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

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Results

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

Table S1) Comparison of concentration values (± 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)
Test	102 ± 3.9	97.2 ± 4.9
Ando	54.5 ± 3.0	52.6 ± 3.7
Bold	61.6 ± 7.0	59.0 ± 3.8
5-Andro	1620 ± 40	1260 ± 120*
Meta	215 ± 9.0	216 ± 9.0
6-Meta	1630 ± 130	997 ± 130*
Stan	218 ± 6.7	215 ± 15
16-Stan	209 ± 11	202 ± 12
Tren	203 ± 15	188 ± 7.5
Epi Tren	196 ± 11	188 ± 4.5
Mete	191 ± 6.7	189 ± 17
CH_3-Test	209 ± 6.6	204 ± 5.7
Nand	214 ± 11	208 ± 17
THG	199 ± 9.4	204 ± 20
Ox	790 ± 33	775 ± 11
Epi-Ox	1580 ± 77	1360 ± 120*

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

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Table S2) RSDs calculated from a one-way ANOVA ar
stability study data.[3]

	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

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

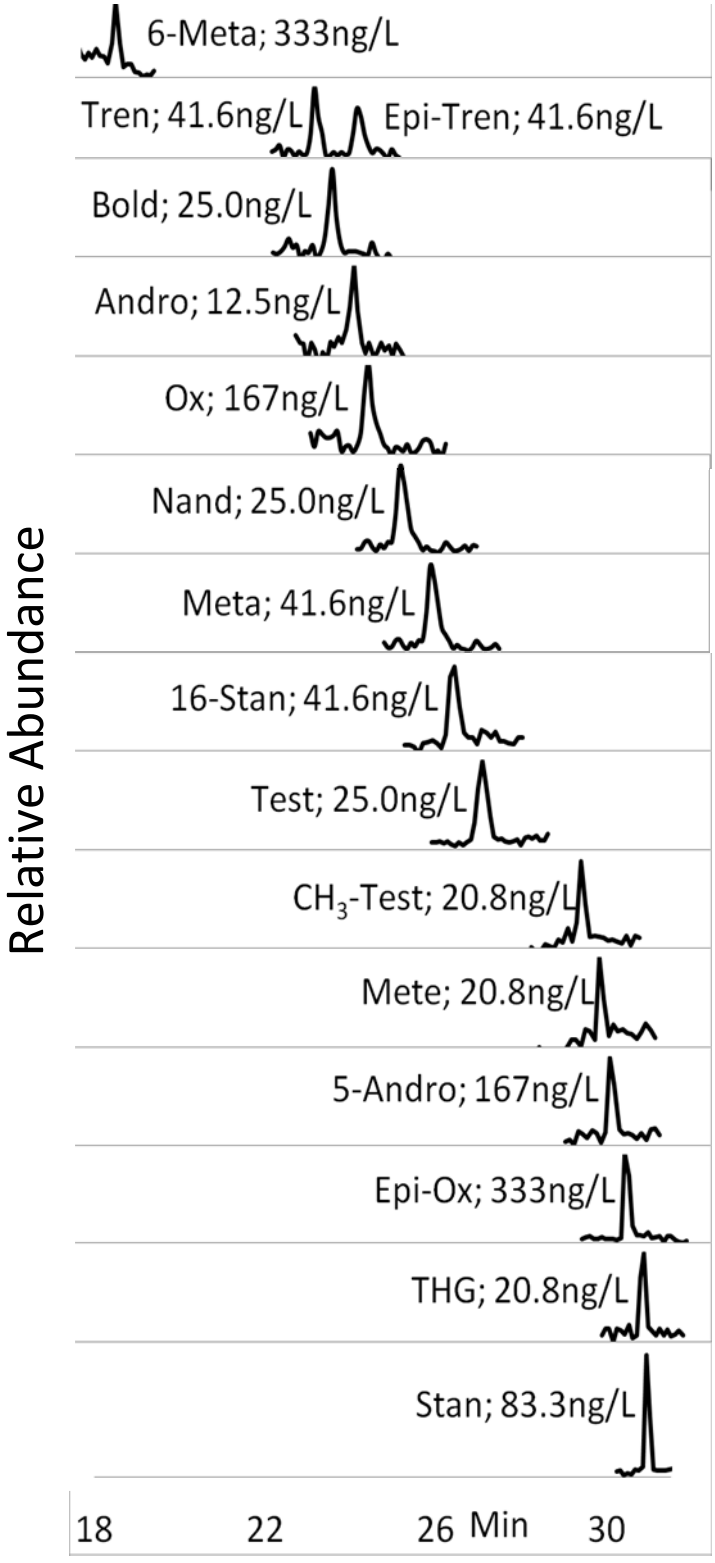

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Figure S2) Analytes spiked into river water matrices near their LOD or LOQ.

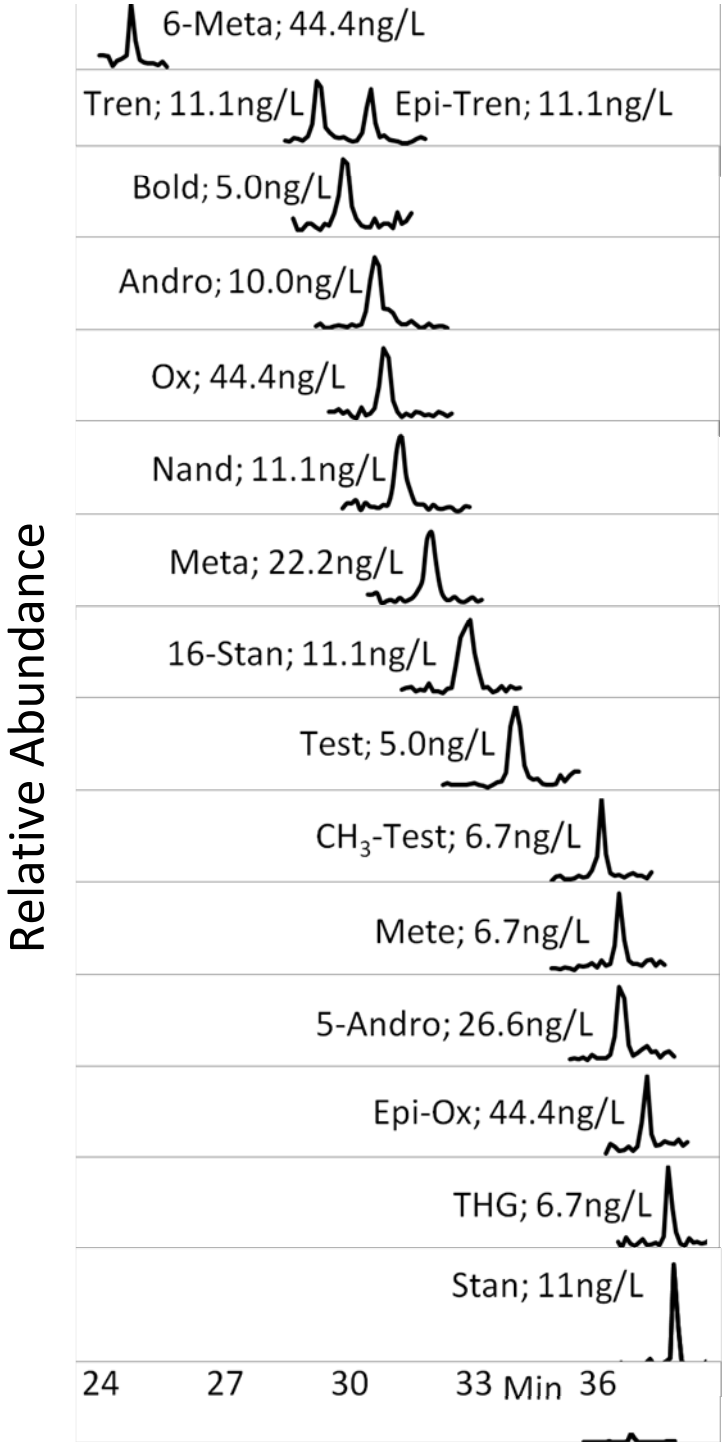


Figure S3) Signal-to-noise as a function of injection volume for selected analytes in wastewater influent. Analyte concentration was kept constant at 250 ng/L.

