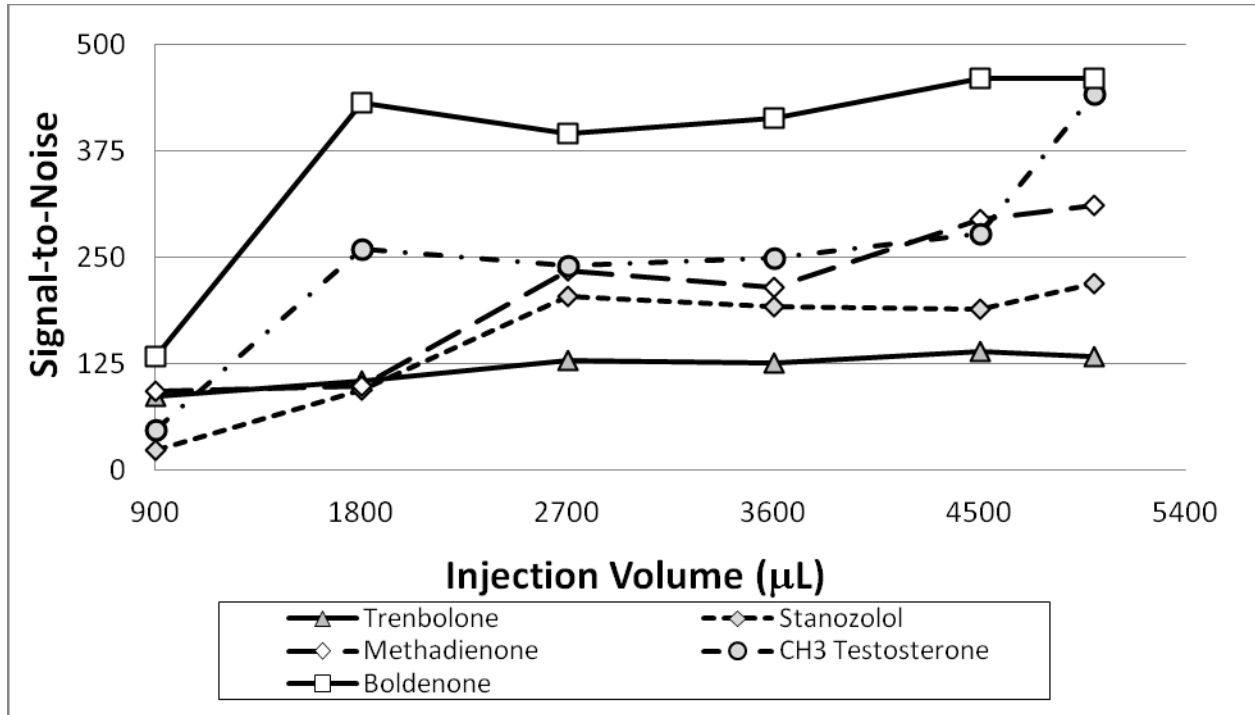
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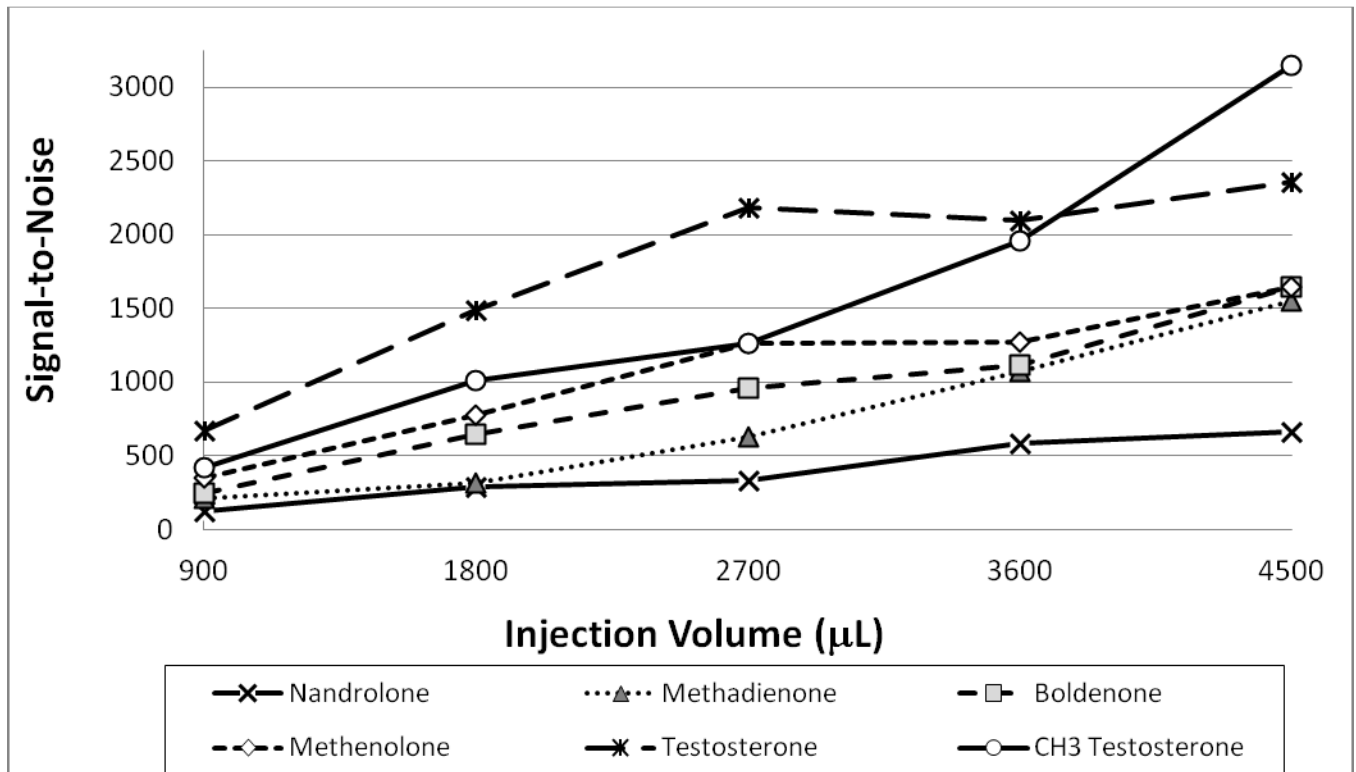
**Figure S4)** Signal-to-noise as a function of injection volume for selected analytes in wastewater effluent. Analyte concentration was kept constant at 150 ng/L.



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45 **Figure S5)** Signal-to-noise as a function of injection volume of selected analytes in fish-housing water. Analyte  
