

# Targeted and non-targeted screening of biomarkers in wastewater: towards a unique analytical methodology for sample preparation

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## 1. Supplementary materials

Table S1: properties of targeted compounds

|                                             | Compound name    | Molecular weight | Log D at pH 7.4* | Log p* | CAS         | Supplier      | Strongest basic pKa** | Strongest acidic pKa** | (+/-) at pH 7 |
|---------------------------------------------|------------------|------------------|------------------|--------|-------------|---------------|-----------------------|------------------------|---------------|
| <b>Pharmaceuticals</b>                      |                  |                  |                  |        |             |               |                       |                        |               |
| <i>Antibiotics</i>                          |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Clarithromycin   | 749.0            | 2.4              | 3.2    | 81103-11-9  | Cluzeau       | 9                     | 12.46                  | +             |
|                                             | Sulfamethoxazole | 253.3            | -0.6             | 0.9    | 723-46-6    | Cluzeau       | 1.97                  | 6.16                   | -             |
|                                             | Trimethoprim     | 290.3            | -1.2             | 0.4    | 738-70-5    | Cluzeau       | 7.16                  | (-)                    | +             |
| <i>Analgesics &amp; anti-inflammatories</i> |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Acetaminophen    | 151.2            | 0.4              | 0.3    | 103-90-2    | Cluzeau       | (-)                   | 9.46                   | 0             |
|                                             | Diclofenac       | 296.2            | 1.4              | 4.1    | 15307-79-6  | Cluzeau       | (-)                   | 4                      | -             |
|                                             | Ibuprofen        | 206.3            | 0.5              | 3.7    | 15687-27-1  | Cluzeau       | (-)                   | 4.85                   | -             |
|                                             | Ketoprofen       | 254.3            | 0.1              | 2.8    | 22071-15-4  | Cluzeau       | (-)                   | 3.88                   | -             |
| <i>β-blockers</i>                           |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Metoprolol       | 267.4            | -0.3             | 1.8    | 51384-51-1  | Cluzeau       | 9.67                  | (-)                    | +             |
|                                             | Propanolol       | 259.3            | 1.2              | 3.1    | 525-66-6    | Sigma-Aldrich | 9.67                  | (-)                    | +             |
|                                             | Salbutamol       | 239.3            | -1.5             | 0.0    | 18559-94-9  | CIL           | 9.4                   | 10.12                  | +             |
| <i>Anticonvulsants</i>                      |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Carbamazepine    | 236.3            | 2.3              | 2.7    | 298-46-4    | Cluzeau       | (-)                   | (-)                    | 0             |
|                                             | Gabapentin       | 171.2            | -1.4             | 1.2    | 60142-96-3  | Cluzeau       | 9.91                  | 4.63                   | z (+/-)       |
| <i>Antidepressants</i>                      |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Amisulpride      | 369.5            | -0.4             | 1.6    | 71675-85-9  | LGC           | 8.28                  | (-)                    | +             |
|                                             | Citalopram       | 324.4            | 1.3              | 2.5    | 59729-32-7  | Cluzeau       | 9.78                  | (-)                    | +             |
|                                             | Fluoxetine       | 309.3            | 1.8              | 4.1    | 54910-89-3  | Cluzeau       | 9.8                   | (-)                    | +             |
|                                             | Venlafaxine      | 277.4            | 1.4              | 2.9    | 99300-78-4  | Cluzeau       | 8.91                  | (-)                    | +             |
| <i>Antidiabetics</i>                        |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Glibenclamide    | 494.0            | 1.9              | 3.8    | 10238-21-8  | Cluzeau       | (-)                   | 4.32                   | -             |
|                                             | Metformin        | 129.2            | -3.4             | -2.3   | 657-24-9    | Cluzeau       | 12.33                 | (-)                    | -             |
| <i>Antihistamines</i>                       |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Cetirizine       | 388.9            | -0.6             | 2.2    | 83881-52-1  | CIL           | 7.42                  | 3.59                   | z (+/-)       |
|                                             | Desloratadine    | 310.8            | 2.1              | 6.8    | 100643-71-8 | CIL           | 9.73                  | (-)                    | +             |
|                                             | Fexofenadine     | 501.7            | 2.4              | 4.8    | 153439-40-8 | CIL           | 9.01                  | 4.04                   | z (+/-)       |
| <i>Hormones</i>                             |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Levonorgestrel   | 312.5            | 3.3              | 3.9    | 797-63-7    | LGC           | (-)                   | (-)                    | 0             |
| <i>Anti-hypertensive</i>                    |                  |                  |                  |        |             |               |                       |                        |               |
|                                             | Candesartan      | 440.5            | 0.0              | 5.0    | 139481-59-7 | CIL           | 1.5                   | 3.44                   | -             |

