

Supplementary Materials

Online In-Tube Solid-Phase Microextraction Coupled with Liquid Chromatography–Tandem Mass Spectrometry for Automated Analysis of Four Sulfated Steroid Metabolites in Saliva Samples

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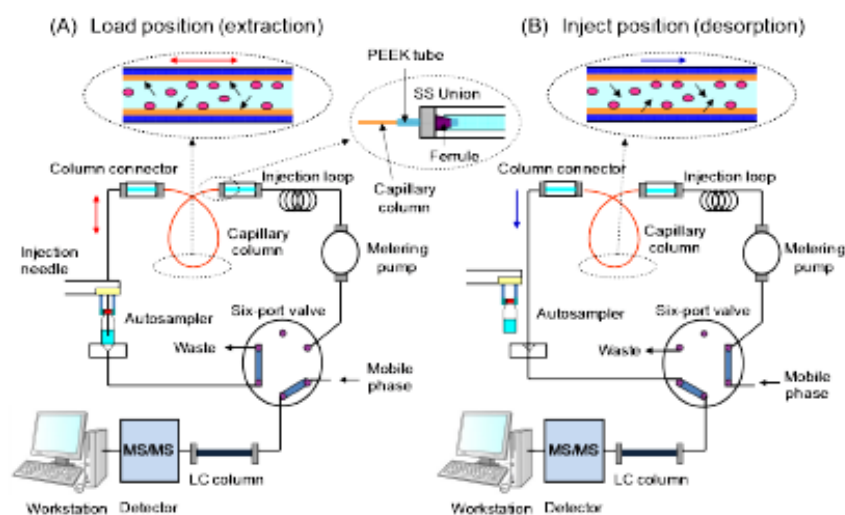


Figure S1. Schematic diagrams of the automated online IT-SPME/LC–MS/MS system.

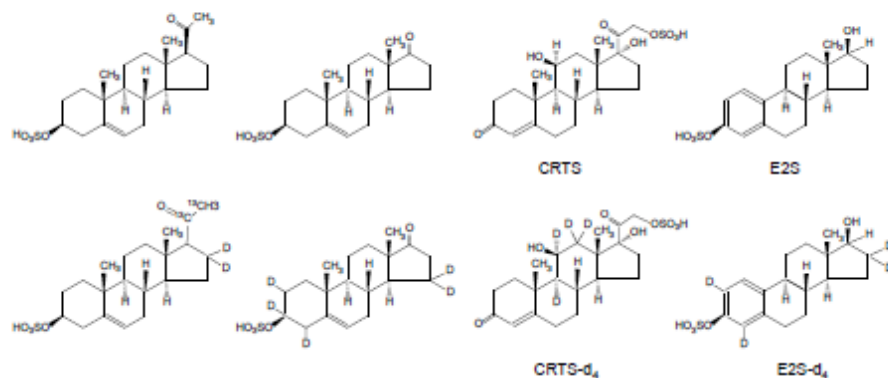


Figure S2. Structures of sulfated steroid metabolites and their respective stable isotope-labeled compounds as internal standards.

Table S1. Program for the IT-SPME process.

| Sequence | Event | Switching valve | Vial | Draw/ejection | | |
|----------|--|-----------------|--------|--------------------|-------------|-------------------------------|
| | | | | Cycle ¹ | Volume (μL) | Speed (μL min ⁻¹) |
| 1 | Conditioning of the capillary | Load | MeOH | D/E (2) | 40 | 200 |
| 2 | Drawing of air into the capillary | Load | Empty | D (1) | 30 | 200 |
| 3 | Conditioning of the capillary | Load | Water | D/E (2) | 40 | 200 |
| 4 | Extraction of analytes into the capillary | Load | Sample | D/E (30) | 40 | 200 |
| 5 | Needle washing | Load | MeOH | D/E (1) | 2 | 200 |
| 6 | Desorption of analytes from the capillary | Inject | – | – | – | – |
| 7 | HPLC separation of analytes and return to sequence 1 | Load | – | – | – | – |

¹ D: draw, E: ejection.**Table S2.** MRM transitions and setting parameters for sulfated steroid metabolites and their stable isotope-labeled compounds.

| TSNA | RT ¹ (min) | Mass transition (m/z) | Dwell time (msec) | DP ² (V) | EP ³ (V) | CE ⁴ (V) | CXP ⁵ (V) |
|--|-----------------------|-----------------------|-------------------|---------------------|---------------------|---------------------|----------------------|
| PREGS | 3.9 | 395.0 →96.8 | 125 | -65 | -8 | -65 | -10 |
| DHEAS | 2.7 | 367.0 →97.0 | 125 | -60 | -8 | -60 | -5 |
| CRTS | 2.1 | 441.2 →97.0 | 125 | -65 | -8 | -65 | -15 |
| E2S | 2.3 | 351.3 →80.1 | 125 | -65 | -8 | -60 | -5 |
| PREGS- ¹³ C ₁₇ -d ₃ | 3.9 | 399.0 →96.8 | 125 | -65 | -8 | -65 | -10 |
| DHEAS-d ₃ | 2.7 | 372.4 →97.4 | 125 | -60 | -8 | -60 | -5 |
| CRTS-d ₄ | 2.1 | 445.2 →96.8 | 125 | -65 | -8 | -65 | -15 |
| E2S-d ₄ | 2.3 | 354.7 →80.0 | 125 | -65 | -8 | -60 | -5 |

¹ Retention time (min)² Declustering potential (V)³ Entrance potential (V)⁴ Collision energy (V)⁵ Collision cell exit potential (V)