

Supplementary material (ESI) for Journal of Environmental Monitoring

**Development of immunoassays to determinate sulfamethoxazole residues in wastewaters**

Ester Gallego-Iglesias, Eva M. Brun, Nuria Pastor-Navarro, Ángel Maquieira, Rosa Puchades

**Supporting information**

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## S1. Spectral data of the synthesized compounds

$\delta$  values are given in ppm and  $J$  values in Hz.

### Hapten S10:

*(4-acetylamino-benzenesulfonylamino)-acetic acid*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 10.31 (1H, s, NH-CO), 7.90 (1H, t,  $J$  6.0, NH), 7.80-7.70 (4H, m, Ar-H), 3.56 (2H, d,  $J$  6.0, CH<sub>2</sub>), 2.09 (3H, s, CH<sub>3</sub>);  $\delta_{\text{C}}$ (75.4 MHz; DMSO- $d_6$ ) 170.3 (COOH), 169.0 (CO), 142.8 (C-Ar), 134.3 (C-Ar), 127.7 (2CH-Ar), 118.5 (2CH-Ar), 43.8 (CH<sub>2</sub>), 24.1 (CH<sub>3</sub>).

*(4-amino-benzenesulfonylamino)-acetic acid*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 7.38 (2H, d,  $J$  8.7, 2CH-C-SO<sub>2</sub>), 6.58 (2H, d,  $J$  8.7, 2CH-C-NH<sub>2</sub>), 5.90 (2H, br s, NH<sub>2</sub>), 2.94 (2H, s, CH<sub>2</sub>);  $\delta_{\text{C}}$ (75.4 MHz; DMSO- $d_6$ ) 169.9 (COOH), 152.5 (C-Ar), 128.6 (2CH-C-SO<sub>2</sub>), 124.5 (C-Ar), 112.6 (2CH-C-NH<sub>2</sub>), 46.1 (CH<sub>2</sub>).

### Hapten S11:

*6-(4-nitro-benzenesulfonylamino)-hexanoic acid*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 8.42 (2H, d,  $J$  9.0, 2CH-C-NO<sub>2</sub>), 8.05 (2H, d,  $J$  9.0, 2CH-C-SO<sub>2</sub>), 7.98 (1H, t,  $J$  5.7, NH), 2.79 (2H, q,  $J$  6.9, CH<sub>2</sub>-NH), 2.14 (2H, t,  $J$  7.2, CH<sub>2</sub>-COOH), 1.47-1.30 (4H, m, 2CH<sub>2</sub>), 1.28-1.15 (2H, m, CH<sub>2</sub>);  $\delta_{\text{C}}$ (75.4 MHz; DMSO- $d_6$ ) 174.3 (COOH), 149.5 (C-Ar), 146.2 (C-Ar), 128.0 (2CH-C-SO<sub>2</sub>), 124.6 (2CH-C-NO<sub>2</sub>), 42.4 (CH<sub>2</sub>-NH), 33.5 (CH<sub>2</sub>-CO), 28.7 (CH<sub>2</sub>), 25.5 (CH<sub>2</sub>), 24.0 (CH<sub>2</sub>).

*6-(4-amino-benzenesulfonylamino)-hexanoic acid*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 11.95 (1H, s, COOH), 7.40 (2H, d,  $J$  8.7, 2CH-C-SO<sub>2</sub>), 7.04 (1H, t,  $J$  6.0, NH), 6.60 (2H, d,  $J$  8.7, 2CH-C-NH<sub>2</sub>), 5.85 (2H, br s, NH<sub>2</sub>), 2.62 (2H, q,  $J$  6.6, CH<sub>2</sub>-NH), 2.15 (2H, t,  $J$  7.2, CH<sub>2</sub>-COOH), 1.47-1.27 (4H, m, 2CH<sub>2</sub>), 1.27-1.19 (2H, m, CH<sub>2</sub>);  $\delta_{\text{C}}$ (75.4 MHz; DMSO- $d_6$ ) 174.4 (COOH), 152.3 (C-Ar), 128.4 (2CH-C-SO<sub>2</sub>), 125.6 (C-Ar), 112.6 (2CH-C-NH<sub>2</sub>), 42.3 (CH<sub>2</sub>-NH), 33.5 (CH<sub>2</sub>-CO), 29.6 (CH<sub>2</sub>), 25.7 (CH<sub>2</sub>), 24.1 (CH<sub>2</sub>).

