Qualitative & Quantitative Analysis of Heroine and other adulterants

INTRODUCTION

Heroin (diacetylmorphine) is a semisynthetic drug first made from morphine at St, May's hospital (London) in 1874 . it was introduce in 1898 as remedy for cough and for morphine addiction . Some years passed , however before it was appreciated that it cured morphine addiction by substituting itself as the addicting agent . It is converted to morphine in the body .

It is commonly stated that heroin is the most potent of all dependence-producing drugs .

The great advantage of heroin, from the point of view of illicit traffickers, is that it seems capable of almost unlimited dilution with other substances, while still the addict obtains from it some gratification for his desire. Nearly every small peddler who resells heroin first mixes his supply with at least an approximately equal amount of lactose or powdered sugar. As his heroin is usually highly adulterated and diluted even before he gets it, it frequently reaches the final user containing no more than 5% of the actual alkaloid, or even sometimes as little as1% . This illicit "heroin" is actually a salt of the base, namely the hydrochloride.

The impure heroine, containing small amounts of acetylcodeine and papaverine derived from the original opium, and of poorly acetylated heroine, containing more or less monoacetylmorphine and residual morphine.

CLASSIFICATION OF OTHER SUBSTANCES

The various substances that may be found in a heroin sample, besides diacetylmorphine, the substances may be classified as follows:

A. DILUENTS:

Generally lactose or powdered sugar, but sometimes mannitol or some other substance may be used.

B. ADULTERANTS:

While the diluents may also be called adulterants in the usual meaning of the term, it is convenient to distinguish the substances which are merely diluents from the drugs of more or less activity which are added either to conceal the comparative lack of real heroine, or to enhance its effect. **Quinine** is the most common adulterant and probably occurs in more than half the heroin samples in the United States and Canada. It is probably added, for one reason, *to enhance the bitter taste of the mixture, in case the addict tries to assay the strength by tasting*. It is most often added as the hydrochloride; sometimes as the sulfate. Some years ago, **procaine** was very commonly used. The amount of quinine

usually equals or exceeds that of heroine, but the procaine was only about one-sixth to one-third the amount of the heroine, and possibly it was used chiefly f*or its effect as a local anaesthetic*. Other adulterants that have been found are **barbiturates**, **caffeine**, **acetphenetidin**, **methadone**, and **amphetamine**.

C. IMPURITIES OF MANUFACTUR:

These include monoacetylmorphine and morphine, remaining from imperfect acetylation. The absence of such impurities may be significant of diversion from legal manufacture.

D. IMPURITIES OF ORIGIN:

These include codeine (present as acetylcodeine after the acetylation), papaverine, meconic acid, and brown coloring matter. The absence of these substances may not mean much, but their presence proves that a rather crude process was used, not only in the manufacture and purification of the heroine, but before that, in the extraction of the morphine.

LAB WORK

Lab work includes the qualitative and of heroin by color tests, Microscopically tests and TLC and quantitative analysis by HPLC.

◈ Colour Tests :

1. Marquis' reagent (Formaldehyde in concentrated or (6 + 1) H_2SO_4) -purple red changing to purple.
2. Frohde's reagent (Molybdate in concentrated H_2SO_4) strikes violet , quickly changing to strong purplish red, fading out to weaker brown or brownish, then developing green.
3. Mecke's (or Lafon's) reagent (Selenious acid in concentrated H_2SO_4) green,quickly greenish blue, changing to blue, slowly to bluish green with yellow-brown edge, then olivaceous green.

   Comment on tests 1 to 3: These sulfuric acid reagents provide highly characteristic colour tests for the spot-plate, and most adulterants and diluents do not seriously interfere. Rarely, a sample may contain so little heroine that charring of the diluent sugar with the concentrated acid will obscure the colours. Of course in that case, a separation is necessary. Imperfect acetylation does not matter as morphine and monoacetylmorphine give the same colours. The initial colour with the hydrochloride is slightly different from that with the free alkaloid or sulfate, bluer with Frohde's reagent and more yellow-brown with Mecke's; and this effect is increased if additional chloride is present.

4. Nitric acid-light yellow solution, gradually bright green. Concentrated HNO_3 is usually used, but a (4 + 1) acid is somewhat better (4 parts concentrated HNO_3 mixed with 1 part H_2O).

   Comment: This highly characteristic test is not very sensitive and often enough it may not be obtained on adulterated, diluted samples. Morphine gives an orange red color fading to yellow; sometimes with a little morphine present a red-orange is obtained at first and later the green of diacetylmorphine develops. Monoacetylmorphine is similar to morphine.

5. Copper test. To a little of the powder on the spot plate add several drops of water, 2 or 3 drops of 3% H_2O_2, a drop or two of NH 4OH and stir with a piece of copper. A pink to red color is produced. This test is given by various phenols and their acetyl derivatives including morphine and diacetylmorphine.

◈ Microscopic Tests :

1. Platinum chloride, H_2PtCl_6 . A little of the powder being examined is dissolved in a drop of water, or better in diluted acetic acid, on the microscpoe slide, and a drop of the 5% reagent solution is added, then crystals are looked for under the microscope. They form gradually and are needles in rosettes.The crystals grow larger and form blades in rosettes in the presence of acetic acid, which also diminishes the interface of quinine, and may be used up to (1+1) strength. (Lews, New York International Revenue Laboratory.)
2. Mercuric iodide in HCl. (The solvent solution contains about 27% by volume of concentrated HCl, or 10% by weight of HCl, and is saturated with Hgl_2) This reagent is applied to a little of the dry substance on the microscope slide. The crystals are branching threads and splinter-plates.