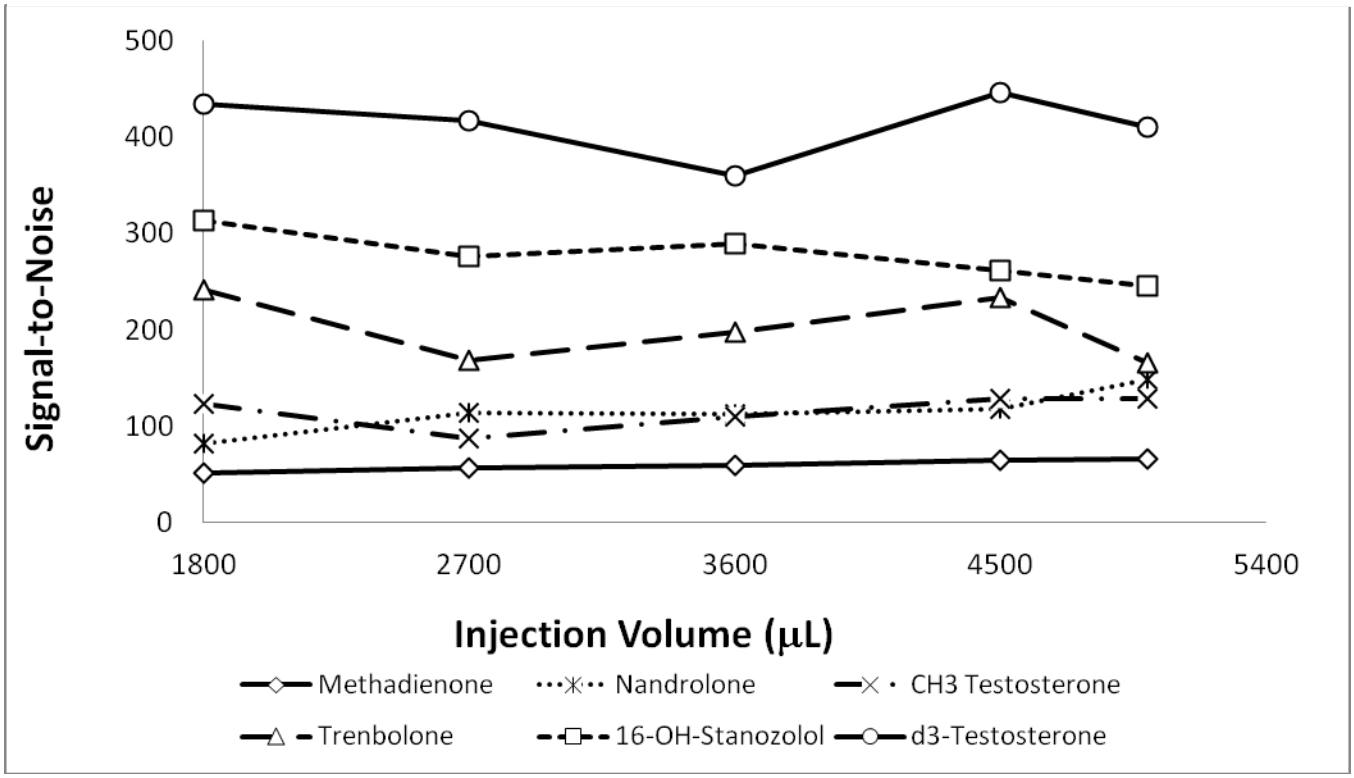


Figure S4) Signal-to-noise as a function of injection volume for selected analytes in wastewater effluent. Analyte concentration was kept constant at 150 ng/L.

