



K36 *In Vitro* Formation of Acetylmorphine From Morphine and Aspirin in Gastric Contents and Water

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After attending this presentation, attendees will understand that it is possible to form acetylmorphine *in vitro* by incubating human gastric contents or Deionized (DI) water with morphine and aspirin.

This presentation will impact the forensic science community by suggesting that detection of 6-acetylmorphine (6-AM) may no longer solely be an indicator of heroin use.

Forensic toxicologists across the world have considered detection of 6-AM to be definitive evidence of heroin use. 6-AM was detected in an 85-year-old female with a history of a witnessed arrest in bed at a nursing home. The decedent was under hospice care for failure to thrive, and had a history of multiple strokes, syncope, hyperkalemia, osteoporosis, anemia, and osteomyelitis. There was no history of illicit drug use and the decedent was prescribed morphine sulfate elixir (Roxinol®). Whether she was taking aspirin (acetylsalicylic acid) was not recorded and salicylates were not detected by colorimetry. The manner of death was natural and the cause of death was ruled as bronchopneumonia due to hypertensive atherosclerotic cardiovascular disease with remote myocardial and cerebral infarcts.

What about the 6-AM? Is it possible that an individual may be taking morphine (pain management) and aspirin (anticoagulant), and the aspirin may acetylate morphine to produce acetylmorphine? In the present study, the possibility of formation of acetylmorphine when morphine is mixed in solution with aspirin was investigated.

Two opioid negative, postmortem gastric specimens were selected for this study, along with morphine sulfate-Extended Release (ER) tablets (15mg) and coated-aspirin tablets (325mg). Morphine and aspirin tablets were placed into 50mL samples of the two separate gastric specimens, as well as deionized water. The three morphine/aspirin solutions were incubated at 37°C for increasing lengths of time. A separate experiment was run in gastric contents using 15mg morphine sulfate powder in lieu of morphine extended-release tablets. One milliliter aliquots were taken from all samples at 10 min intervals up to one hour, and then at 90 min, two hours, and ultimately 26 hrs. Aliquots were extracted using a previously published UCT solid phase opiate procedure, and analyzed by GC/MS in SIM mode.

Acetylmorphine was detected in all of the samples containing morphine and aspirin in combination. Levels of acetylmorphine were greater in gastric contents than in DI water during the same incubation period. After 120 min, the 6-AM concentrations for the samples containing aspirin and an ER tablet were 21ng/mL and 25ng/mL in the gastric solutions, compared to 7ng/mL in water. After 26 hrs at room temperature, the gastric concentrations were 124ng/mL and 121ng/mL, and in water 27ng/mL. The increase in concentration of acetylmorphine in gastric was linear ($R^2 = 0.99$ and 0.98), while formation in water was non-linear ($R^2 = 0.63$). The results for morphine sulfate powder were essentially identical to those observed for ER tablets. The initial pH of the two gastric samples were 4.74 and 5.27, respectively; following the addition of the morphine/aspirin tablets and two hours incubation, final pH values were 3.86 and 3.92. The final pH of the water solution was 2.88. This study demonstrates that it is possible to form acetylmorphine *in vitro* by combining morphine and aspirin tablets in both postmortem gastric contents and deionized water. The compound produced in this study was identified as 6-AM by GC/MS. Further investigation must be done to determine whether the compound is actually 6-acetylmorphine, 3-acetylmorphine, or a mixture of the two compounds.

Does acetylmorphine form *in vivo*? In addition to the case described above, 10,602 specimens were assayed for opioids by a pain management laboratory using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Three cases containing acetylmorphine were found to be inconsistent with heroin usage. A single specimen was listed as having a prescription for morphine and contained codeine, morphine, and 6-AM; the other two specimens contained 6-AM but not morphine or codeine. Although *in vitro* formation of acetylmorphine has been demonstrated, these data indicate that *in vivo* formation from the co-administration of aspirin and morphine is unlikely to occur. This may be attributed to inconsistencies in elimination half-lives; half-lives are 13 – 20 minutes and 1.3 – 6.7 hours for aspirin and morphine, respectively.

Morphine, Acetylsalicylic Acid, Acetylmorphine