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Handbook of FORENSIC ANALYTICAL TOXICOLOGY

SECOND EDITION

AK Jaiswal
Tabin Millo

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Chapter 3

Extraction/Isolation and Clean-up Methods

EXTRACTION/ISOLATION METHODS FOR PESTICIDES/INSECTICIDES

Pesticides/insecticides are extensively used in agriculture and household remedies for the control of insects and pests. Due to their easy availability, inadvertent knowledge, and quick action, these pesticides are being largely used for suicidal purposes. Basically, all these pesticides/insecticides are broadly classified as organophosphorus (OP) compound, organochlorine (OC) compound, carbamates, and pyrethroids.

Organophosphorus compounds are the derivatives of the oxoacids of phosphorus or thiophosphoric acid. The different acids from which organophosphates are being derived are phosphoric acid, thiophosphoric acid, dithiophosphoric acid, and miscellaneous OP compound. The important derivatives of the phosphoric acid having insecticidal activity are dichlorvos, naked, phosphamidon, phosphinon, phosdrin, bidrin, birlane, gardona, dimefox, mipafox, avenin, and cyolane. The replacement of one of the oxygen atoms by sulfur in the derivatives of phosphoric acid decreases the toxicity of the compounds related to mammals without substantial changes in the insecticidal or acaricidal activity. Thiophosphoric acid derivatives are more toxic to mammals compared to thiono derivatives. The common thiophosphoric acid are parathion, methyl parathion, paraoxon, thiophos ME, fenitrothion, chlorthion, dicapthon, ronnel, bromophos, fenthion, dasanit, diazinon, demeton, methyldemeton, dursban, potasan, vamidothion, acetophos, chlorpyrifos, etc. Derivatives of dithiophosphoric acid under the class are more stable but less toxic than the corresponding compound of thiophosphoric acid. These derivatives are found effective as insecticide in agricultural applications. The list of some important compounds are malathion, dimethoate, morphothion, formothion, thimet, ethion, ekatin, disyston, tetrathion, phosalone, imidan, guthion, menazon, edifenphos, etc. Other miscellaneous OP compound includes trichlorfon, O-ethyl O-(4-nitrophenyl) phenylphosphonothioate (EPN), etc.

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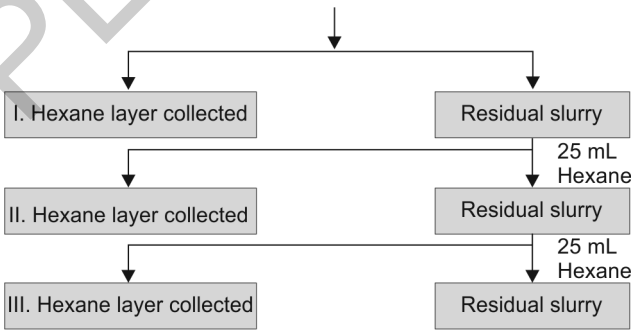
Organochlorine insecticides such as dichlorodiphenyltrichloroacetic acid (DDT), benzene hexachloride (BHC), lindane, endrin, dieldrin, endosulfan, heptox, chlordan, toxaphene, kelthane, heptachlor, methoxychlor, etc., are being extensively used in agriculture and are also familiar in domestic applications. New varieties of these insecticides are emerging every year. Owing to easy availability, these insecticides are frequently used in suicidal cases. Accidental poisoning cases are also known. Carbamates such as propoxur, carbaryl, carbofuran, zineb, etc., are also used for homicidal/suicidal cases. The mechanism of toxic manifestation in mammalian system is similar to OP insecticides, but the toxicity of the former is comparatively lesser. The pyrethroids have also emerged as a major class of synthetic organic insecticides from the time of its use for agricultural applications. Commonly used pyrethroids are fenvalerate, permethrin, dichlorovinyl, cyfluthrin, deltamethrin, fenpropathrin, bifenthrin, etc. These compounds are comparatively less toxic than other classes in respect of mammalian toxicity. Owing to their availability, these compounds are misused in criminal poisoning cases.