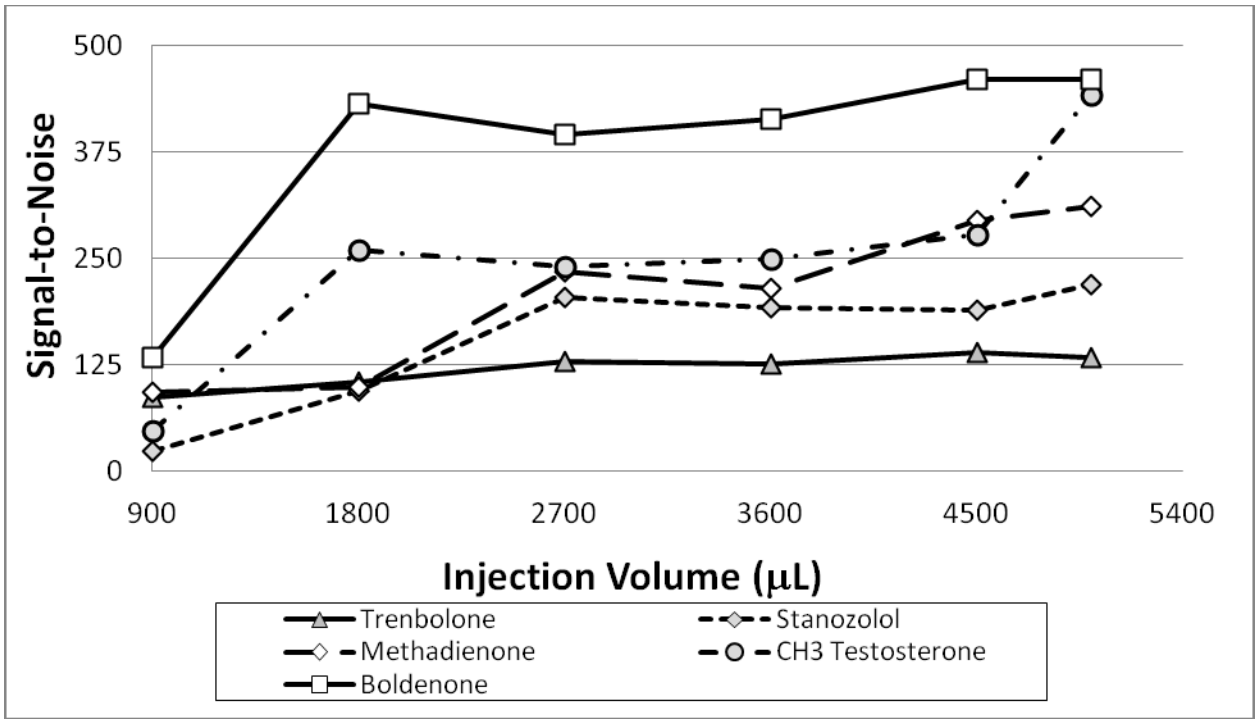
