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# EXTRACTION OF DRUGS FROM BIOLOGICAL MATERIALS SUCH AS VISCERA, BLOOD, URINE AND VITREOUS HUMOUR

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**ABSTRACT:** Sample preparation is a basic step before any chemical or instrumental analysis. Liquid-liquid extraction is one of the basic steps for extraction of drug from any biological matrix such as blood, vitreous humour, urine etc. Among the highly sophisticated extraction procedures like solid phase extraction (SPE), solid phase micro extraction (SPME), liquid phase extraction (LPE), liquid phase micro extraction (LPME) and distillation, liquid-liquid is still the basic preferred method. The paper explains the basic extraction procedure for extraction of acidic, basic and neutral drugs in variety of biological matrix. Other techniques have disadvantage of being costly because it requires specific infrastructure; chemicals of high purity and expertise while handling. The advantage of using liquid –liquid extraction over other procedures is that it can be attempted with basic laboratory setup.

**KEYWORDS**: Extraction, sample preparation, biological matrix.

## INTRODUCTION:

Extraction is a very common laboratory procedure using for isolating or purifying a product. Extraction methods are used to extract the different substance like various types of drugs, poisonous substances like insecticide, pesticide, rodenticides, herbicides, alcohol, vegetable poison, plant poison and alkaloids etc from the biological samples /spiked sample/post mortem sample like body fluids and viscera. Extraction can be done by various methods/techniques like solid phase extraction (SPE), solid phase micro extraction (SPME), liquid-liquid extraction (LLE), liquid phase extraction (LPE), liquid phase micro extraction (LPME) and distillation. Distillation is the process of making steam of volatiles poisons and distillate is collected at different volume for analysis of different volatile poisons. Extraction methods like solid phase and micro extraction are highly expensive but less time consuming. Technique like solvent extraction, liquid –liquid extraction is also called partitioning separation. This is economical and easy to perform. Before going for the liquid –liquid extraction (LLE) sample preparation method are done by methods like deproteinization, hydrolysis and digestion. By these methods the coloured sample are converted into the decolorized form by filtering. Liquid–liquid extraction is also known as solvent extraction and partitioning, separation of compounds based on their relative solubility in two different immiscible liquids, usually water and an organic solvent. The extraction of the analyte is based on the

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transfer of a solute substance from one liquid phase into another liquid phase according to the solubility. The extraction solvents are organic, volatile and miscible into another organic but immiscible in the aqueous layer and the two layers are distinctly separated. The organic layer is passed through anhydrous sodium sulphate to remove impurity[1-3]. The drugs/poison which dissolve into the organic solvent are left for evaporation thus the pure drug/poison is extracted[4].

## MATERIALS AND METHOD:

1. **Glassware:** 100 ml Beaker, conical/round bottom flask, separating funnel, funnel, evaporating bowl, measuring cylinder, Florisil column etc.

2. **Chemical and reagents:** Anhydrous sodium sulphate, anhydrous ammonium sulphate, acetic acid, diethyl ether, sodium tungstate, ammonium hydroxide, chloroform etc. All the chemical and reagent should be of analytical grade.

3. **Miscellaneous:** Filter paper, scissor, tripod stand etc.

4. **Extraction of drugs from viscera** [5-7]

**Pre-treatment**

A 50 g tissue was taken in the 100 ml beaker which was cut into small pieces and macerated properly. The material was then transferred to the conical flask/round bottom flask with 10 g of anhydrous sodium sulfate and 10 ml of glacial acetic acid. This conical flask/round bottom flask was placed on boiling water bath at 60$^{o}$C for three to four hours. Contents are then cooled and filtered using filter paper. The filtrate was used for extraction of different drugs such as acidic, basic and neutral drugs as per following method.

**Acidic extraction**

The filtrate is transferred to separating funnel and 50 ml diethyl ether was added to it and shaken for 5-10 minutes. The ether layer (organic layer) was separated (AE1). The aqueous layer was again extracted with 40 ml diethyl ether and 30 ml diethyl ether respectively. The ether layers were separated (AE2 and AE3). All three organic layers AE1, AE2 and AE3 were pooled and passed through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through Florisil column).

