

Concentration Ratios of Morphine to Codeine in Blood of Impaired Drivers as Evidence of Heroin Use and not Medication with Codeine

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Background: Both the illicit drug heroin and the prescription drug codeine are metabolized to morphine, which tends to complicate interpretation of opiate-positive samples. We report here the concentrations of morphine and codeine, the morphine/codeine ratios, and 6-acetylmorphine (6-AM) in blood specimens from individuals arrested for driving under the influence of drugs (DUID) in Sweden. The results were compared with positive findings of 6-AM in urine as evidence of heroin intake.

Methods: In 339 DUID suspects, both blood and urine specimens were available for toxicologic analysis. In another 882 cases, only blood was available. All specimens were initially analyzed by immunoassay, and the positive results were verified by isotope-dilution gas chromatography–mass spectrometry. In routine case-work, the limits of quantification (LOQs) for unconjugated opiates were 5 ng/g for blood and 20 µg/L for urine. **Results:** The median concentration of morphine in blood was 30 ng/g with 2.5 and 97.5 percentiles of 5 and 230 ng/g, respectively (n = 979). This compares with a median codeine concentration of 20 ng/g and 2.5 and 97.5 percentiles of 5 and 592 ng/g, respectively (n = 784). The specific metabolite of heroin, 6-AM, was identified in only 16 of 675 blood specimens (2.3%). This compares with positive findings of 6-AM in 212 of 339 urine samples (62%) from the same population of DUID suspects. When 6-AM was identified in urine, the morphine/codeine ratio in blood was always greater than unity (median, 6.0; range, 1–66). In 18 instances, 6-AM was present in urine, although morphine and codeine were below the LOQ in blood. The morphine/codeine ratio in blood was greater than unity in 85% of DUID cases when urine was not available (n = 506), and the

median morphine and codeine concentrations were 70 ng/g and 10 ng/g, respectively. When morphine/codeine ratios in blood were less than unity (n = 76), the median morphine and codeine concentrations were 10 ng/g and 180 ng/g, respectively.

Conclusions: Only 2.3% of opiate-positive DUID suspects were verified as heroin users on the basis of positive findings of 6-AM in blood. A much higher proportion (62%) were verified heroin users from 6-AM identified in urine. When urine was not available for analysis, finding a morphine/codeine concentration ratio in blood above unity suggests heroin use and not medication with codeine. This biomarker indicated that 85% of opiate-positive DUID blood samples were from heroin users.

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Determining whether a person has taken heroin as opposed to a prescription drug containing codeine is not always easy because both opiates undergo metabolism into morphine (1–4). Moreover, clandestine heroin preparations contain acetylcodeine as an impurity, and this opiate is readily deacetylated to produce codeine (5, 6). Accordingly, forensic toxicology reports showing morphine and codeine in blood or urine could have several possible explanations. Heroin has a specific metabolite, 6-acetylmorphine (6-AM),¹ but its very short half-life of 10–20 min means that the window of detection in blood is only 1–2 h after administration (3, 4). For this reason, 6-AM is seldom identified in forensic blood samples above the limits of quantification (LOQs) by current methods of gas chromatography–mass spectrometry (GC-MS) (7, 8). However, evidence of heroin intake is easier to obtain by analysis of urine specimens because 6-AM remains in urine for much longer than in blood (8–11).

The traffic police in Sweden try to obtain specimens of

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¹ Nonstandard abbreviations: 6-AM, 6-acetylmorphine; LOQ, limit of quantification; GC-MS, gas chromatography–mass spectrometry; and DUID, driving under the influence of drugs.

both blood and urine for toxicologic analysis, especially from individuals suspected of driving under the influence of drugs (DUID). This gave us the opportunity to compare the concentrations of morphine, codeine, and 6-AM in blood with the frequency of finding 6-AM in urine as unequivocal evidence of heroin intake.

Materials and Methods

All blood and urine specimens from impaired drivers apprehended in Sweden are submitted for analysis to one central laboratory, the National Laboratory of Forensic Chemistry located at the University Hospital in Linköping. Drivers are apprehended for committing a moving traffic violation, during routine sobriety controls, or after traffic crashes. The material for this study was taken from the routine case files and toxicologic reports of blood or urine or both. Table 1 summarizes the numbers of DUID suspects involved in the study and whether blood or urine was available for analysis.