   Comment: The test is extremely sensitive and usually succeeds even with highly adulterated samples. The reagent can also be applied to the aqueous solution.

◈ Thin Layer Chromatography (TLC) :

Sample preparation :

- If the sample provided ,suspected to contain heroin, is in powdered from : It is boiled with dichloromethane (CH_2Cl_3) , Filtered then the residue (after evaporation of solvent) is dissolved in 1 ml $CHCl_3$:MeOH .
- If the sample is provided in solution , it should be alkalinizing (using NH_4OH) then extracted with CH_2Cl_2 as mentioned before.

Condition :

- Stationary phase : Silica gel 60 F_{254} (5X10)
- Mobile phase :MeOH (10ml) :NH_4OH (5 drops) OR 5% MeOH/$CHCl_3$ (using 4-5 drops)
- Detection : by UV at 254 nm then spray with Dragendorff's reagent
- References : Heroin 0.05% in $CHCl_3$/MeOH .

N.B. Heroin, distributed in the market is usually mixed with adulterants and diluents. The average purity of wholesale samples (45%) was only slightly higher than the purity of retail samples (30%). It may contain: Paracetamol, phenobarbitone, caffeine, which a can be detected easily on TLC plate by using references of these compounds.

Tests for adulterants :

1. Quinine or other fluorescent compounds. A little of the powder is dissolved in dilute sulfuric acid in a test tube or on the microscope slide and observed under ultraviolet light. Nupercaine also fluoresces strongly.
2. Sanchez test for procaine and other primary aromatic amines. A solution of furfural in acetic acid, applied to the dry powder on the spot plate, yields a bright red colour with any speck of procaine or other primary aromatic amine.
3. Chromium sulfate-chloride reagent for methadone. This reagent can be applied either to a drop of solution or directly to a little of the dry powder on the microscope slide, and will readily yield the methadone crystals even in the presence of much more heroine or quinine or both.

High Performance Liquid Chromatogarphy (HPLC)

Conditions:

Column : μ-porasil [Normal phase] (150 X 3.9 mm)
Mobile phase : 3% MeOH in $CHCl_3$.
Detector : UV at 254 nm
Flow rate : 2 ml/ min
CS : 1 cm / min
Volume injection : 20 μl

Procedure:

1. For standard curve:

Heroin is prepared in concentration (0.05%) in CH_2Cl_2 (0.5 mg/1 ml). Then dilute different volumes : 4, 3, 2, and 1 ml to each to 5 ml (in a volumetric flask) with CH_2Cl_2 , representing: 0.4, 0.3, 0.2, and 0.1 mg/ml.

Inject 20 ml, in duplicate, into the HPLC system. The standard curve is plotted using different concentrations of heroin (mg/1mg) versus area under the peak.

A linear relationship confirm the possible use of this curve for quantization.

2. For the Sample:

For determination of heroin in the sample provided : prepare 0.1 – 0.2 % in CH_2Cl_2 and inject 20μl, in duplicate, into the HPLC system. Obtain from the read-out sheat the area under the peak corresponding to the injected samples refer to the standard curve to calculate the percentage of heroin in the sample.

Opiates

Trade Names	Morphine, Heroin, Codeine, Hydromorphone, Hydrocodone
Classification	Narcotic analgesic
Physical/ Psychological Dependence:	Morphine-high; Heroin-high; Codeine-moderate; Hydromorphone-high
Methods of Administration:	Swallowed or injected
Physical Appearance:	White, brown, or black powder, liquids (injectable), tablets, capsules of various sizes and colors
Approximate Detection Time in Urine:	3 days
Clinical Effects/ Symptoms:	Euphoria, analgesia, drowsiness, respiratory depression

The Basics

Opiates are drugs such as heroin, codeine and morphine, which can produce high physical and/or psychological dependency by their users. Samples may be tested for codeine and morphine (a metabolite of both codeine and heroin), and follow-up testing for 6 acetylmorphine, also a metabolite of heroin, is allowed on morphine-positive samples.

Testing for the opiate hydrocodone (Vicodin, Lortabs), hydromorphone (Dilaudid), is performed on nonregulated samples. Feelings of euphoria, analgesia, drowsiness, and respiratory depression are reported by users.

Sample

Random urine specimen in a properly sealed and labeled urine container.

Method and Instrument

Preliminary screening for drugs of abuse in urine is performed by immunoassay, Bayer ADVIA 2400. Confirmation by Gas Chromatography/Mass Spectroscopy.

Stability

After proper collection, concentration of codeine, morphine or other opiates in urine will not change significantly for several days at room temperature, for several weeks at refrigerated temperature or indefinitely when frozen.

Purpose:

Urine positive indicates recent usage.

Normal Results

Negative.

Abnormal Results

The original immunoassay has been confirmed by GC/MS. The screening cutoff for opiates in regulated samples is 2000 ng/mL. The confirmation cutoff for either codeine or morphine in these samples is 2000 ng/mL. This concentration is consistent with ingestion of the analyte or the analyte-producing medication sometime within the 72-hour period preceding the urine collection.

Interferences

Though no compounds have been tested that cannot be separated from codeine and morphine by GC/MS analysis, there are numerous prescription medications which contain codeine. In addition, poppy seeds contain morphine and codeine in varying amounts, so careful evaluation of confirmed opiate-positive samples by an experienced professional can be essential in avoiding a false accusation of drug abuse.