**Basic extraction**

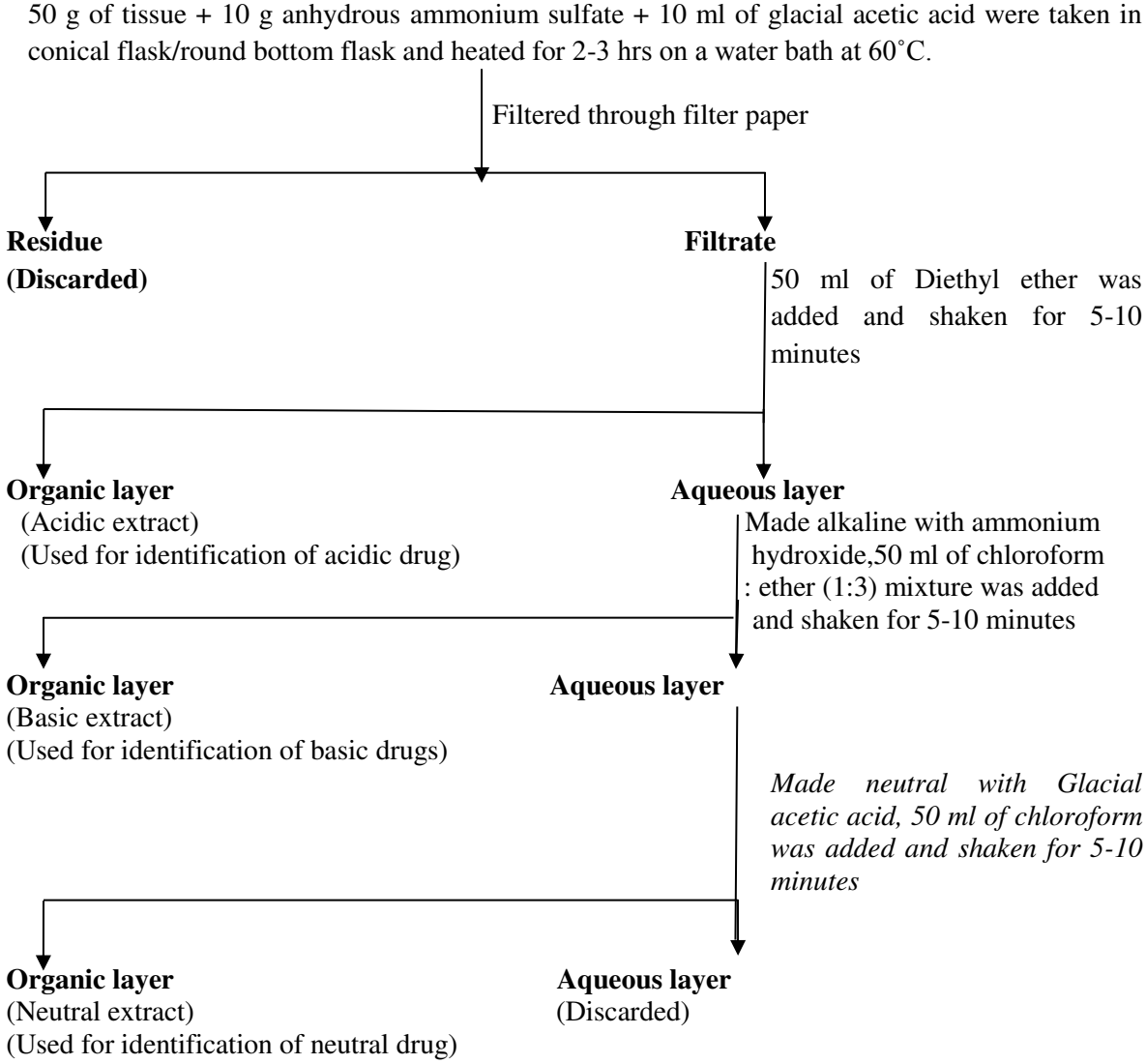
The aqueous acidic layer obtained after acidic extraction was made alkaline by adding ammonium hydroxide (pH should be approximately 9-10) in a separating funnel and extracted with 50 ml of ether: chloroform (3:1) mixture and shaken for 5-10 minutes. The ether layer was separated (BE1). The aqueous layer was again extracted with 40 ml and 30 ml ether: chloroform (3:1) mixture respectively. The ether layers were separated (BE2 & BE3). All three organic extract BE1, BE2 and BE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Neutral extraction**