46 concentration was kept constant at 150 ng/L.



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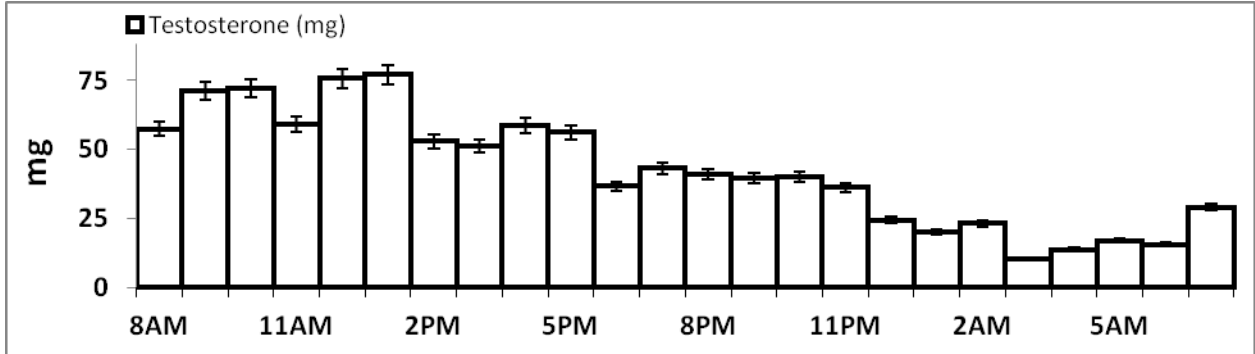
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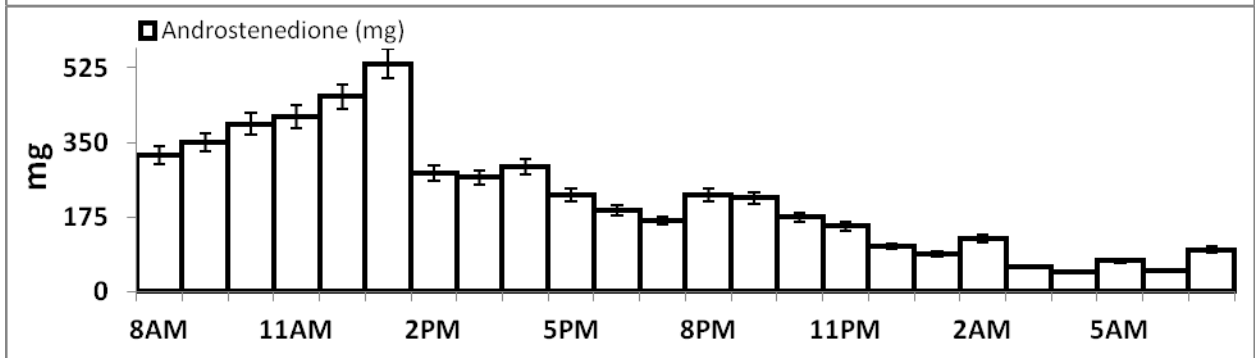
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**Figure S6)** Diurnal profiles of analyte concentrations ( $\pm$  