The Analysis of Thebaine in Urine for the Detection of Poppy Seed Consumption

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Abstract

The consumption of poppy seeds in various foods may lead to a positive opiate result in urine subjected to testing for drugs of abuse. As a natural constituent of poppy seeds, thebaine was investigated as a possible marker for poppy seed consumption. Poppy seeds were examined for opiate content by gas chromatography–ion trap mass spectrometry (GC–MS) after extraction with methanol. Urine samples spiked with thebaine and urine from subjects given 11 g of poppy seeds were tested for the presence of thebaine, codeine, and morphine. Street heroin, one morphine and one codeine tablet, and urine from individuals who had used heroin were also examined for thebaine. Urine specimens were screened by enzyme immunoassay (EMIT) and confirmed for thebaine by GC–MS using a solid-phase extraction method. The GC–MS assay showed a linear response over a range of 1–100 ng/mL and a limit of detection of 0.5 ng/mL. Thebaine was detectable in the urine of poppy seed eaters in concentrations ranging from 2 to 81 ng/mL. Because thebaine was absent in powdered drugs and the urine of true opiate drug users, thebaine is proposed as a direct marker for poppy seed use.

Introduction

Several studies have shown that the consumption of foods containing poppy seeds may lead to a positive test result for opiates (1,2). This has become an increasingly important issue considering the large role drug testing has taken in current workplace and drug rehabilitation programs. In a recent report, ElSohly and Jones (3) suggested guidelines for differentiating sources of morphine and codeine use based on total morphine and codeine concentrations in urine and the ratio of concentrations relative to each other. Analysis for the presence of 6-monoacetylmorphine (6-MAM) (4) is helpful in confirming heroin use. At present, however, there is no direct marker to rule in poppy seed ingestion. Therefore, if an individual claims consumption of poppy seeds, Medical Review Officers are inclined to overturn the results of urine tests that are positive for opiates.

The concentration of illicit opiates from commercial poppy seed products have been documented by several investigators. Preinger et al. (5) used thin-layer chromatography (TLC) to identify the presence of morphine, codeine, thebaine, papaverine, and narcotine. The concentration of these opiates varied depending on the growing, washing, and processing conditions of the seeds (6). The opiate concentrations in poppy seeds have been reported as 0.1–3.8 µg/g for codeine, 5.1–106 µg/g for morphine, and 0.3–14.0 µg/g for thebaine (1). ElSohly et al. (1) first suggested that thebaine be used as a marker for poppy seed consumption. Unfortunately, these investigators were unsuccessful in detecting thebaine in urine samples that were positive for opiates. Thebaine is present in low concentrations in urine following poppy seed use. Moreover, there are no active hydrogen groups that can be targeted for derivatization to produce silyl or acyl derivatives. Thus, thebaine analysis is difficult by the typical gas chromatographic–mass spectrometric (GC–MS) procedures used for codeine and morphine. In the current study, thebaine was re-examined as a possible marker of poppy seed consumption through use of a modified extraction method followed by a sensitive GC–MS assay.

Materials and Methods

Drugs and reagents

Spice Time® Foods, Inc. (Hoboken, NJ) poppy seeds were obtained from Christmas Tree Shoppes® (Manchester, CT). Australian poppy seeds were obtained from World of Nutrition (Newington, CT). A dozen poppy seed muffins were prepared from a boxed mix (Krusteaz® low-fat lemon poppy seed mix) to which a half cup (132 g) of Spice Time poppy seeds were added (11 g poppy seeds per muffin).