The aqueous basic layer obtained after basic extract was neutralized by adding glacial acetic acid (pH should be approximately 7) in a

separating funnel and extracted with 50 ml of chloroform and shaken for 5-10 minutes. The chloroform layer was separated (NE1). The aqueous layer was again extracted with 40 ml and 30 ml chloroform respectively. The ether layers were separated (NE2 & NE3). All three organic extract NE1, NE2 and NE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel , then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Flow chart 1: Extraction process of drug from viscera**



## 5. Extraction of drug from Blood [8-9]

### Pre-treatment

A 10ml of blood was taken in the conical flask with 100 mg of sodium tungstate and 1 ml of conc. Sulfuric acid was added and mixed. This mixture was then heated for 2-3 minute at 60˚C. Contents were then cooled and filtered using filter paper. The filtrate was used for extraction of different drugs such as acidic, basic and neutral drugs as per following method.

### Acidic extraction

The filtrate was added to separating funnel and 25 ml of diethyl ether was added to it and shaken for 5-10 minutes. The ether layer (organic layer) was separated (AE1). The aqueous layer was again extracted with 20 ml diethyl ether and 15 ml diethyl ether respectively. The ether layers were separated (AE2 and AE3). All three organic layers AE1, AE2 and AE3 were pooled and passed through a pad of anhydrous sodium sulfate over a funnel , then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

