

Abstract

The performance characteristics of a method for detecting methadone in oral fluid specimens were examined and compared with urine specimens. Oral fluid was obtained using a simple device that collects between 1 and 1.5 mL of fluid for laboratory analysis. When specimen or standard is added to an EIA well containing an oral fluid specimen positive for methadone, there is a competition between the drug and the enzyme-labeled hapten to bind the antibody fixed onto the EIA well. The EIA wells are then washed, substrate is added, and color is produced.

Specimens collected simultaneously from 102 methadone non-users and users from a drug treatment center were first tested using an immunoassay cutoff of 5 ng/mL in oral fluids and 300 ng/mL in urine. Using a second aliquot, methadone confirmation in urine was performed by GC/MS and in oral fluids by GC/MS. The combined immunoassay and GC/MS procedures were completed with less than 500 µL of oral fluid.

The immunoassay was tested for precision, stability, and the effects of potential cross-reactants and interferences. The total precision for 20 days of testing calculated using the NCCLS EP5-T2 protocol yielded CV's less than 15%. The assay is specific for methadone but also has 18% cross-reactivity with LAAM. The assay exhibited no cross-reactivity to compounds such as acetylsalicylic acid, alprazolam, benzoylcegonine, caffeine, cotinine, d-amphetamine, ibuprofen, morphine, naproxen, penicillin, pseudoephedrine, and Δ⁹-THC. The following adulterants did not interfere with the assay: sugar water, toothpaste, antacid, cola, and orange juice.

The results yielded 87.3% agreement between oral fluid and urine and 97.1% agreement between oral fluid and GC/MS, suggesting that oral fluid may be a reliable matrix for methadone detection.

Background

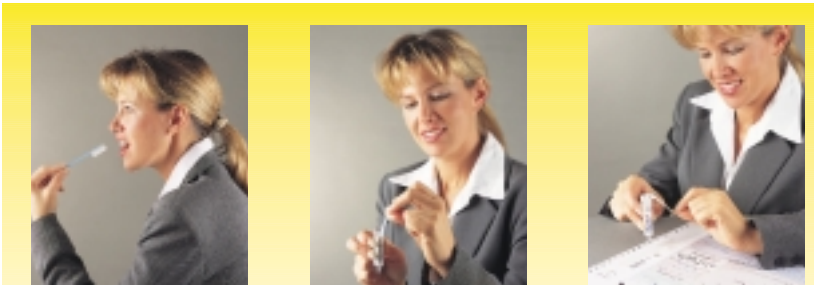
Methadone is a synthetic narcotic that acts as an analgesic and a sedative. Its major therapeutic use is for narcotics detoxification or in methadone maintenance programs.⁽¹⁾ Methadone has been identified in saliva following administration and may be present in saliva at detectable levels for as long as 70 hours.⁽²⁾ The length of time following drug use for which a positive result may occur in saliva is dependent upon several factors including the frequency and amount of drug. Methadone undergoes extensive metabolism, resulting in only about 10% of the dose appearing unchanged in the urine.⁽¹⁾

Oral Fluid Collection



The Intercept™ DOA Oral Specimen Collection Device was developed for the purpose of collecting oral fluid for diagnostic testing. The collection device consists of a treated absorbent cotton fiber pad affixed to a nylon stick (Collection Pad) and a

preservative solution in a plastic container (Specimen Vial). The Collection Pad is impregnated with a mixture of common salts and gelatin which creates a hypertonic environment and an increased osmotic pressure wherever it contacts oral mucosal cells. The pad is placed in contact with the gingival mucosa (between the lower gum and cheek) which enhances the flow of mucosal transudate across the mucosal surfaces onto the absorptive cotton fibers of the pad. Following the collection period, the Collection Pad is placed into a vial containing a preservative solution which serves to inhibit the growth of oral micro-organisms recovered on the Collection Pad. The vial is sealed with a plastic cap and transported to a laboratory for processing and testing. Following processing, a fluid containing a mixture of saliva components and the preservative solution is recovered which is suitable for testing for the presence of methadone in the OTI Methadone Intercept™ MICRO-PLATE EIA manufactured by OraSure Technologies, Inc., Bethlehem, PA with GC/MS confirmation.

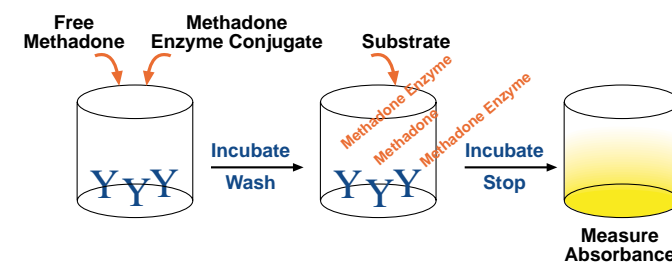


1. Peel open pad package far enough to allow easy removal of the Collection Pad.
2. Place pad between lower cheek and gum and gently rub back and forth until moist.
3. Keep the pad in place for 2 minutes (maximum 5 minutes) while timing.
4. Open vial in upright position.
5. Insert pad into the blue liquid at the bottom of the vial.
6. Break the pad handle by snapping it against the side of vial.
7. Replace the cap with a snap.
8. Place seal over top of vial and send sample to a laboratory for processing and testing.

Assay Format and Procedure

The OTI Methadone Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) is a competitive immunoassay for the qualitative detection of methadone in oral fluid (see diagram below).

