

(a)

(Takeo Iwamoto et al.)
Extraction with alumina

Human urine (2 mL)

+Alumina (0.25 g)
+2.0 M Tris buffer (pH 8.6, 2 mL)

Shake for 1 min and
centrifuge at 2,000 x g
for 10 min

Supernatant

Alumina

Wash with methanol and water
and centrifuge at 2000 x g for 5 min

Supernatant

Alumina

+0.2 N HCl (0.5 mL)

Filtrate and get
the HCl layer

Analyze HCl layer by HPLC
system (10 μ L injection)

Exhausted solvent: 2.5 mL
Sample volume: 2 mL
Pretreatment time: 16 min

(b)

(G. Grossi et al.)
Extraction with Bond Elut C18

Activate the C18 cartridge
with 2 X 1 mL of methanol

Equilibrate with 2 X 1 mL 0.2 M NH_4Cl -
 NH_4OH buffer, pH 8.5 (0.05 % EDTA)

Sample mix: 0.5 mL urine+ 10 μ L IS+ 1
mL 2.0 M NH_4Cl - NH_4OH (pH 8.5) buffer
(0.2 % DPBA complex and 0.5 % EDTA)

Apply 1 mL of sample

Wash with 2 X 1 mL of buffer

Wash with 1 mL of methanol-
buffer pH 8.5 20 : 80

Elute with 2 mL of 0.08 M acetic acid

Analyze elute by HPLC
system (50 μ L injection)

Exhausted solvent: 5 mL
Sample volume: 0.5 mL
Pretreatment time: 8 min

(c)

(This work)
Extraction with nanofibers

Activate the PFSPE cartridge with 100
 μ L of methanol 100 μ L of water

Load 100 μ L urine sample, 100 μ L 2
mg/mL DPBA solution and 30 μ L 100
ng/mL IS solution to the PFSPE column

Wash 3 times with 100 μ L
2 mg/mL DPBA solution

Elute with 50 μ L eluant

Analyze elute by HPLC-ECD
system (20 μ L injection)

Exhausted solvent: 0.5 mL
Sample volume: 0.1 mL
Pretreatment time: 5 min