within-day RSD\*) present in the one hour composite samples of influent. Nandrolone was excluded because most values were below the LOQ.

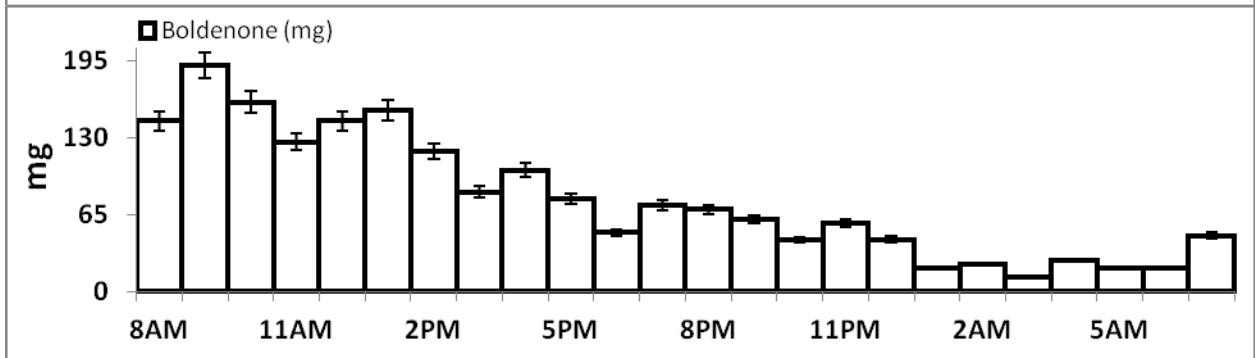
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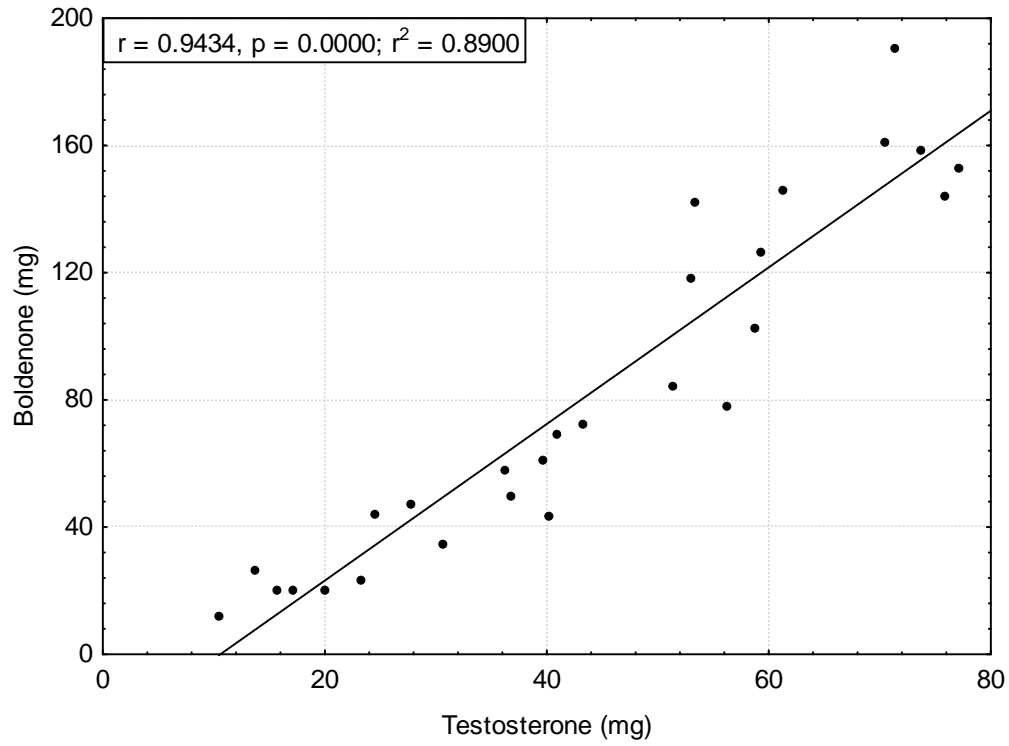
\*Error bars are given in mg  $\pm$  (mass load (mg) x within-day RSD%)

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**Figure S7)** Correlation