The Determination of Morphine in Urine and Oral Fluid Following Ingestion of Poppy Seeds*

Timothy P. Rohrig†
Sedgwick County Regional Forensic Science Center, 1109 N. Minneapolis, Wichita, Kansas 67214-3129

Christine Moore
U.S. Drug Testing Laboratories, 1700 S. Mount Prospect Road, Des Plaines, Illinois 60018

Abstract

In workplace drug-testing programs, the use of heroin, morphine, and codeine is currently determined by the analysis of urine specimens. It has been shown that ingestion of poppy seeds can cause a positive test result for morphine. In an attempt to differentiate positive results caused by poppy seed ingestion from those caused by heroin or morphine abuse, the screening cutoff concentration for urine opiates in the federal workplace drug-testing program was raised to 2000 ng/mL from 300 ng/mL. Currently, oral fluid is under consideration as a possible alternative to urine for drug testing. The suggested cutoff for oral fluid morphine is 40 ng/mL; however, the effect of poppy seed ingestion on morphine concentrations in this specimen type has not been widely investigated. Volunteers at two separate sites ingested commercially available poppy seeds and/or poppy seed bagels. Oral fluid and urine samples were collected at both sites. Oral fluid samples were collected for 24 h; urine was collected for 2 days. The samples were analyzed for the presence of codeine and morphine using gas chromatography–mass spectrometry. Morphine concentrations greater than the suggested cutoff concentrations were detected in oral fluid up to 1 h and in urine for up to 8 h. This study has demonstrated that a positive result for morphine in oral fluid may be due to the ingestion of poppy seeds.

Introduction

In workplace drug-testing programs, the use of heroin, morphine, and codeine is generally detected through the analysis of urine. However, the presence of both morphine and codeine in most poppy seeds (1,2) has caused the ingestion of foods containing poppy seeds to be used as the basis for the explanation of positive codeine and/or morphine results in urine (3,4). In November 1998, the federal governmental raised the cutoff concentration for urine drug testing from 300 ng/mL to 2000 ng/mL to try to eliminate this defense (5,6).

Oral fluid is currently under consideration by the Drug Testing Advisory Board as an alternative specimen for use in federal drug-testing programs. A proposed cutoff of 40 ng/mL for opiates is currently under consideration (7).

This study was conducted to determine the concentrations of morphine in oral fluid and urine following the consumption of commercially available poppy seeds, and whether such ingestion is a viable explanation for a positive oral-fluid opiate drug-testing result.

Because there are differences in morphine and codeine content in various poppy seeds and differences in collection devices, one site (Site #1) used poppy seed bagels only with an Epitope® collection device, and the other (Site #2) used loose poppy seeds and neat oral fluid collectors.

Experimental

Site #1

Four adult volunteers ingested three commercially prepared poppy seed bagels. The bagels were consumed by the volunteers within a 1 h. Specimens of oral fluid and urine were collected prior to and at 1, 4, 6, 8, and 24 h after ingestion. The oral fluid samples were collected using the Epitope oral fluid collection device.

Site #2

Three volunteers each ate one poppy seed bagel (820 mg of poppy seeds) and as many poppy seeds as possible from a commercially available jar within about an hour of giving the baseline samples. The total amount of poppy seeds consumed by Volunteer #1 was 14.82 g (315 mg/kg), by Volunteer #2 was 9.82 g (130 mg/kg), and by Volunteer #3 was 20.82 g (161 mg/kg). Urine and oral fluid samples were collected prior to ingestion.

Post-ingestion oral fluid specimens were collected at 15

* Presented in part at the SOFT-TIAFT meeting in Albuquerque, NM in October 1998.
† Author to whom correspondence should be addressed. E-mail: trohrig@sedgwick.gov.

min, 30 min, 1 h, 2 h, 4 h, and 8 h using collector-free oral fluid kits. Post-ingestion urine specimens were collected at 1 h, 2 h, 4 h, 8 h, 24 h, and 48 h.

