Electronic Supplementary Material (ESI) for Analytical Methods.


# A systematic approach of method development for analysis of multiple classes of Emerging contaminants in wastewater: A case study of a biological nutrient removal based plant

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## Details of the Treatment Plant:

The WWTP is based on advanced activated sludge process also aimed to remove nitrogen and phosphorus. It consists of a coarse screen of 20 mm diameter and fine mechanical and manual screen of 10 mm diameter, a cyclonic grit and grease chamber, primary clarifier. The effluent of the primary clarifier enters the anaerobic zone for phosphorus removal followed by anoxic zone where denitrification process reduces nitrate to nitrogen gas. Aeration converts ammonical nitrogen to nitrate, which is recycled to the anoxic zone. The SRT of the aeration tank is 20 days.

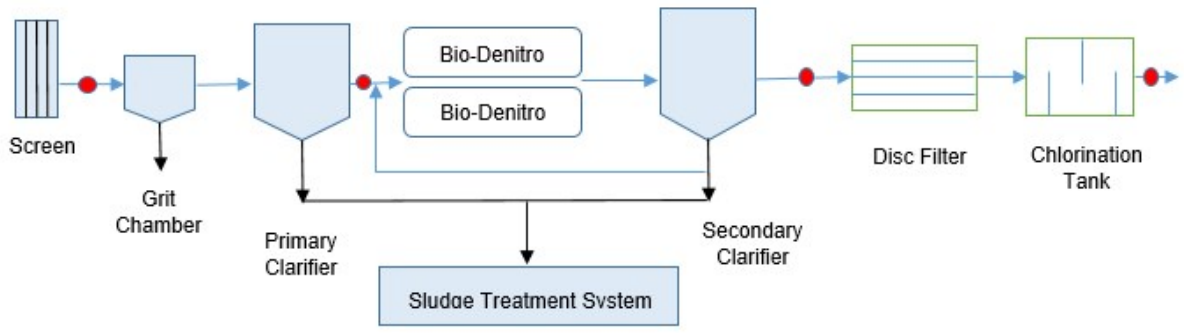



Fig. S1: Schematic diagram of the WWTP with sample collection points.

Table S1: 4 hourly flow variation over 24 hours in the treatment plant

| Time | Average Flow ($m^3$/hr) |
|---|---|
| 11:00 | 2348 |
| 15:00 | 4251 |
| 19:00 | 2127 |
| 23:00 | 1949 |
| 03:00 | 659 |
| 07:00 | 269 |

## 1. LCMS method optimization

Table S2: Signal Intensity at different flow rate of the mobile phase S1, A:B (Water + 0.1 % FA : ACN + 0.1% FA); Capillary voltage – 3.5kV.

| Compound | Flow = 0.300 mL/min | Flow = 0.500 mL/min | Flow = 0.700 mL/min |
|---|---|---|---|
| Estrone | 19802832 | ND | ND |
| Sulfamethoxazole | 72627463 | 46454640 | 42294373 |
| Progesterone | ND | 39669868 | 43542157 |
| Trimethoprim | ND | ND | ND |
| 2-hydroxybenzothiazole | 61269459 | 26951726 | 20193144 |
| Ketoprofen | 202583547 | 92178124 | 59193763 |
| Caffeine | 44615006 | 28815545 | 22786547 |
| Naproxen | 48509773 | 14914008 | 8446238 |
| Testosterone | 185657837 | 85034777 | 53201361 |
| Carbamazepine | Peak distorted | 164886342 | 126603318 |
| Enrofloxacin | 68723536 | 53231546 | 42137894 |
| Gemfibrozil | 5264896 | 4951279 | 3742562 |
| Triclosan | 4445132 | 3716425 | 2819821 |
| Diclofenac | 5124953 | 4172596 | 3346389 |

Table S3: Signal Intensity at different capillary voltage for mobile phase, A:B (Water + 0.1 % FA : ACN + 0.1% FA).

