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Development of a New Methodology for Extraction and Analysis of Pesticides, Drugs, Heavy Metals and Various Toxins from Viscera Samples

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Abstract

Viscera analysis plays a major and important role in forensics, Scientists use their own tried and tested methods to avoid excess time consumption and errors, to analyse the viscera samples for the presence of various illicit drugs, pesticides, heavy metals, or any other poisonous substances that may be the reason of death. In this article, the current scenario of viscera handling in forensic science laboratories in India is mentioned in detail with a step-by-step procedure. Tests for phosphine and various pesticides are performed in the stomach and small intestine. Drugs are extracted according to different procedures depending upon the affinity of the drug i.e., acidic, basic, or neutral. After that, Tests for drugs and other toxic substances are usually performed in blood, liver, spleen, and kidney. Blood usually comes in the third jar, and is tested for drugs, alcohol, and maybe for pesticides, phosphine and for any other suspected toxin. Viscera sample needs to be digested before the analysis of heavy metals. Any part of the viscera can be digested and tested for heavy metal. Usually, a mixture of all the viscera parts is digested and analysed. The techniques mentioned in this article are rigorously used in the chemistry and toxicology division in forensic laboratories in India.

Keywords: Drugs, Heavy Metals, Pesticides, Poison, Toxicology, Viscera

1. Introduction

Viscera analysis plays a major and important role in forensics, Scientists use their own tried and tested methods to avoid excess time consumption and errors, to analyse the viscera samples for the presence of various illicit drugs[1,2], pesticides[3,4], heavy metals[5,6] or any other poisonous substances that may be the reason of death[7]. From time to time, from laboratory to laboratory, the methods of handling viscera samples[8,9] change, depending upon the forensic scientist handling and assessing the cases. With the basic methodology remaining the same,

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one can vary the number of samples, solvents taken, and steps performed and can also modify it to get ease in manpower, time, and cost efficiency.

Viscera exhibits[9] majorly include the stomach, small intestine, liver, spleen, kidney, and blood samples, and in some special poisoning cases, other organs can be there too, packed and labelled separately. This research article discusses the current practices followed for handling and analysis of viscera[10,11]. Tests for phosphine and various pesticides[12] are performed in the stomach and small intestine. Drugs are extracted according to different procedures depending upon the affinity of the drug[11,13] i.e., acidic, basic, or neutral. After that, tests for drugs and other toxic substances are usually performed in the blood, liver, spleen, and kidney. Blood usually comes in the third jar, and is tested for drugs, alcohol, and maybe for pesticides, phosphine and for any other suspected toxin. Viscera sample needs to be digested before the analysis of heavy metals. Any part of the viscera can be digested and tested for heavy metal. Usually, a mixture of all the viscera parts is digested using a microwave digester system[14] and analysed. Sophisticated analytical instruments like LC-MS, HPLC, GC, GC-MS, UV and IR Spectrophotometers, etc., are used for viscera analysis depending upon the necessity. Viscera preservation guidelines in the Indian scenario are given as a book chapter[15], but no discussion or protocols of visceral analysis were given. Only case studies, after receiving viscera reports[16,17], Sample preservation protocols[15,18,19], and false positive vs. false negative guidelines, can be seen in the literature, but none was found to be about how to analyze viscera samples. This research article aims for the development of a new protocol and methodology to isolate, extract and analyze pesticides, drugs, heavy metals and various toxins from viscera samples.

2. Viscera Examination

Cases involving Viscera, any biological sample analysis, or Arson are mainly handled in the forensic chemistry and toxicology division, in any forensic laboratory.

2.1 Examination of Case Exhibits

A physical examination of the case exhibit is done i.e., the whole of the box and jars are checked so that there is no leakage or signs of rotting on it. After that, details mentioned in the case exhibit are matched with the details on the case examination forms, etc.

2.2 Case Opening

Case exhibits usually come in a corrugated box/wooden box/metallic box (Figure 1), enclosing 4 jars inside it. Two of them are big jars, and the other two are small ones. Jars could be of glass or plastic, depending on the hospital from which the viscera have been sent. The large jars contain the whole of the stomach, small intestine, liver, spleen, kidney, blood, and a preservative i.e., usually a saturated salt solution or solid salt or could be rectified spirit for the future preservation of the viscera (Figure 2).

Jar 1 (one of the big jars) contains the stomach and small intestine which is named 1; Jar 2 (the other big jar) contains the liver, spleen, and kidney which is named 2; Jar 3 (one of the small jars) blood and named as 3 and Jar 4 (the other small jar) contains preservative, and it is named as 4, respectively.

