

Stability of Cocaine, Heroin and Metabolites in Oral fluid collected with the Intercept® Collector

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Abstract

There is increasing interest in the use of oral fluids for detection of drugs of abuse in the workplace and as a possible substitute for blood in driving under the influence cases. Oral fluids often have higher concentrations of parent drugs that are detectable for longer periods of time than in urine and blood. This increased window of detection of the parent compounds can help provide evidence as to the time of use of the drug. However, some of these parent compounds such as Cocaine and Heroin are less stable than their corresponding metabolites. In this study, Cocaine, Benzoylgonine (BE), Heroin, 6-monoacetylmorphine (MAM) and Morphine were spiked into oral fluid samples collected with the Intercept® oral specimen collection device from volunteers. The Intercept® collection device utilizes a collection pad impregnated with dried salts that increase osmotic pressure, facilitating increased collection efficiency. Following collection, the pad is stored in a preservative solution. The pooled spiked volunteer samples were incubated at -20°C, 4°C, room temperature (RT) and 37°C for 14 days. The samples were then extracted by solid phase extraction (SPE) using the Zymark RapidTrace SPE Workstation. The SPE extraction used Bond Elute Certify® extraction columns that were washed with methanol, deionized water and 0.1 M pH 6 phosphate buffer. Samples were applied, followed by rinsing with 1M acetic acid and methanol. The drugs were extracted with 78/20/2 (methylene chloride/isopropanol/ammonium hydroxide), evaporated to dryness, derivatized with BSTFA+1%TCMS and then analyzed by GC/MS/MS using the Varian 1200 GC/MS/MS.

The stability of Cocaine and Heroin spiked into oral fluid samples collected with the Intercept® collection device was tested during the extraction procedure. In addition, Cocaine, Benzoylgonine (BE), Heroin, 6-monoacetylmorphine (MAM) and Morphine were spiked into oral fluid samples collected with the Intercept® collection device at 20ng/mL and tested on day 0,1,2,3,7,10 and 14 at the four different temperatures. We compared our method to previous publications which utilized several changes to reduce this conversion of Heroin to MAM^{14,5,6} and found that both methods produced no measurable loss of Heroin over the time period required to sequentially extract ten samples using the RapidTrace Workstation. For the temperature stability study, Heroin also showed the quickest decline out of all of the analytes tested with a complete conversion to MAM in 1 day at 37°C. Cocaine was next with all converted to BE by 7 days at 37°C. MAM showed a decline of 50% at 37°C by 14 days. Morphine and BE remained relatively stable during the entire 14 day testing period at all temperatures. Reduction of temperature did slow down the breakdown of Heroin, Cocaine and MAM. Cocaine and MAM were stable the entire 14 days at 4°C, but Heroin required -20°C to maintain stability for the 14 day time period tested.

This is the first reported stability data of Cocaine, Heroin and metabolites in oral fluid collected with the Intercept® collection device. The stability of Cocaine in oral fluid was similar to that of cocaine in blood, as reported in the literature.⁷

Background

Heroin and Cocaine can be detected in saliva following administration due to a pH-dependent exchange between the blood system and salivary glands.^{11,2,3} The presence of Heroin or MAM in the sample is very important in distinguishing between a Heroin user and poppy seed ingestion. In addition, the amounts of MAM and Cocaine present in saliva samples can be used to help determine the level of impairment and time of drug use. Previous studies have indicated that there may be an increased window of detection of Heroin and Cocaine in oral fluids versus blood after smoking.¹⁴ Both drugs undergo degradation upon storage. Cocaine hydrolyzes to BE, Heroin to MAM, and MAM to Morphine. The rate of hydrolysis is dependent on several factors including pH and storage temperature.¹⁴ Storage temperature can be controlled to some extent, however pH differences between samples can be more extreme depending on the sample matrix. Freshly collected blood has the most constant pH compared to urine pH which ranges from pH 4 to 9. Oral fluid pH has a narrower pH range of 5 to 8 which is influenced to a large extent upon the method of collection. Stimulated methods of collection tend to produce oral fluids with lower pH's.^{11,2,3} The Intercept® collection device collects approximately 400 microliters of oral fluid using a collector stick impregnated with dried salts which is then placed into 800 microliters of a preservative solution at a pH of approximately 7.

Our research involved investigation of the effects of storage temperature over a period of 14 days and extraction methods on the degradation of Heroin, MAM, Morphine, Cocaine and BE.

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 opiates and cocaine in the Intercept® Opiates or Cocaine MICRO-PLATE EIA manufactured by OraSure Technologies, Bethlehem, PA and by GC/MS/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.