The procedures for extraction, isolation, and clean-up for analysis of pesticides in different biological materials have been shown in flowchart manner, which can be of handy reference for student/analyst/scientist/forensic toxicologist. Solvent extraction procedure is the most frequent method of choice, which is simple and easily available. It serves a threefold purpose, namely as a concentration step, as a clean-up procedure, and to render the sample in a form suitable for analysis.

From Tissues

Up to 50 g tissues + anhydrous sodium sulfate (Na_2SO_4) + 50 mL n-hexane is taken in a round bottom or in a conical flask fitted with air condenser and heated in water bath for 2–3 hours. The contents are filtered.



Three hexane layers I, II, and III are pooled + 15 mL of acetonitrile saturated with n-hexane is taken in a separating funnel and shaken vigorously for 2–3 minutes.

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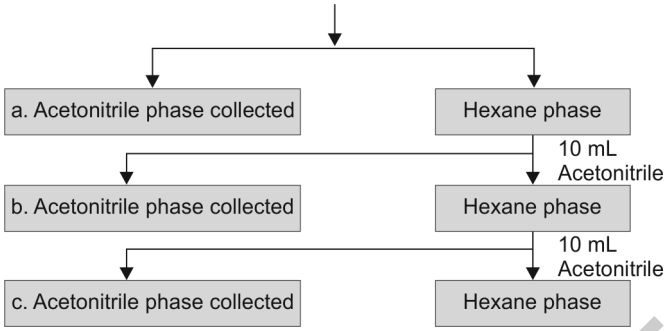
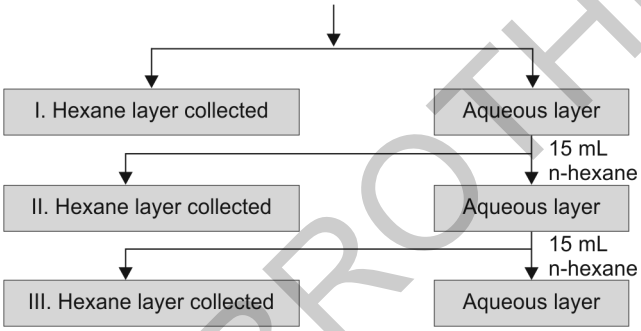
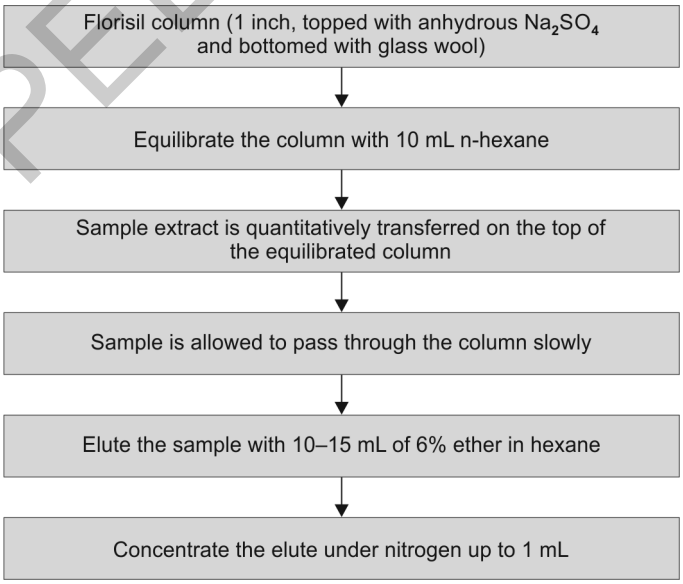


Acetonitrile phases a, b, and c are pooled + 50 mL water + 15 mL hexane is taken in a separating funnel and shaken vigorously for 2–3 minutes.



Hexane layer is pooled and concentrated to 1 mL either by nitrogen or in water bath.