All specimens were analyzed according to the laboratory's Standard Operating Procedures for Opiates. The gas chromatography–mass spectrometry (GC–MS) instrumentation was operated at the limit of quantitation for all samples. At Site #1, the limit of quantitation for codeine and morphine in urine was 5 ng/mL; for oral fluid 3 ng/device. At Site #2, the limit of quantitation for codeine and morphine in urine and oral fluid was 5 and 2 ng/mL, respectively.

Urine

Hydrolysis. Urine (1 mL) and required controls were transferred to polypropylene tubes. Internal standard (trideuterated codeine and morphine, 50 µL) was added to each sample and all calibrators/controls to give an internal standard concentration of 200 ng/mL. β-Glucuronidase solution (100 µL) and 2 mL 2M acetate buffer (pH 4.8) were added to each tube. Each tube was capped, mixed, and heated at 55°C for 2 h. The specimens were then allowed to cool and centrifuged (2500 rpm for 5 min).

Extraction. Solid-phase extraction columns (CleanScreen ZSDAU020) were conditioned by sequentially passing the following through each column without allowing the column to dry out: methanol (3 mL), deionized water (3 mL), and 0.1M phosphate buffer (pH 6.0, 1 mL). Each sample was poured into the appropriately labeled extraction column and allowed to flow through the column. Each column was rinsed with 2 mL deionized water, dried for 1 min at full vacuum, rinsed with 2 mL 2M acetate buffer (pH 4.8), dried for 1 min at full vacuum, rinsed with 3 mL methanol, and dried for 5 min at full vacuum. The drugs were eluted from the column with 3 mL methylene chloride/isopropanol/ammonium hydroxide (80:20:5, v/v). The extracts were evaporated to dryness (17 psi air, 60°C), reconstituted with ethanol (50 µL), and transferred to autosampler vials. The extracts were re-evaporated to dryness.

Derivatization. BSTFA (50 µL) was added to the dried residue. The vial was capped and heated for 20 min at 80°C in dry heating block, then allowed to cool to room temperature.

Analysis. At Site #1, the analysis was run on a Hewlett-Packard 5890 GC coupled to a 5971 mass selective detector. The GC column was an HP Ultra (12.5 m) The injector temperature was 250°C, and the transfer line temperature was 300°C. The oven was programmed from 100°C and ramped at 15°C/min to 300°C, and the injection mode was splitless.

At Site #2, the analysis was also run on a Hewlett-Packard 5890 GC coupled to a 5971 mass selective detector. The GC column was a DB-5 MS (5% phenyl-95% methyl silicone, 0.20-mm i.d., 0.33-µm film thickness, 12-m length). The injector temperature was 250°C, and the transfer line temperature was 250°C. The oven was programmed from 100°C, held for 1 min, ramped at 25°C/min to 230°C, and ramped at 3°C/min to 250°C, and the injection mode was splitless.

At both sites, mass spectral data was monitored in two groups (quantitation ion is underlined). Group 1: <u>374</u>, 346 (codeine-d_3) and <u>371</u>, 343, 234 (codeine), and Group 2: <u>432</u>, 417, 327 (morphine-d_3) and <u>429</u>, 430, 401, 196 (morphine).

Oral fluid

Sample preparation. At Site #1, the Epitope collection device was centrifuged and the collection pad was removed. The resulting volume was approximately 1 mL of fluid. The extraction procedure was the same as Site #2, as described, with the exception of the target analytes. Site#1 assayed only for codeine and morphine, whereas the standard procedure for Site #2, included the analysis of hydrocodone, hydromorphone, oxycodone, codeine, morphine, and 6-acetylmorphine (8).

Specifically, 250 mL oral fluid was transferred into a silanized glass tube. Deuterated internal standard (25 µL) was added, yielding final internal standard concentrations of 10 ng/mL for codeine, morphine, and 6-acetylmorphine and 20 ng/mL for hydrocodone and oxycodone. The solutions were allowed to stand for 5 min at room temperature. Methanol (100 mL) was added; the specimen was mixed and centrifuged (2500 rpm, 10 min). The supernatant was transferred to a fresh tube using a glass pipette. Then 1 mL 0.1M sodium acetate buffer (pH 4.5), 500 mL 2.4N hydrochloric acid, and 250 mL 10% methoxyamine hydrochloride (aqueous) were added and the specimen mixed. The samples were incubated at room temperature for at least 1 h, then 3 mL 0.1M phosphate buffer (pH 6.0) was added.