Viscera exhibit is divided into 4 different parts namely 1, 2, 3, and 4.

2.3 Viscera Exhibits and Analysis

2.3.1 Analysis of Jar 1

1 - Stomach and Small Intestine

Typically, Jar 1 is used to test for all sorts of pesticides (including Organochlorine, Organophosphorus (with and without the group), Carbamates, and Pyrethroids), phosphine gas, or any ingested substance that may induce fatality[3,4,20], or acute death. A sudden death can occur within twenty-four hours of the first sign of illness, even in the absence of any illness or previous medical history. These tests are performed in Jar 1 because substances that would have resulted in a sudden or abrupt death would not have been metabolized and would not be present



Figure 1. Sealed corrugated box with sample seal.

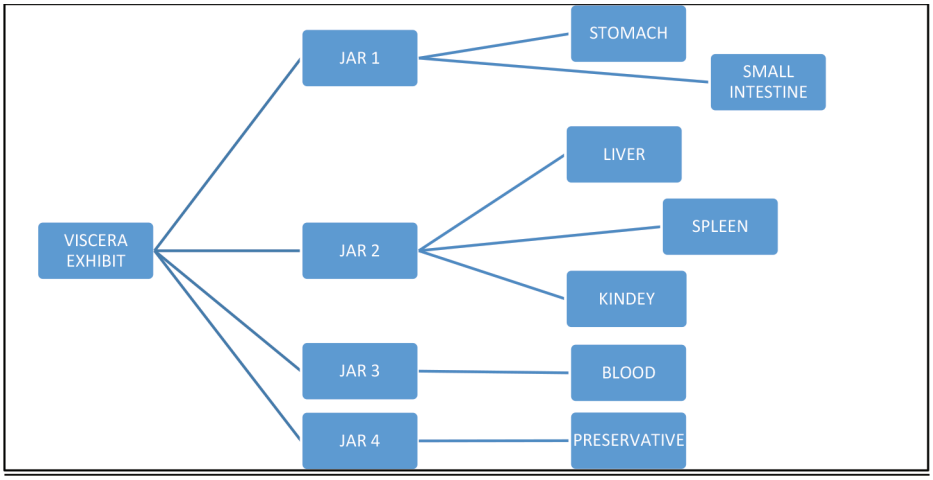


Figure 2. Viscera exhibits.

in the stomach or the small intestine. As a result of the fact that the liver is responsible for metabolizing any foreign material that is consumed or absorbed and that the kidneys are responsible for excreting it, prescribed or illicit drugs are not usually identified in Jar 1 testing; rather, they are more likely to be found in Jar 2. Even though tests for any such toxin can be performed on any biological material, these tests are conducted on specific parts, organs, or samples where there is a high likelihood of discovering the toxin.

The following steps are taken during the opening of the exhibit:

- First, the contents of Exhibit Jar 1 (the stomach and small intestine) are removed and placed in a large surgical plate that has been previously sterilized and rinsed with 4 (the saturated salt solution).



Figure 3. All Viscera separated after opening the case.

- The pH of the sample of viscera is then assessed.
- Next, the stomach and small intestine are divided into three equal pieces, and the three pieces are then placed in three conical flasks labelled, Phos, Pest, and Spare (which stands for tests for phosphonate gas, pesticides, and one saved for future uses, i.e., Spare) (Figure 3).
- The apparatuses are all labelled, 1A Phos, 1B Pest, and 1C Spare as appropriate.

1 is further divided into 3 parts:

- 1A Phos
- 1B Pest
- 1C Spare

2.3.1.1 1A Phos

In this section of 1, the Phosphine gas (PH_3) test is conducted. A colourless gas, phosphine is very hazardous even at deficient concentrations[21,22]. In India's rural markets, phosphide salts are typically found as pesticides under the trade names: Celphos and Quickphos, which typically include aluminium phosphide and zinc phosphide[23]. When taken orally, whether intentionally or unintentionally, it combines with the stomach's HCl and water to form phosphine gas. The lungs' epithelium absorbs phosphine from the circulation after it is generated in the gastrointestinal tract[23,24]. Oxyhemoglobin and other enzymes necessary for humans to breathe and break down foods are denaturized by phosphorus, and it may also have negative effects on cellular membranes. A lethal dose of Phosphine is approximately 150-500 mg for an average 70 kg human[23].

The interaction of phosphine gas with mercury bromide (HgBr) transforms HgBr's colour into parrot-

yellow, which is used to detect the gas. As heated viscera contain many contaminants and must be passed through lead acetate to release phosphine gas, this might interfere with the test's ability to produce the right colour[20].

