

Table SI. Procedure for sample preparation in the modified LC-MS/MS method and other LC-MS/MS protocols used in the present and previous studies.

| First author, year            | Sample preparation                                 | Procedures  | Reference |
|-------------------------------|--|---|-----------|
| Present study                 | One-step protein precipitation                     | i) Prepare sample pretreatment reagent: Zinc sulfate and CSA-d12 in methanol/water.<br>ii) Mix sample with sample pretreatment reagent.<br>iii) Vortex, mix, and centrifuge.  | -         |
| Liu <i>et al</i> , 2019       | Two-step protein precipitation                     | i) Mix with zinc sulfate.<br>ii) Vortex.<br>iii) Added internal standard cyclosporine D and methanol-acetonitrile.<br>iv) Vortexed and centrifuged.   | (5)       |
| Watanabe <i>et al</i> , 2021  | Solid-phase extraction                             | i) Mix sample with ultrapure water, internal standard solution, and formic acid in acetonitrile.<br>ii) Add zinc sulfate.<br>iii) Vortex, incubate and centrifuge.<br>iv) Condition Oasis HLB $\mu$ Elution plate with MeOH and equilibrate with ultrapure water.<br>v) Add supernatant to a well of the $\mu$ Elution plate and wash the well with ultrapure water and MeOH.<br>vi) Elute analytes with MeOH into a 96-well collection plate.<br>vii) Dilute the water, then seal the collection plate with a sealing cap. | (21)      |
| Mei <i>et al</i> , 2018       | Four-step protein precipitation                    | i) Spike sample with internal standard and methanol-water.<br>ii) Add ZnSO <sub>4</sub> .<br>iii) Vortex.<br>iv) Add methanol.<br>v) Vortex and centrifuge.   | (14)      |
| Koster <i>et al</i> , 2009    | Two-step protein precipitation                     | i) Mix sample with methanol and ascomycin.<br>ii) Add zinc sulfate to water.<br>iii) Vortex and centrifuge.   | (6)       |
| Meinitzer <i>et al</i> , 2010 | One-step protein precipitation and online clean up | i) Prepare sample pretreatment reagent: Methanol and ZnSO <sub>4</sub> containing CSA-d4.<br>ii) Centrifuge.<br>iii) Perform online sample clean up (a perfusion chromatography column with macroporous structure).   | (26)      |
| Salm <i>et al</i> , 2008      | Two-step protein precipitation                     | i) Mix sample with zinc sulphate.<br>ii) Add acetonitrile (containing ascomycin and cyclosporin D).<br>iii) Mix and centrifuge.   | (9)       |

LC-MS/MS/ liquid chromatography-tandem mass spectrometry; CSA, cyclosporin A.