Extraction. Solid-phase extraction columns (CleanScreen ZSDAU020) were conditioned by sequentially passing the following through each column: 3 mL methanol, 3 mL deionized water, and 1 mL 0.1M phosphate buffer (pH 6.0) without allowing the column to dry out. Each sample was poured into the appropriately labeled extraction column and allowed to flow through the column. Each column was rinsed with 2 mL deionized water, dried for 1 min at full vacuum, rinsed with 3 mL 0.1M HCl, dried for 1 min at full vacuum, rinsed with 3 mL methanol, and dried for 5 min at full vacuum. The drugs were eluted from the column with 3 mL methylene chloride/isopropanol/ammonium hydroxide (80:20:5, v/v). The extracts were evaporated to dryness (17 psi air, 60°C), reconstituted with ethanol (100 µL), and transferred to autosampler vials. The extracts were re-evaporated to dryness.

Derivatization. To the dried residue, 20 µL BSTFA was added. The vial was capped and heated for 30 min at 80°C in dry heating block, then allowed to cool to room temperature.

Analysis

At Site #1, the analysis was identical to that of urine with the exception that the analysis was run on a Hewlett-Packard 5890 GC coupled to a 5972 series 2 Plus MS detector. The oven program ran from 150°C for 1 min and was then ramped at 25°C/min to 300°C where it was held for 2 min.

At Site #2, the analysis was run on a Hewlett-Packard 6890 GC coupled to a 5973 mass selective detector. The GC column was a DB-5 MS (5% phenyl-95% methyl silicone, 0.20-mm i.d., 0.33-µm film thickness, 25-m length). The injector temperature was 280°C, and transfer line temperature was 290°C. The injection volume was 3 µL, and the injection mode was splitless. The helium carrier gas flow rate was 1.5 mL/min, and the purge time on was 2 min. The back inlet pressure was 34.35 psi, and the purge flow was 20 mL/min. The oven was programmed from 150°C, held for 1 min, ramped at 20°C/min

to 245°C, held for 8 min, and ramped at 50°C/min to 290°C for a total run time of 14.65 min. The MS source temperature was 230°C, and the MS quadrupole was 150°C. The following ions were monitored: 374.3, 346.3 (codeine-d_3); 371.3, 343.3 (codeine); 432.2, 417.20 (morphine-d_3); 429.2, 401.2, 414.2 (morphine); 331.3, 300.3 (hydrocodone-d_3); 328.3, 297.2 (hydrocodone); 386.3, 355.3 (hydromorphone); 402.4, 343.3 (6-acetylmorphine-d_3); 399.3, 340.3, 287.2 (6-AM); 419.4, 420.4 (oxycodone-d_3); and 416.4, 417.4 (oxycodone).

Quantitative curves were constructed utilizing matrix matched (Site #1: buffer and Site #2: negative saliva) calibrators. The target analyte concentrations were determined by utilizing the internal standard calibration procedure.

Results

Site #1

Oral fluid. Morphine and codeine were not detected in any of the oral fluid specimens (LOD: 3 ng/device). The first collection was 1 h post-ingestion.

Urine. Trace concentrations of codeine, less than 40 ng/mL, were detected. The concentrations of morphine detected in the urine samples are consistent with previous reports (2,4) and are shown in Figure 1. The highest morphine concentration was achieved 1–4 h post-ingestion. The urine$_{max}$ concentrations ranged from 314 to 603 ng/mL.

Site #2

Oral fluid. Codeine and 6-acetylmorphine were not detected in the oral fluid samples. The results of morphine analysis are shown in Figure 2A. The highest concentration of morphine detected was 205 ng/mL. The OF$_{max}$ occurred at 15 min (1st collection) post-ingestion. The oral fluid concentration of morphine in this study remained above 50 ng/mL for 30 min after dosing, and in two cases, the levels were close to 40 ng/mL after 1 h (Volunteers #1 and #3 were 39 and 33 ng/mL, respectively). Although volunteer #1 received a higher dose than the others according to weight, this individual was below the proposed limit after 1 h. The highest concentrations of morphine in oral fluid were obtained after 15 min (the first collection) and declined thereafter. In urine, the highest levels were obtained after 2 h.