The police typically conduct a breath-alcohol screening test at the roadside with the aid of a hand-held instrument (12), either an Alcolmeter S-D2 or Alcolmeter 400 (Lion Laboratories). Performing various field-sobriety tests is unnecessary before making the preliminary breath-alcohol analysis. If the breath-alcohol test is negative and the police suspect impairment by drugs other than alcohol, specimens of blood and urine are taken for toxicologic analysis. The police make this decision on the basis of the general appearance and behavior of the suspect. More recently, the police also examine pupil size and eye movements, as well as other signs and symptoms of drug impairment. Examination of DUID suspects by drug recognition experts is not an option in Sweden. However, in some situations, a physician makes a clinical examination in conjunction with obtaining blood and urine samples for forensic toxicology.

Venous whole blood was submitted in two 10-mL gray-stoppered Vacutainer tubes (Becton Dickinson) containing 100 mg of sodium fluoride and 25 mg of sodium oxalate as preservatives. Urine specimens were sent for analysis in two 10-mL, plastic screw-capped tubes, each containing 100 mg of sodium fluoride as preservative. A qualitative screening analysis of urine and/or blood was made for five classes of abused drugs (opiates, cannabinoids, amphetamine analogs, cocaine metabolites, and benzodiazepines) by immunoassay techniques such as Emit or CEDIA with the Hitachi 717. The most commonly prescribed sedative-hypnotics and analgesics were ana-

lyzed in blood by capillary column gas chromatography with nitrogen-phosphorous detection (13). All positive immunoassay results were verified by more specific methods (liquid chromatography-mass spectrometry or GC-MS). After solid-phase extraction, opiates were determined by GC-MS with the use of deuterium-labeled internal standards and selected-ion monitoring (14). The blood aliquots were measured by weight and concentrations of drugs reported in mass/mass units (ng/g) because the fluidity of specimens differs widely, especially in postmortem bloods where it is not always practical to dispense aliquots by volume. After extraction and before gas chromatography, the opiates were converted to their pentafluoropropionic acid anhydride derivatives. For analysis of urine, the aliquots were measured by volume and results were thus reported mass/volume ($\mu\text{g/L}$). Before GC-MS, the trimethylsilyl ester derivatives were prepared by treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide. The concentrations of opiates in blood and urine (morphine, codeine, and 6-AM) were quantitatively determined in a single GC-MS analytical run. The specimens were not hydrolyzed; therefore, the concentrations of unconjugated opiates could be reported. The LOQs for codeine, morphine, and 6-AM in blood were 5 ng/g and 20 $\mu\text{g/L}$ for urine. Calibration curves were constructed up to a maximum concentration of 1000 $\mu\text{g/L}$ in urine and values above this concentration were reported as >1000 $\mu\text{g/L}$.

The frequency distributions of the concentrations of morphine and codeine in blood were markedly skewed, so the median value and 2.5 and 97.5 percentiles were used to characterize the data.

Results

Table 2 presents the drugs verified positive in blood from individuals apprehended for DUID in Sweden during 2000. Many individuals had several different drugs present in their blood, including scheduled narcotic drugs, prescription medications (mainly benzodiazepines), as well as various alcohol and drug combinations. Amphetamine and cannabis are the most commonly encountered drugs in DUID suspects in Sweden, but morphine and codeine are also high on the list of substances confirmed present in blood specimens.

Table 3 gives the concentrations of morphine, codeine, and 6-AM in blood samples from DUID suspects apprehended between 1992 and 2000. As expected, the concentrations span a wide range in this population, which comprises people taking therapeutic doses of opiates, as well as many drug addicts. The heroin metabolite 6-AM was determined 43 times at a median concentration of 8 ng/g (2.5 and 97.5 percentiles, 5 and 104 ng/g). The highly skewed distributions of morphine and codeine concentrations in blood are shown in Figs. 1 and 2 with medians of 30 ng/g for morphine and 20 ng/g for codeine.

In 675 opiate-positive blood samples, 6-AM was verified above the LOQ only 16 times (2.3%). By contrast,

Table 1. Distribution of DUID cases in Sweden before and after introduction of zero-tolerance legislation (July 1999).