|                                  |                                     |       |      |      |              |               |      |       |         |
|----------------------------------|-------------------------------------|-------|------|------|--------------|---------------|------|-------|---------|
|                                  | Irbesartan                          | 428.5 | 1.2  | 4.5  | 138402-11-6  | CIL           | 4.12 | 5.85  | -       |
| <b>Licit &amp; illicit drugs</b> |                                     |       |      |      |              |               |      |       |         |
| <i>Stimulants</i>                | Anhydroecgonine Methyl Ester (AEME) | 181.2 | 0.6  | 1.7  | 43021-26-7   | LGC           | 9.0  |       | +       |
|                                  | Benzoylecgonine                     | 289.3 | -0.2 | 2.7  | 519-09-5     | Sigma-Aldrich | 9.5  | 3.1   | z (+/-) |
|                                  | Cocaine                             | 303.4 | 1.2  | 3.1  | 50-36-2      | Sigma-Aldrich | 8.9  | (-)   | +       |
| <i>Hallucinogens</i>             | MDMA                                | 193.2 | -0.8 | 1.8  | 42542-10-9   | Sigma-Aldrich | 10.1 | (-)   | +       |
| <i>Opioids</i>                   | 6-acetyl-morphine                   | 327.0 | 0.8  | 1.3  | 2784-73-8    | Sigma-Aldrich | 9.1  | 10.2  | +       |
|                                  | Codeine                             | 299.4 | 0.3  | 1.2  | 76-57-3      | Sigma-Aldrich | 9.2  | 13.8  | +       |
|                                  | EDDP ☆                              | 277.9 | 4.4  | 5.5  | 30223-73-5   | Sigma-Aldrich | 9.6  |       | +       |
| <i>Psychiatric drugs</i>         | Methadone                           | 309.5 | 2.8  | 4.2  | 76-99-3      | Sigma-Aldrich | 9.1  | (-)   | +       |
|                                  | Morphine                            | 285.3 | -0.1 | 0.4  | 57-27-2      | Sigma-Aldrich | 9.1  | 10.3  | +       |
|                                  | Naloxone                            | 327.4 | 1.4  | 1.5  | 465-65-6     | LGC           | 7.8  | 10.1  | +       |
|                                  | Tramadol                            | 263.4 | 0.5  | 2.5  | 27203-92-5   | Cluzeau       | 9.2  | 13.8  | +       |
|                                  | Diazepam                            | 284.7 | 2.9  | 2.9  | 439-14-5     | Sigma-Aldrich | 2.9  | (-)   | -       |
|                                  | Oxazepam                            | 287.7 | 1.5  | 1.8  | 604-75-1     | Sigma-Aldrich | (-)  | 10.6  | 0       |
| <i>Cannabinoid</i>               | THC-COOH                            | 343.0 | 3.1  | 6.2  | 56354-06-4   | LGC           | 4.0  | (-)   | -       |
| <i>Coffee</i>                    | Paraxanthine ☆                      | 180.2 | -0.4 | -1.6 | 611-59-6     | Cluzeau       | 8.6  | (-)   | +       |
|                                  | Caffeine                            | 194.0 | 0.3  | -0.1 | 58-08-2      | Cluzeau       | 14   | (-)   | +       |
| <i>Tobacco</i>                   | Cotinine                            | 176.2 | 0.2  | -0.2 | 486-56-6     | Cluzeau       | 4.8  | (-)   | 0       |
|                                  | Nicotine                            | 162.2 | -0.4 | 0.7  | 54-11-5      | Cluzeau       | 8.6  | (-)   | +       |
| <i>Alcohol</i>                   |                                     |       |      |      |              |               |      |       |         |
|                                  | Ethyl-sulfate ☆                     | 125.9 | -4.5 | 0.1  | 540-82-9     | Sigma-Aldrich | (-)  | (-)   | -       |
| <b>Others</b>                    |                                     |       |      |      |              |               |      |       |         |
| <i>Dietary intake</i>            | Acesulfame                          | 201.2 | -2.8 | -0.3 | 55589-62-3   | Cluzeau       | (-)  | 3.0   | -       |
|                                  | Saccharin                           | 183.2 | -1.3 | 0.9  | 81-07-2      | Cluzeau       | (-)  | 1.9   | -       |
|                                  | B3-2PY ☆                            | 152.2 | -1.5 | -1.1 | 701-44-0     | Sigma-Aldrich | 1.4  | (-)   | 0       |
|                                  | E - αCEHC ☆                         | 278.3 | (-)  | 3.1  | 4072-32-6    | Santa Cruz    | (-)  | 4.4   | -       |
|                                  | 4-Pyridoxic acid ☆                  | 183.0 | -2.2 | 0.6  | 82-82-6      | Sigma-Aldrich | 4.1  | 2.6   | -       |
|                                  | CMPF                                | 240.3 | -2.1 | 2.6  | 86879-39-2   | LGC           | (-)  | 3.9   | -       |
|                                  | Daidzein                            | 254.3 | 2.4  | 2.8  | 486-66-8     | Cluzeau       | (-)  | 6.5   | -       |
|                                  | Enterodiol                          | 302.4 | 2.0  | 1.6  | 80226-002    | Sigma-Aldrich | (-)  | 9.8   | 0       |
|                                  | Enterolactone                       | 298.3 | 2.5  | 2.3  | 78473-71-9   | Sigma-Aldrich | (-)  | 9.2   | 0       |
|                                  | 8-iso-prostaglandin F2α             | 354.5 | *    | 2.2  | 27415-26-5   | Santa Cruz    | (-)  | 4.4   | -       |
| <i>Oxidative stress</i>          | Dinor-11β-Prostaglandin F2α         | 326.4 | -1.0 | 2.1  | 221664-05-07 | Santa Cruz    | (-)  | (-)   | ?       |
|                                  | Prostaglandin E2                    | 352.5 | -0.1 | 1.9  | 363-24-6     | Sigma-Aldrich | (-)  | 4.3   | -       |
| <i>Demographic marker</i>        | 5-HIAA                              | 191.2 | -2.0 | 0.3  | 54-16-0      | LGC           | (-)  | 4.22  | -       |
| <i>Anti-corrosive</i>            | 5-methyl-1H-benzotriazole           | 136.2 | 1.7  | 1.8  | 136-85-6     | LGC           | 8.74 | (-)   | +       |
| <i>Mycotoxin</i>                 | Deoxynivalenol                      | 296.3 | -0.9 | -1.4 | 51481-10-8   | CIL           | (-)  | 12.68 | 0       |
| <b>ILIS</b>                      |                                     |       |      |      |              |               |      |       |         |
|                                  | 4-Methylbenzotriazole-d3            |       |      |      | 1219151-49-1 | CIL           |      |       |         |
|                                  | 5-HIAA-d5                           |       |      |      | 1219802-93-3 | LGC           |      |       |         |
|                                  | Acesulfame potassium salt-d4        |       |      |      | 1623054-53-4 | Cluzeau       |      |       |         |
|                                  | Acetaminophen-d4                    |       |      |      | 64315-36-2   | Sigma-Aldrich |      |       |         |
|                                  | Amisulpride-d5                      |       |      |      | 1216626-17-3 | CIL           |      |       |         |