Study Design Section

- All oral fluids were prepared by collecting samples from volunteers in the laboratory using the Intercept® collection device. The samples were pooled and then tested by GC/MS/MS for the drugs of interest prior to spiking.
- All drugs were purchased from Cerilliant and were stored according to the manufacturer's recommendations. All oral fluid samples were spiked with standards prepared the same day at a concentration of 20ng/mL.
- For the method comparison study, a sample of pooled oral was spiked with Heroin and then split into 20 aliquots, 10 for each method. The samples were then extracted on two RapidTrace Workstations which sequentially extract 10 samples per module. The samples were then analyzed by GC/MS/MS.
- 7 pooled oral fluid samples were spiked with Heroin and Cocaine, a second set of 7 samples were spiked with Morphine and BE, and a third set of 7 samples were spiked with MAM only for each storage temperature. The spiked oral fluid samples were tested immediately after spiking and then again on day 1, 2, 3, 7, 11 and 14 at storage temperatures of 37°C, room temperature, 4°C, and -20°C. All extractions were performed using method #1 followed by GC/MS/MS analysis.

GC/MS/MS Acquisition Parameters

Varian 3800 GC with 1177 Injector

Column: Chrompack CP-SIL 8 CB Low Bleed, 15 meter, 0.25 mm
Oven: 130°C for 1 min., 20°C/min. up to 300°C
Flow rate: 1mL/min Helium
Injector: 280°C
Transfer line: 280°C

Varian 1200 MS/MS

Ion source: 200°C
Requested Scan Time: 0.300
Ion Polarity: Positive
SIM Width: 1.00
Scan Mode: Centroid
CID gas : 2 mitorr Argon

Analyte	Description	Parent Ion	Collision Energy (Volts)	Quant Ions	Qualifier Ions
PCP d-5	Internal Std	205	-25	122	84
PCP	Std	200	-25	117	84
BZE d-3	Internal Std	243	-25	125	111
BZE	Std	240	-25	122	108
Cocaine	Std	303	-15	83	198
Codeine d-3	Internal Std	374	-25	237	149
Codeine	Std	371	-25	234	146
Morphine d-3	Internal Std	432	-20	237	223
Morphine	Std	429	-20	234	220
6-MAM	Std	399	-15	287	340
Heroin	Std	369	-15	310	204

Oral Fluid Extraction Method #1

1. Add d-3 Morphine, d-3 MAM and d-3 BE internal standard to 200µL of oral fluid sample and then dilute to 4 mLs with 0.1M phosphate buffer (pH 6) and mix.
2. This solution is then extracted on a RapidTrace under the following conditions using Varian Bond Elute Certify 130mg 3mL columns:
 - Condition 1.3 mL Elution buffer*
 - Condition 1.3 mL Methanol
 - Condition 1.3 mL Water
 - Condition 1.3 mL 0.1M phosphate buffer (pH 6)
 - Load 4 mL Sample
 - Rinse 1.3 mL Water
 - Rinse 1.3 mL 1M 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
3. The solvent is dried under nitrogen at 55°C.
4. 50µL of BSTFA + 1% TCMS is added to the dried down extracts and heated at 70°C for 15 minutes.
5. 1µL of the derivatized extract is injected into the Varian 1200 GC/MS/MS.

Oral Fluid Extraction Method #2 (4,5,6)

1. Add d-3 Morphine, d-3 MAM and d-3 BE internal standard to 200mL of oral fluid sample and then dilute to 4 mLs with 0.1M acetate buffer (pH 6) with 5% sodium fluoride and mix.
2. This solution is then extracted on a RapidTrace under the following conditions using Varian Bond Elute Certify 130mg 3mL columns:
 - Condition 1.3 mL Elution buffer*
 - Condition 1.3 mL Methanol
 - Condition 1.3 mL Water
 - Condition 1.3 mL 0.1M acetate buffer (pH 6)
 - Load 4 mL Sample
 - Rinse 1.3 mL Water

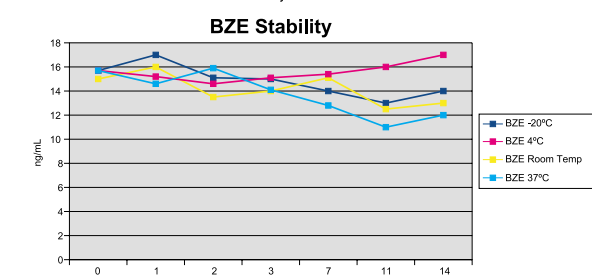
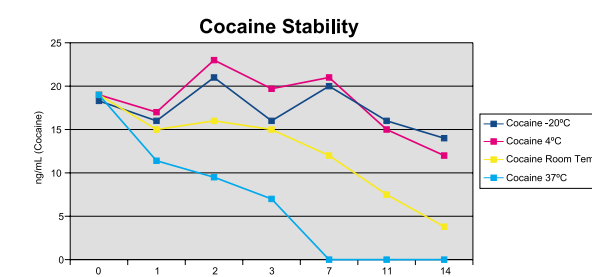
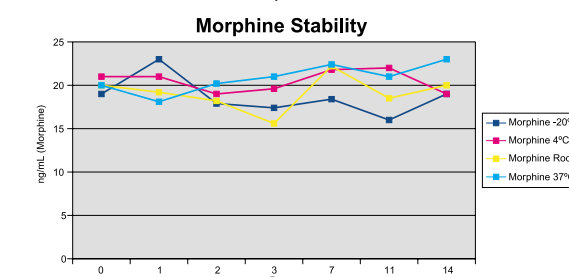
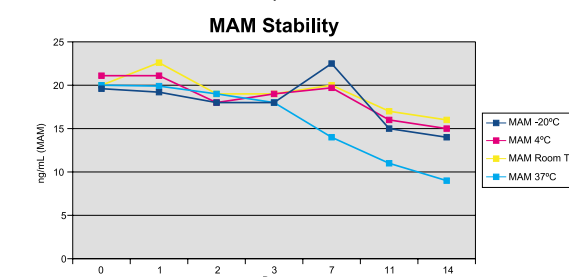
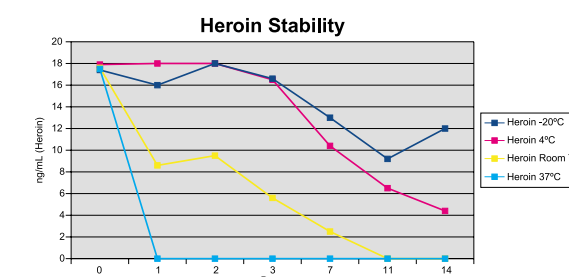
Rinse 1.3 mL 1M acetic acid
Rinse 1.7 mL Acetonitrile
Dry 1 minute
Purge 2 mL Methanol
Collect 1.3 mL Elution buffer*
Collect 1.3 mL Elution buffer*
Rinse 2 mL Methanol
Rinse 2 mL Methanol
Purge 2 mL Water