Clean-up Process



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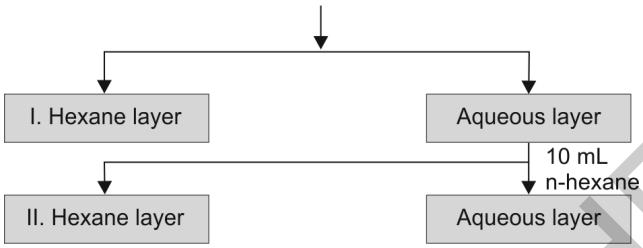
From Stomach Wash, Urine, Vomit, Etc.

20 mL of biological fluid (stomach wash, urine, vomit, etc.) + 50 mL of n-hexane is taken in a round bottom flask fitted with air condenser and heated in a water bath for half an hour. The contents are cooled, filtered, and mixed with 20 mL of hexane and taken in a separating funnel.

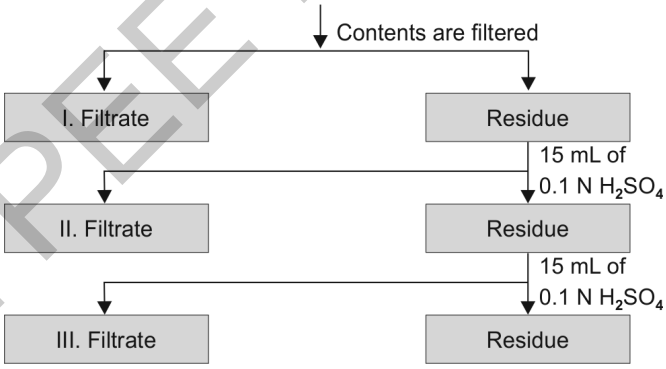


Hexane layer is collected, passed through anhydrous Na_2SO_4, and evaporated up to 1 mL. Loaded on a column of florisil already equilibrated with 10 mL n-hexane, sample allowed to percolate down the column and then eluting with 10 mL n-hexane:ether mixture (2:1) in a graduated tube and again concentrated up to 1 mL.

From Blood

Method I

10 mL blood + 10 mL of 10% sodium tungstate solution + 15 mL of 0.1 N sulfuric acid (H_2SO_4) and shaken for 2 minutes.



(H_2SO_4: sulfuric acid)

Filtrates I, II, and III are pooled + 20 mL of n-hexane is taken in a separating funnel and shaken for 2 minutes.

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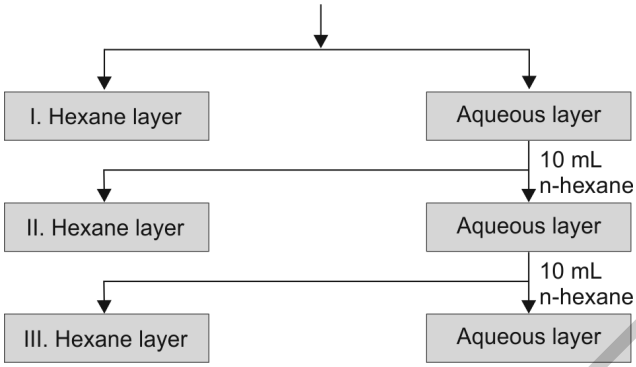
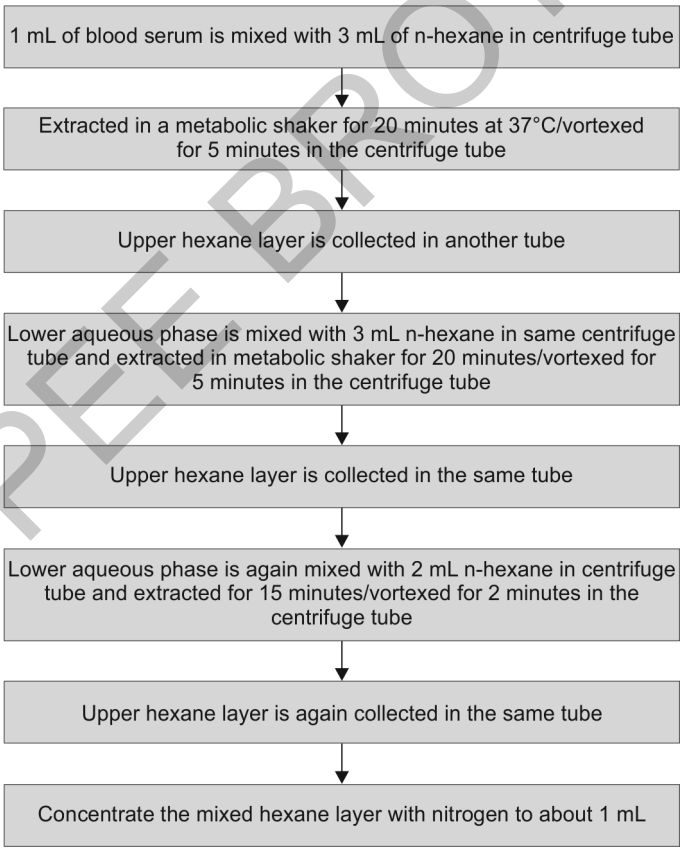