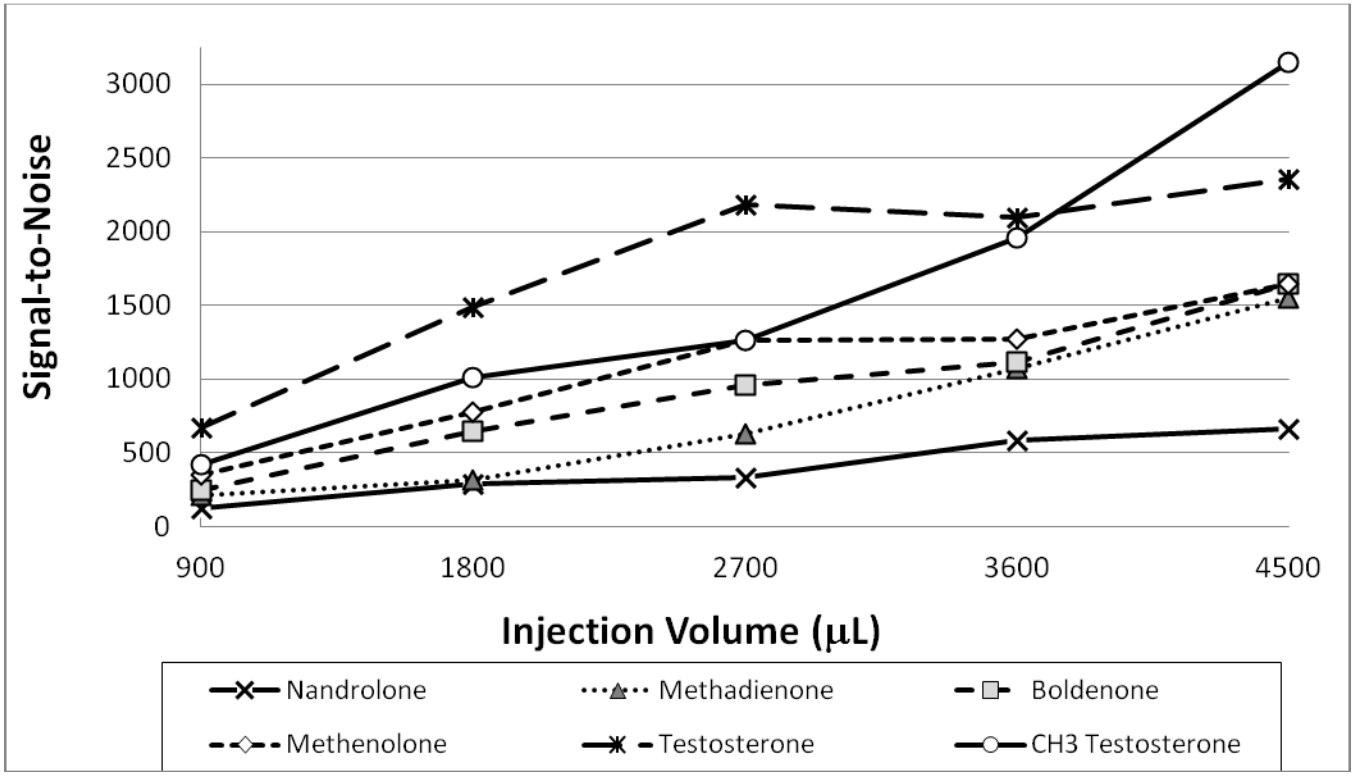


