Published in final edited form as:

Drug Test Anal. 2015 July ; 7(7): 586–591. doi:10.1002/dta.1742.

Morphine and Codeine in Oral Fluid after Controlled Poppy Seed Administration

Marta Concheiro¹, Matthew N. Newmeyer^{1,2}, Jose Luiz da Costa^{1,3}, Ron Flegel⁴, David A. Gorelick^{1,5}, and Marilyn A. Huestis¹

¹Chemistry and Drug Metabolism Section, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA

²Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, USA

³Forensic Toxicology and Chemistry Laboratory, Criminalistics Institute of Sao Paulo, Sao Paulo, SP, Brazil

⁴Division of Workplace Programs, Substance Abuse Mental Health Services Administration, Department of Health and Human Services, Rockville, MD, USA

Abstract

Opiates are an important drug class in drug testing programs. Ingestion of poppy seeds containing morphine and codeine can yield positive opiate tests and mislead result interpretation in forensic and clinical settings. Multiple publications evaluated urine opiate concentrations following poppy seed ingestion, but only 2 addressed oral fluid (OF) results; neither provided the ingested morphine and codeine dosage. We administered two 45g raw poppy seed doses, each containing 15.7mg morphine and 3.1mg codeine, 8h apart to 17 healthy adults. All OF specimens were screened by on-site OF immunoassay Draeger DrugTest 5000, and confirmed with OF collected with Oral-Eze® device and quantified by liquid chromatography tandem mass spectrometry (1µg/L morphine and codeine limits of quantification). Specimens (n=459) were collected before and up to 32h after the first dose. All specimens screened positive 0.5h after dosing and remained positive for 0.5-13h at Draeger 20µg/L morphine cutoff. Maximum OF morphine and codeine concentrations (C_{max}) were 177 and 32.6µg/L, with times to C_{max} (T_{max}) of 0.5-1h and 0.5-2.5h post-dose, respectively. Windows of detection after the second dose extended at least 24h for morphine and to 18h for codeine. After both doses, the last morphine positive OF result was 1h with 40µg/L 2004 proposed US Substance Abuse and Mental Health Services Administration cutoff, and 0.5h with 95µg/L cutoff, recently recommended by the Driving Under the Influence of Drugs and Medicines project. Positive OF morphine results are possible 0.5-1h after ingestion of 15.7mg of morphine in raw poppy seeds, depending upon the cutoff employed.

Keywords

oral fluid; poppy seeds; screening; morphine; codeine

Corresponding Author Professor Dr. Dr. (h.c.) Marilyn A. Huestis Chief, Chemistry and Drug Metabolism, Intramural Research Program National Institute on Drug Abuse, National Institutes of Health 251 Bayview Boulevard, Suite 200 Room 05A-721 Baltimore, MD 21224 Tel: 1-443-740-2524 Fax: 1-443-740-2823 mhuestis@intra.nida.nih.gov.

⁵currently at Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA

1. Introduction

The seeds of the opium poppy plant, *Papaver somniferum* L., are commonly consumed in food worldwide. The seeds, consumed raw or cooked, contain opiate alkaloids, primarily morphine (0-450 µg/g seeds) and codeine (0-57.1 µg/g), but also lower concentrations of thebaine (0.3-41 µg/g), noscapine (0.84-230 µg/g) and papaverine (0-67 µg/g) [1]. Thus, poppy seeds consumption can yield a positive opiate test in biological matrices [2-4], and mislead interpretation of clinical and forensic results. Despite multiple efforts, no unambiguous markers were identified, nor has a poppy seed consumption limit or analytical cutoff been developed to differentiate poppy seed ingestion from heroin or morphine use. A recent study suggested that urinary ATM4G, a glucuronide metabolite of a byproduct from the synthesis of illicit heroin (thebaine with a 2(N-methylacetamido)ethyl side-chain), can differentiate the source of opiate intake [5].

Poppy seed morphine content is highly variable, depending upon poppy seed variety, geographical origin, harvesting and processing procedures [2,6]. Morphine content is substantially reduced after grinding (34%), baking (90%), or washing the seeds (70%) [7].

Morphine and codeine concentrations were reported in urine, serum, blood and oral fluid (OF) following poppy seeds ingestion. Urine cases were recently reviewed [2], with morphine concentrations as high as 18,000µg/L after poppy seed cake ingestion [8]. In serum, total morphine maximum concentration was 100µg/L 2h after ingestion of 7.4mg morphine in poppy seeds [9], and in blood, up to 24µg/L after ingestion products containing 50µg morphine/g poppy seeds. We are aware of only two reports of OF opiates following poppy seed ingestion, but neither provided the amount of morphine or codeine ingested. One study detected morphine for up to 15min [10]; the other reported morphine positive specimens for up to 1h [11].

