Electronic Supporting Information (ESI)

A twin enrichment method based on dispersive liquid-liquid microextraction and field-amplified sample injection for the simultaneous determination of sulfonamides

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**Bernoulli equation**

\[ u = \frac{\Delta P \cdot r^2}{8 \eta L} \]

\( \Delta P = 3.45 \text{kPa} \), \( r \) is the capillary radius, \( \eta \) is the viscosity of water, \( L \) is the effective length of the capillary. The experimental results were shown in Fig. 3A. When the water plug length is 0.507 cm (3.45 kPa × 3 s), the enrichment efficiency of the four SAs reaches the maximum. When the water injection time is less than 3 s, the subject to be measured will be rapidly pushed out of the capillary tube by electric seepage, and the detection sensitivity of the analytes cannot be greatly improved. When the water injection time is longer than 3 s, serious peak broadening and overlap will occur. Therefore, the optimum length of water plug is 0.507 cm.

**Fig. S1** Molecular structures of the four SAs analyzed in this work.
Fig. S2 Effect of NaH$_2$PO$_4$ concentration on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.

Fig. S3 Effect of buffer pH on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.
**Fig. S4** Effect of MeOH and ACN on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.

**Fig. S5** Effect of ACN contents on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.
**Fig. S6** Effect of separation voltage on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.

**Fig. S7** Effects of (A) volume of extraction solvent and (B) volume of disperser solvent on the peak area of the four SAs. Extraction conditions: (A) sample volume, 5 mL; dispersive solvent, 800 μL DMSO and (B) sample volume, 5 mL; extraction solvent, 400 μL C₆H₅Cl. CE conditions: 20 mmol/L NaH₂PO₄ containing 10% (v/v) ACN at pH=7.8, injection 5 s with 0.5 psi, +20 kV applied voltage.
**Fig. S8** Effect of NaCl contents on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.

**Fig. S9** Effect of type of the redissolved solvent on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.
**Fig. S10** Effect of volume of the redissolved solvent on the separation of the four SAs. Peak identification: (1) SMZ; (2) SMR; (3) SDZ; (4) SFA.
Fig. S11 Effects of (A) water injection time, (B) injection voltage, (C) injection time, and (D) assisted pressure on the peak area of the four SAs. Sample conditions: SA standard solution at $1.0 \times 10^4$ ng/mL individual. FASI conditions: the assisted pressure of electric injection is 0.5 psi (ca. 3.45 kpa), 3 s injection time of water plug, pressure injection at -10kV voltage, and injection time of 0.5 min.
Table S1 Analytical performance of the DLLME-FASI-CE method for the determination of four SAs in standard solution.

|          | Calibration equation<sup>a</sup> | Correlation coefficient<sup>r</sup> | Linear range (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) | DLLME | DLLME-FASI |
|----------|----------------------------------|-------------------------------------|----------------------|-------------|-------------|-------|-------------|
|          | k (mean ± SD)                    | b (mean ± SD)                       |                      |             |             |       |             |
| SMZ      | 7915.9±12.300                    | 1153.80±837.0                       | 0.9938               | 2.0–20.0    | 0.67        | 2.23  | 29.3        | 206         |
| SMR      | 930.9±10.400                     | 455.31±231.7                        | 0.9904               | 1.0–20.0    | 0.34        | 0.94  | 31          | 166         |
| SDZ      | 12734.0±7.804                    | 367.00±198.2                        | 0.9942               | 1.0–20.0    | 0.33        | 1.10  | 46.2        | 185         |
| SFA      | 12028.0±5.100                    | 5429.00±1250.0                      | 0.9931               | 2.0–20.0    | 0.75        | 2.50  | 27          | 150         |

<sup>a</sup> $y = kx + b$; based on peak area.
Table S2 Method precision of migration time and peak area in standard solution.

| SAs | Intra-day Migration time | RSDa ( %, n=5 ) | Inter-day Migration time | Peak area |
|-----|--------------------------|-----------------|--------------------------|-----------|
| SMZ | 0.19                     | 2.02            | 1.09                     | 5.17      |
| SMR | 0.25                     | 1.86            | 1.14                     | 3.76      |
| SDZ | 0.29                     | 2.16            | 1.74                     | 3.97      |
| SFA | 0.32                     | 2.82            | 1.05                     | 4.34      |

a At individual concentrations of 1.0×10^4 ng/mL.

