

## **ALKALOIDAL DRUG ASSAY**

### **Alkaloids**

Alkaloids are the cyclic organic compounds normally with basic chemical properties, containing nitrogen in its negative oxidation state and occurring chiefly in many vascular plants and some fungi.

### **“ALKALOIDAL DRUG ASSAY”**

- Alkaloids are slightly or very slightly soluble in water but soluble in certain organic solvents immiscible with water such as chloroform, ether, amylalcohol and benzene or mixture of these. Salts of alkaloids however are usually practically insoluble in nearly all of the organic solvents. The process of assay by immiscible solvents, generally known as the “**shaking out**” process is based on these partitioning properties of alkaloids
- It is carried out by treating the drug or a concentrated liquid extract of it with a solvent immiscible with water, in the presence of an excess of alkali, which liberates the alkaloid. The immiscible solvent from which it is subsequently removed by means of an excess of dilute aqueous acid dissolves the free alkaloid
- The acid solutions are then extracted with an immiscible solvent in the presence of an excess of alkali, and immiscible solvent evaporated to obtain the alkaloid, which is either weighed or determined volumetrically.

The amount of alkaloids is calculated by multiplying the factor under individual monograph with the volume of standard acid used

### **PREPARATION OF DRUG FOR ASSAY “**

- Grind the drug to be extracted to a powder of fineness designated. Care should be taken to avoid the loss of water during the powdering of the drug.

### **WEIGHING FOR ASSAY**

- In weighing bulky, crude drugs for the assay, accuracy to within 10mg for quantities of 5g and over is sufficient.
- Portions of pilular (soft) extracts or ointments may be weighed on a tared piece of waxed paper and transferred in to the vessel containing the solvent. In transferring weighed portions to a separator, thoroughly rinse the vessel in which the material to be assayed was weighed, and add the rinsing to the separator.

### **EXTRACTION OF DRUGS”**

- Because of the structural diversity of alkaloids, there is no single method of their extraction from natural raw materials. Most methods exploit the property of most alkaloids to be soluble in organic solvents but not in water, and the opposite tendency of their salts.
- Most plants contain several alkaloids. Their mixture is extracted first and then individual alkaloids are separated.
- Plants are thoroughly ground before extraction. Most alkaloids are present in the raw plants in the form of salts of organic acids. The extracted alkaloids may remain salts or change into bases.

### **METHODS OF EXTRACTION”**

The alkaloid content of alkaloid bearing drugs is usually extracted by one of the following methods;

- **Maceration**
- **Percolation**
- **Continuous extraction**

**“MACERATION”**

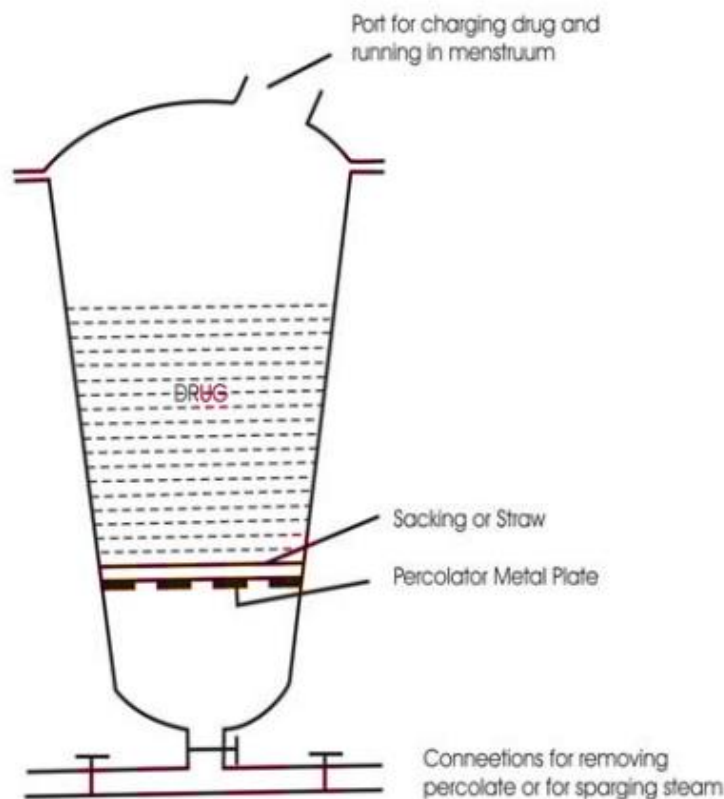
- The term maceration comes from the latin “macerare” which means “to soak”.
- It is a process in which the properly comminuted drug is permitted to soak in the menstrum until the cellular structure is softened and penetrated by the menstrum, as well as nearly all the constituents are dissolved in the menstrum.
- In the maceration process, the drug to be extracted is generally placed in a wide mouth container with a prescribed menstrum, the vessel is stoppered tightly, and contents are agitated repeatedly over a period usually ranging between 2 to 14 days.
- The agitation permits the repeated flow of fresh menstrum over the entire surface area of comminuted drug. An alternative to repeated shaking is to place the drug in a porous cloth bag i.e tied and suspended in the upper portion of menstrum, much the same as a tea bag is suspended in water to make a cup of tea. As the soluble constituents dissolve in the menstrum ,they tend to settle to the bottom because of an increase in the specific gravity of liquid due to its added weight. The extractive is separated from the marc by expressing the bag of drug and washing it with additional fresh menstrum, these washings being also added to the extractive. If maceration is performed with loose drug then marc may be removed by straining or filtration.
- **Maceration Process for Organized and Unorganized Crude Drugs**
- Organized drugs have a defined cellular structure whereas unorganized drugs are non-cellular. Bark and roots are examples of organized crude drugs, while gum and resin are unorganized crude drugs. The processes of maceration for organized and unorganized drugs are slightly different, as shown in Table 1.
- **Table 1: Four differentiating steps of the maceration process, for organized and unorganized crude drugs**