of Boldenone loads to Testosterone loads in one-hr composite waste water influent samples



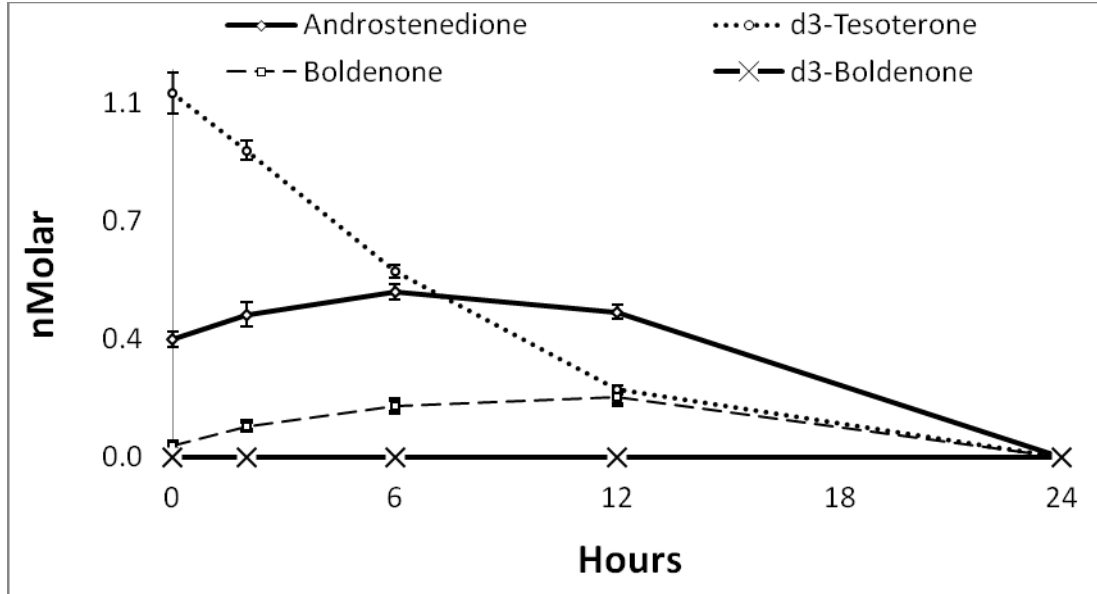
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**Figure S8)** Analyte concentrations in wastewater influent over time. d<sub>3</sub>-Test was spiked into wastewater influent to test if d<sub>3</sub>-Bold would be produced.



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