Specimens available for analysis	January 1992 to June 1999	July 1999 to December 2000
Blood only	4569 (77%)	2922 (56%)
Total blood and urine	1348 (23%)	2248 (44%)
Total	5917 (100%)	5176 (100%)

Table 2. Analytical results for the most commonly encountered drugs in blood samples from DUI suspects in Sweden during 2000.^a

Drug	Year 2000 (n = 3808)
Amphetamine	2395 (63%)
Tetrahydrocannabinol	1119 (29%)
Diazepam	743 (20%)
Morphine	382 (10%)
Methamphetamine	364 (10%)
Flunitrazepam	347 (9%)
Codeine	245 (6%)
Paracetamol (acetaminophen)	129 (3%)
MDMA	116 (3%)
Phenazone	109 (3%)
Benzoylcegonine	89 (2%)
Nitrazepam	81 (2%)
Alprazolam	80 (2%)
d-Proxyphe	67 (2%)

^a Note that many specimens contained several different drug classes.

6-AM was verified in 212 of 339 urine samples analyzed (62%). In 89 urine specimens containing 6-AM, the median morphine/codeine ratio in the corresponding blood sample was 6.0 (range, 1–66). In 18 instances, 6-AM was present in urine, although morphine and codeine were below the LOQ in blood. Another 105 urine samples contained 6-AM when morphine was above the LOQ in blood (median concentration, 10 ng/g) but the concentration of codeine in blood was below the LOQ.

The frequency distribution of morphine/codeine ratios in blood when urine was not available for analysis, and when morphine and codeine were both above the LOQ (n = 506), is shown in Fig. 3. In 76 instances (15%), the morphine/codeine ratio was less than unity (median, 0.057), and the corresponding median blood-morphine concentration was 10 ng/g compared with 180 ng/g for codeine, which gives a median codeine/morphine ratio of 18:1. This high codeine/morphine ratio most likely reflects intake of the prescription drug codeine. In 430 cases, the morphine/codeine ratio was greater than unity (median, 5.0; range, 1.1–67). For these cases, the median concentration of morphine was 70 ng/g (range, 7–620 ng/g) compared with 10 ng/g (range, 1–100 ng/g) for codeine, indicating heroin use. These results are summarized in Table 4.

Table 3. Concentrations of morphine, codeine, and 6-AM in opiate-positive blood specimens from motorists apprehended for DUI in Sweden between 1992 and 2000.

Opiate	Number	Concentration of opiates in blood, median (2.5 and 97.5 percentiles)
Morphine	979	30 ng/g (5 and 230)
Codeine	784	20 ng/g (5 and 592)
6-AM	43	8 ng/g (5 and 104)

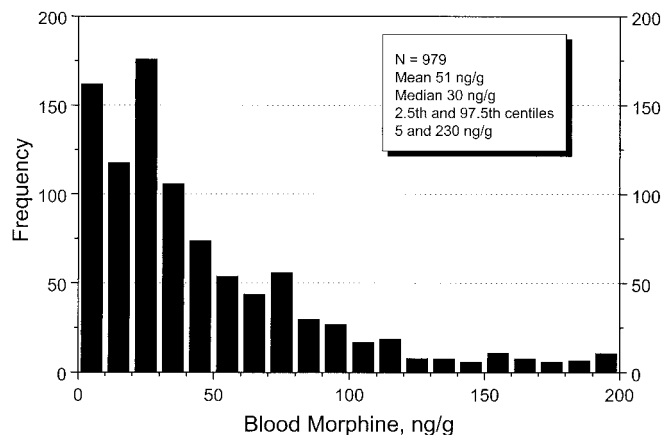


Fig. 1. Frequency distribution of the concentrations of unconjugated morphine in whole blood from DUI suspects in Sweden (n = 979).

Discussion

Since July 1999, Sweden has enforced a zero-tolerance law for DUI offenses, which means that the presence of a scheduled narcotic drug in blood is sufficient for prosecution regardless of whether the person exhibits any signs and symptoms of impairment. For the use of prescription drugs (e.g., benzodiazepines) without clear-cut evidence of impairment, the prosecution is required to prove that the recommended dose was exceeded. This requires an expert opinion to relate the blood drug concentration to the expected therapeutic concentration for the particular medication, which is not always easy.