| <b>Organized drugs</b>  | <b>Unorganized drugs</b>   |
|---|--|
| ▪ (i) Drug + entire volume of menstrum  | (i) Drug + four-fifths of menstrum (in most cases)                   |
| ▪ (ii) Shake occasionally for 7 days  | (ii) Shake occasionally on days 2 to 7, as specified                 |
| ▪ (iii) Strain liquid, press the marc   | (iii) Decant the liquid. Marc is not pressed                         |
| ▪ (iv) Mix the liquids, clarify by subsidence for Filtrate is not adjusted for volume | (iv) Filter the liquid and add remaining menstrum through the filter |

**PERCOLATION**

Organized vegetable drug in a suitably powdered form. Uniform moistening of the powdered vegetable drugs with menstrum for a period of 4 hours in a separable vessel (Imbibition). Packed evenly into the percolator. A piece of filter paper is placed on

surface followed by a layer of clean sand so that top layers of drugs are not disturbed. Sufficient menstruum is poured over the drug slowly and evenly to saturate it, keeping the tap at bottom open for passing of occluded gas to pass out. Sufficient menstruum is also added to maintain a small layer above the drug and allowed to stand for 24 hours. After maceration, the outlet is opened and solvent is percolated at a control rate with continuous addition of fresh volume. 75% of the volume of the finished product is collected. Marc is pressed and expressed liquid is added to the percolate giving 80% to 90% of the final volume. Volume is adjusted with calculated quantities of fresh menstruum. Evaporation and concentration to get finished products by applying suitable techniques and apparatus.

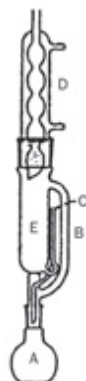


Commercial scale (about 1 ton capacity) percolator

### **CONTINUOUS EXTRACTION**

In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of

time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.



**Soxhlet apparatus**

### **Purification**

The extraction of the alkaloid from the bulk of the crude alkaloid solution in immiscible organic solvent is invariably carried out by shaking with an acid solution. In usual practice, the use of HCl is restricted when chloroform remains as the solvent because of the fact that quite a few alkaloidal hydrochlorides are distinctly soluble in the latter. However, dilute  $H_2SO_4$  is always preferred over HCl for general use in the extraction of alkaloids. Subsequently, the acid solution is rendered alkaline with dilute  $NH_4OH$  solution to liberate the alkaloids which is then extracted with an organic solvent. The solvent is removed under reduced pressure and the traces of moisture is removed with anhydrous sodium sulphate.

### **DETERMINATION OF ALKALOIDS**

- Evaporate the solution of the purified alkaloids in the immiscible solvent to dryness on a steam bath or with a current of air. When the alkaloid residue is to be determined volumetrically. Soften it by the addition of about 1ml of neutralized alcohol or ether; add an accurately measured volume of the standard acid. Equivalent to about one and one half to two times the volume estimated for the quantity of alkaloid present. Warm the mixture gently to ensure the complete solution of the alkaloid. If preferred. Dissolve the alkaloid residue in chloroform. Add the standard acid of higher normality and remove the chloroform completely by evaporation. Then add a sufficient quantity of water to make the volume of the mixture measure at least 25 ml. Titrate the excess of acid with standard alkali, using the appropriate indicator. If the alkaloidal residue is to be weighed, dry it at  $105^\circ C$  to a constant weight. If the solvent has been chloroform remove last traces of the solvent by addition of 5 ml of neutralized ether or alcohol followed by evaporation.

### **INDICATOR**

Unless otherwise specified in the individual monograph use methyl red TS as the indicator in volumetric determinations and for standardizing volumetric solutions.

