

Supplementary information

Sensitive method to determine melatonin in saliva by
automated online in-tube solid-phase microextraction
coupled with stable isotope-dilution liquid
chromatography-tandem mass spectrometry

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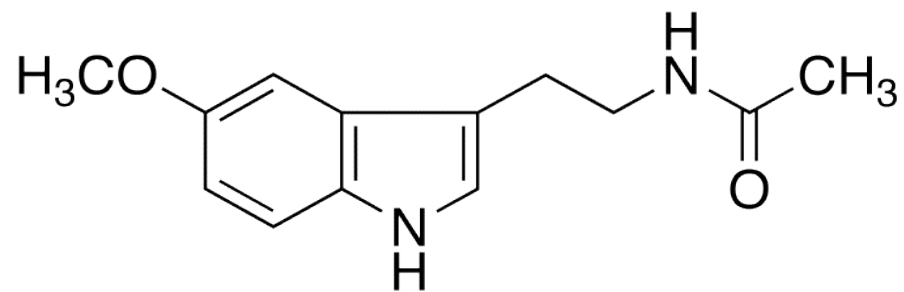
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Supplementary figures

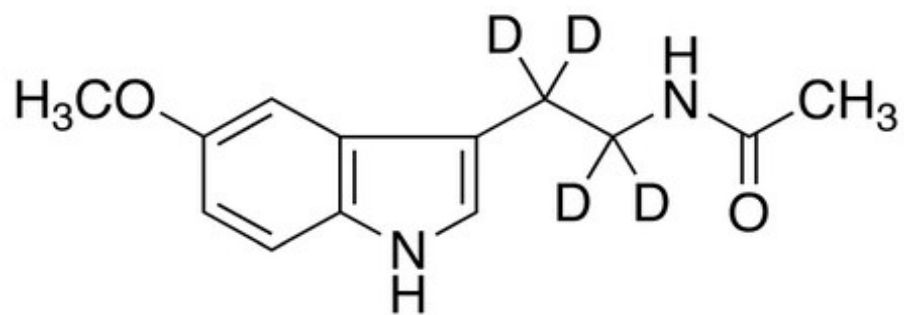
Fig. S1 Structures of MLT and MLT-d₄ used as an internal standard.

Fig. S2 Schematic diagram of the automated online in-tube SPME/LC–MS/MS system.

Fig. S1 Structures of melatonin and melatonin-d₄ used as an internal standard.



Melatonin



Melatonin-d₄

Fig. S2 Schematic diagram of the automated online in-tube SPME/LC-MS/MS system.