Figure S5) Signal-to-noise as a function of injection volume of selected analytes in fish-housing water. Analyte concentration was kept constant at 150 ng/L.



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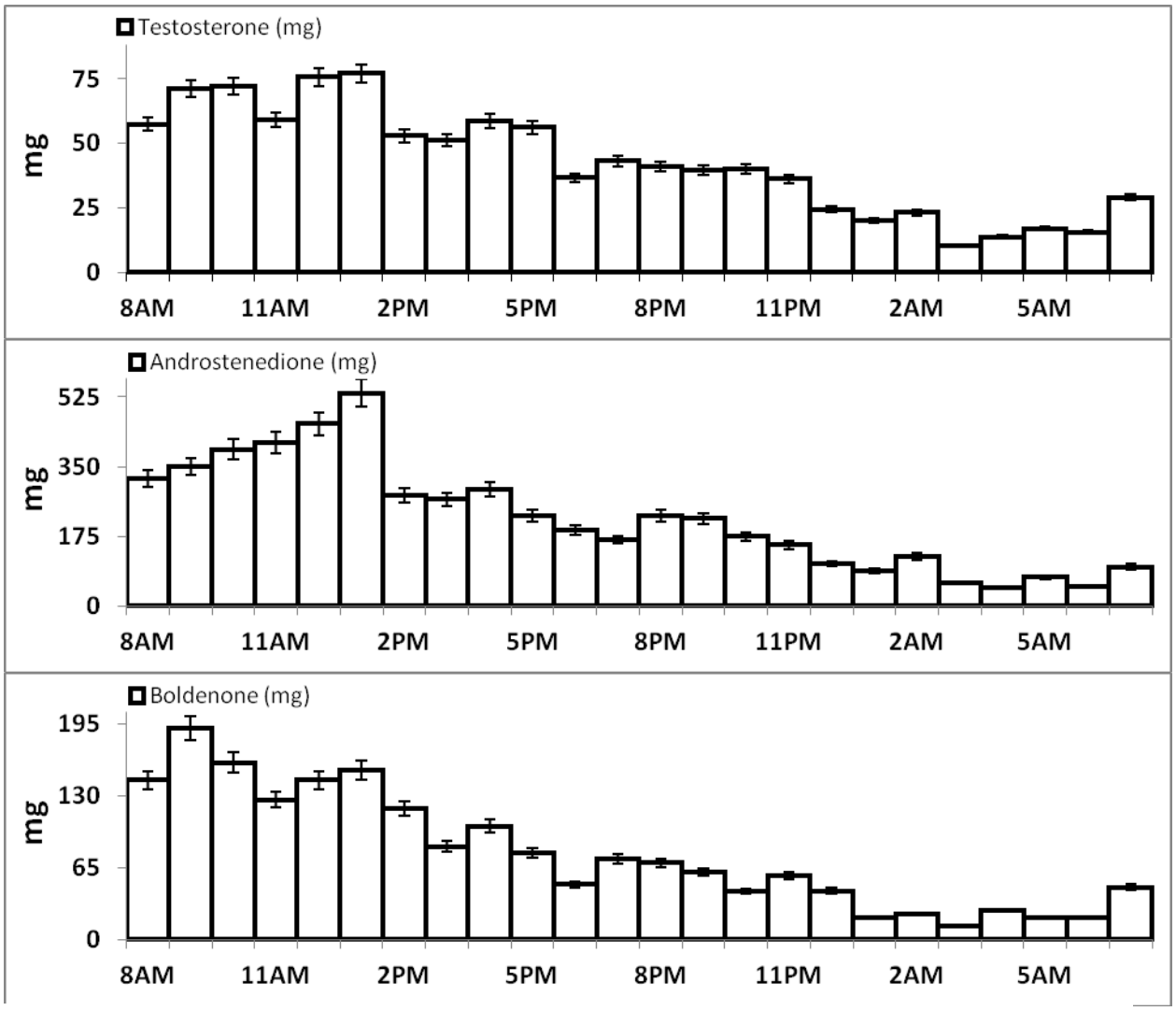
50 **Figure S6)** Diurnal profiles of analyte concentrations (± within-day RSD*) present in the one hour composite samples of influent. Nandrolone was excluded because most values were below the LOQ.