| Compound | Cap vol = 1 | Cap vol = 2 | Cap vol = 3 | Cap Vol = 3.5 | Cap Vol = 4 |
|---|---|---|---|---|---|
| Estrone | ND | ND | ND | 19802832 | ND |
| Sulfamethoxazole | 94631451 | 82551319 | 74719932 | 72627463 | 62950765 |
| Progesterone | ND | ND | ND | ND | ND |
| Trimethoprim | 210905785 | 153313121 | 133272580 | ND | 166450253 |
| 2-hydroxybenzothiazole | 52809821 | 67366561 | 70411348 | 61269459 | 69798550 |
| Ketoprofen | 150953997 | 211620758 | 245323277 | 202583547 | 236610342 |
| Caffeine | 45589382 | 37316618 | 33948483 | 44615006 | 36389053 |
| Naproxen | 14091561 | 31779626 | 46900032 | 48509773 | 53894363 |

| | | | | | |
|---|---|---|---|---|---|
| Testosterone | 198138294 | 209986236 | 207923341 | 185657837 | 185058080 |
| Carbamazepine | 437705465 | 363394390 | 327037773 | Peak distorted | Peak distorted |
| Enrofloxacin | 53824561 | 74258463 | 85465778 | 68723536 | 59565432 |
| Gemfibrozil | 12089743 | 10843562 | 7426232 | 5264896 | 4179364 |
| Triclosan | 4125482 | 4145675 | 4468731 | 4445132 | 4235625 |
| Diclofenac | 7434658 | 6548721 | 5418735 | 5124953 | 4953134 |

Table S4: Signal Intensity at different capillary voltage for mobile phase, A:B (Water + 0.1 % FA : MeOH)

| **Compound** | **Cap vol = 1** | **Cap 3** | **Cap vol = 4** |
|---|---|---|---|
| Estrone | ND | Peak distorted | Peak distorted |
| Sulfamethoxazole | 51155743 | 51424673 | 51659500 |
| Progesterone | ND | 56101209 | ND |
| Trimethoprim | 1507951861 | 827953518 | 361079979 |
| 2-hydroxybenzothiazole | 30856382 | 53892104 | 62346454 |
| Ketoprofen | 29432870 | 72453827 | 102278437 |
| Caffeine | 68750508 | 70163964 | Peak distorted |
| Naproxen | 57264835 | 88207930 | Peak distorted |
| Testosterone | 30821542 | 89261573 | 106385058 |
| Carbamazepine | 207566556 | 219784325 | 236630034 |
| Enrofloxacin | 75248628 | 141056213 | 68457216 |
| Gemfibrozil | 8465251 | 18519754 | 6561862 |
| Triclosan | 3491278 | 4128418 | 4628471 |
| Diclofenac | 3458231 | 3612194 | 4282359 |

Table S5: Signal Intensity at different capillary voltage for mobile phase, A:B (10mM $NH_4Ac$: ACN + 0.1% FA)

| **Compound** | **Cap vol = 1** | **Cap vol = 3** |
|---|---|---|
| Estrone | ND | ND |
| Sulfamethoxazole | 67558617 | 99879892 |
| Progesterone | 174983328 | 217849544 |
| Trimethoprim | Peak distorted | Peak distorted |
| 2-hydroxybenzothiazole | 14993600 | 25641856 |
| Ketoprofen | 152093250 | 66263818 |
| Caffeine | Peak distorted | Peak distorted |
| Naproxen | Peak distorted | Peak distorted |
| Testosterone | 504728426 | 64794345 |
| Carbamazepine | 1415831887 | 408142383 |
| Enrofloxacin | ND | ND |
| Gemfibrozil | ND | ND |
| Triclosan | 5428254 | 5640873 |