OF is an alternative matrix of increasing interest in workplace, drug treatment, pain management, and driving under the influence of drugs programs. Collection is easy, non invasive, gender-neutral and difficult to adulterate because of the observed collection. In 2004, the US Substance Abuse and Mental Health Services Administration (SAMHSA) proposed a 40µg/L OF screening and confirmation cutoff for morphine and codeine for workplace drug testing [12]. The Driving Under the Influence of Drugs and Medicines (DRUID) project recommended analytical OF morphine and codeine cutoffs of 20µg/L each. Additionally, the DRUID project recommended OF cutoffs of 95µg/L confirmation for morphine and 94µg/L for codeine as equivalent concentrations to 10µg/L in whole blood for drug prevalence studies [13].

We evaluated morphine and codeine OF concentrations at different cutoffs after controlled oral administration of two doses 8h apart of 45g raw poppy seeds containing 15.7mg morphine and 3.1mg codeine. All OF specimens were collected by the Oral-Eze® device and then screened by an onsite immunoassay device; OF morphine and codeine collected from the confirmatory device were quantified by liquid chromatography tandem mass spectrometry (LC-MSMS).

2. Materials and Methods

2.1. Participants

Healthy volunteers, 18-65 years old, were recruited from the community by advertising and word-of-mouth and received a comprehensive medical and psychological evaluation. Exclusion criteria included current medical condition precluding safe study participation, current physical dependence on any psychoactive substance other than caffeine or tobacco, inability to tolerate orally administered poppy seeds, women who were pregnant or nursing, history of psychosis or any current major psychiatric disorder, or inadequate peripheral venous access. All participants provided written informed consent for this National Institute on Drug Abuse Institutional Review Board-approved study.

2.2. Poppy Seed Administration

Participants resided on the secure clinical unit 2h before and 32h after the first poppy seed administration. Each participant consumed two doses of 45g raw, uncooked poppy seeds purchased from Brugger's Bagels (Raleigh, NC). Morphine and codeine content were determined by Research Triangle International (NC, USA). The amount of codeine and morphine in the poppy seeds was determined in triplicate using the method of standard additions. An aqueous solution containing codeine and morphine at 0.5 mg/mL was added to glass tubes containing 1 ± 0.03 g of ground poppy seeds to provide standard amounts of codeine and morphine at 0, 100, 200 and 400 $\mu\text{g/g}$. The internal standard, hydromorphone, was added to each tube at 100 $\mu\text{g/g}$. The samples were dried, and 3mL extraction solvent (methanol with 0.1% acetic acid) was added to each sample. The samples were vortexed, sonicated for one hour and centrifuged at 3500rpm for 5 min. The solvent layer was removed and transferred into an autosampler vial for analysis. For each analyte (codeine and morphine), the relative response (analyte response/internal standard response) for each sample was plotted versus the concentration of analyte added ($\mu\text{g/g}$) and a linear trendline was fit to the data. The analyte concentration in each sample was calculated by extrapolating the line back to the x- axis intercept and taking the absolute value. Average codeine and morphine concentrations were calculated from the three analyses. The black seeds average (SD) morphine and codeine concentrations were 348 (31) $\mu\text{g/g}$ and 70 (4) $\mu\text{g/g}$, respectively; each dose contained 15.7mg morphine and 3.1mg codeine. A dose consisted of 4 plastic bottles each holding 11.25g poppy seeds suspended in 40mL Ora-Plus® suspension vehicle and 10mL Ora-Sweet® sweetener (both from Paddock Laboratories, Inc., MN, USA). After consuming the entire dose, each bottle was rinsed up to three times if needed with 50mL water to collect residual seeds. Within a 15 minute time period, all participants drank all the rinses to ensure delivery of the entire dose. Doses were administered at 0900h and 1700h on admission day.