Preparations:

- Two (02) Saturated solutions are prepared, one of Lead acetate in water and the other of Mercuric Bromide (HgBr) in Ethanol.
- Whatman (02) Whatman filter papers were taken; each filter paper was rinsed with a single solution and then left for about 2-3 hours for drying.



Figure 4. Positive test for Phosphine Gas.

Procedure for Phos test

- Add 0.5 mL of concentrated HCl to the flask.
- The conical flask's mouth was lined with lead-acetate Whatman filter paper, followed by filter paper washed with Mercury bromide.
- Using a thread or a rubber band, both filter papers were snugly secured at the lip of the conical flask.
- Heat the flask until the solution begins to boil.
- The heating of the flask is halted once the solution begins to boil.
- The conical flask is left at room temperature for 8 hours to cool.
- A Parrot-Yellow hue will appear on the filter paper rinsed with HgBr if phosphine gas is present in the sample[20] (Figure 4).

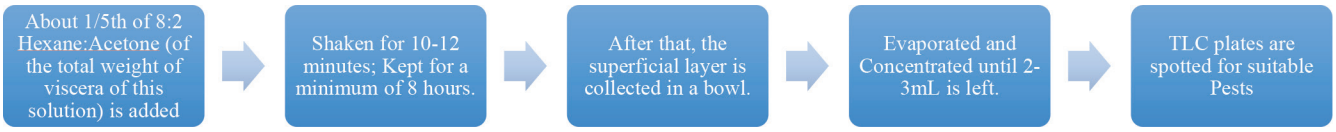
2.3.1.2 1B Pest

In this section of 1B Pest, a test for pesticides is conducted. Any chemical used to eradicate undesirable pests, rodents, germs, fungi, weeds, etc. is considered a pesticide. The test is done to check for pesticide consumption of any sort in cases of suicide, homicide, or accidental poisoning. Pesticides are widely available in local stores, both in rural and urban regions, for agricultural and domestic use[12].

Pesticides are of 4 types:

- Organochlorine Pesticides[25,26] (Examples: DDT, Endosulfan, Endrin),
- Organophosphorus Pesticides[27] (Examples: Parathion, Dichlorvos, Chlorpyrifos, Malathion),
  - with thio group
  - without thio group
- Carbamates[28] (Examples: Aldicarb, Carbaryl), and
- Pyrethroids[29] (Examples: Permethrin, deltamethrin, cypermethrin).

Procedure



TLC Solvents for Pesticides

Solvent systems are used for the analysis of different types of pesticides[30] (Table 1).

Pesticides with suitable spray reagents

- Organochlorine Pesticides: Zinc-chloride with Diphenylamine is used as a spraying reagent, and then the TLC plate is exposed to UV-336nm in a UV Chamber[25] (Figure 5).
- Organophosphorus Pesticides:
  - With Thio-Group: 4,4-nitrobenzylpyridine is used as a spraying reagent and then the TLC plate is

Table 1. Solvent systems used for different types of Pesticide

Pesticides	Solvent System
Organophosphorus	• Hexane: Acetone (8:2) • Chloroform: Acetone (7:3)
Organochlorine	• Hexane: Acetone (9:1) • Cyclohexane: Acetone (8:2)
Carbamate	• Hexane: Acetone (8:2) • Chloroform: Acetone (7:3)
Pyrethroids	• Petroleum Ether: Diethyl Ether (9:1) • Cyclohexane: Toluene (7:3) • Hexane: Acetone: Acetic Acid (25:25:1) • Hexane: Chloroform: Acetic Acid (9:5:0.5)

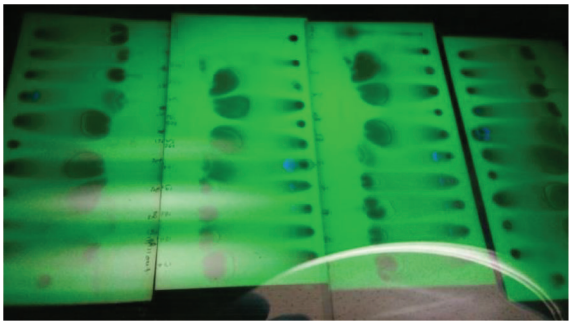


Figure 5. TLC being observed under UV Lamp.



Figure 6. TLC spray chamber.

heated at 120 degrees Celcius for 5-10 min. and then again sprayed with tetraehtylpentamine[27].
- Without Thio-Group: Palladium chloride is used as a spraying reagent[27].