If scheduled drugs like amphetamine, methamphetamine, cocaine, and tetrahydrocannabinol are present in blood above the LOQs by GC-MS, this is sufficient for prosecution, regardless of whether the person's driving ability was impaired. Zero-tolerance laws for narcotic drugs are similar in principle to the widely used per se laws for driving under the influence of alcohol. Because all specimens are sent to one forensic toxicology laboratory in Sweden (population 8.8 million), the LOQs for scheduled drugs in blood determined by GC-MS are

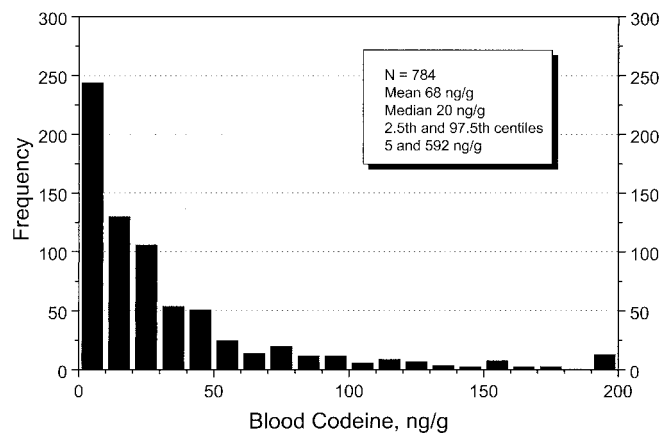


Fig. 2. Frequency distribution of the concentrations of unconjugated codeine in whole blood from DUI suspects apprehended in Sweden (n = 784).

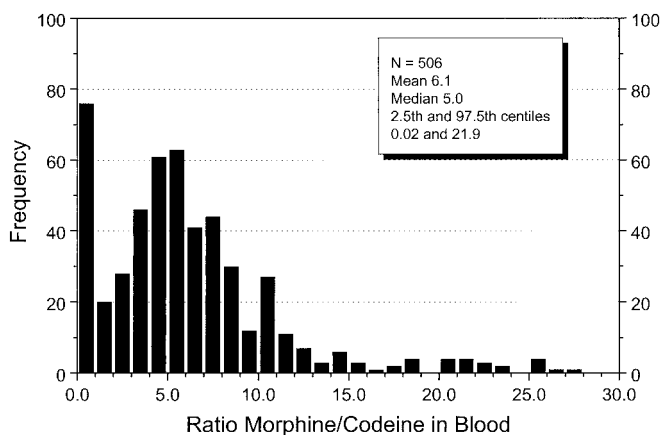


Fig. 3. Frequency distribution of the concentration ratios of unconjugated morphine to codeine in specimens of whole blood from DUID suspects apprehended in Sweden ($n = 506$).

effectively the threshold concentration limits for prosecution. Since the introduction of this zero-tolerance law for narcotics, the number of DUID cases submitted by the police has increased approximately fivefold. This sharp increase after July 1999 stems from an increased activity and enthusiasm by the police and prosecutors, who are now more likely to win a conviction. Moreover, the police are trained to recognize signs and symptoms of drug abuse and are allowed to monitor pupil size and look for nystagmus. This dramatic increase in the number of DUID cases can also be explained, at least in part, by prior knowledge about criminal elements and drug abusers in the community.

The ultimate evidence of heroin use is finding 6-AM in blood or urine. However, only 2.3% of opiate-positive DUID suspects were verified as heroin users based on the analysis of the 6-AM metabolite in blood. This compares with 62% confirmed users of heroin after the analysis of 6-AM in urine. The window of detection for 6-AM in blood and urine depends on the dose of heroin taken, the route of administration, and the frequency of heroin intake (10). The concentrations of opiates excreted in urine, including 6-AM, vastly exceeds the concentration in blood (15, 16). Interpreting the concentrations of morphine and codeine measured in urine is more complicated, owing to the irregular frequency of urination and

pooling of urine in the bladder, as well as variable diuresis and fluctuations in pH, which will affect the results of toxicologic analysis (16–19). Moreover, cleavage of glucuronides is often a tricky problem depending on whether acid hydrolysis or enzymatic methods are used and the source of β -glucuronidase (20). The unconjugated drug concentrations of opiates in blood, more so than urine, reflect fairly recent intake (17).