|                       |              |               |
|-----------------------|--------------|---------------|
| Benzoyllecgonine-d3   | 115732-68-8  | Sigma-Aldrich |
| Caffeine-13C3         | 78072-66-9   | Sigma-Aldrich |
| Carbamazepin-13C6     | 67-56-1      | Sigma-Aldrich |
| CMPF-d3               | 86879-39-2   | LGC           |
| Cotinine-d3           | 110952-70-0  | Sigma-Aldrich |
| Diclofenac-d4         | 153466-65-0  | Cluzeau       |
| Fexofenadine-13C6     | 67-56-1      | Sigma-Aldrich |
| Fluoxetine-d6         | 2319722-07-9 | Sigma-Aldrich |
| Ibuprofen-d3          | 121662-14-4  | Sigma-Aldrich |
| Irbesartan-d6         | 2375621-21-7 | CIL           |
| Metformin-d6          | 1185166-01-1 | LGC           |
| Oxazepam-d5           | 65854-78-6   | LGC           |
| Propanolol-d7         | 344298-99-3  | Sigma-Aldrich |
| Sulfamethoxazole-13C6 | 1196157-90-0 | CIL           |
| THC-COOH-d3           | 136844-96-7  | LGC           |
| Tramadol-13C-d3       | 67-56-1      | LGC           |
| Trimethoprim-d3       | 1189923-38-3 | Cluzeau       |

\*Information from ChemSpider (Royal Society of Chemistry), Predicted data is generated using the [ACD/Labs Percepta Platform - PhysChem Module](#), version: 14.00, Log D value at pH 7.4.

