

EXPERIMENT NO: 7

DETERMINATION AND RECOGNITION OF NARCOTICS WITH THIN LAYER CHROMATOGRAPHY (TLC)

A) General Information:

Substance addiction is a common problem today, especially among young people. The main character of a substance addiction is the presence of a group of symptoms indicating that the person is constantly using the substance in spite of significant substance -related problems.

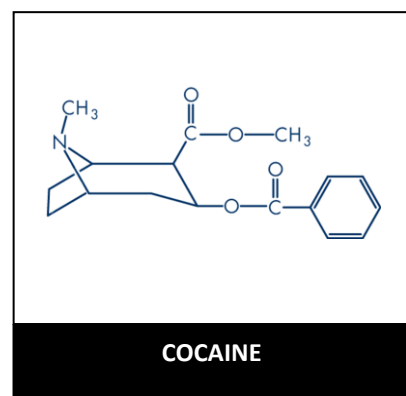
- Tolerance is defined as a person's diminished response to a substance that is the result of repeated use.
- Behavioral changes (= abstinence) are observed when the levels of substance in blood and tissue are decreased. To alleviate or avoid symptoms of withdrawal, the person needs to take the drug.
- The person takes the substance more amount or longer time than he or she intended.
- There are unsuccessful attempts to interrupt substance use.
- He/She expends a considerable amount of time to find, use, and get rid of its effects.
- His/her daily activities focus on substance.

In addition, the risk of transmission of disease agents such as hepatitis B, hepatitis C and HIV viruses during injector sharing in parenteral use should be kept in mind.

In our laboratory study, we will examine cocaine and opiates (eg morphine and codeine) from narcotics.

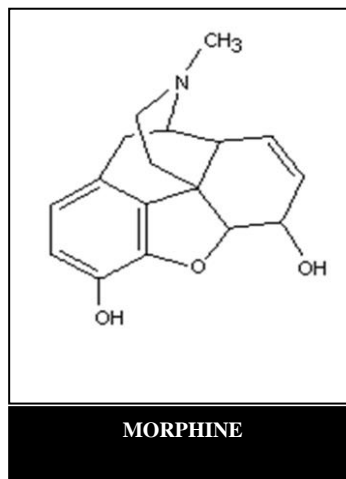
Cocaine

- Cocaine is obtained from the leaves of the Erythroxylon coca plant.
- Although it has local anesthetic properties, its use is not preferred due to its toxicity and misuse potential.
- In the case if abuse or dependence, cocaine is used as a HCl salt by inhalation or parenteral route. It is also used in the form of cigarette.
- It shows stimulating effect on brain cortex. The euphoria effect is stronger than other psychoactive drugs.
- Nasal septum perforation and sinonasal structural damages may also be the presenting signs of addiction in cocaine users.



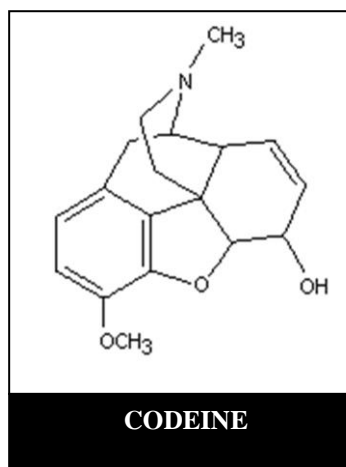
Morphine

- Opium is obtained by solidification of the leaked liquid by drawing the fruit capsules of the *Papaver somniferum*.
- Morphine is the major opium alkaloid.
- Morphine has strong analgesic effect; however, it is used in a controlled manner because of its addiction potential.
- It is usually administered by intramuscular or subcutaneous injection. Oral administration is also possible in patients with painful terminal cancer. The analgesic effect begins within 1-2 minutes when administered intravenously.
- Acute Poisoning Symptoms: Myosis, hypotension, hypothermia, coma, convulsions, respiratory depression.
- Death may occur due to respiratory depression.
- Naloxone (N-allyl normorphine) antagonizes the central toxic effects of morphine.
- It may cause bronchoconstriction and asthma attack in people with bronchial asthma because of causing histamine release.