2.3. OF Collection

OF specimens were screened and collected approximately 0.25h before, and 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, 6, 6.5, 7, and 8h after the first and 0.5, 1, 2, 2.5, 3, 4, 5, 7, 13, 16, 18, 20, 22 and 24h after the second dose. OF for confirmation analysis was collected with the Oral-Eze® device (Quest Diagnostics, Inc., Lenexa, KS) by placing the collector between the lower cheek and teeth, with the plastic shield against the cheek with the mouth closed. Due to the

complexity of the study timeline, the collector remained in the mouth for 5min or until the volume-adequacy indicator turned blue, indicating approximately 1mL OF collection, whichever occurred first. The pad was removed and placed in a plastic tube containing the elution/stabilization buffer, and stored laying down at room temperature for 12h to allow analyte elution from the pad. The weight supplied with the Oral-Eze collection tube was inserted into the tube, followed by centrifugation at 4°C for 5min at 2,200Xg; during centrifugation the weight depresses the collection pad and improves release of the OF-buffer mixture. The OF-buffer solution was decanted into a 3.6mL Nunc™ CryoTube™ (Thermo Scientific, Waltham, MA) and refrigerated at 4°C until analysis. Low-volume specimens (volume-adequacy indicator did not turn blue) were recorded at the time of collection and analyzed as collected, without applying weight corrections.

OF was screened by the on-site Draeger DrugTest 5000 device (Draeger, Lübeck, Germany) with a 20µg/L morphine cutoff. The assay's antibody cross-reactivities reported by the manufacturer with common co-occurring compounds are summarized in Table 1. The OF collector was continuously moved around the participant's mouth until the indicator turned blue. After inserting the collector and test cartridge into the analyzer and closing the door, results were displayed in approximately 8min. Invalid tests (specimen collected correctly but no reading) were recorded at the time of screening.

2.4. Opiate Analysis

We quantified OF morphine and codeine by a fully validated LC-MS/MS method. To 0.75mL Oral-Eze (0.25mL OF and 0.5mL buffer) specimen, we added 25µL methanolic d₆-morphine and d₆-codeine internal standard solution at 1µg/mL, followed by 2mL 1% formic acid in water. Specimens were centrifuged at 4°C and 4,000Xg for 5 min. Supernatants were subjected to solid phase extraction and analyzed on an ABSciex 3200 QTrap® mass spectrometer. Analytes were quantified by two MRM transitions and their ratio; transitions for morphine (quantification transition is underlined) were m/z 286→152 and m/z 286→128, and for codeine m/z 300→152 and m/z 300→128.

Validation parameters included linearity, accuracy, inter- and intra-day imprecision, extraction efficiencies, and matrix effects. Linearity was determined on 5 different days and determined acceptable if r^2 was 0.99 and calibrators quantified within ±20% of target. Limit of detection (LOD) and limit of quantification (LOQ) were determined by fortifying matrices with decreasing analyte concentrations. LOD was defined as the lowest concentration with acceptable chromatography, two MRM transitions with appropriate ion ratio, and a signal-to-noise 3. LOD was evaluated in samples from three different sources. The LOQ was the lowest concentration with two MRM transitions with appropriate ion ratio, signal-to-noise ratio 10, and imprecision <20% and bias ±20%. LOQ was evaluated by five replicates on three different days and three different matrix sources. Imprecision was determined at low, medium and high QC concentrations with five replicates per concentration on four different days (n = 20). Imprecision was expressed as coefficient of variation (%CV) and was expected to be <20%. Inter-day, intra-day, and total imprecision were calculated according to Chesher [14]. Accuracy was calculated as percent target concentration (n = 20) with required criteria of 80–120%. Matrix effect and extraction and

efficiency were evaluated by preparing specimens in blank matrix before ($n = 5$) and after ($n = 10$) the extraction for all QC concentrations. Neat samples were prepared in the elution solvent ($n = 5$). All replicates were prepared in blank matrix from different donors. Matrix effect was calculated as $([\text{average peak area from after set}/\text{average peak area from neat set}] - 1) \times 100$. Extraction efficiency was calculated as $(\text{average peak area from before set}/\text{average peak area from after set}) \times 100$. Linear ranges for morphine and codeine were 1-500 $\mu\text{g/L}$, with limits of detection of 0.5 $\mu\text{g/L}$ and of quantification of 1 $\mu\text{g/L}$. Intra- and inter-day imprecision and accuracy/bias were 16.5% CV and 95.3-101.3%, respectively; extraction efficiencies and matrix effects were 84.4-105.6% and -46.1 to -28%, respectively. Figure 1 shows an extracted ion chromatogram for morphine and codeine at the LOQ in Oral-Eze-collected OF.