\*\*Information from Drugbank Online, Predicted data is generated using ChemAxon

☆ Results marked with a star (☆) should be considered with caution

Table S2: Source parameters for LC-MS<sup>2</sup> analysis

|   | Parameter Id                | Positive Value | Negative Value |
|---|-----------------------------|----------------|----------------|
| 1 | Gas Temperature (°C)        | 300            | 300            |
| 2 | Gas Flow (L/min)            | 7              | 7              |
| 3 | Nebulizer (psi)             | 15             | 15             |
| 4 | Capillary Voltage (V)       | 4000           | 4000           |
| 5 | Sheath Gas Temperature (°C) | 250            | 250            |
| 6 | Sheath Gas Flow (L/min)     | 11             | 11             |
| 7 | Nozzle Voltage (V)          | 1500           | 1500           |

Table S3: Gradient used for UPLC-HRMS analysis

| Vion-UPLC-IMS-QTOF gradient |       |       |
|-----------------------------|-------|-------|
| Time (min)                  | A (%) | B (%) |
| 0                           | 98    | 2     |
| 1                           | 98    | 2     |
| 25                          | 2     | 98    |
| 30                          | 2     | 98    |
| 31.5                        | 98    | 2     |
| 34                          | 98    | 2     |

A : Milli-Q water + 0.1% formic acid and B : ACN + 0.1% formic acid

Table S4: Absolute recoveries of targeted compounds following each SPE protocol (HLB, MC, HAC) on the three investigated matrices (IWW, PW, SW)