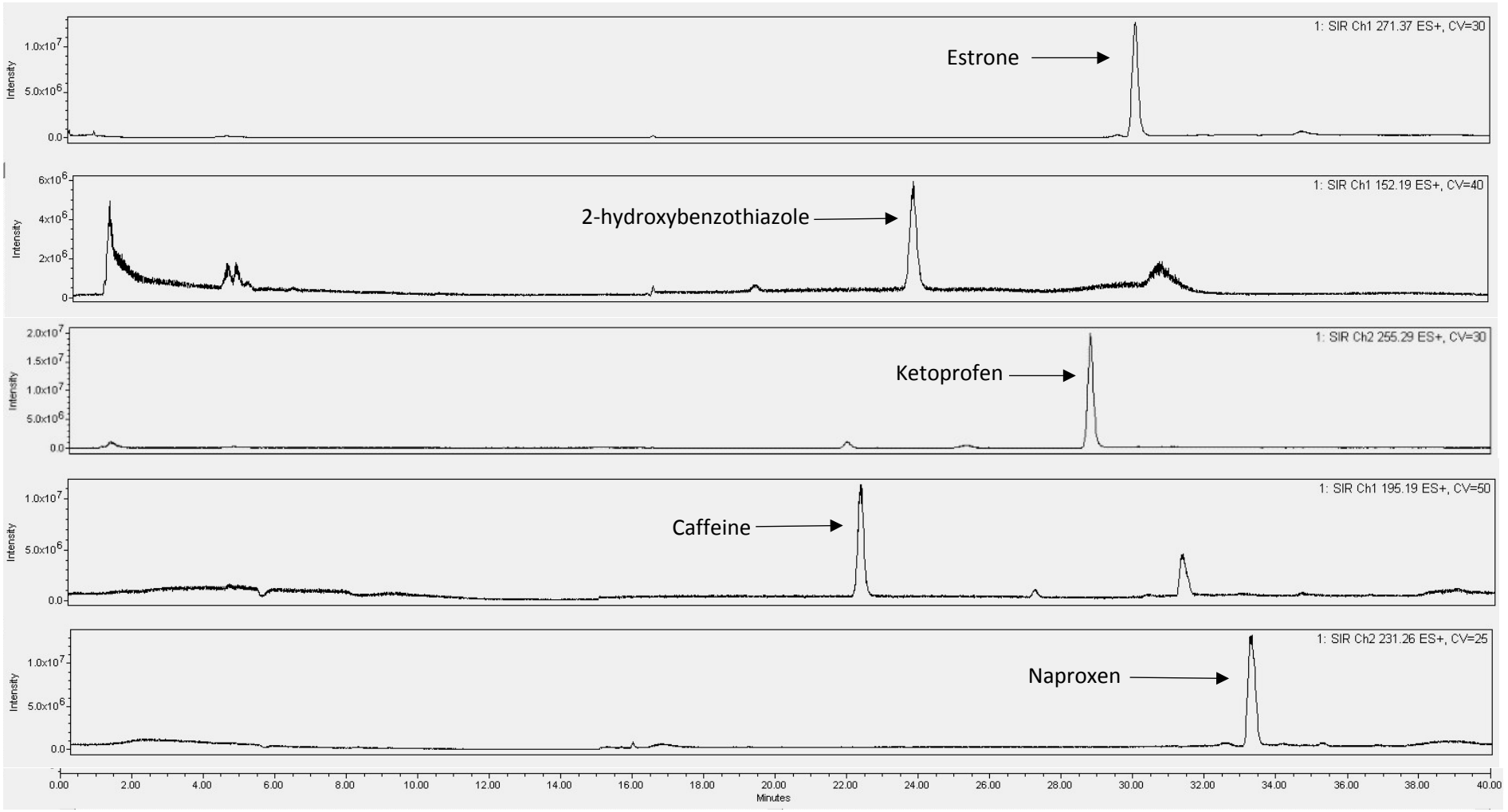
| | | |
|---|---|---|
| Diclofenac | 28435617 | 43208795 |