Seven crude heroin samples were obtained from the streets of Hartford (each equivalent to one “street fold”) as discarded confiscated evidence from the Department of Consumer
Protection, Drug Control Division, Hartford, CT. Seven urine samples from individuals who were admitted to Hartford Hospital (Hartford, CT) because of heroin use, as confirmed by history and medical examination, were tested. Morphine (Restek, Bellefonte, PA), codeine (Restek), thebaine (Sigma, St. Louis, MO), and 6-MAM (Alltech, Deerfield, IL) were all received as 1-mg/mL standards in methanol. The chemical structures for codeine, morphine, heroin, and thebaine are shown in Figure 1. The standards were diluted to concentrations ranging from 1 to 300 ng/mL with drug-free urine. Codeine and morphine tablets were obtained from Roxane Labs, Inc. (Columbus, OH) and Purdue Frederick (Norwalk, CT), respectively. High-purity high-performance liquid chromatography (HPLC)-grade solvents used for extractions were methanol (Baxter, San Fernando, CA), methylene chloride (Baxter), isopropanol (Baxter), ammonium hydroxide (Mallinckrodt, Paris, KY), and ethyl acetate (Mallinckrodt).

In the initial studies, cocaine (Restek) was used as the internal standard. Because cocaine is prevalent in urine tested for drugs of abuse, 6-ethylcodeine (6-ECO) was later synthesized and used as an internal standard. 6-ECO was synthesized using the procedure of Johnstone and Rose (7). Powdered KOH (75 mg) was mixed with 0.67 mL of dimethylsulfoxide in a tube and allowed to stand at room temperature for 5 min with intermittent mixing. Codeine (101 mg) was added and mixed on a vortex mixer; 54 mL of iodoethane was then added. The reaction proceeded at room temperature for 1 h. Reagent-grade water (5 mL) was added, and the mixture was extracted three times with 5 mL of dichloromethane. The solvent was evaporated to dryness, the residue was dissolved in 10 mL of methanol (Baxter, San Fernando, CA), methylene chloride (Baxter), isopropanol (Baxter), ammonium hydroxide (Mallinckrodt, Paris, KY), and ethyl acetate (Mallinckrodt).

An aliquot of this solution was diluted in 0.1 mol/L HCl and extracted twice with 30 mL of methanol. The methanol was evaporated to dryness, the residue was dissolved in 15 mL of CH₂Cl₂, and the solution was passed through filter paper to remove any remaining silica gel particles. The solvent was evaporated to dryness, and the residue was dissolved in 10 mL of methanol. A 0.05 dilution of this solution was analyzed by temperature-programmed GC–MS (HPS, 0.25-mm x 30-mm column, 15–320°C at 15°C/min). One peak was observed in the total ion chromatogram, and the base ion (m/z 327) in the mass spectrum of this peak was consistent with the molecular weight of 6-ECO. An aliquot of this solution was diluted in 0.1 mol/L HCl and quantitatively assayed at 285 nm using the extinction coefficient for codeine (55 A·dL/g) (8). The concentration of 6-ECO in the methanolic solution was approximately 2.5 mg/mL.

**Assays**

The EMIT II immunoassay for opiates (Behring Diagnostics, San Jose, CA) was used according to the manufacturer's recommendation on a BM/Hitachi-717 analyzer (Boehringer Mannheim, Indianapolis, IN) to screen all urine specimens obtained for this study. The cross-reactivity of thebaine to the EMIT opiate assay was determined by spiking drug-free urine with thebaine to final concentrations of 300, 450, 600, 750, 1500, and 3000 ng/mL.

Specimens positive for opiates by immunoassay and some negative urine samples were assayed by GC–MS for the presence of thebaine. A Varian 3300 GC interfaced with a Finnigan Mat Witness* ion trap MS was used for the confirmation analysis using a 0.25-mm diameter DB-5 column (J&W Scientific, Folsom, CA). Ultra-high purity (99.999%) helium was used as the carrier gas at a flow rate of 1 mL/min. Samples were introduced into the GC using the splitless injection technique. The initial oven temperature of 50°C was held isothermally for 1 min and was ramped to 300°C at 25°C/min and held for 1 min. The temperatures of the injector and transfer line were 275 and 280°C, respectively. The MS was operated in the electron ionization (EI) mode with mass-to-charge (m/z) data collected from 70 to 450 amu at a rate of 6.7 scans/s. Ion chromatograms of the molecular ions of thebaine (m/z 311), codeine (m/z 299), morphine (m/z 285), cocaine (m/z 182), and 6-ECO (m/z 327) were reconstructed using the Finnigan Magnum software program.