Hexane layers I, II, and III are pooled and passed through Na_2SO_4 and evaporated up to 1 mL. Clean-up process will be same as discussed in tissues.

Method II



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EXTRACTION/ISOLATION METHODS FOR VOLATILE POISONS

Poisons, which are volatile in nature, are called as volatile poisons such as acetaldehyde, acetone, aniline, benzene, benzyl alcohol, carbon disulfide, carbon tetrachloride, chloral hydrate, chloroform, cresols, ethyl alcohol, hydrocyanic acid, formaldehyde, isopropyl alcohol, methyl alcohol, naphthalene, paraldehyde, phenol, nitrobenzene, etc. But mainly ethanol and methanol are the most commonly used volatile poisons.

Acetaldehyde: It is colorless, inflammable liquid with boiling point (bp) 20.2°C. It is miscible in water, ether, and ethanol and obtained as byproduct of alcoholic fermentation.

Acetone: It is a clear colorless volatile inflammable liquid with bp 56°C. It is miscible in water, ether, chloroform, and ethanol and obtained as a product of destructive distillation of wood/byproduct of starch fermentation.

Aniline: It is colorless but with time turns to brown color due to nitroso compound formation with bp 184°C. It is miscible in alcohol, benzene, chloroform, and other organic solvents, but insoluble in water and forms salts with mineral acids.

Benzene: It is a clear colorless, inflammable liquid with characteristic aromatic smell with bp 80°C. It is partially miscible in water, miscible in alcohol, acetone, ether, and glacial acetic acid, and obtained in light oil fraction of coal tar distillation.

Benzyl alcohol: It is a colorless liquid with faint aromatic odor with bp 203°C. It is soluble in water, methanol, chloroform, and ether and obtained as ester of cinnamic acid and benzoic acid in Balsam of Tolu and Peru.

Carbon disulfide: It is a colorless liquid with faint aromatic odor with bp 203–208°C and inflammable liquid with characteristic aromatic smell with bp 80°C. It is soluble in water, miscible in ethanol, ether, and chloroform, and obtained from coal tar.

Carbon tetrachloride: It is a heavy clear colorless liquid with chloroform-like odor and is volatile. It is almost insoluble in water, miscible in dehydrated alcohol, chloroform, and ether, and synthesized by chlorination of hydrocarbon.

Chloral hydrate: It is colorless/white crystal, which liquefies between 50°C and boils at 98°C. It is soluble in ether and chloroform and partially soluble in water and it is a synthetic compound.

Chloroform: It is colorless volatile liquid with bp 61°C. It is insoluble in water and miscible with dehydrated alcohol and ether.

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Cresols: They are almost colorless to pale brownish-yellow liquid, which becomes darker with age/light exposure. It is soluble in water, miscible in ethanol, ether, and chloroform, and occurs in coal tar and in pine and bleached wood tar.

Ethyl alcohol: It is transparent, colorless, and volatile liquid having spirituous odor and burning taste with bp 78.4°C. It is hygroscopic and found as free alcohol in some fruit juices/as ester in some eucalyptus oil and produced by starch, molasses, and grapes fermentation.

Formaldehyde: It is colorless, inflammable gas. It is soluble in water and slightly soluble in ethanol and ether.