### Hapten S12:

*N-[4-(3-hydroxypropylsulfamoyl)-phenyl]-acetamide*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 10.31 (1H, s, NH-CO), 7.76 (2H, d,  $J$  9.0, Ar-H), 7.70 (2H, d,  $J$  9.0, Ar-H), 7.37 (1H, t,  $J$  6.0, NH), 3.35 (2H, t,  $J$  6.3, CH<sub>2</sub>-OH), 2.75 (2H, q,  $J$  6.6, CH<sub>2</sub>-NH), 2.08 (3H, s, CH<sub>3</sub>), 1.49 (2H, quintet,  $J$  6.9, CH<sub>2</sub>);  $\delta_{\text{C}}$ (75.4 MHz; DMSO- $d_6$ ) 169.0 (COOH), 142.6 (C-Ar), 134.1 (C-Ar), 127.6 (2CH-Ar), 118.6 (2CH-Ar), 58.0 (CH<sub>2</sub>OH), 39.9 (CH<sub>2</sub>NH), 32.3 (CH<sub>2</sub>), 24.1 (CH<sub>3</sub>).

*4-amino-N-(3-hydroxypropyl)-benzenesulfonamide*:  $\delta_{\text{H}}$ (300 MHz; DMSO- $d_6$ ) 7.40 (2H, d,  $J$  8.7, 2CH-C-SO<sub>2</sub>), 6.99 (1H, t,  $J$  6.0, NH), 6.61 (2H, d,  $J$  8.7, 2CH-C-NH<sub>2</sub>), 5.89 (2H, s, NH<sub>2</sub>), 3.35 (2H, t,  $J$  6.3, CH<sub>2</sub>OH), 2.69 (2H, q,  $J$  6.6, CH<sub>2</sub>NH), 1.49 (2H, m,

$J$  6.9, CH<sub>2</sub>);  $\delta_C$ (75.4 MHz; DMSO- $d_6$ ) 152.3 (C-Ar), 128.4 (2CH-C-SO<sub>2</sub>), 125.4 (C-Ar), 112.6 (2CH-C-NH<sub>2</sub>), 58.2 (CH<sub>2</sub>-OH), 39.9 (CH<sub>2</sub>-NH), 32.2 (CH<sub>2</sub>).

**Hapten S13:**

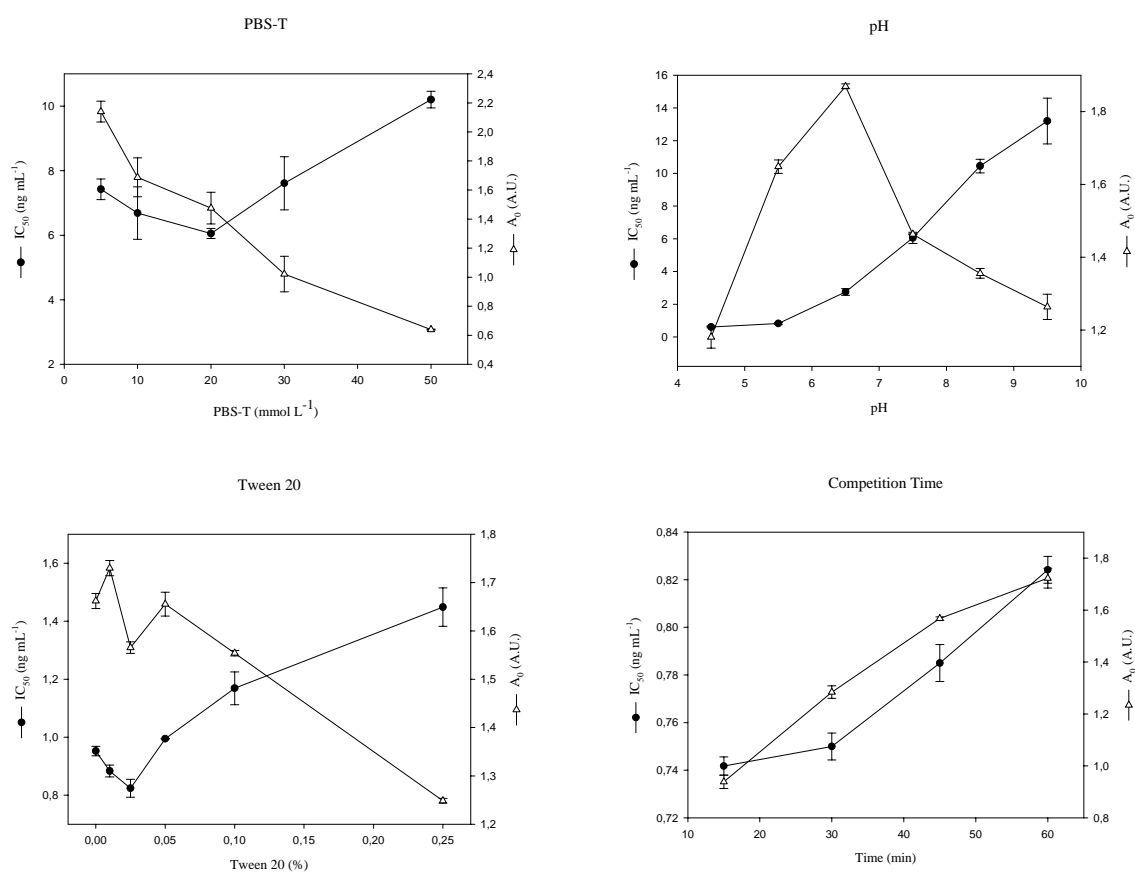
*4-(4-acetylamino-benzenesulfonylamino)-butanoic acid*:  $\delta_H$ (300 MHz; DMSO- $d_6$ ) 12.03 (COOH), 10.30 (1H, s, NH-CO), 7.76 (2H, d,  $J$  9.0, Ar-H), 7.69 (2H, d,  $J$  9.0, Ar-H), 7.47 (1H, t,  $J$  6.0, NH), 2.72 (2H, q,  $J$  6.3, CH<sub>2</sub>NH), 2.20 (2H, t,  $J$  7.5, CH<sub>2</sub>CO), 2.08 (3H, s, CH<sub>3</sub>), 1.57 (2H, quintet,  $J$  7.2, CH<sub>2</sub>);  $\delta_C$ (75.4 MHz; DMSO- $d_6$ ) 174.0 (COOH), 169.0 (CO), 142.7 (C-Ar), 134.1 (C-Ar), 127.6 (2CH-Ar), 118.6 (2CH-Ar), 41.9 (CH<sub>2</sub>NH), 30.6 (CH<sub>2</sub>CO), 24.5 (CH<sub>2</sub>), 24.1 (CH<sub>3</sub>).