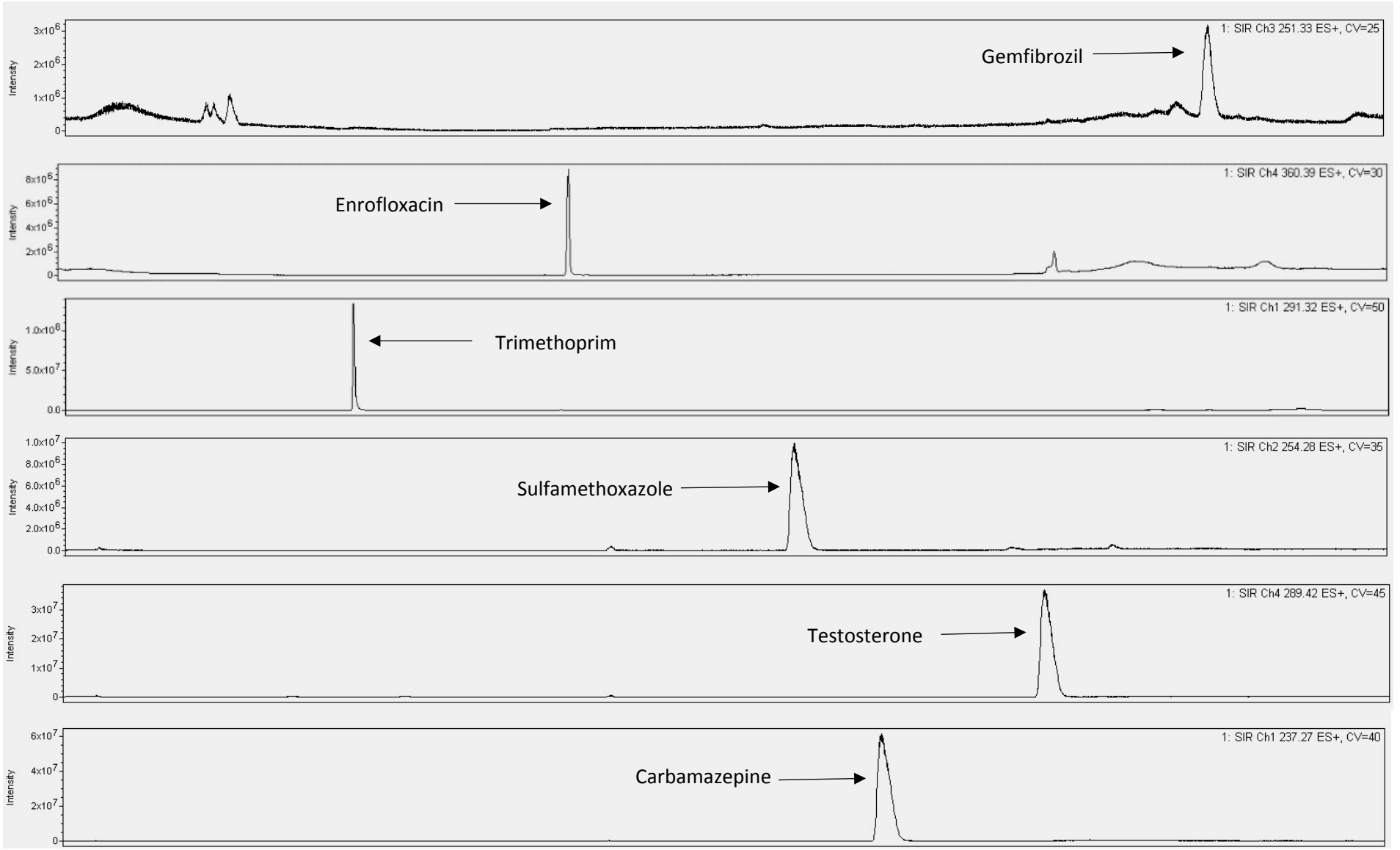
**2. SPE Optimization of eluent**

Table S6: % Recovery of the analytes for different eluents:

| **Analytes** | **ACN [A]** | **% RSD [A]** | **MeOH [B]** | **% RSD [B]** | **$NH_4OH$:MeOH (5:95, v/v) [C]** | **% RSD [C]** | **FA:MeOH (2:98, v/v) [C]** | **% RSD [D]** |
|---|---|---|---|---|---|---|---|---|
| 2 hydroxy | 103.13 | 2.4 | 116.10 | 1.2 | 123.71 | 6.1 | 80.41 | 1.6 |
| Estrone | 94.17 | 1.8 | 96.89 | 0.8 | 100.56 | 5.0 | 85.67 | 0.4 |
| KTP | 99.75 | 3.6 | 101.20 | 0.4 | 98.12 | 12.3 | 114.83 | 0.2 |
| Diclofenac | 31.66 | 8.6 | 72.32 | 2.2 | 289.76 | 2.1 | 69.93 | 2.5 |
| Triclosan | 5.68 | 2.3 | 60.62 | 2.1 | 112.86 | 0.4 | 18.64 | 0.9 |
| Progesterone | 80.76 | 2.9 | 76.25 | 2.3 | 105.92 | 0.6 | 75.04 | 2.0 |
| CZP | 56.95 | 0.41 | 96.01 | 4.6 | 100.95 | 0.2 | 101.50 | 0.1 |
| Testosterone | 42.87 | 0.87 | 54.30 | 3.5 | 36.89 | 0.3 | 59.78 | 0.8 |
| SMZ | 18.54 | 5.3 | 19.54 | 5.4 | 82.81 | 7.2 | 17.82 | 4.2 |
| Caffiene | 79.81 | 1.7 | 87.28 | 4.1 | 98.96 | 6.4 | 97.19 | 7.6 |
| Enrofloxacin | 89.78 | 0.62 | 89.15 | 0.7 | 92.48 | 0.3 | 79.71 | 0.3 |
| Gemfibrozil | 66.32 | 2.5 | 100.95 | 0.2 | 120.30 | 1.3 | 97.67 | 1.5 |
| Naproxen | 79.75 | 12.2 | 77.49 | 3.4 | 83.76 | 2.7 | 102.41 | 20.8 |
| Trimethoprim | 96.07 | 1.9 | 96.50 | 0.6 | 93.85 | 1.50 | 97.85 | 0.4 |

