

Poppy Seeds: Differences in Morphine and Codeine Content and Variation in Inter- and Intra-Individual Excretion

REFERENCE: Pelders, M. G. and Ros, J. J. W., "Poppy Seeds: Differences in Morphine and Codeine Content and Variation in Inter- and Intra-Individual Excretion," *Journal of Forensic Sciences*, JFSCA, Vol. 41, No. 2, March 1996, pp. 209-212.

ABSTRACT: Poppy seeds from seven different origins (Dutch, Australian, Hungarian, Spanish, Czech, and two Turkish) were analyzed for the amount of opiates present. Four grams of each kind of seeds, equivalent to the amount of seeds on two bagels, were ingested by volunteers. One volunteer also ingested four times the same amount of poppy seeds from the same origin (Spanish). During 24 hours urine samples were obtained and screened for the presence of morphine and codeine using the FPIA technique (cut-off = 200 ng/mL) and a GC/MS confirmation with a limit of detection (LOD) of 25 ng/mL for codeine and morphine. Poppy seeds from different origins contain a wide variation of morphine (2-251 µg/g) and codeine (0.4-57.1 µg/g) content. No other opiate could be detected. After ingestion a large interindividual variation of excretion of opiates exists. The testing results from the same kind of seeds ingested four times with a one week interval by the same volunteer also show a poor reproducibility. Several kinds of poppy seeds can give positive testing results (Australian, Hungarian, Spanish and one kind of Turkish seeds). Within 24 hours all testing results became negative.

KEYWORDS: forensic science, toxicology, codeine, interindividual variation, intraindividual variation, morphine, poppy seeds

It is well known that eating of poppy seeds can lead to positive opiate findings in urine drug-screening programs (1-5). Most opiate screening programs include morphine and codeine, which are also present in poppy seeds. In a drug screening program alleged to eliminate drug abuse, the opiates screened are primarily morphine and codeine. It is not always possible to discriminate between opiates originating from drug abuse or poppy seeds consumption.

In studies reporting the results of opiate screening after poppy seeds consumption, it is not often described what source the seeds come from and whether seeds from different sources can result in different screening results. Furthermore, in some studies the amount ingested by volunteers (25 g or more) is unrealistically high (2,5). For these reasons this study was started. Healthy volunteers ingested poppy seeds of six different origins and inter- and intra-individual variation was assessed. A correlation between the amount of opiates excreted by the volunteers and the amount of opiates in the poppy seeds was investigated.

Materials and Methods

Subjects and Schedule

Several bakers were interviewed on the amount of poppy seeds they use on their bagels. Four gram of poppy seeds, corresponding

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with 1 to 2 bagels, was taken by each of six healthy volunteers. The time of ingestion was 5 to 6 p.m., which corresponds with dinner time. During the 24 hours after ingestion urine samples were obtained. In order to correct for dilution of urine samples (for example, because of differences in drinking patterns the ratio between opiate concentration and urine creatinine concentration was assessed). Using this protocol, the volunteers ingested with one week intervals, poppy seeds from six different origins (Dutch, Australian, Hungarian, Spanish, Czech and two Turkish seeds). The amount of morphine and codeine of each kind of seeds was quantified. To obtain data on the intra-individual variation of excretion of opiates from poppy seeds one volunteer ingested four times four gram of Spanish poppy seeds with a one week interval.

Extraction of Codeine and Morphine from Poppy Seeds

To 1 gram of crushed seeds 20 mL 0.1 N HCl and 500 µL of internal standard solution (25 µg/mL nalorfine (OPG, Utrecht, The Netherlands) was added. The solution was refluxed for 1 hour. After cooling down to room temperature the sample was centrifuged 5 minutes at 3000 rpm. To 1 mL of the centrifuged sample 3 mL 0.5 M KH₂PO₄ buffer (pH 7.0) was added.