Analysis of drugs of abuse in urine provides evidence of intake but does not permit conclusions to be drawn about coexisting concentrations in blood or impairment of the individual at the time of voiding. Even the concentrations of unconjugated opiates in blood or serum are difficult to relate to impairment, because of the development of tolerance. The introduction of so-called zero-tolerance laws for scheduled drugs in traffic cases sidesteps these problems and offers an effective means of combating the problem of DUID.

The forensic blood samples that are sent for analysis to our laboratory contain 1% sodium fluoride as preservative, which tends to cause hemolysis. Accordingly, the concentrations of drugs and poisons are determined in whole blood and not plasma or serum, which are the specimens more commonly used for therapeutic drug monitoring. Because the distribution ratio of morphine between plasma and whole blood is close to unity (21), finding a morphine/codeine ratio >1.0 in plasma or serum also furnishes evidence of heroin use. Studies on the pharmacokinetics of codeine have shown that morphine/codeine ratios in plasma remain less than unity at all times after administration (1, 2, 18). When 100 mg of codeine phosphate was given to healthy volunteers, the peak plasma morphine concentration was only 3.2% of the peak codeine concentration (19). Furthermore, the morphine/codeine ratios in plasma remained less than unity for up to 23 h postdosing (19). Similar results have been reported after a single oral dose of 60 mg of codeine and also after repeated intake (1, 18). Morphine is also a metabolite of ethyl morphine, the active ingredient in various antitussive medications (22, 23). However only a small fraction of the ethyl morphine is seemingly converted to morphine, and both parent drug and metabolite are easily detected in urine for up to 24 h after intake (22). Since the first report in Sweden in 1982 (24), the presence of various opiates in poppy seeds and other food products is well recognized as a confounding factor in urine drug testing programs. Finding 6-AM in urine can be used to dismiss the allegation that the source of morphine and codeine in urine was from foods laced with poppy seeds (25). The concentrations of unconjugated morphine and codeine in blood samples after eating poppy seed cakes will hardly be expected to exceed the LOQ of the GC-MS method of analysis (26).

Because of polymorphisms of the CYP2D6 enzyme, large interethnic differences in the rate and extent of demethylation of codeine exist (27). This leads to slow and rapid metabolizers of codeine depending on the

Table 4. Concentrations of morphine and codeine in blood samples from motorists apprehended for DUID in Sweden when both opiates were above the LOQs (5 ng/g) by GC-MS.

Opiate in blood	Number	Median (2.5 and 97.5 percentiles)
Morphine	506	50 ng/g (6 and 268)
Codeine	506	10 ng/g (5 and 451)
Morphine/Codeine ratio >1	430	Morphine, 70 ng/g; codeine, 10 ng/g
Morphine/Codeine ratio <1	76	Morphine, 10 ng/g; codeine, 180 ng/g

particular genotype inherited (27, 28). Most Caucasians seem to be rapid metabolizers of codeine, and morphine/codeine ratios within this population are always much less than unity (27). In a group of Chinese volunteers with low capability of metabolizing codeine, the morphine/codeine concentration ratios after a dose of 50 mg of codeine were even less than in Caucasians. Accordingly, finding a high morphine/codeine ratio in forensic blood specimens cannot be attributed to a person's inherent ability to convert codeine into morphine.

Many DUID suspects with morphine and codeine identified in blood claim they took the prescription drug codeine or received morphine for relief of pain (e.g., from injuries resulting from a traffic crash). However, the results reported here, based on finding 6-AM in urine, suggests that when morphine/codeine ratios in blood are greater than unity, the person has more likely used heroin than taken the legal drug codeine. This allowed us to conclude that in 506 DUID suspects with morphine and codeine verified in blood above the LOQ, 85% had used heroin. Intake of mixtures of codeine and morphine or codeine and heroin obviously complicates the interpretation of morphine/codeine ratios in blood. The rapid increase of immigration into Sweden has seen new drug cultures emerging. An example is the abuse of raw opium, which is likely to give a different pattern of morphine and codeine concentrations in body fluids. However, experience with two documented cases of raw opium abuse gave morphine/codeine ratios in blood of 1.5 and 2.0. Accordingly, such cases would not be confused with the intake of codeine, for which morphine/codeine ratios are always much less than unity (19).

From the results of this study, we recommend that if urine is not available for toxicologic analysis, the finding of a morphine/codeine concentration ratio in blood exceeding unity is strong evidence that the person had used heroin, as opposed to having taken a prescription drug containing codeine.

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