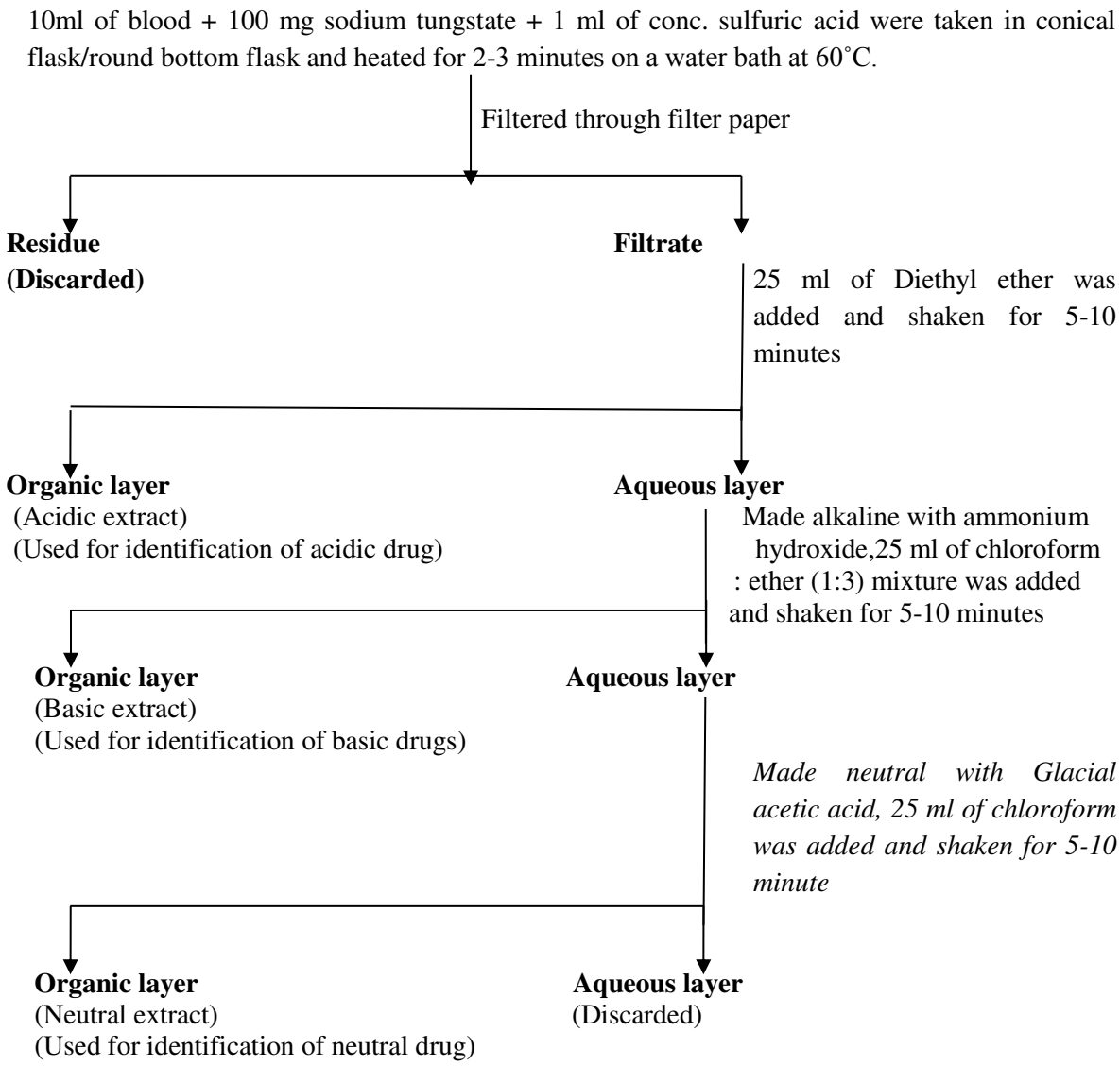
### Basic extraction

The aqueous acidic layer obtained from acidic extraction was made alkaline by adding ammonium hydroxide (pH should be app- 8-10) in a separating funnel and extracted with 25ml diethyl ether: chloroform (3:1) mixture and shaken for 5-10 minutes. The ether layer was separated (BE1). The aqueous layer was again extracted with 20 ml and 15 ml ether: chloroform (3:1) mixture respectively. The ether layers were separated (BE2 & BE3). All three organic extract BE1, BE2 and BE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel , then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

### Neutral extraction

The aqueous basic layer was made neutral by adding glacial acetic acid (pH should be app-7) in separating funnel, extracted with 25 ml of chloroform and shaken for 5-10 minutes. The chloroform layer was separated (NE1). The aqueous layer was again extracted with 20 ml and 15 ml of chloroform respectively. The chloroform layers were separated (NE2 & NE3). All three organic layers extract NE1, NE2 and NE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column)

**Flow chart 2: Extraction process of drug from blood**




## 6. Extraction of drug form Urine[8-9]

### Acidic extraction

The 10 ml urine sample was made acidic by adding phosphoric acid or tartaric acid in to it then it is added to separating funnel and 30 ml of diethyl ether was added to the separating funnel and shaken for 5-10 minutes. The ether layer (organic layer) was separated (AE1). The aqueous layer was again extracted with 25 ml diethyl ether and 20 ml diethyl ether respectively. The ether layers were separated (AE2 and AE3). All three organic layers AE1, AE2 and AE3 were pooled and passed through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