| Pharmaceuticals                  | Compounds         | Ultrapure water (n =3) |             |             | Surface water (n =3) |             |             | IWW (n=3)   |             |             |
|----------------------------------|-------------------|------------------------|-------------|-------------|----------------------|-------------|-------------|-------------|-------------|-------------|
|                                  |                   | HLB                    | HAC         | MC          | HLB                  | HAC         | MC          | HLB         | HAC         | MC          |
|                                  |                   | Rec Abs (%)            | Rec Abs (%) | Rec Abs (%) | Rec Abs (%)          | Rec Abs (%) | Rec Abs (%) | Rec Abs (%) | Rec Abs (%) | Rec Abs (%) |
| Antibiotics                      | Clarithromycin    | 42.4                   | 60.4        | 65.2        | 76.9                 | 45.7        | 58.8        | 58.8        | 46.2        | 47.0        |
|                                  | Sulfamethoxazole  | 90.8                   | 76.6        | 72.2        | 73.0                 | 64.8        | 66.9        | 37.3        | 30.3        | 30.4        |
|                                  | Trimethoprim      | 159.8                  | 101.1       | 108.8       | 156.4                | 108.7       | 118.6       | 124.2       | 84.4        | 84.2        |
| Analgesics & anti-inflammatories | Acetaminophen     | 69.6                   | 28.8        | 29.9        | 68.1                 | 29.3        | 31.4        | *           | *           | *           |
|                                  | Diclofenac        | 80.7                   | 58.8        | 59.6        | 83.3                 | 48.7        | 56.9        | 50.8        | 27.7        | 29.6        |
|                                  | Ibuprofen         | 88.7                   | 59.1        | 56.9        | 73.9                 | 53.8        | 58.1        | 28.0        | 13.2        | 15.4        |
|                                  | Ketoprofen        | 92.8                   | 72.6        | 64.1        | 81.6                 | 65.0        | 68.8        | 41.8        | 29.7        | 29.9        |
| $\beta$ -blockers                | Metoprolol        | 102.8                  | 98.6        | 99.2        | 100.8                | 90.1        | 100.6       | 74.7        | 58.9        | 60.6        |
|                                  | Propanolol        | 43.4                   | 53.7        | 49.4        | 56.1                 | 42.0        | 42.2        | 39.3        | 32.9        | 31.9        |
|                                  | Salbutamol        | 231.3                  | 71.3        | 71.9        | 184.0                | 66.9        | 70.6        | 143.8       | 45.8        | 46.3        |
| Anticonvulsants                  | Carbamazepine     | 98.4                   | 94.1        | 96.0        | 99.0                 | 84.9        | 94.0        | 55.0        | 44.3        | 46.5        |
|                                  | Gabapentin        | 18.9                   | 45.3        | 46.9        | 14.0                 | 32.7        | 40.0        | 12.5        | 22.4        | 34.8        |
| Antidepressants                  | Amisulpride       | 176.6                  | 113.9       | 132.8       | 208.8                | 161.5       | 166.6       | 150.4       | 110.5       | 116.8       |
|                                  | Citalopram        | 51.6                   | 55.3        | 54.9        | 62.3                 | 44.9        | 50.8        | 55.1        | 45.9        | 44.3        |
|                                  | Fluoxetine        | 25.4                   | 44.3        | 28.2        | 16.9                 | 9.4         | 8.6         | 14.7        | 18.1        | 20.9        |
|                                  | Venlafaxine       | 69.7                   | 58.9        | 56.9        | 79.4                 | 59.6        | 59.6        | 56.1        | 37.3        | 39.4        |
| Antidiabetics                    | Glibenclamide     | 61.6                   | 61.2        | 59.3        | 83.8                 | 31.8        | 47.5        | 65.0        | 49.1        | 44.6        |
|                                  | Metformin         | 7.6                    | 36.6        | 27.9        | 3.1                  | 42.3        | 28.3        | *           | *           | *           |
| Antihistamines                   | Cetirizine        | 75.4                   | 60.8        | 67.2        | 80.1                 | 49.6        | 56.8        | 49.8        | 37.0        | 36.1        |
|                                  | Desloratadine     | 35.6                   | 45.6        | 47.5        | 51.7                 | 35.3        | 40.5        | 45.1        | 36.7        | 37.3        |
|                                  | Fexofenadine      | 74.1                   | 76.6        | 69.2        | 90.0                 | 59.7        | 61.2        | 62.7        | 50.3        | 50.5        |
| Hormones                         | Levonorgestrel    | 52.9                   | 54.2        | 56.0        | 84.3                 | 40.1        | 54.9        | 72.4        | 44.8        | 41.1        |
| Anti-hypertensive                | Candesartan       | 99.6                   | 74.9        | 77.9        | 86.6                 | 72.3        | 77.1        | 71.1        | 51.2        | 50.7        |
|                                  | Irbesartan        | 63.6                   | 55.6        | 61.7        | 82.0                 | 48.3        | 63.0        | 57.9        | 38.1        | 40.5        |
| Licit & illicit drugs            |                   |                        |             |             |                      |             |             |             |             |             |
| Stimulants                       | AEME              | 237.6                  | 144.9       | 137.7       | 213.9                | 126.6       | 125.0       | 163.6       | 60.2        | 60.4        |
|                                  | Benzoylcegonine   | 112.6                  | 68.5        | 70.2        | 106.3                | 64.1        | 70.5        | 72.9        | 35.7        | 44.2        |
|                                  | Cocaine           | 104.9                  | 88.9        | 92.3        | 93.7                 | 96.6        | 104.1       | 91.1        | 65.4        | 65.6        |
| Hallucinogens                    | MDMA              | 137.6                  | 99.5        | 99.3        | 121.1                | 90.0        | 97.9        | 101.3       | 61.8        | 62.8        |
| Opioids                          | 6-acetyl-morphine | 203.3                  | 125.2       | 127.8       | 176.1                | 129.9       | 140.5       | 196.8       | 117.4       | 113.8       |
|                                  | Codeine           | 210.1                  | 130.3       | 131.6       | 226.9                | 146.3       | 149.7       | 204.6       | 100.3       | 99.3        |
|                                  | EDDP ☆            | 45.2                   | 46.3        | 34.3        | 40.9                 | 38.6        | 32.2        | 36.1        | 28.6        | 26.1        |
|                                  | Methadone         | 67.6                   | 59.5        | 59.0        | 70.9                 | 48.1        | 55.1        | 42.4        | 33.3        | 34.5        |
|                                  | Morphine          | 55.7                   | 49.9        | 55.1        | 66.2                 | 40.8        | 54.7        | 47.5        | 33.2        | 29.5        |
|                                  | Naloxone          | 161.1                  | 112.3       | 115.4       | 178.5                | 100.4       | 114.9       | 159.7       | 83.7        | 85.0        |
|                                  | Tramadol          | 96.2                   | 76.1        | 80.1        | 106.6                | 82.7        | 89.2        | 81.6        | 50.0        | 53.3        |
|                                  | Diazepam          | 58.6                   | 59.7        | 61.2        | 82.6                 | 44.3        | 59.2        | 52.2        | 38.8        | 38.6        |
| Psychiatric drugs                | Oxazepam          | 66.4                   | 65.4        | 66.3        | 78.1                 | 51.6        | 59.5        | 39.5        | 26.3        | 29.1        |
|                                  | THC-COOH          | 40.0                   | 21.0        | 5.1         | 57.6                 | 5.8         | 5.5         | 40.0        | 30.9        | 28.0        |
| Cannabinoid                      | THC-COOH          | 40.0                   | 21.0        | 5.1         | 57.6                 | 5.8         | 5.5         | 40.0        | 30.9        | 28.0        |
| Coffee                           | Paraxanthine ☆    | 56.6                   | 19.1        | 20.2        | 60.6                 | 20.6        | 23.1        | 56.7        | 5.9         | 7.2         |
|                                  | Caffeine          | 98.3                   | 49.0        | 52.8        | 90.9                 | 49.2        | 53.5        | *           | *           | *           |
| Tobacco                          | Cotinine          | 226.8                  | 77.6        | 78.9        | 152.9                | 69.4        | 67.2        | *           | *           | *           |