Urine. Codeine was measured in trace amounts and the morphine levels are shown in Figure 2B. The concentrations are somewhat higher than those found in the volunteers from Site #1, but the amount of poppy seeds ingested at Site #2 was significantly higher.

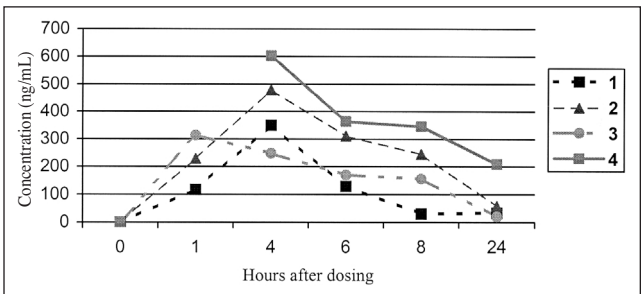

Figure 1. Morphine levels in urine: Site #1.

Discussion

There are several publications reporting the detection of morphine in urine following the ingestion of poppy seeds. Following the ingestion of a meal containing poppy seeds, morphine levels of 120–1270 ng/mL were measured (2). Hayes et al. (3) detected morphine and codeine in serum and urine following ingestion of four brands of poppy seeds, and urine levels greater than 300 ng/mL were reported for as long as 48 h after ingestion. Meadway et al. (4) reported that morphine urine concentrations varied widely depending upon the variety of poppy seeds ingested, and reached maximum concentrations of 832 ng/mL 2–4 h after dosing. Further, urine concentrations of morphine following the ingestion of up to 6 g of poppy seed showed the highest concentrations 3–8 h after dosing. Only 16 of 264 samples collected exceeded 300 ng/mL at any time in the experiment, and in all cases the levels were less than 150 ng/mL after 24 h (5).

In our study, at Site #1, the results for urine specimens are similar to those previously reported in the literature; however, the concentrations detected at Site #2 were markedly higher. The amount of poppy seeds ingested at Site #2 was greater than at Site #1, but all volunteers were naïve users and were still above the 2000 ng/mL level for at least 4 h.

There is a less information as to whether or not poppy seed ingestion will cause a positive oral fluid drug test. Kopecky et al. (9) reported a brief study in which they had "several" vol-

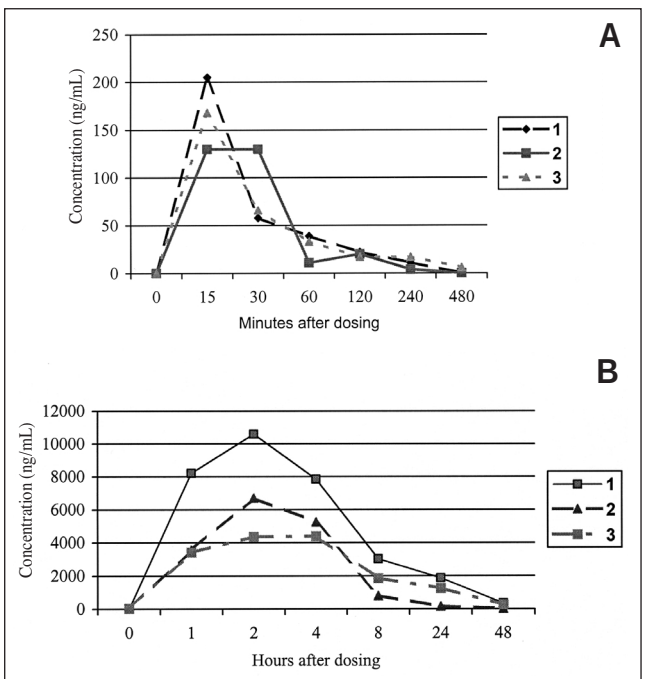

Figure 2. Morphine levels in oral fluid: Site #2 (A). Morphine levels in urine: Site #2 (B).

unteers consume one bagel with poppy seeds. Samples were collected 4 h post-ingestion and assayed by morphine-specific RIA. No morphine was found in the oral fluid of these volunteers. Niebdbala et al. (10) reported that, in one individual, following the ingestion of 40 g of poppy seeds, the oral fluid samples were positive at concentrations greater than 10 ng/mL for only 15 min after ingestion. The urine samples were greater than 300 ng/mL for 4 h.