- Carbamates Pesticides: Tollen's Reagent is used as a spraying reagent[28].
- Synthetic Pyrethroids: Silver nitrate is sprayed over TLC and then TLC is heated at 120 degrees Celcius[29] in a TLC Visualisation Chamber (Figure 6), Black spots are seen.

2.3.1.3 1C Spare

The contents within this conical flask (1C Spare) are preserved without any alterations. This allows for the possibility of repeating the tests on this specimen in the event of ambiguity or a false positive or negative outcome.

2.3.2 Analysis of Jar 2

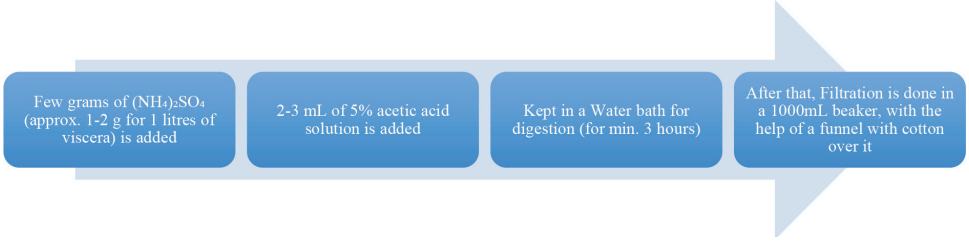
2 - Liver, Spleen, and Kidney

The liver, spleen, and kidney are kept in Jar 2. Drug tests are typically performed in this part of the viscera exhibit. Even though drugs can be tested in any of the visceral exhibitions. However, as drugs are only processed and expelled through the liver and kidney, there is an excellent chance of discovering drugs, and particularly their metabolites, in Jar 2. Both acutely and chronically abused medications can be found in this exhibit. Some medicines may stay up to 4-5 days in a non-habitual user's system, while others can take months to eliminate from the body. Drug tests can also be performed in 1, however, this is normally not chosen due to the significant risk of drug skipping[11].

The following steps are taken during the opening of the exhibit:

- A Large surgical plate is cleaned with ultrapure/distilled water and then again rinsed with contents of Jar 4 (preservative solution).
- Jar 2 is poured on the surgical plate.
- Equal halves the liver, spleen, and both kidneys are cut into 2 equal halves. Half of the cut portion is kept back in the original viscera jar in which it came as a specimen, and the other part is kept in a 1000 mL beaker for further analysis.