### Basic extraction

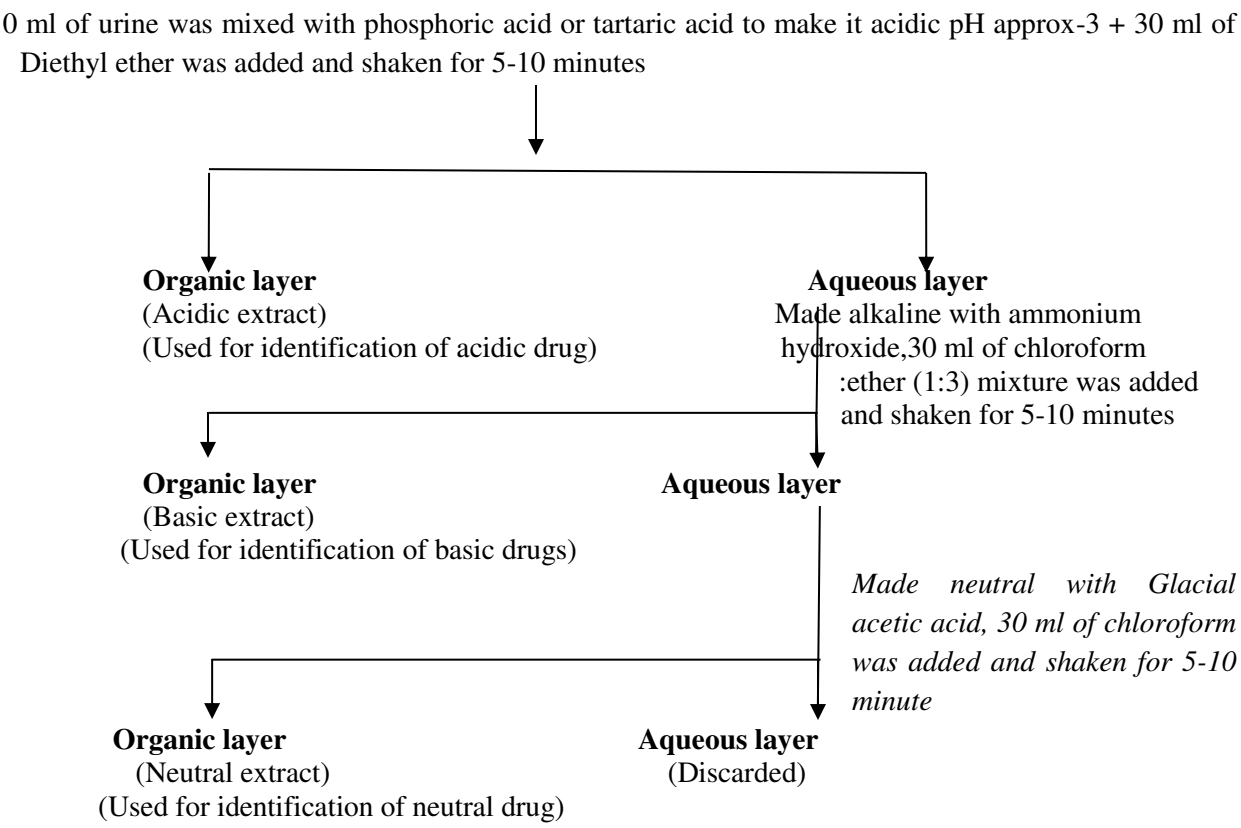
The aqueous acidic layer obtained from acidic extraction was made alkaline by adding ammonium hydroxide (pH should be app- 8-10) in a separating funnel and extracted with 30 ml

diethyl ether: chloroform (3:1) mixture and shaken for 5-10 minutes. The ether layer was separated (BE1). The aqueous layer was again extracted with 25 ml and 20 ml ether: chloroform (3:1) mixture respectively. The ether layers were separated (BE2 & BE3). All three organic extract BE1, BE2 and BE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Neutral extraction**

The aqueous basic layer obtained after basic extract was neutralized by adding glacial acetic acid (pH should be approximately 7) in a separating funnel and extracted with 30 ml of chloroform and shaken for 5-10 minutes. The chloroform layer was separated (NE1). The aqueous layer was again extracted with 25 ml and 20 ml chloroform respectively. The ether layers were separated (NE2 & NE3). All three organic extract NE1, NE2 and NE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Flow chart 3: Extraction process of drug from urine**




**7. Extraction from Vitreous Humour fluid**[8-9]

No pre-treatment is required for this fluid because most of the part of this fluid is water.

**Acidic Extraction**

The 4 ml vitreous humour sample was added to separating funnel and 15 ml of diethyl ether was added to the separating funnel and shaken for 5-10 minutes. The ether layer (organic layer) was separated (AE1). The aqueous layer was again

extracted with 10 ml diethyl ether and 10 ml diethyl ether respectively. The ether layers were separated (AE2 and AE3). All three organic layers AE1, AE2 and AE3 were pooled and passed through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