2.5. Immunoassay evaluation

We evaluated the Draeger Drug Test 5000 device comparing its valid results to Oral-Eze morphine and codeine confirmation results in all specimens ($n=450$) and in full volume specimens ($n=301$). We evaluated the Draeger test at different confirmation cutoffs (1, 5, 10, 15 and 20 $\mu\text{g/L}$) for morphine in the Oral-Eze specimens, and also taking into account codeine cross-reactivity (morphine and 80% codeine at 0.07 μM equivalent to morphine 20 $\mu\text{g/L}$). We determined true positive (TP, screen and confirmation positive), true negative (TN, screen and confirmation negative), false positive (FP, screen positive but confirmation negative), and false negative (FN, screen negative but confirmation positive). Sensitivity was $\text{TP}/(\text{TP} + \text{FN}) \times 100$, specificity $\text{TN}/(\text{TN} + \text{FP}) \times 100$ and efficiency $(\text{TP} + \text{TN})/(\text{TP} + \text{TN} + \text{FP} + \text{FN}) \times 100$.

Results

Seventeen healthy adults aged 19-54 years old (12 male, 5 female; 11 African-American, 3 Caucasian, and 3 multi-racial), body weight 52.2-88.9 kg, were admitted to the secure clinical research unit. Participants provided 459 OF specimens collected with the Oral-Eze device, and 459 that were screened by the Draeger Drug Test 5000. Nine Draeger tests were invalid (2%), 6 from participant L (8h post-first dose and 0.5, 1, 3, 4 and 5h post-second dose), 2 for participant U (before dosing and 6.5h post-first dose) and one for participant S (18h post-second dose). Among the Oral-Eze specimens, 155 were low volume (33.8%), collected throughout the study timeline after both doses (median 4.5h, range 0.5-24h post-dosing).

At the method LOQ (1 $\mu\text{g/L}$), 288 Oral-Eze OF specimens were positive for morphine (62.8%) and 306 for codeine (66.9%). Median (interquartile range) OF concentration-time curves for morphine and codeine are presented in Figure 2. For morphine, the maximum concentration (C_{max}) after the first dose ranged from 11.9-99.9 $\mu\text{g/L}$ (median 34.0 $\mu\text{g/L}$), and after the second dose from 7.8-177 $\mu\text{g/L}$ (median 46.5). Time to reach C_{max} (T_{max}) after both doses was 0.5-1h (median 0.5, the first OF sample). Six of 17 participants were still positive at the time of the second dose (1.1-2.7 $\mu\text{g/L}$); therefore, C_{last} and T_{last} could not be determined for the first dose at the method LOQ. For the other 11 participants, C_{last} was 1-3.5 $\mu\text{g/L}$ (median 1.3 $\mu\text{g/L}$) and T_{last} 4-7h (median 5h). All participants except participant O

(1.1µg/L) were negative after the second dose prior to the last specimen (24h after the second dose). For these 16 participants, C_{last} was 1-3.8µg/L (median 1.5µg/L) and T_{last} was 3-22h (median 13h).

Codeine C_{max} after the first dose was 3.8-31.8µg/L (median 8.6) and after the second dose from 1.1-32.6µg/L (median 9.5). Median T_{max} after the first and second doses was 0.5h (0.5-7h). Eight participants were positive for codeine at the time of the second dose (1.3-3.7µg/L) and nine were negative. C_{last} in these 9 participants was 1.1-1.6µg/L (median 1.2) and T_{last} 3-7h (median 6.5). After the second dose and before the end of the study, all participants were negative for codeine. C_{last} was 1.1-4.1µg/L (median 1.4µg/L), and T_{last} was 4-18h (median 13). Pharmacokinetic data are summarized in Table 2.

At the DRUID OF opiate cutoff of 95µg/L for morphine and 94µg/L for codeine equivalent prevalence to 10µg/L in whole blood, 7 specimens were positive for morphine (1.5%) and none for codeine. Morphine T_{last} after the first (n=1) and the second dose (n=6) was 0.5h. At the SAMHSA proposed 40µg/L OF opiate cutoff, 16 specimens were positive for morphine (3.5%) and none for codeine. Morphine T_{last} was 0.5h after the first (n=5) and 0.5-1h after the second dose (n=10). Another suggested cutoff is 30µg/L for the opiate screen and 15µg/L for the morphine and codeine confirmation cutoffs. At a 30µg/L screening cutoff, 32 specimens were positive for morphine (6.9%) and 2 for codeine (0.4%). Morphine T_{last} after the first (n=11) and second (n=14) doses was 0.5-1h, and codeine T_{last} was 0.5h after the first (n=1) and the second (n=1) doses. At a 15µg/L confirmation cutoff, 50 specimens were positive for morphine (10.9%) and 11 for codeine (2.4%). Morphine T_{last} after the first (n=13) and second (n=16) doses was 0.5-2.5h. For codeine, T_{last} after the first dose was 0.5-2h (n=3) and after the second dose 0.5-2.5h (n=5). At the DRUID analytical OF opiate cutoff of 20µg/L, 45 specimens were positive for morphine (9.8%) and 6 for codeine (1.3%). For morphine, T_{last} after the first dose was 0.5-2h (n=12 participants), and after the second dose 0.5-2.5h (n=16 participants). For codeine, T_{last} after the first dose was 0.5h (n=2) and after the second dose 0.5-2.5h (n=4).

