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 introduced in 1898 as a 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 as 1%. This illicit "heroin" is actually a salt of the base, namely the hydrochloride.

The impure heroine, containing small amounts of acetylcocodeine 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 for
its effect as a local anaesthetic. Other adulterants that have been found are
barbiturates, caffeine, acethphenetidin, 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\textsubscript{2}SO\textsubscript{4}) -purple red
   changing to purple.
2. Frohde's reagent (Molybdate in concentrated H\textsubscript{2}SO\textsubscript{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\textsubscript{2}SO\textsubscript{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 HNO₃ is usually used, but a (4 + 1) acid is somewhat better (4 parts concentrated HNO₃ mixed with 1 part H₂O).

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% H₂O₂, a drop or two of NH₄OH 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, H₂PtCl₆. A little of the powder being examined is dissolved in a drop of water, or better in diluted acetic acid, on the microscope 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 HgI₂) 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 form: It is boiled with dichloromethane ($\text{CH}_2\text{Cl}_2$), filtered then the residue (after evaporation of solvent) is dissolved in 1 ml $\text{CHCl}_3$:MeOH.
- If the sample is provided in solution, it should be alkalinizing (using $\text{NH}_4\text{OH}$) then extracted with $\text{CH}_2\text{Cl}_2$ as mentioned before.

**Condition:**
- **Stationary phase**: Silica gel 60 F$_{254}$ (5X10)
- **Mobile phase**: MeOH (10 ml):NH$_4$OH (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 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 heroin or quinine or both.
**High Performance Liquid Chromatography (HPLC)**

**Conditions:**
- **Column:** µ-porasil [Normal phase] (150 X 3.9 mm)
- **Mobile phase:** 3% MeOH in CHCl₃
- **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 CH₂Cl₂ (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 CH₂Cl₂, 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 CH₂Cl₂ 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

The Basics

Opiates are drugs such as heroin, codeine and morphine, which can produce a very high physical and/or psychological dependence 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 in heroin-positive samples is allowed. Testing for the opiates, hydrocodone (Vicodin, Lortabs), hydromorphone (Dilaudid), is performed on nonregulated samples. Feelings of euphoria, analgesia, drowsiness, and respiratory depression are reported by Swallowed or injected.

Sample

Random urine

Physical Appearance:

White, brown, or black powder 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.