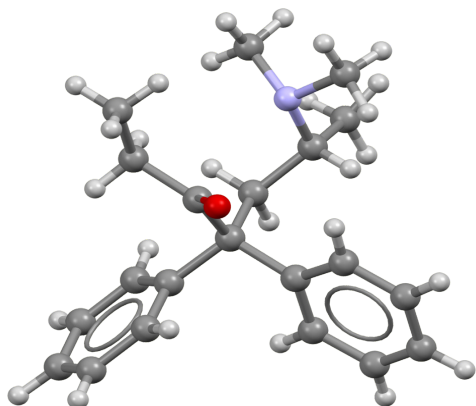


Methadone / EDDP In Whole Blood, Plasma / Serum, Tissue and Urine Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

ZSDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
Without Tips

Procedure:

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= 6.0).
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load at 1-2 mL/minute.

4. Wash Column

- 1 x 3 mL D.I. 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. Reconstitute

- Reconstitute sample in 100 µL of CH₃OH.
- Inject 5 µL.

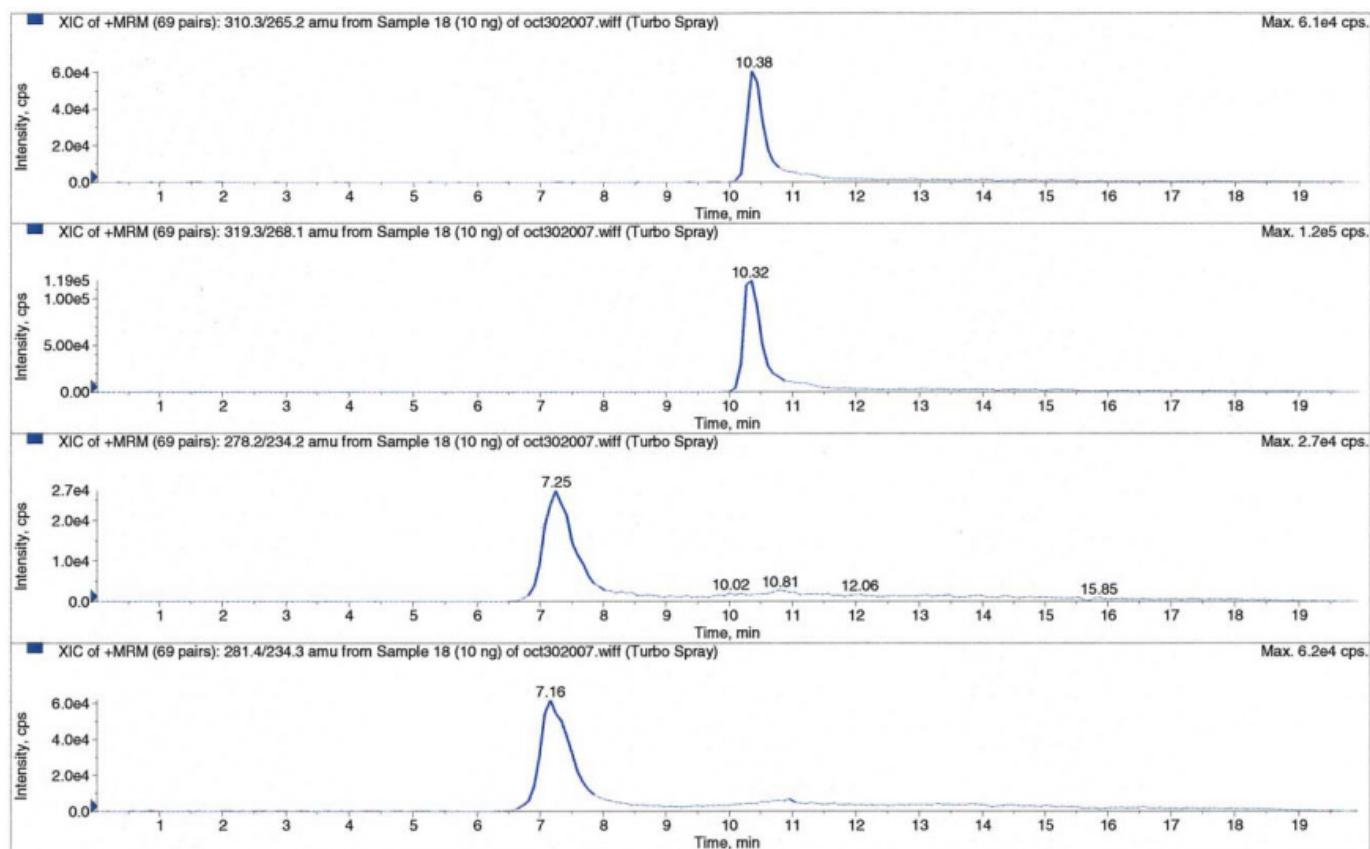
Instrument Conditions

Column	150 x 2.1 mm (3 µm) SB-Aq (Agilent Technologies)
Flow Rate	0.35 mL/minute
Column Temperature	ambient
Detector	API 2000 MS/MS



Mobile Phase		
Time	% Acetonitrile	% 0.1% Formic Acid
0	25	75
5	25	75
14	90	10
15	25	75
20	25	75

Compound	MRM Transition	Cerilliant #
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



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