Codeine

- Codeine is also an opiate alkaloid.
- Most of the licit morphine produced is used to make codeine by methylation.
- Codeine reveals the antitussive effect primarily via the μ -opioid receptor in the central nervous system (CNS). It is included in pharmaceutical formulations due to its antitussive effect.
- Can be used alone or in combination with non-narcotic analgesics in mild to moderate pain.
- Codeine can potentiate the effect of CNS depressants and alcohol.
- During the evaluation of laboratory findings, there is a point to consider: Morphine is one of the products formed during the metabolism of codeine and heroin (diacetylmorphine). The presence of metabolites in body fluids should be evaluated in order to determine whether morphine occurs during codeine or heroin metabolism.



Urine is most often the preferred test substance for narcotic substance screening and verification tests because of the ease of collection. Screening tests were performed by TLC and immunoassay. Verification tests were performed by gas and liquid chromatography and mass spectroscopy. Sensitivity in screening tests and specificity in validation tests are important.

B) Materials And Solutions Required For The Test:

- Standards: Morphine, Codeine, Cocaine (concentration: 20 mg / mL, in methanol)
- Samples
- Analytical balance
- Silicagel G
- Distilled water
- Graduated cylinder
- Erlenmeyer flask
- Spatula
- TLC plates
- Plaque preparation system
- Filter paper
- Oven
- Pasteur pipette, capillary or plastic pipette tip
- TLC tank
- Vaseline
- Carrier, template, ruler
- Fume hood

• Color reagent:

Dragendorf Reagent

(10mL Reagent A + 10 mL Reagent B + 20mL acetic acid + 100mL distilled water)

<u>Reagent A:</u>	Bismuth subnitrate in acetic acid solution:
	Bismuth subnitrate 2 g
	Acetic acid..... 25 mL
	Distilled water 100 mL

<u>Reagent B:</u>	Potassium iodide solution (in water)
	KI 40 g
	Distilled water 100 mL

Protect from the light. Resistant for two weeks at 4 ° C.

- **Solvent System:** Ethyl acetate: methanol: 25% ammonium hydroxide (85: 10: 5)

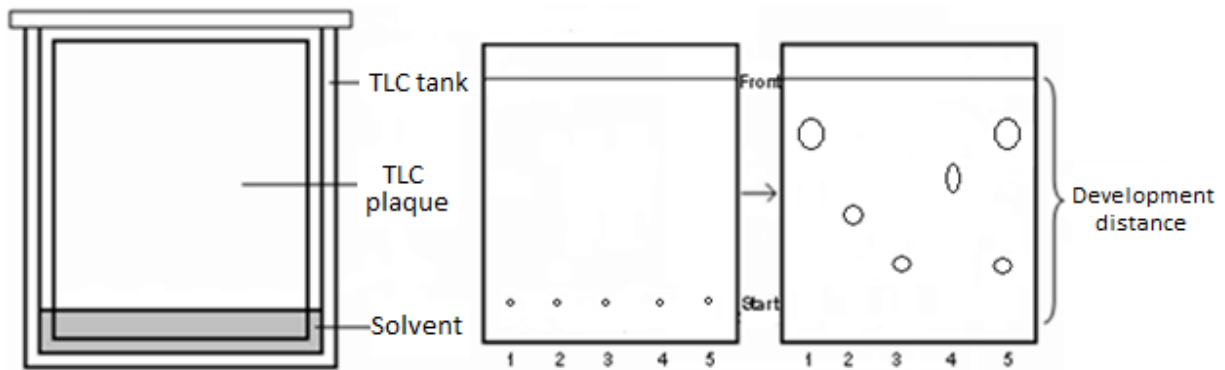
C) Experimental Procedure:

1. Plates are prepared in 250µm thickness with silicagel G and activated at 105 ° C for 1 hour.
2. Plaque is placed on the template. Front is drawn with the apparatus. The standards and sample are applied to plate. Be careful not to spread the sample excessively and not to damage the adsorbent (silica gel) during the application.
3. The plates are placed in the tank saturated with a mixture of ethyl acetate: methanol: 25% ammonium hydroxide (85: 10: 5). The development solution is expected to reach the front, and the development time is recorded.
4. The plates removed from the tank and are dried at room temperature under the fume hood.
5. Plates are sprayed with color reagent to make stains on the chromatogram visible. Application with color reagent should also be carried out on fume hood. Morphine, codeine and cocaine, in the presence of Dragendorf reagent gives orange color on yellow color background.

6. Retention factor (Rf) values are calculated.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

7. The calculated Rf values for the samples examined are compared with the standards and made a comment.



In the example given above, (1-4) standards; (5) sample.

It can be said that the sample contains substances (1) and (3).