Hydrocyanic acid: This smells like bitter almonds. Salts are highly soluble in water and alkaline in nature and are obtained naturally from cherry, apricot, peach, pears, and bitter almond.

Isopropyl alcohol: It is a clear colorless, inflammable, and volatile liquid with bp 81–83°C. It is miscible in water, ethanol, chloroform, and ether and obtained by fermentation.

Kerosene: It is an inflammable oily liquid of characteristic odor with boiling range of 150–300°C. It is obtained by petroleum fractionation.

Methyl alcohol: It is a colorless extremely poisonous liquid of peculiar odor with bp of 67°C. It is soluble in water and organic solvents and synthesized commercially/obtained from the liquid fraction pyroligneous acid in the destructive distillation of wood.

Naphthalene: It is a colorless with melting point of 80°C and bp of 208°C. It is practically insoluble in water, miscible in ethanol and chloroform and highly soluble in ether, and obtained in middle oil fraction of coal tar distillation.

Nitrobenzene: It is a pale yellow oily liquid with bp of 206°C, having odor of bitter almonds. It is practically insoluble in water, miscible in ether and methanol, and produced by nitration of benzene.

Paraldehyde: It is a clear colorless or pale yellow liquid with bp of 123–126°C. It is soluble in water, miscible in ethanol, chloroform, and ether, and produced by polymerization of acetaldehyde.

Phenol: It is a colorless or faintly pink, deliquescent crystals, or crystalline mass with bp of 181°C. It is partially soluble in water, soluble in ethanol, chloroform, and ether, and obtained in light oil fraction of coal tar distillation.

Extraction/isolation of volatile poison is done by distillation, which may be acidic, basic, or neutral.

The procedures for extraction/isolation for analysis of volatile poisons in different biological materials have been shown in a flowchart manner, which can be of handy reference for student/analyst/scientist/forensic toxicologist.

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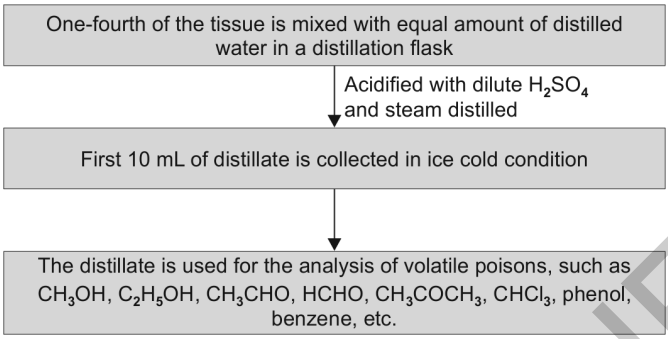
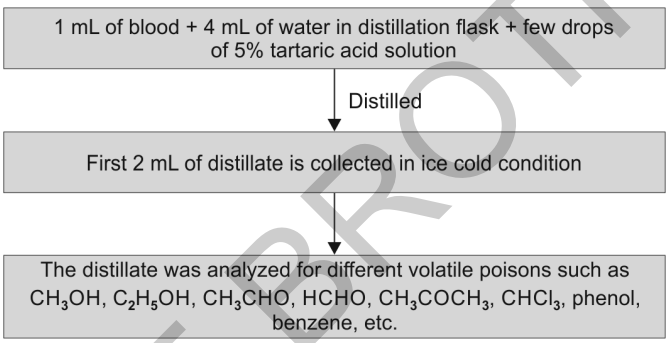
Neutral and Acid Distillation