Table S3 Method precision of migration time and peak area in seawater sample.

| SAs | Intra-day Migration time | RSDa ( %, n=5 ) | Inter-day Migration time | Peak area |
|-----|--------------------------|-----------------|--------------------------|-----------|
| SMZ | 0.60                     | 7.13            | 4.21                     | 5.36      |
| SMR | 0.77                     | 5.30            | 3.09                     | 7.68      |
| SDZ | 0.80                     | 6.79            | 3.99                     | 10.47     |
| SFA | 0.47                     | 2.04            | 3.72                     | 11.15     |

a At individual concentrations of 1.0×10^4 ng/mL.
Table S4 Recoveries obtained for the determination of four SAs in spiked tap, lake and river water samples (n=5).

| SAs    | Spiked (ng/mL $10^3$) | Tap water | Lake water | Seawater |
|--------|-----------------------|-----------|------------|----------|
|        | Found±SD (10^3 ng/mL) | Recovery (%) | RSD (%) | Found±SD (10^3 ng/mL) | Recovery (%) | RSD (%) | Found±SD (10^3 ng/mL) | Recovery (%) | RSD (%) |
| SAs    |                       |            |           |          |                       |            |           |                       |            |        |
| SMZ    | 0.1                   | 0.093±0.004 | 93.0      | 4.30     | 0.097±0.005 | 97.0      | 5.15     | 0.105±0.003 | 105.0      | 2.85    |
|        | 2.0                   | 1.950±0.037 | 97.5      | 1.89     | 2.010±0.033 | 101.7     | 1.65     | 2.130±0.180 | 106.5      | 7.45    |
|        | 10.0                  | 10.120±0.310 | 101.2   | 3.06     | 10.230±0.270 | 102.0     | 2.64     | 9.930±0.400 | 99.0       | 4.06    |
| SMR    | 0.1                   | 0.095±0.003 | 95.0      | 3.15     | 0.097±0.002 | 97.0      | 2.06     | 0.102±0.003 | 102.0      | 2.94    |
|        | 2.0                   | 1.920±0.040 | 96.0      | 2.08     | 2.030±0.220 | 102.3     | 6.80     | 2.160±0.130 | 108.0      | 6.01    |
|        | 10.0                  | 10.120±0.310 | 87.7   | 3.56     | 9.930±0.135 | 99.3      | 1.36     | 10.130±0.370 | 101.3      | 3.74    |
| SDZ    | 0.1                   | 0.091±0.003 | 91.0      | 3.29     | 0.095±0.002 | 95.0      | 2.10     | 1.080±0.050 | 108.0      | 4.62    |
|        | 2.0                   | 1.930±0.090 | 96.5      | 4.67     | 1.980±0.170 | 102.0     | 5.58     | 2.160±0.154 | 108.0      | 7.12    |
|        | 10.0                  | 9.810±0.320 | 98.0     | 3.24     | 10.300±0.140 | 103.0     | 1.37     | 10.430±0.730 | 104.3      | 7.08    |
| SFA    | 0.1                   | 0.093±0.002 | 93.0      | 2.15     | 0.102±0.003 | 112.0     | 2.94     | 0.108±0.004 | 108.0      | 3.70    |
|        | 2.0                   | 1.950±0.060 | 97.5      | 3.07     | 2.070±0.160 | 103.5     | 6.72     | 2.010±0.022 | 100.5      | 1.09    |
|        | 10.0                  | 10.270±0.210 | 102.7 | 2.03     | 9.980±0.320 | 99.8      | 3.24     | 10.190±0.410 | 102.0      | 4.06    |

*Not detected.*
Table S5 Analytical performance comparison of DLLME-FASI-CE with other reported methods for determination of SAs.