**Experimental studies**

**Controlled consumption.** A total of nine volunteer subjects consumed 1–3 muffins containing poppy seeds. A baseline (preconsumption) urine specimen was collected from each subject along with one specimen 6–8 h after consumption. In addition, urine from one subject was collected every 2 h postconsumption for 12 h, and at 24- and 32-h postconsumption. Table I gives the number of muffins consumed and timing of urine specimens for all of the subjects studied.

**Stability, linearity, and limit of detection (LOD).** Several urine samples of poppy seed eaters were

Figure 1. Chemical structures of codeine (A), morphine (B), heroin (C), and thebaine (D).
stored for one month at −20°C following the initial analysis. These samples were removed from the freezer, thawed at room temperature, reanalyzed for thebaine, and the recovery of thebaine was determined. Thebaine was also subjected to the heat used in derivatization for codeine and morphine to determine the stability of thebaine at these temperatures. For linearity, aqueous standards were prepared from thebaine stock standard in triplicate at 1, 5, 10, 25, 50, and 100 ng/mL. The LOD was determined as 3 times the standard deviation of the noise (n = 10). Aqueous standards were also prepared from thebaine stock standard for determination of the precision. Samples were analyzed five times each at 10 and 25 ng/mL. The coefficient of variation (%CV) was calculated.

Sample processing

Poppy seeds. Seeds were ground to a powder using a mortar and pestle. One half gram of poppy seeds was weighed and transferred to a plastic test tube. Ten milliliters of methanol was added, the test tube was capped, agitated on a RIA-pac rotator (Pharmacia Diagnostics, Piscataway, NJ), and centrifuged for 5 min. The methanol layer was transferred to a clean test tube, and the solvent was evaporated under a stream of dry nitrogen at 40°C. The residue was reconstituted with 5 mL of HPLC-grade water and extracted using the solid-phase extraction method described previously for the extraction of urine samples. An aliquot of the poppy seed extracts was derivatized with 50 µL N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS, Pierce Chemical, Rockford, IL) under nitrogen at 65°C for 20 min to produce a trimethylsilyl (TMS) derivative.

Drugs. Street-heroin samples (0.5 mg) and portions of the codeine and morphine tablets (0.04 g) were placed into volumetric flasks (50 or 100 mL) and diluted to volume with methanol. Five milliliters of the resulting solutions were removed, and the solvents were evaporated. The dried residues were reconstituted in 50 µL of ethyl acetate, and 1 µL was analyzed separately for presence of thebaine by GC–MS. As these analyses were conducted qualitatively, no internal standard was used.

Urine. Thebaine was extracted directly from urine (as described above) without hydrolysis and injected into the GC–MS without derivatization. Five-milliliter urine aliquots obtained from spiking studies, baseline and postconsumption from volunteer subjects, and true opiate users, as well as extracts from poppy seeds, were transferred to 12-mL screw-capped tubes. Fifty microliters of cocaine or 6-ECO internal standard was added to each tube to give a final concentration of 50 ng/mL. The pH of the samples was adjusted to 6.5 ± 0.5 using 1.0 mol/L NaOH or 0.1 mol/L HCl accordingly. Solid-phase columns (10 mL, 200 mg, Clean Screen DAU, United Chemical Technologies, Horsham, PA) were prepared by washing with 3 mL of methanol, 3 mL of HPLC-grade water, and 1 mL of 100 mmol/L phosphate buffer (pH 6.0).

![Figure 2. Reconstructed ion gas chromatograms and partial mass spectra of the contents of 0.5 g Australian poppy seed extracts for thebaine, 20.7 µg/g; codeine, 31.8 µg/g; and morphine, 107 µg/g, found in the seeds. Note the presence of an unknown material substance eluting after codeine with a molecular ion at m/z 299.](image-url)
Table 1. Results of GC–MS Analysis of Urine from Poppy Seed Consumption Study

