**Supplemental Materials**

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| **De-conjugation** |
| Urine + 40µl β-glucuronidase in phosphate buffer (pH 7.2,250 units/µl), 37 ̊C overnight |

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| **Solid-phase extraction with TSNA-specific cartridge** |

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| Pre-condition |
| 1. 10 ml methanol/dichloromethane (9:1) 2. 1 ml methanol 3. 1 ml water |

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| **Addition of Urine** |
| 6 ml urine treated with β-glucuronidase |

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| Wash |
| 1. 4 ml ammonium acetate (10 mM) 2. 2 ml heptane 3. 1 ml hexane |

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| **Eluted** |
| 3 ml dichlonomethane/toluene (1:1) |

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| **Evaporation and reconstitution** |
| Dried in a speed vacuum concentrator (80 °C, 90 min) and re-dissolved with 1 mL PBS |

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| **Solid-phase extraction with MCX cartridge** |

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| Precondition |
| 1. 2 ml methanol 2. 1 ml methanol/25% ammonium hydroxide (9:1) 3. 2 ml water |

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| **Addition of extract** |
| 1 mL of TSNA purified extract |

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| Wash |
| 1. 2 ml water 2. 2 ml 0.1 N HCl 3. 2 ml methanol |

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| **Eluted** |
| 2 ml methanol / 25% ammonium hydroxide |

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| **Evaporation and reconstitution** |
| Dried in a speed vacuum concentrator (65 °C, 40 min) and re-dissolved with 100 µl 0.1% ammonium acetate in water/0.1% Formic acid in acetonitrile (9:1) |

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| Add internal standard (IS) |
| + 5µl 25ng/mL NNAL-d3, + 5µl 25ng/mL NNN-d4 |

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| **LC-MS** |

Fig. S1. Sample preparation for determination of urinary NNN and NNAL metabolites.