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

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

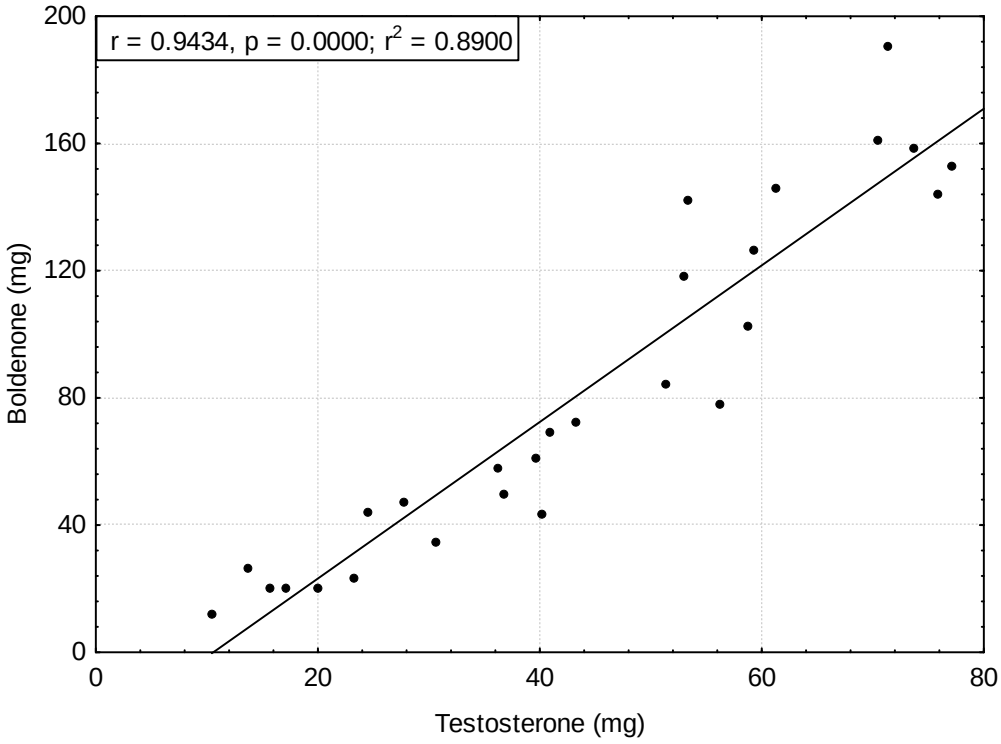

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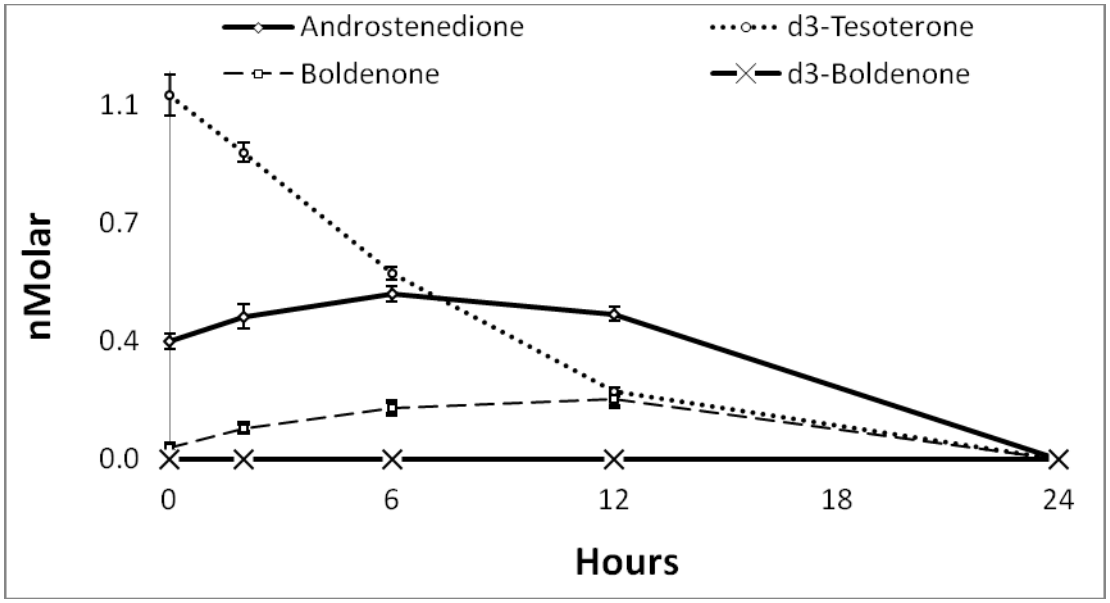
Figure S8) Analyte concentrations in wastewater influent over time. d_3-Test was spiked into
60 wastewater influent to test if d_3-Bold would be produced.



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(1) Chiaia, A. C.; Banta-Green, C.; Field, J. *Environ. Sci. Technol.* **2008,** *42*, 8841-8848.

(2) Schroder, H. F.; Gebhardt, W.; Thevis, M.; *Anal. Bioanal. Chem.* **2010,** *398*, 1207-1229.

(3) Anderson, R. L., *Practical Statistics for Analytical Chemists*. Van Nostrand Reinhold Company Inc.: New York, 1987.