



**METHADONE / EDDP IN WHOLE BLOOD,
PLASMA / SERUM, TISSUE AND URINE USING: 200 mg
CLEAN SCREEN[®] EXTRACTION COLUMN**
PART #: ZSDAU020
LC-MSMS

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH= 6) add internal standard.*
Add 1 mL of whole blood, serum/ plasma urine or tissue (1 g of 1:4 homogenate).
Add 2 mL of 100 mM phosphate buffer (pH= 6).
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Mix/vortex.
Centrifuge as appropriate.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
1 x 1 mL 100 mM phosphate buffer (pH= 6).
Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. APPLY SAMPLE

Load sample at 1-2 mL / minute.

4. WASH COLUMN

1 x 3 mL DI H₂O
1 x 3 mL 100 mM acetic acid.
1 x 3 mL CH₃OH
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE METHADONE/EDDP

1 x 3 mL ethyl acetate; acetonitrile: ammonia (78: 20: 2 v/v)
Collect eluate at 1-2 mL /minute.
NOTE: Prepare elution solvent daily

6. EVAPORATION

Evaporate eluates under a gentle stream of nitrogen < 40°C.

7. Reconstitue sample in 100 µL of CH₃OH.

Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) SB-Aq (Agilent Technologies)

Mobile phase:

<u>Time</u>	<u>% Acetonitrile</u>	<u>%0.1% Formic acid</u>
0	25	75
5	25	75
14	90	10
15	25	75
20	25	75

Flowrate: 0.35 mL/ minute

Column Temperature: ambient

Detector: API 2000 MS/MS

<u>Compound</u>	<u>MRM Transition</u>	<u>Cerilliant #</u>
Methadone	310.2/105.1	M-007
*Methadone-D9	319.2/268.3	M-088
EDDP	278.2/234.2	E-022
*EDDP-D3	281.4/234.3	E-021