|                         |                             |       |       |       |       |      |      |       |      |      |
|-------------------------|-----------------------------|-------|-------|-------|-------|------|------|-------|------|------|
|                         | Nicotine                    | 138.1 | 60.6  | 90.4  | 121.2 | 76.3 | 63.5 | *     | *    | *    |
| <i>Alcohol</i>          |                             |       |       |       |       |      |      |       |      |      |
|                         | Ethyl-sulfate ☆             | 0.2   | 4.6   | 2.6   | *     | 0.6  | 0.3  | *     | *    | *    |
| <b>Others</b>           |                             |       |       |       |       |      |      |       |      |      |
| <i>Dietary intake</i>   | Acesulfame                  | 14.3  | 22.9  | 23.2  | 20.4  | 23.6 | 23.1 | *     | *    | *    |
|                         | Saccharin                   | 36.6  | 44.8  | 41.7  | 62.7  | 30.7 | 30.0 | 22.7  | 12.0 | 24.5 |
|                         | 2PY ☆                       | 108.8 | 54.0  | 48.3  | 95.9  | 45.7 | 60.3 | *     | *    | *    |
|                         | αCEHC ☆                     | 29.7  | 63.4  | 47.7  | 28.4  | 26.5 | 15.1 | 18.2  | 8.4  | 9.3  |
|                         | 4-Pyridoxic acid ☆          | 60.1  | 40.4  | 40.8  | 108.8 | 75.9 | 58.6 | 108.8 | 75.9 | 58.6 |
|                         | CMPF                        | 88.4  | 83.4  | 70.9  | 16.3  | 17.8 | 17.4 | 30.9  | 4.4  | 5.6  |
|                         | Daidzein                    | 80.1  | 49.3  | 36.5  | 54.1  | 22.1 | 24.4 | 18.5  | 8.1  | 9.8  |
|                         | Enterodiol                  | 136.9 | 123.1 | 111.4 | 101.3 | 70.3 | 80.0 | 25.8  | 22.0 | 21.7 |
|                         | Enterolactone               | 61.0  | 61.4  | 54.4  | 54.2  | 31.2 | 39.4 | 16.1  | 12.4 | 12.0 |
|                         | 8-iso-prostaglandin F2α     | 98.0  | 62.8  | 54.3  | 61.2  | 47.4 | 51.8 | 61.2  | 47.4 | 51.8 |
|                         | Dinor-11β-Prostaglandin F2α | 97.1  | 78.1  | 71.3  | 61.3  | 55.9 | 54.1 | 19.9  | 21.1 | 19.7 |
| <i>Oxidative stress</i> | Prostaglandin E2            | 88.3  | 51.9  | 53.9  | 68.4  | 49.0 | 53.8 | 31.5  | 18.6 | 18.0 |
|                         |                             |       |       |       |       |      |      |       |      |      |
| Demographic marker      | 5-HIAA                      | 64.5  | 26.1  | 23.1  | 12.1  | 37.3 | 21.4 | 7.2   | 30.4 | 36.2 |
|                         | 5-methyl-1H-benzotriazole   | 82.7  | 72.4  | 68.9  | 69.8  | 65.7 | 71.0 | 67.1  | 47.1 | 45.6 |
| Anti-corrosive          |                             |       |       |       |       |      |      |       |      |      |
| Mycotoxin               | Deoxynivalenol              | 29.7  | 10.5  | 10.7  | 44.6  | 17.5 | 18.3 | 26.4  | 10.6 | 9.6  |