Procedure

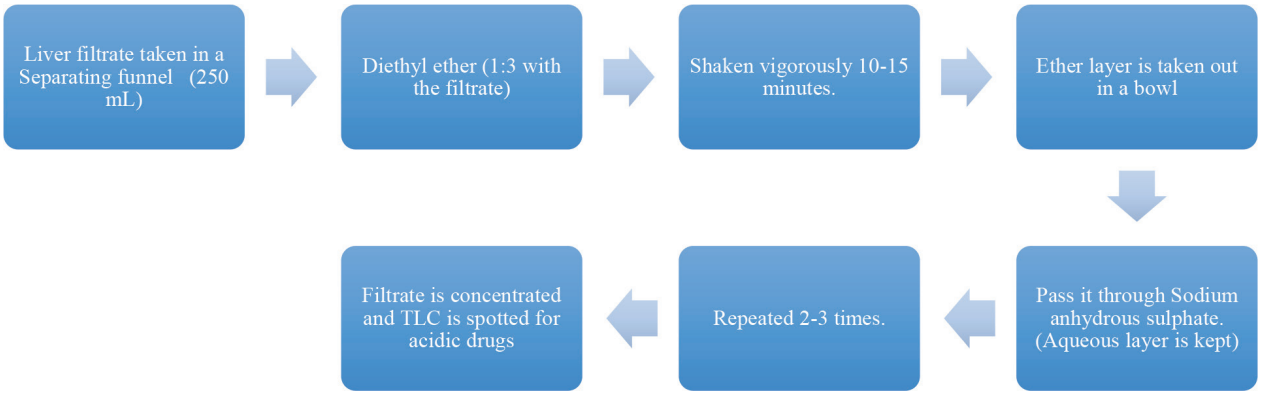


Extraction procedure

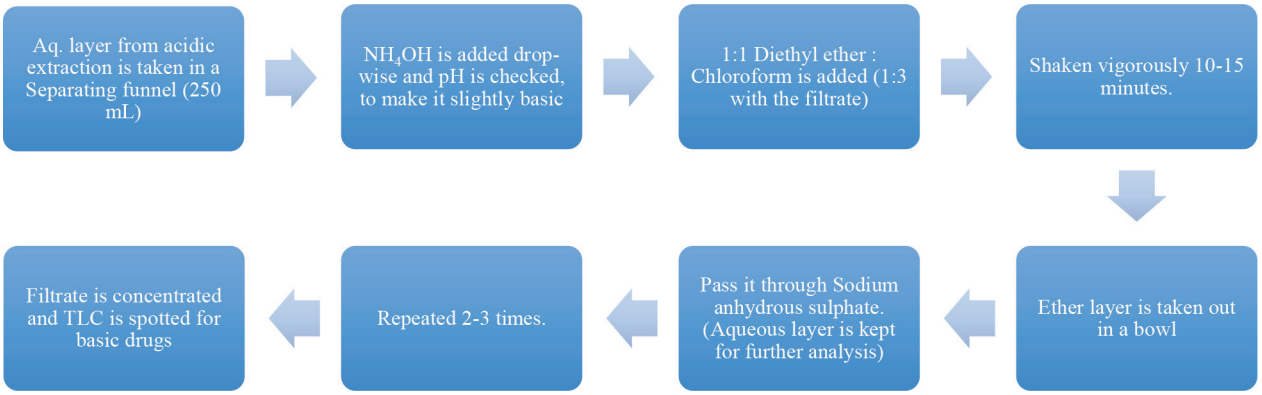
Numerous medications are sensitive to pH, which means that certain of them can only be found in acidic, basic, and neutral environments, respectively. The extraction process is carried out by the drug's pH sensitivity, which must be extracted[11].

The liver filtrate is obtained, and it is then processed further using different extraction techniques (Figure 7). The extracted liquid is next processed via HPTLC or another chromatographic process like GC-MS, GC-HS-MS, LC-MS, etc.

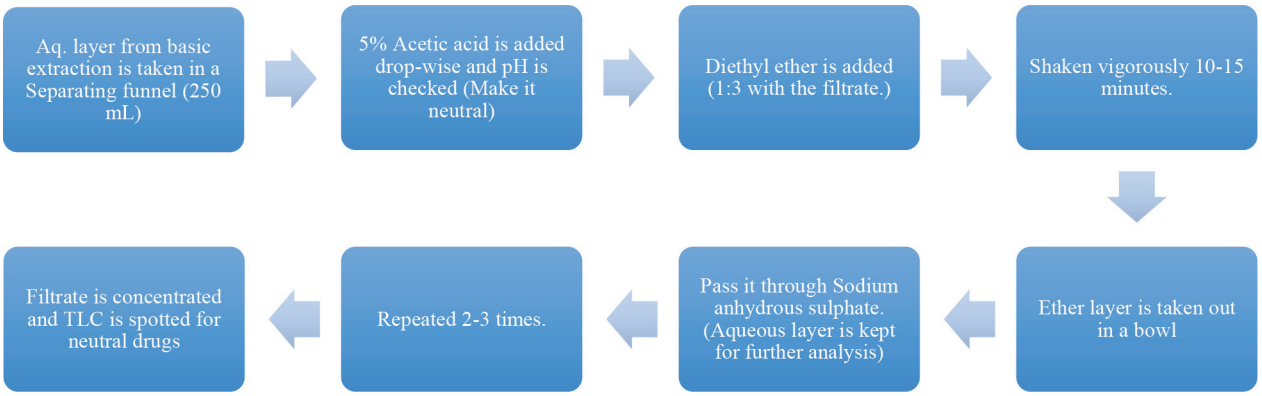
2.3.2.1 Acidic Extraction



2.3.2.2 Basic Extraction



2.3.2.3 Neutral Extraction



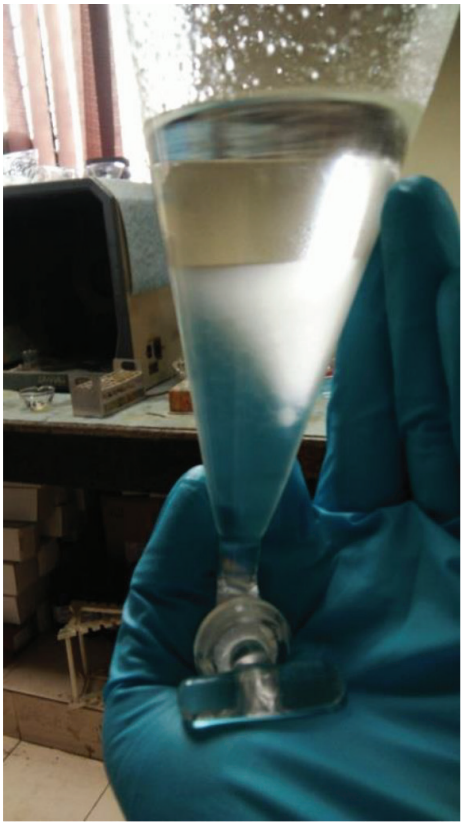


Figure 7. Drug extraction: Layer separation.