* Elution solvent = Ethyl acetate/Diethylamine (78/2)

3. The solvent is dried under nitrogen at 55°C.
4. 50µL of BSTFA + 1% TCMS is added to the dried down extracts and heated at 70°C for 15 minutes.
5. 1µL of the derivatized extract is injected into the Varian 1200 GC/MS/MS.

Method Comparison

Rapid Trace Position	Heroin (ng/mL)	
	Method #1	Method #2
1	24	18.3
2	22.5	16.9
3	18.5	27.1
4	19.5	19
5	22	20.3
6	20	22.8
7	22.5	34
8	18.5	20.5
9	20.5	18.5
10	19.5	26.7
Mean	20.8	22.4
Standard Deviation	1.9	5.3
% CV	9.1	23.9



Conclusion

- The major differences between the two methods were all designed to minimize degradation of Heroin to MAM and Morphine. These included using pH 6 acetate buffer containing sodium fluoride in place of the pH 6 phosphate buffer, acetonitrile instead of methanol as the rinse solvent and ethyl acetate/diethylamine in place of methylene chloride/isopropanol/ammonium hydroxide. Another step, published previously, was to split the extract into two, derivatizing one and not derivatizing the portion that would be used for Heroin analysis. We did not try this last step since we were using d-3 MAM as the internal standard for Heroin.
- Both methods took 3 hours each to run 10 samples on their respective RapidTrace module, so the first sample extracted was sitting for almost 3 hours in elution solvent. Prior to derivatization the samples were then evaporated to dryness and derivatized simultaneously. Recoveries were similar for both methods, but Method 1 had a 9.3% CV as compared to a 23.9% CV for Method 2. There was no measurable degradation of Heroin using either method.
- For the 14 day study, Heroin showed the quickest decline out of all of the analytes tested with a complete conversion to MAM in 1 day at 37°C. Cocaine was next with all converted to BE by 7 days at 37°C. MAM showed a decline of 50% at 37°C by 14 days. Morphine and BE remained relatively stable during the entire 14 day testing period at all temperatures. Reduction of temperature did slow down the breakdown of Heroin, Cocaine and MAM. Cocaine and MAM were stable the entire 14 days at 4°C, but Heroin required -20°C to maintain stability for the 14 day time period tested.

REFERENCES

1. Kidwell, D., Holland J., and Athanaselis, S., "Testing for Drugs of Abuse in Saliva and Sweat," *Journal of Chromatography*, 1998; 713: 111-135.
2. Schramm, W., Smith, R.H., and Craig, P.A., "Drugs of Abuse in Saliva: A Review," *Journal of Analytical Toxicology*, 1992; 16:1-9.
3. Cone, E.J., "Saliva Testing for Drugs of Abuse," Presented at the NY Acad. Sciences meeting Oct. 22-25, 1992.
4. Jenkins, A.J., Oyler, J.M., and Cone, E.J., "Comparison of heroin and Cocaine Concentrations in Saliva with Concentrations in Blood and Plasma," *Journal of Analytical Toxicology*, 1995: 359-374.
5. Wang, W., Darwin, W.D., and Cone, E.J., "Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by GA-MS," *Journal of Chromatography*, 1994: 279-290.
6. Goldberger, B.A., Darwin, W.D., Grant, T.M., Allen, A.C., Caplan, Y.H., and Cone, E. J., " measurement of Heroin and its Metabolites by Isotope-Dilution Electron-Impact Mass Spectrometry," *Clinical Chemistry*, 1993: 670-675.
7. Isenschmidt, D.S., Levine, B.S., and Caplan, Y.H., "A Comprehensive Study of the Stability of Cocaine and Its Metabolites," *Journal of Analytical Toxicology*, 1989: 250-256.