Among the 459 OF specimens screened by Draeger with a 20µg/L morphine cutoff, 9 were invalid and 149 were positive (33%). Among the 9 invalid test, 3 were confirmed negative for morphine and codeine (one specimen from participant S and 2 from participant U), and 6 from participant L were confirmed positive with concentrations ranging from 1 to 177µg/L for morphine and from 1.1 to 22µg/L for codeine. Among the positive tests, 71 were after the first and 78 after the second dose. All specimens were positive in the first OF specimen 0.5h after both doses. The median and range times of last OF positive results were 3 (1-5) and 3 (0.5-13) h after the first and second doses, respectively. The Draeger DrugTest 5000 had excellent sensitivity (95-98%) at morphine confirmation cutoffs >5µg/L, but only moderate specificity (74-79%) and accuracy (76-82%) (Table 3).

Discussion

We evaluated the validity of the so-called “poppy seed defense” for a positive oral fluid opiate test by having healthy adults ingest two 45g raw poppy seed doses 8h apart, each

containing 15.7mg morphine and 3.1mg codeine. We employed raw poppy seeds to avoid any morphine or codeine loss due to food processing (from 34 to 90% loss) [7].

Niedbala et al [10] reported opiate positive OF specimens for only 15 min after ingesting 40g poppy seed. OF was collected with the Intercept collection device and analyzed by the immunoassay OraSure Technologies, Inc. (10µg/L morphine cutoff; high level of cross-reactivity with 6-acetylmorphine, diacetylmorphine and codeine). Rohrig and Moore [11] administered 3 commercially prepared poppy seed bagels to 4 participants at one site. OF specimens were collected with the Epitope® OF device and analyzed by GC-MS. Morphine and codeine were not detected in any of the specimens. At a second location, 3 participants consumed one bagel containing 820mg seeds and 9.8-20.8g seeds from a jar (not fully explained). OF specimens were collected by expectoration, and also analyzed by GC-MS. Morphine C_{max} was 205µg/L 15 min after dosing, with concentrations >50µg/L 30 min post-dose, and <40µg/L at 1h. Codeine was not detected in any specimen.

In the present study, morphine C_{max} were 11.9-99.9µg/L after the first and 7.8-177µg/L after the second dose. Codeine C_{max} ranged from 3.8-31.8µg/L after the first, and 1.1-32.6µg/L after the second dose. Rohrig et al [11] did not detect codeine in any specimen. Different codeine content in the administered poppy seeds and/or different method sensitivities (present method LOQ 1µg/L vs. 3µg/L) might explain these results.

We observed slightly more specimens positive for codeine than for morphine (306 vs. 288). In these 18 codeine-positive and morphine-negative specimens, codeine concentrations were around the method LOQ (1µg/L). Morphine and codeine have similar pKa (8.2), as well as pharmacokinetic parameters such as half-life (2-4h) and volume of distribution (1-6 L/Kg). However, codeine is more lipophilic than morphine (codeine LogP 1.19, morphine LogP 0.89) and less bound to proteins (codeine 7-25%, morphine 30-40%). It is possible additional plasma codeine crossed into the oral cavity and was trapped via ionization in OF.

In the present study, morphine maximum OF concentration occurred 0.5-1h post-dose. Niedbala et al [10] and Rohrig and Moore [11] reported a T_{max} at 15min, most likely due to oral contamination. In the present study, the first collection time point was 0.5h post-dose. One participant after the first and 2 after the second dose had T_{max} of 1h. The initial oral contamination may have partially cleared in these individuals.