*4-(4-amino-benzenesulfonylamino)-butanoic acid*:  $\delta_H$ (300 MHz; DMSO- $d_6$ ) 12.02 (1H, s, COOH), 7.39 (2H, d,  $J$  8.7, 2CH-C-SO<sub>2</sub>), 7.09 (1H, t,  $J$  6.0, NH), 6.60 (2H, d,  $J$  8.7, 2CH-C-NH<sub>2</sub>), 5.90 (2H, br s, NH<sub>2</sub>), 2.66 (2H, q,  $J$  6.6, CH<sub>2</sub>-NH), 2.19 (2H, t,  $J$  7.2, CH<sub>2</sub>-COOH), 1.57 (2H, quint.,  $J$  7.2, CH<sub>2</sub>);  $\delta_C$ (75.4 MHz; DMSO- $d_6$ ) 174.0 (COOH), 152.3 (C-Ar), 128.3 (2CH-C-SO<sub>2</sub>), 125.5 (C-Ar), 112.7 (2CH-C-NH<sub>2</sub>), 41.8 (CH<sub>2</sub>-NH), 30.7 (CH<sub>2</sub>-CO), 24.4 (CH<sub>2</sub>).

**Hapten S14:**

*4-[4-(4-acetylamino-benzenesulfonylamino)-benzenesulfonylamino]-benzoic acid*:  $\delta_H$ (300 MHz; DMSO- $d_6$ ) 10.84, 10.66, 10.31 (3H, s, NH), 7.78, 7.12 (4H, d, AA'BB',  $J$  8.7, Ar-H), 7.76-7.68 (4H, Ar-H), 7.67, 7.20 (4H, d, AA'BB',  $J$  8.7, Ar-H), 2.09 (3H, s, CH<sub>3</sub>);  $\delta_C$ (75.4 MHz; DMSO- $d_6$ ) 169.1 (CO), 166.7 (CO), 143.5 (C-Ar), 142.3 (C-Ar), 141.9 (C-Ar), 133.3 (C-Ar), 132.4 (C-Ar), 130.7 (2CH-Ar), 128.3 (2CH-Ar), 128.0 (2CH-Ar), 125.6 (C-Ar), 118.7 (2CH-Ar), 118.3 (2CH-Ar), 118.1 (2CH-Ar), 24.1 (CH<sub>3</sub>).