2.3.3 *Analysis of Jar 3*

3 - Blood

The jar containing blood is named 3. The ideal biological sample to test for any type of medication or toxin is blood because it is the only fluid that the body uses for circulation. Blood can be tested for heavy metals, narcotics, pesticides, and volatile poisons/drugs like alcohol, toluene, etc[31] (Figure 8).



Figure 8. 3-Blood.

The following steps are taken during the opening of the exhibit:

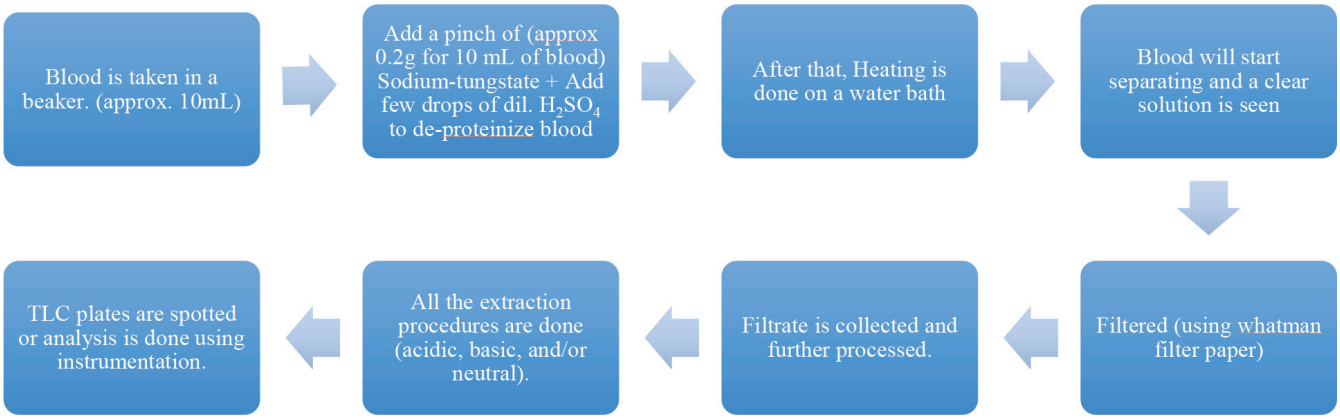
- Blood is present as an exhibit in Jar 3, which is measured, and noted.
- Then divided in half and maintained in the original viscera jar and a 250 mL conical flask.

Extraction procedure for the extraction of pesticides and drugs from blood is mainly processed by using one of the 2 methods:

- Liquid-Liquid Extraction (LLE) Method – When blood is present in ample amounts,
- Small Volume Liquid Extraction (SVLE) Method – When blood is in a small amount usually less than 2 mL.

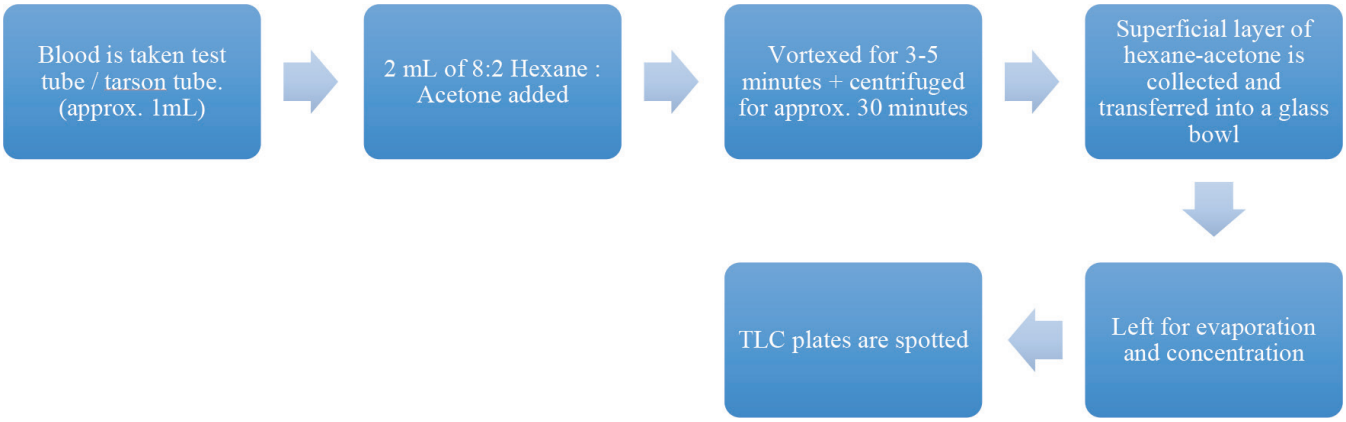
2.3.3.1 *Liquid-Liquid Extraction (LLE) Technique*