As expected, the morphine window of detection (T_{last}) was inversely associated with the cutoff limit. At the method's LOQ (1µg/L), morphine was positive for more than 24h post-dose and codeine up to 18h. At 15 and 20µg/L cutoffs, morphine and codeine were positive up to 2.5h after dosing. At 30µg/L, morphine was positive up to 1 h and codeine up to 0.5h. With the SAMHSA cutoff (40µg/L), no specimen was positive for codeine, and for morphine up to 1h, and with the DRUID cutoff (95µg/L for morphine and 94µg/L for codeine), morphine was positive for 0.5 h and all the specimens were negative for codeine. These results suggest that fewer poppy seed OF results are possible with the higher SAMHSA (40µg/L) cutoffs than with the lower DRUID analytical 20µg/L cutoffs; however, true positive morphine tests due to heroin or morphine consumption might be missed. The establishment of morphine OF cutoff from poppy seed ingestion is difficult because the

morphine threshold value depends on the origin and quantity of poppy seeds ingested and on inter-individual variability.

OF confirmation specimens were collected with the Oral-Eze device, and 33.8% of these were low volume specimens. This high percentage of low volume specimens might be due to Oral-Eze collection occurring second in the collection process, suggesting that when more than one OF specimen is collected, additional time may be needed for full collection of the second OF specimen.

OF specimens were screened at each time point by the Draeger immunoassay. Similar results were obtained when Draeger performance was compared to all OF confirmation samples and also to only full volume samples' results. At the Draeger morphine 20µg/L cutoff, the test showed good sensitivity above 95%, but specificity and accuracy were below 90%. Taking into account codeine cross-reactivity, test specificity and accuracy were still below 90% (Table 3). The T_{last} for the Draeger Drug Test was 0.5-13h post-dose, longer than that observed for the 20µg/L morphine confirmation cutoff (up to 2.5h). The best results were obtained at 5µg/L morphine cutoff (Table 3). All the Draeger unconfirmed positive specimens contained morphine and/or codeine above the confirmation method LOQ (1µg/L), except for 2 cases. Cross-reactivity with other alkaloids in the specimen, and/or a true cutoff below 20µg/L could explain this longer window of detection and the unconfirmed positive screening results.

Urine also was collected during this investigation, and data describing urine opiate pharmacokinetics were recently published [15]. Urine opiate data were evaluated according to the current US federal 2,000µg/L and the former 300µg/L cutoffs. At morphine 2,000µg/L the median (range) first positive urine specimen was detected 6.6 (1.2-12.1)h after the first dose, and the T_{last} after the second dose was detected at 12.2 (2.6-18.3)h. At morphine 300µg/L, all participants were positive within 3h of the first dose, and most were still positive at the last collection (24h after the second dose). No participant was codeine positive at 2,000µg/L. At codeine 300µg/L, participants' first positive urine specimen was observed 6.6 (1.6-19.5)h after the first dose, and they remained positive for 8.8 (2.6-14.0)h after the second dose. In comparison, the longest morphine and codeine T_{last} were observed in OF according to any of the proposed cutoffs was 2.5h at a 15µg/L cutoff.

Urine collected from the present study was screened with different immunoassays, and their performances were characterized [15]. The KIMS opiate II immunoassay (Roche Diagnostics) at both 300 and 2,000µg/L had sensitivities, specificities, and efficiencies >90%. The CEDIA 6-acetylmorphine (6-AM) immunoassay (Thermo Fisher Scientific) and 6-AM enzyme immunoassay (Lin-Zhi International) each had specificity and efficiency >90; no 6-AM was confirmed in any sample so sensitivities could not be calculated. The Draeger DrugTest 5000 did not achieve sensitivities, specificities, and efficiencies (accuracy) simultaneously >90%, even when considering multiple cutoffs and codeine cross reactivity.

Morphine and codeine were detected in OF after ingestion of 45g raw poppy seeds containing 15.7mg morphine and 3.1mg codeine. The morphine ingested was equivalent to the amount found in commonly prescribed opiates analgesics, and the poppy seeds were

raw, to guarantee that there was no morphine or codeine loss due to food processing. These high morphine and codeine poppy seed doses were designed to evaluate a worst case scenario for opiate exposure. The OF concentrations were never above 177µg/L for morphine and 33µg/L for codeine. The low method LOQ (1µg/L) permits evaluation of many different cutoffs. The time of the last morphine positive specimen after poppy seed consumption is dependent upon the cutoff employed.