| SAs                                                                 | Pretreatment method | Detection technique | Migration time (min) | Linearity range          | LODs                  | LOQs                  | EF/SEF | Sample                  | Ref.   |
|--------------------------------------------------------------------|---------------------|---------------------|----------------------|--------------------------|------------------------|------------------------|--------|-------------------------|--------|
| Sulfadiazine, sulfathiazole, sulfamethazine, sulfamethoxypyridazine | MSPE                | HPLC                | ~14                  | 3.97–1000 (ng/g)         | 1.73–5.23 (ng/g)       | 3.97–15.89 (ng/g)       | –      | Meat sample              | [1]    |
| Sulfaguanidine, sulfadiazine, sulfathiazole, sulfamethazine, sulfamethoxypyridazine, sulfadoxine, sulfadimethoxine, sulfasalazine | SPE                 | LC-MS               | ~25                  | 50–500 (µg/kg)           | 25 (µg/kg)             | –                      | –      | Milk and chicken muscle | [4]    |
| Sulfadiazine, sulfathiazole, sulfamethazine, Sulfapyridine sulfamethoxyypyridazine, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime | microextraction     | HPLC                | ~30                  | 0–100 (ng/g)             | 0.008–0.0019 (µg/L); 0.077–0.350 (µg/L) | 0.23–1.05 (µg/L); 0.025–0.057 (µg/L) | –      | Milk and water sample    | [5]    |
| Sulfadiazine, sulfathiazole, sulfamethazine, sulfanilamide, sulfamethoxyypyridazine, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime, sulfadoxime | Salting-out-LLE     | HPLC-fluorescence   | ~35                  | 1.5–2 (ng/g)             | 5–100 (ng/g)           | –                      |        | Honey                   | [7]    |
| Analytes                                                                 | Method                      | Limits                        | Matrix     | Ref. |
|-------------------------------------------------------------------------|-----------------------------|-------------------------------|------------|------|
| Sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfadimethoxine, sulfisoxazole, sulfadoxine | polymer monolith microextraction LC/MS | ~14 (ng/mL); 0.4–5.7 (ng/mL); 0.9–9.8 (ng/mL); 1.8–19 (ng/g); 2.9–32.8 (ng/g); 9.4–19.6 (ng/g) | Milk and egg | [8] |
| Sulfadimethoxine, sulfadiazine, sulfapyridine, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfachloropyridazine, sulfamethoxazole | MIP-SPE HPLC | ~14 | Blood | [9] |
| Sulfadimethoxine, sulfadiazine, sulfapyridine, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfachloropyridazine, sulfamethoxazole | MSPE UV | ~15 | 1–100 (µg/L) | Waters | [10] |
| Sulfathiazole, sulfamethoxazole, sulfadiazole, sulfadoxine hydrochloride, oxytetracycline, chlortetracycline, tiamulin fumarate | SPE | LC-MS | ~30 | 1–1000 (µg/L) | 0.5–8 (µg/L) | Swine wastewater [11] |
| --- | --- | --- | --- | --- | --- | --- |
| Sulfonamides, sulfadimidin, sulfadoxin, sulfadiazine, sulfamerazin, sulfapyridine | DLLME | CE-UV | ~5 | 0.5–50 (µg/L) | 0.020–0.570 (µg/mL) | – | – | Water [12] |
| Sulfaguanidine, sulfanilamide, sulfamerazine, sulfadiazine, sulfadoxin, sulfapyridine, sulfamethoxazole, sulfisoxazole, sulfaquinoxaline | SPE | Nano-LC/MS | ~35 | 50–1800 (µg/kg) | 2–40 (µg/kg) | 8–96 (µg/kg) | – | Honey, milk [33] |
| Sulfadiazine, sulfathiazole, sulfamethazine, Sulfapyridine sulfamethoxypyridazine, sulfachloropyridazine, sulfadimethoxine, sulfisoxazole, sulfaquinoxaline | Microextraction | HPLC | ~30 | 0.05–150 (µg/L) | 0.008–0.350 (µg/L) | 0.025–0.057 (µg/mL) | 0.23–1.05 (µg/mL) | 25–463 | Lake water, Milk [34] |
| Substance                          | Method   | Detection Limit | Linear Range | spiked | Matrix | Reference |
|-----------------------------------|----------|-----------------|--------------|--------|--------|-----------|
| Sulfachloropyridazine, sulfaquinoxaline, sulfadiazine, sulfamethoxazole, sulfapyridine, sulfathiazole, sulfadiazine, sulfamerazine, sulfamethoxydiazine | MSPE     | UHPLC-MS/MS     | ~9          | 5.0–1000.0 (ng/L) | 0.49–1.59 (µg/L) | 1.64–5.29 (ng/L) | – | Water | [35] |
| Sulfaguanidine, sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfapryidine, sulfamethazine, sulfamethoxydiazine, sulfachloropyridazine, sulfadoxine | LLE-SPE  | HPLC-fluorescence | ~60         | 2–100 (ng/g) | 1–2 (ng/g) | 2–5 (ng/g) | – | Honey | [45] |
| Sulfamethazine, sulfamethazine free acid, sulfadizine, sulfacetamide | DLLME    | CE              | ~6          | 0.01–10 (µg/mL) | 0.0020–0.063 (µg/mL) | 0.0067–0.201 (µg/mL) | 150–206 | Water | This work |