In this study, Site #1 reported no positive oral fluid specimens. The volunteers at Site #2 were positive above the proposed cut-off of 40 ng/mL for almost an hour after ingestion. The differences between Site #1 and Site #2 were in the amount of poppy seeds ingested, timing of the first collection and the method of oral fluid collection (Epitope device versus neat oral fluid collector). It is possible that there was some absorption of drug onto the collection pad at Site #1. Dose also played a role in the difference in the morphine oral fluid concentrations, as demonstrated by the urinary morphine concentrations. Comparison of the data from the two sites at the 1 h post-ingestion are consistent, that is, all subjects were negative utilizing the proposed oral fluid morphine cutoff of 40 ng/mL.

Conclusions

By measuring opiates in urine specimens, we have shown the deposition of morphine into the body, following the administration of poppy seeds. The high morphine urine concentrations detected at Site #2 show that ingestion of poppy seeds can cause a positive result higher than the 2000 ng/mL cutoff mandated by SAMHSA in all three volunteers up to 4 h after ingestion; and in one case up to 8 h after ingestion. The concentrations detected in oral fluid at Site #2, showed that positive results can be obtained for almost an hour after ingestion. Using the proposed cutoff of 40 ng/mL for oral fluid morphine, the argument of poppy seed ingestion as an explanation for the positive test result cannot be ruled out. This study has shown that positive oral fluid morphine results may be obtained after a reasonable ingestion of poppy seeds for up to 1 h post-ingestion.

References

1. M.G. Pelders and J.J. Ros. Poppy seeds: differences in morphine and codeine content and variation in inter- and intra-individual excretion. *J. Forensic Sci.* **41(2):** 209–212 (1996).
2. D.S. Lo and T.H. Chua. Poppy seeds: implications of consumption. *Med. Sci. Law* **32(4):** 296–302 (1992).
3. L.W. Hayes, W.G. Krasselt, and P.A. Mueggler. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. *Clin. Chem.* **33(6):** 806–808 (1987).
4. C. Meadway, S. George, and R. Braithwaite. Opiate concentrations following the ingestion of poppy seed products—evidence for 'the poppy seed defence'. *Forensic Sci. Int.* **96(1):** 29–38 (1998).
5. H.N. ElSohly, M.A. ElSohly, and D.F. Stanford. Poppy seed ingestion and opiates urinalysis: a closer look. *J. Anal. Toxicol.* **14:** 308–310 (1990).
6. A.D. Fraser and D. Worth. Experience with a urine opiate screening and confirmation cutoff of 2000 ng/mL. *J. Anal. Toxicol.* **23:** 549–551 (1999).
7. Mandatory Guidelines for Federal Workplace Drug Testing Programs. Draft #4 9/5/2001. Division of Workplace Programs, SAMHSA.
8. J. Jones, K. Tomlinson, and C. Moore. The simultaneous determination of codeine, morphine, hydrocodone, hydromorphone, 6-acetylmorphine, and oxycodone in hair and oral fluid. *J. Anal. Toxicol.* **26:** 171–175 (2002).
9. E.A. Kopecky, S. Jacobsen, J. Klein, B. Kapur, and G. Koren. Correlation of morphine sulfate in blood plasma and saliva in pediatric patients. *Ther. Drug Monit.* **19(5):** 530–534 (1997).
10. R.S. Niedbala, K. Kardos, J. Waga, D. Fritch, L. Yeager, S. Doddamane, and E. Schoener. Laboratory analysis of remotely collected oral fluid specimens for opiates by immunoassay. *J. Anal. Toxicol.* **25:** 310–315 (2001).