<table>
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<tr>
<th>Sample No.</th>
<th>Subject No.</th>
<th>Muffins consumed</th>
<th>Collection time (h)</th>
<th>Screen result</th>
<th>Codeine (ng/mL)</th>
<th>Thebaine (ng/mL)</th>
<th>Morphine (ng/mL)</th>
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<td>4</td>
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<td>51</td>
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*Abbreviations: nt, not tested; na, not available.
*Result near the cutoff concentration.
Samples were passed through the columns at a rate of 1–2 mL/min. The columns were washed with 2 mL of HPLC-grade water, 2 mL of 100 mmol/L acetate buffer (pH 4.5), and 3 mL of methanol. The columns were vacuum aspirated for 5 min. The columns were then eluted with 3 mL of a methylene chloride, isopropanol, and ammonium hydroxide (78:20:2). The eluent was evaporated. The dried residues were reconstituted with 50 µL of ethyl acetate. Dried residues were found to be stable for thebaine at 0–8°C for at least two weeks.

The quantitation for morphine in urine was performed following derivatization using 50 µL of ethyl acetate and 50 µL of BSTFA with 1% TMCS. Five microliters of 1 mg/mL thebaine stock standard was transferred to a glass test tube. The solvent was dried, and the residue was derivatized as described previously for morphine.

Results

The antibody used in the EMIT II assay for opiates produced a negative result (ΔA/min rate that was near that of the cutoff calibrator) when urine was spiked with 450 ng/mL of thebaine and a positive result at all urine concentrations exceeding 600 ng/mL. A thebaine concentration of approximately 480 ng/mL gave a response equivalent to the 300-ng/mL cutoff for free morphine (63% cross-reactivity). This was similar to the response that hydromorphone had to the EMIT II assay (9).

The GC–MS assay for thebaine was linear from 1 to 100 ng/mL \((y = 4.23 \times 10^{-3}x + 4.63 \times 10^{-3}, r = 0.9998)\) and had a LOD of 0.5 ng/mL. The %CV for the quantitation of thebaine was 5.2% at 25 ng/mL and 15.5% at 10 ng/mL. The average recovery of thebaine from urine that underwent one freeze/thaw cycle was 96%, which indicated no degradation after storage at −20°C.

In contrast, when a thebaine standard was heated under conditions ordinarily used to derivatize other opiates, the thebaine peak diminished in peak height and area. Other unknown peaks appeared in the chromatogram, which suggested a degradation of the opiate under these conditions. Thebaine should not be subjected to derivatization procedures because of its instability and because this opiate does not form TMS derivatives.

The opiate concentrations of the Australian poppy seeds were as follows: thebaine, 20.7 µg/g; codeine, 31.8 µg/g; and morphine, 107 µg/g. For the Spice Time product, the corresponding values were 8.2, 17.7, and 164 µg/g, respectively. A chromatogram from one of the poppy seed extracts with corresponding mass spectra for codeine, morphine, and thebaine is shown in Figure 2. Although the Spice Time seeds contained less than half the thebaine content than the Australian seeds, the Spice Time seeds were used in preparing the poppy seed muffins for the controlled consumption studies because they were more readily available. In addition to the presence of codeine, morphine, and thebaine, both poppy seed extracts also showed an unknown compound that eluted just after codeine and had the same molecular ion as codeine with \(m/z\) of 299 (Figure 2). The mass spectrum of this compound does not match that of hydrocodone, which also has a molecular ion of

![Figure 4. Gas chromatogram and mass spectrum of an unextracted methanolic thebaine standard (100 µg/mL).](image-url)
m/z 299. The identity and relevance of this substance endoge-
nous to poppy seeds are currently unknown.

Samples of powdered street heroin, pharmaceutical prepa-
rations of morphine and codeine, and urine from known heroin
users were qualitatively assayed by GC–MS to determine if the-
bane was present in any of these materials. If thebaine were
present, it would not be a useful marker for poppy seed use.
Figure 3 shows a representative gas chromatogram and partial
mass spectrum of an extracted crude heroin sample. The pre-
dominate peak was heroin with trace amounts of acetylcodeine
and 6-MAM. The codeine and morphine tablets and urine from
true heroin users also produced expected results, with no extra-
neous opiate peaks. Thebaine was not detected in any of these
samples.