Acknowledgements

We acknowledge funding provided by the Division of Workplace Programs, Substance Abuse and Mental Health Services Administration and NIDA Intramural Research Program (IRP), NIH; contributions by the clinical staff of the NIDA IRP, Behavioral Pharmacology Research Unit, and Clinical Research Unit, Johns Hopkins Bayview Medical Center. The authors also thank Megan Taylor, Clinical Protocol Coordinator, CDM, NIDA IRP, for her valuable assistance.

References

1. Trafkowski J, Madea B, Musshoff F. The significance of putative urinary markers of illicit heroin use after consumption of poppy seed products. *Ther. Drug Monit.* 2006; 28(4):552. [PubMed: 16885724]
2. Lachenmeier DW, Sproll C, Musshoff F. Poppy Seed Foods and Opiate Drug Testing-Where Are We Today? *Ther. Drug Monit.* 2010; 32(1):11. [PubMed: 19901868]
3. Jankovicová K, Ulbrich P, Fuknová M. Effect of poppy seed consumption on the positive results of opiates screening in biological samples. *Leg. Med.* 2009; 11(Suppl. 1):S416.
4. Moeller MR, Hammer K, Engel O. Poppy seed consumption and toxicological analysis of blood and urine samples. *Forensic. Sci. Int.* 2004; 143(2-3):183. [PubMed: 15240041]
5. Chen P, Braithwaite RA, George C, Hylands PJ, Parkin MC, Smith NW, Kicman AT. The poppy seed defense: a novel solution. *Drug Test Anal.* 2014; 6(3):194. [PubMed: 24339374]
6. Chang J, Wang M, Appleton C. Headache bread - A case of high codeine containing variety of poppy seed. *J Anal Toxicol.* 2012; 36(4):288. [PubMed: 22511702]
7. Sproll C, Perz RC, Lachenmeier DW. Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. *J. Agric. Food Chem.* 2006; 54(15):5292. [PubMed: 16848508]
8. Fritschi G, Prescott WR Jr. Morphine levels in urine subsequent to poppy seed consumption. *Forensic Sci. Int.* 1985; 27:111. [PubMed: 3979930]
9. Hayes LW, Krasselt WG, Mueggler PA. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seed. *Clin. Chem.* 1987; 33:806. [PubMed: 3594820]
10. Niedbala RS, Kardos K, Waga J, Fritch D, Yeager L, Doddaman S, Schoener E. Laboratory analysis of remotely collected oral fluid specimens for opiates by immunoassay. *J. Anal. Toxicol.* 2001; 25:310. [PubMed: 11499883]
11. Rohrig TP, Moore C. The determination of morphine in urine and oral fluid following ingestion of poppy seeds. *J. Anal. Toxicol.* 2003; 27(7):449. [PubMed: 14606998]
12. Substance Abuse and Mental Health Services Administration (SAMHSA). Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs. 2004; 69(71):19673.
13. Schulze H, Schumacher M, Urmeew R, Auerbach K. Druid Final Report: Work performed, main results and recommendations. 2012
14. Chesher D. Evaluating assay imprecision. *Clin. Biochem. Rev.* 2008; 29(Suppl. 1):S23.
15. Smith ML, Nichols DC, Underwood P, Fuller Z, Moser MA, LoDico C, Gorelick DA, Newmeyer MN, Concheiro M, Huestis MA. Morphine and codeine concentrations in human urine following controlled poppy seed administration of known opiate content. *Forensic. Sci. Int.* 2014; 241:87. [PubMed: 24887324]

Figure 1.
Morphine and codeine extracted ion chromatogram at the limit of quantitation (1 μ g/L) in Oral-Eze.

Figure 2.

Median (range) oral fluid concentration-time curves for morphine (panel A) and codeine (panel B) after the ingestion of 45g raw poppy seeds (15.7mg morphine and 3.1mg codeine) at $t=0$ and $t=8$ h (vertical dash lines). SAMHSA (40 $\mu\text{g/L}$) and DRUID (95 $\mu\text{g/L}$ and 20 $\mu\text{g/L}$) opiate cutoffs are indicated (horizontal dash lines).

Table 1

Draeger DrugTest 5000 antibody cross-reactivities reported by the manufacturer with common co-occurring compounds.