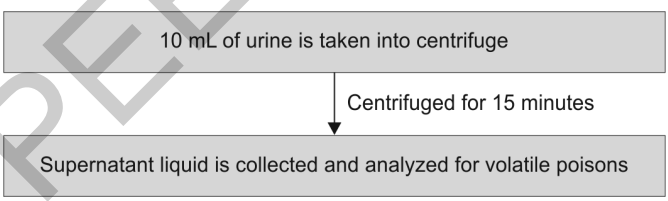
From Viscera



From Blood

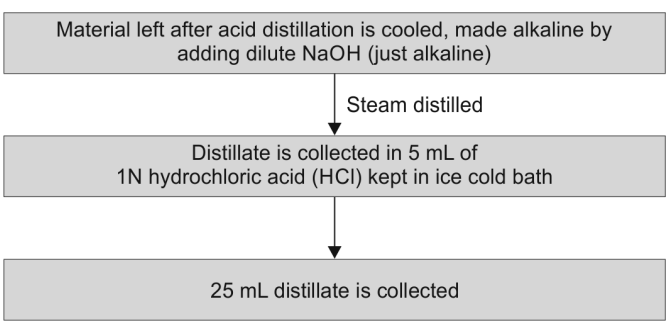


From Urine



Alkaline Distillation

Method I



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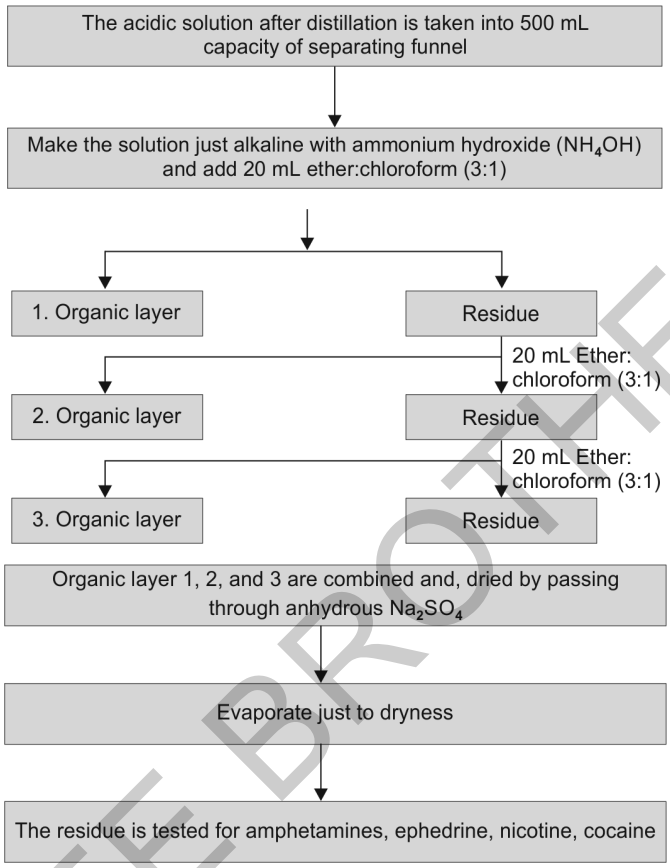
Method II



EXTRACTION/ISOLATION METHOD FOR CYANIDE

Cyanides are compound, containing the CN group such as cyanogens, the cyanogen halides, hydrogen cyanide, and the metal cyanides. The metal cyanides include the compounds that contain cyanide as an anion such as KCN and compounds in which cyanide is complexed to a metal as a ligand. Cyanide is one of the most rapidly acting of all poisons and in the form of the hydrocyanic acid and its sodium and potassium salts, it is one of the most deadly. The hydrocyanic acid is extremely volatile producing the deadly gas hydrogen cyanide, which has the distinctive odor of bitter almonds. Inhalation causes severe toxic effects that lead to death within minutes. The chemist who discovered cyanide, Carl Wilhelm Steele, was killed by its vapors. Exposure to cyanide can also occur by inhalation of cyanogen gas. The determination of cyanide in body fluids requires the separation of cyanide from thiocyanate, usually by distillation of cyanides or microdiffusion into an absorber solution.

The procedures for extraction/isolation of cyanide from different biological materials have been shown in a flowchart manner, which can be used by student/analyst/scientist/forensic toxicologist.