For Drugs:

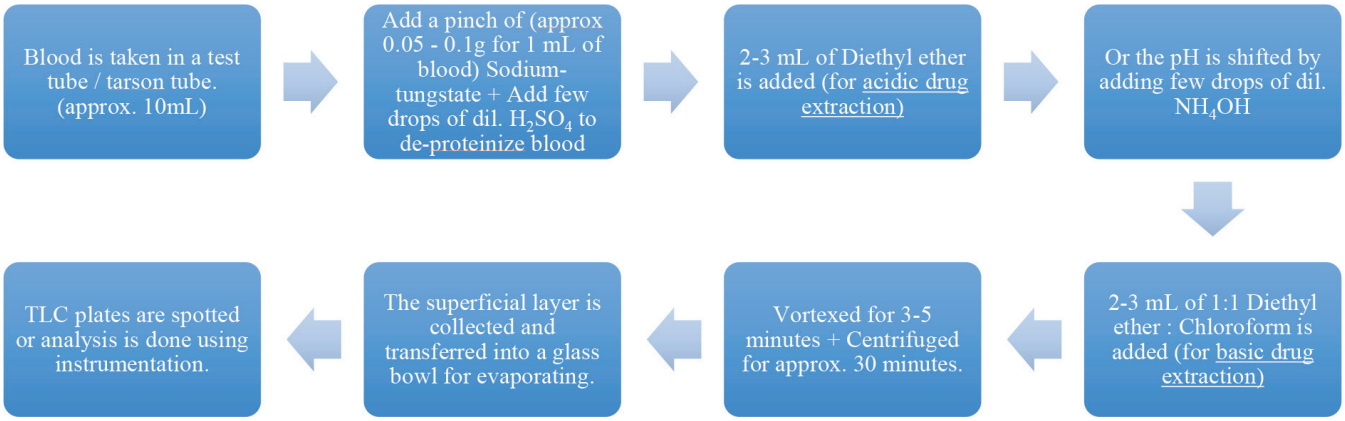


2.3.3.2 Small Volume Liquid Extraction (SVLE) Technique

For Pesticides:



For Drugs:



The best way is to analyse blood on any instrument, as per the pesticides/drugs needed.