Solid Phase Extraction (SPE) of Morphine and Codeine

SPE was performed with Bond-Elut Certify® columns (Varian, Houten, The Netherlands). The columns were conditioned with 3 mL methanol (Merck, Darmstadt, Germany) and 3 mL 0.5 M phosphatebuffer (pH 7.0). After conditioning, the sample was slowly poured down the column. The column was then washed successively with 1 mL 0.1 M acetatebuffer (pH 4.3), 2 mL of deionized water and 3 mL methanol. The column was then dried under full vacuum for 5 minutes. The samples were eluted with 3 mL of a mixture of dichloromethane/2-propanol/triethylamine (80:20:2) (Merck, Darmstadt, Germany). The extracts were dried under nitrogen at 40°C. To the dried extracts 50 µL of bistrimethylsilyltrifluoroacetamide (BSTFA) (Pierce, Rockford, U.S.A.) was added and the tube was closed and heated for 15 minutes at 100°C. After cooling down 1 µL of sample was injected into the GC/MSD.

Screening for Opiates

All urine samples were screened for opiates with fluorescence polarization immuno assay ADx®-FPIA (Abbott, Illinois, U.S.A.).

Extraction of Morphine, Codeine and 6-monoacetylmorphine from Urine Samples

To 1 mL of urine (5 mL for 6-monoacetylmorphine) 2500 U β-glucuronidase type IX-A from *E. Coli* (Sigma, Axel, Belgium)

TABLE 1—Amounts of morphine and codeine in poppy seeds of different origin, ADx opiates screening results (at a cut-off of 200 ng/mL), total number of samples collected over 24 hours, number and percentage of positive results.

Country of origin	Seeds		Total number of urinary samples collected	Urine			
	Morphine ($\mu\text{g/g}$ seed)	Codeine ($\mu\text{g/g}$ seed)		Positive Samples (>200ng/mL)	%	opiates (ng/mL) [range]	creatinine (mmol/L) [range]
Australia	90	6.5	31	13	42	0–694	1.50–27.6
Hungaria	46	3.7	15	1	7	0–200	1.94–25.1
Czech Republic	2	0.5	22	0	0	0–33	3.65–24.3
Spain	251	57.1	42	25	60	0–2034	1.29–22.8
Turkey I	5	1.2	29	0	0	0–124	2.53–27.9
Turkey II	27	15.5	39	3	8	7–314	0.80–26.9
The Netherlands	4	0.4	30	0	0	0–30	2.05–20.4

in 0.075 M phosphatebuffer (pH 6.8), 25 μL of internal standard solution (25 $\mu\text{g/mL}$ nalorfine) and 3 mL 0.5 M phosphatebuffer (pH 7.0) was added. The samples were placed in a waterbath at 37°C and were incubated overnight. After incubating the samples were extracted by SPE as earlier described.

GC/MS Analysis of Morphine, Codeine and 6-monoacetylmorphine

GC/MS analysis was carried out with an HP 5890 GC (Hewlett Packard, Palo Alto, U.S.A.) equipped with a HP 7673A automatic injector and interfaced to a HP 5971A MSD. The column was a HP-1 12 m capillary with an internal diameter of 0.22 mm and with a 0.33 μm film thickness (Hewlett Packard, U.S.A.).

Helium was used as carrier gas at a initial pressure of 21.4 psi for 1 minute after which a constant flow rate was maintained of 0.8 mL/min. Injections were made in the splitless mode. The injection temperature was 250°C and the transfer line temperature was 280°C. The oven temperature was maintained at 160°C for 1 min and then programed at 10°C/min to 260°C after which it was programed at 50°C/min tot 300°C.

Quantitative analyses were performed operating the mass selective detector in the selective ion monitoring (SIM) mode. The following ions were monitored for each compound: morphine 429, 414, 371, 401; codeine 371, 343, 313, 372; 6-monoacetylmorphine m/z 399, 340, 342, 400; nalorfine 455, 440, 414, 371; with the first ion mentioned used for quantification. Identification was based on comparison of retention times and relative abundances of the other 3 ions with values of authentic standards run on a daily basis. Calibration curves were linear (25–1000 ng/mL for morphine, 25–2500 ng/mL for codeine and 0.5–50 ng/mL for 6-monoacetylmorphine) and the limit of quantification (LOQ) for morphine and codeine was 25 ng/mL, for 6 monoacetylmorphine 0.5 ng/mL.