Results of the controlled poppy seed consumption study are
shown in Table I. Thebaine was detected in 11 of 15 samples
screened positive for opiates and 1 of the 7 samples that were
screened negative for opiates. In the one negative screen with
a positive GC–MS result, the codeine concentration was 1.5
ng/mL, morphine was 51 ng/mL, and thebaine was 3 ng/mL.
The EMIT absorbance rate was near the cutoff value. Repre-
sentative chromatograms and mass spectra of an unextracted
standard and urine of a poppy seed eater are shown in Figures
4 and 5, respectively. Thebaine concentrations ranged from 2 to
81 ng/mL. Codeine, morphine, and another unidentified poppy
seed-related compound (which also had a molecular weight of
299) were also detected in the urine samples of poppy seed
eaters (1). The temporal excretion of codeine, morphine, and
thebaine from one subject who ate poppy seeds is illustrated in
Figures 6A and B. The urine specimens began to test positive by
the EMIT II opiate immunoassay as early as 2 h postadminis-
tration and remained positive for 12 h after consumption. The
maximum urinary concentration for thebaine and codeine did
not occur at the same time, but instead were at 4 and 2 h,
respectively. Morphine was above the 300 ng/mL immunoassay
threshold for 12 h and remained detectable by EMIT for up to
32 h after consumption.

Discussion

In this study, a common opiate extraction procedure (10) was
modified to exclude the hydrolysis and derivatization steps in
order to extract thebaine from urine at measurable concentra-
tions. The exposure of thebaine to heat, especially under the
acidic conditions used in derivatization with BSTFA, resulted in
considerable decomposition of thebaine (11). Underivatized
morphine was not monitored because of its adherence to the
column. Morphine peaks tailed severely and suffered a loss in
height. Better morphine chromatography and quantitation
could be accomplished by the use of a TMS derivative.

A critical component of interpreting positive results when
testing for drugs of abuse in urine is the distinction between
actual drug abuse and consumption of certain foods or over-
the-counter medications. Results from this study suggest that
a more direct means may be available to determine if poppy seeds were consumed, which could be used to corroborate claims made by donors when discussing results with Medical Review Officers. It is important to recognize that the absence of thebaine in a urine sample screened positive for opiates does not exclude the possibility of poppy seed consumption as a cause of positive results, which occurred in four of the controlled cases. Figure 6 shows that thebaine has a faster clearance than morphine in urine. Thus, a positive screen and negative confirmation for thebaine can be produced from a poppy seed eater if urine is collected 12-24 h after consumption. It is likely that use of a more sensitive GC–MS assay for thebaine will result in a higher detection of poppy seed use. Results for thebaine analysis will certainly depend on the amount of poppy seeds eaten, their thebaine content, time since the seeds were eaten, and individual rate of metabolism. More data are required to establish the range of results expected during drug screening following poppy seed consumption.

A positive result for thebaine does not exclude the possibility of concurrent opiate drug use. The presence of thebaine in the urine of an opiate user could be caused by either inadvertent consumption of poppy seed-containing foods by the opiate abuser or through purposeful adulteration in an attempt to disguise opiate abuse. However, it may be possible that the calculation of a ratio between thebaine and morphine may provide information as to the likelihood of opiate drug abuse. It may be possible that a second endogenous poppy seed constituent, such as the unknown peak eluting after codeine with an m/z of 299, may also provide additional information if it can be identified and reliably measured.

Currently, the threshold concentration for a positive screening test for opiates established by the Department of Health and Human Services (HHS) for the testing of Federal employees is 300 ng/mL. HHS has recently proposed a revision to the mandatory guidelines by increasing the immunoassay screening threshold to 2000 ng/mL for opiates and adding a requirement that 6-MAM be confirmed by GC–MS at a threshold of 10 ng/mL (12). If these proposals are adopted, it will reduce the incidence of positive results caused by the inadvertent consumption of opiates through poppy seeds. However, it will also reduce the incidence of positive urine results produced by true opiate drug users.

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