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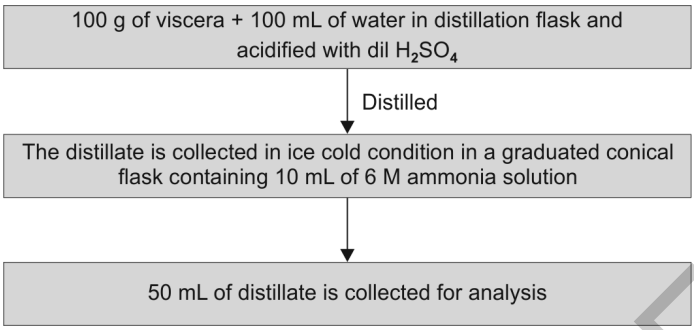
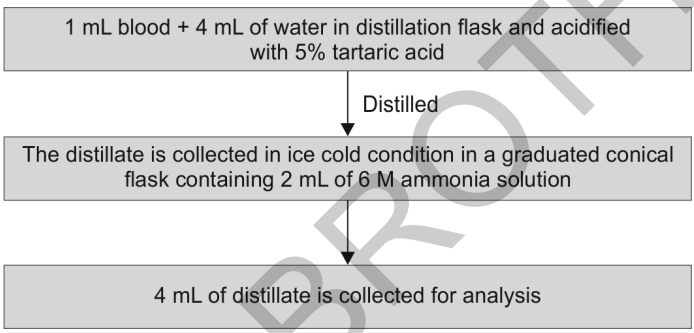
From Tissues



From Blood



EXTRACTION/ISOLATION METHODS FOR TRANQUILIZERS

Tranquilizer is an old term that means a drug which reduces mental tension and produces calmness without inducing sleep or depressing mental faculties. Common tranquilizers are chlorpromazine, promazine, promethazine, prochlorperazine, mepazine, ethopropazine, trimeprazine, perphenazine, thiopropazate, triflupromazine, trifluoperazine, fluphenazine, methoxypromazine, thioridazine, reserpine, benactyzine, azacyclonol, hydroxyzine, imipramine, etc. Tranquilizers are divided into major tranquilizer and minor tranquilizer groups. Major tranquilizers include phenothiazines, indoles, thioxanthenes, butyrophenones, piperazine, and piperidine compounds. Minor tranquilizers are more common of the tranquilizers. These include benzodiazepines, which are very commonly prescribed as antianxiety drugs or anxiolytics. They are often referred to as sedatives/hypnotics. They are central nervous system (CNS) depressants with specific sites of action. The primary route of administration for these medications is oral, swallowed as a tablet, capsule, or liquid. They are also available in solution form for intravenous use. Tranquilizers are the most widely prescribed psychotherapeutic agents in the world and are most involved in suicide attempts and accidental overdoses.

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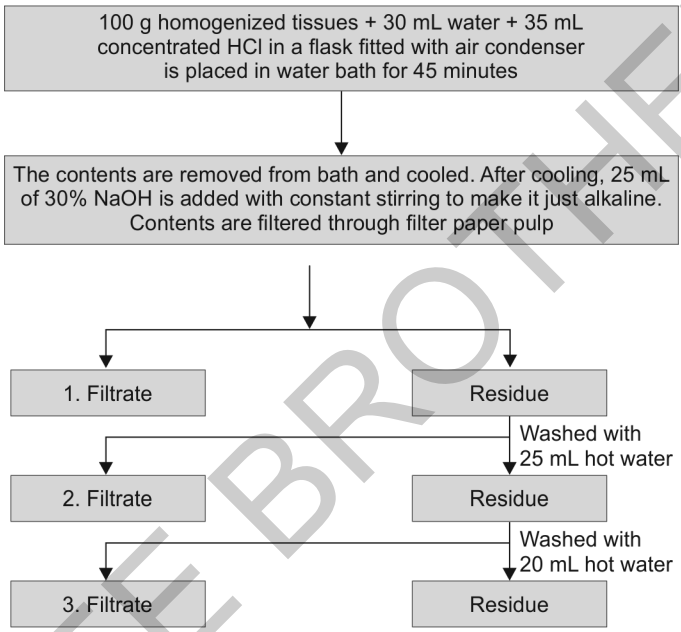
The procedures for extraction/isolation and clean-up procedures for analysis of tranquilizers in different biological materials have been shown in a flowchart manner, which can be of handy reference for student/analyst/scientist/forensic toxicologist. Solvent extraction procedure is the most frequent method of choice, which is simple and easily available.