Analysis of Creatinine Levels in Urine Samples

Creatinine was analyzed for each urine sample spectrophotometrically using a RA 1000 (Technicon, New York, U.S.A.).

Results

Morphine and Codeine in Poppy Seeds

The amount of morphine from the poppy seeds of different origin ranged from 2–251 $\mu\text{g/g}$ seeds, codeine from 0.4–57.1 $\mu\text{g/g}$ seeds (Table 1).

TABLE 2—Excretion of morphine and codeine after ingestion of four grams of Spanish poppy seeds by seven different volunteers, Morphine and codeine concentrations result from GC/MS analysis.

Volunteer number →	1	2	3	4	5	6	7
Hour after ingestion	0	0	0	0	0	0	0
Morphine (ng/mL)	0	0	0	0	0	0	0
Codeine (ng/mL)	0	0	0	0	0	0	0
Creatinine (mmol/L)	6.15	11.96	11.72	12.16	20.31	9.84	20.20
Hour after ingestion	1	3.5	1.75	1.5	2.5	3.25	1
Morphine (ng/mL)	304	454	348	300	3674	645	352
Codeine (ng/mL)	5	16	22	0	67	49	6
Creatinine (mmol/L)	5.04	11.76	5.21	2.82	15.61	14.24	12.89
Hour after ingestion	9.5	10.75	5.5	3.75	13	5.25	3.75
Morphine (ng/mL)	602	1091	633	446	1434	446	434
Codeine (ng/mL)	37	50	9	6	70	23	16
Creatinine (mmol/L)	6.75	13.11	5.50	2.58	17.14	14.61	5.81
Hour after ingestion	12.5	14.5	12.75	4.25	18.5	13.25	13.5
Morphine (ng/mL)	350	540	430	182	529	3255	409
Codeine (ng/mL)	48	25	0	7	16	5	5
Creatinine (mmol/L)	7.8	14.66	10.60	1.29	12.76	17.86	11.78
Hour after ingestion	17.5	16.5	16.75	6.25	21.5	19.75	18.5
Morphine (ng/mL)	400	160	45	235	33	50	200
Codeine (ng/mL)	11	0	0	8	0	0	0
Creatinine (mmol/L)	16.08	8.64	4.42	4.66	13.32	22.78	15.19
Hour after ingestion	21.5	18.5	20.5	12.75	25.5	24	20
Morphine (ng/mL)	136	106	33	196	272	19	152
Codeine (ng/mL)	14	0	0	5	0	0	0
Creatinine (mmol/L)	11.84	5.96	4.77	3.46	19.09	19.15	16.25
Hour after ingestion	24	21	24	19			23.5
Morphine (ng/mL)	107	174	38	85			24
Codeine (ng/mL)	0	0	0	0			0
Creatinine (mmol/L)	14.56	13.07	8.17	12.01			10.16
Hour after ingestion		23.5		24			
Morphine (ng/mL)		27		78			
Codeine (ng/mL)		0		0			
Creatinine (mmol/L)		4.87		13.22			

Screening Urine Samples for Opiates

The Abbott-ADx obtains a threshold for positive opiates screening of 200 ng/mL. Morphine is used as calibration compound at levels from 0–1000 ng/mL. Morphine standards of 200, 250 and 800 ng/mL show variation coefficients of 3.24, 2.89 and 5.52%, respectively. In Table 1, the results of this screening for all urine samples are reported.

Because of the large amount of data this study produced we present only the results obtained after ingestion of Spanish seeds.

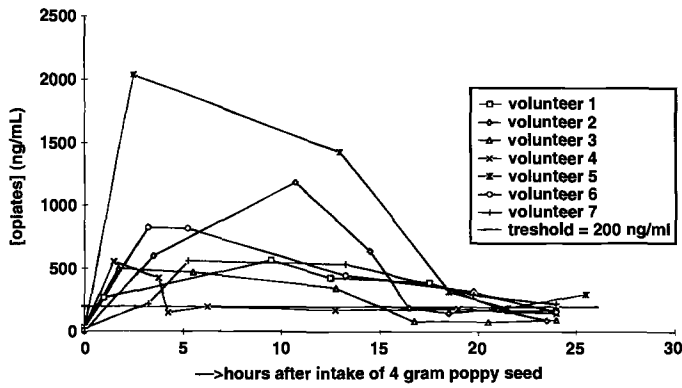


FIG. 1—ADx results, screening urines of seven volunteers after poppy seed (Spanish) ingestion.