*4-[4-(4-amino-benzenesulfonylamino)-benzenesulfonylamino]-benzoic acid*:  $\delta_H$ (300 MHz; DMSO- $d_6$ ) 10.67, 10.55 (2H, s, 2 SO<sub>2</sub>NH), 7.78, 7.14 (4H, d, AA'BB',  $J$  9.0, Ar-H), 7.66, 7.17 (4H, d, AA'BB',  $J$  8.7, Ar-H), 7.42, 6.53 (4H, d, AA'BB',  $J$  8.7, Ar-H), 6.02 (2H, br s, NH<sub>2</sub>);  $\delta_C$ (75.4 MHz; DMSO- $d_6$ ) 166.7 (COOH), 153.2 (C-Ar), 142.9 (C-Ar), 142.0 (C-Ar), 132.7 (C-Ar), 130.7 (2CH-C-COOH), 128.8 (2CH-C-SO<sub>2</sub>), 128.2 (2CH-C-SO<sub>2</sub>), 125.5 (C-Ar), 123.7 (C-Ar), 118.1 (2CH-C-NH), 117.8 (2CH-C-NH), 112.7 (2CH-C-NH<sub>2</sub>).



**Figure S1.** Influence of different parameters on the SMX ELISA-plate performance

**Table S1.** Cross-reactivity values for different sulfonamides in both ELISA-plate and CD format

| OVA S8 // S12-II                            | ELISA-plate format                      |                     | CD format                               |        |
|---|---|---------------------|---|--------|
| Compound                                    | IC <sub>50</sub> (ng mL <sup>-1</sup> ) | CR (%) <sup>a</sup> | IC <sub>50</sub> (ng mL <sup>-1</sup> ) | CR (%) |
| Sulfamethoxazole                            | 0.75                                    | 100                 | 1.09                                    | 100    |
| Sulfasalazine                               | 71                                      | 1.06                | > 10,000                                | <0.01  |
| Sulfamethizole                              | 200                                     | 0.38                | > 10,000                                | <0.01  |
| <i>N</i> <sup>4</sup> -Phtalylsulfathiazole | 266                                     | 0.28                | > 10,000                                | <0.01  |
| Sulfacetamide                               | 509                                     | 0.15                | > 10,000                                | <0.01  |
| Sulfadiazine                                | 644                                     | 0.12                | > 10,000                                | <0.01  |
| Sulfathiazole                               | 898                                     | 0.08                | > 10,000                                | <0.01  |
| Sulfapyridine                               | 1,203                                   | 0.06                | > 10,000                                | <0.01  |
| Sulfanilamide                               | 1,719                                   | 0.04                | > 10,000                                | <0.01  |
| Sulfisoxazole                               | 3,072                                   | 0.02                | > 10,000                                | <0.01  |
| Sulfaguanidine                              | 6,817                                   | 0.01                | > 10,000                                | <0.01  |
| Sulfamerazine                               | 9,861                                   | 0.01                | > 10,000                                | <0.01  |
| Sulfadimethoxine                            | >10,000                                 | <0.01               | > 10,000                                | <0.01  |
| Sulfamethazine                              | >10,000                                 | <0.01               | > 10,000                                | <0.01  |
| Sulfamethoxypyridazine                      | >10,000                                 | <0.01               | > 10,000                                | <0.01  |

<sup>a</sup> Cross-reactivity was calculated with the equation: [IC<sub>50</sub>(SMX)/IC<sub>50</sub>(interferent)]x100

**Table S2.** Influence of the studied parameters on the sensitivity of the SMX microarray

| Parameter                   | IC <sub>50</sub> (ng mL <sup>-1</sup> ) <sup>a</sup> |
|-----------------------------|--|
| PBS (mmol L <sup>-1</sup> ) |  |
| 2.5                         | 13.35 ± 2.02   |
| 5                           | 18.65 ± 1.51   |
| 10                          | 3.96 ± 1.03  |
| 20                          | 23.49 ± 6.82   |
| pH                          |  |
| 6.5                         | 164 ± 7  |
| 7.5                         | 3.96 ± 1.21  |
| 8.5                         | 3.60 ± 0.71  |
| 9.5                         | 45.91 ± 6.51   |
| Tween 20 (%)                |  |
| 0                           | nc   |
| 0.01                        | 3.60 ± 0.71  |
| 0.025                       | 12.88 ± 1.05   |
| 0.05                        | 12.79 ± 5.99   |
| Competition time (min)      |  |
| 20                          | 3.60 ± 0.71  |
| 15                          | 1.09 ± 0.41  |
| 10                          | 2.35 ± 0.98  |
| 5                           | nc   |
| [GAR-Gold]                  |  |
| 1/15                        | 12.23 ± 3.60   |
| 1/25                        | 1.09 ± 0.41  |
| 1/50                        | 1.12 ± 0.43  |
| 1/75                        | 4.98 ± 1.89  |
| t GAR-Gold (min)            |  |
| 60                          | 1.09 ± 0.41  |
| 45                          | 17.65 ± 2.25   |
| 30                          | 57.93 ± 5.77   |
| 15                          | nc   |

<sup>a</sup> Mean value ± standard deviation (three replicates)

nc = no competition

OVA-S8 // S12-II (assay A)