2.3.4 Analysis of Jar 4

4 - Preservative

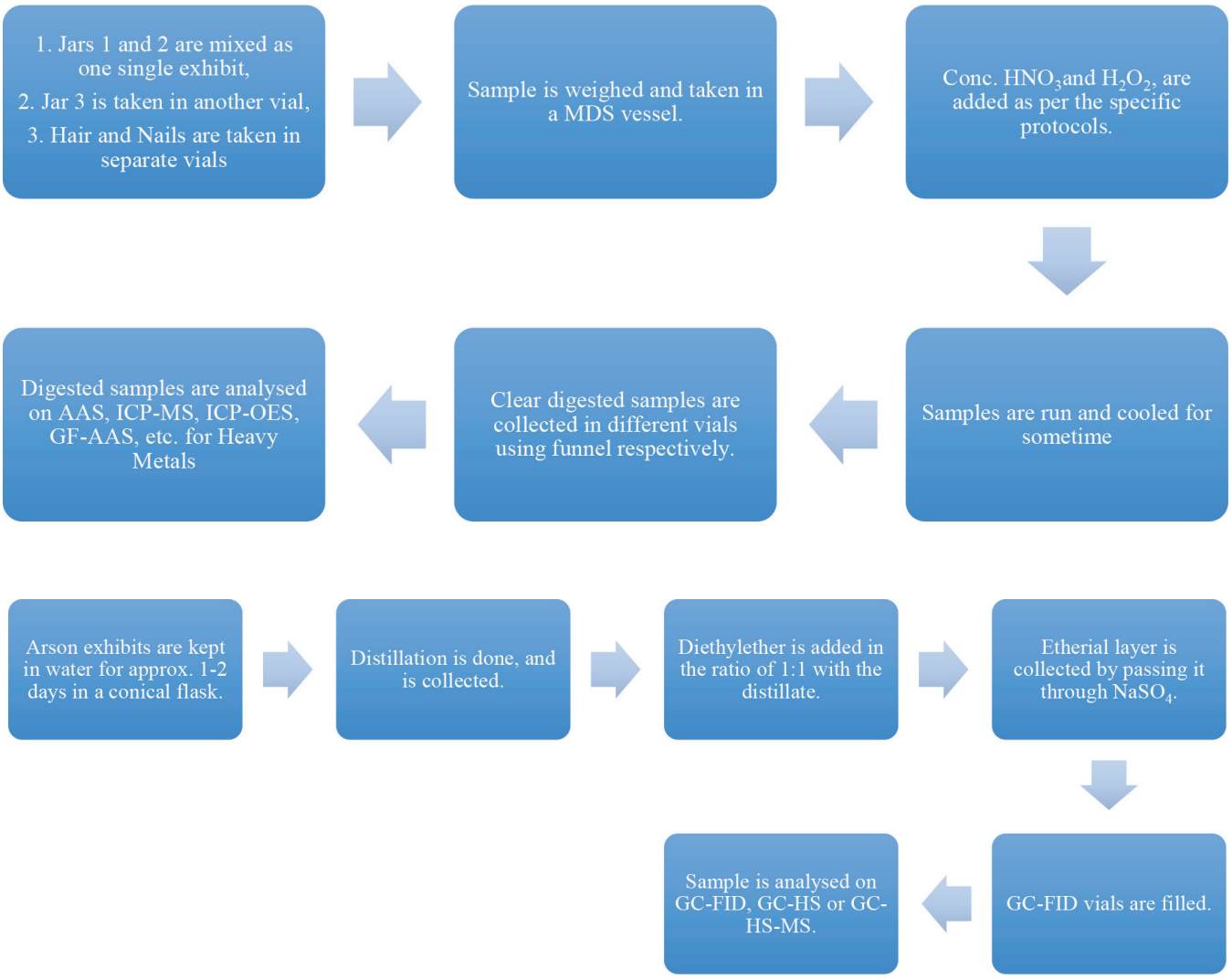
A preservative usually comes along with viscera exhibits. Preservatives often come in a small jar, though they can also be sent in packets. It could be solid, like alum crystals, or liquid, like a saturated salt solution. This is used to keep viscera samples safe and preserved for a longer time at room temperature.

2.4 Test for Heavy Metals

Test for Heavy Metals can be done in any biological exhibit provided. Acute exposure to heavy metals can be examined in blood and/or urine samples, and chronic exposure from samples from the viscera, hair, and nail samples. Heavy metals build up in the body and are difficult to eliminate, even minute amounts of them can be fatal for humans. Heavy metals include As, Hg, Cd, Pb, and several others. Typically, samples of urine, nails, and hair are given for the analysis. Such samples are frequently

digested using the microwave digestion technique, which requires a Microwave Digestion System (MDS). When utilizing MDS, different settings can be used to digest several samples[14].

Digestion Procedure



Arson exhibits are marked according to the number of exhibits that came up in the case. Each exhibit has its significance and can tell the seat of fire (origin point). If an exhibit from nearby the opening shows a large amount of fuel, then it is sure that it is the place least burnt, and hence could not be the seat of the fire[30].

Procedure:

2.5 Examination of Arson Exhibits

Arson exhibits are also received and analysed in the Toxicology/Chemistry division. Queries in arson cases generally include finding out whether the fire caught was accidental or intentional. And if intentional then what kind of fuel was used for the purpose i.e., petrol, kerosene, or diesel[20].

3. Summary and Conclusion

The above-mentioned viscera examination scenario is not exclusively followed in every forensic laboratory in India, as these protocols are confidential as well as modified according to the ease of the respective laboratories. Most of the protocols/steps are designed by applying their own hit and trial methods to the

standard protocols, Hence, a forensic scientist should always perform the procedures on controlled samples before applying them to case samples, to validate them accordingly.

Moreover, the time consumed in the analysis of a single case depends upon how simple or tedious the case is, the amount of viscera available in that case, how much experience the forensic scientist has, and how much manpower is available in the laboratory. Hence, a new protocol and methodology to isolate, extract and analyze pesticides, drugs, heavy metals, and various toxins from Viscera samples is developed.

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