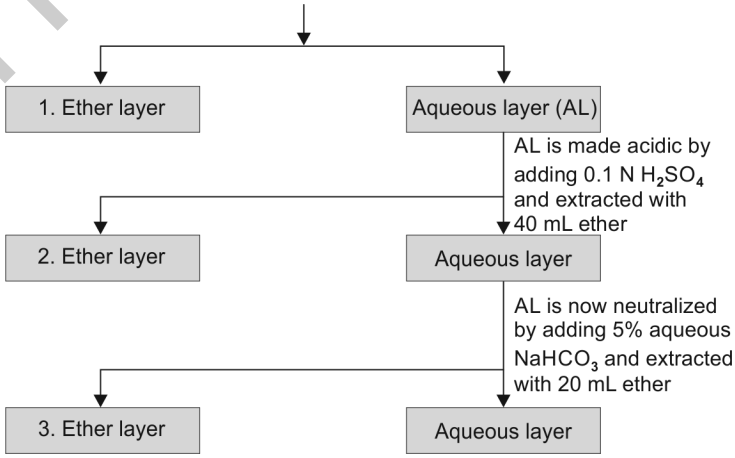
From Tissues

Step 1



Step 2

The filtrates 1, 2, and 3 are combined, taken into a separating funnel. Up to 40 mL of ether is taken into a separating funnel and shaken for 5 minutes.



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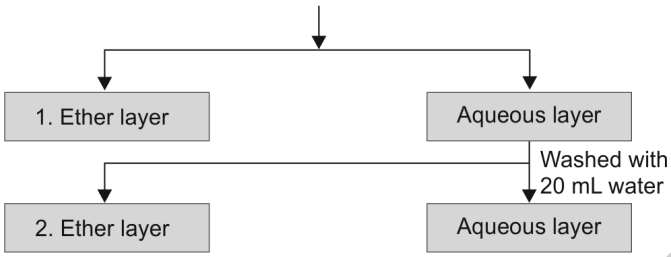
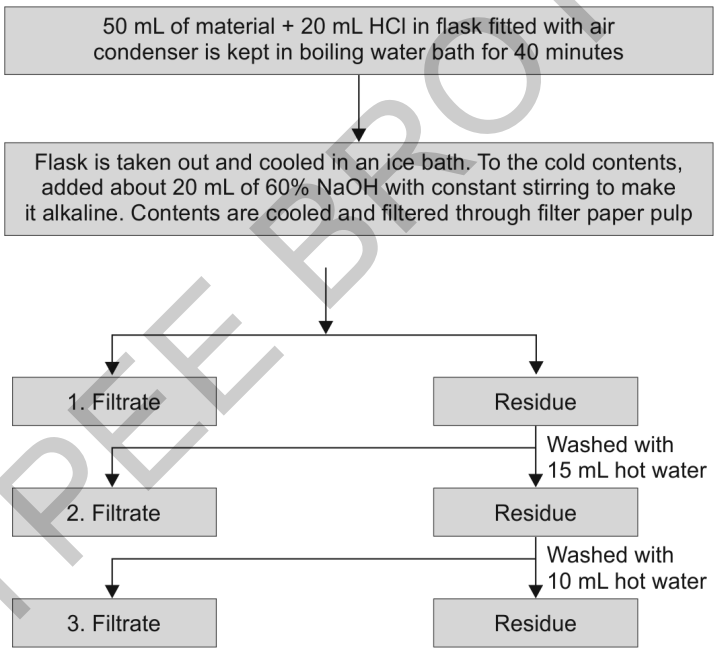
The entire three ether layers 1, 2, and 3 are combined and taken into a separating funnel and washed with 25 mL portion of water.



Ether layer is combined and passed through anhydrous Na_2SO_4 and evaporated to dryness. The residue contains tranquilizers.

From Stomach Wash/Vomit/Urine

Step 1



Step 2

The filtrates 1, 2, and 3 are combined and remaining method of extraction is same as discussed in tissues.

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