In Table 2 the amount of morphine, codeine and creatinine in the urine samples of the volunteers after ingesting Spanish poppy seeds is shown. In Fig. 1, the results of the ADx analyses is shown. Figure 2 depicts the obtained morphine levels in urine. In Fig. 3 the morphine to creatinine ratios are shown.

The amount of morphine, codeine, and creatinine in the urine samples of one person who ingested four times 4 grams of Spanish with a one week interval is shown in Table 3.

Discussion and Conclusions

Poppy seeds from different origins contain different amounts of morphine and codeine. Even the two Turkish samples contain

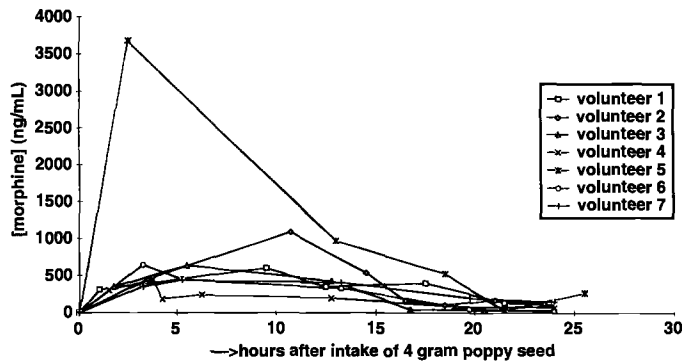


FIG. 2—Urinary morphine levels of seven volunteers after intake of Spanish poppy seeds.

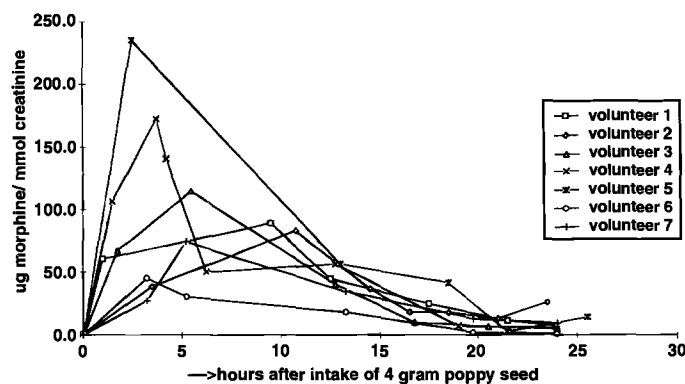


FIG. 3—Morphine to creatinine ratio in urines of seven volunteers after intake of Spanish poppy seeds.

TABLE 3—Excretion of morphine and codeine after ingestion of four grams of Spanish poppy seeds by one volunteer, 4 times, with a one week interval. Morphine and codeine concentrations result from GC/MS analysis.

	1	2	3	4
Hour after ingestion	0	0	0	0
Morphine (ng/mL)	0	0	0	0
Codeine (ng/mL)	0	0	0	0
Creatinine (mmol/L)	12.16	15.1	8.6	4.7
Hour after ingestion	1.5	3.5	3	1.5
Morphine (ng/mL)	300	1180	529	69
Codeine (ng/mL)	0	24	33	8
Creatinine (mmol/L)	2.82	2.5	17.2	8.2
Hour after ingestion	3.75	13	13	2.75
Morphine (ng/mL)	446	539	425	144
Codeine (ng/mL)	6	37	33	8
Creatinine (mmol/L)	2.58	3.1	19	9.1
Hour after ingestion	4.25	17	17.5	12
Morphine (ng/mL)	182	54	68	208
Codeine (ng/mL)	7	3	2	21
Creatinine (mmol/L)	1.29	13.3	9.9	13.3
Hour after ingestion	6.25	22	24	17
Morphine (ng/mL)	235	40	79	55
Codeine (ng/mL)	8	0	6	4
Creatinine (mmol/L)	4.66	9.2	7.9	8.1
Hour after ingestion	12.75	24		24
Morphine (ng/mL)	196	13		33
Codeine (ng/mL)	4	0		0
Creatinine (mmol/L)	3.46	10		15.6
Hour after ingestion	19			
Morphine (ng/mL)	85			
Codeine (ng/mL)	0			
Creatinine (mmol/L)	12.01			
Hour after ingestion	24			
Morphine (ng/mL)	78			
Codeine (ng/mL)	0			
Creatinine (mmol/L)	13.22			