**Chromatograms of analytes in wastewater sample.**




Intensity
1: SIR Ch3 251.33 ES+, CV=25
Gemfibrozil
1: SIR Ch4 360.39 ES+, CV=30
Enrofloxacin
1: SIR Ch1 291.32 ES+, CV=50
Trimethoprim
1: SIR Ch2 254.28 ES+, CV=35
Sulfamethoxazole
1: SIR Ch4 289.42 ES+, CV=45
Testosterone
1: SIR Ch1 237.27 ES+, CV=40
Carbamazepine

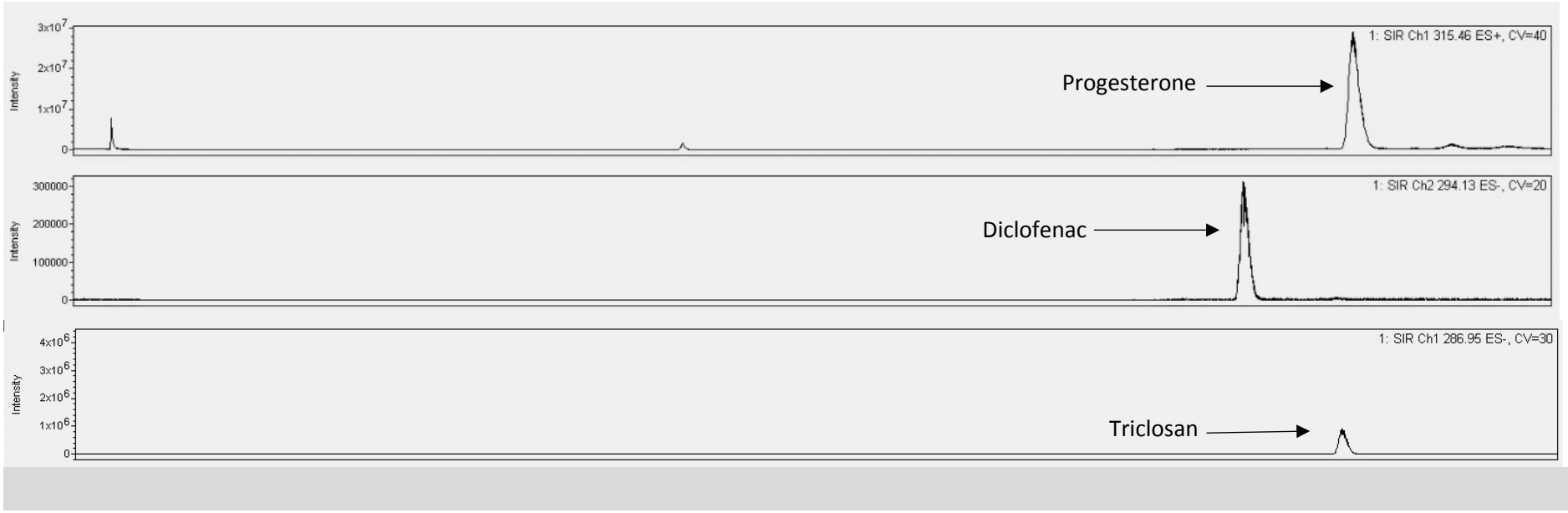



Fig. S2: Chromatogram of the wastewater sample spiked at 100 µg/L of analytes.