1. Hold Collection Vial upright with the tip pointed up. Move the pad away from the vial tip by gently tapping the vial.
2. Break the pointed tip of the vial off with your thumb, place a tube over the vial, and invert the tube and vial.
3. Centrifuge at 600 - 800 x g for 15 minutes.
4. Add 25 µL of sample or calibrator to each well. Test all samples in duplicate.
5. Add 100 µL of Enzyme Conjugate to each test well and incubate for 30 minutes at room temperature (15-27°C) in the dark.
6. Wash the plate using a suitable plate washer; wash each well 6 times with 300 mL of distilled water.
7. Add 100 µL of Substrate Reagent to each well and incubate for 30 minutes at room temperature (15-27°C) in the dark.
8. Add 100 µL of Stopping Reagent to each well and then measure the absorbance (A) at 450 nm. If the A is ≤cutoff, the sample is positive; if the A is >cutoff, the sample is negative.



Cross-Reactivity of the OTI Methadone Intercept™ MICRO-PLATE EIA

Compound	Tested Concentration (ng/mL)	Methadone Equivalents (ng/mL)	Cross-Reactivity %
EMDP	10,000	2.2	0.02
EDDP	5,000	4.6	0.09
LAAM	25	4.4	17.6

Precision of the OTI Methadone Intercept™ MICRO-PLATE EIA

The precision of the OTI Methadone Intercept™ MICRO-PLATE EIA was assessed by testing Oral Fluid Diluent containing 0, 2.5, 5.0, and 7.5 ng/mL methadone. The intra-assay precision was determined by analyzing each level 16 times per run for 4 runs. Inter-assay precision was determined by analyzing 2 samples at each level twice per day for 20 days. The results of this testing are described in the following table:

Methadone (ng/mL)	Mean O.D.	Intra-Assay % CV(n=64)	Inter-Assay % CV(n=4/day 20 days)
0	1.932	6.3	9.9
2.5	0.687	6.6	12.3
5.0	0.507	6.7	12.9
7.5	0.426	6.8	13.6

Oral Fluid GC/MS Method

1. Add Δ⁹ Methadone internal standard to 400 µL of oral fluid sample and then dilute to 3.6 mL with 0.1 M phosphate buffer (pH 6) and mix.
2. This solution is then extracted on a RapidTrace under the following conditions using Varian Bond Elute Certify 130 mg 3mL columns:

Condition	1.3 mL Elution Buffer*
Condition	1.3 mL Methanol
Condition	1.3 mL Water
Condition	1.3 mL 0.1 M Phosphate Buffer (pH 6)
Load	4 mL Sample
Rinse	1.3 mL Water
Rinse	1.3 mL 1 M Acetic Acid
Rinse	1.7 mL Methanol
Dry	1 minute
Purge	2 mL Methanol
Collect	1.3 mL Elution Buffer*
Collect	1.3 mL Elution Buffer*
Rinse	2 mL Methanol
Rinse	2 mL Methanol
Purge	2 mL Water

* Elution solvent = Methylene chloride/Isopropanol/Ammonium hydroxide (78/20/2)

3. The solvent is dried under nitrogen at 55°C.
4. 50 µL Ethyl Acetate is added to the dried-down extracts.
5. 3 µL is injected into the Saturn 2000 Ion Trap and analyzed under the following conditions:

Column:	Chrompack CP-SIL 5 CB Low Bleed, 15 meter, 0.25 mm
Oven:	80°C for 1 min., 20°C/min. up to 300°C
Flow rate:	1 mL/min.
Injector:	250°C
Transfer line:	290°C
Detector:	250°C

Study Design Section

The clinical accuracy of the OTI Methadone Intercept™ MICRO-PLATE EIA was determined from specimens collected from individuals in a drug rehabilitation clinic. For oral fluid testing, the cutoff for EIA and GC/MS was 5.0 ng/mL. For urine testing, the cutoff was 300 ng/mL.

A total of 102 oral fluid and urine specimen pairs were tested by EIA. Of the 102 samples tested, all oral fluid samples and all urine EIA positives were confirmed by GC/MS. The oral fluid and urine results compare as follows:

		GC/MS of Intercept™ Specimens (5.0 ng/mL cutoff)	
		+	-
OTI Intercept™ EIA (5.0 ng/mL cutoff)	+	49	3
	-	0	50
		% Agreement = 97.1%	
		Urine EIA (300 ng/mL cutoff)	
		+	-
OTI Intercept™ EIA (5.0 ng/mL cutoff)	+	41	11
	-	0	50
		% Agreement = 89.2%	

Conclusions

- Butalbital concentrations for each subject peaked between 0.5 and 2 hours and were still detectable after 48 hours in oral fluid after a single dose of 50 mg. Also Butalbital concentrations for urine peaked between 0.5 and 6 hours and could be detected for up to 48 hours after the single dose.
- The OTI Methadone Intercept™ MICRO-PLATE EIA has a broad cross-reactivity profile which includes Allobarbitol, Amobarbitol, Aprobarbitol, Barbitol, Butabarbitol, Butalbital, Pentobarbitol, Phenobarbitol, and Talbutal.
- After a ten-minute waiting period all adulterants screened were cleared from the oral cavity and did not interfere with samples containing drug concentrations greater than 40 ng/mL.
- Twenty patient samples from known methadone users were collected and screened in the OTI Methadone Intercept™ MICRO-PLATE EIA. Concentrations of samples containing drug ranged from 90 ng/mL to 2910 ng/mL.
- Oral fluid screening has shown acceptable agreement with urine screening.

REFERENCES

1. Kwong, et al., "Critical Issues in Urinalysis of Abused Substances: Report of the Substance Abuse Testing Committee," *Clin. Chem.*, Vol. 34, No. 3 1988.
2. Nilsson, M., et al., "Effect of Urinary pH on the Disposition of Methadone in Man," *Eur. J. Clin. Pharmacol.*, Vol. 22, 1982, 337-342.