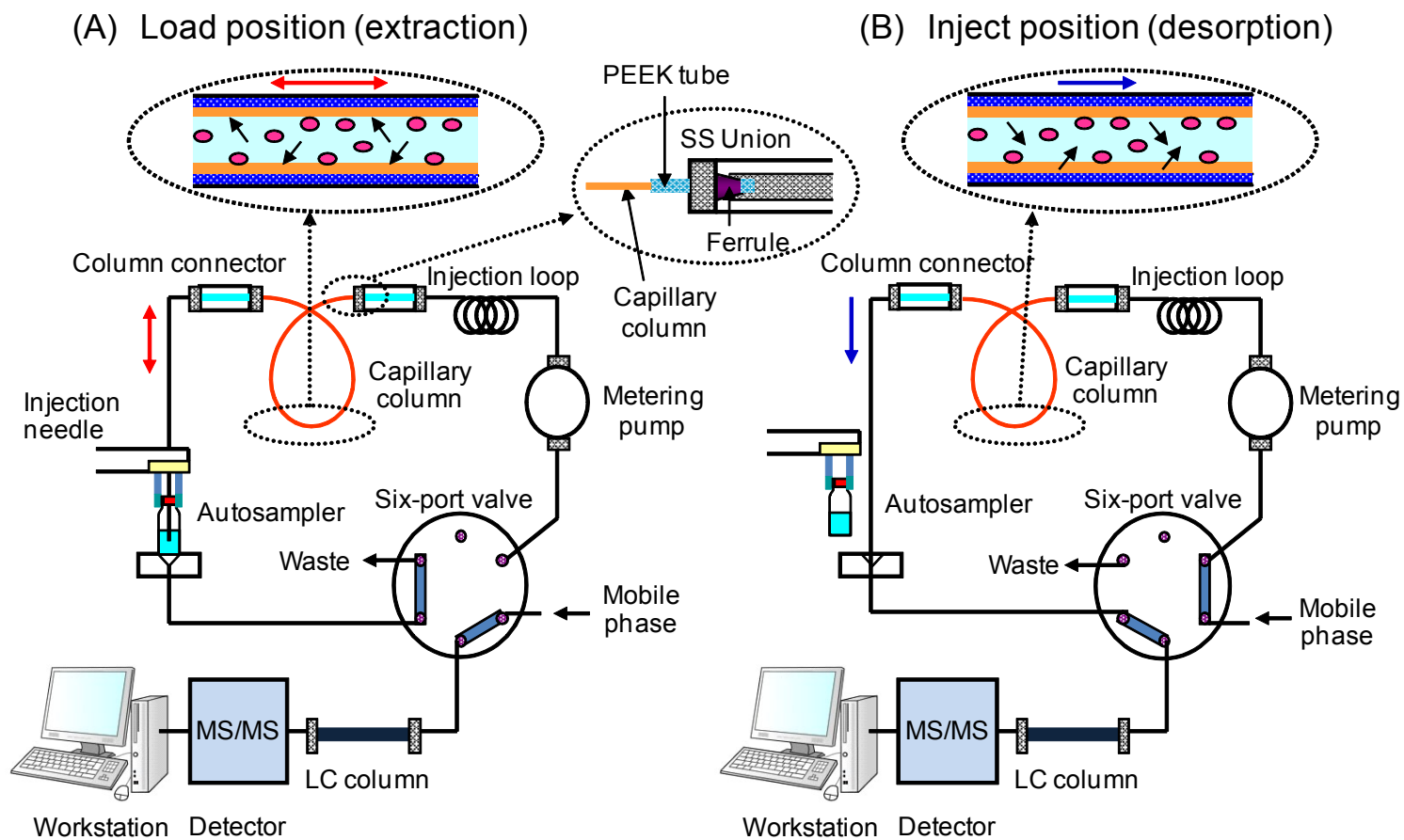


Table S1 MRM conditions for MLT and MLT-d₄.

| Compound | Q1 (precursor ion) | Q3 (product ion) | Dwell time (ms) | DP (V) | EP (V) | CE (eV) | CXP (V) |
|---|-----------------------|---------------------|--------------------|--------|--------|---------|---------|
| MLT (Quantitation) | 233.3 | 174.3 | 333 | 60 | 15 | 20 | 5 |
| MLT (Confirmation) | 233.3 | 159.1 | 333 | 60 | 15 | 30 | 15 |
| MLT-d ₄ (Internal standard) | 237.3 | 178.3 | 333 | 60 | 15 | 20 | 5 |

Table S2 Program for the in-tube SPME process.

| Sequence | Event | Switching valve | Vial | Draw/eject | | |
|----------|--|-----------------|--------|---------------------|-------------|-------------------------------|
| | | | | Cycle ^{a)} | 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) | 50 | 200 |
| 3 | Conditioning of the capillary | Load | Water | D/E (2) | 40 | 200 |
| 4 | Extraction of analytes into the capillary | Load | Sample | D/E (25) | 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 | – | – | – | – |

^{a)} D: draw, E: ejection.

Table S3 Precision of MLT measurements by in-tube SPME LC–MS/MS.

| Compound | Concentration | Precision (RSD %) ^{a)} | |
|----------|--------------------------|---------------------------------|-----------|
| | | Intra-day | Inter-day |
| MLT | 1.0 pg mL ⁻¹ | 4.1 | 5.8 |
| | 10.0 pg mL ⁻¹ | 3.0 | 3.6 |

^{a)} $n = 5$.

Table S4 Recoveries of MLT spiked into saliva samples.

| Compound | Concentration (pg mL ⁻¹ saliva) | | Recovery (%) |
|----------|--|--------------------------|--------------|
| | Spiked | Mean ± SD (<i>n</i> =3) | |
| MLT | 0 | 3.8 ± 0.1 | |
| | 10.0 | 13.4 ± 1.0 | 96 |
| | 100.0 | 102.1 ± 3.9 | 98 |