Compound	Positive result at a given concentration (µg/L)	% Cross-reactivity
6-acetylmorphine	35	57.14
Amobarbital	100,000	0.02
Atropine	80,000	0.03
Codeine	25	80
Delta-9-tetrahydrocannabinol	55,000	0.04
Diazepam	50,000	0.04
Dihydrocodeine	20	100
Ecgonine methyl ester	1,500	1.33
Hydrocodone	20	100
Hydromorphone	30	66.67
Methadone	85,000	0.02
Methylenedioxyethylamphetamine	10,000	0.2
Morphine-3-glucuronide	35	57.14
Nalorphine	35	57.14
Naloxone	1,000	2
Norcodeine	4,000	0.5
Normorphine	8,000	0.25
Ofloxacin	55,000	0.04
Oxycodone	1,000	2
Procaine	70,000	0.03
S(+)-methamphetamine	20,000	0.1

Table 2

Morphine and codeine oral fluid pharmacokinetic parameters after controlled oral administration of poppy seeds

	Morphine		Codeine	
	Dose 1	Dose 2	Dose 1	Dose 2
$C_{\max}$ (µg/L)	34 (11.9-99.9) n=17	46.5 (7.8-177) n=17	8.6 (3.8-31.8) n=17	9.5 (1.1-32.6) n=17
$T_{\max}$ (h)	0.5 (0.5-1) n=17	0.5 (0.5-1) n=17	0.5 (0.5-2.5) n=17	0.5 (0.5-7) n=17
C_{last} (µg/L)	1.3 (1-3.5) n=11	1.5 (1-3.8) n=16	1.2 (1.1-1.6) n=9	1.4 (1.1-4.1) n=17
T_{last} (h)	5 (4-7) n=11	13 (3-22) n=16	6.5 (3-7) n=9	13 (4-18) n=17
T_{last} (h) DRUID OF cutoff (94-95 µg/L equivalent to 10 µg/L in whole blood)	0.5 (0.5) n=1	0.5 (0.5) n=6	N/A	N/A
T_{last} (h) SAMHSA OF cutoff (40 µg/L)	0.5 (0.5) n=5	0.5 (0.5-1) n=10	N/A	N/A
T_{last} (h) 30 µg/L cutoff	0.5 (0.5-1) n=11	0.5 (0.5-1) n=14	0.5 (0.5) n=1	0.5 (0.5) n=1
T_{last} (h) DRUID analytical OF cutoff (20 µg/L)	0.8 (0.5-2) n=12	1 (0.5-2.5) n=16	0.5 (0.5) n=2	0.5 (0.5-2.5) n=4
T_{last} (h) 15 µg/L cutoff	1 (0.5-2.5) n=13	1 (0.5-2.5) n=16	2 (0.5-2) n=3	1 (0.5-2.5) n=5

17 participants ingested 2 doses 8h apart of 45g raw poppy seeds, each containing 15.7mg morphine and 3.1mg codeine. Results are expressed as median (range), and n participants. Limits of quantification (LOQ) were 1µg/L for morphine and codeine. N was less than 17 for C_{last} and T_{last} because some participants were still positive at method LOQ at the time of last sample collection. N was less than 17 for the different cutoffs evaluated because some participants were never positive at those concentrations.

Table 3

Draeger DrugTest 5000 opiates on-site screening results compared to all Oral-Eze confirmation specimens (n=450) and to full volume Oral-Eze confirmation specimens (n=301).

N specimens	Confirmation cutoff (µg/L)	TP	TN	FP	FN	Sensitivity %	Specificity %	Accuracy %
450	Morphine 1	146	164	3	137	51.6	98.2	68.9
	Morphine 5	104	283	45	18	85.2	86.3	86.0
	Morphine 10	60	299	89	2	96.8	77.1	79.8
	Morphine 15	47	300	102	1	97.9	74.6	77.1
	Morphine 20	42	300	107	1	97.7	73.7	76
	Morphine & 80% Codeine at 20	50	300	99	1	98.0	75.2	77.8
301	Morphine 1	94	108	2	97	49.2	98.2	67.1
	Morphine 5	72	190	24	15	82.8	88.8	87.0
	Morphine 10	43	203	53	2	95.6	79.3	81.7
	Morphine 15	32	204	64	1	97.0	76.7	78.4
	Morphine 20	29	204	67	1	96.7	75.3	77.4
	Morphine & 80% Codeine at 20	35	204	61	1	97.2	77.0	79.4

The Draeger test was evaluated at different confirmation cutoffs (1, 5, 10, 15 and 20µg/L) for morphine, and also taking into account codeine cross-reactivity (morphine and 80% codeine at 0.07µM equivalent to morphine 20µg/L). TP, true positive; TN, true negative; FP, false positive; FN, false negative.