\*Not estimated due to the amount present in “blank” sample

☆ Results marked with a star (☆) should be considered with caution

Table S5: Limits of quantification (LOQ) in ng L<sup>-1</sup> in surface water (SW) and influent wastewater (IWW)

| Pharmaceuticals                             | Compounds        | SW (n=3)                  |       |       | IWW (n=3)                 |        |       |
|---------------------------------------------|------------------|---------------------------|-------|-------|---------------------------|--------|-------|
|                                             |                  | LOQ (ng L <sup>-1</sup> ) |       |       | LOQ (ng L <sup>-1</sup> ) |        |       |
| <i>Antibiotics</i>                          |                  | HLB                       | HAC   | MC    | HLB                       | HAC    | MC    |
|                                             | Clarithromycin   | 9.3                       | 9.6   | 15.8  | 41.9                      | 33.8   | 126.5 |
|                                             | Sulfamethoxazole | 25.6                      | 18.7  | 120.0 | 119.3                     | 17.4   | 66.6  |
|                                             | Trimethoprim     | 2.6                       | 0.8   | 8.6   | 7.0                       | 3.8    | 5.6   |
| <i>Analgesics &amp; anti-inflammatories</i> | Acetaminophen    | 25.6                      | 104.0 | 69.5  | 844.6                     | 1359.1 | 392.6 |
|                                             | Diclofenac       | 17.8                      | 51.1  | 131.4 | 16.5                      | 19.2   | 122.1 |
|                                             | Ibuprofen        | 24.8                      | 11.1  | 104.7 | 40.8                      | 569.2  | 646.4 |
|                                             | Ketoprofen       | 8.2                       | 23.6  | 17.2  | 44.1                      | 122.6  | 137.0 |
| <i>β-blockers</i>                           | Metoprolol       | 6.4                       | 6.2   | 10.7  | 2.7                       | 30.7   | 10.7  |
|                                             | Propanolol       | 2.4                       | 1.4   | 17.3  | 25.5                      | 22.1   | 4.9   |
|                                             | Salbutamol       | 2.1                       | 1.5   | 0.8   | 1.4                       | 1.4    | 3.5   |
| <i>Anticonvulsants</i>                      | Carbamazepine    | 7.6                       | 4.3   | 18.9  | 25.6                      | 7.6    | 2.7   |
|                                             | Gabapentin       | 0.9                       | 18.5  | 13.3  | 8.0                       | 15.7   | 17.1  |
| <i>Antidepressants</i>                      | Amisulpride      | 0.6                       | 0.7   | 0.2   | 3.4                       | 1.8    | 1.6   |
|                                             | Citalopram       | 13.6                      | 10.3  | 10.3  | 36.1                      | 53.2   | 39.4  |
|                                             | Fluoxetine       | 3.8                       | 2.9   | 3.0   | 9.0                       | 5.1    | 1.7   |
|                                             | Venlafaxine      | 2.9                       | 3.2   | 3.2   | 4.1                       | 6.6    | 0.8   |
| <i>Antidiabetics</i>                        | Glibenclamide    | 13.9                      | 1.4   | 18.2  | 7.4                       | 3.5    | 5.6   |
|                                             | Metformin        | 88.4                      | 0.7   | 5.2   | 1091.6                    | 309.3  | 327.3 |
| <i>Antihistamines</i>                       | Cetirizine       | 8.4                       | 4.9   | 3.0   | 15.8                      | 13.6   | 12.6  |