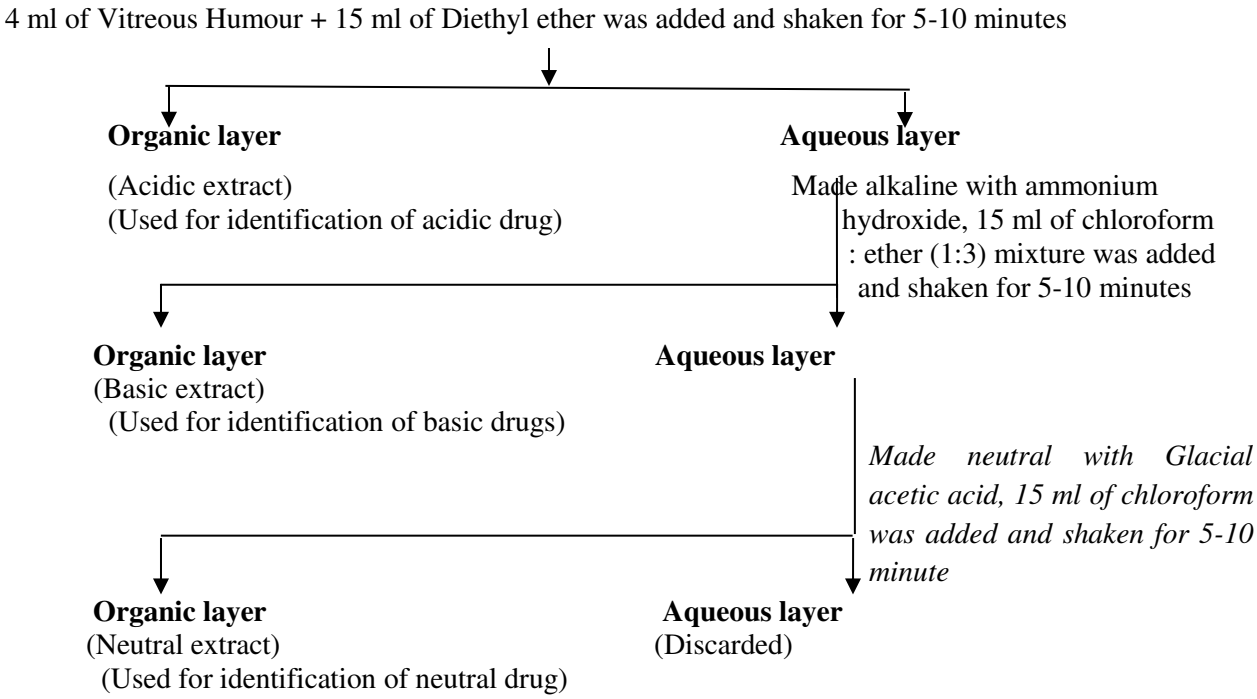
**Basic Extraction**

The aqueous acidic layer obtained from acidic extraction was made alkaline by adding ammonium hydroxide (pH should be app- 8-10) in a separating funnel and extracted with 15 ml diethyl ether: chloroform (1:3) mixture and shaken for 5-10 minutes. The ether layer was separated (BE1). The aqueous layer was again extracted with 10 ml and 10 ml ether: chloroform (3:1) mixture respectively. The ether layers were separated (BE2 & BE3). All three organic extract BE1, BE2 and BE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel, then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Neutral Extraction**

The aqueous basic layer obtained after basic extract was neutralized by adding glacial acetic acid (pH should be approximately 7) in a separating funnel and extracted with 15 ml of chloroform and shaken for 5-10 minutes. The chloroform layer was separated (NE1). The aqueous layer was again extracted with 10 ml and 10 ml chloroform respectively. The ether layers were separated (NE2 & NE3). All three organic extract NE1, NE2 and NE3 were combined and pass through a pad of anhydrous sodium sulfate over a funnel , then evaporated to dryness and used for TLC (for instrumentation purpose organic layer should be passed through florisil column).

**Flow chart 4: Extraction process of drug from vitreous humour**



## CONCLUSION:

In the toxicological analysis the most important step is the sample preparation. The result of analysis is depending on the quantity and purity of sample extracted. There is no single method of extraction which can be applicable to all drugs/poisons, so every drug has to be separated using suitable extraction method. These extraction techniques have been developed after repeated trial and testing. These techniques are being improved as time passes. It is important for forensic toxicologist to known the best available method for detecting the drugs in biological sample. The drugs classified as acidic, basic and neutral easily get separated by these procedures from the Blood, Urine, vitreous Humour and Viscera.

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**CONFLICT OF INTEREST**: Authors declared no conflict of interest