different amounts of opiates. The reason for this is may be that they can be harvested in different years or that they may stem from distinct race cultures. Because of these facts the chance for positive screening results depends on the origin of the poppy seeds. Poppy seeds containing large amounts of morphine and codeine increase the chance of a subject screening positive. In comparison with the amount of morphine, the codeine content of the poppy seeds contributes very little to the screening results.

The screening results depend on the method of analysis: FPIA and GCMS both give rise to positive tests in the same urine samples, but the exact concentrations differ; the main reason for this is that the FPIA-test adds the concentration of conjugated and non conjugated opiates together with a certain cross-reactivity, while with GC/MS morphine and codeine concentrations are measured separately. Both methods of analysis show that the opiates are excreted fast after ingestion of the seeds. Peak concentrations were seen within the first 6 to 8 hours or already in the first void. A large interindividual variation exists in concentrations recovered and in percentages of the opiates which theoretically could be excreted. The maximum amount of opiates to be excreted was calculated from the analysis of the seeds from different origins. Apart from the interindividual variation of excretion, the mean percentage of opiates excreted varied widely from one kind of seeds to another. Positive screening results (ADx > 200 ng/mL) could be found only when seeds that contain a large amount of morphine and codeine are ingested. Seeds originating from the Czech Republic, Turkey, and The Netherlands, which contain almost no

morphine and codeine, did never give rise to positive screening results. Seeds from Spain and Australia with the largest morphine and codeine content, gave one or more positive screening results for each of the volunteers.

Apart from the large interindividual variation a large intra-individual variation in testing results exists: the percentage of morphine excreted within 24 hours varies from 21–52%, of codeine from 4–11%. Because only one volunteer ingested the seeds four times with a one week interval, it does not fully describe the expected range of intra-individual variation in opiate urinary excretion.

No urine specimen gives rise to positive testing results after 24 hours. When two urine samples were produced within a short period of time, (within 1 hour) the creatinine concentration could become very low (<1 mmol/L). This means that in detoxification centers or in prisons where negative testing results are important, one should be careful in suggesting illegal dilution of urine when urine creatinine concentrations are very low. The calculated ratio between opiates and creatinine concentration were less reliable for low creatinine concentrations.

In order to get a better picture of the data of the intra-individual variation, the ratios between opiate concentration and urine creatinine concentration were calculated. However, this did not significantly improve the correlation.

6-mono-acetyl-morphine could not be detected in any sample. It is well known that it is very difficult to detect 6-MAM in urine. From our study it is concluded that the presence of this compound in a urine sample cannot be the result of poppy seeds ingestion, but it must be the result of heroin consumption. This is consistent with the work of others who studied the effects of larger amounts of poppy seeds and who came to the same conclusion (1,2,5). It is always difficult to discriminate between different sources for

morphine and codeine in urine. Especially in workplace/employment drug screening situations, forensic toxicologists grapple with the interpretation of urinary morphine and codeine concentration. The results of this study confirm the conclusions of ElSohly et al. who stated that poppy seeds as the sole source of morphine and codeine in urine could be ruled out when: (1) codeine levels exceed 300 ng/mL, (2) morphine—codeine ratio is less than 2 (3) high levels of morphine (>1000 ng/mL) are detected without codeine being present (1).

Acknowledgment

We would like to thank Mr. O.C. Knottnerus for providing the different kind of seeds and the volunteers who ingested the seeds.

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