|                                  |                             |       |       |        |        |         |         |
|----------------------------------|-----------------------------|-------|-------|--------|--------|---------|---------|
|                                  | Desloratadine               | 1.5   | 3.2   | 3.7    | 9.2    | 1.6     | 2.6     |
|                                  | Fexofenadine                | 65.5  | 11.1  | 15.1   | 39.4   | 40.7    | 10.5    |
| <i>Hormones</i>                  | Levonorgestrel              | 16.4  | 20.4  | 24.0   | 291.2  | 876.8   | 553.4   |
| <i>Anti-hypertensive</i>         | Candesartan                 | 2.0   | 5.9   | 0.5    | 9.4    | 9.8     | 6.9     |
|                                  | Irbesartan                  | 1.7   | 0.6   | 1.0    | 5.6    | 1.3     | 3.6     |
| <b>Licit &amp; illicit drugs</b> |                             |       |       |        |        |         |         |
| <i>Stimulants</i>                | AEME                        | 3.1   | 0.7   | 13.9   | 47.3   | 3.4     | 19.6    |
|                                  | Benzoyllecgonine            | 0.3   | 1.3   | 2.2    | 6.0    | 0.9     | 4.9     |
|                                  | Cocaine                     | 8.6   | 5.2   | 34.8   | 8.0    | 3.0     | 3.7     |
| <i>Hallucinogens</i>             | MDMA                        | 1.5   | 1.9   | 14.0   | 28.8   | 0.7     | 7.3     |
| <i>Opioids</i>                   | 6-acetyl-morphine           | 11.4  | 24.8  | 16.2   | 126.2  | 77.8    | 26.6    |
|                                  | Codeine                     | 4.1   | 22.0  | 15.1   | 33.0   | 10.0    | 40.2    |
|                                  | EDDP ☆                      | 5.1   | 1.3   | 1.6    | 0.7    | 1.5     | 0.5     |
|                                  | Methadone                   | 7.6   | 0.5   | 6.5    | 4.5    | 0.5     | 1.5     |
|                                  | Morphine                    | 64.7  | 84.8  | 86.6   | 136.4  | 549.6   | 105.2   |
|                                  | Naloxone                    | 7.8   | 14.5  | 2.1    | 13.3   | 9.4     | 11.9    |
|                                  | Tramadol                    | 0.6   | 32.2  | 7.0    | 22.2   | 0.6     | 4.5     |
| <i>Psychiatric drugs</i>         | Diazepam                    | 8.2   | 14.5  | 9.1    | 43.3   | 17.7    | 16.4    |
|                                  | Oxazepam                    | 29.7  | 23.7  | 26.9   | 57.1   | 34.6    | 57.4    |
| <i>Cannabinoid</i>               | THC-COOH                    | 94.9  | 882.7 | 1342.9 | 128.0  | 89.1    | 105.2   |
| <i>Coffee</i>                    | Paraxanthine ☆              | 61.6  | 21.0  | 34.0   | 92.2   | 136.6   | 158.6   |
|                                  | Caffeine                    | 17.0  | 31.6  | 35.1   | 448.7  | 393.4   | 62.3    |
| <i>Tobacco</i>                   | Cotinine                    | 15.2  | 5.0   | 22.0   | 176.9  | 343.6   | 136.6   |
|                                  | Nicotine                    | 19.9  | 23.4  | 26.8   | 470.1  | 97.3    | 84.2    |
| <i>Alcohol</i>                   |                             |       |       |        |        |         |         |
|                                  | Ethyl-sulfate               | 27.8  | 9.9   | 2.2    | 43.9   | 12.6    | 76.2    |
| <b>Others</b>                    |                             |       |       |        |        |         |         |
| <i>Dietary intake</i>            | Acesulfame                  | 3.5   | 7.0   | 16.1   | 37.3   | 36.6    | 19.9    |
|                                  | Saccharin                   | 22.1  | 43.4  | 28.1   | 81.8   | 328.9   | 38.8    |
|                                  | 2PY ☆                       | 344.6 | 113.4 | 595.6  | 1702.3 | 15228.5 | 22575.5 |
|                                  | αCEHC ☆                     | 336.2 | 216.8 | 208.7  | 3073.3 | 445.9   | 266.4   |
|                                  | 4-Pyridoxic acid ☆          | 86.1  | 112.5 | 172.4  | 127.4  | 111.1   | 1102.9  |
|                                  | CMPF                        | 15.6  | 18.3  | 18.9   | 45.4   | 30.7    | 34.2    |
|                                  | Daidzein                    | 13.0  | 17.0  | 11.2   | 11.7   | 258.9   | 44.2    |
|                                  | Enterodiol                  | 0.6   | 2.0   | 1.8    | 4.5    | 19.5    | 14.7    |
|                                  | Enterolactone               | 305.9 | 49.0  | 78.1   | 584.8  | 262.5   | 361.4   |
| <i>Oxidative stress</i>          | 8-iso-prostaglandin F2α     | 28.7  | 94.9  | 22.7   | 63.5   | 120.0   | 157.0   |
|                                  | Dinor-11β-Prostaglandin F2α | 32.1  | 18.1  | 103.3  | 345.5  | 547.1   | 1058.4  |
|                                  | Prostaglandin E2            | 33.6  | 51.9  | 111.6  | 292.2  | 714.1   | 68.7    |
| <i>Demographic marker</i>        | 5-HIAA                      | 112.1 | 24.8  | 50.7   | 118.5  | 65.9    | 71.7    |
| <i>Anti-corrosive</i>            | 5-methyl-1H-benzotriazole   | 18.2  | 5.8   | 2.6    | 3.5    | 1.9     | 1.8     |
| <i>Mycotoxin</i>                 | Deoxynivalenol              | 29.6  | 23.5  | 18.0   | 169.6  | 199.1   | 20.8    |

☆ Results marked with a star (☆) should be considered with caution